Heat Shock Transformation Protocol

Needed Materials

Media (for two heat shock cultures)

We are performing 8 heat shock transformations so we need to multiply these amounts by 4

- sterile LB (18ml)
- filtered 0.1M CaCl2 (22ml)
- sterile 50% glycerol (100 uL)

Flasks

- Sterile flask for growing culture after heat shock

Removing Biobrick Parts from 360 Well Registry Plates

Adapted from the Registry of Biological Parts

This procedure should be performed at the end of the comp cells are growing up for 4 hours or during the washing cycle (see comp cell preparation below). Place the DNA on ice until needed for the transformation.

1. With a pipette tip, punch a hole through the foil cover into the corresponding well to the Biobrick™-standard part that you want. Make sure you have properly oriented the plate. We recommend that you do not remove the foil cover, as it could lead to cross contamination between the wells.
2. Add 15uL of ultrapure water
3. Remove from well and transfer into an eppendorff tube
4. Place 10 uL of DNA into new chilled eppendorff tube to mix with comp. cells
5. Place the remaining 5 uL into the -20C freezer to store

The biobricks we are taking are

Number	Resistance	Well	Plate (2010)
I719015	A	15P	1
I719005	A	15N	1
K145001	A	2F	2
K117008	A	14L	2
K117002	A	14H	2
K103006	A	22P	2

Preparation of Competent Cells

1. Inoculate 3ml of LB and cultivate it overnight.

   We need to multiply all amounts by 4 so Alex and Jennifer inoculated a 12 mL culture

2. Inoculate 15ml of LB (a conical tube) with the seed culture and cultivate it until OD600 reaches between 0.4 and 0.9.

   This step should be done approximately 4 hours before the washing begins and was done by Ann at 2:30pm. Make sure the chill all pipets and media that are going to touch the bacteria when the culture is started.

3. Chill it for 10min.

   Since we are making a large amount of comp cells, divide the culture evenly between two 50 mL falcon tubes by decanting

   Submerge both falcon tubes in an icebath, this suspends the bacteria in their exponential state of growth and from this point forward until sticking the cultures in the ice bath everything that touches the cultures needs to be chilled to maintain this state

   Also chill the ependorff tubes (1.5 mL tubes) with the DNA that the heat shock will be performed in. Add 5 uL of DNA to each tube. Make sure the save the remaining biobrick mix from the registry in the -20C freezer in case the transformation does not go according to plan

   Set temperature of centrifuge to 4C

   Set temperature of the water bath to 42C

4. Harvesting - Centrifuge it @ 3500rpm for 15min. Discard supernatant.

   Discard the supernatant by decanting with the cell pellet facing upward so it does not get washed off.

5. Washing 1 - Resuspend cell pellet with 5ml of cold sterile 0.1M $CaCl_2$. Centrifuge it @ 3500rpm for 15min. Discard supernatant.

   When growing in LB media the cell pellet will be very dense and the cell pellet should be resuspended in 1 mL of salt solution first before adding the rest.

6. Washing 2 - Resuspend cell pellet with 5ml of cold sterile 0.1M $CaCl_2$. Chill it for 30min. Centrifuge it. Discard supernatant.

   The cell pellet will be much loser so be careful while decanting!

7. Resuspend cell pellet with 100 uL of cold sterile 0.1M CaCl2 and Check the density of the comp cells

   The residual liquid may be enough and only glycerol needs to be added

   a. For each sample, prepare a 1:100 dilution
      i. 10 μL of competent cells
      ii. 990 μL dH2O
   b. Measure OD600 w/ cuvette on spectrophotometer
   c. Use dH2O blank
   d. Net OD600 should be ~0.14.

8. Add 100ul of cold sterile 50% glycerol.
9. Let comp cells chill for 20 minutes

Transformation

1. Mix100 uL of comp cells with pre chilled DNA in 1.5 mL falcon tubes
2. Give heat shock for 1min.

   Only perform one heat shock at a time to make sure all cultures are saved after 1 minute

3. Immediately add 1ml of LB to it.
4. Cultivate 1hr. Spread 200ul of cell on a plate.

   We will be performing 8 transformations, 6 biobrick parts, 1 positive control (pSIM with proper antibiotic resistance) and 1 negative control (cells only)

*note: the cells grew out too much (OD600 = ~1.2) they were diluted and when the experiment began the OD600 was ~.506

OD600 after preparing the competent cells was 0.348 and 0.355 for batch 1 and 2 respectively.

7/9/2010

1. Only the cells with DNA from plate 1 grew (1 plate had over 100 colonies the other had 4)-see Ann's notes!