Transfection of HeLa cells

For transient transfection of HeLa cells, we used either electroporation or lipofection. For both, HeLa cells grown on a 100 mm cell culture dish in growth medium were harvested for the experiment (see protocol “Passaging of HeLa cells”, steps 1-12).

Electroporation

For electroporation, we used the Ingenio® EZporator® Electroporation System (Mirus Bio) and self-prepared electroporation buffer (see protocol “Preparation of Electroporation buffer) as described in [1].

Material

- HeLa cells to be transfected
- Growth medium (see protocol “Preparation of growth medium”)
- Electroporation buffer
- Plasmid DNA
- 24-well plate
- Electroporation cuvette
- Electroporator

Procedure

All steps are performed in a sterile environment – we always worked within a biosafety cabinet, but using a laminar floow hood or clean bench is also possible.

1. Add 1 mL growth medium per well of a 24 well plate.
2. Harvest HeLa cells as described in the protocol “Passaging of HeLa cells”, steps 1-11.
3. Resuspend cell pellet in 0.5 mL electroporation buffer by pipetting up and down.
4. Count cells as described in the protocol “Counting cells”.
5. Further dilute cell suspension to a concentration of $3 * 10^6$ cells/mL using electroporation buffer.
6. For each plasmid DNA to be introduced into the cells, prepare an Eppendorf tube containing 250 µL cell suspension (equal to $7.5 * 10^5$ cells) and 5 µg DNA and mix well.
7. Transfer transfection mix to electroporation cuvette, thereby avoid producing bubbles.
8. Cap cuvette and transfer to electroporator.
9. Electroporation is performed at a voltage of 260 V.
   Note: Foaming is usual.
10. Gently pipette electroporated cell suspension up and down, rinsing especially the electrodes.
11. Transfer 40 µL of electroporated cell suspension (corresponds to $1.2 * 10^5$ cells) to one well of the prepared 24-well plate.
12. Incubate cells at 37°C, 5 % CO_2 and a humidified atmosphere.

Lipofection

For lipofection, we used ViaFect™ Transfection Reagent (Promega).

Material

- Growth medium (see protocol “Preparation of growth medium”)
- ViaFect™ Transfection Reagent
- OptiMEM
- Plasmid DNA
- HeLa cells to be transfected
- 48-well plate

Procedure

All steps are performed in a sterile environment – we always worked within a biosafety cabinet, but using a laminar floow hood or clean bench is also possible.

Seeding of HeLa cells on the day before transfection

1. Harvest HeLa cells as described in the protocol “Passaging of HeLa cells”, steps 1-12.
2. Count cells as described in the protocol “Counting cells”.
3. Per well of a 48-well plate, seed 7 * 10^4 cells in a total volume of 300 µL growth medium.
   Note: Making a cell suspension master mix makes things easier! If seeding of 48 wells is desired, prepare master mix for 50 wells:
   Dilute
   50 * 7 * 10^4 cells = 3.5 *10^6 cells
   in a total volume of
   50 * 0.3 mL = 15 mL.
   Mix well by inverting tube a few times.
   Add 300 µL of this cell suspension to each well.
4. Incubate cells at 37°C, 5 % CO_2 and a humidified atmosphere over night to let cells adhere.

Transfection of adherend HeLa cells

1. Warm up ViaFect Transfection Reagent and OptiMEM to room temperature and thaw plasmid DNA.
2. For each plasmid DNA to be introduced into the cells, prepare two Eppendorf cups A and B.
3. Use the following amounts per well to be transfected:
   - **solution A**: Add 0.6 µL ViaFect™ Transfection Reagent in a total volume of 15 µL OptiMEM.
   - **solution B**: Add 2.0 µg plasmid DNA in a total volume of 15 µL OptiMEM.
     Note: Depending on the plasmid DNA concentration, DNA might have to be pre-diluted in OptiMEM. We used 1:10 pre-dilutions in most cases.
4. Let stand at room temperature for 5 minutes.
5. Add all of solution B to solution A. Mix by gently pipetting up and down once.
6. Let stand at room temperature for 20 minutes.
7. In the meantime, change media of HeLa cells seeded the day before.
   - Remove and discard old media.
   - Add 270 µL of fresh growth medium per well.
8. Add 30 µL of the transfection mix dropwise to the cells. Do not pipette up and down.
9. Incubate cells at 37°C, 5 % CO_2 and a humidified atmosphere.

Notes

- Always wear a labcoat and gloves.
- Clean the hood, your gloves and all the material which you are about to use with 70 % ethanol before starting your work/putting consumables under the sterile hood to prevent any possible contamination.
- Only open the consumables within a sterile environment to keep them sterile and to prevent any possible contaminations.

Citations

[1] Chicaybam, L., Barcelos, C., Peixoto, B., Carneiro, M., Limia, C. G., Redondo, P., Lira, C., Paraguassú-Braga, F., Vasconcelos, Z. F., Barros, L., & Bonamino, M. H. (2017). An Efficient Electroporation Protocol for the Genetic Modification of Mammalian Cells. *Frontiers in bioengineering and biotechnology*, *4*, 99.