NVBEC Workshop
December 9-10, 2011

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North Valley Biotechnology Education Collaborative

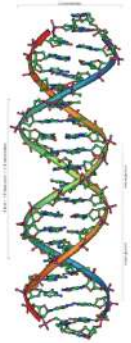
ARC BIOTECHNOLOGY PROGRAM

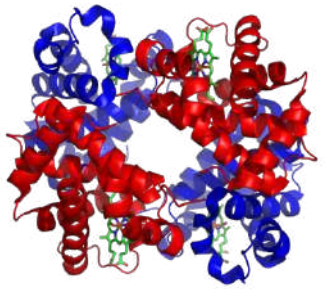
NVBEC Workshop Activities

- Day 1
  - Micropipette practice
  - Bacterial transformation
- Day 2
  - Analyze transformation results
  - PCR
  - Sickle cell anemia – protein (dye electrophoresis) and DNA profiling

What is molecular biology and biotechnology?

- Molecular biology is the study of macromolecules and their activity in living things



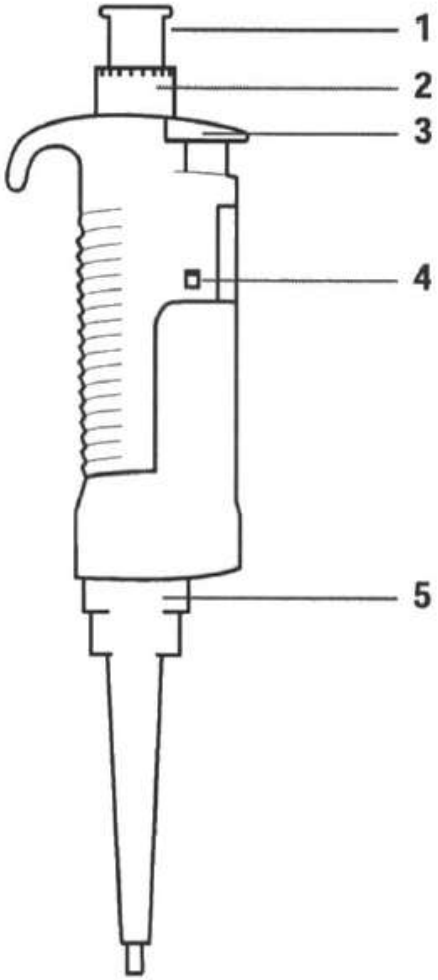


- Biotechnology is the use of cells and biological molecules to solve problems or make useful products

Herceptin®
Trastuzumab
anti-HER2 monoclonal antibody

AquaBounty TECHNOLOGIES

Micropipette



CEPRAP 2002
http://ceprap.ucdavis.edu

1 Control button
First stop (measuring stroke); the aspirated volume is dispensed.
Second stop (blow-out); the liquid remaining in the tip is blown out.

2 Setting ring
To set the volume
For fixed-volume pipettes, this ring is for adjustment purposes only.

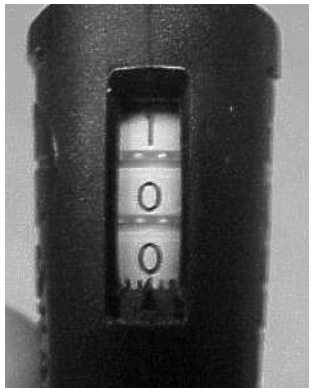
3 Ejection button
Tip ejection.

4 Adjustment opening
For inserting the wrench to make volume adjustments.

5 Ejection sleeve
To extract liquids from long vessels, the ejection sleeve may be pulled off when the ejection button is held down.

Figure 1: Illustration of a generic micropipette.

Micropipette volumes



P-20

1	Tens of microliters
0	Ones of microliters
5	Tenths of microliters

P-200

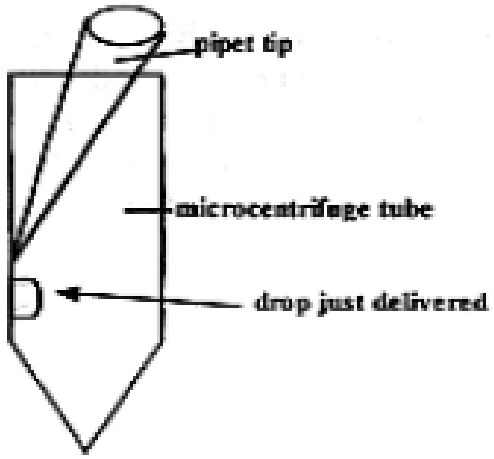
1	Hundreds of microliters
5	Tens of microliters
1	Ones of microliters

P-1000

0	Thousands of microliters
5	Hundreds of microliters
1	Tens of microliters

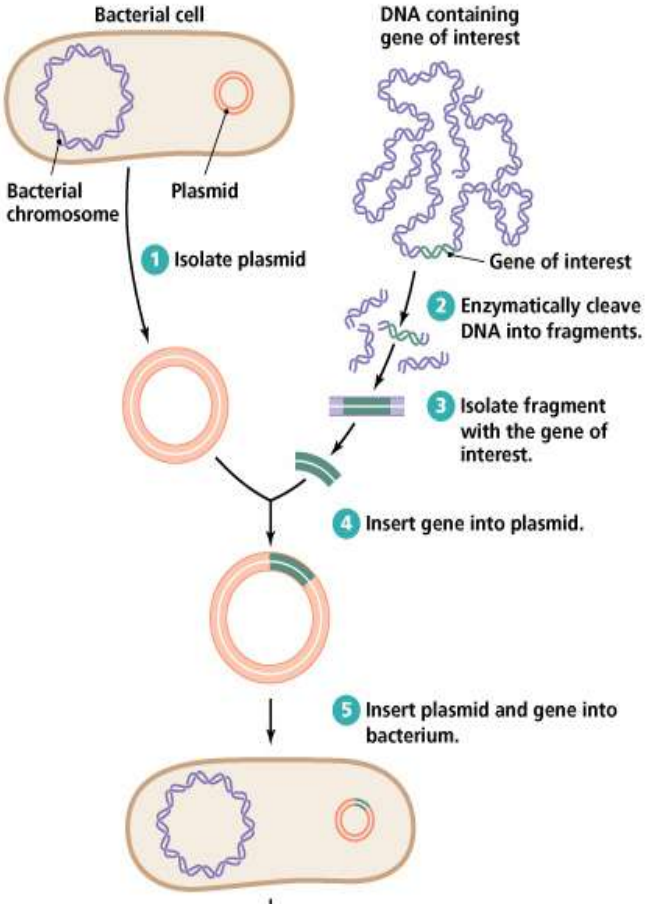
Micropipette

E. Small Volumes Technique: With small volumes, especially the 1-10 ul range used in molecular biology protocols, you must keep track of the droplets you pipet. Carefully expell the liquid droplet *on the side wall* of the tube so that you can see it, drawing the tip away/out carefully BEFORE releasing the plunger.



Recombinant DNA

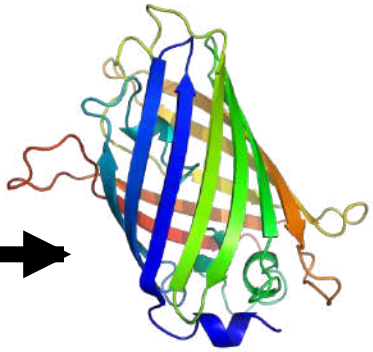
- Recombinant DNA technology: the process of cutting and combining DNA that contains genetic information from two different species of organisms
- Restriction enzyme: an enzyme that recognizes a specific short DNA sequence and cuts DNA internally on both strands
- DNA ligase: an enzyme that joins DNA molecules



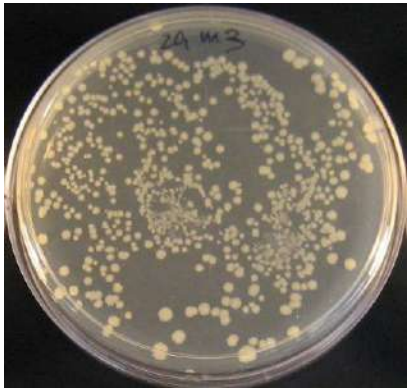
Green Fluorescent Protein (GFP)

Protein from jellyfish *Aequoria victoria*

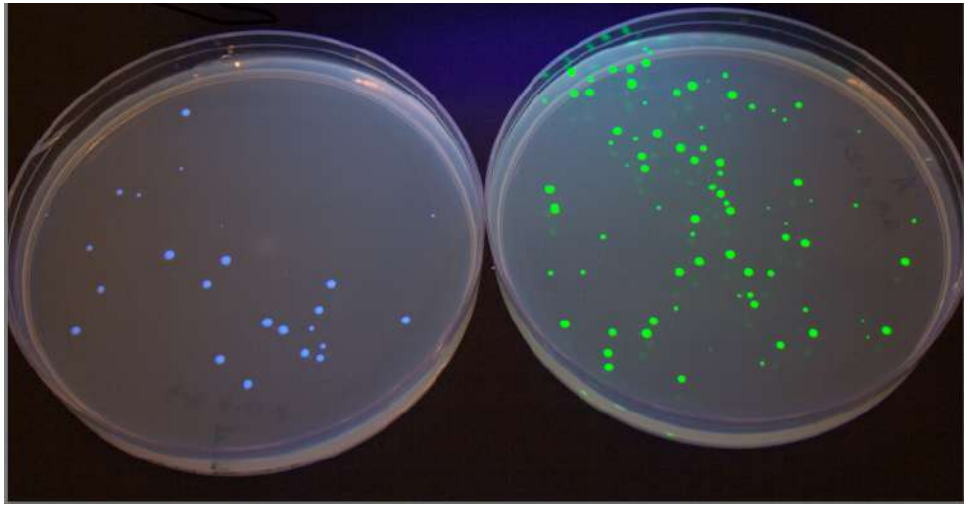
GFP



E. coli

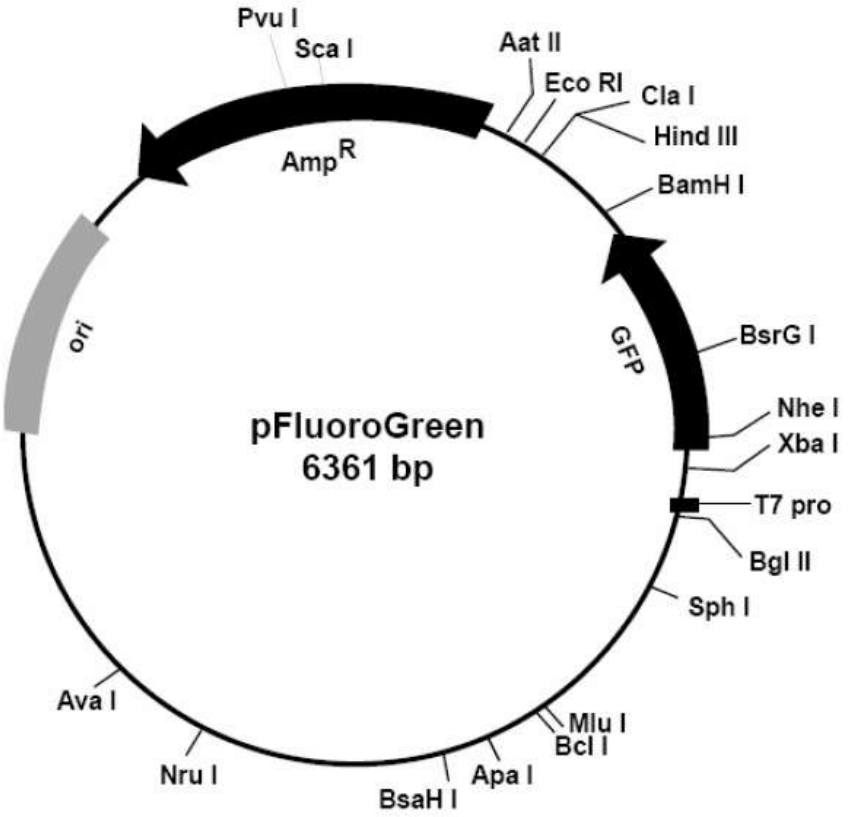


Aequoria victoria



Plasmid

- circular DNA molecule found in bacteria
- contains antibiotic resistance gene
- can contain genes from foreign organisms



