REFERENCES

1. Dzubak, P., et al., *Pharmacological activities of natural triterpenoids and their therapeutic implications.* Natural Product Reports, 2006. **23**(3): p. 394-411.
2. Salvador, J.A.R., et al., *Ursane-type pentacyclic triterpenoids as useful platforms to discover anticancer drugs.* Natural Product Reports, 2012. **29**(12): p. 1463-1479.
3. K. U. Wendt, et al., *Enzyme Mechanisms for Polycyclic Triterpene Formation.* Angewandte Chemie International Edition, 2000. **39**: p. 2812-2833.
4. Phillips, D.R., et al., *Biosynthetic diversity in plant triterpene cyclization.* Current Opinion in Plant Biology, 2006. **9**(3): p. 305-314.
5. Gupta, N.C., B. Singh, and D.S. Bhakuni, *Steroids and triterpenes from Alangium lamarckii, Allamanda cathartica, Abrus precatorius and Holoptelea integrifolia.* Phytochemistry, 1969. **8**(4): p. 791-792.
6. Abe, I., M. Rohmer, and G.D. Prestwich, *Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes.* Chemical Reviews, 1993. **93**(6): p. 2189-2206.
7. Xu, R., G.C. Fazio, and S.P.T. Matsuda, *On the origins of triterpenoid skeletal diversity.* Phytochemistry, 2004. **65**(3): p. 261-291.
8. Wang Z., et al., *Two oxidosqualene cyclases responsible for biosynthesis of tomato fruit cuticular triterpenoids.* Plant Physiology, 2011. **155**(1): p. 540-552.
9. Wang, Z., et al., *Cloning and Characterization of Oxidosqualene Cyclases from Kalanchoe daigremontiana.* Journal of Biological Chemistry, 2010. **285**(39): p. 29703–29712.
10. Wendt, K.U., et al., *Crystallization and preliminary X-ray crystallographic analysis of squalene-hopene cyclase from Alicyclobacillus acidocaldarius.* Protein Science, 1997. **6**(3): p. 722-724.
11. Thoma, R., et al., *Insight into steroid scaffold formation from the structure of human oxidosqualene cyclase.* Nature, 2004. **432**(7013): p. 118-122.
12. Siedenburg, G. and D. Jendrossek, *Squalene-Hopene Cyclases.* Applied and Envisonmental Microbiology, 2011. **77**(12): p. 3905–3915.
13. Schulz–Gasch, T. and M. Stahl, *Mechanistic insights into oxidosqualene cyclizations through homology modeling.* Journal of Computational Chemistry, 2003. **24**(6): p. 741-753.
14. Haralampidis, K., M. Trojanowska, and A. Osbourn, *Biosynthesis of Triterpenoid Saponins in Plants*, in *History and Trends in Bioprocessing and*

Biotransformation, N.N. Dutta, et al., Editors. 2002, Springer Berlin Heidelberg. p. 31-49.

15. Kushiro, T., et al., *Mutational Studies on Triterpene Synthases: Engineering Lupeol Synthase into β-Amyrin Synthase.* Journal of the American Chemical Society, 2000. **122**(29): p. 6816-6824.
16. Shibuya, M., et al., *Origin of structural diversity in natural triterpenes: direct synthesis of seco-triterpene skeletons by oxidosqualene cyclase.* Journal of the American Chemical Society, 2007. **129**(5): p. 1450-1455
17. Herrera J.B.R., et al., *Cloning and characterization of the Arabidopsis thaliana lupeol synthase gene.* Phytochemistry, 1998. **49**(7): p. 1905-1911.
18. Kushiro T., et al., *A novel multifunctional triterpene synthase from Arabidopsis thaliana.* Tetrahedron Letter, 2000. **41**: p. 7705-7710.
19. Corey E. J., Matsuda S. P., and B. B., *Isolation of an Arabidopsis thaliana gene encoding cycloartenol synthase by functional expression in a yeast mutant lacking lanosterol synthase by the use of a chromatographic screen.* Proceedings of the National Academy of Sciences of the United States of America, 1993. 90(24): p. 11628–11632.
20. Suzuki M., et al., *Lanosterol synthase in dicotyledonous plants.* Plant Cell Physiology, 2006. **47**(5): p. 565-571.
21. Kolesnikova, M.D., et al., *Lanosterol biosynthesis in plants.* Archives of Biochemistry and Biophysics, 2006. **447**(1): p. 87-95.
22. Shibuya, M., et al., *Identification of a product specific β-amyrin synthase from Arabidopsis thaliana.* Plant Physiology and Biochemistry, 2009. 47(1): p. 26-30.
23. Shibuya, M., et al., *Two branches of the lupeol synthase gene in the molecular evolution of plant oxidosqualene cyclases.* European Journal of Biochemistry, 1999. **266**(1): p. 302-307.
24. You, S., et al., *Molecular cloning and sequencing of an Allium macrostemon cDNA probably encoding oxidosqualene cyclase.* Plant Biotechnology, 1999. **16**: p. 311-314.
25. Zhang, H., et al., *Oxidosqualene cyclases from cell suspension cultures of Betula platyphylla var. japonica: molecular evolution of oxidosqualene cyclases in higher plants.* Biological and Pharmaceutical Bulletin, 2003. **26**(5): p. 642-650.
26. Suzuki, H., et al., *A genomics approach to the early stages of triterpene saponin biosynthesis in Medicago truncatula.* The Plant Journal, 2002. **32**(6): p. 1033-1048.

27. Cammareri, M., et al., *Molecular characterization of ß-amyrin synthase from Aster sedifolius L. and triterpenoid saponin analysis.* Plant Science, 2008. **175**(3): p. 255-261.

28. Basyuni, M., et al., *Molecular cloning and functional expression of a multifunctional triterpene synthase cDNA from a mangrove species Kandelia candel (L.) Druce.* Phytochemistry, 2006. **67**(23): p. 2517-2524.

29. Morita, M., et al., *Molecular cloning and functional expression of triterpene synthases from pea (Pisum sativum).* European Journal of Biochemistry, 2000. **267**(12): p. 3453-3460.

30. Hayashi, H., et al., *Molecular cloning and characterization of isomultiflorenol synthase, a new triterpene synthase from Luffa cylindrica, involved in biosynthesis of bryonolic acid.* European Journal of Biochemistry, 2001. **268**(23): p. 6311-6317.

31. Hayashi, H., et al., *Differential Expression of Three Oxidosqualene Cyclase mRNAs in Glycyrrhiza glabra.* Biological and Pharmaceutical Bulletin, 2004. **27**(7): p. 1086-1092.

32. Kushiro, T., M. Shibuya, and Y. Ebizuka, *ß-Amyrin synthase.* European Journal of Biochemistry, 1998. **256**(1): p. 238-244.

33. Tansakul, P., et al., *Dammarenediol-II synthase, the first dedicated enzyme for ginsenoside biosynthesis, in Panax ginseng.* FEBS Letters, 2006. **580**(22): p. 5143-5149.

34. Saimaru, H., et al., *Production of triterpene acids by cell suspension cultures of Olea europaea.* Chemical and Pharmaceutical Bulletin 2007. **55**(5): p. 784-788.

35. Iturbe-Ormaetxe, I., et al., *Molecular cloning and characterization of triterpene synthases from Medicago truncatula and Lotus japonicus.* Plant Molecular Biology, 2003. **51**(5): p. 731-743.

36. Sawai, S., et al., *Plant Lanosterol Synthase: Divergence of the Sterol and Triterpene Biosynthetic Pathways in Eukaryotes.* Plant and Cell Physiology, 2006. **47**(5): p. 673-677.

37. Huang, L., et al., *Molecular characterization of the pentacyclic triterpenoid biosynthetic pathway in Catharanthus roseus.* Planta, 2012. **236**(5): p. 1571-1581.

38. Sawai, S., et al., *Functional and structural analysis of genes encoding oxidosqualene cyclases of Lotus japonicus.* Plant Science, 2006. **170**(2): p. 247-257.

39. The Botanical Garden Organization Plant Database. ปรู๋. [online] 2011 [cited 2013 16 January]; Available from: http://www.qsbg.org/database/botanic_book%20full%20option/search_detail.asp?botanic_id=1057.

40. เสรมสิริ วินิจฉัยกุล, et al., ปรู๋. 1 ed. สมุนไพร ไม้พื้นบ้าน. Vol. 2. 2541, กรุงเทพมหานคร: บริษัท ประชาชน จำกัด.

41. Itoh, A., et al., *Two Alangium Alkaloids from Alangium lamarckii.* Journal of Natural Products, 2000. **63**(5): p. 723-725.

42. Pailee, P., et al., *Protoberberine Alkaloids and Cancer Chemopreventive Properties of Compounds from Alangium salviifolium.* European Journal of Organic Chemistry, 2011. **2011**(20-21): p. 3809-3814.

43. Dasgupta, B., *Chemical investigations of Alangiun lamarckii I. Isolation of a new alkaloid, ankorine, from the leaves.* Journal of Pharmaceutical Sciences, 1965. **54**(3): p. 481-483.

44. Gietz, R.D. and A.R. Woods, *Yeast Transformation by the LiAc/SS Carrier DNA/PEG Method*, in *Yeast Protocol*. 2006, Springer. p. 107-120.

