Identifying efficient chemical-based nucleic acid transfection compound for primary neurons and neuronal cell lines

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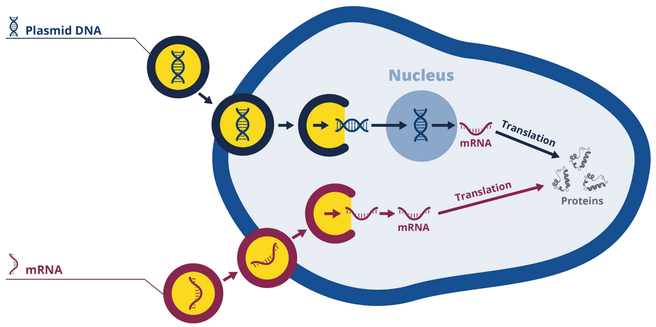
Abstract

Efficient gene expression in neurons is indispensable for the study of neuronal cell biology, such as investigating gene and protein function, cell behavior and/or cell morphology. The need for more physiologically relevant cellular models has become a requirement to further validate studies performed in neuron cell lines that are easier to transfect compared to primary neurons. Primary neurons are fragile and difficult to transfect, and with currently available transfection method either results in low transfection efficiency or low cell viability. Currently, the most efficient methods for exogenous gene delivery into slow to non-dividing neurons are electroporation or viral-based transduction methods. These methods are often associated with side-effects on cellular viability and morphology. Here we describe the screening of a new library of chemical compounds to identify candidates as potent DNA transfection reagents in different primary neurons (such as primary hippocampal or cortical neurons from rat) and neuronal cell lines. Following the optimization of hits-to-lead, we selected the best candidate based on its high transfection efficiency and its ability to maintain excellent cell viability and morphology.

Introduction

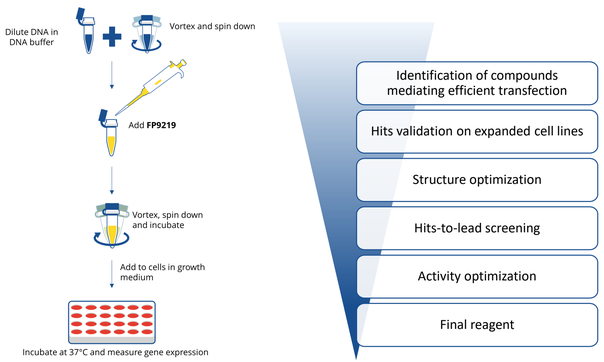
Transfection of primary neurons and neuronal cells is a challenge for many researchers. The available DNA transfection reagents usually results in low transfection efficiency and are toxic for the cells. Taking into consideration the difficulties encountered and based on our expertise in transfection, we are currently developing a novel DNA transfection reagent, **FP9219**. This compound is promising, as it improves DNA delivery and intracellular transport, leading to an efficient gene expression.

As an alternative, mRNA transfection using **jetMESSENGER®** can be an efficient approach to transfect primary neurons and neuronal cells. This poster will present the results obtained with both approaches.

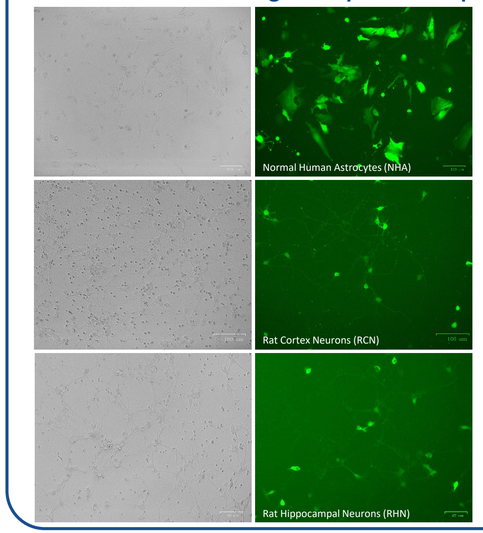


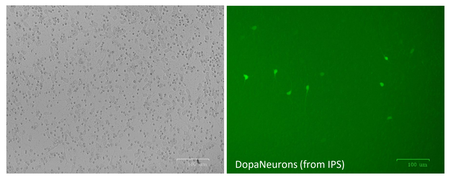
FP9219 development

A screening of a proprietary chemical compounds library was performed to select new molecules leading to superior DNA transfection efficiency. After hits identification and validation, we optimized their chemistry through structure/activity relationship (SAR) studies to select the best one, FP9219. The easy and fast transfection protocol is an advantage for this ready-to-use compound.



