Universitas Esa Unggul

Smart, Creative and Entrepreneurial

www.esaunggul.ac.id

Enzim Restriksi Endonuklease

Kemampuan Akhir yang Diharapkan

- Mahasiswa dapat menjelaskan prinsip kerja enzim restriksi endonuklease
- Mahasiswa dapat menjelaskan pengelompokan enzim restriksi
- Mahasiswa dapat menjelaskan macam-macam enzim restriksi pada vektor

Restriction Enzymes Mapping

What are restriction enzymes?

- Molecular scissors that cut double stranded DNA molecules at specific points.
- Found naturally in a wide variety of prokaryotes
- An important tool for manipulating DNA.

Discovery

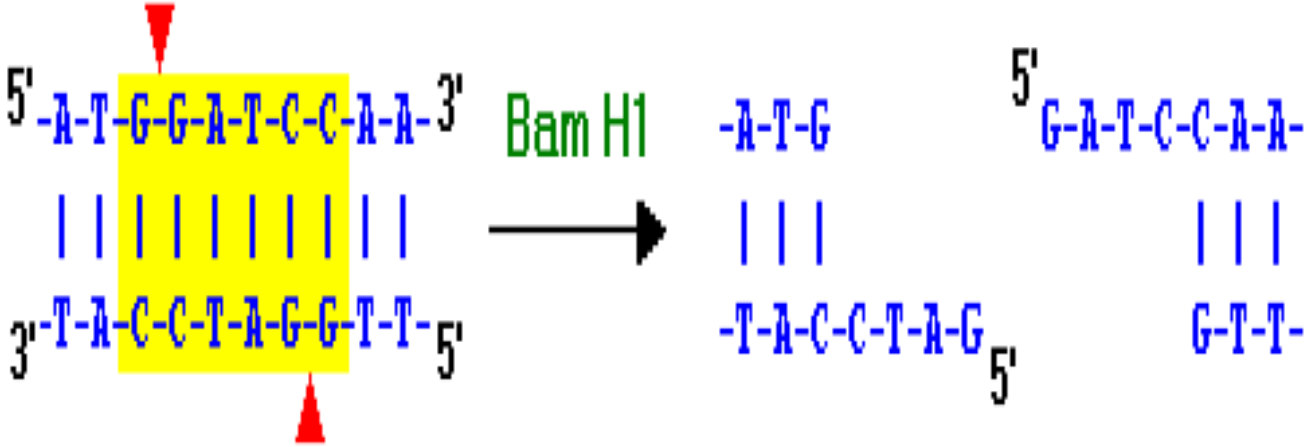
- Arbor and Dussoix in 1962 discovered that certain bacteria contain Endonucleases which have the ability to cleave DNA.
- In 1970 Smith and colleagues purified and characterized the cleavage site of a Restriction Enzyme.
- Werner Arbor, Hamilton Smith and Daniel Nathans shared the 1978 Nobel prize for Medicine and Physiology for their discovery of Restriction Enzymes.

ENZYMES USED IN MOLECULAR BIOLOGY

Restriction enzymes	Cut the DNA
Alkaline phosphatase	Removes phosphate groups from 5' ends of DNA (prevents unwanted re-ligation of cut DNA)
DNA ligase	Joins compatible ends of DNA fragments (blunt/blunt or complementary cohesive ends). Uses ATP
DNA polymerase I	Synthesises DNA complementary to a DNA template in the 5'-to-3'direction. Starts from an oligonucleotide primer with a 3' OH end
Exonuclease III	Digests nucleotides progressiviely from a DNA strand in the 3' -to-5' direction
Polynucleotide kinase	Adds a phosphate group to the 5' end of double- or single-stranded DNA or RNA. Uses ATP
RNase A	Nuclease which digests RNA, not DNA
Taq DNA polymerase	Heat-stable DNA polymerase isolated from a thermostable microbe (*Thermus aquaticus*)

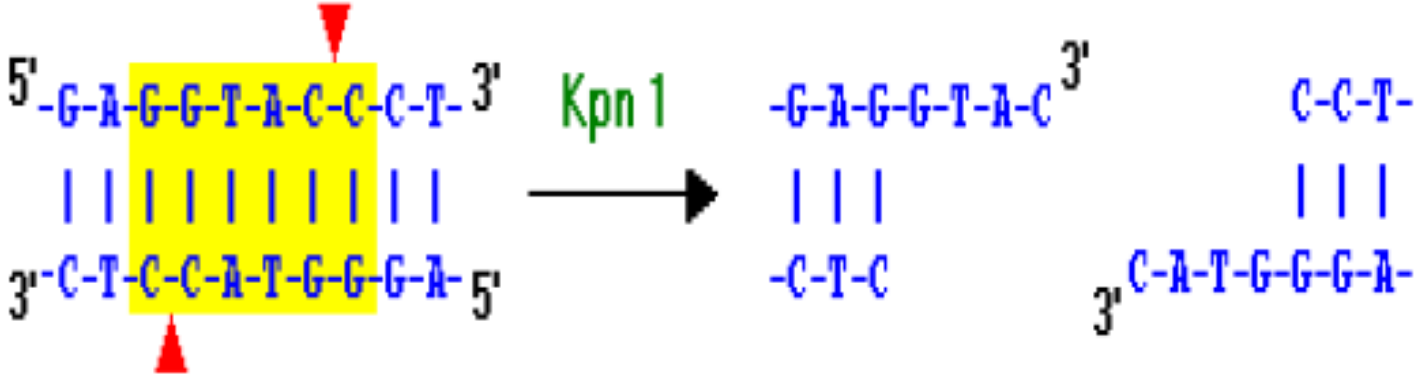
5' OVERHANGS

- **5' overhangs:** The enzyme cuts asymmetrically within the recognition site such that a short single-stranded segment extends from the 5' ends. *Bam* HI cuts in this manner.



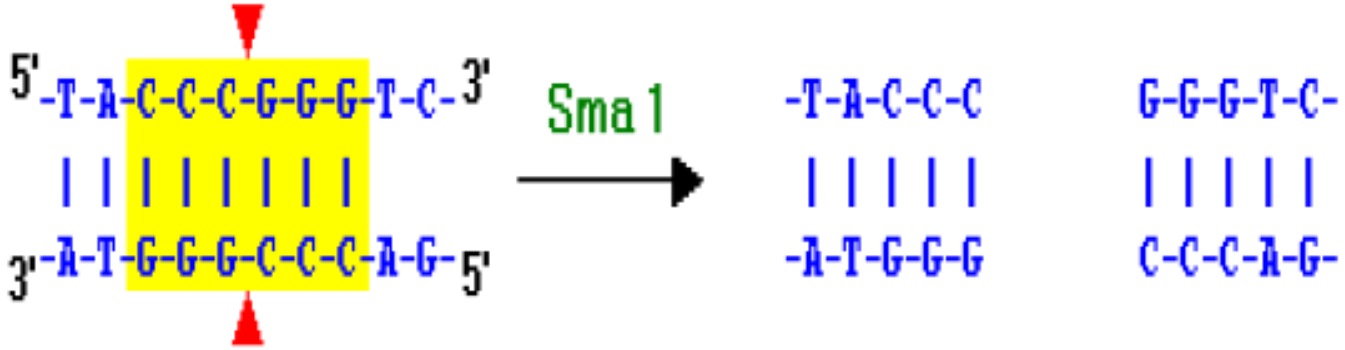
3' OVERHANGS

- **3' overhangs:** Again, we see asymmetrical cutting within the recognition site, but the result is a single-stranded overhang from the two 3' ends. *Kpn*I cuts in this manner.



BLUNT ENDS

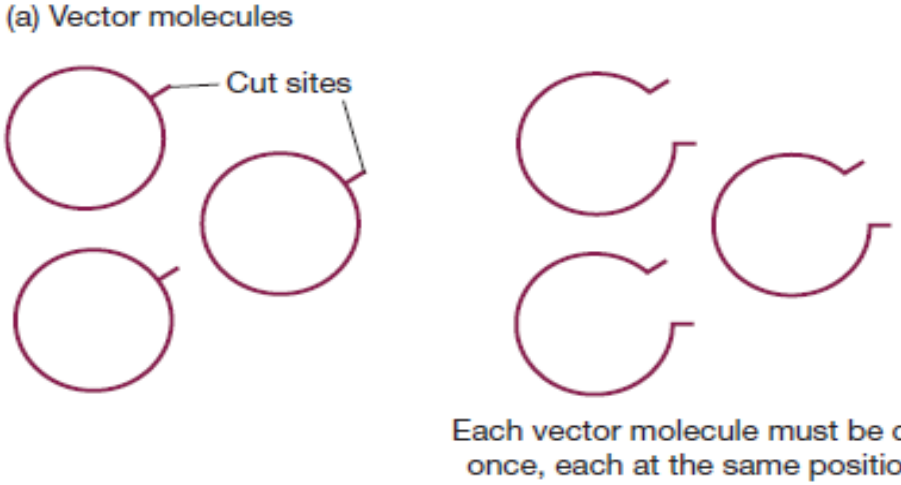
- **Blunts:** Enzymes that cut at precisely opposite sites in the two strands of DNA generate blunt ends without overhangs. *Sma*I is an example of an enzyme that generates blunt ends.



