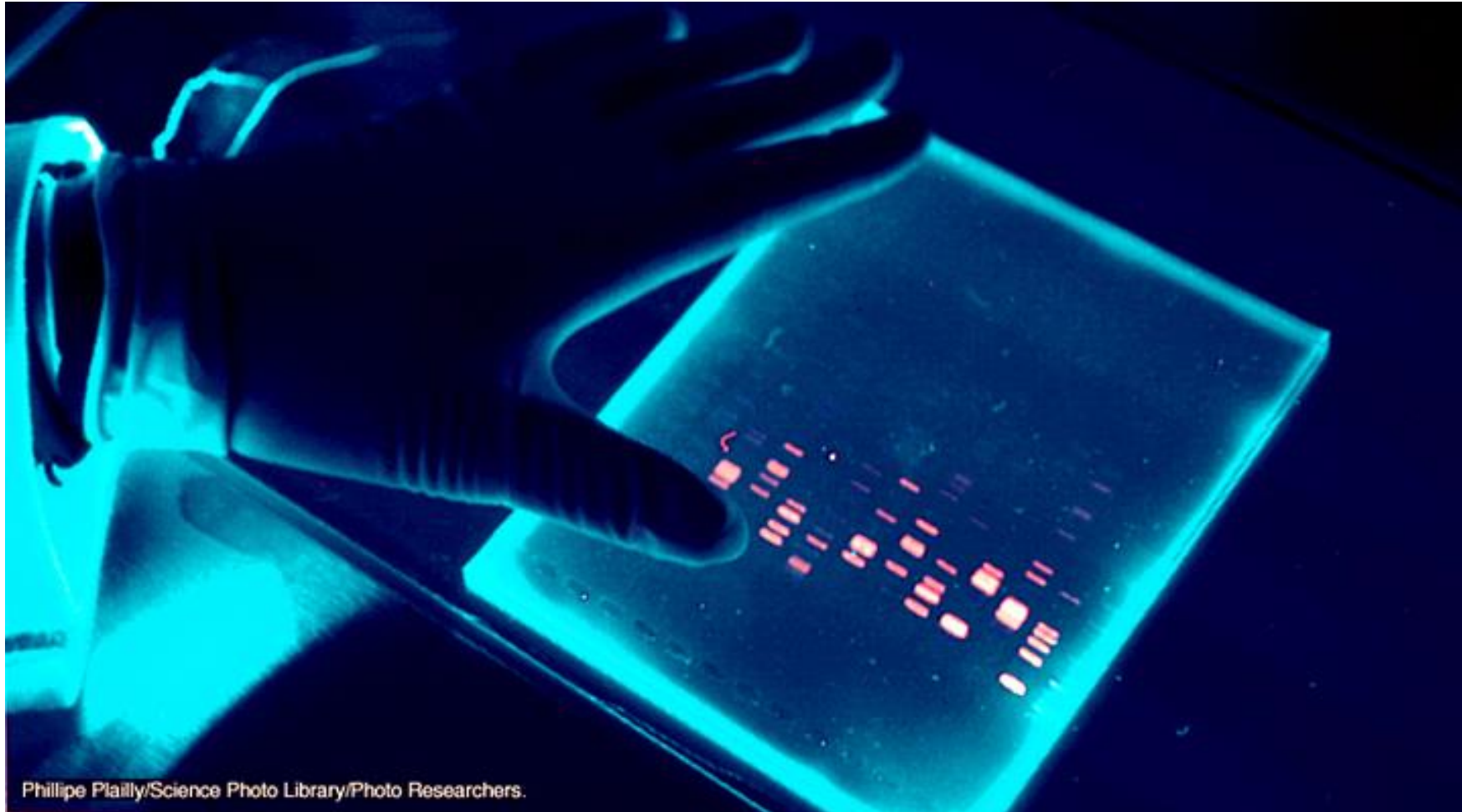


Lab # 9 : Restriction enzyme mapping



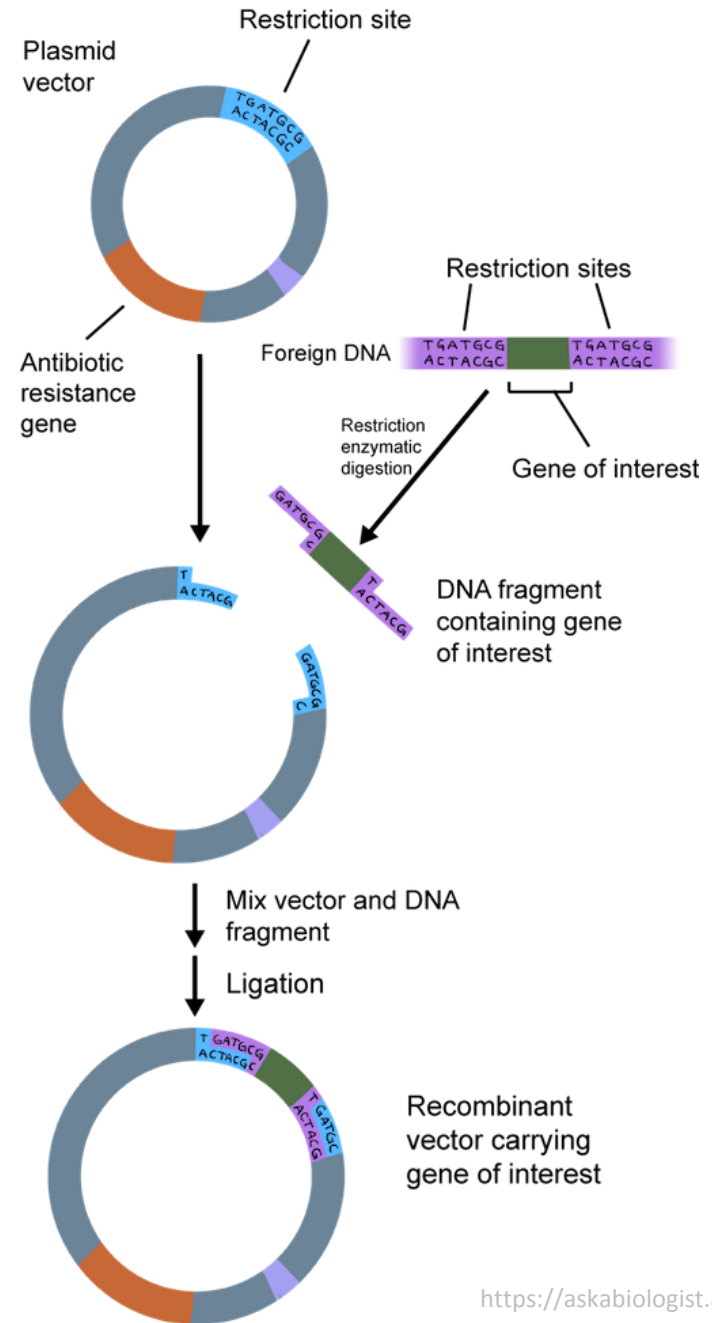
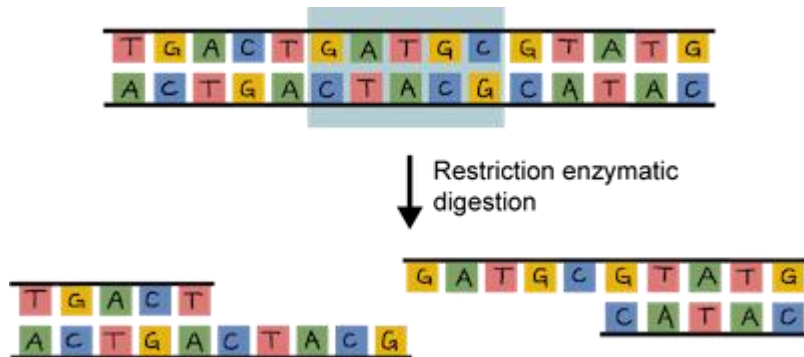
Restriction Enzymes

Restriction enzymes, also known as restriction endonucleases, are enzymes that cut a DNA molecule at a particular place (=recognition site).

