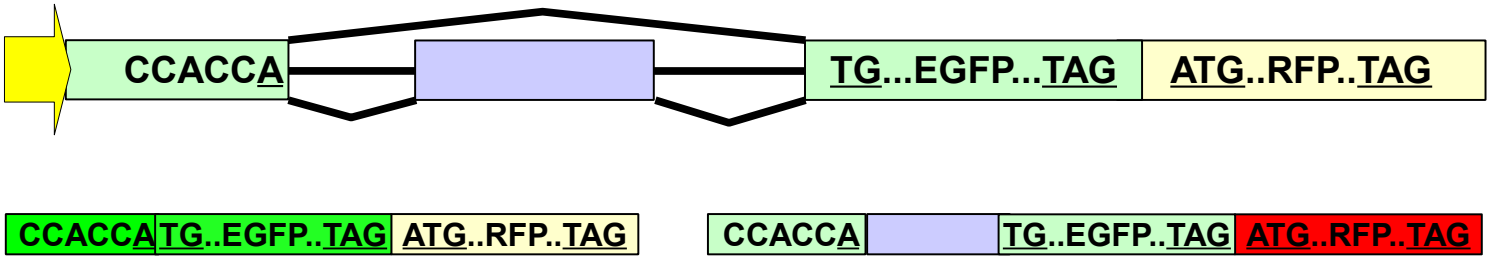
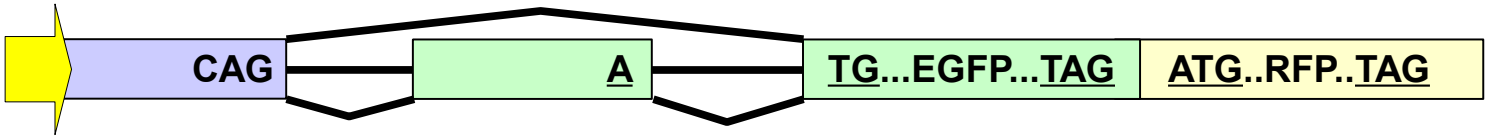
A


