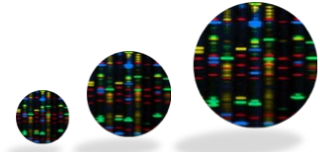


Discovering DNA

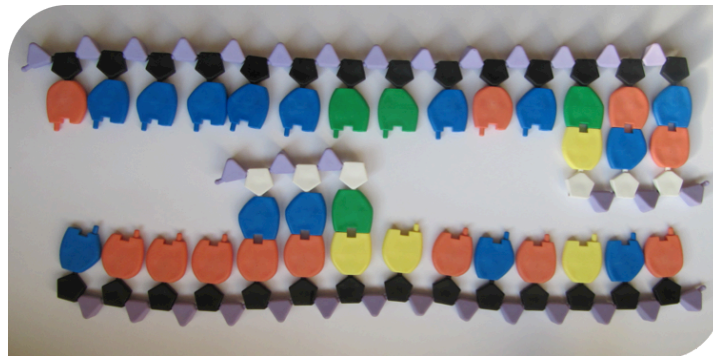


Molymod® miniDNA®

Discovering DNA Fingerprinting

Instructions and teacher's guide

Cat no MDNA-STR-408



We'd love to hear any feedback, comments or questions you have!

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Discovering DNA Fingerprinting

MDNA-STR-408

Contents

Black sugar	112	Cytosine (Yellow)	22
White sugar	24	Guanine (Green)	22
Phosphate (Purple)	136	Adenine (Blue)	50
		Thymine (Orange)	42

Introduction

DNA fingerprinting has revolutionized forensics, paternity testing, ancestry studies, and many other aspects of science that depend on identifying individuals and finding genetic relationships from DNA. The technique was invented by Professor Sir Alec Jeffreys, of Leicester University in 1984.

Repeated sequences in DNA

Alec Jeffreys used a type of repeated DNA sequence in his invention. Such “tandem repeats” have the same short sequence repeated over and over in a row and these repeated sequences occur in the non-coding regions of DNA – often called junk DNA. These regions of DNA have more variation than coding DNA as any changes do not have an impact on survival in the way a genetic mutation would have. The reason for these repeated regions is not fully understood. Sometimes they are found in pseudo genes that have lost their function or in non-coding introns within genes. A general term for these repeats is variable number tandem repeats (VNTRs) because they are repeats of the same sequence in a row in a varying number.

Whilst searching for the human myoglobin gene, Alec Jeffreys stumbled upon a type of tandem repeat called a minisatellite in an intron in the seal myoglobin gene. Seals were useful because they have a lot of myoglobin as it helps them stay underwater for so long. He noticed it had some similarity in sequence to other published variable repeats, so decided to use it to see if it could act as a hook (a probe) to find other minisatellites in the human genome.

To do this, DNA was extracted from several individuals and cut with restriction enzymes. The DNA fragments were then separated using an electrophoresis gel and analysed using a radioactively labeled DNA probe. This is essentially a Southern blot (see Restriction Mapping & Southern Blotting Made Simple for more details).

The first DNA fingerprint!

When this was done, Alec Jeffreys found numerous regions containing a similar sequence to the probe. However, on closer inspection, he noticed you could see unique banding patterns for the individuals on the gel. His technician Vicky Wilson and her parents had provided samples and you could see her bands were inherited from either one of her parents giving her a unique pattern but one that showed biological relatedness. Even the individual seals could be identified!

As Alec Jeffreys said, “At first the images looked like a complicated mess. Then the penny dropped. We had found a method of DNA based biological identification.” Thus, at 9.05am on Monday 10th September 1984, DNA fingerprinting was born!

At the time it was not known if DNA was stable enough to be collected from forensic type samples, so Alec Jeffreys put smears of his blood all over his lab to see if useable DNA could be recovered!

In subsequent years, the technique was refined and improved.

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

DNA profiling

By 1987, Alec Jeffreys had found that by focusing on a smaller number of highly variable minisatellites, the DNA fingerprint could be seen with fewer bands than on the early patterns. The results could be identified and interpreted more easily by non-experts (such as judges and juries) so it was more useful for forensic and other uses. This development was called DNA profiling.

