

REFERENCES

- Adachi, O., Ano, Y., Toyama, H. and Matsushita, K. (2006a). Enzymatic preparation of metabolic intermediates, 3-dehydroquinate and 3-dehydroshikimate, in the shikimate pathway. Bioscience, Biotechnology, and Biochemistry **70**: 3081-3083.
- Adachi, O., Ano, Y., Toyama, H. and Matsushita, K. (2006b). High shikimate production from quinate with two enzymatic systems of acetic acid bacteria. Bioscience, Biotechnology, and Biochemistry **70**: 2579-2582.
- Adachi, O., Tanasupawat, S., Yoshihara, N., Toyama, H. and Matsushita, K. (2003a). 3-dehydroquinate production by oxidative fermentation and further conversion of 3-dehydroquinate to the intermediates in the shikimate pathway. Bioscience, Biotechnology, and Biochemistry **67**: 2124-2131.
- Adachi, O., Moonmangmee, D., Toyama, H., Yamada, M., Shinagawa, E. and Matsushita, K. (2003b). New developments in oxidative fermentation. Applied Microbiology and Biotechnology **60**: 643-653.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (2002). Short Protocols in Molecular Biology : A Compendium of Methods from Current Protocols in Molecular Biology. J. Wiley, [Hoboken, N.J.].
- Barrosoa, J. B., Peragóna, J., García-Salguerob, L., Higuerac, M. and Lupiáñez, J. A. (1999). Variations in the kinetic behaviour of the NADPH-production systems in different tissues of the trout when fed on an amino-acid-based diet at different frequencies. The International Journal of Biochemistry & Cell Biology **31**: 277-290.

- Campbell, S. A., Richards, T. A., Mui, E. J., Samuel, B. U., Coggins, J. R., McLeod, R. and Roberts, C. W. (2004). A complete shikimate pathway in *Toxoplasma gondii*: an ancient eukaryotic innovation. International Journal for Parasitology **34**: 5-13.
- Chandran, S. S., Yi, J., Draths, K. M., von Daeniken, R., Weber, W. and Frost, J. W. (2003). Phosphoenolpyruvate availability and the biosynthesis of shikimic acid. Biotechnology Progress **19**: 808-814.
- Chang, Y. C., Almy, E. A., Blamer, G. A., Gray, J. I., Frost, J. W. and Strasburg, G. M. (2003). Antioxidant activity of 3-dehydroshikimic acid in liposomes, emulsions, and bulk oil. Journal of Agricultural and Food Chemistry **51**: 2753-2757.
- Deppenmeier, U., Hoffmeister, M. and Prust, C. (2002). Biochemistry and biotechnological applications of *Gluconobacter* strains. Applied Microbiology and Biotechnology **60**: 233-242.
- Draths, K. M., Knop, D. R. and Frost, J. W. (1999). Shikimic acid and quinic acid: Replacing isolation from plant sources with recombinant microbial biocatalysis. Journal of the American Chemical Society **121**.
- Evans, L. D., Roszak, A. W., Noble, L. J., Robinson, D. A., Chalk, P. A., Matthews, J. L., Coggins, J. R., Price, N. C. and Lapthorn, A. J. (2002). Specificity of substrate recognition by type II dehydroquinases as revealed by binding of polyanions. FEBS Letters **530**: 24-30.
- Fonseca, I. O., Magalhaes, M. L., Oliveira, J. S., Silva, R. G., Mendes, M. A., Palma, M. S., Santos, D. S. and Basso, L. A. (2006). Functional shikimate dehydrogenase from *Mycobacterium tuberculosis* H37Rv: purification and characterization. Protein Expression and Purification **46**: 429-437.

- Herrmann, K. M. (1995). The Shikimate Pathway: Early Steps in the Biosynthesis of Aromatic Compounds. The Plant Cell **7**: 907-919.
- Kataoka, M., Yamamoto, K., Kawabata, H., Wada, M., Kita, K., Yanase, H. and Shimizu, S. (1999). Stereoselective reduction of ethyl 4-chloro-3-oxobutanoate by *Escherichia coli* transformant cells coexpressing the aldehyde reductase and glucose dehydrogenase genes. Applied Microbiology and Biotechnology **51**: 486-490.
- Lehninger, A. L., Nelson, D. L. and Cox, M. M. (2000). Lehninger principles of biochemistry. Worth Publishers, New York.
- Li, K. and Frost, J. W. (1999). Microbial synthesis of 3-dehydroshikimic acid: a comparative analysis of D-xylose, L-arabinose, and D-glucose carbon sources. Biotechnology Progress **15**: 876-883.
- Li, K., Mikola, M. R., Draths, K. M., Worden, R. M. and Frost, J. W. (1999). Fed-Batch Fermentor Synthesis of 3-Dehydroshikimic Acid Using Recombinant *Escherichia coli*. Biotechnology and Bioengineering **64**: 61-73.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry **193**: 265-275.
- Michel, G., Roszak, A. W., Sauve, V., Maclean, J., Matte, A., Coggins, J. R., Cygler, M. and Lapthorn, A. J. (2003). Structures of shikimate dehydrogenase AroE and its Paralog YdiB. A common structural framework for different activities. The Journal of Biological Chemistry **278**: 19463-19472.
- Novagen (2003). pET System Manual. Novagen, United States.

