

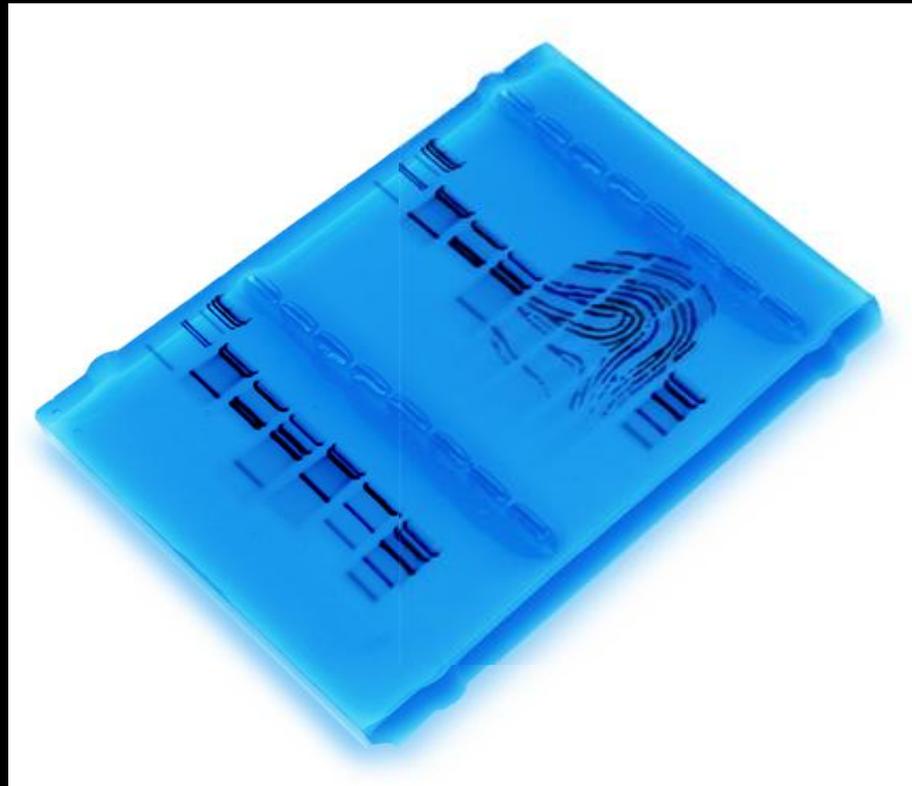
Biotechnology Explorer™

CAPTIVATING SCIENCE EDUCATION

BIO-RAD

Professional Development

Forensic DNA Fingerprinting: Using Restriction Enzymes



Forensic DNA Fingerprinting Kit

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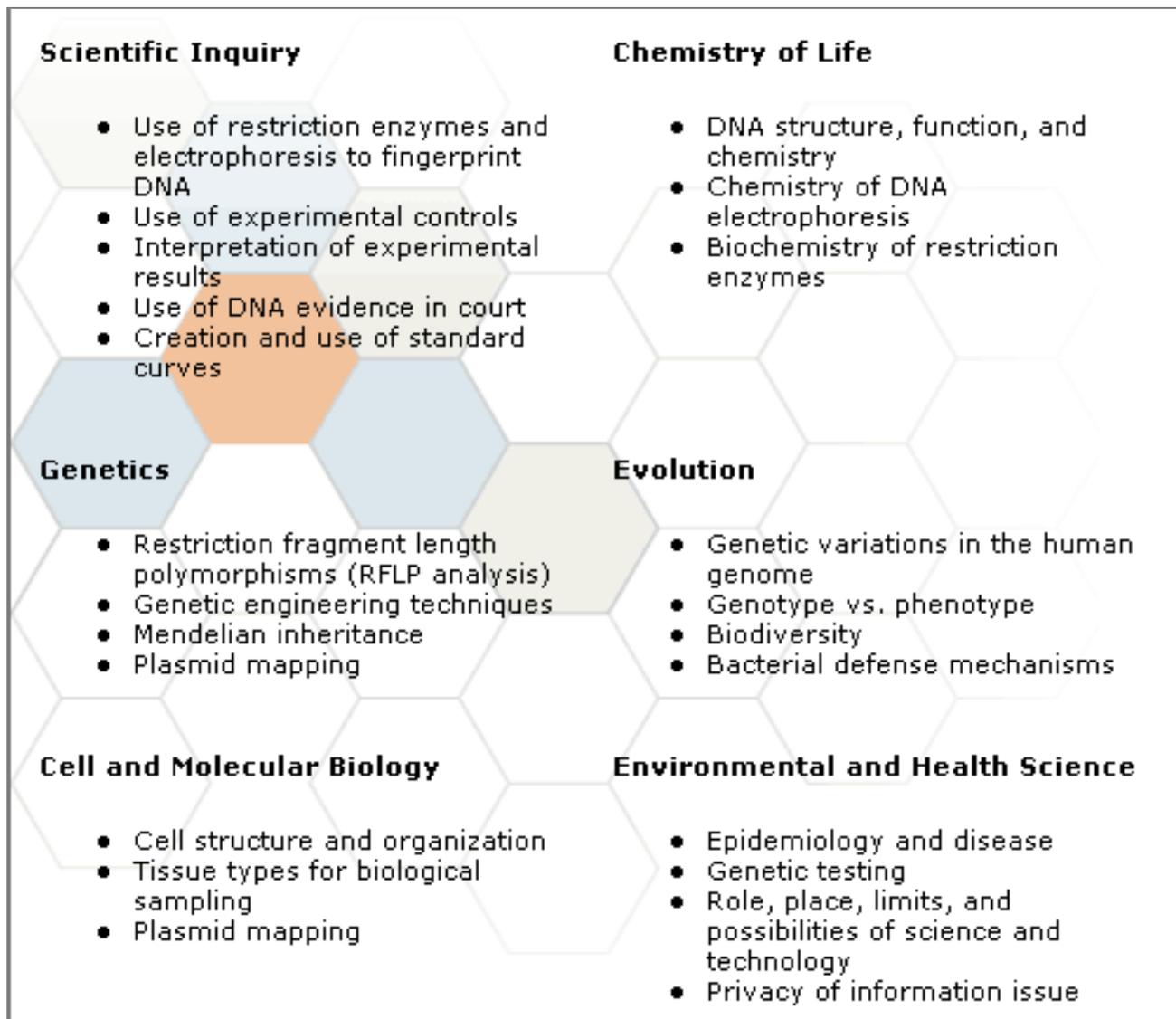
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Why Teach DNA Fingerprinting?



- **Real-world connections**
- **Tangible results**
- **Link to careers and industry**
- **Laboratory extensions**
- **Standards-based**



Forensic DNA Fingerprinting

Kit Advantages



- **Standards Based**

Aligns with AP Biology Lab 6

- **Use of real restriction enzymes and electrophoresis of real DNA fragments**

- **Lab can completed in two 45 minute sessions**

- **Sufficient materials for 8 student workstations**

The Forensic DNA Fingerprinting Kit **Can Help** **You Teach:**



- **DNA structure**
- **DNA restriction analysis (RFLP)**
- **Agarose gel electrophoresis**
- **Molecular weight determination**
- **Simulation of DNA Fingerprinting**
- **Plasmid mapping**

