

Application Note

Detection of alternative Tra2 β regulated splicing

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This application note describes how the QIAxcel® system was used to successfully determine the splicing pattern of exonic sequences targeted by Tra2 β protein isoforms.

Introduction

Tra2 β (*Sfrs10*) is an evolutionarily conserved splicing protein that is crucial for mouse embryogenesis (1), but its biological role is not fully understood. It has a modular structure with domains rich in arginine and serine (RS1 and RS2) and a central RNA recognition motif (RRM) that binds to target RNA sequences (2, 3). Furthermore, at least 3 isoforms of Tra2 β have been identified. Tra2 β is known to splice the *Nasp* histone chaperone gene, which monitors DNA double strand breaks (4). An evolutionarily conserved cassette exon (annotated *Nasp-T*) may play a crucial role in developmental processes. Tra2 β splices *Nasp* via a number of binding sites, but the exact role of these interactions is not known.

Because of the high levels of splicing inclusion observed for the wild type *Nasp-T* exon at endogenous cellular concentrations of Tra2 β , we tested a mutated exon ("M3+M4"), which is less efficiently spliced, to find out whether the Tra2 β binding sites are necessary for splicing activation. We also investigated the need for the Tra2 β RRM and RS1 domains in these interactions (5).

The QIAxcel system provides rapid, sensitive, and reproducible analyses of Tra2 β regulated splicing. This system may also prove advantageous for studying the role of other splicing proteins and their target sequences.

Materials and Methods

HEK 293 cells were cotransfected with a mutated *Nasp-T* construct (M3+M4) and one of 3 Tra2 β -GFP constructs encoding full length Tra2 β , Tra2 β Δ RRM, or Tra2 β Δ RS1. Control cells were cotransfected with the *Nasp-T* construct (M3+M4) and GFP only. ►

The extracted pre-mRNAs were subjected to RT-PCR and subsequently analyzed using the QIAxcel system. Samples of low DNA concentration were analyzed using Method OL400. Samples were injected at 8 kV for 20 s, and separation was performed at 6 kV for 400 s. Alignment marker, with fragments of 15 bp and 3 kb, was injected at 4 kV and 20 s and run simultaneously with the samples. QX DNA size marker, with fragments ranging from 50–800 bp, was used for size and concentration estimation.

High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) was performed as previously described (6) using an antibody specific to Tra2 β (7).

Results and Discussion

As expected, analyses on the QIAxcel system demonstrated very high splicing activation by full length Tra2 β , but also significant percentage splicing inclusion (PSI) activation by the Tra2 β Δ RRM–GFP protein (Figure 1). These results indicate that for some exons, Tra2 β can act as a coactivator as well as a splicing activator.

Interestingly, Tra2 β Δ RS1 seems to behave as a potent splicing repressor. This indicates that the endogenous Tra2 β Δ RS1 isoform acts a splicing repressor and/or that the RS1 domain plays a central role in splicing activation.

Conclusions

- The QIAxcel system is a valuable tool for revealing exon splicing patterns.
- Analyses of the splicing patterns using the QIAxcel system were both qualitative and quantitative.
- The QIAxcel system can help to identify the roles of other splicing proteins as activators, coactivators, or repressors.

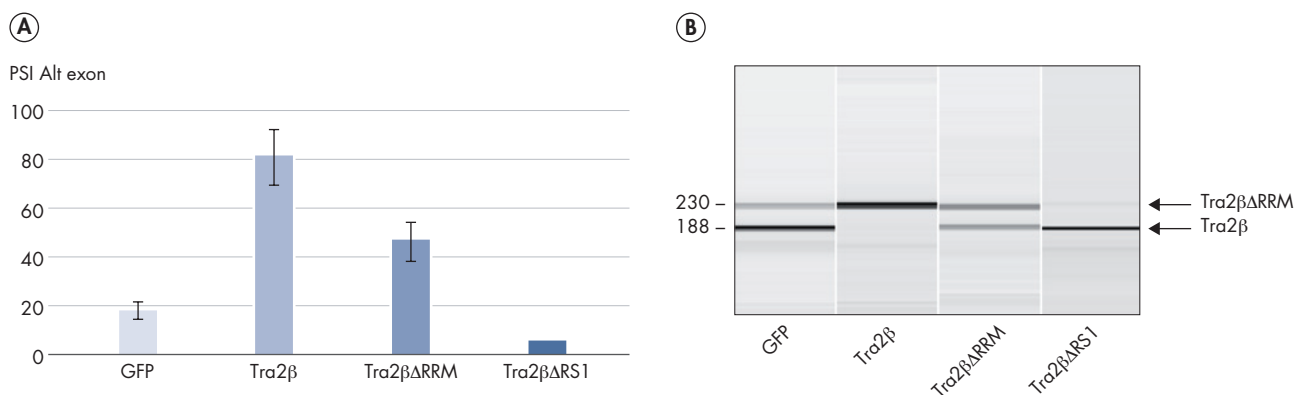


Figure 1. Splicing of *Nasp-T M3+M4* (mutant) exon. A. Percentage splicing inclusion (PSI) of a panel of exons identified through HITS-CLIP in response to GFP and Tra2 β -GFP fusion proteins. Data represent at least 3 biological replicates, and the error bars are shown as standard errors. **B.** Representative image from RT-PCR analysis on the QIAxcel system. Probability (*p*) values were calculated using an independent two-sample T-test between the PSI levels for cells cotransfected with GFP and each of the different Tra2 β -GFP constructs ($p \leq 0.05$, $p \leq 0.01$) (adapted from reference 5).

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