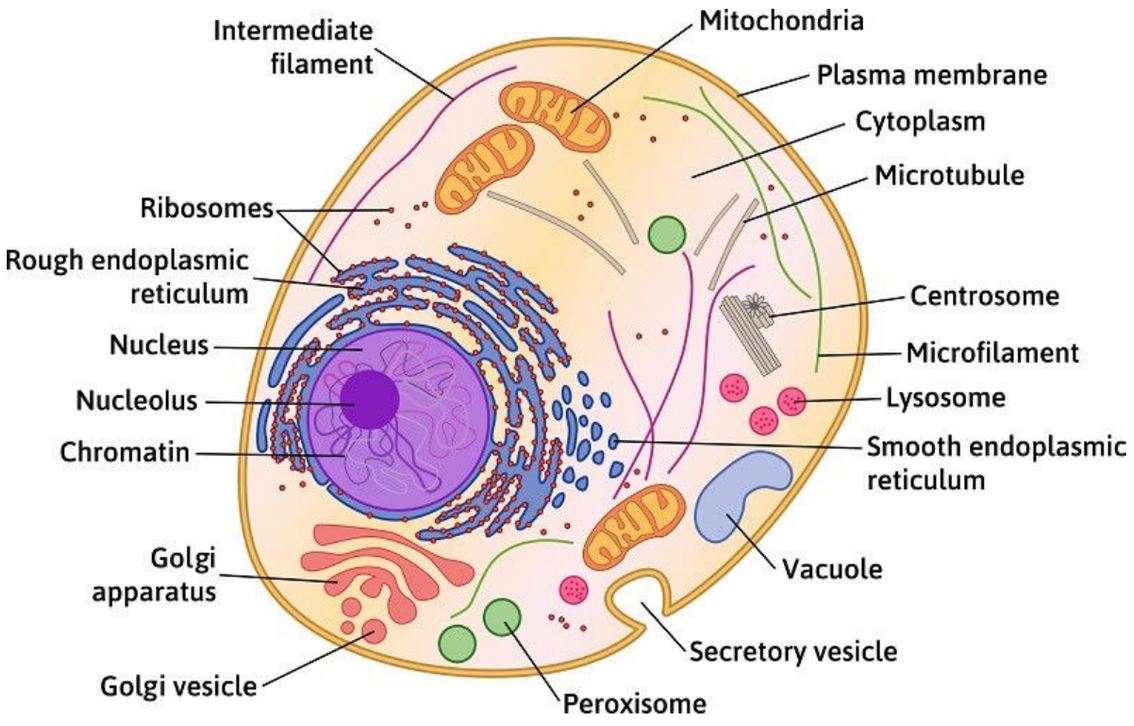
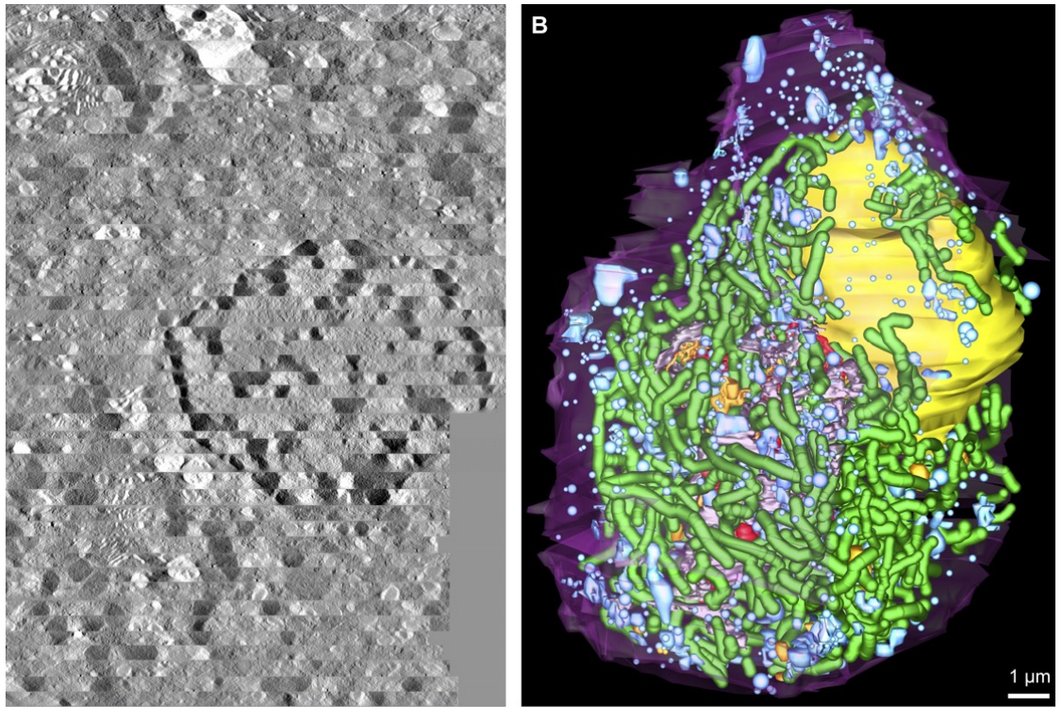
THE INTERACTOME STUDY

October 2022

Cells are complex





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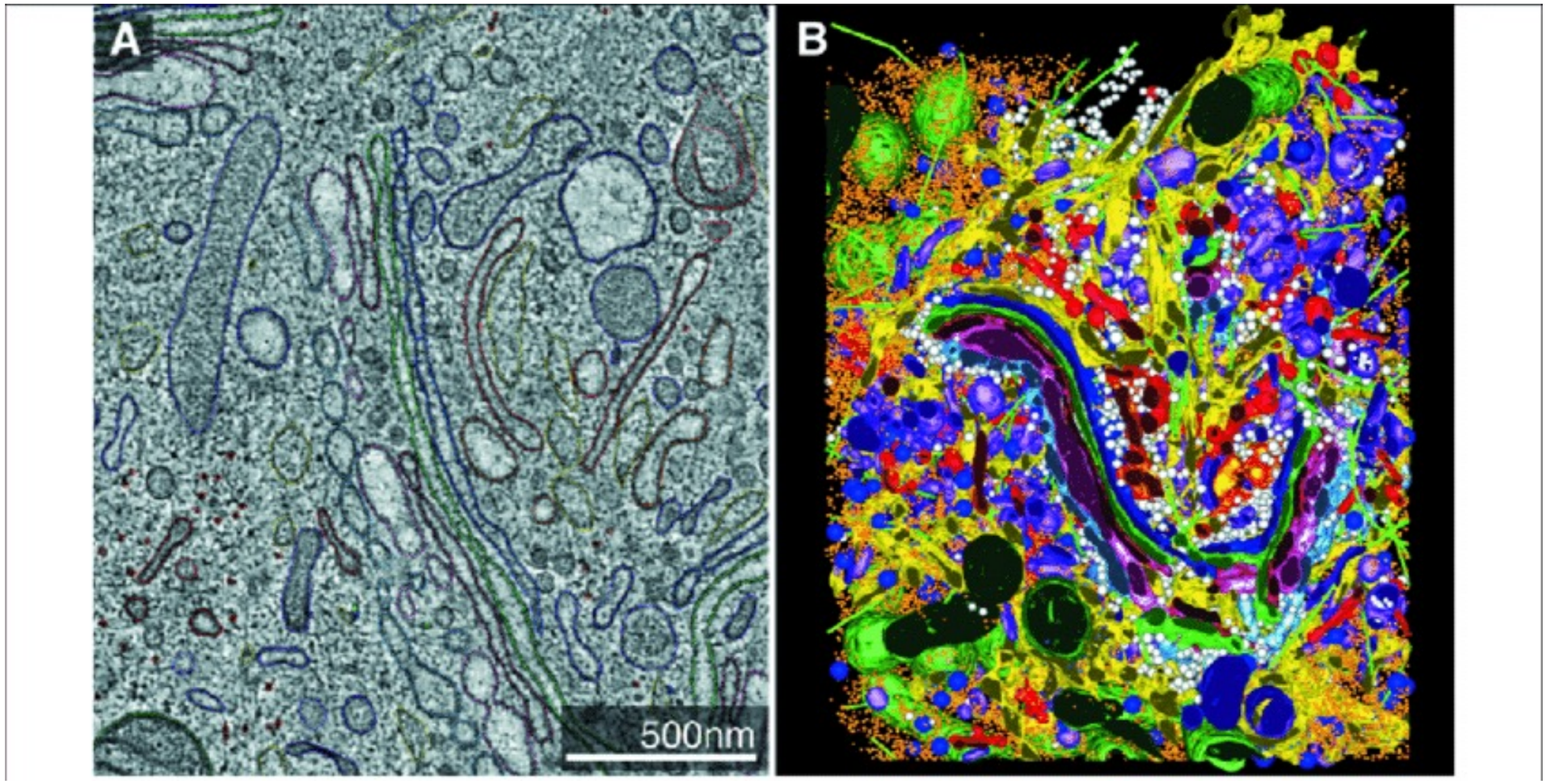
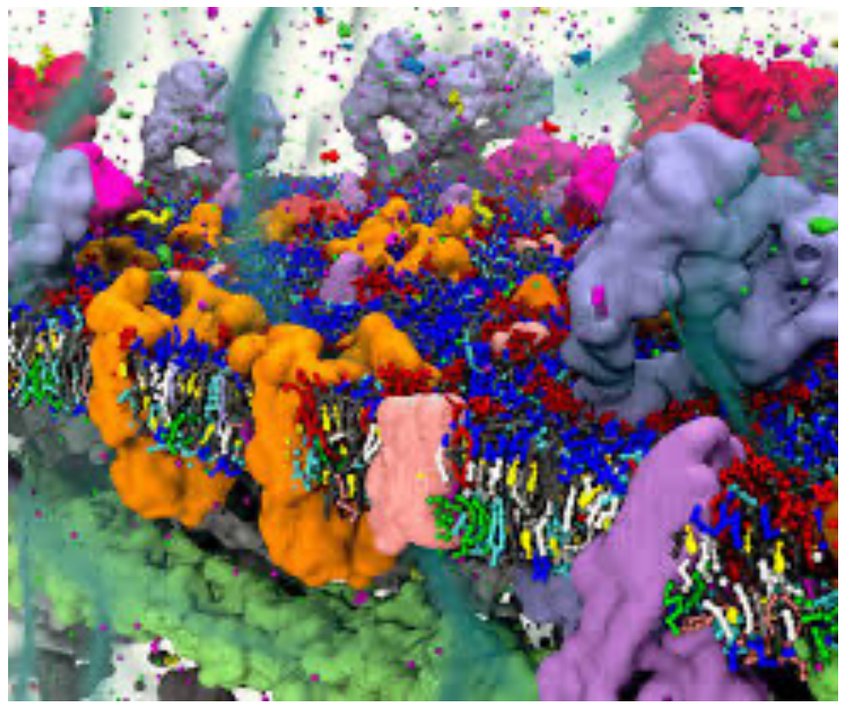
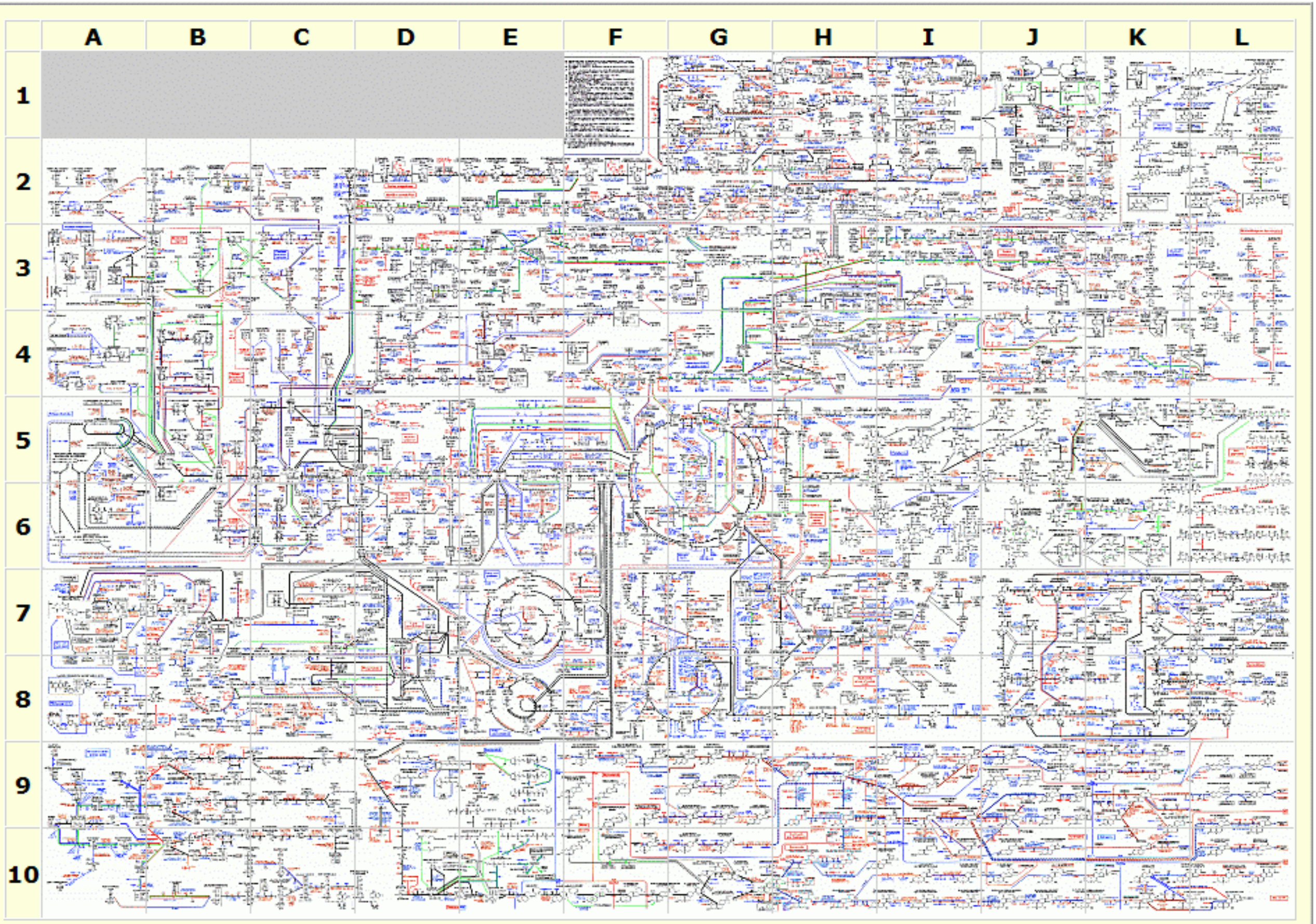
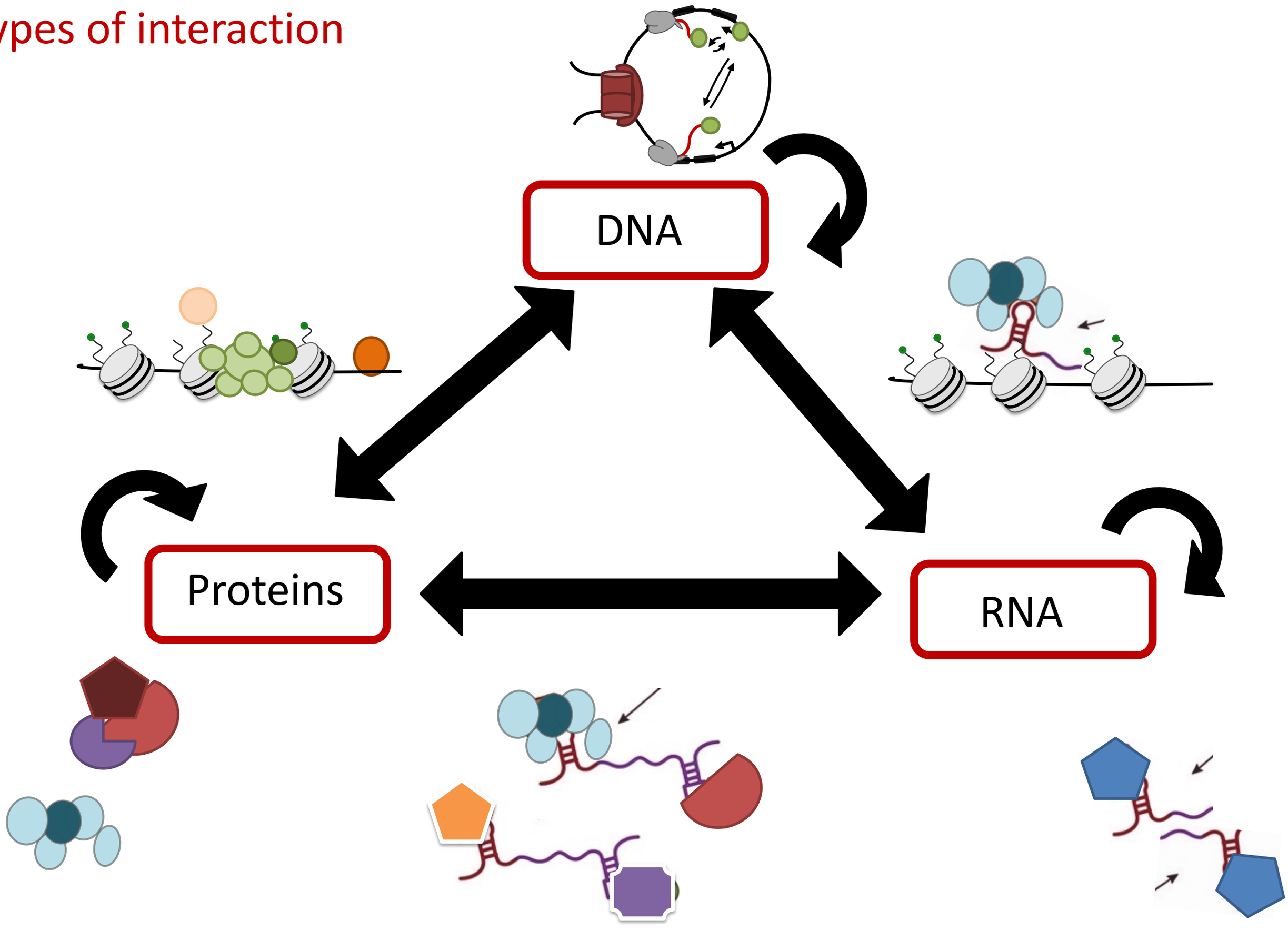




The molecular level is not better



Types of interaction



Enrichment methods: the basics

Co-IP

Prepare

Protein complex

Enrich

Primary antibody

Protein A/G magnetic beads

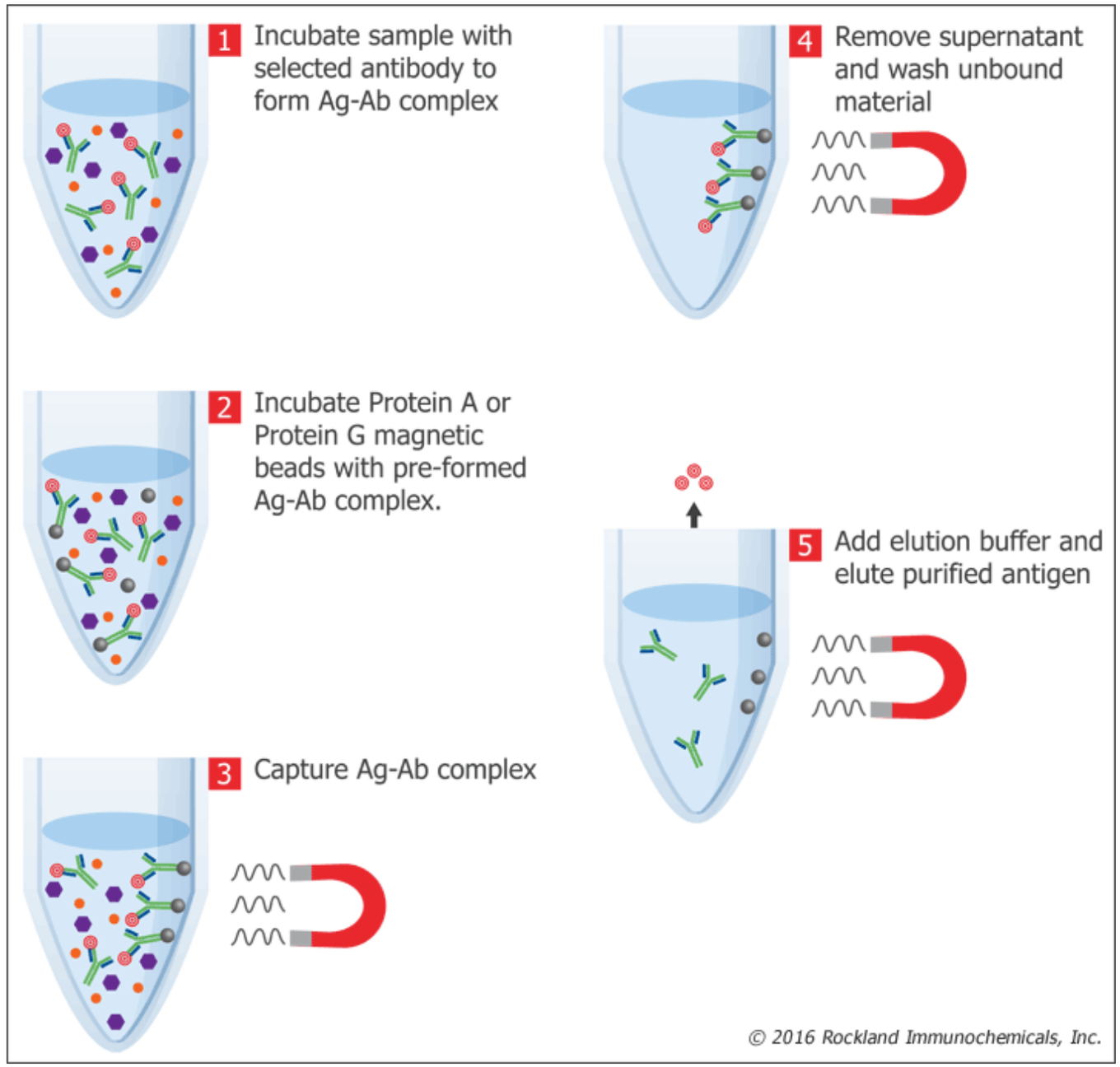
Wash

Washing

Analyse

Elution

Western Blot or proteomics



Enrichment methods

Finding molecular parterns:

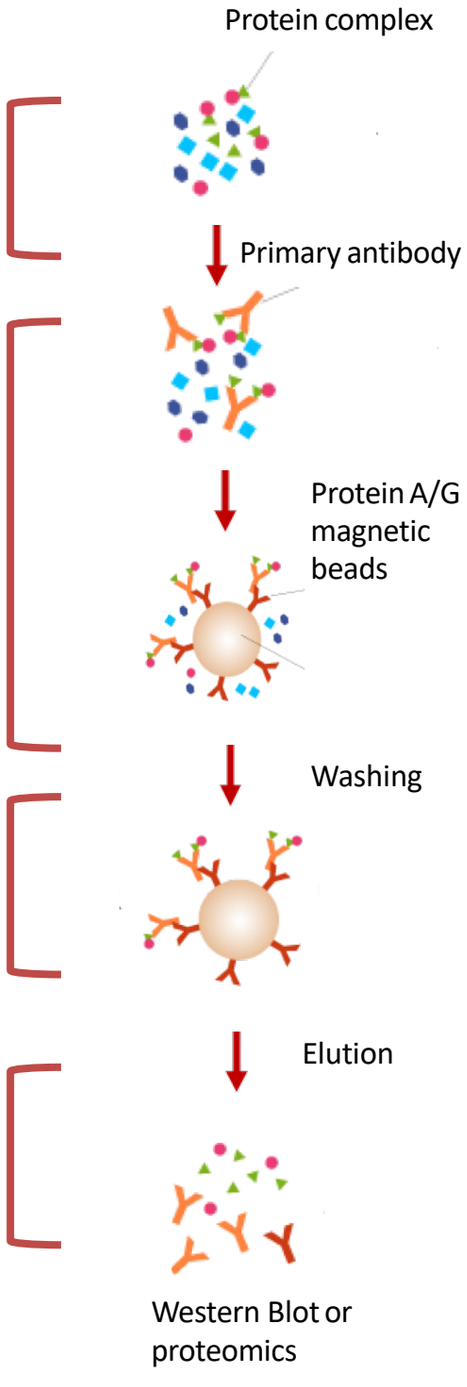
Prepare

Enrich

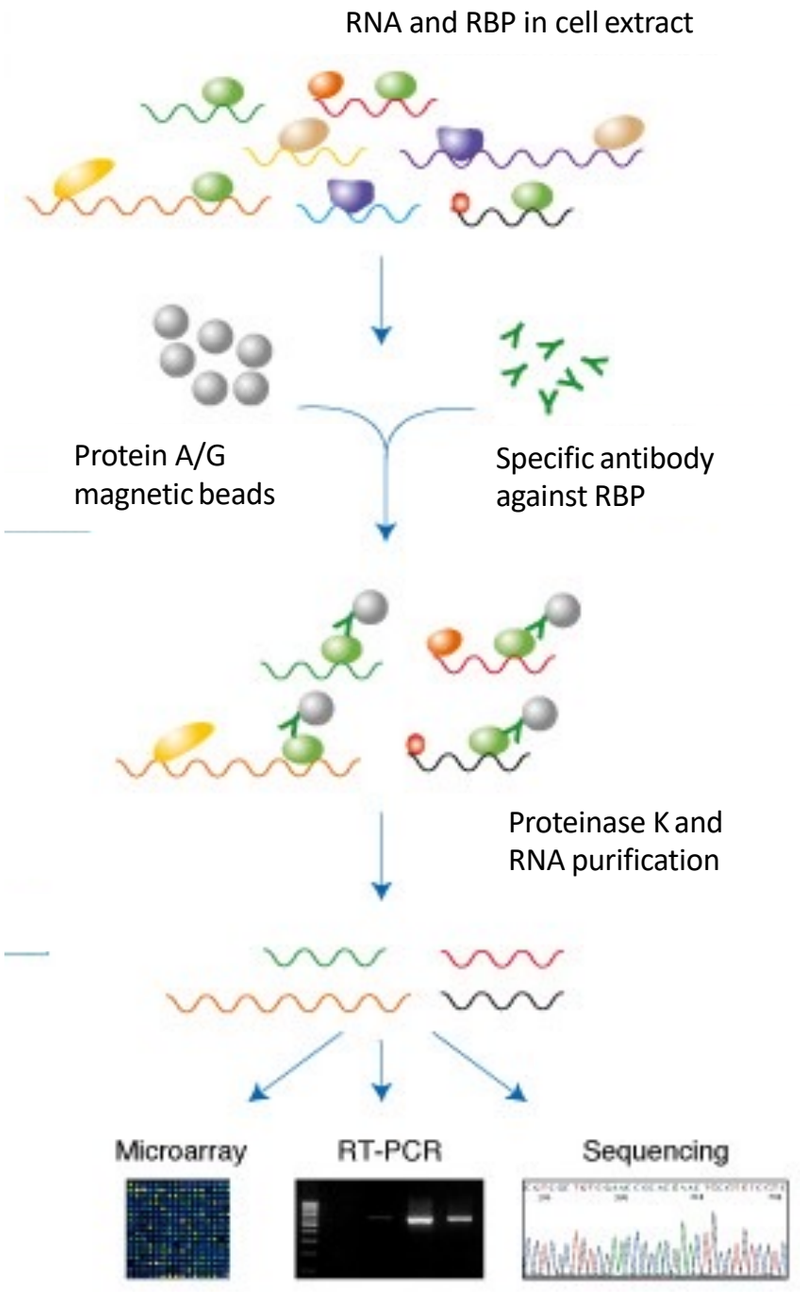
Wash

Analyse

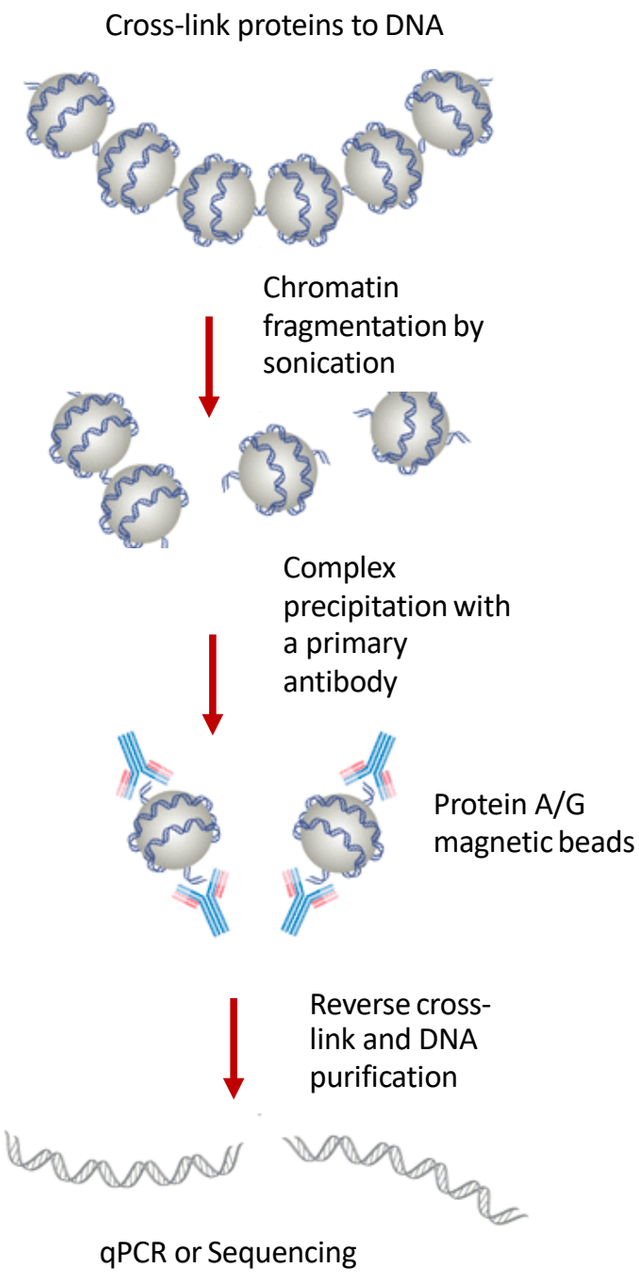
Co-IP



RIP/CLIP



CHIP



Types of interaction

		OUTPUT (what we analyse)		
		Protein	RNA	DNA
BAIT (What we enrich)	Protein	**CO-IP** (co-immunoprecipitation)	**RIP/CLIP** (RNA-Immunoprecipitation)	**ChIP** (Chromatin Immunoprecipitation)
	RNA	**Exogenous RNA pull Down RAP-Protein** (RNA antisense purificaton)	**RAP-RNA** (RNA antisense purificaton)	**ChIrP** (Chromatin isolation by RNA purification)
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Considerations:

Crosslinked vs native.

