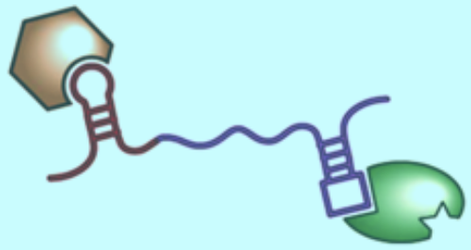
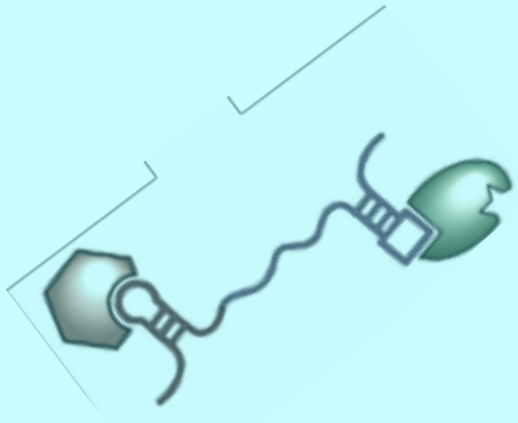
# THE INTERACTOME STUDY

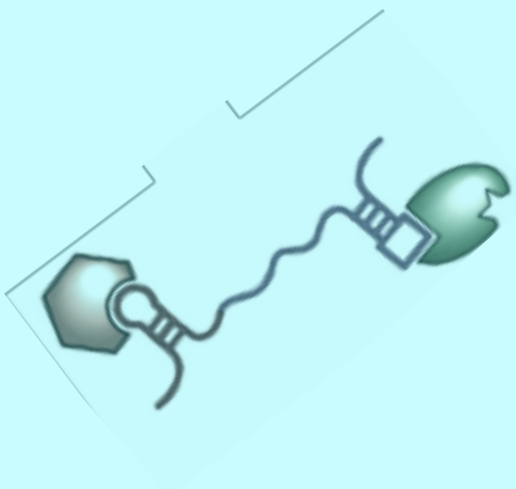
REGE

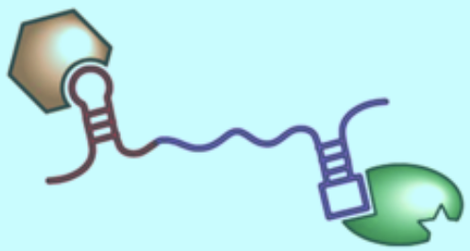
# Inside a cell...

Cytoplasm












Nucleus

# Molecular techniques for the study of the interaction between:

**DNA-protein:**

**ChIP** (Chromatin immunoprecipitation)

**RNA-DNA:**

**ChIRP** (Chromatin isolation by RNA purification)

**RNA-Protein (RNA centric):**

**Exogenous RNA pulldown,**

**Endogenous RNA pulldown**

**RAP (**RNA antisense purification)

**Protein-RNA (Protein centric):**

**RIP,** (RNA immunoprecipitation)

**CLIP**(Cross-linked immunoprecipitation)

# Molecular techniques for the study of the interaction between:

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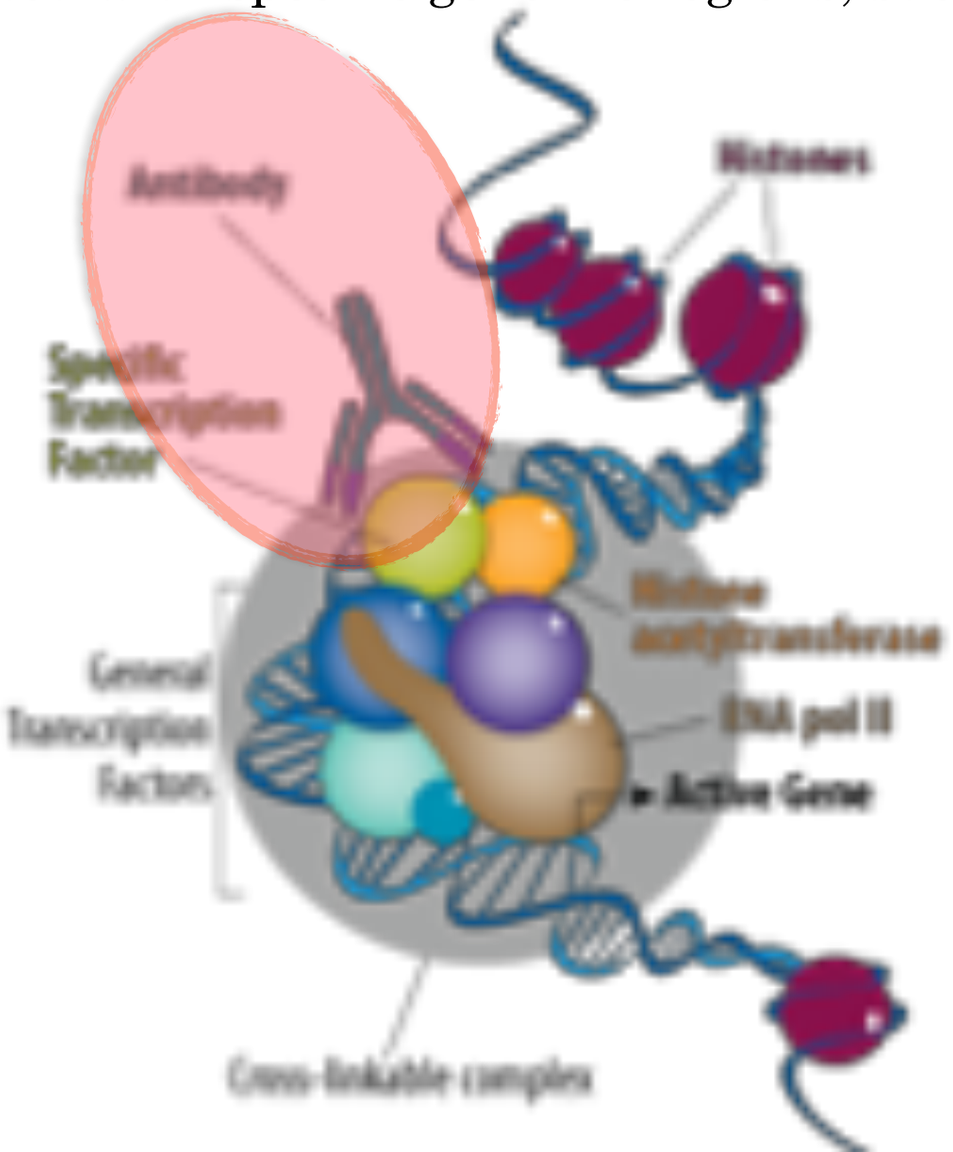
# ChIP

