Plasmid Transformation into DH5alpha *E.coli* cells using Heat Shock
(by Manish, summarized from LIFE technologies protocol)

In advance:
Prepare dry ice/ethanol.
Prepare 42C water bath.
Prechill 1.5 ml tubes on wet ice.
Prepare LB+ antibiotic plates +/- IPTG/X-gal. Prepare plates in advance, or else, spread 50uL of antibiotic on top of the media with a spreader in sterile conditions.

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1. Thaw cells from -70 onto wet ice. Prechill 1.5mL tubes on ice.

2. Aliquot 50uL of competent cells into 1.5mL tubes.

3. Refreeze any unused cells in a dry ice/ethanol bath for 5 minutes and return to -70C freezer. Do not use liquid nitrogen.

4. Add 1-10 ng of plasmid DNA to one tube of competent cells; Gently tap tube to mix. 25 ng of (uncut) plasmid will generate >10 000 colonies on a single plate. We want <1000 colonies per plate to be able to distinguish individual colonies.

5. Incubate on ice for 30 minutes

6. Heat shock for 20 seconds at 37C.

7. Place back on ice for 2 minutes.

8. Add 950 uL of SOC medium (or LB is OK). SOC can be purchased directly from LIFE tech. Transfer to 2-5 ml tubes.

9. Shake at 225 rpm for 37C for 1 hour to express the antibiotic resistance gene.

10. Spread 100 uL of the reaction and/or serial dilutions onto LB plates using a plate spreader under sterile conditions. If necessary to use more transformed cells, then centrifuge in the 1.5mL tube for 5 seconds and resuspend in 100 uL of SOC.

11. Let petri plates dry (covered) for 10 minutes.

12. Incubate plates overnight at 37C. Then store at 4C under needed. Store remaining transformed cells at 4C and then throw away if the transformation was successful.