Restriction enzymes (p 718)
Highly specific, purpose is to destroy foreign DNA, the sensitive sites in "self" being protected by methylation of cytosine and adenine. Discovered by Arber. Ham Smith, 1970, discovered HindII fr H. influenzae, cut T7 into 40 specific fragments. (Blunt ends here). Site specific. (methylation protects).

Restriction enzymes recognize unique palindromic restriction sites on DNA at which they hydrolyze the phosphodiester linkage, leaving palindromic sequences (palindrome = again or back, to run) (ABLE WAS I ERE I SAW ELBA.): sticky ends
(Construct: any series of bases, add reverse complimentary bases)

E. coli has plasmid R (p 718)  EcoRI,  G’AATTC
Hemophilus influenzae,  Hind III  A’AGCTT
Bacillus amyloliquifaciens  BamHI  G’GATCC

Endonucleases now crucial in genetic analysis and engineering.

CLONING INTO VECTORS: USING ENDONUCLEASES: (p. 723) Produce a chimera (goat, monster):
1) Purify plasmid from bacterium containing an antibiotic resistant gene. (ex: pBR 322: p 723, 375)
2) digest plasmid with endonuclease which cuts in a single point on the plasmid, not in Ab resist.
3) purify and digest genomic DNA with endonuclease which does not cut in the middle of the target gene. (To prevent self-annealing: treat with exonuclease, then terminal transferase vector with dATP. Do same with genomic, but tail with dTTP.)
4) anneal digests of the plasmid and genomic DNA
5) close the hybrid plasmid with ligase.
6) Using electroporation to inject plasmid into a host bacterium.
7) plate out the culture on medium with the antibiotic (only bacteria with plasmid will grow.)
8) Identify colonies containing target gene (ZB; with fluorescent-tagged antibodies).

DIGESTION, END LABELING:
1) cleave DNA with specific endonuclease
2) remove 5' PO₄ on cleaved end with alkaline phosphatase
3) use polynucleotide kinase to label 5' end with ³²P
4) digest longest fragment with various nucleases, analyze labeled fragments.

RESTRICITON FRAGMENT MAPPING (p. 148):
Restriction length fragment polymorphism:
1) Partial (1/50 sites) digestion of end-labeled with Alu
2) select longest fragment
3) partially digest this fragment (1/50 sites again) produce families of labeled subfragments.
4) Electrophoresis, Southern blot (p 728), autoradiography, indicates the location of the restriction sites.
5) Repeat with second restriction enz, produce DNA cleavage map.