Types of baits.

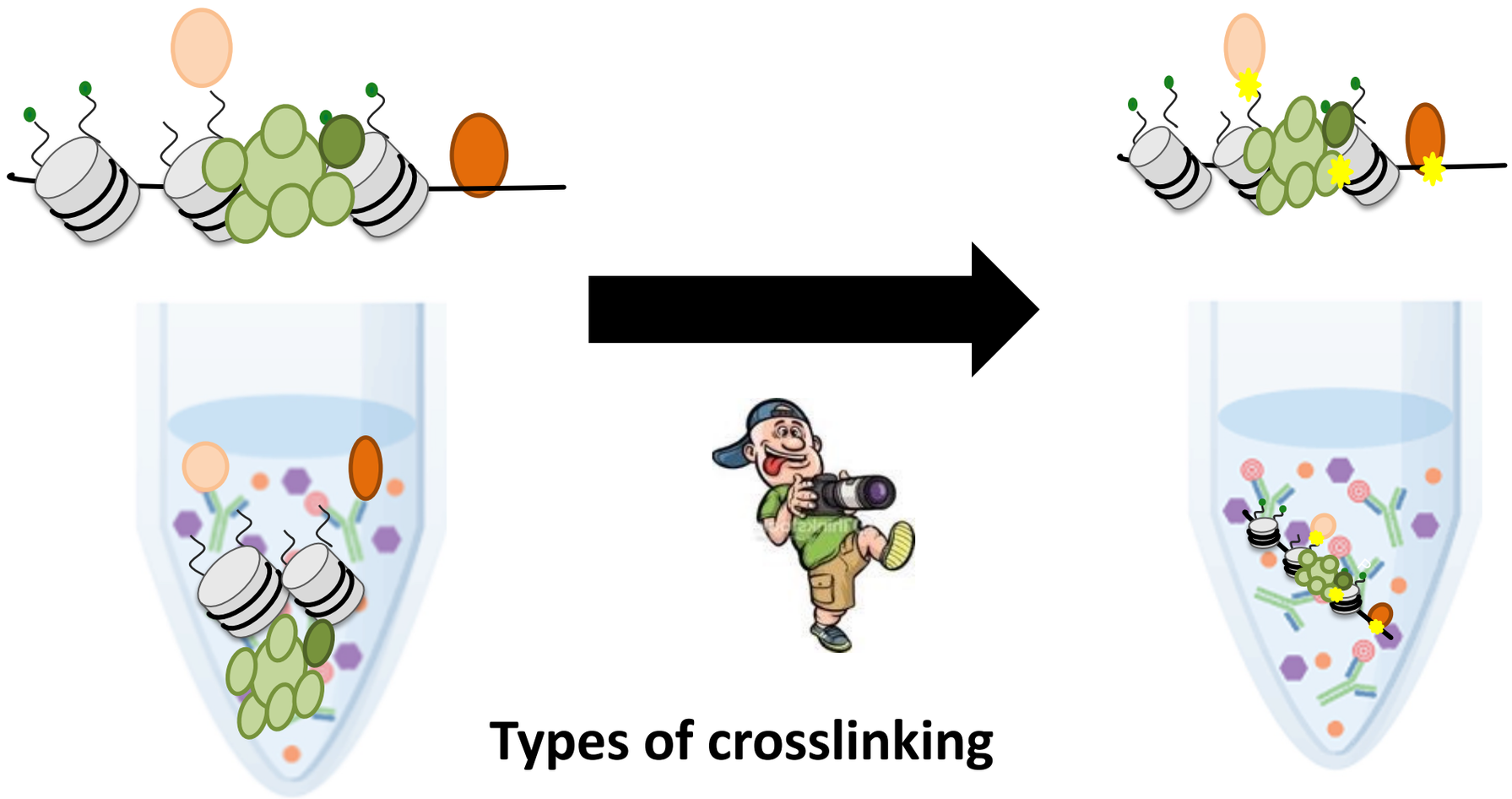
Types of output.

Crosslinked vs native

Crosslinking: establishing molecular bonds between molecules.

Avoid non specific interaction

Capture interaction in a timeframe



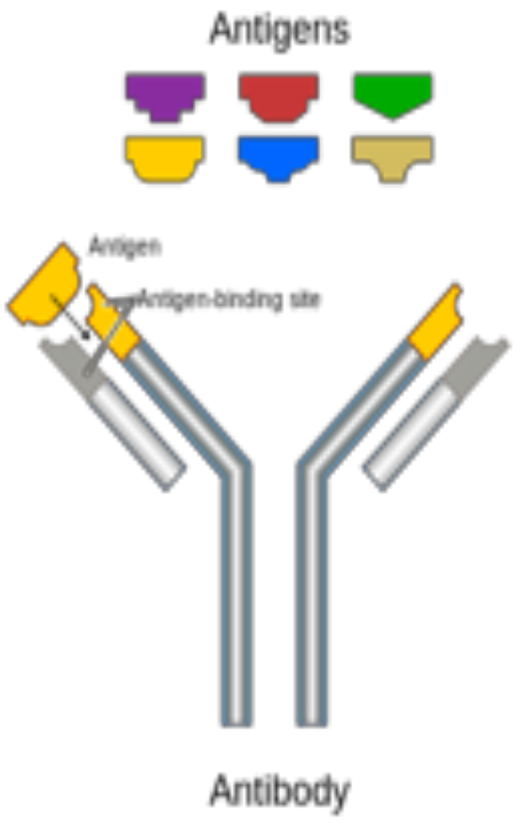
Types of crosslinking

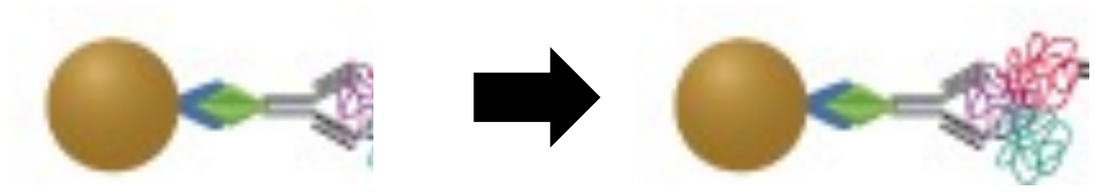
- **UV light**
- **Formaldehyde**
- **Gluthaladehyde**
- **Psoralen**

Types of baits.

Antibodies + prot G → to catch proteins

- Monoclonal: 1 clone, 1 epitope
- Polyclonal: several clones, several epitopes

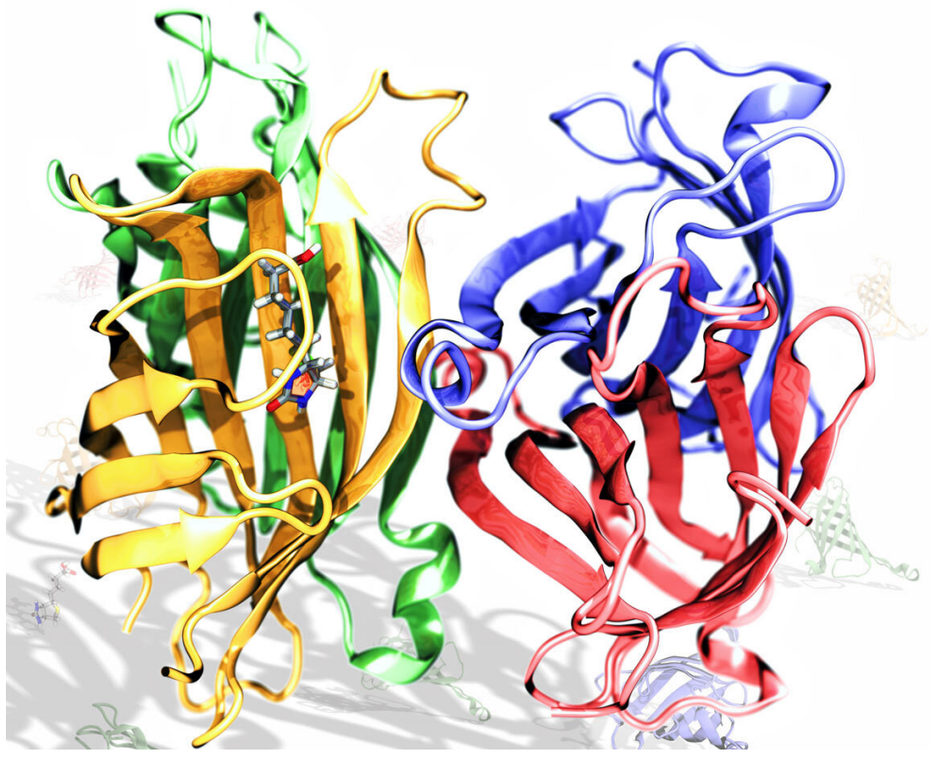




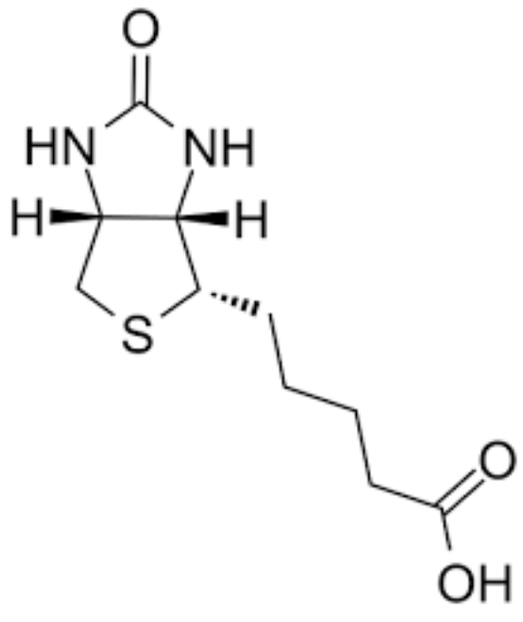
Types of baits.

Biotinylated Nucleic acid + streptavidin beads

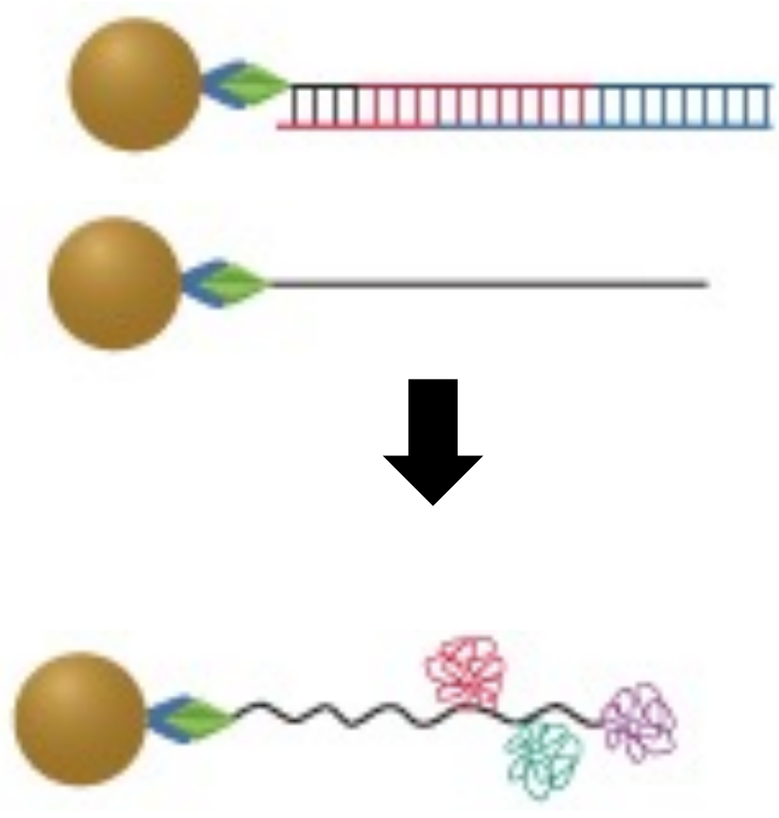
- Biotinylated DNA/RNA
- We can modify nucleotides :LNA, sulfur bonds..
- Specific base-base interaction.



Streptavidin



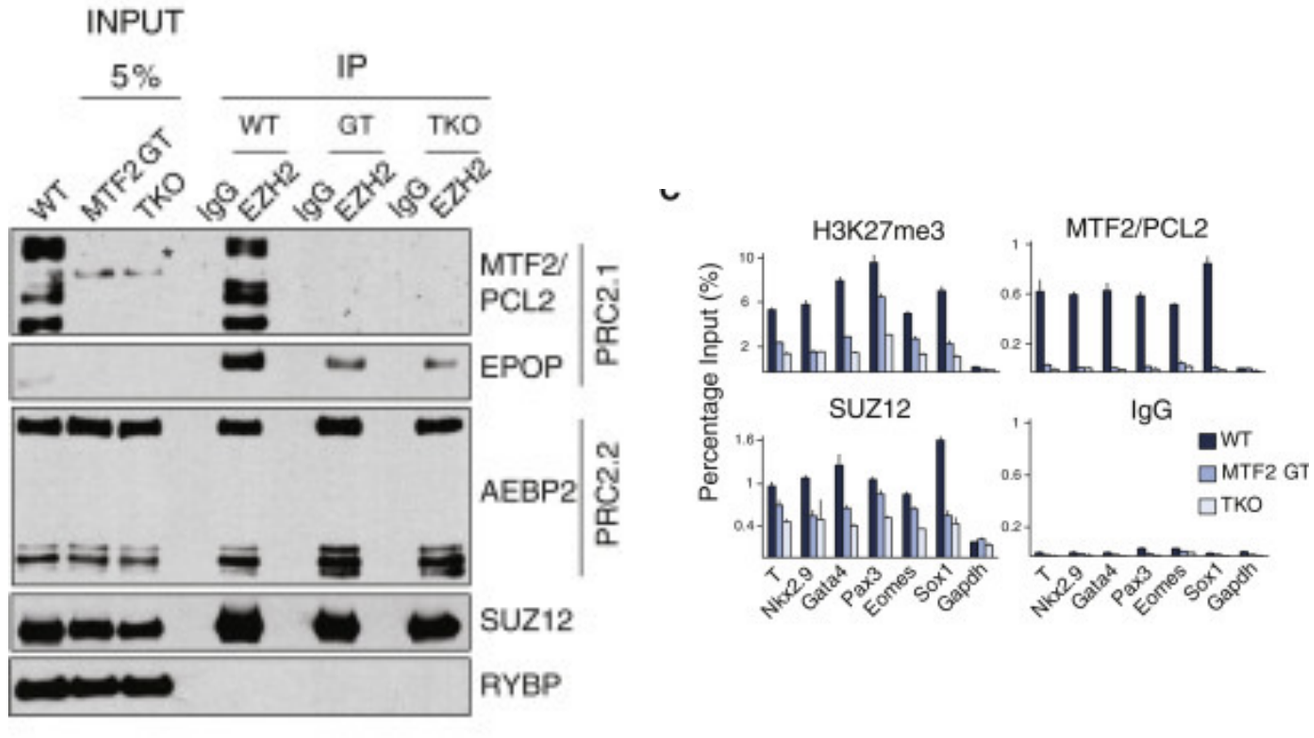
Biotin



Types of outputs.

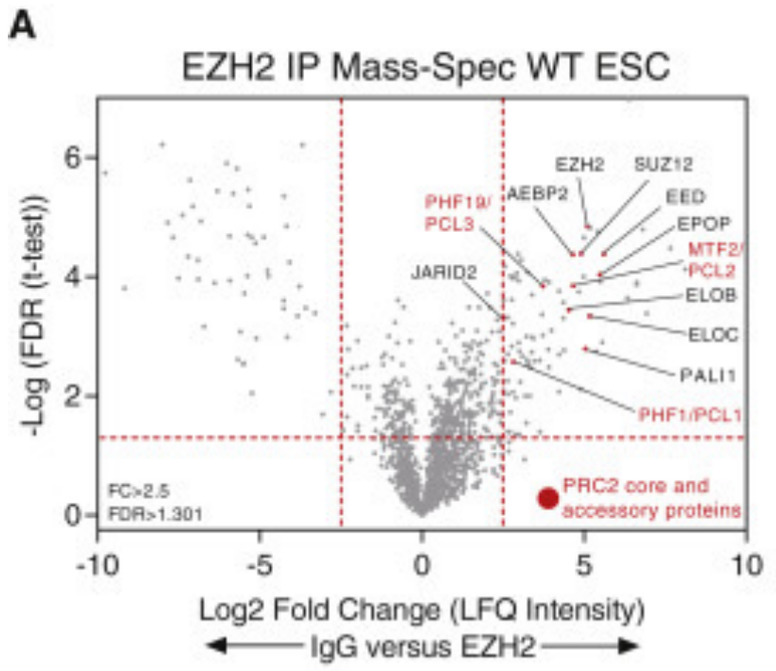
Low throughput: we analyse the interaction of our bait with few genes

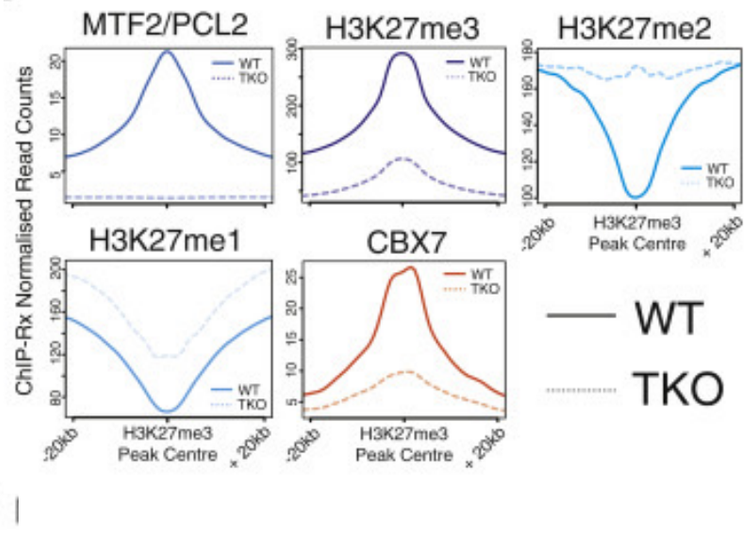
Western Blot/PCR



High throughput: we analyse the interaction of our bait with all the genome/proteome in the cell.

Mass spectrometry/ Sequencing





Evan Healy et al. 2020

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Co-immunoprecipitation

AIM: Identification of protein interactions. Bait: protein/output: protein)

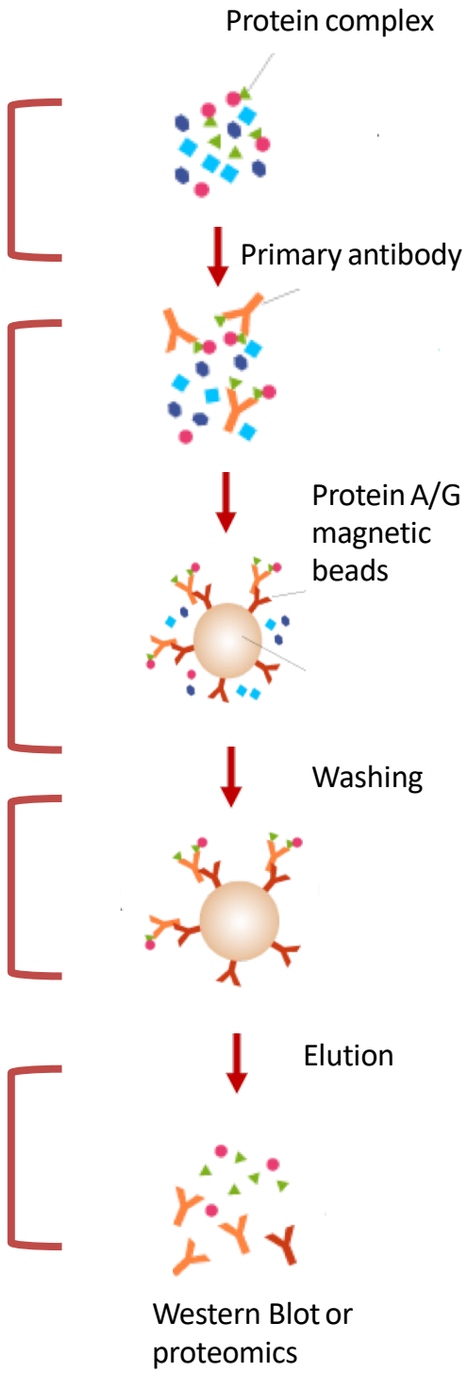
Co-Immunoprecipitation (COIP) is an immunoprecipitation technique used to investigate the interaction between proteins.

Prepare

Enrich

Wash

Analyse



Preparation:

We prepare a protein cell lysate

Enrichment (immunoprecipitation)

Using an antibody or a tagged protein

Washing

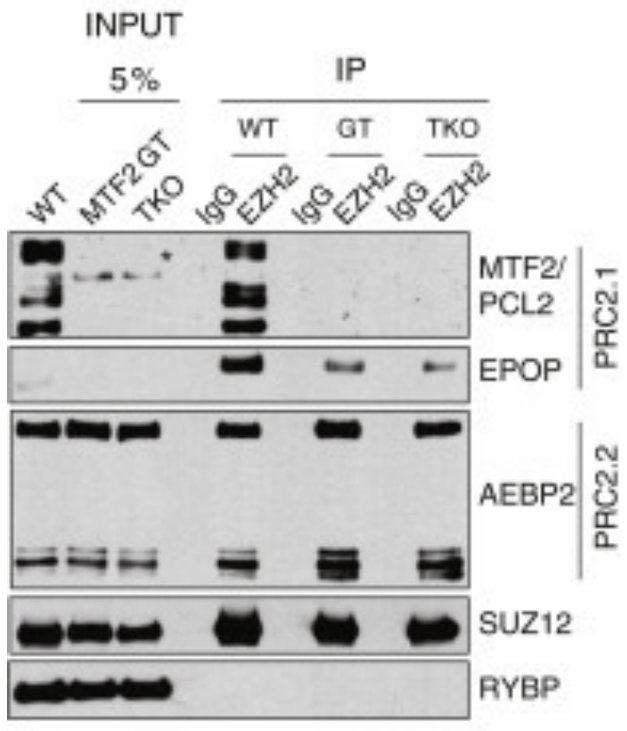
essential to remove non specific proteins

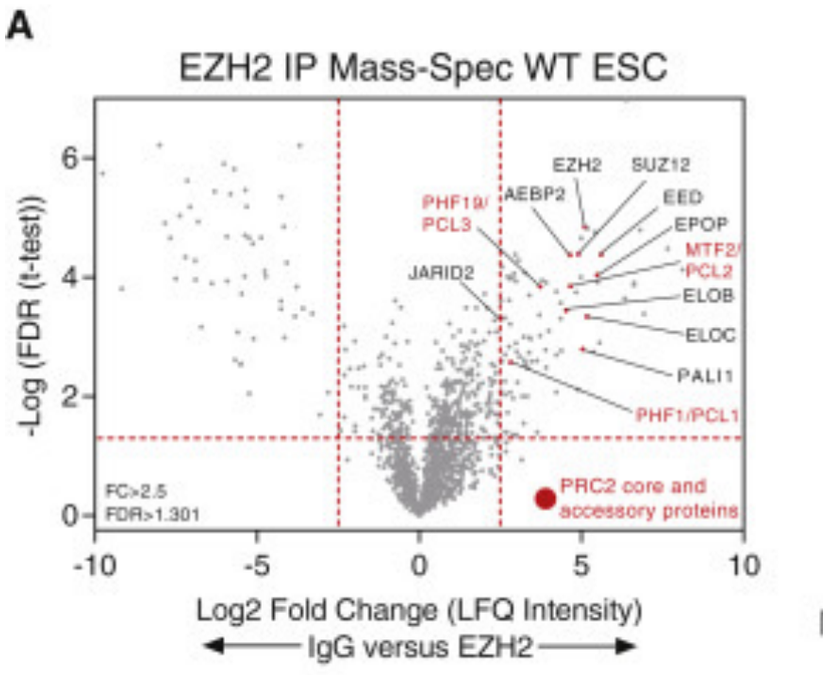
Analysis

Western blot or mass spectometry

Co-immunoprecipitation

Example: PRC2 complex





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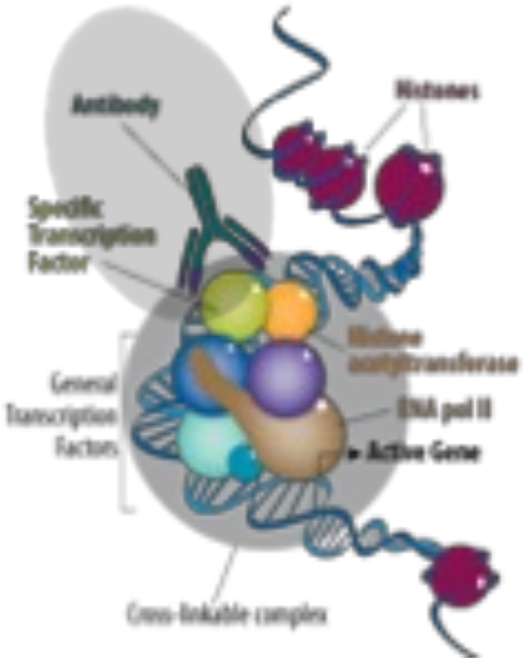
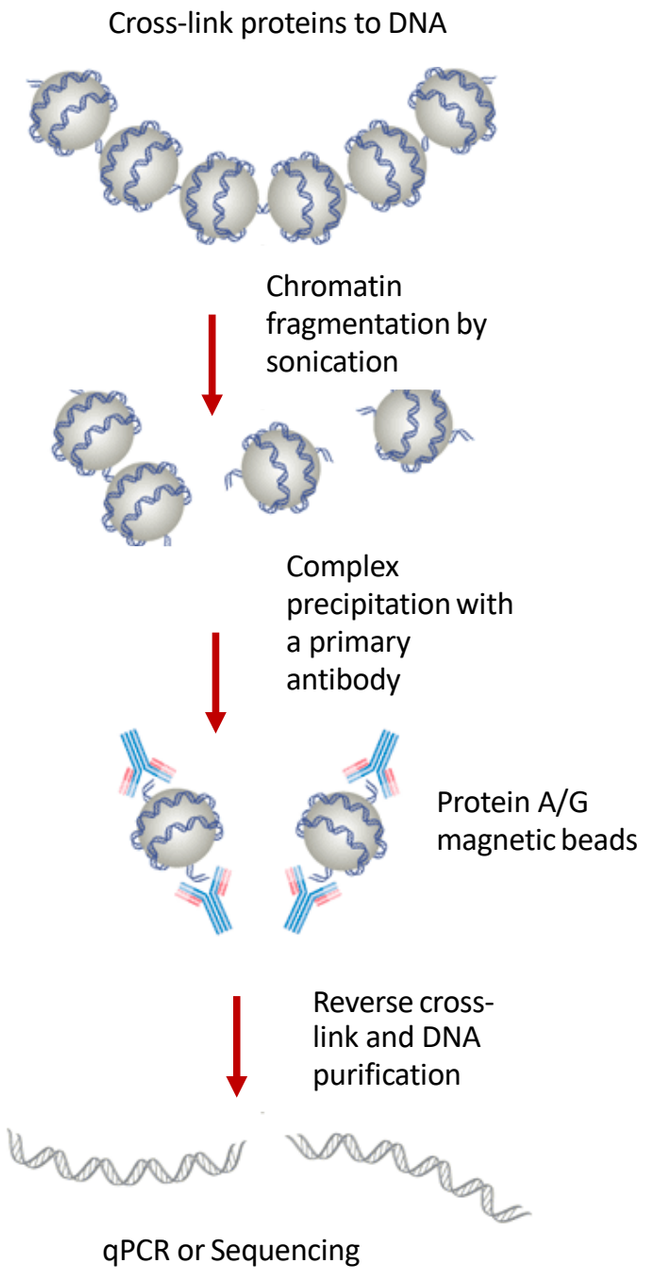
ChIP

AIM: Identification of the genomic loci bound to a DNA binding protein. (Bait Protein/ output :DNA)

Chromatin Immunoprecipitation (ChIP) is an immunoprecipitation technique used to investigate the interaction between proteins and DNA in the cell. It aims to determine whether specific proteins are associated with specific genomic regions, such as transcription factors on promoters.





