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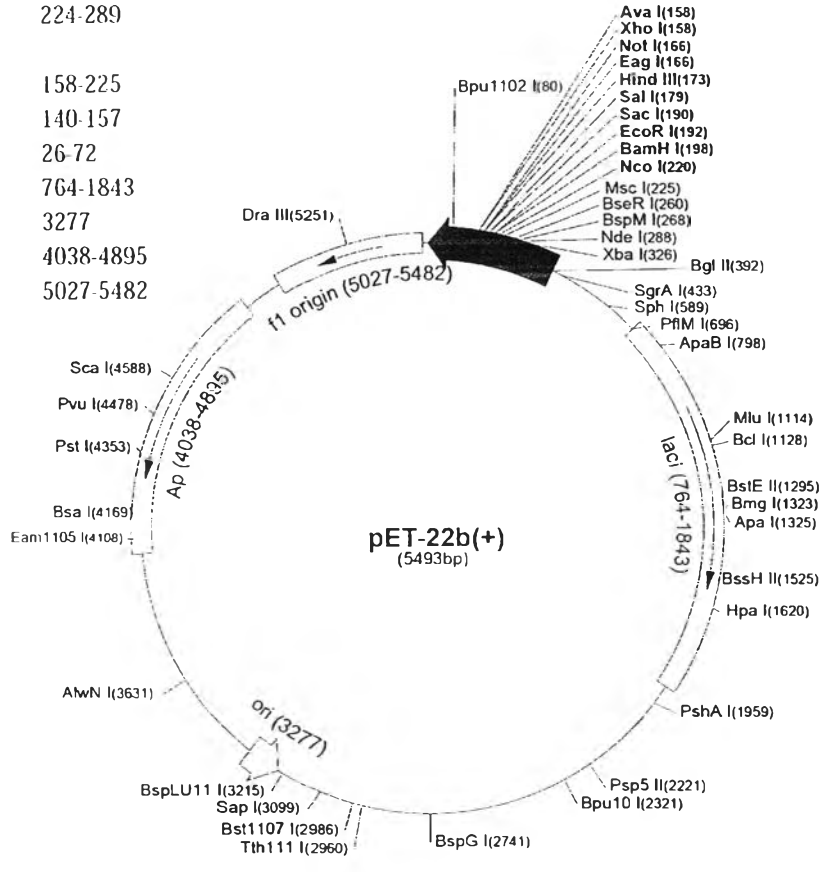
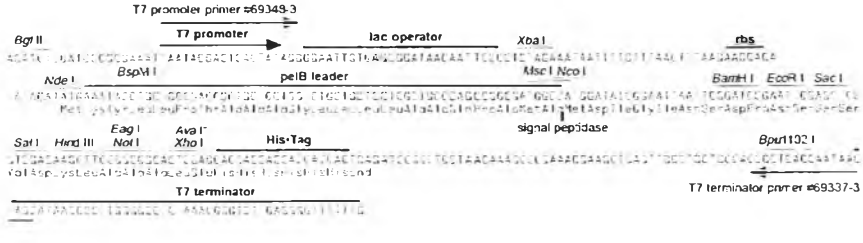
APPENDICES

APPENDIX A

Restriction map of pET-22b(+)

pET-22b(+) sequence landmarks	
T7 promoter	361-377
T7 transcription start	360
pelB coding sequence	224-289
Multiple cloning sites (*Nco* I - *Xho* I)	158-225
His• Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	764-1843
pBR322 origin	3277
bla coding sequence	4038-4895
f1 origin	5027-5482