DNA Fingerprinting Real World Applications



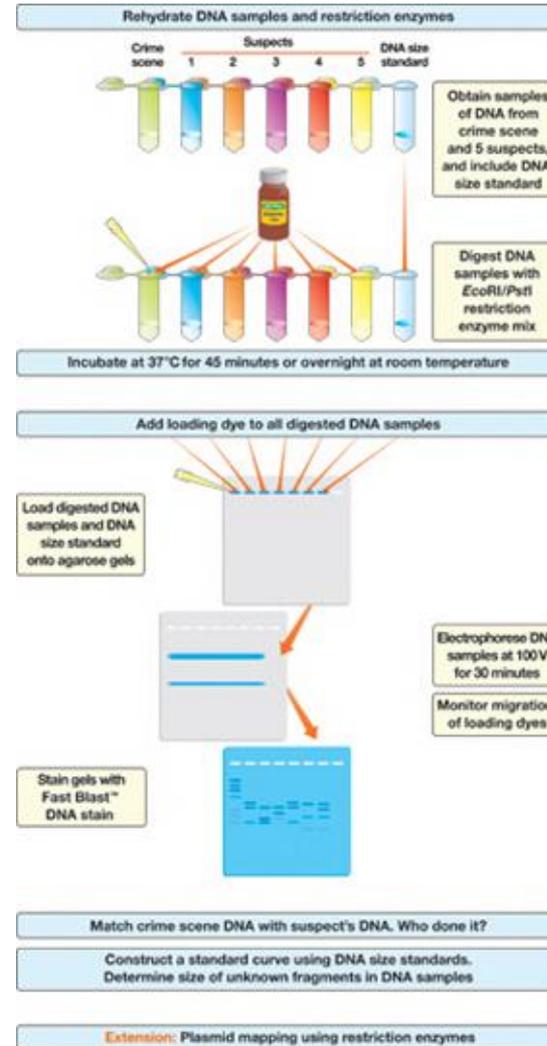
- **Crime scene**
- **Human relatedness**
- **Paternity**
- **Animal relatedness**
- **Anthropology studies**
- **Disease-causing organisms**
- **Food identification**
- **Human remains**
- **Monitoring transplants**

Workshop Time Line



- **Restriction digest of DNA samples**
- **Introduction to DNA Fingerprinting and RFLP analysis**
- **Electrophoresis on Agarose gels**
- **Analysis and interpretation of results**

DNA Fingerprinting Procedure Overview



Laboratory Quick Guide

Quick Guide for DNA Fingerprinting Kit

Lesson 2 Restriction Digestion

1. Place the tube containing the restriction enzyme mix, labeled ENZ, on ice.



2. Label one of each colored micro test tubes as follows:

- green tube CS (crime scene)
- blue tube S1 (suspect 1)
- orange tube S2 = suspect 2
- violet tube S3 = suspect 3
- red tube S4 = suspect 4
- yellow tube S5 = suspect 5



Label the tubes with your name, date, and lab period. Place the tubes in the foam micro test tube holder.

3. Using a fresh tip for each sample, pipet 10 µl of each DNA sample from the stock tubes and transfer to the corresponding colored micro test tubes. Make sure the sample is transferred to the bottom of the tubes.



4. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube.

5. Tightly cap the tubes and mix the components by gently flicking the tubes with your finger. If a microcentrifuge is available, pulse-spin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, gently tap the tube on the table top.



6. Place the tubes in the foam micro tube holder and incubate for 45 min at 37°C or overnight at room temperature in a large volume of water heated to 37°C.

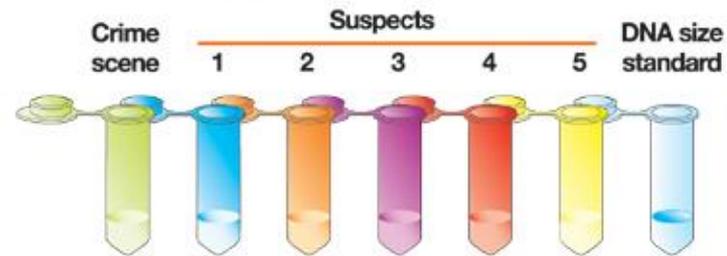


7. After the incubation period, remove the tubes from the water bath and place in the refrigerator until the next laboratory period. If there is sufficient time to continue, proceed directly to step 2 of Lesson 3.

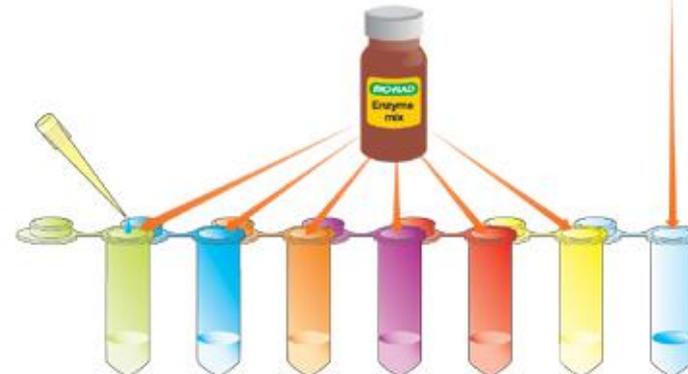


DNA Fingerprinting Procedures Day One

Rehydrate DNA samples and restriction enzymes



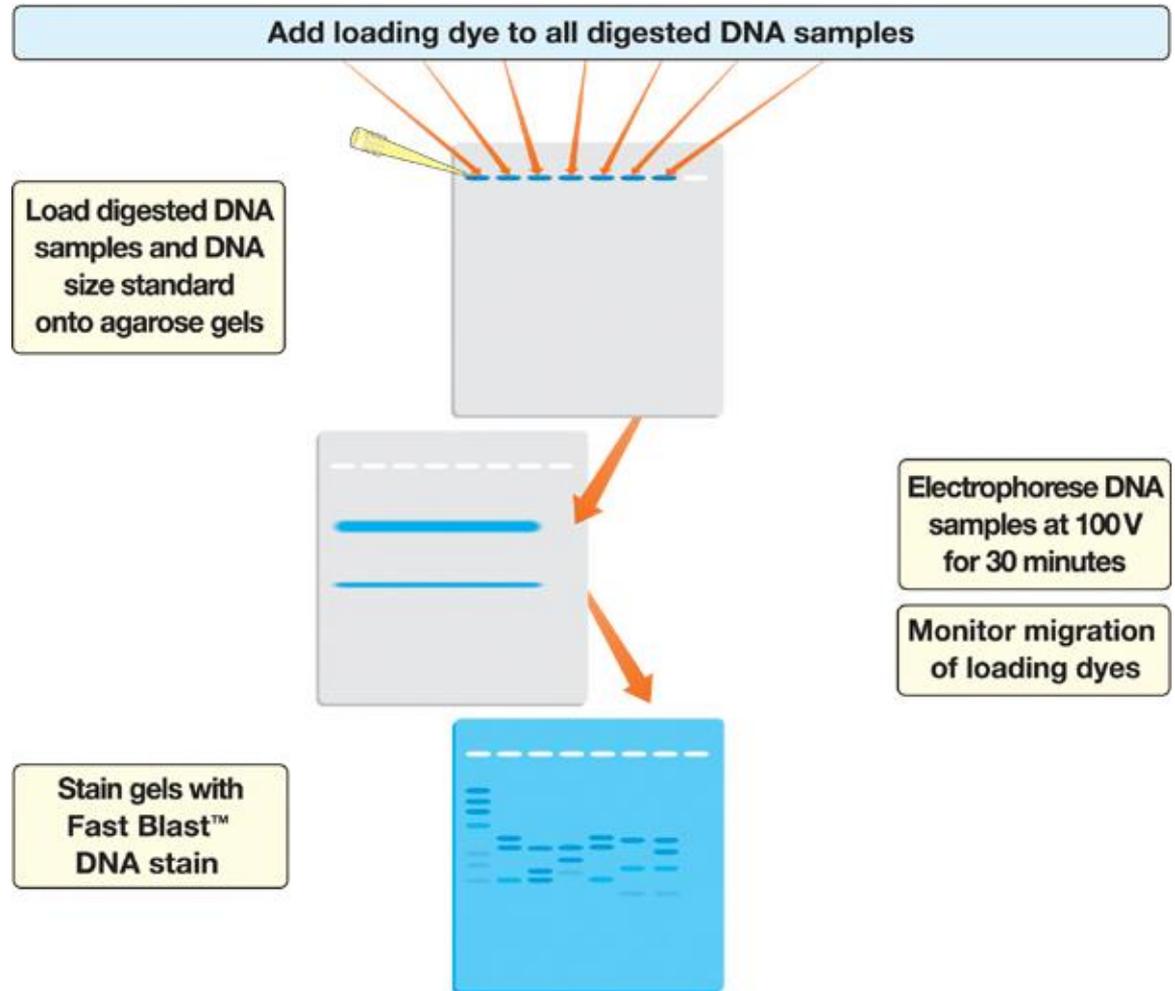
Obtain samples of DNA from crime scene and 5 suspects, and include DNA size standard



