Restriction enzyme

Restriction Endonucleases

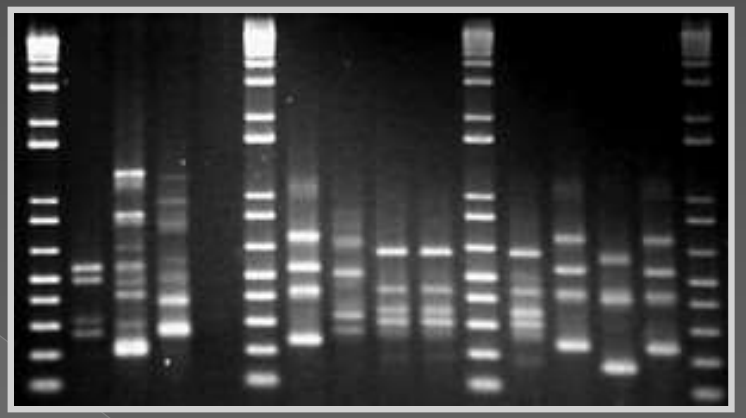
- Restriction endonucleases RESTRICT viruses
  - Viral genome is destroyed upon entry
- Restriction endonuclease = Restriction enzymes
  - Endo (inside), nuclease (cuts nucleic acid)
- Restriction endonuclease recognizes a short and specific DNA sequence and cuts it from inside.
- The specific DNA sequence is called recognition sequence

Biological Role of RE

- Restriction Modification System -restriction enzymes are paired with methylases.
- Methylases are enzymes that add methyl groups to specific nucleotides within the recognition sequence. The methylation prevents recognition by the restriction enzyme.
- Therefore, the restriction enzyme within a cell doesn't destroy its own DNA. However the restriction enzyme can destroy foreign DNA which enters the cell such as bacteriophage.

Definition

http://previews5.nvtech.com/75/tf05310/NVTech_vc063467.jpg



http://www.esemag.com/0105/dna2.jpg

- Restriction enzyme:

  A protein that recognizes a particular sequence of DNA and cuts the DNA at that site (the restriction site)

- Digestion:

  the act of breaking down into pieces

Discovery

- 1952-53: Luria and Human discovered the phenomenon of restriction and modification
- Named as host-induced, or host-controlled, variation.

Restriction enzymes

- Recognize short specific sequences, often palindromes
- Isolated from bacteria as endogenous "restrictors" of bacterial pathogens

Enzyme	Recognition Sequence
BamH I	GGATCC CCTAGG
Not I	GCGGCCGC CGCCGGCG
Sau3A I	GATC CTAG
Sac I	GAGCTC CTCGAG
Sst I	GAGCTC CTCGAG
Hinf I	GANTC CTNAG
Xho II	PuGATCPy PyCTAGPu

http://arbl.cvmbs.colostate.edu/

Enzyme Activity

Scanning

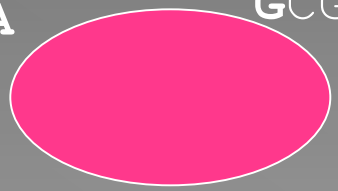
GGACGCTAGCTGAT**GAATTC**GCATCGGATCCGAATCCGCTCTTTCAA
CCTGCGATCGACTA**CTTAAG**CGTAGCCTAGGCTTAGGCGAGAAAGTT

Recognition Sequence

GGACGCTAGCTGAT**GAATTC**GCATCGGATCCGAATCCGCTCTTTCAA
CCTGCGATCGACTA**CTTAAG**CGTAGCCTAGGCTTAGGCGAGAAAGTT

Cleavage

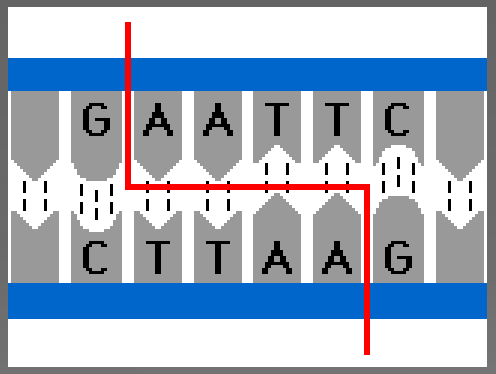
GGACGCTAGCTGAT**G**
CCTGCGATCGACTA**CTTAA**



AATTCGCATCGGATCCGAATCCGCTCTTTCAA
GCGTAGCCTAGGCTTAGGCGAGAAAGTT

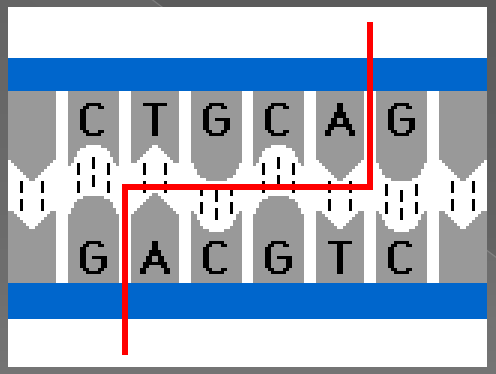
Different enzymes…different sites…different cuts

http://www.phschool.com/science/



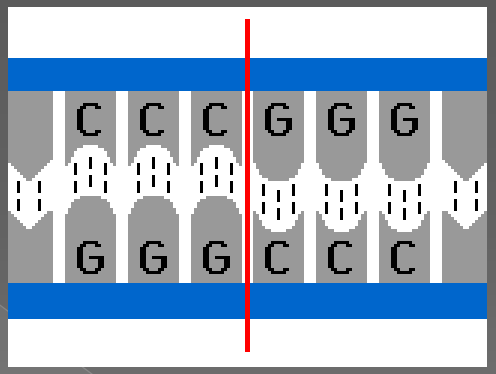
Eco RI

http://www.phschool.com/science/



Pst I

http://www.phschool.com/science/



Sma I

Diversity of Enzymes

EcoRI	*Esherichia coli* R	G/AATTC
BamHI	*Baccilu amyloliquefaciens* H	G/GATCC
HindIII	*Haemophilus influenzae* Rd	A/AGCCT
PstI	*Providencia stuartii*	CTGCA/G
PmeI	*Psuedomonas mendocina*	GTTT/AAAC

