Ultra Competent *E. coli* Cells

- Inoculate 250 mL **SOB** media with 10 - 12 colonies from a freshly streaked plate.
- Grow to an OD_{600} of 0.94 at 18°C (or RT). (Doubling time is 4-10 hours). (1/04: 9.7 hr for INVaF')
- Chill on ice for about 10 minutes

****From this point on, it is critical to keep cells below 4°C****

****Work in cold room when possible****

- Pellet cells at 2500 x g for 10 minutes at 4°C.
- Resuspend cells gently in 80 mL of *ice cold* **TB**.
- Leave on ice for ~10 min.
- Pellet cells as before
- Resuspend cells gently in 10 mL *ice cold* **TB**.
- Add 10 mL *ice cold* **TB + 14% DMSO**.
- Wait 10 minutes, then aliquot into *pre-cooled* 1.5 mL tubes, and freeze them in liquid nitrogen.
- Store in -70 freezer.

Recipes

SOB+glycine

1 L
20 g Tryptone
5 g Yeast Extract
0.5 g NaCl
625 µL 4 M KCl
10 g glycine
pH to 7 with 1 M HCl (~800 µL)
Autoclave
10 mL 1 M $MgCl_2$

TB

100 mL
*Use the purest water available, and pure-water-rinsed glassware in preparation
0.30 g (10 mM) PIPES
0.22 g (15 mM) $CaCl_2 \cdot 2H_2O$
1.86 g (250 mM) KCl
pH to 6.7 with 150-200(?) µL 10 M KOH
1.09 g $MnCl_2 \cdot 4H_2O$
Filter sterilize

Things to autoclave

Centrifuge bottles
100 mL Graduated Cylinder (Chill)
1.5 or 2 mL eppendorf tubes (Chill)

Efficiency test:

Transform 0.1 pg pUC19: 100 colonies ⇨ 10^9 cfu/µg
1 pg pUC19: 100 colonies ⇨ 10^8 cfu/µg
10 pg pUC19: 100 colonies ⇨ 10^7 cfu/µg

Based upon Inoue, Nojima, and Okayama. 1990. *Gene* **96:** 23-28 with modifications suggested by Tang *et al*. 1994. *NAR* **22:** 2857-2858 and Akhtar, et al. 2000 *Anal. Biochem.* **277:** 273-276.

Transformation

•Thaw cells on ice. (If desired, split comp cells to pre-cooled tubes. I have gotten by with 12.5 µL/transformation.)

- *•(Optional for ~2-3X efficiency increase) Add 4 µL of 0.5* ***M*** *(or 0.14 µL of 100%) β-mercaptoethanol to cells per 100 µL competent cells. (20 m**M** final)*

•Add DNA *(I add <3 µL per 100 µL comp cells)*

Incubate in ice for ~30 minutes. *(Longer incubations seem to help for plasmids >10 kb.)*

•Heat shock at 42°C for 30 seconds then place on ice for another 2 minutes.

•Add 0.9 mL pre-warmed SOC media. *(LB works but might affect efficiency)*

• 37°C for 45 minutes (or longer). *(Some people suggest shaking, others say it kills the cells)*

•Plate.

From Inoue, Nojima, and Okayama. 1990. *Gene* **96:** 23-28;
β–ME from Invitrogen's and Stratogene's protocols

"five minute" protocol

notes: This protocol is reportedly sensitive to salt in the DNA mix (50% less colonies at 5m**M** relative to 0m**M**). Although others claim to get twice the efficiency with this fast protocol, in my hands it was the same as that from above.
For transforming supercoiled DNA, I squirt the transformation towards edge of plate and streak for single colonies.
Do not use for kanamycin selection—only amp.

•Prewarm plates to 37°C for ≥ 30 minutes.
•Thaw cells and add DNA.
•After >5 minutes, spread onto plate From Pope & Kent *NAR* 24:536-7