# Nov 2015 H2 Biol Paper 3

## N15P3Q1

The polymerase chain reaction (PCR) is a three-stage process.Describe what occurs in

**(a) (i)** The first stage at 95°C, [2]

1. heating to 95°C separates two strands of the DNA double helix;
2. by breaking the hydrogen bond between complementary bases through increased molecular vibrations, denaturing the DNA;

**(ii)** the second stage. [2]

1. Cool to around 64°C, for DNA primers to anneal;
2. Primers anneal to complementary 3' end of each template/single strand;

**(b)**

**(i)** Suggest and explain why Taq DNA polymerase is now obtained from genetically modified *E.coli*. [2]

1. The use of genetically modified *E.coli* would allow for mass production of Taq DNA polymerase;
2. Taq polymerase can be easily obtained;

**(ii)** With reference to your knowledge of PCR, explain why a half-life of 40 minutes at 95°C allows many cycles of PCR before the enzyme needs to be replaced. [3]

A single cycle of PCR is very short/much shorter than 40 minutes;

1. Many cycles of PCR can occur within a single half-life of Taq polymerase;
2. As sufficient amounts of enzyme are present to carry out many cycles of PCR before replacement is required;

**(c)** Explain how RFLP analysis of DNA samples from imported grain and non-GM grain can show whether or not the imported grain has been genetically modified. [6]

1. Cut the DNA samples from the imported grain and non-GM grain with ***same restriction enzyme**** to obtain different-sized DNA fragments;
2. DNA is separated according to size in gel electrophoresis where ***negatively-charged DNA**** migrates towards the positive electrode/anode when subjected to an electric field / current;
3. Meshwork of agarose fibres impedes movement of longer fragments more than shorter fragments resulting in smallest fragments moving furthest/largest fragments least far from well;
4. Carry out Southern blotting using ***radioactive probe**** for VNTR/STR repeat sequence followed by visualising the banding pattern using autoradiography / x-ray film
5. Banding pattern in DNA fingerprint is due to different alleles/markers producing different bands in gel resulting in the unique banding pattern for different DNA samples;
6. Different bands arise due to polymorphic nature of different DNA samples, there will be variations in number and location of restriction sites and number of tandemly repeated nucleotide sequence among different DNA samples;
7. Genetic fingerprint of imported grain can be compared against fingerprint of non-GM grain to see how closely related they are;
8. If fingerprint pattern is similar between the two different DNA samples, then imported grain is non-GM;

[Total: 15 ]

**N15P3Q2 (OUT OF SYLLABUS)**

**N15P3Q3 (OUT OF SYLLABUS)**

**N15P3Q4**

**Planning Question**

Suggested answer scheme:

**Part 1: Aim**

To investigate the time taken for the milk droplet of different concentrations to sink, and hence determine the concentration of the milk provided by the supplier.

**Part 2: Theory**

(Main Theory): **[T1: 1 mark for any 2 points from 1 to 5]**

1. Milk is denser than the standard copper sulfate solution, and hence will sink to the bottom.
2. Density of the milk can be determined by the rate taken for the drop of milk to sink to the bottom of standard copper sulfate solution, measured by the time taken **(dependent / measurable variable)**
3. When milk is diluted with water, the density of the diluted milk will decrease and hence take a longer time for the drop of milk to sink to the bottom. (predicted trend)
4. Milk of the same density will sink at the same rate.
5. Hence the density of the sample milk can be determined by comparing the rate it sinks to the standard graph plotted using results of the rate of sinking of a set of standard concentrations of milk.

**Independent variable :**
**(at least 5 concentrations of milk solution at equal interval) AND description of how it is controlled :** 20%, 40%, 60%, 80% and 100% milk solution [2]

6. Prepare 10 $cm^3$ 20%, 40%, 60%, 80% and 100% milk solution by mixing appropriate volumes of undiluted milk and distilled water in a test-tube as shown in the table below. Use a 10$cm^3$ syringe to measure the volume stated in the table.

| Concentration of milk /% | Volume of undiluted milk /$cm^3$ | Volume of distilled water used /$cm^3$ | Total volume /$cm^3$ |
|---|---|---|---|
| 20 | 2 | 8 | 10 |
| 40 | 4 | 6 | 10 |
| 60 | 6 | 4 | 10 |
| 80 | 8 | 2 | 10 |
| 100 | 10 | 0 | 10 |

**Part 3: Procedure**

1. **Pilot Test**

   Conduct a pilot experiment to determine suitable range of independent variables used, suitability of apparatus, and concentration of solution (e.g. copper sulfate solution). **[P: 1 mark]**
2. **Annotated diagram**

   Set-up simple, diagram probably not needed.
3. **Numbered steps in procedure**

4. Fill a 100$cm^3$ measuring cylinder with 100 $cm^3$ of copper sulfate solution. [constant variable]
5. Using the syringe with the attached needle, draw up 1$cm^3$ of the 100% milk
6. Insert the tip of the syringe into the copper sulfate solution. Ensure the tip of the needle is about 1cm into the copper sulfate solution. [constant variable]
7. In a controlled manner, slowly release 1 drop of the milk [constant variable] from the syringe into the copper solution. Start the stopwatch.

