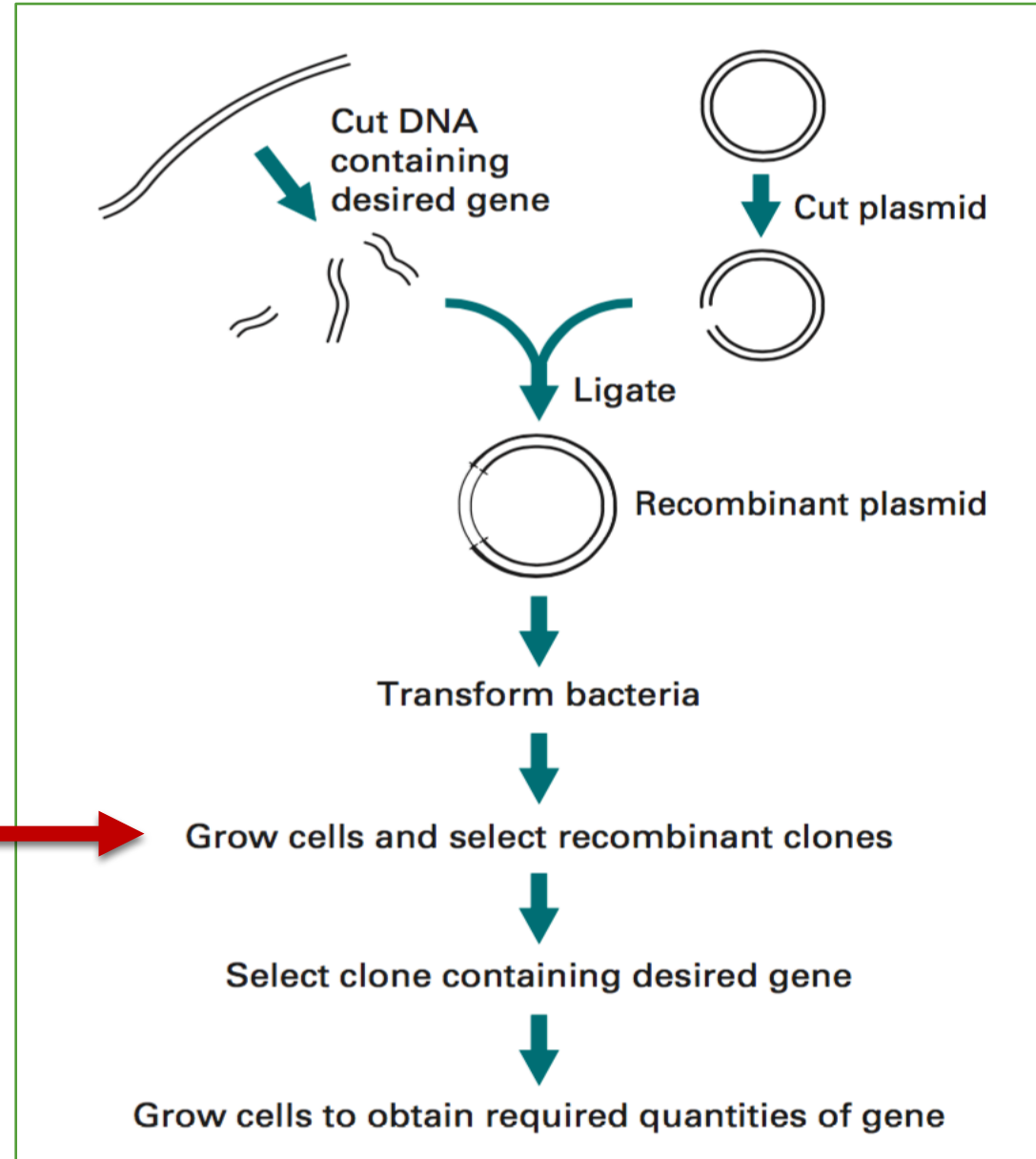
