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PENGANTAR BIOINFORMATIKA

IBT 431

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Meeting 11

Restriction Enzymes Mapping

Sasaran Perkuliahan

- Mahasiswa mampu menganalisis dan memetakan enzim restriksi pada sebuah sekuen DNA
- Mahasiswa Dapat mengenali situs pemotongan enzim serta pola pemotongannya

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.

Biological Role

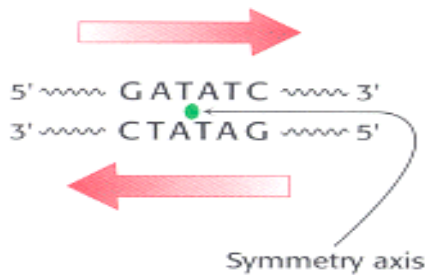
- Most bacteria use Restriction Enzymes as a defence against bacteriophages.
- Restriction enzymes prevent the replication of the phage by cleaving its DNA at specific sites.
- The host DNA is protected by Methylases which add methyl groups to adenine or cytosine bases within the recognition site thereby modifying the site and protecting the DNA.

Types of Restriction Enzymes

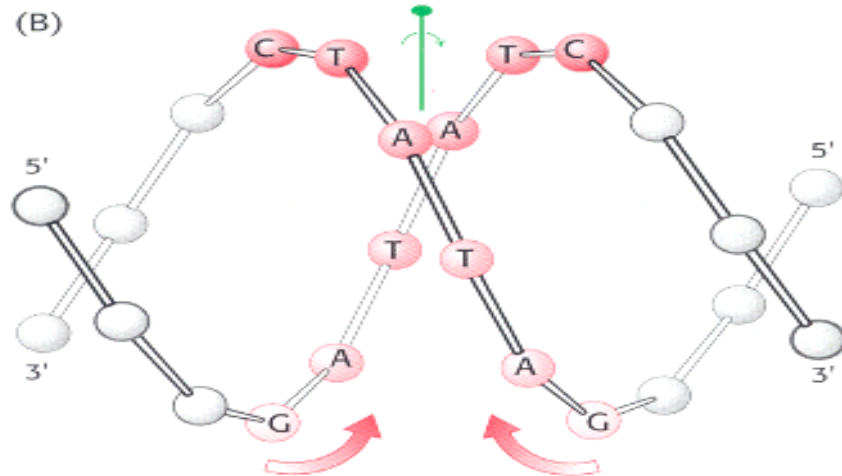
	Cleavage site	Location of methylase	Examples
Type I	Random Around 1000bp away from recognition site	Endonuclease and methylase located on a single protein molecule	EcoK I EcoA I CfrA I
Type II	Specific Within the recognition site	Endonuclease and methylase are separate entities	EcoR I BamH I Hind III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

Recognition sites of most restriction enzymes have a twofold rotational symmetry

(A)



(B)

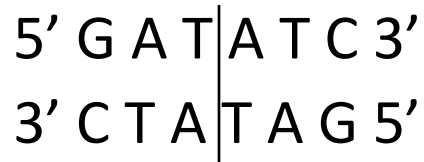


Restriction enzymes have corresponding symmetry to facilitate recognition and usually cleave the DNA on the axis of symmetry

Restriction fragments can be blunt ended or sticky ended



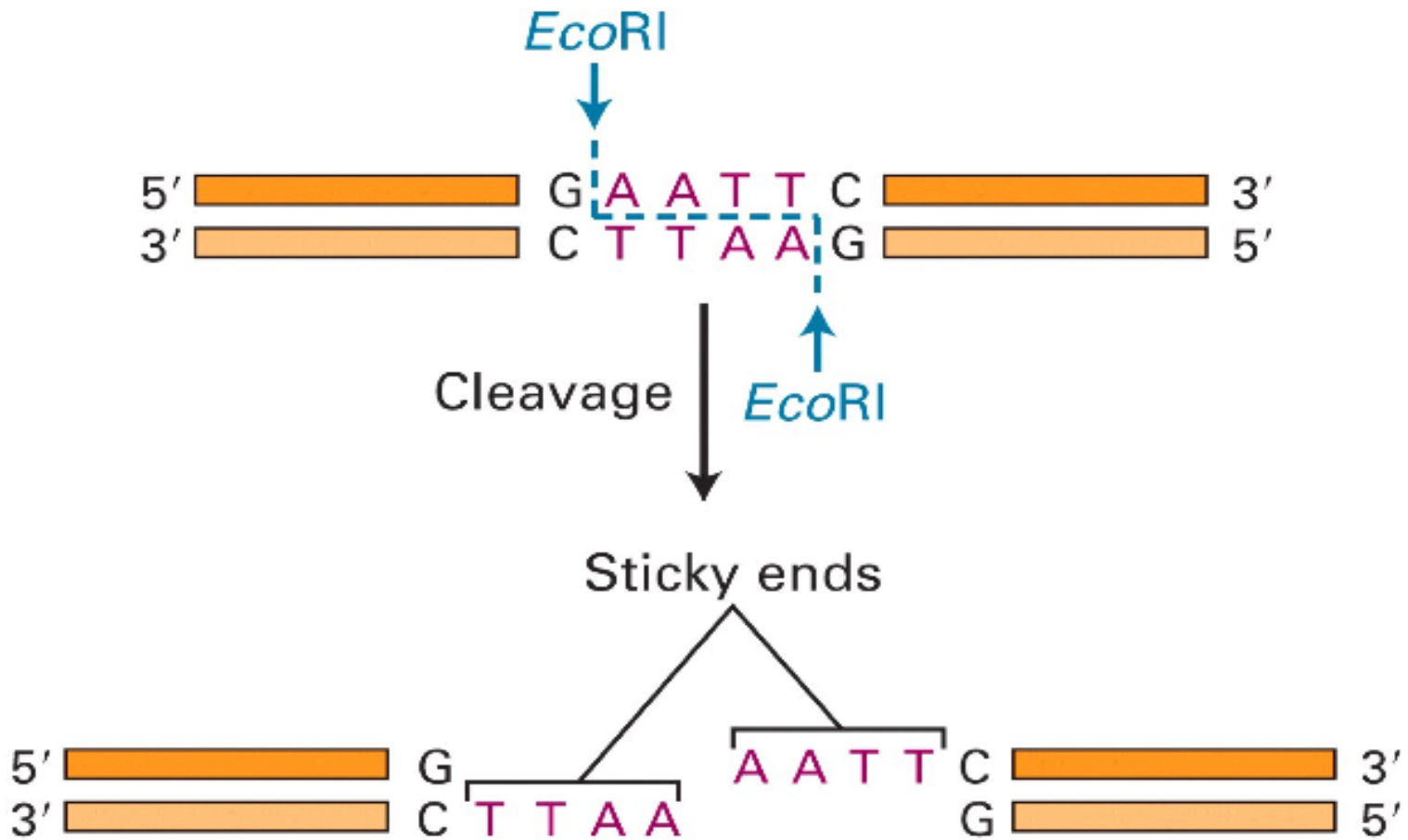
Sticky Ends



Blunt Ends

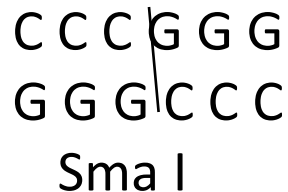
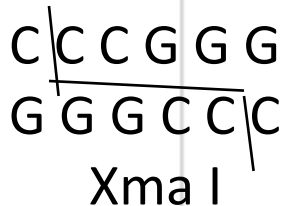
Sticky ends or blunt ends can be used to join DNA fragments. Sticky ends are more cohesive compared to blunt ends.

