ArunA BIOMEDICAL

Application Note

Genetic Modification

of hNP1™ Neural Progenitor Cells

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Introduction

ArunA Biomedical's hNP1™ Human Neural Progenitor Cells are derived from human embryonic stem cells (hESC; WA09 line) using defined, serum-free conditions (1). hNP1™ Human Neural Progenitor Cells (Figure 1) proliferate as adherent monolayers, maintain a stable karyotype for multiple (>10 passages) and express proneural markers (nestin, CD133, musashi1 and SOX2). hNP1™ Human Neural Progenitor Cells are readily scalable for high content imaging and high throughput format (96- and 384-well) assays, as well as capable of differentiating into various neural phenotypes (2-4).

Effective genetic modification of hNP1™ Human Neural Progenitor Cells broadens their potential further – enabling selection, directed differentiation and live cell tracking and monitoring, etc. Plasmid DNA transfection and recombinant lentivirus transduction are two common gene transfer technologies currently available. The following application note describes both lipid-based transfection with non-integrating plasmid DNA and transduction with integrating recombinant lentivirus of ArunA Biomedical's **hNP1™ Human Neural Progenitor Cells (hNP1™ cells)**.

Seven different commercially available transfection reagents and recombinant lentivirus with two different promoters at two different concentrations were evaluated to determine efficiency and expression in hNP1™ cells.

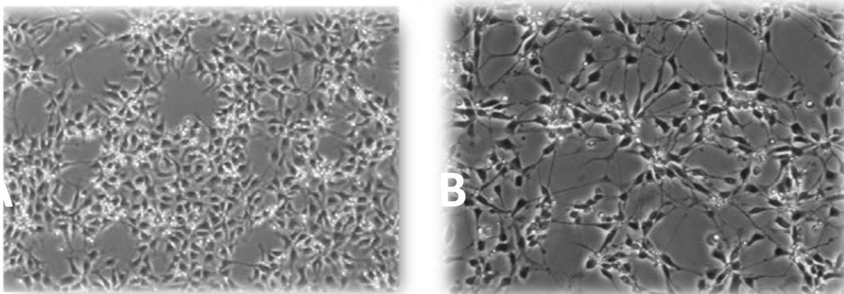

Figure 1: Representative phase contrast image (100X) of **hNP1™ Neural Progenitor Cells** (A). hNP1™ cells can readily be differentiated into a mature neural phenotype, **hN2™ Neural Cells** (B).

Methods

A. Plasmid DNA Transfection

hNP1™ cells (ArunA Biomedical) were plated at 1 x 10^6 cells/well (80-100% confluence) in 6-well plates coated with BD Matrigel™ (BD Biosciences) in AB2™ Neural Basal Medium supplemented with ANS™ (ArunA Biomedical). Five or twenty-four hours later (depending on transfection reagent), hNP1™ cells were transfected in AB2™/ANS™ medium with the **pZsGreen1-N1** (Clontech) plasmid using various commercial transfection reagents per manufacturer's recommendations and instructions (see Table 1 for transfection conditions).

B. Recombinant Lentivirus Transduction

hNP1™ cells (ArunA Biomedical) were plated at 5 x 10^5 cells/well (50-60% confluence) in 6-well plates coated with BD Matrigel™ (BD Biosciences) in AB2™ Neural Basal Medium supplemented with ANS™ Neural Supplement (ArunA Biomedical). Twenty-four hours later, hNP1™ cells were transduced with replicative-incompetent, recombinant HIV-1 lentivirus particles pseudotyped with the vesicular-stomatitis virus G (VSV-G) envelope protein (Thermo Fisher Scientific). Lentivirus particles encoded enhanced green fluorescent protein (eGFP) with expression driven by the cytomegalovirus immediate-early (CMV) or TurboGFP driven by the elongation factor 1 alpha (EF1α) constitutive promoter (**TZV-CMV-eGFP** or **TZV-EF1α-TurboGFP**). hNP1™ cells were transduced in AB2™/ANS™ medium in the presence of 8 µg/mL Polybrene (Sigma).

