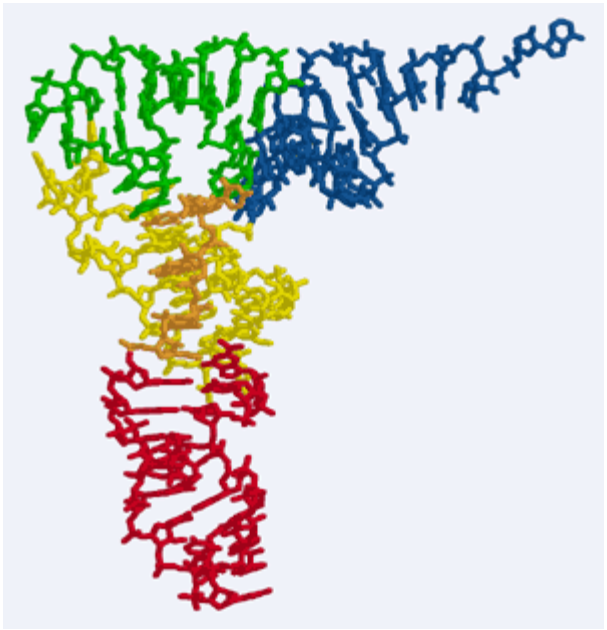
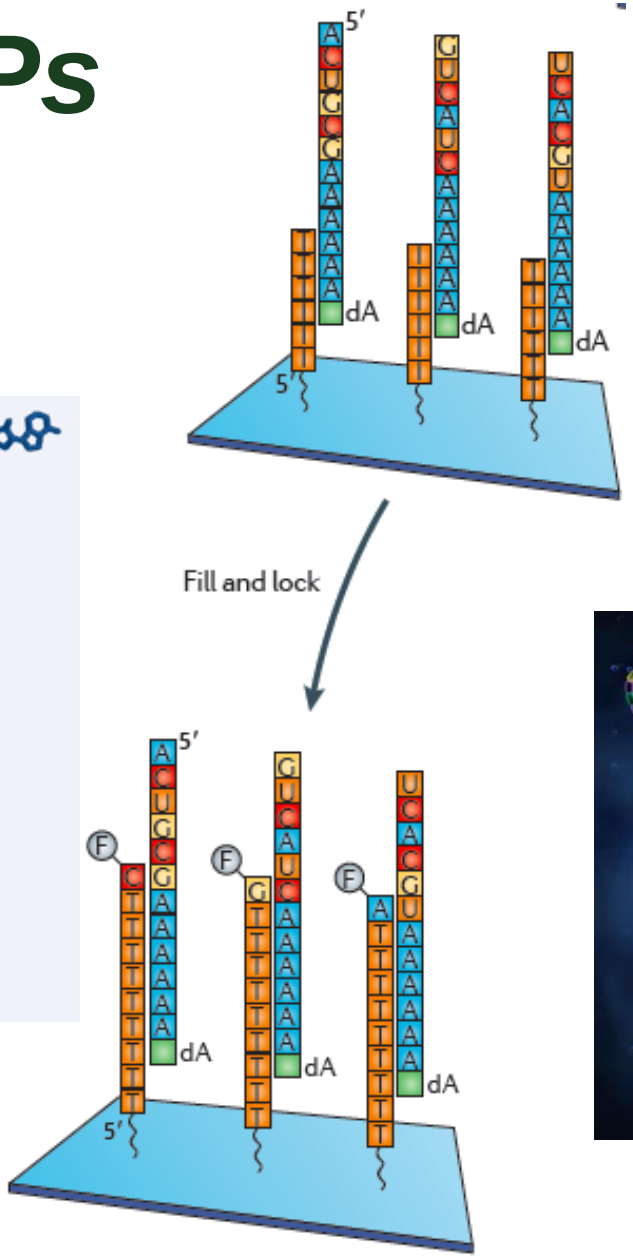
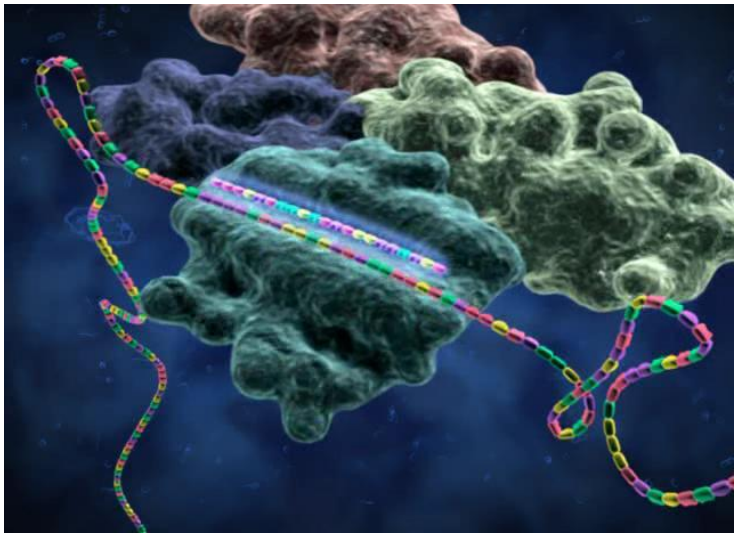
GLOBAL ANALYSES of RNAs and RNPs



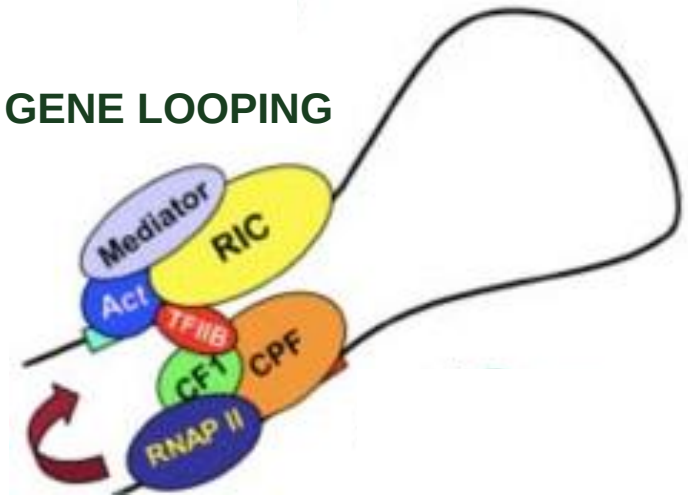


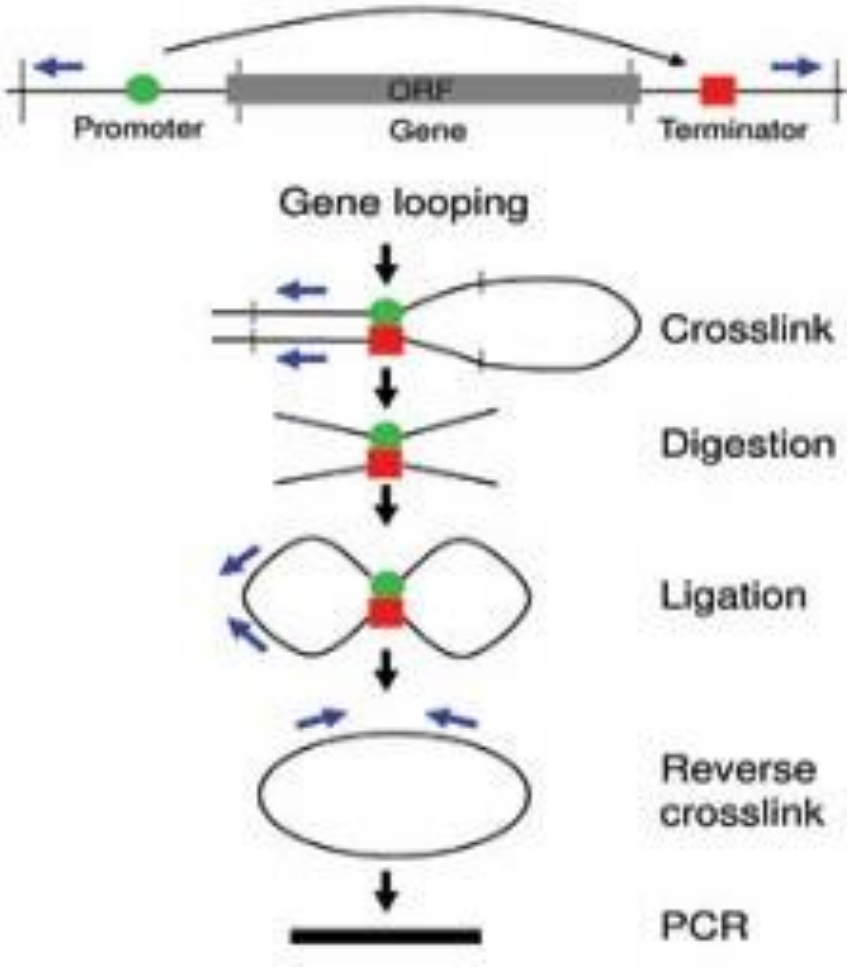


Chromatin/Transcription

3C Chromosome Conformation Capture

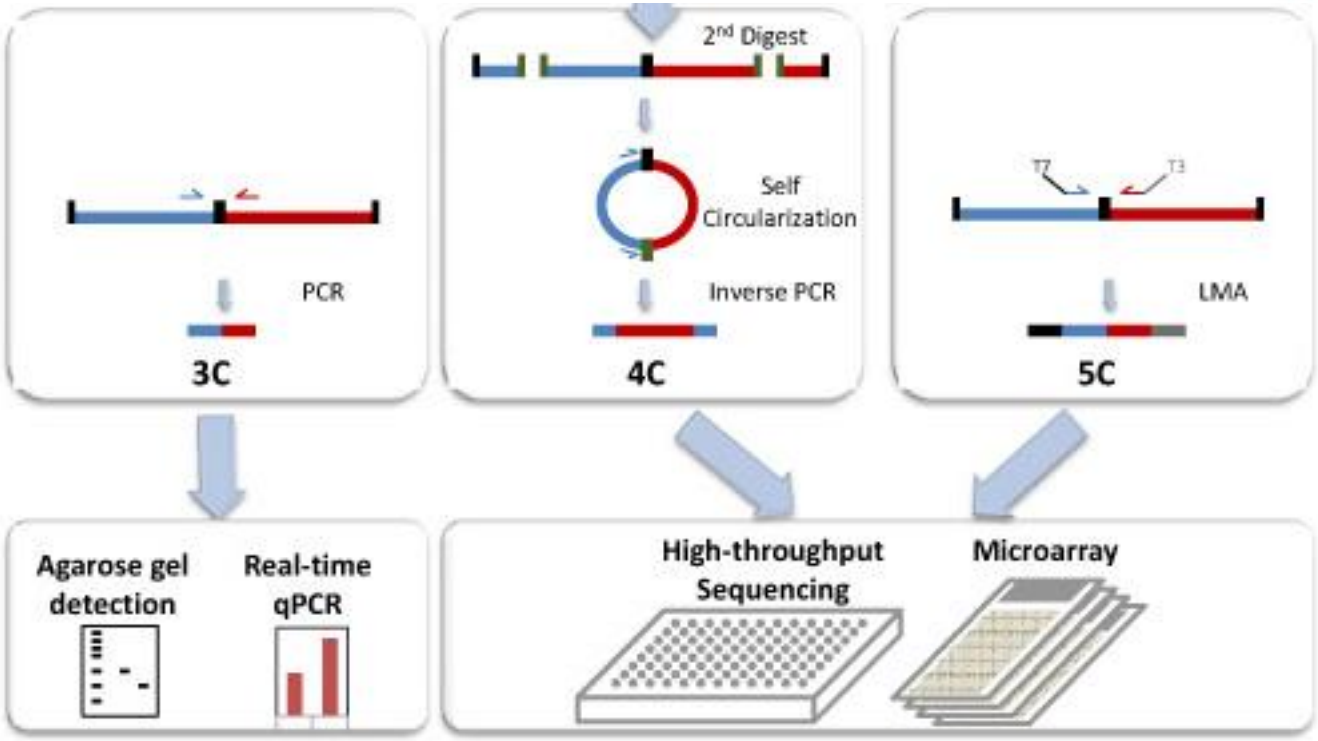
based on Proximity Ligation Assay (PLA)

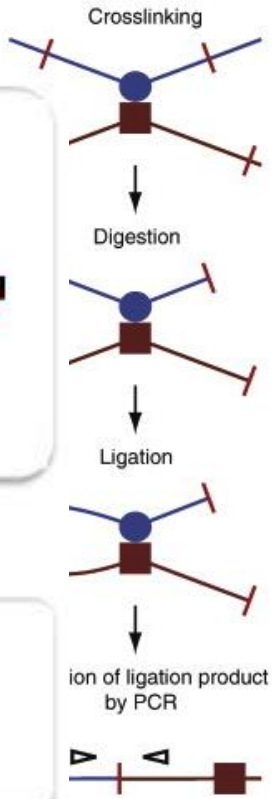


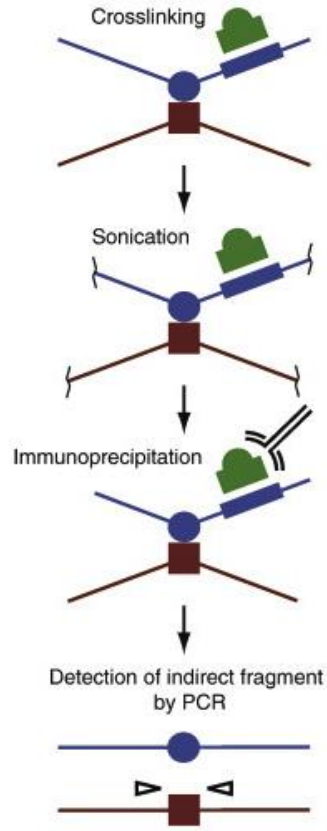


Ansari and Hampsay, GDev, 2005; El Kaderi et al., JBC, 2009

3C 4C 5C .....







3C

Indirect ChIP

3C Chromosome Conformation Capture

4C Circularized CCC (enhanced 3C)

5C Carbon-Copy CCC with multiple ligation-mediated amplification (LMA)

GCC Genome CC (Hi-C – deep Seq); ChIP version of GCC as 6C

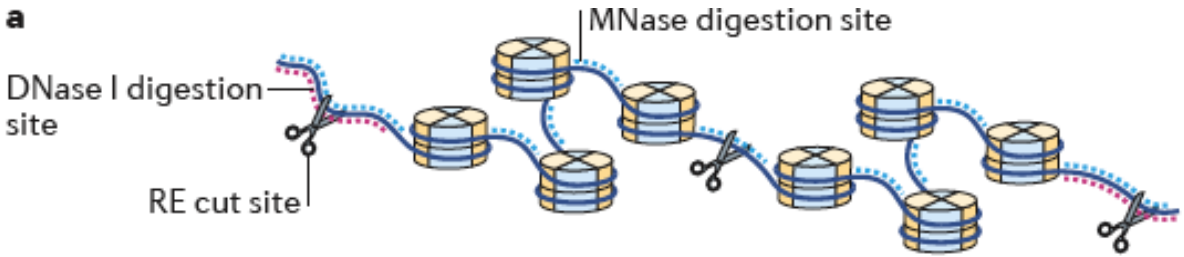
ChIP Loop (indirect ChIP); **6C ChIP GCC**

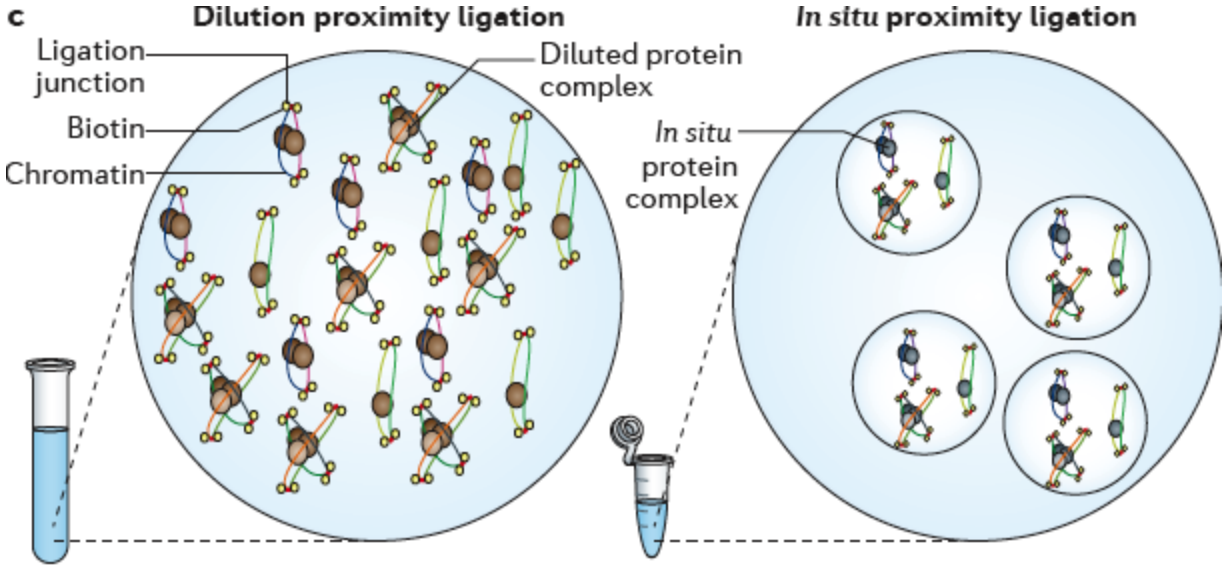
3C | Open-ended 3C | 3C-DSL | e4C | Capture-3C | 3C-seq

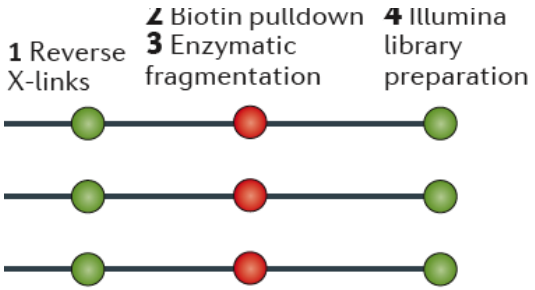
4C | 4C-seq | TLA | e4C | ACT | TCC | GCC | ELP | Hi-C | 5C

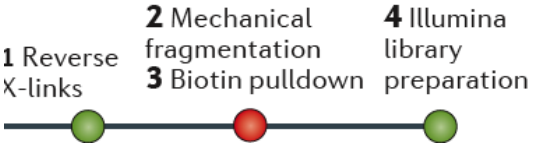
ChIA–PET | Capture-HiC | Single-cell Hi-C | *In situ* Hi-C | DNase Hi-C | Micro-C

3C 4C Hi-C





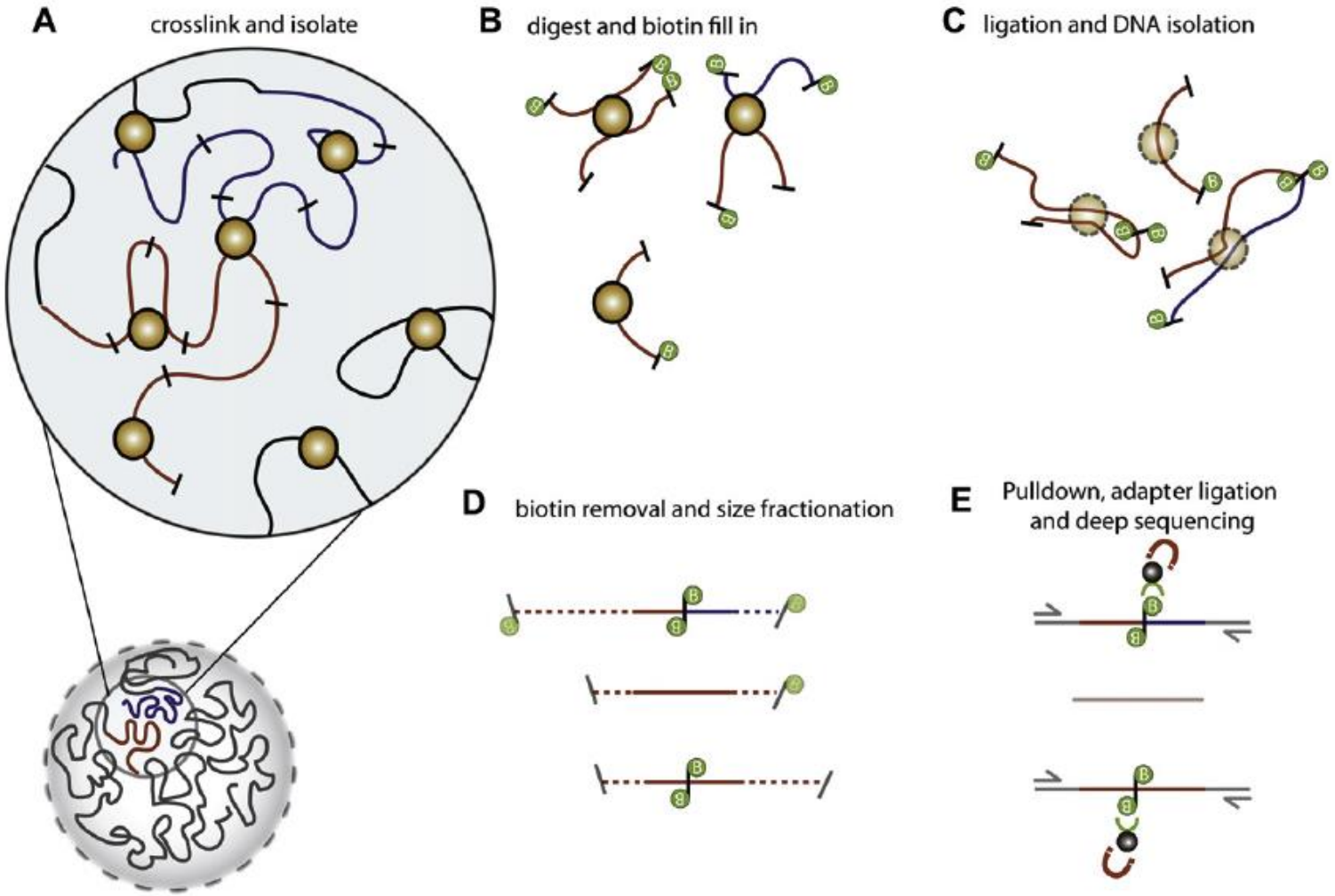




Schmit et al., Nat Rev Mol Cell Biol, 2016

Hi-C **- chromatin crosslinking, digestion, re-ligation, PCR amplification/deep sequencing**

