BME

MOLECULAR BIOLOGY EXPERIMENT

MINIPREP & ENDONUCLEASE RX

SKKU BME

3RD GRADE, 2ND SEMESTER

TODAY

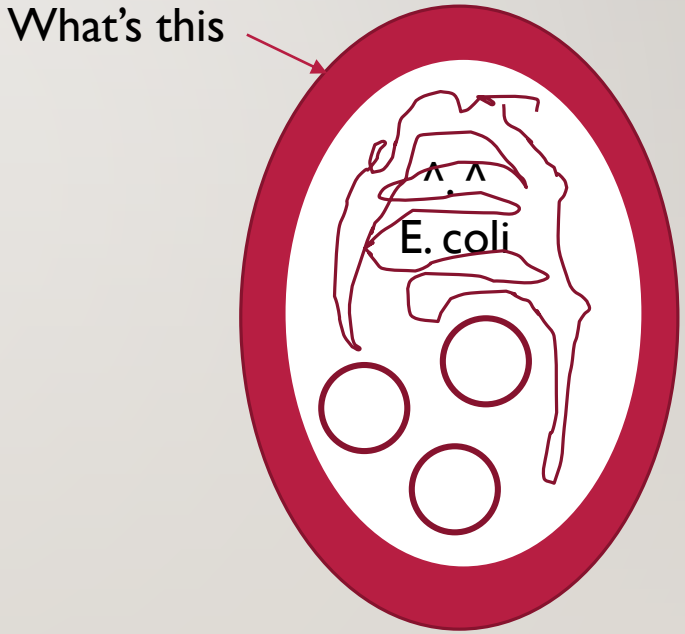
- Mini-preparation of plasmid DNA
- How to confirm whether the plasmid DNA is correct or not?

MINIPREP

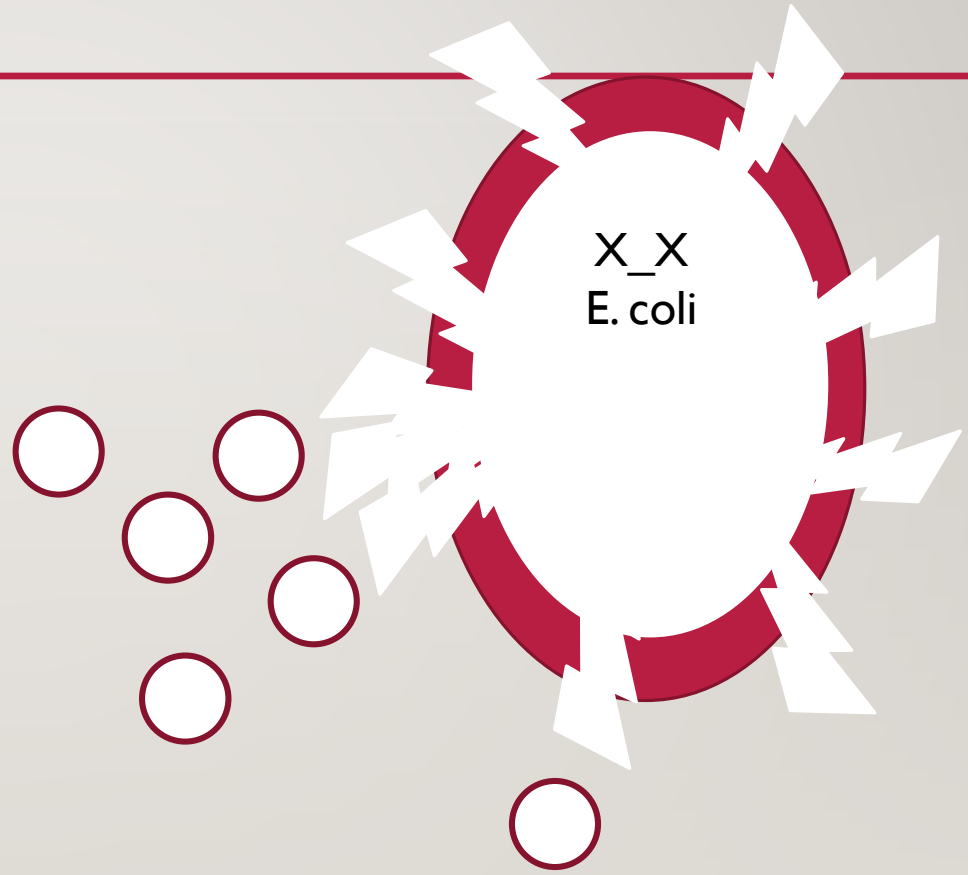
- Prep. the plasmid DNA from E. coli

- **Minipreparation** of plasmid DNA is a rapid, small-scale isolation of plasmid DNA from bacteria. It is based on the alkaline lysis method. The extracted plasmid DNA resulting from performing a **miniprep** is itself often called a "**miniprep**".

WHAT'S THE FIRST STEP?



WHAT'S THE FIRST STEP?



