Synthetic Biology for

iGEM

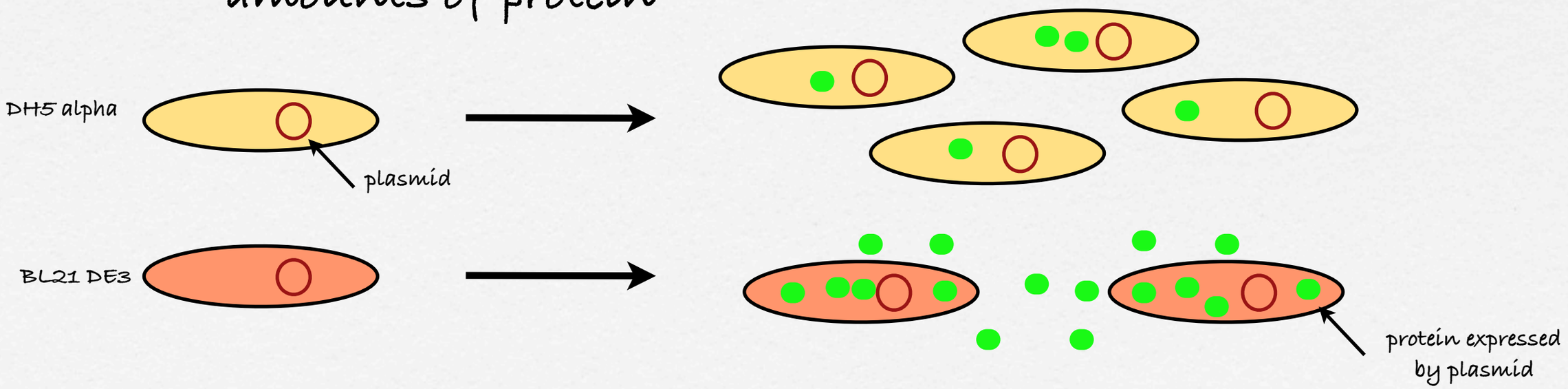
Transformations, Cloning

Transforming Bacteria

- Transforming is the process of introducing foreign DNA (plasmid) into host cells
- Competent bacterial cells are those that are able to take in foreign DNA
- E. coli is not naturally competent, and is made competent using calcium chloride and heat shock treatment

Transformations: Cloning vs. Expression

- Different types of E. Coli are good for different purposes
- DH5-alpha and similar strains are good for replicating DNA (they have low error rates)
- Strains such as BL21 DE3 are good for expression; they do not copy DNA well, but can be induced to express large amounts of protein



Experimental Methods: Transformation

- Incubate bacterial colony on ice in calcium chloride solution
- Heat shock at 42 C for 30 seconds

Heat bath incubating eppendorf tubes.

Experimental Methods: Incubation

- After heat shock, bacteria are transferred to a culture tube of LB (lysogeny broth) solution
- LB is a solution containing ingredients that promote bacterial growth (peptides, vitamins, minerals, and trace elements of nitrogen, sulfur, magnesium, etc.)
- Bacteria are cultured in broth for about an hour to increase colony size

