KING'S College LONDON

INSTITUTE OF PSYCHIATRY,
PSYCHOLOGY & NEUROSCIENCE

Module:
Techniques in Neuroscience

Week 5:
Molecular biology: Going inside the cell

Dr Elizabeth Glennon

Topic 1:
An introduction to molecular biology methods
Part 2 of 3

Part 2

Part 2 outline

In this section, we will study the SDS-PAGE process:

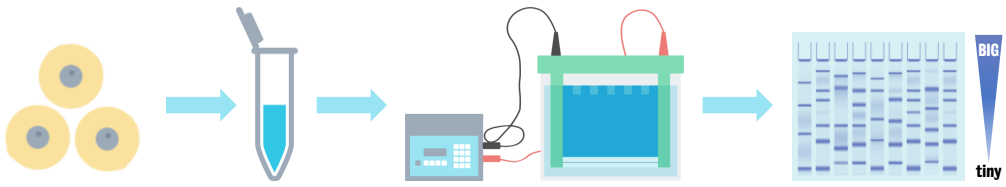
1. Process overview
2. Sample preparation
3. Principles of separation
4. The electrophoresis set up

Click **Next** to continue

SDS-PAGE overview

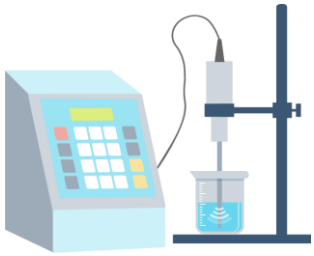
SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis):

- separates proteins in a sample by molecular weight
- works by applying an electric field across a polyacrylamide gel



1. **Sample preparation:**
   - break down tissue sample
   - denature the proteins
   - add electric charge to proteins
2. **SDS-PAGE:**
   - separates protein molecules using an electric field
3. **Protein separation**

Sample preparation: tissue breakdown



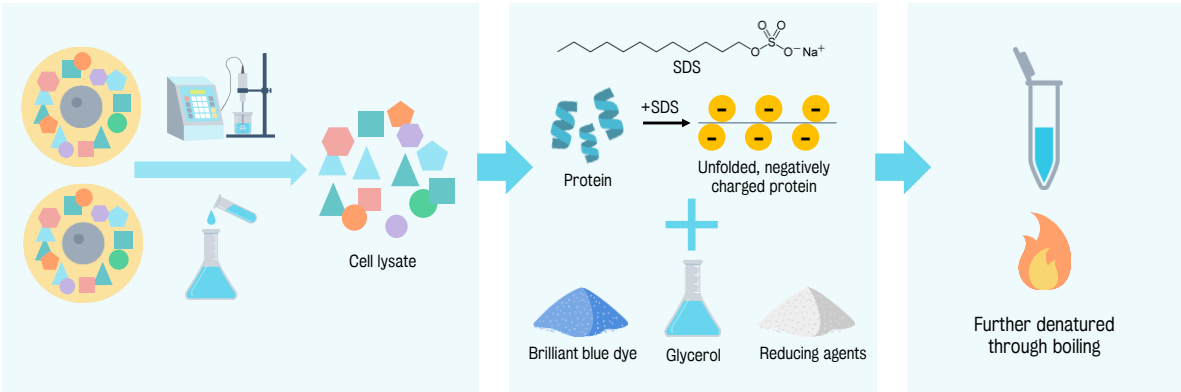
Mechanical breakdown:

- uses homogeniser or ultrasonic vibration
- tears tissues apart, preserving some cell structure

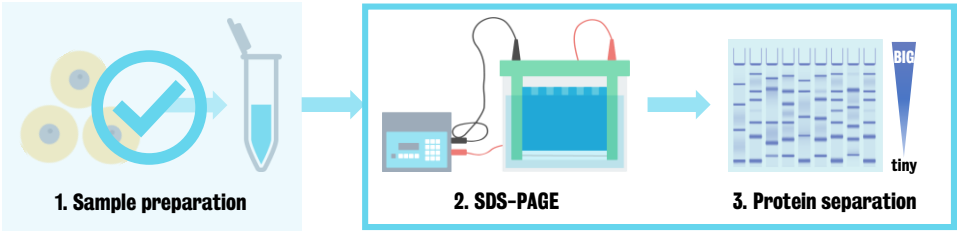
Chemical breakdown (Lysis):

