TECHNIQUES IN MOLECULAR BIOLOGY

Course: Molecular Biology (02022312)

Instructor: Dr. M A Srour

Textbook:
Watson J, Baker TA, Bell SP, Gann A, Levine M, Losick R (2008). Molecular Biology of the Gene, 6th ed. Chap 21/ pp739-82

Lec # 2 Wed 25.01.2012

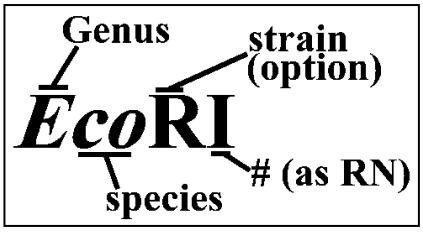
Restriction Enzymes (RE)

- RE: Site-specific endonucleases of prokaryotes
- Restriction enzymes = restriction endonucleases
- Recognize short (4-8 bp) target sequences called **Restriction site**, typically ***Palindromic*** sites

EcoRI restriction site

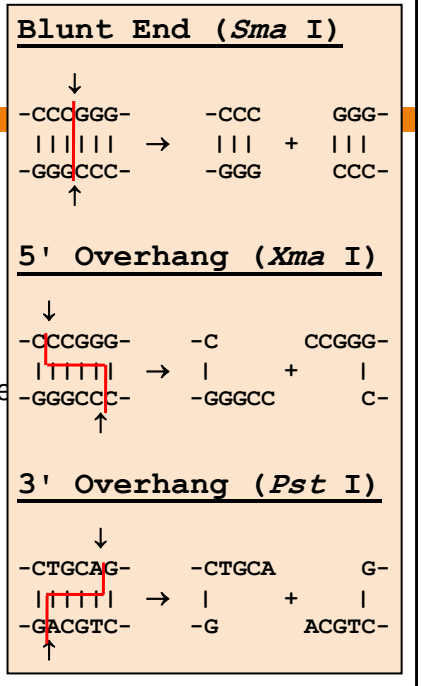
Restriction Enzymes (RE)

- Type II REs cuts adjacent or within restriction sites
- Type II enzymes are powerful tools in molecular biology
- RE are named after the bacterial species/strain from which it was isolated



Features of Restriction Sites

- Typically 4-8 bp & palindromic
- Frequency of RS: 4^4= 256 bp, 4^6=4096 bp,4^8=~65000 bp
- Degeneracy permitted by some enzymes
- Cleavage produces 5'-PO_4 & 3'-OH
- Both strands cleaved between same residues:
  - Blunt ends (flush ends)
  - Staggered /sticky ends at RT
    - 5'-overhangs
    - 3'-overhangs



REs

Isoschizomers

- ***Sma* I** CCC↓GGG
- ***Xma* I** C↓CCGGG

Compatible Ends

- ***Pst* I** CTGCA↓G
- ***Nsi* I** ATGCA↓T

TABLE 5-1 Selected Restriction Enzymes and Their Recognition Sequences

ENZYME	SOURCE MICROORGANISM	RECOGNITION SITE*	ENDS PRODUCED
*Bam*HI	*Bacillus amyloliquefaciens*	↓ -G-G-A-T-C-C- -C-C-T-A-G-G- ↑	Sticky
*Sau*3A	*Staphylococcus aureus*	↓ -G-A-T-C- -C-T-A-G- ↑	Sticky
*Eco*RI	*Escherichia coli*	↓ -G-A-A-T-T-C- -C-T-T-A-A-G ↑	Sticky
*Hin*dIII	*Haemophilus influenzae*	↓ -A-A-G-C-T-T- -T-T-C-G-A-A- ↑	Sticky
*Sma*I	*Serratia marcescens*	↓ -C-C-C-G-G-G- -G-G-G-C-C-C- ↑	Blunt
*Not*I	*Nocardia otitidis-caviarum*	↓ -G-C-G-G-C-C-G-C- -C-G-C-C-G-G-C-G- ↑	Sticky

*Many of these recognition sequences are included in a common polylinker sequence (see Figure 5-13).

