



“New toolkits to see, study, and drug RNA”

**Spinach splice sensor:  
A cell-based drug discovery  
platform for splicing-related  
diseases**



[lucernatechnologies.com](http://lucernatechnologies.com)



# RNA splicing as a drug target

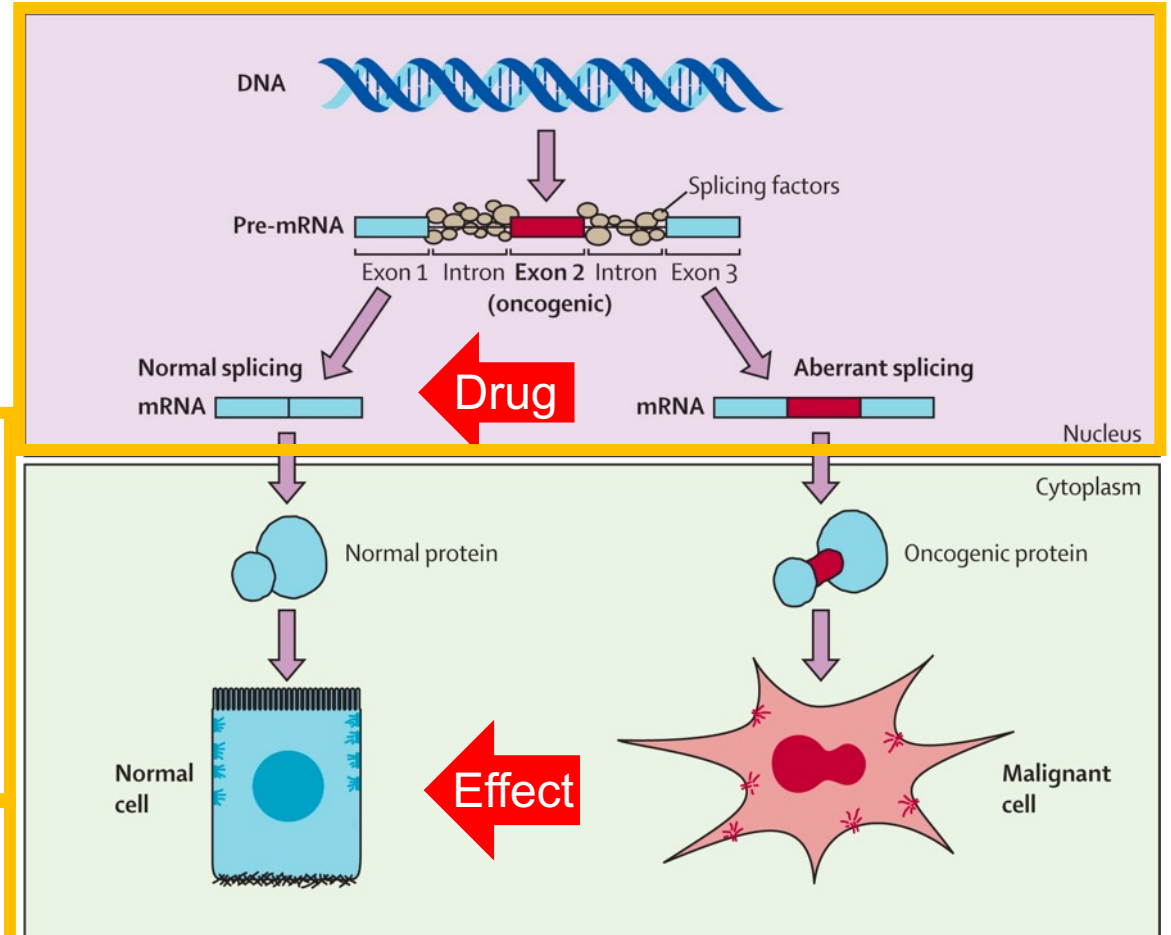
## RNA splicing:

- 40-60% of human genes have alternative splice forms
- Defects in splicing have been linked to many diseases

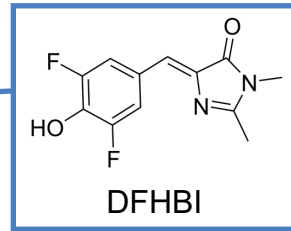
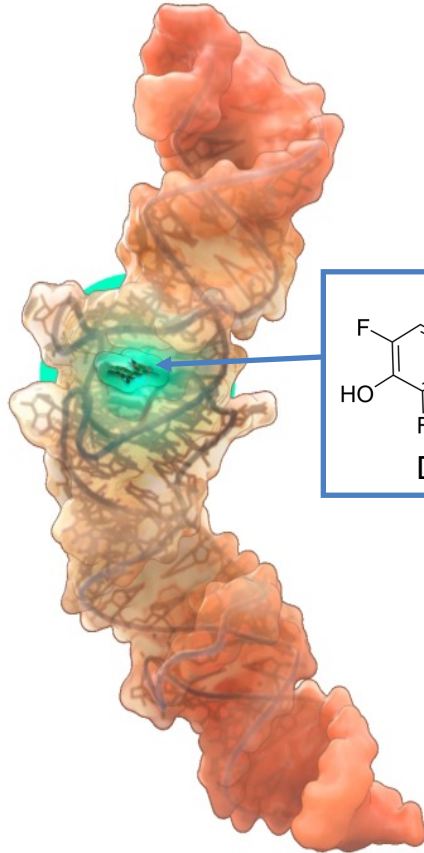
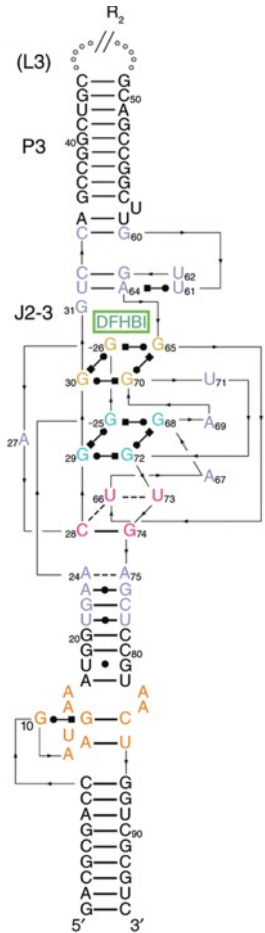
## First-in-class drugs:

- Nusinersen (Biogen) – ASO for SMA
- Eteplirsen (Sarepta) – ASO for DMD
- Risdiplam (Roche/PTC) – SMs for SMA

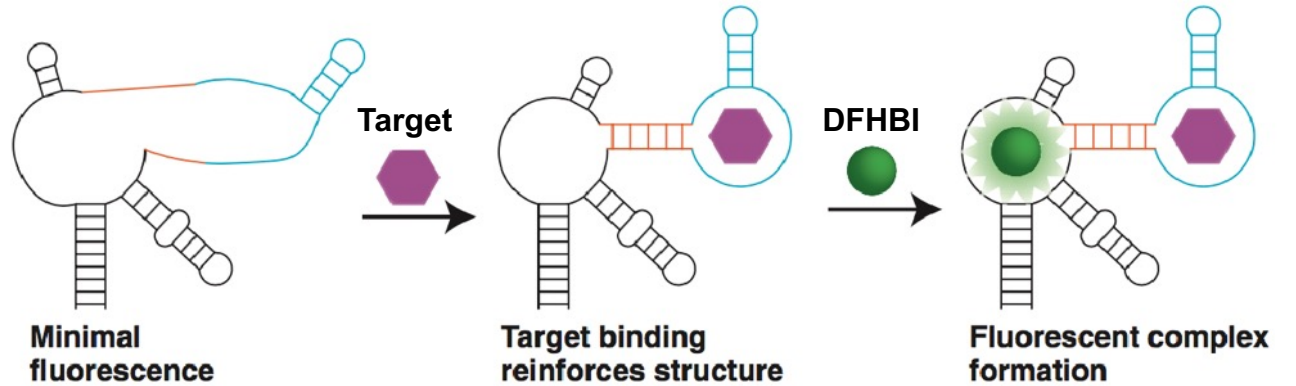
**Lucerna's Splice Sensor Platform:** Enabling technology that allows for rapid identification of small molecules/ASOs that modulate RNA splicing



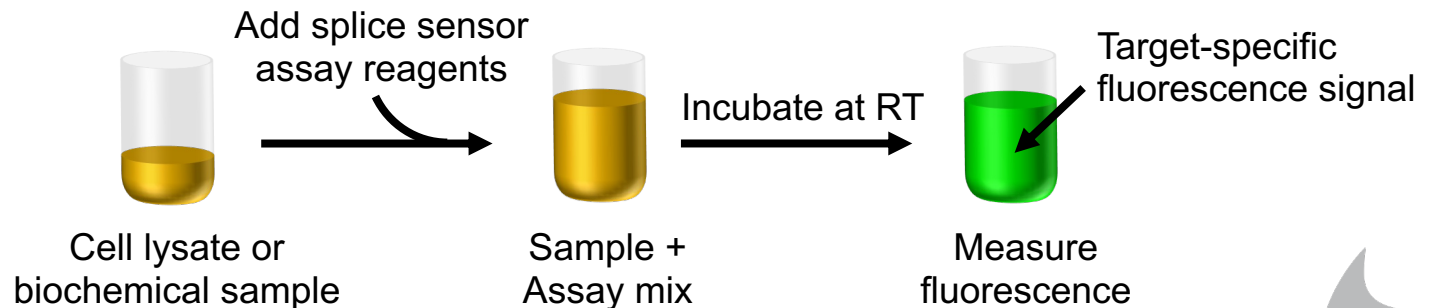
# Spinach™: A fluorescent RNA technology



Spinach sensor fluoresces only upon target detection



Homogenous assay format suitable for HTS

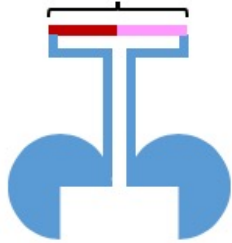


Spinach is a RNA aptamer that turns on the fluorescence of an otherwise non-fluorescent dye (DFHBI).

