mESC ssODN Electroporation

Adapted after WTCHG Transgenic Services.
Zoe Holloway and Philip Hublitz, WIMM, July 2017.

- IDT ssODN ultramers are supplied lyophilized at 4nmol.
- Suspend into 40µl of embryo transfer water (100mM final).
- For the electroporation we use

  ssODN (100microM) - 2 microlitres
  px459sgRNA - 5 microgram

  The total DNA volumes should not exceed 10 microliters.
  Cell suspension – 1000000 cells in 110microlites buffer.
  Selection: 2 days in Puro (concentration for JM8)
  4 days in G418 subsequently (concentration for JM8)

mESC plasmid electroporation

Adapted after WTCHG, Transgenics Service
Philip Hublitz and Zoe Holloway, WIMM, July 2017

- Plasmids are Qiagen Maxiprep grade and must have a ratio higher than 1.90
- For the electroporation we use

  pTC donor – 5 microgram
  px459sgRNA – 5 microgram

  The total DNA volumes should not exceed 10 microliters.
  Cell suspension – 1000000 cells in 110microlites buffer.
  Selection: 2 days of no selection for recovery
  4 days in G418 subsequently (concentration for JM8)