Heat shock transformation

Introduction

Heat shock plasmid transformation to competent E.coli.

Materials

- Competent E.coli cells
  - 50 ul For each DNA construct / 100 ul For ligation.
- 50 ng of circular DNA
- Ice
- Water bath at 42°C
- 1.5 ml tube per sample
  - (Eppendorf or similar)
- 1 ml of LB per sample
  - (with no antibiotic added)
- LB+antibiotics plates
  - 2 or 3 per sample for dilutions
- Drigalski spatula

Procedure

Heat shock

1. Take competent E.coli cells from –80° C freezer. Use Top10 cells in most cases.
2. Turn on water bath to 42°C.
3. Put competent cells in a 1.5 ml tube (Eppendorf or similar). For transforming a DNA construct, use 50 ul of competent cells. For transforming a ligation, use 100 ul of competent cells. You may need more or less cells, depending how competent they are.
4. Keep tubes on ice.
5. Add 50 ng of circular DNA into E.coli cells. Incubate **on ice** for **20 min**. to thaw competent cells.
6. Put tube(s) with DNA and E.coli into water bath at **42°C** for **1.5 min**.
7. Put tubes back **on ice** for **5 minutes** to reduce damage to the E.coli cells.

8. Add 1 ml of LB (with no antibiotic added). Incubate tubes for **1 hour** at **37°C**. (Can incubate tubes for 30 minutes, unless trying to grow DNA for ligation which is more sensitive. For ligation, leave tubes for 1 hour).

9. Spread about 100 ul of the resulting culture on LB plates (with appropriate antibiotic added). Grow overnight.

10. Pick colonies about 12-16 hours later.