ELECTROPORATION

1. DNA

- Linearize vector DNA or purify a DNA fragment (75 - 100 µg per experiment) from an agarose gel (Pharmacia NA) by electro-elution (see Sambrook et al. 6.28). Extract DNA with Phenol, Phenol/Chloroform and Chloroform, and precipitate with alcohol.
- Dry pellet in flow cabinet and dissolve in 300 µl sterile PBS
- Check DNA concentration by agarose gel electrophoresis of 1 - 2 µl.

2. ES cells

- Trypsinize ES cells; use 50 cm^2 per experiment.
- Wash cells with small volume of PBS.
- Add TVP and incubate 5 min at 37 °C.
- Detach cells.
- Add complete medium and resuspend to single cells with blue tip
- Count cells, approx. 4×10^7 cells are present.
- Spin down cells for 5 min at 1200 rpm at RT.
- Resuspend pellet in 300 µl of PBS.

3. Electroporation

- Mix cells (≈ 4×10^7) with DNA (≈ 50-75 µg) → ± 600 µl: 5' RT.
- Transfer mixture to 0.4 cm Biorad cuvet.
- Electroporate cells with Biorad gene pulser model 1652078:
  - Voltage 0.8 kV
  - Capacitance: 3 µF
  - 1 pulse gives a time constant of 0.1 ms
- 5' RT.
- Resuspend cells in 64 ml of 60% BRL medium and spread 8 x 8 ml in 10 cm gelatin-coated TC dishes.
- Incubate ON at 37 °C/ 5% CO_2.

<u>NOTE</u> : Electroporation can be done at smaller scale, e.g.:
. mix cells from 10 cm^2 with 25-30 µg DNA in a total volume of 200 µl PBS.
. electroporate with capacitance of 1 µF giving a time constant of 0.1.
. spread cells onto two 10 cm dishes.

4. Drug selection

- Refeed cells with 8 ml 60% medium plus appropriate drug per dish:

G418:	200 µg/ml
Hygromycin B:	150 µg/ml
L-Histdinol:	1.5-2.5 mM
HAT:	Hypoxanthine 0.1mM
	Aminopterin 0.8 µM
	Thymidine 20 µM
Puromycin:	1.8 µg/ml

- Refeed cells with selective medium every 2-3 days.
- After 6 - 8 days individual colonies can be seen.