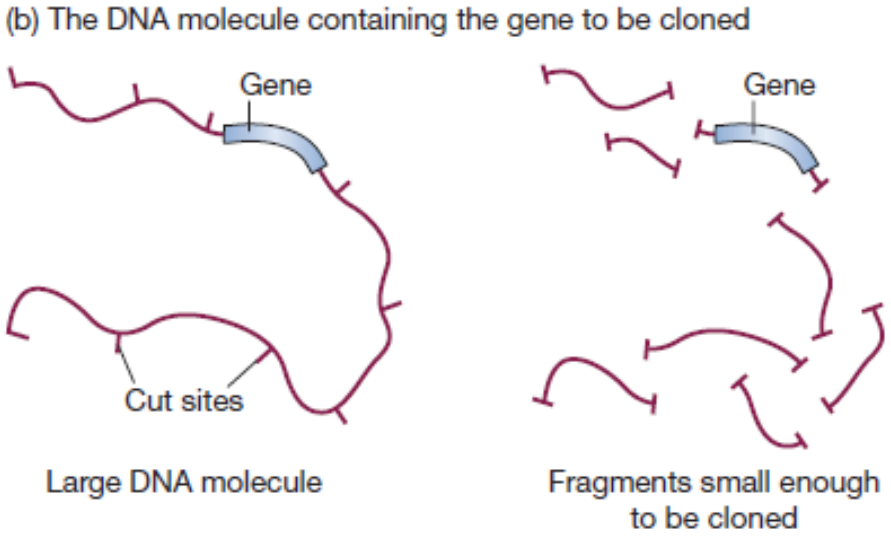
Types of Restriction Enzymes

	Cleavage site	Location of methylase	Examples
Type I	Random Around 1000bp away from recognition site	Endonuclease and methylase located on a single protein molecule	EcoK I EcoA I CfrA I
Type II	Specific Within the recognition site	Endonuclease and methylase are separate entities	EcoR I BamH I Hind III
Type III	Random 24-26 bp away from recognition site	Endonuclease and methylase located on a single protein molecule	EcoP I Hinf III EcoP15 I

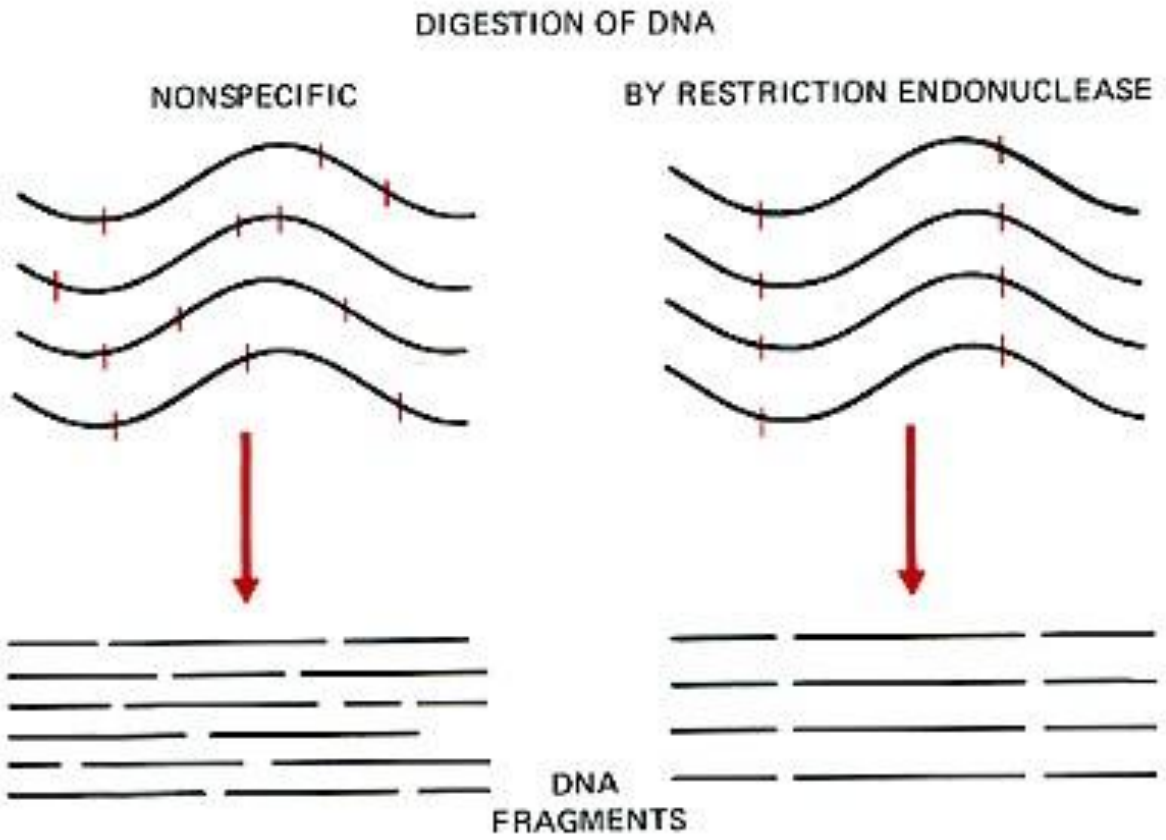
Pemotongan DNA pada Teknologi DNA Rekombinan



