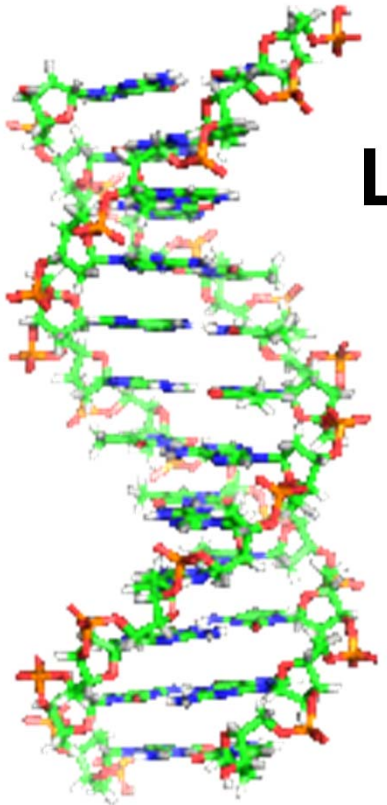
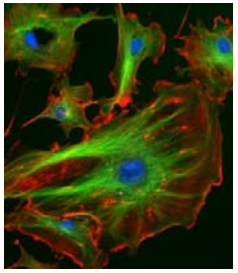


Lecture 9: From Mutation to Crime Scene Investigation



Section B: From Concepts to Techniques & Applications

Genes & Society
LSM 1302 / GEK 1527

With the advent of DNA, we know that people have been convicted and sentenced to death who later proved not to be guilty of the crime.

- Kamala Harris, Attorney General

Overview

- **Mutations & DNA fingerprinting**
- **Extracting & Amplifying DNA**
- **Separating & Visualizing DNA fragments**
- **DNA fingerprinting in Society**

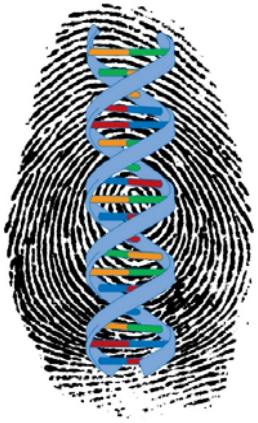
http://www.forensicmag.com/articles/2014/04/processing-touch-dna-crime-scene

http://crimesceneinvestigationstoday.blogspot.com/2011/03/dna-crime-scene-investigations.html

Mutations & DNA Fingerprinting

- Over 98% of our genome do not code for any protein.
- These non-coding regions help to buffer for mutation, i.e. in the event of a random mutation, there is a greater chance (>98%) that it will occur in non-coding region so that it will not affect an encoded protein that can potentially be harmful to an individual.
- Therefore, these non-coding regions become sites where mutations can accumulate and pass on from one generation to another.
- Two major forms of mutations are frequently accumulated and inherited:

1. Single Nucleotide Polymorphisms (SNPs)
2. Tandem Repeats: Variable Number of Tandem Repeats (VNTRs) and Short Tandem Repeats (STRs)



Non-coding region (intergenic)	Regulatory Region (promoter)	Coding region (exon1)	Non-coding region (intron1)	Coding region (exon2)	Non-coding region (intron2)	Coding region (exon3)

SNPs
5'-GA**A** TTC-3'
5'-GA**T** TTC-3'
5'-GA**C** TTC-3'
5'-GA**G** TTC-3'
(can occur anywhere in the genome)

R1	R2	R3	R…

Tandem Repeats (R)
R = 5'–GATCTTTAAACCCATC-3' (VNTR)
R = 5'-CATG-3' (STR)
(usually do not occur in coding regions)

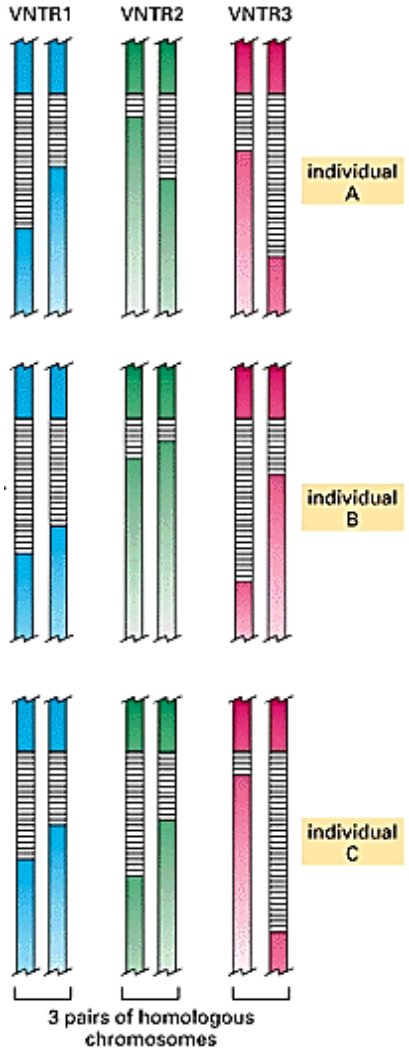
Watch how repeats are generated during DNA replication
http://www.hhmi.org/biointeractive/tri-nucleotide-repeat

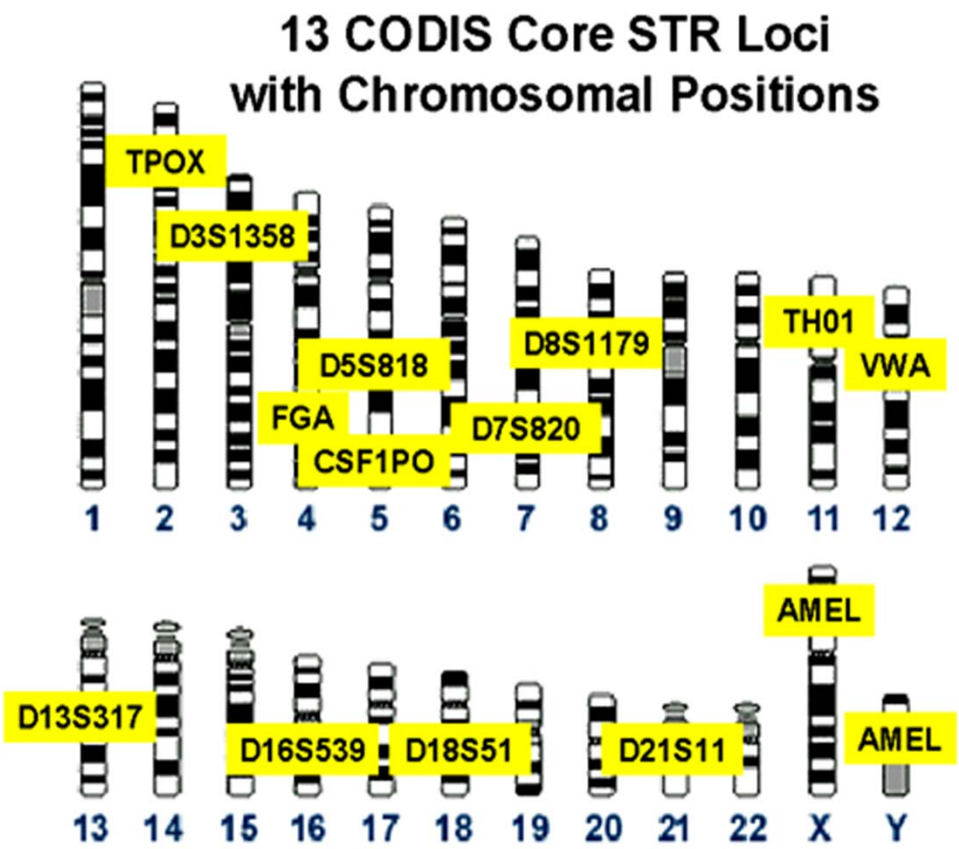
http://www.robsonpiresxerife.com/notas/nova-lei-cria-banco-de-dna-de-criminosos-sexuais-na-paraiba/

DNA Fingerprinting: Detecting Inherited Mutations in Individuals

The principle is based on detecting the different number of tandem repeats (VNTRs and STRs) in certain regions of the genome that are highly polymorphic.

The differences in the number of tandem repeats in specific are highly individual (inherited from both parents as a result of ancestral accumulated mutation) hence they can be used as a basis for identifying individuals (a DNA fingerprint).

