

IX. APPENDIXES

APPENDIX A

Nucleotide Sequences of Clones Encoding Actin and Hsp70

A.1) Five-prime nucleotide sequence of clone encoding actin

CACACACACA CACATCCTTC ATCATGGAAG AGGAAGTCGC TGCTCTCGTT
ATTGACAATG GTTCGGGCAT GTGCAAGGCC GGTTCGCCG GTGATGACGC
ACCCCGAGCC GTGTTCCCTT CGATCGTTGG TCGTCCCCGT CACCATGGTA
TCATGATTGG TATGGGCCAA AAGGACTCCT ATGTCGGTGA TGAGGCACAG
TCTAAGCGTG GTATCCTCAC CTTGAGATAC CCCATCGAGC ACGGTAGTCG
TCACCAACTG GGATGACATG GA

A.2) Five-prime nucleotide sequence of clone encoding Hsp70

GCAGTTCACA CCTGAGGAAA TCTCTTCCAT GGTCTTGATC AAGATGCGTG
AGACTGCTGA GGCCTACCTC GGTGGTACCG TTAACAACGC TGTCATCACT
GTCCCCGCCT ACTTCAACGA CTCCCAGCGT CAGGCCACCA AGGATGCTGG
TCTCATTGCC GGTTTGAACG TCCTCCGTAT CATCAACGAA CCTACTGCTG
CCGCCATTGC CTACGGTCTC GACAAGAAGG TTGAGGGTGA GCGCAACGTT
CTCATCTTCG ATCTTGGTGG TGGTACCTTC GATGTTTCTC TCCTCACCAT
CGAGGACGGT ATCTTCGAGG TCAAGGCCAC CGCCGGTGAC ACTCACTTGG
GTGGTGAGGA CTTCGACTCT CGCCTTGTC ACCACTTTGC CTCCGAGTTC
AAGAGGAAAT ATAAGAAGGA TTTGACCACC AATGC

APPENDIX B

Restriction Map of P1, P3, P23, P6, and P7

B.1) Alphabetical map of P1

MluI
 CCGACGCGTGGGCGTACTCGAATCCCCCAGCTGTCTGAACTATCTTATTGAAGCAAACCAACAGATGGCTGAGAGC
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 80
 GCCTGCGCACCCGCATGAGCTTAGGGGGTGTGACAGACTTGATAGAATAAACTCGTTTGGTTTGTCTACCGACTCTCG
 M A E S

BsrBI **KpnI**
Acc65 **MscI**
 AAGTGTCCCGCTCACCAGCATGTGTTGAAGGCCAACGTCGGCGGTGCTGGTACCAGCAACCAAGATTGGTGGCCAGACCG
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 160
 TTCACAGGGCGAGTGGTTCGTACACAACCTTCCGGTTGCAGCCGCCACGACCATGGTCGTTGGTTCTAACCACCGGTCTGGC
 K C P A H Q H V L K A N V G G A G T S N Q D W W P D R

EcoRI **BsaXB** **BsmBI** **BstXI** **BsaXA**
Hind3
 CTTGAAGCTTAACATCCTCCGCCAAAACAACCCGCTCTCAACCCCTCTGGGCGAGGAATTTGACTATGCCGCCGCTTCA
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 240
 GAACTTCGAATTGTAGGAGGCGGTTTTGTTGGGGCAGAGGTTGGGAGACCCGCTCCTTAACTGATACGGCGGCGGAAGT
 L K L N I L R Q N N P V S N P L G E E F D Y A A A F

BpuEI **BglII** **NaeI**
 ACAGCCTAGATTACTTTGCGCTCAAGAAGGATATTCAAGATCTGATGACTGACTCCCAGGACTGGTGGCCGGCTGACTTT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 320
 TGTGCGATCTAATGAAACGCGAGTTCTTCTATAAGTTCTAGACTACTGACTGAGGGTCCTGACCACCGCGGCGACTGAAA
 N S L D Y F A L K K D I Q D L M T D S Q D W W P A D F

BstXI **KpnI**
MscI **AleI** **BsaI** **Acc65**
 GGCCACTATGGTGGTCTCTTTATTGCTATGGCCTGGCATAGTGCCGGTACCTACCGAGTCGCCGACGGTCGAGGCGGCGG
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 400
 CCGGTGATACCAACAGAGAAATAAGCATACCGGACCGTATCACGGCCATGGATGGCTCAGCGGCTGCCAGCTCCGCCGCC
 G H Y G G L F I R M A W H S A G T Y R V A D G R G G G

AfeI **BseRI** **PvuII** **PflFI** **BsaI**
 TGGCGGCGGCCAACAGCGCTTTGCTCCTCTCAACAGCTGGCCCGACAATGTCGGTCTCGACAAGGCCCGCGCTTTGTGT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 480
 ACCGCCGCGGTTGTCGCGAAACGAGGAGGTTGTCGACCGGGCTGTTACAGCCAGAGCTGTTCCGGGCGGCAAACAACA
 G G G Q Q R F A P L N S W P D N V G L D K A R R L L

BssSI **BglII** **EcoRI** **NcoI**
 GGCCCATCAAGCAGAAATACGGAACAAGATCTCGTGGGCGGATCTCCTATTGCTCACTGGTAACGTCGCCCTTGAGTCC
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 560
 CCGGGTAGTTCGTTCTTTATGCCTTTGTTCTAGAGCACCCGCTAGAGGATAACGAGTGACCATTGCAGCGGGAACCTCAGG
 W P I K Q K Y G N K I S W A D L L L L T G N V A L E S

BpuE1 | Bmr1
 ATGGGTTTCAAGACCTTTGGTTTCTCTGGCGGTCTGTGCCGACACATGGGAAGTGGATGAGTCAGCCAACCTGGGGAGGGGA
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 640
 TACCCAAAGTTCTGGAAACCAAGAGACCGCCAGCACGGCTGTGTACCTTCACCTACTCAGTCGGTTGACCCCTCCCT
 M G F K T F G F S G G R A D T W E V D E S A N W G G E

 Aat2 | BsrD1 | EcoRV | Acl1
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 720
 TTGGTGGACCGATCCGTTACTGACGGCGATGAGGCCGCAATCCGACTAGTGTTCCTATAGGTGTTGGCACTGAACCTGT
 T T W L G N D V R Y S G G K A D H K D I H N R D L D

 AGCCACTGGCGCTGCCCACATGGGTTTGATCTATGTCAACCCCGAAGGTCTGTATGGAAACCCGACCCCATCGCCGCT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 800
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 K P L A A A H M G L I Y V N P E G P D G N P D P I A A

 Bcg1b | Bcg1a | Msc1 | BsmB1 | BseR1 | Nae1
 GCCAAAGATATTCGACCCACCTTCGGTCTGTATGGCCATGAACGACGAGGAGACGGTTGCCCTTATTGCCGGCGGTACAC
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 880
 CGGTTTCTATAAGCGTGGTGAAGCCAGCATACCGGTACTTGCTGCTCCTCTGCCAACGGGAATAACGGCCGCCAGTGTG
 A K D I R T T F G R M A M N D E E T V A L I A G G H T

 Dra3 | BseY1 | AlwN1 | Pst1 | EcoK
 CTTGGTAAGACACACGGTGTGCTGCCAGCAGACAAGCTCGGCCCGGAACAGAGGCTGCAGACATGGCACAACAGGGTT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 960
 GAAGCCATTCGTGTGCCACGACCGGTCGTCTGTTCGAGCCGGGCTTGGTCTCCGACGCTGTACCGTGTGTGCCAA
 F G K T H G A G P A D K L G P E P E A A D M A Q Q G

 BsaXb | BsaI
 TAGGCTGGACCAATAGCTTCAAAGCGCAAGGGTCTGATACCACAACAGCGGTCTCGAAGTTACCTGGACCAAGACT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1040
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 L G W T N S F K S G K G P D T T T S G L E V T W T K T

 BsaXa | PflM1 | Ear1 | Bmr1 | Nar1 | Nae1
 CCTACTAAATGGAGTAACCAATTCTTGGAGTACCTCTTCCGCTACGACTGGGAACCTACTAAGAGTCTGCCGGCGCCCA
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1120
 GGATGATTTACCTCATTTGTTAAGAACCTCATGGAGAAGGCGATGCTGACCTTGAGTGATTCTCAGGACGGCCGCGGT
 P T K W S N Q F L E Y L F R Y D W E L T K S P A G A H

