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PENGANTAR BIOINFORMATIKA
IBT 431

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Meeting 11

Restriction Enzymes Mapping

Universitas Esa Unggul

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.

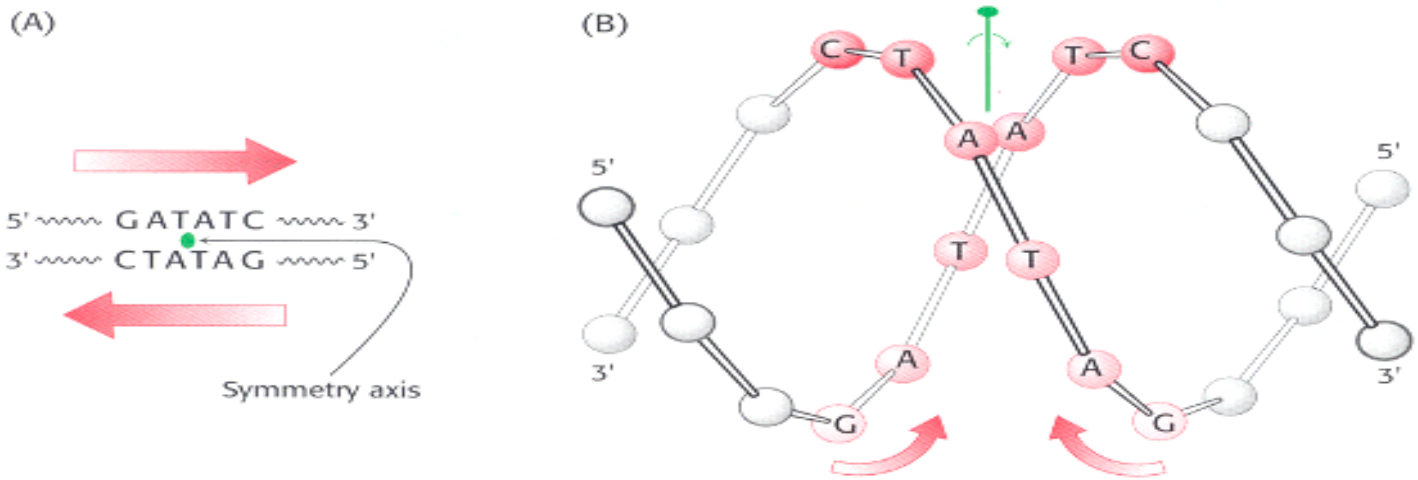
Biological Role

- 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



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

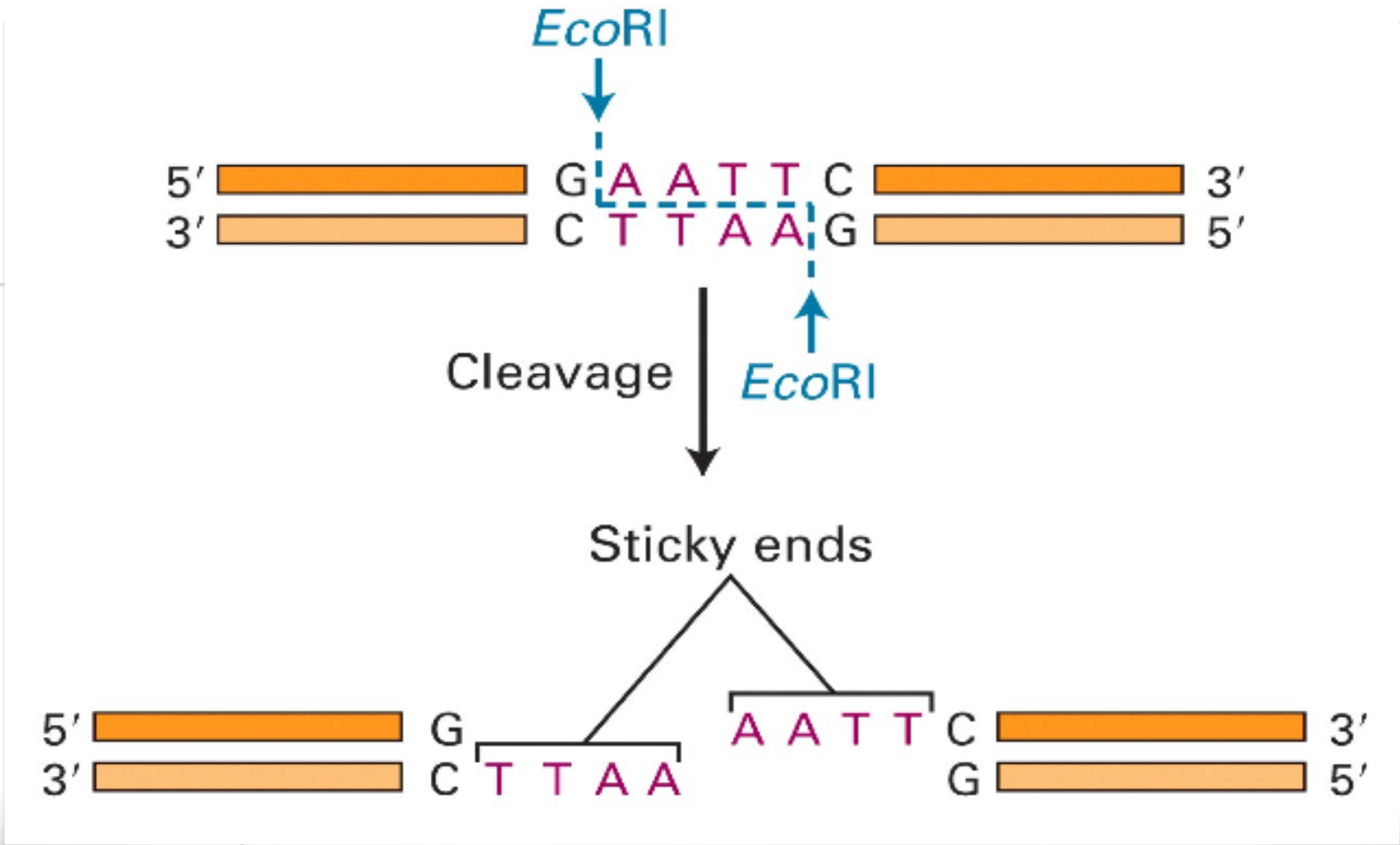
Restriction fragments can be blunt ended or sticky ended

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 Neochischizomers

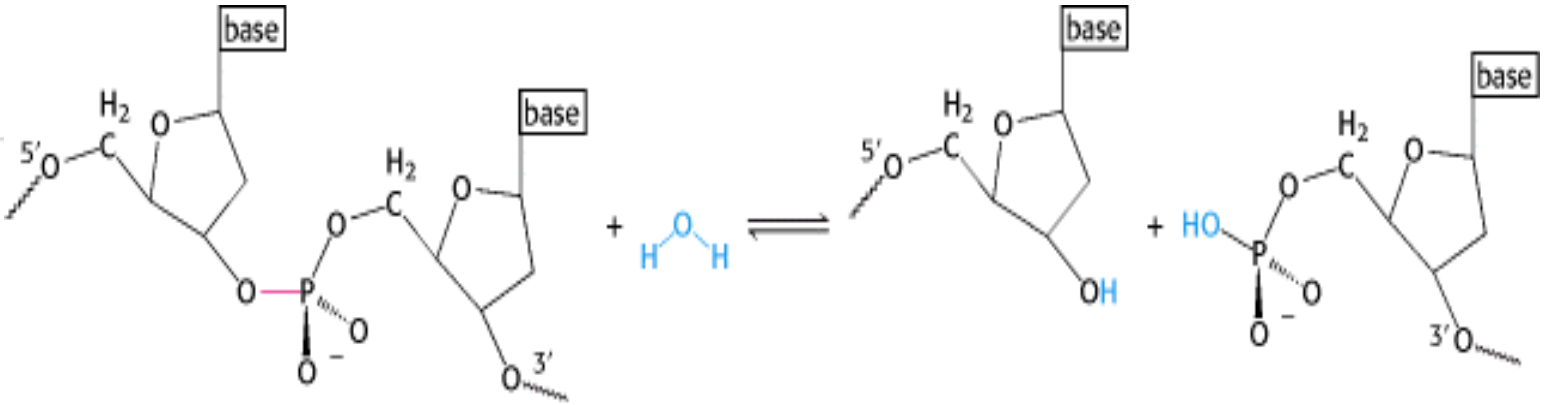
- Restriction enzymes that have the same recognition sequence as well as the same cleavage site are **Isoschizomers**.
  Ex : HpaII (C↓CGG) dan MspI (C↓CGG)

- Restriction enzymes that have the same recognition sequence but cleave the DNA at a different site within that sequence are **Neochizomers**. 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 phoshphodiester 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 → *Eco*RI
  - *Bacillus amyloliquefaciens* H → *Bam*HI
  - *Providencia stuartii* → *Pst*I