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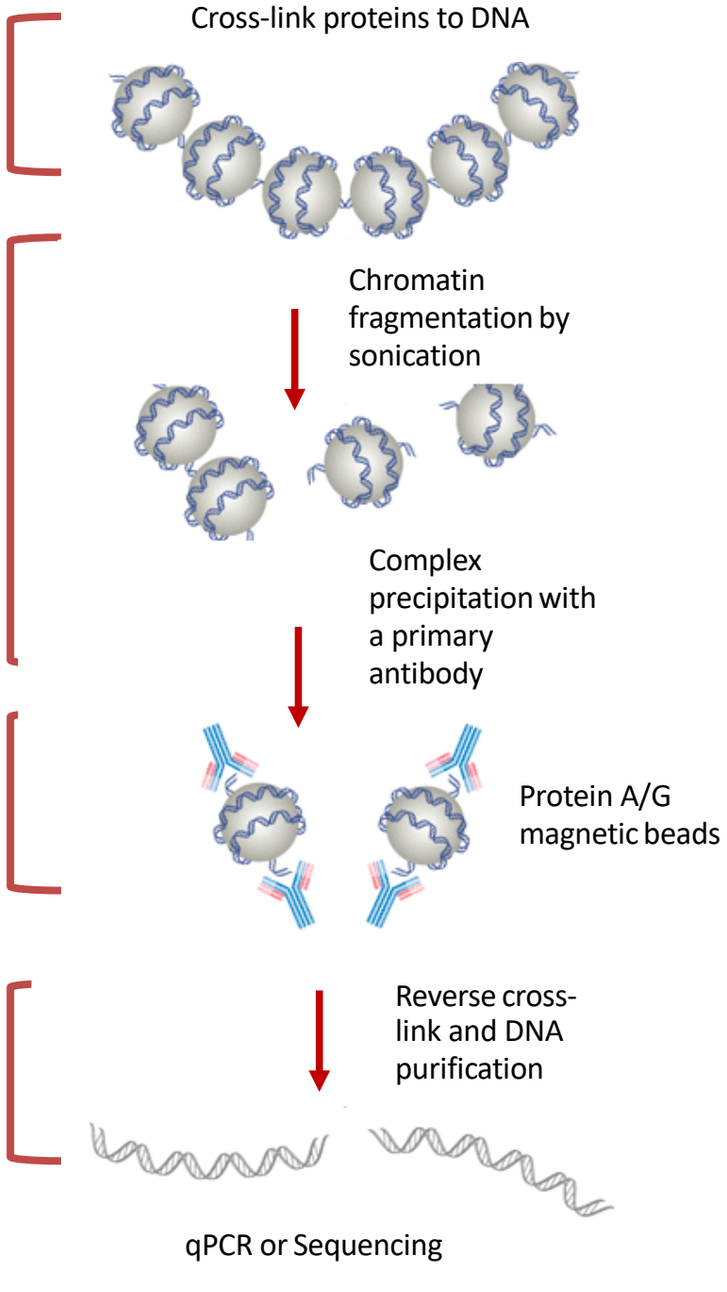
Analyse



Preparation: Possible crosslinking

Native-ChIP: Native chromatin is used as substrate

- only proteins tightly associated with DNA can be immunoprecipitated
- antigens cannot be occurred or modi9ied by chemical cross-inking
- the speci9icity of the antibody binding to un9ixed chromatin is more predicta

X-ChIP: cross-linked chromatin is used as substrate

- also proteins weakly or not directly associated with DNA
- antigens can be obscured or modified by the formaldehyde cross-linking
- more widely used than Native ChIP

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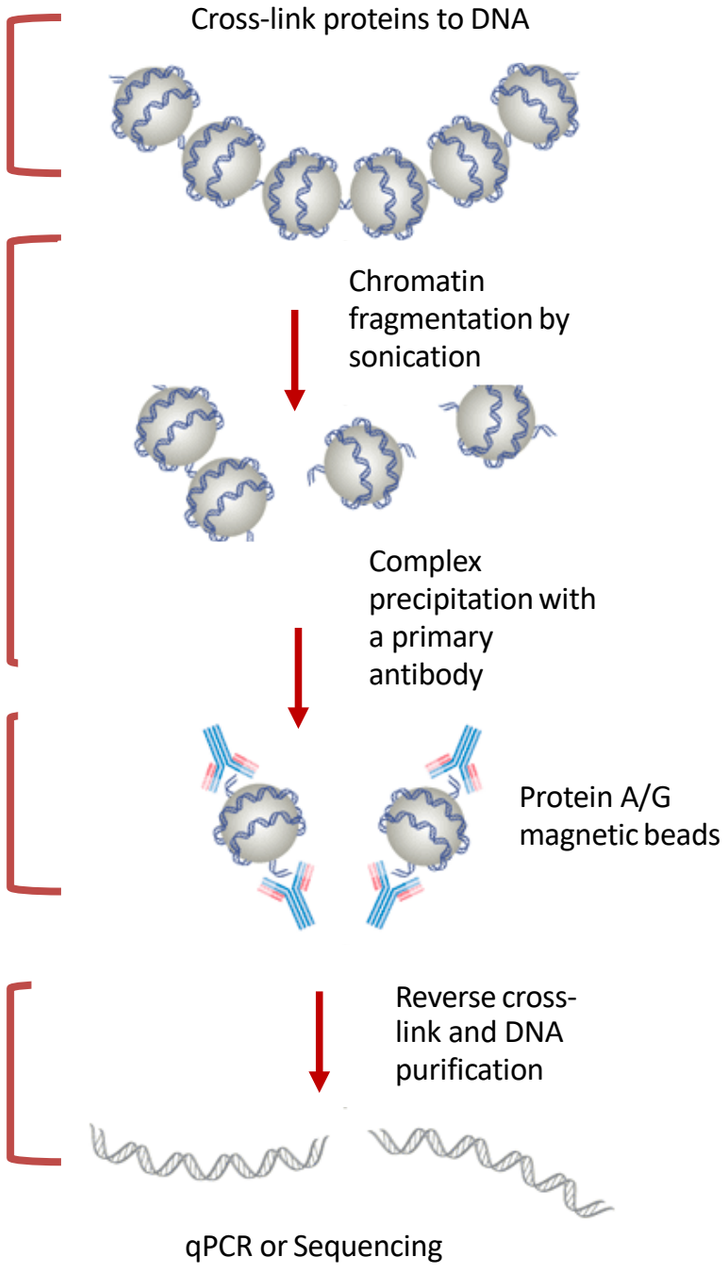
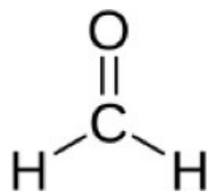


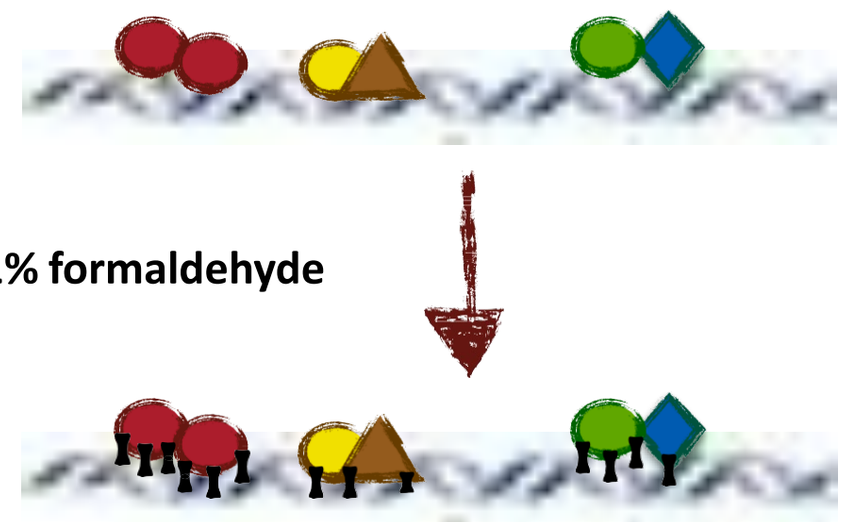
1.Preparation: crosslinking

The cross-linking is an experimental procedure that convert in **covalent** all thethe weak and non-covalentinteractions between DNA - PROTEINS and PROTEIN-PROTEIN

UV-Crosslinking
Formaldehyde
Glutaraldehyde



1% formaldehyde



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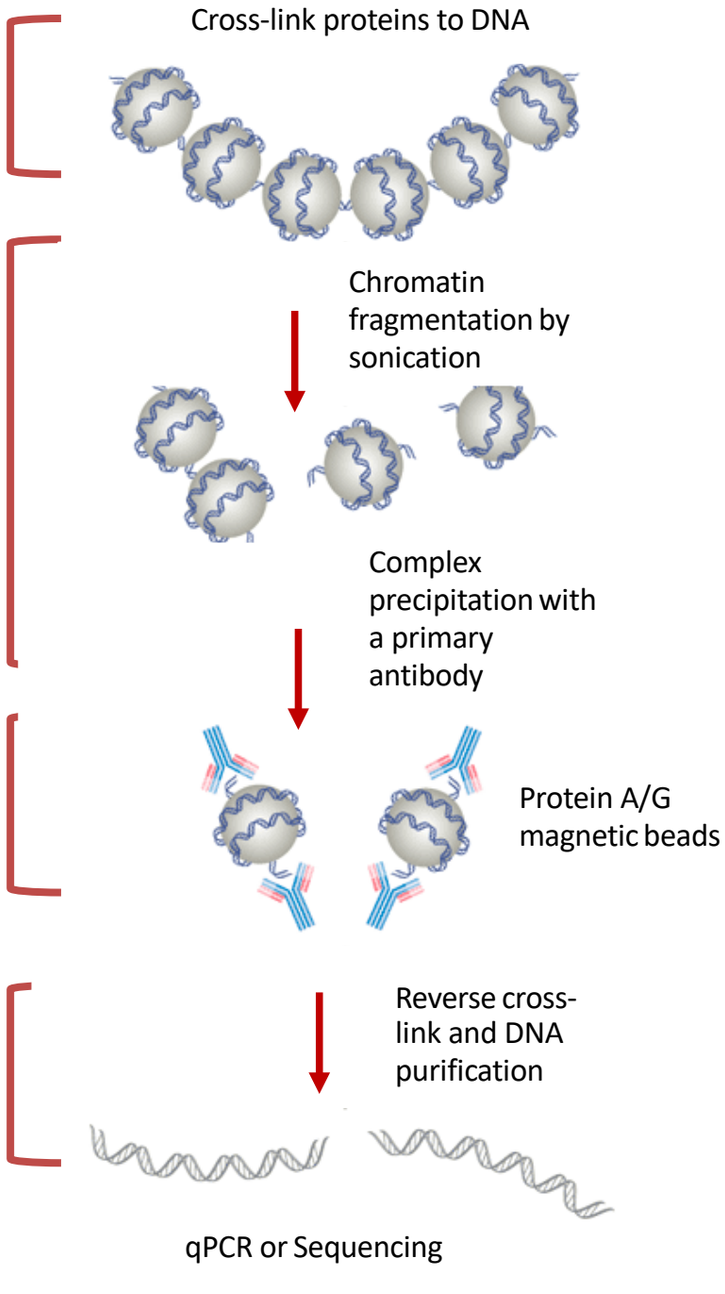

Formaldehyde Cross-linking

- Formaldehyde is an organic compound. It is water soluble and penetrates biological membranes It targets **primary aminogroups** (i.e. lysines in proteins, side chains of A,C,G in DNA)
- It crosslinks both **protein-nucleic acids**, **nucleic acids-nucleic acids** and **protein-protein**
- The crosslinking is reversible (65.C reverse protein-DNA; 100.C reverse protein-protein)
- Reaction is stopped by providing an excess of primary amino groups (0.125M glycine)

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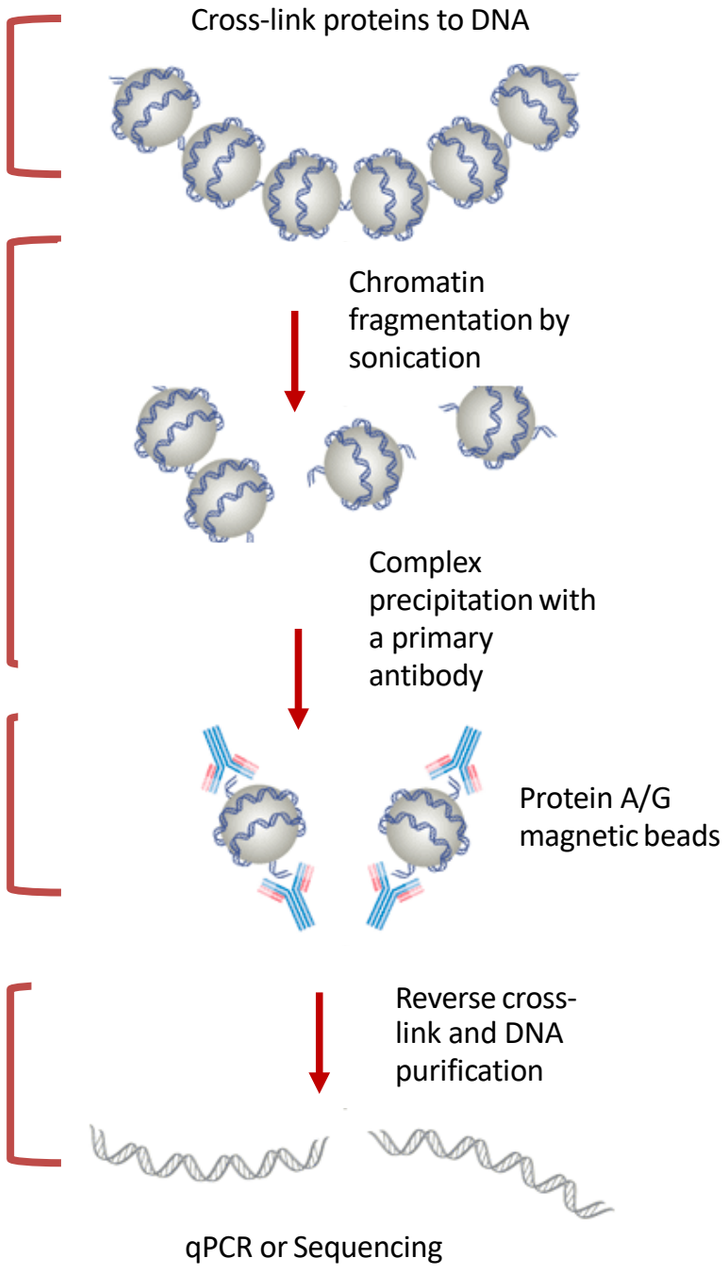
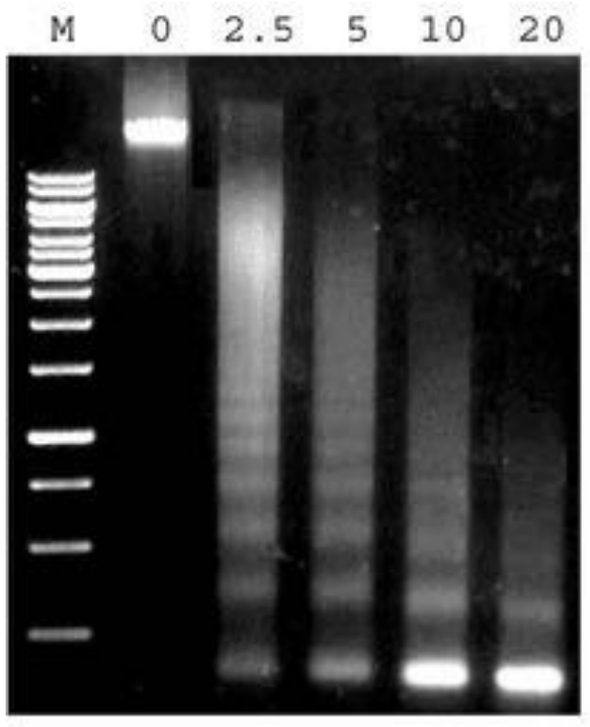
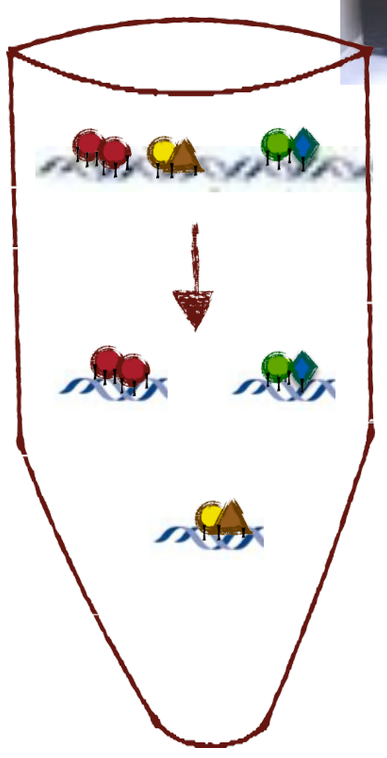


2. Preparation: sonication

The DNA-protein complexes (chromatin-protein) are then sheared into ~500 bp DNA fragments by **sonication** or (nuclease digestion).



size range: 100-500 bp



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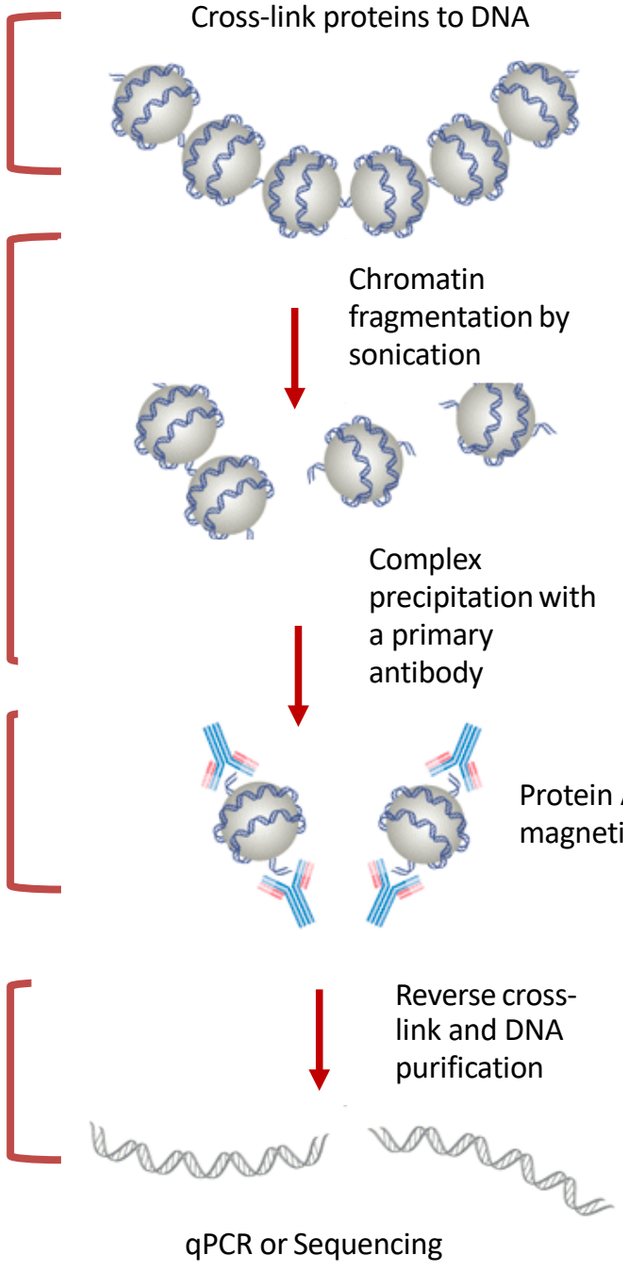
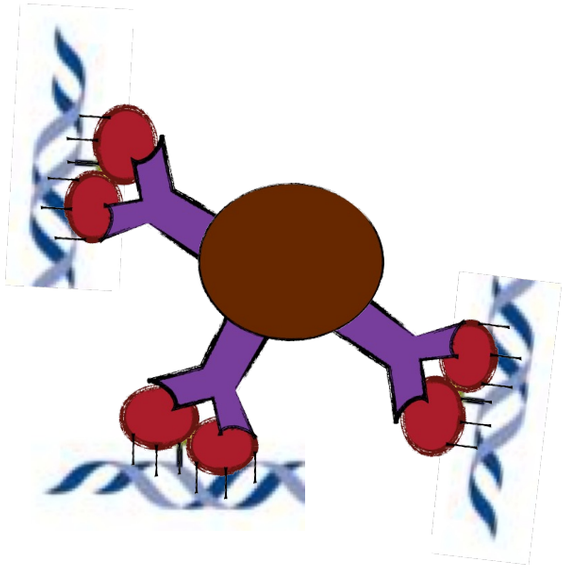
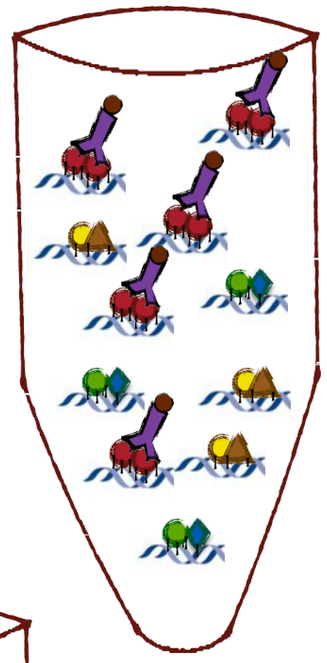


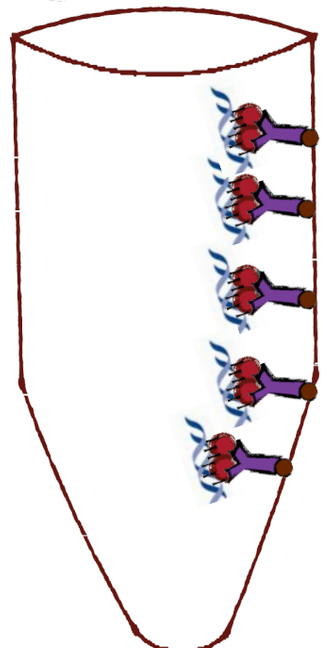
2. Antibody binding

Antibody binding several hours (or over night) at 4 degrees.





3. Wash



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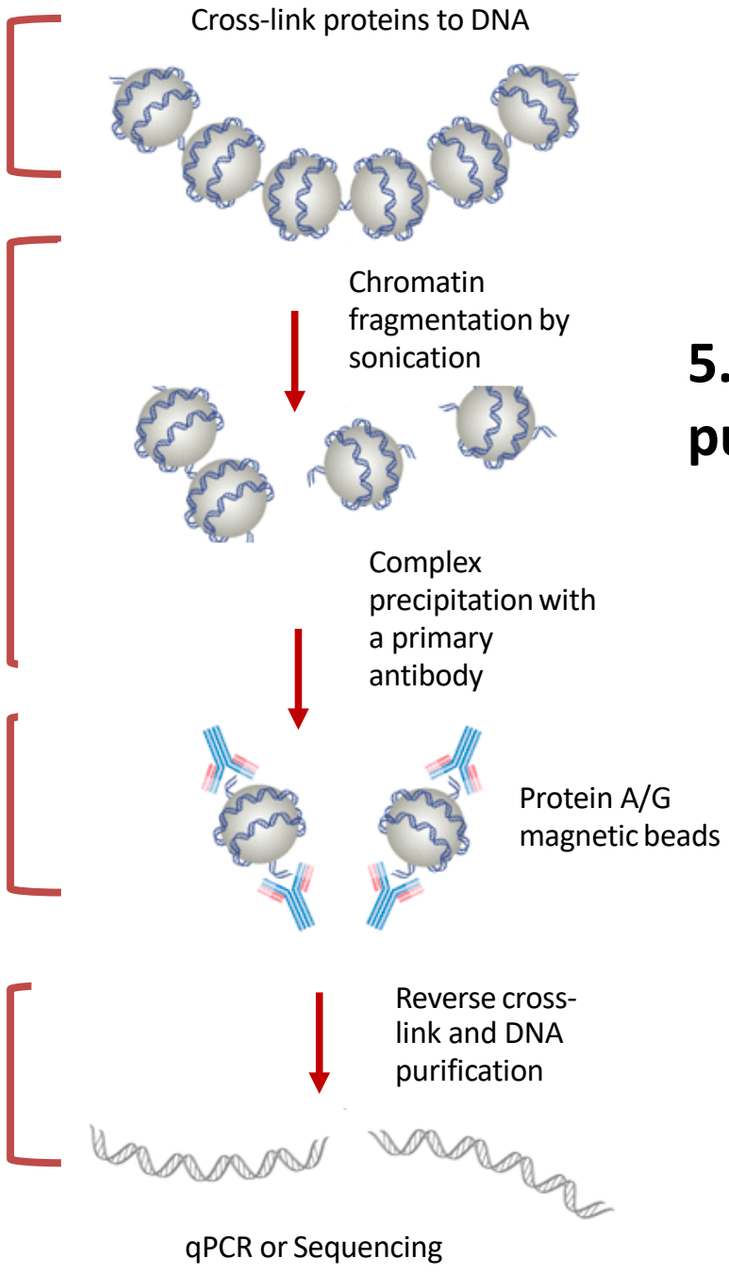
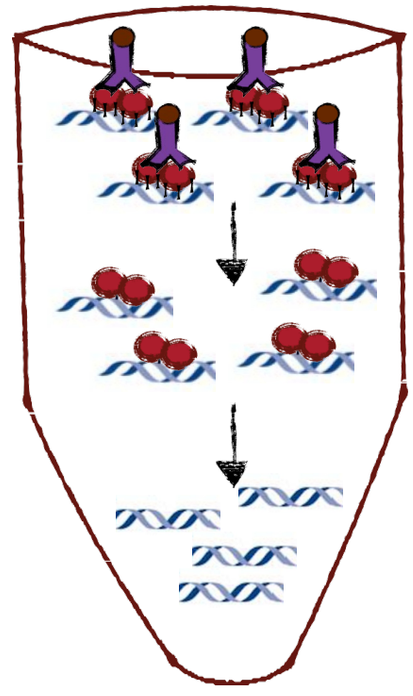


4. Reverse crosslinking

The cross-linking with formaldehyde is able to be removed through the incubation of the extract at High temperature. (65.C reverse protein-DNA; 100.C reverse protein-protein this step allows the detach of the protein from the DNA that is subsequently purified and analyzed

5. Proteinase K treatment, Rnase A treatment and DNA purification



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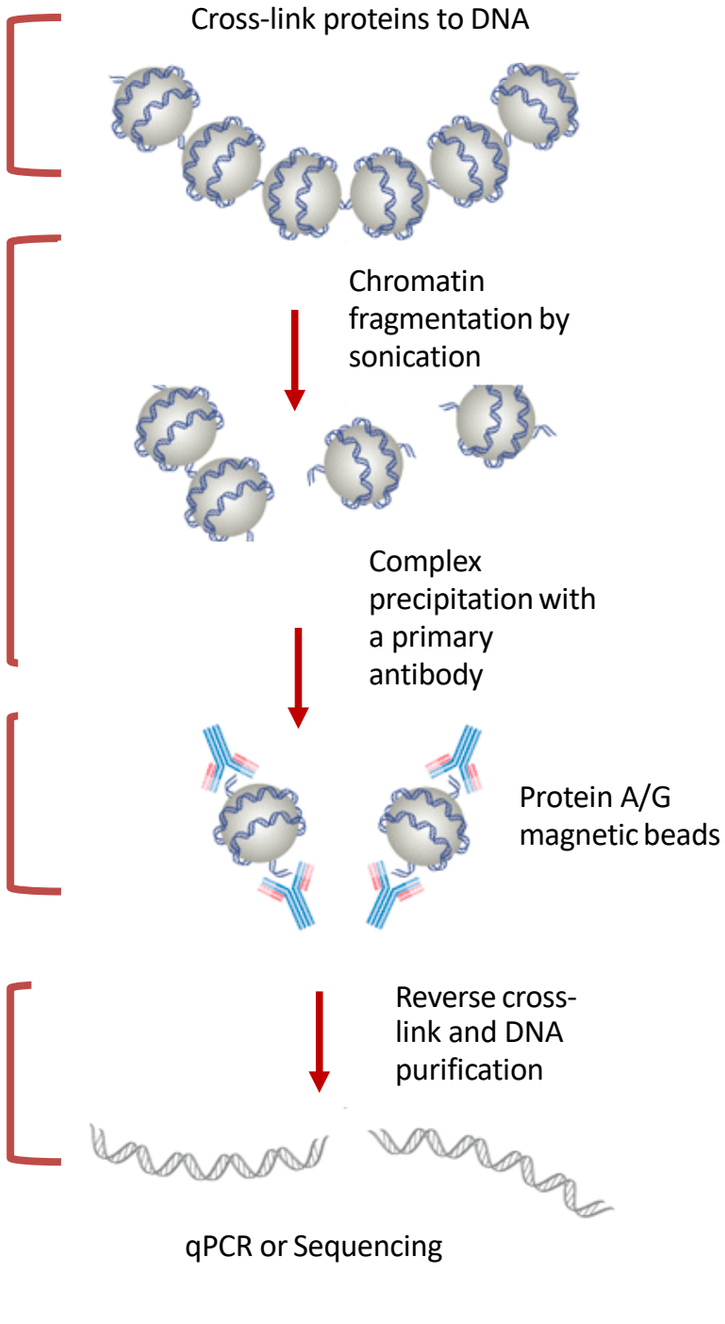
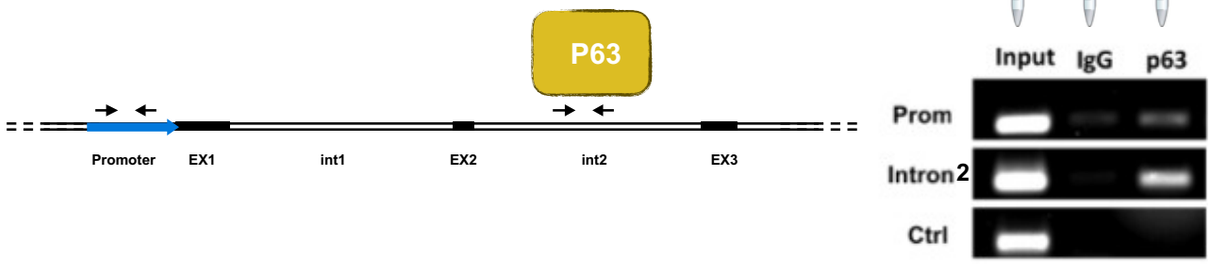
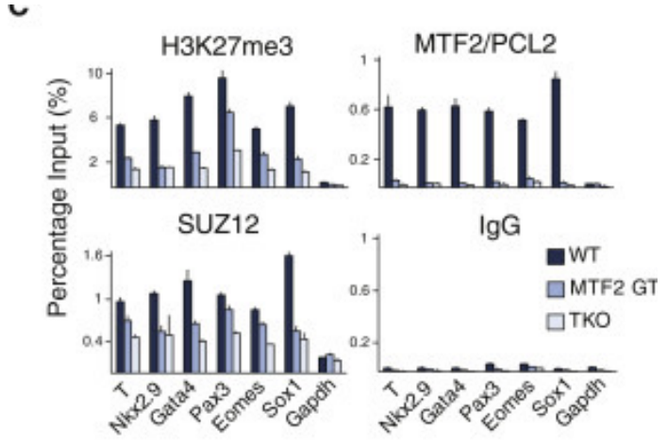


