Real-Life Research

THE UNIVERSITY of EDINBURGH
Easter Bush
Science Outreach Centre

Get hands-on with real-life science

ANSWER KEY

PCR MASTERCLASS: A QUESTION OF TASTE

Control of Gene Expression: *Genotype to Phenotype*

1. What did you do in the workshop? Number 1 to 7.

2	I extracted DNA from my cheek cells.
1	I determined my phenotype for PTC, a bitter chemical by doing a taste test.
3	I added the ingredients required for PCR to a small tube then put it in a thermo-cycler.
5	I loaded my PCR samples on a gel and carried out gel electrophoresis.
4	I added the enzyme HaeIII to my PCR product then put it in a water bath at 37°C for 20 minutes to allow it to cut the taster allele.
6	I analysed my gel on a blue light to see the fragments of DNA generated by the PCR reaction and restriction digestion by the Hae III enzyme.
7	I compared my newly discovered genotype with my phenotype.

2. Why were only some able to taste the PTC? Use these words in your answer.

phenotype, alleles, homozygous, heterozygous, dominant, recessive

Student's own answers. Example answer.

We see different phenotypes in our class as we have different genotypes.
The TAS2R38 gene has two different alleles, one allele encodes a functional taste receptor (R) the other allele encodes a non-functional taste receptor (r).

The taster allele is dominant and the non-taster allele is recessive.

Strong tasters are homozygous dominant (RR), weak tasters are heterozygous (Rr) and non-tasters are homozygous recessive (rr).

3. How do we taste things?

   Molecule binds to a receptor located in the cell membrane of cells that make up taste buds. The receptor conveys information and the information goes to brain through a nerve impulse.

4. What is a receptor?

   A protein that sits on surface of cell that monitor change in the environment (detect stimuli)

5. This is the mRNA sequence of one part of the TASR38 gene, write out the DNA sequence.

   mRNA 5'GUCAGGCCU 3'

   DNA *5'GTCAGGCCT3' (coding strand)*

   3' CAGTCCGGA5' (template strand)

   Is this a taster or a non-taster? Reminder: GGCC (Taster), GGGC (Non-Taster)

   They are a taster (weak or strong taster depending on whether they have one or two T alleles).

6. How could a change in one nucleotide of a taste receptor gene, change the protein structure of the receptor?

   A change in one nucleotide could lead to a different codon that codes for a different amino acid, therefore a different amino acid is incorporated during translation, this could change the overall structure of the receptor.

7. There are 43 human TAS2R genes, they don't have introns. What does that mean?

   There are no non-coding regions in these genes.

8. In a class of 18 pupils, 20% are non-tasters. How many students could taste PTC?

*(18/100)*20= 3.6 = 4*

18 - 4 = 14 pupils

Polymerase Chain Reaction

9. How does PCR differ from replication in our cells and how is it similar?

PCR	*DNA Replication*
Synthetic – only in the lab	*Natural – happens in cells*
Requirements: *Template DNA* *Primers* *Nucleotides* *Heat-Tolerant DNA Polymerase*	*Requirements:* *Template DNA* *Primers* *Nucleotides* *DNA Polymerase* *Ligase* *ATP*
Very Fast	*Fast*
Very Hot	*Steady Temperature*

10. What do we need to run PCR?

Template DNA

Primers

Heat-tolerant DNA polymerase

Nucleotides

Thermo-cycler/PCR machine or water baths of different temperatures

11. What is a primer? How many do you need?

A primer is a short sequence of nucleotides that binds to the template strand by, complementary base paring, and provides the starting point. Two primers are needed for both forward and reverse DNA strands.

12. What is special about the DNA polymerase we used for PCR?

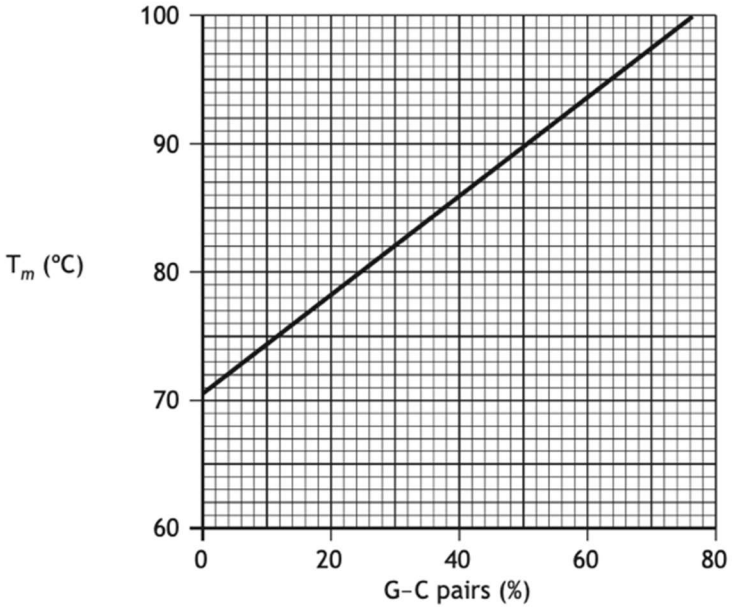
It is heat-tolerant, so it won't degrade in the high temperatures required for PCR. It comes from bacteria that live in hot springs.

