Application Note:

Use of RJH Transfection Reagents in Antisense Oligonucleotide (ASO) Delivery

BACKGROUND

The convergences of sophisticated delivery systems, oligonucleotide chemistry and genetic bioinformatics has significantly enhanced the exploration of antisense oligonucleotides (ASO) for therapeutic purposes, which has been reflected in many clinical studies *(Nat. Rev. Drug Discov.* 11, 125–140, 2012*; Annu. Rev. Pharmacol. Toxicol.* 50, 259–293, 2010). ASO is a single stranded nucleic acid first introduced in 1978 by Stephenson and Zamecnik to silence target genes (PNAS, 75, 285-288, 1978). Since then, it has been developed as a potential tool for diverse application ranging from restoration of protein expression to modification of mutant protein. RNaseH-mediated degradation or the modulation of splicing of complementary mRNA is the fundamental mode of action in ASO-based therapy. Studies have explored ASO mediated experimental and therapeutic approaches to modulate biological function at molecular level in both *in vitro* and *in vivo* model (*Hum. Gene Ther.* 26, 475–485, 2015). A variety of transfection reagents have been used to deliver ASO to human cells. Complexes of ASO with polycations (e.g., peptides, cationic polymers and dendrimers) are convenient to prepare and offer safe alternatives for use. The transfection reagents developed by ***RJH Biosciences*** have been tested and found suitable the ASO delivery. This note summarizes the technical experience for ASO delivery for ASO-mediated modulation of splicing in muscle cells by an independent researcher.

PROCEDURE

- Immortalized human myoblasts were differentiated to myotubes and seeded in 12-wells for transfection.
- Cells were transfected with ASO skipping *DMD* exon 51 or random control (mock) ASO complexes by using complexes formed at 1:5 and 1:10 ratios.
- Polymer solutions (100 or 200 μg) were added to ASO solution (20 μg) to get complexes at 1:5 and 1:10 ratios. The complexes were incubated for 30 min at room temperature before addition to the myotubes.
- Transfection medium was replaced after 2 days with medium and cells were incubated for 2 additional days.
- Cells were harvested 4 days post-transfection (or 8 days post-differentiation). RNA was collected using Trizol, cDNA synthesized using SuperScript IV, and RT-PCR done using GoTaq for primer in *DMD* exon 49/50 and exon 52. PCR products were resolved in 2% agarose gels, and post-stained with GelRed.

RESULTS

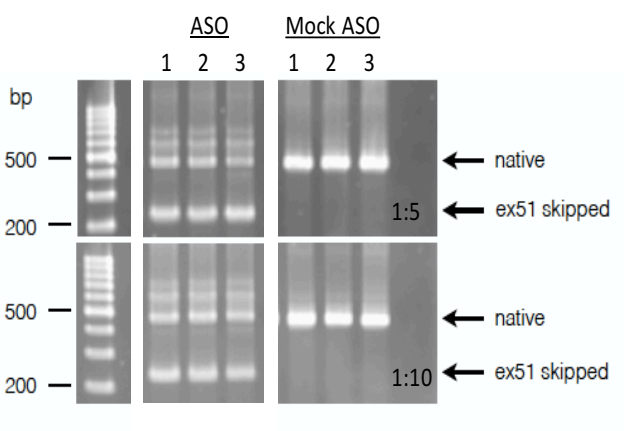

Figure 1. Exon skipping in myoblast cells using RJH transfection reagents. Three different RJH reagents (**1**: Leu-Fect A, **2** and **3**: 2 proprietary formulations) were used for ASO delivery to myotubes cells. The transfection reagents were formulated at ASO:carrier ratios of 1:5 and 1:10 (w/w) using exon-51 specific and mock ASO. The positive responses of myotubes to ASO-complexes are indicated by the appearance of exon-51 skipped band. Approximately 70-80% exon51 skipping was obtained in this experiment, based on RT-PCR and densitometric analysis of the bands.

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