Nucleofection Protocol.

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

Materials

- □ 70% Ethanol
- □ Complete basic media CD FortiCHO (Cat # A 11483-01), 8mM L-Glutamine
- □ SF Nucleofector solution (LONZA kit; Cat#PBC2-02250)
- □ NF GFP Control Solution
- □ Post-nucleofection medium: Glutamine free CD FortiCHO (Cat # A 11483-01)
- □ Trypan blue
- □ Hemocytometer Slides
- □ 15mL and 50mL tubes
- □ Low attachment 6 well or 96 well plate
- □ NF Cuvettes or NF Strip
- □ NF pipettes
- □ Cells
- □ DNA, RNA, or protein construct
- □ 1000, 200, 20, and 10 uL pipettes + tips

General Cas-Clover Protocol for Cuvettes

Left Guide (1ug/ul)	*Right Guide (1ug/ul)*	*Cas-CLOVER mRNA (1ug/ul)*	*EGFP mRNA (Trilink; 1ug/ul)*
2.5ug	2.5ug	2.5ug	-
-	-	-	2ug

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 and Cas-CLOVER because they are capable of up to 10ul (10ug) of substrate volume. If using Harbor-IN or Cas-CLOVER 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).

Detailed instructions for Nucleofection

Culturing of cells before nucleofection.

Passage 2 days before nucleofection

High cell densities may cause lower nucleofection efficiencies.

Low cell densities may cause high toxicity.

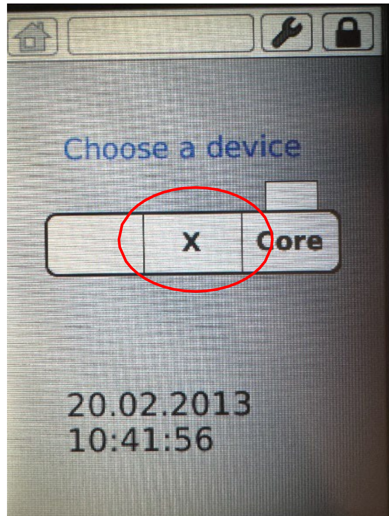
DNA or mRNA preparation

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

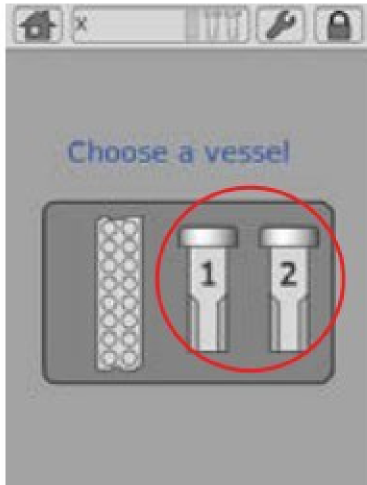
0.5µg for Strip and 2µg for cuvettes EGFP mRNA

- ◻ Choose X on touch screen.

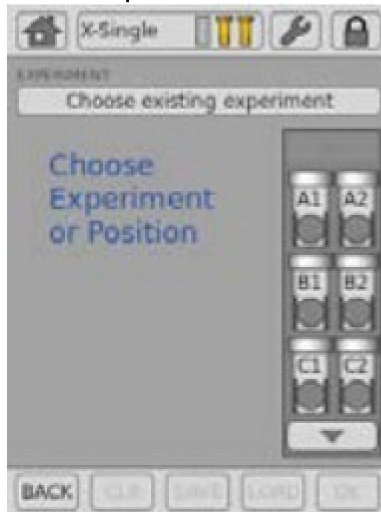


Nucleocuvette Protocol

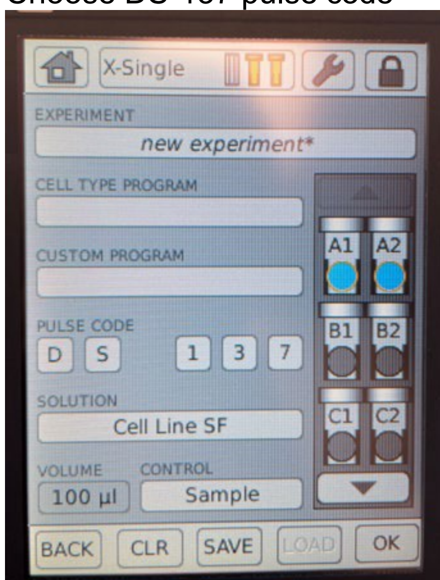
- ◻ Choose a vessel(cuvettes)



- Choose position A1 and or A2.



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



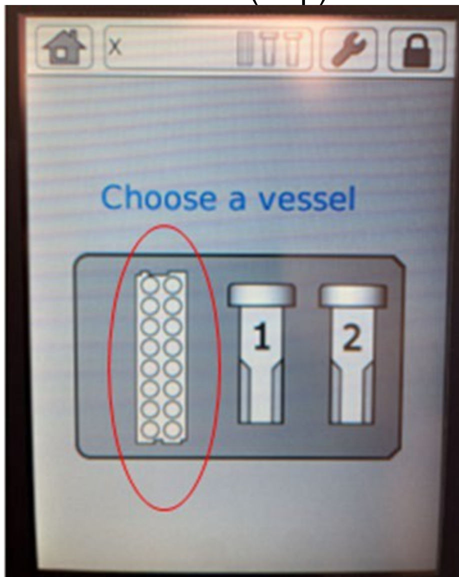
- Click Save, OK, OK

- ◻ Pre-warm the supplemented SF Nucleofector solution (LONZA kit; Cat#PBC2-02250) recommended by Amaxa at room temperature. **Table 1**.
- ◻ Pre-warm nucleofection medium CD FortiCHO (Cat # A 11483-01) with 8mM L-Glutamine.
- ◻ 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 Countess 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, mRNA or protein 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.