An advantage of DNA profiling is that the results could be digitized as you would have a finite number of repeats of a sequence at each position. This meant the data could be stored in a database. The DNA database raises important ethical questions as in the UK there are now over 5.1 million people on it, many of whom are innocent. Conversely, the database has been used to solve many crimes that would have been difficult or impossible to solve without using DNA evidence.

Polymerase chain reaction arrives

A further innovation was to take advantage of the development of the polymerase chain reaction (PCR). PCR was invented by Kary Mullis in 1983 and offered for the first time a way to quickly and easily amplify (copy) small amounts of DNA which could then be used for DNA fingerprinting. PCR also offered the potential to automate the previously laborious process of DNA fingerprinting. Kary Mullis won a Nobel prize for his creation in 1993 which uses the enzyme DNA polymerase that normally copies DNA during mitosis. Mullis had the idea to use short DNA primers to specify what is copied rather than copying the entire genome as during mitosis. See PCR Puzzle for more details on PCR.

PCR could only copy up to 1000 bases of DNA reliably so the minisatellites in use for DNA profiling were too long. Thus, a shorter type of repeat sequence had to be identified that gave the same unique patterns but were suitable for amplification by PCR. These sequences were identified and called microsatellites or short tandem repeats (STRs). They are repeats of sequences of 5 or fewer bases in length which give PCR products of 100 to 400 bases in length that can be visualised on an electrophoresis gel.

By using PCR primers that are specific to the outer region of each STR region, you get a measure of the length of DNA between each primer set, thus you see the number of copies of the repeat at each location. By combining the results of several STRs, you can compile a DNA profile that is specific to the individual.

DNA fingerprinting today

STRs are used in the latest DNA fingerprinting systems in use today – SGM plus and CODIS. The STRs used are based on 4 base pair repeats that occur at a standard set of regions. SGM plus uses 10 STRs whereas CODIS uses 13 STRs with much overlap between them:

STR name	Location	Repeat	CODIS	SGM Plus
TPOX	Chromosome 2	[AATG]	✓	
D2S1338	Chromosome 2	[TGCC] _n [TTCC] _n		✓
D3S1358	Chromosome 3	[TCTA]	✓	✓
FGA	Chromosome 4	[TTTC] ₃ TTTTTCT[CITT] _n CTCC[TTCC] ₂	✓	✓
D5S818	Chromosome 5	[AGAT]	✓	
CSF1PO	Chromosome 5	[AGAT]	✓	
D7S820	Chromosome 7	[GATA]	✓	
D8S1179	Chromosome 8	[TATC]	✓	✓
TH01	Chromosome 11	[AATG]	✓	✓
VWA	Chromosome 12	[TCTA] with [TCTG] and [TCCA] inserts	✓	✓
D13S317	Chromosome 13	[TATC]	✓	
D16S539	Chromosome 16	[GATA]	✓	✓
D18S51	Chromosome 18	[GAAA]	✓	✓
D19S433	Chromosome 19	(AAGG)(AAAG)(AAGG)(TAGG)[AAGG] _n		✓
D21S11	Chromosome 21	[TCTA]	✓	✓
Amelogenin	X or Y chromosome	6 base pair deletion in X absent in Y	✓	✓

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

Finding gender from DNA

Gender can be identified from a DNA sample by looking at a gene called *amelogenin*. *Amelogenin* is involved in enamel formation during tooth development but because it is found in two forms (alleles) that differ between X and Y chromosome it is also useful as a way of determining gender from a DNA sample.

In the first intron of the *amelogenin* gene, there is a 6 base pair deletion in the X chromosome but not in the Y chromosome version of the gene. Thus, after PCR of the region around the first intron, the PCR product with the 6 base pair deletion (X) will run more quickly during electrophoresis than the product without the deletion (Y). A female will have one band (XX) and a male will have two bands one for Y and one for X.

