AON transfection with Lipofectamine 2000/3000

Using this protocol, we have successfully transfected AONs into the following cell lines: A549, Hela, RPE, THP-1, RS4:11, SHSY5Y, SEM, LnCap, PC-3 and U87MG.

AON transfection for adherent cells (2-days protocol)

1. 24h before transfection, seed 150,000-250,000 cells/well (in 2 ml growth medium) in 6-well plate and incubate overnight. Cells shall reach 40-60 % confluence by the time of transfection.
2. Replace the medium with growth medium without antibiotics before transfection (1.8 ml/well).
3. Prepare the 2 transfection mixes in 2 separate Eppendorf tubes or falcon tubes
    a. 100 µl Opti-MEM medium + 2 µl AON (from 100 µM stock, for a final concentration of 100 nM)
    b. 100 µl Opti-MEM medium + 2-5 µl Lipofectamine 2000 or lipofectamine 3000 (Different ratio of AON : Lipofectamine must be tested to determine the optimal ratio for different cell lines)
4. Mix a. in b. gently.
5. Leave the transfection mixture for 20 minutes at room temperature.
6. Add dropwise the 200 µl mixture into the well with cells.
7. (Optional) Medium can be changed 5 hours after transfection.

AON transfection for adherent cells and suspension cells (1-day protocol)

1. Seed 150,000-300,000 cells/well (in 1.8 ml growth medium without antibiotics) in 6-well plate.
2. Prepare the 2 diluted reagents in 2 separate Eppendorf tubes or falcon tubes
    a. 100 µl Opti-MEM medium + 2 µl AON (from 100 µM stock, for a final concentration of 100 nM)
    b. 100 µl Opti-MEM medium + 4-5 µl Lipofectamine 2000 or lipofectamine 3000 (Different ratio of AON : Lipofectamine must be tested to determine the optimal ratio for different cell lines)
3. Mix a. in b. gently.
4. Leave the transfection mixture for 20 minutes at room temperature.
5. Add dropwise the 200 µl mixture into the well with cells.

AON transfection with Lipofectamine RNAiMAX

Using this protocol, we have successfully transfected AONs into LnCap and PC-3 cells with higher efficiency as compared to Lipofectamine 2000 or 3000.

1. 24h before transfection, seed 150,000-250,000 cells/well (in 2 ml growth medium) in 6-well plate and incubate for overnight.
2. Replace the medium with Opti-MEM medium (700 µl/well) before transfection.
3. Prepare the 2 diluted reagents in 2 separate Eppendorf tubes or falcon tubes
    a. 150 µl Opti-MEM medium + 1 µl AON (from 100 µM stock, for a final concentration of 100 nM)
    b. 150 µl Opti-MEM medium + 2 to 4 µl of RNAiMAX (optimization must be performed)
4. Mix a. in b. gently.
5. Leave the transfection mixture for 10 minutes at room temperature.
6. Add dropwise the 300 µl mixture into the well with cells.
7. Change to normal growth medium 6 hours after transfection or the day after.