

ENCORE: Using the ENCODE RNA binding protein resource to study RNA processing regulation

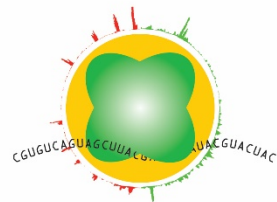
Eric Van Nostrand

elvannostrand@ucsd.edu

Yeo Lab, UCSD

10/16/18

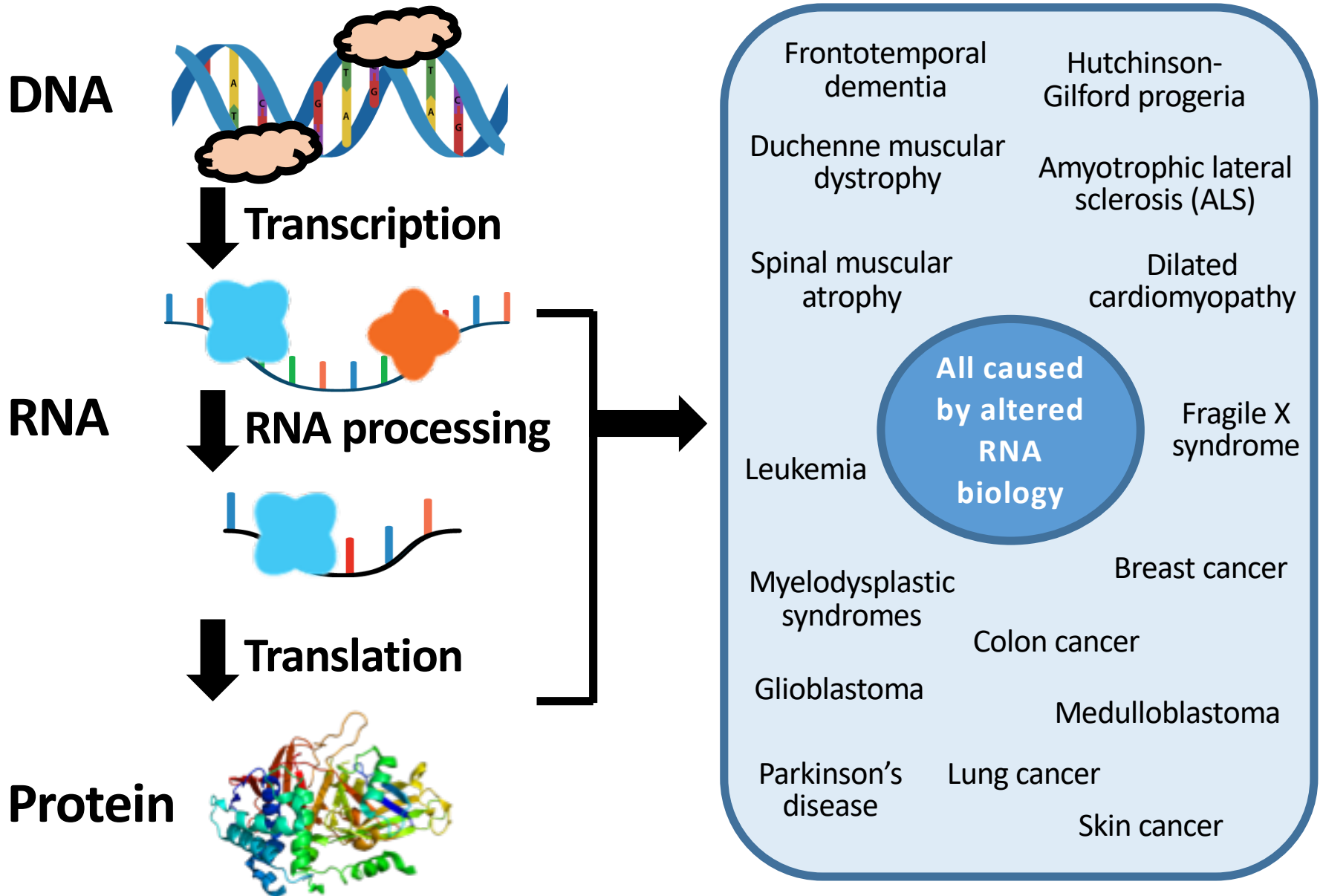
Disclosure



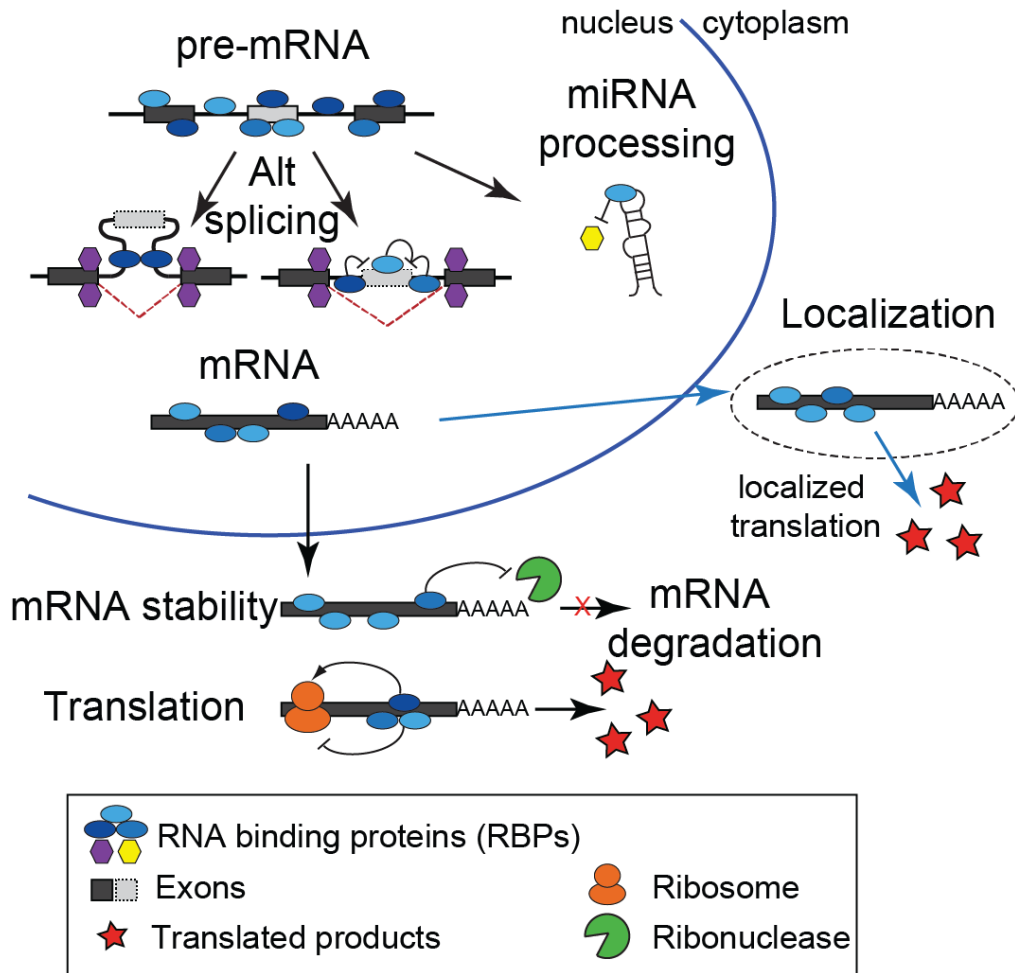
ECLIPSE
BIOINNOVATIONS

Co-founder & consultant for
Eclipse BioInnovations Inc.

The RNA world is controlled by RBPs



Each step of RNA processing is highly regulated



- RNA binding proteins (RBPs) act as trans factors to regulate RNA processing steps
- Estimated >1000 RBPs in human
- RNA processing plays critical roles in development and human physiology
- Mutation or alteration of RNA binding proteins plays critical roles in disease

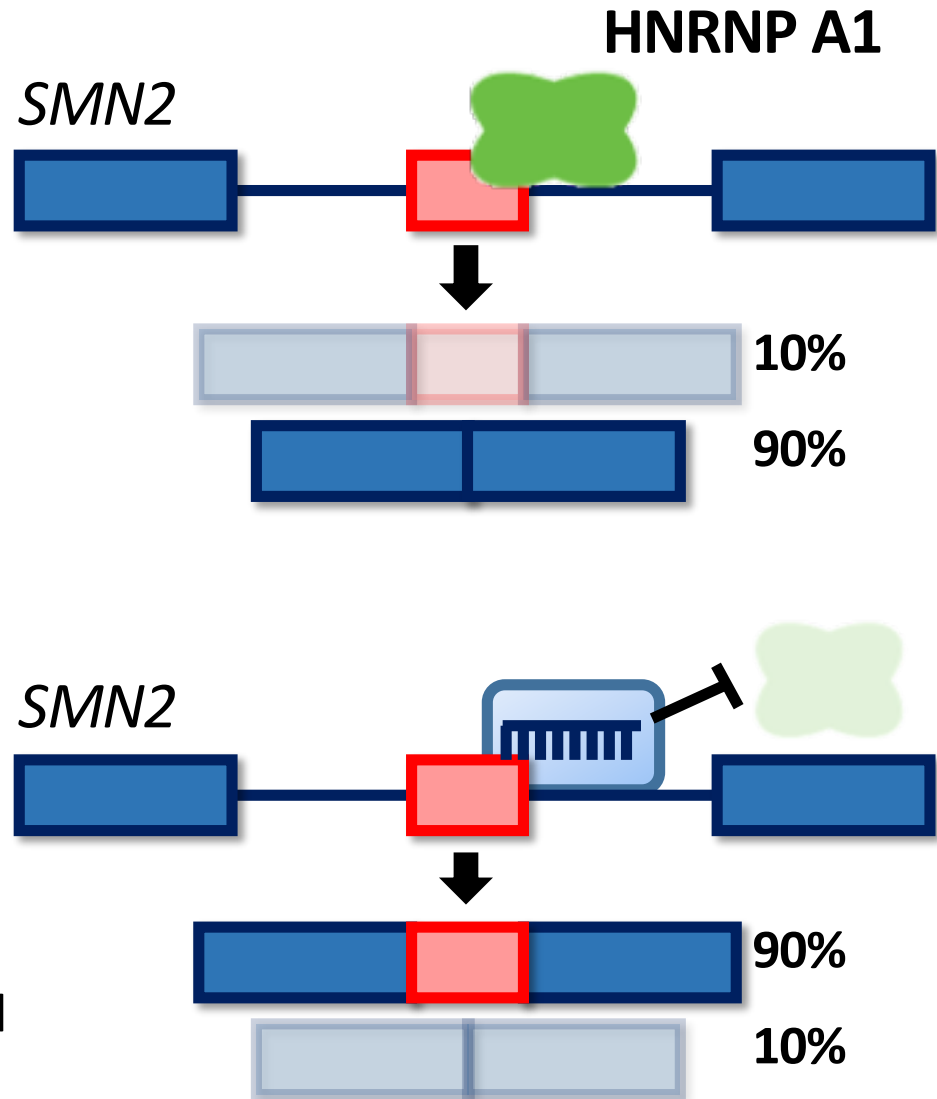
RBP targets are therapeutic targets

Spinal Muscular Atrophy



Example: Spinal Muscular Atrophy

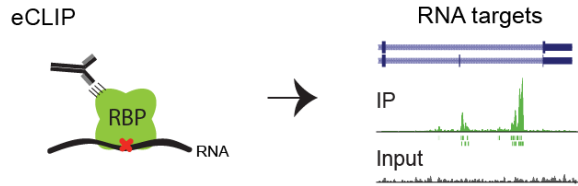
- Antisense oligo therapy targeting
- 1/8000 births
- HNRNP A1 binding site in *SMN2*
- Causes motor neuron death and
- Developed by Ionis
- system-wide muscle wasting
- Pharmaceuticals & Biogen
- Caused by HNRNP A1 binding and
- FDA approved in 2016
- suppressing exon in *SMN2*
- >\$1 billion annual sales by 2025



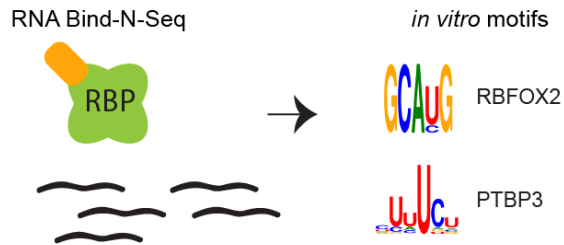
Outline

- Data overview for ENCORE experiments
 - Available data types
 - Example: a deeper dive into accessing eCLIP data
- Incorporating ENCORE data into analyses
 - Using eCLIP to identify potential regulators of an RNA of interest
 - Integrating *in vivo* and *in vitro* motifs to study RBP regulation
 - Integrating RBP-responsive and *in vivo* RNA targets to map regulation of RNA stability and splicing
 - Using localization to predict RBP function (and vice versa)

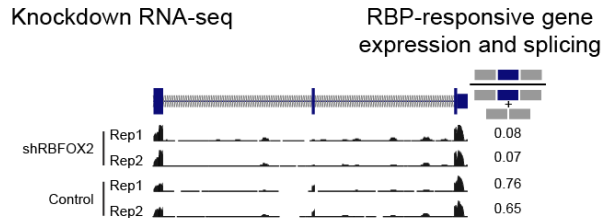
ENCORE: the ENCODE RNA regulation group



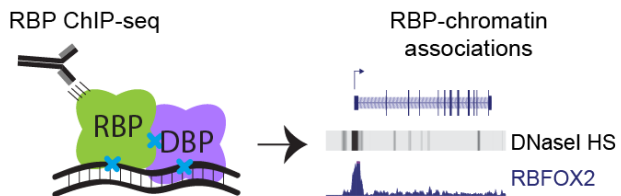
⇒ Yeo Lab, UCSD



⇒ Burge Lab, MIT



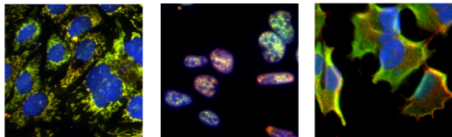
⇒ Graveley Lab, UConn



⇒ Fu Lab, UCSD

Immunofluorescence

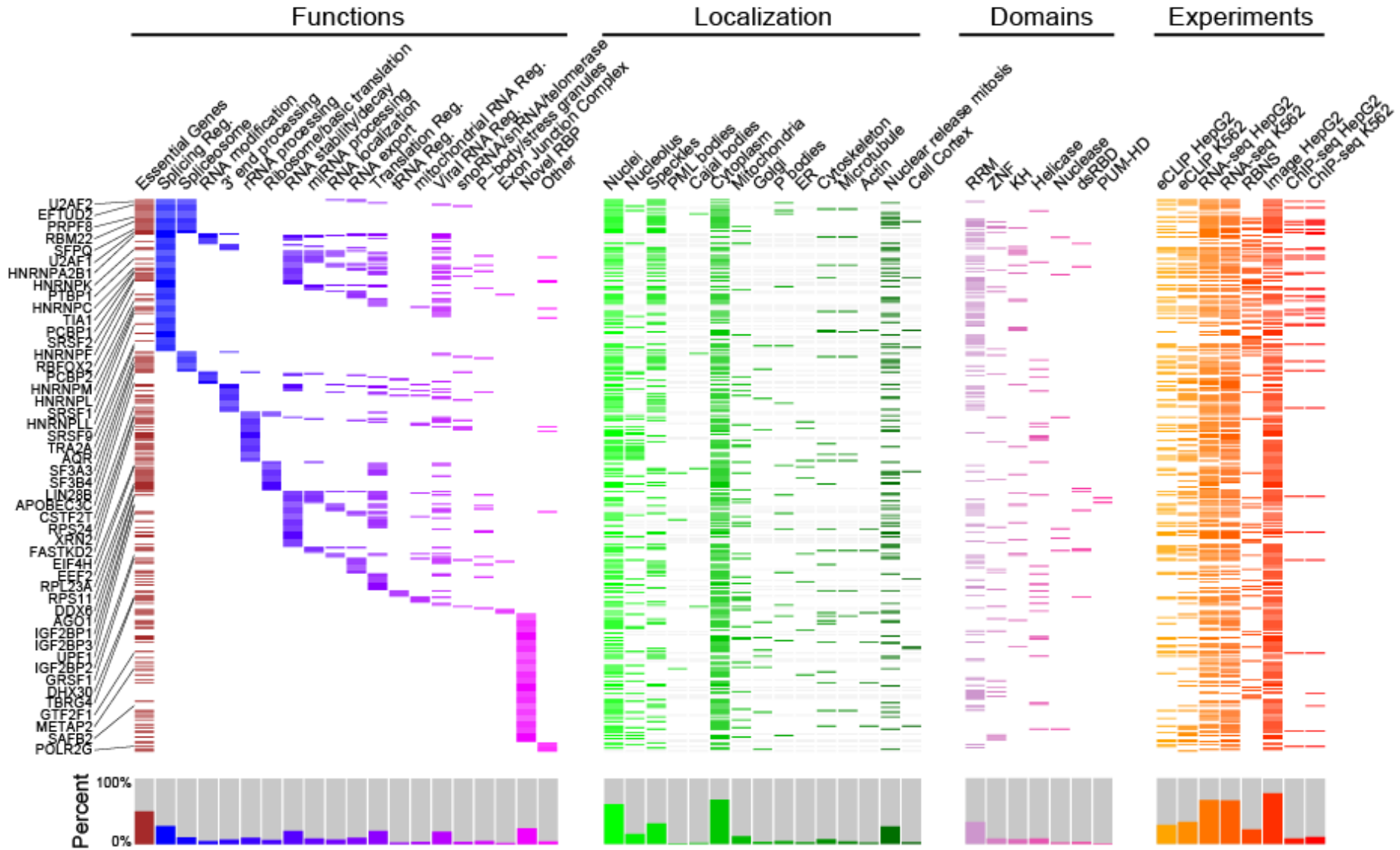
RBP localization



⇒ Lécuyer Lab, IRCM

RBP Data Production Overview

(ENCODE Phase 3)



ENCORE data availability

<https://www.encodeproject.org/>

The screenshot displays the ENCODE Experiment Matrix interface. At the top, there is a navigation bar with 'ENCODE', 'Data', 'Encyclopedia', 'Materials & Methods', and 'Help', along with a search bar. The main content area is titled 'Experiment Matrix' and includes a search input field. Below this, there are several filter panels: 'Organism' (Homo sapiens: 818), 'Biosample type' (cell line: 816, cell-free sample: 79, tissue: 2), 'Organ' (blood: 431, bodily fluid: 431, liver: 385, adrenal gland: 2), 'Project' (ENCODE: 897), and 'Genome assembly (visualization)' (hg19: 799, GRCv38: 798). The central part of the page shows '897 results' for the 'ASSAY' filter, with a 3D bar chart and a table. The table lists results for 'cell line' (K562: 246/124, HepG2: 232/105) and 'cell-free sample' (adrenal gland: 79). A 'Download' button and a 'Filter to 500 to visualize' button are at the bottom.

