DYS SEE

Transformation.

Protocols

Transformation Protocol

Estimated time: 1.5h

1. Take competent cells out of -80°C and thaw on ice (approximately 20 mins).
2. Mix 1µl of DNA (usually 10pg - 100ng) into 50-100µl of competent cells in a microcentrifuge or falcon tube. GENTLY mix by flicking the bottom of the tube with your finger a few times.

   Note: Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid.

3. Incubate the competent cell-DNA mixture on ice for 20-30 mins.
4. Heat shock each transformation tube by placing the bottom of the tube into a 42°C water bath for 60 secs
5. Put the tubes back on ice for 5 min.
6. Add 400-900µl LB (without antibiotic) to the bacteria and grow in 37°C shaking incubator for 1h.

   Note: This outgrowth step allows the bacteria time to generate the antibiotic resistance proteins encoded in the plasmid backbone so that they will be able to grow once plated on the antibiotic containing agar plate. This step is not critical for Ampicillin resistance but is much more important for other antibiotic resistances.

7. Plate some or all of the transformation onto a 10 cm LB agar plate containing the appropriate antibiotic.

Note: We recommend that you plate 50µl on one plate and the rest on a second plate. This gives the best chance of getting single colonies, while allowing you to recover all transformants.

Note: If the culture volume is too big, gently collect the cells by centrifugation (9,000 x g for 1 min) and resuspend in a smaller volume of LB so that there isn't too much liquid media on the agar plates. If the agar plate doesn't dry adequately before the cells begin dividing, the bacteria diffuse through the liquid and won't grow in colonies.

8. Incubate plates at 37°C overnight.

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