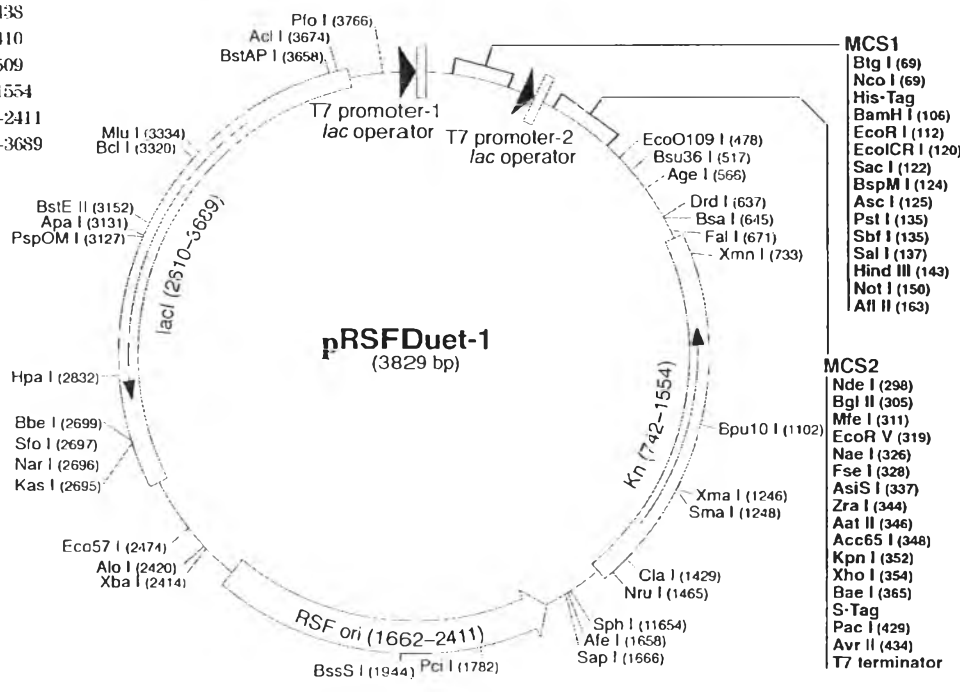


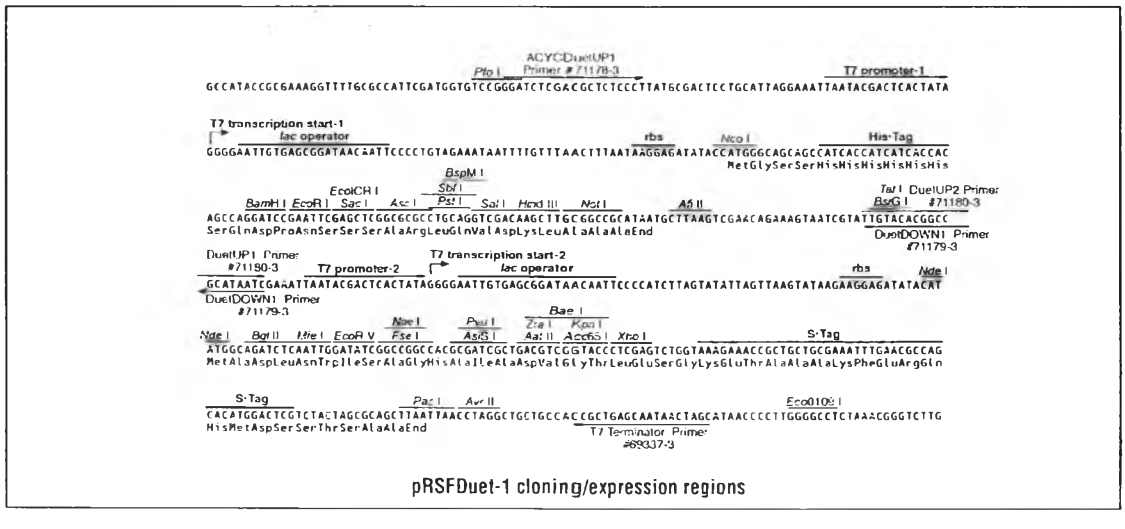
pET-22b(+) cloning/expression region

APPENDIX B

Restriction map of pRSFDuet-1

pRSFDuet-1 DNA	71341-3
pRSFDuet-1 sequence landmarks	
T7 promoter-1	3582–3598
T7 transcription start-1	1
His•Tag® coding sequence	83–100
Multiple cloning sites-1 (*Nco* I–*Afl* II)	69–168
T7 promoter-2	214–230
T7 transcription start-2	231
Multiple cloning sites-2 (*Nde* I–*Avr* II)	297–438
S•Tag™ coding sequence	366–410
T7 terminator	462–509
kan (Kn^R) coding sequence	742–1554
RSF origin	1662–2411
lacI coding sequence	2610–3689





