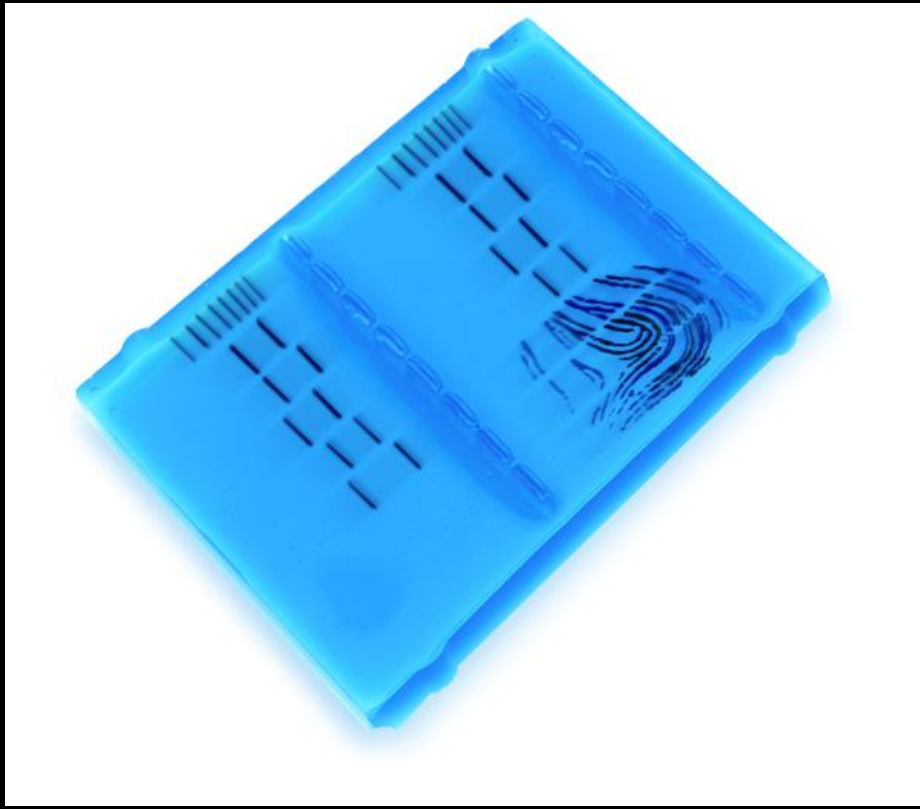

Biotechnology
CAPTIVATING SCIENCE EDUCATION
Explorer™
BIO-RAD
Professional Development

Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

BIO-RAD

Crime Scene Investigator PCR Basics™



Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

BIO-RAD

Crime Scene Investigator PCR Basics™ Kit

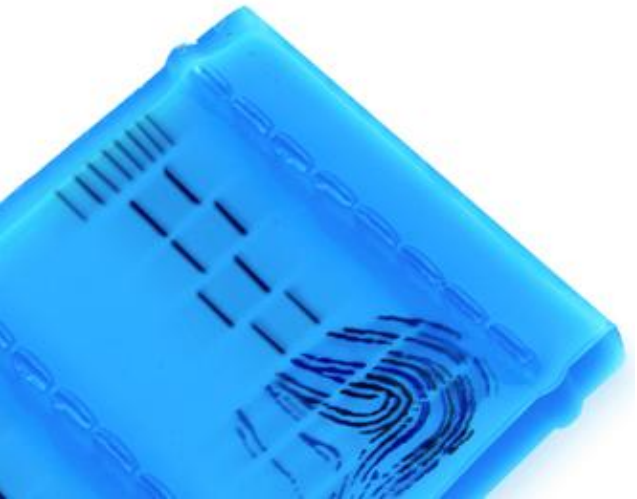
Instructors

Stan Hitomi
Coordinator – Math & Science
San Ramon Valley Unified School District
Danville, CA

Kirk Brown
Lead Instructor, Edward Teller Education Center
Science Chair, Tracy High School
and Delta College, Tracy, CA

Sherri Andrews, Ph.D.
Curriculum and Training Specialist
Bio-Rad Laboratories

Essy Levy, M.Sc.
Curriculum and Training Specialist
Bio-Rad Laboratories



Why Teach Crime Scene Investigator PCR Basics™ Kit ?

- Exciting real-world connections
- Tangible results
- Statistical Analysis
- Standards-based

Environmental and Health Science

- Population genetics
- Genetic screening
- Genetic databases
- Role, place, limits of science and technology
- Privacy of information issues

Genetics

- Mendelian genetics
- Polymorphic loci and multiple alleles
- Genetics of noncoding DNA
- Short tandem repeats (STRs)
- Genetic identity
- Structure and function of the human genome

Evolution

- Genetic diversity and individual identification
- Allele frequencies in populations
- Statistics, probabilities, and the power of discrimination

Cell and Molecular Biology

- Eukaryotic cell structure and organization
- Tissue types and biological sampling

Chemistry of Life

- DNA replication and the polymerase chain reaction (PCR)
- Chemical properties of biological molecules
- DNA structure and function
- Chemistry of electrophoresis

Scientific Inquiry

- Use of PCR and gel electrophoresis for DNA profiling
- Use of experimental controls
- Interpretation of experimental results
- Use of forensic evidence in the courts
- Use of bioinformatics

Target Audience

- **The Crime Scene Investigator PCR Basics™ Kit is intended to be an introduction to the polymerase chain reaction (PCR)**
- **Students will have a much better appreciation of the kit if they have some understanding of DNA structure and function**

Crime Scene Investigator PCR Basics™ Kit Advantages

- Standards Based
- Perform real-world DNA profiling
- Use PCR to amplify multiple DNA samples
- Use electrophoresis to visualize results
- Complete in two 45 minute lab sessions
- Sufficient materials for 8 student workstations

Workshop Time Line

- **Introduction to DNA profiling**
- **Set up PCR reactions**
- **Electrophorese PCR products**
- **Analysis and interpretation of results**

What is DNA profiling?

DNA profiling is the use of molecular genetic methods to determine the exact genotype of a DNA sample in a way that can basically distinguish one human being from another

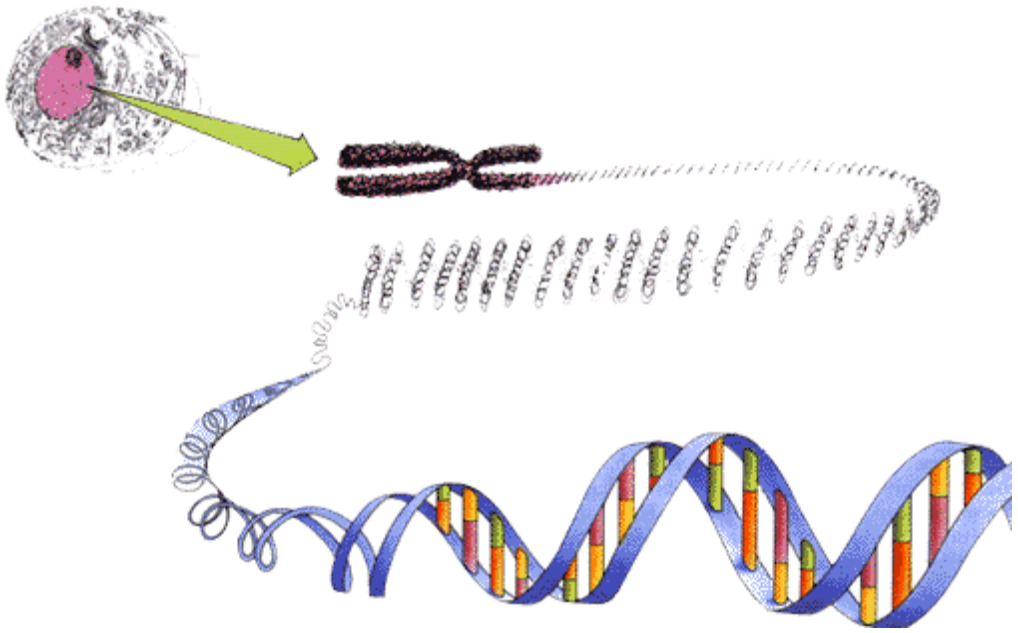
The unique genotype of each sample is called a DNA profile.

How do crime scene investigators create a DNA profile?

