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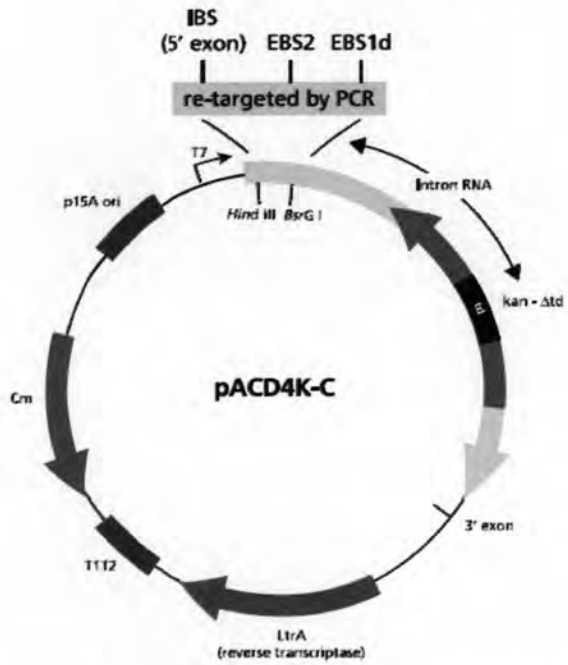
APPENDICES

APPENDIX A

pACD4K-C (7,678 bp)



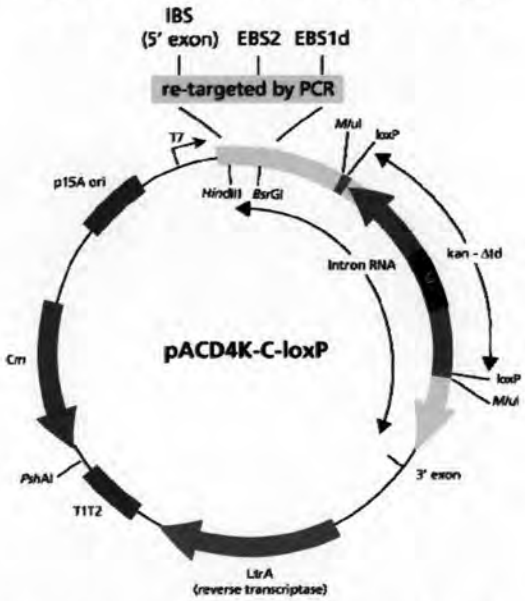
Description:

pACD4K-C is designed for use in gene knockout mutation. Plasmid is propagated in medium containing chloramphenicol (25μg/ml). The kanamycin marker located within the group II intron on pACD4K-C is interrupted by *td* group I intron is excised, activating the kanamycin marker.

bp	Feature
585-1497	p15Aori
1741-1762	T7 promoter
1802-1826	5' exon (IBS)
1827-4115	Intron RNA
2049-2053	EBS2 (Exon binding site sequence 2)
2102-2111	EBS1d
2524-3711	kanamycin RAM marker (for chromosomal insertion selection, not plasmid propagation)
2820-3122	td group I intron (interrupts kan ORF)
4116-4125	3' exon
4362-6161	LtrA ORF
6297-6587	T1/T2 transcription terminator
7238-219	chloramphenicol (Cm) resistance (for plasmid propagation)

APPENDIX A (continued)

pACD4K-C-loxP (7,745 bp)



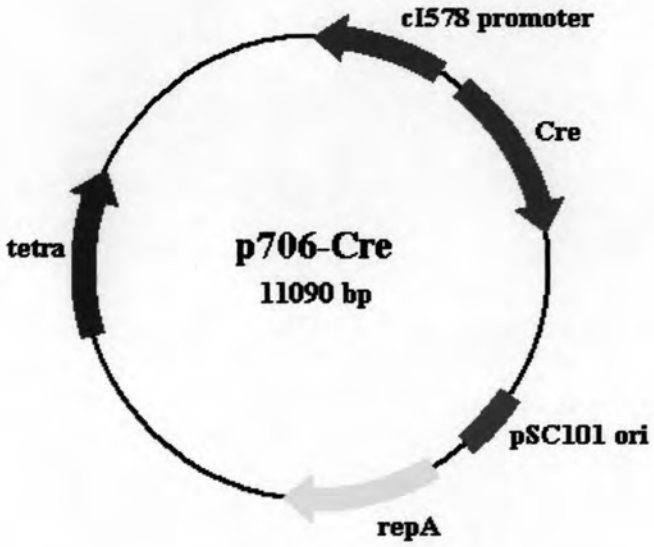
Description:

pACD4K-C-loxP is differs from the pACD4K-C in that it has loxP sites flanking each end of the kanamycin ORF. The addition of the loxP sites on this vector allow for multiple created a multiple gene knockout mutation. After chromosomal insertion, the kanamycin resistance marker used to selection for insertion can be removed via Cre-loxP mediated recombination.

