DEVELOPING AND EVALUATING THAI STUDENTS' UNDERSTANDINGS OF BACTERIAL CONJUGATION AND PROTEIN PURIFICATION USING PRACTICAL WORKS

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
(SCIENCE AND TECHNOLOGY EDUCATION)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2007

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Thesis
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was submitted to the Faculty of Graduate Studies, Mahidol University
For the degree of Doctor of Philosophy (Science and Technology Education)
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This study was financially supported by the scholarship under
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the Institute for the Promotion of Teaching Science Technology (IPST),
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This work is dedicated to the memory of Prof. Dr. Simon Baumberg.
Through his kindness to me and my fellow students' life,
he helped shape me to be a good science teacher.

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ACKNOWLEDGEMENTS

I would like to thank my advisor Associate Professor Dr. Bhinyo Panijpan, Director of Institute for Innovation and Development of Learning Process, Mahidol University for giving me the opportunity to work in both science and education. I am greatly indebted to my co-advisor Assistant Professor Dr. Arinthip Thamchaipenet, Department of Genetics, Faculty of Science, Kasetsart University for her continuous support, guidance and encouragement throughout my research. They painstakingly commented on the drafts of each aspect of this study and suggested valuable discussions during their supervision sessions. Thanks also to Associate Professor Dr. Pintip Ruenwongsa, Chair of Science and Technology Education Programme, for her helpful management along my Ph.D. study.

My sincere thanks to Professor Dr. Edward J. Wood, School of Biochemistry and Molecular Biology, University of Leeds, UK, for his kindness in examining the research instruments and providing suggestions throughout my dissertation involving on manuscripts for publication and who was the chair of the thesis defence.

I owe a huge debt of gratitude for Dr. Kenneth J. McDowall for hosting me to do researches in gene expression in *S. coelicolor* and purification of His-tagged AtrA by Ni^{2+} affinity chromatography at the Institute of Integrative and Comparative Biology, Faculty of Biological Sciences, University of Leeds, UK. My special thanks are also expressed to the late Professor Dr. Simon Baumberg for his excellent comments on the conjugation experiment for teaching Thai secondary school students.

I adequately express my gratitude for Professor Dr. Andrew G. Booth, School of Biochemistry and Molecular Biology, University of Leeds, UK, who offered valuable project on Protein Purification Program translated into Thai for teaching Thai students. Especially, I would like to say thanks to Professor Dr. Phillip Scott and Professor Dr. John Leach, Faculty of Education, University of Leeds, UK, for giving very helpful knowledge in science education. Special thanks to Dr. Jenny Lewis, Faculty of Education, University of Leeds, UK, for her healthful comments on my manuscript.

I would like to acknowledge Associate Professor Dr. Piroh Pinphanichakarn, Department of Microbiology, Faculty of Science, Chulalongkorn University for her kindness to approve my teaching instruments on bacterial conjugation. Thanks to Dr. Chaovaree Ruangvilaisap, Department of Microbiology, Faculty of Science, Silpakorn University as well as Dr. Chanan Phonprapai and Thirawatthana Pharamat, Department of Biotechnology, Faculty of Science, Thammasart University, to give their permissions for using Protein Purification Program in their classes. I am delightful to thank Sataporn Wantanawijarn and her academic staffs for helping me to step into teaching students using the learning model on bacterial conjugation in Mahidol Wittayanusorn School. My special thanks are expressed to all students for giving their attentions to learn with my practical works.

For the friendly and stimulating everyday working environment in Leeds, I would like to thank my friends Dr. Bin Hong, Dr. Gabriel Uguru, Jane Towle and Stephanie Jourdan. I should express my special thanks to my friends in Mahidol University who gave my enjoyable Ph.D. life and helpful discussions on my research, particularly Arun Chanchaichaovivat and Assistant Professor Dr. Joompot Poomsripanon and in Kasetsart University, especially Nirinya Sudtachat, Apanthee Tanittaisong, Sirirat Mienkerd, Aiyada Aroonsri and Ratchaniwan Jeamseang. I am also thankful to staffs at the Institute for Innovation and Development of Learning Process for their mindful helps.

Words cannot sufficiently express my sincere gratitude to my family for their love and patience during my academic studies. This dissertation is dedicated to the memory of my father whose strong thought-provoking insights on my life and spirit were never diminished eternally.

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DEVELOPING AND EVALUATING THAI STUDENTS' UNDERSTANDINGS OF BACTERIAL CONJUGATION AND PROTEIN PURIFICATION USING PRACTICAL WORKS

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ABSTRACT

Streptomyces are filamentous Gram-positive soil bacteria that can produce abundantly industrial secondary metabolites and enzymes. The first part of this research describes the appropriate condition for conjugal transfer of plasmid pIJ8600 from *E. coli* to *S. rimosus* 593, R7 and M4018. The suitable condition for pre-germination of spores of *S. rimosus* R7 and M4018 was the heat treatment at 40 ºC for 10 min. The mycelia without heat treatment can be used as recipients for the non-spore forming strain *S. rimosus* 593. The suitable conjugative media is Oxoid TSA containing 10 mM $MgCl_2$. The chromosomal attachment site (*attB*) of *S. rimosus* R7 is identical to those of *S. rimosus* M4018 and *S. avermitilis*. The second part of this study describes the nature of the post-translational modification of AtrA in *S. coelicolor*. This modification is brought about purifying the AtrA by Ni^{2+} affinity chromatography and analysing the DNA binding activity of complexes between AtrA and *act*II-ORF4 using electrophoretic mobility shift. DNA binding activity of AtrA after post-translational modification in *S. coelicolor* could not be detected. However, DNA binding activity in non-modification form from *E. coli* was 140.6 nM.

The knowledge from first two parts of this study were used to construct the practical works in 'bacterial conjugation' and 'protein purification' to teach at the senior secondary school and undergraduate levels, respectively. The findings showed that having a teaching lab before lecture can assist secondary school students to develop a better understanding of bacterial conjugation compared to teaching by lecture only and teaching lecture before lab. In teaching protein purification by using a virtual class, teaching with a Thai/English version can help undergraduates to have a better construction of the purification concepts than teaching with the English version only. From these findings, teaching in a practical hands-on way, by using Kolb's and Winn's constructivist learning models, is required for students to construct biological concepts.

KEYWORDS: BACTERIAL CONJUGATION / PROTEIN PURIFICATION / PRACTICAL WORK / EXPERIENTIAL LEARNING MODEL

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การพัฒนาและประเมินความเข้าใจของผู้เรียนไทยเรื่องการคอนจูเกชันของแบคทีเรียและการทำโปรตีนให้บริสุทธิ์ด้วยบทปฏิบัติการ (DEVELOPING AND EVALUATING THAI STUDENTS' UNDERSTANDINGS OF BACTERIAL CONJUGATION AND PROTEIN PURIFICATION USING PRACTICAL WORKS)

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บทคัดย่อ

Streptomyces เป็นแบคทีเรียแกรมบวกในดินที่มีลักษณะเป็นเส้นใย สามารถผลิตสารเมแทบอไลต์ทุติยภูมิและเอนไซม์ที่ใช้ในอุตสาหกรรมต่าง ๆ ส่วนแรกของการวิจัยเป็นการหาภาวะที่เหมาะสมสำหรับการส่งถ่ายพลาสมิด pIJ8600 จาก *E. coli* ไปยัง *S. rimosus* 593, R7 และ M4018 พบว่า ภาวะที่เหมาะสมสำหรับกระตุ้นให้สปอร์ของ *S. rimosus* R7 และ M4018 งอกก่อนการคอนจูเกชัน คือ ที่อุณหภูมิ 40 °ซ นาน 10 นาที ขณะที่เส้นใยของ *S. rimosus* 593 สายพันธุ์ที่ไม่สร้างสปอร์ สามารถใช้เป็นผู้รับในการคอนจูเกชันได้อาหารที่เหมาะสมสำหรับคอนจูเกชัน คือ Oxoid TSA ที่มี $MgCl_2$ เข้มข้น 10 มิลลิโมลาร์ และเมื่อเปรียบเทียบลำดับเบสของ attachment site บนโครโมโซม (*attB*) พบว่า *S. rimosus* R7 มีลำดับเบสของ *attB* เหมือนกับ *S. rimosus* M4018 และ *S. avermitilis* ส่วนที่สองของการวิจัยเป็นการติดตามการเปลี่ยนแปลงของโปรตีน AtrA หลังการแปลรหัสใน *S. coelicolor* โดยทำโปรตีน AtrA ให้บริสุทธิ์ด้วยวิธี Ni^{2+} affinity chromatography และวิเคราะห์ DNA binding activity ระหว่าง AtrA กับ *act*II-ORF4 ด้วยวิธี electrophoretic mobility shift พบว่า AtrA ที่มีการเปลี่ยนแปลงไปหลังการแปลรหัสจาก *S. coelicolor* ไม่สามารถตรวจวัดได้ ส่วน AtrA ใน *E. coli* ซึ่งไม่มีการเปลี่ยนแปลงหลังการแปลรหัส มีค่า DNA binding activity เท่ากับ 140.6 nM

จากการนำองค์ความรู้ในสองส่วนแรกของงานวิจัยมาใช้สร้างบทปฏิบัติการเรื่อง 'การคอนจูเกชันในแบคทีเรีย' และ 'การทำโปรตีนให้บริสุทธิ์' และนำไปสอนในระดับมัธยมศึกษาตอนปลายและระดับปริญญาตรี ตามลำดับ พบว่า เมื่อสอนด้วยบทปฏิบัติการก่อนบรรยายช่วยให้ผู้เรียนระดับมัธยมศึกษาตอนปลายพัฒนาความเข้าใจเรื่องคอนจูเกชันของแบคทีเรียได้ดีที่สุด เมื่อเปรียบเทียบกับการสอนแบบบรรยายอย่างเดียวและการสอนแบบบรรยายก่อนทำปฏิบัติการ สำหรับการสอนการทำโปรตีนให้บริสุทธิ์ด้วยห้องปฏิบัติการเสมือนรุ่นภาษาไทยและภาษาอังกฤษ ช่วยให้ผู้เรียนสามารถสร้างมโนทัศน์ของการทำโปรตีนให้บริสุทธิ์ได้ดีกว่าการสอนด้วยรุ่นภาษาอังกฤษอย่างเดียว จากผลดังกล่าวสรุปได้ว่า การสอนบทปฏิบัติการด้วยการสร้างองค์ความรู้ของคอล์ปและวินน์สามารถช่วยให้ผู้เรียนสามารถสร้างมโนทัศน์ทางชีววิทยาได้

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LIST OF ABBREVIATIONS

AtrA	Actinorhodin-associated transcriptional regulator
bp	base pair(s)
BSA	bovine serum albumin
DMSO	dimethylsulfoxide
kb	kilobase pair(s)
MW	molecular weight
Ni-NTA	Ni^{2+}-nitrilotriacetate
OD	optical density
PCR	polymerase chain reaction
PEG	polyethylene glycol
ppGpp	guanosine 5'-diphosphate-3-diphosphate
rpm	revolutions per minute
RT	room temperature
SARP	*Streptomyces* antibiotic regulatory protein
SDS	sodium dodecyl sulphate
V	volt
v/v	volume by volume
w/v	weight by volume

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CHAPTER I
INTRODUCTION

The trends in the postgenomic era to explain various biological events in cells and tissues, e.g. the genetic transfers, gene expressions, dynamics of protein networks, nucleic acid-interacting proteins and metabolic systems, require a plethora of “omics” (i.e. genomics, proteomics and metabolomics), analysis and interpretation (Kwangwon et al., 2006; Stead et al., 2006) including bioinformatics tools (Shachak et al., 2003). Secondary school students are limited in genetic knowledge and available time to participate in advanced research and their knowledge does not correlate with higher levels (Hanauer et al., 2006). The significance of recent advances in molecular genetics, moreover, is often lost on undergraduate students (Griffin et al., 2005). Many students do not understand how research scientists use omics to investigate biological events, for example, the use of bioinformatics and functional genomics to determine the genetics of human disease and development (van Omen, 2002). Therefore, teaching the basic knowledge such as genetic transfers and protein purification should be considered in order to reinforce students’ understanding at senior secondary school and undergraduate levels of advanced knowledge of genomics and proteomics.

Learning science is essentially an “enculturation” rather than a discovery of scientific knowledge and ways of thinking. Science learning is viewed as developing new ways of thinking about natural phenomena that are different from the everyday ways (Driver, Asoko, et al., 1994). To learn science is not only learning what previous generations of scientists thought about the world, but it also means understanding how a new generation of scientists re-makes our view of the world and how they may have some degree of participation in the process of invention and discovery (Millar & Osborne, 1998). The main objective of learning science is to rationally understand the basic concepts of any field being studied. The key terms here related to how to learn science are ‘concepts’ and ‘understanding’ which are mental objects and a mental process, respectively. Both belong to a new terminology referred to as a cognitive

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model of science education. Therefore, teachers have an important role to play in constructing students' scientific knowledge of the natural world from students' previous ideas on learning science in the classroom (Driver, Asoko, et al., 1994; Driver, Squires, et al., 1994; Lemke, 2000).

'Learning is not separated from reality' (Papert, 1993), so the teaching approaches in this study are designed to take accounts of students' existing ideas and to help students to develop the target science ideas using an experiential learning model (ELM). Hands-on experiments are experiential learning tools in science education (Ma & Nickerson, 2006) and laboratory experience makes science come alive (Clough, 2002). Hands-on experiments or 'wet labs' have traditionally been used in order to allow students, using their experiences, to learn with understanding, to reconstruct basic concepts and ideas, to demonstrate concepts taught in the classroom as well as to teach concepts in the case of enquiry-based science curricula (Kirschner & Huisman, 1998; Tobin, 1990). Besides students' cognitive growth, wet practicals have the potential to enhance constructive social relationships and positive attitudes by cooperative learning (Hofstein & Lunette, 1982; Lazarowitz & Tamir, 1994). 'Dry labs' or non-laboratory practicals, particularly using multimedia, are used to help students achieve high-order thinking in cognitive skills (e.g. analysis, synthesis and evaluation), which are needed to practise science and to carry out science enquiry (Kirschner & Huisman, 1998; Skinner & Hoback, 2003).

Very often researchers, however, have failed to use hands-on experiences to reconstruct scientific knowledge of natural world (Clough, 2002; Lunetta, 1998; Nakleh et al., 2002) since there are insufficient data to indicate whether laboratory instructions are effective or whether the teaching media are ineffective for attaining the goal of teaching and learning science. Appropriate hands-on experiments are sufficient in helping students reconstruct their knowledge and develop logical skills such as enquiry-type skills and problem-solving skills (Gunstone, 1991; Tobin, 1990). In addition, they can assist students to develop psychomotor skills (i.e. manipulative and observational skills) and promote positive attitudes and skills regarding cooperation and communication. They also have the potential to provide science teachers with opportunities to vary their instructional techniques and avoid a monotonous classroom learning environment (Hofstein, 2004).

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Bransford et al. (2000) claimed that using hands-on experiments is a powerful way of grounding emergent knowledge; however, students have to learn with a good teaching sequence to evoke the underlying conceptual understanding that aids generalisation. Tobin (1990) also suggested that meaningful learning is possible in the laboratory where the students are given opportunities to manipulate equipment and materials in an environment appropriate for them to construct their knowledge of phenomena and scientific concepts. According to these claims, the best way to learn biology is through direct hands-on experiences that can help foster the empathy and understanding necessary for sustainable development in education (e.g. Marrs & Novak, 2004; Huxham et al., 2006; Weston et al., 2002). Hands-on experiments, moreover, are used to understand the concepts, to connect theory to practice, to stimulate interest and enjoyment and to practise laboratory skills and techniques (Jenkins, 1999; Ottander & Grelsson, 2005). The student's prior knowledge in areas studied is, therefore, necessary for analysis of the differences between existing ideas and the target science ideas. These differences between the everyday-life view and the school science view give rise to a learning demand associated with trying to understand scientific explanations (Leach & Scott, 1995, 2002; Mortimer & Scott, 2003).

Although education has been reformed in Thailand, there are still problems in science teaching and learning. In general, the budget given to each school is inadequate to facilitate the purchase of chemical reagents, equipment and instruments in the laboratories (Ministry of Education [MOE], 2005). Although science education in Thailand has developed considerably during the past decade, the effort has not yet been all that successful, particularly in implementing the laboratory approach to learning scientific knowledge in schools. In addition, most secondary schools do not have laboratories provided in every class. Since almost all examinations in Thailand are multiple choice questions (e.g. final exams and national tests including the entrance exams for higher education in the former educational system), students generally accept and rarely complain about the traditional lecture approach. Furthermore, there is high number of students per class (on average 36-45 students; MOE, 2006) that inhibits teachers from offering laboratory classes. The number of various science teachers has also decreased in many schools and universities. Some

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teachers have to teach various fields of science. Science teachers tend to accumulate in big or famous schools and universities located in big towns.

This research was carried out so that its outcomes can help rectify problems related to manipulative skills and exposure to real problems in the students' learning by giving them an opportunity to learn science by doing it directly or by solving scenario problems. We have provided easy hands-on experiments to help students to construct scientific knowledge from their experiences by designed teaching interventions.

The focus of the present study is to investigate the feasibility of improving senior secondary school (aged 14-17) and undergraduate Thai students' conceptual understanding of bacterial conjugation (a natural process) and protein purification (a succession of manipulative steps) following planned teaching interventions. Because there are few details on genetic transfers and protein purification taught in Thai curricula, providing the conceptual underpinnings can help students to make sense of these. However, if the key concepts are not provided, students may simply memorise things taught to them rather than being able to understand, recognise and apply the concepts appropriately. For example, without the concepts of the properties of proteins, students might not be able to choose a suitable technique to purify a protein, and without the concepts of the mechanism of conjugation students might think that all bacteria have to use sex pili to transfer genetic material.

The study was, therefore, started initially to teach how scientists understand genetic transfer and protein purification by using the intergeneric conjugation from *E. coli* to *Streptomyces* and purifying the AtrA protein from *E. coli* and *Streptomyces* as models. The practicals, a laboratory exercise of conjugation in *E. coli* and Thai-translated Protein Purification Program involving teaching sequences and assessment tools, were then developed to promote the conceptual understanding of bacterial conjugation and protein purification. The simple conceptual models were designed to be accessible for students in explaining not only phenomena, techniques and mechanisms but also applying their knowledge to unknown situations such as purifying a protein from an unknown mixture composed of twenty proteins or conjugative step of intergeneric conjugation between Gram-positive and Gram-negative bacteria. In this way of teaching approaches aimed to provide the bases for future learning and researching in genomics and proteomics.

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CHAPTER II
OBJECTIVES

The work involved first a scientific study of plasmid transfer as well as the purification of AtrA protein and second the investigation of how this sort of information can be transmitted to students.

2.1 Rationales in scientific aspects

2.1.1 Conjugal transfer of plasmid pIJ8600 from *E. coli* to *S. rimosus*

Streptomycetes are 'boundary organisms' between prokaryotes and eukaryotes that show complex morphological differentiation resembling that of filamentous fungi. It makes this genus a model prokaryote for studying multicellular differentiation (Horinouchi, 2007). They are a group of soil bacteria producing about two thirds of natural occurring antibiotics as well as a broad range of biologically active secondary metabolites, which have medical, pharmaceutical and agricultural importance such as anti-bacterials, anti-fungals, anti-tumours, anti-parasitics, anti-cholesterolemics, immunosuppressants, animal growth promotants, insecticides and herbicides (Baltz, 1998; Hopwood, 1999). A large family of secondary metabolites produced by *Streptomyces* are polyketides, which are structurally diverse natural products synthesised by polyketide synthases (PKSs) (Challis & Hopwood, 2003). *S. rimosus* produces polyketide antibiotics, both the large macrolactone type I, and the aromatic type II. The wild type, *S. rimosus* R7 (ATCC 10970), predominantly produces rimocidin, an anti-fungal polyene antibiotic (Davisson et al., 1951), while the production strains from Pfizer, *S. rimosus* M4018 and from Pliva, *S. rimosus* R6, produce only oxytetracycline (Hunter & Hill, 1997; Petković et al., 2006).

Plasmid transfer from *E. coli* to *Streptomyces* is an important method used for studying morphology, physiology and genetics in this genus including the function of PKSs. Normally the plasmid transferred is integrated into the genome of

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Streptomyces by integrases derived from bacteriophages, such as ϕC31 integrase. The bacteriophage integrases are highly sequence specific, generally requiring pairing of attachment site sequences, or *att* site, to enable recombination (Calos, 2006). The appropriate condition for intergeneric conjugation, therefore, could be applied to manipulate rimocidin- and oxytetracycline-producing strains of *S. rimosus*. The *S. rimosus* strains used in this work are (i) *S. rimosus* R7, the rimocidin producer, that has never been envisaged as a host; (ii) *S. rimosus* M4018, the industrial oxytetracycline-producing strain that is a well-characterised host and can be manipulated by PEG-mediated protoplast transformation and electroparation (Butler et al., 1989; Pigac & Schrempf, 1995); and (iii) *S. rimosus* 593, a non-spore forming mutant derived from *S. rimosus* R6 that produces lower yield of oxytetracyline (Gravinus et al., 1994).

2.1.2 Comparison of the activity of AtrA purified from *S. coelicolor* and *E. coli*

A plethora of genes affecting the actinorhodin biosynthesis have been identified in the model streptomycete, *S. coelicolor* (Bibb, 1996; Hopwood et al., 1995). Recently, a member of the TetR family of transcription factors called AtrA (actinorhodin-associated transcriptional regulator) has been characterised (Uguru et al., 2005). AtrA is required for maximum transcription of *act*II-ORF4, the pathway-specific activator of actinorhodin biosynthetic genes in *S. coelicolor*, as dimers or dimers of dimers (Uguru et al., 2005). Disruption of *atrA* can reduce actinorhodin production; however, it had neither effect on the production of undecylprodigiosin nor the calcium-dependent antibiotics. An orthologue of *atrA* was also present in the genome of *S. avermitilis*. AtrA can bind *in vitro* to the promoter of *strR*, the final regulator of streptomycin production in *S. griseus* (Beyer et al., 1996; Horinouchi & Beppu, 1994; Retzlaff & Distler, 1995). Therefore, AtrA is likely to be evolutionarily conserved and played important role in *Streptomyces* antibiotic production pathways.

Although AtrA is required for normal levels of transcription of *act*II-ORF4 and production of actinorhodin, there is no evidence to explain the mechanism by which the activity of *atrA* is regulated. It is speculated that (i) the expression of *atrA* is regulated at the level of transcription or translation, (ii) the AtrA polypeptide is subject to post-translational modification, (iii) regulation of *act*II-ORF4 is triggered by another factor that functions only in concert with AtrA, or (iv) a combination thereof.

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If (ii) is correct, it is likely that AtrA purified from *E. coli* will have a different activity from AtrA purified from *S. coelicolor*.

2.2 Rationales for the teaching interventions

2.2.1 Learning about 'bacterial conjugation' through laboratory using different teaching sequences at secondary school level

A knowledge of genetics is important in biology curricula for all students at secondary and university levels. It is however one of the areas of the maximum difficulty for both students and their teachers (Bahar et al., 1999; Finley et al., 1982; Johnstone & Mahmoud, 1980). The difficulties in the meaningful learning of genetics concepts are both the study of patterns of inheritance and their mechanisms (Wood-Robinson et al., 2000), for example, cell division, the chromosome theory of heredity, Mendel's laws of genetics, multiple alleles, sex-linkage and genetic engineering (Bahar et al., 1999; Longden, 1982; Pashley, 1994; Steward, 1982; Thomson & Stewart, 1985; Walker et al., 1980). Recently, the domain-specific difficulties have been classified into five major categories (Knippels, 2002):

(i) the domain-specific vocabulary and terminology,
(ii) the mathematical content in carrying out Mendelian genetics tasks,
(iii) understanding cytological processes,
(iv) the abstract nature of the subject in biology curriculum, and
(v) the complex nature of genetics: a macro-micro problem.

There are two reasons: teachers have chosen an inappropriate teaching mode of biological organisation, and the level of conceptual abstraction is unsuitable for students' learning abilities (Lazarowitz & Penso, 1992; Lazarowitz & Lieb, 2006). There are confusions in basic terms, such as organisms, cells, nucleus, genes, DNA, alleles, chromatids and chromosome, and thus students find it difficult to understand genetic relationship between cells and the concepts of mitosis, meiosis and DNA replication (Lewis et al., 2000a, 2000b, 2000c). Students also have difficulties in explaining genetic phenomena at the macro (organismal) level by using knowledge from the micro (cellular and molecular) level (Marbach-Ad & Stavy, 2000).

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Bacterial conjugation is a horizontal gene transfer from donor cells bearing one or more conjugative plasmids to plasmid-free recipient cells in order to achieve plasmid survival and spread at the population level. Conjugative plasmids in most bacteria can even be transferred to distantly related or even unrelated microorganisms. For this reason, antibiotic resistances can spread among pathogenic bacteria, and bacterial DNA can be transferred into eukaryotic host cells (Bingle & Thomas, 2001; Llosa et al., 2002). Conjugation is an important topic in genetics in school curricula. However, it has been taught by lecture under the topic of 'Sexual reproduction in bacteria' in secondary school by using the mechanism in *E. coli* as a model to explain how DNA transfer from one cell to another (Clegg & Mackean, 2000). Teaching by lecture only has drawbacks for not helping students to gain a better understanding and to visualise the symbolic and mathematic aspects involved in the abstract concepts of genetics (Longden, 1982; Rotbain et al., 2006). Models, analogies and illustrations have frequently been used to teach genetics for students in the secondary school classroom. Although models and analogies may have their drawbacks, they are powerful cognitive tools for assisting students to obtain a better understanding in genetics in the classroom. Thus, accurate models and analogies can be presented to clarify some important aspects in genetics lessons (Rothhaar et al., 2006; Wood-Robinson et al., 2000).

Laboratory experiences are powerful cognitive learning tools that provide students with opportunities to interact directly with the material world (or with data drawn from material world), using the tools, data collection techniques, models and theories of science. However, there is a lack of challenging and meaningful laboratory experiments for secondary school students (National Research Council [NRC], 2005). In general, a concept-based laboratory experiment requires a combination of "hands-on" and "minds-on" activities (Figure 2-1), and students should design (minds-on) the experimental plan according to their prior experiences as well as perform (hands-on) controlled experiments in achieving experiment goals (Halme et al., 2006; Rotbain et al., 2006). From the basic knowledge of genetic transfer in bacteria, a laboratory exercise 'conjugation in *E. coli*' was set up as a hands-on experience to help students in understanding the conjugation process. As previously stated, this exercise which

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includes hands-on and minds-on activities should enable students themselves to construct the concepts of bacterial conjugation.

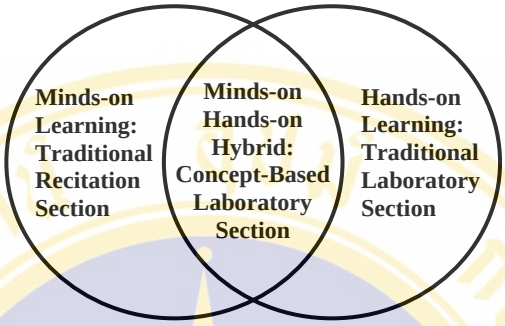

Figure 2-1 A hybrid of hands-on and minds-on learning in concept-based laboratory section (Halme et al., 2006).

Therefore, this study was designed in order that a teaching approach within the experiential learning could be developed and evaluated with the aims of promoting a conceptual understanding of bacterial conjugation, by designing an appropriate laboratory exercise for secondary school students so that they can use it to give mechanistic explanations of conjugation phenomena. By constructivist learning, two teaching sequences, i.e. lecture before lab and lab before lecture, were provided to construct the concepts for students, compared with the traditional instruction.

2.2.2 Improving Thai students' understanding of concepts in 'protein purification' by using a simulation program in Thai and English versions

Protein purification is very important topic to teach life-science students in their first biochemistry classes. However, Thai undergraduates learn this topic only in a theoretical way and carry out no experiments. Students have to learn all topics in biochemistry in the limited time; therefore, they cannot go in each concept deeply. In general, this topic has been taught by an explanation of some techniques in the classrooms, which leaves most students in passive and receptive role. In such situations, students can obtain the fragmentary knowledge, which is too abstract to be integrated into a larger important concept and unspecific to be flexible used and transferred (Lave, 1996; Linn, 1998). This does not necessarily imply that learning

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with tradition education is ineffective; however, it cannot assist students to incorporate their knowledge into the complexity of real world. Nowadays, hands-on experiences on the computer, such as computer-based learning, multimedia-based learning, web-based learning, virtual classroom, have been adapted to science practice in the classroom. These have high potential for dealing with authentic science in a manner that can be adapted to the needs of students at various stages of science literacy (Zumbach et al., 2006). Such practical work can also be powerful in boosting the teaching in any science areas which are difficult to teach and learn through traditional methods (Wekesa et al., 2006). They also help students develop problem-solving, critical thinking and decision-making skills to focus on scientific knowledge, tools and techniques and scientists' attitudes involving their social interactions (Edelson, 1998; Lee et al., 2002; Skinner & Hoback, 2003).

The understanding of protein purification comes from students' experience with the concepts studied. If students carry out the virtual laboratory class after the lecture, their experience might be increased in advance. Furthermore, each purification technique uses different properties of proteins in the separation and the cost of the procedure can be decreased if students choose appropriate techniques in each step of purification scheme. In 1986, Andrew G. Booth, a professor in the School of Biochemistry and Molecular Biology at the University of Leeds, UK, developed the protein purification program written with Java language to teach his students in the first biochemistry class after lecture and a laboratory experiment (Booth, 1986). In 2005, the Python version of protein purification program was prepared for international virtual class including one in the Thai language. The program includes the general purification techniques as well as help files to give the additional information in strategies, theories and cost. Moreover, students can assess their understanding of the concepts of each technique by means of progress reports at each purification step, which show total protein, units of enzyme, yield, enrichment and cost, and the summarised progress report, which indicates whether or not students can choose appropriate techniques in the purification scheme. In this way, students can make their purification plans for unknown mixture. The achievements in the classes using either the Thai/English or the single language (English) program were

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compared, and preferences towards the two-language simulation program were also assessed in classes of both undergraduates and graduates.

2.3 Research questions and intentions

The main aim of the study is to explore, clarify and improve understanding of the issues involved in the theoretical bases in science and education. The practicals of bacterial conjugation and protein purification are implemented for in senior secondary school and undergraduate levels, respectively. The research questions central to this study addressed were as follows:

(1) Can both spore and mycelium of *S. rimosus* be efficiently used as recipients for intergeneric conjugation with *E. coli*?

(2) Which sequence was a chromosomal integration site of *S. rimosus* that was specific to ϕC31 *attP* in the plasmids?

(3) Was the activity of AtrA regulated by a post-translational modification in *S. coelicolor*?

(4) Did providing the practical work assist students to understand the concepts of bacterial conjugation and on the simulation of protein purification?

(5) Was there any difference between the students that used the laboratory exercise in 'bacterial conjugation' after teaching interventions and the students that learned by traditional methods?

(6) Was there any difference amongst students who used the Protein Purification Program in a single language (English) compared with the bi-language (Thai/English) version?

2.4 The aims of this study

In line with the rationales mentioned in the previous section, the aims of this study in scientific aspects can be stated as follows. The first aim of the aspect was whether both spore and mycelium of *S. rimosus* strains can be efficiently used as recipients for intergeneric conjugal transfer of plasmid pIJ8600 with *E. coli*. The exconjugants showed phenotypic changes in morphology, sporulation and antibiotic production which indicated that plasmid integration at *attB* is acceptable, and this

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system could be conveniently applied for gene manipulation in rimocidin- and oxtetracycline-producing strains of *S. rimosus*.

Streptomyces is a prokaryote having many processes like eukaryotes, particularly post-translational modifications following protein synthesis. Thus the activity of any proteins in *Streptomyces* before and after modification should be different. Since AtrA is not modified in *E. coli*, the activities of AtrA from *E. coli* and *S. coelicolor* were also compared to investigate a post-translational modification in wild type of *S. coelicolor*. By this assumption, another aim in this regard was to test the possibility by producing and purifying a recombinant form of AtrA from both *E. coli* and *S. coelicolor*.

With respect to the educational aspects, the aims of this study were: (i) to design a laboratory exercise in 'bacterial conjugation' for senior secondary school level and develop the Protein Purification Program in a Thai version for use at the undergraduate level; (ii) to examine and compare the outcomes of the designed teaching approaches using both practical experiences with the outcomes of typical teaching sequences; and (iii) to qualitatively and quantitatively explore whether students have a better conceptual understanding of bacterial conjugation and protein purification following teaching interventions by using practical works.

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CHAPTER III
LITERATURE REVIEW

This chapter highlights the theoretical perspectives taken in this study in both scientific and educational aspects. Relevant literature was examined with three main foci, relating to the objectives of the study in Chapter 2. Firstly, the literature relating to the background of *Streptomyces* and the genetic transfer system in *Streptomyces* is discussed in section 3.1-3.2 and will be used in Chapter 4.1 as a framework to analyse the condition of intergeneric conjugation and attachment site (*attB*) for integration in the chromosome of *S. rimosus*. Secondly, the literature relating to the regulation of antibiotic production in *Streptomyces* in section 3.3 is examined as an introduction to analyse the activity of AtrA protein purified from *E. coli* and *S. coelicolor* in Chapter 4.2. The constructivist, experiential learning and practical work in science education are reviewed in section 3.4-3.6 to provide a background of the third focus of the study and literature relating to hands-on experiments using the multimedia program as well as the laboratory is also given in this chapter.

3.1 *Streptomyces*

Streptomycetes are the most intensively studied in the actinomycete group. The genus *Streptomyces* is generally characterised as non-motile, filamentous, aerobic, saprophytic, spore-forming Gram-positive bacteria that have high G+C content (74 mol%) in their DNA (Goodfellow et al., 1992; Waksman & Henrici, 1943; Williams et al., 1989). They are classified into Order Actinomycetales, Family Streptomycetaceae (Tewfik & Bradley, 1967; Williams et al., 1989). They are largely responsible for secreting the earthy odorous chemicals in soil called geosmins (1,10 dimethyl-9-decalols; Gust et al., 2003). The optimum growth temperature and pH are 25-35 ºC and 6.5-8.0, respectively (Williams et al., 1989). *Streptomyces* are significant producers of secondary metabolites used in pharmaceutics and agriculture including anti-bacterials, anti-fungals, anti-cancers, immuosuppressants, herbicides and insecticides. Moreover,

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a large number of enzymes are produced by *Streptomyces*, for instance, cholesterol oxidase, amylase, chitinase and endonuclease (Tokala et al., 2002; Williams et al., 1989). According to a mathematic modelling, only 3% of all bioactive metabolites produced by *Streptomyces* have been identified so far (Watve et al., 2001).

Streptomyces have a complex life cycle (Figure 3-1) consisting of filamentous growth producing multigenomic and branching hyphae as well as the formation of aerial filaments with long chains of exospores (Chater, 1998; Keleman & Buttner, 1998; Paradkar et al., 2003). During the *Streptomyces* life cycle, the growth of aerial hyphae is initiated by responses to nutrient limitation or other physiological stresses (Bibb, 1996, 2005; Chakraburtty & Bibb, 1997) and/or to cell density via extracellular signals (Horinouchi & Beppu, 1994; Willey et al., 1993). The spores, moreover, can impart resistance to low nutrient and water availability and survive in soil for long periods (Ensign, 1978; Elliot & Talbot, 2004). The large size (8-9 Mb) *Streptomyces* chromosome is linear (Hopwood, 2006) and can be translated into more predicted genes than that of simple eukaryotes as in *Saccharomyces cerevisiae* (6,203 genes), e.g. 7,825 predicted genes for *S. coelicolor* as well as 7,574 predicted genes for *S. avermitilis* (Bentley et al., 2002; Hopwood, 2003; Ikeda et al., 2003).

3.1.1 *Streptomyces coelicolor* - the model organism

Owing to its complex life cycle, *S. coelicolor* A3(2) serves as a model streptomycete to study the morphological and physiological development (Claessen et al., 2006) as well as to investigate the genetic control of antibiotic production (Hopwood et al., 1995). This strain is able to produce four known antibiotics, i.e. the acidic lipopeptide calcium-dependent antibiotic (CDA), a grey spore pigment encoded by the *whiE* locus (Keleman et al., 1998), the deep blue pigmented aromatic polyketide actinorhodin (Act), whose colour is responsible for the *S. coelicolor* name, and the red-pigment tripyrrole undecylprodigiosin (Red) (Hopwood, 1999). The biosynthetic gene clusters for the first three are found in the chromosome and for the latter, in plasmid SCP1.

The complete genome sequence of *S. coelicolor* strain M145, which is a prototrophic derivative of strain A3(2) lacking its two plasmids (SCP1, linear, 365 kb; and SCP2, circular, 31 kb) has been reported (Bentley et al., 2002). Interestingly, the

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distribution of different types of gene reveals a central core comprising approximately half the chromosome and a pair of chromosome arms (Figure 3-2). Nearly all unconditionally essential genes, such as those for cell division, DNA replication, transcription, translation and amino-acid biosynthesis, are located in the core, and non-essential functions, particularly secondary metabolites, lie in the arms. Its genome sequence now predicts 18 clusters that code for enzymes characteristic of secondary metabolites including type I and type II polyketide synthases, chalcone synthases, non-ribosomal peptide synthases (NRPSs), terpene, geosmin, and others (Bentley et al., 2002).

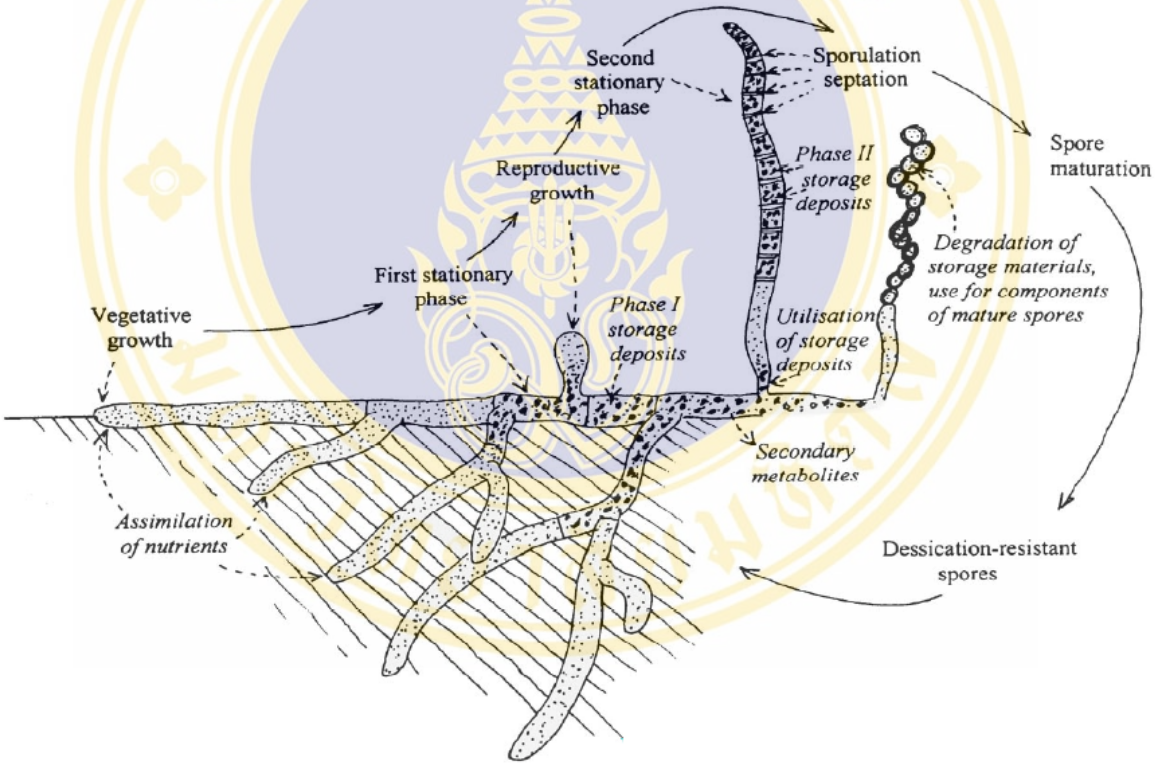

Figure 3-1 Streptomycete life cycle (Chater, 1998). An idealised diagrammatic representation summarising the complex life cycle of *S. coelicolor* A3(2) from spore germination, through vegetative growth, to erection of aerial mycelium and spore formation.

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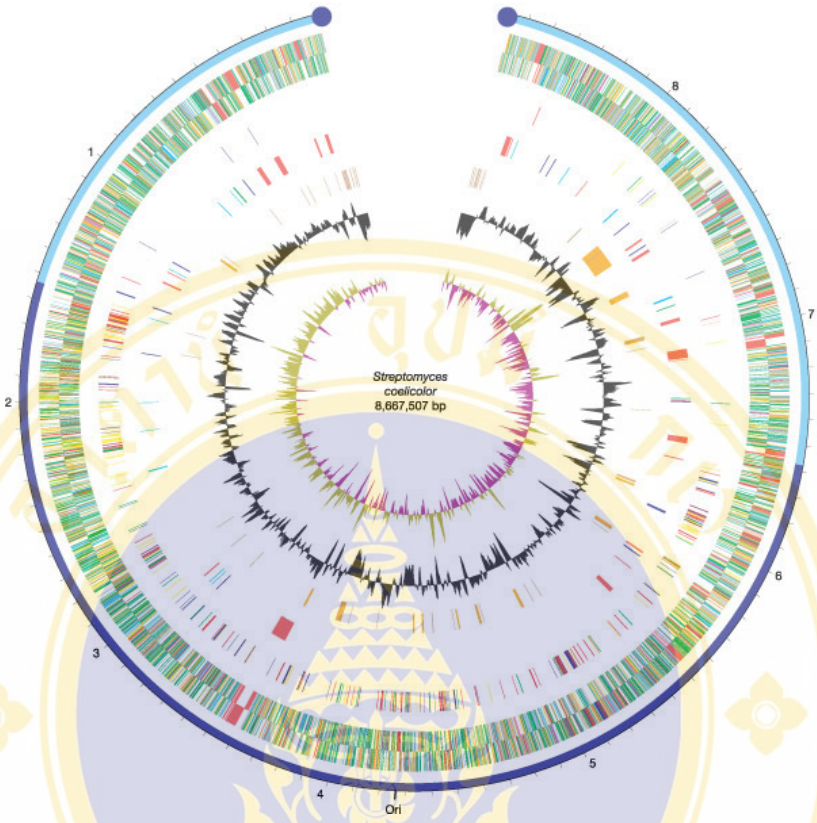

Figure 3-2 Circular representation of the *S. coelicolor* chromosome (Bentley et al., 2002). The outer scale is numbered anticlockwise in megabase and indicates the core (dark blue) and arm (light blue) regions of the chromosome. Circles 1 and 2 (from the outside in), all genes (reverse and forward strands, respectively) colour-coded by function: black, energy metabolism; red, information transfer and secondary metabolism; dark green, surface associated; cyan, degradation of large molecules; magenta, degradation of small molecules; yellow, central or intermediary metabolism; pale blue, regulators; orange, conserved hypothetical; brown, pseudogenes; pale green, unknown; grey, miscellaneous. Circle 3 indicates selected essential genes for cell division, transcription, translation and amino-acid biosynthesis, colour coding as for circles 1 and 2. Circle 4 denotes selected contingency genes: red, secondary metabolism; pale blue, exoenzymes; dark blue, conservon; green, gas vesicle proteins. Circle 5 indicates mobile elements: brown, transposases; orange, putative laterally acquired genes. Circle 6 denotes the G + C content and circle 7 indicates the GC bias [(G – C/G + C), khaki indicates values >1, purple <1]. The origin of replication (Ori) and terminal protein (blue circle) are also indicated.