Experiment Matrix
Click or enter search terms to filter the experiments included in the matrix.

Enter search term(s)

Assay

shRNA RNA-seq	478
eCLIP	231
RNA Bind-n-Seq	79
ChIP-seq	71
siRNA RNA-seq	57
CRISPR RNA-seq	38
RIP-seq	29
iCLIP	7

Assay category

Transcription	516
RNA binding	310
DNA binding	71

Target of assay

RNA binding protein	897
control	412
transcription factor	235

Date released

August, 2015	84
March, 2016	81
December, 2014	80
October, 2014	80
August, 2018	55

+ See more...

Available data

fastq	885
bam	808
bigWig	808
tsv	591
tar	472

+ See more...

ASSAY

BIOSAMPLE

897 results

Clear Filters

cell line

K562	246/124	38	23
HepG2	232/105	33	15

cell-free sample

cell-free sample	79
------------------	----

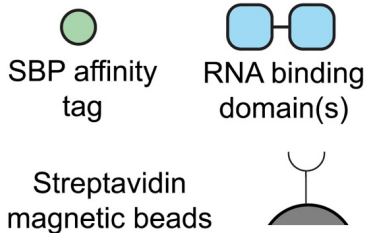
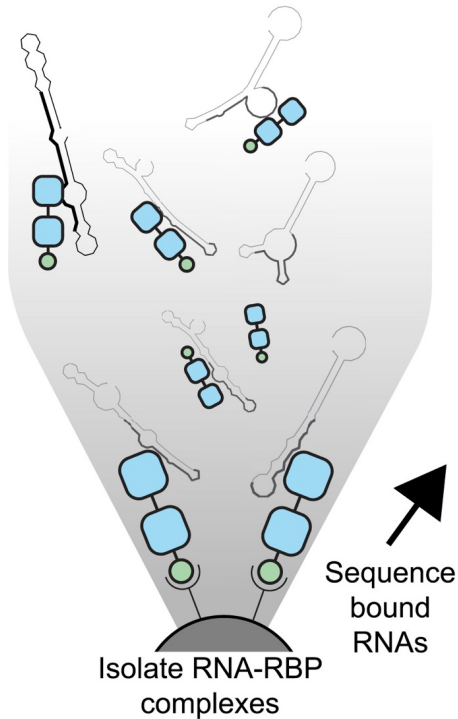
tissue

adrenal gland	2
---------------	---

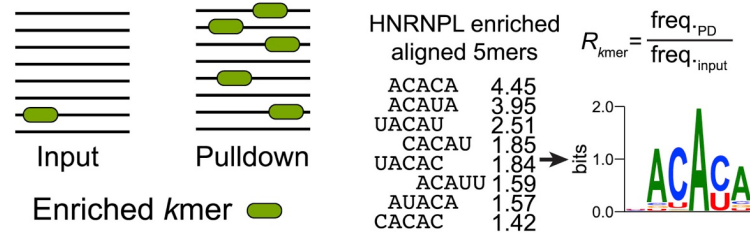
Download Filter to 500 to visualize

RNA Bind-N-Seq – *in vitro* binding motifs

Binding reactions
at 5 RBP
concentrations



Linear Sequence Features



Available files:

FASTQ

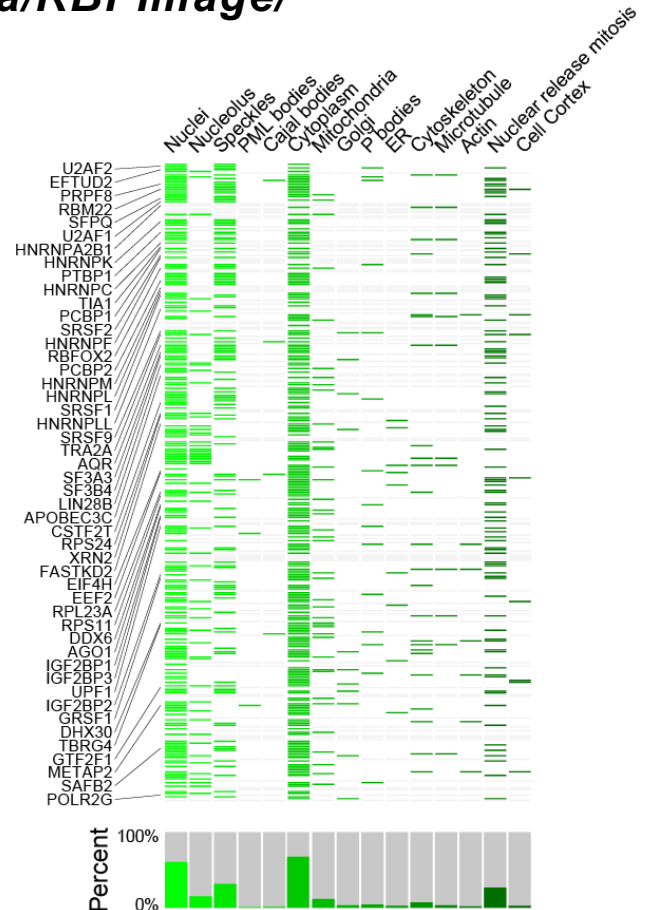
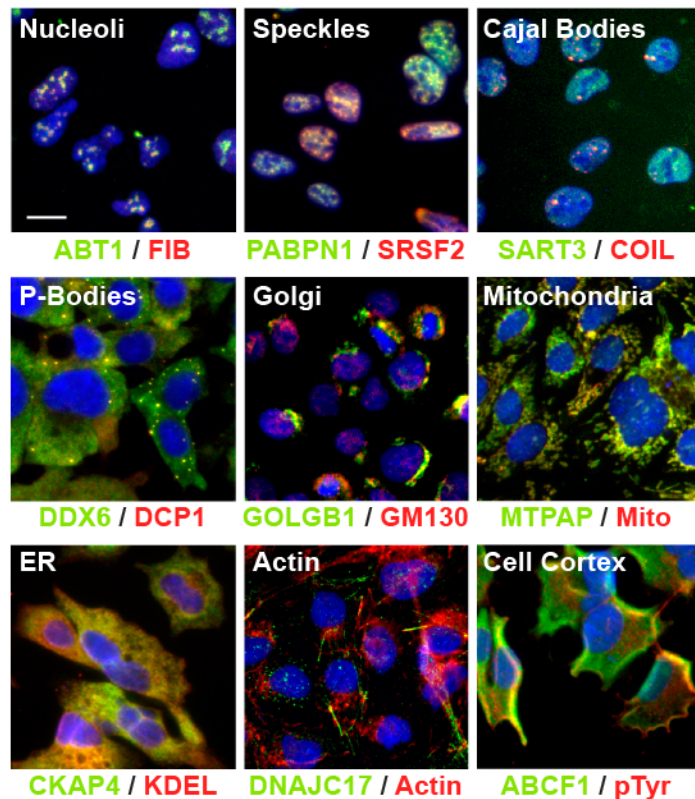
- Sequencing files containing reads from various RBP concentration

TSV

- Enrichment 'R' values for 5, 6, and 7-mers
- Estimated binding fraction values for 5, 6, and 7-mers

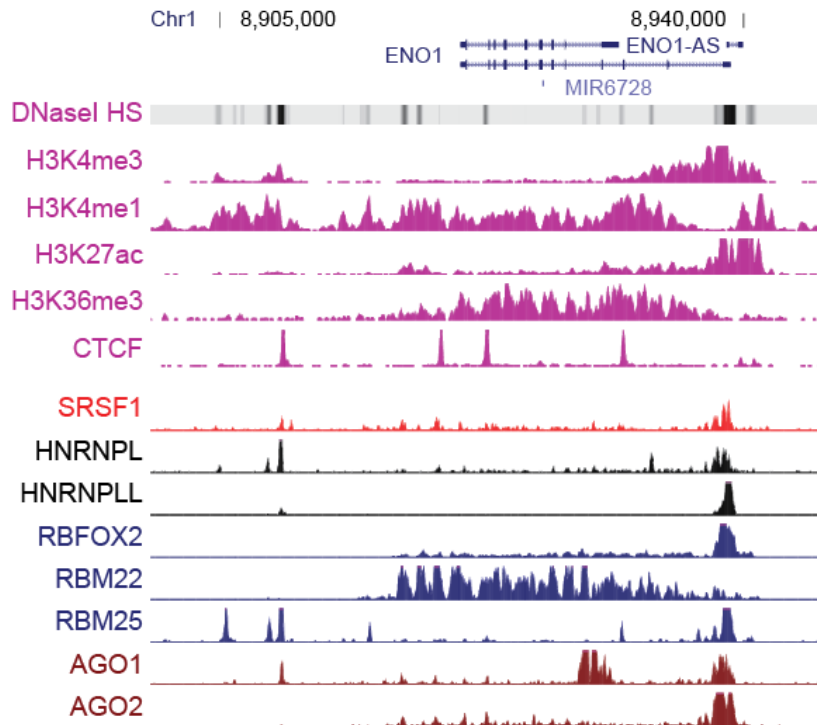
Immunofluorescence - RBP subcellular localization

Searchable images available at:
<http://rnabiology.ircm.qc.ca/RBPImage/>

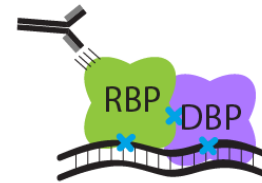


Data table available as supplementary information for Van Nostrand E, *et al. Manuscript submitted*.
 Preprint available at: <https://www.biorxiv.org/content/early/2018/10/05/179648>

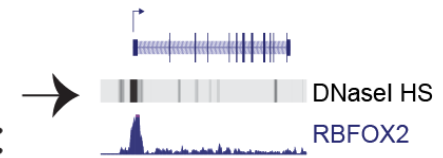
RBP DNA associations – ChIP-seq



RBP ChIP-seq



RBP-chromatin associations



Available files:

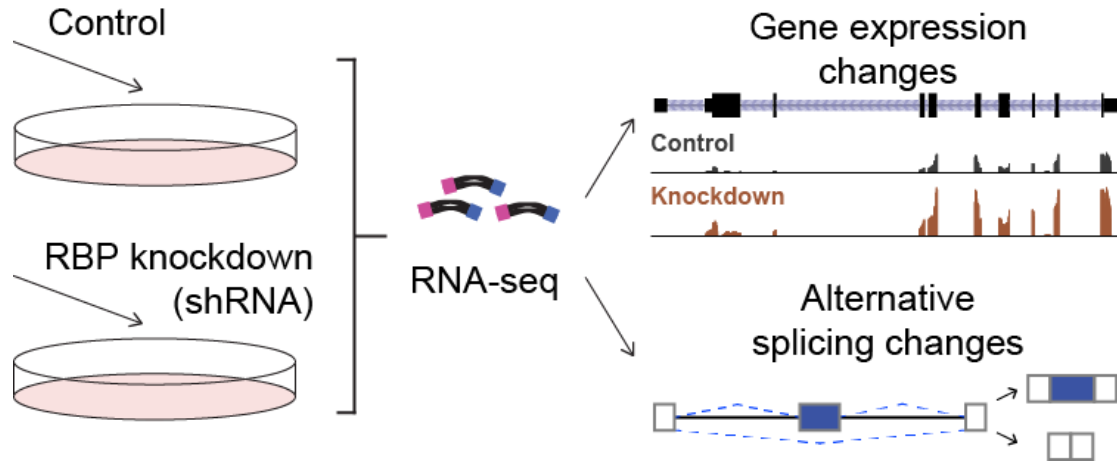
FASTQ

BAM
bigWig

narrowPeak
(bed + bigBed)

- Sequenced reads for IP (and paired input controls)
- Genome-mapped reads (*hg19* & *hg38*)
- Peak calls for individual replicates
- Reproducible (IDR) peak calls across replicates

RBP-responsive targets – knockdown/RNA-seq



Available files:

FASTQ

- Sequenced reads for knockdown (and paired non-target controls)

BAM
bigWig

- Genome-mapped reads (*hg19* & *hg38*)

tsv

- Differential expression (DEseq2) against paired control

tsv

- Differential splicing (rMATS) against paired control

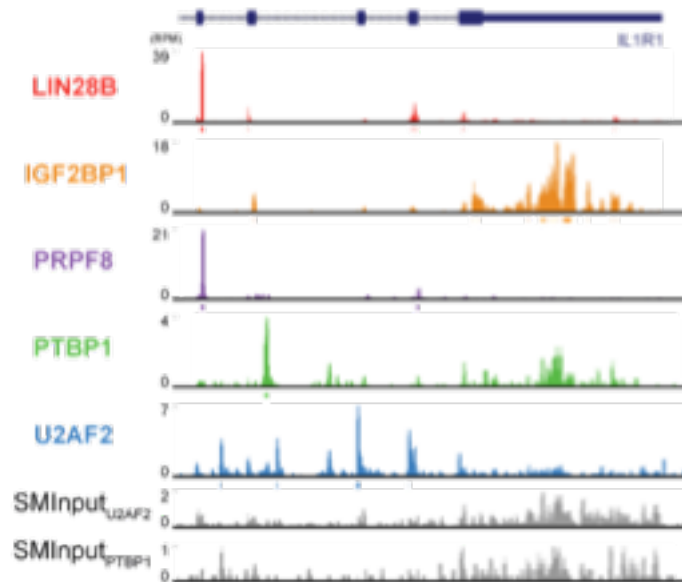
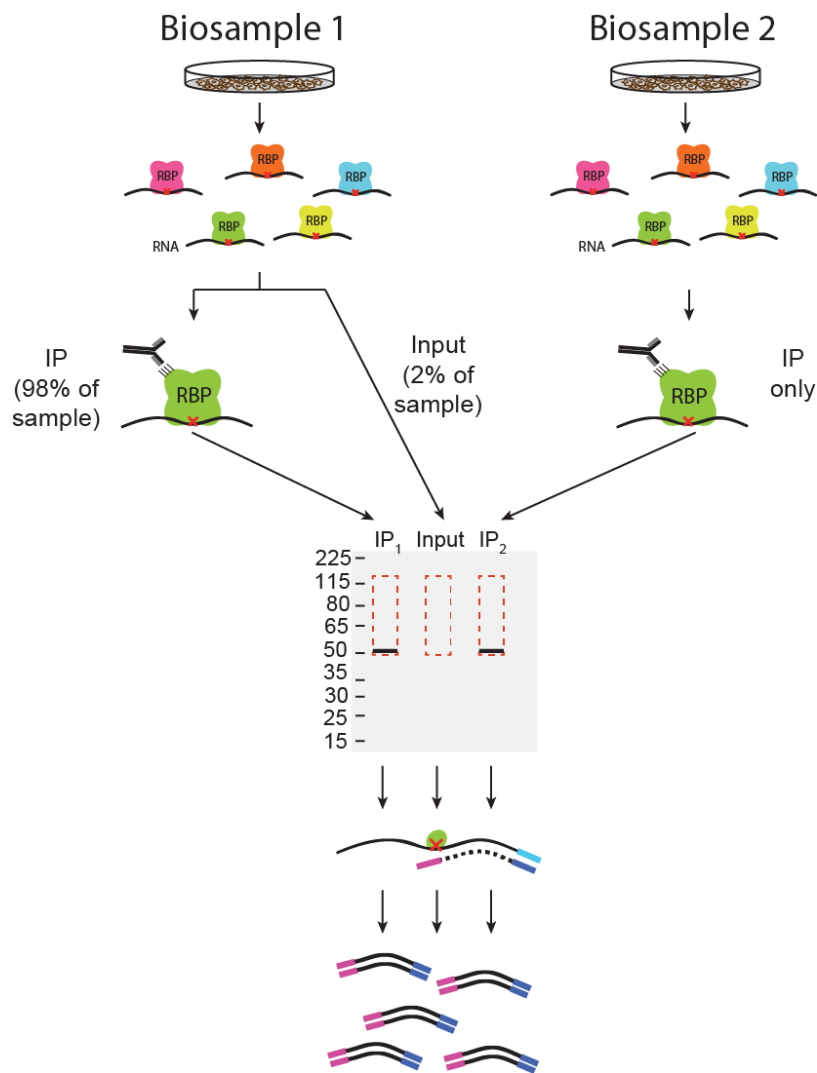
tsv