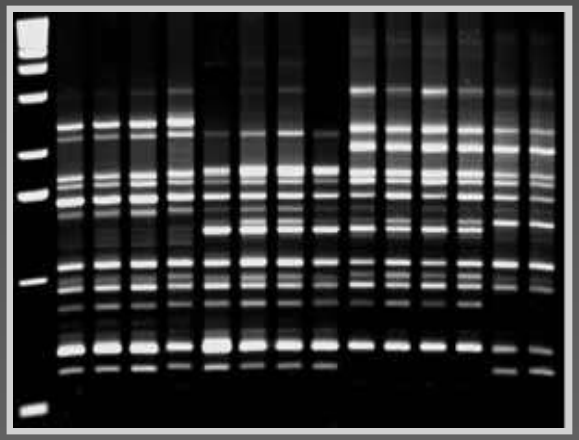
Recognition Sequences

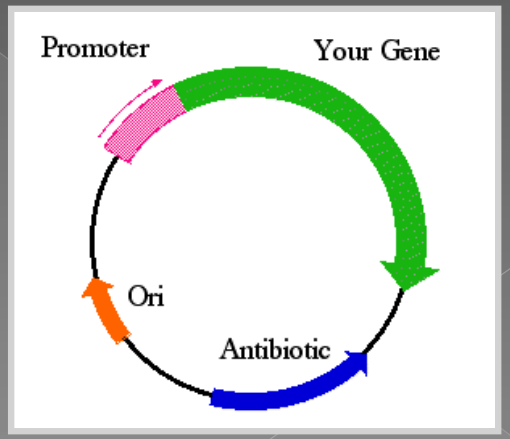
EcoRI	G/AATTC
BamHI	G/GATCC
HindIII	A/AGCCT
PstI	CTGCA/G
PmeI	GTTT/AAAC
HincII	GTY/RAC
FunII	G/AATTC

Features
Palindromic
Length
4 cutters, 6 cutters etc
Site of cleavage
Sticky ends
3' overhang
5' overhang
blunt end
Compatibility
Multiple Recognition sequence
Isoschisomers
Type II vs Type III RE

Examples

- Restriction enzyme digestion is used for…
  - Identifying individuals (DNA fingerprinting)
  - Identifying species (e.g. *Mytilus)*
  - Cloning (moving genes in and out of plasmids)





http://www.nottingham.ac.uk/biology
http://www.bio.davidson.edu/courses/genomics/method/inducepromoter.gif

Digestion Procedure

Prepare Master Mix
Add buffer and enzyme in correct proportions.

⬇

Add restriction digest master mix to DNA
Mix thoroughly by flicking tube.

⬇

Incubate
Temperature and time depend on enzyme.

⬇

View result by gel electrophoresis

Digestion Conditions

- XbaI
  - Buffer 2: (10 mM Tris-HCl, 10 mM MgCl2, 50 mM NaCl, 1 mM DTT, pH 7.9 at 25°C.
  - 100 µg/ml BSA
  - Incubate at 37°
  - 1 Unit digest 1 µg DNA in 1 hour
  - Heat inactivate 65° for 20min

Typical RE Reaction

20 µl reaction.

10 µl DNA (~1 µg total)
7 µl water
2 µl 10X reaction buffer
1 µl RE 10units/µl

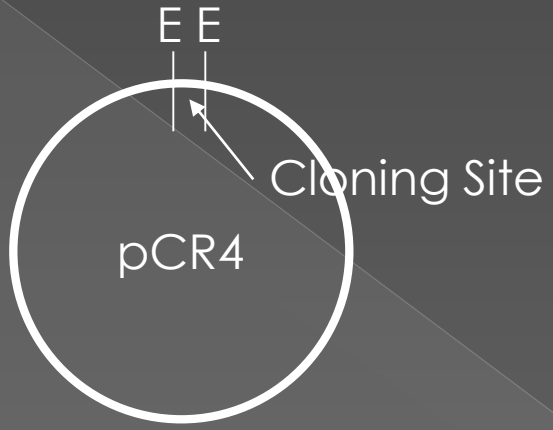
Incubate 1 hour at appropriate temperature

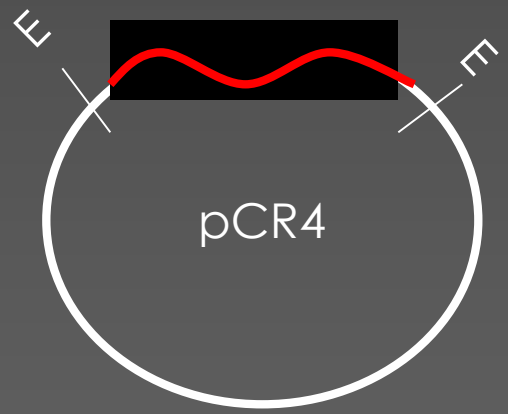
Note:

1. 10 fold excess enzyme ensures complete digestion.
2. Enzyme should never exceed 1/10th of reaction.
3. BSA is often recommended because it stabilizes the enzyme. add 0.2 µl of BSA stock for 20 µl reaction.
4. For plasmid minipreps – add 1 µl RNase the last 5 min of digestion.