45. Tansakul, P., et al. *cDNA Cloning of Triterpene Synthase from Alangium lamarckii Leaves*. in *The 5th International Conference on Plant Metabolomics*. 15-18 July, 2008. Pacifico Yokohama.

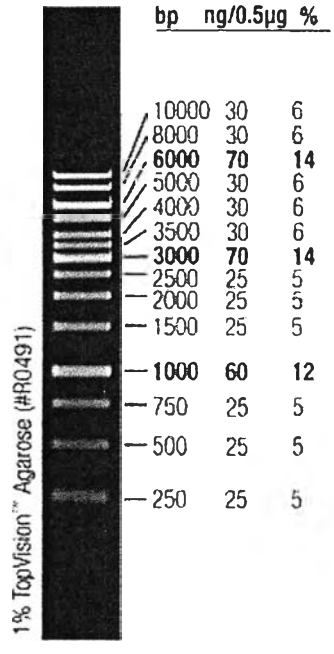
46. Sambrook, J., E.F. Fritsch, and T. Maniatis, *Molecular Cloning: A Laboratory Manual*. 2 ed. 1989, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

47. Baker, C.H., et al., *Molecular-Cloning of the Human Gene Encoding Lanosterol Synthase from a Liver cDNA Library.* Biochemical and Biophysical Research Communications, 1995. **213**(1): p. 154-160.

APPENDIX

Appendix A

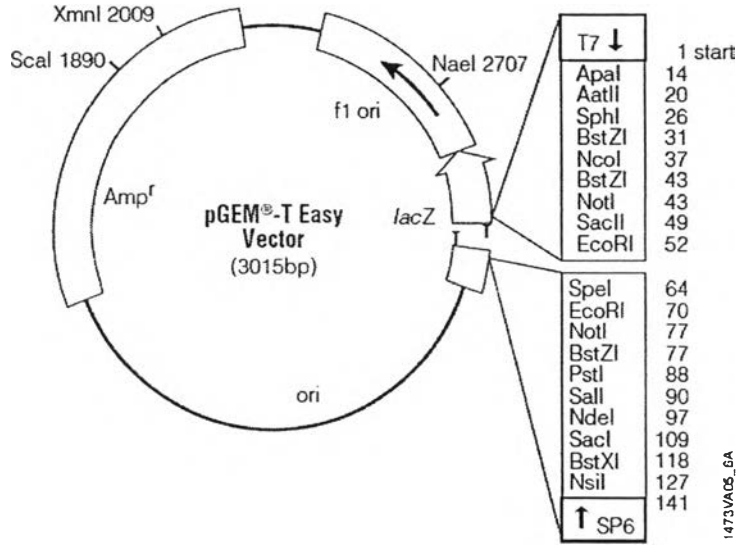
GeneRuler™ 1 kb DNA Ladder



0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

1 kb DNA marker (FERMENTAS)

Appendix B

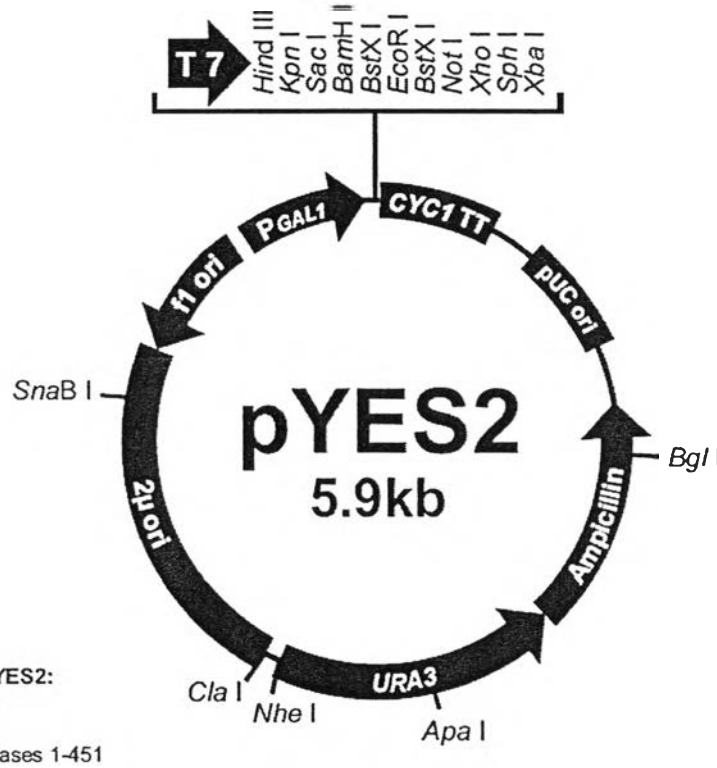


pGEM®-T Easy Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–128
SP6 RNA polymerase promoter (–17 to +3)	139–158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176–197
lacZ start codon	180
lac operator	200–216
β-lactamase coding region	1337–2197
phage f1 region	2380–2835
lac operon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA polymerase promoter (–17 to +3)	2999–3

pGEM-T Easy Vector map and sequence reference points.

Appendix C

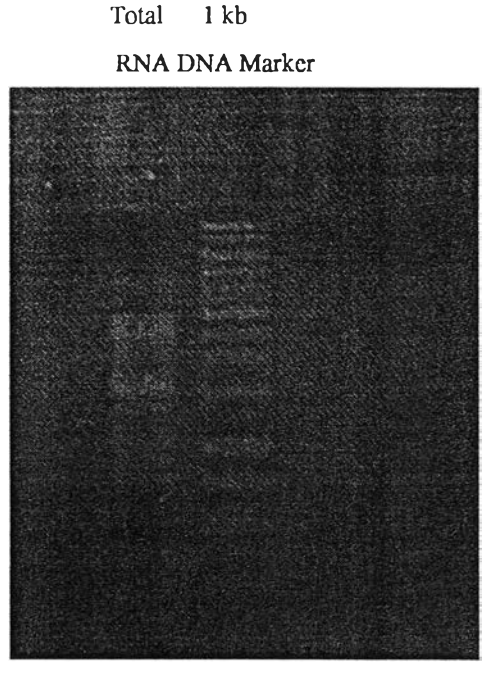


Comments for pYES2:
5856 nucleotides

GAL1 promoter: bases 1-451
T7 promoter/priming site: bases 475-494
Multiple cloning site: bases 501-600
CYC1 transcription terminator: bases 608-856
pUC origin: bases 1038-1711
Ampicillin resistance gene: bases 1856-2716 (C)
URA3 gene: bases 2734-3841 (C)
2 micron (µ) origin: bases 3845-5316
f1 origin: bases 5384-5839 (C)
(C) = complementary strand

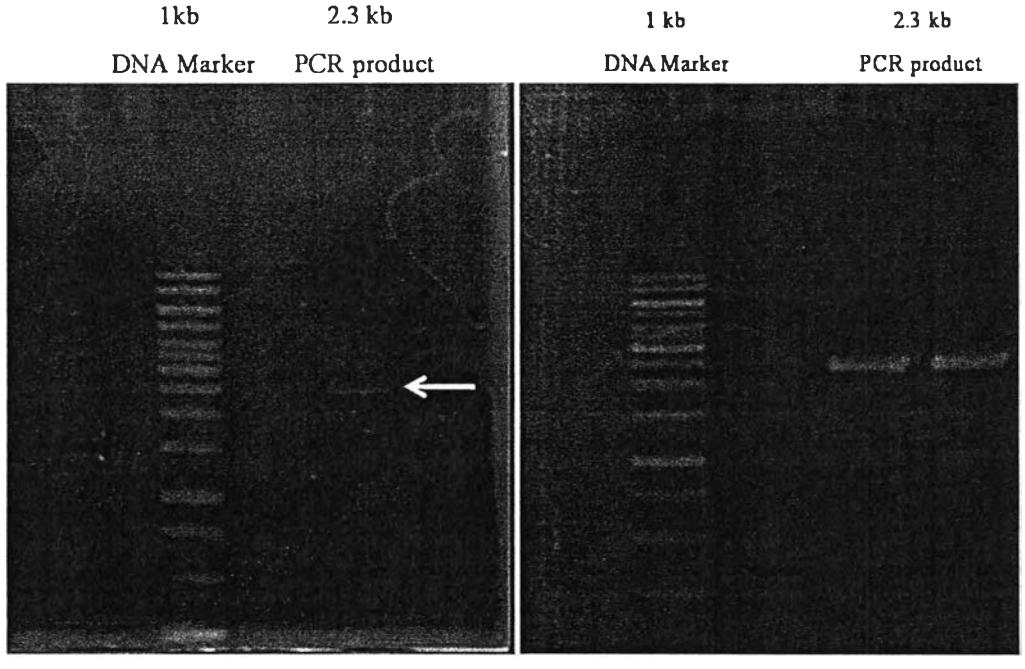
pYES-2 Vector map and sequence reference sites.