- Batch corrected expression levels

tsv

- Batch corrected splicing changes

RBP *in vivo* RNA targets - eCLIP



Available files:

FASTQ

BAM
bigWig

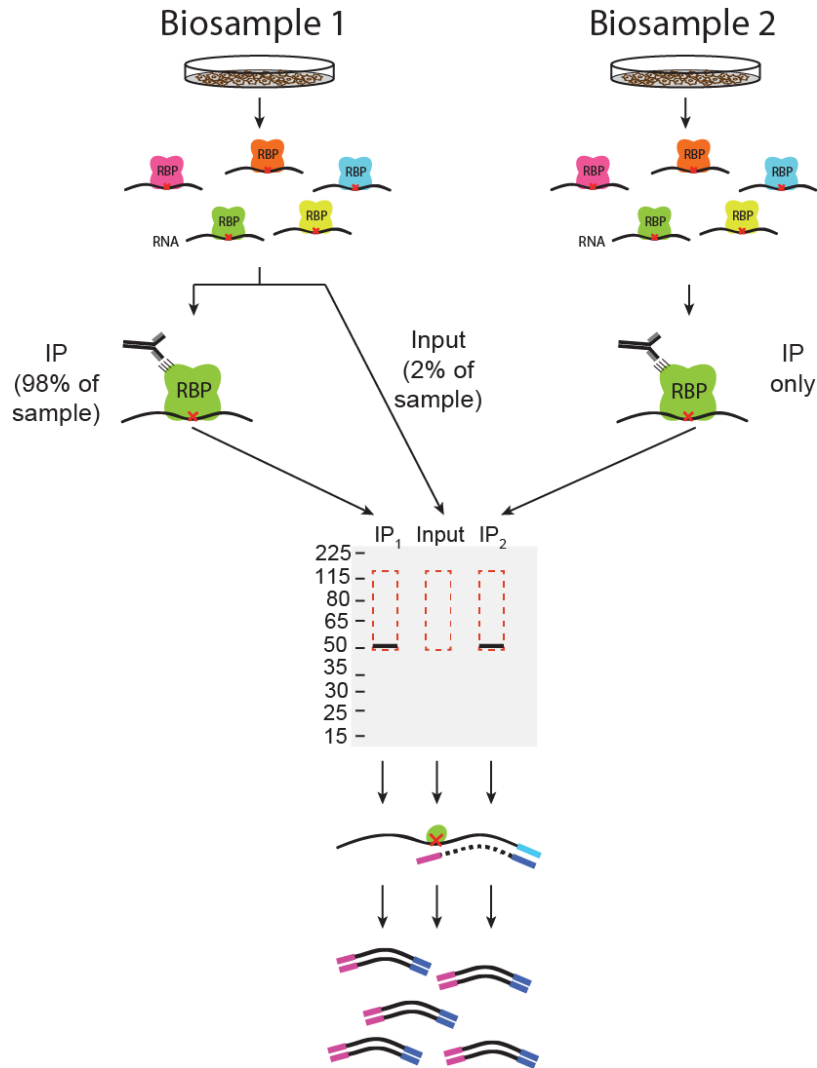
narrowPeak
(bed + bigBed)

- Sequenced reads for IP and paired input controls)
- Genome-mapped, PCR duplicate-removed reads (*hg19* & *hg38*)
- Peak calls for individual replicates
- Reproducible (IDR) peak calls across replicates

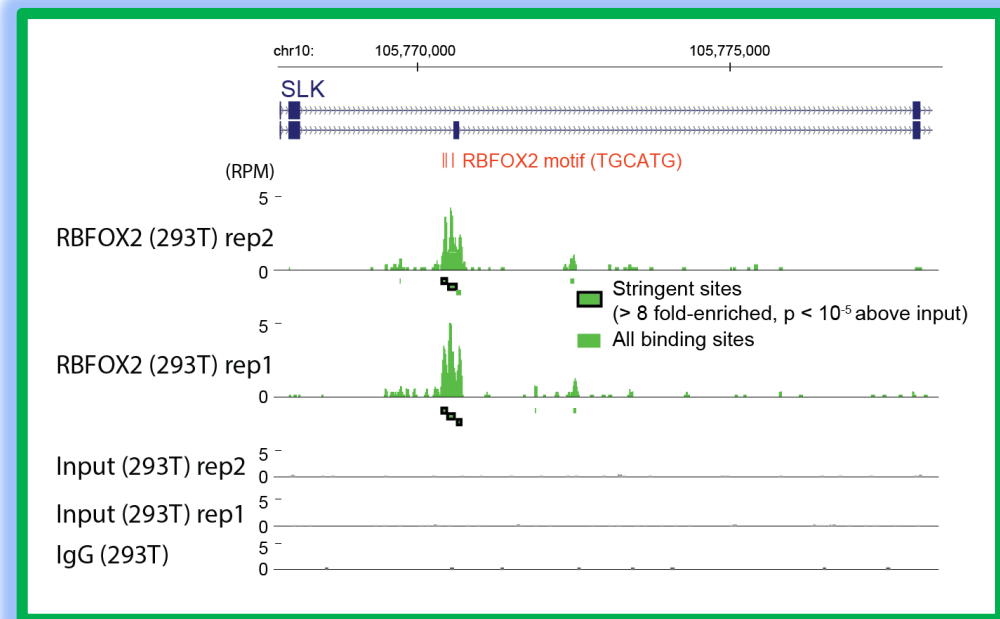
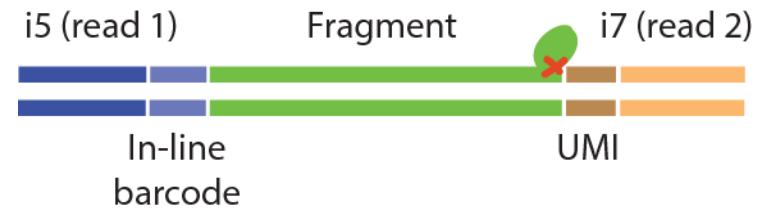
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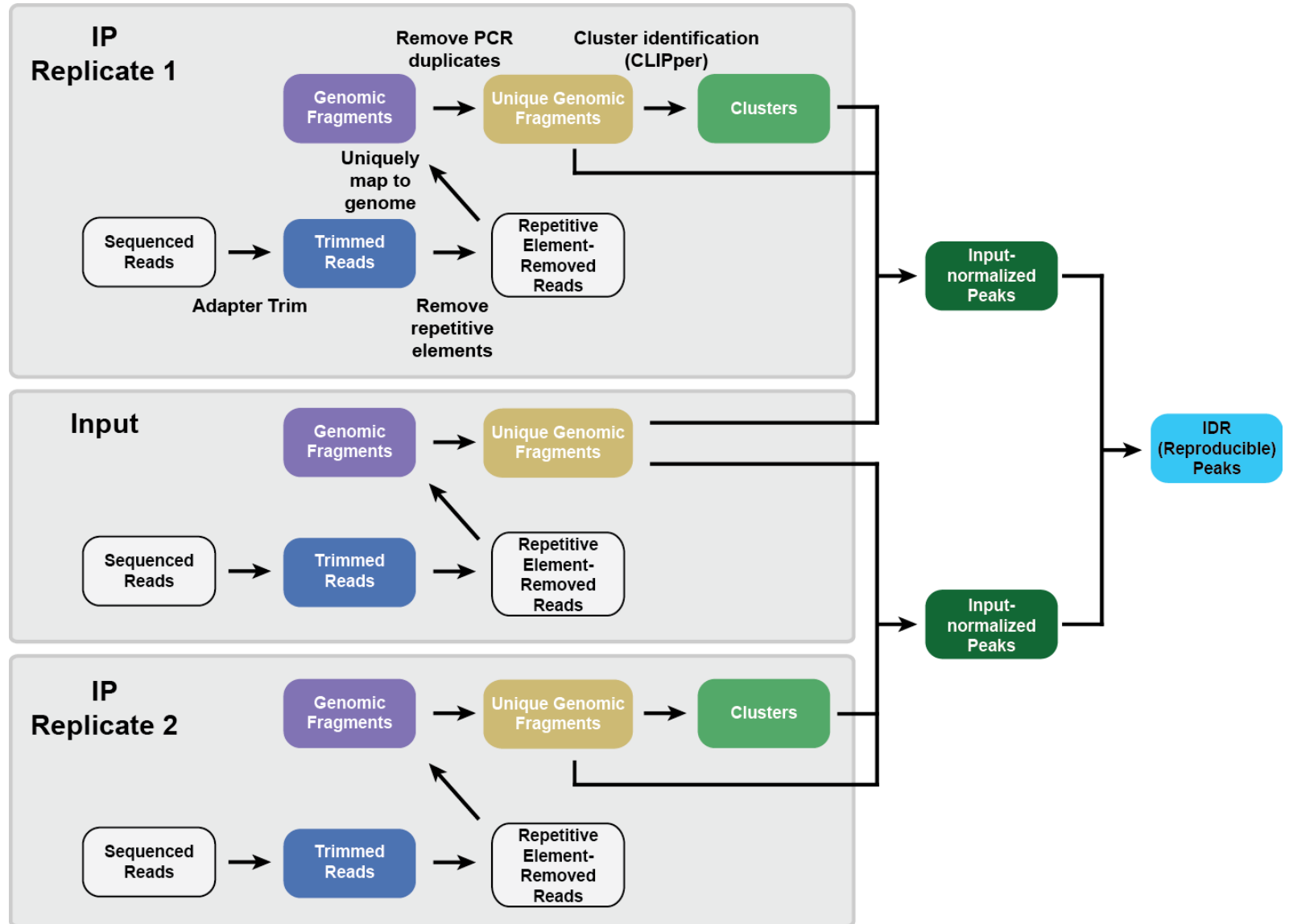
A deeper dive into eCLIP



Final library structure:



eCLIP: data processing overview



eCLIP: data access overview

ENCODE Data Encyclopedia Materials & Methods Help

RBFOX2

Data Type

- Dataset 7
- Experiment 7
- Biosample 4
- AntibodyLot 2
- Publication 2

+ See more...

Showing 18 of 18 results

RBFOX2 (*Homo sapiens*) Target
External resources: ENSEMBL:ENSG00000100320 HGNC:FOI2 HGNC:RBM9 GeneID:23543 HGNC:HRNBP2 UniProtKB:O43251
HGNC:RTA

RBFOX2 eCLIP mock input (*Homo sapiens*) Target
External resources: None submitted

RNA Bind-n-Seq Experiment
Target: RBFOX2
Lab: Chris Burge, MIT
Project: ENCODE
ENCSR441HLP released

RBFOX2 (*Homo sapiens*) Antibody
Source: GeneTex
Product ID / Lot ID: GTX116327 / 40555
ENCAB507HJJ

RBFOX2 (*Homo sapiens*) Antibody
Source: Bethyl Labs
Product ID / Lot ID: A300-854A / 2
ENCAB592TEY

K562 (*Homo sapiens*, adult 53 year) Biosample
Type: immortalized cell line
Summary: Homo sapiens K562 immortalized cell line transient RNAi knockdown shRNA...
RNAi target: RBFOX2
Culture harvest date: 2015-03-05
Source: ATCC
ENCBS677KBE released

eCLIP: data availability

ENCODE Data Encyclopedia Materials & Methods Help RBFOX2

Showing 2 of 2 results

Download Visualize

Assay category
RNA binding 2

Assay
RNA Binding Seq 2
eCLIP 2

Project
ENCODE 2

RFA
ENCODE3 2

Experiment status
released 2

Genome assembly (visualization)
hg19 1

Organism
Homo sapiens 2

Target of assay
RNA binding protein 1
control 1

Biosample type
immortalized cell line 2

Life stage
child 2

Target: RBFOX2 eCLIP mock input
Exp: Gene 100, 0000
Project: ENCODE

Experiment
ENCSR799EKA released

Target: RBFOX2
Exp: Gene 100, 0000
Project: ENCODE

Experiment
ENCSR987FTF released

Biosample 1

Biosample 2

eCLIP Replicate 1

Size-matched input

eCLIP Replicate 2


eCLIP: data processing overview

ENCODE Data Encyclopedia Materials & Methods Help

EXPERIMENTS / ECLIP / HOMO SAPIENS / HEPG2

Experiment summary for ENCSR987FTF

(:)

Summary	Attribution
Status: ● released	Lab: Gene Yeo, UCSD
Assay: eCLIP	Award: U54HG007005 (Brenton Graveley, UConn)
Target: RBFOX2	Project: ENCODE
Biosample summary: Homo sapiens HepG2	External resources: RBPImage:RBFOX2 GEO:GSE92211
Biosample Type: cell line	Aliases: gene-yeo:204
Replication type: isogenic	Date submitted: March 21, 2016
Description: eCLIP experiment on HepG2 against RBFOX2	Date released: July 15, 2015
Nucleic acid type: RNA	Submitter comment: Fastq files ENCF591SSP, ENCF172GUS, ENCF289OFA, and ENCF647KDW contain reads of mixed lengths. Read length followed by number of reads: ENCF591SSP (28, 24096), (29, 29994), (30, 36373), (31, 44027), (32, 52186), (33, 62212), (34, 72148), (35, 83327), (36, 95836), (37, 111283), (38, 124658), (39, 145860), (40, 173927), (41, 207859), (42, 257173), (43, 286977), (44, 325622); ENCF172GUS (28, 30109), (29, 37058), (30, 44623), (31, 54327), (32, 64359), (33, 76121), (34, 88715), (35, 100926), (36, 115541), (37, 131323), (38, 148448), (39, 168373), (40, 192382), (41, 220921), (42, 229070), (43, 264478), (44, 311656); ENCF289OFA (30, 101628), (31, 43075), (32, 50755), (33, 59105), (34, 68593), (35, 79699), (36, 90230), (37, 99313), (38, 111706), (39, 126182), (40, 136622), (41, 156824), (42, 186932), (43, 226418), (44, 282322), (45, 2032984), (46, 5788540); and ENCF647KDW (30, 88964), (31, 46645), (32, 54434), (33, 64706), (34, 75349), (35, 87425), (36, 100155), (37, 111358), (38, 125762), (39, 140030), (40, 156664), (41, 177459), (42, 208190), (43, 306399), (44, 396621), (45, 4798999), (46, 5699706).
Lysis method: see document	Tags: 
Extraction method: see document	
Fragmentation method: see document	
Size selection method: agarose gel extraction	
Strand specificity: Strand-specific	
Platform: Illumina HiSeq 2000	
Controls: ENCSR799EKA	

Isogenic replicates

Isogenic replicate	Technical replicate	Summary	Biosample	Antibody	Library
1	1	Homo sapiens HepG2 immortalized cell line	ENCBS547JWV	ENCAB592TEY	ENCLB180GIG
2	1	Homo sapiens HepG2 immortalized cell line	ENCBS537ADD	ENCAB592TEY	ENCLB696TLV

ENCAB592TEY

Antibody against *Homo sapiens* RBFOX2

(-)

Homo sapiens K562, HepG2

Characterized to standards

Status: released
Source (vendor): Bethyl Labs
Product ID: A300-864A
Lot ID: 2
Targets: RBFOX2 (Homo sapiens)
Host: Rabbit
Clonality: Polyclonal
Purification: Affinity
Isotype: IgG
Aliases: xiang-dong-fu-RBFOX2
External resources: ARIAB_2815817

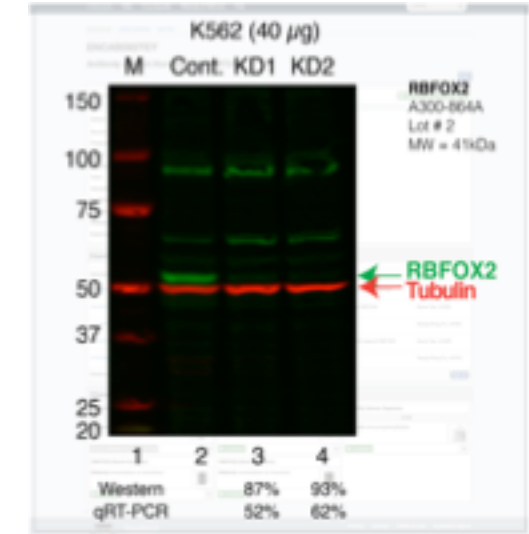
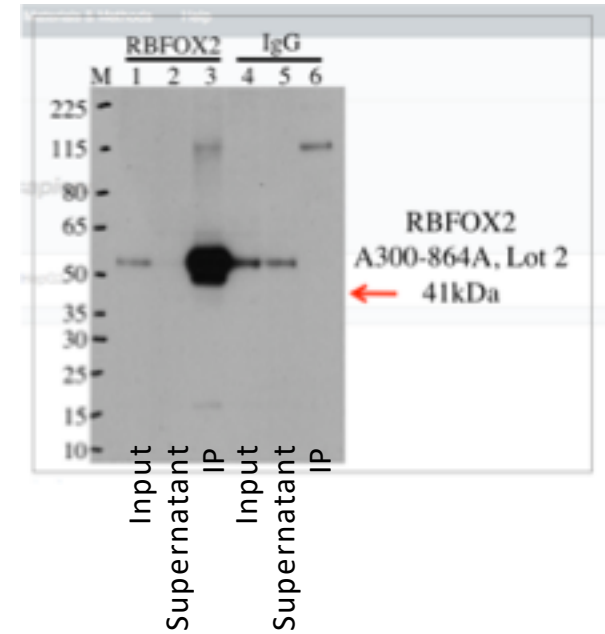
Experiments using this antibody

