Genetics
& Cell biology

◉ Sheet ○ Slides

Number

8

Done by:

Mustafa Alshekhly

Corrected by:

Lubna Allawi

Doctor

Mamoun

Revision: PCR is the process of DNA pieces amplification, the primers determine which regions of the DNA to be amplified, and we must have a prior knowledge about the DNA sequence; based on which we will choose the primers. The annealing temperature determines the binding specificity between the primers and the DNA:

As the temperature increase ⟶ higher specificity

As the temperature decrease ⟶ non-specific binding

Let's say that while doing PCR we placed the primers at annealing temperature of 55°c and the result was formation of 2 bands, what does this mean? It means that the primer binds to 2 locations, so we increased the temperature (to increase the binding specificity) to 62°c and the result did not change (2 bands), what could be the reason behind primer binding to 2 locations? The reason is that we have gene families, just like there are similarities between histones (2A, 2B; they have very similar sequences but with minor variations) so the primer of one gene could bind to another gene of the same family resulting in the formation of 2 bands, we also have actin gene family.

We can also use PCR in case we discovered a new gene in mice and we want to check if it is found in humans as well, by designing primers that are based on the genome of the mouse and do PCR using them on human genes, assuming there is homology between the DNA sequence of mice and humans, if binding occurs then humans have this gene.

the coming part is copied from slides

Advantages of PCR

1-Easy, fast, sensitive, robust

2-Discovery of gene families

3-Disease diagnosis

Disadvantages of PCR

1-Primers must be known

2-Contamination

3-Product length is limited (usually <5 Kb)

4-Accuracy is an issue

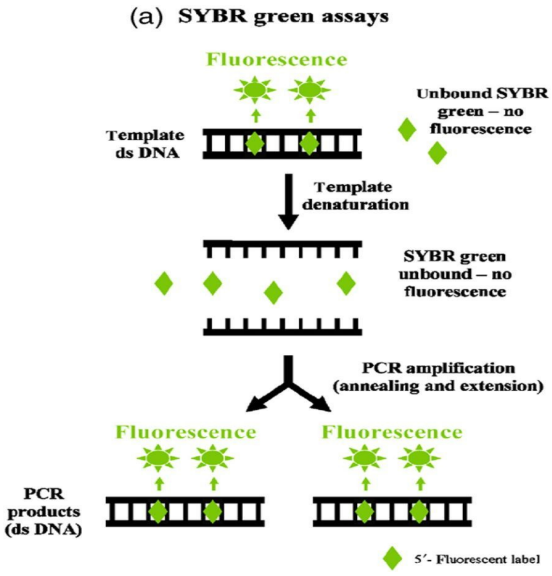
5-Not quantitative by itself (the doctor only mentioned this point)

Uses of PCR:

1- Forensic medicine, just like RFLP (Restriction fragment length polymorphism) in which we use electrophoresis to separate the DNA fragment. Every person has a genetic profile based on which we can determine to whom the unknown DNA sample belong (this was discussed in the previous lectures), Also used with paternity cases.

2- Knowing whether the individual has specific virus/bacteria by designing a primer that binds to it. If we want to know the viral load (how much of the virus are there) we use quantitative PCR.

Quantitative PCR (qPCR) is used to know how much of the DNA copies are there, it depends on SYBR green, which is a molecule that binds to double-stranded DNA and fluoresces only when bound, for example if I want to do PCR with a person that has 1000 viruses per 1 ml of blood and another person has 10 viruses/ml.