Molecular Scissors



Isoschizomers and Neochisizomers

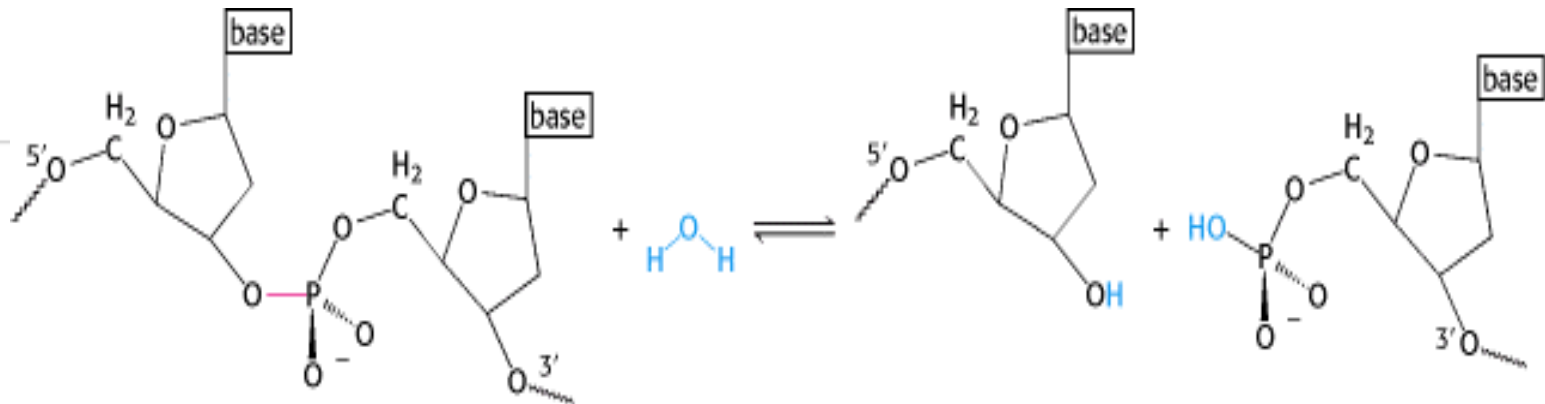
- Restriction enzymes that have the same recognition sequence as well as the same cleavage site are **Isoschizomers**.
Ex : HpaI (C↓CGG) dan MspI (C↓CGG)
- Restriction enzymes that have the same recognition sequence but cleave the DNA at a different site within that sequence are **Neochisizomers**. Eg:SmaI and XmaI



Mechanism of Action

- Restriction Endonuclease scan the length of the DNA , binds to the DNA molecule when it recognizes a specific sequence and makes one cut in each of the sugar phosphate backbones of the double helix – by hydrolyzing the phosphodiester bond. Specifically, the bond between the 3' O atom and the P atom is broken.

Direct hydrolysis by nucleophilic attack at the phosphorous atom



3'OH and 5' PO_4^{3-} is produced. Mg^{2+} is required for the catalytic activity of the enzyme. It holds the water molecule in a position where it can attack the phosphoryl group and also helps polarize the water molecule towards deprotonation.

Restriction Enzymes

- > 3,500 different restriction enzymes
- > 270 different specificities
- Named for species and strain from which they were originally isolated:
 - *Escherichia coli* R → *EcoRI*
 - *Bacillus amyloliquefaciens* H → *BamHI*
 - *Providencia stuartii* → *PstI*

Recognition Sites of Restriction Enzymes

Enzyme	Source	Recognition Sequence	Cut
EcoRI	Escherichia coli	5'GAATTC 3'CTTAAG	5'---G AATTC---3' 3'---CTTAA G---5'
BamHI	Bacillus amyloliquefaciens	5'GGATCC 3'CCTAGG	5'---G GATCC---3' 3'---CCTAG G---5'
HindIII	Haemophilus influenzae	5'AAGCTT 3'TTCGAA	5'---A AGCTT---3' 3'---TTCGA A---5'
MstII	Microcoleus species	5'CCTNAGG 3'GGANTCC	5'---CC TNAGG---3' 3'---GGANT CC---5'
TaqI	Thermus aquaticus	5'TCGA 3'AGCT	5'---T CGA---3' 3'---AGC T---5'
NotI	Nocardia otitidis	5'GANTC 3'CTNAG	5'---GC GGCCGC---3' 3'---CGCCGG CG---5'
HinfI	Haemophilus influenzae	5'GANTC 3'CTNAG	5'---G ANTC---3' 3'---CTNA G---5'
AluI*	Arthrobacter luteus	5'AGCT 3'TCGA	5'---AG CT---3' 3'---TC GA---5'

* = blunt ends

Restriction Enzyme Examples

MseI

5' A/T A A 3'

3' T A T/A 5'

4 cutter

BamHI

5' G/G A T C C 3'

3' C C T A G/G 5'

EcoRI

5' G/A A T T C 3'

3' C T T A A/G 5'

HindIII

5' A/A G C T T 3'

3' T T C G A/A 5'

