

Efficient mRNA Delivery in difficult-to-transfect cells with jetMESSENGER® Transfection Reagent

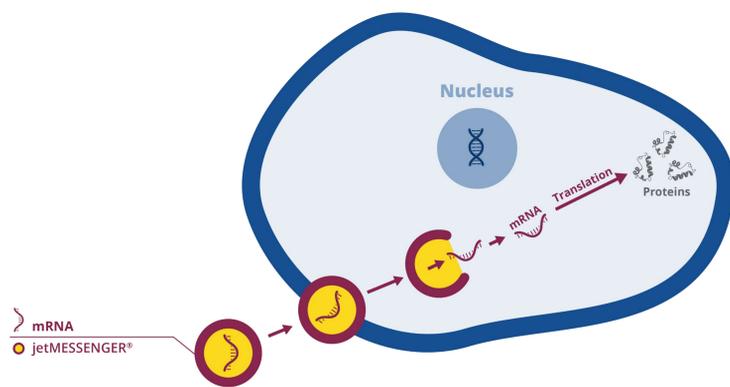
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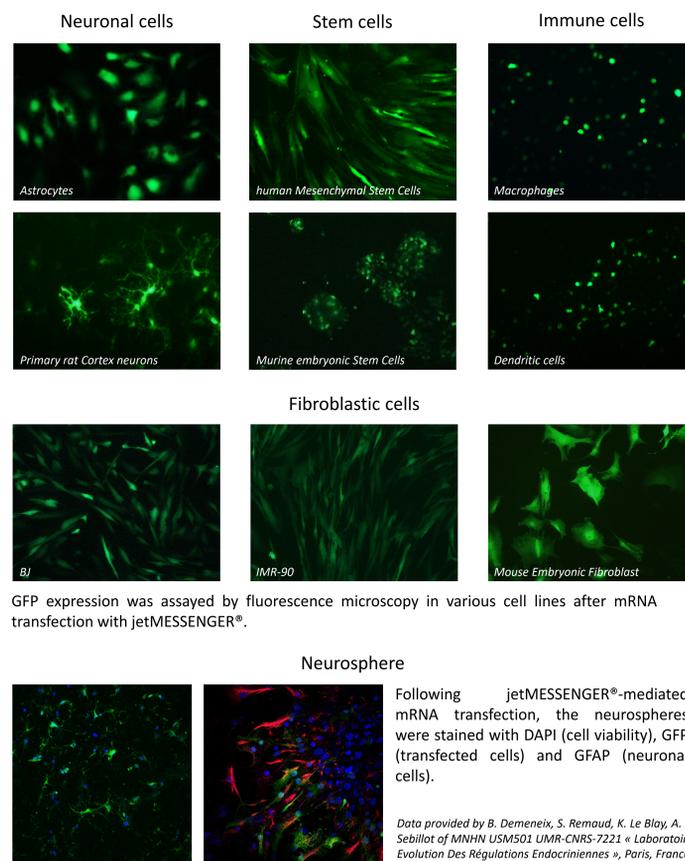
Abstract

Messenger RNA transfection is a new solution for a wide variety of cells that are difficult to transfect with plasmid DNA including adherent (neurons or primary cells) and suspension cells (lymphocytes). This process has many advantages over DNA transfection: high percentage of transfected cells, faster protein expression following transfection, and no risk of insertional mutagenesis in contrast to plasmid or viral vectors. In fact, mRNA is delivered and expressed in the cytoplasm and does not require to cross the nuclear membrane, one of the limiting steps in plasmid transfer. Furthermore, the last generation of modified mRNA reduces the toxicity and immune response usually induced by mRNA entry into the cytosol (1, 2). The transient nature of mRNA transfection is desirable for a number of applications, including cellular reprogramming, genome editing (CRISPR/Cas9) and vaccines. Polyplus-transfection® has developed a novel mRNA transfection reagent (jetMESSENGER®) which outperforms the efficiency of DNA transfection. This poster will present the results obtained with various cell lines, fragile and primary cells.

