Protocol A (*Seed COS cells 16 to 20 hours earlier at 8 X 10^5 per 100 mm diameter dish).*

1. Prepare cells by plating the day before the transfection .

2. Prepare the wash solution (1×PBS(phosphate-buffered saline)).
Warm wash solution and the cationic graft-copolymer to 37 □.

3. Using the 10 ×PBS prepared, dilute to a 1×solution. Prepare transfection solutions as outlined:
100mm plate: In a sterile tube, dilute 20 μg of DNA (plasmid corded Luciferase activity) to 540μl in 1×PBS. Add 28μl of the cationic graft-copolymer having the 10mg/ml as the starting polycation to the DNA solution(Do not reverse its order). Tap the tube to mix.

4. Remove culture medium* from the cells. Wash cells twice with 2×10ml per 100mm plate.

5. Add the mixture between DNA and the cationic graft-copolymer to cells. Swirl plate to distribute †.

6. Incubate plates at 37℃ for 30 minutes with occasional rocking.

7. Gently add 6ml of growth medium* per 100mm plate.
Incubate for up to 2.5 hours or until cytotoxicity is apparent.
Change medium. Cells are generally ready to harvest 48-72 hours post- transfection † and assayed for luciferase activity.

8. The luciferase activity was determined by a Luciferase assays kit.
For example, using a kit of Promega(Promega,Madison,WI) and a Turner model TD-20e luminometer, the luciferase activity was reported in Turner light units(TLU). Cells were lysed in the culture plate wells with 200μl of lysis buffer per well and the cell lysates transfered to microfuge tubes. The cell lysates were centrifuged to pellet insoluble cellular debris and 20μl aliquots of the cell lysates were assayed in 100μl of luciferase activity.
The approximation of a Turner light units(TLU) was done by assaying serial dilutions of recombinant luciferase (cat. # E170A,Promega,Madison,WI) as recommended.

*Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 0.1 mM nonessential amino acids, 5 mM l-glutamine, and antibiotics (100 μg/mL streptomycin, 100 U/mL penicillin)

† An aq. solution of cationic graft-copolymer having a thixotropy property, a strong sheer stress is needed for its solution to flow and wet the cell. It is better to use Incubator shaker.

† If necessary, 0.1μ l －5μ l of SDS supplement for toxi-blocking can be added to medium in 3-7 hours post- transfection until cytotoxicity is apparent.

Protocol B (Seed HEK293 cells 16 to 20 hours earlier at 3 X 10^5 per 35 mm diameter dish)

1. Prepare cells by plating the day before the transfection
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2. Prepare the wash solution (not supplied; either 1×PBS or 1×HBSS)
Warm wash solution and the cationic graft-copolymer 37 □.

3. Using the 10 ×PBS prepared, dilute to a 1 ×solution. Prepare transfection solutions as outlined:
Per 35mm plate: In a sterile tube, dilute 10μg of DNA to 270μl in 1×PBS. Add 14μl of the cationic graft-copolymer having the 10mg/ml as the starting polycation to the DNA solution(Do not reverse its order). Tap the tube to mix.

4. Remove culture medium* from the cells. Wash cells twice with 2×2.0ml per 35mm plate.

5. Add the DNA/ (the cationic graft-copolymer) mixture to cells. Swirl plate to distribute †.

6. Incubate plates at 37℃ for 30 minutes with occasional rocking.

7. Gently add 3.0ml of growth medium* per 35mm plate.
Incubate for up to 2.5 hours or until cytotoxicity is apparent.
Change medium. Cells are generally ready to harvest 48-72 hours post transfection ‡
and assayed for transfection activity.

*Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 0.1 mM nonessential amino acids, 5 mM l-glutamine, and antibiotics (100 μg/mL streptomycin, 100 U/mL penicillin)
† An aq. solution of cationic graft-copolymer having a thixotropy property, a strong sheer stress is needed for its solution to flow and wet the cell. It is better to use Incubator shaker.
‡ If necessary, 0.1μl －5μl of SDS supplement for toxi-blocking can be added to medium in 3-7 hours post- transfection until cytotoxicity is apparent.