NotI

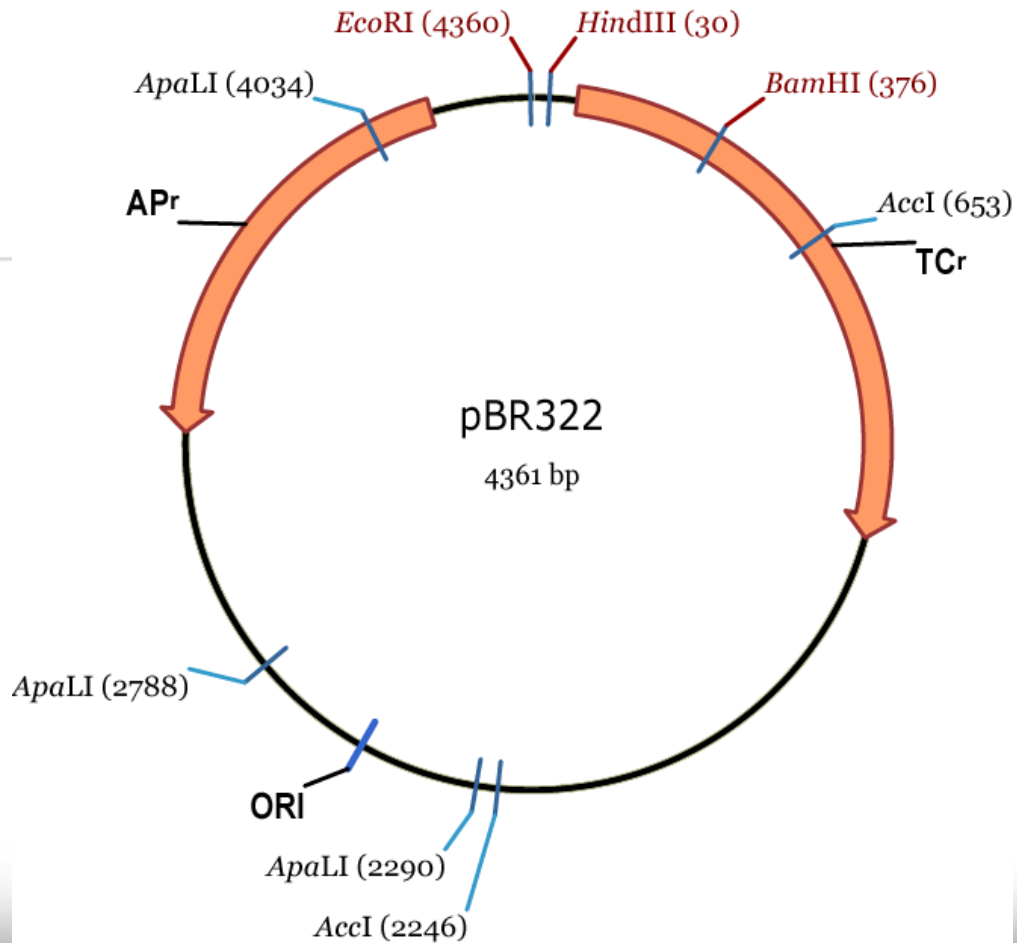
5' G C/G G C C G C 3'

3' C G C C G G/C G 5'