- Pflug, S., Richter, S. M. and Urlacher, V. B. (2007). Development of a fed-batch process for the production of the cytochrome P450 monooxygenase CYP102A1 from *Bacillus megaterium* in *E. coli*. Journal of Biotechnology **129**: 481-488.
- Prust, C., Hoffmeister, M., Liesegang, H., Wiezer, A., Fricke, W. F., Ehrenreich, A., Gottschalk, G. and Deppenmeier, U. (2005). Complete genome sequence of the acetic acid bacterium *Gluconobacter oxydans*. Nature Biotechnology **23**: 195-200.
- Schrodel, A., Volz, J. and Marco, A. (2005). Fusion tags and chaperone co-expression modulate both the solubility and the inclusion body features of the recombinant CLIPB14 serine protease. Journal of Biotechnology **120**: 2-10.
- Singh, S., Korolev, S., Koroleva, O., Zarembinski, T., Collart, F., Joachimiak, A. and Christendat, D. (2005). Crystal structure of a novel shikimate dehydrogenase from *Haemophilus influenzae*. The Journal of Biological Chemistry **280**: 17101-17108.
- Strandberg, L. and Enfors, S. O. (1991). Factors influencing inclusion body formation in the production of a fused protein in *Escherichia coli*. Applied and Environmental Microbiology **57**: 1669-1674.
- Studier, F. W. and Moffatt, B. A. (1986). Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. Journal of Molecular Biology **189**: 113-130.
- Thanaraj, T. A. and Pandit, M. W. (1989). An additional ribosome-binding site on mRNA of highly expressed genes and a bifunctional site on the colicin fragment of 16S rRNA from *Escherichia coli*: important determinants of the efficiency of translation-initiation. Nucleic Acids Research **17**: 2973-2985.

- Thomas, J. G. and Baneyx, F. (1996). Protein misfolding and inclusion body formation in recombinant *Escherichia coli* cells overexpressing Heat-shock proteins. The Journal of biological chemistry **271**: 11141-11147.
- Tonouchi, N., Sugiyama, M. and Yokozeki, K. (2003). Construction of a vector plasmid for use in *Gluconobacter oxydans*. Bioscience, Biotechnology, and Biochemistry **67**: 211-213.
- Vangnai, A. S., Toyama, H., De-Eknamkul, W., Yoshihara, N., Adachi, O. and Matsushita, K. (2004). Quinate oxidation in *Gluconobacter oxydans* IFO3244: purification and characterization of quinoprotein quinate dehydrogenase. FEMS Microbiology Letters **241**: 157-162.
- Voet, D. and Voet, J. G. (1995). Biochemistry. J. Wiley & Sons, New York.
- Wen, Y., Feng, M., Yuan, Z. and Zhou, P. (2005). Expression and overproduction of recombinant penicillin G acylase from *Kluyvera citrophila* in *Escherichia coli*. Enzyme and Microbial Technology **37**: 233-237.
- Xu, Z., Jing, K., Liu, Y. and Cen, P. (2007). High-level expression of recombinant glucose dehydrogenase and its application in NADPH regeneration. Journal of Industrial Microbiology & Biotechnology **34**: 83-90.
- Yi, J., Li, K., Draths, K. M. and Frost, J. W. (2002). Modulation of phosphoenolpyruvate synthase expression increases shikimate pathway product yields in *E. coli*. Biotechnology Progress **18**: 1141-1148.
- Zhong, Z., Xu, Z., Peng, L., Huang, L., Fang, X. and Cen, P. (2006). Tandem repeat mhBD2 gene enhance the soluble fusion expression of hBD2 in *Escherichia coli*. Applied Microbiology and Biotechnology **71**: 661-667.

APPENDICES

APPENDIX A : dqd primer position

10 20 30 40 50 60 70 80 90
 GATCGCACCA GATAGCACAA AAATGCGGCC CTGTCCCCTT TTGGACACCC TGAAGACGGC AGCAATAGGG CAATGACGCT TTCATGACGG
¹dqd_F →
 100 110 120 130 140 150 160 170 180
 CTCCGAAAGT GCTATCGCGC GGCCAGATGA AACGCCCTCT GATCACCGTT CTCAACGGTC CGAATCTCAA CATGCTGGGT CTTCGCCAGC
²dqdpET_F →
 190 200 210 220 230 240 250 260 270
 CCGGAATCTA TGGTCACGCC ACGCTCGATG ATGTCGAGCA GGTGTGCATT CAGGCTGCCG AACGGCTTGA TGTCGCCATT GATTTCGTC
 280 290 300 310 320 330 340 350 360
 AGACGAACGG AGAGGGTGAA CTCGTGTCCT GGGTGCAGGA ATGTCGCGGC CGTGCAGACG GTATCGTGAT CAATCCTGCC GCTTACGGGC
 370 380 390 400 410 420 430 440 450
 ATACCTCGAT TGCCCTGCTC GATGCGCTTC TGGCCGTCGA GCTTCCCCTG ATTGAGGTTTC ATATTTCCAA TATCCATCGC AGGGAGCCGT
 460 470 480 490 500 510 520 530 540
 TCCGTCATCA CACCTACGTC TCGCAGGCCG CCATCGGGGT GATCTGCCGC CTCGGCGTCA GGGGATAACGC GCACGCGCTT CAGGCAATAA
 ←
 550 560 570 580 590 600 610 620 630
 CCGACATGAT CGAAGACGAA GGATGAGCCG TATGCTCGTG GACAAGGATG CCATTGCCGC ATTGGCCGAT ATTCTGACGG
²dqdpET_R ← GAGCTC →
¹dqd_R ←

1 = forward and reverse primers used to clone gene from *G. oxydans* 621H

2 = forward and reverse primers used to subclone gene into pET-21a vector; dqdpET_F was tagged with CAT, *Nde*I restriction site, while dqdpET_R was tagged with GAGCTC, *Xho*I restriction site.

