Application Note for RmesFect Genome Edition using the CRISPR-Cas9 system

IMPORTANT NOTES – Before you begin

- ✓ RmesFect must be stored at -20°C. Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ **Medium or buffer without serum & supplement** must be used for the preparation of the complexes. Culture medium such as DMEM or OptiMEM or buffers such as HBS or PBS are recommended.
- ✓ Dilute RmesFect with deionized water for doses less than 1µL.
- ✓ For sensitive cells, medium can be replaced with fresh complete culture medium 4 to 6h after transfection or right after the magnetofection procedure.
- ✓ Cells should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. The optimal confluence has to be adjusted according to the cells and the vessel used. We recommend using regularly passaged cells for transfection. Do not use cells that have been cultured for too long (> 2 months).
- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

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Any questions?

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1. Cells preparation

It is recommended to seed or plate the cells the day prior transfection. The suitable cell density will depend on the growth rate and the cells conditions. Cells should be 80-90% confluent at the time of transfection (refer to table 1).

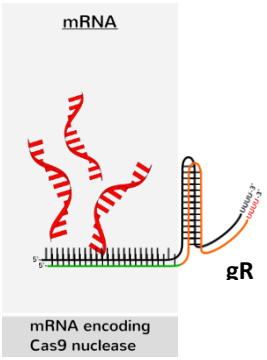
Tissue Culture Dish	Adherent Cell Number	mRNA quantity (µg)	RmesFect volume (µL)	Dilution Volume (µL)	Transfection volume
96 well	0.5 – 2 x 10^4	0.25	0.75 – 1.0	2 x 25	150µL
24 well	0.5 – 1 x 10^5	0.5	1.5 - 2	2 x 50	500µL
6 well	2 – 5 x 10^5	2.0	6 – 8	2 x 150	2mL

Table 1: Suggested transfection conditions

2. Option 1: Cas9 encoding mRNA and gRNA are delivered together

Rmesfect transfection reagent is dedicated to mRNA delivery and can be used as well to transfect complexes of mRNA and sgRNA together; use the following protocol:

a. *mRNA encoding Cas9 endonuclease:* Dilute the indicated quantity of Cas9 mRNA in 25 to 100 µL of culture medium without serum and supplement (refer to Table 1).
b. *Short guide RNA (sgRNA)*: prepare a solution of gRNA for a final concentration of 50-125 nM.
c. *RmesFect*: dilute the indicated quantity of RmesFect (refer to Table 1) in 25 to 100 µL of culture medium without serum and supplement.
d. *Complexes formation*: Mix Cas9 mRNA solution with gRNA solution, gently pipette up and down several times and add the mix to RmesFect solution.
e. Incubate the mixture for 20 min at room temperature.



3. Option 2: Cas9 encoding mRNA delivery only

a. *mRNA encoding Cas9 endonuclease:* dilute the indicated quantity of Cas9 mRNA in 25 to 100 µL of culture medium without serum and supplement (refer to Table 1).
b. *RmesFect*: dilute the indicated quantity of RmesFect (refer to Table 1) in 25 to 100 µL of culture medium without serum and supplement.
c. *Short guide RNA (sgRNA)*: prepare a solution of gRNA for a final concentration of 50-125 nM.
d. Lullaby*: prepare a tube containing the required amount of Lullaby, refer to Lullaby protocol
e. *Formation of complexes*: Add Cas9 mRNA suspension to RmesFect solution and Mix sgRNA solution with Lullaby

f. Incubate the mixtures for 20 min at room temperature.

* Lullaby transfection reagent (#LL70500) is the ideal transfection reagent to deliver short RNA into cells such as siRNA or sgRNA.

NOTES:

- OZBiosciences provides also Cas9 mRNA as a ready-to-use stabilized mRNA encoding for Cas9 endonuclease, concentrated at 1.0 mg/mL in 1 mM Sodium Citrate (pH 6.4) that has been designed to produce high expression level of wild-type endonuclease CRISPR associated (Cas) protein 9.
- Proceed quickly to complex formation to avoid any mRNA degradation or surface adsorption.
- Proceed to transfection within 30 minutes.

4. Transfection

a. Add the complexes in a drop wise manner onto the cells growing in complete culture medium and homogenize by rocking the plate back and forth to ensure a uniform distribution of the mixture.

b. Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the genome edition.

c. Gently rock the plate to ensure a uniform distribution.

NOTES:

- In case of cells very sensitive to transfection, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium.
- Reverse transfection can also be performed: add complexes first, and then add cells at twice the recommended cell density.

Optimization Protocol

To achieve the highest efficiency, optimize the transfection conditions as follows:

- Vary the RmesFect (µL) / mRNA (µg) ratio from 1/1 to 6/1.
- Once the optimal mRNA/RmesFect ratio is found, adjust the mRNA quantity according to Table 2.
- Finally, culture medium compositions (for preparing the complexes), cell density, total culture medium volume and incubation times can also be optimized.

Tissue Culture Dish format	mRNA Quantity (µg)
96 well	0.125 to 1
24 well	0.250 to 2
6 well	1 to 8

Table 2: Suggested range of mRNA amounts for optimization (per well)

NOTES

Additional transfection reagents for CRISPR/Cas9 Genome Editing

- **Pro-deliverIN CRISPR** for Cas9 protein delivery
- **PolyMag Neo** for the co-transfection of plasmids encoding Cas9 and guide RNA
- **ViroMag R/L** to enhance transduction efficiency of CRISPR/Cas9 viruses

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