Nucleofection Protocol.

This protocol describes nucleofection using Demeetra technologies (i.e. Harbor-IN with CleanCut GS CHO cell line).

Materials

- □ 70% Ethanol
- □ Complete basic media CD FortiCHO (Cat # A 11483-01), 8mM L-Glutamine, 1:1000 Anti-Clumping Agent (ACA, Gibco, 0010057DG)
- □ SF Nucleofector solution (LONZA kit; Cat#PBC2-02250)
- □ NF GFP Control Solution
- □ Recovery medium: Glutamine-free CD FortiCHO (Cat # A 11483-01) and 1:1000 ACA
- □ Trypan blue
- □ Hemocytometer Slides
- □ 15mL and 50mL tubes
- □ Low attachment 6 well
- □ NF Cuvettes
- □ NF pipettes
- □ Cells
- □ DNA and mRNA
- □ 1000, 200, 20, and 10 uL pipettes + tips

General Harbor-IN Protocol for Cuvettes

ID Set	Transposon plasmid (1ug/ul)	Harbor-IN transposase mRNA (1ug/ul)	EGFP mRNA (Trilink; 1ug/ul)
CleanCut GS CHO	8ug	2ug	-
CleanCut GS CHO	-	-	2ug

Note* Demeetra typically uses the cuvette protocol for Harbor-IN because they are capable of up to 10ul (10ug) of substrate volume. If using Harbor-IN for strip protocol, which is limited to 2uL of volume, adjust volume accordingly while keeping a 4:1 ratio of transposon to transposase (1.6ug to 0.4ug).

Demeetra

Detailed instructions for Nucleofection

Culturing of cells before nucleofection.

Seed cells at $1x10^6$ cells/mL 1 day prior to transfection

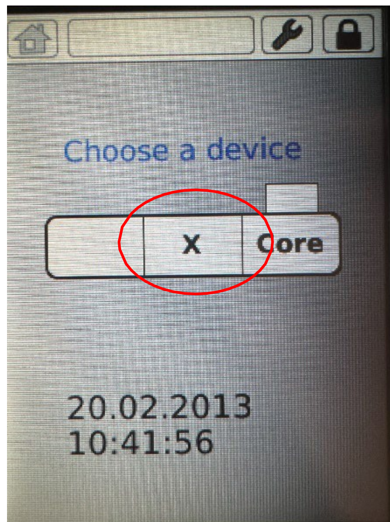
DNA or mRNA preparation

The quality and concentration of DNA or mRNA for nucleofection plays a central role for the efficiency of gene transfer. Keep all substrate on ice prior to use for NF.

Positive controls

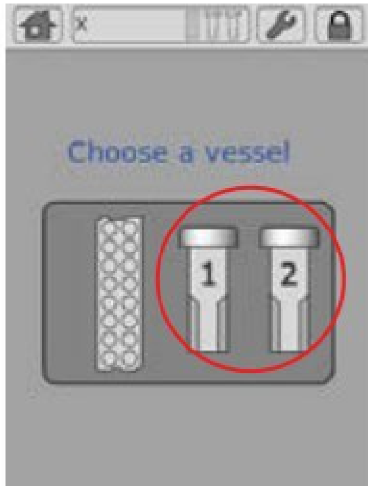
2µg for cuvettes EGFP mRNA

□ Choose X on touch screen.

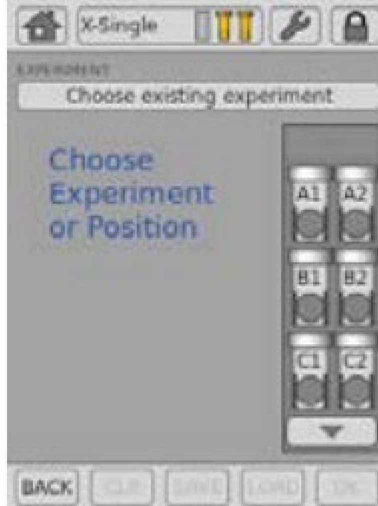


<u>Nucleocuvette Protocol</u>

□ Choose a vessel(cuvettes)



- Choose position A1 and or A2.