Bacterial transformation

- Process in which bacteria takes up and expresses DNA from outside the cell

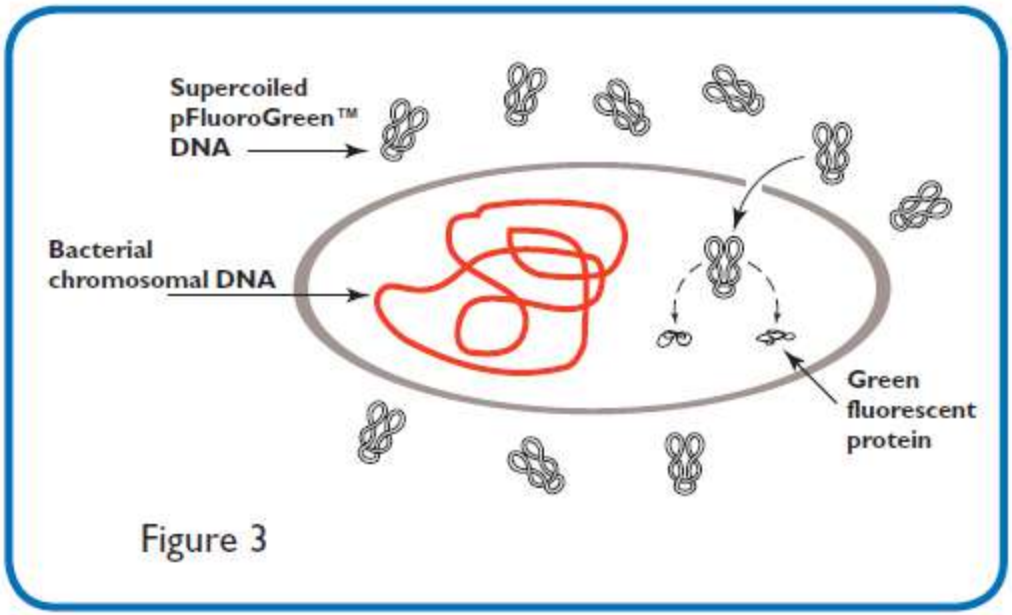
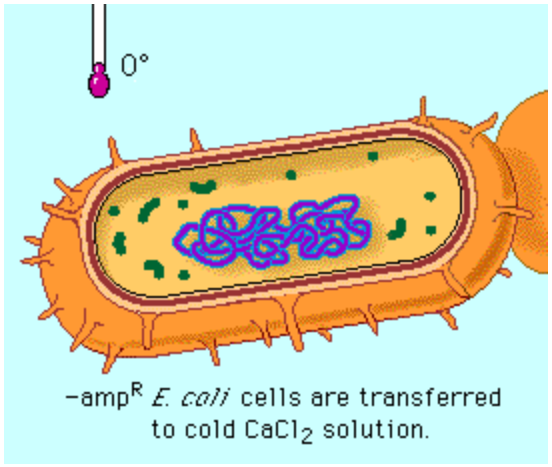
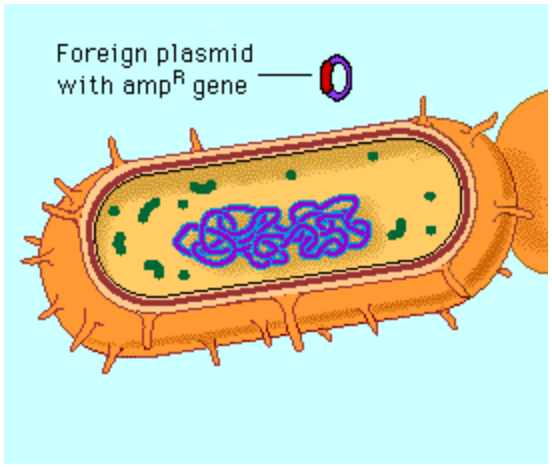


Figure 3

Bacterial Transformation



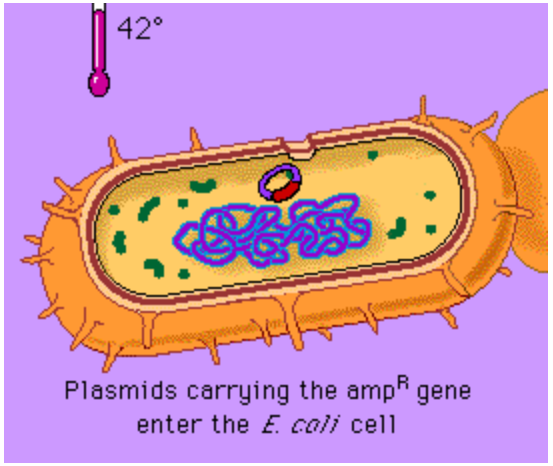
Mix E. coli cells in $CaCl_2$



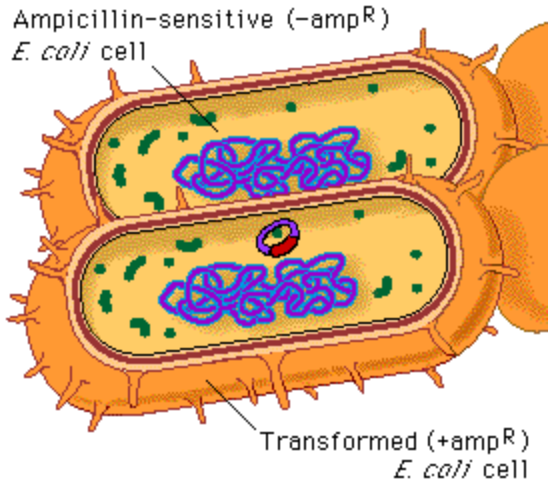
Add plasmid DNA

Pearson LabBench Activity
http://www.phschool.com/science/biology_place/labbench/lab6/tranproc.html

Bacterial Transformation



Heat shock: short, sudden exposure to heat to induce intake of DNA



Recovery: allow E. coli cells to grow

Pearson LabBench Activity
http://www.phschool.com/science/biology_place/labbench/lab6/tranproc.html

Bacterial Transformation

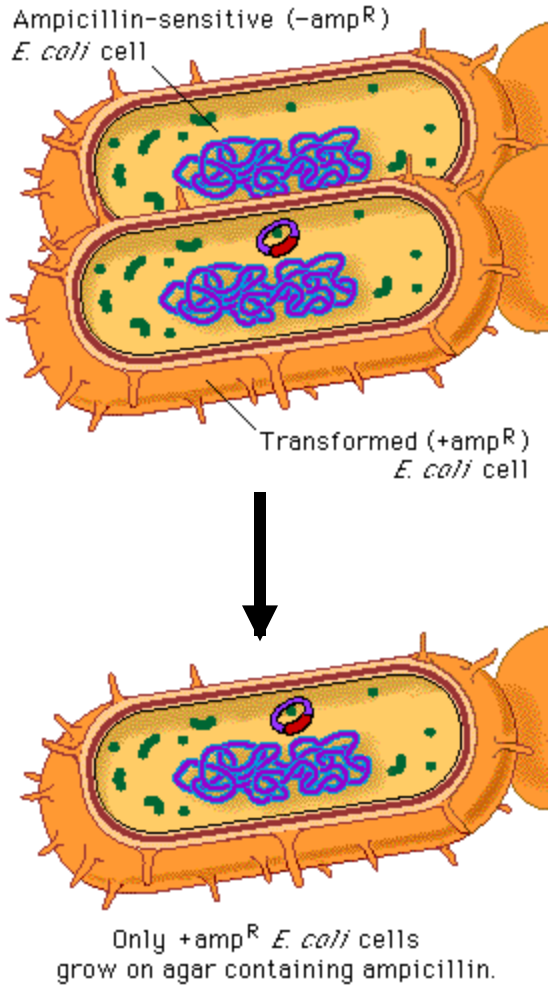
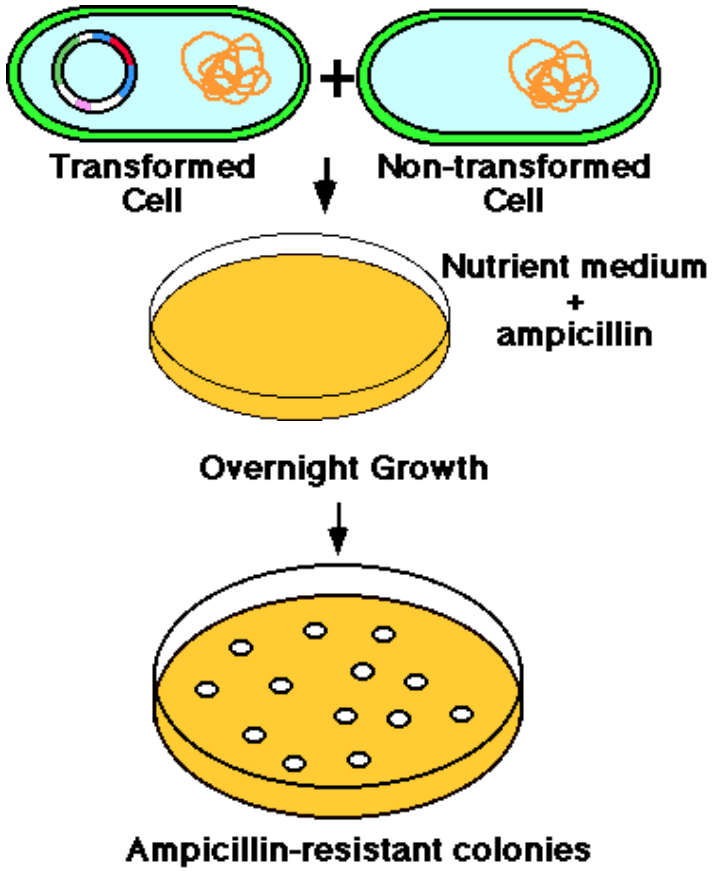
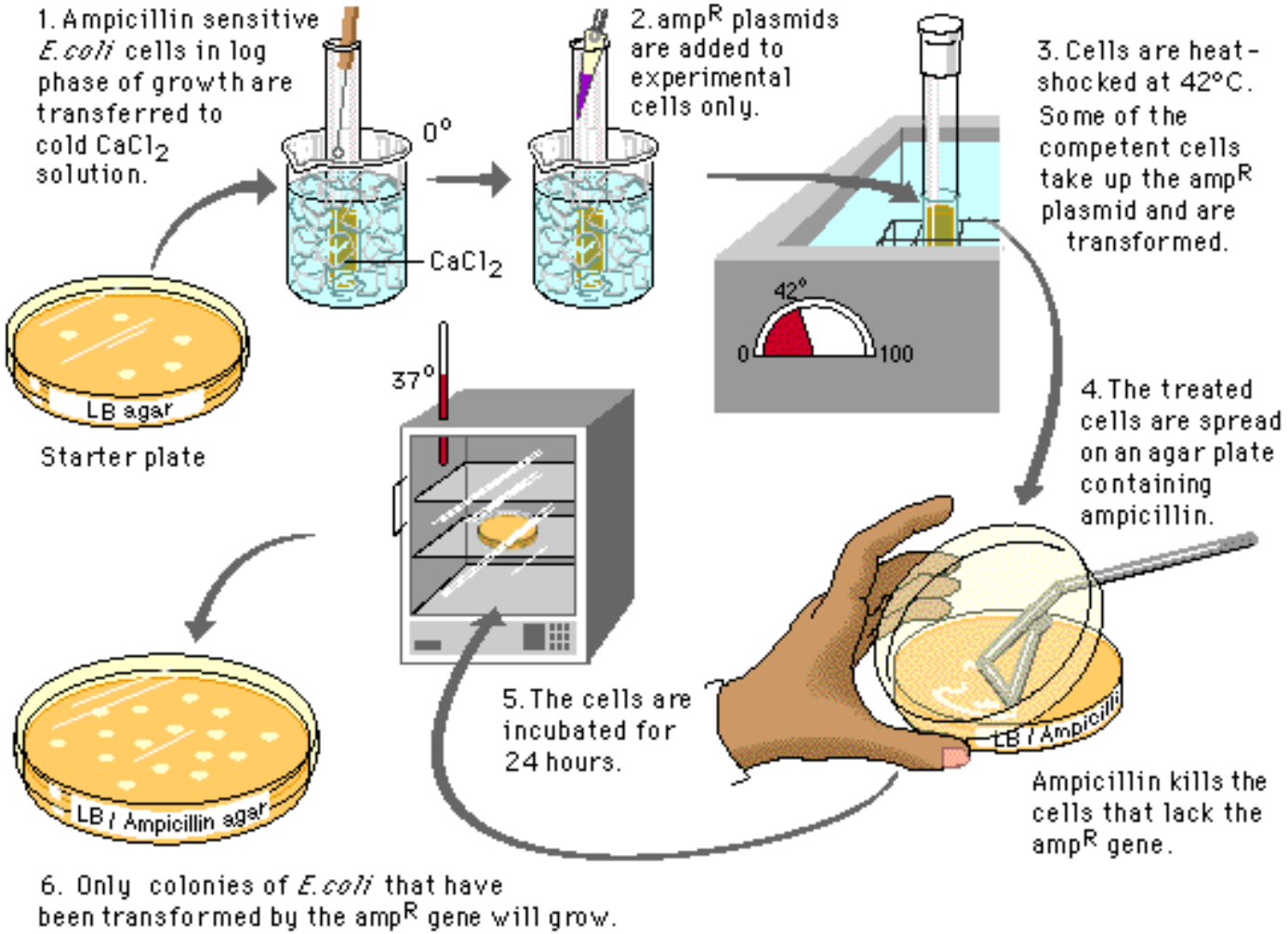