Recognition Sites of Restriction Enzymes

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

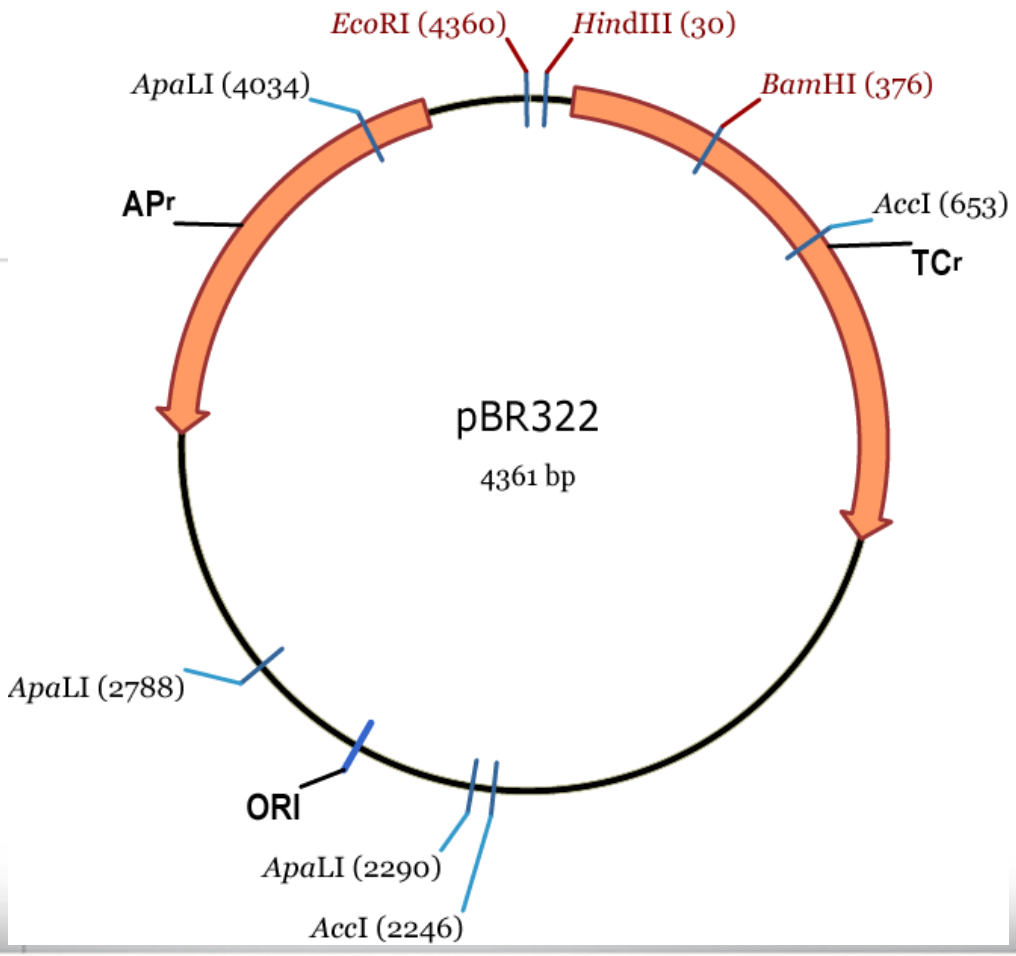
* = blunt ends

Molecular Cell Biology, 4th edition

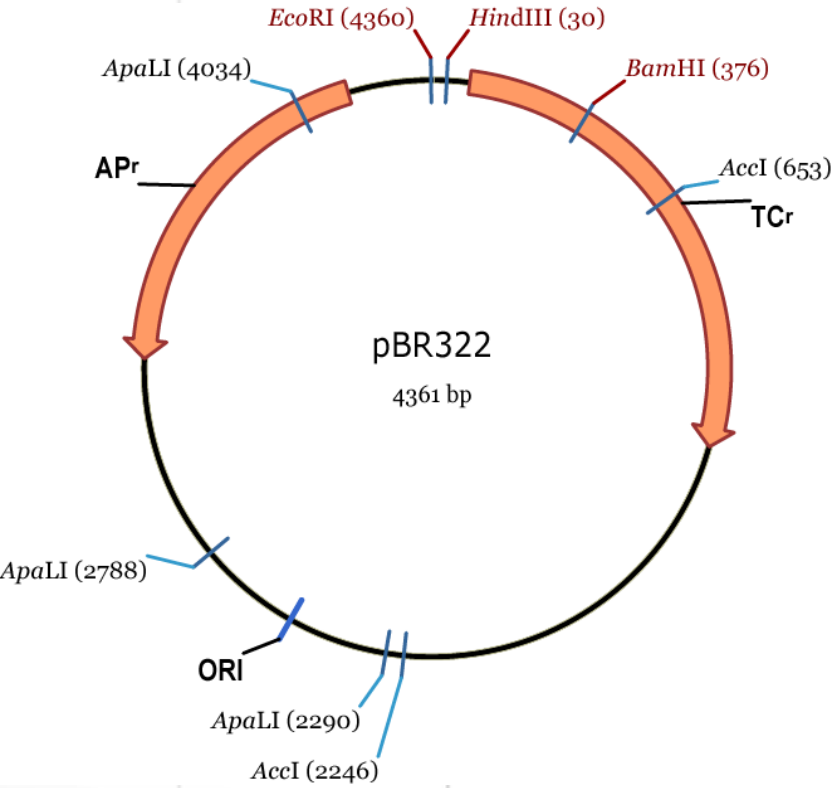
Restriction Enzyme Examples

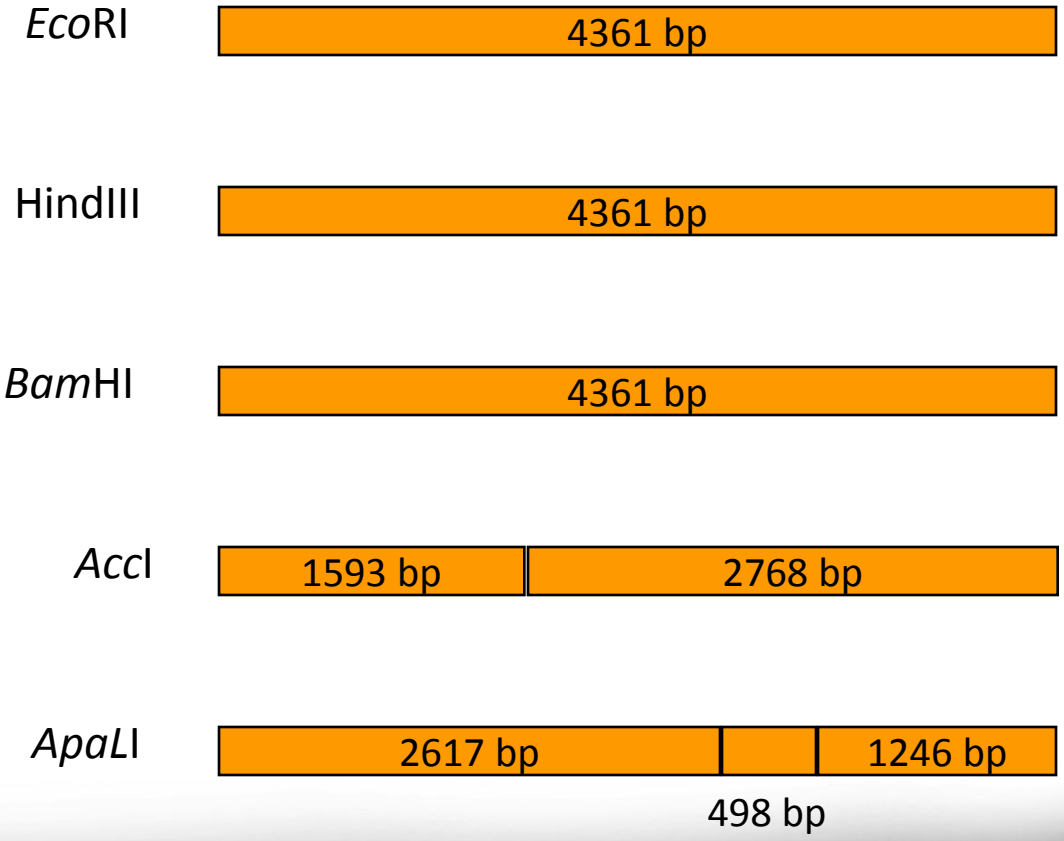
***Mse*I**	5′ A/T A A 3′ 3′ T A T/A 5′	4 cutter
***Bam*HI**	5′ G/G A T C C 3′ 3′ C C T A G/G 5′	6 cutters
***Eco*RI**	5′ G/A A T T C 3′ 3′ C T T A A/G 5′	
***Hin*dIII**	5′ A/A G C T T 3′ 3′ T T C G A/A 5′	
***Not*I**	5′ G C/G G C C G C 3′ 3′ C G C C G G/C G 5′	8 cutter

