Defining the roles of RNA binding proteins with CLIP-seq

Patryk Konieczny, PhD

12th Poznan Summer School of Bioinformatics; 06.09.2017

- RNA binding proteins
- CLIP-seq (HITS-CLIP, iCLIP, PAR-CLIP, eCLIP, irCLIP)
- CLIP-seq related methods (interactome capture, CLASH, hiCLIP)
- CLIP-seq in paraneoplastic opsoclonus-myoclonus-ataxia (POMA), myotonic dystrophy (DM), fragile X syndrome (FXS)
- Regulatory networks

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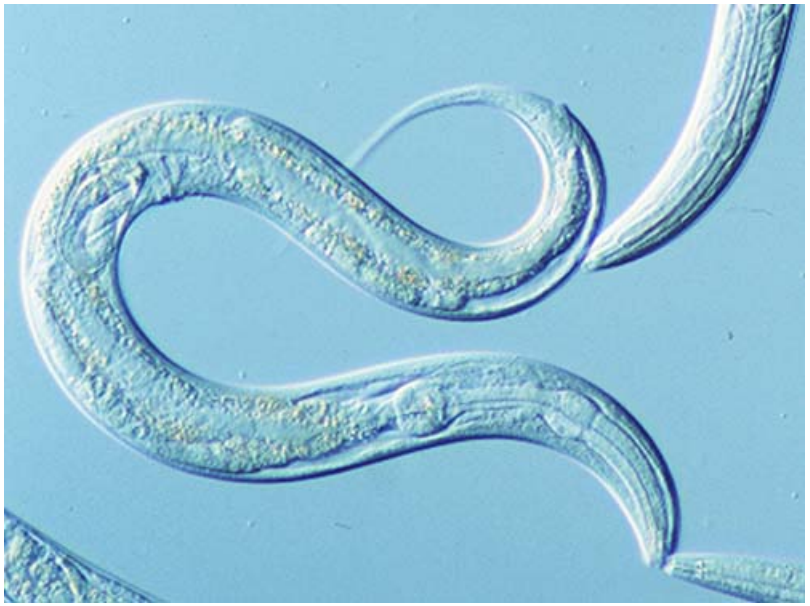
Caenorhabditis elegans



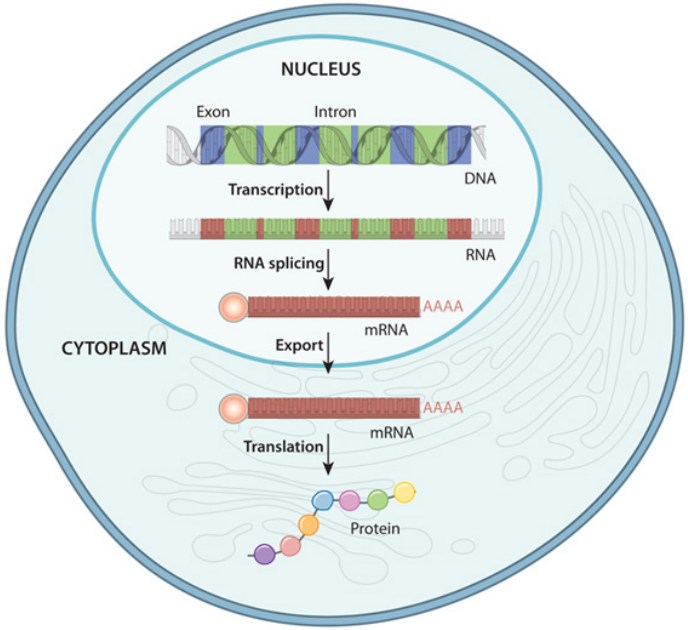
Organism type: Nematode
Genome: 100,300,000 bp (100 Mb)
No. of genes: ~19000

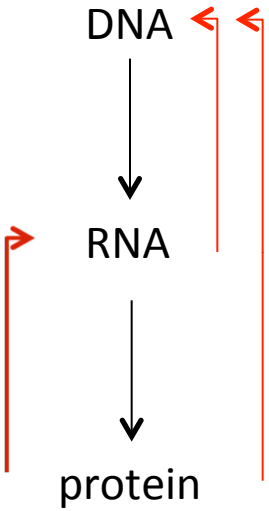
Homo sapiens

Organism type: Mammal
Genome: 3,289,000,000 bp (3.3 Gb)
No. of genes: ~20000

GENE: the basic physical unit of heredity; a linear sequence of nucleotides along a segment of DNA that provides the coded instructions for synthesis of RNA, which, when translated into protein, leads to the expression of hereditary character.

http://www.dictionary.com

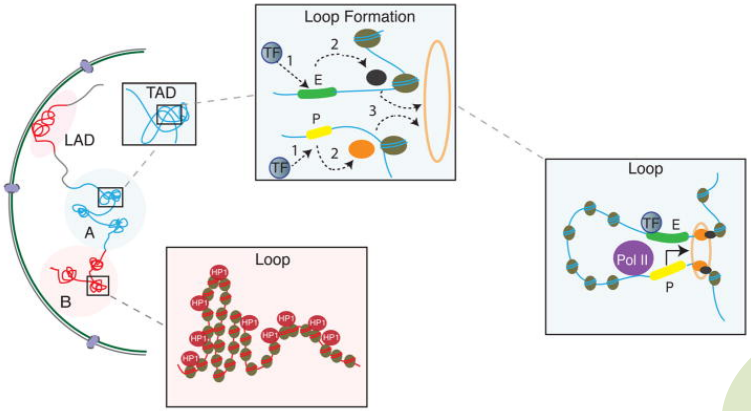


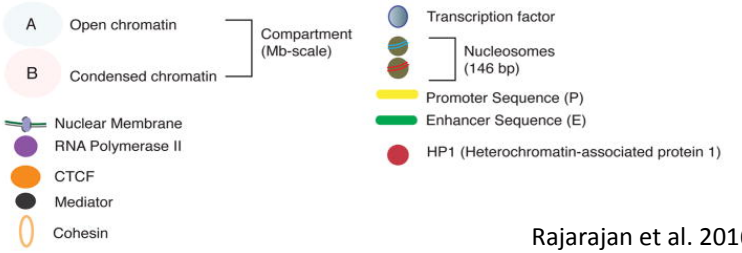


one gene = one protein

one gene ~~=~~ one protein

spatial genome organization

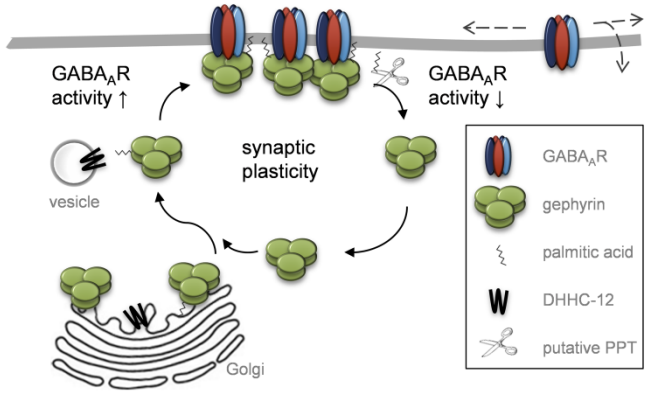




Rajarajan et al. 2016

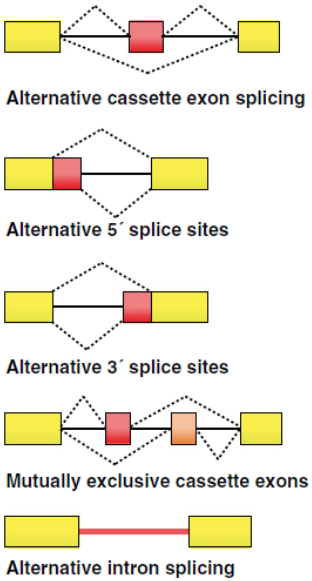
phenotypic diversity

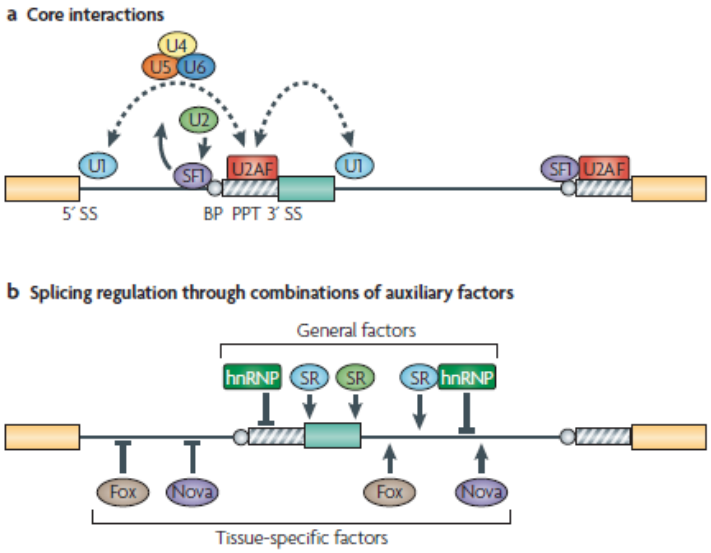
post-translational modification



Dejanovic et al. 2017

post-transcriptional regulation



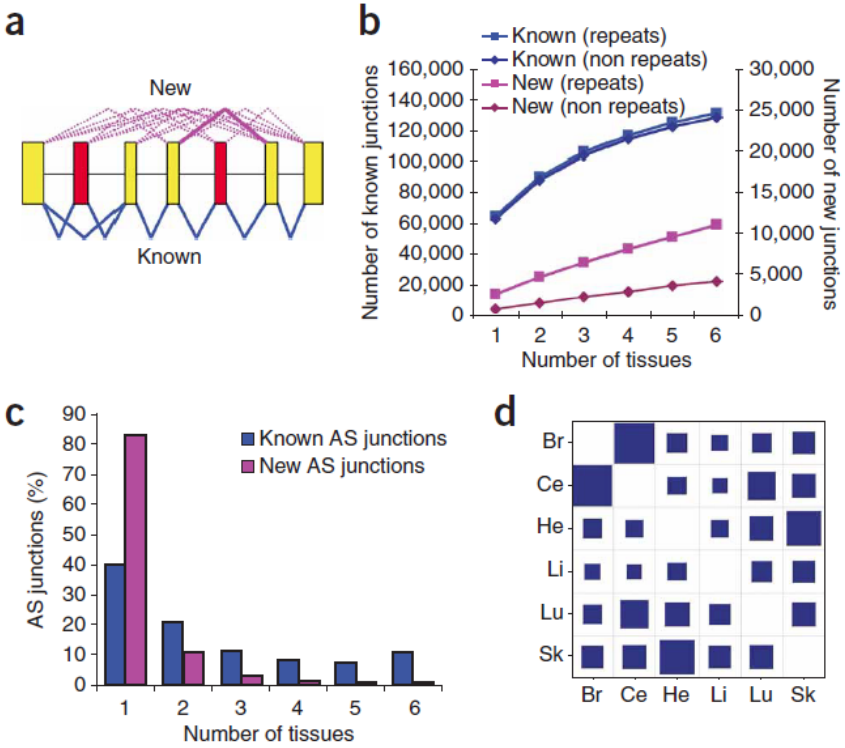


Irmia and Blencowe 2012; Licatalosi and Darnell 2010

Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing

Qun Pan[1], Ofer Shai[1,2], Leo J Lee[1,2], Brendan J Frey[1,2] & Benjamin J Blencowe[1,3]

We carried out the first analysis of alternative splicing complexity in human tissues using mRNA-Seq data. New splice junctions were detected in ~20% of multiexon genes, many of which are tissue specific. By combining mRNA-Seq and EST-cDNA sequence data, we estimate that transcripts from ~95% of multiexon genes undergo alternative splicing and that there are ~100,000 intermediate- to high-abundance alternative splicing events in major human tissues. From a comparison with quantitative alternative splicing microarray profiling data, we also show that mRNA-Seq data provide reliable measurements for exon inclusion levels.



nature Vol 456 | 27 November 2008 | doi:10.1038/nature07509

ARTICLES

Alternative isoform regulation in human tissue transcriptomes

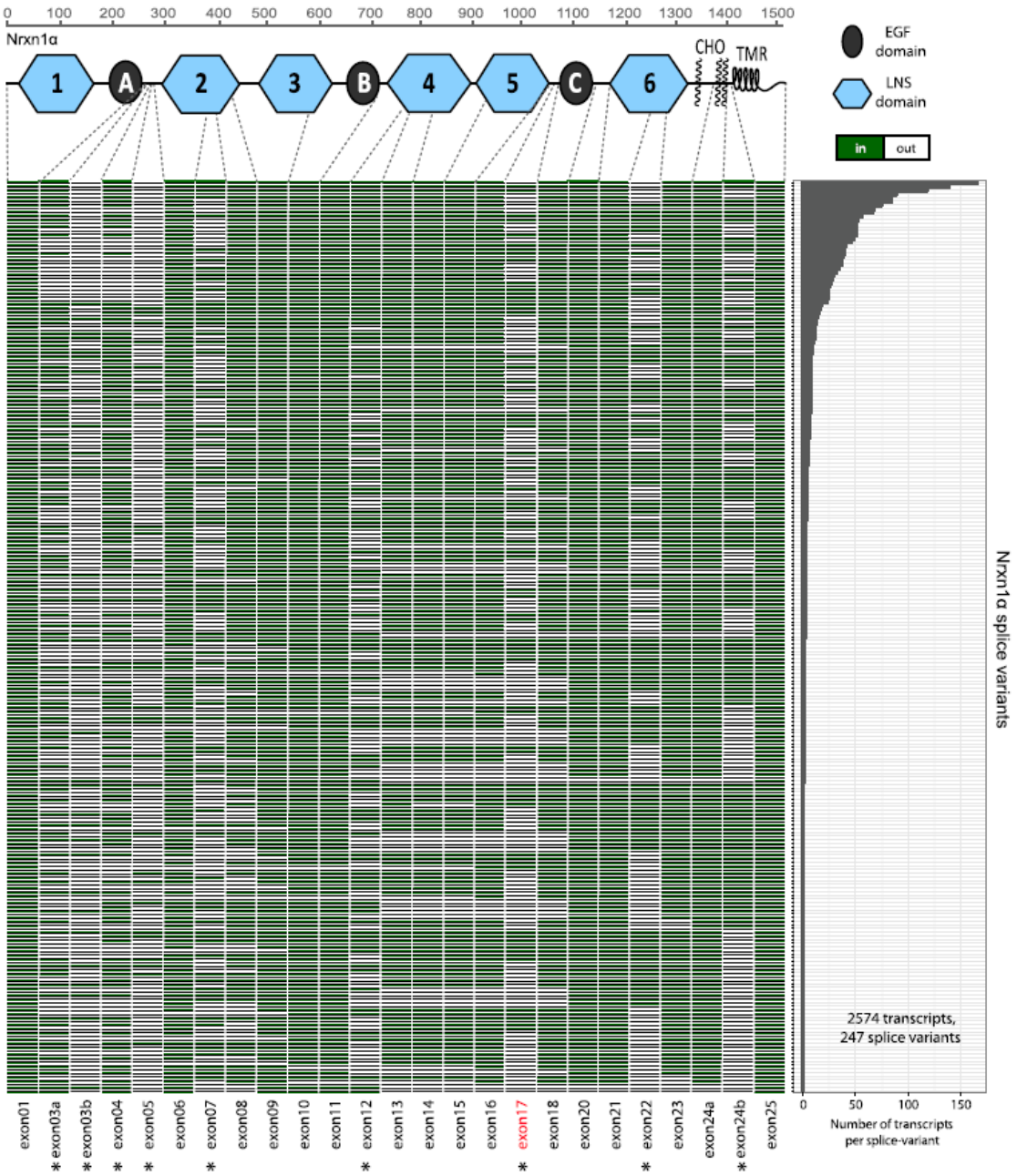
Eric T. Wang[1,2]*, Rickard Sandberg[1,3]*, Shujun Luo[4], Irina Khrebtukova[4], Lu Zhang[4], Christine Mayr[5], Stephen F. Kingsmore[6], Gary P. Schroth[4] & Christopher B. Burge[1]

Through alternative processing of pre-messenger RNAs, individual mammalian genes often produce multiple mRNA and protein isoforms that may have related, distinct or even opposing functions. Here we report an in-depth analysis of 15 diverse human tissue and cell line transcriptomes on the basis of deep sequencing of complementary DNA fragments, yielding a digital inventory of gene and mRNA isoform expression. Analyses in which sequence reads are mapped to exon–exon junctions indicated that 92–94% of human genes undergo alternative splicing, ~86% with a minor isoform frequency of 15% or more. Differences in isoform-specific read densities indicated that most alternative splicing and alternative cleavage and polyadenylation events vary between tissues, whereas variation between individuals was approximately twofold to threefold less common. Extreme or 'switch-like' regulation of splicing between tissues was associated with increased sequence conservation in regulatory regions and with generation of full-length open reading frames. Patterns of alternative splicing and alternative cleavage and polyadenylation were strongly correlated across tissues, suggesting coordinated regulation of these processes, and sequence conservation of a subset of known regulatory motifs in both alternative introns and 3′ untranslated regions suggested common involvement of specific factors in tissue-level regulation of both splicing and polyadenylation.

Alternative transcript events		Total events ($\times 10^3$)	Number detected ($\times 10^3$)	Both isoforms detected	Number tissue-regulated	% Tissue-regulated (observed)	% Tissue-regulated (estimated)
Skipped exon		37	35	10,436	6,822	65	72
Retained intron		1	1	167	96	57	71
Alternative 5′ splice site (A5SS)		15	15	2,168	1,386	64	72
Alternative 3′ splice site (A3SS)		17	16	4,181	2,655	64	74
Mutually exclusive exon (MXE)		4	4	167	95	57	66
Alternative first exon (AFE)		14	13	10,281	5,311	52	63
Alternative last exon (ALE)		9	8	5,246	2,491	47	52
Tandem 3′ UTRs	pA pA	7	7	5,136	3,801	74	80
Total		105	100	37,782	22,657	60	68

Constitutive exon or region — Body read — Junction read — pA Polyadenylation site
Alternative exon or extension — Inclusive/extended isoform — Exclusive isoform — Both isoforms

Splice landscape of neurexin Nrxn1α



Treutlein et al. 2014

alternative splicing

alternative transcription

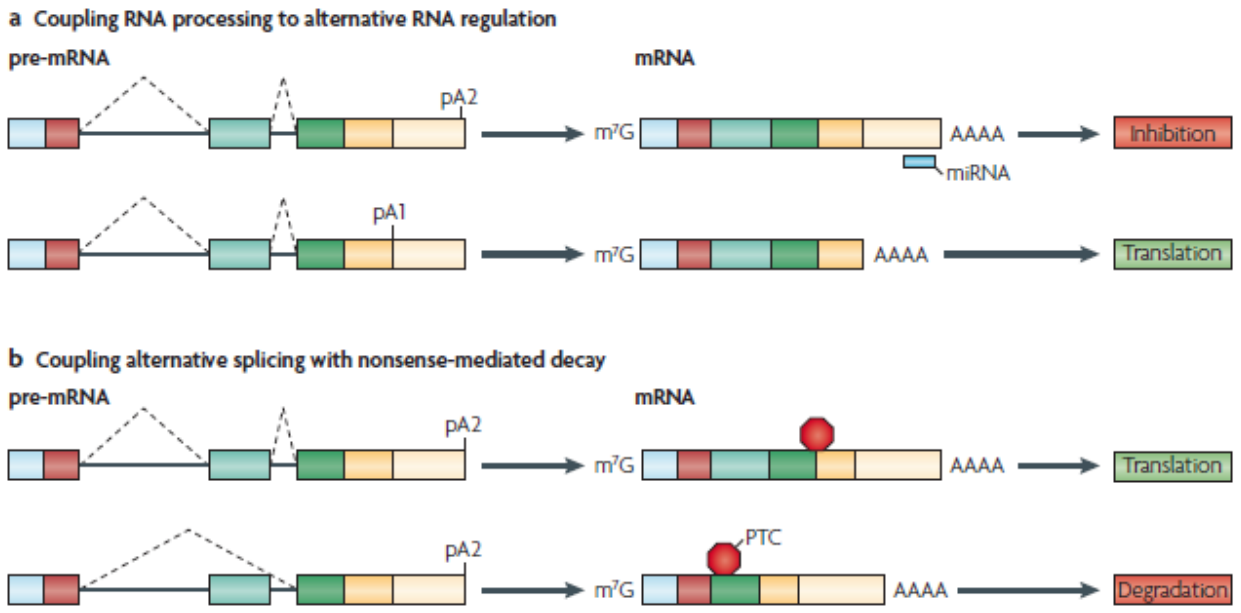
post-transcriptional modification
(e.g. pseudouridylation)