Plate cells on ampicillin medium



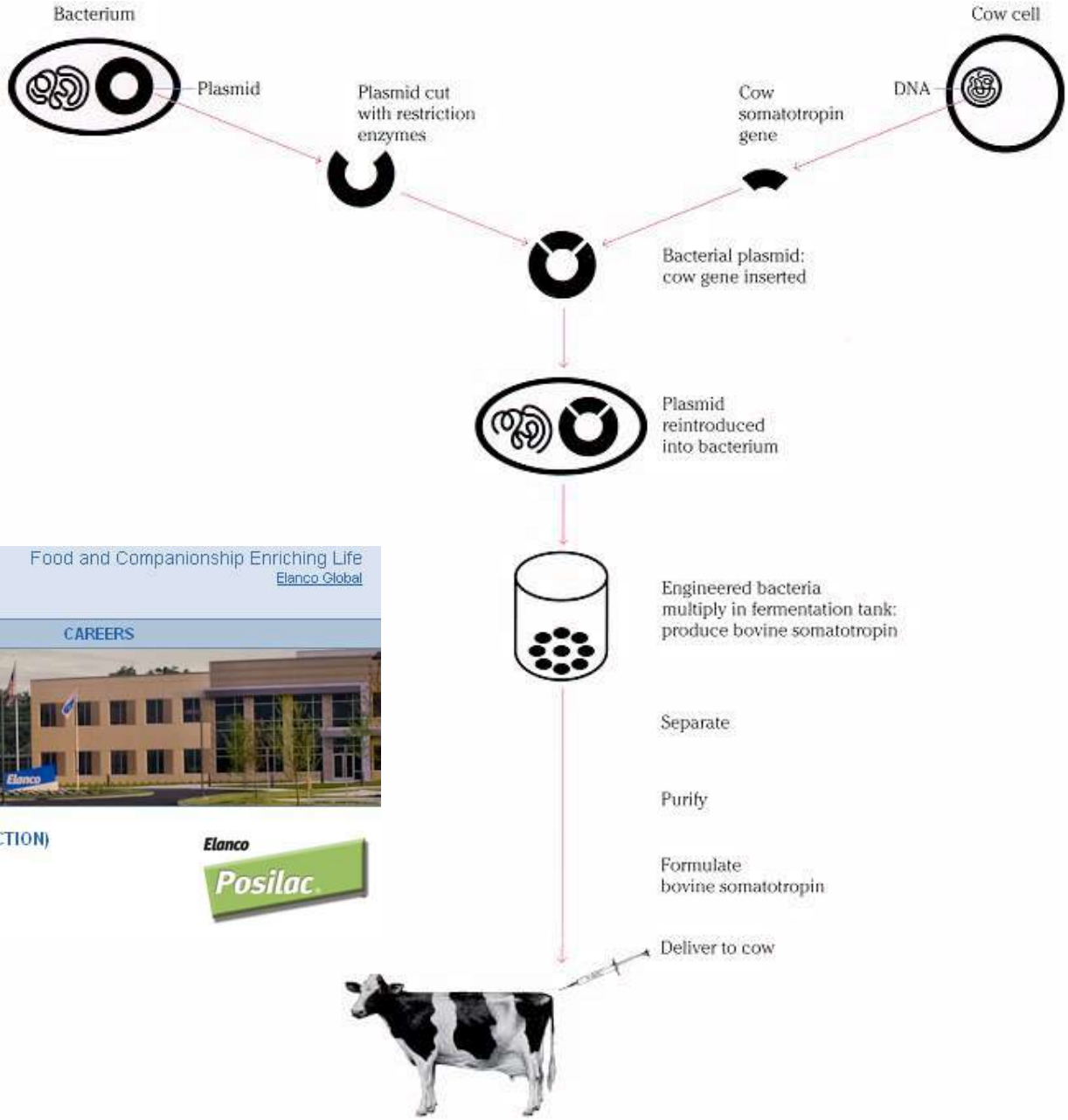
Pearson LabBench Activity
http://www.phschool.com/science/biology_place/labbench/lab6/tranproc.html

Bacterial Transformation



http://www.phschool.com/science/biology_place/labbench/lab6/tranproc.html

Recombinant bovine growth hormone (rBGH)



Transformation efficiency

$$\frac{\text{Number of transformants}}{\mu\text{g of DNA}} \times \frac{\text{final vol at recovery (ml)}}{\text{vol plated (ml)}} = \text{Number of transformants per } \mu\text{g}$$

Example:
40 colonies
0.05 ug (50 ng used)
0.5 mL total volume
0.25 mL plated

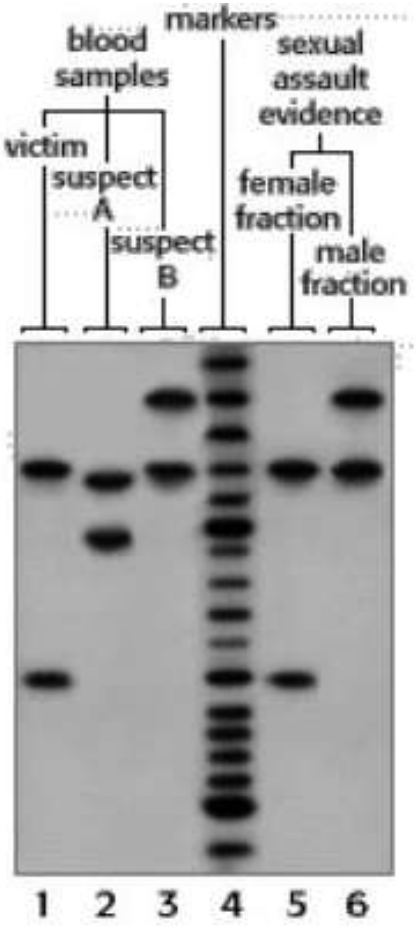
Example:

Assume you observed 40 colonies:

$$\frac{40 \text{ transformants}}{0.05\ \mu\text{g}} \times \frac{0.5\ \text{ml}}{0.25\ \text{ml}} = 1600\ (1.6 \times 10^3)\ \text{transformants per } \mu\text{g}$$

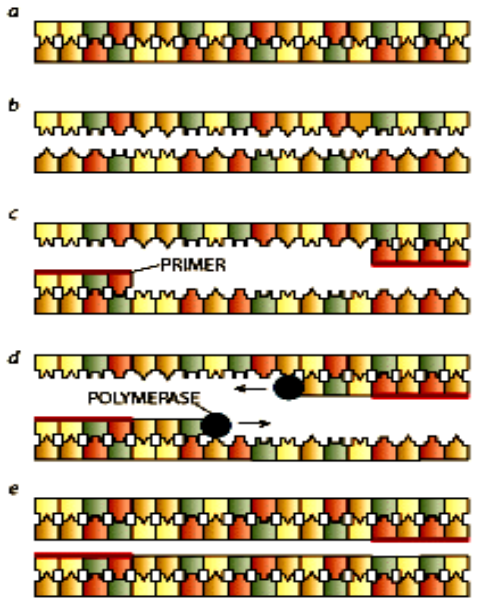
DNA Profiling (DNA fingerprinting)

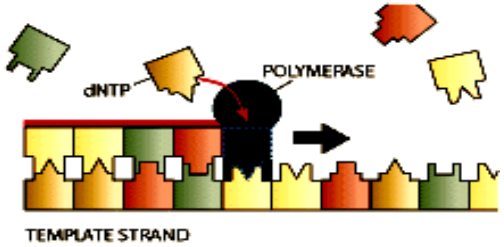
- Analysis of fragments prepared from the DNA of an individual, which can then be used to distinguish that individual from another.



DNA Profiling by PCR

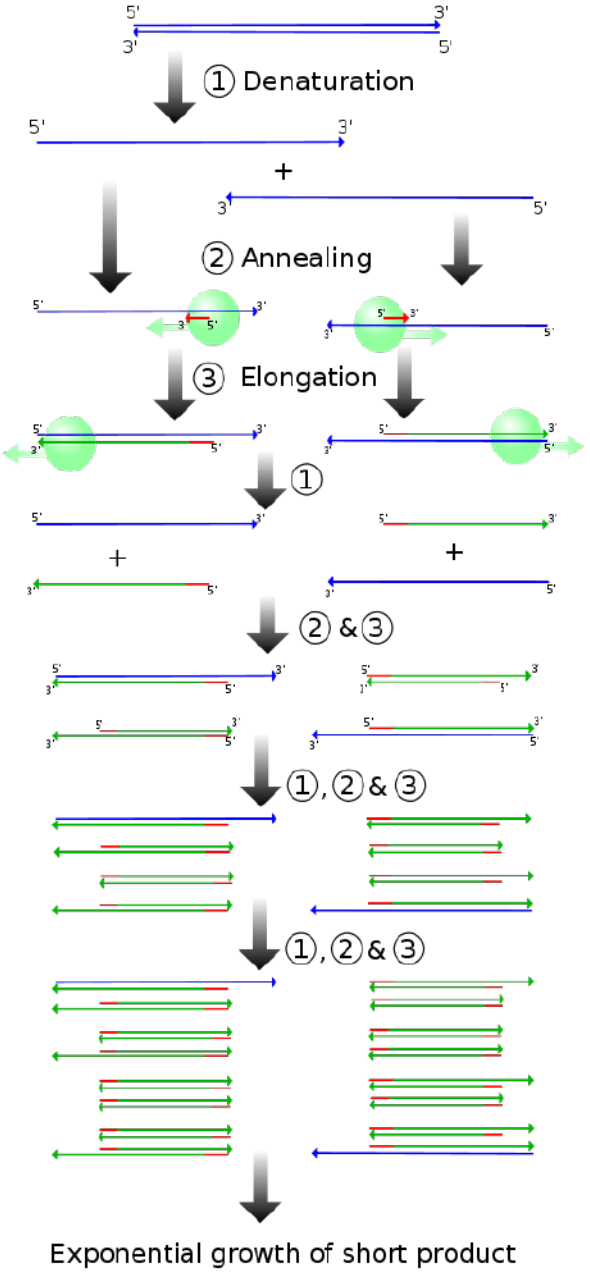
- PCR = polymerase chain reaction
  - Analysis of DNA sample by making many copies of specific DNA sequences