- biotin-labeled nucleotide incorporated at the ligation junction for selective purification of chimeric DNA ligation junctions

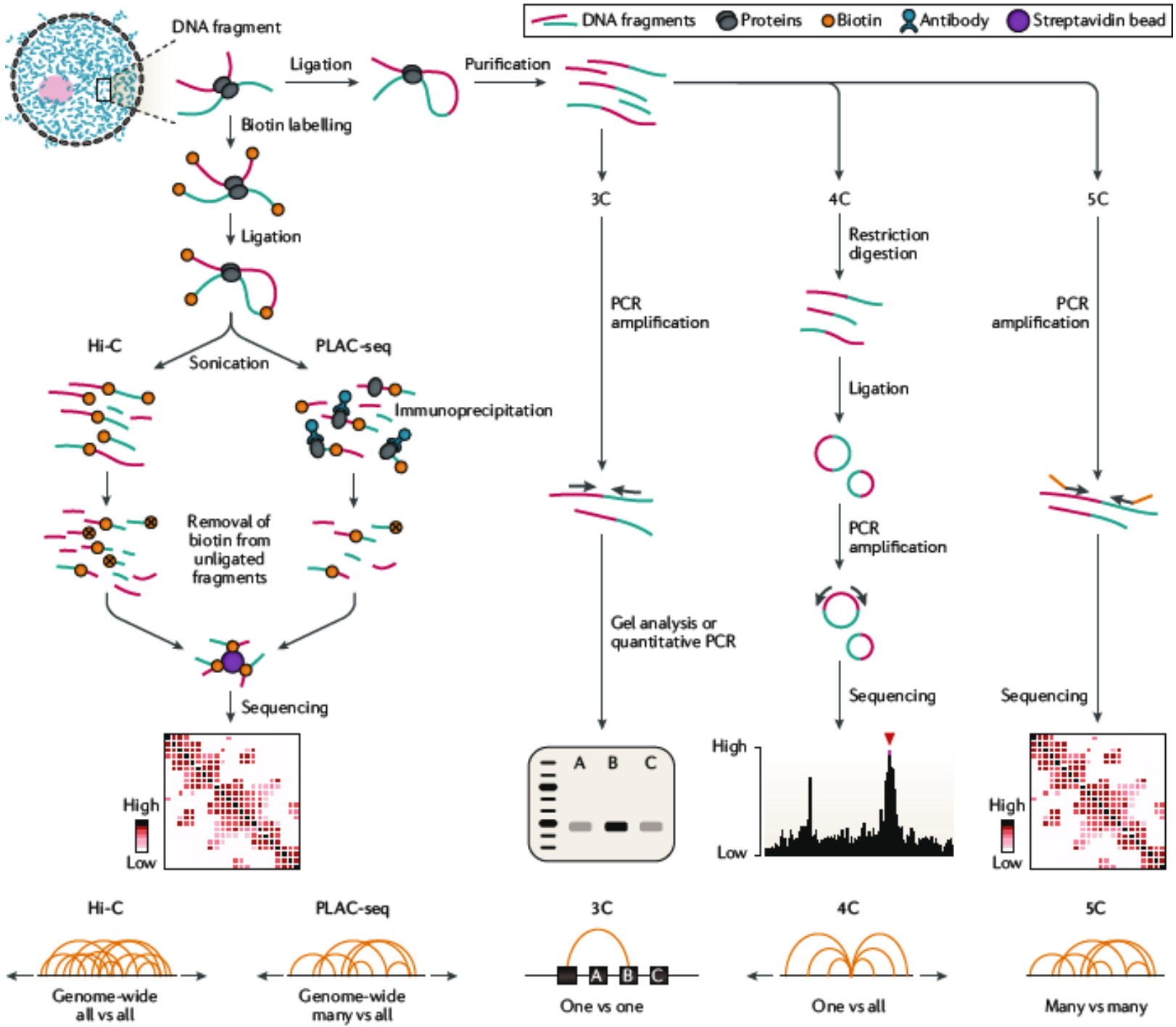


Belton et al., Methods, 2012

Methods to detect chromatin contacts

Assay	Description	Number of contacts per experiment	Multiplicity of contacts	Single-cell information	Number of cells	Detectable contacts	Protocol
3C-based methods							
3C	Proximity ligation and selection of target regions with primers, detection by quantitative PCR	One versus one	Pairwise	No	100 million[192]	Protein-mediated	192
4C	Proximity ligation and enrichment for contacts with one bait region by inverse PCR, detection by sequencing	One versus all	Pairwise	No	Robust: 10 million[193], low input: 340,000 (REF.[194])	Protein-mediated	193
5C	Proximity ligation and enrichment for larger target region with primers, detection by sequencing	Many versus many	Pairwise	No	Robust: 50–70 million[195], low input: 2 million[196]	Protein-mediated	195,196
Hi-C	Proximity ligation and enrichment for all ligated contact pairs, detection by sequencing	All versus all	Pairwise	No	Robust: 2–5 million[64], low input: 100,000–500,000 (REFS[70,197])	Protein-mediated	64,197
TCC	Tethered proximity ligation and enrichment for all ligated contact pairs, detection by sequencing	All versus all	Pairwise	No	25 million[57]	Protein-mediated	57
PLAC-seq, ChIA-PET	Proximity ligation and pull-down of specific protein-mediated contacts, detection by sequencing	Many versus many	Pairwise	No	Robust: 100 million[198], low input: 500,000 (REF.[81])	Protein-mediated (specific)	81,198
Capture-C, C-HiC	Proximity ligation and target enrichment using probes for genomic regions of interest, detection by sequencing	Many versus all	Pairwise	No	Robust: 100,000 (REF.[199]), low input: 10,000–20,000 (REF.[97])	Protein-mediated	199
Single-cell Hi-C	Proximity ligation and enrichment for all ligated contact pairs, detection by sequencing	All versus all	Pairwise	Yes	Hundreds	Protein-mediated	71
Imaging							
2D-FISH	Fixation to flatten cells, hybridization of fluorescent probes to target regions, measurement of 2D spatial distances	Between 2 and 52 regions[a]	Pairwise or more	Yes	Hundreds	All in spatial proximity	200
3D-FISH	Fixation of cells, hybridization of fluorescent probes for target regions, measurement of 3D spatial distances	Between 2 and 52 regions[a]	Pairwise or more	Yes	Hundreds	All in spatial proximity	201
Cryo-FISH	Fixation of cells, cryosectioning, hybridization of fluorescent probes for target regions, measurement of 2D spatial distances	Between 2 and 52 regions[a]	Pairwise or more	Yes	Hundreds	All in spatial proximity	101
Live-cell imaging	Fluorescent labelling of genomic loci in living cells, measurement of spatial distances over time	Between 2 and 12 regions	Pairwise or more	Yes	Hundreds	All in spatial proximity	9,99,202,203
Ligation-free methods							
GAM	Cryosectioning of fixed cells, DNA extraction from nuclear sections and sequencing, inferring spatial distances from co-segregation of genomic regions in nuclear sections	All versus all	Pairwise or more	Yes	Hundreds[10]	All in spatial proximity	10
SPRITE	Fixation of cells, identification of crosslinked chromatin fragments by split-pool barcoding and sequencing	All versus all	Many	No	10 million[11]	Protein-mediated	11
ChIA-Drop	Fixation of cells, identification of crosslinked chromatin fragments by droplet-based and barcode-linked sequencing	All versus all	Many	No	10 million[12]	Protein-mediated	12

Kempfer and Pombo, NatRevGenet 2019



Kempfer and Pombo, NatRevGenet 2019

METHODS TO STUDY TRANSCRIPTOMES

- **SAGE - serial analysis of gene expression**

sequencing of small cDNA tags generated by type II restriction enzymes

- **CAGE - cap analysis of gene expression**

sequencing of small cDNA tags derived from capped transcripts

- **3' long SAGE**

identification of SAGE tags that originate from 3' ends of transcripts

- **RNA Seq - high throughput sequencing of cDNAs**
- **GRO-seq - genomic run-on sequencing**

sequencing of cDNA tags extended from nascent transcripts

- **tiling arrays**

microarrays with overlapping probes that cover the complete genome

METHODS TO STUDY TRANSCRIPTOMES

- **ChIP (ChIP-chip, ChIP-Seq) - chromatin immunoprecipitation**

indirectly reveal unknown ncRNAs

- **RIP-Seq - RNA immunoprecipitation-sequencing**

- **ChIRP – Chromatin isolation by RNA Purification (+RNA-Seq)**

- **ChART - Capture Hybridization Analysis of RNA targets (+RNA-Seq)**

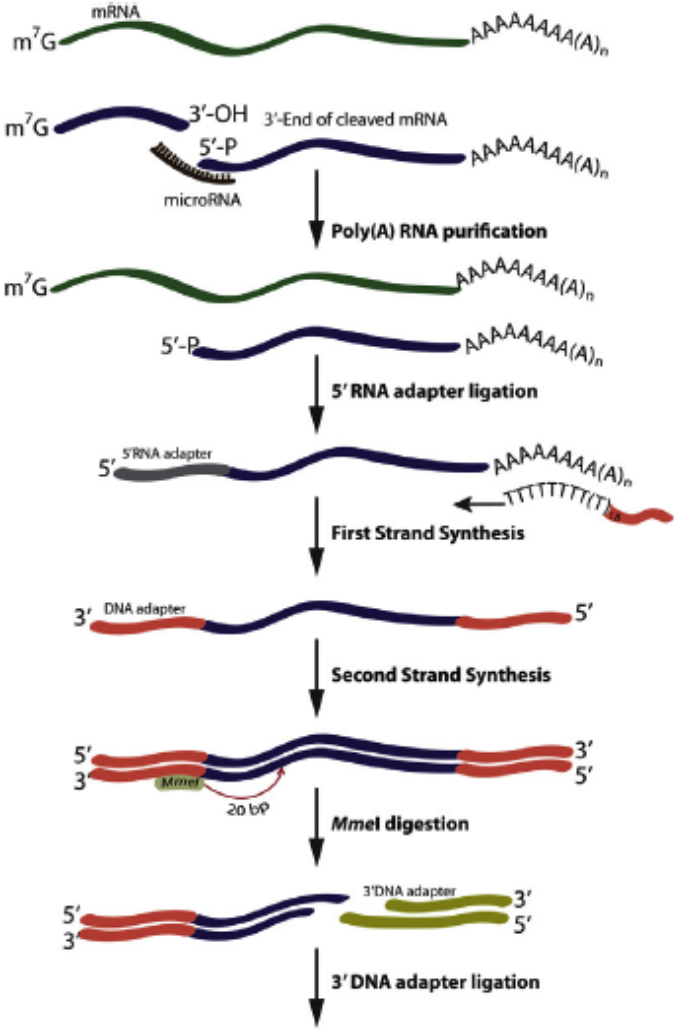
biotinylated oligonucleotides used to enrich for DNA sequences associated with a particular RNA

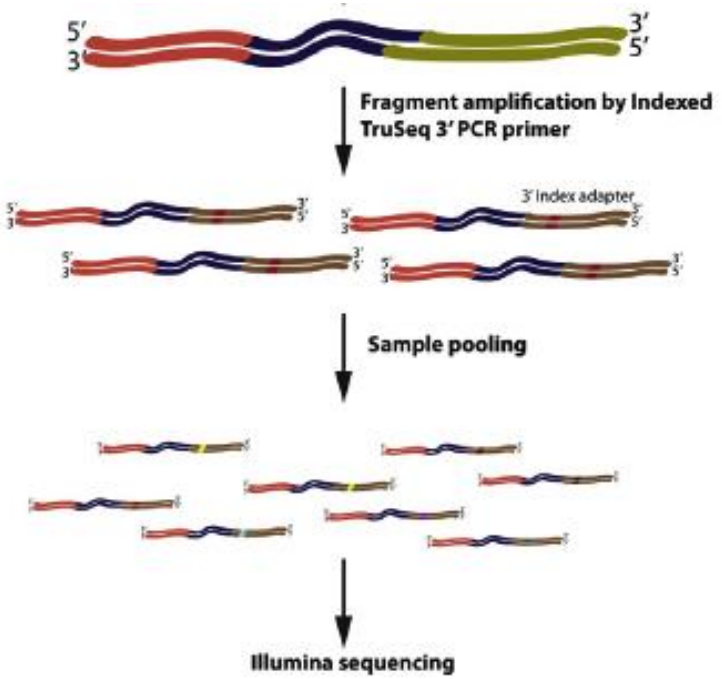
- **CRAC - CRosslinking and Analysis of cDNA**

- **PAR-CLIP - PhotoActivatable ribonucleoside–enhanced CrossLinking and ImmunoPrecipitation**

- **HITS-CLIP - High-Throughput Seq CLIP**

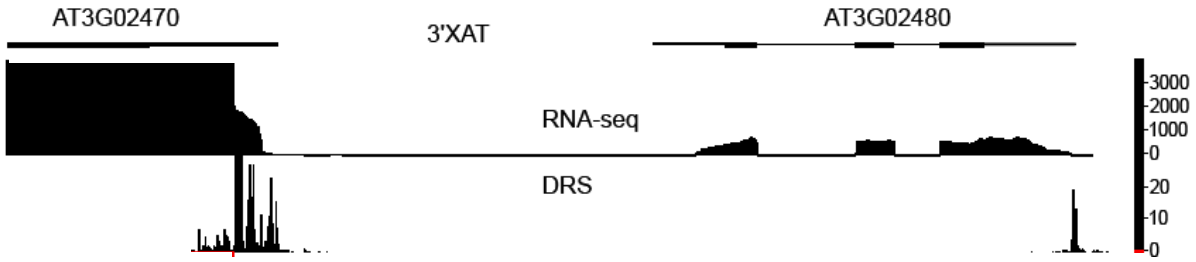
PARE: Parallel Analysis of RNA End
mRNA DEGRADOME RNA-seq





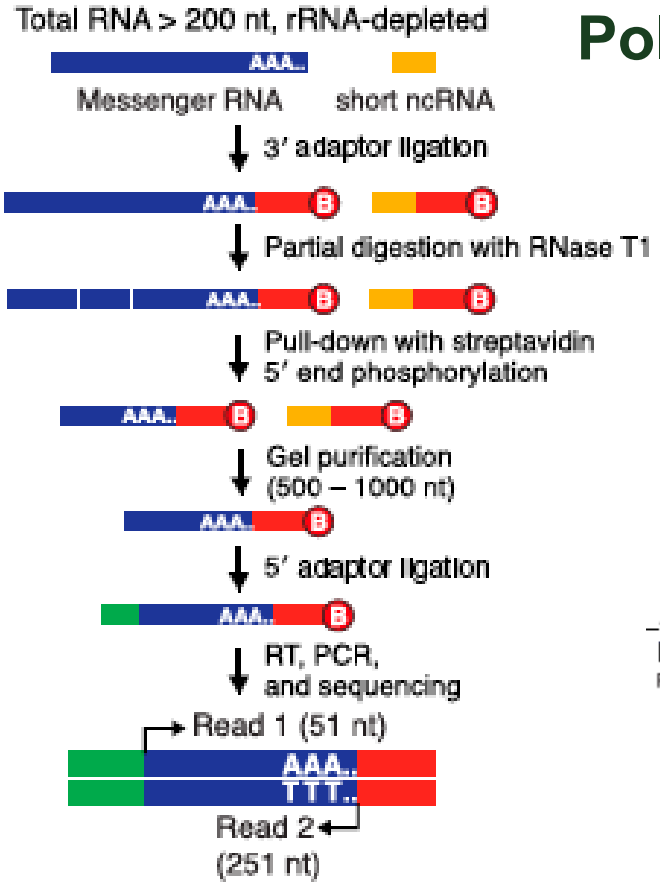
Zhai et al., Methods, 2014

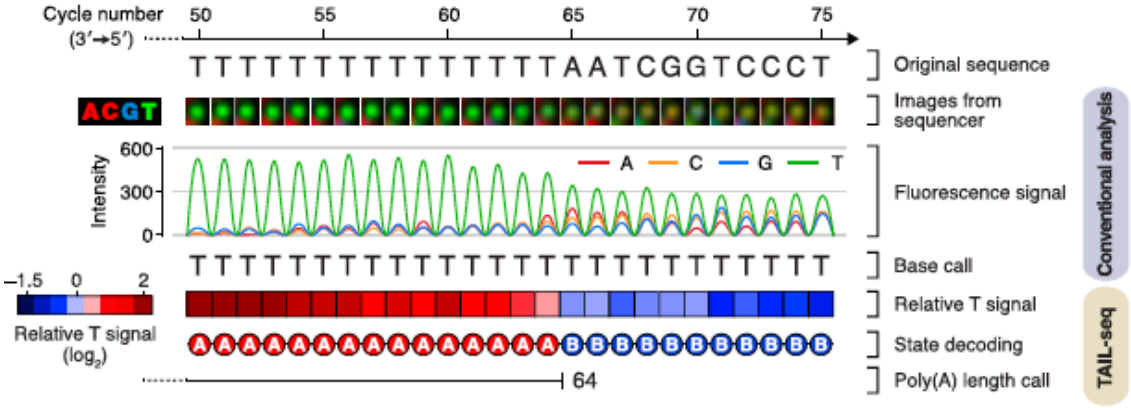
DRS: Direct RNA sequencing of Poly(A) sites



TAIL-seq: RNA 3' end sequencing

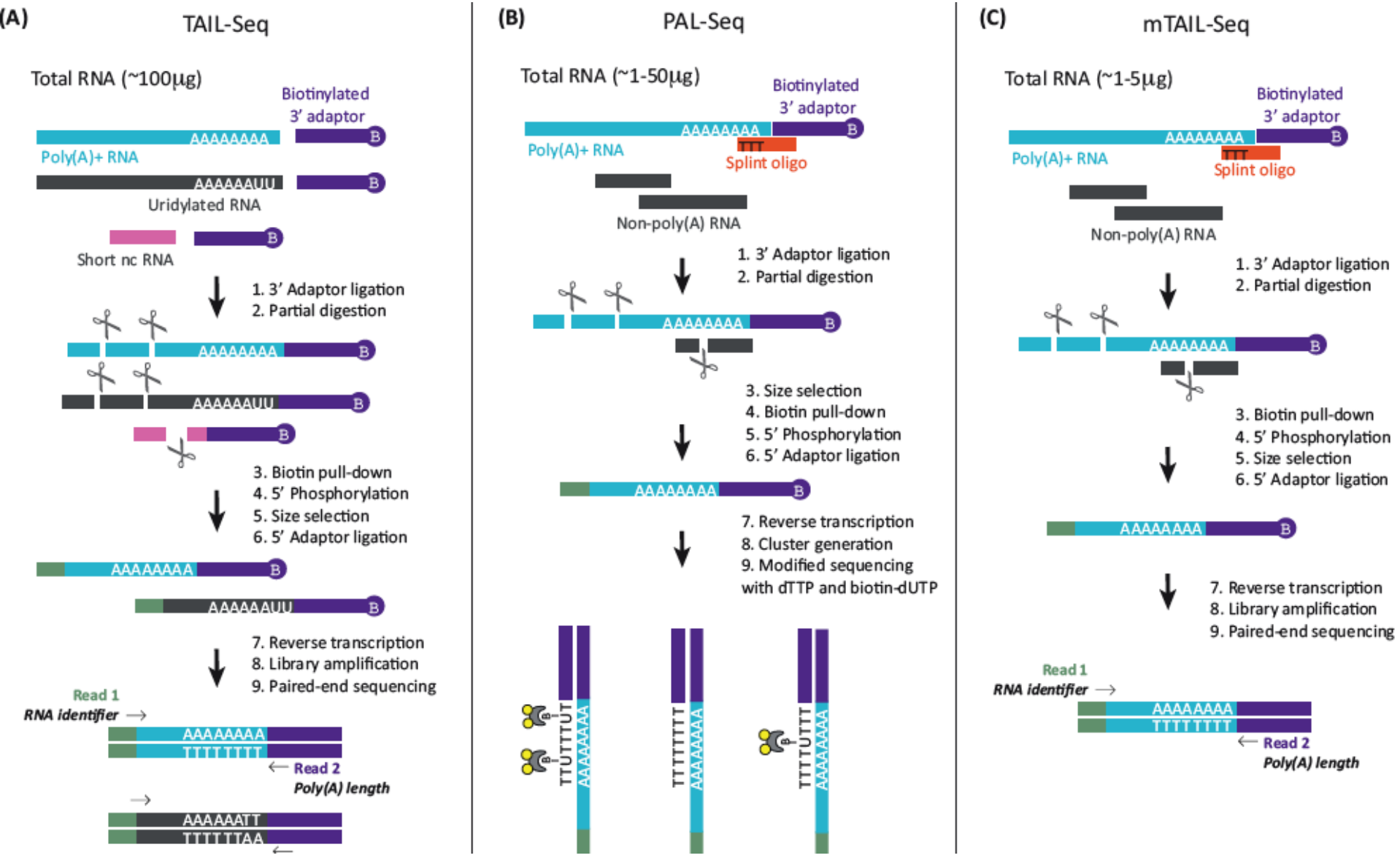
Poly(A) tail length and 3' end modifications (e.g. U-tailing)