post-transcriptional regulation

alternative polyadenylation

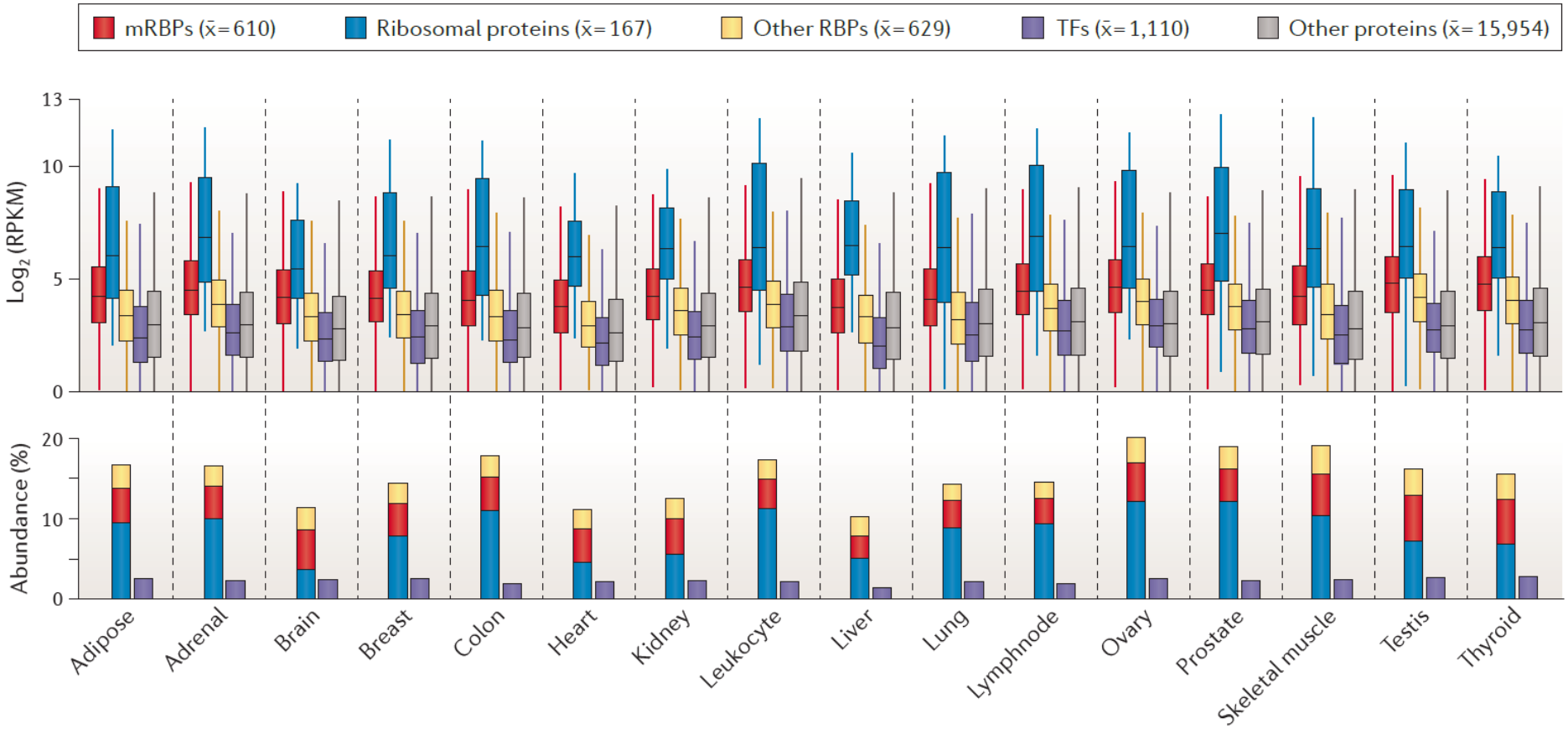
RNA editing
(e.g. adenosine to inosine)

maturation, transport, stability and
translation of coding and non-coding RNAs



Licatalosi and Darnell 2010

Transcript abundance of RNA binding proteins

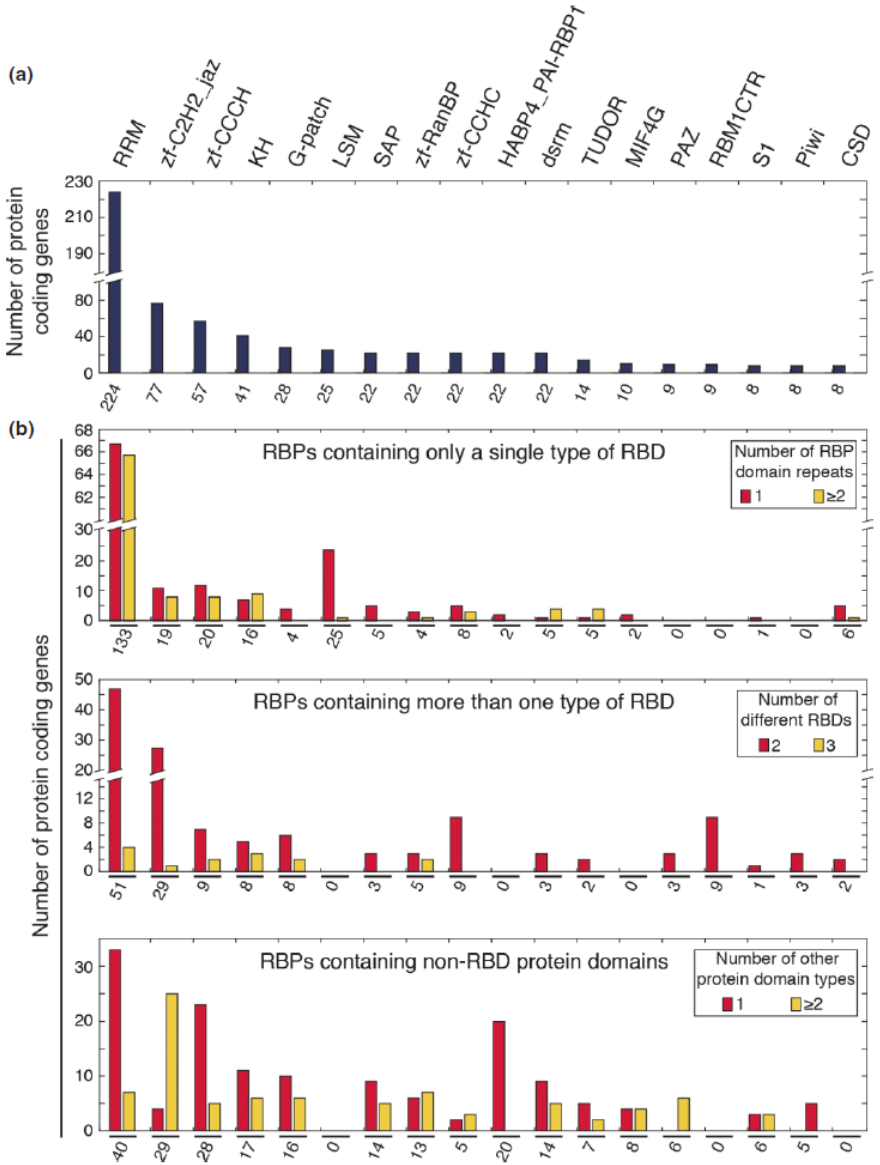


Gestberger et al. 2014

RNA binding proteins and their RNA binding domains

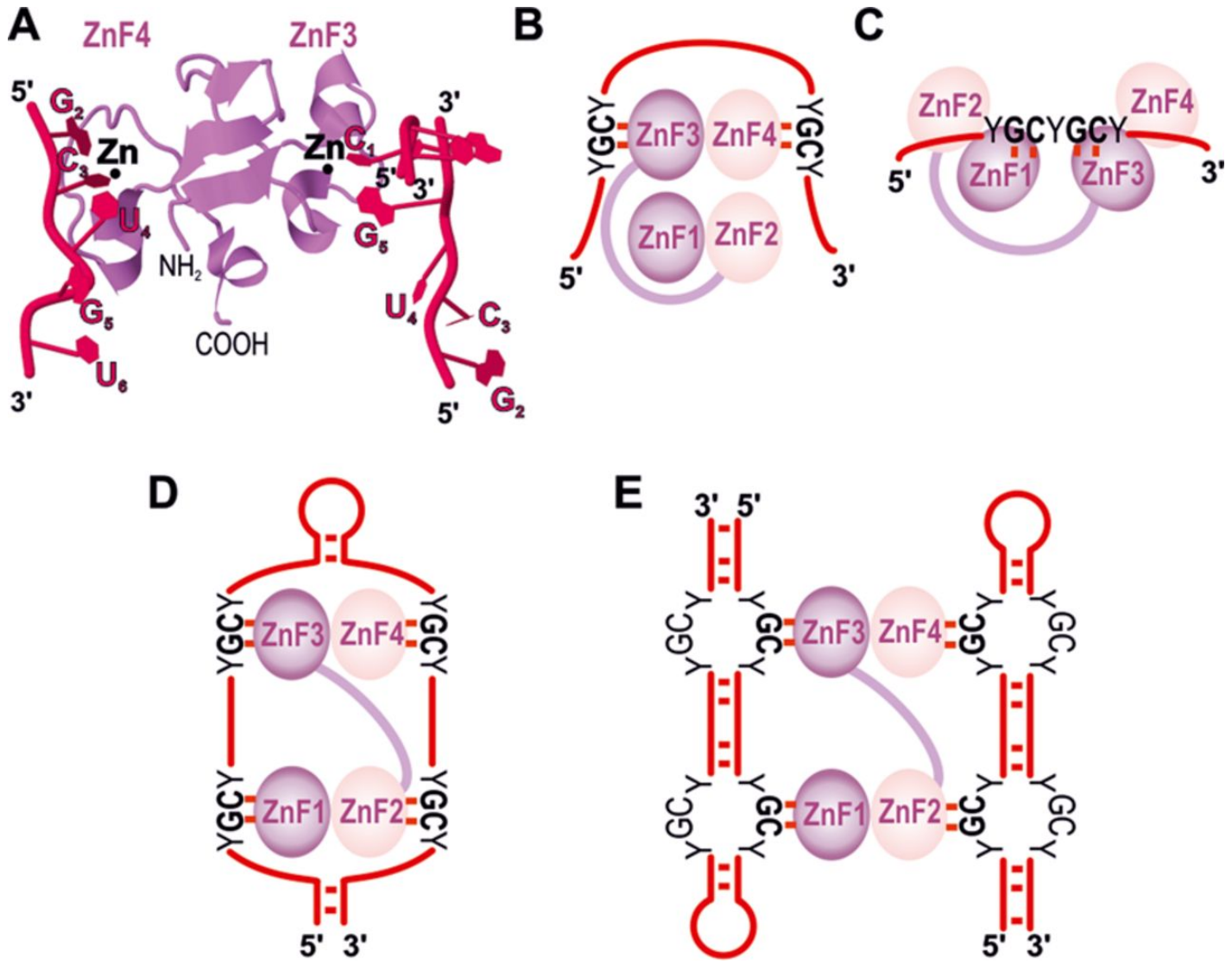
- the human genome encodes **at least 600 RNA binding proteins** based on the presence of known RNA-binding domains

- there are about **75 annotated RNA binding domains** and those that have been molecularly characterized mostly bind short 4–6 nt segments in a sequence and/ or structural specific manner



Ascano et al. 2012

MBNL proteins bind to YGCY motifs through CCCH domains



[Konieczny et al. 2014 (based on Teplova and Patel 2008)]

RNA binding proteins in health and disease

CLIP studies on RBPs implicated in neurological function in health and disease. Abbreviations: NOVA1/2 — neuro-oncological ventral antigen 1/2, TARDP — TAR DNA binding protein, FMRP — fragile-X mental retardation protein, MBNL1/2 — muscleblind-like protein 1/2, FUS/TLS — fused in sarcoma/translocated in liposarcoma, PTBP2 — polypyrimidine tract binding protein 2, PARK7 — Parkinson protein 7, ELAVL1 — embryonic lethal, abnormal vision, Drosophila-like 1, CELF4 — CUGBP Elav-like family member 4.

Symbol	Disease	Key findings	Reference
CELF4	Epilepsy	Binds UGU motifs in 3′UTRs.	Wagnon et al. (2012)
	Hyperactivity	Controls stability of mRNAs encoding synaptic proteins.	
ELAVL1	Epilepsy	Recognises U-rich stretches interspersed with Gs.	Ince-Dunn et al. (2012)
		Regulates transcript stability.	
		Controls the synthesis of glutamate.	
FMR1	Fragile-X mental retardation	Represses the translation of target mRNAs.	Darnell et al. (2011)
	Autism spectrum disorders	Preferred binding to the coding region of exons.	Ascano et al. (2012)
		Increased association with transcripts encoding synaptic proteins.	
		Binds ACUK and WGGA (in which K = G or U and W = A or U) motifs.	
FUS	Frontotemporal lobar degeneration, amyotrophic lateral sclerosis	Binds along the full length of pre-mRNAs.	Ishigaki et al. (2012)
		Regulates alternative splicing of many neuronal development genes.	Rogelj et al. (2012)
		Knockdown leads to decreased expression of long genes in the brain.	Lagier-Tourenne et al. (2012)
MBNL1/2	Myotonic dystrophy (DM)	Recognises UGC or GCU-containing 4-mer clusters.	Wang et al. (2012)
		Regulates DM-related alternative splicing.	Charizanis et al. (2012)
		Contributes to mRNA localisation and translation by binding to 3′UTRs.	
NOVA1/2	Paraneoplastic opsoclonus-myoclonus-ataxia (POMA)	Binds YCAY clusters to regulate alternative splicing.	Licatalosi et al. (2008)
		Controls synaptogenesis and neuronal migration via specific mRNAs.	Ruggiu et al. (2009)
		Regulates alternative poly-adenylation in the brain.	Yano et al. (2010)
PARK7	Parkinson's disease	Recognises CC/GG rich regions.	van der Brug et al. (2008)
		Inhibits translation of target mRNAs.	
PTBP2		Recognises UCU-rich motifs to regulate alternative splicing.	Licatalosi et al. (2012)
		Regulates neural stem cell polarity in developing brain.	
		Involved in mRNA trafficking stability and translation.	
TARDBP	Frontotemporal lobar degeneration, amyotrophic lateral sclerosis	Recognises UG repeats and UG-rich motifs in introns and 3′ UTRs.	Tollervey et al. (2011a)
		Regulates alternative splicing of many neuronal development genes.	Polymenidou et al. (2011)
		Knockdown leads to decreased expression of long genes in the brain.	

Modic et al. 2013

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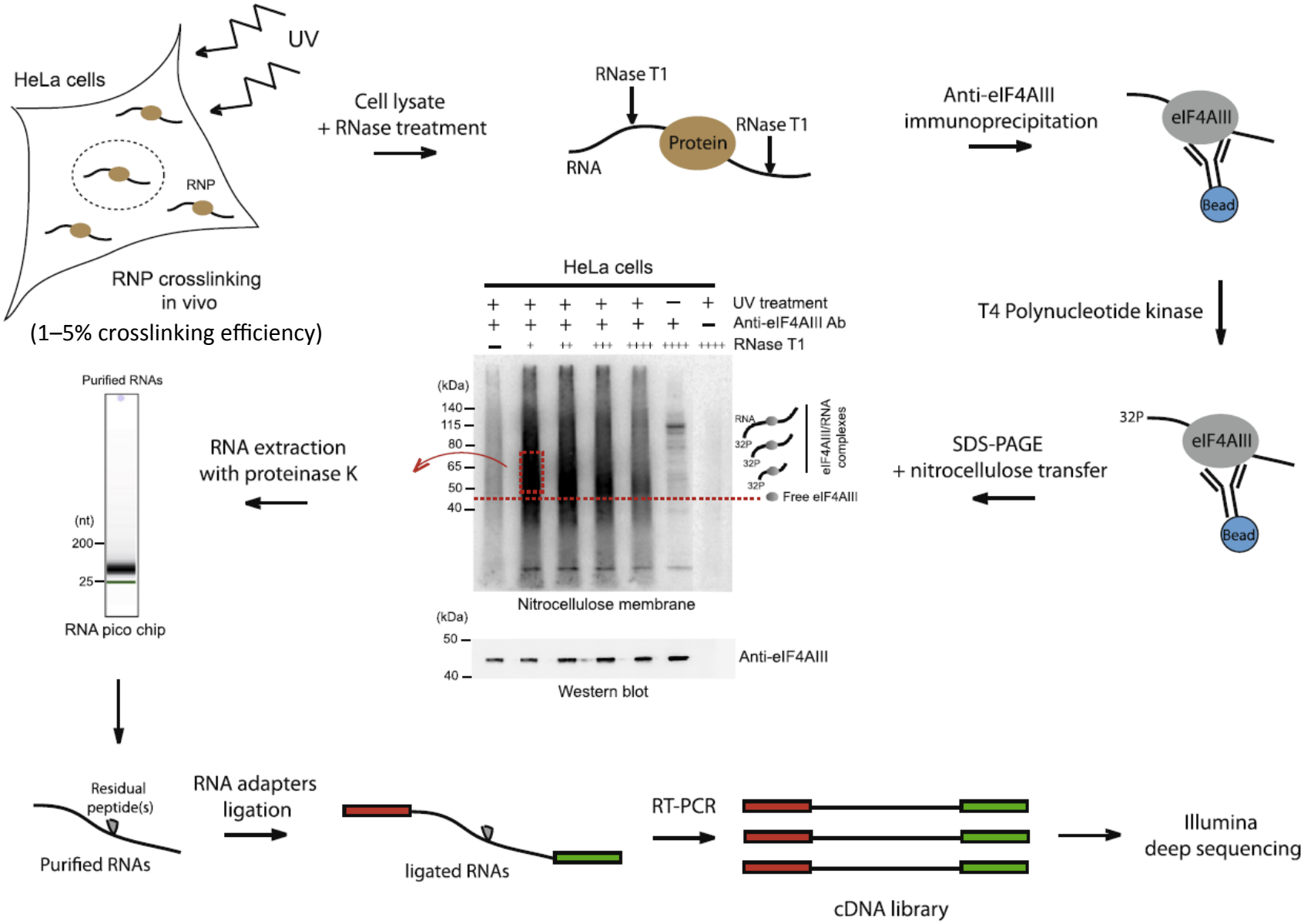
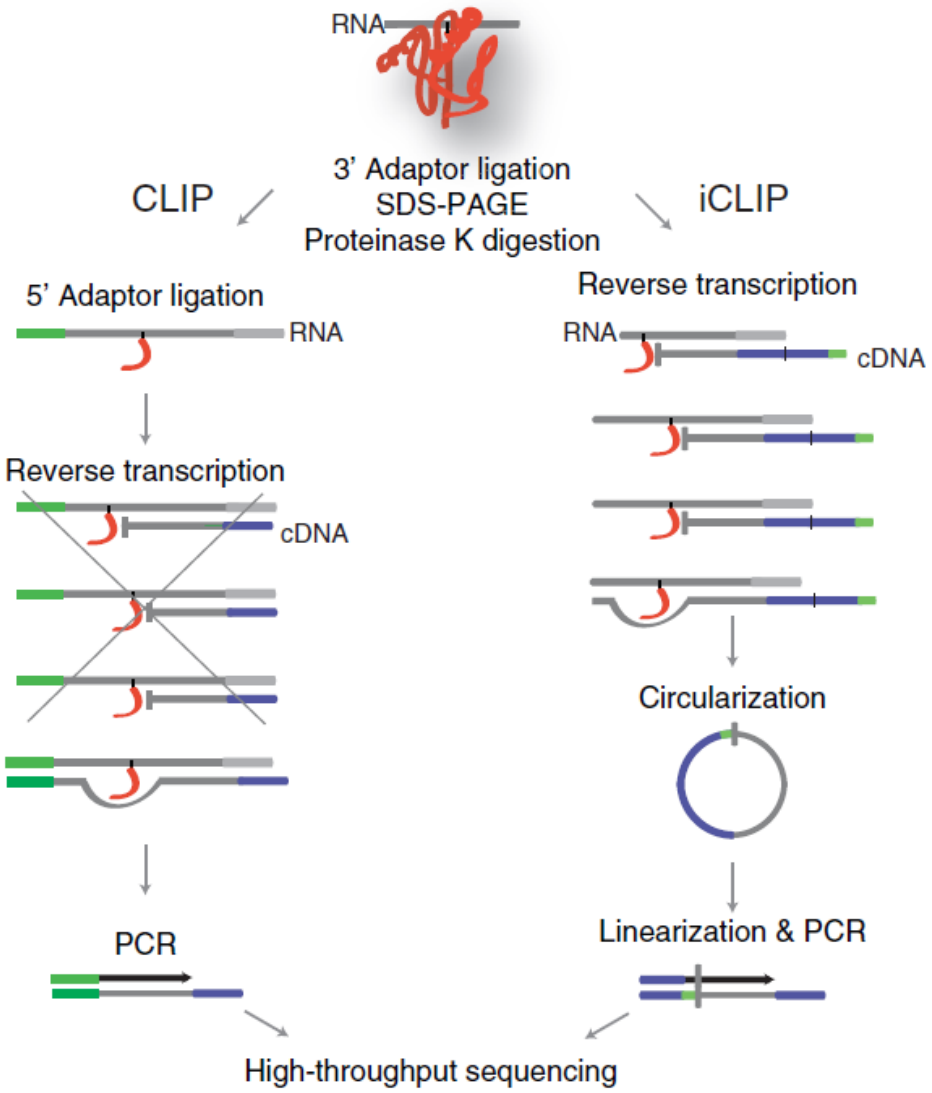
High throughput sequencing (HITS-CLIP)