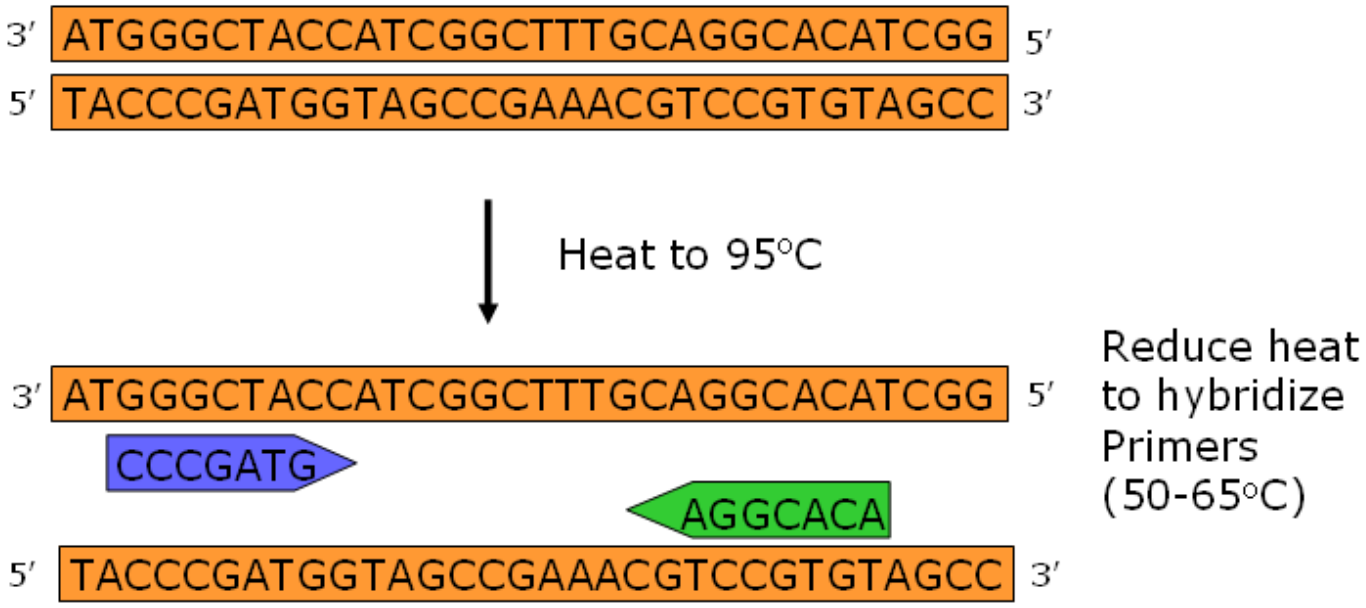
PCR

- Denaturation
- Annealing
- Elongation
- Repeat cycle



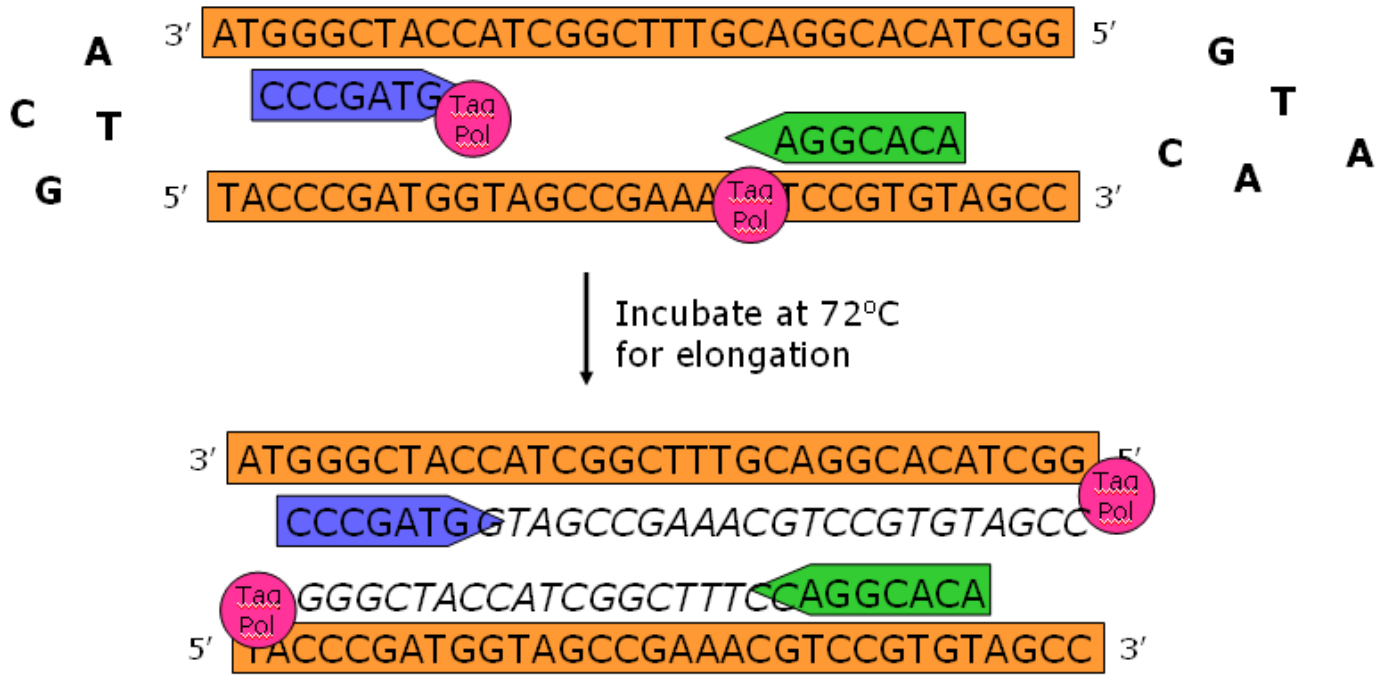
PCR Steps

1. Denaturation of Template DNA
2. Annealing of Primers

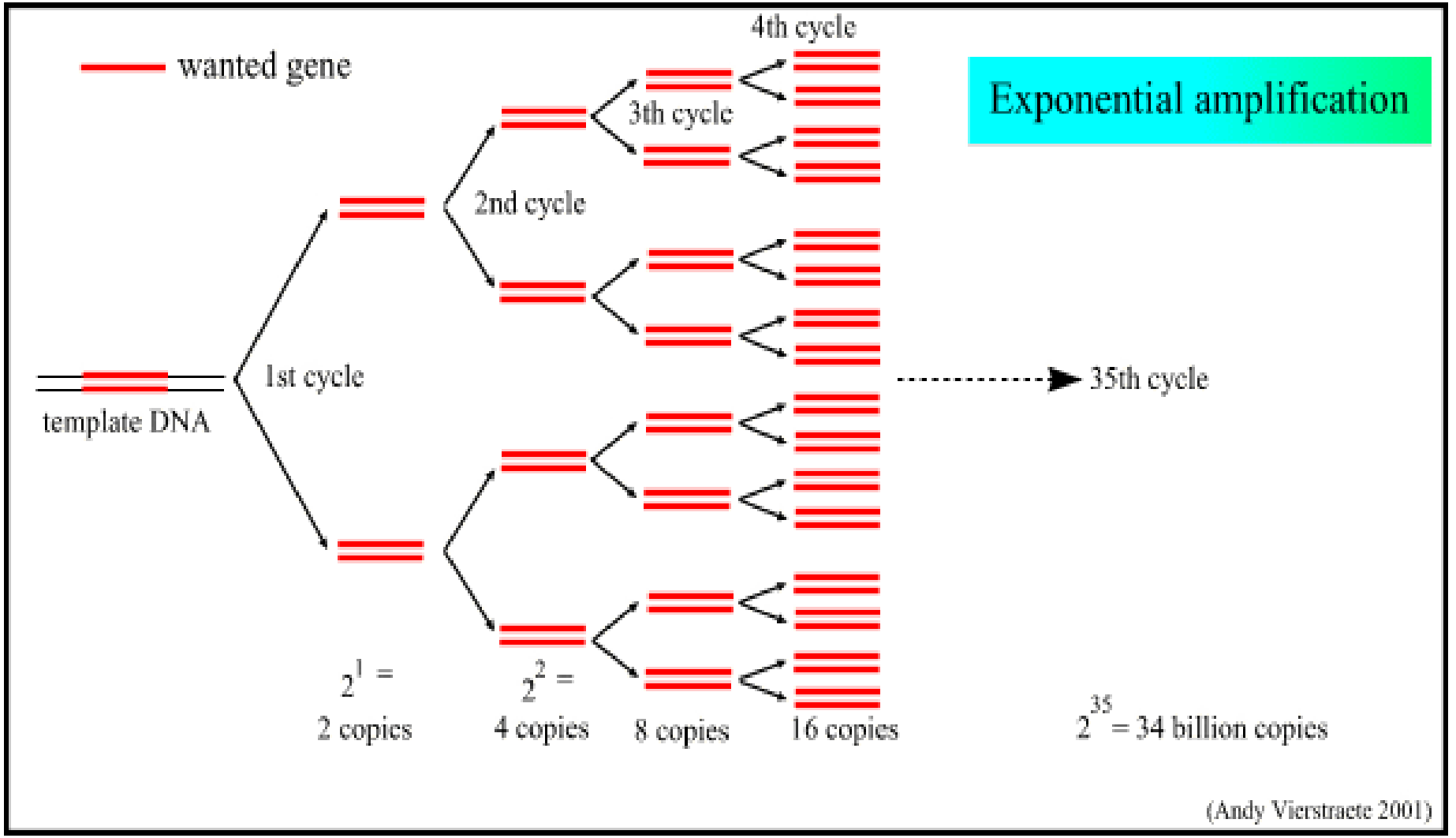


PCR Steps

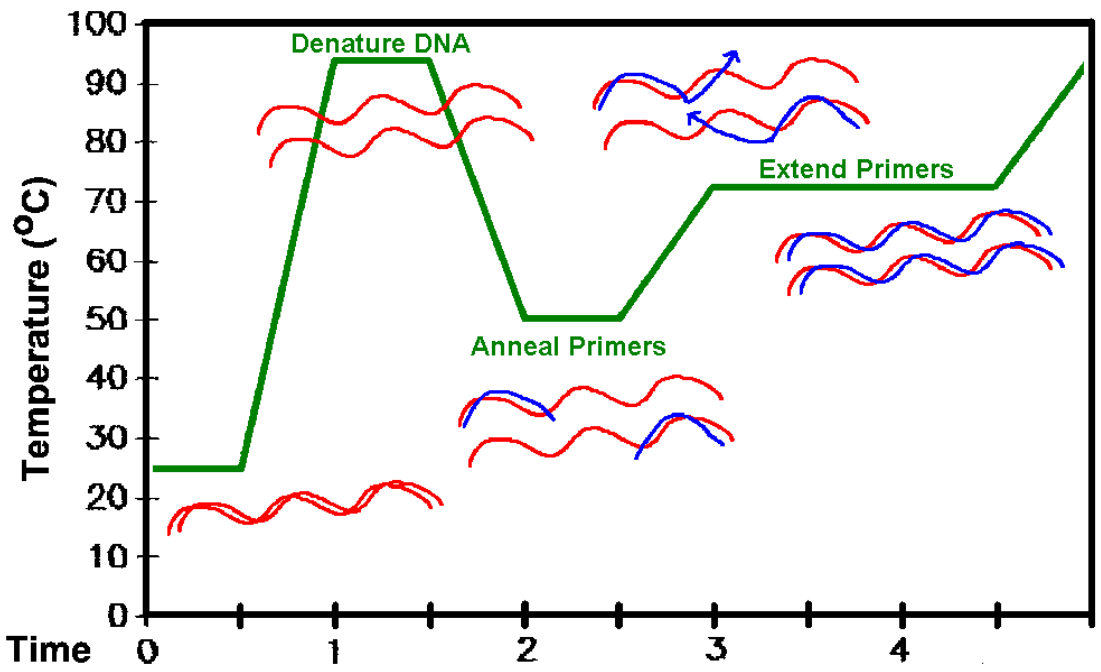
3. Incubate with DNA Polymerase and Nucleotides
4. Repeat Cycle