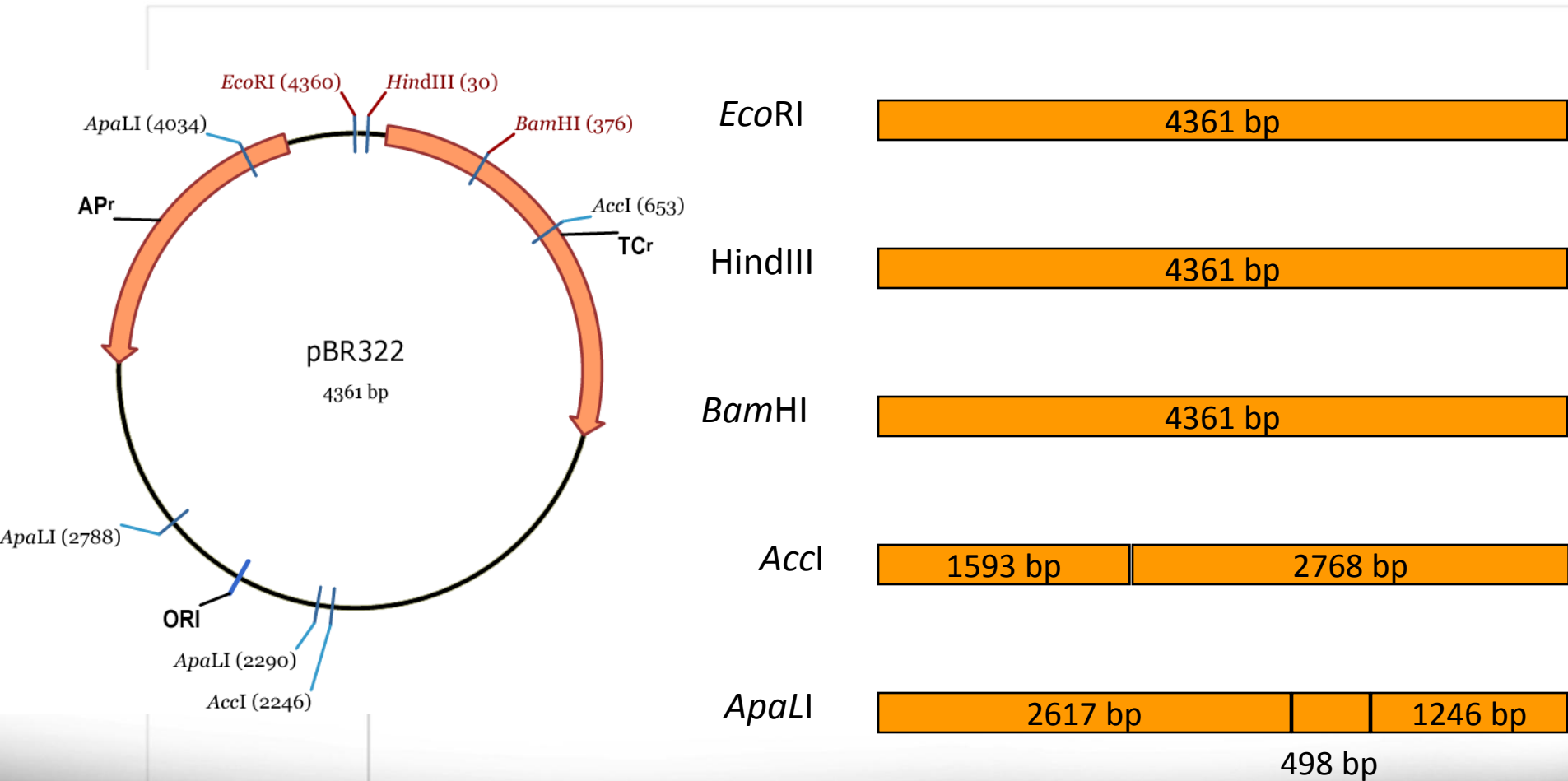
8 cutter

6 cutters

Restriction Map



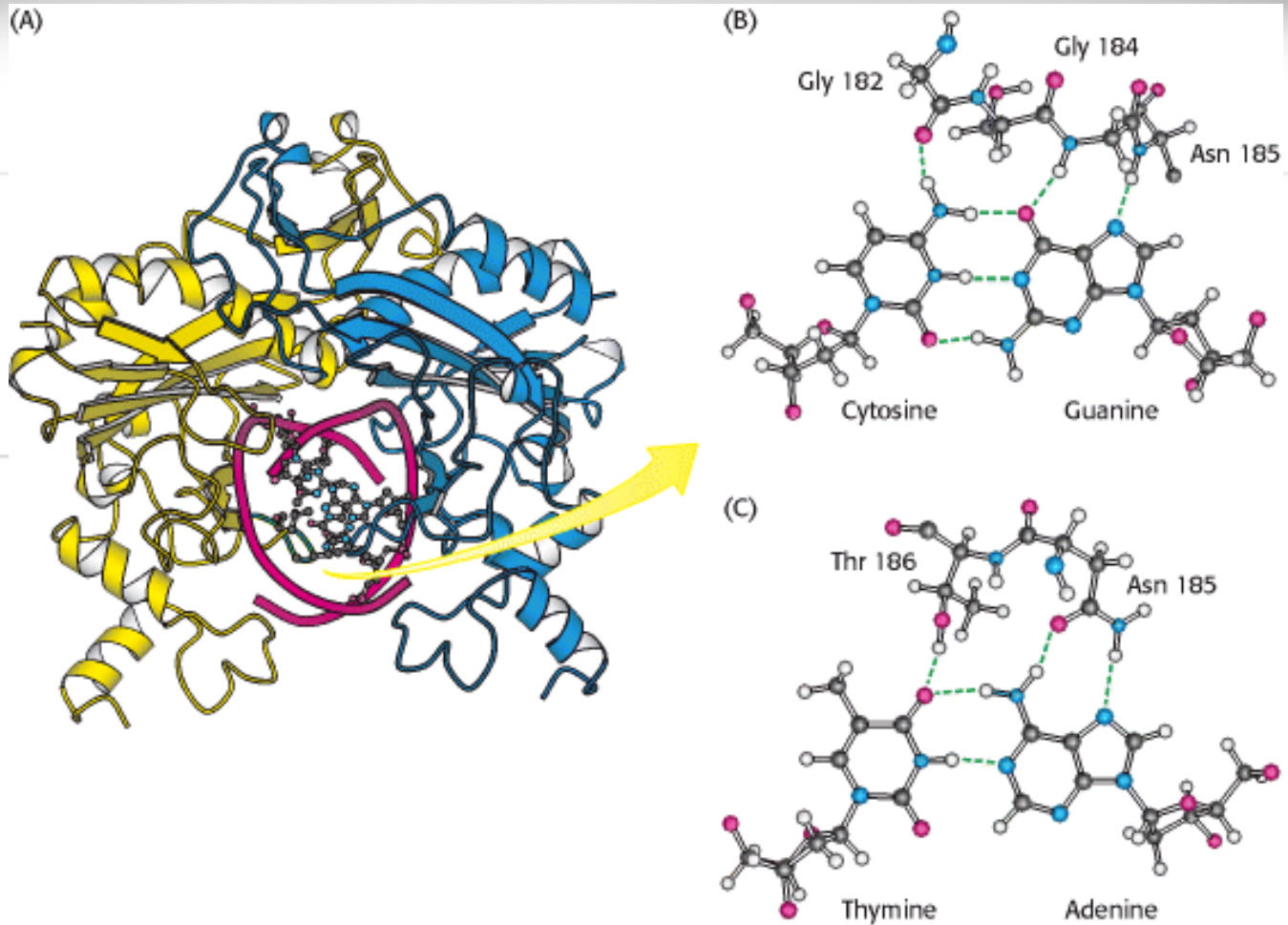
Restriction Digest



Structure of EcoR V endonuclease



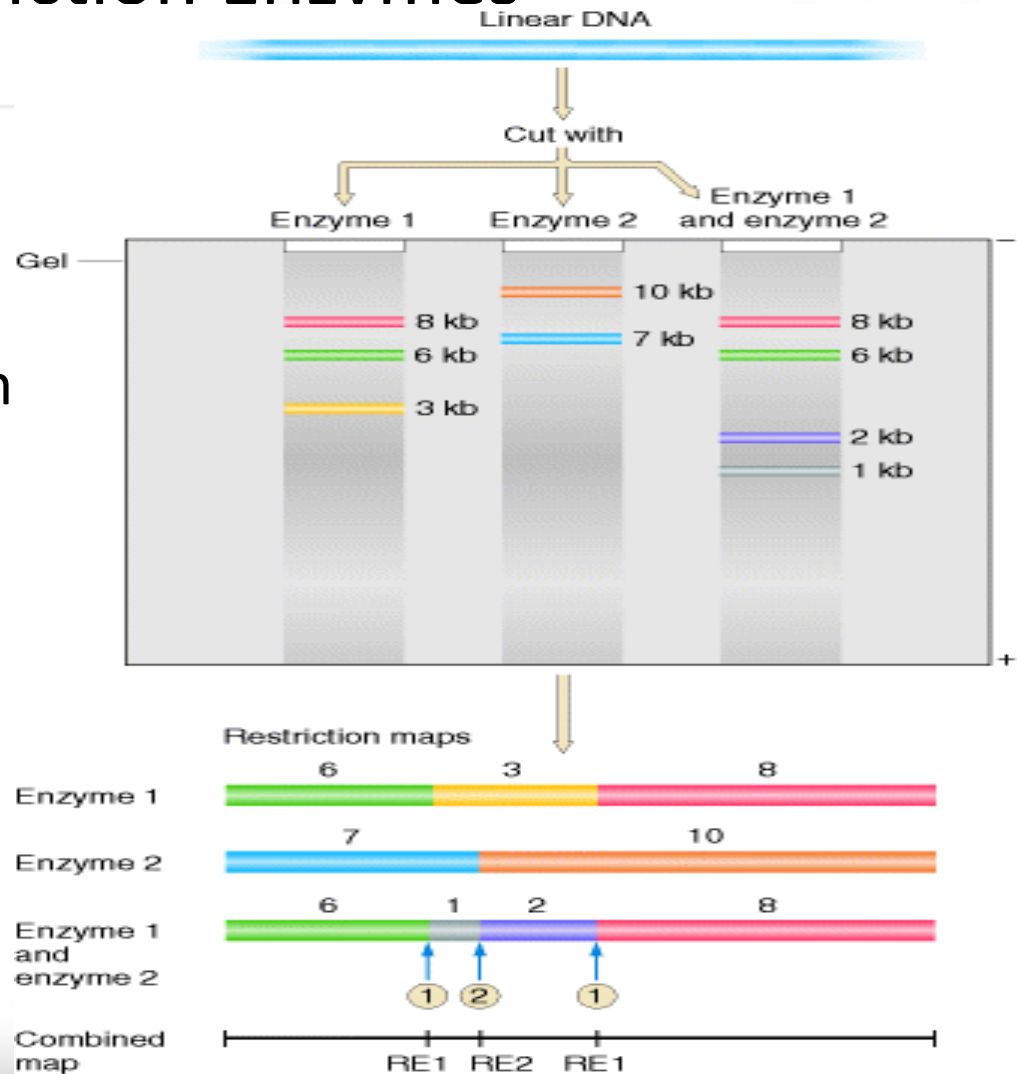
- Consists of two subunits – dimers related by two fold rotational symmetry.
- Binds to the matching symmetry of the DNA molecule at the restriction site and produces a kink at the site.