Table 5-1
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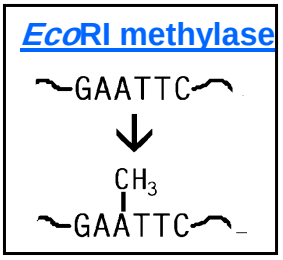
Functions of Restriction Enzymes

- Function to protect bacteria from phage (virus) infection
- Why REs do not destroy the host cell's own DNA?
- Almost all REs are paired with Methylases that recognize & methylate the same DNA sites
- The two enzymes RE & Methylase are collectively called a Restriction-Modification system (R-M system)



Applications of REs

- Restriction mapping & RFLP analysis (discussed later??)
- Cloning: Insertion of DNA fragments into cloning vectors
- Restriction or digestion of DNA by RE
  - Usually done in the appropriate buffer and temperature, in a small volume (~20µl)
  - Digested DNA fragments are analyzed by agarose gel electrophoresis

Restriction mapping

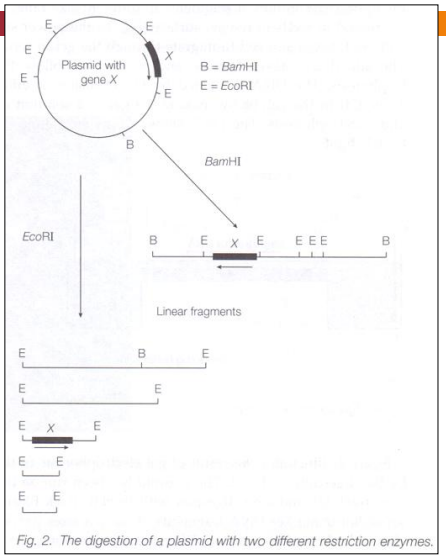
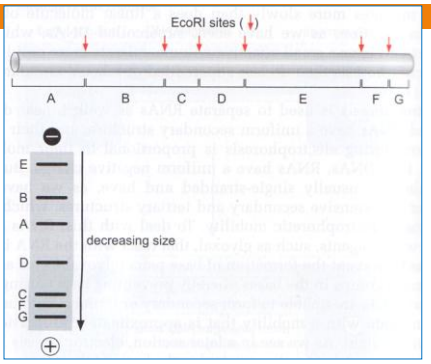


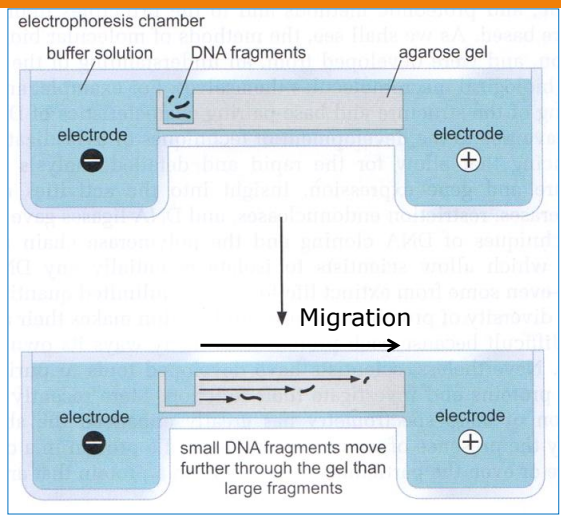
Fig. 2. The digestion of a plasmid with two different restriction enzymes.



Gel Electrophoresis

- Horizontal Agarose gel electrophoresis
- Electrophoresis separates DNA and RNA molecules according to size
- Gel:
  - Agarose 200 – 20,000 bp
  - Polyacrylamide 10- 1,000 bp
- Electrophoresis (constant voltage)
- Detection: with fluorescent dye (eg., ethidium bromide)

Horizontal Agarose Gel Electrophoresis



Voltage: up to 150 V DC

Horizontal Agarose Gel Electrophoresis

DNA ladder (Molecular weight marker)