LYSIS

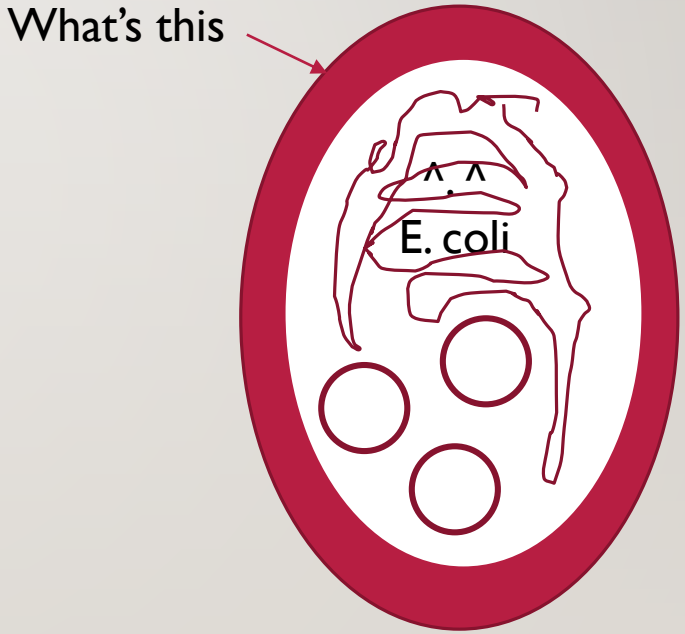
- Traditional method

Lysis buffer

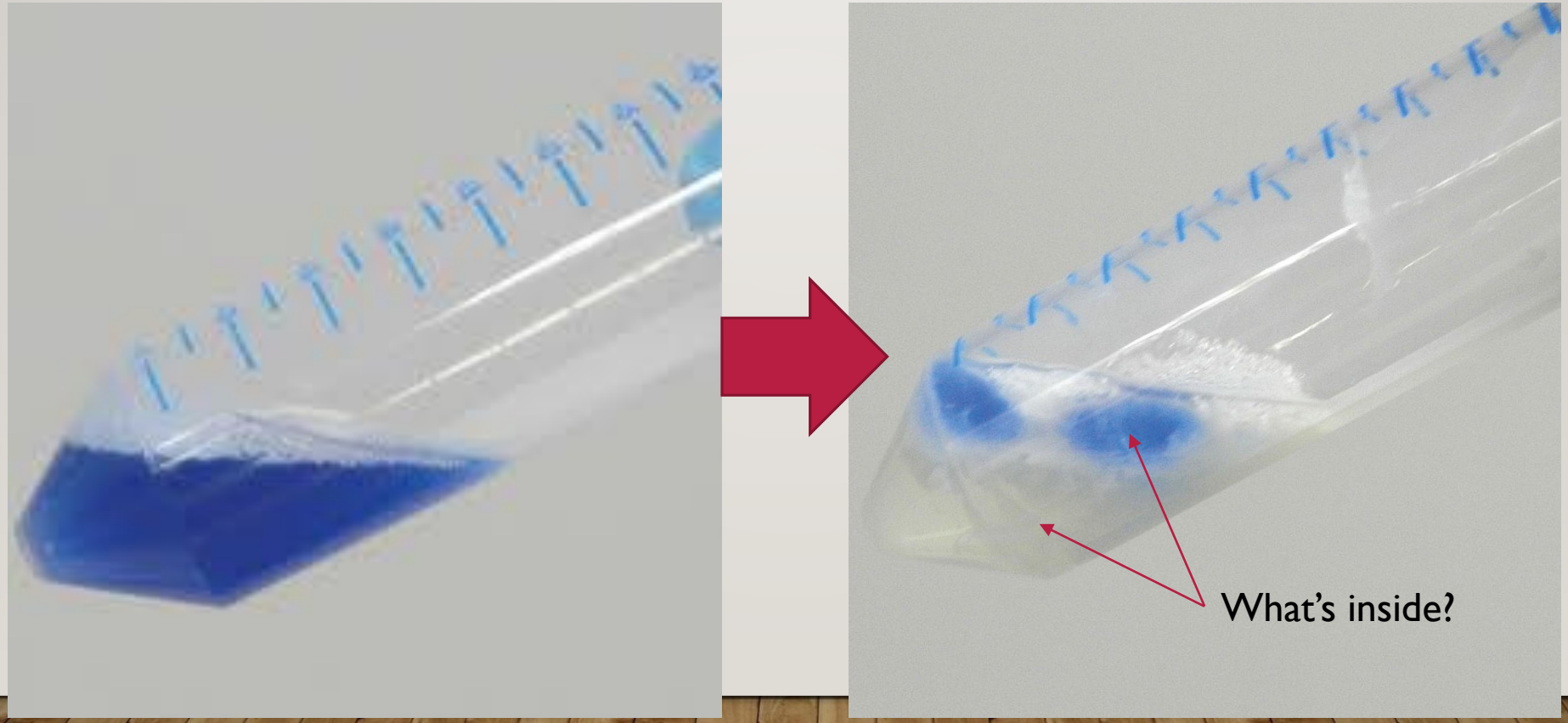
NaOH (0.2 N) and SDS (1%)

Neutralization buffer

3 M NaoAC pH to 4.8 with glacial acetic acid

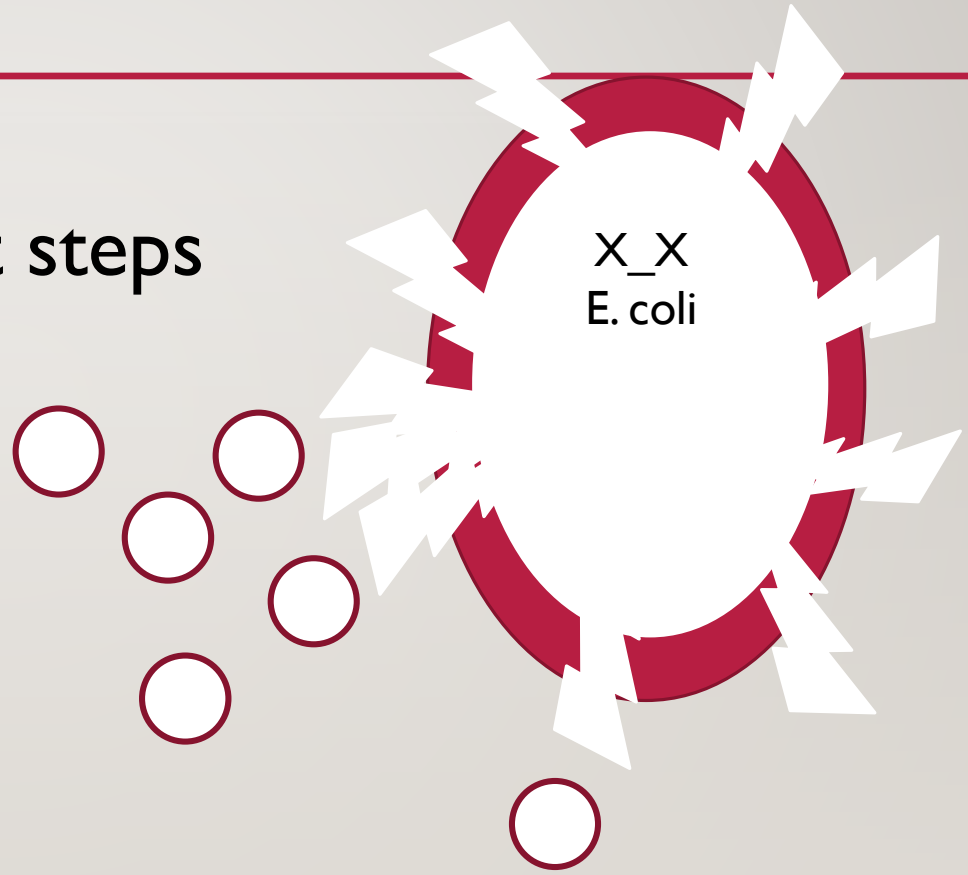


LYSIS!