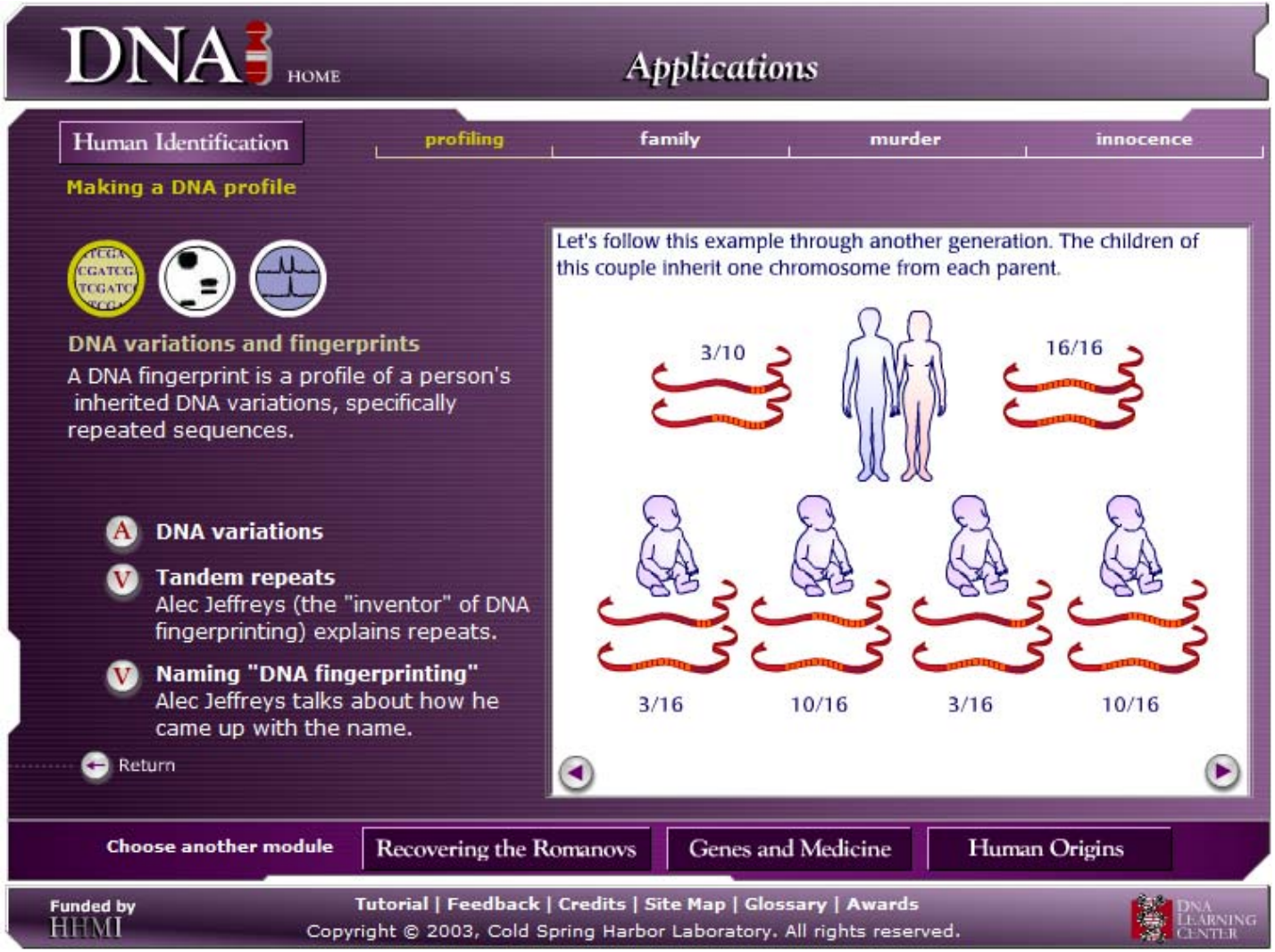




DNA Fingerprinting

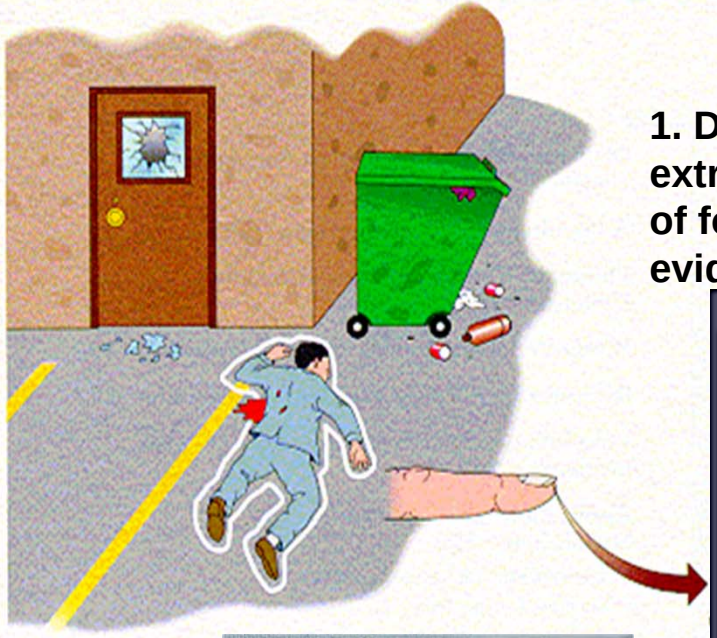
Due to random mutations in our ancestors' genome which are passed down to the germ cells (in the eggs or sperms), each of us will inherit differences in DNA sequence in our genome including different type and amount of Tandem Repeats (VNTRs and STRs).

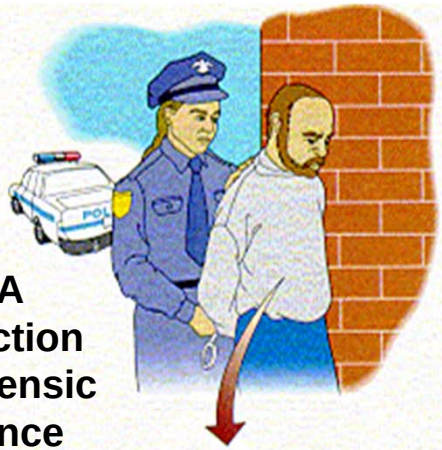
The Tandem Repeats (VNTRs and STRs) are inherited in a Mendelian fashion. Therefore the closer biological relation between two individuals, the more similar the Tandem Repeats (VNTRs and STRs) they would share.



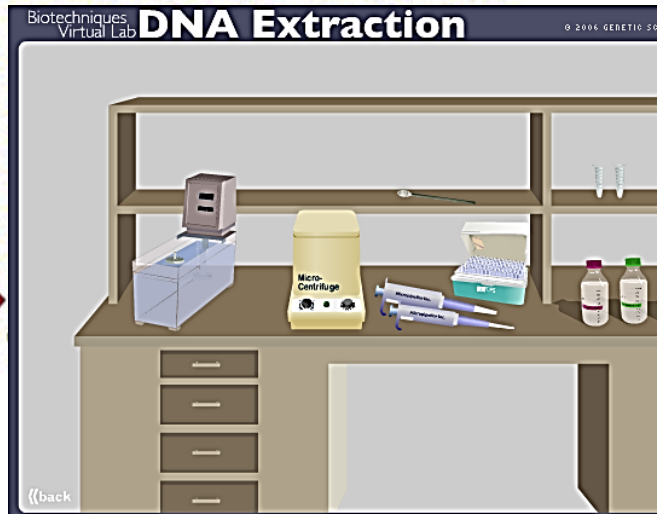
http://www.dnai.org/d/index.html

Crime Scene Investigation





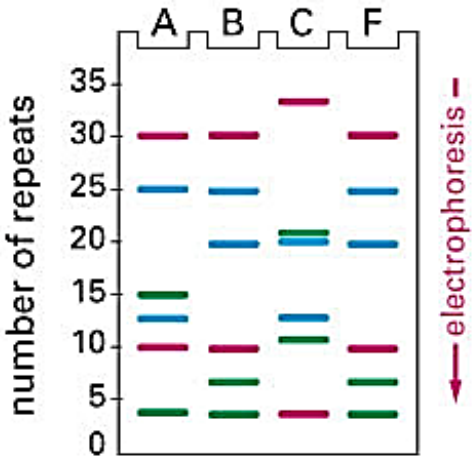
1. DNA extraction of forensic evidence



http://learn.genetics.utah.edu/content/labs/extraction/

2. PCR Amplification of Tandem Repeats

3. Visualization & Analysis of Tandem Repeats



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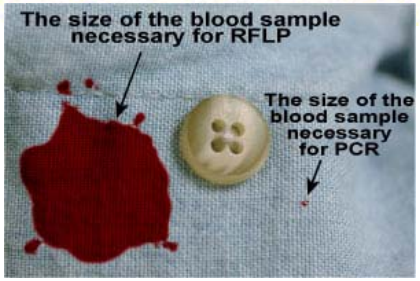


http://projects.nfstc.org/otc/module4/4.1.009.htm

How to Extract DNA?

In order to use or manipulate DNA for various applications, DNA need to be extracted from cells.

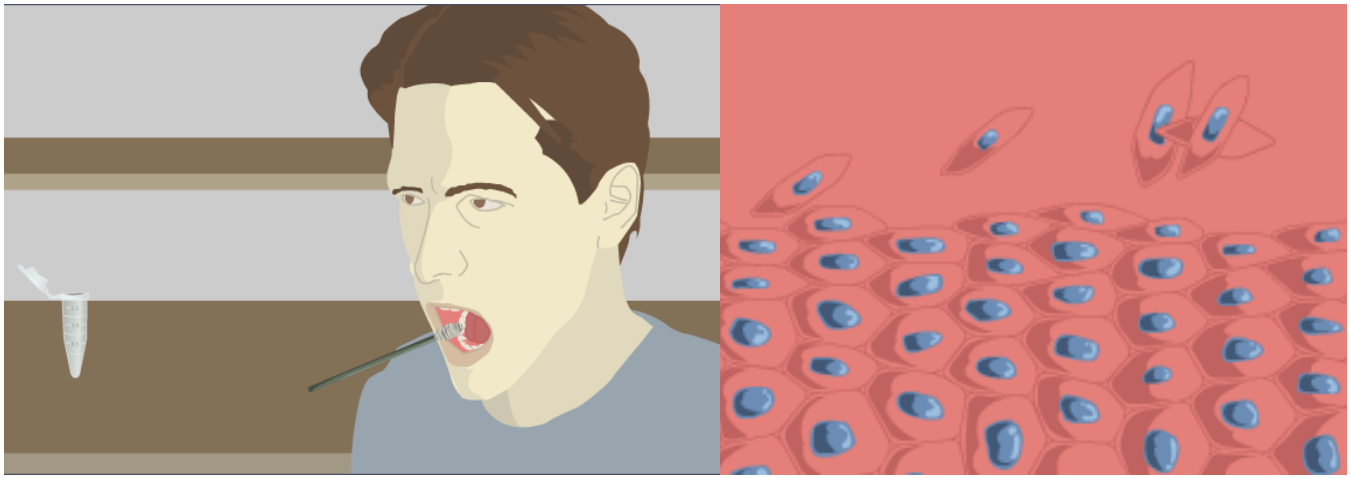
DNA needs to be purified from proteins and other cellular contaminants so that it can be useful.

