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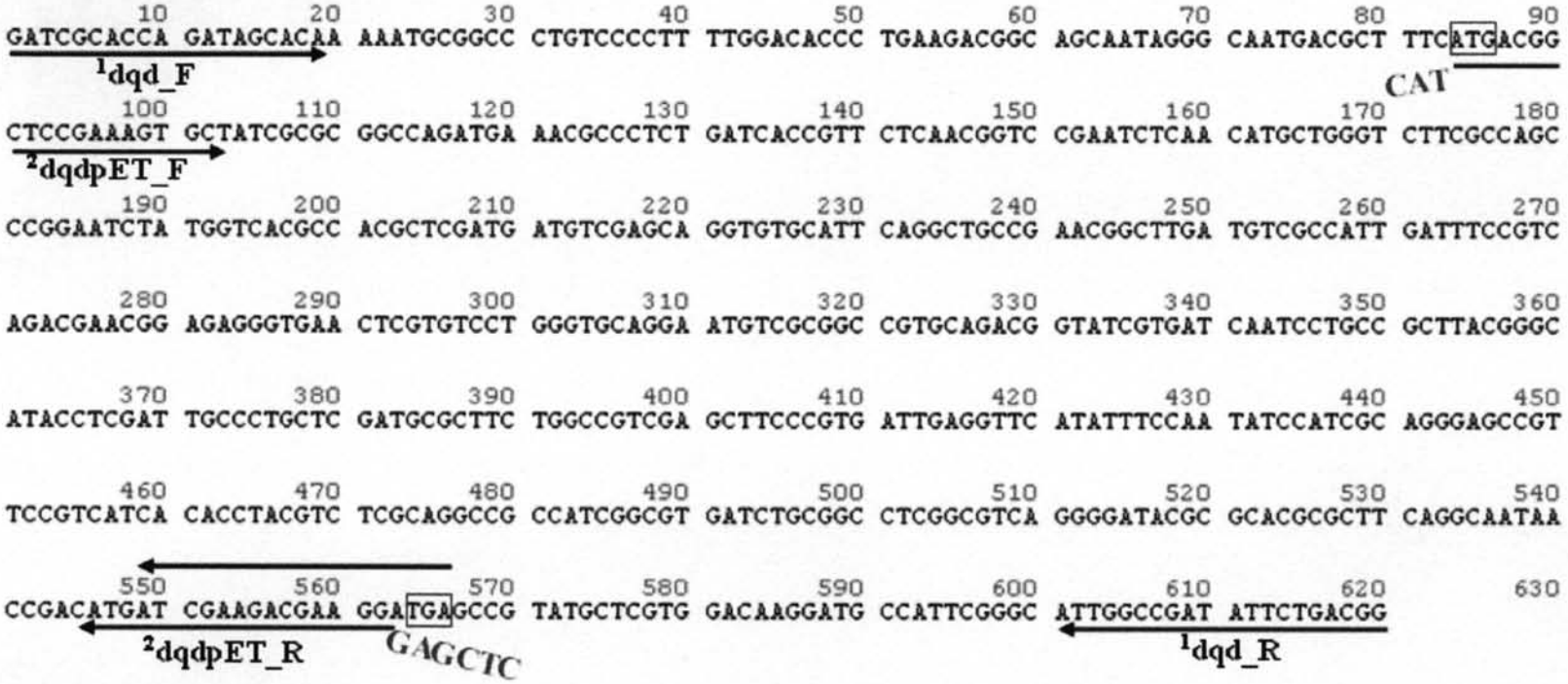
APPENDICES

