Transformation Protocol Using Heat Shock

Mary Teruel, 9/7/00

1) Take out competent E.coli cells from –80^{o}C freezer.

2) Turn on water bath to 42^{o}C.

3) Put 30-50 ul of competent cells in a 1.5 ml tube (Eppendorf or similar) – 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 10 min to thaw competent cells.

6) Put tube(s) with DNA and E.coli into water bath at 42^{o}C for 45 seconds.

7) Put tubes back on ice for 2 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^{o}C. (Can incubate tubes for 30 minutes, unless are trying to grow DNA for ligation which is more sensitive. For ligation DNA, leave tubes for 1 hour)

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

10) Pick the colonies about 12-16 hours later.

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