Exp. 7. Plasmid Ligation and Transformation

What we will do today

Exp.5. Polymerase Chain Reaction(PCR)

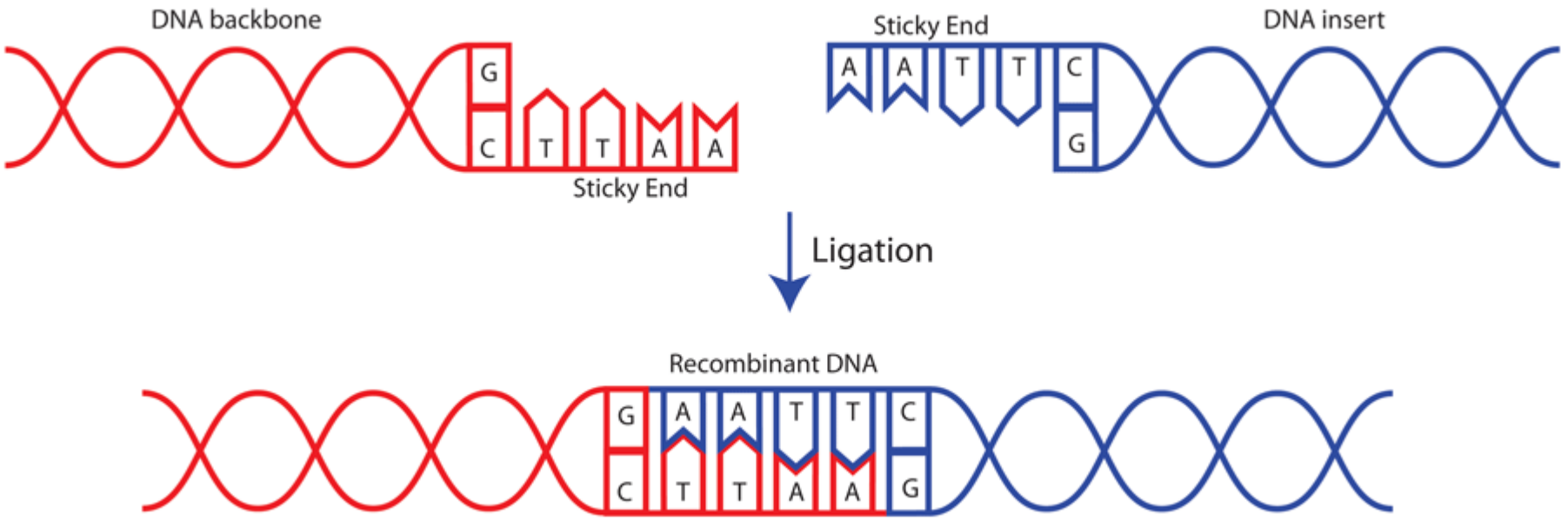
Exp.6. DNA Purification

Exp.7. Plasmid Ligation and Transformation

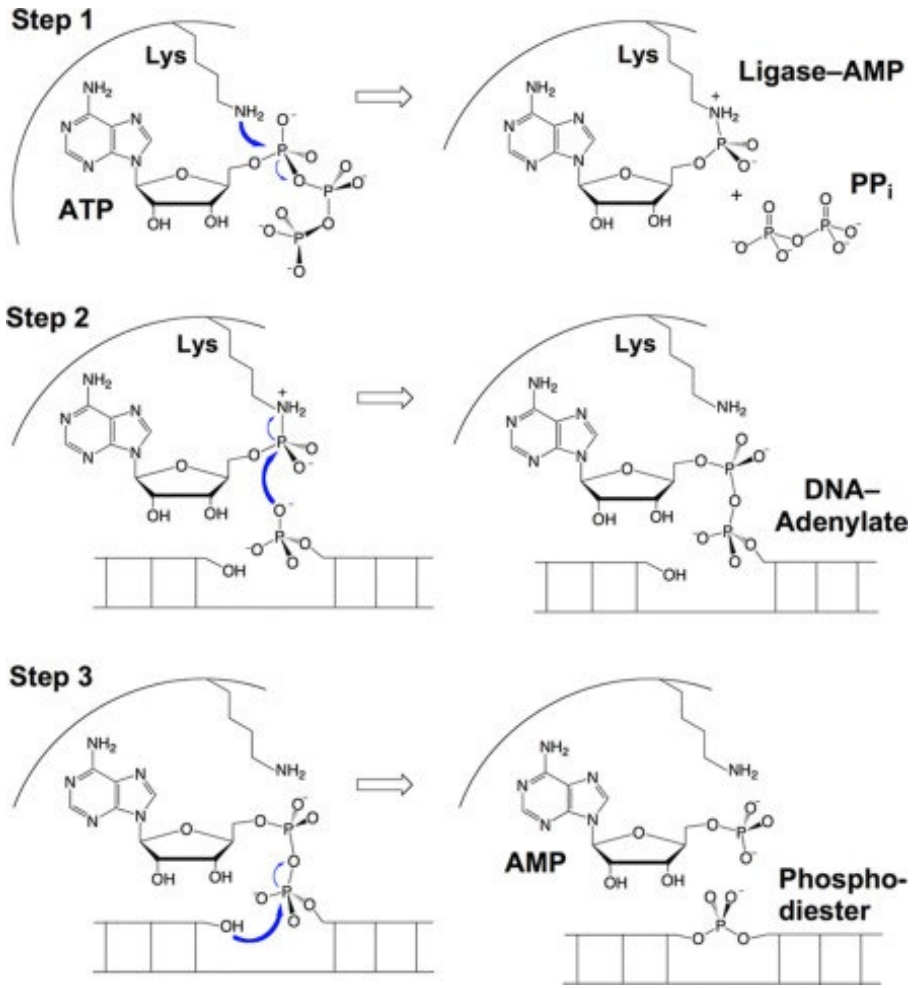
