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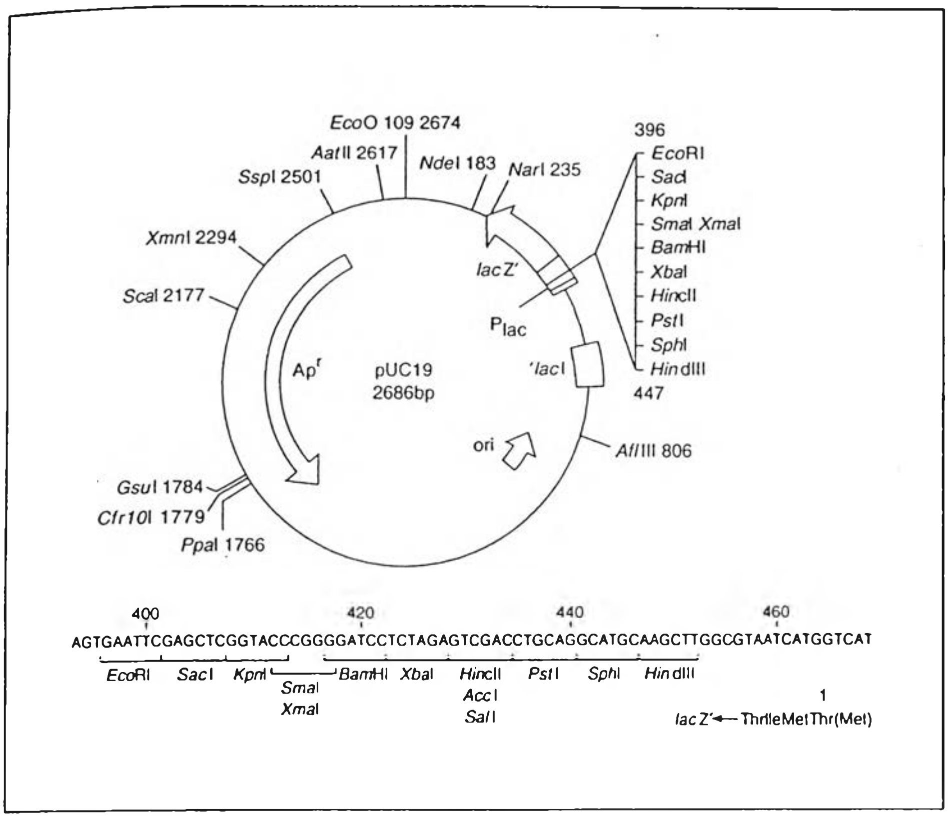
APPENDIX A

Restriction map of pUC 19



pUC 19 DNA: Location of some restriction endonuclease sites in multicloning site

Enzyme	**Location**
*Eco*R I	396
Sma I	412
*Bam*H I	417
*Pst*I	435
*Hin*dIII	447

APPENDIX B

Nucleotide sequence of *pcpD* gene from *Sphingomonas chlorophenolica* ATCC 39723

NUCLETIDE SEQUENCE 28-999 = *pcpD* gene

PRODUCT: pentachlorophenol 4-monooxygenase reductase

ORGANISM: *Sphingomonas chlorophenolica* strain ATCC 39723

REFFERENCE: Lange, C. C. and Orser, C. S.