Our task today

In this activity, we look at the STR D16S539 that is used in both the SGM Plus and CODIS systems to show how DNA fingerprints are created. We use a smaller number of repeats than in reality but use the real sequence for the STR. We also use the real *amelogenin* gene sequence to show how gender is determined in both CODIS and SGM Plus. Our PCR primers are shorter than in reality as they are typically 10 – 20 bases long.

By the end of the session, your students will have learnt:

- DNA fingerprinting uses STR repeats
- STRs are repeats of short sequences on DNA
- Number of STR copies varies between individuals
- The primers must match only one region in the template DNA
- Primers must match each end of the target
- Complementary base pairing is critical for DNA fingerprinting
- How gender is determined from DNA

Time requirements

Preparation

The first time will take about 25 minutes but after this preparation is no more than 5 minutes as you can keep the components assembled in the storage box for future use.

Lesson

20 minutes is sufficient to carry out the activity with more time to explain the stages and analyse the outcomes as required.

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

Preparation

DNA fingerprinting - Preparation

1 Assemble template bases

You only need to do this once as the bases can be kept for future lessons!

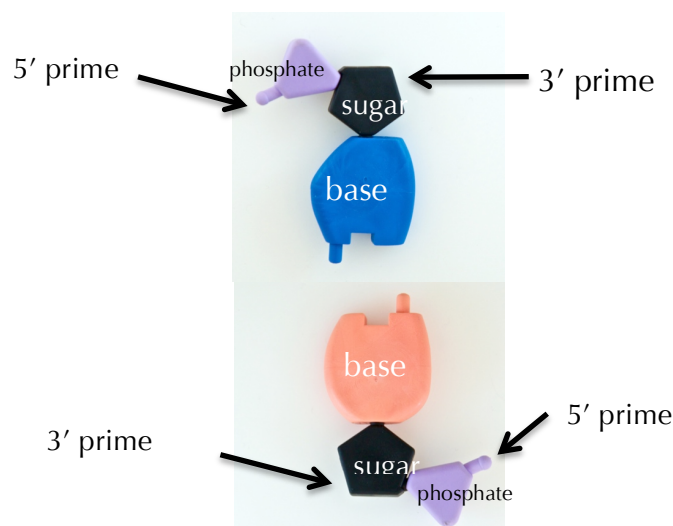
The bases comprise of a sugar (black or white), a phosphate (purple) and a base (yellow, green, blue or orange). Please note the different sugar colours are included to make it easier to see the primer and template they do not represent different chemicals.

Use black sugars for the template bases.

Attach the purple phosphate to the black sugar by pushing the bent knob from the sugar into the hole in the purple phosphate.

Make sure you add the sugar to the 5' prime end as shown so the knob still sticks out of the purple phosphate not out of the sugar.

Push the coloured base (green, orange, blue or yellow) onto the straight knob on the sugar.



Assemble the following bases with black sugars:

Cytosine (Yellow)	18
Guanine (Green)	18
Adenine (Blue)	38
Thymine (Orange)	38

Helpfully, C, G, A, and T are embossed on the edge of the base.

2 Assemble primer bases

Use white sugars for the primer bases. Assemble the following bases:

Cytosine (Yellow)	4
Guanine (Green)	4
Adenine (Blue)	12
Thymine (Orange)	4

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

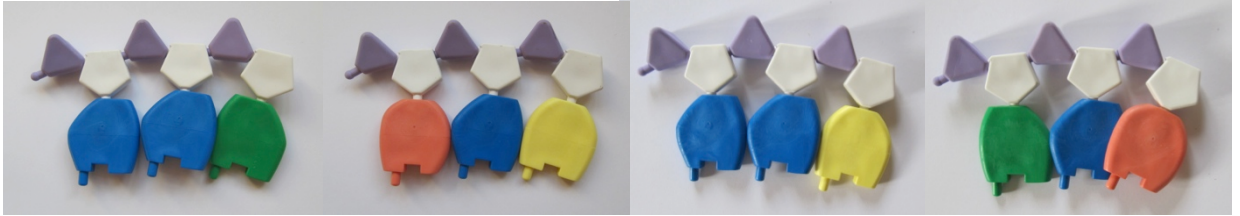
Put them into this sequence:

5' - AAG - 3' x 2

5' - TAC - 3' x 2

5' - AAC - 3' x 2

5' - GAT - 3' x 2



Typically, PCR primers are between 10 and 20 bases long. Remember PCR primers are single stranded!