Location on complement strand (461603 to 462085), locus tag=GOX0437, EC number=4.2.1.10, product=3-Dehydroquinate dehydratase

Appendix B : *skdh* (GOX0859) primer position

10 20 30 40 50 60 70 80 90
 ACAGGCACAG ATCCGAGGAG CCTCTCATGA GCCAGCAGAA TTTCCGCAGC ATCCTGACCG GATCGTTCTC CACGCCATGC GATGACAACC
¹GOX0859_F → CAT ²GOX0859pET_F
 100 110 120 130 140 150 160 170 180
 CGACCGTCGC CATGATCGAG GCCGCGTACCG GCACACGA CATCGATGCG CGTTACATCA ACTGTGACGT CAAGGGGAC GGTCTGAAGG
 190 200 210 220 230 240 250 260 270
 ACGCGGTCGC GGGTGCACGG GCCATGGAGT GGGTCGGGTT CAACTGCTCC CTGCCGCACA AGGTTGCGGT GATCGAGCAT CTGGACGAAC
 280 290 300 310 320 330 340 350 360
 TGGCGGAGTC CGCCCCGGATT ATCGGGTCGG TGAACTGCGT CTCCATCCGG GACGGGGCGCC TGATGGCGA CAATAACGGAC GGGAAAGGGCT
 370 380 390 400 410 420 430 440 450
 TTCTGGCGTC CCTGAACAAAG GTGGGGGATC CGTCCGGAAA GAAGGTCCTG CTTCTGGGCG CGGGCGGGGC TCGCGGTGCG ATCGCCGTGG
 460 470 480 490 500 510 520 530 540
 AACTGGGGCT CGTTTCCGCC GCCCATATCA TGTCATGAA CCGCGATCCC AAAAAAGCGG AAACCATTCG TGCACTGGTG CGGGACAAACA
 550 560 570 580 590 600 610 620 630
 CCTCCGCCAA AGCCGATGTT CAGGCATGGG ACGGCGAGGC CAGCGTGCG GAAGACGTGG ACATCCTGAT CAACGCCACG TCAATCGGTC
 640 650 660 670 680 690 700 710 720
 TGGGGGATGC GGACGCCATG CCGCCGCTGA AGGTCGAGAC CCTGCGCAAG GGCTTGATCG TCGCCGATGT CATTCCGAAC CCGCCTGCTG
 730 740 750 760 770 780 790 800 810
 CGGGAAAGCAG AAAACAGGGG CTGCACCGTG CTGGACGGGC TCAGGATGCT GGTCAATCAG GGCCTGATCG GCGTGGAGCA CTGGCTGGGC
 820 830 840 850 860 870 880 890 900
 AGGACGTTGG ACGCCGGGGT GATGGAGCAG ACCCTGAAGG ATATTTTCGG CGCGGCCCTGA CATGA AAAAA CCCGGTGTCT CCGAAGAGAG
 910 920 930 940 950 960 970 980
 CACCGGGTTT TTGCAGAGCT GAAAGATCAG CGGCCTTCGA AGAAAGTCGG GACCTTGGCG AAGAAGCCGC T
¹GOX0859_R ← ²GOX0859pET_R GAGCTC
¹GOX0859_R

1 = forward and reverse primers used to clone gene from *G. oxydans* 621H

2 = forward and reverse primers used to subclone gene into pET-21a vector; GOX0859pET_F was tagged with CAT, *Nde*I restriction site, while GOX0859pET_R was tagged with GAGCTC, *Xba*I restriction site.

Location on complement strand (928465 to 929313), locus tag=GOX0859, EC number=1.1.1.25, product=Shikimate 5-dehydrogenase

APPENDIX C : skdh (GOX1959) primer position

Sequence diagram of the GOX1959 gene showing forward and reverse primers. The sequence starts at position 100 and ends at position 870. Primers are indicated by arrows above the sequence:

- 1. GOX1959pSG8_F
- 2. GOX1959pET_F
- 3. GOX1959_F
- 4. GOX1959pSG8_R
- 5. GOX1959pET_R
- 6. GOX1959_R

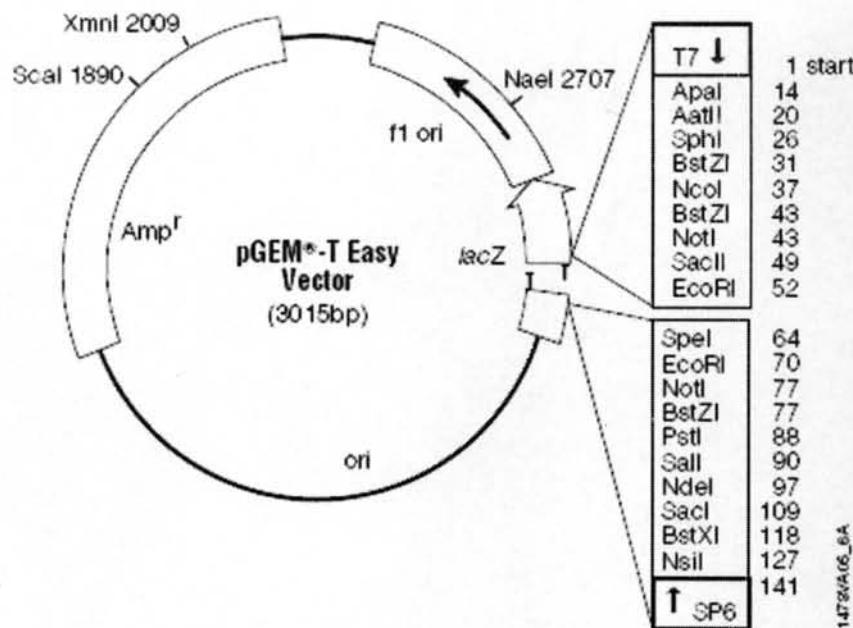
1 = forward and reverse primers used to clone gene from *G. oxydans* 621H

2 = forward and reverse primers used to subclone gene into pET-21a vector; GOX1959pET_F was tagged with CAT, *Nde*I restriction site, while GOX1959pET_R was tagged with GAGCTC, *Xba*I restriction site.

3 = forward and reverse primer used to subclone gene into pSG8 vector; GOX1959pSG8_F was tagged with TTCGAGCTCG, *Sac*I restriction site (underlined), while GOX1959pSG8_R was tagged with GACTCTAGA, *Xba*I restriction site (underlined).

Location on sense strand (2147831 to 2148670), locus tag=GOX0859, EC number=1.1.1.25, product=Shikimate 5-dehydrogenase

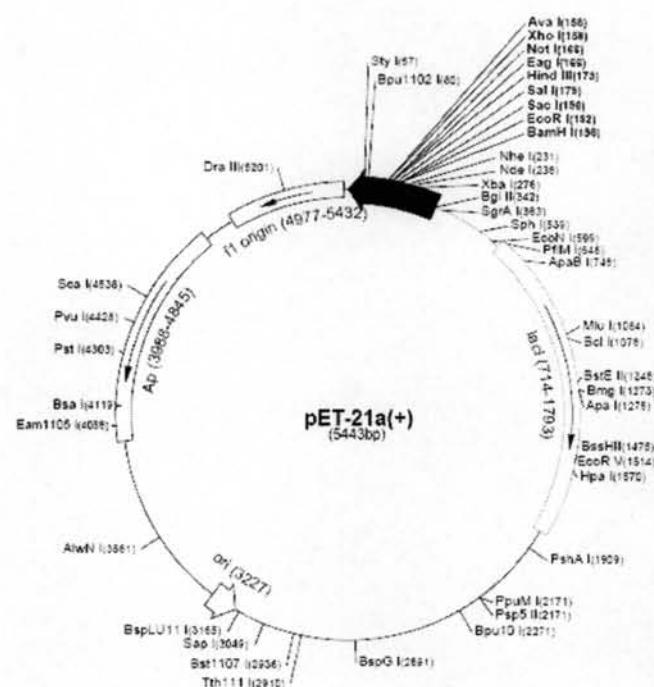
APPENDIX D : Restriction map of pGEM-T® Easy vector (Promega)



Specialized applications of pGEM-T® Easy vector

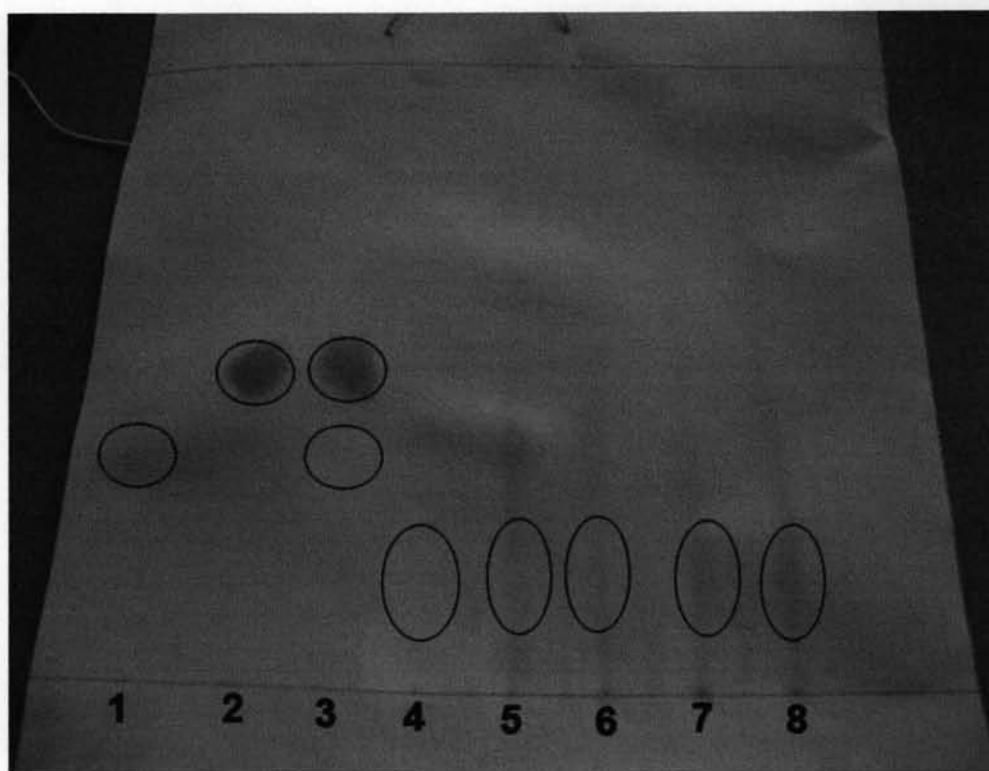
- Cloning PCR products.
- Construction of unidirectional nested deletions with the Erase-a Base® System.
- Production of ssDNA.
- Blue/white screening for recombinants.
- In vitro transcription from dual opposed promoters.

