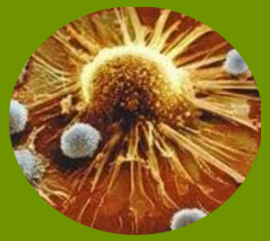
Cryphonectria parasitica tendrils on chestnut tree bark (Photo: Ministry of Agriculture and Regional Development Archive, Ministry of Agriculture and Regional Development, Bugwood.org)



6/22/2014

Enzymes and Restriction Digestion of DNA

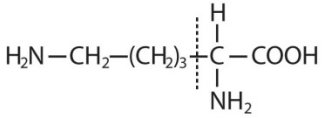
B3 Summer Science Camp at Olympic High School

Dr. Jennifer Weller

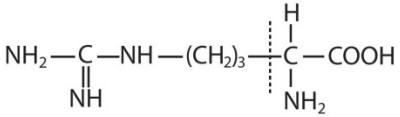
Enzymes

- Enzymes are proteins – polymers of amino acids.
  - They carry out chemical reactions for cells.
  - They are chemical catalysts because they emerge unchanged from each reaction: they are neither reactants (substrates) nor products as usually defined.
  - They speed up the reactions – cells need this because a lot of times the amount of reactant is tiny so the reaction would proceed very slowly without the enzyme.

