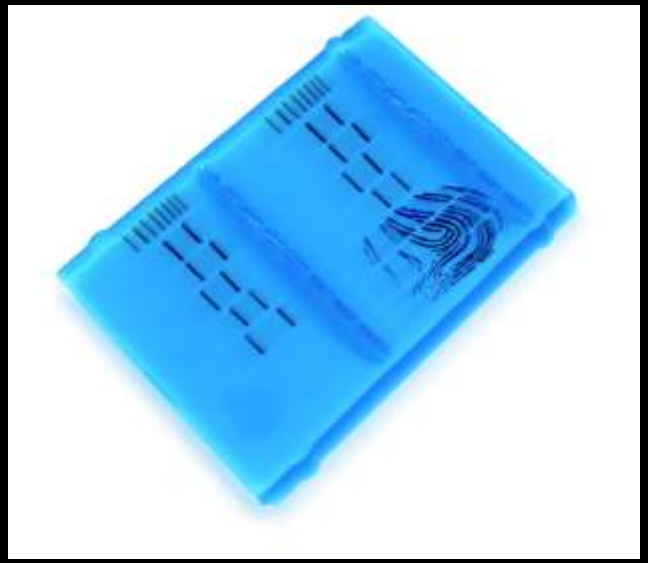
CSI:

CRIME SCENE INVESTIGATION



BRIGHTON HIGH

Overview

- Day 1: Tuesday
  - Introduction to DNA profiling
    - How do we use DNA to solve crimes?
  - Background
    - Polymerase Chain Reaction (PCR)
    - Gel Electrophoresis
  - Set up PCR
- Day 2: Wednesday
  - Make and Run Agarose Gel
  - More Background
    - Polymerase Chain Reaction (PCR)
    - Gel Electrophoresis
  - Stain gel overnight
- Day 3: Thursday
  - Analysis and interpretation of results

Overview

- Background ~30 min
  - DNA profiling
  - Polymerase Chain Reaction (PCR)
  - Gel Electrophoresis
- Lab activity ~50 min
  - PCR

What is DNA profiling

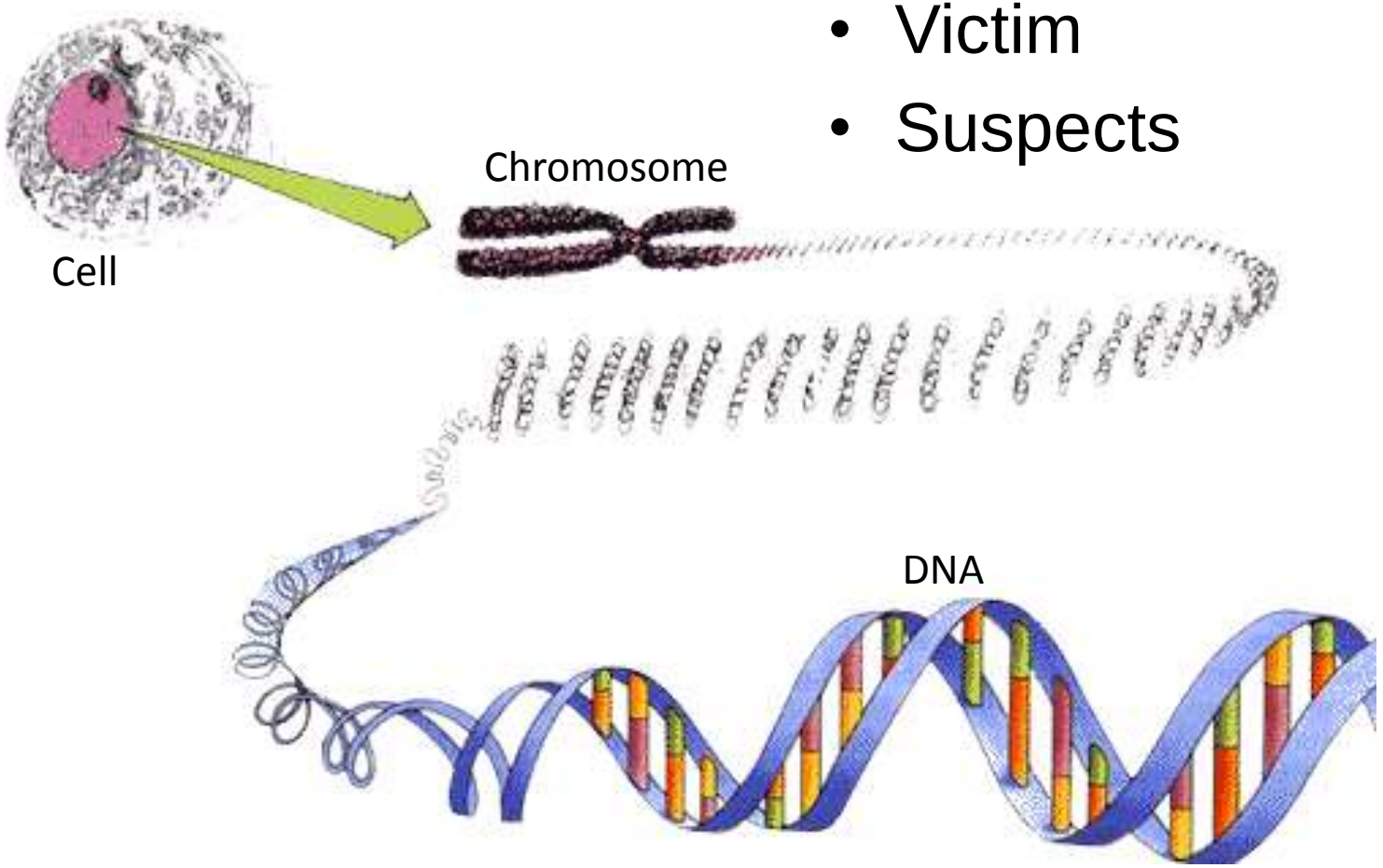
- The use of molecular genetic methods to determine the exact genotype of a DNA sample in a way can distinguish one human being from another.

