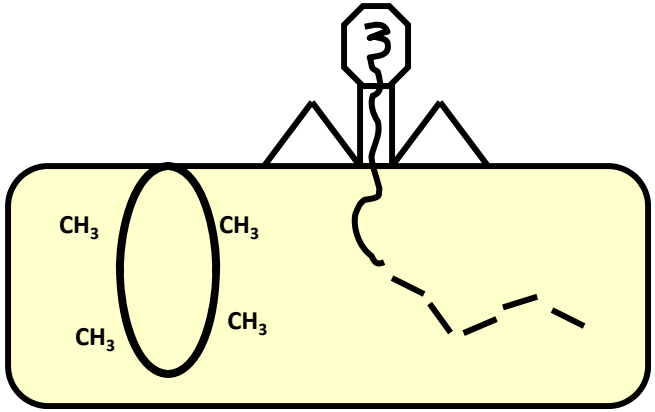
Restriction Enzymes

Biological function of restriction endonucleases is to protect cells from foreign DNA

Infecting DNA is cleaved (restricted) by the restriction enzymes, preventing it from successfully replicating and parasitizing the cell



most lab strains are completely "domesticated" (R-M systems have been inactivated)

some examples in the genotypes:

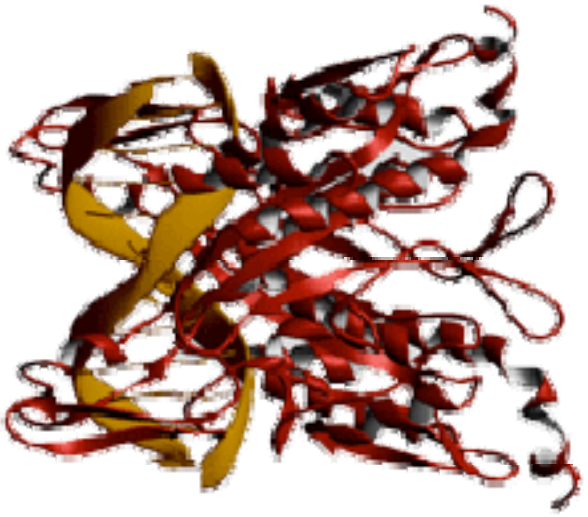
- *hsd*
- *mcr*A
- *mcr*BC

Restriction Endonucleases

- Over 10,000 bacteria and archaea have been screened for restriction enzymes

- Restriction enzymes are not confined exclusively to bacteria

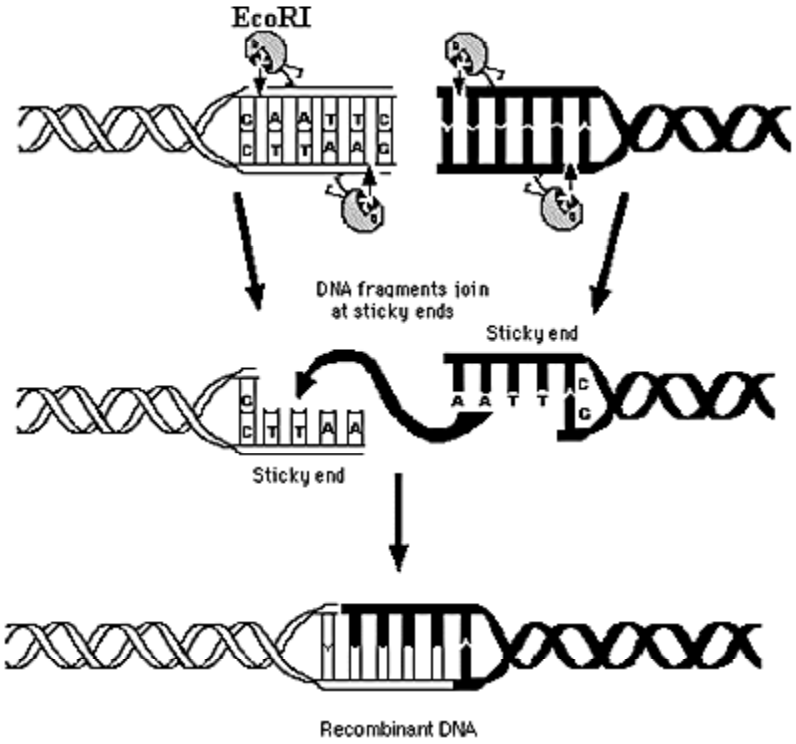
- Nearly 3,000 enzymes have been found, exhibiting over 200 different specificities (many of the 3000 are isoschizomers; different enzyme, same recognition site)