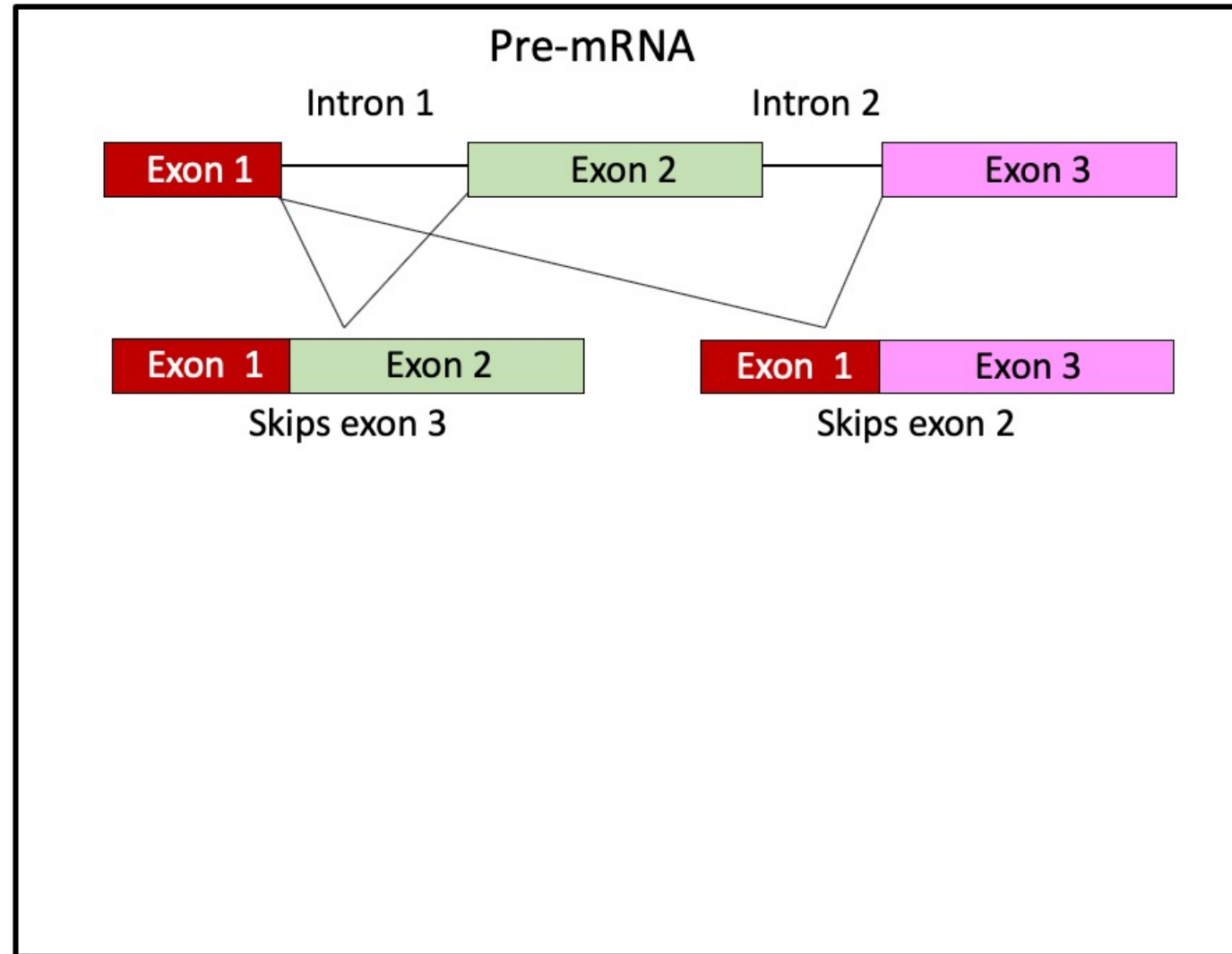
# Splice sensor design

## Splice sensor:

Target recognition region

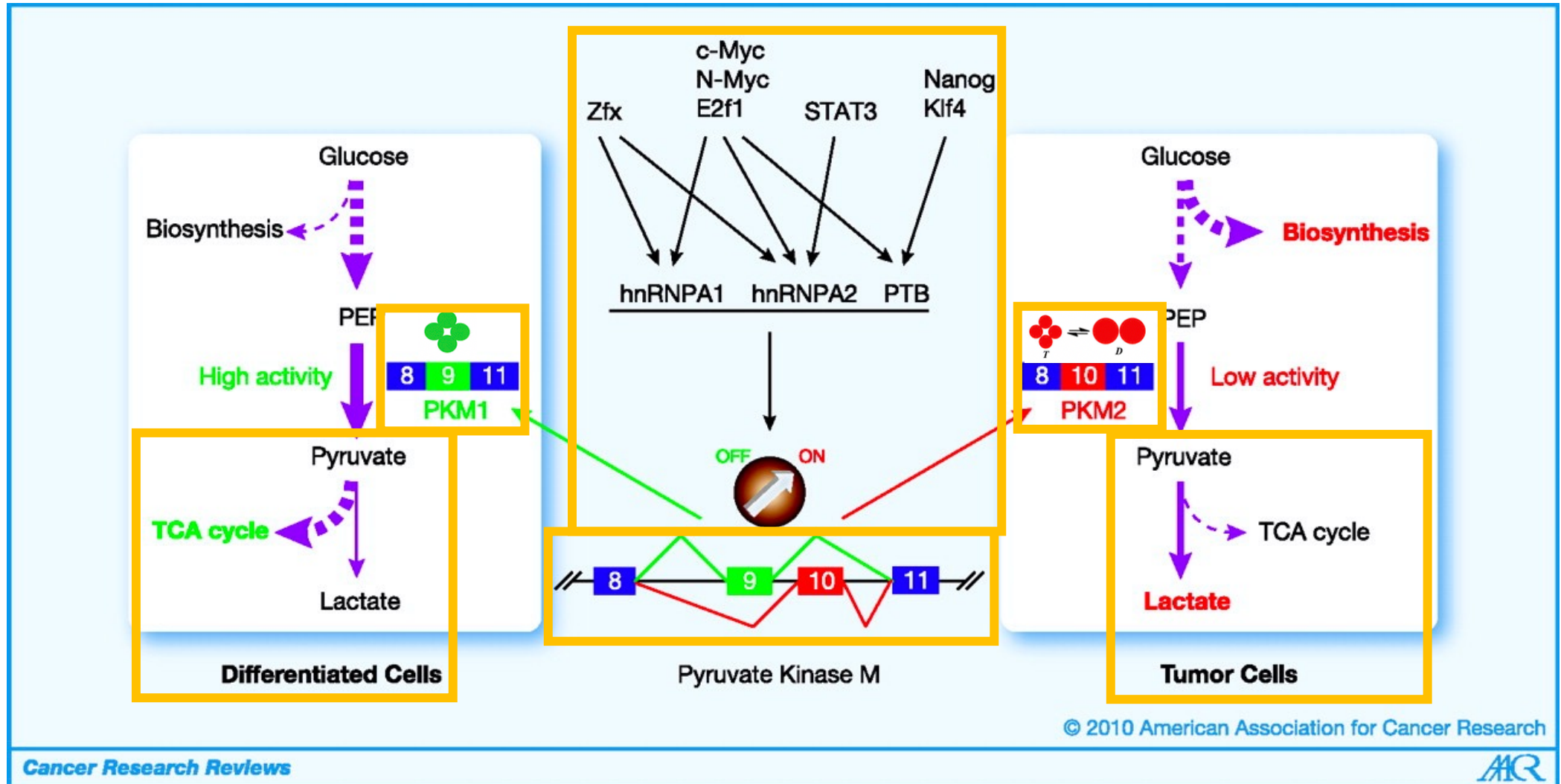