APPENDIX E : Restriction map of pET-21a vector (Novagen)



pET-21a vector characteristics

- T7 lac promoter
 - C-terminal His tag
 - Expression in *E. coli* BL21 (DE3)

APPENDIX F : Dehydroquinate detection by paper chromatography

Lane 1 : standard quinate, $R_f = 0.33$

Lane 2 : standard shikimate, $R_f = 0.45$

Lane 3 : standard shikimate and standard quinate, $R_f = 0.32$ and 0.46 , respectively

Lane 4 : sample at time = 0 hour, $R_f = 0.15$

Lane 5 : sample at time = 7 hour, $R_f = 0.15$

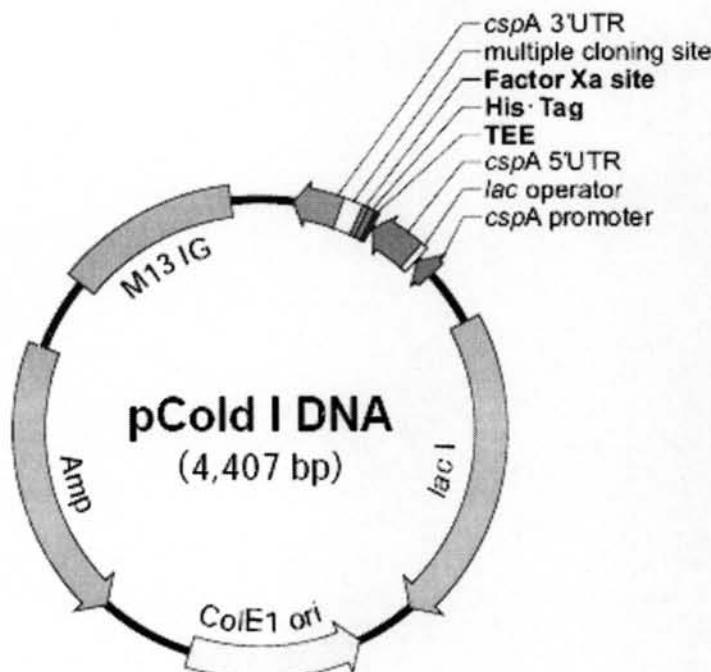
Lane 6 : sample at time = 12 hour, $R_f = 0.15$

Lane 7 : sample at time = 12 hour, $R_f = 0.15$

Lane 8 : sample at time = 12 hour, $R_f = 0.15$

In most cases, quinate of which R_f value of 0.23 gave a spot of pale pink, dehydroquinate ($R_f = 0.28$) gave yellow, shikimate ($R_f = 0.43$) gave red and dehydroshikimate ($R_f = 0.54$) gave yellow.

APPENDIX G : Restriction map of pCold I vector (Takara)



pCold-F Primer →

5' TAACGCTTCAAAATCTGTAAAGCACGCCATATGCCGAAAGG

TEE	His·Tag	Factor Xa
CACACTTAATTATTAAG <u>GAGG</u> TAATACACCATGAATCACAAAGTG	CATCATCATCATCATCAT	ATCGAAGGTAGG
SD Met Asn His Lys Val	His His His His His	Ile Glu Gly Arg

← pCold-R Primer

NdeI SacI KpnI XbaI BamHI EcoRI HindIII SalI PstI XbaI

CATATG GAGCTC GGTACC CTCGAG GGATCC GAATTC AAGCTT GTCGAC CTGCAG TCTAGA TAGGTAATCTCTGCT

His Met Glu Leu Gly Thr Leu Glu Gly Ser Glu Phe Lys Leu Val Asp Leu Gln Ser Arg End

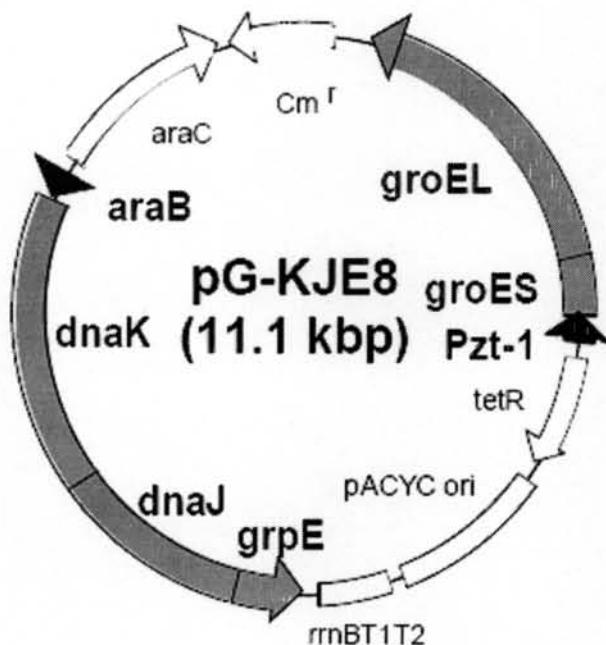
TAAAAGCACAGAACATAAGATCCCTGCCATTGGCGGGGATTTTTTATTTGTTTCAAGGAAATAATAATCGAT 3'

transcription terminator

pCold I vector characteristics

- The promoter derived from cspA gene, one of the cold-shock genes from *E. coli*.
- Expression at 15°C.
- Most *E. coli* strains can be utilized as an expression host.
- N-terminal His-tag.

