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THE USE OF A MODIFIED U1 snRNA AS A THERAPEUTIC STRATEGY TO CORRECT A 5' SPLICE-SITE MUTATION IN MUCOPOLYSACCHARIDOSIS IIIC: *IN VITRO* STEPS TOWARDS AN *IN VIVO* APPROACH

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Genetic therapy directed towards the correction of RNA missplicing is being investigated not only at basic research level but even in late-stage clinical trials. Many mutations that change the normal splicing pattern and lead to aberrant mRNA production have been identified in Lysosomal Storage Disorders (LSDs). The Mucopolysaccharidosis IIIC (MPS IIIC) is a LSD caused by mutations in the *HGSNAT* gene, encoding an enzyme involved in heparan sulphate degradation. Splicing mutations represent one of the most frequent (~20%) genetic defects in MPS IIIC. Approximately 55% corresponds to 5' splice-site mutations which thus constitute a good target for mutation specific therapeutic approaches. Recently, we demonstrated in fibroblast cells that a modified U1snRNA vector designed to improve the definition of exon 2 5'ss of the *HGSNAT* can restore splicing impaired by the mutation c.234+1G>A (Matos *et al.*, 2014). Presently our goal is to evaluate *in vivo* the therapeutic potential of the modified U1snRNA by testing it in mice expressing the human splicing defect. For this, in a first step we tried to generate full-length splicing competent constructs of wild-type (wt) and c.234+1G>A *HGSNAT* by cloning the wt or the mutated *HGSNAT* splicing-competent cassettes into the pcDNA 3.1 backbone. According to the protocol reported by other researchers (Pinotti *et al.*, 2009), plasmid vectors will be used to promote transient expression of the human *HGSNAT* wt or mutant alleles in mice.

Here, we describe the cloning process followed to obtain the aforementioned splicing constructs. During the cloning steps different difficulties were found as, for example, in fragments amplification, ligation, and obtainment of bacterial transformants. Even so, positive bacterial colonies were obtained, selected, and amplified by colony PCR. However, DNA sequencing data showed the presence of different nucleotide point alterations in the obtained clones, invalidating its use for further steps. Therefore, plasmid constructs were ordered commercially. Now we are performing its transfection in COS-7 cells to confirm that they recapitulate the splicing process observed in wt and patient cDNA being thus ready to be expressed in mice to test the therapeutic effect of the modified U1snRNA.

This work shows the different steps and difficulties of the cloning process to obtain *HGSNAT* expression constructs towards testing of an in vivo U1snRNA therapeutic approach.

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