*(Chromatin Immunoprecipitation)*

## AIM: Identification of the genomic loci bound to a DNA binding protein.

**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.



# ChIP

*(Chromatin Immunoprecipitation)*

## What is an antibody?

An **antibody** also known as an **immunoglobulin** (Ig), is a large protein produced mainly by plasma cells (B linphocyte) and used by the immune system to identify and neutralize pathogens such as bacteria and viruses. The antibody recognizes a unique molecule of the pathogen, called **epitope (or antigene)**.

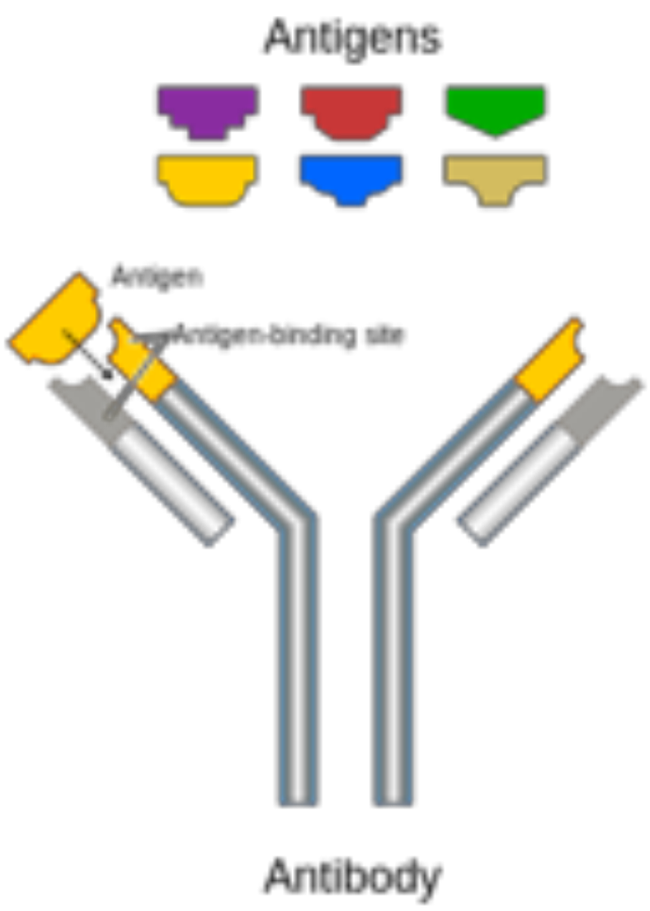
# ChIP

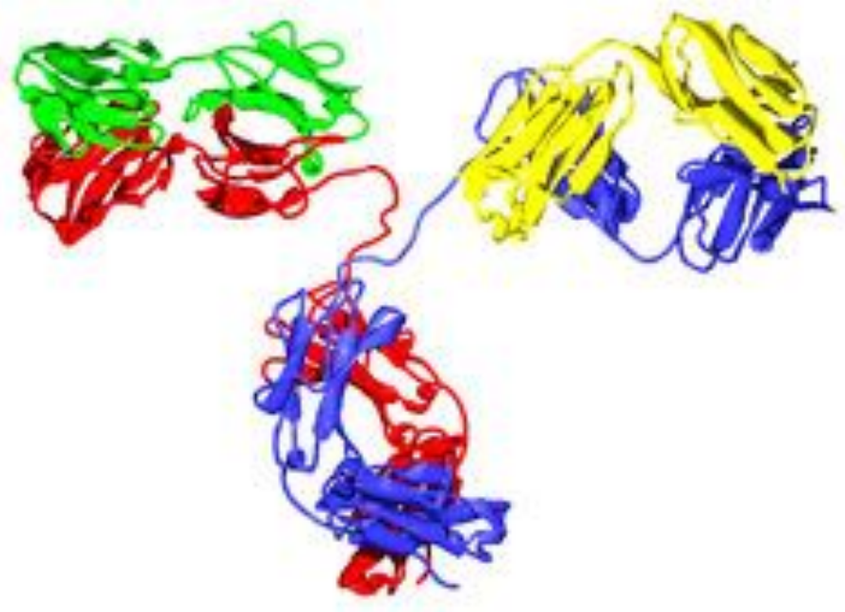
*(Chromatin Immunoprecipitation)*

## How an antibody works?

Each tip of the "Y" of an antibody contains a **paratope** (analogous to a lock) that is specific for one particular **epitope** (similarly analogous to a key), allowing these two structures to bind each other

Using this binding mechanism, an antibody is able to recognize and selectively bind a particular  protein with a very high affinity.



# ChIP

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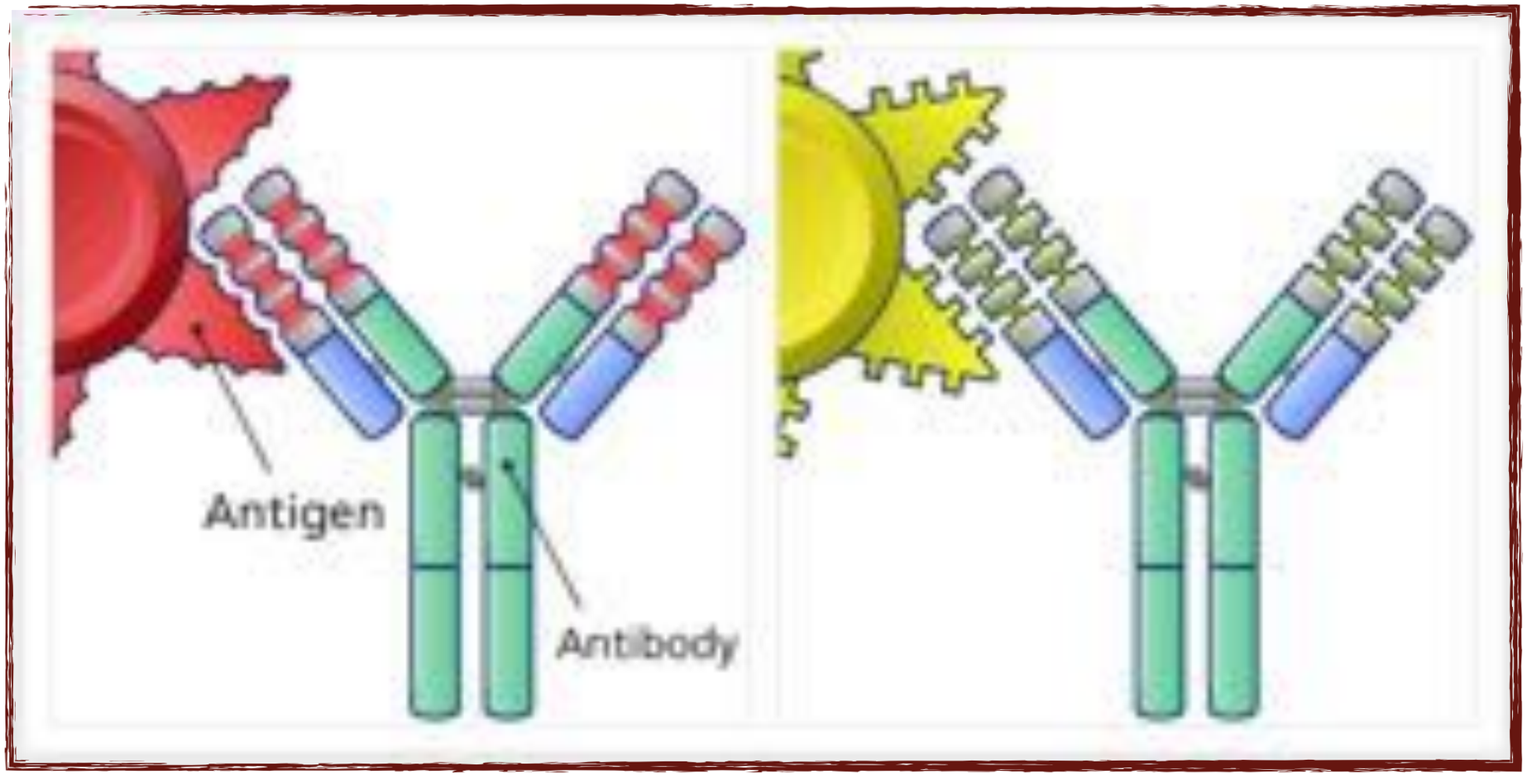
## How an antibody works?