Appendix D



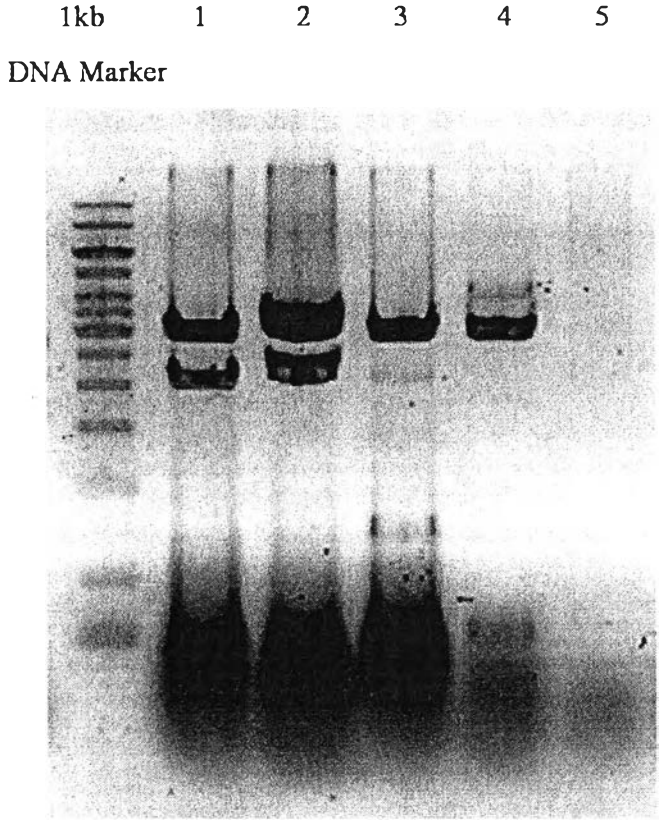
Total RNA isolated from *A. lamarckii* leaves

Appendix E



2.3 kb PCR product using cDNA from *A. lamarckii* leaves as template. Left. First PCR and right, second PCR using the same condition.

Appendix F

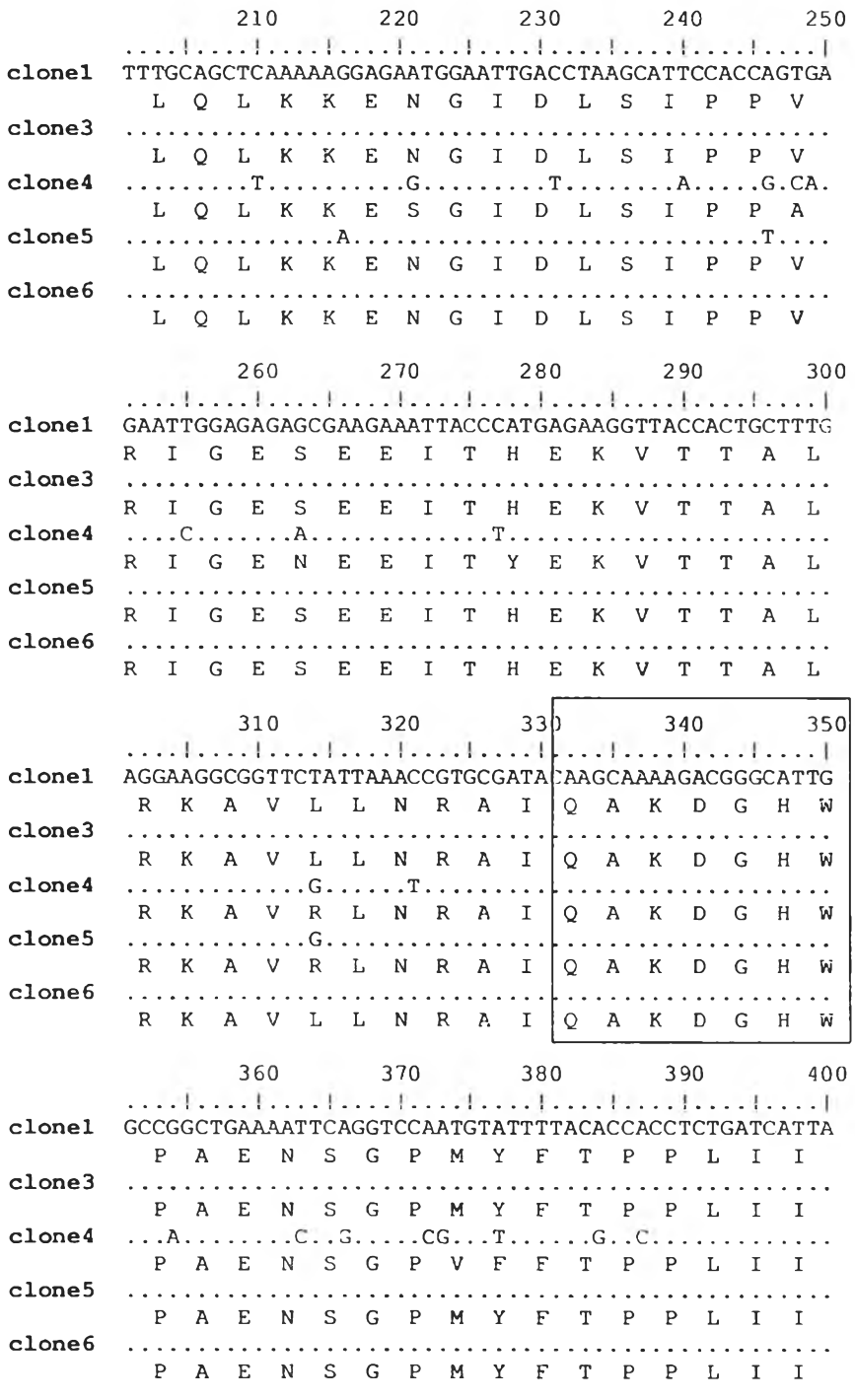


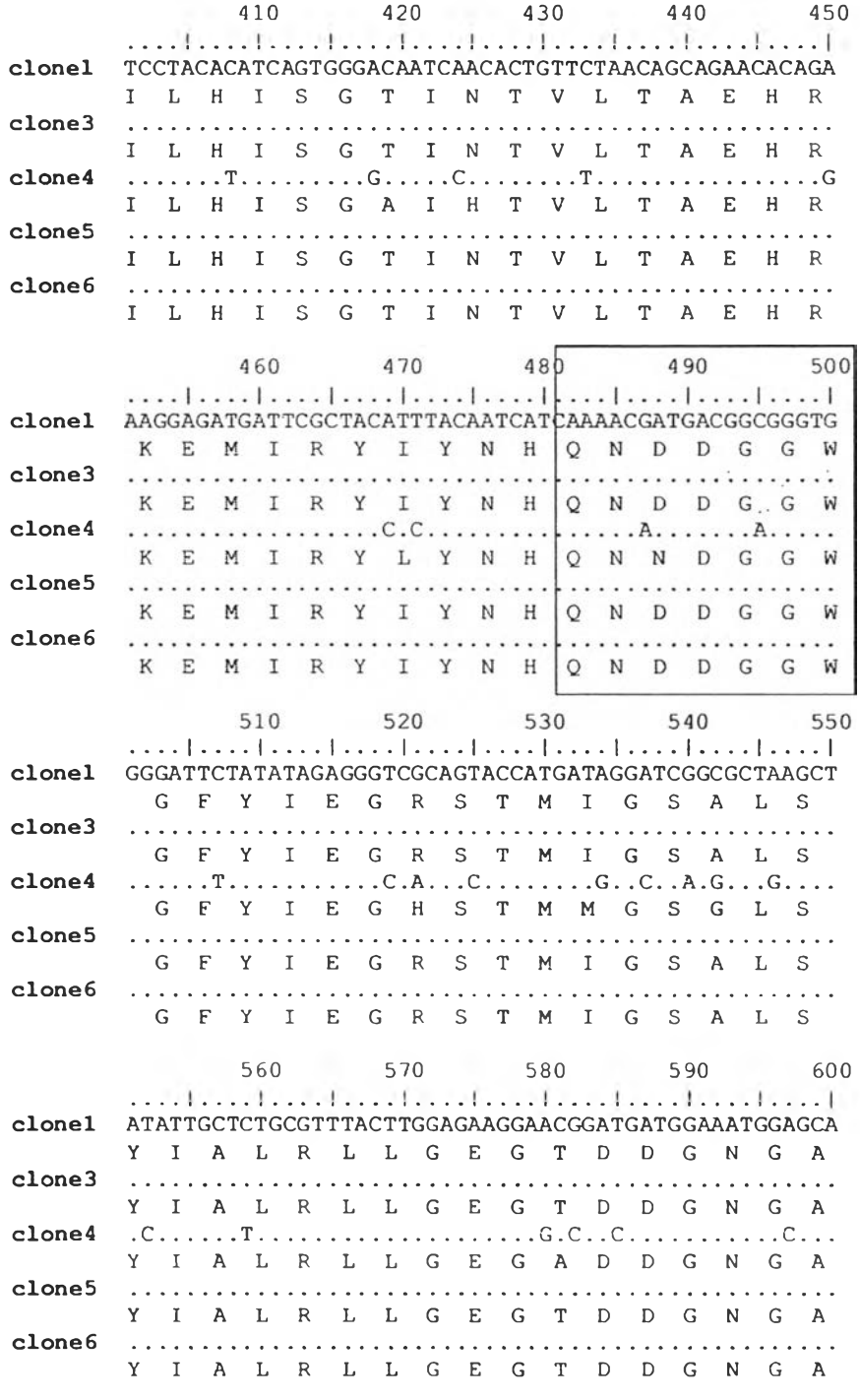
Example of plasmid liagation checking by using *EcoR*I digestion, lane 1 and lane 2 showed expected insertion of the bands at 2.3 kb, while lane 3, 4 and 5 are false positive results of white colonies.

