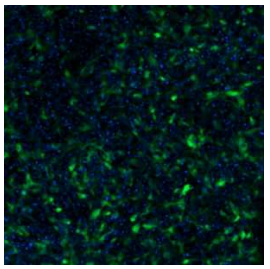
May 07, 2024 Version 2

Efficient transfection protocol of rainbow trout gills epithelial (RTgill-W1) cell line through nucleofection system. V.2



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We use this protocol and it's working

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Abstract

In this protocol, we optimize the experimental condition to express plasmid DNA in salmonid fish rainbow trout gills epithelial (RTgill-W1), using a nucleofection system. The principle of nucleofection is a combination of electrical parameters that ensures efficient DNA delivery into the nucleus, combined with low toxicity and high cell viability. Using the Neon Transfection System (Invitrogen™ MPK5000; code: 10431915) and the Invitrogen™ Neon™ Transfection System Kit 10 µl (brand: Invitrogen™ MPK1096; code: 10124334) we were able to express over 60% of GFP in RTgill W 1 line cells. These results were achieved after trying different optimization steps voltage (ranges between 850 - 1600 volts), pulse exposure (times 10 30 ms), and number of pulses, being the condition **1600 volts, 20 ms 1 pulse, and 1 µg µL of plasmid** the most effective compared to the other conditions, presenting a higher percentage of GFP expression and lower mortality rate.

Image Attribution

The image was taken by Matías Escobar-Aguirre, and corresponds to the RTgill-W1 image under a Cytation 5 microscope. Cell expressing GFP and staining the nucleus with Hoechst.

Materials

- PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Thermo Fisher Scientific Catalog #**AM9625
- Trypan Blue Solution 0.4% Sterile-filtered **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8154
- Trypsin EDTA **Gibco - Thermo Fischer Catalog #**25-051-CI.
- Leibovitz's L-15 Medium **Thermo Fisher Catalog #**11415049
- Hoechst 33342, Trihydrochloride, Trihydrate - 10 mg/mL Solution in Water **Thermo Fisher Scientific Catalog #**H3570

Equipment

NAME	TYPE	BRAND	SKU
Neon Transfection system	electroporation	Invitrogen	MPK5000; code: 10431915

Equipment

NAME	TYPE	BRAND	SKU
Neon Transfection system	Pipette 10 ul	Invitrogen	MPK1096; code: 10124334)

Safety warnings

The protocol will be completely under a laminar flow hood and for the centrifugations the tubes will be closed in order to preserve sterility.

Before start

Cells were grown as a monolayer at 20 °C without CO 2 in Leibovitz L 15 medium (Cityva, USA) supplemented with 10 % heat-inactivated fetal bovine serum (FBS) (Biological Industries, Israel) and maintained at 1 x antibiotics solution ().
The culture must be at 70 - 80% confluency to ensure the exponential growth phase of the cells.

The electroporations will be using 10 µL tips and dispensed in 500 µL of fresh media in a 48- well plate.

Cell preparation

1h

1 Before electroporation, cells should be between about **70 - 90%** confluent (i.e 6x10e^6 cells/ml in a 175 cm^2 flask). 5m

2 Wash cells monolayer 2 times with 5 mL of 1X PBS and 1 mL of trypsin 0.25%- EDTA 10m

3 Neutralize cells with growth medium 9 mL into the flask. 5m

4 Take an aliquot of trypsinized cell suspension, and register the viable total cell number by trypan blue 1:1 or similar. 10m

5 From the total cell number (step 4), split **10^5 cells/well** (48-well plates) in a new tube (i.e 4 reactions = 4 wells, we would have 4×10^5 cells). 5m

6 Centrifuge it 500 x g, 4°C, 00:05:00 to pellet the cells. 5m

7 Remove the supernatant and wash with 5 mL of 1X PBS and repit step 6. 10m

8 Remove all PBS and resuspend the cells in 10 µL per well of **Neon R buffer.** 5m

9 Prepare a **48-well plate with** 500 µL of medium WITHOUT ANTIBIOTIC where the cells will be seeded post-electroporation. 10m

NEON System Preparation

10m

10 Prepare the Neon™ electroporation tubes with 3 mL of electroporation buffer E and place it inside the Neon Pipette Station.