APPENDIX A : *dqd* primer position



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

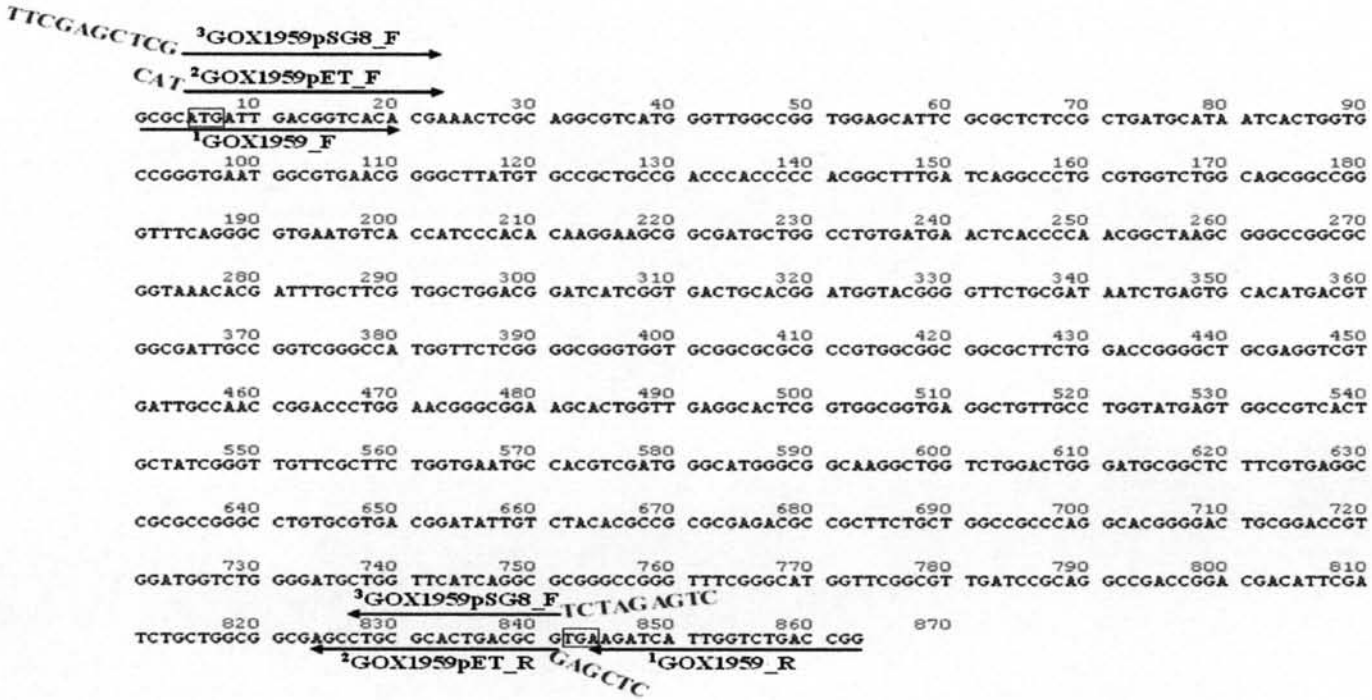
Appendix B : *skdh* (GOX0859) primer position

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, *Xho*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



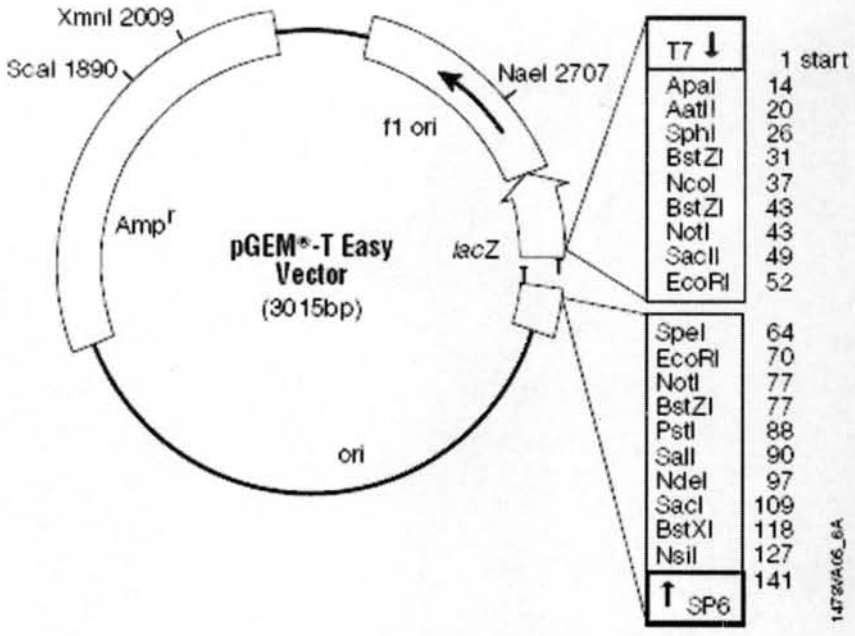
1 = forward and reverse primers used to clone gene from *G. oxydans* 621H

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, *Xho*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.

