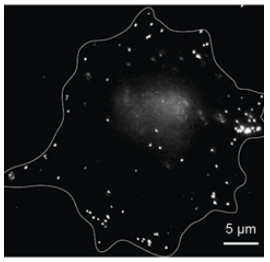
Jan 23, 2017

Electroporation of COS-7 cells and functionalization of QDs



DOI
dx.doi.org/10.17504/protocols.io.g2mbyc6

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DOI: dx.doi.org/10.17504/protocols.io.g2mbyc6

External link: http://biorxiv.org/content/early/2016/11/23/089284

Protocol Citation: Eugene A Katrukha, Marina Mikhaylova, Hugo X van Brakel, Paul M van Bergen en Henegouwen, Anna Akhmanova, Casper C Hoogenraad, Lukas C Kapitein 2017. Electroporation of COS-7 cells and functionalization of QDs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.g2mbyc6

Protocol status: Working

Created: January 23, 2017

Last Modified: March 23, 2018

Protocol Integer ID: 4909

Abstract

Detailed cell electroporation protocol from paper:
Probing cytoskeletal modulation of passive and active intracellular dynamics using nanobody-functionalized quantum dots
Eugene A Katrukha, Marina Mikhaylova, Hugo X van Brakel, Paul M van Bergen en Henegouwen, Anna Akhmanova, Casper C Hoogenraad, Lukas C Kapitein

Guidelines

From our experience, the number of incorporated QDs per cell mainly is proportional to the pouring pulse voltage, length (duration) and repetition. At the same time increase in these values leads to the overall decreased cell survival.
Another improvement can come from increasing QD concentration in the final electroporation solution. For example, by reducing Ringer's volume. But in this case air discharge can happen if electrodes are not immersed in solution.
18mm coverslips and 12 well cell culture plates can be also used.

General advices if using different electroporator:
Specific CUY900-13-3-5 cell-culture-plate electrode has 5 mm distance between electrodes. What is important, is voltage per cm value. In our case, for pouring voltage V=200 V, l=0.5 cm, i.e. V/l = 400 V/cm.

Materials

STEP MATERIALS

- PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Thermo Fisher Scientific Catalog #**AM9625
- Qdot 625 Streptavidin Conjugate **Thermo Fisher Scientific Catalog #**A10196
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Plate cells

1 Plate COS-7 cells using 24 or 25 mm coverslips and 6-well Corning Costar plates (Sigma #CLS3516) .
Perform this step one day in advance for non-functionalized QD electroporation.
If using GFP-proteins transfection, plate them 2 days in advance and 1 day in advance transfect them to allow GFP-protein expression.

Prepare QD - GFP-nanobody mix

2 One day in advance prepare mix of QD with biotinylated GFP-nanobody (bio-VHH_GFP):

Per each cell coverslip/well prepare one vial with:
- 2 µL of Qdot 625 streptavidin conjugate (Thermo Fisher #A10196, stock concentration 1 µM)
- 20 µL of bio-VHH_GFP (purified, concentration 0.7-0.8 µg/µl)
- 187 µL of PBS

In case of electroporation without GFP transfection, prepare 2 µL of Qdot 625 with 198µL PBS mix immediately before step 5.

Instead of biotinylated GFP-nanobody purification, one can perform biotinylation of commercially available GFP-nanobody (Chromotek gt-250)
http://www.chromotek.com/products/nano-traps/gfp-trap/gfp-binding-protein/

PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Thermo Fisher Scientific Catalog #**AM9625

Qdot 625 Streptavidin Conjugate **Thermo Fisher Scientific Catalog #**A10196

Incubate QD - GFP-nanobody mix at RT

3 Incubate vials for 1 hour at room temperature.
01:00:00

Incubate QD - GFP-nanobody mix at 4C

4 Incubate vials at 4°C overnight (~12 hours)
12:00:00

Preparation of electroporation (plate

5 Pre-heat Ringer's solution (10 mM Hepes, 155 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, 2 mM NaH2PO4, 10 mM glucose, pH 7.2) at 37C water bath.

In the absence of Ringer's, cell culture medium or PBS can be used, but with lower electroporation efficiency.

In one well of new 6-well Corning plate add 1.8 ml of warm Ringer's solution and 200 µl of QD - GFP-nanobody mix.

It is convenient to put 70% ethanol and water in the neighbor well to wash electrode.

Preparation of electroporation (Electroporator)

6 We used NEPA 21 electroporator
http://www.nepagene.jp/e_products_nepagene_0001.html
with CUY900-13-3-5 cell-culture-plate electrode
http://www.nepagene.jp/e_products_nepagene_0008.html

Setup electric pulse parameters:
For poring pulse:
Voltage (V) 200
Length (ms) 5
Interval (ms) 50
Number 2
Decay Rate (%) 10

For transfer pulse:
Voltage (V) 50
Length (ms) 50
Interval (ms) 10
Number 10
Decay Rate (%) 40

Make sure your electrode is not covered with rust, clean it.

Preparation of electroporation (Cells)

7 With tweezers remove a coverslip with cells and put it into plate with well with warm Ringer's and QD-nanobody mix.

Preparation of electroporation (impedance check)

8 Insert electrode into well with cells coverslip, immersing it in Ringer's solution, so it touches the coverslip.
Press **Ω** button to measure impedance.
Measured impedance will be displayed in the Impedance indow (in kΩ).
Make sure that it is less then 1 kΩ (0.1-0.2 is fine).
If values of impedance around 30-60 kΩ, add more RInger's solution.

This step is to make sure that all surfaces of electrode are fully immersed into water and electroporator will not discharge into air.

Electroporation (1)

9 Once impedance is below threshold, press the "Start" button (or pedal, if it is equipped) and hold electrode still for the program to finish (1-2 seconds).

During charging and pulsing, a warning beep will sound. When program is complete, a short double beep will sound.

Electroporation (2)

10 Rotate electrode 90 degrees around its axis, measure impedance (step 8) and run electroporation program again ('Start' button/pedal).

This step sends charge in the perpendicular direction, increasing electroporation efficiency.

Cells wash

11 Using tweezers, move cells to an empty well of 6-well plate and wash it 3-4 times with warm PBS or cell culture medium to remove QD in solution.

Electrode wash

12 Wash electrode in neighbor wells (or any other volume) in water, ethanol and another round of water. Dry it with a napkin and wrap in plastic to prevent rusting.

Cell mounting for imaging

13 Mount cells in any live-imaging chamber and they are ready for microscope stage!

Maximum mobility is usually reached in 15-20 minutes, since QDs need to penetrate outer actin level (see paper for details).

Next well

14 For the next well with cells use another (fresh) vial of QD/nanobody mix.