How do crime scene investigators create a DNA profile?

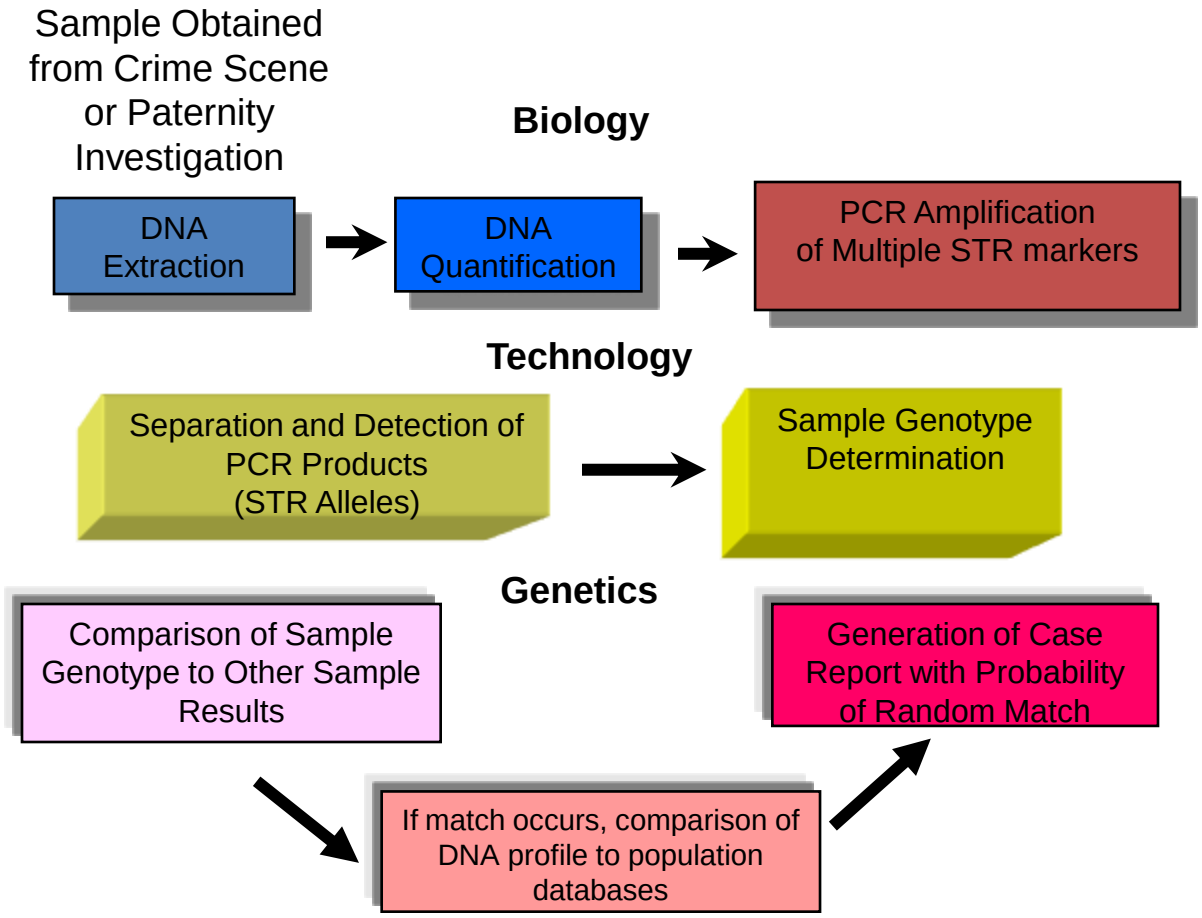
- Step #1 Evidence is collected at the crime scene
- Biological sources: anything that has DNA!

Step #2 DNA is Extracted

- Sources at scene
- Victim
- Suspects



Step #3 DNA samples are processed



How are suspects included or excluded from an investigation?

- Suspects are included in an investigation if their DNA profile matches with genotypes found at the crime scene

- Suspects can be excluded if their DNA profile does not match genotypes found at the crime scene

What part of DNA is used?

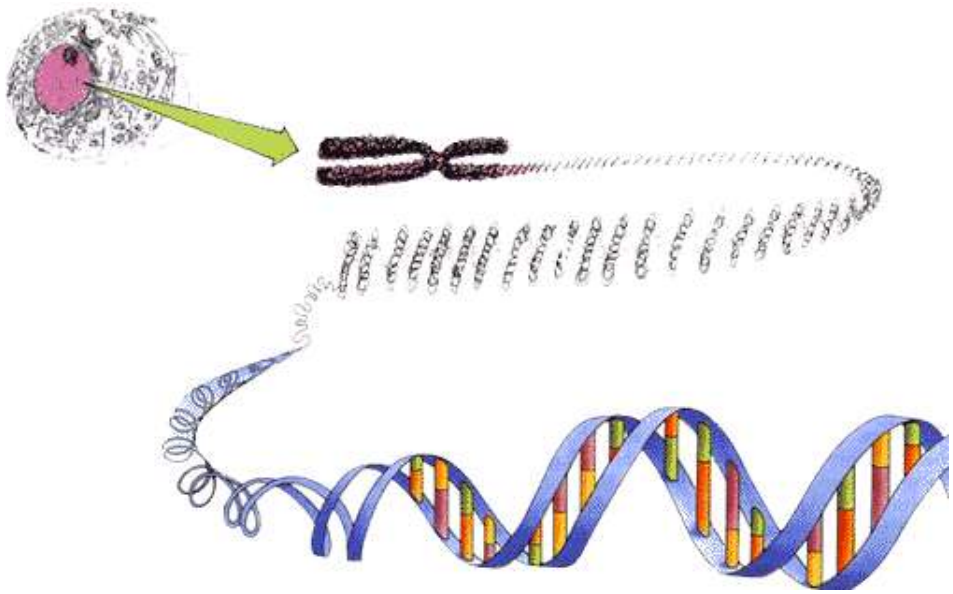
- There are ~3 billion base pairs in the human genome
- >99.5% of human DNA sequences are the same in every person
- Small percentage of DNA is different allowing us to distinguish one individual from another
- Polymorphic DNA – “many forms”
- Do not control any traits, and have no function