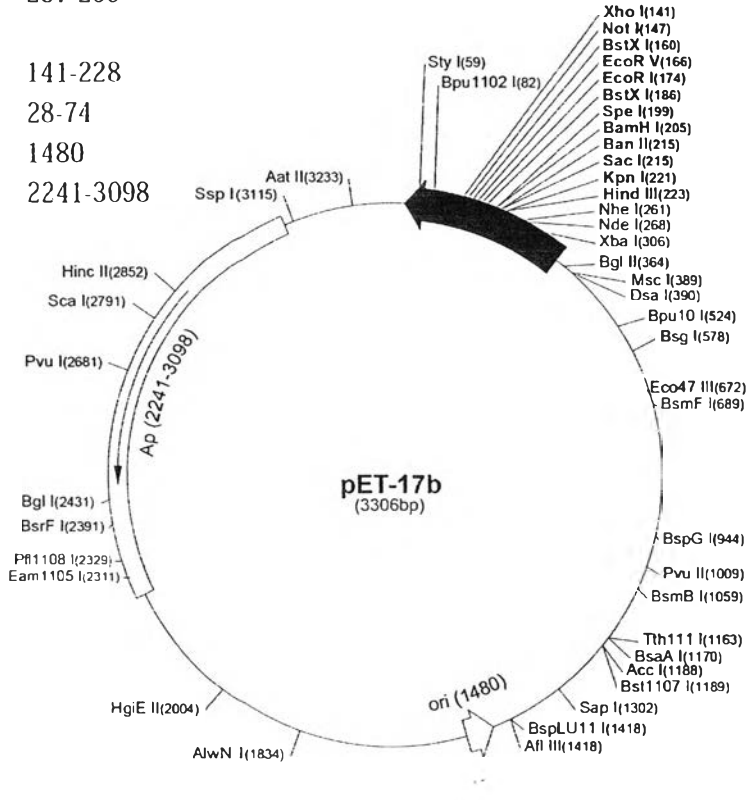
pRSFDuet-1 cloning/expression regions

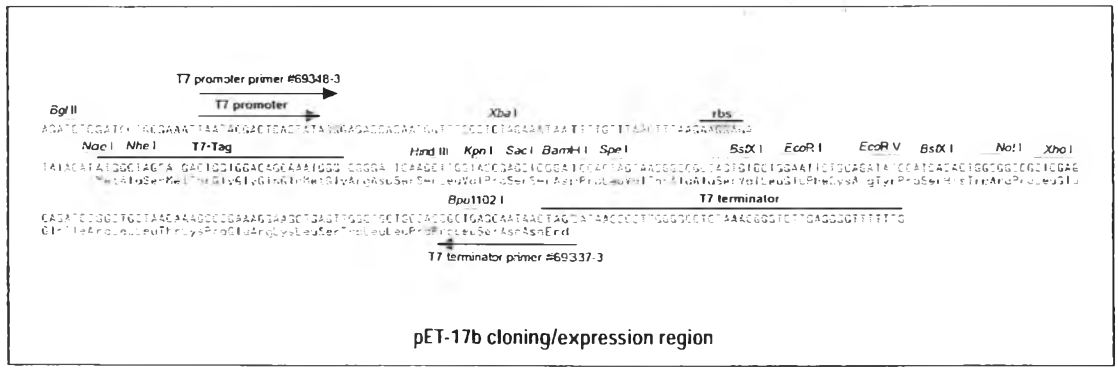
APPENDIX C

Restriction map of pET-17b

pET-17b sequence landmarks

T7 promoter	333-349
T7 transcription start	332
T7• Tag coding sequence	237-269
Multiple cloning sites	
(*Hin*d III - *Xho* I)	141-228
T7 terminator	28-74
pBR322 origin	1480
bla coding sequence	2241-3098





pET-17b cloning/expression region

APPENDIX D

Preparation of reagents for rapid selection of recombinant clone by agarose gel electophoresis

1. Stock solutions

1 M Tris-HCl (pH 8.0)

Tris (hydroxymethyl)-aminomethane 12.1 g

Dissolved with distilled water, adjusted pH to 8.0 with 1 N HCl and made up to 100 mL with distilled water

0.5 M EDTA (pH 8.0)

Ethylene diamine tetra-acetic acid 18.6 g

Dissolved, adjusted pH to 6.8 with 10 N HCl and filled up with distilled water to get a final volume of 100 mL

5 M NaCl

Sodium chloride 29.2 g

Dissolved in distilled water to 100 mL

0.25 M Boric acid

Boric acid 1.6 g

Dissolved in distilled water to 100 mL

25% (w/v) SDS

Sodium dodecyl sulfate (SDS) 25 g

Dissolved in distilled water by warming in a water bath set to 68 ^{o}C and adjusted a final volume to 100 mL

25% (w/v) Sucrose

Sucrose 25 g

Dissolved in distilled water to a final volume of 100 mL

20% (w/v) Bromophenol blue

Bromophenol blue 2 g

Dissolved in 10 mL of distilled water and stirred until dissolved

These solutions were autoclaved at 121 °C for 15 min, except 25% sucrose was done at 110 °C for 15 min.