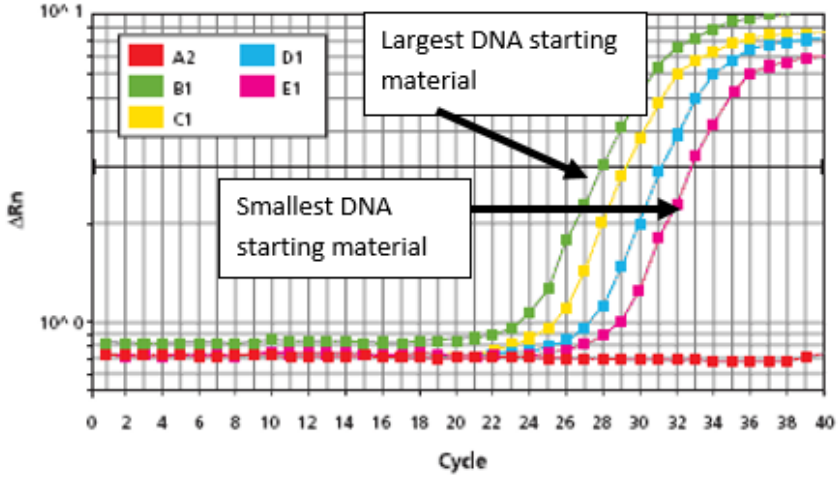


The one with 1000 viruses/ml will have faster amplification since the starting material is larger thus the amplification is faster and greater

Mechanism: usual PCR components (DNA, primers, DNA polymerase) in addition of SYBR green are placed in a special PCR device that can detect fluorescence.

There are 2 types of qPCR:

1- Relative qPCR in which we compare the starting DNA material between different samples, how?