APPENDIX H : pG-KJE8 chaperone vector map (Takara)



pG-KJE8 vector characteristics

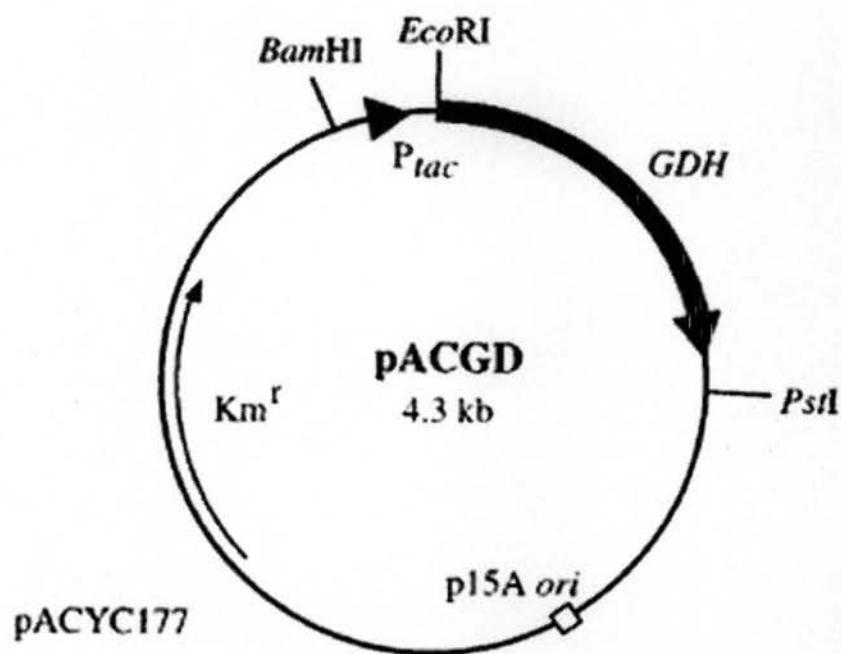
- Expression of dnaK, dnaJ, grpE, groES and groEL chaperone proteins by adding inducers, i.e. L-arabinose and tetracyclin
- Use with *E. coli* expression systems that utilize ColE1- type plasmids containing the ampicillin resistance gene as a marker

APPENDIX I: *gdh* primer position

TCTCGAG 10 20 30 40 50 60 70 80 90
 TAACAAGGAG AGGTGAGGCC AGATGCCTGC CCCTTACAAA GACC GTTTCG CCGGCAAGAA AGTCCTCGTC ACCGGGGCCT CCCAGGGAAAT
***l* GOX2015_F**
 100 110 120 130 140 150 160 170 180
 TGGCGAGGCC ACCCGCCTTC GTTTGCCGA AGAAGGCGCG CAGGTGCCC TCAACGGCG CAAGGAAGAC AAGCTGATCG CGTCCCGA
 190 200 210 220 230 240 250 260 270
 GAAGCTGCC AAGGTTCCG GCGGAGAGCA CCCGATCGCC ACGGGTGACA TTTCAAAGA AGACGACGTC AACGTCTGG TTGCCGAGAG
 280 290 300 310 320 330 340 350 360
 CATCAAGGCC ATGGGTGGTC TCGACGTTCT GGTCTGCAAT GCGGGCTATC AGATCCCCTC CCCGTCAGAA GACATCAAGC TCGAAGATT
 370 380 390 400 410 420 430 440 450
 TGAGGGCGTG ATGCCGTCA ACGTCACGGG GGTGATGCTG CCCTGTCGCG AAGTCATCCG CTACTGGCTG GAAAACGGCA TCAAGGGCAC
 460 470 480 490 500 510 520 530 540
 GATCATTGTG AACTCCTCCG TTCACCAAGAT CATCCCGAA CGGCATTATC TGGGCTATTG CGCCTCCAAG GGTGCCGTG GCAACATTGT
 550 560 570 580 590 600 610 620 630
 CCGCACGCTG GCAC TGGAAAT ATGCCACCCG CGGCATCCGG GTGAATGCCG TGGCGCCCGG CGCCATCGTG ACGCCGATCA ACATGTCGTG
 640 650 660 670 680 690 700 710 720
 GATCGACGAT CCCGAACAGT ACAAGGCCGT TTCGAGCCAC ATCCCGATGA AGCGCCCCGG CGAAAGCCGC GAAATCGCGG ATGCCATCAC
 730 740 750 760 770 780 790 800 810
 CTTCCCTCGCC GCCGAGGACA GCACCTACAT CACGGTCAG ACCCTGTATG TCGATGGTGG TCTGACGCTC TACGGCGATT TCGAAAACAA
 820 830 840 850 860 870
 CTGGTCCTCG TAACTTATA TGGCCCTTC CCTTACCGTT CTGCTGATCG ***R*** *TCTCGAGA*

l= forward and reverse primer used to clone gene from *G. oxydans* 621H; GOX2015_F was tagged with TCTCGAG, *Xba*I restriction site (underlined), while GOX2015_R was tagged with AGAGCTC, *Xba*I restriction site (underlined).

