TRANSFORMATION OF COMPETENT CELLS

Created/updated by: Kyle Novakowski Date: September 6th 2012
Bowdish Lab, McMaster University
Hamilton, ON, Canada
www.bowdish.ca

BACKGROUND

- This protocol is used to transform DNA into competent bacterial cells for the purpose of DNA amplification. 1.5 – 2mg of DNA can be obtained from a 250mL batch of bacteria, depending on plasmid size, growth conditions, etc. For lysis and purification of transformed DNA, see the appropriate MIDI prep kit guidebook. For smaller volumes (such as 5mL) see the MINI prep kit guidebook.

NOTES

- Prepare growth media the day before, if possible.
- Pre-set water bath to 42°C.
- Bacteria needs to incubate overnight. **Best to start at 3PM to prevent culture from overgrowing**.

EQUIPMENT

- Sterile LB
- Competent Cells (DH5α in our case)
- Plasmid DNA
- Water bath
- Ice
- Ampicillin (A 1000x stock is usually kept or can easily be made by dissolving 0.1g ampicillin in 1mL ddH2O)
- Shaker set to 37°C

PROTOCOL

1. Thaw a 50uL frozen aliquot of DH5α competent cells from the -80°C freezer on ice for approximately 15 minutes.
2. Add 1ug of plasmid DNA. The concentration is not critical, but a higher concentration helps.
3. Let the cells rest on ice for 30 minutes. Turn on the water bath now if this has not been done.
4. Place the tube in a 42° water bath for 45 seconds.
5. Place the tube back on ice for 2 minutes.
6. Pipette the volume into 250mL sterile LB.
7. Add a 1:1000 concentration of ampicillin. For 250mL this is 250uL.
8. Incubate cells overnight in a 37°C shaker.
9. Next day, begin the MINI or MIDI prep procedure to isolate and purify the plasmid DNA.

LINKS AND REFERENCES

- www.bowdish.ca/lab