Exp.8. Protein Expression

Exp.9. Protein Purification and SDS-PAGE

DNA Ligation



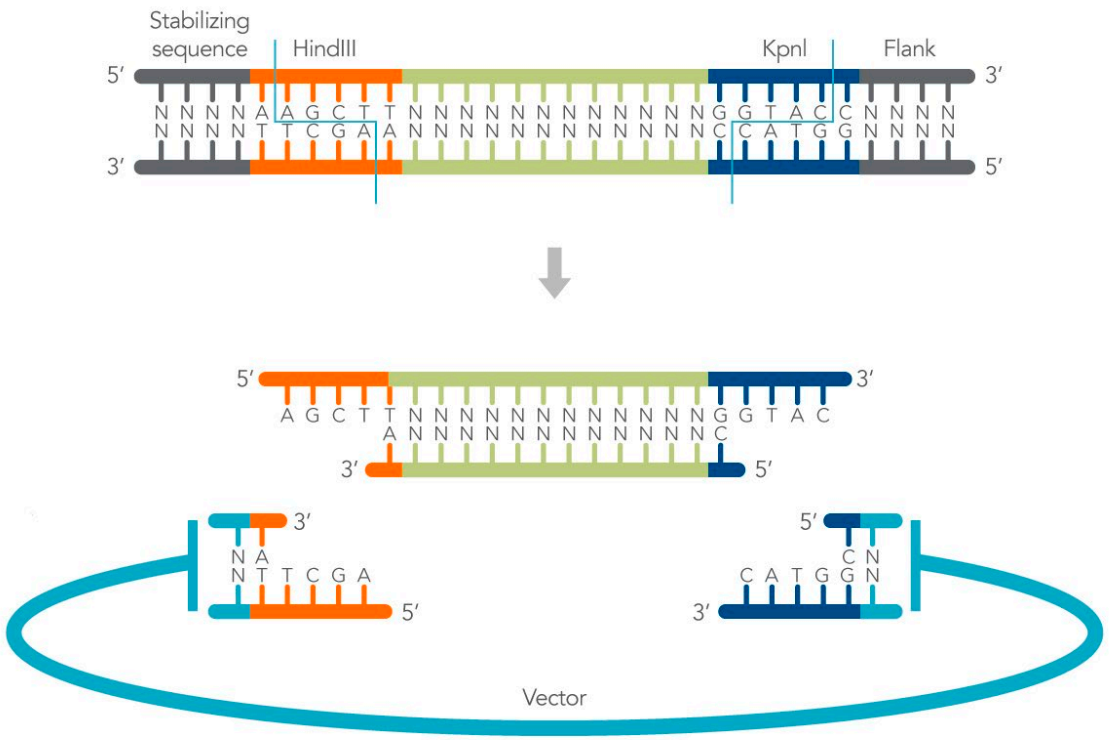
DNA Ligase

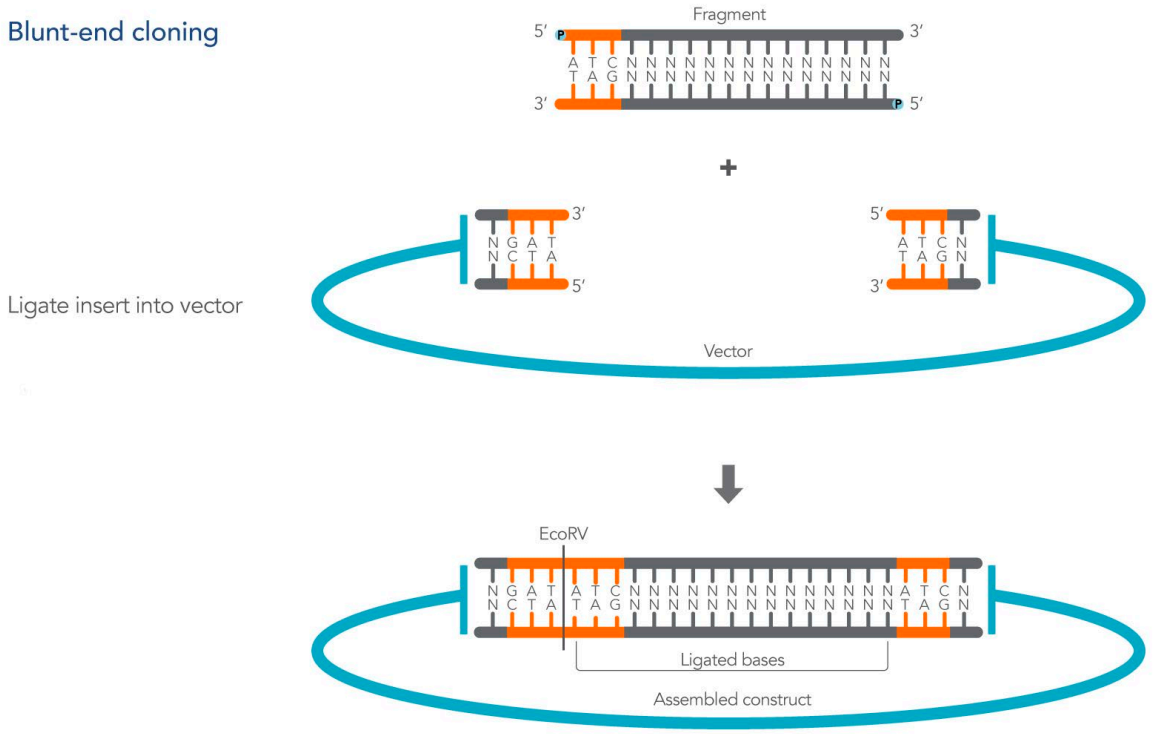


- ✓ Ligation is catalyzed by DNA Ligase.
- ✓ T4 DNA ligase will be used.
- ✓ Ligation requires energy (why?)

(Shuman, *JBC,* 2009)

Sticky End vs Blunt End





Ligation Reaction

Standard reaction conditions

When a restriction fragment as insert DNA is ligated to a vector DNA.