Murigneux et al. 2013

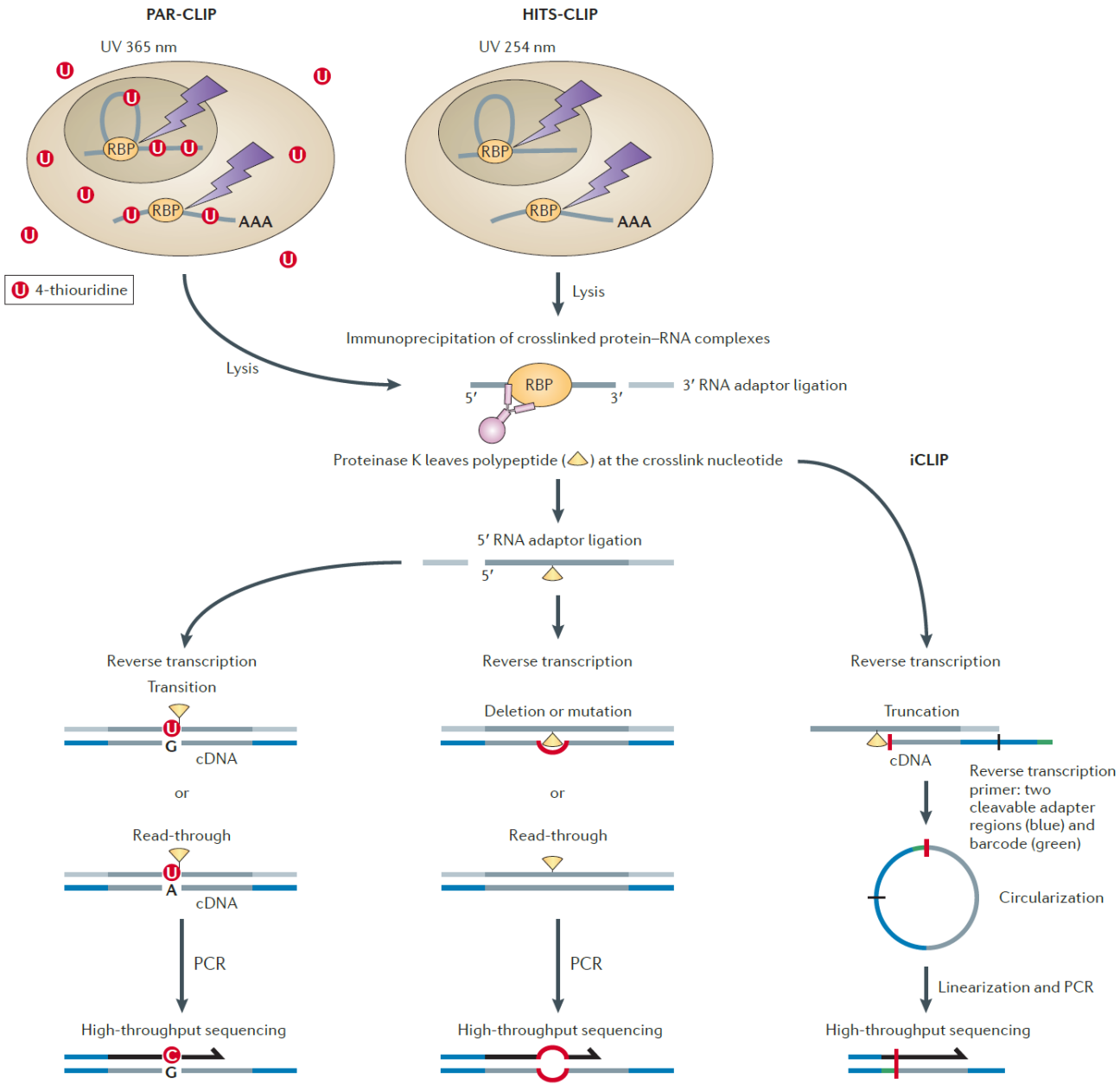
Individual nucleotide resolution CLIP (iCLIP)



Sugimoto et al. 2012

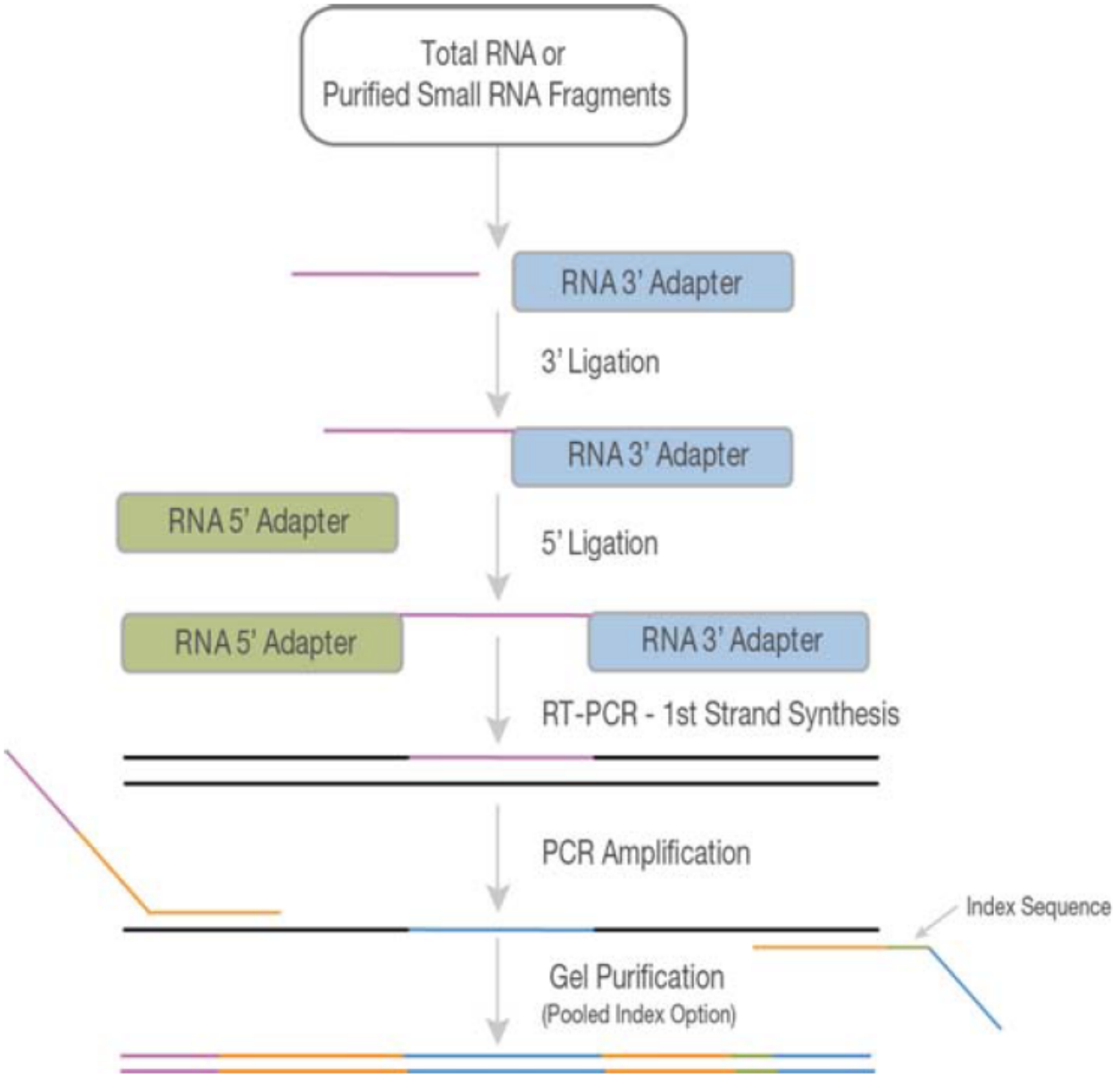
Photoactivatable ribonucleoside-enhanced CLIP (PAR-CLIP)

UV > 310 nm - natural nucleotides no longer crosslink



Koenig et al. 2012

Primers carrying indexes allow for library pooling



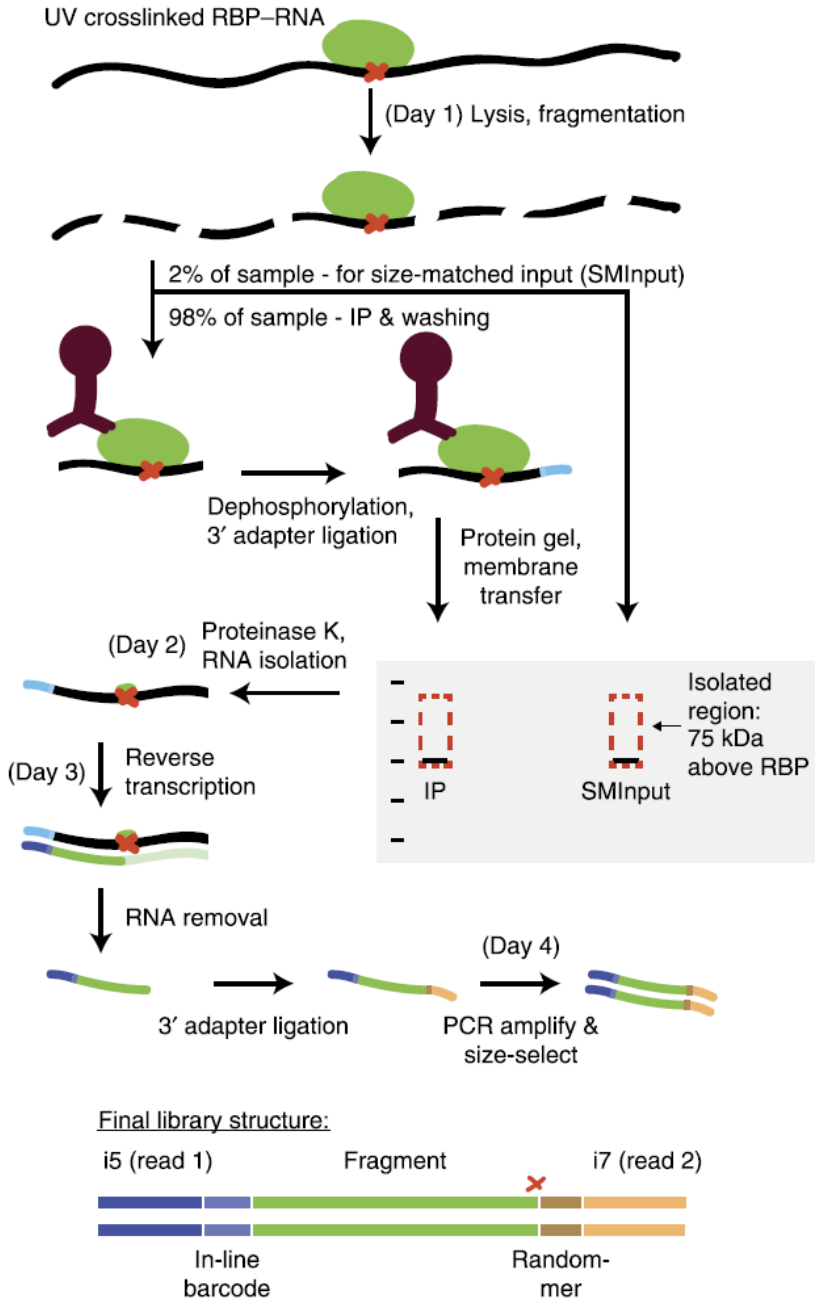
Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP)

Eric L Van Nostrand[1–3], Gabriel A Pratt[1–4], Alexander A Shishkin[5], Chelsea Gelboin-Burkhart[1–3], Mark Y Fang[1–3], Balaji Sundararaman[1–3], Steven M Blue[1–3], Thai B Nguyen[1–3], Christine Surka[5], Keri Elkins[1–3], Rebecca Stanton[1–3], Frank Rigo[6], Mitchell Guttman[5] & Gene W Yeo[1–4,7,8]

As RNA-binding proteins (RBPs) play essential roles in cellular physiology by interacting with target RNA molecules, binding site identification by UV crosslinking and immunoprecipitation (CLIP) of ribonucleoprotein complexes is critical to understanding RBP function. However, current CLIP protocols are technically demanding and yield low-complexity libraries with high experimental failure rates. We have developed an enhanced CLIP (eCLIP) protocol that decreases requisite amplification by ~1,000-fold, decreasing discarded PCR duplicate reads by ~60% while maintaining single-nucleotide binding resolution. By simplifying the generation of paired IgG and size-matched input controls, eCLIP improves specificity in the discovery of authentic binding sites. We generated 102 eCLIP experiments for 73 diverse RBPs in HepG2 and K562 cells (available at https://www.encodeproject.org), demonstrating that eCLIP enables large-scale and robust profiling, with amplification and sample requirements similar to those of ChIP-seq. eCLIP enables integrative analysis of diverse RBPs to reveal factor-specific profiles, common artifacts for CLIP and RNA-centric perspectives on RBP activity.

PUBLISHED ONLINE 28 MARCH 2016; DOI:10.1038/NMETH.3810

NATURE METHODS | ADVANCE ONLINE PUBLICATION |



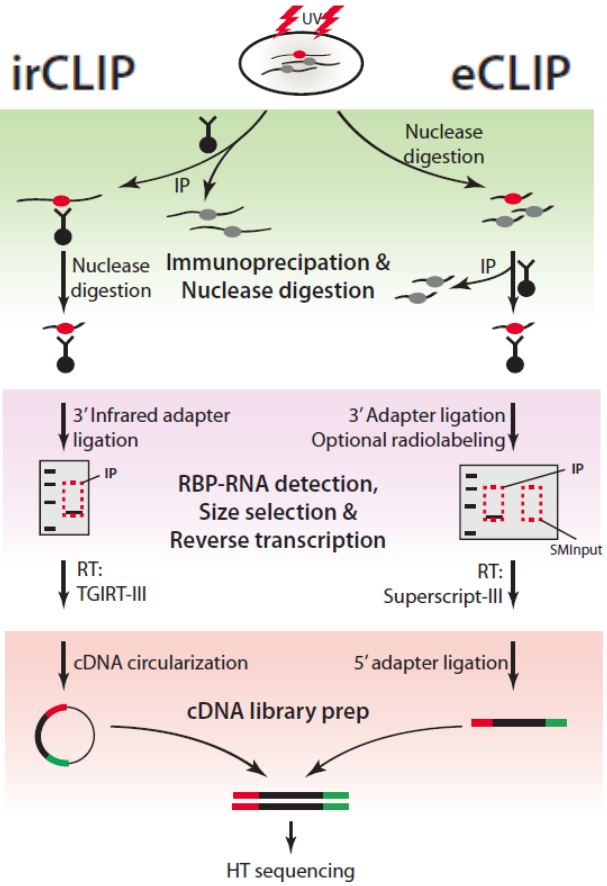
irCLIP platform for efficient characterization of protein–RNA interactions

Brian J Zarnegar[1], Ryan A Flynn[1,2], Ying Shen[1], Brian T Do[1,2], Howard Y Chang[1,2] & Paul A Khavari[1,3]

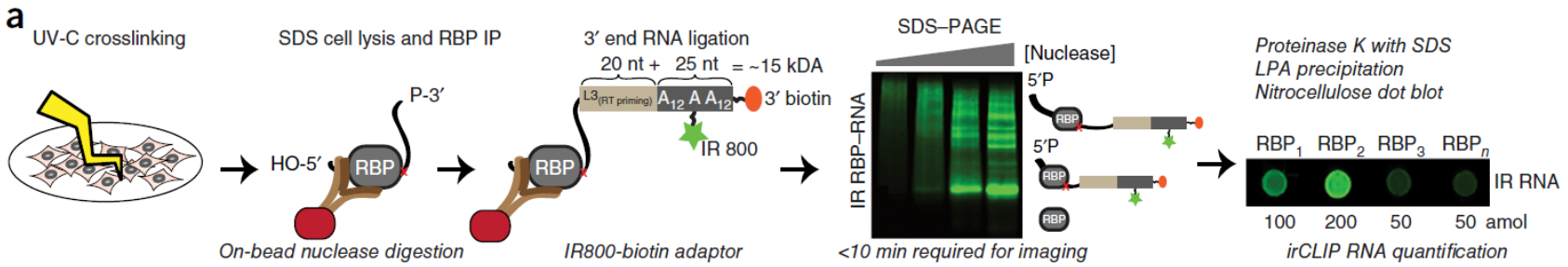
The complexity of transcriptome-wide protein–RNA interaction networks is incompletely understood. While emerging studies are greatly expanding the known universe of RNA-binding proteins, methods for the discovery and characterization of protein–RNA interactions remain resource intensive and technically challenging. Here we introduce a UV-C crosslinking and immunoprecipitation platform, irCLIP, which provides an ultraefficient, fast, and nonisotopic method for the detection of protein–RNA interactions using far less material than standard protocols.

PUBLISHED ONLINE 25 APRIL 2016; DOI:10.1038/NMETH.3840

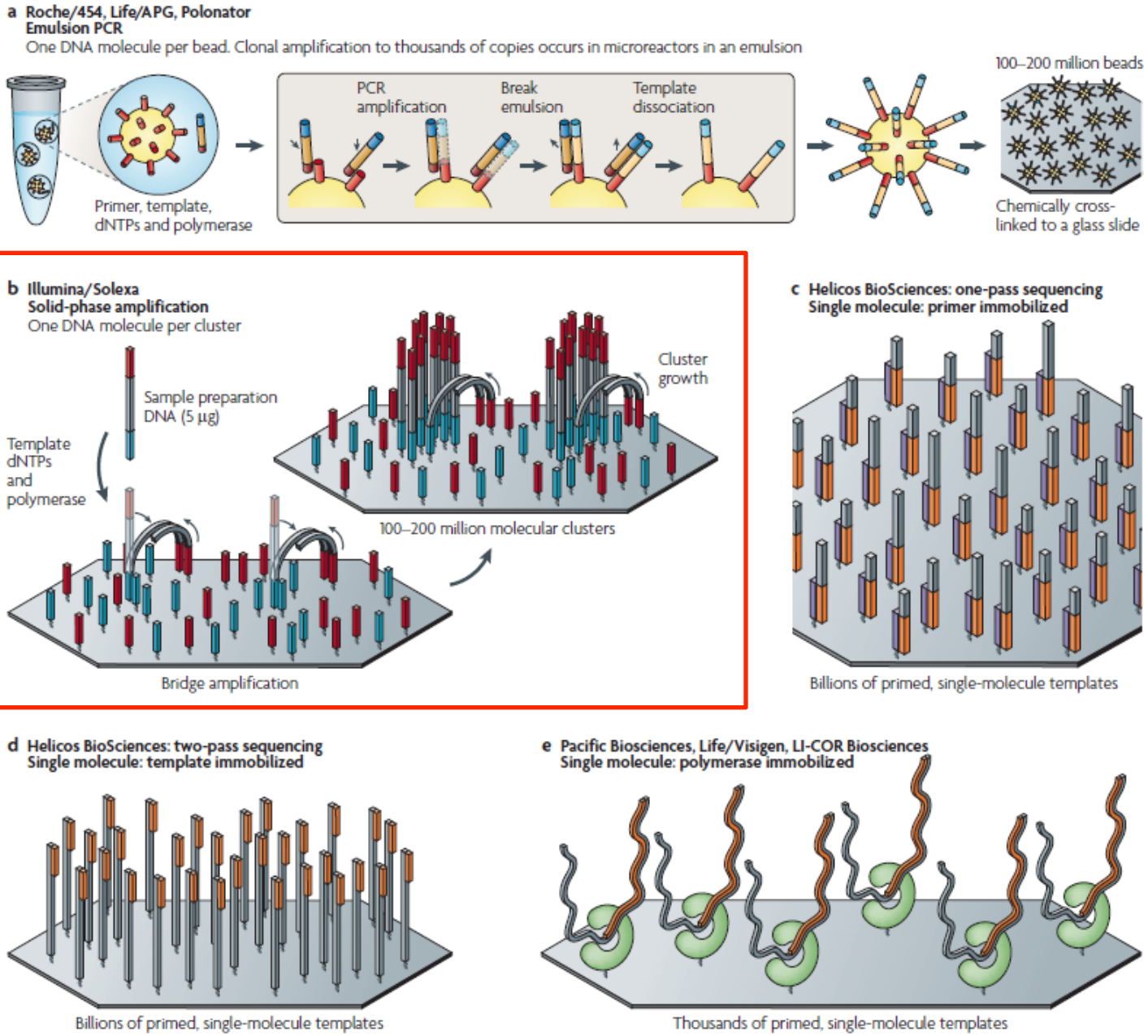
NATURE METHODS | ADVANCE ONLINE PUBLICATION |



Haque and Hogg 2016

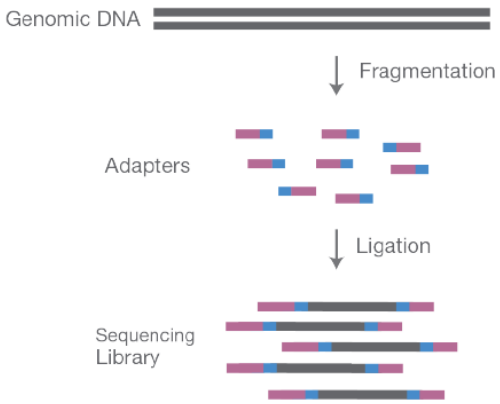


Zarnegar et al. 2016



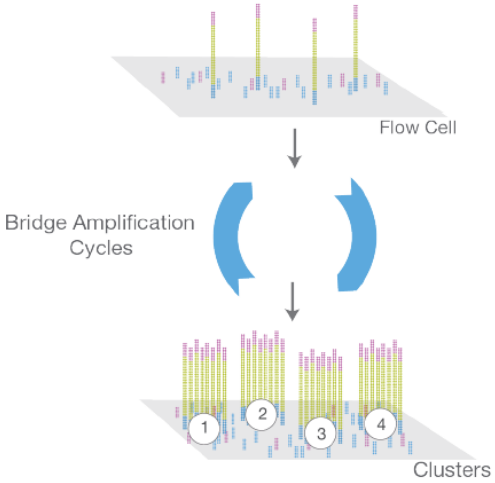
Metzker 2010

A. Library Preparation



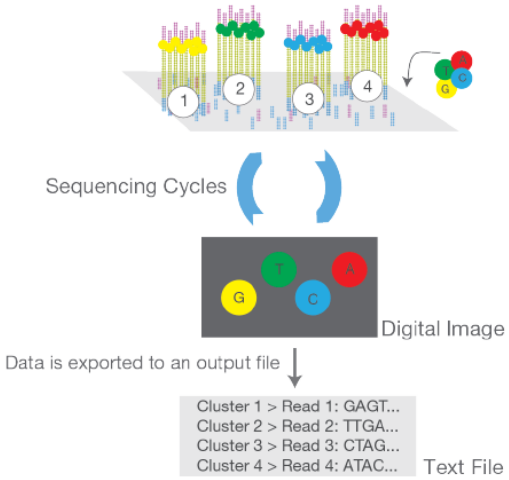
NGS library is prepared by fragmenting a gDNA sample and ligating specialized adapters to both fragment ends.