In general it contain the following major steps:

1. **Obtain source (cells) for DNA**
2. **Burst cells to release DNA**
3. **Precipitate protein with high salt**
4. **Precipitate DNA with isopropanol**

http://learn.genetics.utah.edu/content/labs/extraction/

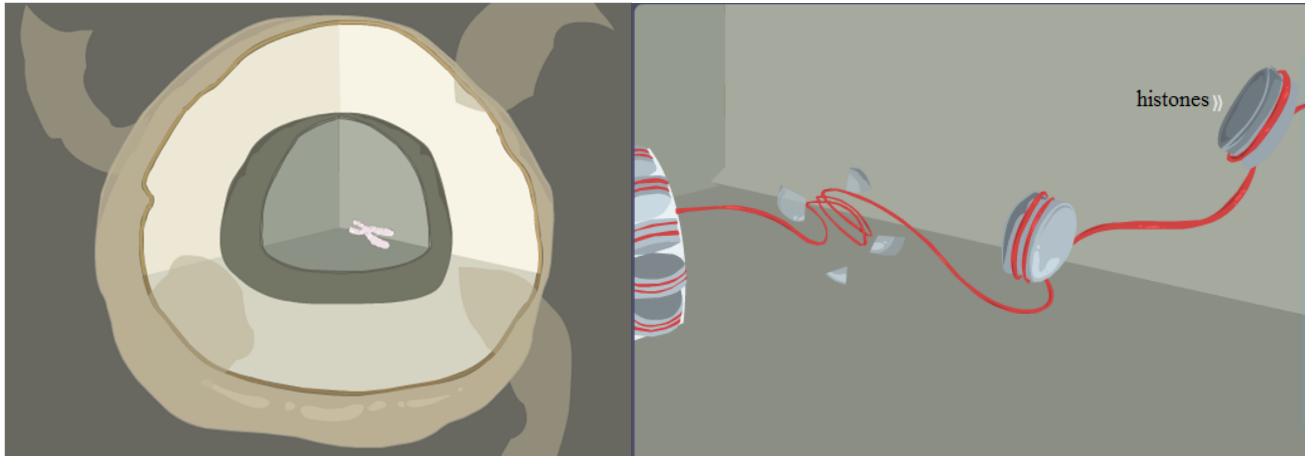
1. Obtain source for DNA extraction



http://learn.genetics.utah.edu/content/labs/extraction/

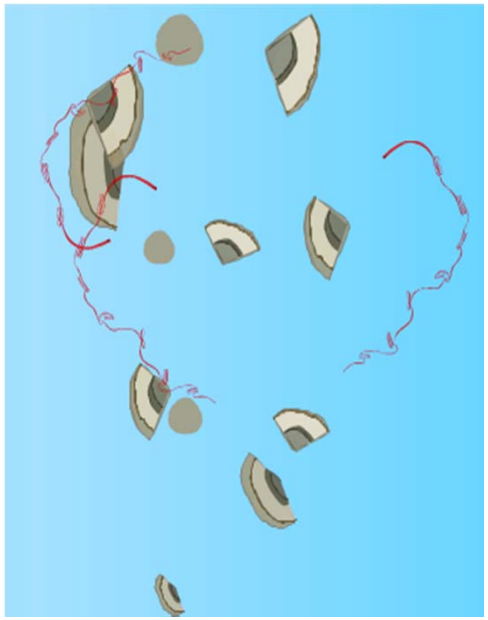
The source of DNA are usually from cells of tissue or organisms (including bacteria and viruses). For humans, cheek cells are usually used because is less invasive. Collection of cheek cells can be easily done with a cotton bud.

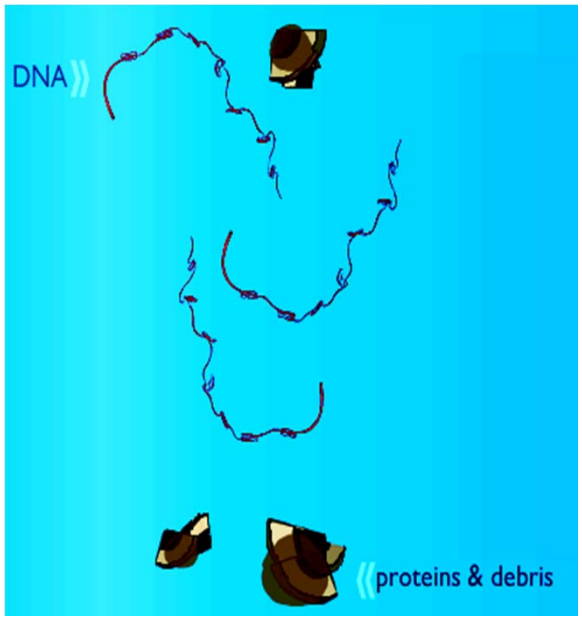
2. Burst cells and cut proteins to release DNA

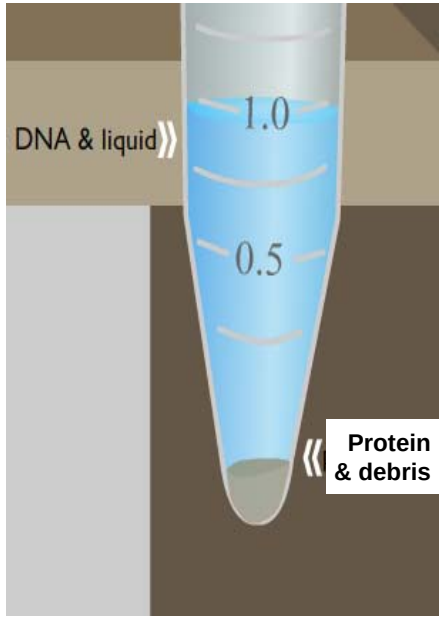


The cell has to be lysed (burst opened) using a solution that contain detergent that will disrupt the cell and nuclear membranes to release the DNA. DNA are usually associated with histone proteins and therefore the proteins need to be digested by an enzyme proteinase-K to free the DNA.

3. Precipitate protein with high salt





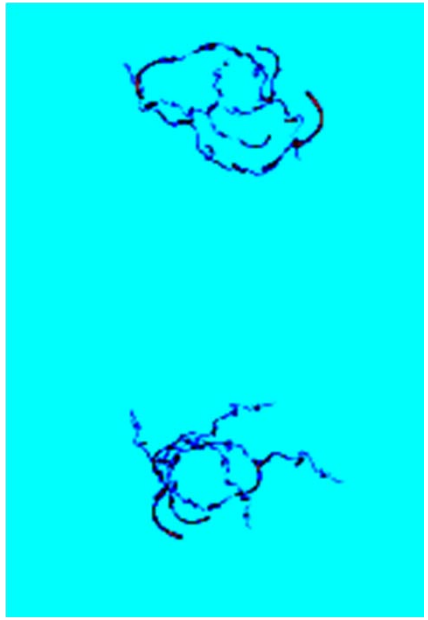


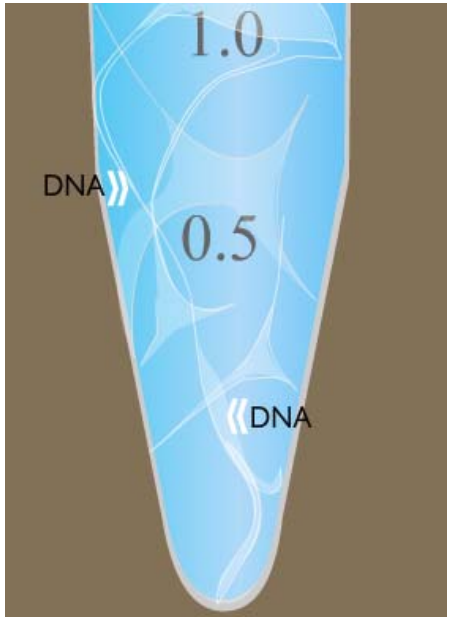
The solution contain mixture of DNA with proteins and other cellular debris. High concentration of salt is added to cause the proteins and cellular debris to clump together as precipitate due to hydrophobic and non-polar regions in these components. DNA which is negatively charged will remain in the solution.

