



Preparing DNA for electroporation

Linearising DNA: Before you start

Measure the concentration of DNA in your final targeting construct DNA samples.
Prepare a 5µg sample of each final targeting construct.
Defrost BSA & NEB buffer 3. Place AsiSI enzyme on ice.

Things you'll need

AsiSI enzyme (NEB: R0630L, includes buffer and BSA)
10x Buffer 3 (NEB)
100x BSA (NEB)
37°C incubator
P20, P200 Gilson pipettes and filtered tips
MilliQ or Tissue Culture (TC) grade water
Ice

Linearising DNA for electroporation

- Decide on a final reaction volume (*Sanger uses 100µl*)
- To 5µg of the final targeting construct add:
 - 1x Buffer 3 (*10µl*)
 - 1x BSA (*1µl*)
 - 4U/µg AsiSI enzyme (*20U = 2 µl of stock enzyme at 10,000U/ml*)Add MilliQ or tissue culture (TC) grade water to the final required reaction volume
Mix well
- Incubate overnight (or a minimum of 4 hours) at 37°C.
- Store at -80°C.

DNA precipitation: Before you start

- Defrost the linearised final targeting constructs
- Set the centrifuge to chill to 4°C
- Make up 70% ethanol using 100% stock and MilliQ/TC water (*chill on ice*)
- Chill an aliquot of 100% ethanol on ice

Things you'll need

Ethanol (99.7-100% v/v)
MilliQ or tissue culture grade water
Tissue culture grade PBS
Tissue culture hood
P20, P200 Gilson pipettes and filtered tips
4°C centrifuge
Ice

DNA precipitation (*use good sterile technique throughout*)



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- Add 2-3 volumes of 100% ethanol to the linearised final targeting constructs (*e.g. 200-300µl ethanol to a 100µl digestion*)
 - Seal the tube or plate carefully to prevent evaporation
 - Incubate on ice for a minimum of 30 minutes
 - Spin for 15 minutes, 3700rpm at 4°C
 - Discard ethanol and check for evidence of precipitated DNA (*white precipitate in bottom of well*)
 - Add 200µl of 70% ethanol, spin plate for 5 minutes, 3700rpm, 4°C
 - Discard ethanol
 - Add 200µl of 70% ethanol, spin plate for 5 minutes, 3700rpm, 4°C
 - Discard ethanol
 - Add 200µl of 100% ethanol, spin plate for 5 minutes, 3700rpm, 4°C
 - Discard ethanol
 - Place samples in tissue culture hood and allow to dry for 5-10 minutes,
 - Add 110µl PBS, seal vessel, label and chill overnight at 4 degrees
 - Store linearised, precipitated DNA at -80°C until required for electroporation