B. Cluster Amplification



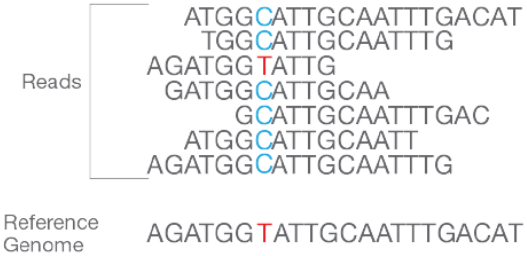
Library is loaded into a flow cell and the fragments are hybridized to the flow cell surface. Each bound fragment is amplified into a clonal cluster through bridge amplification.

C. Sequencing



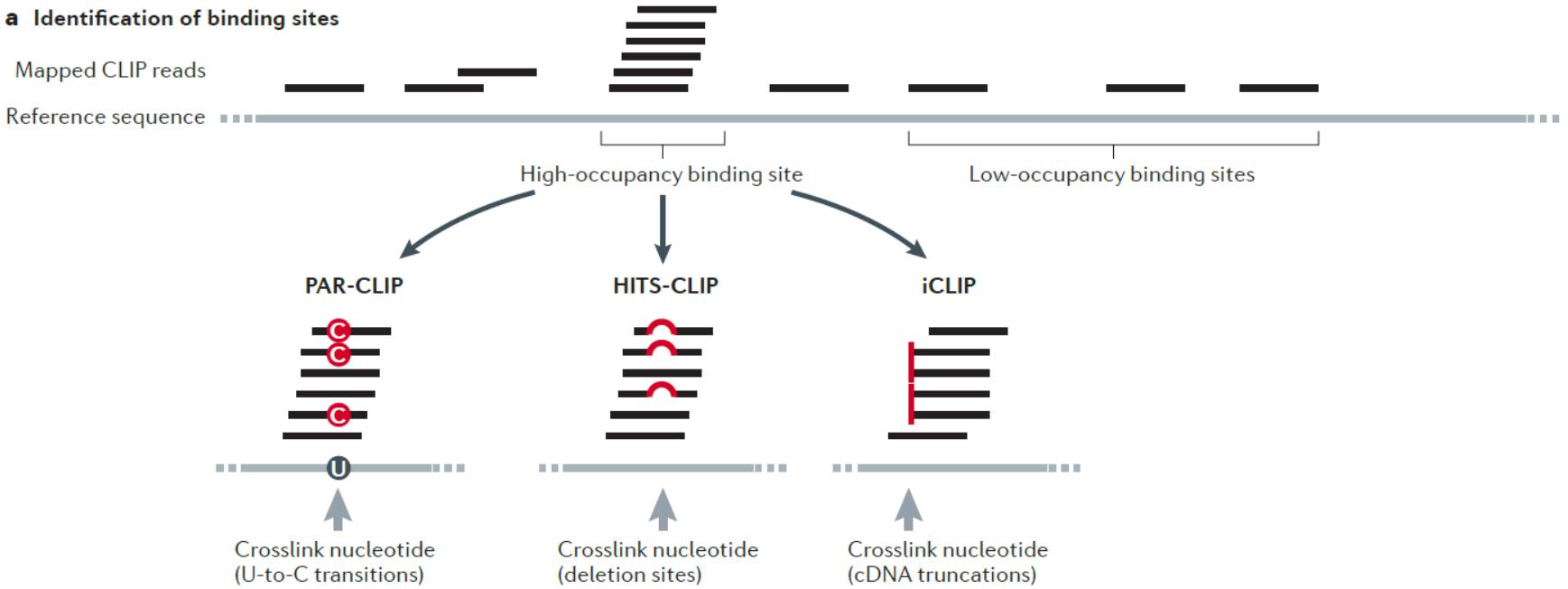
Sequencing reagents, including fluorescently labeled nucleotides, are added and the first base is incorporated. The flow cell is imaged and the emission from each cluster is recorded. The emission wavelength and intensity are used to identify the base. This cycle is repeated "n" times to create a read length of "n" bases.

D. Alignment and Data Anaylsis

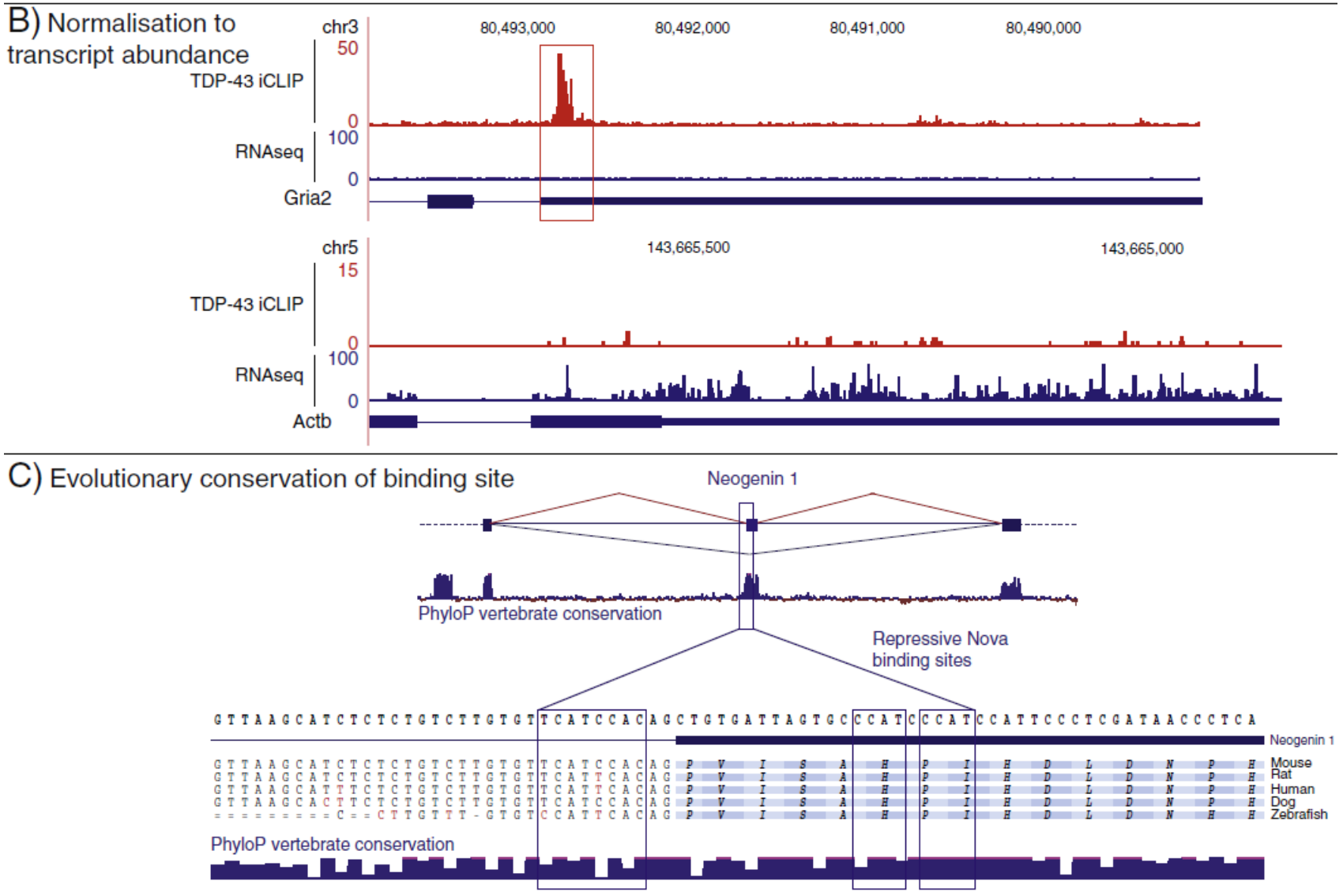


Reads are aligned to a reference sequence with bioinformatics software. After alignment, differences between the reference genome and the newly sequenced reads can be identified.

Crosslink induced mutation sites (CIMS)

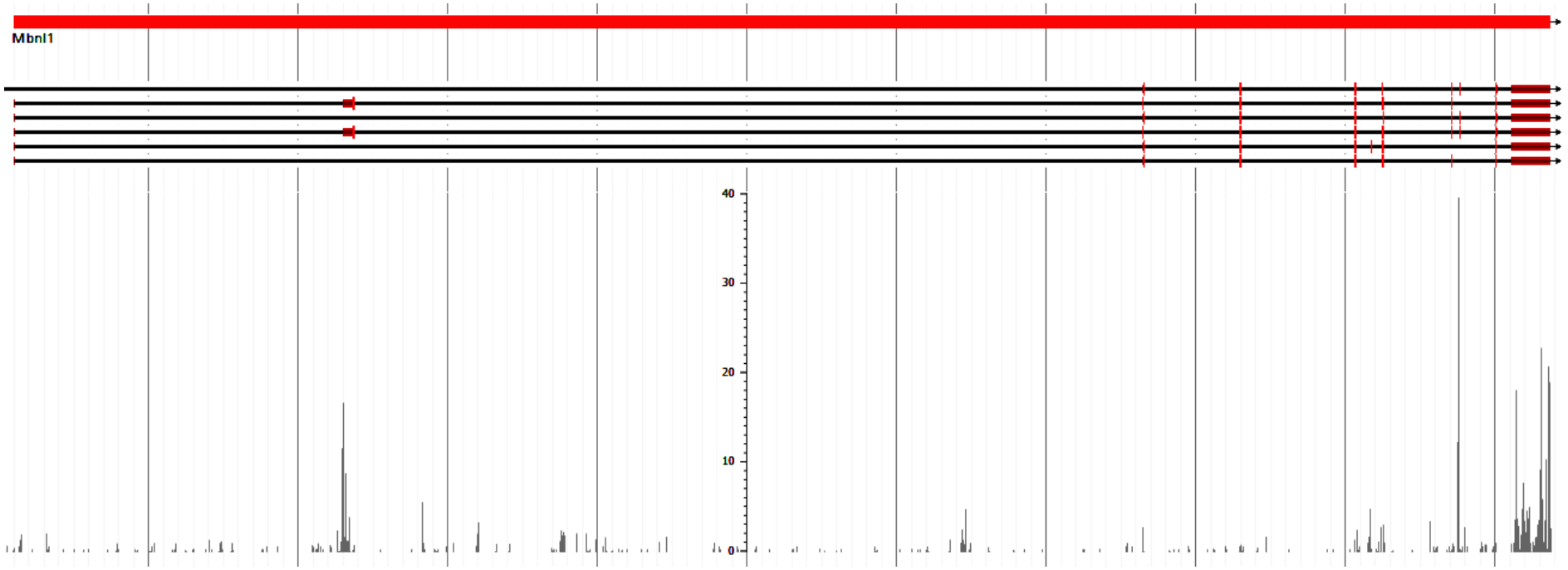


Koenig et al. 2012



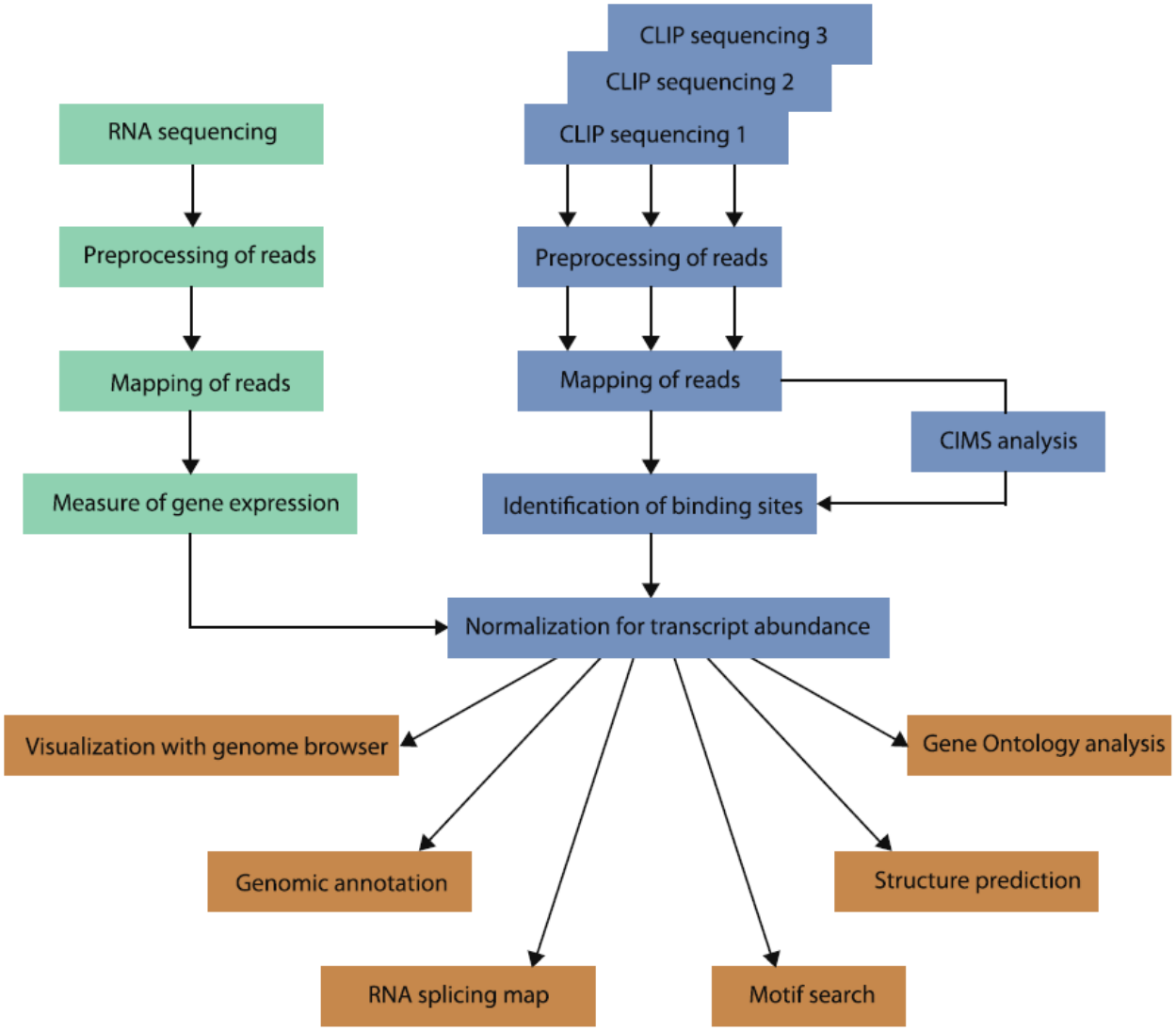
Modic et al. 2013

MBNL Interactome Browser

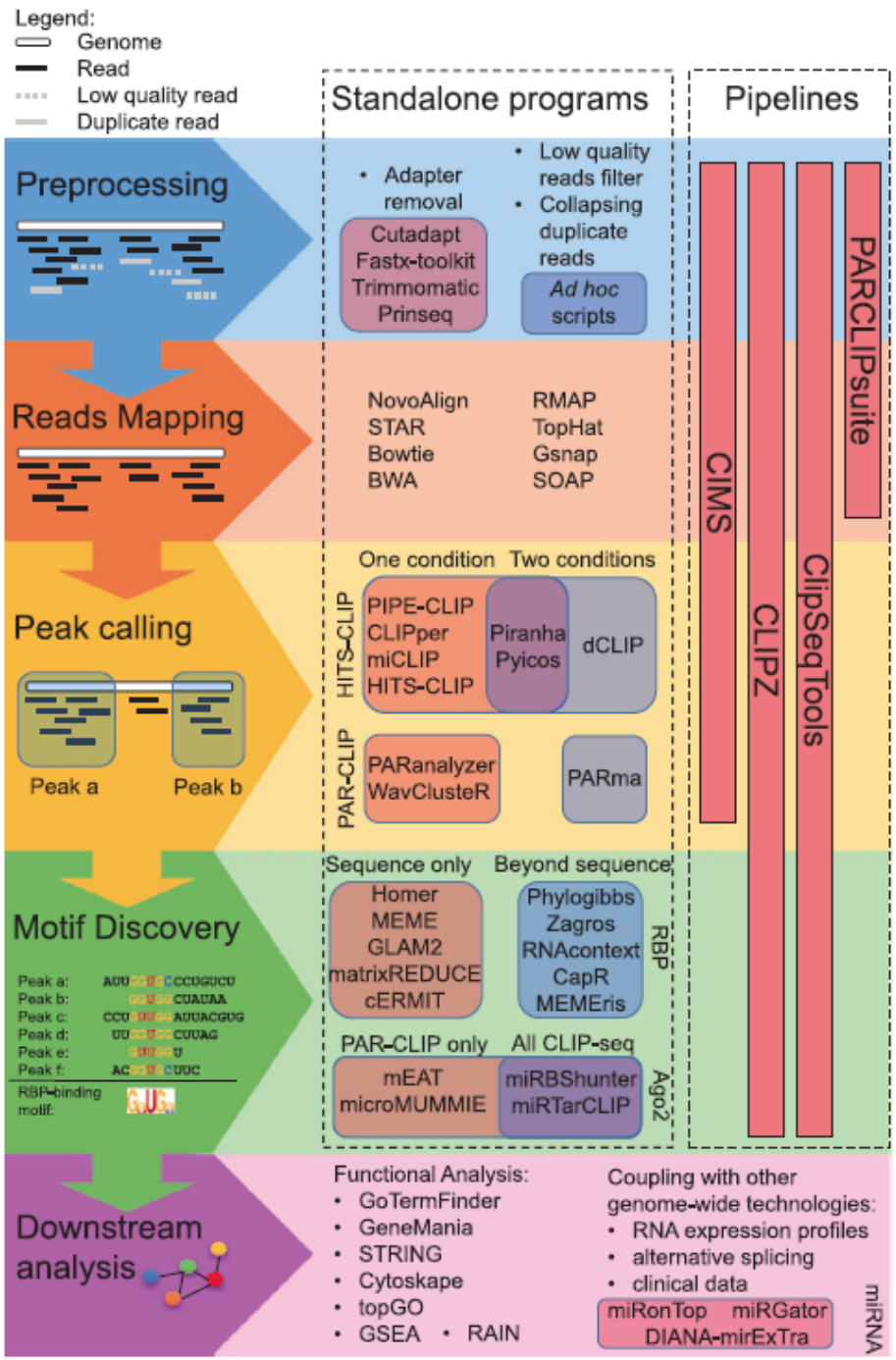


Sznajder et al. 2016; MBNL Interactome Browser (MIB.amu.edu.pl)

Bioinformatic pipeline for CLIP-seq data analysis



Murigneux et al. 2013



Bottini et al. 2017

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- Regulatory networks

Insights into RNA Biology from an Atlas of Mammalian mRNA-Binding Proteins

Alfredo Castello,[1,4] Bernd Fischer,[1,4] Katrin Eichelbaum,[1] Rastislav Horos,[1] Benedikt M. Beckmann,[1] Claudia Strein,[1] Norman E. Davey,[1] David T. Humphreys,[2] Thomas Preiss,[2,3] Lars M. Steinmetz,[1] Jeroen Krijgsveld,[1,*] and Matthias W. Hentze[1,2,*]

