RFLP

(Restriction Fragment Length Polymorphism)

RFLP

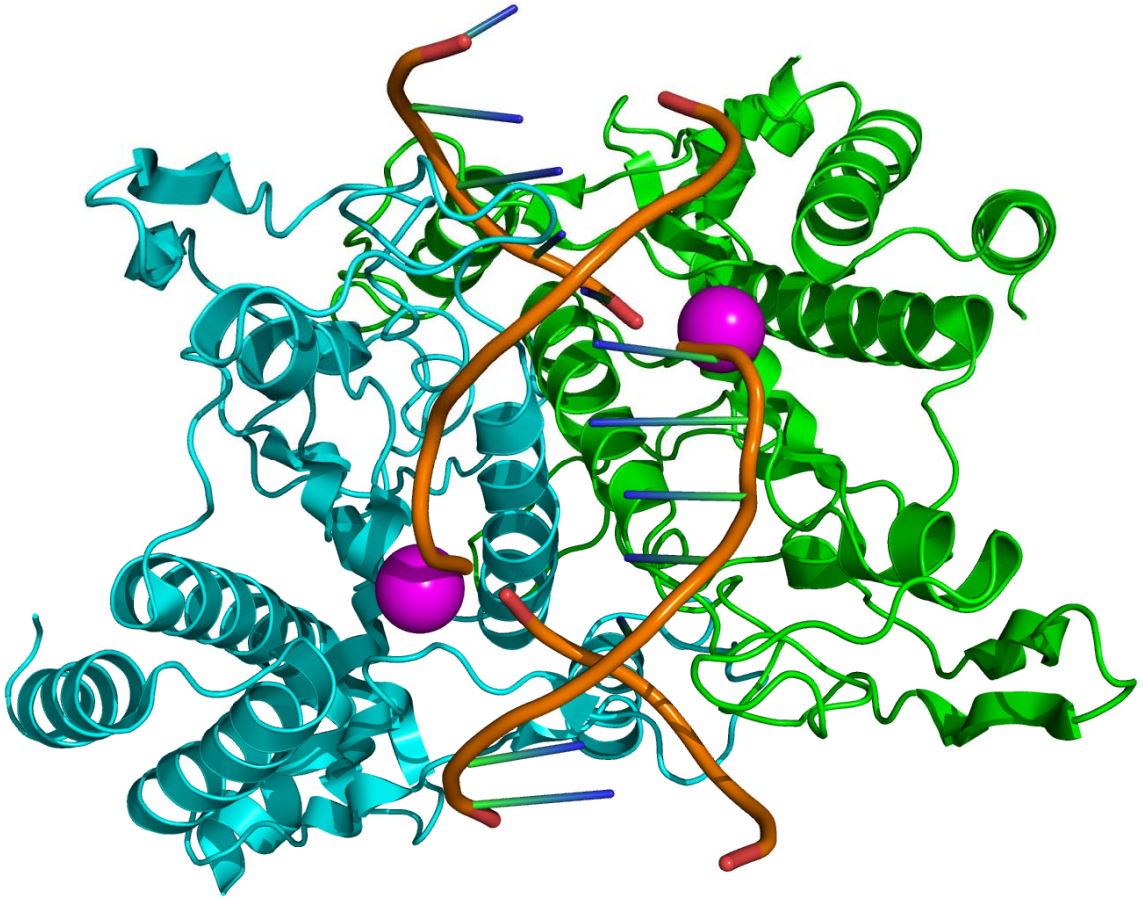
- **RFLP was developed at the late 70's due to the discovery of restriction enzymes (REs; or called as restriction endonucleases) from bacteria.**
- **RE acts as molecular scissors to cut DNA molecules at specific sequence.**
- **e.g. *Eco*RI recognizes sequence GAATTC.**
- **DNA genome of pine tree restricted by *Eco*RI can generate 5 million different restricted fragments.**
- **Daniel Nathans and Hamilton Smith received Nobel Prize in Medicine (1978) for the discovery of restriction endonucleases, leading to the development of recombinant DNA technology.**

Naming of REs

- **Restriction enzymes are named based on bacteria in which they are isolated in the following manner:**
- **e.g. *Eco*RI:**

E	***Escherichia* (genus)**
co	***coli* (species)**
R	**RY13 (strain)**
I	**First identified (order identified in bacterium)**

- ***Bam*HI (*Bacillus amyloliquefaciens*); *Hind*III (*Haemophilus influenzae*); *Taq*I (*Thermus aquaticus*)**



Structure of the homodimeric restriction enzyme *Eco*RI(cyan and green cartoon diagram) bound to double stranded DNA (brown tubes). Two catalytic manganese ions (one from each monomer) are shown as magenta spheres and are adjacent to the cleaved sites in the DNA made by the enzyme (depicted as gaps in the DNA backbone).