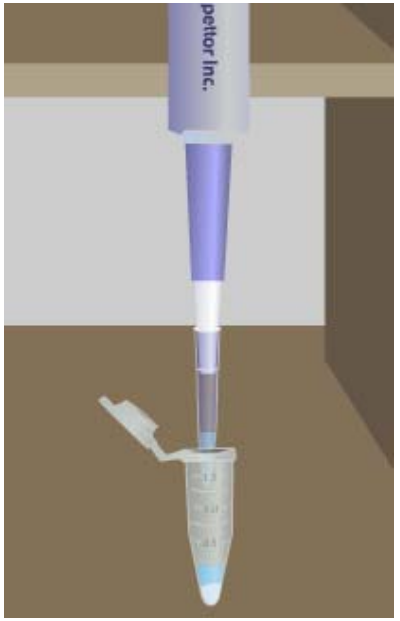
These precipitates are larger in mass and heavier, hence they can be spun down under centrifugation force using a microcentrifuge. DNA that remains in the solution can now be removed into a clean tube and is now separated from the proteins and cellular debris.

http://learn.genetics.utah.edu/content/labs/extraction/

4. Precipitate DNA with isopropanol





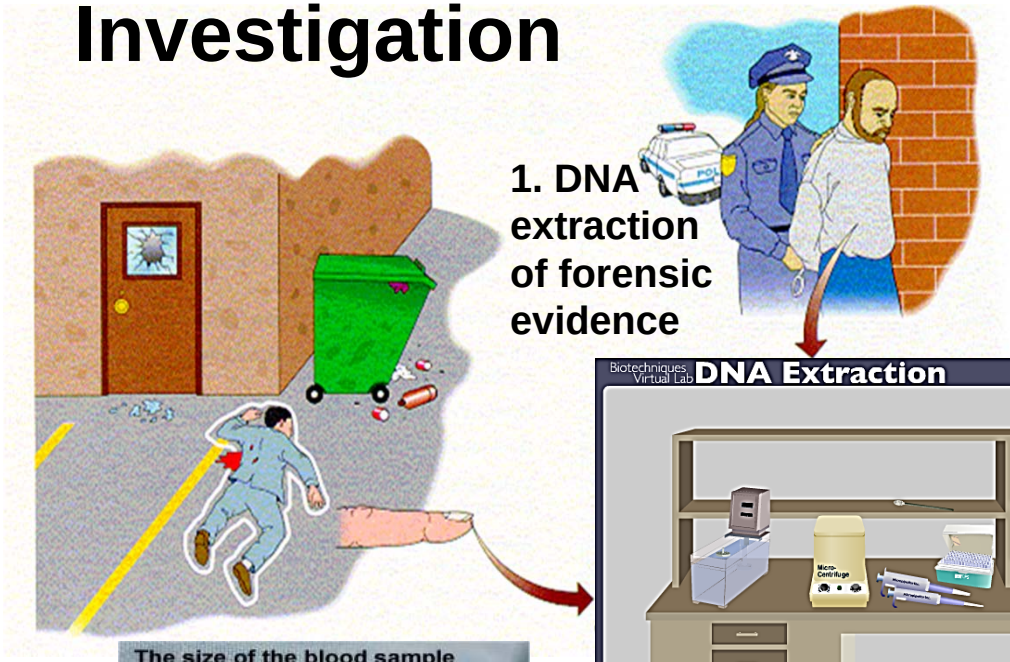


DNA in a high salt environment remain diluted and not suitable for use. Since DNA is not soluble in isopropanol, addition of isopropanol will cause the DNA high salt environment to clump together until they precipitate out.

These DNA precipitates are larger in mass and heavier, hence they can be spun down under centrifugation force using a microcentrifuge. The solution is removed leaving the DNA at the bottom of the tube. The DNA can be resuspended in a small quantity of water or buffer. This will result in a more concentrated solution of pure DNA that can be used for PCR and other applications.

http://learn.genetics.utah.edu/content/labs/extraction/

Crime Scene Investigation



1. DNA extraction of forensic evidence

http://learn.genetics.utah.edu/content/labs/extraction/

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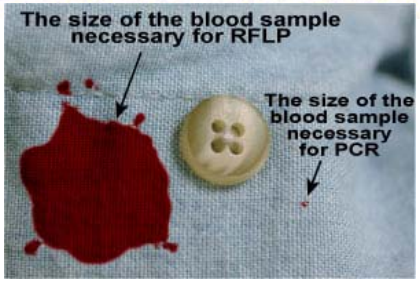
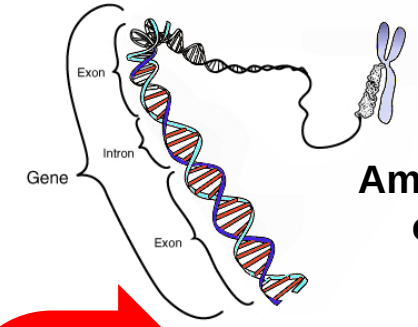
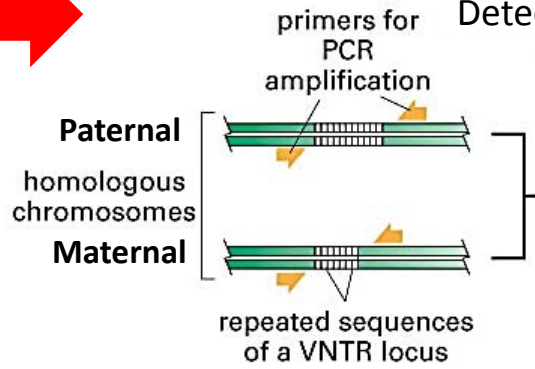


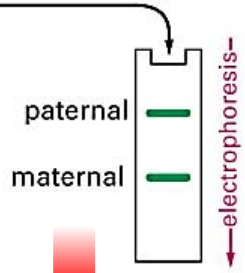
http://projects.nfstc.org/otc/module4/4.1.009.htm