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3.1.2 *Streptomyces rimosus*

S. rimosus is a known industrial producer of oxytetracycline (Finlay et al., 1950). It produces polyketide antibiotics both large macrolactone type I and aromatic type II. The wild type, *S. rimosus* R7 (ATCC 10970) predominantly produces rimocidin, an anti-fungal polyene antibiotic (Davisson et al., 1951), while the production strains from Pfizer, *S. rimosus* M4018, and from Pliva, *S. rimosus* R6 produce only oxytetracycline (Hunter & Hill, 1997; Petković et al., 1999, 2006). While the genetics of *S. coelicolor* has been elucidated, there is paucity of information for *S. rimosus*. The restriction map of chromosome of *S. rimosus* R6-501 (Figure 3-3) was constructed by pulsed-field gel electrophoresis (PFGE) for rarely digesting enzymes *Ase*I and *Dra*I. The map is linear in size of 8 Mb, which is similar to those of *S. coelicolor* A3(2), *S. lividans* 66, *S. griseus* and *S. ambofaciens* (Pandza et al., 1997). The antibiotic biosynthesis clusters, moreover, lies on the end of chromosome like that of *S. coelicolor* A3(2) (Pandza et al., 1997, 1998).

To increase oxytetracycline yield in *S. rimosus* R6 by a strain selection program, there was the extreme phenotypic instability in five traits: sporulation, pigmentation, colony morphology, oxytetracycline production and oxytetracycline resistance. Propagation via spores, furthermore, gave much higher instability than did propagation via mycelial fragments (Gravius et al., 1993). The present reviews on *S. rimosus* genetics point to the application of molecular approaches to develop new tetracyclines by combinatorial biosynthesis as well as novel recombination approach (Hranueli et al., 2005; Petković et al., 2006).

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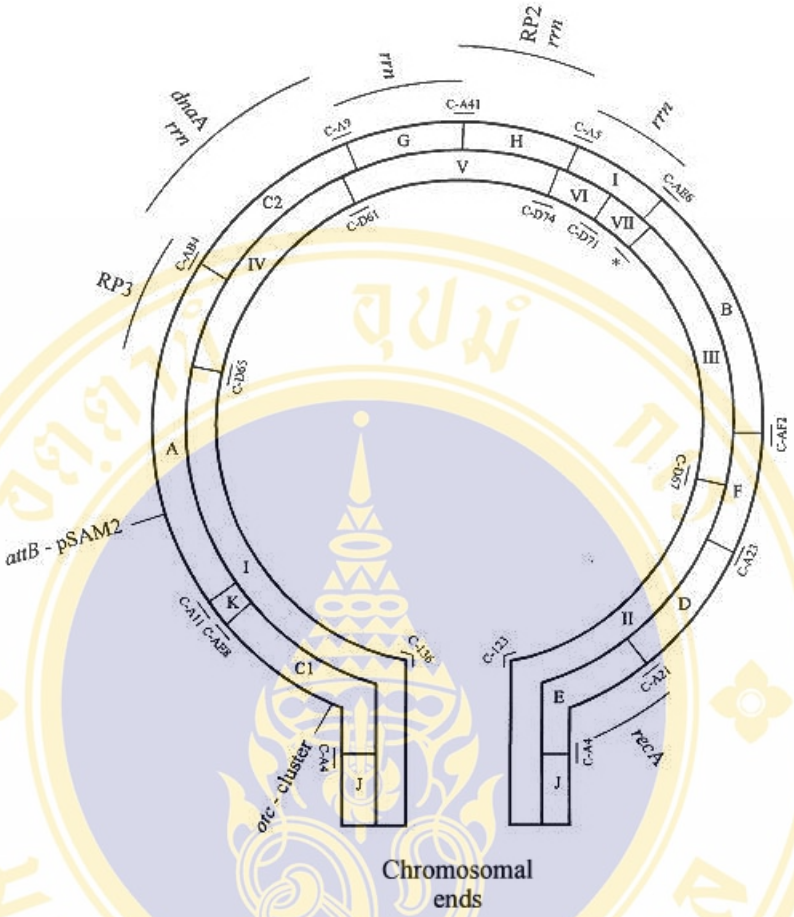

Figure 3-3 Restriction map of the chromosome of *S. rimosus* R6-501 for the enzymes *Ase*I (outer arc) and *Dra*I (inner arc) (Pandza et al., 1997). The terminal inverted repeats are drawn as a stem structure. The numbers of the linking clones are indicated adjacent to the corresponding restriction sites; the missing *Dra*I linking clone is indicated by an asterisk. The cosmid clones carrying the ends of the terminal inverted repeats (C-136 and C-123) are also indicated. The OTC-cluster and the *attB*-pSAM2 gene have been localised precisely. The other markers have been localised only to particular *Ase*I and *Dra*I fragments.

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3.2 Introduction of plasmid into *Streptomyces*

There are many different ways to transfer plasmid DNA into the genus *Streptomyces* in order to study gene function or increase antibiotic production, for instance, transformation with polyethylene glycol (PEG) mediated protoplast (Bibb et al., 1978), transduction with actinophage (Matsushima et al., 1989) and conjugation with transmissible plasmids (Mazodier et al., 1989). The transformation, however, gave low efficiency of recombinant plasmid into competent cells in Gram-positive bacteria since the presence of restriction-modification systems in *Streptomyces* (Kwak et al., 2002; Trieu-Cuot et al., 1987). To reduce the methyl specific restriction barrier, the recombinant DNA isolated from methylation deficient *E. coli* strains, such as *E. coli* ET12567, was found to transform into *Streptomyces* efficiently (Flett et al., 1997; MacNeil et al., 1992). Moreover, the heat treatment of protoplasts prior to transformation has been used to reduce the activity of restriction enzymes that could attack to the foreign DNA (Bailey & Winstanley, 1986; Engel, 1987). In the case of transduction, there is no actinophage that can be used in every strain of *Streptomyces* (Cox & Baltz, 1984). Plasmid-mediated conjugation is a widespread technique to transfer DNA into various bacteria including genus *Streptomyces* since the protocol is simpler and less time consuming than transformation methods (Gormley & Davies, 1991). In general, conjugative plasmids introduced into *Streptomyces* have to be transmissible, for instance, pIJ6902 (Huang et al., 2005), pIJ8600 (Sun et al., 1999) and pSET152 (Bierman et al., 1992).

Conjugation that occurs naturally in both Gram-negative and Gram-positive bacteria requires the close physical contact between donor and recipient cells and is usually mediated by plasmid-encoded proteins which provide transfer (*tra*) functions (Errington et al., 2001; Zechner et al., 2000). In most cases, conjugation is initiated by creating a single-stranded nick at the site-specific endonucleolytic cleavage within the plasmid namely origin of transfer (*oriT*); the unnicked DNA single strand is replicated in the donor cells, while 5'-end of the nicked DNA strand is transported linearly through the donor and recipient envelopes into the recipient cell where it is replicated and the double-stranded DNA is circularised (Lanka & Wilkins, 1995). The conjugation mechanisms in the Gram-positive bacteria including the genus *Streptomyces* and their

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relatives differ in that (i) plasmid replication apparently occurs in the donors, a double-stranded molecule being transferred, and (ii) a single plasmid-encoded protein is sufficient to drive plasmid transfer between mating cells (Errington et al., 2001; Llosa & de la Cruz, 2005; Sprague, 1991). In general, conjugative transfer of plasmid is a transport of single-stranded molecule from the donor to the recipient. However, the plasmid transfer between streptomycetes is a conjugative process of double-stranded DNA molecules (Figure 3-4) to prevent a type II restriction modification (RM) system. The first evidence of double-stranded plasmid transfer was the conjugation between *S. lividans* in the presence of *Sal*I RM system (Possoz et al., 2001). These RM systems comprised of two separate enzymes: the methyltranferase modifies DNA at a specific recognition site and the endonuclease cleaves unmodified DNA at the same site (Wilson, 1991).

Conjugal transfer from *E. coli* to Gram-positive bacteria was first demonstrated by Trieu-Cuot et al. (1987). Later, Mazodier et al. (1989) developed intergeneric conjugation from *E. coli* to *Streptomyces* by using methylation deficient *E. coli* hosts, such as *E. coli* ET12567, to reduce the restriction barriers of foreign DNA and increase high efficiency of DNA introduction (Flett et al., 1997; MacNeil, 1988). In *Streptomyces*, only a single *tra* gene which differs from that in Gram-negative bacteria was first indicated for plasmid transfer and chromosomal mobilisation during mating (Kieser et al., 1982), e.g. *traB* for pSN22 (Kosono et al., 1996), *traSA* for pSAM2 (Possoz et al., 2001). The *tra* gene product (Tra) has also been studied for setting up efficient plasmids in the conjugative system. For example, Tra2 core of the IncPα plasmid was used for mating between *E. coli* and *S. lividans* (Giebelhaus et al., 1996), Tra protein of plasmid pIJ101 was used for *S. lividans* (Pettis & Cohen, 2001) and using for various Tra systems for novel binary vectors in *Streptomyces* spp. (Blaesing et al., 2005).

Two *E. coli* donor strains generally used for intergeneric conjugation are methylation proficient strains, S17-1 harbouring RP4 *tra* gene on chromosome (Mazodier et al., 1989) and a methylation deficient ET12567 containing *tra* gene in a self-transmissible plasmid pUB307 (Flett et al., 1997) or a non-transmissible plasmid pUZ8002 (Sia et al., 1996). Nowadays, conjugative vectors used for facilitating genetic analysis of *Streptomyces* are non-replicative plasmids which can integrate into the genome by homologous

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recombination (Bierman et al., 1992) and by site-specific recombination using the ϕC31 integrase to pair a phage attachment site (*attP*) with an *attB* site on the host genome (Calos, 2006; Kuhtoss & Rao, 1991).

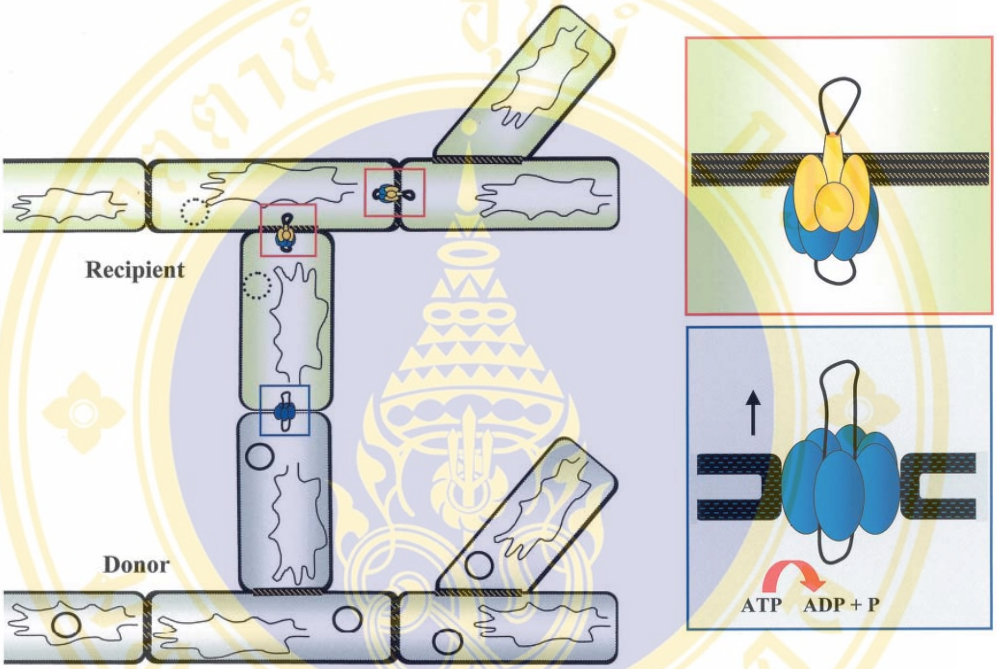

Figure 3-4 The model for conjugative plasmid transfer in *Streptomyces* (Grohmann et al., 2003). The hyphal tips of plasmid carrying donor and a recipient mycelium grow together without the need for a plasmid-encoded aggregation system. In the presence of a Tra protein, the hyphae fuse. Tra multimers from a ring-shaped structure around the double stranded plasmid (lower inset). Depending on ATP hydrolysis, the unprocessed plasmid molecule is translocated through the Tra pore into the recipient. The newly incoming plasmid is subsequently distributed to the neighbouring mycelial compartments via hydrophobic Spd proteins, which form a pore-like structure in the septal crosswalls (upper set).

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In general, intergeneric conjugation is performed by mating an appropriate ratio of the *E. coli* donor to recipient spores of *Streptomyces* and selecting the exconjugants on the agar medium with a suitable antibiotic(s) (Keiser et al., 2000). The advantages of this genetic transfer system from *E. coli* into actinomycetes (Blaesing et al., 2005) exemplifies to allow: (i) identification and functional analysis of biosynthetic pathways for pharmaceutically interesting metabolites, such as antibiotics (Courvalin, 1994), extracellular regulatory factor (Kitani et al., 2000); and (ii) the target manipulation of the biosynthesis by pathway engineering of homologous and heterologous genes, a strategy, which is called combinatorial biosynthesis (Rix et al., 2002). Therefore, an efficient gene transfer system based on conjugation has to be developed in an appropriation for each *Streptomyces* strain (Enŕiquez et al., 2006; Kitani et al., 2000).

The ratio of recipient to donor is a factor affected to the efficiency of conjugation, for example, after conjugal transfer of pSET152 from *E. coli* ET12567 (pUZ8002) to *S. lavendulae* FRI-5 on ISP2 medium supplemented with 10 mM $MgCl_2$, the efficiency of conjugation was increased (in a range of 3.9×10^{-8} to 1.6×10^{-5} exconjugants/recipient) when decreasing the donor/recipient ratio (Kitani et al., 2000). The recipient/donor ratio, furthermore, depends on the type of medium. For instance, the recipient/donor ratio did not affect the frequency of conjugation on MS medium containing 10 mM $MgCl_2$ (Kitani et al., 2000), but it affected the efficiency of conjugation between *E. coli* S17-1 (pOJ436) and *S. toyocaensis* on R2 medium (Matsushima & Baltz, 1996).

Another factor affecting the intergeneric conjugation is the type of conjugative agar medium. After conjugation between *E. coli* ET12567 (pUZ8002/pSET152) and *S. lavendulae* FRI-5, the exconjuants were found on the MS + 10 mM $MgCl_2$ and ISP2 + 10 mM $MgCl_2$, but there was no exconjugant on R5 medium. When comparing three conjugation media (TS, AS1 and R2) for the genetic transfer from *E. coli* to *S. fradiae*, the highest efficiency of conjugation was obtained on AS1 agar plates (Bierman et al., 1992). However, R2 medium gave the highest frequency in conjugation between *E. coli* S17-1 (pOJ436) and *S. toyocaensis* on R2, AS-1 and Bnt (Matsushima & Baltz, 1996). The liquid medium was used as a conjugation medium, but it was not successful (Mazodier et al., 1989).

In case of conjugation with *Streptomyces* spores as recipients, the temperature and time of spore pre-germination affect to the efficiency of conjugation in individual

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species (Table 3-1). In general, the pre-germination of *Streptomyces* spores has been performed at 50 ºC for 10 min (Keiser et al., 2000). Under these conditions, the pre-germination of *S. lividans* spores increased the efficiency of conjugation by 5-10 times (Mazodier et al., 1989). The appropriate condition for conjugation with *S. lavendulae* FRI-5 was spore germination at 40 ºC for 10 min (Kitani et al., 2000). However, a temperature-sensitive strain *S. virginiae* gave the good efficiency without a pre-germination step before mating (Voeykova et al., 1998). The incubation temperature after mating on agar medium also effected the growth of exconjugants. Incubation at 37 ºC of conjugation plates between *E. coli* S17-1 and *S. fradiae* gave three times higher efficiency than at 29 ºC (Mazodier et al., 1989).

Vegetative mycelia were used as recipients instead of spores to expedite the preparation of recipient culture, for example, the mycelium of *S. fradiae* used as a recipient gave no different efficiency from its recipient spores, when conjugated with *E. coli* S17-1 (Bierman et al., 1992). However, the conjugation of recipient mycelia of *S. toyocaensis* with *E. coli* S17-1 gave lower efficiency than those of recipient spores (Matsushima & Baltz, 1996). In case of poorly sporulated strains as *S. peucetius*, the mycelial fragments were used as recipients to conjugate with *E. coli* ET12567 (pUZ8002/pSET152) and the efficiency of conjugation was 1.5×10^{-4} exconjugants per recipient (Paranthaman & Dharmalingam, 2003). Vector size also affected the efficiency of conjugation. In general, the conjugation with small plasmids (approx. 5.5 kb) has 100-1000 times higher efficiency than that with large (approx. 20 kb) or cosmids (40-50 kb) (Bierman et al., 1992).

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Table 3-1 Heat treatment conditions for pre-germinated *Streptomyces* recipient spores before mating step of intergeneric conjugation

Heat treatment condition	Strain	Reference
50 ºC for 10 min	*S. albus* G	Voeykova et al. (1998)
	S. anibioticus ATCC 23879	Voeykova et al. (1998)
	S. aureofaciens VKPM AC 755	Voeykova et al. (1998)
	S. bambergiensis ATCC 13879	Voeykova et al. (1998)
	S. clavuligerus	Fouces et al. (2000)
	S. coelicolor A(3)2	Flett et al. (1997)
	S. conglobatus	Nikodinovic & Priestley (2006)
	S. fradiae B-45 VKPM AC 542	Voeykova et al. (1998)
	S. gresius Kr.15 VKPM AC 115	Voeykova et al. (1998)
	S. hygroscopicus ATCC 10976	Voeykova et al. (1998)
	S. hygroscopicus ATCC 21705	Voeykova et al. (1998)
	S. lividans 66	Flett et al. (1997)
	S. peucetius ATCC 29050	Paranthaman & Dharmalingam (2003)
	S. purpureus ATCC 19777	Voeykova et al. (1998)
	S. rimosus ATCC 23955	Voeykova et al. (1998)
	S. routienii	Nikodinovic & Priestley (2006)
	S. toxytrimycin	Chen et al. (2006)
	S. venezuelae ATCC 21405	Voeykova et al. (1998)
	S. viridochromogenes Tu 494	Voeykova et al. (1998)
	Streptomyces sp. strain M095	Hou et al. (in press)
50 ºC for 25 min	*S. nodosus* ATCC 14899	Nikodinovic et al. (2003)
45 ºC for 10 min	*S. natalensis* ATCC 27448	Enŕiquez et al. (2006)
40 ºC for 10 min	*S. lavendulae* FRI-5	Kitani et al. (2000)
at RT, no heat treatment	*S. virginiae* ATCC 13161	Voeykova et al. (1998)

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3.3 Regulation of antibiotic production in *Streptomyces*

As throughout the actinomycetes, antibiotic production in *Streptomyces* is generally dependent on growth phase and involves the expression of physically cluster regulatory and biosynthetic genes (Bibb, 1996; Chater, 1990; Demain & Fang, 1995; Hopwood, 1988). For instance, the production of the anti-fungal candicidin in liquid-medium cultures of *S. gresius* (Martín & McDaniel, 1975) and oleandomycin production on agar surface-grown cultures of *S. antibioticus* (Méndez et al., 1985) occurred after vegetative growth had ceased. In wild-type *S. coelicolor* M145, the calcium-dependent antibiotic (CDA) and undecylprodigiosin (Red) are produced during the transition phase, whereas actinorhodin (Act) production is not detectable until stationary phase (Figure 3-5) (Hobbs et al., 1990; Huang et al., 2001). The growth rate is accepted to be a major factor in determining the onset of antibiotic production in *Streptomyces* growing in batch culture (Demain & Fang, 1995). However, the relationship between growth-rate control and expression of genes encoding antibiotic synthases in *Streptomyces* are less well understood (Jones et al., 1997). In much published literature, the reduction of growth rate with no growth cessation is an important signal for triggering secondary metabolisms (Bibb, 2005), for example, a decrease of the growth rate was the trigger for actinorhodin production (Doull & Vining, 1990).

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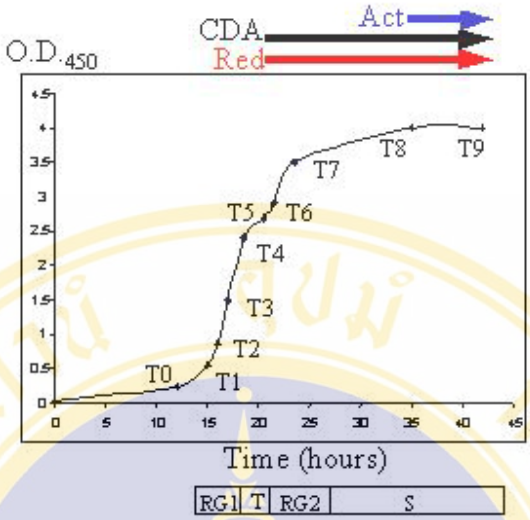

Figure 3-5 Growth and antibiotic production of *S. coelicolor* M145 in liquid R5 medium (Huang et al., 2001). Arrows indicate when antibiotics were detected in cultures at the harvested times T0 to T9. An initial period of rapid growth (RG1) beginning ~14 h after inoculation of liquid cultures was followed by a brief growth slowdown starting ~18.5 h after inoculating (transition [T] phase). After 2-3 h, cultures briefly resumed another rapid growth phase (RG2) before entering stationary (S) phase.

The structural genes responsible for the antibiotic production in *Streptomyces* were readily identified by mutations causing altered pigment production. The gene clusters encoding different metabolic enzymes for a particular biosynthetic pathway usually included specific regulatory genes, for instance, *redD* for undecylprodigiosin (Narva & Feitelson, 1990), *act*II-ORF4 for actinorhodin (Fernández-Moreno et al., 1991), *dnrI* for daunorubicin (Stutzman-Engwall et al., 1992), *mtmR* for mithramycin (Lombó et al., 1999), and *nvoG* for novobiocin (Eustáquio et al., 2005). The architecture of regulatory gene located within antibiotic gene clusters has common features found in actinobacterial genomes. Antibiotic biosynthetic and resistance genes are clustered together (Cundliffe, 1989; Malpartida & Hopwood, 1984; Martín & Liras, 1989) and are regulated by pathway-specific regulatory genes (Wietzorrek & Bibb, 1997) such as *act* cluster (Figure 3-6). The pathway-specific regulatory proteins

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involved in the transcriptional regulation of antibiotic biosynthetic genes that controlled secondary metabolism in streptomycetes were identified into the *Streptomyces* antibiotic regulatory protein (SARP) family, which binds to heptameric direct repeats with the consensus sequence TCGAGCG/C located close to their transcriptional start site (Arias et al., 1999; Sheldon et al., 2002; Wietzorrek & Bibb, 1997). It is also interconnected with other physiological aspects of *Streptomyces*, for example, morphological differentiation and carbon metabolism (Bibb, 1996, 2005; Chakraburtty et al., 1996; Martínez-Costa et al., 1996; Sun et al., 2001). However, the activator StrR is not SARP and regulation of streptomycin biosynthesis in *S. gresius* is SARP-independent (Retzlaff & Distler, 1995).

Figure 3-6 Open reading frame organisation of the actinorhodin biosynthetic cluster. The minimal PKS consists of ketosynthase subunits and acyl carrier protein (ACP). KR, ARO, CYC and SARP are ketoreductase, aromatase, cyclase and *Streptomyces* antibiotic regulatory protein, respectively. Open reading frame organisation of *act*VI was determined by Fernández-Moreno et al. (1994), *act*VA by Caballero et al. (1991), *act*II by Fernández-Moreno et al. (1991) and *act*III to *act*VB by Fernández-Moreno et al. (1992).

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The transcriptional activators have a putative winged helix-turn-helix (HTH) motif towards their N-termini that commonly occurs in microbial DNA-binding regulatory proteins. By bioinformatically comparative analysis, DNA-binding domain of the SARPs is similar to the C-terminal DNA-binding domain of the OmpR family of regulatory proteins (McDowall et al., 1999; Sheldon et al., 2002; Wietzorrek & Bibb, 1997). SARPs appear to be functionally interchangeable between *Streptomyces* species. For instance, the actinorhodin production in an *act*II-ORF4 defective *S. coelicolor* mutant can be stimulated by DnrI which controls daunorubicin production in *S. peuticeus* and MtmR which controls mithramycin in *S. argillaceus* (Stutzman-Engwall et al., 1992; Lombó et al., 1999). However, non-SARPs can also function after exchanging between species, for example, introduction of StrR, which regulate streptomycin production in *S. gresius*, into *S. coelicolor* also results in overexpression of actinorhodin, undecylprodigisin, CDA and methylenomycin (Horinouchi & Beppu, 1994).

A large number of genes that affect antibiotic biosynthesis as part of a wider pleiotropic effect have been discovered through mutational analysis. While SARPs act as pathway-specific regulatory proteins to regulate antibiotic production, at least one pleiotropic regulator incorporates a SARP domain (Bibb, 2005). In case of actinorhodin biosynthesis, most of pleiotropic genes (Table 3-2) have been demonstrated to operate through transcriptional regulation of *act*II-ORF4. For instance, *blaA* encodes the $tRNA^{leu}$ necessary for translation of the TTA codon found in the beginning of the *act*II-ORF4 (Chater, 1991), and level of *act*II-ORF4, *act*VI-ORF1 and *redD* transcripts are elevated in *absA* disruptants which hyperproduce antibiotics (Aceti & Champness, 1998). Besides these regulators, gene expression is related to nutritional concentrations (Bibb, 2005; Doull & Vining, 1990; Hobbs et al., 1990), stringent factors (Hesketh et al., 2001; Strauch et al., 1991) and γ-butyrolactone (Takano et al., 2001, 2005, 2006; Kato et al., 2007).

AtrA is an important transcriptional factor binding to the promoter of *act*II-ORF4, in the model species *S. coelicolor* (Uguru et al., 2005). Through electrophoretic mobility shift assay (EMSA), the AtrA bound *act*II-ORF4 as dimers or dimers of dimers in *S. coelicolor* and bound *in vitro* to the promoter of *strR*, the streptomycin cluster-situated regulator in *S. gresius* (Stead et al., 2006; Uguru et al., 2005). The

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orthologue of *atrA* presented in *S. avermitilis* genome which was expected to act *atrA* as in *S. coelicolor* (Uguru et al., 2005). Furthermore, *S. gresius* carries a single homologue of *atrA* and the expression of *S. coelicolor* AtrA in *S. gresius* causes a DNA binding-dependent reduction in streptomycin production and in the mRNA levels of *strR* and biosynthetic genes. AtrA also stimulates the actinorhodin production in *S. lividans*, a close relative of *S. coelicolor*, but no effect was detected on the production of oxytetracycline or rimocidin by strains of *S. rimosus* (Hong et al., 2007).

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Table 3-2 Effect of pleiotropic regulatory genes on antibiotic biosynthesis in *Streptomyces* spp.

Gene	Effect	Reference
abaA	Disruption eliminates actinorhodin production in *S. coelicolor*, but simulates actinorhodin production in *S. lividans*.	Fernández-Moreno et al. (1992)
absA	Either negative regulation or hyperproduction of all four antibiotics in *S. coelicolor* are found in disruptants.	Adamidis et al. (1990); Brian et al. (1996)
absB	Disruptants cannot produce all four antibiotics in *S. coelicolor*.	Adamidis & Champness (1992)
afsB/ *hrdB*	Mutants cannot produce actinorhodin or undecylprodigiocin.	Hara et al. (1983)
afsK	Disruption of *afsK* reduces level of actinorhodin production.	Matsumoto et al. (1994)
afsR	Multiple copies stimulate antibiotic production in *S. coelicolor* and *S. lividans*. AfsR appears to play a key role as an integrator of multiple physiological and environmental signals transduced by phosphorylation cascades.	Horinouchi et al. (1983); Horinouchi (2003)
afsS	Multiple copies stimulate antibiotic production in *S. coelicolor* and *S. lividans*.	Matsumoto et al. (1995); Vögtli et al. (1994)
bldA	Produces leucyl-tRNA and needed to transcribe the TTA codon in the *act*II-ORF4 gene.	Fernández-Moreno et al. (1991)
cutRS	Disruptants overproduce actinorhodin.	Chang et al. (1996)
eshA	Disruption prevents actinorhodin production.	Kawamoto et al. (2001)
mia	Inhibited production of all four antibiotics when cloned at high-copy number.	Champness et al. (1992)
relC	Deletion prevents ppGpp accumulation and actinorhodin production when nitrogen is depleted.	Ochi et al. (1997)
sigE	Mutants require high levels of magnesium for growth and overproduce actinorhodin.	Paget et al. (1999)

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3.4 Learning theory

Epistemology, or the study of knowledge, encompasses a broad range of learning theories, which are three paradigms in the theory of cognition and learning: the nature of knowing, learning and transfer, as well as motivation and engagement (Greeno et al., 1996). This section will focus on some current theories about learning that provides an adequate background for understand the context of this study.

3.4.1 Constructivism

Constructivism, or the constructivist view of learning, is a theory of learning or meaning making that students create their new own understandings on the basis of an interaction between knowledge that they already know, or prior knowledge, and new knowledge that they receive (Figure 3-7) (Resnick, 1989). By definition, knowledge construction through constructivists' view is an active, rather than a passive process. The individual knowledge was constructed by a process of (i) cognitive and perceptual skills (Cunningham, 1993); (ii) physical construction of meaning (Harel & Papert, 1991), through student's understanding or mental models (Johnson-Laird, 1980); and (iii) physical or virtual representations of knowledge (Papert, 1993; Winn et al., 1997). Thus, 'learning' in the constructivist perspective is the product of self-organisation (von Glasersfeld, 1989), and the constructivism focuses on the learners, the ways people learn, quality experiences and lifelong learning processes, rather than on teaching (DeVries, 2002).

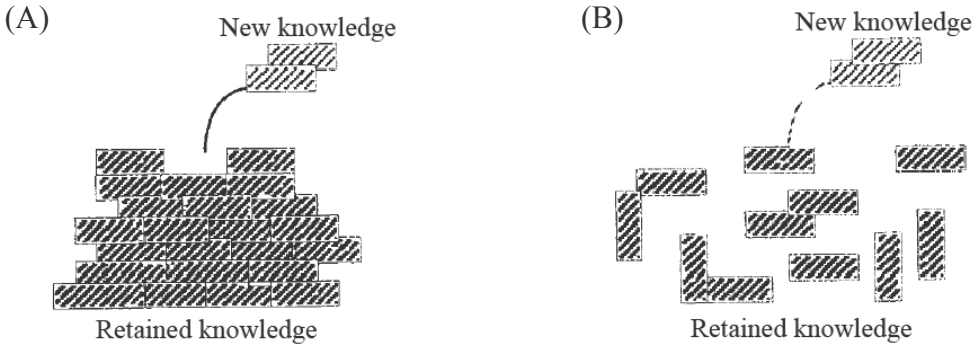

Figure 3-7 Comparison of (A) the constructivist view of knowledge retention (strong foundation) and (B) the traditional view of knowledge retention (no foundation) (Lord, 1997).

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The constructivism was initiated in part of Piaget's idea (1964) of different stages of cognitive structures. In Piaget's view, knowledge is actively constructed by students, not passively received from the environment. The knowledge cannot be simply transferred from teacher to student who must actively interpret and develop understanding from information given to them (Stage et al., 1998). Piaget also views learning as the construction of a scheme which consists of three parts: recognition of a certain situation, association of the situation with specific activities, as well as expectations of a certain result from the activities. In contrast, Vygosky's theoretical framework is based on the ideas of every function in the child's cultural development both on the social level (between people or inter-psychological) and individual level (inside the child or intra-psychological) (Vygosky, 1978). Vygotsky also conceptualises learning as a process involving a subject (the learner), an object (the task or activity) and mediating artefacts (Vygotsky, 1986). Activity in Vygosky's view is based on work from human actions to include a minimal meaningful context (Issroff & Scanlon, 2002).

Constructivist theories have dominated research in science education since the 1980s (Duit & Treagust, 1998; Fensham et al., 1992) to answer the five important questions in all aspects of science education (Ogborn, 1988):

- What do we know? (The ontological question)
- How do we know? (The epistemological question)
- What makes it happen? (The causal question)
- What can we do with our knowledge? (The technological question)
- How can we communicate these ideas? (The communicative question)

The former constructivism investigates learning primarily as a process of actively cognitive reorganisation to answer these first three questions, whereas the latter tends to examine the process of enculturation into a practical community to expand the answers for the whole questions (Cobb, 1996). Within science educational contexts, personal constructivism (Piaget, 1964), social constructivism (Vygotsky, 1978), radical constructivism (von Glasersfeld, 1989) and educational constructivism (Mathews, 1997, 1998) had been used in teaching and learning science. Social constructivism and educational constructivism involving theories of learning and

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pedagogy have had the greatest impact on instruction and curriculum design since they seem to be the most conducive to integration into current educational approaches. Social constructivism is a transition from social contexts to individual understanding (Mortimer & Scott, 2003; Osborne, 1996). Learning science with social constructivism has been initiated into the culture of science. Students have been given access to the knowledge systems of science, and the process of knowledge construction must go beyond personal enquiry. Students need to be given access not only to physical experiences but also to the concepts and models of conventional science. The knowledge can never be transferred directly to the mind of the student; however, teachers have to assist students to construct proper understandings by themselves from their social contexts (Driver, Asoko, et al., 1994; Driver, Squires, et al., 1994).

Radical constructivism was developed by von Glasersfeld (1984) as part of constructivists who viewed constructivism only in terms of learning being built on prior knowledge (Tobin & Tippins, 1993; Tobin et al., 1994). This constructivist theory had impacts on science and mathematics in 1990s (Matthews, 2000) and emphasised the knowledge does not reflect an object ontological reality, but exclusively an ordering and organisation of a world constituted by our specific experience of the world (von Glasersfeld, 1987). It is impossible for one person to know exactly the constructions of the world as constructed internally by another person. Radical constructivist perspective used in science education is thus not only thought of knowledge as being built up by the individual in a particular social context, but also recognised that in the teaching and learning context, what is being talked about is a particular view of the world, rather than the world as such (von Glasersfeld, 1993).

Radical constructivism is a part of Hein's learning model (Figure 3-8). Hein (1996) stated that learning is an active and a social process whereby people learn as they learn. Students use sensory input to construct meaning but physical action alone is insufficient since action that constructs meaning is mental, minds-on and hands-on (Hein, 1999). New knowledge needs to be related to prior knowledge and experience to be accessible, to contextualise what is already known and move into what is unknown. The important outcomes of science education research based on constructivist learning theory have been recognition of the role of prior knowledge in knowledge

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construction and learning. Thus, students' prior knowledge related to the teaching concepts should be explored before starting the lessons.

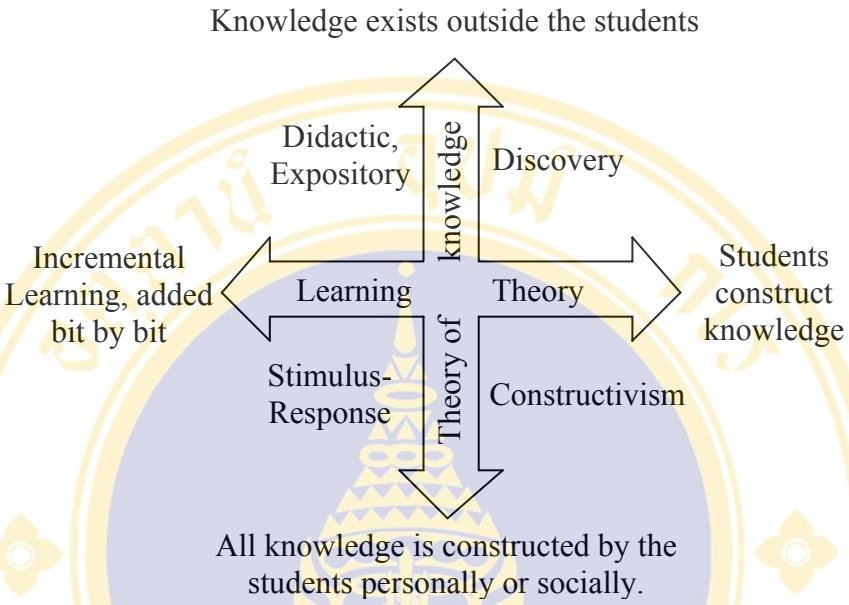

Figure 3-8 Hein's educational theories model for the appropriate pedagogy (Hein, 1994).

3.4.2 Experiential learning theory

Experiential learning theory (ELT) has its origin in critique of "blueprint models for learning and teaching" (Wildemeersch et al., 1998): Dewey's model of learning process, Lewinian model of action research and laboratory training and Piaget's model of learning and cognitive development. ELT defines learning as "the process whereby knowledge is created through the transformation of experience. Knowledge results from the combination of grasping and transforming experience" (Kolb, 1984). Unlike other learning theories, ELT emphasised the central role that experience plays in the learning process. Kolb's learning cycle composes of four steps to complete long-term learning (Figure 3-9): concrete experience (CE), abstract conceptualisation (AC), reflective observation (RO) and active experimentation (AE). By definition, CE and AC are related to modes of grasping experience, whereas RO and AE are related to modes of transforming experience (Kolb et al., 2000).

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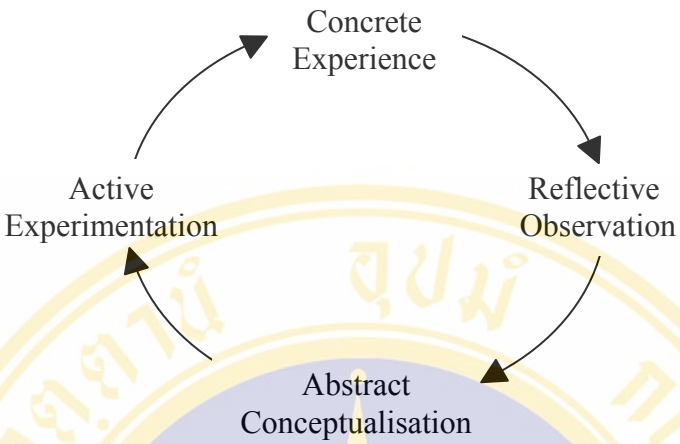

Figure 3-9 Kolb's learning style (Kolb, 1984).

Tell me, and I will forget.
Show me, and I may remember.
Involve me, and I will understand.

As stated in this Chinese proverb which is a guideline principle of experiential education (Hovelynck, 2001), students will progress through a learning cycle in which experience will lead to observation and reflection, which will then lead to concept formation. The conceptual development in turn will lead to new experiences and further experimentation in an experiential learning cycle. The concrete experience stage needs to be engaging by focusing on 'doing' from the students' previous experiences. The next stage, reflection observation, is about 'understanding the doing' by synthesising of meanings and organising of reflection. Students themselves have to ask rhetorical questions to investigate the knowledge they receive, for instance, 'How does this fit together?' or 'How does this fit with what I already know?'. The abstract conceptualisation stage focuses on the "understanding" part while the stage of active is about 'doing the understanding'. This stage allows application and links to theory and practice (Kolb, 1984, 2000, 2005).

Experiential learning theory, nowadays, emphasises current cognitive theory on constructivism, knowledge-dependent learning, and situated knowledge (Tuss, 1996;

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Kolb & Kolb, 2005). The experiential learning approach comprises of basic features of any learning context (Barnes, 1976): (i) Learning occurs by means of the reception of complex information through experience with the world, and (ii) Learning occurs within a social context as the social constructivism. Experiential learning is a good model in science education and the current researches in science education with this learning model have focus on both a lack of authenticity or appropriate contextualisation in classroom learning and the effectiveness of experiential learning, pertaining to empirical evaluations of experience-based programmes and curricula (Tuss, 1996). Students might have experiences from any contexts including the appropriate practical works such as hands-on experiments, interactive multimedia (Tsoi et al., 2002, 2005), research participation programmes, science-technology-society curricula (Tuss, 1996), or implementation of Kolb's learning styles into online distance education (Richmond & Cummings, 2005).

3.5 Practical work in science education

Past researches support the practice of laboratory-based works for students as practical for the following five major reasons (Friedler & Tamir, 1990):

(a) Science involves highly complex and abstract subject matter. Many students fail to comprehend such concepts without the concrete props and opportunities for manipulation afforded in the laboratory.

(b) Students' participation in actual investigations developing their enquiry and intellectual skills is an important component of learning science as enquiry.

(c) Practical experiences, whether manipulative or intellectual, are qualitatively different from other experiences and are essential for the development of skills and strategies with a wide range of generalisable effects.

(d) The laboratory has been found to offer unique contexts for the identification, diagnosis and remediation of students' misconceptions.

(e) Students usually enjoy activities and practical work and when offered a chance to experience meaningful and non-trivial experiences they become motivated and interested in science.

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In order to be encompass all currently available objects, materials or their representations, practical work is newly defined 'all those kinds of learning activities in science which involve students at some points in handling or observing the objects or materials (or direct representations of these, in a simulation or video-recording)' (Millar et al., 1999). The current researches have been required to develop teaching approaches to enable the practical works to be more effective element in science teaching (Friedler & Tamir, 1990). The practical works should more often be used to provide each student opportunity to: (i) explore, manipulate and reflect on findings; (ii) clarify understanding and misunderstanding with peers; and (iii) develop the concepts on their own (Hodson, 1992; Tobin, 1990).

To achieve the good outcomes of learning with any practical tasks, Millar et al. (1999, 2002) proposed an effective model involving the process of designing and evaluating a practical work in Figure 3-10. The starting point is the teacher's (or curriculum developer's) objectives for the task by specifying what the students are intended to learn from the tasks. The teacher then designs the practical task according to the decided learning objectives. Both the objectives and the practical are influenced by the teacher's views of science and learning as well as by practical and institutional considerations. After implementation in the classroom, students could be observed what they actually do on the task, and teacher should access what the students actually learn. The effectiveness will be assessed on both of these questions influenced by the students' view of science and of learning and by practical and institutional setting (Millar, 2002).

Practical work is best seen as communication in context of teaching scientific knowledge. It is not discovery or construction of something new and unknown; in contrast, it is making what others already know your own called 'constructivism' (Millar, 2004). The fundamental purpose of any practical tasks is to assist students to understand the natural world (following the aim of science education) by making links between two domains: the domains of real objects and observable things and the domain of ideas (Millar, 1999). Students value the practical work when: (i) it provides a cognitive challenge, (ii) it has a clear purpose and works and (iii) it gives them sufficient control and independence (Hodson, 1990). Thus, the success of learning

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with any practical works depends on the intended learning objectives of the tasks (Table 3-3) (Millar, 2002).