Introduction: Mechanism of transfection of jetMESSENGER®



High EGFP expression in various cell lines

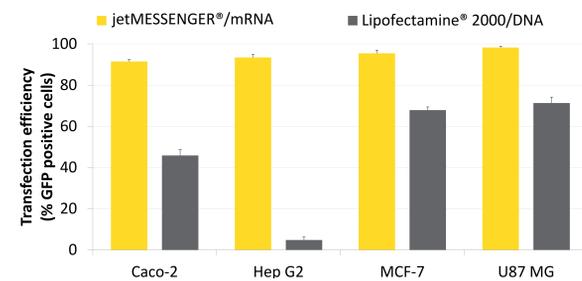


GFP expression was assayed by fluorescence microscopy in various cell lines after mRNA transfection with jetMESSENGER®.

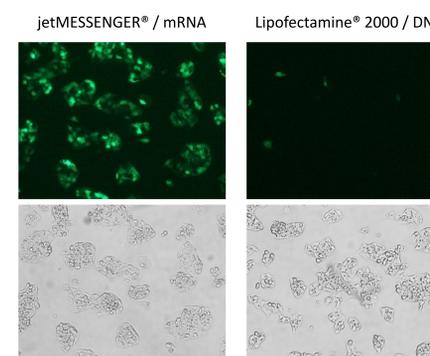
Following jetMESSENGER®-mediated mRNA transfection, the neurospheres were stained with DAPI (cell viability), GFP (transfected cells) and GFAP (neuronal cells).

Data provided by B. Demeneix, S. Remaud, K. Le Bloy, A. Sebillat of MNHN USM501 UMR-CNRS-7221 « Laboratoire Evolution Des Régulations Endocriniennes », Paris, France.

jetMESSENGER® transfection efficiency versus competitor DNA transfection reagent

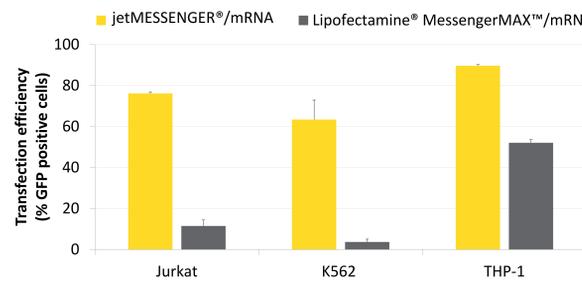


Transfection efficiency was assayed by FACS analysis in cancer cell lines 24 h after transfection of EGFP mRNA (L-6101, Trilink™) with jetMESSENGER® or plasmid DNA encoding for EGFP (pCMV-EGFP) with Lipofectamine® 2000.

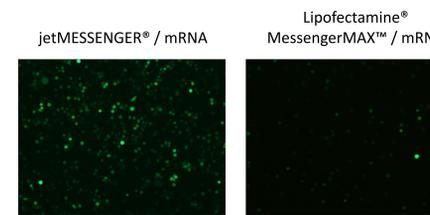


GFP expression was assayed by fluorescence microscopy in Hep G2 cells 24 h after transfection. Cell morphology is maintained during transfection with jetMESSENGER®.

jetMESSENGER® transfection efficiency versus competitor mRNA transfection reagent

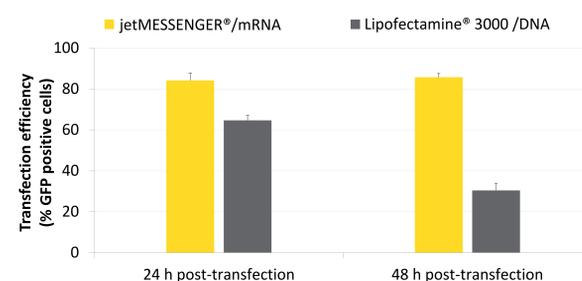


Transfection efficiency was assayed by FACS analysis in suspension cells 24 h after transfection of EGFP mRNA (L-6101, Trilink™) with jetMESSENGER® or Lipofectamine® MessengerMax™.

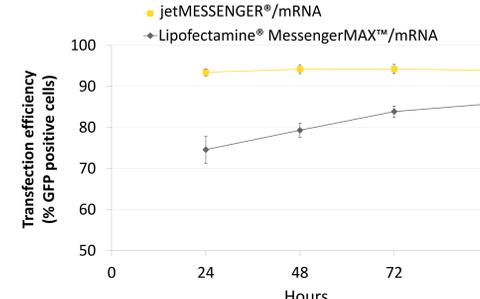


GFP expression was assayed by fluorescence microscopy in Jurkat cells 24 h after transfection.