DAN

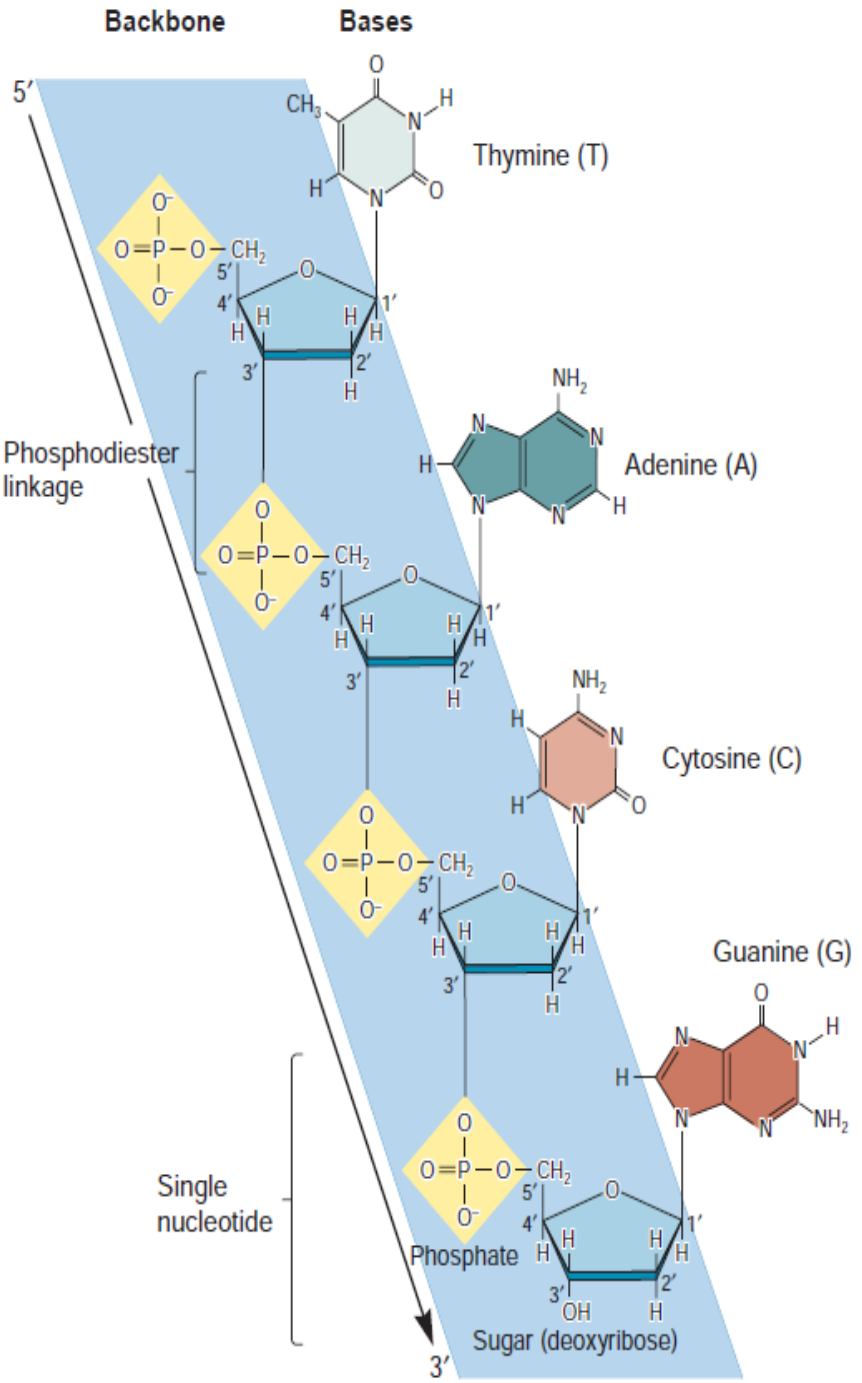


Pemotongan DNA pada Teknologi DNA Rekombinan Tidak Acak



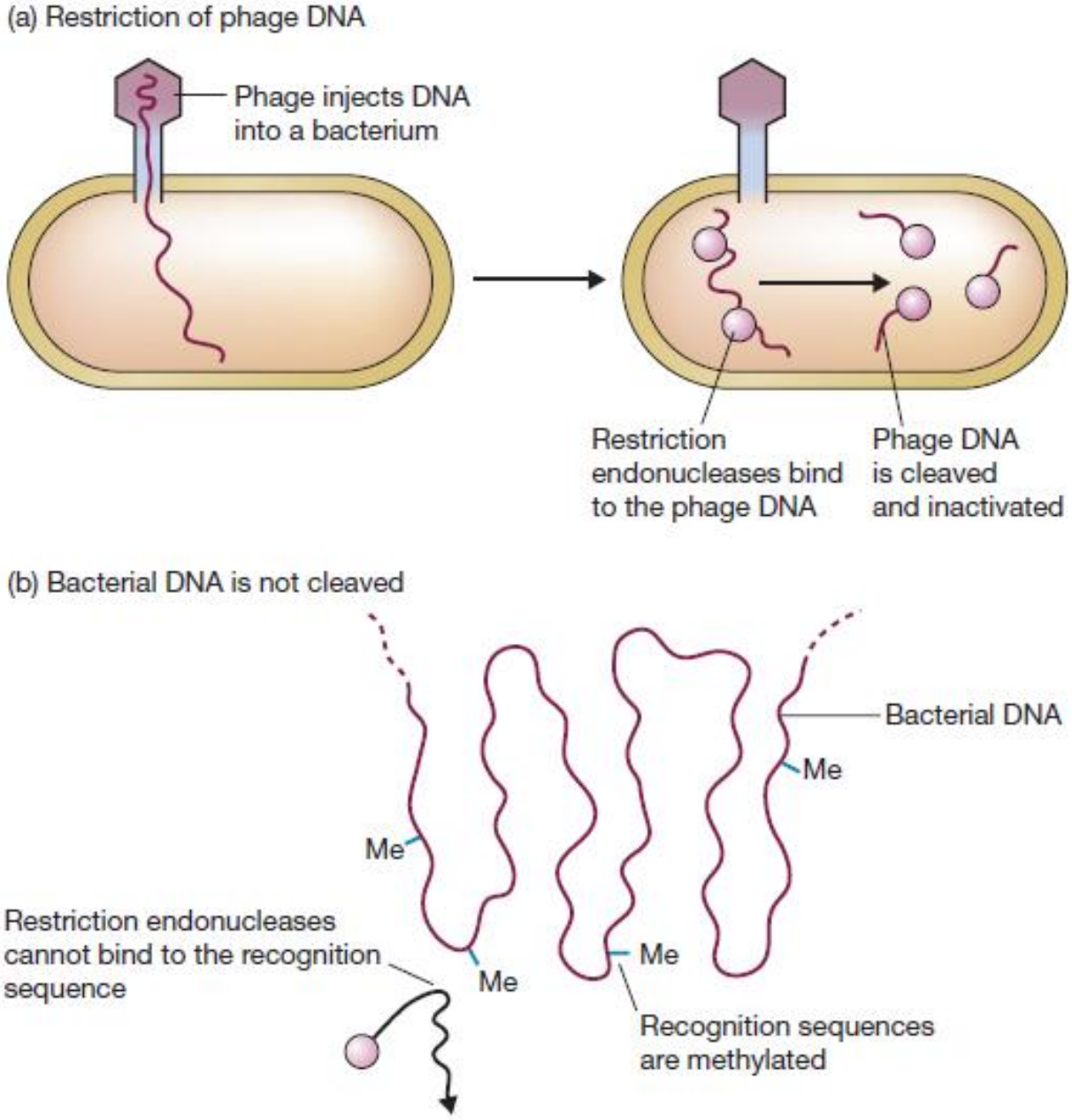
Enzim Restriksi Endonuklease

- Dapat memotong DNA → memotong pada ikatan fosfodiester
- Memotong di bagian dalam → endonuklease
- Disebut juga dengan enzim restriksi
- Dihasilkan oleh bakteri dan Archaea



Sejarah Penemuan Enzim Restriksi

- Beberapa peneliti menemukan bahwa bakteriofaga tidak dapat menginfeksi bakteri
- Hal ini dipelajari lebih lanjut oleh W. Arber, H. Smith dan D. Nathans
- Bakteri menghasilkan enzim yang dapat memotong DNA virus

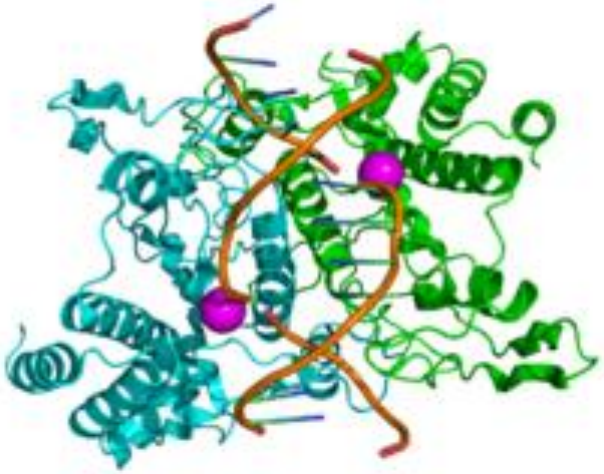

(a) Restriction of phage DNA
Phage injects DNA into a bacterium
Restriction endonucleases bind to the phage DNA
Phage DNA is cleaved and inactivated
(b) Bacterial DNA is not cleaved
Bacterial DNA
Me
Me
Me
Me
Restriction endonucleases cannot bind to the recognition sequence
Recognition sequences are methylated

Sejarah Penemuan Enzim Restriksi

- Menghasilkan enzim restriksi yang pertama yaitu HindIII
- Pada tahun 1978 ketiga peneliti tersebut mendapatkan hadiah Nobel dalam Fisiologi atau Kedokteran

Tipe-tipe Enzim Retriksi Endonuklease

- Tipe I → *akan memotong DNA di luar daerah pemotongan spesifik, tidak digunakan dalam rekombinasi DNA*
- Tipe II → *memotong DNA pada situs yang spesifik, digunakan dalam rekombinasi DNA*
- Tipe III → *akan memotong DNA di luar daerah pemotongan spesifik, memerlukan 2 sekuen yang sama, tidak digunakan untuk rekombinasi DNA*
- Tipe IV → *akan mengenali DNA dengan metilasi, tidak digunakan dalam rekombinasi DNA*



TYPES AND ACTIVITIES OF RESTRICTION ENZYMES

Type I
Cleaves DNA at random sites far from its recognition sequence

Type II
Cleaves DNA at defined positions close to or within its recognition sequence

Type IIG
Cleaves outside its recognition sequence with both REase and MTase enzymatic activities in the same protein

Type IIP
Cleaves symmetric targets and cleavage sites

Type IIS
Recognizes asymmetric sequences

Type III
Cleaves outside its recognition sequence and require two sequences in opposite orientations within the same DNA

Type IV
Cleaves modified (e.g., methylated) DNA

Penamaan Enzim Restriksi

- Huruf yang digunakan tidak dimiringkan
- Huruf pertama merupakan nama genus bakteri yang menghasilkan
- Huruf kedua dan ketiga merupakan nama spesies bakteri yang menghasilkan
- Huruf keempat merupakan strain dari bakteri yang menghasilkan
- Nomor romawi menunjukkan urutan penemuan enzim tersebut

Penamaan Enzim Restriksi

- Contoh : enzim EcoRI
  → Berasal dari *Escherichia coli*
  → strain RY

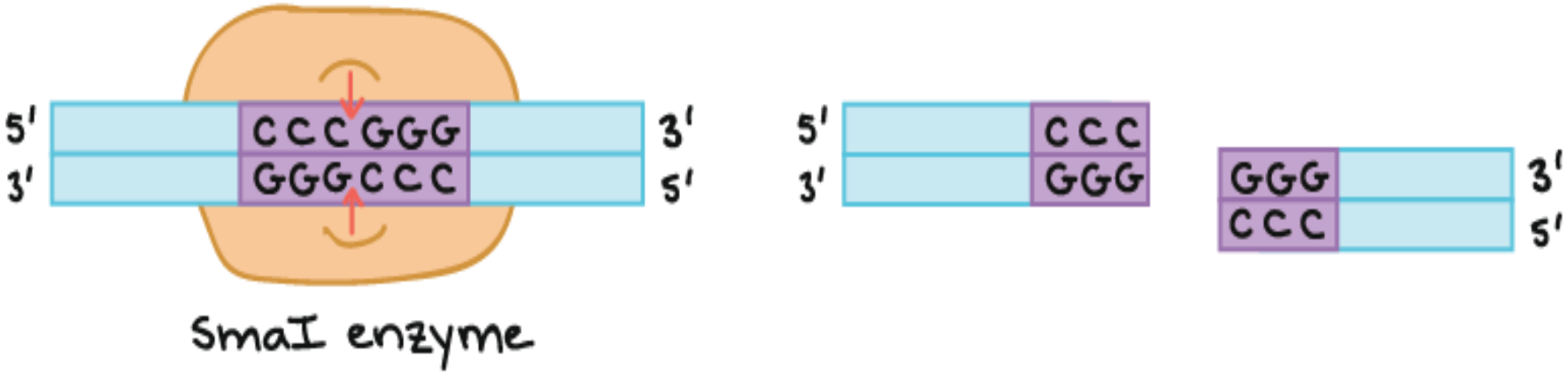
- Contoh : enzim HindIII
  → Berasal dari *Haemophilus influenza*
  → Serotipe d

Enzim Restriksi Tipe II Memotong Pada Situs Pemotongan yang Spesifik

ENZYME	ORGANISM	RECOGNITION SEQUENCE*	BLUNT OR STICKY END
*Eco*RI	*Escherichia coli*	GAATTC	Sticky
*Bam*HI	*Bacillus amyloliquefaciens*	GGATCC	Sticky
*Bgl*II	*Bacillus globigii*	AGATCT	Sticky
*Pvu*I	*Proteus vulgaris*	CGATCG	Sticky
*Pvu*II	*Proteus vulgaris*	CAGCTG	Blunt
*Hind*III	*Haemophilus influenzae* R_d	AAGCTT	Sticky
*Hin*fI	*Haemophilus influenzae* R_f	GANTC	Sticky
*Sau*3A	*Staphylococcus aureus*	GATC	Sticky
*Alu*I	*Arthrobacter luteus*	AGCT	Blunt
*Taq*I	*Thermus aquaticus*	TCGA	Sticky
*Hae*III	*Haemophilus aegyptius*	GGCC	Blunt
*Not*I	*Nocardia otitidis-caviarum*	GCGGCCGC	Sticky
*Sfi*I	*Streptomyces fimbriatus*	GGCCNNNNNGGCC	Sticky

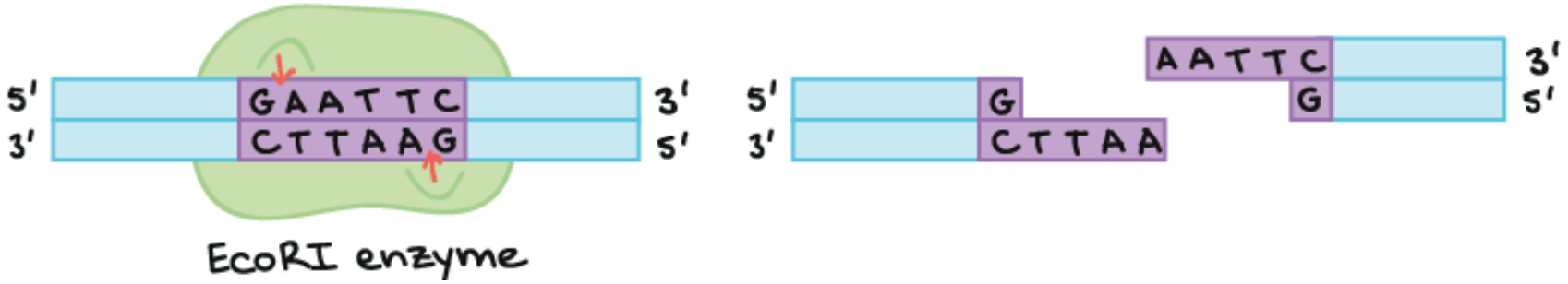
Hasil Pemotongan Enzim Restriksi

- Ujung-ujung tumpul (*Blunt ends*)



