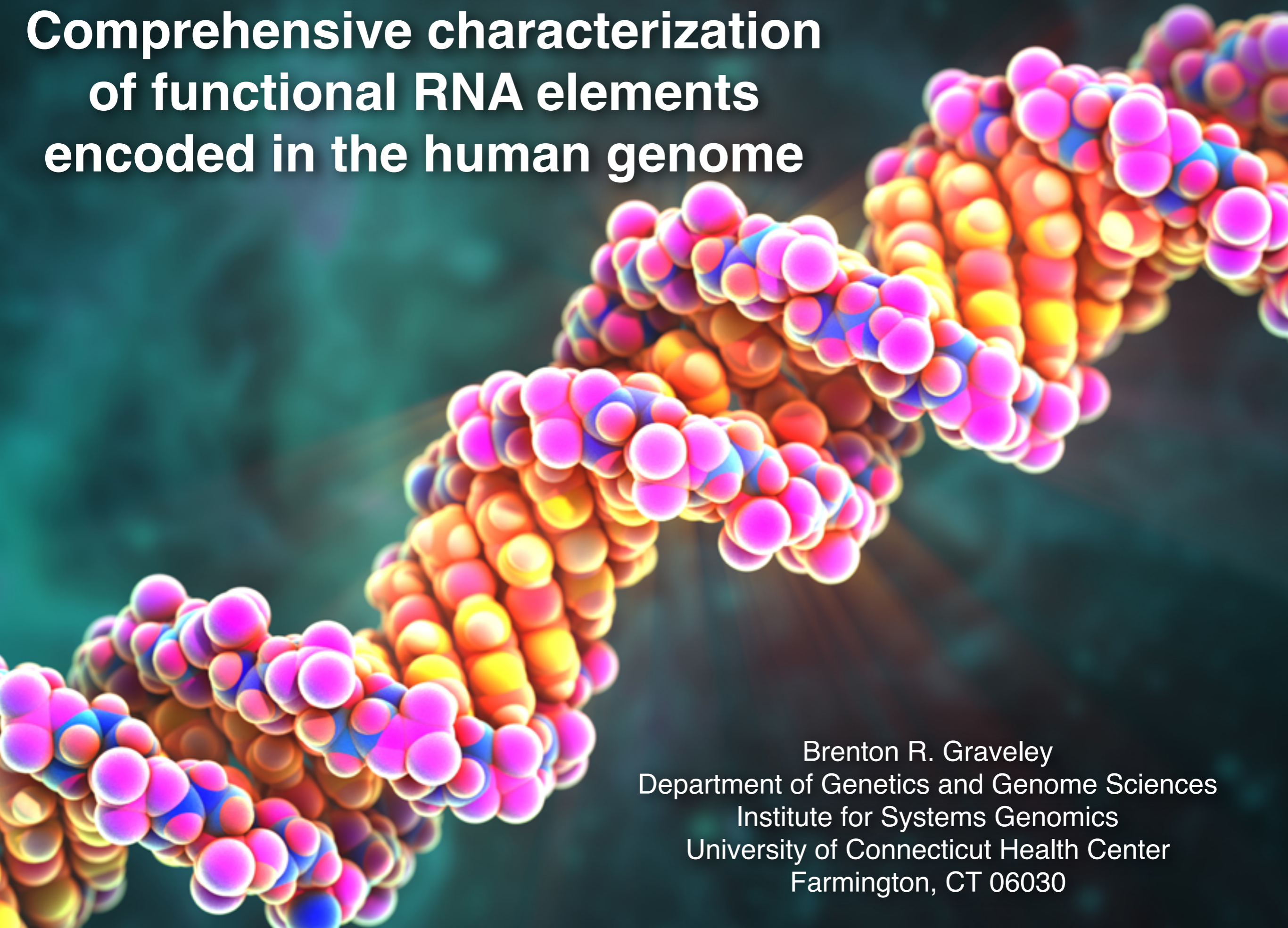
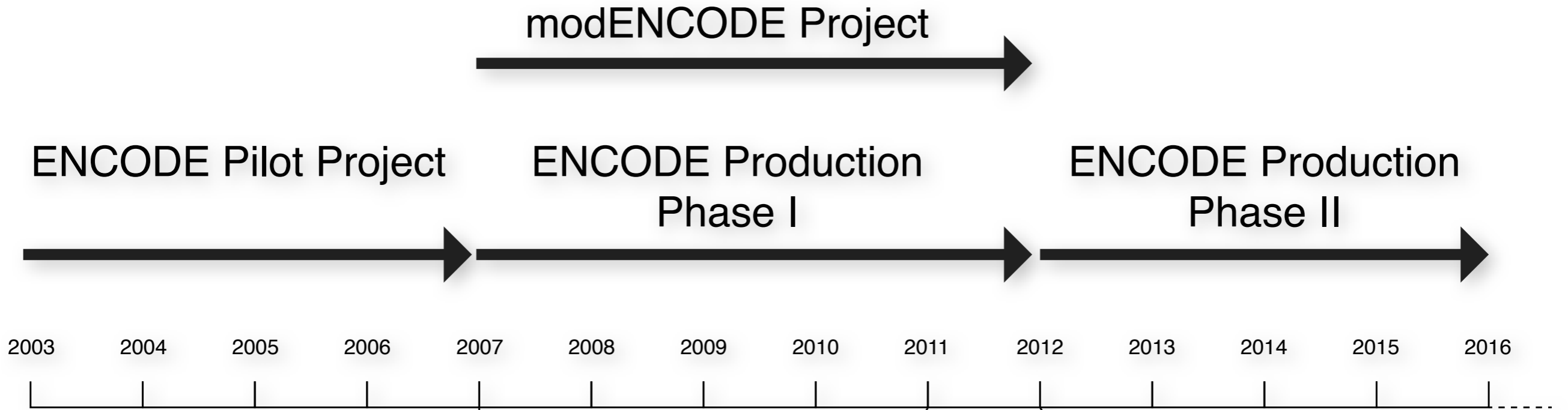


# Comprehensive characterization of functional RNA elements encoded in the human genome



Brenton R. Graveley  
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University of Connecticut Health Center  
Farmington, CT 06030

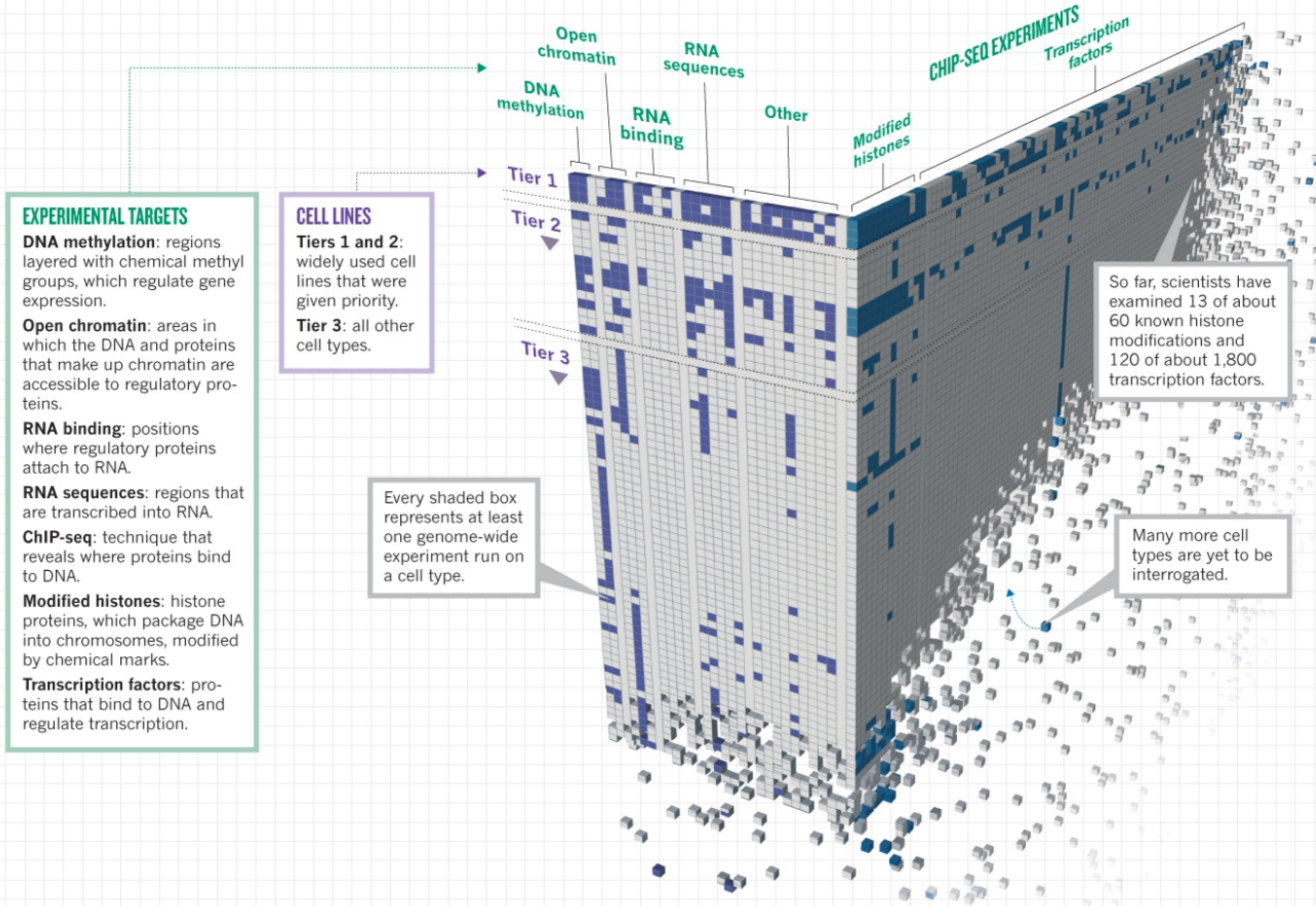
# The ENCODE and modENCODE Projects





# MAKING A GENOME MANUAL

Scientists in the Encyclopedia of DNA Elements Consortium have applied 24 experiment types (across) to more than 150 cell lines (down) to assign functions to as many DNA regions as possible — but the project is still far from complete.





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14



## REPORT

# RNA splicing is a primary link between genetic variation and disease

Yang I. Li<sup>1</sup>, Bryce van de Geijn<sup>2</sup>, Anil Raj<sup>1</sup>, David A. Knowles<sup>3,4</sup>, Allegra A. Petti<sup>5</sup>, David Golan<sup>1</sup>, Yoav Gilad<sup>2,\*</sup>, Jonathan K. Pritchard<sup>1,6,7,\*</sup>

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## RNA splicing links genetics to disease

Many genetic variants associated with disease have no apparent effect on any specific protein coding sequence. Li *et al.* systematically analyzed the effects of DNA variants on the main steps of gene regulation, from the chromatin state through protein function. One-third of expression quantitative trait loci (QTLs) are mediated through transcriptional processes, not chromatin. Splice QTLs and expression QTLs are about comparable in their complex disease risk. Posttranscriptional mechanisms therefore play a large role in translating genotype to phenotype.



## Science

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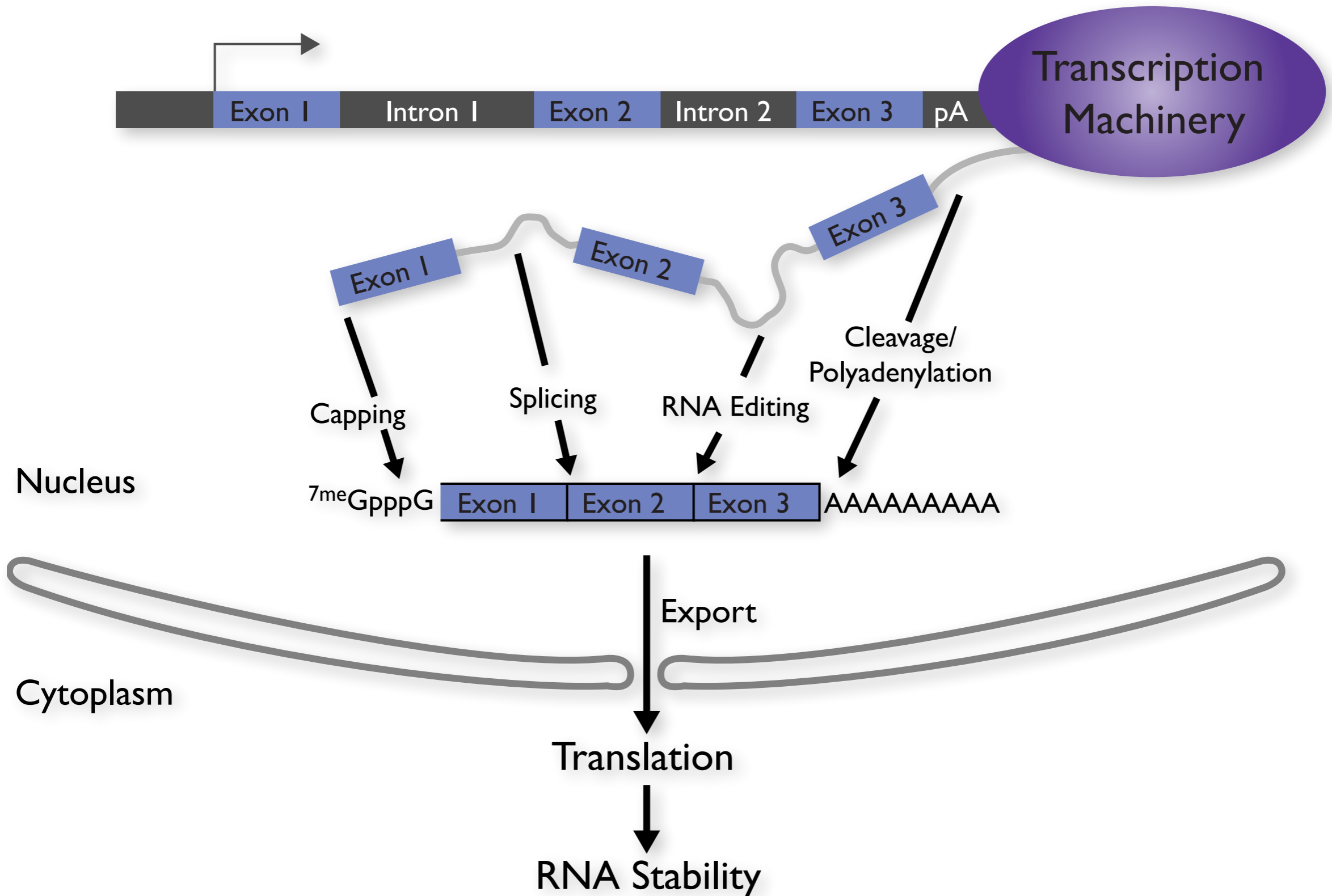
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Howard Hughes Medical Institute, the Bill & Melinda Gates Foundation, the Wellcome Trust, and the Calouste Gulbenkian Foundation announce the International Research Scholars Program which aims to



# Eukaryotic mRNA Synthesis

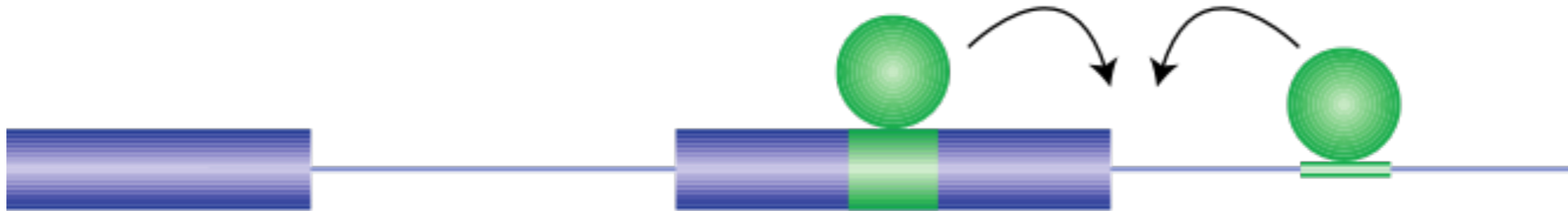


# Functionally Important RNA Elements

Splicing



Polyadenylation



Translation, Localization, or Stability





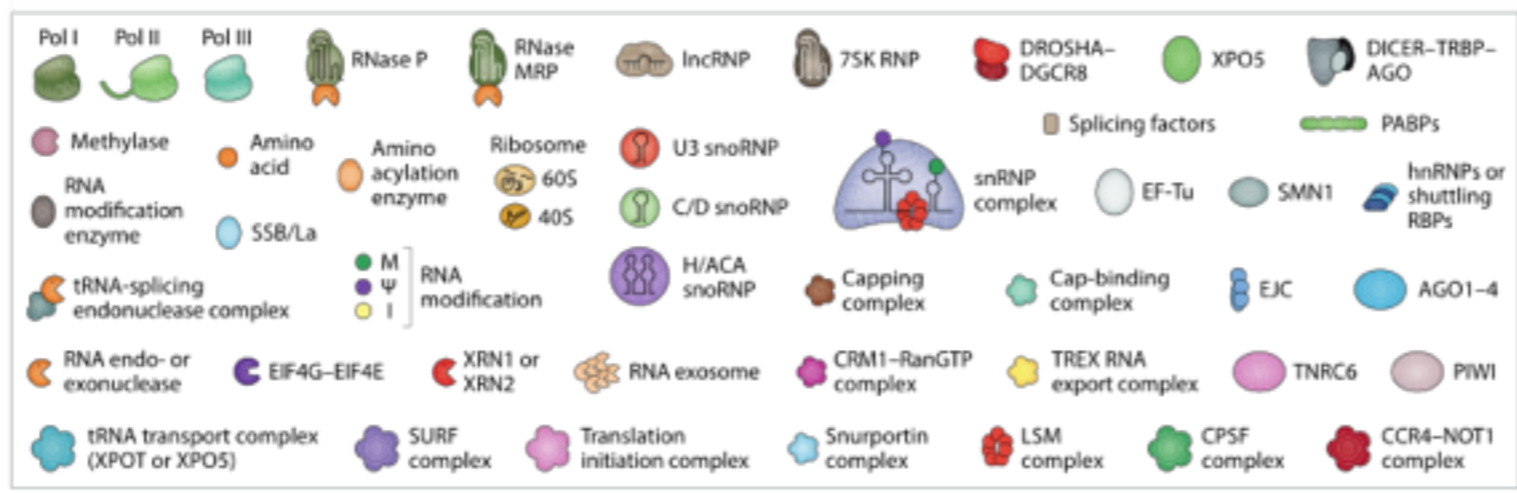
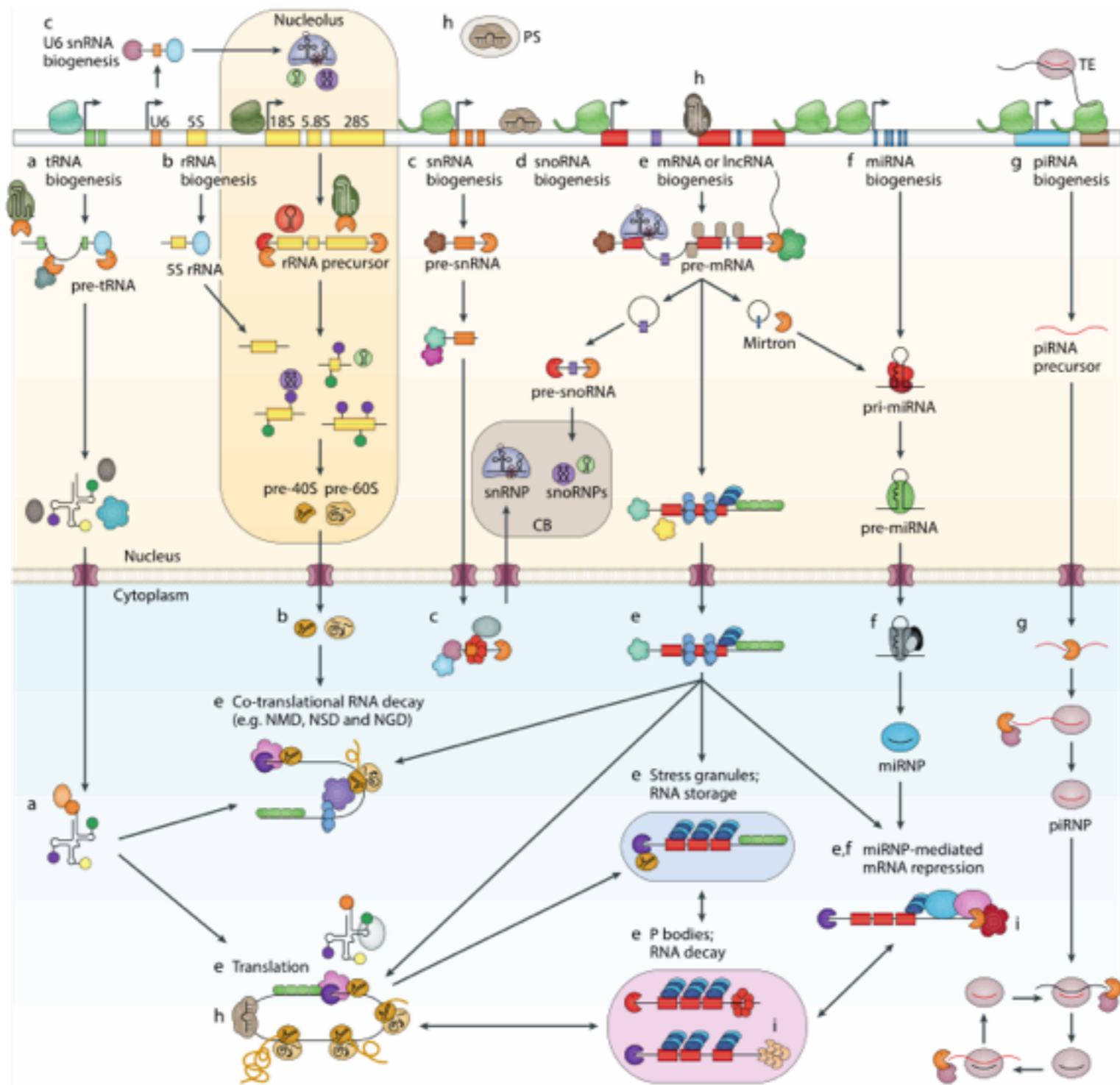