Bacterial culture suspended in LB solution

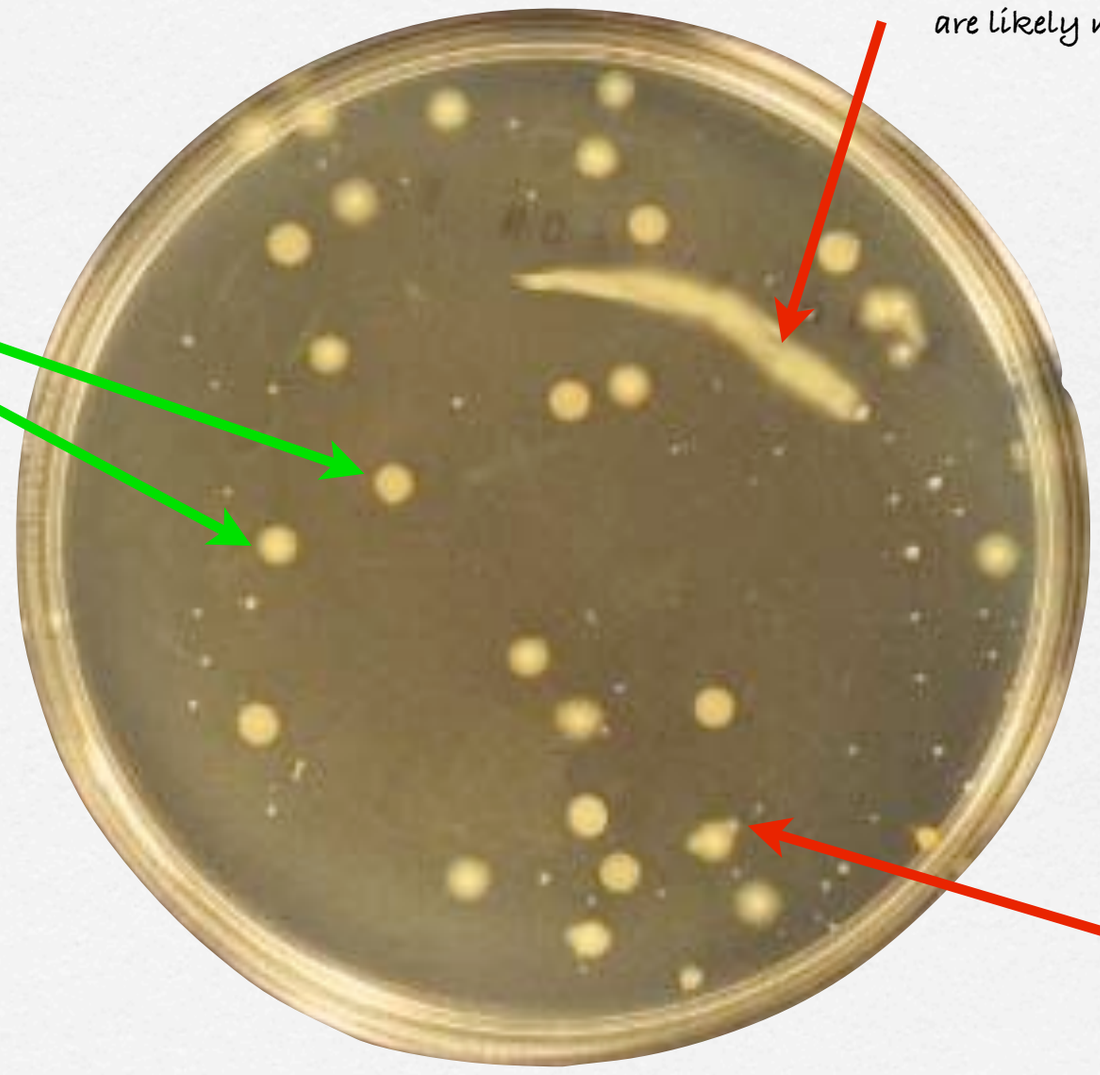
Experimental Methods: Selection

- After incubating the colonies, we spread them on an agar plate.
- Like LB, agar plates have all the necessary ingredients for bacteria to grow
- Plates also have an antibiotic, which the plasmid we introduced should have
- We incubate bacteria on selection plates for 12-16 hours

Selection: Choosing a Colony

These colonies are in a streak. They are likely not uniform in DNA.

Good colonies. These colonies is isolated and healthy.



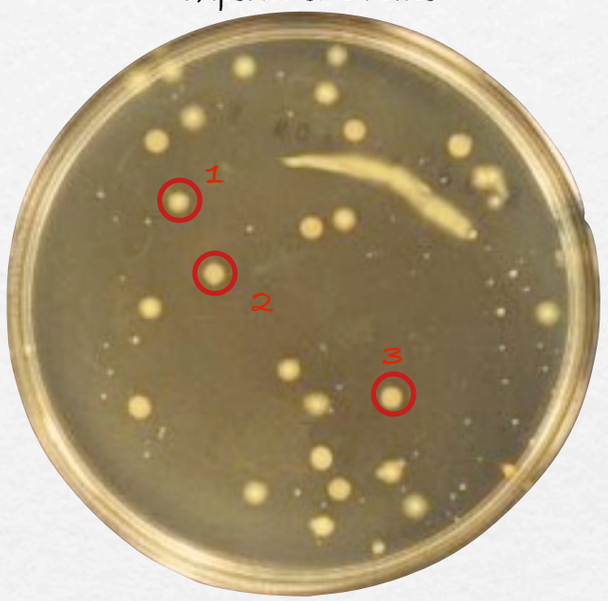
The small colony next to the larger colony is known as a "satellite colony". Satellite colonies are not good to choose from, because that usually means that the antibiotic has run out near the colony, and the satellite has thrived anyway.

