

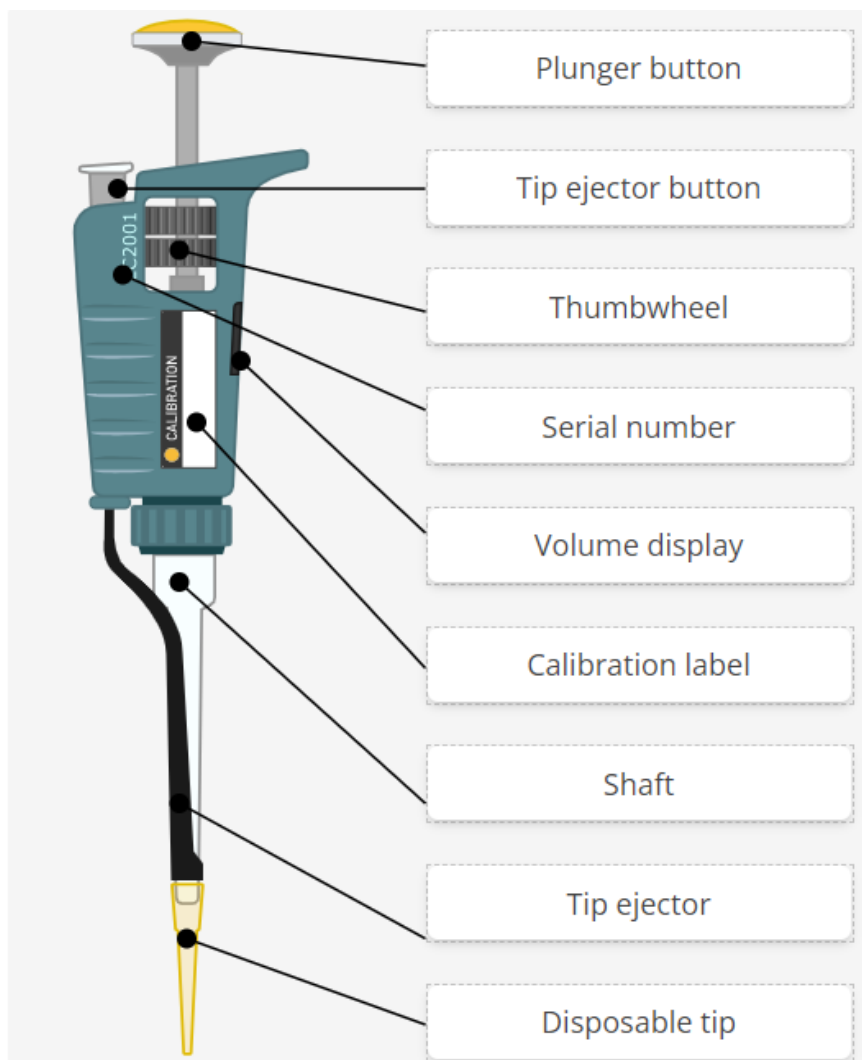
Practical skills: Pipetting



What is a pipette?



