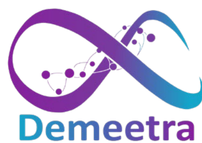


Demeetra – CleanCut GS and Harbor-IN NEON Electroporation

Prepare CleanCut cells for NEON NxT Electroporation:

- Seed CleanCut cells at $1x10^6$cells/mL 1 day prior to transfection.
- **Media:** CD-FortiCHO, 8mM L-Glutamine, and 1:1000 Anti-Clumping Agent (ACA)

Note* Cell viability should be >90%

- o The preferred range for 100ul NEON NxT tip → 1.0 x 10^6 to 2.0 x 10^6 cells

- Pre-warm culture media and aliquot into plates, keep in incubator until transfection.
- Count cells to determine the cell density of the suspension. Add 20ul of cell suspension to 20ul 0.04% Trypan Blue solution, mix by pipetting up and down 5-7 times.
- Transfer cells (number of cells per transfection) to a 1.5 mL reaction tube or 15 mL falcon tube and centrifuge the cells at 250 x g for 5 minutes at room temperature.
- Resuspend the cell pellet 5-7 times in Resuspension Buffer R considering the range information for the 100 uL Neon NxT tip
- After transfecting, immediately place the cells in a 6-well plate containing the corresponding culture media **WITHOUT antibiotics.**

Note* Avoid storing cells in Resuspension Buffer R for more than 10 minutes at room temperature.

- Demeetra recommends aliquoting cells into separate tubes according to volumes of mRNA and transposon cargo.

Note* Keep volume of Harbor-IN reagents to a minimum to maintain consistent transfection conditions (10% of total volume). Can dilute transposon in Buffer R if necessary.

Note* Cell Number accounts for extra volume of the reaction which helps to avoid air bubbles during electroporation.

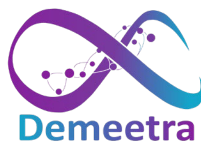
- See below table for typical reaction Harbor-IN set ups for 100 uL NxT tip. Each condition is set up for 1.1 (110ul T.V.) transfections so that no air bubbles will be present.

Recommended Condition

	NTC	Ex.1 (1:1)	Ex.2 (2:1)	Ex.3 (4:1)
Transposase	2.2ul eGFP mRNA	4ul (4ug) Harbor-IN mRNA	4.4ul (4.4ug) Harbor-IN mRNA	2.2ul (2.2ug) Harbor-IN mRNA
Transposon	-	4ug (Xul)	8.8ug (Xul)	8.8ug (Xul)
PBS/Buffer R				
Cells	2.2 x 10^6	2.2 x 10^6	2.2 x 10^6	2.2 x 10^6
Cell Volume	108ul	102ul	98ul	99ul
Total Volume	110ul	110ul	110ul	110ul

- Once all components have been aliquoted in their respective tubes, pipet up and down 3-5 times to mix and fill wells with pre-warmed culture medium and supplements **WITHOUT antibiotics** if not done so already.
  - o Recommended volumes
    - ▪ 6-well → 2mL

Set up NEON NxT Pipette Station according to manufacturer's instructions (buffer should be at RT)



- Buffer E100/E2 for 100 uL NxT tips → 2 mL for NxT Tubes or 3 mL for old version

Load the Pipette according to manufacturer's instructions

- Press the plunger on the NEON NxT Pipette to the first stop and immerse the tip into the cell-payload mixture previously prepared (see table). Slowly release the plunger to aspirate the cell-payload mixture into the NEON NxT Tip.
  - 100uL volume is a total of 2.0 x 10^6 cells per transfection (or well)
  - Avoid air bubbles as they will cause arcing during electroporation
- Dock the NEON NxT Pipette with the sample vertically into the NEON NxT Tube placed in the NEON NxT Pipette Station until you hear a click sound. Ensure that the pipette projection is inserted into the groove of the pipette station, and that the tip is submerged in electrolytic buffer.

Run electroporation protocol

- For CleanCut GS cells,
  - Run program → **1,650V, 10ms, 3 pulses for 10uL tip**
- After successful electroporation, directly add content from NxT tip to the culture plate

Allow cells to recover for 72 hours, while monitoring transfection efficiencies and/or cell viabilties.

Note* Cell viability greatly influences downstream workflows.

After 72 hours recovery, check viability, and if viability is >80% proceed with antibiotic/ glutamine selection to enrich integration-positive pool of cells for single cells cloning.

Example of CLD workflow using CleanCut GS cells and Harbor-IN

- Initial seeding and preparation are the same as above, however, for commercial or scale purposes, here are our recommendations.
- Prepare enough reagents for 4 replicate transfections, T.V. approx. 440ul
  - A single transfection is 2.2x10^6 cells, for 4 transfections, use 8.8x10^6 cells.
  - A single transfection is 2.2ug of Harbor-IN mRNA, use 8.8ug.
  - A single transfection is 8.8ug of transposon, use 35.2ug.
- Resuspend 8.8x10^6 cells in Xul → Xul = 440ul – (mRNA + transposon DNA)
  - Approx. 396ul
- Add resuspended cells to the mRNA and transposon DNA and mix by pipetting up and down 3-5 times.
- Take 100ul with the 100ul NxT Tip and perform electroporation. Add to 2mL warmed media in a 6-well plate.
- Repeat with new 100ul NxT tip for the next three electroporations.
- For CLD, there will be around 8x10^6 cells per condition. 24 hours post transfection, 1-2mL fresh media can be added. 72-96 hours post-transfection, cells from all wells can be pooled and checked for viability prior to glutamine selection. The cells can also be transferred into T25 or E125 flasks to be shaken at 90rpm or 120rpm, respectively.

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