10X T4 DNA Ligase buffer		2 µl
T4 DNA Ligase (400 units/µl)	For sticky ends	1 µl
	For blunt ends	2 µl
Vector DNA (50~400 ng/µl)		1 µl
Insert DNA (3X molar excess of vector DNA)		X µl
Distilled water		up to 20 µl
→ Incubate at room temperature for 1hr.		

$$\frac{\text{ng of vector} \times \text{kb size of insert}}{\text{kb size of vector}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}} = \text{ng of insert}$$

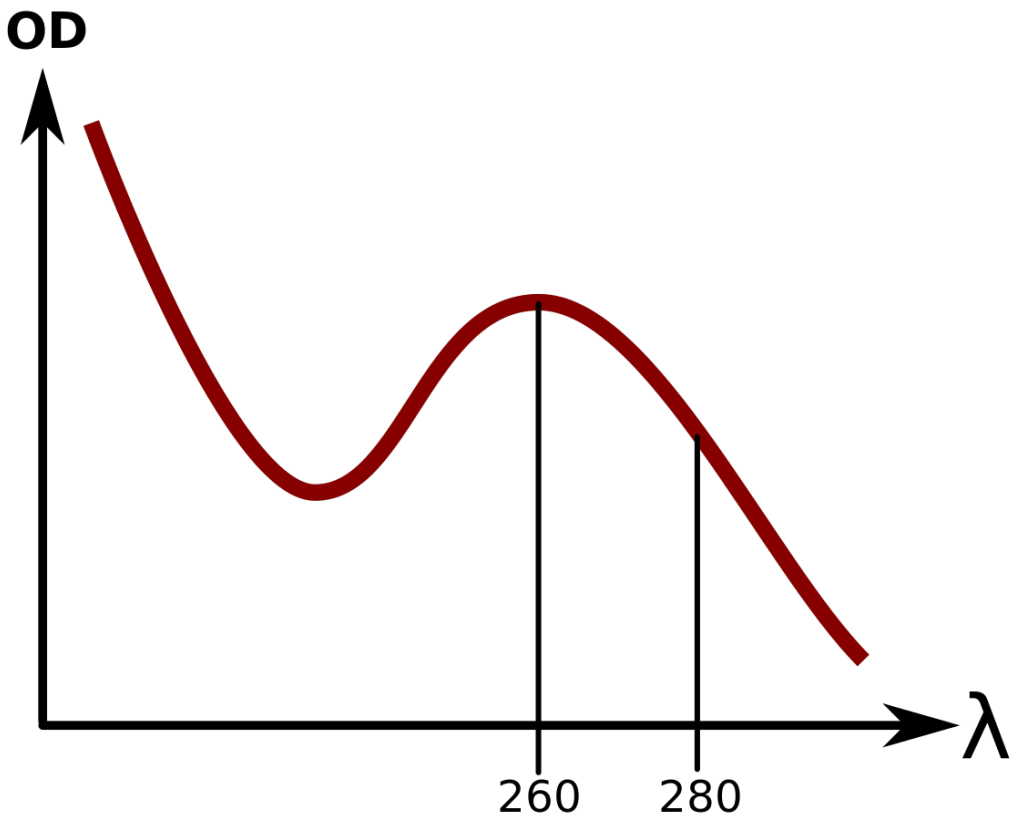
Example:

How much 0.5kb insert DNA should be added to a ligation in which 100ng of 3kb vector will be used? The desired vector:insert ratio will be 1:3.

$$\frac{100\text{ng vector} \times 0.5\text{kb insert}}{3\text{kb vector}} \times \frac{3}{1} = 50\text{ng insert}$$