6. DNA anlaysis PCR

The isolated DNA can be quantified by PCR using specific probes. This allows the analysis of a specific region in multiple samples





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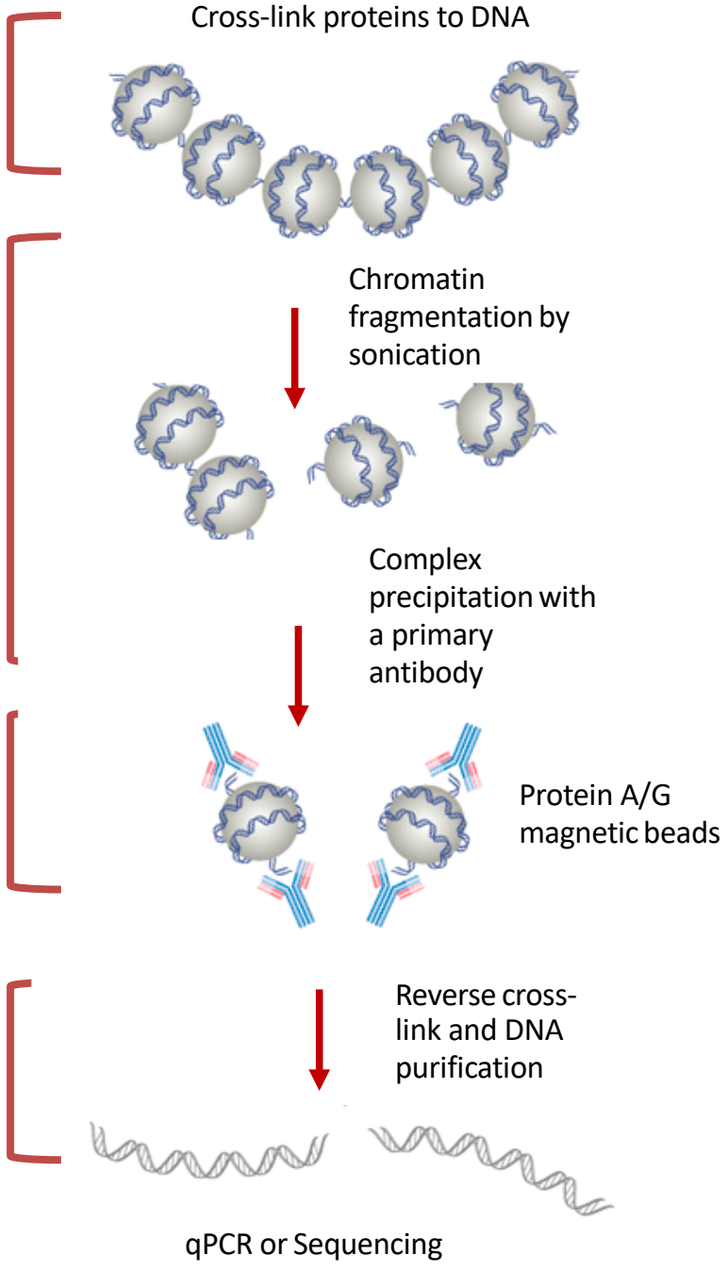
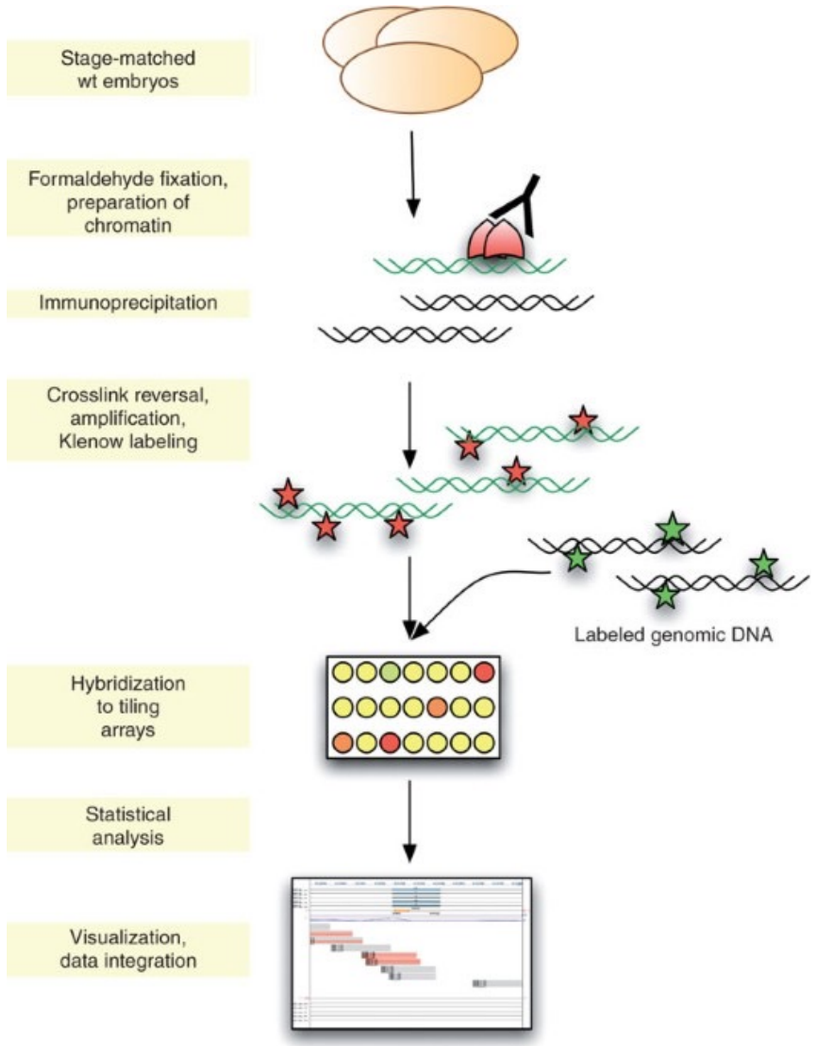


6. DNA anlaysis ChIP on chip

The isolated DNA can be quantified by PCR using specific probes. This allows the analysis of a specific region in multiple samples

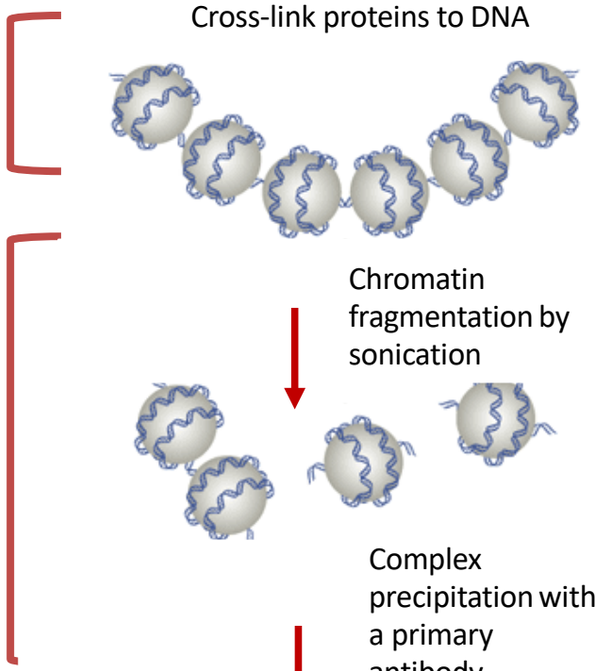


ChIP

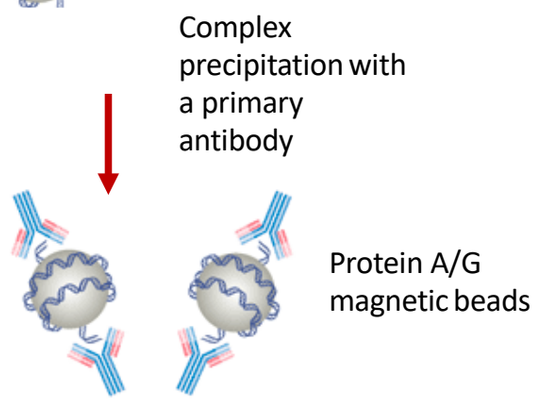
AIM: Identification of the genomic loci bound to a DNA binding protein. (Bait Protein/ output :DNA)

Chromatin Immunoprecipitation (ChIP) is an immunoprecipitation technique used to investigate the interaction between proteins and DNA in the cell. It aims to determine whether specific proteins are associated with specific genomic regions, such as transcription factors on promoters.

Prepare

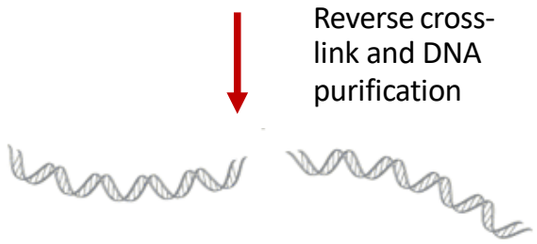


Enrich



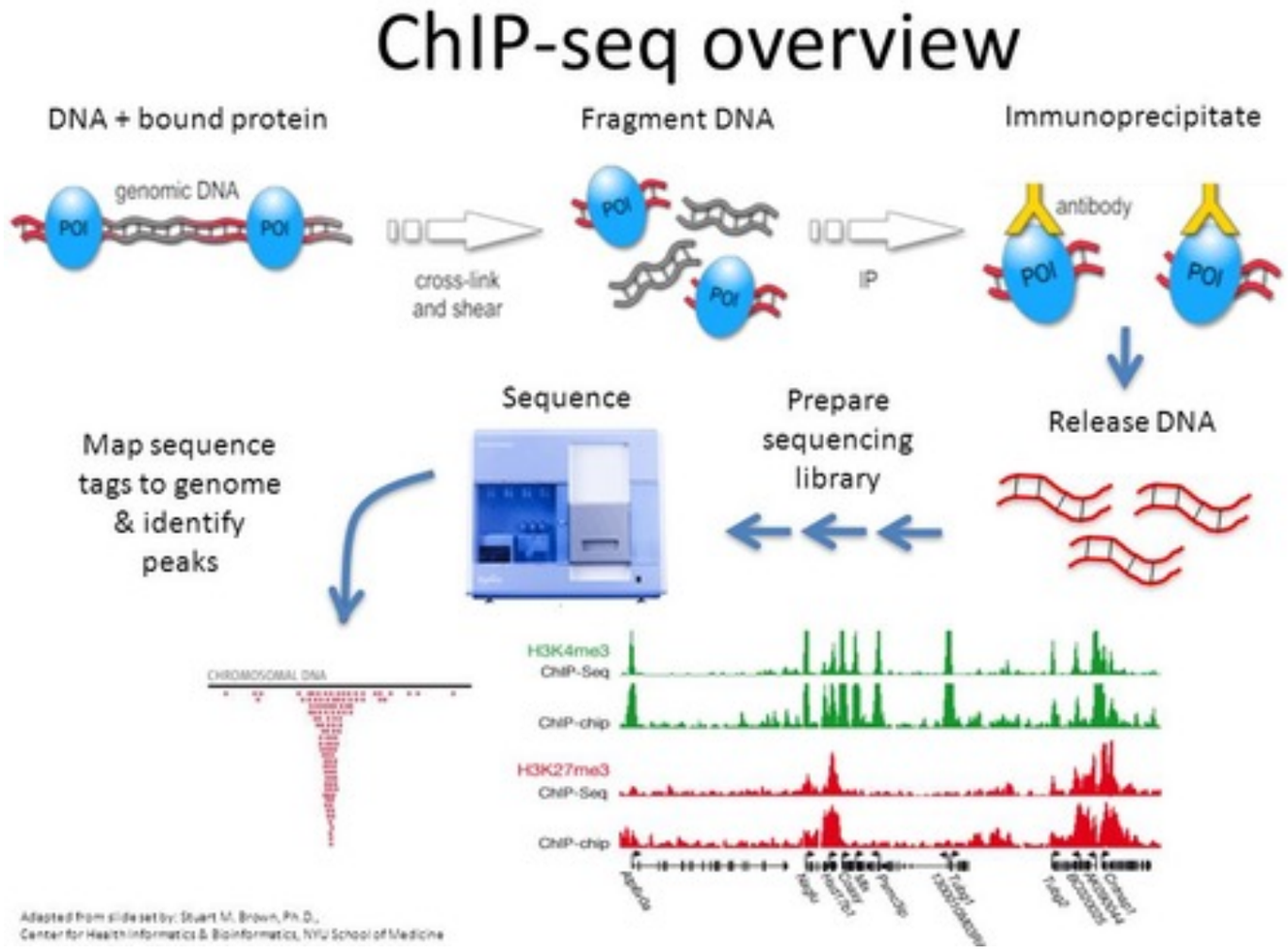
Wash

Analyse



6. DNA anlaysis ChIP seq

We can isolate the DNA and sequence every each single piece of DNA attached to this protein



Types of interaction

		OUTPUT (what we analyse)		
		Protein	RNA	DNA
BAIT (What we enrich)	Protein	**CO-IP** (co-immunoprecipitation)	**RIP/CLIP** (RNA-Immunoprecipitation)	**ChIP** (Chromatin Immunoprecipitation)
	RNA	**Exogenous RNA pull Down RAP-Protein** (RNA antisense purificaton)	**RAP-RNA** (RNA antisense purificaton)	**ChIrP** (Chromatin isolation by RNA purification)
	DNA	**DNA pull down**		**Conformation capture 3C**

ChIRP

AIM: Identification of RNA interactions with the chromatin. Bait: RNA output: DNA/protein)

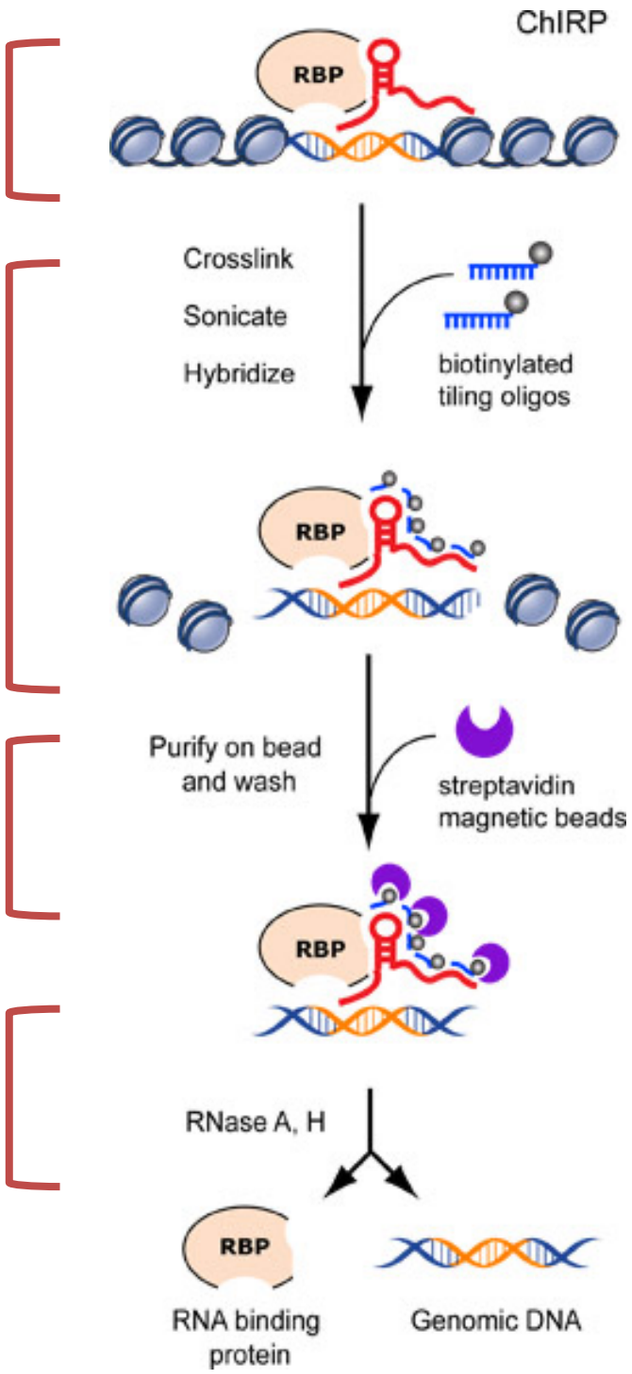
ChIRP (Chromatin Isolation by RNA Purification) a pull down technique used to investigate the interaction between RNA (usually ncRNAs and chromatin). Not only serve to determine interaction but also place of the interaction.

Prepare

Enrich

Wash

Analyse



- Chromatin associated lncRNAs
- Discrimination between ***cis*** and ***trans*** action
- The amount of cellular extract depends on the abundance of the lncRNA

Ci Chu et al. 2011

ChIRP

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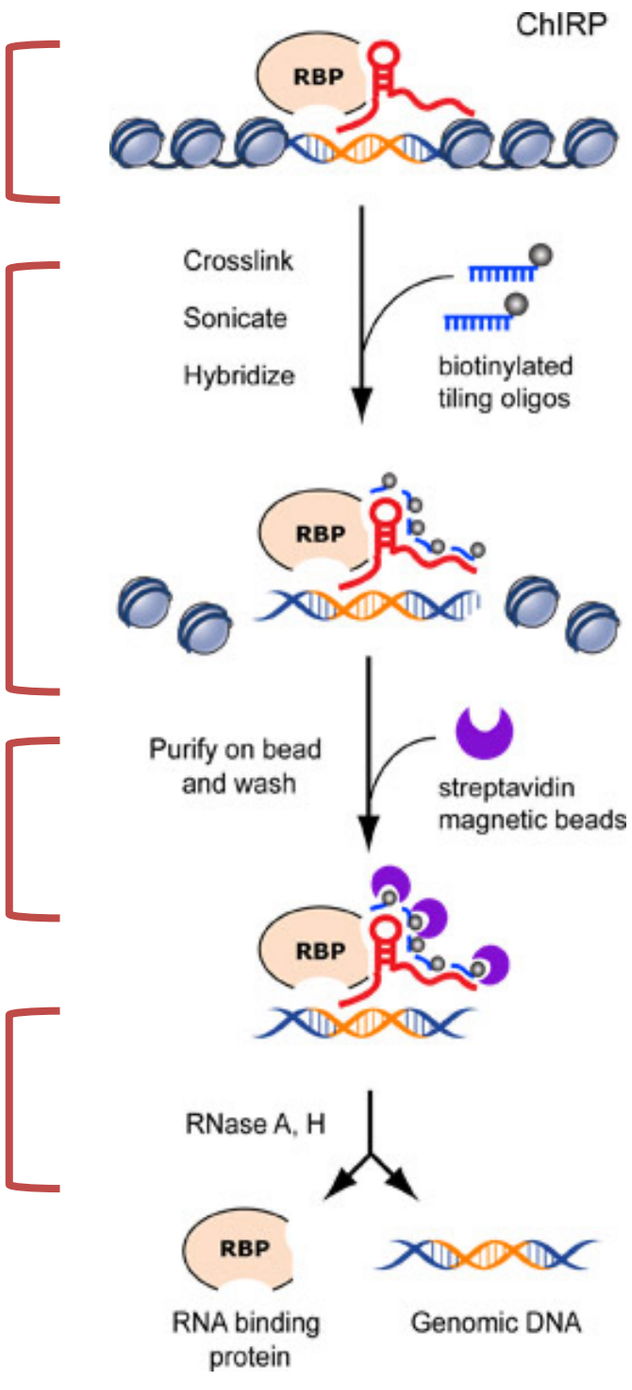
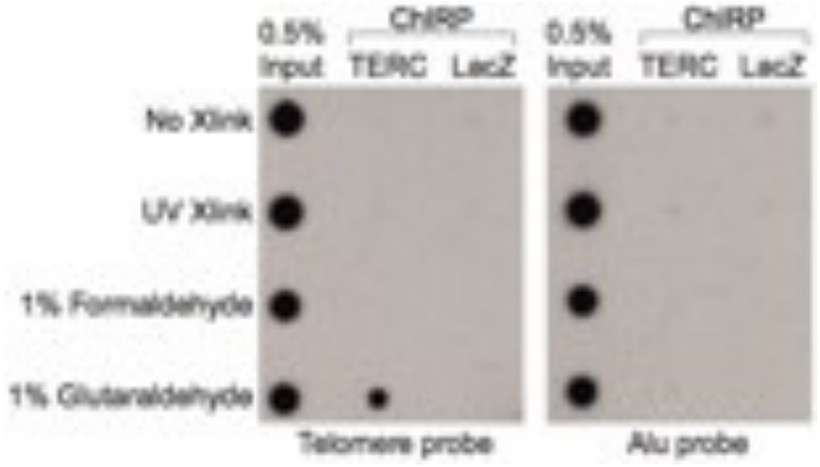


1. Crosslinking

1% of glutaraldehyde

Keep RNA



Ci Chu et al. 2011

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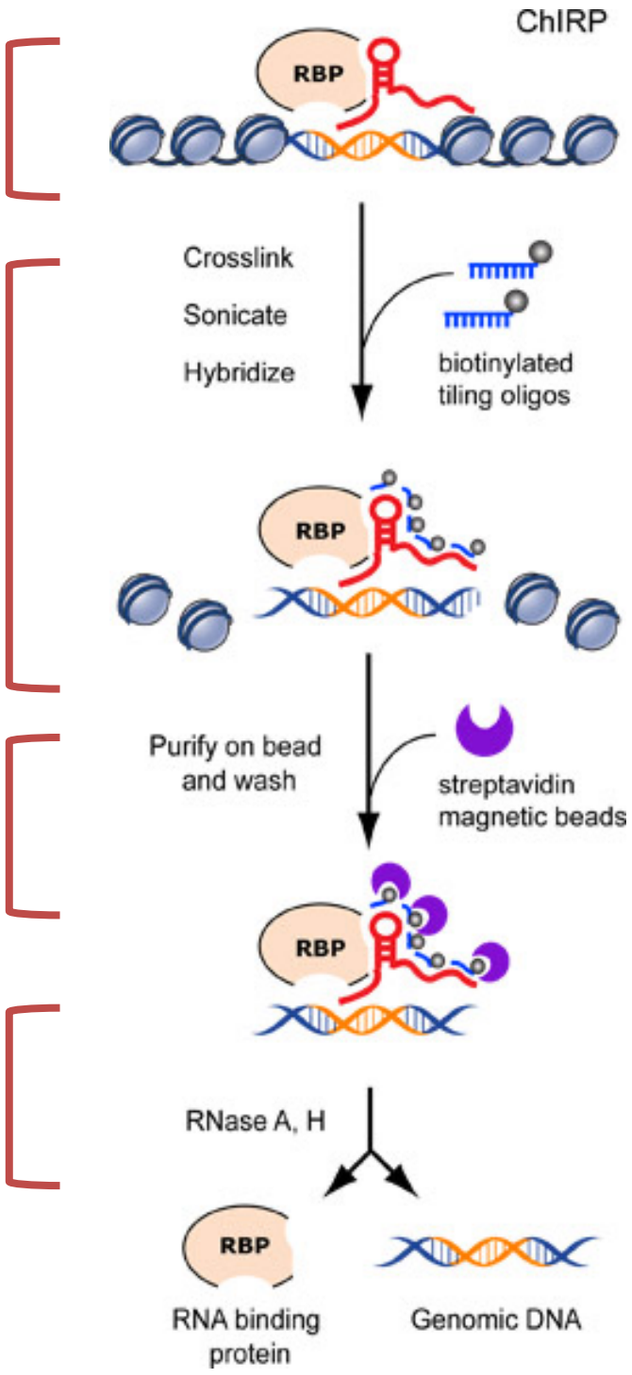
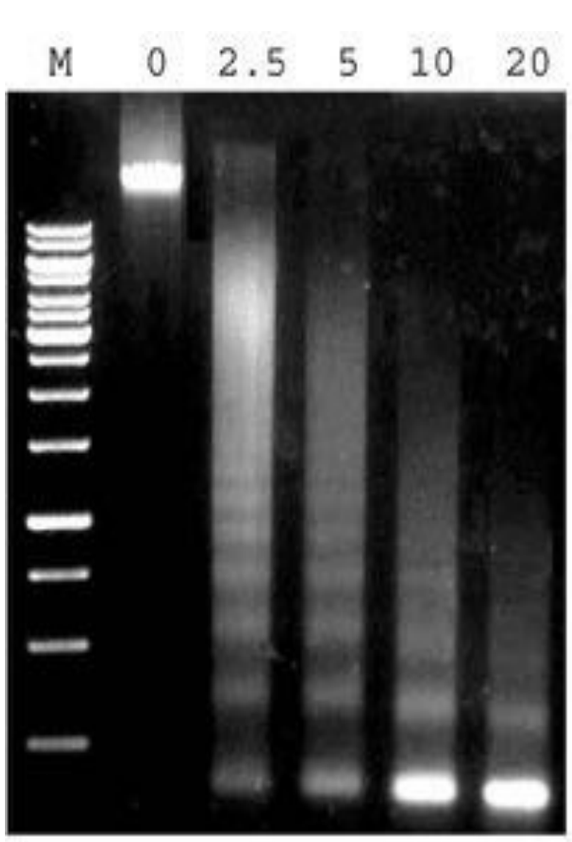
Enrich

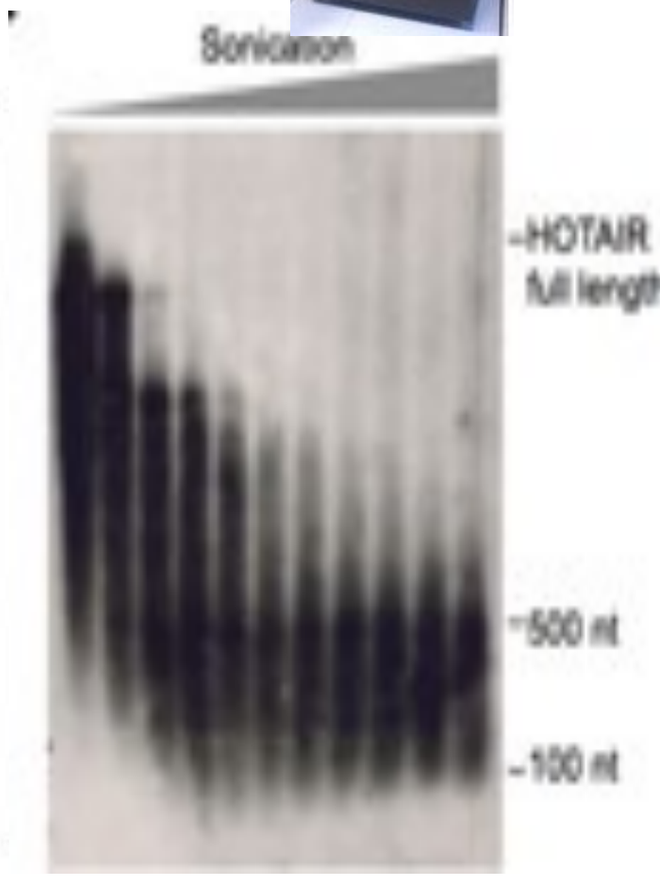
Wash

Analyse



1. Sonication





Northen Blot

Ci Chu et al. 2011

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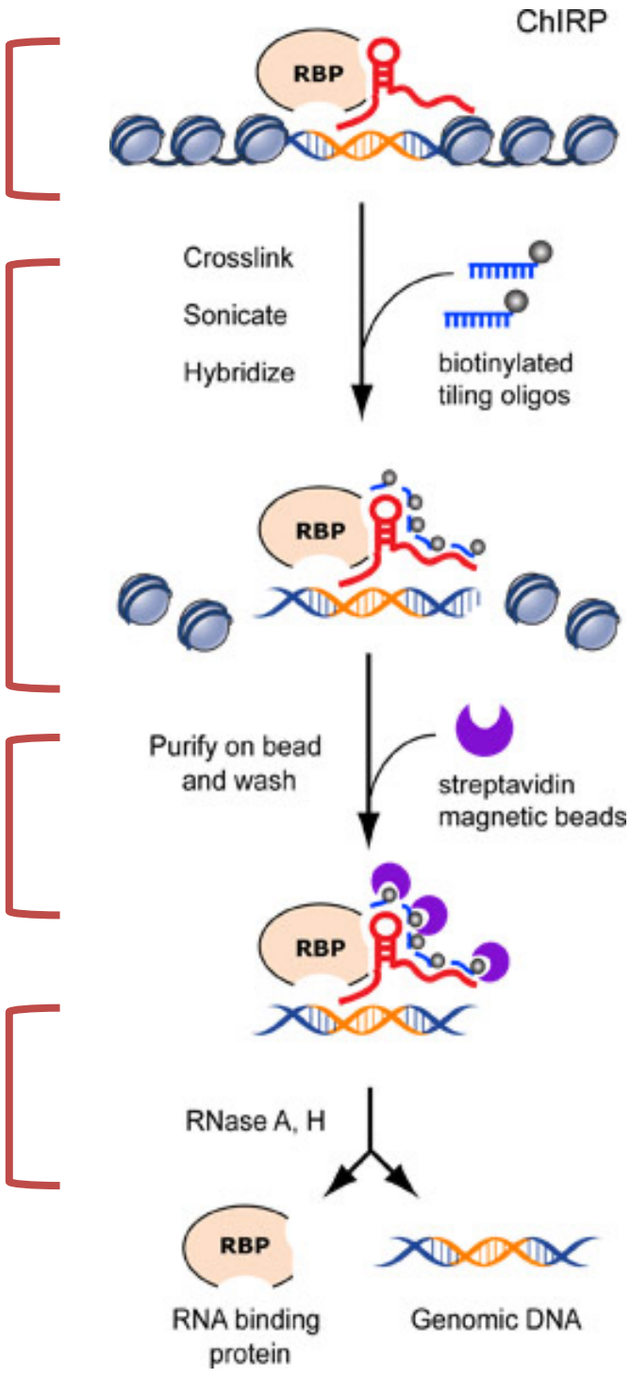
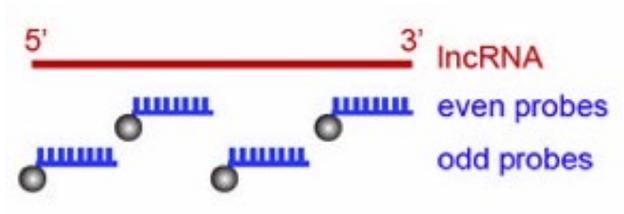
Enrich

Wash

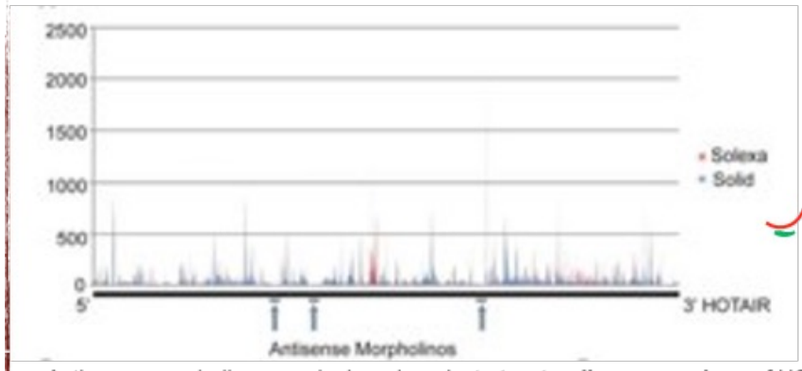
Analyse



1. Probe design



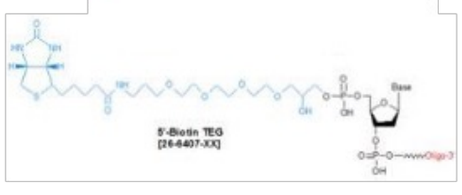
HOTAIR ChIRP

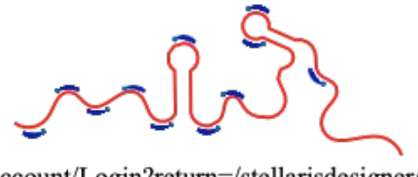


Antisense morpholinos are designed against **structurally open regions** of HOTAIR RNA. Peaks denote secondary structures previously determined by Parallel Analysis of RNA Structure (PARS) (Kertesz et al., 2010).