Digest DNA samples with *EcoRI/PstI* restriction enzyme mix

Incubate at 37°C for 45 minutes or overnight at room temperature

DNA Fingerprinting Procedures Day Two



DNA Fingerprinting Procedures Day Three

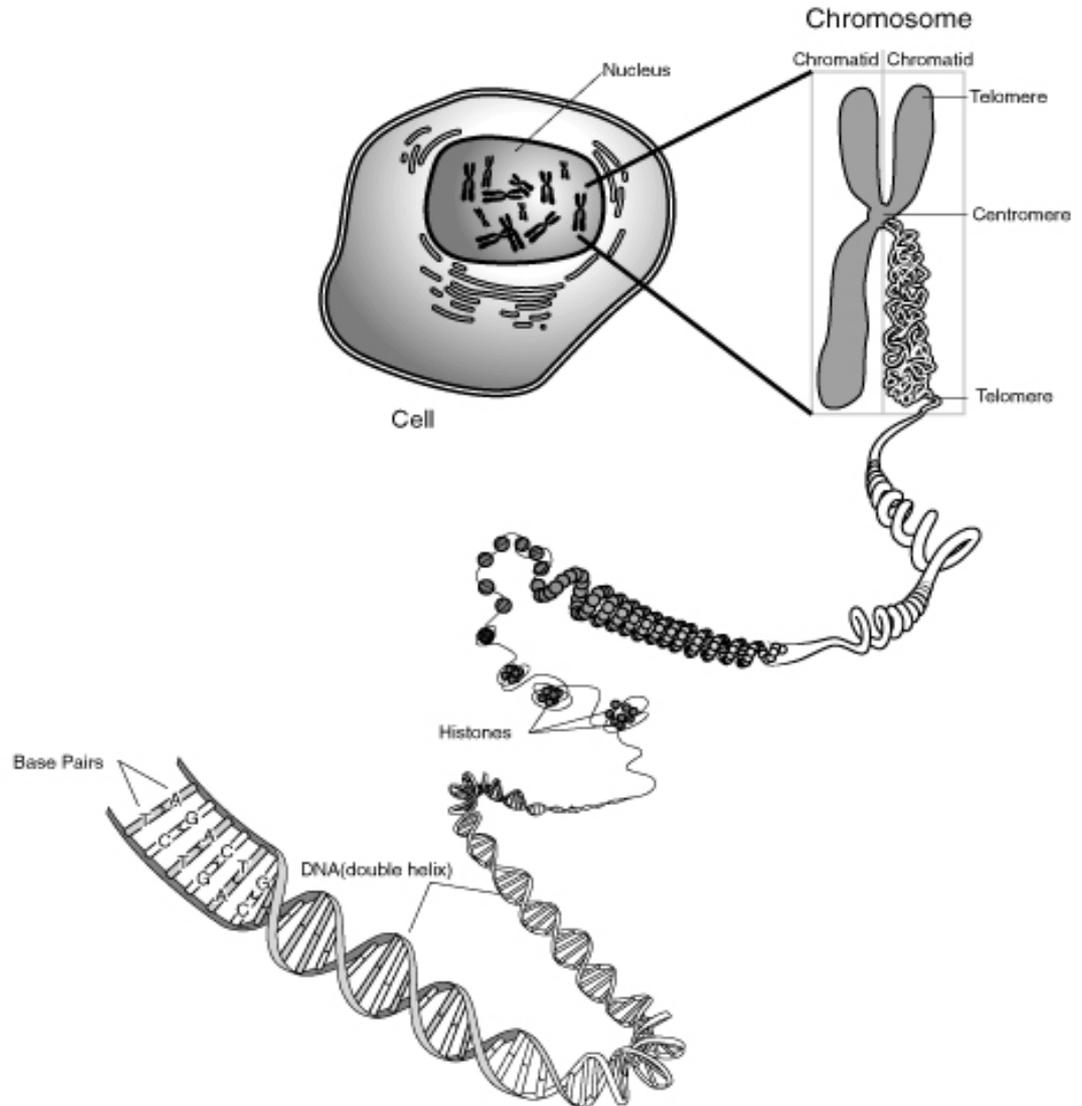


Match crime scene DNA with suspect's DNA. Who done it?

Construct a standard curve using DNA size standards.
Determine size of unknown fragments in DNA samples

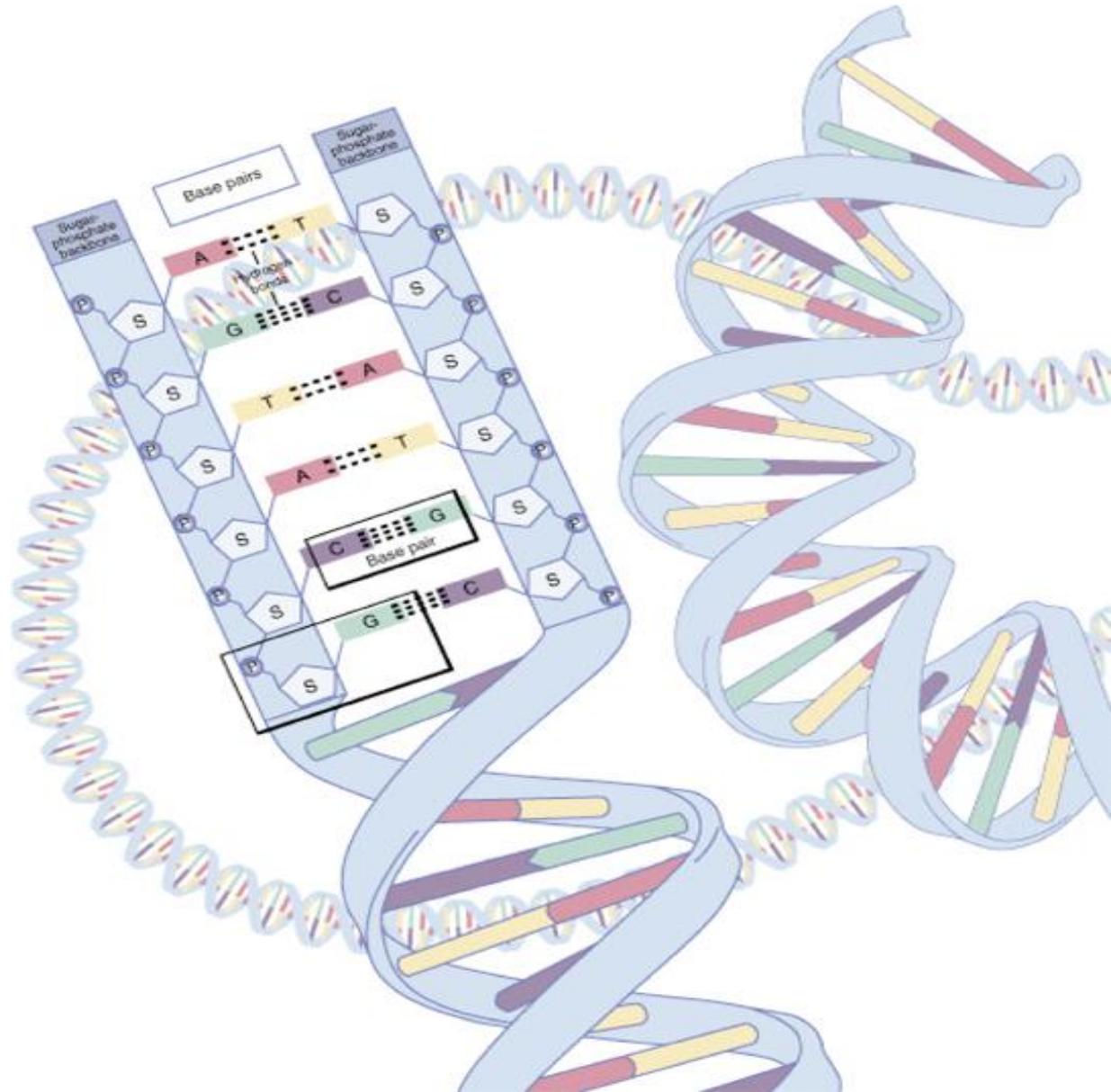
Extension: Plasmid mapping using restriction enzymes