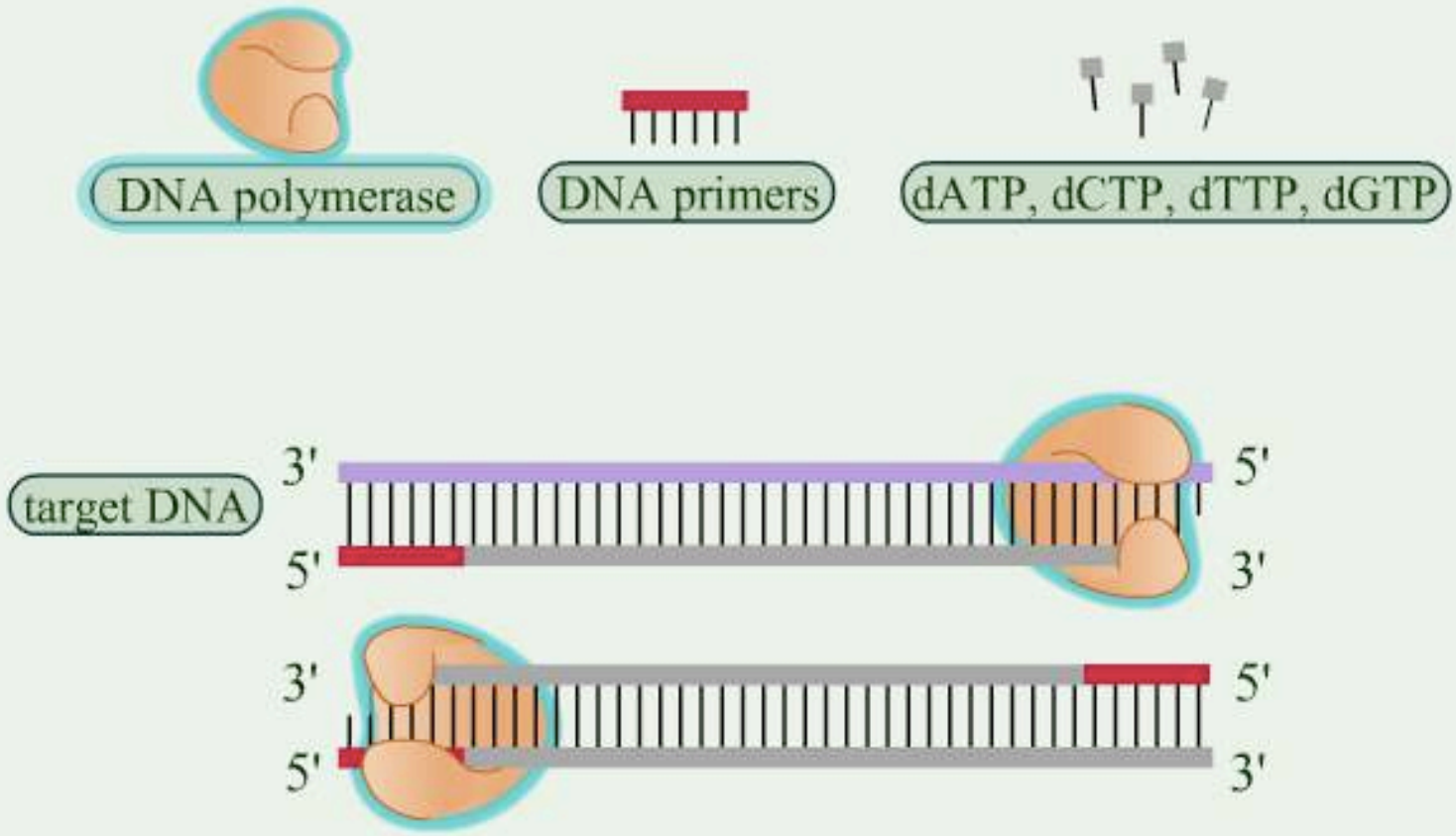
PCR amplification



PCR temperature cycling



PCR components

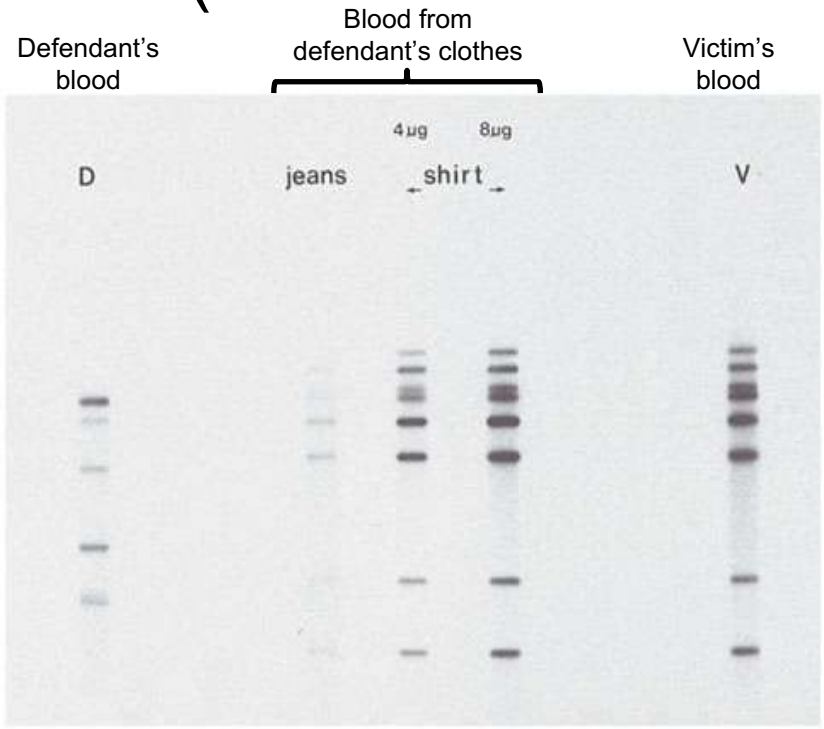


PCR components

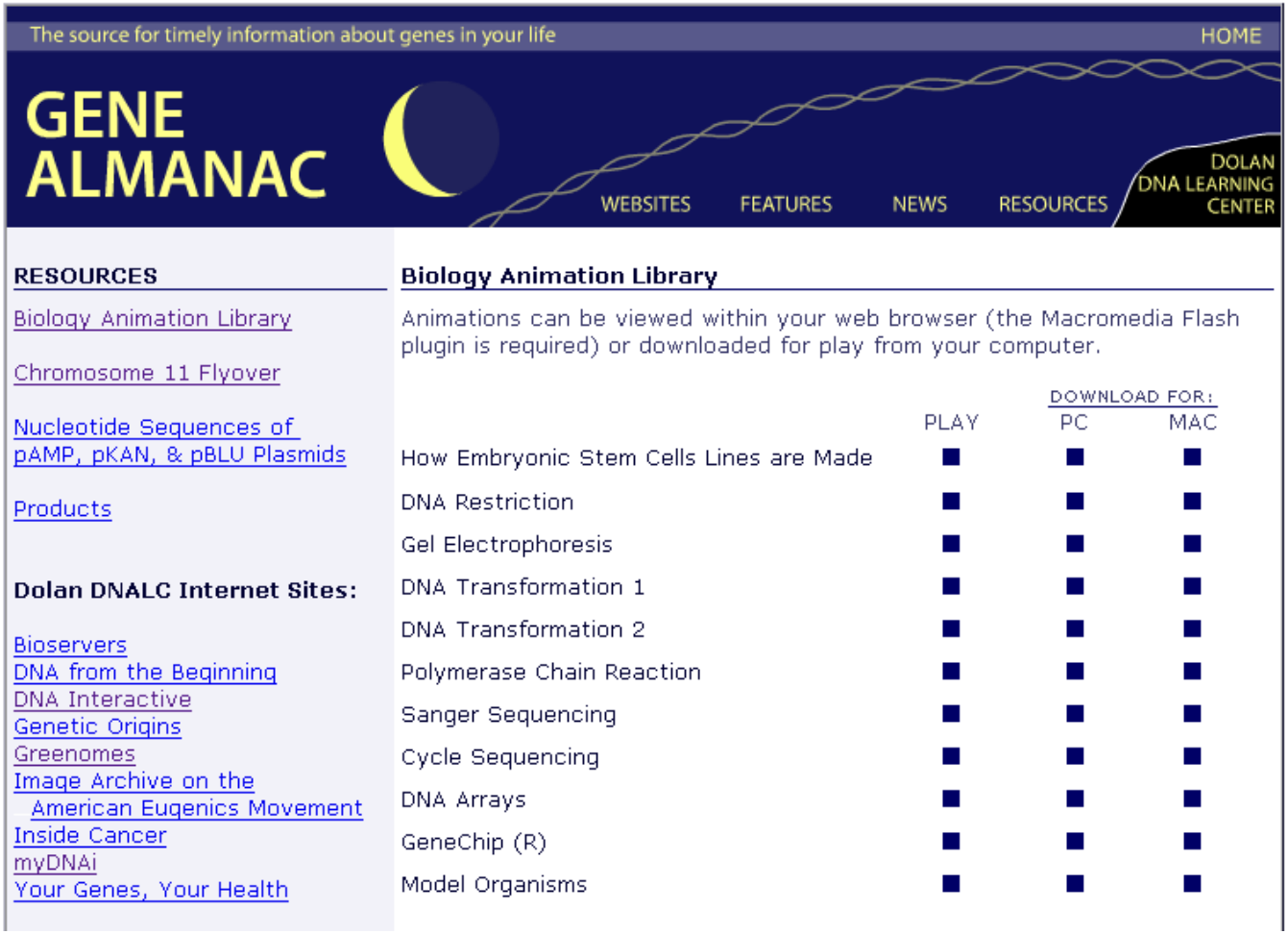
- DNA polymerase – enzyme involved in replication (heat-stable Taq polymerase - from *Thermus aquaticus*)
- Target DNA - DNA molecule to be replicated
- Primers – short, single-stranded DNA sequences complementary to the ends of the region to be amplified
- Nucleotides – bases incorporated into new strand of DNA (A,T,C,G)

PCR Applications

- Cloning
- Sequencing
- Forensics
- Diagnostics (disease and infection)



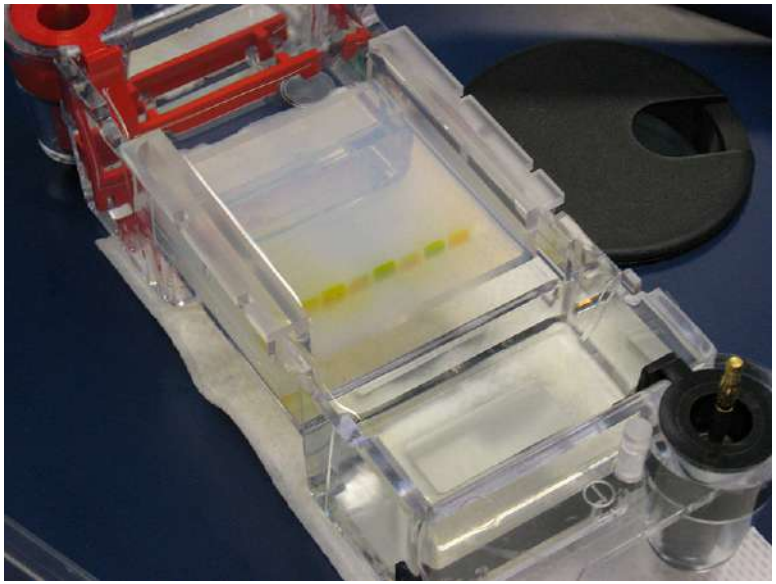
Dolan DNA Learning Center – Biology Animation Library



http://www.dnalc.org/ddnalc/resources/animations.html

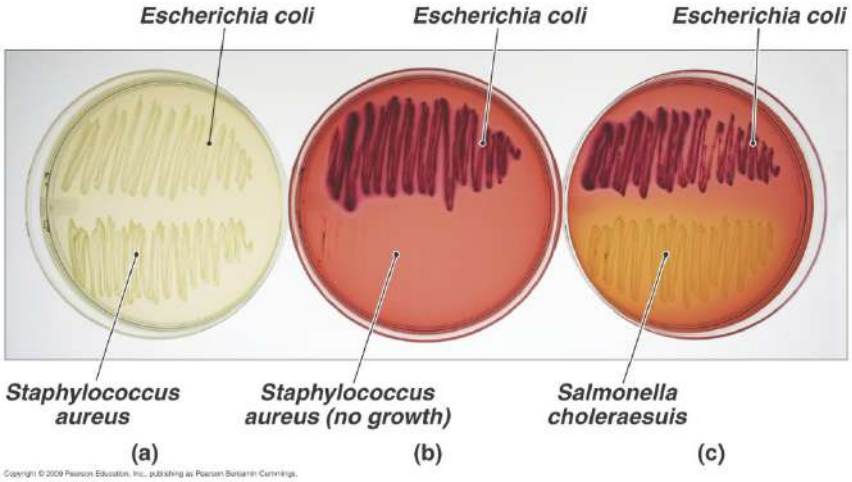
Electrophoresis

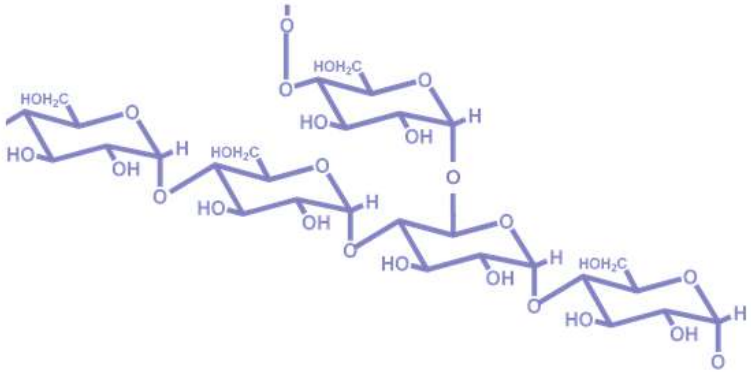
- Technique that uses an electric current to separate molecules based on charge, size, or shape.
- Semi-solid matrix through which molecules migrate