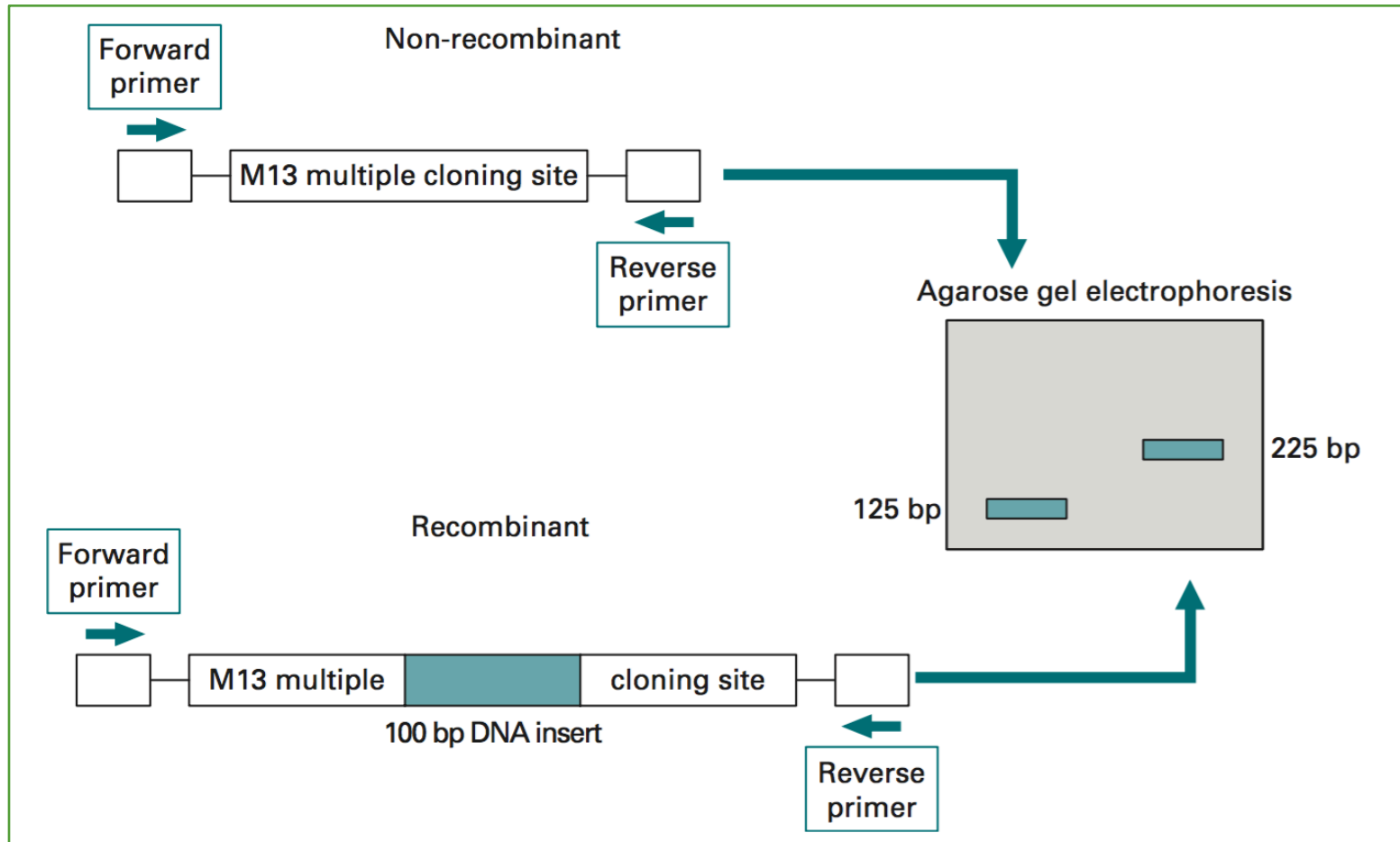


