ELECTROPHORESIS

Overview of the course

Date	1st Lecture	2nd Lecture
16th July	Icebreakers, ground rules and maybe and Intro to Molecular Biology and NGS	
17th July	Intro to Molecular Biology and NGS	Data analysis 1
18th July	Experiment: Nucleic acid extraction	Data analysis 2
19th July	Single-cell DNA, RNA and protein technologies	Proteomics, spatial technologies and epigenomics Data analysis 3
20th July	Data analysis 4	Experiment: Staining our own cells
21st July	Experiment: Gel electrophoresis	Data analysis 5 Data analysis 6
22nd July	Preparation for the final presentation	Final presentation and closing ceremony

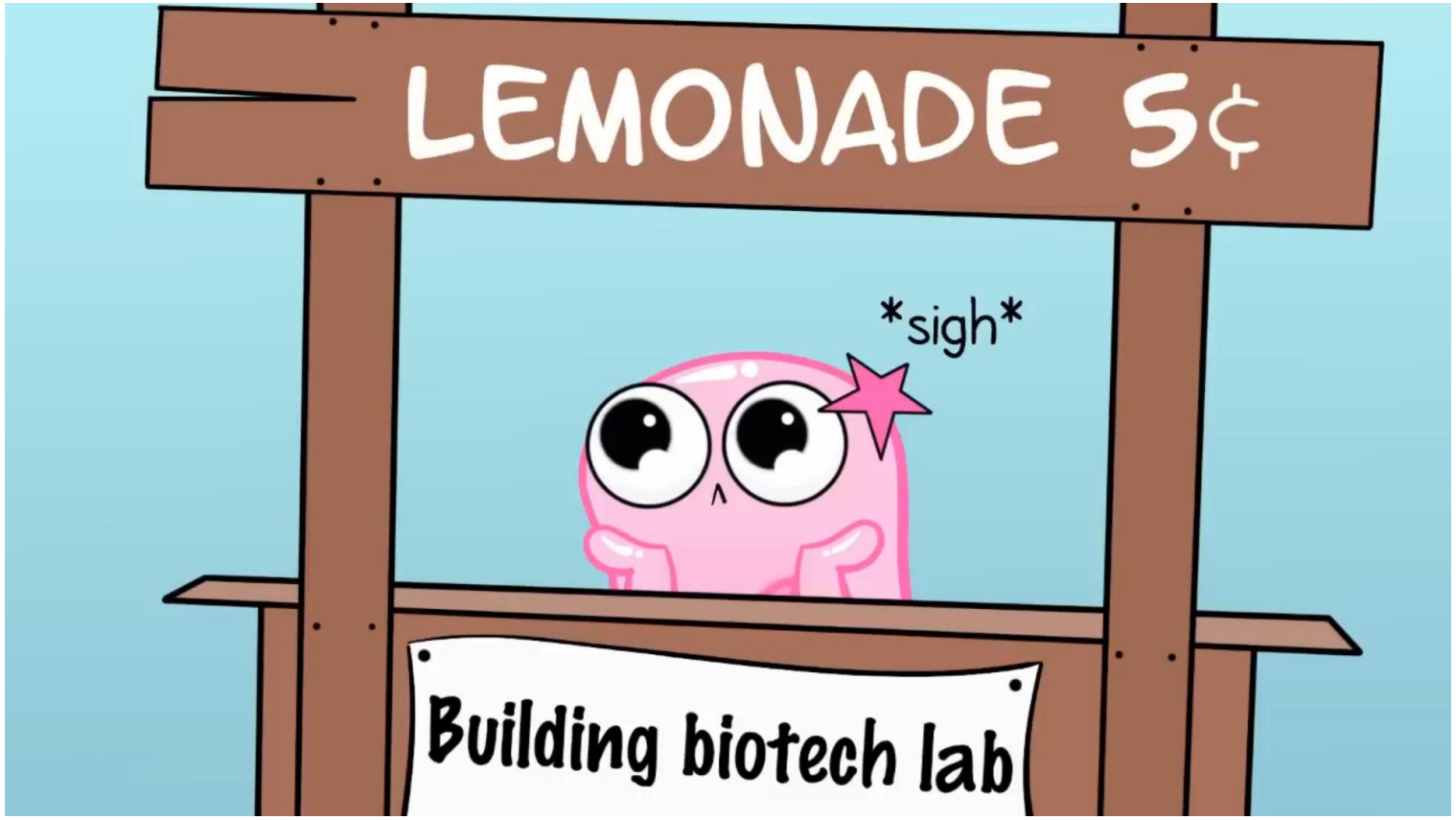
Objectives

1. Understand the principles and process of electrophoresis.
2. Understand the applications of gel electrophoresis
3. Prepare for gel electrophoresis experiment

What is gel electrophoresis?

- Gel electrophoresis is a laboratory technique used to separate macromolecules like DNA, RNA, and proteins based on their size and charge
- When an electric current is passed through a gel matrix charged molecules migrate through the gel at different speeds depending on their size and charge. Smaller molecules move faster and farther than larger ones.
- Staple and well established method.

What is gel electrophoresis?



