| PROTOCOL | | |
|---|---|---|
| **Date:**<br>02-JUL-09 | **Written by:**<br>Chen Guttman | **Laboratory:**<br>Raz Zarivach |
| **Title:**<br>Protocol#3 - Preparation of electrocompetent cells | | **Page**<br>**Page 1 of 2** |

# *Preparation of electrocompetent cells*

### *Aim*

To describe the preparation of **0.5L** of competent cells for electroforation transformation assay. This protocol is specified for the above culture volume – adjust volumes as needed.

### *Materials & Equipment*

*Reserve place in Sorval Centrifuge for 1.5hr!*

- 4X250ml sterile buckets for Sorval centrifuge
- Plastic Cuvette (recycle!)
- 2XSterile 250ml graduated cylinders
- Chilled Sterile eppendorf tubes
- **500ml LB** in a 2L Erlenmeyer
- **20ml LB** in a 100ml Erlenmeyer
- **100ml LB** in a separate bottle for general use
- **500ml** chilled Glycerol 10%
- Liquid Nitrogen

### *Experiment procedure*

1. Seed bacteria on LB agar plate with antibiotics and grow O.N at 37°C.
2. Next afternoon pick a single colony and mix into a culture tube precontaining 5ml LB with antibiotics; grow O.N at 37°C shaking at 230rpm.
3. Transfer 1ml of culture into 500ml LB and incubate w/o antibiotics till the suspension reaches $O.D_{560}$=0.6-0.8 (approximately 1.5-2hrs).
4. Optional: Prepare glycerol stock from the rest of the suspension (see protocol #5)
5. Split suspension into 2X250ml buckets and centrifuge at 4060rpm, 4°C for 20' (*Don't forget to MARK the buckets before addition of suspension!).*

6. Discard of soup and resuspend pellet of one bucket in 250ml Glycerol 10%; transfer suspension and resuspend the second pellet.
7. Centrifuge at 4060rpm (2500g), 4°C for 20'; discard of soup.
8. Resuspend pellet with 125ml of Glycerol 10%.
9. Centrifuge at 4060rpm, 4°C for 20'; discard of soup.
10. Resuspend pellet with 10ml of Glycerol 10%.
11. Centrifuge at 4060rpm, 4°C for 20'; discard of soup.
12. Resuspend pellet with 1.5ml of Glycerol 10%.
13. Aliquot 50µl into 30 prechilled eppendorf tubes.
14. Flash-freeze tubes with liquid nitrogen and store at -80°C.

***Test Preps***

1. Grow competent cells without antibiotics at 5ml LB volume
2. Grow competent cells with antibiotics at 5ml LB volume
3. Perform electroporation with two different selection plasmids (Amp and Kan, for example) and plate each on half a plate of each antibiotic (2 plates).