11 Add cells to the tube containing 1 undetermined of pEGFPC1 plasmid por well and gently mix. This plasmid encodes GFP (green fluorescent protein) as a reporter gene.

12 Insert the Neon tip into the Neon pipette (it is necessary to reach the second stop to open the clamp)

Electroporation

10m

13 Aspirate the cell/plasmidial DNA mix with the Neon tip. **BUBBLES INSIDE THE TIP SHOULD BE AVOIDED AS THESE AFFECT THE VOLTAGE TRANSFER.**

13.1 Insert the Neon pipette together with the Neon tip into the pipette station vertically until a click is heard

14 Apply 1600 volts for 00:20:00 and 1 pulse

20m

15 Carefully remove the Neon pipette from the station and immediately transfer the cells to a **48-well plate with medium WITHOUT ANTIBIOTIC** and gently mix.

16 Culture in monolayer at 20 °C without CO^2 in Leibovitz L 15 medium supplemented with 10% fetal bovine serum **without antibiotic for 24 hours. Then change to fresh culture medium.**

1d

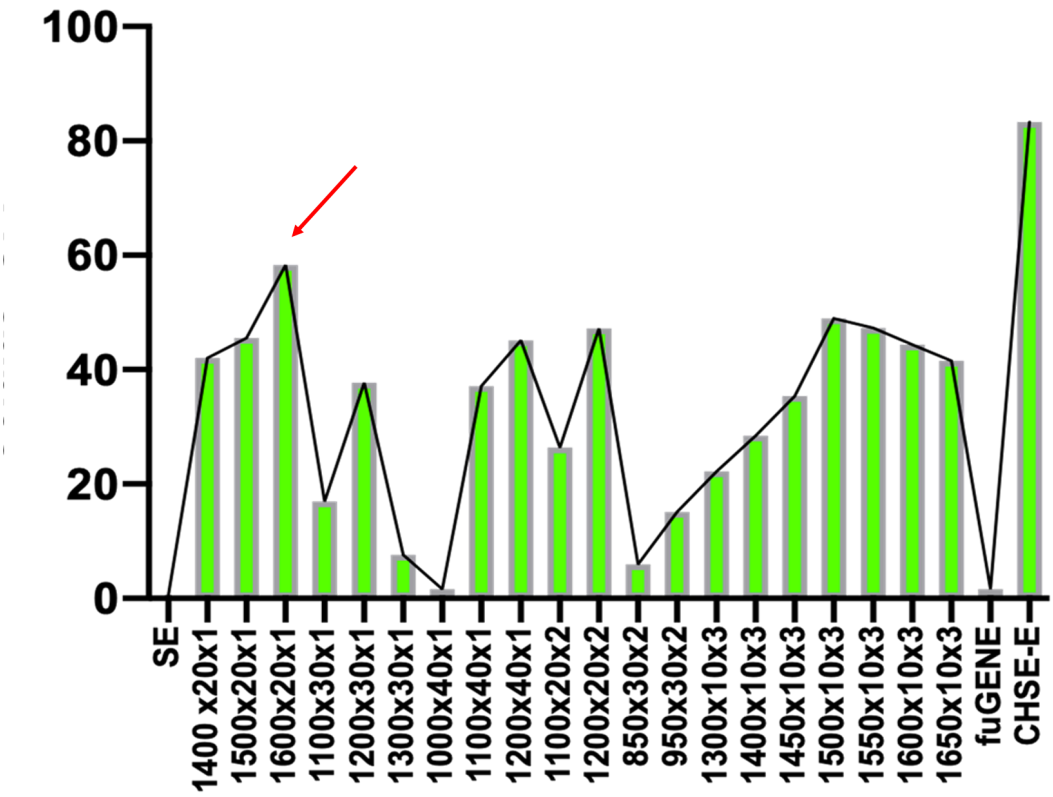
Determination of transfection efficiency