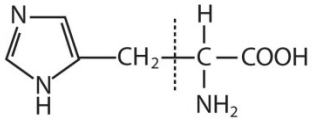
Lysine (K)
Residue Mass 128

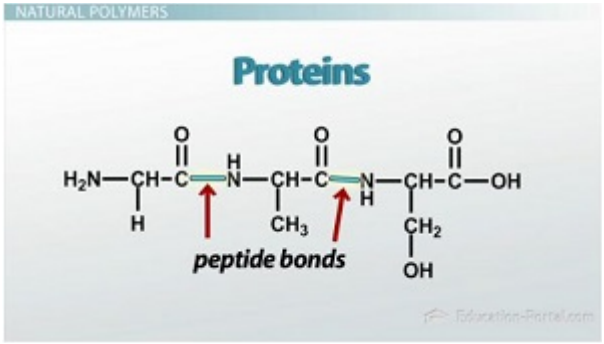


Arginine (R)
Residue Mass 156

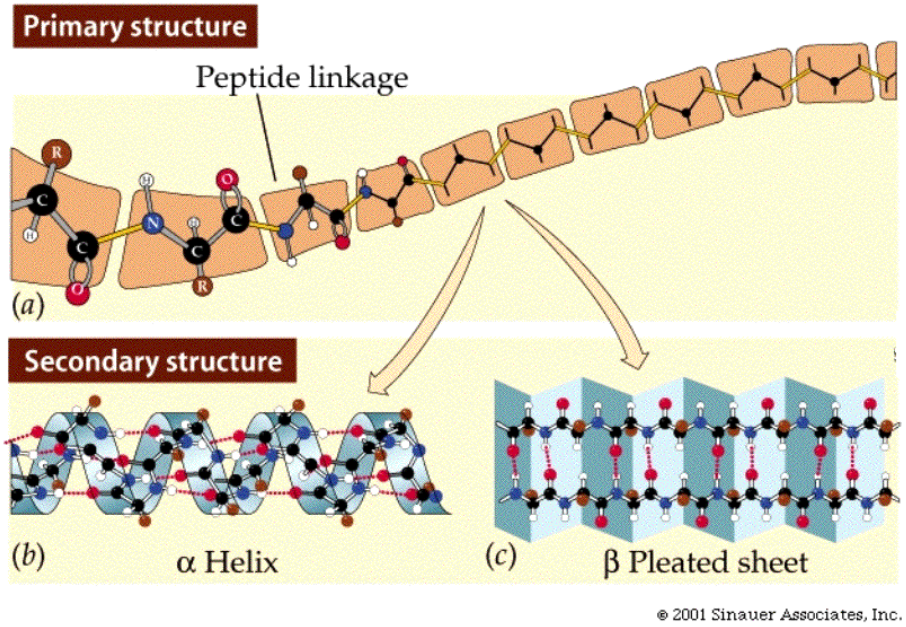


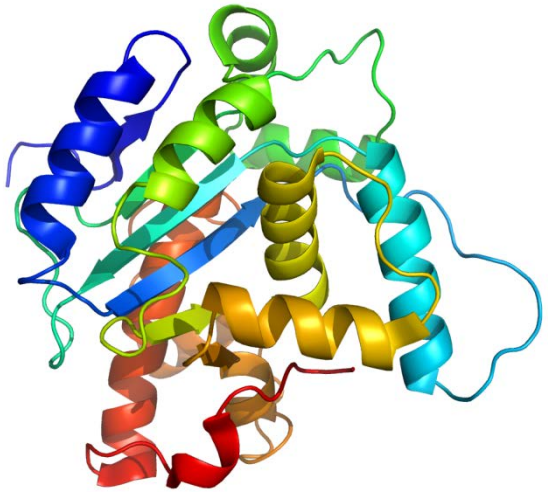
Histidine (H)
