

Linearizing gene-targeting (knockout or knockin) constructs for electroporation into ES cells

Modified, from Kayoko Kimbabra, Ginsberg Lab, August 2004

Isolate enough targeting vector/plasmid:

To get about 400ug of construct:

Culture One Liter of bacteria (LB/Amp or other selective drug)

Purify plasmid DNA by Promega Wizard Plus Megapreps (Cat #A7300)
or Qiagen Mega Kit (cat# 12181)

To get about 150ug of construct:

Culture 250mL of bacteria (LB/Amp or other selective drug)

purify with Promega Wizard Plus Maxipreps (Cat# A7270)
or Qiagen Maxi Kit (cat# 12162)

1. **Digest** 150-400ug plasmid (50ug/tube: total 3-8 tubes)

(300 ul scale digestion per tube)

50 ug construct DNA

30 ul appropriate Buffer

30 ul 10xBSA

5 ul Enzyme (NEB:20 units/ul)

-----Total 300 ul/tube

2. Mix well and incubate them for 4hrs at 37°C

3. **Test** 2ul on an agarose gel to make sure that the digestion is completed

4. **Purify** the linealized DNA

a. Add 300ul Phenol/Chloroform to each tube and shake gently

b. Spin tubes for 15"

c. Transfer the upper to a fresh tube (no contamination of organic phase)

d. Repeat steps a-c twice

(Start using a cell culture hood)

e. Add an equal volume of chloroform (no phenol) and repeat step b and c

f. Estimate the volume of the DNA solution and add sodium acetate to a final concentration of 0.25M. Mix well. Add 2 volumes of 100% ethanol.

g. Mix well (no vortex) and chill to -20°C for 1hr-O/N

h. Centrifuge at 4°C for 15min

i. Wash DNA pellet with 1ml 70-80% ethanol twice

j. Leave the tube open and dry DNA pellet inside a cell culture hood for 10-30min

5. **Collect** precipitated DNA to one tube with 100ul TE

6. **Measure** the concentration and the ratio of OD260nm:OD280nm

For example:

Concentration: 3ug/ul (total about 300ug) in TE

260/280nm: 1.764