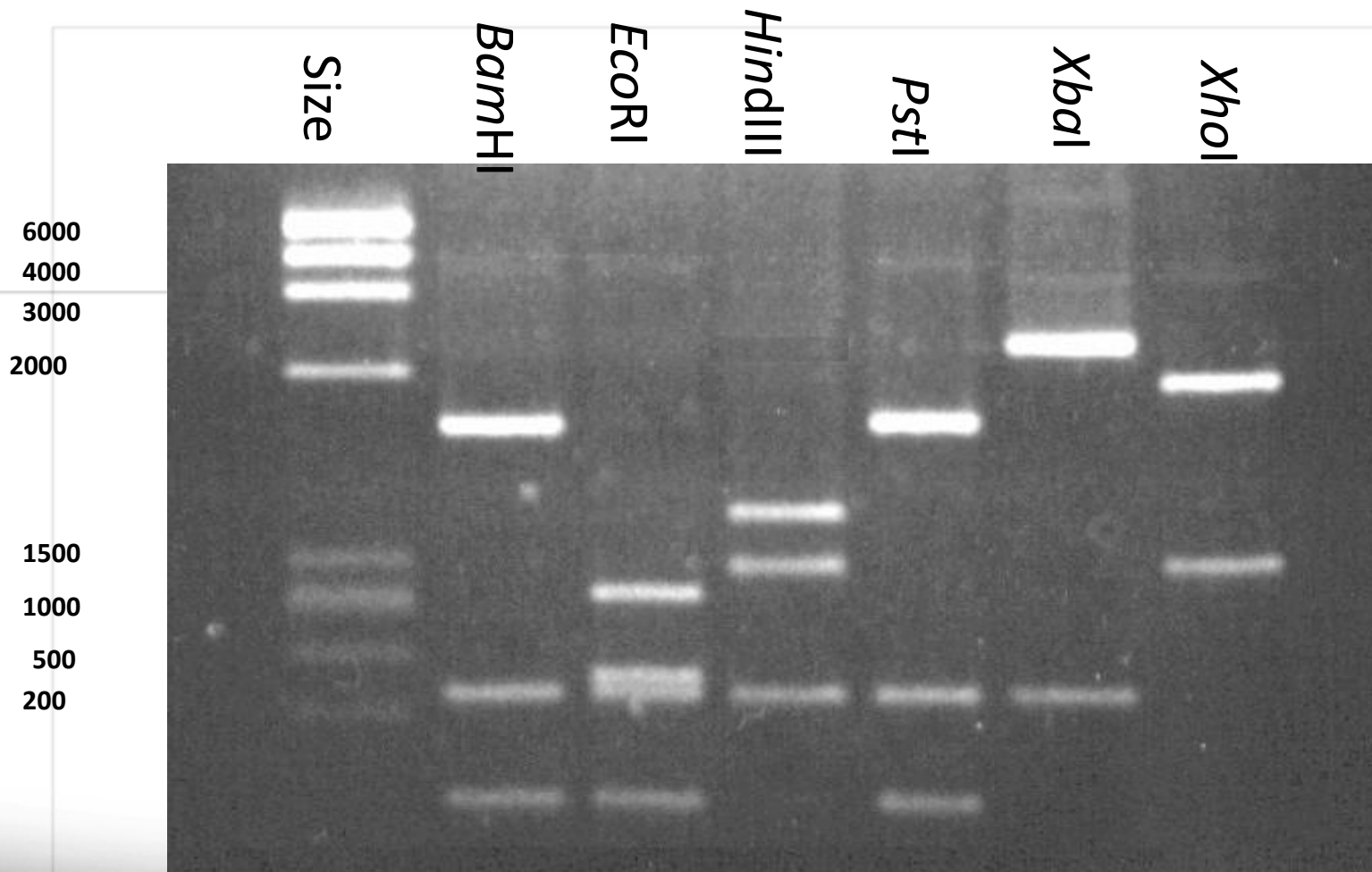
Hydrogen bonding interactions between EcoRv and its DNA substrate

Uses of Restriction Enzymes

Restriction Enzymes can be used to generate a restriction map. This can provide useful information in characterizing a DNA molecule.

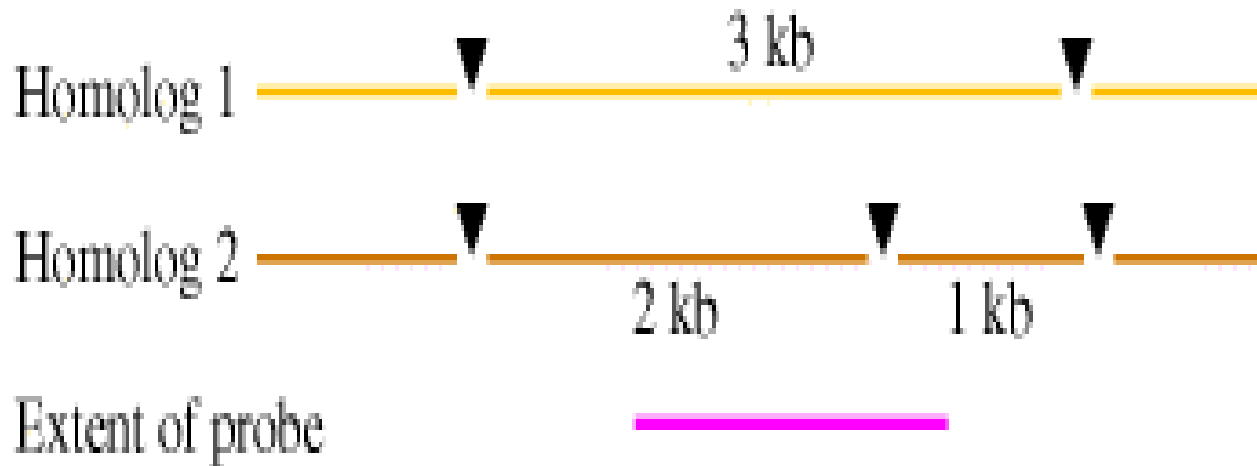


Gel Visualized Under UV Light



Uses....

Restriction Fragment Length Polymorphism is a tool to study variations among individuals & among species



Langkah – Langkah Pencarian Peta Enzim Restriksi

Secara online

1. Selain NCBI, terdapat pula database yang dapat digunakan untuk mengetahui letak enzim restriksi pada suatu Gen, Buka situs NEB (*New England Biolabs*) di www.neb.com sehingga muncul halaman utama dari NEB sebagai berikut

Tampilan halaman utama NEB

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What's New?

New England Biolabs. TECHNICAL SUPPORT

Episode 22 Technical Support

NEB TV Ep. 22 - Technical Support at NEB
Go behind the scenes into NEB's technical support program. Hear from our scientists as they describe memorable tech support moments and interesting collaborations that have resulted from tech support calls.