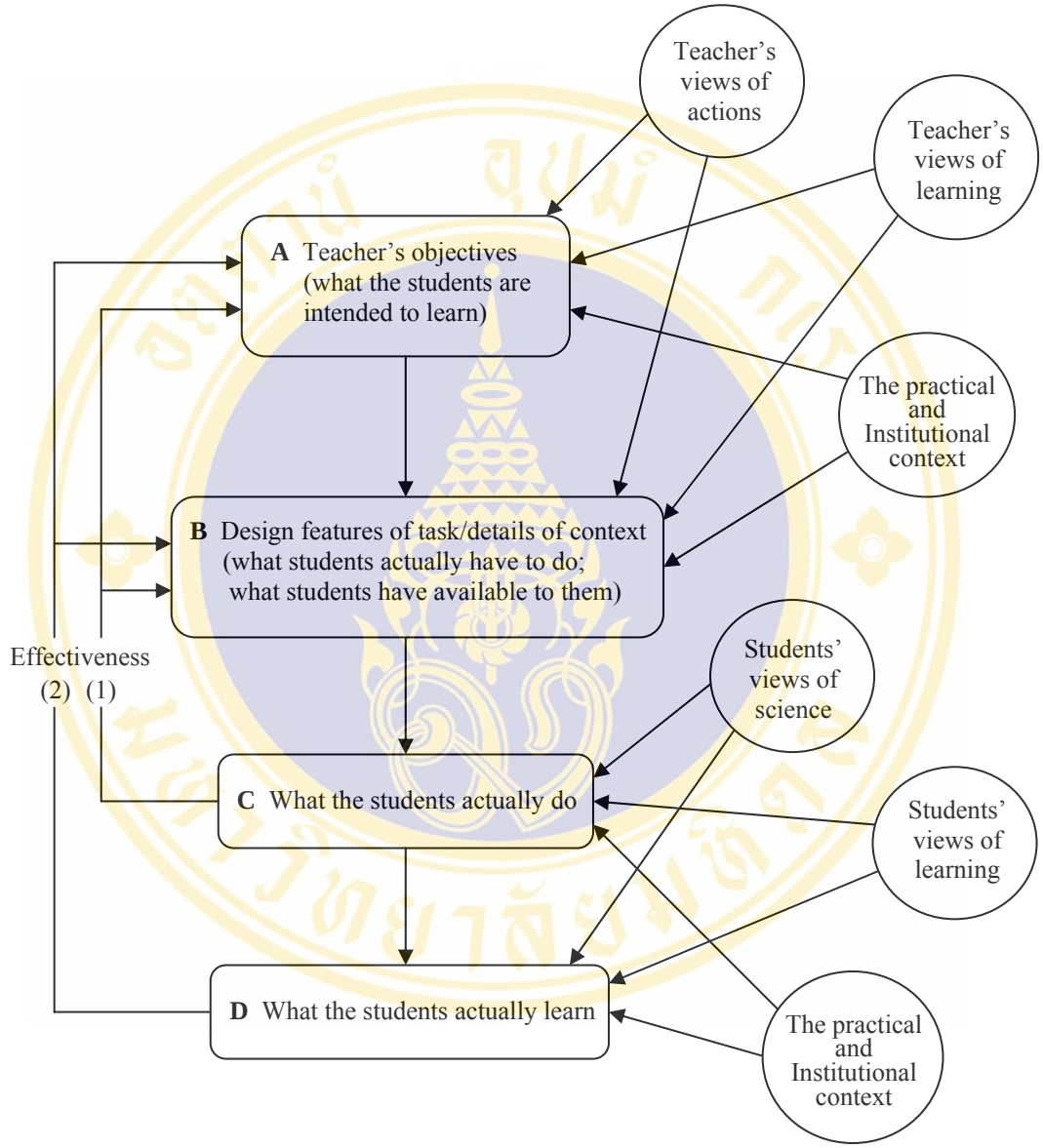

Figure 3-10 A model of process of design and evaluation of a teaching and learning with a practical task (Millar et al., 1999)

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Table 3-3 Possible intended learning outcomes (learning objectives) of a practical work (NRC, 2005)

Goal for practical work	**Description**
1. Enhancing mastery of subject matter	Enhance student understanding of specific scientific facts and concepts of the way in which these facts and concepts are organised in the scientific disciplines.
2. Developing scientific reasoning	Promote a student's ability to identify questions and concepts that guide scientific investigations; to design and conduct scientific investigations; to develop and revise scientific explanations and models; to recognise and analyse alternative explanations and models; to make and defend a scientific argument.
3. Understanding the complexity and ambiguity	Help students to address the challenge inherent in directly observing and manipulating the material world, including troubleshooting equipment used to make observations, understanding measurement error, and interpreting and aggregating in resulting data.
4. Developing practical skills	Learn to use the tools and conventions of science. For example, they may develop skills in using scientific equipment correctly and safely, making observations; taking measurements and carrying out well-defined scientific procedures.
5. Understanding of the nature of science	Help students to understand the values and assumptions inherent in the development and interpretation of scientific knowledge, such as the idea that science is a human endeavour that seeks to understand the material world and that scientific theories, models, and explanations change over time on the basis of new evidence.
6. Cultivating interest in science and interest in learning science	Become interested in learning more about science and see it as relevant to everyday life as a result of hands-on experiences that make science "come alive".
7. Developing teamwork abilities	Promote a student's ability to collaborate effectively with others in carrying out complex tasks, to share the work of the task, to assume different roles at different times, and to contribute and respond to ideas.

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In the context of biology, the purposes of practical work when resources are used in activities performed by small student groups are: (i) developing an understanding of structures and process in living organisms, and (ii) developing skills in manipulation, the process of science and conducting whole scientific investigations (Brown, 1995). A learning model for achieving such purposes of practical work in biology consists of four successive learning stages from facts, information, knowledge to action (Figure 3-11) (Chan et al., 2002; Montelpare & Williams, 2000; Winn, 1997). This model begins with the *facts* stage that students are in contact with basic facts and data of learning subject. Students then move to the *information* stage by complying with facts and data to collect into information. Students in the *knowledge* stage start to understand and contextualise the information obtained and acquired knowledge. In the final *action* stage, students can develop the ability to establish actions in the response to the acquired knowledge (Winn, 1997). This learning cycle is completed when students use the knowledge in the action stage to be facts or data in the first stage again as in the Kolb's learning cycle.

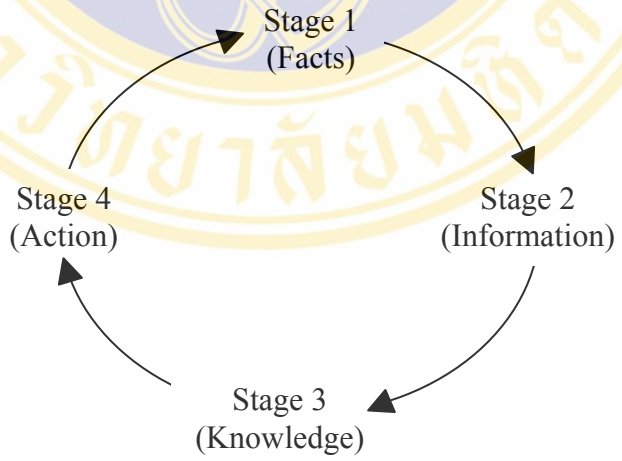

Figure 3-11 The design of practical learning model (adapted from Winn, 1997). The students' learning outcomes are expected from the purposes of practical works in the stage 2 to 4.

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3.6 Practical work based researches in biology education related to learning models in this study

As previously stated in Chapter 2, genetics is the most difficult topic in secondary biology (Table 3-4) since it is an area with a complex and large vocabulary (Bahar et al., 1999) and students lack understanding of the terms used (Wendelberger et al., 1998). Some areas are also moderately difficult to secondary school students such as photosynthesis, cellular respiration, Mendelian genetics, immune system, physiological homeostasis, antibiotics and biological detergents (Bahar et al., 1999; Finley et al., 1982).

Table 3-4 Topics of highest difficulty (Bahar et al., 1999)

Topic	Difficulty index*
Monohybrid and dihybrid crosses and linkage	22.2
Genetic engineering	13.4
Genetic control of development and metabolic processes	13.3
Meiosis	11.9
Central nervous system, sense organs and co-ordination	10.8
Gametes, alleles and genes	10.4

Note: * The difficulty index is the percentage of a number of students who still do not understand that topic to the total number of students in the sample.

Biochemistry is one of difficult subjects in undergraduate levels (Table 3-5) (Vella, 1990), for example, in the topics of carbohydrate chemistry, enzyme metabolism, integration of carbohydrate and lipid metabolism, lipids, molecular biology, nutrition, as well as protein structure and modification (Wendelberger et al., 1998). Rogers (1987) claimed this is because (i) the current teaching emphasises force feeding of facts; (ii) a great deal of knowledge is available on how people learn, yet few teachers use this knowledge for their teaching processes; and (iii) teachers still use lectures which are one of the poorest teaching methods to promote any knowledge for students.

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Table 3-5 Situations which make learning of biochemistry difficult (Vella, 1990)

Biochemistry (or almost any other subject) is experienced as difficult in the following seven situations:

(i) When teaching of Biochemistry addresses the learner's left mind and ignores the right mind.

(ii) When teaching of Biochemistry emphasises detailed descriptions of models and terminology rather than the principles and processes on which these descriptions and models are constructed.

(iii) When the teaching of Biochemistry emphasises transfer to, and acquisition by, the learner of factual information rather than the development of the learner's intellectual skills. Whereas factual information is very closely linked to verbal memory, the latter is frequently context-related and is short-lived, intellectual skills are an important component of 'intelligence' and once acquired are much more permanent and of more general application.

(iv) When teaching of Biochemistry is concerned with delivery to, and acquisition by, the learner of 'information' and ignores the 'formation' and 'transformation' of the learner. The 'information transfer' model of teaching ignores, and absolves itself of any responsibility for, the 'formation' and 'transformation' components that are integral to the education process.

(v) When the teaching of Biochemistry uses didactic methods that are not appropriate for the stated educational objectives.

(vi) When the teaching of Biochemistry is not related to the real-life experience of the learners and to their career objectives. This relates to the frequent complaint about relevance of much of the biochemistry taught especially to medical and other students of the health sciences.

(vii) When teaching of Biochemistry does not recognise the hierarchies of knowledge and understanding. The biochemical knowledge that the learner is to acquire can be viewed as having a hierarchical organisation.

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There are also some explanations why biology and related subjects are difficult to understand easily, for example, inappropriate the existing gap between the formal learning level demands of learning material and students' cognitive development (Lazarowitz & Penso, 1992), a lack of interest towards molecular biology or molecularphobia (Costa & de Brito, 2000; Wood, 1996), teaching mode of concept and principle in biology, as well as the misconceptions of prior knowledge which could obstruct the acquisition of new knowledge (Kindfield, 1991; Lazarowitz & Leab, 2006). To complete three learning objectives: content (illustration of the lecture material), application (becoming competent with technical skills and other aspects of experimentation) and method (relating to how scientific enquiry is carried out), students need to learn with good practical work both wet and dry practical works (Wood, 1996). Some researches using the practical work to construct the new knowledge in biology and related subjects are reviewed as the following details.

Haigh (1993) used hands-on and minds-on to introduce senior secondary students to give the initiation for finding solution to problems in practical work. However, the result indicated the mismatch between the students' declared confidence of their ability to plan open investigations in biology and their demonstrated competence. Therefore, the practical skills should have been developed and practised in the junior secondary school, and students require appropriate guidance regarding what is expected to them, and support while they are engaged in open investigation and feedback regarding their techniques and findings. Schulz & McRobbie (1994) suggested the science laboratory can be more effective mode of learning if experimental activities are designed from a constructivist perspective on conceptual change. By learning with this approach, students find the experiments more meaningful to learn concepts in the laboratory, find the ideas of other students to be helpful while doing the experiments in group and require good safety in the experiments. Besides the labworks, the learning and teaching biology should involve other practical activities to construct any concepts and enhance students' communication and teamwork skills such as concept mapping activities (Brown, 2003; Kinchin, 1999; Marbach-Ad, 2001), drawing-based activities (Rotbain et al., 2005), problem-solving exercises (Dobos et al., 1999), any models (Rothhaar et al., 2006; Rotbain et al., 2006), web-based activities (Skinner & Hoback, 2003).

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Schlenker & Tierney (2006) used practical works involving the integration of constructivism, discovery and enquiry to enhance students to learn bacterial population growth, population densities and some forecast of ramifications of unchecked population expansion upon available living space requirement. These practical activities were designed to integrate science, mathematics, sociology and public health ideas. By these practicals with the good teaching intervention, student can examine the bacterial expansion pattern and attempt to relate what they see and discover to their real-life situations.

In a series of young's people understanding of the nature of genetics (Lewis et al., 2000a, 2000b, 2000c; Wood-Robinson et al., 2000), genetics learning in secondary students aged 14-16 should focus on not only the pattern of heritance but also its mechanism. Students had the most basic ideas related to function, structure and location of genes, and many students hold the misconceptions that cells of different types will contain different genetic information. They also lack understanding of the physical link between chromosome and genetic material as well as the relationship between the behaviour of chromosome at cell division and the continuity of genetic information. These made it difficult for students to understand the more complex genetic concepts demanded by the UK National Curriculum, such as genetic engineering. Marbach-Ad & Stavy (2000) suggested to make a bridge between macroscopic and microscopic levels, for example, to explain the appearance of phenotypic traits (macroscopic level) using concepts like genes or chromosome (microscopic level) or apply the genetic knowledge to genetic engineering issues (Sadler & Zeidler, 2004).

Both wet laboratory and dry simulation practicals have been used to solve the students' understanding in many areas including in genetics and biochemistry (Wood, 1996). Many wet laboratories are based on various learning/teaching interventions and some assessments have been designed to be suitable in any curricula. For instance, Longtin et al. (2004) developed a classroom exercise to detect genotype of antibiotics resistance in *E. coli* by using the polymerase chain reaction, Hamilton et al. (2006) created a protocol for secondary students to investigate genetic variation using PCR method, and Zion et al. (2006) designed a simple hands-on laboratory procedure for secondary school students in studying both radiation damage and DNA repair

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systems in bacteria, which the experimental system might serve as a basis of open enquiry. Furthermore, Goodman et al. (2006) designed and implemented a hands-on course in introductory biology for elementary education majors which students appreciated how the relevance of science and conducting their own scientific experimentation could help them learn and analyse scientific concepts. Students enjoyed working and studying in a small group, and also valued the design of the course, which offered class time with both content discussion and hands-on experiments. Throughout the course, the students were likely to have experienced the course material as long-term learning instead of concentrating on memorisation and recall learning.

Nowadays, computer-based practicals have been used to improve the preparedness of students for laboratory exercises (Pamula et al., 1996), to assist the areas of science that are difficult to teach and learn using regular methods (Wekesa et al., 2006), and to develop novel web-based learning environment in bioinformatics and other 'omic' sciences (Gelbart & Yarden, 2006). Lee et al. (2002) achieved to implement the *Lateblight* computer simulation designed by Arnerson & Ticknor (1990) in the general biology laboratory and the science methods course for elementary teachers to reinforce the scientific process and to allow the students to learn by enquiry methods of building scientific concepts. This simulation was taught by using the constructivist instruction and cooperative learning approach. Aegerter-Wilmsen et al. (2006) introduced molecular life science students in the Netherlands and Switzerland to build the model of natural phenomena via computer simulation to increase the students' understanding of biological systems. Zumbach et al. (2006) claimed the hands-on experience with virtual molecular biology learning lab, lifelab®, can assist students to activate prior knowledge, understand basic concepts of new domains, to acquire domain specific declarative/procedural knowledge, to explore and to experiment, and to exchange research questions and findings by various instructional methods.

Up-to-date knowledge in genomics and bioinformatics has been taught by not only wet lab simulation (e.g. Campbell et al., 2006) but also computer-based practicals (e.g. Gelbart & Yaden, 2006). Thus, both labwork in bacterial conjugation and virtual class of protein purification have been proposed to teach with designed experiential model on constructivism in senior secondary school and undergraduate levels, respectively.

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CHAPTER IV
MATERIALS AND METHODS

This chapter focuses on the materials and methods used in both scientific and educational aspects. The scientific aspects include bacterial strains, chemicals, reagents, instruments as well as microbiological and molecular methods, whereas the educational aspects describe the research designs, the samples, the instruments used (together with their modifications and validations), data collections, statistics used during intervention phases.

4.1 Scientific aspect

4.1.1 Bacterial strains and vectors

Bacterial strains and vectors used in this study are shown in Table 4-1.

Table 4-1 Sources of bacterial strains and vectors used in this study

Strain or plasmid	Relevant characteristics	Source/reference
E. coli BL21(DE3)	F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm*(DE3)	Stratagene
E. coli ET12567	F^- *dam-13*::Tn*9 dcm-6 hsdM hsdS* Cm^r (pUB307 or pUZ8002)	MacNeil et al. (1992)
E. coli S17-1	*recA pro* (RP4-2Tet::Mu Kan::Tn*7*)	ATCC 47055; Simon et al. (1983)
E. coli JM109	*e14*$^-$ (*mcrA*$^-$) *recA1 endA1 gyrA96* (Nal^r) *thi-1 hsdR17* (rK^- mK^+) *supE44 relA1* Δ(*lac-proAB*) [F′ *traD36 proAB lacI*qZΔ*M15*]	Yanisch-Perron et al. (1985)
E. coli XL1-Blue	*recA1 endA1 gyrA96* (Nal^r) *thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*qZΔ*M15* Tn*10* (Tet^r)]	Bullock et al. (1987)

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Table 4-1 Sources of bacterial strains and vectors used in this study (continued)

Strain or plasmid	Relevant characteristics	Source/reference
S. coelicolor A(3)2 M145	Actinorhodin and undecylprodigiosin producer	Hopwood et al. (1985)
S. rimosus 593	Non-sporulating strain derived from *S. rimosus* R6	Gravius et al. (1994)
S. rimosus M4018	Industrial oxytetracycline production strain	Butler et al. (1989)
S. rimosus R7	Wild type, rimocidin producer	ATCC 10970; Hranueli et al. (1979)
pARO181	pMB1*ori oriT* (RP4) *lacZα* Ap^r (6,070 bp)	Parke (1990)
pARO190	pMB1*ori oriT* (RP4) *lacZα* Km^r (5,460 bp)	Parke (1990)
patrASet	*atrA* (M145) in pICLSet (6,300 bp)	Constructed by Jonathan A. Stead
pET16b	T7*lac* N-his•tag, FactorXa Ap^r (5,711bp)	Studier et al. (1990); Novagen
pET21c	T7*lac* C-his•tag N-T7•tag Ap^r (5,441bp)	Studier et al. (1990); Novagen
pGEM-T easy	3′-Oligothymine overhanged linear vector, *lacZ* f1*ori* Ap^r (3018 bp)	Promega
pICLSet	*aceA ermE*ptuf* in pSET152 (8,619 bp)	Tilley (2005)
pIJ6902	*tipA*p *tfd to* pUC18*ori oriT* (RK2) *int* ϕC31 *attP* ϕC31 *aac(3)IV tsr* (7,406 bp)	Huang et al. (2005)
pIJ8600	*tipA*p *tfd to* pUC18*ori oriT* (RK2) *int* ϕC31 *attP* ϕC31 *aac(3)IV tsr* (8,100 bp)	Sun et al. (1999)
pJAS407	*atrA* (M145) in pET16b	Uguru et al. (2005)

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Table 4-1 Sources of bacterial strains and vectors used in this study (continued)

Strain or plasmid	Relevant characteristics	Source/reference
pL644	R71A in pICLSet	Constructed by Jane E. Towle
pL646c	0.9-kb C-His_{10} *Nde*I-*Bam*HI *atrA* fragment in pICLSet	This work
pL646n	0.9-kb N-His_9 *Nde*I-*Bam*HI *atrA* fragment in pICLSet	This work
pSET152	pUC18*ori lacZα oriT* (RK2) *int* ϕC31 *attP* ϕC31 *aac(3)IV* (5,500 bp)	Bierman et al. (1992)
pYP001c	0.9-kb C-His_{10} *Nde*I-*Bam*HI *atrA* fragment in pET21c	This work
pYP002n	0.9-kb N-His_9 *Nde*I-*Bam*HI *atrA* fragment in pET16b	This work

4.1.2 Chemicals, media and enzymes

The Majority of routinely used chemicals and media components were purchased from Ameresco (USA), Amersham Pharmacia Biotech (UK), APS Finechem (Australia), Becton Dickinson (USA), Fluka (Switzerland), FMC Bioproduct (USA), Himedia (India), Hispanlab (Spain), Macherey-Nagel (Germany), Merck (Germany), Promega (USA), Scharlau (Spain), Sigma (Germany), Difco (UK), Oxoid (UK) and Doi Khum (Thailand). DNA markers were obtained from Gibco BRL (USA), Fermentus (USA), Biolabs (UK) and Bioline (UK) and protein makers were supplied by Fermentus (USA).

Kits for DNA mainupulation were obtained from QIAGEN (Germany) and Promega (USA). The chemicals for protein work and dialysis tubes were purchased from Bio-Rad (UK) and Spectrum Laboratories (UK), respectively.

Restriction enzymes were supplied by Gibco BRL (USA), Fermentus (USA) and New England BioLabs (USA). Calf Intestine Alkaline Phosphatase (CIAP) was obtained from Amersham Pharmacia Biotech (UK). Lysozyme, RNase A, T4 DNA

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ligase and *Taq* polymerase were purchased from Ameresco (USA), Fluka (Switzerland), Gibco BRL (USA), Fermentus (USA), Promega (USA) and Invitrogen (USA).

4.1.3 Culture media

(1) Mannitol soya flour agar (MS) consisted of 20 g mannitol, 20 g soya flour and 20 g agar per litre (Hobbs et al., 1989).

(2) R5 medium consisted of 103 g sucrose, 0.25 g K_2SO_4, 10.12 g $MgCl_2 \cdot 6H_2O$, 10 g glucose, 0.1 g Difco casamino acids, 5 g yeast extract, 5.73 g TES buffer, 22 g agar and 2 ml trace element solution [4 mg $ZnCl_2$, 20 mg $FeCl_3 \cdot 7H_2O$, 1 mg $CuCl_2$, 1 mg $MnCl_2 \cdot 4H_2O$, 1 mg $NaHB_4O_7 \cdot 10H_2O$ and 1 mg $(NH_4)_2MoO_4 \cdot 10H_2O$ per 100 ml] per litre. After autoclaving pre-sterilised additions of 10 ml KH_2PO_4 (0.5% w/v), 4 ml $CaCl_2 \cdot 2H_2O$ (5 M), 15 ml L-proline (20% w/v) and 7 ml NaOH (1 N) were added (Kieser et al., 2000).

(3) Luria-Bertani (LB) broth consisted of 10 mg tryptone and 5 g yeast extract, 10 g NaCl per litre and was adjusted to pH 7.2 with NaOH.

(4) LB agar was prepared as LB broth with the addition of 1.5 g agar per 100 ml LB broth.

(5) 2 × YT consisted of 10 g yeast extract and 5 g NaCl per litre.

4.1.4 Sterilisation of culture media and solutions

Antibiotic solutions were sterilised by filtration through a millipore 0.2 µm dispensable filter. All culture media and various additions were sterilised by autoclaving at 121 ºC for 20 min.

4.1.5 Growth and maintenance of *Streptomyces*

Unless otherwise stated, the methods for *Streptomyces* growth were as detailed in Kieser et al. (2000).

Growth conditions: Streptomycetes were grown by streaking spore suspension on the surface of agar media and incubated at 30 ºC for 3-5 days. In liquid culture, spores were typically grown in 50 ml of media in 250 ml Erlenmeyer flask having spring coil rounded at the bottom of the flask at 30 ºC, 200-250 rpm. Spores

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suspensions of 10^9-10^{10} spores/ml were used to inoculate in Tryptic soya broth (TSB) in the ratio of 1:100. The appropriate antibiotic concentrations added in the media were as shown in Table 4-2.

Table 4-2 Concentrations of antibiotics used for growing *Streptomyces* and *E. coli*

Antibiotic stock solution (mg/ml)	Final concentration in media (μg/ml)				
	Streptomyces			*E. coli*	
	Agar	Broth	Flooding (μg/plate)	Agar	Broth
Ampicillin (100)	–	–	–	100	50
Apramycin (50)	100	50	100 (2000)	100	50
Chloramphenicol (200 in ethanol)	–	–	–	50	25
Kanamycin (50)	–	–	–	50	25
Nalidixic acid (25 in 0.15 M NaOH)	25	–	25 (500)	–	–
Thiostrepton (50 in DMSO)	50	5	50 (1000)	–	–

Preparation of spores: Streptomyces were grown on MS medium at 30 ºC for 3-5 days or until the surface was fully covered by a mass of spores. The spores were harvested by adding 10 ml of sterilised water and rubbing the surface gently with a sterilised needle. Mycelium fragments and contaminating agar were removed by a single passage through a cotton wool filter (Kieser et al., 2000). The collected spores were precipitated by centrifugation at 4000-6000 rpm for 2-5 min and resuspended in 20% glycerol. The spores were stored at -20 ºC.

Spore count: Colony forming units were determined by plating spore samples on MS agar. The numbers of colonies were counted after incubation at 30 ºC for 5 days.

4.1.6 Growth and maintenance of *E. coli*

Unless otherwise stated, all methods for *E. coli* cultivation were obtained from Sambrook & Russell (2001).

Growth conditions: E. coli was grown on LB agar at 37 ºC overnight or until colonies were clearly visible. Plates could then be stored at 4 ºC for a month. In

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liquid culture, *E. coli* was grown in LB broth at 37 ºC, 200-250 rpm. Appropriate antibiotic concentrations were used as shown in Table 4-1.

Preservation of E. coli strains: E. coli were maintained for long periods by transferring 0.5 ml of a dense bacterial culture to a cryogenic storage tube. An equal volume of sterilised 50% (w/v) glycerol was then added to the bacterial culture. The contents were mixed and then stored at -80 ºC.

4.1.7 Introduction of plasmid DNA into *E. coli* by transformation

E. coli competent cells were prepared as described by Chung et al. (1989) and *E. coli* transformation procedure by heat-shock was obtained from Sambrook & Russell (2001).

Preparation of competent cells: 500 µl of an overnight culture of *E. coli* was added in 50 ml of LB broth and incubated at 37 ºC, 250 rpm till *E. coli* reached on early exponential phase (OD_{600} at 0.3-0.4). The culture was then transferred to 50 ml centrifuge tube and incubated on ice for 30 min. The cells were harvested by centrifugation at 4000 rpm, 4 ºC for 5 min and resuspended in 5 ml ice-cold transformation and storage solution [TSS; LB broth containing 10% PEG (MW 8000), 5% (v/v) DMSO and 50 mM $MgCl_2$, at pH 6.5]. Cells were harvested again and resuspended in 2 ml ice-cold TSS. Cells were aliquoted in 100 µl each and stored at -80 ºC or directly used for transformation.

Transformation procedure: 100-250 ng of plasmid DNA (at a volume of no more than 10 µl) was added to 100 µl of competent cells. The samples was gently mixed and incubated on ice for 20 min. The sample was then heat-shocked at 42 ºC for 90 min, and immediately transferred the tube to ice for 5 min. 900 µl of LB broth was added and the tube was incubated at 37 ºC for 1 h. The cells were harvested by centrifugation at 12000 rpm for 2 min and the supernatant was decanted. The pellet was then resuspended and plated onto LB agar containing the appropriate antibiotics/ chromogenic substances. In case of selection by the function of gene *lacZ*, 40 µl of 20 mg/ml X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) in dimethyl formamide (DMF) and 4 µl of 200 mg/ml IPTG (isopropylthio-β-D-galactoside) were spread on

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the LB agar plates to select the recombinant clones. The plates were incubated overnight at 37 ºC.

The transformation efficiency of the competent cells could be calculated as follows:

Transformation efficiency = number of colonies obtained × dilution × 1/amount of DNA used (μg)

Transformation efficiency should reach 1×10^{-6} to 1×10^{-7}.

4.1.8 Method of intergeneric conjugation

The protocol using spores as recipients described by Kieser et al. (2000) as followed. Spores of *Streptomyces* were suspended in 500 μl 2 × YT medium and treated at an appropriate temperature in each strain for 10 min. The donor *E. coli* ET12567 containing pUZ8002 or pUB307 was grown in LB until OD_{600} reached 0.4-0.6 in the presence of 50 μg/ml chloramphenical, 25 μg/ml kanamycin and 50 μg/ml apramycin. *E. coli* cells were pelleted by centrifugation and washed twice with LB broth to remove the remaining antibiotics, then resuspended in final volume of 500 μl LB broth (at the concentration 10^8 cells/ml). The *E. coli* cells and heat-treated spore suspension were mixed in an equal volume and spread on MS, ISP4 and Oxoid TSA media containing 10 mM $MgCl_2$ and incubated at 30 ºC for 16-20 h. The plated were overlaid with 1 ml sterilised water containing 500 μg/ml nalidixic acid and either apramycin or thiostrepton and incubated further at 30 ºC for 5-10 days.

For conjugation with mycelia, spores were inoculated in TSB and incubated at 30 ºC, 250 rpm for 24 h. The equal volume of mycelia was used to mix with the donor cells and further conjugation was performed in the same way as described previously. The conjugation frequency was calculated by the number of exconjugants per recipient.

4.1.9 General DNA manipulation methods

Unless otherwise stated, all methods used for the manipulation of recombinant DNA for *E. coli* and *Streptomyces* were obtained from Sambrook & Russell (2001) and Kieser et al. (2000), respectively.

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Commonly used buffers for DNA manipulation:

(1) Equilibrate phenol: 500 g phenol and 0.5 g 8-hydroxyquinoline saturated with 50 mM Tris-HCl pH 8.0

(2) 2 × Kirby mix: 2 g sodium tri-isopropylnaphthalene sulphate (TPNS, Kodak P3513; SDS can be used instead), 12 g sodium 4-aminosalicilate (BDH), 5 ml 2 M Tris-HCl pH 8.0 and 6 ml equilibrated phenol pH 8.0 in 100 ml.

(3) 6 × Loading dye: 0.25% bromophenol blue, 0.25% xylene cyanol and 30% (v/v) glycerol.

(4) Lysozyme solution: 0.3 M sucrose, 25 mM Tris-HCl and 25 mM EDTA pH 8.0. 10 µg/µl lysozyme and RNaseA 50 µg/ml were added before use.

(5) Solution I: 50 mM glucose, 10 mM EDTA and 25 mM Tris-HCl pH 8.0.

(6) Solution II: 0.2 M NaOH and 1% (w/v) SDS. Make fresh before use.

(7) Solution III: 3 M potassium acetate and 5 M glacial acetic acid.

(8) 50 × TAE buffer (electrophoresis buffer): 302.4 g of Tris-base, 57.1 ml of glacial acetic acid and 100 ml of 0.5 M EDTA pH 8.0 were added to 1 litre of deionised water.

(9) 10 × TBE buffer (electrophoresis buffer): 890 mM Tris-base, 890 mM boric acid and 0.25 mM EDTA, adjusted to pH 8.3 with NaOH.

(10) 10 × TE buffer: 100 mM Tris-HCl pH 8.0 and 1 mM EDTA.

Isolation of plasmid DNA from cells by alkaline lysis with SDS: Plasmid-containing *E. coli* cells were grown in 3-5 ml of LB broth overnight at 37 ºC. The cells were harvested by centrifugation at 12000 rpm and resuspended in ice-cold 100 µl Solution I. The sample was mixed and incubated on ice for 10-15 min. The bacteria were lysed by the addition of 200 µl of freshly prepared solution II and mixing by inversion of the tube several times. After no more than 5 min, precipitation of protein and chromosomal DNA was achieved by the addition of ice-cold 150 µl of solution III. The tube was inverted several times to disperse the solution III through the viscous bacterial lysate. After centrifugation at 12000 rpm for 5 min, the supernatant was transferred to a fresh tube and equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added. The solution was then mixed by inversion of the tube for 1 min

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before re-centrifugation. The top layer of the supernatant was transferred to another fresh tube, to which two volumes of absolute ethanol was added. After final centrifugation for 10 min, the supernatant was removed without disturbing the pellet and the tube was allowed to dry. The DNA was then re-dissolved in 20-50 µl of TE containing 20 µg/ml RNaseA. The DNA sample was kept at -20 ºC.

Preparation of plasmid DNA from cells for sequencing: High quality plasmid DNA for sequencing was achieved using QIAprep Miniprep (QIAGEN, Germany). The method was obtained from the manual of QIAprep Miniprep.

Extraction of total DNA from Streptomyces: Extraction of total DNA from *Streptomyces* was achieved using the Fisher method described in Hopwood et al. (1985) or the modified Kirby mix procedure (Kieser et al., 2000; Soliveri et al., 1999).

(1) Extraction from liquid culture by Fisher method: After growing *Streptomyces* in TSB at 30 ºC for 3 days, 1.5 ml of mycelia were harvested by centrifugation at 12000 rpm for 2 min and supernatant was decanted. The pellet of mycelia was resuspended in 500 µl of lysozyme solution and mixed thoroughly. The sample was incubated at 37 ºC until the suspension appeared clear (approx. 45-60 min). 250 µl of 2% SDS was added and immediately mixed by inversion of the tube several times. 250 µl of phenol/chloroform/isoamyl alcohol was then added and the sample was subjected to vortexing for 30 s. After centrifugation at 12000 rpm for 5 min, the aqueous layer was removed and placed into a fresh tube. The phenol/chloroform/ isoamyl alcohol might be repeated 2-3 times to remove a large amount of protein contaminants. 0.1 volume of 3.0 M sodium acetate pH 4.8 was added and mixed thoroughly before adding 1 volume of isopropanol was then added. The sample was mixed thoroughly once again before centrifugation at 12000 rpm for 10 min. The pellet was washed with 200 µl of 70% ethanol and dried before dissolved in 20-50 µl of 1 × TE buffer. The chromosomal DNA was stored at 4 ºC or -20 ºC for a long-period use.

(2) Extraction from a colony by the modified Kirby mix procedure: The 4-day-old colonies from solid media were crumbled with a toothpick in 50 µl of 1 × TE and then vortexed for 1 min. The samples were added 1 × TE to a final volume of 100 µl and 3 µl of 100 mg/ml lysozyme. After incubation at 37 ºC for 30-45 min, 133 µl 2 ×

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Kirby mix was added and mixed by vortexing for 1 min. 200 µl of phenol/chloroform/ isoamyl alcohol was added and mixed before centrifugation at 12000 rpm for 10 min. The upper phase was transferred to a fresh tube and 20 µl of 3 M sodium acetate was added before mixed gently by the inversion of the tube several times. 150 µl of isopropanol was added and then mixed thoroughly before centrifugation at 12000 rpm for 10 min. The pellet was washed with 150 µl of 70% ethanol and dried before dissolved in 20-50 µl of 1 × TE buffer.

Digestion of DNA with restriction enzymes: DNA was digested with a restriction endonuclease(s) to generate compatible ends for cloning and to verify newly created constructs (Roberts, 1988). The reactions were set up according to instructions provided by the manufacturers. Typically, digestion conditions consisted of 200 ng - 0.5 µg DNA, and 10 unit/µl restriction endonuclease, 2 × manufacturer buffer, which were made up to a total volume of 20 µl with sterilised deionised water. The reaction mixtures were incubated at 37 ºC or at proper temperature for 1 h up to overnight.

The 5' terminal phosphate groups on linearised vectors were removed using a single restriction enzyme with calf intestinal alkaline phosphatase (CIAP) when to prevent self ligation. After setting up the digestions, 1 µl of CIAP (1 unit/µl) was then added and incubated at 37 ºC for 1 h. The reactions with CIAP were terminated by incubation at 70 ºC for 20 min.

Ligation of DNA fragment: Digested DNA fragment was annealed with plasmid using T_4 DNA ligase to create recombinant plasmids. Typically, 25 ng of linear vector was used. The quantity of the ligation mixture was calculated using insert: vector ratio 2:1 when performing cohesive end ligations, and 1:1 when ligating blunt ends according to the equation below:

$$\text{ng of insert} = \frac{\text{vector size (kb)}}{\text{insert size (kb)}} \times \text{insert: vector molar ratio} \times \text{ng of vector}$$

Standard ligation reactions consist of x ng of vector and insert, 4 µl of 5 × ligation buffer and 1 unit/µl of T4 DNA ligase in a final volume of 20 µl. The ligation mix was then incubated at room temperature for 2 h or 16 ºC (or 4 ºC) overnight.

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Agarose gel electrophoresis: The DNA samples were mixed with 6 × loading dye at ratio 5:1 and run on 0.8% agarose gel in 1 × TAE or 1 × TBE at 100 V for 30-45 min. The agarose gel was stained with 0.5 μg/ml ethidium bromide for 10 min and de-stained with tap water for a further 5 min. The specific sizes of interest DNA was compared to 1 kb DNA ladder under UV illumination and then photographed.

Recovery of DNA fragments from agarose gels: DNA extraction from agarose gel was achieved using a freeze-squeeze method (Tauz & Renz, 1983) and a QIAquick Gel Extraction Kit (QIAGEN, Germany) that the reaction conditions were set up according to manufacturer instructions. By freeze-squeeze method, the agarose gel portions containing the DNA fragment were sliced out and transferred to a cotton wool filtration unit. After frozen at -80 ºC for 1 h, the samples were centrifuged at 4 ºC 12000 rpm for 10 min. 100 μl of phenol/choloroform/isoamyl alcohol was added into the filtrates and the samples were mixed by vortexing for 30 s. After centrifugation at 12000 rpm for 5 min, the aqueous layer was transferred to a fresh tube. 20 μl of 3.0 M sodium acetate pH 4.8 was added and mixed thoroughly. 2 volume of absolute ethanol were added and mixed by the inversion of the tubes. The samples were frozen at -20 ºC for 30 min before centrifugation at 12000 rpm for 5 min. The pellet was washed with 200 μl of 70% ethanol and dried before dissolving in 10-20 μl of 1 × TE buffer.

Purification of PCR product: PCR products were purified using the QIAquick purification kit (QIAGEN, Germany). The purified DNA was quantified on agarose gel electrophoresis and stored at -20 ºC for a long-period use.

DNA sequencing: DNA was extracted from liquid culture using QIAprep Miniprep and QIAquick purification kit (QIAGEN, Germany) and sent for sequencing at the Bio-Technology Service Unit (BSU), National Science and Technology Development Agency (NSTDA), Thailand and DNA Services, Astbury Building, Faculty of Biological Sciences, University of Leeds, UK.

Polymerase chain reaction (PCR): A PCR reaction mixture consisted of 20 ng/μl template DNA, 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 10% DMSO, 0.5 unit/μl *Taq* DNA polymerase and 0.05 pmol/μl forward and reverse primers (Table 4-3) to a total volume of 20 μl with sterilised deionised water. 100 μl of sterilised

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mineral oil was added on the top of reaction mixtures to prevent the evaporation of the reactants. The standard protocol entailed a denaturation step of 94 ºC for 5 min, and a 30-cycle amplification step consisted of a denaturing step of 94 ºC for 30-60 s followed by a 30-s annealing step at the temperature specific to each primer pair as shown in Table 4-3. The cycle completed with an elongation step at 72 ºC for 1 min per kb of the template DNA. The reaction completed with a polishing step of 72 ºC for 10 min. Approximately, 10% of PCR products were run on 0.8% agarose gel to determine if amplification of the DNA was successful.

Table 4-3 Primer sequences and reaction conditions for regions amplified by PCR

Target fragment	Primer sequence	Annealing temp. (°C)	Extension time (min)
tsr	**ATT012:** 5' TGA CTG AGT TGG ACA CCA TCG C 3' **ATT013:** 5' CCT GTC GAT CCT CTC GTG CAG 3'	68	1.30
attP	**ATT016:** 5' AGC CGC ATA ATC AGG TGA 3' **ATT017:** 5' AGG TGC GAA TAA GGG ACA 3'	53	1.30
attP/B	**attLF:** 5' GTT CAC CCA CAG CTG GAG GC 3' **attPR:** 5' ATT TCG CGC TCG CGC GA CTA 3'	62	0.30
attP/B	**attPF:** 5' GCC CTG GGT GGG TTA CAC GA 3' **attRR:** 5' CGT CAT GCC CCC AGT GAC C 3'	62	0.30
atrA	**AtrAF:** 5' GGA TCC TCA GGA TTC TCA TTG GTC GTC 3' **AtrAR:** 5' GGA TCC GTG GCT ATG ACC AGC AGC AC 3'	55	1.00
atrA (insert ten histidine codons at the 3′)	*Nde*I **AtrAC_For:** 5' CG CAT ATG CAT GTT CAG GAT TCT CAT TGG 3' *Bam*HI **AtrAC_Rev:** 5' CG GGA TCC TTA ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG CAC CGG CCG CGA CCG CAG 3'	60	10.00
atrA (disrupt *Nde*I site)	**NdeIcF:** 5' GAT CGA AGT CGT CAC ATG CAT GTT CAG GAT 3' **NdeIcR:** 5' ATC CTG AAC ATG CAT GTG ACG ACC TCC GAT 3'	55	7.00

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Table 4-3 Primer sequences and reaction conditions for regions amplified by PCR (continued)

Target fragment	Primer sequence	Annealing temp. (°C)	Extension time (min)
atrA (insert ten histidine codons at the 3′)	*Nde*I **AtrAC_For:** 5' CG CAT ATG CAT GTT CAG GAT TCT CAT TGG 3' *Bam*HI **AtrAC_Rev:** 5' CG GGA TCC TTA ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG CAC CGG CCG CGA CCG CAG 3'	60	10.00
atrA (disrupt *Nde*I site)	**NdeIcF:** 5' GAT CGA AGT CGT CAC ATG CAT GTT CAG GAT 3' **NdeIcR:** 5' ATC CTG AAC ATG CAT GTG ACG ACC TCC GAT 3'	55	7.00
atrA (change *Nde*I site to *Nco*I site)	*Nco*I **NdeI_NcoIF:** 5' AGG AGG ACC CCA ACC ATG CAT GTT CAG GAT 3'	55	7.00
	NdeI_NcoIR: 5' ATC CTG AAC ATG CAT GGT TGG GGT CCT CCT 3'		
attL	**ScC31attFor:** 5' CAC TGG GTG TCG CCG TTG 3' **pSET:** 5' TTC GGC GGC TTC AAG TTC GG 3'	56	1.50
attL	**attB_gresius:** 5' CGG TGC GGG TGC CAG GGC 3' **pSET:** 5' TTC GGC GGC TTC AAG TTC GG 3'	64	1.30
Oligohistine-tagged *atrA* in pGEM-T easy	**Gemup:** 5' TCC CAG TCA CGA CGT TGT AAA ACG 3' **Gemrt:** 5' AGC TAT GAC CAT GAT GAT TAC GCC AAG 3'	60	0.30
M13	**M13F:** 5' TTG TAA AAC GAC GGC CAG TGA 3' **M13R:** 5' CAG GAA ACA GCT ATG ACC ATG 3'	sequencing	
T7 promoter	5' TAA TAC GAC TCA CTA TAG GG 3'	sequencing	

Southern blotting analysis: Analysis of DNA on agarose gel by Southern blotting (Southern, 1975) was performed on Hybond-N^+ membrane using the Gene Image Random-Prime Labelling and Detection Kit (Amersham, UK). The buffers and solutions used for Southern blot hybridisation were described in Table 4-4.

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Table 4-4 Buffers and solutions for Southern blot analysis

Buffer/solution	Component	Final concentration	Preparation
Denaturing solution	NaOH NaCl	0.5 M 1.5 M	
Alkaline transfer buffer	NaOH NaCl	0.25 M 1.5 M	
SDS stock	SDS	10% (w/v)	Add to deionised water.
20 × SSC buffer	NaCl Sodium citrate	3.0 M 0.3 M	Adjust the pH to 7.0
Hybridisation buffer	Liquid block SDS from 10% stock Dextran sulphate (or PEG MW 8000)	5.0% (v/v) 0.1% (w/v) 5.0% (w/v) (1.25% w/v)	Add to 5 × SSC before use.
2 × and 0.1 × Washing buffer	SDS from 10% stock	0.1% (w/v)	Add to 2 × SSC before use.
Buffer A	Tris-HCl NaCl	10 mM 300 mM	Adjust the pH to 9.5.
Tween® washing buffer	Tween® 20	0.3% (v/v)	Add to Buffer A before use.
Blocking solution	Liquid block	10% (v/v)	Add to Buffer A before use.
Antibody solution	BSA Anti-fluorescein alkaline phosphatase conjugate	0.5% (w/v) 1:5000	Add to Buffer A before use.