HIGH PH

Neutralization for the next steps

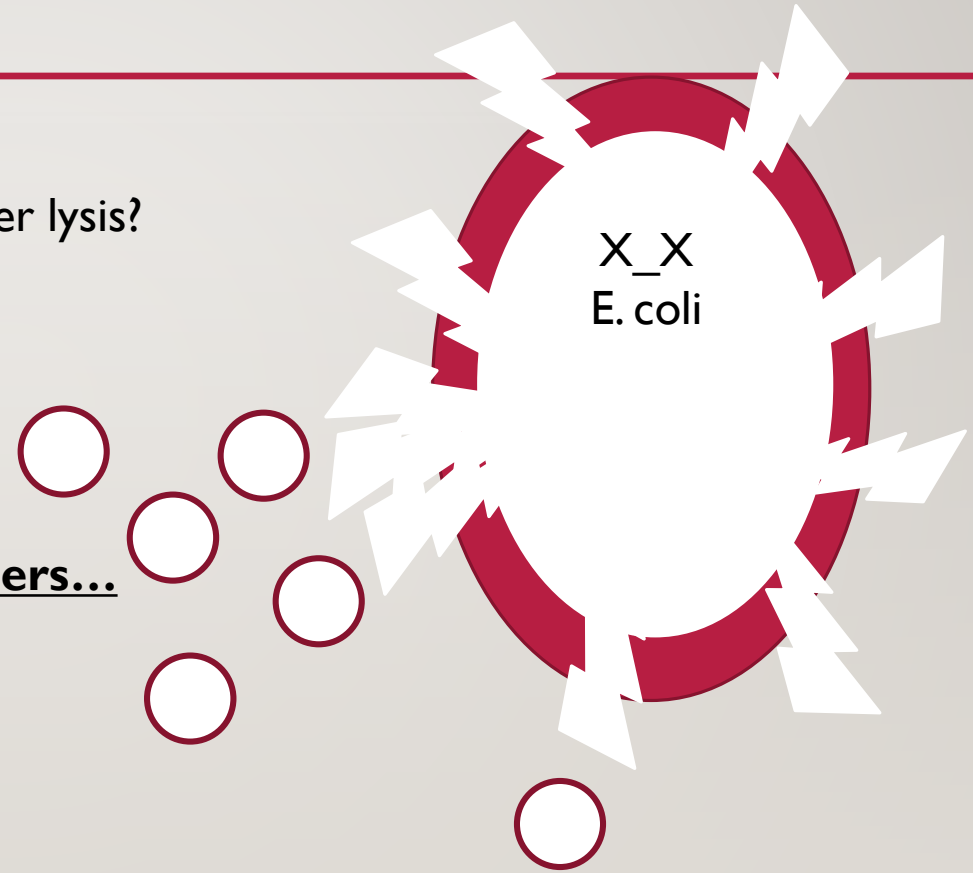


AFTER THE LYSIS…

What is the main question with this situation after lysis?

What is your purpose?

<u>Separate the plasmid DNA from others…</u>

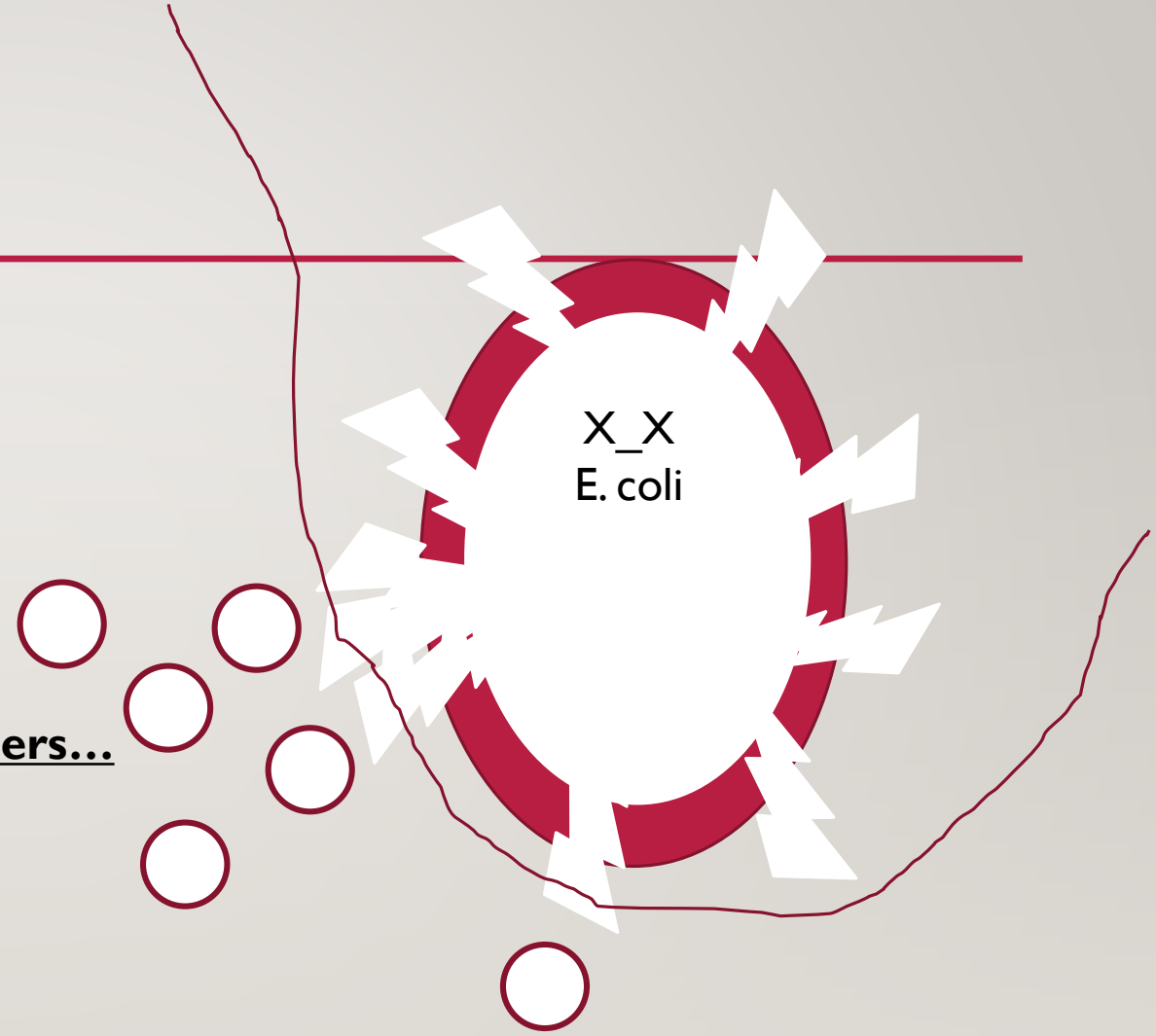


AFTER THE LYSIS…

How?

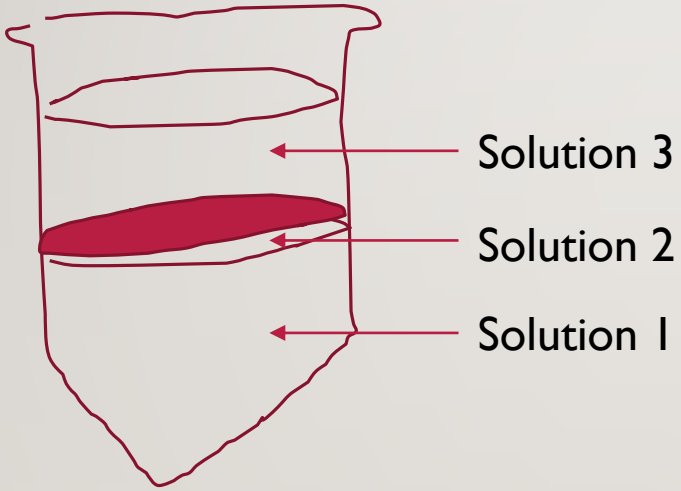
1. Phenol-chloroform extraction
2. Using column

Separate the plasmid DNA from others…



Idea> something capturing the plasmid DNA!!!

PHENOL-CHLOROFORM EXTRACTION METHOD



Homework!

