13. Protocol for Cell transfection and stimulation

·Material

24-well cell culture plates
DMEM
polyethylenimine (PEI) solution (1 mg/mL)
Vortex oscillator

·Steps

① Seed approximately 5 x 10^4 cells into 24-well cell culture plates.
② Culture for 16 h before transfection.
③ Total plasmid mixes of 500 ng per well are mixed thoroughly in serum-free DMEM before a polyethylenimine (PEI) solution (1 mg/mL) is added into the plasmid mixture in a ratio of 1:3 (plasmid weight/PEI weight).
④ The plasmid–PEI mixture is vortexed and incubated at room temperature for 15 min. The mixture is then added into the cells and incubated for at least 6 h.
⑤ Cells are then changed into fresh medium and applied with stimulus for before sampling and analysis assay.

·Note

To validate and optimize the gene circuits to reach the intended functionality and performance with high throughput, transient transfections are preferred. Also, reporters that are easy to be detected (such as SEAP and Luciferase) are also preferred during this process to evaluate the dynamics of the circuits.