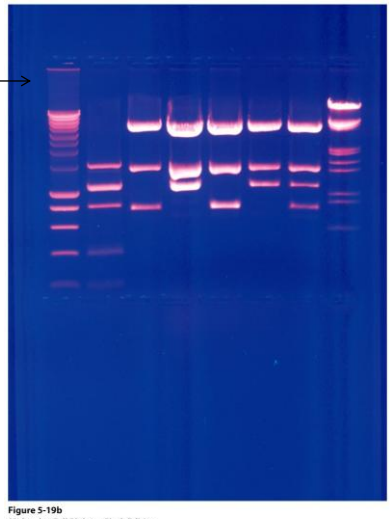


Figure 5-19b
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Gel electrophoresis separates DNA molecules of different length

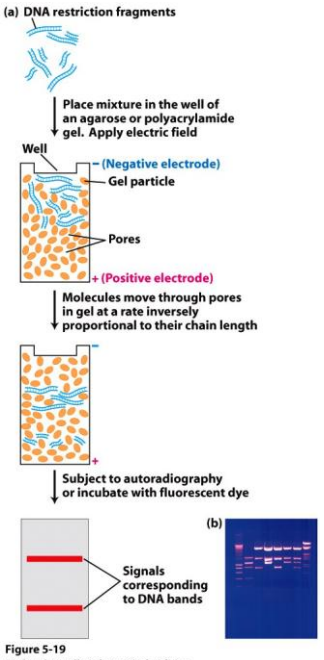

Figure 5-19
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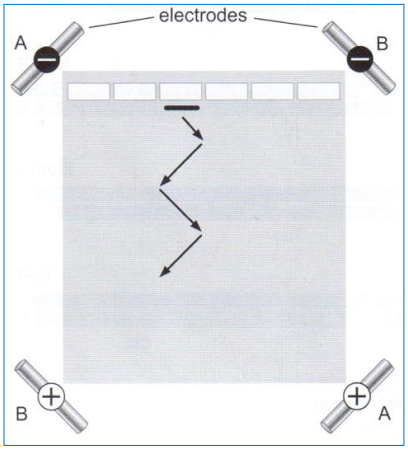
Pulsed-field gel electrophoresis

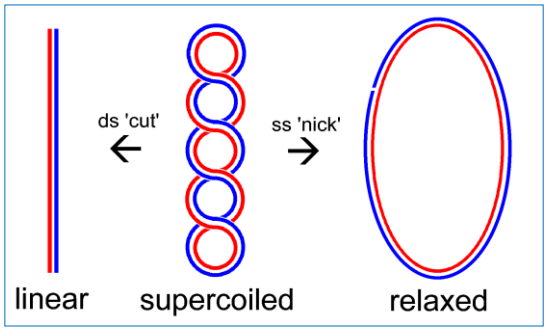
Use two electrodes oriented orthogonally to each other, and are switched on & off alternately.

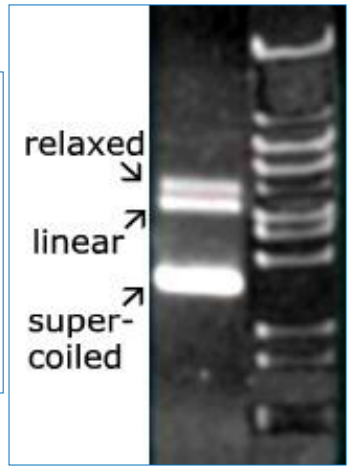
It's used to determine the size of entire bacterial chromosomes & chromosomes of lower eukaryotes



Circular vs. Linear DNA

- Electrophoresis separates DNA not only according to size but also according to shape and topology
- Linear DNA and circular DNA exhibit different mobility in gel electrophoresis





DNA hybridization

- Hybridization: the technique wherein renatured DNA is formed from separate single-stranded samples
- Applications> nucleic acid blotting
  - Southern blot hybridization
  - Northern blot hybridization