Variable Number Tandem Repeat (VNTR)

- DNA profiling uses repetitive ("repeat") sequences that vary a lot
- VNTR loci are very similar between closely related humans. (relatives)
- However, they are so variable that unrelated individuals are extremely unlikely to have the same VNTRs.
- Amplify loci and visualize to see differences

Short Tandem Repeats (STR's)

- Type of VNTR
  - Variable number tandem repeat
- Unique from individual to individual and are "anonymous"
- Control no known trait or function



Example of an STR

- TH01 locus: Tyrosine hydroxylase intron 01
- Repeats of TCAT
- CCC TCAT TCAT TCAT TCAT TCAT TCAT AAA
- This example has 6 TCAT repeats.
- There are more than 20 known TH01 alleles.
- Each individual inherits 1 allele from each parent.
- These make up the genotype. Ex. 6,2

Example of an STR

- Ms. Smith's TH01 locus for her two chromosomes is given below.
- What is her genotype?
- MOM'S CHROMOSOME

  CCC TCAT TCAT TCAT TCAT TCAT TCAT AAA

- DAD'S CHROMOSOME

  CCC TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT AAA

Example of an STR

- Ms. Smith's TH01 locus for her two chromosomes is given below.
- MOM'S CHROMOSOME
  - CCC TCAT TCAT TCAT TCAT TCAT TCAT AAA
- DAD'S CHROMOSOME
  - CCC TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT TCAT AAA
- What is her genotype?
- How can we visualize this difference?

Polymerase Chain Reaction (PCR)

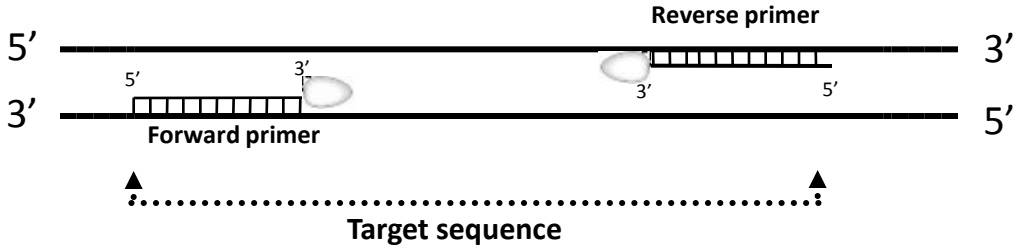
- Replication in a test tube
- Makes copies of a specific piece of DNA
- Amplify DNA so we can visualize it
- Allows you to look at one specific piece of DNA by making copies of *only* that piece of DNA
- Can amplify a very small amount of DNA
- PCR is like looking for a needle in a haystack, and then making a haystack out of the needle

What do you need for PCR?

- What do you need for replication?
- 1
- 2
- 3
- 4

The PCR Reaction
What do you need?

- Template (the STR you want to amplify for the study)
- Sequence-specific primers flanking the target sequence

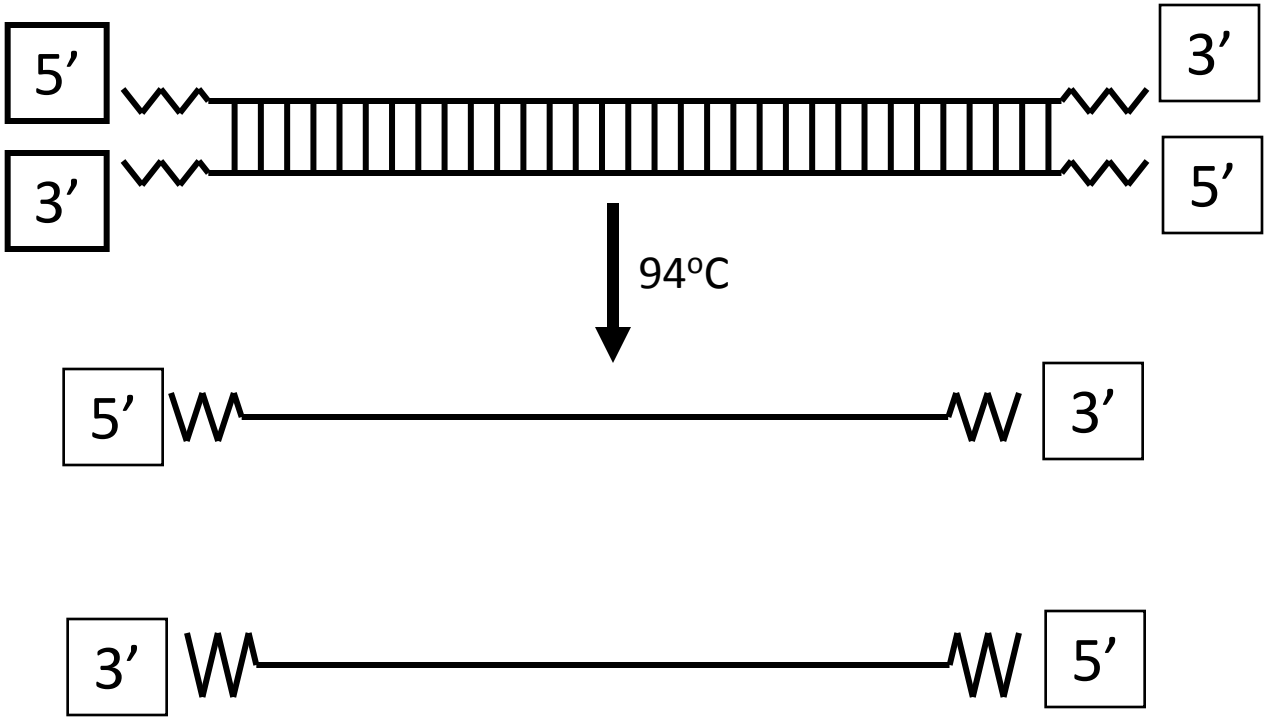


- Nucleotides (dATP, dCTP, dGTP, dTTP)
- Magnesium chloride (enzyme cofactor)
- Buffer, containing salt
- *Taq* polymerase: *Thermus aquaticus*