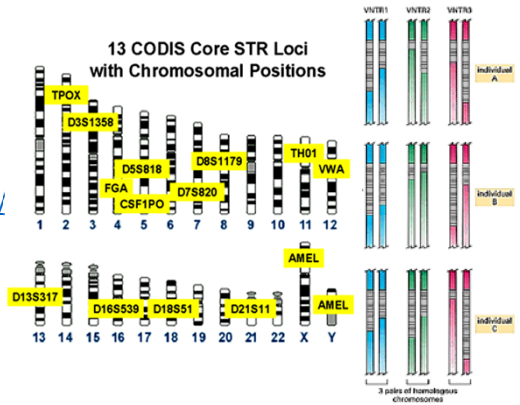


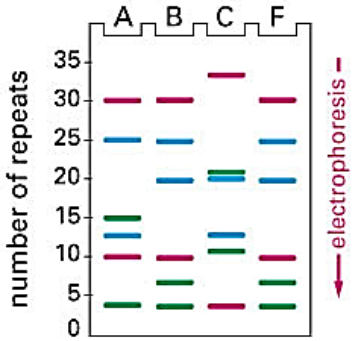
2. PCR Amplification of Tandem Repeats

Detect amplified VNTR on gel



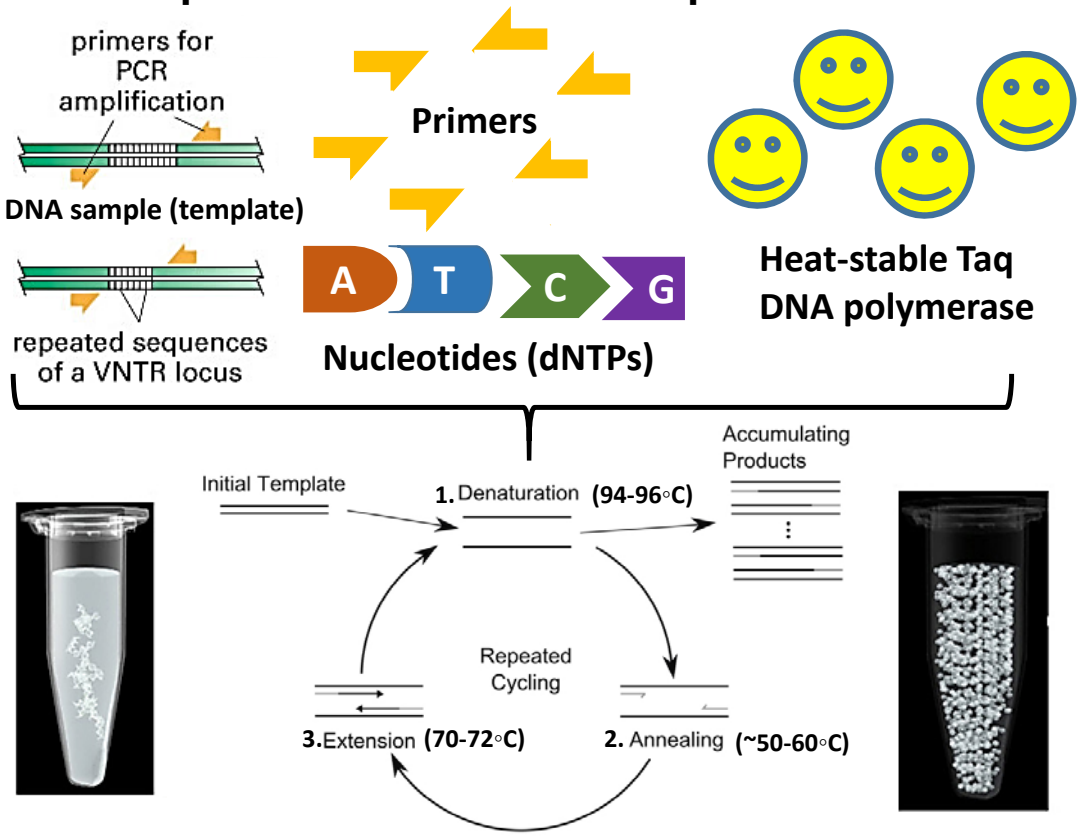


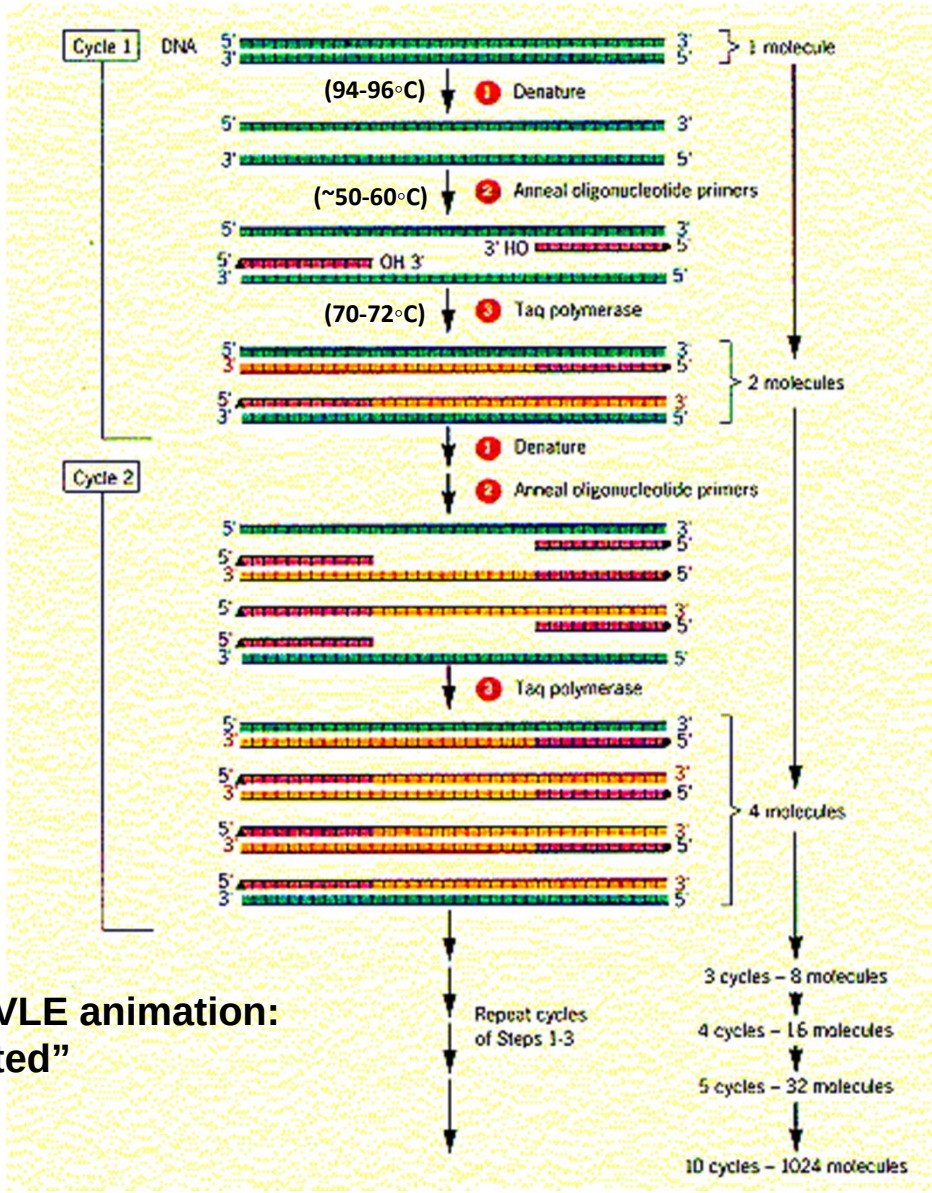




3. Visualization & Analysis of Tandem Repeats

2. PCR Amplification of Tandem Repeats



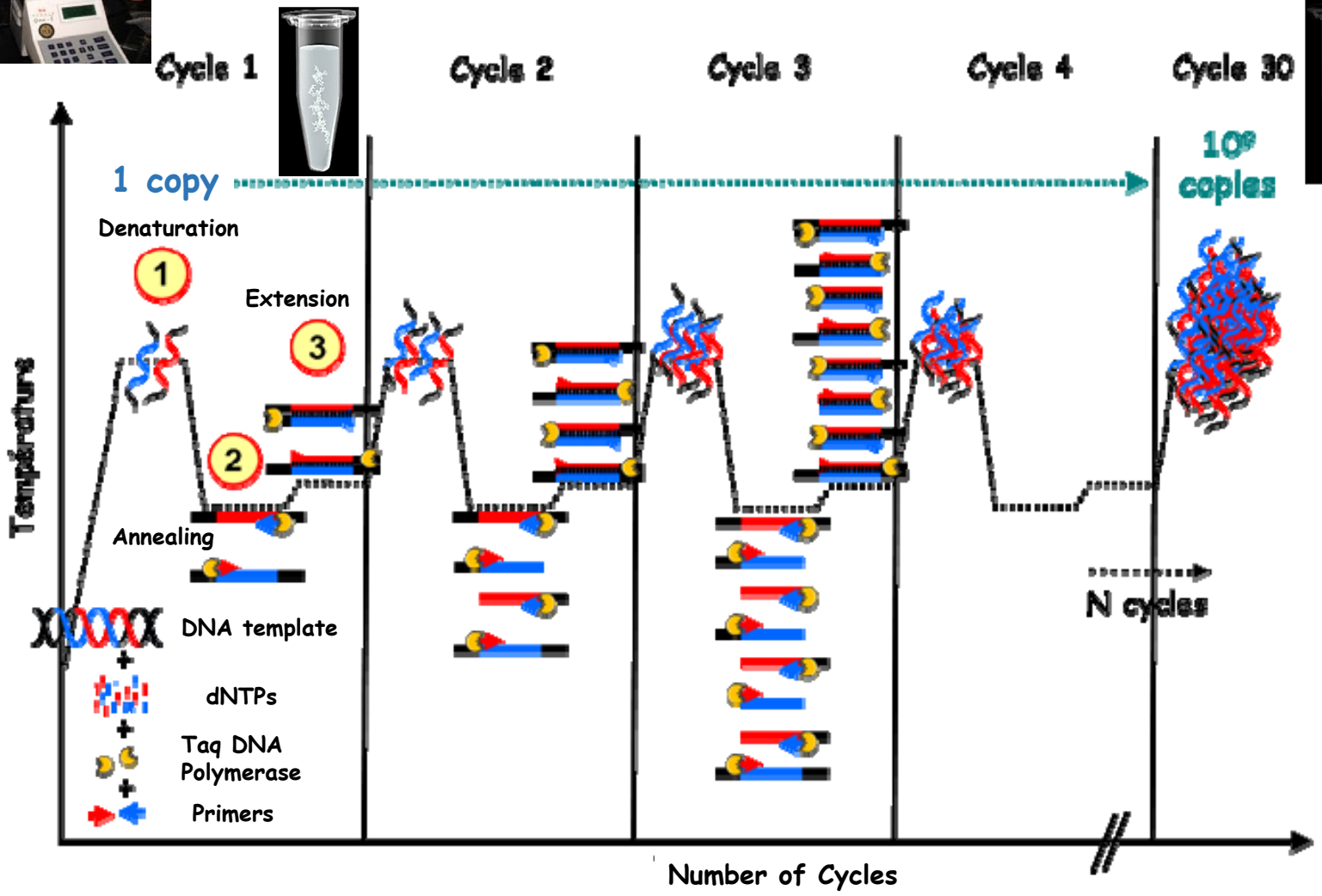


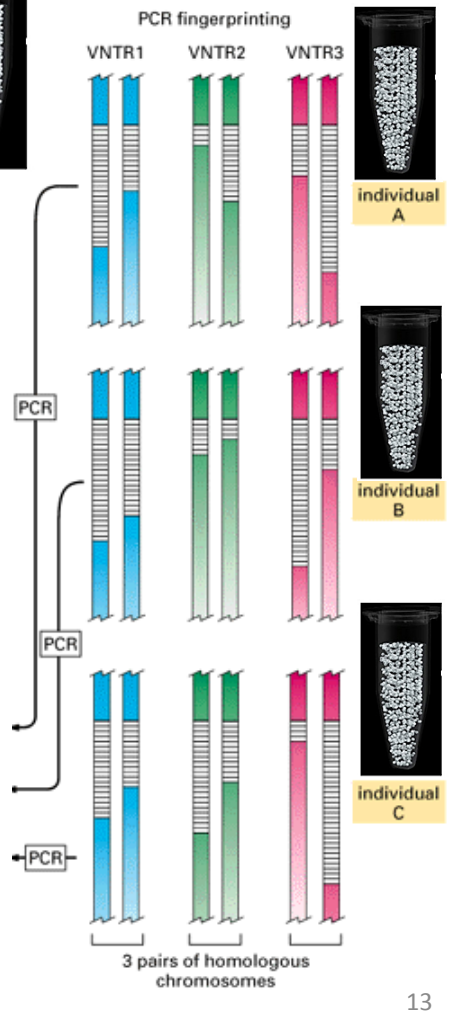
Thermocycler (PCR machine) provide rapid change in temperature within a PCR cycle

Check out IVLE animation: "PCR narrated"

http://www.bio-rad.com/en-es/applications-technologies/droplet-digital-pcr-ddpcr-technology

2. PCR Amplification of Tandem Repeats





https://ed414-openlab.unistra.fr/les-tp/adn-et-genetique-2009-2012/pour-preparer-le-tp/la-pcr-quest-ce-que-cest/

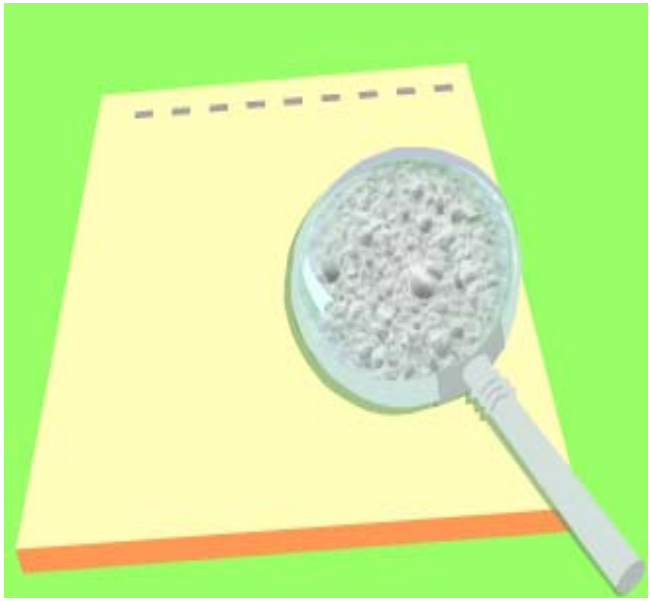
3. Visualization & Analysis of Tandem Repeats

Gel Electrophoresis technique allows separation and visualization of a mixture of DNA fragments of various sizes (length) e.g. PCR amplified Tandem Repeats.

