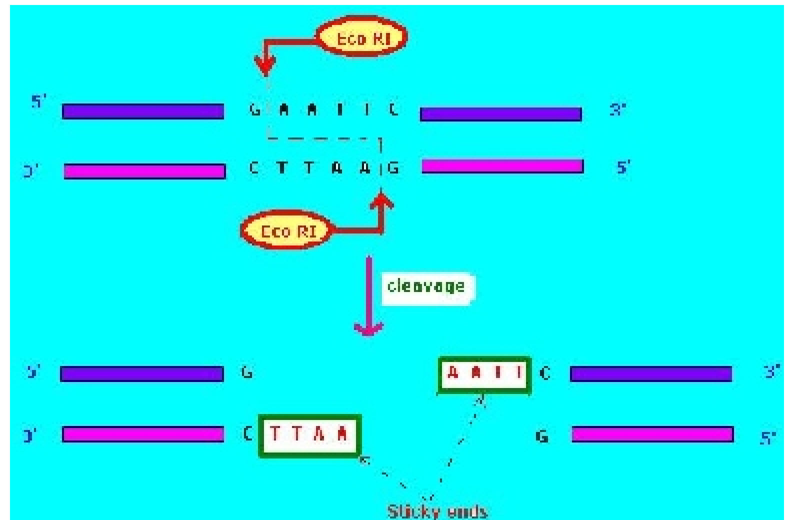
MANIPULATION OF DNA: RESTRICTION ENZYMES, CLONING

rvsd 24 Feb 1993, 23 Feb 1994, 24 Feb 95, 26 Feb 96, 23 Feb 00, 25 Feb 04, 25 Feb 05, 1 Mar 06, 29Feb08, 27Feb09, 1Mar10, 25Feb11
SGML p388-, gmslg: p. 416-434, GMSLG 7th: pp365- , 9th: pp715-755

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)

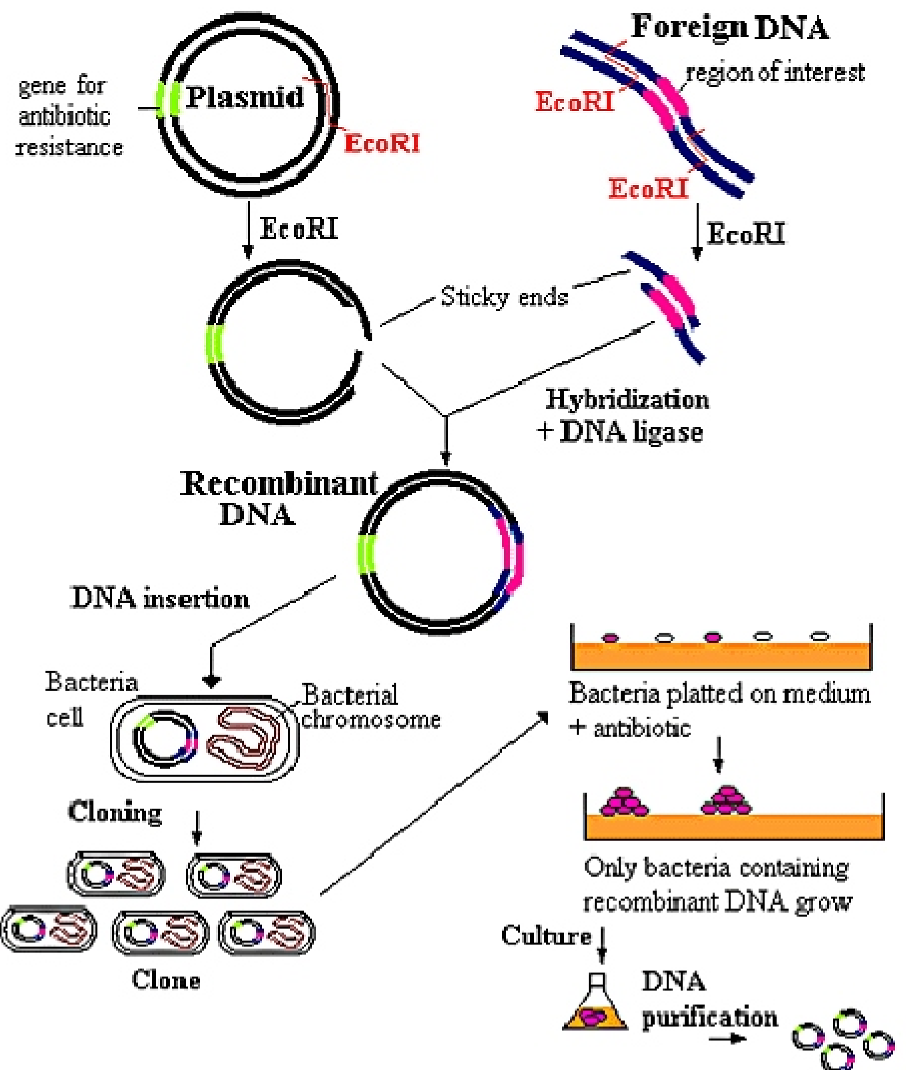
E coli has plasmid R (p 718)	EcoRI,	GˇAATTC
Hemophilus influenzae,	Hind III	AˇAGCTT
Bacillus amyloliquefaciens	*BamHI*	GˇGATCC



Endonucleases are now crucial in the fields of genetic anaylsis and engineering.

CLONING INTO VECTORS: USING ENDONUCLEASES: (p. 723) ProduceA chimera (goat, monster):

1) Purify plasmid from bacterium containin an antibiotic resistant gene.(ex: pBR 322: p 723, 375)
2) digest plasmid with endonucleas which cuts in a single point on the plasmid, not in Ab res.
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 thehybrid 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).



Cloning into a plasmid

DIGESTION, END LABELING:

1) cleave DNA with specific **endonuclease**
2) remove 5' PO_4 on cleaved end with **alkaline phosphatase**
3) use **polynucleotide kinase** to label 5' end with ^{32}P
4) digest longest fragment with various nucleases, analyze labeled fragments.

RESTRICTION 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.