 Nsi1
 CCAGTGGGTCGCCAAAATGCAGAGGCTTTCATCCCGATGCATTTGACCCATCCAAGAAGCGCAAGCCAATGATGCTCA
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1200
 GGTCAACCGCGGTTTTTACGTCTCCGAAAGTAGGGCTACGTAAACTGGGTAGGTCTTTCGCGTTCGGTTACTACGAGT
 Q W V A K N A E A F I P D A F D P S K K R K P M M L

 Bgl2
 CGACCGATCTTTCCCTTCGCTATGACCTATCTACGAGAAGATCTCTCGTCTGCTTCTTGGAGCACCTGACCAAGTTGCT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1280
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 T T D L S L R Y D P I Y E K I S R R F L E H P D Q F A

BssS1 Bcg1a Sac1

GATGCGTTTGCCCGTGCCTGGTTCAAGTTACTTCACCGTGACCTTGGCCACGAGCTCTCTACATTGGTCCCGAAGTGCC
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1360
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 D A F A R A W F K L L H R D L G P R A L Y I G P E V P

Bbs1

Pst1 Bcg1b BamH1 Sal1 BsrD1 Mlu1

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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1440
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 A E V L P W Q D P V P A V D H P L I S N E D A S A L

Bts1 PflM1

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 K Q R I L A S G V K P S S L I S T A W A S A S T F R G

Eci1 BseR1 Bpu10 BbvC1 Hpa1

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 S D K R G G A N G A R I R L S P Q R E W A V N N Q P W

Bsa1 Xmn1 Bmr1

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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1680
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 L R E T L S V L E A I Q K Q F N T S Q S G G K K V S

BciV1

Bpm1 Nru1

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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1760
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 I A D L I V L A G V A A V E K A A R D A G Y A V T V P

Aat2

PshA1 Eco57 BsrD1

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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1840
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 F T P G R T D A S Q E Q T D V Q S F S D M E P I A D G

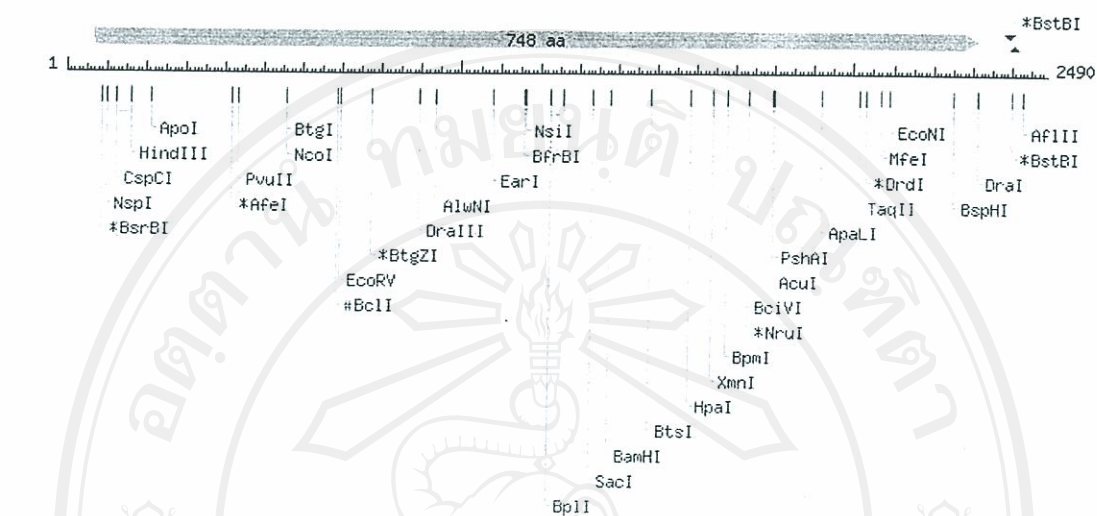
BseR1

BbvC1 Bpu10 Cla1

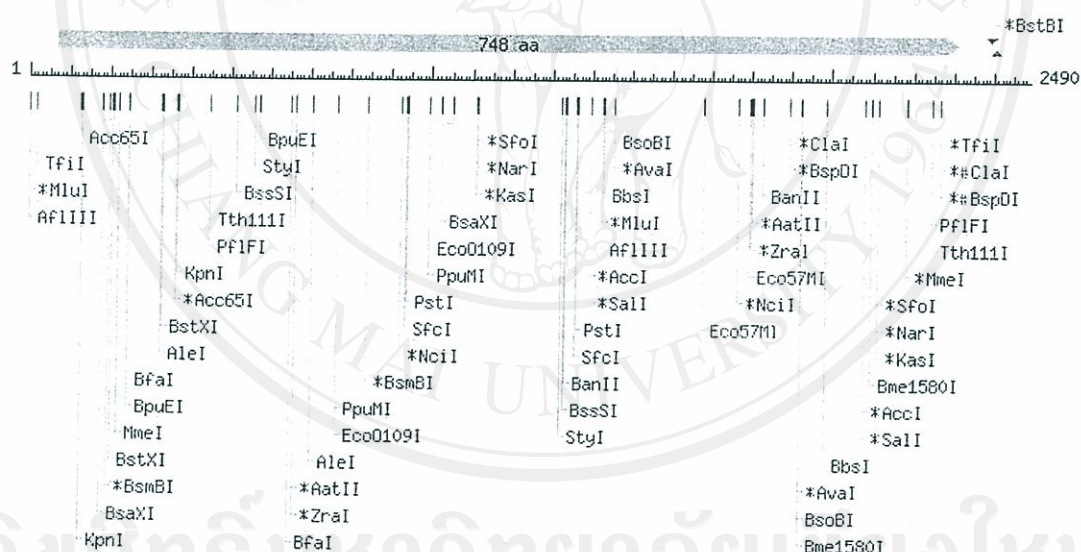
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 1920
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 F R N Y G S S T S R V R A E E W L I D K A Q L L T L