1. Evidence is collected at the crime scene:

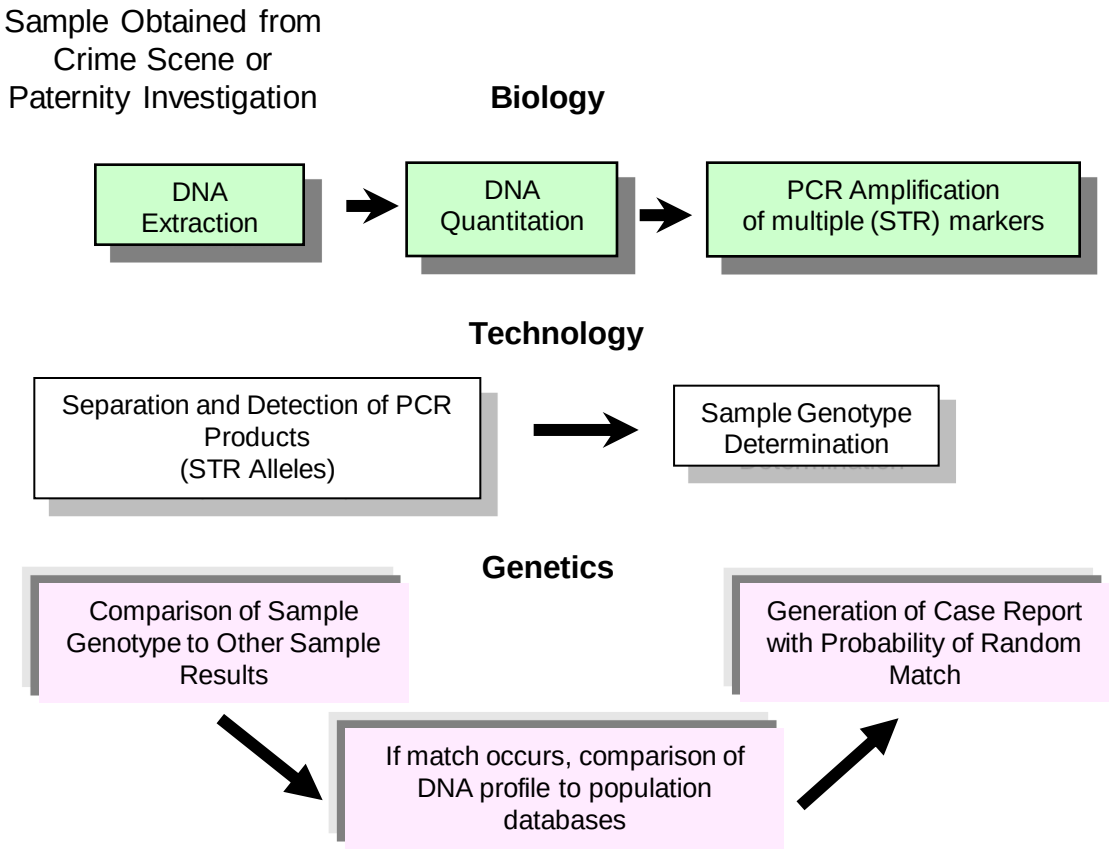
How do crime scene investigators create a DNA profile?

2. DNA is extracted from sources at the crime scene and from victim and suspects



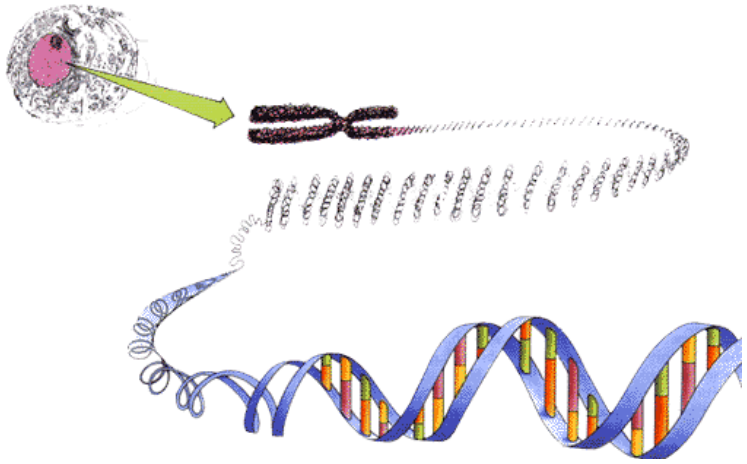
How do crime scene investigators create a DNA profile?

3. DNA samples are processed



Since humans are 99.9% identical where do crime scene investigators look for differences in DNA profiles?

4. Crime Scene Investigators search in areas of the genome that are unique from individual to individual and are "anonymous" (control no known trait or function) The areas examined are Short Tandem Repeats or STR's



STR region

Example of an STR: TH01

The TH01 locus contains 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.

Determining genotypes for individuals using STRs

Ms. Smith's TH01 locus for her two chromosomes is given below.

What is her genotype?

MOM'S CHROMOSOME

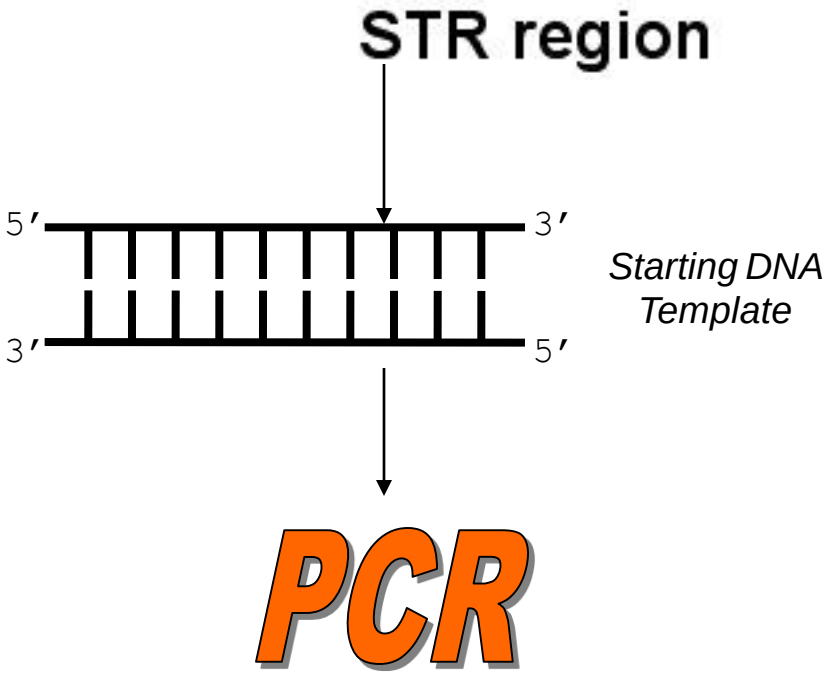
CCC TCAT TCAT TCAT TCAT TCAT TCAT AAA

DAD'S CHROMOSOME

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

To determine the genotype (DNA profile) Crime Scene Investigators make billions of copies of the target sequence using PCR

Target DNA



What's the point of PCR?

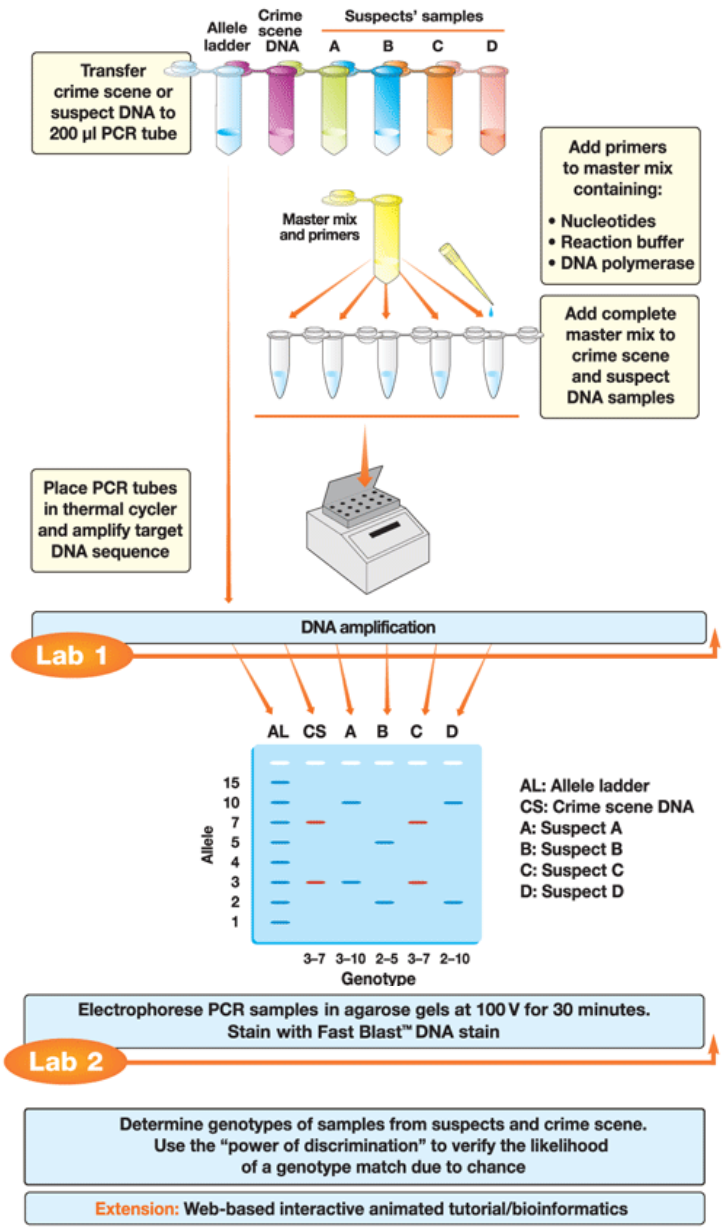
- PCR, or the polymerase chain reaction, makes copies of a specific piece of DNA
- PCR allows you to look at one specific piece of DNA by making copies of *only* that piece of DNA
- PCR is like looking for a needle in a haystack, and then making a haystack out of the needle

DNA profiling is used to determine which suspect can not be excluded from suspicion.