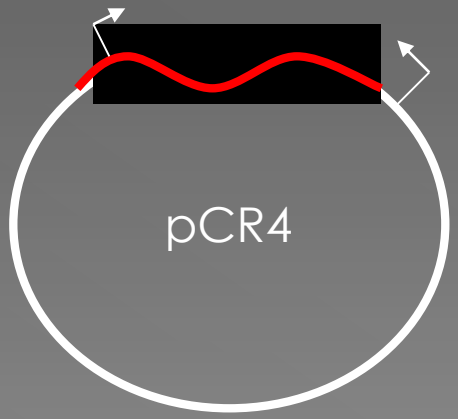
Class Project

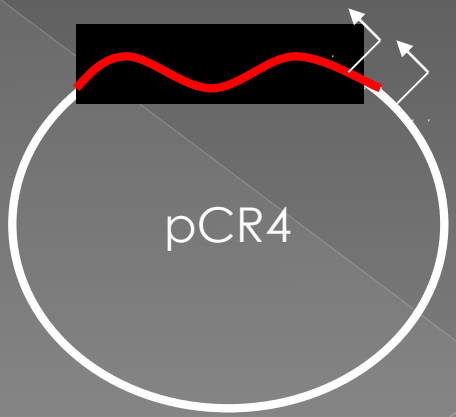
1. Digest with EcoRI to release cloned insert





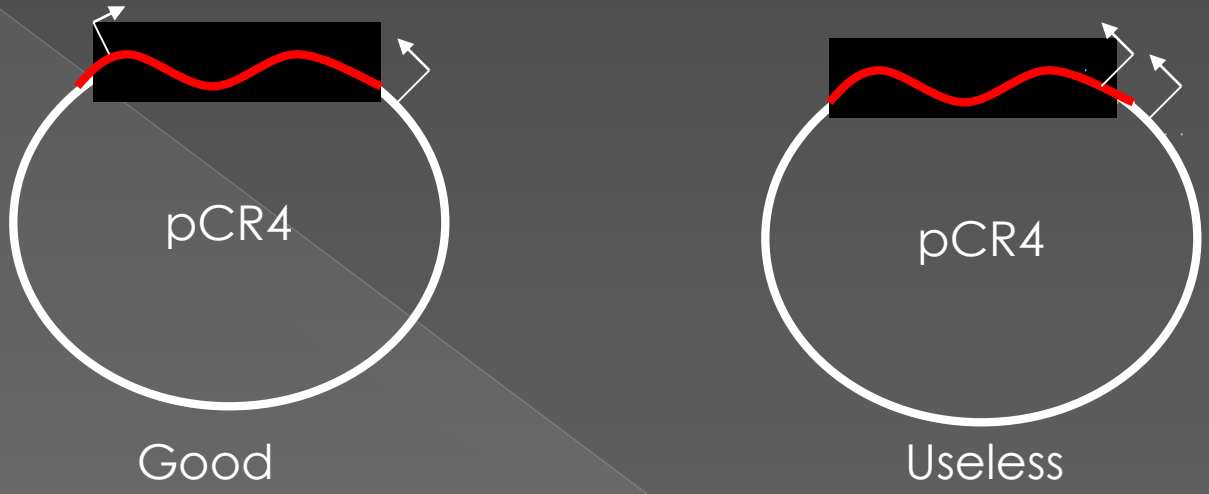
2. Orientation of Insert





Class Project

2. Orientation of Insert

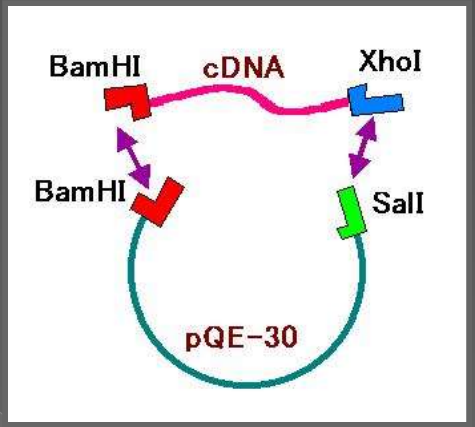


1. Identify internal restriction sites
   - Best internal sites will match one of the external sites

2. Digest with enzymes to cut internal site and external site – analyze by electrophoresis

Downstream Applications

- Ligation and…..



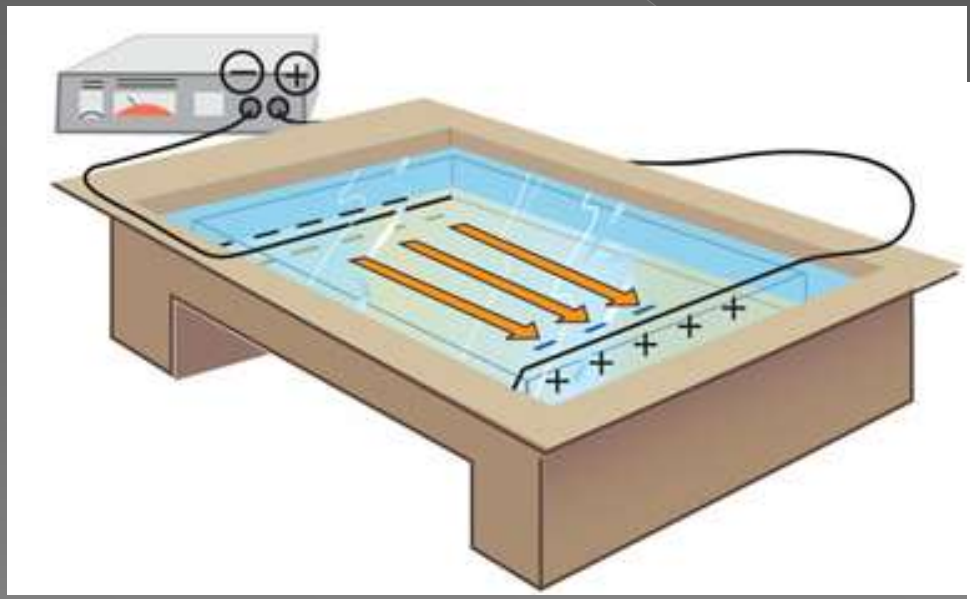
http://www.kochi-u.ac.jp/~tatataa/tech2/gene/ligation.jpg