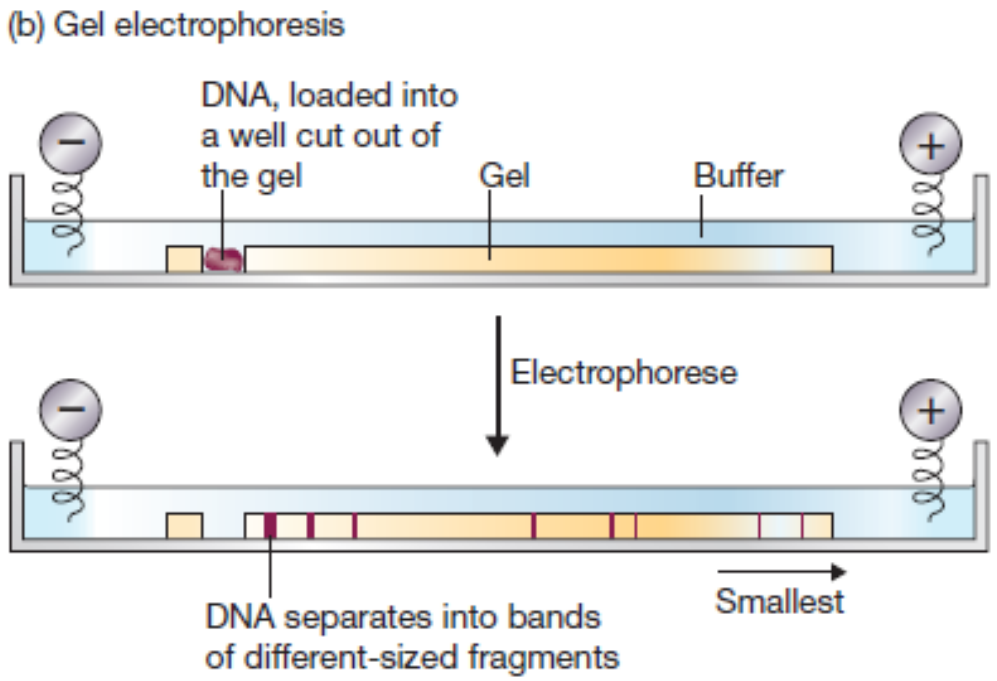
Hasil Pemotongan Enzim Restriksi

- Ujung-ujung menggantung (*Sticky ends*)

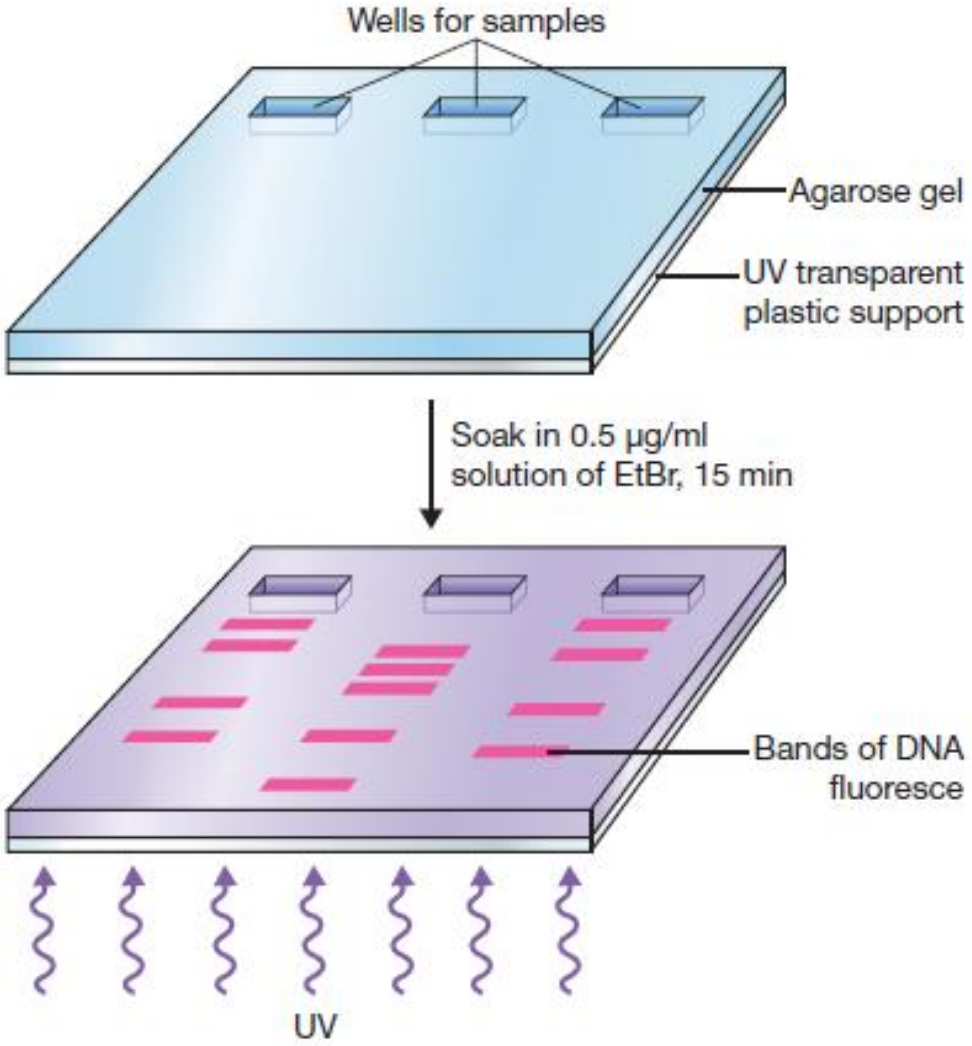


Analisa Hasil Restriksi DNA

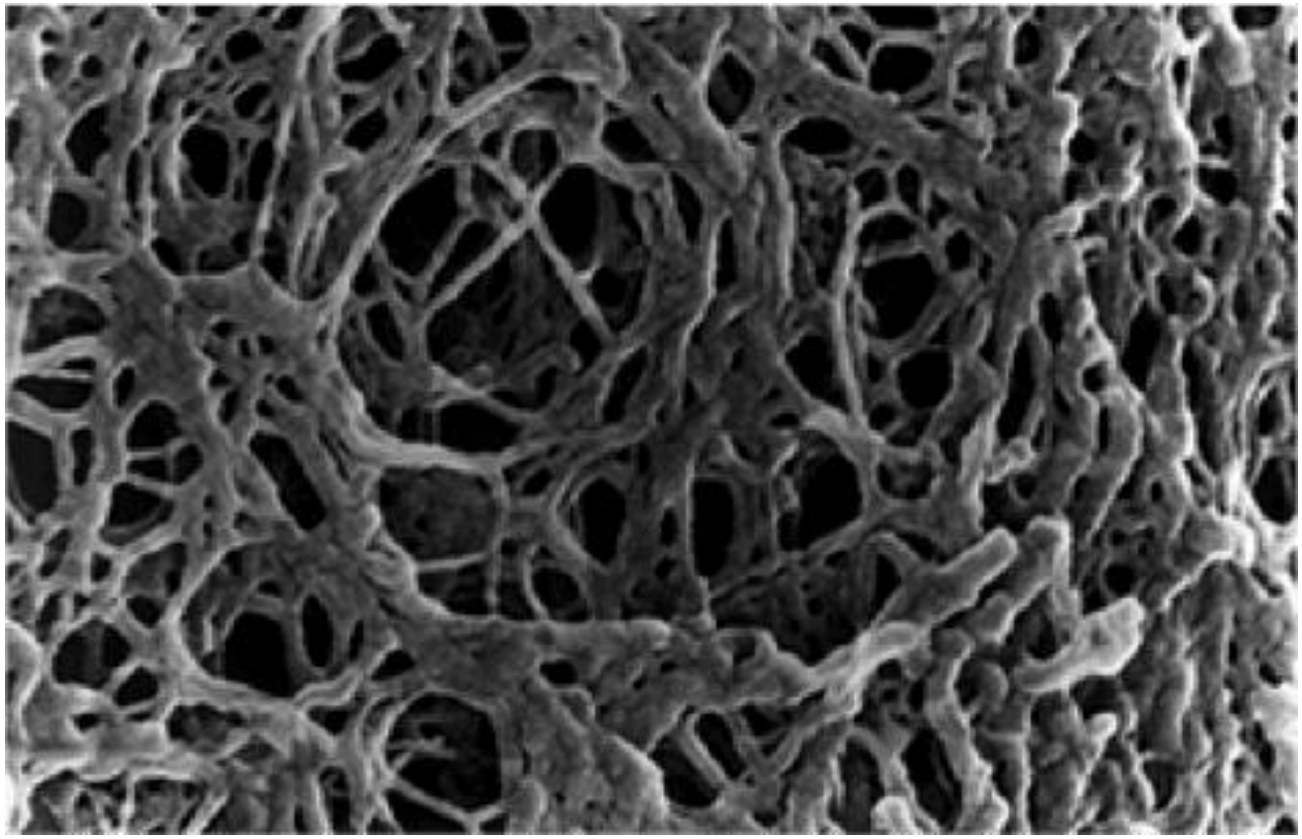
- Dengan elektroforesis gel
- Baik gel agarose maupun poliakrilamid