**Antibody A** **Antibody B**

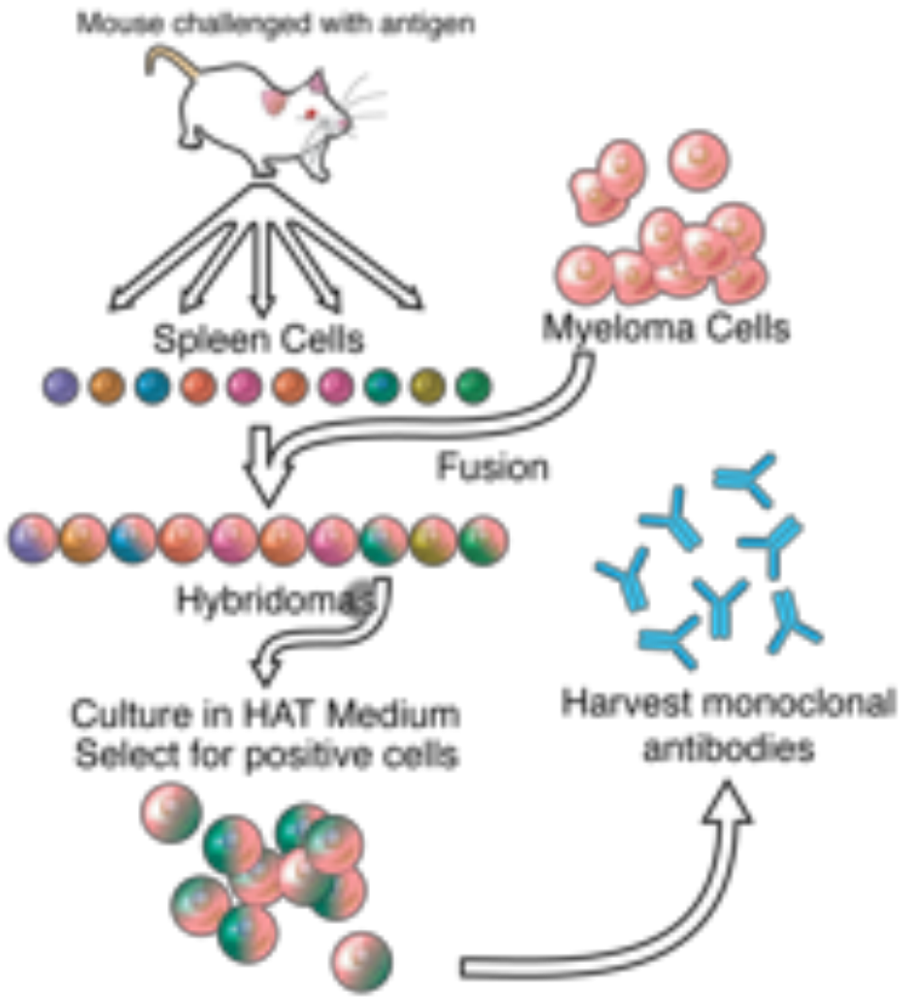
# ChIP

*(Chromatin Immunoprecipitation)*

## Polyclonal vs Monoclonal antibodies

***Polyclonal antibodies*** are produced by injecting a protein into a mammal, such as a **mouse**, **rat**, **rabbit**, **goat**, **sheep**, or **horse**. These animal will produce different antibodies against the same protein
Blood isolated from these animals contains antibodies that are able to recognize **different epitopes of the same protein.**

***Monoclonal antibodies*** are antibodies that are made by identical immune cells that are all clones of a unique parent cell, in contrast to polyclonal antibodies, which are made from several different immune cells. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope



# ChIP

*(Chromatin Immunoprecipitation)*

## Monoclonal vs Polyclonal antibodies

**Polyclonal antibody**


Regognize several epitopes

More experimental variability

**Monoclonal antibody**


Regognize a single epitope

Less experimental variability

# ChIP

*(Chromatin Immunoprecipitation)*

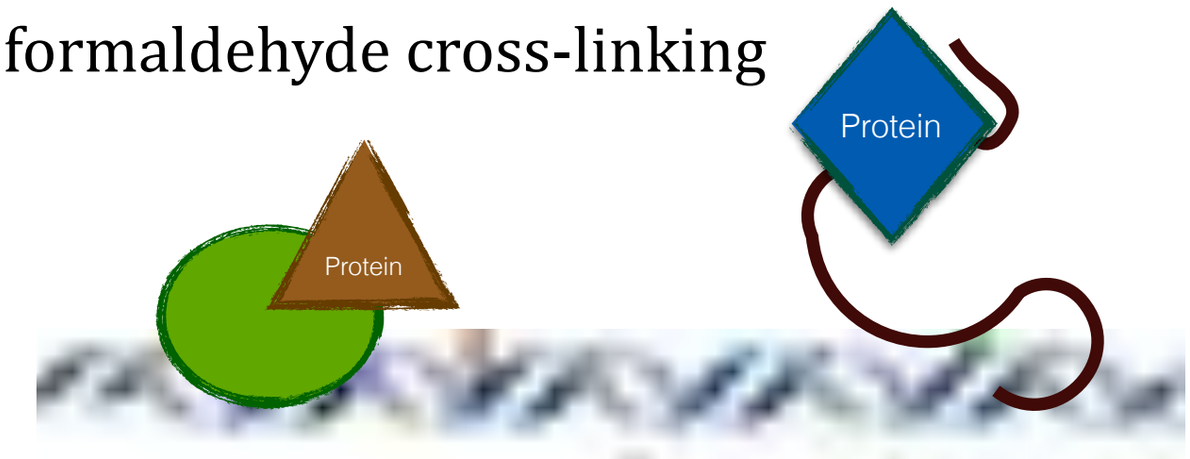
## Native versus Cross-linked ChIP

**Native-ChIP**: Native chromatin is used as substrate

- only proteins tightly associated with DNA can be immunoprecipitated
- antigens cannot be occurred or modified by chemical cross-inking
- the specificity of the antibody binding to unfixed chromatin is more predictable