3 Steps of PCR

- Step #1 Denaturation
  - Heat (94°C) to denature DNA template strands
- Step #2 Annealing
  - Cool (52°C) to anneal primers to template
- Step #3 Extension
  - Warm (72°C) to activate Taq polymerase, which extends primers and replicates DNA
- Repeat 35 cycles

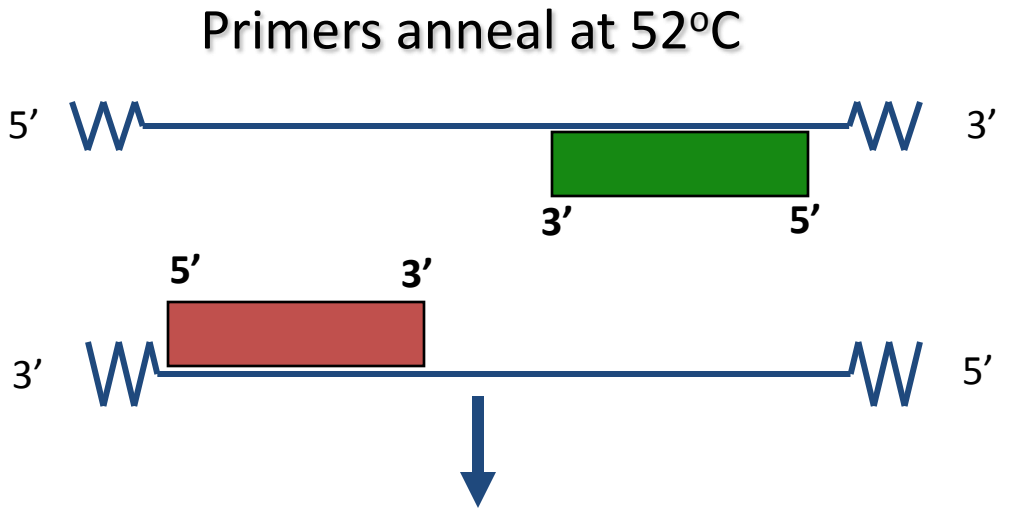
Step #1 Denaturation



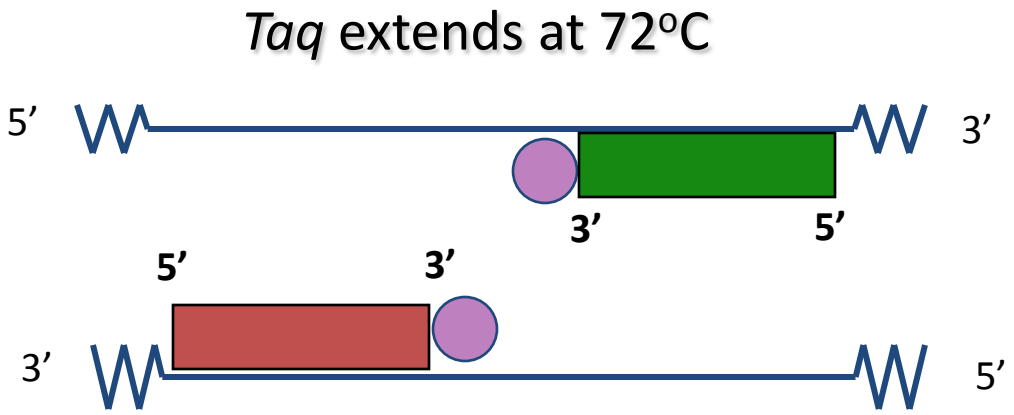
Heat causes DNA strands to separate

Primers Anneal

- Primers bind to the template sequence



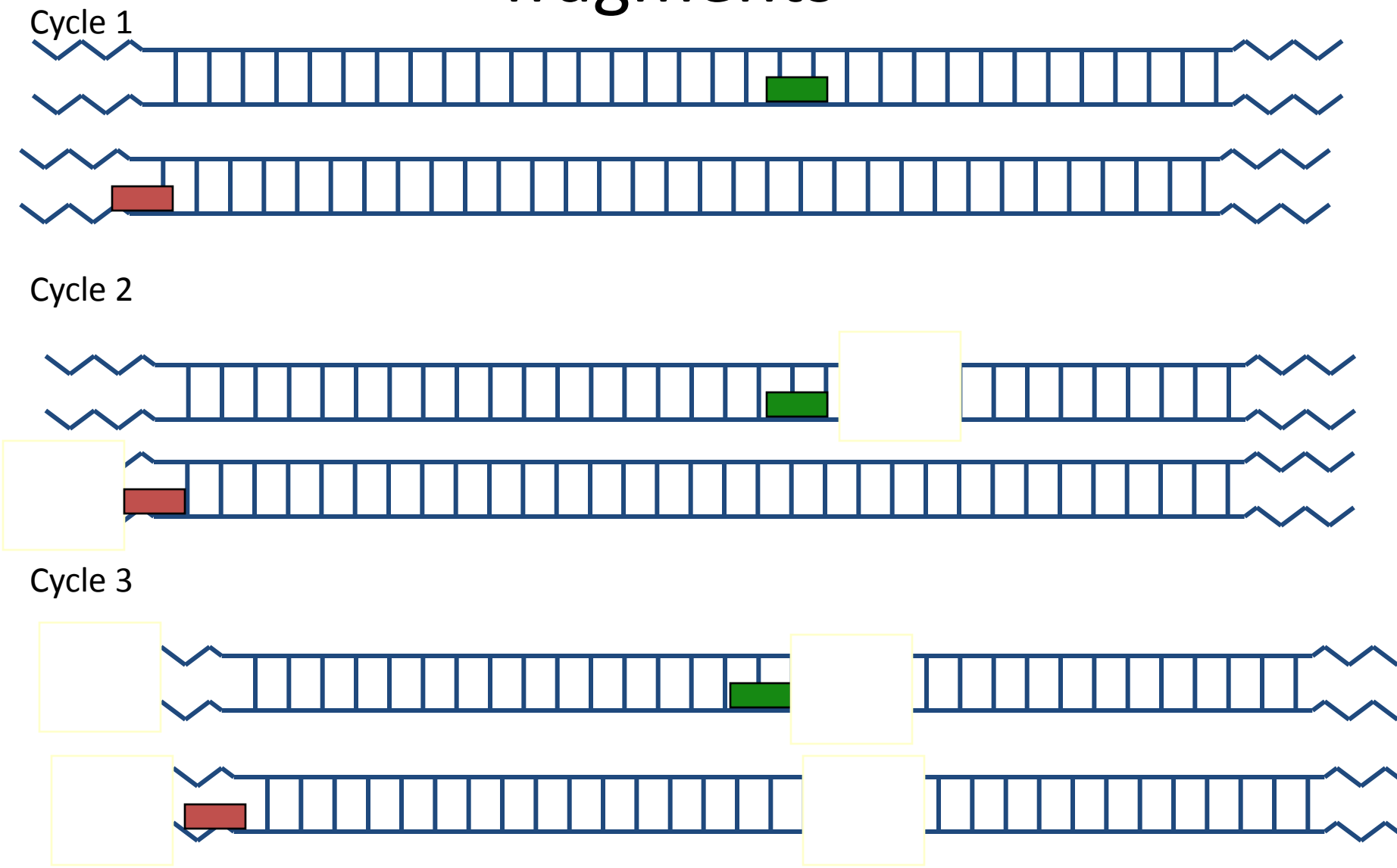
- *Taq* polymerase recognizes 3’ end of primer + template strand