(1) Southern blot preparation: DNA digested with an appropriate enzyme(s) and the molecular weight marker were analysed by agarose gel electrophoresis and stained with ethidium bromide and photographed. The DNA's were depurinated by soaking with 0.25 M HCl at 50 rpm for 15 min. After rinsed briefly with deionised water, the gel was then immersed completely in denaturing solution twice at 50 rpm for 15 min. Southern blot was carried out by capillary transfer using alkaline transfer buffer. The gel was placed on pre-wet filter papers (in alkaline transfer buffer), overlaid with pre-wet Hybond-N^+ membrane (in alkaline transfer buffer) and pre-wet filter papers (in alkaline transfer buffer). About 10-cm paper towels and a 1-kg weight were stacked on the top. The transfer was carried out overnight and the membrane was used immediately or stored at 4 ºC after air dry.

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(2) Probe preparation: Probe for Southern hybridisation was prepared by the Gene Image Random-Prime Labelling kit (Amersham, UK) as described by manufacturer manual. 50 ng of the DNA fragment in 34 µl of deionised water was denaturised by boiling for 5 min with subsequent cooling on ice for 5 min. 10 µl of nucleotide mix, 5 µl of random primer and 1 µl (2U) of Klenow enzyme were added in a final volume of 50 µl. After thoroughly mixed, the reaction mixture was incubated at 37 ºC for overnight. The probe in a light-protected tube was stored at -20 ºC before use.

(3) Hybridisation: The membrane was incubated in hybridisation solution (20 ml/100 cm^2) at 68 ºC for 1-2 h with gentle agitation. 5-10 µl of appropriate probe was added to the hybridisation solution and denaturised by heating in a boiling water bath for 5 min and immediately chilled on ice for 5 min before use. The prehybridisation solution was replaced with hybridisation solution containing the denaturised probe and incubated at 68 ºC overnight.

(4) Detection: The membrane was washed twice with 2 × washing buffer at RT, 50 rpm for 10 min and washed twice with 0.1 × washing buffer at 68 ºC for 15 min with gentle agitation. After equilibration in Tween® washing buffer at RT, 50 rpm for 5 min, the membrane was incubated with antibody solution for 1 h at RT, 50 rpm. The membrane was washed three times with Tween® washing buffer at RT, 50 rpm for 5 min to remove excess of antibody. Transferred the membrane and dropped to plastic sheet, CDP-star detection agent (Amersham, UK) onto the membrane (0.5ml/100 cm^2) before incubated for 3-5 min. The membrane was immediately placed between two sheets and removed the excess solution and air bubble. The membrane was exposed to a Kodak Medical X-ray Film (Kodak, Japan) for suitable time depending on the strength of the signal. The film was developed in Kodak Developer and Replenisher (Kodak, Australia) for 1 min and briefly washed with tap water. The film was placed in Kodak Fixer and Replenisher (Kodak, Australia) for 1 min and then briefly washed with tap water before air dry.

4.1.10 General protein manipulation methods

Commonly used buffers for protein manipulation:

(1) Lysis buffer: 50 mM NaH_2PO_4, 300 mM NaCl and 10 mM imidazole, pH 8.0.

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(2) Wash buffer: 50 mM NaH_2PO_4, 300 mM NaCl and 20 mM imidazole, pH 8.0.

(3) Elution buffer: 50 mM NaH_2PO_4, 300 mM NaCl and 250 mM imidazole, pH 8.0.

(4) 10 × Tris-glycerine buffer: 1% (w/v) SDS, 0.25 M Tris-base and 1.92 M glycerine.

(5) Protein gel staining solution: 0.3% (w/v) Coomasie brilliant blue R in 10% (v/v) acetic acid and 25% (v/v) ethanol.

(6) Protein gel destaining solution: 10% (v/v) acetic acid and 25% (v/v) ethanol.

(7) Dialysis buffer for AtrA: 50 mM Tris-HCl (pH 7.6), 300 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol (DTT) and 40% (v/v) glycerol in 5 litres. The buffer was chilled to 4 ºC before use.

(8) 1 × Transfer buffer: 25 mM Tris-base, 157 mM Glycerine and 20% (v/v) methanol. The buffer was chilled to 4 ºC before use.

(9) 5 × Tris-buffered saline with Tween (TBST): 50 mM Tris-HCl (pH 8.0), 0.75 M NaCl and 0.25% (v/v) Tween-20. The buffer was chilled to 4 ºC before use.

(10) Ponceau S stain: 0.1% (w/v) Ponceau S in 5% (v/v) acetic acid.

(11) Milk blotto: 7% (w/v) nonfat dry instant milk in cool 1 × TBST.

(12) ECL solution 1: 1 ml of 1 M Tris-HCl pH 8.5, 100 µl of 250 mM luminol, 44 µl of 90 mM p-coumaric acid and 8.85 ml deionised water.

(13) ECL solution 2: 6 µl of 30% H_2O_2, 1 ml of 1 M Tris-HCl pH 8.5 and 9 ml deionised water.

(14) 5 × Tris-glycerine-EDTA (TGED) buffer: 250 mM Tris-HCl pH 8.5, 1.9 M glycerine and 0.25 mM EDTA adjusted to pH 8.3 with NaOH.

(15) 5 × Tris-glycerine-EDTA-KCl (TGEK) buffer: 10 mM Tris-HCl pH 7.9, 10% (v/v) glycerine, 0.1 mM EDTA and 50 mM KCl.

Preparation of protein from cells: Protein extracts were prepared according to a modified method from pET system manual (Novagen, US) and protein purification manual under native condition (QIAGEN, Germany). The *E. coli* cells with the

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recombinant plasmid were grown in LB containing appropriate antibiotics until OD_{600} reached 0.4-0.6. The expression was induced by the addition of IPTG at a final volume of 1 mM. After 3-h growth at 37 ºC, 250 rpm, the cells were harvested by centrifugation at 3000 rpm for 10 min in a Sorvall RC-SB Refrigerated Superspeed Centrifuge. The pellets were stored at -20 ºC before use. The cells were thawed for 15 min and resuspended in 1 ml lysis buffer per 50 ml culture. Lysozyme was added to 1 mg/ml and then incubated on ice for 30 min. The cells were lysed by sonication (six times for 10 s, each time with 5 s between) and then centrifuged at 12000 rpm, 4 ºC for 20 min to remove cell debris.

In case of *Streptomyces*, 10 µl of concentrated spores were grown in 50 ml of TSB containing appropriate antibiotics for 3 days at 30 ºC, 250 rpm to prepare the seed culture. 10% (v/v) of seed culture was inoculated into two flasks of 250 ml TSB and continued to grow for 2 days at 30 ºC, 250 rpm. The cells were harvested by centrifugation at 3000 rpm for 10 min in a Sorvall RC-SB Refrigerated Superspeed Centrifuge. The pellets were stored at -20 ºC before use. The cells were thawed for 15 min and resuspended in 20 ml lysis buffer per 500 ml culture. The cells were lysed in a pre-cooled French pressure cell (SLM-AMINCO®, Spectronic Instruments) and then centrifuge at 100000 × *g* (32000 rpm) for 30 min at 4 ºC using a Beckman 45Ti rotor to remove cell debris.

Determination of protein concentration: The amount of protein present in a sample was determined using the Bio-Rad Protein Assay (München, Germany) calibrating against bovine serum albumin (BSA) standards of 0.1-0.5 µg/ml. This dye-binding method following the techniques developed by Bradford (1976) which is reliant on the maximum absorbance of Coomassie Brilliant Blue G-250 in an acidic solution shift from 465 nm to 595 nm when protein binding occurs.

SDS polyacrylamide gel electrophoresis: The SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a 12% concentration gel, made by the method described by Laemmli (1970). Gels were poured between Mini-protean II glass plates (Bio-Rad, UK). The running gel contained 12% (w/v) (29: 1) acrylamide: bis-acrylamide (Bio-Rad, UK), 400 mM Tris-HCl (pH 8.8), 0.1% (w/v) SDS, 0.1% (w/v) ammonium persulphate (APS) and 0.1% (v/v) N,N,N',N'-tetramethyl-ethylene

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diamine (TEMED). Polymerisation occurred under a layer of water-saturated isobutanol. The stacking gel contained 4.2% (w/v) (29:1) acrylamide: bis-acrylamide, 120 mM Tris-HCl (pH 6.8), 0.1% (w/v) SDS, 0.1% (w/v) APS and 0.1% (v/v) TEMED. 1 × Laemmli buffer (Bio-Rad, UK) was loaded to each sample prior to loading on the gel and 10 µl of PageRuler^{TM} unstained protein ladder (Fermentus, USA) run alongside the samples as markers. Gel electrophoresis was carried out in a Bio-Rad minigel tank in 1 × Tris-glycerine buffer at 100 V. Protein bands were visualised by staining with protein gel staining solution then destained in a protein gel destaining solution.

Protein purification using Ni^{2+} affinity chromatography: Proteins prepared from cells were purified using Ni-NTA spin column (QIAGEN, Germany). The column was equilibrated with 600 µl lysis buffer and 700 × *g* centrifuged (approx. 2000 rpm) for 2 min. 600 µl of the cleared lysate containing the histidine-tagged proteins was loaded onto the pre-equilibrated column before at 700 × *g* centrifugation for 2 min. The column was washed twice with 600 µl wash buffer and centrifuged at 700 × *g* for 2 min. The proteins were eluted twice with 200 µl elution buffer before centrifugation at 700 × *g* for 2 min. 40 µl of cleared cell lysate, unbounded protein, first wash, second wash and elute samples were collected and added with 1 volume of 1 × Laemmli buffer. After sonication and boiling for 2 min, all samples were run on 12% SDS-PAGE gel at 120 V.

Dialysis: Dialysis was performed using a dialysis pouch (Spectra/Por® Cellulose Ester, Spectrum). 400 µl of samples were loaded into the dialysis pouch, which floated in 5 litres of agitated dialysis buffer at 4 ºC overnight. The dialysed elute sample was stored at -20 ºC. 20 µl of dialysed sample was collected and added with 1 volume of 1 × Laemmli buffer. After sonication and boiling for 2 min, the sample was run on 12% SDS-PAGE gel at 120 V.

Western blot analysis: The electrophoretic transfer of proteins from SDS-PAGE gel to PVDF or nitrocellulose membrane and immunoblotting were obtained using a modified procedure from Burnette (1981) and Towbin et al. (1979). After gel electrophoresis of the samples, the gel was equilibrated in 1 × transfer buffer for 30 min using agitation. Separated protein bands were transferred onto PVDF (soaked in

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methanol before use) or nitrocellulose membranes using a submerged-type apparatus at 100 V, 4 ºC for 1 h. The protein transfer was verified by staining the membrane with Ponceau S stain for 1 min and briefly washing with tap water. The membrane was used immediately or stored at RT after drying with filter papers. Blots were blocked with milk blotto for 30 min at RT using agitation. Blotting proceeded with the monoclonal anti-His primary antibody (Invitrogen, USA) in 1 × TBST (in dilution of 1:7500) at RT for 30 min. Blots were washed three times in 1 × TBST for 5 min at RT to remove excess primary antibodies. For detection purpose, a goat anti-mouse antibody conjugated with horseradish peroxide (HRP) (Sigma, USA) was applied in 1 × TBST (in dilution of 1:7500) at RT for 30 min. Blots were also washed three times in 1 × TBST at RT for 5 min to remove excess secondary antibodies. To visualise the protein-antibody complex by chemiluminescence, the membrane was incubated with a mixture of ECL solution 1 and solution 2 (Amersham, UK) for 1 min and exposed together with an X-ray film for 45-60 s. The X-ray film was then developed and scanned.

4.1.11 Analysis of DNA: protein interaction using electrophoretic mobility shift

The electrophoretic mobility shift assays (EMSA) were obtained using a method of Vujaklija et al. (1993) and Stead et al. (2006). To determine the interaction between DNA and protein, DNA and protein were mixed in a final volume of 20 µl with 1 × TGEK containing 2 µg of sonicated Herring sperm DNA. After incubation at 30 ºC for 20 min, samples were run in native 5% acrylamide: bis-acrylamide gel (80:1) with, as the re-circulating running buffer 1 × TGED. DNA was labelled at the 5' end fluorescein incorporated during primer synthesis and complexes within the gel were visualised using a Fluorescence Molecular Imager (Bio-Rad FX Pro) with an exitation wavelength of 488 nm in combination with a 523-nm filter. The DNA probe used was a double-stranded oligonucleotide corresponding to the *atrA* Footprinting region 2 of *actII*-ORF4 (Uguru et al., 2005), the sequence of which was 5' Flu-CGA TGC GGG ATG TGT ATT TCC GCT TAA ATC C 3'.

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4.2 Educational aspect: Learning 'bacterial conjugation' through laboratory work using different teaching sequences at secondary school level

4.2.1 Instrumentation

Based on the knowledge from previous section, a laboratory exercise 'Conjugation in *E. coli*' (Appendix B) was designed to assist students in understanding the concepts of bacteria conjugation by their experiences. Owing to the difficulty of conjugation mechanism, a drawing-based activity (Appendix C) was designed to use the lecture class. The instruments to assess students' understanding of the concepts were 20 multiple choice questions (MCQ) used as pre- and post-tests (Appendix D, Section D.1) as well as six open-ended questions used as a conceptual tests (Appendix D, Section D.2). All tests were validated by using the index of item-objective congruent (IOC) (Hambleton, 1980; Rovinelli & Hambleton, 1977). The IOC was processed by rating procedures for measuring the content experts' judgements (Appendix E, Section E.1) following the specific objectives listed by the test developer. The scores using for item evaluation in each objective were: a +1 if there was a strong match between an item and an objective; a 0 if a judge was uncertain whether congruence exists; and a –1 if the item did not match the objective. The IOC was then calculated by the summation of the experts' judgement scores divided by the number of experts. The index score for each item was in a range of –1 and +1, and the acceptable score to be test items is not less than 0.5. In this study, all item average scores of five experts were higher than 0.65.

4.2.2 Pilot test

The pre-, post- and conceptual tests were field tested by 116 third-year students in senior secondary school. The field test provided an opportunity for this test designer to consider possible questions concerning their content, grammar and readability. Appropriate changes were made based on the field test. The internal consistencies of the test items of MCQ and open-ended questions analysed through Cronbach's alpha coefficient were 0.89-0.90 and 0.82-0.86 (Appendix F, Section F.1 and F.2), respectively. Both MCQ and open-ended questions had a good reliability of

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0.90. As such, both can be used to assess students in understanding bacterial conjugation.

4.2.3 Research design

There was a practical time constraint of three months for data collection that influenced the scale of the study. The study involved three comparison groups of different teaching sequences: a control group learned by only lecture and two experimental groups learned by lecture before doing experiment and experiment done before lecture. The method used to answer the research questions was a multi-method approach utilising quantitative and qualitative approaches. A pre-test/post-test quasi-experimental design (Dawson, 1997) was employed in this study. The semi-structured conceptual interview after teaching sequences was used in the qualitative approach.

4.2.4 The subjects

Considering the conceptual area of bacterial conjugation in Thai curriculum, the study involved subjects who were senior secondary school students aged 14 to 17. Three separate student groups in a 10th grade science class in Mahidol Wittayanusorn School were selected to serve as the experimental and control groups. This school promotes mathematics and science teaching and learning at the senior secondary level and is located in the group of Mahidol University on Salaya Campus in Nakorn Pathom Province. A total of 141 students took part in this study. The member of students in experimental and control groups was 47.

4.2.5 Data collection

The instruments were constructed during a previous year and validated on five classes in 12th grade of senior secondary school. The study took place during the second semester of two-semester session. The three student groups in 10th grade were then exposed to the instructional procedures by the researcher, who undertook the teaching process to ensure a good measure of intra-lecture reliability, which if not maintained could be a potential source of experimental error (Frick & Semmel, 1978). Three instructional models and instructional plans were shown in Figure 4-1 to 4-3

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and Appendix G. The scoring rubrics (Appendix H) had been used to score responses to MCQ, open-ended questions, concept map and lab reports. The semi-structured conceptual interviews were audio-taped to probe the deeper understanding from all groups of students. The questionnaires (Appendix I) were given to student and teacher participants to evaluate the attitude towards learning in this concept using the laboratory exercise with an experiential learning model.

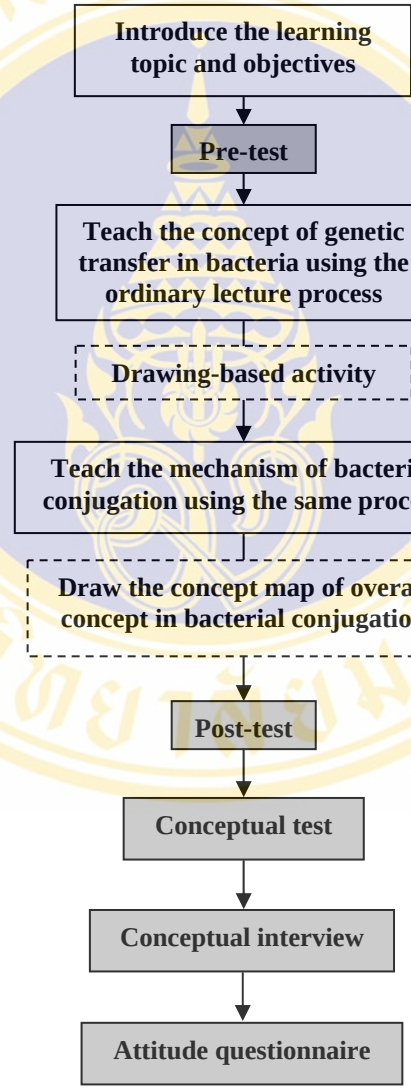

Figure 4-1 The teaching sequence, activities and assessments of bacterial conjugation for the control group. The dash-lined box showed the activity in the classrooms, whereas the opaque box indicated the assessment.

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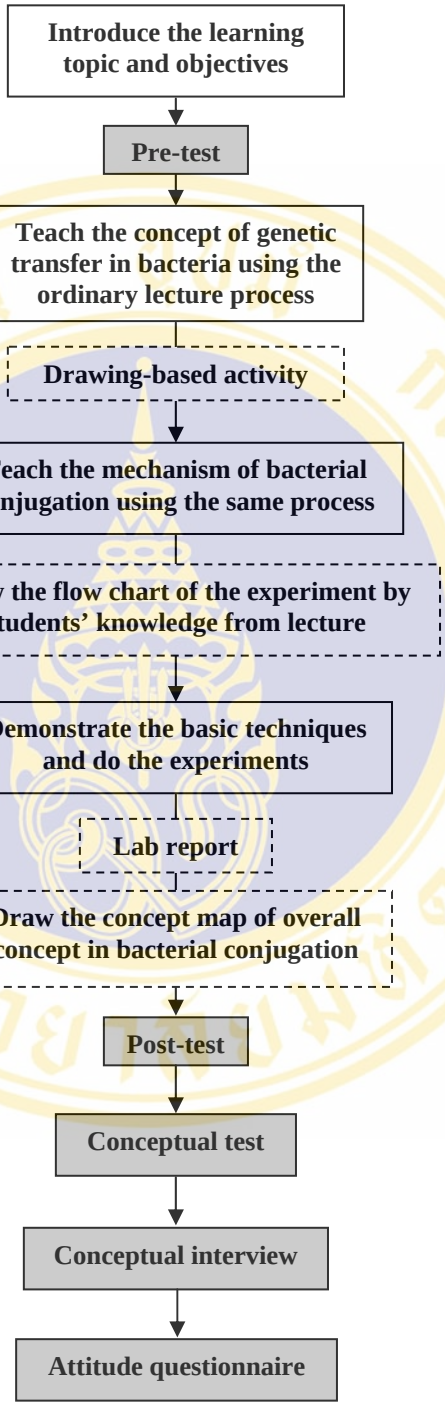

Figure 4-2 The teaching sequence, activities and assessments of bacterial conjugation for the experimental group using lecture before laboratory. The dash-lined box showed the activity in the classrooms, whereas the opaque box indicated the assessment.

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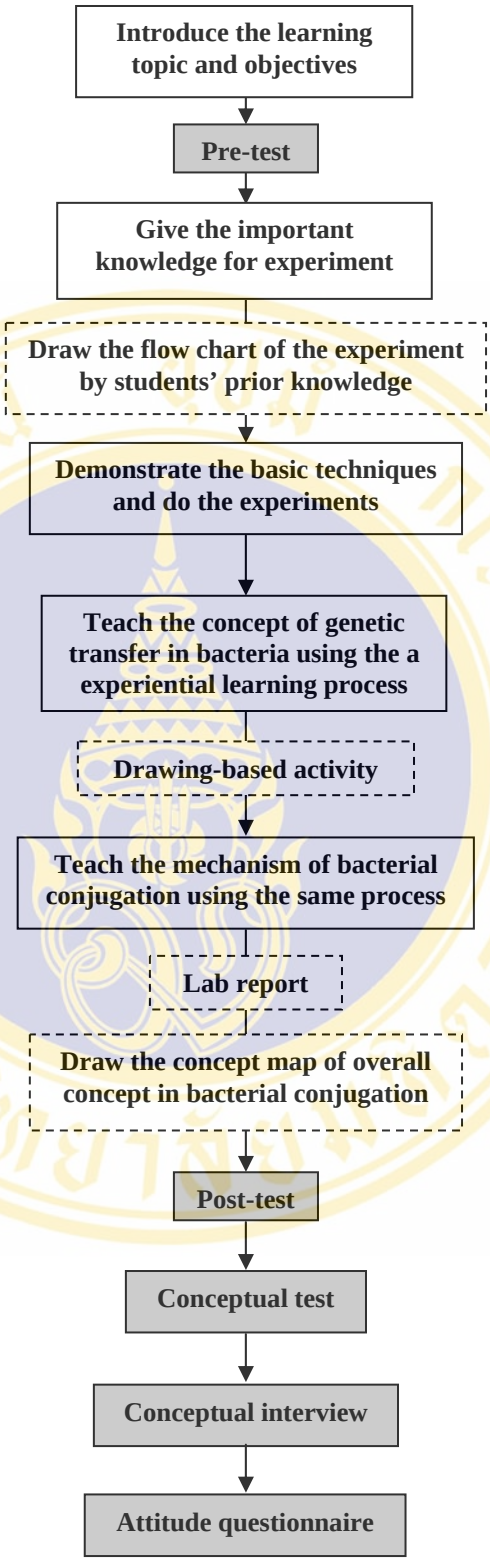

Figure 4-3 The teaching sequence, activities and assessments of bacterial conjugation for the experimental group using laboratory before lecture. The dash-lined box showed the activity in the classrooms, whereas the opaque box indicated the assessment.

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4.2.6 Data analysis and interpretation

The data were analysed using the Analysis of Covariance (ANCOVA) based on scores of post-test with the pre-test score as covariate to adjust for initial perceived difference on knowledge among three student groups at $p < 0.05$. The gain percentage (Hake, 1998) can be calculated according to the equation below. The improving students' conceptual understanding in the medium level is $0.7 >$ gain percentage ≥ 0.3. The gain percentage scores of ≥ 0.7 and < 0.3 are the high and low development level of conceptual understanding, respectively.

$$\text{Gain percentage} = \frac{(\%\text{post-test} - \%\text{pre-test})}{(100 - \%\text{pre-test})}$$

The gain percentage and conceptual test scores were performed using the One-Way Analysis of Variance and all comparison tests in this study were investigated through Scheffe's Multiple Comparison test at $p < 0.05$. The concept interviews were analysed to increase the accuracy of the quantitative results, and the questionnaire were coded as well as analysed to interpret whether this design with all instruments and this learning model was appropriate for assisting students in understanding the bacterial conjugation.

4.3 Educational aspect: Improving Thai students' understanding of concepts in 'protein purification' by using a simulation program in Thai and English versions

4.3.1 Instrumentation

Based on the knowledge of protein purification from *Streptomyces* in Section 4.1 and the difficulty of this topic to teach Thai undergraduates by traditional method, a simulation Protein Purification Program was translated and developed into an appropriate Thai version. The five open-ended questions were designed with respect to Thai context to be pre- and post-tests (Appendix D, Section D.3). The test was validated by using the IOC, which processed by rating procedure for measuring the content experts' judgements (Appendix E, Section E.2) according to the specific objectives

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listed by the test developer. The rating score and calculation of IOC were used as those in section 4.2.1. In this case, all item average scores of five experts were higher than 0.79.

4.3.2 Pilot test

The pre- and post-tests were field tested by 32 students in undergraduate and graduate levels in Mahidol University. The field test provided an opportunity for this test designer to consider possible questions concerning their content, grammar and readability. Appropriate changes were made based on the field test. The internal consistency of the test items of five open-ended questions analysed through Cronbach's alpha coefficient was 0.79-0.89 (Appendix F, Section F.3). The open-ended questions had a good reliability of 0.90. As such, it can be used to assess students in understanding the protein purification.

4.3.3 Research design

This study had a practical time constraint of three months for data collection and involved two comparison groups of different program learned: a control group learned by only single (English) language program and an experimental group learned with a two-language (Thai/English) program. There was a multi-method approach utilising quantitative and qualitative approaches to answer the research questions. A pre-test/post-test quasi-experimental design was employed in this study and followed to the semi-structured conceptual interview as the qualitative approach. The design of the study also concerned about the difficulty of language in translation of any concepts of protein purification.

4.3.4 The subjects

Considering the conceptual area of protein purification in Thai biological science curricula, the subjects used were undergraduate students in Kasetsart University, Silpakorn University and Thammasart University. Two separate students groups were selected to serve as the experimental and control groups. A total of 56 students

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participated in this study and the number of students in experimental and control groups was 28.

4.3.5 Data collection

The instruments were constructed during a previous year and validated on a mixed group of undergraduates and graduates. The study was done during both semester sessions in a year. The two groups of undergraduate students were then exposed to the instructional procedures by the researcher, who undertook the teaching process to ensure a good measure of intra-lecture reliability, which if not maintained could be a potential source of experimental error. The instructional models were shown in Figure 4-4, and the scoring rubrics (Appendix H) were used to score responses to activities prepared in the programs and open-ended questions. The semi-structured conceptual interviews were audio-taped to probe the deeper understanding from both student groups. The questionnaires (Appendix J) were given to both student and teacher participants to evaluate the attitude towards learning the protein purification using the simulation programs with an experiential learning model.

4.3.6 Data analysis and interpretation

The data were analysed using the Analysis of Covariance (ANCOVA) based on scores of post-test with the pre-test score as covariate to adjust for initial perceived difference on knowledge between two student groups at $p < 0.05$. The concept interviews were analysed to increase the accuracy of the quantitative results, and the questionnaire were coded as well as analysed to interpret whether this design with all instruments and this learning model was appropriate for assisting students in understanding the protein purification.

4.4 Ethical issues

Ethical issue needs to be addressed in terms of human relationships to be aware of the real purpose of this study. Permission to use the classes involved in the study was obtained from the school directors and class teachers in school and university. Class teachers were invited to discuss this study with the researcher in all steps of

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teaching and assessing students. Students and teachers were given the name, phone number and e-mail address of the researcher. They were also informed the nature of this project, the titles and objectives of the study as well as the reasons for study. Moreover, they were informed the timing and length of interviews, questionnaires and their rights during the study. Pseudonyms were used instead of the names of students and universities to ensure student anonymity. All data collected have securely been stored on computer compact disks as well as the researcher is only person with access to data.

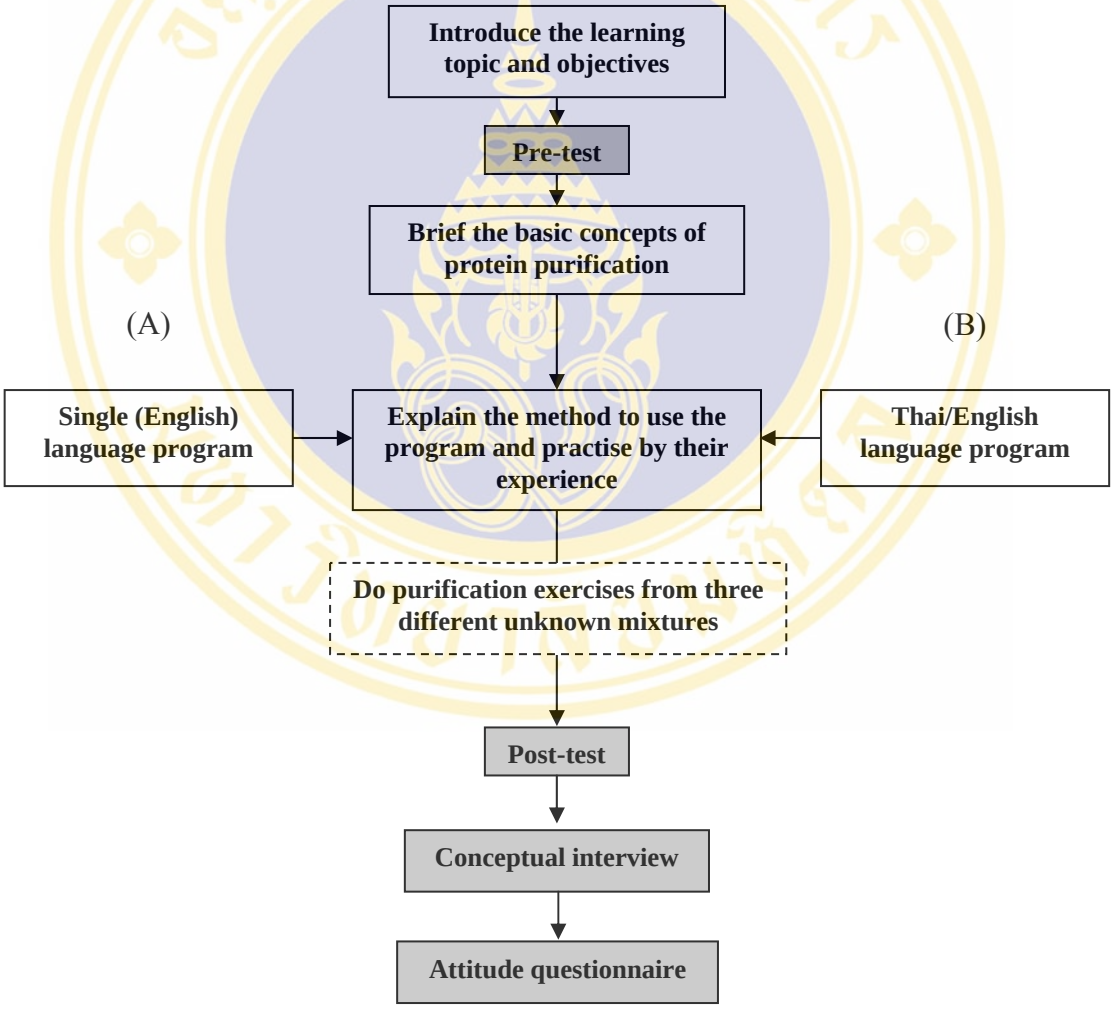

Figure 4-4 The teaching sequence, activity and assessment of protein purification for (A) the control group and (B) the experimental group. The dash-lined box showed the activity in the classrooms, whereas the opaque box indicated the assessment.

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CHAPTER V
RESULTS

This chapter presents two findings related to the **scientific aspects** of the work: (i) the appropriate condition for conjugal transfer between *S. rimosus* and *E. coli* and the sequence of chromosomal attachment site in *S. rimosus* R7, and (ii) the activity of AtrA from *S. coelicolor* comparing to that from *E. coli* by using one-step purification to purify both oligohistidine-tagged AtrA. Following the scientific investigation the findings were developed into some practical classes in order to try to answer some education research questions related to the teaching interventions using hands-on experiments as well as a multimedia based tool. In the **educational aspects**, data were collected from teachers and students. The demographic information of students was firstly organised and followed by the comparison between control and experimental groups. The findings from qualitative approaches and questionnaires are also presented.

SCIENTIFIC ASPECTS

5.1 Conjugal transfer of plasmid pIJ8600 from *E. coli* to *S. rimosus*

5.1.1 Heat treatment conditions for intergeneric conjugation of *S. rimosus* R7 and M4018

To determine the appropriate temperature for pre-germination of recipient spores, the heat treatment condition of *S. rimosus* spores before conjugation step was assessed. Spore suspensions of the wild type, *S. rimosus* R7, and the industrial strain, *S. rimosus* M4018, were incubated at temperature varying from RT to 55 ºC for 10 min. Spores of *S. rimosus* R7 and M4018 were then mated with *E. coli* ET12567 (pUZ8002/ pIJ8600) to investigate the efficiency of conjugation of pIJ8600 to *S. rimosus*. Conjugative plasmid pIJ8600 carrying a thiostrepton resistance gene (Sun et al., 1999) was employed in this experiment instead of the commonly used pSET152 (Bierman et al., 1992) since *S. rimosus* is resistant to apramycin that presented as a selective marker in

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pSET152. After flooding on MS plates with nalidixic acid and thiostrepton, exconjugants were counted and the efficiency of conjugation calculated. As shown in Table 5-1, at the same incubation time for 10 min, heat-treated spores of *S. rimosus* R7 and M4018 gave the same efficiency of conjugation from RT up to 50 ºC. Spores of *S. rimosus* M4018 gave significantly higher efficiency of conjugation than that of wild type ($p < 0.05$).

Table 5-1 Effect of temperature on spores of *S. rimosus* R7 and M4018 as recipients for conjugal transfer of pIJ8600 from *E. coli* ET12567 (pUZ8002)

S. rimosus strain	Number of recipient spores	Exconjugants per recipient[1]					
		RT	35 ºC	40 ºC	45 ºC	50 ºC	55 ºC
R7	10^4	$(2.39 \pm 1.85) \times 10^{-3}$	$(2.24 \pm 1.41) \times 10^{-3}$	$(4.21 \pm 3.85) \times 10^{-3}$	$(2.32 \pm 3.01) \times 10^{-3}$	$(8.97 \pm 2.59) \times 10^{-4}$	$(1.96 \pm 1.54) \times 10^{-4}$
M4018	10^3	$(3.93 \pm 5.08) \times 10^{-2}$	$(4.46 \pm 5.66) \times 10^{-2}$	$(5.48 \pm 1.50) \times 10^{-2}$	$(4.00 \pm 4.87) \times 10^{-2}$	$(2.14 \pm 1.25) \times 10^{-3}$	$(3.62 \pm 2.18) \times 10^{-4}$

Note: [1] Value represents average efficiency from three independent experiments.

5.1.2 Efficiency of spores and mycelia of *S. rimosus* as recipients on intergeneric conjugation

In order to investigate an appropriate recipient type for intergeneric conjugation of *S. rimosus*, strain R7 and M4018 were prepared as the recipients by using both spores and mycelia, while only mycelia were prepared in the case of strain 593. Spore suspension of *S. rimosus* R7 and M4018 were heat-treated at the optimum temperature of 40 ºC for 10 min before mating with log-phase cells of *E. coli* ET12567 (pUZ8002/pIJ8600). 24-h young mycelia of *S. rimosus* R7, M4018 and 593 were mated with above *E. coli* donor without the process of fragmentation. Intergeneric conjugation was performed under these conditions on MS medium flooded with thiostrepton for selection of pIJ8600. As shown in Table 5-2, mycelia and spores used as recipients gave the same conjugation frequency in each strain. There was no difference of frequency between production strain M4018 and non-sporulating strain 593. However, the wild type R7 gave the lowest conjugation frequency of 10^{-4} exconjugants per

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recipient ($p < 0.05$). It was demonstrated here that both mycelia and spores could be used for competent recipients in all three strains of *S. rimosus*. From this result, *S. rimosus* wild type and mutant strains could be manipulated as decent recipients either spores or young mycelia to generate relatively high frequency of intergeneric conjugation.

Table 5-2 Effect of recipient type of *S. rimosus* R7, M4018 and 593 on the conjugation frequency of pIJ8600 from *E. coli* ET12567 (pUZ8002)

S. rimosus strain	Recipient type[1]	Number of recipients	Exconjugants per recipient[2]
R7	Mycelium	10^4	$(9.93 \pm 5.38) \times 10^{-4}$
	Spore	10^4	$(9.68 \pm 5.11) \times 10^{-4}$
M4018	Mycelium	10^4	$(3.78 \pm 5.72) \times 10^{-2}$
	Spore	10^4	$(2.02 \pm 3.67) \times 10^{-2}$
593	Mycelium	10^4	$(1.25 \pm 1.41) \times 10^{-2}$

Notes: [1] Recipient spores were heat-treated at 40 ºC for 10 min before mating.
[2] Value indicates average efficiency from four independent experiments.

5.1.3 Conjugative media affecting on the intergeneric conjugation of *S. rimosus* R7 and M4018

To determine the effect of conjugative media on intergeneric conjugation, heat-treated spores of *S. rimosus* R7 and M4018 at 40 ºC for 10 min were mixed with *E. coli* (pUZ8002/pIJ8600) and spread on three different conjugative media, MS, ISP4 and Oxoid TSA, containing 10 mM $MgCl_2$ to increase the efficiency of conjugation (Choi et al., 2004). Comparing the solid conjugative media as shown in Table 5-3, the exconjugants of *S. rimosus* R7 and M4018 on Oxoid TSA containing 10 mM $MgCl_2$ showed better growth than those on MS and ISP4 ($p < 0.05$). The exconjugants of *S. rimosus* R7 on MS gave no difference of conjugation frequency from that on ISP4, whereas the exconjugants of *S. rimosus* M4018 on MS containing 10 mM $MgCl_2$ gave higher conjugation frequency than that on ISP4. To check the viability loss of exconjugants on the different media, the exconjugants on each conjugative medium were

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restreaked on MS or TSA media for several generations. In this study, Oxoid TSA (7.0 g casein, 3.0 g soybean meal, 5.0 g NaCl, 2.5 g K_2HPO_4, 2.5 g glucose, 15 g agar in 1 litre, pH 7.3) containing different ingredients from the general used TSA for growing *Streptomyces* was selected for the conjugation. From this result, the suitable conjugative media for intergeneric conjugal transfer of pIJ8600 from *E. coli* ET12567 (pUZ8002) to *S. rimosus* R7 and M4018 was Oxoid TSA containing 10 mM $MgCl_2$.

Table 5-3 Effect of different conjugative media on efficiency of intergeneric conjugal transfer of pIJ8600 from *E. coli* ET12567 (pUZ8002) to *S. rimosus* R7 and M4018

S. rimosus strain	Number of recipient spores[1]	Exconjugants per recipient[2]		
		MS+$MgCl_2$	ISP4+$MgCl_2$	Oxoid TSA+$MgCl_2$
R7	10^4	$(3.86 \pm 2.76) \times 10^{-4}$	$(3.95 \pm 2.30) \times 10^{-4}$	$(4.21 \pm 3.85) \times 10^{-3}$
M4018	10^3	$(2.13 \pm 9.52) \times 10^{-3}$	$(2.16 \pm 1.14) \times 10^{-5}$	$(5.48 \pm 1.50) \times 10^{-2}$

Notes: [1] Recipient spores were incubated at 40 °C for 10 min before mating.

[2] Value indicates average efficiency from three independent experiments.

5.1.4 Integration of pIJ8600 into the chromosome of *S. rimosus* R7 by site-specific recombination

To determine the integration of pIJ8600 into the chromosome of *S. rimosus* R7 by site-specific recombination, the *tsr* gene on exconjugant chromosomes were confirmed by PCR using *tsr* primers: ATT012 and ATT013. Comparing with *tsr* gene in pIJ8600, the expected 0.8-kb PCR products were obtained from all exconjugants (Figure 5-1A, Lanes 3-5). To investigate site-specific recombination at the attachment site, the exconjugant chromosomes were confirmed by PCR using *attP* primers: ATT016 and ATT017. There were no 0.91-kb PCR products from all exconjugants (Figure 5-1B, Lanes 3-5).

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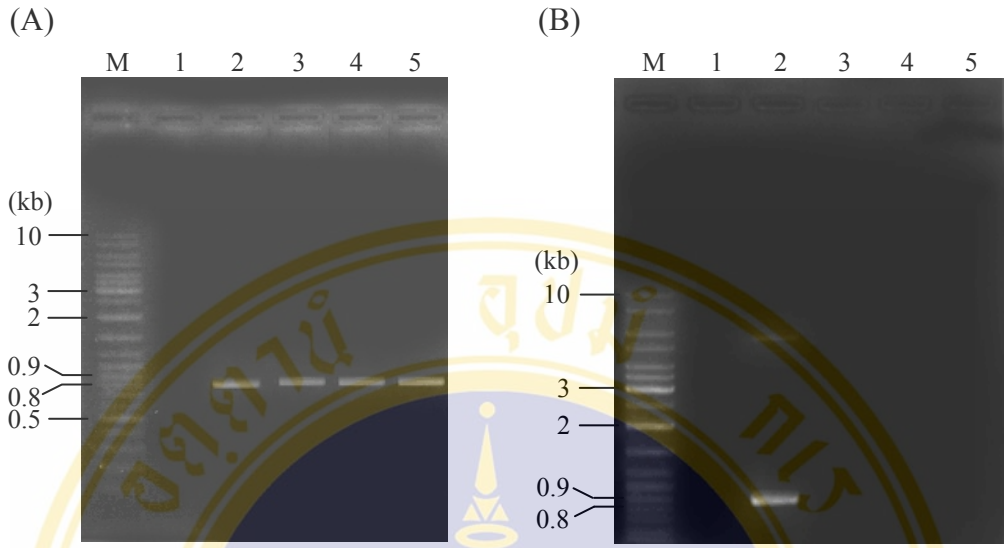

Figure 5-1 The integration of pIJ8600 in exconjugants of *S. rimosus* R7. (A) The integration was determined by PCR using *tsr* primers, ATT012 and ATT013. (B) The integration at attachment site was confirmed by PCR using *attP* primers, ATT016 and ATT017. Lane M, DNA size marker (1-kb DNA ladder); Lane 1, *S. rimosus* R7, as a negative control; Lane 2, pIJ8600, as a positive control; Lanes 3-5, three independent exconjugants.

Chromosomal DNA's of exconjugants from *S. rimosus* R7 were digested with *Eco*RV and Southern hybridisation at 68 °C was performed using labelled *tsr* gene as a probe. Digestion of the exconjugant chromosome with *Eco*RV that located as a single site on *tsr* gene gave two bands of 10 kb and larger than 10 kb (Figure 5-2B, Lane 2; Figure 5-3). From the PCR results and Southern blot pattern implied that there was a single copy of pIJ8600 and was recombined at the attachment site (*attB*) of *S. rimosus* R7 chromosome.

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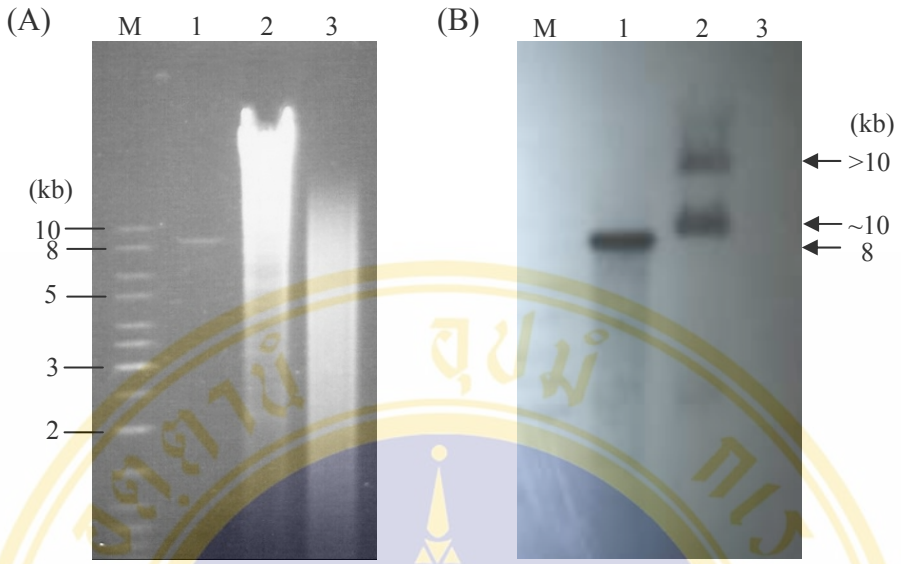

Figure 5-2 The integration of pIJ8600 in the exconjugant of *S. rimosus* R7 confirmed by Southern blot analysis. (A) Total DNA digested with *Eco*RV was run on 0.8% agarose gel in 1 × TAE. (B) Southern blot and hybridisation of total DNA digested with *Eco*RV with a labelled *tsr* probe. Lane M, 1-kb DNA ladder; Lane 1, pIJ8600/*Eco*RV; Lane 2, the exconjugants of *S. rimosus* R7/*Eco*RV; Lane 3, *S. rimosus* R7/*Eco*RV.