## Sensor dye:

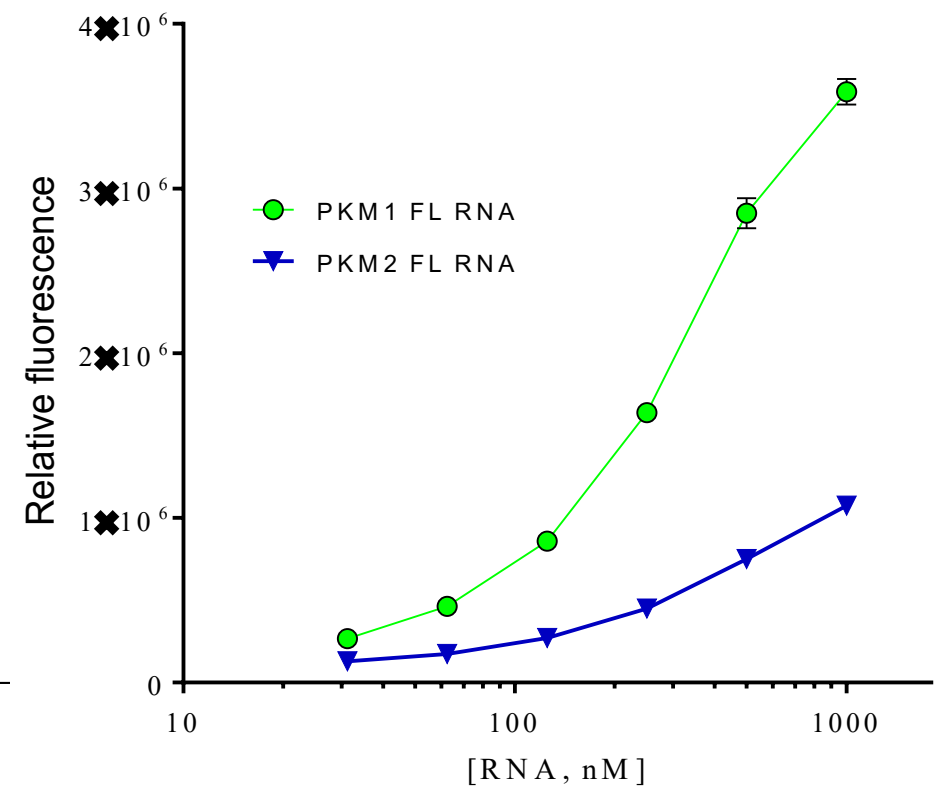
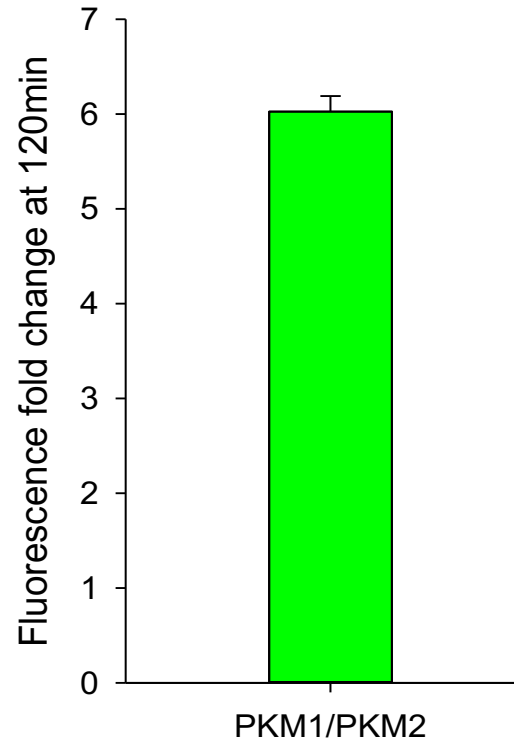
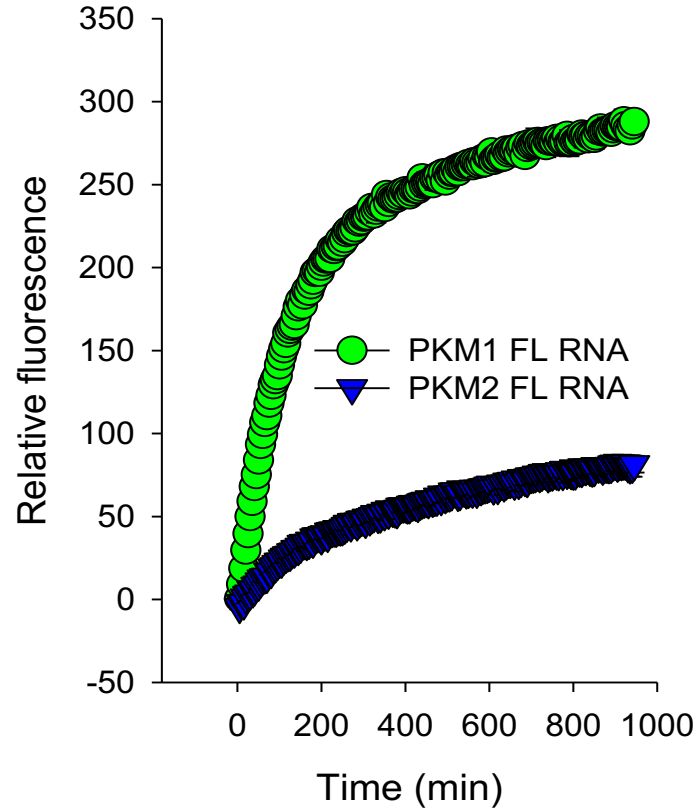




