Transformation of competent cells

Introduction

In order to transform our competent *E.coli* cells with our vector we will use the "Transformation Protocol" from AddGene.

For more information check the following link: [https://international.neb.com/protocols/2012/05/21/transformation-protocol]

Materials

Materials

- Competent *E.coli* cells
- Plasmid pSB1C3
- Plates with Chloramphenicol
- Ice
- LB

Equipment

- Incubator
- Shaking incubator
- Microcentrifuge tubes
- Tips
- Pipettes

Procedure

Transformation

1. Take competent cells out of -80°C and thaw on ice (approximately 20-30 mins).
2. Remove agar plates (containing the appropriate antibiotic) from storage at 4°C and let warm up to room temperature and then (optional) incubate in 37°C incubator.
3. Mix 1 - 5 µl of DNA (usually 10 pg - 100 ng) into 20-50 µL of competent cells in a microcentrifuge or falcon tube. GENTLY mix by flicking the bottom of the tube with your finger a few times.
4. Incubate the competent cell/DNA mixture on ice for 20-30 mins.
5. Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 30-60 secs (45 secs is usually ideal, but this varies depending on the competent cells you are using).
6. Put the tubes back on ice for 2 min.

7. Add 250-1,000 µl LB or SOC media (without antibiotic) to the bacteria and grow in 37°C shaking incubator for 45 min.

   Pro-Tip 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.

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

   Pro-Tip We recommend that you plate 50 µL 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.

9. Incubate plates at 37°C overnight.

Table1

	A	B
1	Reagents	Volume
2	pSB1C3	1 ul (50 ng)
3	Nuclease free water	3.56 ul
4	Competent cells	50 ul
5	LB media	945 ul
6	**Total**	**1000 ul**