bp	Feature
585-1497	p15Aori
1741-1762	T7 promoter
1802-1826	5' exon (IBS)
1827-4182	Intron RNA
2049-2053	EBS2 (Exon binding site sequence 2)
2102-2111	EBS1d
2523-2555	5' loxP site
2556-3923	kanamycin RAM marker (for chromosomal insertion selection, not plasmid propagation)
2853-3245	td group I intron (interrupts kan ORF)
3924-3957	3' loxP site
4183-4192	3' exon
4429-6228	LtrA ORF
6365-6644	T1/T2 transcription terminator
7305-219	chloramphenicol (Cm) resistance (for plasmid propagation)

APPENDIX A (continued)

p706-Cre (11,090 bp)



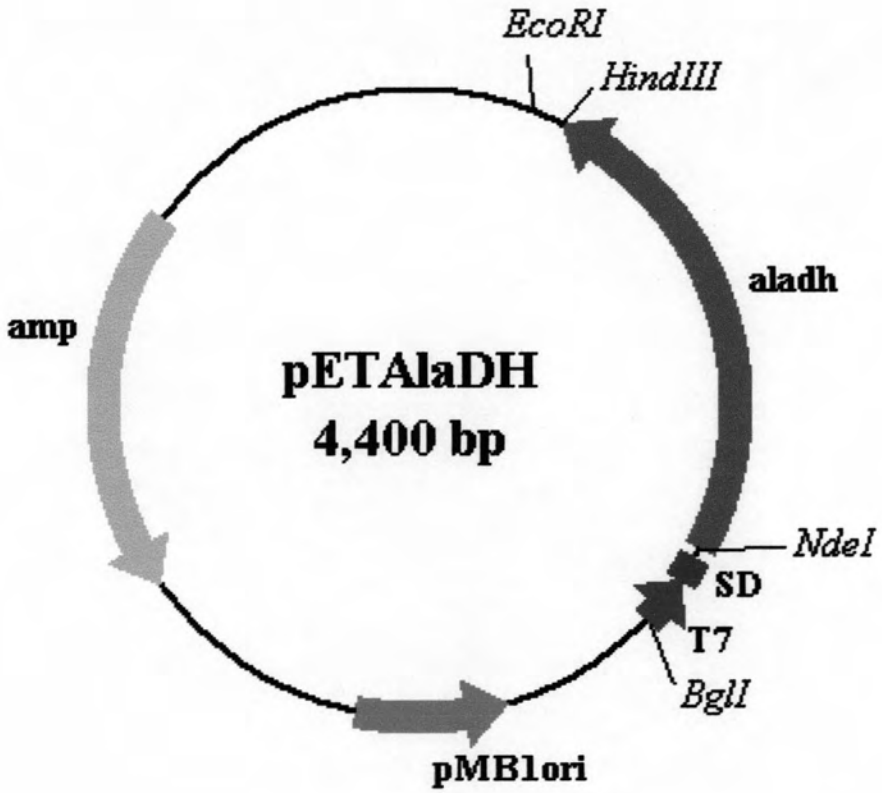
Description:

p706-Cre is designed for use in Cre-mediated genomic manipulations. The plasmid has a pSC101 origin which maintains low copy and replicates at 30°C. The plasmid will not propagate and will get lost when incubated at 37°C. The expression of the Cre-recombinase is driven by the thermosensitive promoter cI578 (λ_{PR} promoter). Therefore, the expression of Cre is repressed at 30°C and induced between 37-42°C. The plasmid carried a tetracycline resistance.

The plasmid has not been completely sequenced.

APPENDIX A (continued)

pETAlaDH



APPENDIX B

Preparation for protein determination

Reagent for determination of protein concentration (modified from Lowry *et al.*, 1951)

Solution A (0.5% copper sulfate and 1% potassium tartate, pH 7.0)

Potassium tartate	1	g
Copper sulfate	0.5	g

Adjusted pH to 7.0 and adjust the solution volume to 100 ml.