# Case study: Pyruvate kinase isoforms



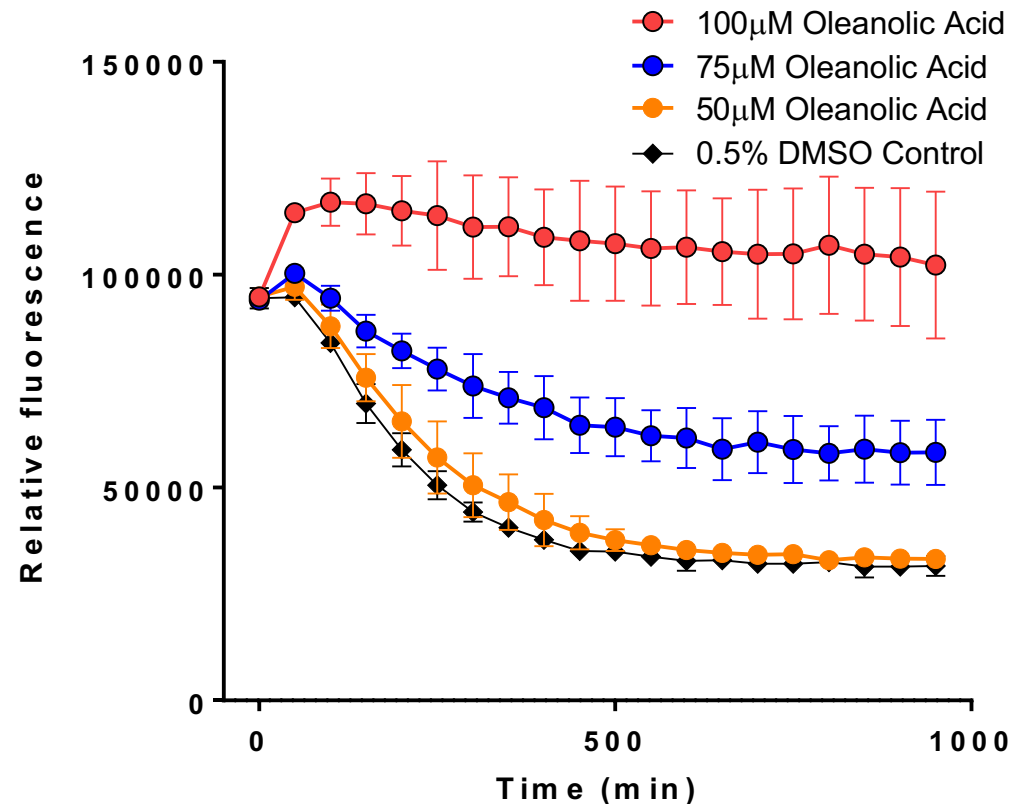
# Splice sensor performance



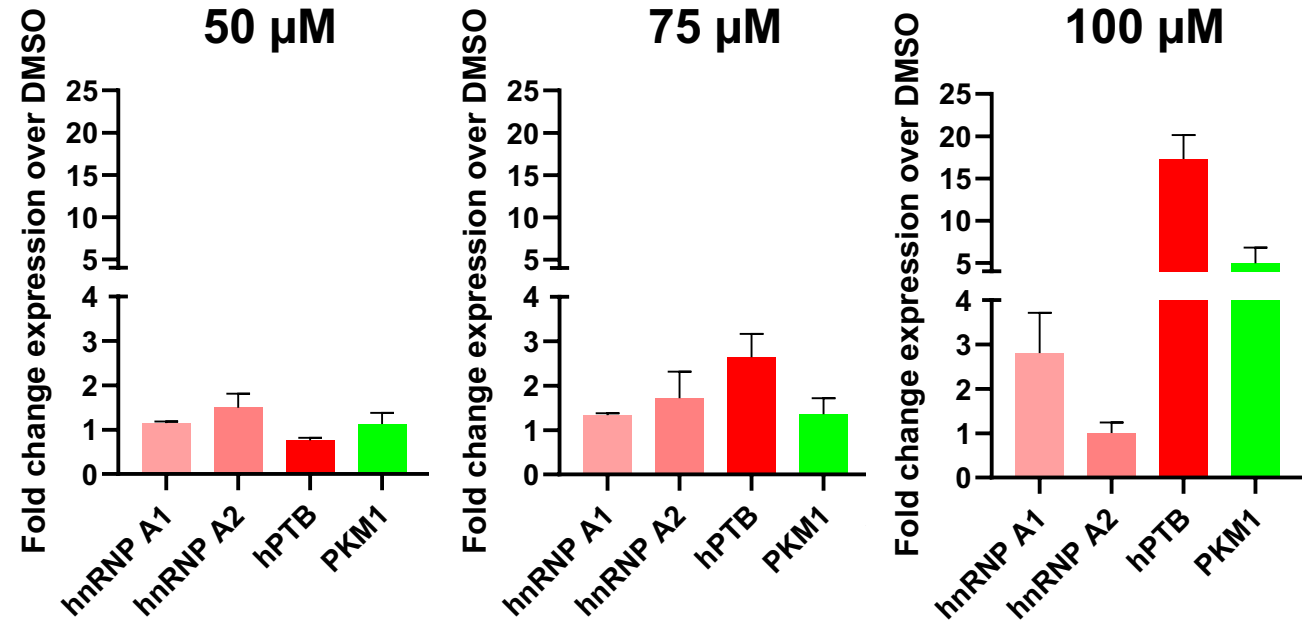
- PKM1 sensor exhibits ~6-fold specific fluorescence in the presence of PKM1 FL RNA.
- Sensor response is fast and stable for over 16h.
- Sensors discriminate between PKM1 and PKM 2 RNA as low as 30 nM.

