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)
37ºC 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 37ºC.
- Store at -80ºC.

DNA precipitation: Before you start

- Defrost the linearised final targeting constructs
- Set the centrifuge to chill to 4ºC
- 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
4ºC centrifuge
Ice

DNA precipitation *(use good sterile thechnique throughout)*

- 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 4ºC
- 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, 4ºC
- Discard ethanol
- Add 200µl of 70% ethanol, spin plate for 5 minutes, 3700rpm, 4ºC
- Discard ethanol
- Add 200µl of 100% ethanol, spin plate for 5 minutes, 3700rpm, 4ºC
- 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 -80ºC until required for electroporation