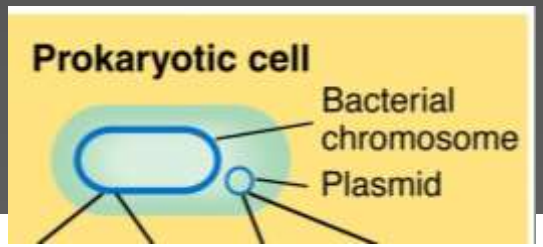
- Bacterial transformation

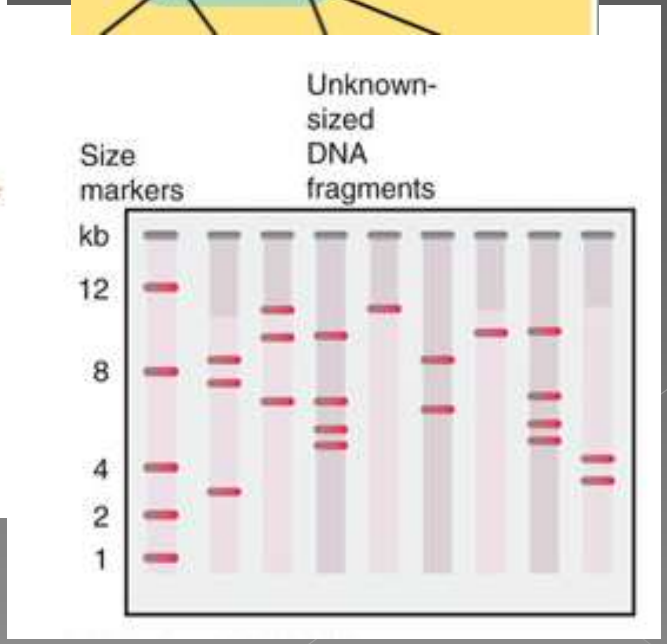
http://hthbiotech.sandiegostc.org/tr-2a-b.html

Restriction Fragment Analysis

Gel electrophoresis separates DNA fragments primarily on the basis of size/length



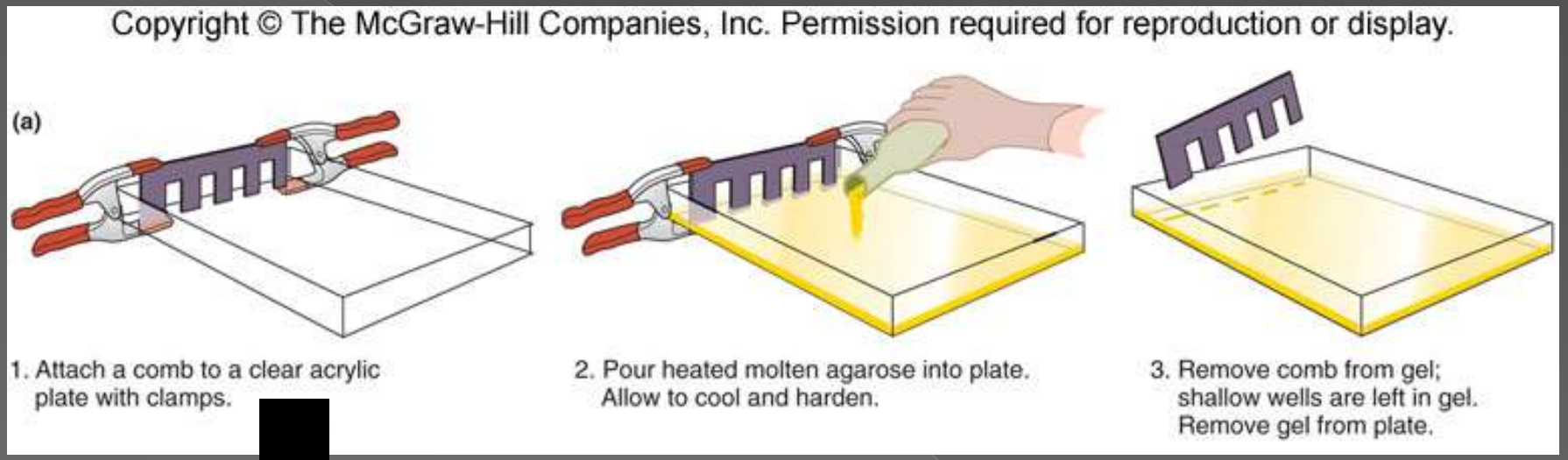




Restriction Enzyme digest of plasmid DNA

Fig. 9.5.a

DNA is electrophoresed through a polymer



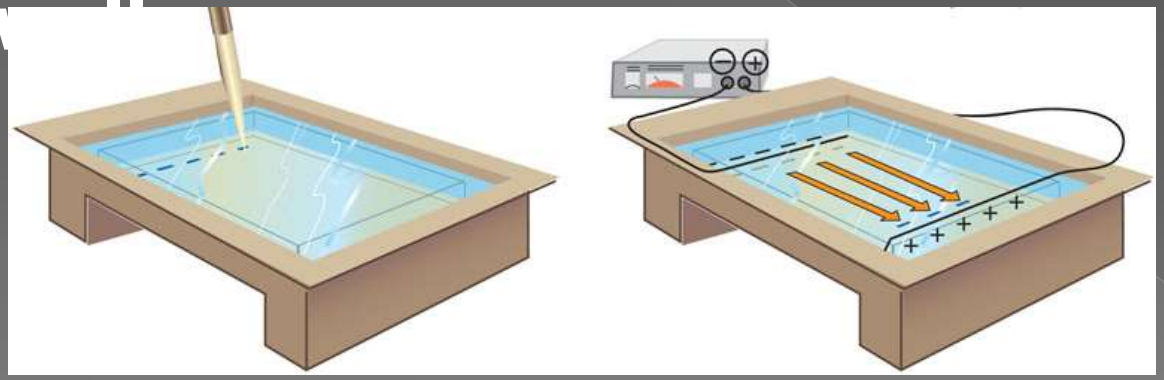
Agarose vs. Acrylamide Gels

Agarose: larger migration space for DNA

Polyacrylamide: smaller migration space for DNA

Fig. 9.5.a

- DNA is loaded (pipetted) into the wells of the gel
- Sucrose or glycerol provide density so the DNA sample sinks into the wells of the submerged gel
- A dye helps to see the sample fall into the