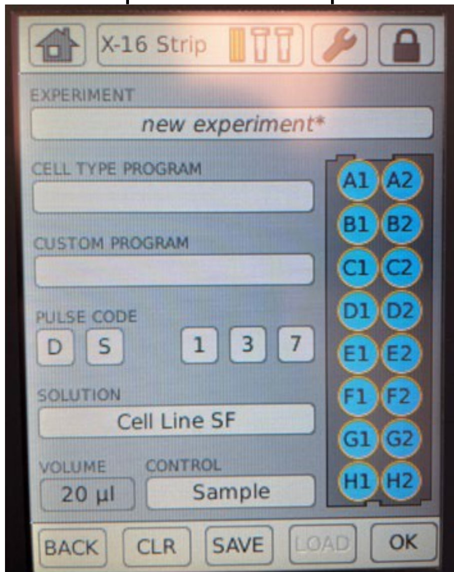
- ◻ Press the ‘’START’’ button to start the nucleofection process.
- ◻ When nucleofection process completed, vessel tray will come out.
- ◻ Transfer cuvettes to BSC and keep them at RT for 10 minutes. Add 500µl Pre-warm nucleofection medium CD FortiCHO (Cat # A 11483-01) with 8mM L-Glutamine to cuvette 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.

Nucleocuvette Strip (strip in protocol)

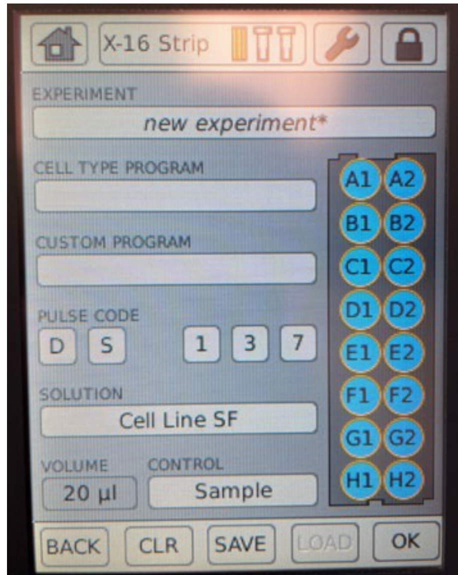
- ◻ Choose a vessel (strip)



- ◻ Choose position on strip.



- ◻ 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 complete basic media CD FortiCHO (Cat # A 11483-01) w/no L-Glutamine
- ☐ Pre-warm nucleofection medium CD FortiCHO (Cat # A 11483-01) with 8mM L-Glutamine.
- ☐ Label plates with project ID, cell line, date of nucleofection and Initials.
- ☐ Numerically label the wells according to design of experiment.
- ☐ Prepare corresponded 96 well plates by filling the appropriate number of wells with 150µl 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 Countess automatic cell counter.
- ☐ Aliquot cell suspension into 15 ml conical tubes with numbers of cells for experiment cells per tube
- ☐ Centrifuge the 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 SF kit/ Nucleofector solution required for the cells number in RT.
- ☐ Transfer 20ul cell suspension in each well of strip.
- ☐ Add DNA, mRNA or protein to cell suspension in volume up to 2ul in each well of strip. Process each strip separately to avoid storing the cells longer than 15 min in Nucleofection Solution
- ☐ Avoid air bubbles in strip by tapping on BSC’s floor 5 times.
- ☐ Insert the strip into the sample tray that the yellow pin at the rear end of the strip must be visible. If the strip is mounted in the wrong orientation its rear end will stay above the strip holder and yellow pin is hardly visible.

- ☐ Make sure that strip is mounted correctly.

C Right orientation

D Wrong orientation

- ☐ Press the ‘’START’’ button to start the nucleofection process.
- ☐ When nucleofection process completed, vessel tray will come out.
- ☐ Transfer strip to BSC and keep them at RT for 10 minutes. Add 80µl Pre-warm nucleofection medium CD FortiCHO (Cat # A 11483-01) with 8mM L-Glutamine to each well in strip using multichannel P200 pipette. **Table 3**.
- ☐ Resuspend three times. Switch to 50 µl setting of multichannel P200 pipette and transfer the sample into 96 well plate. One strip-well per one well in 96 well plate.
- ☐ Incubate cells in incubator with 37˚C and 8% CO2.
- ☐ On Day 2-3 after nucleofection switch to post-nucleofection medium.

Volume required for a single reaction

	100ul Single Nucleocuvette	*20ul Nucleocuvette Strip*
Volume of NF solution	*82ul*	*16.4ul*
Volume of supplement	*18ul*	*3.6ul*

Table 1.

Required amount of cells, medium and program.

	100ul Single Nucleocuvette	*20ul Nucleocuvette Strip*
CHO cells	*2x10^6*	*2x10^5*
**Control Substrate for nucleofection efficiency (EGFP mRNA)*	*2ug*	*0.5ug*
SF 4D-Kit	*100ul*	*20ul*
Program	*DS-137*	*DS-137*
6-well culture plate(post-NF)	*1.5ml*	-
96 well culture plate(post-NF)	-	*150ul*
Culture medium to be added to the sample post-NF	*500ul*	*80ul*

Table 2.

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

Recommended volumes for sample transfer into culture plate.

	100ul Single Nucleocuvette	*20ul Nucleocuvette Strip*
Culture medium to be added to the sample post-NF	*500ul*	*80ul*
Volume of sample transferred to culture plate	*Complete sample (using supplied pipettes)*	*50ul*

Table 3.