What is happening in the PCR tube while in the thermocycler?

- PCR ANIMATION
- http://highered.mcgraw-hill.com/olc/dl/120078/micro15.swf

Generation of Precise-length fragments



Food Fight !!!

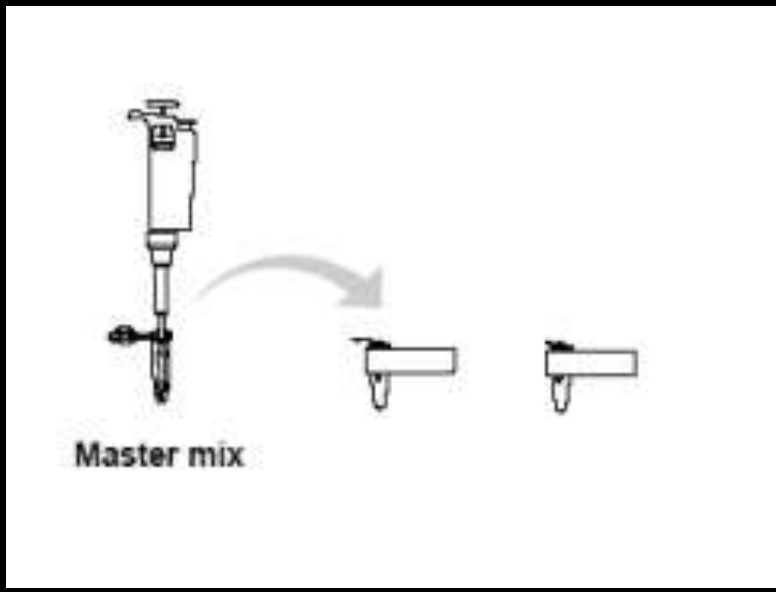
- Crime Scene Evidence (CS)
  - DNA isolated from a half eaten sandwich
- Suspects
  - A: Dylan
  - B: Sabrina
  - C: Aristides
  - D: Tarialas

How to use a micropipette

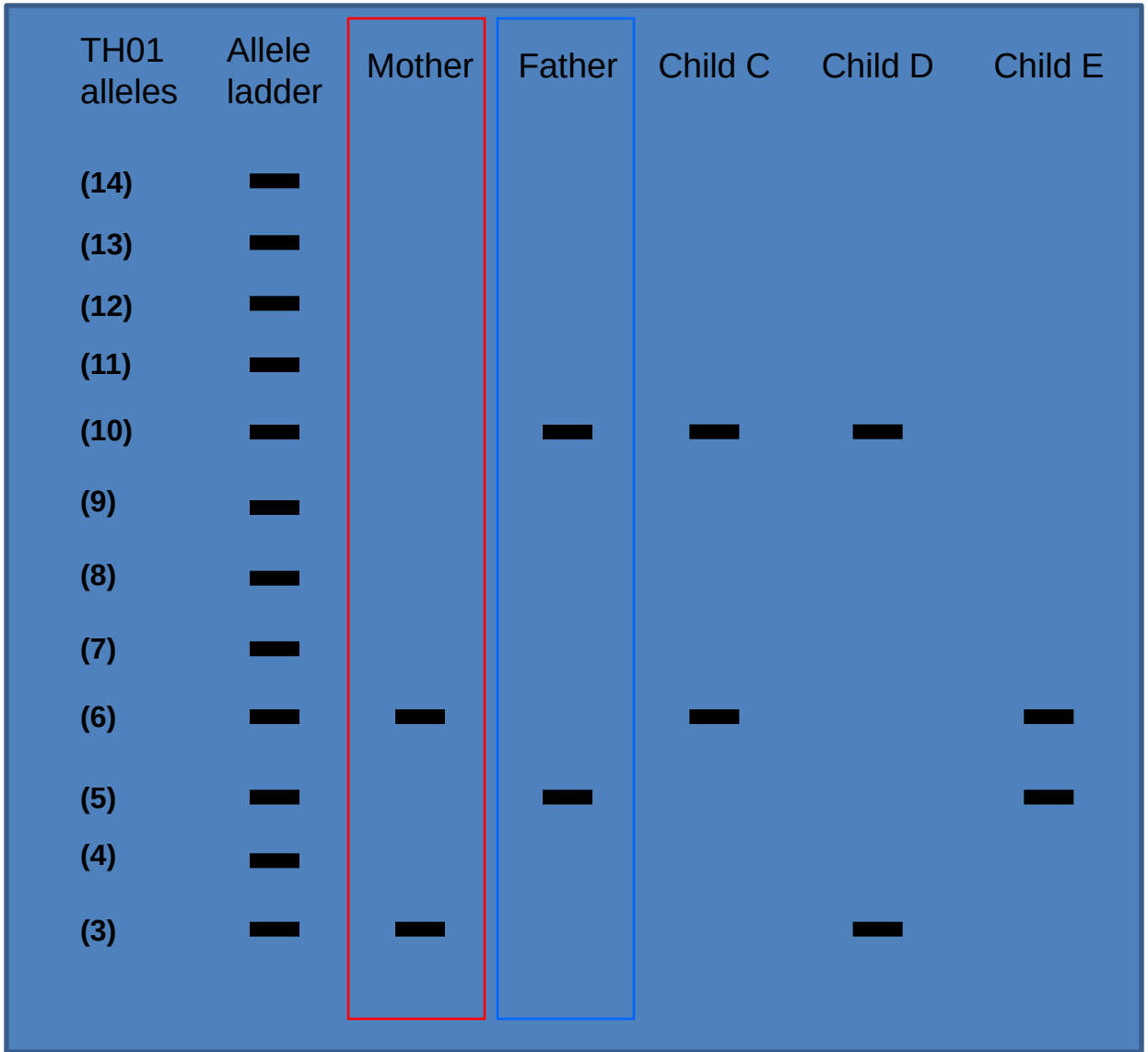
- Press the plunger down to the first soft stop. You will feel it stop naturally
- Lower the tip into the loading dye and slowly let the plunger come back up and fill the pipet.
- Press down to the first soft stop to deliver the loading dye. If it all doesn't come out you can push down to the hard stop to give it one last chance to come out.
- Eject tip