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NEB Literature

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expressions
PCR & RT-PCR
Builder 1XP DNA Assembly

16:23
05/06/2018

2. Pada kontak menu Online Tools & Mobile Apps, pilih *NEBcutter* yang memiliki icon gunting (Lingkar merah)

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Online Tools & Mobile Apps




NEBcutter V2.0
Use this tool to identify the restriction sites within your DNA sequence


[View All Tools](#)




Tm Calculator
Use this tool when designing PCR reaction protocols to help determine the optimal annealing temperature for your amplicon



NEBioCalculator
Use this tool for your scientific calculations and conversions for DNA and RNA



NEBcloner
Use this tool to find the right products and protocols for each step (digestion, end modification, ligation and transformation) of your next traditional cloning experiment



NEB Tools
NEB Tools brings New England Biolabs' most popular web tools to your iPhone, iPad or Android phone, allowing you to plan your experiments from anywhere

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<https://www.neb.com/external-links/nebcutter>

16:31 05/06/2018

3. Maka akan muncul tampilan *NEBcutter*, pada kotak *GenBank number*, isikan Accession number dari gen yang diinginkan, misalnya mTGase (Y08820.1) atau langsung mengcopy sekuen gen pada kolom



NEBcutter V2.0



This tool will take a DNA sequence and find the large, non-overlapping open reading frames using the E.coli genetic code and the sites for all Type II and commercially available Type III restriction enzymes that cut the sequence just once. By default, only enzymes available from NEB are used, but other sets may be chosen. Just enter your sequence and "submit". Further options will appear with the output. **The maximum size of the input file is 1 MByte, and the maximum sequence length is 300 Kbases.**

[What's new in V2.0](#) [Citing NEBcutter](#)

Local sequence file: No file chosen

GenBank number:

or paste in your DNA sequence: (plain or FASTA format)

```
AGTGGTCTGCCGGGTACGCGGACTTCGGCGCTACGTGATCACGTTACATACCAAGAGCTGGAACA
CCGC
CCCCGCCAAGGTGGAGCAAGGCTGGCCGTGACAGGCTGGTACTACGACCTCTGACTGATTTCTTGA
CCCC
GTCAGTCCACGTACCTCTCGACTGAACCGTGGTACAGTGACAACCTGGTACTGACTGAGTTCGGGT
GTGA
TGCGAGATGATGACTGGACTACA
```

NEB enzymes
 All commercially available specificities
 All specificities
 All + defined oligonucleotide sequences
 Only defined oligonucleotide sequences
[\[define oligos\]](#)

The sequence is: Linear Circular

Enzymes to use:

Minimum ORF length to display: a.a.

Standard sequences:
Plasmid vectors ▾
Viral + phage ▾

Name of sequence: (optional)

Earlier projects:

Note: Your earlier projects will be deleted 2 days after they were last accessed.

4. Sebelum si submit, pilih tampilan sekuens gen dengan mencentang tanda linear atau circular dan pilih NEB enzymes, klik Submit



NEBcutter V2.0



This tool will take a DNA sequence and find the large, non-overlapping open reading frames using the E.coli genetic code and the sites for all Type II and commercially available Type III restriction enzymes that cut the sequence just once. By default, only enzymes available from NEB are used, but other sets may be chosen. Just enter your sequence and "submit". Further options will appear with the output. **The maximum size of the input file is 1 MByte, and the maximum sequence length is 300 KBases.**

[What's new in V2.0](#) [Citing NEBcutter](#)

Local sequence file: No file chosen

GenBank number: [\[Browse GenBank\]](#)

or paste in your DNA sequence: *(plain or FASTA format)*

```

>Streptomyces baldaccii tg gene for transglutaminase
CCCCGCCACCCGGACCGGGCCCGCTCGTCCGTCGGCAACGACGCTGATGAGCGCCGGCCCCG
CCGG
CCCCGGCCGACCAAGCCACGCGGGCCGGCGACCGAGTCGCGCCGGCCCGCTCGGCGACCAACGCG
GCCA
TGACCGGCGACGAGCGCGCCGCCACCGCCATCTCCCCGATTGCCGATCTCCTCCGCCCGCGCGC
GGCG
GCCCTCCTGTCGTGGCACAGTGACGCGAGGCCAGGGCCGCCAAGGCCCTGACCGGCAATCTCAA
                    
```

Standard sequences:

Plasmid vectors

Viral + phage

The sequence is: Linear Circular

Enzymes to use:

- NEB enzymes
- All commercially available specificities
- All specificities
- All + defined oligonucleotide sequences
- Only defined oligonucleotide sequences


[\[define oligos\]](#)

Minimum ORF length to display: a. a.

Name of sequence: *(optional)*

Earlier projects:
[S.cinnamoneum_TGase](#)

4. Maka tampilan pada laman web, gen lengkap dengan enzim restriksi yang terdapat pada gen (Linear).



Display: - NEB single cutter restriction enzymes
- Main non-overlapping, min. 100 aa ORFs

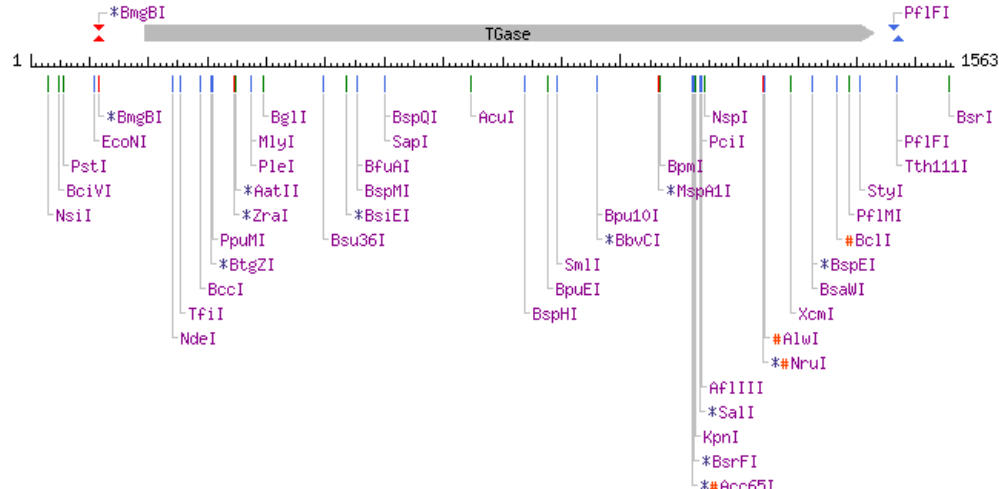
GC=60%, AT=40%

Linear Sequence: S.cinnamoneum TGase

Help

Comments

Cleavage code	Enzyme name code
▲ blunt end cut	Available from NEB
▼ 5' extension	Has other supplier
▲ 3' extension	Not commercially available
▼ cuts 1 strand	*: cleavage affected by CpG meth.
	#: cleavage affected by other meth.
	(enz.name): ambiguous site



Main options

- New DNA
- Custom digest
- View sequence
- ORF summary
- Translate GB file
- Save project

Availability

All commercial

All

Display

2 cutters

3 cutters

Zoom

Zoom in

More...

List

0 cutters

1 cutters

All sites

Save all sites

Flanking enzymes

Minimum ORF length to display: aa.

4. tampilan gen lengkap dengan enzim restriksi yang terdapat pada gen (sirkular.)