# Sensor measurement in drug treated cells

## Splice sensor assay

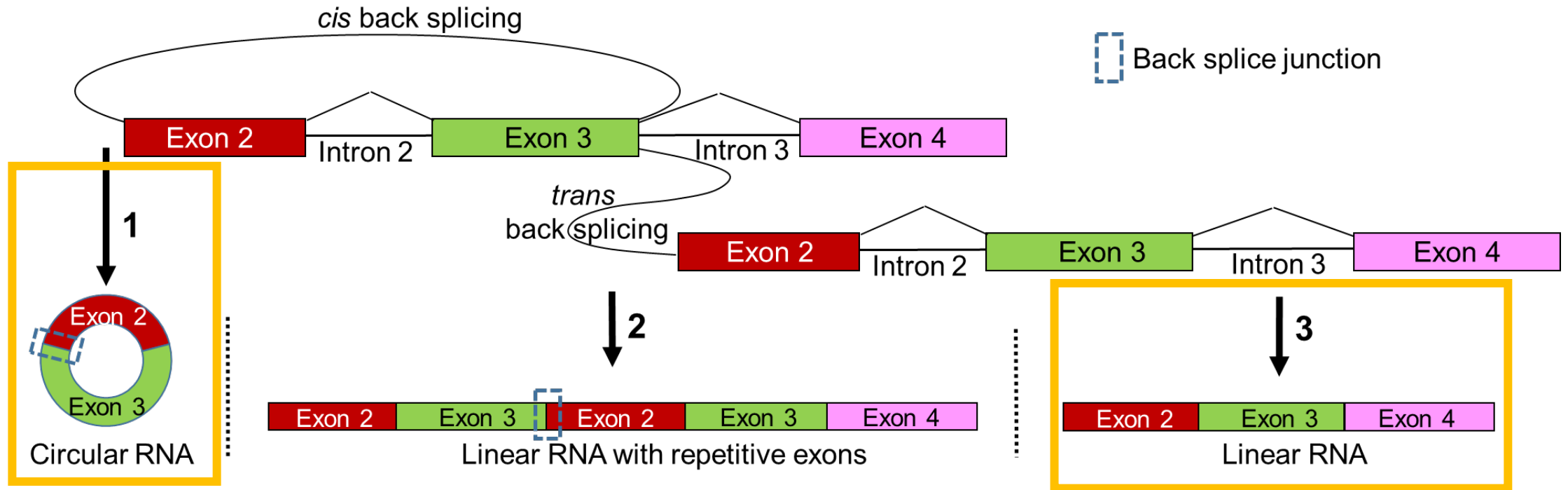


## Relative mRNA Levels (qPCR)



- HeLa cells in 384-wells were treated with oleanolic acid (6h) to increase PKM1 expression.
- Sensor signal shows a dose-response curve and is consistent with RT-qPCR data.
- RT-qPCR data comparable to assay with hnRNP A1, A2, and hPTB KD cells (not shown).