JOURNAL: Thesis (1994) Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow.

```
tgccgtgcgc ctgaggagac ccgtgcgatg acaaaccccg tttcgacaat cgacatgacg 61
gtcacgcaga tcacccgcgt ggccaaggac atcggctctt acgaacttcg cccggaaccc 121
ggcgtgatat tgccggagtt caccgcgggg gcgcatatcg gcgtttcgct tcccaacggg 181
atccagcgca gctattcgct cgtcaacccg cagggcgaga gggaccgtta cgtgatcacg 241
gtcaacctcg accgcaacag ccgggggcggt tcgcgctacc tccacgagca gttgcgggtc 301
gggcagcgcc tgtccatcgt accgcccgcc aataatttcg ccctggtgga gacagccccc 361
cactccgtcc tgttcgcggg cggcatcggc atcacgccga tctggtcgat gatccaacgg 421
ttgcgggaac tcggttccac ctgggagctt caggacgcct gtcgcggcaa ggatttcgtc 481
gcctaccgcc aggaactgga gcaggcggcg gcggaggctg gagcgagatt ccacctccac 541
ctcgatgaag aggccgacgg caaattcctg gacctggcgg gccccgtggc gcaggcgggc 601
caggacagca tcttctattg ctgcggccct gaggcgatgc tccaggccta taaggcggcg 661
acgggcacct gccgtccgaa cgggtgcggt tcgaacattc tgcgcggcct tgacggcgaa 721
ccggcggacg acgtgttcac ggtcgtcgtg gcgcggcggt ccggccagga attcacggtc 781
gaaccgggga tgacgatcct ggagacgctg ctccagaacg gcataagccg gaattactcc 841
tgcacccagg gcgtctgcgg cacctgcgag accaaggtgc tggagggcga acccgatcat 901
cgcgactggg tcttgtccga cgagaagaag gcgtcaaatt cgaccatgtt gatctgctgt 961
tcgctgagca aatccccgcg gctggtgctg gacatctgaa tcgaccgctc gaaggacgac 1021
```

APPENDIX C

Transposon Tn 903 fragment encodes for kanamycin resistance

DNA sequence 1-955: coordinates from transposon Tn 903 sequence are 1083-2038

NUCLEOTIDE SEQUENCE: 80-895 code for product neomycin phosphotransferase

SOURCE: *E. coli.*

1	ttacattgca	caagataaaa	atatatcatc	atgaacaata	aaactgtctg	cttacataaa	cagtaataca	aggggtgtta
81	tgagccatat	tcaacggga	acgtcttgct	cgaggccgg	attaaattcc	aacatggatg	ctgatttata	tgggtataaa
161	tgggctcgcg	ataatgtcgg	gcaatcaggt	gcgacaatct	actgattgta	tgggaagcc	gatgcgccg	agttgtttct
241	gaaacatgc	aaaggtagg	ttgccaatga	tattacagat	gagatggtca	gactaaactg	gctgacgga	tttatgcctc
321	ttccgaccat	caagcatttt	atccgtactc	ctgatgatga	atggttactc	accactgca	tccccggga	aacagcattc
401	caggtattag	aagaatatcc	tgattcaggt	gaaaatattg	ttgatgcgct	ggcagtgttc	ctgcgccggt	tgcattcgat
481	tcctgtttgt	aattgtcctt	ttaacagca	tcgcgtattt	cgtctcgctc	aggcgcaac	acgaatgaat	aacggtttgg
561	ttgatgcgag	tgattttgat	gacgagca	atggcaggc	agttgaacaa	gtctcgaaag	aaatgcataa	acttttgcca
641	ttctcaccgg	attcagtcgt	cactcatggt	gatttctcac	ttgataacct	tatttttgac	gaggggaat	taataggttg
721	tattgatgtt	ggacgagtg	gaatcgcaa	ccgataccg	gatcttgcca	tcctatggaa	ctgcctcggt	gagttttctc
801	ttcattaca	gaaacggctt	tttcaaaaat	atggtattga	taatcctgat	atgaataaat	tgcagtttca	tttgatgctc
881	gatgagtttt	tctaatcaga	attggttaat	tggttgtaac	actggcagg	cattacgctg	acttgacggg	acggc

APPENDIX D

Transposon Tn 5 fragment encodes for neomycin and kanamycin resistance

NUCLEOTIDE SEQUENCE: 151-945 code for neomycin phosphotransferase

NUCLEOTIDE SEQUENCE: 13-62; function: aminoglycoside phosphotransferase promotor.