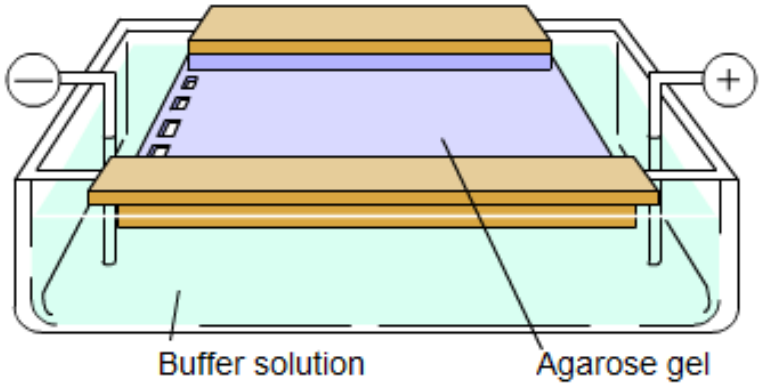
Basic steps:

1) **Make the gel.**
2) **Set up gel apparatus**
3) **Load DNA sample into the wells of the gel**
4) **Hook up electrical current to run gel**
5) **Stain the gel to visualize and analyze results.**

http://learn.genetics.utah.edu/content/labs/gel/

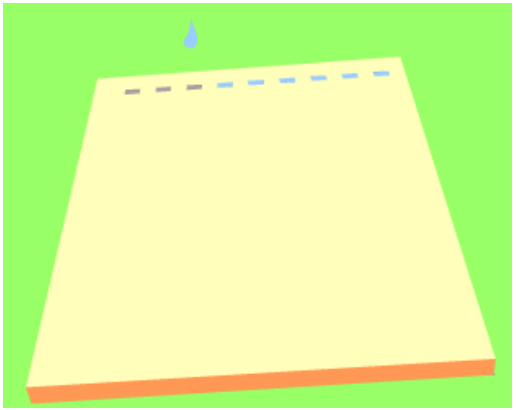


1. Make the Gel
An agarose gel is made by melting some agar in a volume of buffer using heat (in a microwave oven). Before the agar solution has solidified, it is poured into a cast/mold. Once solidified, the agar gel slab has wells on one end to contain the DNA to be loaded. A closer examination within the gel reveal a mesh-like network of agar particles that will act as a sieve or filter to separate the DNA fragments according to their size when they migrate within the gel under the influence of an electrical current.

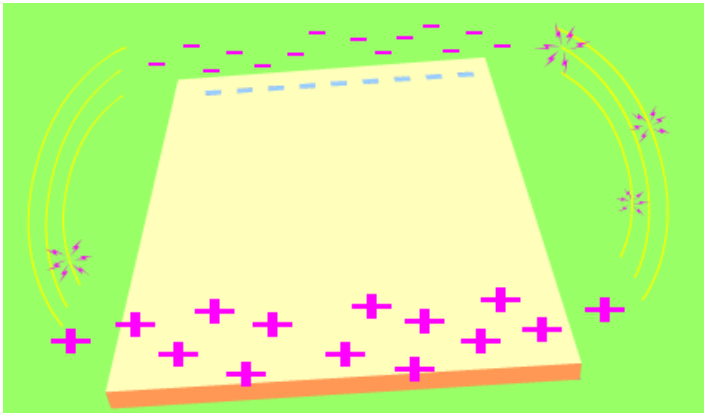


2. Set up the gel apparatus
The gel is placed in a tank containing buffer and the tank can be hooked to an electrical current that will cause the DNA fragments to migrate within the gel. Which electrode (positive or negative) would DNA migrate towards?

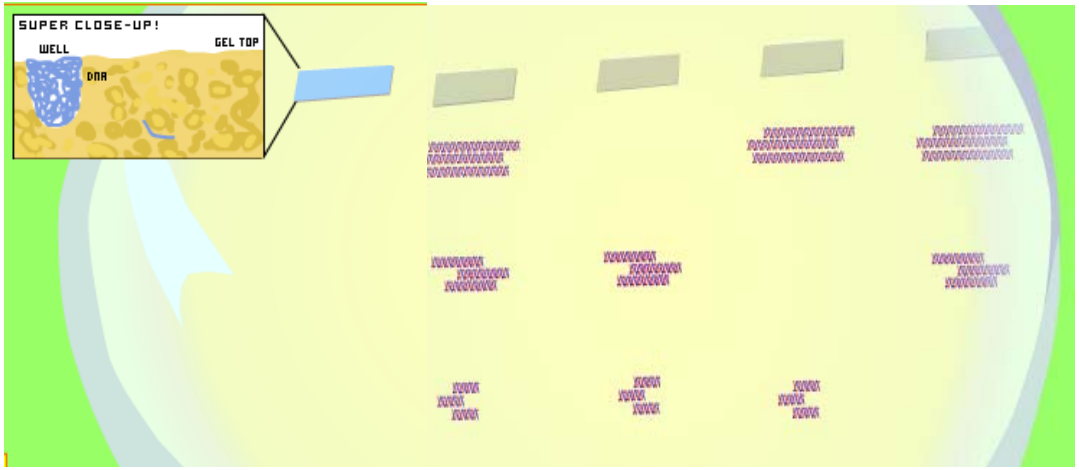
http://learn.genetics.utah.edu/content/labs/gel/



3. Load the DNA samples into the wells of the gel.

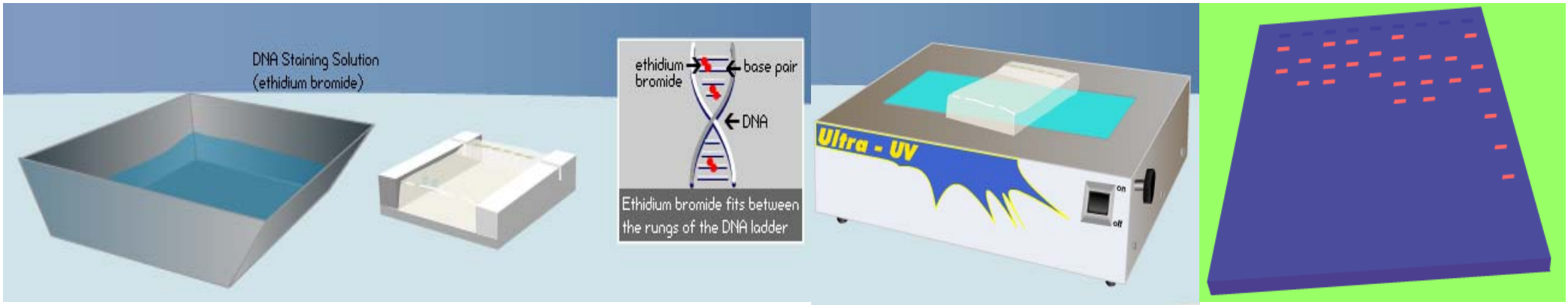


4. Hooked up the electric current and run the gel. The wells loaded with DNA is placed closer to the negative electrode.

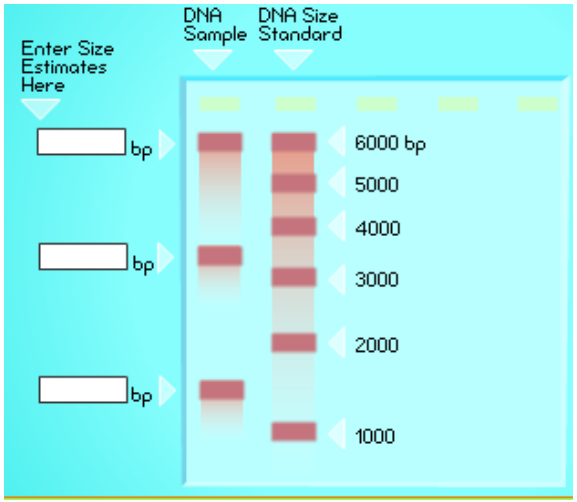


The negatively-charged DNA will migrate from the negative electrode towards the positive electrode. Shorter fragments will migrate faster through the mesh network of agar compared to longer fragments. The different DNA fragment size (VNTRs) are separated.