Accession	Assay	Biosample term name	Target	Description	Lab
ENC5R0519X	eCLIP	K562	RBFOX2	eCLIP control experiment on K562 against RBFOX2	Gene Yeo, UCSD
ENC5R75M0KJ	eCLIP	K562	RBFOX2	eCLIP experiment on K562 against RBFOX2	Gene Yeo, UCSD
ENC5R754FY5	ChIP-seq	HepG2	RBFOX2	RBFOX2 ChIP-seq in HepG2	Xiang-Dong Fu, UCSD
ENC5R7590XA	eCLIP	HepG2	RBFOX2	eCLIP control experiment on HepG2 against RBFOX2	Gene Yeo, UCSD
ENC5R825L8D	ChIP-seq	K562	RBFOX2	RBFOX2 ChIP-seq in K562	Xiang-Dong Fu, UCSD

Displaying 5 of 5 View all

Characterizations

<p>RBFOX2 (Homo Sapiens) K562 Method: Immunoprecipitation Completed</p>	<p>RBFOX2 (Homo Sapiens) HepG2 Method: Immunoprecipitation Completed</p>	<p>RBFOX2 (Homo Sapiens) K562 Method: Immunoprecipitation Completed</p>
<p>RBFOX2 (Homo Sapiens) Method: knockdown or knockout Completed</p>	<p>RBFOX2 (Homo Sapiens) Method: knockdown or knockout Completed</p>	



For more info on antibody validation see Sundaraman B, et al. Molecular Cell (2016)

Isogenic replicates

Isogenic replicate	Technical replicate	Summary	Biosample	Antibody	Library
1	1	Homo sapiens HepG2 immortalized cell line	ENCBS547JWV	ENCAB592TEY	ENCLB180GIG
2	1	Homo sapiens HepG2 immortalized cell line	ENCBS537ADD	ENCAB592TEY	ENCLB696TLV

File summary

Visualize Data

Raw data files

Accession	File type	Biological replicate	Library	Run type	Read	Lab	Date added	File size	Audit status	File status
ENCFF647KDW	fastq	1	ENCLB180GIG	PE 50-nt	R2	Gene Yeo, UCSD	2016-03-22	335 MB	✓	released
ENCFF172GUS	fastq	1	ENCLB180GIG	PE 50-nt	R1	Gene Yeo, UCSD	2016-03-22	313 MB	✓	released
ENCFF289OFA	fastq	2	ENCLB696TLV	PE 50-nt	R2	Gene Yeo, UCSD	2016-03-22	338 MB	✓	released
ENCFF591SSP	fastq	2	ENCLB696TLV	PE 50-nt	R1	Gene Yeo, UCSD	2016-03-22	315 MB	✓	released

R1 + R2 fastq files

Processed data files

Accession	File type	Output type	Biological replicate	Mapping assembly	Lab	Date added	File size	Audit status	File status
ENCFF735HJV	bed narrowPeak	peaks	1	hg19	Gene Yeo, UCSD	2016-04-05	784 kB	⚠	In progress
ENCFF475KXE	bigBed narrowPeak	peaks	1	hg19	Gene Yeo, UCSD	2016-03-24	2.67 MB	✓	released
ENCFF994WPX	bam	alignments	1	hg19	Gene Yeo, UCSD	2016-03-23	365 MB	✓	released
ENCFF030USB	bed narrowPeak	peaks	2	hg19	Gene Yeo, UCSD	2016-04-05	665 kB	⚠	In progress
ENCFF026CVE	bigBed narrowPeak	peaks	2	hg19	Gene Yeo, UCSD	2016-03-24	2.7 MB	✓	released
ENCFF154BQS	bam	alignments	2	hg19	Gene Yeo, UCSD	2016-03-23	365 MB	✓	released

Input-normalized peaks

Paired-end mapping (STAR)

File details: bed narrowPeak (input-normalized peaks)

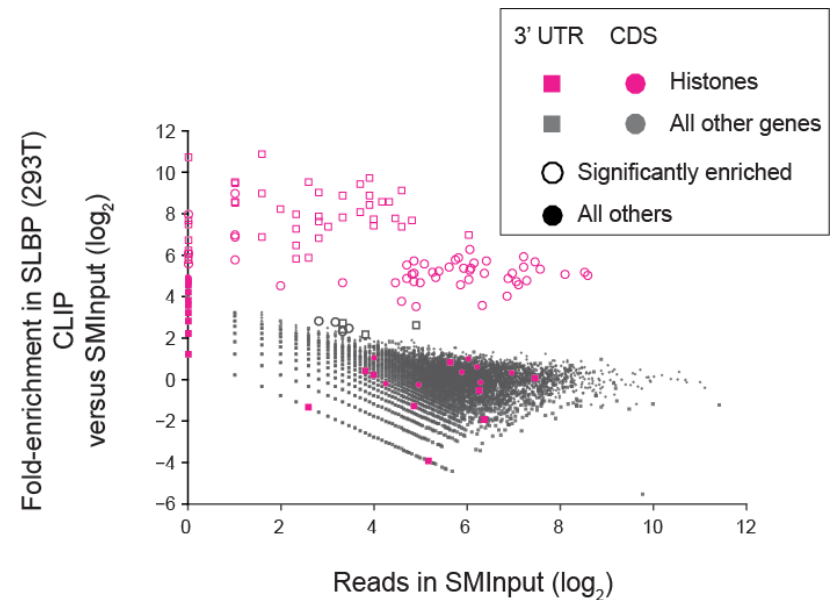
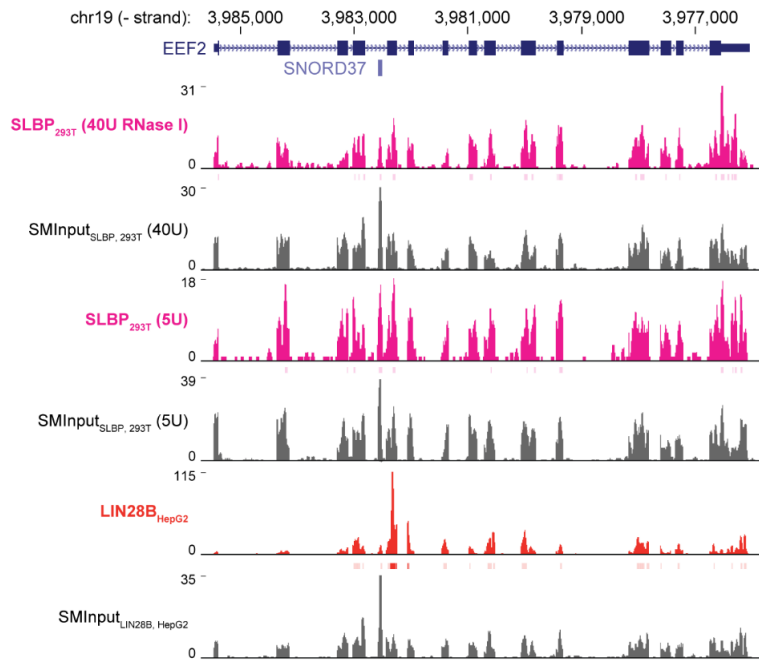
```
chr \t start \t stop \t dataset_label \t 1000 \t strand \t log2(eCLIP fold-enrichment  
over size-matched input) \t -log10(eCLIP vs size-matched input p-value) \t -1 \t -1
```

- Note: p-value is calculated by Fisher's Exact test (minimum p-value 2.2×10^{-16}), with chi-square test ($-\log_{10}(\text{p-value})$ set to 400 if p-value reported == 0)
- Our typical 'stringent' cutoffs: require $-\log_{10}(\text{p-value}) \geq 3$ and $\log_2(\text{fold-enrichment}) \geq 3$

```
track type=narrowPeak visibility=3 db=hg19 name="RBFOX2_HepG2_rep01" description="RBFOX2_HepG2_rep01  
input-normalized peaks"
```

Chr7	4757099	4757219	RBFOX2_HepG2_rep01	1000	+	6.539331235	400	-1	-1
Chr7	99949578	99949652	RBFOX2_HepG2_rep01	1000	+	5.233511963	400	-1	-1
Chr7	1027402	1027481	RBFOX2_HepG2_rep01	1000	+	5.243129966	69.5293984	-1	-1

Why input normalize?

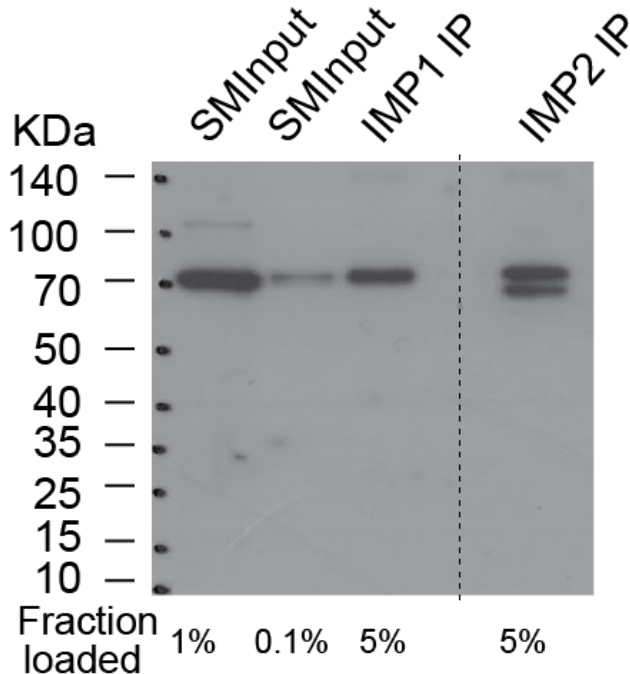


- We see mRNA background at nearly all abundant genes...

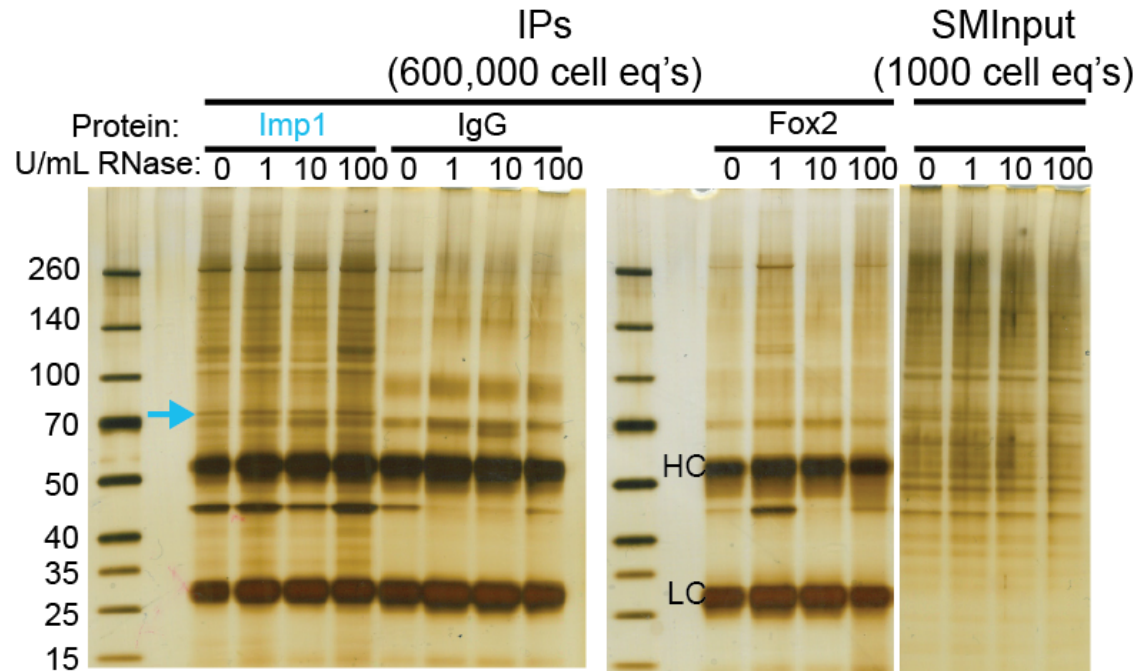
... but true signal is highly enriched above this background

Why input normalize?

IP-Western



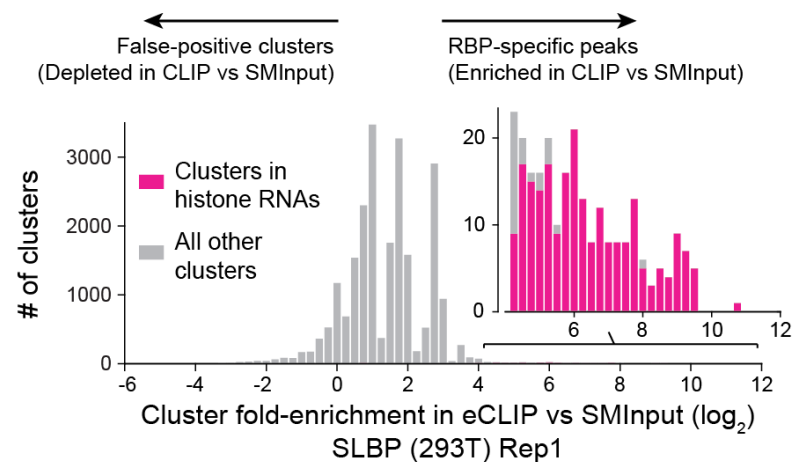
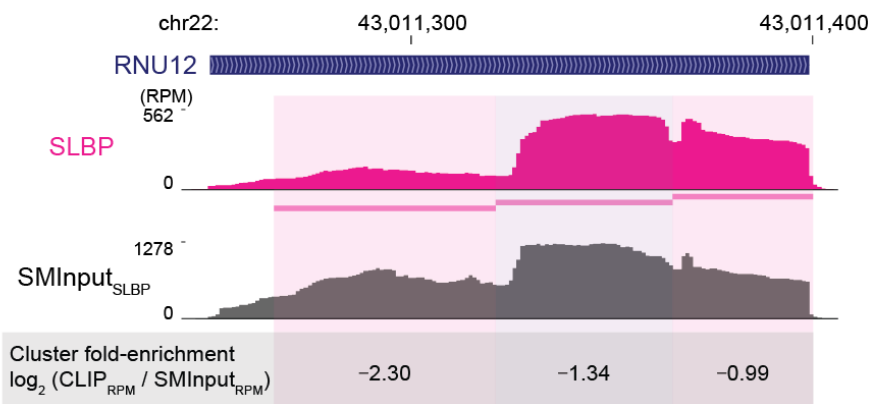
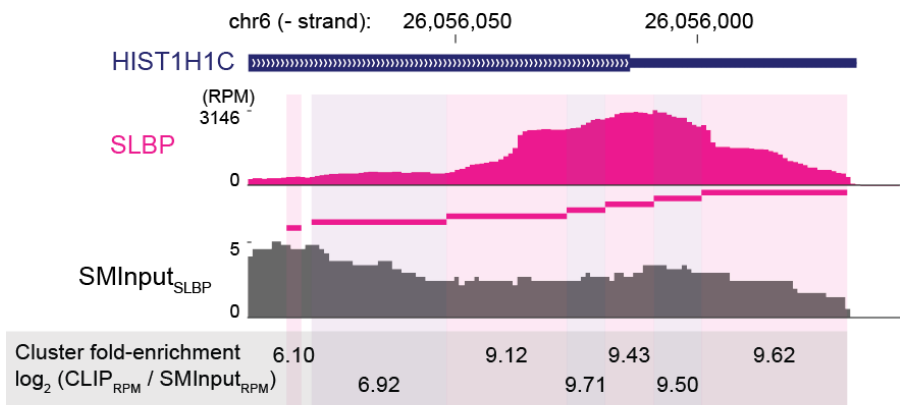
Silver stain (all protein)



- IP-western indicates immunoprecipitation success...