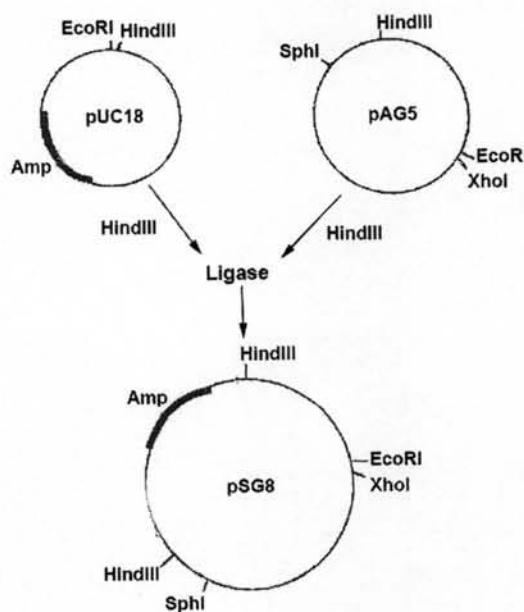
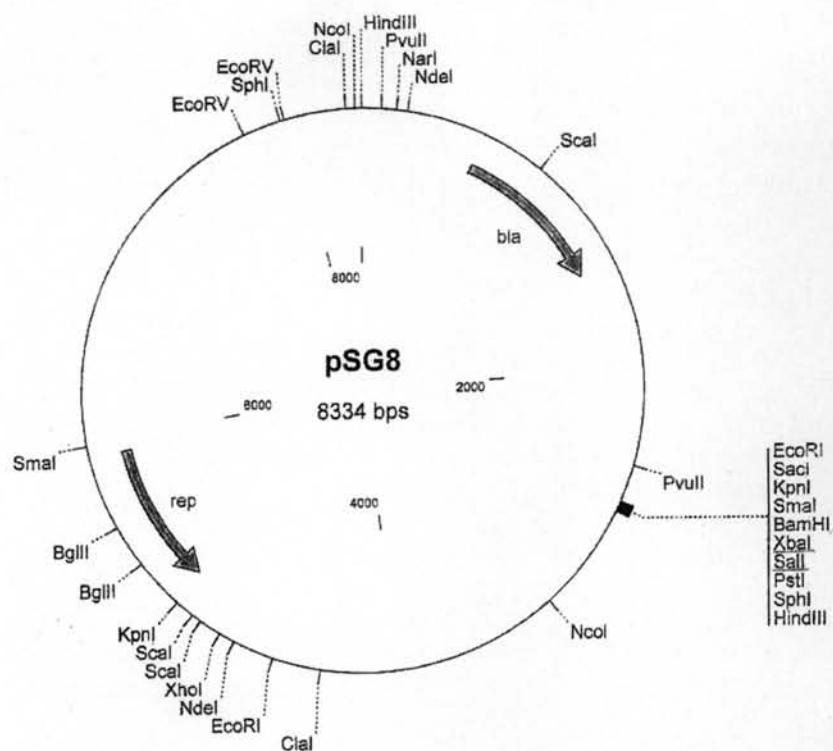
Location on complement strand (2210486 to 2211286), locus tag=GOX2015, EC number=1.1.1.47, product=NAD(P)-dependent glucose 1-dehydrogenase.

APPENDIX J : pACGD vector map (Kataoka *et al.*, 1999)

pACGD vector characteristics

- Vector harboring 1-kb of gdh gene from *Bacillus megaterium*
- tac promoter

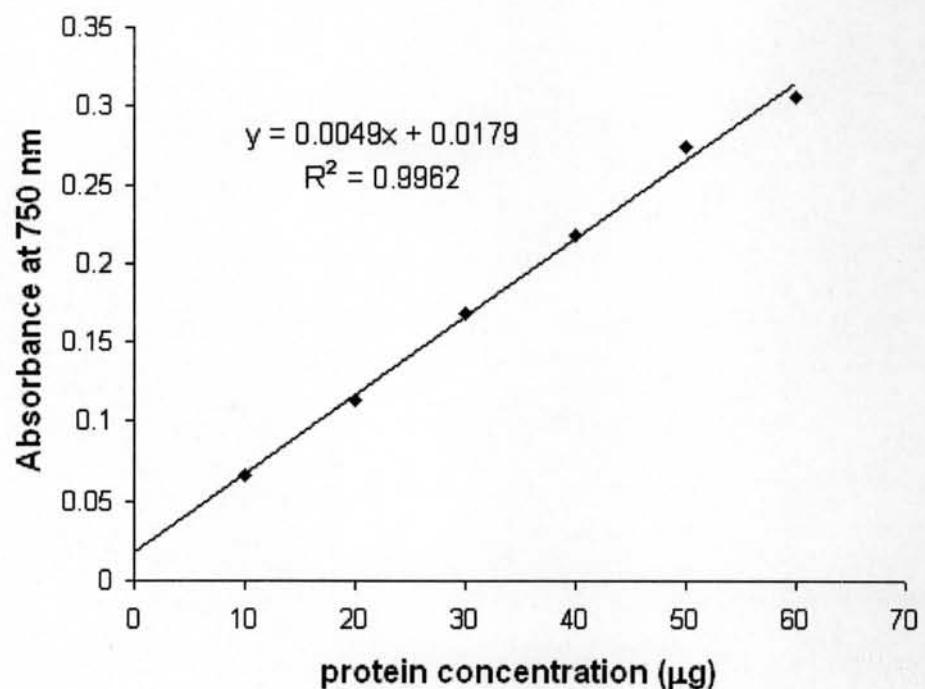
APPENDIX K : Restriction map of pSG8 vector (Tonouchi *et al.*, 2003)



pSG8 vector characteristics

- Shuttle vector constructed from pUC18 vector and pAG5, *G. oxydans* vector
- *lac* promoter
- Ampicillin resistance