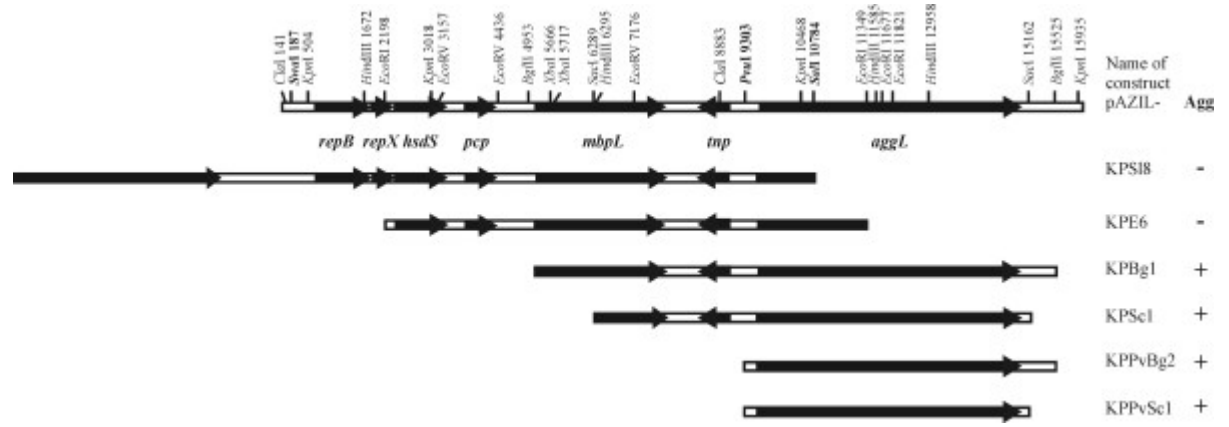
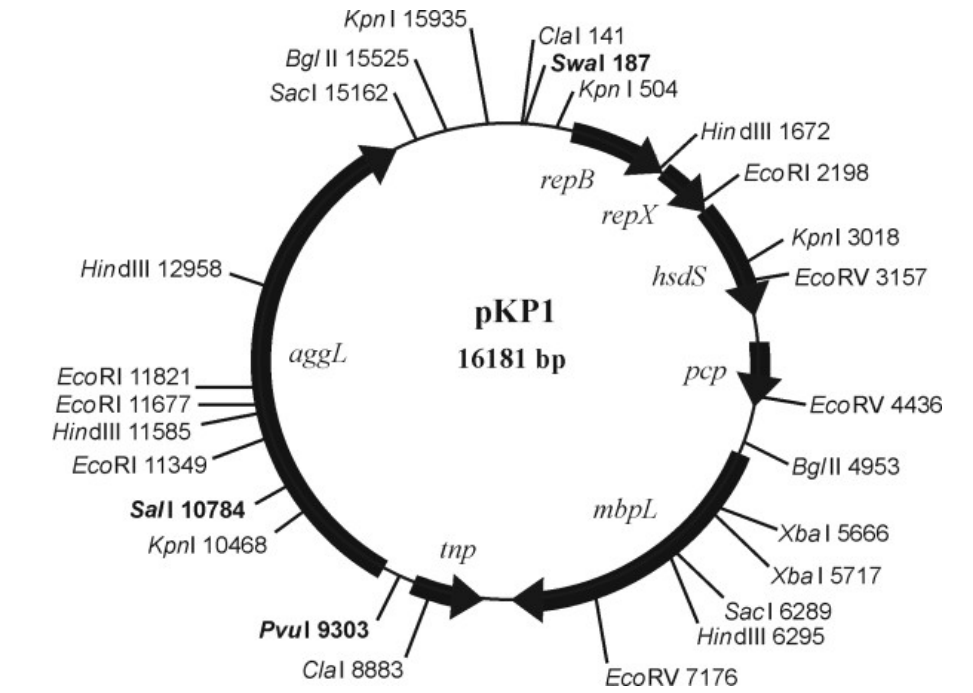