COLUMN METHOD

DNA binding beads inside the column

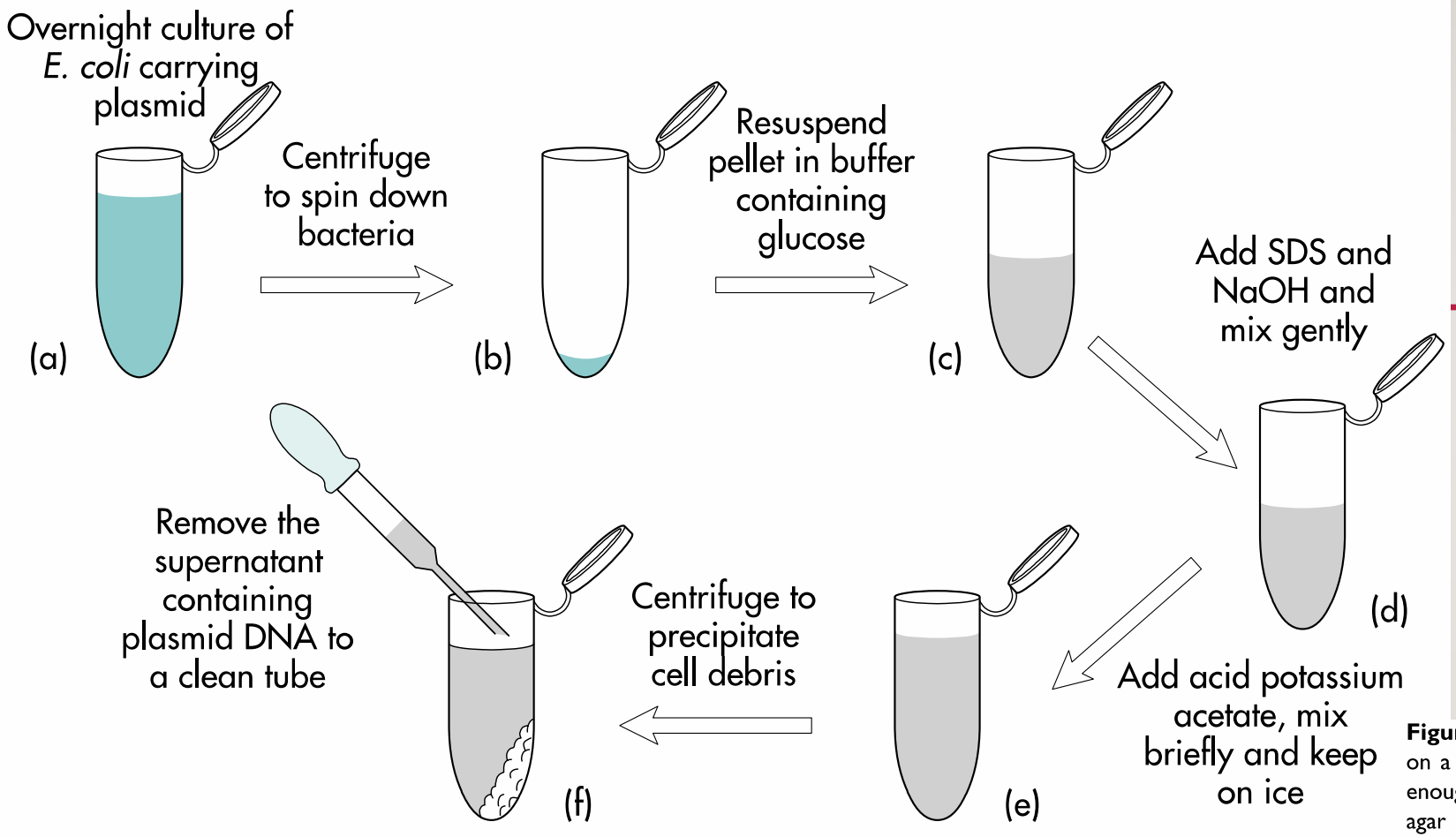


Figure. 3.7 Procedure for "miniprep" of plasmid DNA. The procedure is done on a small volume of cells (typically 1.5 mL of an overnight culture) and yields enough DNA for several restriction digests. a) A single colony is selected from an agar plate and is inoculated into growth medium, with appropriate antibiotics, and grown up overnight. A small sample (usually 1.5 mL) is used in the preparation. b) The bacteria are harvested from the culture by centrifugation, c) resuspended in buffer and d) then are lysed by addition of a solution containing sodium hydroxide and a detergent (sodium dodecyl sulfate or SDS). The DNA is denatured in these alkaline conditions, and the sample becomes very viscous as the bacteria lyse and the DNA is released. e) The addition of ice-cold potassium acetate causes cell debris, including pieces of bacterial membrane, to aggregate; f) these impurities are then removed by centrifugation to produce a "cleared lysate" containing mostly plasmid DNA, RNA, and protein. The bacterial chromosome, which is often attached to the membrane, is also removed in this step. Although plasmid DNA prepared in this way is heavily contaminated with RNA, mainly from ribosomes, the RNA does not interfere with many of the subsequent manipulations that you may wish to do, and indeed helps the DNA to precipitate.