Restriction Map

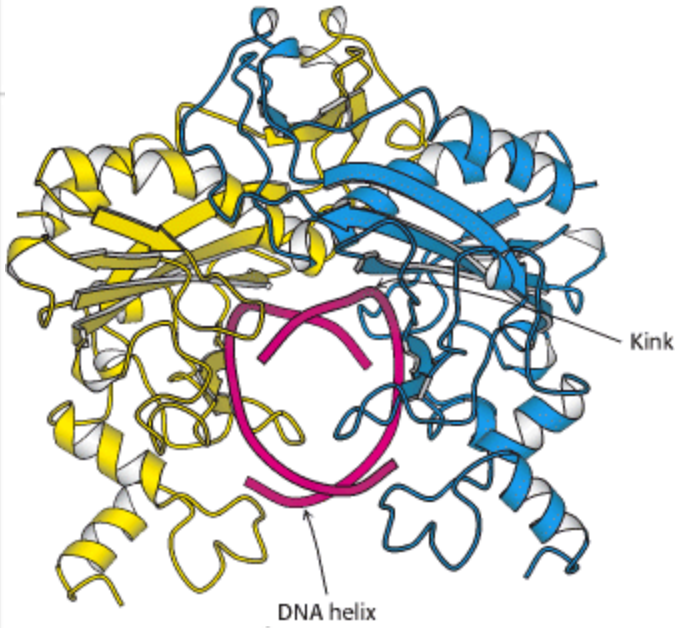


Restriction Digest

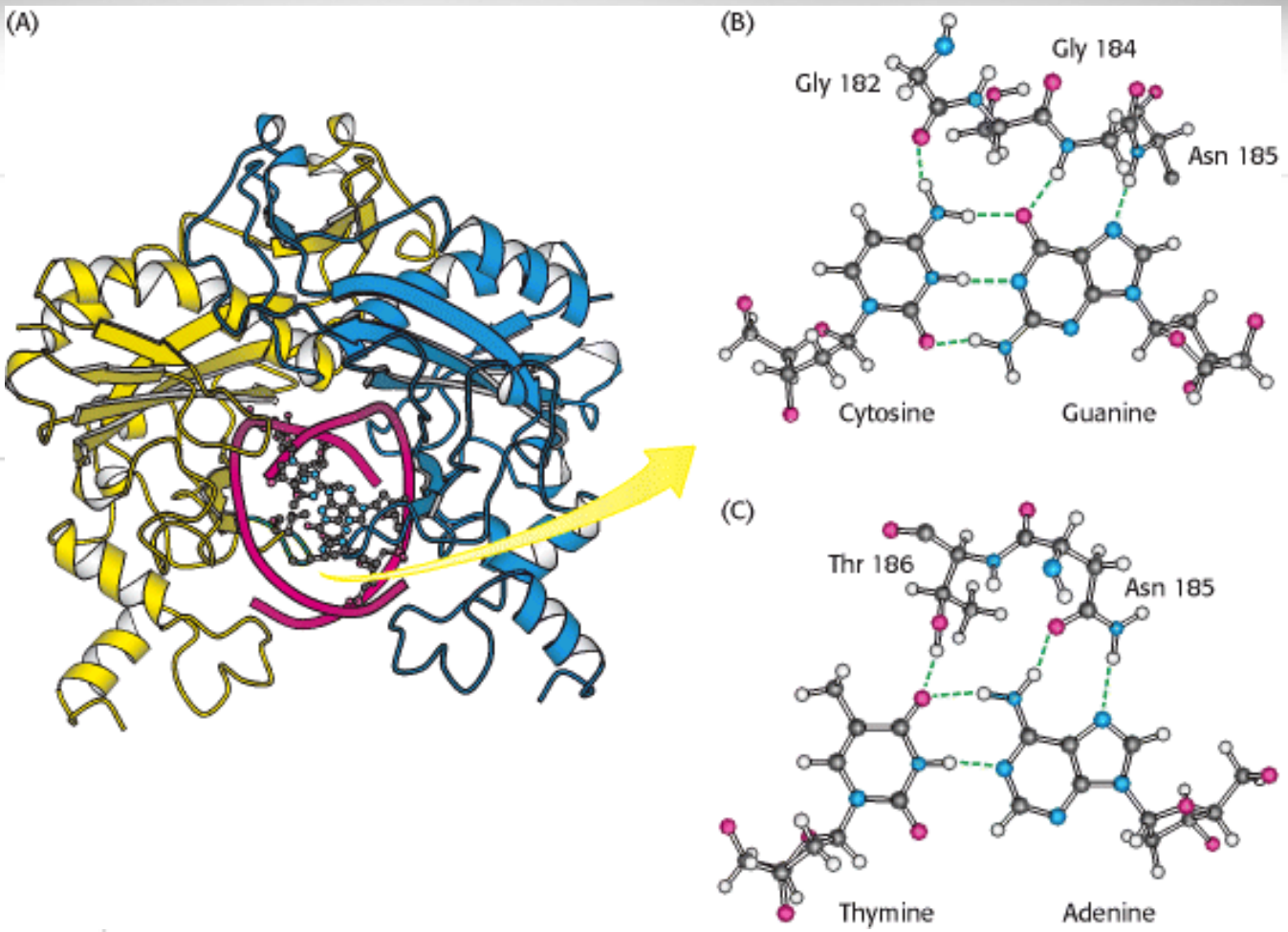




Structure of EcoR V endonuclease



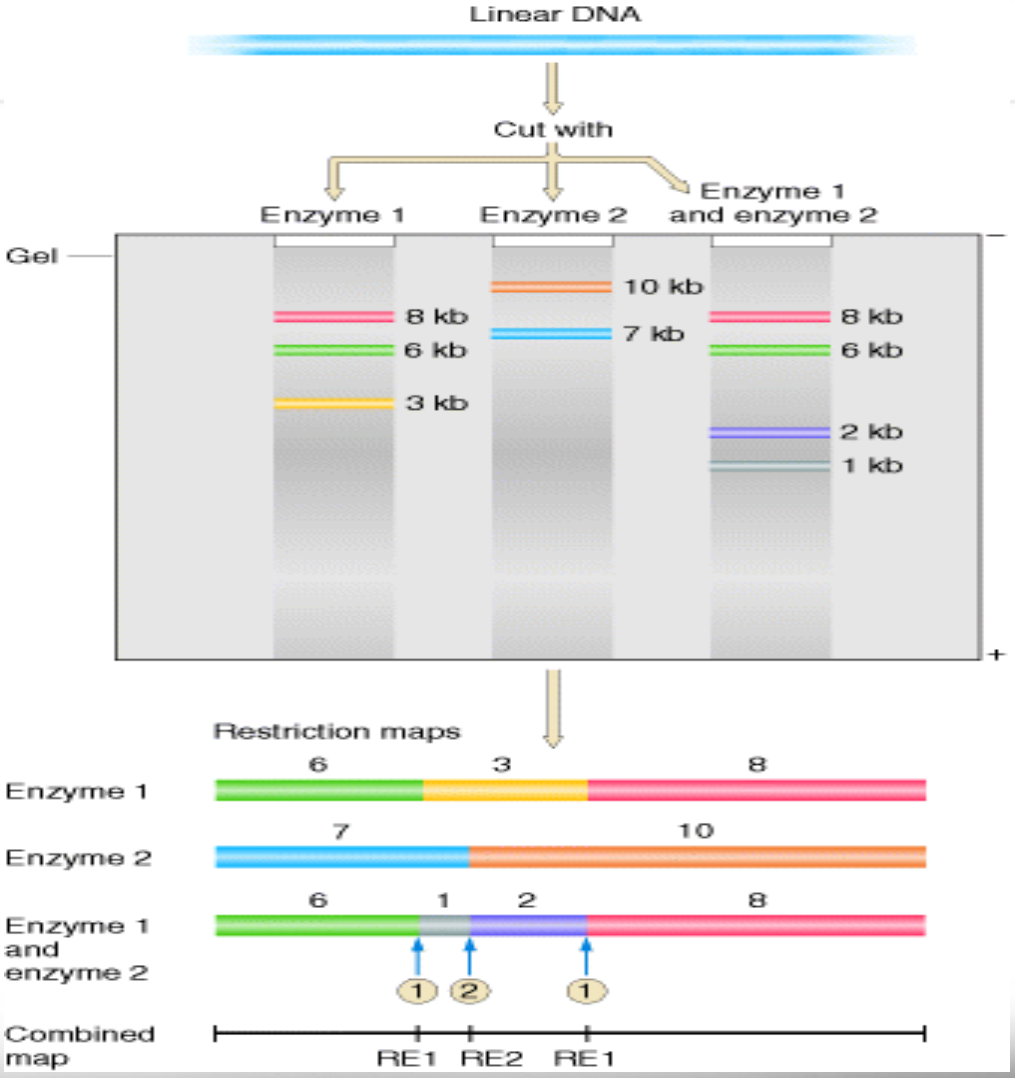
- Consists of two subunits – dimers related by two fold rotational symmetry.
- Binds to the matching symmetry of the DNA molecule at the restriction site and produces a kink at the site.



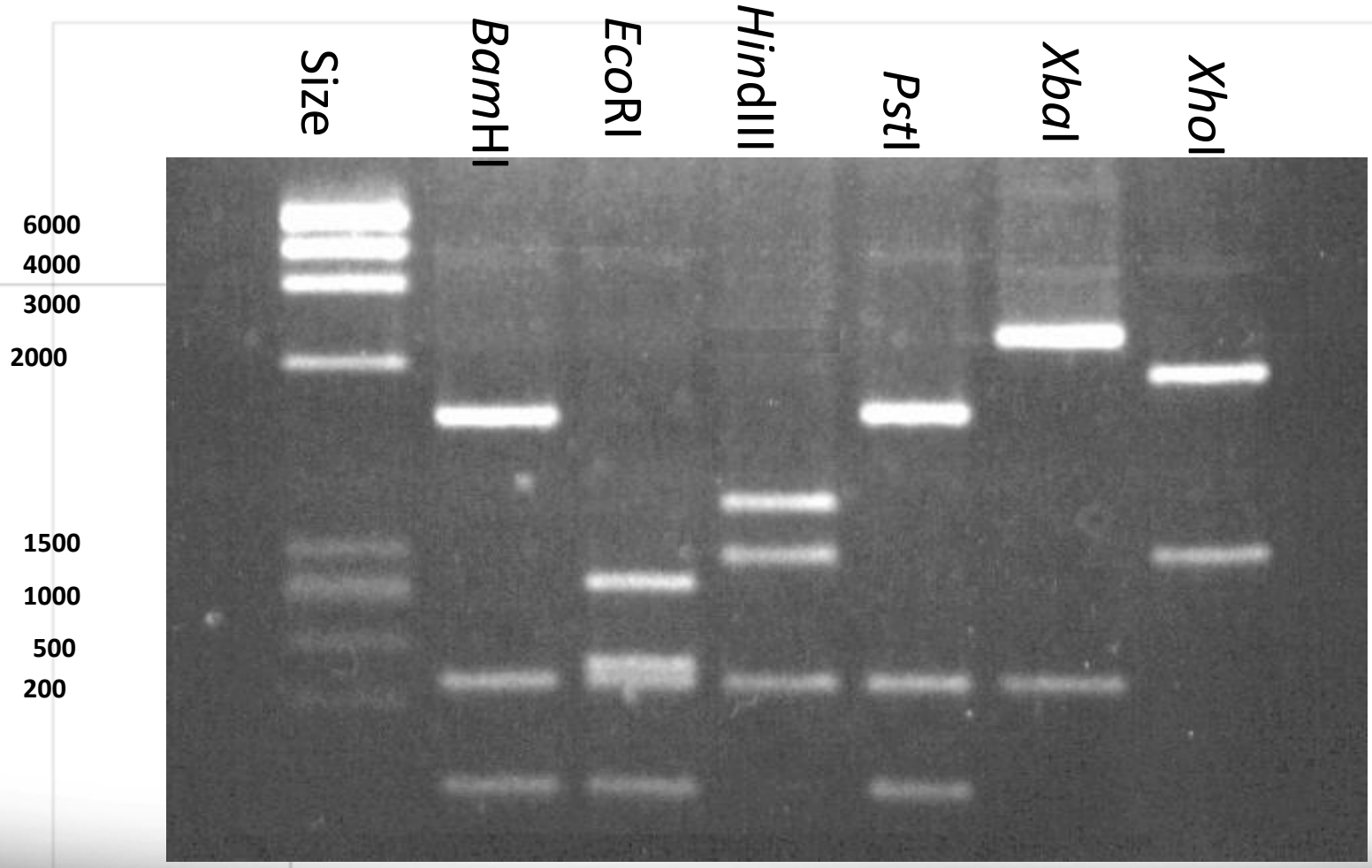
Hydrogen bonding interactions between EcoRv and its DNA substrate