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**

Crime Scene Investigator PCR Basics™ Procedures Overview



Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

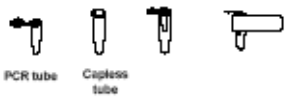
BIO-RAD

Laboratory Quick Guide

Quick Guide

Lesson 1: Setting up the PCR Reactions

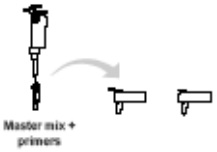
1. Label 5 PCR tubes CS, A, B, C, or D, and include your group name or initials as well. Place the PCR tube in a capless tube in the foam float on ice.



2. Using the chart below as a guide, transfer 20 µl of the appropriate template DNA into the correctly labeled tube. **Important: use a fresh aerosol barrier pipet tip for each DNA sample.**

Tube label	DNA	Master mix + primers
CS + your initials	20 µl Crime Scene DNA	20 µl MMP (blue)
A + your initials	20 µl Suspect A DNA	20 µl MMP (blue)
B + your initials	20 µl Suspect B DNA	20 µl MMP (blue)
C + your initials	20 µl Suspect C DNA	20 µl MMP (blue)
D + your initials	20 µl Suspect D DNA	20 µl MMP (blue)

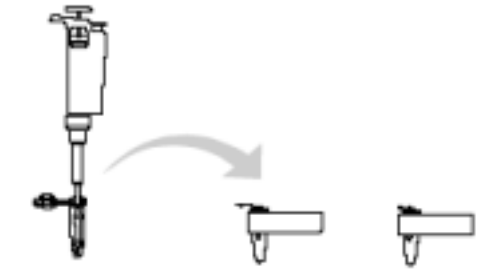
3. Transfer 20 µl of the blue MMP into each of the 5 PCR tubes containing template DNA. Pipet up and down to mix. Cap each tube after adding blue MM. **Important: use a fresh aerosol barrier pipet tip each time. Immediately cap each tube after adding MMP.**



4. Place your capped PCR tubes in their adaptors on ice.

5. When instructed to do so, place your tubes in the thermal cycler. Your instructor will program it for PCR.

Set up PCR reactions

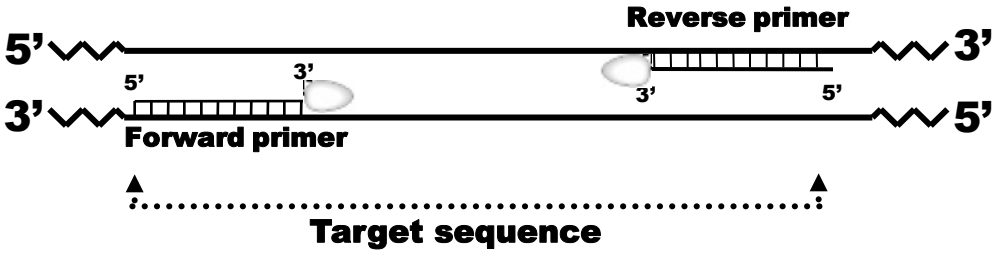


1. Find the PCR tubes at your station. Label them ‘CS’ for Crime Scene DNA, ‘A’ for Suspect A DNA, ‘B’ for Suspect B DNA, ‘C’ for Suspect C DNA, and ‘D’ for Suspect D DNA.
2. Keeping the tubes on ice, add 20 µl of Master Mix + blue primers to each tube.
3. Keeping the tubes on ice, add 20 µl of each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

The PCR Reaction

What do you need?

- **Template (containing 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**

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

PCR Animation

http://www.bio-rad.com/flash/07-0335/07-0335_PCR.html

The PCR Reaction

How does it work?

Heat (94°C) to denature DNA strands

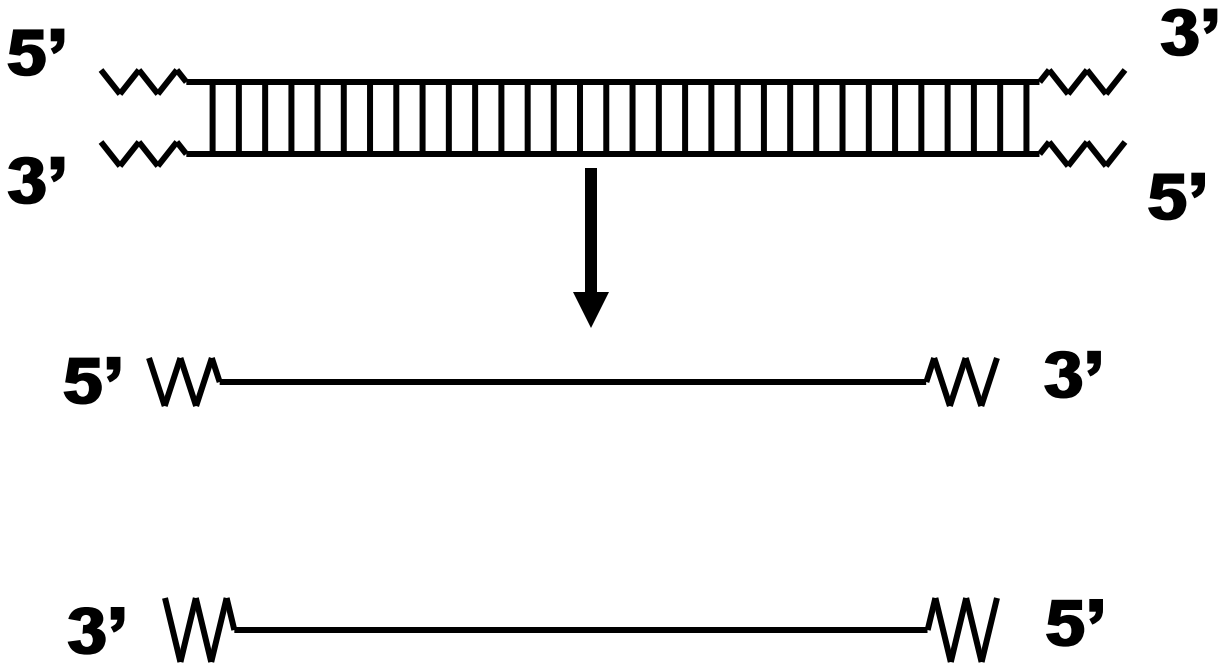
Cool (52°C) to anneal primers to template

Warm (72°C) to activate *Taq* polymerase, which extends primers and replicates DNA