B.2) Graphical map of P1

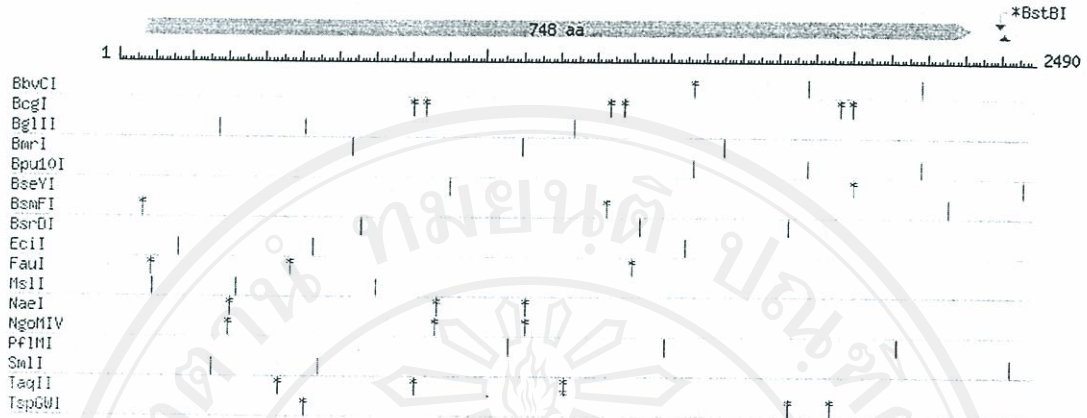
Single cutter



Double cutter



Triple cutter



List of 0 cutter

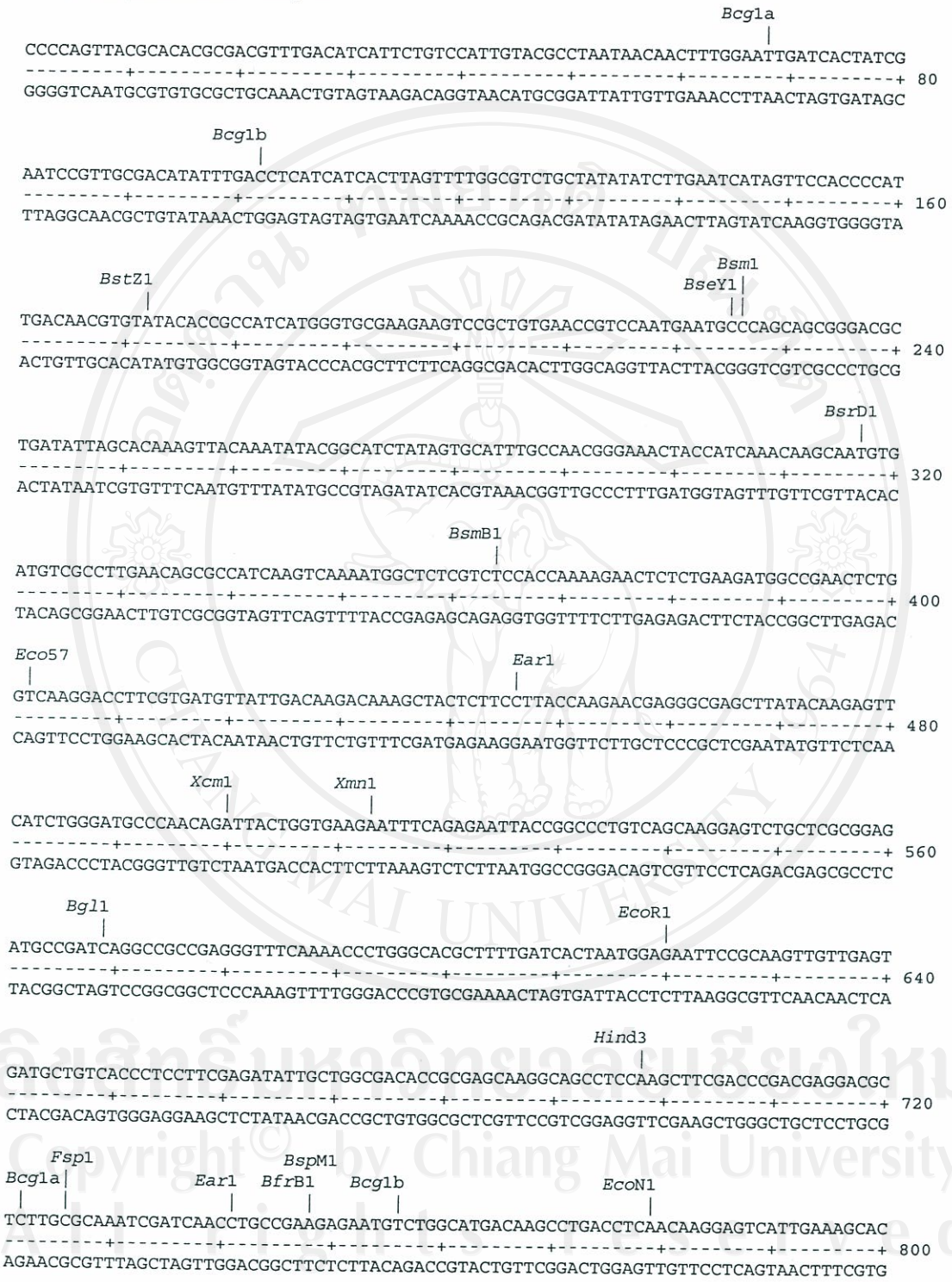
AarI AclI AgeI AhdI AlfI AloI ApaI AscI AseI AsiSI AvrII BaeI BfuAI
BglI BlpI BmgBI BmtI BsaAI BsaBI BsaWI BsgI BsiWI BsmI BspEI
BspMI BsrGI BssHII BstAPI BstEII BstZ17I Bsu36I EagI EcoRI FaeI
FseI FspAI FspI NdeI NheI NotI PacI PaeR7I PciI PfoI PmeI PmlI
PpiI PsiI PspOMI PspXI PsrI PvuI RsrII SacII SanDI SapI SbfI ScaI
SexAI SfiI SgrAI SmaI SnaBI SpeI SphI SrfI SspI StuI SwaI TatI TliI
XbaI XcmI XhoI XmaI

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B.3) Alphabetical map of P3



Dra1 Nhe1 BfrB1 BstAP
 AATTCAAGGAACAGACCGACCGATTAAACCTGCTAGCAAGCAAGATGTTCAAGAAGCAGCTAATGCTGCTACTACTGCT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 880
 TTAAGTTCCTTGTCTGGCTGGCTAAATTTGGACGATCGTTTCGTTCTACAAGTTCTTCGTCGATTACGACGATGATGACGA
 M F K K Q L M L L L L L L

Eco57 Pml1 Bsa1
 GCAACTGGTGGTCAGCAAGATGCTTCAGTCTCTCAGATTGACGCACGTGCTGGTGTCAATGCTGCCAAGGGAGACCCCTGC
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 960
 CGTTGACCACCAGTCGTTCTACGAAGTCAGAGAGTCTAACTGCGTGCACGACCACAGTTACGACGGTTCCCTCTGGGACG
 Q L V V S K M L Q S L R L T H V L V S M L P R E T L

BssH2 Bbs1 Eco57
 AACAGCGCGCCGAGCAGAATGTTGCACCTGAAGACCGGGACCAAGTTCGACAGGTTACTGAACAAGCACAGCGGTATCT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1040
 TTGTCGCGCGGCTCGTCTTACAACGTGGACTTCTGGCCCTGGTTCAAGCTGTCCAATGACTTGTTCGTGTTTCGCCATAGA
 Q Q R A E Q N V A P E D R D Q V R Q V T E Q A Q A V S

TCTGAATATAATCGGCGTATTAAAGATTCTTGGCCTCCAAGATGCCCAAAGAAGCTCGTGAACAGATCGTTTGGCGTTT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1120
 AGACTTATATTAGCCGCATAATTCTTAAAGAACCGGAGGTTCTACGGGTTTCTTGCAGCACTTGTCTAGCAAACCGCAAA
 S E Y N R R I K D F L A S K M P K E R R E Q I V W R L

Nde1
 GAAGAAGATGATTGTTGAGATCCAGGTCACCTCTGATTACCAACAAGCCATCGAGACTCTCTTGAGTTTGGCTGAGTCAT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1200
 CTTCTTCTACTAACAACCTCTAGGTCCCAGTGAGACTAATGGTTGTTCCGGTAGCTCTGAGAGAACTCAAACCGACTCAGTA
 K K M I V E I Q G H S D Y Q Q A I E T L L S L A E S

BpuE1 Ear1 EcoRV Ear1 BpuE1
 ATGCTGGACATGGAAGAGATATCTCTTCGCAAGGAACGACCGCTACTAAAGGGTTCATGGATAGCAAGAAGGACATGCTG
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1280
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 Y A G H G R D I S S Q G T T A T K G F M D S K K D M L

Bcgla BsaB1 Bcg1b
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1360
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 M R L K I L I E R F A N S T S T D D F F D S L N T I Y

Nru1 Sac2 Sal1 BstB1
 TCGCGATGCCGACCAGGACCCGCGGTTGAAGGAATGGTTTAGGGGTGTCGACACTTACATTCGAAAATGCCTGCGCGAGC
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1440
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 R D A D Q D P R L K E W F R G V D T Y I R K C L R E

Baelb Bsa1 Age1
 AAGGGTTCATCATGCAAGATGAAGCTAATGACCAGTGGAAACAGCTGTACGATGAGGGTCGCTTCTTGCTTCGAGACCGG
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1520
 TTCCCAAGTAGTACGTTCTACTTCTGATTACTGGTCACTTGTTCGACATGCTACTCCAGCGAAGAACGAAGCTCTGGCC
 Q G F I M Q D E A N D Q W N K L Y D E G R F L L R D R

Baela MscI
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 -----+ 1600
 ATGTCTTCAGTGTGTCTAGCATAACGGCTACTTCGGTTCAAAAACCGGTGAGTTAAACTGCTTCTAGGTGTCTTGGCTCG
 Y R S H T D R I A D E A K F L A T Q F D E D P Q N R A

EarI
 TTTCGGTCAATCTTTGGAGAGACTCTTCAAGGATCTGGGTCAGGACCAGTATGGAAAGCCGACGTTCAAGCCCCATTGGA
 -----+ 1680
 AAAGGCAGTTAGAAACCTCTCTGAGAAGTTCCTAGACCCAGTCTCGGTACATACCTTTCGGCTGCAAGTTCGGGGTAAACT
 F R Q S L E R L F K D L G Q D Q Y G K P T F K P H L