Residue Mass 137



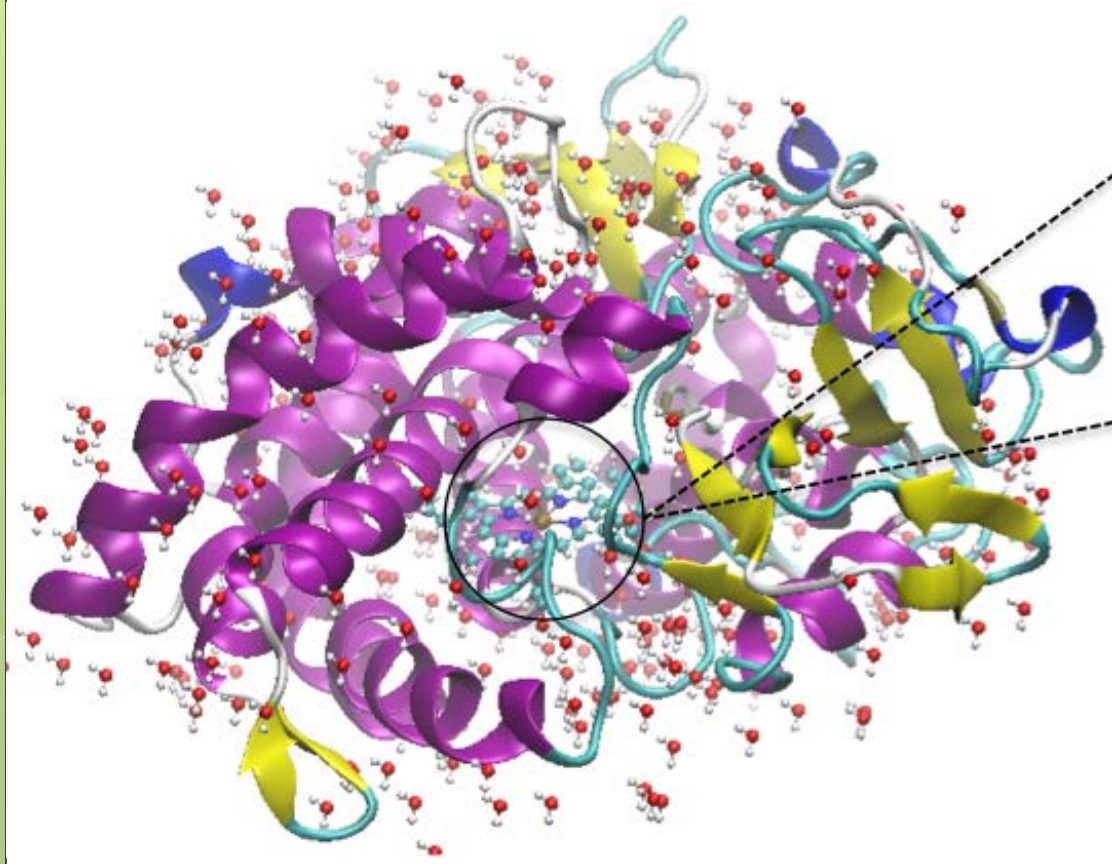


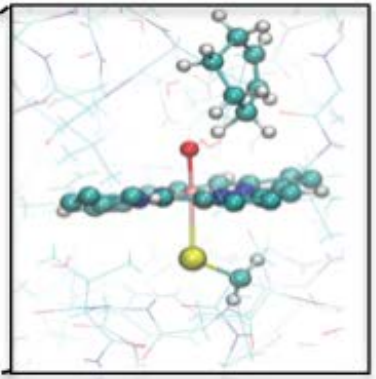
Enzymes

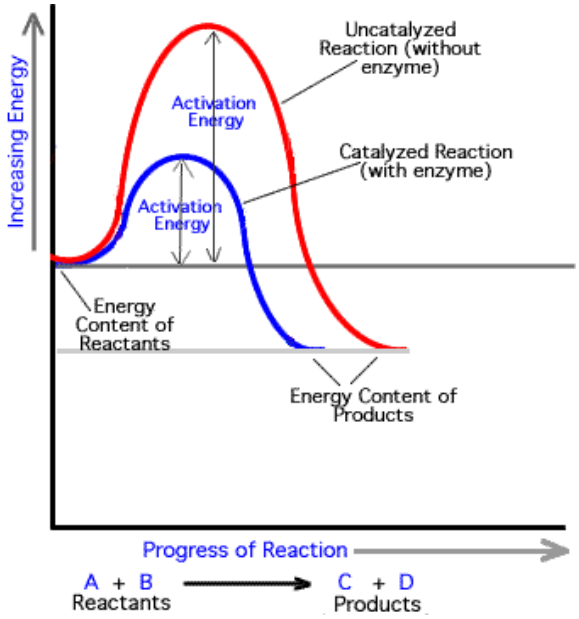




Enzymatic Reaction

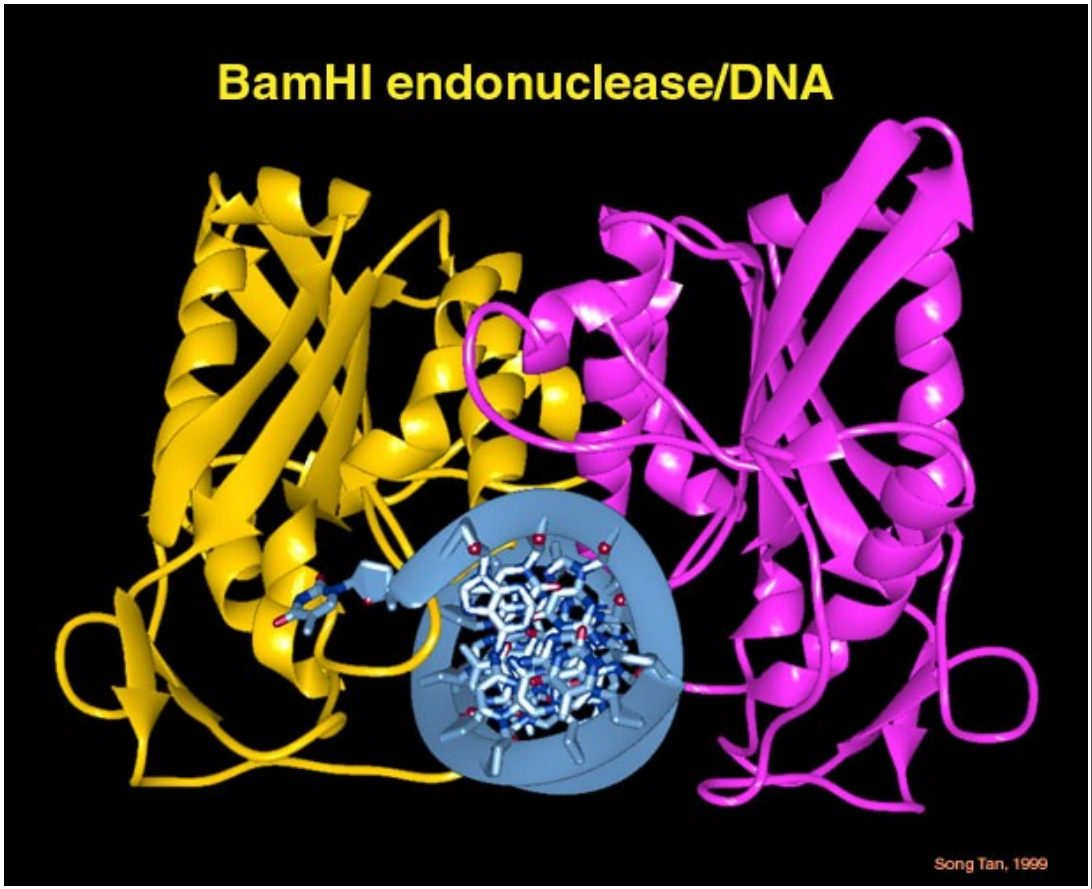