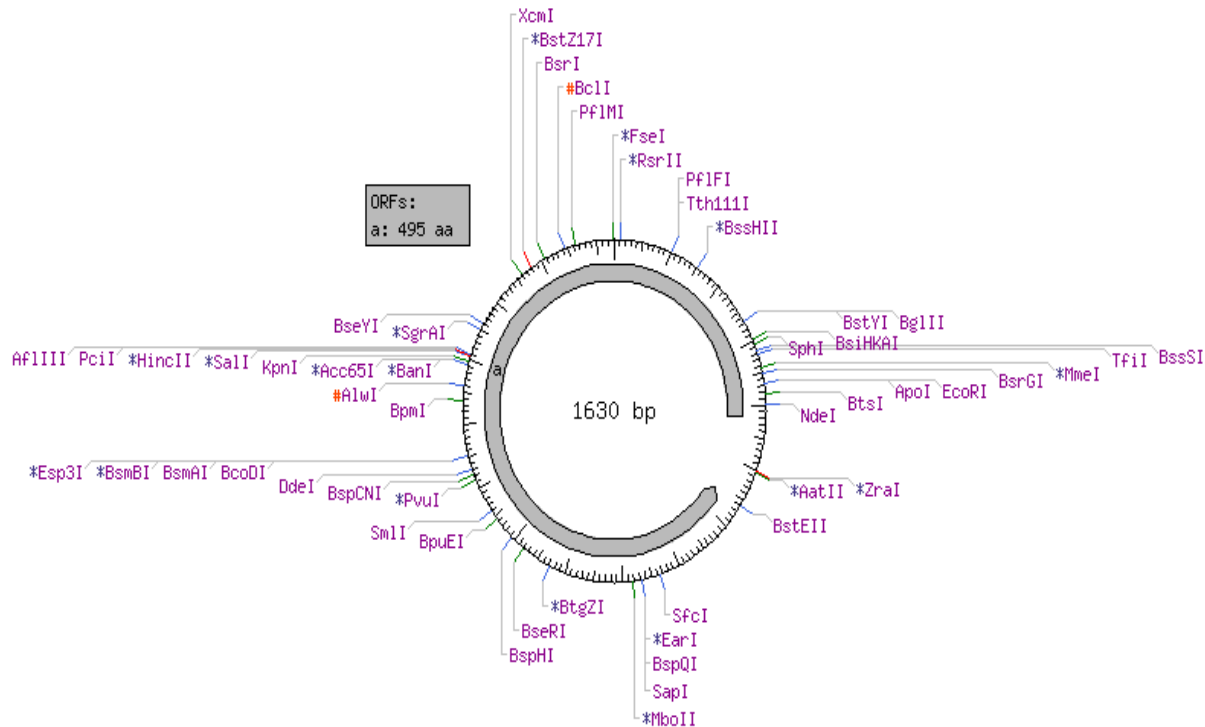
Circular Sequence: *Streptomyces baldac*

[Help](#) [Comments](#)

Display: - NEB single cutter restriction enzymes
- Main non-overlapping, min. 100 aa ORFs

GC=64%, AT=36%

Cleavage code	Enzyme name code
▬ blunt end cut	Available from NEB
▬ 5' extension	Has other supplier
▬ 3' extension	Not commercially available
▬ cuts 1 strand	*: cleavage affected by CpG meth.
	#: cleavage affected by other meth.
	(enz.name): ambiguous site

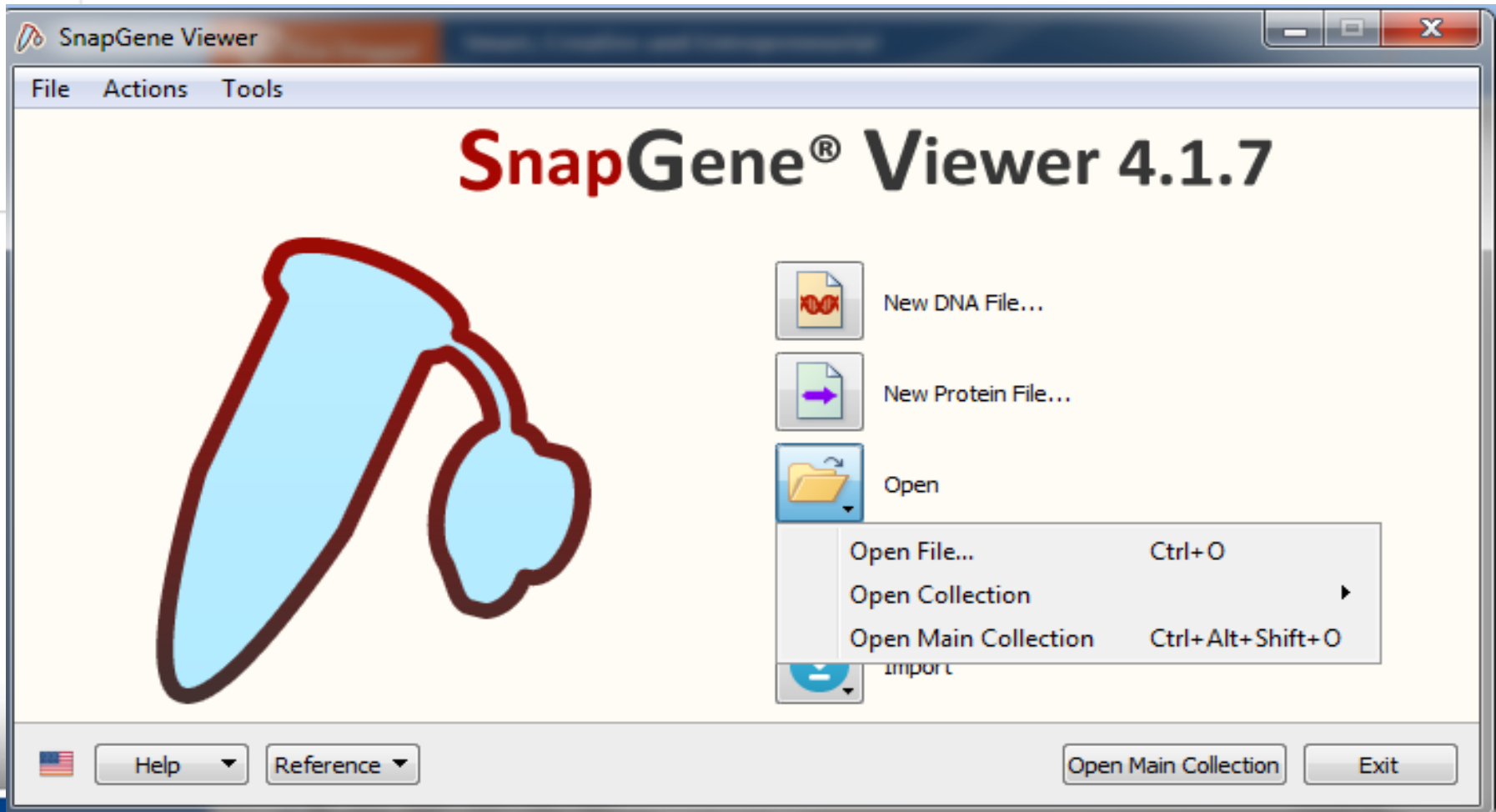


Langkah – Langkah Pencarian Peta Enzim Restriksi

Secara offline

- Selain NEB, terdapat pula database yang dapat digunakan untuk mengetahui letak enzim restriksi pada suatu Gen, menggunakan software offline dengan menggunakan SnapGene

1. Buka software SnapGene. Pilih open file, pilih gen yang sudah disimpan dengan format fasta.



- Tampilan pada sekuens gen (linear) pada Snapgene. Pilih **sequence** (Tanda merah) yang terletak bagian bawah untuk melihat sekuen utuh serta situs restriksinya

The screenshot displays the SnapGene software interface. The main workspace shows a linear DNA sequence of 1251 bp with various restriction enzyme sites marked. The sites are labeled with their names and positions, such as NdeI (49), BspQI - SapI (410), and BspHI (650). The sequence starts at (0) Start and ends at End (1251). The right-hand panel contains fields for Synthetic DNA, Laboratory Host, Methylation (Dam+, Dcm+, EcoKI+), Description, Created/Last Modified (Today), Accession Number, Code Number, Sequence Author, and Comments. A red box highlights the 'Sequence' tab in the bottom-left corner.