Outline of gene cloning:



Stopped here last lab

PCR screening of recombinant vectors:



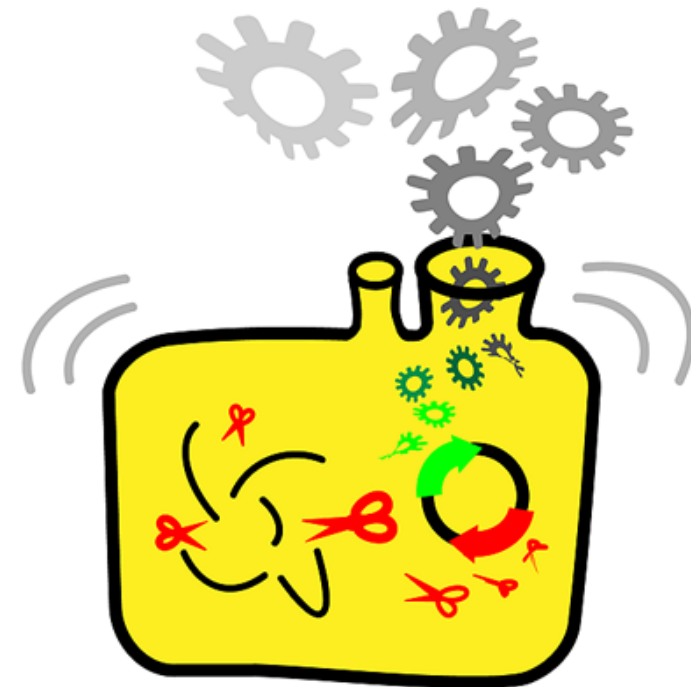
This will give an indication of the size of the cloned insert rather than the sequence of the insert; however, PCR primers that are specific for a foreign DNA insert may also be used. This allows a more rigorous characterization of clone DNA itself.



EXTRACTION AND DETERMINATION OF BACTERIAL PROTEINS

Bacterial proteins:

- Either part of the structure of the bacterium or produced by bacterium as a part of its cycle.
- Why it is important to extract bacterial proteins ?



Lab #1 [Plasmid Isolation]	Lab #2 [Transformation of Competent Cells]	Lab #3 [Estimation of Protein Concentration]
<p>1) Growth of the bacterial culture.</p> <p>2) Harvesting and lysis of the bacteria</p> <p>3) Purification of plasmid DNA</p>	<p>1) Growth of the bacterial culture.</p> <p>2) Harvesting.</p> <p>3) Using CaCl₂ solution and brief heat shock to transform the competent cells.</p>	<p>1) Growth of the bacterial culture.</p> <p>2) Harvesting and lysis of the bacteria.</p> <p>3) Estimation of protein concentration using Biuret method.</p>



PRACTICAL PART



Aims:

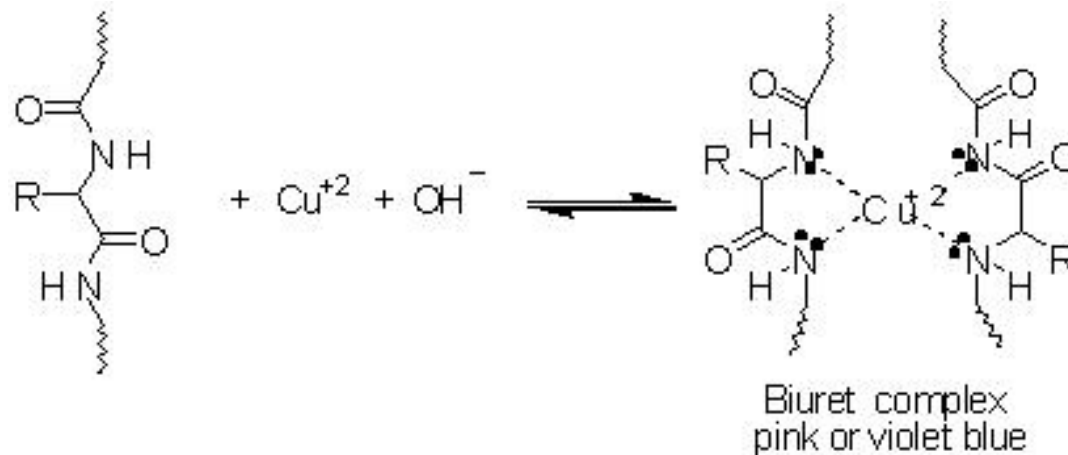
- Extraction of total bacterial proteins.
- Determination of bacterial proteins using biuret method.