CCACCA
TG...EGFP...TAG
ATG..RFP..TAG

CCACCA
TG..EGFP..TAG
ATG..RFP..TAG

CCACCA
TG..EGFP..TAG
ATG..RFP..TAG

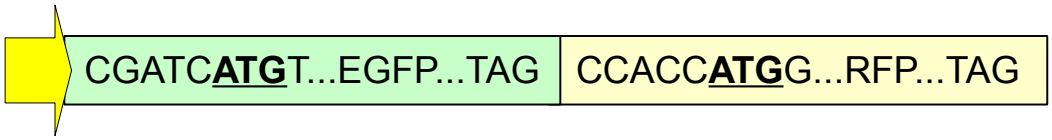
B

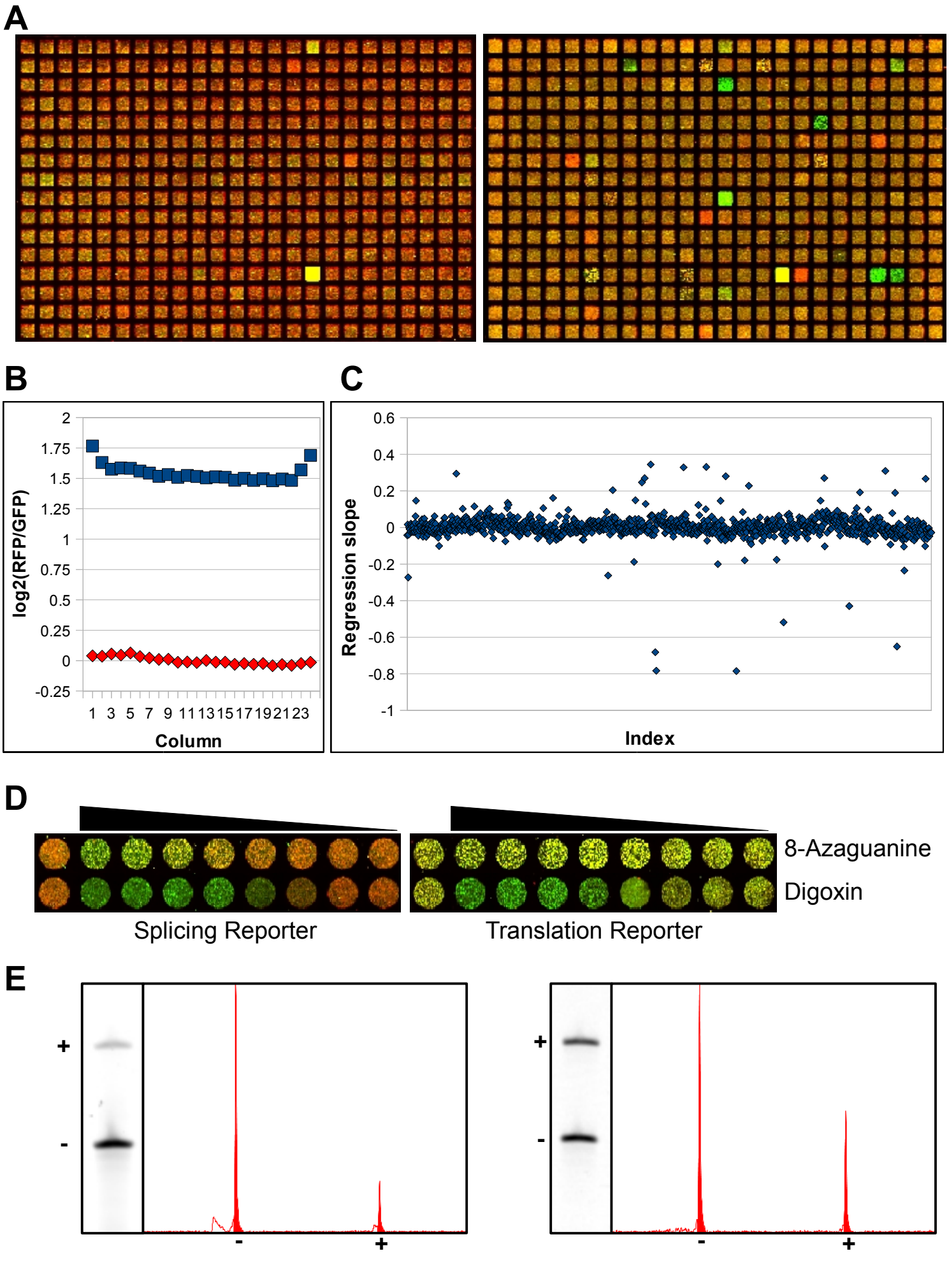

CAG
A
TG...EGFP...TAG
ATG..RFP..TAG


CAG
TG..EGFP..TAG
ATG..RFP..TAG


CAG
A
TG..EGFP..TAG
ATG..RFP..TAG

C


CGATCATGT...EGFP...TAG
CCACCATGG...RFP...TAG


A
B
log2(RFP/GFP)
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
-0.25
1 3 5 7 9 11 13 15 17 19 21 23
Column
C
Regression slope
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
Index
D
8-Azaguanine
Digoxin
Splicing Reporter
Translation Reporter
E
+
-
-
+
+
-
-
+

Overview: Screening for alternative splicing modulators

Add compounds and cells carrying the splicing reporter to 384 well plates.

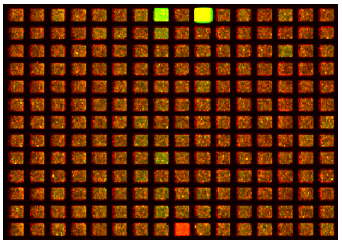
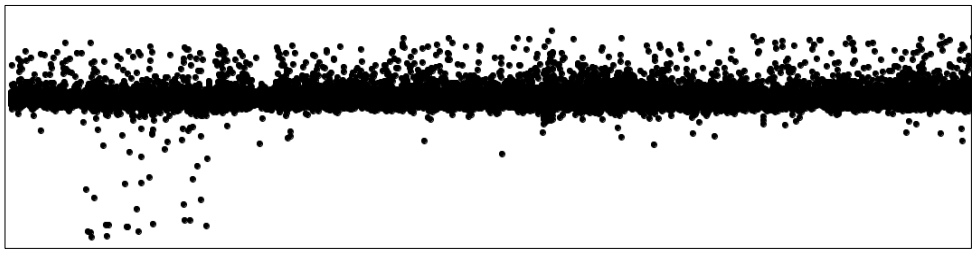
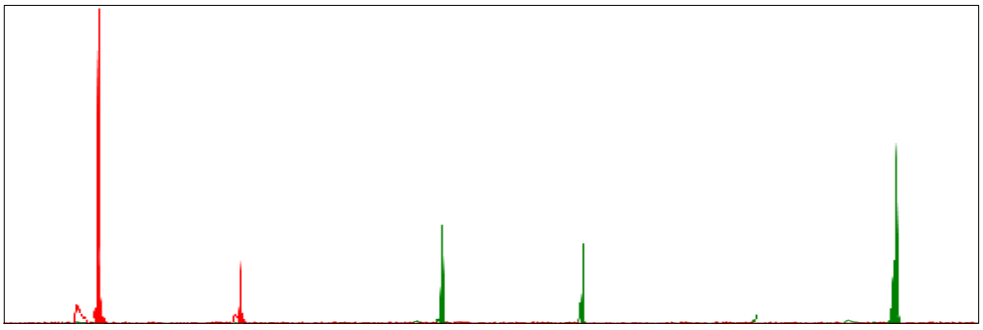


Image plates over the course of several days.



Analyze the images and identify the active compounds, fluorescent compounds or suspected false negatives.

Perform RT-PCR on RNA isolated from the wells containing the active compounds, fluorescent compounds or suspected false negatives.



Analyze the PCR products by capillary electrophoresis to confirm the effect on splicing.

Outcome: Small molecules that modulate the splicing of an alternative exon.

Questions answered: If the screens is performed on annotated library of compounds with known targets it can identify proteins and signaling pathways involved in the regulation of the exon.