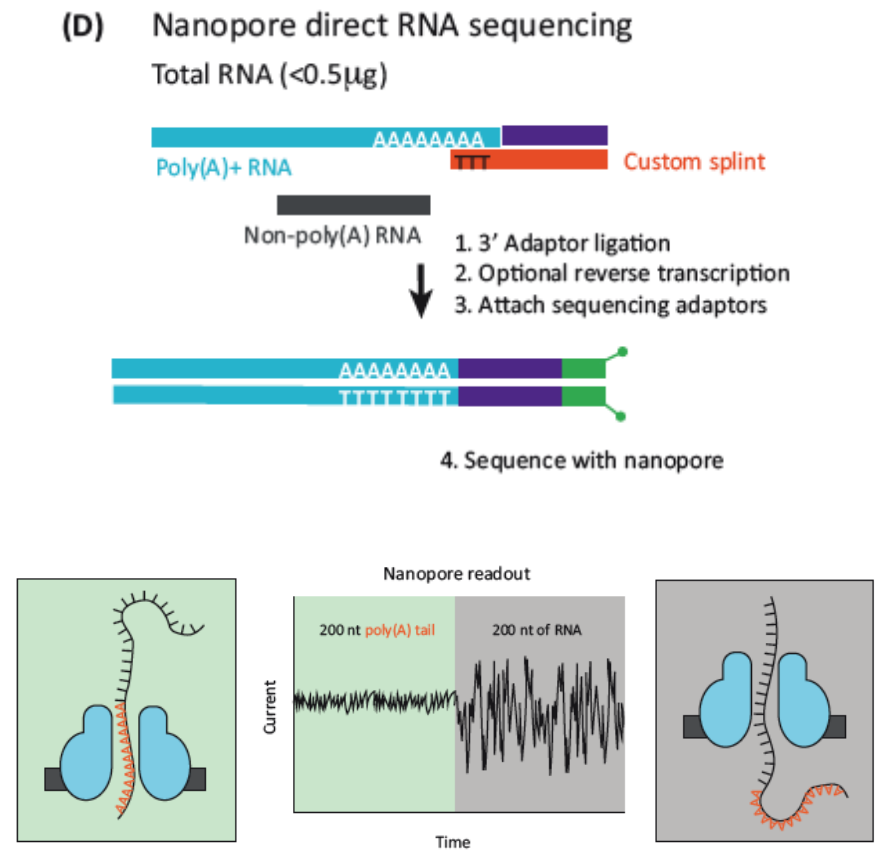
Sherstnev et al., Nat Str Mol Biol, 2012; Chang et al., Mol Cell, 2014

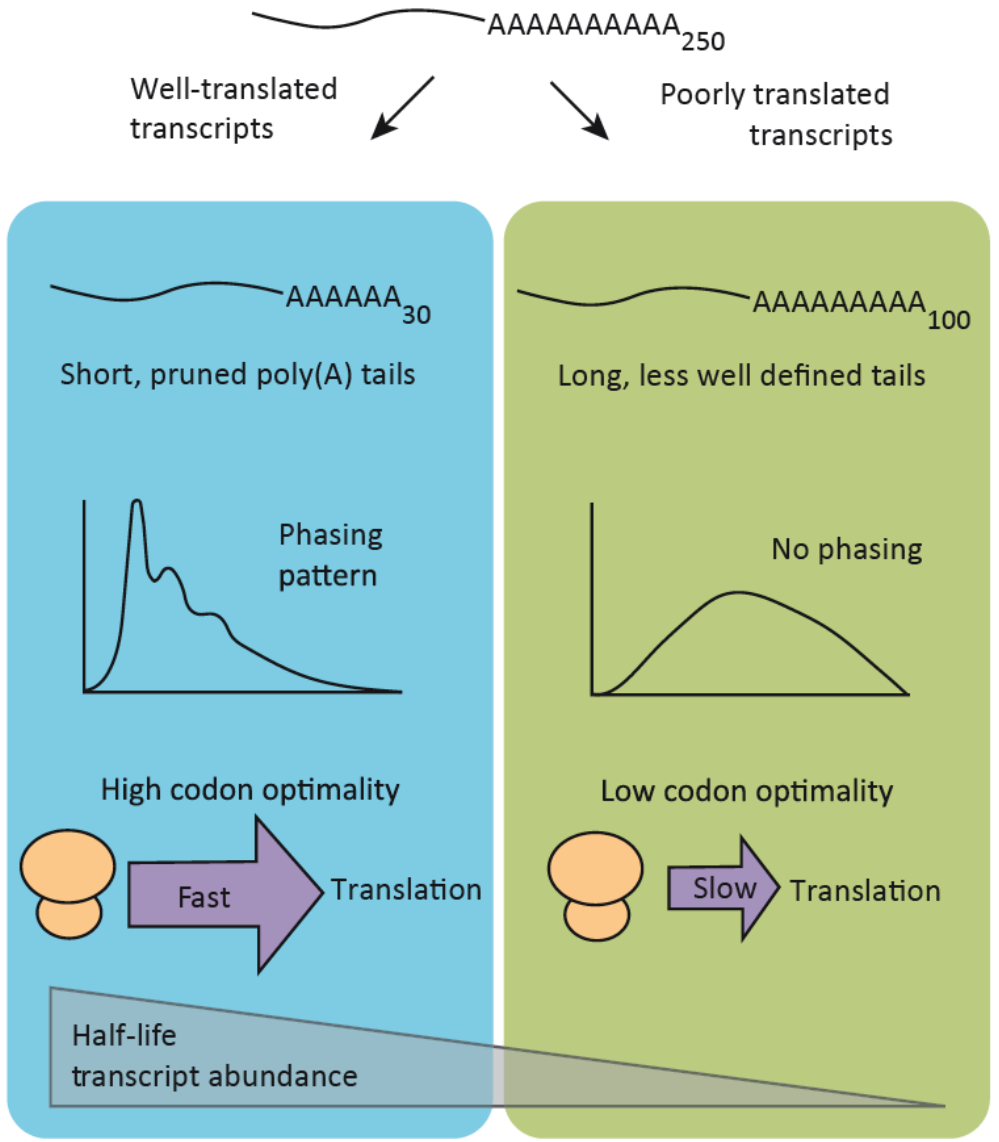
Poly(A) tail analyses



Nicholson and Pasquinelli TiCB 2018

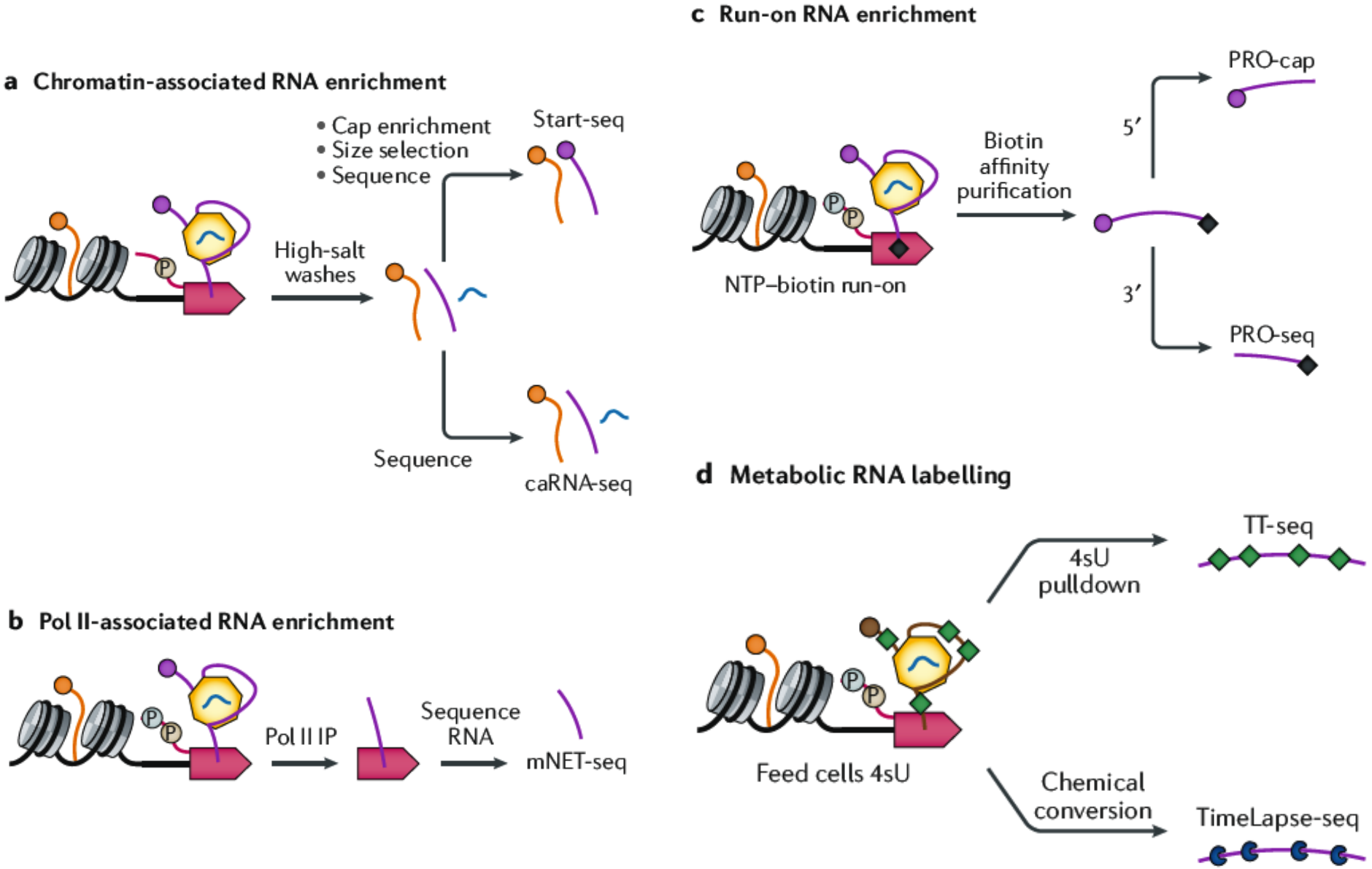
Poly(A) tail analyses Nanopore





Nicholson and Pasquinelli TiCB 2018

Nascent RNA analyses



Wissink et al, Nat Rev Genet, 2019

Nascent RNA methods

caRNA- seq chromatin-associated RNAseq
CoPRO coordinated precision run-on and sequencing
FISH fluorescence in situ hybridization
mNET-seq mammalian native elongating transcript seq
NET-seq native elongating transcript seq
PRO-cap precision run- on with cap selection
PRO-seq precision run- on seq
SL AM-seq thiol (SH)-linked alkylation for the metabolic sequencing of RNA
SMIT-seq single-molecule intron tracking seq
TT- seq transient transcriptome seq

Method	Advantages	Considerations
caRNA-seq	• Can be used to isolate all chromatin-associated RNA species • Can be combined with methods that assay co-transcriptional processes, including RNA methylation and editing	Also sequences non-nascent RNAs that stably associate with chromatin
Start-seq	• Simultaneously identifies initiation and pausing sites • Allows de novo calling of putative enhancers	Does not report transcription beyond the first ~100 nucleotides
Yeast NET-seq	• Is Pol II specific (antibody enrichment) • Identifies Pol II positions at nucleotide resolution genome-wide	Is limited to cells with epitope-tagged Pol II
mNET-seq	• Is Pol II specific (antibody enrichment) • Identifies Pol II positions at nucleotide resolution genome-wide • Can isolate Pol II with different post-translational modifications	• Includes RNAs that are stably associated with Pol II • Does not currently include RNA <30 nucleotides in length • Has detected eRNA transcription from previously called enhancers
PRO-cap	• Identifies transcription initiation sites • Allows de novo calling of putative enhancers	Does not report transcription beyond the first ~100 nucleotides
PRO-seq	• Captures RNAs from transcriptionally competent polymerases • Identifies positions of active transcription at nucleotide resolution genome-wide • Allows de novo calling of putative enhancers	• Does not measure polymerase backtracking • Also captures RNAs being transcribed from Pol I and Pol III
CoPRO	• Simultaneously identifies initiation and pausing sites • Measures RNA capping status	Does not measure transcription beyond promoter-proximal pause site
SMIT-seq	Measures splicing status during transcription	Limited to species with short introns
TT-seq	• Captures RNAs from actively transcribing polymerases • Can be used to determine RNA stability • Identifies transcription termination sites	• Does not detect Pol II pausing • Has detected eRNA transcription from previously called enhancers
SLAM-seq and TimeLapse-seq	• Captures RNAs from actively transcribing polymerases • Can be used to determine RNA stability	• Requires deep sequencing to measure chemical conversion rate • Long labelling times do not capture newly synthesized RNA
Intron sequential FISH	• Detects transcription of thousands of genes in single cells • Contains positional information of transcribed genes in the 3D space of the nucleus	• Does not report chromosomal positions of active Pol II complexes • Does not distinguish different steps of transcription • Requires a library of intron-targeting probes and series of hybridizations

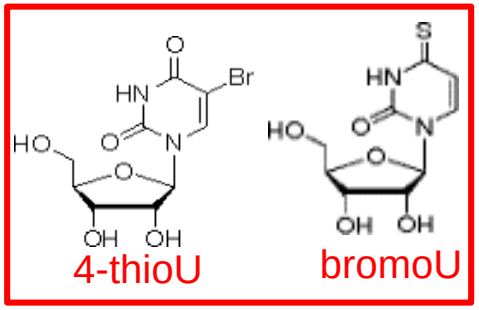
Wissink et al, Nat Rev Genet, 2019

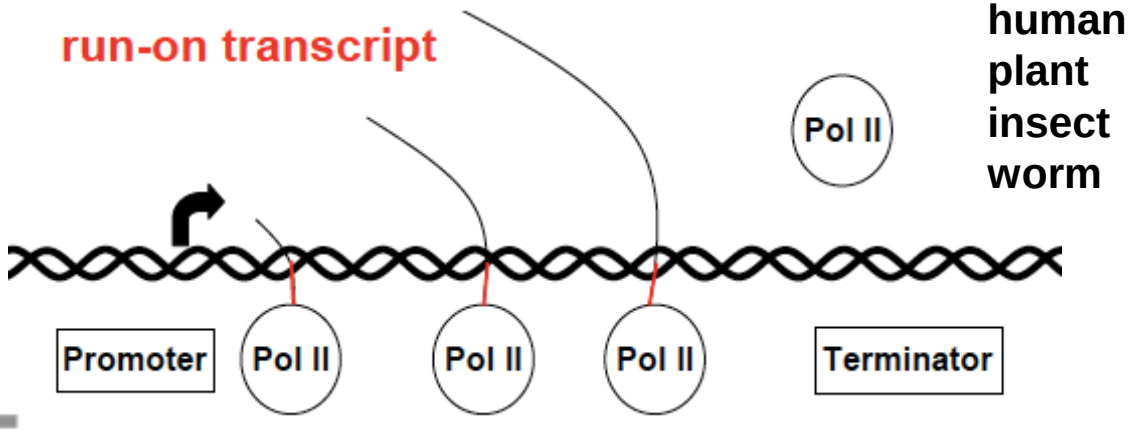
Nascent RNA methods

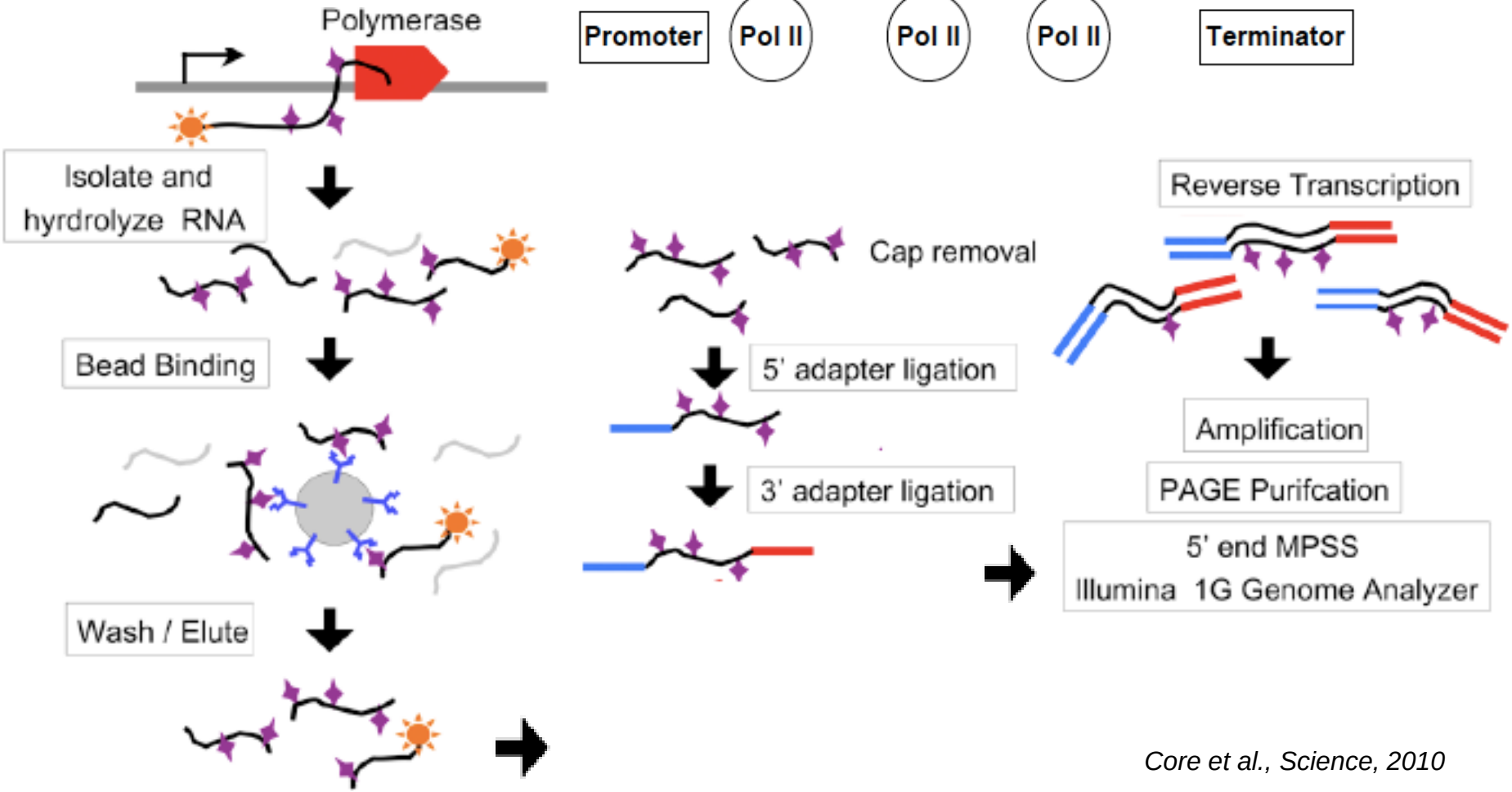
Method	Transcription step						
	TSS[a]	RNA capping	Promoter-proximal pausing	Co-transcriptional RNA processing	Transcription termination	Pol II CTD modification	Transcription bursting
Chromatin isolation-based methods							
caRNA-seq	No	No	No	Yes[42,105–107]	No	No	No
Start-seq	Yes[43]	No	Yes[43]	No	No	No	No
mNET-seq	No	No	Yes[41,73]	Yes[41,63,64]	Yes[41]	Yes[41,63]	No
SMIT-seq	No	No	No	Yes[159,160]	No	No	No
Run-on methods							
GRO-cap and PRO-cap	Yes[4,42]	No	No	No	No	No	No
GRO-seq, PRO-seq and ChRO–seq	No	No	Yes[42,48,74]	Yes[166]	Yes[42]	No	No
CoPRO	Yes[49]	Yes[49]	Yes[49]	No	No	No	No
Metabolic labelling methods							
TT-seq	No	No	No	No	Yes[47]	No	No
Imaging-based methods							
Intron sequential FISH	No	No	No	No	No	No	Yes[55]

Wissink et al, Nat Rev Genet, 2019

Analysis of Nascent Transcripts- GRO-seq



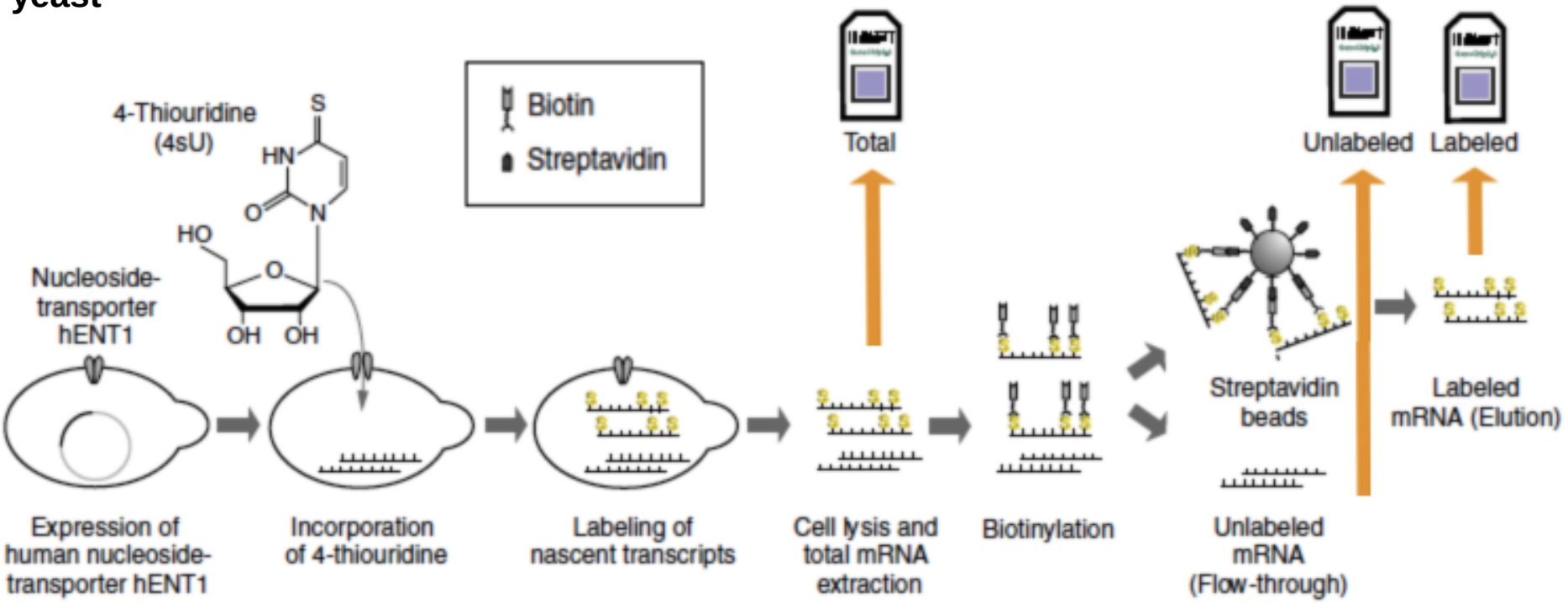




Core et al., Science, 2010

Analysis of Nascent Transcripts

yeast



Expression of hENT1 nucleoside transporter enables uptake of UTP derivatives

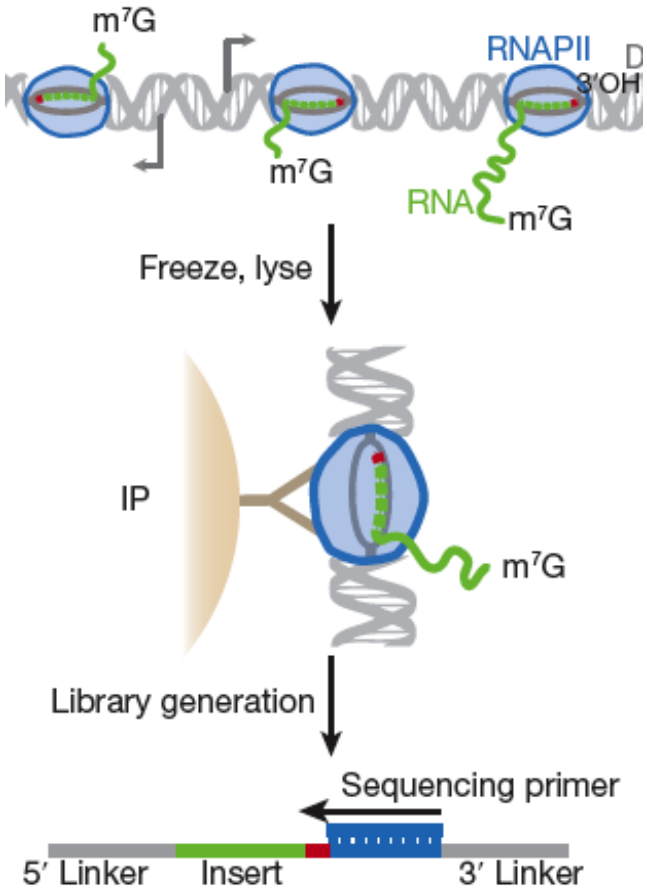
Non-perturbing RNA labeling in yeast
Allows dynamic transcriptome analysis: sythesis and decay rates and the study of nascent transcripts