Components of gel electrophoresis

- Gel box (electrophoresis chamber)
- Gel
- Buffer solution
- Nucleic acid pr protein samples
- Loading dye
- Power supply.

Steps of gel electrophoresis

1. **Preparing the Gel:** Mix agarose powder with buffer, heat to dissolve, pour into the gel tray, insert combs to create wells.

2. **Loading Samples:** Mix DNA or protein samples with loading dye, load into wells using a micropipette.

3. **Running Electrophoresis:** Connect the gel box to the power supply, apply an electric field, molecules migrate through the gel based on size and charge.

4. **Staining and Visualization:** After electrophoresis, stain the gel with a dye and visualize under UV light or using a gel documentation system.

What is blotting?

- Blotting is is used to transfer biomolecules (such as DNA, RNA, or proteins) separated by gel electrophoresis onto a solid membrane for subsequent detection and analysis
- Types of blotting:
  - Western blotting : Protein
  - Northern blotting : RNA
  - Southern blotting : DNA

Objectives

✓Understand the principles and process of electrophoresis.

2. Understand the applications of gel electrophoresis
3. Prepare for gel electrophoresis experiment

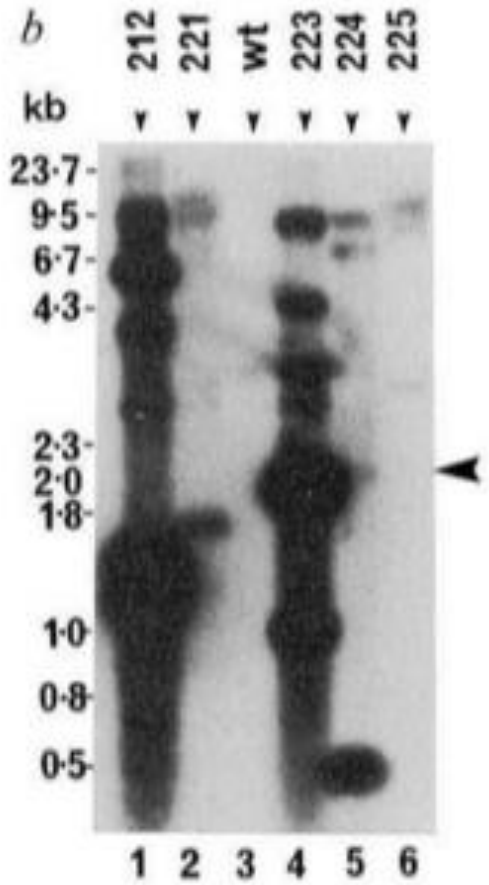
Role of gel electrophoresis in cancer research?