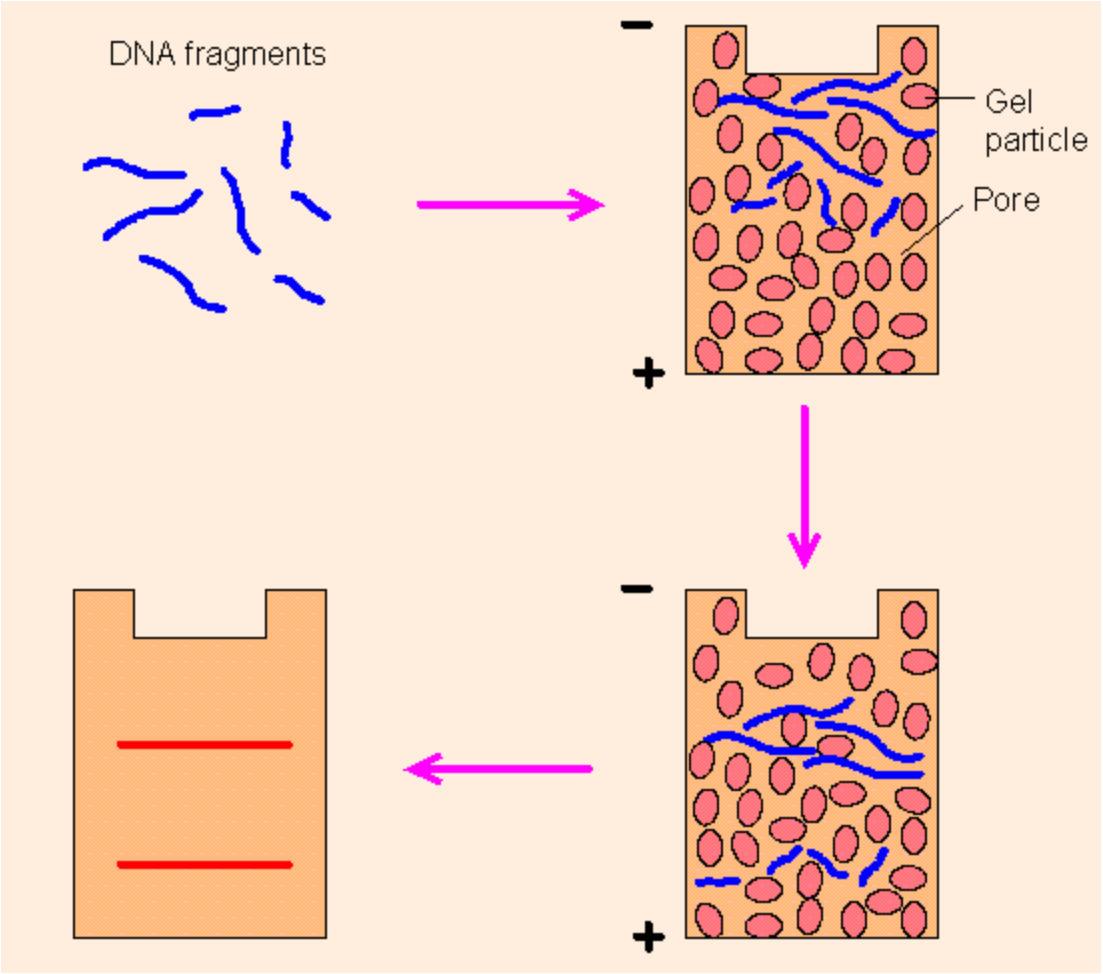
Electrophoresis

- Agarose: polysaccharide derived from red algae.
  - Purified form of agar



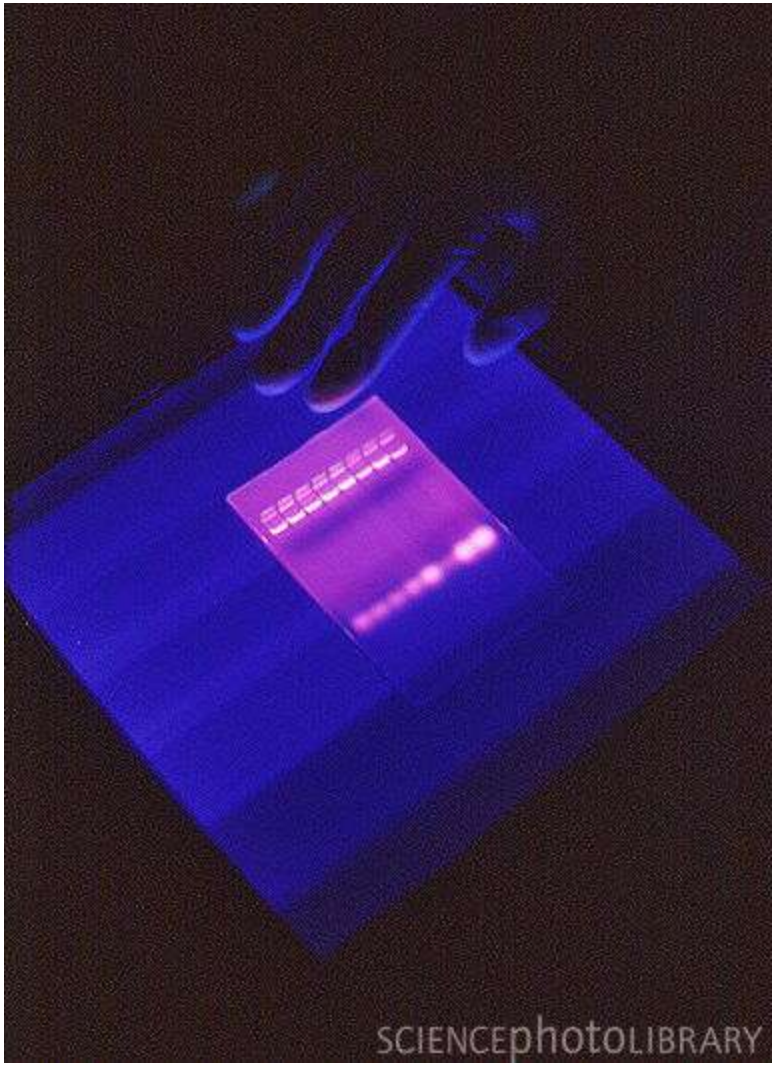


Agarose gel electrophoresis

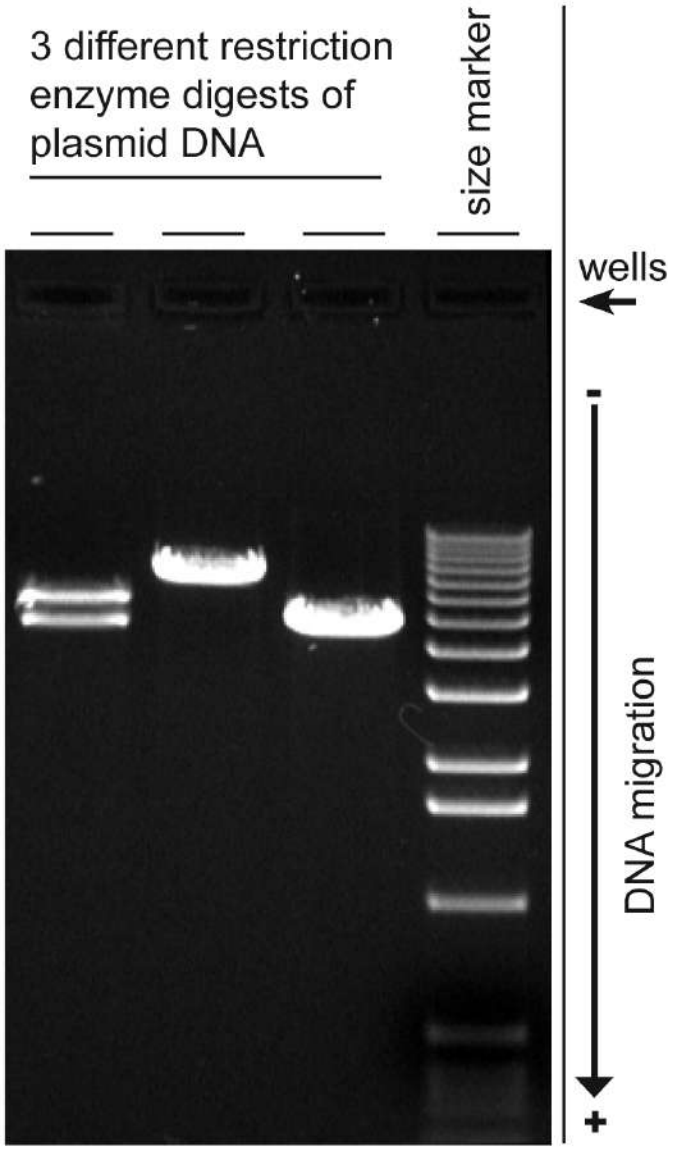


Agarose gel electrophoresis

- Visualization of DNA
  - Ethidium bromide
    - Intercalates between DNA bases
    - Fluorescence by UV light
  - OR other fluorescent dyes (EZ Vision Dye)



DNA Electrophoresis

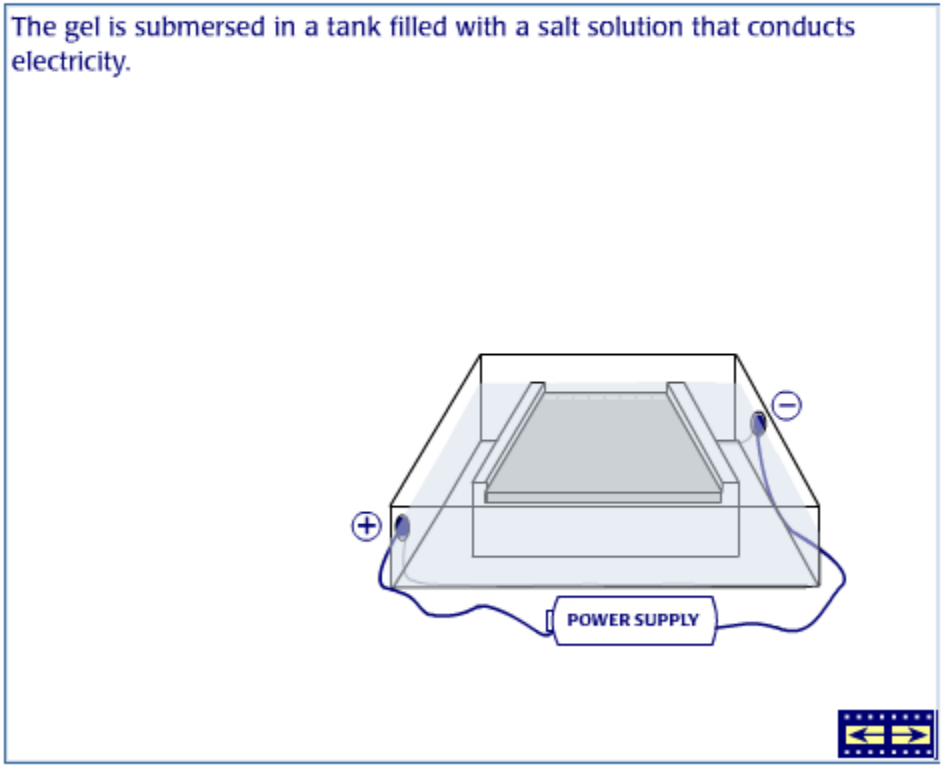


Dolan DNA Learning Center
Gel electrophoresis

Biology Animation Library

Gel Electrophoresis

In the early days of DNA manipulation, DNA fragments were laboriously separated by gravity. In the 1970s, the powerful tool of DNA gel electrophoresis was developed. This process uses electricity to separate DNA fragments by size as they migrate through a gel matrix.



Download for PC Download for Mac

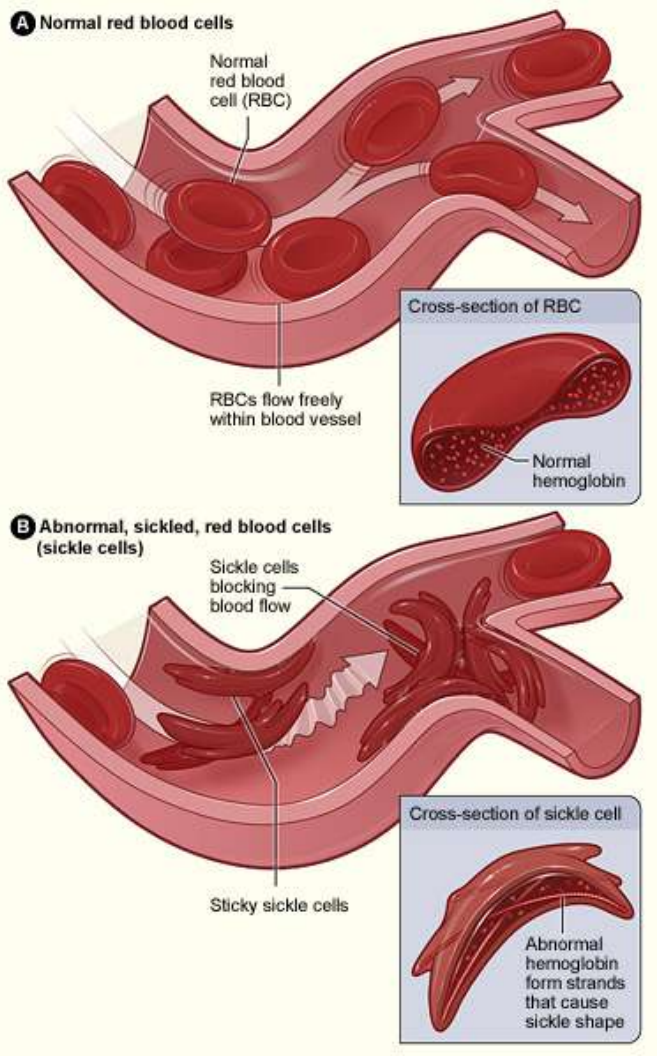
http://www.dnalc.org/resources/animations/gelelectrophoresis.html

Sickle cell anemia

- Most common inherited blood disorder in the United States
- Red blood cells form sickle cells
- Sickle cells caused by abnormal form of hemoglobin (hemoglobin S)
- Hemoglobin S results from mutation in hemoglobin beta gene

Sickle Cells

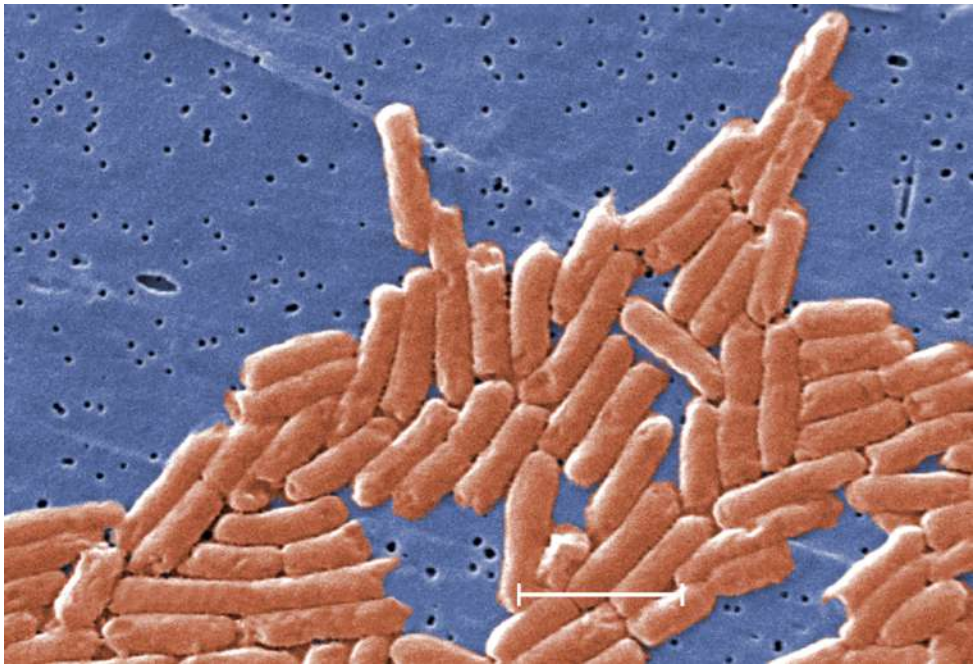
- Normal red blood cells vs. sickle cells in blood vessels