Commercial Reagent	Transfection Conditions		
	µL Reagent	**µg DNA**	**Ratio**
FuGENE® HD	3	2	1.5:1 (or 3:2)
	8	2	4:1
	12	2	6:1
Xfect™	0.75	2.5	0.3µL reagent per µg DNA
	1.5	5	0.3µL reagent per µg DNA
	2.25	7.5	0.3µL reagent per µg DNA
GeneJammer	1.5	2	3:4
	3	2	3:2
	6	2	3:1
***Trans*IT®-2020**	2	2.5	1:1
	5	2.5	2:1
	7.5	2.5	3:1
***Trans*IT-Neural®**	2.5	2.5	1:1
	5	2.5	2:1
	7.5	2.5	3:1
Lipofectamine™ LTX and PLUS™	15µL LTX 0µL PLUS	5	3:0:1
	15µL LTX 5µL PLUS	5	3:1:1
	22.5µL LTX 7.5µL PLUS	7.5	3:1:1
TurboFect™	2.6	2	2.6: 2 (or 13:10)
	4	2	2:1
	6	2	3:1

Table 1: Summary of commercial transfection reagents and transfection conditions used to genetically modify **hNP1™ Neural Progenitor Cells** with the pZsGreen1-N1 (Clontech) plasmid containing the CMV promoter and encoding enhanced green fluorescent protein (eGFP).

C. Flow Cytometry

The percentage of GFP-positive hNP1™ cells was determined three days post plasmid DNA transfection using flow cytometry. Briefly, cells were collected nonenzymatically using cell scrapers, pelleted, washed and resuspended in 1x PBS^{++} with 10% FBS to achieve a single-cell suspension. Non-transfected hNP1™ cells were used to monitor autofluorescence and set detection thresholds for expression.

D. Fluorescence Microscopy

Three days post transfection or transduction, epifluorescent images (100X) were taken with an inverted epifluorescence microscope.

Transfection Reagent	Efficiency (% GFP +)
FuGENE® HD 6:1 (12µL reagent, 2µg DNA)	31
Xfect™ 0.3 µLreagent/ µg DNA (1.5µL reagent, 5µg DNA)	69
GeneJammer 3:1 (3µL reagent, 1µg DNA)	31
Lipofectamine™ LTX and PLUS™ 3:1:1 (15µL LTX, 5µL PLUS, 5µg DNA)	36
***Trans*IT®-2020** 3:1 (3µL reagent, 1µg DNA)	18
***Trans*IT-Neural®** 3:1 (3µL reagent, 1µg DNA)	3.8
TurboFect™ 3:1 (3µL reagent, 1µg DNA)	5.7

Table 2: Comparison of Transfection Efficiencies Determined by Flow Cytometry Using Different Commercial Reagents.

Note: Table above reflects the best results for each transfection reagent

Results

To evaluate non-integrating transient transfection, each commercial reagent was used at various concentrations or with different amounts of DNA, according to individual manufacturer's recommendations and instructions, in attempts to optimize conditions for each reagent (see Table 1). Thus transfection conditions were not uniform across experimental wells.

Using Clontech's Xfect™ transfection reagent resulted in the highest transfection efficiency (69%) of ArunA's hNP1™ cells, yet some cell death was apparent (Table 2, Figure 1B). Most of the transfections resulted in some level of cytotoxicity to hNP1™ cells, indicating the need for optimization that balances efficiency against cytotoxicity for this cell type. The reagent which showed the highest transfection efficiency coupled with low cell toxicity was Promega's FuGENE® HD (31%) (Table 2, Figure 1A).

