Transformation of E. coli

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This is a protocol for transforming *E. coli* with plasmid DNA.

Keywords: bacteria E. coli transformation

Expand

<u>Reagents, supplies, and equipment</u>:

- *E. coli* competent bacteria
- 1.5 ml Eppendorf tubes
- 42°C water bath
- LB broth without ampicillin (no Amp)
- LB + Amp plates
- 100 mM IPTG
- 2% X-gal
- 37°C shaker rack
- microcentrifuge

<u>Procedure</u>: *After ligation of vector and insert is complete, the ligation mixture is ready to be transformed into bacteria.*

1. Add 200 µl *E. coli* competent bacteria to all of the plasmid ligation mixture in a 1.5 ml Eppendorf tube. Place on ice for 30 min.
2. Heat shock at 42°C X 1 min.
3. Add 1 ml of LB broth (No Amp) to cells. Incubate 1 hr at 37°C.
4. Pre-warm 2 LB + Amp plates per transformation at 37°C.
5. Transfer a 200 µl aliquot of transformation mixture to a clean Eppendorf tube (low dilution).
6. Microfuge to pellet the cells (**Be careful since too much centrifugation will destroy the cells)**. Quickly turn microfuge ON and OFF 3-4X. Pipette off and discard all but 200 µl of solution (high dilution).
7. If blue/white color selection can be used, to the low and high dilutions, add:
   10 µl 100 mM IPTG
   50 µl 2% X-gal
8. Transfer mixtures to separate, pre-warmed LB + Amp plates. Spread with a bent glass rod.
9. Incubate 8-12 hr, inverted, at 37°C.
10. Pick white colonies and suspend in 2.5 ml LB + Amp broth (1 colony per tube). Incubate on shaker rack at 37°C 8 hr to overnight.
11. Suspensions are ready for mini-prep assay.