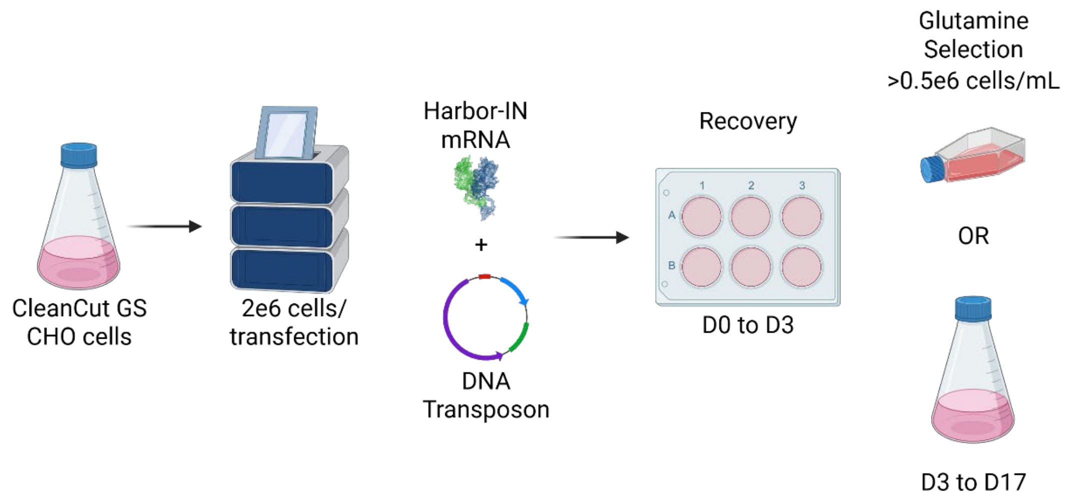
- Choose experiment.
- Choose SF program from the custom program list.
- Choose DS-137 pulse code

- Click Save, OK, OK

- ☐ Pre-warm the supplemented SF Nucleofector solution (LONZA kit; Cat#PBC2-02250) recommended by Amaxa at room temperature. **Table 1**.
- ☐ Pre-warm nucleofection (NF) medium CD FortiCHO (Cat # A 11483-01) with 8mM L-Glutamine and 1:1000 ACA.
- ☐ Prepare corresponded plates by filling the appropriate number of wells with 1.5ml NF medium and pre-incubate plates in incubator 37˚C and 8% CO_2.
- ☐ Using a serological pipette, transfer the cell suspension from E-125 flask into an 50ml sterile conical tube.
- ☐ Count cells using automatic cell counter.
- ☐ Aliquot cell suspension into 15 ml conical tubes with 2x10^6 cells per tube
- ☐ Centrifuge the 2x10^6 cells at 90xG at RT for 10 min. **Table 2**.
- ☐ Connect a Pasteur pipette to the vacuum flask tube and turn on vacuum motor
- ☐ Unscrew cap of the conical tube and carefully aspirate supernatant completely so that no residual medium covers the cell pellet.
- ☐ Resuspend the cell pellet with 100ul of SF kit/ Nucleofector solution in RT.
- ☐ Add DNA and mRNA to cell suspension in volume up to 10ul. Process each sample separately to avoid storing the cells longer than 15 min in Nucleofection Solution
- ☐ Transfer cell suspension to Amaxa certified cuvette.
- ☐ Avoid air bubbles in cuvette by tapping cuvette on BSC's floor 5 times.
- ☐ Insert the cuvette into the sample tray that the opening clip should be at the front.
- ☐ Make sure that 100ul cuvettes are mounted correctly.

- ☐ Press the ''START'' button to start the nucleofection process.
- ☐ When nucleofection process completed, vessel tray will come out.
- ☐ Transfer cuvettes to BSC and keep them at RT for 10 minutes. Add 500μl Pre-warm nucleofection medium CD FortiCHO (Cat # A 11483-01) with 8mM L-Glutamine and 1:1000 ACA to cuvette using P1000 pipette. **Table 3**.
- ☐ Resuspend three times and transfer the sample using nucleofector pipet into one well of a low attachment 6-well plate. One nucleofection cuvette per one well.
- ☐ Incubate cells in incubator with 37˚C and 8% CO_2.
- ☐ On Day 2-3 after nucleofection switch to post-nucleofection medium and scale (see below)
- ☐ Typical CLD workflows, Demeetra recommends performing 4 replicate nucleofections per condition and plating them into separate wells of a 6-well plate. 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.

Demeetra



Volume required for a single reaction

	100ul Single Nucleocuvette
Volume of NF solution	*82ul*
Volume of supplement	*18ul*

Table 1.

Required amount of cells, medium and program.

	100ul Single Nucleocuvette
CHO cells	*2x10^6*
**Control Substrate for nucleofection efficiency (EGFP mRNA)*	*2ug*
SF 4D-Kit	*100ul*
Program	*DS-137*
6-well culture plate(post-NF)	*1.5ml*
96 well culture plate(post-NF)	*-*
Culture medium to be added to the sample post-NF	*500ul*

Table 2.

*Note: For nucleofections with Harbor-IN please refer to the tables at the beginning of this protocol.

Recommended volumes for sample transfer into culture plate.

	100ul Single Nucleocuvette
Culture medium to be added to the sample post-NF	*500ul*
Volume of sample transferred to culture plate	*Complete sample (using supplied pipettes)*

Table 3.