- Identification of genetic mutations associated with cancer susceptibility.
- Discovery of biomarkers for early detection and prognosis.
- Evaluation of treatment responses and drug efficacy.

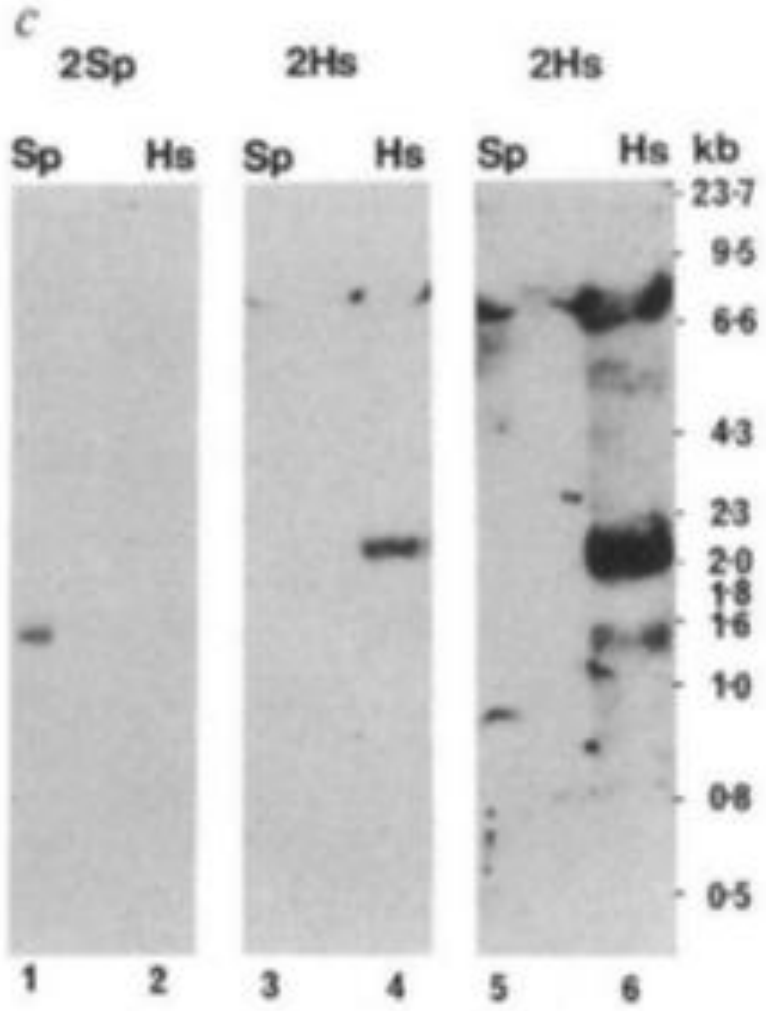
All 3 blotting methods in on paper

- Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2 (Le et al., 1987)
- Cloned human cDNA into mutated fission yeast and identified the human homologue of cdc2 (CDK1)
- Used southern, northern and western blotting to validate their cloning finding
- This discovery propelled the discovery of the other CDKs and cyclins
  - And eventually CDKis