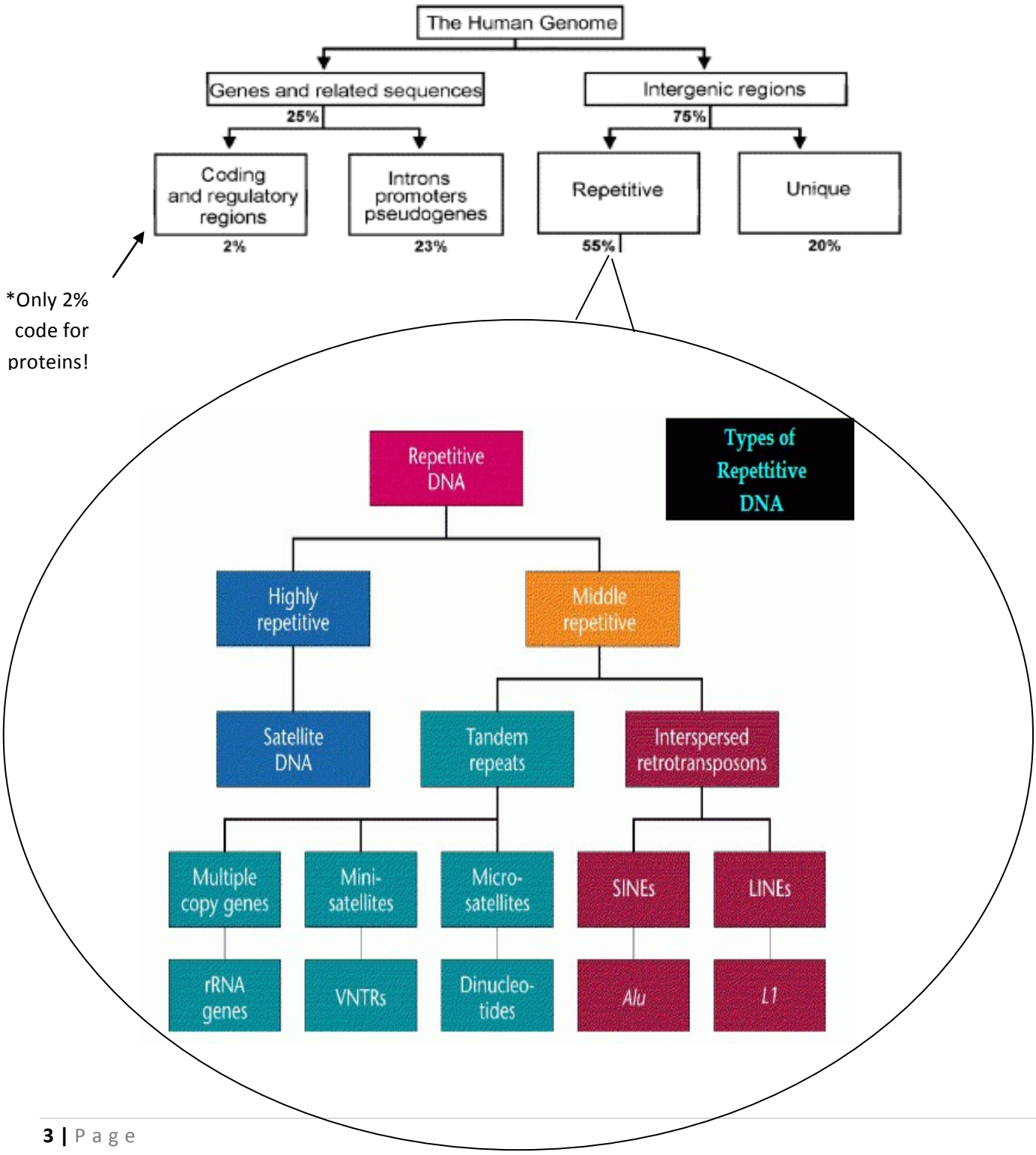


We do multiple qPCR cycles until the amount of fluorescence is detected by the device (as the DNA sample doubles, the amount of fluorescence doubles) the DNA sample that is detected first is the sample which has the largest starting material (largest viral load for example)

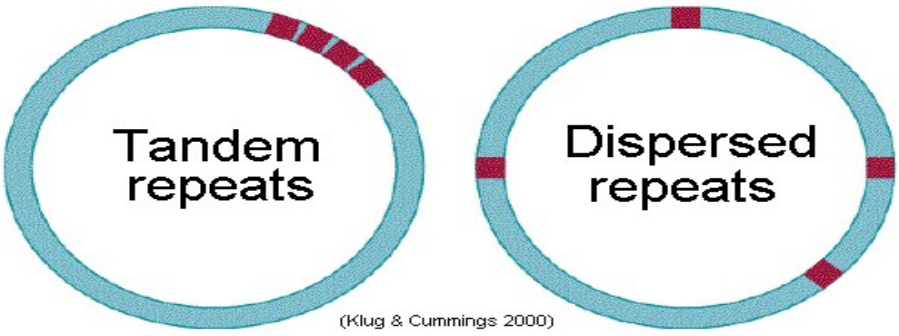
2- **Real** (**absolute) qPCR** in which we have controls with known amounts of DNA starting material, and we can compare the other DNA samples that we want to test (unknown amounts) with them to get an estimate of how much (amount) of DNA starting material is there.

(0-10 Mins)

We finished talking about PCR, now we will talk about the human genome.



Tandem repeats are ordered one after the other, while interspersed repeats are placed on different locations in the genome (separated).



We will discuss each repetitive DNA type:

1-Satellite (macro-satellite) DNA

Large repeats of 100 to 6500 BP located at the peripherals, which are found in

A- Centromeres (171 BP) and are unique to each chromosome, it is also possible to make DNA probes specific to them.

B- Telomeres

2-VNTRs (mini-satellite)

VNTRs (variable number of tandem repeats) are composed of 20 to 100 BP repeats.

3-STRs (microsatellites)

STRs (short tandem repeats) composed of 2 to 10 BP.

The number of repeats in micro and mini satellites is highly variable, they are polymorphic (they are different from one person to another) which means they are useful in:

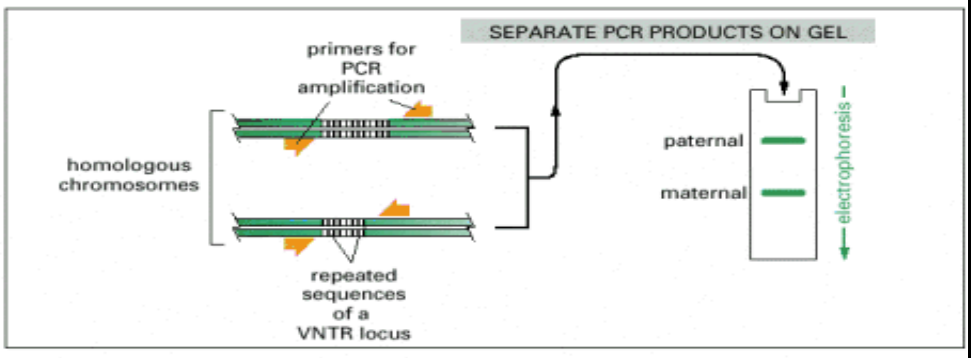
1-Locating genes in DNA (gene mapping)

2-DNA profiling for paternity testing, forensic testing, confirmation of relatedness and dead body identification.

