Cloning – Invitrogen TOPO-TA kit

Ligation

1. Add to the reaction mixture:
   - 4 μl Fresh PCR product
   - 1 μl Salt Solution
   - 1 μl TOPO vector
2. Incubate at room temperature for 5 minutes
3. Store on ice until needed, or overnight at 4° C for maximum number of transformants

Transformation

1. Get cells out of freezer, thaw on ice for 5 minutes
2. Add 2 μl of ligation mixture
3. Incubate on ice for 5 minutes
4. Heat-shock the cells for 30 seconds at 42° C
5. Immediately put the cells on ice
6. Add 250 μl of SOC recovery media
7. Shake at 37°C for 1 hour
8. Spread 50 μl on LB plates with X-gal and ampicillin
9. Incubate plates overnight at 37° C and you should get colonies
10. Proceed to colony PCR to verify your results

Materials necessary

- Cleaned PCR product
- TOPO-TA cloning system
- TOP-10 competent cells
- SOC media
- LB + amp + IPTG + X-gal plates
- 10 μl pipette tips
- 1000 μl pipette tips
- 1.5 ml microcentrifuge tube
- Ice

Equipment necessary

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