Miller et al., Mol Syst Biol, 2010;
Barrass et al, Genome Biol, 2015

Analysis of Nascent Transcripts

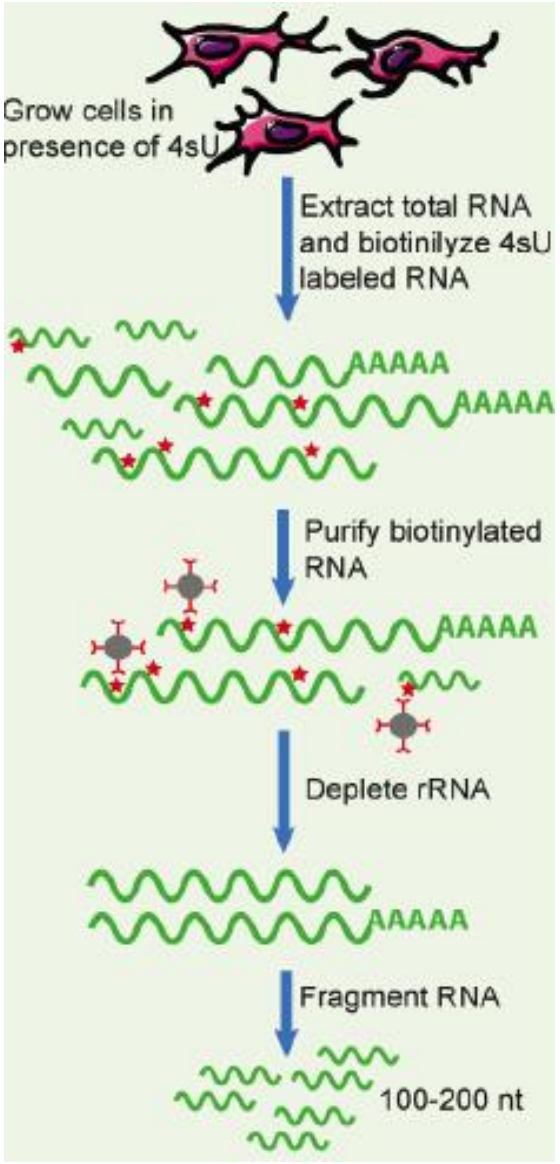
I.

NET-Seq



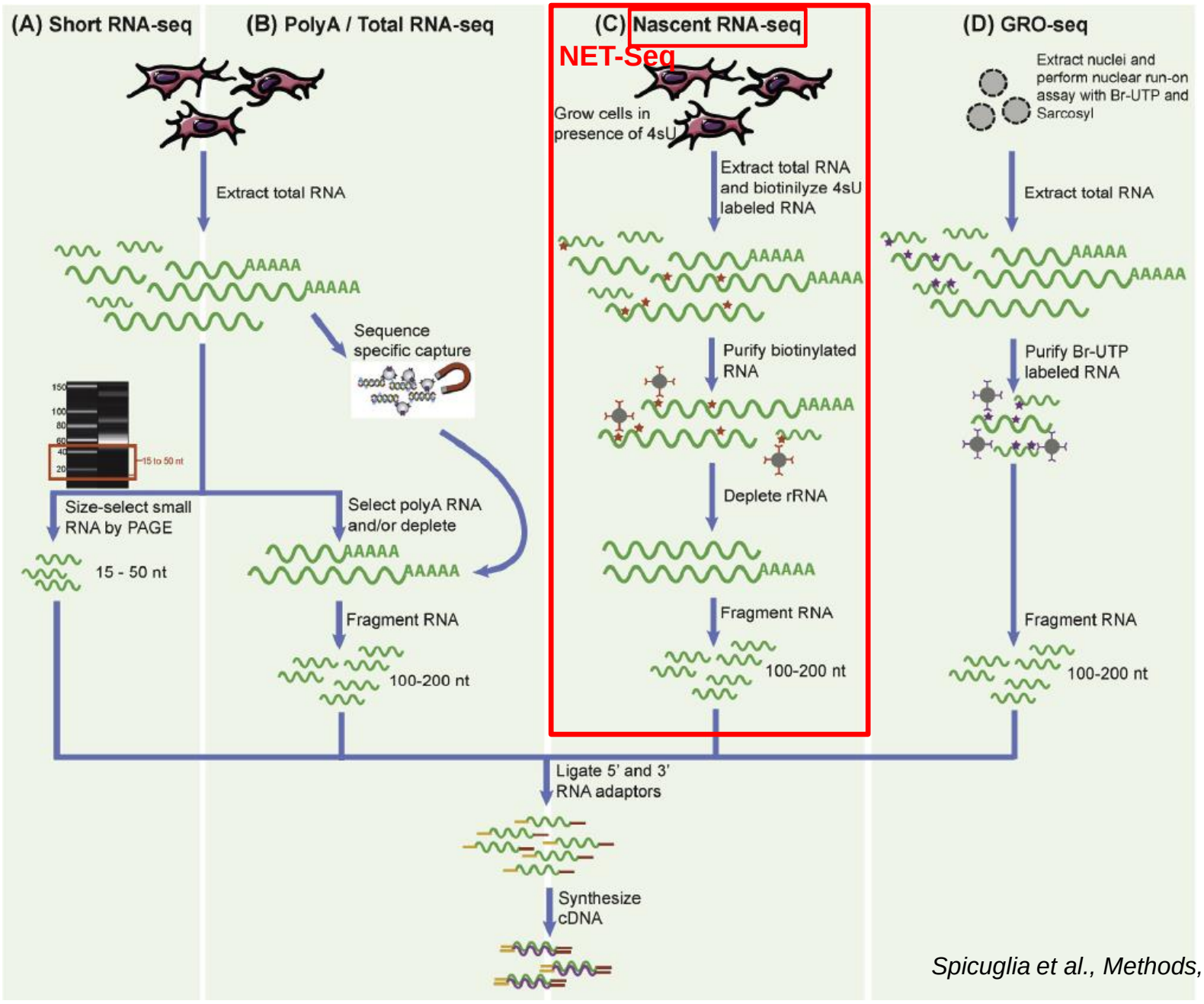
Churchman and Weissman, Nature, 2011

II.



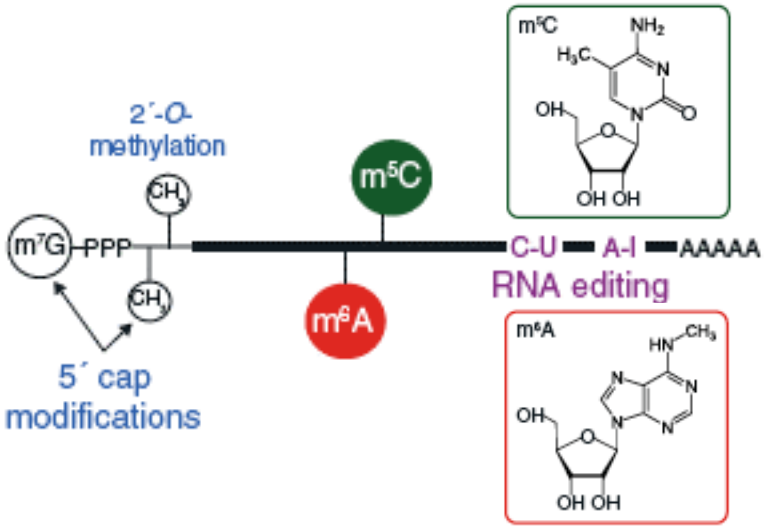
Spicuglia et al., Methods, 2013

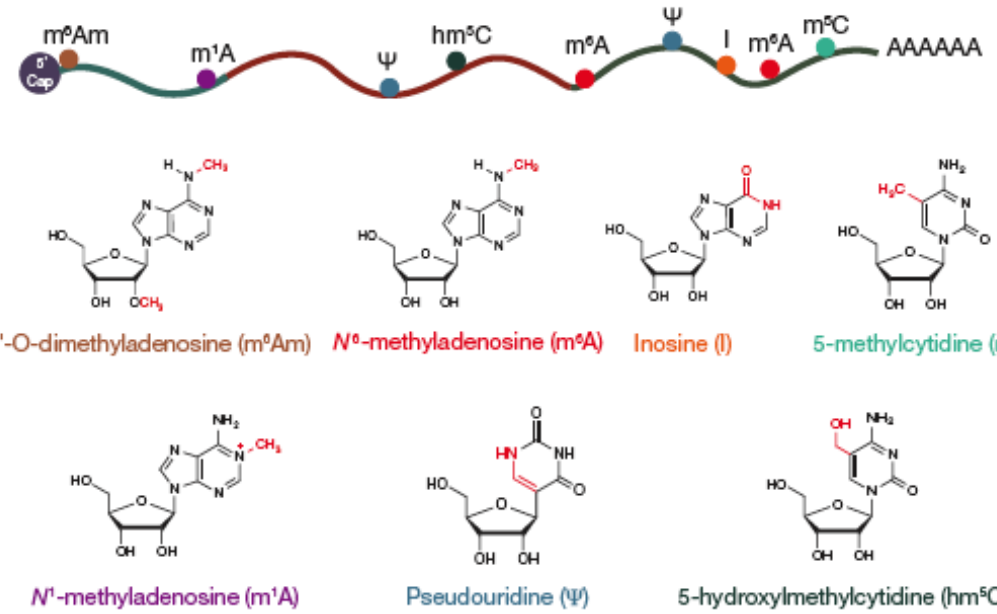
Comparison of different RNA-Seq approaches