Set up PCR reactions

1. Find the PCR tubes at your station.
2. Label them ‘CS’ for Crime Scene DNA, ‘A’ for Suspect A DNA, ‘B’ for Suspect B DNA, ‘C’ for Suspect C DNA, and ‘D’ for Suspect D DNA.
3. Keeping the tubes on ice, add 20 µl of Master Mix + blue primers to each tube.
4. Keeping the tubes on ice, add 20 µl of each DNA to the appropriately labeled tube.
5. USE A FRESH TIP EACH TIME!
6. Mix and put in thermal cycler
7. Cycle ~3 hours (OVERNIGHT)



To visualize PCR products Crime Scene investigators use gel electrophoresis



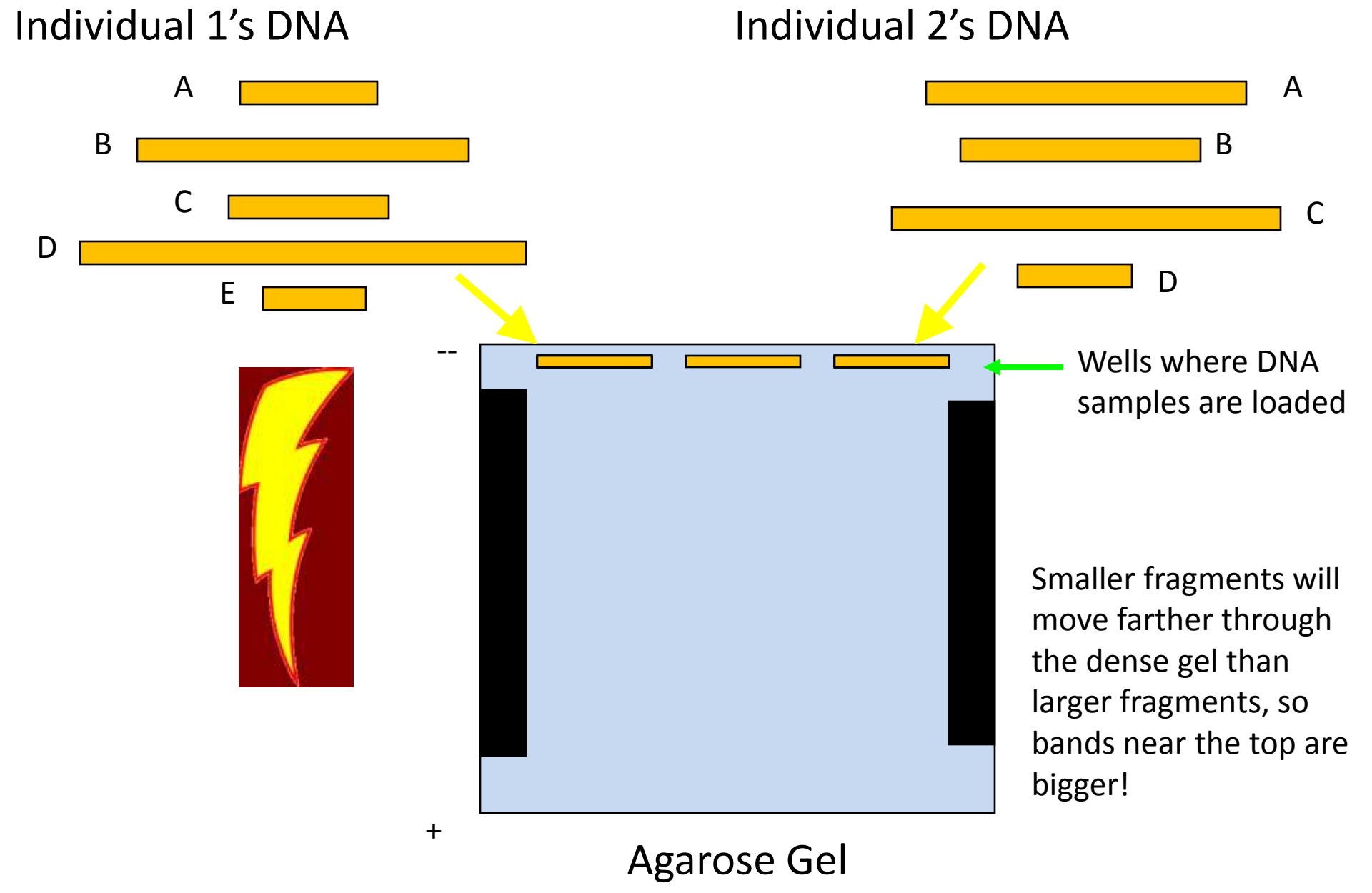
Load Gel

- Obtain 5 PCR Reactions
  - CS
  - A
  - B
  - C
  - D
- Add 10µl of Orange Loading Dye (LD) to each tube

