Technic: HEK293T cells (transient) transfection

Goal: Inserting DNA (e.g. a plasmid) into a host cell to make it express a protein of interest.

⚠️There are no standard protocols for transfection, as this depends on the transfection agent used.

However, all cells intended for transfection were seeded the day before at 50% to achieve a final confluence of 60 to 80%. All transfections were performed in the absence of antibiotics in accordance with recommendations. We tested several ratios [DNA:transfection agent] for each agent, keeping a batch of untransfected cells as a negative control.
We used DreamFect™ from Oz Biosciences, jetPEI® Polyplus transfection (ref 10110N), and IcaFectin®441 from InCellArt.We followed the supplier's protocols and tested several ratios [DNA:transfection agent] for each agent.

We also used Polysciences' 1mg/mL linear 25kDa PEI (ref 23966-100), provided by the IBS (Structural Biology Institute in Grenoble), using the following protocol.

All analyses were performed 24 hours and/or 48 hours after transfection.
We took pictures of the cells in white light and in fluorescence and then proceeded to count the cells of the two conditions by hand, to calculate the transfection rate.

Procedure:

The recommended ratio being [1:2], we also tested [1:3], [1:4], and [1:6]. The final transfection volume is 500µL in 24 well plates, so the transfection mix must have a final volume of 100µL.

1. Prepare PEI solutions in 1.5 mL tubes according to the tested ratios. Vortex 3 x 1 sec.
   For example, for the [1:2] ratio, we took 1µL of PEI at 1µg/µL to which we added 49µL of serum-free DMEM medium (qs 50µL).

2. Prepare the DNA solutions in 1.5mL tubes according to the tested ratios. Vortex 3 x 1 sec.
   For example, for the [1:2] ratio, we took 2.1µL of DNA at 240ng/µL and added 47.9µL of serum-free DMEM medium (qs 50µL).

3. Transfer the PEI solution to the DNA solution (following this order carefully) and vortex 3 x 1 sec.
4. Incubate for 3 min at room temperature.
5. Gently add 100 µL of the PEI/DNA mix to a well of a 24-well plate containing 400µL of DMEM medium with serum but without antibiotics.

6. Incubate at 37°C for 24 to 48 hours.

Reagents and Solutions:
- Polysciences' 1mg/mL linear 25kDa PEI (ref 23966-100)
- DMEM serum-free medium
- DNA (for example, the plasmid coding for the protein of interest)

Equipment:
- Eppendorf tubes
- Serological pipettes
- Pipetboy
- Micropipette and tips

Estimate time: ~1h

Controls and Troubleshooting for every transfection reagents:

As a negative control, you need to create a condition of non-transfected cells, which will have undergone the same changes in conditions (with the exception of the transfection step) as the transfected cells.

To give you an idea of the scale involved, here are the results we obtained with each of the transfection agents tested with HEK293 adherent cells:
- DreamFect™ from Oz Biosciences : 30-50%
- IcaFectin®441 from InCellArt : 13%
- Polysciences' 1mg/mL linear 25kDa PEI : 6%
- jetPEI® Polyplus : 0%

If you wish to analyse proteins in the medium (and also to improve cell viability), we recommend you to change the medium containing the transfection reagent several hours after transfection. For us, that was 6 hours with DreamFect™ but it depends on the reagent.

When the medium turns yellowish, check:
- Cell confluence: they might be too crowded. This can happen quickly as you inoculate the cells at a high confluence.
- Possible contamination. This can be seen with the naked eye or under the microscope. Perform additional tests if nothing is visible after a few days.