5.1.5 Effect of integration at the attachment site on phenotypes of *S. rimosus*

Exconjugants of *S. rimosus* R7, M4018 and 593 were subcultured on MS or TSA without thiostrepton selection for several generations. The exconjugants showed constant phenotypes including colony morphology, spore formation, growth rate and antibiotic productions (data not shown). The result indicated that the integration at the attachment site was stable and had no effect on phenotypes of all exconjugants.

5.1.6 DNA sequence of the attachment region in *S. rimosus* R7

To determine the *attB* in the chromosome of *S. rimosus* R7, chromosomal DNA's of exconjugants were amplified by PCR using two different specific primer pairs of attLF-attPR and attPF-attRR (provided by Iain S. Hunter; Figure 5-3). The expected approximate 0.35 and 0.38-kb PCR products were obtained from the exconjugant chromosomes amplified using two primer pairs of attLF-attPR and attPF-

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attRR, respectively (Figure 5-4). The PCR products were sequenced by using the same specific primer pairs. The *attB* sequence of 449 bp from *S. rimosus* R7 (Figure 5-5) was identified by the alignment of *attP* of ϕC31, left and right *attP*/*B* sequences (Figure 5-6A) and compared with *attB* sequences of various streptomycetes (Figure 5-6B) as well as non-*Streptomyces* actinomycetes (Figure 5-6C) from the NCBI database. The *attB* sequence was submitted into Genbank database under accession number EF670587. The *attB* core region of *S. rimosus* R7 is 5′-CGG TGC GGG TGC CAG GGG GTG CCC TTG GGC TCG CCC GGC GCG TAC TCC ACC-3′. The base-pair homologue 5′TT in the middle indicates that it is the site for recombination. There are high nucleotide identity of *attB* sequences in *Streptomyces* and some non-*Streptomyces* species. *attB* sequences of *S. rimosus* R7 and *S. rimosus* M4018 are identical. Moreover, both are identical to that of *S. avermitilis*.

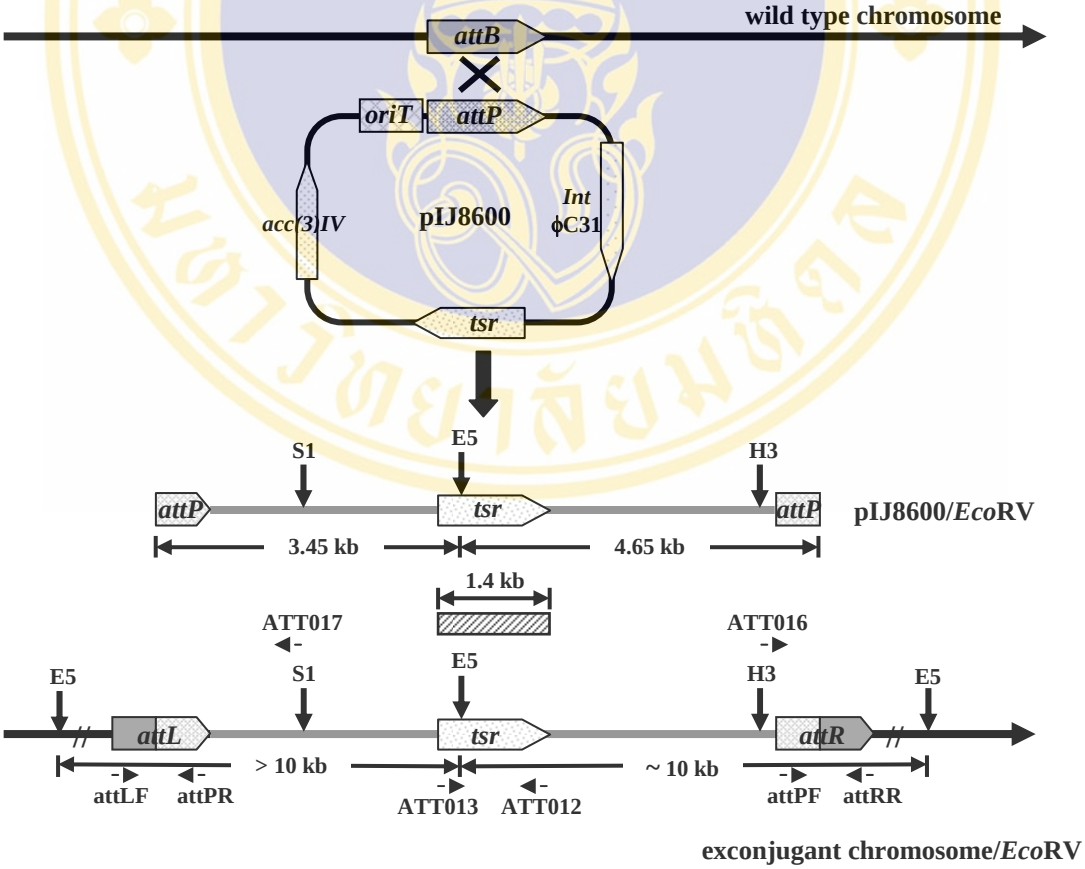

Figure 5-3 Schematic representation of the integration of pIJ8600 in the chromosome of *S. rimosus* R7 by site-specific recombination. E5, *Eco*RV; H3, *Hin*dIII; S1, *Sac*I.

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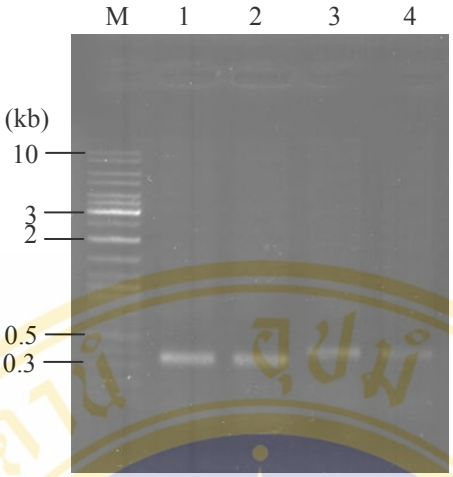

Figure 5-4 PCR amplification of the attachment site in exconjugant chromosomes using two different specific primers. Lane M, 1-kb DNA ladder; Lanes 1-2, PCR products of primers attLF and attPR; Lanes 3-4, PCR products of primers attPF and attRR.

```
        attLF
  1 GTTCACCCAC AGCTGGAGGC CGTGGAAGAG CCCGCCGGTC ATGACCAGCT CCTCCGGCGG
 61 CGCCTCGATG TGCAGCAGGC CGGAGCCGGC CGTCATCCAC TGGGTGTCGC CGTCGTTGAT
121 CACGCCACCG CCGCCGGTGC GAGTCGCGGT GGATGAAGCT GCCGTCCGAT CAGGTACGTG
                                attB core sequence
181 ACGGTCTCGA AGCCGCGGTG CGGGTGCCAG GGGGTGCCCT TGGGCTCGCC CGGCGCGTAC
                                          integration site
241 TCCACCTCGC CCATCTGGTC CATCATGATG AACGGGTCCA GGTACTTGTA GTCGATGCCG
301 GCGAAGGCAC GGCGTACCGG GAAGCCCTCC CCCTCGAAAC CGCTGGGCGC GGTGGACACC
361 GCGAGCACGG CGCGCGGCCG GGCGTCGGCT GGCGCCGCCA CACGCGGCAG GCTCAGCGGA
421 TTCTCTACGG TCACTGCGGG GCATGACGA
```

Figure 5-5 Sequencing result of *attB* in *S. rimosus* R7 chromosome using two pairs of primers: attLF-attPR and attPF-attRR.

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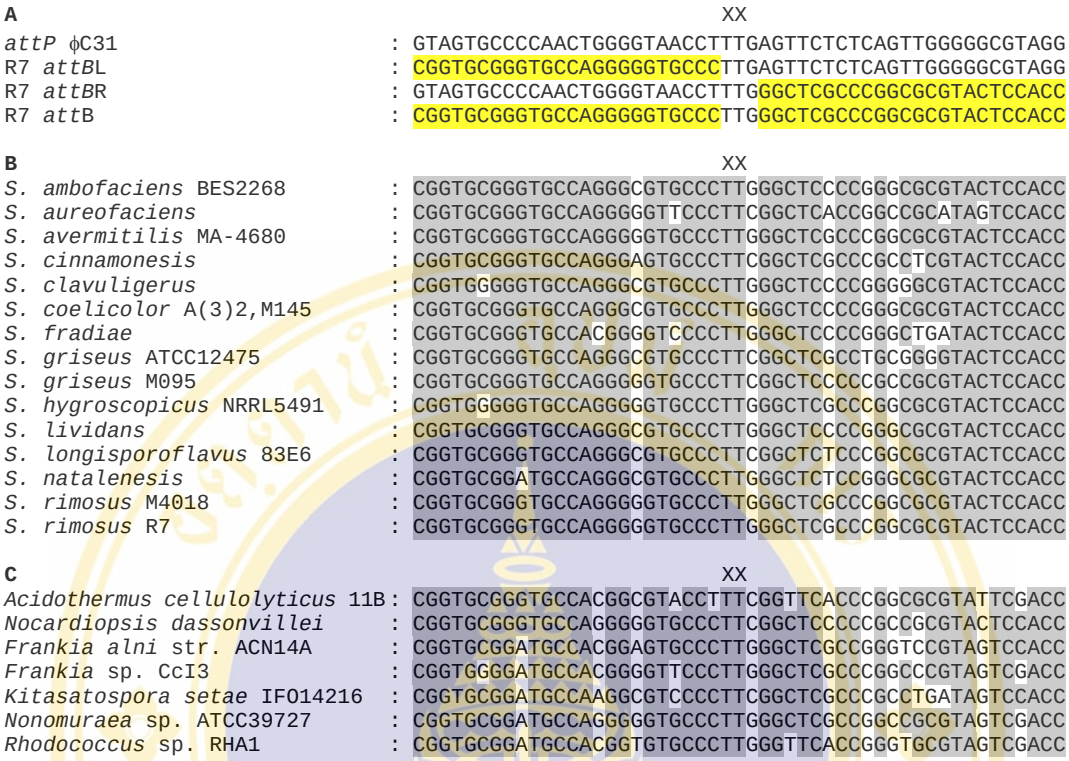

Figure 5-6 Sequence of attachment sites in ϕC31 integration. *attP/B* sequences of *S. rimosus* R7 were aligned with *attP* of ϕC31 in panel A. The complete *attB* sequence of *S. rimosus* R7 was aligned with *attB* of various species of (B) *Streptomyces* and (C) non-*Streptomyces*.

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5.2 Comparison of the activity of AtrA purified from *S. coelicolor* and *E. coli*

5.2.1 Construction and expression of oligohistidine-tagged AtrA at the C-terminus in *S. coelicolor* M145 and *E. coli*

In order to investigate whether the activity of AtrA is regulated by a post-translational modification in *S. coelicolor* M145, the oligohistidine-tagged *atrA* at the C-terminus was constructed and expressed in both *S. coelicolor* M145 and *E. coli*. The *Nde*I restriction site on the ATG start site at the 5′ of *atrA* and ten histidine codons in front of the translational stop codon of *atrA* with the *Bam*HI restriction site after it at the 3′ were inserted to generate a C-terminally oligohistidine-tagged *atrA*. The *atrA* in a pICLSet-based construct called patrASet was amplified by PCR using primers AtrAC_For and AtrAC_Rev to add the *Nde*I before *atrA* and His_{10}-stop codon-*Bam*HI after *atrA*, respectively. The resulted 0.9-kb fragment of *atrA* containing ten histidine codons at the 3′ (Figure 5-7) was ligated into pGEM-T easy. The recombinant plasmid was transformed into *E. coli* JM109 and determined by PCR using primers Gemup and Gemrt. The expected 1.092-kb *Nde*I-*Bam*HI fragment carrying *atrA* gene inserted with ten histidine codons at the 3′ was obtained in recombinant plasmids (Figure 5-8A). The correct recombinant plasmids were confirmed by digestion with *Nde*I and *Bam*HI. The 0.9-kb C-terminally deca-histidine *atrA* gene and 5.4-kb pICLSet were identified in all correct recombinant plasmids (Figure 5-8B). The 0.9-kb *Nde*I-*Bam*HI fragment of C-terminally deca-histidine *atrA* gene was subcloned into expression vectors, pET21c and pICLSet, at the corresponding sites and resulted in plasmid pYP001c and pL646c, respectively. Both plasmids were transformed into *E. coli* JM109 and XL1-Blue, respectively, and confirmed by PCR using primers AtrAF and AtrAR (Figure 5-9) as well as digestion with *Nde*I and *Bam*HI (Figure 5-10). The results showed 0.9-kb fragment of PCR products from pYP001c and pL646c. 0.9-kb fragment and linear vector were shown after digested with *Nde*I and *Bam*HI. The pICLSet-based constructs were then sequenced using M13 forward primer. The results showed deca-histidine codon, stop codon and *Bam*HI restriction site as expected (Figure 5-11).

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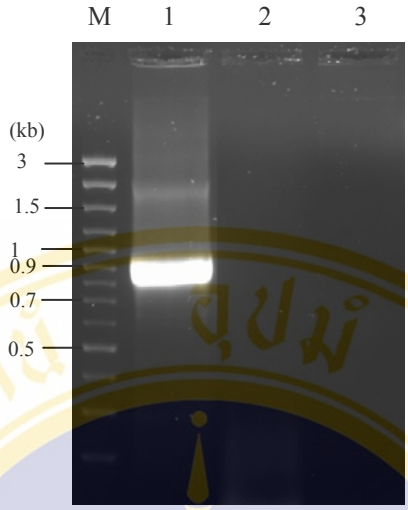

Figure 5-7 PCR amplification of *atrA* gene in patrASet using primers AtrAC_For and AtrAC_Rev. Lane M, 100-bp DNA ladder; Lane 1, PCR product of C-terminally oligohistidine *atrA*; Lanes 2 and 3, PCR reaction without template and primers, respectively.

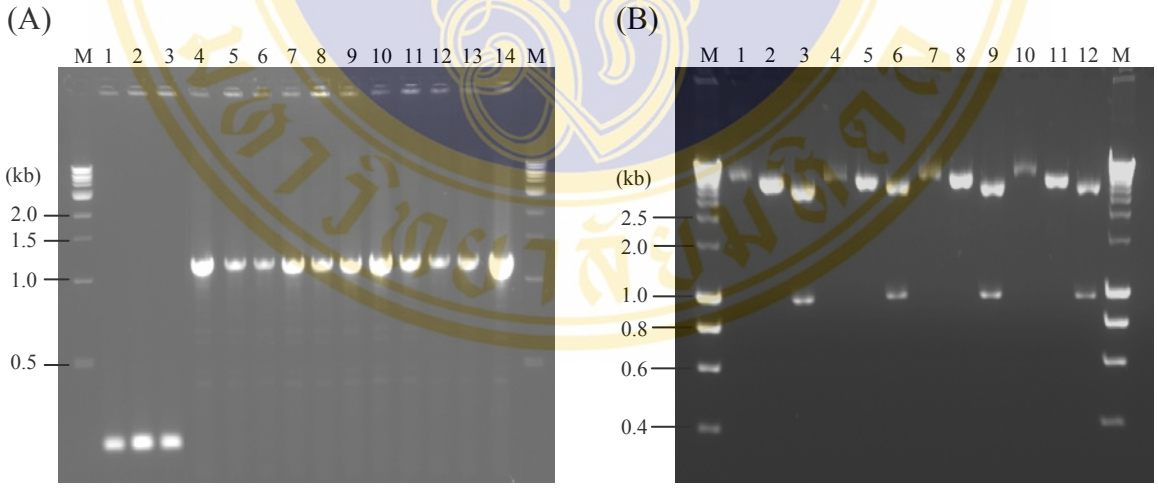

Figure 5-8 Determination of *atrA* gene in pGEM-T easy by (A) PCR using primers Gemup and Gemrt and (B) digestion with *Nde*I and *Bam*HI. Lane M, 1-kb DNA size markers. In panel A, Lanes 1-3, PCR products of *E. coli* without plasmids; Lanes 4-14, PCR products of *atrA* gene in pGEM-T easy clones. In panel B, Lanes 1, 4, 7 and 10, uncut recombinant plasmids; Lanes 2, 5, 8 and 11, recombinant plasmids digested with *Bam*HI; Lanes 3, 6, 9 and 12, recombinant plasmids digested with *Nde*I and *Bam*HI.

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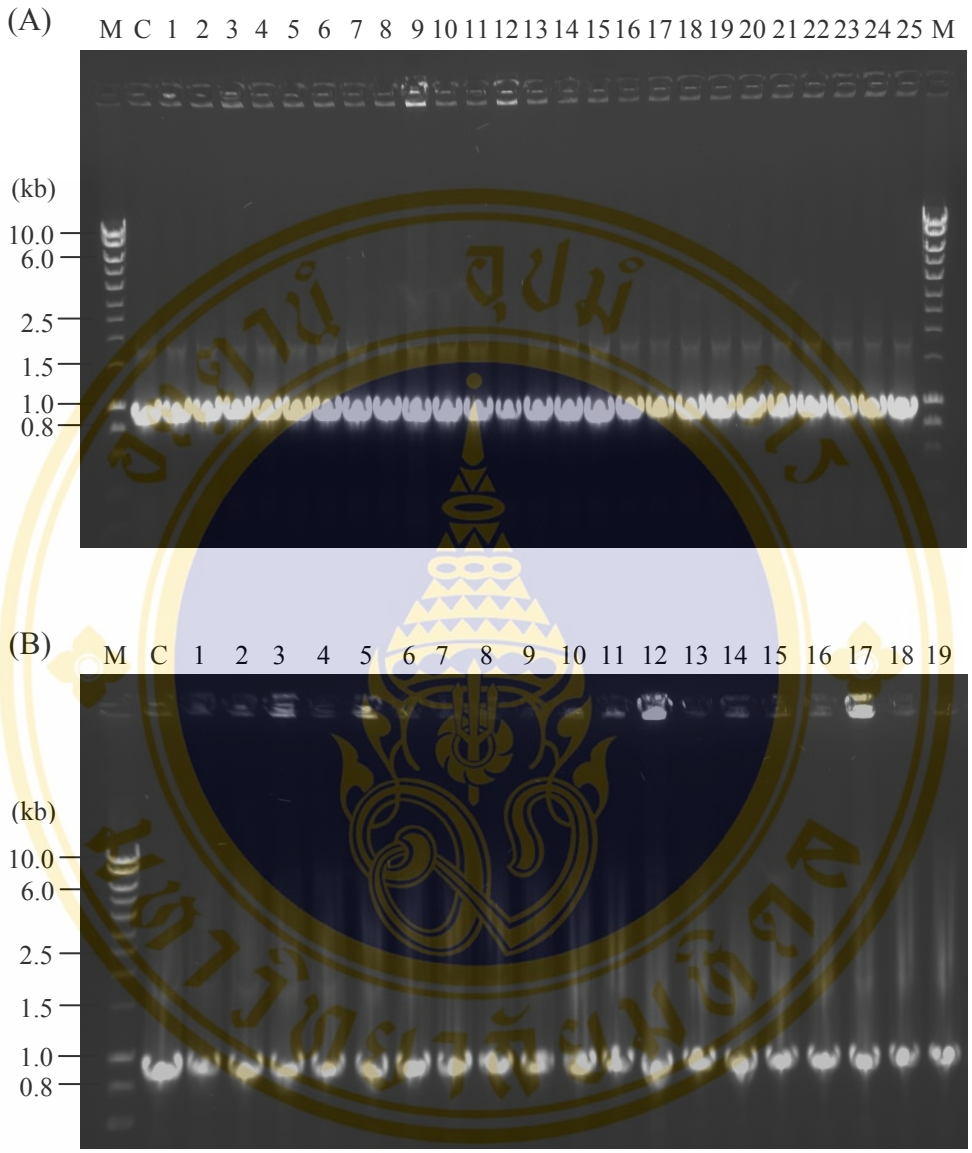

Figure 5-9 Determination of C-terminally deca-histidine *atrA* gene in (A) pET21c and (B) pICLSet by PCR using primers AtrAF and AtrAR. Lane M, 1-kb DNA size markers; Lane C, PCR products of *atrA* in patrASet; Lanes 1-25 (panel A) and Lanes 1-19 (panel B), PCR products of separate isolates of C-terminally deca-histidine *atrA* in pET21c and pICLSet, respectively.

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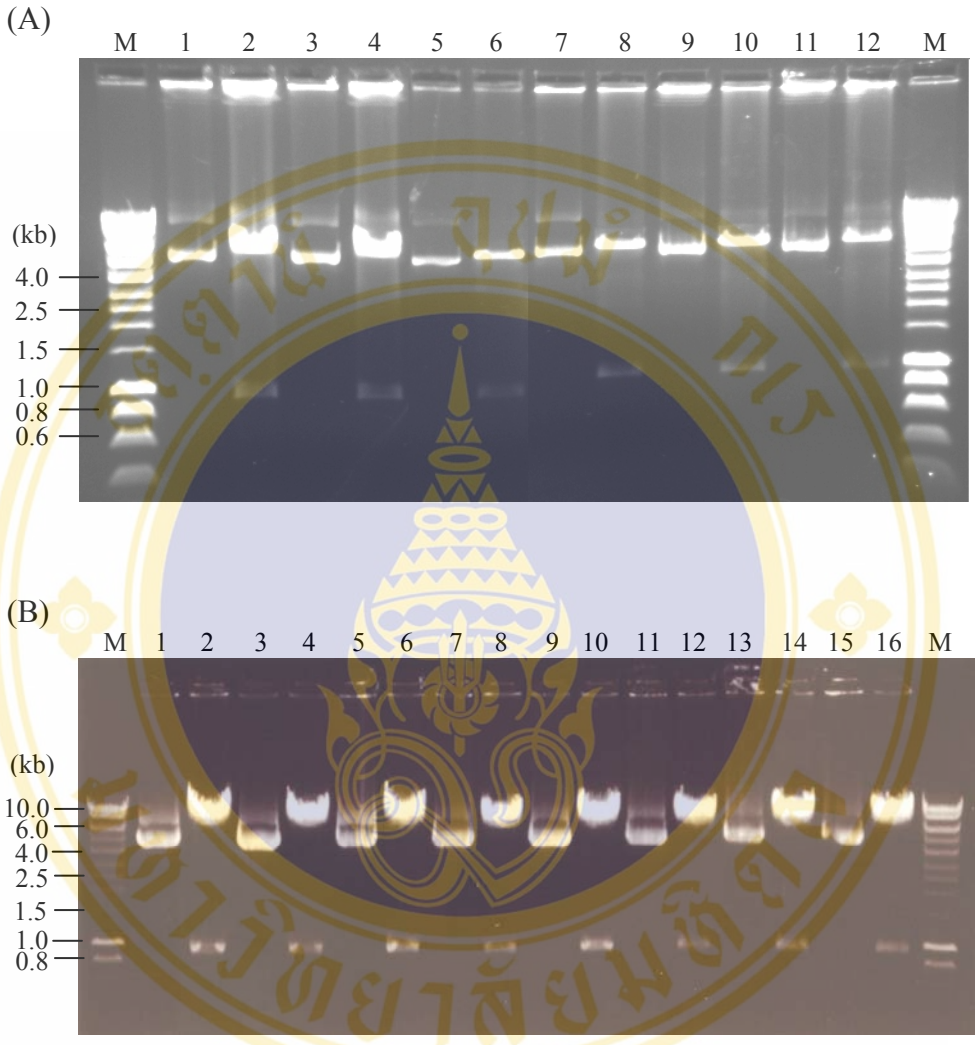

Figure 5-10 Determination of C-terminally deca-histidine *atrA* gene in (A) pET21c and (B) pICLSet by digestion with *Nde*I and *Bam*HI. Lane M, 1-kb DNA size markers; Odd number lanes, uncut plasmid DNA; Even number lanes, plasmid DNA digested with *Nde*I and *Bam*HI.

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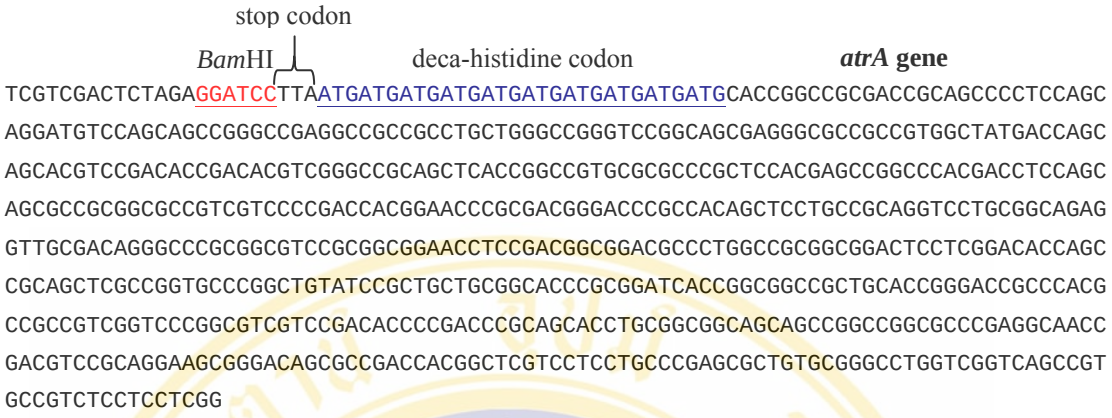

Figure 5-11 Sequencing result of C-terminally deca-histidine *atrA* gene using M13 forward primer.

5.2.2 Determination of the expression of oligohistidine-tagged *atrA* at the C-terminus in *S. coelicolor* M145 and *E. coli*

The pET21c-based construct, pYP001c, was transformed into *E. coli* BL21(DE3). *E. coli* BL21(DE3) transformants containing pYP001c were grown to late exponential phase and the expression of *atrA* gene was induced with 1 mM IPTG. Cell samples of pre- and 3-h post-induction were harvested in the equal volume and suspended in 1 × Laemmli buffer (Laemmli, 1970). After breaking cells by sonication and boiling at 100 °C for 2 min, the pre- and post-induction samples were analysed by 12% SDS-PAGE (Figure 5-12). The overexpression of RNaseE (~60-kDa, Figure 5-12, Lane 2) from *E. coli* D346N and AtrA (31.5 kDa, Figure 5-12, Lane 4) from *E. coli* 6C containing the pJAS407, which served as positive controls, were shown on 12% SDS-PAGE. However, the AtrA bands were not found on SDS-PAGE from separate clones of *E. coli* BL21(DE3) containing pYP001c (Figure 5-12, Lanes 5-10).

The pICLSet-based constructs pL646c were transformed into *E. coli* ET12567 (pUB307) and introduced by intergeneric conjugation into *S. coelicolor* M145 to produce strains L646c. Plasmids pL644 and patrASet were also introduced into *S. coelicolor* M145 as negative and positive controls to produce strain L644 and L646, respectively. To confirm the integration of each construct at the *attB* site, the exconjugant chromosomes were isolated using Kirby mix and amplified by PCR using

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primers ScC31attFor and pSET. The expected 1.75-kb fragments were shown in most exconjugants (Figure 5-13). The phenotypes of selected L646c containing deca-histidine-tagged *atrA* gene at the C-terminus were compared with overproducing wild-type AtrA strain L646 and dominant-negative strain L644 by inoculating patches on R5 agar medium. After incubation at 30 ºC for 3 days, the blue pigment of actinorhodin was detected in the agar surrounding L646 patches, but no blue halo was detected around L644 and L646c. However, a faint blue halo of actinorohodin was shown surrounding L646c after 4-day incubation (Figure 5-14).

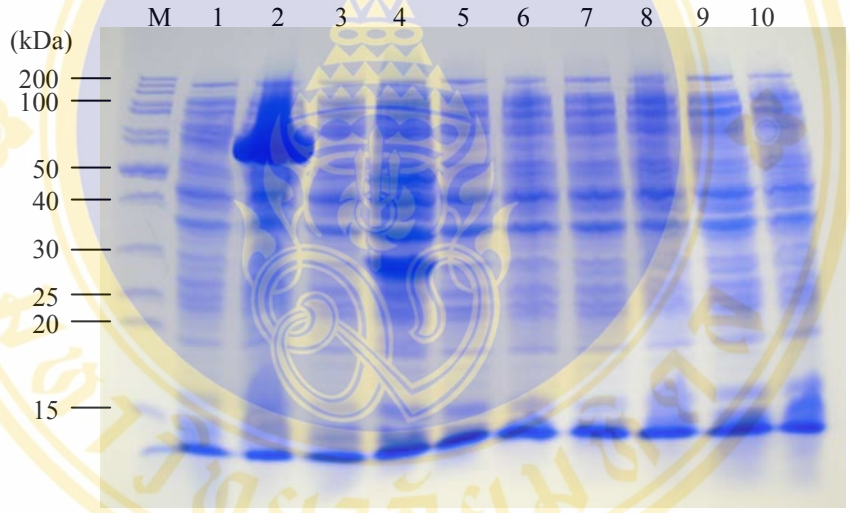

Figure 5-12 SDS-PAGE analysis of proteins from separate *E. coli* clones containing pYP001c. Lane M, standard protein markers; Lanes 1-2, pre- and post-induction from *E. coli* D346N (high-level of RNaseE mutant); Lanes 3-4, pre- and post-induction from *E. coli* 6C containing pJAS407; Lanes 5-6, 7-8 and 9-10, pre- and post-induction proteins from *E. coli* BL21(DE3) containing pYP001c from separate transformants.

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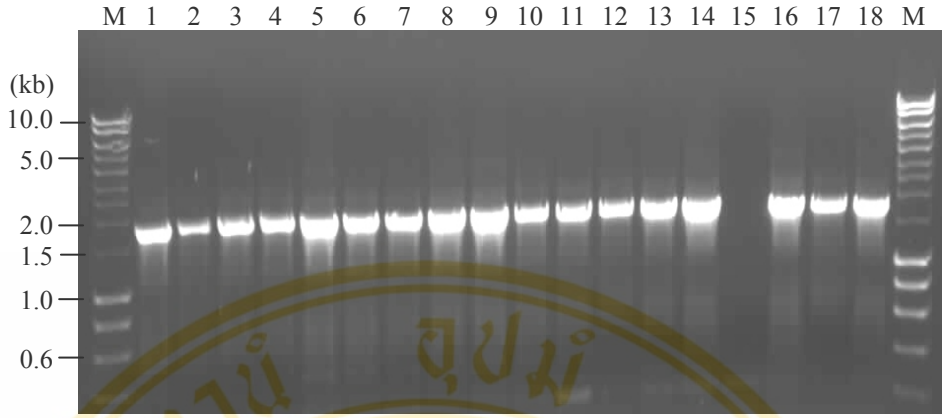

Figure 5-13 Determination of the integration at the attachment site of pL644, pL646 and pL646c in *S. coelicolor* M145 by PCR using primers ScC31attFor and pSET. Lane M, 1-kb DNA size markers; Lanes 1-5, PCR products of separate isolates of dominant-negative strain L644; Lanes 6-10, overproducing wild-type AtrA strain L646; Lanes 11-18, C-terminally oligohistidine-tagged AtrA strain L646c.

Figure 5-14 Effects on actinorhodin production of deca-histidine-tagged *atrA* gene at the C-terminus compared to overproducing wild-type AtrA strain L646 and negative-dominant strain L644. The equal numbers of spores (5×10^4 spores/μl) of each strain in a volume of 10 μl were spotted on the surface of R5 agar plates. After 4-day incubation at 30 ºC, the mycelial patches were exposed to ammonia fumes and photographed. The blue halo around certain patches corresponds to actinorhodin diffusing into the agar.

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5.2.3 Addition of oligohistidine at the N-terminus of AtrA

To achieve the goal of investigating the post-translational modification of AtrA in *S. coelicolor* M145, the oligohistidine-tagged AtrA at the N-terminus was constructed. A pET16b-based construct pJAS407 that producing wild-type AtrA in *E. coli* was site-specifically mutated to create mutants comprising of oligohistidine-tagged AtrA. Site-directed mutagenesis by Quick Change® site-directed mutagenesis kit (Stratagene) using primers NdeIcF and NdeIcR was performed at the *Nde*I site in front of the *atrA* gene from CATATG to CACATG (Figure 5-15). The resulting plasmids were transformed into *E. coli* XL1-Blue and confirmed by digestion with *Nde*I and *Bam*HI. As illustrated in Figure 5-15, the control plasmid pJAS407 digested with *Nde*I and *Bam*HI contained 0.9-kb *atrA* gene and 5.7-kb pET16b, while the mutated pJAS407 gave only one band of 6.6-kb. The previous *Nde*I site, therefore, was disrupted and this plasmid was used for the next mutation step.

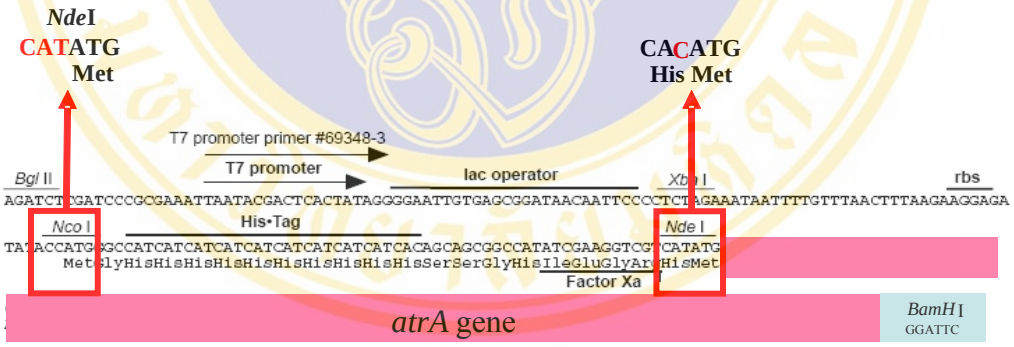

Figure 5-15 Site-directed mutagenesis scheme of pJAS407 to create *Nde*I in front of oligohistidine and disrupted *Nde*I in front of *atrA* gene.

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The *Nde*I-disrupted pJAS407 was site-specifically mutated by changing *Nco*I in front of the oligohistine *atrA* gene from ACCATG to CATATG (*Nde*I recognition site) (Figure 5-15). By Quick Change® site-directed mutagenesis kit (Stratagene), the *Nde*I-disrupted pJAS407 was amplified by PCR using primers NcoI_NdeIF and NcoI_NdeIR. The resulting plasmids were transformed into *E. coli* XL1-Blue and confirmed by double digestions with *Nco*I and *Bam*HI. All mutated plasmids digested with *Nco*I and *Bam*HI gave a single band differ from the control that showed 2 bands (Figure 5-16). The plasmids subsequently digested with *Nde*I and *Bam*HI resulting in plasmid pYP002n (Figure 5-17). pYP002n was then sequenced using T7 promoter and M13 forward primers. The results revealed that *Nde*I site was created in front of nine histidine codons.

Figure 5-16 Confirmation of plasmids disrupted at the *Nde*I recognition site by digestion of *Nde*I and *Bam*HI. Lane M, 1-kb DNA size markers; Lanes 1-2, pJAS407; Odd number lanes, uncut mutated plasmids; Even number lanes, mutated plasmids digested with *Nde*I and *Bam*HI.

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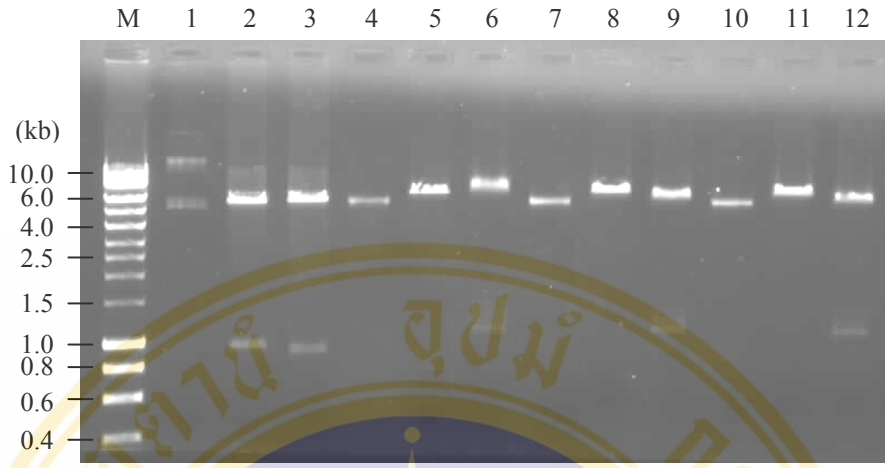

Figure 5-17 Confirmation of mutated plasmids at the position of *Nco*I by restriction analysis. Lane M, 1-kb DNA size markers; Lane 1, mutated pJAS407; Lane 2, pJAS407+*Nco*I/*Bam*HI; Lane 3, pJAS407+*Nde*I/*Bam*HI; Lanes 4, 7 and 10, uncut mutated plasmids; Lanes 5, 8 and 11, mutated plasmid+*Nco*I/*Bam*HI; Lanes 6, 9 and 12, mutated plasmid+*Nde*I/*Bam*HI.

The 0.9-kb *Nde*I-*Bam*HI fragment of N-terminally nona-histidine *atrA* gene was subcloned into the corresponding sites of expression vector pICLSet, resulting in plasmids pL646n, and transformed into *E. coli* XL1-Blue. After confirmation by PCR using primers AtrAF and AtrAR as well as digestion with *Nde*I and *Bam*HI, the pL646n was confirmed the correct sequences by DNA sequencing using M13 forward and reverse primers.

5.2.4 Determination of the expression of oligohistidine-tagged *atrA* at the N-terminus in *S. coelicolor* M145 and *E. coli*

The pET16b-based construct pYP002n was transformed into *E. coli* BL21(DE3) to express AtrA encoded in pYP002n with T7 RNA polymerase promoter. *E. coli* BL21(DE3) transformants containing pYP0002 were cultured and collected the pre- and post-induction samples to analyse on 12% SDS-PAGE. As illustrated in Figure 5-18, the AtrA bands were shown in separate clones of *E. coli* BL21(DE3)

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containing pYP002n as normal AtrA of 31.5 KDa from *E. coli* 6C. Therefore, N-His AtrA would be expressed in *E. coli*.

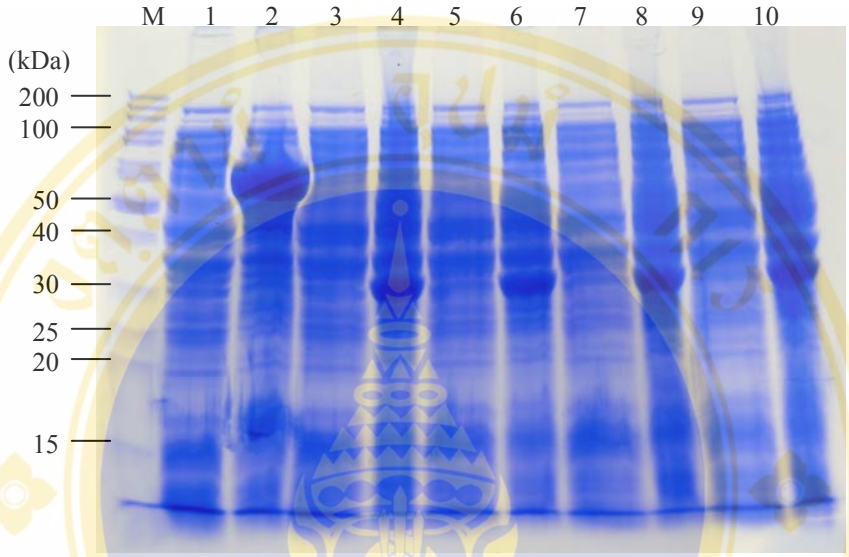

Figure 5-18 SDS-PAGE analysis of proteins from separate *E. coli* clones containing pYP002n. Lane M, standard protein markers; Lanes 1-2 and 3-4, pre- and post-induction proteins from *E. coli* D346N and *E. coli* 6C containing pJAS407, respectively. Lanes 5-6, 7-8 and 9-10, pre- and post-induction proteins from *E. coli* BL21(DE3) containing pYP002n from separate transformants.

The pICLSet-based constructs pL646n were transformed into *E. coli* ET12567 (pUB307) and introduced by intergeneric conjugation into *S. coelicolor* M145 to produce strain L646n. L646 and L644 also served as positive and negative controls. The exconjugant chromosomes were isolated and confirmed by PCR using primers ScC31attFor and pSET. The expected 1.75-kb PCR fragments were obtained in all exconjugants (data not shown). The phenotypes of same L646n clones were compared with L646 and L644 by inocultating patches on R5 agar medium. After incubation at 30 ºC for 3 days, a faint blue halo of actinorhodin was shown surrounding L646n patches (Figure 5-19A). Since the variation of phenotypes was observed on R5

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agar medium, TSB agar plates were used for comparing the phenotypes instead. The blue pigment of actinorhodin could be seen in the agar of L646n patches after incubation at 30 ºC for 4 days (Figure 5-19B).

Since the L646n was not overexpressed as expected, the N-terminally nona-histidine *atrA* was also cloned into another expression vector pIJ6902, a thiostrepton-inducible plasmid, for higher expression level of AtrA. The 0.9-kb *Nde*I-*Bam*HI fragment of N-terminally nona-histidine *atrA* gene was subcloned into the *Nde*I and *Bam*HI sites of pIJ6902, resulting in plasmid pL647n, and transformed into *E. coli* XL1-Blue. The wild-type *atrA* fragment were also subcloned into pIJ6092, resulting in pL647, which served a positive control. The plasmid pIJ6902 was also introduced into *S. coelicolor* by conjugation to create a negative control called L6902. After confirmed by PCR using primers AtrAF and AtrAR as well as digested with *Nde*I and *Bam*HI, the plasmids pL646 and pL647n were transformed into *E. coli* ET12567 (pUB307) and introduced by intergeneric conjugation into *S. coelicolor* M145. The exconjugants were confirmed by PCR using primers attB_gresius and pSET (Hong et al., 2007) and the results of 1.6-kb fragments were shown as expected in both L647 and L647n (Figure 5-20). The phenotypes of same L647n clones were compared with L647 and L6902 on R5 and TSB agar media. 6 µl of 1 mg/ml thiostrepton was added on the top of spots after the growth for 2-4 days (Sun et al., 2001). The blue pigment of actinorhodin was not observed in the agar surrounding L647n patches (data not shown). However, a blue halo was detected in the TSB agar containing 50 µg/ml apramycin surrounding the patches as in overproducing L647 strain (Figure 5-21).