DNA is Tightly Packaged into Chromosomes Which Reside in the Nucleus

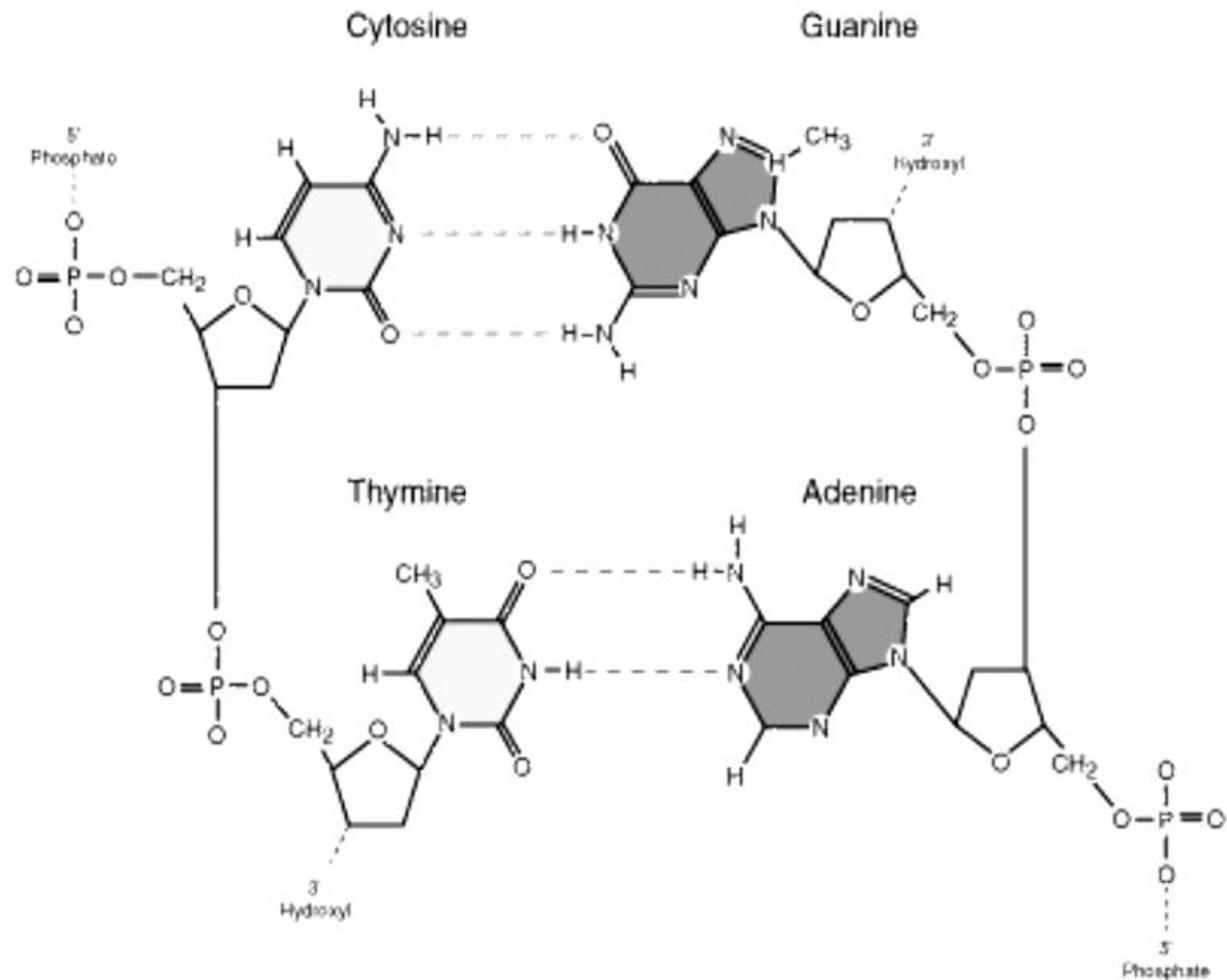
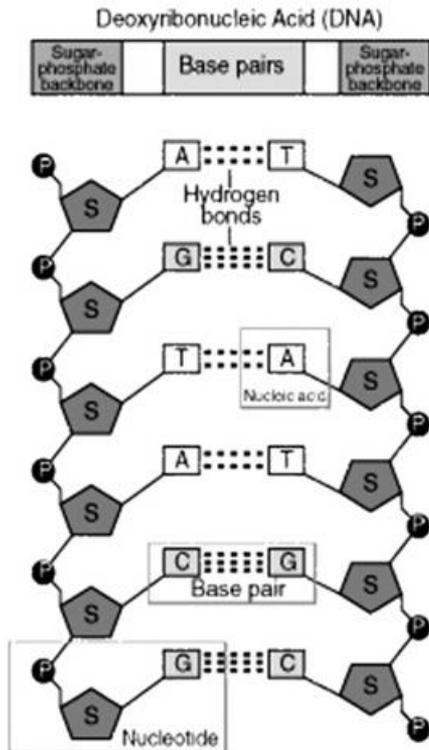


Model of DNA

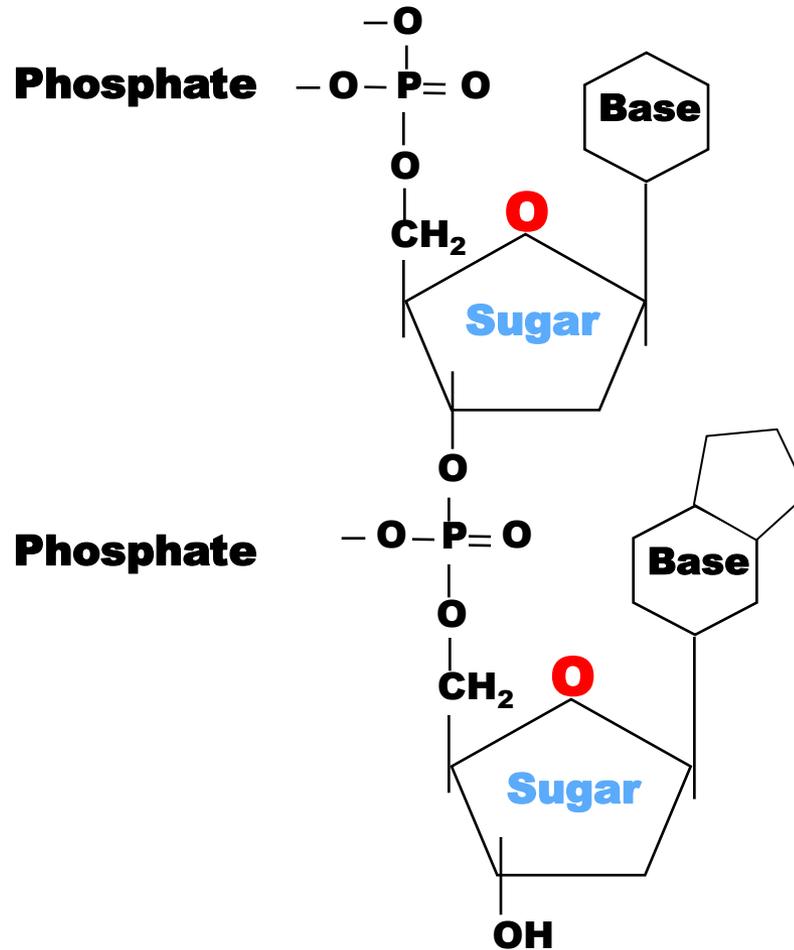
**DNA is
Comprised of
Four Base Pairs**



Deoxyribonucleic Acid (DNA)