http://learn.genetics.utah.edu/content/labs/gel/



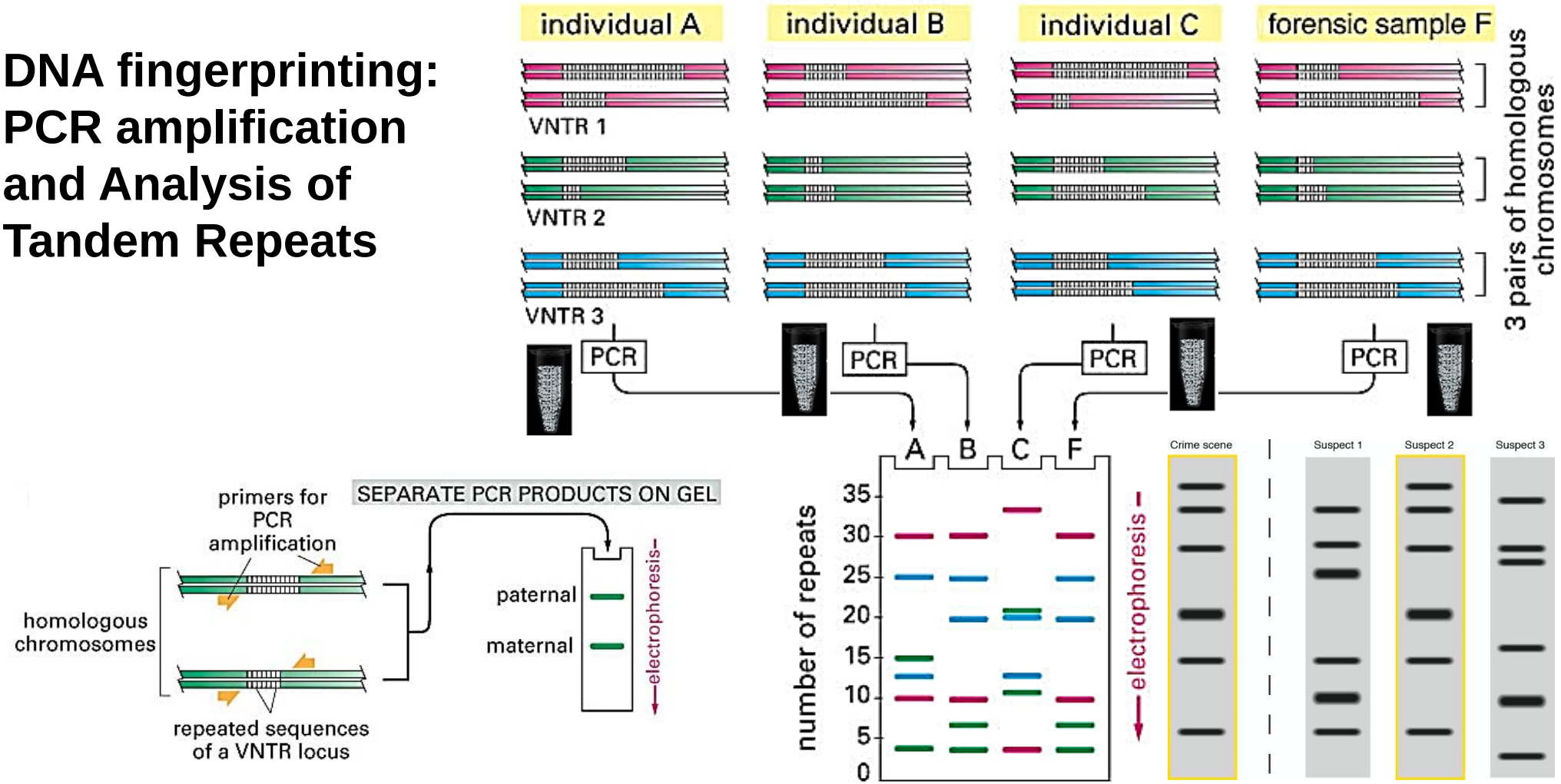
5. Stain the gel with ethidium bromide (EB) to visualize and analyze results.
EB will intercalate and bind to the DNA fragments in the gel. When view under ultraviolet (UV) light, the many DNA fragments bound with EB will fluoresce bright orange. The intensity of the bands correspond with the amount of DNA. A photograph of the bands can be taken for a record purpose.



Usually a commercially produced standard size DNA markers are run side by side with our DNA samples of interest. By comparing with DNA bands of known sizes from the commercial DNA markers, we can estimate the sizes of the bands observed in our DNA sample of interest.

http://learn.genetics.utah.edu/content/labs/gel/

DNA fingerprinting: PCR amplification and Analysis of Tandem Repeats



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Cases

http://en.wikipedia.org/wiki/Genetic_fingerprinting [edit]

In the 1920s, Anna Anderson claimed that she was Princess Anastasia Romanov of Russia; in the 1980s her cremated remains were tested and seemed to show that she was no relation to the Romanovs.

In 1987, British baker Colin Pitchfork was the first criminal caught using DNA fingerprinting in Leicester, the city where it was first discovered.

In 1987, Florida rapist Tommie Lee Andrews was the first person in the United States to be convicted as a result of DNA evidence, for raping a woman during a burglary; he was convicted on 6 November 1987 and sentenced to 22 years in prison. [3] [4]

In 1988, Timothy Spencer was the first man in the United States to be sentenced to death through DNA Testing for several rape and murder charges, He was dubbed "The South Side Strangler" Because he killed all his victims on the southside of Richmond, Virginia. He was later charged with rape and 1st degree murder and was sentenced to death. He was executed on April 27, 1994.

In 1989, Chicago man Gary Dotson was the first person whose conviction was overturned using DNA evidence.

In 1991, Allan Legere was the first Canadian to be convicted as a result of DNA evidence, for four murders he had committed while an escaped prisoner in 1989. During his trial, his defense argued that the relatively shallow gene pool of the region could lead to false positives.

In 1992, DNA evidence was used to prove that Nazi doctor Josef Mengele was buried in Brazil under the name Wolfgang Gerhard.

In 1993, Kirk Bloodsworth was the first person to have been convicted of murder and sentenced to death, whose conviction was overturned using DNA evidence.

The science was made famous in the United States in 1994 when prosecutors heavily relied on — and through expert witnesses exhaustively presented and explained — DNA evidence allegedly linking O.J. Simpson to a double murder. The case also brought to light the laboratory difficulties and handling procedure mishaps which can cause such evidence to be significantly doubted.

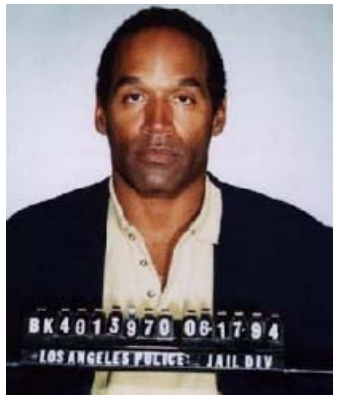
In 1994, RCMP detectives successfully tested hairs from a cat known as Snowball, and used the test to link a man to the murder of his wife, thus marking for the first time in forensic history the use of non-human DNA to identify a criminal.

In 1998, Dr. Richard J. Schmidt was convicted of attempted second-degree murder when it was shown that there was a link between the viral DNA of the human immunodeficiency virus (HIV) he had been accused of injecting in his girlfriend and viral DNA from one of his patients with full-blown AIDS. This was the first time viral DNA fingerprinting had been used as evidence in a criminal trial.

Fake DNA evidence

[edit]

The value of DNA evidence has to be seen in light of recent cases where criminals planted fake DNA samples at crime scenes. In one case, a criminal even planted fake DNA evidence in his own body: Dr. John Schneeberger of Canada raped one of his sedated patients in 1992 and left semen on her underwear. Police drew Schneeberger's blood and compared its DNA against the crime scene semen DNA on three occasions, never showing a match. It turned out that he had surgically inserted a Penrose drain into his arm and filled it with foreign blood and anticoagulants.



http://law2.umkc.edu/faculty/projects/ftrials/Simpson/simpson.htm

The OJ Simpson's case(1995)
Monica Lewinsky's famous dress (1998)

http://law2.umkc.edu/faculty/projects/ftrials/clinton/lewinskydress.html

Blood-sucking leech leads Australian police to armed robber with extraordinary DNA match

By RICHARD SHEARS
Last updated at 1:41 AM on 21st October 2009

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An armed robber has been caught after his DNA was matched with blood from a leech found at the scene of the crime he committed eight years ago.

The creature was the only piece of evidence recovered from a farmhouse ransacked by Peter Cannon and an accomplice.

But it was only when Cannon was arrested for a separate crime last year that the DNA match was discovered by Australian police.

The pair targeted an isolated property in northern Tasmania, stealing around £200 from the safe and tying up 71-year-old Fay Olson.

Their faces hidden under black hoods, and clutching pieces of wood, they forced their way into the victim's home.

herself and when police arrived they were unable to find any obvious clues.

One officer eventually spotted the leech, and as there were no bites on the victim deduced it must have been attached to one of the robbers.

'We took it from the scene because it didn't belong there,' one officer said.

The creature was examined and the DNA extracted from the blood. All police needed then was a person to match it with.

It was seven years before Cannon was arrested and charged with drug offences - and when his DNA was compared with that taken from the leech a match was found.

Crown prosecutor John Ransom told a judge in Tasmania that the chances of the DNA from the leech matching someone else were one in a 100million.