Optional: You can make the PCR products for each group using the parts from PCR Puzzle.

After completion of the lesson

Dissassemble the template strands (with black sugar) and store in the storage box.

Remove the primers (with white sugar) and keep them intact in the storage box.

Preparation for subsequent uses (about 5 minutes)

Sort out the bases (with black sugars) by colour (yellow, green, blue or orange)

Separate the 4 types of primers (with white sugars)

Then you are ready to go!

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

Teachers notes

Lesson tips

Important - ensure students have bases correctly orientated so 5' and 3' are opposite each other when bases are paired (shown on page 2) & don't let your students take the bases apart!

Divide class into 4 groups

Possible scenarios:

Forensic analysis

Disaster victim identification

Historic DNA sample eg Richard III

Ancient DNA eg ice age human

Human ancestry studies eg Neanderthals

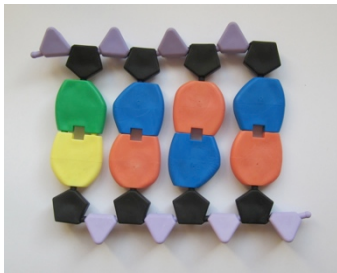
Each group does the profile of one chromosome (thus 2 groups do both alleles of a STR). Two groups use sex chromosomes to work out gender of DNA sample.

STR determination

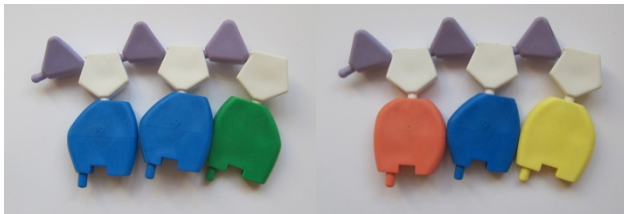
Group 1 and 2 will work out the number of repeats at one STR - D16S539 which is located on chromosome 16.

At this loci people have between 5-16 copies of GATA giving products with a size range of 234-274 base pairs. The length of the PCR product allows you to count how many copies of the GATA repeat are present.

In our scenario, we have either one copy or two copies of the 4 base pair GATA repeat in D16S539 which give either a 10 or 14 base pair PCR product. You could have +/+, +/-, -/- so could show all genotypes with this STR and students can work out what you would see on a gel for the different combinations. This is the how GATA repeat appears:



Groups 1 and 2 use these PCR primers:

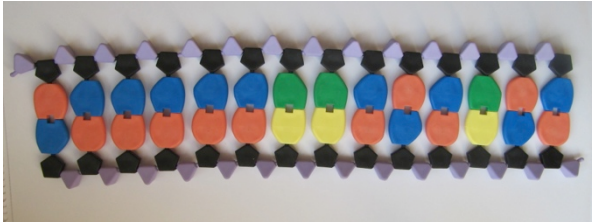


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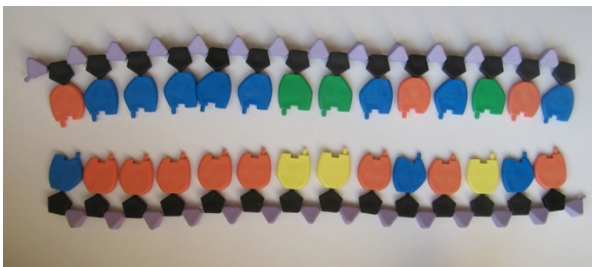
Group 1 – one copy of D16S539 repeat