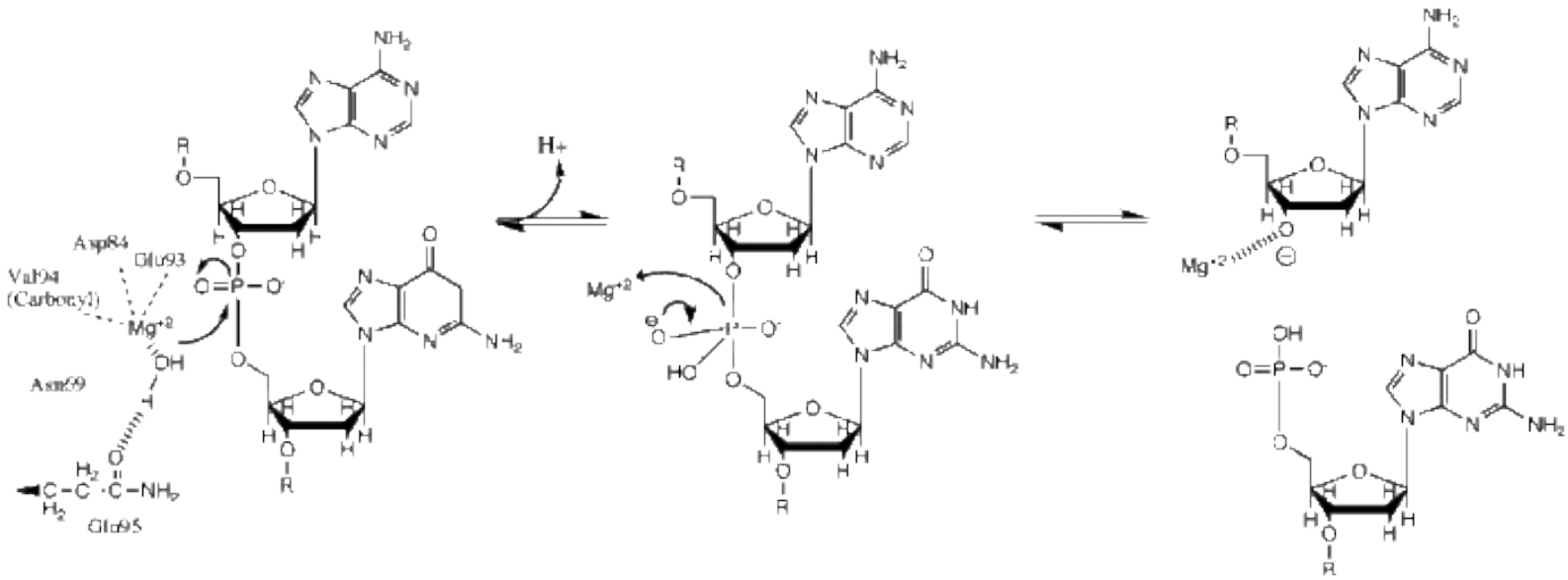


Restriction Endonucleases

- Restriction endonucleases are enzymes.
  - The reaction they catalyze is to cut (cleave) the phosphate backbone of DNA so you end up with 2 pieces when you started with one