Efficient gene expression in primary neurons and neuronal cells with FP9219

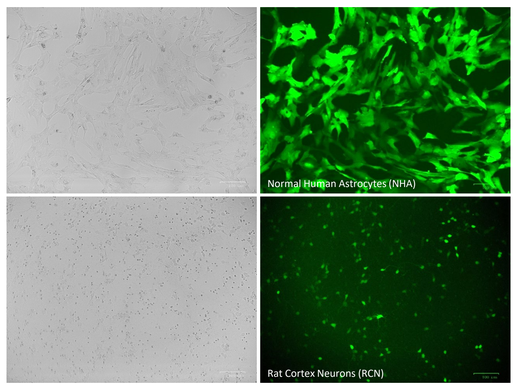


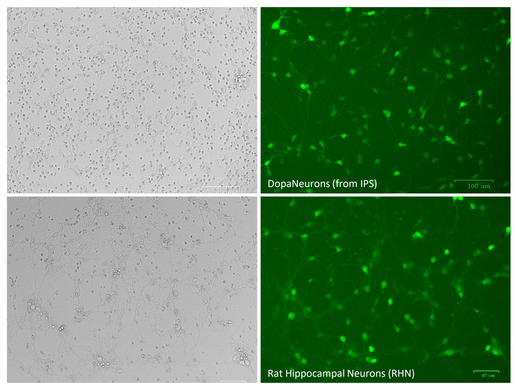


Cell types	DNA Transfection efficiency with FP9219
Normal Human Astrocytes (NHA)	57.7%
Rat Cortex Neurons (RCN)	20.3%
Rat Hippocampal Neurons (RHN)	21%
DopaNeurons (from IPS differentiation)	3.3%

GFP expression was assayed by fluorescence microscopy in different cell types 24h post-transfection of plasmid encoding GFP (pCMV-EGFP) with FP9219. Cell morphologies are maintained and neurites networks fluorescence are clearly visible following transfection with FP9219.

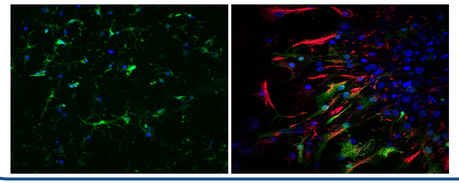
Increased gene expression when switching to mRNA transfection using jetMESSENGER®





GFP expression was assayed by fluorescence microscopy in various cell lines 24h post-transfection of mRNA encoding GFP (EGFP-mRNA) with jetMESSENGER®. High efficiencies and viabilities are obtained. jetMESSENGER® is an efficient transfection reagent and preserves cell morphologies.

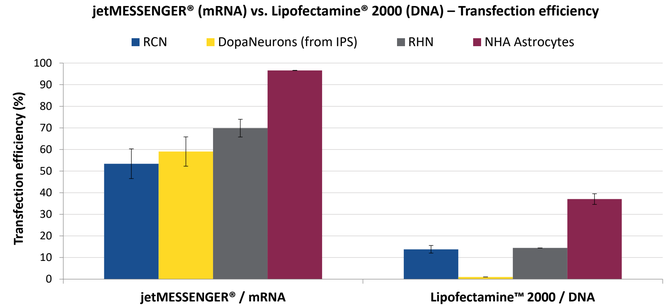
mRNA-EGFP expression in Neurospheres with jetMESSENGER®



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

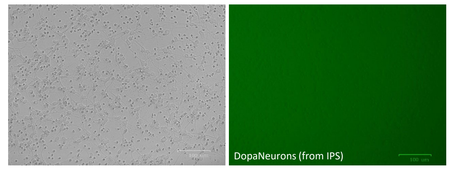
Data provided by K. Le Blay, A. Sebillot, B. Demeneix, S. Remaud of MNHN USM501 UMR-CNRS-7221 « Laboratoire Evolution Des Régulations Endocriniennes », Paris, France.

jetMESSENGER® transfection efficiency versus competitor DNA transfection reagent

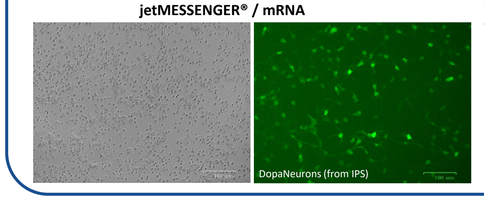


Transfection efficiencies were assayed by fluorescence microscopy or flow cytometry analysis 24h post-transfection of EGFP-mRNA (L-7202, Trilink™) with jetMESSENGER® or pCMV-EGFP with Lipofectamine® 2000.

Lipofectamine® 2000 / DNA



jetMESSENGER® / mRNA



DopaNeurons from Induced Pluripotent stem Cells (IPS) are very difficult to transfect. mRNA-EGFP transfection with jetMESSENGER® is highly efficient in these cells, without inducing any cell toxicity. This approach emphasizes transfection efficiency in comparison to DNA transfection with Lipofectamine® 2000.

Conclusion

Transfection with FP9219 and jetMESSENGER® preserves cell viability and morphology of sensitive cells as it requires low amount of nucleic acid and volume of reagent, while reaching high transfection efficiency in physiological conditions. Transfection using these reagent is straightforward and provides reproducible results.

- Approaches: Two different solutions to transfect Neurons or Neuronal cells (DNA or mRNA)
- Highly efficient: Reach high gene expression in several cells' types
- Cost-effective: Use low reagent volume and DNA/mRNA amounts
- Biologically relevant: Keep a high cell viability & preserve morphology
- Simplicity: Transfect with an optimized easy and fast protocol