Elektroforesis Gel Agarose

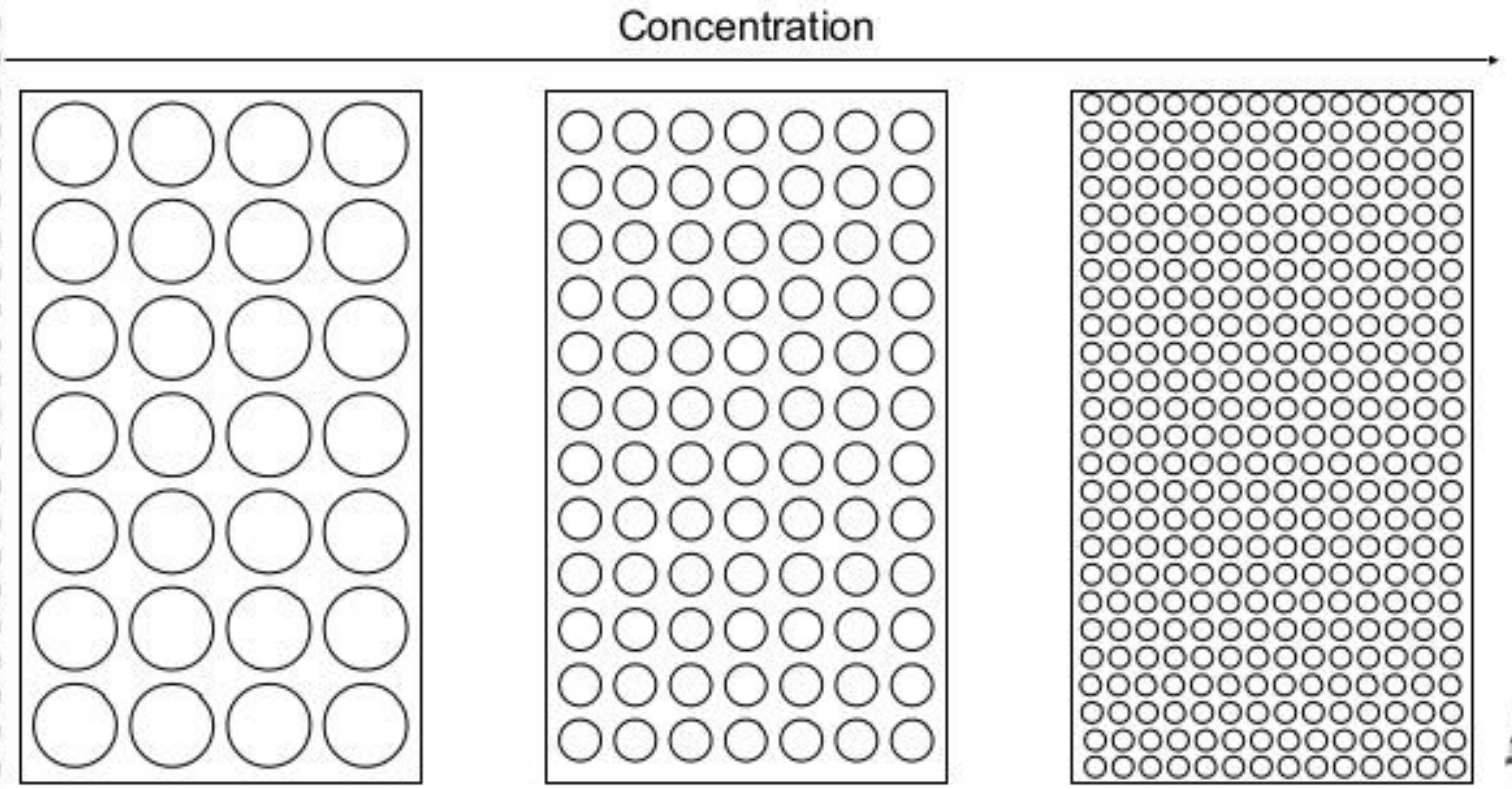


Pori-pori pada Gel Agarose dan Poliakrilamid



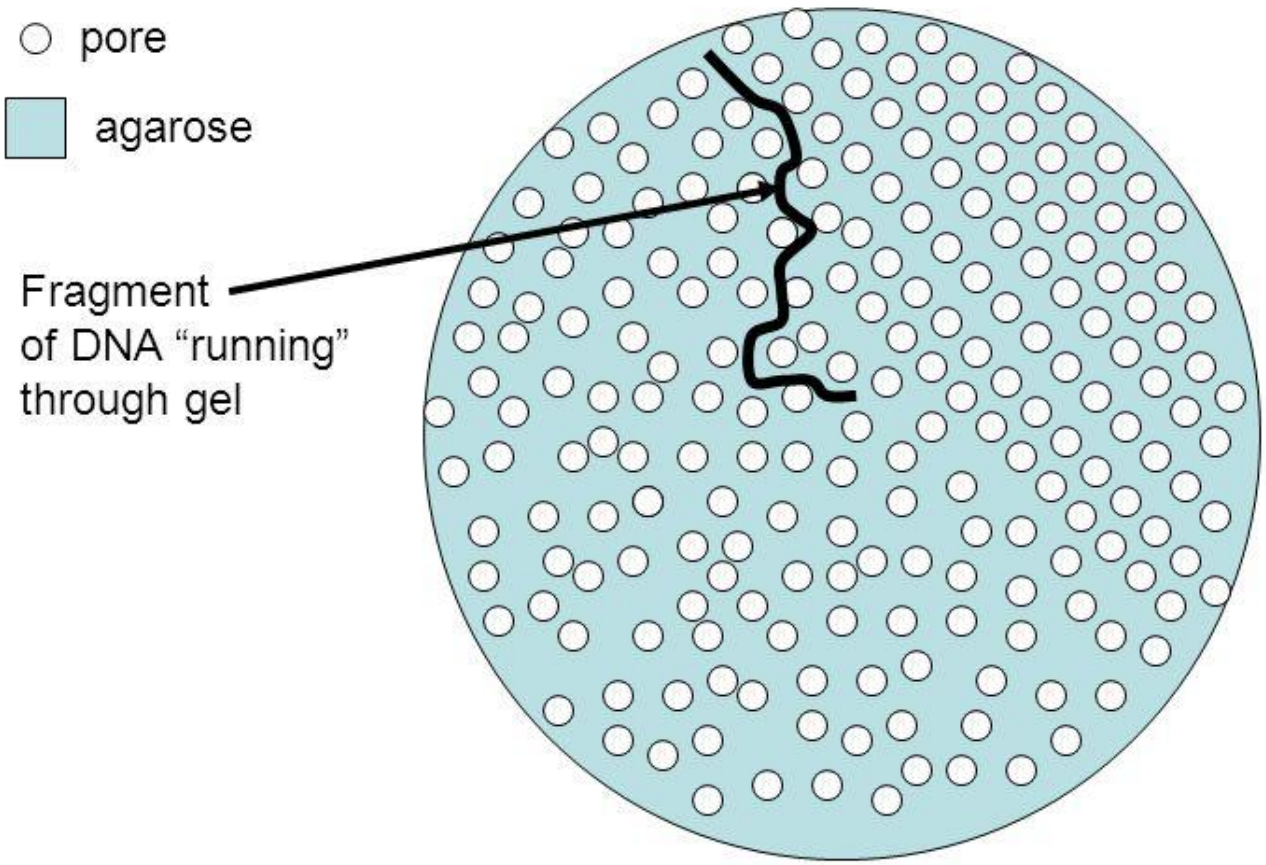
Agarose concentration

As we increase the concentration of agarose (g/ml) the pores gets smaller!



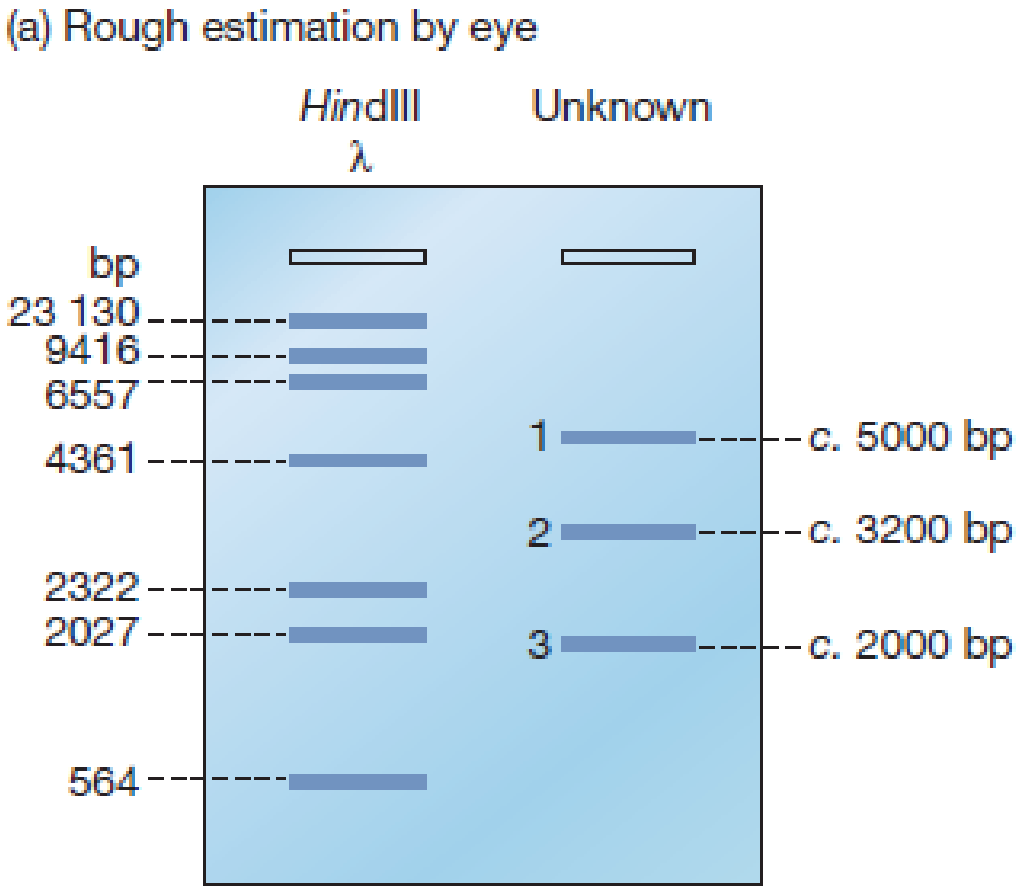
(https://www.slideshare.net/hhalhaddad/281-lec30-moltech2)