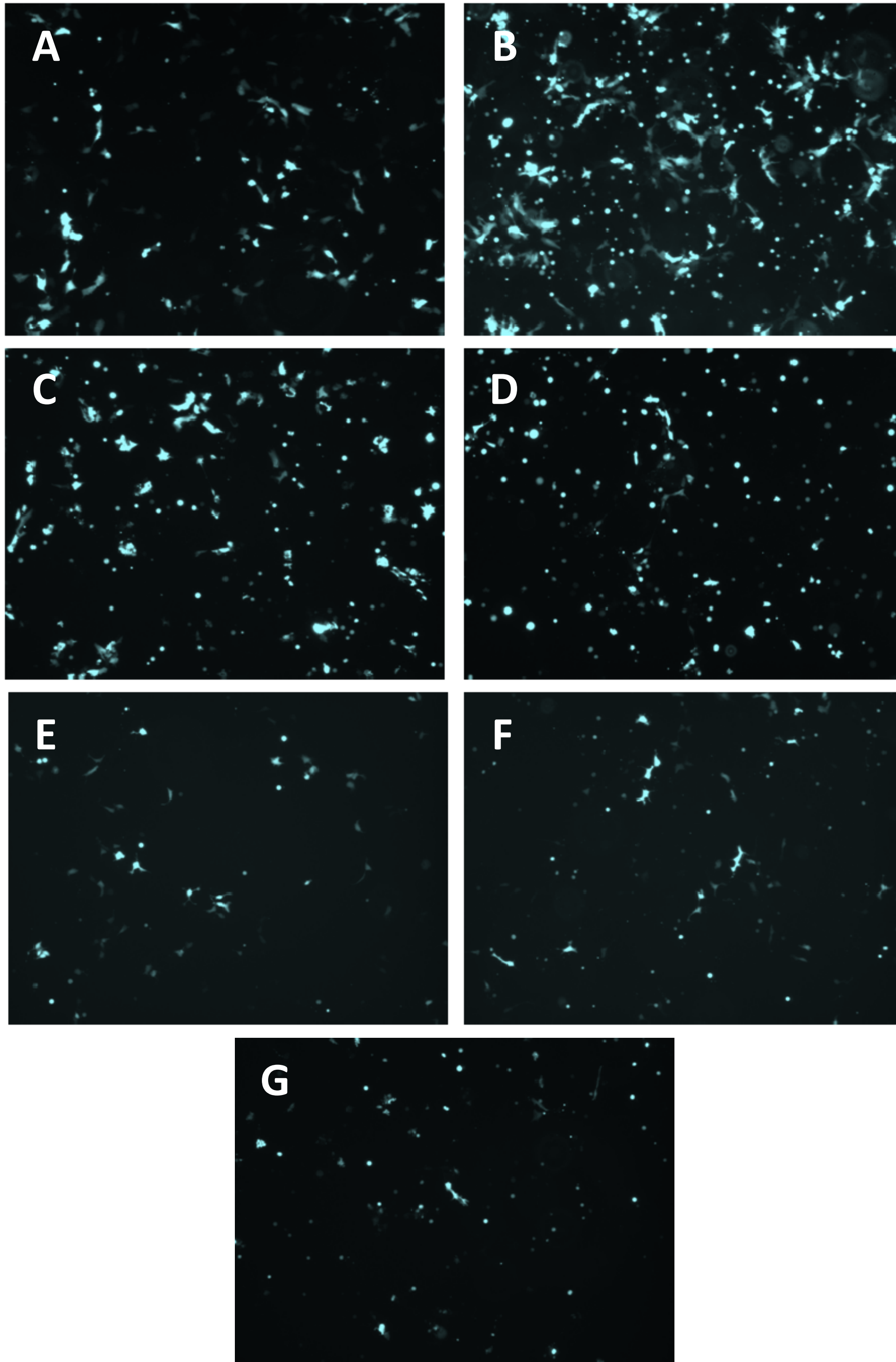

Figure 1: Epifluorescent and phase contrast images (10X objective) ArunA Biomedical's hNP1™ Human Neural Progenitor Cells transfected with pZsGreen1-N1 control GFP plasmid using either FuGENE® HD (A), Xfect™ (B), GeneJammer (C), Lipofectamine™ LTX and PLUS™ (D), *Trans*IT®-2020 (E), *Trans*IT-Neural® (F) or TurboFect™ (G) transfection reagents.