Uses of Restriction Enzymes

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

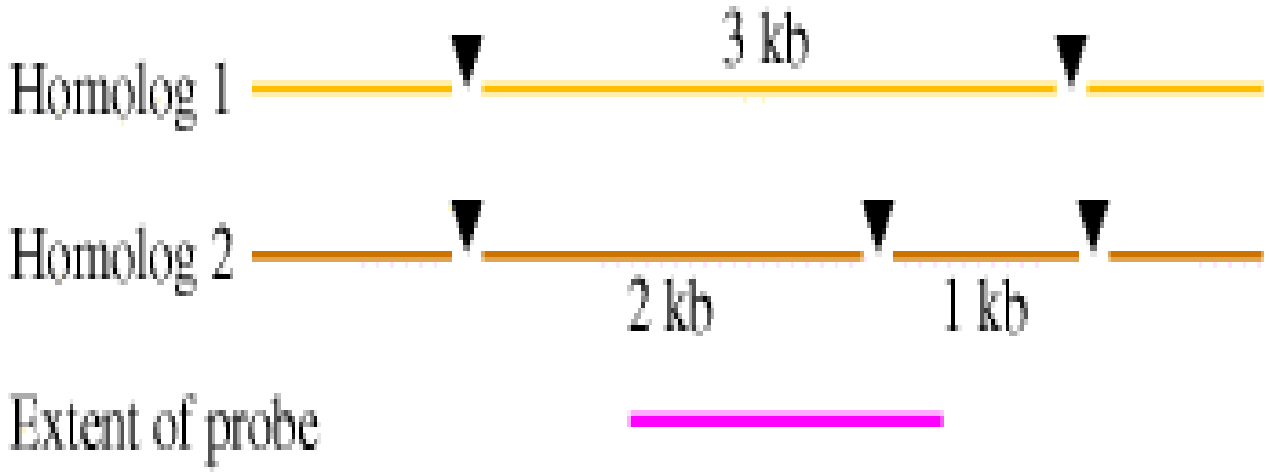


Gel Visualized Under UV Light



Uses….