Microscopic view of a gel

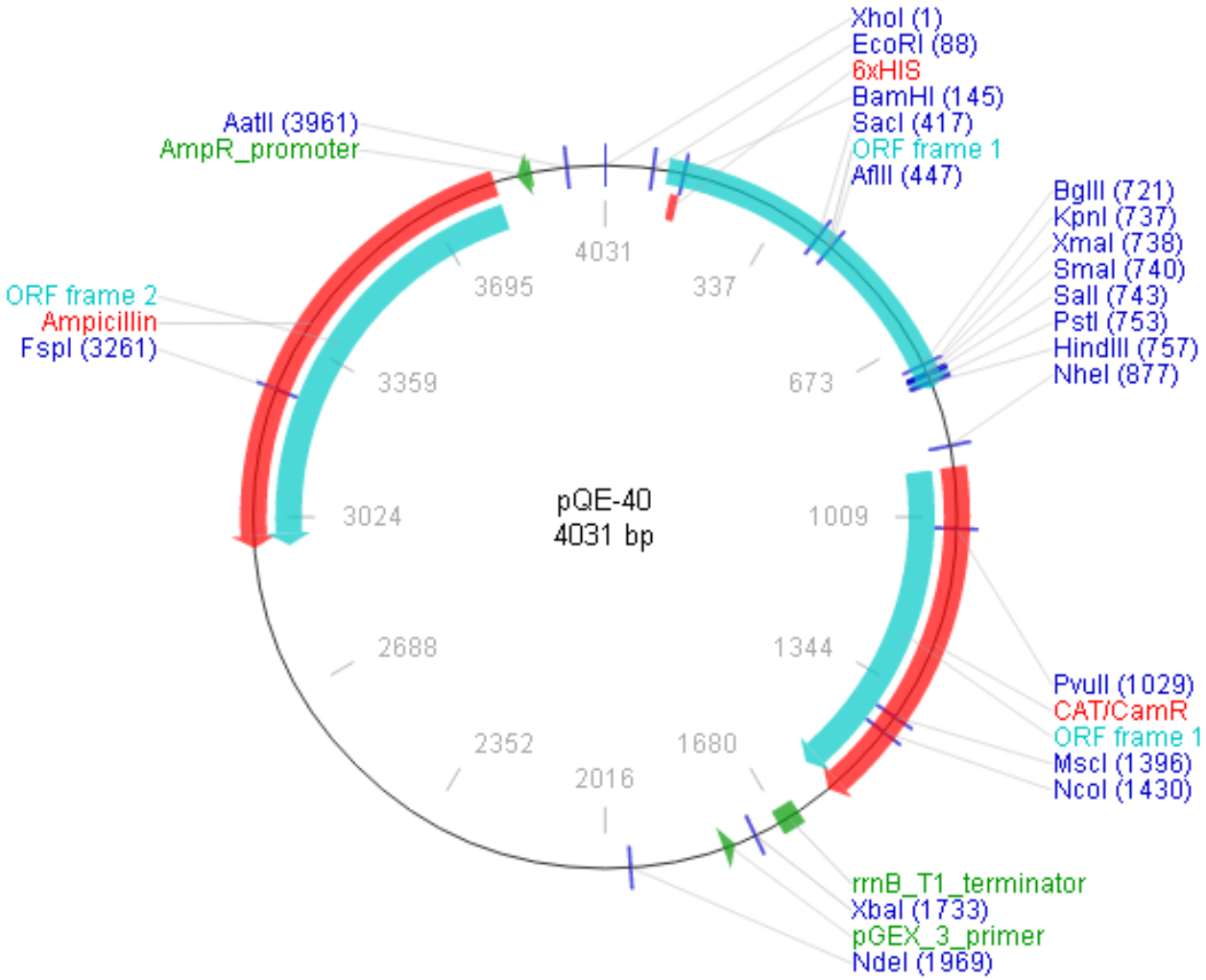


(http://slideplayer.com/slide/7876390/)

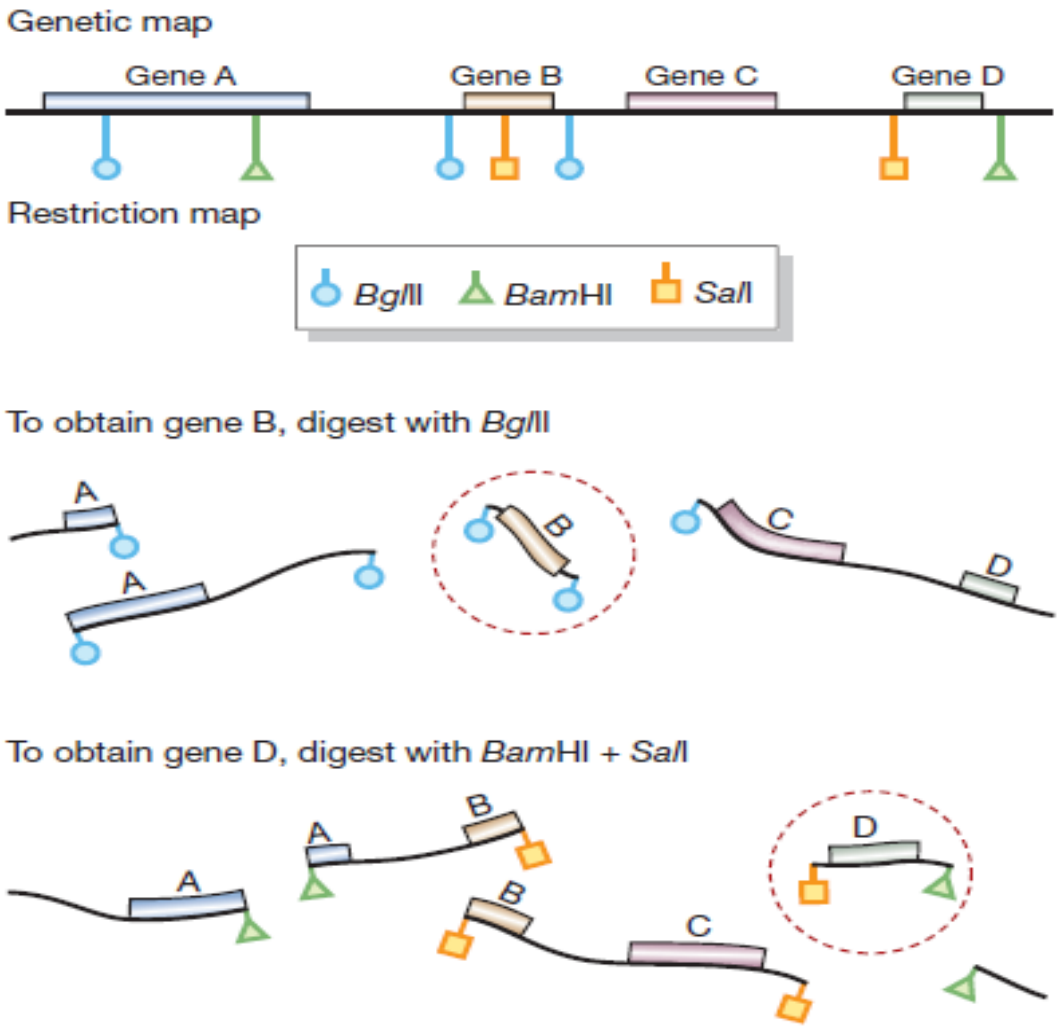
Analisa Hasil Restriksi



Peta Enzim Restriksi pada Vektor



Cara Mengetahui Situs Restriksi Pada Gen atau Genom



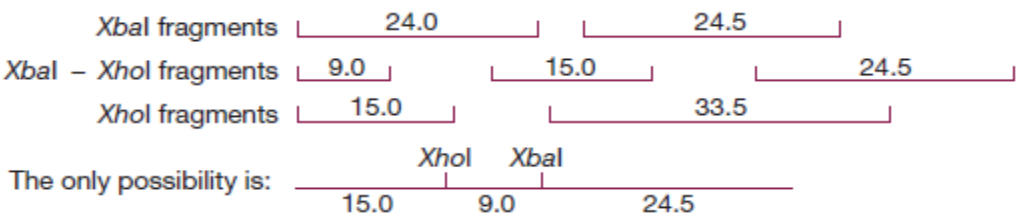
Single and double digestions

Enzyme	Number of fragments	Sizes (kb)
*Xba*I	2	24.0, 24.5
*Xho*I	2	15.0, 33.5
*Kpn*I	3	1.5, 17.0, 30.0
*Xba*I + *Xho*I	3	9.0, 15.0, 24.5
*Xba*I + *Kpn*I	4	1.5, 6.0, 17.0, 24.0

Conclusions:

(1) As λ DNA is linear, the number of restriction sites for each enzyme is *Xba*I 1, *Xho*I 1, *Kpn*I 2.

(2) The *Xba*I and *Xho*I sites can be mapped:



(3) All the *Kpn*I sites fall in the 24.5 kb *Xba*I fragment, as the 24.0 kb fragment is intact after *Xba*I–*Kpn*I double digestion. The order of the *Kpn*I fragments can be determined only by partial digestion.

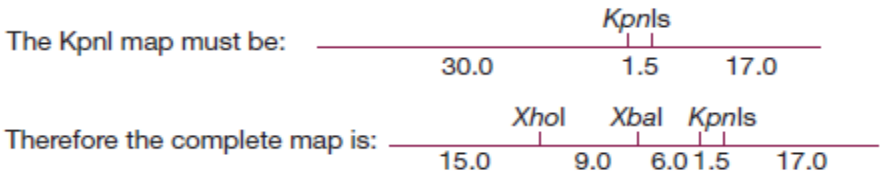
ZAMAN OLD

Partial digestion

Enzyme	Fragment sizes (kb)
*Kpn*I – limiting conditions	1.5, 17.0, 18.5, 30.0, 31.5, 48.5

Conclusions:

(1) 48.5 kb fragment = uncut λ.
(2) 1.5, 17.0 and 30.0 kb fragments are products of complete digestion.
(3) 18.5 and 31.5 kb fragments are products of partial digestion.



Welcome to RestrictionMapper - on line restriction mapping the easy way.

Maps sites for restriction enzymes, a.k.a. restriction endonucleases, in DNA sequences. Also does virtual digestion.

- Help
- FAQ
- What`s New?
- Code
- Contact
- Automate RestrictionMapper
- Other Free Molecular Biology Resources
- Dilution Calculator

Conformation

Circular ○
Linear ◉

Sort By

1. frequency
2. overhang
3. name

Filter By

Maximum Cuts: all

Minimum Site Length: 5

Include

Select Individual Enzymes

All Enzymes
AarI
AasI
AatI
AatII
AccI
AccII

All Commercial ◉
NEB only ○

5' overhang ☑
3' overhang ☑
blunt ☑

Prototypes Only ◉
All Isoschizomers ○

Sequence Info

No non-base letters.
Numbers and spaces OK.