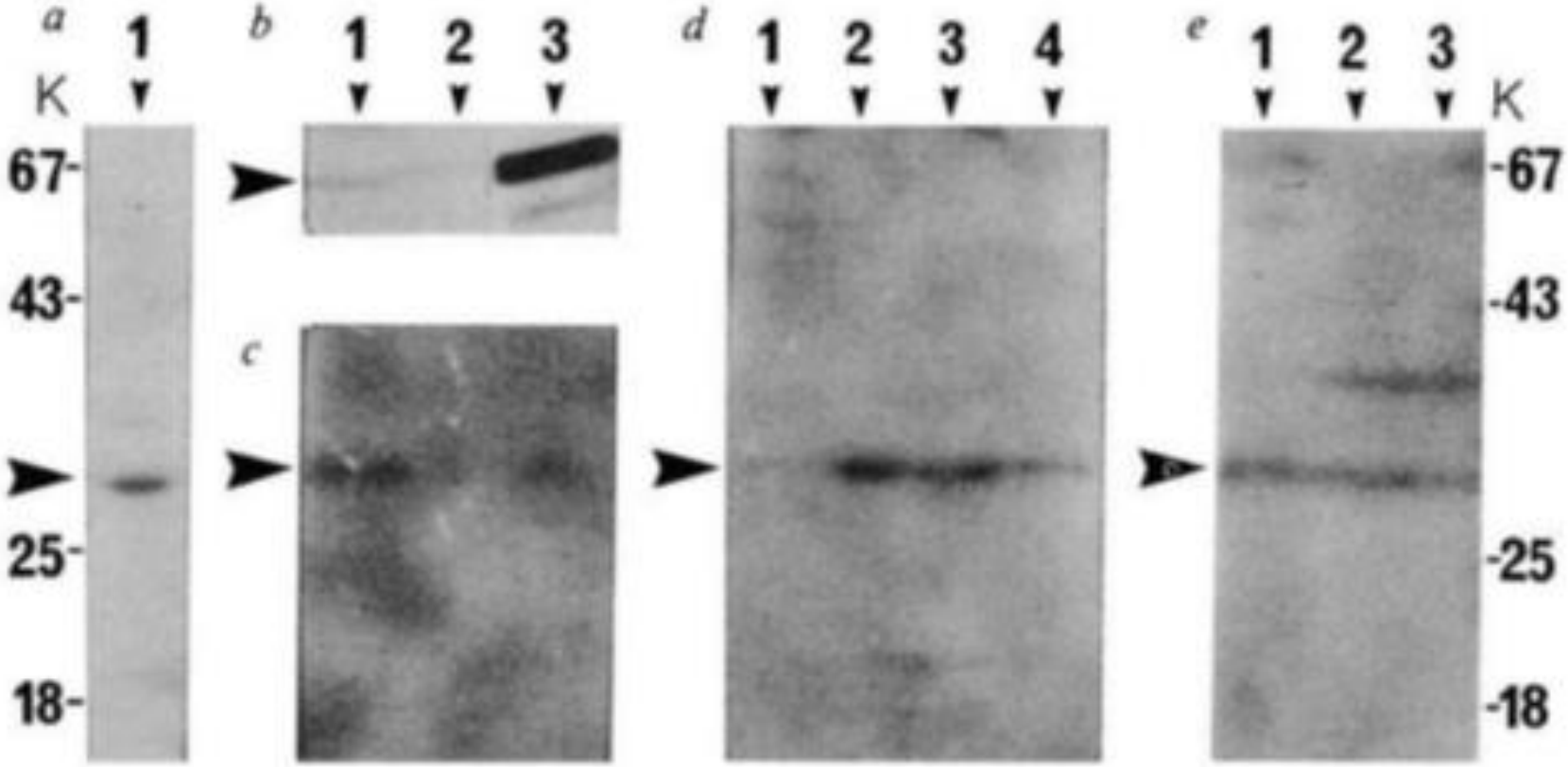
Southern blotting



Northern blotting



Western blots



Objectives

✓Understand the principles and process of electrophoresis.

✓Understand the applications of gel electrophoresis

3. Prepare for gel electrophoresis experiment

In person experiment

In-person experiment

While waiting for gel to set…

First, we'll try this online interactive laboratory protocol of gel electrophoresis:

https://www.labxchange.org/library/items/lb:LabXchange:9548bee3:lx_simulation:1

Quiz!

https://create.kahoot.it/details/3f7e8411-ce78-47b7-a3bc-244633a73253

Objectives

- ✓Understand the principles and process of electrophoresis.
- ✓Understand the applications of gel electrophoresis
- ✓Prepare for gel electrophoresis experiment

Any questions?