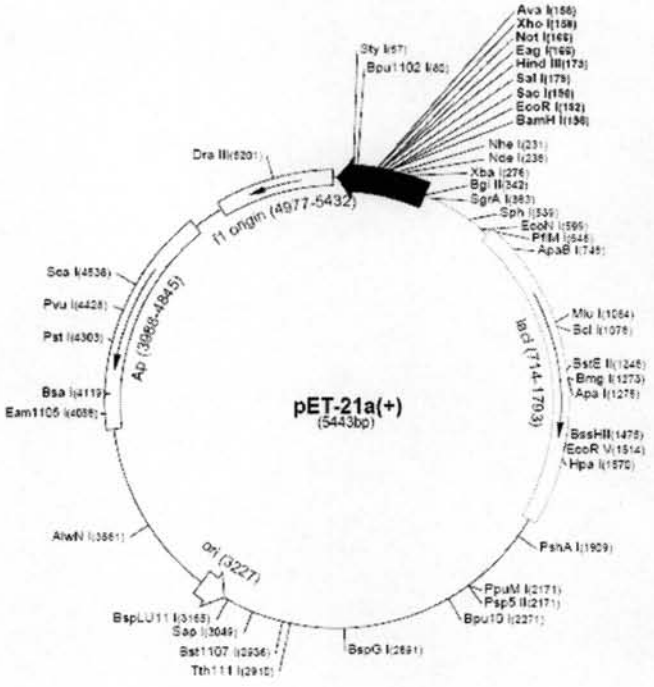
- Construction of unidirectional nested deletions with the Erase-a Base® System.

- Production of ssDNA.

- Blue/white screening for recombinants.

- In vitro transcription from dual opposed promoters.