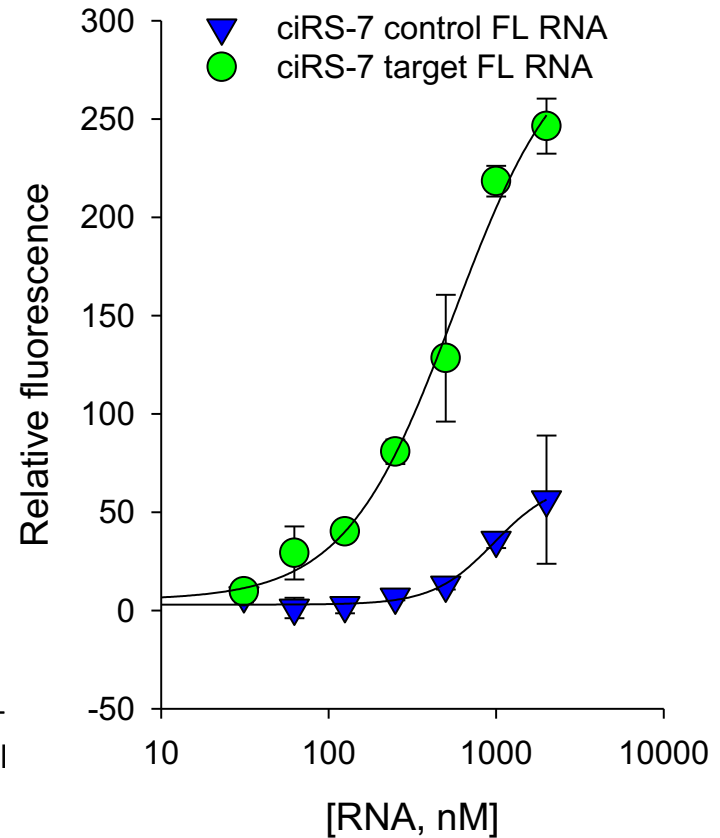
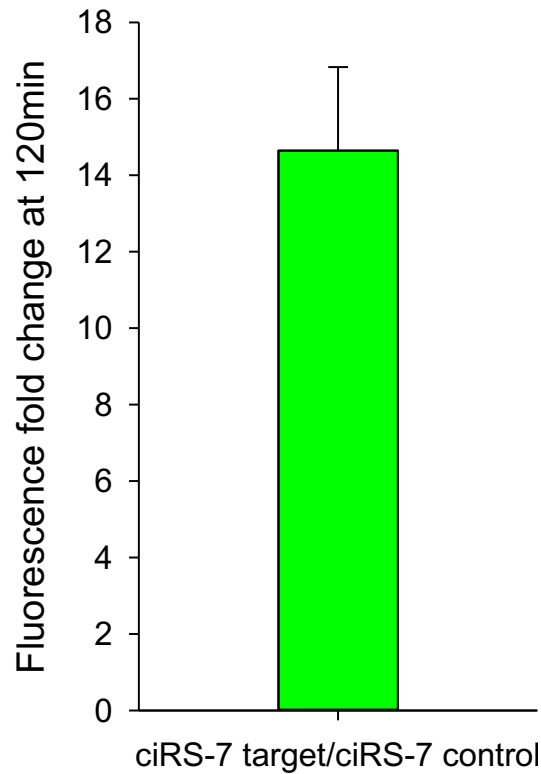
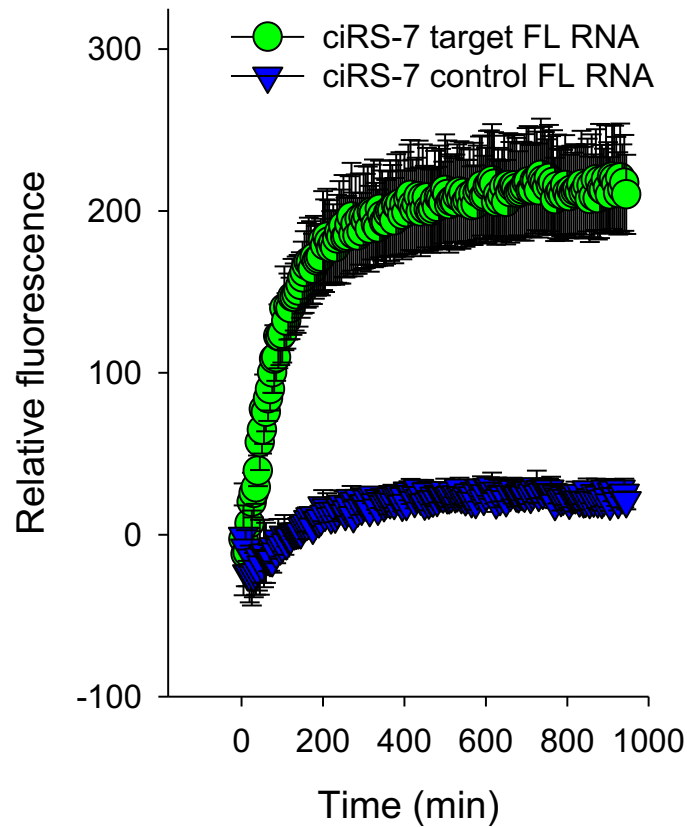
# Circular RNA forms a unique splice site



- 🌱 Circular RNAs (circRNAs) are a diverse family of covalently-closed, single-stranded RNA species that are formed by a special type of splicing called *cis* back splicing.
- 🌱 Studies indicate circRNAs are prognostic and diagnostic biomarkers.