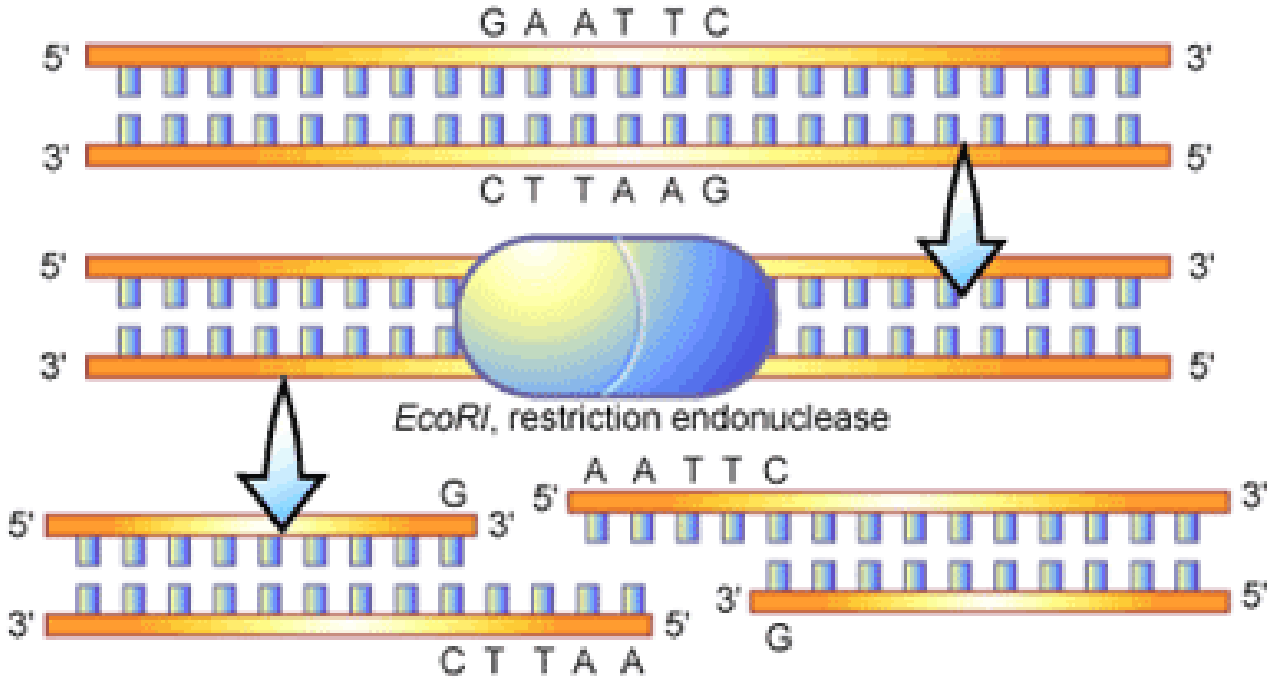


Restriction Endonuclease mechanism



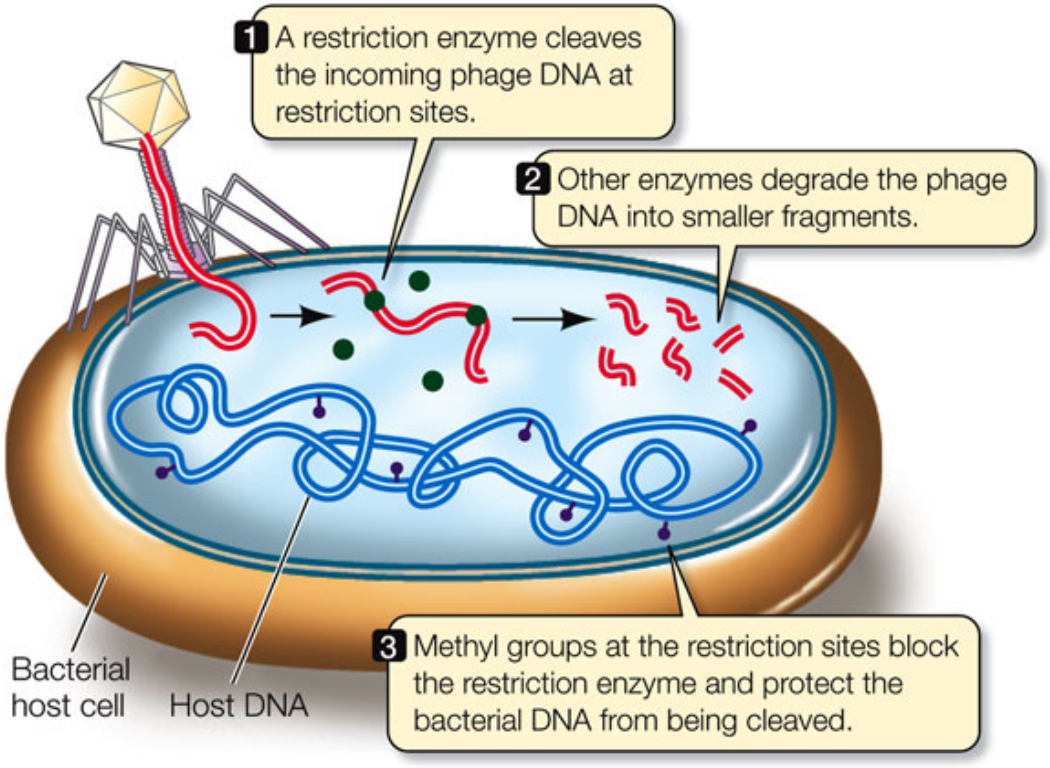
- Many Restriction Endonucleases require a co-factor (the Mg^{2+} above)
- Endonuclease means it cuts in the middle, not from the end of the DNA
- The enzymes are very accurate and can be very complete

Restriction Endonuclease binding site



- The enzyme *recognizes* a particular pattern of nucleotides
- For many enzymes the pattern reads the same on the opposite strands – a palindrome
- They enzymes cut in the neighborhood of (within or just beyond) the recognition site and can give even (blunt) ends or asymmetric ends
  - In this case the overhang could be in either direction

Why did restriction endonucleases evolve?

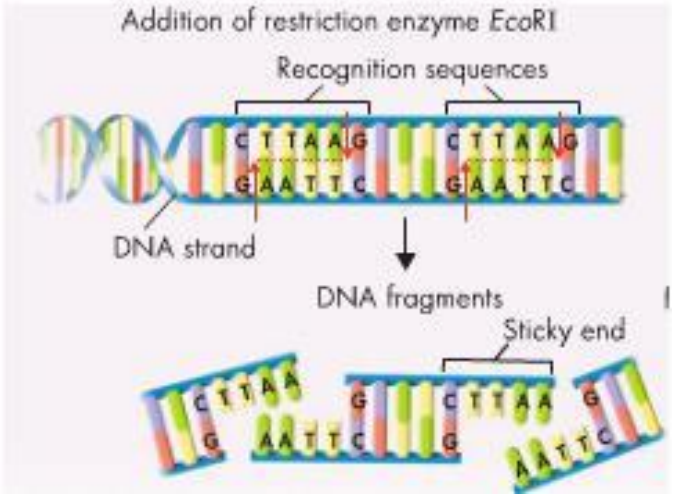


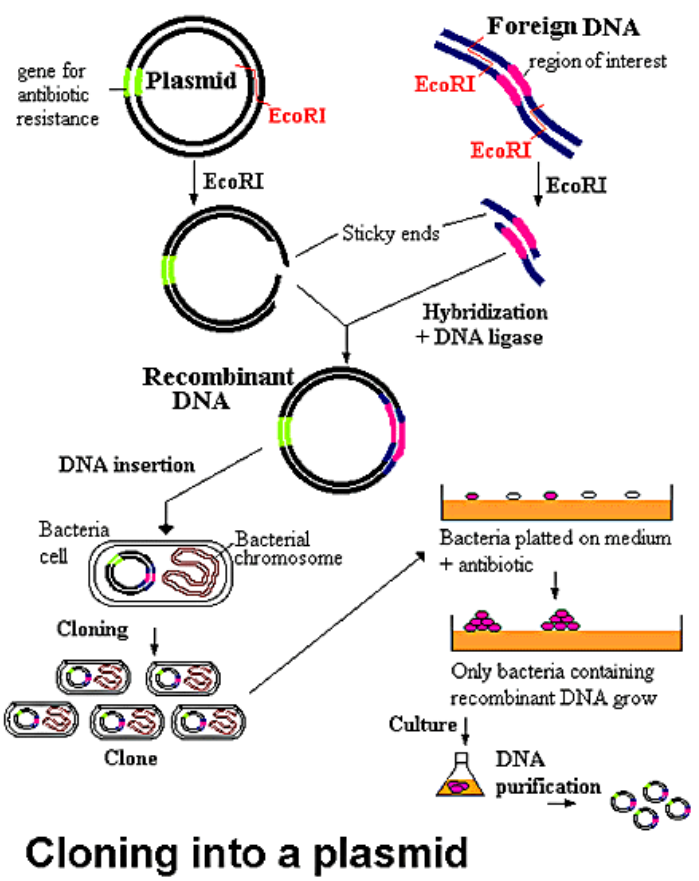
LIFE 8e, Figure 16.1

- Bacteria are also subject to viral infections (Called phage in this case)