BsmI
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 -----+ 1760
 AGTTTCTGTAATGGTTACAGTAGCAAGGACTCTATAAACTCTTACGGTCGATGTAAGGATAAGGAGCTTAAGTCCAAAGG
 I K D I T N V I V P E I F E N A S Y I P I P R I E V S

BseY1 Pvu2 EcoR1
 GACCCAGCTGTTGACATGGTTATCGAAAACCTTATCATCGAGAGCGACAACCTTGATGCCAATGTTTTAGAATTCGGCAC
 -----+ 1840
 CTGGGTCGACAACTGTACCAATAGCTTTTGAATAGTAGCTCTCGCTGTTGAACTACGGGTTACAAAATCTTAAGCCGTG
 D P A V D M V I E N L I I E S D N L M P N V L E F G T

TGACAACACTGCGGTGGGGTCGTAAGAAGATCAGCAGCTTCGACGACCACAAGGTTATGATTTCTGCATCTGGCATCC
 -----+ 1920
 ACTGTTGATGACCGCCACCCAGCATTCCTTAGTCGTCGAAGCTGCTGGTGTCCAATACTAAAGACGTAGACCGTAGG
 D N Y W R W G R K K I S S F D D H K V M I S A S G I

Drd1
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 -----+ 2000
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 Q A D L R D V S Y Y F K K K Q G F P S L T D I G V M D

Hind3
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 -----+ 2080
 TAAGAAGAACCACCAAGTCCGAAGCCGAAGTTCTAACGACGGAGGTTTCGTGTCTTCTTACTGGTGTGCGTCAAAAATT
 I L L G G S G F G F K I A A S K A Q K N D H N A V F K

Eco57 BstAP
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 -----+ 2160
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 L D S V K V N V K N L T I K L K K S K H K M L F I I

Bsu36 BsmI
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 -----+ 2240
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 F R P M L L N V V R P V L E K C W R P T F L R H S K R

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Btr1 Bsa1 BamH1
 Xmn1 BmgB1 Bpm1
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 I S S P A T P M L L V T S S L E K K K Q A E A I A E

Nde1 Xcm1 BsrD1
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Bpu10 Xcm1 Bmr1
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Bts1 Pst1
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2640
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 T S N F P K A S A V S R K P H T A A E S H L Q E K S

Bsg1 BamH1
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2720
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Bpm1 BsrD1 PflM1
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2800
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 S S G K A M L P T N G V T N G Q S A T N G T F Q K E V

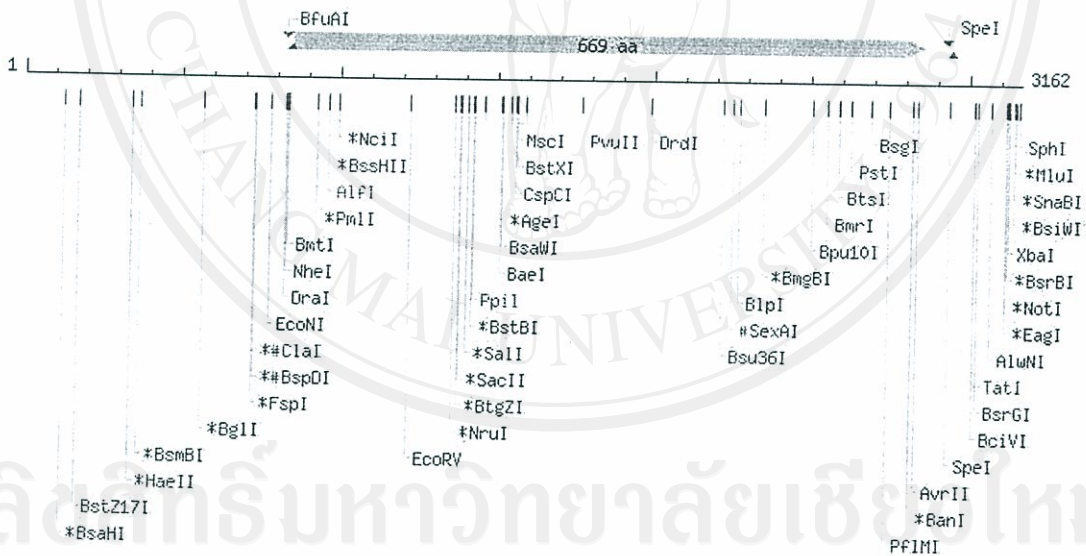
Avr2 Ear1
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2880
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 E R A F D A N G T T L P T L G G V *

EcoR1 Bpm1 SpeI
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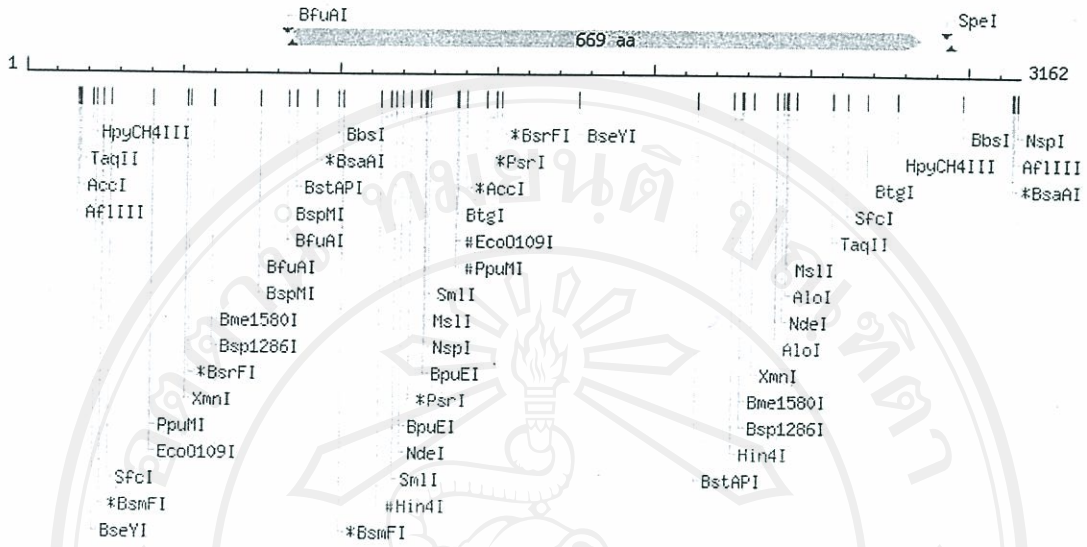
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 AlwN1
 GTCTAGTCTCTAGTTTCCTTTTGTGAGATACTGATACTTAATAAAATAATTACTATTTTCCAAAAAAGGGC 3120
 CAGATCAGAGATCAAAGGAAACAGTCTATGACTATGAATTATTTTATTAATGATAAAAGGTTTTTTTTTTTTTCCCG
 Not1 Mlu1
 Eag1 Xba1 SnaB1
 BsrB1 BamH1 Hind3 BsiW1 Sph1
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B.4) Graphical map of P3

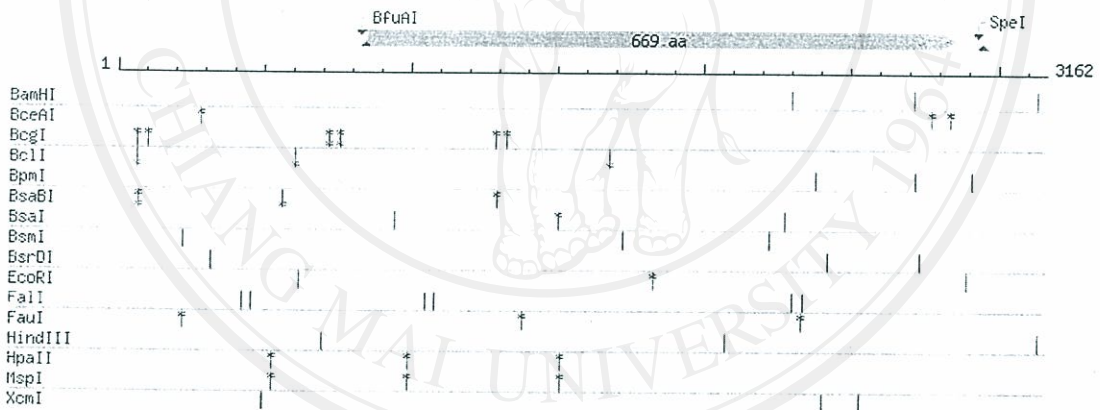
Single cutter



Double cutter



Triple cutter



List of 0 cutter

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 BspEI BspHI BssSI BstEII DraIII EciI FseI FspAI HpaI KasI KpnI
 MfeI NaeI NarI NcoI NgoMIV NsiI PacI PaeR7I PciI PjFI PfoI PmeI
 PshAI PsiI PspOMI PspXI PvuI RsrII SacI SandI SapI SbfI ScaI SfiI
 SfoI SgrAI SmaI SrfI SspI StuI SwaI TliI Tth111I XhoI XmaI ZraI