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

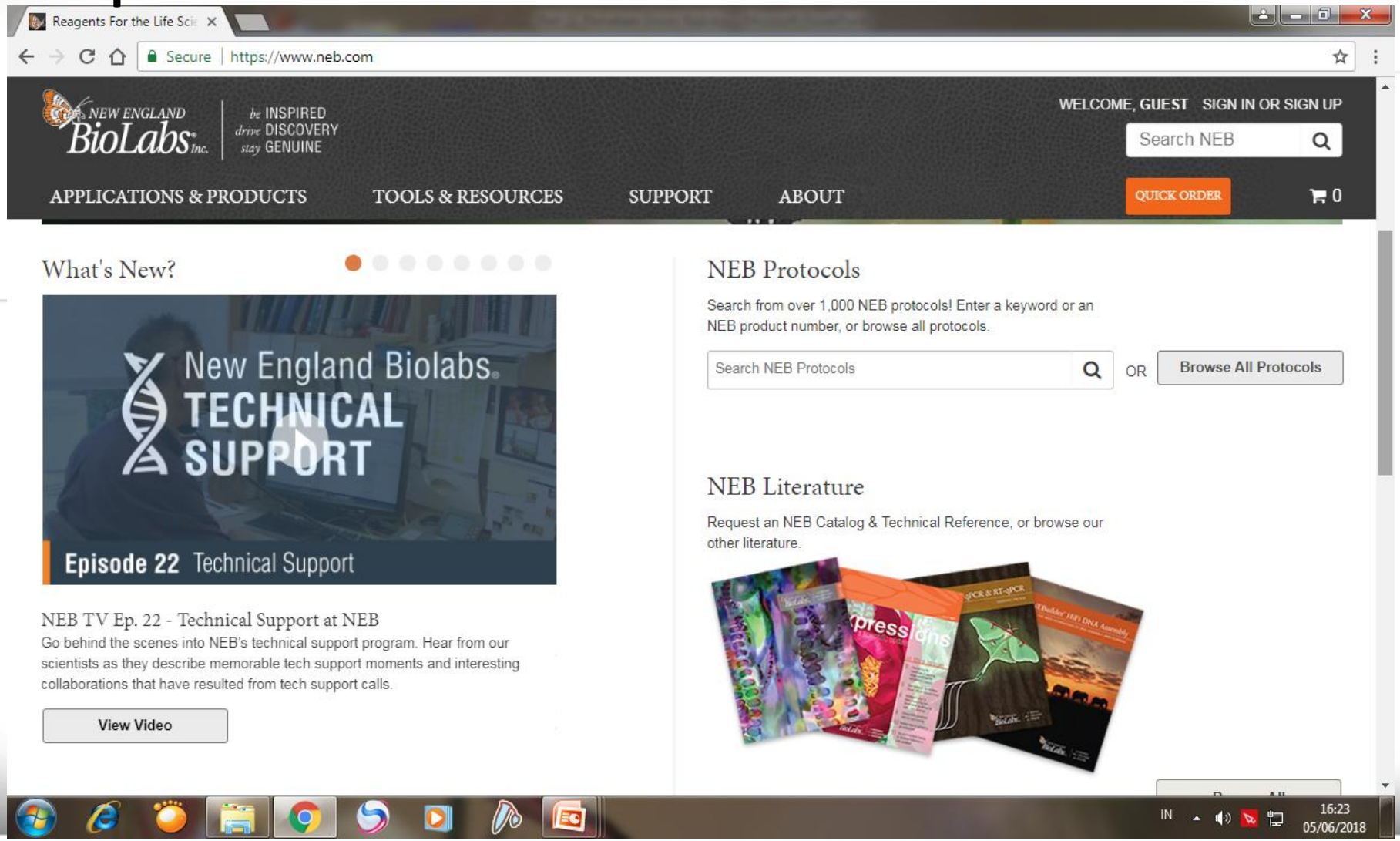


Langkah – Langkah Pencarian Peta Enzim Restriksi

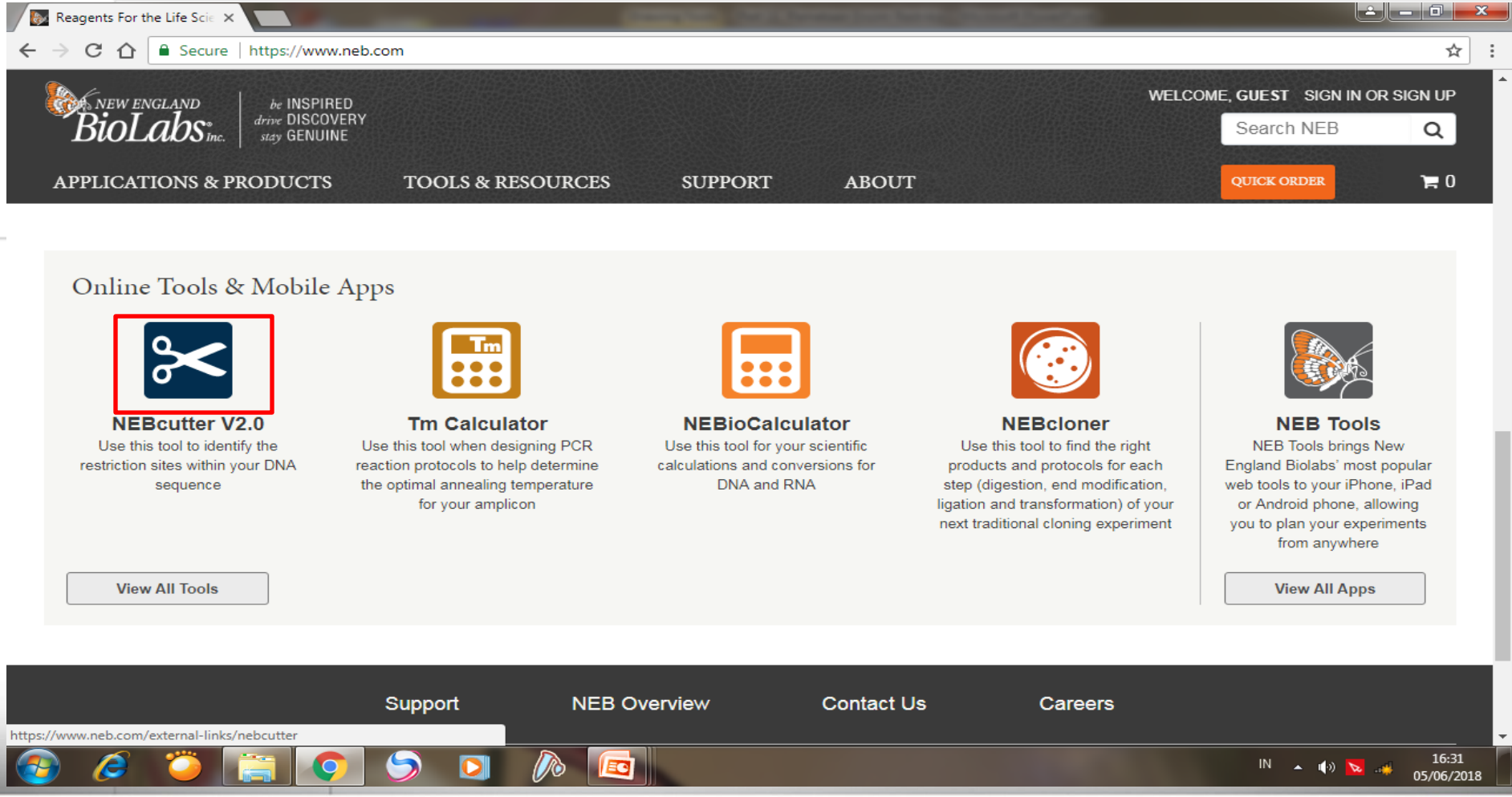
Secara online

1. Selain NCBI, terdapatpula database yang dapat digunakaan 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

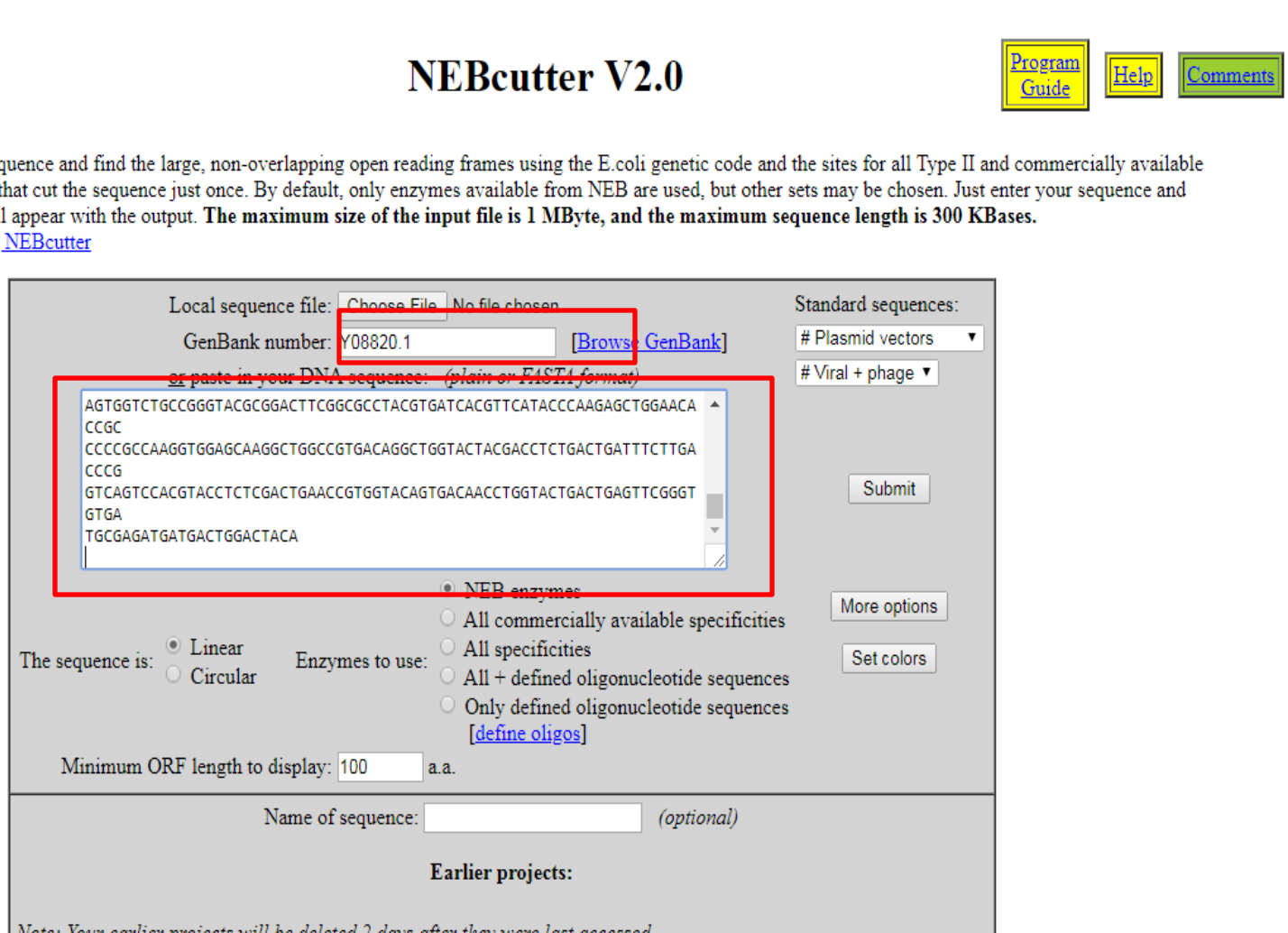
Tampilan halaman utama NEB



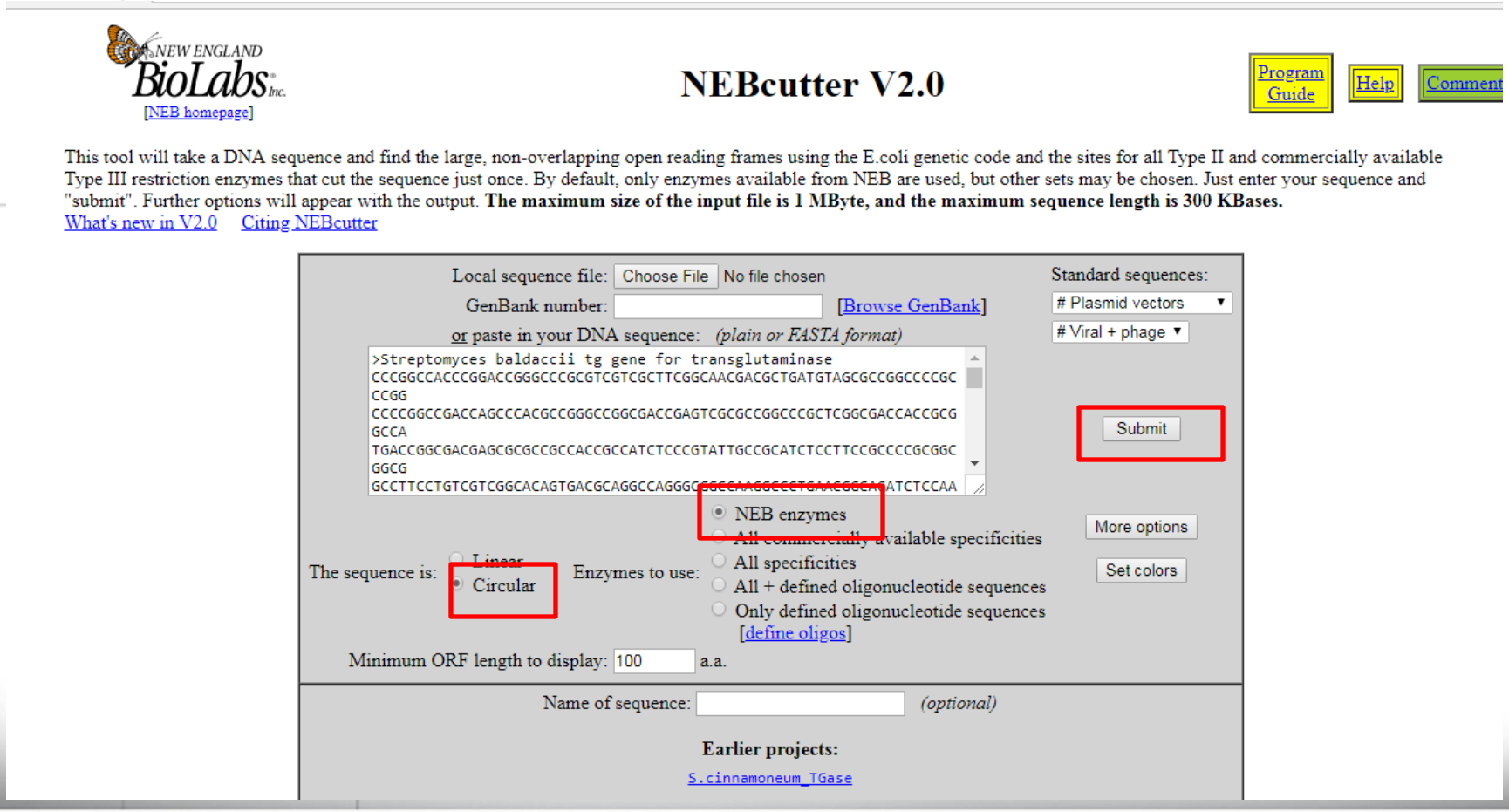
2. Pada kontak menu Online Tools & Mobile Apps, pilih *NEBcutter* yang memiliki icon gunting (Lingkar merah)