DNA is negatively charged and migrates at a rate relative to its size/length

Fig. 9.5.b.2

Anatomy of a DNA Ge

Ethidium Bromide is a dye that intercalates with DNA and fluoresces upon UV exposure

Size markers assist in determining fragment length

UV Light

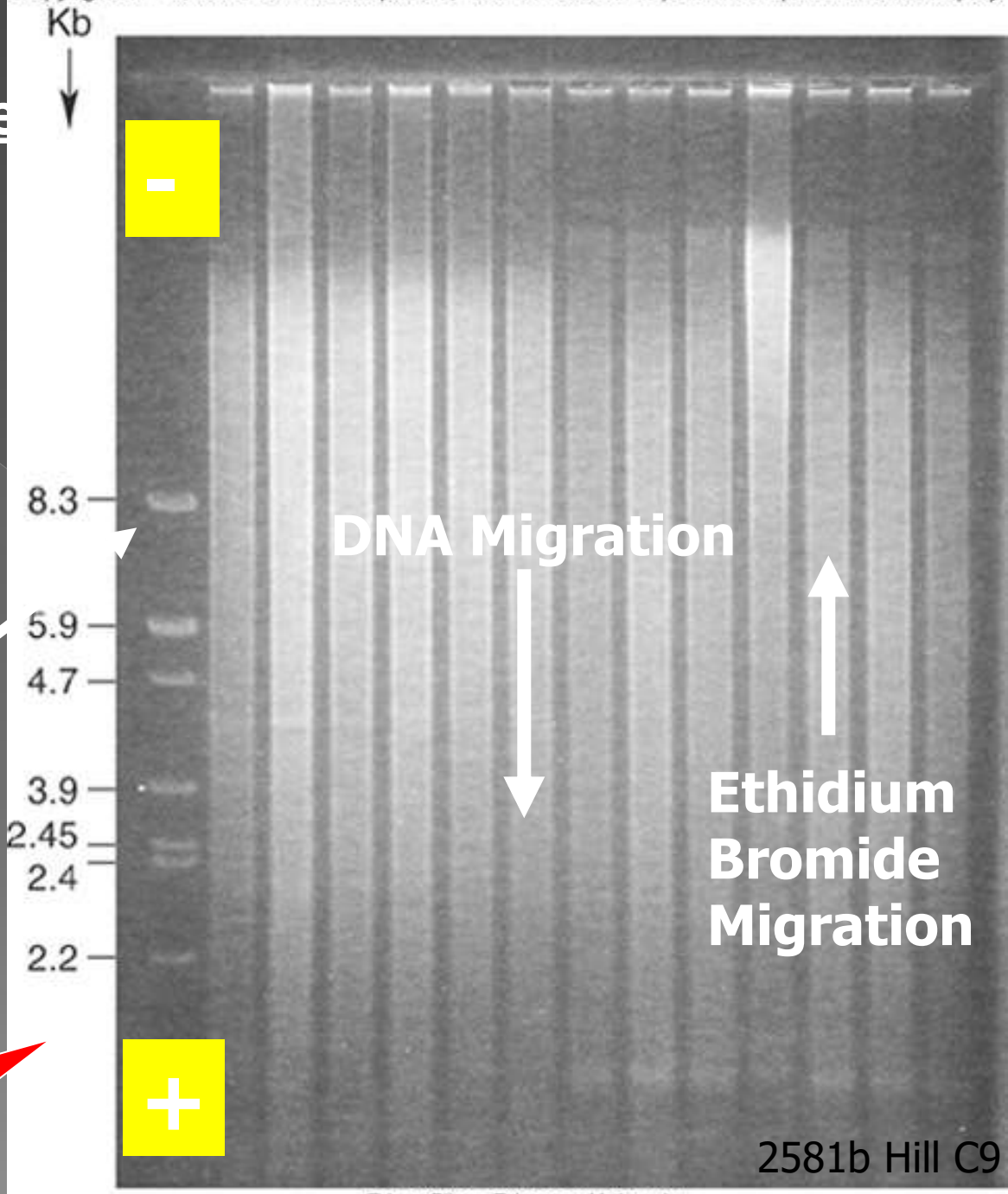
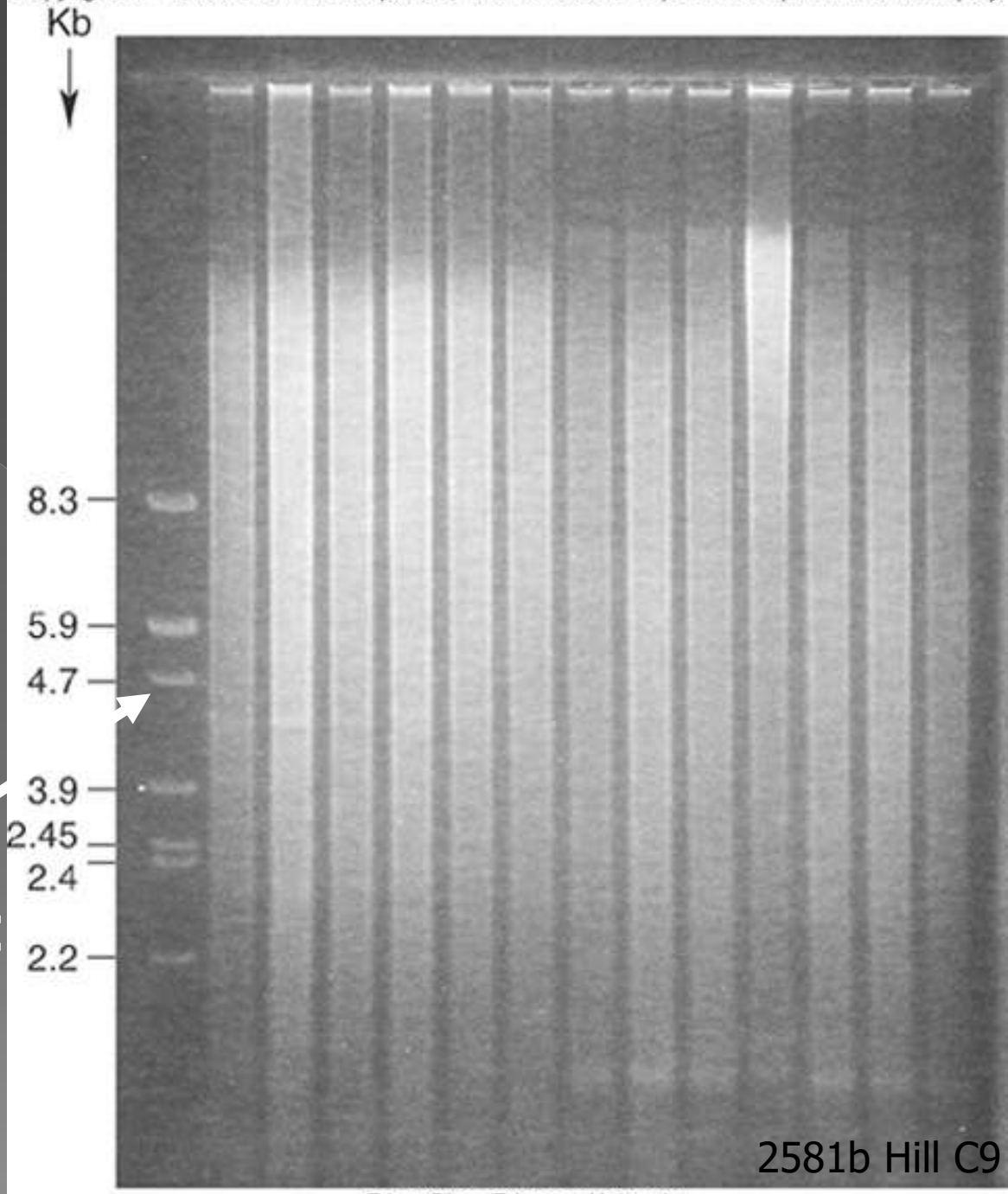