... but there remains substantial background of other proteins after IP

Input normalization removes false-positives and identifies confident binding sites



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223 eCLIP experiments (150 RBPs) released in K562 and HepG2 cell lines

HepG2 (103 RBPs)

BCCIP BCLAF1 CDC40
CSTF2 DDX59 DKC1
EIF3D EIF3H FKBP4
FUBP3 G3BP1 GRSF1
IGF2BP3 NIP7 NKRF
NOL12 PABPN1 PCBP2
POLR2G PPIG PRPF4
RBM5 SF3A3 SFPQ
SRSF9 STAU2 SUB1
SUGP2 TIAL1 XPO5

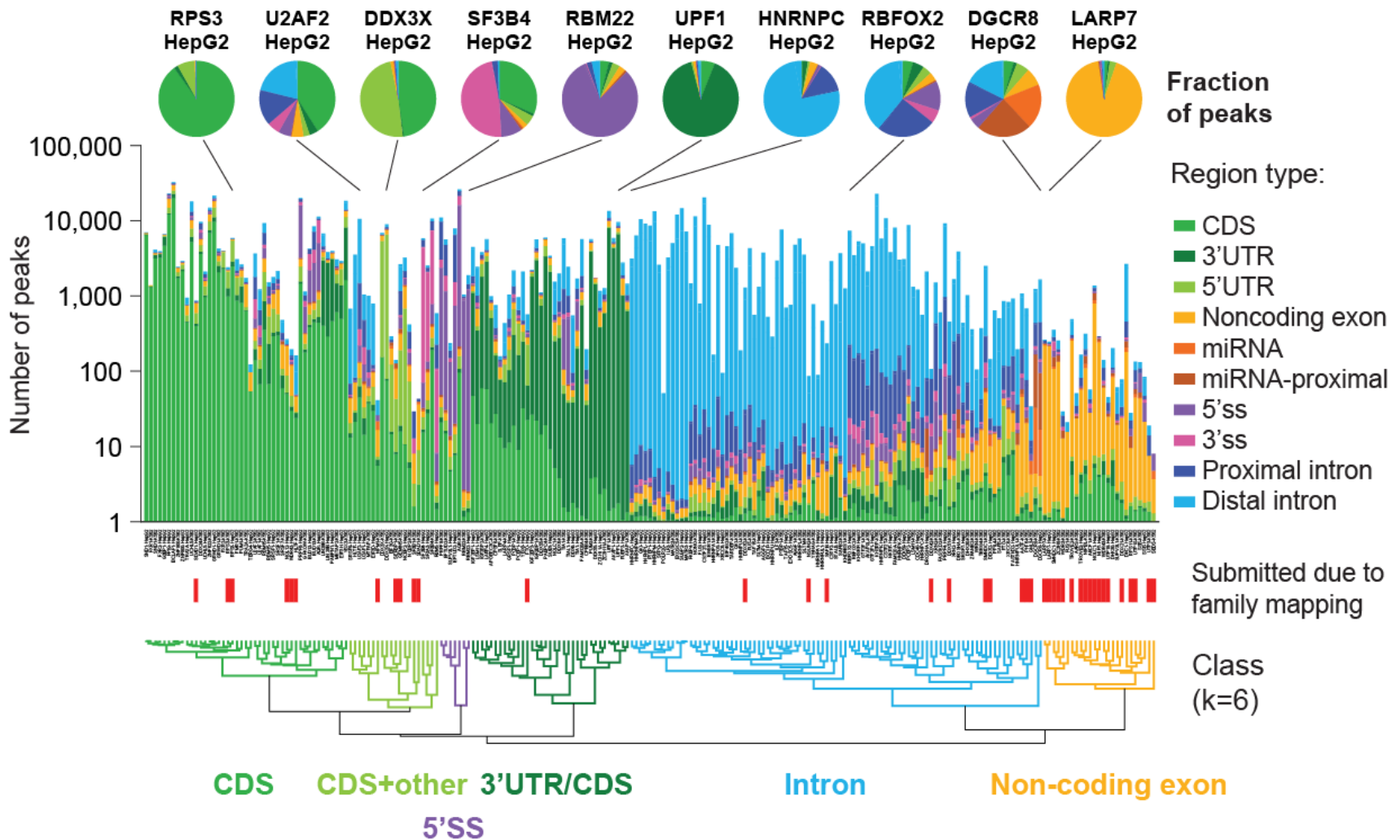
Both (73 RBPs)

AGGF1 AKAP1 AQR BUD13 CSTF2T DDX3X
DDX52 DDX55 DDX6 DGCR8 DHX30
DROSHA EFTUD2 EXOSC5 FAM120A
FASTKD2 FTO FUS FXR2 GRWD1 GTF2F1
HLTF HNRNPA1 HNRNPC HNRNPK HNRNPL
HNRNPM HNRNPU HNRNPUL1 IGF2BP1 ILF3
KHSRP LARP4 LARP7 LIN28B LSM11 MATR3
NCBP2 NOLC1 PCBP1 PRPF8 PTBP1 QKI
RBFOX2 RBM15 RBM22 RPS3 SAFB SDAD1
SF3B4 SLTM SMNDC1 SND1 SRSF1 SRSF7
SSB SUPV3L1 TAF15 TBRG4 TIA1 TRA2A
TROVE2 U2AF1 U2AF2 UCHL5 UPF1 UTP18
WDR43 XRCC6 XRN2 YBX3 ZC3H11A ZNF800

K562 (120 RBPs)

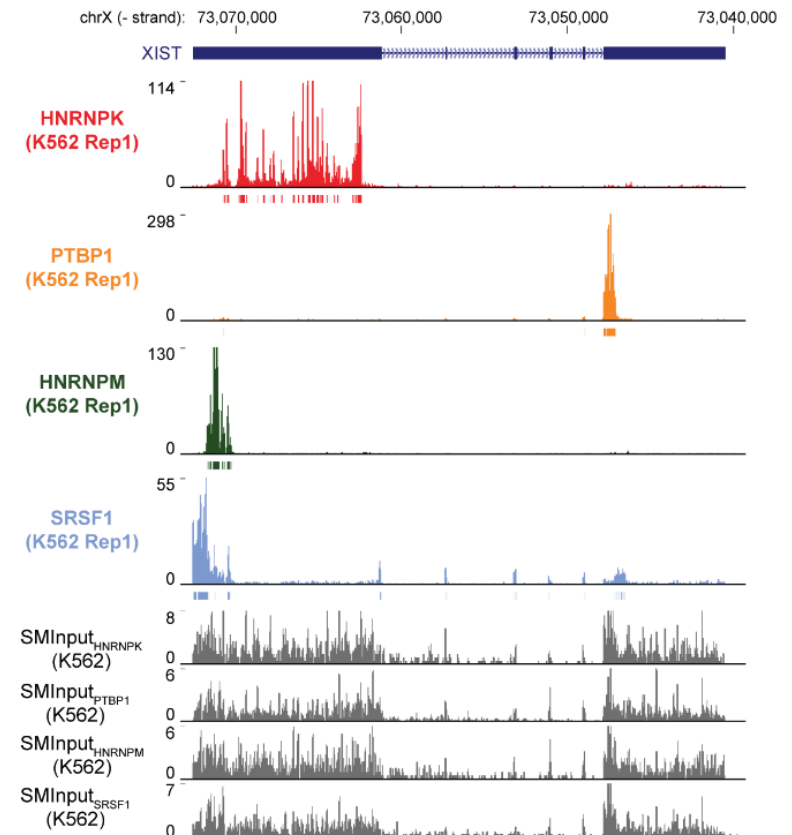
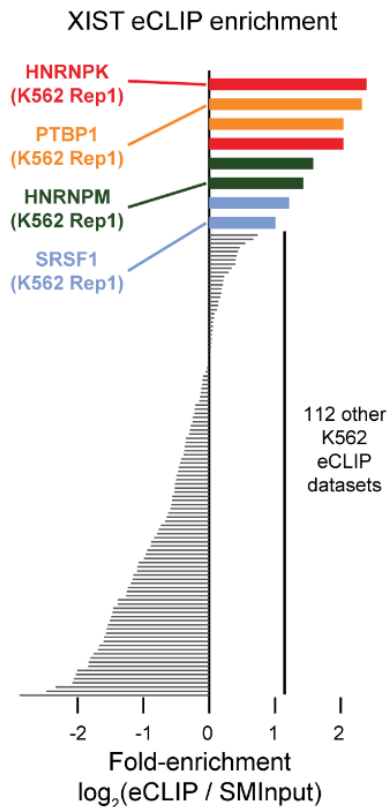
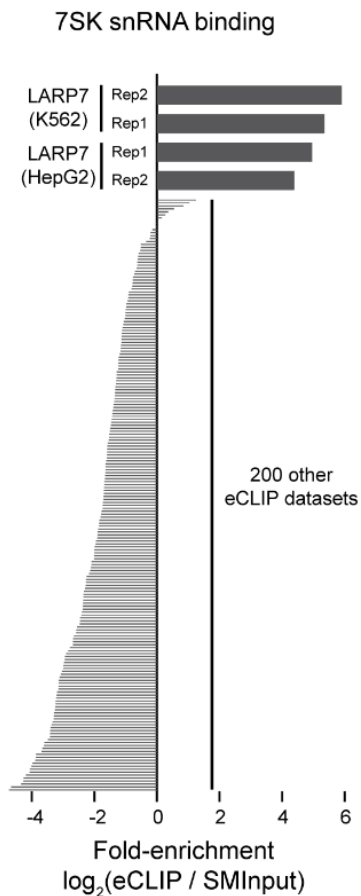
AARS AATF ABCF1 AKAP8L
APOBEC3C CPEB4 CPSF6 DDX21
DDX24 DDX42 DDX51 EIF3G
EIF4G2 EWSR1 FMR1 FXR1
GEMIN5 GNL3 GPKOW IGF2BP2
KHDRBS1 METAP2 MTPAP
NIPBL NONO NPM1 NSUN2
PABPC4 PHF6 PPIL4 PUM1
PUM2 PUS1 RPS11 SAFB2 SBDS
SERBP1 SF3B1 SLBP TARDBP
UTP3 WDR3 WRN YWHAG
ZC3H8 ZNF622 ZRANB2

A '10,000-foot view' of each eCLIP experiment: Summary by distribution of peaks



An “RNA-centric” view of RBP-binding

‘*in silico* screen’ of a desired RNA against all CLIP datasets to identify the best-binding RBPs



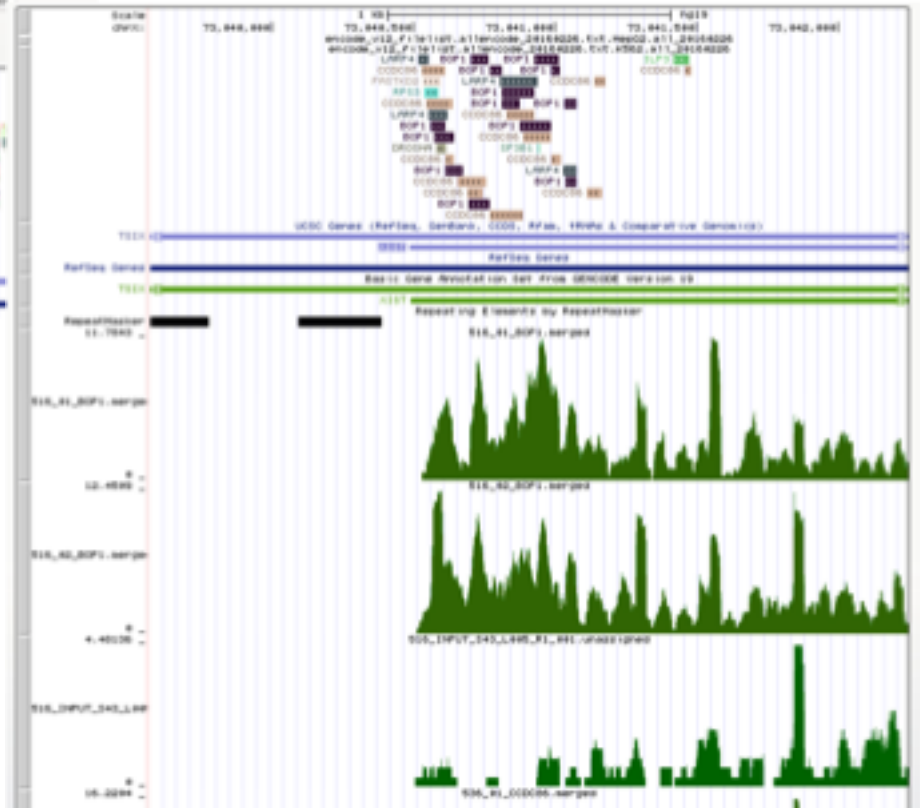
An “RNA-centric” view of RBP-binding

‘*in silico* screen’ of a desired RNA against all CLIP datasets to identify the best-binding RBPs

UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x

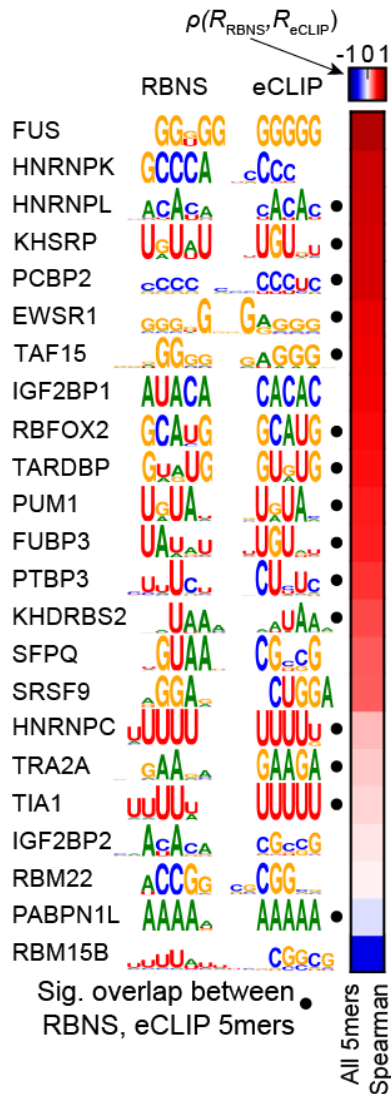
chrX:73,040,486-73,072,588 32,103 bp. enter position, gene symbol or search terms



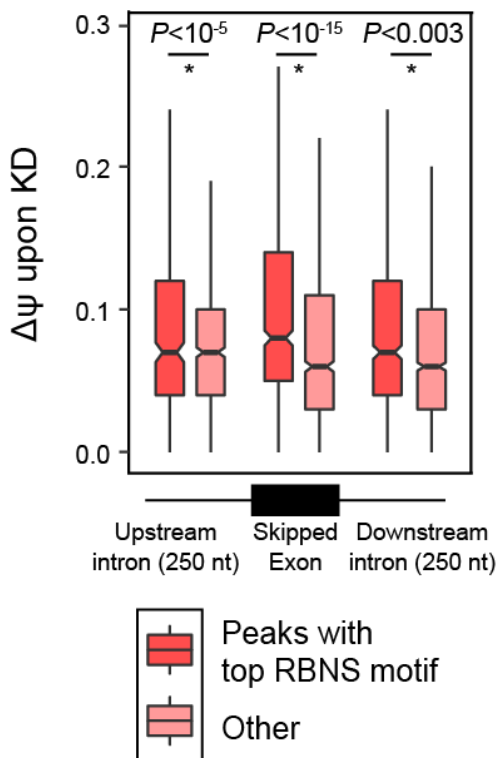
Outline

- Data overview
 - Available data types
 - Example: a deeper dive into accessing eCLIP data
- **Incorporating ENCODE data into analyses**
 - Using eCLIP to identify potential regulators of an RNA of interest
 - Integrating *in vivo* and *in vitro* motifs to study RBP regulation
 - Integrating RBP-responsive and *in vivo* RNA targets to map regulation of RNA stability and splicing
 - Using localization to predict RBP function (and vice versa)

Incorporating *in vitro* motifs can give better insight into regulatory targets



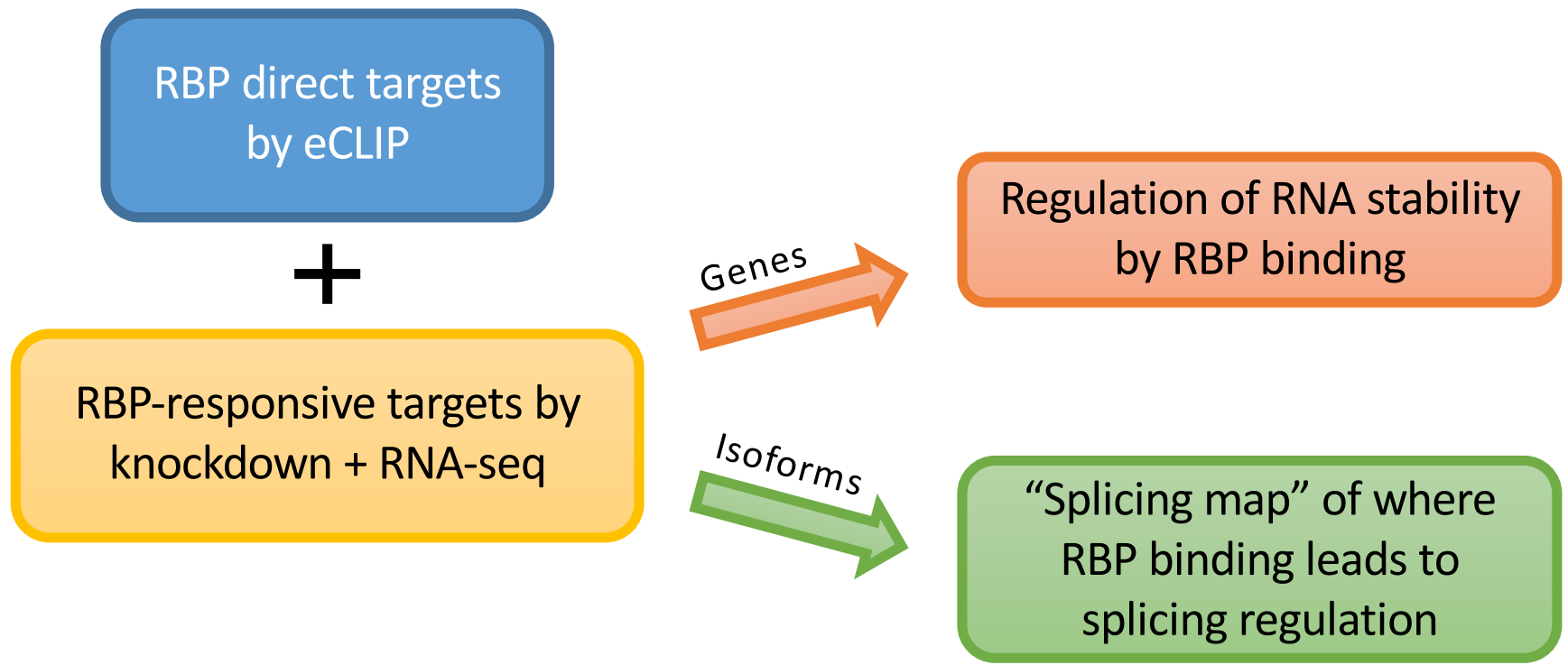
RBP-repressed cassette exons (across all RBPs)