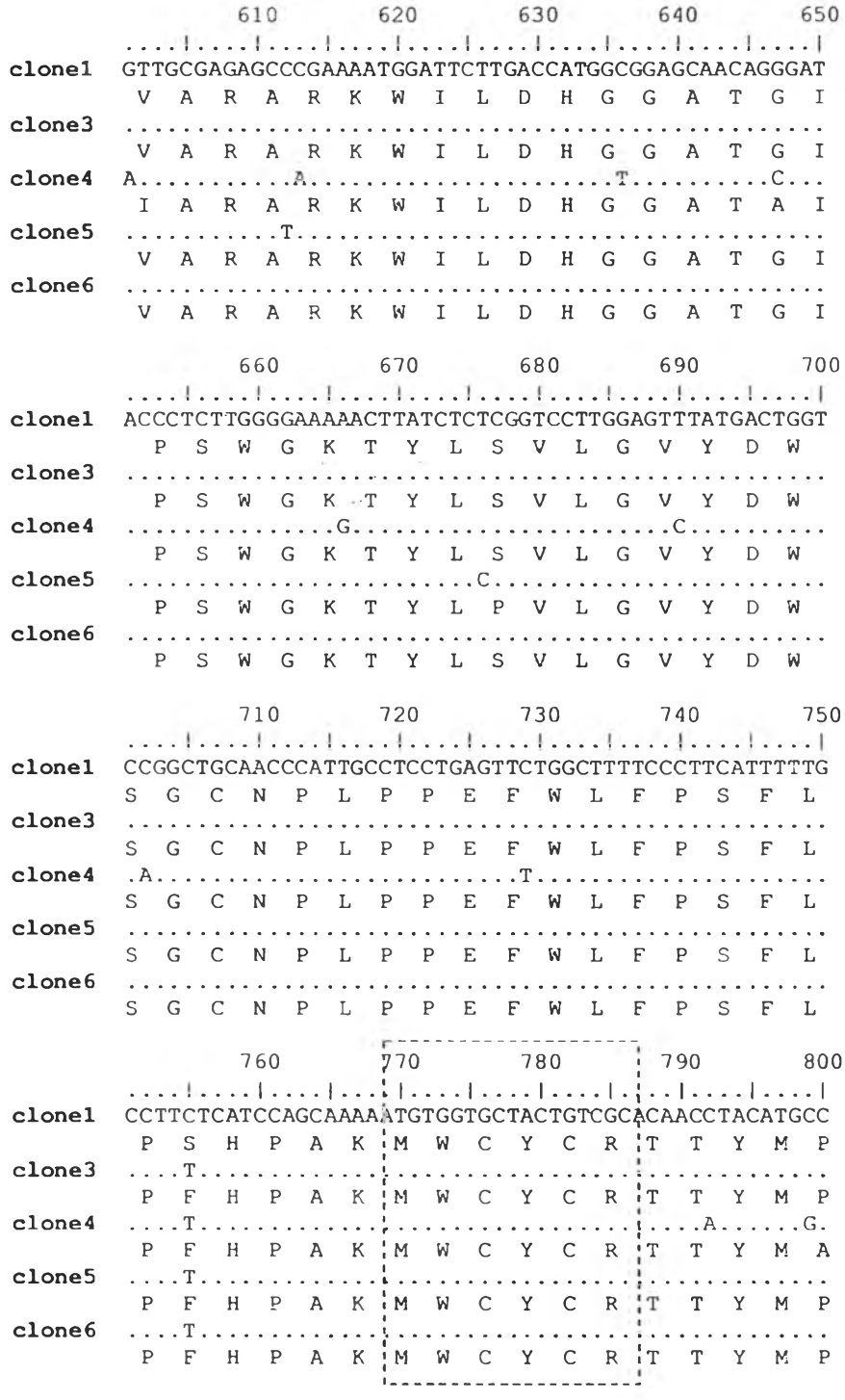
Appendix G

Nucleotide sequence and translated amino acid sequence alignments of *AlOSCs*. QW motif is squared with single line. MWCYCR motif is squared with dot line. And DCTAE motif is squared with double line.

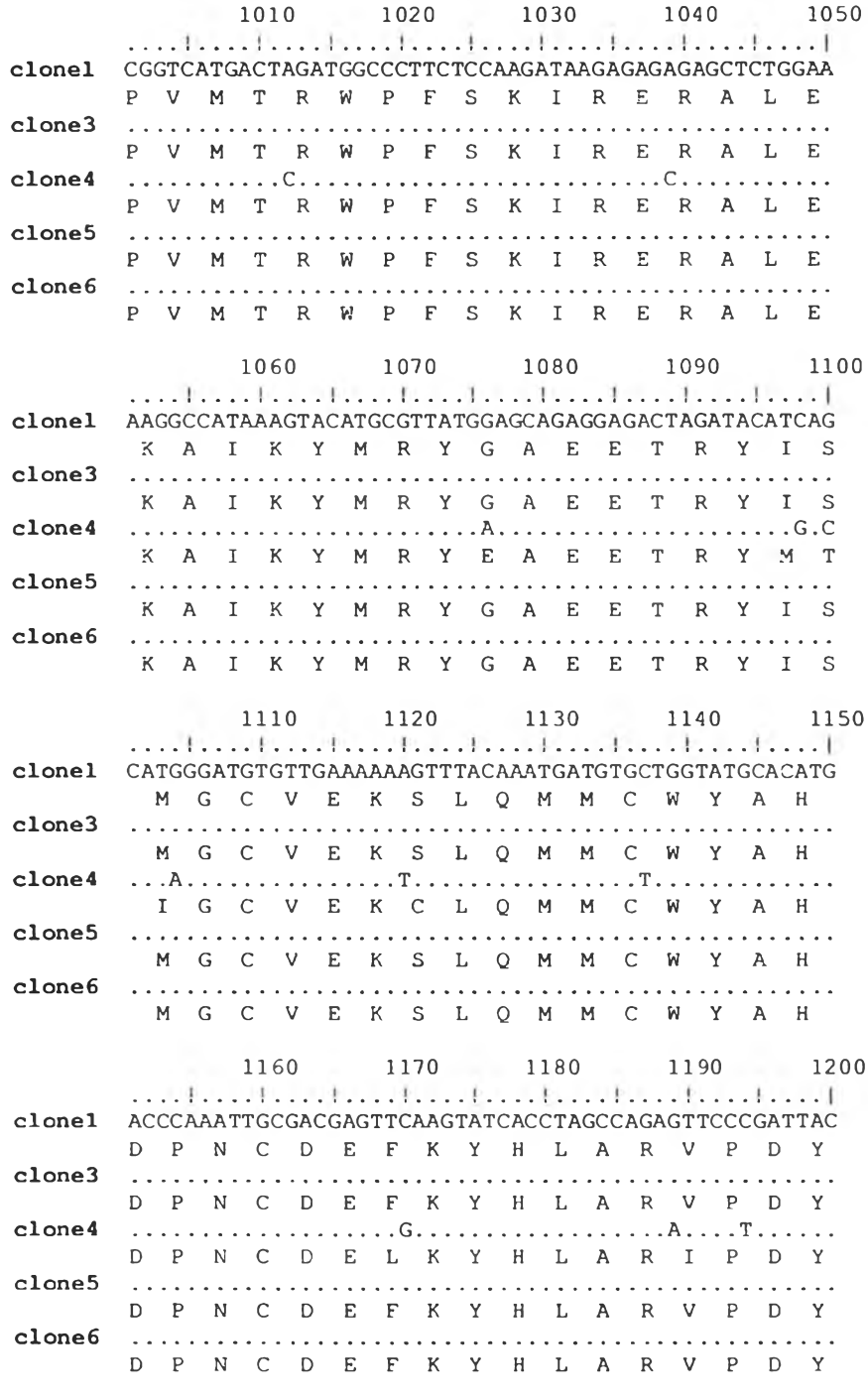
```
                10        20        30        40        50
          ....|....|....|....|....|....|....|....|....|....|
clone1    ATGTGGAGGCTGAAAGTAGCAGAAGGGCATGGGCCATGGTTGTACAGCAC
           M  W  R  L  K  V  A  E  G  H  G  P  W  L  Y  S  T
clone3    ..................................................
           M  W  R  L  K  V  A  E  G  H  G  P  W  L  Y  S  T
clone4    ..........................C.....G...C..........
           M  W  R  L  K  V  A  E  G  H  G  P  W  L  Y  S  T
clone5    ..................................................
           M  W  R  L  K  V  A  E  G  H  G  P  W  L  Y  S  T
clone6    ..................................................
           M  W  R  L  K  V  A  E  G  H  G  P  W  L  Y  S  T

                60        70        80        90       100
          ....|....|....|....|....|....|....|....|....|....|
clone1    CAACAACTTTGTTGGTAGACAAATATGGGAATTTGACCATAAAGCAGGCA
            N  N  F  V  G  R  Q  I  W  E  F  D  H  K  A  G
clone3    ..................................................
            N  N  F  V  G  R  Q  I  W  E  F  D  H  K  A  G
clone4    ..............C.......C............T.CCG.G.......
            N  N  F  V  G  R  Q  T  W  E  F  D  P  E  A  G
clone5    ..................................................
            N  N  F  V  G  R  Q  I  W  E  F  D  H  K  A  G
clone6    ..................................................
            N  N  F  V  G  R  Q  I  W  E  F  D  H  K  A  G

               110       120       130       140       150
          ....|....|....|....|....|....|....|....|....|....|
clone1    CTCCAGAGGAGCGAGCAGAGGTCGACAAAGCTCGAGAGAGCTACCGAAAA
           T  P  E  E  R  A  E  V  D  K  A  R  E  S  Y  R  K
clone3    ..................................................
           T  P  E  E  R  A  E  V  D  K  A  R  E  S  Y  R  K
clone4    ..................A.....G...CT.......GA.....AG...
           T  P  E  E  R  A  E  V  E  K  L  R  E  D  Y  Q  K
clone5    ..................................................
           T  P  E  E  R  A  E  V  D  K  A  R  E  S  Y  R  K
clone6    ..................................................
           T  P  E  E  R  A  E  V  D  K  A  R  E  S  Y  R  K

               160       170       180       190       200
          ....|....|....|....|....|....|....|....|....|....|
clone1    AACAGGAAAAATATGTCTGGTGCTGAAGCTTGTGGTGATCTCATAATGCG
            N  R  K  N  M  S  G  A  E  A  C  G  D  L  I  M  R
clone3    ..................................................
            N  R  K  N  M  S  G  A  E  A  C  G  D  L  I  M  R
clone4    .....A......C.A........C...C......................
            N  R  K  N  L  S  G  A  E  P  C  G  D  L  I  M  R
clone5    ..................................................
            N  R  K  N  M  S  G  A  E  A  C  G  D  L  I  M  R
clone6    ..................................................
            N  R  K  N  M  S  G  A  E  A  C  G  D  L  I  M  R
```