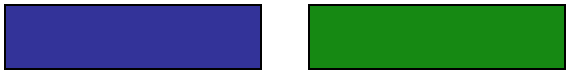
Repeat 35 cycles

Heat causes DNA strands to separate

Denaturation of DNA at 94°C

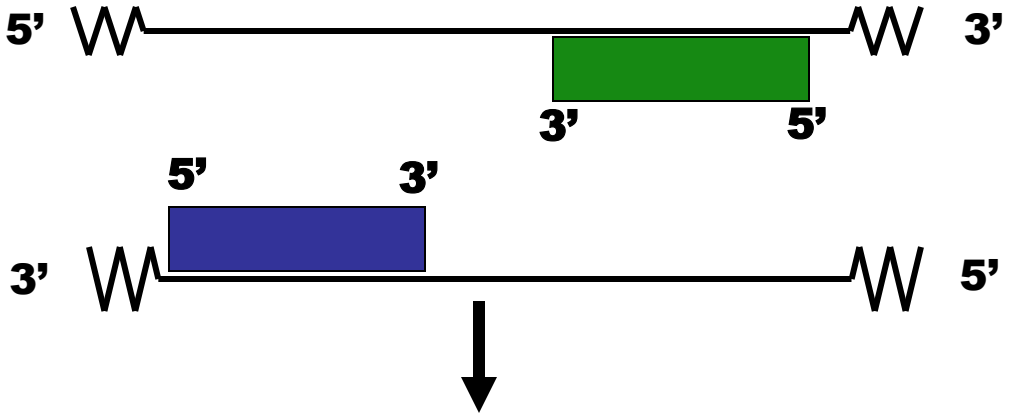


Primers bind to the template sequence

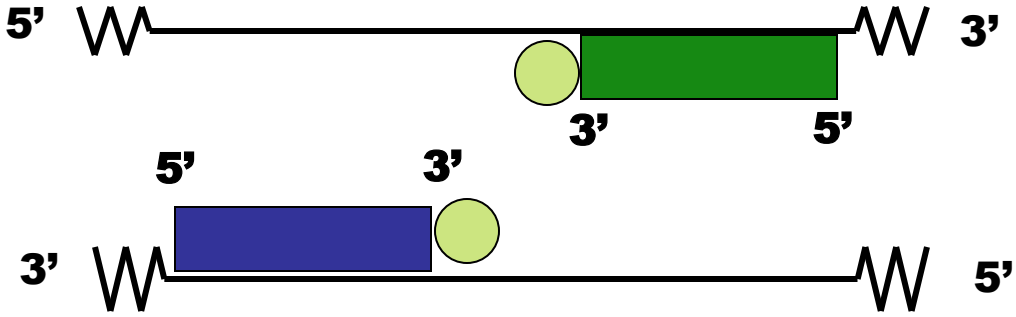


***Taq* polymerase recognizes 3' end of primer + template strand**

Primers anneal at 52°C



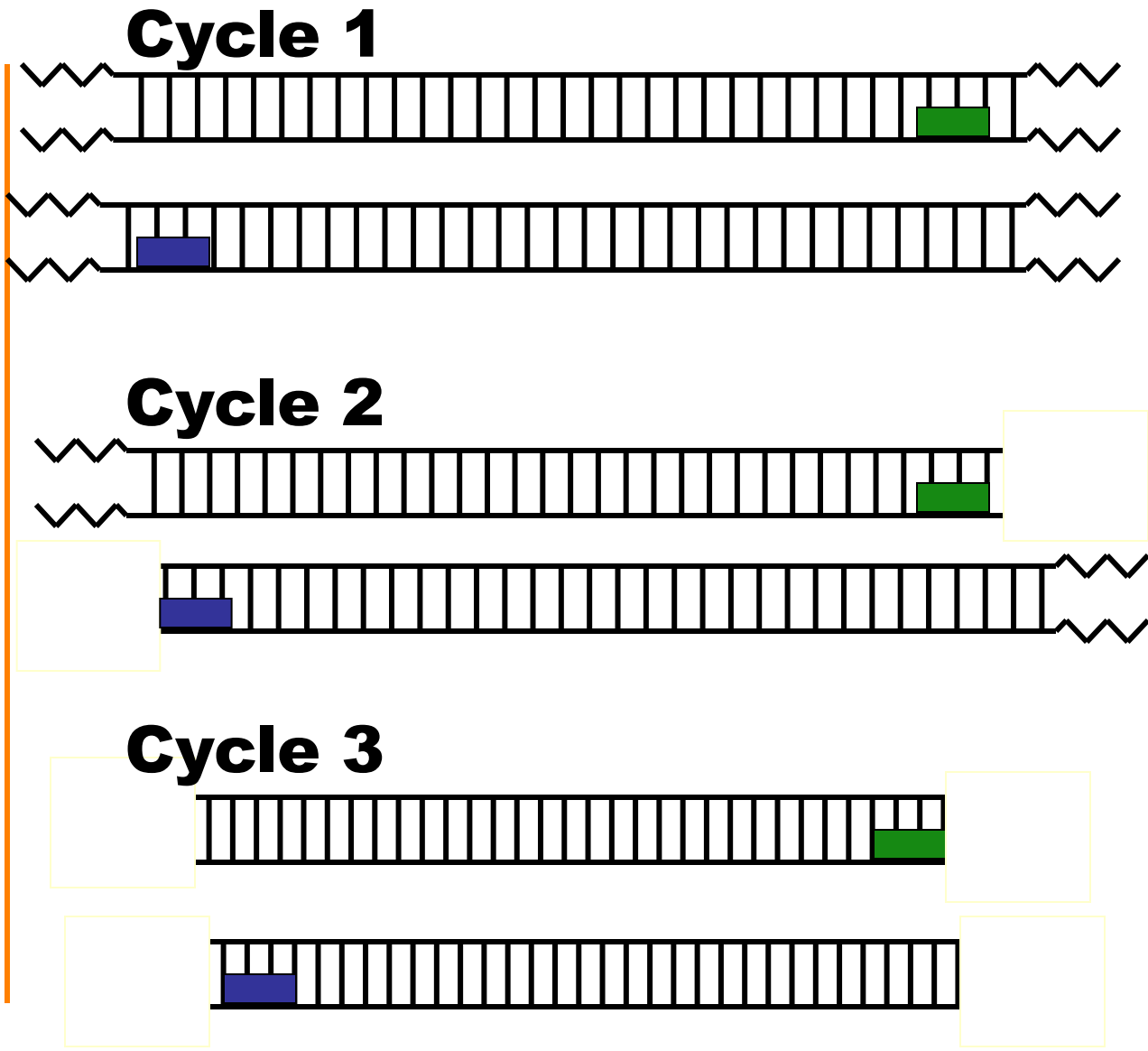
***Taq* extends at 72°C**



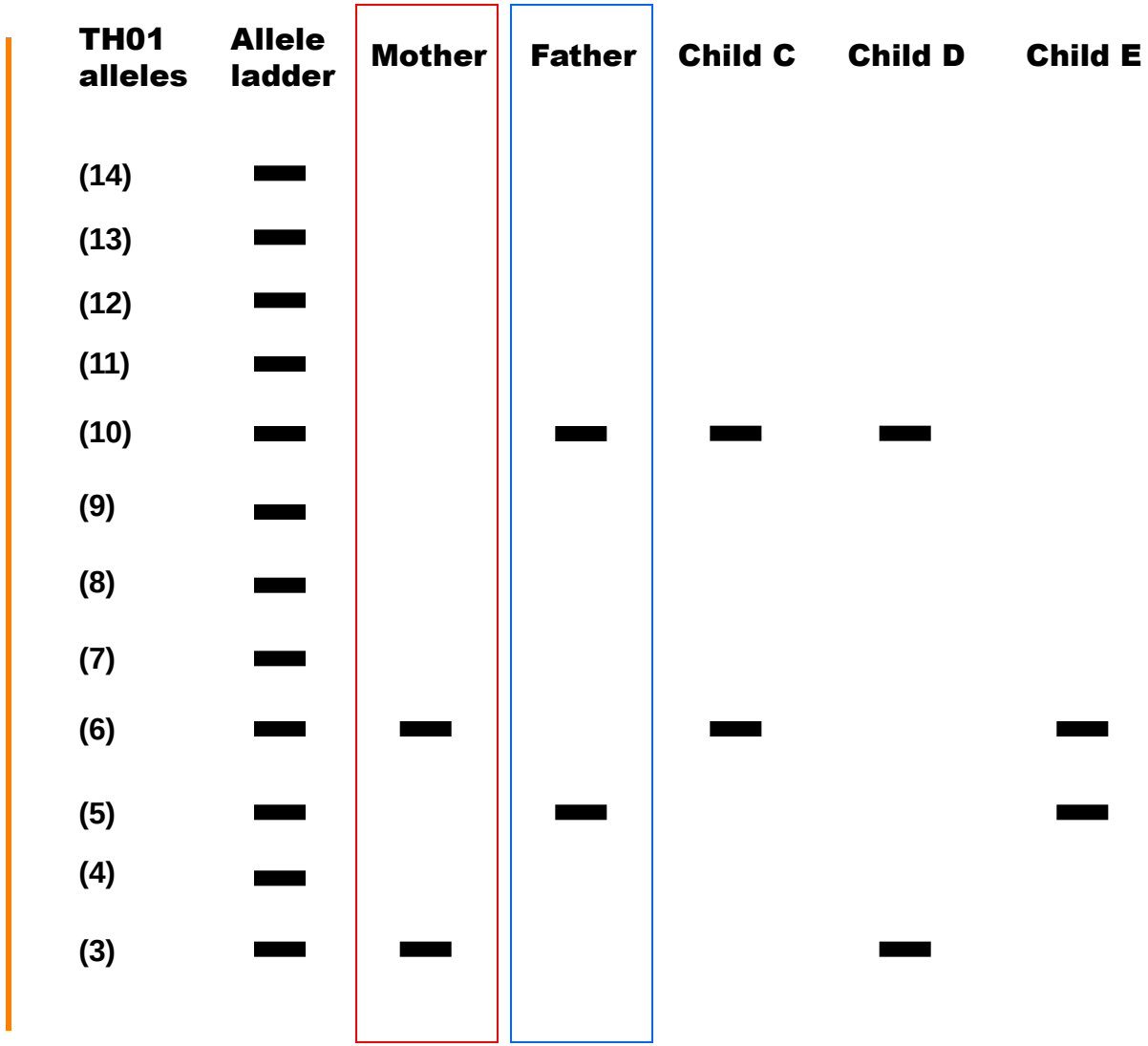
***STR* DNA is replicated**

Repeat denaturing, annealing, and extending 35 cycles

The exact-length target product is made in the third cycle



To visualize PCR products Crime Scene investigators use gel electrophoresis



Electrophorese PCR products

1. Add 10 ul of Orange G Loading Dye to each PCR tube and mix
2. Set up gel and electrophoresis equipment
3. Load 20 ul of CSI allele ladder to Lane 1
4. Load 20 ul of your PCR reactions in lanes 2 to 6
5. Electrophorese samples
6. Stain gel with Fast Blast DNA Stain
7. Analyze results