Fig. 9.5.b.2

Genomic DNA after Digestion

Cutting a complex DNA sample with a frequent cutter results in a smear

Size markers assist in determining fragment length



Nomenclature

- Smith and Nathans (1973) proposed enzyme naming scheme
  - three-letter acronym for each enzyme derived from the source organism
  - First letter from genus
  - Next two letters represent species
  - Additional letter or number represent the strain or serotypes
- For example. the enzyme *Hind*II was isolated from *Haemophilus influenzae* serotype d.

Few Restriction Enzymes

Enzyme	Organism from which derived	Target sequence (cut at *) 5' -->3'
Bam HI	*Bacillus amyloliquefaciens*	G* G A T C C
Eco RI	*Escherichia coli RY 13*	G* A A T T C
Hind III	*Haemophilus inflenzae Rd*	A* A G C T T
Mbo I	*Moraxella bovis*	*G A T C
Pst I	*Providencia stuartii*	C T G C A * G
Sma I	*Serratia marcescens*	C C C * G G G
Taq I	*Thermophilus aquaticus*	T * C G A
Xma I	*Xanthamonas malvacearum*	C * C C G G G

Classification

- Synonymous to Restriction Endonuclease
- Endonuclease: Cut DNA from inside
- Highly heterogeneous
- Evolved independently rather than diverging form a common ancestor
- Broadly classified into four Types

R-M System

- Restriction-modification (R-M) system
  - Endonuclease activity: cuts foreign DNA at the recognition site
  - Methyltransferase activity: protects host DNA from cleavage by the restriction enzyme.
  - Methyleate one of the bases in each strand
- Restriction enzyme and its cognate modification system constitute the R-M system

Protection of Self DNA

- Bacteria protect their self DNA from restriction digestion by methylation of its recognition site.
- Methylation is adding a methyl group (CH_3) to DNA.
- Restriction enzymes are classified based on recognition sequence and methylation pattern.

Type I

- Multi-subunit proteins
- Function as a single protein complex
- Contain
  - two R (restriction) subunits,
  - two M (methylation) subunits and
  - one S (specificity) subunit
- Cleave DNA at random length from recognition site

Type III

- Large enzymes
- Combination restriction-and-modification
- Cleave outside of their recognition sequences
- Require two recognition sequences in opposite orientations within the same DNA molecule
- No commercial use or availability

Type IV

- Cleave only modified DNA (methylated, hydroxymethylated and glucosyl-hydroxymethylated bases).
- Recognition sequences have not been well defined
- Cleavage takes place ~30 bp away from one of the sites.
- Sequence similarity suggests many such systems in other bacteria and archaea.