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

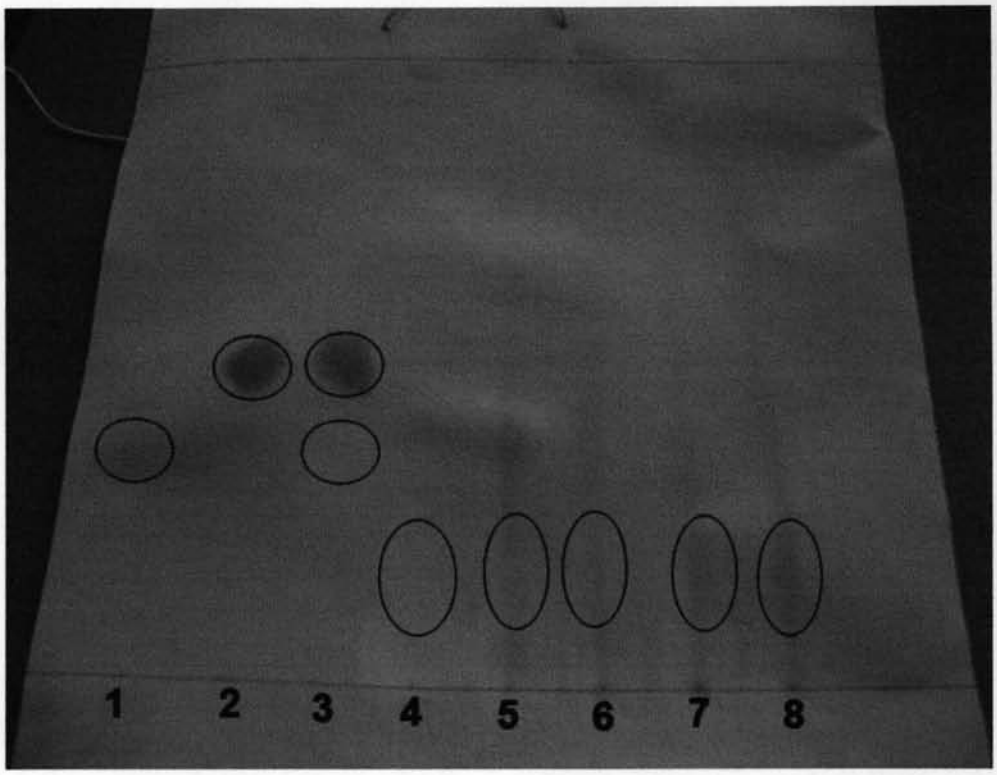


pET-21a-d(+) cloning/expression region

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, $R_f = 0.15$

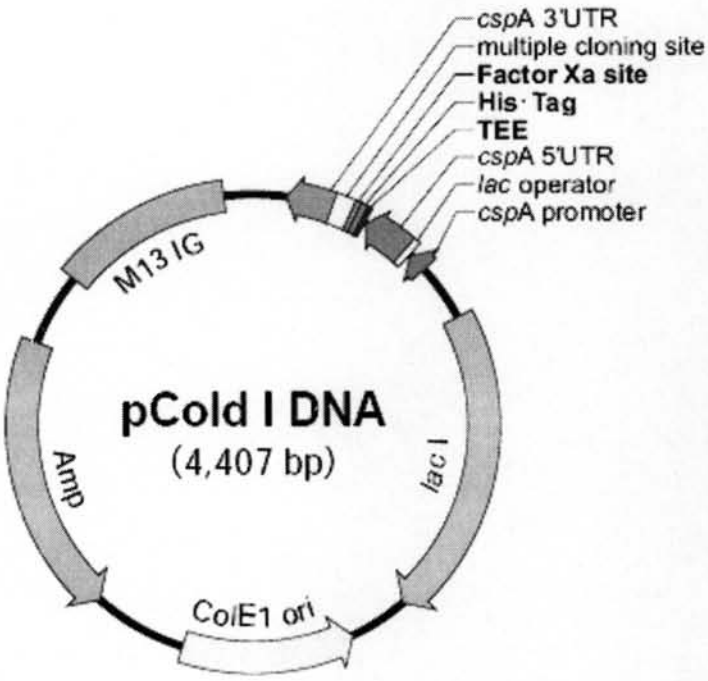
Lane 6 : sample at time = 12 hour, $R_f = 0.15$

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' TAACGCTTCAAAATCTGTAAAGCACGCCATATCGCCGAAAGG

TEE His · Tag Factor Xa

CACACTTAATTATTAA**GAGG**TAATACACCATGAATCACAAAGTG CATCATCATCATCATCAT ATCGAAGGTAGG

SD Met Asn His Lys Val His His His His His His Ile Glu Gly Arg

Nde I *Sac* I *Kpn* I *Xho* I *Bam*H I *Eco*R I *Hind* III *Sal* I *Pst* I *Xba* I

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**

pCold-R Primer

TAAAAGCACAGAATCTAAGA**TCCCTGCCA**TTT**GGCGGGGA**TTTTTTTTATTTGTTTTCAGGAAATAAATAATCGAT 3'

transcription terminator

pCold I vector characteristics

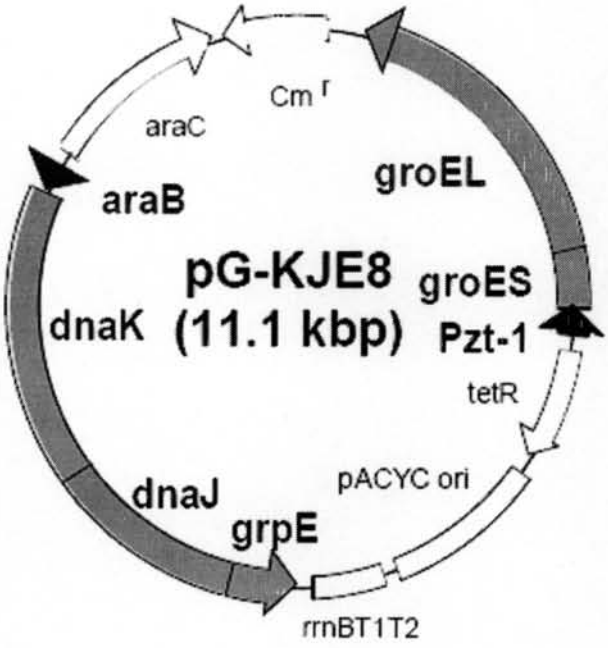
- The promoter derived from cspA gene, one of the cold-shock genes from *E. coli*.

