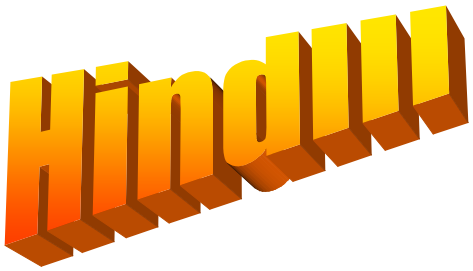
Restriction Enzymes



Restriction Enzymes

- They are proteins produced in a bacteria cell that cut DNA at a specific site.
- Also known as restriction endonucleases
- We can use these to manipulate DNA in the lab.

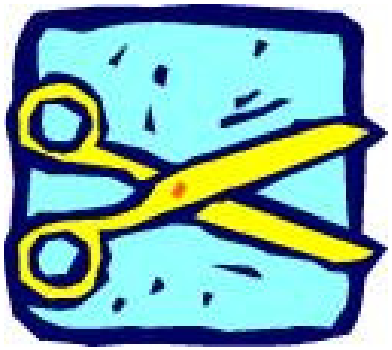
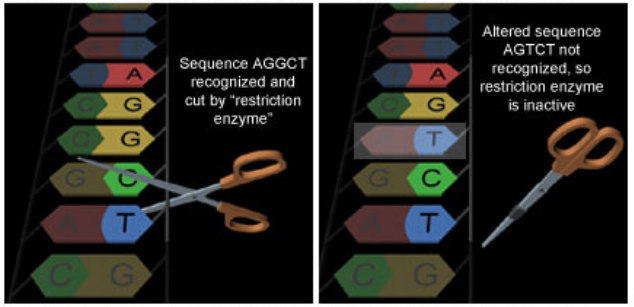


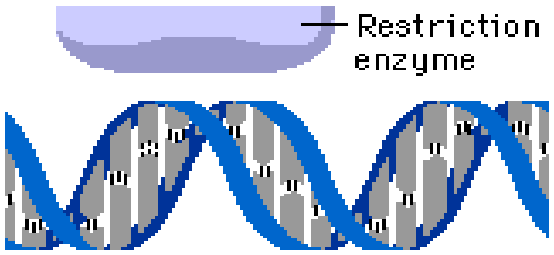
Figure Y-3: Restriction Enzymes Are Sequence Specific



Since the restriction enzyme only cuts at a particular DNA sequence, in this case "AGGCT", the enzyme will only cut when it recognizes this exact sequence. Even if as little as one letter is changed (for example, from G to T), the restriction enzyme will no longer cut.

Discovery and Naming

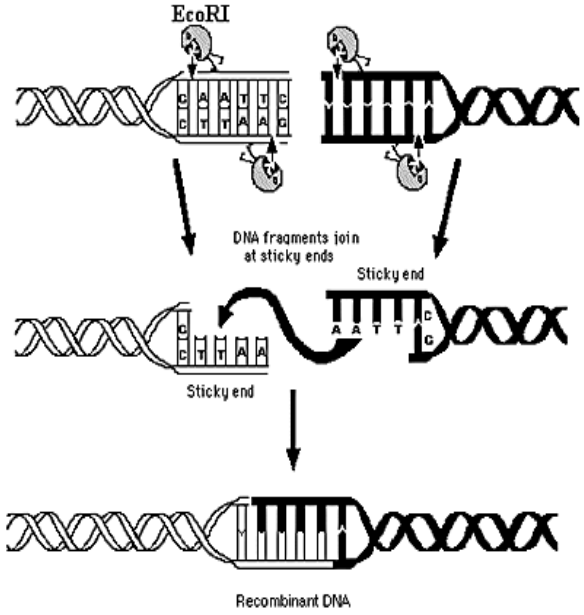
- They were discovered in the late 1960's.
- More than 2,500 type II restriction enzymes have been identified from a variety of bacterial species.
- These enzymes recognize about 200 distinct sequences, which are four to eight bases in length.



