Heat-Shock Transformation (Regular method)

2002-09-16

1. Thaw $CaCl_2$ competent cells on ice. Transfer 100 uL of cells in to 10 mL culture tubes.

2. Start a hot water bath (or heat block) going at 42 ^{0}C. Place LB plates (with selection) in 37 ^{o}C incubator to dry them.

3. Add 2 uL plasmid DNA (or your entire ligation mix) to the cells in the culture tube. Make SURE your pipette tip goes all the way down in to the cells, so that you are adding DNA to the cells.

4. Flick tube gently to mix.

5. Incubate on ice for 20 minutes.

6. Heat-shock cells at 42 ^{0}C for 30-45 sec. *Do not heat shock more than 45 secs.*

7. IMMEDIATELY add 900 μL SOC (or LB) media to cells. *SOC gives greater efficiency; do not hesistate to add SOC after heat shocking; even 1 minute delay lowers transformation efficiency 2-3 fold.*

8. Incubate tubes at 37 ^{0}C for 1 hour. *Do not shake, just incubate stationary tubes.*

9. *OPTIONAL: For plasmids with blue-white screening (bGal, ie, pCR2.1 TA-cloning), add to plates:*

   a. *40 μL IPTG (100 mM).*

   b. *80 μL Bluo-Gal (20 mg/mL in DMF).*

10. Spread 10-100 μL cells from each transformation onto pre-warmed LB plates (with appropriate selection, i.e. ampicillin).

    a. OR, spin down cells (1 min at max. speed in microfuge tubes), discard most of the media, then resuspend and plate entire transformation. *This is not normally necessary, and should only be done as a last resort if a transformation isn't working or if you need high-efficiency (ie, ligations). It will result in a plate with lots of satellite colonies, which aren't nice to have.*

11. Incubate overnight at 37 ^{0}C.

12. Pick colonies.

Heat-Shock Transformation (Quick method)

2004-06-30

- ONLY use this method to transform plasmids in to E. coli. Do NOT use it for ligations, etc). It is an order of magnitude less efficient than the standard method.

- As tempting as it is to use this method routinely, remember that it's much more likely to fail than the standard method. So if you have time, use the standard method.

1. **Place LB-Agar plate in 37 ^{0}C incubator. This is really, really important because the plates need to be warm for the quick method to work. Put the plate in the incubator at least 1 hour before continuing on to step 2.**

2. Thaw $CaCl_2$ competent cells on ice. Transfer 100 ul cells in to a new microfuge tube.

3. Add 2 ul plasmid DNA to cells. Mix gently by flicking tube.

4. Incubate on ice for 5 minutes.

5. Quickly spread entire cell mixture (100 uL) onto **pre-warmed** LB plates (with selection). *It is vital that your plates be warm for this to work, since the warm plates act as the heat shocking step.*

6. Incubate overnight at 37 ^{0}C.

7. Pick colonies.