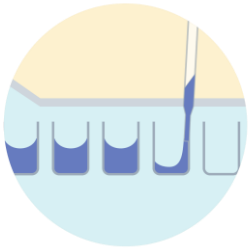
- mixing cells with a buffer to break the membranes apart

Sample preparation

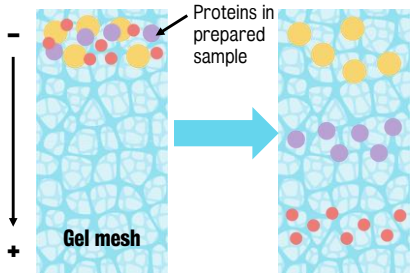


Principles of separation



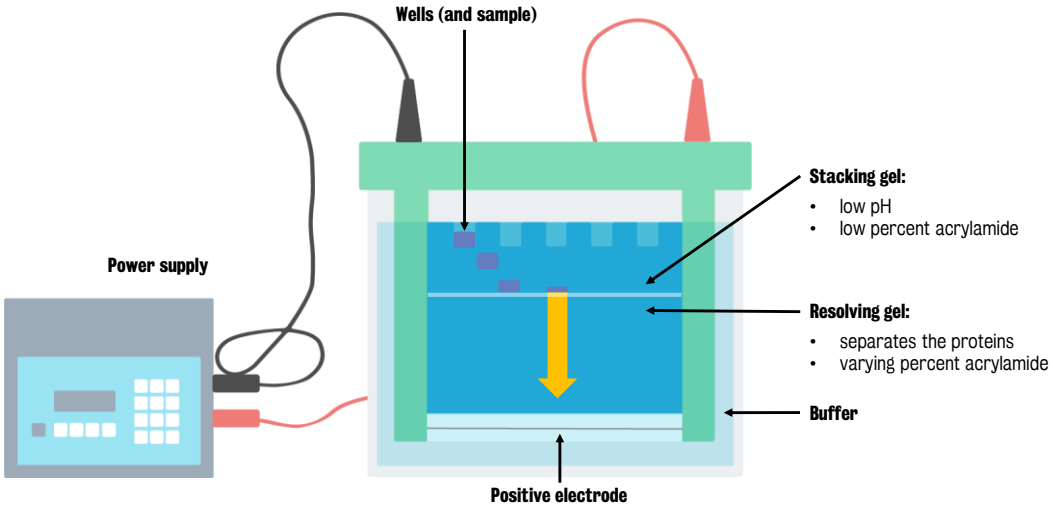


Prepared samples are pipetted into wells on the gel.

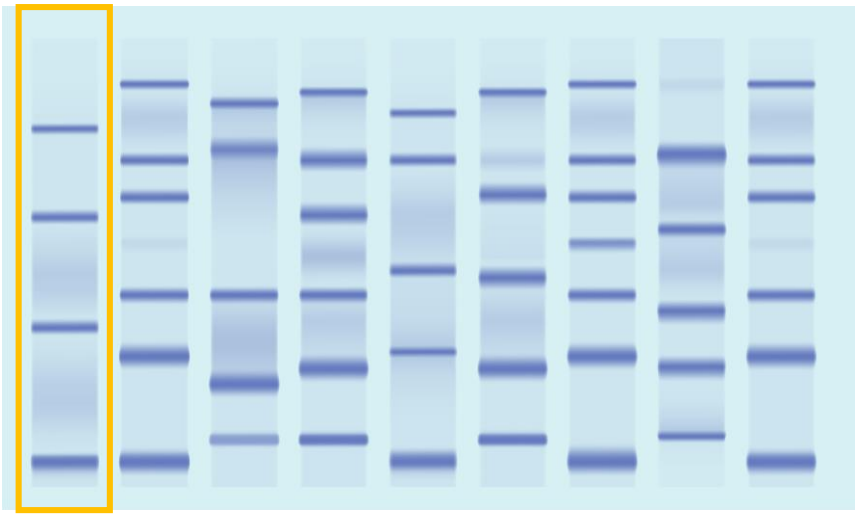


SDS-PAGE process

Electrophoresis set up



SDS-PAGE results



Standard size markers **Proteins spread through the gel after SDS-PAGE.**

End of part 2