2. Working solutions

Resuspension buffer (30 mM Tris-HCl, 5 mM EDTA, pH 8.0, and 50 mM NaCl containing 2.5 mg/mL of lysozyme and 1 mg/mL of RNase A)

1 M Tris-HCl (pH 8.0)	30	µL
0.5 M EDTA (pH 8.0)	10	µL
5 M NaCl	10	µL
25% (w/v) Sucrose	800	µL
Sterile water	150	µL

Each of these components was put into a sterile 1.5 mL microcentrifuge tube and finally 50 µL of 20 mg/mL of RNase A and 50 µL of 50 mg/mL of lysozyme were added. The resuspension buffer should be kept at -20 °C until used.

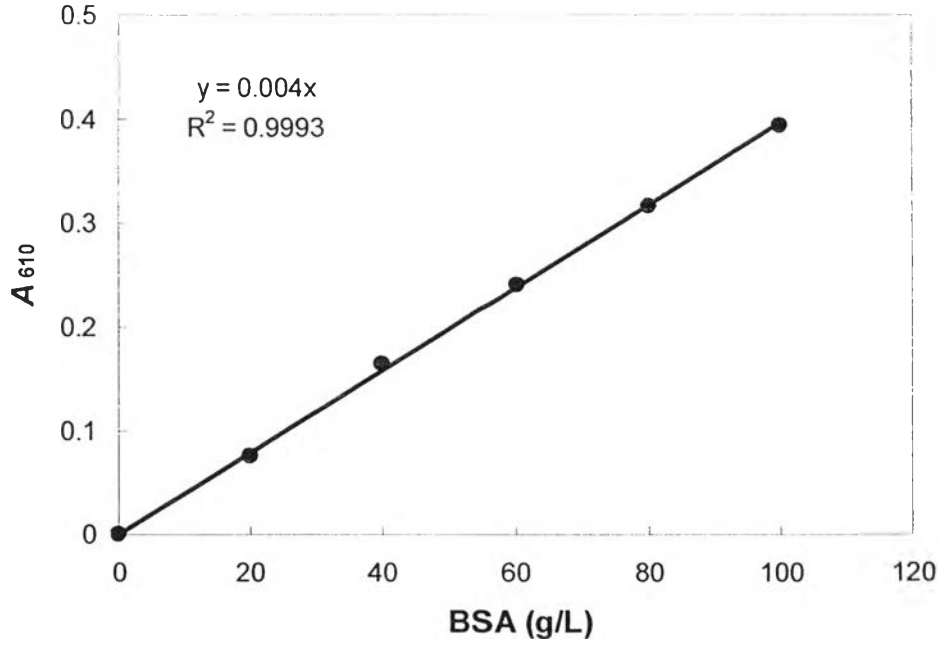
Lysis buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.0, 2% SDS, 5% sucrose and 0.04% bromophenol blue)

1 M Tris-HCl (pH 8.0)	89	µL
0.25 M Boric acid	356	µL
0.5 M EDTA (pH 8.0)	5	µL
25% (w/v) SDS	80	µL
25% (w/v) Sucrose	200	µL
20% (w/v) Bromophenol blue	2	µL
Sterile water	268	µL

Each of these components was put into a sterile 1.5 mL microcentrifuge tube and should be stored at -20 °C until used.

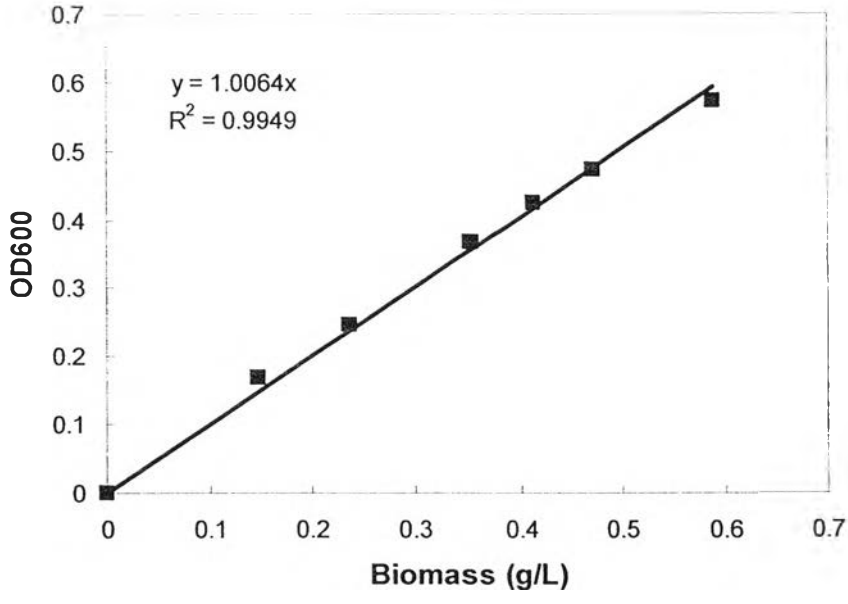
APPENDIX E

Standard curve for protein determination by Lowry's method



APPENDIX F

Calibration curve for determination of dry biomass concentration



APPENDIX G

Preparation for glycerol determination (Bondioli and Bella, 2005)