- ✓ Need to measure DNA concentration

DNA Quantification

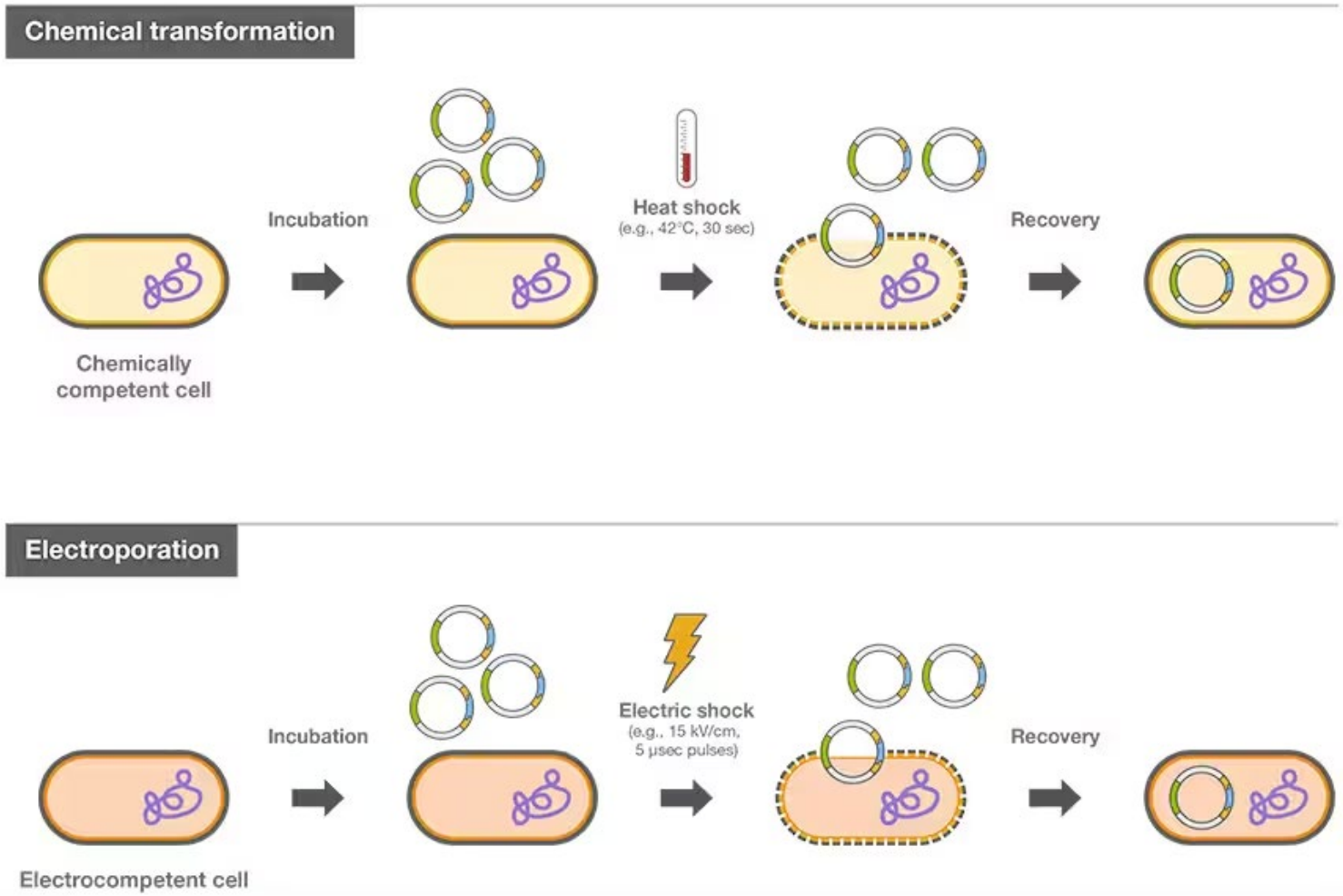


- ✓ DNA absorbs UV light due to the presence of *base*
- ✓ DNA concentration is determined by measuring UV absorbance (optical density) at 260nm
- ✓ Aromatic residues of protein (tryptophan, phenylalanine, etc.) also absorb UV 280nm maxima.