Outline

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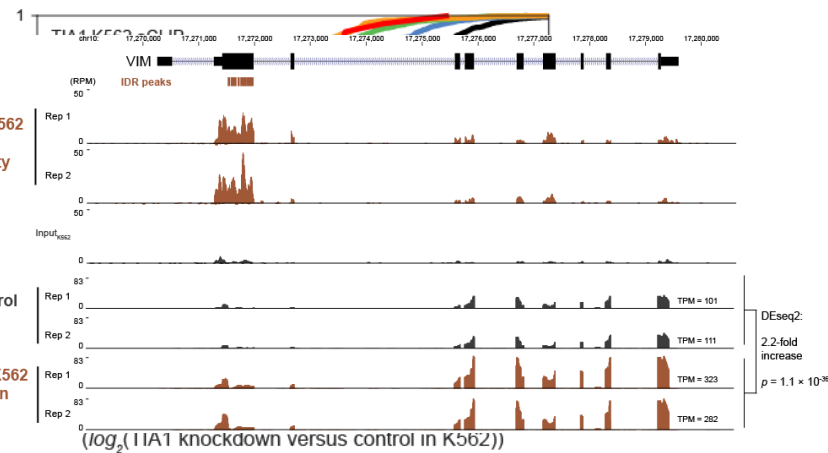
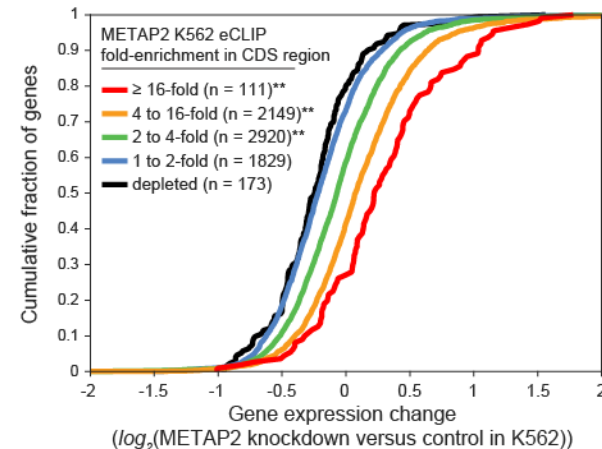
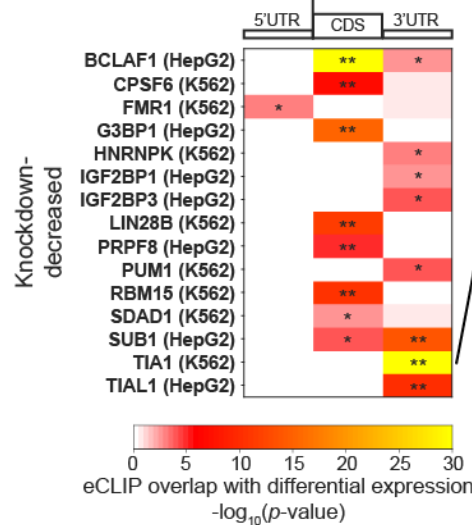
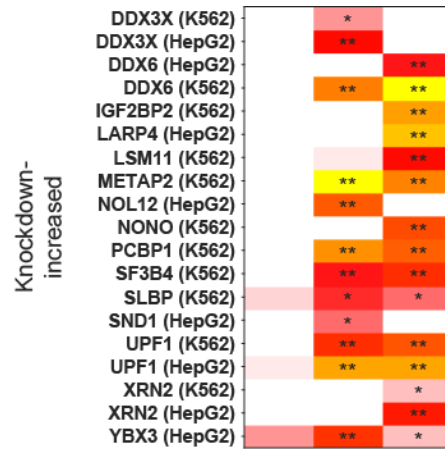
Identifying functional targets by integrating eCLIP with knockdown/RNA-seq



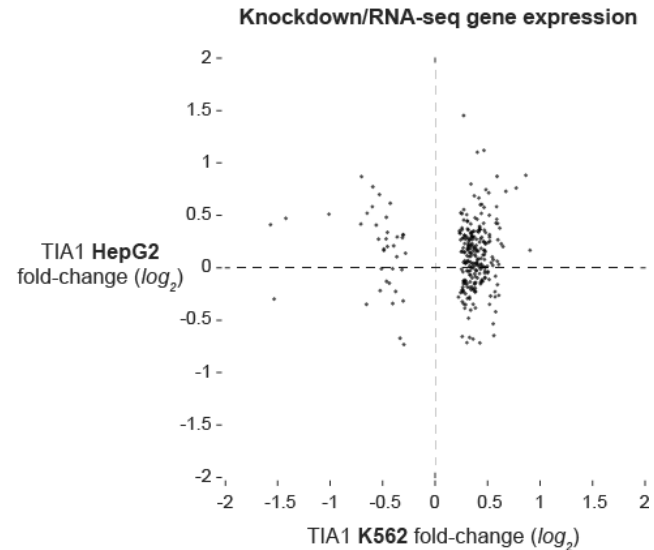
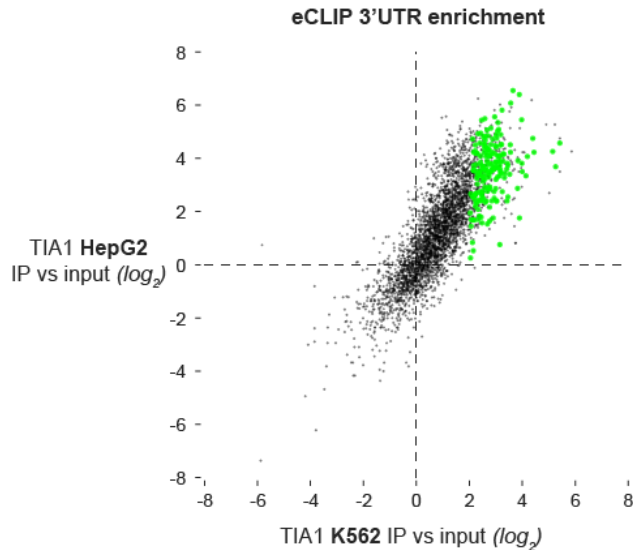
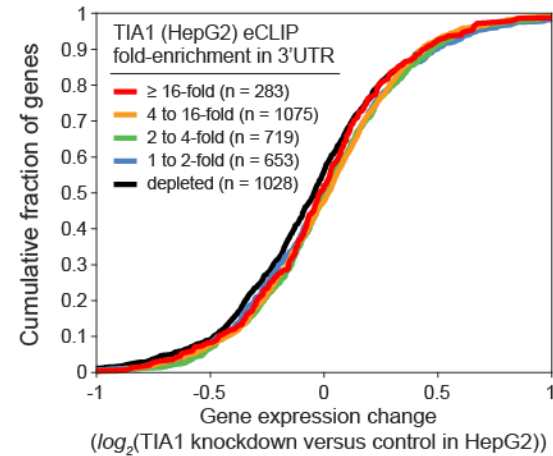
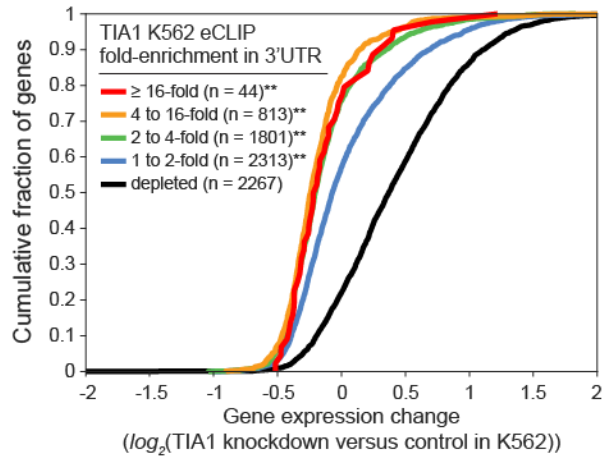
Identification of new candidate regulators of RNA stability

Genes bound by RBP (eCLIP)

RBP-responsive genes (knockdown/RNA-seq)