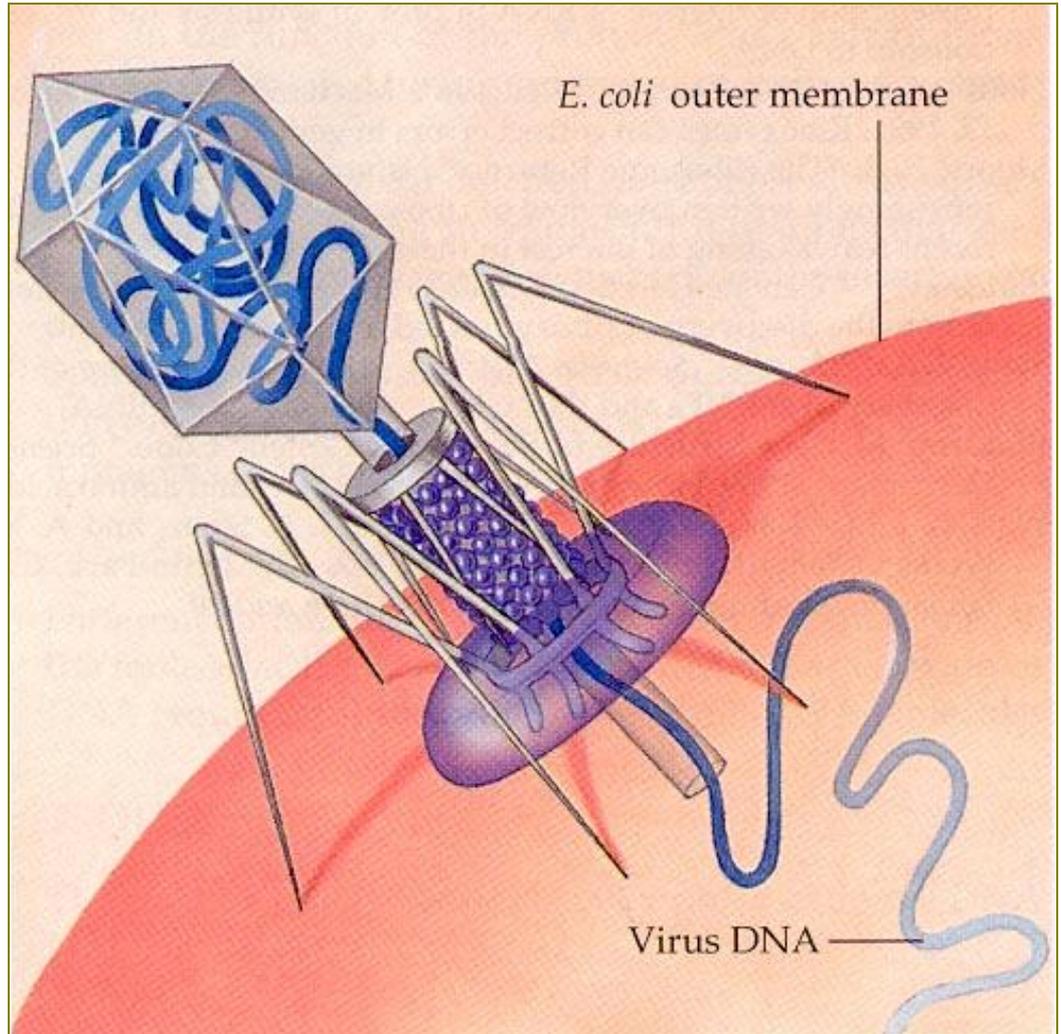


DNA Schematic



DNA Restriction Enzymes

- Evolved by bacteria to protect against viral DNA infection
- Endonucleases = cleave within DNA strands
- Over 3,000 known enzymes



Enzyme Site Recognition

- Each enzyme digests (cuts) DNA at a specific sequence = restriction site
- Enzymes recognize 4- or 6- base pair, palindromic sequences (eg GAATTC)

Restriction site

Palindrome

G T A G G A A T T C A T T T C A C G C A
C A T C T T A A G T A A G T G C G T

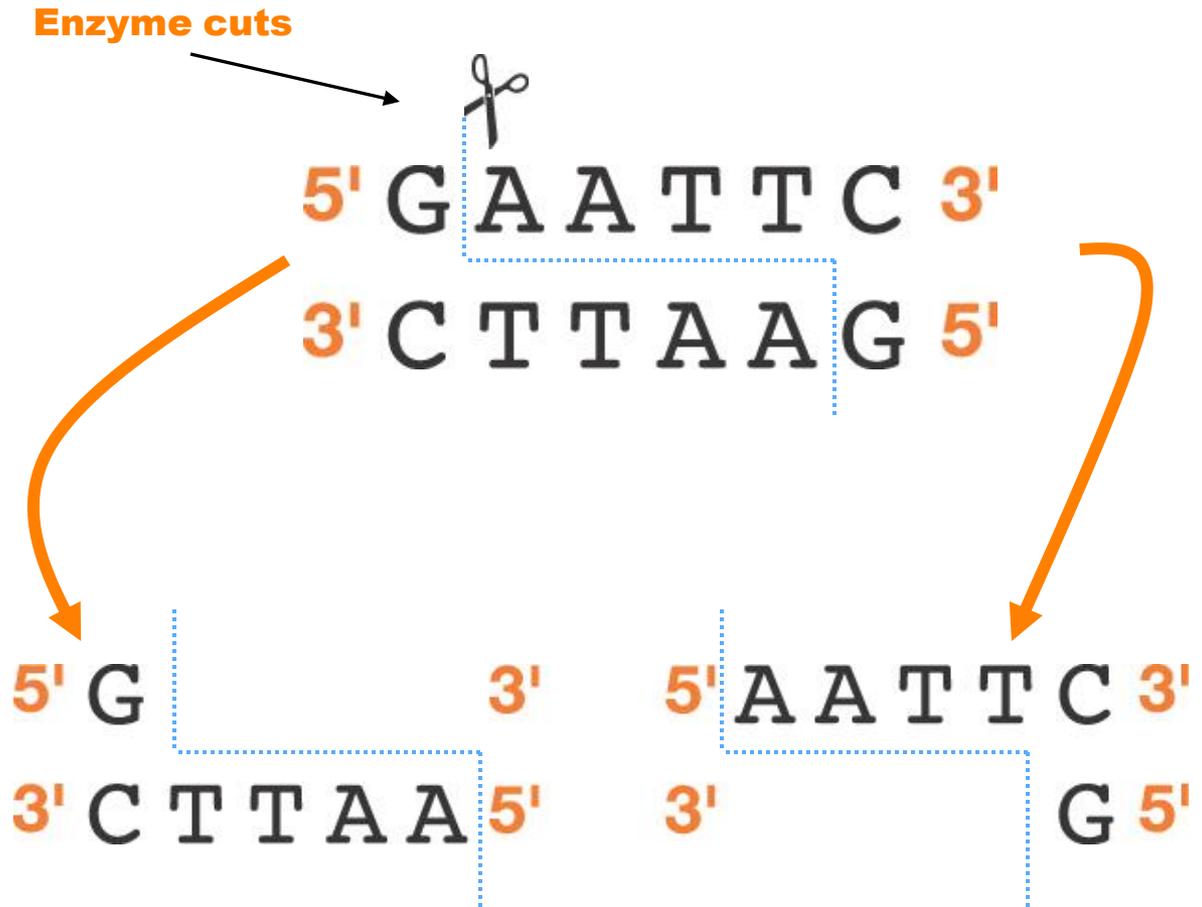
G T A G A A T T C A T T T C A C G C A
C A T C T T A A G T A A G T G C G T