Solution B (2% sodium carbonate and 1 N sodium hydroxide)

Sodium carbonate	20	g
Sodium hydroxide	4	g

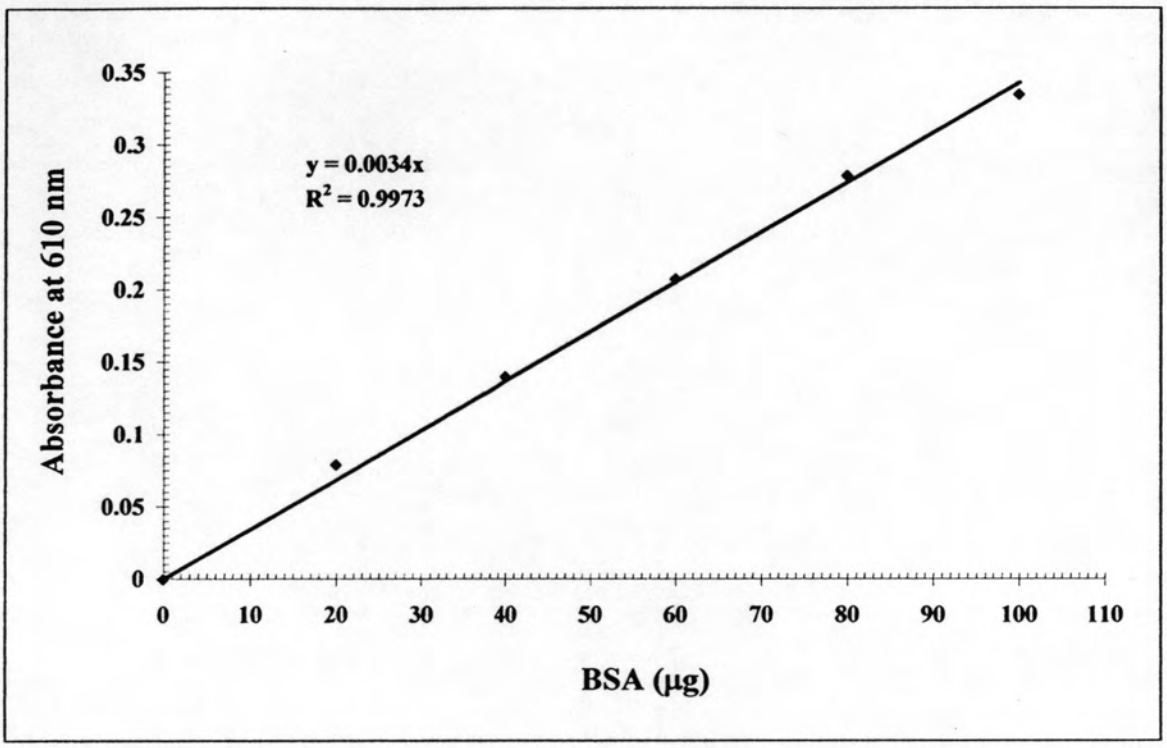
Dissolved in distilled water to 1 liter.

Solution C (phenol reagent)

Folin-Ciocalteu phenol reagent used in this work was reagent grade from Carlo Erba, Italy.