Key parts of a pipette



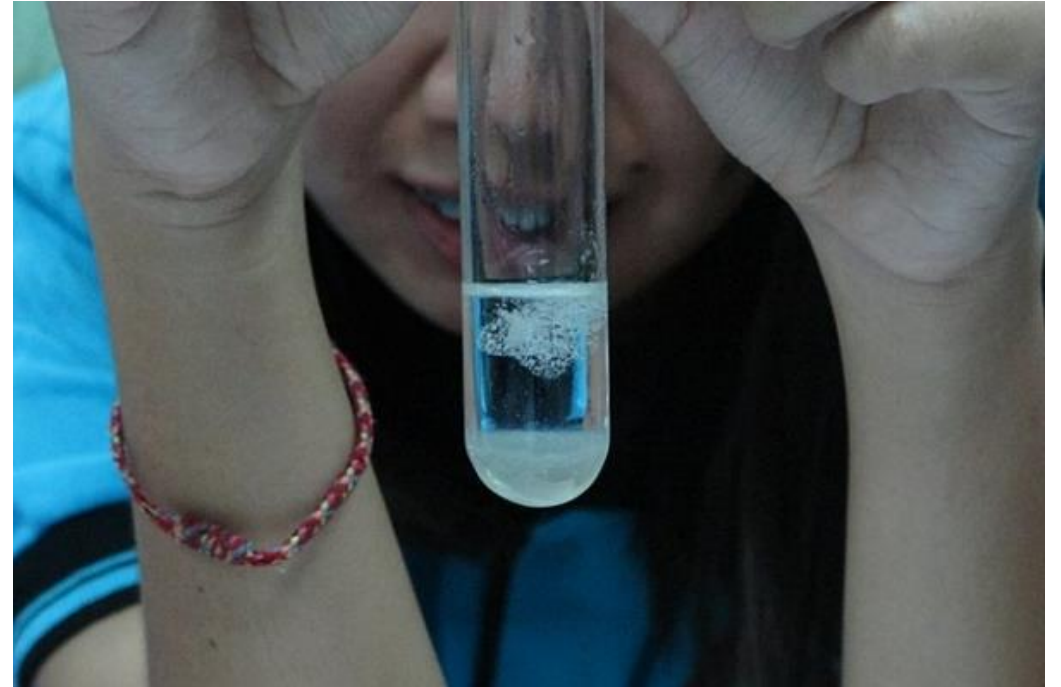
Sizes of pipette



Practical 1: DNA extraction

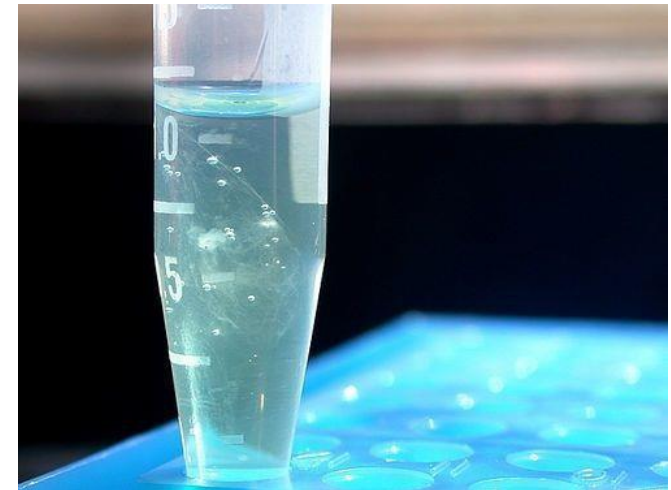
Aim:

- Understand three main stages involved in extracting DNA from plant cells and animal cells.



Stages of DNA extraction

- The process of DNA extraction is fairly straightforward, incorporating the following basic steps:
 1. Breaking cells open
 2. Separating DNA from proteins and other cellular debris
 3. Precipitating the DNA with an alcohol
 4. Cleaning the DNA (purification)
 5. Confirming the presence and quality of the DNA



Practical 2: PCR

Aim:

- Understand the processes involved in carrying out a PCR reaction.



Practical 2: PCR

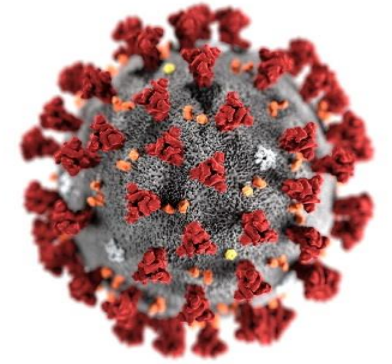
Polymerase Chain Reaction

Uses cell's mechanism of DNA replication to make lots of copies of small sections of DNA

Why would that be useful?

