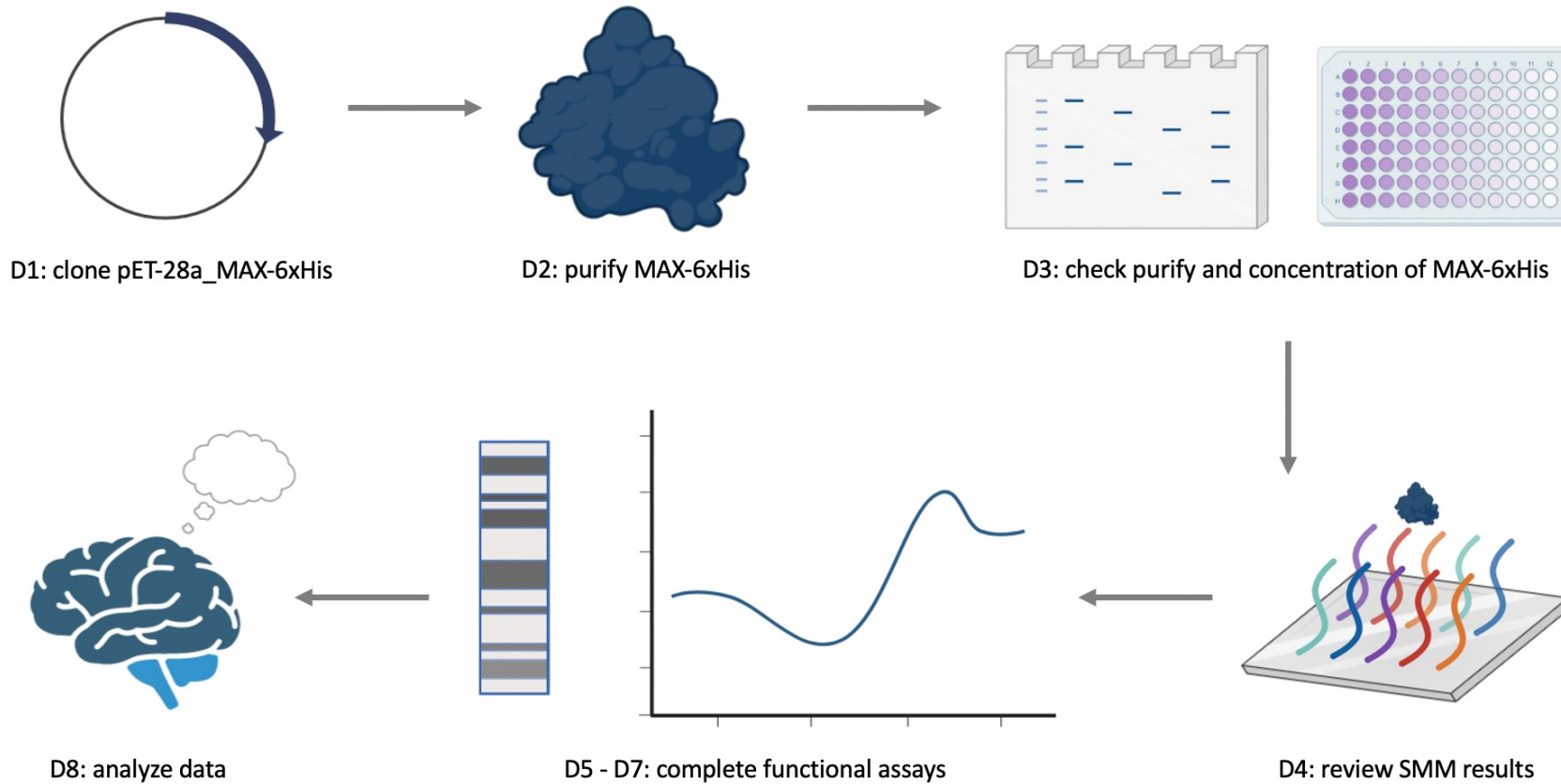


M1D7: Complete EMSA experiment

1. Prepare and electrophorese DNA complexes
2. Transfer DNA complexes onto membrane



Overview of Mod 1 experiments:



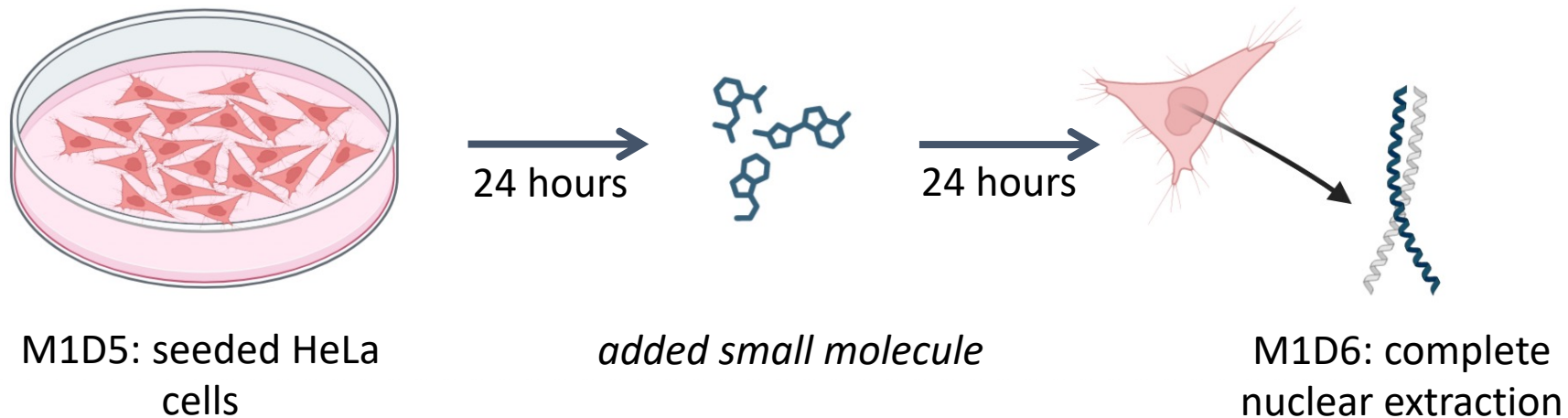
Workflow for secondary assays

	M1D5	M1D6	M1D7	M1D8
DSF	prepare samples and setup assay run DSF experiment	plot data to identify shifts in melting temperature		apply statistics to data interpret results
EMSA	seed cells	extract nuclear proteins	complete electrophoresis and transfer nuclear proteins onto membrane	image EMSA experiment to assess binding interpret results

What are we testing with each experiment?

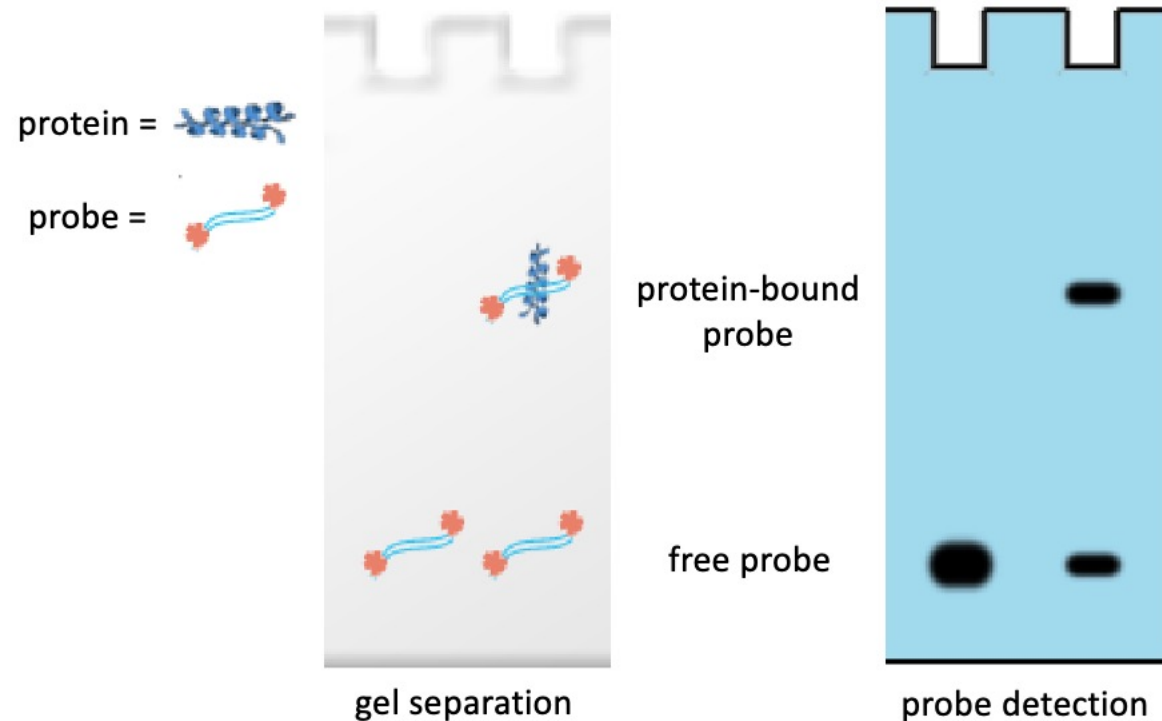
Let's review...