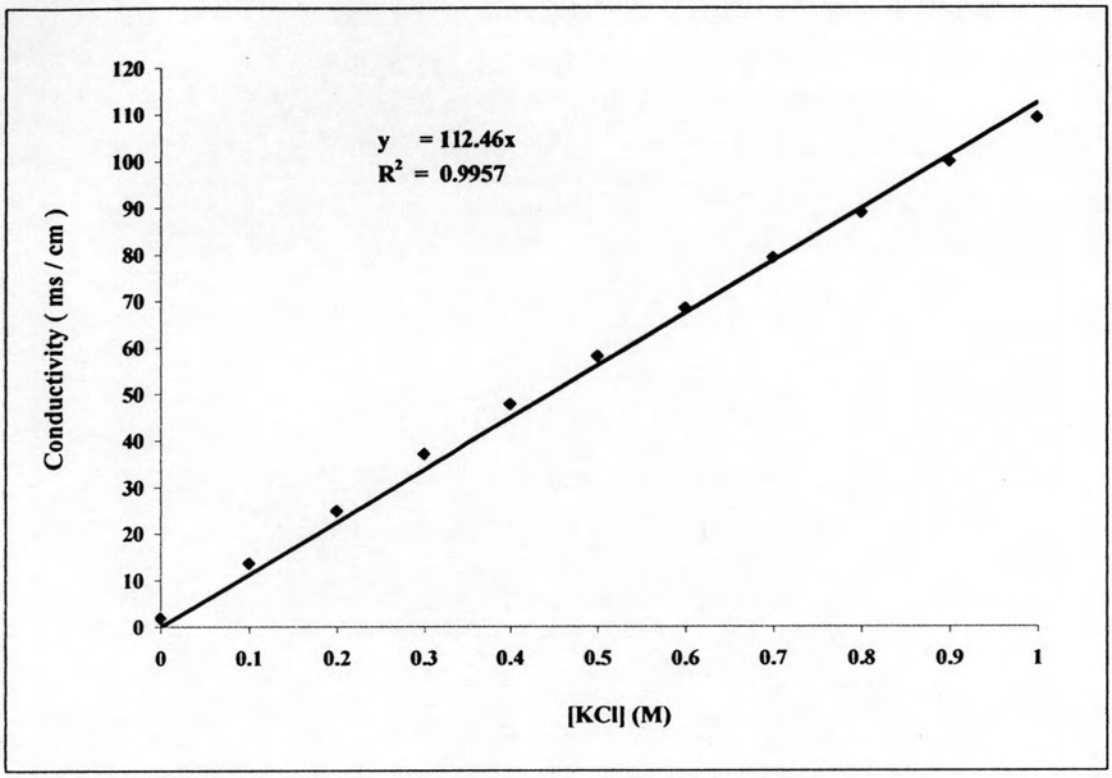
APPENDIX C

Standard curve for protein determination by Lowry' s method



APPENDIX D

Calibration curve for conductivity of potassium chloride



APPENDIX F

Preparation for denaturing polyacrylamide gel electrophoresis

1. Stock solution

2 M Tris-HCl (pH 8.8)

Tris (hydroxymethyl)-aminomethane 24.2 g

Adjusted pH to 8.8 with 1 N HCl and adjusted volume to 100 ml with distilled water.

1 M Tris-HCl (pH 6.8)

Tris (hydroxymethyl)-aminomethane 12.1 g

Adjusted pH to 6.8 with 1 N HCl and adjusted volume to 100 ml with distilled water.

10% (w/v) SDS

Sodium dodecyl sulfate (SDS) 10 g

Added distilled water to a total volume of 100 ml.

50% (w/v) Glycerol

100% Glycerol 50 ml

Added distilled water to a total volume of 100 ml.

1% (w/v) Bromophenol blue

Bromophenol blue 100 mg

Brought to 10 ml with distilled water and stirred until dissolved.

The aggregated dye was removed by filtration.

APPENDIX F (continued)

2. Working solutions

Solution A (30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide)

Acrylamide	29.2	g
N, N'-methylene-bis-acrylamide	0.8	g

Adjusted volume to 100 ml with distilled water.

Filtered and stored in dark (brown bottle) at 4°C

Solution B (1.5 M Tris-HCl, pH 8.8 and 0.4% SDS)

2 M Tris-HCl (pH 8.8)	75	ml
10% (w/v) SDS	4	ml
Distilled water	21	ml

Solution C (0.5 M Tris-HCl, pH 6.8, 0.4% SDS)

1 M Tris-HCl (pH 6.8)	50	ml
10% (w/v) SDS	4	ml
Distilled water	46	ml

10% (w/v) Ammonium persulfate

Ammonium persulfate	0.5	g
Distilled water	5.0	ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine and 0.1% (w/v) SDS)

Tris (hydroxymethyl)-aminomethane	3.0	g
Glycine	14.4	ml
SDS	1	g

Dissolved and adjusted to total volume to 1 liter with distilled water

(final pH should be approximately 8.3)

APPENDIX F (continued)

5x Sample buffer (312.5 mM Tris-HCl pH 6.8, 50% (v/v) glycerol, 1% (w/v) bromophenol blue)

1 M Tris-HCl (pH 6.8)	0.6	ml
50% (v/v) Glycerol	5.0	ml
10% (w/v) SDS	2	ml
1% (w/v) Bromophenol blue	1	ml
2-Mercaptoethanol	0.5	ml
Distilled water	1.4	ml

3. SDS-PAGE

10% Separating gel

Solution A	3.3	ml
Solution B	2.5	ml
Distilled water	4.2	ml
10% (w/v) Ammonium persulfate	50	µl
TEMED	5	µl

5.0% Stacking gel

Solution A	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10% (w/v) Ammonium persulfate	30	µl
TEMED	5	µl

APPENDIX F (continued)

4. Protein staining solution

Staining solution, 1 liter

Coomassie brilliant blue R-250	1.0	ml
Methanol	450	ml
Distilled water	450	ml

Destaining solution, 1 liter

Methanol	100	ml
Glacial acetic acid	100	ml
Distilled water	800	ml

APPENDIX G

Preparation for Southern blot analysis

Reagent for Southern blot analysis (Sambrook *et al.*, 1989)

Depurination solution (250 mM hydrochloric acid)

37%(w/w) hydrochloric acid	10.3	ml

Dissolved in distilled water to 500 ml.