Selection: Choosing a Colony

- Ultimately, we want a colony whose cells have the plasmid, and no colonies that support cells without the plasmid (choose isolated colonies)

- The number of colonies for the next round is determined by the health of the control plate

- The control plate does not have cells with the plasmid, and theoretically should be blank



Experiment Plate

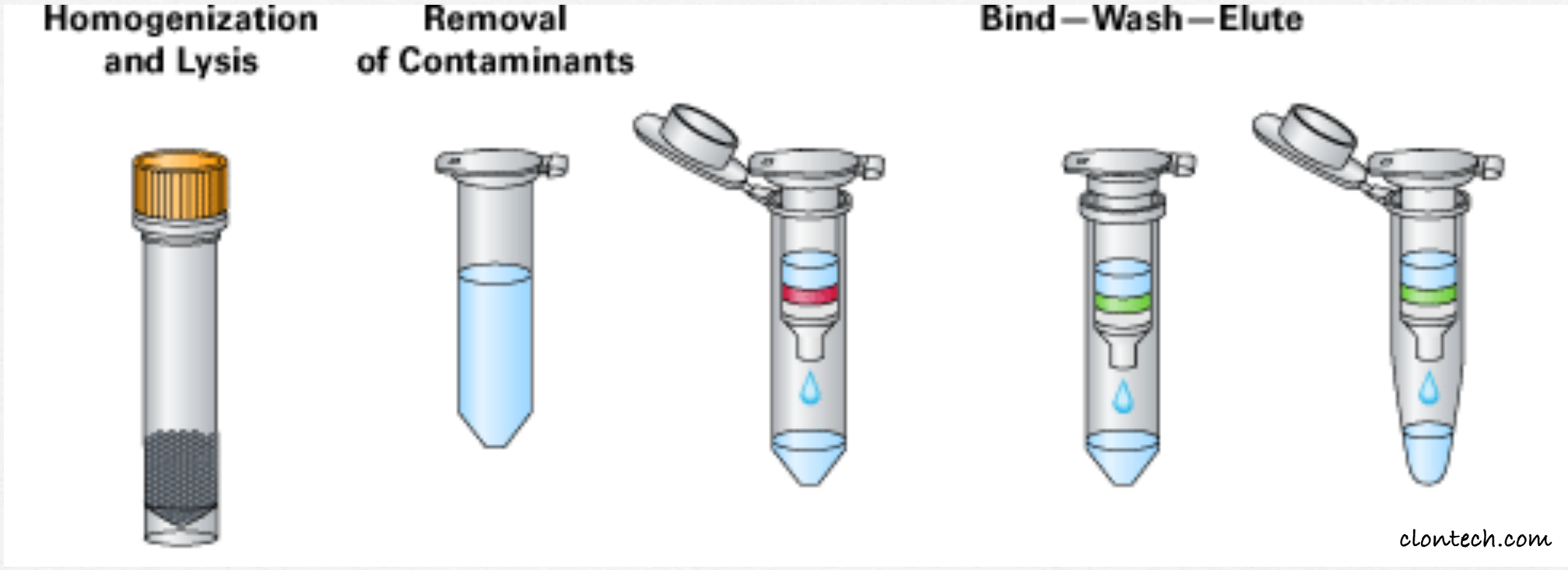
Circle and number your colonies. This will make it easier to know which colonies you have selected and will help you if you need to look at the plate again.

Control Plate

Experimental Methods: Isolate Cloned DNA

- For each colony, prepare in 5 mL of LB/antibiotic; incubate 12 hours at 37 C
- Isolate Plasmid Kit using mini-prep
- Measure the 280/260 absorbance in nanodrop to get the concentration of plamid DNA
- Sequence using any number of sequencing methods

DNA Purification Minipreps



Breaks open cells

Separates out heavier contaminants, easier for plasmid DNA to bind column. Centrifuge.