REs

- **over 3000 REs have been studied in detail, and more than 600 of these are available commercially.**

4-base cutters		6-base cutters	
*Hpa*II	CCGG	*Hind*III	AAGCTT
*Rsa*I	GTAC	*EcoR*I	GAATTC
*Taq*I	TCGA	*Dra*I	TTTAAA
*Alu*I	AGCT	*Bam*HI	GGATCC
*Hinf*I	GANTC	*Bgl*I	AGATCT
*Dde*I	CTNAG	*Cla*I	ATCGAT

REs

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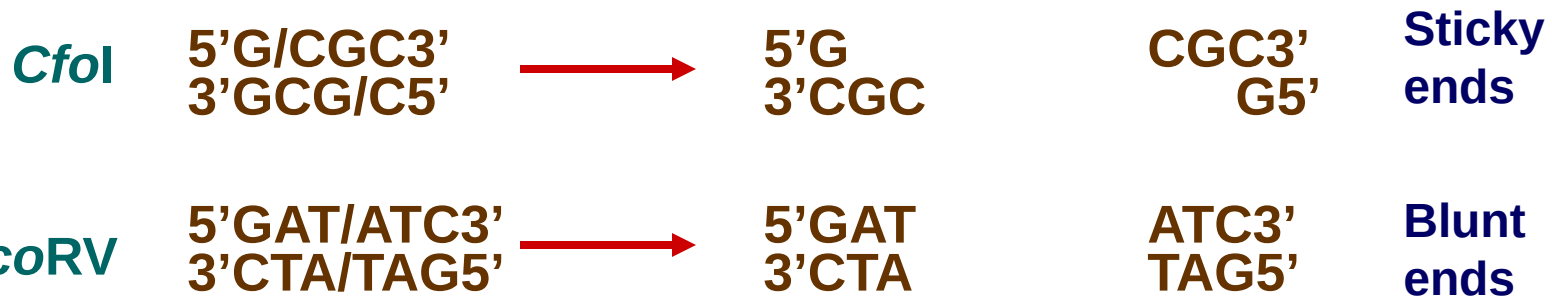
*Hpa*II	CCGG	*Hind*III	AAGCTT
*Rsa*I	GTAC	*EcoR*I	GAATTC
*Taq*I	TCGA	*Dra*I	TTTAAA
*Alu*I	AGCT	*Bam*HI	GGATCC
*Hinf*I	GANTC	*Bgl*I	AGATCT
*Dde*I	CTNAG	*Cla*I	ATCGAT

Some REs produce blunt ends and others produce sticky ends.



REs

4-base cutters			6-base cutters		
*Hpa*II	CCGG	S	*Hind*III	AAGCTT	S
*Rsa*I	GTAC	B	*EcoR*I	GAATTC	S
*Taq*I	TCGA	S	*Dra*I	TTTAAA	B
*Alu*I	AGCT	B	*Bam*HI	GGATCC	S
*Hinf*I	GANTC	S	*Bgl*I	AGATCT	S
*Dde*I	CTNAG	S	*Cla*I	ATCGAT	S

B = blunt ends; S = sticky ends (overhang)

*Cfo*I 5’G/CGC3’ / 3’GCG/C5’ → 5’G / 3’CGC + CGC3’ / G5’ — Sticky ends

*Eco*RV 5’GAT/ATC3’ / 3’CTA/TAG5’ → 5’GAT / 3’CTA + ATC3’ / TAG5’ — Blunt ends

Isoschizomers

- **Restriction enzymes that recognize the same sequence and are derived from different organisms**
- **They may have different sites of specific cleavage.**

***Sma*I (*Serratia marcescens* SB)**
CCC^GGG

***Xma*I (*Xanthomonas malvacaerum*)**
C^CCGGG

***Psp*AI (*Pseudomonas* species)**
C^CCGGG

Tutorial

Give the specific sequences recognized by the REs. What type of ends produced by the REs?