Denaturation solution (1.5 M sodium chloride, 0.5 N sodium hydroxide)

Sodium chloride	21.9	g
Sodium hydroxide	5.0	g

Dissolved in distillation water to 250 ml.

Neutralization solution (0.5 M tris-hydrochloric acid, 1.5 M sodium chloride, pH7.5)

Trizma base	30.3	g
Sodium chloride	87.6	g

Adjusted pH to 7.5 with hydrochloric acid and adjust the solution volume to 500 ml with distilled water.

SSC, 20x (0.3 M sodium citrate, 3 M sodium chloride, pH7.0)

Sodium citrate	88.2	g
Sodium chloride	175.3	g

Adjusted pH to 7.0 with citric acid and adjusted the solution volume to 1 l with distillation water.

Tris-hydrochloric acid (1 M tris-hydrochloric acid, pH8.0

Trisma base	60.5	g

Adjusted pH to 8.0 with hydrochloric acid and brought volume to 500 ml and autoclaved

APPENDIX G(continued)

Denhardt's solution (100x)

Bovine serum albumin	2.0	g
Polyvinyl pyrrolidone	2.0	g
Ficall, type 400	2.0	g

Dissolved in distilled water to 100 ml. Filtered through a sterile, 0.22 μm PVDF filter and stored at 4°C. Do not freeze.

Denature DNA stock (5 mg/ml)

Calf thymus DNA	500.0	mg

Dissolved in TE buffer, pH 8.0 to 75 ml. Stored at 4°C.

SDS, 20%(w/v)

Sodium dodecylsulfate	20.0	g

Dissolved in sterile distilled water to 100 ml.

Prehybridization solution [50%(v/v) formamide, 0.75 M sodium salt, 5x Denhardt's solution, 100 μg/ml denature DNA, 0.2%(w/v) sodium dodecylsulfate]

Formamide	50.0	ml
20x SSC	25.0	ml
100x Denhardt's solution	5.0	ml
Denature DNA stock	2.0	ml
20%(w/v) SDS	1.0	ml

Adjusted volume with distilled water to 100 ml. The solution could stored at 4°C for short period time.

APPENDIX G (continued)

Hybridization solution [50%(v/v) formamide, 0.75 M sodium salt, 1x Denhardt's solution, 0.2%(w/v) sodium dodecylsulfate]

Formamide	50.0	ml
20x SSC	25.0	ml
100x Denhardt's solution	1.0	ml
20%(w/v) SDS	1.0	ml

Adjusted volume with distilledwater to 100 ml. The solution could stored at 4°C for short period time.

Klenow buffer, 10X (500 mM Tris-hydrochloric acid, 100 mM magnesium sulfate , 1 mM dithiothreitol, 600 μM each of dNTP)

1 M Tris-hydrochloric acid, stock	0.5	ml
1 M magnesium sulfate, stock	100.0	μl
1 M dithiothreitol, stock	1.0	μl
100 mM each of dNTP, stock	6.0	μl

Adjusted volume to 1 ml with sterile distilled water.

Scintillation fluid

2,5-diphenyloxazole (POP)	2.0	μl
1,4-bis[2-(5-phenyloxazole)]benzene (POPOP)	25.0	mg
toluene	333.5	ml
Triton X100	1 66.5	ml

APPENDIX H

SOC medium

Tryptone	2.0	g
Yeast extract	0.5	g
1 M Sodium chloride	1.0	ml
1 M Potassium chloride	0.25	ml
1 M Magnesium chloride	0.40	ml
1M Magnesium sulfate	0.40	ml
2M Glucose	1.0	ml

Adjusted pH to 7.0, brought to 100 ml with distilled water and autoclave.

