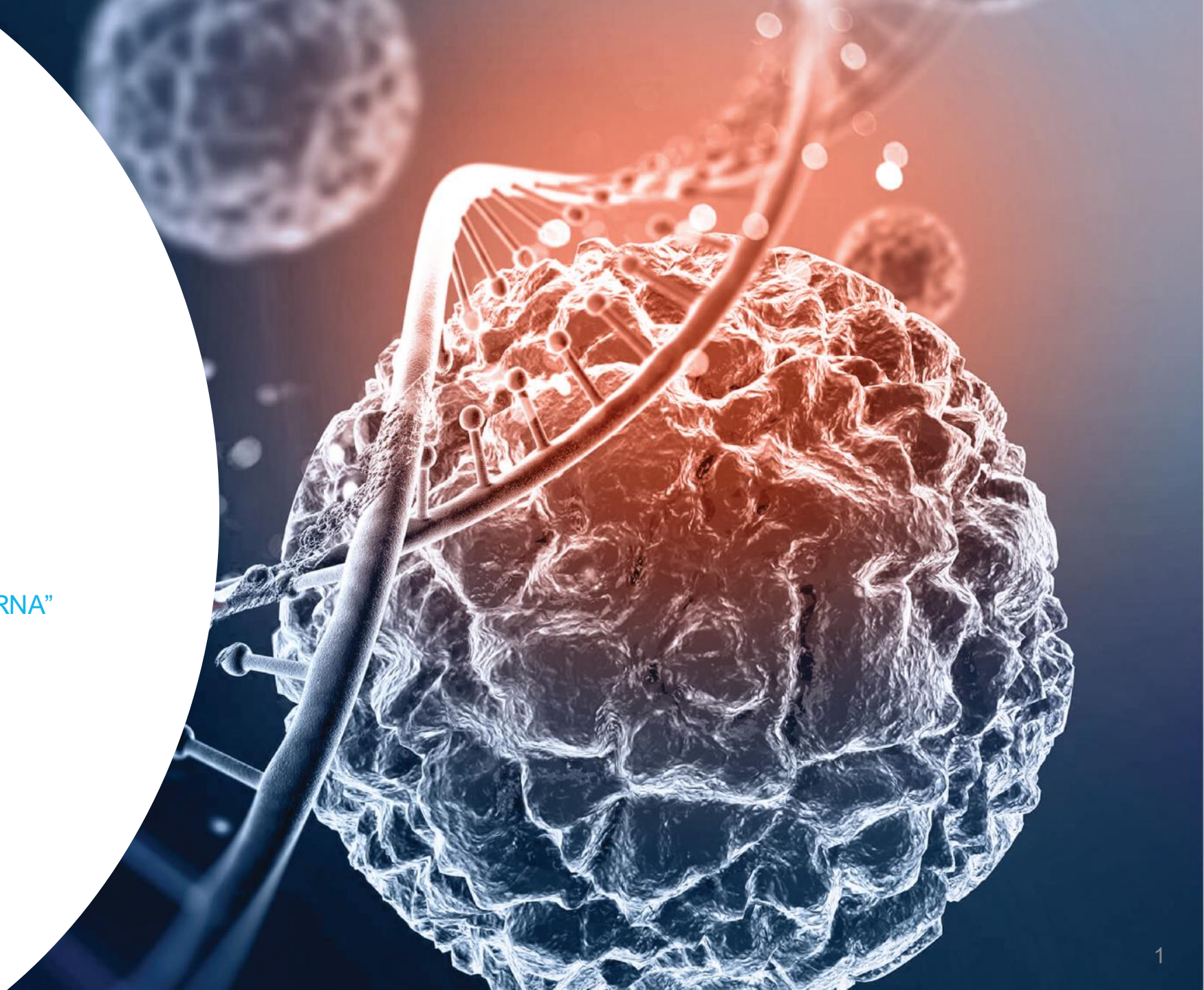
Technology Capability Presentation

"New toolkits to see, study, and drug RNA"

Non-confidential information only

lucernatechnologies.com



About us

Who

Lucerna, Inc. is a biotech company with a proprietary fluorescent aptamer technology developed from Dr. Samie Jaffrey's laboratory at Cornell University.

How

Spinach™ is the RNA version of green fluorescent protein (GFP). We also have **Corn**, **Squash**, and **Broccoli**.

What

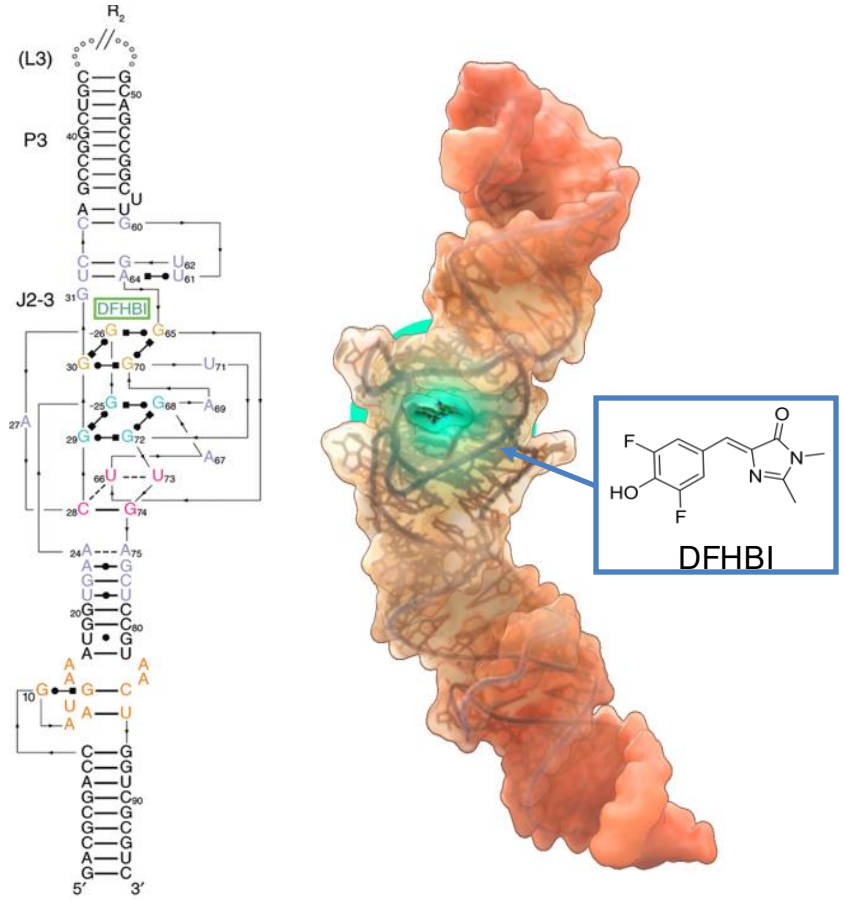
The **Spinach™ Technology** has highly versatile applications:

- **Drug Discovery** – Modular platform, mix-and-read, HTS assays
- **RNA Imaging** – Live-cell imaging of endogenous mRNAs and non-coding RNAs
- **R&D Toolkits** – Customized RNA tools for molecular detection and diagnostics

New

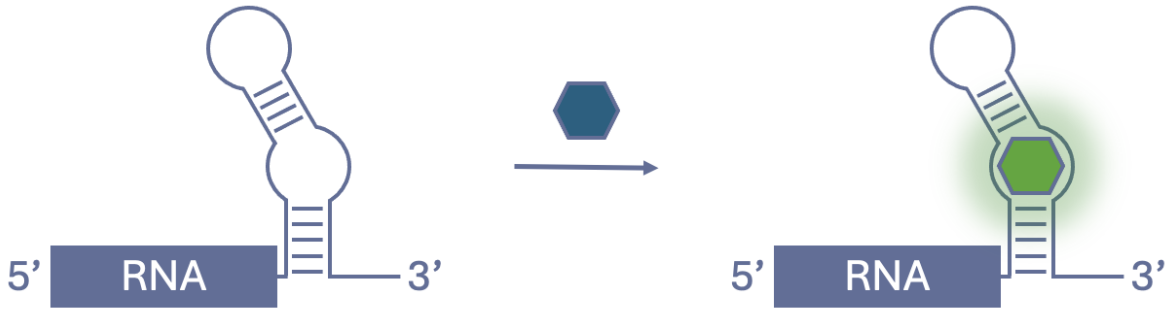
We have now achieved **single-molecule mRNA imaging resolution** and has developed high-throughput assay platforms for **RNA-targeted drug discovery**.