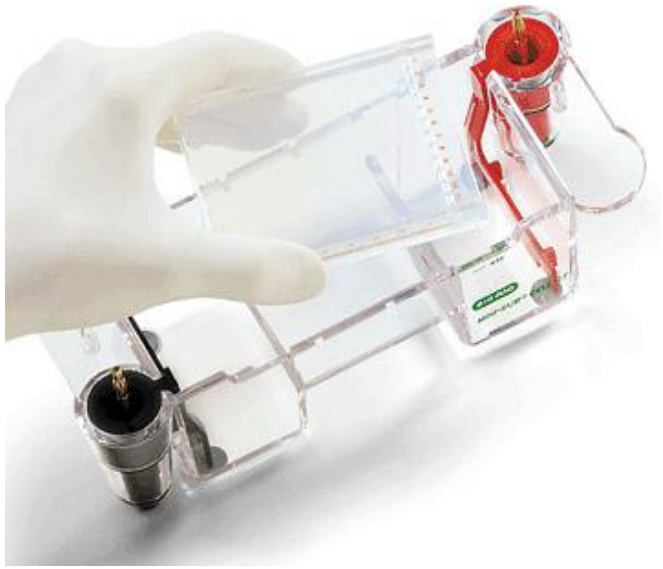
Using the digital micropipet

Add 10ul of loading dye to each microtube

Agarose Electrophoresis

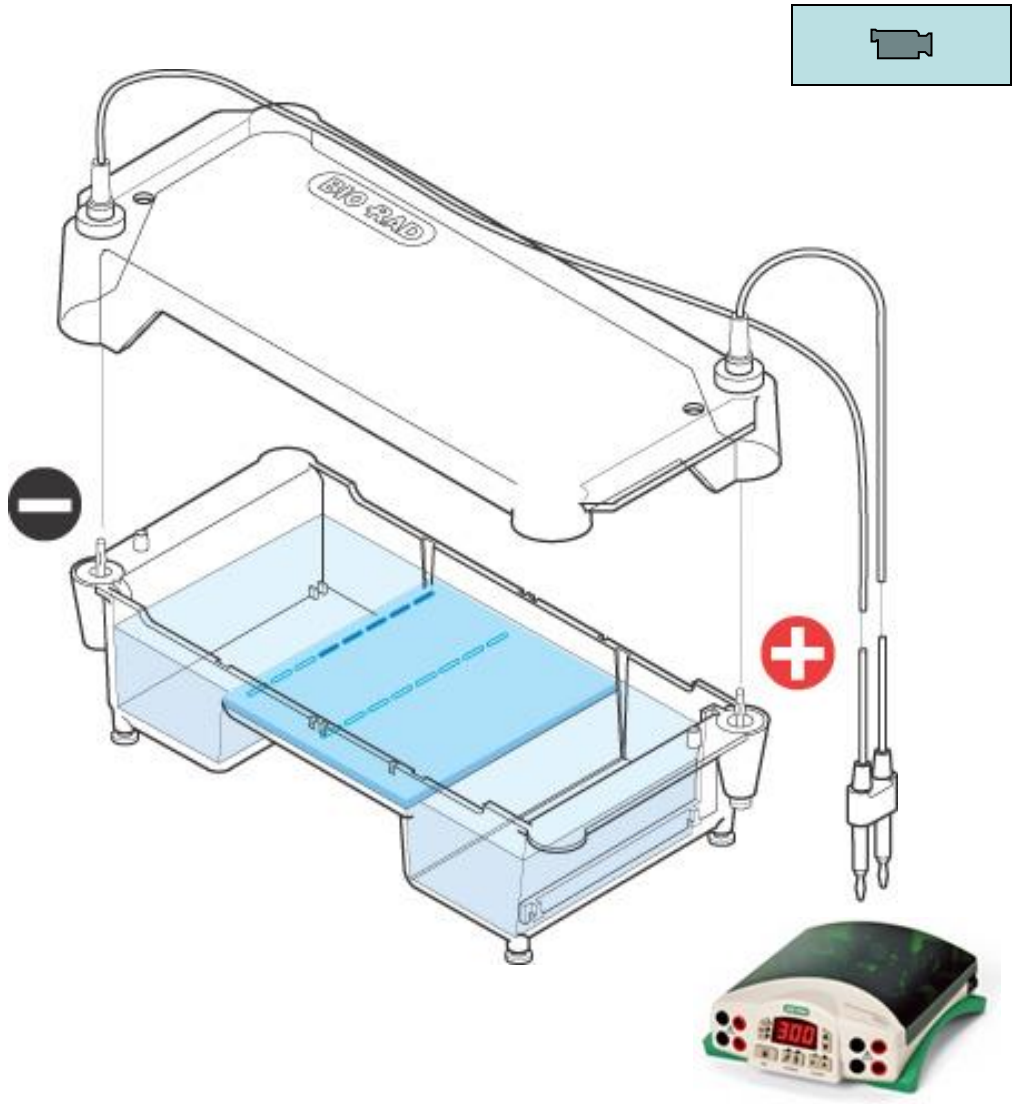
Place gel in gel box

Pour buffer in box until gel wells are covered.



Place 20ul of samples into appropriate wells

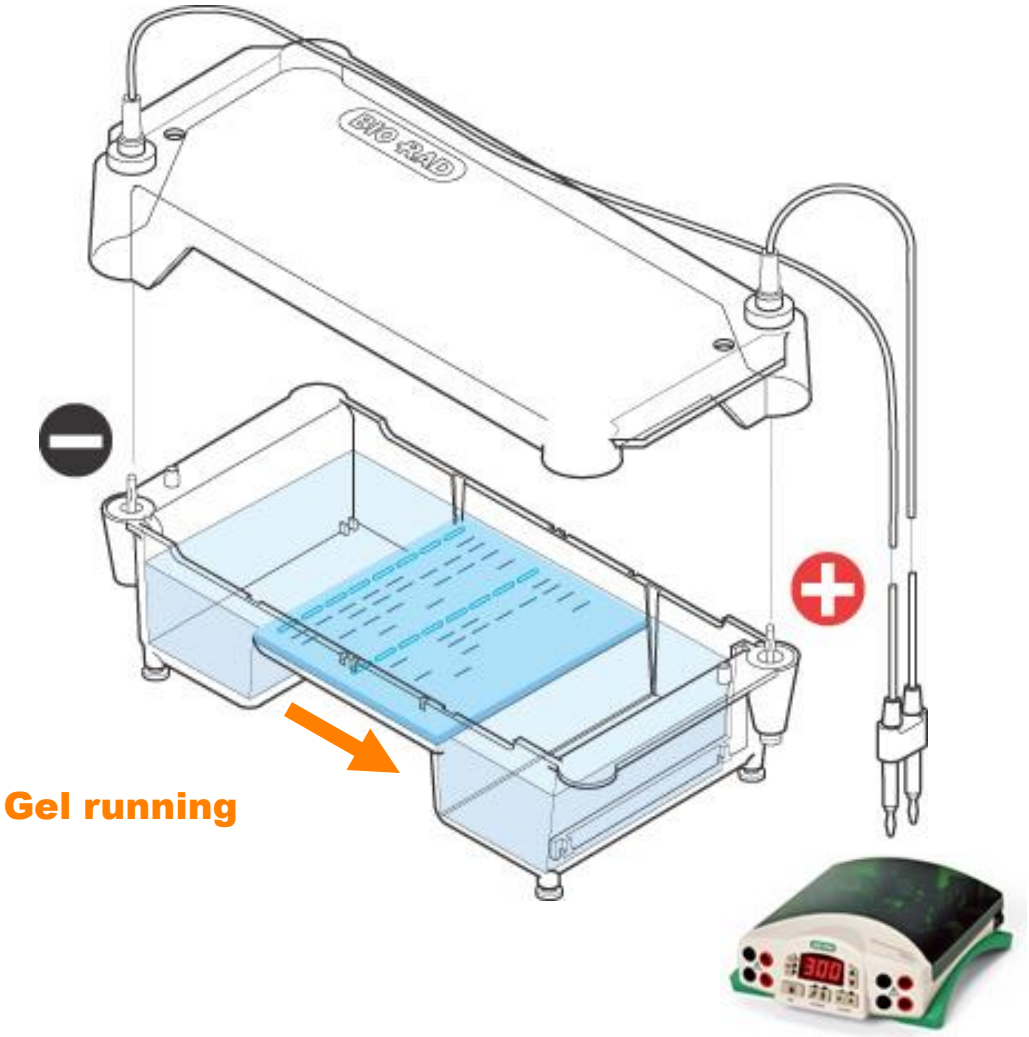
Set up electrophoresis chamber by putting top in place and connecting it to the power supply