The VNTRS are more informative and accurate thus we usually use VNTRs more than STRs

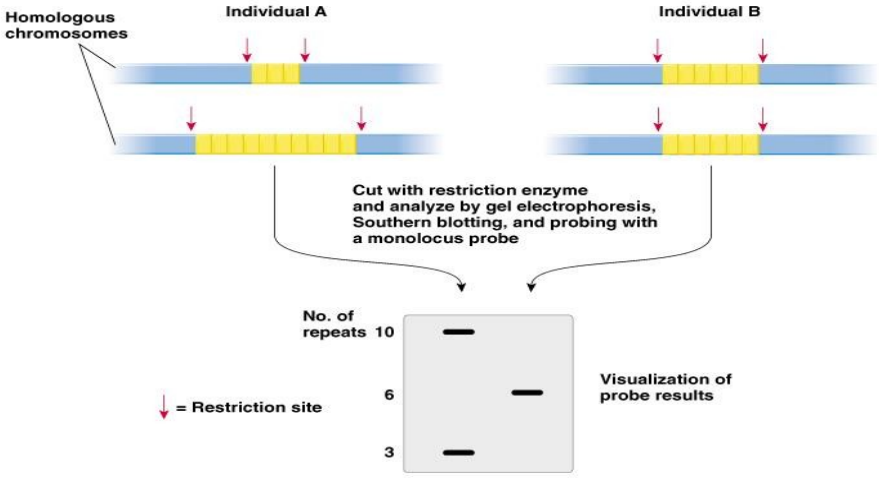
(10-20 mins)

PCR of VNTRs is done to get the DNA profile, by placing a primer that binds to the VNTRs from the outside of both chromosomes (homologous pair), and according to the number of tandem repeats on each chromosome (maternal and paternal) there will be variation among individuals in the length of the fragment (PCR product) which will allow us to assign a DNA profile for every individual.



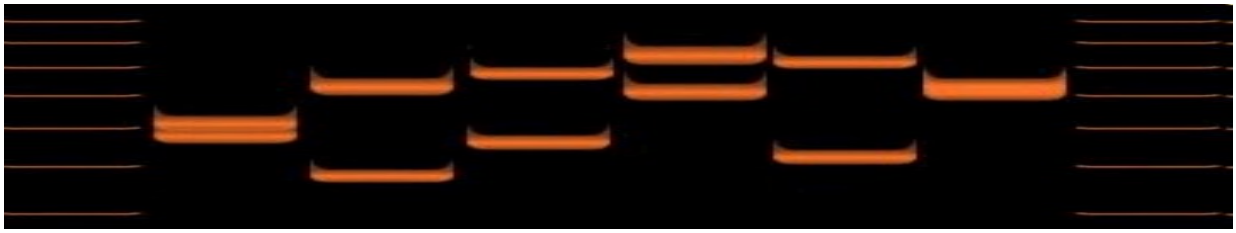
The locations of VNTRs and STRs are known from the *human genome project*, and based on their location the primers are designed.

Ex 1: individual A has different number of tandem repeats on each chromosome (homologous chromosomes), so the result of the PCR is 2 fragments with different number of repeats (3 and 10 repeats), while individual B has the same number of repeats on the 2 homologous chromosomes, so the result is 1 fragment (6 repeats).



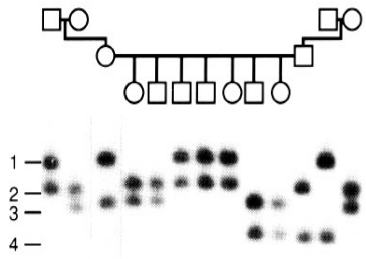
Ex 2: VNTR allelic length variation among 6 individuals (genetic profiling/fingerprinting)

The likelihood of 2 unrelated individuals having same allelic pattern is extremely improbable. When we do forensic testing or paternity testing we don't rely on one VNTR; because DNA polymerase could make mistakes resulting in changing the number of tandem repeats, instead we test more than one VNTR on different locations to confirm the identity of individual.