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



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



NEW ENGLAND BioLabs Inc.
[NEB homepage]

NEBcutter V2.0

Program Guide | Help | Comment

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: Choose File No file chosen
GenBank number: [Browse GenBank]
or paste in your DNA sequence: *(plain or FASTA format)*

```
>Streptomyces baldaccii tg gene for transglutaminase
CCCGGCCACCCGGACCGGGCCCGCGTCGTCGCTTCGGCAACGACGCTGATGTAGCGCCGGCCCCGC
CCGG
CCCCGGCCGACCAGCCCACGCCGGGCCGGCGACCGAGTCGCGCCGGCCCGCTCGGCGACCACCGCG
GCCA
TGACCGGCGACGAGCGCGCCGCCACCGCCATCTCCCGTATTGCCGCATCTCCTTCCGCCCCGCGGC
GGCG
GCCTTCCTGTCGTCGGCACAGTGACGCAGGCCAGGGC[redacted]ATCTCCAA
```

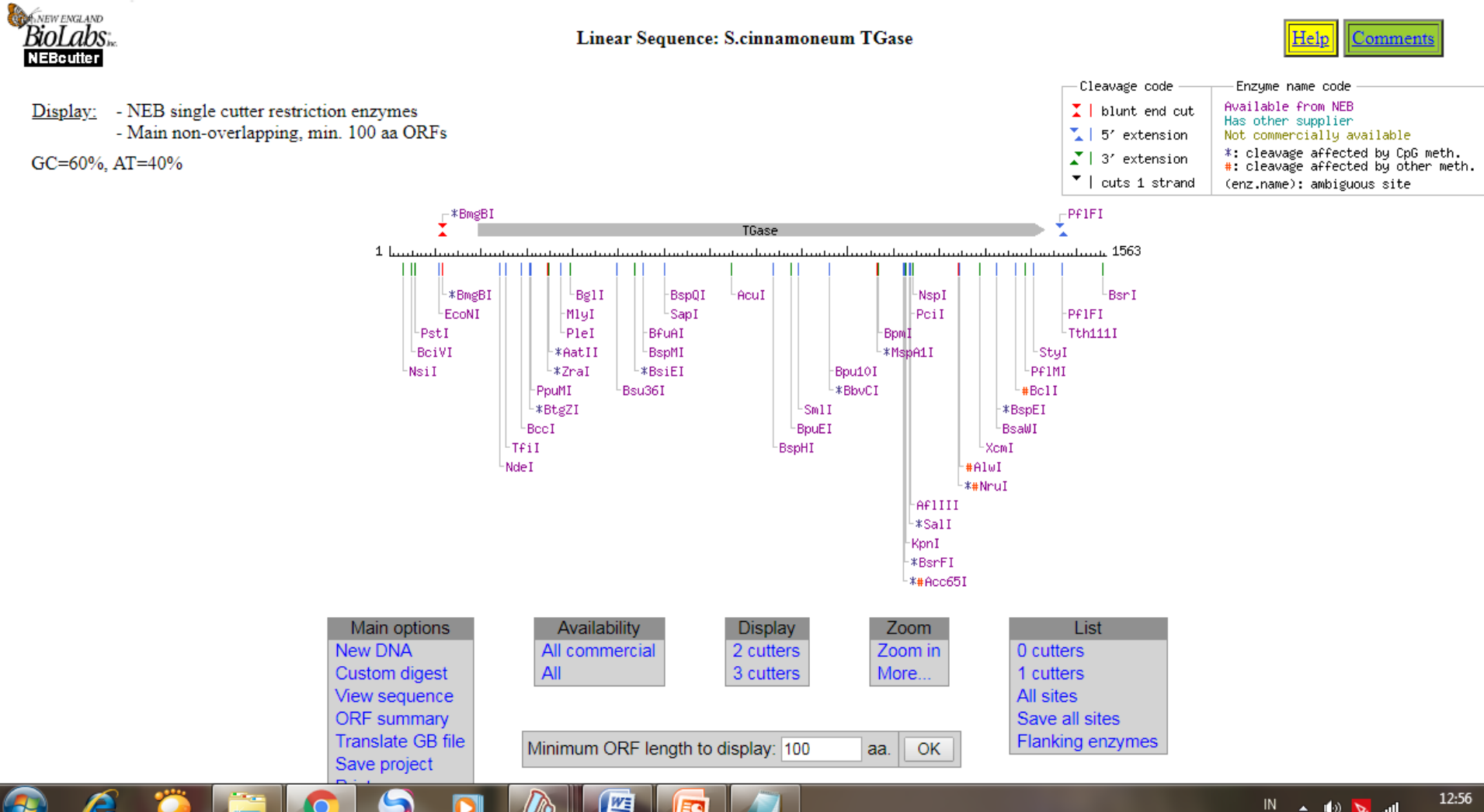
Standard sequences:
Plasmid vectors
Viral + phage
Submit
More options
Set colors

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: 100 a.a.