MTGase. fasta.txt (Linear / 1251 bp)

File Edit View Enzymes Features Primers Actions Tools Window Help

New Open Save Print Undo Redo Cut Copy Paste

MTGase. fasta

1251 bp

Unique 6+ Cutters Nonredundant

Map Sequence Enzymes Features Primers History

Sequence Author: Click above to import from PubMed.

- Tampilan pada sekuens gen (Sirkular) pada Snapgene. Pilih **sequence** (Tanda merah) yang terletak bagian bawah untuk melihat sekuen utuh serta situs restriksinya

MTGase. fasta.txt (Circular / 1251 bp)

File Edit View Enzymes Features Primers Actions Tools Window Help

New Open Save Print Undo Redo Cut Copy Paste

(1208) PflMI
 (1187) BclI*
 (1176) SacII
 (1139) BsaWI - BspEI
 (1126) BssSai
 (1104) XcmI
 (957) NspI
 (953) AflIII - PciI
 (949) SalI
 (941) KpnI
 (940) BsrFI
 (937) Acc65I - Bani
 (882) BpmI
 (775) BbvCI - Bpu10I
 (703) SmlI
 (688) BpuEI
 (650) BspHI
 (1200) NdeI (49)
 (200) BtsaI (99)
 (116) PpuMI
 (153) ZraI
 (155) AatII
 (203) BglI
 (239) BssHII
 (248) TspMI - XmaI
 (250) SmaI
 (305) Bsu36I
 (363) BfuAI - BspMI
 (378) SfcI
 (410) BspQI - SapI
 (400) TatI (521)
 (556) AcuI

MTGase. fasta
1251 bp

Unique 6+ Cutters (Nonredundant)

Map Sequence Enzymes Features Primers History

1. Pada laman sequence, tampilan sebagai berikut, Pada bagian ini juga dapat menentukan anotasi gen, untuk menentukan lokus gen pada sebuah genom.

The screenshot displays the MTase software interface for editing a DNA sequence. The main window shows a sequence of 1251 base pairs (bp) with several restriction enzyme sites annotated. The sequence is displayed in a standard font with vertical lines indicating the positions of the enzymes. The enzymes shown are NdeI, BtsaI, PpuMI, ZraI, AatII, BglI, BssHII, XmaI, TspMI, SmaI, and Bsu36I. The sequence is shown in a standard font with vertical lines indicating the positions of the enzymes. The sequence is shown in a standard font with vertical lines indicating the positions of the enzymes.

MTase, fasta.txt (Linear / 1251 bp)

File Edit View Enzymes Features Primers Actions Tools Window Help

New Open Save Print Undo Redo Cut Copy Paste

Order

1251 bp

Synthetic DNA Confirmed experimentally

Laboratory Host:

Methylation: Dam⁺ Dcm⁺ EcoKI⁺ Change...

Description:

Created: Today Last Modified: Today

Accession Number: Code Number:

Sequence Author:

Comments:

References: Click above to import from PubMed.

Unique 6+ Cutters (Nonredundant)

Map Sequence Enzymes Features Primers History

13:18 19/06/2018

1. Untuk menghilangkan tampilan situs RE, dapat di klik tanda lingkaran merah, anotasi dapat dilakukan setelah tanda enzim dihilangkan

MTGase.fasta.txt (Circular / 1251 bp)

File Edit View Enzymes Features Primers Actions Tools Window Help

New Open Save Print Undo Redo Cut Copy Paste

Try SnapGene if you wish to align DNA sequences with a reference sequence. Order

1251 bp

Synthetic DNA Confirmed experimentally

Laboratory Host:

Bacterial Transformation Strain: Unspecified

Methylation: Dam⁺ Dcm⁺ EcoKI⁺

Description:

Created: Today Last Modified: Today

Accession Number: Code Number:

Sequence Author:

Comments:

References:

Map Sequence Enzymes Features Primers History Description Panel

5' ATGCACAAACGTCGGAGACTTCTCGCCTTCGCCACTGTGGGTGCGGTCATATGCACCGCAGGATTCACAC
...
3' TACGTGTTTGCAGCCTCTGAAGAGCGGAAGCGGTGACACCCACGCCAGTATACGTGGCGTCTTAAGTGTG

CTTCGGTCAGCCAGGCCGCCAGCAGTGGCGATGGGGAAGAGAAGGGTCTACGCCGAAACGCACGGCCT
140
GAAGCCAGTCGGTCCGGCGGTCTGTCACCGCTACCCCTTCTCTCCCCAGGATGCGGCTTTGCGTGCCGGA

GACGGCGGATGACGTCGAGAGCATCAACGCACTGAACGAAAGAGCTCTGACTCTGGGCCAACCTGGCAAG
210
CTGCCGCTACTGCAGCTCTCGTAGTTGCGTGACTTGCTTTCTCGAGACTGAGACCCGGTTGGACCBTTC

CCTCCGAAGGAATTACCTCCGAGCGCCAGCGCGCCCTCCCGGGCCCCCTCCGATGACCGGGAAACTCCTC
280
GGAGGCTTCTTAATGGAGGCTCGCGGTCTGCGCGGGAGGGCCCGGGGGAGGCTACTGGCCCTTTGAGGAG

CCGCCGAGCCGCTCGACAGGATGCCTGAGGGCTACCGGGCTACGGAGGCAGGGCCACTACGGTCGTCAA
350
GGCGGCTCGGCGAGCTGTCTACGGACTCCGCATGGCCGGATGCCTCCGTCCCGGTGATGCCAGCAGTT

CAACTACATACGCAAGTGGCAGCAGGTCTACAGTCACCGCGACGGAAAGAAACAGCAAATGACCGAAGAG
420
GTTGATGATGCGTTACCGTCTGTCAGATGTCAGTGGCGCTGCCTTTCTTTGTCGTTACTGGCTTCTC

CAGCGAGAAAAGCTGTCTACGGTTGCGTTGGCGTCACTGGGTCAACTCGGGCCCTACCCGACGAACA

TUGAS PRAKTIKUM ---- KUMPULKAN MINGGU DEPAN

1. Buat peta enzim restriksi pada sebuah gen Tertentu (masing - masing individu gennya berbeda) dengan menggunakan NEB cutter dan SnapGene
2. Laporan dikumpul minggu depan

*gen dapat di searching di NCBI

THANK
YOU



607132.wordpress.com

Noviani's Blog