1. Acetic acid stock solution (1.6 M acetic acid)

Acetic acid 96 g

Added distilled water to a final volume of 1 L. Solution is stable over time.

2. Ammonium acetate stock solution (4.0 M ammonium acetate)

Ammonium acetate 308 g

Dissolved in distilled water to 1 L. The solution is stable over time. As mixed in equal volumes of acetic acid stock solution and ammonium acetate stock solution, the resulting solution is a buffer solution at pH 5.5.

3. Acetylacetone solution (0.2 M acetylacetone)

Acetylacetone 2 mL

Dissolved in 50 mL of acetic acid stock solution and 50 mL of ammonium acetate stock solution. This reagent must be prepared daily.

4. Sodium periodate solution (10 mM sodium periodate)

Sodium meta periodate 210 mg

Completely dissolved in 50 mL of acetic acid stock solution first and then dissolved in 50 mL of ammonium acetate stock solution. This reagent must be prepared daily.

5. Working solvent (1:1 (v/v) 95% ethanol: distilled water)

95% ethanol 500 mL

Added distilled water to a final volume of 1 L

6. Glycerol reference stock solution (3 mg/mL of glycerol)

Glycerol 150 mg

Weighed glycerol into a 50 mL volumetric flask and dissolved with the working solvent and filled up to the mark. The solution is stable for some weeks.

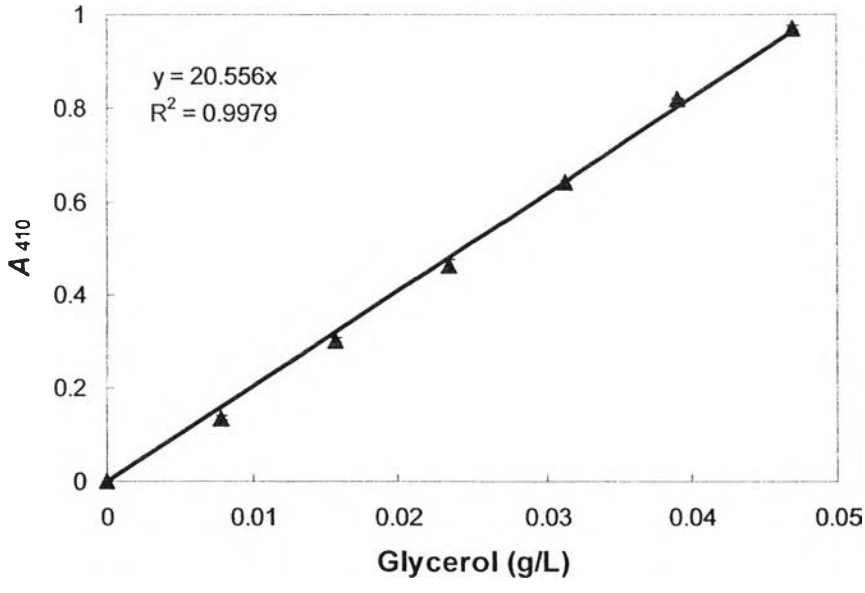
7. Glycerol reference working solution (0.03 mg/mL of glycerol)

Glycerol reference stock solution 1 mL

Transferred into a 100 mL volumetric flask and filled up to the mark using the working solvent. The solution is stable for some weeks.