210 220 230 240 250
clone1 TTTGCAGCTCAAAAAGGAGAATGGAATTGACCTAAGCATTCCACCAGTGA
L Q L K K E N G I D L S I P P V
clone3 .....
L Q L K K E N G I D L S I P P V
clone4 .....T.....G.....T.....A.....G.CA.
L Q L K K E S G I D L S I P P A
clone5 .....A.....T....
L Q L K K E N G I D L S I P P V
clone6 .....
L Q L K K E N G I D L S I P P V
260 270 280 290 300
clone1 GAATTGGAGAGAGCGAAGAAATTACCCATGAGAAGGTTACCACTGCTTTG
R I G E S E E I T H E K V T T A L
clone3 .....
R I G E S E E I T H E K V T T A L
clone4 ....C.....A.....T.....
R I G E N E E I T Y E K V T T A L
clone5 .....
R I G E S E E I T H E K V T T A L
clone6 .....
R I G E S E E I T H E K V T T A L
310 320 330 340 350
clone1 AGGAAGGCGGTTCTATTAAACCGTGCGATACAAGCAAAAGACGGGCATTG
R K A V L L N R A I Q A K D G H W
clone3 .....
R K A V L L N R A I Q A K D G H W
clone4 .....G.....T.....
R K A V R L N R A I Q A K D G H W
clone5 .....G.....
R K A V R L N R A I Q A K D G H W
clone6 .....
R K A V L L N R A I Q A K D G H W
360 370 380 390 400
clone1 GCCGGCTGAAAATTCAGGTCCAATGTATTTTACACCACCTCTGATCATTA
P A E N S G P M Y F T P P L I I
clone3 .....
P A E N S G P M Y F T P P L I I
clone4 ...A.....C..G.....CG...T.....G..C.....
P A E N S G P V F F T P P L I I
clone5 .....
P A E N S G P M Y F T P P L I I
clone6 .....
P A E N S G P M Y F T P P L I I

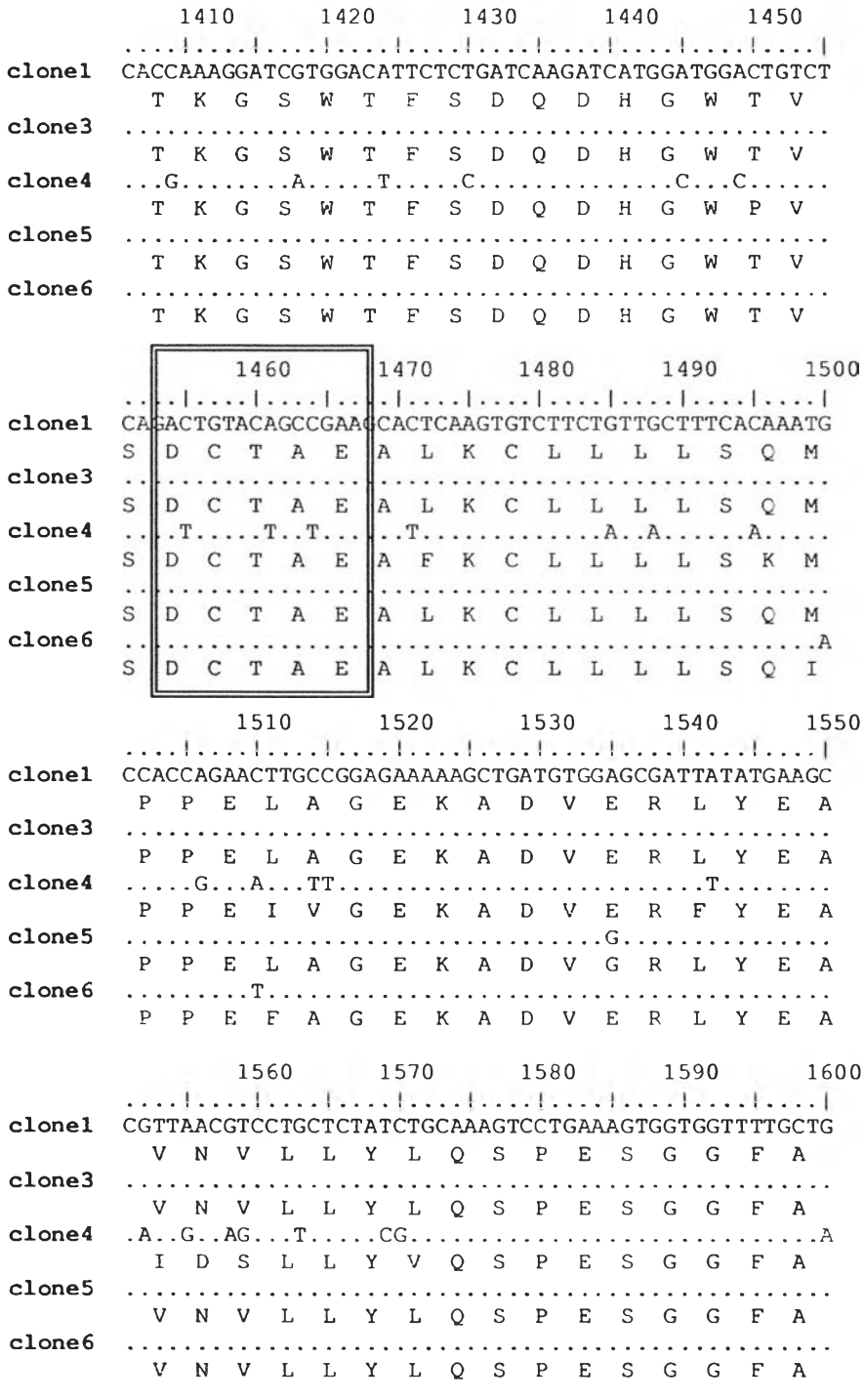

410 420 430 440 450
clone1 TCCTACACATCAGTGGGACAATCAACACTGTTCTAACAGCAGAACACAGA
I L H I S G T I N T V L T A E H R
clone3 .....
I L H I S G T I N T V L T A E H R
clone4 .....T.....G.....C.....T.....G
I L H I S G A I H T V L T A E H R
clone5 .....
I L H I S G T I N T V L T A E H R
clone6 .....
I L H I S G T I N T V L T A E H R
460 470 480 490 500
clone1 AAGGAGATGATTCGCTACATTTACAATCATCAAAACGATGACGGCGGGTG
K E M I R Y I Y N H Q N D D G G W
clone3 .....
K E M I R Y I Y N H Q N D D G G W
clone4 .....C.C.....A.....A.....
K E M I R Y L Y N H Q N N D G G W
clone5 .....
K E M I R Y I Y N H Q N D D G G W
clone6 .....
K E M I R Y I Y N H Q N D D G G W
510 520 530 540 550
clone1 GGGATTCTATATAGAGGGTCGCAGTACCATGATAGGATCGGCGCTAAGCT
G F Y I E G R S T M I G S A L S
clone3 .....
G F Y I E G R S T M I G S A L S
clone4 .....T.....C.A...C.....G..C..A.G...G....
G F Y I E G H S T M M G S G L S
clone5 .....
G F Y I E G R S T M I G S A L S
clone6 .....
G F Y I E G R S T M I G S A L S
560 570 580 590 600
clone1 ATATTGCTCTGCGTTTACTTGGAGAAGGAACGGATGATGGAAATGGAGCA
Y I A L R L L G E G T D D G N G A
clone3 .....
Y I A L R L L G E G T D D G N G A
clone4 .C.....T.....G.C..C.....C...
Y I A L R L L G E G A D D G N G A
clone5 .....
Y I A L R L L G E G T D D G N G A
clone6 .....
Y I A L R L L G E G T D D G N G A