***Eco*RI binding DNA**

http://www.biophysics.pitt.edu/john_m_rosenberg.htm

Activity of a typical Restriction Enzyme



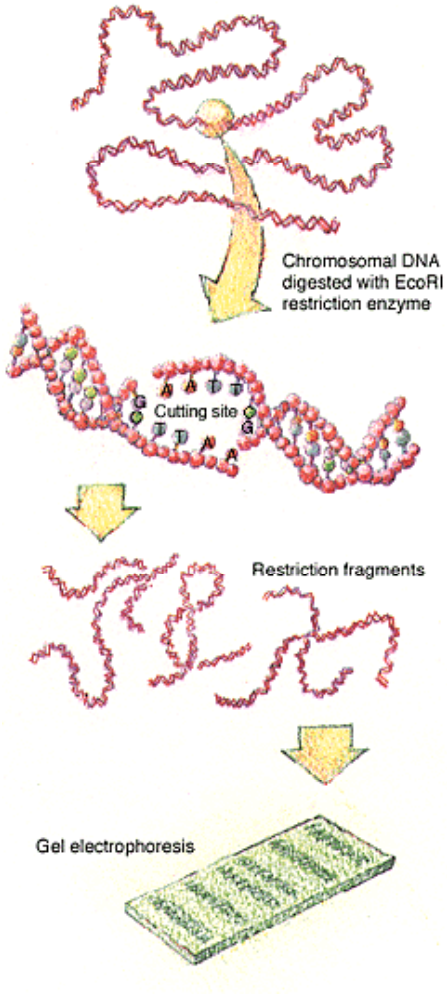
Restriction Enzyme Action of EcoRI

ends generated from a Type II restriction endonucleases

	enzyme	recognition site	end generated	
defined ends	*Pvu*II	5′CAGCTG 3′GTCGAC	5′CAG CTG 3′GTC GAC	blunt
	*Bam*HI	5′GGATCC 3′CCTAGG	5′G GATCC 3′CCTAG G	5′ overhang
	*Pst*I	5′CTGCAG 3′GACGTC	5′CTGCA G 3′G ACGTC	3′ overhang
variable ends	*Xmn*I	5′GAANNNNTTC 3′CTTNNNNAAG	5′GAANN NNTTC 3′CTTNN NNAAG	blunt
	*Ban*I	5′GGPyPuCC 3′CCPuPyGG	5′G GPyPuCC 3′CCPuPyG G	5′ overhang
	*Bst*XI	5′CCANNNNNNTGG 3′GGTNNNNNNACC	5′CCANNNNN NTGG 3′GGTN NNNNNACC	3′ overhang