Restriction Endonucleases

Named for bacterial genus, species, strain. and type:

Example: EcoR1
Genus: Escherichia
Species: coli
Strain: R
Order discovered: 1



http://www.accessexcellence.org/AB/GG/restriction.html

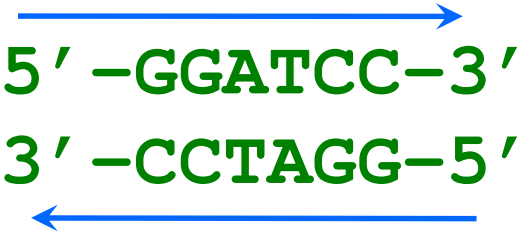
Restriction Enzyme Action of EcoRI

Restriction Endonucleases

Recognition sites have symmetry (palindromic)

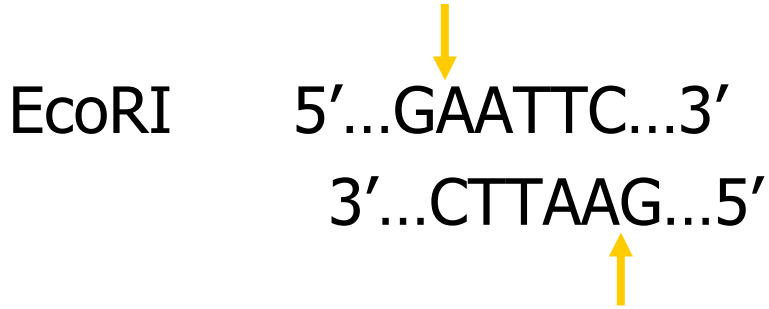
"Able was I, ere, I saw Elba"

Bam H1 site:



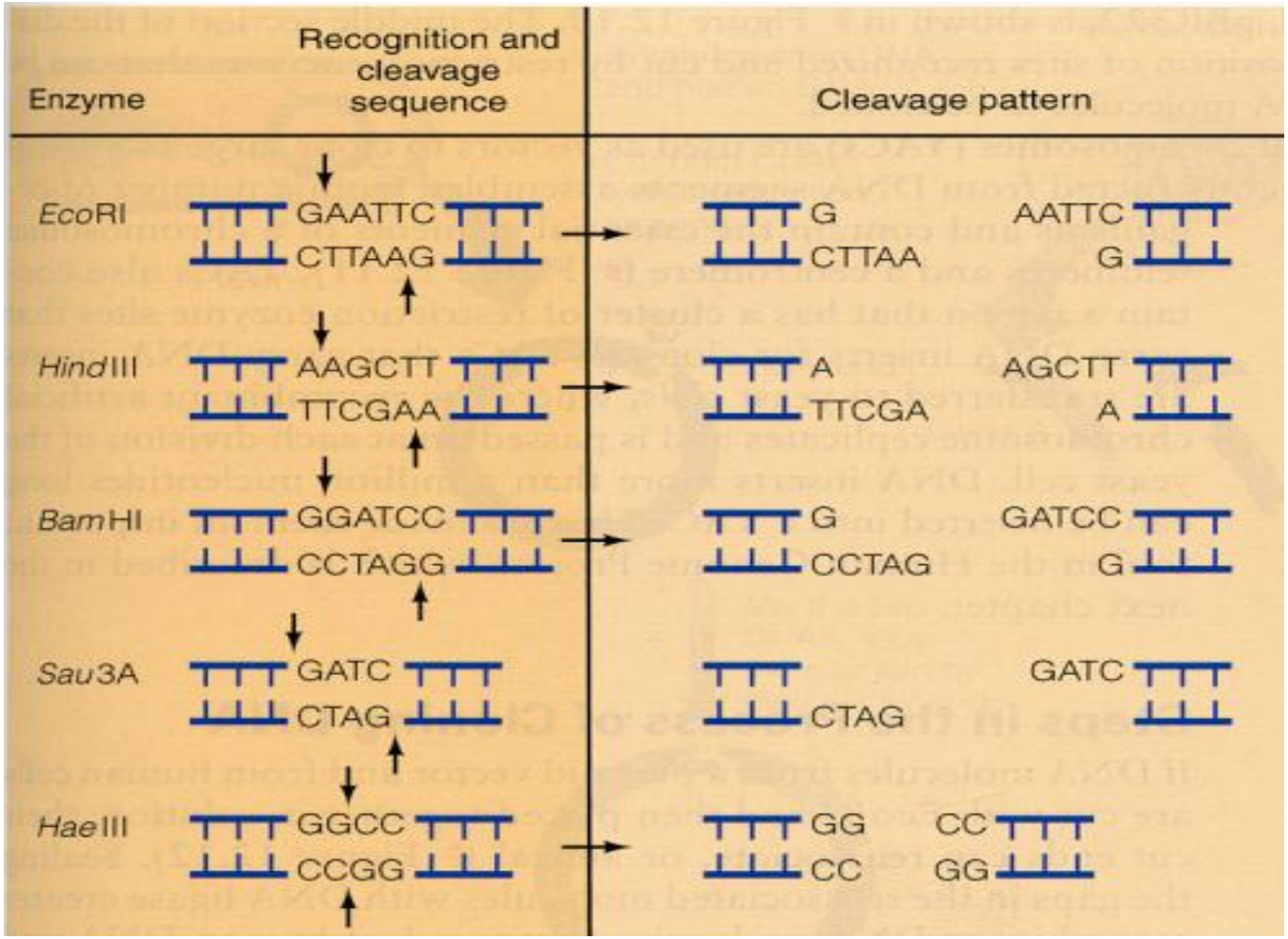
Restriction Endonucleases

Some enzymes cut in a staggered fashion - "sticky ends"

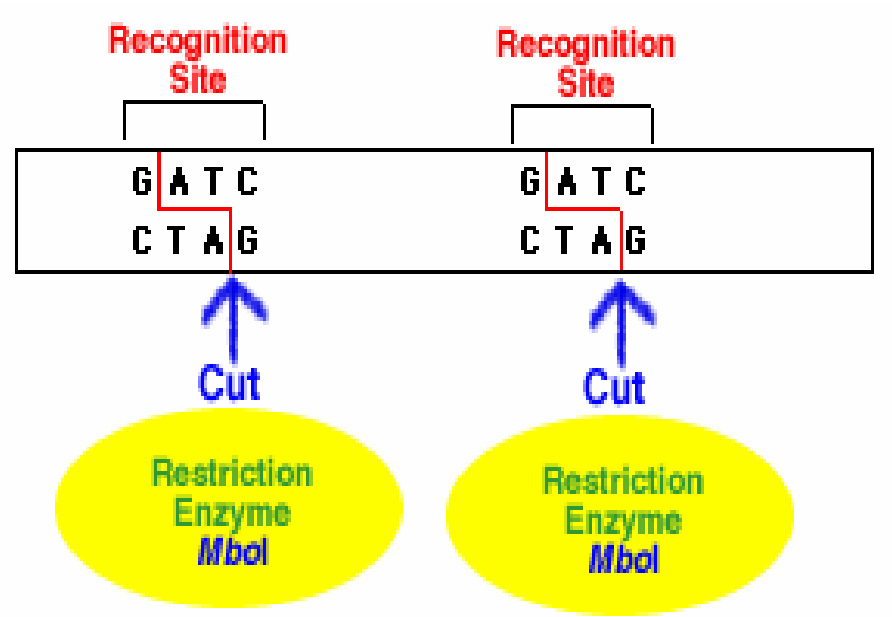


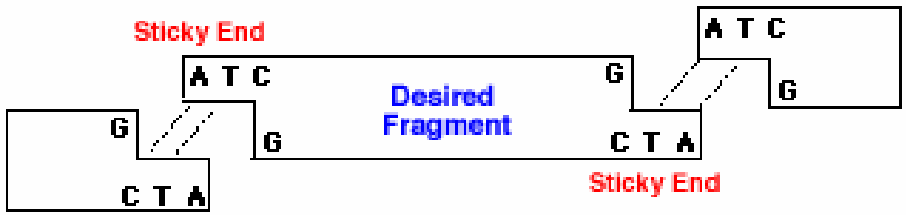
Some enzymes cut in a direct fashion – "blunt ends"