Sticky ends are very useful in biotechnology

- Allow effective study of DNA variation for PCR and sequencing assays
- Can create recombinant products – ‘cloning’
  - Use bacteria to produce a gene of interest in large quantities
  - *Human insulin*
  - *Protein to make cheese*
  - *Enzyme to fade blue jeans*
  - *Insect resistance (potato)*
  - *Pesticide resistance (corn, soybean, cotton)*





Cloning into a plasmid

There are thousands of known REs and hundreds are available

Palindromic sequence	Name of restriction enzyme that recognizes the palindrome
GAATTC CTTAAG	*Eco*RI
AAGCTT TTCGAA	*Hind*III
CTGCAG GACGTC	*Pst*I

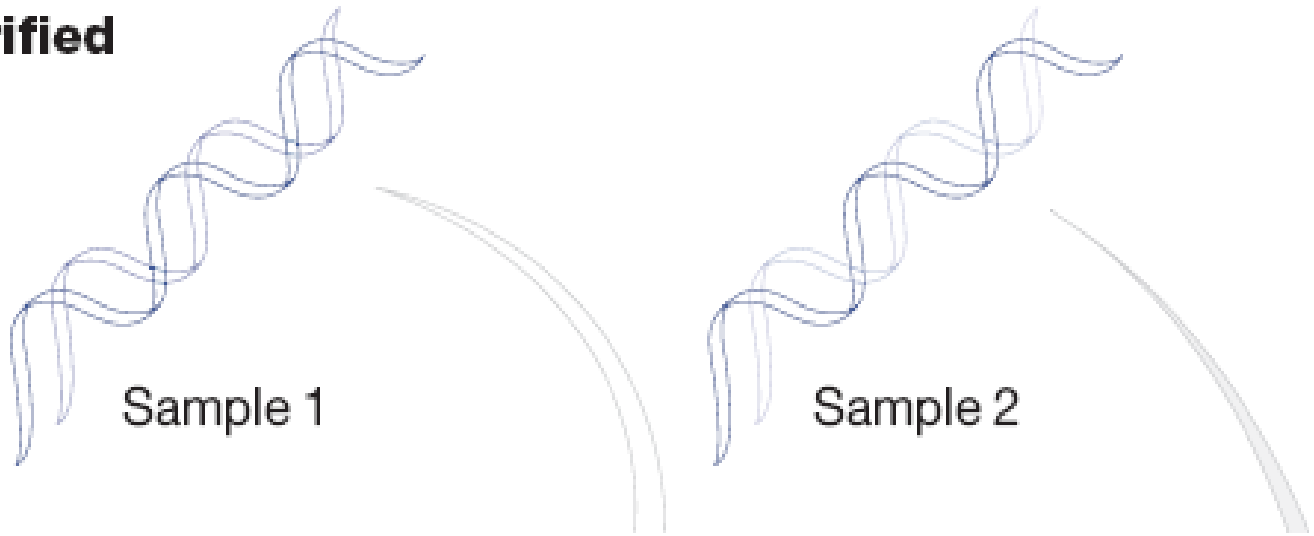
ENZYME	SEQUENCE
EcoRV	GAT/ATC
EcoRV-HF	GAT/ATC
EcoRV-HF RE-Mix	GAT/ATC
FatI	/CATG
BspDI	AT/CGAT
BspEI	T/CCGGA
BspHI	T/CATGA
PacI	TTAAT/TAA
PacI RE-Mix	TTAAT/TAA

For example, visit the New England Biolabs web site for a very large list.