Type II

- Most useful for gene analysis and cloning
- More than 3500 REs
- Recognize 4-8 bp sequences
- Need Mg^{2+} as cofactor
- Cut in close proximity of the recognition site
- Homodimers
- ATP hydrolysis is not required

Recognition Sequences

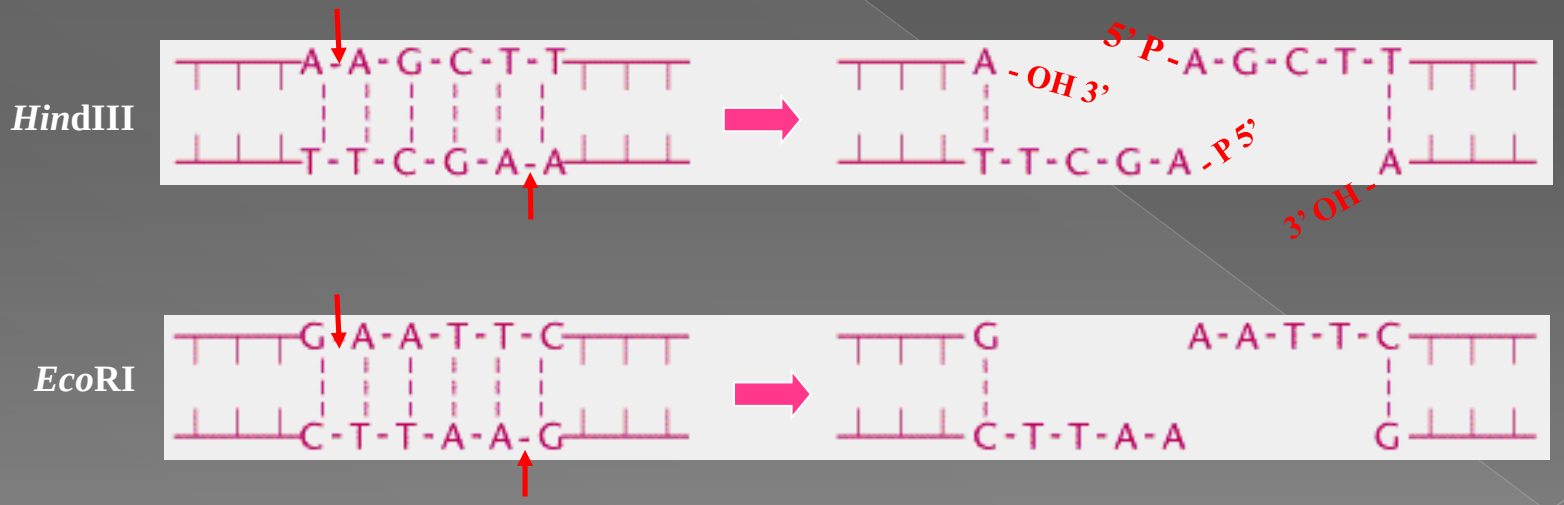
- Each restriction enzyme always cuts at the same recognition sequence.
- Produce the same gel banding pattern (fingerprint)
- Many restriction sequences are palindromic. For example,

5' GAATTC 3'
3' CTTAAG 5'

(Read the same in the opposite direction (eg. madam, race car…)

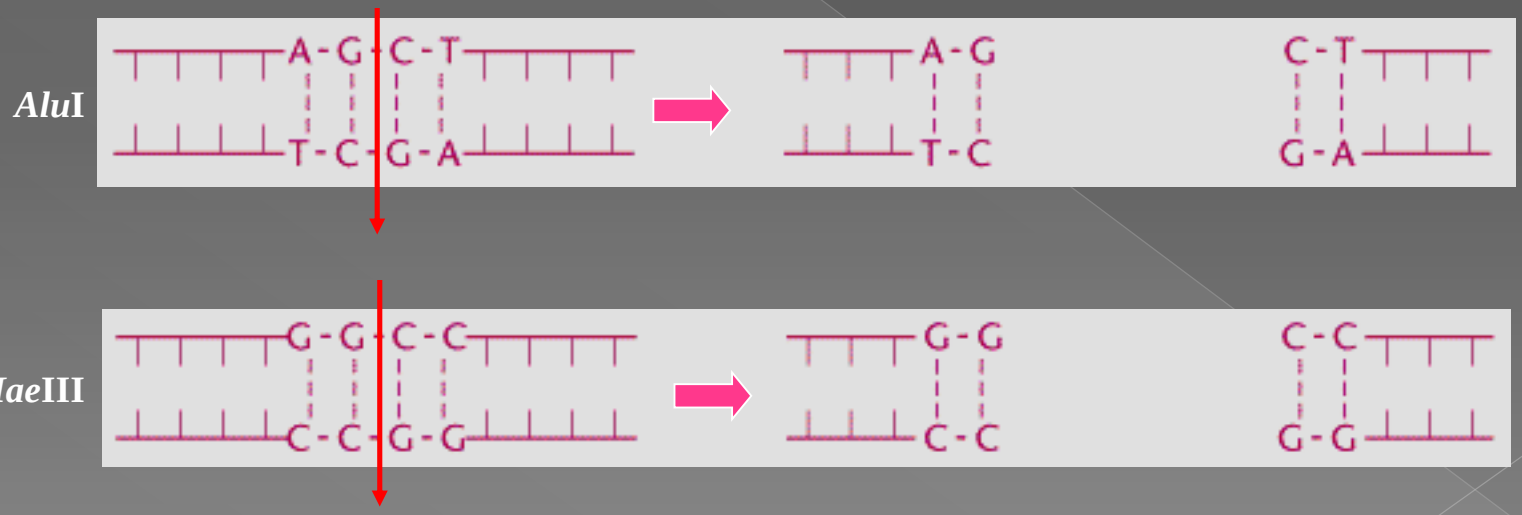
Sticky End Cutters

- Most restriction enzymes make staggered cuts
- Staggered cuts produce single stranded "sticky-ends"
- DNA from different sources can be spliced easily because of sticky-end overhangs.