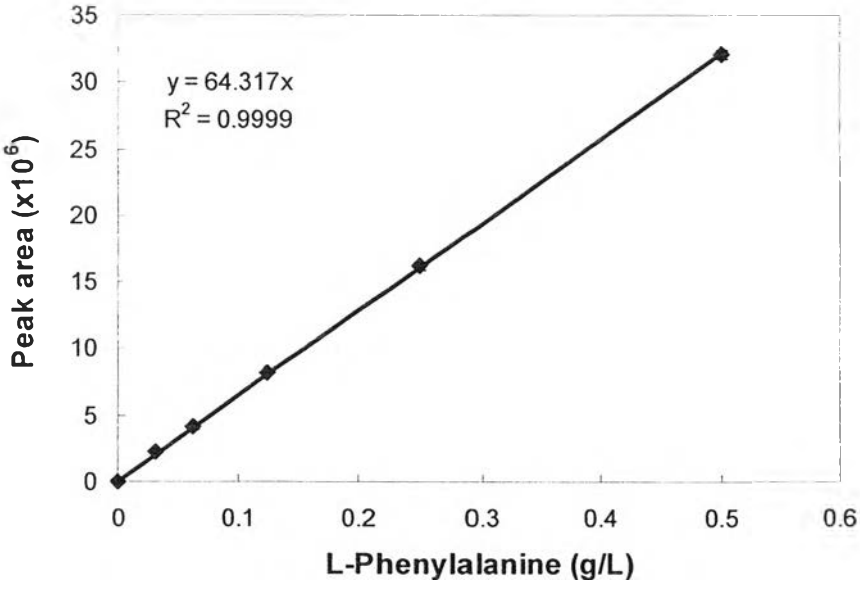
APPENDIX H

Calibration curve for glycerol determination



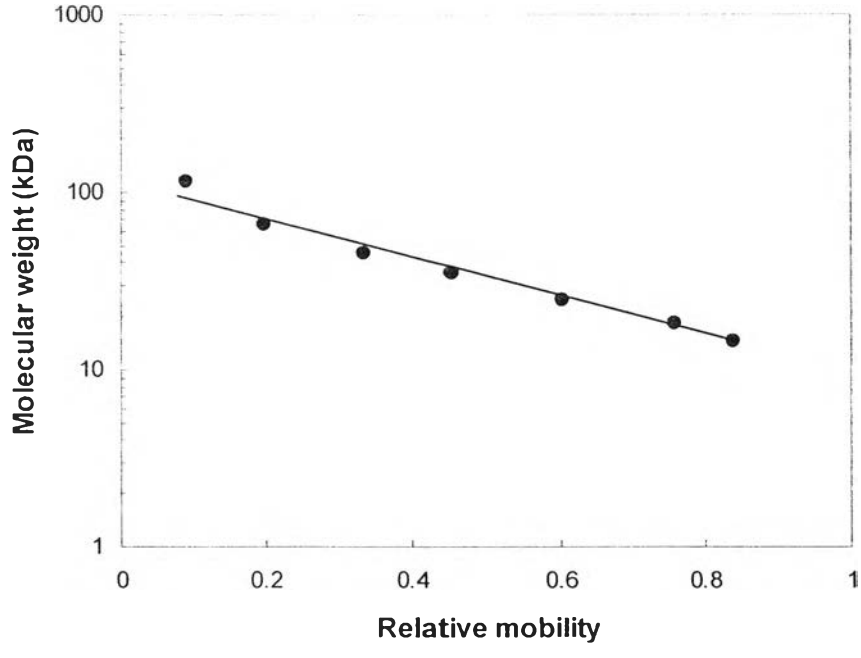
APPENDIX I

Calibration curve for determination of L-Phe concentration



APPENDIX J

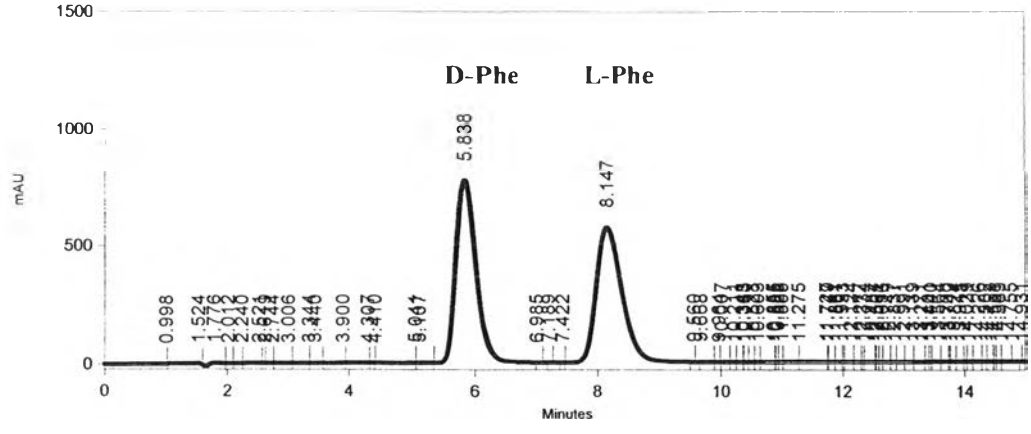
Calibration curve for molecular weight estimation by SDS-PAGE