# 1072 “RNA binding proteins”

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BTF3 BZW1 BMS1 DAZ2 DDX1 DUT ENO1 EEF2 FCF1 H1FO KTN1 IFI6 MLL3 NXF3 PLEC PRR3 RALY PUS7 RPL3 RPS6 RRP9 RPL8 SND1 TBL2 TNS1 URB1  
AIMP1 BAT4 CLK3 CSTB DDX17 DIEXF DDX6 EIF3H EIF5B LSM2 LSM4 NOL6 MTDH PNP1 PTRH1 PTPN1 RNF17 RPL10 RPP21 RPL21 SRRT TBL3 STIP1 XRN2 XPO1  
ACIN1 BICC1 CNBP CSDA CSTF1 DDX51 DDX41 ERAL1 EIF3D LSM3 MDH2 LSM11 MTF2 OASL PIWIL1 RBM41 RBM13 RPL22 RPS12 SF3B1 RRP12 UBE2I U2AF1 YARS ZFR  
AARS CELF1 CSD2 DDX31 DDX42 DDX47 DHX16 ESRP1 LSM7 HSPE1 MKI67 MFAP1 KR11 NONOPDIA3 RBM14 RBM15 RPL36 RPL35 RPP25 RTN4 SREK1 SSBP1 TFB1M UBF1  
CCT4 CANX CSRP1 CSDE1 DIAPH1 DPPA5 EIF1AX FTSJ3 LAS1L LSM10 NAT10 NOL10 MSI2 PPRC1 RC3H1 RPL7A RPP30 SF3B2 TCF20 SYNE1 UHMK1 UTP20  
ADAD1 CELF2 CELF4 CPSF6 DDX56 DHX33 DUS2L EIF2C1 LARP7 LTA4H MRPL4 NOP10 NOP14 NOP16 PRPF8 PTC1 RPS24 RPS20 SF3B4 TES TCOF1 TNPO1 UPF3B TFI1  
AICDA CPEB2 CPEB3 CEBPZ DDX43 DDX53 DDX50 DDX59 FKBP3 HABP4 HDLBP HSP88 LAR6 MAZMYEF2 PAPD5 PHF5A RBM22 RBM47 SART1 SF3A2 SRSF9 SRSF8 TDRD7 ZRSR2  
ASCC1 ANXA2 CHERP CDC40 DSP EIF2C2 EIF2C4 DHX58 ESRP2 LARP6 MRPL11 MTPAP PCBP4 PIWIL3 RBM24 RBM42 PTBP1 SF3A3 SRSF4 SURF6 TDRD5 TRA2B ZFP106  
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ADD1 ABT1 DND1 EDF1 FMR1 FXR1 IMP3 LRP1 KRR1 NME1 PES1 PINX1 PHF6 PTRF PSIP1 RPL5 ROD1 RPL7 RPS9 SON SUB1 TIAL1 UPF2 XIRP1 YBX1





# Comprehensive Identification of Functional RNA Elements in the Human Genome

- Identify RNA Elements Recognized by 250 (All) Human RNA Binding Proteins *in vivo*
- Characterize the Binding Affinity of each RNA Binding Protein to all possible RNA Sequences
- Determine the functions of the Protein-RNA Interactions

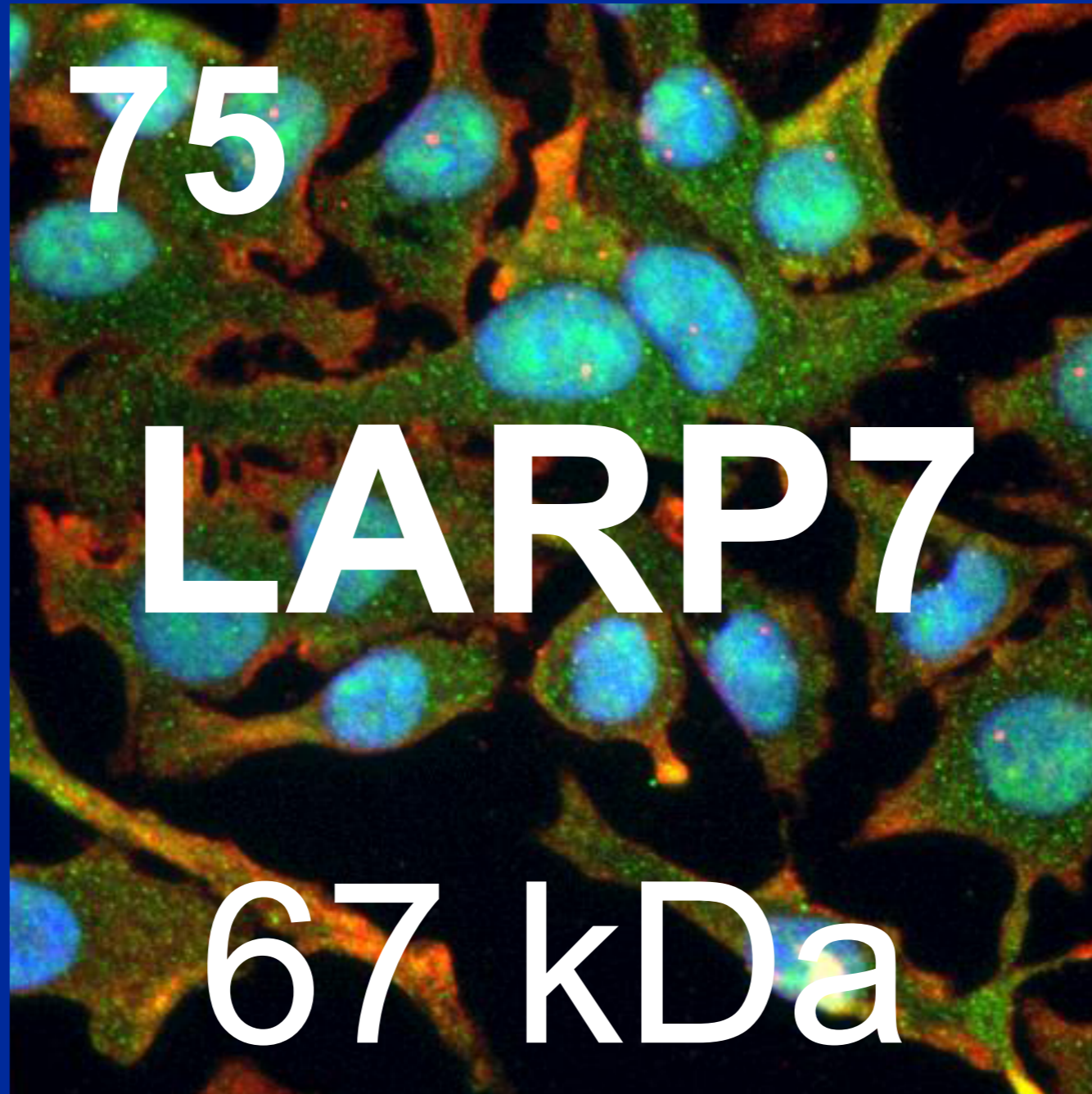




# The Periodic Table of Human RNA Binding Proteins

RNA localization

1 QKI 38 kDa			
3 RBFOX2 41 kDa	4 SMN1 32 kDa		
11 TIAL1 42 kDa	12 TARDBP 45 kDa		
19 PTBP1 58 kDa	20 FUS 53 kDa	21 TRA2A 33 kDa	22 SRSF9 26 kDa
37 RAVER1 64 kDa	38 FXR1 70 kDa	39 SCAF4 126 kDa	40 SRSF7 27 kDa
55 PUM2 114 kDa	56 FXR2 74 kDa	57-71	72 SRSF5 31 kDa
87 PUM1 126 kDa	88 ATXN1 87 kDa	89-103	104 SRSF4 56 kDa
57 DHX3 47 kDa			
89 RPS2 15 kDa			



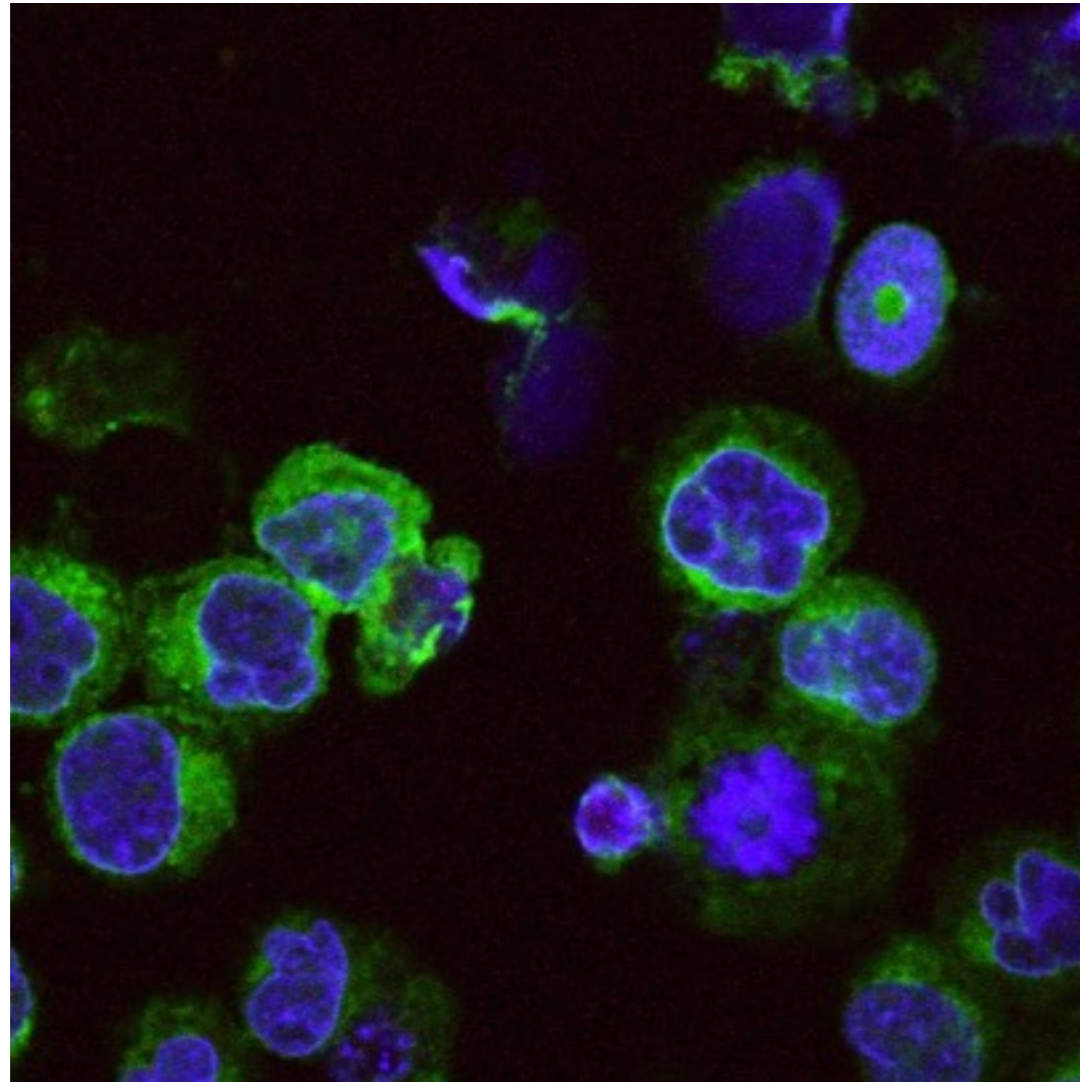
				2 RBM17 45 kDa
4 HNRNPC 34 kDa	8 CSTF50 48 kDa	9 SLBP 31 kDa	10 RBM22 47 kDa	
14 KHDRBS2 39 kDa	16 CPSF7 52 kDa	17 PABPN1 33 kDa	18 RBM5 92 kDa	
33 KHDRBS1 48 kDa	34 CPSF6 59 kDa	35 RBM34 48 kDa	36 RBM25 100 kDa	
51 HNRNPH1 49 kDa	52 CSTF2 61 kDa	53 MTPAP 66 kDa	54 RBM15 107 kDa	
83 EWSR1 68 kDa	84 CSTF2T 64 kDa	85 PABPC4 71 kDa	86 RBM27 119 kDa	
115 HNRNPU 91 kDa	116 FIP1L1 67 kDa	117 CPEB4 80 kDa	118 RBM6 129 kDa	
<ul style="list-style-type: none"> <li><span style="color: magenta;">■</span> DDX/Helicase</li> <li><span style="color: yellow;">■</span> Translation</li> </ul>		<ul style="list-style-type: none"> <li><span style="color: black;">■</span> SRP</li> <li><span style="color: purple;">■</span> Modification</li> </ul>		
67 DDX20 92 kDa	69 DDX24 96 kDa	70 DHX30 134 kDa	71 DHX29 155 kDa	
100 EIF4G2 102 kDa	101 EFTUD2 109 kDa	102 EIF3A 167 kDa	103 EIF4G1 175 kDa	

# What can be done with these data?

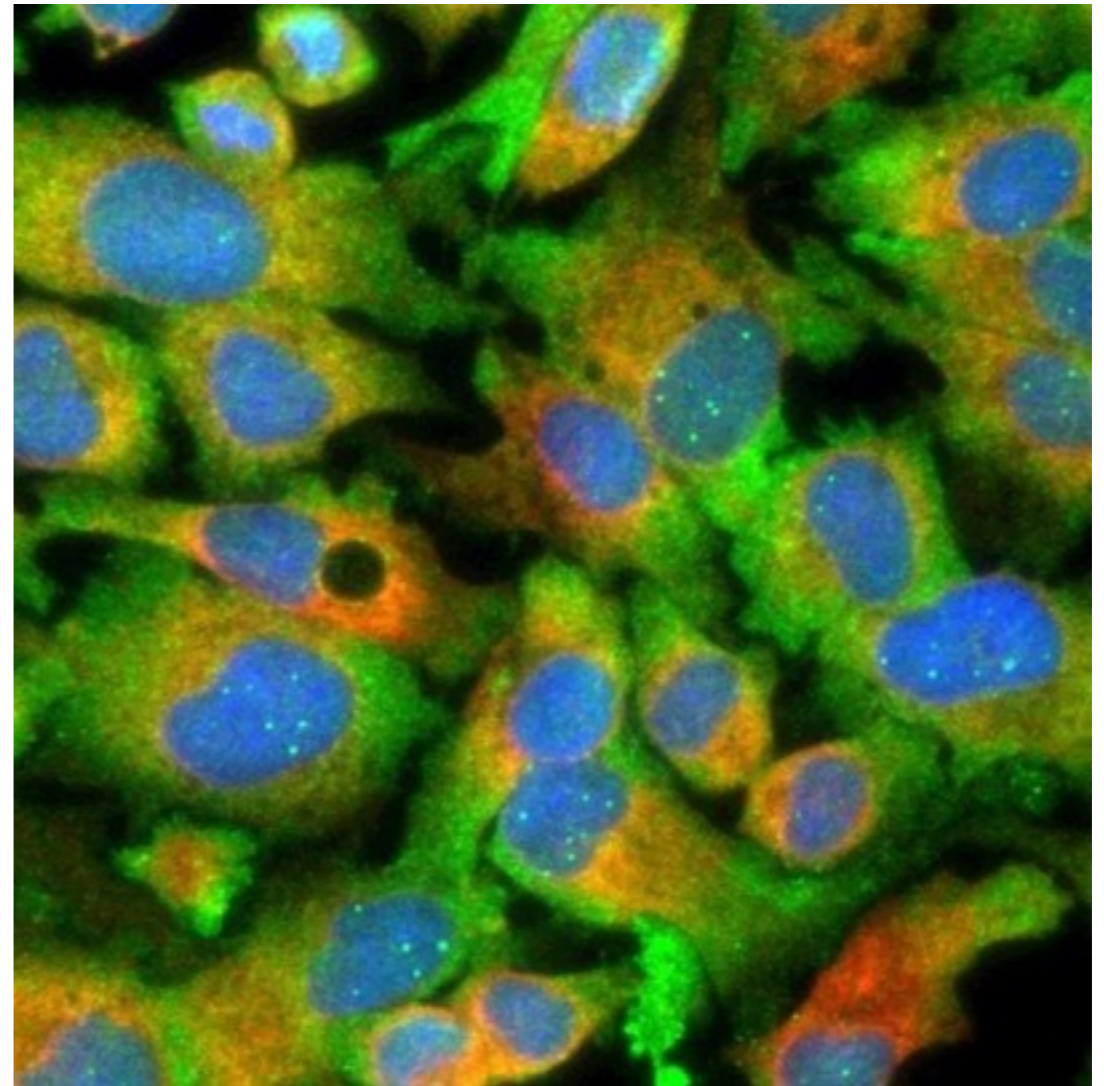
- Identify binding sites for all RBPs
- Determine the function of RBP binding sites
  - Determine RNP composition
  - Predict how mutations will impact RNA processing
  - New insights into RNA biology