1 Make the double stranded DNA template using bases with black sugars as follows:

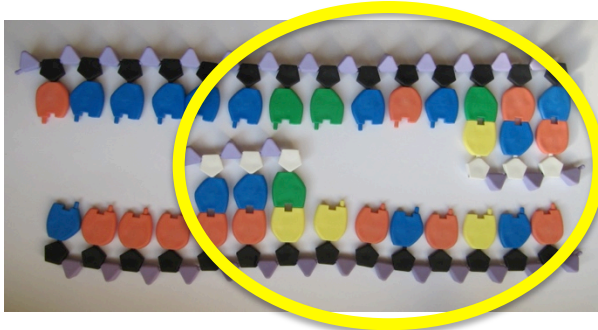
TAAA AAG GATA GTA
ATTT TTC CTAT CAI



2 Denature the two strands

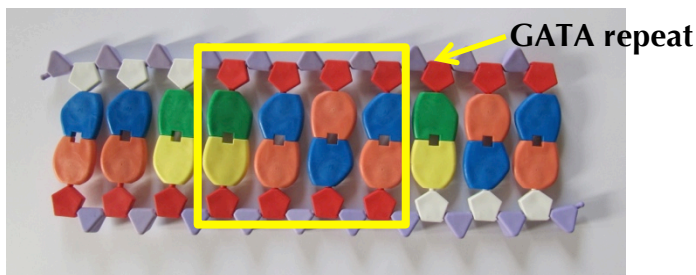


3 Anneal primers



4 Students should work out length of the final PCR product.

5 Optional extension – use parts from PCR Puzzle to make a model of the double stranded PCR product using bases with red sugars as shown:



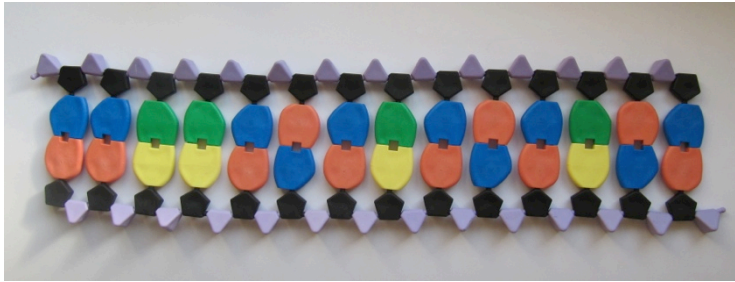
6 We end up with one copy of the 4 base pair GATA repeat in D16S539 that gives a 10 base pair product.

Group 2 – two copies of D16S539 repeat

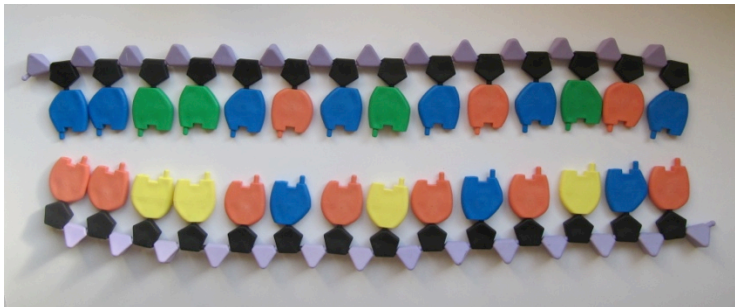
Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

1 Make the double stranded DNA template using bases with black sugars as follows:

AAG GATA GATA GTA
TTC CTAT CTAT CAT



2 Denature the two strands

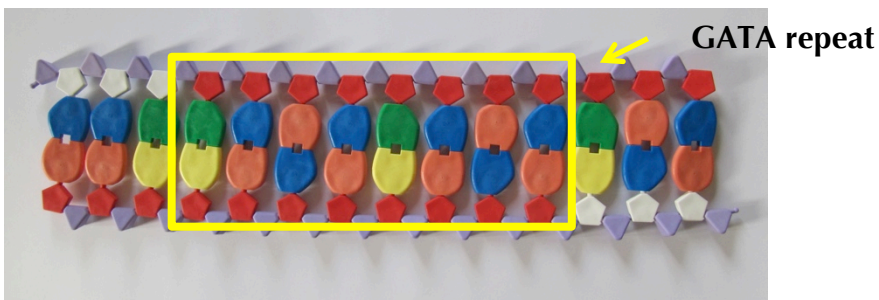


3 Anneal primers



4 Students should work out length of the final PCR product.

5 Optional extension – use parts from PCR Puzzle to make a model of the double stranded PCR product using bases with red sugars as shown:



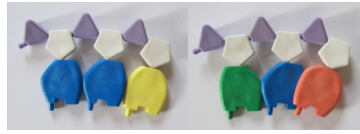
6 We have two copies of the 4 base pair GATA repeat in D16S539 that gives a 14 base pair product.