Spinach™: A fluorescent RNA technology



The system comprises of a RNA aptamer (Spinach) that turns on the fluorescence of an otherwise non-fluorescent dye (DFHBI).

With the Spinach™ system, the entire life cycle of any RNA of interest can be visualized in real time.



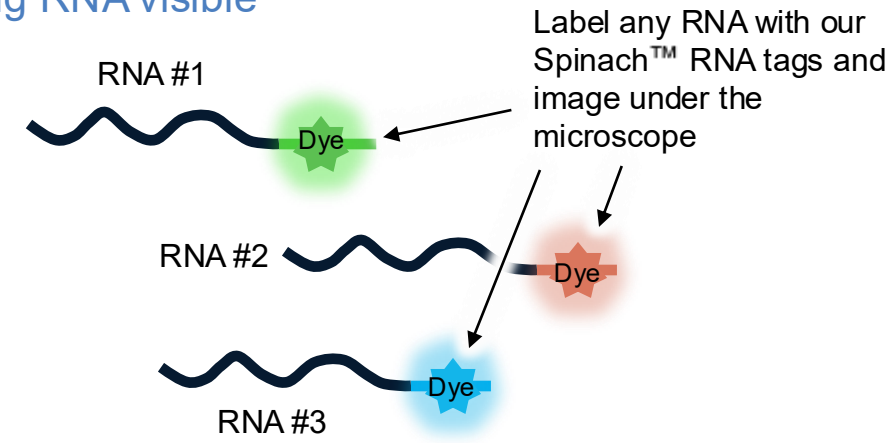
Advantages over protein-based imaging systems:

- On/off fluorescence control for precise visualization
- Small tag size minimizes cellular interference
- Enables tagging of non-coding RNAs
- Allows direct measurement of RNA activity, delivering more accurate data

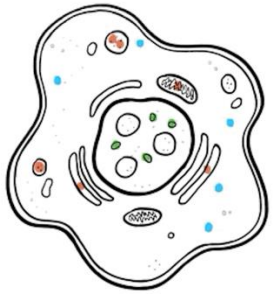
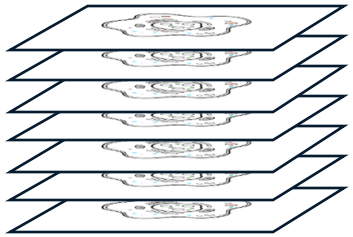
LUCERNA™

Spinach™ capabilities

1 "Making RNA visible"

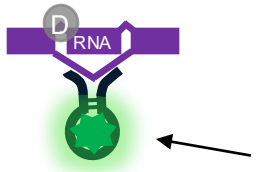


Label any RNA with our Spinach™ RNA tags and image under the microscope

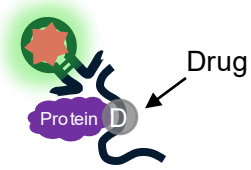
Us		Competitors
	Vs.	
Data gathered from 1 experiment		Same data requires > 9 experiments
(Movies)		(Photographs)

2 "Making RNA druggable"

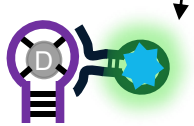
Drugs that control RNA splicing (e.g. nusinersen)



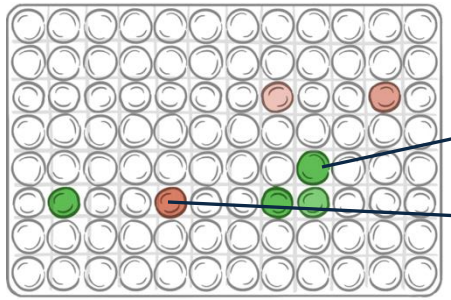
Drugs that regulate RNA-protein interactions (e.g. risdiplam)



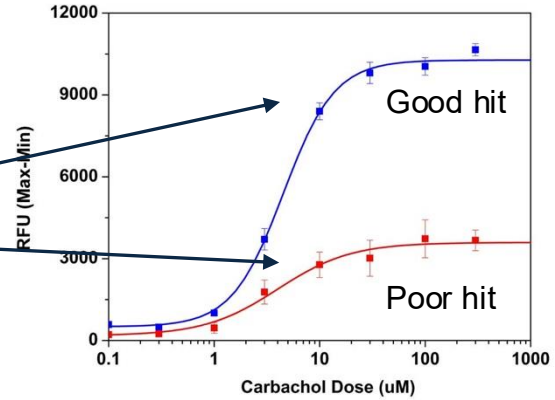
Spinach™ sensors



Drugs that bind RNA structures (e.g. tetracycline)