Ci Chu et al. 2011

random design





https://www.biosearchtech.com/Account/Login?return=/stellarisdesigner/

Ci Chu et al. 2011

ChIRP

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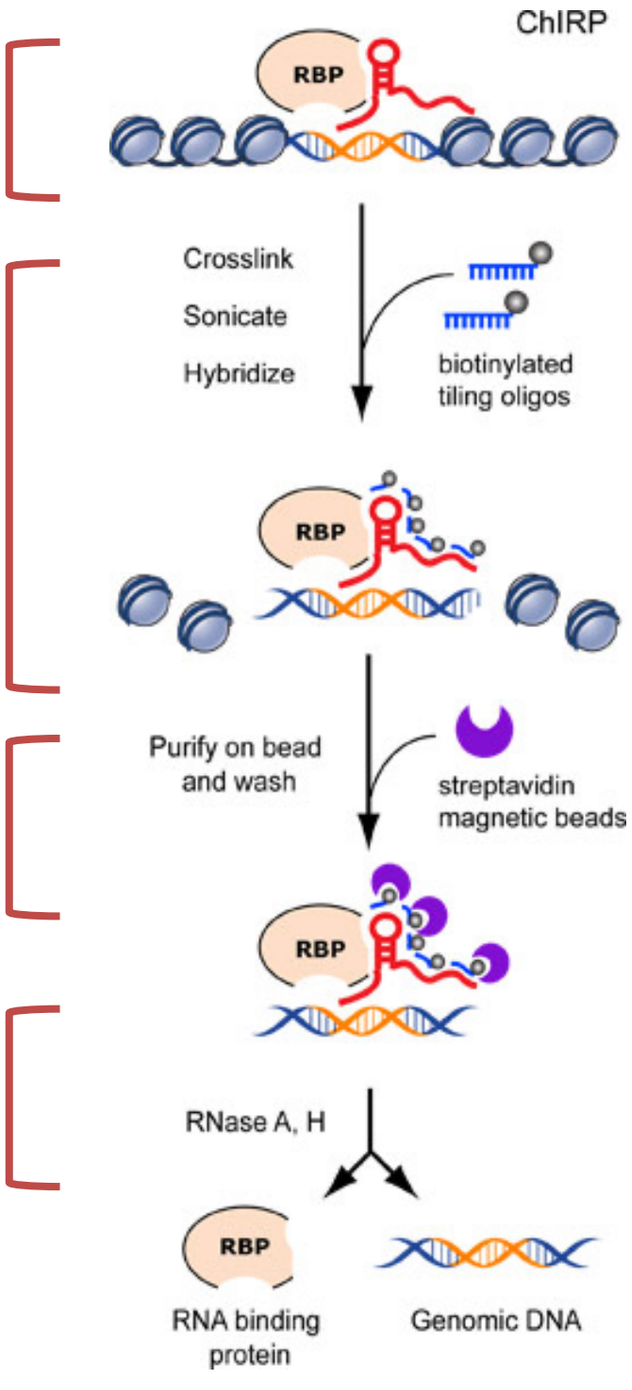

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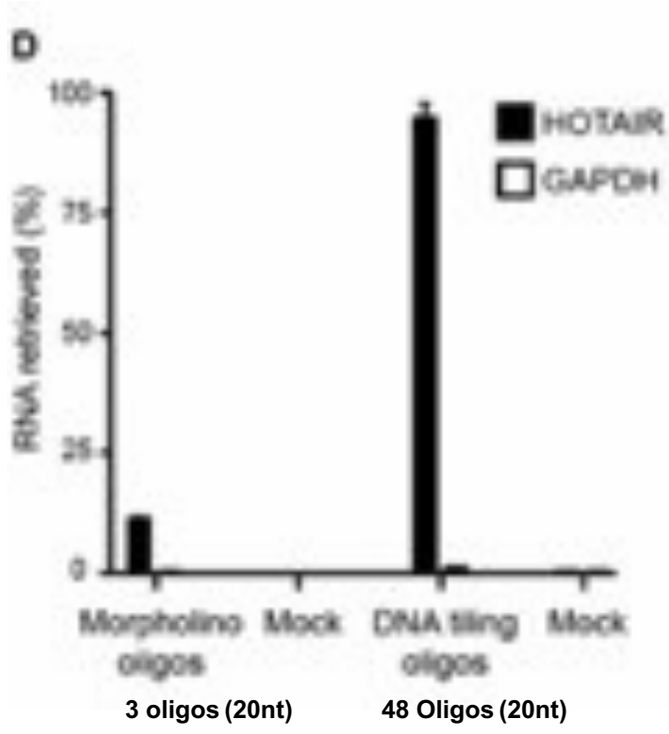

The more probes you have, the better.

Ci Chu et al. 2011

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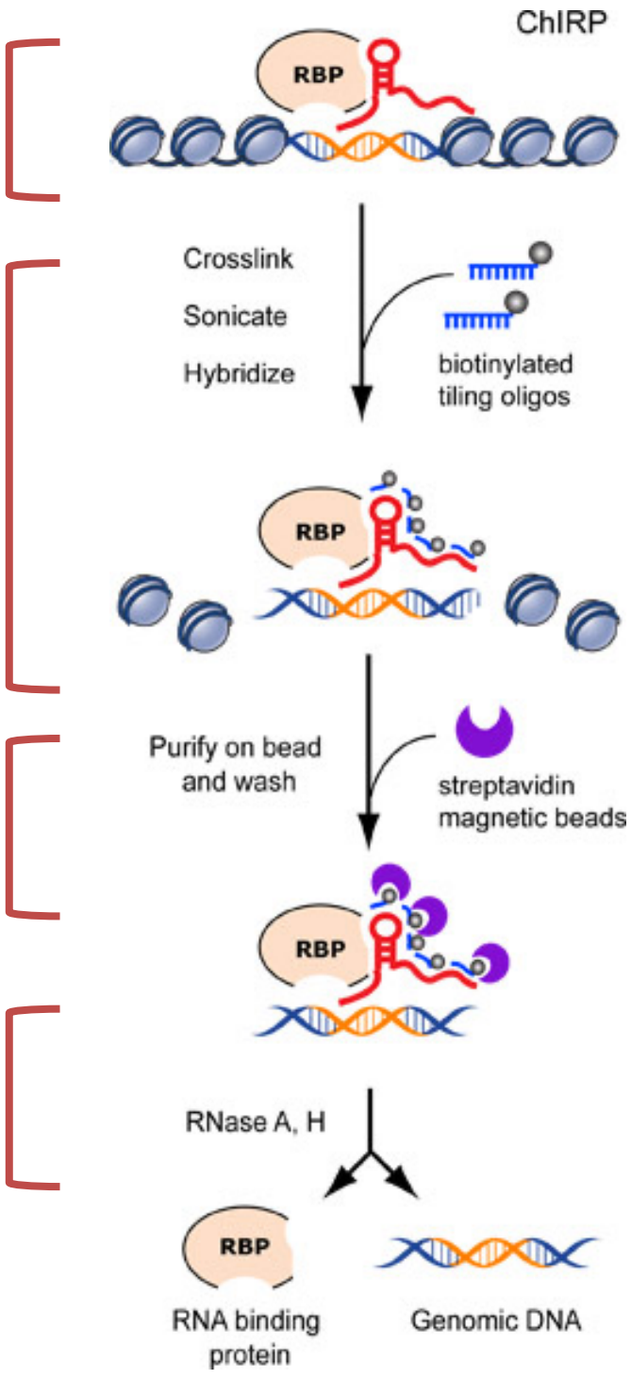
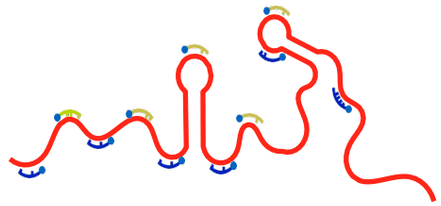
Enrich

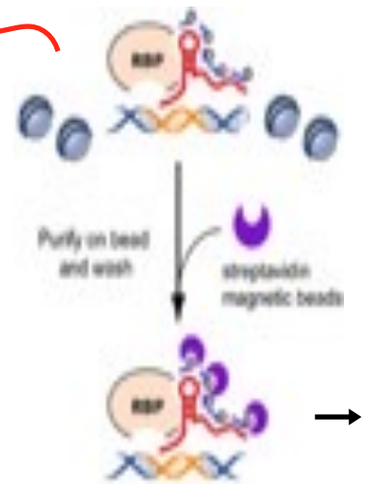
Wash

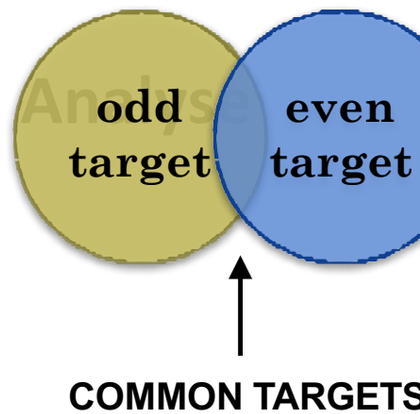
Analyse



2. Precipitation and wash







Dot BLOT ← → **ChIRP-seq**

Ci Chu et al. 2011

ChIRP

AIM: Identification of RNA interactions with the chromatin. Bait: RNA output: DNA/protein)

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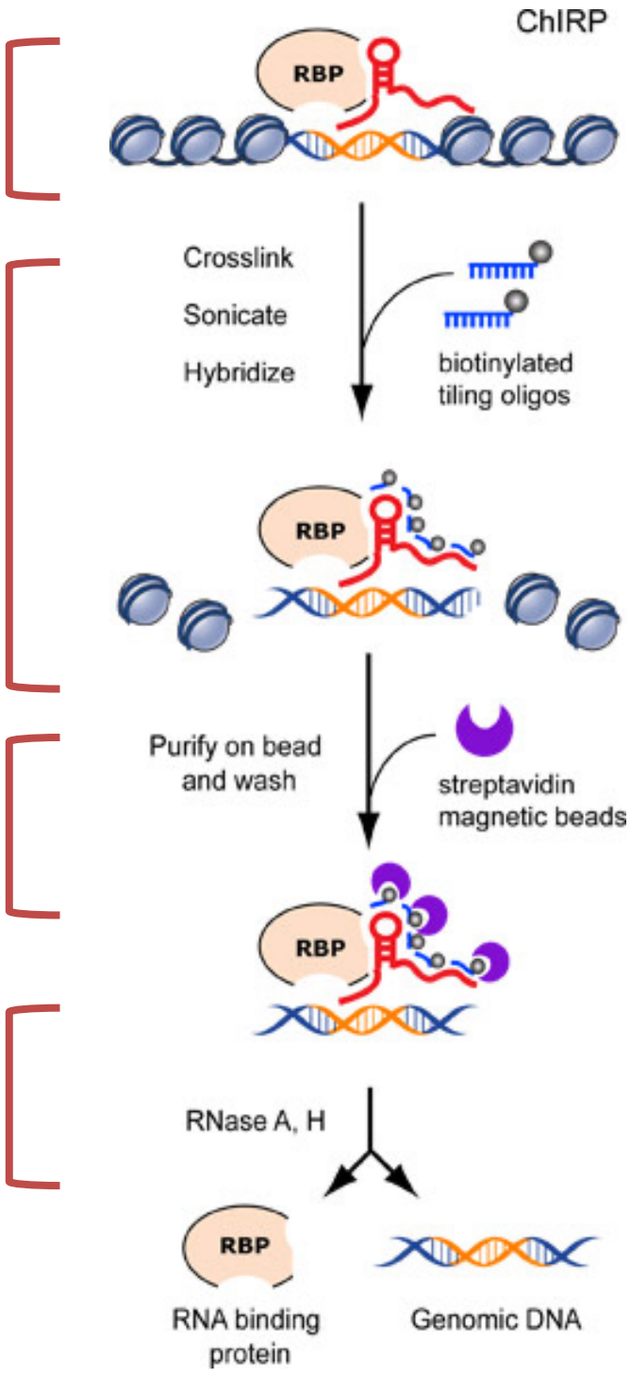
Prepare

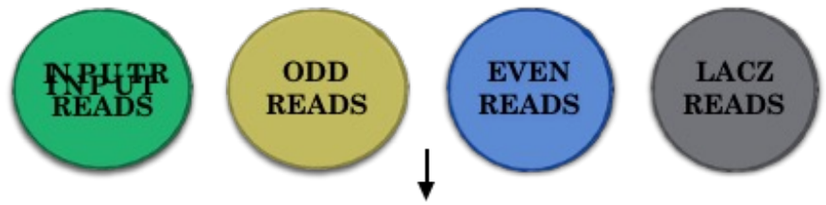
Enrich

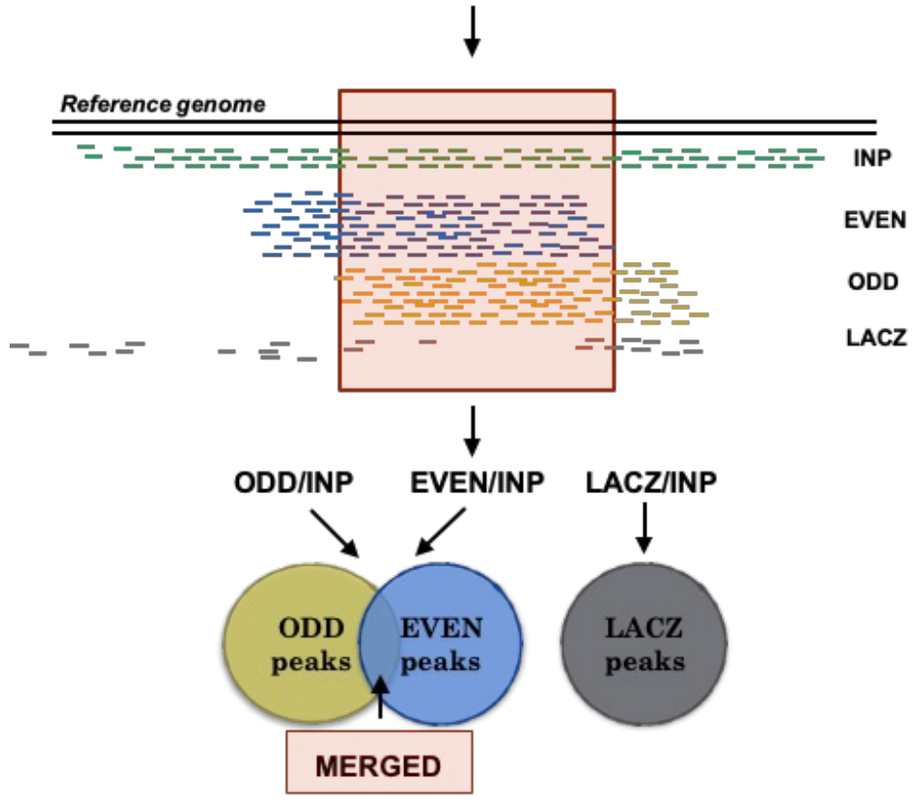
Wash

Analyse



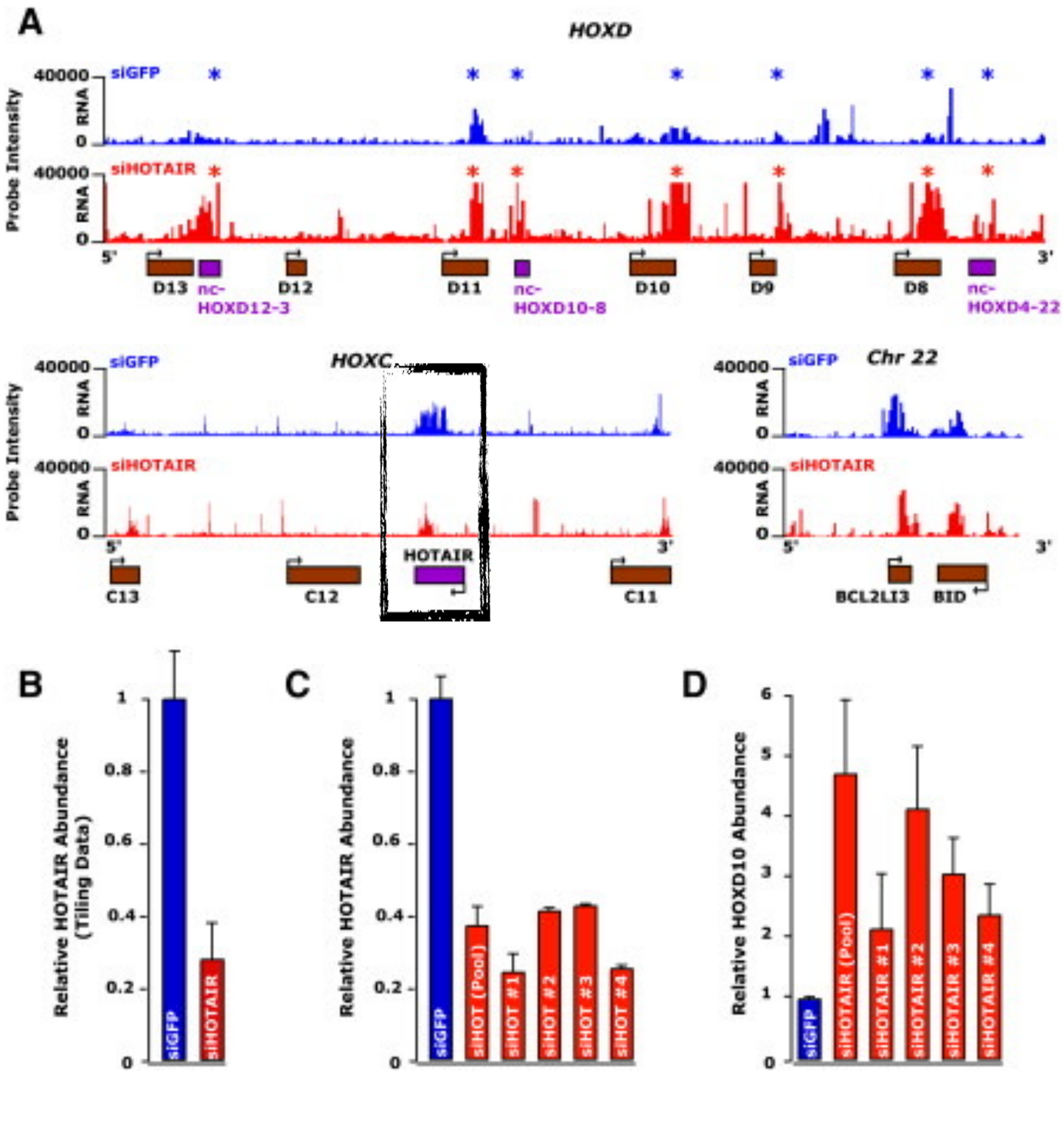
3. Analysis ChIRP-Seq





Ci Chu et al. 2011

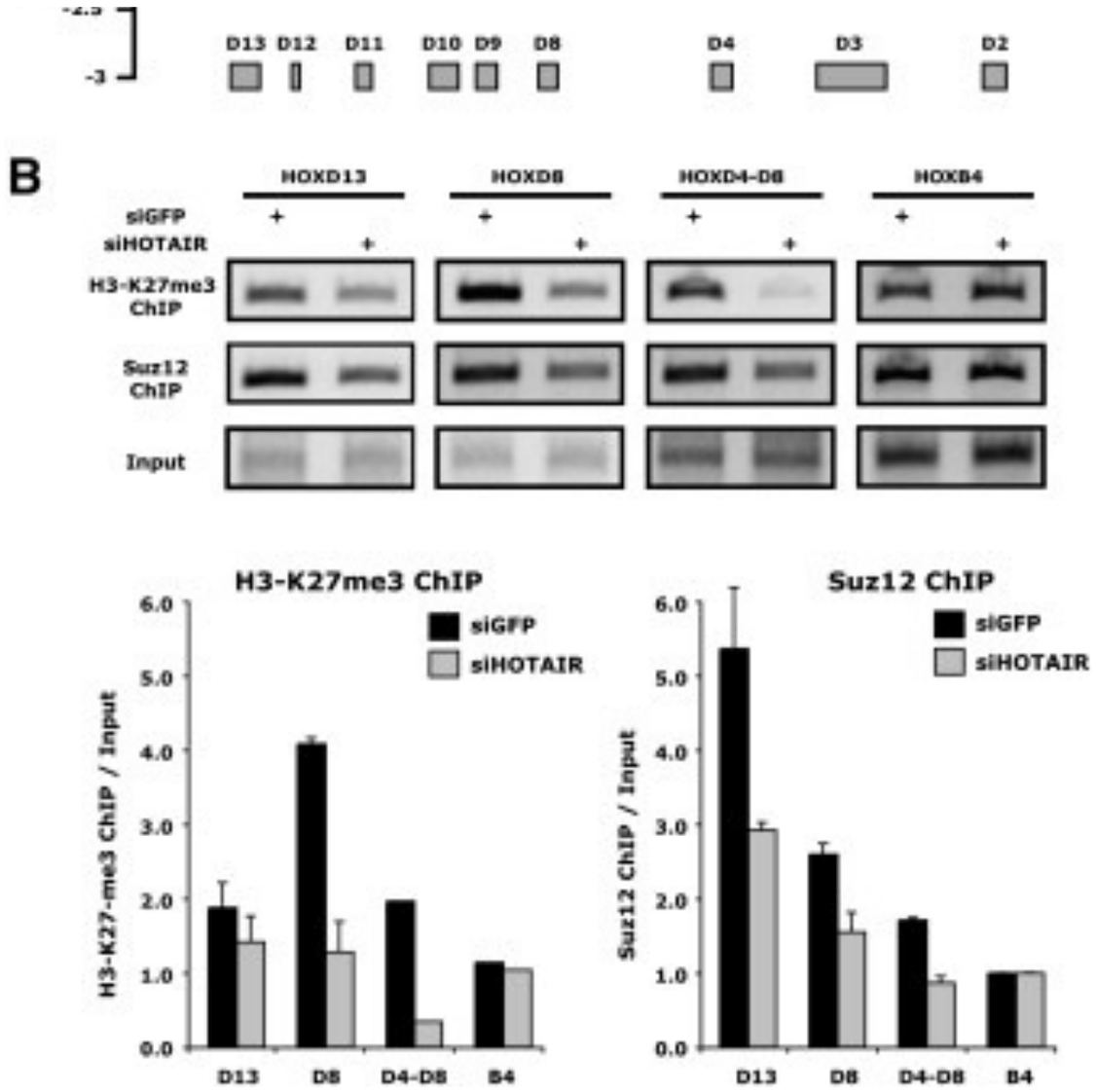
ChIRP: example HOTAIR



Functional data

Rinn et al. 2007

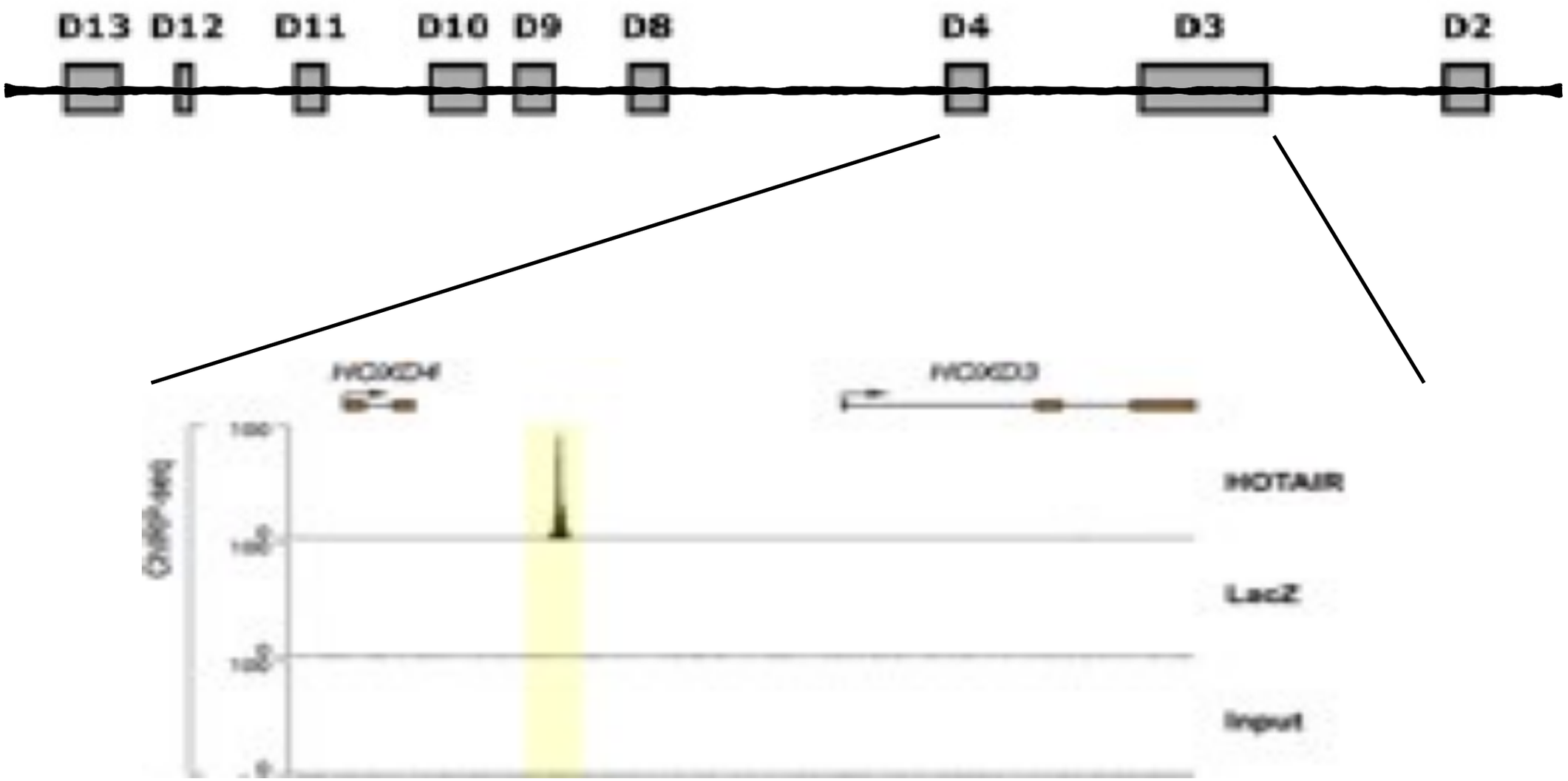
ChIRP: example HOTAIR

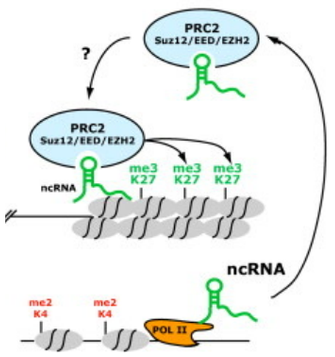


Rinn et al. 2007

ChIRP: example HOTAIR

HOTAIR ***ChIRP***





Ci Chu et al. 2011

Types of interaction

		OUTPUT (what we analyse)		
		Protein	RNA	DNA
BAIT (What we enrich)	Protein	**CO-IP** (co-immunoprecipitation)	**RIP/CLIP** (RNA-Immunoprecipitation)	**ChIP** (Chromatin Immunoprecipitation)
	RNA	**Exogenous RNA pull Down RAP-Protein** (RNA antisense purificaton)	**RAP-RNA** (RNA antisense purificaton)	**ChIrP** (Chromatin isolation by RNA purification)
	DNA	**DNA pull down**		**Conformation capture 3C**

RAP: RNA pull down techniques

AIM: Identification of the protein interactors of an RNA Bait: RNA output: Protein/RNA

RAP (RNA affinity pull down) a pulldown technique used to investigate the interaction between RNA and proteins.