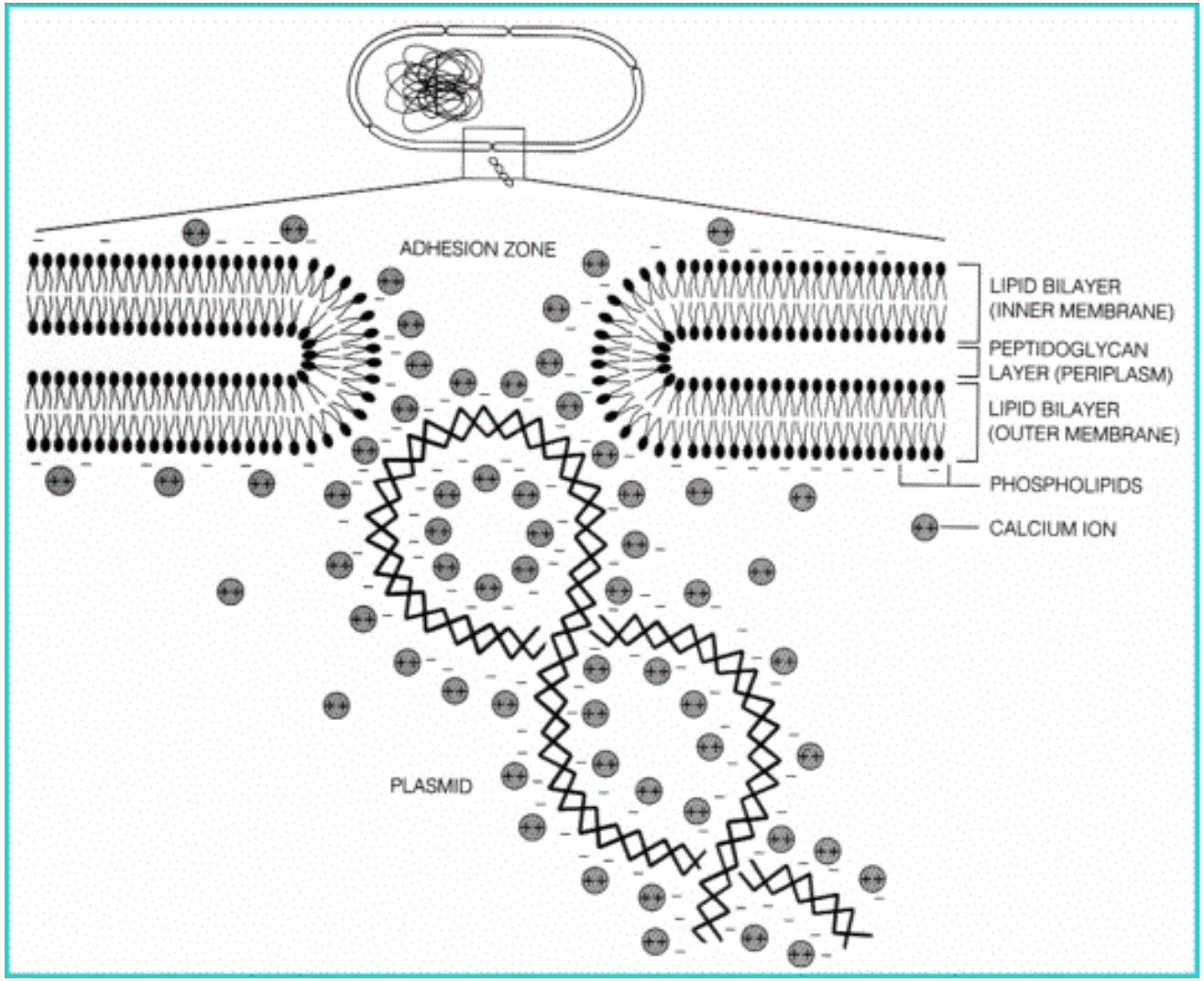
DNA Purity

Ratio	Low reading	High reading
A260/A230	• Carbohydrate carryover (often a problem with plants) • Residual phenol from nucleic acid extraction • Residual guanidine (often used in column-based kits) • Glycogen used for precipitation.	• Making a blank measurement on a dirty pedestal. • Using an inappropriate solution for the blank measurement. The blank solution should be the same pH and of a similar ionic strength as the sample solution. Example: using water for the blank measurement for samples dissolved in TE may result in low 260/230 ratios.
A260/A280	• Residual phenol or other reagent associated with the extraction protocol. • A very low concentration (< 10 ng/µl) of nucleic acid.	• Residual RNA from nucleic acid extraction. * High 260/280 purity ratios are not normally indicative of any issues.