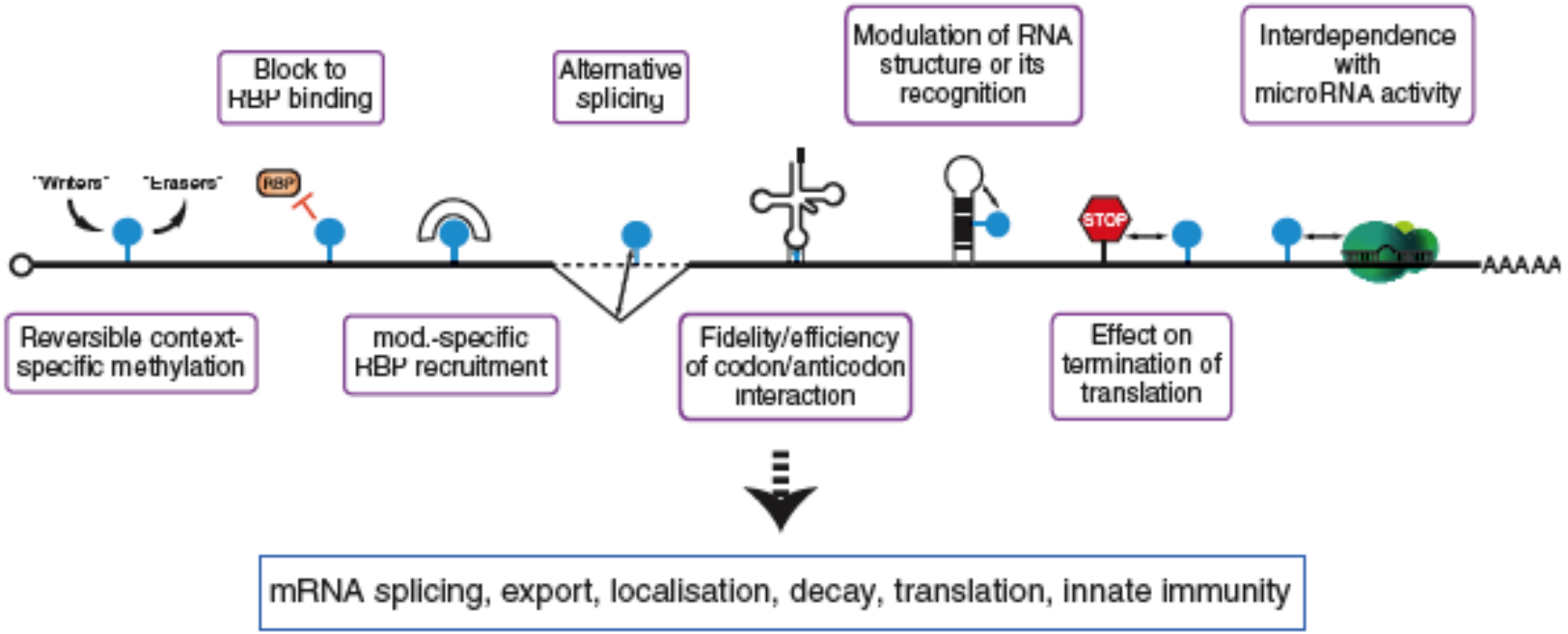
Spicuglia et al., Methods, 2013

RNA MODIFICATIONS



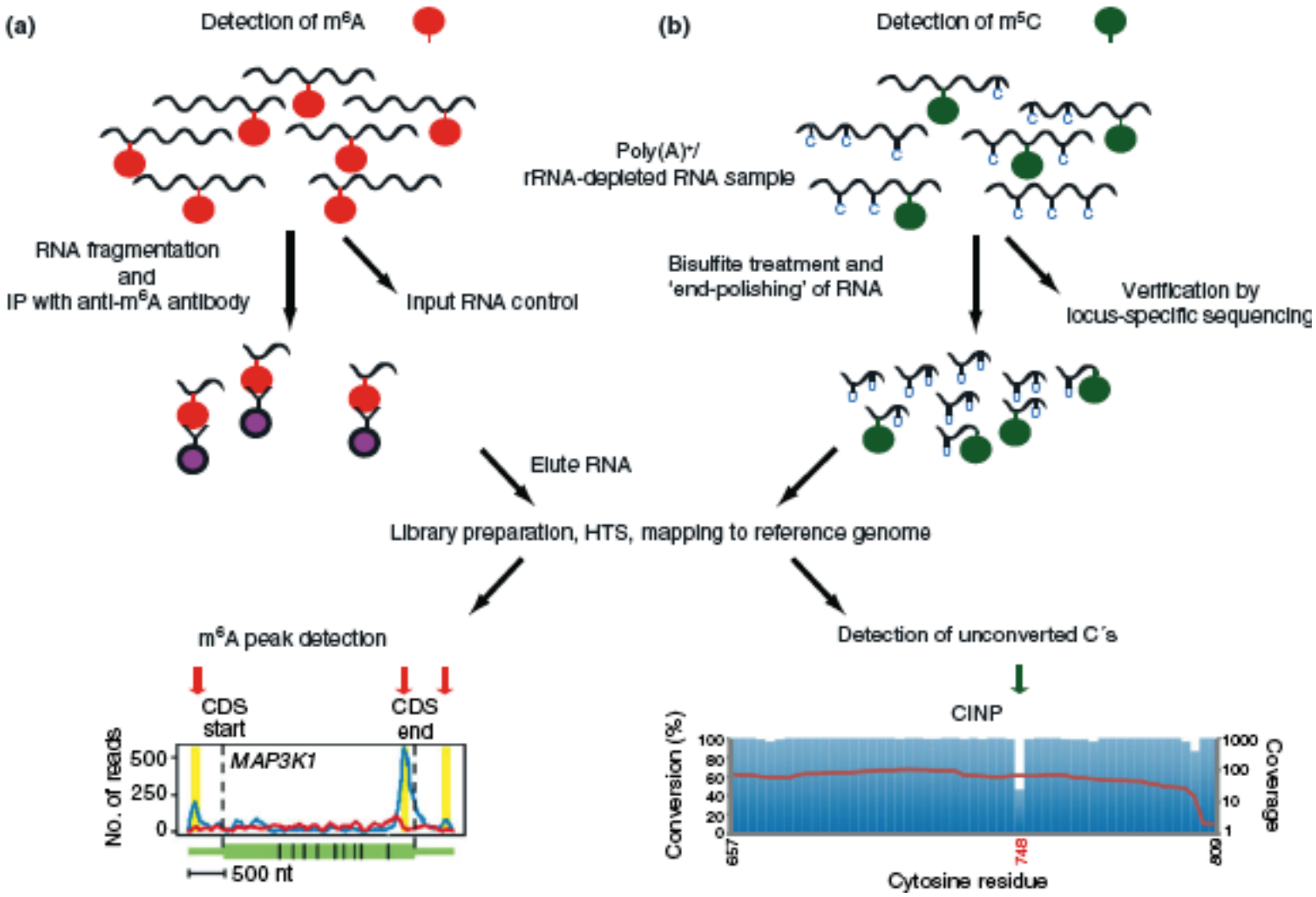


FUNCTIONS



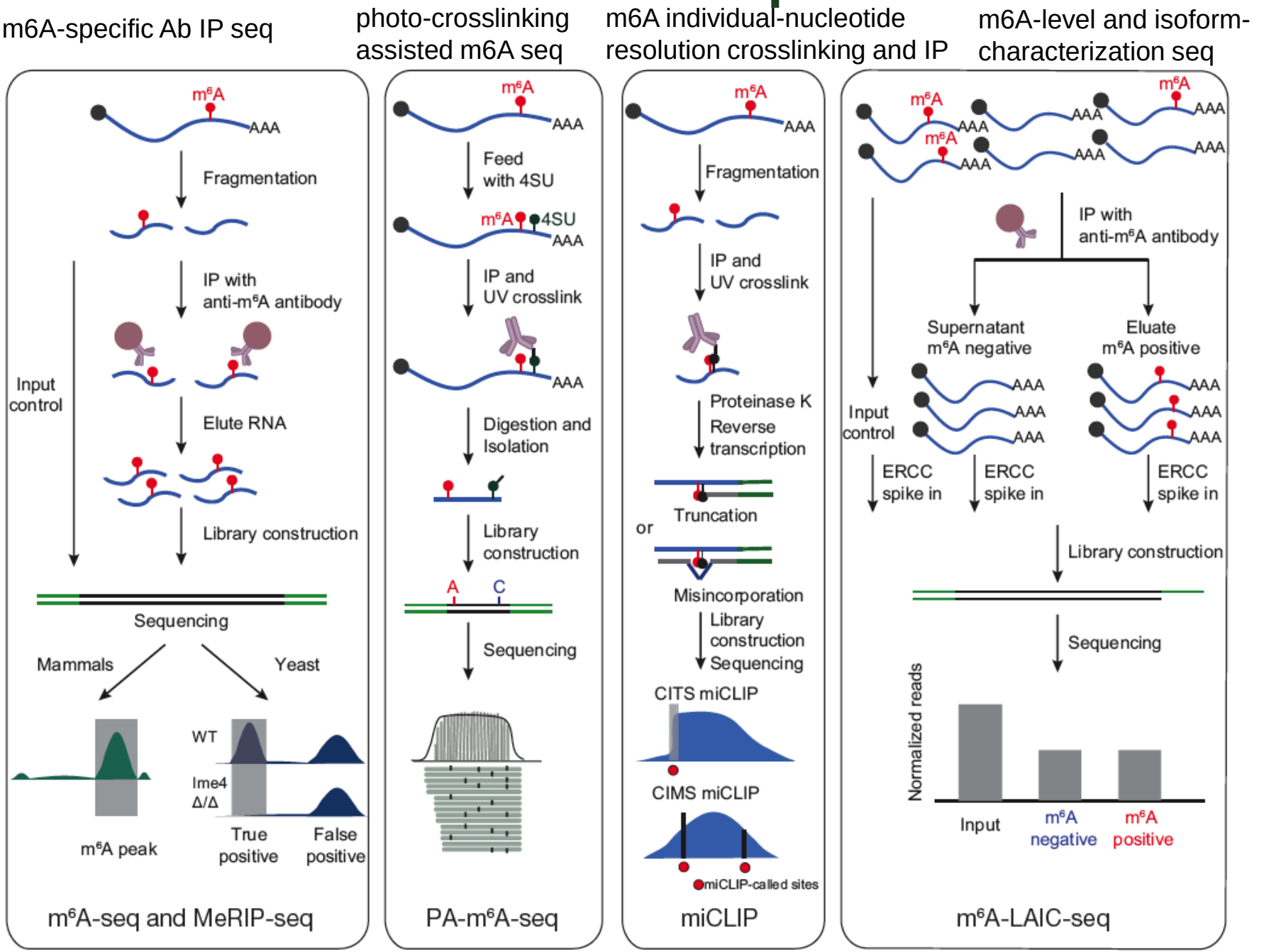
Sibbrrit et al, WIREsRNA 2013

RNA MODIFICATION



Sibbrrit et al, WIREsRNA 2013

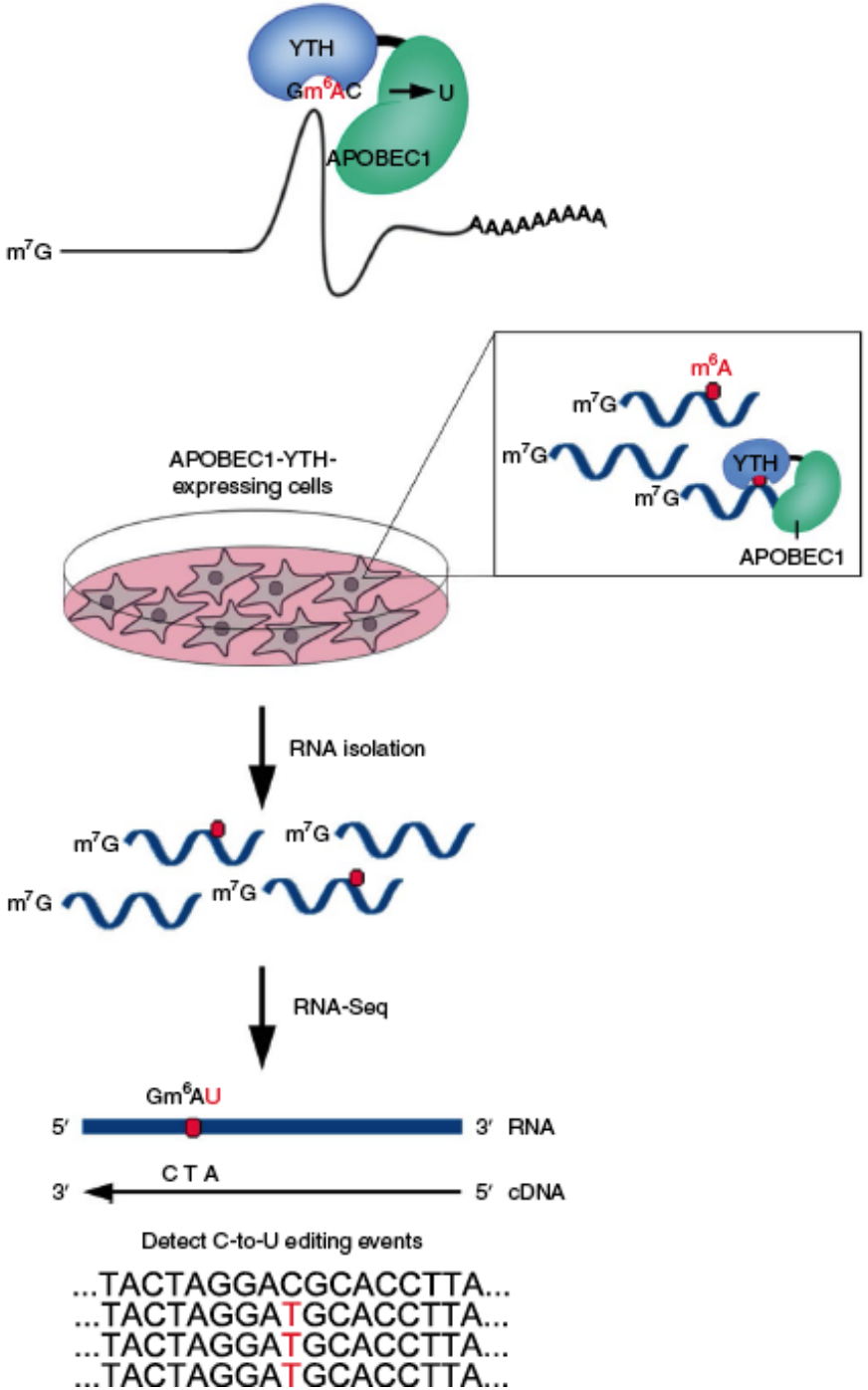
m⁶A RNA-seq



Li at al, Nat Methods, 2017

antibody-free m6A-seq DART-seq

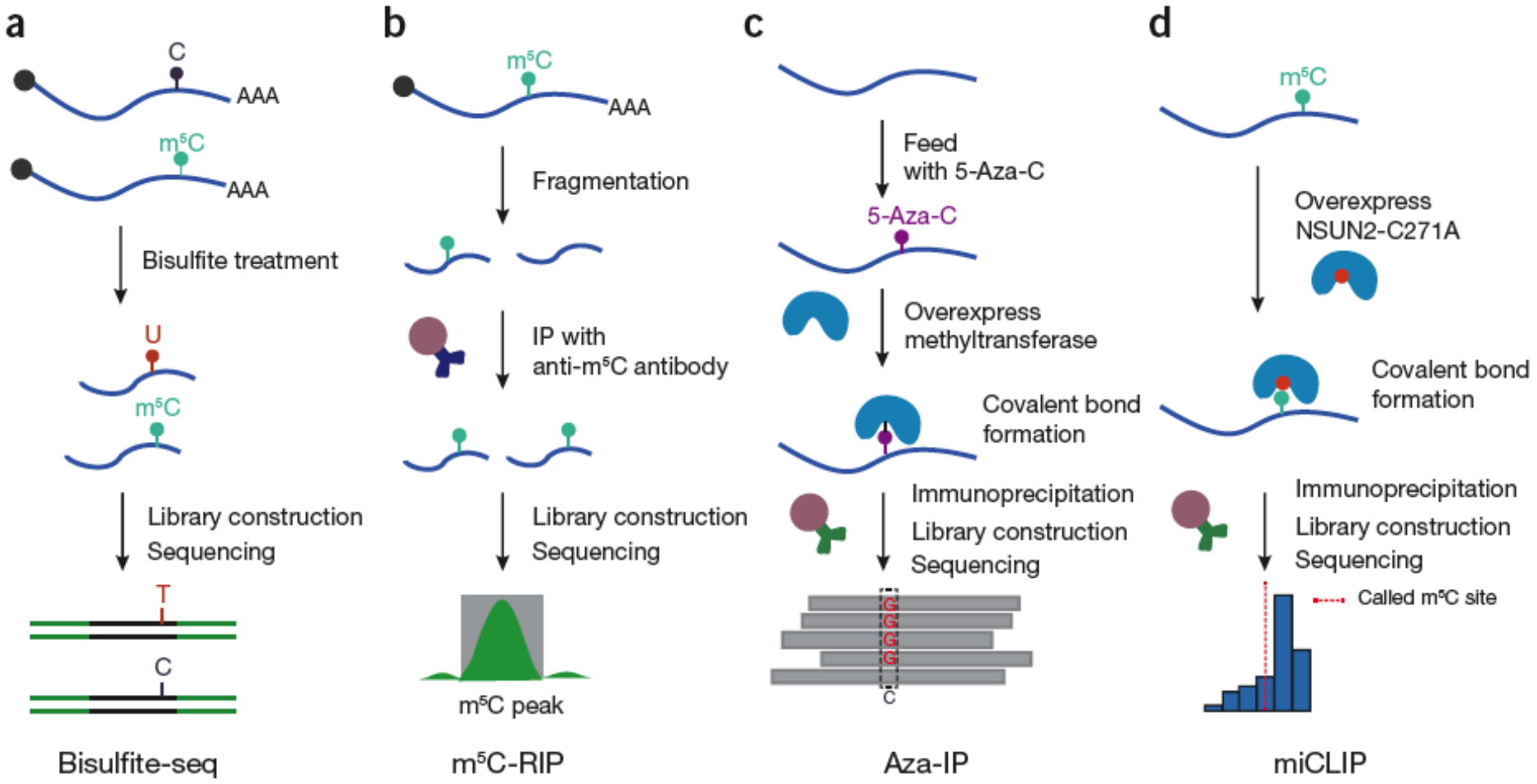
deamination adjacent to RNA modification targets



- **Cytidine deaminase APOBEC1 fused to m^{6}A-binding YTH domain**
- **APOBEC1-YTH induces C-to-U deamination at sites adjacent to m6A**
- **detected using RNA-seq**

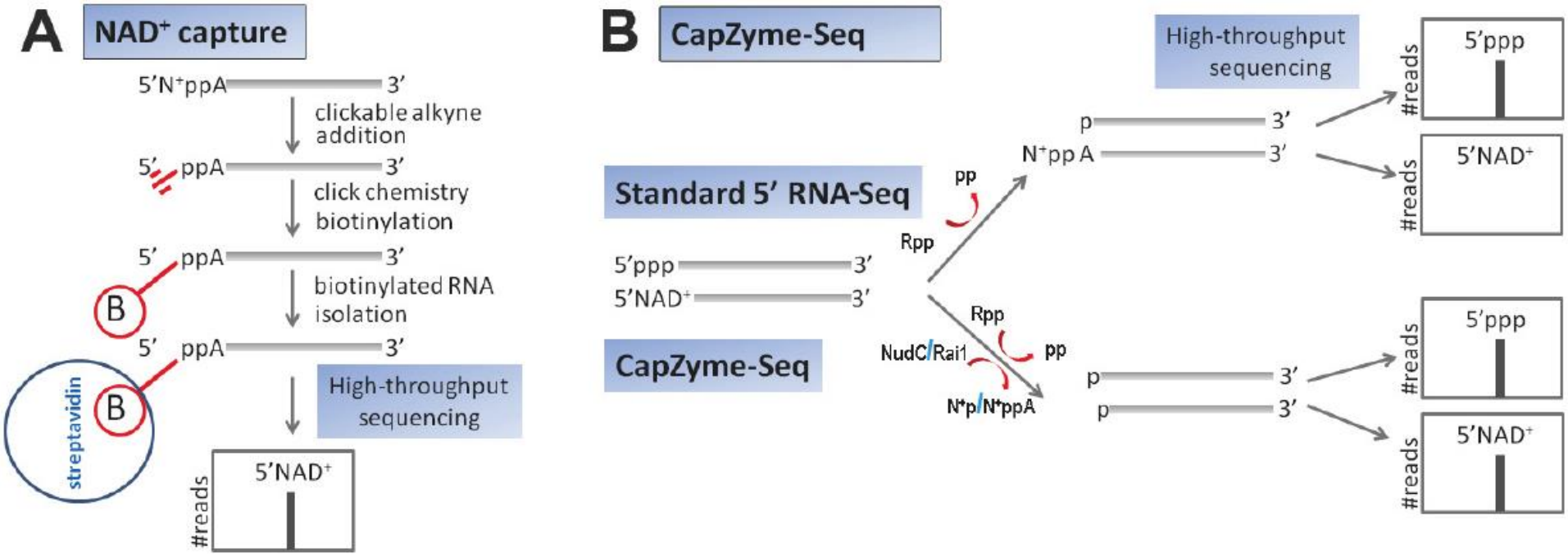
Meyer, Nature Methods 2019

m^{5}C RNA-seq



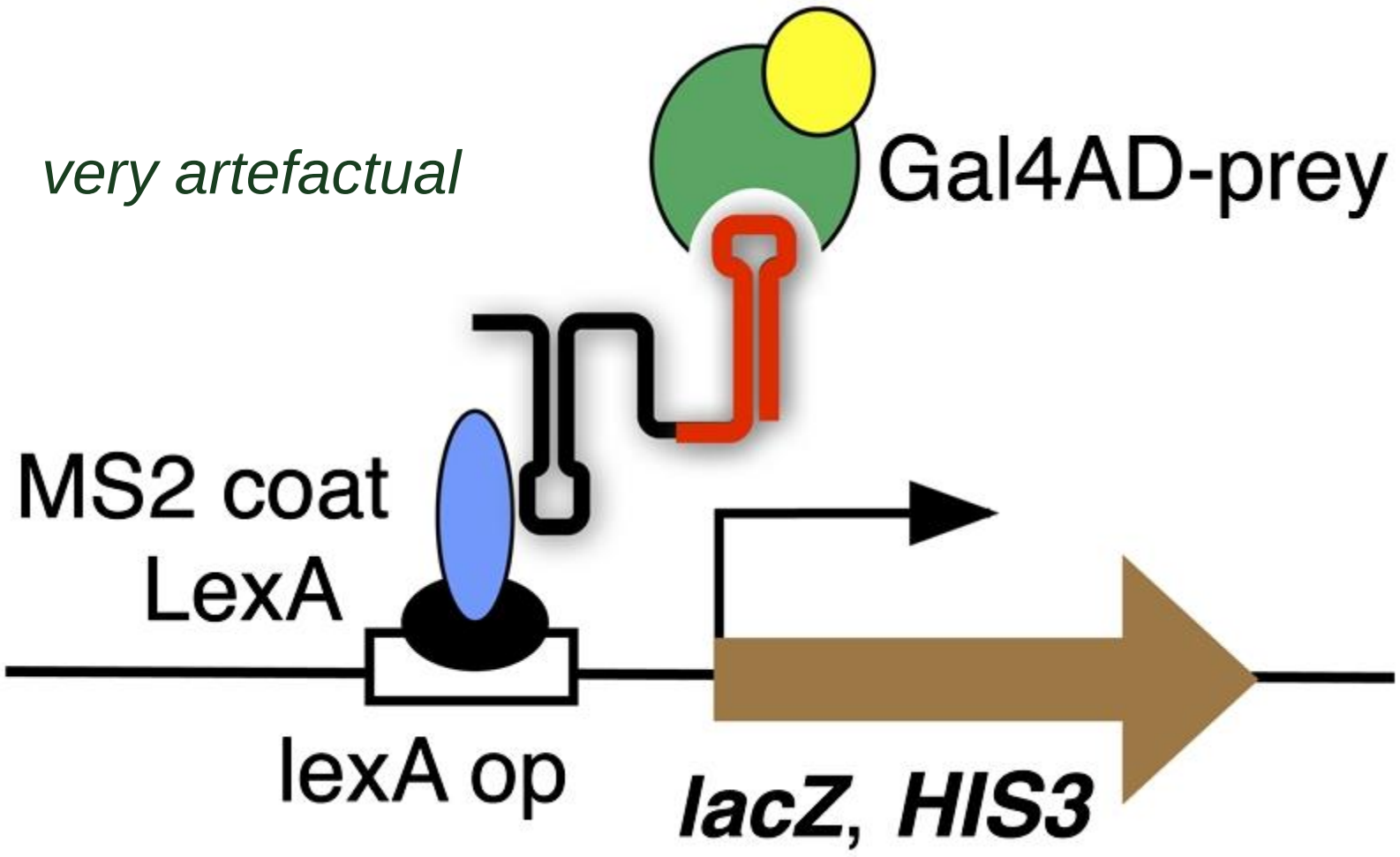
Li at al, Nat Methods, 2017

Identification of NAD+ capped RNAs



INTERACTIONS: RNA-proteins
RNA-DNA
RNA-RNA
RNA structure

GENETIC SCREEN- YEAST THREE HYBRID



The RNA insert (red) is expressed in the context of RNA vector sequences (black) tethered upstream of *lacZ* (brown) and *HIS3* reporter genes via a MS2 coat–LexA fusion protein (blue and black). Gene activation depends on binding of the Gal4 activation domain (yellow) –prey fusion protein (green).

OLD-FASHIONED BIOCHEMICAL PURIFICATION

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 249, No. 18, Issue of September 25, pp. 5963–5970, 1974
Printed in U.S.A.

Isolation, Structure, and General Properties of Yeast Ribonucleic Acid Polymerase A (or I)

(Received for publication, December 28, 1973)

JEAN-MARIE BUHLER, ANDRÉ SENTENAC, AND PIERRE FROMAGEOT

From the Service de Biochimie, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, 91 190 Gif-sur-Yvette, France

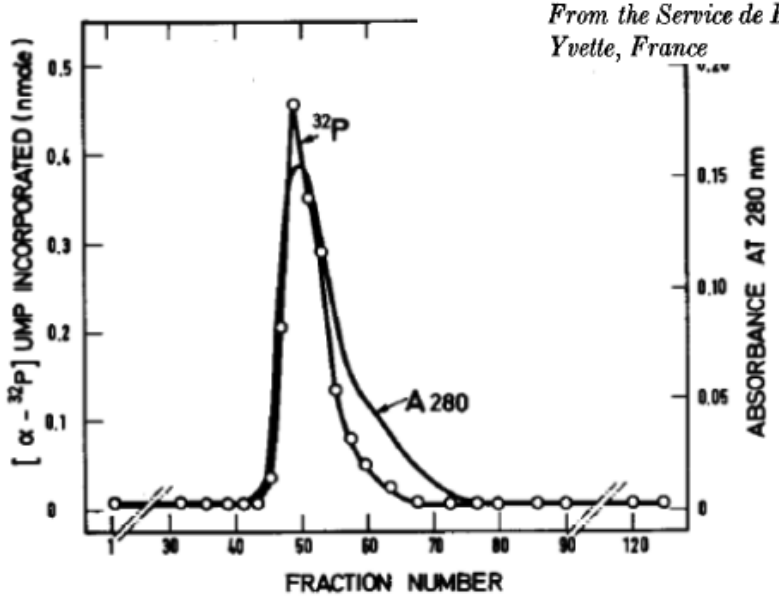


FIG. 1. DEAE-cellulose column chromatography. Fraction 3 (15 ml, $A_{280\ nm}$ 0.8) was applied to a column (5 cm^2 × 16 cm) of DEAE-cellulose and eluted as described in the text. Fractions of 3 ml were collected and assayed for RNA polymerase activity on 10-μl aliquots for 10 min under standard conditions.

TABLE I

Summary of RNA polymerase A purification

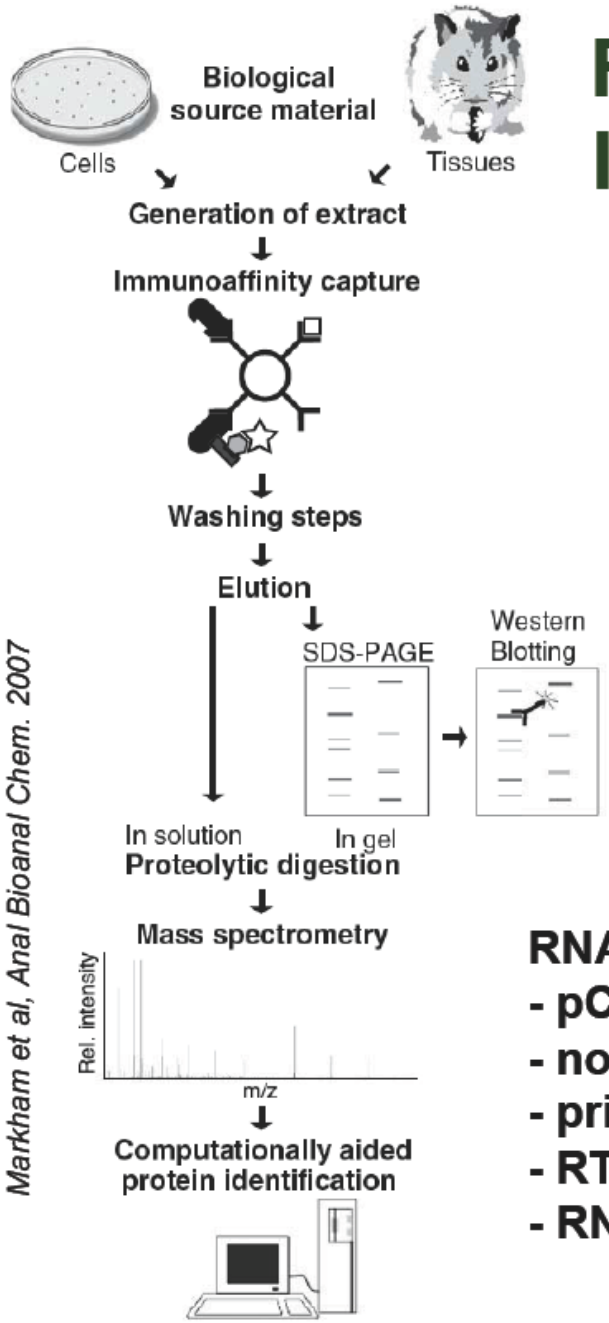
Values are given for 300 g of yeast cells.

Fraction or step in purification	Volume	Proteins	Total activity	Specific activity
	ml	*mg*	*units*	*units/mg*
1. High speed centrifugation..	530	2,300	21,000	0.9[a]
2. Phosphocellulose batch....	290	185	38,000	203
3. DEAE-cellulose batch.....	300	21	25,000	1,200
4. DEAE-cellulose chromatography.....	30	2.5	3,000	1,200
5. Glycerol gradient.....	5	0.5	900	1,800

[a] RNA polymerase A and B are not separated at this stage.