3d

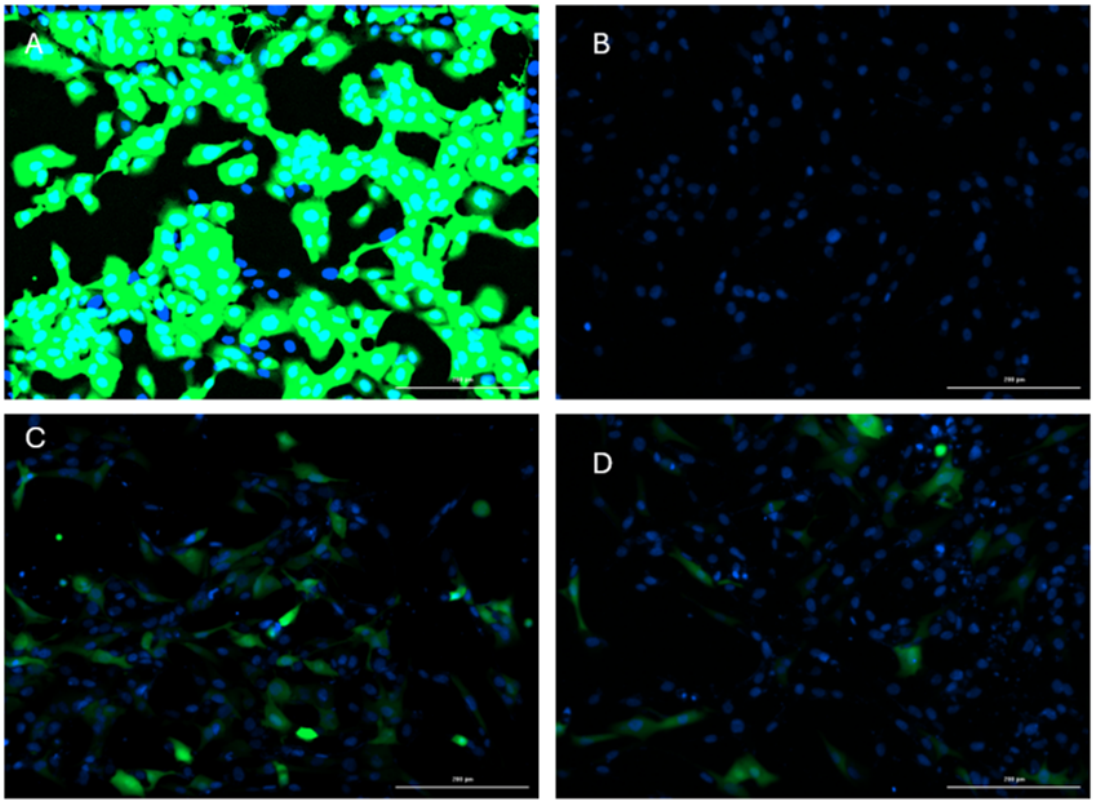
17 Quantify/Estimate the number of live cells after **72 hours** post electroporation.

3d

18 Determined transfection efficiency by measuring GFP fluorescence using the Cytation 5 platform from Agilent Technologies (excitation 469 emission 525).
It is recommended to use Hoechst staining (excitation 377 emission 447) to evaluate cell viability



Screening of cells electroporated 72 hours after electroporation with Hoechst solution. CHE−E cells correspond to cells that constitutively express GFP, as positive control.



Cells electroporated 72 hours after electroporation with Hoechst solution. **A** GFP stably expresses CHSE E cells used as GFP positive control. **B** Non-electroporated RTgill W 1 cells used as electroporation toxicity control. **C** RTgill W 1 cells electroporated using the conditions 1600 V, 20 ms and 1 pulse. **D** RTgill W 1 cells electroporated using the conditions 1500 V, 10 ms and 3 pulses

Protocol references

We would like to thank Dr. Yehwa Jin for their help in setting up the condition.