B.5) Alphabetical map of P23

Pvu2
|
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Ear1 BseR1 Ear1 BsmB1
| | | |
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-----+-----+-----+-----+-----+-----+-----+-----+-----+ 160
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M S L F H R S G D F A P L F R L L D D Y D

BspE1 Bpm1 BpuE1 Hind3 Aat2
| | | | |
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L H R S G R D G Q T P A S S S I S S F A P R F D V R

BsrD1 EcoR1
| |
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-----+-----+-----+-----+-----+-----+-----+-----+-----+ 320
TCAGTTCTTACGAATGGTAGACCTGCCGCTTGAGGGGCCGTAACGAGTTTCTGCAACTTTAGCTTAAGAGGCTGGGC
E S K D A Y H L D G E L P G I A Q K D V E I E F S D P

Bpu10
|
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-----+-----+-----+-----+-----+-----+-----+-----+-----+ 400
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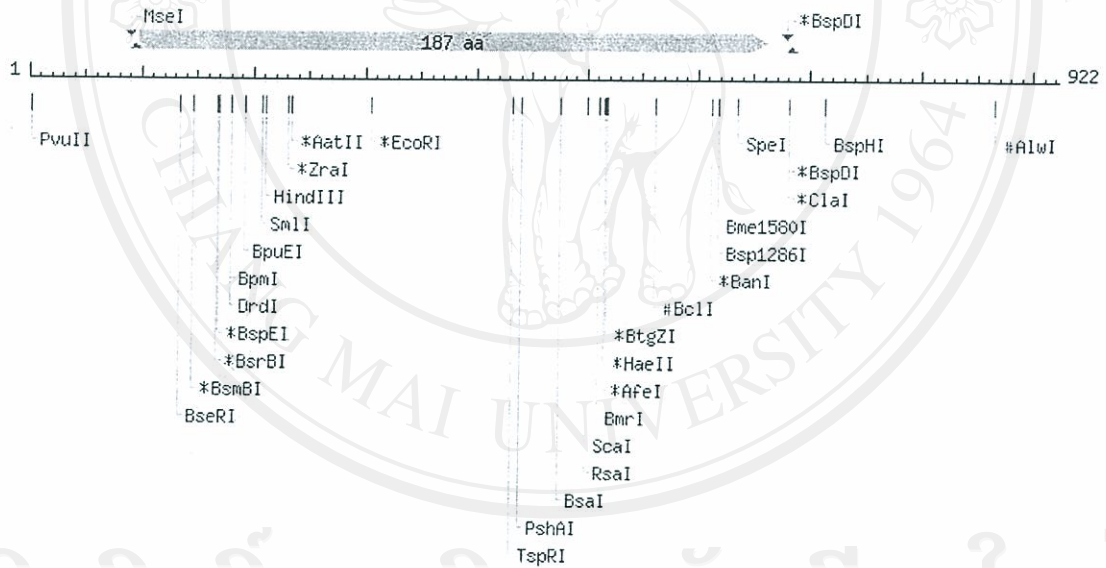
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A S V E D A P E S S D E T A V Q K S S D K K E V S K

Bpu10 Sca1 Bmr1 Afe1
| | | |
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-----+-----+-----+-----+-----+-----+-----+-----+-----+ 560
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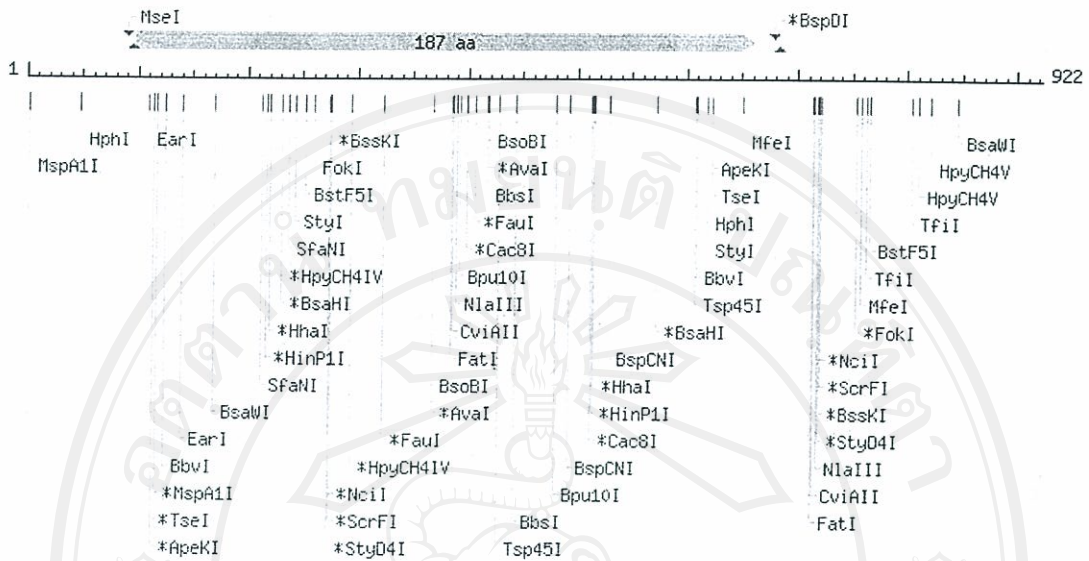
Spe1
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V D H N G V K A S L K N G V L T V T V P K A A P P T S

Mfe1 Cla1 BspH1
| | |
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-----+-----+-----+-----+-----+-----+-----+-----+-----+ 720
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R K I T I E *

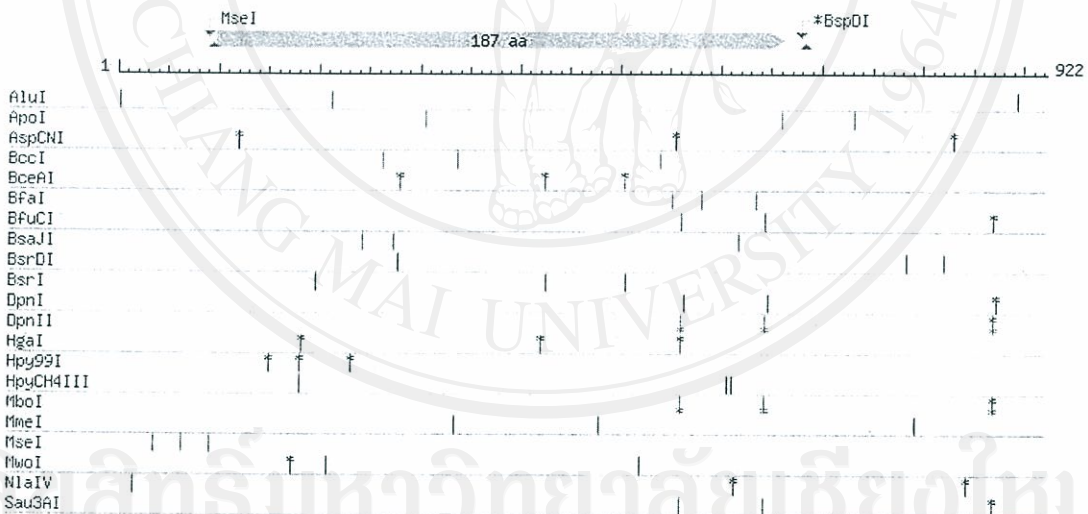
Single cutter



Double cutter



Triple cutter



List of 0 cutter

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BfrBI BfuAI BglI BglII BlpI BmgBI BmtI BsaAI BsaBI BsaXI BseYI
BsgI BsiEI BsiHKAI BsiWI BslI BsmFI BsmI BspMI BsrFI BsrGI
BssHII BssSI BstAPI BstBI BstEII BstNI BstXI BstYI BstZ17I Bsu36I
BtgI BtsI CspCI DraI DraIII EaeI EagI EciI EcoNI EcoO109I EcoRV
FseI FspI HaeIII HincII HpaI KasI KpnI MluI MscI MslI NaeI NarI
NcoI NdeI NgoMIV NheI NotI NruI NsiI NspI PacI PaeR7I PciI PflFI
PflMI PhoI PmeI PmlI PpuMI PsiI PspGI PspOMI PspXI PstI PvuI
RsrII SacI SacII SalI SapI Sau96I SbfI SexAI SfcI SfiI SfoI SgrAI
SmaI SnaBI SphI SspI StuI SwaI TliI Tth111I XbaI XcmI XhoI XmaI
XmnI

