# 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: 5' NNNNNNNNNN AATTCNNNNNNNN 3' 3' NNNNNNTTAA CNNNNNNNNNNNN 5'





### **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 Hind III	Hind III
Diluted Bgl I	Bgl I

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#### Each pair does 4 reaction tubes

Sequence for Restriction Enzyme Reactions										
Rxn Tube	Reaction Buffer (µl)	DNA (µl)	E Qualified Water (µl)	A Hind III (µl)	B Bgl I (µl)	Reaction Volume (µl)	37°C Incubation (minutes)	I0x Gel Load (μl)	Total Sample Volume (µl)	
Т	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





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



https://passel.unl.edu/pages/printinformationmodule.php?idinformationmodule=1065724861 http://tymkrs.tumblr.com/post/3069263517/gel-electrophoresis-negative-to-positive

# Types of DNA Molecules



supercoiled



circular

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

http://laneccgenetics.pbworks.com/w/page/58169624/Chromosomes%20Overview https://pixabay.com/en/photos/dna/ amanaimages.com

http://www.bioch.ox.ac.uk/aspsite/index.asp?pageid=1118