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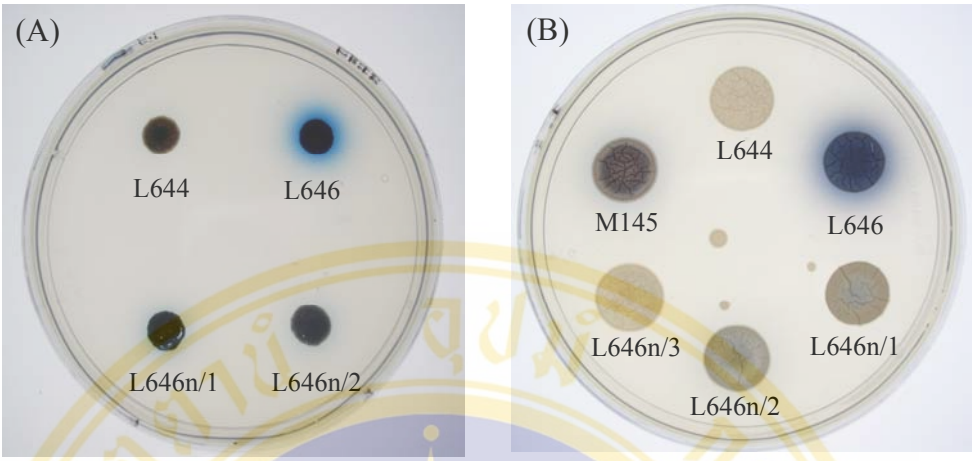

Figure 5-19 Effects on actinorhodin production of nona-histidine-tagged *atrA* gene at the N-terminus compared to overproducing wild-type AtrA strain L646 and negative-dominant strain L644. The equal numbers of spores (5×10^4 spores/μl) of each strain in a volume of 10 μl were spotted on the surface of R5 agar (panel A) and TSB agar (panel B) plates. After 4-day incubation at 30 ºC, the mycelial patches were exposed to ammonia fumes and photographed. The blue halo around certain patches corresponds to actinorhodin diffusing into the agar.

Figure 5-20 Determination of the integration at the attachment site of pIJ6902-based constructs pL647 and pL647n in *S. coelicolor* M145 by PCR using primers attB_gresius and pSET. Lane M, 1-kb DNA size markers; Lanes 1-8 and 9-16, PCR products of separate isolates of overproducing wild-type AtrA strain L647 and N-terminally oligohistidine-tagged AtrA strain L647n, respectively.

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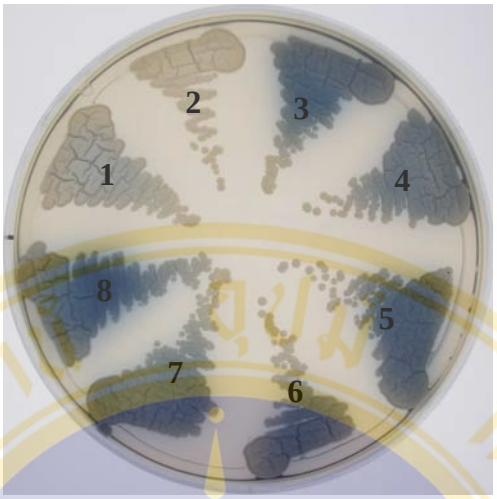

Figure 5-21 Effects on actinorhodin production of nona-histidine-tagged *atrA* gene at the N-terminus compared to L647 served as a positive control and L6902 on TSB containing 50 µg/ml apramycin. After 3-day incubation at 30 ºC, the mycelial patches were exposed to ammonia fumes and photographed. The blue pigment around certain patches corresponds to actinorhodin diffusing into the agar. Numbers 1-2, 3-4 and 5-8 correspond to L6902, L647 and L647n, respectively.

5.2.5 Purification of N-terminally His-tagged AtrA from *E. coli* and *S. coelicolor* M145 by Ni^{2+} affinity chromatography

The *E. coli* BL21(DE3) containing pYP002n were grown to the late exponential phase before the expression of *atrA* gene was induced with 1 mM IPTG. Cell samples of pre- and 3-h post-induction were harvested in the equal volume and dissolved in 1 × Laemmli buffer. The 3-h-induced cells were harvested and resuspended in lysis buffer containing 10 mM imidazole. Cells were incubated with lysozyme on ice and broken by sonication. The clear lysates after centrifugation were loaded into Ni-NTA spin column. Loosely bound proteins were washed off with wash buffer containing 20 mM imidazole, and the tightly bound proteins were eluted with elution buffer containing 250 mM imidazole. The eluted proteins were dialysed with 31.5 kDa MWC dialysis tubes (Spectrum Laboratories) against AtrA buffer [50 mM Tris-HCl

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pH 7.6, 300 mM NaCl, 1 mM EDTA, 1 mM DTT, 40% (v/v) glycerol]. The protein concentrations were determined by Bradford assay (Bio-Rad). The purities of each purification step were judged by 12% SDS-PAGE (Figure 5-22).

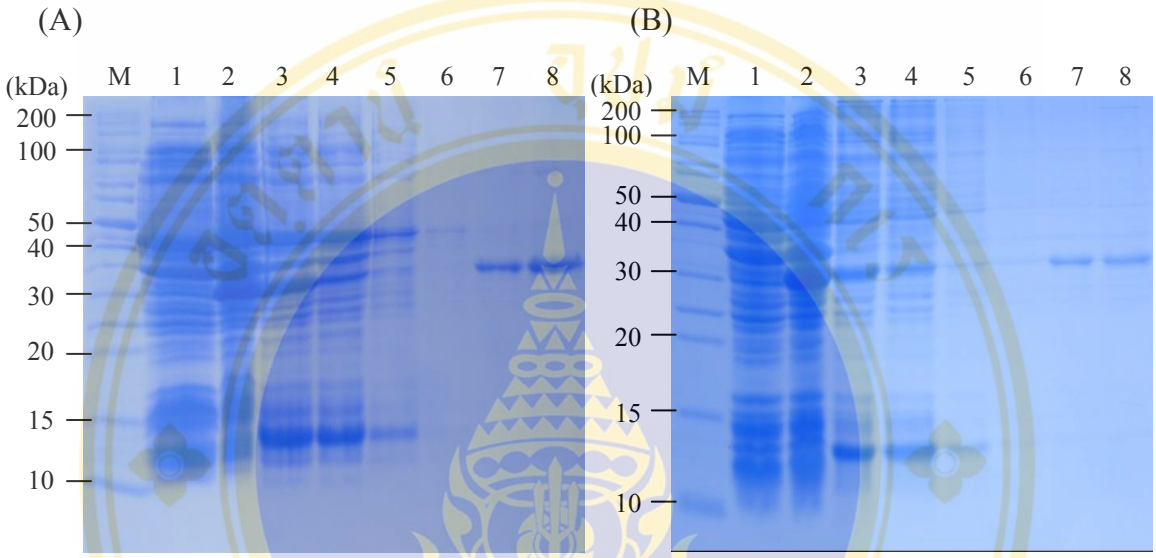

Figure 5-22 Purification of AtrA after overexpression as a fusion protein with an N-terminal His_9 tag in *E. coli* BL21(DE3) containing pJAS407 (panel A) and pYP002n (panel B). Lane M, standard protein markers; Lane 1, pre-induction; Lane 2, post-induction; Lane 3, cell lysate; Lane 4, unbound protein; Lane 5, first wash; Lane 6, second wash; Lane 7, elute; Lane 8, dialysed elute.

The AtrA from *S. coelicolor* L646n was purified using the same buffer as *E. coli* protocol. After being grown in TSB medium at 30 ºC for 3 days, mycelia of L646n were harvested and resuspended in lysis buffer containing 10 mM imidazole. Cells were broken by French pressure cell and the clear lysates after centrifugation were loaded into Ni-NTA spin column. Resins in the column were washed to clean loosely bound proteins with wash buffer containing 20 mM imidazole, and eluted with elution buffer containing 250 mM imidazole. The purities at each step were analysed by 12% SDS-PAGE (Figure 5-23) and immunostained using anti-His-antibodies. AtrA from L646n could not be detected after purification by Ni-NTA spin column by

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comparing with the AtrA from the exconjugant of *S. coelicolor* containing pSET152, named L152, served as a negative control. After 2 days of growth, L647n was induced with thiostrepton in a final concentration of 12 µl/ml (Sun et al., 1999). The AtrA from L647n was purified and compared with the proteins from the exconjugant of *S. coelicolor* containing pIJ6902, called L6902, which served as a negative control. By the Western blot analysis, the AtrA of L647n could not be detected as that of L646n (data not shown).

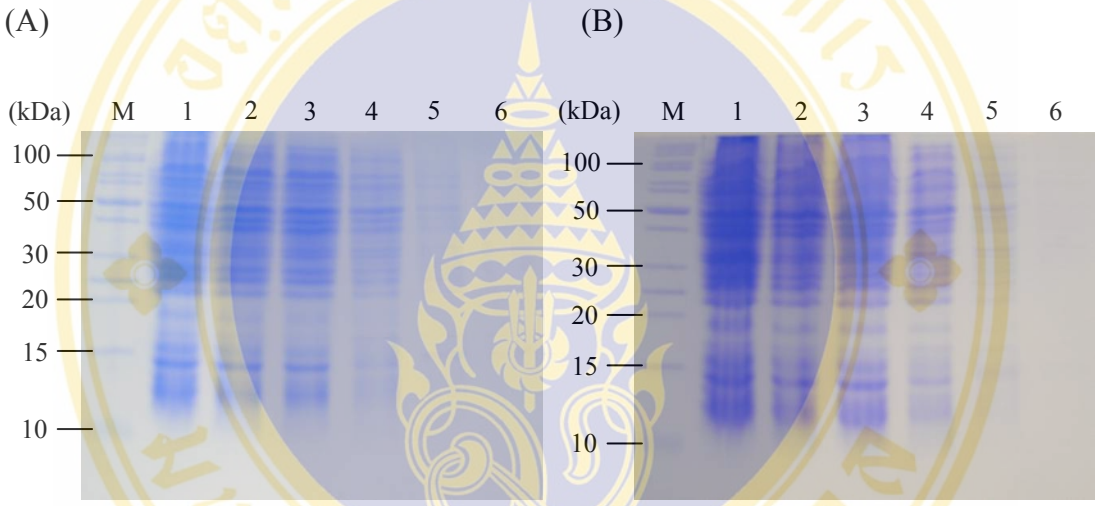

Figure 5-23 SDS-PAGE analysis of AtrA purified from *S. coelicolor* L6902 (panel A) and L647n (panel B). Lane M; standard protein markers; Lane 1, cell lysate; Lane 2, supernatant; Lane 3, unbound proteins; Lane 4, first wash; Lane 5, second wash; Lane 6, elute.

5.2.6 Analysis of DNA-binding activity of AtrA purified from *E. coli*

To compare the DNA-binding activity of purified recombinant gene products tagged at the N-terminus with a His_9 oligopeptide, the protein were purified using Ni^{2+} affinity chromatography and then assayed using an electrophoretic mobility shift assay (EMSA). Double-stranded oligonucleotide substrates from *E. coli* BL21(DE3) containing pJAS407 and pYP002n had the affinity corresponding to region 2 within the *act*II-ORF4 promoter region (Figure 5-24). From the protein concentration at which 50% of the region 2 bound with AtrA (Lane 7), the equilibrium dissociation

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constants (K_d') of complexes between AtrA and region 2 of *act*II-ORF4 from *E. coli* BL21(DE3) containing pJAS407 and pYP002n were 140.6 nM.

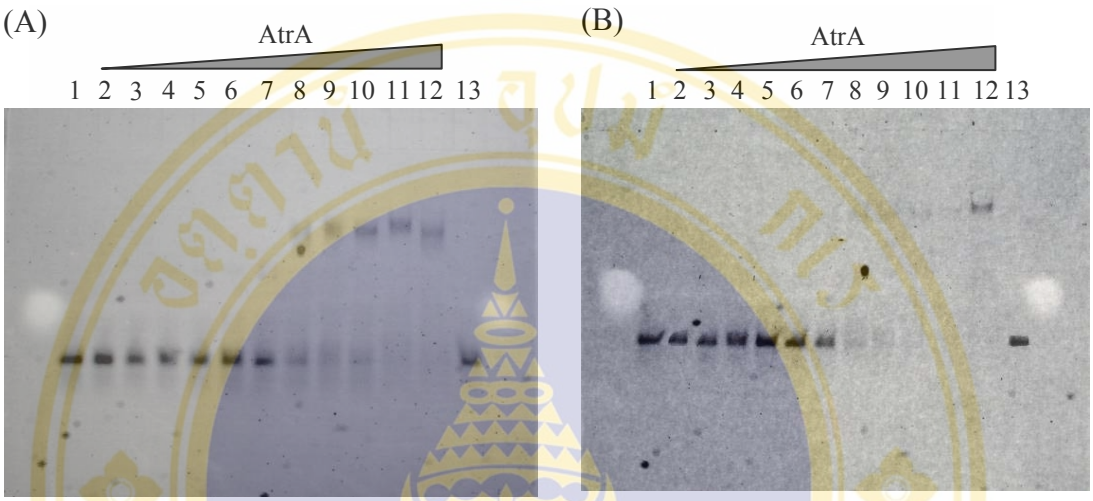

Figure 5-24 Electrophoretic mobility shift assay of AtrA from *E. coli* BL21(DE3) containing pJAS407 (panel A) and pYP002n (panel B) binding to region 2 of *act*II-ORF4. Each lane contains 2 pmol of 5' fluorescently labelled region 2 DNA. Lanes 1 and 13 contain only 5' fluorescently labelled region 2 DNA. In panel A, Lanes 2-12 contain 3, 6, 12, 24, 47, 188, 375, 750, 1500 and 3000 nM of purified AtrA. In panel B, Lanes 1-12 contain 2, 4, 8, 16, 32, 65, 125, 250, 500, 1000 and 2000 nM of purified AtrA.

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EDUCATIONAL ASPECTS

5.3 Learning 'bacterial conjugation' through laboratory work using different teaching sequences at secondary school level

5.3.1 Demographics

The data analysis process began with a study of the demographics of the first year of senior secondary school. The demographics of experimental and control students, aged 14-17, with regards to gender are shown in Table 5-4. Normally, the number of male is more than that of female in all levels of this school. The gender inequality did not affect to the study because of two reasons: the gender pattern was the same trend in each study group as well as there was an equality of the pre-test variances in both male and female groups analysed through Levene's test at $p < 0.05$.

Table 5-4 Frequencies of the senior secondary school students in this study with respect to gender

Gender	Student group			Total
	Lecture (control)	Lecture before lab	Lab before lecture	
Male	28 (60)	31 (66)	27 (57)	86 (61)
Female	19 (40)	16 (34)	20 (43)	55 (39)
Total	47 (100)	47 (100)	47 (100)	141 (100)

Note: Values in parentheses are percentages.

5.3.2 The understanding of bacterial conjugation in different teaching interventions

To investigate the experiential learning model through a laboratory exercise, conjugation in *E. coli*, was used to determine whether or not students' understanding of bacterial conjugation in the first year of senior secondary level. Students were taught by the three different teaching interventions: lecture only (control), lecture before doing experiment, and experiment done before lecture. The different understandings of bacterial conjugation with three teaching sequences were

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first assessed by observing students' concept maps in each student group. The concept maps were scored by using the holistic scoring rubric (Appendix H, Section H.2). However, the scores are insufficient to indicate any differences among three student groups. All three comparison groups worked on pre-test and post-test before and after the teaching sequences. The pre-test scores of three comparison groups, analysed by using Analysis of Variance (ANOVA) at $p < 0.05$, had an equality of variance and were not significantly different, thus they can be used for comparison groups in this study. The post-test scores of all student groups were compared by using Analysis of Covariance (ANCOVA) with pre-test scores as covariate to reduce the extraneous variable from students' prior knowledge (Table 5-5). To explore the significance of the effective teaching sequences, a post-hoc test, the Scheffe's Multiple Range Comparison test was performed separately on the three teaching sequences. The results showed the experimental group taught by doing the labwork before lecture had the highest mean score (15.38) when compared with other groups taught by other teaching sequences. Teaching sequences using the laboratory exercise were also able to assist students to understand the bacterial conjugation better than the traditional teaching sequence.

Table 5-5 Statistical evaluation of the post-test scores for conceptual understanding of bacterial conjugation by ANCOVA with the pre-test scores as covariate.

Group	n	Pre-test $\overline{X} \pm SD$	Post-test[1] $\overline{X} \pm SD$	SS	MS	F	P
Lecture	47	6.81 ± 2.07	10.14 ± 1.96[c]	640.99	320.49	108.51	0.00*
Lecture before lab	47	6.89 ± 2.27	12.82 ± 2.47[b]				
Lab before lecture	47	6.45 ± 1.76	15.38 ± 1.55[a]				

Notes: [1] Values, adjusted means of post-test, with different superscript letters are significantly.

* Significant difference at $p < 0.05$

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Moreover, gain percentage scores were analysed by using One-Way Analysis of Variance and compared with Scheffe's Multiple Range Comparison test at $p < 0.05$ (Table 5-6 and 5-7). The results indicated that student groups taught by lab before lecture had better gain percentage scores and thus learning with the laboratory exercise can help students to achieve the gain percentage better than learning with the traditional method. All groups of students were also probed for their understanding of bacterial conjugation by working with the conceptual test after all the teaching sequences for 3 weeks. The conceptual test scores were analysed by using the same statistical design as the gain percentage scores (Table 5-8 and 5-9). The conceptual test scores had the same achievement trends as the pre-test and gain percentage scores.

Table 5-6 Descriptive statistics of gain percentage scores with different teaching sequences

Group	n	$\overline{X}$ [1]	SD	Min	Max
Lecture	47	0.254[c]	0.101	0.000	0.462
Lecture before lab	47	0.458[b]	0.167	0.077	0.857
Lab before lecture	47	0.649[a]	0.104	0.438	0.909

Note: [1] Values with different superscript letters are significantly.

Table 5-7 ANOVA results of the gain percentage scores for conceptual understanding of bacterial conjugation

Source of variance	df	SS	MS	F	P
Between groups	2	3.666	1.833	111.859	0.00*
Within group	138	2.261	0.016		
Total	141	5.927			

Note: * Significant difference at $p < 0.05$

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Table 5-8 Descriptive statistics of conceptual test scores with different teaching sequences

Group	n	$\overline{X}$ [1]	SD	Min	Max
Lecture	47	8.83[c]	2.54	0	13
Lecture before lab	47	10.43[b]	2.16	4	14
Lab before lecture	47	13.45[a]	2.02	10	18

Note: [1] Values with different superscript letters are significantly.

Table 5-9 ANOVA results for conceptual test scores in understanding the bacterial conjugation

Source of variance	df	SS	MS	F	P
Between groups	2	516.87	258.43	50.97	0.00*
Within group	138	669.75	5.07		
Total	141	1216.61			

Note: * Significant difference at $p < 0.05$

5.3.3 Qualitative data analysis of students' understanding of bacterial conjugation

To try to judge the extent of understanding students had developed with respect to conceptually-related ideas of bacterial conjugation, ten random students in each group were interviewed in Thai by an in-depth semi-structured process. The extracts from interview with some students illustrated the misconceptions and confusion when learning with lecture only (R is the researcher and S is the student):

R: *Which keywords are involved in the concepts of conjugation?*

S_1: *I think… pilus, plasmid, gene, two cells.*

S_4: *Um… F^+, F^-, genetic material, muscle.*

S_9: *recipient, donor, sex pilus, chromosome, plasmid, replication.*

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R: *Well… What is the difference between conjugation and binary fission in bacteria?*

S_2: *Conjugation is a process to transfer only DNA. The binary fission is not a DNA transfer process, but it is a process to increase the number of cells.*

S_5: *Conjugation is a process of vertical transfer process, but binary fission is a process of horizontal gene transfer.*

S_9: *Conjugation helps bacterial survival in various environments; however, binary fission is not use for this purpose.*

R: *Could you please briefly explain about a process of conjugation in bacteria?*

S_7: *Donor's pilus contacts to recipient's cell, DNA is transferred into recipient cells by rolling-circle mechanism, and cells were separated after conjugation.*

S_4: *Ah… The process comprises of mating pair of bacterial cells, the construction of sex pilus, DNA transfer process and the separation of mating pair.*

S_9: *A single-stranded plasmid DNA is transferred into recipient cell by rolling-circle mechanism.*

[…]

R: *Do Gram-positive bacteria use the same mechanism of conjugation as Gram-negative bacteria?*

S_3: *Yeah, all steps are similar.*

S_6: *No, it's different in the steps of mating pair. Gram-positive bacteria use sex pilus, but mating pair in Gram-negative bacteria is formed by adhesin protein.*

[…]

R: *Do you think bacterial conjugation is the same as other sexual reproductions in higher eukaryotes?*

S_3: *Yes. Because it consists of different cells called donor and recipient like male and female of animals.*

S_{10}: *No. The conjugation helps bacteria to transfer DNA and other molecules such as proteins, enzymes from a cell to another. However, there is the*

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fertilisation between sperm from male and egg from female in higher organisms to be a zygote. All are not transferred from males to females like bacteria.

The same questions were also asked with students taught by lecture before lab and lab before lecture. The student group taught by lecture before lab still had some confusion about bacterial conjugation. The extracts from students taught by lecture before lab are as follows:

R: *Which keywords are involved in the concepts of conjugation?*

S_2: *Ah… sex pilus, plasmid, donor, recipient cell, transconjugant and mating pair.*

S_7: *Well… donor, recipient, transconjugant, pilus, pheromone, replication and episome.*

S_{10}: *Bacteria, mating pair, male, female, antibiotic resistance genes, transconjuant, sex pilus and transfer protein.*

R: *Well… What is the difference between conjugation and binary fission in bacteria?*

S_1: *I think… There are the same characteristics of offspring cells after binary fission, but the recipient cells in conjugation process has the foreign genetic materials.*

S_8: *Ahha… Conjugation does not increase the number of cells in the process like the binary fission.*

R: *Could you please briefly explain about a process of conjugation in bacteria?*

S_2: *First, a mating pair of donor and recipient forms by contraction of sex pilus, and then DNA is transferred into recipient cells… DNA transferred is replicated before the separation of mating pair.*

S_3: *After contraction of sex pilus, the cell walls of donor and recipient cells are a contact. Then single-stranded DNA of plasmid is transferred into the recipient and the linear plasmid is formed to circular DNA and duplicated by rolling-circle mechanism. Finally, the cells are separated.*

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S_6: *Ah... There are 4 steps in the process: the preparation of mating pairs, the transfer process, the replication of transferred DNA and the separation of mating pairs.*

[…]

R: *Do Gram-positive bacteria use the same mechanism of conjugation as Gram-negative bacteria?*

S_2: *Some are different, particularly the formation of mating pairs. Gram-negative bacteria use the contraction of sex pilus, but Gram-positive bacteria the adhesin protein from donor cell to form a mating pair.*

S_5: *No. Gram-positive recipient releases sex pheromone to stimulate the donor to encode the adhesin protein for coating both cells. But Gram-negative bacteria use sex pilus in stead.*

[…]

R: *Do you think bacterial conjugation is the same as other sexual reproductions in higher eukaryotes?*

S_4: *No, it's different. Though both have the process of mating pairs, the results are different. Conjugation has the DNA transferred from donor cell to recipient cells in the same generation, but there are offspring after sexual reproductions of higher living things.*

S_9: *I think conjugation looks like the sexual reproductions only in the mating pairs. Other processes are different.*

However, there was very little confusion and misconceptions when the topic was taught by lab before lecture. The extracts given below showed students taught by this teaching sequence had a clearer understanding of bacterial conjugation than those taught by other methods.

R: *Which keywords are involved in the concepts of conjugation?*

S_3: *Um… donor, recipient, transconjugant, transfer gene, origin of transfer, pilus and pheromone.*

S_4: *F^+, F^-,transconjugant, oriT, tra gene, sex pilus, mating pair, intrageneric conjugation and intergeneric conjugation.*

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S_{10}: *Bacterial cell, Paramecium, donor, recipient, transconjugant, transfer gene, origin of transfer, pilus and mating pair.*

R: *Well… What is the difference between conjugation and binary fission in bacteria?*

S_2: *Oh... Binary fission is a vertical gene transfer, but conjugation is a horizontal gene transfer.*

S_7: *Conjugation does not increase the number of cells like binary fission and occurs in the same generation.*

S_9: *Conjugation is a horizontal gene transfer like transformation and transduction. However, the result of binary fission is shown in the next generation.*

R: *Could you please briefly explain about a process of conjugation in bacteria?*

S_1: *It starts from the formation of mating pair by contraction of pilus or adhesin coating. The nicked single-strand of plasmid DNA is transferred into recipient cell. Single-stranded plasmids of both donor and recipient cells are replicated to be double-stranded. Donor and recipient separate to be free cells, and the recipient cell is able to be a new donor.*

S_6: *Um… It comprises of the formation of mating pairs, the plasmid transfer into a recipient cell, the duplication of single-stranded plasmid DNA in both donor and recipient cells and the separation of mating pairs.*

[…]

R: *Do Gram-positive bacteria use the same mechanism of conjugation as Gram-negative bacteria?*

S_4: *Nope. The formation of mating pairs is different between them. In Gram-positive, the recipient cell encoded the sex pheromone to stimulate the donor cell to release the adhesin protein for coating the donor and recipient cells. In Gram-negative, the mating pairs are formed by the contraction of sex pilus.*

S_7: *Yep, except the formation of mating pairs. Other details are the same.*

S_{10}: *No. Gram-positive bacteria use two proteins, sex pheromone and adhesin to form the mating pairs, but Gram-negative bacteria use the contraction*

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of fimbria. Um… I don't know how mating pairs between Gram-positive and Gram-negative bacteria are formed.

[…]

R: *Do you think bacterial conjugation is the same as other sexual reproductions in higher eukaryotes?*

S_3: *No. The conjugation has the mating pair like the sexual reproduction of higher living things. But it's a horizontal transfer, whereas the sexual reproduction of higher organisms is a vertical transfer.*

S_5: *Um… I don't think it looks similar because the conjugation is a DNA transfer process in the same generation, while sexual reproduction of higher organisms gave the offspring in the next generation.*

After this, 30 students who had been interviewed were asked which teaching sequence they would like any teachers to use in the classes. Almost all students would like to learn by doing the experiment before any lectures since they were so bored when they had previously known the answers from lectures and this was not challenging. Only one student liked to learn with lecture before lab because following the protocol is easier than thinking by herself!

5.3.4 Preferences towards learning in bacterial conjugation with the laboratory exercise

A questionnaire to assess students' general information, prior knowledge and preferences towards the concepts of bacterial conjugation was given to students learning with both teaching sequences using the laboratory exercise. The researcher asked students to fill in the form in two sections before starting the lesson and to complete the remaining sections after finishing the conceptual tests. Students rated their own knowledge background and preferences on a five-point Likert scale arranged in ascending order. The sample group composed of 54 males and 38 females. The range in age was 14 to 17 years with a mean of 15.53. Students' prior knowledge (self-assessed) in both theory and practical skills is shown in Table 5-10: students believed that they had achieved a moderate level in all of the required knowledge. Five teacher

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participants thought they taught all required concepts to students, thus they expected the students to have better prior knowledge than what they found out from assessment.

Table 5-10 Teacher overestimation of their students' knowledge as shown by discrepancy of level of knowledge estimated by students and teachers

Description	Level of knowledge (Mean ± SD) from	
	students	teachers
1. Theory		
1.1 Basic knowledge of cells (involving the bacterial cells)	3.05 ± 0.84	4.40 ± 0.55
1.2 Bacterial reproduction	2.84 ± 1.01	3.00 ± 0.71
1.3 DNA, gene and DNA replication	3.14 ± 0.93	4.80 ± 0.45
1.4 Protein synthesis	2.99 ± 0.97	4.80 ± 0.45
2. Practical skills		
2.1 Chemical calculation and data analysis	2.73 ± 0.83	4.00 ± 0.71
2.2 Sterilised techniques	2.68 ± 0.99	2.60 ± 1.14
2.3 Design and outline the experiment plan	2.80 ± 0.88	4.00 ± 0.71
2.4 General laboratory techniques and safety	3.02 ± 0.91	4.20 ± 0.84

The preferences towards learning in the concepts of bacterial conjugation were assessed from both students and teachers. Students' overall preference (Table 5-11) was at the "satisfied" level with a mean of 4.41, and students recorded "satisfied" to link their prior knowledge to the concept of bacterial conjugation using these teaching methods, assessment tools as well as the teaching sequences (items 1-5 in Table 5-11). They enjoyed learning the new concepts by using their experience with laboratory techniques, lectures and activities including concept maps which were design in appropriate for senior secondary school level (items 6-8 in Table 5-11). This practical work was also appreciated for consolidating the students' understanding of conjugation (item 9 in Table 5-11).

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Table 5-11 Students' preferences for learning of bacterial conjugation with the laboratory exercise

Description	Preference (Mean ± SD)
1. Students can link their prior knowledge to the concept of bacterial conjugation.	4.14 ± 0.59
2. Students are able to define any technical terms in conjugation.	4.13 ± 0.54
3. Students can describe the importance of conjugation in bacteria.	4.20 ± 0.54
4. Students can explain the mechanism of bacterial conjugation.	4.28 ± 0.52
5. Students can design a simple experiment to investigate the conjugation in bacteria.	4.22 ± 0.68
6. Students have an additional techniques e.g. sterilised techniques, serial dilution, ability to use some scientific instruments.	4.28 ± 0.54
7. The lecture and labwork is easy to understand at the senior secondary school level.	4.25 ± 0.60
8. Activities and the concept map can help student to understand overall concepts and details better.	4.38 ± 0.55
9. This laboratory exercise can help student to have a better understand in the concept.	4.35 ± 0.60
Overall preference	4.41 ± 0.56

In order to confirm whether the concepts of conjugation are appropriate for the science curriculum at the senior secondary level, the preferences towards this study were assessed from senior secondary students. They thought the concepts of bacterial conjugation should be provided for the secondary science curriculum with a mean of 4.40. They also agreed with students about the appropriateness of teaching materials, assessment tools and other activities to be able to support the behavioural objectives of this lesson (items 1-3 in Table 5-12). The teaching sequences using students' experiences in the practical work can help students to understand the concepts of bacterial conjugation and integrate them with other concepts at secondary school level (items 4-6 in Table 5-12).

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Table 5-12 Teachers' preferences for learning of bacterial conjugation with the laboratory exercise

Description	Preference (Mean ± SD)
1. Lecture and labwork are designed by following the behavioural objectives.	4.60 ± 0.55
2. Activities and the concept map can help student to understand overall concepts and details better.	4.80 ± 0.45
3. Assessment tools can assess and evaluate students with respect to the objectives.	4.60 ± 0.55
4. From students' experience in labwork, students are able to design any simple experiments to investigate the conjugation in bacteria.	4.60 ± 0.55
5. The teaching sequence, lab before lecture, can stimulate students to have a better understand of the bacterial conjugation.	4.20 ± 0.45
6. This topic can be applied to any other concepts in the senior secondary curriculum.	4.00 ± 0.71
Overall preference: the concept of bacterial conjugation should be filled in the senior secondary curriculum.	4.40 ± 0.55

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5.4 Improving Thai students' understanding of concepts in 'protein purification' by using a simulation program in Thai and English versions

5.4.1 The difficulties of translating Protein Purification Program into Thai

The Python version of this program written by A. G. Booth has been translated into various languages to provide the international version including Thai (Figure 5-25). The developer used the knowledge from purifying AtrA protein from *E. coli* and *Streptomyces* (in Section 5.2) to improve the texts about purification techniques to be appropriate for the conceptual understanding at Thai university levels. In the translated program, students can switch between languages in Thai/English version by changing the command line in Prolab.ini file (i.e. language=EN for English and language=TH for Thai) before starting the program. Students thus have the choice of language and can flip between Thai and English when there are words or concepts that are not clear to them. The reason for this is that translation from English into Thai for specific/technical purposes is difficult since there are many differences between the languages particularly in their syntax and semantics, as well as the existence of somewhat less appropriate Thai words in place of English scientific technical terms. In general, translation from the original version into other languages by word-to-word alignment strategies is limited since the direct alignment approach considers only morphological formation (Naruedomkul et al., 1999; Tiedenman, 2004) and there are no appropriate exact words to help students understand in the scientific/technical concepts. Most of the Thai basic technical terms are used following as the Thai Royal Institute website (http://rirs3.royin.go.th/coinages/webcoinage.php) and most chemical and reagent names are rendered from their English sounds by transcription using the criteria from the Royal Institute website (http://www.royin.go.th). However, many specific terms are not included in this website and some words rooted from other languages particularly Pali and Sanskrit are difficult to understand by Thai speakers. The words occurring in students' learning life were used instead of these, but some of the words might confuse students who do not have sufficient background in chemistry and biochemistry. Moreover, their contexts are very important to give word(s) of appropriate meaning for Thai students' understanding of protein purification. Indeed

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some words might need to be changed to more appropriate ones after the implementation of this program in some courses.

(A) (B)

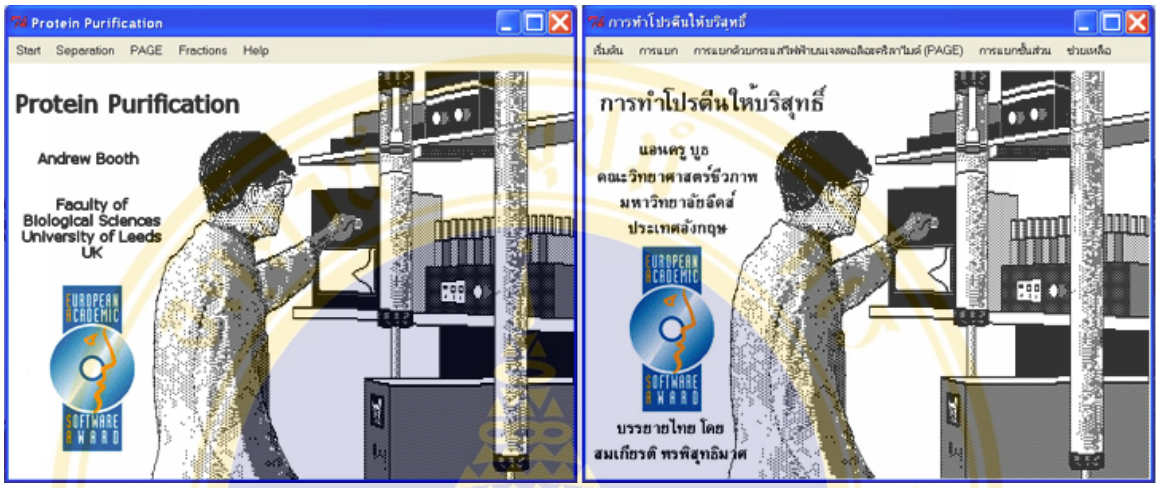

Figure 5-25 The first window of the Python version of Protein Purification Program in (A) English and (B) Thai

5.4.2 Comparison of responses between students using the single and Thai/ English language versions

To investigate the experiential learning by student through a virtual class the Protein Purification Program in Thai and English versions were used to determine whether or not student's understanding of protein purification at the undergraduate level had improved, a student group was taught by the two-language program compared with another group taught by single language (English) program, which served as a control. Three activities in the program about purifying from different unknown mixtures were prepared to serve as a formative assessment. Students can assess themselves from single-step and the overall progress reports (Figure 5-26). After learning with the programs, teachers can evaluate students' understandings of protein purification by their progress reports and score by using the scoring rubric in Appendix H, Section H.4. The pre- and post-tests consisted of five open-ended questions designed using the key concepts of protein purification (Appendix D, Section D.3) and approved by the five experts in science and education (Appendix E, Section E.2). A scoring rubric (Appendix H, Section H.1) has been used to score

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open-ended responses to the tests. The data were analysed using the Analysis of Covariance (ANCOVA) based on the post-test scores from student taught using the English and the Thai/English programs, respectively, with pre-test scores as covariate to adjust for initial perceived differences on knowledge between two student groups at $p < 0.05$ (Table 5-13). Undergraduates using the Thai/English program scored significantly higher than those using only the English language program ($p < 0.05$).

(A) (B)

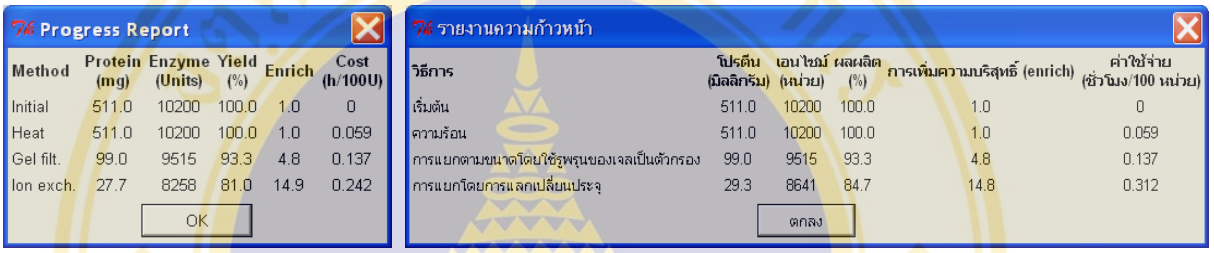

Figure 5-26 Examples of the overall progress report (purification table) in the help file of the Protein Purification Program in the (A) English and (B) Thai versions

Table 5-13 ANCOVA results of the post-test scores for conceptual understanding of protein purification with the pre-test as a covariate.

Group	n	Pre-test $\bar{X} \pm SD$	Post-test $\bar{X}^{a} \pm SD$	SS	MS	F	P
Taught using a single language program	28	3.61 ± 1.37	8.74 ± 1.75	409.08	409.08	223.82	0.00*
Taught using a two-language program	28	3.36 ± 1.79	14.16 ± 1.17				

Notes: [1] Values, adjusted means of post-test.

* Significant difference at $p < 0.05$

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To discover students' opinions about the Protein Purification Program and whether the availability of the program in the two languages had helped their understanding, 10 out of 28 students in each group were interviewed and audio-taped. An extract from an interview with NJ illustrates the confusion when using the single language (English) program (R is the researcher):

R: *What is the first quantity you should measure before starting to purify an enzyme from a mixture of proteins?*

NJ: *I think the total protein should be quantified first.*

R: *What method would you use in the laboratory for this?*

NJ: *I would use column chromatography and measure the protein in each fraction at 280 nm.*

[…]

R: *How will you know which fractions contain the enzyme you would like to purify?*

NJ: *It is all the peaks that you can get at 280 nm.*

R: *Well, why do you think it's your enzyme?*

NJ: *At that wavelength, we can get the enzymes as well.*

[…]

R: *How will you know whether your enzyme has been purified?*

NJ: *I think we should run a 2-D SDS-PAGE gel (sic). If it shows only one spot, it means the enzyme has been purified.*

R: *Can you be sure that that spot is your enzyme?*

NJ *Yeah! That spot should comprise one enzyme.*

The same questions were also asked with KA, a student who used the two-language version of the program:

R: *What is the first quantity you should measure before starting to purify an enzyme from a mixture of proteins?*

KA: *We should do a total protein assay to compare what is happening in each purification step.*

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R: *What method would you use in the laboratory for this?*

KA: *We can use any methods such as the Lowry method or the Bradford method.*

[…]

R: *How will you know which fractions contain the enzyme you would like to purify?*

KA: *If we want to know which is our enzyme, or not, we should measure the concentration of the product when adding substrate.*

[…]

R*: How will you know whether your enzyme has been purified?*

KA: *Indeed, proteins in each step of the purification scheme are separated out. In the last step, a few proteins remained and we can use the specific technique to purify our protein (or enzyme) according to the characteristics of remaining proteins. We also confirm our enzyme in the last step with SDS-PAGE.*

All students interviewed who learned using English the program said they would like to learn with the program translated into Thai since they thought it would help them to achieve a better understanding.

5.4.3 Preferences towards learning in protein purification by using simulation program in Thai and English versions

A questionnaire was designed to assess students' general information, prior knowledge and preferences towards this program. Students rated their own knowledge background and preferences on a five-point Likert scale arranged in ascending order (Appendix J, Section J.1). The sample group comprised of 15 males and 56 females (this approximates the current general gender balance in Thailand universities). The range in age was 19 to 40 years with a mean of 23.8. Most students were taking Microbiology, Genetics as well as Biochemistry: the characteristics of the sample with their frequencies are shown in Table 5-14. Students' prior knowledge (self-assessed) in both theory and practical skills is presented in Table 5-15: students believed that they achieved a moderate level in all of the required knowledge. The

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preferences towards the Thai/English version of the program were assessed (Table 5-16). Their overall preference was at the "satisfied" level with a mean of 4.15 and students recorded "satisfied" for materials provided and format of results (items 1-4 in Table 5-16). The program aims to prepare students to make rational choices of the methods and their sequence for protein purification and encourages them to think about their strategy at each step of a protein purification in real situations (items 5-6 in Table 5-16). Students to some extent agreed with the advantage of a two-language version which they believed helped them to read various kinds of documents in English (item 7 in Table 5-16). They also suggested providing an "Undo" button in each step of the purification but the researchers told them that this situation does not occur when real experiments such as heat denaturation are carried out and that they should learn this.

Another questionnaire for teachers' evaluation of this program (Appendix J, Section J.2) was prepared to ask them about developing the program for biological science curricula in the university level. The sample group composed of 2 males and 5 females from 4 universities: Burapha University, Kasetsart University, Silpakorn University and Thammasart University. The range in age was 32 to 43 years with a mean age of 38.14. All teachers taught in various areas of biological sciences. Their overall preference was at the "satisfied" level with a mean of 4.29 and teachers thought the use of both programs could help students to understand the concepts of protein purification from the materials, techniques, help files and students' experiences provided in these programs (items 1-4 in Table 5-17). All texts, vocabularies and information used in this program were suitable for university levels, and particularly for biological science students (items 5-6 in Table 5-17). The Thai/English program can assist students to be accustomed to read any related documents such as manuals, procedures and other documents in English (item 7 in Table 5-17).

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Table 5-14 Demographics of the sample from the questionnaire with respect to their major, gender and studying degree

Variable	Levels	Frequency
Major	Agricultural science	8
	Biochemistry	13
	Biology	1
	Biotechnology	1
	Genetics	22
	Genetic Engineering	4
	Microbiology	20
	Science education	2
Sex	Female	56
	Male	15
Studying degree	Bachelor	29
	Master	27
	Doctoral	15
Age (in range)	19-40 years	

Table 5-15 Students' estimation of their prior background knowledge

Description	Level of knowledge (Mean ± SD)
1. Theory	
1.1 Structure and function of proteins	2.75 ± 0.77
1.2 Chemical purification	2.52 ± 0.73
1.3 Techniques in protein purification	2.42 ± 0.87
1.4 Drawing and analysis of graphs and charts	2.55 ± 0.86
2. Practical skills	
2.1 Chemical calculation and data analysis	2.51 ± 0.86
2.2 Chemical purification	2.49 ± 0.81
2.3 Techniques in protein purification	2.32 ± 0.84
2.4 General laboratory techniques and safety	3.11 ± 0.82

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Table 5-16 Students' preferences for using the Protein Purification Program as an aid to learning

Description	Preference (Mean ± SD)
1. Instruction and practices are clear enough to be followed.	3.96 ± 0.57
2. Languages are clear enough to understand and follow in the experiments.	4.08 ± 0.61
3. This program helped you to understand the concepts of protein purification.	4.04 ± 0.57
4. The simulation of the experiments in this program including contexts, tables and charts helped you to understand protein purification better.	4.10 ± 0.59
5. After use of the program, students are able to choose the suitable techniques of protein purification.	4.00 ± 0.66
6. This program helps students in systematic thinking and prepares them to do experiments in real situation.	4.06 ± 0.65
7. The two-language program helps students to be accustomed to manuals, procedures and other documents in English.	4.08 ± 0.67
Overall preference	4.15 ± 0.55

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Table 5-17 Teachers' preferences towards the Protein Purification Program

Description	Preference (Mean ± SD)
1. This program is a learning tool to assist students in understanding the concepts of protein purification.	4.57 ± 0.54
2. Students are able to design their purification plans by choosing the suitable steps and techniques.	4.71 ± 0.49
3. Students can apply the knowledge and thinking skills in protein purification for their future researches (if they will get the manuals in each technique).	4.43 ± 0.54
4. Students can read and interpret the results in various sources better, e.g. graph, chart.	4.29 ± 0.49
5. Texts, information and vocabulary used in this program are suitable for understanding the protein purification.	4.14 ± 0.69
6. This program can be applied to the knowledge in biological curricula to teach or supplement in the concepts of protein purification.	4.86 ± 0.38
7. The two-language program helps students to be accustomed to manuals, procedures and other documents in English.	4.71 ± 0.49
Overall preference	4.29 ± 0.49

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CHAPTER VI
DISCUSSION

This chapter draws together many threads that have emerged in previous chapters and provides answers to the research questions in both scientific and educational aspects of the work. The **scientific purpose** of this study was to investigate the optimal condition for intergeneric conjugal transfer of pIJ8600 from *E. coli* ET12567 (pUZ8002) to *S. rimosus*. A second purpose was to investigate a post-translational modification of AtrA in *S. coelicolor*.

