
**Plasmid DNA preparation:** Grow E.coli cells transformed with pF/R-wt plasmid DNA in appropriate volume of LB or 2XYT media with ampicillin overnight and isolate DNA using standard Qiagen Mini or Maxiprep protocol.

**Cells:** Plate HeLa cells (or other appropriate cell culture) on the day before transfection. Incubate the cells at 37°C in a CO2 incubator overnight. For the maximal transfection efficiency, calculate the number of cells plated to obtain 80-90% confluence (~1.1 x 10^6 HeLa cells per well in 6-well plate in 2 ml of the appropriate growth medium supplemented with serum). For some cell lines, optimal cell density may vary.

All subsequent steps are for the transfection of cells seeded in 6-well plates. Amounts of DNA, liposomes and media must be adjusted for the other plate formats.

1. For each well, dilute 2 μg of DNA in 0.5 ml of growth medium without serum and mix.
2. For each well, dilute 10 μl of Lipofectamine 2000 (Invitrogen) in 0.5 ml of growth medium without serum and mix. (Different types of transfection reagents can be used for transfection, optimize the amounts of DNA and liposomes for each type).
3. Combine diluted DNA and Lipofectamine, mix and incubate at room temperature for 20 min.
4. Wash cell monolayers with 1 ml of prewarmed growth medium without serum.
5. Overlay the diluted DNA-liposome complexes (1 ml per well) onto rinsed cells and incubate for the desired time at 37°C in a CO2 incubator.
6. Following 5 h incubation, add 1 ml of growth medium containing twice the normal concentration of serum without removing the transfection mixture.
7. Assay cell lysates at desired time after transfection for the luciferase expression using Dual-Luciferase Assay System (Promega) according to manufacturer’s instructions.