CRISPR/Cas9 plasmid transfection method for GSC and hNSC lines

General notes:
Cell culture protocols for GSC and hNSC lines are available from the GCGR website.
We use the following equipment and reagents:

- Lonza 4D-Nucleofector Core and X unit (AAF-1003B and AAF-1003X)
- SG cell line transfection kit with cuvettes (X Kit L) Lonzo V4XC-3024 or SG cell line transfection kit with microcuvettes (X Kit S) Lonzo V4XC-3032 for smaller scale transfection
- Plasmids prepped using Plasmid Midi kit (Qiagen 12143) or Mini kit (Qiagen 12123)

We use 1 x 10^6 cells for transfections, this may be scaled up or down.
We use a maximum of 4 μg of DNA for X Kit L cuvette transfections.

2 - 4 hr before transfection:
Pre-coat T25 flask (Corning Costar 430639) with Laminin for at least 2 hr 37°C (see cell culture protocol for details). Remove laminin immediately before adding conditioned media (details below).

40 min before transfection:
Bring SG cell line nucleofection buffer to room temperature. Ensure the provided supplement has been added to the buffer before use. The buffer will last 3 months from when the supplement was added.

20 min before transfection:
Prewarm cell culture media to 37°C.

Transfection:
Prepare plasmid mix (if using multiple plasmids) using as small a volume as possible, to a max 10 ul.
Use up to a total maximum of 4 ug of DNA for X Kit L cuvette transfections. For multiple plasmids, use plasmids in equal molar ratios. We have successfully used up to 8 plasmids/transfection.
Collect media from cells (conditioned media), remove laminin from the T25 flask and add 8 ml of conditioned media.
Dissociate cells using Accutase, resuspend in wash buffer and count.
Transfer 1 x 10^6 cells to new tube, centrifuge, aspirate media and resuspend cells in 100 ul SG cell line buffer.
Add cells to the plasmid mix. Flick the tube gently to mix and carefully pipette into a microcuvette avoiding bubbles.
Place the cuvette in the 4D nucleofector (position and program selected) deliver 1 pulse using the DN-100 programme.
Immediately return to TC hood and promptly add 500 ul pre-warmed complete media to the transfections and gently mix using the provided pipettes. Transfer the transfections to the flask containing 8 ml conditioned media.
Change the media the following day then every 7-10 days or until passage.