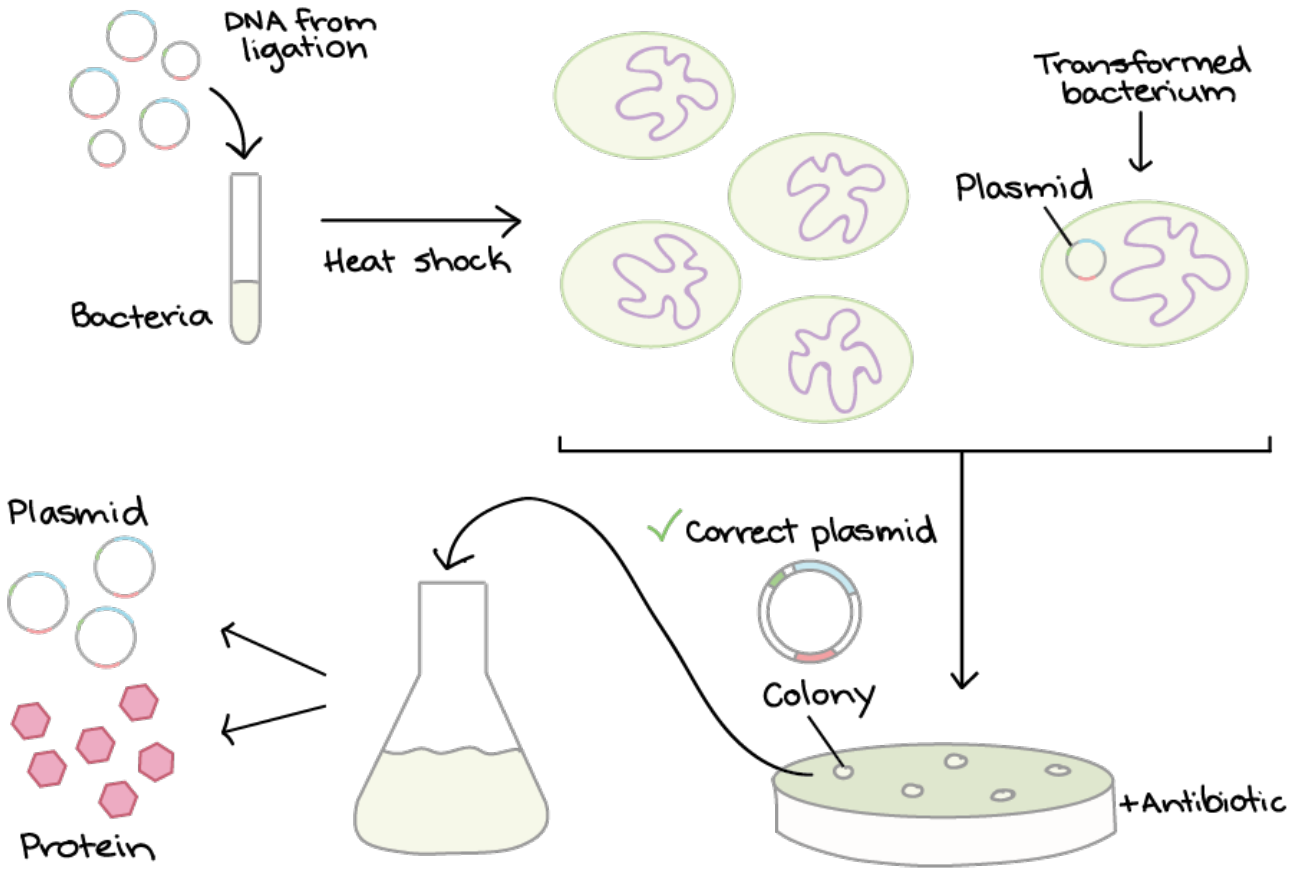
Bacterial Transformation

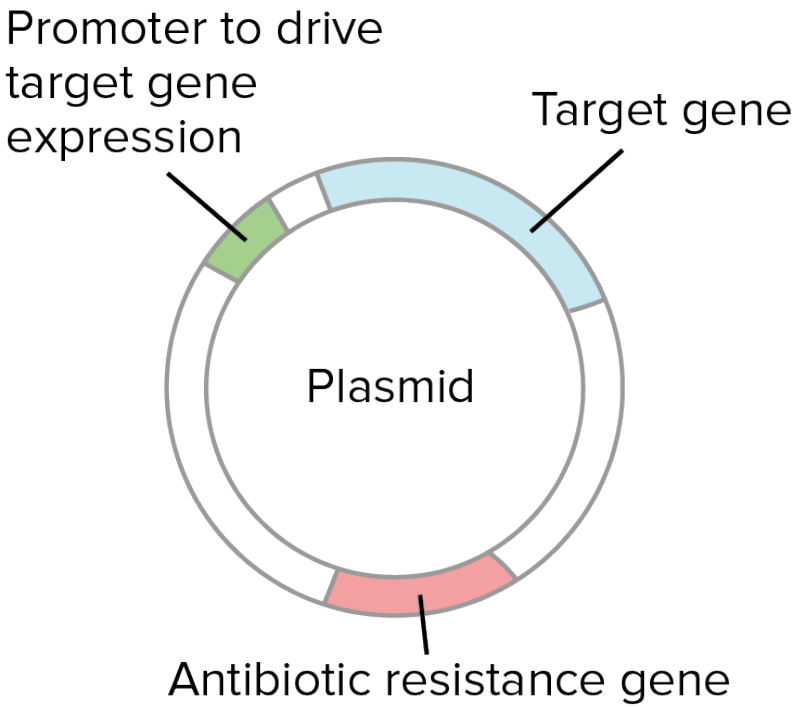


Bacterial Transformation



Colony Selection





Procedure – Ligation

1. Thaw the materials (10x T4 DNA ligase buffer, digested DNA)
2. Move to the Kang lab
3. Measure the concentration of the digested DNA (both vector and insert).
4. Calculate the volume of the DNA for the reaction. Vector:Insert should be approximately 1:3. Ideally, the vector should be ~100ng in total.
5. Calculate the volume of DW required.
6. Add DW and 2ul of 10x T4 DNA ligase buffer.
7. Transfer vector and corresponding volume of insert into a microtube.
8. Add 1ul of T4 DNA ligase. Mix well with pipet and incubate at room temperature for an hour.

Procedure – Ligation

Order to add	Component	Volume
1	3rd distilled water	X μℓ (up to final volume)
2	10x ligation buffer	2 μℓ
3	digested plasmid DNA	2 μℓ (subject to change)
4	digested insert DNA	4 μℓ (subject to change)
7 (add later)	T4 ligase	1 μℓ
	Total volume	**20 μℓ**

Procedure – LB Preparation

1. Measure and pour 200mL of DW into a beaker (w/ stirring bar).
2. Measure and add 2g NaCl, 2g tryptone, and 1g yeast extract.
3. Stir until the powder is well dissolved.
4. Transfer the solution to a 500mL flask.
5. Put the flask into autoclave and start autoclave.

■ Pause point: LB broth can be stored at room temperature for one week.

Procedure – Transformation

1. Thaw DH5α & BL21(DE3) competent cells on ice.