Blunt End Cuts

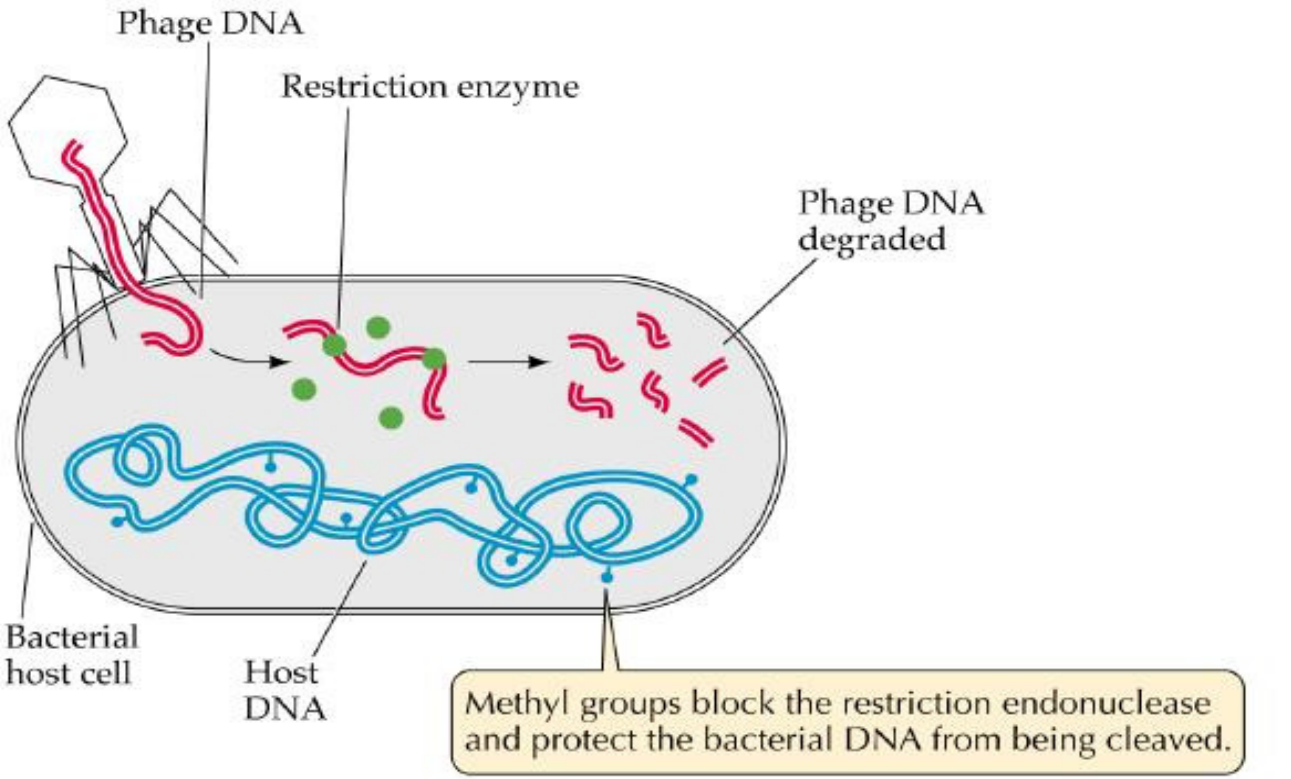


Sticky End Cuts

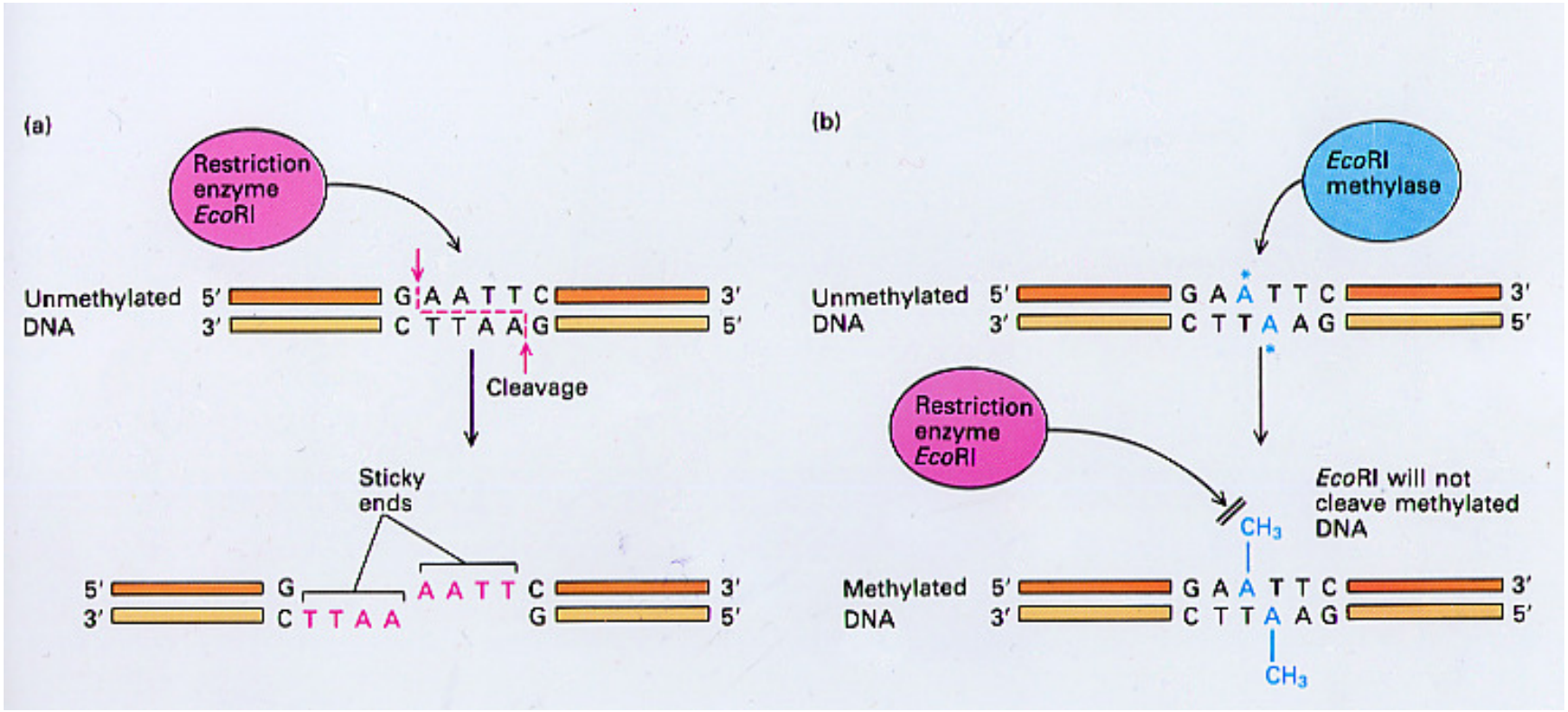




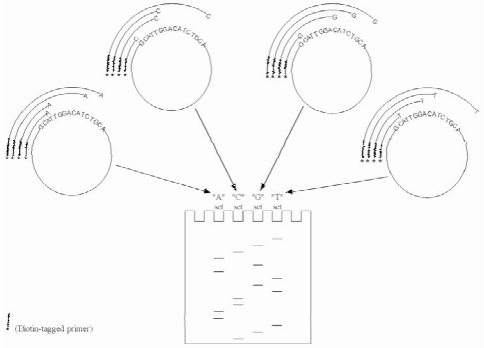
Why don't bacteria destroy their own DNA with their restriction enzymes?