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[4]These authors contributed equally to this work
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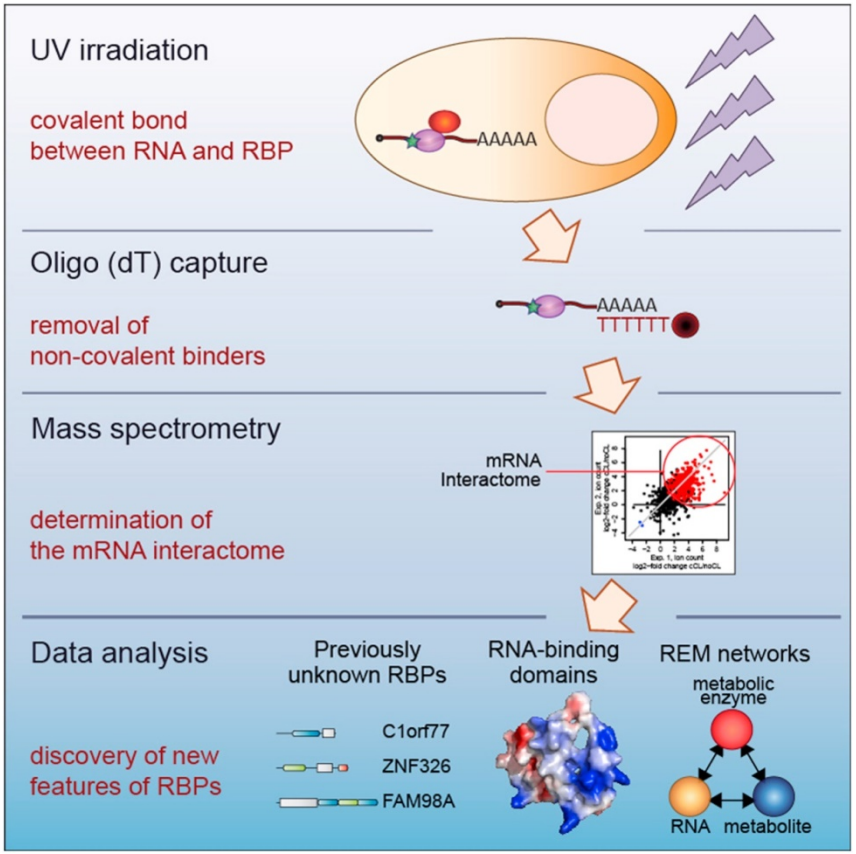
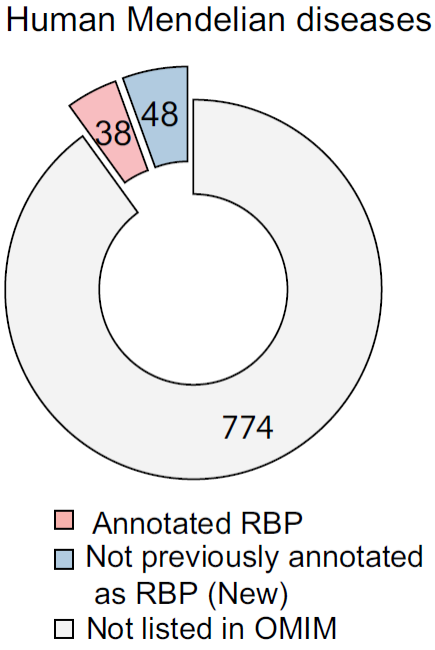
DOI 10.1016/j.cell.2012.04.031

Interactome capture



Number of proteins of the mRNA interactome listed in the OMIM database



Castello et al. 2012

PROTOCOL

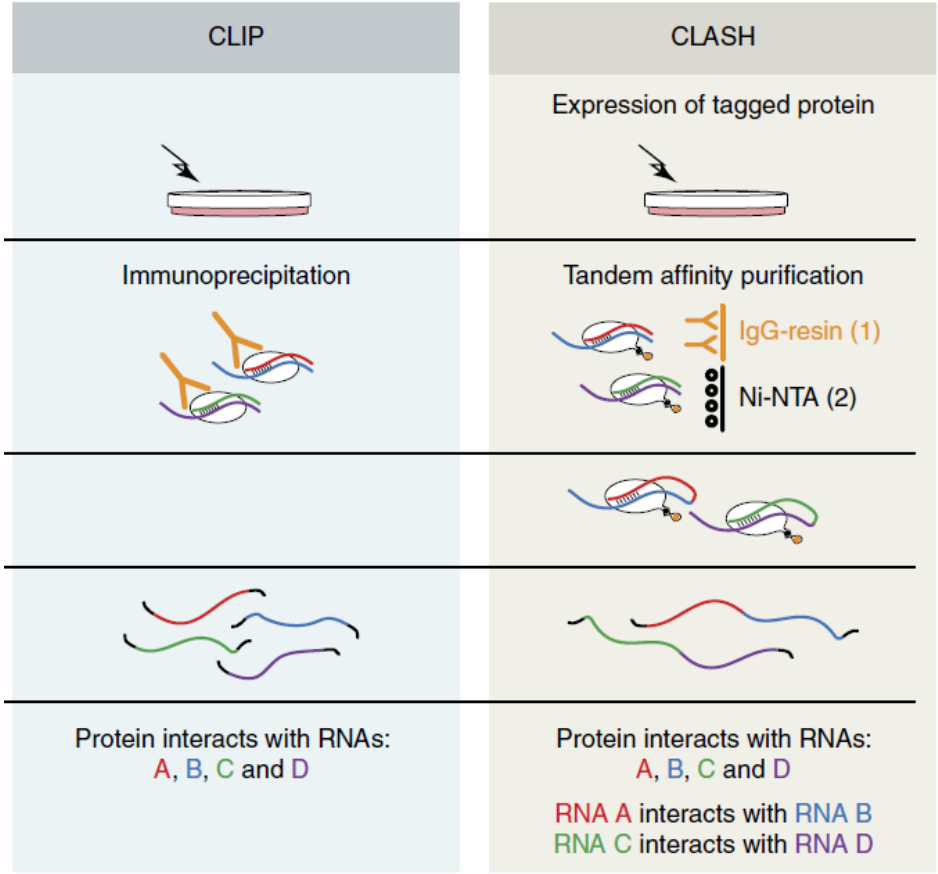
Mapping the miRNA interactome by cross-linking ligation and sequencing of hybrids (CLASH)

Aleksandra Helwak & David Tollervey

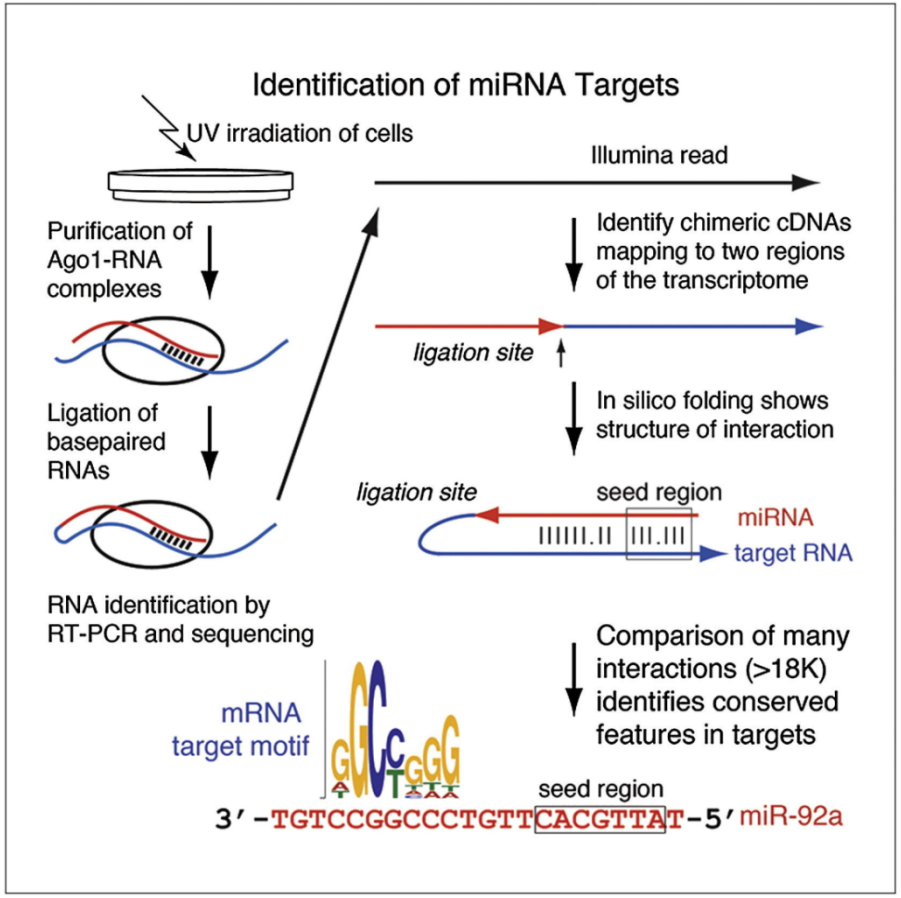
Wellcome Trust Centre for Cell Biology, The University of Edinburgh, Edinburgh, UK. Correspondence should be addressed to A.H. (olahelwak@yahoo.com) or D.T. (d.tollervey@ed.ac.uk).

Published online 27 February 2014; doi:10.1038/nprot.2014.043

RNA-RNA interactions have critical roles in many cellular processes, but studying them is difficult and laborious. Here we describe an experimental procedure, termed cross-linking ligation and sequencing of hybrids (CLASH), which allows high-throughput identification of sites of RNA-RNA interaction. During CLASH, a tagged bait protein is UV–cross-linked in cell cultures to stabilize RNA interactions, and it is purified under denaturing conditions. RNAs associated with the bait protein are partially truncated, and the ends of RNA duplexes are ligated together. After linker addition, cDNA library preparation and high-throughput sequencing, the ligated duplexes give rise to chimeric cDNAs, which unambiguously identify RNA-RNA interaction sites independent of bioinformatic predictions. This protocol is optimized for studying miRNA targets bound by Argonaute (AGO) proteins, but it should be easily adapted for other RNA-binding proteins and classes of RNA. The protocol requires ~5 d to complete, excluding the time required for high-throughput sequencing and bioinformatic analyses.



Helwak and Tollervey 2014



Helwak et al. 2013

A experimental approach identifies in vivo targets for miRNAs

miRNAs show frequent noncanonical targeting of mRNAs

Nonseed interactions are common and functional

miRNA base pairing shows distinct patterns and overrepresented motifs

PROTOCOL

Using hiCLIP to identify RNA duplexes that interact with a specific RNA-binding protein

Yoichiro Sugimoto[1,2], Anob M Chakrabarti[2,3], Nicholas M Luscombe[2–4] & Jernej Ule[1,2]

[1]Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK. [2]The Francis Crick Institute, London, UK. [3]Department of Genetics, Evolution and Environment, UCL Genetics Institute, University College London, London, UK. [4]Okinawa Institute of Science and Technology Graduate University, Onna-son, Okinawa, Japan. Correspondence should be addressed to J.U. (jernej.ule@crick.ac.uk).

Published online 23 February 2017; doi:10.1038/nprot.2016.188

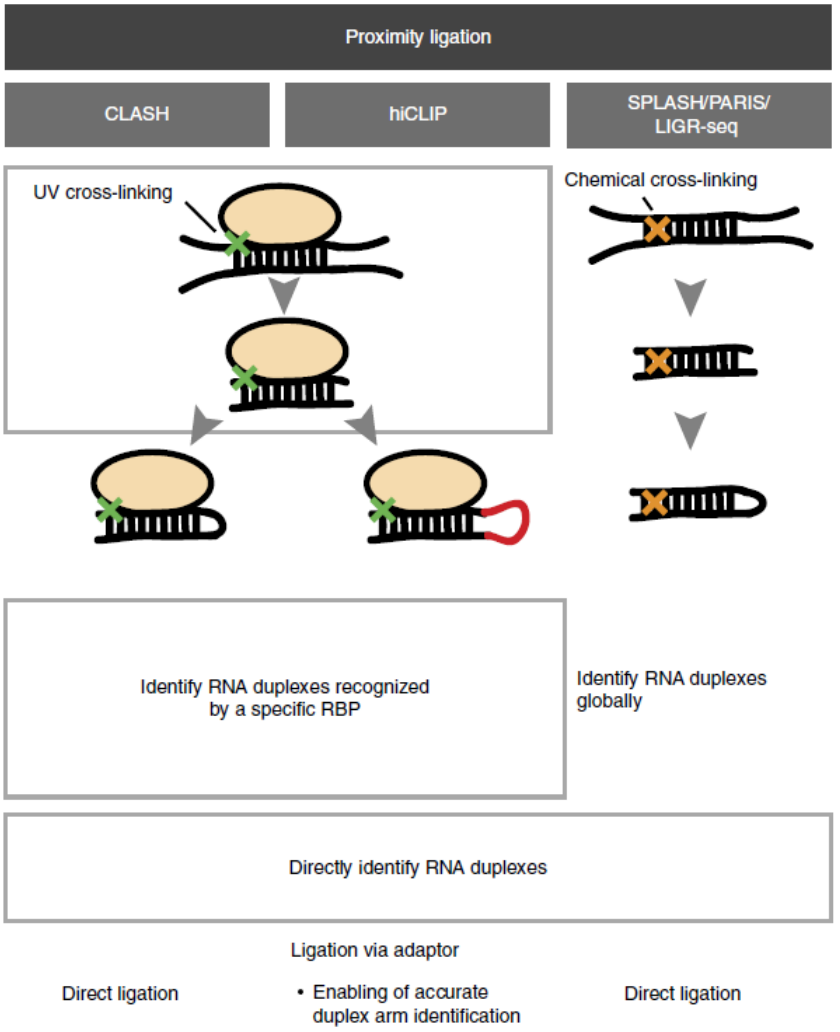
RNA hybrid and individual-nucleotide resolution ultraviolet crosslinking and immunoprecipitation (hiCLIP)

LETTER

doi:10.1038/nature14280

hiCLIP reveals the *in vivo* atlas of mRNA secondary structures recognized by Staufen 1

Yoichiro Sugimoto[1], Alessandra Vigilante[3,4]*, Elodie Darbo[3]*, Alexandra Zirra[2], Cristina Militti[2], Andrea D'Ambrogio[1,2], Nicholas M. Luscombe[3,4,5] & Jernej Ule[1,2]



Sugimoto et al. 2017

- RNA binding proteins
- CLIP-seq (HITS-CLIP, iCLIP, PAR-CLIP, eCLIP, irCLIP)
- CLIP-seq related methods (interactome capture, CLASH, hiCLIP)
- CLIP-seq in paraneoplastic opsoclonus-myoclonus-ataxia (POMA), myotonic dystrophy (DM), fragile X syndrome (FXS)
- Regulatory networks

Paraneoplastic opsoclonus-myoclonus-ataxia (POMA)

autoimmune disorder characterized by involuntary, irregular, repetitive, **rapid conjugated eye movements** that occur in all directions and brief multifocal **myoclonic muscle jerks** usually accompanied by cerebellar dysfunction with dysarthria and ataxia

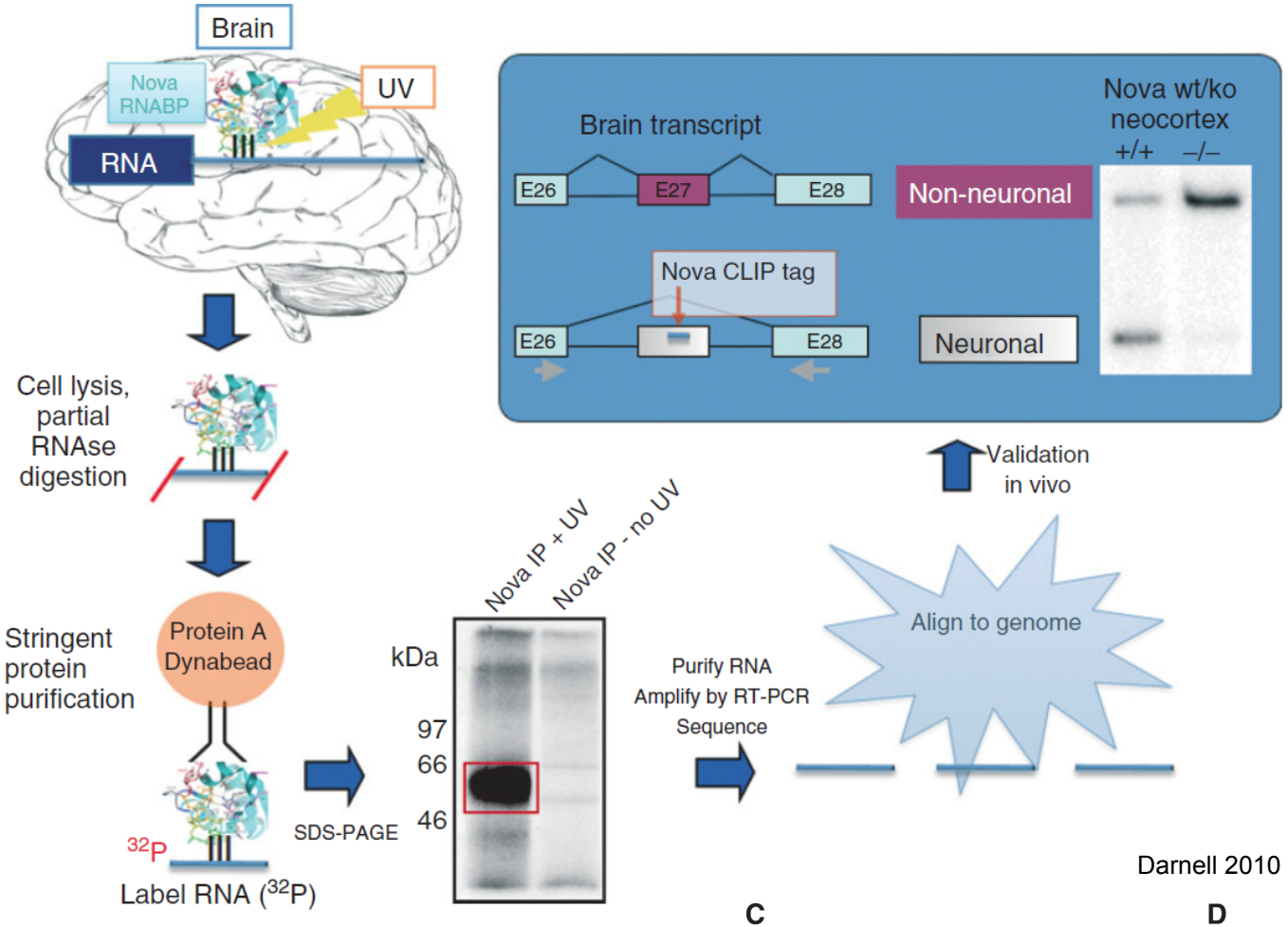
rare syndrome that arise as a result of an **anti-tumor/anti-neuronal immune response** in the setting of a tumor expressing proteins

associated with a variety of tumors but most often with small cell lung cancer and breast cancer, while in children OMA is usually associated with neuroblastoma

high-titer **autoantibodies in blood and/or cerebrospinal fluid (CSF)** that recognize two closely related neuronal RNA-binding proteins designated **Nova-1 and Nova-2**

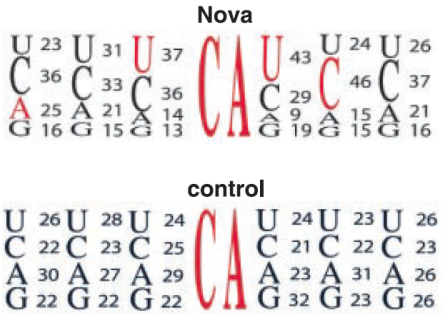
Van Diest et al. 2008

NOVA CLIP-seq



Darnell 2010

C

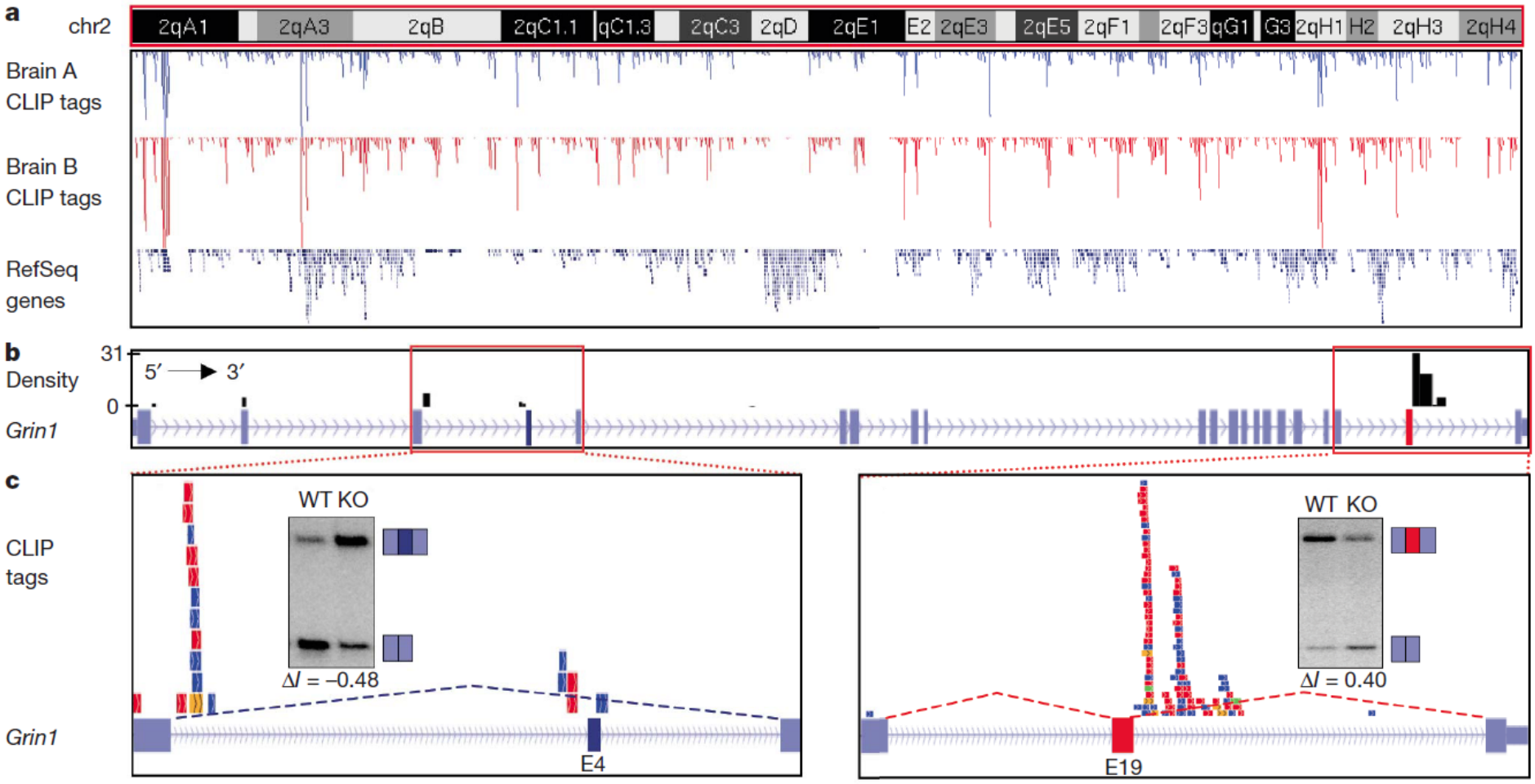


D

hexamer	Nova tags obs/exp	control tags obs/exp
UCCAUC	30.1	1.3
CCAUCC	27.3	1.2
AUCCAU	25.0	0.9
CAUCCA	23.7	1.7
UCAUCC	14.7	0.9