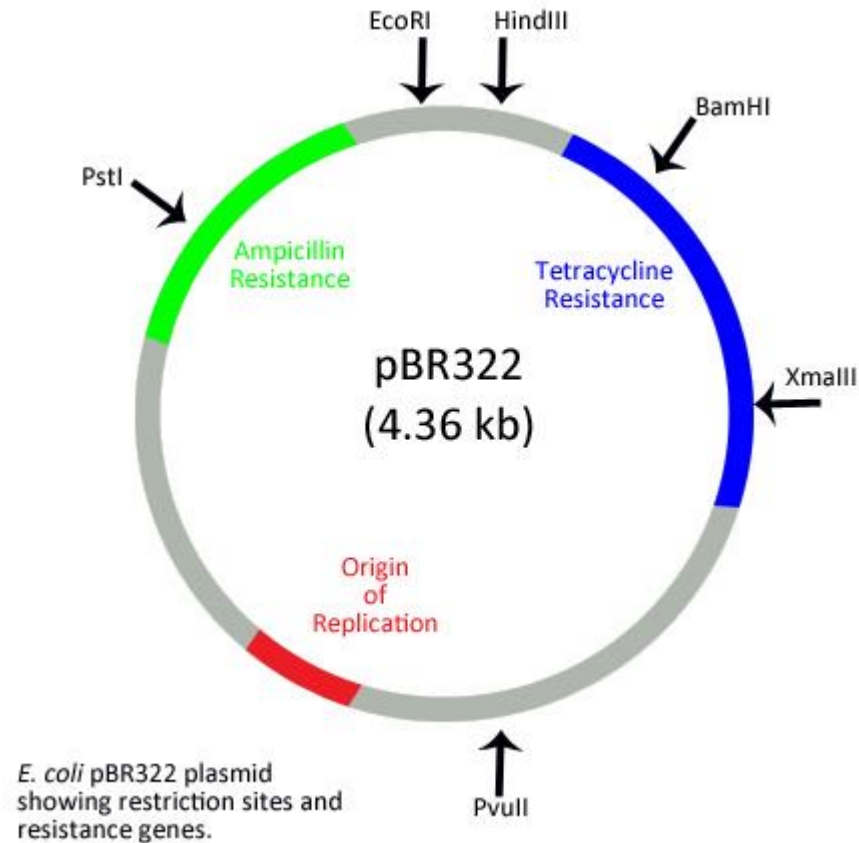
A blunt end :