- Why did we extract nuclear proteins for EMSA experiment?
- What are we testing with the EMSA experiment?



How will we use EMSA to test Myc:MAX binding to DNA?

- Nuclear extract mixed with labelled probe
 - Probe = proprietary DNA sequence presumably specific for Myc:MAX binding
- Free probe is smaller and migrates more quickly
- Protein-bound probe is larger and migrates more slowly
- Difference is a shift



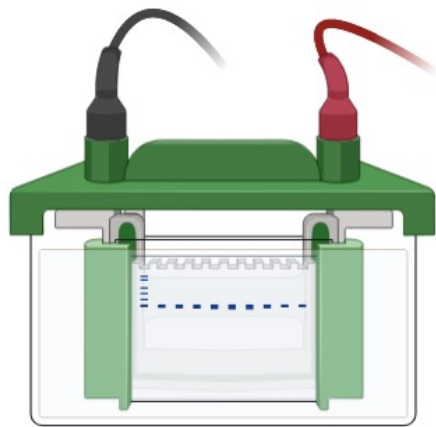
Preparing DNA complexes

- Nuclear extract incubated with labelled probe and assigned small molecule
- What is in the nuclear extract?
- What is the labelled probe?
- Why is the small molecule included?

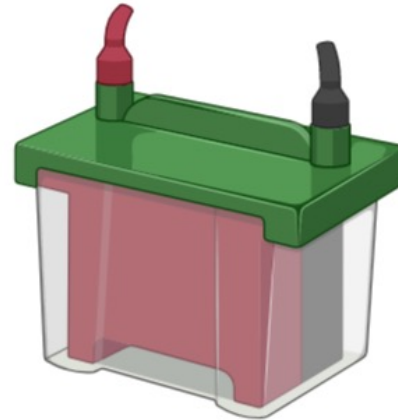
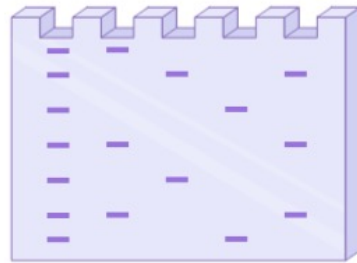


Electrophoresis and transfer of DNA complexes

- Why is it necessary to transfer protein complexes onto a membrane?



1. DNA complexes electrophoresed to separate free probe from bound probe



2. DNA complexes transferred from polyacrylamide gel to nitrocellulose membrane



Differences between SDS-PAGE and electrophoresis for EMSA

	SDS-PAGE	non-denaturing PAGE
Buffer components:	TGS; tris-glycine-SDS	TBE; tris-borate-EDTA
Gel matrix:	4-20% TGX	5% TBE
Sample preparation:	laemmli sample buffer + boiling	loading buffer

For today...

- Class will be divided into two groups for paper discussion
 - During electrophoresis: Pink, Blue
 - During transfer: Green, Yellow, Orange
- Use downtime to complete homework assignments 😊

For M1D8...

- Use feedback to improve methods homework
 - Include protocols for SDS-PAGE and BCA assay
- Answer Conclusion & Future works prompts