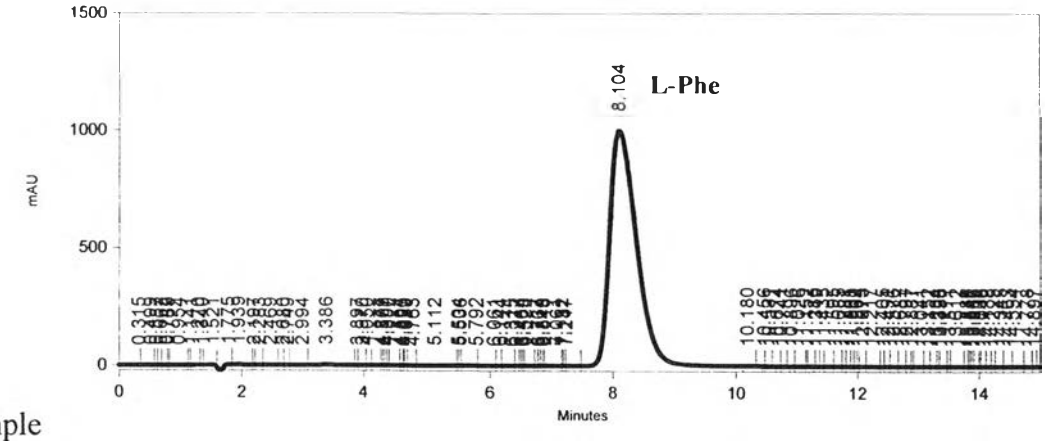
APPENDIX K

Chromatograms of separation of L-phenylalanine by HPLC

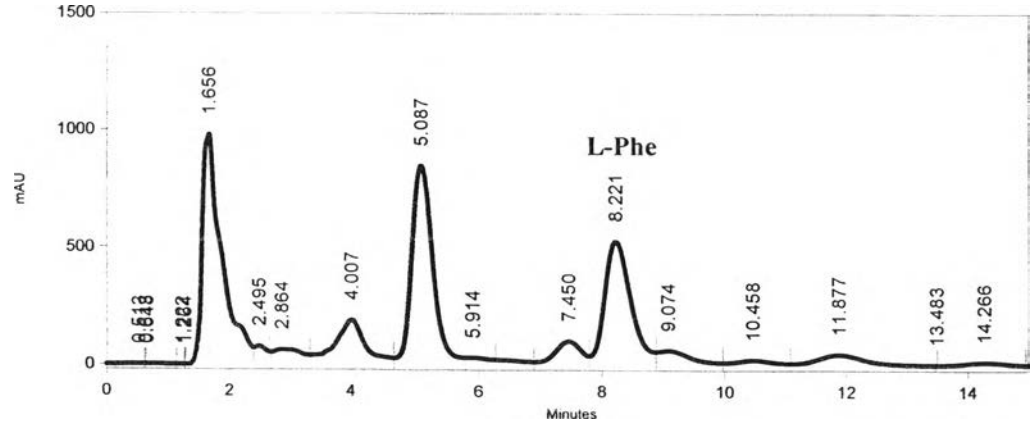
A) DL-Phenylalanine



B) L-Phenylalanine

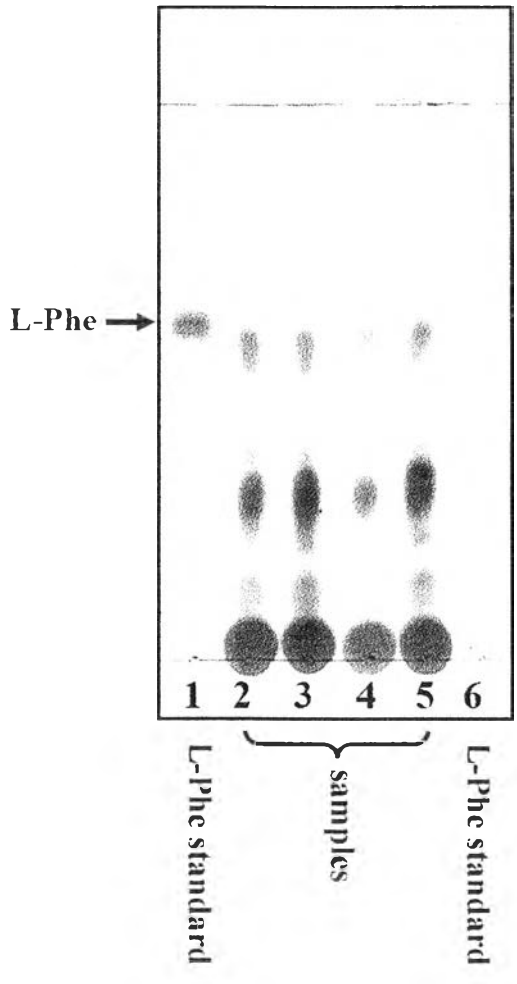


C) Sample



APPENDIX L

Chromatogram of separation of L-phenylalanine by TLC



APPENDIX M

Personal information

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Telephone Number:	0 3555 1714
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Marital Status:	Single