COLUMN METHOD

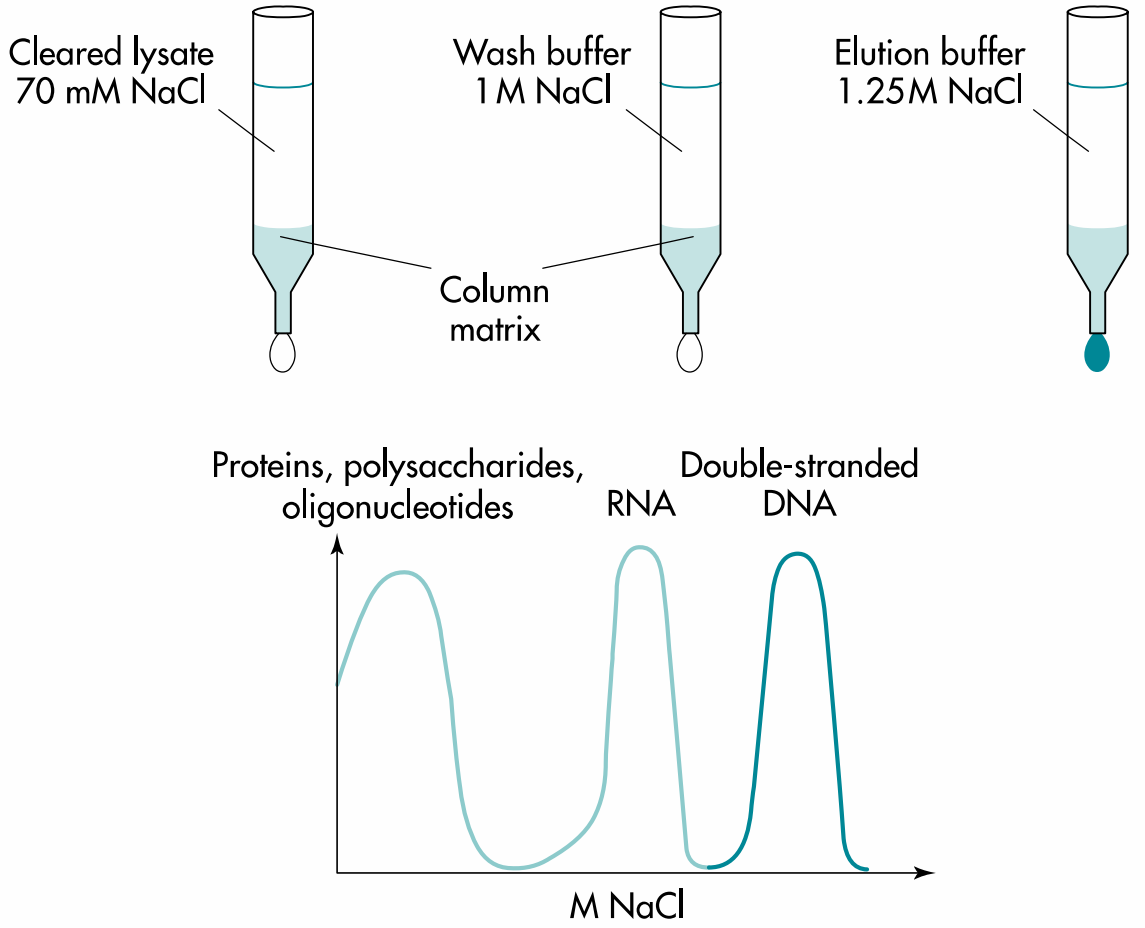


Figure 3.8 Purification of plasmid DNA on an ion exchange column. The cleared lysate is passed down an ion exchange column. Contaminating protein and carbohydrate do not bind to the column. The column is then washed with a solution containing 1M salt, which removes RNA. The double-stranded plasmid DNA is then eluted from the column with a buffer containing 1.25 M salt.

DNA CONCENTRATION

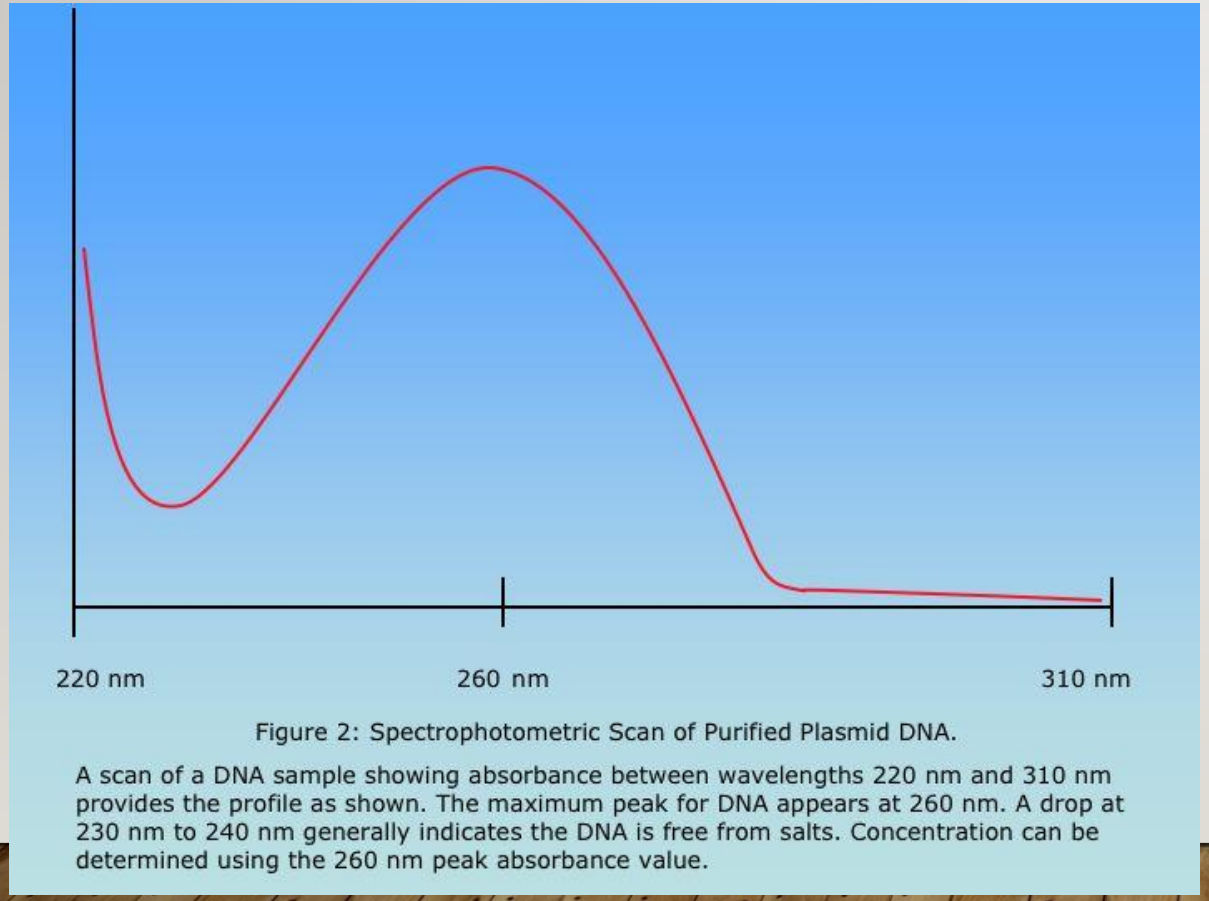
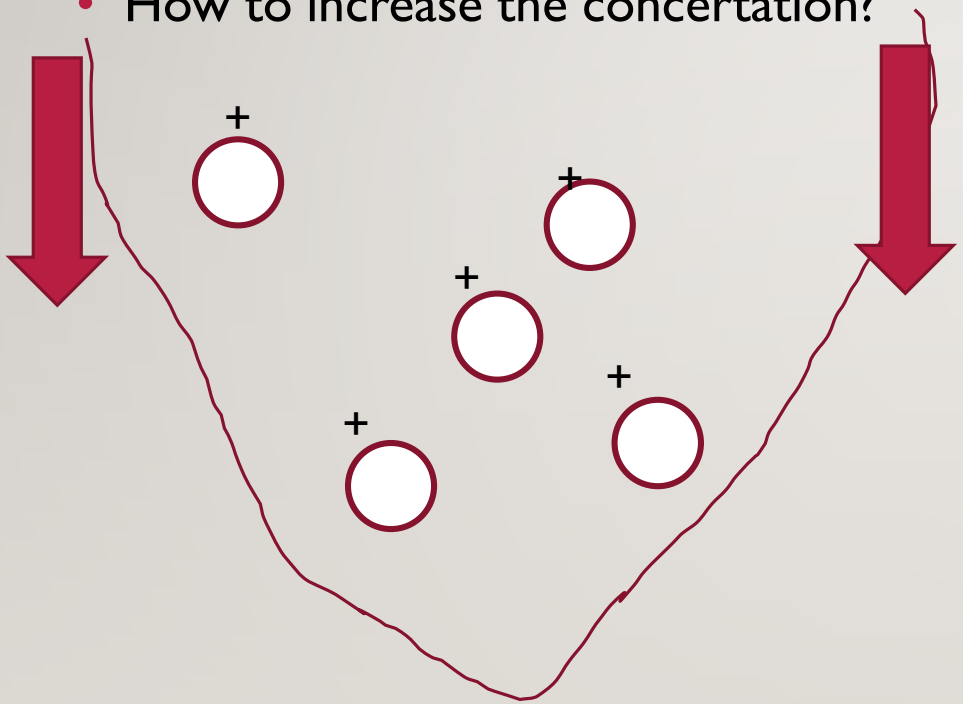


Figure 2: Spectrophotometric Scan of Purified Plasmid DNA.