APPENDIX H

alr gene sequence

LOCUS AE014075 1080 bp DNA linear BCT 20-APR-2006
DEFINITION *Escherichia coli* CFT073, complete genome.
ACCESSION AE014075 REGION: 4804186..4805265
VERSION AE014075.1 GI:26111730
KEYWORDS .
SOURCE *Escherichia coli* CFT073
ORGANISM Escherichia coli CFT073
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia.
REFERENCE 1 (bases 1 to 1080)
AUTHORS Welch,R.A., Burland,V., Plunkett,G.D. III, Redford,P., Roesch,P., Rasko,D.A., Buckles,E.L., Liou,S.-R., Boutin,A., Hackett,J., Stroud,D., Mayhew,G.F., Rose,D.J., Zhou,S., Schwartz,D.C., Perna,N.T., Mobley,H.L.T., Donnenberg,M.S. and Blattner,F.R.
TITLE Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 99 (26), 17020-17024 (2002)
PUBMED 12471157
REFERENCE 2 (bases 1 to 1080)
AUTHORS Welch,R.A., Burland,V., Plunkett,G.D. III, Redford,P., Roesch,P., Rasko,D.A., Buckles,E.L., Liou,S.-R., Boutin,A., Hackett,J., Stroud,D., Mayhew,G.F., Rose,D.J., Zhou,S., Schwartz,D.C., Perna,N.T., Mobley,H.L.T., Donnenberg,M.S. and Blattner,F.R.
TITLE Direct Submission
JOURNAL Submitted (20-JUN-2002) Genetics Laboratory, University of Wisconsin - Madison, 445 Henry Mall, Madison, WI 53706, USA
COMMENT On or before Jan 19, 2006 this sequence version replaced gi:26106314, gi:26106632, gi:26106912, gi:26107213, gi:26107527, gi:26107941, gi:26108223, gi:26108544, gi:26108838, gi:26109116, gi:26109395, gi:26109707, gi:26110054, gi:26110397, gi:26110701, gi:26111017, gi:26111307, gi:26111641.
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APPENDIX I

dadX gene sequence

LOCUS	AE014075 1071 bp DNA linear BCT 20-APR-2006
DEFINITION	*Escherichia coli* CFT073, complete genome.
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VERSION	AE014075.1 GI:26111730
KEYWORDS	.
SOURCE	*Escherichia coli* CFT073
ORGANISM	Escherichia coli CFT073 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia.
REFERENCE	1 (bases 1 to 1071)
AUTHORS	Welch,R.A., Burland,V., Plunkett,G.D. III, Redford,P., Roesch,P., Rasko,D.A., Buckles,E.L., Liou,S.-R., Boutin,A., Hackett,J., Stroud,D., Mayhew,G.F., Rose,D.J., Zhou,S., Schwartz,D.C., Perna,N.T., Mobley,H.L.T., Donnenberg,M.S. and Blattner,F.R.
TITLE	Extensive mosaic structure revealed by the complete genome sequence of uropathogenic Escherichia coli
JOURNAL	Proc. Natl. Acad. Sci. U.S.A. 99 (26), 17020-17024 (2002)
PUBMED	12471157
REFERENCE	2 (bases 1 to 1071)
AUTHORS	Welch,R.A., Burland,V., Plunkett,G.D. III, Redford,P., Roesch,P., Rasko,D.A., Buckles,E.L., Liou,S.-R., Boutin,A., Hackett,J., Stroud,D., Mayhew,G.F., Rose,D.J., Zhou,S., Schwartz,D.C., Perna,N.T., Mobley,H.L.T., Donnenberg,M.S. and Blattner,F.R.
TITLE	Direct Submission
JOURNAL	Submitted (20-JUN-2002) Genetics Laboratory, University of Wisconsin - Madison, 445 Henry Mall, Madison, WI 53706, USA
COMMENT	On or before Jan 19, 2006 this sequence version replaced gi:26106314, gi:26106632, gi:26106912, gi:26107213, gi:26107527, gi:26107941, gi:26108223, gi:26108544, gi:26108838, gi:26109116, gi:26109395, gi:26109707, gi:26110054, gi:26110397, gi:26110701, gi:26111017, gi:26111307, gi:26111641.
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ORIGIN

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หอสมุดกลาง ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

BIOGRAPHY

Ms. Duangporn Ungsupravate was born on September 11, 1978 in Suratthani. She finished high school at Suratpittaya school, Suratthani. She graduated with the B.Sc. in Chemistry-Biology from Faculty of Science and Technology, Prince of Songkla University in 2000. She has studied for Master degree in Biochemistry, Faculty of Science at Chulalongkorn University since 2003.