# Cell lines being studied



K562 (bone marrow)  
chronic myelogenous leukemia (CML)  
53 year old female



HepG2 (Liver)  
Hepatocellular carcinoma  
15 year old male



# 1,072 “RNA binding proteins”

API5 BOP1 BAT1 DAZ1 DCN EZR FUS ILF2 IFIH1 IFIT2 MSH1 NFX1 NXF2 NXF5 PRR3 RALY PUM1 RDX RPF2 RPL6 RRS1 RTF1 TXN WRN  
BTF3 BZW1 BMS1 DAZ2 DDX1 DUT ENO1 EEF2 FCF1 H1F0 KTN1 IFI6 MLL3 NXF3 PLEC PKM2 PURB RBM7 RPL3 RPS6 RRP9 RPL8 SND1 TBL2 TNS1 URB1  
AIMP1 BAT4 CLK3 CSTB DAZ3 DDX5 EIF4B FIP1L1 EIF4H HFM1 LLPH LYAR PARN NPM3 NSA2 PWP2 RBMT1 RIMS1 RPL17 RPS11 RPS5 TBL3 STIP1 XRN2 XPO1  
ACIN1 BICC1 CNBP CSDA DDX17 DIEXFDDX6 EIF3H EIF5B LSM2 LSM4 NOL6 MTDH PNPT1 PTRH1 PTPN1 RNF17 RPL10 RPP21 RPL21 SRRT SNIP1 UTP15 XPO5 ZYX  
AARS CELF1 CSD2 DDX31 CSTF1 DDX51 DDX41 ERAL1 EIF3D LSM3 MDH2 LSM11 MTIF2 OASL PIWIL1 RBM41 RPL13 RPL22 RPS12 SF3B1 RRP12 TRAP1 U2AF1 YARS ZFR  
CCT4 CANX CSRP1 CSDE1 DDX42 DDX47 DHX16 ESRP1 LSM7 HSPE1 MKI67 MFAP1 KR11 NONOPDIA3 RBM14 RBM15 RPL36 RPL35 RPP25 RTN4 SREK1 SSBP1 TFB1M UBFD1  
ADAD1 CELF2 CELF4 CPSF6 DDX56 DHX33 DUS2L EIF2C1 LARP7 LTA4H MRPL4 NOP10 NOP14 NOP16 PTCD1 RPS24 RPS20 SF3B4 TEST TCOF1 TNPO1 UPF3B TFIPI1  
AICDA CPEB2 CPEB3 DDX43 DDX53 DDX50 DDX59 FKBP3 HABP4 HDLBP LSM10 NAT10 NOL10 MSI2 PPRC1 RC3H1 RPL7A RPP30 SF3B2 TCF20 SYNE1 UHMK1 UTP20  
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ALDH18A1 C10RF77 CCDC55 DDX39A FNDC3A G3BP2 FAM46A GRSF1 HNRNPR LUC7L3 MRPL27 PABPN1 RPS7 RBM8A RPL18A S100A4 SFRS15 TDRD10 ZNF326 ZRANB2  
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AC010724.1 APOBEC4 C3ORF77 DROSHA EIF4G2 GLRX3 HNRNPH3 HNRNPC METAP2 MRPL39 PABPC1 PNLD C INCL RBMY1J RPS15A SETD1A TIA1 SUPT5 HUBAP2L YTHDF3  
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AC004381.6 APOBEC3B C10RF68 CCDC124 FASTKD1 GTF2E2 HSP90AA1 KHDRBS3 LRPPRC PABPN1 POLR3E RBMXL2 PPIE SEC23IP SNRPE1 UNK TNRC6B ZC3H11A  
CNOT1 APOBEC3D C14ORF166 CCDC108 GRN GTF2F1 GPKOW GTPBP4 LSM1 MAGOH NANOS2 PAPOLB PPRC2B PPIG RBPMS2 RPPGR SAMD4A SNRNP40 SUGP2 SRFBP1 ZFP36L2  
AC092037.3 APOBEC3F C7ORF50 FASTKD2 GOLGB1 HNRNPA1L2 LGALS3 KIAA1324 PABPC1 PURA RBFOX2 RECQL4 RPL13A SERPINH1 SPATS2L TNRC6C ZC3HAV1  
AGGF1 ARHGAP1 CCDC9 CDC42EP4 EMG1 FASTKD5 GLTSCR2 HNRNPA2B1 MPHOSPH10 QKI PABPC1L2A POLRMT RALYL RECQL5 SFSWAP SNRPG SYNCRIPT SR1 WBSR16  
ANKHD1-EIF4EBP3 CR759786.10 EBNA1BP2 DAZ1 GPATCH2 IGF2BP2 MAGOH MYBBP1A PAPOLG PRPF38B RECQLRP4-697K14.7 SF1 SLC4A1 APSUCLG JTPD52L2 XXBAC  
CD3EAP APOBEC3GESF1 CR388220.8 DMGDH FLYWCH2 GPATCH4 IGF2BP3 MAGOH3PEIF3L PABPC1L2B PPARGC1A PURG RG9MTD2 RPS19BP1 SNRNP200 TWF2 YWHAQ  
AC008073.5 BOLLAQR CR759761.2 DGCR8 GTPBP1 GPATCH8 HNRNPUL2 LGALS1 NANOS3 PA2G4 PPARGC1B QARS RG9MTD1 SCG3 SECISBP2 SLC25A5 THUMP1 ZGPAT  
ATXN2L APOBEC3A C14ORF156 CCDC137 FBL GANAB GPATCH3 ISG20L2 NA KHDRBS1 PAPOLA POLR2G RBMY1A1 RP11-658F2.1 SLBP SNORD84 TBRG4 TPT1 ZCCHC7  
BCDIN3D APOBEC3C C22ORF28 CCDC137 EXOSC10 GEMIN5 GTPBP10 IGF2BP1 METT10D PABPC5 POLDIP3 RBFOX3 RBMXL3 SSB SLC16A3 SNRNP35 SUPT16H TUT1 ZCCHC11  
CAPRIN1 APOBEC3H C15ORF52 DYNCL1 FASTKD3 GTSF1L HNRNPL1 KIAA0802 MTHFSD PABPC3 PPRC2C SRSF2 RPUSD3 SKIV2L2 SNRNP70 SUPV3L1 TEP1 ZCCHC8  
AC009729.1 C16ORF88 C15ORF52 DYNCL1 FASTKD3 GTSF1L HNRNPL1 KIAA0802 MTHFSD PABPC3 PPRC2C SRSF2 RPUSD3 SKIV2L2 SNRNP70 SUPV3L1 TEP1 ZCCHC8  
DDX28AL844527.1 C17ORF85 C3ORF63 HDGF FAM120C GRWD1 HNRNPA0 KIAA0020 MRPS30 NCBP2L PEG10 RBFOX1 RBMX2 RPUSD2 SMNDC1 SORBS2 TMSB4X ZCCHC7  
AC116353.1 C14ORF93 C10RF131 EIF4G1 FAM120A HIST1H4H HMGB2 HNRNPH2 ILF3 MRPS23 MRPL43 POU5F1 RBM15B RBMY1D RPL35A S100A16 SAMSNI SNRPD2 SUGP1 TRMT2A ZCCHC6  
AC013461.1 C14ORF21 C10RF35 GARI DYNCL1 HIST1H1C HNRNPAB HNRNPM MRPS24 NUSAP1 NUDT21 RBM12B RBMY1B RPL35A SLC3A2 SPATS2 TOP1 ZC3H7B ZCCHC9  
AC011611.1 AMAC1L3 C4ORF14 CORO1A EIF3G GSPT2 HIST1H1B HTATSF1 LRRC59 MRPL37 MKI67IP NOVA2 RBMXL1 RBMY1F SEC61B SETD1B TDRKHT ARBP2 ZCCHC3  
AKAP8L APOBEC1BUD13 COL14A1 DZIP3 DNTTIP2 FAM98A GSPT1 HNRNPK HSPA1B MRPL32 NGRN MRPL41 PSMD4 SRPRXO4 RSL1D1 SNRPA THOC4 TRMT1L YWHAE YTHDF2  
ACAA2 ARL6IP4 CLNS1ACT NNA1 DDX60L ELAVL4 FANCM HNRNPL HNRPLL MRPS31 MRPS11 NOC4L PSMC4 SRPRXO4 RSL1D1 SNRPA THOC4 TRMT1L YWHAE YTHDF2  
DEKATXN1L BCLAF1 DAZAP1 DDX19A GFM1 GNL3 HELQ HEATR1 HRSP12 NOL7 MRPL15 MEPCE NAP1L4 PPHLN1 RBM38 RNPC3 SART3 SNRPB SRRM2 TDRD12 ZC3H15 ZNF598  
AHNAK ASCC3 CASC3 CNOT8 CNOT4 AHNAC ASCC3 CASC3 CNOT8 CNOT4 AHNAC ASCC3 CASC3 CNOT8 CNOT4 AHNAC ASCC3 CASC3 CNOT8 CNOT4 AHNAC ASCC3 CASC3 CNOT8 CNOT4  
ARCN1 ATXN2 C4BPA CWC15 DUSP11 FASTK GNL2 HERC5 KHSRP MATR3 MEX3C NOC2L NHP2L1 PTCD3 PTCD2 RBM23 RCC2 SNRPE SNRPN TRIM25 TRIM56 ZFC3H1  
DCD CELF5 CCAR1 CPEB4 CPNE3 EIF2C3 DICER1 ERCC3 HUWE1 HSPA9 LIN28A MEX3B MRPS5 NCBP2 NOP58 RBM46 PPA NRPS4X SRSF11 STRBP TOP3B TRIM56 ZFC3H1  
ABCF1 CSTF2 DDX49 DDX60 DDX39 DDX58 ELAC2 EIF2S2 ENOX1 SY1 MRPL3 MBNL3 NUP56 NOVA1 PCBP3 RBM26 RBM4B RBMS1 SEC63 SNTB2 TDRD6 TDRD9 ZMAT3  
AKAP1 CELF6 DDX20 DDX46 DDX23 DHX30 DHX36 EIF4A2 FUBP3 HBP1 MBNL2 MOV10 MRPL2 NOLC1 PCBP2 RBM27 RBM45 RAN RRP7A SRSF7 SRSF3 TRUB2 TRA2A UCHL5  
BYSL BCCIP CELF3 CPSF7 DDX25 DDX52 DHX29 DIMIT1L FRG1 FSCN1 LARP4 NAA15 NLRP11 NUP35 RBM18 PUS7L RBM44 RTCD1 SRP54 SRP68 SRSF6 ZC3H4 ZC3H8  
CALR ATXN1 DDX10 DDX27 DDX54 DDX55 DHX57 EEF1A1 FKBP4 HSPB1 MBNL1 NOL12 NOSIP PIWIL4 PRPF6 RBM10 RPS28 RRP36 SRBD1 SRP72 STAU1 USP36 UTP1L  
ADAR CAST CIRBP DDX18 DCP1B DDX24 EIF2S1 EIF4A1 FUBP1 LARP1 KRT18 MRPL1 PARP1 PRDX1 PSCP1 RNPS1 REPIN1 RPL30 RPL23 TPR SRSF1 TRMT1 WDR3 ZCRB1  
AATF CHD3 CRKL DARS DDX21 DHX15 EIF3A FASN KIF1C LSM6 LSM5 NOP2 NMD3 NOL11 PEBP1 RBMX RPL31 RPS21 RPS10 RRP1B SLTMRPS2 TAF15 TRIP6 UTP18  
BRIX1 APEH BST2 CHD2 DHX9 DHX8 EIF3B FDPS FLNA HELZ MAP4 NKRF NOL8 PDIA4 PUM2 RBM6 RBM3 PNN RPL15 RPL14 SPEN TFAM TFRC TUFM ZNF1  
A1CF ASS1 BLM DBR1 DAZ4 DDX4 DKC1 FXR2 HLTF IMMT NHP2 MRM1 NOB1 PKN2 PIL4 RBM4 RBM5 RPS3 RPS8 RRP8 TOE1 SYF2 USO1 UTP3  
ADD1 ABT1 DND1 EDF1 FMR1 FXR1 IMP3 LRP1 KRR1 NME1 PES1 PINX1 PHF6 PTRF PPS1 RPL5 ROD1 RPL7 RPS9 SON SUB1 TIAL1 UPF2 XIRP1 YBX1

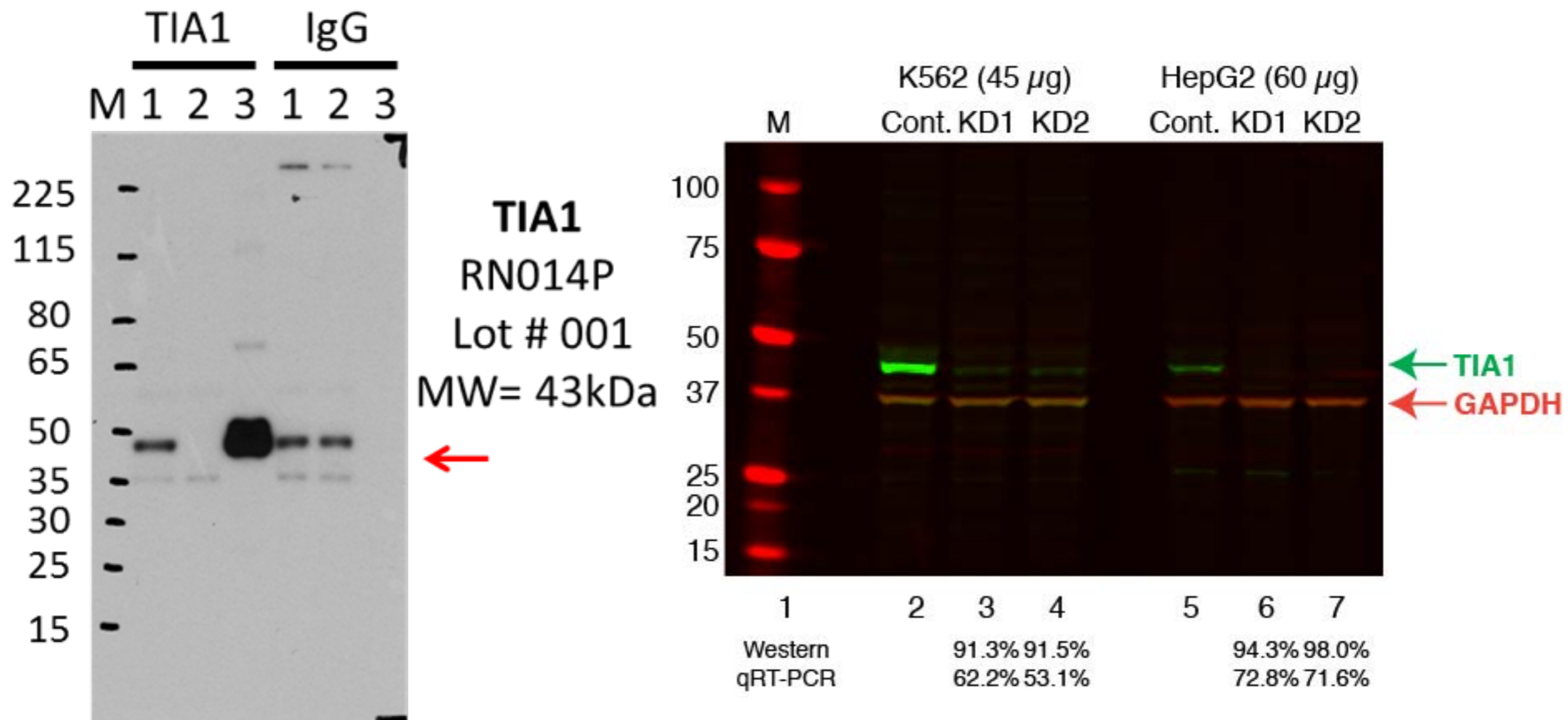






# Antibody Validations

Acquired 852 antibodies and >1,000 shRNAs  
 Tested 701 antibodies against 538 unique RBPs



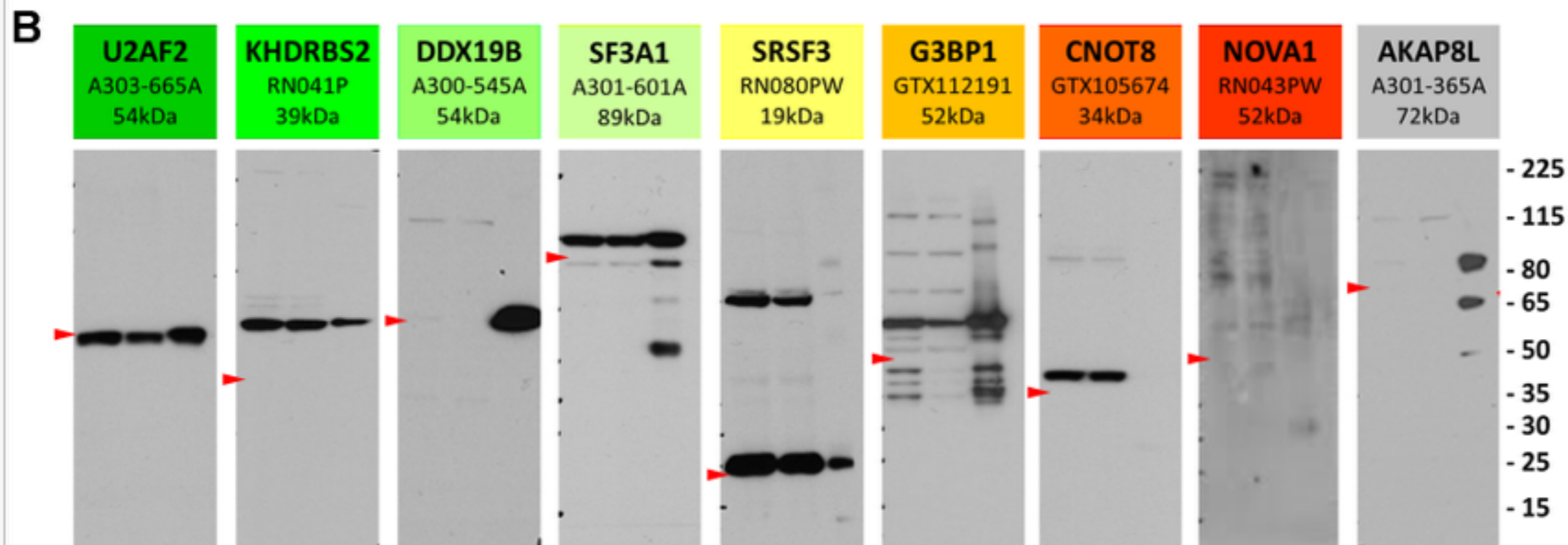
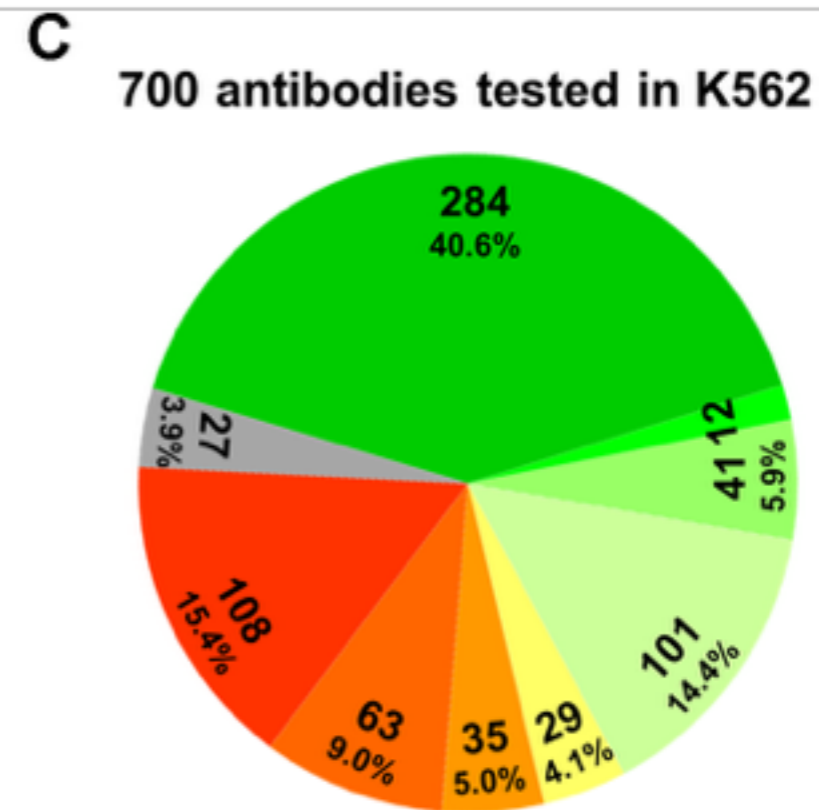
438 antibodies against 385 RBPs  
 362 shRNAs against 276 RBPs