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

Gender determination

Group 3 and 4 look for one of two alleles of the *amelogenin* gene that allows the determination of gender from a DNA sample.

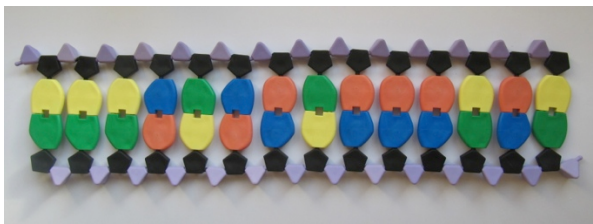
Use these primers for groups 3 and 4:



Group 3 – X chromosome - deletion in *amelogenin* gene

1 Make the double stranded DNA template using bases with black sugars as follows:

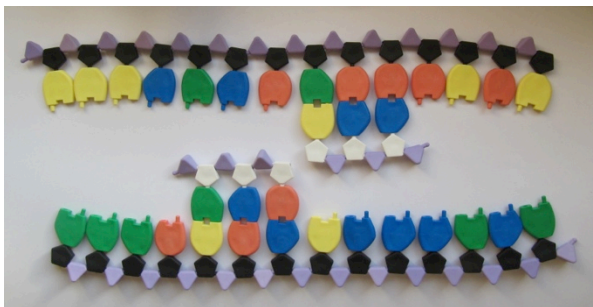
X Sequence	CCCAGAT	GTTTCTC
	GGGTCTA	CAAAGAG



2 Denature the template

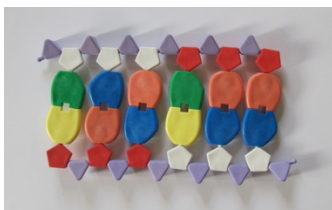


3 Anneal primers



4 Students should work out length of the final PCR product.

5 Optional extension – use parts from PCR Puzzle to make a model of the double stranded PCR product using bases with red sugars as shown:



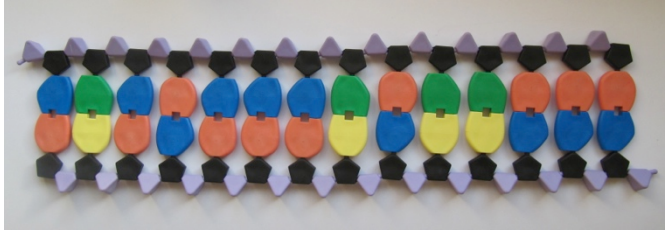
6 The result shows this is a chromosome X as you get a shorter 6 base pair product due to the deletion.

Key: Green = Guanine Yellow = Cytosine Blue = Adenine Orange = Thymine

Group 4 – Y chromosome - no deletion in *amelogenin* gene

1 Make the double stranded DNA template using bases with black sugars as follows:

Y sequence AGAT AAAGTG GTTT
 TCTA T T TCAC CAAA



2 Denature the template

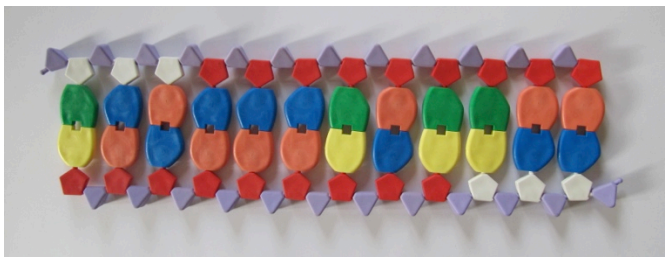


3 Anneal primers



4 Students should work out length of the final PCR product.

5 Optional extension – use parts from PCR Puzzle to make a model of the double stranded PCR product using bases with red sugars as shown:



6 The result shows this is a chromosome Y as you get a longer 12 base pair product as there is no deletion.

How to determine gender from these results

A female will have XX chromosomes so you get one band on a gel as both give the same size PCR product. A male will have XY chromosomes so you get two bands of different sizes on a gel.

