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Non-invasive and high-throughput monitoring of exon-specific isoform expression

Keywords: exon usage, alternative splicing, isoform, Alzheimer's Disease, tau, MAPT, exon 10, FOXP1, Cas13

INVENTION NOVELTY

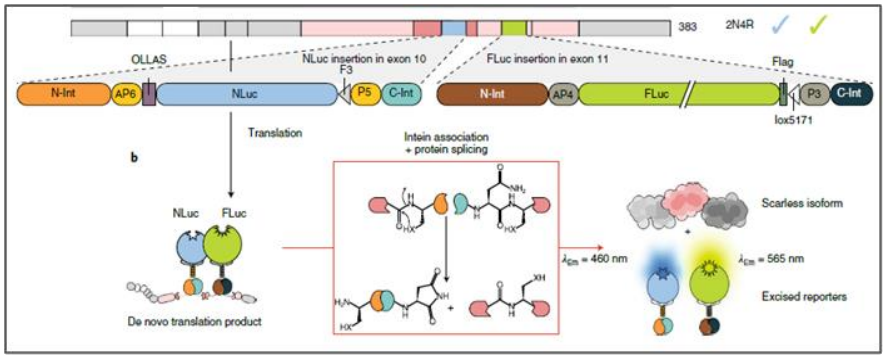
This novel versatile isoform-specific expression reporter system (EXSISERS) is non-invasively quantifying cellular isoform splicing and exon usage of endogenous genes without affecting the natural splicing pattern. It signals upon translation of a tagged exon-specific isoform by a scarless excision and reconstitution of the reporter protein from the nascent polypeptide chain through highly efficient, intein-mediated protein splicing. The approach offers cellular resolution for real time monitoring of native splice isoforms and is enabling high-throughput screening applications.

VALUE PROPOSITION

Alternative pre-mRNA splicing is a fundamental mechanism in eukaryotes for producing several protein isoforms from one gene and its impairment is associated with a variety of diseases. Approximately 15% of point mutations causing genetic diseases affect splicing, which makes alternative splicing a potential target for novel drug development approaches. Conventional methods and molecular tools to assess alternative splicing patterns are either consumptive and work-intensive or do not allow a quantitative analysis of isoform expression at the protein level (Fig. 1a). In contrast, EXSISERS (**ex**on-**s**pecific **is**oform **e**xpression **r**eporter **s**ystem) enables a non-disruptive, real time and multimodal monitoring of exon-specific isoform expression with high sensitivity and cellular resolution and empowers high-throughput screening applications for exon-specific therapeutic interventions. Based on its superior performance it is the first established non-invasive molecular tool to track alternative spliced protein isoforms with the potential to significantly improve and ease the analysis of exon-specific isoform expression in research and drug discovery.

	EXSISERS	minigenes	immuno-blot	immunocyto-chemistry	RT-qPCR	RNA-FISH
Non-consumptive	☑	☑				
Reflects complex regulation at the endogenous sites	☑		☑	☑	☑	☑
Does not compete with splice factor binding	☑		☑	☑	☑	☑
Cellular resolution	☑	☑		☑		☑
Detection at protein-level	☑		☑	☑		
Longitudinal readout	☑	☑				
Sensitive & high dynamic range	☑	☑			☑	
High-throughput compatible	☑	☑				
Enables survival screen for endogenous regulators	☑					
Does not require cell line establishment			☑	☑	☑	☑

a) Features of EXSISERS compared to current methods for the analysis of splice isoforms



b) EXSISERS components for the analysis of an exon of interest

TECHNOLOGY DESCRIPTION

As proof-of-concept a split-intein-mediated NanoLuc luciferase-based reporter system was established in HEK293T cells for analysis of exon 10 usage of the disease-associated microtubule-associated protein tau (MAPT; Fig. 1b). EXSISERS was further applied to patient-derived induced pluripotent stem cells to quantify MAPT exon 10 usage. Cas13d systems were evaluated for the development of RNA-guided splice suppressors or enhancers. In addition, the inclusion of exon 18b of FOXP1, which is involved in maintaining pluripotency of embryonic stem, was monitored and a selection strategy was established to identify splice regulators of FOXP1, demonstrating that MBNL1 is the dominant suppressor of exon 18b inclusion.

COMMERCIAL OPPORTUNITY

The technology is available for licensing or further co-development and includes access to material.

DEVELOPMENT STATUS

Established reporter systems are available for research and drug discovery applications. Further reporter systems could be developed in collaboration or by licensee.

PATENT SITUATION

A PCT application (WO2020161236A1) has been filed which is entitled “Detection of splice events of a gene of interest” (priority: 06.02.2019). National phases were entered in US and EP.

FURTHER READING

Truong, DJ.J., Phlairaharn, T., Eßwein, B. *et al.* Non-invasive and high-throughput interrogation of exon-specific isoform expression. *Nat Cell Biol* **23,** 652–663 (2021); https://doi.org/10.1038/s41556-021-00678-x

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