Fragment 1

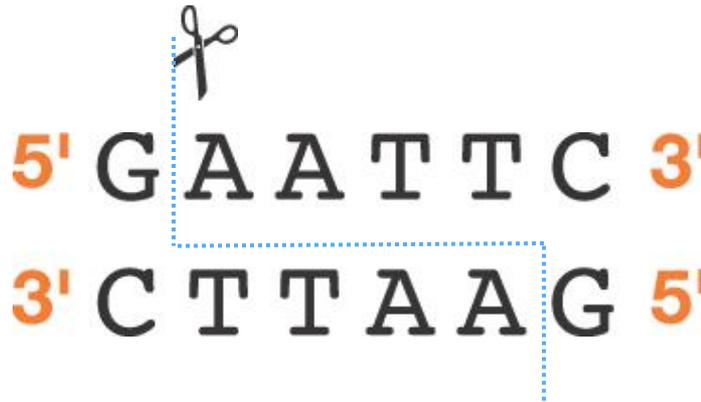
Fragment 2

5 vs 3 Prime Overhang

- Generates 5 prime overhang

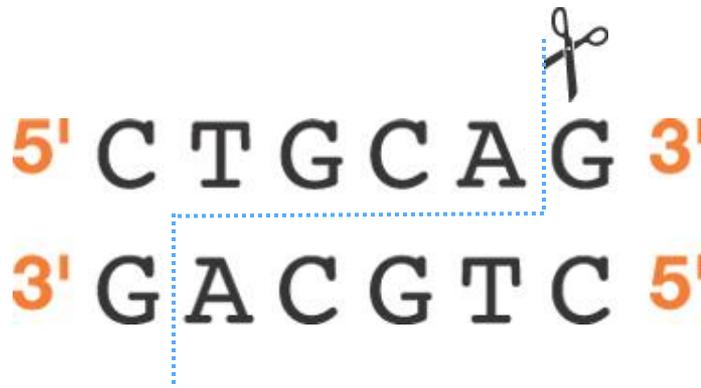


Common Restriction Enzymes



EcoRI

- Escherichia coli
- 5 prime overhang



PstI

- Providencia stuartii
- 3 prime overhang

The DNA Digestion Reaction

Restriction Buffer provides optimal conditions

- **NaCl** provides the correct ionic strength
- **Tris-HCl** provides the proper pH
- **Mg²⁺** is an enzyme co-factor

DNA Digestion Temperature

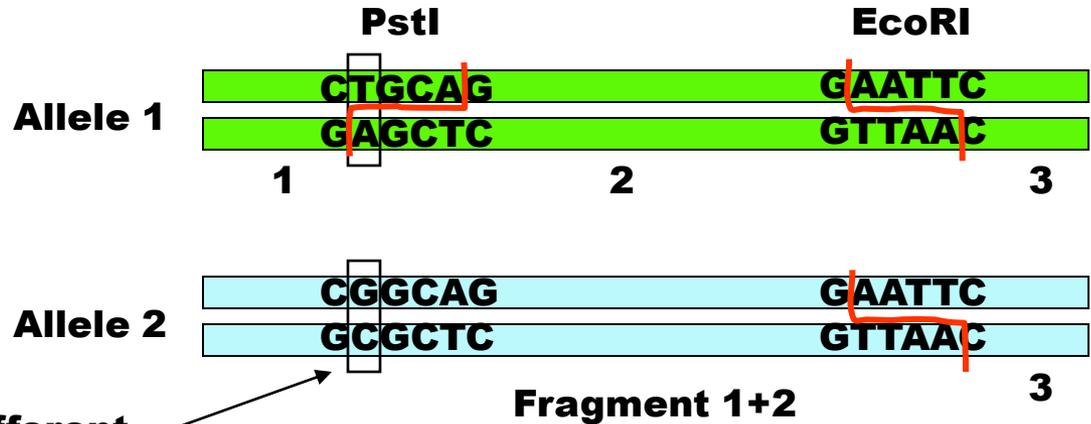
Why incubate at 37°C?

- Body temperature is optimal for these and most other enzymes

What happens if the temperature is too hot or cool?

- *Too hot* = enzyme may be denatured (killed)
- *Too cool* = enzyme activity lowered, requiring longer digestion time

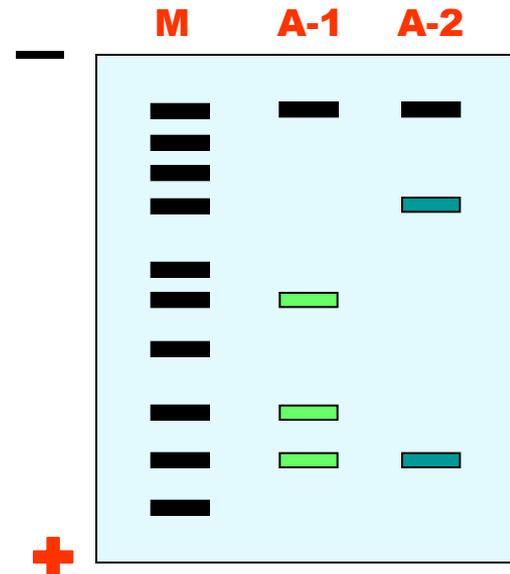
Restriction Fragment Length Polymorphism RFLP



Different
Base Pairs
No restriction site

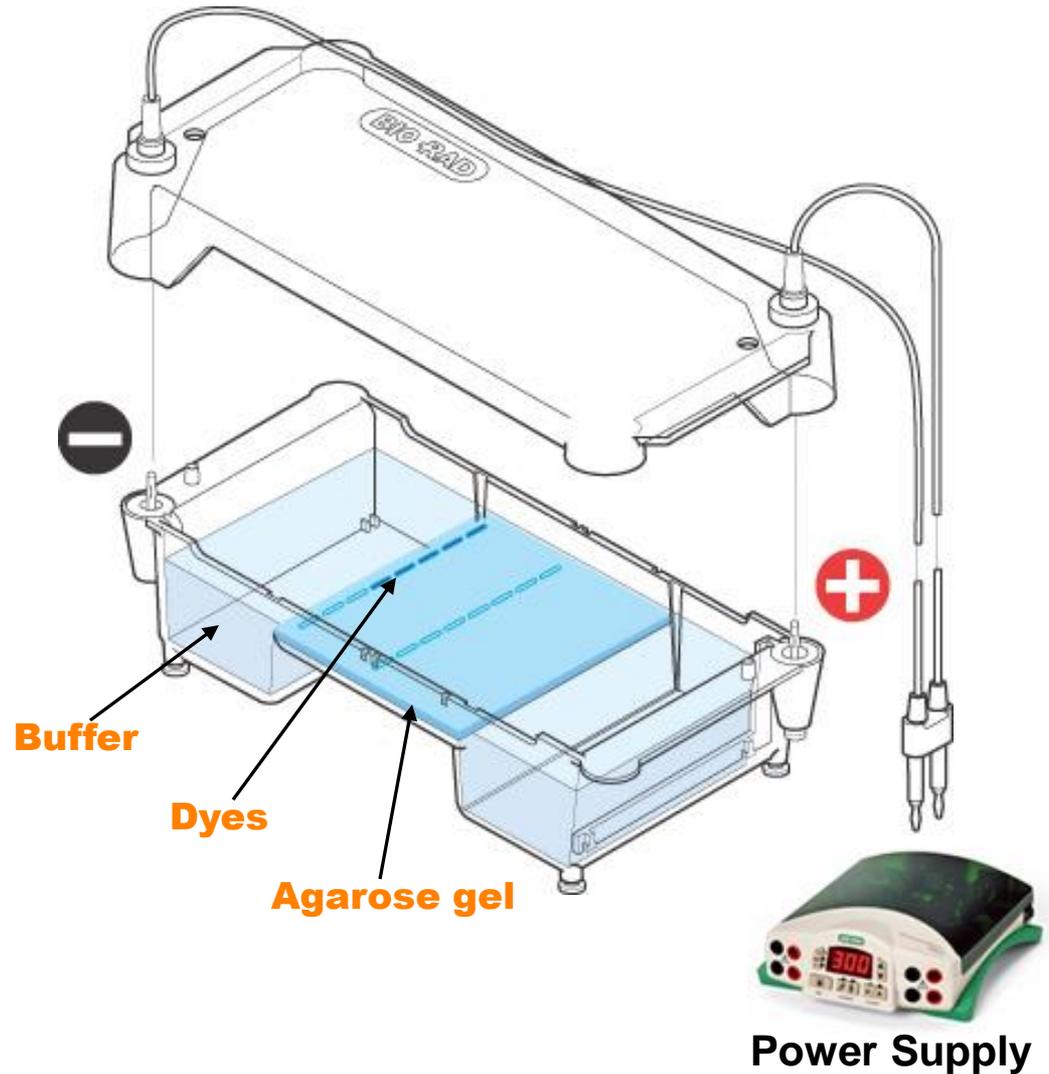
Electrophoresis of restriction fragments

