Electroporation

Cell/DNA prep:

(1) Thaw frozen competent cells on ice.

Meanwhile:

(2) Chill electroporation cuvette on ice.

(3) Aliquot 1ml SOC to culture tubes (to add to the cells after electroporation).

(4) Aliquot #DNA to be transformed to m/f tube; place on ice.

(5) Mix 50-100µL of the thawed competent cells with the DNA to be transformed.

(6) Transfer competent cell/DNA mix to electroporation cuvette. Avoid introducing air bubbles into the cell mix in the cuvette as this can cause arcing during electroporation.

Electroporation:

(7) Activate electroporator by pressing the ON/STDBY key.

(8) Set the desired voltage by pressing the UP/DOWN keys. Pressing both keys simultaneously will cause the voltage to cycle between 0, 1800 and 2500. Optimum voltage for E. coli is 12,000 to 19,000 V/cm; using 0.1 cm cuvettes this means a desired voltage of 1,200 to 1,900 V. (Try 1500V or 1,800 V)

(9) Remove the electroporation cuvette containing the competent cells/DNA mix from the ice, wipe off any excess ice/moisture from the cuvette with a kimwipe and place it into the electroporator cuvette holder (the cuvettes have a notch protruding from them and as such can only fit into the cuvette holder in one direction).

(10) Slide the cuvette holder all the way back until it is flush with the front of the unit.

(11) Press the PULSE key **twice** (within 2 seconds). The "Chg" light will be displayed; after another ~5sec a "beep" will sound indicating that electroporation has occurred.

(12) **Immediately** remove the cuvette and add ~1ml SOC (or LB if you are out of SOC).

(13) Repeat the process for another competent cells/DNA mix or press the ON/STDBY key to switch the electroporator to standby mode.

(14) Outgrow (recover) the transformed cells by gentle shaking at 37°C for ~1h.

(15) *Plate a serial dilution of the transformed cells on selective medium:

(a) Transfer the cells to a 1ml m/f tube.
(b) Quick spin (~10 – 15 sec) the cells down in the m/f. Decant the s/n.
(c) Resuspend cells in 200µl. This now becomes the 10^0 dilution.
(d) Add 10µl of the transformation mix to 90µl SOC (or LB). This is 10^{-1} dilution.
(e) Add 10µl of the 10^{-1} dilution to 90 µl SOC. This is 10^{-2} dilution. Etc.

Soln's and stuff:

(1) SOC

Stock	For 100ml
(a) Bacto Tryptone	2g
(b) Yeast Extract	0.5g
(c) 5M NaCl	200µL
(d) 1M KCl	250µL
(e) 1M MgCl	1ml
(f) 1M $MgSO_4$	1ml
(g) dH_20	to 100ml
(h) A/C	
(i) 1M Glucose	**1ml (or 360µL 50% glucose)

(2) LB Medium (500 ml)

(a) Bacto Tryptone	5.0g
(b) Yeast extract	2.5g
(c) NaCl	2.5g
(d) pH with NaOH	to ~7 (I add one pellet of NaOH per 500 ml which adjusts the pH to ~7)
(e) dH_2O	to 500ml
(f) Agar	15g
(g) A/C	20-30 min

Cool media to ~50°C
Add antibiotic and pour plates (it helps if a stir bar was included in the flask when autoclaved).
Let plates cool. Invert plates after media solidifies. Incubate on bench o/n (helps plates to dry).
Store plates in 4°C. Amp plates only last 2-4 weeks. Kan will stay good for ~ year. Chl = ?

(3) Antibiotics

Prepare antibiotic by dissolving it in the appropriate solvent. Filter-sterilize each antibiotic by passing the solution through a syringe with attached 0.2µm filter sterilization unit. Collect the supernatant into sterile microfuge tubes or a 15ml sterile plastic conical tube. Antibiotics dissolved in MeOH or EtOH do not require filter sterilization. Store all antibiotics at –20°C. The final concentration of antibiotic in the media is actually dependant upon the plasmid and the host. The following are general guidelines based on frequently used plasmids and the hosts, when in doubt ask first.

Antibiotic	Solvent	Stock conc	For 10ml of stock	Final conc in media
(a) Ampicillin	H_2O	50mg/ml	0.5g	100µg/ml
(b) Chloramphenicol	EtOH	34mg/ml	0.34g	34µg/ml
(c) Gentamycin	H_2O	50mg/ml	0.5g	50µg/ml (Agro)
(d) Kanamycin	H_2O	50mg/ml	0.5g	50µg/ml
(e) Rifampicin	MeOH	50mg/ml	0.5g	
(f) Streptomycin	H_2O	300mg/ml	3.0g	10µg/ml *E. coli* 300µg/ml Agro
(g) Tetracycline	EtOH	5mg/ml	0.5g	
(h) Timenton	H_2O	300mg/ml	3.0g	300µg/ml Agro

Notes:

Read Sambrook (P 1.75) for further information.

To make competent cells see **Competent Cell** protocol.

#DNA for electroporation must essentially be free of ions otherwise arcing will occur and kill the cells. If it is a ligation mix it is wisest to either perform an EtOH ppt before electroporating or perform ligations in very small volumes (e.g., 5µl) and transform only ~1 to 2 µl. Or dilute the DNA in H_2O first.

The electroporation event heats up the cells significantly; it is wise to prechill everything (work on ice).

Be gentle with the frozen/thawed cells.

This protocol assumes use of the eppendorf Electroporator 2510 apparatus.

0.1cm cuvettes are generally used for E.coli transformation.

*__As a control__, 50ng of supercoiled plasmid DNA should yield ~10^6 to 10^8 cfu. If you transformed 50ng of supercoiled plasmid DNA into 100µl of cells you would need to dilute the transformation mix to the dilutions 10^{-4}, 10^{-6} and 10^{-6} in order to obtain 100, 10 and 1 colonies for 10^6 competent cells, respectively and 10^{-6}, 10^{-7} and 10^{-8} for ~10^8 competent cells, respectively. Ligations will be one to two orders of magnitude below this. Plate serial dilutions accordingly. Hint: it is much more accurate (and better) to pipette 10µl into 90µl than it is to pipette 1µl into 10µl.

Cuvettes may be used numerous times. Simply wash out the cuvette w/ 70% EtOH, rinse several times w/ hot water, dI water and invert to dry. Autoclaving cuvettes is not necessary but can be done if so desired (not all brands are autoclavable; I know that BioRad brand is), however this shortens their useful life span.

**Glucose is generally A/C separately from any media or solutions containing Mg^{2+} ions because Mg^{2+} causes sugars to caramelize during heating (i.e., autoclaving) and this is generally believed to reduce the quality/effectiveness of the media.