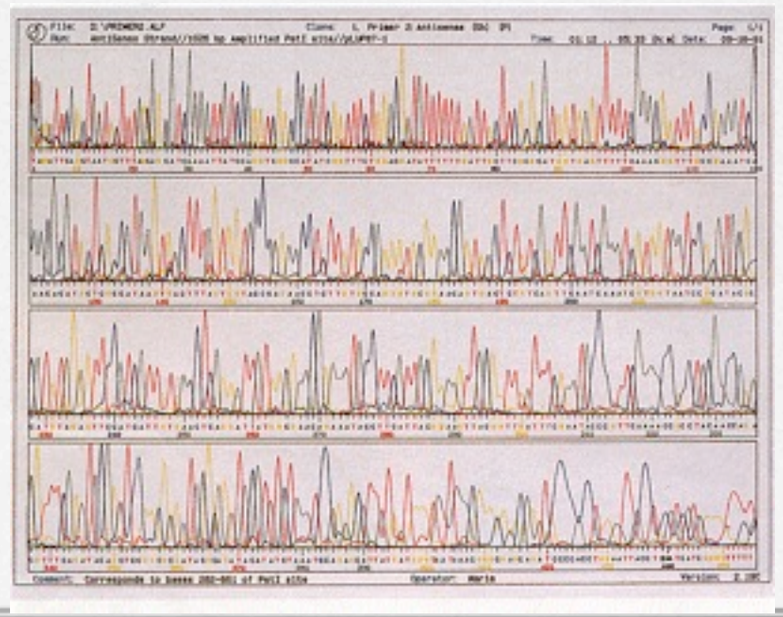
Bind the plasmid DNA to the column

Use wash step to remove contaminants that are bound nonspecifically

Elute out plasmid DNA using special buffer to break bonds between DNA and column

DNA Sequencing

- Any number of places will do DNA sequencing for you (takes a day or two);
- Average cost $3-20 depending on accuracy of sequencing method
- Align the sequences using BLAST or similar program



Example of DNA sequencing results

Using a DNA Sequencing Result

- Compare the DNA sequencing results of each colony to the sequence from your plasmid design (it should be on file)
- The colony who has the least mutations in the target gene should be used

Sequence on file	ACGTAACCTTGGGATCTCGGAATTAATTGATGCCATAATGGTTTTCAATCATG
Colony 1	ACGTAACCTTGGGAGCTCGGAATTAATTGATGGCATAATGGATTTCAATCATG
Colony 2	ACGTAACCATGGGACCTCGGAATGAATTGATGCCATATTGGTTTTCAATCATG
Colony 3	ACGTAACCTTGGGATCTCGGAATTAATTGATGCCATAATGGTTTTCAATCATG
Colony 4	ACGTACCCTTGAGATCTGGGAATTAATGGATGCCATAATGATTTTTAATCATG

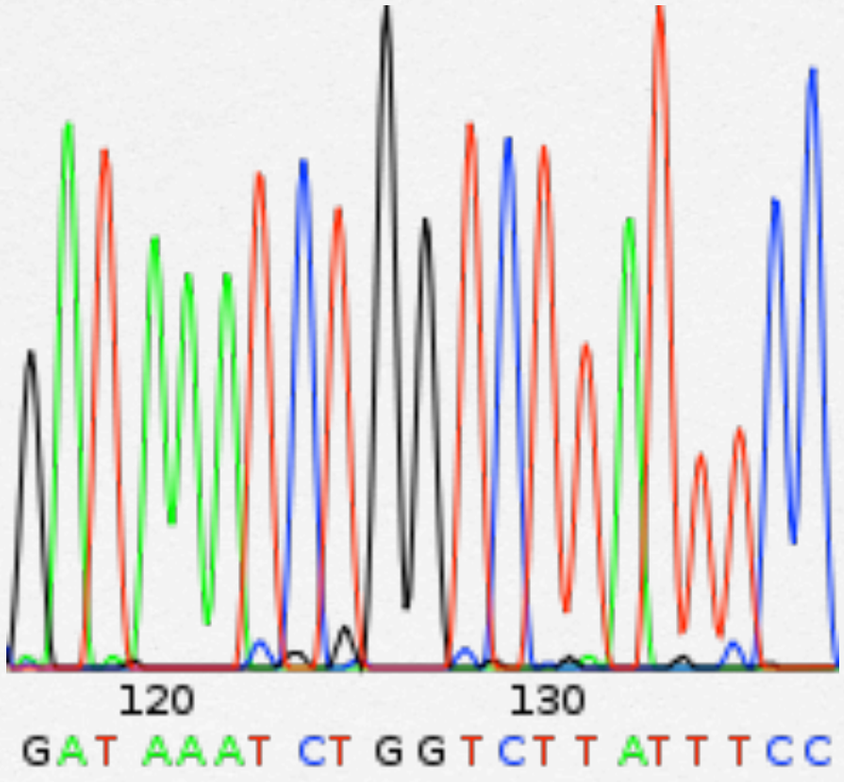
Since colony 3 has the fewest errors, we should use the DNA from that colony for future steps