Discovering DNA fingerprinting class activity – student's notes

Important - ensure you have bases correctly orientated so 5' and 3' are opposite each other when bases are paired & don't take the bases apart!

Remember in base pairing the following match:

T pairs with A (orange with blue)

C pairs with G (yellow with green)

PCR primers are typically 10 - 20 bases long so we have scaled them down for this activity.

Lesson Part One – STR identification

Group 1 and 2 will work out the number of repeats at one STR - D16S539 which is located on chromosome 16.

At this loci people have between 5-16 copies of GATA giving products with a size range of 234-274 base pairs. The length of the PCR product allows you to count how many copies of the GATA repeat are present.

Group 1 – D16S539 repeat

- 1 Make the double stranded DNA template using bases with black sugars as follows:
5' - TAAA AAG GATA GTA - 3'
3' - ATTT TTC CTAT CAT - 5'
Remember to get the 5' to 3' orientation correct!
- 2 Denature the two strands by separating them.
- 3 Anneal single stranded primers to the complementary bases - one primer per strand.
5' - AAG - 3' and 5' - TAC - 3'
- 4 Work out the length of the final PCR product. How many copies of the STR repeat (5' - GATA - 3') do you have?
- 5 Optional extension – use parts from PCR Puzzle to make a model of the double stranded PCR product using bases with red sugars.

Group 2 – D16S539 repeat

- 1 Make the double stranded DNA template using bases with black sugars as follows:
5' - AAG GATA GATA GTA - 3'
3' - TTC CTAT CTAT CAT - 5'
- 2 Denature the two strands by separating them.
- 3 Anneal single stranded primers to the complementary bases - one primer per strand.
5' - AAG - 3' and 5' - TAC - 3'
- 4 Work out the length of the final PCR product. How many copies of the STR repeat (5' - GATA - 3') do you have?
- 5 Optional extension – use parts from PCR Puzzle to make a model of the double stranded PCR product using bases with red sugars.

Lesson Part Two - Gender determination

Group 3 and 4 look for one of two alleles of the *amelogenin* gene that allows the determination of gender from a DNA sample. The X chromosome has a 6 base pair deletion in *amelogenin* but the Y chromosome does not, so they give different size bands on an electrophoresis gel after PCR amplification.

Group 3 – Gender chromosome

- 1 Make the double stranded DNA template using bases with black sugars as follows:
 5' - CCCAGATGTTTCTC - 3'
 3' - GGGTCTACAAAGAG - 5'
- 2 Denature the template by separating the strands.
- 3 Anneal single stranded primers to the complementary bases - one primer per strand.
 5' - AAC - 3' and 5' - GAT - 3'
- 4 Work out length of the final PCR product.
- 5 Optional extension – use parts from PCR Puzzle to make a model of the double stranded PCR product using bases with red sugars.

Group 4 – Gender chromosome

- 1 Make the double stranded DNA template using bases with black sugars as follows:
 5' - AGAT AAAGTG GTTT - 3'
 3' - TCTA T T TCAC CAAA - 5'
- 2 Denature the template
- 3 Anneal single stranded primers to the complementary bases - one primer per strand.
 5' - AAC - 3' and 5' - GAT - 3'
- 4 Work out length of the final PCR product.
- 5 Optional extension – use parts from PCR Puzzle to make a model of the double stranded PCR product using bases with red sugars.

How to determine gender from these results

A female will have XX chromosomes so you get one band on a gel as both give the same size PCR product. A male will have XY chromosomes so you get two bands of different sizes on a gel.

Think about how male and female DNA will appear on an electrophoresis gel.