Transfer of eGFP-mRNA into *Bone Marrow-derived Dendritic Cells (BMDCs)* Using Fuse-It-mRNA

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An adapted protocol for the use of ibidi Fuse-It-mRNA with LPS-matured murine bone marrow-derived dendritic cells. This protocol stains approximately 1.75x10^6 cells with 70-80% mRNA-expression efficiency.

1. Materials and Reagents

a. Fuse-It-mRNA, 60500, ibidi GmbH, Martinsried Germany
b. eGFP-mRNA, 0.5 µg/µl, L-6301, TriLink Biotechnologies, Inc.
c. RNase-free 1.5 ml tubes
d. 15 ml tubes
e. 1x PBS (pH 7.4-8.0; osmolarity 250-320 mOs/kg) room temperature, with 10 ml warmed to 37°C.
f. 35 mm cell culture dishes, untreated (e.g. Corning 430588)
g. Mature bone marrow-derived dendritic cells grown according to Lutz et al. (see Note 1).

2. Equipment

a. Cell culture incubator (humidified, 37°C, 5% CO_2)
b. Centrifuge capable of holding 15 ml tubes.
c. Vortex
d. Cell counter (e.g. Beckman Coulter Z2) or haemocytometer + microscope.
e. Ultrasonic bath (e.g. Bandelin Sonorex Digitec, 120 W, 35 kHz) filled with icy water.
f. Water bath set to 37°C.

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3. Procedure

All centrifugation steps are performed at room temperature, 300 xg for 5 minutes.

1. Add 4 µg mRNA to 8 µl of Neutralization Buffer (NB) in a 1.5 ml tube and mix thoroughly. Incubate for 20 minutes at room temperature.
2. Vortex the Fusogenic Solution (FS) until the solution is homogeneous. If necessary, mix by pipetting.
3. Transfer 10 µl of the FS to a new 1.5 ml tube and sonicate it in an ultrasonic bath for 5 minutes in icy water.
4. Add the neutralized mRNA (mRNA + NB) to the sonicated FS and mix thoroughly. Sonicate the mixture in an ultrasonic bath for 5 minutes in icy water.
5. Meanwhile, collect non- and semi-adherent cells and transfer to 15 ml tube. Centrifuge cells and dispose of plate.
6. Add 1x PBS to the fusogenic mixture to make a final volume of 1 ml, and mix thoroughly. Sonicate the mixture in an ultrasonic bath for 5 minutes in icy water.
7. Meanwhile, dispose of cell supernatant and resuspend pellet in 1 ml fresh culture media (without LPS). Quantify cells and transfer approximately $1.75x10^6$ cells into a new 15 ml tube containing 5 ml pre-warmed PBS (see Note 2). Centrifuge.
8. Bring fusogenic mixture back to room temperature. Dispose of cell supernatant and resuspend pellet in 1 ml fusogenic mixture. Transfer to 35 mm dish and incubate for 20 minutes at 37°C with 5% CO_2. Gently swirl the plate every 5 minutes to ensure thorough mixing.
9. Remove cells from plate and transfer to 15 ml tube with 5 ml pre-warmed PBS. Centrifuge. Dispose of supernatant and resuspend cells in fresh media as per downstream protocol (e.g. ibidi µ-Slide Chemotaxis).

The result of 70-80% mRNA-expression efficiency was achieved by using flow cytometry. A fluorescent image was taken in the µ-Slide Chemotaxis after fixation.

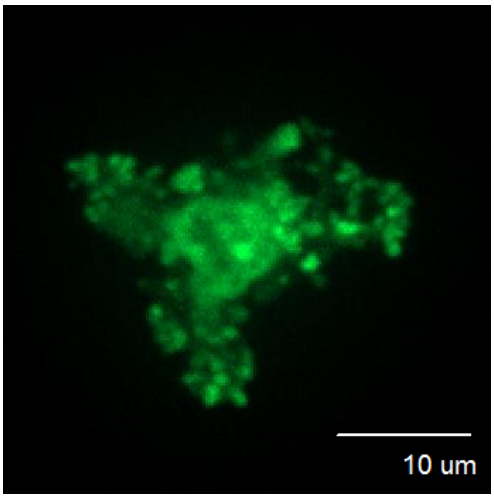

Figure 1: Fluorescent image of BMDC expressing eGFP-mRNA.

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4. References

An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow.
M. Lutz, N. Kukutsch, A. Ogilvie, S. Rössner, F. Koch, N. Romani and G. Schuler.
Journal of Immunological Methods, 1999, doi:10.1016/S0022-1759(98)00204-X

5. Notes

1. Brief protocol and media recipes for BMDC culture:

   Day 0: Plate 2x10^6 cells in 10 ml culture media (Table 1), 20 ng/ml GM-CSF, in 10 cm dish.
   Day 3: Add 10 ml culture media with 20 ng/ml GM-CSF.
   Day 6: Remove 10 ml media and centrifuge 300 xg for 5 minutes at room temperature. Dispose of supernatant and resuspend in 10 ml fresh cell culture media with 20 ng/ml GM-CSF. Add back to plate.
   Day 8: Repeat day 6.
   Day 10: Collect non- and semi-adherent cells with gentle pipetting and centrifuge 300 xg for 5 minutes at room temperature. Dispose of supernatant and resuspend in 10 ml fresh cell culture media with 10 ng/ml GM-CSF and 200 ng/ml LPS. Plate in 6 cm dish overnight.
   Day 11: Mature BMDCs ready to use.

Table 1: Material and reagents for the cell culture of murine BMDCs.

	Reagent	Concentration	Company	Order No.
Culture	Bone marrow (murine)	-	-	-
	RPMI 1640	1x	ThermoFisher	31870025
	Heat-inactivated FCS	10%	ThermoFisher	10500064
	L-Glutamine	1%	Sigma-Aldrich	G7513
	Penicillin/Streptomycin	1%	Sigma-Aldrich	P4333
	β-Mercaptoethanol	50 µM	ThermoFisher	31350010
	GM-CSF	10-20 ng/ml	Peprotech	315-03
Stimulation	LPS	200 ng/ml	Sigma-Aldrich	L4516

2. Approximate yield per plate after stimulation is 6-8x10^6 cells.

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