Optimization of an exon trapping vector to study alternative splicing of neuronal genes in mammalian cells

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Introduction

Alternative splicing is highly regulated through complex interactions between *cis*-acting elements and *trans*-acting factors. The pSPL3 exon trapping vector contains a small artificial gene with an exon-intron-exon cassette. The presence of a multiple cloning site within the intron allows for the insertion of a genomic fragment of interest to create a minigene expression construct that can be transfected in mammalian cells to dissect the molecular mechanism regulating inclusion or skipping of a specific exon present in that genomic fragment.

Study Objective

Recent efforts to investigate the role of the splicing factor Ptbp2 using pSPL3 constructs have been hampered by the fact that Ptbp2 promotes robust inclusion of a cryptic exon present in the vector intron, independently of the minigene sequence. Because Ptbp2 acts in concert with other splicing factors to co-regulate alternative splicing of specific exons, this confounding factor diminishes the ability to study their regulatory roles. This project aims at optimizing pSPL3 function by eliminating the Ptbp2-dependent inclusion of the cryptic exon sequence from the original pSPL3 vector.

Methods

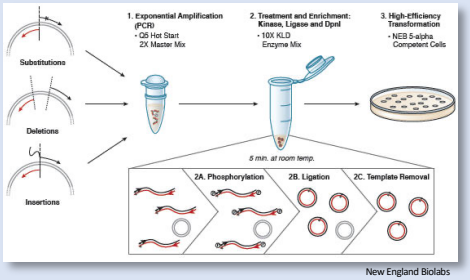

Fig 1. The Q5® Site-Directed Mutagenesis Kit was used to efficiently incorporate deletions and substitutions into vector DNA. First, the plasmid DNA was amplified exponentially using the desired primers and a master mix of Q5 Hot Start High-Fidelity DNA Polymerase. This solution was then incubated with an enzyme mix, allowing for rapid circularization of the PCR product and removal of template DNA. Finally, competent cells were transformed with the mutated DNA.

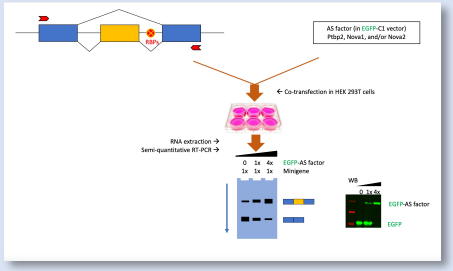

Fig 2. HEK 293T cells were co-transfected with a pSPL3 minigene construct and increasing amounts of the EGFP-tagged splicing factor. HEK 293T cells were used on account of their efficiency for transfection and the fact that they do not express many of the neuron-specific splicing factors studied. After RNA extraction, alternative splicing was monitored via semi-quantitative RT-PCR and gel electrophoresis. The use of EGFP allows for the monitoring of transfection efficiency and splicing factor expression by western blot. Dose-dependent increase in exon inclusion or skipping suggests a direct role for a specific splicing factor in regulating alternative splicing of that minigene.

Results

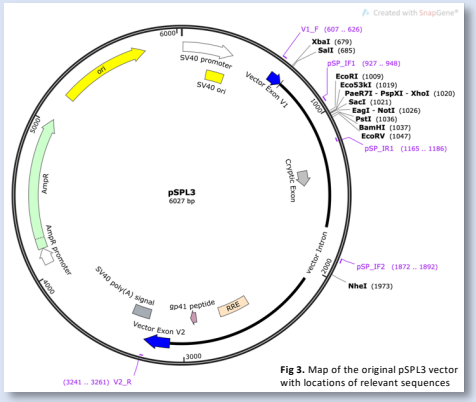

Fig 3. Map of the original pSPL3 vector with locations of relevant sequences

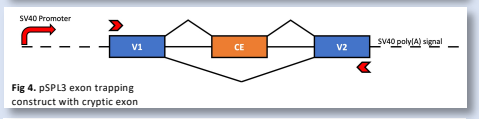

Fig 4. pSPL3 exon trapping construct with cryptic exon

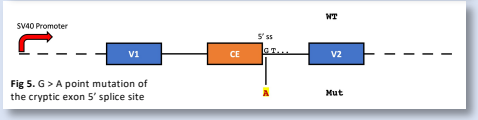

Fig 5. G > A point mutation of the cryptic exon 5' splice site

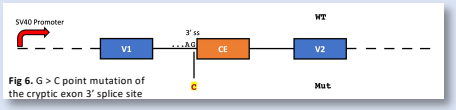

Fig 6. G > C point mutation of the cryptic exon 3' splice site

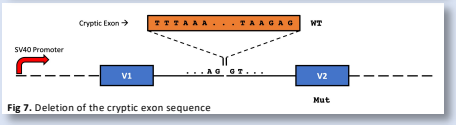

Fig 7. Deletion of the cryptic exon sequence

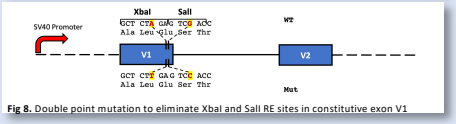

Fig 8. Double point mutation to eliminate XbaI and SalI RE sites in constitutive exon V1

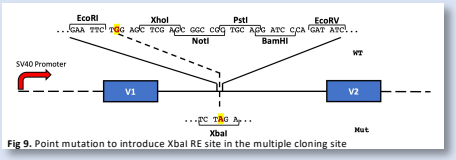

Fig 9. Point mutation to introduce XbaI RE site in the multiple cloning site

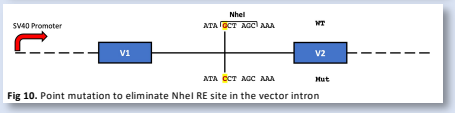

Fig 10. Point mutation to eliminate NheI RE site in the vector intron

Conclusion

We successfully eliminated the Ptbp2-dependent cryptic exon from the original pSPL3 vector. Additional point mutations and deletions were generated for improved cloning. Collectively, these mutations will give us the flexibility to clone the exon trapping cassette into a different vector for further study of the regulatory mechanisms of alternative splicing. Optimization of this exon trapping tool for minigene splicing assays will help improve our understanding of how alternative splicing events critical for neuron biology are regulated in normal conditions and in disease.

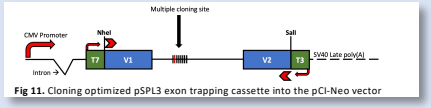

Fig 11. Cloning optimized pSPL3 exon trapping cassette into the pCI-Neo vector

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