# Antibody Validations

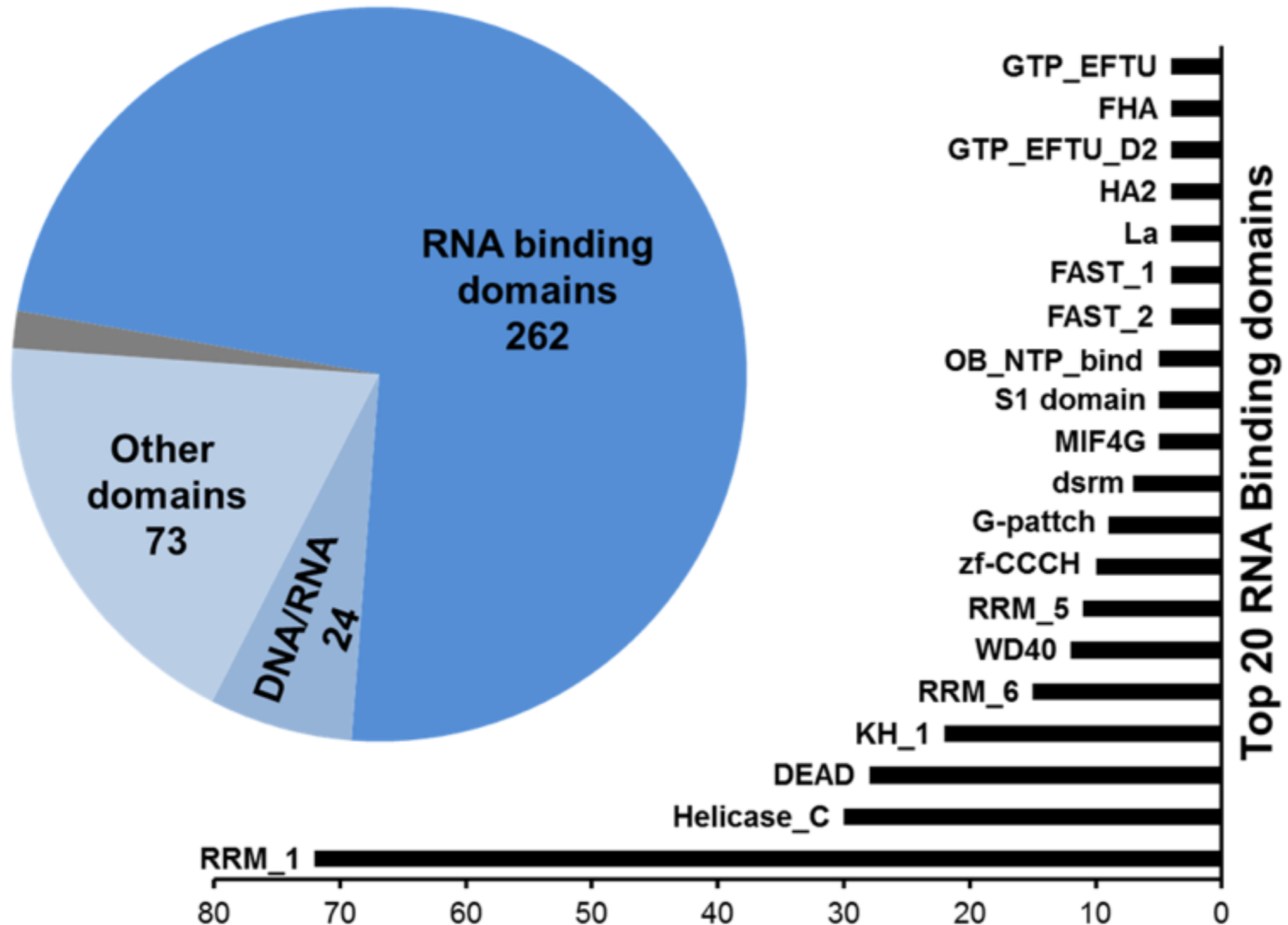
**A**

Score	WB Protein Species Detected	IP Efficiency
1	Single band; MW deviation < $\pm 20\%$	>10x
1MW	Single band; MW deviation > $\pm 20\%$	>10x
1IP	Single band (undetectable in Input)	>>10x
1MB	Multiple bands below MW	>10x
0.5	Single band; MW deviation < $\pm 20\%$	<10x
0.5MB	Multiple bands above MW	>10x
0WB	One or more bands detected in Input	0
0	Undetectable*	Undetectable*
Others	Variable	Variable



# Antibody Validations

Domain analysis of RBPs with IP-grade antibodies





# Antibody Resource

The screenshot shows the ENCODE Antibody Resource interface. The top navigation bar includes 'ENCODE', 'Data', 'Methods', 'About ENCODE', and 'Help'. A search bar labeled 'Search ENCODE' and a 'Sign in' link are on the right. The left sidebar contains filter categories: 'Eligibility status', 'Target Organism', 'Target of antibody', and 'Characterization method'. The main content area displays a list of antibodies with details such as name, source, and product/lot IDs. Annotations include a 'Dropdown menus' box pointing to the navigation bar, a 'Search box' box pointing to the search bar, and a 'Filters' box pointing to the sidebar filters.

**ENCODE** Data Methods About ENCODE Help Search ENCODE Sign in

Showing 25 of 1144 antibodies

**Eligibility status**

- not pursued 509
- awaiting lab characterization 313
- not eligible for new data 264
- eligible for new data 53
- pending dcc review 6

**Target Organism**

- Homo sapiens 1055
- Mus musculus 113
- Caenorhabditis elegans 38
- Drosophila melanogaster 19
- Aequorea victoria 1

**Target of antibody**

- transcription factor 590
- RNA binding protein 345
- histone 200
- histone modification 192
- control 13

**Characterization method**

- immunoprecipitation 546
- immunoblot 500

**IGF2BP3 (*Homo sapiens*)** ●  
Source: MBLI  
Product ID / Lot ID: RN009) / 002

**EZH2 (*Homo sapiens*)** ●  
Source: Cell Signaling  
Product ID / Lot ID: 07-449 / 2120130

**CHD4 (*Homo sapiens*)** ●  
Source: Abcam  
Product ID / Lot ID: ab70469 / GR104037-5

**IGF2BP2 (*Homo sapiens*)** ●  
Source: MBLI  
Product ID / Lot ID: RN008P / 002

**H3K36me3 (*Mus musculus*)** ●  
Source: Abcam

View All

Antibody ENCAB934MDN

Antibody ENCAB913HCF

Antibody ENCAB036YAO

Antibody ENCAB062MMV

Antibody ENCAB597TIV

Antibody ENCAB592DBV

Dropdown menus

Search box

Filters

# Antibody Resource

ENCODE Data Methods About ENCODE Help Search ENCODE Sign in

**ENCAB934MDN** ← Accession ID


Antibody against *Homo sapiens* IGF2BP3

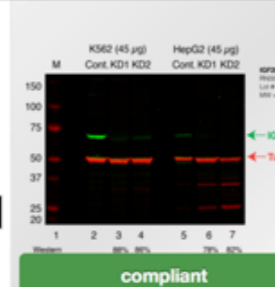
*Homo sapiens* K562 Antibody status → Eligible for new data ●

Source (vendor): MBLI  
 Product ID: RN009  
 Lot ID: 002  
 Targets: IGF2BP3 (*Homo sapiens*)  
 Host: Rabbit  
 Clonality: Polyclonal  
 Isotype: IgA  
 Antigen description: KLH-conjugated synthetic peptide HQQQKALQSGF

Meta data

Characterization status

**IGF2BP3 (*Homo sapiens*)**  
 Method: immunoprecipitation  
 Caption excerpt: IP-Western Blot analysis of HepG2 whole cell lysate using IGF2BP3 specific antibody. Lane 1 is 1% of twenty million whole cell lysate input and lane 2 is 25% of IP enrichment using rabbit normal IgG...  
  
 compliant

**IGF2BP3 (*Homo sapiens*)**  
 Method: knockdown or knockout  
 Caption excerpt: Western blot following shRNA against IGF2BP3 in K562 and HepG2 whole cell lysate using IGF2BP3 specific antibody. Lane 1 is a ladder, lane 2 is K562 non-targeting control knockdown, lane 3 and 4 are...  
  
 compliant

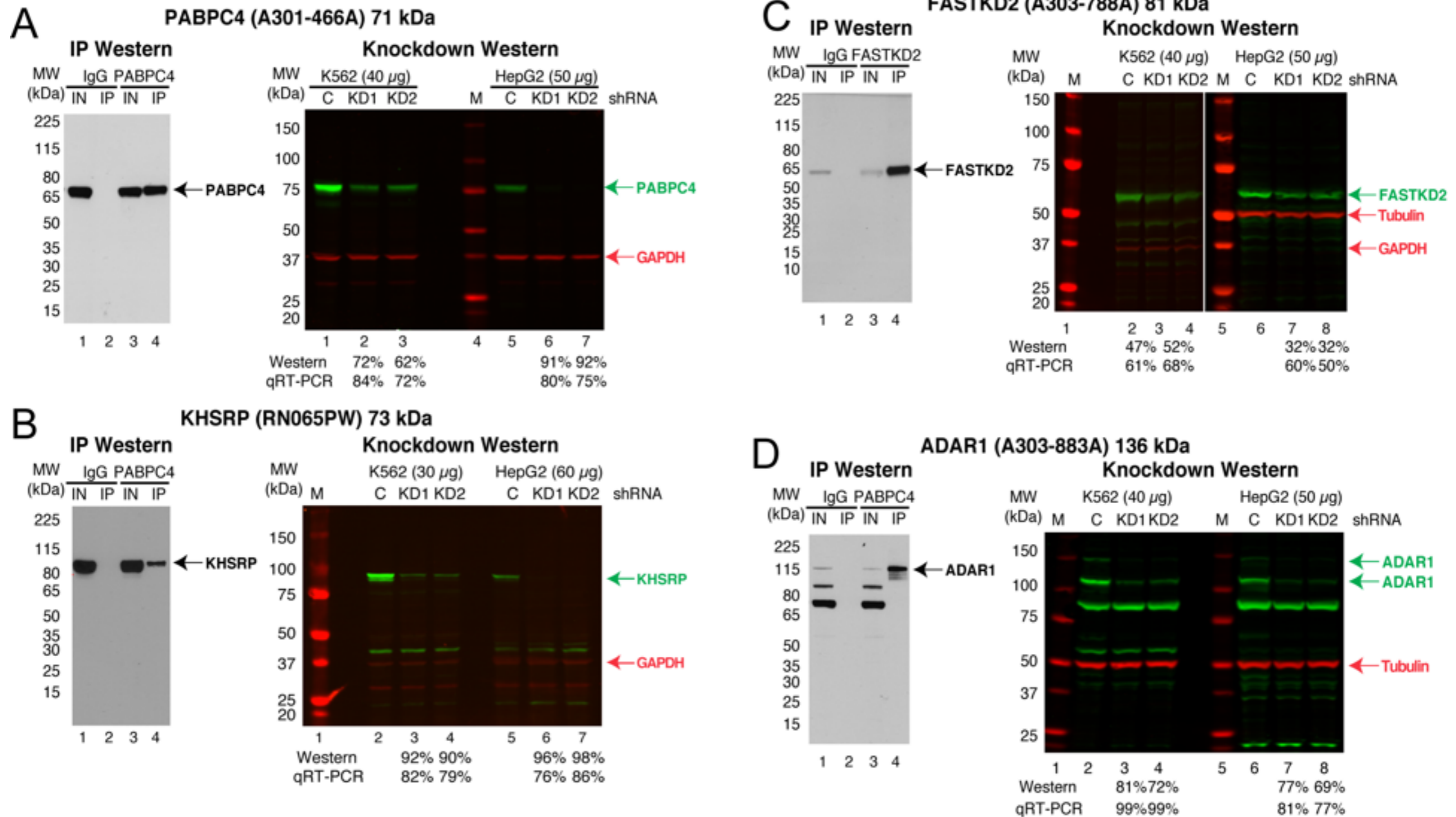
Experiments using antibody ENCAB934MDN

Accession	Assay	Biosample term name	Target	Description	Lab
<a href="#">ENCSR077KVG</a>	eCLIP	HepG2	IGF2BP3 eCLIP mock input	eCLIP control experiment on HepG2 against IGF2BP3	Gene Yeo, UCSD
<a href="#">ENCSR993OLA</a>	eCLIP	HepG2	IGF2BP3	eCLIP experiment on HepG2 against IGF2BP3	Gene Yeo, UCSD
<a href="#">ENCSR096IJV</a>	iCLIP	K562	IGF2BP3		Gene Yeo, UCSD

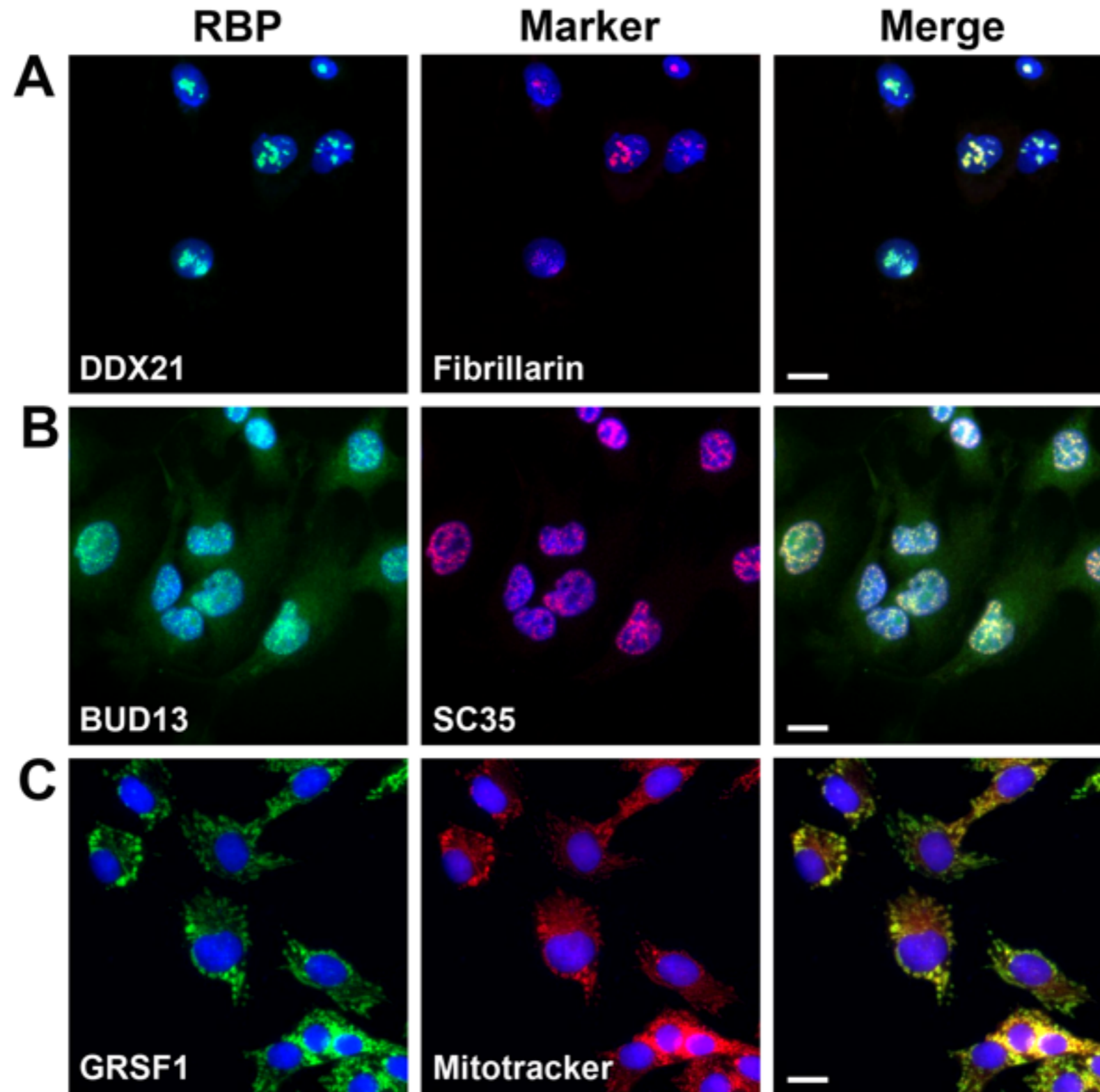
Links to CLIP experiments



# Antibody and shRNA Resources



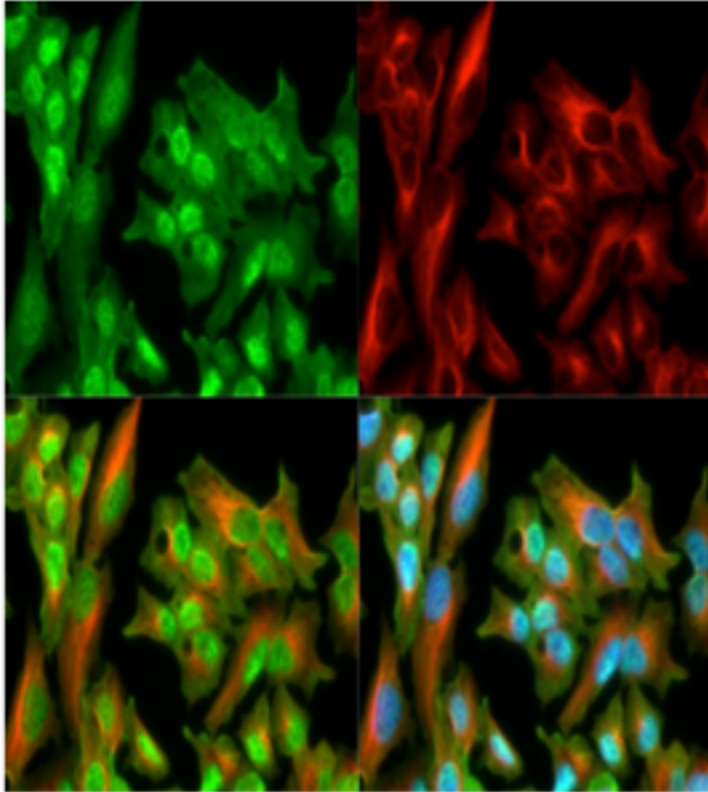
# Protein Localization Studies





# Accessing ENCODE RBP Data

## RBP Image Database



## About the database

This microscopy image database documents the results of immuno-fluorescence (IF) experiments to characterize the subcellular localization properties of human RNA binding proteins (RBPs) studied in the context of the ENCODE team grant entitled "Comprehensive analysis of function RNA elements encoded in the human genome", led by Dr. Brenton Graveley at the UConn Health Center. These studies are being conducted using several human model cell lines prioritized by the ENCODE consortium (e.g. HepG2, MCF7 and HeLa) and validated commercial antibodies targeting over 250 distinct RBPs. The database displays images resulting from co-labeling experiments of individual RBPs in conjunction with a panel of cellular markers for various organelles and subcellular structures. The data can be accessed via different search options, either by searching for individual RBPs or in batch display formats. Antibody validation was performed in the lab of Dr. Gene Yeo at UCSD, while all IF experiments and analyses are being conducted in the laboratory of Dr. Eric Lécuyer at the IRCM in Montreal.

Kindly note that this website is still under active construction. Not all functions are implemented or yet fully functional. Thank you for your comprehension.