RE	Specific Sequence (5'→3')	Type of ends
*Sal*I		
*Hae*III		
*Hha*I		
*Hpa*I		
*Mbo*I		
*Not*I		
*Pst*I		
*Xho*I		
*Xba*I		
*Sac*I		

RE Digestion

What does a restriction enzyme need in order to do its duty?

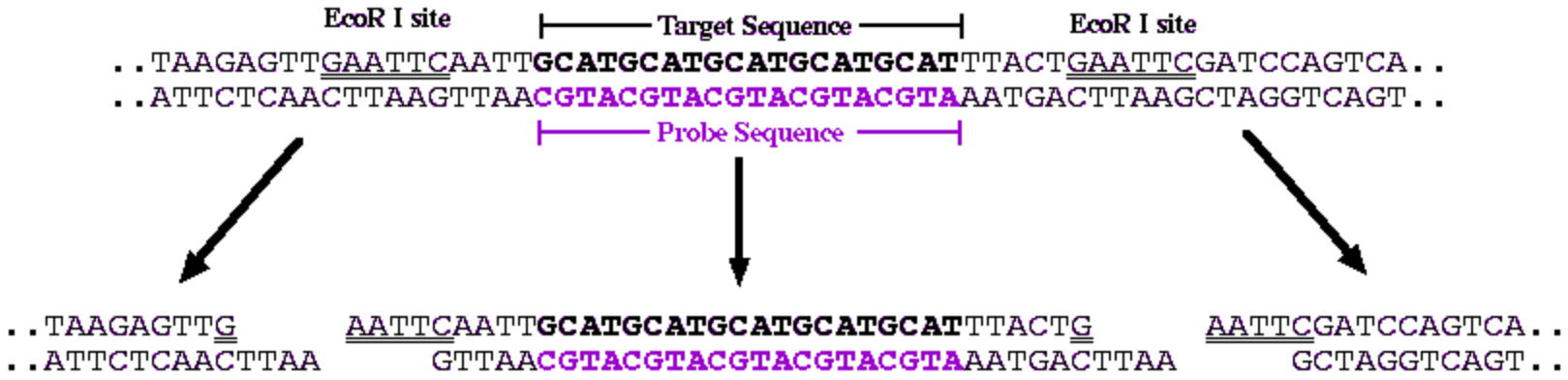
- a double-stranded DNA sequence containing the recognition sequence.
- suitable conditions for digestion.

- Most restriction enzymes are used at 37°C. However, there are exceptional temparatures: *Sma*I (25°C), *Apa*I (30°C), *Bcl*I (50°C), *Bst*EII (60°C) and *Taq*I (65°C).
- *Taq*I is a restriction enzyme from the same type of organism that produces *Taq* DNA polymerase (*Thermophilus aquaticus* or *Thermus aquaticus*).

DNA probes

- Short DNA fragment (0.5 – 3 kb)
- Source of DNA probe (homologous or heterologous)
  - mtDNA, cpDNA, nDNA, microsatellite DNA, minisatellite DNA
- Labeling of DNA probe
  - Radioactive labeling (P^{32}, P^{33}, S^{35})
  - Non-radioactive labeling (biotin, digoxigenin, fluorescent dyes – 5 colours)

DNA probes


EcoR I site
Target Sequence
EcoR I site
..TAAGAGTTGAATTCAATTGCATGCATGCATGCATGCATTTACTGAATTCGATCCAGTCA..
..ATTCTCAACTTAAGTTAACGTACGTACGTACGTACGTAAATGACTTAAGCTAGGTCAGT..
Probe Sequence
..TAAGAGTTG
AATTCAATTGCATGCATGCATGCATGCATTTACTG
AATTCGATCCAGTCA..
..ATTCTCAACTTAA
GTTAACGTACGTACGTACGTACGTAAATGACTTAA
GCTAGGTCAGT..