Reverse transfection:

Before the experiment: Prepare an Excel file with all the calculations for the experiment.

Materials:

- 80% confluent culture vessel containing the cells.
- Complete growth medium, prewarmed to 37° C.
- Phosphate-Buffered Saline (PBS), prewarmed to 37° C.
- Dissociation reagent such as trypsin, prewarmed to 37° C.
- Polyethyleneimine (PEI).
- Low serum medium such as Opti-MEM.
- DNA plasmids in the desirable concentration.

- **The amount of materials suitable for a 10 cm^2 plate.**

Protocol:

1. Clean the hood and all the material you will use with 70% ethanol spray.
2. Prepare the amount of DNA needed in a 1.5 ml Eppendorf.
   ** In case of co-transfection the total amount of DNA split equally between two plasmids.

3. Remove and discard the spent cell culture medium from the culture vessel, using a vacuum pump.

4. Wash cells using PBS (approximately 5 mL per 10 cm2 culture surface area). Gently add the wash solution to the side of the vessel and rock the vessel back and forth several times.

5. Remove and discard the wash solution from the culture vessel.

6. Add 2 mL trypsin enzyme to the center of the plate. Gently rock the container to get complete coverage of the cell layer.

7. Incubate the culture vessel at 37° C for approximately 2 minutes.

8. Observe the cells under the microscope for detachment. When ≥90% of the cells have detached, return the plate into the hood and add 8 mL of complete growth medium to the plate.

9. Disperse the medium by pipetting over the cell layer surface several times. Move all 10 mL of cells to a 15 mL falcon and continue to pipet to avoid spores.

10. Take 10 µl from the cells suspension and mix with and 20 µl of blue dye in an Eppendorf.
11. Count the cells under a microscope using hemocytometer.
12. Dilute the cell suspension stock with complete growth medium to the desired concentration.
13. According to the wanted ratio of DNA:PEI, Mix PEI and OptiMEM and wait 5 minutes
14. Carefully add the PEI-OptiMEM mix on top of the DNA and mix well.
15. Wait 15 minutes for the DNA:PEI complexes to form.
16. Add the needed volume from the DNA:PEI mix to each well in the transfection plate (6 wells plate, 96 wells plate etc.)
17. Add the needed volume of cells to each well in the plate, rock the plate back and forth.
18. Incubate the plate at 37° C.