A sticky end:



Restriction Enzyme Mapping



Today's lab

Quick Reference

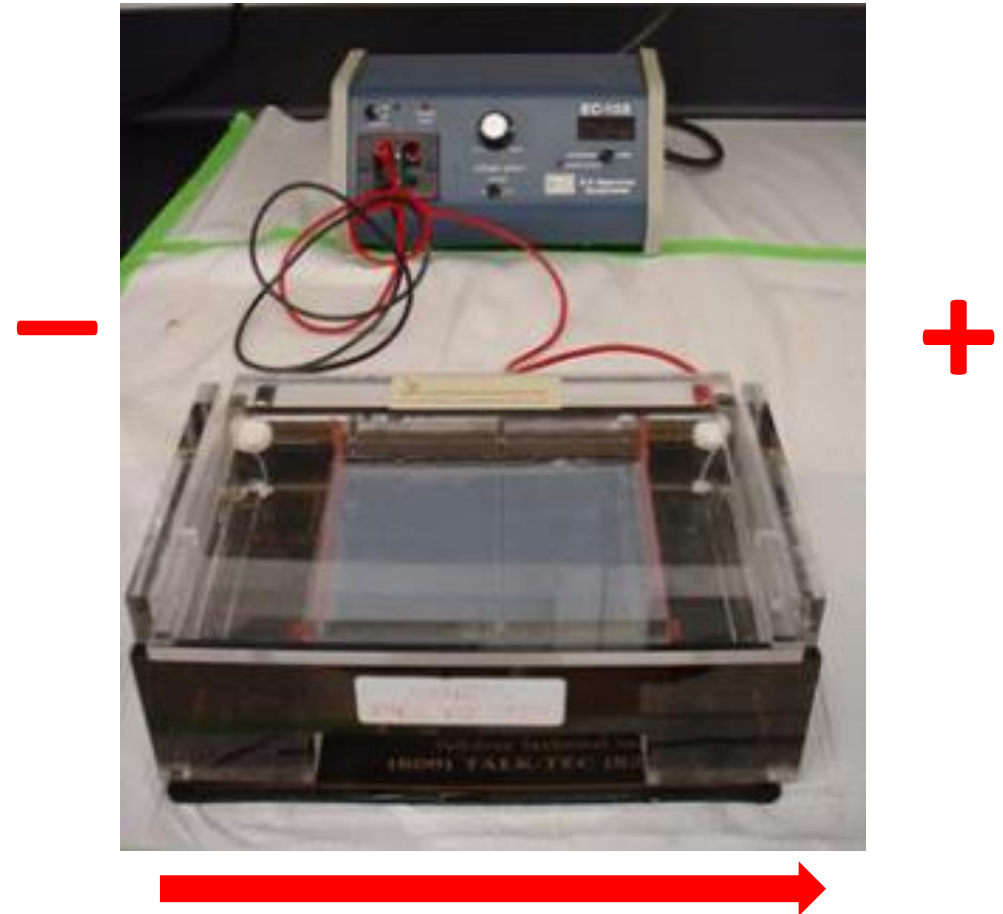
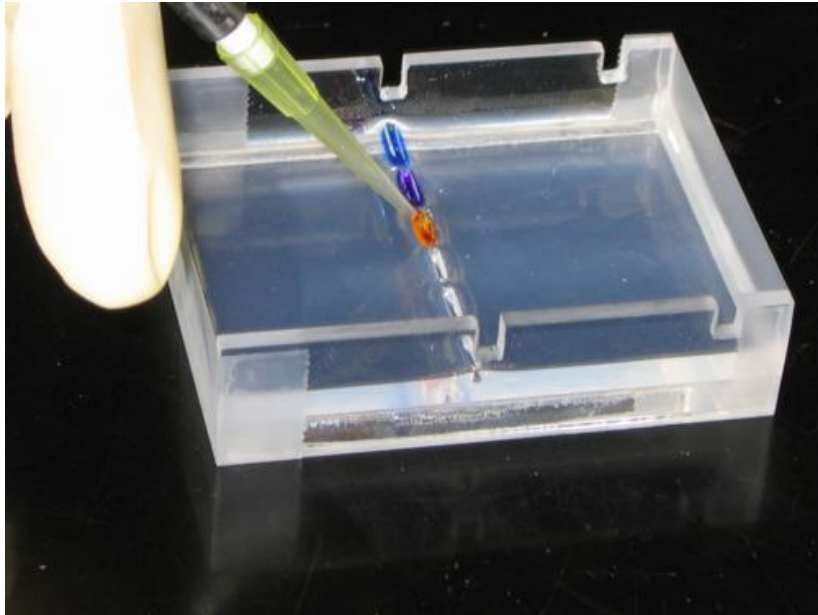
Component	Label
Plasmid DNA	DNA
Restriction Enzyme	
Reaction Buffer	Rxn Buffer
Enzyme Grade water	Water
Standard DNA Fragments	Markers
Diluted <i>Hind</i> III	<i>Hind</i> III
Diluted <i>Bgl</i> I	<i>Bgl</i> I

Each pair does 4 reaction tubes

Sequence for Restriction Enzyme Reactions

Rxn Tube	Reaction Buffer (μl)	DNA (μl)	E Qualified Water (μl)	A <i>Hind</i> III (μl)	B <i>Bgl</i> I (μl)	Reaction Volume (μl)	37°C Incubation (minutes)	10x Gel Load (μl)	Total Sample Volume (μl)
1	30	10	10	-	-	50	30-60	5	55
2	30	10	5	5	-	50	30-60	5	55
3	30	10	5	-	5	50	30-60	5	55
4	30	10	-	5	5	50	30-60	5	55