Agarose Electrophoresis Running

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

Use a 3% gel to separate small fragment sizes



Milestones in Forensic DNA analysis

1985	Alec Jeffries develops RFLP
1990	PCR analysis using single locus STR begins
1992	FBI initiates STR work
1994	DNA Identification Act: provides funding for national DNA database
1995	OJ Simpson trial focuses public attention on DNA evidence
1998	FBI starts CODIS database; Swissair disaster – all remains identified using STR DNA profiling
2001	World Trade Center disaster in NYC – many remains identified using a combination of DNA profiling approaches
2004	Indian Ocean tsunami; Interpol and other world agencies use DNA profiling to identify victims
Today	Trace your Genetic Genealogy; commercially available packages can trace paternal/maternal ancestry

DNA Testing Today

GeneTree.com & Ancestry.com provide DNA tests from $99-$200 to trace genealogy

TUESDAY, OCTOBER 23, 2007 ☆☆☆☆☆ San Francisco Chronicle B5

Sites offer DNA tests, databases to assist with genealogy research

By Anick Jesdanun
ASSOCIATED PRESS

NEW YORK — Two services launching just a week apart tap a growing interest in DNA testing to help people find their ancestors and learn more about their lives.

GeneTree, which opens today, and Ancestry.com, which started its DNA Ancestry service last Tuesday, both sell DNA kits for less than $200. Users can build online family trees and contact others with DNA matches to compare family histories.

Genealogy research has become popular in recent years as online services improve access to vast databases of immigration, military and other records from around the world. According to the Pew Internet and American Life Project, a quarter of Internet users have researched their ancestors online.

Lately, many of them have been turning to DNA testing to uncover additional clues, said Dick Eastman, who writes a newsletter about online genealogy. Although DNA won't provide all the answers, such as names and precise dates, he said, it could open leads.

"Anybody who's got a mystery is going to do this sooner or later, and that's a pretty high percentage of us," Eastman said.

That's particularly true of black Americans, many of whom have trouble tracing roots beyond the slavery era, Eastman said. Eastern Europeans, Jews and certain other groups also find records fragmented, he said.

GeneTree and Ancestry join services from Family Tree DNA and others.

James Lee Sorenson, GeneTree's chief executive, said he believes his site stands out for its exclusive access to records from the nonprofit Sorenson Molecular Genealogy Foundation. The group has collected DNA samples from 100,000 individuals worldwide and conducted ancestry research on them to produce a larger database of 6 million people.

Ancestry, based in Provo, Utah, is building its DNA database largely from scratch; company officials say they are on track to capture the genetic profiles of 50,000 people within six months.

Both GeneTree and Ancestry use DNA test kits from Sorenson Genomics.

GeneTree sends mouthwash that users swirl in their mouths, spit into a container and mail back for $99 or $149, depending on how much DNA the user wants analyzed. With Ancestry, users return a cheek swab. Ancestry offers a greater variety of tests; the one comparable to GeneTree's higher-end offering costs $179.

Besides finding matches, DNA patterns can help assess the likely origins of an individual's ancestors thousands of years ago, allowing the user to then visually trace migration backward to the first humans, widely believed to hail from Africa.

Both sites are incorporating elements of social networking, akin to those at Facebook and MySpace.

With GeneTree, each family member in the online tree — whether a user of GeneTree or not — gets a personal profile page. Users may add photos, video and other documents to their own pages or those of relatives, using free tools from sister company Sorenson Media.

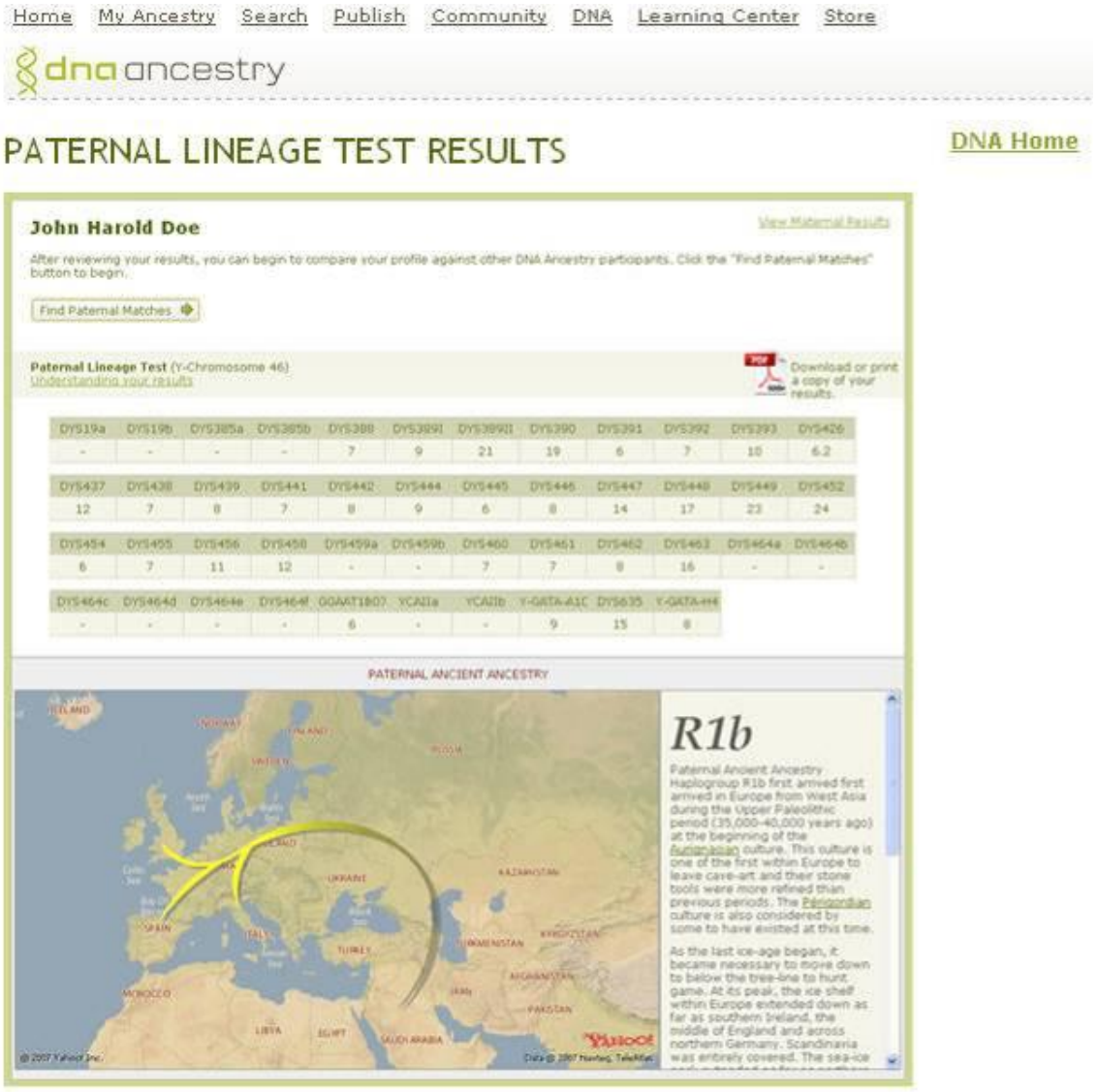
Ancestry already has some tools for adding photos and other files and plans additional features, such as letting users with the same last name DNA results.