**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 N-.-ChIP



# ChIP

*(Chromatin Immunoprecipitation)*

## WORK FLOW

**1. Cell Crosslinking**

**2. Chromatin Sonication**

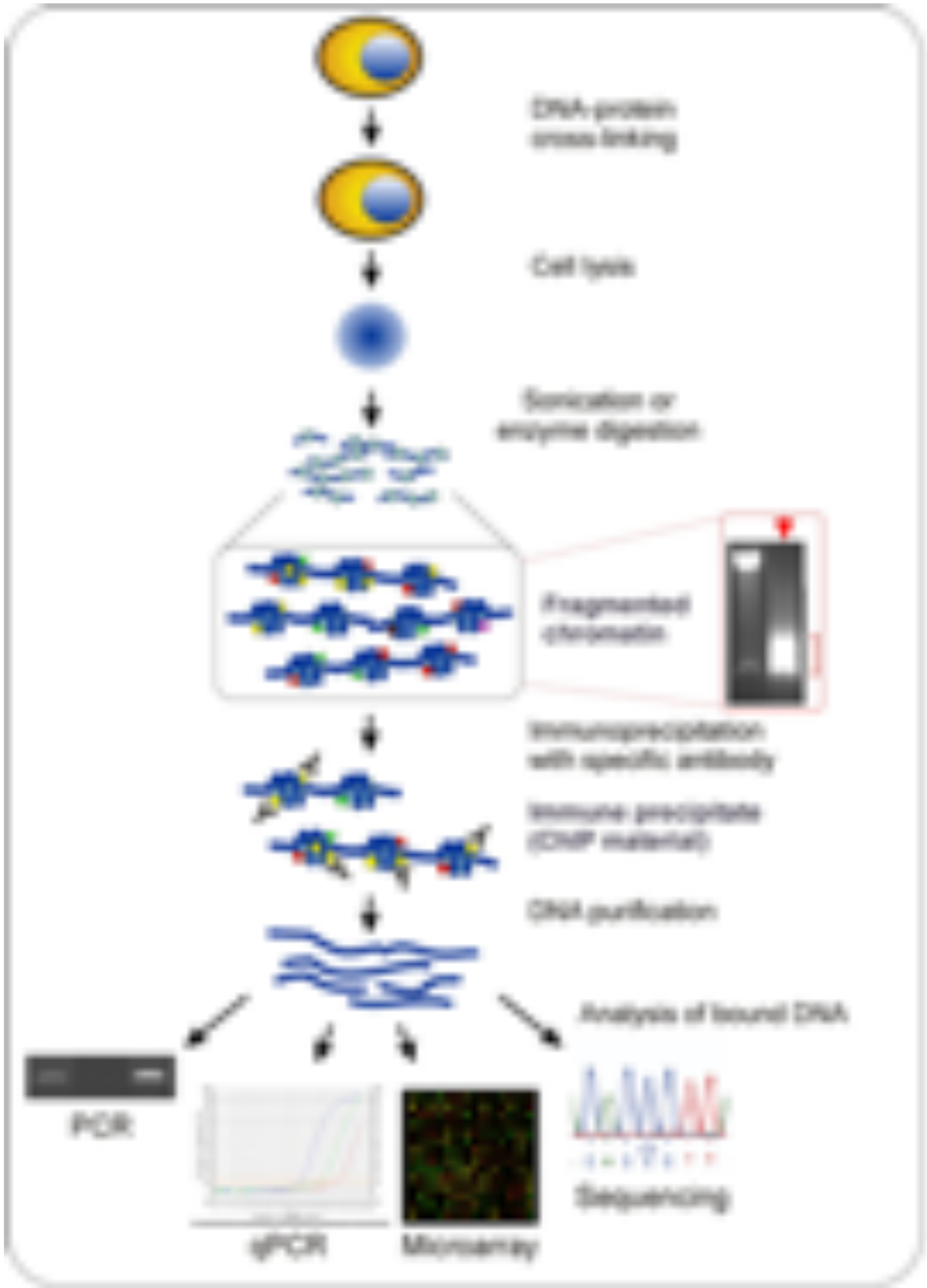
**4. Antibody -Extract incubation**

**5. Binding between Beads and Antibody**

**6. Immunoprecipitation**

**7. Reverse crosslink and DNA purification**

**7. Analysis of DNA**



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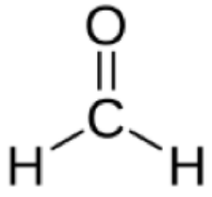
The cross-linking is an experimental procedure that convert in **covalent** all the the weak and non-covalent interactions between DNA - PROTEINS and PROTEIN-PROTEIN

