

Electroporation

1. Place 40 micro-liters of competent, electroporatable cells into cuevette.
2. Place DNA sample into cuevette. (1-3 micro-liters of ligation rxn)
3. Shake cuevette to force all cells to the bottom.
4. Set Volts at **2.5**. Make sure that:
Capacitance=25 micro-farrad
Extended capacitance=960 micro-farrad
Resistance=200 ohms.
5. Pulse by pressing both buttons at the same time. Hold until complete.
6. Add 1ml of SOC or LB to cuevette.
7. Remove media+transformed cells and shake for 45min-1hr.
8. Plate cells.

SOLUTIONS

LB Media

5 g of yeast extract
10 g of NaCl
10 g of tryptone
Add 800 ml of dH₂O; bring the pH to 7.0 with NaOH. Bring the volume up to 1 liter with dH₂O. For plates add 15 g of agar per liter.

Addition of antibiotics:

	Stock Solution(-200C) concentration	Working concentration	
		stringent plasmid	relaxed plasmid
Ampicillin	25-50 mg/ml	20µg/ml	60-100µg/ml
Carbenicillin	50 mg/ml	20µg/ml	60µg/ml
Chloramphenicol	34 mg/ml in EtOH	25µg/ml	170µg/ml
Kanamycin	10mg/ml	10µg/ml	50µg/ml
Streptomycin	10mg/ml	10µg/ml	50µg/ml
Tetracycline	5mg/ml in EtOH	10µg/ml	50µg/ml

Stock solutions of antibiotics dissolved in dH₂O should be filter sterilized through a 0.22 micron filter. Antibiotics in EtOH need not be sterilized. Store solutions in light-tight containers. Magnesium ions are antagonists of tetracycline. Use media without magnesium salts (e.g. LB media) for selection of bacteria resistant to tetracycline.

NOTE: Add filter-sterilized antibiotics to LB liquid before use. Add sterile antibiotics to cooled LB agar after autoclaving before plates are poured. These concentrations are those suggested by Sambrook et.al. Different laboratories/experiments may call for concentrations differing from those listed above.

SOC media

To 950 ml of ddH₂O add:
20 g bacto-tryptone
5g yeast extract
0.5g NaCl

Allow solids to dissolve and add 10ml of 250mM KCL. Adjust the pH to 7.0 with 5M NaOH (~ 2.0ml) . Adjust the volume to 1 liter with ddH₂O . Sterilize by autoclaving. Allow the solution to cool to 60°C or less and add 5 mls of 2M MgCl₂ (19g MgCl₂ per 100ml ddH₂O) and 20 ml of filter sterilized 1M glucose (18g glucose per 100ml of ddH₂O).

Taken from: Sambrook et.al Molecular Cloning, A Laboratory Manual; second edition. Look here for more detailed information.

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