Spinach™ splice sensor: a cell-based drug discovery platform for splicing-related diseases

Ryan O'Hanlon, Balajee Somalinga, Aminoor Rashid, and Karen Wu
Lucerna, Inc., 760 Parkside Avenue, Suite #327C, Brooklyn, NY 11226
Email: ryan@lucernatechnologies.com (or) karen.wu@lucernatechnologies.com

ABSTRACT

RNA splicing plays a central role in the generation of proteome diversity and in gene regulation. Splicing affects cellular processes, such as cell-fate and differentiation, acquisition of tissue-identity, and organ development. For human diseases, it is estimated that up to 50% of all pathogenic genetic mutations may affect RNA splicing. Additionally, defects in splicing are linked to spinal muscular atrophy (SMA), Duchenne muscular dystrophy, Parkinson's disease, and several types of cancer. Recent studies with drugs modulating RNA splicing have demonstrated potential as effective therapeutics for these diseases, and subsequently intensified efforts for drug discovery of novel spliceosome targets. However, monitoring RNA splicing with current gold standard techniques (RT-qPCR and RNA-seq) are not readily adaptable for high-throughput screening (HTS) and is cost-prohibitive at this scale due to their complex and time-consuming methodology. While splice mini-gene systems are higher throughput, they lack the ability to monitor the endogenous target RNA due to its artificial design. Thus, there is an unmet need for simple and robust cell-based, HTS-ready assays to monitor bona fide endogenous RNA splicing.

To address this critical bottleneck for splice modulation drug discovery, Lucerna has developed a simple, HTS-ready splice sensor platform that can be customized to detect any splicing event of interest. In our assay format, sensor reagents are added to target cells in one step and read at room temperature in common fluorescent plate readers. This homogenous workflow significantly reduces sample handling time and error rates compared to other competing assays. Importantly, our assays can detect as little as a 10% change in splicing, further reducing false negative hit rates and increasing valuable hit identification. With this technology, Lucerna is striving to be the market leader in the development of RNA-based research reagents that enable scientific discovery.

Currently, we have developed splice sensors against a variety of splicing targets, including survival of motor neuron 1 (SMN1), pyruvate kinase isoforms M1 (PKM1) and M2 (PKM2), and splicing targets involved in neurodegenerative diseases. Previously, we have demonstrated that against target RNAs, these sensors display rapid response times, high selectivity, excellent sensitivity, and an extended readout window. Building upon this, we have successfully adapted the splice sensor platform into cell-based assays capable of detecting endogenous RNA isoform changes caused by perturbations with small molecules or siRNA. This allows the use of more disease relevant materials, such as patient-derived primary cells and total RNA from tissues extracted from *in vivo* models. In summary, the Spinach™ splice sensor platform offers HTS-ready, disease-relevant, and customizable assays for better splice modulating drug discovery.

ASSAY CONCEPT

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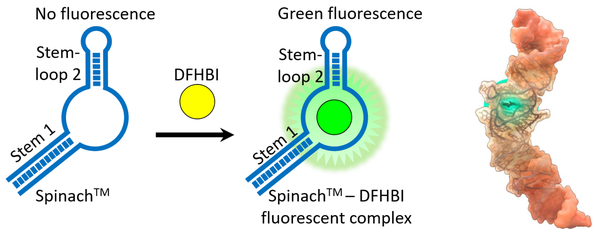
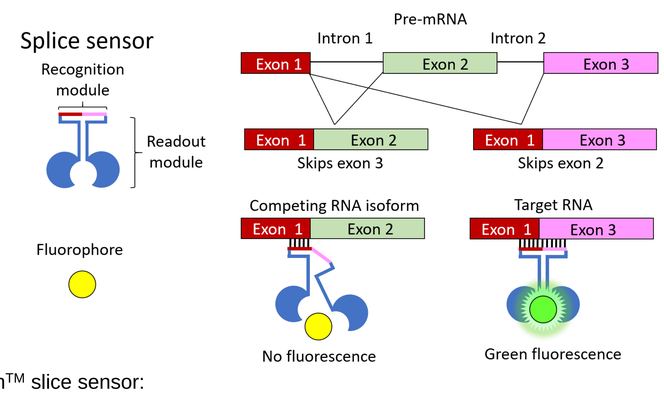


Spinach™ technology:

- An RNA mimic of the green fluorescent protein (GFP).
- Consists of RNA aptamers that bind and turn on the fluorescence of otherwise non-fluorescent dyes, such as DFHBI.
- Is genetically encodable with proven utility for imaging RNA and measuring cellular metabolites in living cells.