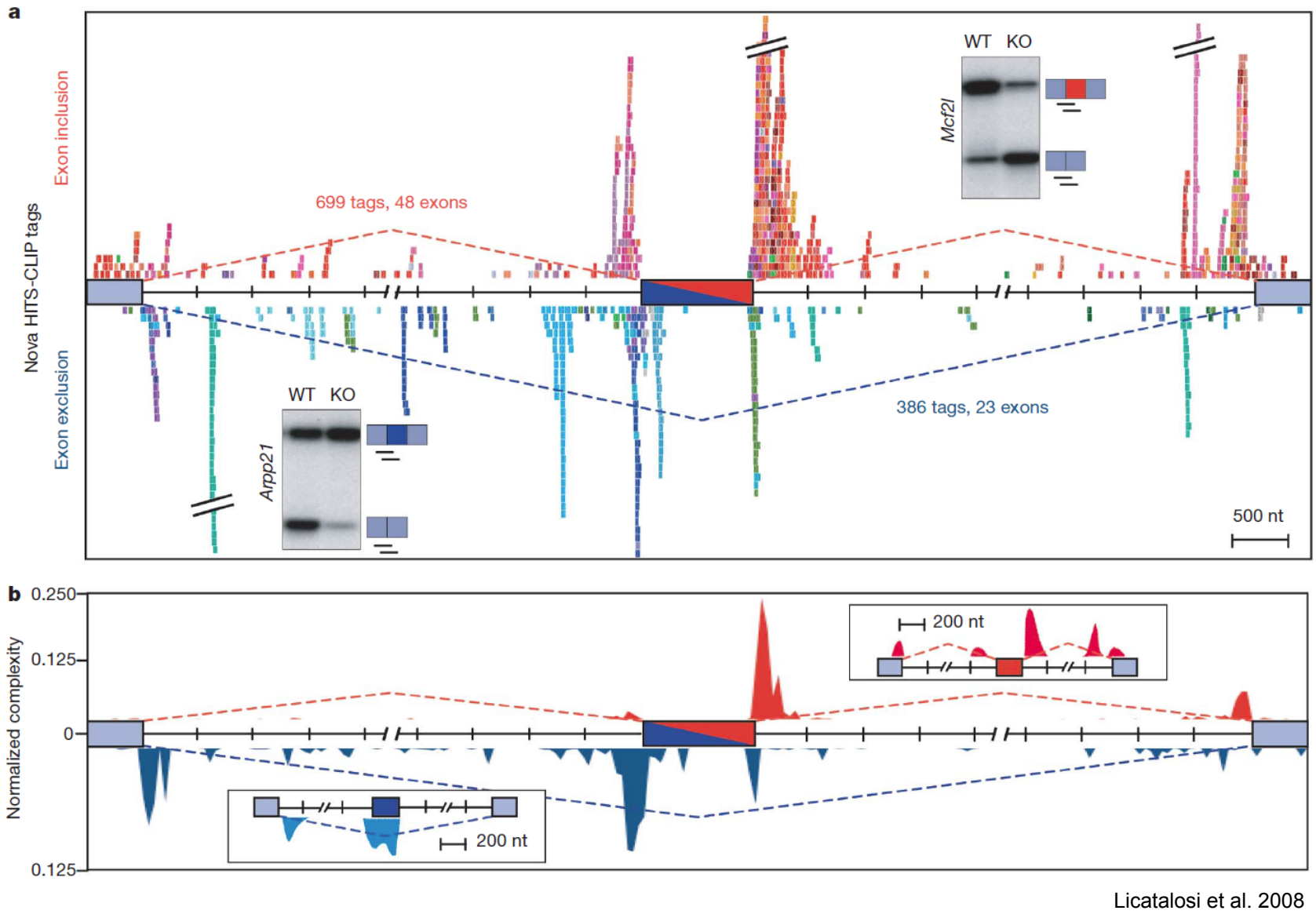
Ule et al. 2003

CLIP-seq genome-wide map of Nova–RNA binding sites



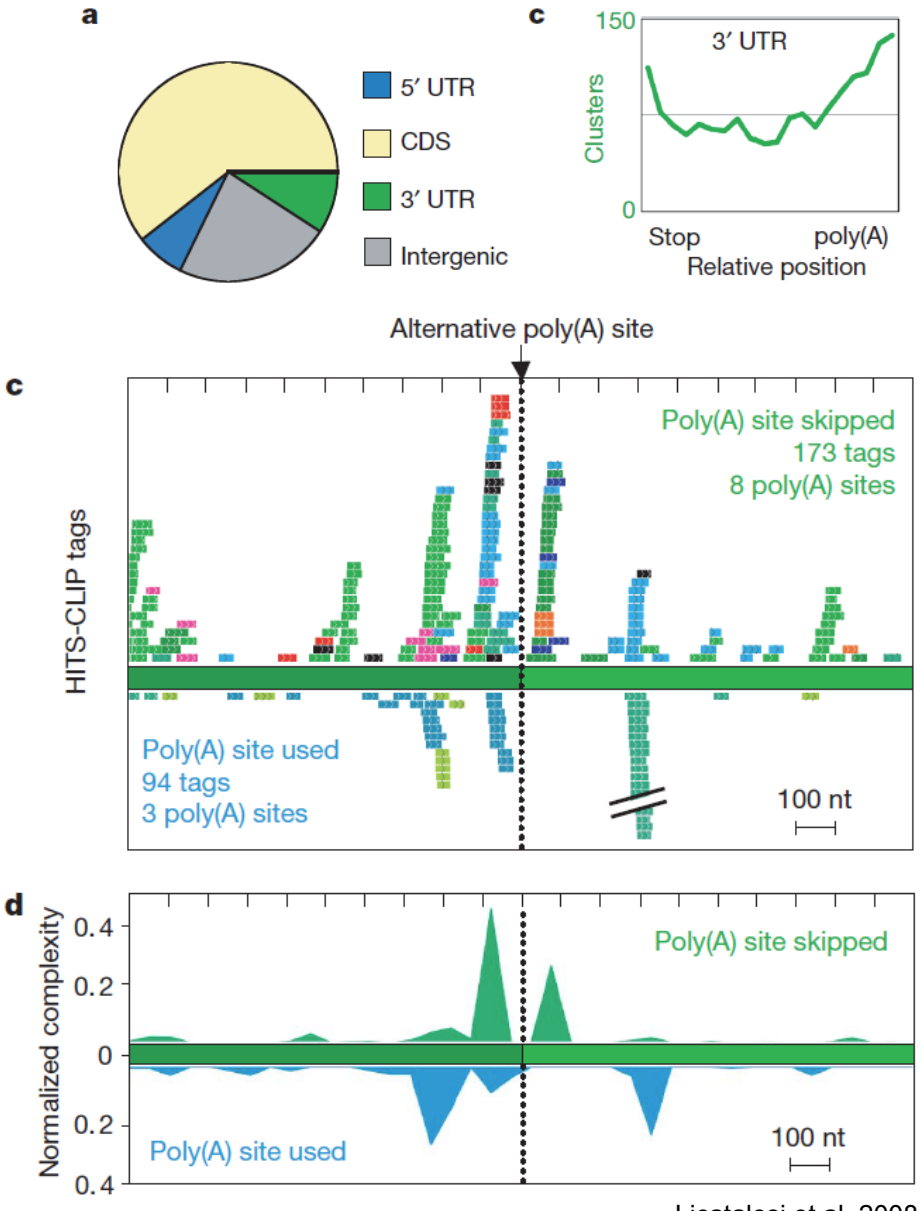
Licatalosi et al. 2008

Nova–RNA interaction maps associated with Nova-dependent splicing regulation



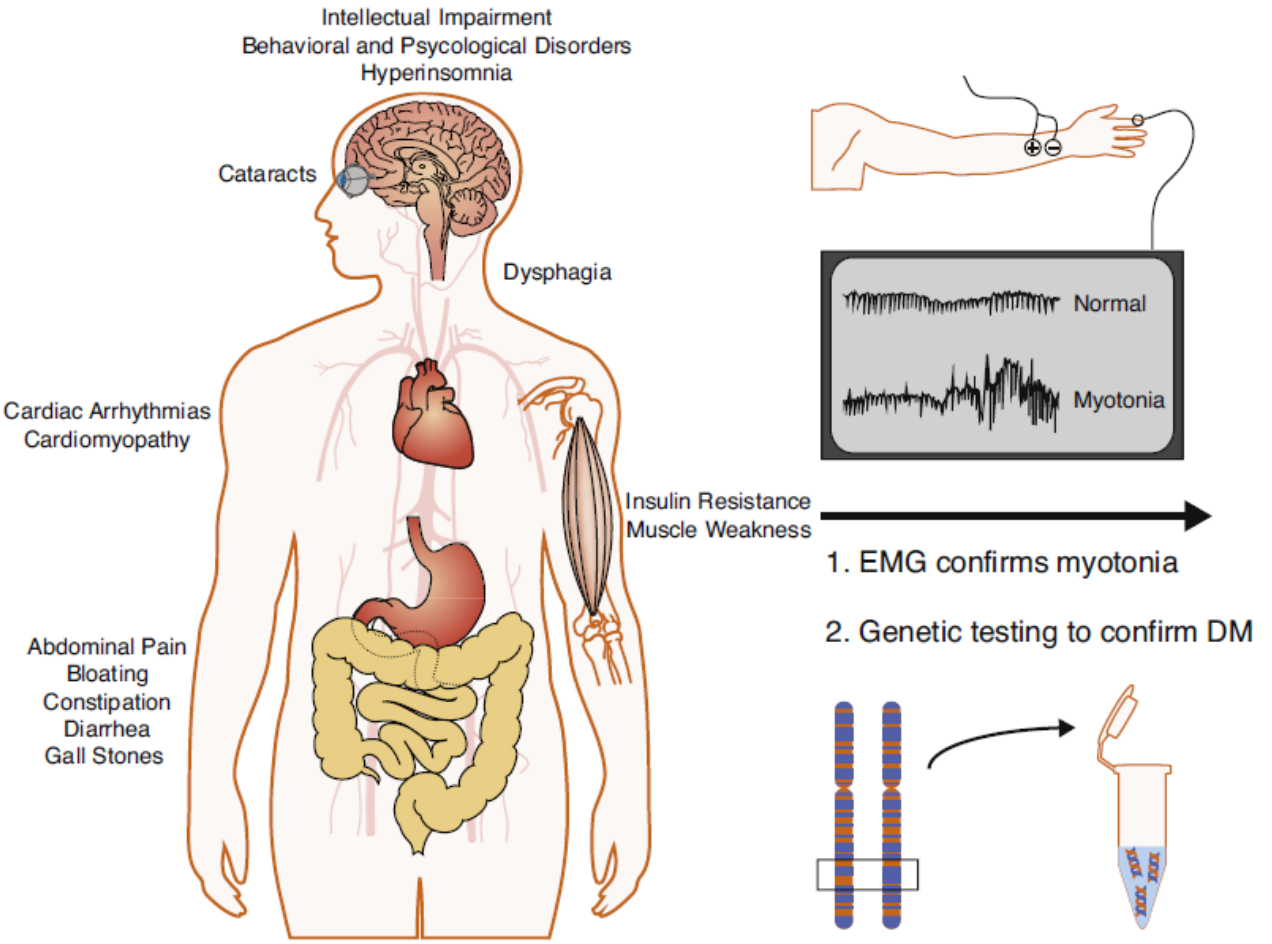
Licatalosi et al. 2008

Nova CLIP tags cluster near polyadenylation sites



Licatalosi et al. 2008

Myotonic dystrophy



Yum et al. 2017

Autosomal dominant disease

DM1 - Expansion of CTG triplets in 3'UTR of ***DMPK*** (*chrom. 19*)
DM2 - Expansion of CCTG repeats in intron 1 of ***ZNF9*** (*chrom. 3*)

Occurrence 1 per 6-10,000 people

DM1 – Myotonic Dystrophy type 1

Normal gene *… CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG …*

Mutated gene *…*

CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG
CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG
CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG
CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG
CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG
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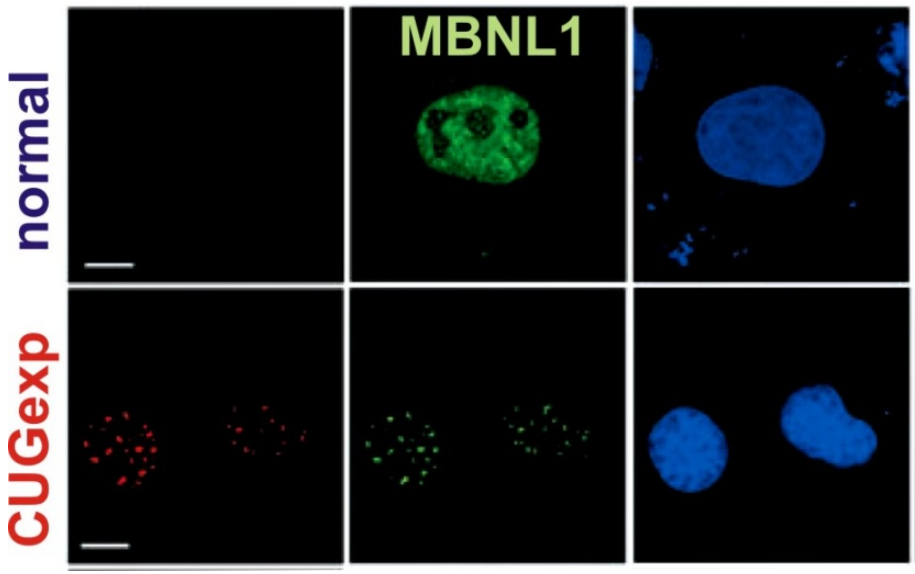
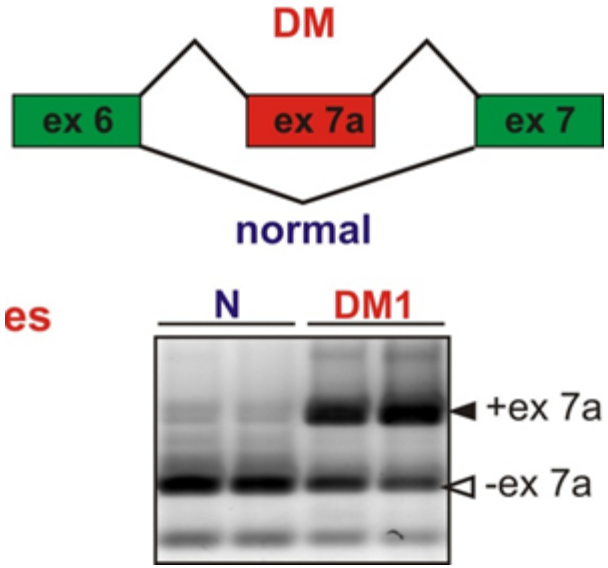
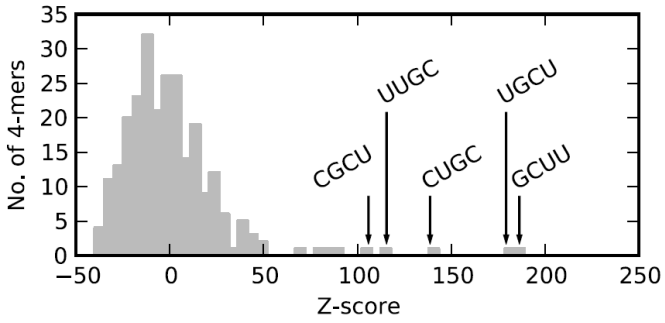
CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG
CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG …

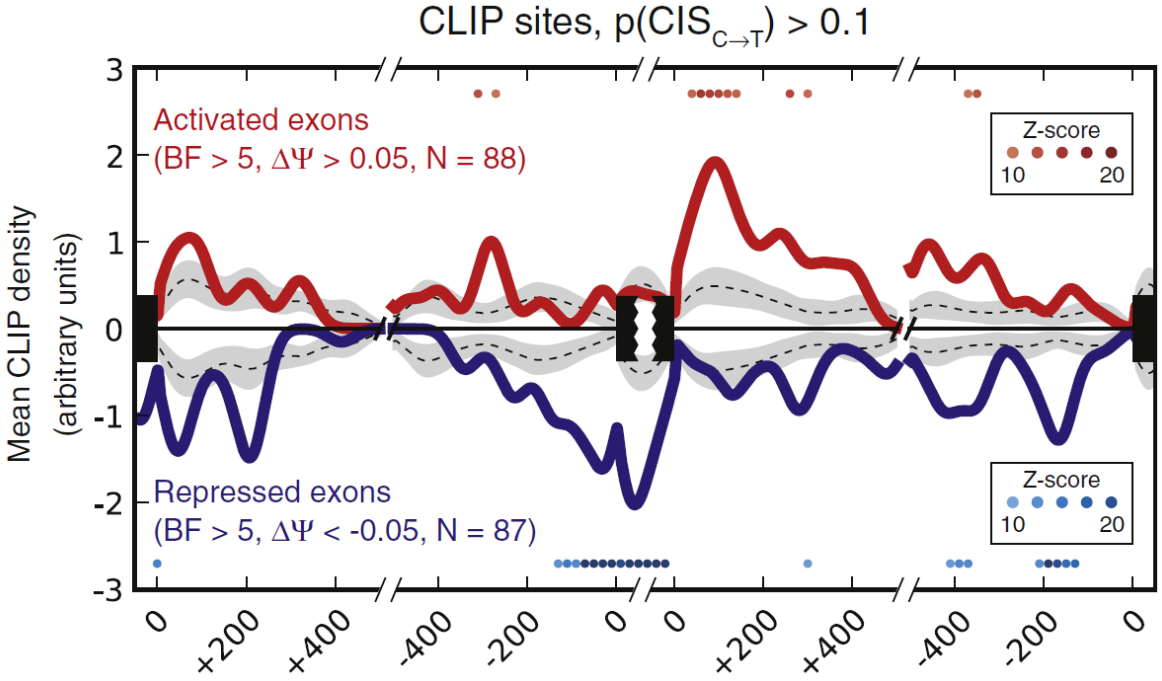
Sequestration of MBNL splicing regulator in DM