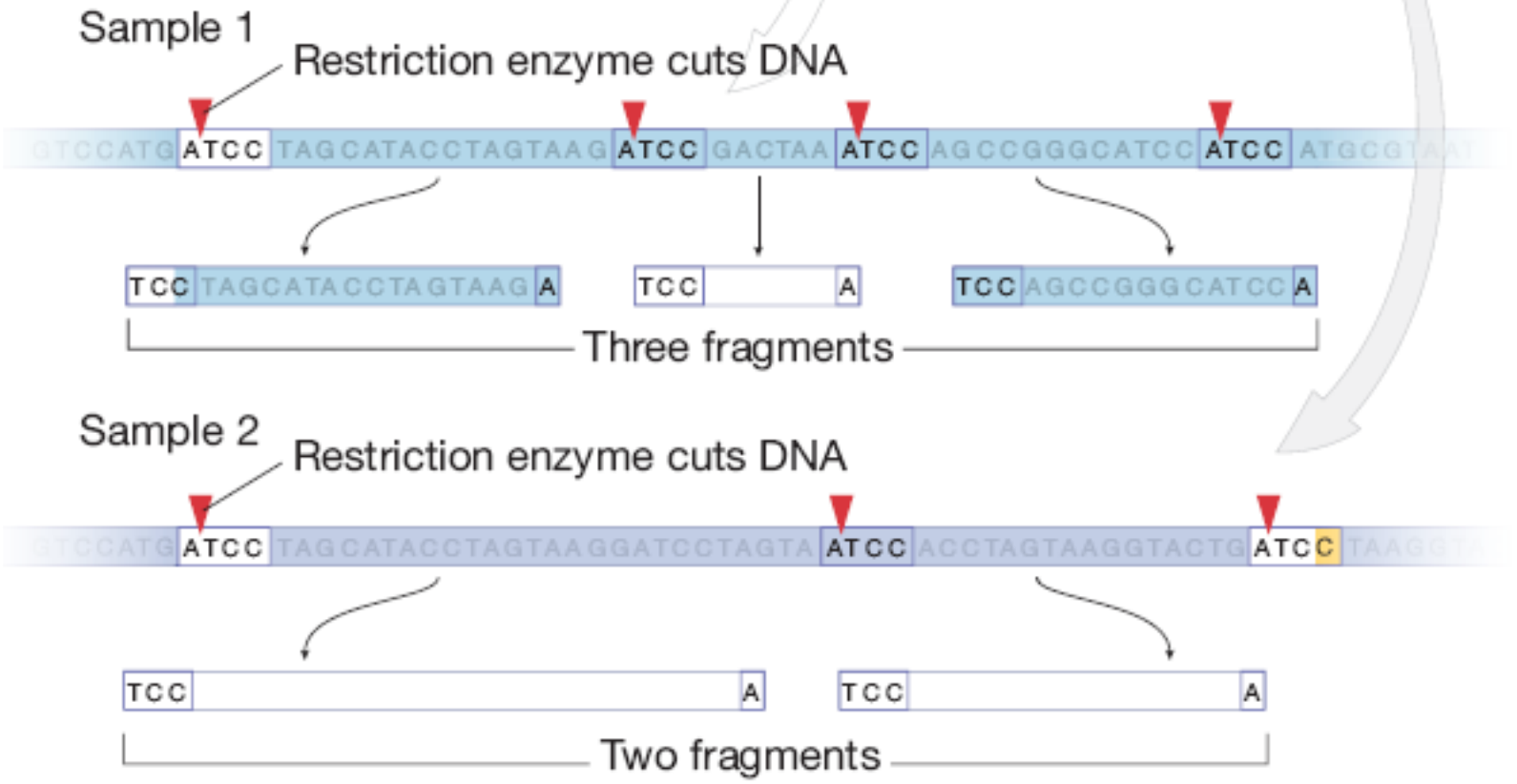
The names are derived from the bacteria in which they were found:
HinfI is from Haemophilus influenzae, I means the first purified.