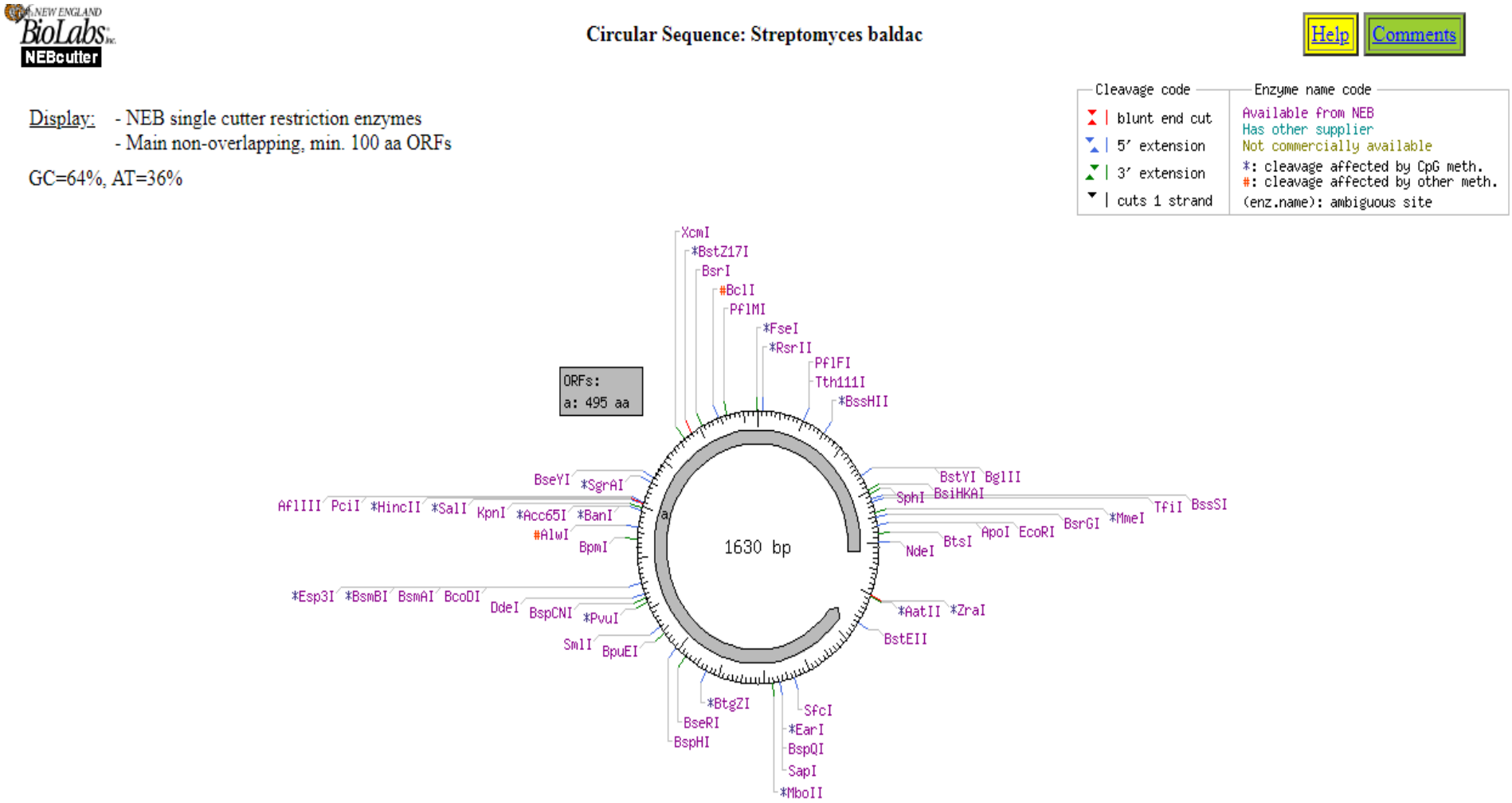
Name of sequence: *(optional)*

Earlier projects:
S.cinnamoneum_TGase

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



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

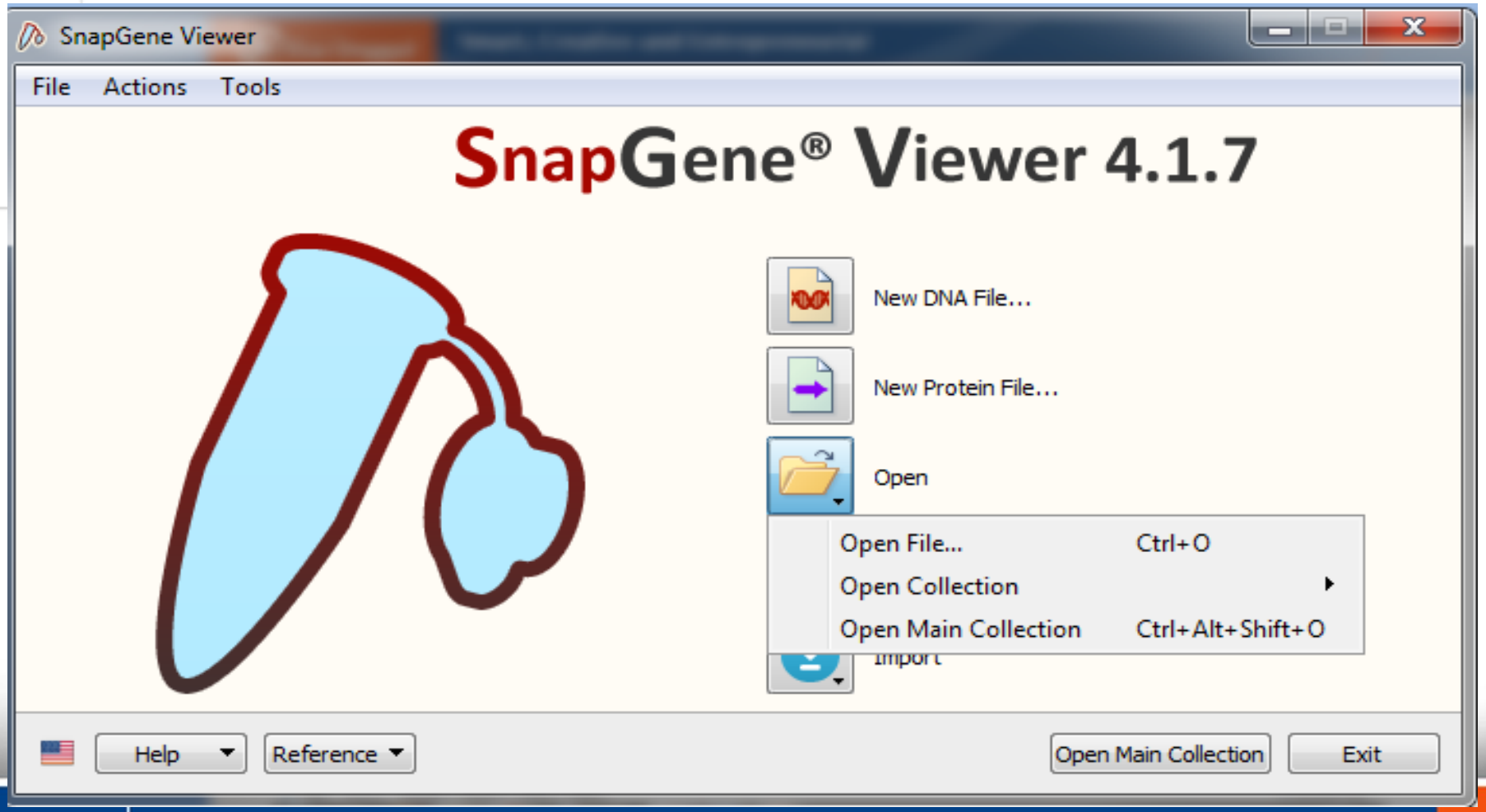


Langkah – Langkah Pencarian Peta Enzim Restriksi

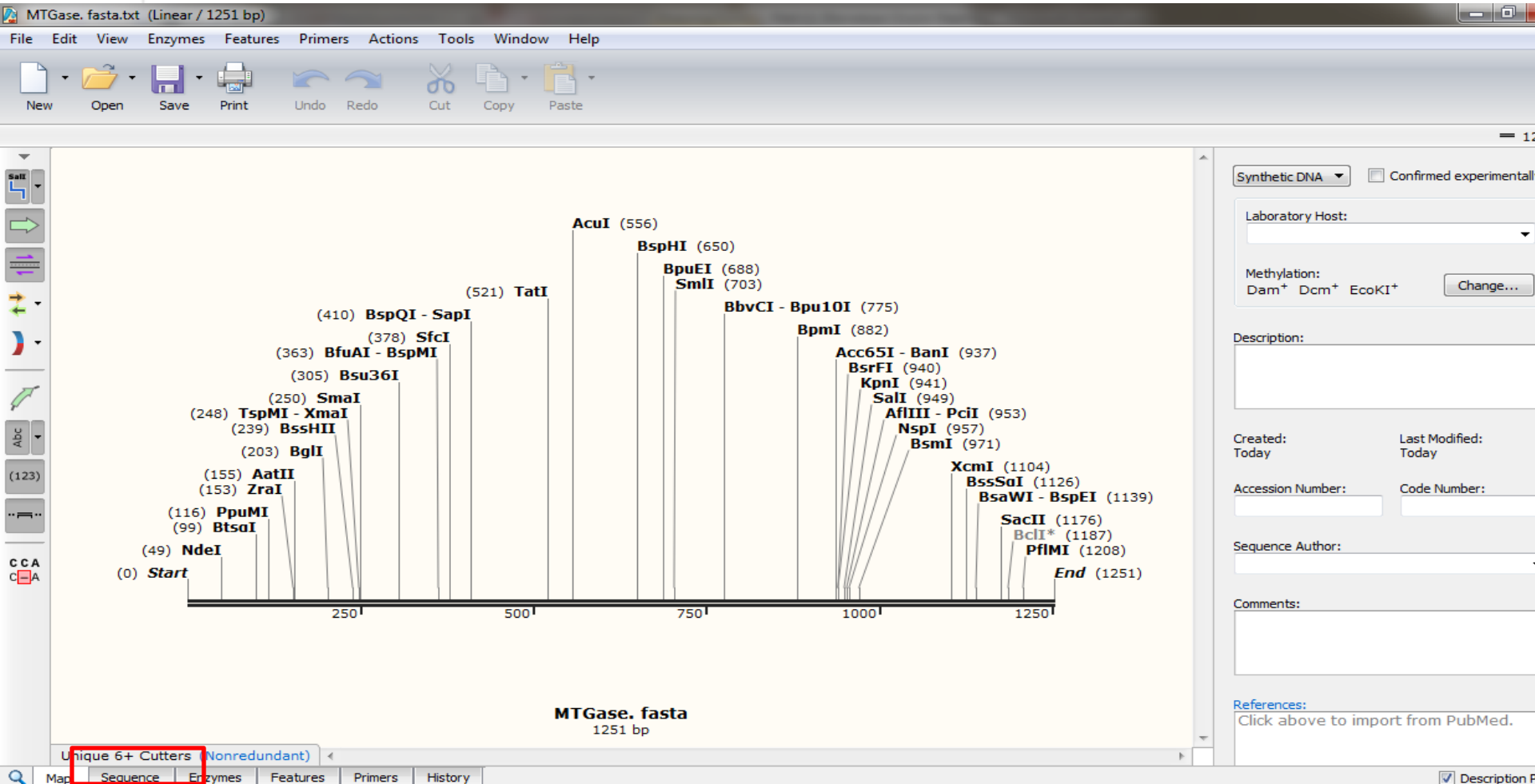
Secara offline

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

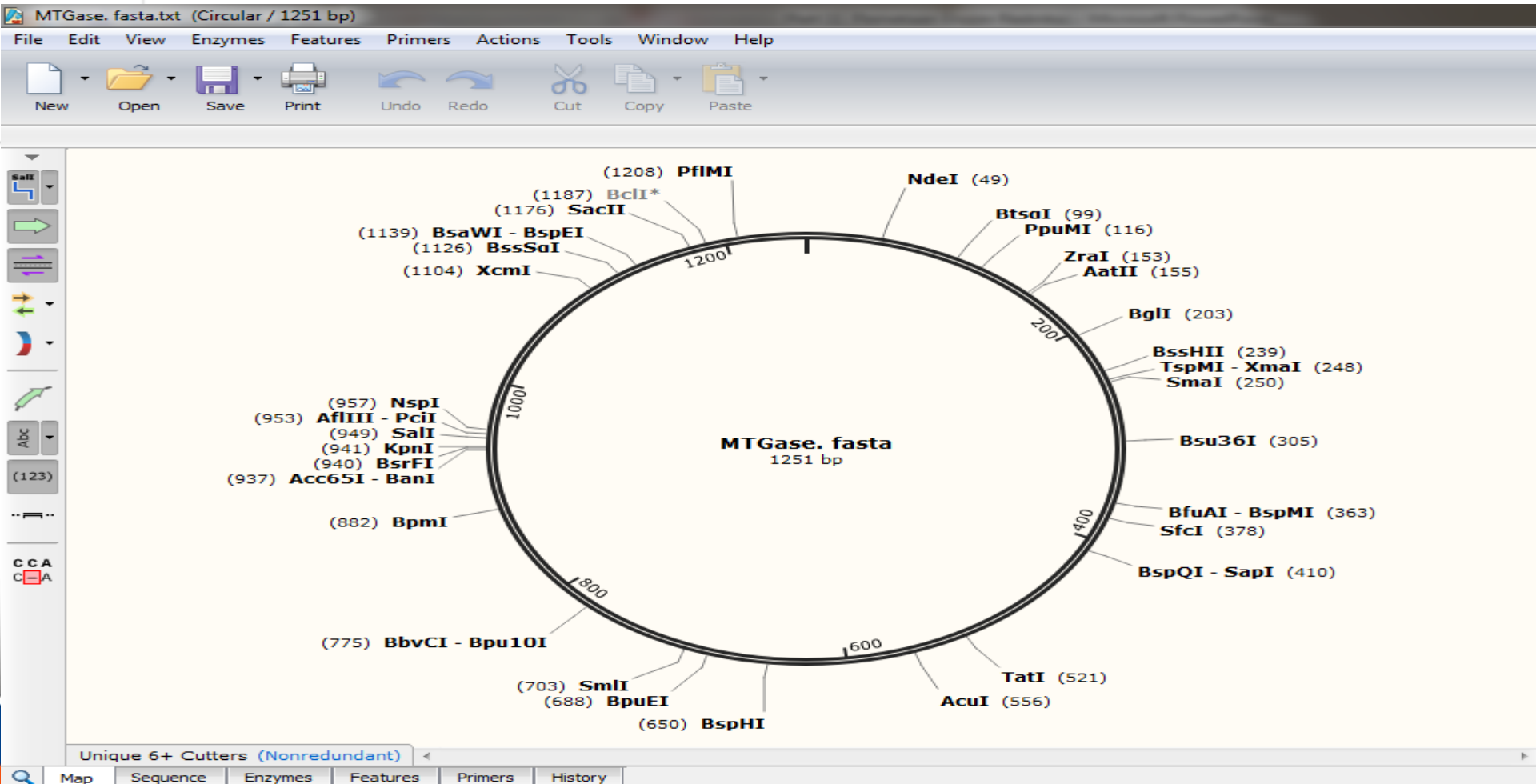
1. Buka software SnapGene. Pilih open file, pilih gen yang sudah disimpan dengan format fasta.