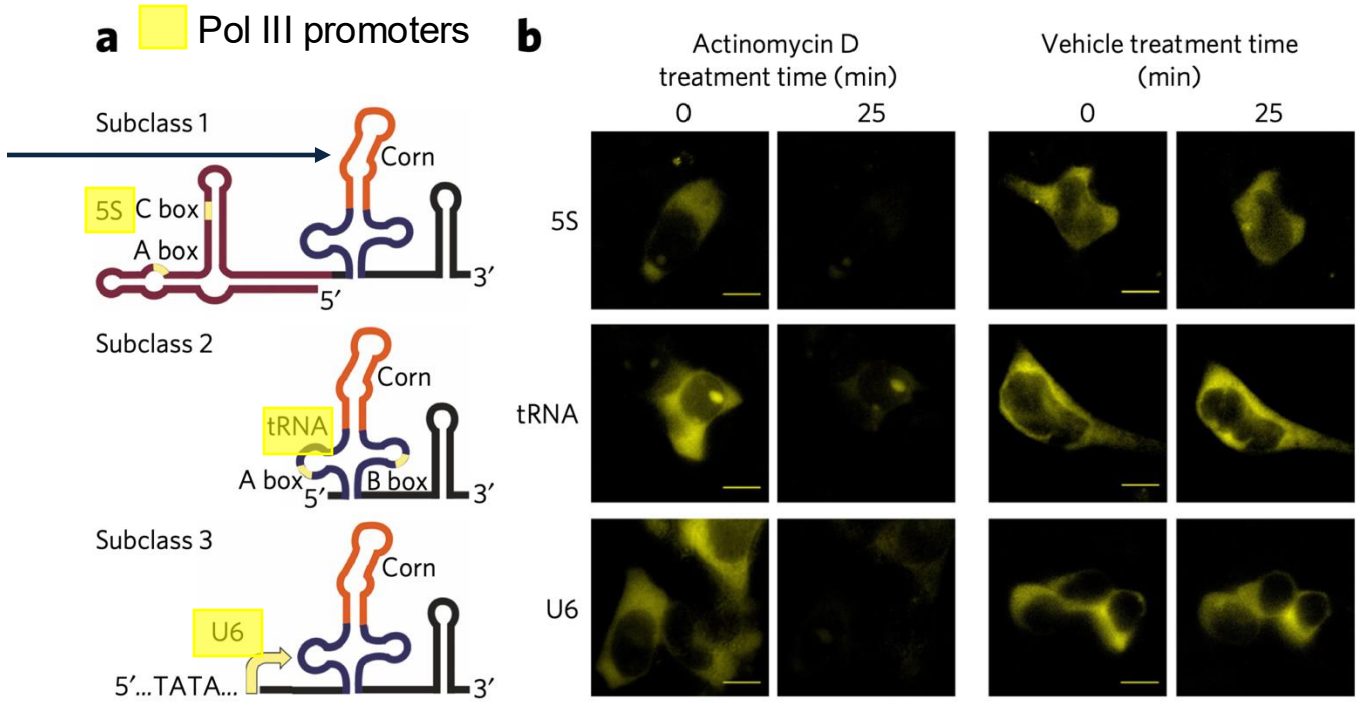


High-throughput assays



Application #1: Imaging transcription activities

Lucerna's Corn tag expressed as part of the Pol III RNA transcripts



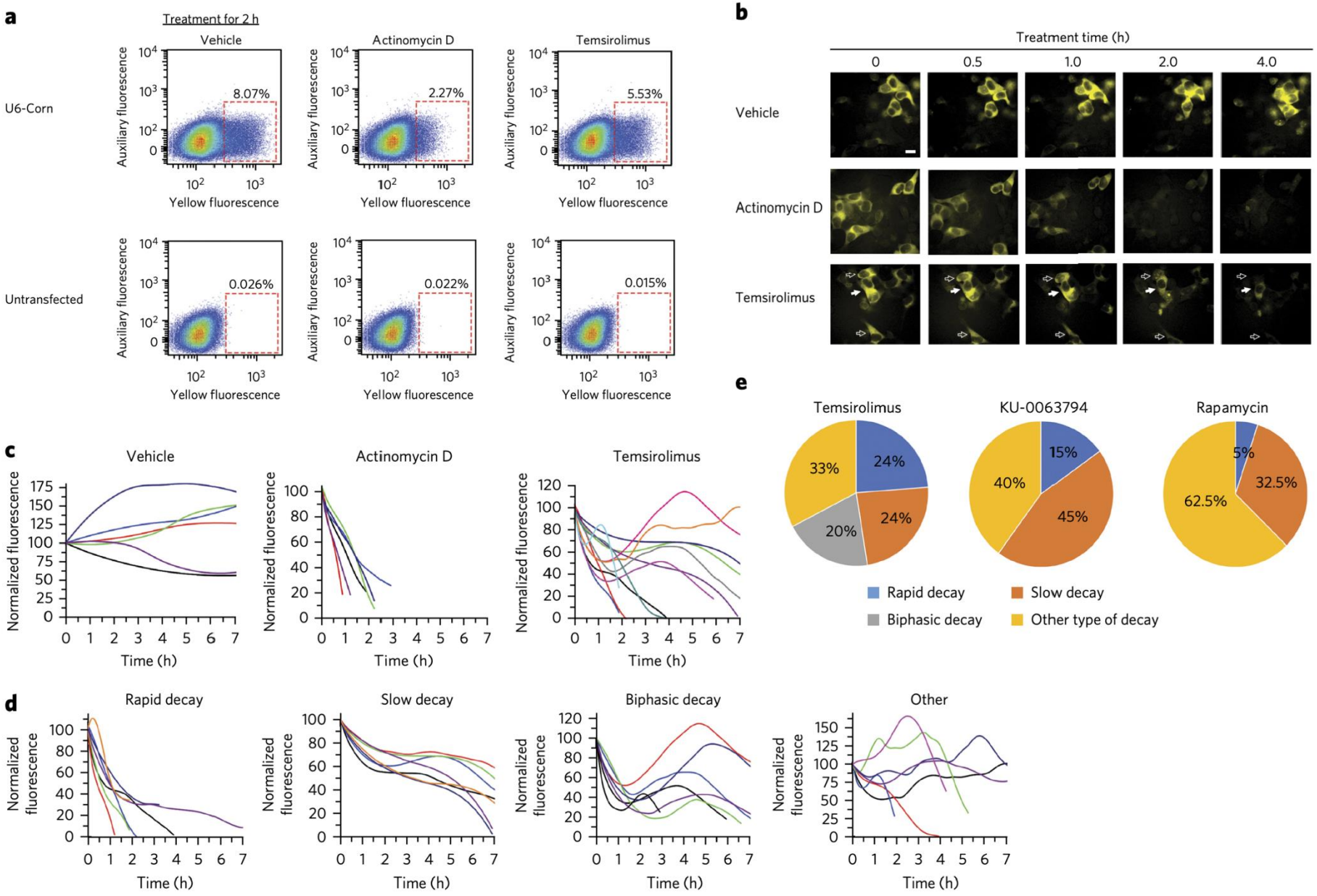
HEK293T cells expressing different Pol III reporter constructs exhibit yellow fluorescence (Corn)

Corn fluorescence exhibits rapid turnover in the presence of actinomycin D, a transcription inhibitor.

Song *et al.*, Nat Cell Biol, 2017.

Whole-population drug response by flow cytometry

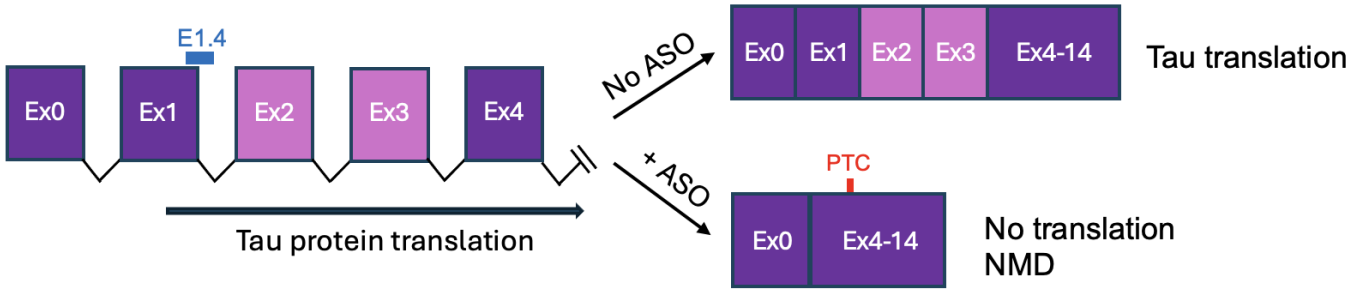
Single-cell Pol III activity analysis revealed heterogenous transcription suppressions by current mTOR inhibitors



Song *et al*., Nat Cell Biol, 2017.

Application #2: RNA degradation assay

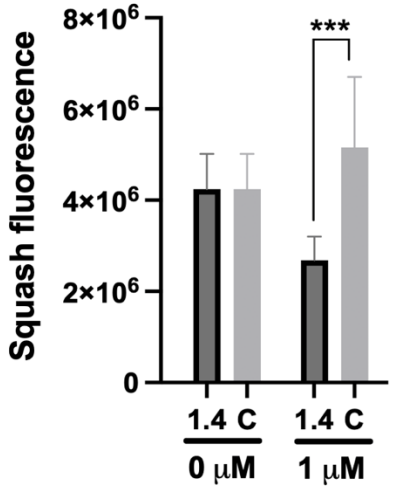
Microtubule-associated protein Tau (MAPT)

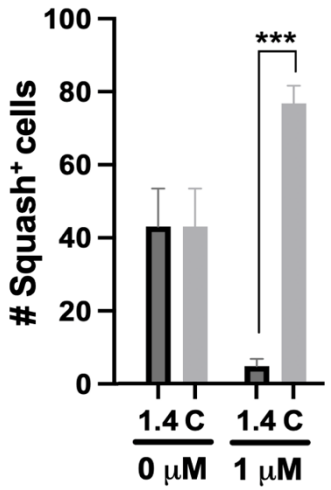


Exon 1.4-targeting ASO (1.4) reduced *MAPT* transcripts by ~55% and tau protein levels by ~70% in SH-SY5Y and IMR32 cells *

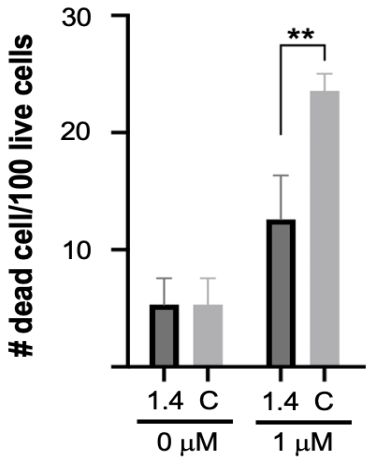
* Sud *et al.*, Mol Ther Nucleic Acids, 2014.