610 620 630 640 650
clone1 GTTGCGAGAGCCCGAAAATGGATTCTTGACCATGGCGGAGCAACAGGGAT
V A R A R K W I L D H G G A T G I
clone3 .....
V A R A R K W I L D H G G A T G I
clone4 A.....A.....T.....C...
I A R A R K W I L D H G G A T A I
clone5 .....T.....
V A R A R K W I L D H G G A T G I
clone6 .....
V A R A R K W I L D H G G A T G I
660 670 680 690 700
clone1 ACCCTCTTGGGGAAAAACTTATCTCTCGGTCCTTGGAGTTTATGACTGGT
P S W G K T Y L S V L G V Y D W
clone3 .....
P S W G K T Y L S V L G V Y D W
clone4 .....G.....C.....
P S W G K T Y L S V L G V Y D W
clone5 .....C.....
P S W G K T Y L P V L G V Y D W
clone6 .....
P S W G K T Y L S V L G V Y D W
710 720 730 740 750
clone1 CCGGCTGCAACCCATTGCCTCCTGAGTTCTGGCTTTTCCCTTCATTTTTG
S G C N P L P P E F W L F P S F L
clone3 .....
S G C N P L P P E F W L F P S F L
clone4 .A.....T.....
S G C N P L P P E F W L F P S F L
clone5 .....
S G C N P L P P E F W L F P S F L
clone6 .....
S G C N P L P P E F W L F P S F L
760 770 780 790 800
clone1 CCTTCTCATCCAGCAAAAATGTGGTGCTACTGTCGCACAACCTACATGCC
P S H P A K M W C Y C R T T Y M P
clone3 ....T.....
P F H P A K M W C Y C R T T Y M P
clone4 ....T.....A.....G.
P F H P A K M W C Y C R T T Y M A
clone5 ....T.....
P F H P A K M W C Y C R T T Y M P
clone6 ....T.....
P F H P A K M W C Y C R T T Y M P

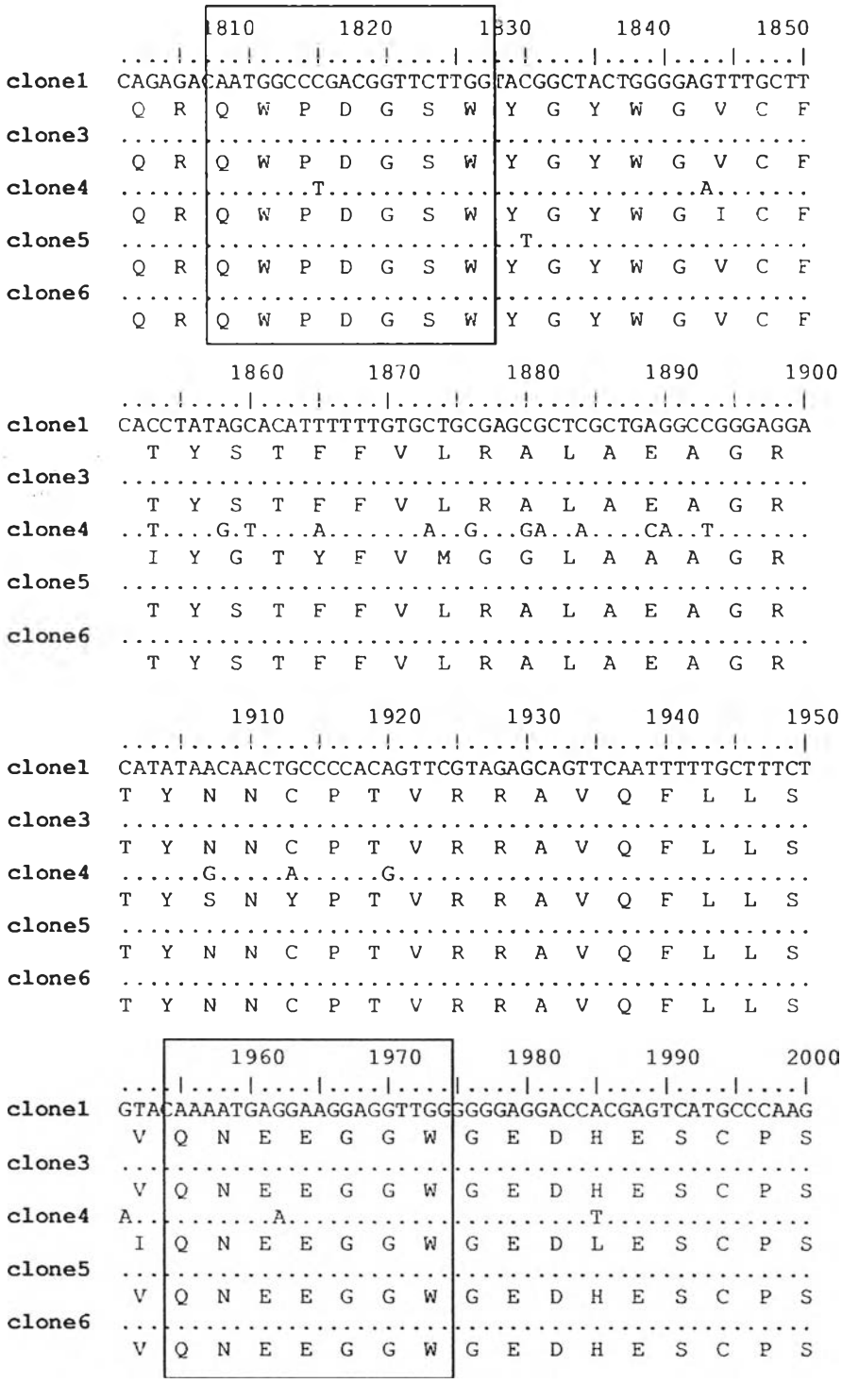
```
                 810        820        830        840        850
          ....|....|....|....|....|....|....|....|....|....|....|
clone1    CATGTCCTATTTGTATGGAAGGAAGTATTCTGGGCCGATCACAGATCTTG
            M  S  Y  L  Y  G  R  K  Y  S  G  P  I  T  D  L
clone3    ..................................................
            M  S  Y  L  Y  G  R  K  Y  S  G  P  I  T  D  L
clone4    ......................G...........................
            M  S  Y  L  Y  G  R  R  Y  S  G  P  I  T  D  L
clone5    ..................................................
            M  S  Y  L  Y  G  R  K  Y  S  G  P  I  T  D  L
clone6    ..................................................
            M  S  Y  L  Y  G  R  K  Y  S  G  P  I  T  D  L

                 860        870        880        890        900
          ....|....|....|....|....|....|....|....|....|....|....|
clone1    TGAAGTCATTGAGGGAAGAAATTCACACCAGGCCATATGACAAAATTGAT
          V  K  S  L  R  E  E  I  H  T  R  P  Y  D  K  I  D
clone3    ..................................................
          V  K  S  L  R  E  E  I  H  T  R  P  Y  D  K  I  D
clone4    ............A..G............AA.....CC..C.G.......
          V  K  S  L  R  E  E  I  H  T  K  P  Y  H  Q  I  D
clone5    ..................................................
          V  K  S  L  R  E  E  I  H  T  R  P  Y  D  K  I  D
clone6    ..................................................
          V  K  S  L  R  E  E  I  H  T  R  P  Y  D  K  I  D

                 910        920        930        940        950
          ....|....|....|....|....|....|....|....|....|....|....|
clone1    TGGAACAGGGCACGCAACGACTGTTGCAAGGAGGATCTCTACTACCCTCA
           W  N  R  A  R  N  D  C  C  K  E  D  L  Y  Y  P  H
clone3    ..................................................
           W  N  R  A  R  N  D  C  C  K  E  D  L  Y  Y  P  H
clone4    .......A.......C.TC..............................
           W  N  K  A  R  H  H  C  C  K  E  D  L  Y  Y  P  H
clone5    ..........................................G.......
           W  N  R  A  R  N  D  C  C  K  E  D  L  Y  C  P  H
clone6    ..................................................
           W  N  R  A  R  N  D  C  C  K  E  D  L  Y  Y  P  H

                 960        970        980        990       1000
          ....|....|....|....|....|....|....|....|....|....|....|
clone1    TAGTTTCGTCCAAGATCTGCTGTGGGACACTCTTCATTACTTCAGCGAGC
            S  F  V  Q  D  L  L  W  D  T  L  H  Y  F  S  E
clone3    ..................................................
            S  F  V  Q  D  L  L  W  D  T  L  H  Y  F  S  E
clone4    ...C...A.............A.................T..........
            S  F  I  Q  D  L  L  W  D  T  L  H  Y  F  S  E
clone5    ..................................................
            S  F  V  Q  D  L  L  W  D  T  L  H  Y  F  S  E
clone6    ..................................................
            S  F  V  Q  D  L  L  W  D  T  L  H  Y  F  S  E
```