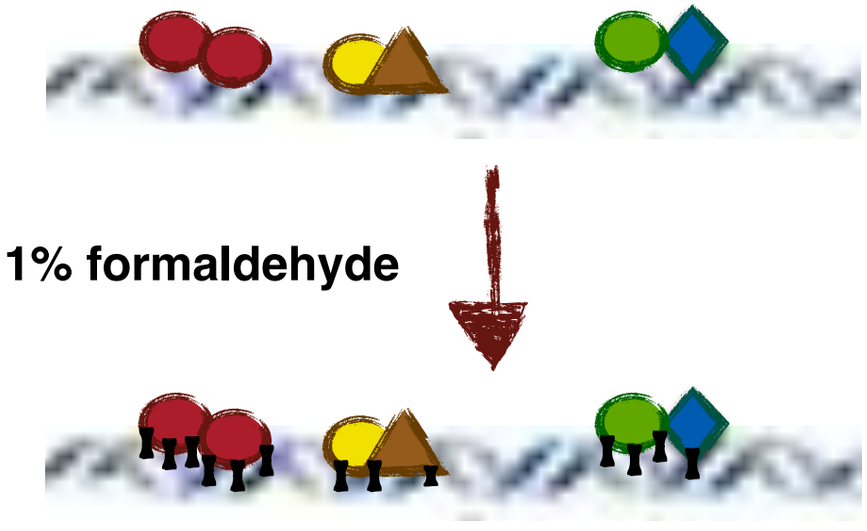
## Crosslinking strategies

UV-Crosslinking

**Formaldehyde**

Glutaraldehyde



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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)

# ChIP

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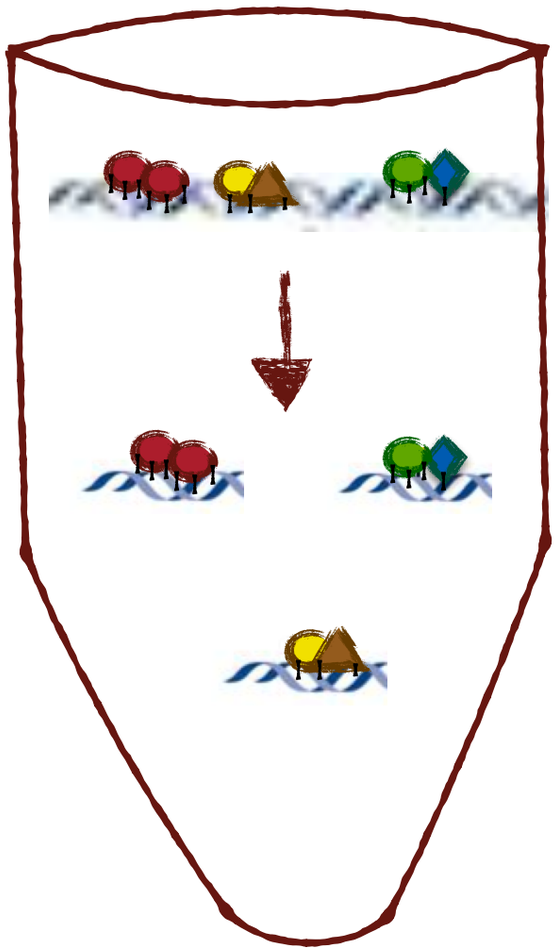
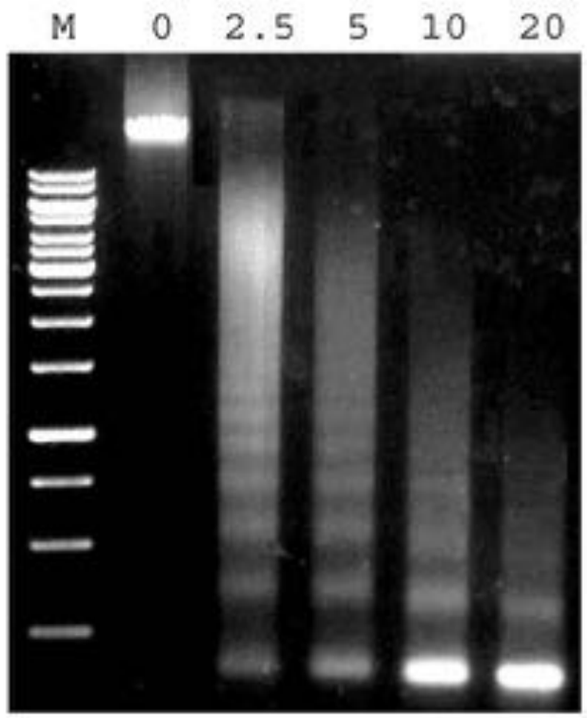
The DNA-protein complexes (chromatin-protein) are then sheared into ~500 bp DNA fragments by **sonication** or (nuclease digestion).






