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LipoMag for CRISPR/CAS9 – Genome Editing

Using expression plasmids on primary and hard-to-transfect cells

The following protocol is given for a single well from a 24-well tissue culture plate containing ~1x10^5 cells/well in 400 µL complete culture serum.

If a different culture plate format is used, adjust cell number and reagent amounts according to the table below:

Tissue Culture Dish	Cell Number per well	DNA Quantity (µg)	Total transfection volume per well
96-well	0.5 – 2.0 x 10^4	0.125	0.2 mL
24-well	0.5 – 1.0 x 10^5	0.5	0.5 mL
12-well	1.0 – 2.0 x 10^5	1	1.0 mL
6-well	2.0 – 4.0 x 10^5	2	2.0 mL
60 mm dish	0.5 – 1.0 x 10^6	5	4.0 mL
90-100 mm dish	1.0 – 2.0 x 10^6	10	8.0 mL
T75 flask	2.0 – 5.0 x 10^6	15	12.0 mL

Table 1: recommended cell number, DNA quantity and transfection volume.

Key parameter before beginning the procedure:

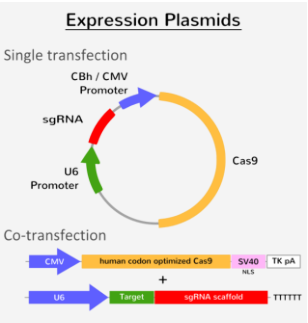
- The vector (DNA, gRNA, mRNA, protein, viral particles) and transfection reagents solutions should have an ambient temperature and be gently vortexed prior to use.
- All the complexes must be prepared in medium without serum and supplement.
- It is not recommended to use RPMI during complex preparation, prefer DMEM or PBS.
- For sensitive cells, medium can be replaced with fresh complete culture medium 4 to 6h after transfection or right after magnetofection procedure.
- The use of Magnetofection technology allows performing sequential transfections: repeat the same protocol 24h after the first transfection assay for: (1) enhancing gene expression in transfected cells and (2) transfecting more cells (3) improving genome editing capacities.

NOTES: DreamFect Gold (**DG**) and CombiMag (**CM**) should be stored respectively at -20°C and +4°C. Use **3 µL** of DG and **1 µL** of CM per µg DNA (ratio 3:1). DNA and DG will be diluted in **50 µL** of medium without any supplement (DMEM) and mixed together before addition onto CM nanoparticles.

1. **DNA solutions**

   A. Single transfection: one Plasmid encoding for both guide RNA and Cas9 endonuclease. Dilute **0.5 µg** DNA in **50 µL** of DMEM without any supplement.

   B. Co-transfection: two plasmids encoding for guide RNA and Cas9 endonuclease. Prepare a mix of gRNA and Cas9 encoding plasmids in a **0.25:1** to **1:1** ratio (respectively gRNA to Cas9) for a final quantity of **0.5 µg** total DNA in **50 µL** DMEM without any supplement.



Expression plasmids encoding Cas9 gene and sgRNA cassette

2. **DreamFect Gold solution:** Add **1.5 µL** of DreamFect Gold in **50 µL** DMEM without any supplement.
3. **CombiMag Solution:** Add **0.5 µL** of CombiMag in a new tube.
4. **Complexes preparation**
   A. Mix the DNA suspension with DreamFect Gold solution, gently pipette up and down several times.
   B. Incubate the mixture for **5 min** at room temperature.
   C. Add the complexes onto CombiMag and mix gently by pipetting up and down.
   D. Incubate the mixture for **20 min** at room temperature.
5. **Transfection**
   A. Add the magnetic complexes dropwise onto the cells and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.
   B. Place the cells on the magnetic device and incubate at **37°C** for **30 min**.
   C. After **30 min** of incubation, remove the magnetic plate.
   D. Incubate the cells under your standard culture conditions for **24** to **72 h**.