B.7) Alphabetical map of P6

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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 80
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 M E T A N D A P S T T L E G Q

 BpmI | BbsI
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 160
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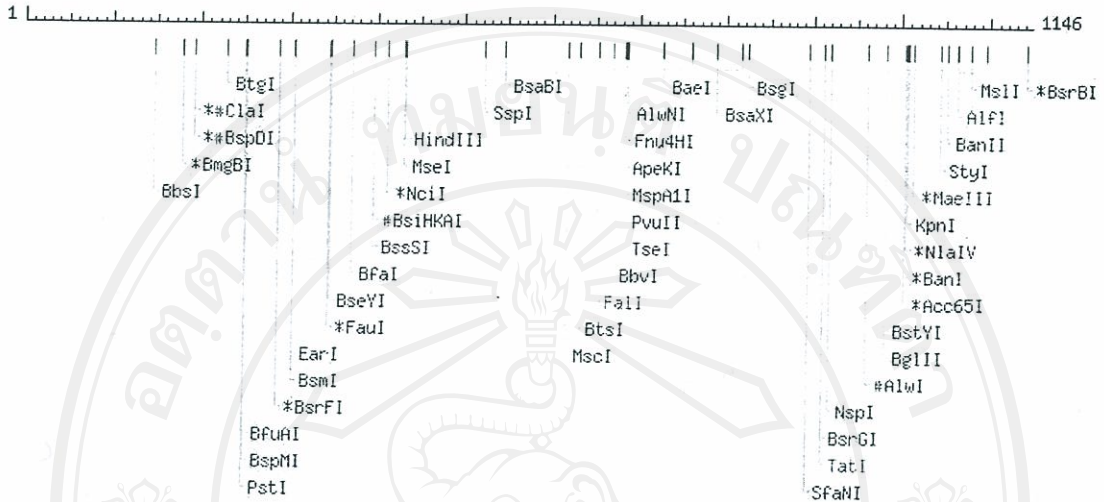
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 BsrDI BmgBI | BsrDI BsaI
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 240
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 R P P L P P R P E T I D L L N E G I A F R T S T A R

 BspMI BsmBI
 BfrBI | EarI
 PstI BsmI |
 CAAACCTGCAGTCGACGCGACAACGGCACTGTCTATTGACAGACATTACCGGCCAAACGAATGCAGATGGAAGAGACGGT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 320
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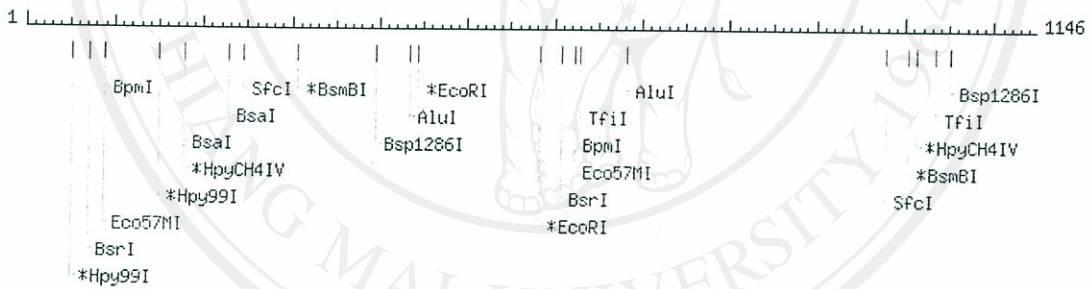
 BseYI | EcoK
 BssSI |
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B.8) Graphical map of P6

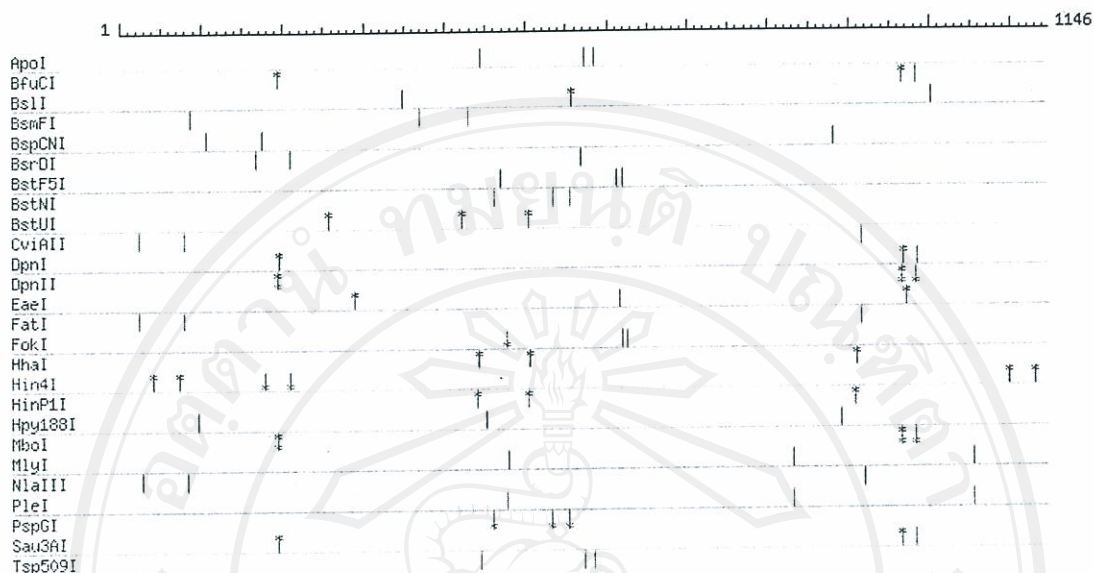
Single cutter



Double cutter



Triple cutter



List of 0 cutter

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 BciVI BclI BfrBI BglI BlpI Bme1580I BmrI BmtI BpI Bpu10I BpuEI
 BsaAI BsaHI BsaWI BseRI BsiEI BsiWI BsoBI BspEI BspHI BssHII
 BstAPI BstBI BstEII BstXI BstZ17I Bsu36I BtgZI CspCI DraI DraIII
 DrdI EagI EciI EcoNI EcoO109I EcoRV FseI FspAI FspI HaeII HincII
 HpaI HphI Hpy8I KasI MfeI MluI MmeI NaeI NarI NcoI NdeI NgoMIV
 NheI NotI NruI NsiI PacI PaeR7I PciI PflFI PflMI PfoI PmeI PmlI
 PpiI PpuMI PshAI PsiI PspOMI PspXI PsrI Pvul RsrII SacI SacII SalI
 SandI SapI Sau96I SbfI ScaI SexAI SfiI SfoI SgrAI SmaI SmI SnaBI
 SpeI SphI SrfI StuI SwaI TaqII TaqII Taul TliI Tsp45I TspGWI Tth111I
 XbaI XcmI XhoI XmaI XmnI ZraI

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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B.9) Alphabetical map of P7

Ear1 Ear1 BssS1
 | | |
 CCTCTTCTCTCTTCACGCTTCCTCTCCCTCGTGAATCTACATTCTTTCATGTGGTGATCTCTCCACACTCCCATCTTT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 80
 GGAGAAGGAGAGAAGTGCGAAGGAGAGGGAGCACCTTAGATGTAAGAAAGTACACCACTAGAGAGGTGTGAGGGTAGAAA