Load Gel

Lane	Description
1	20 µl of CSI allele ladder
2	20 µl of Crime Scene PCR reaction
3	20 µl of Suspect A PCR reaction
4	20 µl of Suspect B PCR reaction
5	20 µl of Suspect C PCR reaction
6	20 µl of Suspect D PCR reaction

Agarose Gel Electrophoresis



Agarose gel

- Agarose
  - gelatanous substance derived from algae
  - polymer of galactose
- Forms a matrix
  - Separate fragments of DNA
  - Smaller fragments travel farther than larger
- Higher percentage of agarose separates small molecules
- To make a gel
  - Grams of agarose/100ml of buffer

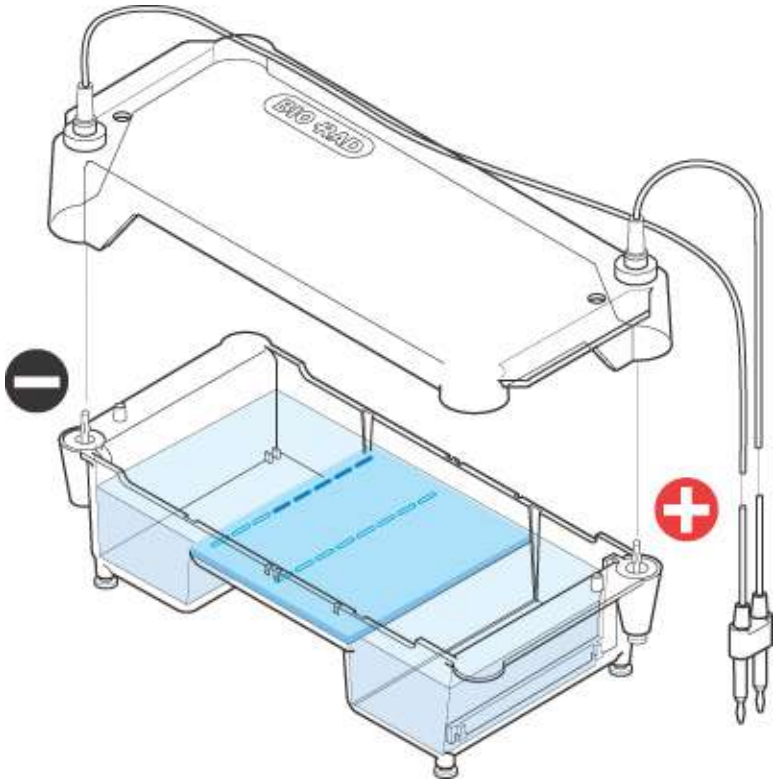
Gel Electrophoresis

- Place gel in gel box
- Pour buffer in box until gel wells are covered.

Gel Electrophoresis

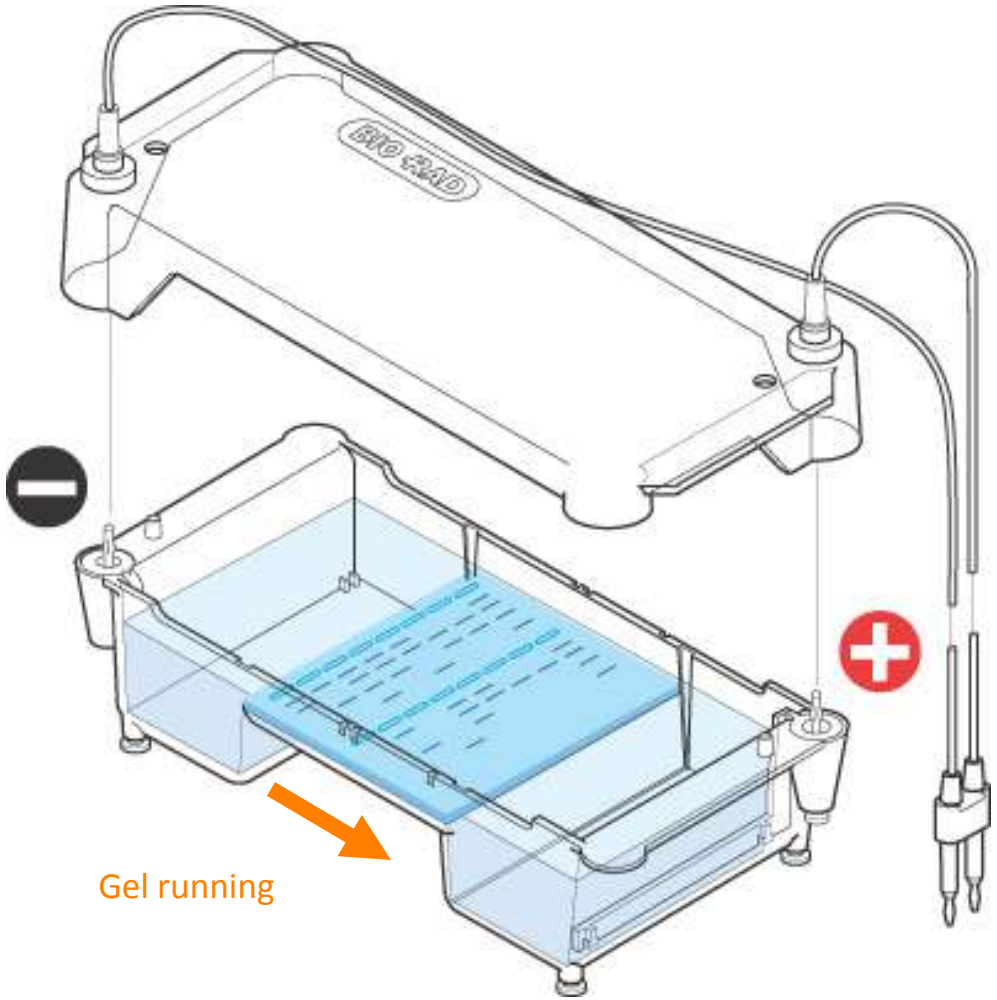
Place 20ul of samples into appropriate wells