1010 1020 1030 1040 1050
clone1 CGGTCATGACTAGATGGCCCTTCTCCAAGATAAGAGAGAGAGCTCTGGAA
P V M T R W P F S K I R E R A L E
clone3 .....
P V M T R W P F S K I R E R A L E
clone4 .....C.....C.....
P V M T R W P F S K I R E R A L E
clone5 .....
P V M T R W P F S K I R E R A L E
clone6 .....
P V M T R W P F S K I R E R A L E
1060 1070 1080 1090 1100
clone1 AAGGCCATAAAGTACATGCGTTATGGAGCAGAGGAGACTAGATACATCAG
K A I K Y M R Y G A E E T R Y I S
clone3 .....
K A I K Y M R Y G A E E T R Y I S
clone4 .....A.....G.C
K A I K Y M R Y E A E E T R Y M T
clone5 .....
K A I K Y M R Y G A E E T R Y I S
clone6 .....
K A I K Y M R Y G A E E T R Y I S
1110 1120 1130 1140 1150
clone1 CATGGGATGTGTTGAAAAAAGTTTACAAATGATGTGCTGGTATGCACATG
M G C V E K S L Q M M C W Y A H
clone3 .....
M G C V E K S L Q M M C W Y A H
clone4 ...A.....T.....T.....
I G C V E K C L Q M M C W Y A H
clone5 .....
M G C V E K S L Q M M C W Y A H
clone6 .....
M G C V E K S L Q M M C W Y A H
1160 1170 1180 1190 1200
clone1 ACCCAAATTGCGACGAGTTCAAGTATCACCTAGCCAGAGTTCCCGATTAC
D P N C D E F K Y H L A R V P D Y
clone3 .....
D P N C D E F K Y H L A R V P D Y
clone4 .....G.....A....T.....
D P N C D E L K Y H L A R I P D Y
clone5 .....
D P N C D E F K Y H L A R V P D Y
clone6 .....
D P N C D E F K Y H L A R V P D Y

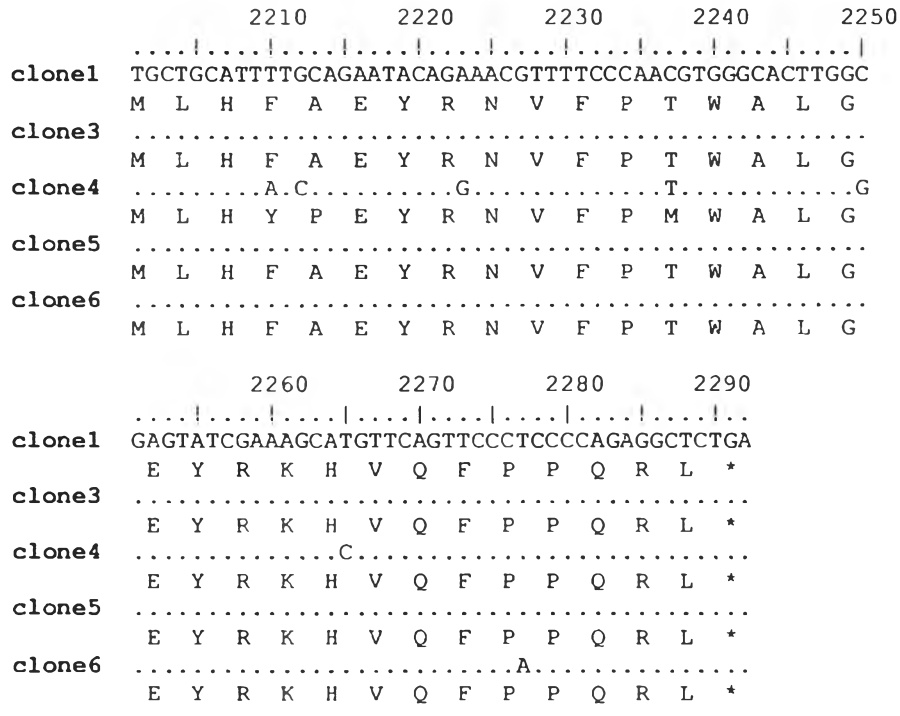
```
                1210        1220        1230        1240        1250
            ....|....|....|....|....|....|....|....|....|....|
clone1      TTGTGGCTTGCCGAAGATGGAATGAAAATGCAGAGCTTTGGGAGTCAGGT
             L  W  L  A  E  D  G  M  K  M  Q  S  F  G  S  Q  V
clone3      ..................................................
             L  W  L  A  E  D  G  M  K  M  Q  S  F  G  S  Q  V
clone4      C.....G.G..T.............C.........C......T......T.
             L  W  V  A  E  D  G  M  T  M  Q  T  F  G  S  Q  L
clone5      ..........T.......................................
             L  W  L  A  E  D  G  M  K  M  Q  S  F  G  S  Q  V
clone6      ..................................................
             L  W  L  A  E  D  G  M  K  M  Q  S  F  G  S  Q  V

                1260        1270        1280        1290        1300
            ....|....|....|....|....|....|....|....|....|....|
clone1      ATGGGACTGTACACTTGCAATTCAGGCACTTATTGCAAGTAATCTTGTTG
              W  D  C  T  L  A  I  Q  A  L  I  A  S  N  L  V
clone3      .....G............................................
              W  G  C  T  L  A  I  Q  A  L  I  A  S  N  L  V
clone4      .......A..G.CT......C.........C..............C....
              W  D  S  A  F  A  T  Q  A  L  I  A  S  N  L  V
clone5      ..................................................
              W  D  C  T  L  A  I  Q  A  L  I  A  S  N  L  V
clone6      ..................................................
              W  D  C  T  L  A  I  Q  A  L  I  A  S  N  L  V

                1310        1320        1330        1340        1350
            ....|....|....|....|....|....|....|....|....|....|
clone1      ATGAGTATGGGGATTCTCTTAAAAAGGCCCACTTTTATCTGAAAGAATCA
            D  E  Y  G  D  S  L  K  K  A  H  F  Y  L  K  E  S
clone3      ..................................................
            D  E  Y  G  D  S  L  K  K  A  H  F  Y  L  K  E  S
clone4      ....A.....A.................G.......TCG.......G..G
            D  E  Y  G  D  S  L  K  K  A  H  F  F  V  K  E  S
clone5      ..................................................
            D  E  Y  G  D  S  L  K  K  A  H  F  Y  L  K  E  S
clone6      ..................................................
            D  E  Y  G  D  S  L  K  K  A  H  F  Y  L  K  E  S

                1360        1370        1380        1390        1400
            ....|....|....|....|....|....|....|....|....|....|
clone1      CAGGTCAAAGAAAACCCAGCTGGTGATTTCACAAGTATGTATCGTCACTT
             Q  V  K  E  N  P  A  G  D  F  T  S  M  Y  R  H  F
clone3      ..................................................
             Q  V  K  E  N  P  A  G  D  F  T  S  M  Y  R  H  F
clone4      ...A....G.........................................
             Q  I  K  E  N  P  A  G  D  F  T  S  M  Y  R  H  F
clone5      ..................................................
             Q  V  K  E  N  P  A  G  D  F  T  S  M  Y  R  H  F
clone6      ................T.................................
             Q  V  K  E  N  P  A  G  D  F  T  S  M  Y  R  H  F
```


1410 1420 1430 1440 1450
clone1 CACCAAAGGATCGTGGACATTCTCTGATCAAGATCATGGATGGACTGTCT
T K G S W T F S D Q D H G W T V
clone3 .....
T K G S W T F S D Q D H G W T V
clone4 ...G.....A.....T.....C.....C...C.....
T K G S W T F S D Q D H G W P V
clone5 .....
T K G S W T F S D Q D H G W T V
clone6 .....
T K G S W T F S D Q D H G W T V
1460 1470 1480 1490 1500
clone1 CAGACTGTACAGCCGAAGCACTCAAGTGTCTTCTGTTGCTTTCACAAATG
S D C T A E A L K C L L L L S Q M
clone3 .....
S D C T A E A L K C L L L L S Q M
clone4 ..T.....T..T...T.....A..A.....A.....
S D C T A E A F K C L L L L S K M
clone5 .....
S D C T A E A L K C L L L L S Q M
clone6 .....A
S D C T A E A L K C L L L L S Q I
1510 1520 1530 1540 1550
clone1 CCACCAGAACTTGCCGGAGAAAAAGCTGATGTGGAGCGATTATATGAAGC
P P E L A G E K A D V E R L Y E A
clone3 .....
P P E L A G E K A D V E R L Y E A
clone4 .....G...A...TT.....T.....
P P E I V G E K A D V E R F Y E A
clone5 .....G.....
P P E L A G E K A D V G R L Y E A
clone6 .....T.....
P P E F A G E K A D V E R L Y E A
1560 1570 1580 1590 1600
clone1 CGTTAACGTCCTGCTCTATCTGCAAAGTCCTGAAAGTGGTGGTTTTGCTG
V N V L L Y L Q S P E S G G F A
clone3 .....
V N V L L Y L Q S P E S G G F A
clone4 .A..G..AG...T.....CG.....A
I D S L L Y V Q S P E S G G F A
clone5 .....
V N V L L Y L Q S P E S G G F A
clone6 .....
V N V L L Y L Q S P E S G G F A

```
                1610        1620        1630        1640        1650
           ....|....|....|....|....|....|....|....|....|....|....|
clone1     CTTGGGAGCCACCAGTTCCACAGCCTTATTTGCAGGTGTTGAATCCTTCT
           A  W  E  P  P  V  P  Q  P  Y  L  Q  V  L  N  P  S
clone3     ..................................................
           A  W  E  P  P  V  P  Q  P  Y  L  Q  V  L  N  P  S
clone4     T.............................AG.............A...
           I  W  E  P  P  V  P  Q  P  Y  L  E  V  L  N  P  S
clone5     ..................................................
           A  W  E  P  P  V  P  Q  P  Y  L  Q  V  L  N  P  S
clone6     ..................................................
           A  W  E  P  P  V  P  Q  P  Y  L  Q  V  L  N  P  S

                1660        1670        1680        1690        1700
           ....|....|....|....|....|....|....|....|....|....|....|
clone1     GAACTTTTTGCTGATATTGTGGTGGAGCAAGAGCATGTTGAATGCACTGC
            E  L  F  A  D  I  V  V  E  Q  E  H  V  E  C  T  A
clone3     ..................................................
            E  L  F  A  D  I  V  V  E  Q  E  H  V  E  C  T  A
clone4     ...T.........C.............T.....................
            E  F  F  A  D  I  V  V  E  H  E  H  V  E  C  T  A
clone5     ..................................................
            E  L  F  A  D  I  V  V  E  Q  E  H  V  E  C  T  A
clone6     ..................................................
            E  L  F  A  D  I  V  V  E  Q  E  H  V  E  C  T  A

                1710        1720        1730        1740        1750
           ....|....|....|....|....|....|....|....|....|....|....|
clone1     ATCTGTAGTCCAAGCTCTACTGTTGTTCAAGCGCTTACATCCCGGGCACA
              S  V  V  Q  A  L  L  L  F  K  R  L  H  P  G  H
clone3     ..................................................
              S  V  V  Q  A  L  L  L  F  K  R  L  H  P  G  H
clone4     ....A...........C..........C.AG........TTG...T.T.
              S  I  V  Q  A  P  L  L  F  Q  G  L  H  L  G  Y
clone5     ......................C...........................
              S  V  V  Q  A  L  L  S  F  K  R  L  H  P  G  H
clone6     ..................................................
              S  V  V  Q  A  L  L  L  F  K  R  L  H  P  G  H

                1760        1770        1780        1790        1800
           ....|....|....|....|....|....|....|....|....|....|....|
clone1     GGGAGAATGAAATAGGCATTTCCGTGAAAAAAGCACTGCATTTTCTTGAG
           R  E  N  E  I  G  I  S  V  K  K  A  L  H  F  L  E
clone3     ..................................................
           R  E  N  E  I  G  I  S  V  K  K  A  L  H  F  L  E
clone4     .......A.......A......T........G.......G..........
           R  E  K  E  I  D  I  S  V  K  K  A  L  R  F  L  E
clone5     ..................................................
           R  E  N  E  I  G  I  S  V  K  K  A  L  H  F  L  E
clone6     ..................................................
           R  E  N  E  I  G  I  S  V  K  K  A  L  H  F  L  E
```