National Heart Lung and Blood Institute
http://www.nhlbi.nih.gov/health/dci/Diseases/Sca/SCA_WhatIs.html

Salmonella

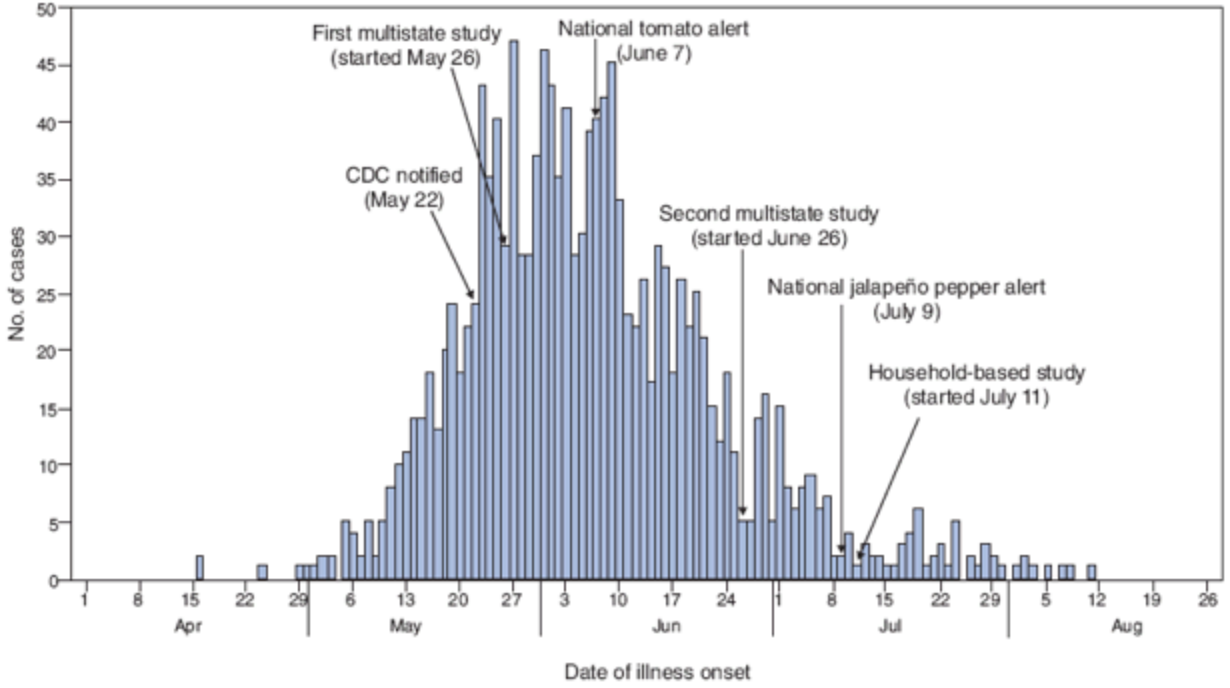
- Salmonella – group of bacteria that can cause gastrointestinal illness
- Most common in US – Salmonella serotype Typhimurium and Salmonella serotype Enteritidis



CDC Public Health Image Library

Salmonella
April – August 2008 Outbreak

- 1442 people infected
- At least 286 hospitalized
- Possibly two deaths



* Includes cases with onset information received as of August 25, 2008. Some illness onset dates (n = 366) were estimated by subtracting 3 days from the specimen date. Illness that began during July 29–August 25 might not yet be reported.

Salmonella outbreak 2008

FDA Statement

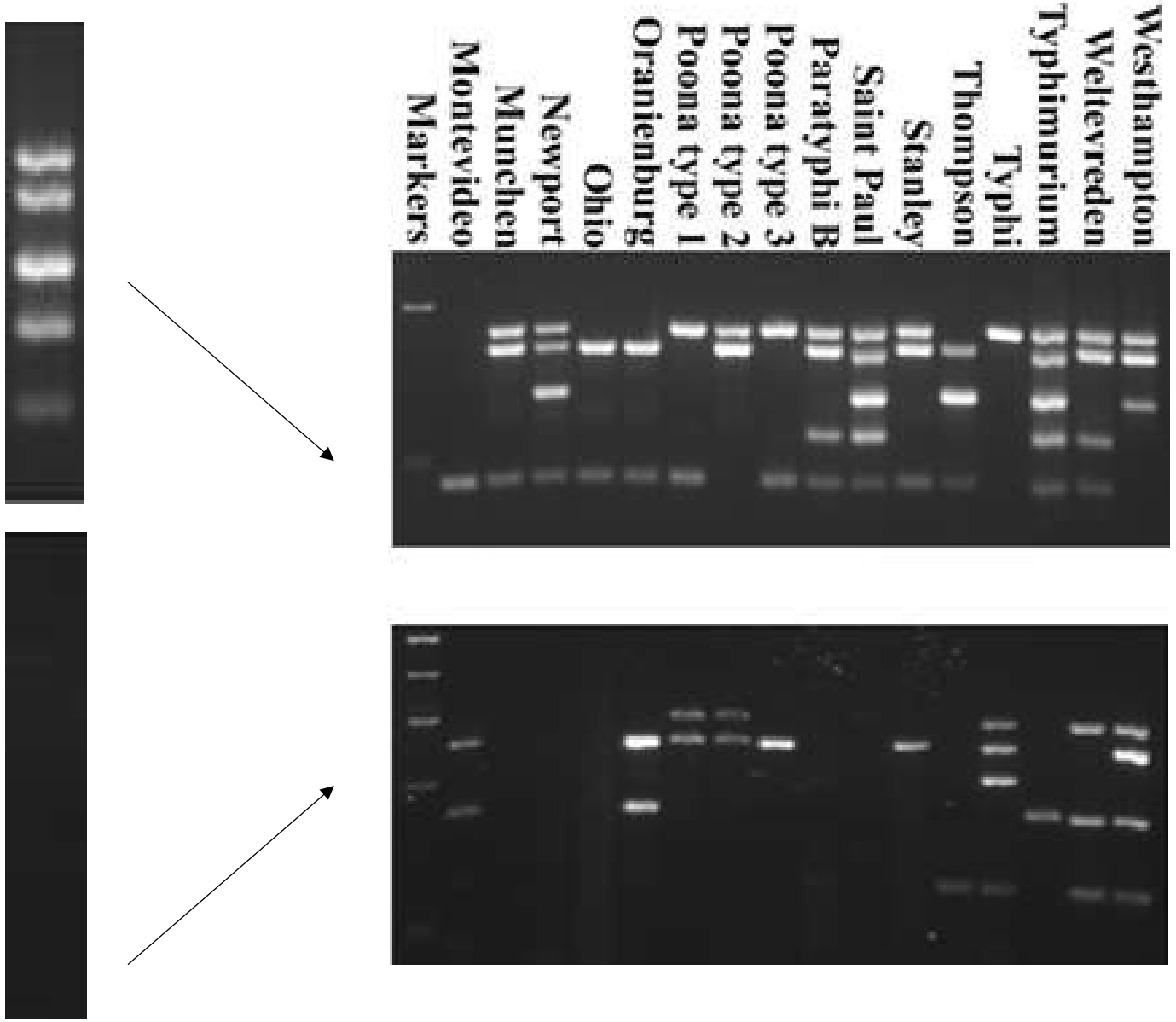
FOR IMMEDIATE RELEASE
Statement
July 30, 2008

Media Inquiries:
Karen Riley, 301-827-6244
Consumer Inquiries:
888-INFO-FDA

FDA Extends Consumer Warning on Serrano Peppers from Mexico

Source: Photos by Luis A. Solorzano, FDA.

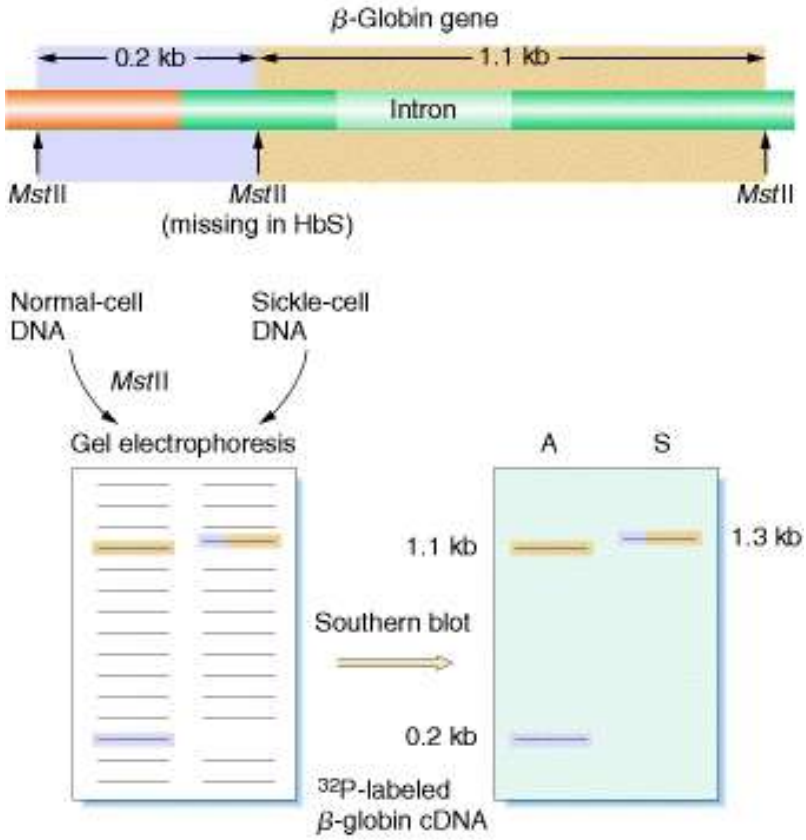
Salmonella DNA profiling



DNA Profiling (DNA fingerprinting)

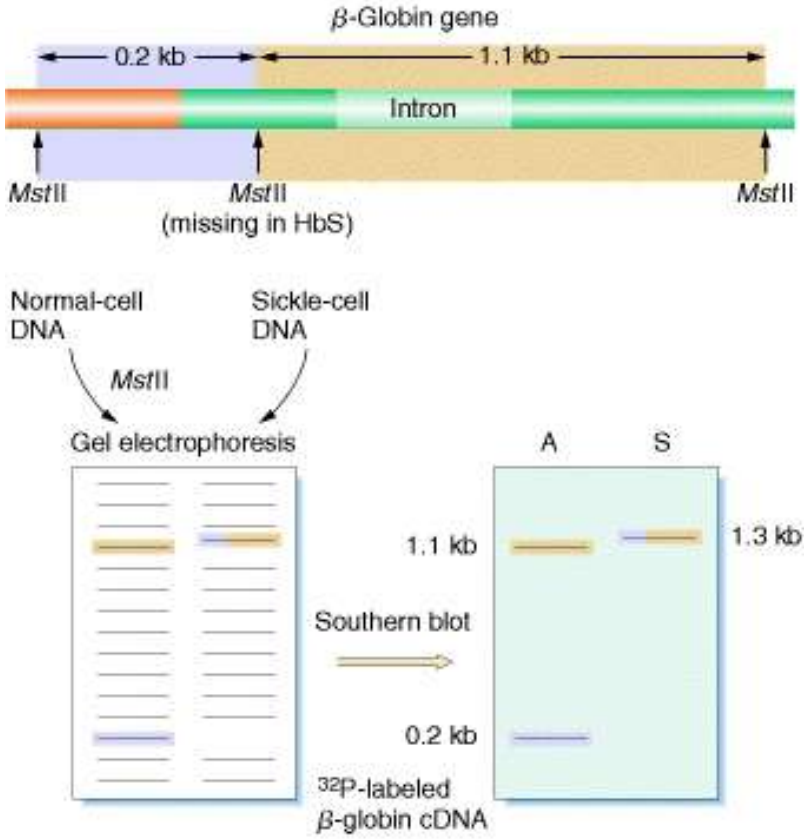
- Analysis of fragments prepared from the DNA of an individual, which can then be used to distinguish that individual from another.

- RFLP = restriction fragment length polymorphism



RFLP = restriction fragment length polymorphism

- Analysis of DNA fragments by using restriction enzymes to cut the DNA.

