Name ……

Class ……

Date ……..

Real-Life Research

THE UNIVERSITY of EDINBURGH
Easter Bush
Science Outreach Centre

Get hands-on with real-life science

PCR MASTERCLASS: A QUESTION OF TASTE

We hope you enjoyed our **PCR Masterclass: A Question of Taste** and that it helped you understand the concepts you have been learning in class! This workbook will build on the foundation of the experiments you carried out and will give you some more practical experience of interpreting real data. The key concepts included in this workbook are:

- The structure of DNA
- Replication of DNA
    - DNA replication
    - Polymerase chain reaction (PCR)
- Gene expression
    - Amino acids form polypeptides
- Mutations
- Genetic control of metabolism
    - Restriction enzymes

This workbook is also a good exercise if you are preparing for exams!

Control of Gene Expression: *Genotype to Phenotype*

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

	I extracted DNA from my cheek cells.
	I determined my phenotype for PTC, a bitter chemical by doing a taste test.
	I added the ingredients required for PCR to a small tube then put it in a thermo-cycler.
	I loaded my PCR samples on a gel and carried out gel electrophoresis.
	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.
	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.
	I compared my newly discovered genotype with my phenotype.

2. Why were only some able to taste the PTC? Use these words to describe

phenotype, alleles, homozygous, heterozygous, dominant, recessive

3. How do we taste things?

4. What is a receptor?

5. This is the mRNA sequence of one part of the TASR38 gene, write out the DNA sequence, show the top (coding) and bottom (template) strands.

mRNA 5'GUCAGGCCU 3'

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

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

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

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

Polymerase Chain Reaction

1. How does PCR differ from replication in our cells and how is it similar? Draw a table to show your answer.

2. What do we need to run PCR?

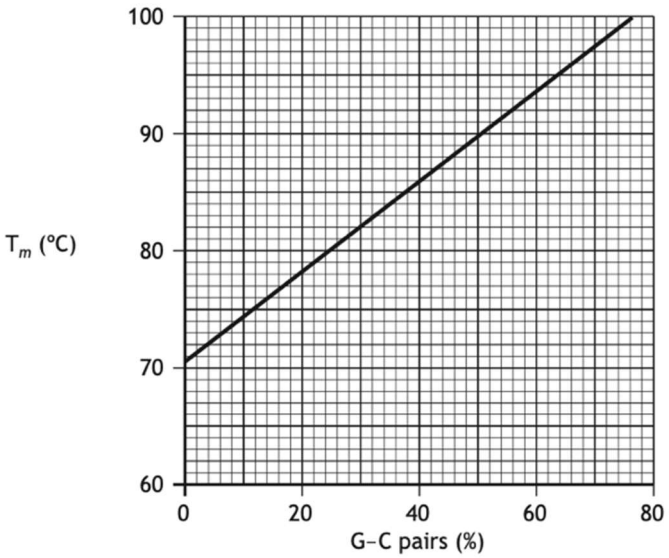
3. What is a primer? How many do you need to do PCR?

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

5. 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?

   A 15min B 35 min C 50 min D 55 min

6. 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?

A 78°C B 86°C C 94°C D 96°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?

2. What is a restriction enzyme?

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?

AAGCGGGCCATTTCGGCTGGCCTTGCAGGCCTT

4. How does gel electrophoresis work?

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?

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

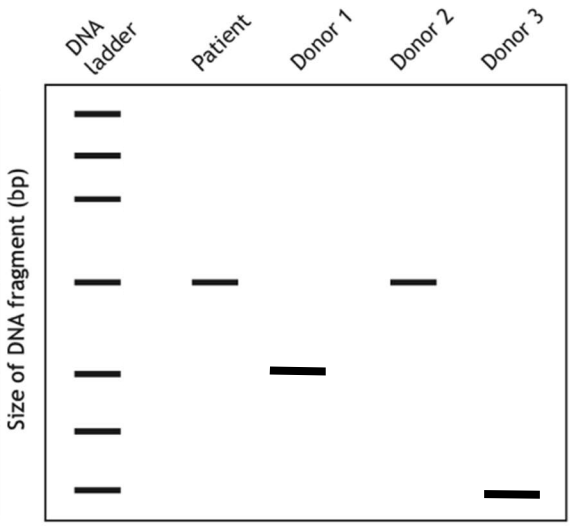
    a. The gel also contains a dye called SYBER SAFE. It is used at a concentration of 1:10 000. How much SYBR SAFE (in µl) do you need to use if you make up 300ml of gel?

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?

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

   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).