Uses of PCR

- Detection of Virus or bacteria
- Identification of Individuals (DNA fingerprinting)
- Identification of species (DNA barcoding)
- Diagnosis of genetic disorders



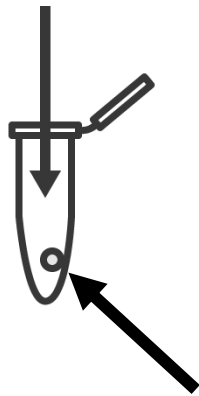
What do you need for a PCR reaction?

- A **DNA template** to be copied
- A **primer** – a short piece of DNA that is designed to bind with the DNA you want to copy
- **DNA bases** - the building blocks of the DNA molecule
- **DNA polymerase** – enzyme to build the new DNA fragments
- **Buffer** – this ensures the conditions remain stable for the reaction to take place.
- A **thermal cycler** – a machine that heats and cools the sample

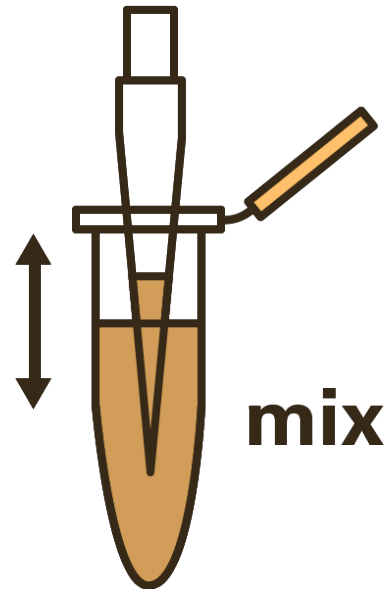
What will we be doing in the lab?