13. Each cycle of a polymerase chain reaction (PCR) takes 5 minutes.
If there are 1000 DNA fragments at the start of the reaction, how long will it take for the number of fragments produced by the reaction to be greater than 1 million?

C 50 min

The number of DNA fragments doubles every 5 minutes, after 50minutes there will be 1,024,000 fragments of DNA.

14. The melting temperature of a molecule of DNA (T_m) is the temperature at which half of its base pairs separate. T_m is proportional to the percentage of the guanine to cytosine (G–C) base pairs in the molecule as shown on the graph below.



The numbers of base pairs present in a DNA molecule are shown in the table below.

Number of base pairs present	
A-T	G-C
1200	800

What is T_m for this molecule?

B 86°C

First calculate the percentage of G-C:

1200+800= 2000

800/2000= 40%

Then, use the graph to determine the temperature 40% GC content correlates with at Tm of 86°C

Working in the lab

1. A section of double-stranded DNA contains 80 guanine and 40 adenine molecules. What is the total number of deoxyribose sugars in this section?

   Remind students that guanine pairs with cytosine, and adenine pairs with thiamine.

   80G + 80C

   40A + 40T

   = 240 deoxyribose sugars

2. What is a restriction enzyme?

Restriction enzymes are used to cut DNA at specific DNA sequences called restriction sites. The enzyme recognises specific sequences (4-8 nucleotides in length) and only cuts there.

3. We used a restriction enzyme called HaeIII, which cuts at GGCC. How many fragments would generated if this piece of DNA was cut with HaeIII?

   AAGCGGG|CCATTTCGGCTGG|CCTTGCAGG|CCTT

   There are three restriction sites so 4 fragments of DNA will be generated.

4. How does electrophoresis work?

   DNA is loaded into wells of an agarose gel, placed in a buffer, and connected to power supply. DNA will run down gel from the negative to the positive electrode because DNA is slightly negatively charged. DNA fragments will be separated by size – larger DNA fragments move slower, so stay closer to the top.

5. The buffer used is called TAE and was at 0.25x concentration, made using 50x TAE stock solution and water. How much 50x TAE stock solution do you need to use to make 1L of 0.25x TAE? How much to make 300ml?

   1L of 1xTAE:

   20ml of 50x TAE

   980ml of H_2O

   1L of 0.25xTAE:

   250ml 1x TAE

   750ml of H_2O

6. What is the mass of agarose needed to make a 30ml, 2% gel?

   To make a 1% gel that is 30ml:

   30ml/100% = 0.3g

   To make a 2% gel that is 30ml

   0.3g x 2 = 0.6g

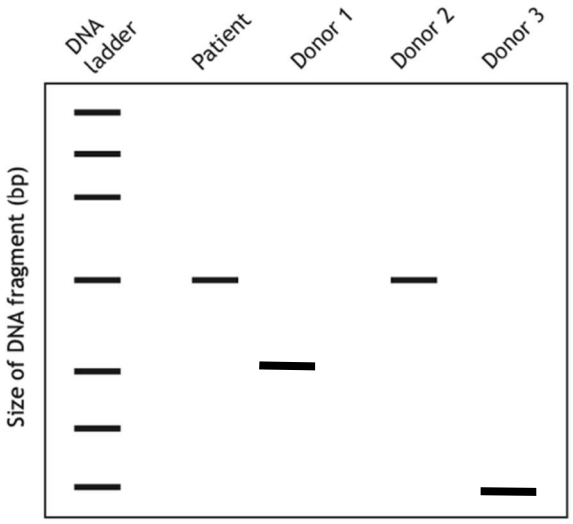
   a. The gel also contains a dye called SYBER SAFE. It is used at a concentration of 1:10 000. How much SYBR SAFE (in µl) do you need to use if you make up 300ml of gel?
      300ml / 10 000 = 0.03ml = 30µl

Analysing Results

7. Patients requiring an organ transplant are tissue typed to match with potential donors. Polymerase chain reaction (PCR) and gel electrophoresis are used to compare DNA sequences of the patient with those of donors. Gel electrophoresis separates mixtures of DNA fragments according to size. The presence of a specific DNA band indicates that a donor is a suitable match.

   The DNA ladder contains fragments of DNA, separated by gel electrophoresis, which are of a known size and measured in base pairs (bp). The distances the DNA fragments travelled were measured and are shown in the table below. The diagram below shows the result of the gel electrophoresis.

Size of DNA fragment (bp)	*Distance travelled* (mm)
200	72
300	58
550	32
700	18
800	12
1000	10
1300	8



a. Which donor matches the patient?

Donor 2, the fragments are both 700bp.

b. Which donor has the smallest DNA fragment? What is its size?

Donor 3, it is 200bp.

c. Using information in the **table and diagram** give the distance travelled by the fragment from Donor 1 in the DNA ladder in millimetres (mm).
The fragment is the same size as the 550bp fragment in the DNA ladder, so it has travelled 32mm.