Restriction Enzymes I

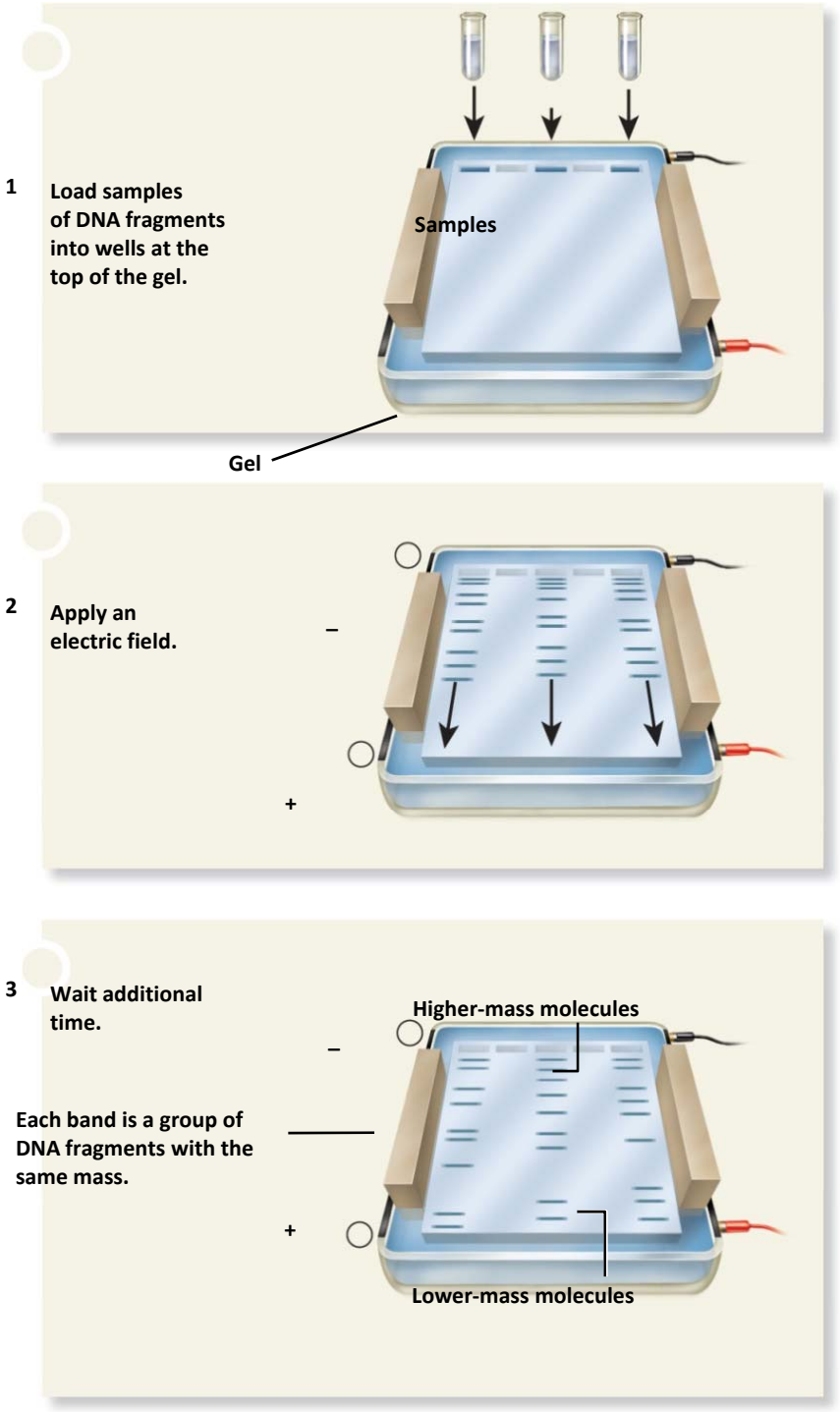
- Restriction enzymes can be used:
  - to cut DNA at specific nucleotide sequences.
    - Example – to cut chromosomes into smaller pieces for analysis by gel electrophoresis.
  - for cloning or the generation of genetic libraries.