Precipitation of the RNA and PROTEINS checking

Total Cytoplamic or Nuclear extract

Huge amount of cellular extract is needed

The efiiciency depends on the abundance of the RNA

Exogenous RNA caputure

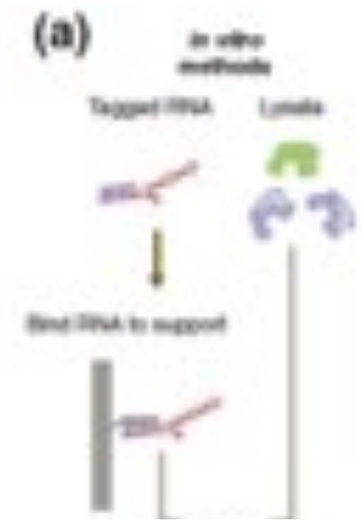
RNA affinity capture methods

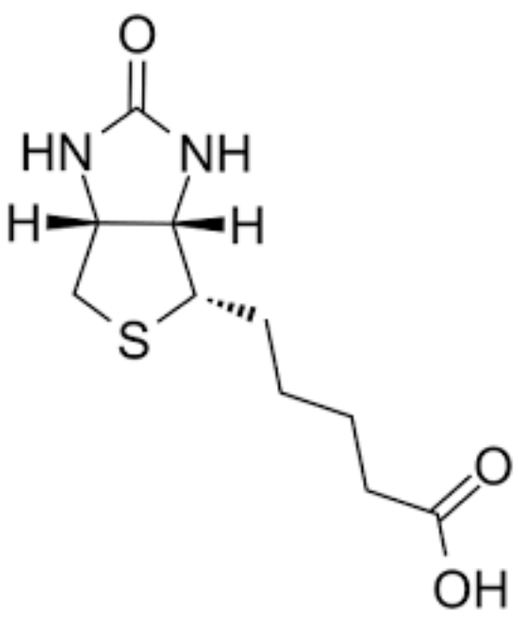
Different Tags

MS2 viral protein —> Loop stem loop

Cy4 —> RNA aptamer

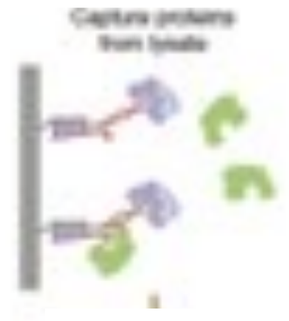
STREPTAVIDIN —> S1 aptamer





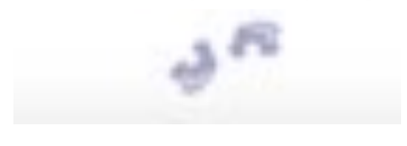
Biotin

Capture protein from lysate



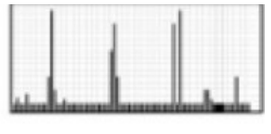
Washing

Boil with SDS



Western Blot

Mass spectrometry



Exogenous RNA caputure

Work flow

1. **RNA tagging**

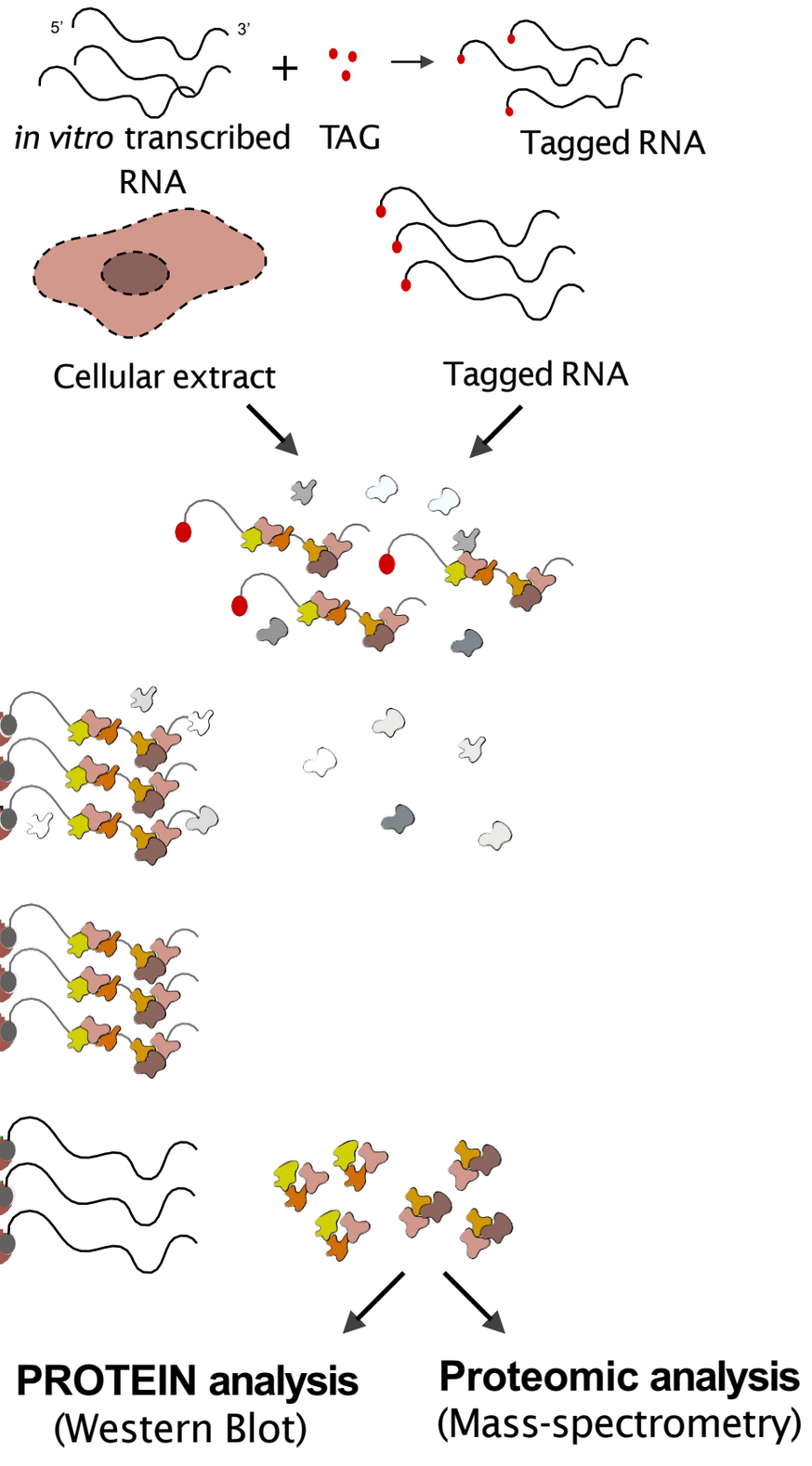
2. **Cell lysis**

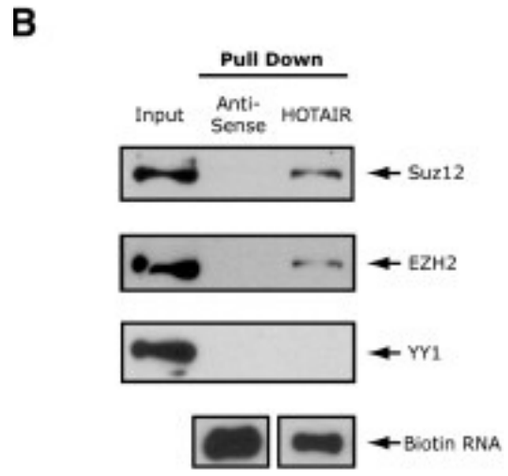
3. **Cell extract/tagged RNA incubation**

4. **RNA/resin binding**

5. **Washing**

6. **PROTEIN elution**





Rinn et al. Cell 129, 1311–1323, June 29, 2007

HOTAIR exogenous pulldown

Cipriano and Ballarino, FMB 2018

Exogenous RNA caputure

The SDS boiling phase will dissociate bound material from the resin, including complexes bound specifically through the tag and those bound non-specifically directly to the resin

NEGATIVE CONTROL

With the exogenous RNA pulldown Is difficult discriminate between real and fake interactions since many interactions can occur just *in vitro*

Exogenous RNA caputure. It might not be specific

Article

SOURCE DATA | TRANSPARENT PROCESS | OPEN ACCESS

THE EMBO JOURNAL

PRC2 is dispensable for *HOTAIR*-mediated transcriptional repression

Manuela Portoso[1,2], Roberta Ragazzini[1,2], Živa Brenčič[1,2], Arianna Moiani[1,2], Audrey Michaud[1,2], Ivaylo Vassilev[1,2], Michel Wassef[1,2], Nicolas Servant[1,3], Bruno Sargueil[4] & Raphaël Margueron[1,2,*]

Abstract

Long non-coding RNAs (lncRNAs) play diverse roles in physiological and pathological processes. Several lncRNAs have been suggested to modulate gene expression by guiding chromatin-modifying complexes to specific sites in the genome. However, besides the example of Xist, clear-cut evidence demonstrating this novel mode of regulation remains sparse. Here, we focus on *HOTAIR*, a lncRNA that is overexpressed in several tumor types and previously proposed to play a key role in gene silencing through direct recruitment of Polycomb Repressive Complex 2 (PRC2) to defined genomic loci. Using genetic tools and a novel RNA-tethering system, we investigated the interplay between *HOTAIR* and PRC2 in gene silencing. Surprisingly, we observed that forced overexpression of *HOTAIR* in breast cancer cells leads to subtle transcriptomic changes that appear to be independent of PRC2. Mechanistically, we found that artificial tethering of *HOTAIR* to chromatin causes transcriptional repression, but that this effect does not require PRC2. Instead, PRC2 recruitment appears to be a consequence of gene silencing. We propose that PRC2 binding to RNA might serve functions other than chromatin targeting.

regulation of chromatin structure, either through histone modifications or through chromatin compaction (Simon & Kingston, 2009). In *Drosophila*, four PcG complexes have been identified, while in mammals, only two complexes are well characterized so far: Polycomb Repressive Complex 2 (PRC2) and Polycomb Repressive Complex 1 (PRC1). The PRC2 is responsible for histone H3 lysine 27 (H3K27) di- and tri-methylation (Margueron & Reinberg, 2011).

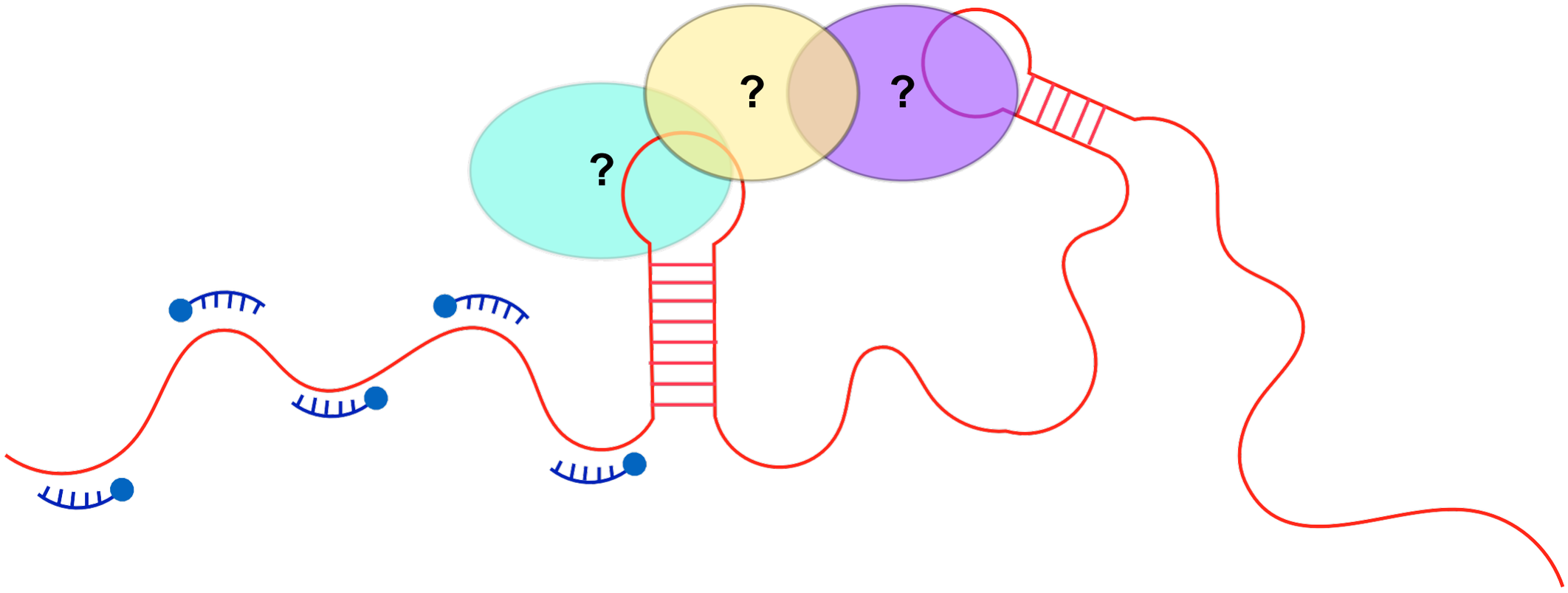
Although our understanding of how PRC2 contacts chromatin has improved, how it is specifically recruited to defined genomic loci is still only partially understood. The core PRC2 has no known sequence-specific DNA-binding domain. In *Drosophila*, DNA sequences known as Polycomb responsive elements (PREs) mediate PcG recruitment through a combination of specific transcription factors. Although similar mechanisms have been proposed in mammals (Arnold *et al*, 2013; Sing *et al*, 2009; Woo *et al*, 2010), they do not appear to be the general rule. Indeed, the specific transcription factors found to bind these putative mammalian PREs do not act consistently as PRC2 genomewide recruiters. Importantly, GC-rich regions are frequently bound by PRC2 components (Ku *et al*, 2008) and they are, in some instances, sufficient to mediate PRC2 recruitment (Mendenhall *et al*, 2010; Jermann *et al*, 2014), although once again this cannot account for the specificity and dynamics of

EPIC FAIL

Endogenous RNA caputure. RAP

AIM: Identification of the protein interactors of an RNA Bait: RNA output: Protein/RNA

RAP (RNA affinity pull down) a pulldown technique used to investigate the interaction between RNA and proteins.



Endogenous RNA caputure. RAP

AIM: Identification of the protein interactors of an RNA Bait: RNA output: Protein/RNA

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2 Cell lysis

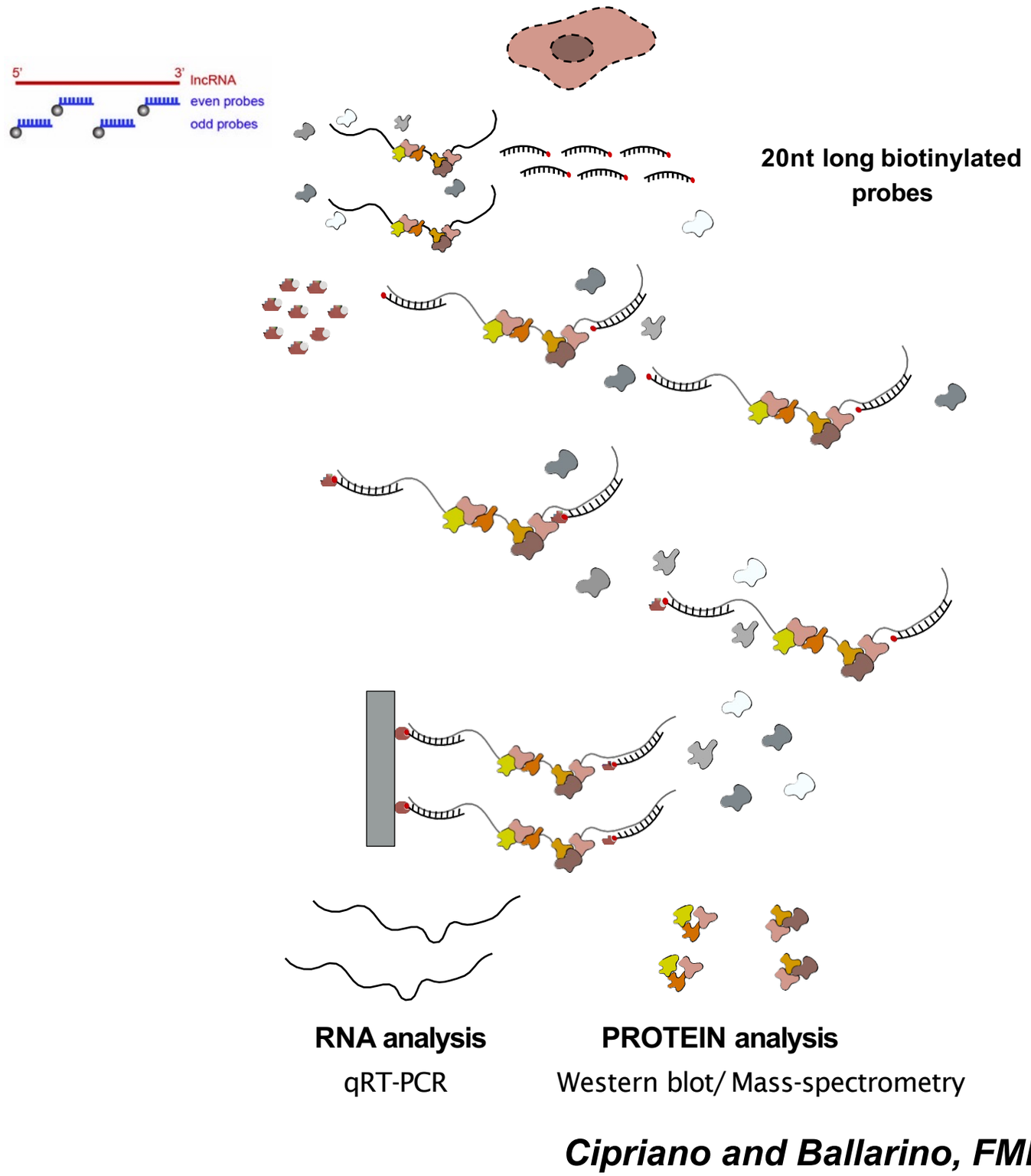
3. Cell extract/probes incubation

3. Binding step

4.Introduction of Streptavidin-magnetic beads and Capture RNA/ probescomplexes from lysate

5.Purilication of RNA/probes complexes and washes with low salt buffers (150mM NaCl)

6. Protein and RNA elution and analysis



Cipriano and Ballarino, FMB 2018

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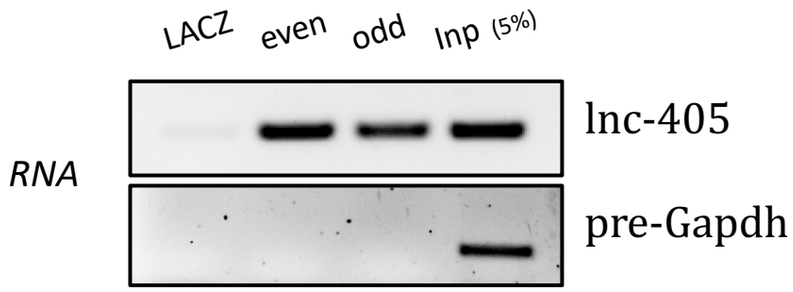
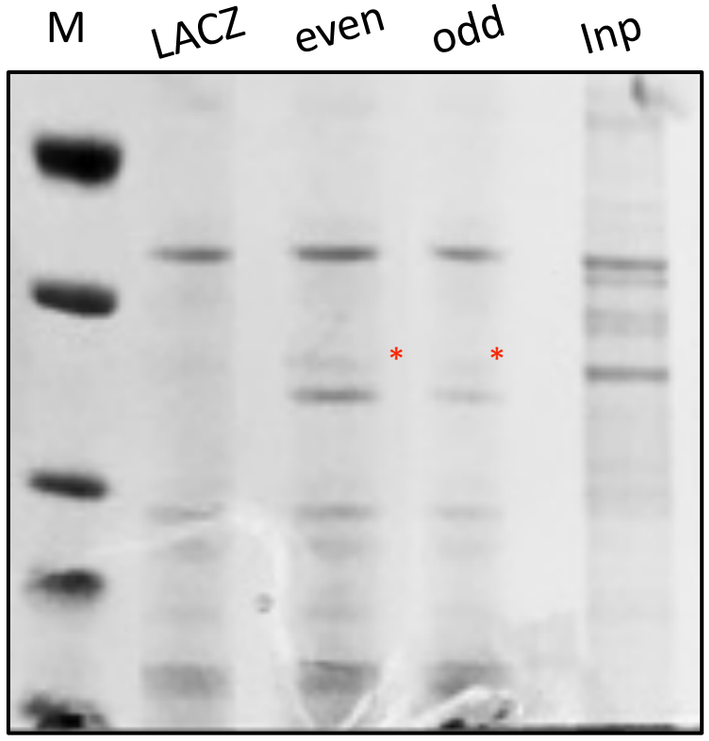
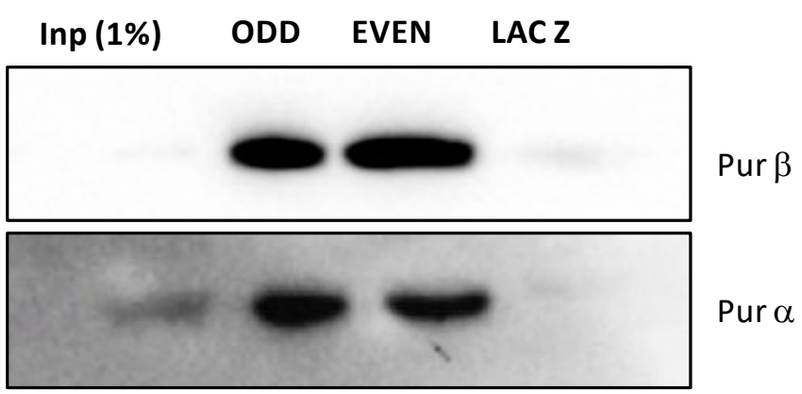
Lnc-405 endogenous pulldown



Semiq RT-PCR



WB

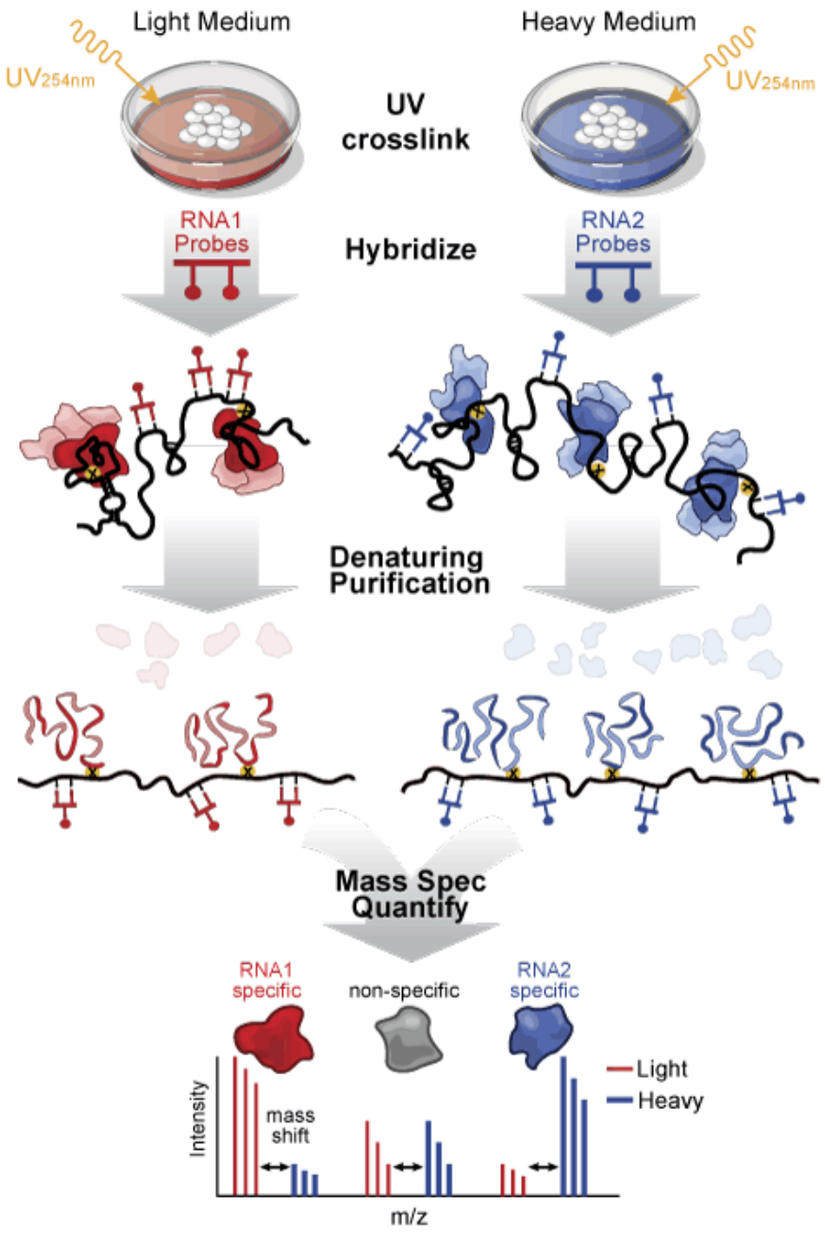


Accession	Description
Q9QXS1	Plectin OS=Mus musculus GN=Plec PE=1 SV=3 - [PLEC_MOUSE]
Q8VDD5	Myosin-9 OS=Mus musculus GN=Myh9 PE=1 SV=4 - [MYH9_MOUSE]
P62843	40S ribosomal protein S15 OS=Mus musculus GN=Rps15 PE=1 SV=2 - [RS15_MOUSE]
P42669	Transcriptional activator protein Pur alpha OS=Mus musculus GN=Pura PE=1 SV=1 - [PURA_MOUSE]
P62301	40S ribosomal protein S13 OS=Mus musculus GN=Rps13 PE=1 SV=2 - [RS13_MOUSE]
E9Q557	Desmoplakin OS=Mus musculus GN=Dsp PE=1 SV=1 - [DESP_MOUSE]
Q6P5H2	Nestin OS=Mus musculus GN=Nes PE=1 SV=1 - [NEST_MOUSE]
P62702	40S ribosomal protein S4, X isoform OS=Mus musculus GN=Rps4x PE=1 SV=2 - [RS4X_MOUSE]
P20152	Vimentin OS=Mus musculus GN=Vim PE=1 SV=3 - [VIME_MOUSE]
P31001	Desmin OS=Mus musculus GN=Des PE=1 SV=3 - [DESM_MOUSE]
O35295	Transcriptional activator protein Pur beta OS=Mus musculus GN=Purb PE=1 SV=3 - [PURB_MOUSE]

Endogenous RNA caputure. RAP

AIM: Identification of the protein interactors of an RNA Bait: RNA output: Protein/RNA

RAP (RNA affinity pull down) a pulldown technique used to investigate the interaction between RNA and proteins.



