Cloning - Promega pGEM-T Easy kit

Ligation

1. Add to the reaction mixture:
    a. 3 µl Fresh PCR product
    b. 5 µl 2x Rapid Ligation Buffer
    c. 1 µl pGEM-T vector
    d. 1 µl T4 DNA Ligase
2. Incubate at room temperature for 1 hour or overnight at 4°C
3. Store on ice until needed

Transformation

1. Get cells out of freezer, thaw on ice for 5 minutes
2. Add 2 µl of ligation mixture into a 1.5 ml tube on ice
3. **Carefully** transfer 50 µl of cells into the tube
4. Incubate on ice for 20 minutes
5. Heat-shock the cells for 45-50 seconds at 42°C
6. Immediately put the cells on ice for 2 minutes to recover
7. Add 950 µl of SOC recovery media
8. Shake at 37°C for 1.5 hours
9. Spread 100 µl onto LB plates with X-gal and ampicillin
10. Incubate plates overnight at 37°C and you should get colonies.

Materials required

Cleaned PCR product
Promega pGEM-T easy cloning kit
Promega JM109 Competent cells
SOC media
LB + amp + IPTG + X-gal plates
10 µl, 200 µl, and 1000 µl pipette tips
1.5 ml microcentrifuge tubes
Ice

Equipment necessary

10 µl micropipette
200 µl micropipette
1000 µl micropipette
37° C incubator
37° C shaking incubator
42° C heat block