Sometimes there are only one or two mutations in your target sequence. If this is the case, you can check the raw output to determine whether the error was a result of sequencing or actually a mutation. See next page for how to read raw sequencing data.

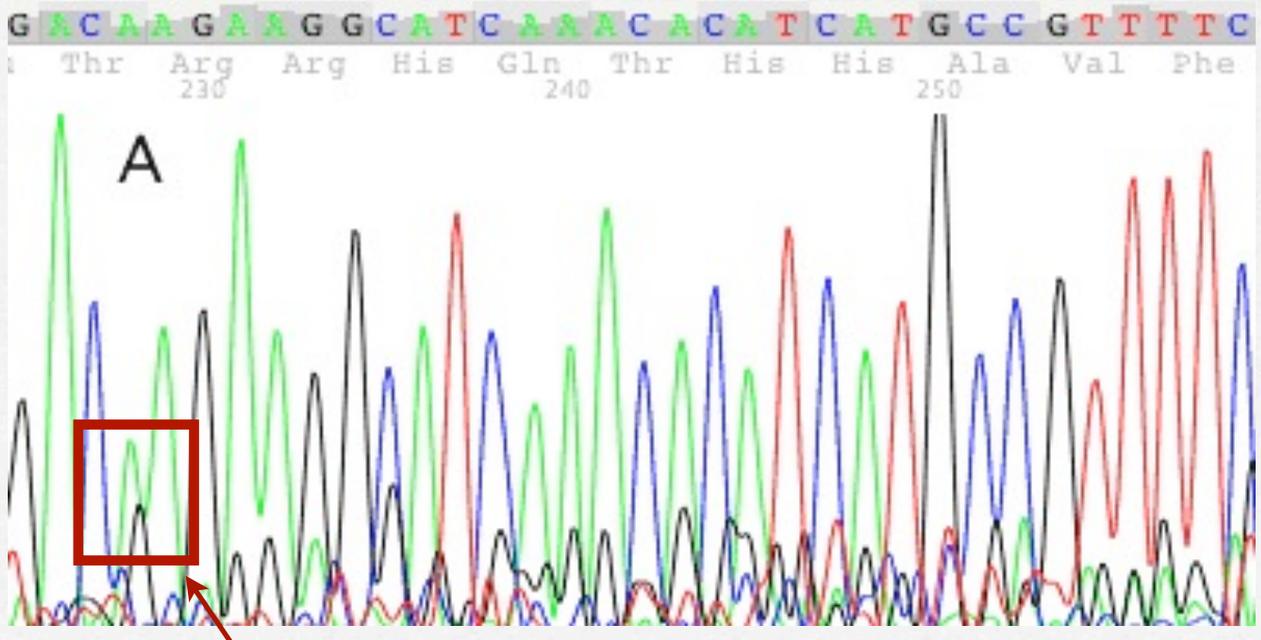
Reading DNA Sequencing Results

- DNA sequencing results are often presented using graphs where each of the four colors represents a base

Ideal DNA Sequencing Results



Noisy DNA Sequencing Results (more realistic)



Example of DNA sequencing noise. This base could have been an A or a G, but was read as A because it had a higher peak

Results of Transformation/Cloning

- We now have isolated plasmid in large quantities
- DNA sequencing has revealed that we have the correct sequence for our target gene (no mutations)
- The next step is to transform bacteria again, this time for expression

Important Terminology

Transformation: The process of introducing exogenous DNA into cells.
Exogenous: Apart from the genome. (Exogenous DNA is DNA not found in the cell's normal genome.
Cloning: Reproduction of DNA by mitosis and cell reproduction.
Expression: Production of proteins coded by plasmid DNA.
Competency: The ability of cells to accept foreign DNA. Some cells are naturally competent, and some must be artificially induced to be competent.
Lysogeny Broth (LB): A special solution that contains nutrients specifically designed to help bacteria grow.