5 μ l DNA template

20 μ l primer mix

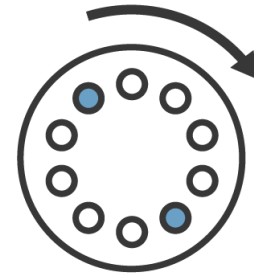


PCR bead



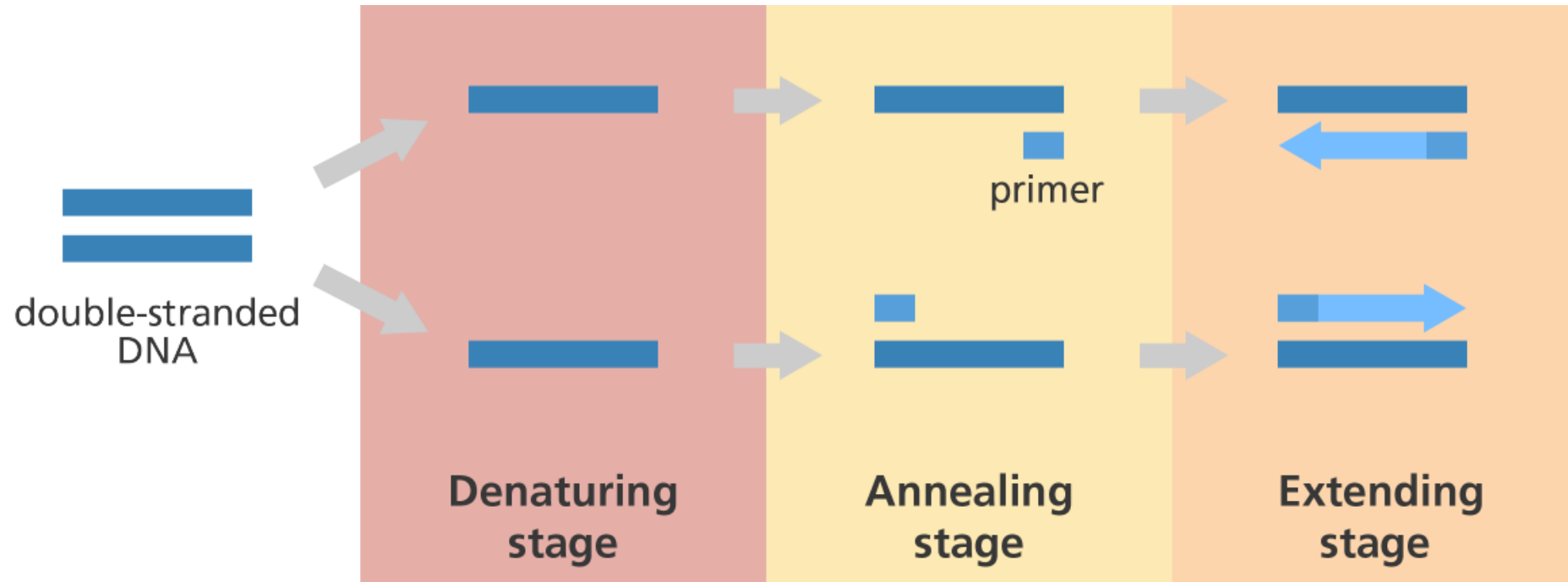
mix

few seconds



centrifuge

PCR cycling conditions



Our protocol

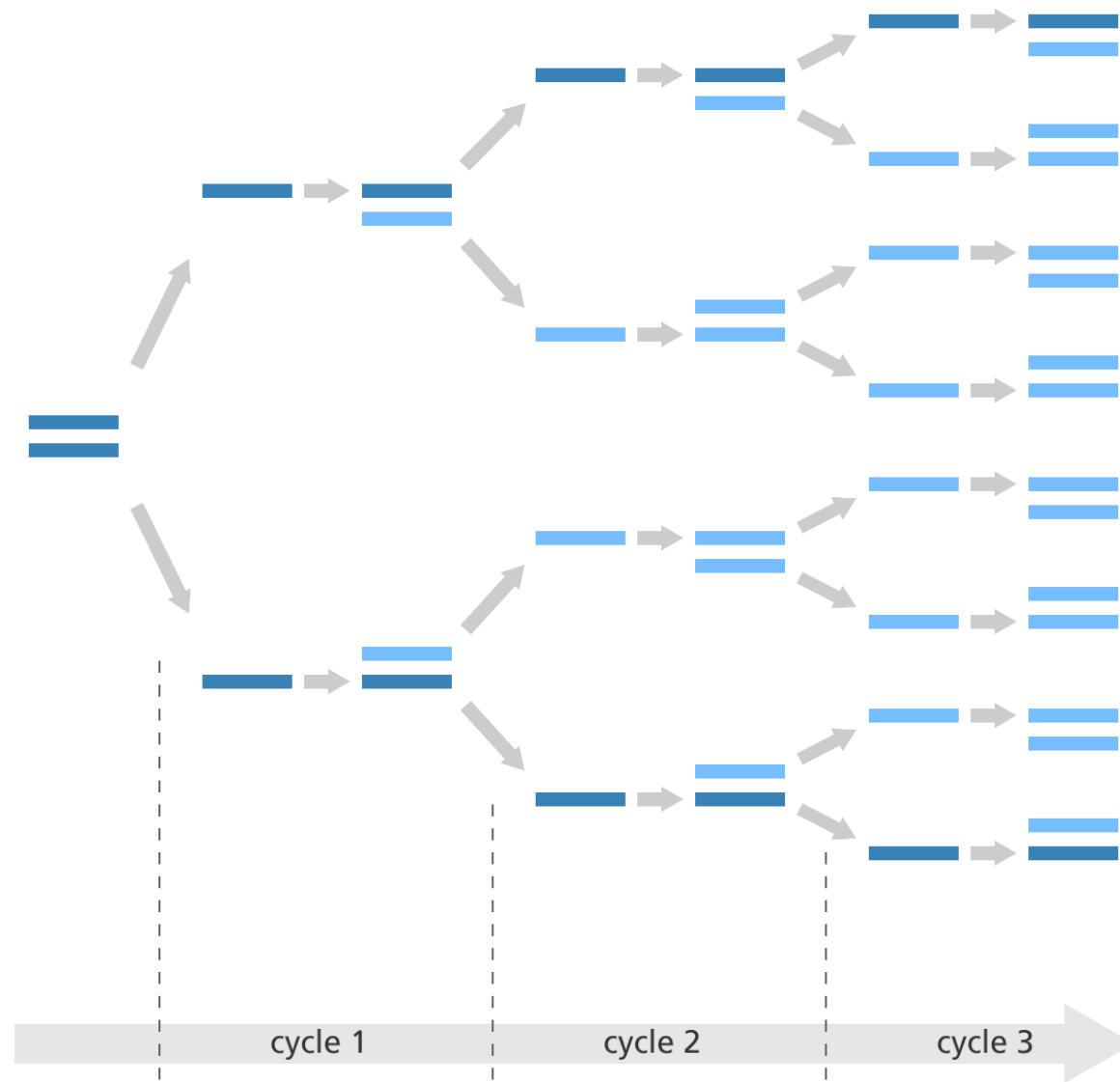
94 °C for 3 mins

94°C for 30 Secs