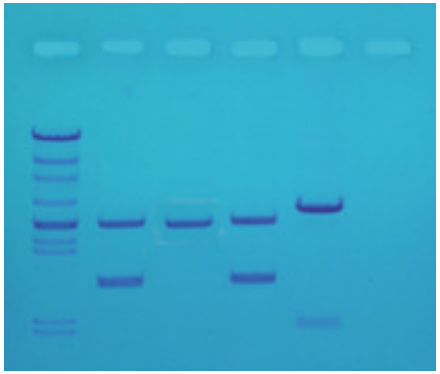
Methylation

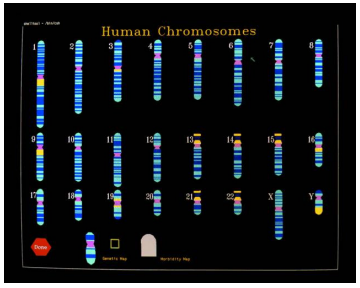


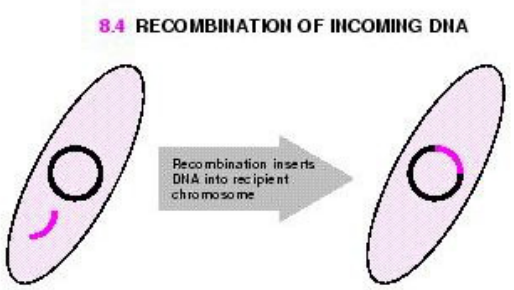
Examples of Uses



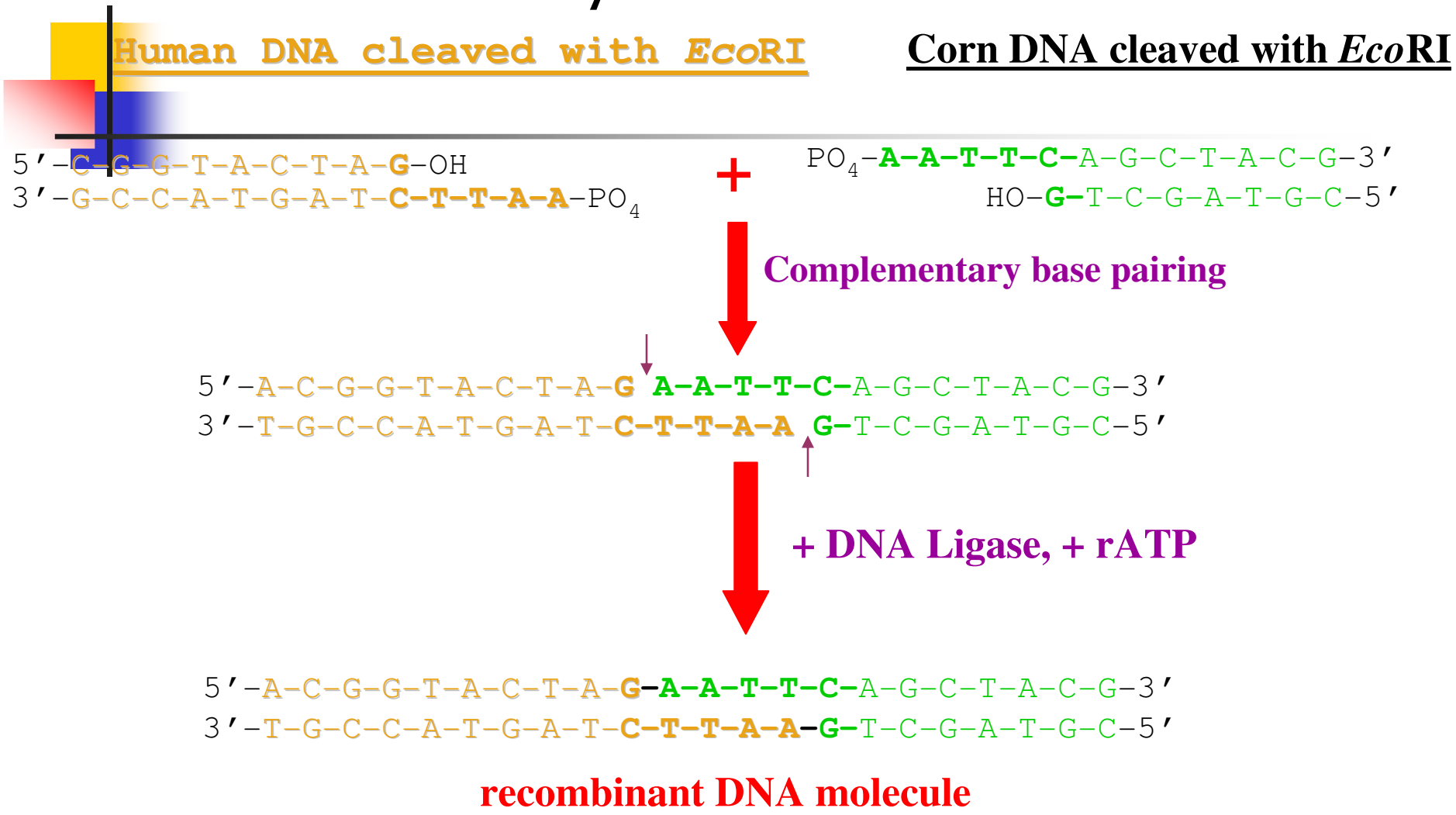
- Restriction Enzymes are used in the following areas:
  - DNA fingerprinting
  - DNA typing/profiling
  - DNA sequencing
  - Gene splicing/recombinant DNA
  - Transformation
  - Human Genome Project



