HOST-PATHOGEN INTERACTIONS LAB
Benjamin Aroeti, Ph.D.
aroeti@mail.huji.ac.il

TITLE: ELECTROPORATION OF EPEC WITH PLASMID cDNA:

Name: Ipsita Nandi

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1. Dilute an over-night starter of bacteria (grown in the appropriate antibiotics) 1:100 into 2 mL of fresh LB (no antibiotics – to allow easy growth). Shake at 37 ºC for 2 hrs to reach a logarithmic phase (OD_{600} should be 0.3-0.5).
2. Centrifuge the bacteria for 2 minutes in 14,000 rpm and aspirate the sup.
3. Pelleted bacteria are washed 3 times in ice-cold DDW (1 ml, sterile 4 ºC) by centrifugation.
4. After the third centrifugation, all supernatant is removed, and cells are re-suspended in 80 µl of cold DDW.
5. 1-2 µl of mini-prep plasmid DNA is added, and the solution transferred to a cold and sterile 1 mm electroporation cuvette (Should be obtained from Tzabam).
6. Cells are electroporated at 1.85 kV/ 25 µF/ 200 Ohm (time constant should be ~5 millisec), using the BioRad electroporator situated at Tzabam.
7. To transfer the cells out of the cuvette, 0.5 ml of warm LB is quickly added to the cuvette. Bacteria are suspended by “up and down”, and then transferred into a snap-cap tube containing 2 ml warm LB.
8. Bacteria are allowed to recover for 1 hour at 37 °C with shaking (250 rpm).
9. Thereafter, 100µl of the cells are plated on LB agar plates containing the appropriate selection antibiotics for the insert and incubated for 16 hours at 37 °C until colonies are developed.
10. Take 1.5-2 ml of the LB with the bacteria centrifuge for 1-2 minutes in 14,000 rpm, aspirate most of the sup, leave out 100 µl of LB, resuspend the bacteria

and spread on LB agar plates containing the appropriate selection antibiotics for the insert and incubated for 16 hours at 37 °C until colonies are developed.

11. Pick 5-6 different colonies and grow as a starter with ALL appropriate antibiotics. You should look for a phenotype in 2 or 3 of picked colonies.

12. Make Glycerol Stocks.