APPENDIX L : Standard curve for protein determination by modified Lowry method



APPENDIX M : Preparation for protein determination**Reagent for determination of protein concentration**(modified from Lowry *et al.*, 1951)**Solution A : 2% sodium carbonate in 0.1 M sodium hydroxide****containing 0.5% sodium lauryl sulphate (SDS)**

Sodium carbonate	20	g
Sodium hydroxide	4	g
sodium lauryl sulphate	5	g

Dissolved in distilled water to 1 litre.

Solution B : 0.5% copper sulfate in 1% potassium sodium tartrate

Copper sulfate	1	g
Potassium sodium tartrate	2	g

Dissolved in distilled water to 200 ml.

Solution C : Phenol reagent (Folin-Ciocalteu's reagent)

Folin-Ciocalteu's reagent used in this work was reagent grade from Carlo Erba Reagenti, France.

APPENDIX N : Preparation for SDS-polyacrylamide gel electrophoresis

1. Stock reagents

30% Acrylamide, 0.8% bis-acrylamide, 100 ml

Acrylamide	29.2	g
<i>N,N'</i> -methyl-bis-acrylamide	0.8	g

Adjust volume to 100 ml with distilled water.

1.5 M Tris-HCl pH 8.8

Tris (hydroxymethyl) aminomethane	18.17	g
-----------------------------------	-------	---

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

2.0 M Tris-HCl pH 8.8

Tris (hydroxymethyl) aminomethane	24.2	g
-----------------------------------	------	---

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl) aminomethane	6.06	g
-----------------------------------	------	---

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

1.0 M Tris-HCl pH 6.8

Tris (hydroxymethyl) aminomethane	12.1	g
-----------------------------------	------	---

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

2. Stock reagents for SDS-PAGE

Solution B

2.0 M Tris-HCl pH 8.8	75	ml
10% SDS	4	ml
Distilled water	21	ml

Solution C

1.0 M Tris-HCl pH 6.8	50	ml
10% SDS	4	ml
Distilled water	46	ml

Calculation for X% separating gel

30% Acrylamide solution	X/3	ml
Solution B	2.5	ml
Distilled water	(7.5-X/3)	ml
10% Ammonium persulfate	50	μl
TEMED	5	μl (10 μl if X<8%)
Total volume	10	ml

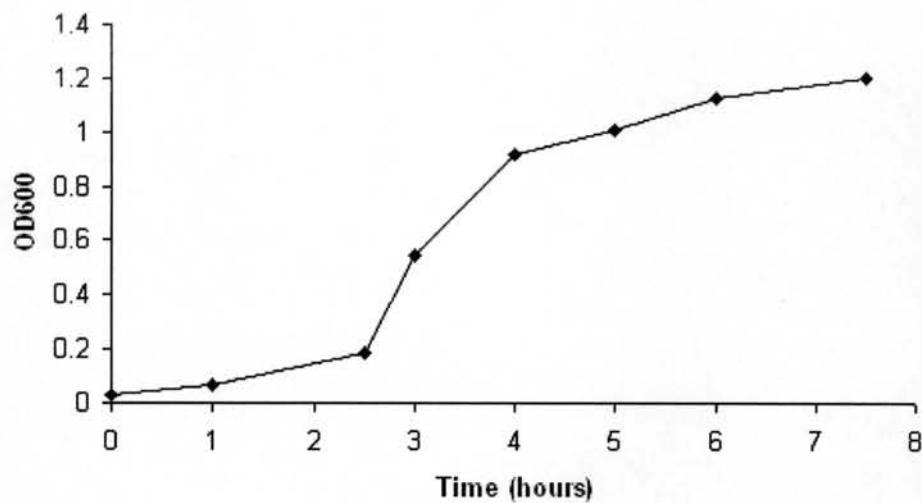
12.5% Separating gel

30% Acrylamide solution	3.33	ml
Solution B	2.5	ml
Distilled water	4.17	ml
10% Ammonium persulfate	50	μl
TEMED	5	μl

5.0% Stacking gel

30% Acrylamide solution	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10% Ammonium persulfate	30	µl
TEMED	5	µl

APPENDIX O : *E. coli* BL21 (DE3)/pET-GOX1959-GOX2015 growth curve when grown at 37°C



BIOGRAPHY

Miss Chayatip Insomphun was born on August 27, 1982 in Chiangmai, Thailand. She finished High School at The Prince Royal's College, Chiangmai and enrolled in the Faculty of Science, Chiangmai University in 1999. She graduated with the Bachelor Degree of Science in Biochemistry and Boichemical Technology in 2004 and continued studying for Master Degree of Science in Biochemistry at Chulalongkorn University in that year. She finished Master Degree of Science in Biochemistry in October 2007.