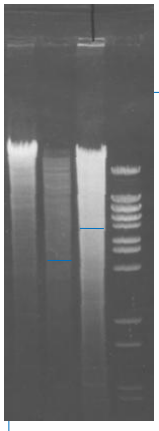
Southern Blot

- Described by Dr. Edward Southern (1975)
- Used to identify particular DNA fragment
- **Method**
- Digest and electrophorese DNA on agarose gel
- dsDNA in gel is denatured using alkali (NaOH)
- Transfer from gel to positively charged membrane > "imprint" or "blot"
- Immobilize the DNA to membrane by UV-cross linking
- Detect with a labeled probe (complementary to a sequence within the gene of interest)> hybridization
- When X-ray film is exposed to hybridized membrane > autoradiogram

Northern Blot

- Used to identify particular RNA fragment
- RNA are short (typically <5 kb) are not digested
- Method is similar to Southern blot
- Applications: Study gene expression or quantify the mRNA level of a specific gene
- Can study one gene at a time

Southern & Northern Blots



Southern Blots

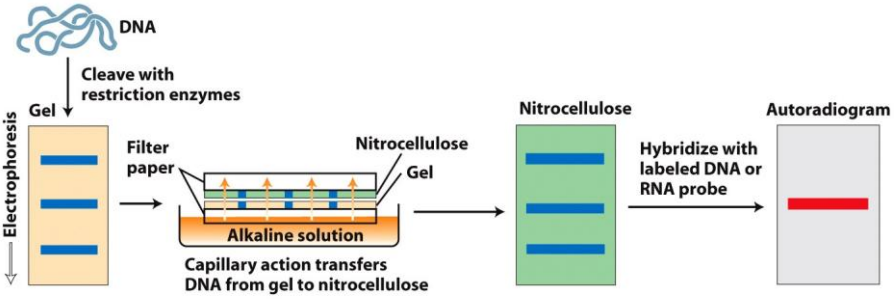


Figure 5-26
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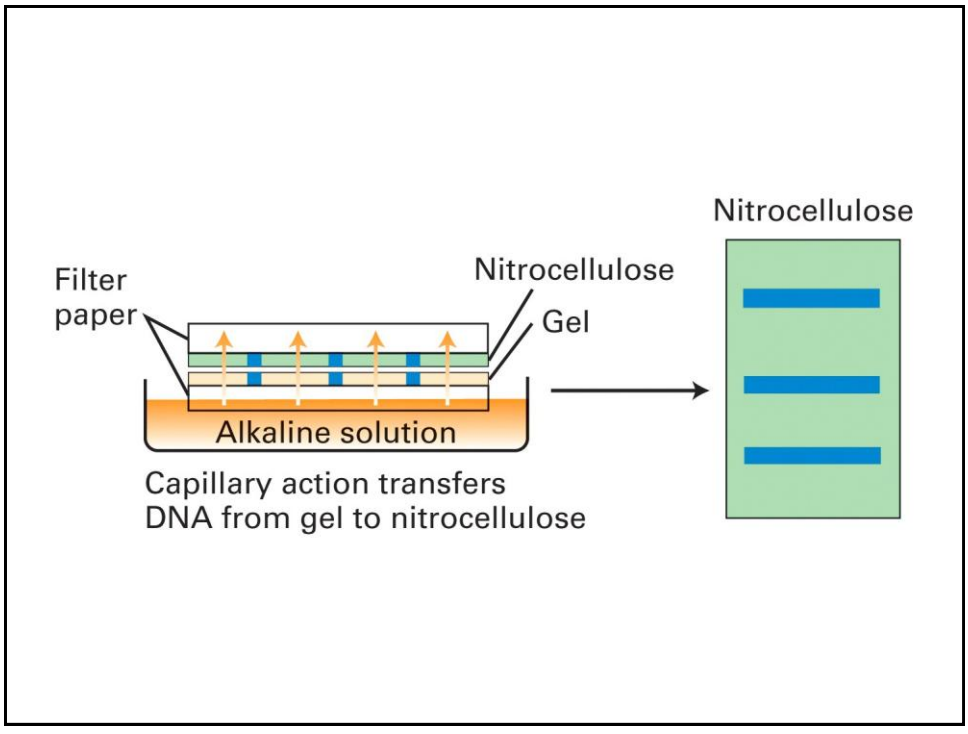
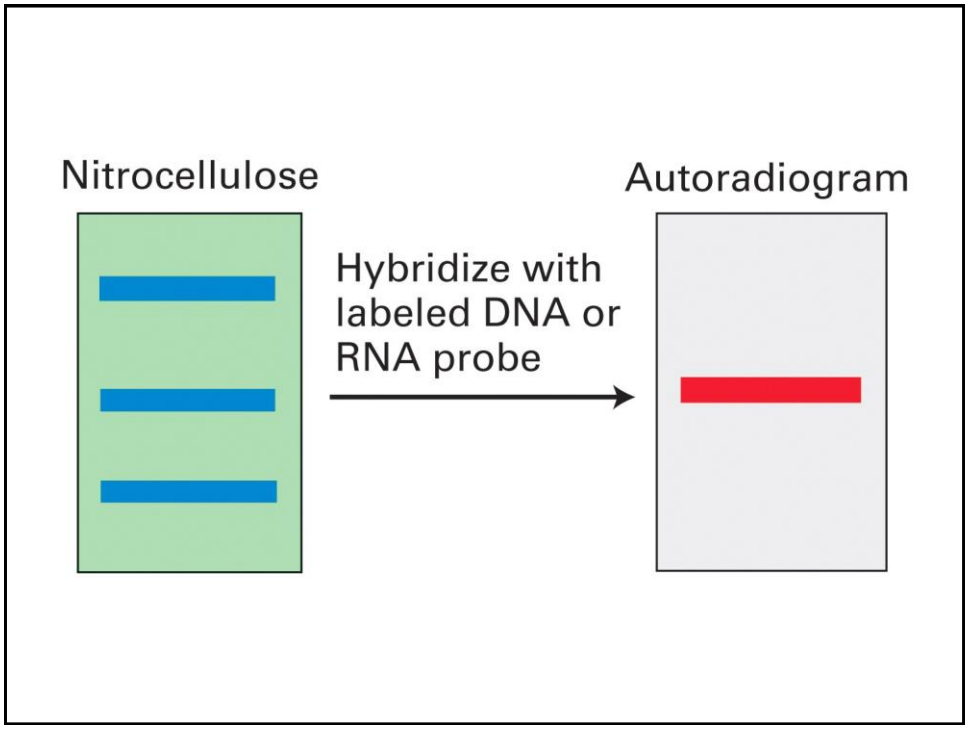

Nitrocellulose
Filter paper
Nitrocellulose
Gel
Alkaline solution
Capillary action transfers DNA from gel to nitrocellulose


Nitrocellulose
Autoradiogram
Hybridize with labeled DNA or RNA probe

Northern blotting>
autoradiogram

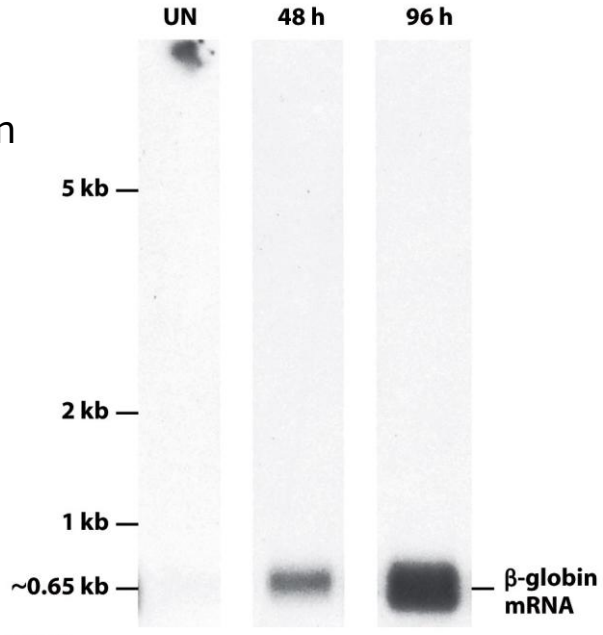


Figure 5-27
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