

ES Electroporation Protocol
for homologous recombination

Plate irradiated MEFs on 10cm dishes at least 2 hours before electroporation.

Change medium to fresh ES medium 1 hour before electroporation.

Prepare 10×10^6 ES cells for electroporation by washing and trypsinizing.

Resuspend ES cells in 800 μ l 2xHEBS and add to electroporation cuvette.

Add 50 μ g linearized target DNA to the cuvette.

Wait 10 minutes (room temperature).

Electroporate at 250V, 975 μ F (BioRad machine).

Time constant should be around 23.9

Wait 5 minutes (room temperature).

Divide into 10cm dishes (about 6) with 10ml of ES medium (no need to spin and wash cells).

Allow 36 hours recovery then start selection or analyze cells for gfp.

2XHEBS:

137 mM NaCl

5 mM KCl

0.75 mM Na₂HP0₄

5.6 mM dextrose

42 mM HEPES free acid

pH to 7.05 with NaOH

sterile filter