Protocol for heat shock transformation of chemically-competent cells

1. Remove one or more aliquots (as required) of chemically competent cells of your *E.coli* strain from the -80°C freezer. Thaw the cells e.g. by rubbing them in your hands or put them briefly in a 37°C waterbath, but don't let them stay warm! As soon as they are thawed, put them onto ice.

2. Divide the cells into the appropriate number of 50 µl aliquots in separate Eppi tubes on ice. Add your DNA samples to each tube; you can use up to ~10 ul of ligation mixture or plasmid here, but note that typically 3 µl of ligation mix or 1 µl of plasmid would be standard.

3. Make sure you include both a positive control and a negative control in the transformation experiment. The positive control should be 1 µl of a plasmid with the correct antibiotic resistance (same resistance as the plasmid used for the ligation), and should also be a plasmid stock that you know is in good condition (based on agarose gel).The negative control is simply no DNA added.

4. Put the cells into a foam 'floatie' and put on ice. Ensure at least the bottom half of the tube (approx 2 cm) is embedded in the ice, don't just rest them on top of the ice. Allow the cell/DNA mixtures to incubate on ice for 15-30 min.

5. Take your esky of ice over to the 42°C waterbath or 42°C heat block. Put the floatie into the waterbath. Allow 45 seconds for heat shock. (Plus or minus 10 seconds, this needs to be exact!). Then transfer the floatie straight back onto ice (embed into ice, as above, don't just rest on top).

6. Allow transformation mixtures to sit for 2 min on ice, then add 1 ml sterile LB broth to each tube. You can also use more fancy media (e.g. SOC or SOB), but there is not that much difference.

7. Incubate on 37°C shaker for 1 hour. Put the tubes horizontal so they get good shaking action. eg. put the tubes laying flat on the shaker platform and masking-tape into place. Make sure the lids are tight! You can incubate without shaking, and you can incubate for less time (30 min), but it won't work as well in these cases. If transforming with an ampicillin-resistant plasmid, you can skip the recovery stage, due to the different mode of action of the 'cillins vs. other antibiotics.

8. Label the LB-antibiotic plates before starting the next bit; you need two plates for each ligation condition or plasmid type, since we will plate out two different cell concentrations of each to ensure we get countable/pickable numbers of colonies. Double check the plates to ensure you are using the correct type of antibiotic(s) for the type of plasmid(s) you are using.

9. Pipette 100 µl of the first cell suspension onto one LB-antibiotic plate (label '100 µl' in addition to other info). Sterilise the glass spreader with ethanol and flame (CAUTION! READ THE **SOP** FIRST!), and spread the cells around the plate with the spreader. Do this by pushing the spreader with a back-and-forth motion, while turning the plate around in a circular motion. Be careful not to touch the spreader on your fingers! Keep spreading for approx 10 seconds. You may also use disposable plastic spreaders if flaming a glass one is not possible or appropriate (i.e. inside a biosafety cabinet). To reduce plastic waste you can reuse the spreader from a 100uL plate for the pellet plate of the same sample, otherwise you will need a new one for each sample.

> If the plates are properly dried, you should feel the spreader start to 'stick' to the agar, this means the liquid has been drawn into the agar. If this doesn't happen after ~20 seconds, stop spreading, but next time, dry plates for longer! If the plates are incubated with a lot of liquid still on them, you may not get nice discrete colonies (the cells will swim around in the liquid, making a mess).

10. Spread 100 µl of the remaining samples, each onto a separate, appropriately-labelled plate.

11. Centrifuge all the tubes at ~15,000 rpm for 1 minute in a micro-centrifuge. Pour off most of the supernatant into culture waste (being careful not to touch the tubes on the edge of the culture waste bottle). Leave a little bit of liquid behind (about one or two drops).

12. Vortex the cells in the remaining liquid for about 10 seconds, or mix by pipetting up and down gently, until they are not sticking to the tube, and you have a nice smooth, even, cell suspension.

13. Pipette the cells from the first cell suspension onto the appropriate pre-labelled LB-antibiotic plate (label with 'pellet' in addition to other info), spread plate as above. Repeat for the remaining samples

14. Incubate all plates at 37°C overnight. Note that for some plasmid constructs, it may be better to use 30°C or room temp for 2-3 days – this lowers the copy number of pUC-type plasmids, and slows transcription and translation – both effects may be useful to allow retrieval of 'toxic' clones.

15. When examining your plates, first check your controls. The positive control should have thousands of colonies, especially on the 'pellet' plate. The negative control should have no colonies at all. If you don't see these results with the controls, anything you see on your experimental plates is questionable. Common problems and their interpretation are summarised in the Table below. "Mix up of labelling" can cause MANY problems – be super careful with your labelling ! (do this BEFORE starting the hands-on bit of the procedure, and double-check everything)

Problem	**Interpretation/solution**
Lots of growth of the negative control (thousands of colonies or lawn)	• Forgot to add antibiotic to the plates • Antibiotic concentration is wrong (too low) • Host bacteria are already resistant to the antibiotic (e.g. TOP10 has chromosomal streptomycin resistance) • Plates incubated too long (especially with LB-ampicillin) • severe contamination with an antibiotic-resistant bacterium (not E.coli) (unlikely!) • Mix up of labelling somewhere – is this actually the positive control? or one of the experimental tests?
Some growth on the negative control (a few colonies)	• Contamination during the procedure, e.g. from one of the other samples or the pipette etc. This may not be a 'deal-breaker' so long as there are lots more colonies on your experimental test plates • Mix up of labelling
No growth or very little growth on the positive control plate	• The cells are not competent • Used the wrong antibiotic in the agar (check the sequence of your plasmid to confirm correct resistance) • Used the wrong concentration of antibiotic (too much) • Agar plates are 'bad' for some other reason (e.g. added mercuric chloride instead of sodium chloride!) • Plasmid stock has gone bad (run a gel to check) • Mix up of labelling • Pipetting error (look at the pipette tip to ensure that you really have 1 µl of plasmid in there!)