RNP IMMUNOPRECIPITATION
IP, co-IP

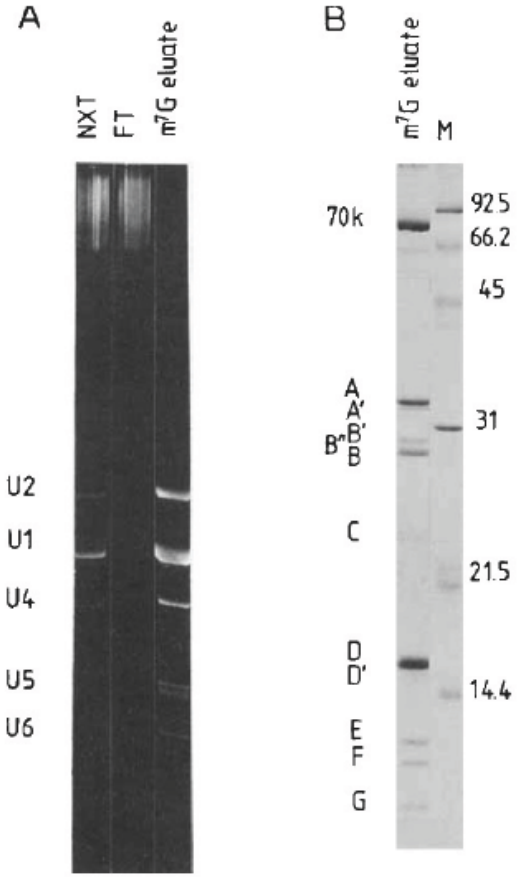
With specific antibodies or using tagged proteins



Markham et al, Anal Bioanal Chem. 2007

RNA analysed by:
- **pCp labeling (3' end)**
- **northern blot**
- **primer extension**
- **RT-PCR**
- **RNASeq**

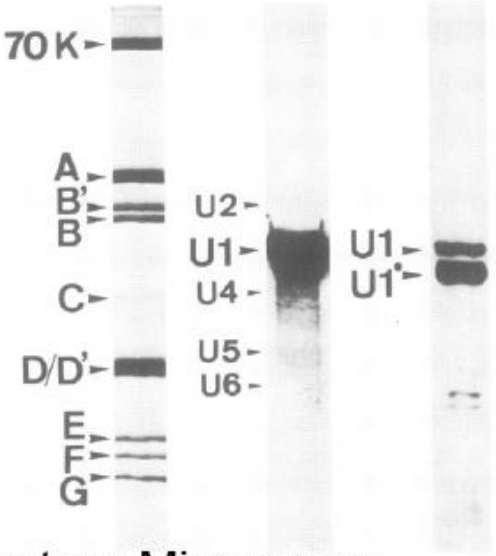
U snRNPs with anti-TMG cap antibody



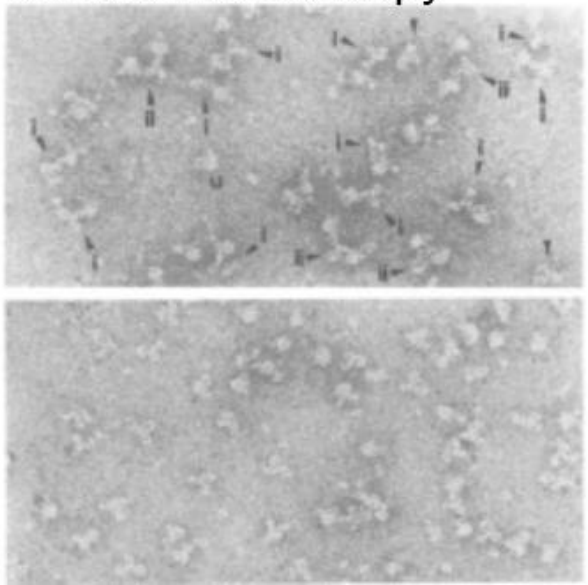
Bochnig et al, Eur. J Biochem. 1987
(Luhrmann's lab)

IP of U1 snRNP with anti-70K Ab (U1 specific protein)

Immunoaffinity +ion exchange



Electron Microscopy



IP of snRNPs with anti-TMG cap Ab

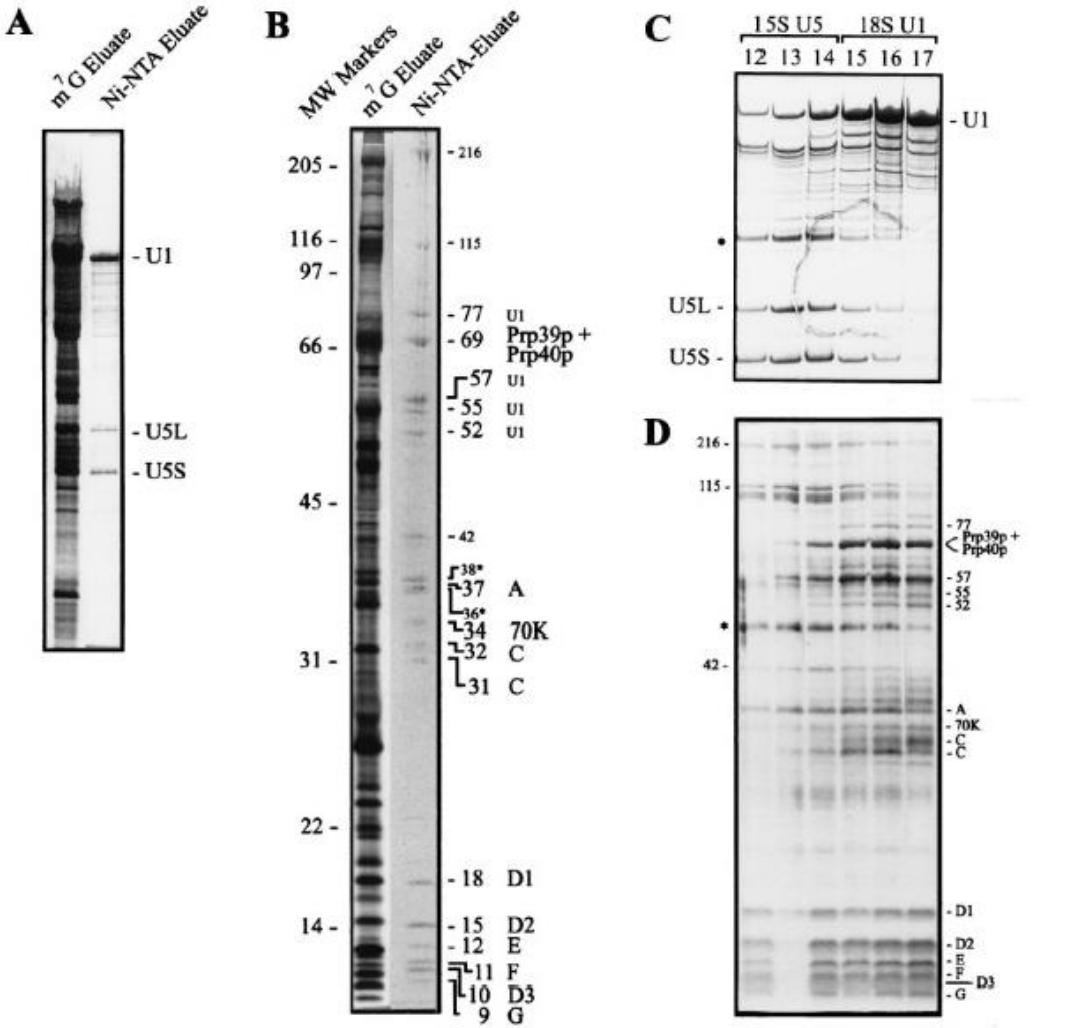
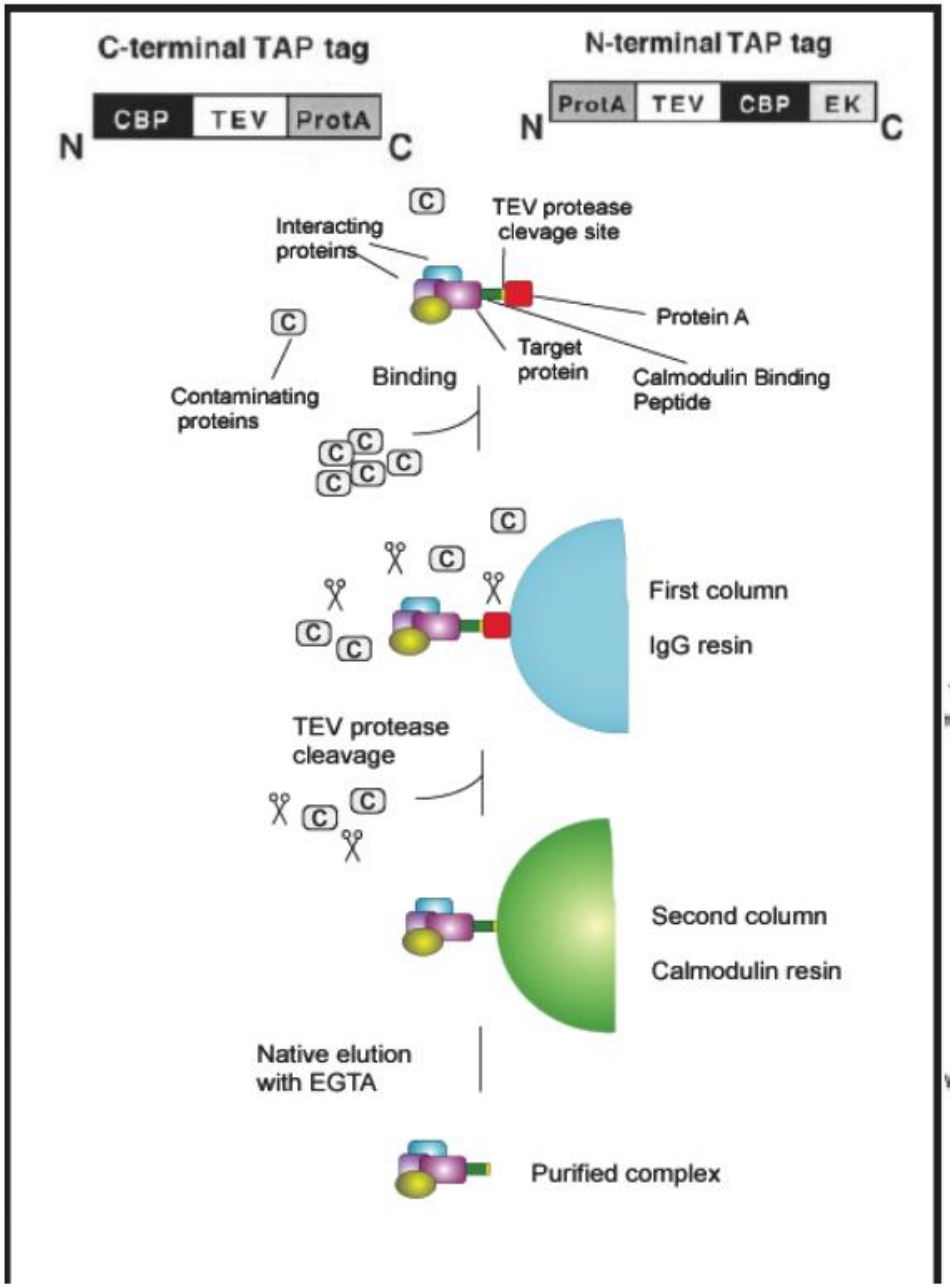
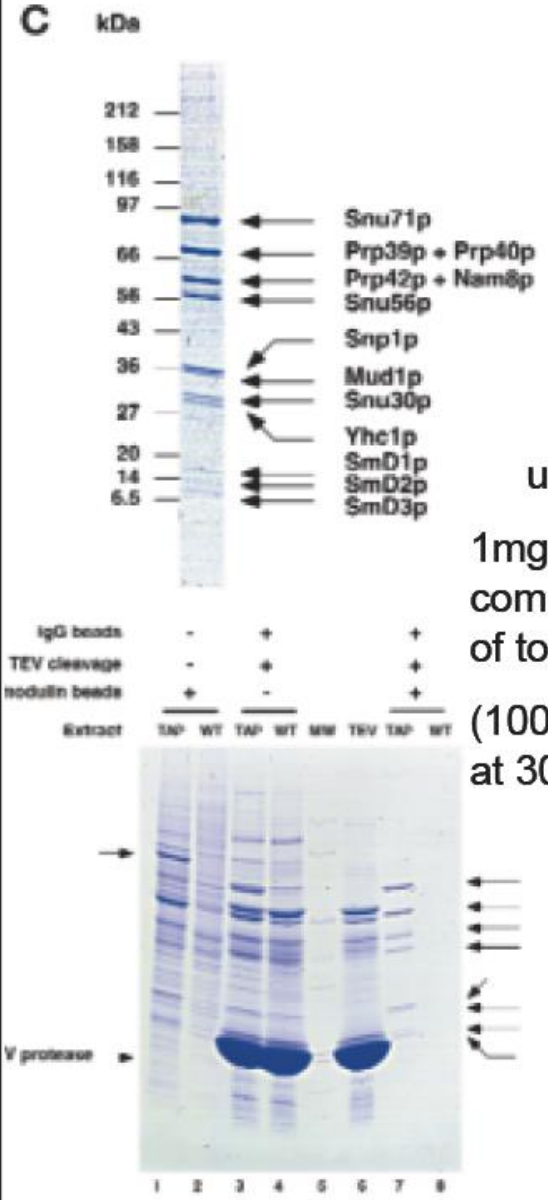


FIG. 1. Purification of U1 snRNPs from *S. cerevisiae*. (*A*) Silver staining of snRNAs eluted from anti-m_3G-cap (m^7G eluate) and Ni-NTA affinity

TANDEM AFFINITY PURIFICATION (TAP)





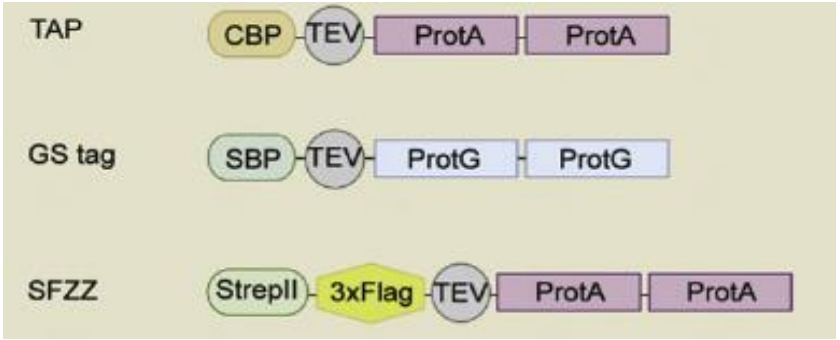
usual yield:

1mg of protein complex from 300mg of total protein

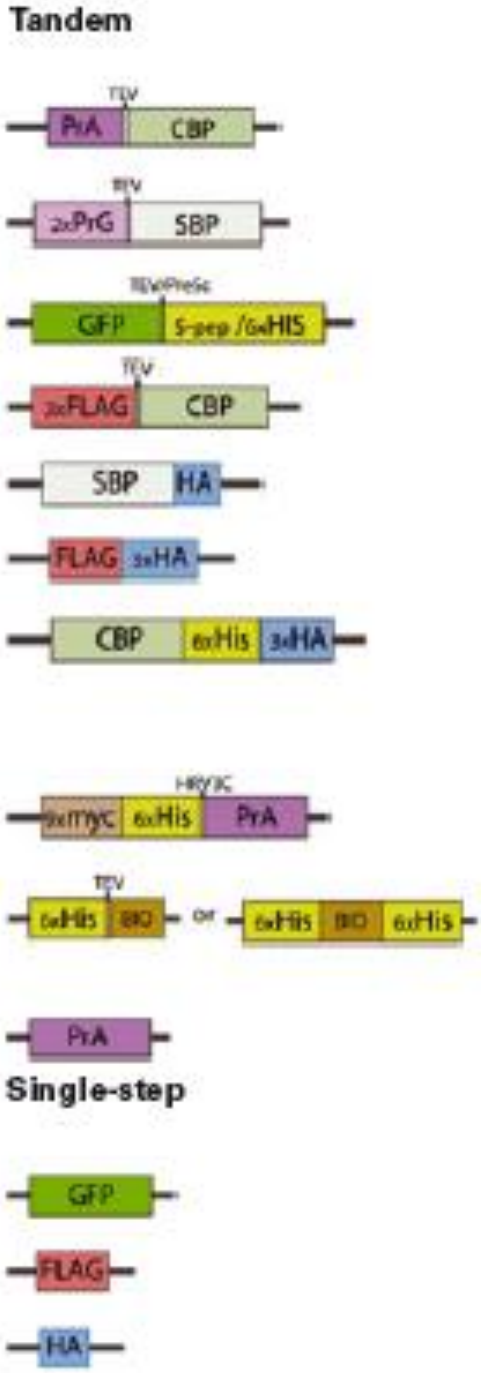
(100 000 X purification at 30% efficiency)

MODIFIED TAP tags

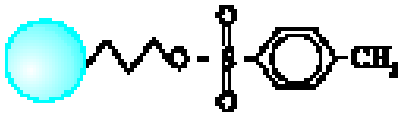
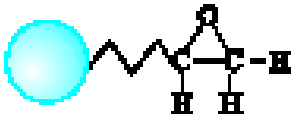
mammalian cells



Drakas et al., Proteomics, 2005
Van Leene et al., TiPlSci, 2008;
Gloeckner et al, Proteomics, 2007
Oeffinger, Proteomics, 2012



MAGNETIC BEADS vs SEPHAROSE

Dynabeads® M-280 Tosylactivated	**Dynabeads® M-270 Epoxy**
	
• Hydrophobic bead. • Surface tosyl groups. • Bead diameter 2.8 µm.	• Hydrophilic bead. • Surface epoxy groups. • Bead diameter 2.8 µm.
• Direct covalent binding to primary amino- or sulfhydryl groups in proteins and peptides. • No further surface activation required. • Binding over night at neutral to high pH and high temperature.	• Direct covalent binding to primary amino and sulfhydryl functional groups in proteins and peptides. • No further surface activation required. • Binding over night at neutral pH, high salt and a wide temperature range.

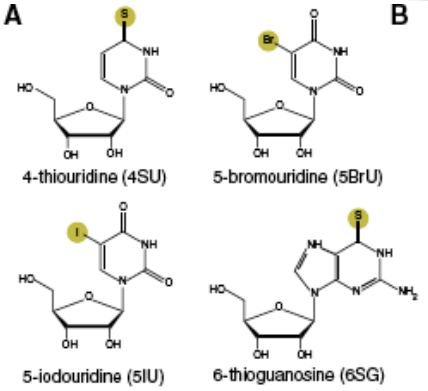
Diameter 2.8 um volume 40 000 smaller than agarose/sepharose

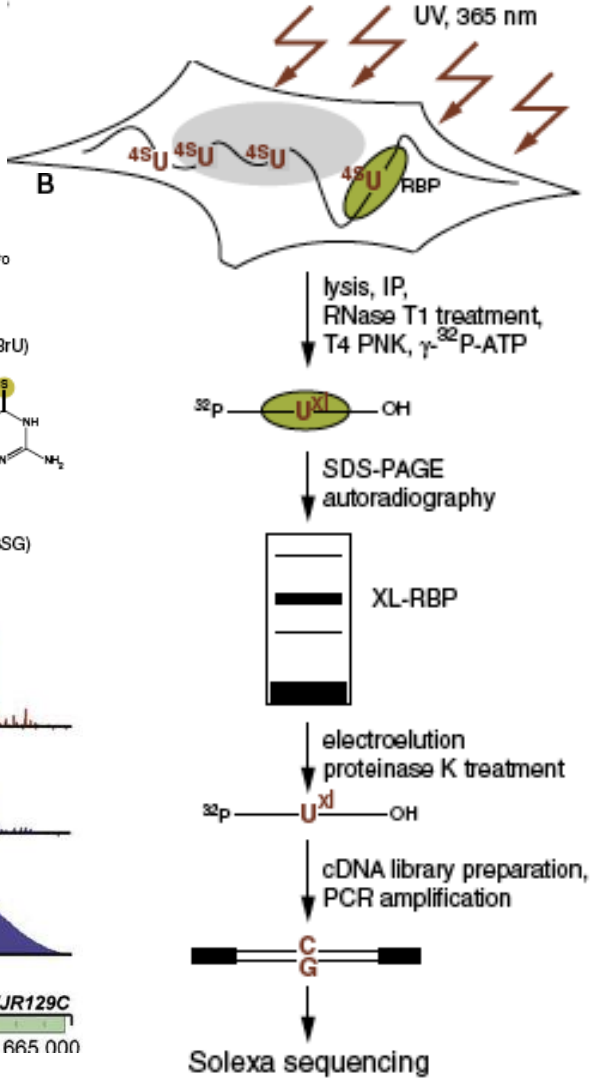
PAR-CLIP

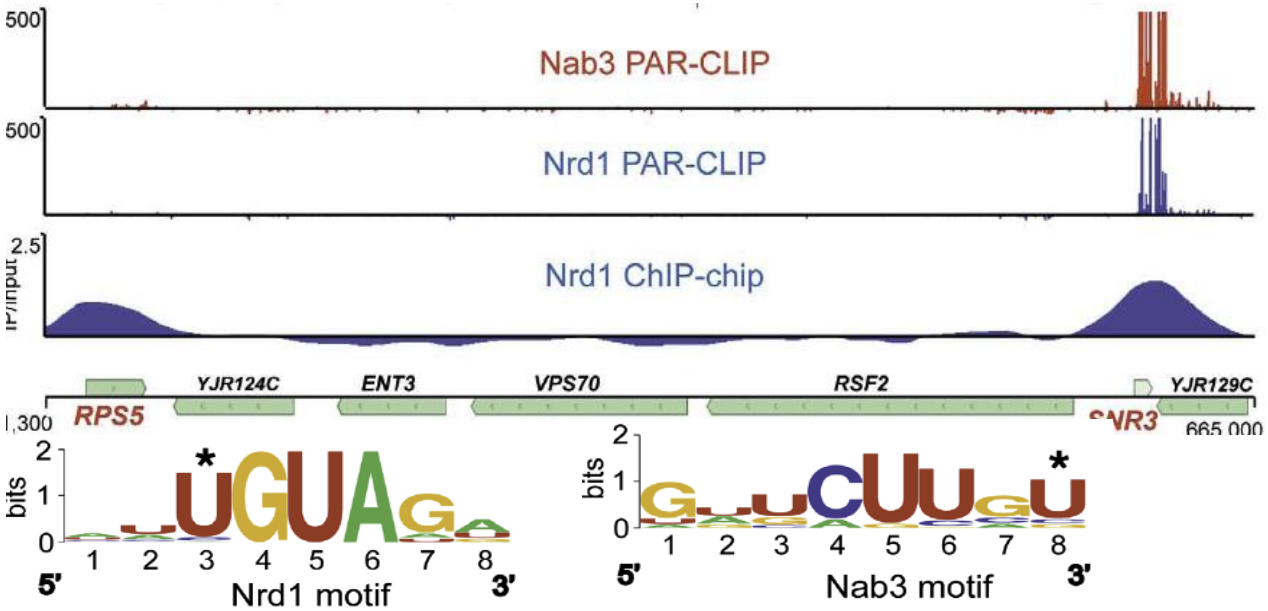
PhotoActivatable ribonucleoside–enhanced CrossLinking and ImmunoPrecipitation

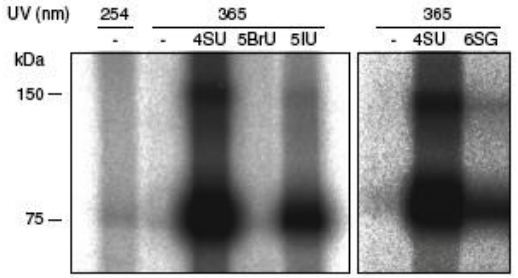
HITS-CLIP:

High-Throughput Seq CLIP





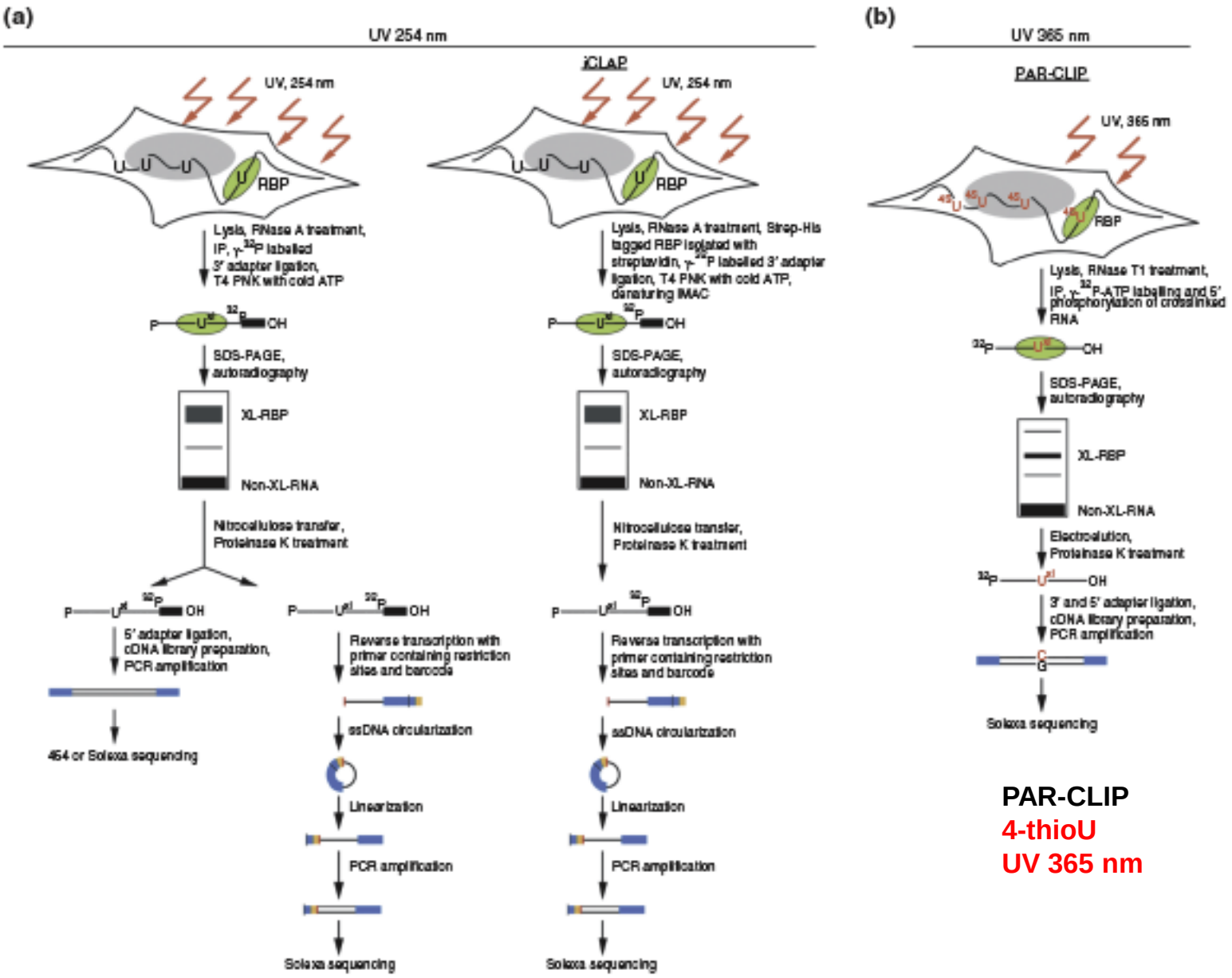




Creamer et al., PLOS Genet, 2011

Hafner et al., Cell, 2010

http://www.jove.com/index/details.stp?ID=2034



PAR-CLIP
4-thioU
UV 365 nm

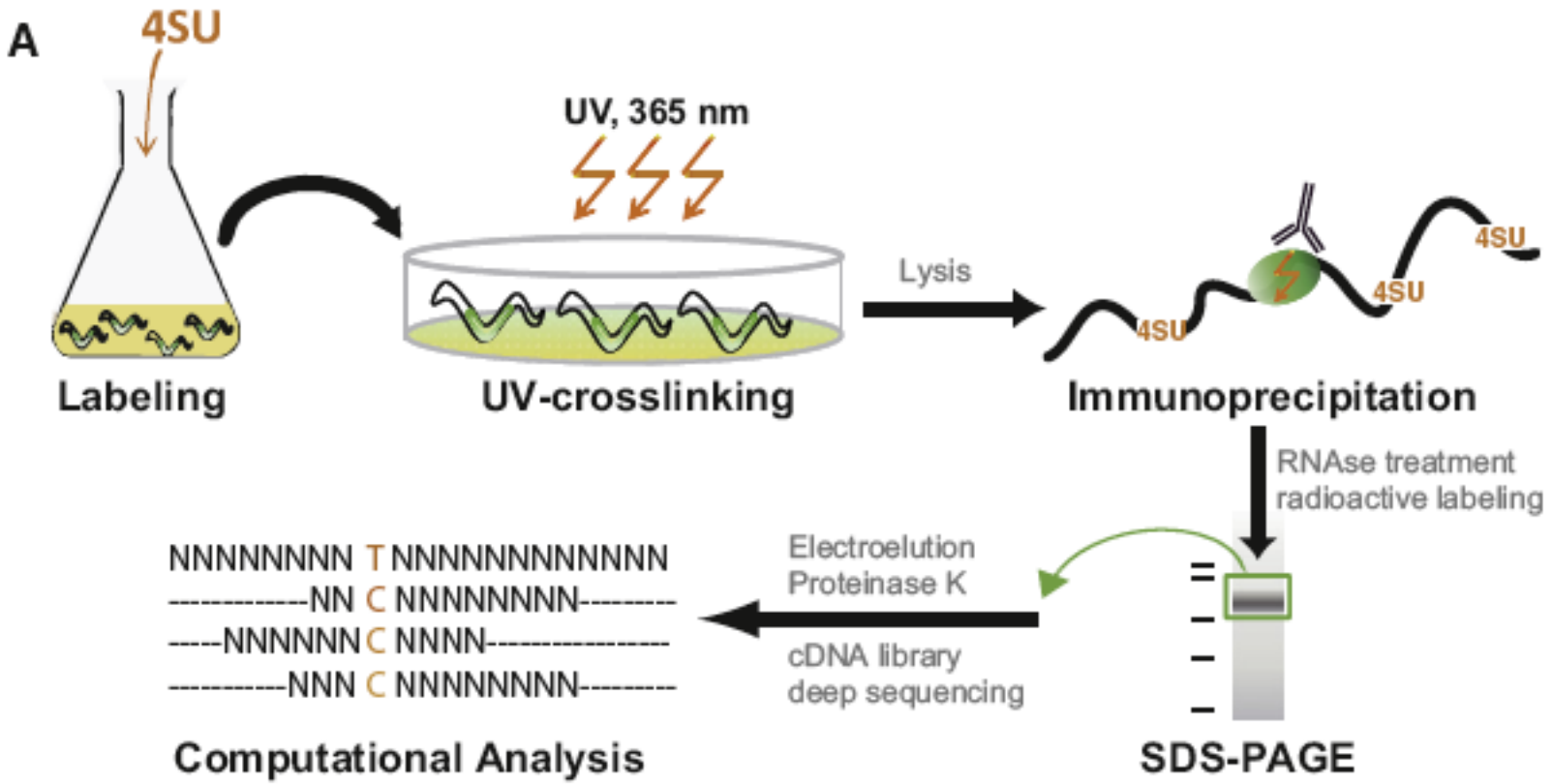
UV 254 nm **HiTS-CLIP**

iCLIP
ssDNA is circularized

iCLAP
RBP is Strep- and polyHis tagged

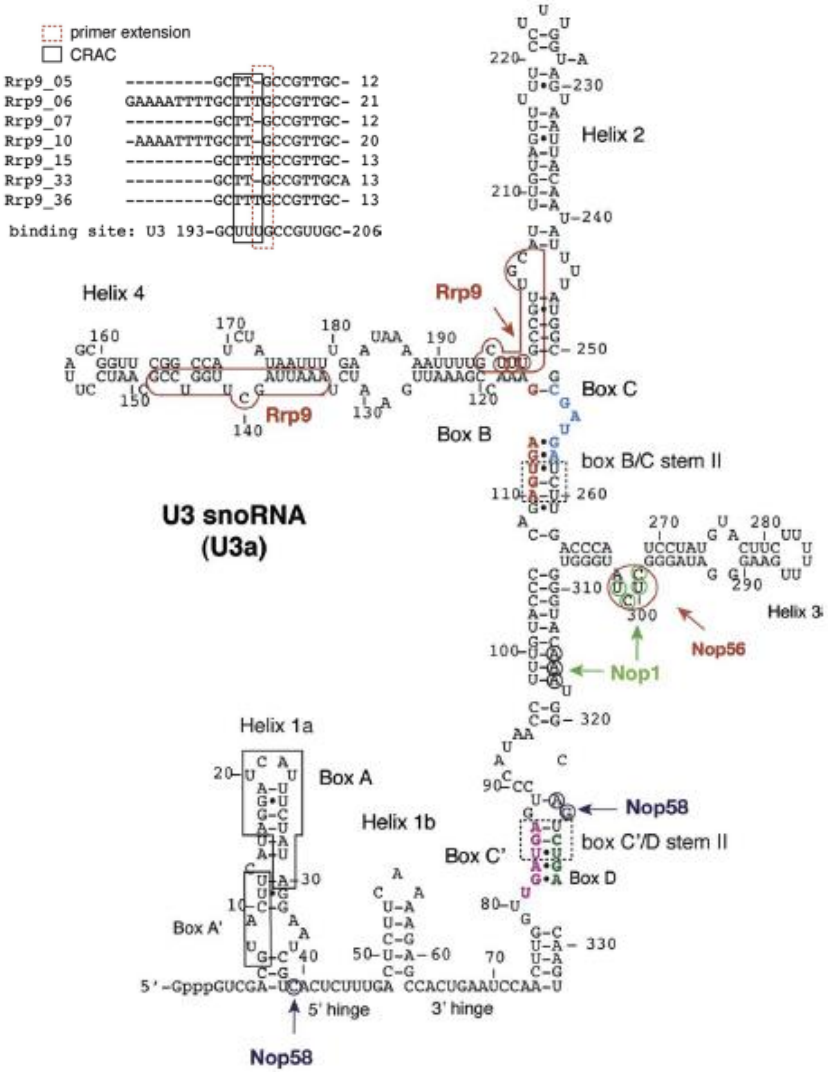
Ascano et al., WIREs RNA, 2012

in vivo PAR-CLIP

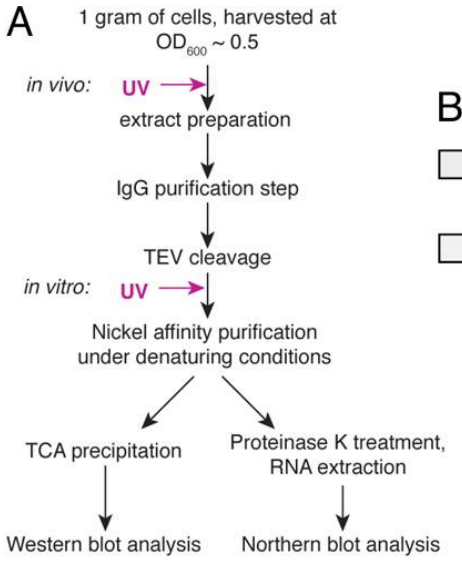


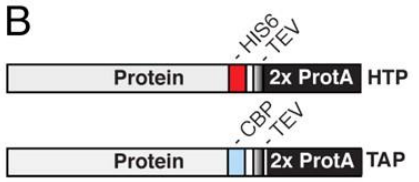
Jungkamp et al., Mol Cell, 2011

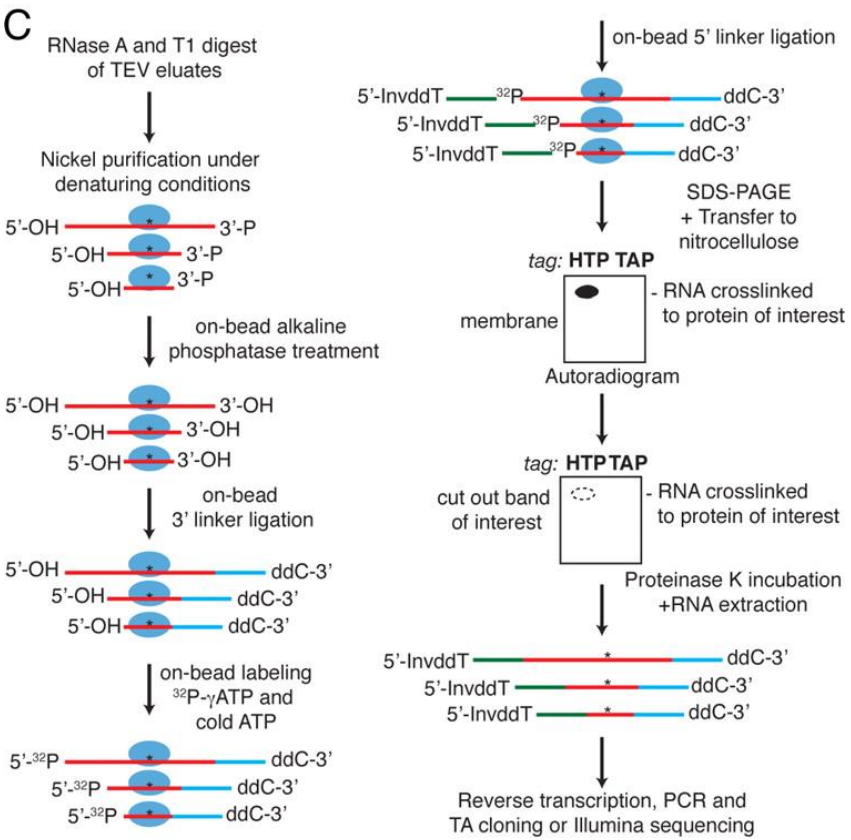
CRAC technique:
CRosslinking and Analysis of cDNA

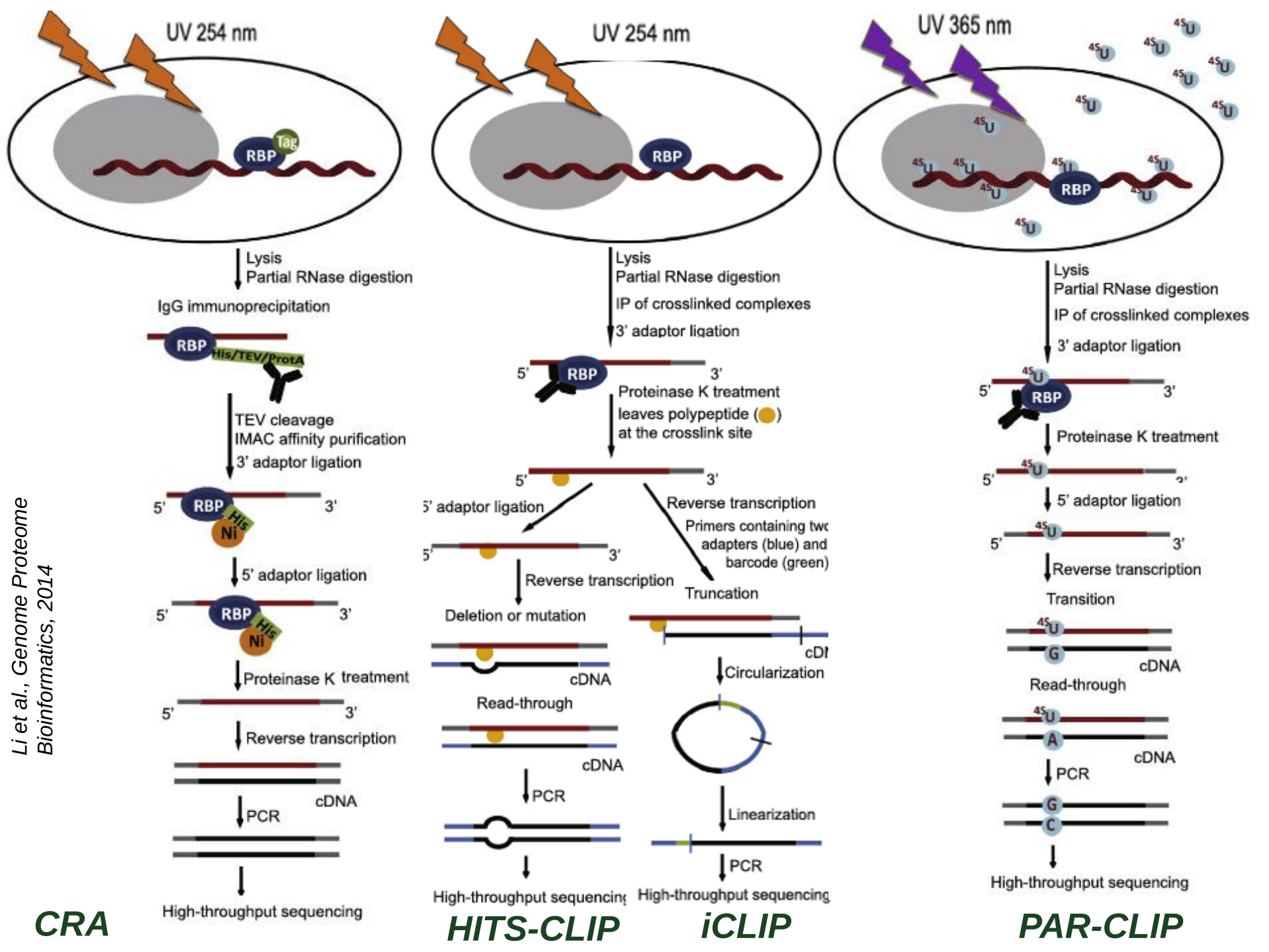


Granneman et al., PNAS, 2009



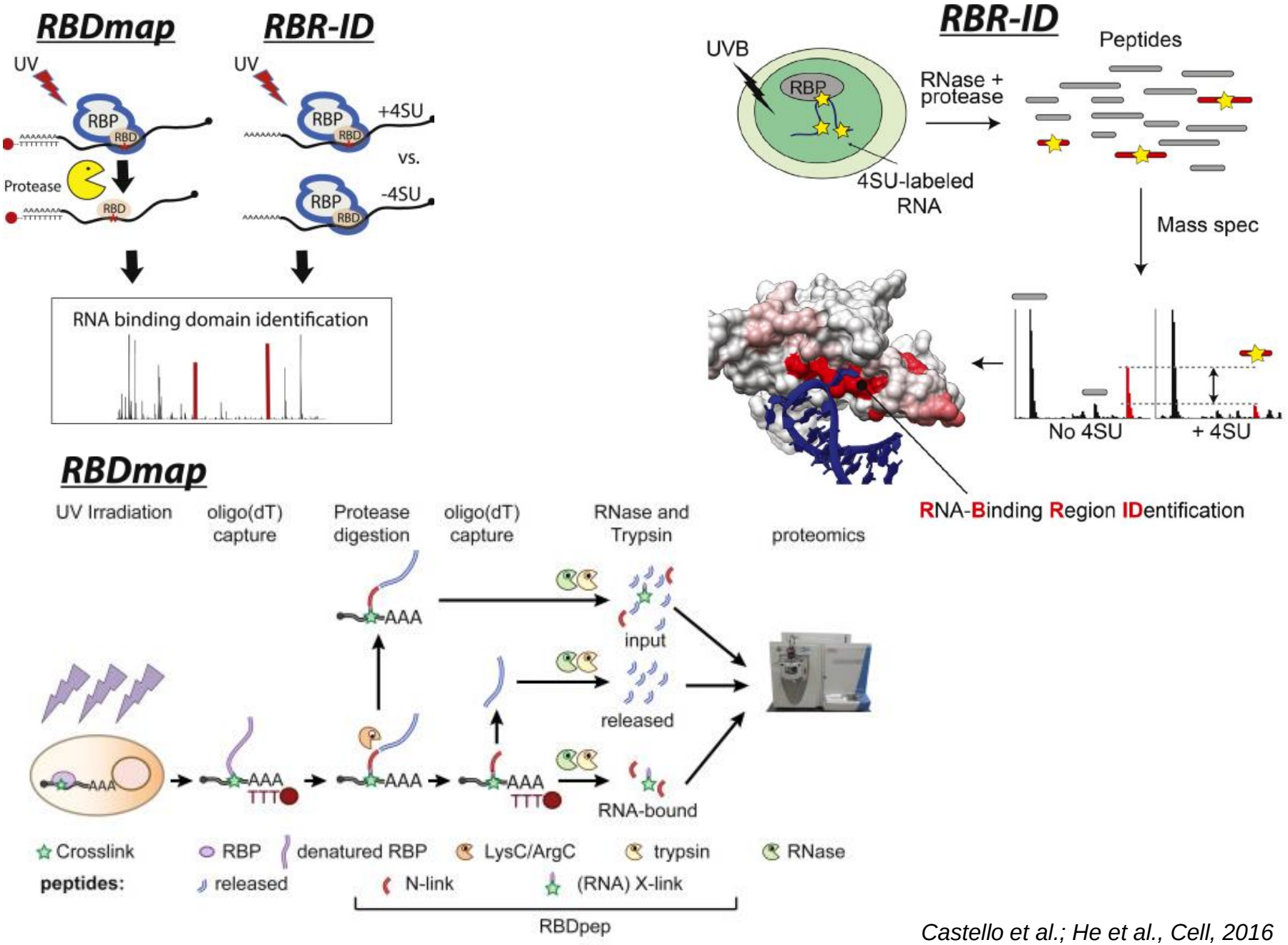






Li et al., Genome Proteome Bioinformatics, 2014

mRNA binding proteome (poly(A) BP)