The **educational purposes** of the study were to design laboratory practical work in bacterial conjugation and a computer program of protein purification to teach students in senior secondary school and university levels respectively and to determine the outcomes of the teaching sequences designed, both qualitatively and quantitatively.

SCIENTIFIC ASPECTS

6.1 Intergeneric conjugation of plasmid pIJ8600 between *E. coli* and *S. rimosus*

Intergeneric conjugation was introduced as an alternative gene transfer protocol in *S. rimosus* R7 and M4018 that produce abundant spores, as well as *S. rimosus* 593, a non-spore-forming strain. *S. rimosus* M4018, an industrial oxytetracycline producer, is a well characterised host for gene manipulation by using PEG-mediated transformation and electroporation (Butler et al., 1989; Pigac & Schrempf, 1995). In contrast, the wild type *S. rimosus* R7 that produces rimocidin and the non-spore-forming mutant, *S. rimosus* 593, which produces less oxytetracycline, have never been used as hosts.

In general, recipient spores of *Streptomyces* species are pre-germinated by heat treatment before mating with *E. coli* donor cells for intergeneric conjugation (Kieser et al., 2000). Young germinated spores are highly efficient at gene transfer by intergeneric conjugation (Flett et al., 1997; Kitani et al., 2000; Mazodier et al., 1989). Heat treatment at 50 °C for 10 min was routinely used for *Streptomyces* spores, whereas different conditions were used for some *Streptomyces* species and no heat treatment

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was used for a heat-sensitive *S. virginiae* (Voeykova et al., 1998). However, some strains need to be given to the heat shock condition and, thus, required optimisation is required to find the appropriate temperature that activates spore germination as well as maintaining a good yield of spore viability. For example, the heat treatment condition of *S. lavendulae* spore was at 40 ºC for 10 min (Kitani et al., 2000) while *S. natalensis* was at 45 ºC for 10 min (Enŕiquez et al., 2006). In this study, the conjugation efficiency from untreated spores (at RT) was as high as from the treated spores varying from 35-45 ºC. Therefore, the heat treatment step for spore pre-germination is not a prerequisite for conjugation of *S. rimosus*. The result was, however, in contrast with *S. natalensis* where the use of untreated spores gave no exconjugants (Enŕiquez et al., 2006). The efficiency of conjugation for untreated *S. rimosus* spores was comparable to the efficiency reported for *S. virginae* that posses heat-sensitive spores (2×10^{-3}, Voeykoya et al., 1998) but was much higher than that of untreated *S. albulus* spores (9×10^{-8}, Hamano et al., 2005). Spores of *S. rimosus* without heat treatment were chosen for conjugation experiments; this condition is routinely used for freshly prepared spore suspensions. In the case of frozen spores, heat treatment at 40-45 ºC for 10 min is recommended to achieve a high efficiency of conjugation in *S. rimosus*.

Although heat treatment was recommended to pre-germinate most recipient spores of *Streptomyces* spp., some species were sensitive to heat. However, for some *Streptomyces* strains it was not possible to use either heat treated or non-heat-treated spores for conjugation experiments because of poor sporulation or highly heat-sensitive spores. Young fragmented mycelia of *S. peucetius* that produces spore poorly were used instead as an alternative recipient at high conjugation frequency of 1.5×10^{-4} exconjugants per recipient (Paranthaman & Dharmalingam, 2003). The efficiency received from *S. rimosus* R7 mycelium was comparable with the result of *S. peucetius* mycelium (Paranthaman & Dharmalingam, 2003) but was quite the opposite to that of *S. natalensis* in which the attempt to use mycelium was unsuccessful (Enŕiquez et al., 2006). The use of mycelia as recipients has been increasingly reported from non-streptomycetes such as *Rhodococcus*, *Nonomuraea* and *Micromonospora* that produce spore poorly or none (Rose & Steinbüchel, 2002; Stinchi et al., 2003; Voeykova et al., 1998). Both mycelia and spores could be used as compatible recipients in spore forming *S. rimosus* R7 and M4018 as well as the non-spore forming strain 593.

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Several solid media reported for intergeneric conjugation between *E. coli* and *Streptomyces*. Some are complete media such as LB, AS1, TSA, SR6, SR12 and YSP (Bierman *et al*., 1992; Mazodier *et al.*, 1989; Voeykova et al., 1998). LB, AS1 and TSA, however, were unsuitable for conjugation as *E. coli* would grow fast and a lawn of *E. coli* could be observed after overnight incubation even flooding with nalidixic acid. Furthermore, scraping the *E. coli* lawn was rather tedious. The recent commonly used *Streptomyces* medium for conjugation was MS supplemented with 10 mM $MgCl_2$. It was used for *S. coelicolor* and *S. lividans* (Flett et al., 1997), *S. lavendulae* FRI-5 (Kitani et al., 2000), *S. clavuligerus* (Fouces et al., 2000), *S. nodosus* (Nikodinovic et al., 2003), *S. peucetius* (Paranthaman & Dharmalingum, 2003) and *S. natalensis* (Enŕiquez et al., 2006). Normally, MS agar containing 10 mM $MgCl_2$ was used as a conjugative medium for *S. rimosus*, but some false exconjugants were occasionally obtained. This might due to the soya in MS agar that is capable of absorbing thiostrepton and, thus, can lower the actual concentration of the antibiotics. ISP4 medium used for *Kitasatospora setae* (Choi et al., 2004) was also selected to investigate the effect on conjugation efficiency. Another selected medium was Oxoid TSA which has a formula different from the general used TSA for growing streptomycetes. The results showed that Oxoid TSA containing 10 mM $MgCl_2$ gave 10-100 times higher efficiency than those of MS and ISP4. The exconjugants of *S. rimosus* R7 gave no difference in conjugation frequency on either MS or ISP4, whereas, the exconjugants of *S. rimosus* M4018 gave a 10 times higher frequency on MS than that on ISP4. When Oxoid TSA was applied for mycelia recipients, similar results were obtained. Therefore, Oxoid TSA is the best conjugative media and yields the highest conjugation efficiency for *S. rimosus* without the variation of exconjugants.

By conjugation, the integration of pIJ8600 at *attB* of *S. rimosus* chromosome had no effect on phenotype of all exconjugants as that of pSET152 in *attB* of *S. lavendulae* FRI-5 (Kitani et al., 2000). Comparing *attB* core sequence with *Streptomyces* and non-*Streptomyces*, it showed consevered sequence that recognised by ϕC31 integrase including minimum core sequence of 5′ TT (Combes *et al.*, 2002), and the *attB* core sequence of *S. rimosus* R7 chromosome is identical to those of *S. rimosus* M4018 and *S. avermitilis*. The three base-pair homologue of integration site is 5′TTG as in other *Streptomyces*, e.g. *S. ambofaciens* and *S. fradiae* (Kushstoss & Rao,

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1991) as well as in non-*Streptomyces*, e.g. *Kitasatospora setae* (Choi et al., 2004). Besides the ϕC31 *attP* which is normally used in various conjugative plasmids (Combes et al., 2002), there is the ϕBT *attP* as alternative for conjugation in several plasmids (i.e. pRT801, pRT802 and PMS82), which gave the same efficiency of conjugation as the ϕC31 *attP* (Gregory et al., 2003).

This is the second report of actinophage attachment site found in *S. rimosus* after *attB* of actinophage RP3 (Gabriel et al., 1995). The *attB* core of ϕC31 on *S. rimosus* genome is completely different from that of RP3. It is evident that individual actinophage is restrict to its host by specific sequence of attachment site on *Streptomyces* genome and each *Streptomyces* can accept more than one kind of phage.

6.2 Comparison of the activity of AtrA purified from *S. coelicolor* and *E. coli*

The transcription factor AtrA that binds within the promoter of the pathway-specific regulator of the actinorhodin cluster, *act*II-ORF4, was first identified in the model species *S. coelicolor* (Uguru et al., 2005). Although AtrA is required for normal levels of *act*II-ORF4 and production of actinorhodin, the mechanism that regulates the activity of *atrA* is not known. Possible lines of evidence that support the regulation mechanism of *atrA* are:

(1) the expression of *atrA* is regulated either at the level of transcription or translation,
(2) the AtrA polypeptide is subjected to post-translational modification,
(3) the regulation of *act*II-ORF4 is triggered by another factor that functions only in concert with AtrA, or
(4) a combination of these.

If the AtrA polypeptide is modified post-translationally, then AtrA purified from *E. coli* should have a different activity from that purified from *S. coelicolor*. A pET21a-based construct, pYP001c, and a pICLSET-based construct, pL646c, containing the *Nde*I site at the 5′ of *atrA* gene and deca-histidine codons in front of the translational stop codon of *atrA* at the 3′ were constructed to express in *E. coli* and *S. coelicolor*, respectively. By the pET system (Studier et al., 1990), the pYP001c was introduced into *E. coli* BL21(DE3) and induced with IPTG. However, the resulting strains did

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not overproduce AtrA as a positive control pJAS407. After the introduction of pL646c into *S. coelicolor* by conjugation, the blue pigment of actinorhodin was not detected on R5 medium. The possible explanation of these results is the oligohistidine tags at the C-terminus might affect to the secondary structures of AtrA.

pJAS407 is a pET16b-based construct containing nonahistidine-tagged *atrA* at its N-terminus (Stead et al., 2006; Uguru et al., 2005); therefore, nonahistidine that added in the N-terminus of this gene might not affect to the polypeptide structure after translation. The pET16b-based construct, pYP002n, was constructed by adding the oligohistidine tags at the 5′ of *atrA* gene and transformed into *E. coli* BL21(DE3) to express in *E. coli*. After induction with IPTG and purification by Ni-NTA spin column, the 30 kDa putative transcriptional regulator AtrA from all transformants in *E. coli* was run on SDS-PAGE and the apparent equilibrium dissociation constant (k_d') of N-terminally oligohistidine-tagged AtrA was ~141 nM assuming as that of normal AtrA from pJAS407 (Uguru et al., 2005). The nonahistidine-tagged *atrA* fragment was cloned into pICLSET and an integrative P_{tipA} expression vector pIJ6902 (Huang et al., 2005) and transformed into *S. coelicolor*. As the variety of *S. coelicolor* phenotypes grew on R5 medium, but not on TSB agar medium (Jane E. Towle, unpublished data), the phenotypes of exconjugants L646n and L647n were compared on both R5 and TSB agar media, supplemented with 6 μg of thiostrepton on the patches in case of L647n (Sun et al., 2001). Actinorhodin production could not be detected in the agar since the L647n cells were decreased by thiostrepton added to induce the production of AtrA. The high concentration of AtrA, therefore, might be toxic to the L647n cells is found in some proteins such as overexpression of RecA in *S. lividans* (Vierling et al., 2000) and factor C in *S. gresius* (Birkó et al., 2001). This conclusion was also supported by the production of actinorhodin from L647n on the TSB agar supplemented with 50 μg/ml apramycin. However, the AtrA was not detected on SDS-PAGE after purification from the L646n and L647n cells by Ni-NTA spin column. The nonahistidine-tagged AtrA at the N-terminus, moreover, could not be detected by Western blot analysis. The possible reasons are:

(1) AtrA is an unstable intermediate of antibiotic biosynthetic pathway, and

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(2) AtrA might be used in very small amounts in *Streptomyces* similar to the expression of C-terminally oligohistidine-labelled *fac*C gene in *S. griseus* (Birkó et al., 2001).

In further investigations using L647n, the amount of AtrA will be detected using microarray experiments, the oligohistidine-tagged AtrA at the N-terminus will be used to elucidate the regulation of antibiotic production in the pathways, and the other factors affected to AtrA will also be determined in *S. coelicolor*.

EDUCATIONAL ASPECTS

6.3 Learning 'bacterial conjugation' through laboratory work using different teaching sequences at secondary school level

One of the aims of this study was to design a labwork activity to promote the conceptual learning by using an appropriate teaching intervention. In the first phase, the prior knowledge of bacteria and genetics analysed from the national standard for Thai secondary sciences and surveyed from the students' as well as teachers' views was used for providing the concept of genetic transfer by conjugation. Owing to the difficulty of genetics (Bahar et al., 1999; Wendelberger et al., 1998), a laboratory class was designed to make simple conceptual model of genetic transfer in bacteria by using the conjugation in *E. coli* as a model. Drawing-based activities and concept map activities were provided for the teaching sequence to reinforce the mechanism of conjugation and investigate the overall concepts that students learned with the labwork. A lesson plan, teacher's manual and lab handout were also designed to be suitable for learning and teaching this topic. This practical allows students to try their hands at constructing the concepts on their own by following their use of the practical learning model (adapted from Winn, 1997), which has the characteristics in the learning cycle similar to the experiential learning model based on constructivism (Kolb, 1984). Pre-instructional data from third-year senior secondary students were used to develop suitable materials such as activities and assessment tools and to validate all instruments that could investigate the students' ideas and try to show that the assumption was true.

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In the second phase, the teaching interventions were implemented in first-year senior secondary students to promote conceptual understanding of bacterial conjugation. Leonard (2000) recommended that using the lab before lecture can assist students to achieve the science concepts since it allows instruction to proceed from concrete to abstract (or from the first stage to third stage in Kolb's learning cycle) and should make learning much more successful. The findings showed that teaching lab before lecture gave the highest post-test scores, gain percentage scores and conceptual scores, which compared with teaching by lecture only and teaching lecture before lab. Thus, this teaching sequence can assist students to obtain the best construction of concepts in bacterial conjugation as in the recommendation of constructivist learning (Leonard, 2000).

Labwork is more useful to help students to achieve an understanding of concepts (Wood, 1996), and there are many research papers that design labwork for biological areas e.g. Hamilton et al. (2006), Longtin et al. (2004) and Zion et al. (2006). Although the concept-based laboratory which designed with a combination of hands-on and minds-on aspects (Halme et al., 2006; Rotbain et al., 2006), students might not achieve the conceptual understanding with lack of suitable teaching sequence. The findings indicated teaching lecture before lab gave higher scores than the traditional method, and yet it is not as effective as doing lab before lecture. From the qualitative data, students cling to their misconception because of their prior knowledge (Lewis, 2000b), for example, cells have muscle as an organelle, and binary fission is a horizontal gene transfer. Students taught by traditional method have much confusion such as that conjugation is vertical gene transfer, and that the conjugal transfer uses the rolling-circle mechanism, whereas in contrast their confusion is less when using the labwork in the teaching interventions. Because of the poor mathematical skills of a large proportion of students (Lake, 1999; Marsh & Anderson, 1989; Tariq, 2002), the difficulties in handling unit conversions and indices (i.e. power of 10) could be discerned from their lab reports. Moreover, students had problems in designing and reading the data from the experiment since there is not this topic dealt with the biology curriculum. Therefore, not only basic mathematical understanding (Tariq, 2002) but also reading data from graphs and tables (Lake, 1999) are required for all biology courses.

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The preferences towards learning the concept of bacterial conjugation were investigated in the final phase to develop an appropriate learning environment for this concept. There were conflicts of prior knowledge rated in the questionnaire between teachers' and students' views, except with respect to the use of sterile technique. Teachers evaluated students' prior knowledge according to details in the biology curriculum, and students rated the prior knowledge that they understood before learning these concepts. When learning this concept, students were probed for their prior knowledge and understanding using various assessment tools and observation in the class. The rating using the topics provided before learning this concept were not correct since students had some misconceptions and confusion in prior knowledge, which teachers thought students should be able to understand after the classes. To probe the understanding of concepts, teachers should use various assessment tools to obtain students' grasp of those concepts. Therefore, triangulating their prior knowledge from teacher, student and researcher is very important for answering the current situation with respect to their prior knowledge. In this study, students' and researcher's views of prior knowledge were different from its teachers' views. Furthermore, students and teachers thought the practical work designed for the teaching intervention of learning lab before lecture can assist students to understand the concept given in the classroom and are appropriate for the secondary biology curriculum.

6.4 Improving Thai students' understanding of concepts in 'protein purification' by using a simulation program in Thai and English versions

In general, there are four educational goals that may result from hands-on practials (Ma & Nickerson, 2006):

(i) conceptual understanding (extent to which practical activities help students understand and solve problems related to key concepts taught in the classroom);

(ii) design skills (extent to which practical activities increases students' ability to solve open-ended problems through the design and construction of new artifacts or processes);

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(iii) social skills (extent to which students learn how to productively perform biology-related activities in groups; and

(iv) professional skills (extent to which students become familiar with the technical skills they will be expected to have when practising in the profession).

By these goals, a Thai virtual class was provided which aimed to positively affect the development of student's understanding and perception of protein purification lessons in undergraduate levels.

The first phase of this study was to translate the protein purification program from original language (English) into Thai. The development of Thai version is very slow since:

(1) there are linguistic differences between English and Thai;

(2) many technical terms are not used in students' everyday life; and

(3) no appropriate words are available to substitute for specific terms.

This virtual class was used to improve the preparedness of students for laboratory exercises and eliminate some difficulty with conceptual understanding as other computer-based mediated learning in laboratory classes (Pamula et al., 1996). The explanations in help files are insufficient to view the concepts; therefore, some animations and pictures were added. The simulation practical allowed students to try their hands at constructing the concepts of protein purification by following a practical learning simulation (adapted from Winn, 1997). When comparing the UK and Thai undergraduate curricula, we decided to try out the first Thai version with a mixed group of undergraduate and graduate students since there is no class on this topic at the undergraduate level. Pre-instructional data used for developing the suitable virtual class and validating the assessment tools can probe the students' idea about protein purification.

In the second phase, the virtual class was implemented with undergraduate students by comparing between single and two-language programs. In the treatment group of two-language program, the subjects reported more positive perceptions of their hands-on learning experience and obtained higher post-test scores than those learning with single language program. In confirmation using qualitative data, students learning with single program showed much more confusion than those learning with

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Thai/English program. Thus, the possibility of codeswitching (Muñoz et al., 1999; Macro, 2005) between two languages can assist students to grasp the knowledge better than using the English version alone. These findings, furthermore, reiterate that the use of computer-based instructional programs tends to improve students' achievement scores when compared to the use of traditional or regular methods of instruction (Chan et al., 2002; Kiboss et al., 2004; Wekesa et al., 2006; Zumbach et al., 2006).

In the final phase, two-language Protein Purification Program was implemented in a biological science class of both undergraduate and graduate levels. From the questionnaire, students and teachers evaluated the attitude towards Thai/English program. The concepts of protein purification cannot only be learned from the simulation program, and the virtual class should be a part of teaching and learning of this topic to reinforce students to produce a better understanding for their future researches. Furthermore, both digital tools and lab bench exercises should be used in the "blended" approaches for teaching and learning any concepts in biology. These blended approaches are used to eliminate the misuse of digital simulation in classes (Scheckler, 2003).

6.5 Summary of the learning model and the educational implications

The teaching interventions using the practical work in this study follow the practical learning cycle adapted from Winn (1997), which has four stages as in Kolb's learning model based on constructivism (Table 6-5). The 'fact-information-knowledge-action' model has a utility with the development of practical-based learning. Findings from the study suggest that the practical works are required for constructing concepts in biology through the Winn's, Kolb's and constructivist learning models.

The appropriate teaching intervention to generate the new knowledge based on students' prior knowledge is constructivism by the use of lab before lecture. Teachers or researchers who design the practical activities for science classes should be concerned about the pedagogical content knowledge (PCK) which is used to assist students to obtain a proper conceptual understanding. Many students do not want to do lab after the lecture since they feel they already know the "answer" to the experiments. If

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teachers are designing the teaching sequences by using the lecture before lab, they should make a plan that can help students construct knowledge from the experiment.

These practicals enhanced the biology curriculum for secondary school students and undergraduates as expected. However, some materials should be selected for each student group, and the Protein Purification Program has been developed to be appropriate for Thai undergraduates in both structures and content translation in help files. Some aspects in the findings, such as gender, concept map based learning, drawing-based activity, should be analysed to probe students' conceptual understandings in the areas of bacterial conjugation and protein purification. Hence, further research is needed to gain greater understanding of the assumptions based on the constructivist learning. In turn, these practicals will enable instructional designers to gain greater awareness of the ways that students can gain a better understanding of concepts.

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Table 6-1 Conclusion of the activities in practical works used in this study

Stage	Learning cycle		Wet practical in bacterial conjugation	Dry practical in Protein purification
	Winn (1997)	Kolb (1984)		
			Pretest	
1	Fact	Concrete experience	The general concepts of bacterial conjugation and lab techniques are given to students before doing the lab, and students design the plan before practice.	A practical simulation given to the students, and the general concepts of protein purification are given to students.
2	Information	Reflect observation	Students do the labwork in small groups with their plans and some teachers' guidelines on their plans.	Students learn with the program and attempt to purify unknowns by various techniques by exercise designed and the purification criteria to grasp the concepts of protein purification.
3	Knowledge	Abstract conceptualisation	Students build up the concepts of conjugation from labwork. A drawing-based activity is given to students. Then, teacher gives the mechanism ideas and application of conjugation.	Students build up the concepts from simulation program. The teacher gives a set of unknown, which are easy to purify.
			Lab report	
4	Action	Active experimentation	Students apply the knowledge to discuss and debate the result in the classroom and via electronic mail with peers and teacher. Students answer some questions that are set for applying knowledge the new situations.	Students apply their knowledge to discuss and purify one protein from the complex unknown (20 proteins). After finishing all practices, the teacher gives the new situations that are different from the unknown in the program for students to debate in the classroom or via electronic mails.
			Concept map, Posttest	*Posttest*
			Conceptual test	*Conceptual interview*
			Conceptual interview	

Note: Italics indicates the assessment tools

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CHAPTER VII
CONCLUSION

To find out the suitable condition for intergeneric conjugation between *E. coli* and *S. rimosus* wild type and mutant strains, the pre-germination of spores of *S. rimosus* R7 and M4018 by heat treatment at 40 ºC for 10 min was recommended for conjugation when using the spores as recipients, and Oxoid TSA containing 10 mM $MgCl_2$ is an effectively conjugative medium. In case of non-spore forming *Streptomyces* as *S. rimosus* 593, the mycelia can use as recipients instead of spores without heat treatment. After conjugal transferring of pIJ8600 to *S. rimosus* R7, the exconjugants were examined by PCR amplification and done by the Southern blot hybridisation to determine the attachment site on the chromosome. The *attB* core sequence of *S. rimosus* R7 is 5′-CGG TGC GGG TGC CAG GGG GTG CCC TTG GGC TCG CCC GGC GCG TAC TCC ACC-3′ which is identical to those of *S. rimosus* M4018 and *S. avermitilis*. The sequence composed of three base-pair homologue 5′TTG in the middle of the DNA sequence indicated the specific recombination site.

In order to investigate the post-translational modification of AtrA protein by comparing the activity of AtrA purified between *E. coli* and *S. coelicolor*, the oligohistidine-tagged AtrA at C- and N-termini was constructed. The oligohistidine tags at C-terminus might affect the secondary structure of AtrA; therefore, it cannot drive the actinorhodin biosynthesis of *S. coelicolor*. However, this situation does not occur in the tags at N-terminus. The AtrA can be easily purified from *E. coli* by Ni-NTA affinity column, but it is not detected when purifying from *S. coelicolor*. Owing to the low amount of AtrA and its function of intermediate in the pathway, the microarray technique will be used to determine the AtrA in the future work.

In the educational aspects, the practical works were designed to teach the concepts of bacterial conjugation and protein purification at the secondary and university levels. When comparing three teaching interventions used in teaching and learning of conjugation, the teaching lab before lecture with the Winn's practical

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learning model, a modified learning cycle based on constructivism can help students to obtain the better achievement than other learning interventions. Students who learned by lab before lecture had the highest posttest scores, gain percentage scores and conceptual test scores because of less confusion, probed by semi-structured conceptual interviews. In the concept of protein purification, two-language program can help students to understand the concepts better than single (English) language program. With qualitative and quantitative data, it indicated the student's achievements by learning with Thai/English language program are better than those by learning with only English program. From the questionnaire, students evaluated the practicals are good for learning with any learning models, particularly constructivist learning model, and teachers also agree to add both concepts in the curriculum to teach in the next year.

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APPENDIX

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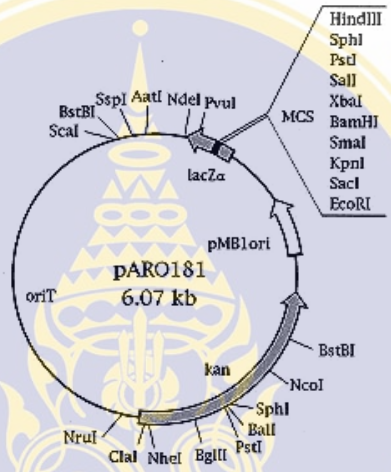
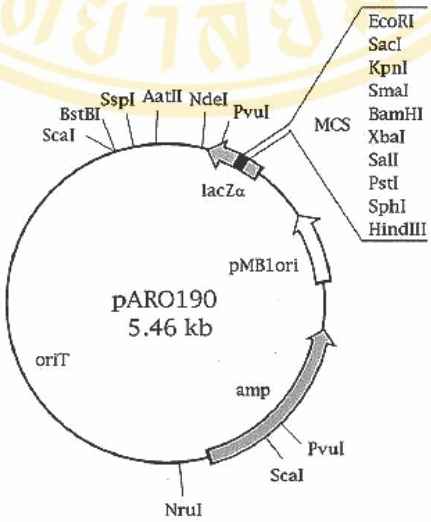
APPENDIX A

Vector Circle Maps

A.1 pARO181 (Parke, 1990)



A.2 pARO190 (Parke, 1990)



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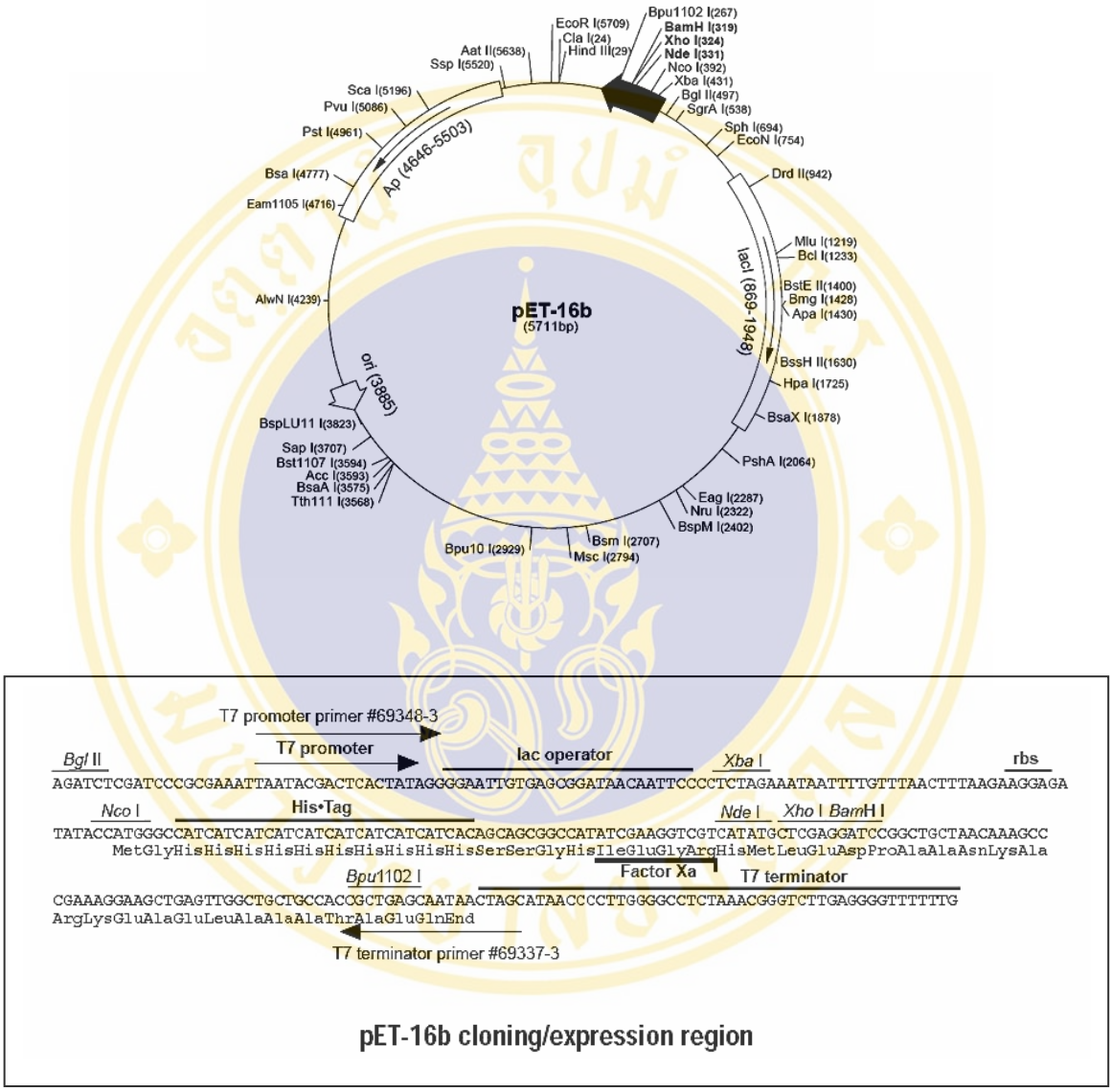
A.3 pET-16b (Novagen)



pET-16b cloning/expression region

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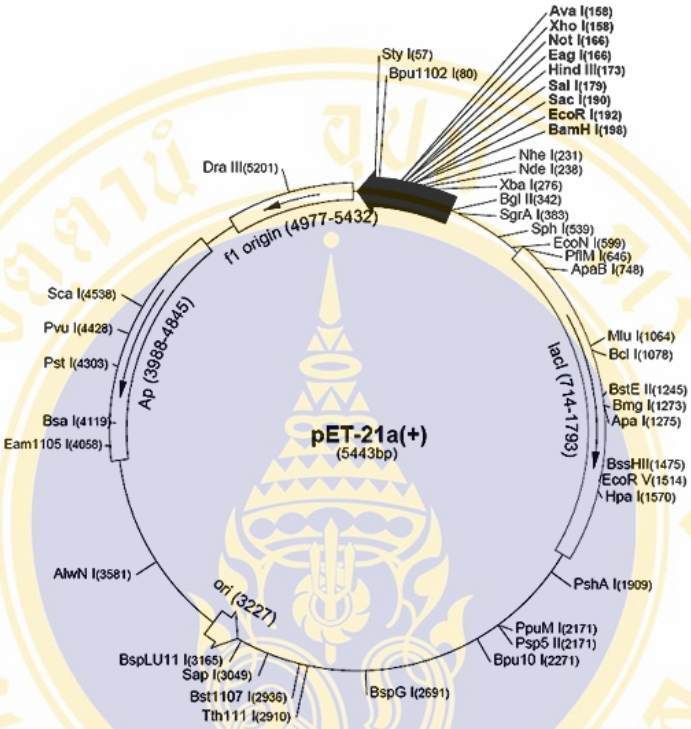
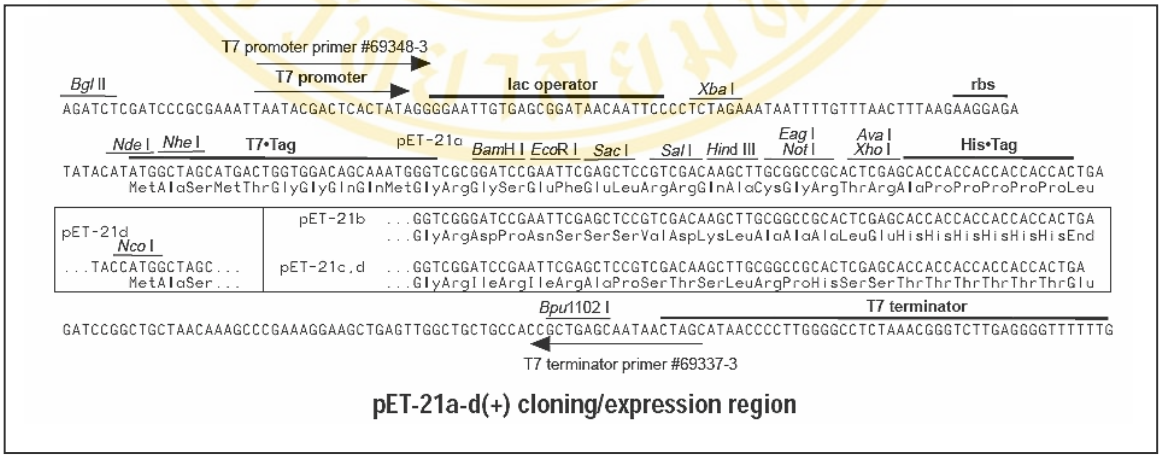
A.4 pET-21a-d(+) (Novagen)





pET-21a-d(+) cloning/expression region

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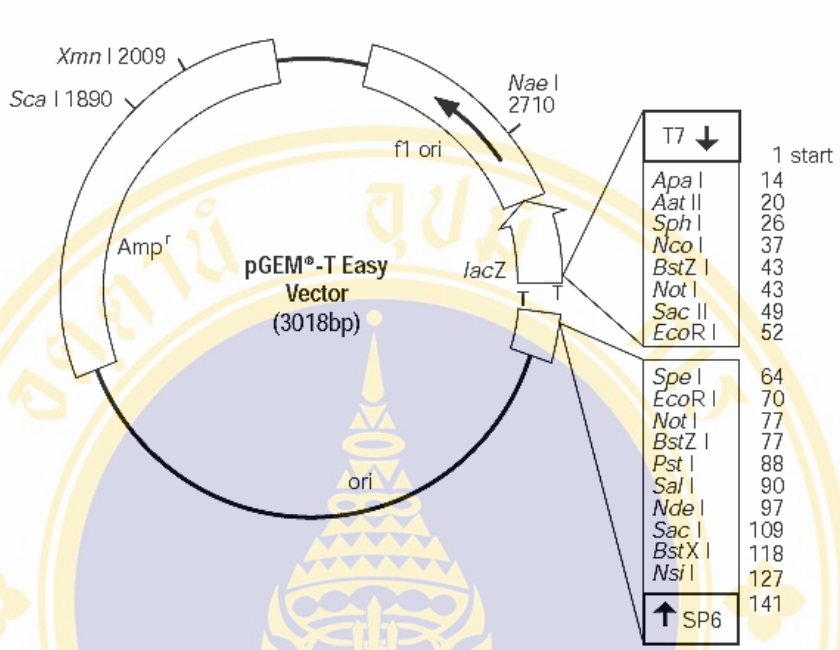
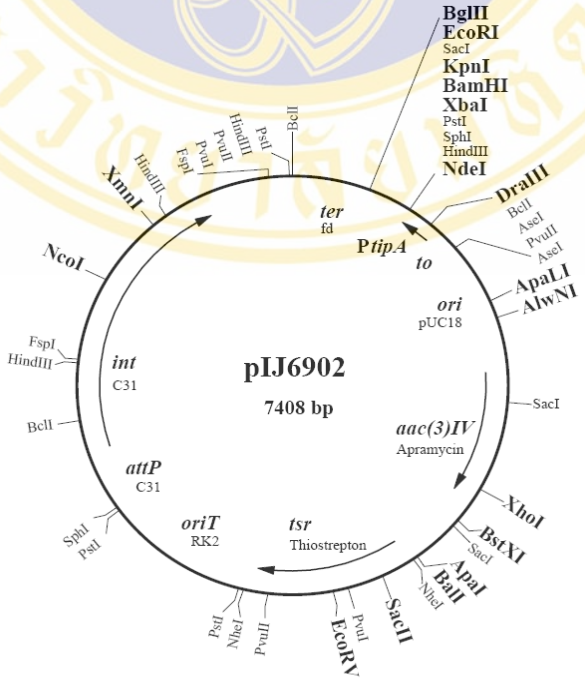
A.5 pGEM-T easy (Promega)



A.6 pIJ6902 (Huang et al., 2005)



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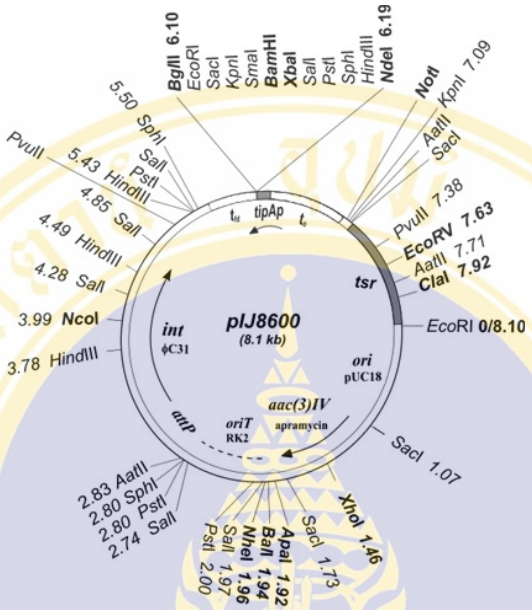
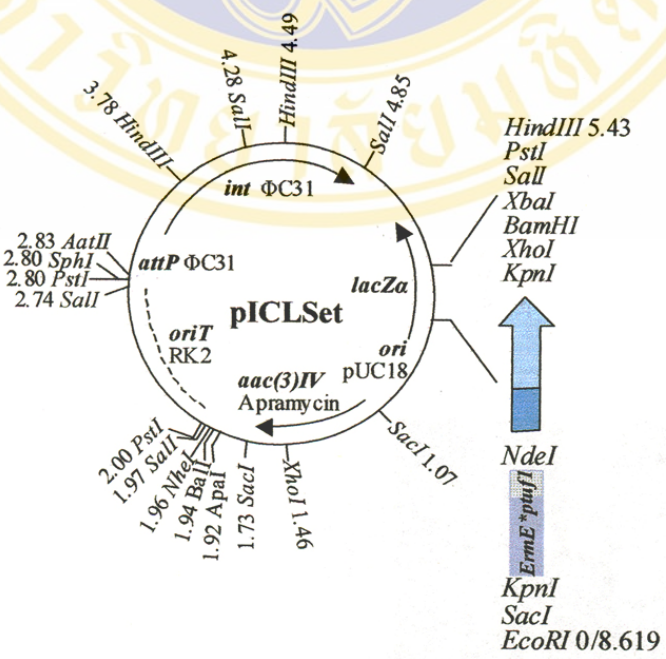
A.7 pIJ8600 (Sun et al., 1999)



A.8 pICLSet (Tilley, 2005)



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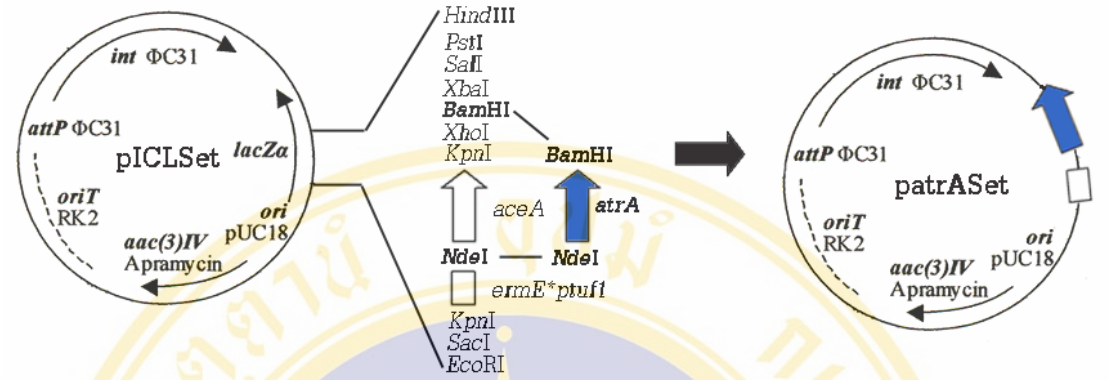
A.9 patrASet (constructed by Jonathan A. Stead)



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APPENDIX B

Conjugation in *Escherichia coli*: A Laboratory Exercise

Introduction

Conjugation in *E. coli* is a horizontal gene transfer from donor cells to recipient cells. Donor cells transfer the DNA after the establishment of mating pairs by the agency of slender thread-like surface structures, following by pilus contraction and physical contact between donor and recipient cells. The recipient cells receiving the DNA after conjugation is a new donor called transconjugant. Each bacterium has different ability to transfer the plasmid, thus we can compare the ability of plasmid transfer from donor to recipient by calculating the efficiency of conjugation (or conjugation frequency) and considering the ratio of donor to recipient used in each conjugation experiment. This experiment is used to describe how plasmids pARO181 and pARO190 (Parke, 1990) transfer from *E. coli* S17-1 to *E. coli* JM109 (Yanisch-Perron et al., 1985). The plasmid pARO181 and pARO190 contains kanamycin and ampicillin resistance genes for selection of the transconjugants, respectively. This protocol additionally provides for the confirmation of transconjugants by agarose gel electrophoresis to demonstrate the presence of plasmids, originally in the donor, after DNA transfer. If the agarose electrophoresis is not available, the confirmation is not included in the student protocol.

To practise the scientific skills, students should previously design their own experiment plan and draw the experiment flow chart. The plan includes the method, control group, experimental group and the importance of using each antibiotic in this exercise.

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Behavioural objectives

After doing the experiment, students are able to:

(1) Describe the meaning of keywords in conjugation, such as conjugation, donor, recipient, sex pilus, transconjugant.

(2) Draw the picture of mechanism of conjugation in *E. coli*.

(3) Calculate the efficiency of conjugation in various bacteria by using the conjugation in *E. coli* as a model.

(4) Draw the diagram of the relation among the function of any genes in conjugation process as well as their RNAs and proteins.

(5) Design an easy experiment to determine the intrageneric and intergeneric conjugation.

Methods

Teacher preparation procedure

Conjugative donor and recipient strains of E. coli

The basic conjugative donor strain is *E. coli* S17-1 (ATCC 47055) containing plasmid pARO181 (ATCC 77125) or pARO190 (ATCC 77124). The donor strain and mobilisable plasmids could be requested from Department of Microbial Genetics at National Institute of Genetics, Japan (http://www.shigen.nig.ac.jp/cvector/cvector.html). The mobilisable plasmids are transformed into *E. coli* S17-1 by standard procedures of DNA manipulation given in Sambrook & Russell (2001). The DNA transformation kit from various biological supply companies (e.g. Bio-Rad, Fermentus) may be used. However, the teachers in UK may use the excellent information on the website of the National Centre for Biotechnology Education (http://www.ncbe.reading.ac.uk/NCBE/SAFETY/dnasafety1.html) to purchase the transformation kit licensed for secondary school such as the pGLO transformation kit from Bio-Rad (Bio-Rad Laboratories) (Longtin et al., 2004). The transformants are grown overnight at 37 °C on Luria-Bertani (LB) agar supplemented with a selectable marker in each mobilisable plasmid: kanamycin (for pARO181) and ampicillin (for pARO190), and then picked to grow in LB broth supplemented with the same antibiotics. The antibiotic concentrations used for growing

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donor strains are shown in Table B-1. The donor strains of *E. coli* can be washed twice with LB broth in the same volume of culture, resuspended in 20% glycerol and stored frozen at -20 °C for years.