- 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)

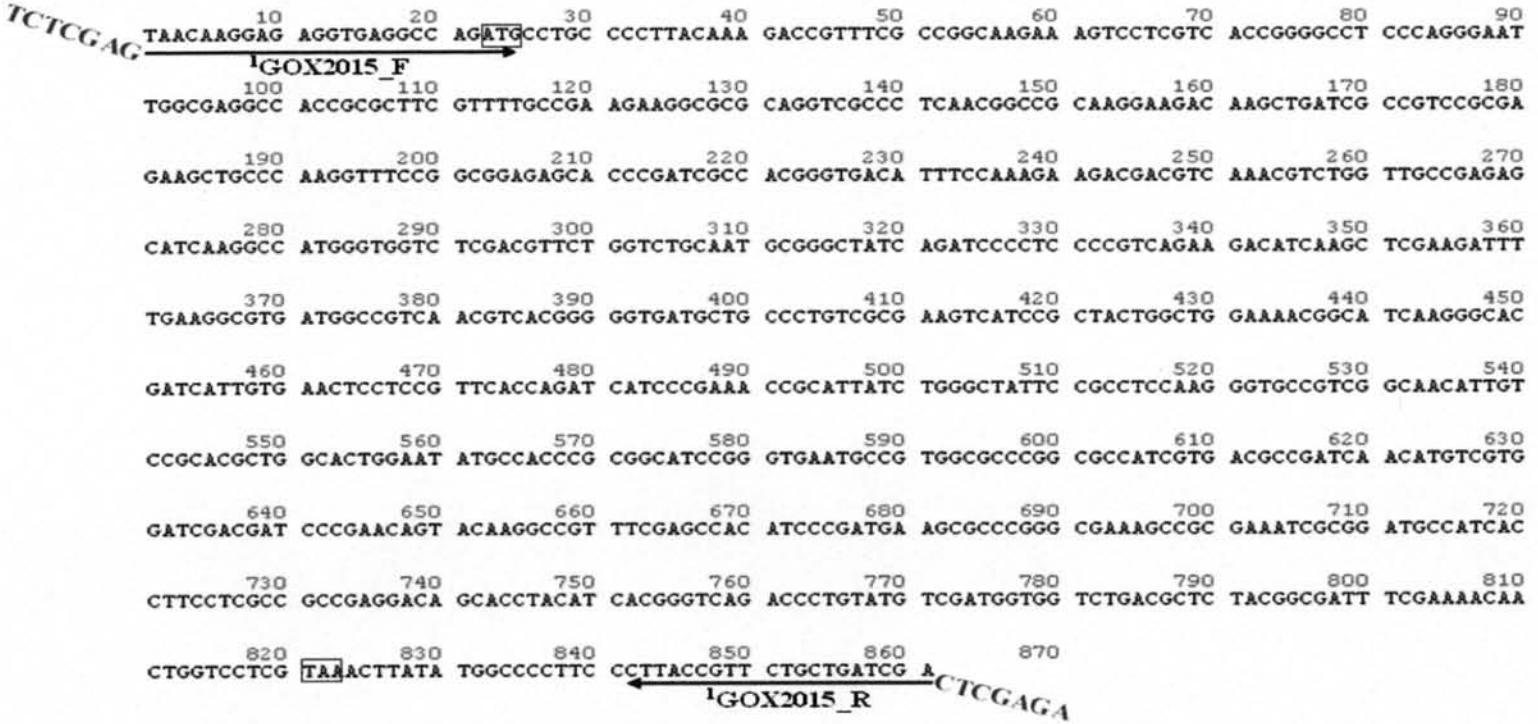


pG-KJE8 vector characteristics

- 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 resistence gene as a marker