Reporter assays

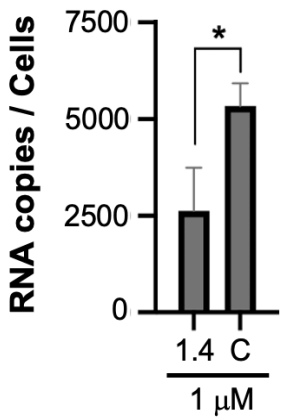




Cell toxicity

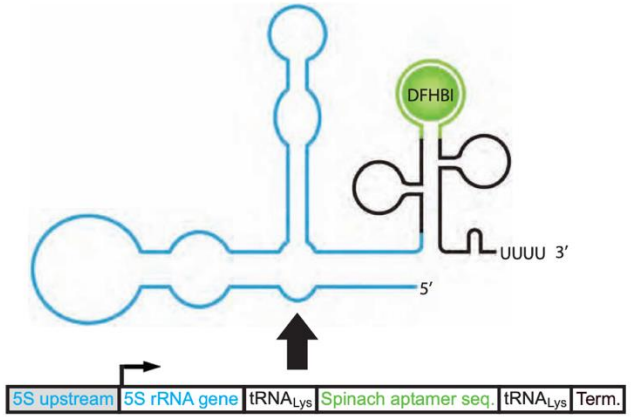


qPCR



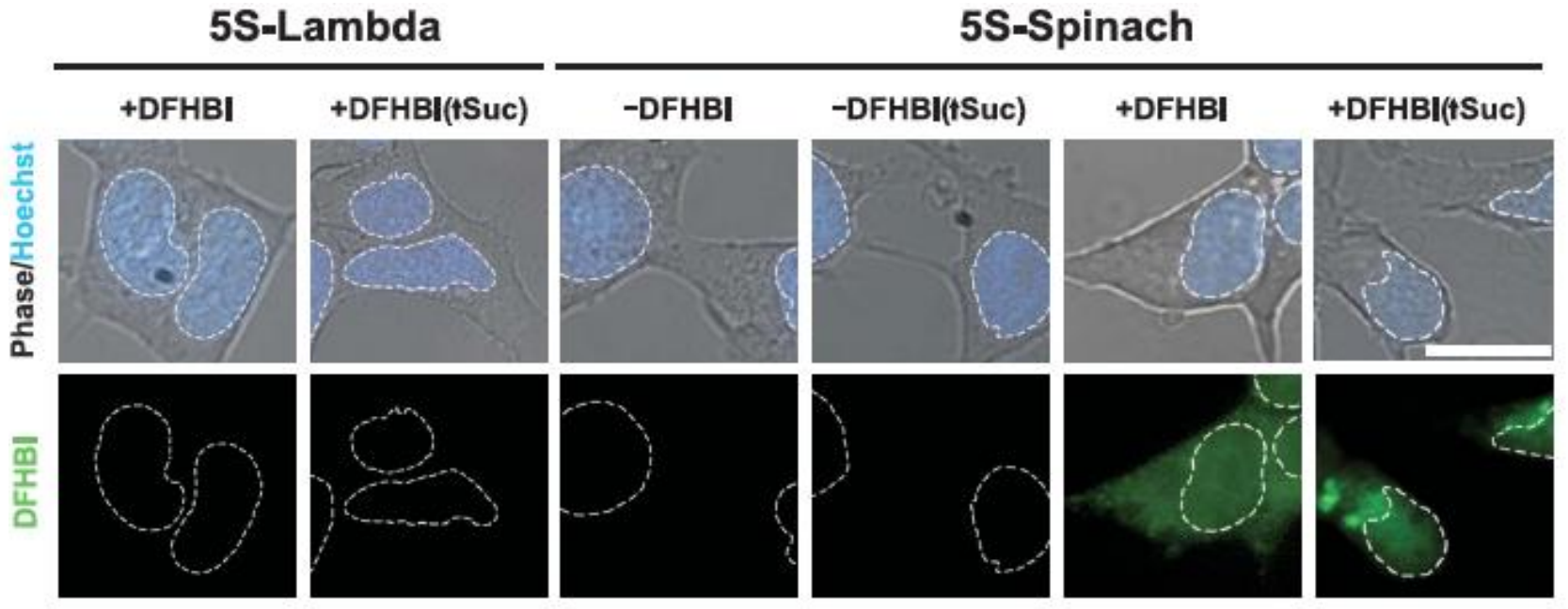
ASO1.4 reduced MAPT transcripts as reflected by reporter assays and qPCR analysis. Further, ASO1.4 was found to have cell protective effects

Application #3a: Imaging of ncRNA molecular condensates

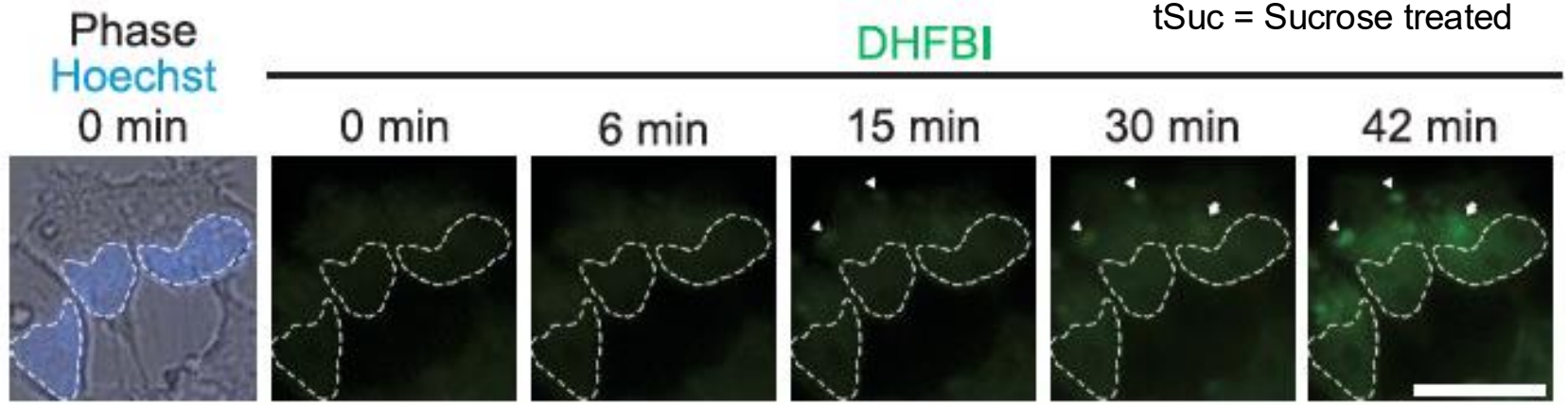


Change to image RNA of interest

Change to image transcription activity



tSuc = Sucrose treated

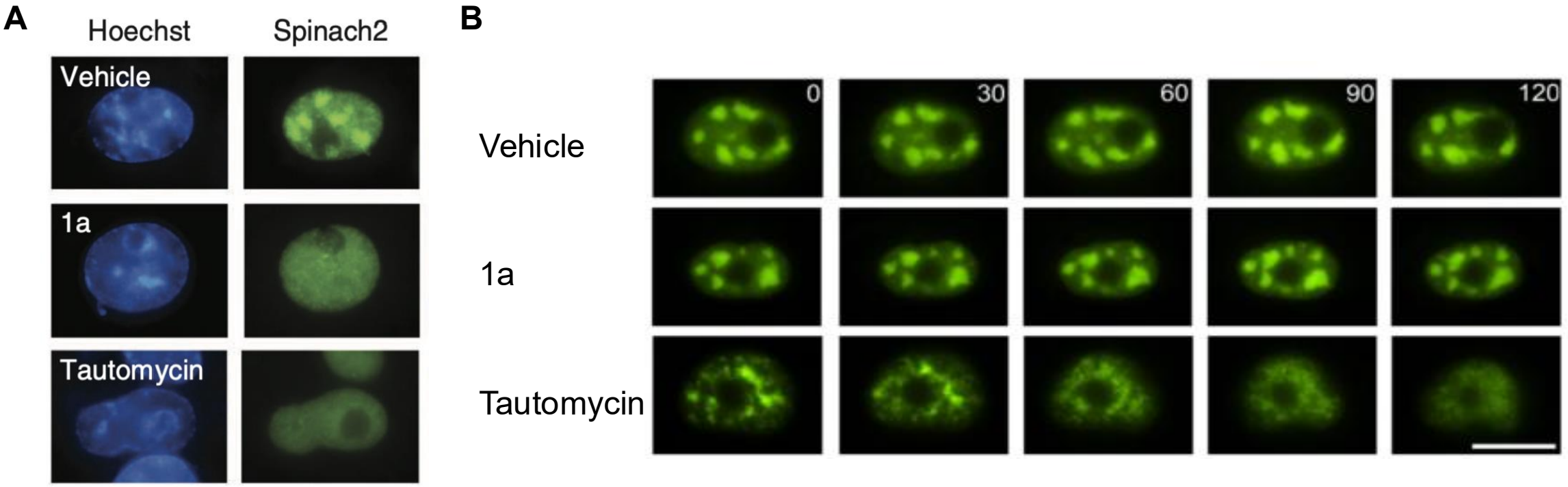


► Denotes 5S rRNA in stress granules

Paige *et al*., Science, 2011.

Application #3b: Imaging of toxic CGG foci for FXTAS drug discovery

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a progressive neurodegenerative disorder. RNA gain-of-function toxicity from expanded CGG repeat aggregation (foci) is the primary cause of FXTAS pathology.



While both 1A and tautomycin prevent CGG foci formation (**A**), only tautomycin dissolves existing foci (**B**).

Strack *et al.*, Nat Meth, 2013.