8. Stop the stopwatch once the drop of milk reaches the bottom of the measuring cylinder.
9. Record the time taken for the drop of milk to reach the bottom of the measuring cylinder **[dependent variable]** in the table below.
10. Calculate the rate taken for the drop of milk to sink using 1/time taken ($s^{-1}$).
**[Dependent Variable: 1 mark – steps 9 and 10, show how to obtain dependent variable including calculation, method of obtaining must be scientifically sound]**

11. Repeats steps 1 to 7 twice. These serve as **replicates** to check that no anomalies are present. **[R1: 1 mark for both replicates and repeats, including how they are carried out and why they are carried out]**
12. **Repeats steps 1 to 8 using** 20%, 40%, 60%, 80% milk solution, as well as the milk sample from the supplier.
13. **Repeat** entire experiment (steps 1-9) twice more to check for reproducibility.
**[R1: 1 mark for both replicates and repeats, including how they are carried out and why they are carried out]**

14. **control**
Keep all variables constant. Set up control experiment using water in place of milk.
This is to show that the presence of milk that causes the sinking of the droplet.
**[Co: 1 mark for either control, including how it is carried out and reason why it is performed]**

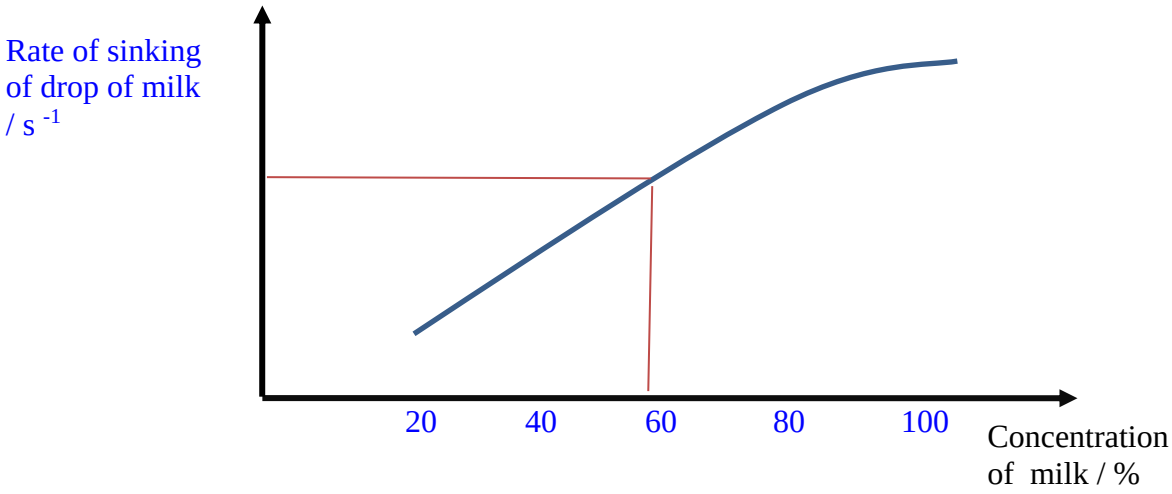
**Part 4: Data recording and processing:**

Table showing rate of sinking of milk droplet

**[T1: 1 mark for any full table including correct units – for time / rate & concentrationeither pH or temperature]**

| Concentration of milk /% | Time taken for drop of milk to sink / s | | | | Rate of sinking of drop of milk / $s^{-1}$ |
|---|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | Replicate 3 | Average | |
| 20 | | | | | |
| 40 | | | | | |
| 60 | | | | | |
| 80 | | | | | |
| 100 | | | | | |
| Sample milk from supplier | | | | | |

Standard graph of rate of sinking of milk droplet for different concentrations of milk




As shown above, use the standard curve to determine the concentration of the milk in the sample from supplier.

**Part 5 : Risks and precautions [1 mark]**

(i) Be careful when using the sharp need, in order to prevent pricking oneself.
Wear gloves and goggle when measuring copper sulphate as they could be are irritants.

[Total: 12]

**N15P3Q5 (OUT OF SYLLABUS)**