Restriction endonucleases and their applications

History of restriction endonucleases and its role in establishing molecular biology

Restriction enzymes

- Over 10,000 bacteria species have been screened for restriction enzymes
- Over 2,500 restriction enzymes have been found
- Over 250 distinct specificities
- Occasionally enzymes with novel DNA sequence specificities are still found while most now prove to be duplicates (isoschizomers) of already discovered specificities.

Restriction Enzyme Function

- It is generally believed that the biological function of restriction enzymes is to protect cells from foreign DNA.
- Infecting DNA is cleaved (restricted) by the restriction enzyme(s) preventing it from successfully replicating and parasitizing the cell.

Why the bacteria does not kill itself?
The Restriction Enzyme Modification Systems

if everything gets cleaved, how come the bacteria does not kill itself?

- **Usually, organisms that make restriction enzymes also make a companion modification enzyme (DNA methyltransferase) that protects their own DNA from cleavage.**

- **These enzymes recognize the same DNA sequence as the restriction enzyme they accompany, but instead of cleaving the sequence, they disguise it by methylating one of the bases in each DNA strand.**

Classification of Restriction enzymes

Class I	Class II (93%)	Class III
Restriction-methylase on the same subunit	Homo-dimers, methylase on a separate subunit	Restriction-methylase on the same subunit
ATP-dependent	Mg++ dependent	ATP-dependent
Binds to DNA recognition site and cuts DNA randomly - any DNA as long as it comes in contact	recognize symmetric DNA sequences and cleave within the sequences	Cut the DNA at the recognition site and then dissociate from the DNA

Type II Restriction enzymes

are endonucleases

that cut DNA at specific sites, and are most useful for molecular biology research

Type II Restriction enzymes

Recognition sites

are

Palindroimes:

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IFFI, ABA

AAGCTT

TTCGAA

How do I know what sequence each enzyme cut?

- Test by cutting DNA of known sequence
- Commercial sources are tested already, and you find a catalog

Some popular Biotechnology Companies

- Life Technologies (BRL/GIBCO)
- New England Biolabs
- Amersham Pharmacia Biotech
- Qiagen
- Promega
- Clonetech
- Invitrogen
- Stratagene
- ...

Nomenclature of restriction enzyme

- ***Eco*** **R1: E coli**
- ***Pst*** **I: Providencia stuartii**
- ***Hin*****d III: Haemophilus influenza**
- ***Not*** **I: Norcardia otitidis-caviarum**

- **What do you name a restriction enzyme isolated from *Xanthomonas graminis*?**

How long is the recognition sequence

- 4 bp: e.g., Taq 1, HpaII, MspI
- 6 bp: e.g., EcoR1, HindIII, BamH1, PstI, sall
- 8 bp: Not I, Sfi I