World first: Blood from the engorged leech was matched to Cannon after he was later arrested and charged with drug offences

http://www.dailymail.co.uk/news/worldnews/article-1221626/Blood-sucking-leech-leads-Australian-police-armed-robber.html

Where DNA fingerprinting fails (human error)

Twins escape hanging over ID confusion

Posted Sat Feb 7, 2009 5:49pm AEDT

A pair of identical twins escaped being convicted and hanged on drugs charges in Malaysia, due to confusion over which one of them was the culprit, reports said Saturday.

The 27-year-old twins, R Sathis Raj and Sabarish Raj, had been charged with trafficking 166 kilos of cannabis and 1.7 kilos of raw opium in 2003. But police had trouble identifying which one was in possession of the drugs.

"This is a very unique case as they are identical twins. Even the DNA evidence could not prove anything as the DNA could be either Sathis' or Sabarish's," said High Court judge Zaharah Ibrahim.

"I can't be calling the wrong twin to enter his defence. I can't be sending the wrong person to the gallows," she said according to the New Straits Times as she acquitted the pair.

The court heard that one of the twins was caught taking a carload of drugs to a house in suburban Kuala Lumpur. After he was apprehended the other twin arrived at the house and was also arrested.

"It is clear that the first twin who was caught was the only one who had access to the house. Therefore, it is impossible for him not to know what was inside the bags," the judge said according to the daily.

http://www.abc.net.au/news/stories/2009/02/07/2485172.htm

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412 criminal cases reviewed after DNA test lab error

Published on Jan 4, 2012

By Salma Khalik , HEALTH CORRESPONDENT

An error in a government laboratory resulted in the solution used in DNA testing in criminal cases to be 10 times stronger than it should have been.

The error had essentially led to a 'marginal reduction in sensitivity' of DNA tests done by the Health Sciences Authority (HSA) between October 2010 and August last year. -- ST PHOTO: ALPHONSUS CHERN

As a result, 412 cases had to be reviewed, and the Attorney-General's Chambers (AGC) now wants DNA tests in 87 of the cases to be redone, as a precaution. But the Government on Tuesday also gave the assurance that the mistake has not resulted in anyone being wrongly convicted.

The error had essentially led to a 'marginal reduction in sensitivity' of DNA tests done by the Health Sciences Authority (HSA) between October 2010 and August last year.

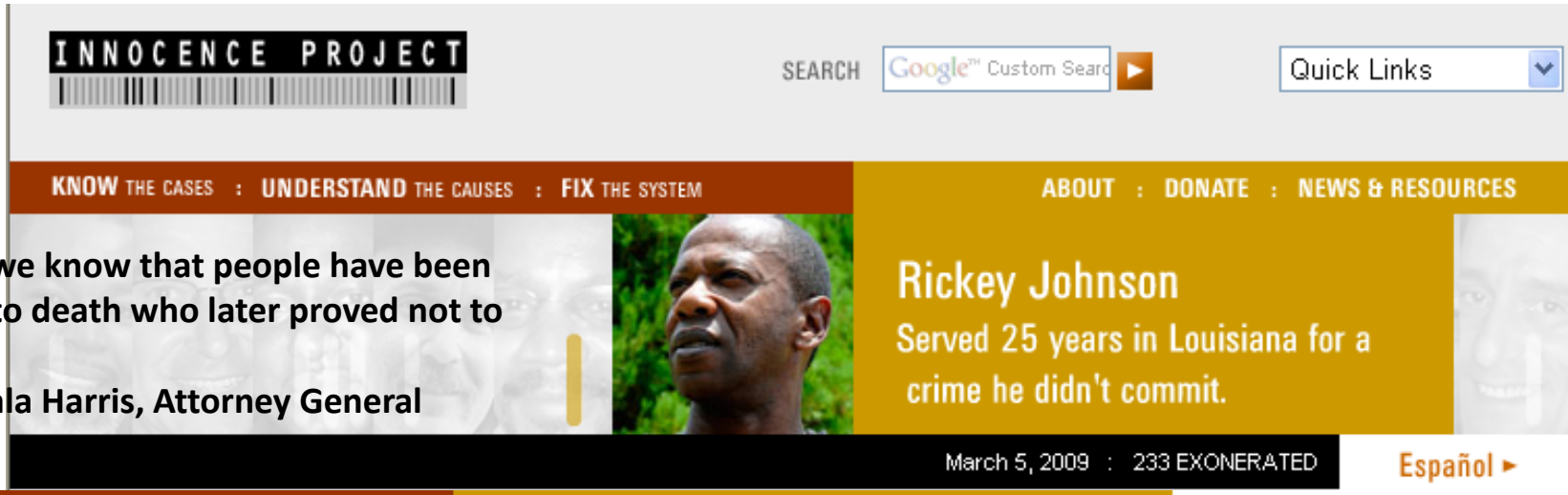
'There was no possibility of falsely indicating any DNA profile that was not present,' said the AGC.

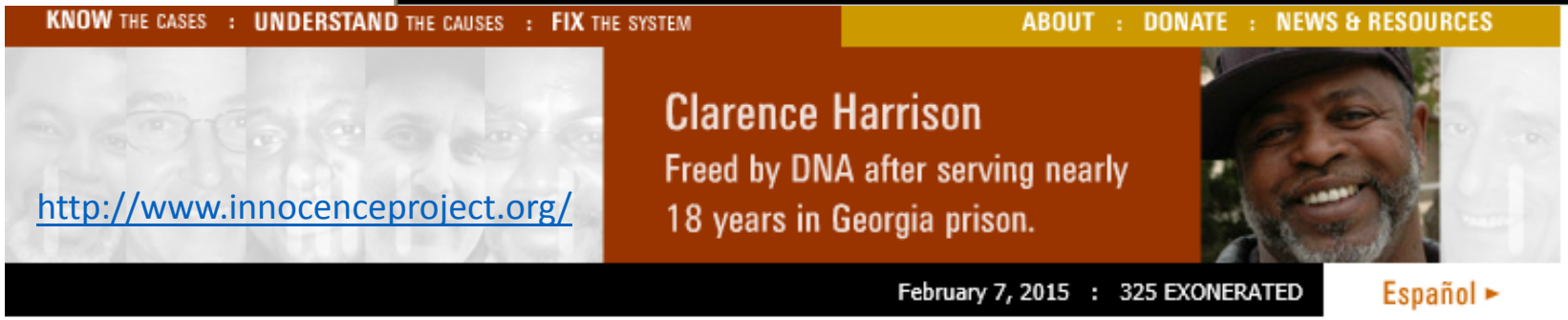
BACKGROUND STORY

A look at the numbers

With the advent of DNA, we know that people have been convicted and sentenced to death who later proved not to be guilty of the crime.

- Kamala Harris, Attorney General





Rickey Wyatt Fully Exonerated, Becoming 325th Person Cleared by DNA

On the request of the District Attorney's Office, a Dallas County judge entered a court order finding Rickey Dale Wyatt innocent of a rape for which he served nearly 31 years.

MORE ►

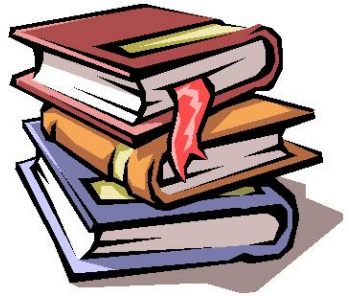
Over the past 6 years, 92 people have been exonerated using DNA evidence.

Read: "Exonerations in 2014 Report"

Summary & Conclusion

- **Two major forms of mutation frequently accumulated and inherited in in a Mendelian fashion are Single Nucleotide Polymorphisms (SNPs) and Tandem Repeats (VNTRs and STRs).**
- **DNA fingerprinting is based on detecting the mutations that are uniquely inherited such as different number of tandem repeats (VNTRs and STRs) in certain non-coding regions of the genome.**
- **In order to perform DNA fingerprinting, DNA sample has to be extracted, and tandem repeats has to be amplified by PCR and subsequently separated and visualized/analyzed using gel electrophoresis.**
- **To extract DNA, the cells have to be lysed to release the DNA, and the proteins have to be digested and precipitated in high salt to separate from the DNA before the DNA itself is precipitated with isopropanol. The DNA pellet can be concentrated by resuspending in small volume of water.**
- **To amplify DNA, the DNA template must be targeted and annealed by specific primers for Taq DNA polymerase to initiate DNA synthesis using available dNTPs in a PCR reaction where multiple cycles of denaturation, annealing and extension are repeated.**
- **To separate and visualize DNA of different sizes, the mixture of DNA fragments has to be separated under an electrical current that will cause the DNA to migrate at different rates based on their size through a gel mesh. The separated DNA fragments in the gel can be stained with ethidium bromide and viewed under UV light.**
- **DNA fingerprinting has impacted society in areas of crime scene investigation, criminal-legal cases, familial relationships, body identification,**

Additional Enrichment Materials



- **Check out IVLE animation "PCR narrated"**

Useful Weblinks:

- **Check out DNA Interactive Applications on "Human Identification: http://www.dnai.org/d/index.html**
- **Check out Learn Genetics Virtual Labs DNA Extraction: http://learn.genetics.utah.edu/content/labs/extraction/**
- **Check out Learn Genetics Virtual Labs PCR: http://learn.genetics.utah.edu/content/labs/pcr/**
- **Check out Learn Genetics Virtual Labs Gel Electrophoresis:**

  http://learn.genetics.utah.edu/content/labs/gel/
- **Check Out Innocence Project: http://www.innocenceproject.org**
- **Read "The Government Wants Your DNA "**
- **Read "Exonerations in 2014 Report"**