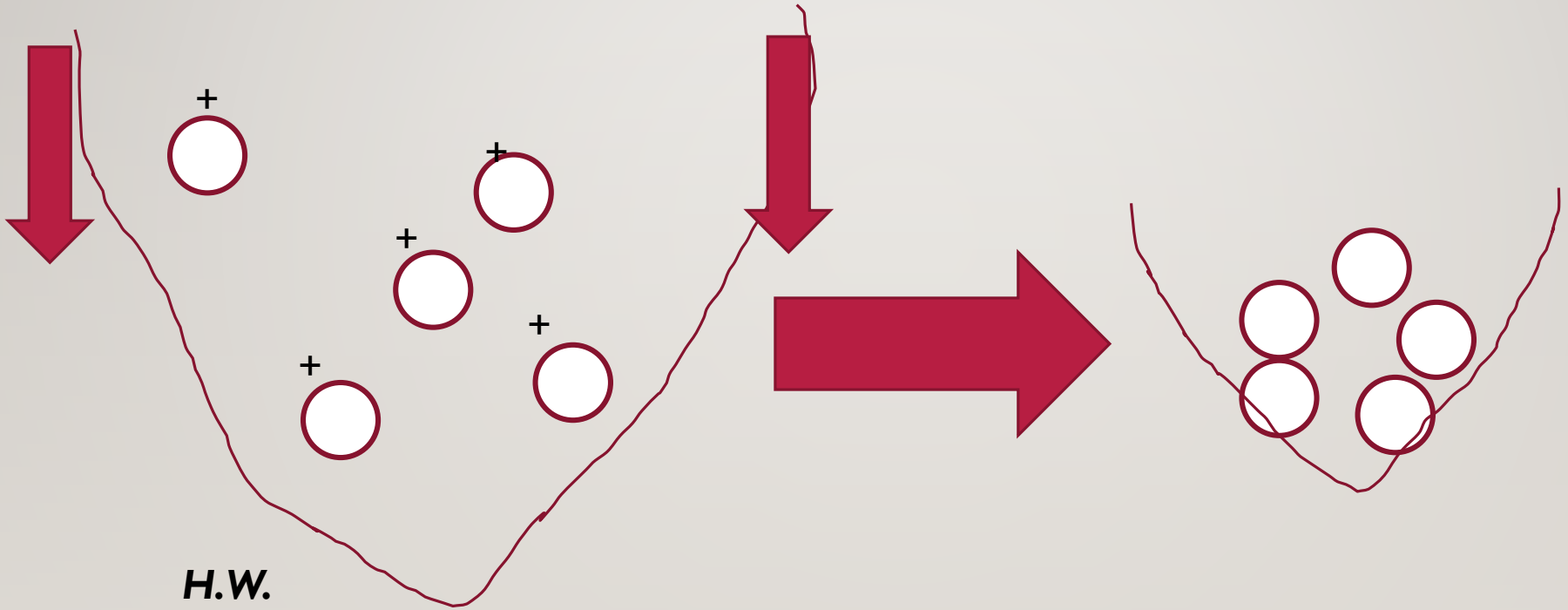
A scan of a DNA sample showing absorbance between wavelengths 220 nm and 310 nm provides the profile as shown. The maximum peak for DNA appears at 260 nm. A drop at 230 nm to 240 nm generally indicates the DNA is free from salts. Concentration can be determined using the 260 nm peak absorbance value.

ONE MORE PROBLEM: LOW LOW CONCENTRATION!!!

- How to increase the concertation?



ETHANOL PRECIPITATION



H.W.

Consider the charge!

Hydrophilic and hydrophobic properties of plasmid DNA and ethanol

MIDIPREP

- Then what?

Maxiprep

Midipreparation
The starting E. coli culture volume is 15-25 mL of lysogeny broth (LB) and the expected DNA yield is 100-350 µg.

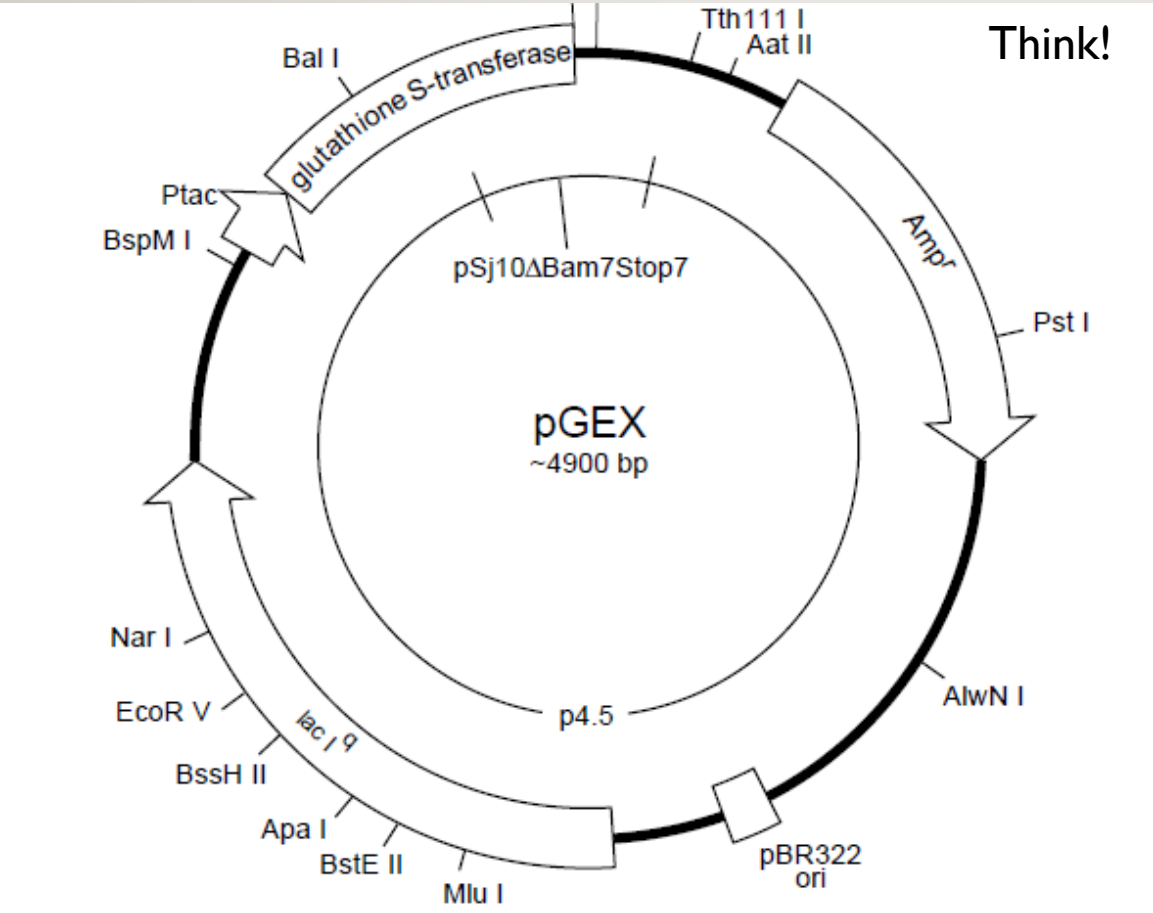
Maxipreparation
The starting E. coli culture volume is 100-200 mL of LB and the expected DNA yield is 500-850 µg.