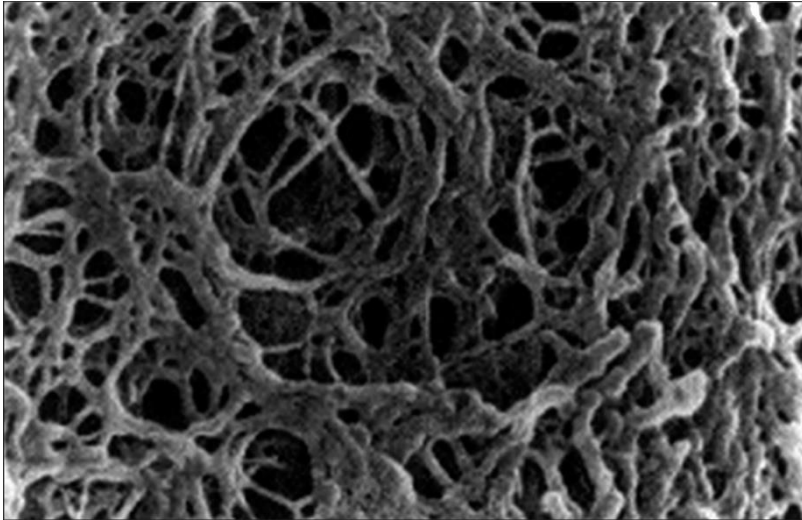
Gel Electrophoresis



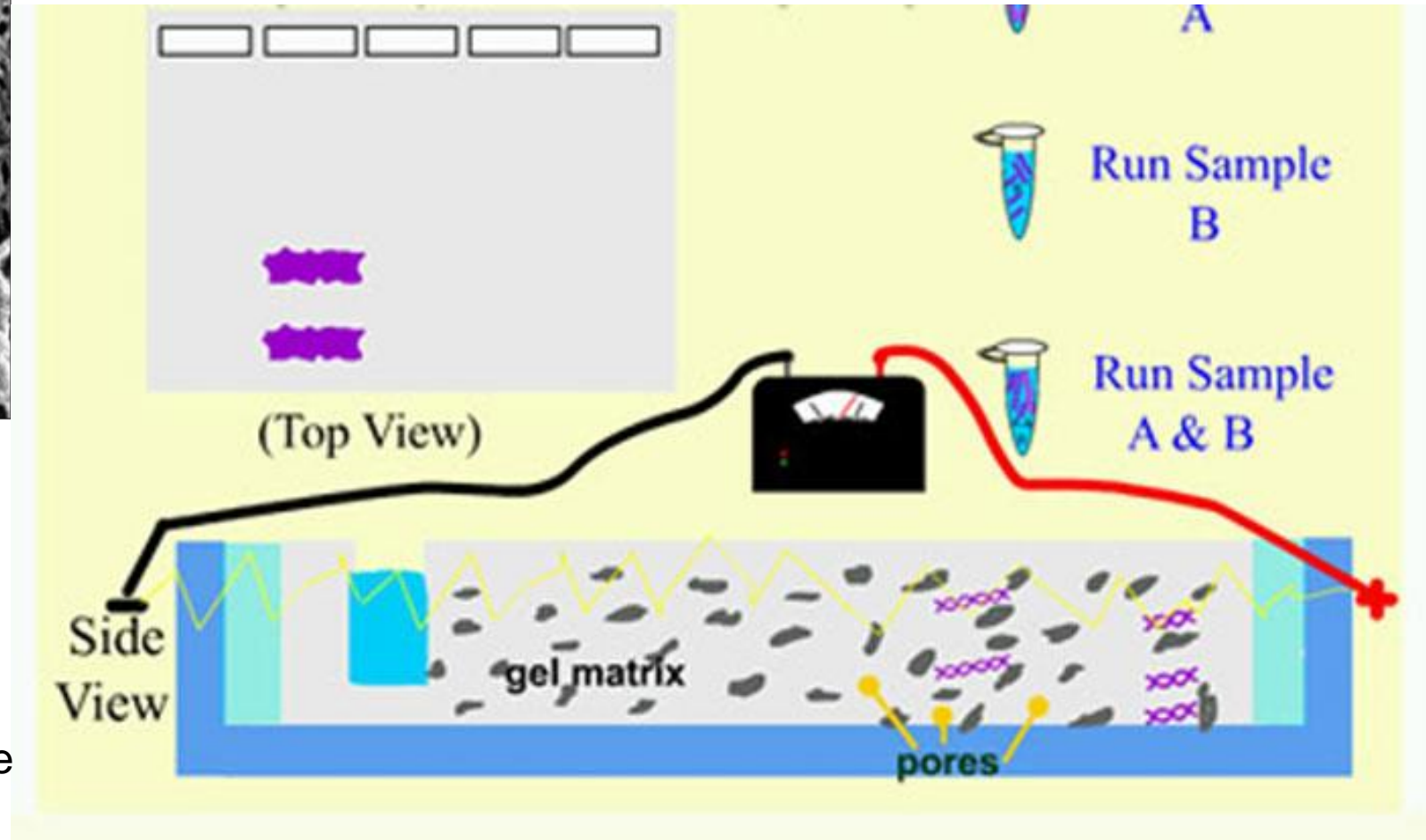
Run to the red!