### Search by gene symbols

Select a Cell Line

Select a Target

Search

### Search by Annotation

Under construction

Search

### Browse by cell lines

HepG2 253 genes

MCF7 22 genes

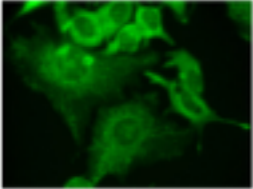
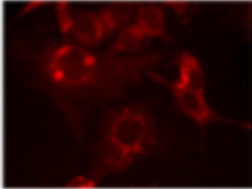
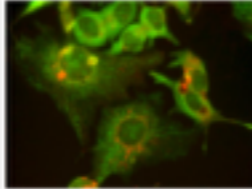
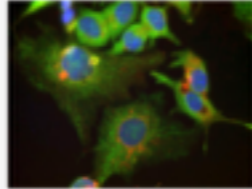
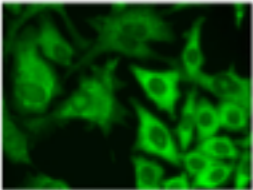
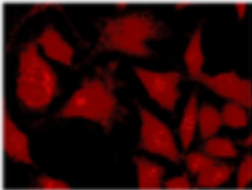
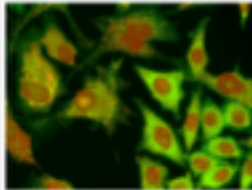
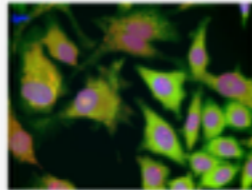
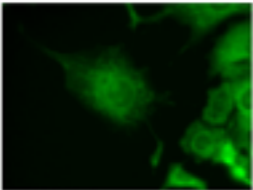
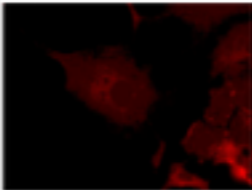
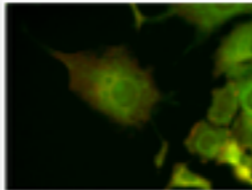
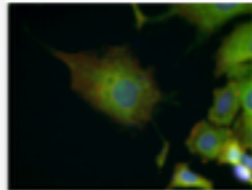
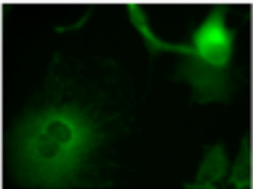
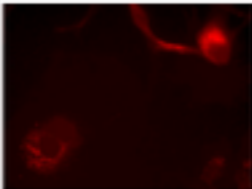
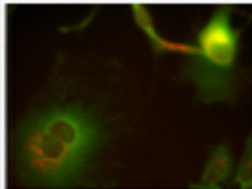
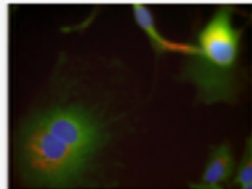
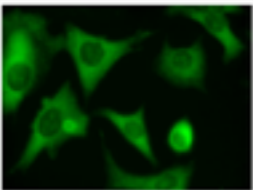
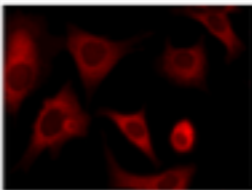
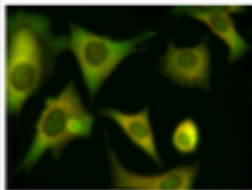
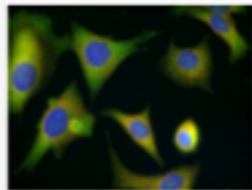
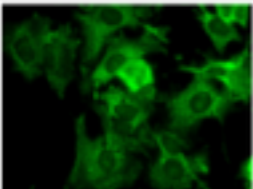
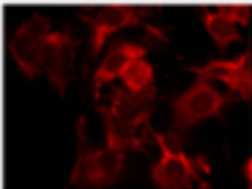
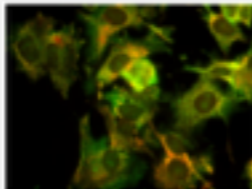
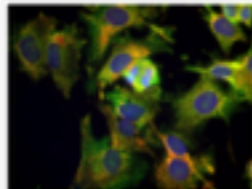
Browse

This database was developed and is maintained by the Lecuyer Lab. Please [email](#) us for any comments, suggestions or bug reports.

<http://rnabiology.ircm.qc.ca/RBPImage/>

# Accessing ENCODE RBP Data

## Gene sets

	AARS	Marker	Co-labelled	Co-labelled with DAPI	Comments
Endosomal Network (anti-CD63)					
Cajal Bodies (anti-Coilin)					
P-Bodies (anti-DCP1a)					
Golgi Apparatus (anti-GM130)					
Endoplasmic Reticulum (anti-KDEL)					
Mitochondria (Mitotracker)					

**Annotation**

Quality: Good  
Exposure time:  
Molecular weight:  
Product:  
Catalogue:  
Notes :  
HepG2  
Marker Co-Localized  
Nuclei  
Nucleolus  
Cytoplasm  
Compartment/Organelle  
Mitochondria  
Cytosolic  
Unidentified  
Cytoplasm (UN)  
Cyto Foci (UN)  
Nuclei (UN)  
Nuci Foci (UN)  
**Additional comments:**  
Mainly cytosolic; though not always as prominent in the mitochondria and nucleoli, it is consistently observed in all the images. .

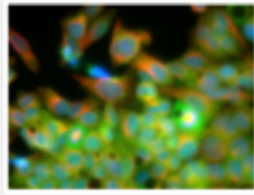
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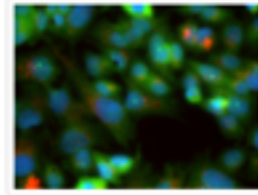
# Accessing ENCODE RBP Data

## ACO1

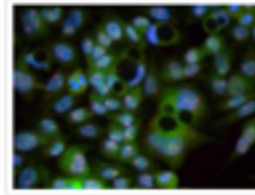
Microtubules  
(anti- $\alpha$ Tubulin)



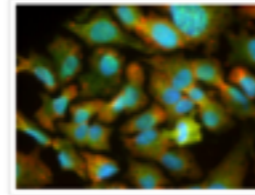
Endosomal Network  
(anti-CD63)



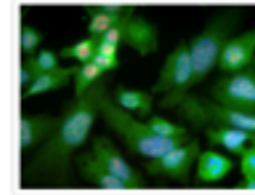
Cajal Bodies  
(anti-Coilin)



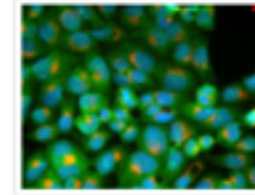
P-Bodies  
(anti-DCP1a)



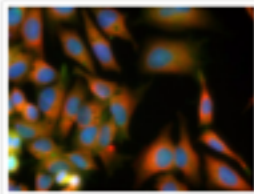
Nucleoli  
(anti-Fibrillarin)



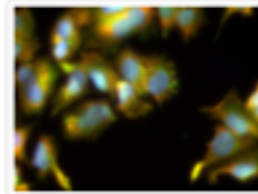
Golgi Apparatus  
(anti-GM130)



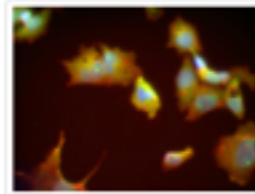
Endoplasmic Reticulum  
(anti-KDEL)



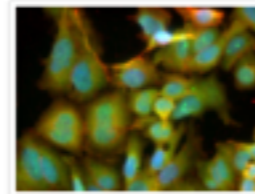
Mitochondria  
(Mitotracker)



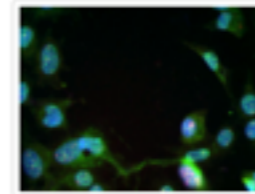
Filamentous Actin  
(Phalloidin)



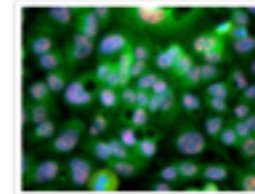
Cell Cortex and Focal  
Adhesions  
(anti-PhosphoTyrosine)



PML Nuclear Bodies  
(anti-PML)

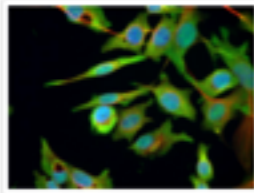


Nuclear Speckles  
(anti-SC35)

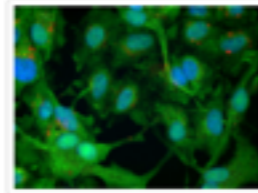


## ADAR

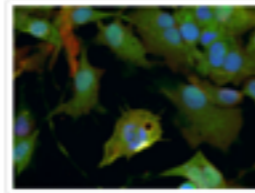
Microtubules  
(anti- $\alpha$ Tubulin)



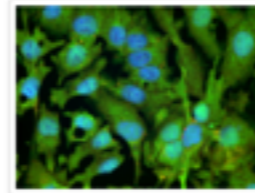
Endosomal Network  
(anti-CD63)



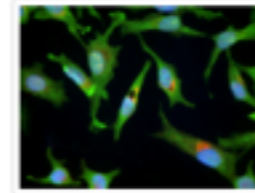
Cajal Bodies  
(anti-Coilin)



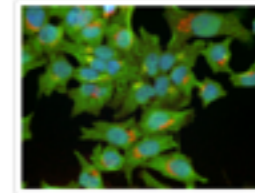
P-Bodies  
(anti-DCP1a)



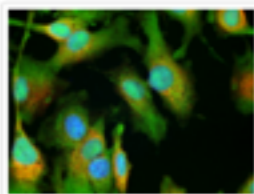
Nucleoli  
(anti-Fibrillarin)



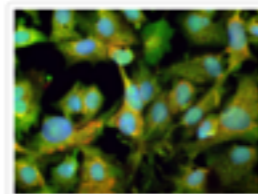
Golgi Apparatus  
(anti-GM130)



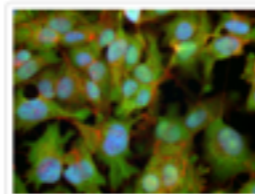
Endoplasmic Reticulum  
(anti-KDEL)



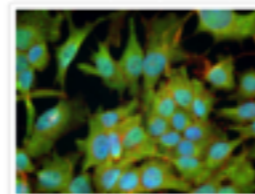
Mitochondria  
(Mitotracker)



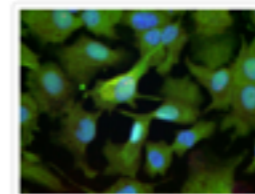
Filamentous Actin  
(Phalloidin)



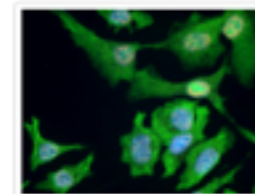
Cell Cortex and Focal  
Adhesions  
(anti-PhosphoTyrosine)



PML Nuclear Bodies  
(anti-PML)

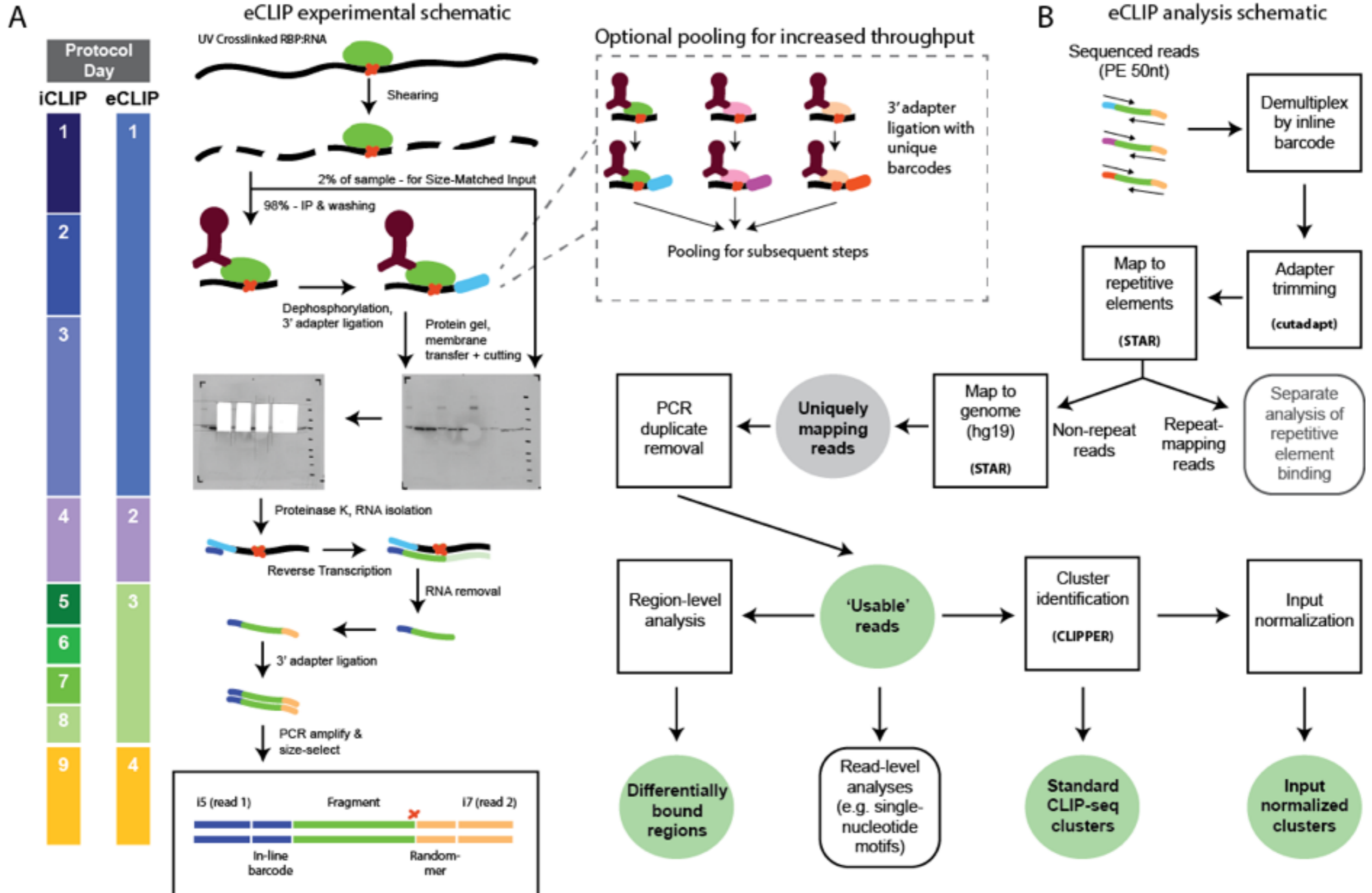


Nuclear Speckles  
(anti-SC35)



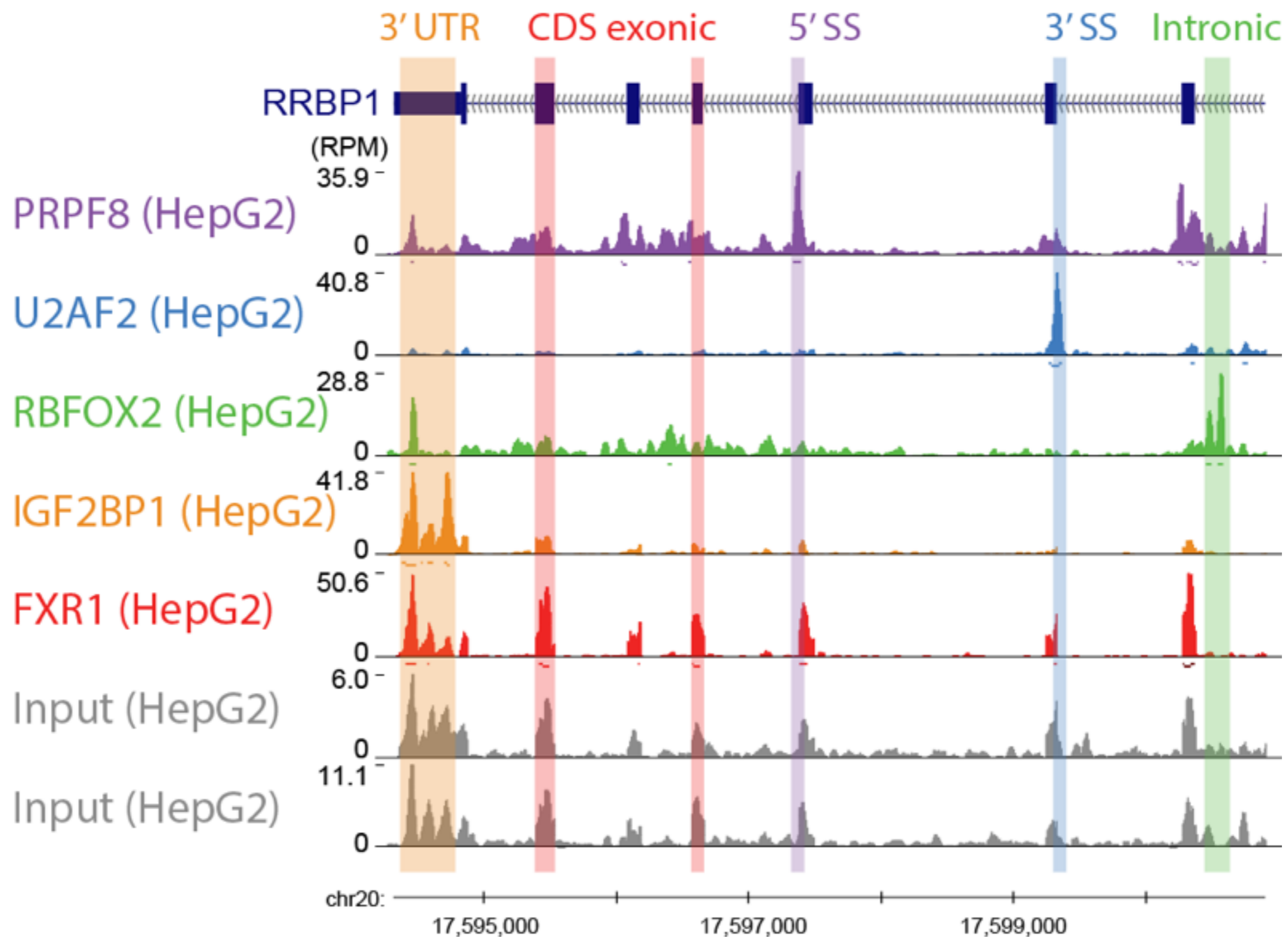
<http://rnabiology.ircm.qc.ca/RBPImage/>