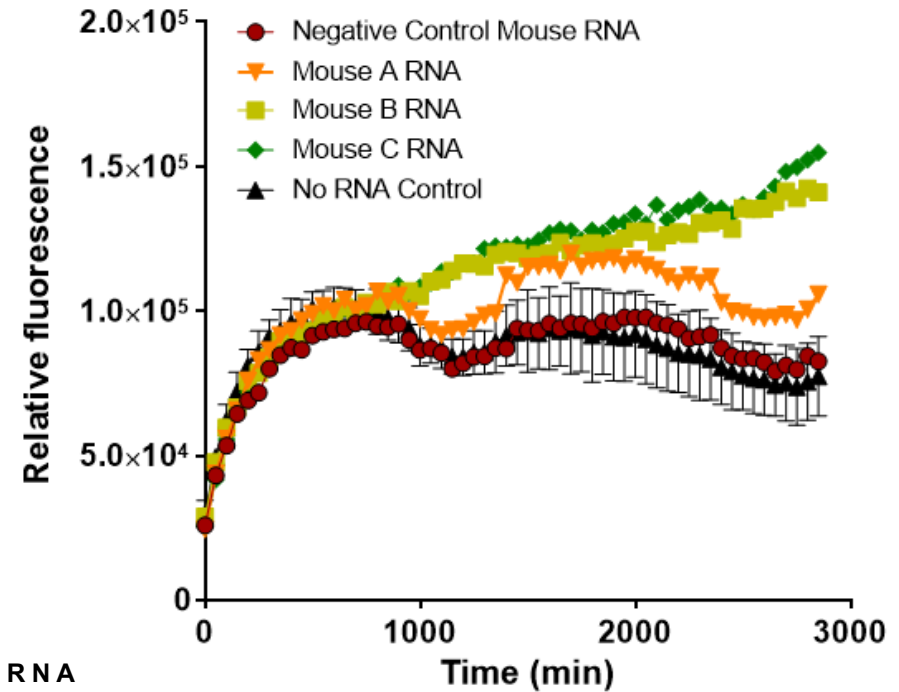
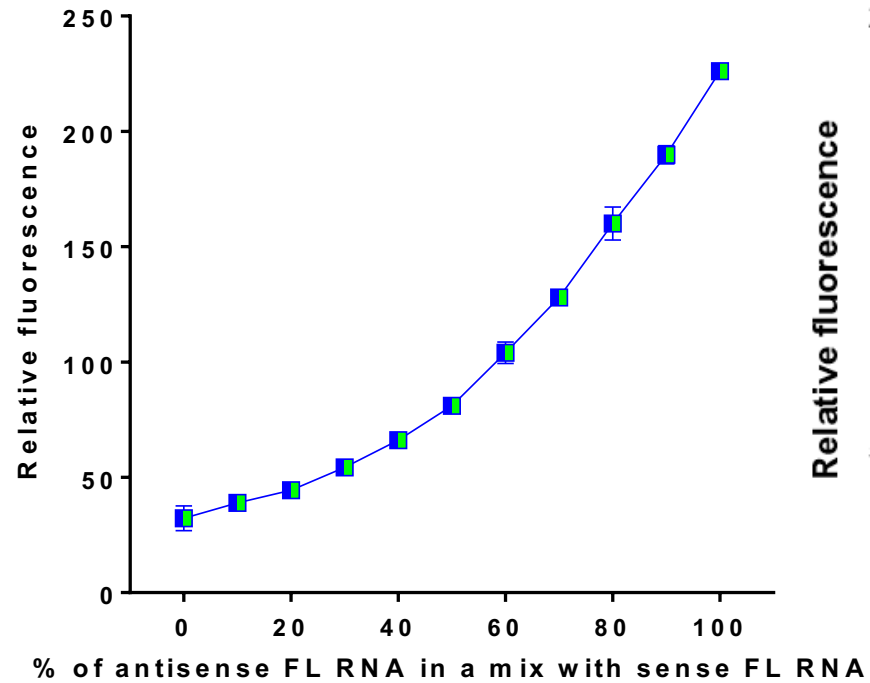
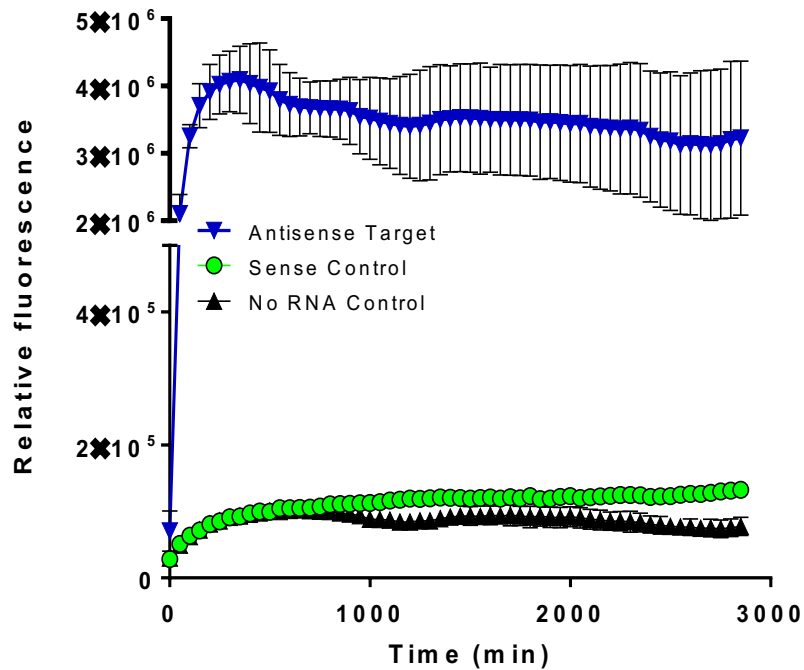


# ciRS-7 circRNA sensor performance



- ⦿ A rapid response (<30 min) in fluorescence with ciRS-7 target FL RNA.
- ⦿ Good selectivity over linear non-target RNA control (~14-fold greater fluorescence).
- ⦿ Sensor sensitivity is as low as 62.5 nM.

# Intronic RNA sensor performance



- ⦿ A rapid response (<30 min) in fluorescence with antisense target FL RNA.
- ⦿ Good selectivity in sense and antisense FL RNA mixtures.
- ⦿ Can detect transcripts as low as 5-10 RNA copies / cell.

# Splice sensor platform summary

## Capabilities:

- High target selectivity and broad dynamic range
- Detects endogenous mRNAs without amplification
- Detects smaller changes in splicing, identifies more viable hits
- Multiplex with sensors containing other fluorescent aptamers
- HTS compatible for small molecule and ASO screening

## Targets:

- Linear mRNA
- Circular RNA
- Intronic RNA



FOR YOUR ATTENTION



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