Date: 02-JUL-09	Written by: Chen Guttman	Laboratory: Raz Zarivach
Title: Protocol#2 - Electroporation of competent cells		Page **Page 1 of 2**

Electroporation of competent cells

Aim

To transfer plasmid DNA into competent cells (see protocol#3 for electrocompetent cell preparation)

Materials & Equipment

- 1ml LB in long glass inoculation tube
- Rack for inoculation tube
- Chilled Electroporation Cuvette
- Minimum 50ng of Plasmid DNA (for ligation take a minimum of 75ng)
- Pipettors (1000µl and 100µl)
- Tips (1000µl and 100µl)
- Ice
- Competent cells

Verify availability of the Electroforation device at Amir Aharoni's lab

For later stage – LB agar plates with antibiotics.

Experiment procedure

1. Overlay plasmid/ligation product on competent cells and incubate for 10' on ice (after 5’ incubation transfer to cuevette).
2. Switch on device (right side)
3. Prepare pipettor 1000µl at 600µl volume setting with tip ready in inoculation tube
4. Wipe dry cuevette and insert into device with notch facing right side.
5. Press "Pulse".
6. Quickly remove cuevette and suspend cells with 600µl of LB medium by pipetting thoroughly.

7. Transfer suspension into inoculation tube and incubate for 1hr at 37°C, shaking for recovery.
8. Spin inoculation at 3800rpm for 3'; Resuspend with leftover of LB (approx. 100µl).
9. At the end of step 7, Seed 100µl of the inoculum onto LB agar plates with antibiotics.