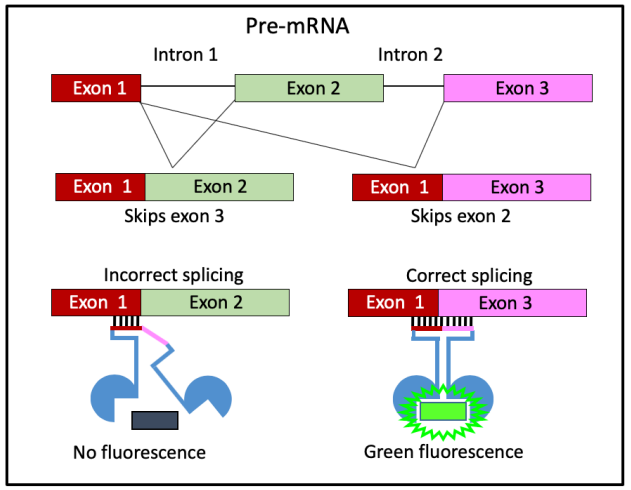
Application #3: Platform for RNA splicing drug discovery

Sensor schematic

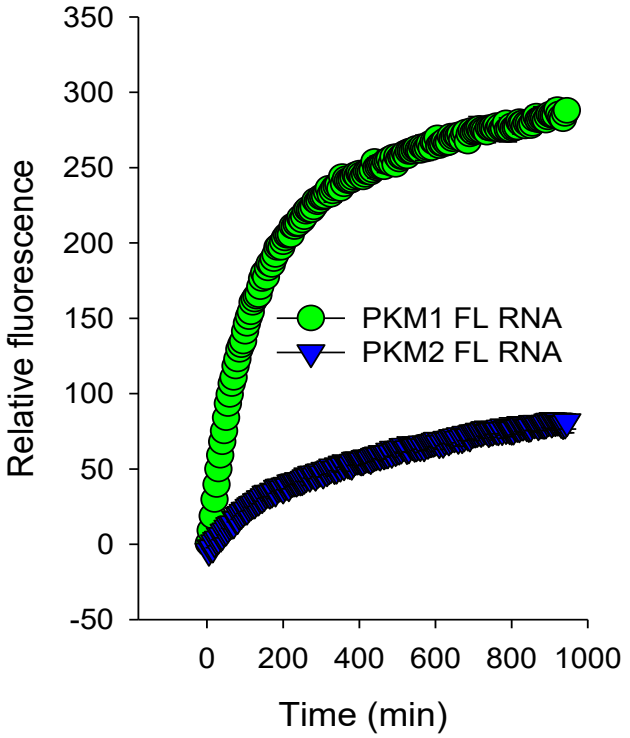
Splice sensor:

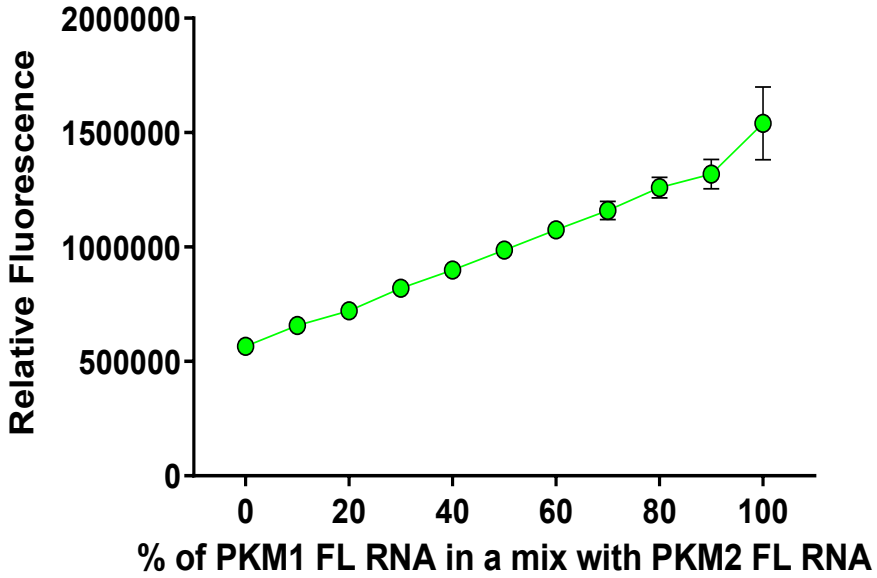
Target recognition region

Sensor dye:



Assay performance



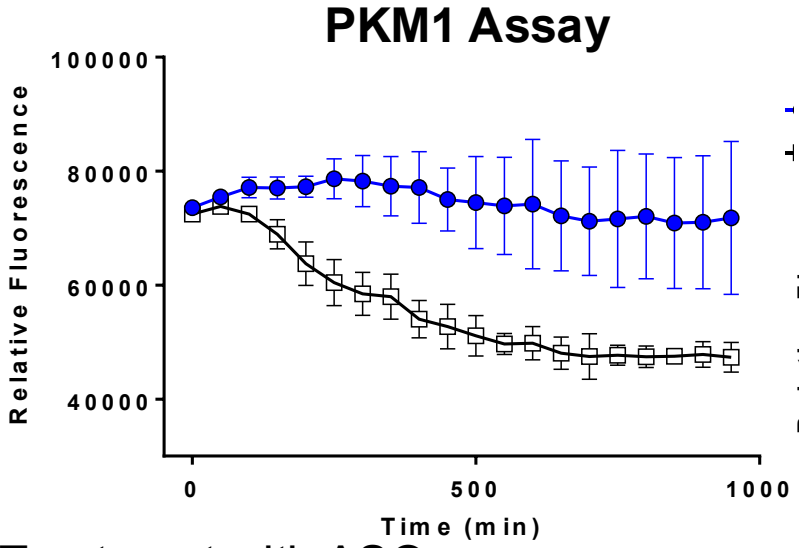


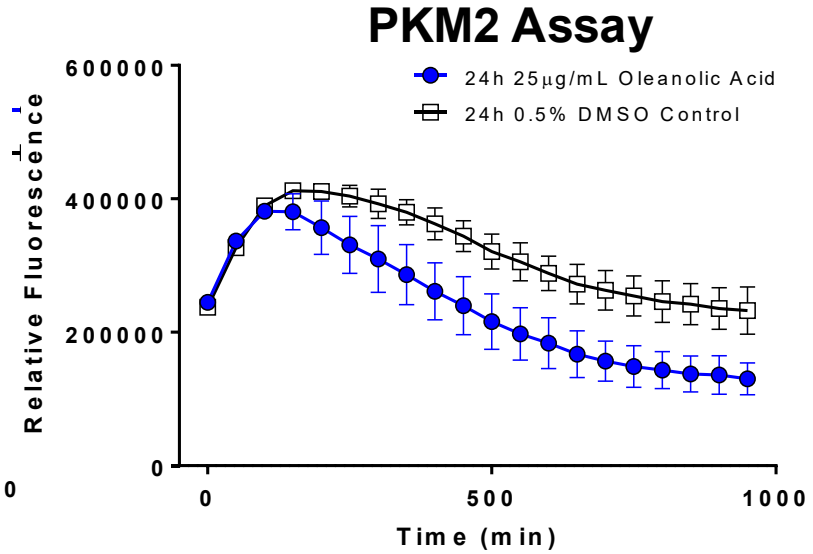
- Pyruvate kinase spliced isoforms PKM1 (Ex8/9) and PKM2 (Ex8/10) regulate cancer cell metabolism
- Significant fluorescence changes are detected in mixtures with only 10% splicing changes
- Sensors discriminate between PKM1 and PKM 2 RNA within a large dynamic range.