Paste Sequence Here

Name your sequence

Untitled

Menu

Map Sites
Virtual Digest
Reset Form

Resources

Technical Resource Centers

Bioinformatics Tools

- Molecular Biology Tools
- Peptide Tools
- Protein Tools

Learning Center

Research Applications

Reference Databases

Immunoprecipitation

Custom stable cell line applications

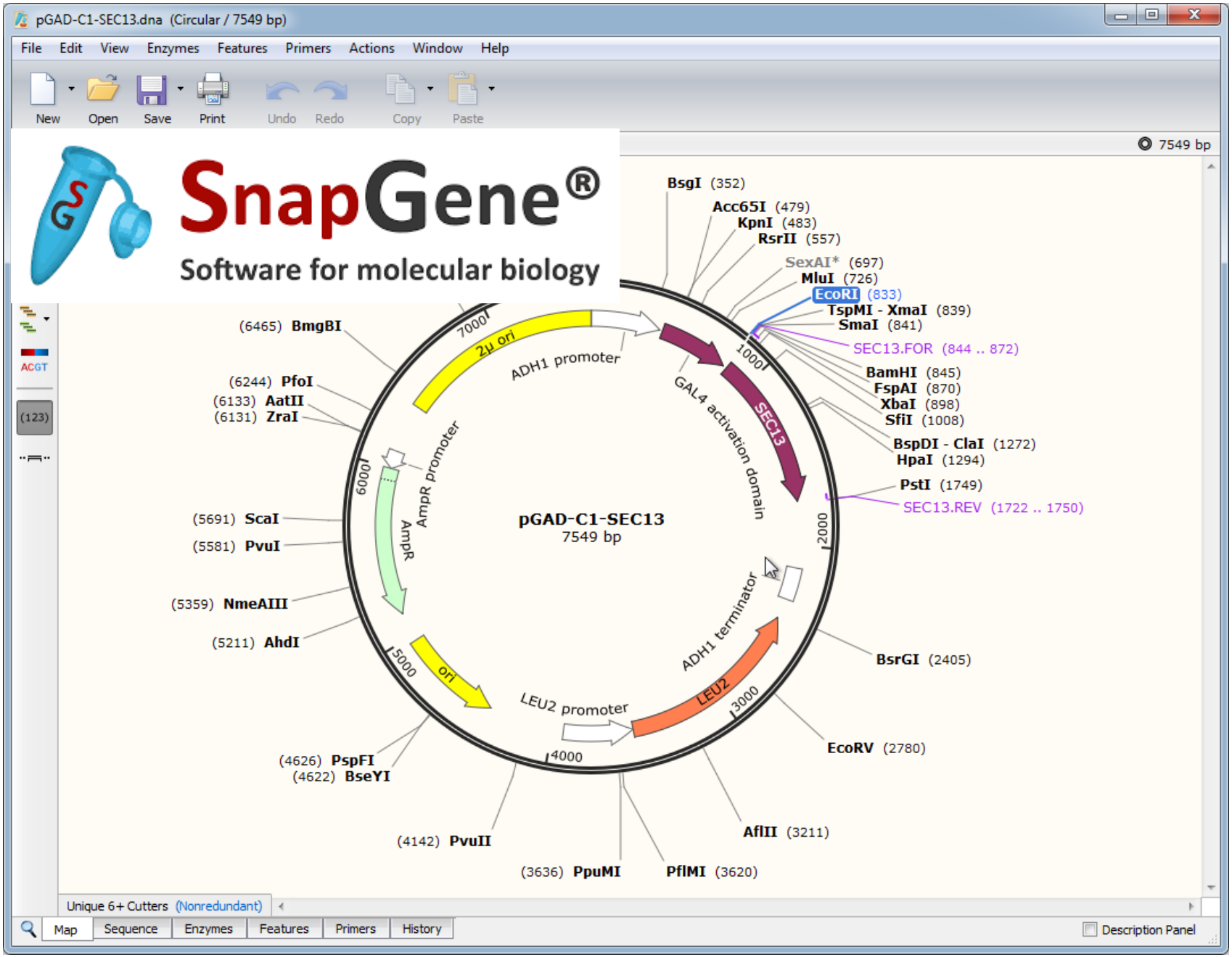
Webinars

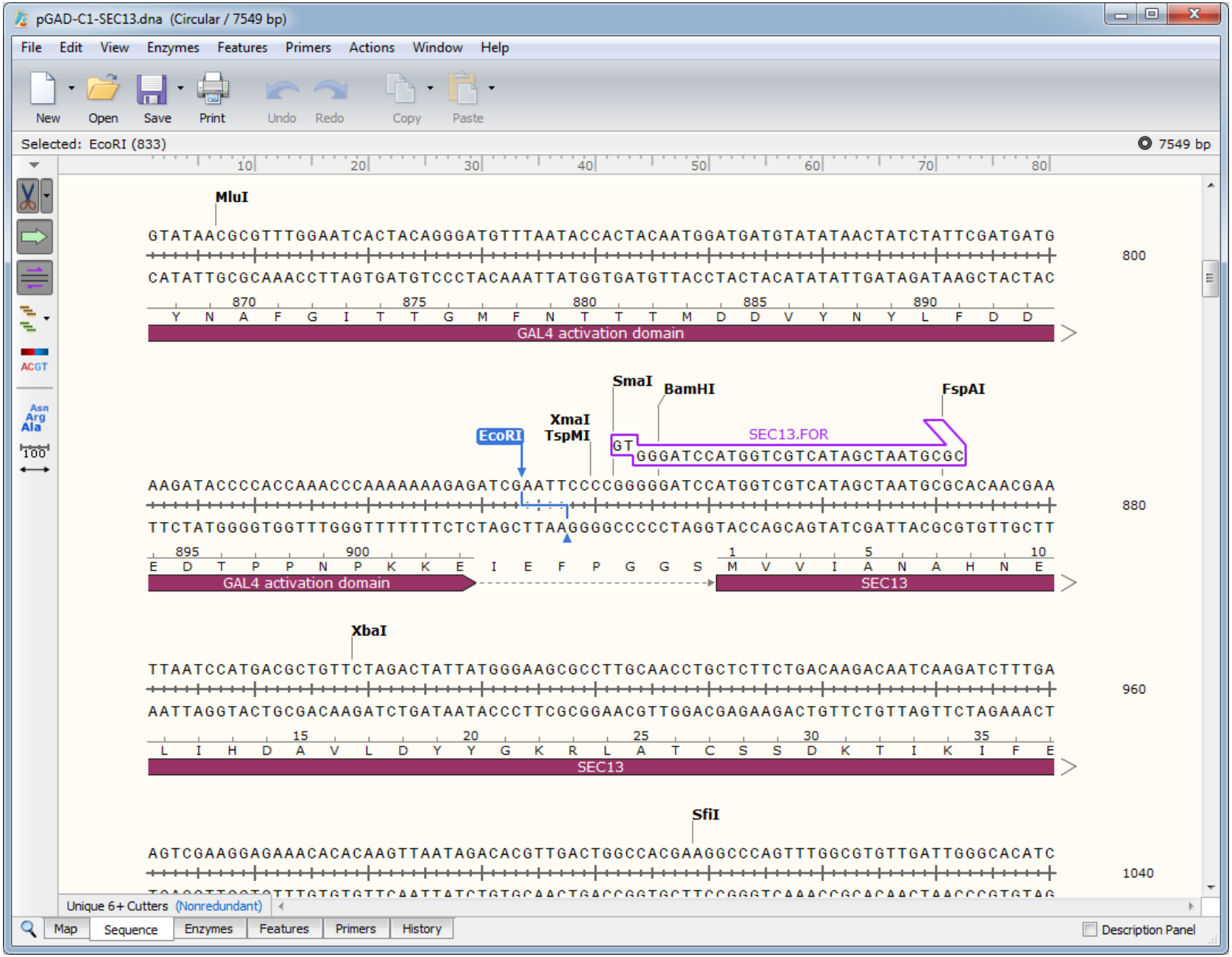
GenScript Restriction Enzyme Map Analysis Tools

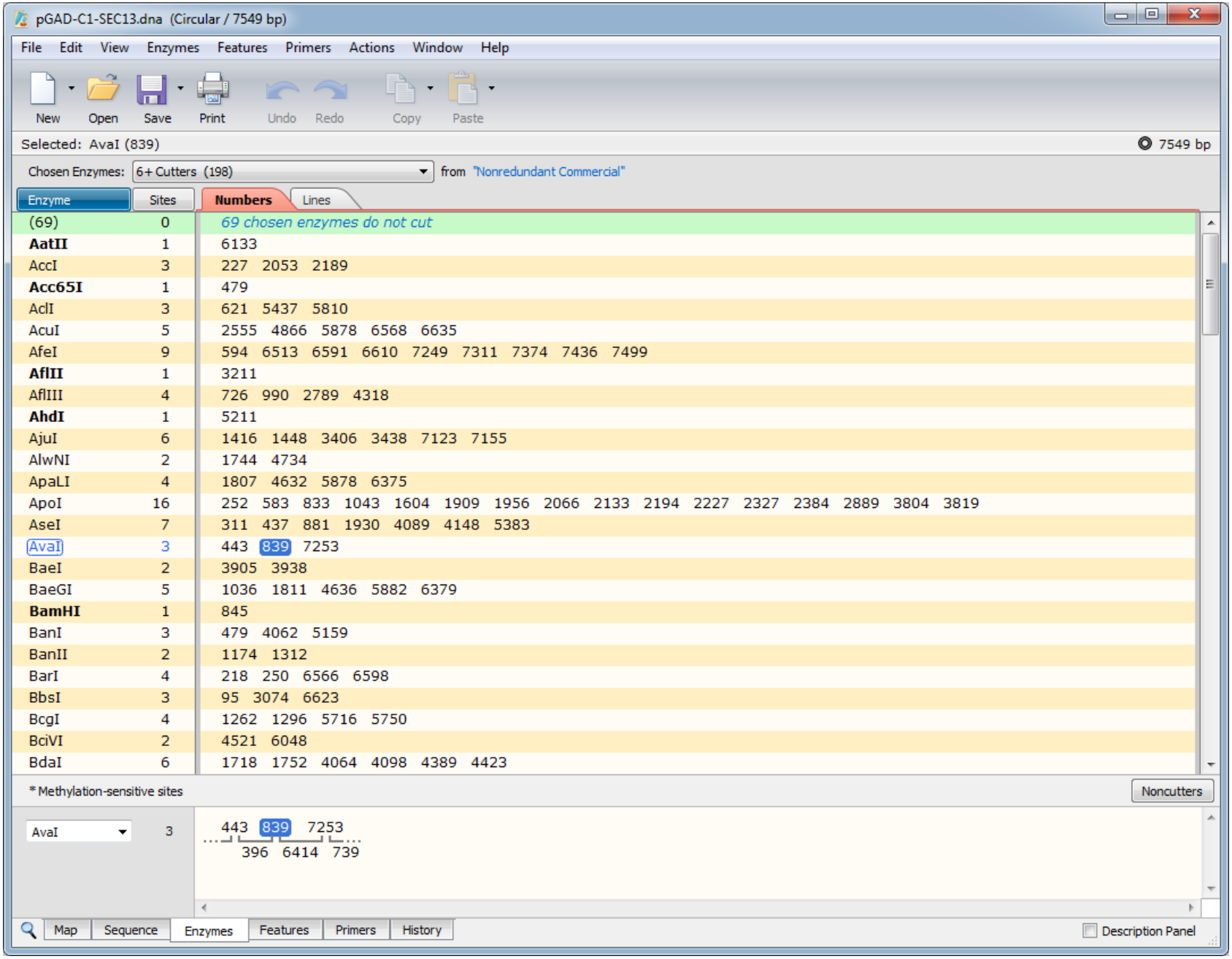
** This online tool helps you analyze restriction enzyme cutting maps.