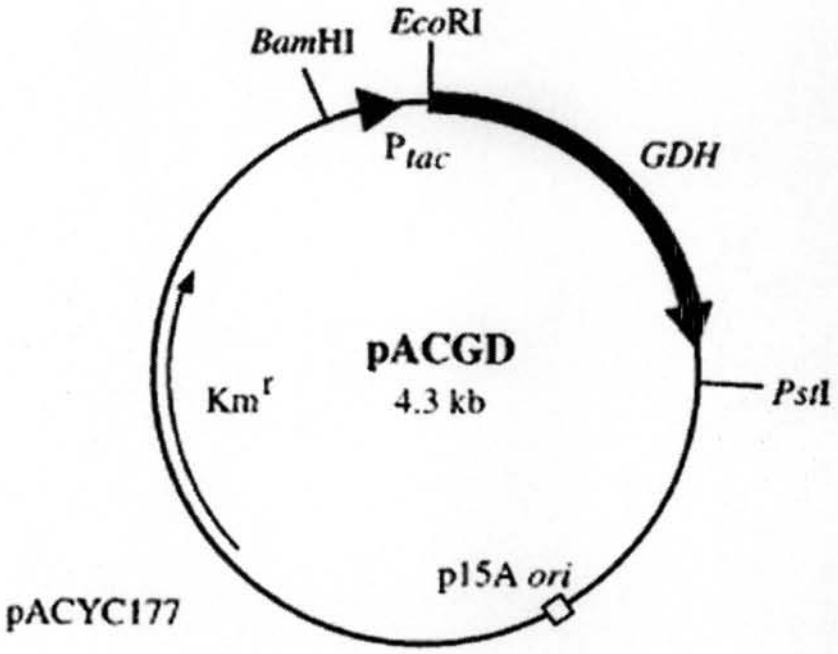
APPENDIX I: *gdh* primer position



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

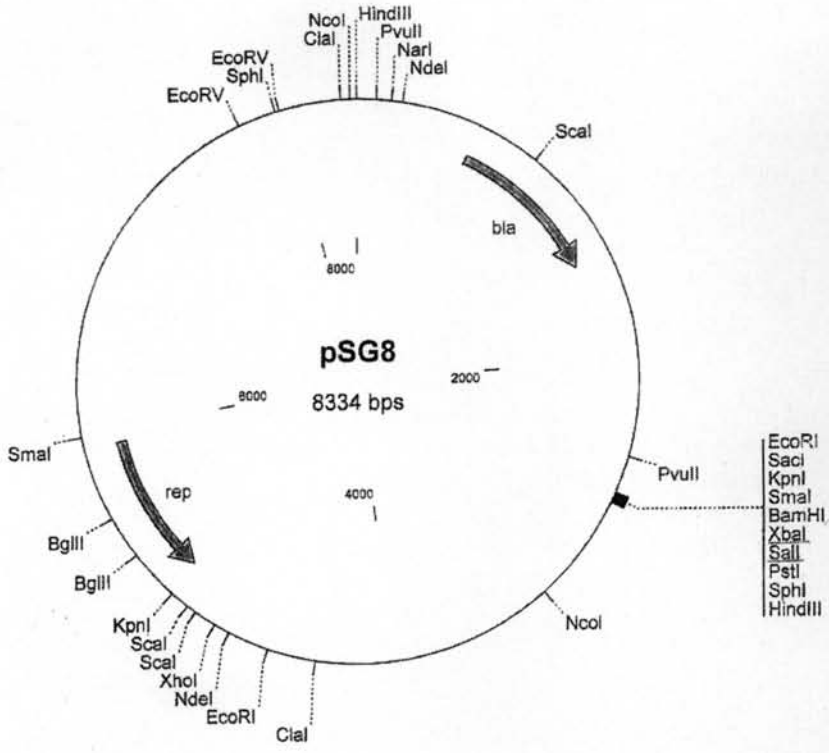


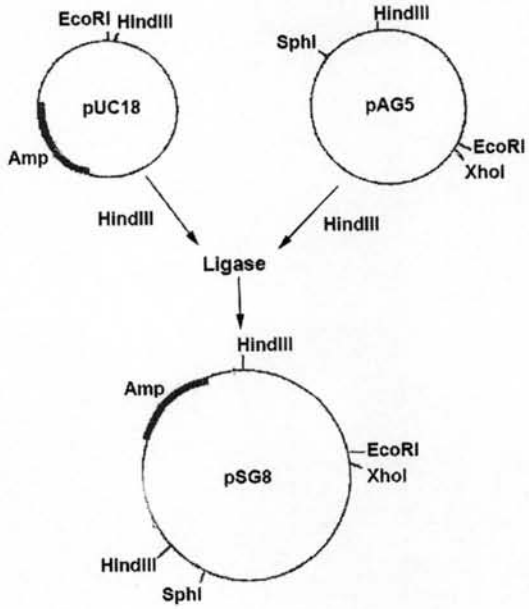
pACGD vector characteristics

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

- tac promoter

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





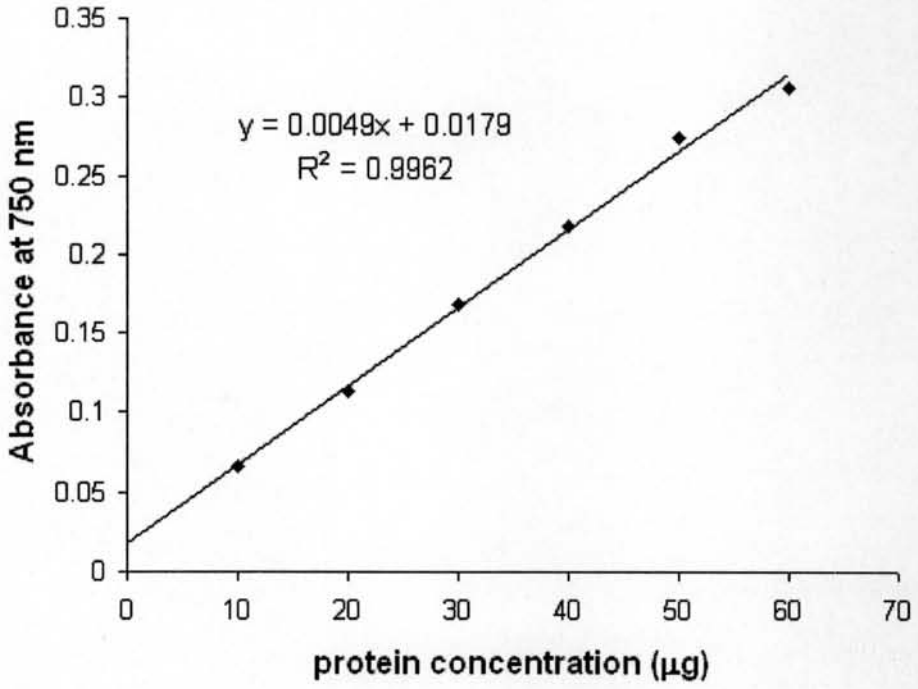