Courtesy of U. S. Department of Energy

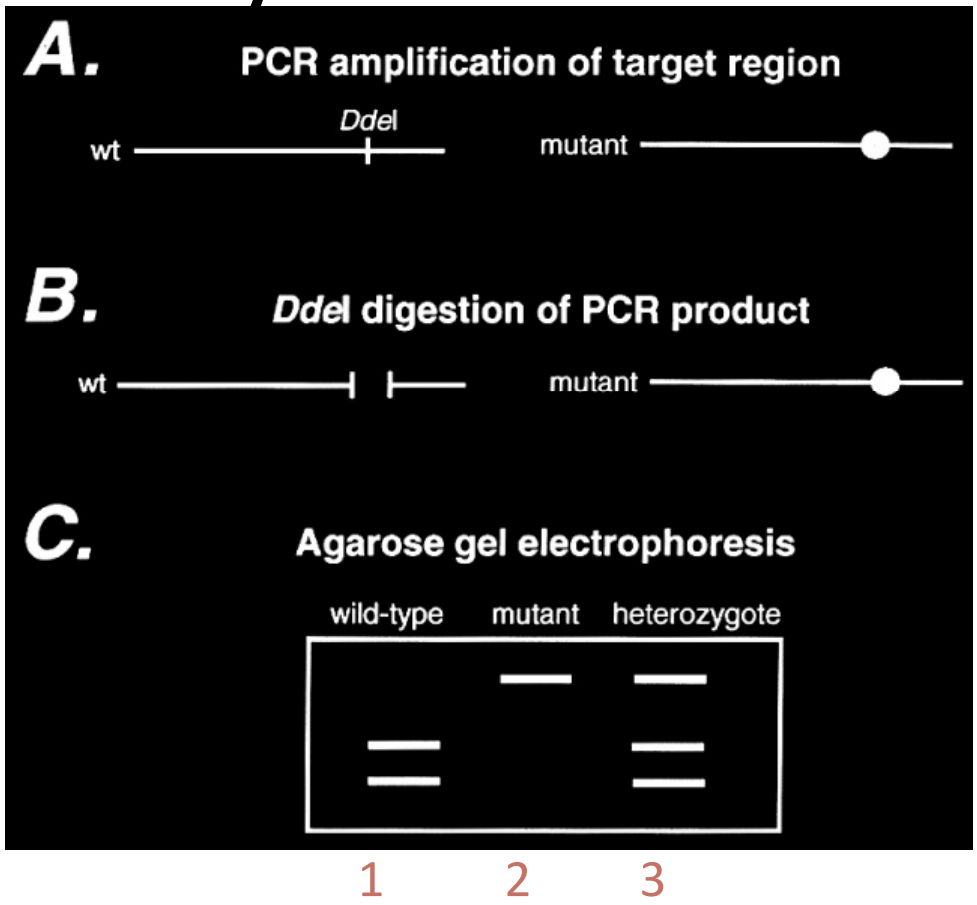
Electrophoresis

- Technique that is used to separate macromolecules, such as DNA and proteins, on a gel
- Can be used to separate molecules based on their charge, size/length, and mass



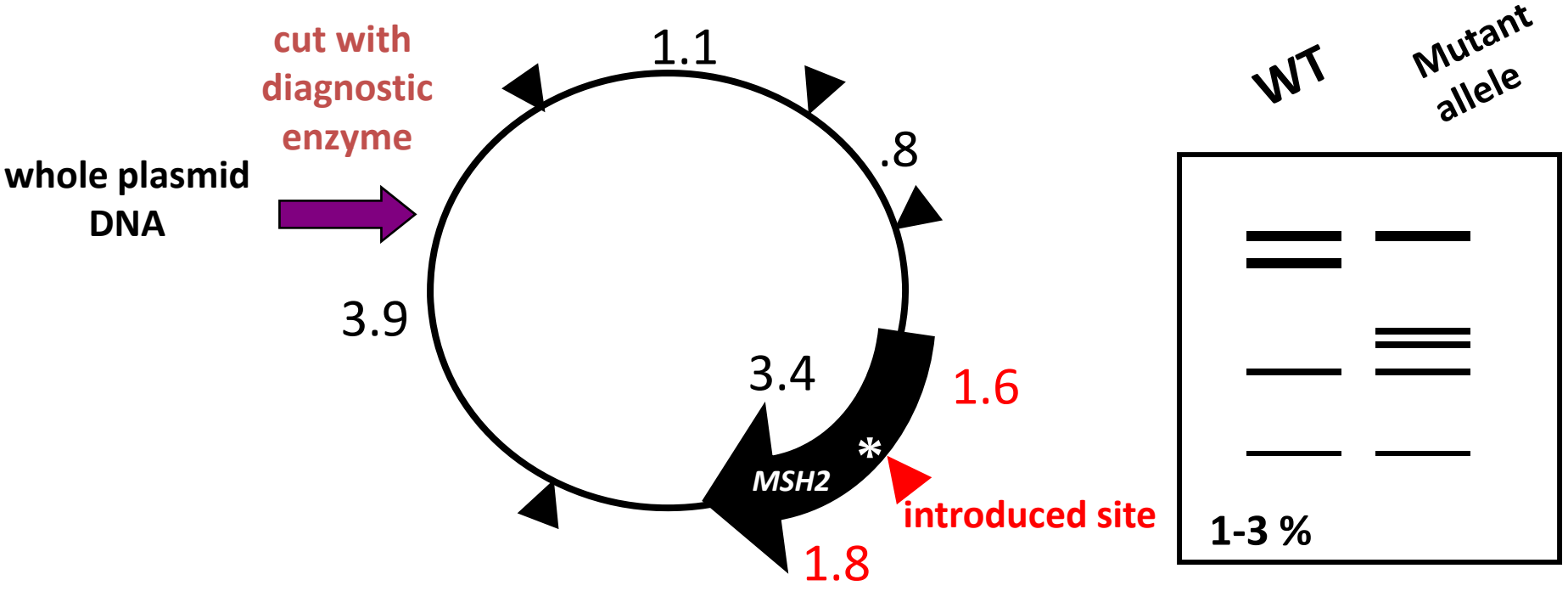
Restriction Enzymes II

- Can be used to detect mutations in DNA.
  - Example – The enzyme called DdeI can identify the mutation that causes sickle cell anemia.
  - The mutation changes the DNA sequence so that DdeI cannot cut the DNA if the mutation is present.