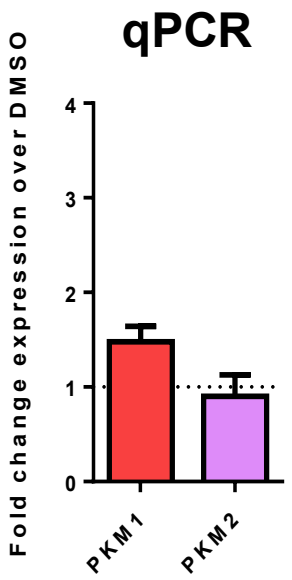
LUCERNA™

PKM assay detects splice switching in cells treated with drug or ASO

Treatment with Oleanolic Acid

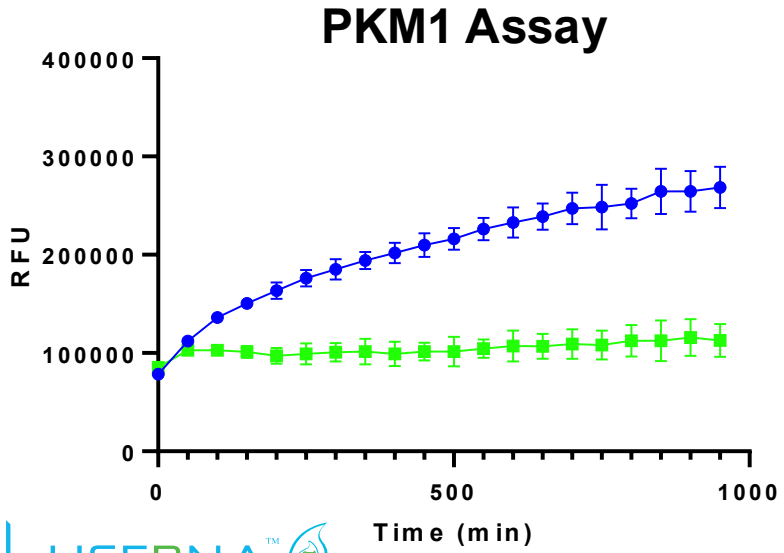


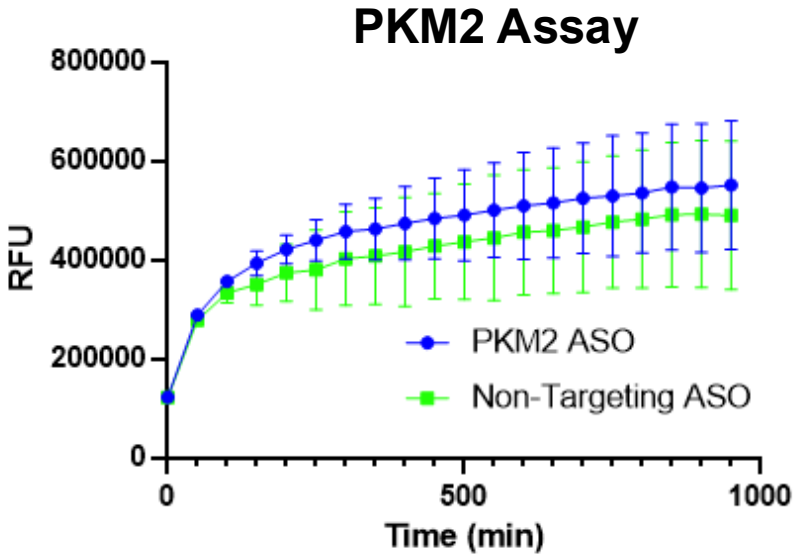


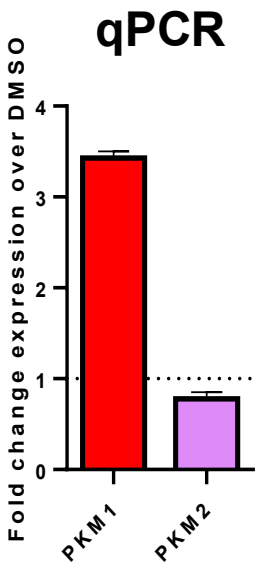


- Oleanolic Acid Treatment: PKM1 up 1.5X in fluorescent assay and qPCR. PKM2 down 30% in fluorescent assay and 10% by qPCR.

Treatment with ASO



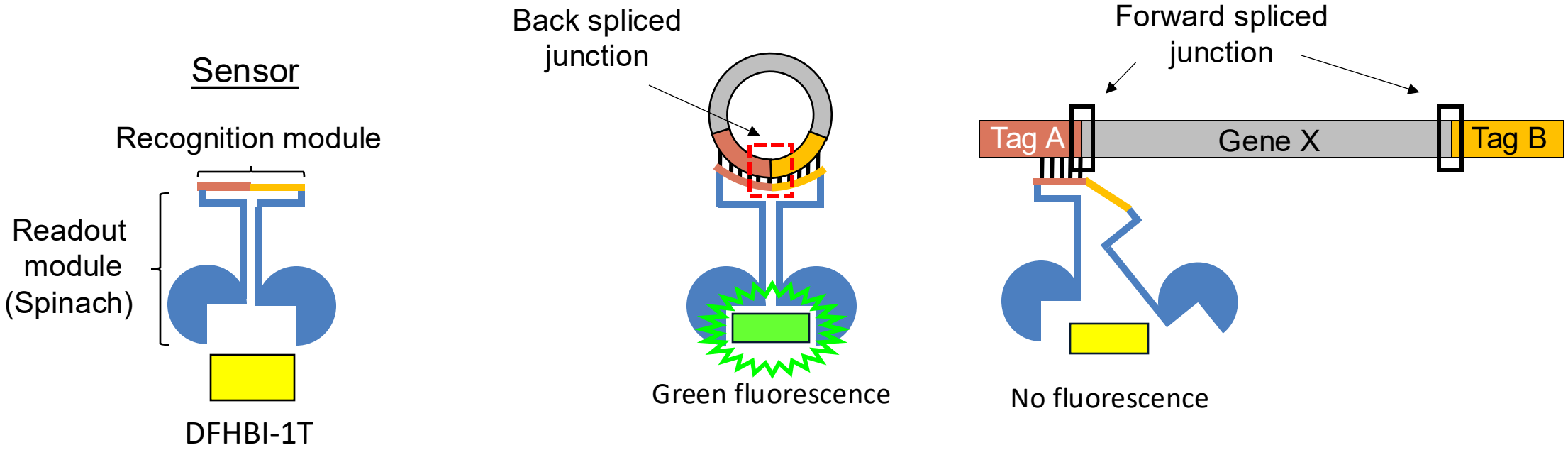




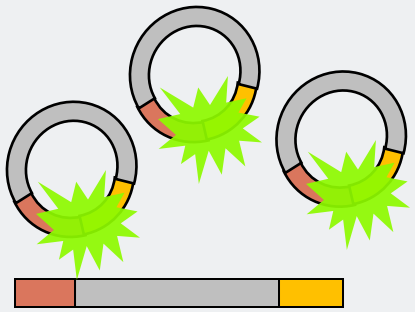
- ASO Treatment: PKM1 up 2.4X in fluorescent assay and 3.5X by qPCR. PKM2 down 0% in fluorescent assay and 20% by qPCR.