Example of using Restriction Endonucleases to characterize samples

1. DNA Purified

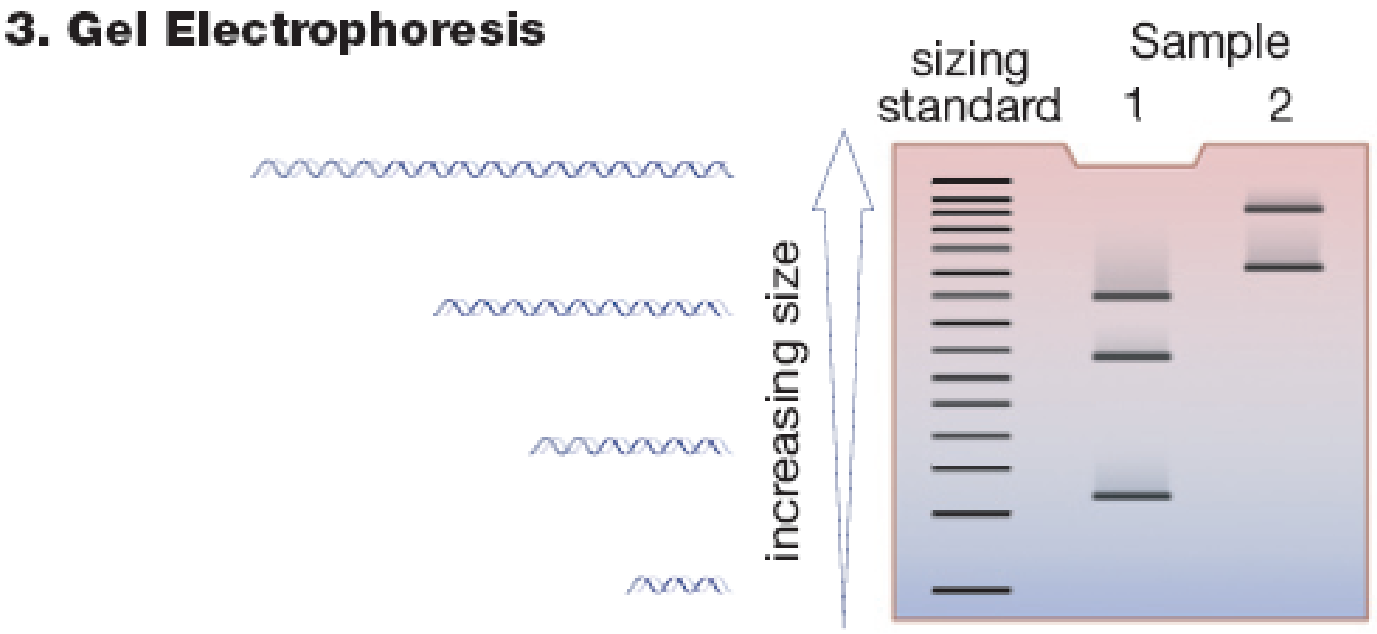


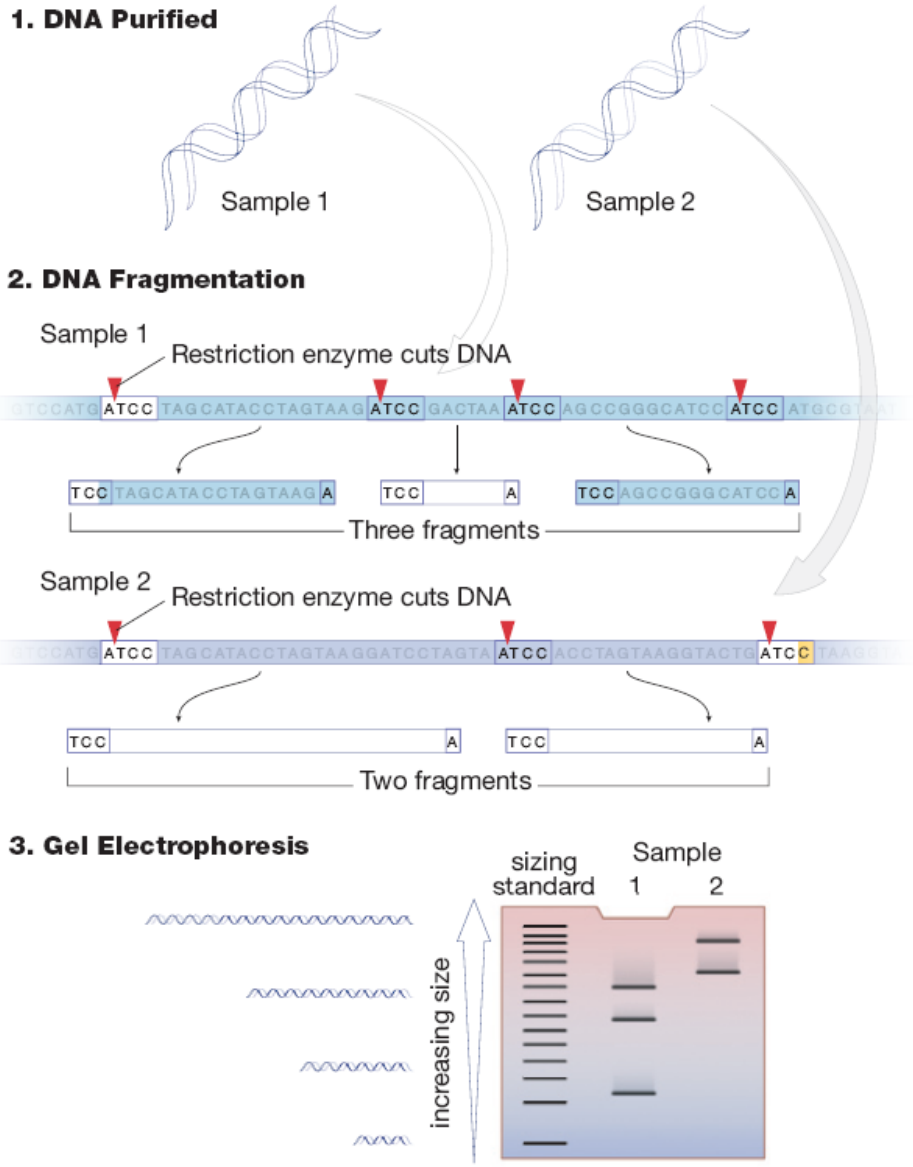
2. DNA Fragmentation



This difference in the two molecular sequence patterns is called a *polymorphism*. A nucleotide change is a sequence polymorphism, if it results in a pattern change then you could get a length polymorphism.

To detect a pattern or length polymorphism you can display the fragments on an agarose gel.




1. DNA Purified
Sample 1
Sample 2
2. DNA Fragmentation
Sample 1
Restriction enzyme cuts DNA
ATCC
TAGCATACCTAGTAAG
GACTAA
AGCCGGGCATCC
TCC
A
Three fragments
Sample 2
Restriction enzyme cuts DNA
ATCC
TAGCATACCTAGTAAGGATCCTAGTA
ACCTAGTAAGGTACTG
ATCC
TCC
A
Two fragments
3. Gel Electrophoresis
sizing standard
Sample
1
2
increasing size

Animation – restriction enzymes

- Restriction Fragment Length Polymorphisms