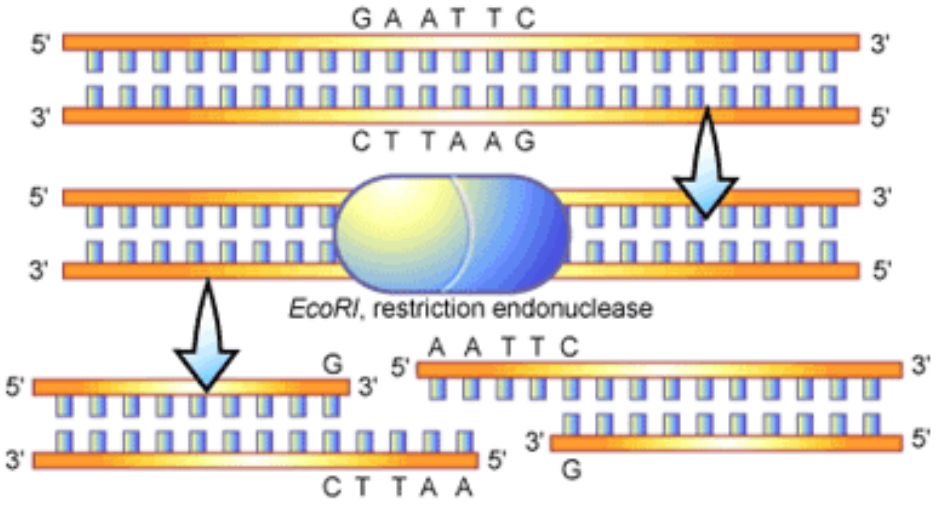


Restriction enzyme

- An enzyme that recognizes a specific short DNA sequence and cuts DNA internally on both strands

Eco RI recognition sequence

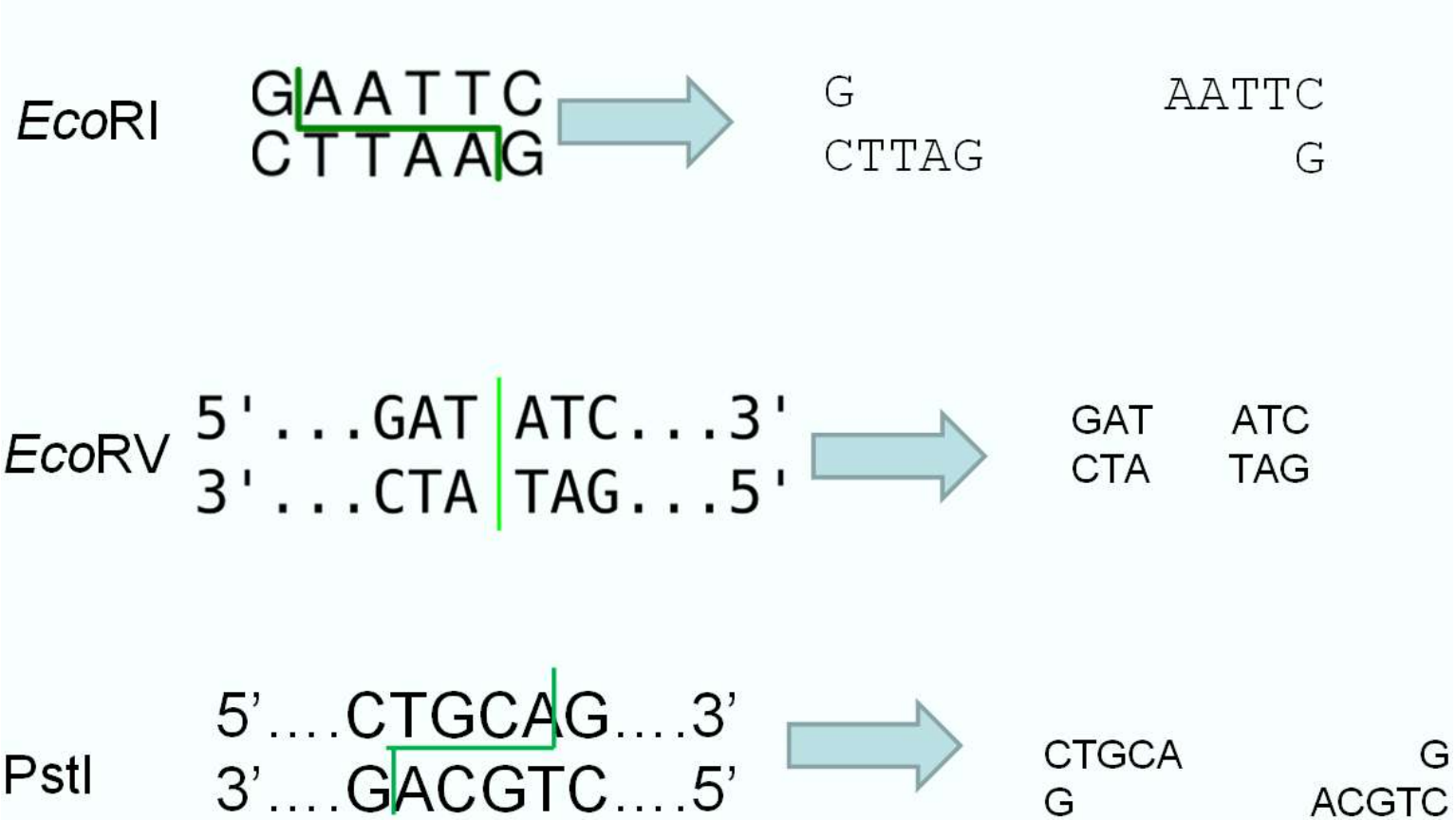
GAATTC
CTTAAG



http://www.scq.ubc.ca/wp-content/endonuclease2.gif

Examples of restriction enzymes

- EcoRI and PstI produce sticky ends
- EcoRV produces blunt ends



Sickle cell anemia mutation

(a)

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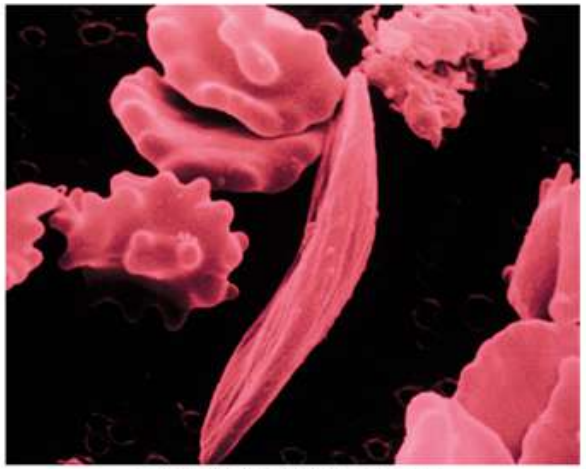

(b)

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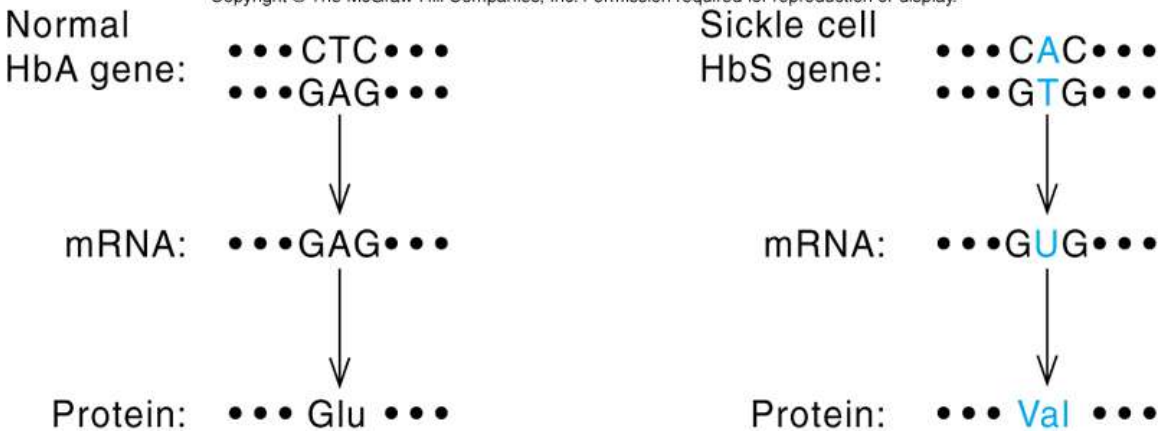
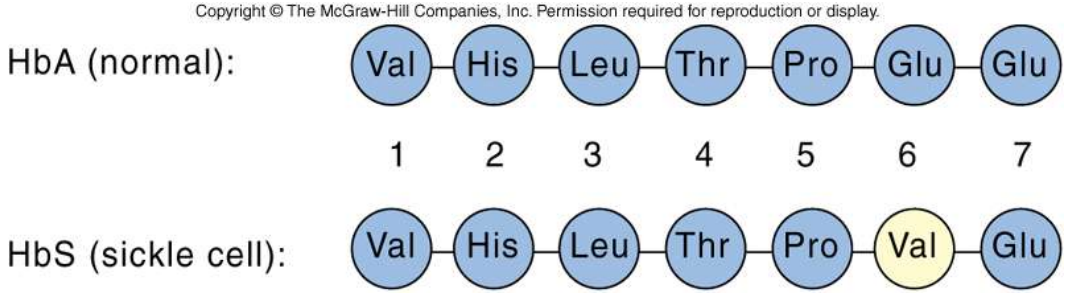
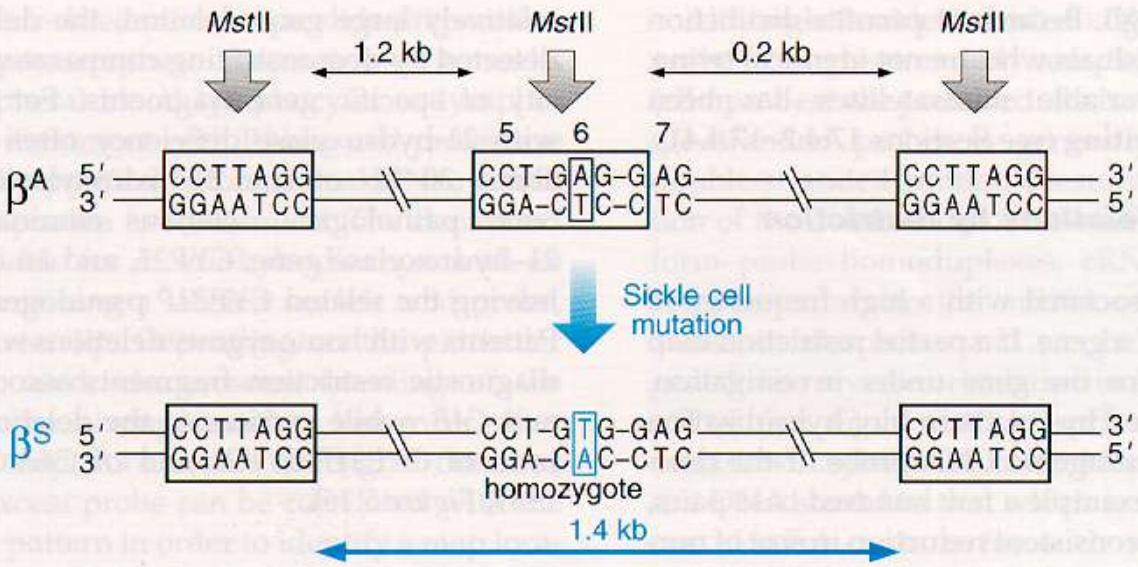
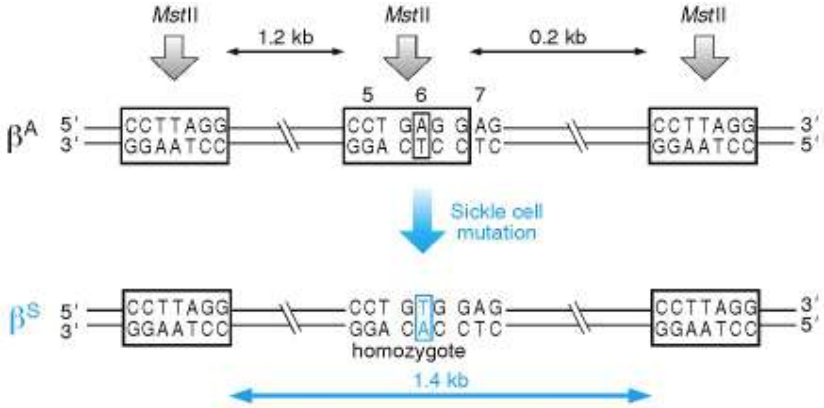




RFLP – Sickle cell anemia

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MstII
0.2 kb
MstII
5 6 7
βA 5′ CCTTAGG CCT GAG GAG CCTTAGG 3′
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Sickle cell mutation
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homozygote
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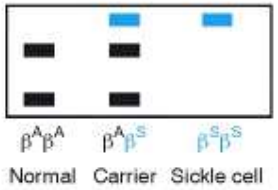
βAβA
βAβS
βSβS
Normal
Carrier
Sickle cell