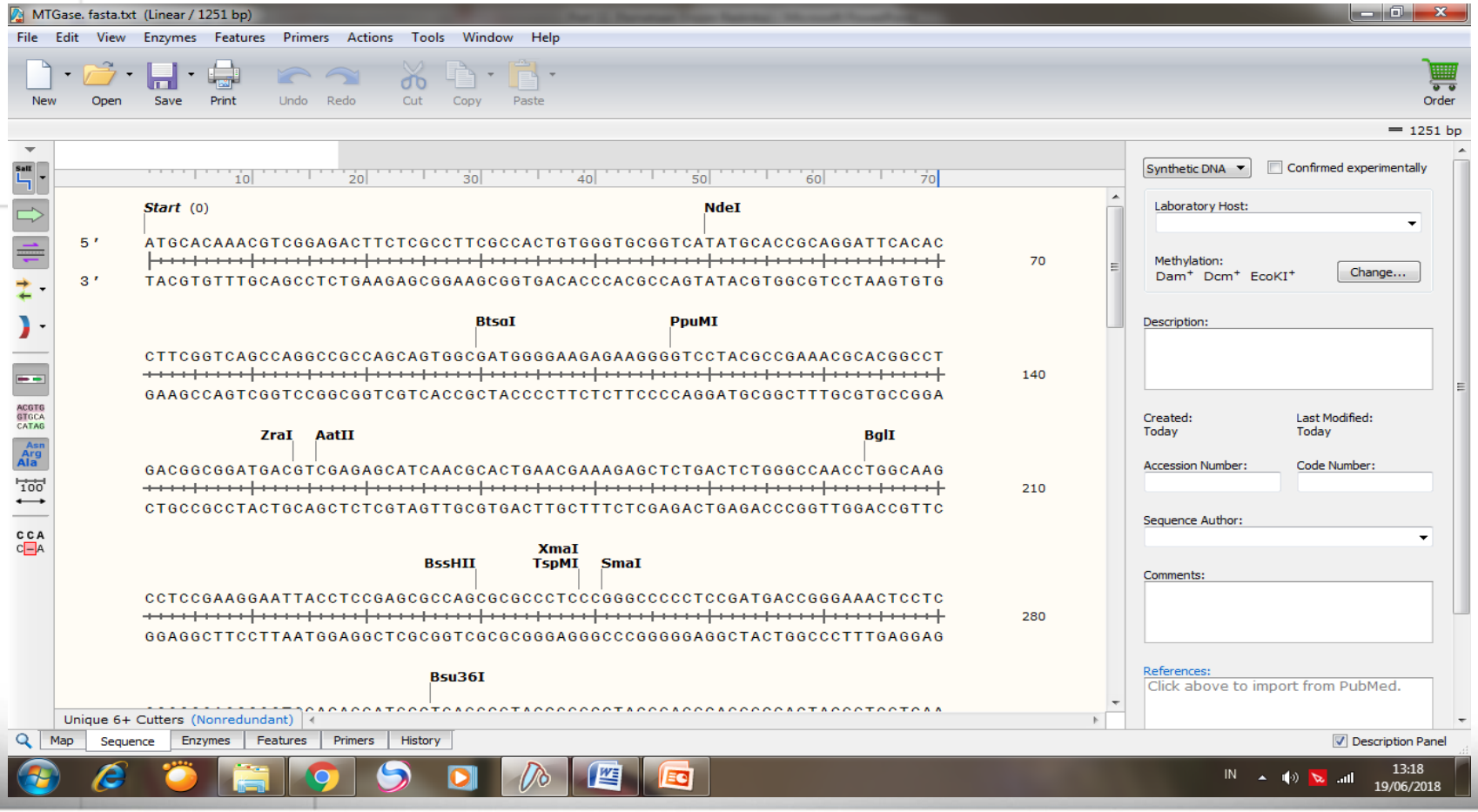
2. Tampilan pada sekuens gen (linear) pada Snapgene. Pilih **sequence** (Tanda merah) yang terletak bagian bawah untuk melihat sekuen utuh serta situs restriksinya



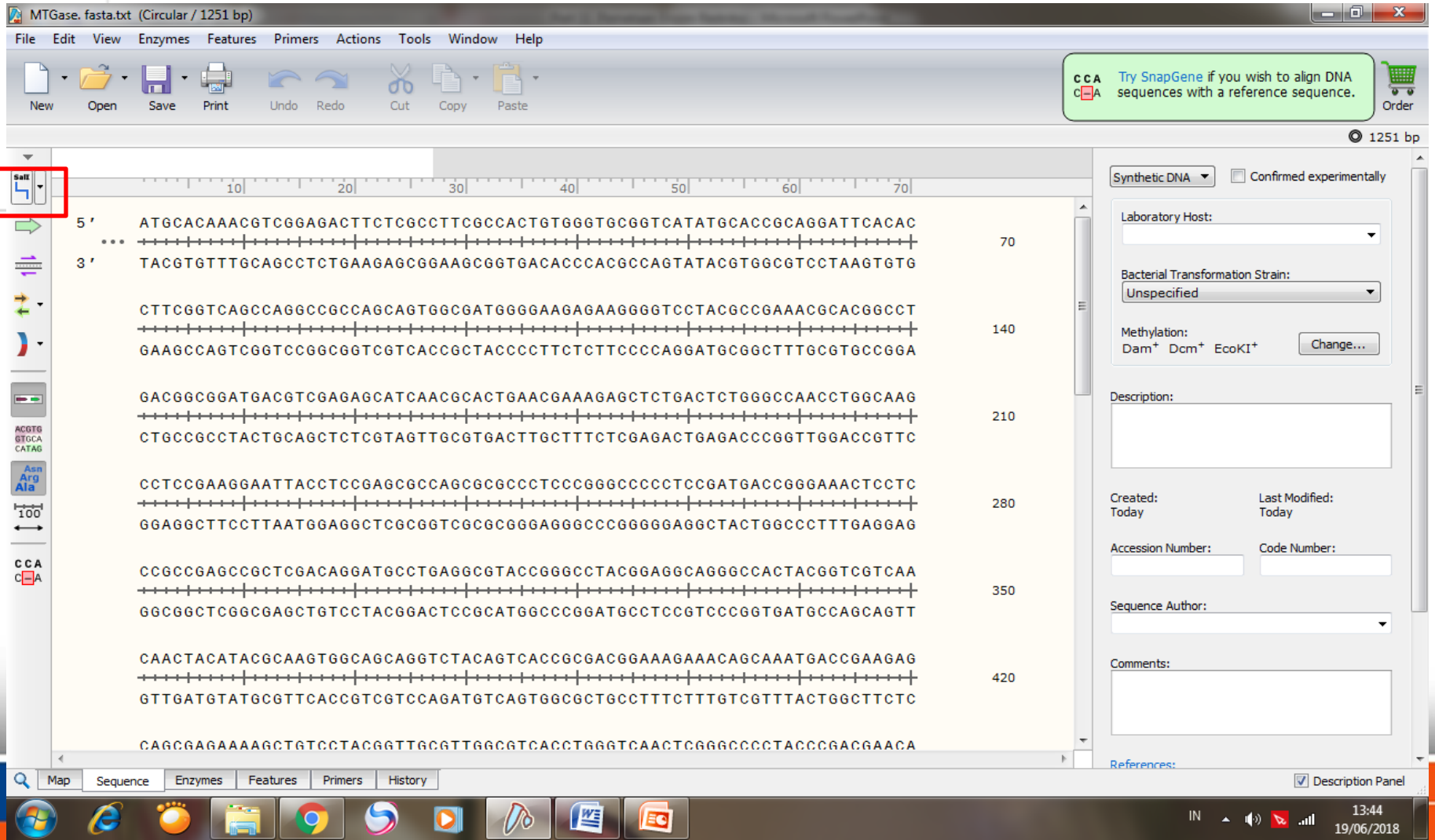
2. Tampilan pada sekuens gen (Sirkular) pada Snapgene. Pilih **sequence** (Tanda merah) yang terletak bagian bawah untuk melihat sekuen utuh serta situs restriksinya



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



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



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
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Novi Jan's Blog