size range: 100-500 bp

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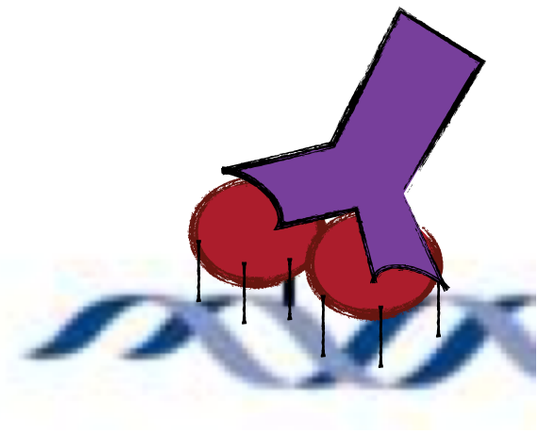
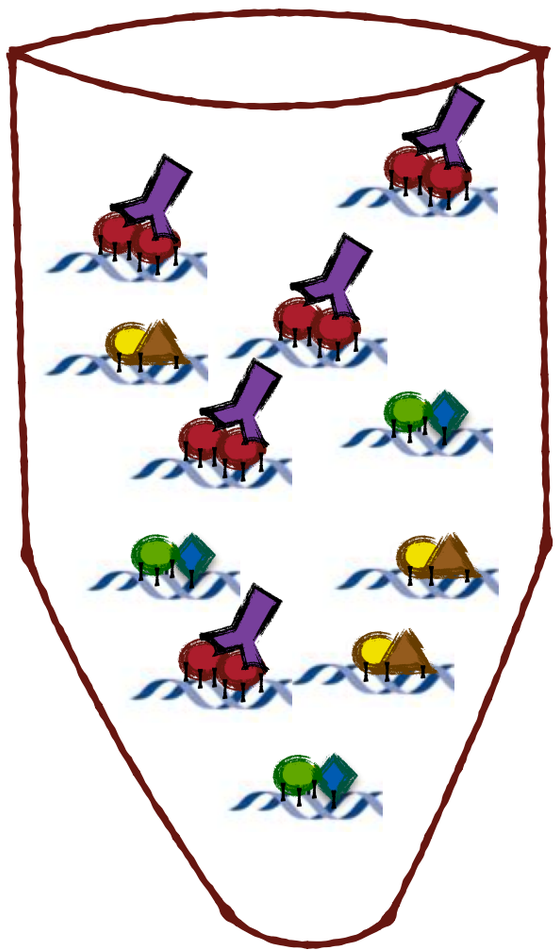
The antibody specific for the protein of interest, is incubated together with the extract, this step allows the formation of strongly interaction between the antibody and the protein of interest

## What is a good Negative controll?





**Incubation at 4°**
**with gentle agitation for 1 hr.**

# ChIP

*(Chromatin Immunoprecipitation)*

## WORK FLOW

1. Cell Crosslinking

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4. Antibody -Extract incubation