Integrating lentiviral transduction of ArunA's hNP1™ cells was also assessed three days post transduction by fluorescence microscopy. hNP1™ cells demonstrated efficient transduction with both TZV-CMV-eGFP and TZV-EF1α-TurboGFP (Figure 2). hNP1™ cells showed similar levels of fluorescent intensity from transduction with both lentivial vectors. hNP1™ cells were able to be transduced with lentiviral vectors with MOI's as low as 1 and 5 to yield GFP+ cell populations with sustainable GFP expression over multiple passages with both lentiviral vectors. MOI of 5 demonstrated greater transduction efficiency, with approximately 70% of cells transduced with either lentiviral vector. hNP1™ cells transduced with TZV-EF1α-TurboGFP at MOI 5 were more extensively cultured, showing GFP expression for greater than 10 passages. In these studies, lentiviral transduction appeared to be the more efficient means of genetically modifying hNP1™ cells. Additional studies on hNP1™ cells have also indicated that lentiviral transduction does not interfere with directed differentiation into mature neural phenotypes (5).

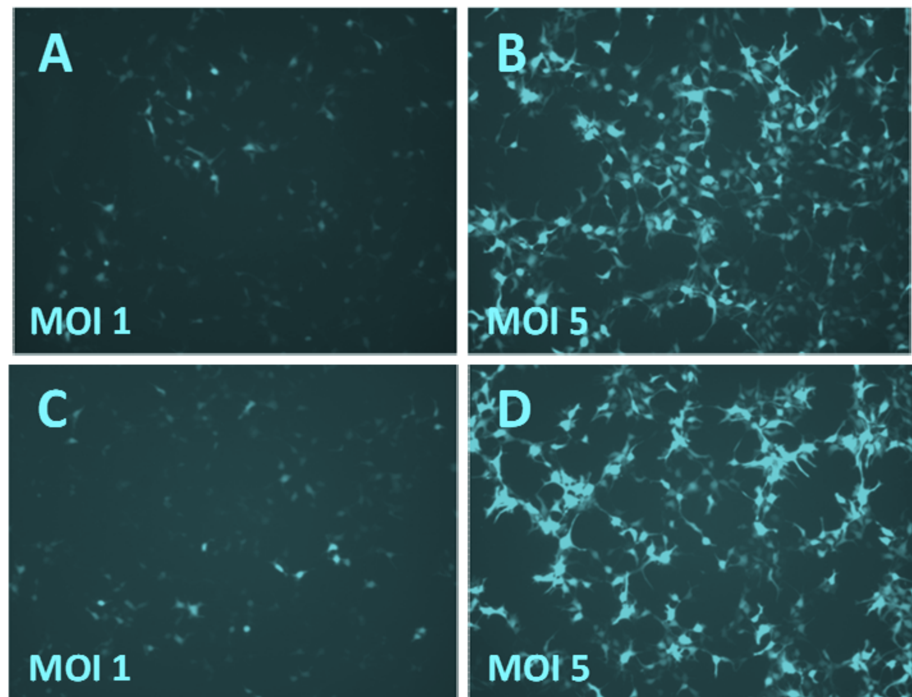

Figure 2: Epifluorescent images (10X objective) of ArunA Biomedical's hNP1™ Neural Progenitor Cells transduced with VSV-G pseudotyped lentiviral vectors with either the CMV (A-B) or EF1α promoters (C-D) at multiplicity of infection (MOI) of 1 and 5.

Together, these results show that different gene transfer strategies can be employed to successfully genetically modify ArunA Biomedical's hNP1™ Human Neural Progenitor Cells and further their use as a cellular model system.

References

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For More Information

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RELATED PRODUCTS

Available from www.arunabiomedical.com.

Description	Catalog No.
hNP1™ Human Neural Progenitor Expansion Kit	**hNP7013.1**
hN2™ Human Neural Discovery Kit	**hN27012.2D**
hN2™ Neural Screening Kit	**hN27012.2S**
AB2™ Neural Expansion Media Kit	**hNP7013.2**
hN2™ Neural Culture Media Kit	**hN2-7011**