In vivo UV crosslinking

Longer probes (90nt)

High stringency binding conditions

High stringency wash conditions

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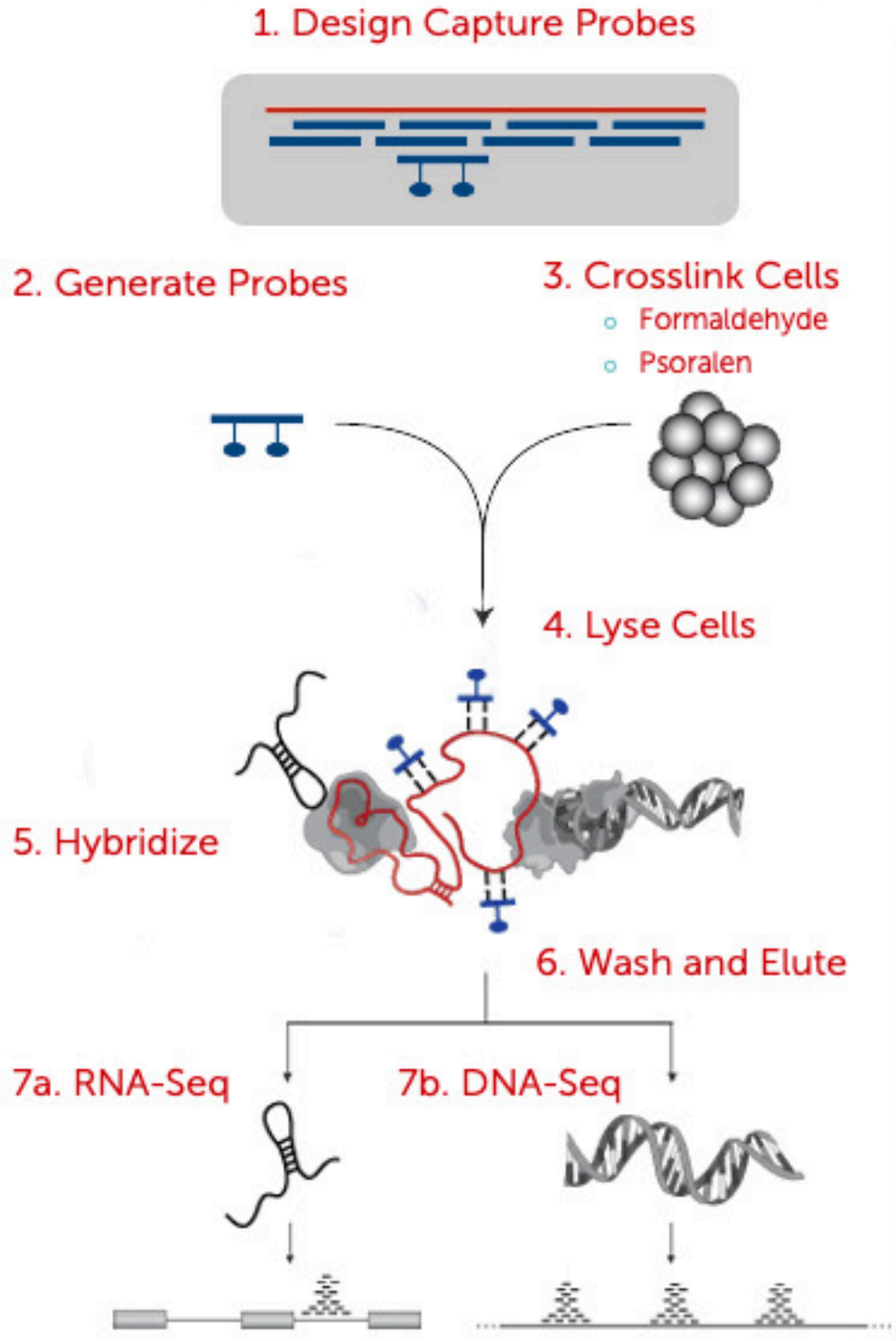


In vivo UV crosslinking

Longer probes (90nt)

High stringency binding conditions

High stringency wash conditions

Endogenous RNA caputure. RAP

Work flow

UV cross-linking

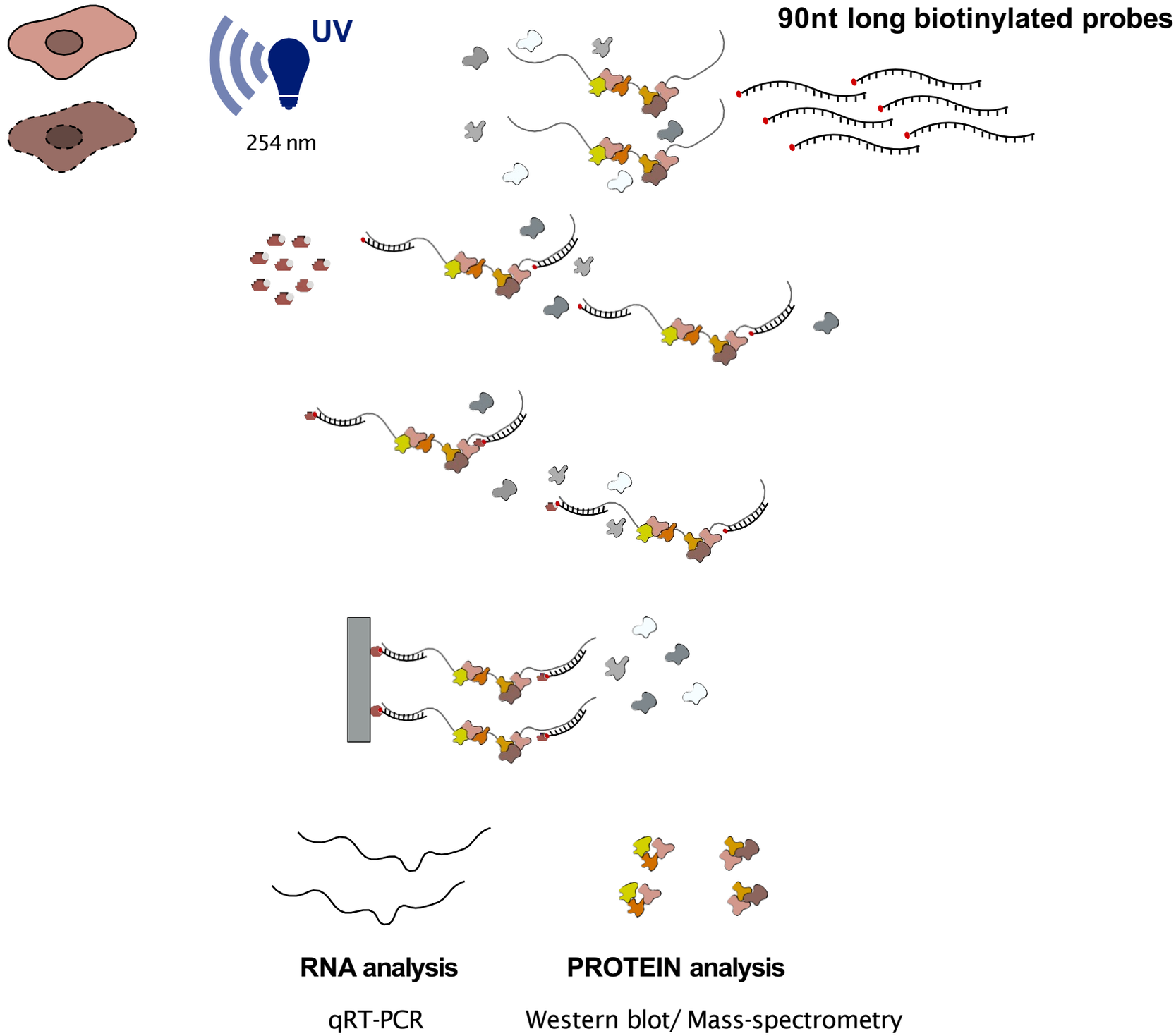
Cell lysis

RNA/probes binding to streptavidin magnetic beads

Capture RNA/probes complexes from lysate

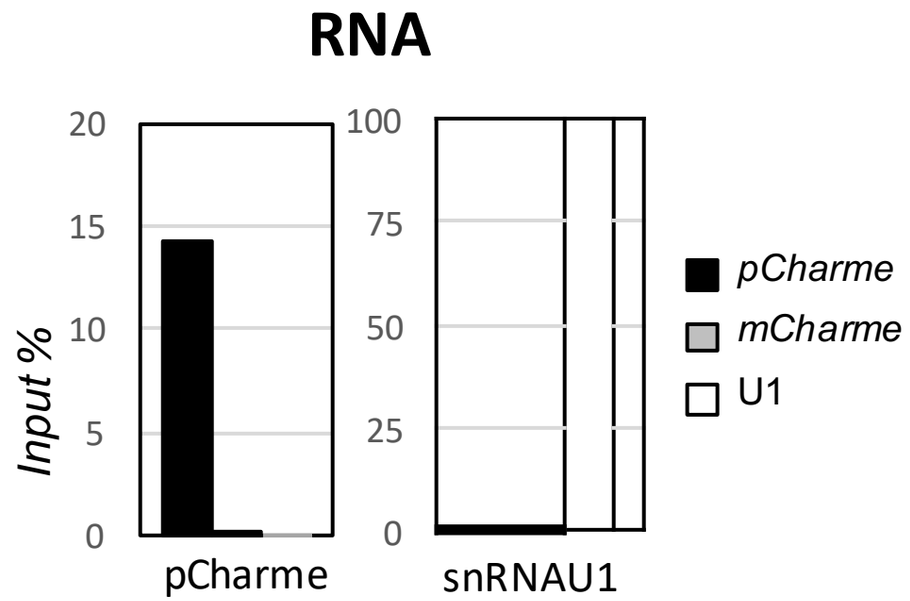
Purification of RNA/probes complexes and washes in high salt buffers (1M LiCl)

RNA and PROTEIN elution



Cipriano and Ballarino, FMB 2018

Endogenous RNA caputure. RAP



PD PD

Accession	Description	pCharme	U1
Q8BGJ5	MCG13402, isoform CRA_a OS=Mus musculus GN=Ptbp1 PE=1 SV=1 - [Q8BGJ5_MOUSE]	273.10	50.91
Q8K310	Matrin-3 OS=Mus musculus GN=Matr3 PE=1 SV=1 - [MATR3_MOUSE]	165.04	35.42
Q61990	Poly(rC)-binding protein 2 OS=Mus musculus GN=Pcbp2 PE=1 SV=1 - [PCBP2_MOUSE]	57.12	19.65
A0A0G2JGW0	Polypyrimidine tract-binding protein 2 OS=Mus musculus GN=Ptbp2 PE=1 SV=1 - [A0A0G2JGW0_MOUSE]	22.18	0.00
B1B0C7	Basement membrane-specific heparan sulfate proteoglycan core protein OS=Mus musculus GN=Hspg2 PE=1 SV=1 -	16.66	0.00
A0A0R4J044	Poly(rC)-binding protein 4 OS=Mus musculus GN=Pcbp4 PE=1 SV=1 - [A0A0R4J044_MOUSE]	16.04	0.00
Q4FK66	Pre-mRNA-splicing factor 38A OS=Mus musculus GN=Prpf38a PE=1 SV=1 - [PR38A_MOUSE]	14.10	0.00
Z4YJF5	Myomesin-1 OS=Mus musculus GN=Myom1 PE=1 SV=1 - [Z4YJF5_MOUSE]	10.38	0.00
Q62376	U1 small nuclear ribonucleoprotein 70 kDa OS=Mus musculus GN=Snrnp70 PE=1 SV=2 - [RU17_MOUSE]	0.00	432.94
Q62189	U1 small nuclear ribonucleoprotein A OS=Mus musculus GN=Snrpa PE=1 SV=3 - [SNRPA_MOUSE]	0.00	304.30
Q8K4Z5	Splicing factor 3A subunit 1 OS=Mus musculus GN=Sf3a1 PE=1 SV=1 - [SF3A1_MOUSE]	0.00	162.87
P62309	Small nuclear ribonucleoprotein G OS=Mus musculus GN=Snrpg PE=1 SV=1 - [RUXG_MOUSE]	0.00	121.09
P62317	Small nuclear ribonucleoprotein Sm D2 OS=Mus musculus GN=Snrpd2 PE=1 SV=1 - [SMD2_MOUSE]	0.00	68.35
Q6P4T2	U5 small nuclear ribonucleoprotein 200 kDa helicase OS=Mus musculus GN=Snrnp200 PE=1 SV=1 - [U520_MOUSE]	10.24	50.45
P62320	Small nuclear ribonucleoprotein Sm D3 OS=Mus musculus GN=Snrpd3 PE=1 SV=1 - [SMD3_MOUSE]	0.00	50.24
Q62241	U1 small nuclear ribonucleoprotein C OS=Mus musculus GN=Snrpc PE=1 SV=1 - [RU1C_MOUSE]	0.00	34.61

Types of interaction

		OUTPUT (what we analyse)		
		Protein	RNA	DNA
BAIT (What we enrich)	Protein	**CO-IP** (co-immunoprecipitation)	**RIP/CLIP** (RNA-Immunoprecipitation)	**ChIP** (Chromatin Immunoprecipitation)
	RNA	**Exogenous RNA pull Down RAP-Protein** (RNA antisense purificaton)	**RAP-RNA** (RNA antisense purificaton)	**ChIrP** (Chromatin isolation by RNA purification)
	DNA	**DNA pull down**		**Conformation capture 3C**

RIP/CLIP

AIM: Identification of RNA interaction with known proteins. Bait: Proteins output: RNA

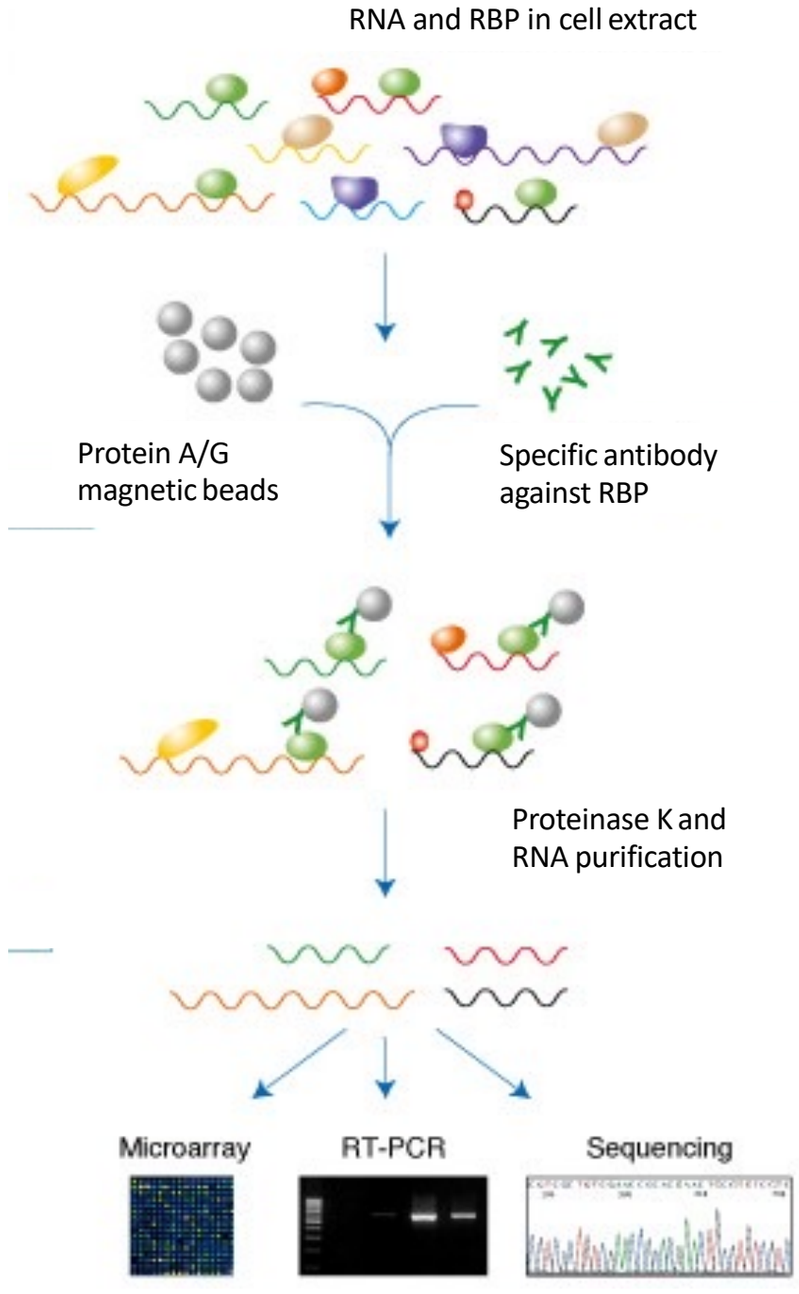
RIP (RNA immunoprecipitation) or CLIP (Crosslinked RNA immunoprecipitation) an immunoprecipitation technique used to investigate the interaction between RNA and preoteins). Not only serve to determine interaction but also place of the interaction.

Prepare

Enrich

Wash

Analyse



Interaction between RNA and Proteins focusing on the proteins (protein focused)

A lot of protocols, same essence.

- Cytoplasmic or Nuclear extract
- Isolation of Ribonucleoprotein complexes
- The resulting data have a low resolution, also **not directly associated** RNAs could be immunoprecipitated, and the **binding site** in the co-purified RNA molecule remained unresolved.
- Variants:

CLIP (UV-RIP)
PAR-CLIP
i-CLIP (CLIP-seq)

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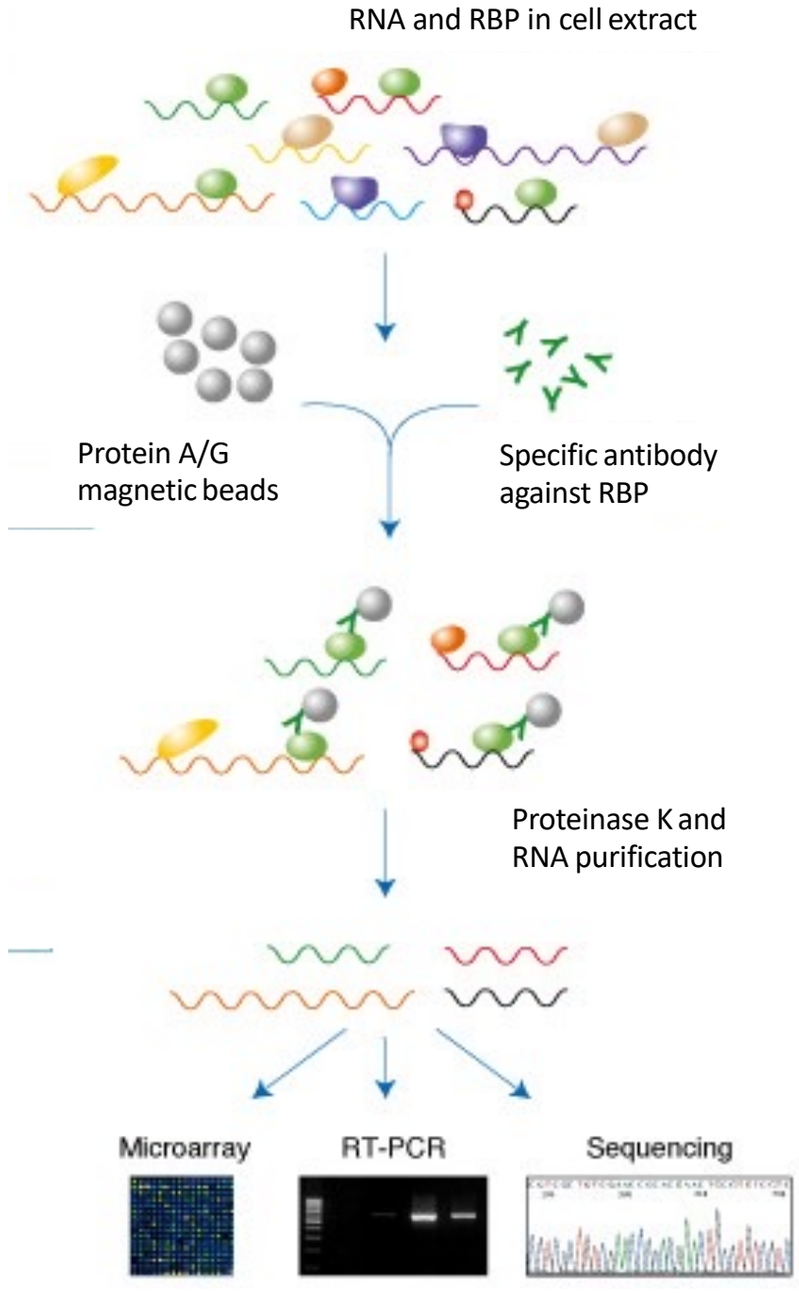

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CLIP (UV-RIP)
PAR-CLIP
i-CLIP (CLIP-seq)

RIP

Work flow

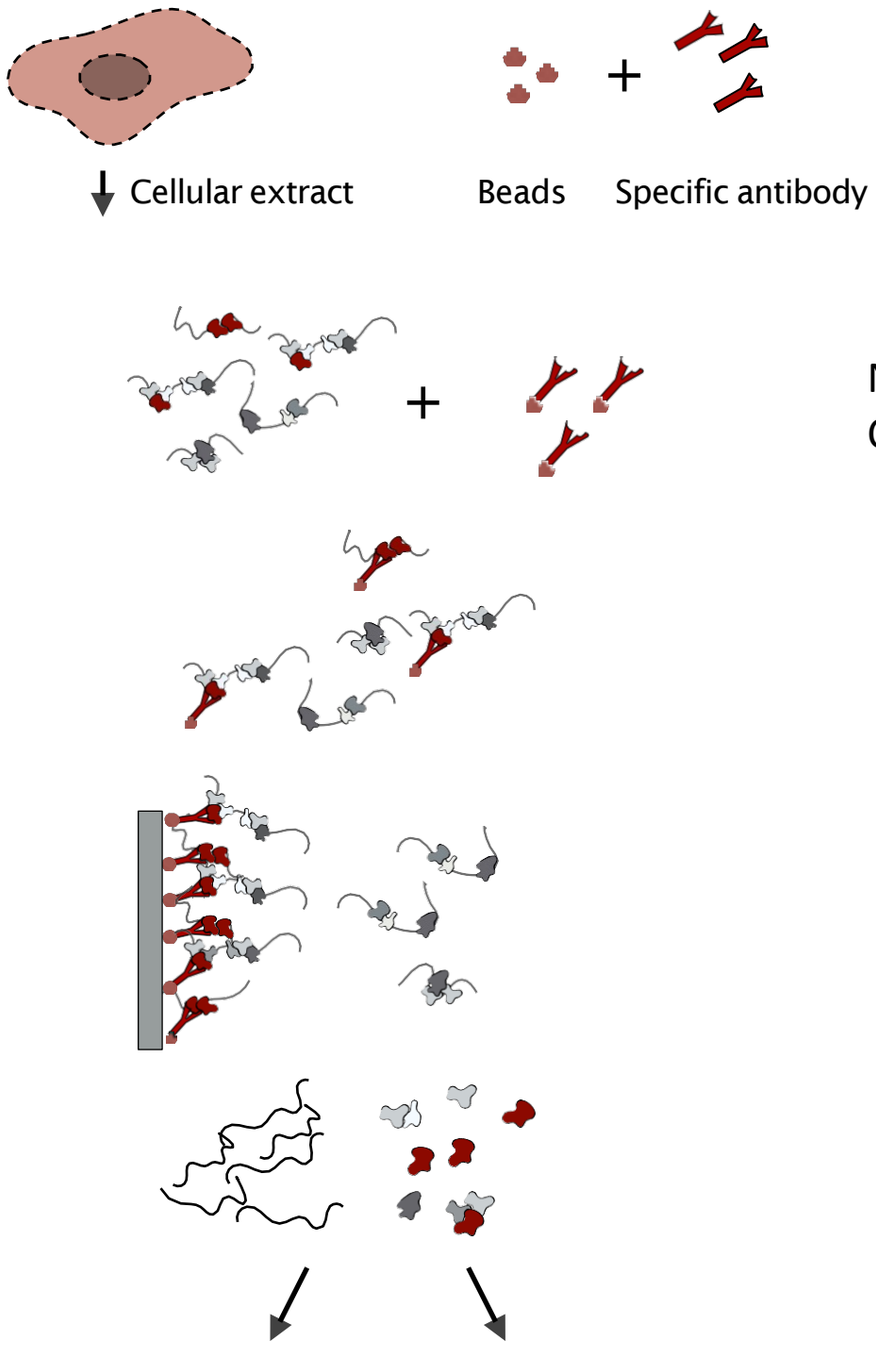
1. Lysis Cells and collect cell extract

2 Prebinding between AntiBody and Beads

3.Introduction of cellular extract (Binding step)

4.Wash and Purilication of RNA-protein complexes

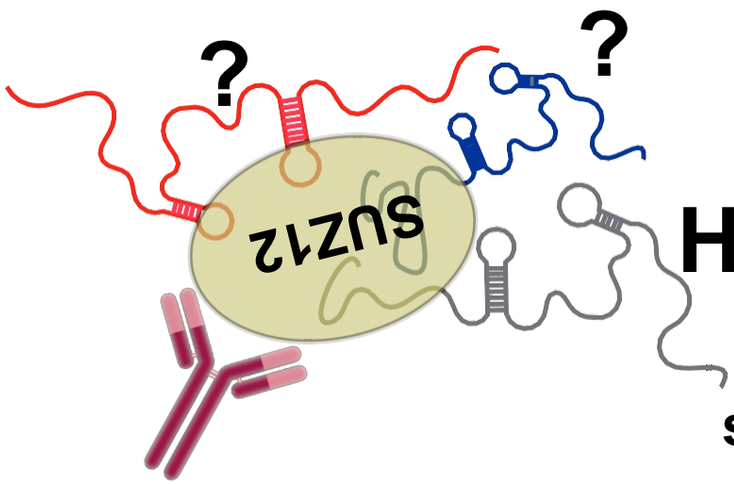
5. Protein and RNA elution



No crosslink
Capture specific and non specific interactions

Cipriano and Ballarino, FMB 2018

RIP/CLIP



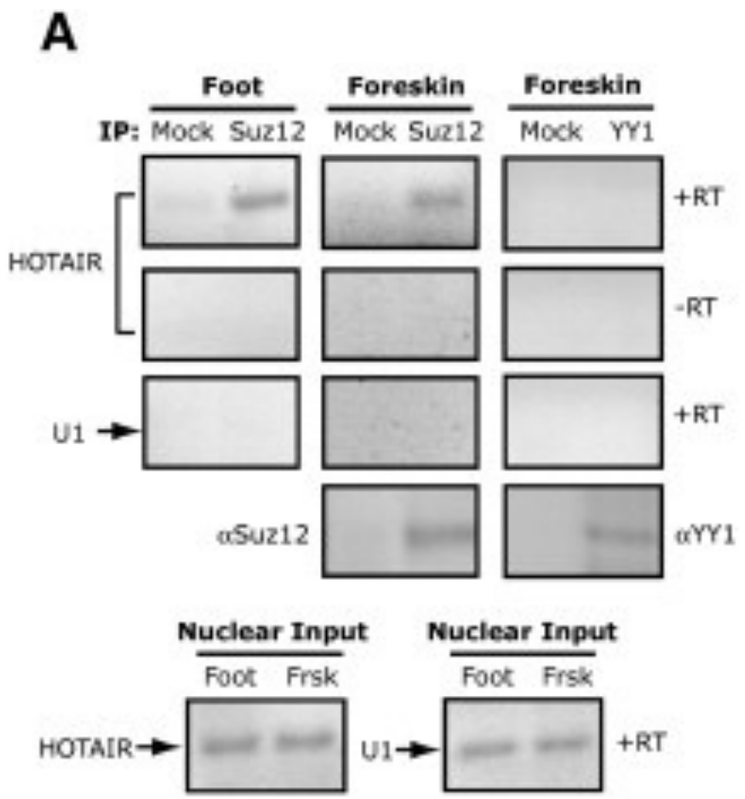
HOTAIR

semiq-rtPCR

Western Blot

semiq-rtPCR

SUZ12 RIP



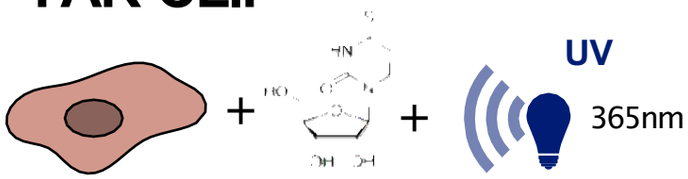
Rinn et al., Cell 2007

CLIP

(in vivo and cross-linked)

HITS-CLIP

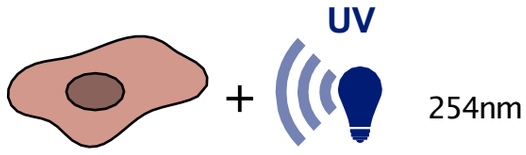
PAR-CLIP



① UV crosslinking

iCLIP/eCLIP

CRAC



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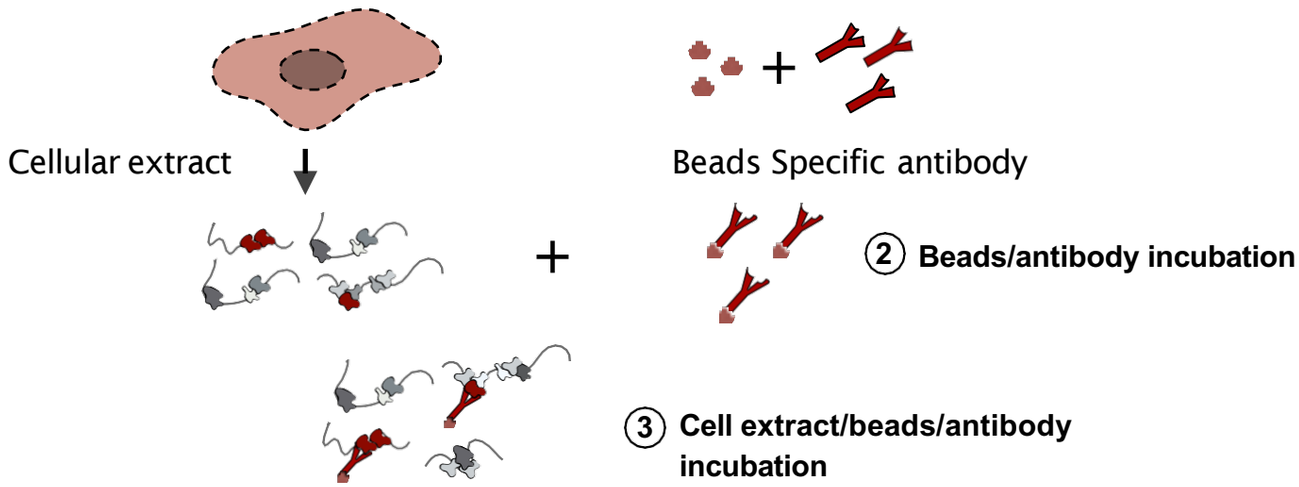
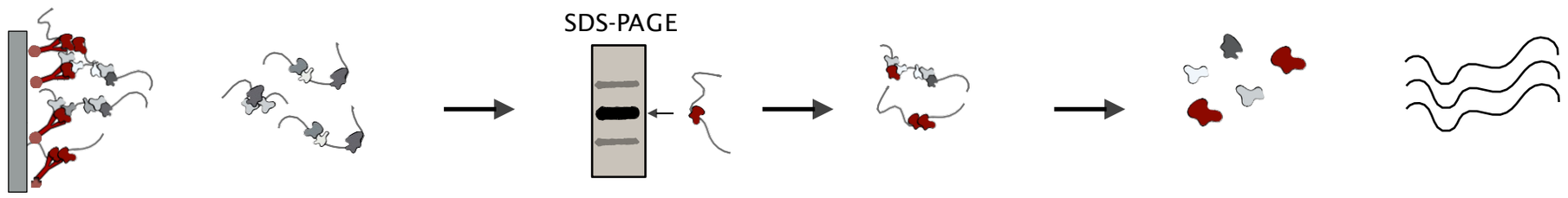