DNA is strongly negative => it will migrate through the gel towards the positive electrode (from cathode to anode)

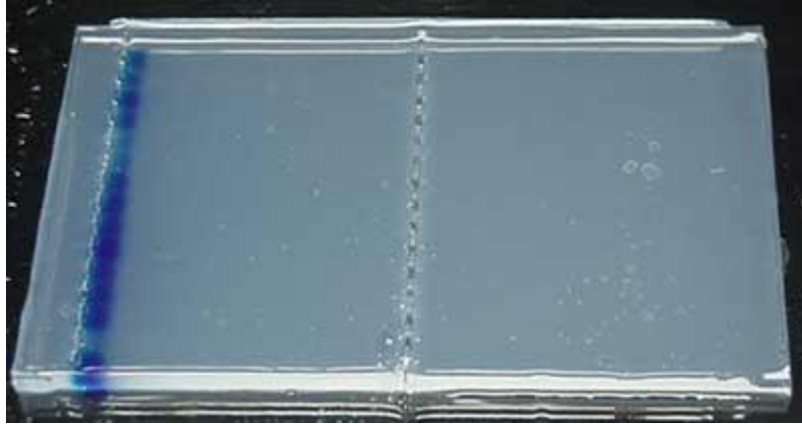
DNA migration in Agarose Gel



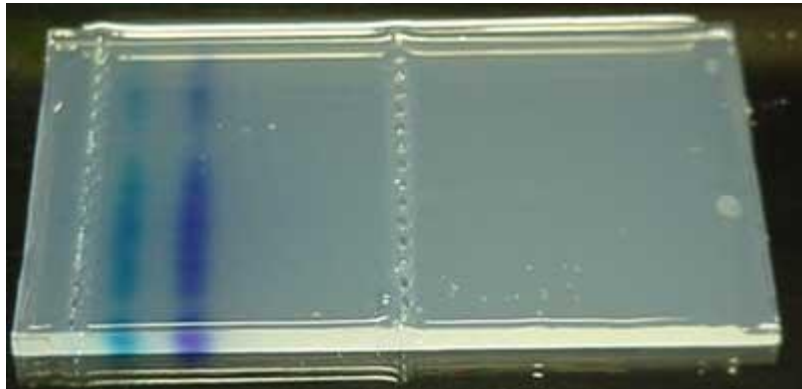
- The pores in the gel separate the DNA molecules according to their size and shape.
- The smaller the DNA molecule, the faster it migrates through the gel.