SOURCE: *E. coli*

```
   1 acagcaagcg aaccggaatt gccagctggg gcgccctctg gtaaggttgg gaagccctgc
  61 aaagtaaact ggatggcttt cttgccgcca aggatctgat ggcgcagggg atcaagatct
 121 gatcaagaga caggatgagg atcgtttcgc atgattgaac aagatggatt gcacgcaggt
 181 tctccggccg cttgggtgga gaggctattc ggctatgact gggcacaaca gacaatcggc
 241 tgctctgatg ccgccgtgtt ccggctgtca gcgcaggggc gcccggttct ttttgtcaag
 301 accgacctgt ccggtgccct gaatgaactg caggacgagg cagcgcggct atcgtggctg
 361 gccacgacgg gcgttccttg cgcagctgtg ctcgacgttg tcactgaagc gggaagggac
 421 tggctgctat tgggcgaagt gccggggcag gatctcctgt catctcacct tgctcctgcc
 481 gagaaagtat ccatcatggc tgatgcaatg cggcggctgc atacgcttga tccggctacc
 541 tgcccattcg accaccaagc gaaacatcgc atcgagcgag cacgtactcg gatggaagcc
 601 ggtcttgtcg atcaggatga tctggacgaa gagcatcagg ggctcgcgcc agccgaactg
 661 ttcgccaggc tcaaggcgcg catgcccgac ggcgaggatc tcgtcgtgac ccatggcgat
 721 gcctgcttgc cgaatatcat ggtggaaaat ggccgctttt ctggattcat cgactgtggc
 781 cggctgggtg tggcggaccg ctatcaggac atagcgttgg ctacccgtga tattgctgaa
 841 gagcttggcg gcgaatgggc tgaccgcttc ctcgtgcttt acggtatcgc cgctcccgat
 901 tcgcagcgca tcgccttcta tcgccttctt gacgagttct tctgagcggg actctggggt
 961 tcgaaatgac cgaccaagcg acgcccaacc tgccatcacg agatttcgat tccaccgccg
1021 ccttctatga aaggttgggc ttcggaatcg ttttccggga cgccggctgg atgatcctcc
1081 agcgcgggga tctcatgctg gagttcttcg cccaccccgg gctcgatccc ctcgcgagtt
1141 ggttcagctg ctgcctgagg ctggacgacc tcgcggagtt ctaccggcag tgcaaatccg
1201 tcggcatcca ggaaaccagc agcggctatc cgcgcatcca tgcccccgaa ctgcaggagt
1261 ggggaggcac gatggccgct ttggtcgacc cggacgggac
//
```

APPENDIX E

Media composition for bacteria culture

Luria-Bertani (LB), per liter for *E. coli*

10 g Bacto-tryptone

5 g Bacto-yeast extract

10 g NaCl

Mineral salt medium, per liter for *S. chlorophenolica*

0.82 g $K_2 HPO_4$ 3HO

0.19 g $KH_2 PO_4$

0.5 g $NaNO_3$

0.1 g $MgSO_4$ $7H_2 O$

20 μM $FeSO_4$

4 g L-glutamaic acid sodium salt

SOC medium

0.5% (w/v) yeast extract

2% (w/v) tryptone

10 mM NaCl

2.5 mM KCl

10 mM $MgCl_2$

20 mM $MgSO_4$

20 mM glucose

APPENDIX F

Solution and buffer for Southern blot hybridization

Hybridization

10X SSC: 0.15 M NaCitrat, 1.5 M NaCl, pH 7.0

SDS, 10%

Standard hybridization buffer

- 5X SSC
- N-lauroylsarcosine, 0.1% (w/w)
- SDS, 0.02%
- Blocking reagent, 1% (1/10 volumn of blocking solution, 10X conc.)

Immunological detection

Maleic acid buffer: 0.1 M maleic acid, 0.15 M NaCl; adjusted to pH 7.5

Blocking solution: diluting the 10X blocking solution 1:10 in maleic acid buffer

Washing buffer: Malic acid buffer plus 0.3% Tween 20 (v/V)

Detection buffer: 0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5

BIOGRAPHY

Miss Panarat Arunrattiyakorn was bone on November 20, 1973 in Bangkok. She graduated with the degree of Bachelor of Science from the Department of Biotechnology at King Moungkut Institute of Technology, Ladkabang (KMITL) in 1995. She had studied for the degree of Master of Science at the Department of Biochemistry, Chulalongkorn University since 1996.