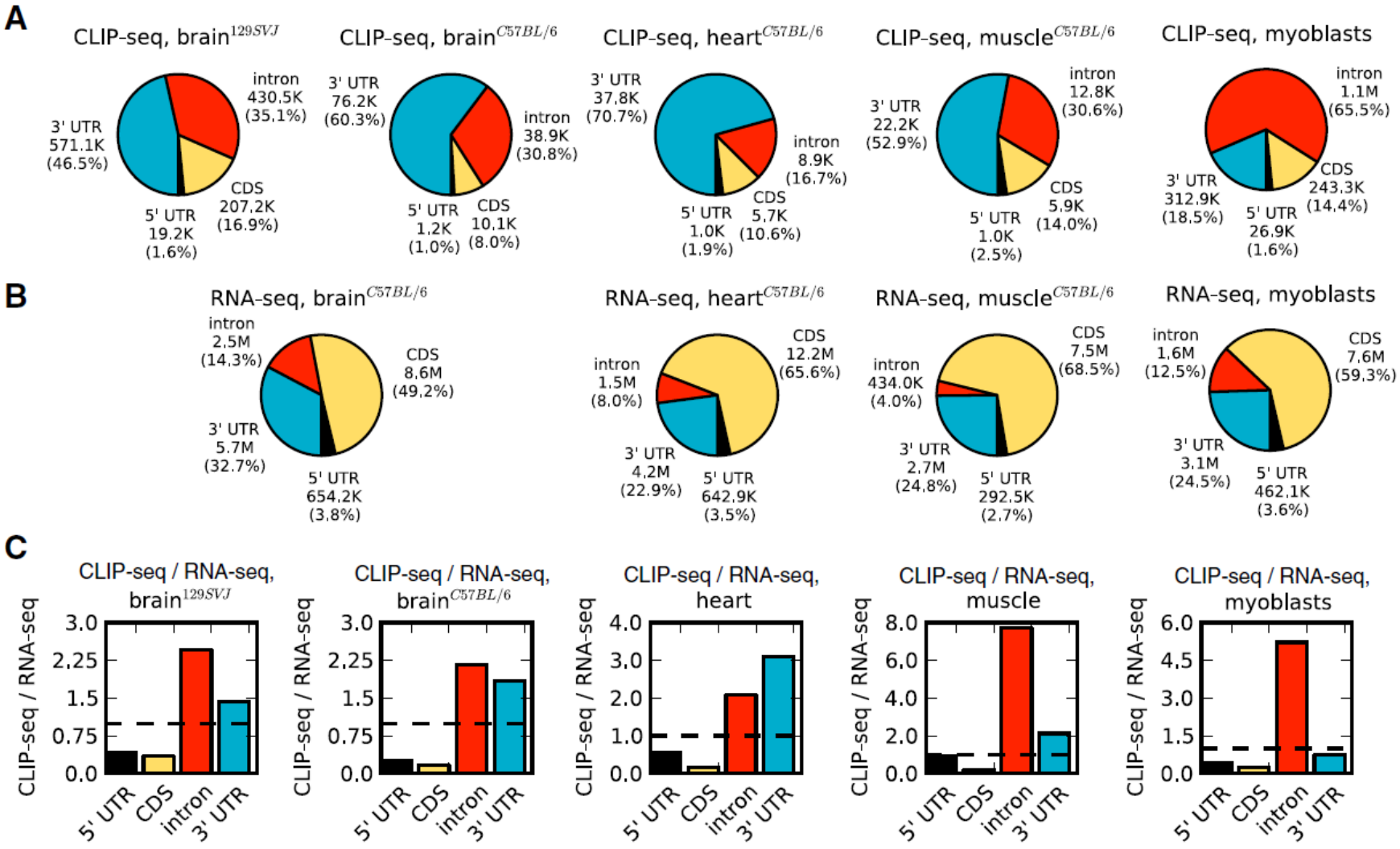
MBNL1 binds preferentially UGCU rich motifs





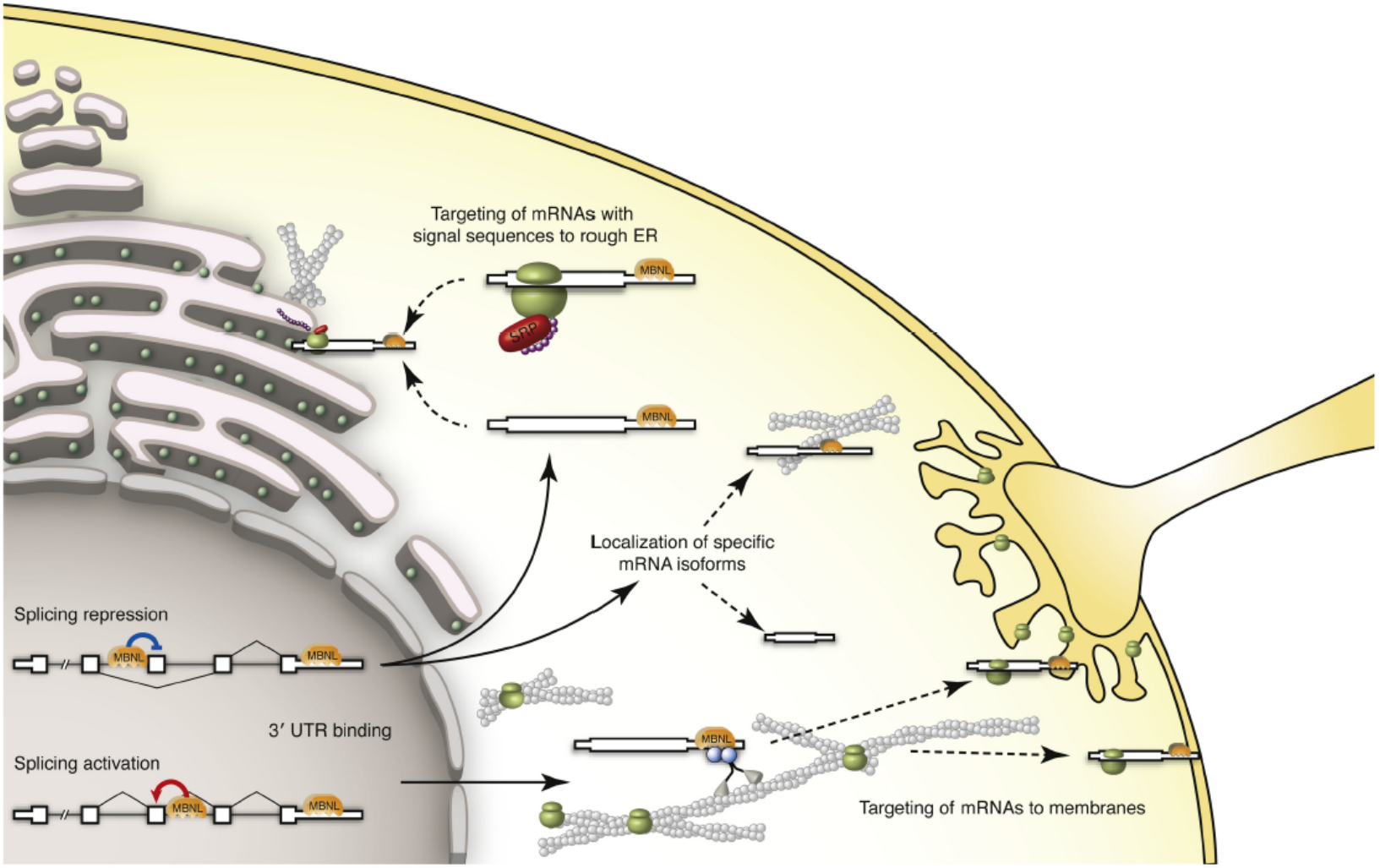
Wang et al. 2012

MBNL1 associates with high frequency with 3'UTRs



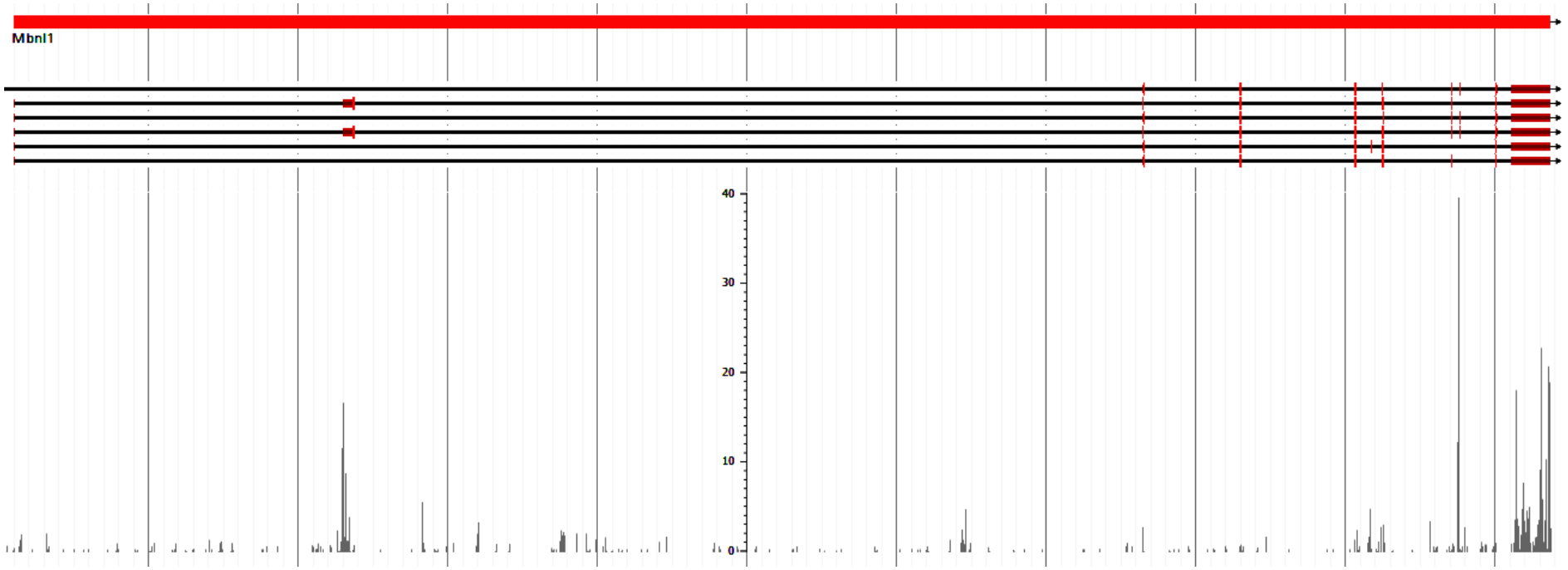
Wang et al. 2012

A Model for Nuclear and Cytoplasmic Functions of Mbnl



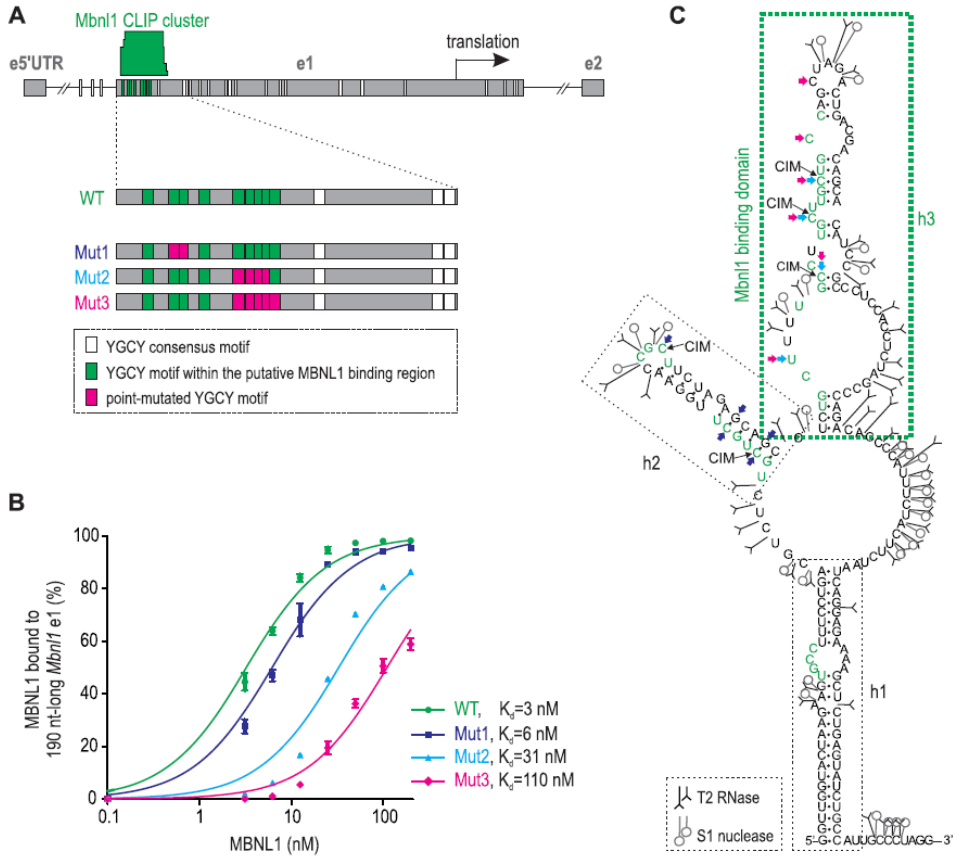
Wang et al. 2012

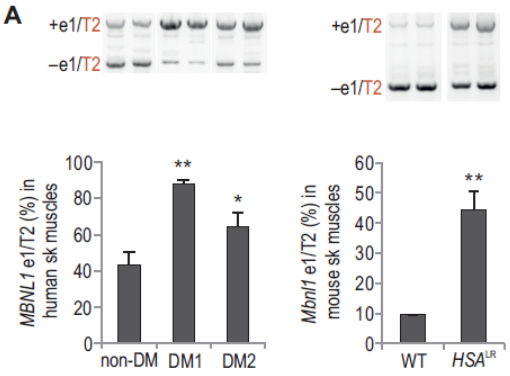
MBNL Interactome Browser



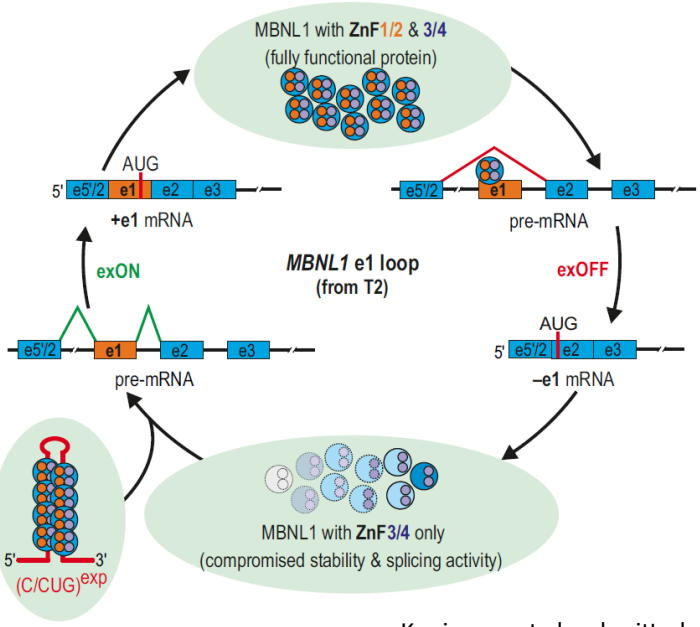
Sznajder et al. 2016; MBNL Interactome Browser (MIB.amu.edu.pl)

Autoregulation of MBNL1 function by exon 1 exclusion from *MBNL1* transcript





Konieczny et al. 2016



Konieczny et al. submitted

Fragile X syndrome (FXS)

(A) Fragile X-associated tremor/ataxia syndrome (FXTAS)

Onset: late (60.6 ± 8.6 years)

Clinical phenotype: intention tremor, cerebellar ataxia, neuropathic pain, parkinsonian features, psychological disorders

Other: brain atrophy and white matter disease; can lead to premature death

Mutation: 55 to 200 CGG repeats (premutation) in 5'UTR of *FMR1* gene

Prevalence: 1 in 150–300 females and 1 in 400–850 males

Penetrance: 16–20% of females and 40–75% of males with premutation

(B) Fragile X syndrome (FXS)

Onset: early (2 years)

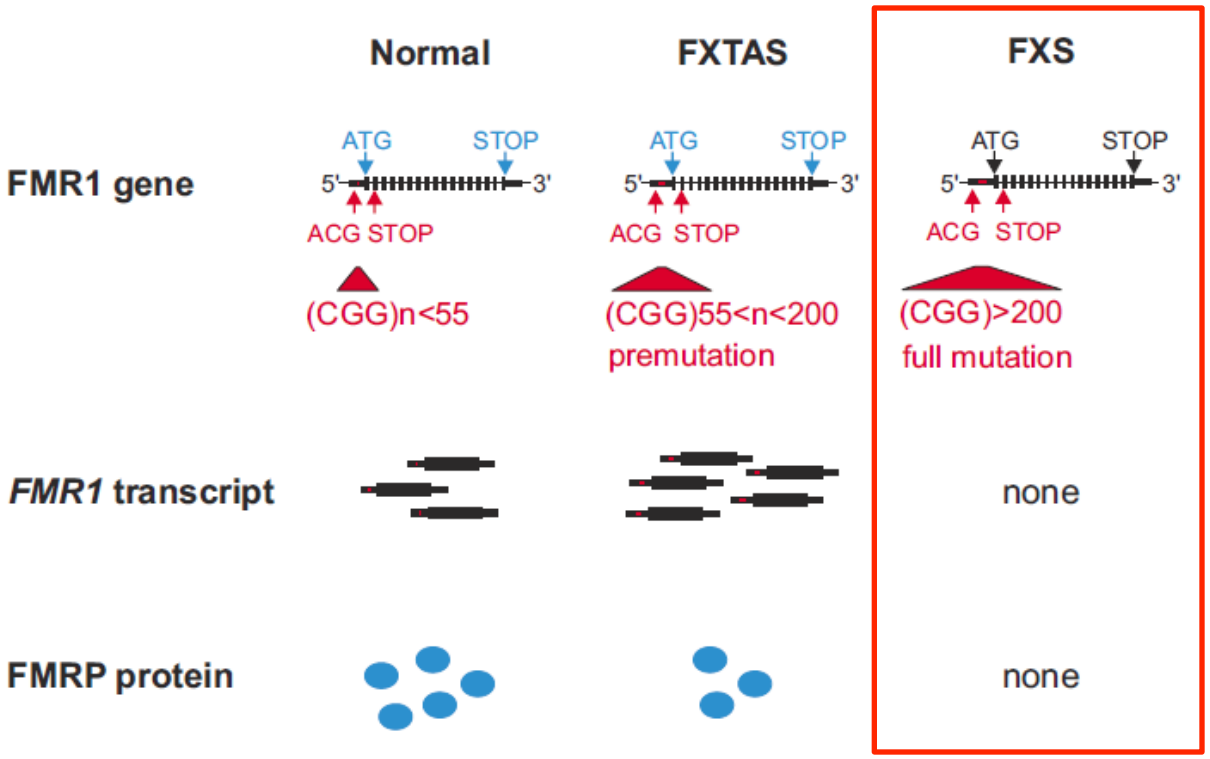
Clinical phenotype: intellectual disability and autism spectrum disorder, postpubertal macroorchism, long face, hyperextensible joints, prominent ears

Other: can lead to shortened lifespan

Mutation: > 200 CGG repeats (full mutation) in 5'UTR of *FMR1* gene

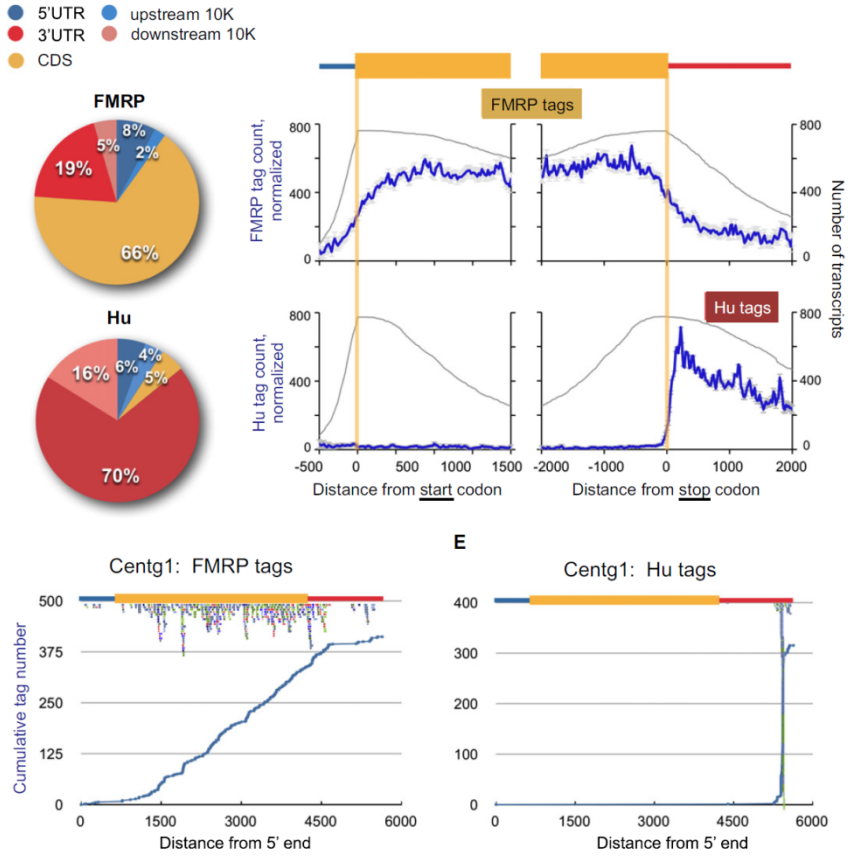
Prevalence: 1 in 7000 females and 1 in 4000 males

