SUPPLEMENTARY NOTE 1

VISUALIZING ALTERNATIVE MRNA SPLICING IN LIVE MAMMALIAN CELLS WITH THE RIBOGLOW-FLIM SENSOR

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Principle of producing two alternatively spliced isoforms in the PIG3 splicing reporter system

Alternative splicing may trigger frameshifts, such that stop codons may be in frame in one isoform but not another, resulting in translation termination in different reading frames. This principle is the foundation to evaluate the splicing of alternative exons in reporter genes. A previously established splicing reporter includes exon skipping and an open reading frameshift, leading to production of differentiable reporter proteins (Gurskaya et al, 2016)[1]. Based on this splicing model, we developed a splicing reporter as follows (see also main Fig. 3). A fusion protein is produced upon normal splicing (Halotag-exon1-exon2-exon3-GFP). Exon 2 has 193 nucleotides (notably not triplicates) and ends with one extra nucleotide. This extra nucleotide (A) is responsible for frame shifts, per Gurskaya et al, 2016. Exon 3 has 185 nucleotides, i.e. can be thought of as having two extra nucleotides that will result in the -GFP gene read in frame. Hence, if exon 2 is retained, there is no frame shift, no stop codon is produced and GFP is translated in frame and a GFP produced (normal splicing, main Fig. 3). Cells producing this fusion protein are positive for the Halotag fluorescence reporter signal and GFP fluorescence reporter signal.

In contrast, UV-exposure results in production of the alternative spliced transcripts that leads to exon skipping. Here, a frameshift in exon 3 results in encoding of a stop codon before the GFP coding region (main figure 3), such that the produced gene product results in a fusion protein that only includes the Halotag and not GFP. Any additional gene modifications of the reporter (such as including the RNA tag fragments or changing reporter proteins) do not alter the reporter system, as long as additions are divisible by three to avoid adding additional unwanted frame shifts.

Normal splicing product (see also main Figure 3):

Exon 2
Exon 3
GFP
TAA / TAG (*stop codon)*

Halotag-linker-Exon1-GCT GGA GTT AAT CTT ATT CTA GAC TGC ATA GGC GGA TCC TAC TGG GAG AAG AAC GTC AAC TGC CTG GCT CTT GAT GGT CGA TGG GTT CTC TAT GGT CTG ATG GGA GGA GGT GAC ATC AAT GGG CCC CTG TTT TCA AAG CTA CTT TTT AAG CGA GGA AGT CTG ATC ACC AGT TTG CTG AGG TCT AGG GAC AAT ***AAG*** *TAC AAG CAA ATG CTG GTG AAT GCT TTC ACG GAG CAA ATT CTG CCT CAC TTC TCC ACG GAG GGC CCC CAA CGT CTG CTG CCG GTT CTG GAC AGA ATC TAC CCA GTG ACC GAA ATC CAG GAG GCC CAT AAG TAC ATG GAG GCC AAC AAG AAC ATA GGC AAG ATC GTC CTG GAA CTG CCC CAG ACG ATG AGC GGG GGC GAG GAG CTG …etc.TAA*

UV-exposure induces alternative splicing where exon 2 is spliced out, resulting in a frame shift and stop codon in exon 3 (see also main Fig. 3):

Exon 3
GFP
Stop codon
Halotag-linker-Exon1-AGT ACA AGC AAA TGC TGG TGA ATG CTT TCA CGG AGC AAA TTC TGC CTC ACT TCT CCA CGG AGG GCC CCC AAC GTC TGC TGC CGG TTC TGG ACA GAA TCT ACC CAG TGA CCG AAA TCC AGG AGG CCC ATA AGT ACA TGG AGG CCA ACA AGA ACA TAG *GCA AGA TCG TCC TGG AAC TGC CCC AGA CGA TGA GCG GGG GCG… etc. TAA*

Reference:

(1) Gurskaya, N. G.; Staroverov, D. B.; Lukyanov, K. A. Chapter Eleven - Fluorescent Protein-Based Quantification of Alternative Splicing of a Target Cassette Exon in Mammalian Cells. In *Methods in Enzymology*; Filonov, G. S., Jaffrey, S. R., Eds.; Academic Press, 2016; Vol. 572, pp 255–268. https://doi.org/10.1016/bs.mie.2016.02.007.