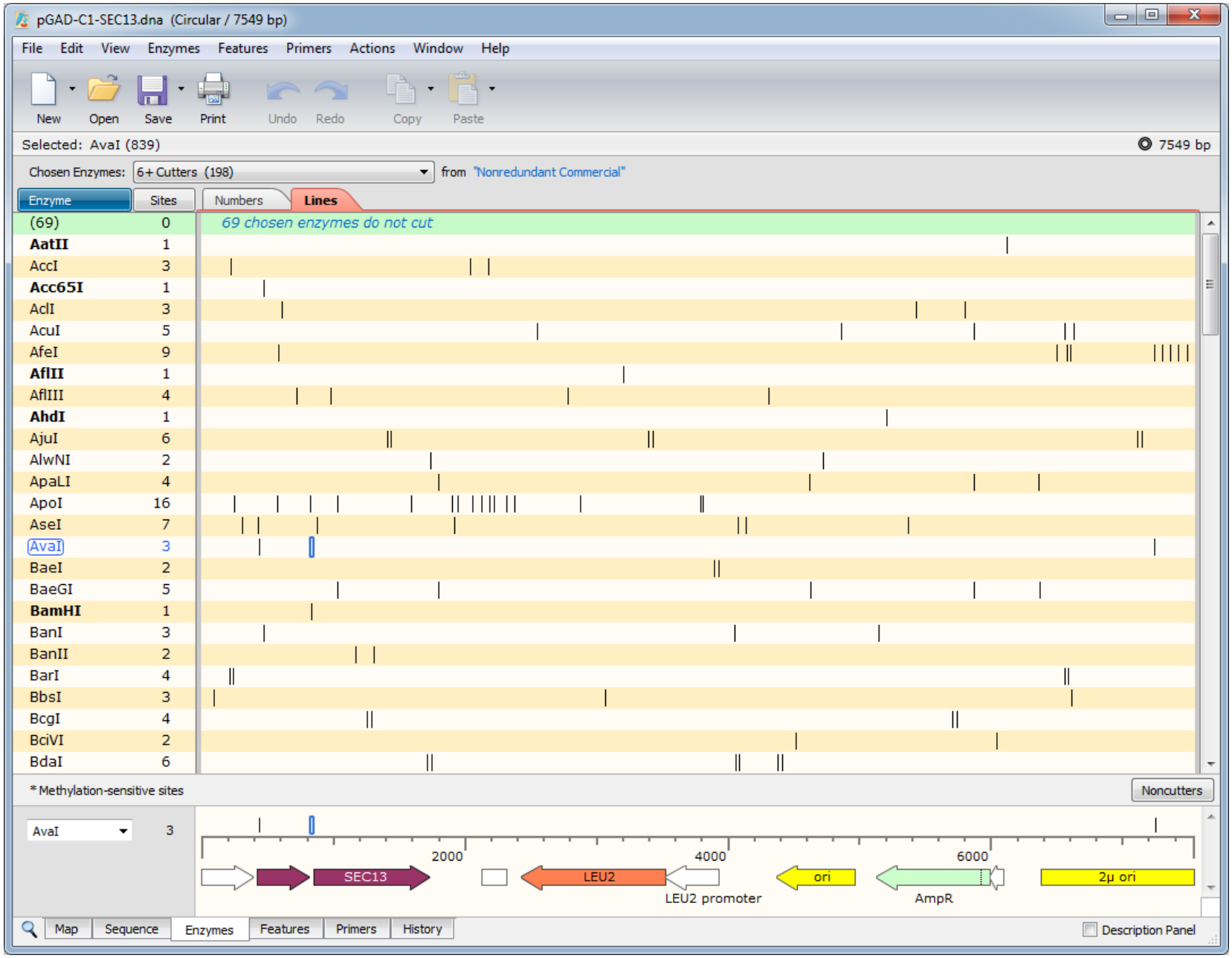
Paste in DNA Sequence:

Submit Reset


pGAD-C1-SEC13.dna (Circular / 7549 bp)
File Edit View Enzymes Features Primers Actions Window Help
New Open Save Print Undo Redo Copy Paste
SnapGene®
Software for molecular biology
7549 bp
BsgI (352)
Acc65I (479)
KpnI (483)
RsrII (557)
SexAI* (697)
MluI (726)
EcoRI (833)
TspMI - XmaI (839)
SmaI (841)
SEC13.FOR (844 .. 872)
BamHI (845)
FspAI (870)
XbaI (898)
SfiI (1008)
BspDI - ClaI (1272)
HpaI (1294)
PstI (1749)
SEC13.REV (1722 .. 1750)
BsrGI (2405)
EcoRV (2780)
AflII (3211)
PflMI (3620)
PpuMI (3636)
PvuII (4142)
PspFI (4626)
BseYI (4622)
NmeAIII (5359)
AhdI (5211)
ScaI (5691)
PvuI (5581)
BmgBI (6465)
PfoI (6244)
AatII (6133)
ZraI (6131)
pGAD-C1-SEC13
7549 bp
2µ ori
ADH1 promoter
GAL4 activation domain
SEC13
AmpR promoter
AmpR
ori
LEU2 promoter
LEU2
ADH1 terminator
1000
2000
3000
4000
5000
6000
7000
Unique 6+ Cutters (Nonredundant)
Map Sequence Enzymes Features Primers History
Description Panel


pGAD-C1-SEC13.dna (Circular / 7549 bp)
File Edit View Enzymes Features Primers Actions Window Help
New Open Save Print Undo Redo Copy Paste
Selected: EcoRI (833)
7549 bp
MluI
GTATAACGCGTTTGGAATCACTACAGGGATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATTCGATGATG
CATATTGCGCAAACCTTAGTGATGTCCCTACAAATTATGGTGATGTTACCTACTACATATATTGATAGATAAGCTACTAC
Y N A F G I T T G M F N T T M D D V Y N Y L F D D
GAL4 activation domain
SmaI
BamHI
FspAI
XmaI
TspMI
EcoRI
SEC13.FOR
GT GGGATCCATGGTCGTCATAGCTAATGCGC
AAGATACCCCACCAAACCCAAAAAAAGAGATCGAATTCCCCGGGGGATCCATGGTCGTCATAGCTAATGCGCACAACGAA
TTCTATGGGGTGGTTTGGGTTTTTTTCTCTAGCTTAAGGGGCCCCCTAGGTACCAGCAGTATCGATTACGCGTGTTGCTT
E D T P P N P K K E I E F P G G S M V V I A N A H N E
GAL4 activation domain
SEC13
XbaI
TTAATCCATGACGCTGTTCTAGACTATTATGGGAAGCGCCTTGCAACCTGCTCTTCTGACAAGACAATCAAGATCTTTGA
AATTAGGTACTGCGACAAGATCTGATAATACCCTTCGCGGAACGTTGGACGAGAAGACTGTTCTGTTAGTTCTAGAAACT
L I H D A V L D Y Y G K R L A T C S S D K T I K I F E
SEC13
SfiI
AGTCGAAGGAGAAACACACAAGTTAATAGACACGTTGACTGGCCACGAAGGCCCAGTTTGGCGTGTTGATTGGGCACATC
800
880
960
1040
Unique 6+ Cutters (Nonredundant)
Map Sequence Enzymes Features Primers History
Description Panel


pGAD-C1-SEC13.dna (Circular / 7549 bp)
File Edit View Enzymes Features Primers Actions Window Help
New Open Save Print Undo Redo Copy Paste
Selected: AvaI (839)
7549 bp
Chosen Enzymes: 6+ Cutters (198) from "Nonredundant Commercial"
Enzyme Sites Numbers Lines
(69) 0 69 chosen enzymes do not cut
AatII 1 6133
AccI 3 227 2053 2189
Acc65I 1 479
AclI 3 621 5437 5810
AcuI 5 2555 4866 5878 6568 6635
AfeI 9 594 6513 6591 6610 7249 7311 7374 7436 7499
AflII 1 3211
AflIII 4 726 990 2789 4318
AhdI 1 5211
AjuI 6 1416 1448 3406 3438 7123 7155
AlwNI 2 1744 4734
ApaLI 4 1807 4632 5878 6375
ApoI 16 252 583 833 1043 1604 1909 1956 2066 2133 2194 2227 2327 2384 2889 3804 3819
AseI 7 311 437 881 1930 4089 4148 5383
AvaI 3 443 839 7253
BaeI 2 3905 3938
BaeGI 5 1036 1811 4636 5882 6379
BamHI 1 845
BanI 3 479 4062 5159
BanII 2 1174 1312
BarI 4 218 250 6566 6598
BbsI 3 95 3074 6623
BcgI 4 1262 1296 5716 5750
BciVI 2 4521 6048
BdaI 6 1718 1752 4064 4098 4389 4423
* Methylation-sensitive sites
Noncutters
AvaI 3
443 839 7253
396 6414 739
Map Sequence Enzymes Features Primers History
Description Panel


pGAD-C1-SEC13.dna (Circular / 7549 bp)
File Edit View Enzymes Features Primers Actions Window Help
New Open Save Print Undo Redo Copy Paste
Selected: AvaI (839)
7549 bp
Chosen Enzymes: 6+ Cutters (198) from "Nonredundant Commercial"
Enzyme Sites Numbers Lines
(69) 0 69 chosen enzymes do not cut
AatII 1
AccI 3
Acc65I 1
AclI 3
AcuI 5
AfeI 9
AflII 1
AflIII 4
AhdI 1
AjuI 6
AlwNI 2
ApaLI 4
ApoI 16
AseI 7
AvaI 3
BaeI 2
BaeGI 5
BamHI 1
BanI 3
BanII 2
BarI 4
BbsI 3
BcgI 4
BciVI 2
BdaI 6
* Methylation-sensitive sites
Noncutters
AvaI 3
2000
4000
6000
SEC13
LEU2
LEU2 promoter
ori
AmpR
2µ ori
Map Sequence Enzymes Features Primers History
Description Panel