Visualization of DNA migration

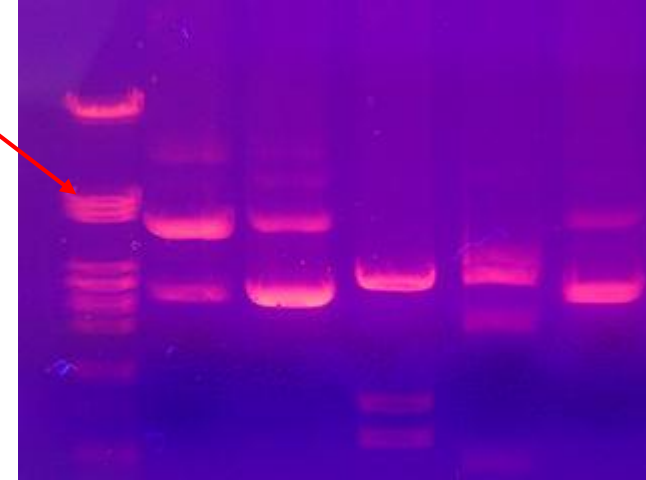


The tracking dye moves along with the DNA sample

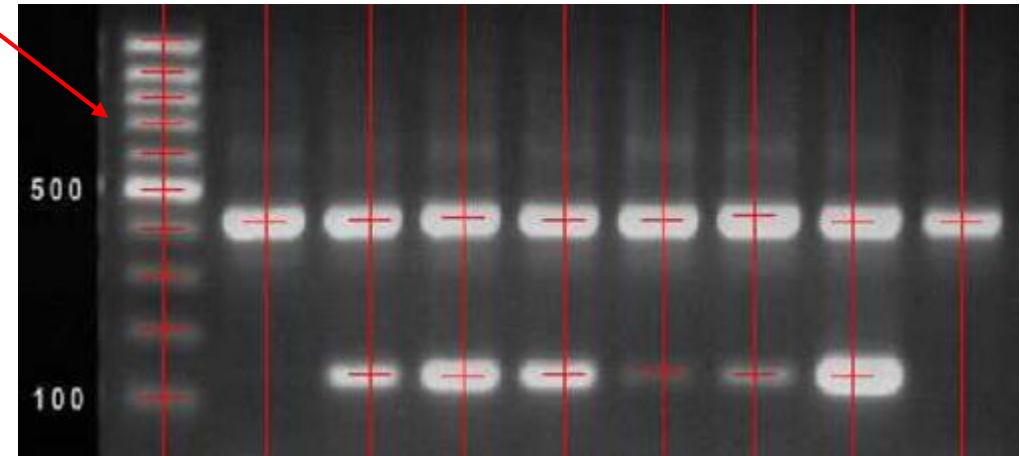


Two dyes can be used

Ladder



Ladder



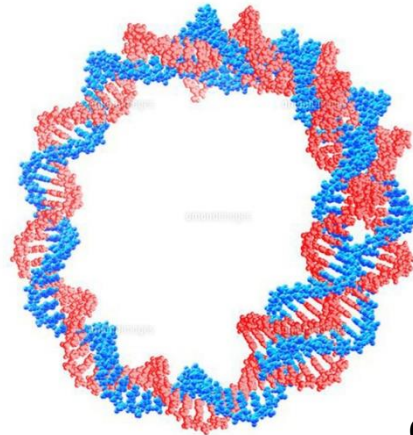
Types of DNA Molecules



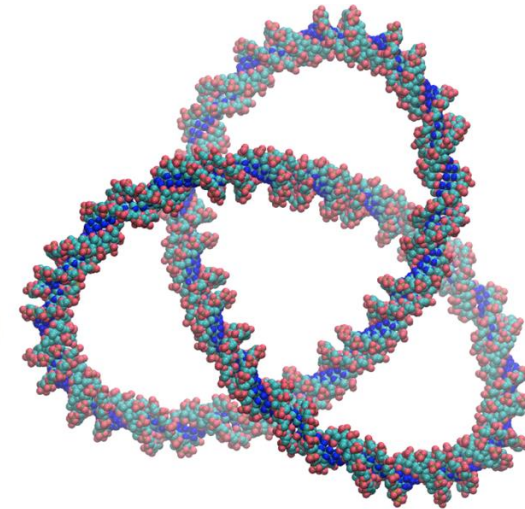
supercoiled



linear



circular



- supercoiled DNA migrates faster than its linear form
- linear DNA migrates faster than its nicked circular form