Degrees:

2003	B.Sc. (Second Class Hons., Biochemistry), Chulalongkorn University, Thailand
2006	M.Sc. (Biotechnology), Chulalongkorn University, Thailand
2007-present	Ph.D. student, Program in Biotechnology, Chulalongkorn University, Thailand

Previous Researches:

Thongchuang, M. 2003. Optimization of *E. coli* JM 109 harboring alanine dehydrogenase gene from *Aeromonas hydrophila*. Senior Project. Department of Biochemistry, Faculty of Science, Chulalongkorn University.

Thongchuang, M. 2006. Cloning and expression of phenylalanine dehydrogenase gene from *Bacillus lentus*. Master's Thesis. Program in Biotechnology, Faculty of Science, Chulalongkorn University.

Activities:

2005-2006 Teaching assistant, Department of Biochemistry, Faculty of Science, Chulalongkorn University

2006 Participant and poster presentation, The 5th JSPS-NRCT Joint Seminar on Development of Thermotolerant Microbial Resources and Their Applications, Pattaya, Thailand

2007 Participant and poster presentation, The 1st Biochemistry and Molecular Biology Conference, Chulalongkorn University, Bangkok, Thailand

2010 Participant and poster presentation, The 22nd Annual Meeting of the Thai Society for Biotechnology, "International Conference on Biotechnology for Healthy Living", Prince of Songkla University, Trang Campus, Thailand

Participant and poster presentation, The 1st Joint Symposium CU-NUT Chulalongkorn University, Bangkok, Thailand

Poster Presentations:

Inkure, S., Thongchuang, M., and Packdibamrung, K. Purification and characterization of phenylalanine dehydrogenase from *Bacillus lentus*. The 5th JSPS-NRCT Joint Seminar on Development of Thermotolerant Microbial Resources and Their Applications, Pattaya, Thailand

Thongchuang, M., and Packdibamrung, K. Cloning and expression of phenylalanine dehydrogenase gene from *Bacillus lentus*. The 1st Biochemistry and Molecular Biology Conference, Chulalongkorn University, Bangkok, Thailand

Thongchuang, M., Packdibamrung, K., and Pongsawasdi, P. Improvement of L-phenylalanine production in *Escherichia coli* containing phenylalanine dehydrogenase gene by aromatic amino acid exporter. The 22nd Annual Meeting of the Thai Society for Biotechnology, "International Conference on Biotechnology for Healthy Living", Prince of Songkla University, Trang Campus, Thailand and The 1st Joint Symposium CU-NUT, Chulalongkorn University, Bangkok, Thailand

Academic Award:

2007 The Outstanding Poster Award (Excellent Poster Presentation), Cloning and expression of phenylalanine dehydrogenase gene from *Bacillus lentus*. The 1st Biochemistry and Molecular Biology Conference, Chulalongkorn University, Bangkok, Thailand

Academic and Research Grants:

2005-2006 Teaching Assistant Fellowship

2005 Graduate School Thesis Grant

2008 Chulalongkorn University Graduate Scholarship to Commemorate the 72nd Anniversary of His Majesty King Bhumibol Adulyadej

2009-present "Strategic Scholarships for Frontier Research Network for the Joint Ph.D. Program, Thai Doctoral Degree" from the Office of the Higher Education Commission, Thailand

BIOGRAPHY

Miss Mayura Thongchuang was born on June 19, 1982 in Suphan Buri. She graduated with the degree of Bachelor of Science from the Department of Biochemistry, Faculty of Science, Chulalongkorn University in 2003. After that, she graduated with the degree of Master of Science from Program in Biotechnology, Faculty of Science, Chulalongkorn University in 2006. She has studied for the degree of Doctor of Philosophy of Science at Program in Biotechnology, Faculty of Science, Chulalongkorn University since 2007.

Her work entitled design of a recombinant *Escherichia coli* for producing L-phenylalanine from glycerol is now under revision after submitting to World Journal of Microbiology and Biotechnology since January 23, 2012.