**Transformation of "Ultra Competent" E. coli (Inoue Method)**

Inoue, H., H. Nojima, and H. Okayama. 1990. High efficiency transformation of Escherichia coli with plasmids. Gene 96:23-28.

Allow 3 days to prep and make competent cells.
(2 day culture prep, 1day purification)

**Day 1:**

1) Streak a LB plate (no ampicillin) with competent cells and let colonies grow overnight (16-20hrs) at 37˚C.

**Day 2:**

2) Pick one colony for start up culture. Be sterile as possible because there is no amp.
Flame lid of SOB
Flame wooden stick and touch agar with no colonies to cool stick
Flame pipette

Fill Nalgene flask (250ml) w/ 25ml of SOB media and dip wooden stick with chosen colony into the 25ml of LB or SOB media.

3) Incubate culture for 6-8hrs at 37˚C with vigorous shaking (250-300 rpm). Adjust other incubator to 18-22˚C in preparation for later in the evening.

4) At the end of the 6-8hrs, seed the starter culture. Each starter culture can be inoculated into a 1L flask containing 250ml of SOB.
Volume of culture to seed:
-1ml of culture expanded into 250mlof SOB will take ~20hrs to reach $OD_{600}$ of 0.55-0.6.
-2ml of culture, 250ml SOB
-4ml of culture, 250ml SOB
-10ml of culture, 250ml SOB

Incubate all flasks overnight at 18-22˚C with moderate shaking (~200rpm).

**Day 3:**

5) In the morning, take an $OD_{600}$ of all the cultures. Continue to monitor at 1hr intervals for the first 3 hours, then monitor at 30min.

-For 1ml starter culture, can monitor at 1.5hr intervals for the first 3 hours. After 3 hrs, monitor at 45-30min intervals.
- For 2ml – 10ml starter cultures, monitor at shorter intervals.

6) Once one of the cultures reaches an $OD_{600}$ of 0.55-0.6, transfer flask into an ice water bath for 10 minutes. Also, chill TB (transformation buffer) solution at this time.

7) Harvest cells by centrifuging at 2500g (5000rpm in Sorvall GSA or 3000rpm in a Beckman J-6B centrifuge) for 10 min at 4˚C.

8) Pour off the media and blot the open centrifuge bottle on a stack of paper towels for 2 mins. Aspirate if necessary to remove any droplets.

9) Resuspend cells gently in 80ml of ice cold TB. Try swirling first, if that does not work, vortex or pipette up & down. Incubate on ice for 10 mins.

10) Spin at 2500g for 10min at 4˚C.

11) Repeat step 8

12) Resuspend cells gently in 20ml of ice cold TB.

13) Add 1.5 ml of DMSO, mix suspension by swirling. Then store on ice for 10 mins.
During this time, get materials ready for the next step 2 people recommended in order to quickly carry it out. Get a bath of liquid nitrogen ready. Place eppendorfs into racks & gather liquid nitrogen storage boxes. Decide how much you want to aliquot into each eppendorf. If you decide to do 50µl aliquots, get about 430 eppendorfs ready. (21.5ml/50µl)

14) Working quickly, one person should aliquot 50µl (use repeater) and the other person should snap close the eppendorfs and immerse tubes into liquid nitrogen bath. Store tubes in -80˚C freezer until needed.

**Solutions:**

| **SOB** | **1L** | **Transformation Buffer** | **500ml** |
|---|---|---|---|
| 2% (w/v) bacto tryptone | 20g | 10mM Pipes | 1.51 g |
| 0.5% (w/v) yeast extract | 5g | 15mM $CaCl_2$ | 1.10g |
| 10mM NaCl | 0.58g or 2ml of 5MNaCl | 250mM KCl | 9.325 |
| 2.5mM KCl | 0.19g or 2.5ml of 1MKCl | pH 6.755mM | pH first than add $MnCl_2$ |
| 10mM $MgCl_2$ | 0.95g or 10ml of 1M $MgCl_2$ | $MnCl_2$ | 5.45g |
| 10mM $MgSO_4$ | 1.2g or 10ml of 1M $MgSO_4$ | Sterile filter! | |
| pH 6.7 – 7.0 | | | |

**Lab ware:**
250ml flask
3 1L flasks
4 250ml centrifuge bottle