2



Spinach™ slice sensor:

- Readout module comprised of Spinach™ and a recognition module that targets the spliced RNA of interest.
- The recognition module is comprised of two RNA probes complementary to the exon sequences flanking the target splice site, i.e. exon 1 (dark red) and exon 3 (pink).
- Binding of the correct target RNA enables the folding of Spinach™, binding to fluorophore, and fluorescence.

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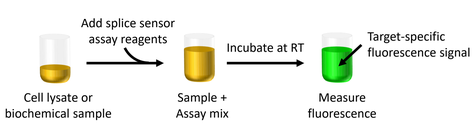

The homogenous assay format of the Spinach™ splice sensor assay makes it easily adaptable for high-throughput screening.

CASE STUDY: PYRUVATE KINASE

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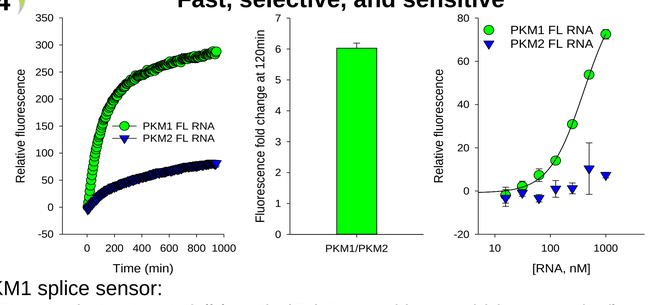
Fast, selective, and sensitive



PKM1 splice sensor:

- Responds to PKM1 full-length (FL) RNA with a rapid increase in fluorescence signal that was stable for over 16h.
- Displays good selectivity (~6-fold fluorescence over PKM2 FL RNA).
- Has sensitivity as low as 62.5nM of PKM1 FL RNA within 120 minutes.

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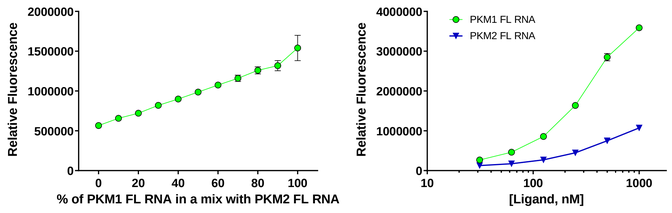
Detects small splicing changes



Within 120 minutes, PKM1 splice sensor:

- Able to detect a significant change in fluorescence in a mixture of only 10% PKM1 FL RNA and 90% PKM2 FL RNA ($p<0.05$).
- Is sensitive to as low as 31nM of the PKM1 FL RNA in when spiked in HeLa cell lysates.

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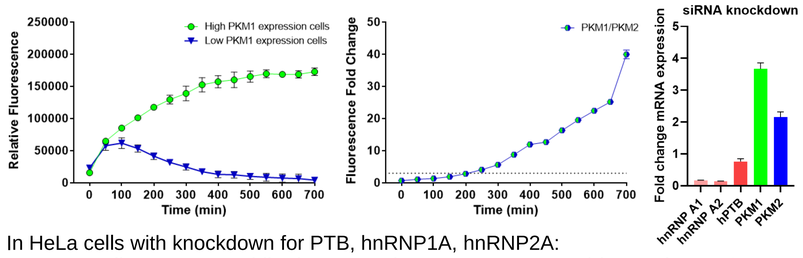
Measuring changes of endogenous PKM1 RNA in HeLa cells



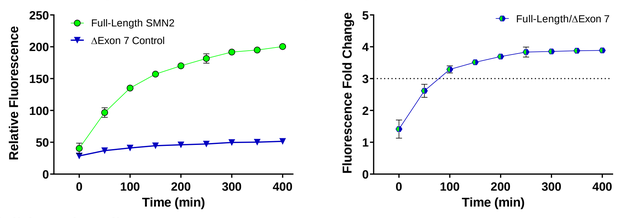
In HeLa cells with knockdown for PTB, hnRNP1A, hnRNP2A:

- PKM1 splice sensor rapidly detects endogenous mRNA, and has at least >3-fold fluorescence over cells transfected with scrambled siRNA after ~200 minutes.
- With proprietary stabilization reagent, can sustain fluorescence for >14h (data not shown).
- Sensor measurements of PKM1 mRNA levels corroborated with qPCR data.