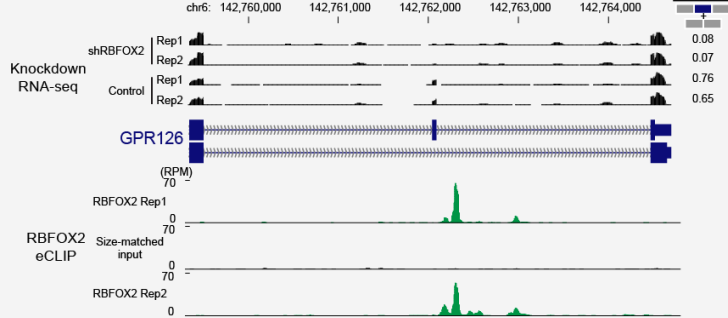


Analysis of TIA1 reveals cell-type specific regulation

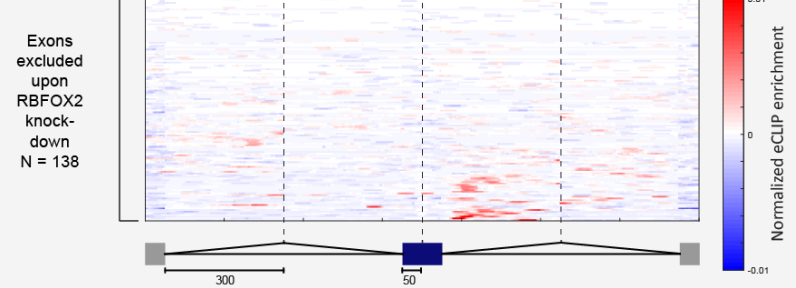


Splicing regulatory maps identify regulatory binding

Individual RBP-regulated event



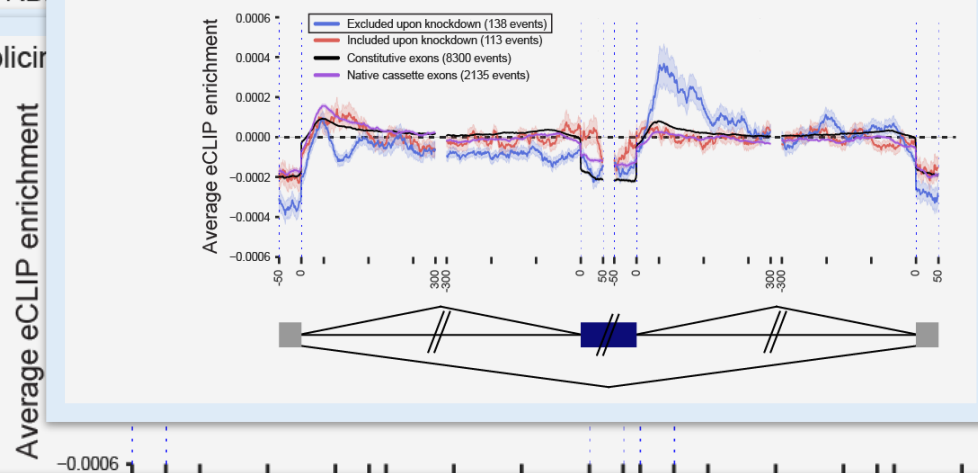
All RBP-regulated events



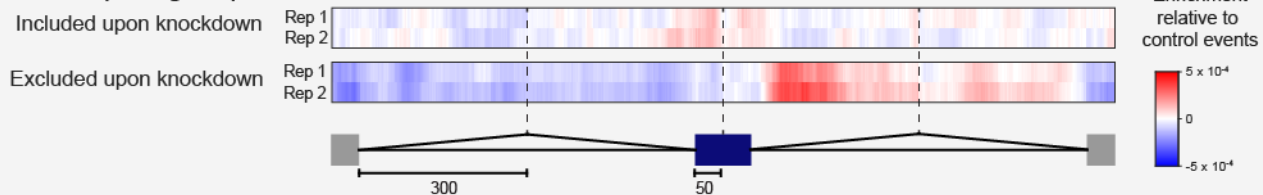
Individual RBP

Normalized splicing

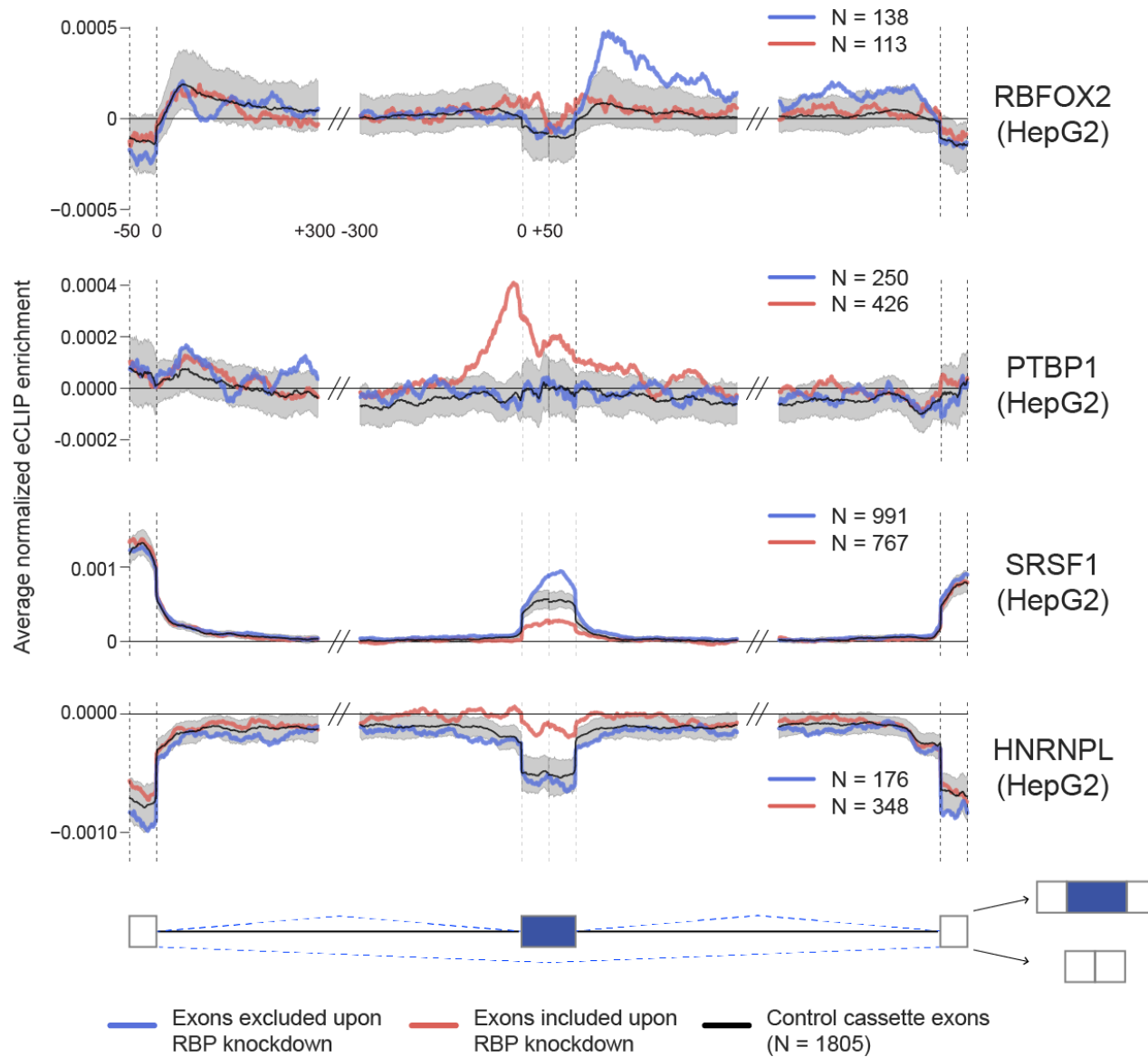
Normalized splicing map



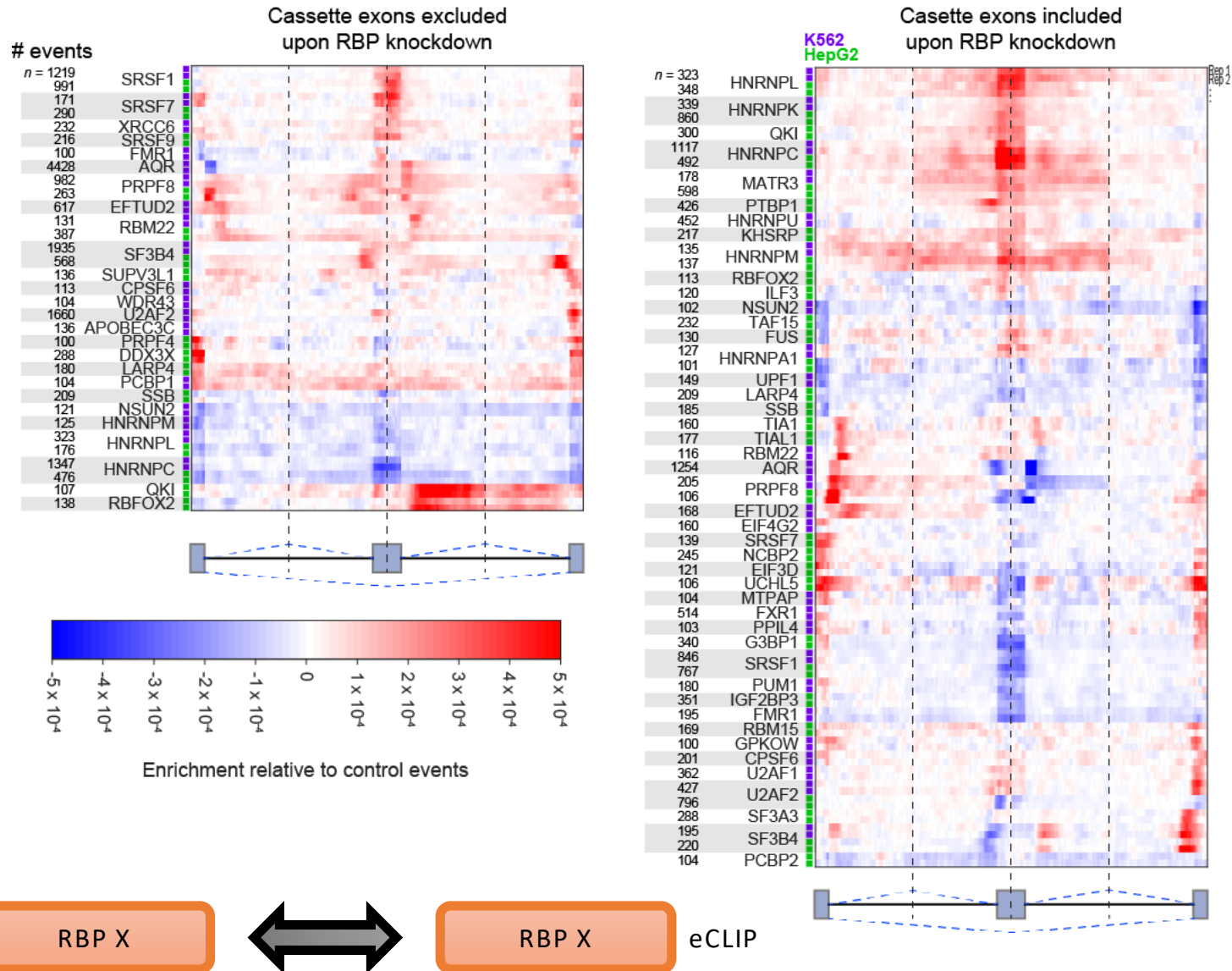
Relative splicing map



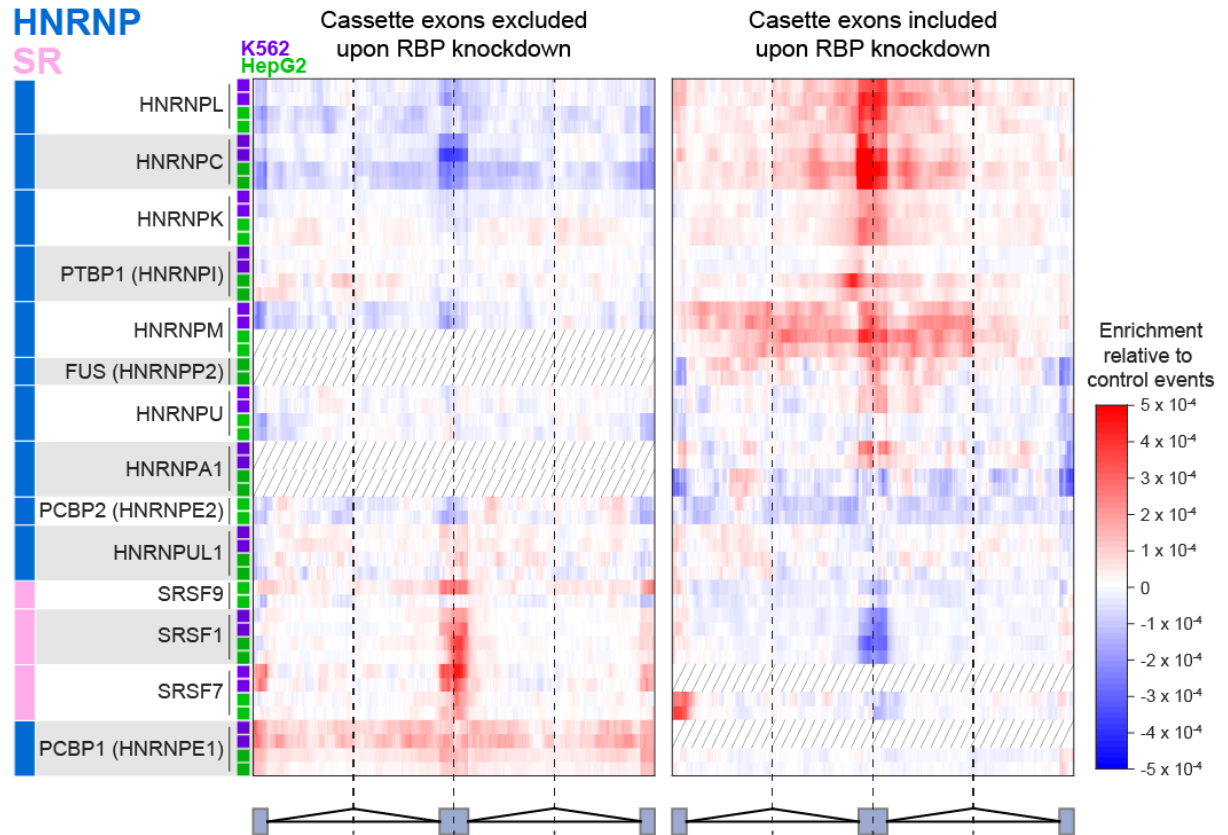
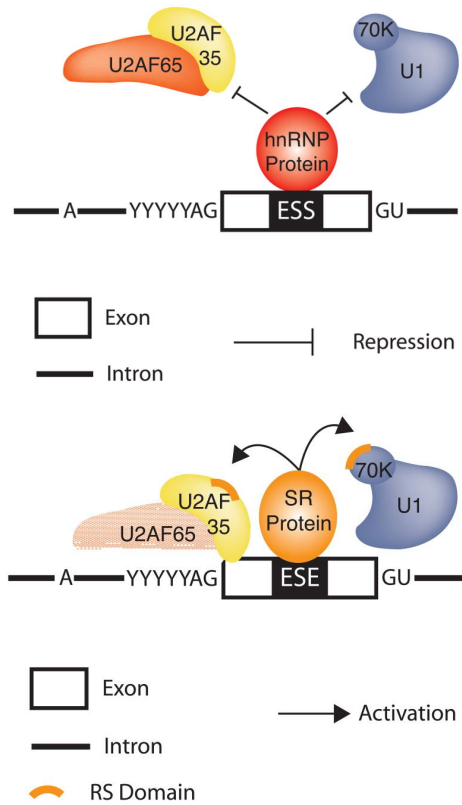
Splicing regulatory maps identify regulatory binding



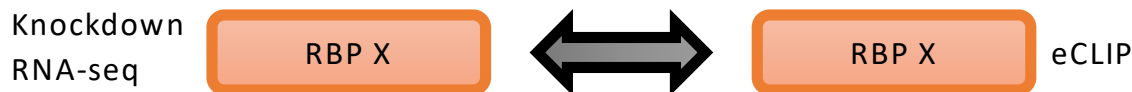
Splicing regulatory maps identify regulatory binding



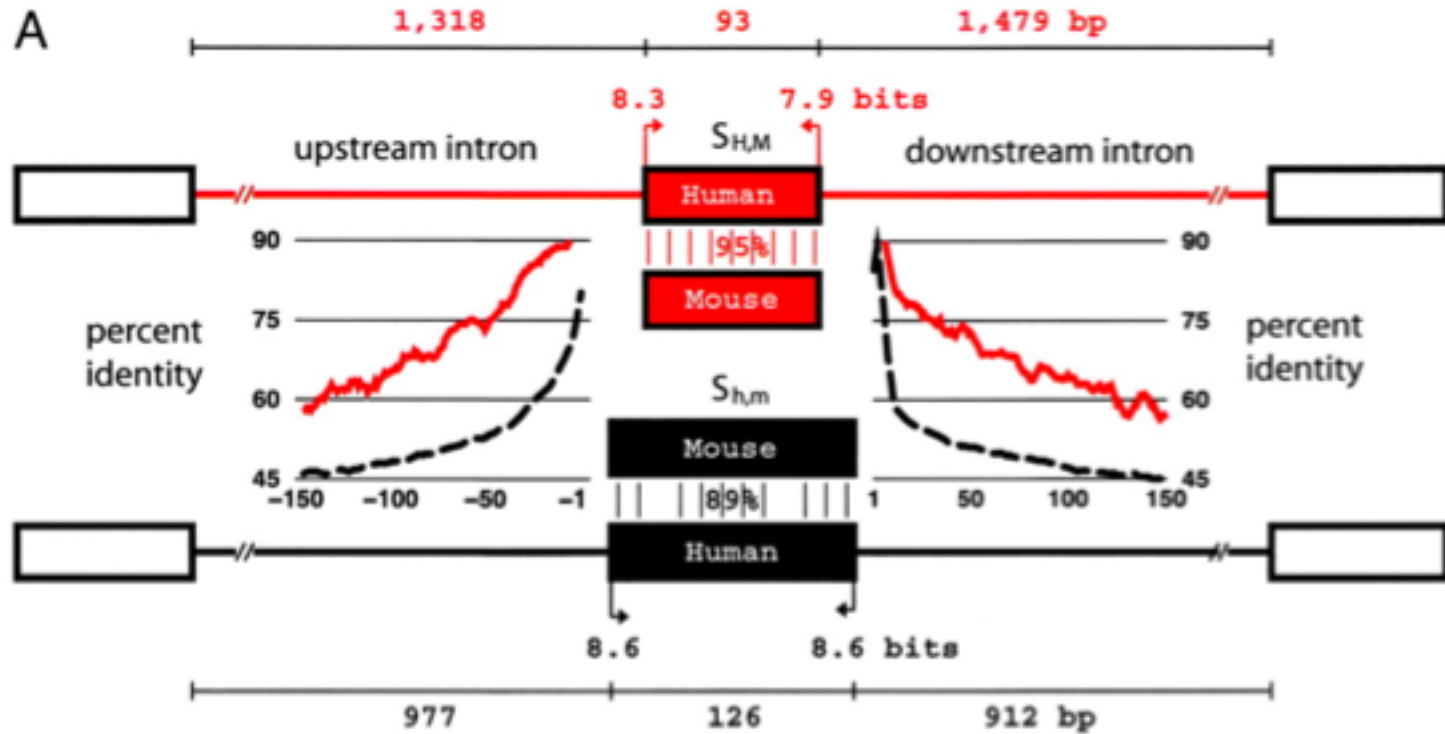
Global analysis recapitulates general principles of alternative splicing regulation



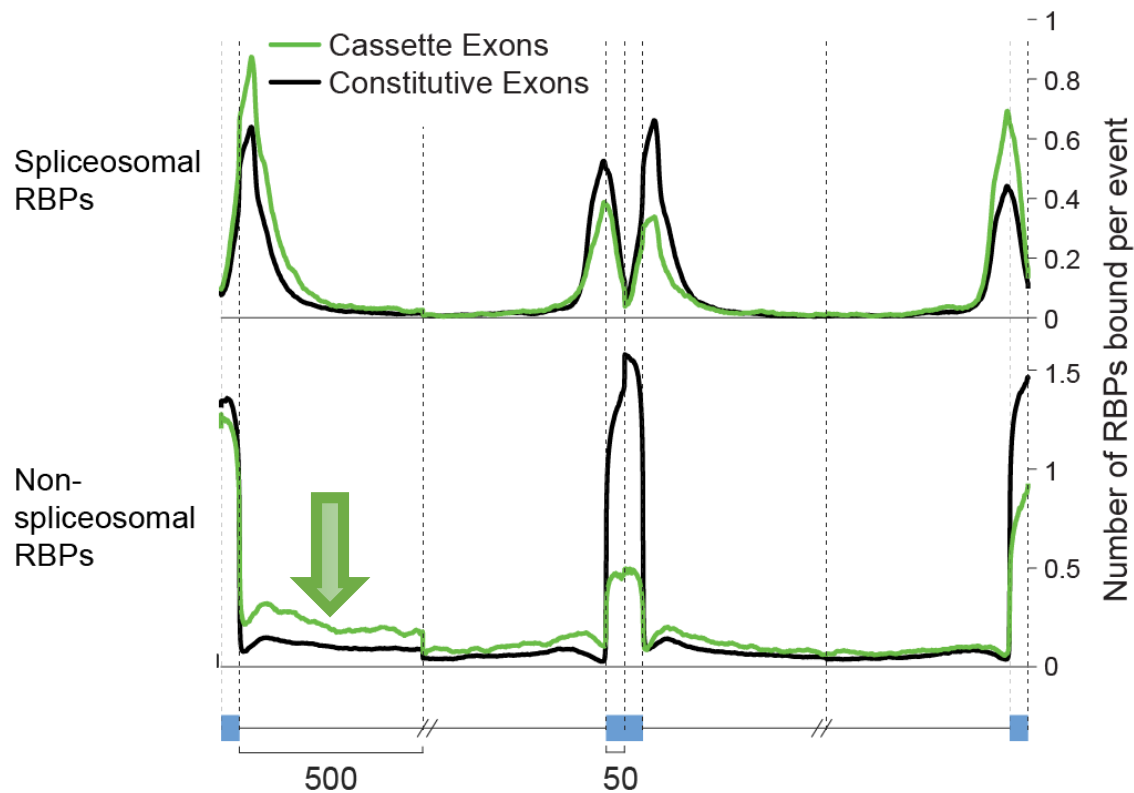
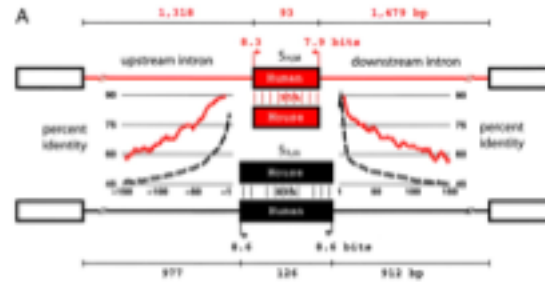
Busch A & Hertel KJ,
Wiley Interdiscip Rev RNA (2012)



Where are key regions for splicing regulation?

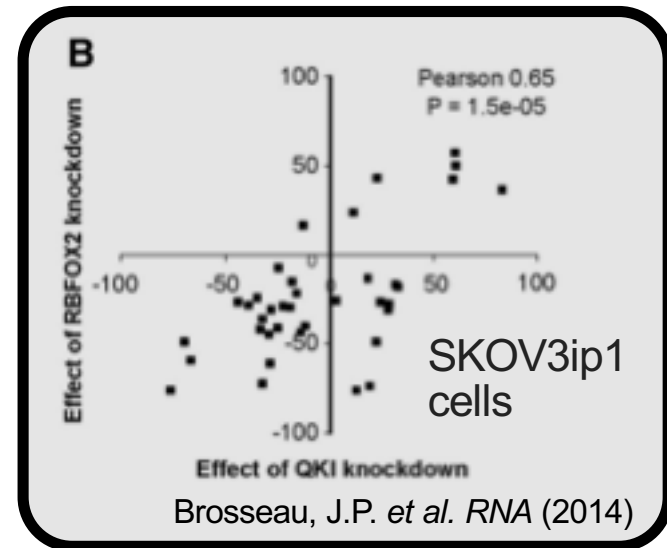
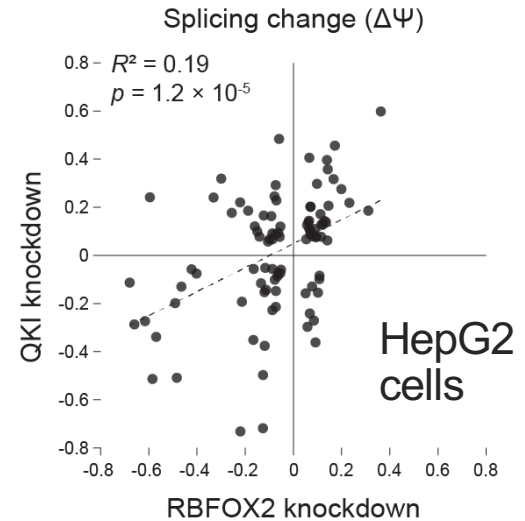
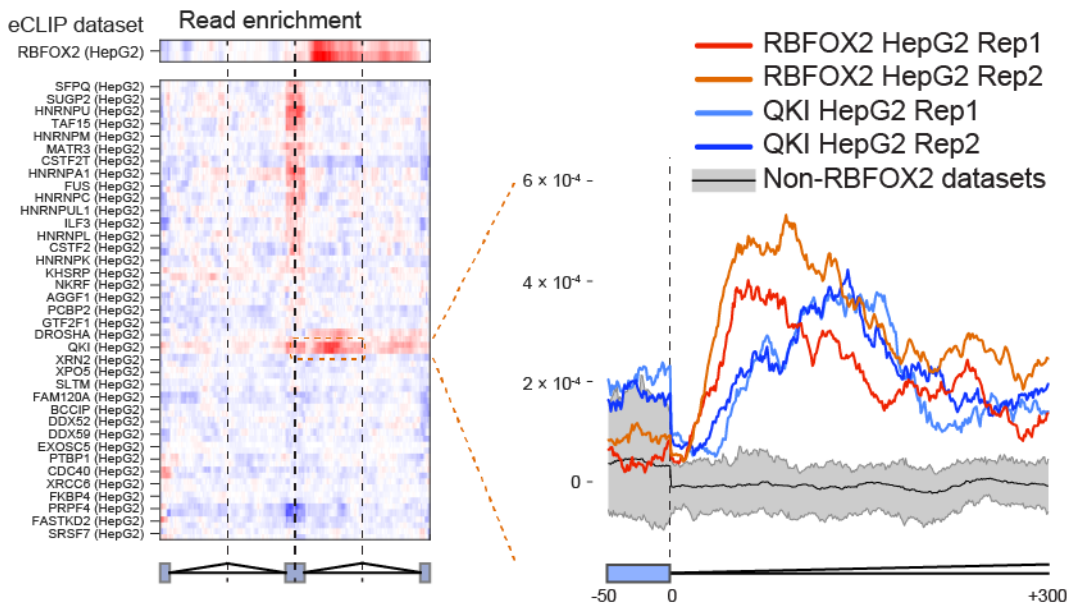


Where are key regions for splicing regulation?