Penetrance: 30-50% of females and 80% of males

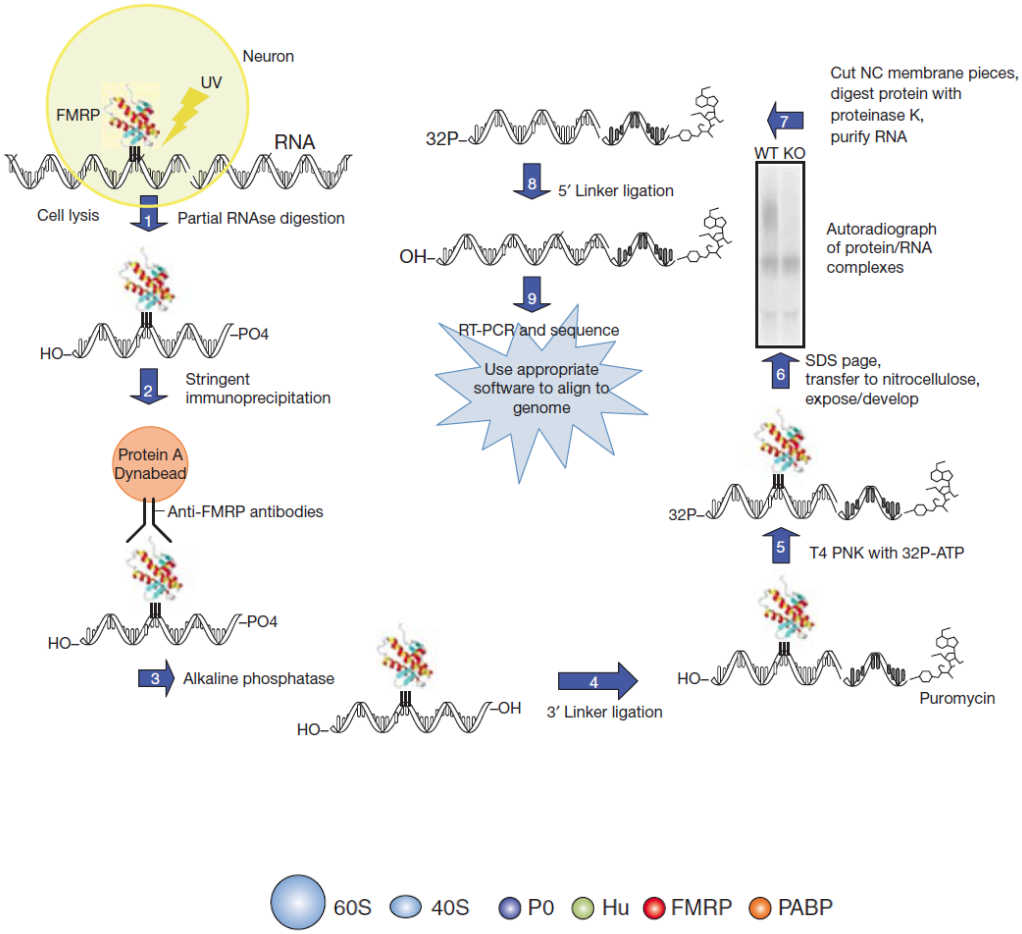


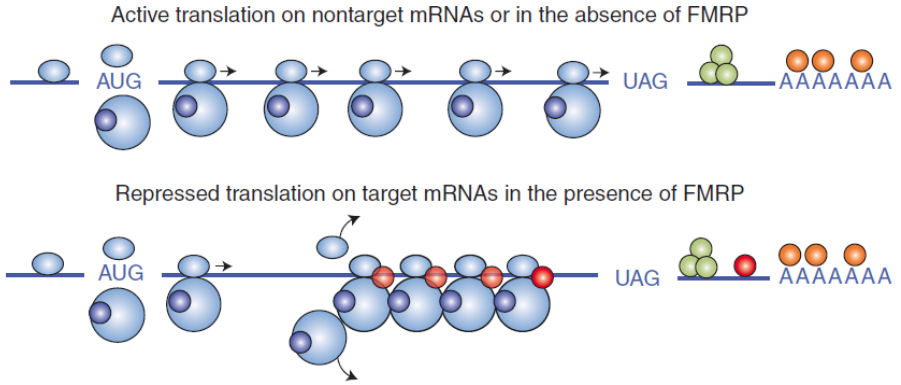
FMRP Stalls Ribosomal Translocation on mRNAs Linked to Synaptic Function and Autism

Jennifer C. Darnell,[1,*] Sarah J. Van Driesche,[1] Chaolin Zhang,[1] Ka Ying Sharon Hung,[1] Aldo Mele,[1] Claire E. Fraser,[1] Elizabeth F. Stone,[1] Cynthia Chen,[1] John J. Fak,[1] Sung Wook Chi,[1,4] Donny D. Licatalosi,[1,5] Joel D. Richter,[3] and Robert B. Darnell[1,2,*]
[1]Laboratory of Molecular Neuro-Oncology
[2]Howard Hughes Medical Institute
The Rockefeller University, New York, NY 10065, USA
[3]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA
[4]Present address: Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, NY 11724, USA
[5]Present address: Case Western Reserve University, Center for RNA Molecular Biology, Wood Building, RT100, 10900 Euclid Avenue, Cleveland, OH 44106, USA
*Correspondence: darneje@rockefeller.edu (J.C.D.), darnelr@rockefeller.edu (R.B.D.)
DOI 10.1016/j.cell.2011.06.013



Darnell et al. 2011





Darnell and Richter 2012

- RNA binding proteins
- CLIP-seq (HITS-CLIP, iCLIP, PAR-CLIP, eCLIP, irCLIP)
- CLIP-seq related methods (interactome capture, CLASH, hiCLIP)
- CLIP-seq in paraneoplastic opsoclonus-myoclonus-ataxia (POMA), myotonic dystrophy (DM), fragile X syndrome (FXS)
- Regulatory networks

Cell Reports
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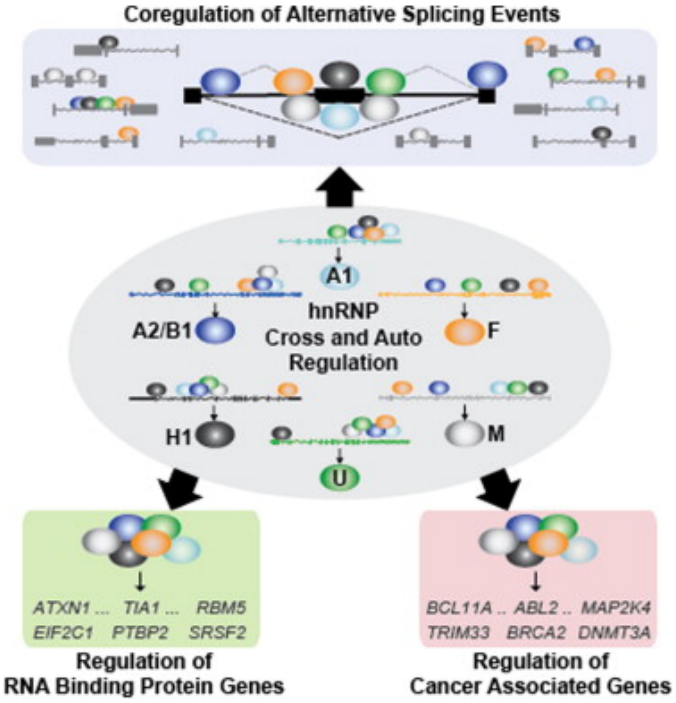
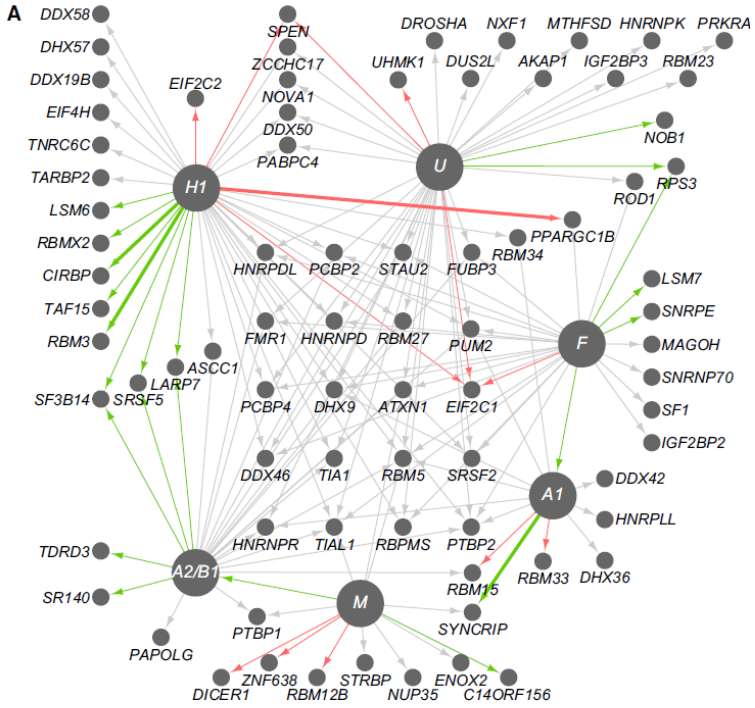
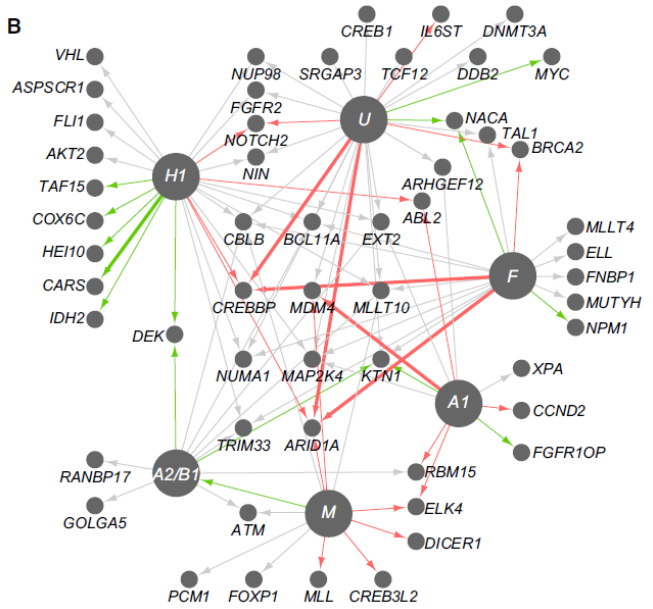
Integrative Genome-wide Analysis Reveals Cooperative Regulation of Alternative Splicing by hnRNP Proteins

Stephanie C. Huelga,[1,2] Anthony Q. Vu,[1,2] Justin D. Arnold,[1,2] Tiffany Y. Liang,[1,2] Patrick P. Liu,[1,2] Bernice Y. Yan,[1,2] John Paul Donohue,[3] Lily Shiue,[3] Shawn Hoon,[4] Sydney Brenner,[4] Manuel Ares Jr.,[3] and Gene W. Yeo[1,2,4,*]
[1]Department of Cellular and Molecular Medicine, University of California at San Diego, La Jolla, California, 92093, USA
[2]Stem Cell Program and Institute for Genomic Medicine, University of California at San Diego, La Jolla, California, 92093, USA
[3]RNA Center, Department of Molecular, Cell and Developmental Biology, Sinsheimer Labs, University of California, Santa Cruz, California, 95064, USA
[4]Molecular Engineering Lab, Agency for Science Technology and Research, Singapore
*Correspondence: geneyeo@ucsd.edu
DOI 10.1016/j.celrep.2012.02.001

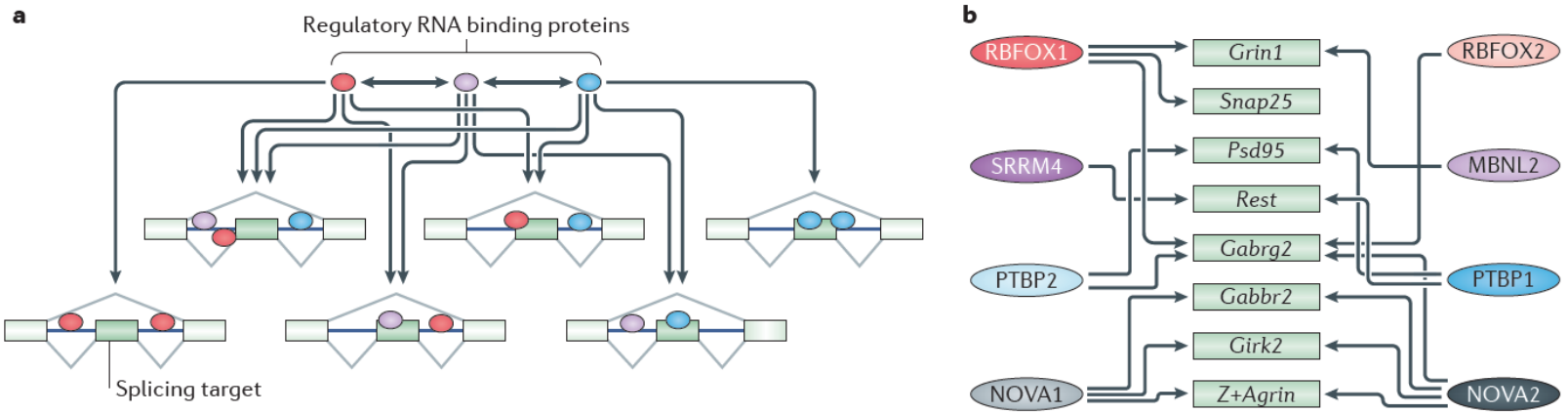


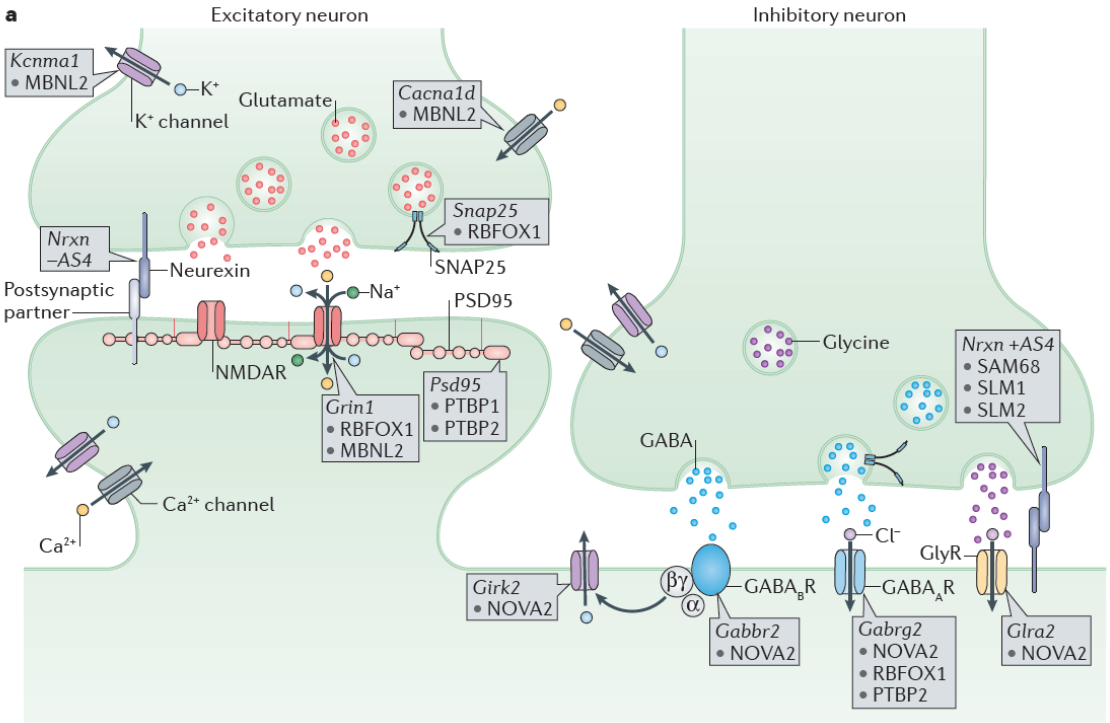




Huelga et al. 2012

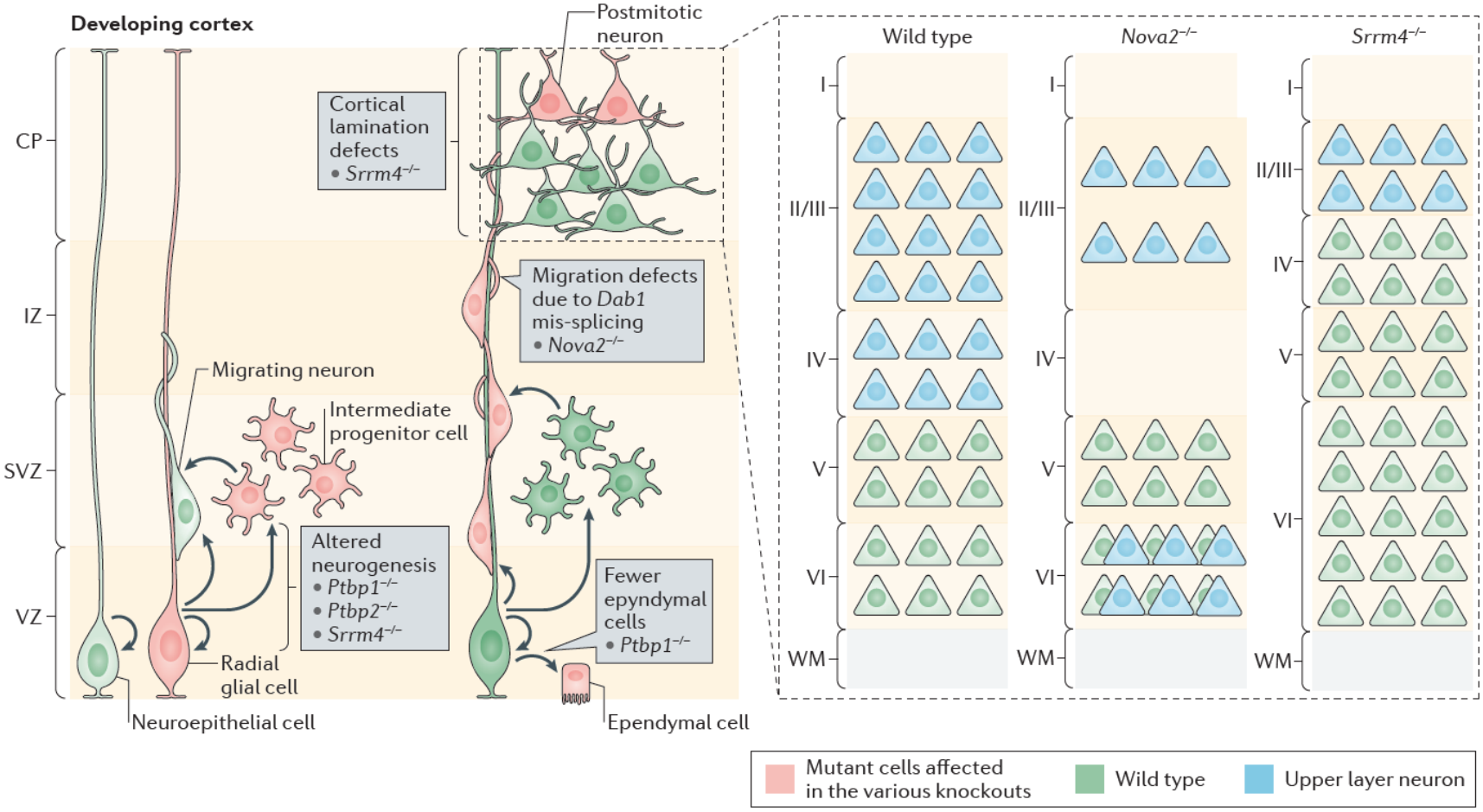
Regulatory networks – splicing regulators in synaptic function





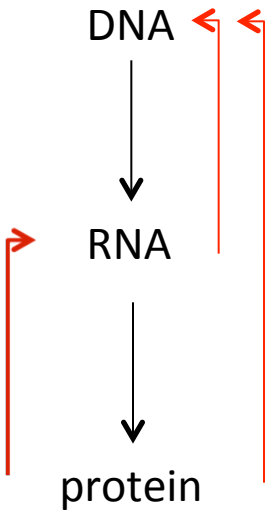
Vuong et al. 2016

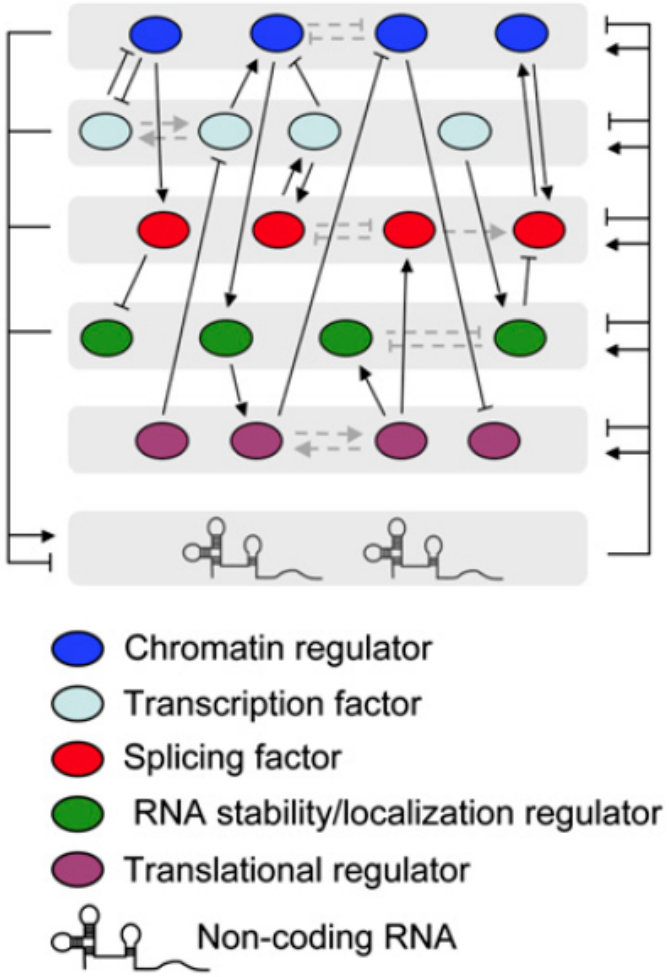
Regulatory networks - splicing regulators in cortical development and function



Vuong et al. 2016

Cross-talk between factors acting at multiple layers of gene regulation





Calarco et al. 2011

Thank you !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Polymorphism
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Gene expression
indel
SNP


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