# Bacterial Transformation and Generation of High Competency Cells using the Low Temperature Method

## Experimental considerations

- A key issue for obtaining high efficiency using this protocol is that the cells must be cultured at room temperature or 48 hrs.
- In the original method, described by Inoue et al. (Gene 1990, 96:23), the culture density at the time of harvest (O.D. at 600 nm ) is very important. In the protocol described below, this parameter is not critical and tranformation efficiencies of 1-3 x $10^9$ colonies/ug DNA can routinely be obtained. It is still helpful to have O.D. values of between 0.3-0.7. However, if the density is higher, the culture can be diluted (note: we have never done this so we don't know how much this may impact the transformation efficiency).
- It is apparently critical that cells be resuspended very gently (while minimizing bubble formation) following each centrifugation step.
- Another critical issue can be the quality of the water employed; although milliQ filtered water is good enough, we typically employ ultrapure water (Aldrich, cat#32,007-2).
- Freezing cells is critical (even if cells are needed right away, they must be frozen). Although it is recommended to keep frozen vials in liquid nitrogen, we usually keep them in the freezer at –70°C.

## Protocol

I. Preparation of Competent Cells

- Plate bacterial strain onto antibiotic-free 2xYT plate and incubate over night at 37°C.
- Place 200 ml SOB medium into 2 liters flask (it is a good practice to grow cells in a container with a volume that is 10 times greater than the volume of the culture, and a wide neck is better; these measures increase aeration of the culture).
- Inoculate five isolated colonies into the flasks containing SOB medium.
- Incubate on shaker (150-250 rpm) at 18°C until culture density is between 0.4-0.6 O.D. (600nm)(it should take longer than 30 hours).
- Spin cells down by centrifugation at 3000 rpm for 15 minutes at 4°C (**see note).
- Take off supernatant and add 1/3 volume (i.e. 67 ml) of ice cold Transformation Buffer (TB).
- Resuspend bacterial pellet very gently at 4°C (using a 25ml pipette) and incubate for 10 minutes on ice.
- Spin cells down again (15 minutes at 4°C).
- Take off supernatant and add 1/12.5 volume (i.e. 16 ml) of ice cold TB.
- Gently suspend cells at 4°C again using a 25ml pipette.
- Add DMSO up to 7% (v/v) (i.e. 1.2 ml) and leave cell suspension on ice for 10 minutes.
- Aliquot cell suspension into ice cold eppy tubes (0.2-1.0 ml per tube).
- Freeze competent cells as fast as possible (we usually drop tubes immediately in a small liquid nitrogen container and leave them until they are completely frozen and all aliquots have been made).
- Transfer tubes to –70°C freezer.

**Note: must use very clean centrifuge tubes since contaminating residual plasmid DNA causes background in cloning experiments.

II. Transformation

- Thaw competent cells by warming vial quickly in hands and place on ice before aliquot is completely melted.
- Place 100 ul competent cells in a polypropylene tube (although eppy tubes can be used, we regularly employ 5 ml tubes from Falcon, cat#352063)
- Add 1-20 ul DNA sample and mix gently (avoiding bubbles).
- Incubate 30 minutes on ice.
- Heat shock: 30 seconds at 42°C
- Immediately put samples back on ice and leave them for 5 minutes
- Add 400 ul SOC media (other bacterial culture media can be used, but efficiency will be lower) and incubate on shaker for 45 minutes.
- Plate 300ul onto an amp containing 100mm 2xYT plate.
- Incubate plate overnight at 37°C (note: 300ul of solution is generally too much to absorb well into agar so we do not invert plate during overnight incubation – this allows the plate to dry slightly and allows the solution to soak in better).

## Solutions

Transformation Buffer (TB) (1 liter)

| Mix the following: | | Final Concentration |
|---|---|---|
| 3.0 g PIPES | (Sigma P-9291) | 10 mM PIPES |
| 2.2 g $CaCl_2$ –2 $H_20$ | (Sigma C-7902) | 15 mM $CaCl_2$ –2 $H_20$ |
| 18.6 g KCl | (Fisher P-217) | 250 mM KCl |

- Dissolve in 975 ml ultrapure water (Aldrich, Cat#32007-2; milliQ and dd$H_20$ are also good, but make sure the filters are working right).
- Bring pH up to 6.7-6.8 by adding drops of 5 N KOH (white precipitates will not go into solution until the pH is close to 6.0).

| - Add: | | Final Concentration |
|---|---|---|
| 10.9 g $MnCl_2$-4$H_2O$ | (Sigma ) | 55 mM $MnCl_2$ |

Add water up to 1 liter and filter it (0.22 um) and keep it at $4^0$C (it lasts for months).

SOB Media

| | 1 liter |
|---|---|
| Tryptone | 20.0 g |
| Yeast Extract | 5.0 g |
| 10mM NaCl | 0.58 g |
| 2.5mM KCl | 0.18 g |
| MilliQ $H_2O$ | Up to 990 ml* |

- Autoclave above mixture and then add 10 ml 2M Mg Solution before using.

| 2M Mg Solution | | 100 ml |
|---|---|---|
| 1M $MgSO_4$-7$H_2O$ | (Fisher M-63) | 24.6 g |
| 1M $MgCl_2$-6$H_2O$ | (Sigma M-9272) | 20.3 g |

SOC Media

| | 50 ml |
|---|---|
| SOB | 49.5 ml |
| 2M Glucose | 0.5 ml** |

- Filter SOC (0.22 um) after adding Glucose.

| 2M Glucose | | 100 ml |
|---|---|---|
| D(+)Glucose | (Sigma G-8270) | 36 g |

Filter and store at $4^0$C