Genealogical Analysis Uses:

DNA sequencing (mtDNA)

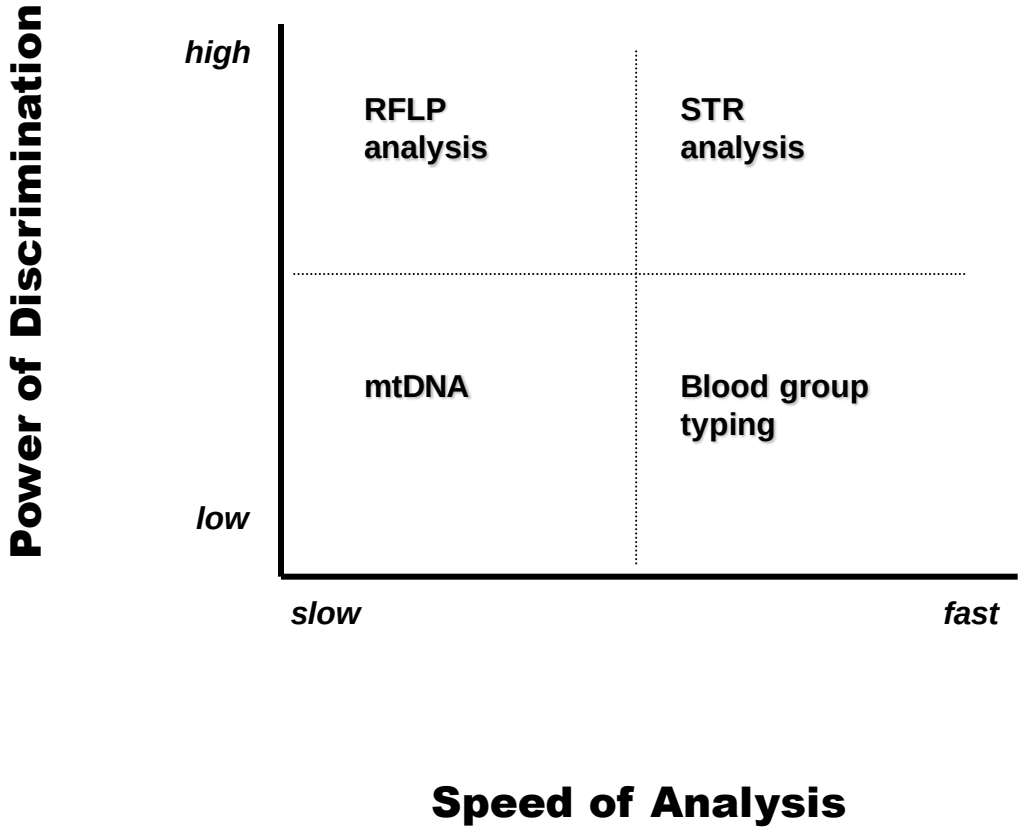
Single Nucleotide Polymorphism testing (SNPs)

Short Tandem Repeat testing (STRs)



Crime scene investigators use techniques that are fast, cost effective, and have a high Power of Discrimination

The Power of Discrimination is the ability of a test to distinguish between different samples (genotypes)



Statistics of Chance: M&M Locus

6 Possible Alleles:

- Green
- Red
- Yellow
- Blue
- Brown
- Orange

Probabilities

- **One allele from each parent means 2 copies of gene/locus**

$$\frac{1}{6} \times \frac{1}{6} = \frac{1}{36}$$

Frequency of any M&M genotype

Probabilities

Jolly Rancher: 5 alleles

Mike & Ikes: 5 alleles

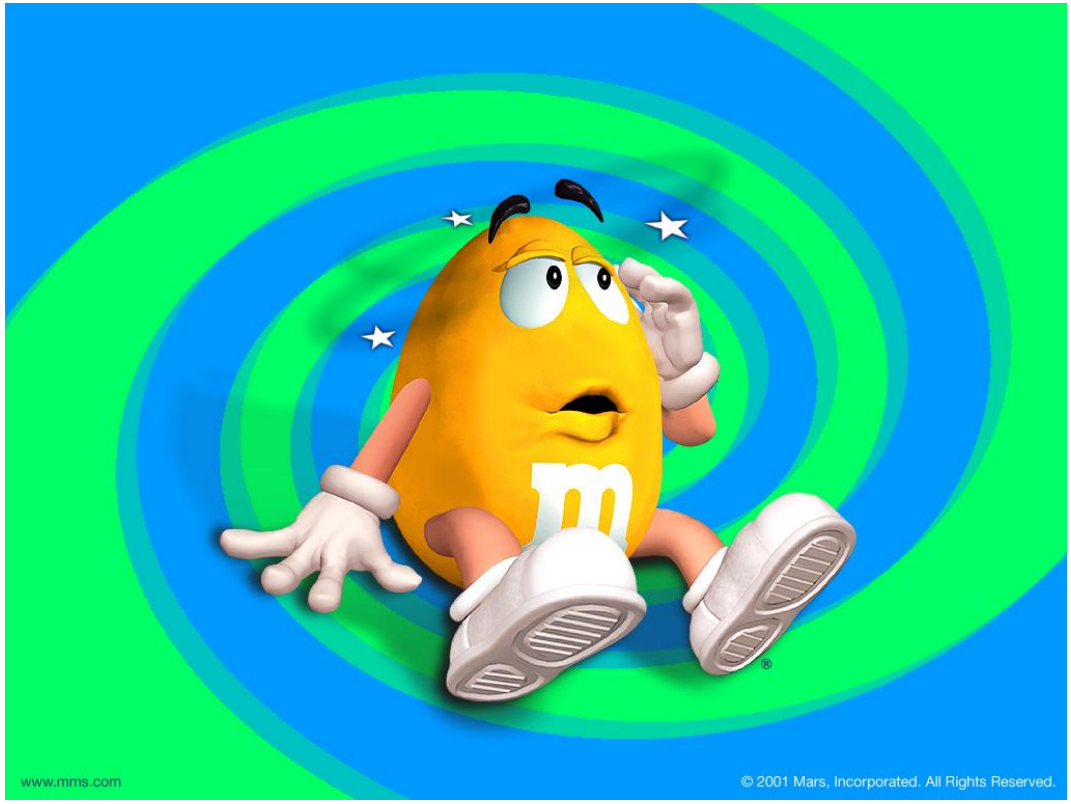
Locus

M&M 6 alleles	$\frac{1}{6} \times \frac{1}{6}$	=	$\frac{1}{36}$
Jolly Rancher 5 alleles	$\frac{1}{5} \times \frac{1}{5}$	=	$\frac{1}{25}$
Mike & Ikes 5 alleles	$\frac{1}{5} \times \frac{1}{5}$	=	$\frac{1}{25}$

Chance an individual has a given genotype = $\frac{1}{22{,}500}$

Chance 2 people have the same genotype = $\frac{1}{506{,}250{,}000}$

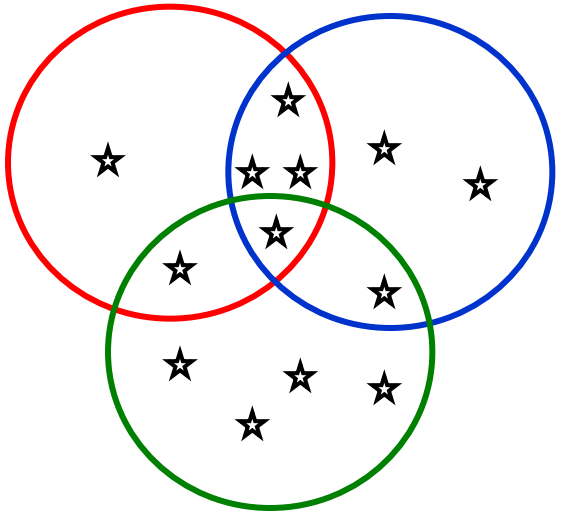
Who can't we exclude from the pool of suspects?



The Power of Discrimination increases with the number of loci profiled

TPOX genotype8-12

0.044



D3S1358 genotype 16-17

0.099

FGA genotype 21-23

0.050

$$0.044 \times 0.099 \times 0.050 = 2.18 \times 10^{-4}$$

$$1 / 2.18 \times 10^{-4}$$

or 1 in 4591

The Power of Discrimination increases with the number of loci profiled

TPOX 8-12	0.044
D3S1358 16-17	0.099
FGA 21-23	0.050
VWA 14-14	0.0088

Random Match Probability

= 1 in 5.3×10^5

Real-World Probabilities

- Forensics labs use 13 different loci with multiple alleles
- Allele frequencies DO NOT follow mathematical principles - allele frequencies vary by population.
- These 13 loci allow for discrimination of any two people in the world (with the exception of identical twins), living or dead.
- Probability of a random match when all 13 loci typed: ~1 in 3 trillion.