Blunt End Cutters

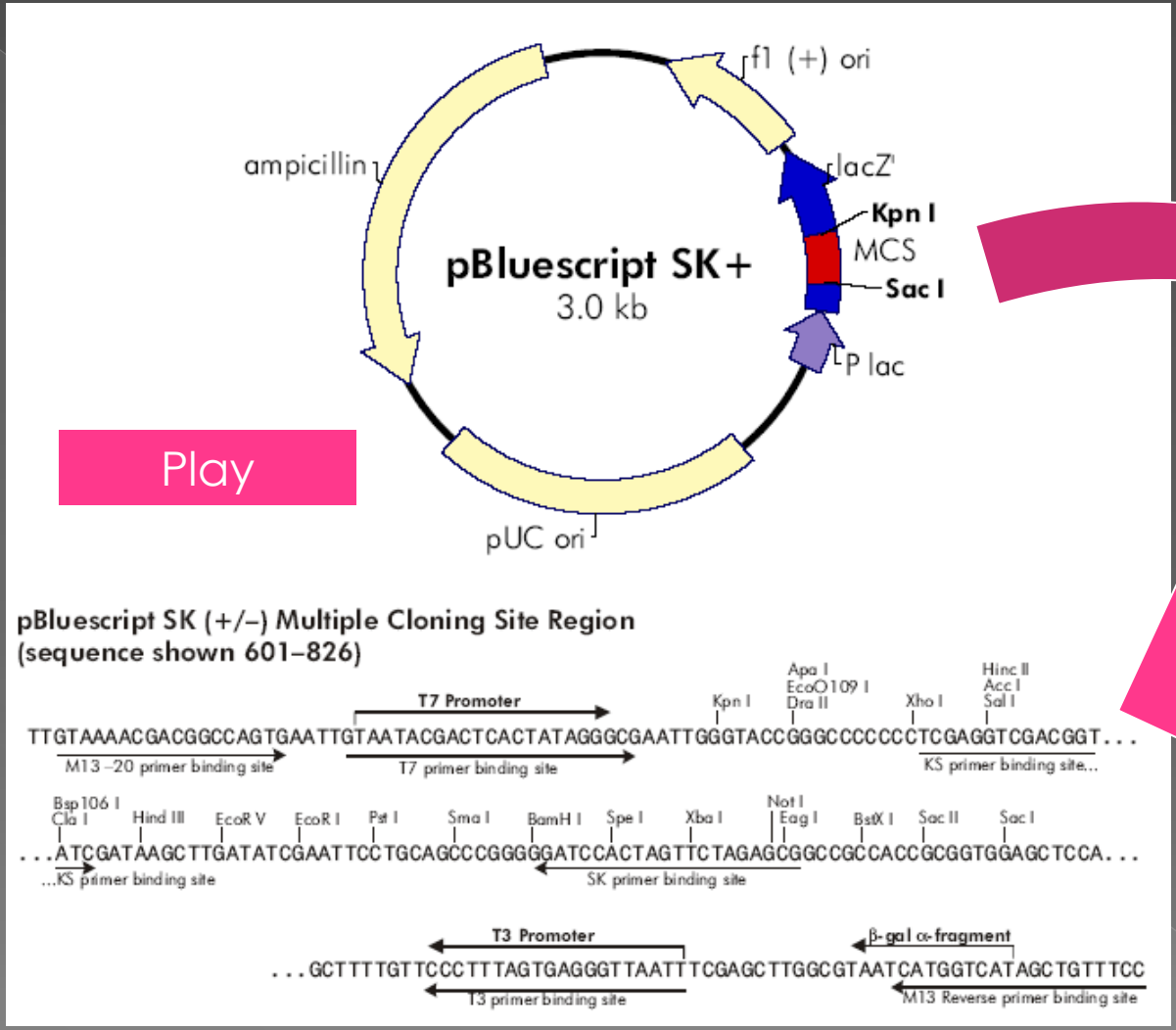
- Some restriction enzymes cut DNA at opposite base
- They leave blunt ended DNA fragments
- These are called blunt end cutters



Restriction Enzyme Use

- Discovery of enzymes that cut and paste DNA make genetic engineering possible.
- Restriction enzyme cuts DNA and generates fragments
- Ligase joins different DNA fragments
- DNA fragments from different species can be ligated (joined) to create Recombinant DNA

Cloning Vectors

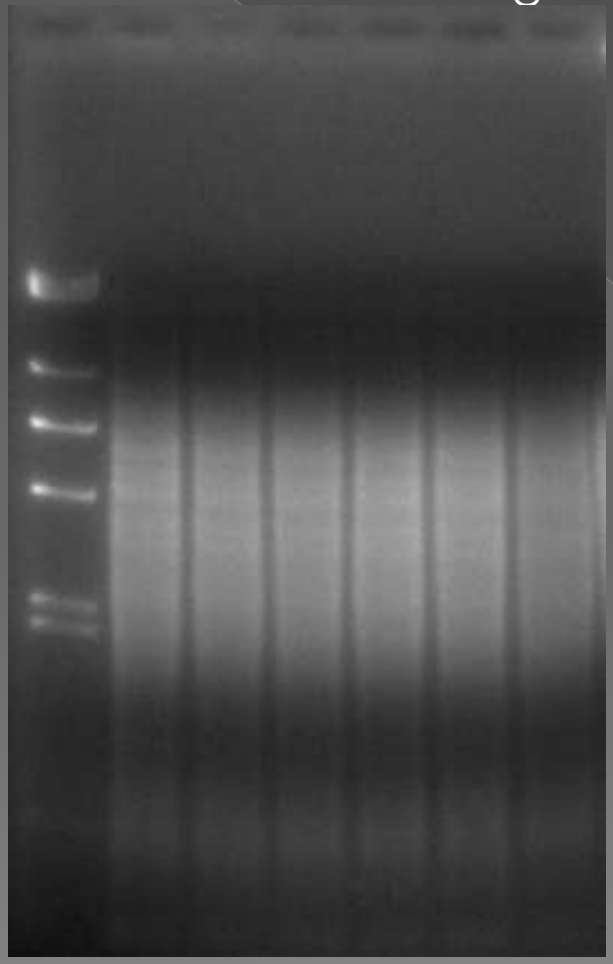


Typical Restriction Digest

Sterile, deionized water	16.3 µl
RE 10X Buffer	2.0 µl
Acetylated BSA, 10µg/µl	0.2 µl
DNA, 1µg/µl	1.0 µl
Mix by pipetting, then add:	
Restriction Enzyme, 10u/µl	0.5 µl
Final volume	20.0 µl

How does it Look after Restriction Digestion?

Genomic DNA Digest



Plasmid DNA Digest