Application Note for PolyMag Neo mediated plasmid-based Genome Edition using the CRISPR-Cas9 system (Primary and hard-to-transfect cells)

IMPORTANT NOTES – Before you begin

- ✓ PolyMag Neo must be stored at 4°C and used at a ratio of 1:1. Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ For co-transfection with 2 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).
- ✓ **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.
- ✓ Order of addition is important: add the DNA solution onto PolyMag Neo magnetic nanoparticles.
- ✓ Dilute PolyMag Neo 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.
- ✓ 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) increasing the number of transfected cells, or (3) improving genome editing capacities.

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

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Any questions?

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Protocol

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 60-90% confluent at the time of transfection (refer to table 1).

Tissue Culture Dish	Adherent Cell Number	DNA amount (µg)	Dilution volume (µL)	PolyMag Neo Volume (µL)	Transfection volume
96 well	$0.5 - 2 \times 10^4$	0.1 – 0.5	50	0.1 – 0.5	200µL
24 well	$0.5 - 1 \times 10^5$	0.25 - 1	100	0.25 - 1	500µL
6 well	$2 - 4 \times 10^5$	2 - 6	200	2 - 6	2mL

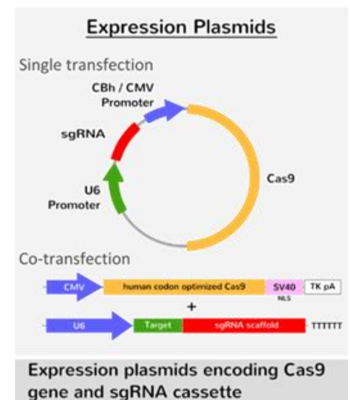
Table 1: Suggested transfection conditions

2. DNA solution preparation

Cas 9 and guide RNA (gRNA) can be encoded by a single plasmid (Single transfection) or two different ones (Co-transfection).

For co-transfections, prepare a mix of gRNA and Cas9 encoding plasmids in a 0.25:1 to 1:1 ratio (respectively gRNA to Cas9) according to your experimental set up.

Dilute the indicated quantity of DNA (see Table 1) in 50 to 200 µL of culture medium without serum and supplement.



Expression plasmids encoding Cas9 gene and sgRNA cassette

3. Complexes preparation

a. *PolyMag Neo*: Vortex the reagent and place the appropriate amounts in an empty microtube (refer to Table 1).

b. Add the DNA solution to the PolyMag Neo solution by vigorous pipetting and incubate at room temperature for 20 minutes. Do not vortex.

4. Transfection

c. Add the PolyMag Neo / DNA complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution. Place the cell culture plate on the magnetic plate during 30 minutes.

d. Remove the magnetic plate.

e. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression (from 24h up to 7 days).

NOTES:

- In case of cells very sensitive to transfection, the medium can be changed right after the Magnetofection procedure
  - ➔ keep cells onto the magnetic plate and replace the transfection medium with fresh pre-warmed complete culture medium.
- Some cell types need medium change 2 - 4h after transfection.

Notes for Co-transfection

Two plasmids will be delivered at the same time into a same cell only if they are first mixed together. For an efficient co-transfection of two plasmids respectively encoding for guide RNA and Cas9 endonuclease, mix the two plasmids and perform transfection as described above. We generally recommend using 0.25µg sgRNA encoding plasmid and 0.75µg Cas9 encoding plasmid with 1µL PolyMag Neo. However, depending on many parameters (promoters, read-out, cell type…) the stoichiometry of each DNA may vary.

Important: the preparation of two different solutions of complexes, one for Cas9 and one for sgRNA, may lead to limited efficiency since some cells would receive one of the plasmids and only a few population, both of them.

Option for Co-transfection

Transfections can be realized sequentially instead of simultaneously. So, cells can be transfected with one plasmid DNA first and 4h to 24h later can be transfected with the other plasmid DNA. Follow the procedure as detailed above for DNA transfection. A medium changed can be also performed between the two transfections.

Optimization Protocol

We strongly advise you to optimize your transfection conditions in order to get the best out of Magnetofection™. Several parameters can be optimized:

- Ratio of Cas9 plasmid over sgRNA plasmid (in case of co-transfection)
- Nucleic acid dose used
- Ratio of *PolyMag Neo* to nucleic acid
- Cell density
- Incubation time

1. Start by optimizing the ratio PolyMag Neo/ DNA. To this end, use a fixed amount of DNA. Vary the amount of PolyMag Neo from 0.25 to 5µL / µg of DNA. The ratio PolyMag Neo / DNA can be changed by doubling or multiplying the volume of the reagent used. Reagent can be pre-diluted in deionized water.

2. Thereafter, change the nucleic acid dose with a fixed ratio of PolyMag Neo / DNA that has been previously optimized. For this purpose, you can perform a serial dilution of a preformed magnetic vector complex.

3. Stoichiometry of each plasmid can also be investigated: vary the proportion of each plasmid DNA within the whole mix.

4. After having identified the correct quantities of PolyMag Neo and nucleic acid, you can pursue the process by optimizing the cell number as well as the incubation times for the complex formation and for the magnetic field application.

Additional transfection reagents for CRISPR/Cas9 Genome Editing

- **Pro-deliverIN CRISPR** for Cas9 protein delivery
- **RmesFect** for Cas9 mRNA transfection
- **ViroMag** to enhance transduction efficiency of CRISPR/Cas9 viruses

Purchaser Notification
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The purchase of the PolyMag Neo kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the PolyMag Neo kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all PolyMag Neo kit reagents and documentation to OZ Biosciences, or by destroying all PolyMag Neo components. Purchasers are advised to contact OZ Biosciences with the notification that a PolyMag Neo kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the PolyMag Neo kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.
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