② Beads/antibody incubation

③ Cell extract/beads/antibody incubation



④ Purification of PROTEIN-antibody complexes, washes and radioactive labelling

⑤ PROTEIN/RNA complexes size selection

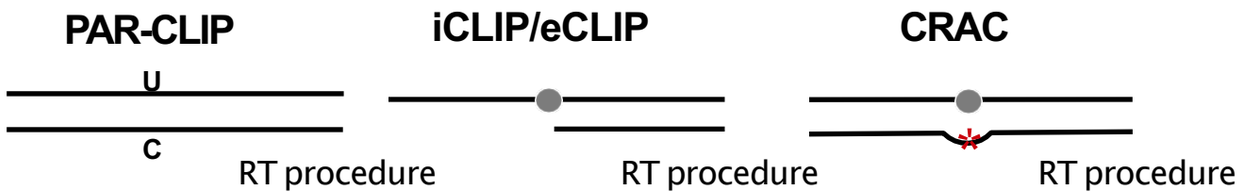
⑥ PROTEIN/RNA complexes purification

⑦ PROTEIN analysis (Western Blot)

⑧ RNA analysis

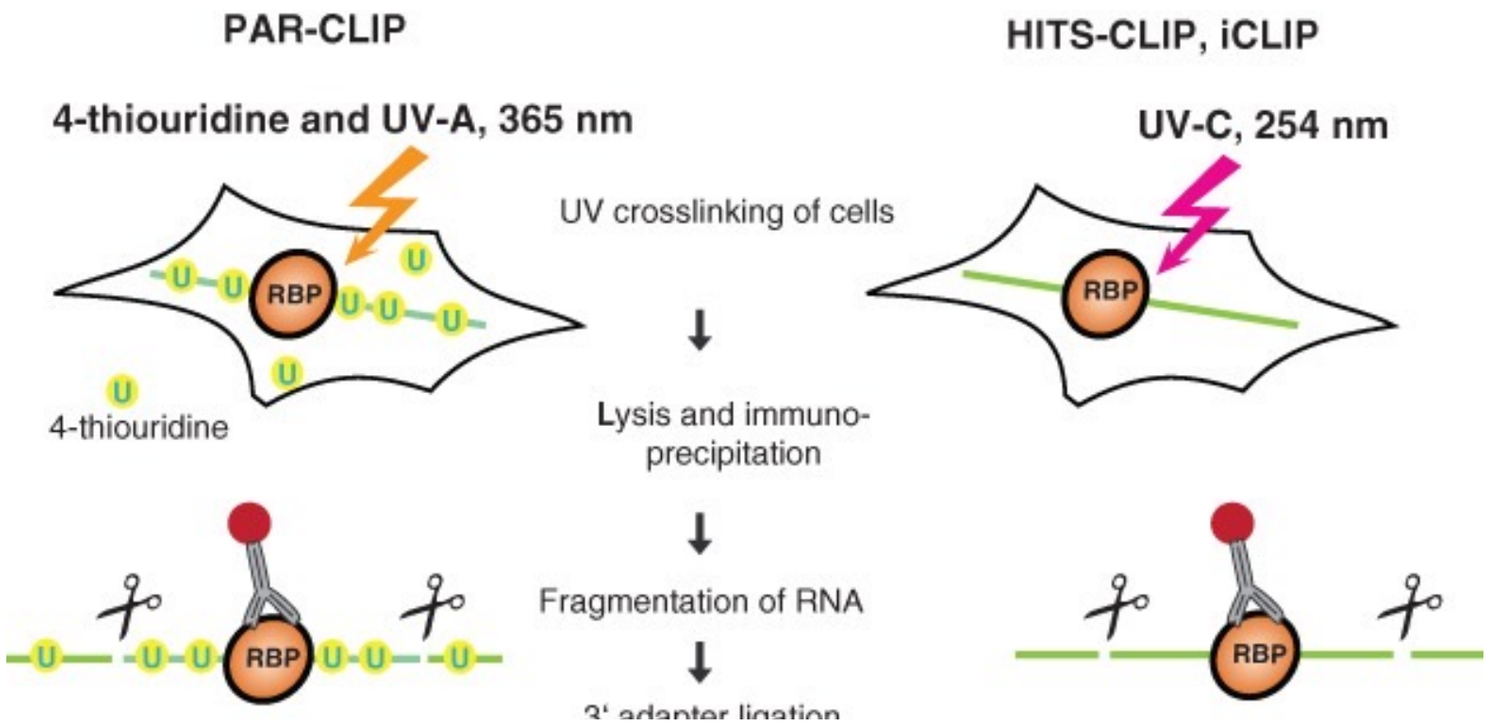
Library preparation and sequencing

qRT-PCR



Cipriano and Ballarino, FMB 2018

CLIP

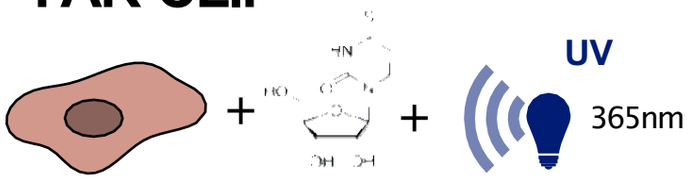

PAR-CLIP
4-thiouridine and UV-A, 365 nm
HITS-CLIP, iCLIP
UV-C, 254 nm
UV crosslinking of cells
RBP
4-thiouridine
Lysis and immuno-
precipitation
Fragmentation of RNA
3' adapter ligation

CLIP

(in vivo and cross-linked)

HITS-CLIP

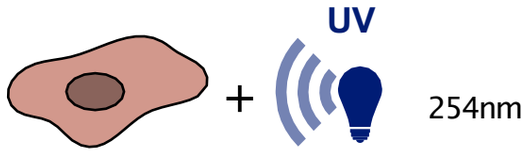
PAR-CLIP



① UV crosslinking

iCLIP/eCLIP

CRAC



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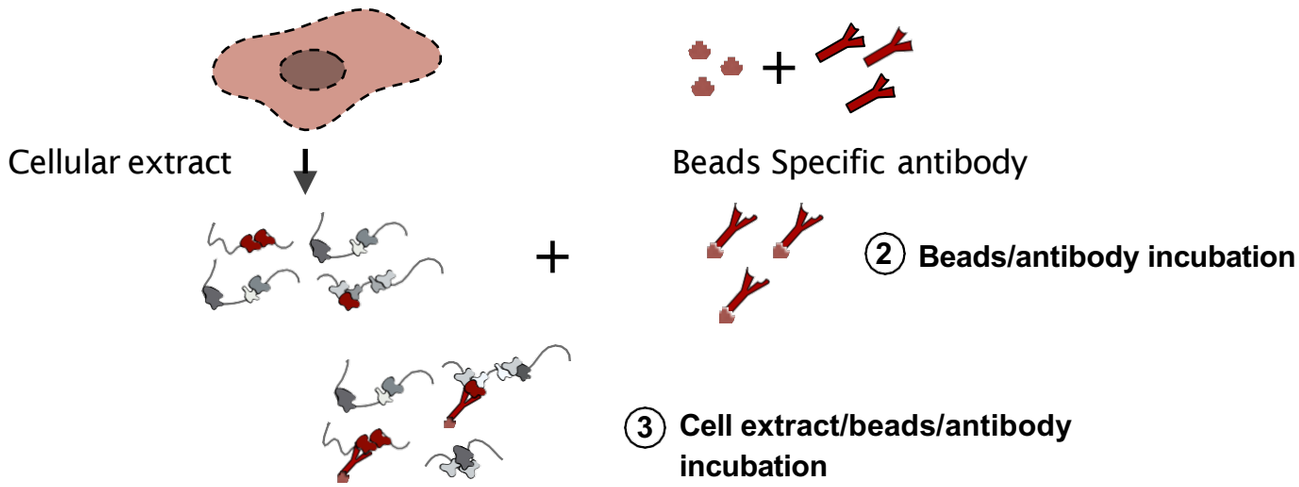

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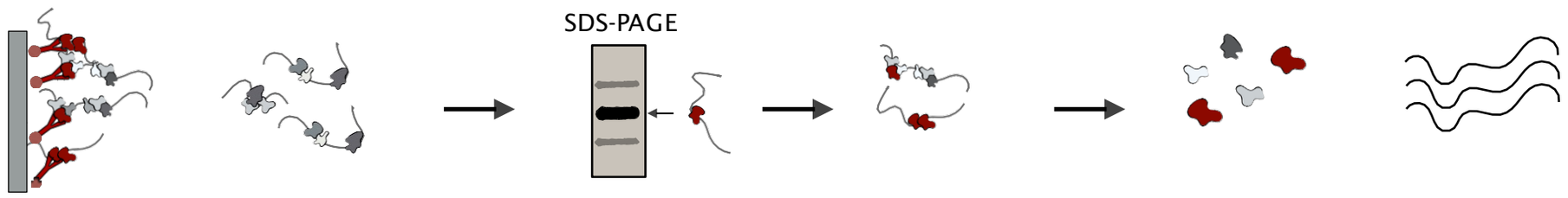

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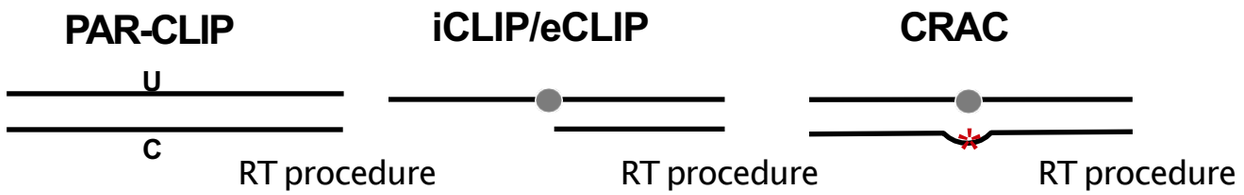
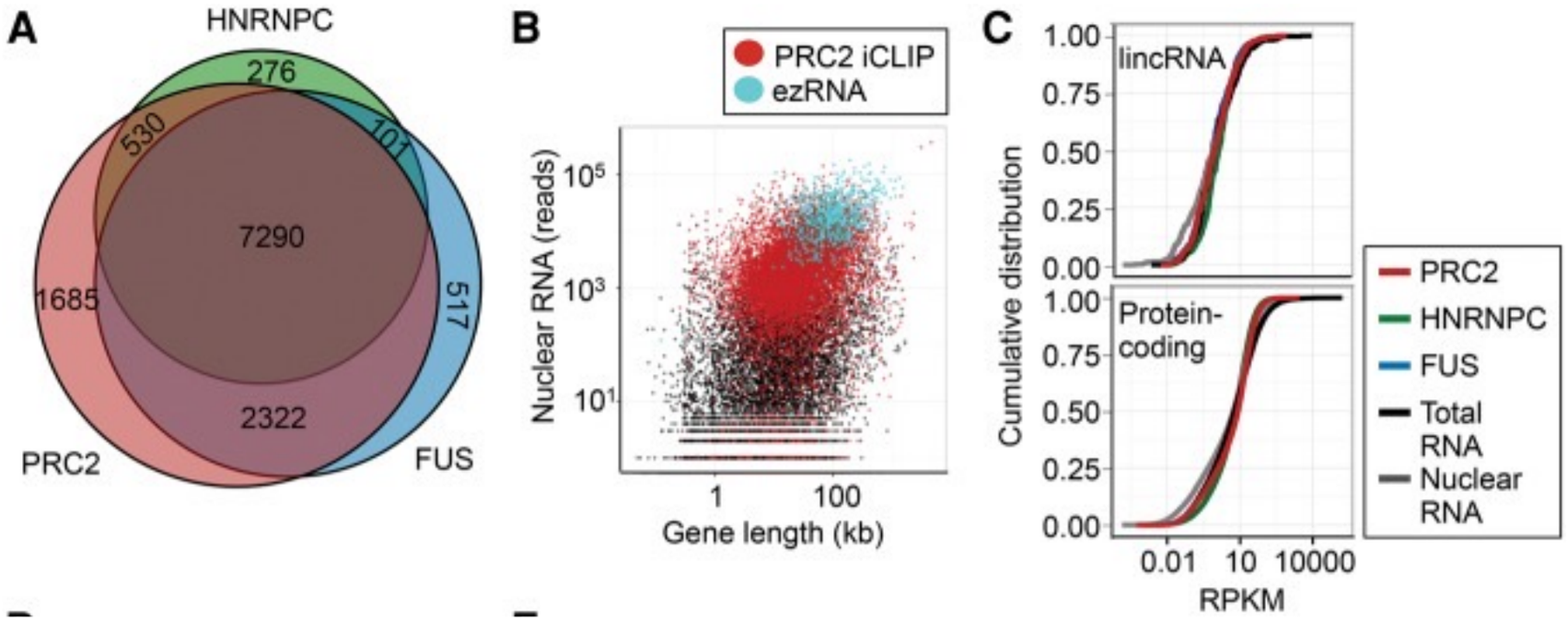
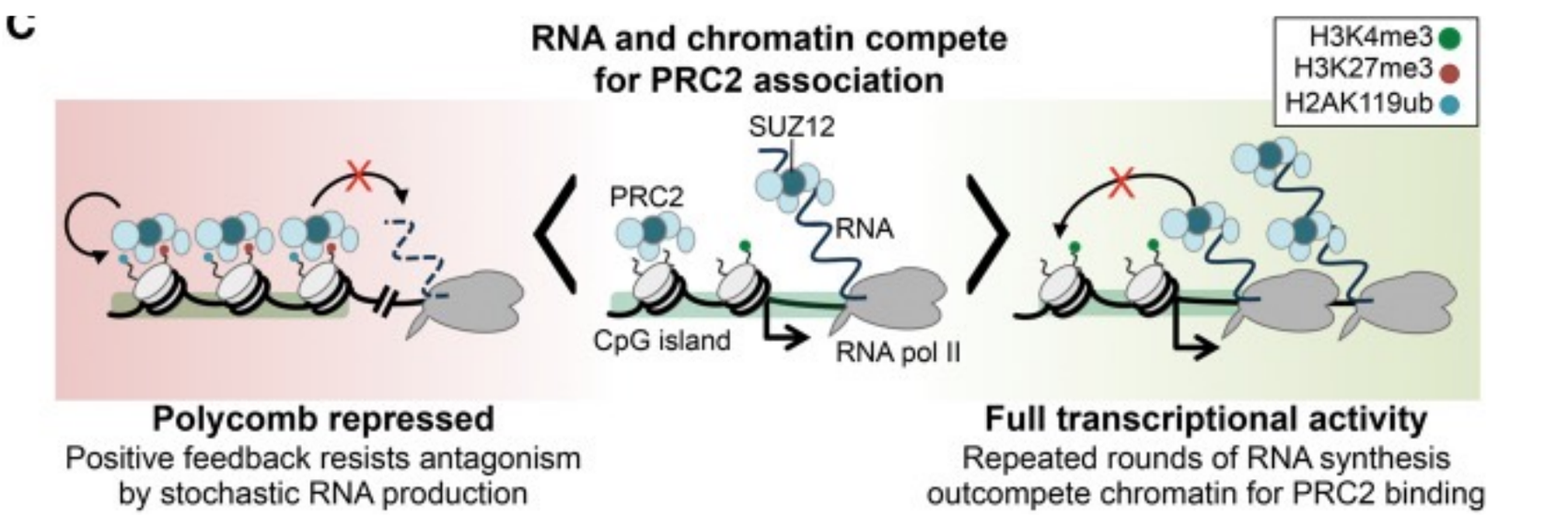


Cipriano and Ballarino, FMB 2018

CLIP example





Beltran et al 2015

Summary

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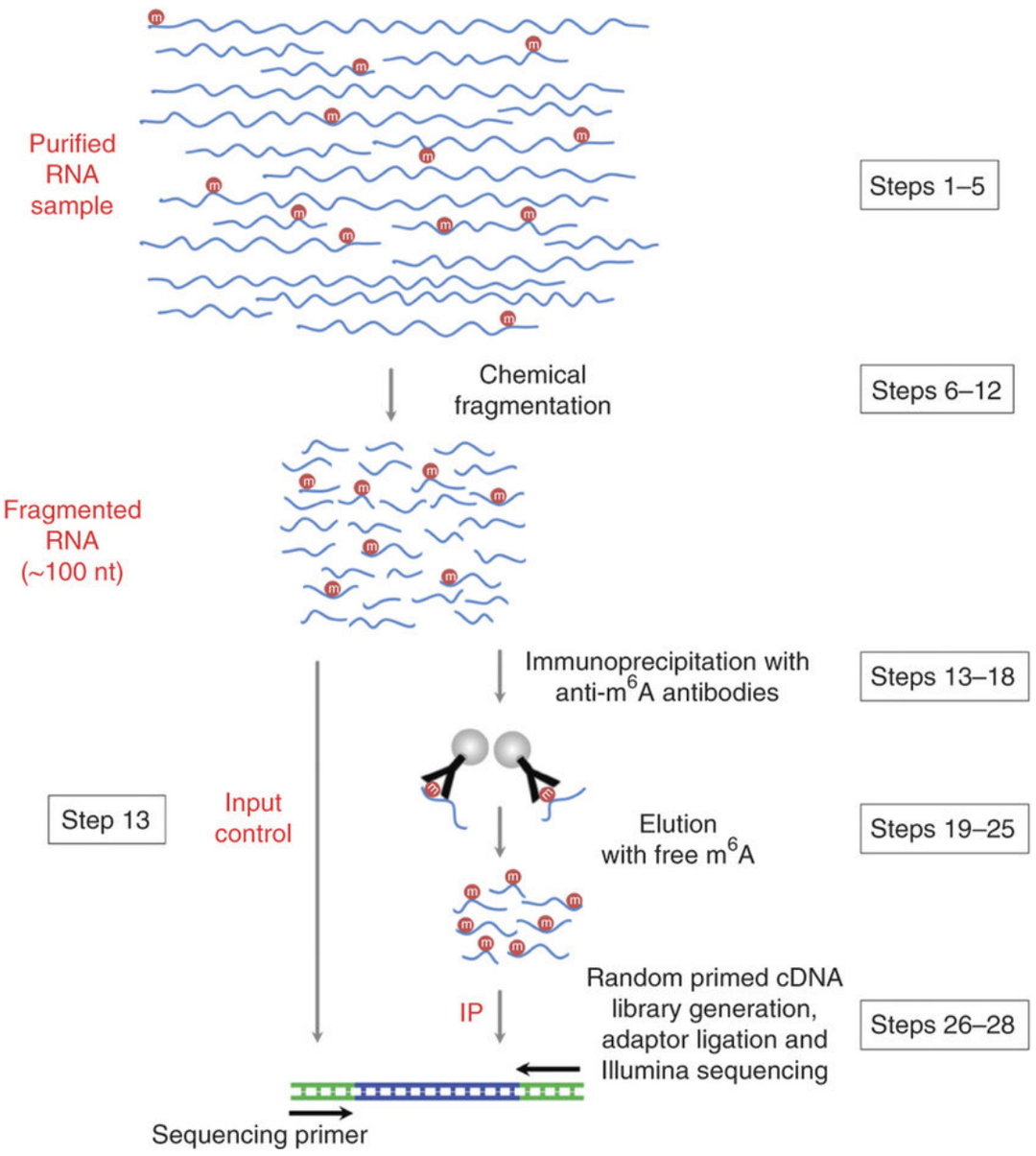
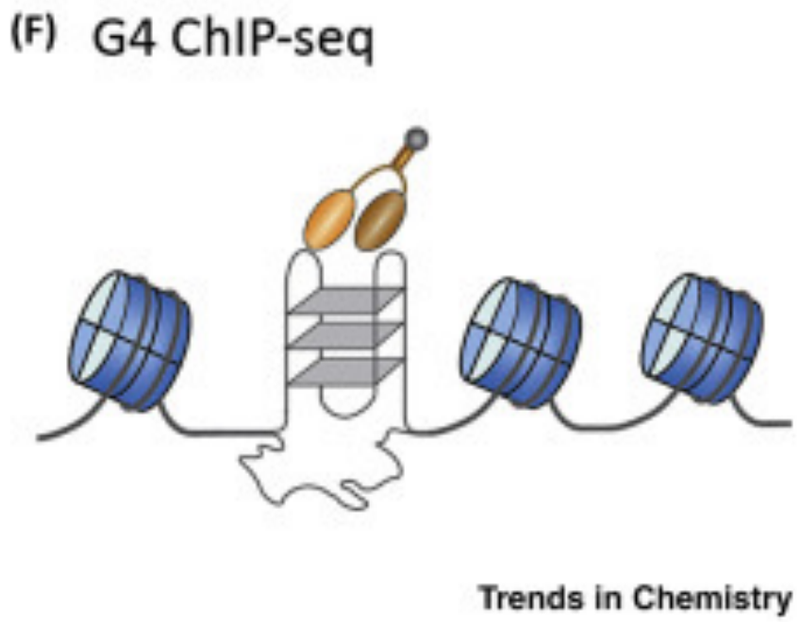
	DNA	**DNA pull down**		**Conformation capture 3C**

ALL of them are useful. But please make proper controls!

Bonus track: Antibodies against nucleic acids



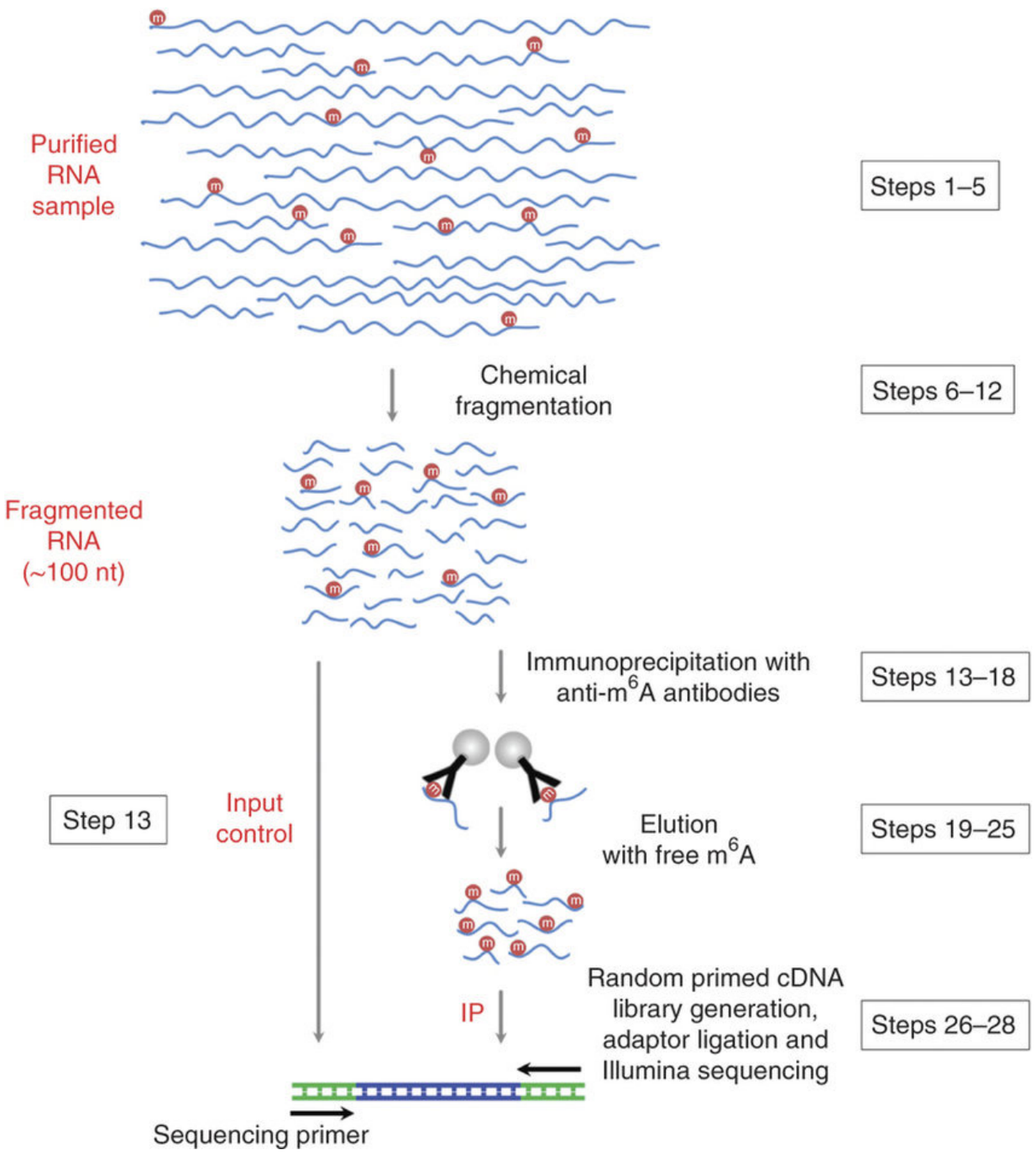


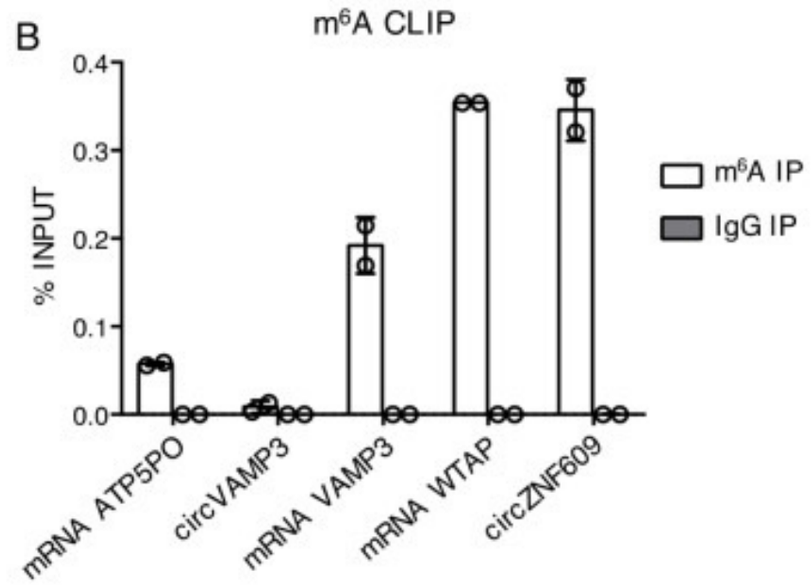
We also can detect modification on the RNA/ DNA structures using antibodies:

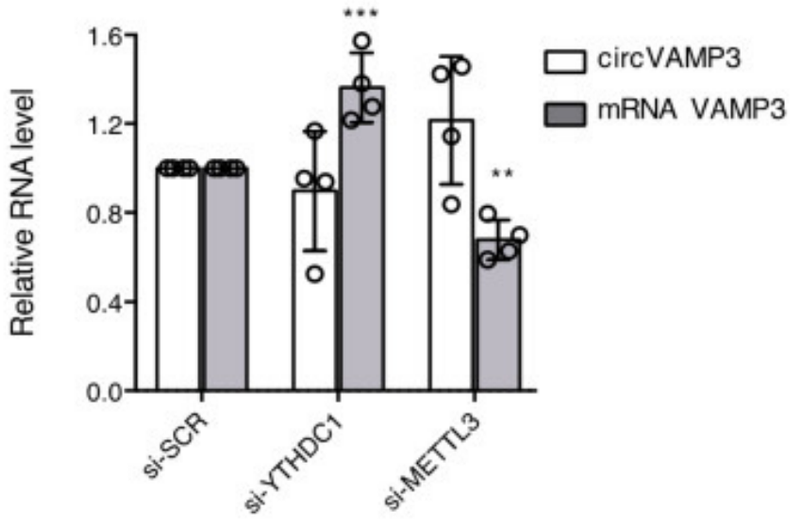
M6A RIP

G4- quadruplex CHIP

Bonus track: Antibodies against nucleic acids







References

SUMMARY

- Cipriano A and Ballarino M. "**The ever-evolving concept of the gene: the use of RNA/Protein experimental techniques to understand genome functions**". Front. Mol. Biosci 2018 Mar 6;5:20 doi:10.3389/fmolb.2018.00020.

COIP And ChiP

Healy et al., 2019, Molecular Cell76, 437–452November 7, 2019ª2019 Elsevier Inc.https://doi.org/10.1016/j.molcel.2019.08.012

CHiRP and RNA pulldown

- Ci Chu, Kun Qu, Franklin L. Zhong, Steven E. Artandi and Howard Y. Chang; **Genomic maps of lincRNA occupancy reveal principles of RNA-chromatin interactions**. Mol Cell *44*, 667–678, November 18, 2011.

- John L. Rinn, Michael Kertesz, Jordon K. Wang, Sharon L. Squazzo, Xiao Xu, Samantha A. Brugmann, L. Henry Goodnough, Jill A. Helms, Peggy J. Farnham, Eran Segal, and Howard Y. Chang; **Functional Demarcation of Active and Silent Chromatin Domains in Human HOX Loci by Non-Coding RNAs**. Cell *129*, 1311–1323, June 29, 2007.

- Colleen A. McHugh1, Chun-Kan Chen, Amy Chow, Christine F. Surka, Christina Tran, Patrick McDonel, Amy Pandya-Jones, Mario Blanco, Christina Burghard, Annie Moradian, Michael J. Sweredoski, Alexander A. Shishkin, Julia Su, Eric S. Lander, Sonja Hess, Kathrin Plath & Mitchell Guttman; **The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3.** *Nature* **521**, 232–236 May 2015

HITS-CLIP AND PAR-CLIP

- Zhen Wang, James Tollervey, Michael Briese, Daniel Turner, Jernej Ule; CLIP: **Construction of cDNA libraries for high-throughput sequencing from RNAs cross-linked to proteins *in vivo*** Methods 48 (2009) 287–293

- Julian König, Kathi Zarnack, Nicholas M. Luscombe and Jernej Ule; **Protein–RNA interactions: new genomic technologies and perspectives**. Nature Reviews Genetics 13, 77-83 February 2012.

- Colleen A McHugh, Pamela Russell and Mitchell Guttman; **Methods for comprehensive experimental identification of RNA-protein interactions.** *Genome Biology,* 15:**203** 2014

Exercise: Interactome in the web

Genome browser

https://genome.ucsc.edu/

https://genome.ucsc.edu/s/mbeltran/G401

STRING

https://string-db.org/