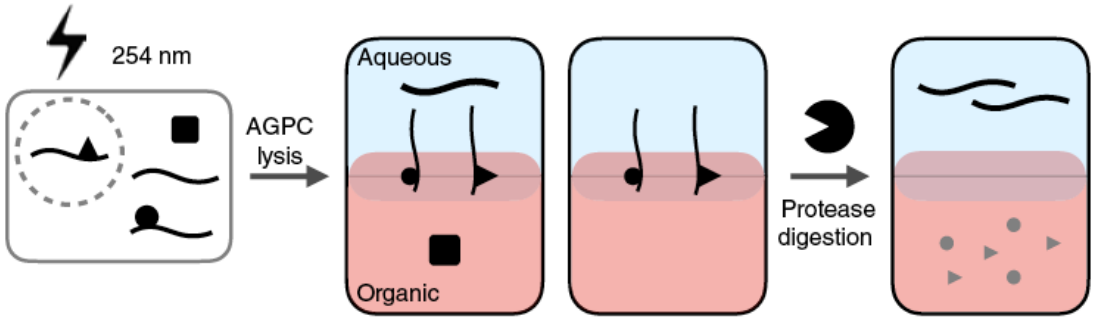
Castello et al.; He et al., Cell, 2016

OOPS, TRAPP, XRNAX RNP interactome, RPBome

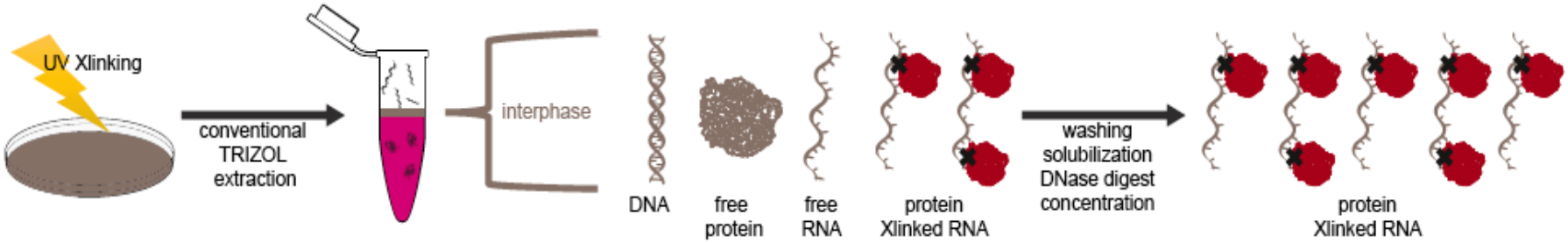
OOPS - orthogonal organic phase separation **XRNAX**

TRAPP/PAR-TRAPP - RNA-associated protein purification

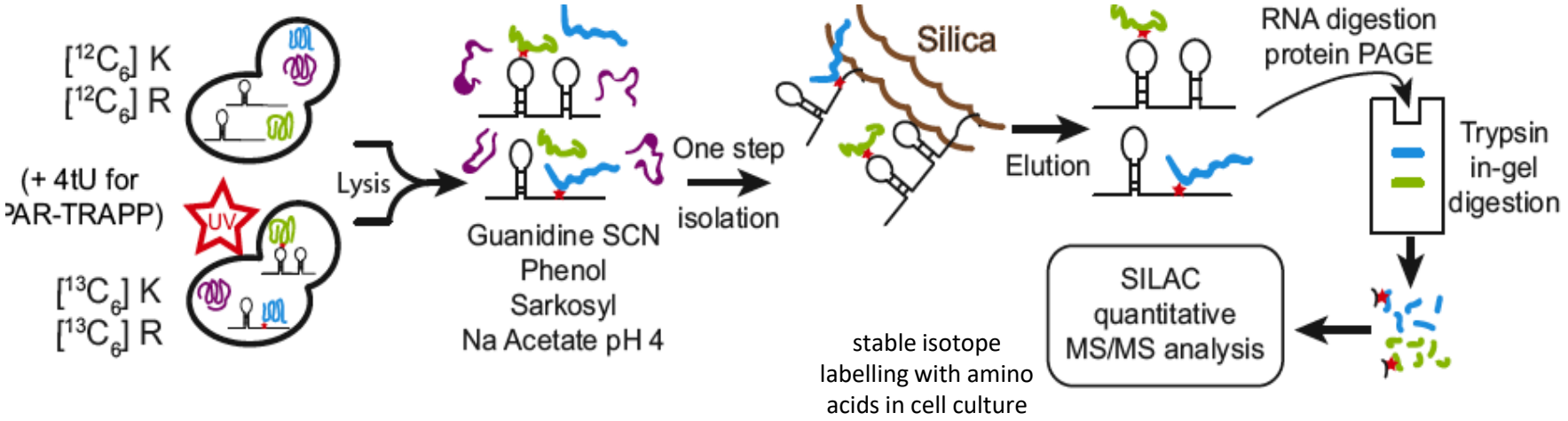
OOPS



XRNAX

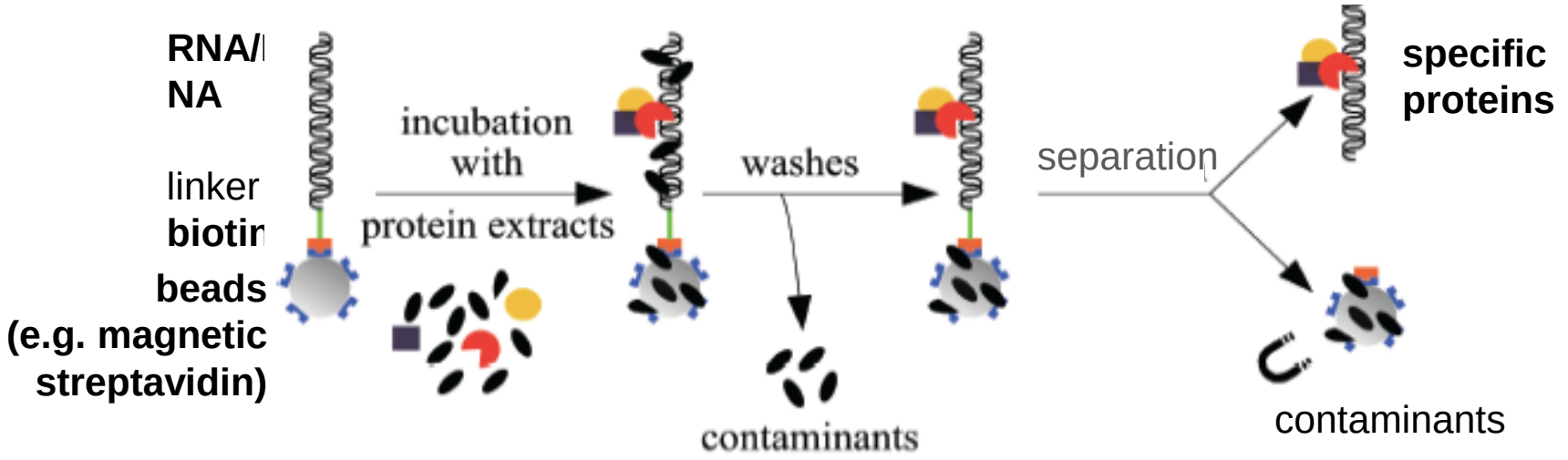


TRAPP/PAR-TRAPP

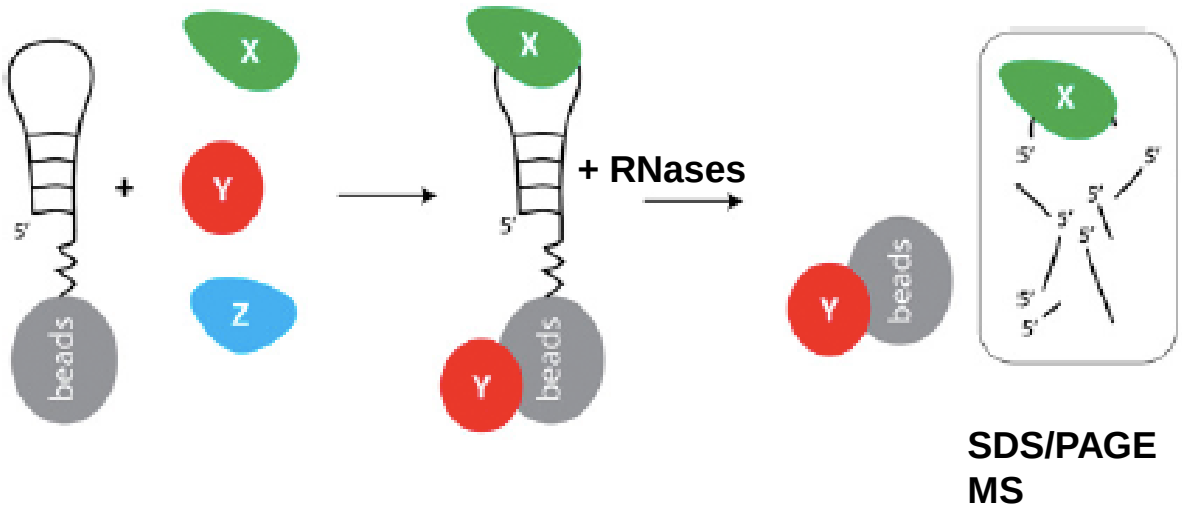


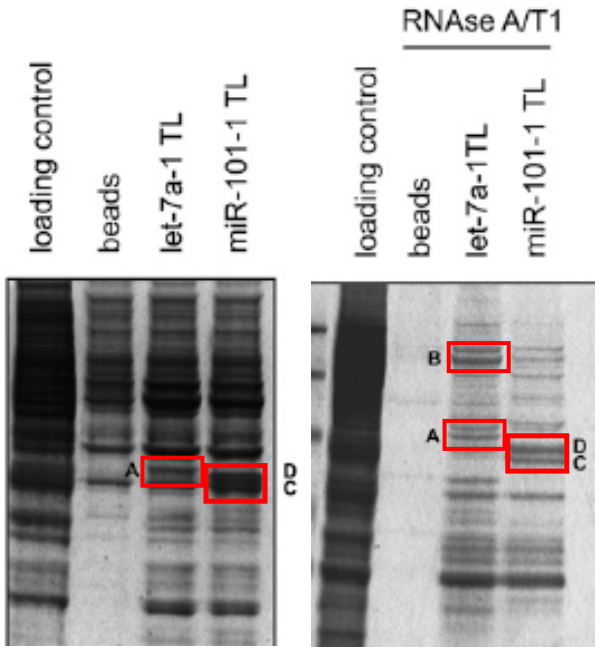
Queiroz et al, Nat Biotech, 2019; Shchepachev et al, Mol Sys Biol, 2019

RNA CHROMATOGRAPHY *in vitro*



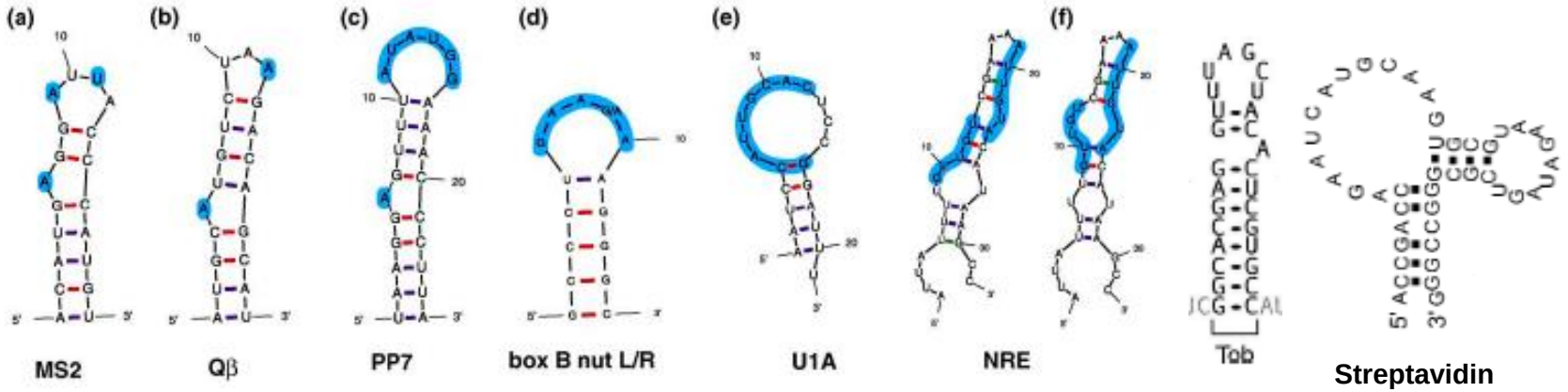
RNase-assisted RNA chromatography



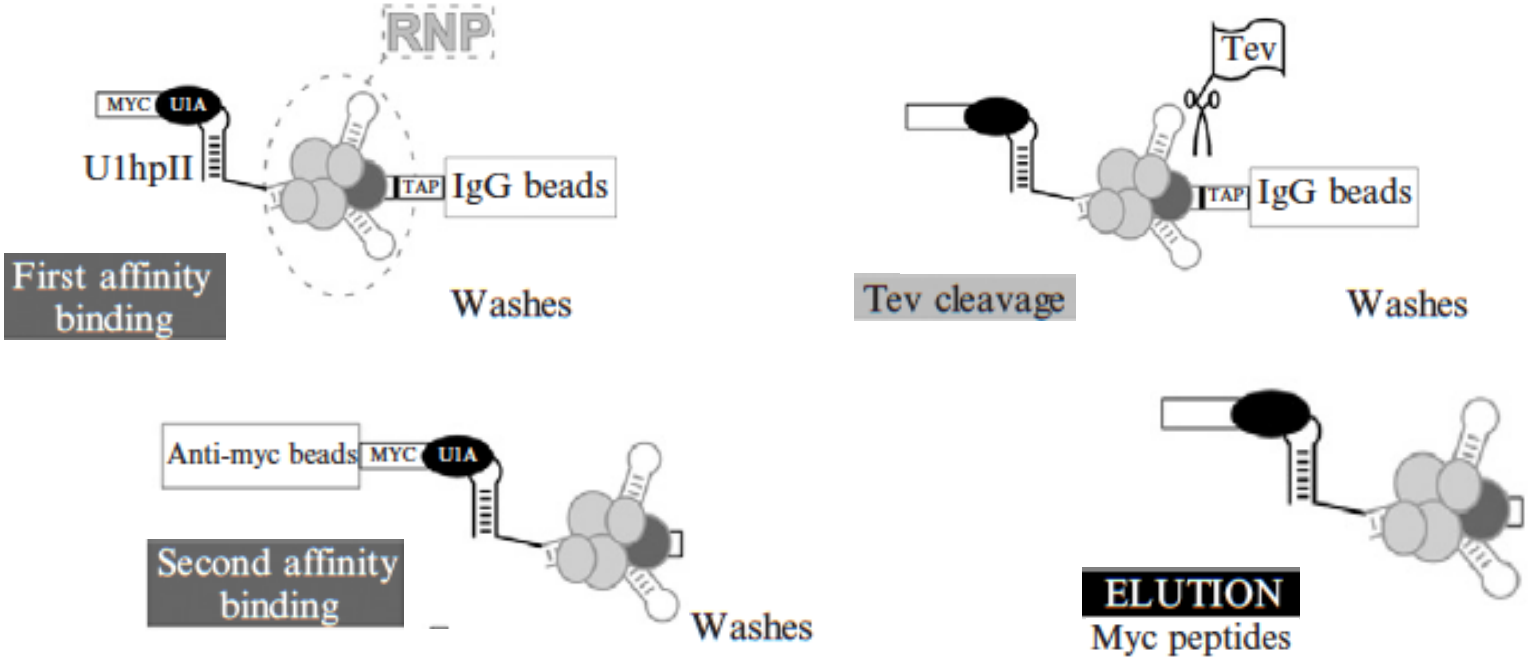


Hegarat et al., NAR, 2010; Michlewski and Caceres, RNA, 2010

RNA CHROMATOGRAPHY *in vivo*

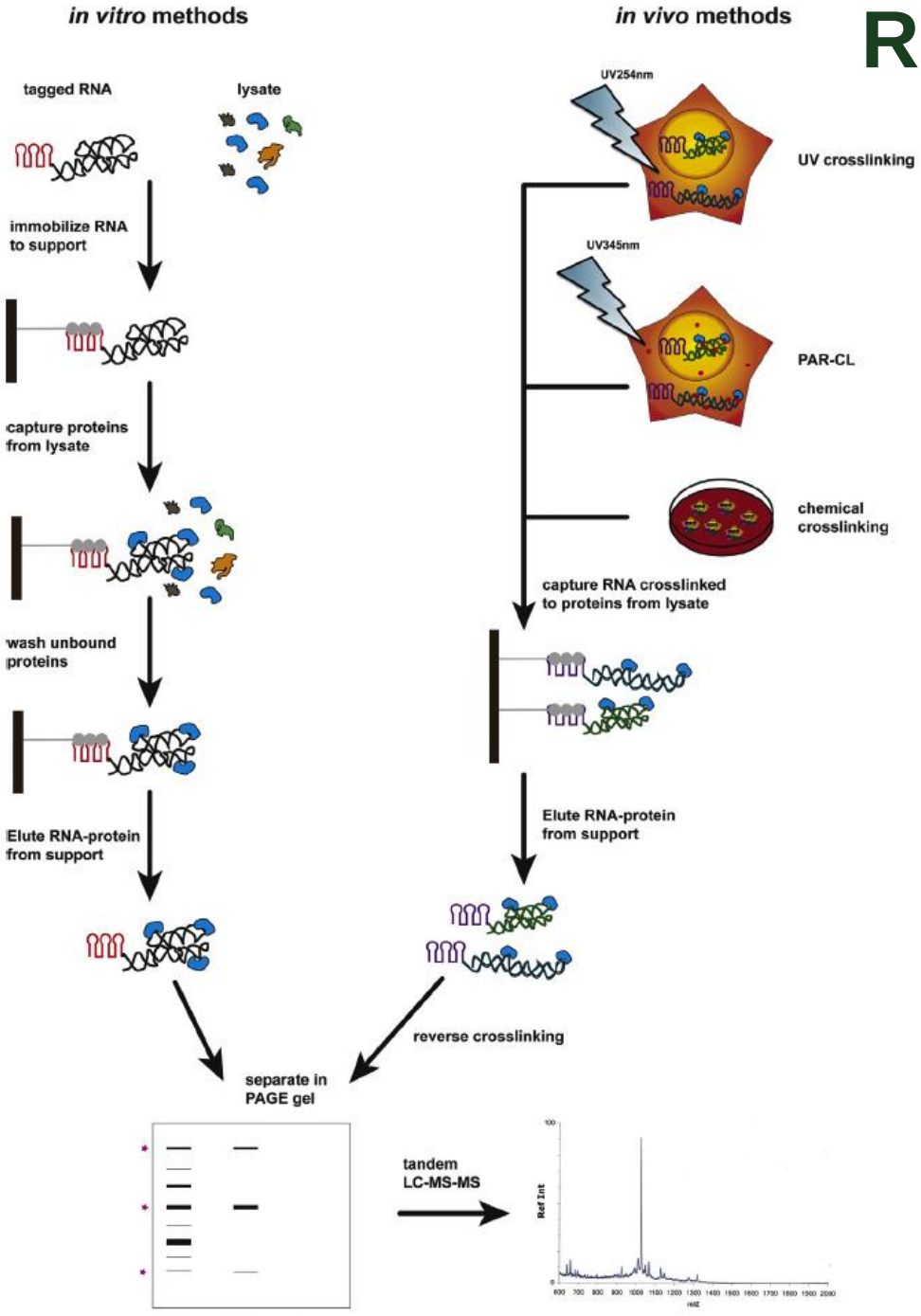


RNP purification from cells expressing RNA with affinity tags

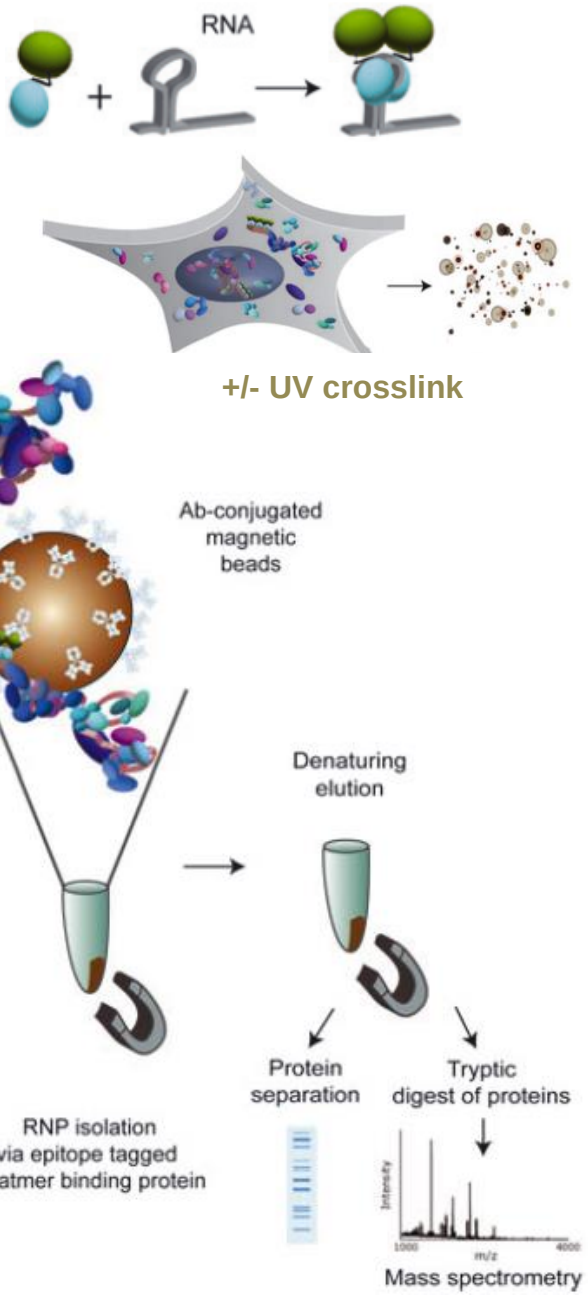


Higg and Collins, RNA, 2007; Srisawat and Engelke, Methods, 2002; Bachler et al., RNA, 1999; Weil et al., TiCB, 2010; Piekna-Przybylska et al., Meth Enzymol, 2007

RNA chromatography



Faoro and Ataide, FEBS Lett, 2014



Oeffinger, Proteomics, 2012