E. coli Competent cell preparation

(This protocol is from Erh-Min Lai, based on Molecular Cloning, 2nd ed.)

1. Inoculate fresh E.coli colony in 3ml SOB+20mM $MgSO_4$ broth, incubate at 37℃, 16hr.
2. Transfer 1ml culture into 99 ml SOB+20mM $MgSO_4$, incubate at 37℃, 2.5-3.0 hr,測 OD_{600}。約 0.35-0.4 即可。
3. Transfer to sterile, ice-cold 50 ml PP tube. Cool the cultures to 0℃ by storing the tubes on ice for 10 minutes.
4. Centrifugation at 3000g for 10 minutes at 4℃.
5. Decant the medium from the cell pellets. Stand the tubes in an inverted position for 1 minute to allow the last traces of medium to drain away.
6. Resuspend the pellets by swiring or gentle vortexing in ~20 ml (per 50 ml tubes) of ice-cold TEB (for fresh) or FSB (for frozen) transformation buffer. Store the resuspended cells on ice for 10 minutes.
7. Centrifugation at 3000g for 10 minutes at 4℃.
8. Decant the medium from the cell pellets. Stand the tubes in an inverted position for 1 minute to allow the last traces of medium to drain away.
9. Resuspend the pellets by swiring or gentle vortexing in 4 ml (per 50 ml tubes) of ice-cold TEB (for fresh use) or FSB (for store at -70℃ and used at a later date).
10. To prepare fresh competent cells
   a. Add 140 µl of DnD solution into the center of each cell suspension. Immediately mix by swirling gently, and store the suspension on ice for 15 minutes.
   b. Add an additional 140 µl of DnD solution to each suspension. Mix by swirling gently, and the store the suspension on ice for a further 15 minutes.
   c. Dispense aliquots of the suspensions into chilled, sterile 17x100 ml PP tubes. Store the tube on ice.
11. To prepare frozen stocks of competent cells
   a. Add 140 µl of DMSO per 4 ml of suspended cells. Mix gently by swirling, and store the suspension on ice for 15 minutes.
   b. Add an additional 140 µl of DMSO to each suspension. Mix gently by swirling, and then return the suspensions to an ice bath.
   c. Dispense 100 µl aliquots of the suspensions into chilled, sterile microfuge tubes or tissue culture vials. 以液態氮迅速冷凍，存放於-70℃使用時再取出。
   d. 使用時由-70℃取出，以手的溫度融解，融了之後立即放於冰上 10 分鐘。
   e. 分裝至 17x100 mm 的 PP tube，放置於冰上
12. 每 100 µl competent cells 加 25-100 ng transforming DNA(5µl)(體積勿超過 5%)，每次作 2 個 control，一個加入已知量的 plasmid DNA，另一個不加，置於冰上 30mins。
13. 42℃水浴 90 秒。
14. 冰浴 1-2 分鐘。
15. 加 800 µl SOC medium，37℃ 水浴加熱，移至 37℃培養 45 分鐘。
16. LB+抗生素的 plate 上加入 100-200 µl, spread evenly(用 sterilized 玻璃珠)。
17. Spread 均勻後倒掉玻璃珠。
18. 37℃培養 12-16 小時可得 transformed colonies。