Recognition sequence may be interrupted or ambiguous

Acc I: GT(at/gc)AC

Bgl I: GCCNNNNNGGC

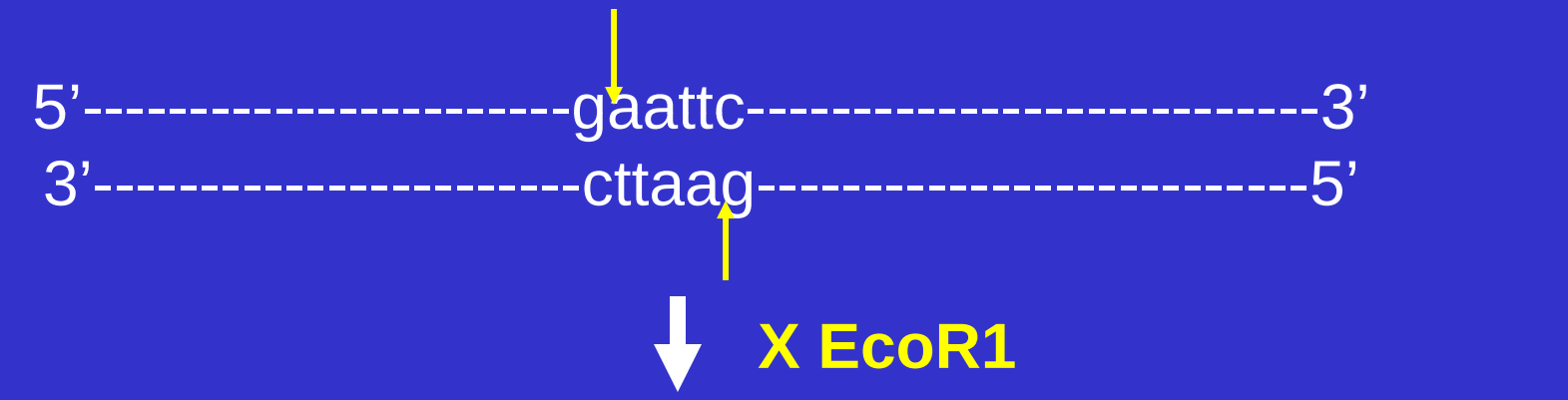
Afl III: ACPuPyGT

Three types of ends produced by type II restriction enzymes

- 3’-overhang (protruding)
- 5’-overhang
- Blunt end

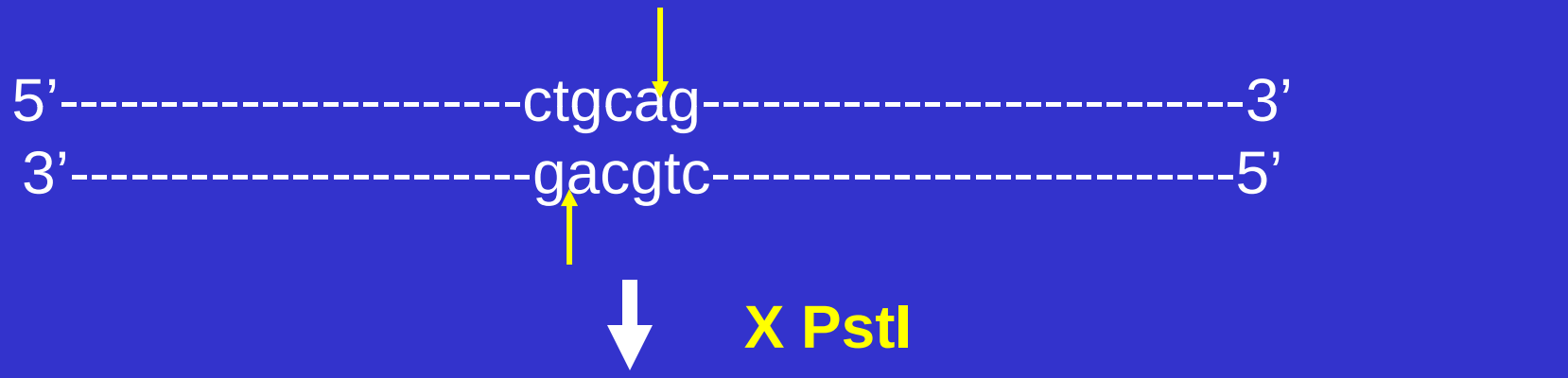
5’-overhang

EcoR I



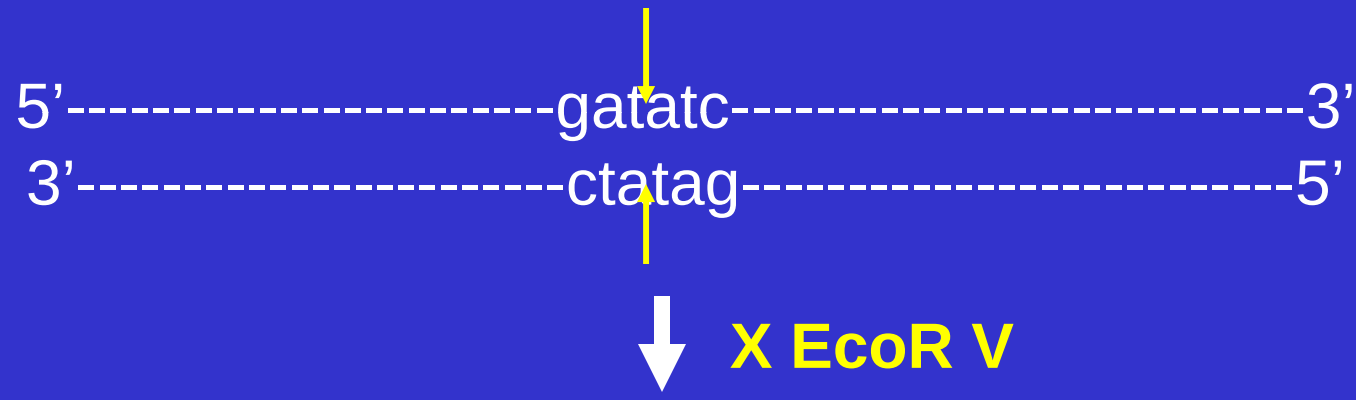
3’-overhang

Pst I:



Blunt end

EcoR V



Only Compatible, base-pairable ends can ligate

Odds of cutting at a segment of DNA

- 4 bp cutter: $4^4 = 256$ bp
- 6 bp cutter: $4^6 = 4$ kb
- 8 bp cutter: $4^8 = 64$ kb

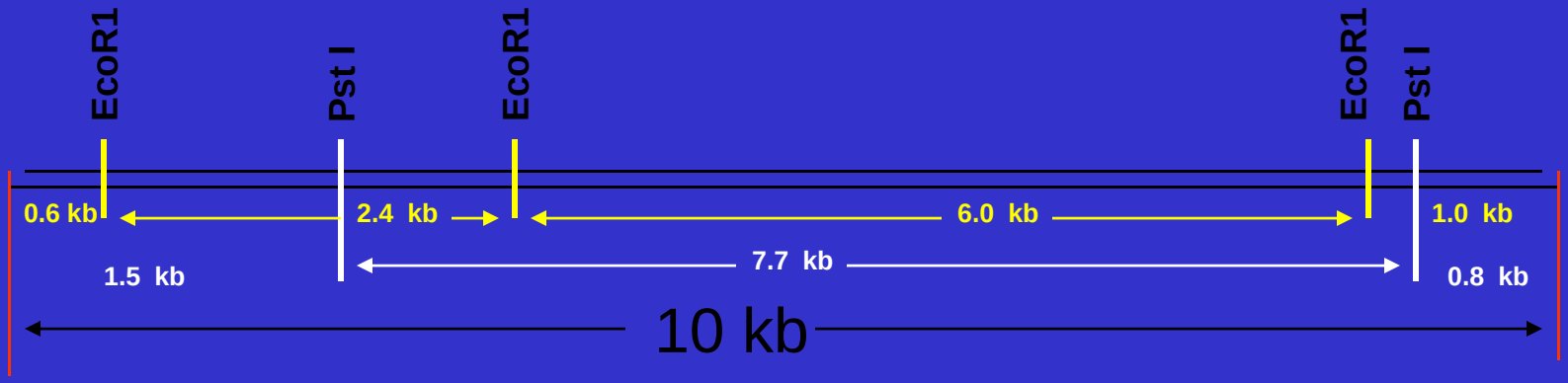
- ??? How many do you predict Eco R1 to cut catfish genome of 8×10^9 bp

What do you expect if you digest with your plasmid DNA with 4-bp cutters

What do you expect if you digest with your plasmid DNA with 6-bp cutters

What do you expect if you digest with your plasmid DNA with 8-bp cutters

Restriction mapping



What do you expect to see with double digest, if reaction is complete?

0.6 kb, 0.9 kb*, 1.5 kb, 6.0 kb, 0.2 kb, 0.8 kb.

What do you expect if you digest with your genomic DNA with 4-bp cutters

What do you expect if you digest with your genomic DNA with 6-bp cutters

What do you expect if you digest with your genomic DNA with 8-bp cutters

Selection of restriction enzymes

Of Over 250 commercially available and over 2,000 total, how do I know what to use?

- Cutting frequency
- Easy to work with
- Economical