Set up electrophoresis chamber by putting top in place and connecting it to the power supply

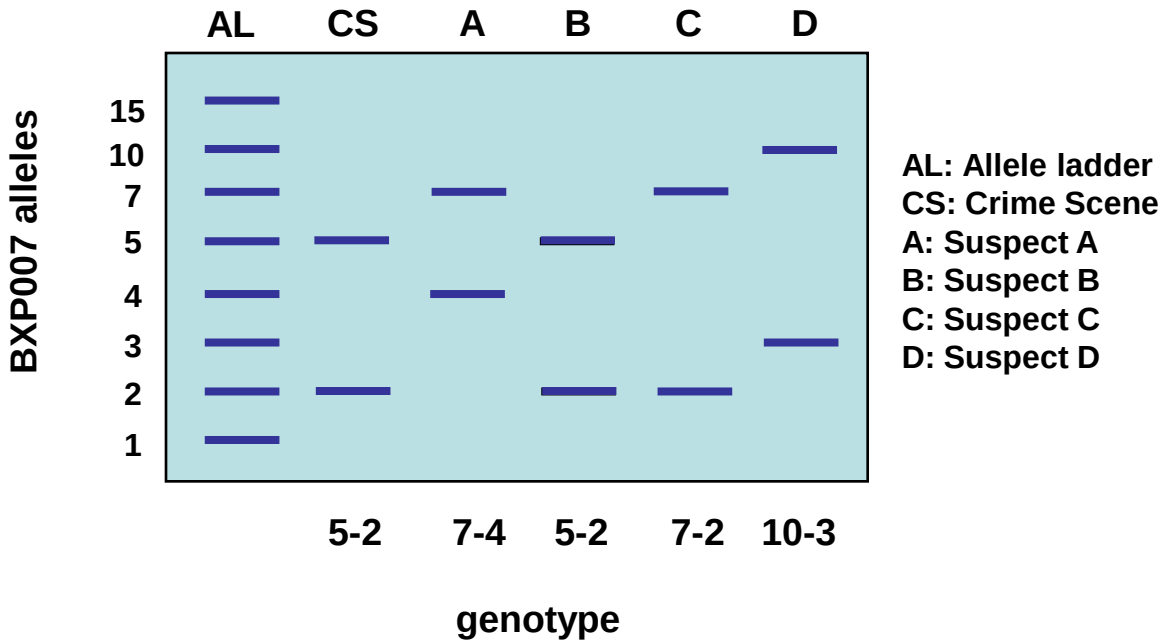


Gel Electrophoresis

- Gel runs from negative to positive
- DNA slightly negative



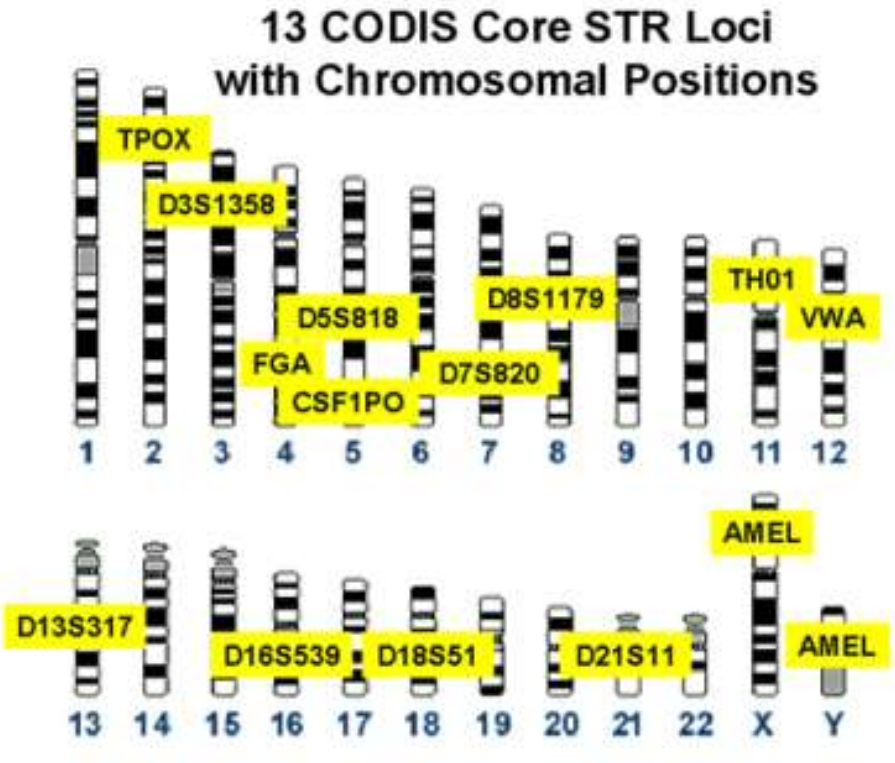
Analysis of Results



- Who can't be excluded?

COmbined DNA Index System (CODIS)

- A federally maintained database used by law enforcement officials



Real STR analysis

- Four different fluorescent tags have been used to identify 7 amplified loci
- Allele ladders are indicated by arrows