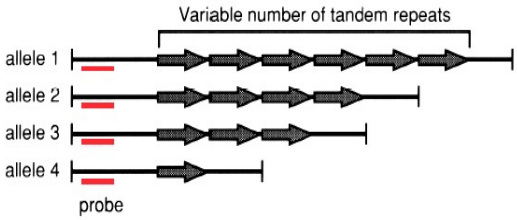


Student's question: does the identical twins have the same number of repeats of VNTRs?
Answer: they will be similar, but there could be differences due to the mistakes that the DNA polymerase makes.

Ex 3: in this example we have a family of 3 generations, there are 4 alleles (different numbers of VNTR repeats: 1, 3, 4 and 6 which are resembled by the number of arrows in the figure) and sons and daughters will take one allele from each parent.



single-locus probe but multiple alleles



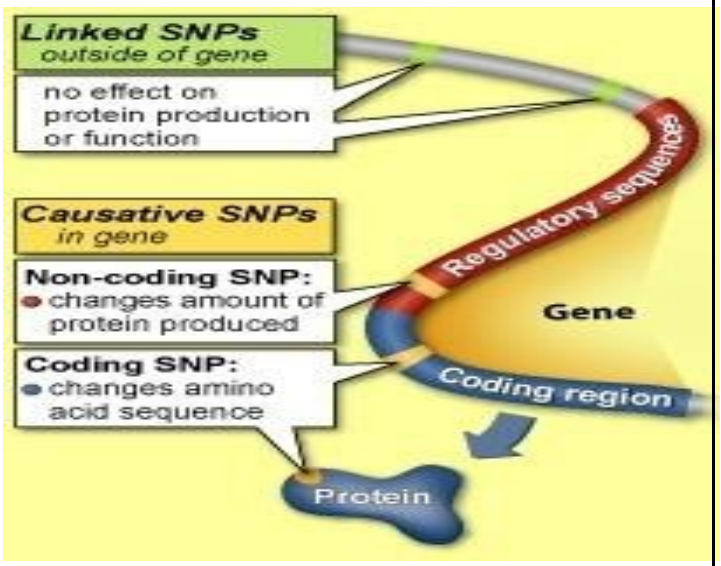
Thompson & Thompson Genetics in Medicine, p. 130, 1991

(20-30 mins)

SNPs

Single nucleotide polymorphism which is a variation among people in 1 nucleotide at a specific location and in order for a variation to be considered a SNP it must be found in at least 1% of the population. If less than 1%, it is considered a mutation, yet there could be a mutation that is found in more than 1% of the population and is still not considered a SNP for example, sickle-cell anemia (which provides protection against Malaria). in Africa, it is found in more than 1% of population and is considered a mutation.

There are about 10 million SNPs in our genome (1 SNP for every 300 BP). Most of the SNPs are of no importance, yet there are 500,000 SNPs that are important either because they are considered **linked** or **causative** SNPs.



A- **Causative SNPs**: can cause a disease; it could either be on a coding region results in changing the amino acid sequence or a non-coding region (ex: promoter region)

Ex: a person has a SNP on a coding region that causes Alanine to be replaced by Valine in an enzyme; this change does not cause a disease since the structure of Alanine and Valine are similar, but the efficiencies of those 2 enzymes would be different, and this leads to the idea of personalized medicine, which means that a person should take drugs and doses according to his genetic profile.

B- **Linked SNPs:** are linked to a disease, individuals who have the disease always have these SNPs, even though they are not directly related to the disease; because these SNPs (linked) are inherited among side the mutative gene. 80% of individuals who have linked SNPs will develop the disease.

(30-40 mins)

Transposons are repetitive sequences that have the ability to jump from one position to another there is DNA Transposons which represent (2-3) % of the human genome, and RNA Transposons which represent 40% of the human genome these could be:

1- **LINES** (long interspersed sequences) which are about 7000 BP in length

2- **SINES** (short interspersed sequences) shorter than 500 BP

Most of these Transposons lost the ability of jumping in humans, yet they could still cause disease if they jumped in front of the gene which may cause over-expression of the gene (promoter region) or within the coding regions causing destruction of the protein to be transcribed. Ex: hemophilia A could be caused because of Transposons jumping within the coding region, severe combined immunodeficiency could also be caused by Transposons jumping.

Luckily most of our genome consists of non-coding regions, so most of these jumps will have no effect on us.

Sorry for any mistakes and good luck.

"The end"