BsmB1 Bsg1 BspM1
 | | |
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 160
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Bpm1 Afe1
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 240
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Sca1 EcoRV
 | |
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 320
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Nde1
 |
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 400
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BspH1 Btr1
 | |
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 480
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 M S H A H F H R H E R R N F I S D V E D F F G V

Sca1 BstZ1 PflF1
 | | |
 CAATTACGCAAAGGTCCACAGAGTACTGTAAGTAAAGTGCCTCGATTGTATACGTGACTGCGTCGCCAACCTTCACCG
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 560
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PflM1 AlwN1 BamH1
 | | |
 GCCCAATCGGTGGTTATATCACGGGCACAGATACTGCCAGCCCTGCGGATCCAACCTACAACCGCAGAAAGGTGCTCCT
 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 640
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 G P I G G Y I T G T D T A S P A D P T T T A G K G A P

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 V A Q S S T K T T A A S S K S D T S T T T K V P A P T

PflF1 *Bcgla* *Xho1*
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 800
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 T H T T S T L S T T S T S L S D L L T T S S D S P A

Bcg1b *Bbs1* *Aat2* *Bbs1* *BsaXb*
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 T T F L T S S S T Q S A S S T S T D T A L D Q L S S S

Bts1 *Pst1* *BsaXa*
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 960
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 P T A S P T T S A A A S T S N G L T G G A K A G I A I

 TGGTGTCTTGTTCGGCGTTGGTCTTATTGCTGGATTGATTCTGTTCTGGCTACATAAGCAGAAGAAGAACAGAGAAGAAG
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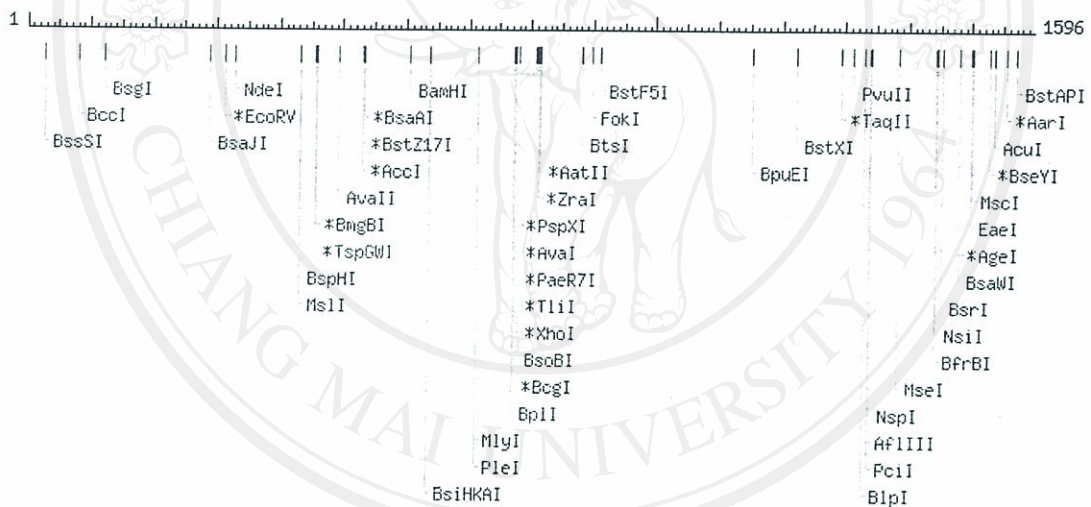
Pst1 *Afe1*
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 A A A A A A A A E N E K F T P S Q P P P M T S A Q S P

Ear1 *Bpm1* *BpuE1* *AlwN1*
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 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1200
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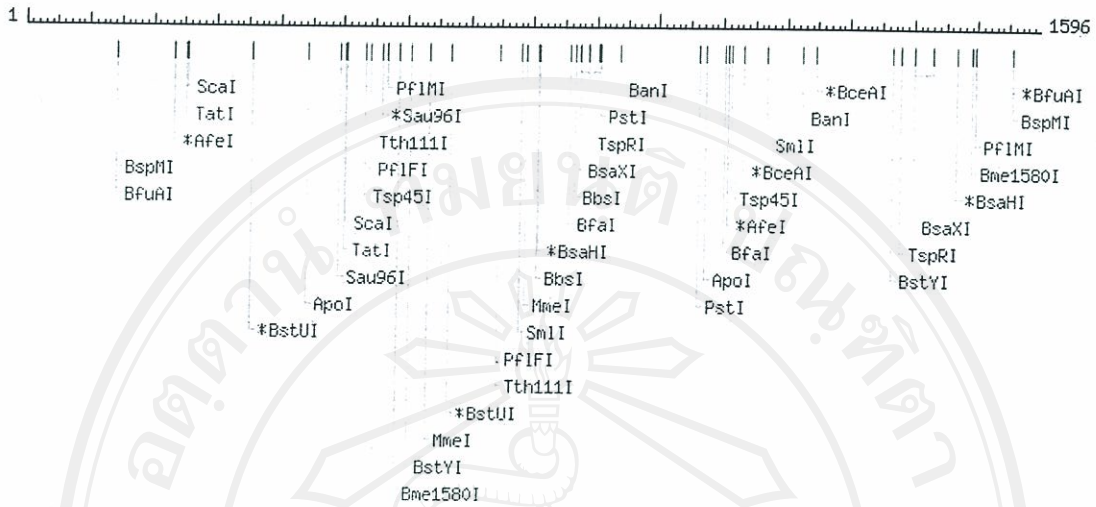
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 N P L L S Q P G G A N P Y A A G A V G A A V G G A A

BspLU *Pvu2* *Blp1* *BseR1* *BseR1*
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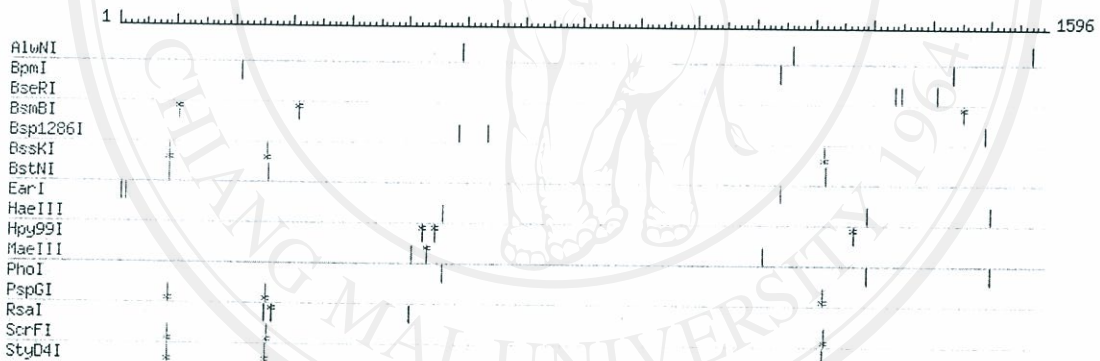
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 R Q D P F T D P V N P F D N G A Q T A S P P M P P A K



Double cutter



Triple cutter



List of 0 cutter

Acc65I AclI AflIII AhdI AleI AlfI AloI ApaI ApaLI AscI AseI AsiSI
 AvrII BaeI BanII BbvCI BciVI BclI BglI BglIII BmrI BmtI Bpu10I BsaBI
 BsaI BsiEI BsiWI BsmFI BsmI BspDI BspEI BsrBI BsrDI BsrGI BssHII
 BstBI BstEII Bsu36I BtgI BtgZI ClaI CspCI DraI DraIII DrdI EagI
 EciI EcoNI EcoO109I EcoRI FalI FauI FseI FspAI FspI HindIII HpaI
 KasI KpnI MfeI MluI NaeI NarI NciI NcoI NgoMIV NheI NotI NruI
 PacI PfoI PmeI PmlI PpiI PpuMI PshAI PsiI PspOMI PsrI PvuI RsrII
 SacI SacII SalI SanDI SapI SbfI SexAI SfiI SfoI SgrAI SmaI SnaBI
 SpeI SphI SrfI SspI StuI StyI SwaI TaqII XbaI XcmI XmaI XmnI

APPENDIX C

Recipes

C.1) Media preparation

LB (Luria-Bertani medium)

Per liter:

To 950 ml of deionized water, add:

Tryptone	10	g
Yeast extract	5	g
NaCl	10	g

Shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH (~0.2 ml). Adjust the volume of the solution to 1 liter with deionized water. Sterile by autoclaving for 20 min at 15 lb/sq. in. on liquid cycle.