Principle:

- **Isolation of bacterial proteins involves several steps :**
 1. Growth and induction of bacterial cultures.
 2. Lysis of cells in a suitable buffer containing a detergent which achieved by sonication (20 kHz) for 30–60
 3. DNase and RNase treatment for the removal of the nucleic acids.
 4. Determine the protein concentration using suitable method.
 5. Passage of the extract through an affinity resin and finally elution of proteins.

Principle cont':

- Biuret method, is used to determine the protein concentration, using standard curve of concentrations.
- Biuret method, is based on copper ions binding to peptide bonds of protein under alkaline condition to give a violet (purple) color.
- The intensity of the color resulting from the (Cu+protein) complex is linearly proportional to the concentration of protein present in the solution.

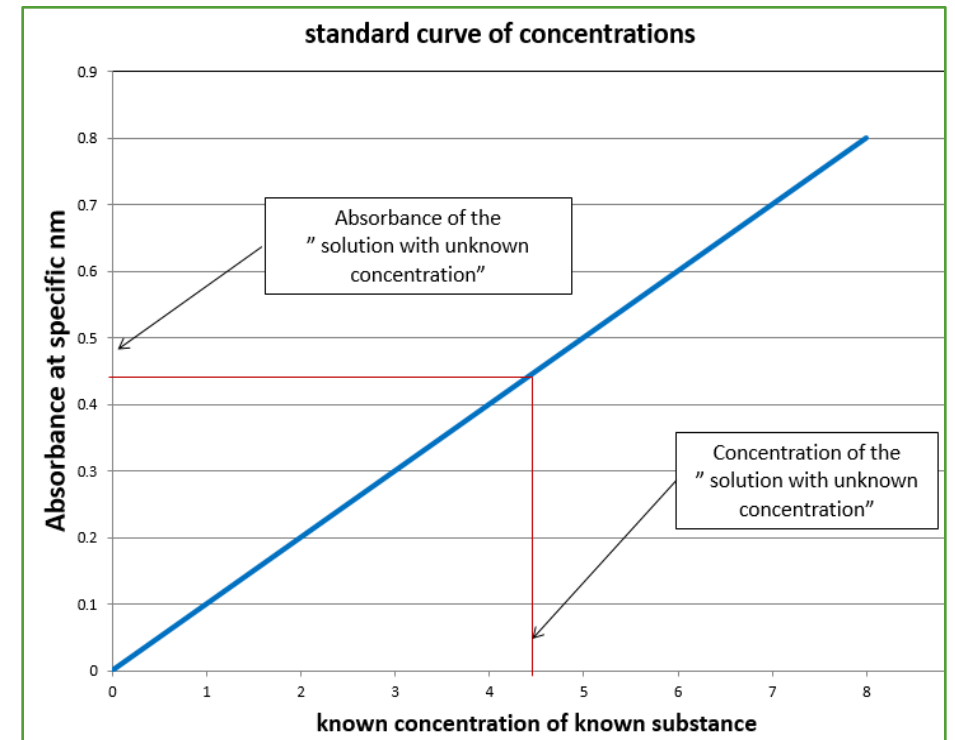


negative biuret
test results

positive biuret
test results

Results:

Test tube	Protein concentration [$\mu\text{g}/\mu\text{l}$]	Absorbance at 540 nm
Blank		
A		
B		
C		
D		
E		
F	_____	
G	_____	



Homework.

- ❖ Understanding bacterial protein could be involving in pharmacology, how ?
- ❖ In this lab we used sonication as a method to disrupt bacterial cells, mention two other common methods?