# The eCLIP Protocol and Analysis Pipeline

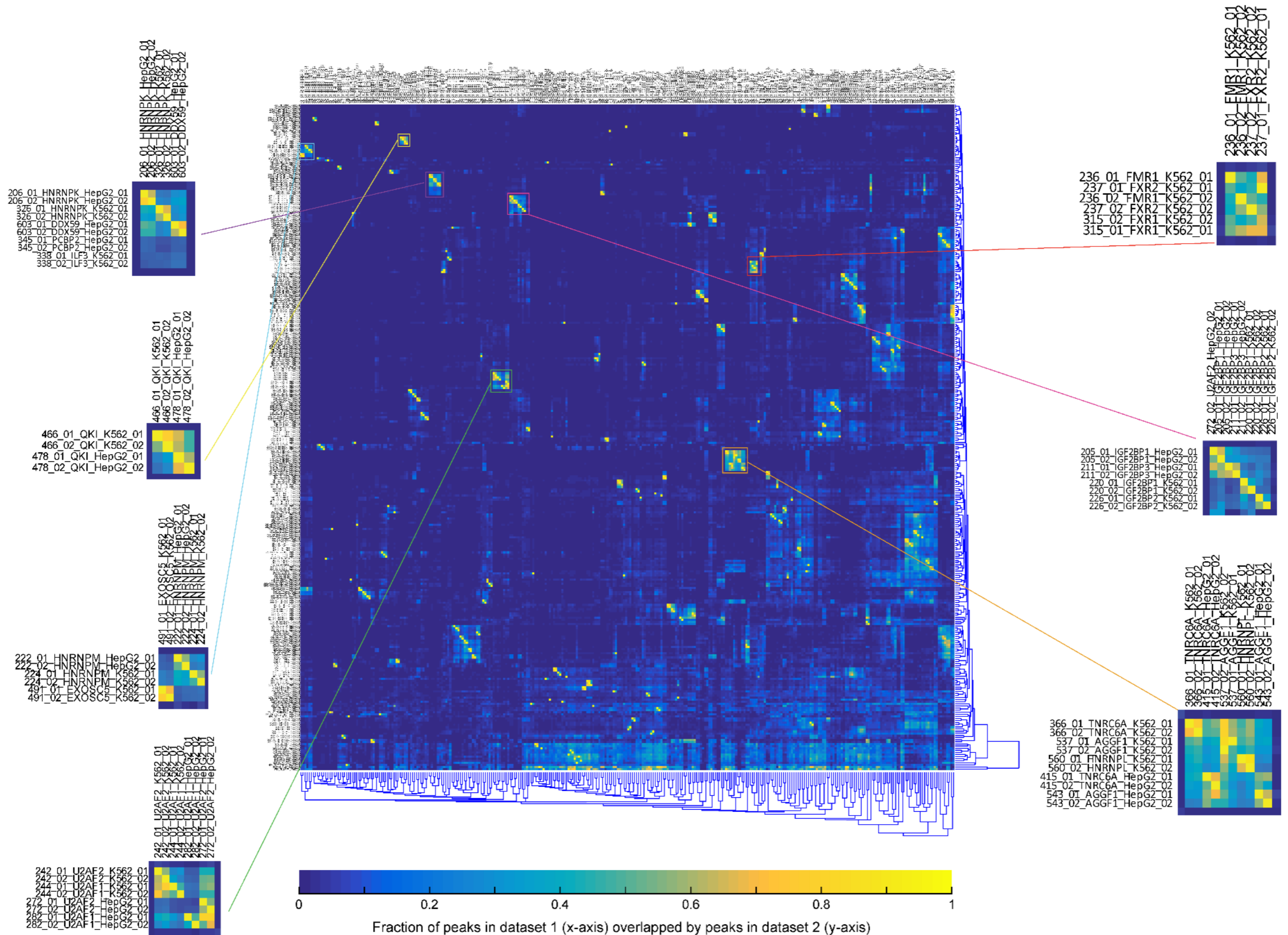




# eCLIP-seq reveals RBP-specific binding profiles

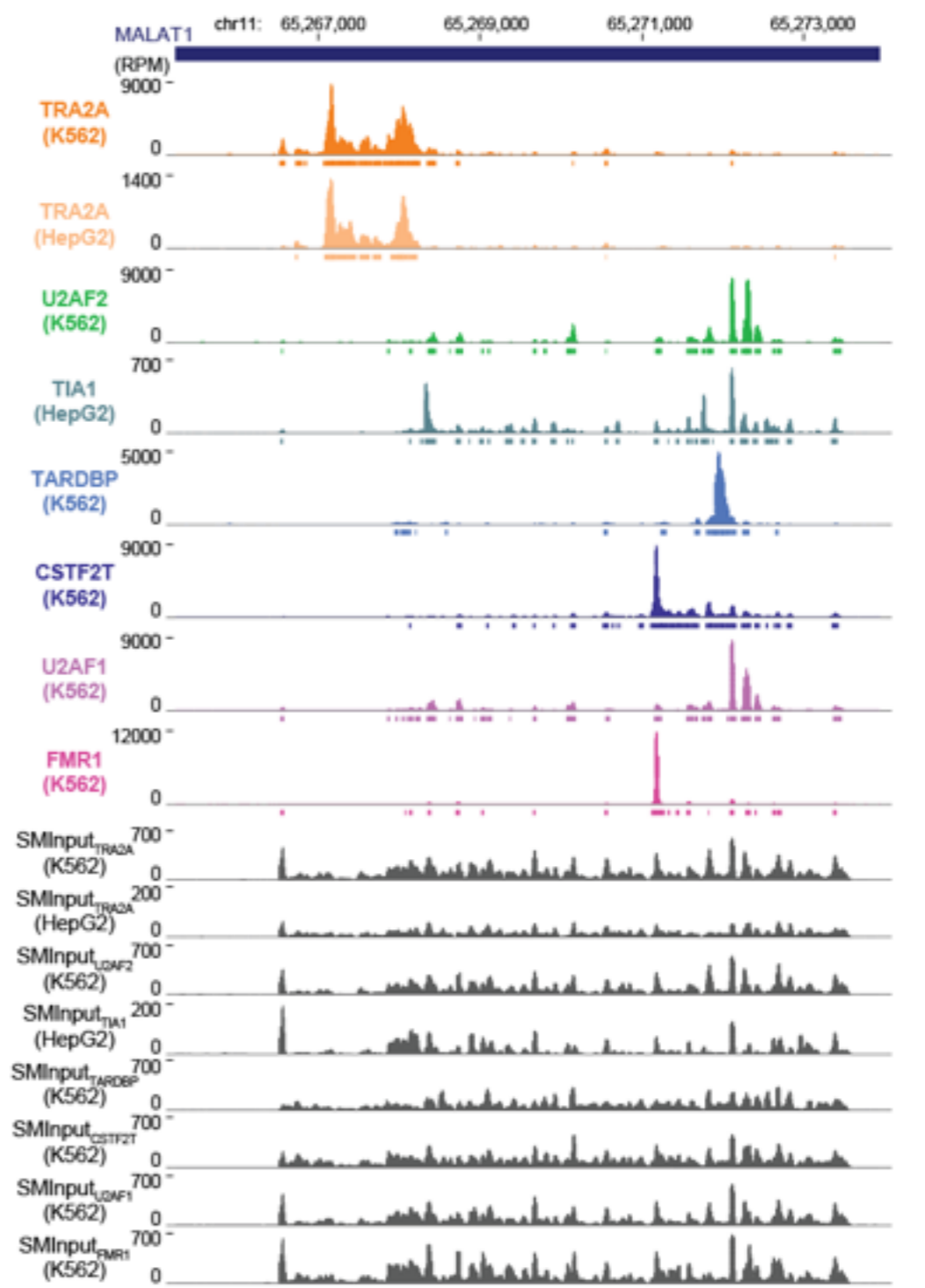
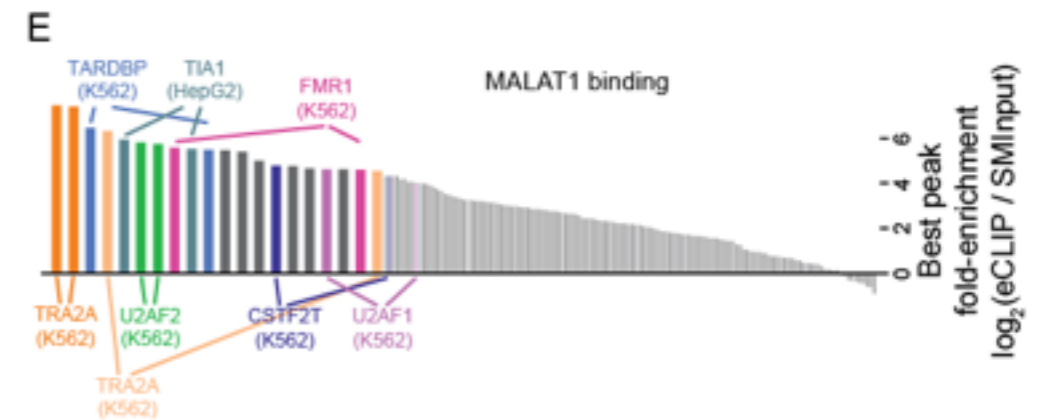
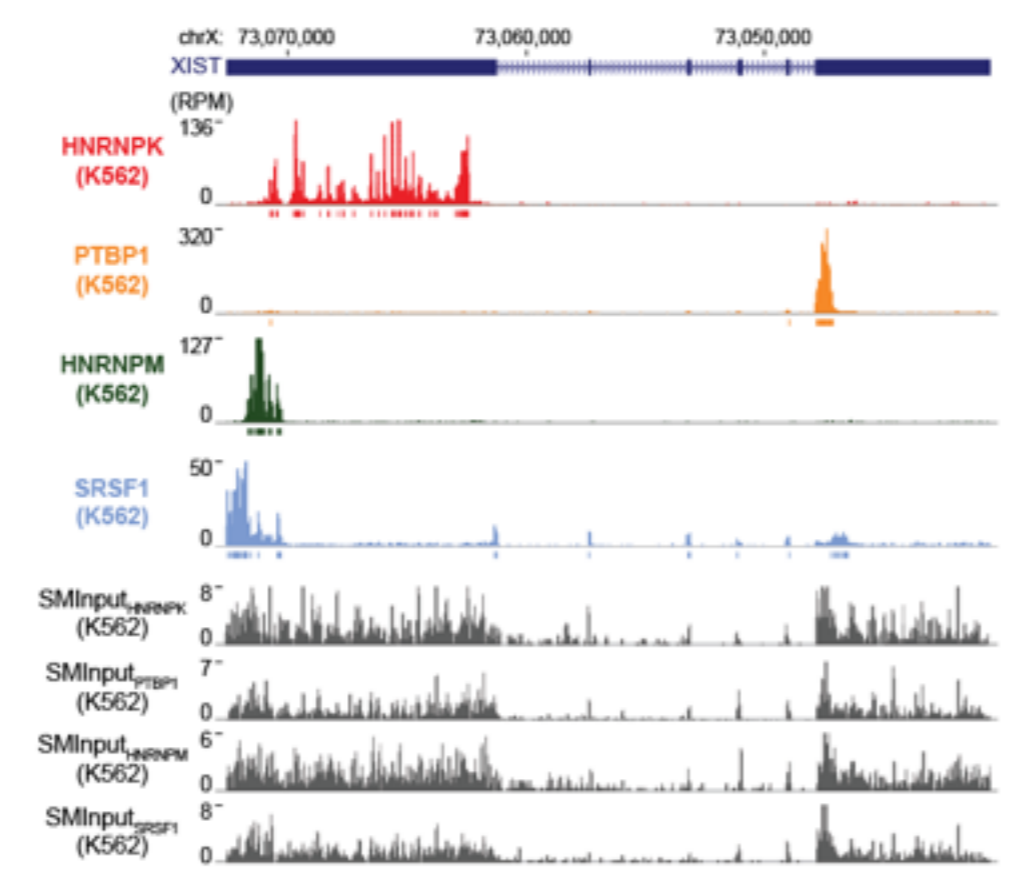
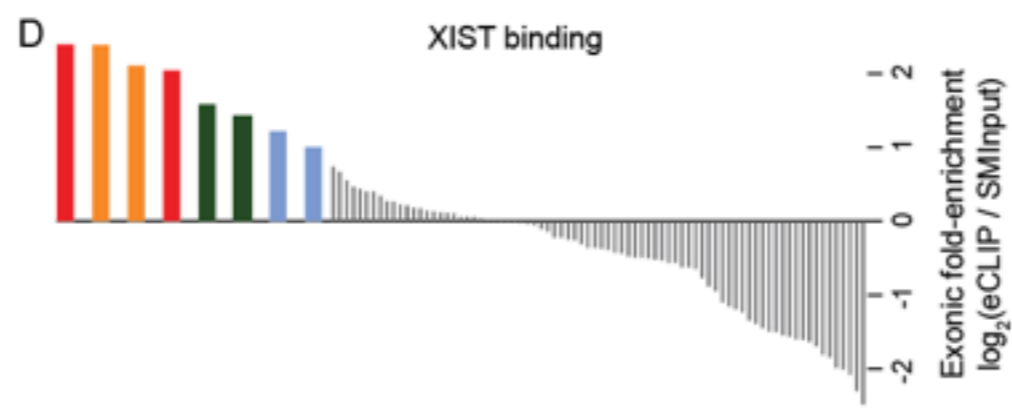
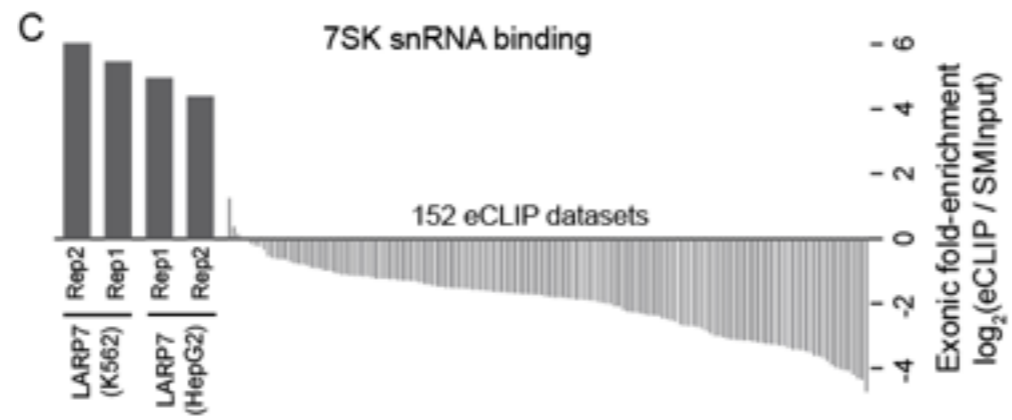


# eCLIP-seq Identifies Co-Associated Proteins

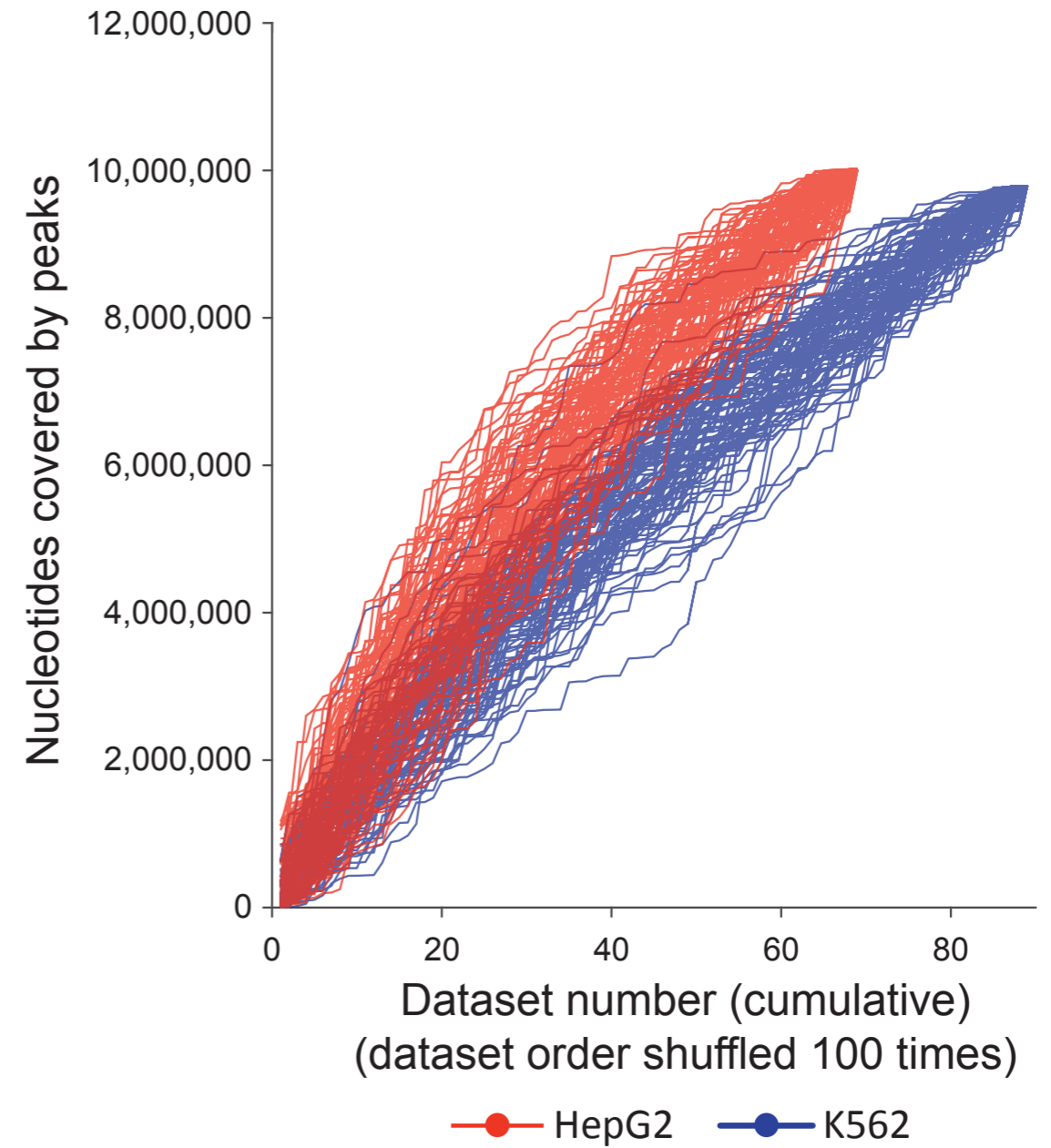
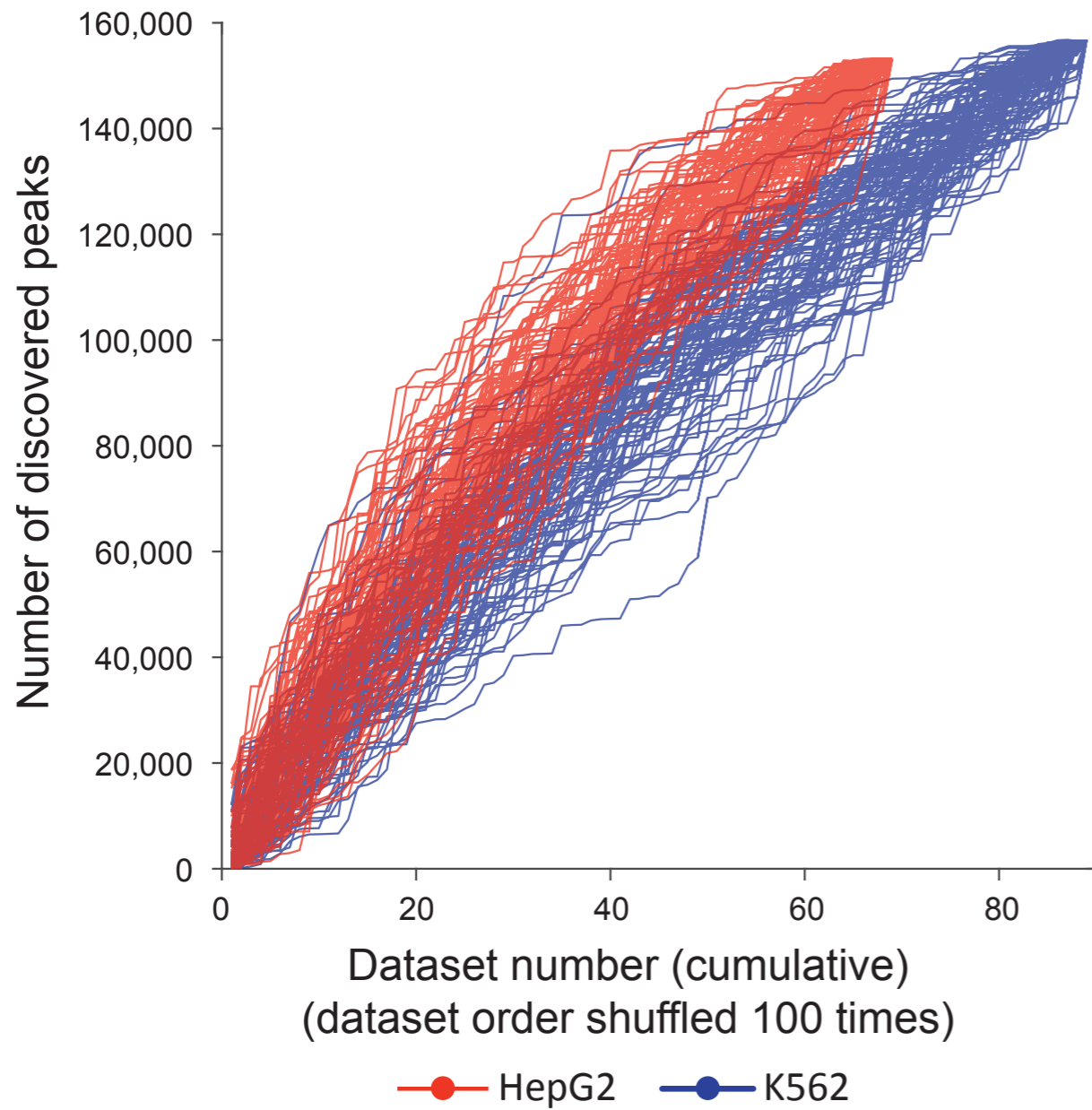