Table B-1 Antibiotic concentration used in this laboratory exercise

Antibiotic concentration of stock solutions (mg/ml)	Final concentration (µg/ml)	Amount of stock solution (µl) in a 5-ml LB broth tube	Amount of stock solution (µl) in a 25-ml LB agar plate
Ampicillin (100 in water)	100*	2.5	25
Kanamycin (50 in water)	25	5	25
Nalidixic acid (25 in 0.15 M NaOH)	25	–	25

*50 µg/ml in liquid media

The recipient strain of *E. coli* which should be resistant to nalidixic acid (Nal^r) [e.g. strain JM109 (ATCC 53323)] can be ordered from biological supply companies (e.g. Stratagene, Promega Biotech) and are grown in LB broth overnight at 37 °C.

Reagent preparation

100 ml Tris-EDTA (TE) buffer, pH 8.0 which is composed of 10 mM Tris-HCl (1 ml of 1 M stock, pH 8.0), 1 mM sodium-EDTA (200 µl of 0.5 M stock, pH 8.0) and distilled water to 100 ml and is sterilised by autoclaving.

Student laboratory procedure

Material required per a student group

- Overnight culture of donor and recipient strains
- A glass spreader in an alcohol container
- Alcohol lamp
- 5 ml sterile LB broth
- 6 plates of LB agar and 2 plates of LB agar containing nalidixic acid and a selectable marker: kanamycin and ampicillin, respectively
- A bottle of sterile tooth-picks
- 30 sterile 1.5-ml microcentrifuge tubes
- 6 ml sterile water

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- A micropipetter (needed for pipetting 1 to 100 µl)
- 300 µl TE buffer, pH 8.0
- 0.8% agarose gel and agarose gel electrophoresis apparatus
- 7 µl gel loading dye
- Ethidium bromide
- 0.5 × TBE buffer (can be diluted from 5 × or 10 × solutions available from biological supply companies)
- 2 µl 1kb DNA ladder and 6 µl loading dye (Gibco BRL®, USA or Fermentus®, USA)
- Incubator shaker or waterbath shaker
- vortex mixer
- UV transilluminator

The number of donor and recipient cells

The overnight culture of donor and recipient strains are serially diluted in 10-fold (by adding 10 µl of culture to 90 µl sterile water). Three dilutions of the donor (e.g. 10^{-7}, 10^{-8} and 10^{-9} ml^{-1}) and the recipient (e.g. 10^{-7}, 10^{-8} and 10^{-9} ml^{-1}) are selected to grown on LB agar plates. After growing overnight at 37 °C, colonies on the plates are counted and the colony forming units calculated (e.g. colony forming units/ml).

Conjugation procedure

The overnight cultures of donor strains are diluted serially 10-fold to 10^{-4}. 100 µl of 10^{3} and 10^{5} ml^{-1} of donor cells are mixed with 100 µl of undiluted recipient cells. The mixtures are incubated at 37 °C for 30 min with very gentle shaking, briefly vortex mixed after incubation to terminate the conjugal transfer and then spread on LB agar containing nalidixic acid and a selectable marker: kanamycin (for pARO181) and ampicillin (for pARO190). All plates are incubated overnight at 37 °C. Colonies grown on the plates are counted and calculate the efficiency of conjugation, which is estimated as the number of transconjugants per donor cell.

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Confirmation of transconjugants by agarose gel electrophoresis

3-5 colonies of transconjugants are selected to confirm plasmid carriage by agarose gel electrophoresis. Each transconjugant colony from LB agar plate is picked with a sterile tooth-pick and dipped into a microcentrifuge tube containing 30 µl of TE buffer. 15 µl of this culture suspension is vortex mixed for 3 min, remixed with 1 µl of loading dye and run on 0.8% agarose gel in 0.5 × TBE buffer (Sambrook & Russell, 2000). Students can find a useful protocol for agarose gel electrophoresis on the websites such as http://www.methodbook.net/dna/agarogel.html.

Expected results

As an example, conjugation was performed using the donor strain S17-1 containing conjugative plasmids pARO181 or pARO190 and recipient strain JM109. After conjugation on LB agar containing nalidixic acid and a selectable marker, kanamycin and ampicillin, following antibiotic selection as above, the transconjugants were counted to calculate the efficiency of conjugation (Table B-2). Total DNA was isolated from some colonies on the conjugation plates by vortex mixing with TE buffer for 1 min and run on 0.8% agarose gel electrophoresis using the conjugative plasmid DNA's isolated from the donor strains as positive controls. As shown in Figure B-1, total DNA from transconjugants should give the same pattern as conjugative plasmids isolated from donor strains.

Table B-2 Efficiency of conjugation of pARO181 and pARO190 between *E. coli* S17-1 and *E. coli* JM109

Conjugative plasmid	Number of donors in 1 ml	Number of recipients in 1 ml	Donor: recipient Ratio	Number of transconjugants in 1 ml	Efficiency of conjugation
pARO181	1.62×10^{11}	2.83×10^{11}	2:3	1.53×10^{6}	9.44×10^{-6}
pARO190	1.73×10^{11}	2.83×10^{11}	2:3	1.84×10^{6}	1.06×10^{-5}

All values were from two replicates in two independent experiments.

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(A) (B)

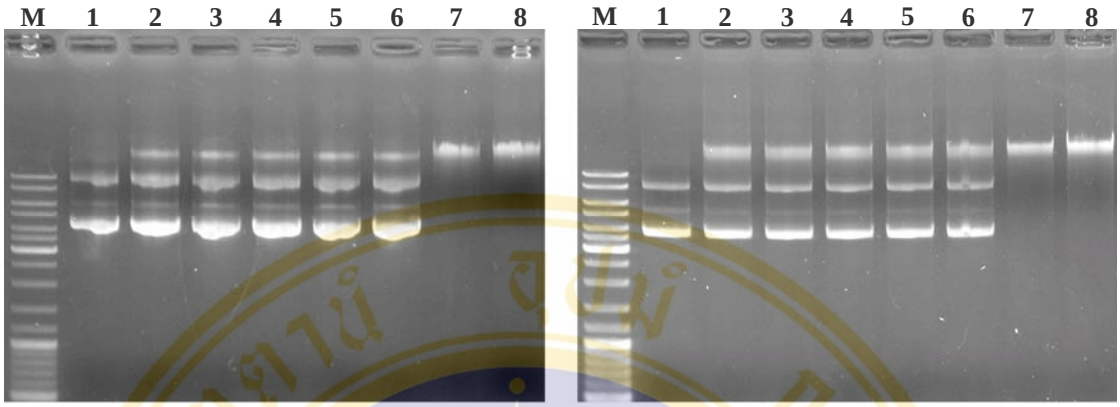

Figure B-1 Agarose gel electrophoresis of transconjugants compared with the conjugative plasmids. Lane M: 1-kb DNA ladder. Lane 1: plasmids (A) pARO181 and (B) pARO190 isolated from the donors using the mini-prep. Lanes 2-3: total DNA involving (A) pARO181 and (B) pARO190 isolated from the donors. Lanes 4-6: total DNA isolated from the transconjugants containing (A) pARO181 and (B) pARO190. Lane 7: total DNA from the recipient. Lane 8: total DNA from donor *E. coli* S17-1. The total DNA extracts from transconjugants are similar to those from the donor S17-1 containing pARO181 or pARO190, but there is no plasmid in the recipient strain JM109. The thick expected bands in the lanes of donors (Lanes 2-3) and transconjugants (Lanes 4-6) are the supercoil plasmid DNA (lower band) and open circular plasmid DNA (higher band). The highest band in the lanes 2-8 is the chromosomal DNA.

Student's self-assessment questions

1. Do you think all bacteria have the same conjugation process as *E. coli*? Why?
2. Why do we use nalidixic acid in this experiment?
3. If you would like to count only transconjugants on the agar medium after conjugation, how should the characteristics of donor cells differ from that of recipient cells?
4. Could you design an experiment to proof the conjugation can occur between different bacterial genera by using your experience from the experiment?
5. Draw the diagram of the mechanism of conjugation in *E. coli* and give the explanations in each step.

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6. Draw the concept map of bacterial conjugation that you learnt from the lecture with the experiment.

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APPENDIX C

Drawing-based Activity in Conjugation of *E. coli*

Figures (a) to (f) represent the steps in the conjugation process. The figures are arranged in their correct order in process.

1. Fill in the missing words (use the keywords given below).
2. The description of each of the following six sentences is compatible to one of the Figures (a) to (f), but not in the correct order. Write the suitable letter in each sentence:

   ____ The cell walls of donor and recipients are closely contacted. Transfer protein (Tra), encoded from transfer (*tra*) gene, breaks the phosphodiester bond between the nucleotides on the origin of transfer (*oriT*) as well as unwind the DNA helix.

   ____ Transferred linear DNA links together to form a close circle, and a complementary strand to the remaining DNA is completely synthesised in the donor cell.

   ____ Nicking protein binds to the 5' end of the nicked DNA which enters to the recipients.

   ____ During the DNA transfer process, a complementary strand to the single-stranded plasmid remaining is synthesised.

   ____ The close physical contact of the cell walls between donor and recipient cells occurs after the contraction of sex pilus.

   ____ Once inside the recipient cells, a complementary strand to the single-stranded plasmid is synthesised. Both cells separates, and the recipient containing the plasmid transferred becomes a new donor.
3. Which step is the termination of conjugation?
4. According to the previous explanation, what is A and B in Figure (a)?
5. Which step should come before Figure (f)?

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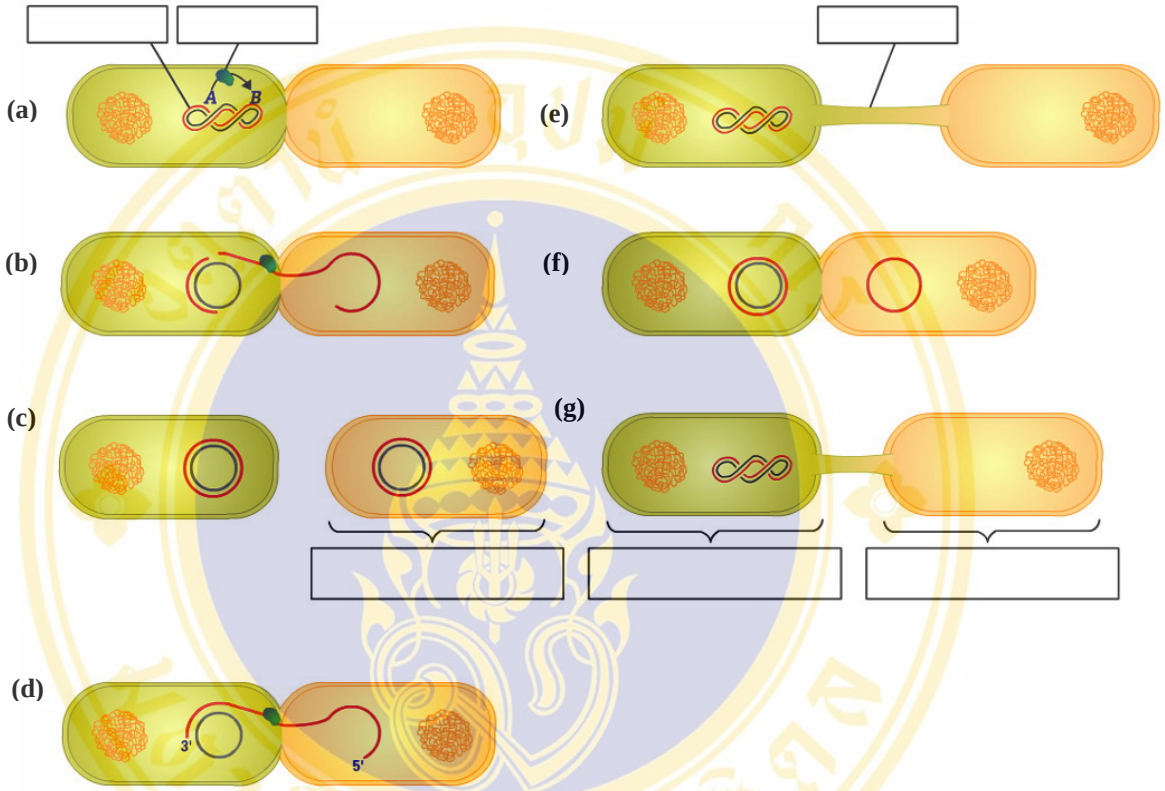
6. Based on the pictures and explanations, write a short paragraph that describes the conjugation process in *E. coli*.



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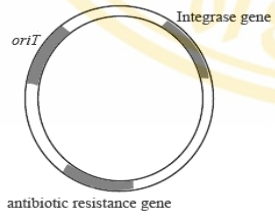
APPENDIX D

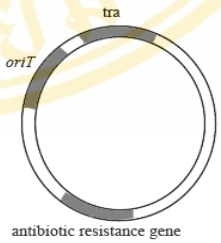
The Instruments to Assess Conceptual Understanding in Bacterial Conjugation and Protein Purification

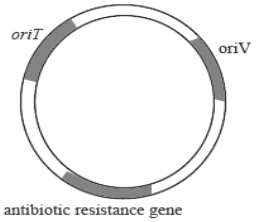
D.1 Multiple choice questions designed as pre- and post-tests to assess the conceptual understanding of bacterial conjugation at the senior secondary level

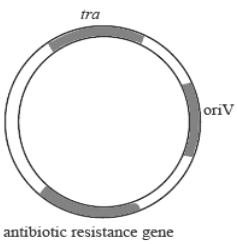
1. Which organism can reproduce the same way as the conjugation in bacteria?
   a. mould b. algae c. *Paramecium* d. virus

2. Which of the following best describes the conjugation?
   a. DNA is transferred between the same or different bacterial species.
   b. DNA is transferred from a bacterial cell to another.
   c. DNA is transferred between the same species of bacteria.
   d. DNA is transferred between the different species of bacteria.

3. Which is a self-mobilisable plasmid?
   a.


   b.


   c.


   d.


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4. Which of the following is **NOT** true about the genetic materials in bacteria?
   a. A group of single stranded DNA can replicate itself.
   b. A group of double stranded DNA can replicate itself.
   c. A circular DNA cannot replicate itself.
   d. A circular DNA can replicate itself.

5. Which part should **NOT** get involved in the plasmid before conjugation if that plasmid cannot be increased its number in the recipient cells?
   a. origin of transfer
   b. origin of replication
   c. transfer gene
   d. integrase gene

6. Which structure is used for transferring the DNA during the conjugation?
   a. flagella
   b. cell membrane
   c. fimbriae
   d. microfilament

7. Which of the following is **NOT** involved in spontaneous bacterial conjugation?
   a. origin of transfer
   b. transfer gene
   c. integrase gene
   d. helicase

8. Which the following best describes the mating pair in the conjugation process?
   a. In Gram-negative bacteria, donor's pilus contacts to the recipient's membrane receptor, and there is a close contact between F^+ and F^- because of the contraction of the pilus.
   b. In Gram-negative bacteria, donor's pilus contacts to any area on the recipient's membrane, and there is a close contact between F^+ and F^- because of the contraction of pilus.
   c. In Gram-positive bacteria, donor cells do not have the construction of pilus but the mating pair is formed by donor's adhesin coat.
   d. In Gram-positive bacteria, recipient's sex pheromone stimulates donor cells to release a protein called adhesion that is used to form their mating pair.

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9. Which is the best order of mechanism of conjugation?
    1. The plasmid DNA is nicked at the specific site within the origin of transfer.
    2. The donor cell makes contact with recipient cell.
    3. Plasmid replicates to be a double strand within recipient cell.
    4. After the contraction of frimbriae, there is a close physical contact between donor and recipient cells.

    a. 1, 2, 3, 4 b. 2, 4, 1, 3 c. 4, 2, 3, 1 d. 4, 2, 1, 3

10. Why is the conjugation a horizontal gene transfer?
    a. Because it is a genetic transfer in the same generation and the genetic change only occurs in recipient cells.
    b. Because it is a genetic transfer in the same species and the genetic change only occurs in recipient cells.
    c. Because it is a genetic transfer in the different species and the genetic change only occurs in recipient cells.
    d. Because it is a genetic transfer between cells and the genetic change only occurs in the next generation.

11. Which mutant can be a donor cell that has a normal conjugation?
    a. Stop codon of transfer gene is changed to be a start codon of transcription.
    b. A set of stop codon of transfer gene is increased after the stop codon of the gene.
    c. Two bases after start codon of transfer gene are deleted.
    d. Two bases after start codon of transfer gene are inserted.

12. Which of the following is **NOT** the importance of bacterial conjugation?
    a. To reduce the possibility of plasmid loss in bacteria.
    b. To maintain the population survival in various surroundings.
    c. To control the function of gene in chromosome by the plasmid transferred.
    d. To prevent the bacterial cells from other cells' antibiotics.

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13. Which of the following is correct about the definition of 'nick'?
    a. A gap of double stranded DNA digested by helicase.
    b. A gap of single stranded DNA digested by helicase.
    c. A gap of single stranded DNA digested by endonuclease.
    d. A gap of double stranded DNA digested by endonuclease.

14. Why does a donor cell not be changed to be a recipient cell after conjugation?
    a. Since only single stranded DNA is transferred into the recipient cell.
    b. Since donor's DNA is replicated before transferring into the recipient cell.
    c. Since some parts of DNA is transferred into the recipient cell.
    d. Since recipient's DNA is transferred back into the donor cell.

15. Which protein is NOT produced from the transfer gene?
    a. endonuclease b. helicase
    c. adhesin d. nicking protein

16. How does the plasmid in cytoplasm integrate into the bacterial chromosome?
    a. Transfer gene produces an enzyme to assist the chromosomal integration of plasmid.
    b. There is a nick on chromosome which is a gap for the integration of plasmid.
    c. The integration site on chromosome is digested and ligated with the plasmid.
    d. Integrase gene encoded an key enzyme for the chromosomal integration.

17. If the 3×10^9 cells/ml of donor cells conjugates with 3×10^{10} cells/ml of recipient cells and the number of mutant is 3×10^4 cells/ml, how many is an efficiency of conjugation (In this case, you would like to know how many recipient cells is changed to be transconjugants.)?
    a. 10 b. 10^{-1} c. 10^{-5} d. 10^{-6}

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18. The experiment of intergeneric conjugation from *E. coli* to *Streptomyces* sp. is designed by using the conjugative plasmid that contains two antibiotic resistance genes: gene A is expressed in *E. coli* and gene B is expressed in *Streptomyces* sp. Which group of cells is a mutant after conjugation? (+ indicates cell growth on medium; – shows the cell growth is not found.)

Group of cells	Medium		
	no antibiotics	antibiotic A	antibiotic B
a	+	+	+
b	+	+	–
c	+	–	–
d	+	–	+

19. A bacterial strain requires 5 growth nutrients: A, B, C, D and E. A donor cell can produce A, B and C as well as is able to resist kanamycin (Kan^r), whereas a recipient cell can produce D and E as well as is able to resist ampicillin (Ap^r) and tetracycline (Tet^r). After conjugation, which group of cells is a donor cell? (+ indicates cell growth on medium; – shows the cells are not grown on medium.)

Group of cells	Minimal medium				
	No antibiotics	Supplemented with A+B+C and kanamycin	Supplemented with D+E and kanamycin	Supplemented with A+B+C and ampicillin	Supplemented with D+E and tetracycline
a	+	+	+	+	+
b	+	–	–	+	+
c	–	–	–	+	–
d	–	–	+	–	–

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20. A researcher's conjugation experiment uses *E. coli* S17-1 containing transfer gene on chromosome. Moreover, this *E. coli* has a plasmid containing resistance genes of antibiotics C and D. The recipient cells do not have the donor's plasmid, but they contain resistance gene of antibiotic F on their chromosome. After mixing both cells and leaving a short period for their conjugation, which group of cells is **NOT** the control group of this experiment? (+ indicates cell growth on medium; – shows the cells are not grown on medium.)

Group of cells	Medium			
	no antibiotics	antibiotic C	antibiotic D	antibiotic F
a	+	+	+	-
b	+	–	–	+
c	+	–	–	–
d	+	+	+	+

D.2 Open-ended questions designed as the conceptual test to assess the conceptual understanding of bacterial conjugation at the senior secondary level

Q1 Why is the conjugation a type of sexual reproduction?

Q2 Which are the characteristics of plasmid that can be used in the conjugation process?

Q3 How does *E. coli* have a mechanism of conjugal transfer of the antibiotic resistance gene?

Q4 How different is the conjugation in Gram-positive and that in Gram-negative bacteria?

Q5 How does the donor cell transfer a piece of chromosome into the recipient cell?

Q6 Design the experiment to proof the conjugation between strain A containing a plasmid that resists kanamycin and strain B resisting ampicillin by indicating the determination of donor, recipient and transconjugant cells.

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D.3 Pre- and post-tests designed to assess the conceptual understanding of protein purification of undergraduate students

Q1 To purify an enzyme form a protein mixture, how do you determine the initial quantity of that enzyme?

Q2 At each step, what is the key index to show how your purification is proceeding?

Q3 At the last step, how do you determine the percentage yield compared with the initial mixture?

Q4 Which properties of proteins are used to develop the techniques of protein purification?

Q5 When you have an unknown mixture, how do you design a procedure to prepare an appropriate amount of enzyme at least 80% purity for later work?

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APPENDIX E

Lists of the Expert Committees in Reviewing the Instruments (Indicated with All Experts' Permissions)

E.1 Expert committee for bacterial conjugation

Prof. Dr. Simon Baumberg	School of Biology, University of Leeds, UK
Assoc. Prof. Dr. Piroh Pinpanichakarn	Department of Microbiology, Faculty of Science, Chulalongkorn University, Thailand
Assist. Prof. Dr. Arinthip Thamchaipenet	Department of Genetics, Faculty of Science, Kasetsart University, Thailand.
Assist. Prof. Dr. Joompot Poomsirpanon	Department of Biology, Faculty of Science and Teachnology, Rajamangala University of Technology Suvarnabhumi, Phranakorn Sriayutthaya, Huntra Campus, Thailand.
Sataporn Wantanawijarn	Department of Biology, Mahidol Wittayanusorn School, Thailand

E.2 Expert committee for protein purification

Prof. Dr. Andrew G. Booth	School of Biochemistry and Molecular Biology, University of Leeds, UK
Prof. Dr. Edward J. Wood	School of Biochemistry and Molecular Biology, University of Leeds, UK
Assoc. Prof. Dr. Bhinyo Panijpan	Institute for Innovation and Development of Learning Process, Mahidol University, Thailand.
Assoc. Prof. Dr. Pintip Ruenwongsa	Institute for Innovation and Development of Learning Process, Mahidol University, Thailand.
Assist. Prof. Dr. Joompot Poomsripanon	Department of Biology, Faculty of Science and Teachnology, Rajamangala University of Technology Suvarnabhumi, Phranakorn Sriayutthaya, Huntra Campus, Thailand.

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APPENDIX F

Test Reliability and Item-Analysis

F.1 Test reliability and item-analysis for multiple-choice questions in 'bacterial conjugation' from SPSS Output

	Number of items	Mean	Variance	Standard deviation
Statistics for scale	116	17.48	14.756	3.841
Item total statistics	Scale mean of item deleted	Scale variance of item deleted	Corrected item-total correlation	Cronbach's alpha if item deleted
Item 1	16.58	13.168	.703	.887
Item 2	16.56	13.414	.645	.889
Item 3	16.56	13.414	.645	.889
Item 4	16.59	13.201	.658	.888
Item 5	16.57	13.221	.710	.887
Item 6	16.55	13.571	.597	.891
Item 7	16.61	13.370	.516	.892
Item 8	16.55	13.571	.597	.891
Item 9	16.65	12.978	.612	.889
Item 10	16.57	13.221	.710	.887
Item 11	16.59	13.236	.641	.889
Item 12	16.64	13.276	.509	.892
Item 13	16.65	13.535	.396	.896
Item 14	16.63	13.835	.301	.898
Item 15	16.65	13.483	.416	.895
Item 16	16.63	13.592	.396	.896
Item 17	16.64	13.398	.460	.894
Item 18	16.63	13.122	.586	.890
Item 19	16.71	13.566	.329	.899
Item 20	16.64	13.624	.372	.897
Reliability coefficient (Cronbach's alpha) for 20 items = 0.897				

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F.2 Test reliability and item-analysis for six open-ended questions in bacterial conjugation from SPSS Output

	Number of items	Mean	Variance	Standard deviation
Statistics for scale	116	11.79	16.096	4.012
Item total statistics	Scale mean of item deleted	Scale variance of item deleted	Corrected item-total correlation	Cronbach's alpha if item deleted
Item 1	10.06	12.979	.590	.849
Item 2	9.80	11.621	.734	.823
Item 3	9.83	11.553	.743	.821
Item 4	9.69	11.138	.644	.837
Item 5	10.06	11.327	.546	.859
Item 6	9.53	10.338	.723	.822
Reliability coefficient (Cronbach's alpha) for 6 items = 0.868				

F.3 Test reliability and item-analysis for five open-ended questions in Protein Purification Program from SPSS Output

	Number of items	Mean	Variance	Standard deviation
Statistics for scale	32	14.28	2.080	1.442
Item total statistics	Scale mean of item deleted	Scale variance of item deleted	Corrected item-total correlation	Cronbach's alpha if item deleted
Item 1	11.47	1.160	.892	.792
Item 2	11.41	1.539	.513	.887
Item 3	11.47	1.225	.795	.821
Item 4	11.38	1.597	.528	.882
Item 5	11.41	1.346	.797	.823
Reliability coefficient (Cronbach's alpha) for 5 items = 0.873				

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APPENDIX G

Lesson Plan in 'Bacterial Conjugation' for the Treatment Group at the Senior Secondary School Level

This lesson is provided for the teacher with materials, activities and assessments to teach the concepts of bacterial conjugation at the senior secondary school level.

Main concept:

Bacterial conjugation is a horizontal gene transfer process from a donor cell bearing one or more conjugative plasmids to a plasmid-free recipient cell to achieve plasmid survival and spread at the population level. Conjugal transfer in both Gram-negative and Gram-positive bacteria requires close physical contact between mating cells and is usually mediated by plasmid-encoded proteins which provide transfer (*tra*) functions. Conjugation involves a special type of replication in which a plasmid DNA molecule replicates during conjugation, with one copy remaining within the donor cell and the other being transferred to the recipient. In most cases, replication is initiated by creating a single-stranded nick at the plasmid's origin of transfer (*oriT*); the unnicked DNA single strand is replicated in the donor cells, while the 5' end of the nicked strand DNA is actively transported and linearly through the donor and recipient envelops into recipient cell where it is replicated and the double-stranded DNA is circularised.

In case of conjugation in *E. coli*, donor cells transfer the DNA after the establishment of mating pairs by the agency of slender thread-like surface structure, following by pilus contraction and physical contact between donor and recipient cells. In some Gram-positive bacteria such as *Streptococcus faecalis*, a recipient cell produce a mating protein called bacterial sex hormone related to the pheromone of female insects. The pheromone stimulates the donor to synthesise a protein coating the donor cells named adhesin and cause them to form mating pairs. Synthesis of pheromone is inhibited when the plasmid is transferred to a recipient cell. The plasmid might be incorporated into the bacterial chromosome called episome to cause

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chromosomal genes to be transferred. The bacterial cell having the chromosomal plasmid or episome is called high-frequency recombinants (Hfr).

Behavioural objectives:

After learning this lesson, students should be able to:

1. Write the definition of key terms in bacterial conjugation: donor cell, recipient cell, transconjugants, pilus or fimbriae, plasmid nick, origin of transfer (*oriT*), transfer (*tra*) gene, rolling-circle replication and efficiency of conjugation.
2. Distinguish donor, recipient and transconjugant.
3. Explain why bacterial conjugation is a horizontal gene transfer.
4. Draw a picture indicating the mechanism of bacterial conjugation.
5. Explain the characteristics of F plasmid.
6. Distinguish the different mechanisms of mating pairs between Gram-negative and Gram-positive.
7. Calculate the efficiency of conjugation.
8. Indicate the function of transfer gene and origin of transfer during the conjugation process.
9. Appraise how important conjugation process is in bacteria.
10. Show the functional relationship among gene, RNA and protein related to the genes in the conjugal transfer process.
11. Design a simple experiment(s) to show how to know the plasmid is transferred from a donor to a recipient.

Reference books:

Baltzing, B. L. (2002). *Microbiology: An introduction*. Stamford: Brooks/Cole.

Clegg, C. J., & Mackean, D. G. (2000). *Advanced biology: Principles and applications* (2nd ed.). London: John Murray.

Dale, J. W., & Park, S. F. (2004). *Molecular genetics of bacteria*. Chichester: John Wiley & Sons.

Maloy, S. R., Cronan, Jr., J. E., & Freifelder, D. (1994). *Molecular genetics* (2nd ed.). Boston: John and Bartlett.

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Student previous knowledge:

Students should understand the basic concepts of bacterial cells, reproduction in bacteria, gene, DNA replication, transcription and translation.

Instructional plan for the treatment group:

Total learning hours: 3 hours

Content/breakdown	**Teacher activity**	**Student activity**	**Day/Time**
1. Introduction	Explain students the topic and objectives of the lesson.	Prepare to learn a new concept: bacterial conjugation.	**First day** 5 min
2. Pre-test	Give the MCQ test to students to use their prior knowledge to answer the questions	Read the MCQ questions, think about the answers and choose the best answers.	15 min
3. Definition and history of bacterial conjugation	Explain the definition of conjugation and tell its history and scientific evidence.	Students learn from the lecture and think how to design a good experiment of conjugation in *E. coli*	25 min
4. Conjugation experiment	Give the lab note to students and explain how to do the experiment.	Students draw the flow chart of experiment, indicate the control of experiment and do experiment that described in the handout. Ask questions during experiment.	45 min
5. Mechanism of conjugation	Give students a drawing-based exercise. After finished, explain about the mechanism of conjugation.	Do the exercise and draw the mechanism of conjugation using its information. Compare the knowledge between the exercise and lecture.	**Second day** 45 min
6. Conclusion	Give students the big picture of conjugation, conclude the experiment and answer the questions that students ask.	Ask the questions about the bacterial conjugation and write the overall concept map about conjugation.	45 min
7. Post-test 8. Conceptual test	After learning conjugation at least a few week, assess students by using the post-test and conceptual test for 30-45 min.		
9. Concept interview	Interview a random sampling group of students to probe the understanding in bacterial conjugation for 10 min each.		

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Assessment tools:

1. MCQ pre- and post-tests
2. Conceptual test
3. Students' lab report
4. A set of interview questions

Teaching materials:

1. PowerPoint instructional presentation with an animation of bacterial conjugation
2. A teacher lecture note
3. A lab handout: Conjugation in *E. coli*
4. A drawing-based exercise of the mechanism of conjugation

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APPENDIX H
Scoring Rubrics

H.1 Holistic rubric score for open-ended questions

The responses for the open-ended questions were categorised in four ways as described below: understanding, partially understanding, no understanding and no response (adapted from Acar & Tarhan, 2007; Arter & NcTighe, 2000).

Score	Description
Understanding (3 points)	The response reflects learning objectives clearly and in detail. The student shows a depth of understanding of the idea to the topic and understands important relationships.
Partially understanding (2 points)	The response is satisfactory, contains some details, is vague or not well developed, and includes some misconceptions or some inaccurate information. The response shows an apparent gap in the student's knowledge and understanding of the topic.
No understanding (1 point)	The response is poor, lacks clarity, and contains misconceptions, inaccurate or irrelevant information.
No responses (0 points)	Answer area left blank.

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H.2 Holistic rubric score for concept maps

The concept map in bacterial conjugation were categorised in five ways as described below (adapted from Bybee, 2002; Moni et al., 2004):

Score	Content	Logic & Understanding	Presentation
5	All relevant concepts of conjugation are correct with multiple connections.	Understanding of facts and concepts of conjugation is clearly demonstrated by correct links.	Concept map is *neat*, *clear*, and *legible*, has *easy-to-follow* links and has no spelling errors.
4	Most relevant concepts of conjugation are correct with multiple connections.	Understanding of facts and concepts of conjugation is demonstrated by a few error links.	Concept map is *neat*, *clear*, and *legible*, has *easy-to-follow* links and has some *spelling errors*.
3	Few relevant concepts of conjugation are correct with two or more connections.	Understanding of facts and concepts of conjugation is demonstrated but some incorrect links.	Concept map is *neat*, *legible* but with some links difficult to follow and has some *spelling errors*.
2	Few relevant concepts of conjugation are correct with no connections.	Understanding of facts and concepts of conjugation with some significant errors.	Concept map is untidy with links difficult to follow and has some *spelling errors*.
1	No submission		

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H.3 Lab report rubric score

The lab report of conjugation in *E. coli* was categorised in five ways as described below (adopted from Schlenker & Tierney, 2006):

	4 Points	3 Points	2 Points	1 Piont	0 Points
Title	Title focuses on the investigation(s) conducted and contains no vague terms	Title focuses on the investigation(s) conducted but contains some vague terms.	Title focuses on the investigation(s) conducted but is vague or non-specific terms.	Contains a title.	Title is missing.
Introduction	Includes all of the following: activity background, predictions about the activity outcome, research question to be answered.	Includes 2 of the following 3 items: activity background, predictions about the activity outcome, research question to be answered.	Includes 1 of the following 3 items: activity background, predictions about the activity outcome, research question to be answered.	Contains an Introduction sections.	Introduction is missing.
Methods and Materials	All methods and materials used are included, methods of data collection and analysis are included, quantity of materials used is included.	Some but not all methods and materials used are included, methods of data collection and analysis are included, and quantity of materials used is included.	Some but not all methods and materials used are included, methods of data collection and analysis are missing, and quantity of materials used is included.	Contains a Method and Materials section.	Methods and Materials section is missing.
Results	Includes all of the following: all results, description of results is clear, results are not discussed, only described.	Includes all of the following: some but not all results, description of results is clear, results are not discussed.	Includes all of the following: some results are included, description of results is not clear, results are not discussed.	Contains a Results section.	Results section is missing.
Discussion	Results are explained based upon predictions and research questions, there is a clear connection between this section and the Introduction section.	Results are explained but connections to either predictions or research questions are not clear, there is a clear connection between this section and the Introduction section.	Results are explained but connections to predictions and research questions are missing or there is no clear connection between this section and the Introduction section.	Contains a Discussion section.	Discussion section is missing.

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H.4 Holistic rubric score of practical activities in the Protein Purification Program

The practical activities in the Protein Purification Program were scored by concerning the key criteria for purifying any proteins to be sufficient amount for doing the later work (developed from Arter & NcTighe, 2000; Wiggins, 1998).

Score	Description
1	- Use too many steps to purify proteins - Choose the specific techniques in the initial steps that make the high cost in the purification scheme - Low product yield that is insufficient to use in the later work, even high purity - High contaminants - Choose inappropriate techniques in each purification step.
2	- Use a few steps to purify proteins - High product yield that is enough to use in the later work - Low contaminants - Choose inefficient techniques in the purification scheme - High cost to purify each protein
3	- Use the appropriate step to purify proteins - Appropriate yield that is sufficient for later work - Acceptable high purity - Low cost in overall purification scheme - Choose the efficient techniques in each steps of purification scheme

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APPENDIX I

Satisfactory Evaluation towards Learning in Bacterial Conjugation by Using Practical Work

I.1 Students' evaluation towards learning in bacterial conjugation

Please fill in this form to evaluate your attitude to learn in bacterial conjugation and place a tick in the circle of the following sections.

General information

1.1 Sex ○ Male ○ Female

1.2 Age …….years

Prior knowledge: please evaluate what you think your level of knowledge of bacterial conjugation was.

Prior knowledge before learning	Very low	low	medium	high	Very high
2.1 Theory					
2.1.1 Basic knowledge of cells (involving the bacterial cells)	○	○	○	○	○
2.1.2 Bacterial reproduction	○	○	○	○	○
2.1.3 DNA, gene and DNA replication	○	○	○	○	○
2.1.4 Protein synthesis	○	○	○	○	○
2.2 Practical skills					
2.2.1 Chemical calculation and data analysis	○	○	○	○	○
2.2.2 Sterilised techniques	○	○	○	○	○
2.2.3 Design and outline the experimental plan	○	○	○	○	○
2.2.4 General laboratory techniques and safety	○	○	○	○	○

Questionnaire given after students have learned the concept of bacterial conjugation

Preferences

Indicate your level of satisfaction after using learning the concept of bacterial conjugation using this labwork.	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
3.1 You can link your prior knowledge to the concept of conjugation.	○	○	○	○	○
3.2 You are able to define any technical terms in conjugation.	○	○	○	○	○

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Indicate your level of satisfaction after using learning the concept of bacterial conjugation using this labwork.	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
3.3 You can describe the importance of conjugation in bacteria.	O	O	O	O	O
3.4 You can explain the mechanism of bacterial conjugation.	O	O	O	O	O
3.5 You can design a simple experiment to investigate the conjugation in bacteria.	O	O	O	O	O
3.6 You have an additional techniques, e.g. sterilised techniques, serial dilution, ability to use some scientific instruments.	O	O	O	O	O
3.7 The lecture and labwork is easy to understand at the senior secondary school level.	O	O	O	O	O
3.8 Activities and the concept map can help you to understand overall concepts and details better.	O	O	O	O	O
3.9 This laboratory exercise can help you to have a better understand in the concept.	O	O	O	O	O

3.10 Other comments and suggestions

Overall satisfaction

	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
What was your overall level of satisfaction in learning the concept of bacterial conjugation in both theory and labwork?	O	O	O	O	O

Thank you for this information

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I.2 Teachers' evaluation towards learning in bacterial conjugation

Please fill in this form to evaluate your attitude to learn in bacterial conjugation and place a tick in the circle of the following sections.

General information

1.1 Sex ○ Male ○ Female

1.2 Age …….years

Surveys of students' prior knowledge

Indicate students' level of knowledge before learning with this concept.	Very low	low	medium	high	Very high
2.1 Theory	○	○	○	○	○
2.1.1 Basic knowledge of cells (involving the bacterial cells)	○	○	○	○	○
2.1.2 Bacterial reproduction	○	○	○	○	○
2.1.3 DNA, gene and DNA replication	○	○	○	○	○
2.1.4 Protein synthesis	○	○	○	○	○
2.2 Practical works	○	○	○	○	○
2.2.1 Chemical calculation and data analysis	○	○	○	○	○
2.2.2 Sterilised techniques	○	○	○	○	○
2.2.3 Design and outline the experimental plan	○	○	○	○	○
2.2.4 General laboratory techniques and safety	○	○	○	○	○

Preferences

Indicate your level of satisfaction after students have learned in this concept.	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
3.1 Lecture and labwork followed the behavioral objectives.	○	○	○	○	○
3.2 Activities and the concept map can help students to understand overall concepts and details better.	○	○	○	○	○
3.3 Assessment tools can assess and evaluate students with respect to the objectives.	○	○	○	○	○
3.4 From students' experience in lab work, students are able to design any simple experiments to investigate the conjugation in bacteria.	○	○	○	○	○

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Indicate your level of satisfaction after students have learnt in this concept.	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
3.5 The teaching sequences, lab before lecture can stimulate students to have a better understand in the bacterial conjugation.	O	O	O	O	O
3.6 Bacterial conjugation is related to any other concepts in the senior secondary curriculum.	O	O	O	O	O

3.7 Other comments and suggestions

Overall satisfaction

	strongly dissatisfied	dissatisfied	neutral	satisfied	Strongly Satisfied
Do you think the concept of bacterial conjugation should be filled in the senior secondary curriculum?	O	O	O	O	O

Thank you for this information

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APPENDIX J

Satisfactory Evaluation towards Protein Purification by Using Simulation Program in Thai and English Versions

J.1 Students' evaluation of Protein Purification Programs

Questionnaire given before students have used the programs

General information

1.1 University …… Major ……..

1.2 Sex O Male O Female

1.3 Age …….years

1.4 Studying degree at O Bachelor O Master O Doctoral level

Prior knowledge: please evaluate what you think your level of knowledge of protein purification was.

Prior knowledge before learning	Very low	low	medium	high	Very high
2.1 Theory					
2.1.1 Structure and function of proteins	O	O	O	O	O
2.1.2 Chemical purification	O	O	O	O	O
2.1.3 Techniques in protein purification	O	O	O	O	O
2.1.4 Drawing and analysis of graphs and charts	O	O	O	O	O
2.2 Practical skills					
2.2.1 Chemical calculation and data analysis	O	O	O	O	O
2.2.2 Chemical purification	O	O	O	O	O
2.2.3 Techniques in protein purification	O	O	O	O	O
2.2.4 General laboratory techniques and safety	O	O	O	O	O

Questionnaire given after students have used the programs

Preferences

Indicate your level of satisfaction after using this program.	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
3.1 Instruction and practices are clear enough to be followed.	O	O	O	O	O

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Indicate your level of satisfaction after using this program.	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
3.2 Languages are clear enough to understand and follow in the experiments.	O	O	O	O	O
3.3 This program helps you to understand the concepts of protein purification.	O	O	O	O	O
3.4 The simulation of the experiments in this program including contexts, tables and charts helps you to understand protein purification better.	O	O	O	O	O
3.5 After use of the program, students are able to choose suitable techniques of protein purification.	O	O	O	O	O
3.6 This program helps students in systematic thinking and prepares them to do the experiments in real situations.	O	O	O	O	O
3.7 The two-language program helps students to be accustomed to manuals, procedures and other documents in English.	O	O	O	O	O

3.8 Other comments and suggestions (including any obstacles while using this program)

Overall satisfaction

	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
What was your overall level of satisfaction in using this program?	O	O	O	O	O

Thank you for this information

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J.2 Teachers' evaluation of Protein Purification Programs

Please fill in this form for developing the appropriate structure and context of the program and place a tick in the circle of the following sections.

General information

1.1 University ……. Teaching in major …….

1.2 Sex O Male O Female

1.3 Age …….years

1.4 Studying degree at O Bachelor O Master O Doctoral level

Preferences towards the program

Indicate your level of satisfaction from the students' practices.	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
2.1 This program is a learning tool to assist students in understanding the concepts of protein purification.	O	O	O	O	O
2.2 Students are able to design their purification plans by choosing the suitable steps and techniques.	O	O	O	O	O
2.3 Students can apply the knowledge and thinking skills in protein purification for their future researches (if they will get the manuals in each technique)	O	O	O	O	O
2.4 Students can read and interpret the results in various sources better, e.g. graph, chart.	O	O	O	O	O
2.5 Texts, information and vocabularies used in this program are suitable for understanding the protein purification.	O	O	O	O	O
2.6 This program can be applied to the knowledge in biological curricula to teach or supplement in the concepts of protein purification.	O	O	O	O	O

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	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
Indicate your level of satisfaction from the students' practices.					
2.7 The two-language program helps students to be accustomed to manuals, procedures and other documents in English.	○	○	○	○	○

2.8 Other comments and suggestions (including any obstacles while using this program)

Overall satisfaction

	strongly dissatisfied	dissatisfied	neutral	satisfied	strongly satisfied
What was your overall level of satisfaction in using this program?	○	○	○	○	○

Thank you for this information

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BIOGRAPHY

NAME	Mr. Somkiat Phornphisutthimas
DATE OF BIRTH	5 July 1972
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Srinakharinwirot University, Bangkhen Campus, 1994: Bachelor of Science (First Class Honours in Biology) Chulalongkorn University, 1997: Master of Science (Biotechnology)
RESEARCH EXPEIENCE	Visiting researcher at the Institute of Integrative and Comparative Biology, Faculty of Biological Science, University of Leeds, UK
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