Megapreparation
The starting E. coli culture volume is 500 mL – 2.5 L of LB and the expected DNA yield is 1.5-2.5 mg.

Gigapreparation
The starting E. coli culture volume is 2.5-5 L of LB and the expected DNA yield is 7.5–10 mg.

HOW TO CONFIRM WHETHER THIS PLASMID IS CORRECT ONE OR NOT?

HOW TO CONFIRM WHETHER THE PLASMID DNA IS CORRECT OR NOT?

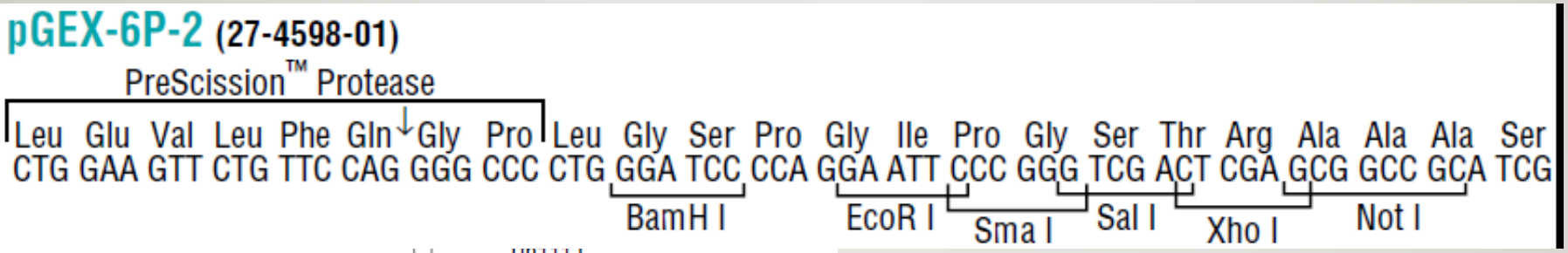


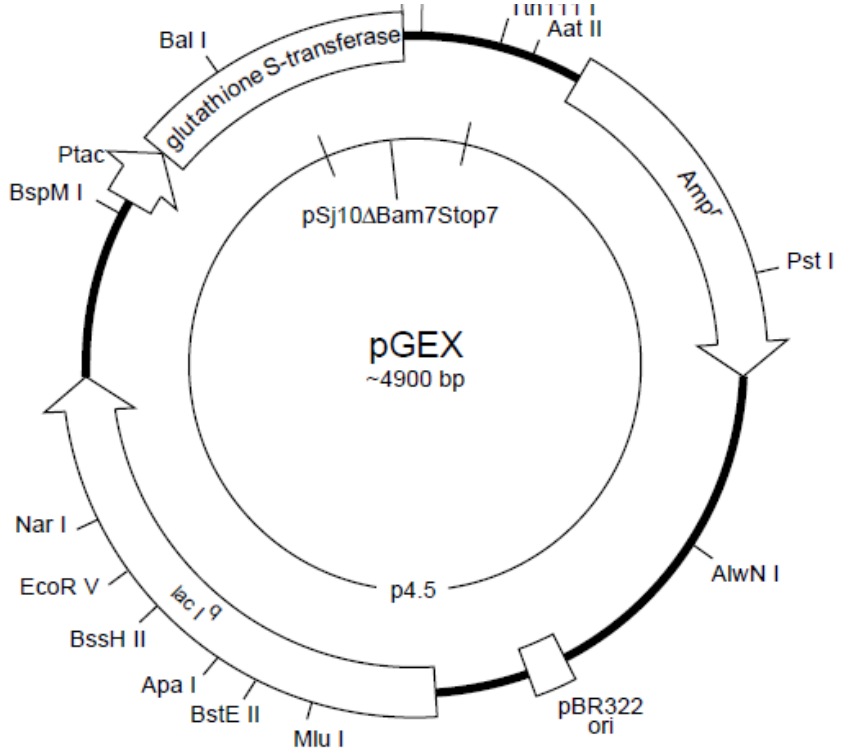
<hint> Plasmid DNA information is DNA sequence…

1. PCR

2. Restriction enzyme cutting & size

HOW TO CONFIRM WHETHER THE PLASMID DNA IS CORRECT OR NOT?





2. Restriction enzyme cutting & size

What's your plan?