An “RNA-centric” view of RBP-binding

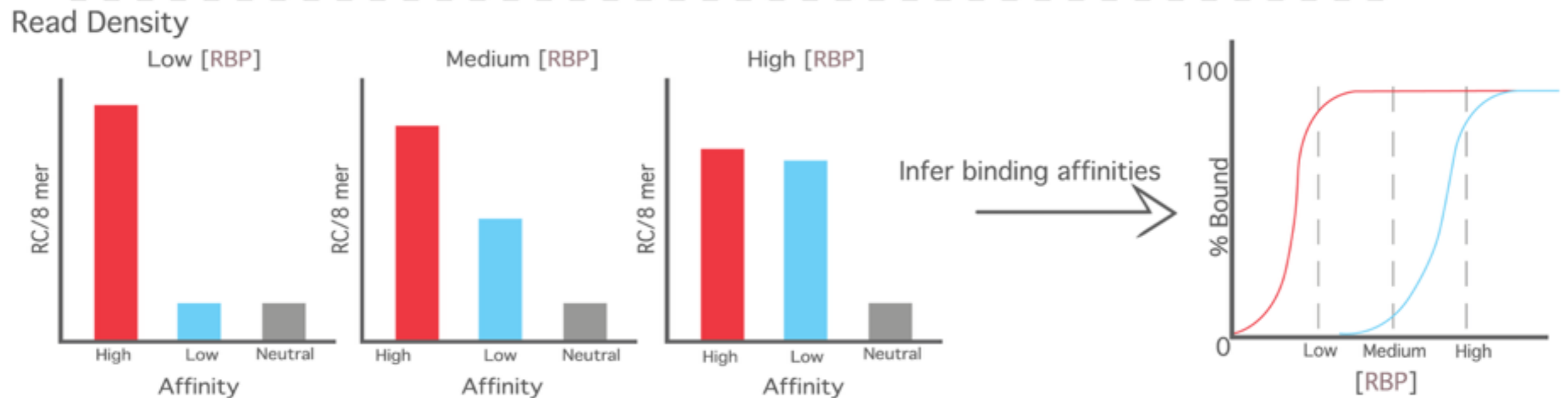
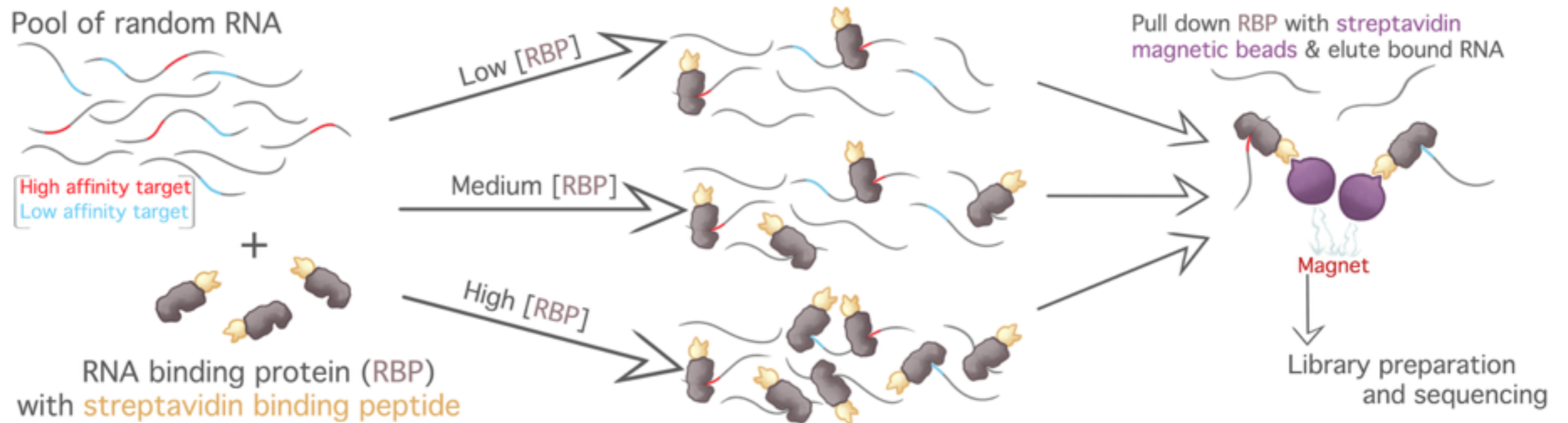


# Discovery of RNA elements in the Human Genome



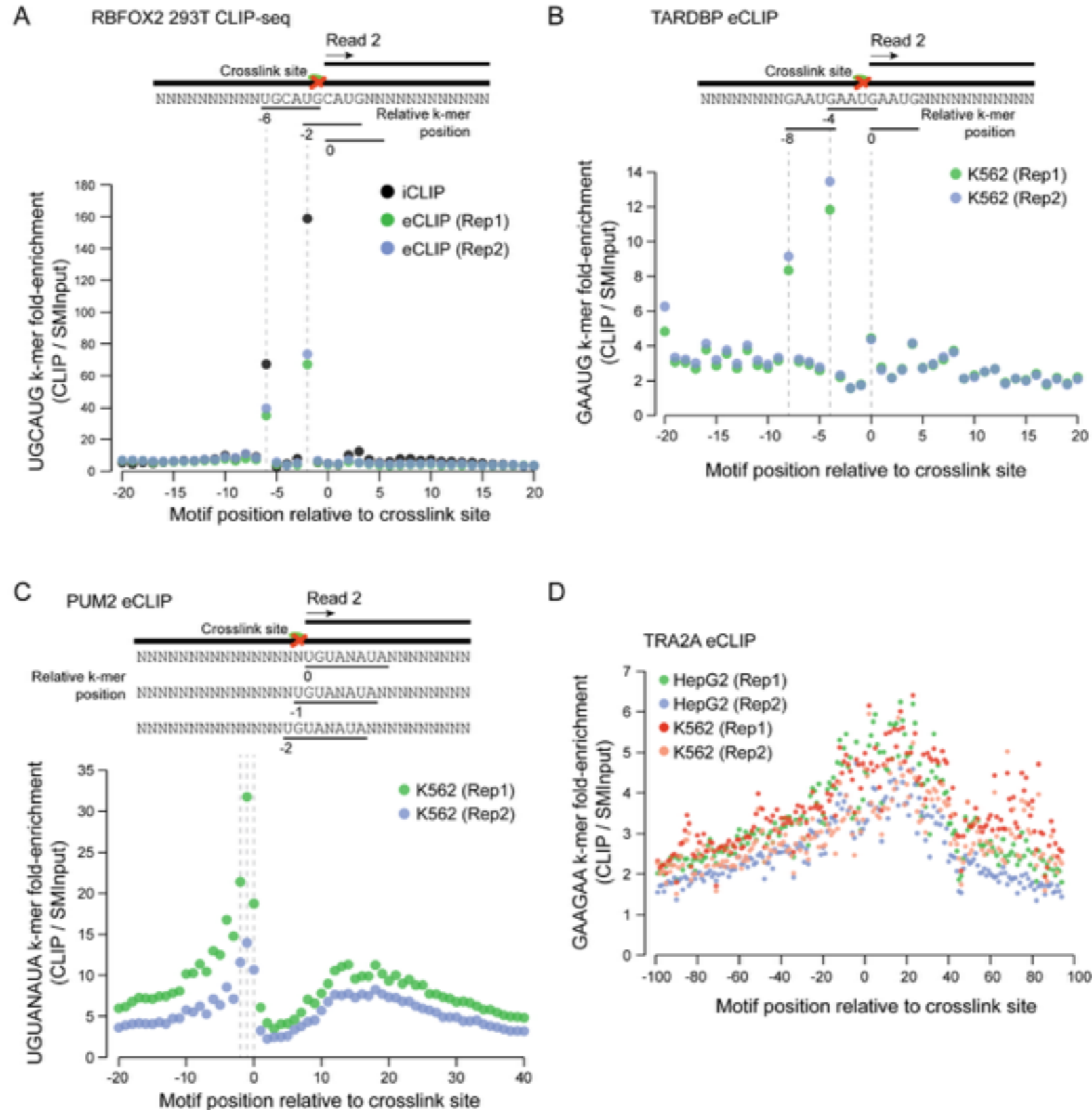


# RNA Bind-n-Seq (RBNS): A method to quantify protein/RNA interactions



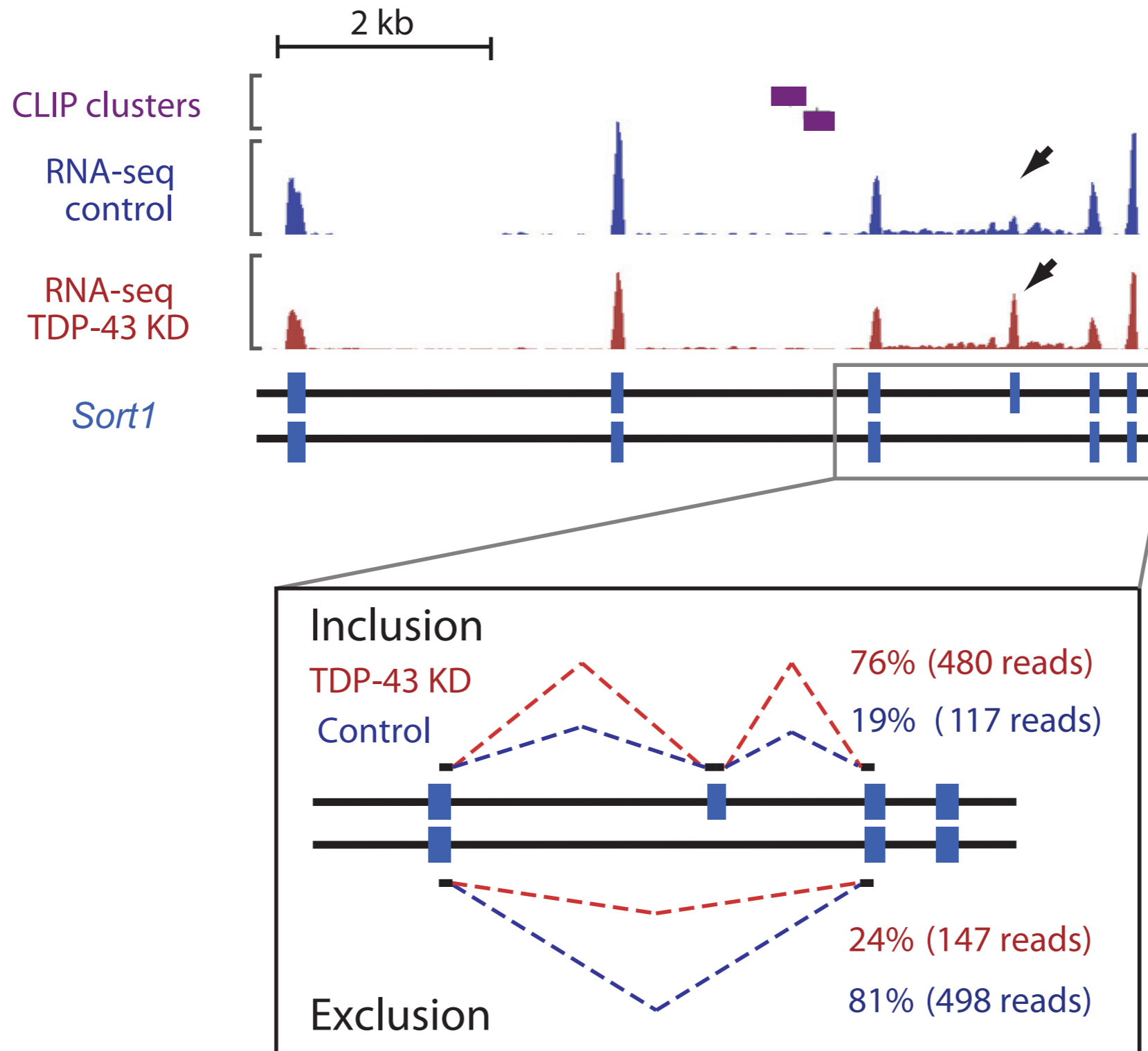
Lambert, N., et al. (2014). RNA Bind-n-Seq: Quantitative Assessment of the Sequence and Structural Binding Specificity of RNA Binding Proteins. *Mol Cell*.