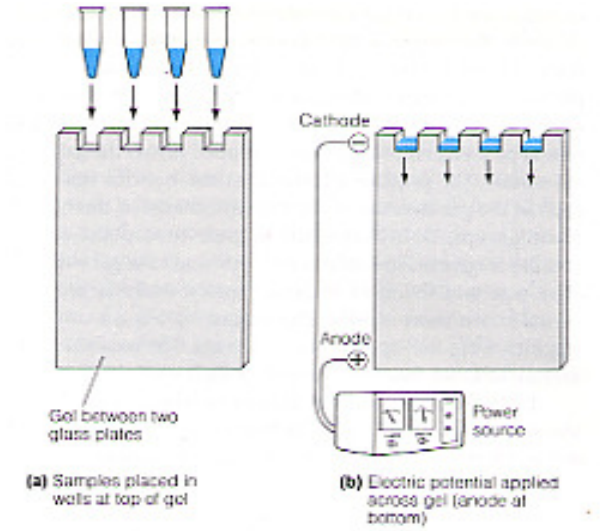


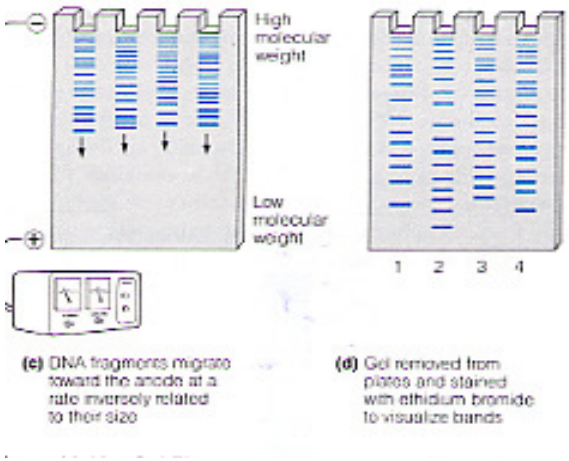


Restriction Enzymes for Transformation



Restriction Enzymes for RFLP



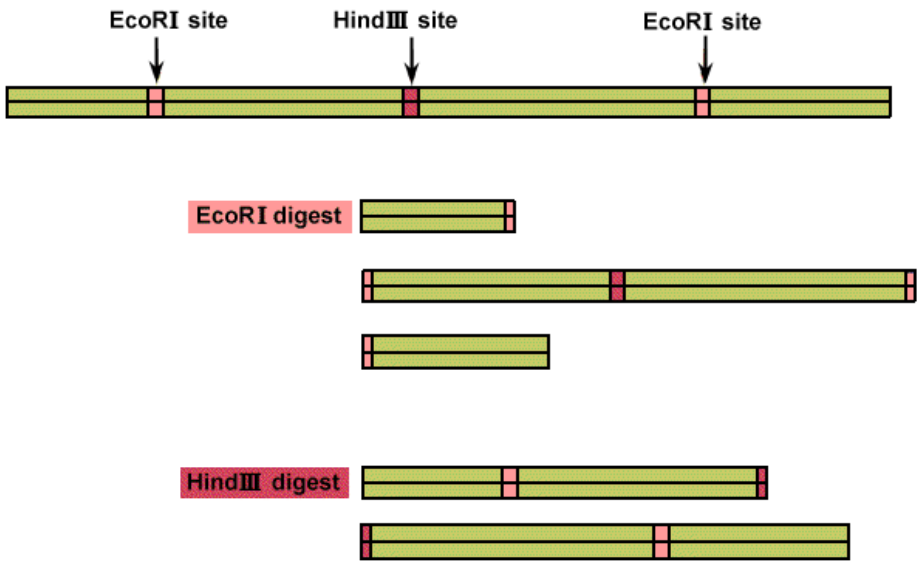


DNA is negatively charged from the phosphate backbone

Visualize DNA with ethidium bromide or SYBR Safe– fluoresces ONLY when bound to DNA

Restriction Enzymes

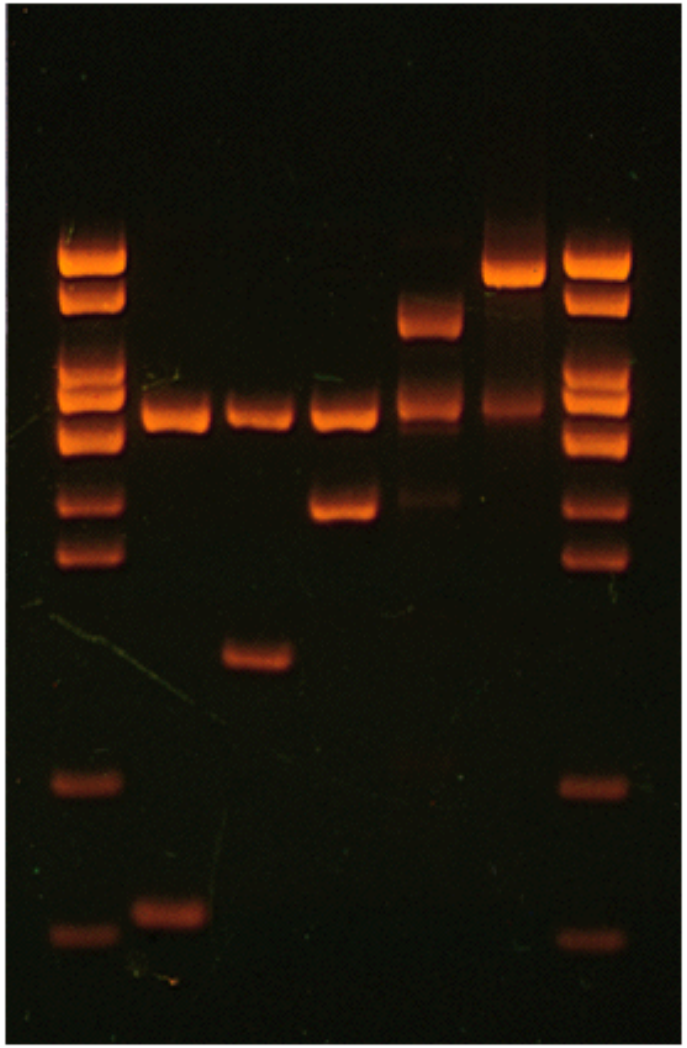
- Since the enzymes cut at a specific site, we end up with different length fragments because each person has a unique pattern of DNA.



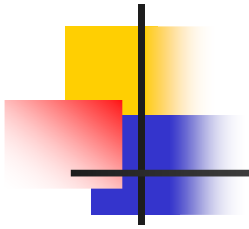
Restriction Enzymes

- The restriction enzymes used work because every one has end-to-end repeats of different short DNA sequences. They can range from 2 bases to 30+ bases long.
- In some regions of the genome, the number of repeats varies highly from individual to individual.
- Restriction enzymes cut at these (VNTR's) variable number tandem repeats.

Gel Electrophoresis



DNA cut with restriction enzymes



Restriction enzyme animation

http://www.dnai.org/b/index.html