Using two enzyme sites

a. BamHI
b. XhoI

Then, check the plasmid size…using DNA electrophoresis

WHAT DO YOU EXPECT TO SEE ON THE GEL? IMAGINE THE PLASMID DNA…

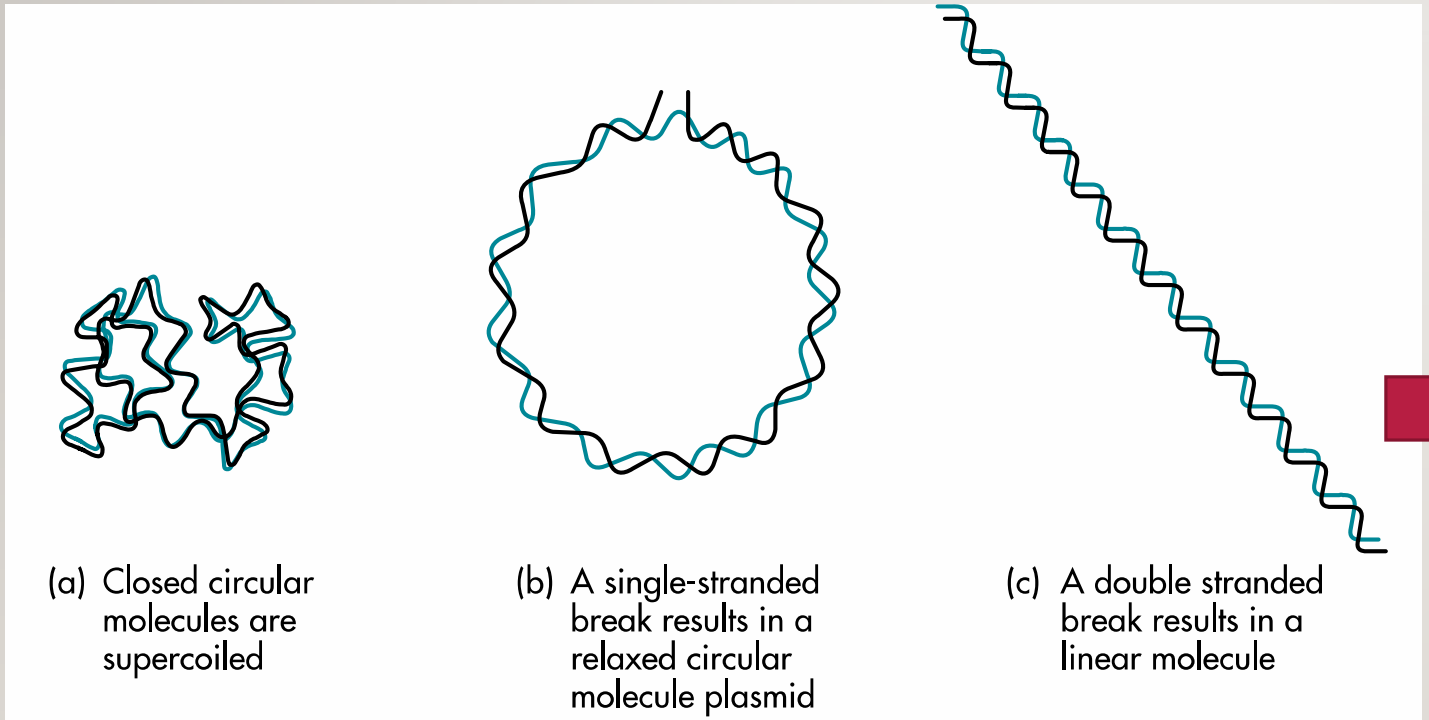
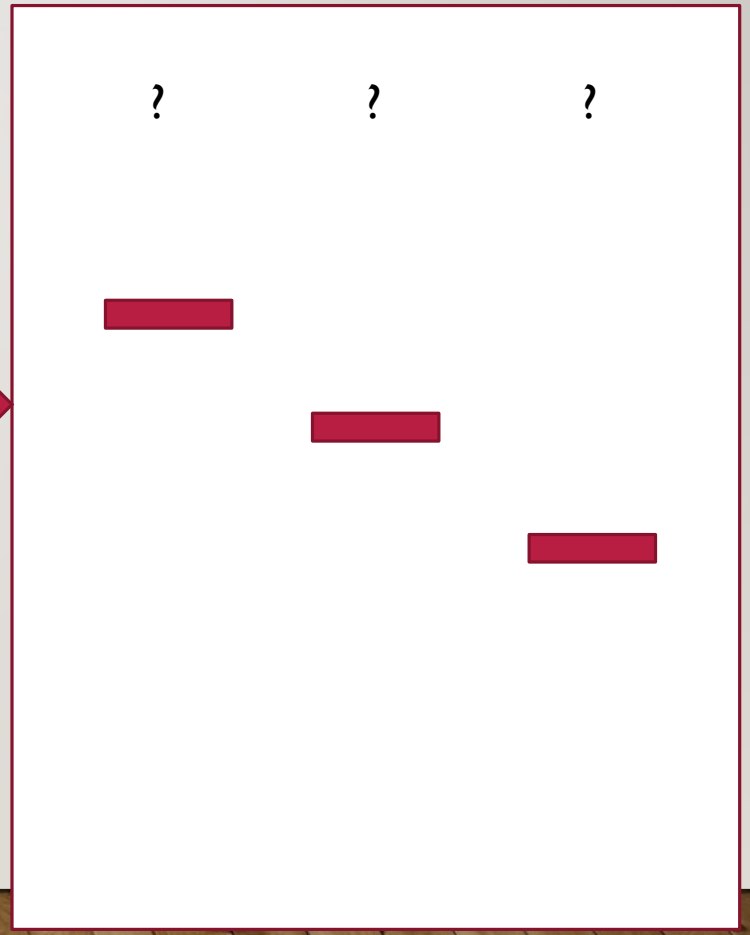


Figure 3.18 The three forms of plasmid DNA: a) closed circular supercoiled; b) relaxed or open circular; and c) linear.

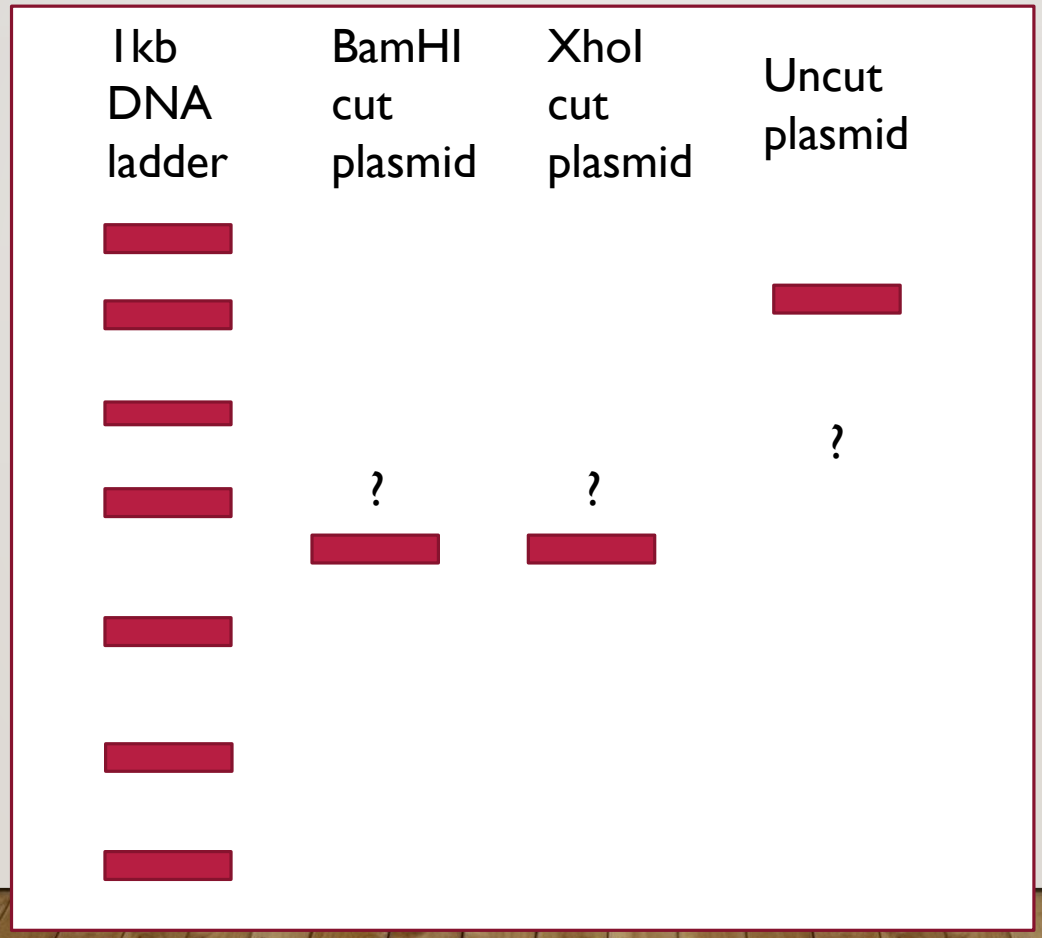


Write down in your result and discussion sessions.

WHAT WE WILL DO TODAY…

- Miniprep using column method
- Plasmid confirmation by enzyme cutting
- DNA electrophoresis

TODAY'S FINAL FIGURE



HOMEWORK

- Please explain the mechanism of ethanol precipitation for DNA
- What is the Phenol-Chloroform extraction? Please explain the mechanism.
- How to measure the protein and cell concentrations? Please find the proper wavelengths and mechanisms.

NEXT WEEK

- DNA cleaning
- Restriction enzyme cutting