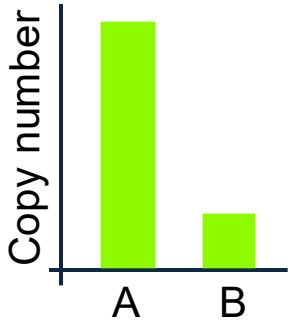
LUCERNA™

Application #4: Circular RNA therapeutics quantification

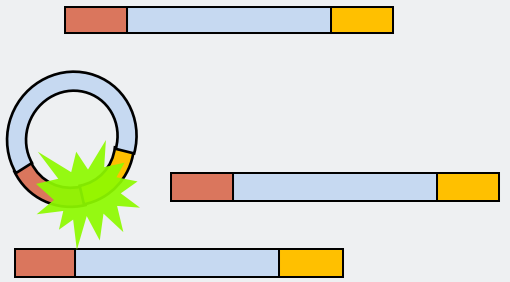


Production A





Production B

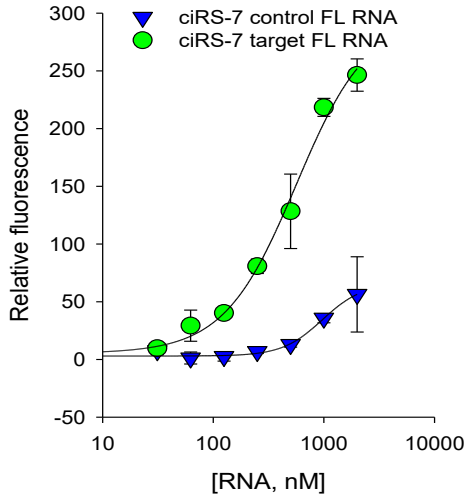


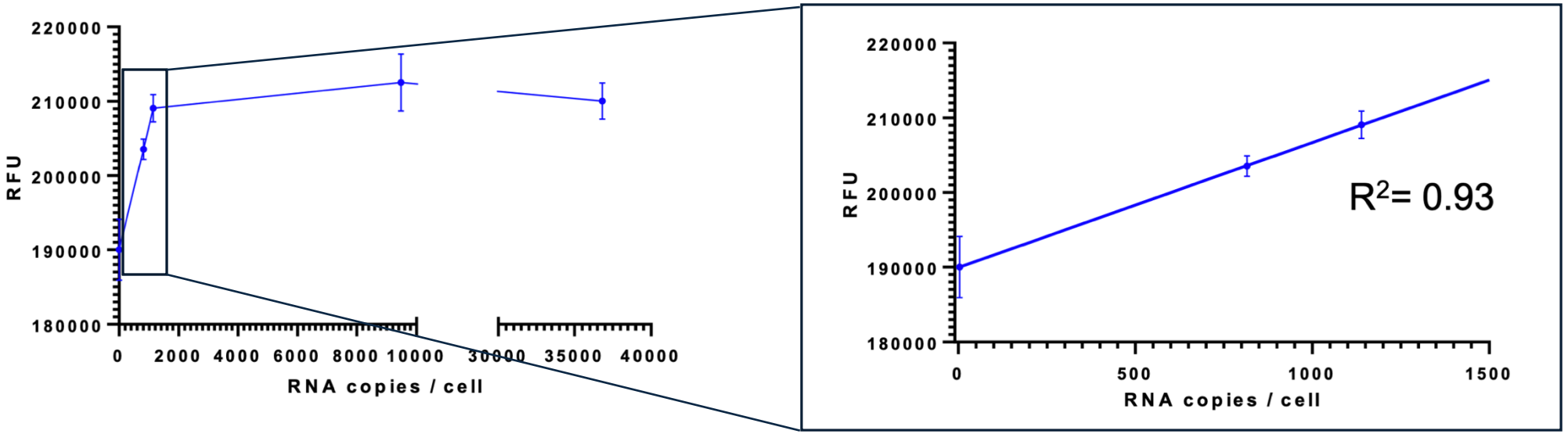
ciRS-7 circRNA sensor detection in cells

ciRS-7 RNA Copies / Cell In Tranfected HeLa Cells

Transfected ciRS-7 circRNA plasmid (µg)	Ct	RNA copies	Number of cells in sample	RNA copies per cell
10	16.87	31,775,554	863	36,820
5	18.5	10,775,554	1,137	9,479
2.5	21.88	1,139,242	1,000	1,139
1.25	22.57	721,001	883	816
Control plasmid	32.04	1,346	307	4

Example of the Fluorescence Assay

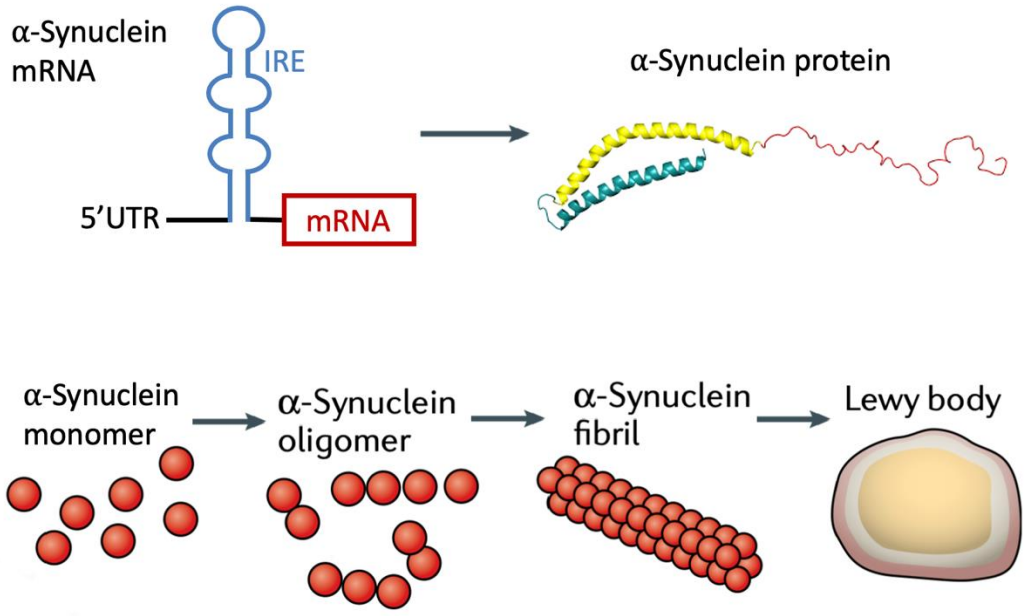




Sensor can detect <1,000 RNA copies / cell with an >1 log dynamic range in a 384-well fluorescence assay

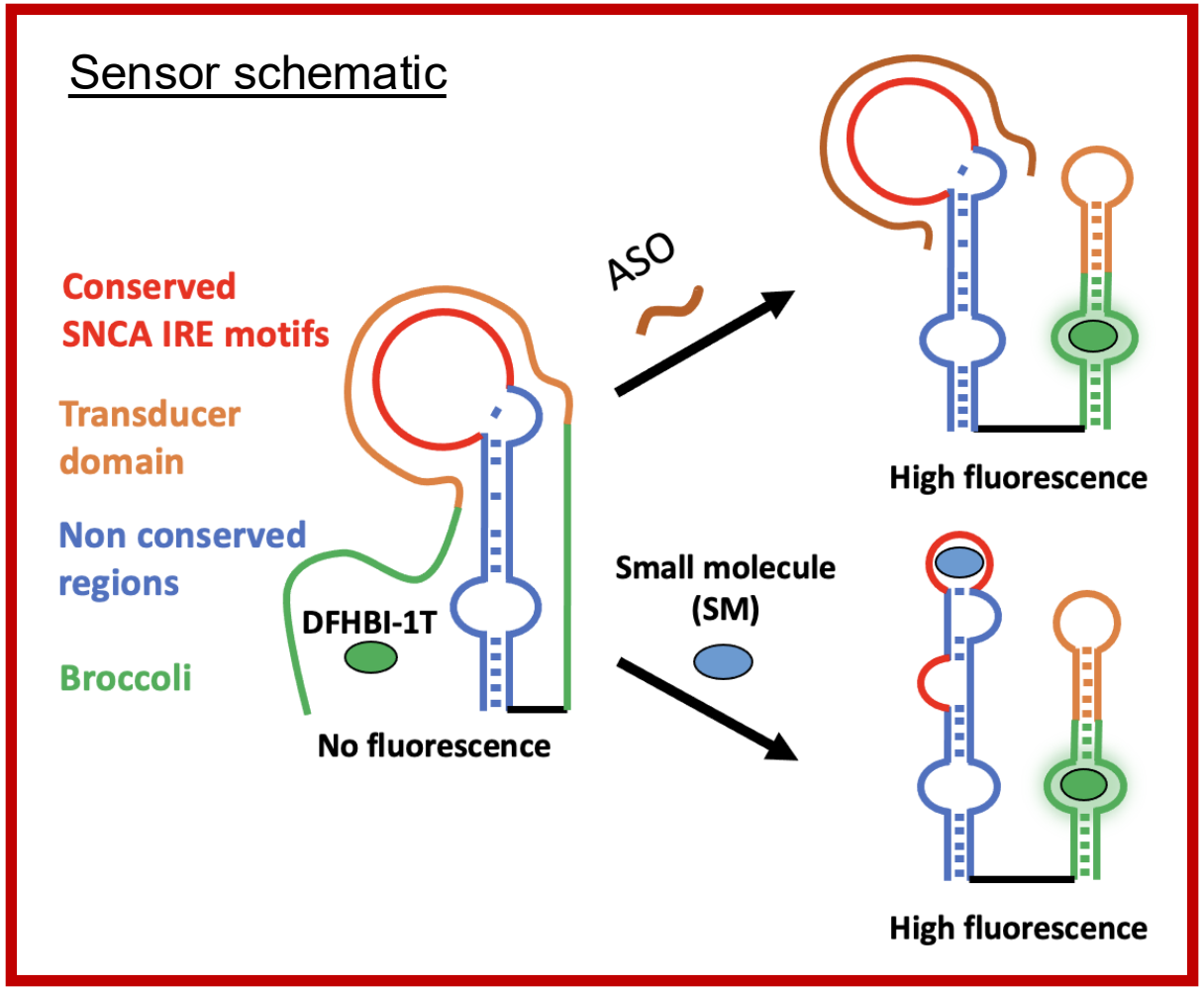
LUCERNA™

Application #5: Platform for RNA structure drug discovery



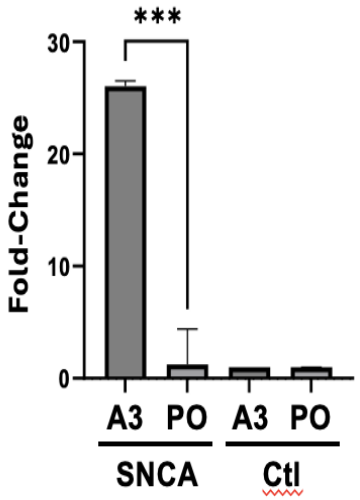
Adopted from Arnaoutoglou et al., *Nat Rev Neurol*, 2019

α-Synuclein (SNCA) is the key protein in PD pathology. 5'UTR of SNCA contains an iron-responsive element (IRE) that regulate its translation.

