

IV. Cloning in Plasmid vectors

A. Molecular Cloning Using TOPO® Cloning

1. Prepare Plates for transformation:

IPTG (100mM): 0.6g IPTG + 25ml water, filter-sterilize and store at 4°C.

X-Gal (40mg/ml): 400mg X-Gal dissolve in 10ml N,N'-dimethyl-formamide. Cover with aluminum foil and store at -20°C.

LB plates with Kanamycin: Add 10g agar, 25g SOC to make 1 liter of medium. Autoclave 20min 15p. Allow the medium to cool to 50°C before adding Kanamycin to a final concentration of 100µg/ml (Stock: 50mg/ml). Pour 20ml of medium into 85mm petri dishes. Let the agar harden. Store at 4°C for up to 1 month or at room temperature for up to 1 week. Prewarm plate at 37°C, add 100µl IPTG (100mM) and 20µl X-Gal (80mg/ml).

2. Ligation

Chemical	Standard Reaction			Check
Salt solution	0.5µl			
TOPO® vector	0.5µl			
PCR insert (100ng)	2µl			
Total	3µl			

- Mix reaction gently and incubate for 30 minutes (30 seconds to 30 minutes) at room temperature (22-23°C).
- Place the reaction on ice for 10 minutes and proceed to Transforming One Shot Competent Cells. Note: You may store the reaction at -20°C overnight. Start:_____.

3. Transformation

- Add 1.5µl of the cloning reaction into a vial of half of One Shot Chemically Competent *E. coli* and mix gently. Do not mix by pipetting up and down.
- Incubate on ice for 10 minute. Start:_____.
- Heat-shock the cells for 30 seconds at 42°C without shaking.
- Immediately transfer the tubes to ice.
- Add 125µl of room temperature SOC medium.
- Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 1 hour. Start:_____.
- Prewarm plate at 37°C, add 40µl IPTG (100mM) and 40µl X-Gal (40mg/ml) separately, and plate immediately. Leave the plates at 37°C for 30 minutes.
- Spread 50 µl from each transformation on a prewarmed selective plate and incubate overnight at 37°C.

4. Blue/white screen for recombinants.

- Use tooth pick to transfer white colony to 5ml SOC medium with Kanamycin in Falcon tube.
- Incubate at 37°C, 200rpm incubator overnight.

- d. Transfer 700µl bacterial culture into 300µl 50% sterile glycerol in 1.5ml microtube, and store at -80°C.
- d. Centrifuge the Falcon tubes at 1000 g for 5min, decant the supernatant. Follow plasmid extraction protocol.

Example data sheet:

No.	PCR#	Gene	White	Blue	Remark	No.	PCR#	Gene	White	Blue	Remark
1						9					
2						10					
3						11					
4						12					
5						13					
6						14					
7						15					
8						16					