RNP 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 microcuvettes (X Kit S) Lonzo V4XC-3032 or scale up to SG cell line transfection kit with cuvettes (X Kit L) Lonzo V4XC-3024.
- tracrRNA from IDT, stock at 100 pmoles/ul
- Cas9 protein prepared in-house but can be purchased commercially

We use 200,000 cells for transfections but this may be scaled up
crRNA purchased from IDT, stock at 100 pmoles/ml, in nuclease-free water or Duplex buffer (IDT)

2 - 4 hr before transfection:
Pre-coat 6 well plate (Corning Costar CLS3516) 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.

For a single transfection mix 1.3 ul of stock crRNA + 1.3 ul of stock tracrRNA
Heat the mix at 95°C for 5 min in a PCR block. Remove and cool at room temperature for 30 min allowing annealing of crRNA and tracrRNA.

20 min before transfection:
Prewarm cell culture media/wash buffer and Accutase to 37°C.

10 min before transfection:
Mix 10 µg of Cas9 protein to the annealed cr/tracrRNA complex (keep total volume under 5 µl).
Incubate at room temperature for 10 min and then place on ice. This will form the RNP complex.

If you also need to use a donor DNA template (e.g for a tag/reporter gene knock-in) then mix 200-250 ng of double-stranded DNA (synthesised DNA block or PCR product) with 40% DMSO (keep total volume under 5 µl). Heat the mixture at 95°C for 5 min to denature the dsDNA and immediately plunge into ice. Keep on ice until transfection.

Transfection:
We use 200,000 cells for transfections (scale up if necessary).
Collect media from cells (conditioned media), remove laminin from 6-well plate and add 2 ml of conditioned media/well.
Dissociate cells using Accutase, resuspend in wash buffer and count.
Transfer 200,000 cells to new tube, centrifuge, aspirate media and resuspend cells in 10 ul SG cell line buffer.
Add cells to the crRNA/tracrRNA/Cas9 complex plus denatured DNA if using. Flick the tube gently to mix and carefully pipette into a microcuvette avoiding bubbles.
Place the microcuvette strip in the 4D nucleofector (well position and program selected) deliver 1 pulse using the DN-100 programme.
Immediately return to TC hood and promptly add 150 ul of pre-warmed complete media to microcuvette. Gently pipette up and down a few times (avoid bubbles) and add to the pre-coated 6 well plate containing 2 ml conditioned media.
Wash the microcuvette with some media to ensure all the cells have been transferred.
Change the media the following day then every 7-10 days or until passage.