To make an agar medium, add 20 g of agar to the 1 L solution and sterile by autoclave.

SOC medium

Per liter:

To 950 ml of deionized water, add:

Tryptone	20	g
Yeast extract	5	g
NaCl	0.5	g

Shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH. Adjust the volume of the solution to 980 ml with the deionized water. Sterile by autoclaving, allow it to cool to 60 °C or less and then add 20 ml of a sterile 1 M solution of glucose.

The glucose solution is made by dissolving 18 g of glucose in 90 ml of deionized water. After the sugar has dissolved, adjust the volume of the solution to 100 ml and sterile by filtration through a 0.2- μ m filter.

C.2) Solutions for working with bacteriophage λ

20 % Maltose

Maltose	20	g
H ₂ O to	100	ml

Sterile the solution by filtration through a 0.2- μ M filter. Store the sterile solution at room temperature.

SM

Per liter:

NaCl	5.8	g
MgSO ₄ • 7H ₂ O	2	g
1 M Tris-Cl (pH 7.5)	50	ml
2 % gelatin solution	5	ml
H ₂ O to	1	L

Sterile the buffer by autoclaving for 20 min at 15 lb/sq.in. on liquid cycle. After the solution has cooled, dispense 50-ml aliquots into sterile containers. SM may be stored indefinitely at room temperature.

A 2 % gelatin solution is made by adding 2 g of gelatin to a total volume of 100 ml of H₂O and autoclaving the solution for 15 min at 15 lb/sq.in. on liquid cycle.

10 mM MgSO₄ (λ diluent)

Per liter:

1 M Tris-Cl (pH 7.5)	10	ml
MgSO ₄ • 7H ₂ O	2	g
H ₂ O to	1	L

Sterile the buffer by autoclaving for 20 min at 15 lb/sq.in. on liquid cycle. After the dilution has cooled, dispense 50-ml aliquots into sterile containers. λ diluent may be stored indefinitely at room temperature.

C.3) Alkaline lysis buffers for minipreparations of plasmid DNA

Solution I

50 mM glucose

25 mM Tris-Cl (pH 8.0)

10 mM ECTA (pH 8.0)

Sterile by autoclaving for 20 min at 15 lb/sq.in. on liquid cycle and stored at 4 °C.

Solution II

0.2 N NaOH (freshly diluted from a 10 N stock)

1 % SDS

Solution III

5 M potassium acetate	60	ml
-----------------------	----	----

glacial acetic acid	11.5	ml
---------------------	------	----

H ₂ O	28.5	ml
------------------	------	----

The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.

C.4) Buffers

Alkaline electrophoresis buffer

10 N NaOH	3	ml
-----------	---	----

0.5 M EDTA	4	ml
------------	---	----

Distilled water	993	ml
-----------------	-----	----

The resulting solution is 30 mM NaOH and 2 mM EDTA

Blocking buffer

Non-fat dry milk	5	g
------------------	---	---

Triton X-100	0.1	ml
--------------	-----	----

PBS, pH 7.2	100	ml
-------------	-----	----

10X MOPS

3-[N-morpholino]propanesulfonic acid	41.8	g
Sodium acetate (anhydrous)	4.1	g
0.5 M EDTA	20	ml
DEPC-treated water	900	ml

Adjust pH to 7.0 with 10 N NaOH and bring the solution up to 1 L

Phosphate buffer saline (PBS), pH 7.2

NaCl	8	g
KCl	0.2	g
Na ₂ HPO ₄	1.15	g
KH ₂ PO ₄	0.2	g
Distilled H ₂ O	800	ml

Adjust pH to 7.2 with 1 N HCl, and then add H₂O to 1 L

PBS-tween (PBS-T)

PBS, pH 7.2	100	ml
Tween 20	0.05	ml

5X SDS-PAGE running buffer

Tris base	6.75	g
Glycine	32.4	g
SDS	2.25	g
Distilled H ₂ O up to	450	ml

0.5 M Tris-HCl, pH 6.8

Tris base	6.05	g
Distilled H ₂ O	60	ml

Dissolve the solute and adjust pH to 6.8 with 5 N HCl. Make the solution volume to 100 ml with distilled water.

10 TEN buffer

1 M Tris-Cl, pH 7.5	50	ml
5 M NaCl	25	ml
0.5 M EDTA	1	ml
Distilled H ₂ O	424	ml

Western blot transfer buffer

Tris base	3.03	g
Glycine	14.4	g
Methanol	200	ml
Distilled H ₂ O up to	1	L

50X Tris-acetate (TAE) buffer

Tris base	242	g
Glacial acetic acid	57.1	ml
0.5 M EDTA	100	ml

The working solution (1X) contains 0.04 M Tris acetate and 0.0001 M EDTA

C.5) Reagents**Chloronaphthol stock solution**

4-Chloro-1naphthol	0.3	g
Absolute ethanol	10	ml

Store at -20 °C for at least 1 year. The working solution can be made by dilute a 0.1-ml of stock solution with 10 ml of 50 mM Tris (pH 7.6). The white procipitate can be removed by filtering through a Whatman no.1 filter paper. Immediately before using, add 10 µl of 30 % H₂O₂ solution.

0.1 % Coomassie brilliant blue R-250

Coomassie brilliant blue R-250	0.1	g
Acid-methanol solution	100	ml

The acid-methanol solution containing 40 ml of methanol, 10 ml of acetic acid, and 50 ml of H₂O

Acrylamide stock solution (30 % T, 2.67 % C)

Acrylamide	29.2	g
N',N'-bis-methylene-acrylamide	0.8	g
Distilled H ₂ O up to	100	ml

Store the solution in a screw-capped dark brown bottle. The solution is stable at least 1 month at 4 °C.

10 % Ammonium persulphate (APS)

APS	0.05	g
Distilled H ₂ O	50	μl

10 % SDS

SDS	1	g
Distilled H ₂ O	10	ml

C.6) Loading buffers**6X agarose gel-loading buffer**

Bromophenol blue	0.025	g
Xylene cyanol FF	0.025	g
Glycerol	3	ml
Distilled water	6.95	ml

Alkaline loading buffer

10 X Alkaline buffer	0.5	ml
Glycerol	1	ml
Bromophenol blue	0.05	g
Distilled water	3.5	ml

RNA sample buffer

Formamide	25	ml
10 X MOPS	5	ml
37 % Formaldehyde	9.5	ml
Bromophenol blue	0.05	g
DEPC-treated water up to	50	ml

2X SDS gel-loading buffer

SDS	1	g
Glycerol	2	ml
0.1 % Bromphenol blue solution	2	ml
1 M Tris-HCl, pH 6.8	1.25	ml
β -mercaptoethanol (β -ME)	1	ml
Distilled H ₂ O up to	10	ml

APPENDIX D

Genotypes of *Escherichia coli* Used in This Study

Strain	Genotype
Y1090	F ⁻ <i>lacU169, lon</i> ⁻¹⁰⁰ , <i>araD139, rpsL, supF, mcrA, trpC22</i> :: <i>Tn10</i> / pMC9
XL1-blue	<i>SupE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac</i> ⁻ F' [<i>proAB</i> ⁺ <i>lacI</i> ^q <i>lacZ</i> M15 <i>Tn10(tet</i> ^r <i>)</i>]
TOP10	<i>lacI</i> ^q <i>Tn10(Tet</i> ^R <i>) mcrA (mrr-hsdRMS-mcrBC) φ80lacZ</i> M15 <i>lacX74 recA1 deoR araD139 (ara-leu)7697 galU</i> <i>galK rpsL (Str</i> ^R <i>) endA1 nupG</i>
BL21(DE3)pLysS	F ⁻ , <i>ompT hsdSB(r</i> _B ⁻ <i>m</i> _B ⁻ <i>) gal dcm (DE3) pLysS (Cam</i> ^R <i>)</i>

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