M: Marker
A-1: Allele 1 Fragments
A-2: Allele 2 Fragments



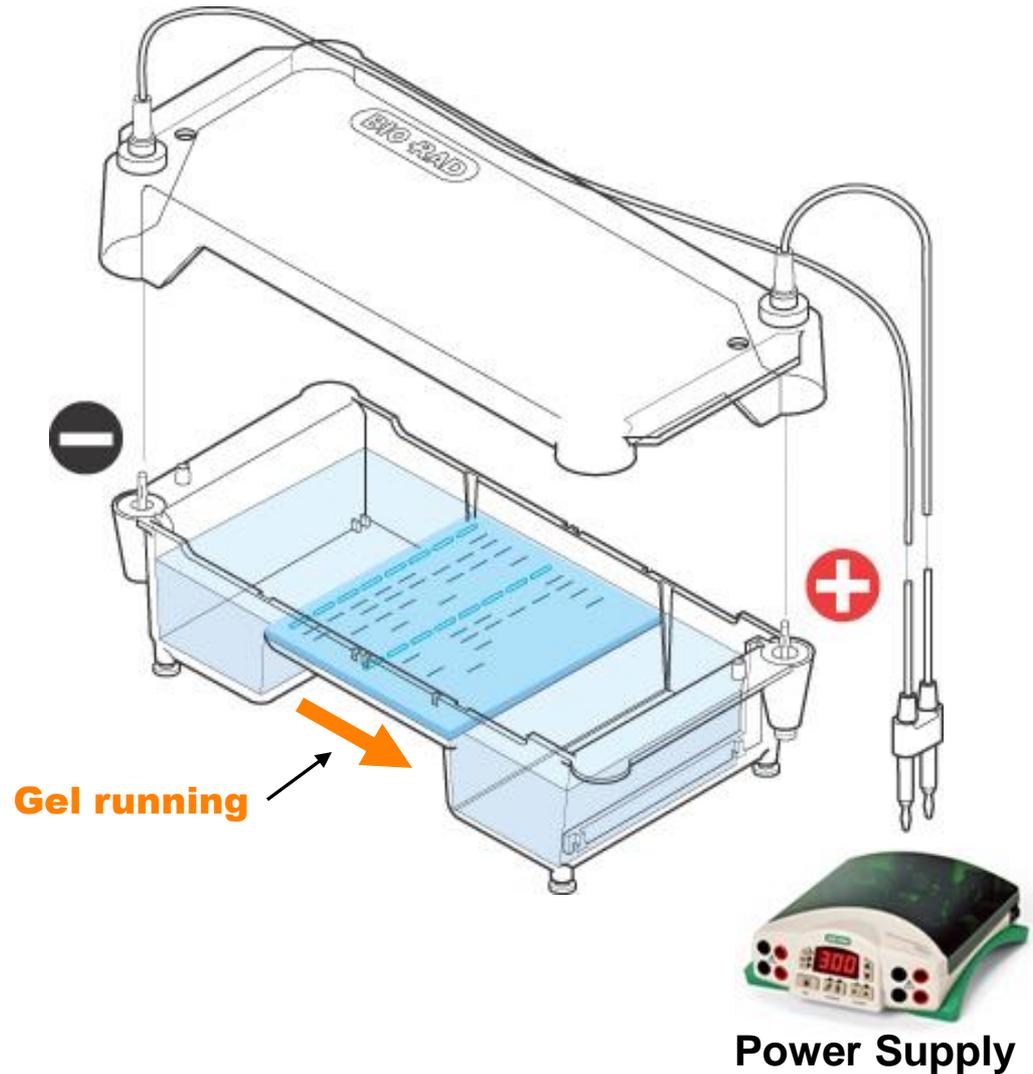
Agarose Electrophoresis Loading

- **Electrical current** carries negatively-charged DNA through gel towards positive (red) electrode



Agarose Electrophoresis Running

- **Agarose gel sieves** DNA fragments according to size
 - Small fragments move farther than large fragments

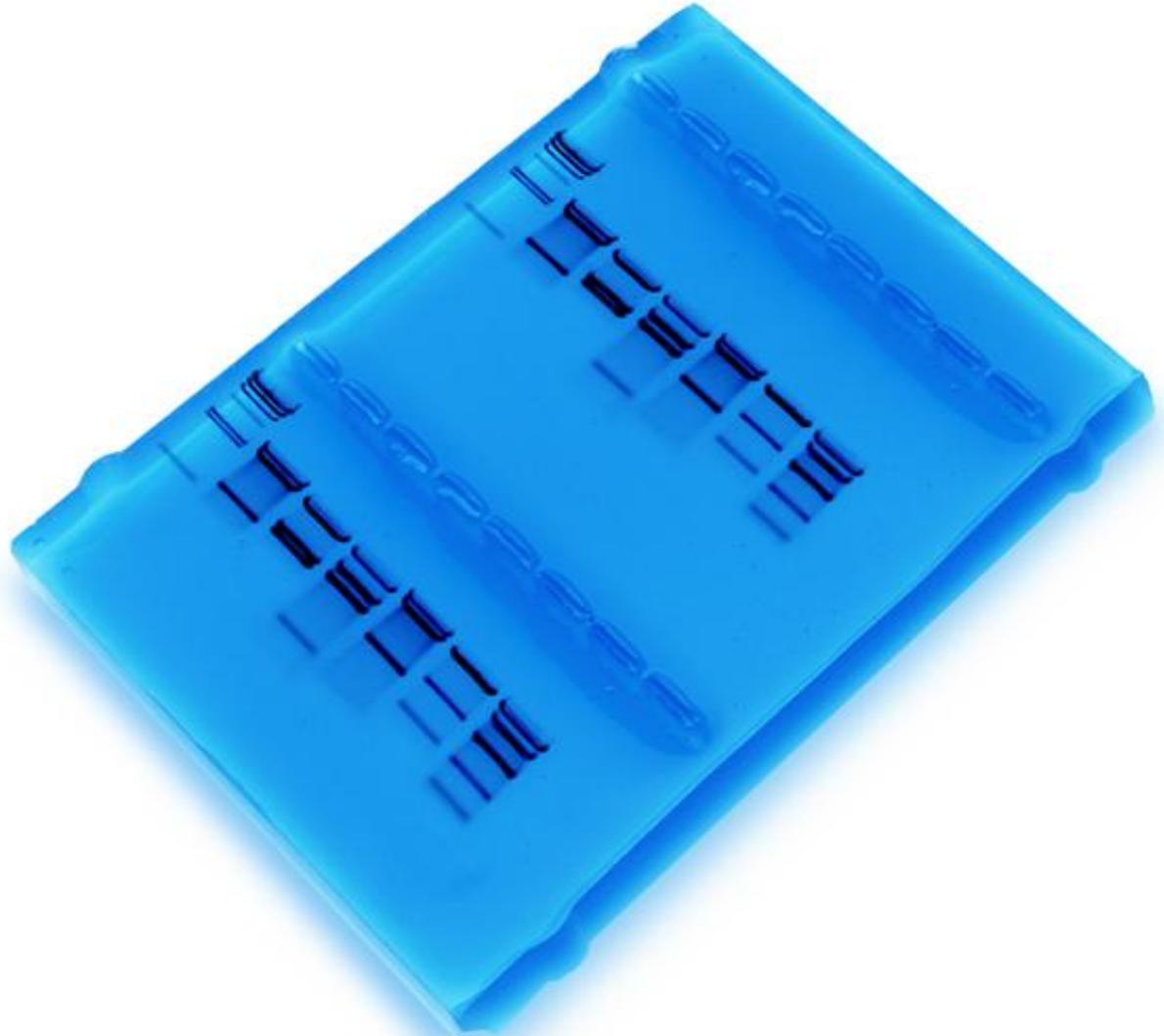


Analysis of Stained Gel

Determine restriction fragment sizes

- Create standard curve using DNA marker
- Measure distance traveled by restriction fragments
- Determine size of DNA fragments