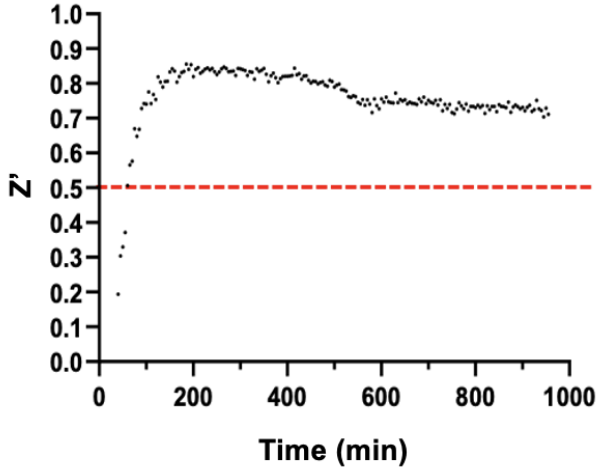


Assay identifies SNCA IRE-specific SM binder

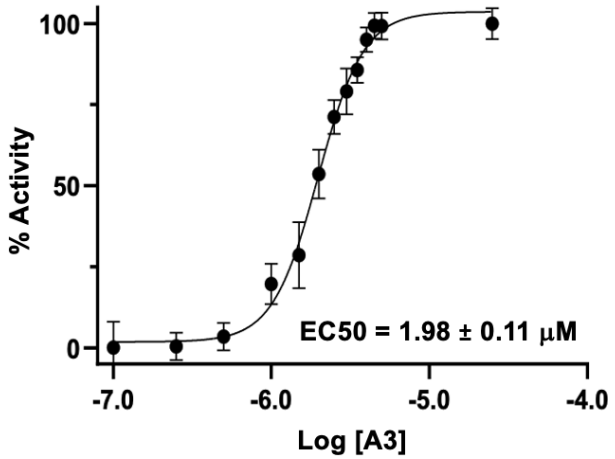
Specific



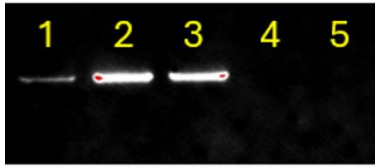
Stable and robust



Dose responsive



Functional



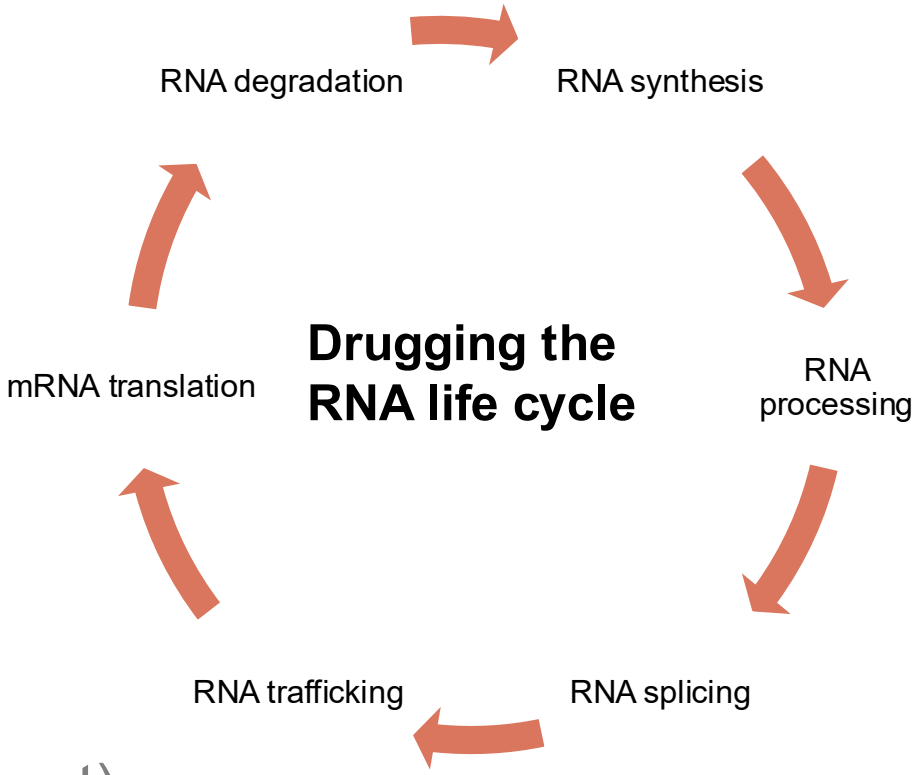
1. IRP1 alone
2. SNCA IRE + IRP1
3. SNCA sensor + IRP1
4. Control sensor + IRP1
5. No RNA + IRP1

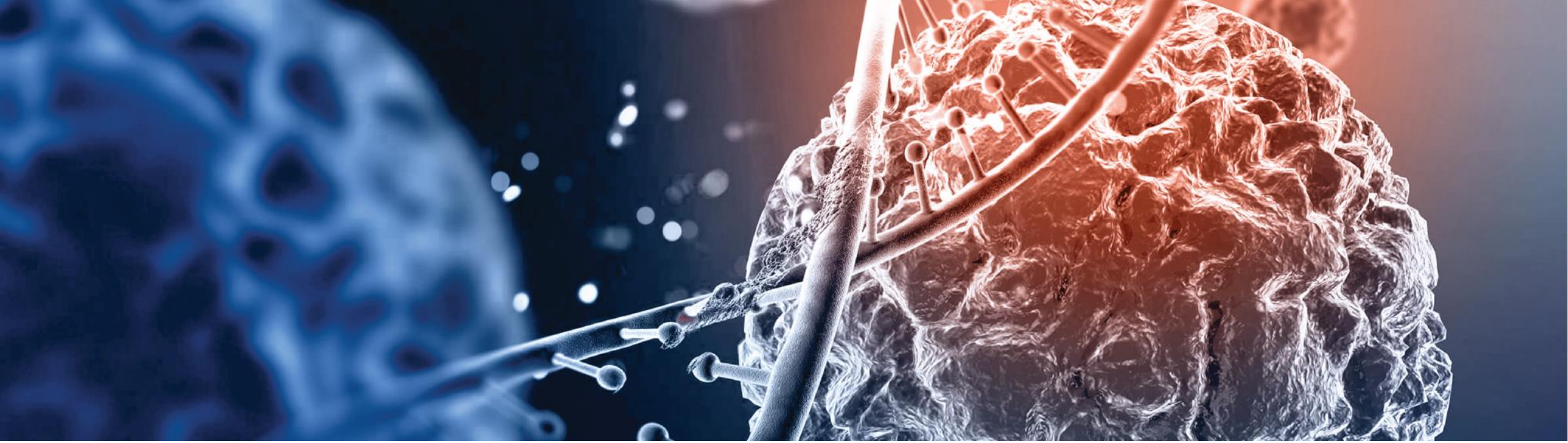
SNCA IRE assay exhibits robust fluorescence only in the presence of A3, a SM previously found to inhibit SNCA translation. Posiphen (PO), a pan IRE binder, does not activate assay signal.

The Spinach™ technology enables both cellular and biochemical RNA-targeted drug discovery applications

Current technology capability:

- Live-cell imaging of mRNA and ncRNA
- Transcription activity assays
- RNA turnover assays
- HTS assays targeting RNA splicing
- Circular RNA quantification assays
- HTS assays targeting RNA structures
- Cellular RNA-protein reporters (in development)





LUCERNA™

FOR YOUR ATTENTION

Downstate Technology Center
760 Parkside Avenue
Brooklyn, NY 11226

+1.646.504.5697

karen.wu@lucernatechnologies.com