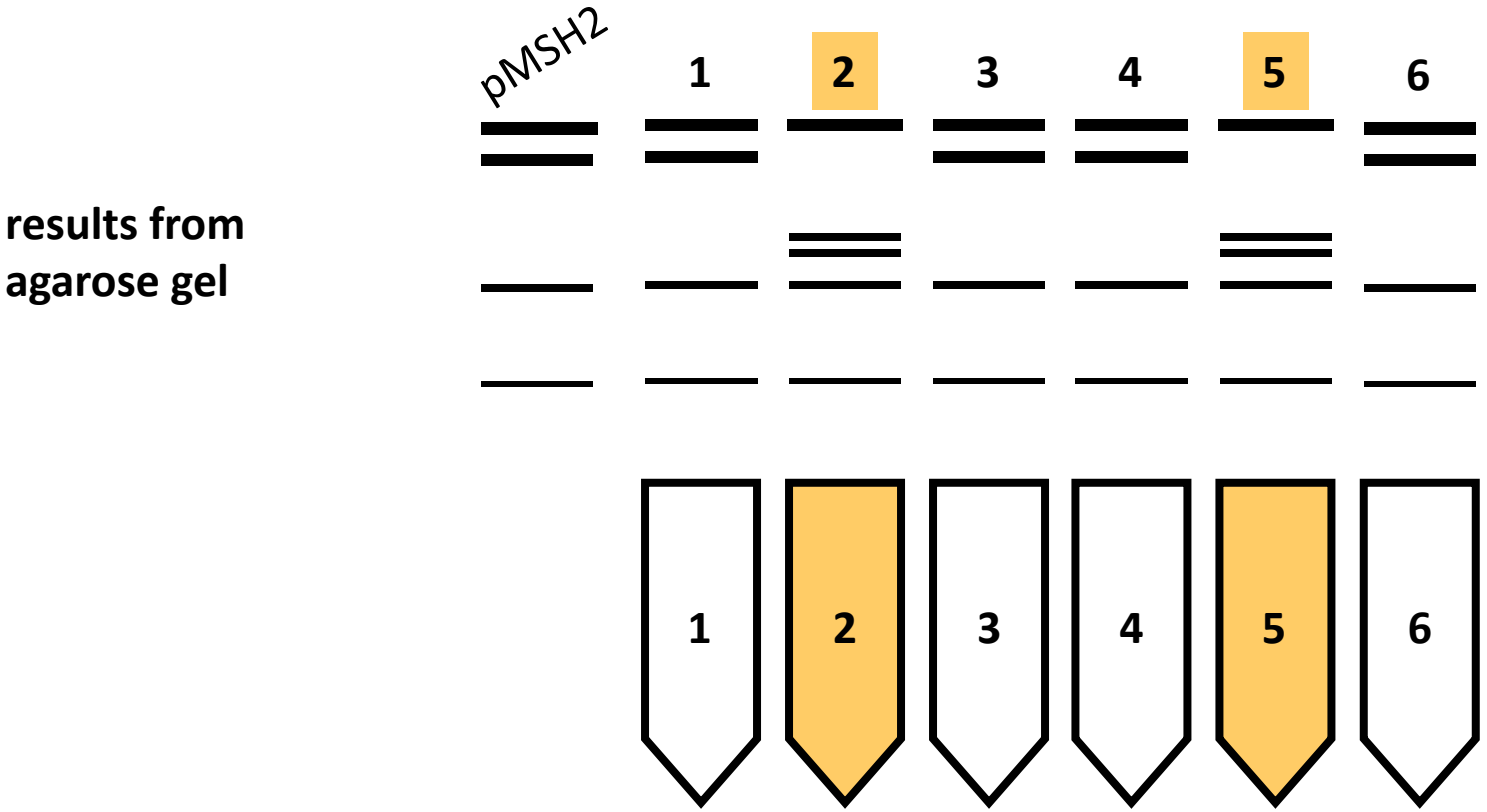


Courtesy of Alford, Rossiter, and Caskey.

We will use restriction digestion to distinguish between WT and mutagenized alleles of our pMSH2 vector



After the diagnostic gel, only proceed with a plasmid showing the altered restriction endonuclease digestion pattern (e.g. 2 or 5)



✓**Inoculate 25 ml of media with the correct bacterial colony for a midi-scale plasmid preparation (anion exchange chromatography)**

✓**Use miniprep DNA to transform yeast to begin the functional analyses**