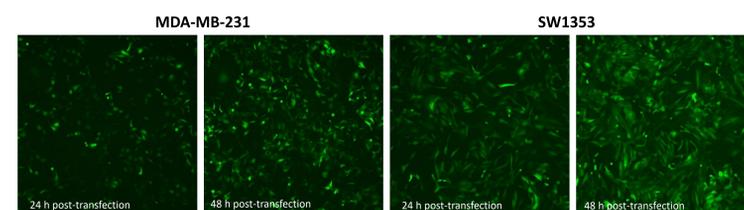
Duration of expression



GFP expression was analysed by FACS in human Astrocytes 24 h or 48 h after transfection of EGFP mRNA (L-6101, Trilink™) with jetMESSENGER® or plasmid DNA encoding for EGFP (pCMV-EGFP) with Lipofectamine® 3000.



GFP expression was analysed by FACS in Human Fibroblast cells 24 h, 48 h, 72 h or 96 h after transfection of EGFP mRNA (L-6101, Trilink™) with jetMESSENGER® or with Lipofectamine® MessengerMax™.



GFP expression was assayed by fluorescence microscopy in human cancer cells of various origins, MDA-MB-231 (Mammary gland cells) and SW1353 (Human bone Fibroblast cells).

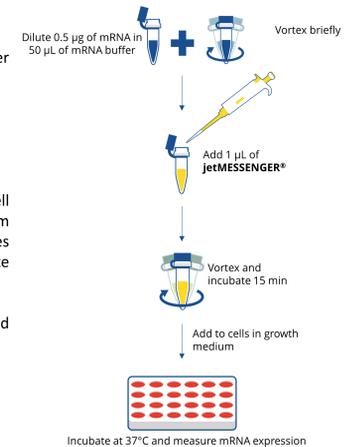
“jetMESSENGER® appears as a very effective reagent for RNA transfection in human cancer cells of various origins. The expression level intensity and the transfected cells proportion were very impressive as compared to conventional DNA transfection reagents, without any sign of cytotoxicity.”

Data provided by Brotin E., Abellard E., Denoyelle C., Poulain L. Inserm U1199 « BioTICLA », Caen, France.

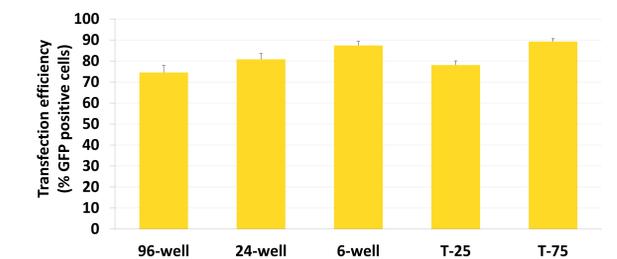
Protocol

mRNA transfection in culture plate 24-wells:

- Dilute 0.5 µg mRNA into 50 µL mRNA buffer and vortex briefly.
- Add 1 µL jetMESSENGER®.
- Vortex and incubate for 15 min at RT.
- Add 50 µL of transfection mix per well dropwise onto the cells in growth medium (containing serum or not) and/or additives (standard culture medium), and distribute evenly.
- Gently rock the plate back and forth and from side to side.
- Analyze at least 24 - 48 h later.



Easy to scale up and down



Transfection efficiency was assessed by FACS analysis in Caco-2 cells 24 h after transfection of EGFP mRNA (L-6101, Trilink™).

Size of culture	Amount of mRNA	Volume of jetMESSENGER®
96-well	150 ng	0,3 µL
24-well	500 ng	1 µL
6-well	2 µg	4 µL
T-25	4 µg	8 µL
T-75	10 µg	20 µL

Conclusion

Advantages of jetMESSENGER®

- High efficiency on a wide variety of difficult to transfect cells
- Outperforms DNA transfection by switching to mRNA
- Extremely gentle on cells
- No risk of genome integration
- Perfectly suited for CRISPR/Cas9 gene editing, iPS generation, stem cell differentiation and immunotherapy assays

References:

- Kariko et al, Mol Ther., 2008.
- Uchida et al, Pharmaceuticals, 2015.