Transformation

1. Transfer 1 µL of the resuspended DNA into 50 µL competent cells. Thaw the recombinant cells on ice for around 20 minutes before use.
2. While waiting, warm the agar plates containing the desired antibiotics by placing them in 37 °C incubator.
3. Place the tubes containing DNA and competent cells on ice for 20 minutes.
4. Heat shock in water bath (42 °C) for 1.5 minutes.
5. Place the tubes containing DNA and competent cells on ice for 2 minutes.
6. Add 1 ml of LB (without antibiotic) into each tube and grow in 37 °C shaking for 1 hour as recovery for bacteria with plasmids that have CHL or KAN resistance. Bacteria with plasmids that have AMP resistance does not need recovery.
7. Centrifuge all tubes at 7000 rpm for 2.5 minutes.
8. Discard the liquid in 100 ml beaker with some left in the tube.
9. Light the Bunsen Burner. Resuspend the tube and transfer 250 µL of each tube onto agar plate with appropriate antibiotics.
10. Incubate overnight at 37 °C.

Digestion (18 µL reaction)

1. Add water (variable volume).
2. Add DNA (variable volume) into the tube. Resuspend.
3. Add 1.8 µL CutSmart buffer. Resuspend it and centrifuge the tube lightly if there is water drop on the wall of the tube.
4. Add restriction enzyme (volume depends on the amount of DNA added). Resuspend the enzyme before use.
5. Incubate the tubes for 1 to 2 hours.

Preparing agarose gel for gel electrophoresis

1. Mix agarose powder and 1X TAE buffer in a flask. The percentage of the gel depends on the DNA sample being added.

2. Melt them in the microwave for 40 seconds to 1miniute. Wear gloves when taking the flask out.

3. Add Midori green (0.001% of the total gel mixture volume) into the gel mixture.

<u>Colony PCR</u>

1. Preparing the sample:

    - Prepare tubes of 20 µL saline solution.

    - Pick 5 to 10 colonies from the transformed plate.

2. Put all reagents into a master mix (18 µL for every reaction)

Component	25 µl reaction
10X Reaction Buffer	2 µl
10 mM dNTPs	0.4 µl
10 µM Forward Primer	0.4 µl
10 µM Reverse Primer	0.4 µl
Colony-containing saline solution	2 µl
Taq DNA Polymerase	0.1 µl
ddH2O	14.7 µl

3. Add 2 µL colony-containing saline solution.

4. Run PCR.

Thermocycler settings:

STEP	TEMP	TIME
Initial Denaturation	95°C	3 miniutes
24 Cycles	95°C 45-68°C (depends on primers used) 68°C	15 seconds 30 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

5. Do gel electrophoresis. Load the 20uL PCR product into the wells.

6. Keep the colony-containing saline solution left at room temperature, which will be used for plate streaking and inoculation.

Ligation

1. Set up the reaction mixture in the micro-centrifuge tubes on ice.
2. Add T4 ligase.
3. Mix the reaction mixture and the DNA fragments gently by pipetting up and down.
4. Incubate the micro-centrifuge tubes at room temperature for 1 hour.
5. Do transformation with the products.

Gibson assembly

1. Prepare the master mix following the recipes below.

Recipes

2X ISO reaction buffer -1.1 ml

Reagent	Stock Concentration	Final Concentration	Volume
PEG 8000	40%	10%	275 ul
Tris, pH 7.5	1M	200mM	220 ul
MgCl2	1M	20mM	22 ul
DTT	1M	20mM	22 ul
dNTP	10 mM (each)	0.4mM	44 ul
NAD*	50mM	2mM	44 ul
Water			473 ul
			Total 1100 ul

*50 mM stocks can be purchased from NEB, cat # B9007S

-Make 133.33 ul aliquots and store at -20. Good for 1 year. Recipe makes 8 aliquots. Scale up or down as you want.

2. Add DNA backbone and inserts with molar ratio 1:5 into a tube of master mix aliquot.
3. Incubate the reaction mixture for 1 hour at 50°C.
4. Do transformation with the products.