1810 1820 1830 1840 1850
clone1 CAGAGACAATGGCCCGACGGTTCTTGGTACGGCTACTGGGGAGTTTGCTT
Q R Q W P D G S W Y G Y W G V C F
clone3 .....
Q R Q W P D G S W Y G Y W G V C F
clone4 .....T.....A.....
Q R Q W P D G S W Y G Y W G I C F
clone5 .....T.....
Q R Q W P D G S W Y G Y W G V C F
clone6 .....
Q R Q W P D G S W Y G Y W G V C F
1860 1870 1880 1890 1900
clone1 CACCTATAGCACATTTTTTGTGCTGCGAGCGCTCGCTGAGGCCGGGAGGA
T Y S T F F V L R A L A E A G R
clone3 .....
T Y S T F F V L R A L A E A G R
clone4 ..T....G.T....A.....A..G...GA..A....CA..T.....
I Y G T Y F V M G G L A A A G R
clone5 .....
T Y S T F F V L R A L A E A G R
clone6 .....
T Y S T F F V L R A L A E A G R
1910 1920 1930 1940 1950
clone1 CATATAACAACTGCCCCACAGTTCGTAGAGCAGTTCAATTTTTGCTTTCT
T Y N N C P T V R R A V Q F L L S
clone3 .....
T Y N N C P T V R R A V Q F L L S
clone4 .....G.....A.....G.....
T Y S N Y P T V R R A V Q F L L S
clone5 .....
T Y N N C P T V R R A V Q F L L S
clone6 .....
T Y N N C P T V R R A V Q F L L S
1960 1970 1980 1990 2000
clone1 GTACAAAATGAGGAAGGAGGTTGGGGGGAGGACCACGAGTCATGCCCAAG
V Q N E E G G W G E D H E S C P S
clone3 ...
V Q N E E G G W G E D H E S C P S
clone4 A..A.....T.....
I Q N E E G G W G E D L E S C P S
clone5 ...
V Q N E E G G W G E D H E S C P S
clone6 ...
V Q N E E G G W G E D H E S C P S

```
                2010      2020      2030      2040      2050
           ....|....|....|....|....|....|....|....|....|....|
clone1     CATGAAATACATTCCATTGAAAGGAAATCGAACGAATTTAGTTCAAACTT
             M  K  Y  I  P  L  K  G  N  R  T  N  L  V  Q  T
clone3     ..................................................
             M  K  Y  I  P  L  K  G  N  R  T  N  L  V  Q  T
clone4     ..........C.....................A.....T.........G
             M  K  Y  T  P  L  K  G  N  R  T  N  F  V  Q  T
clone5     ...................G..............................
             M  K  Y  I  P  L  E  G  N  R  T  N  L  V  Q  T
clone6     ..................................................
             M  K  Y  I  P  L  K  G  N  R  T  N  L  V  Q  T

                2060      2070      2080      2090      2100
           ....|....|....|....|....|....|....|....|....|....|
clone1     CATGGGCTATGCTAGGACTTATATACGGTGGGCAGGCTGAAAGAGATCCA
           S  W  A  M  L  G  L  I  Y  G  G  Q  A  E  R  D  P
clone3     ..................................................
           S  W  A  M  L  G  L  I  Y  G  G  Q  A  E  R  D  P
clone4     ..............CT..C...........A.........G.........
           A  W  A  M  L  A  L  I  Y  G  G  Q  A  E  R  D  P
clone5     ..................................................
           S  W  A  M  L  G  L  I  Y  G  G  Q  A  E  R  D  P
clone6     ..................................................
           S  W  A  M  L  G  L  I  Y  G  G  Q  A  E  R  D  P

                2110      2120      2130      2140      2150
           ....|....|....|....|....|....|....|....|....|....|
clone1     ACGCCTTTACATAGGGCAGCGAAGTTACTAATCAATGCACAGCTAGATGA
            T  P  L  H  R  A  A  K  L  L  I  N  A  Q  L  D  D
clone3     ..................................................
            T  P  L  H  R  A  A  K  L  L  I  N  A  Q  L  D  D
clone4     ..A..........A..G...A........C............T.......
            T  P  L  H  K  G  A  K  L  L  I  N  A  Q  L  D  D
clone5     ..A..........A.....................................
            T  P  L  H  K  A  A  K  L  L  I  N  A  Q  L  D  D
clone6     ..................................................
            T  P  L  H  R  A  A  K  L  L  I  N  A  Q  L  D  D

                2160      2170      2180      2190      2200
           ....|....|....|....|....|....|....|....|....|....|
clone1     TGGTGATTTTCCACAACAGGAAATTACTGGGGTATACATGAAGAATTGCA
             G  D  F  P  Q  Q  E  I  T  G  V  Y  M  K  N  C
clone3     ..................................................
             G  D  F  P  Q  Q  E  I  T  G  V  Y  M  K  N  C
clone4     .........C.....G.....................T..GA.G........
             G  D  F  P  Q  Q  E  I  T  G  V  F  R  R  N  C
clone5     ..................................................
             G  D  F  P  Q  Q  E  I  T  G  V  Y  M  K  N  C
clone6     ..................................................
             G  D  F  P  Q  Q  E  I  T  G  V  Y  M  K  N  C
```


2210 2220 2230 2240 2250
clone1 TGCTGCATTTTGCAGAATACAGAAACGTTTTCCCAACGTGGGCACTTGGC
M L H F A E Y R N V F P T W A L G
clone3 .....
M L H F A E Y R N V F P T W A L G
clone4 .....A.C.....G.....T.....G
M L H Y P E Y R N V F P M W A L G
clone5 .....
M L H F A E Y R N V F P T W A L G
clone6 .....
M L H F A E Y R N V F P T W A L G
2260 2270 2280 2290
clone1 GAGTATCGAAAGCATGTTCAGTTCCCTCCCCAGAGGCTCTGA
E Y R K H V Q F P P Q R L *
clone3 .....
E Y R K H V Q F P P Q R L *
clone4 .....C.....
E Y R K H V Q F P P Q R L *
clone5 .....
E Y R K H V Q F P P Q R L *
clone6 .....A.....
E Y R K H V Q F P P Q R L *

VITA

Ms. Nattaon Tansakul was born on January 7th, 1988 in Nakornratchasima province, Thailand. She graduated with a Bachelor of Pharmaceutical Science Program, Faculty of Pharmaceutical Science, Prince of Songkla University, Songkla, Thailand in 2011. After graduated, she entered in the Master of Science in Pharmacy Program (Pharmacognosy and Pharmaceutical Botany), Faculty of Pharmaceutical Science, Chulalongkorn University during May 2011-June 2014. Her research during the study was submitted to the Proceedings of the 30th Annual Research Conference in Pharmaceutical Sciences, December 6th-8th 2013 at Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok, Thailand.