# eCLIP identifies motifs at crosslink-termination points

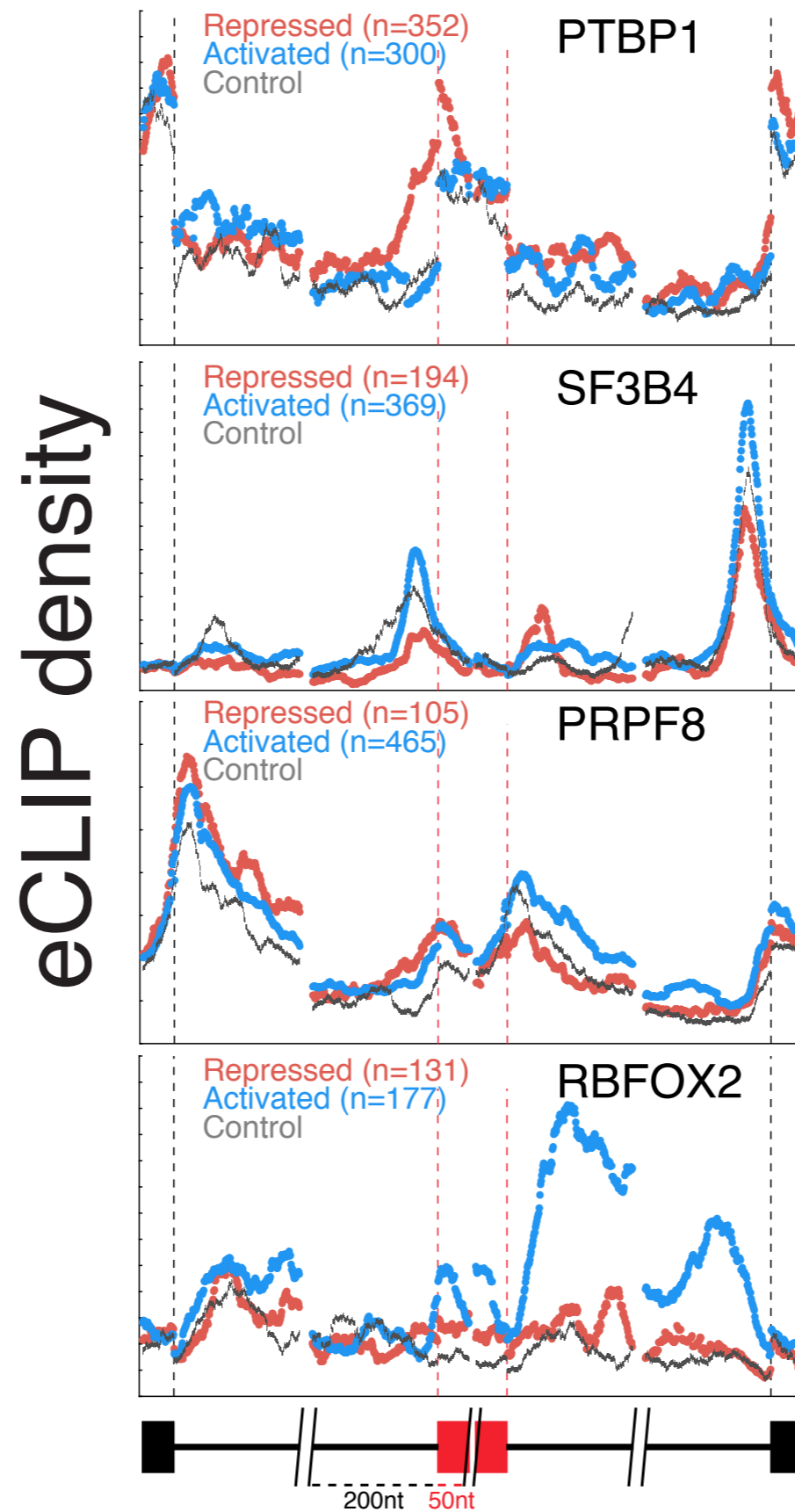




# Assigning Function to Binding Sites

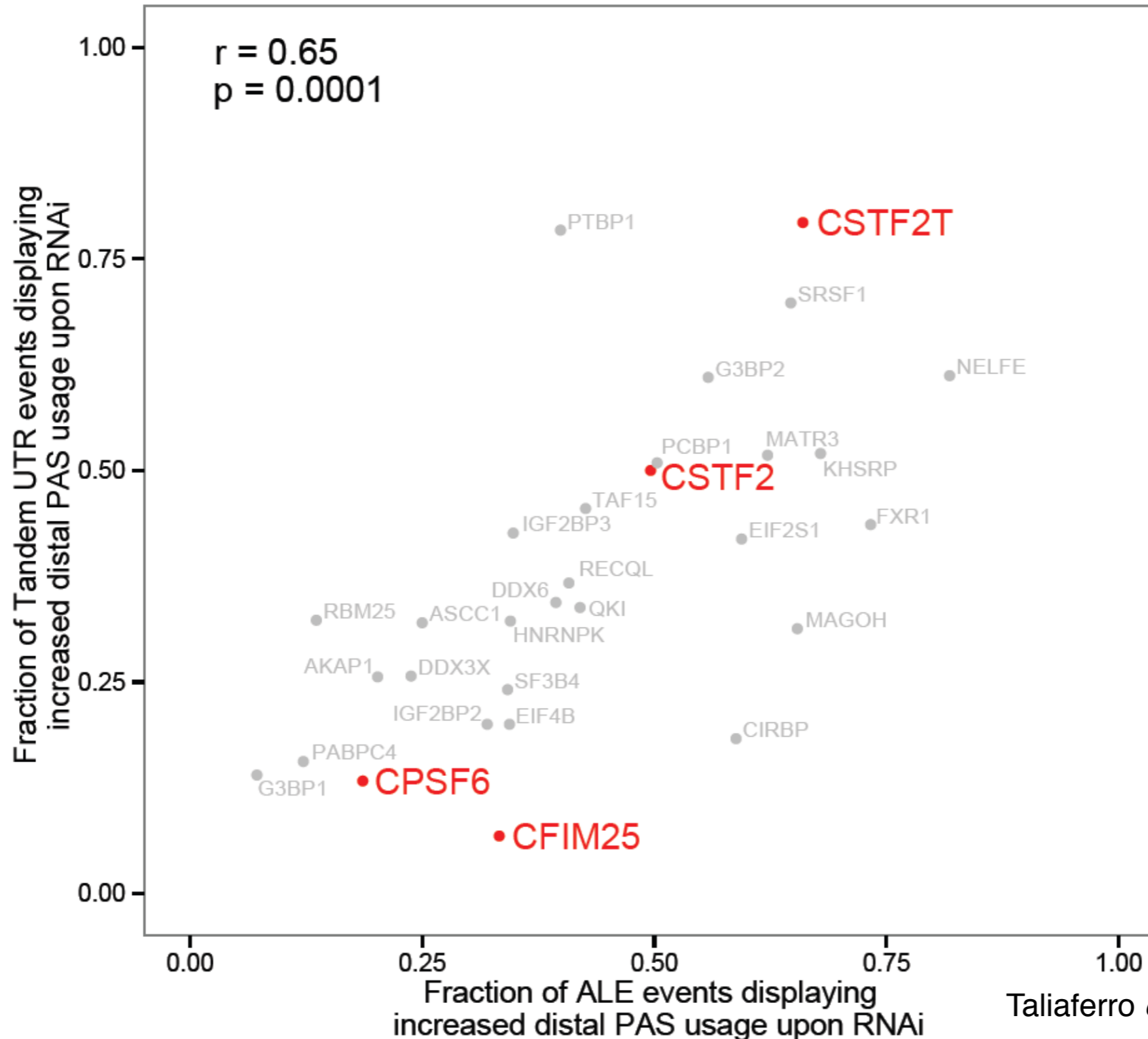


# RNA Maps for RBPs



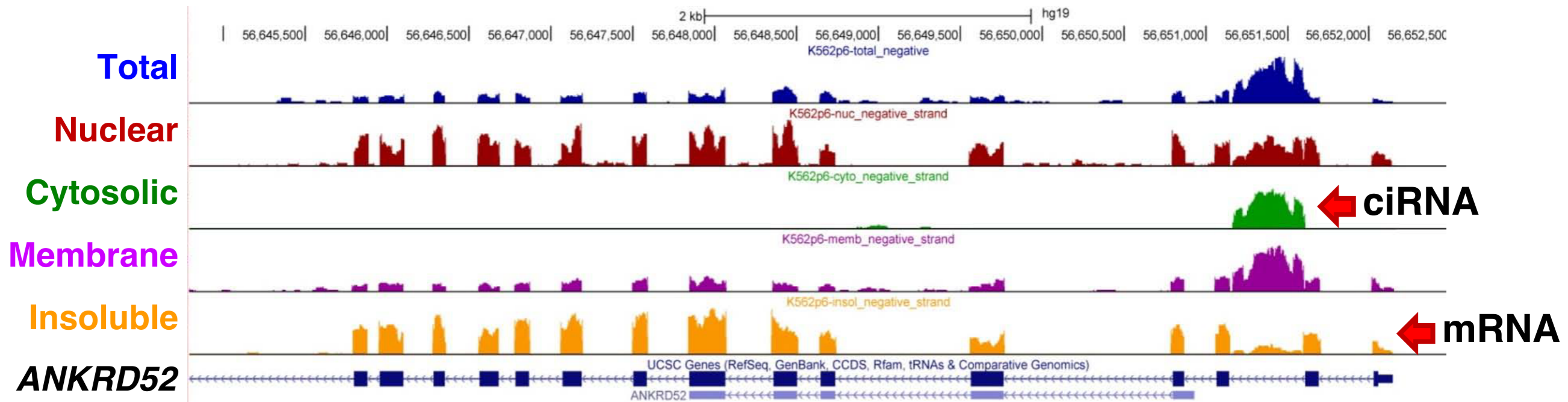


# Identification of RBPs that regulate proximal vs distal polyadenylation site usage

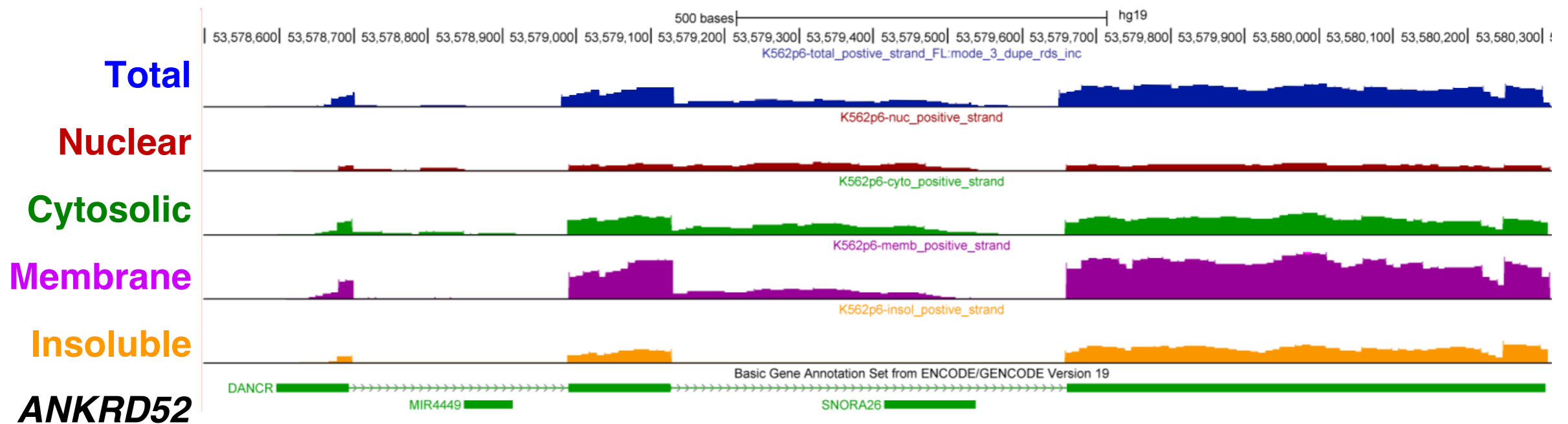


# RNA Localization (Frac-Seq)

## *ANKRD52 (mRNA and ciRNA)*



## *DANCR (lncRNA)*

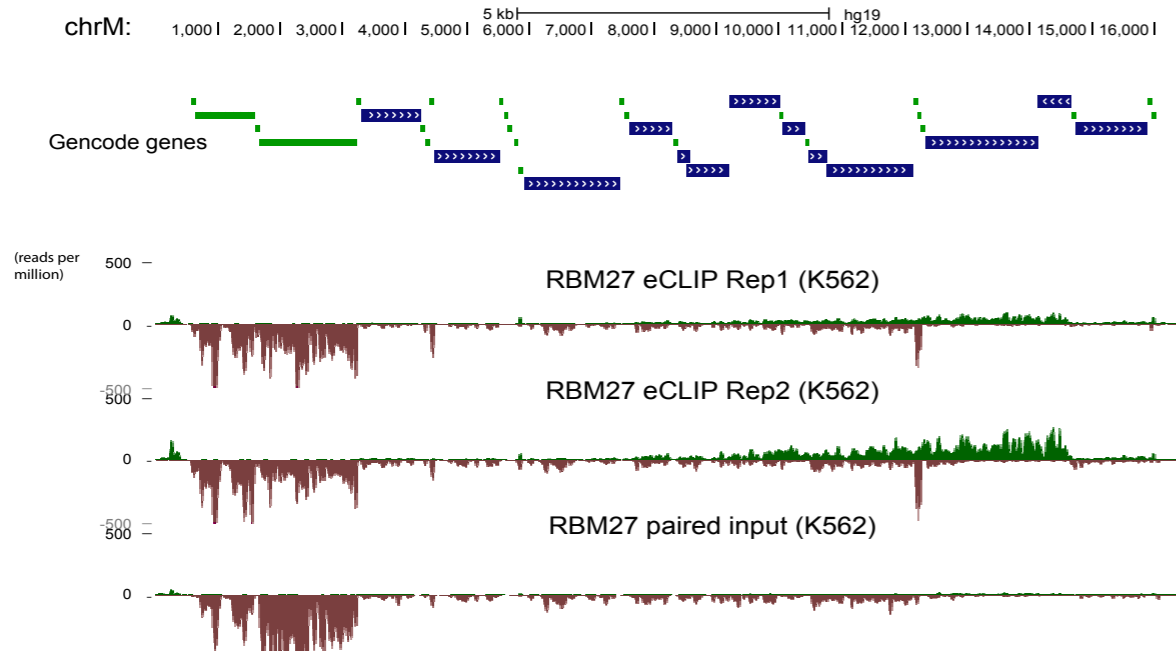


(Neal Cody)

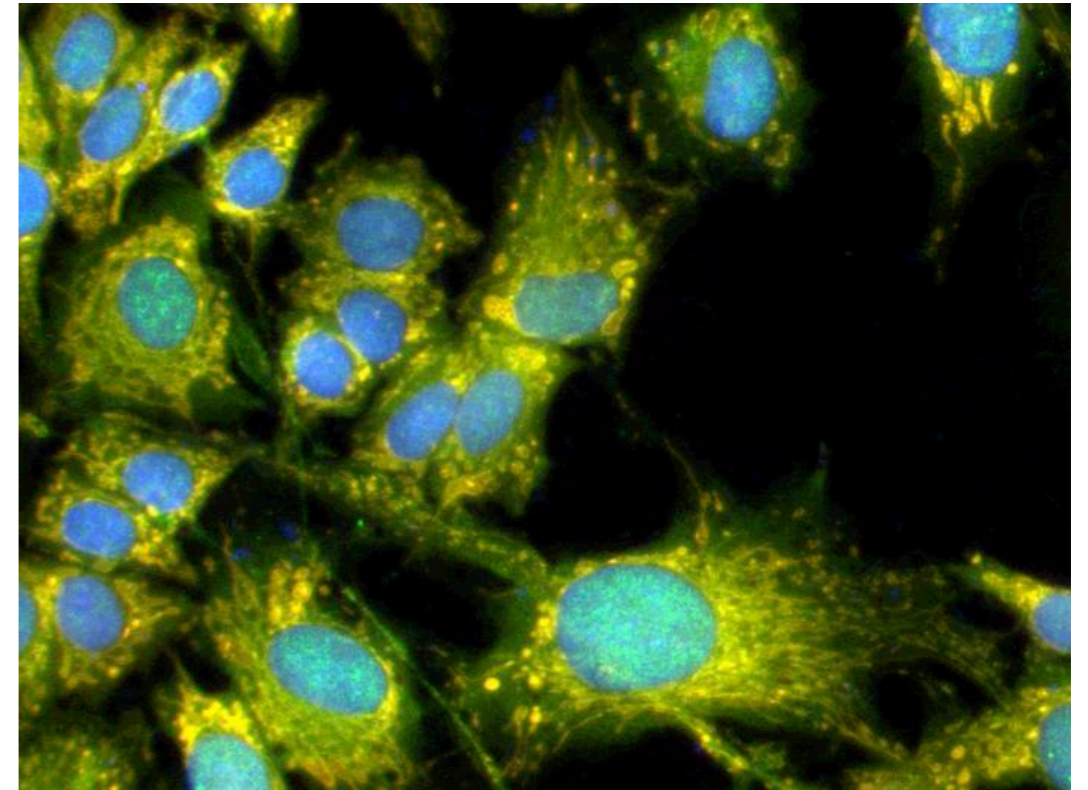


# Integrative Analysis

## eCLIP-Seq

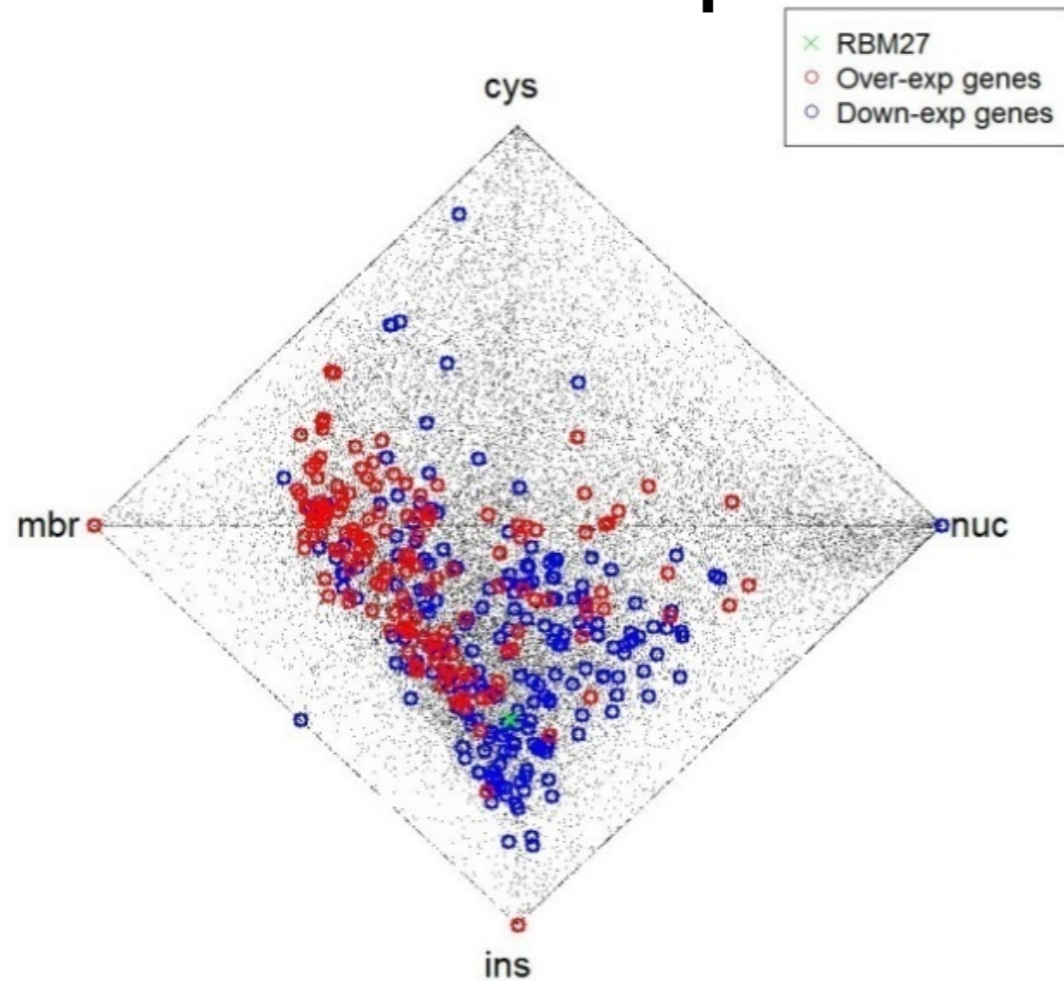


## Imaging

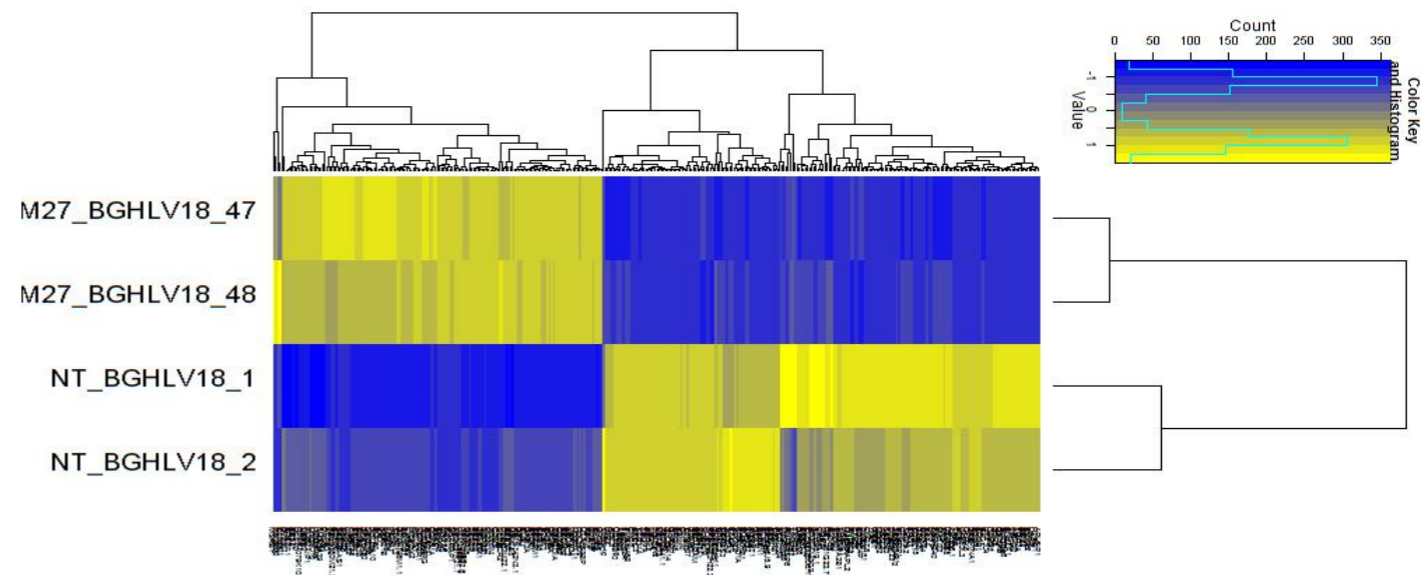


**RBM27** **Mito** **DNA**

## Frac-Seq

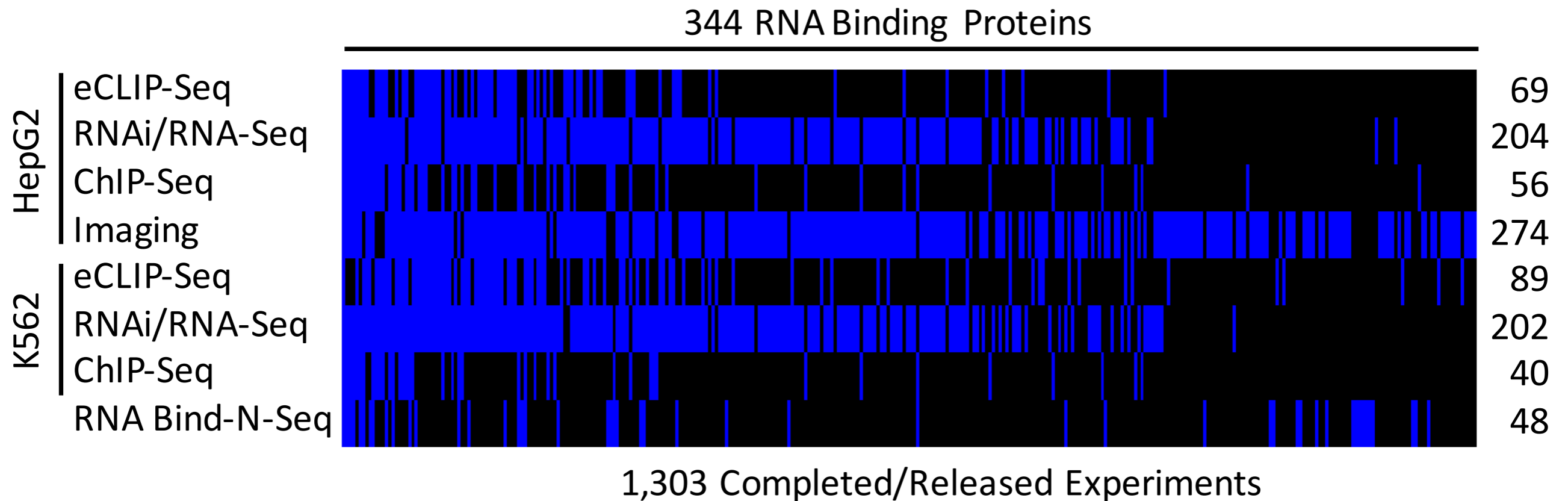


## Gene Expression



# Data Production Status

(as of 06/06/16)





# The Periodic Table of Human RNA Binding Proteins

1 QKI 38 kDa																2 RBM17 45 kDa						
3 RBFOX2 41 kDa	4 SMN1 32 kDa																5 LSM11 40 kDa	6 SF3B4 44 kDa	7 HNRNPC 34 kDa	8 CSTF50 48 kDa	9 SLBP 31 kDa	10 RBM22 47 kDa
11 TIAL1 42 kDa	12 TARDBP 45 kDa																13 U2AF1 28 kDa	14 PRPF4 58 kDa	15 KHDRBS2 39 kDa	16 CPSF7 52 kDa	17 PABPN1 33 kDa	18 RBM5 92 kDa
19 PTBP1 58 kDa	20 FUS 53 kDa	21 TRA2A 33 kDa	22 SRSF9 26 kDa	23 ZC3H8 34 kDa	24 NIP7 20 kDa	25 PUS1 47 kDa	26 LIN28B 27 kDa	27 MAGOH 17 kDa	28 XRCC6 70 kDa	29 IGF2BP1 63 kDa	30 PCBP1 38 kDa	31 U2AF2 54 kDa	32 SF3A3 59 kDa	33 KHDRBS1 48 kDa	34 CPSF6 59 kDa	35 RBM34 48 kDa	36 RBM25 100 kDa					
37 RAVER1 64 kDa	38 FXR1 70 kDa	39 SCAF4 126 kDa	40 SRSF7 27 kDa	41 ZC3H11A 89 kDa	42 NOL12 25 kDa	43 SSB 47 kDa	44 DGCR8 86 kDa	45 PNN 82 kDa	46 XRCC5 83 kDa	47 IGF2BP3 64 kDa	48 PCBP2 39 kDa	49 CAPER 59 kDa	50 PRPF6 107 kDa	51 HNRNPH1 49 kDa	52 CSTF2 61 kDa	53 MTPAP 66 kDa	54 RBM15 107 kDa					
55 PUM2 114 kDa	56 FXR2 74 kDa	57-71	72 SRSF5 31 kDa	73 ZFC3H1 226 kDa	74 NPM1 33 kDa	75 LARP7 67 kDa	76 EIF2C1 97 kDa	77 RENT1 124 kDa	78 XRN2 109 kDa	79 IGF2BP2 66 kDa	80 PCBP3 39 kDa	81 PUF60 60 kDa	82 SNRNP200 244 kDa	83 EWSR1 68 kDa	84 CSTF2T 64 kDa	85 PABPC4 71 kDa	86 RBM27 119 kDa					
87 PUM1 126 kDa	88 ATXN1 87 kDa	89-103	104 SRSF4 56 kDa	105 ZFP106 269 kDa	106 NOLC1 74 kDa	107 LARP4 81 kDa	108 DROSHA 159 kDa	109 UPF2 148 kDa	110 XRN1 194 kDa	111 KHSRP 73 kDa	112 HNRNPK 51 kDa	113 AQR 171 kDa	114 PRPF8 274 kDa	115 HNRNPU 91 kDa	116 FIP1L1 67 kDa	117 CPEB4 80 kDa	118 RBM6 129 kDa					

■ Popular    ■ SR    ■ Nucleolar    ■ miRNA    ■ Turnover/Repair    ■ Spliceosome    ■ 3' End    ■ DDX/Helicase    ■ SRP  
■ Disease    ■ Zinc    ■ La    ■ EJC/NMD    ■ Almost Famous    ■ hnRNPs    ■ RBMs    ■ Translation    ■ Modification

57 DHX33 47 kDa	58 DDX6 54 kDa	59 DDX19B 54 kDa	60 DDX52 67 kDa	61 DDX55 69 kDa	62 DDX59 69 kDa	63 SRP68 71 kDa	64 DDX3X 73 kDa	65 DDX21 87 kDa	66 SUPV3L1 88 kDa	67 DDX27 90 kDa	68 DDX20 92 kDa	69 DDX24 96 kDa	70 DHX30 134 kDa	71 DHX29 155 kDa
89 RPS24 15 kDa	90 RPL23A 18 kDa	91 RPL10 25 kDa	92 RPS3 27 kDa	93 EIF3G 36 kDa	94 EIF2S1 36 kDa	95 EIF3H 40 kDa	96 EIF4A3 47 kDa	97 EIF3D 64 kDa	98 EIF4B 69 kDa	99 EEF2 95 kDa	100 EIF4G2 102 kDa	101 EFTUD2 109 kDa	102 EIF3A 167 kDa	103 EIF4G1 175 kDa



# Acknowledgments

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R01 GM067842

R01 GM095296

R35 GM118140