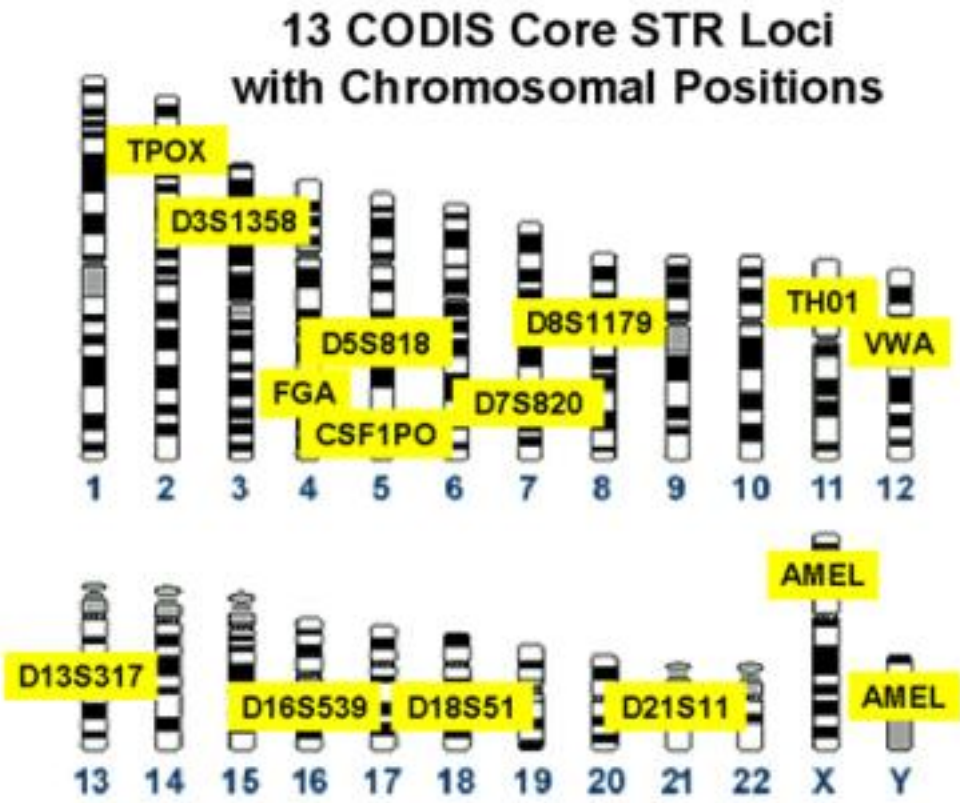
TH01 Published Allele Frequencies by Population

Allele	Caucasians n=302	African American n=258	Latinos n=140
5	.002	.004	
6	.232	.124	.214
7	.190	.421	.279
8	.084	.194	.096
9	.114	.151	.150
10	.008	.002	.014
11	.002		

Butler et al 2003 J Forensic Sci. www.cstl.nist.gov/biotech/strbase/pub_pres/Butler2003a.pdf

CODIS
COmbined DNA Index System

A federally maintained database used by law enforcement officials

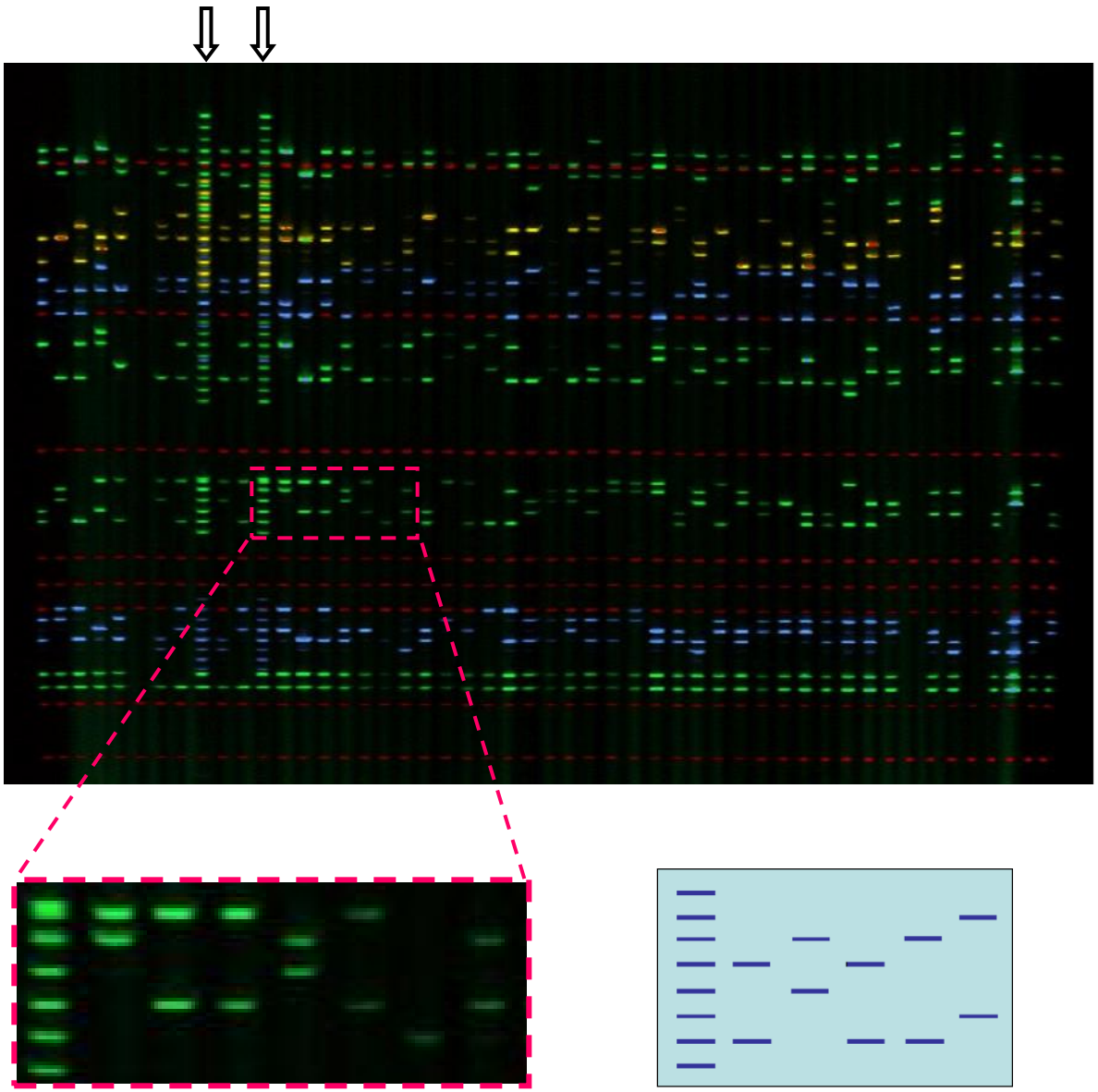


13 loci guarantees high power of discrimination

Real STR analysis

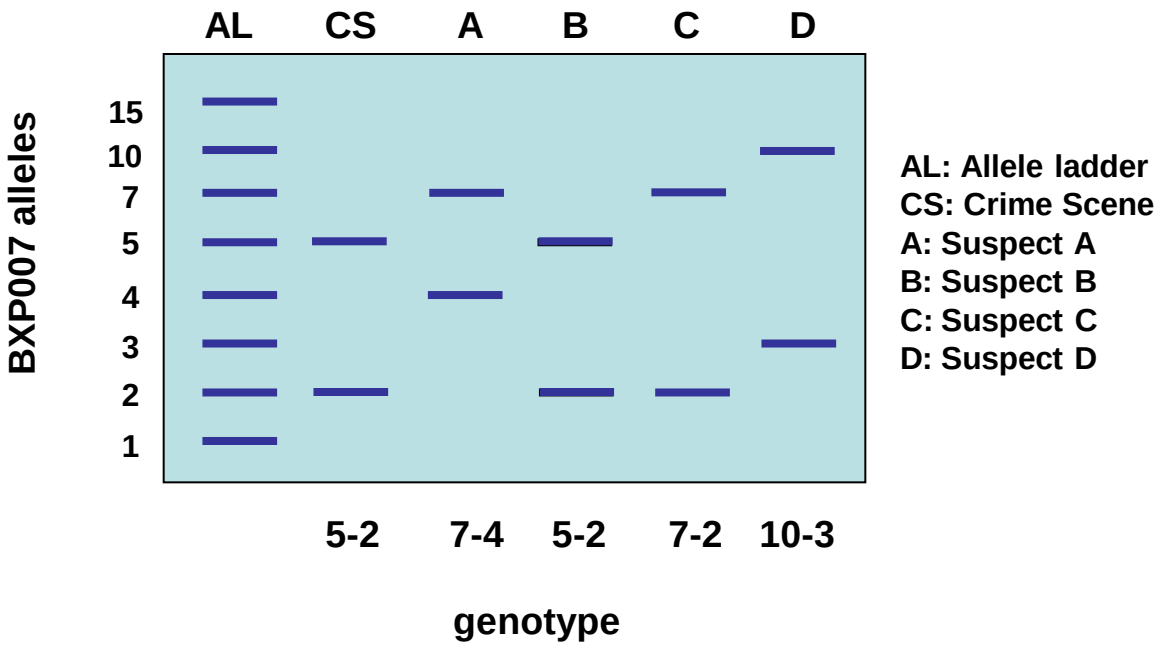
Four different fluorescent tags have been used to identify 7 amplified loci

Allele ladders are indicated by arrows



Analysis of Results:

Who can't be excluded?



Biotechnology Explorer™
CAPTIVATING SCIENCE EDUCATION

BIO-RAD