CASE STUDY: SMA

7

Splice sensor assay adaptable for SMN2



SMN2 full-length splice sensor:

- Responds to SMN2 full-length RNA with a rapid increase in fluorescence.
- Displays good selectivity over Δexon 7 SMN2 (~3-fold greater fluorescence) within 70 minutes.

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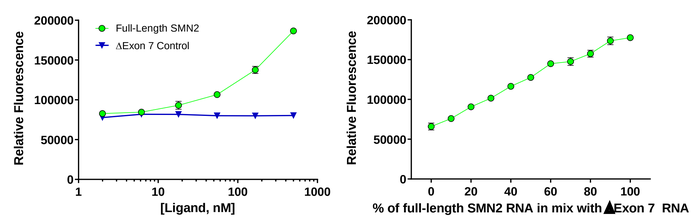
Good sensitivity and specificity in cell lysates



Assays with SMN2 RNA spiked in 293T cell lysates:

- Has sensitivity as low as 18nM of full-length SMN2 RNA within 70 minutes.
- Able to detect a significant change in fluorescence in a mixture of only 10% SMN2 full-length RNA and 90% ΔExon 7 RNA ($p<0.05$) within 70 minutes.

CASE STUDY: INTRONIC RNA

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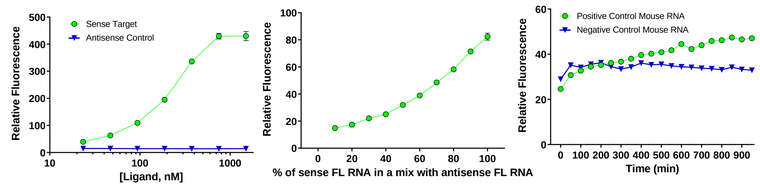
Selectively differentiates sense and antisense RNA



Intronic sensor for a confidential sense strand RNA target (external collaboration):

- Is sensitive to as low as 16 nM of the target within 120 minutes.
- Able to detect a significant change in fluorescence in a mixture of only 10% sense RNA target and 90% antisense RNA ($p<0.05$) within 120 minutes.
- Similar sensitivities and specificities were obtained for an intronic sensor targeting antisense strand of the target (not shown).
- Low copy number sense strand target was detected in total RNA from cells extracted from mouse disease model.

CASE STUDY: CIRCULAR RNA

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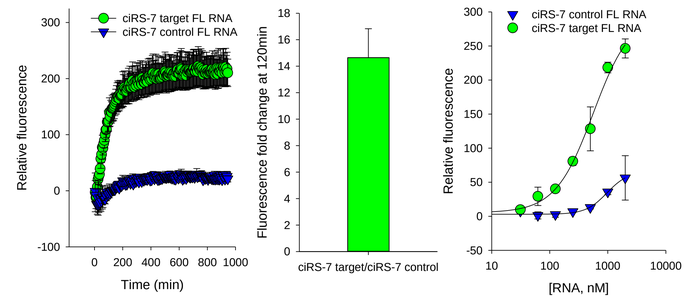
Selectively detects back-spliced transcripts



Splice sensor targeting the back-splice junction of a common circular RNA involved in cancer, ciRS-7:

- Displays a rapid response (<30 min) in fluorescence with ciRS-7 target FL RNA.
- Has good selectivity over linear non-back-spliced RNA control (~14-fold greater fluorescence).
- Sensitivity as low as ~62.5nM of ciRS-7 target FL RNA within 120 minutes.

SUMMARY

Fast, selective, stable	Read in ~1-2h, robust target-specific fluorescence, >16h readout window
Customizable, target any RNA isoform of interest	Detect endogenous mRNAs, circular RNAs, and intronic RNAs.
Super detection resolution	Detect as low as 10% splicing changes and low copy number transcripts.
Ready for cell-based applications	Use cell-lines, primary cells, or total RNA directly in the assay.
Versatile, HTS adaptable, mix-and-read	Cell-based screens with simple workflow, multiplex capabilities

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ACKNOWLEDGEMENTS

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