Cross-RBP splicing maps indicate coordinated regulation

RBFOX2 knockdown-excluded cassette exons (HepG2)



Knockdown
RNA-seq

RBFOX2

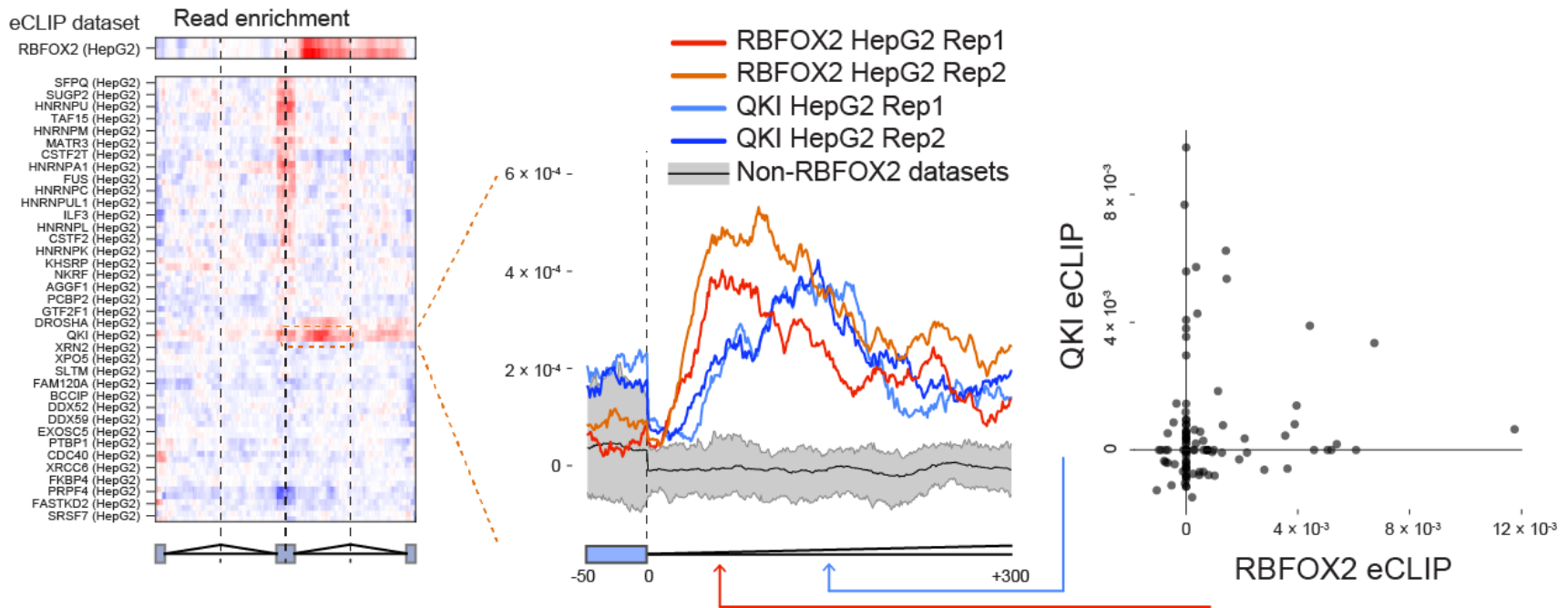


RBP Y

eCLIP

Cross-RBP splicing maps indicate coordinated regulation

RBFOX2 knockdown-excluded cassette exons (HepG2)



Knockdown
RNA-seq

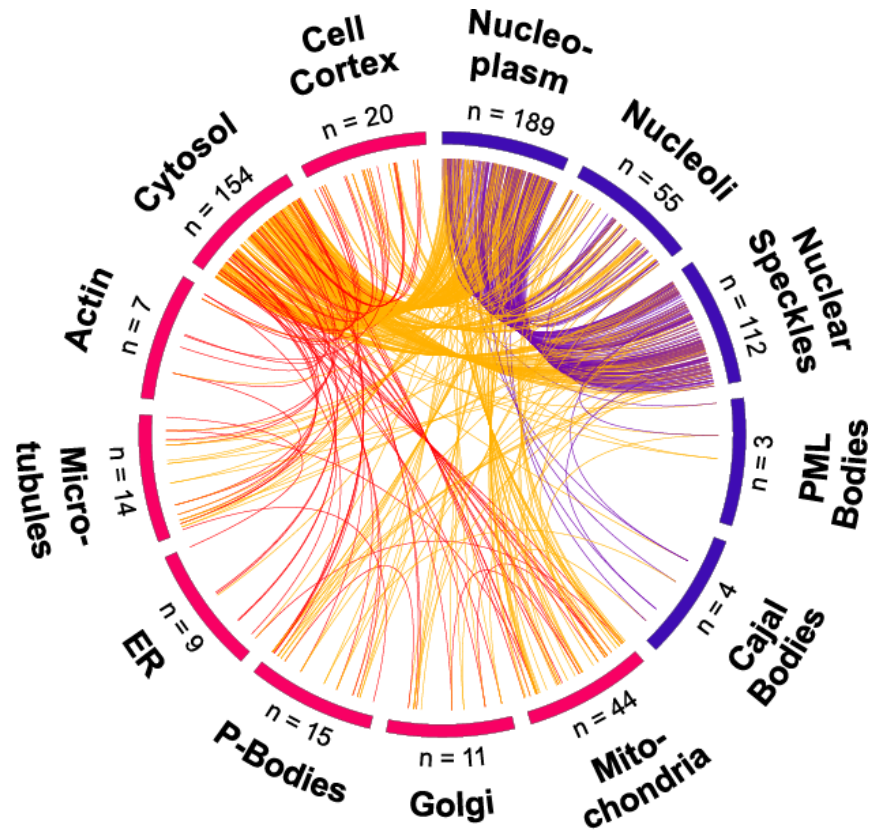
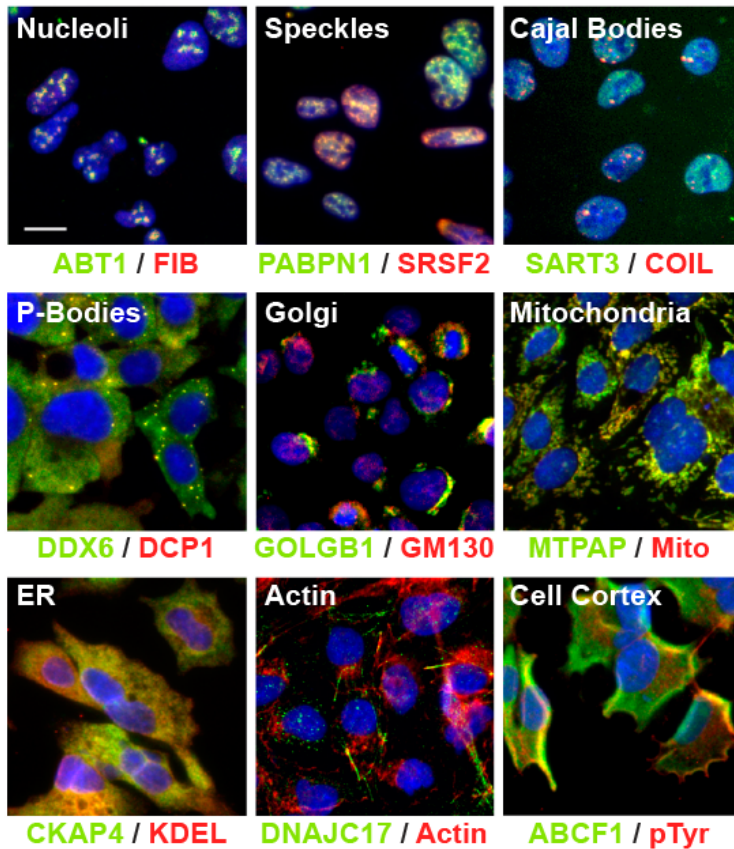


eCLIP

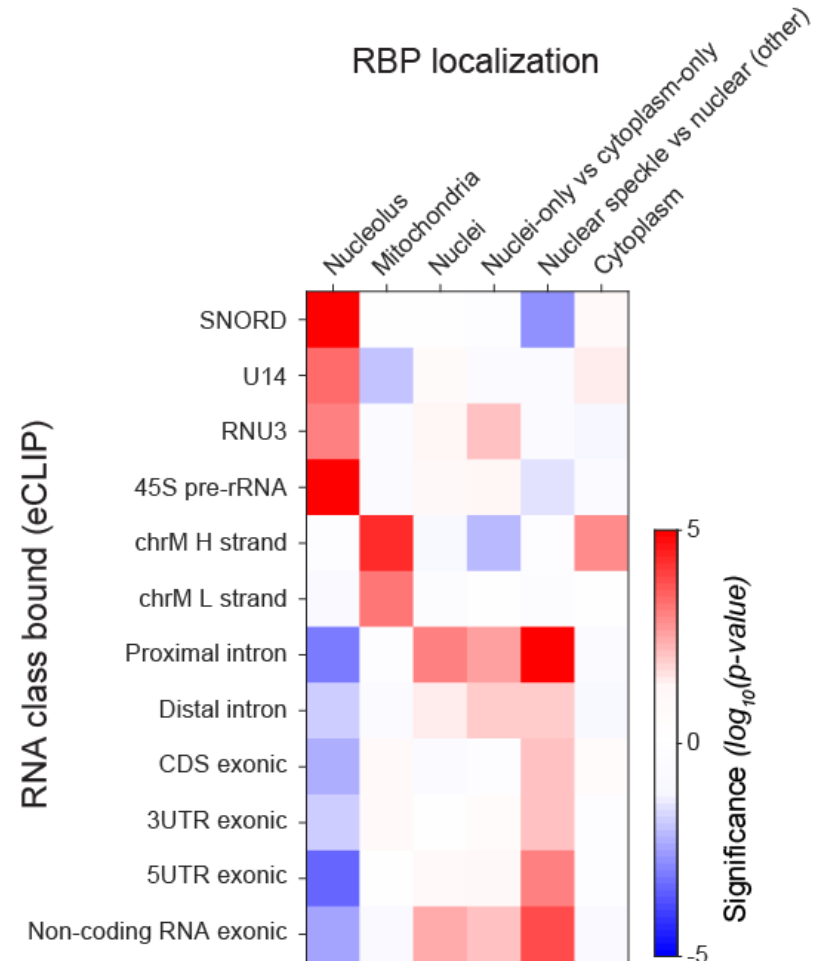
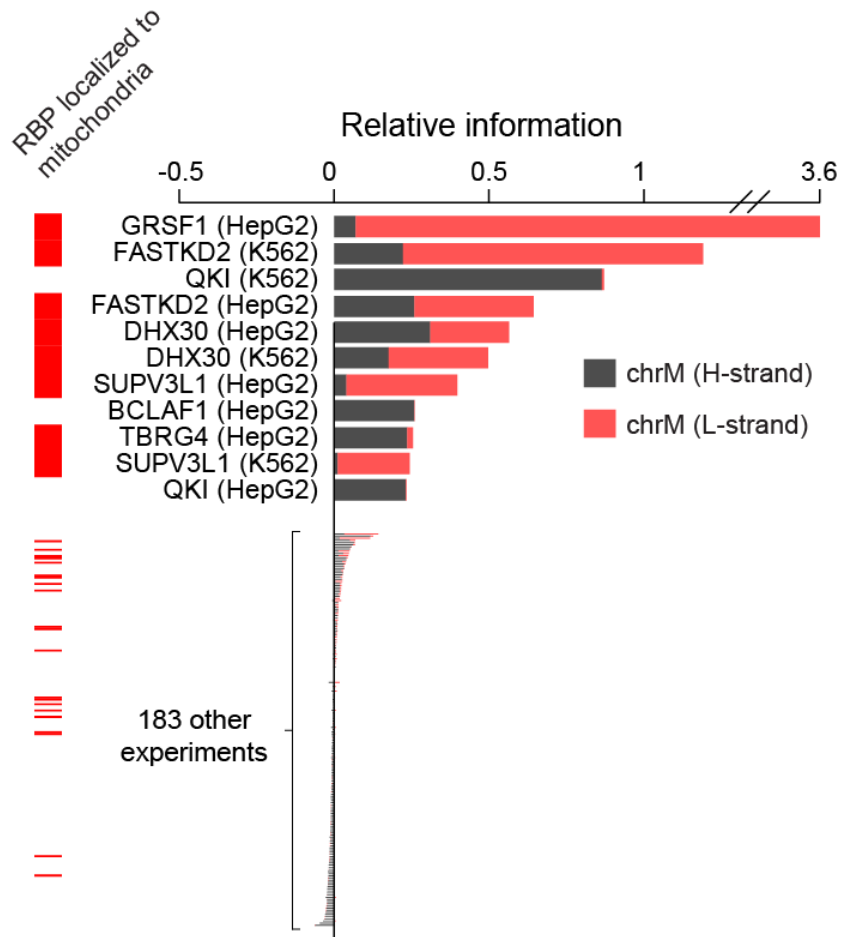
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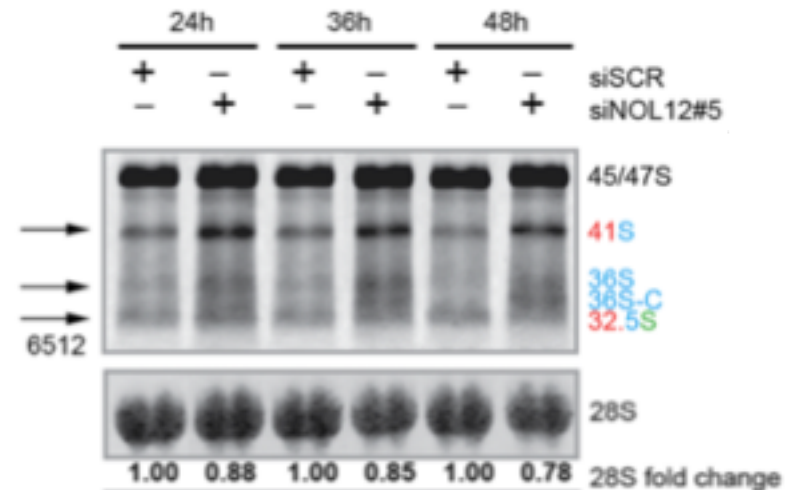
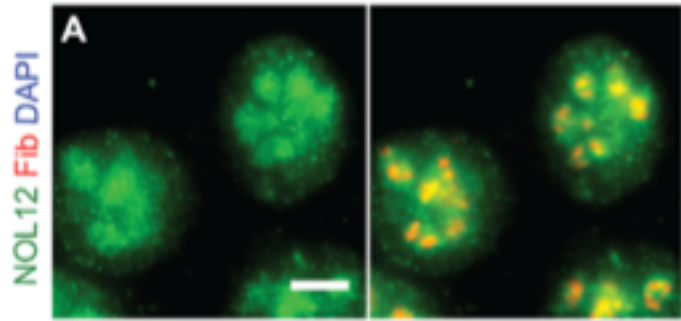
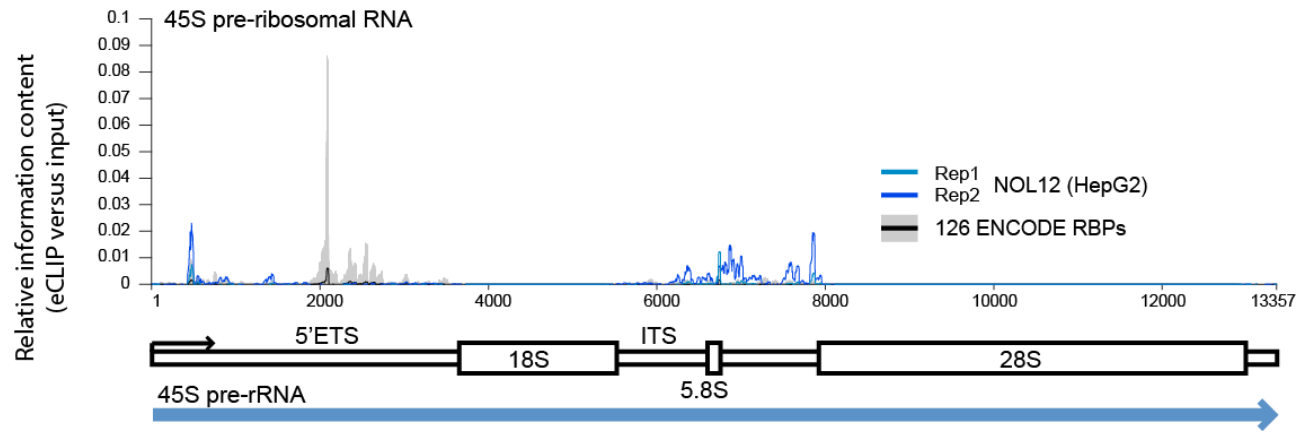
Using RBP localization and binding to infer and confirm RBP functions



Does localization predict binding (and vice versa)?



eCLIP plus localization predicts function



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doi: 10.1093/nar/gkx963

Nol12 is a multifunctional RNA binding protein at the nexus of RNA and DNA metabolism

Daniel D. Scott^{1,2,3}, Christian Trahan^{1,2,3}, Pierre J. Zindy¹, Lisbeth C. Aguilar¹, Marc Y. Delubac^{1,3}, Eric L. Van Nostrand⁴, Srivathsan Adivarahan³, Karen E. Wei¹, Gene W. Yeo^{4,5}, Daniel Zenklusen³ and Marlene Oeffinger^{1,2,3,*}

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K99 Pathway to Independence Award