71°C for 30 Secs

Repeat for 20 cycles

What will be happening in the PCR machine

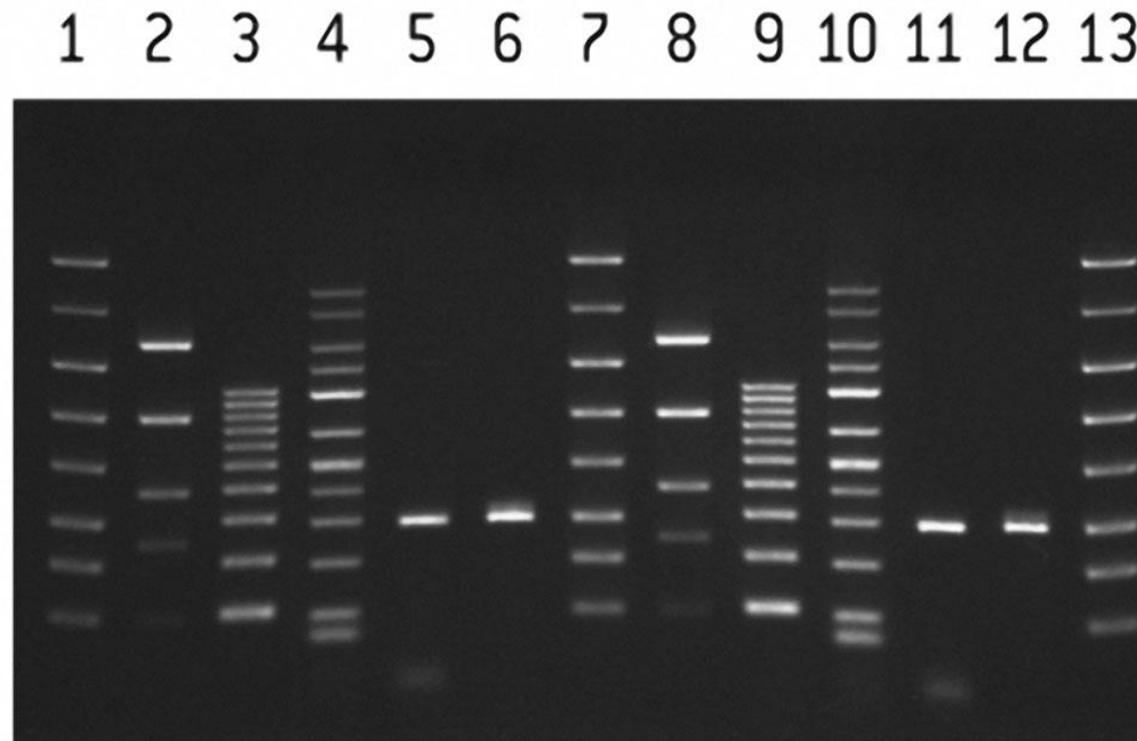


Using the Lonza Flashgel system



Visualising results

Gel electrophoresis can be used to view whether your PCR has been successful



Lonza Flashgel (Gel electrophoresis)