pSG8 vector characteristics

- 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 laulyl sulphate (SDS)

Sodium carbonate	20	g
Sodium hydroxide	4	g
sodium laulyl 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
N,N'-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

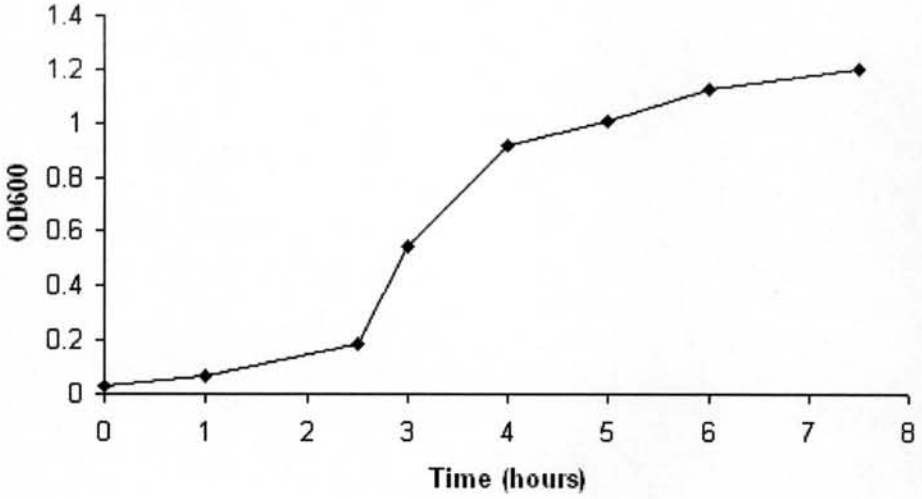
12.5% Separating gel

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.