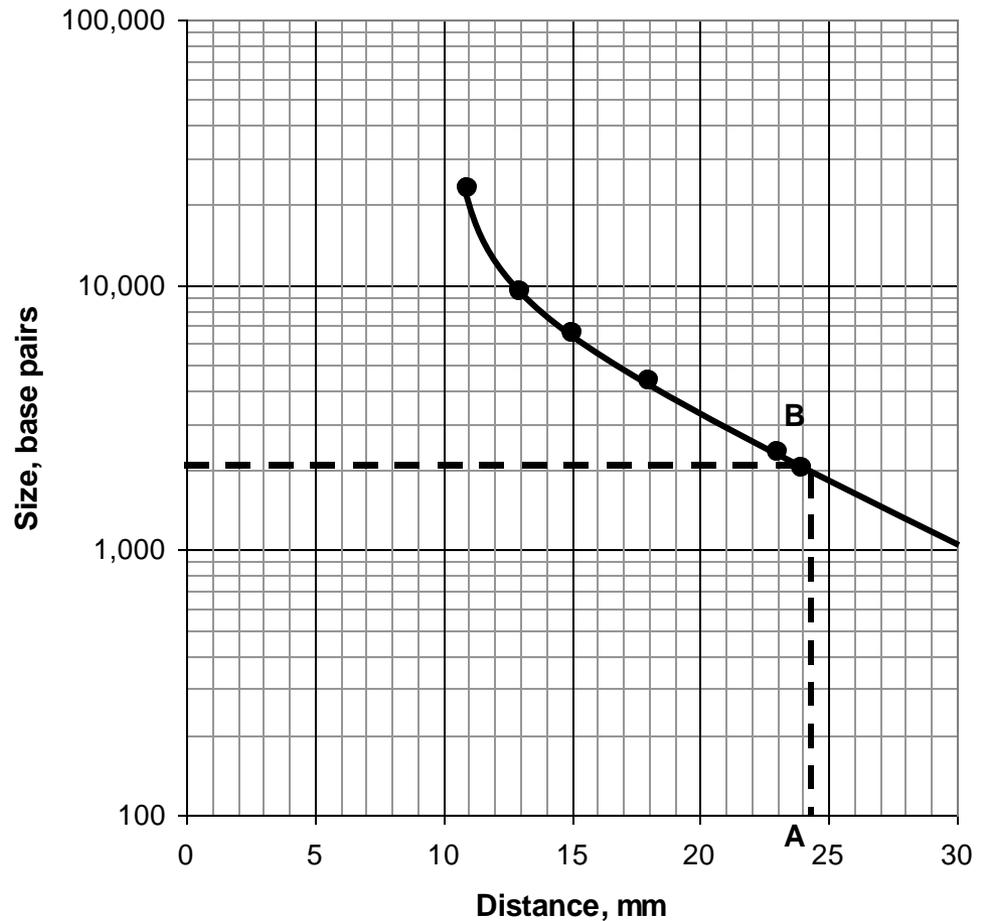
Identify the related samples



Molecular Weight Determination

Size (bp)	Distance (mm)
23,000	11.0
9,400	13.0
6,500	15.0
4,400	18.0
2,300	23.0
2,000	24.0

Fingerprinting Standard Curve: Semi-log

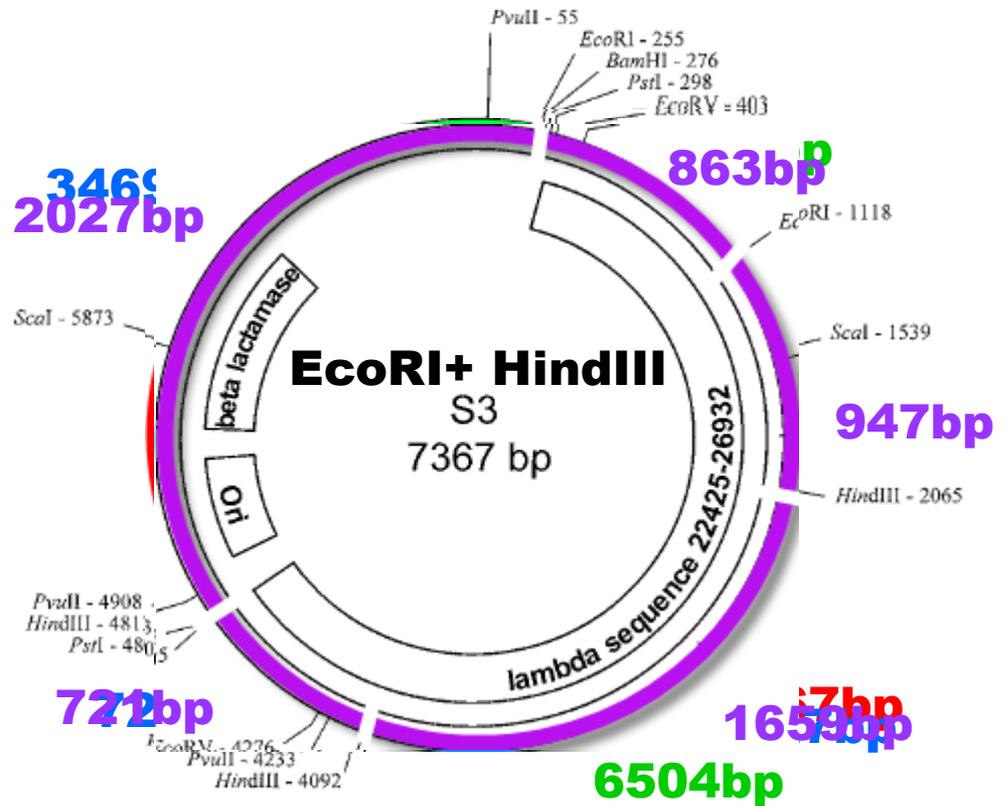


DNA Fingerprinting Lab Extensions

- **Independent studies**
- **Plasmid DNA isolation (mini-preps)**
- **Plasmid mapping using restriction enzymes**
- **Southern blot analysis**
- **Introductory labs to electrophoresis:**
 - Kool-Aid/FastBlast**
 - pH indicator in buffer**

Plasmid Map and Restriction Sites

Laboratory Extensions



BamHI: 1 linear fragment; 7367bp
EcoRI: 2 fragments; 863bp / 6504bp
HindIII: 3 fragments; 721bp/2027bp/3469bp
**EcoRI+Hind III: 5 fragments;
 721bp/863bp/947bp/1659bp/2027bp**

Bio-Rad's Electrophoresis Equipment

- Electrophoresis Cells
- Power Supplies
- Precast Agarose Gels



PowerPac™ Mini



PowerPac™ Basic



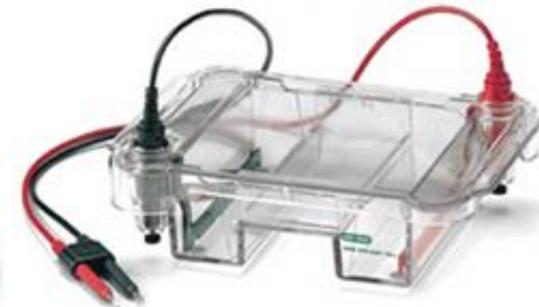
PowerPac™ HC



PowerPac™ Universal



Mini-Sub® Cell GT



Wide Mini-Sub Cell GT