2. Add 20µL of ligated sample solution into DH5α competent cell microtube.
3. Add 1µL of the cloned plasmid (provided) into 20 µL of BL21(DE3) competent cell.
4. Incubate both the competent cells on ice for 20~30 min.
5. Warm-up two LB agar plates that contain appropriate antibiotics at 37℃ incubator (each for DH5α and BL21(DE3)).
6. Place both the competent cells in a 42℃ heat block, and incubate for 40~50 sec.
7. Move the microtubes to ice and incubate for 1 min.
8. Add 800µL of LB medium into the DH5α cell and 150µL of LB medium into the BL21(DE3) cell.
9. Incubate the DH5α cells at 37℃ with shaking for 1 hr (BL21(DE3) cells can skip this step).
10. Spread the cells on the LB agar plate with a spreader. Spread the cell until the agar plate surface dries
11. Incubate the plates at a 37℃ incubator for 12~18hr.

Post-lab Questions

1. We have used DH5α and BL21(DE3). What are the differences between the two stains? When do we use each strain?
2. Calculate the appropriate volume of insert DNA under the following conditions.

Measured plasmid concentration: 50ng/µL

Measured insert DNA concentration: 10ng/µL

Size of the plasmid: 6000bp

Size of insert DNA: 600bp

Target molar ratio: 5:1

3. Heat shock transformation is also called chemical transformation because this competent cell generally contains $CaCl_2$. Search the role of the $CaCl_2$ and the principle of heat shock transformation.

4. In the case of this experiment, which antibiotics would be appropriate? Why?