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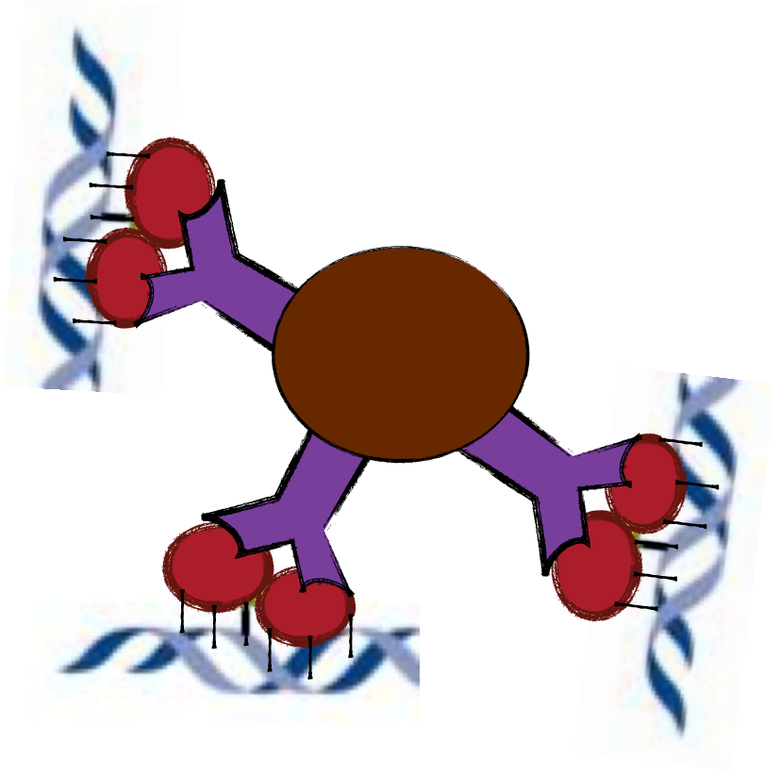
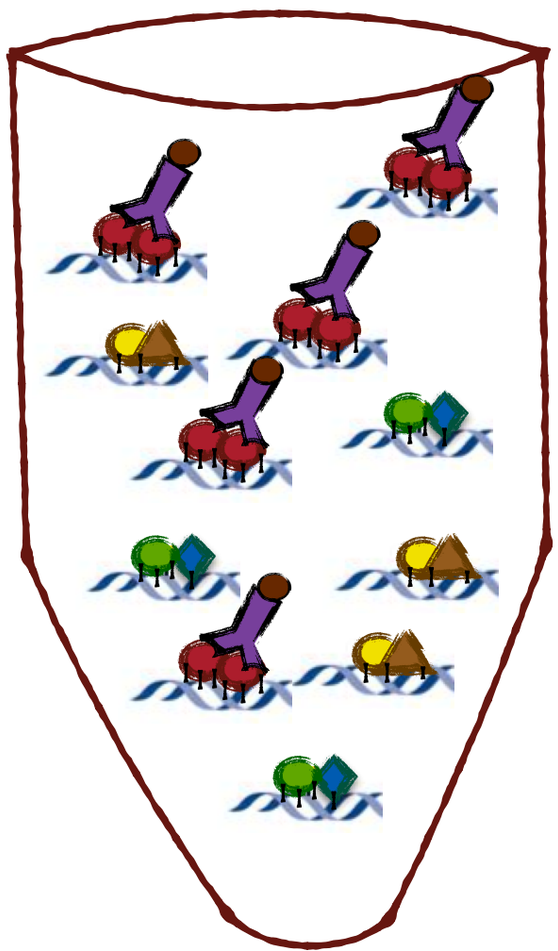
7. Analysis of DNA

Agarose or Magnetic beads (that are able to bind the antibody) are added to the extract.

This step is fundamental for the precipitation of the complex **Bead-Antibody-Protein-DNA**





# ChIP

*(Chromatin Immunoprecipitation)*

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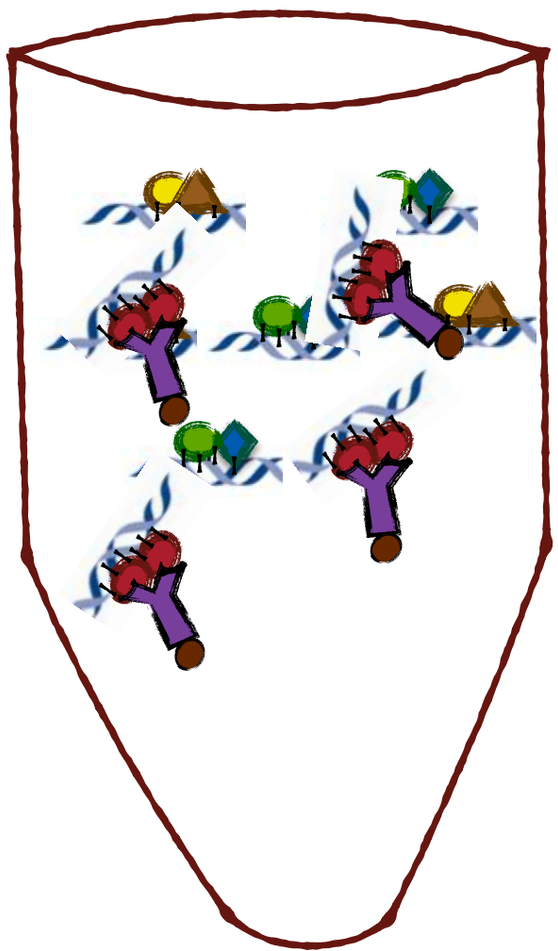
The immuniprecipitation step allows the purification of the complexes that are bound to the antibody

**Centrigugation (Agarose Beads)**
**Using a Magnetic Rec (Magnetic Beads)**



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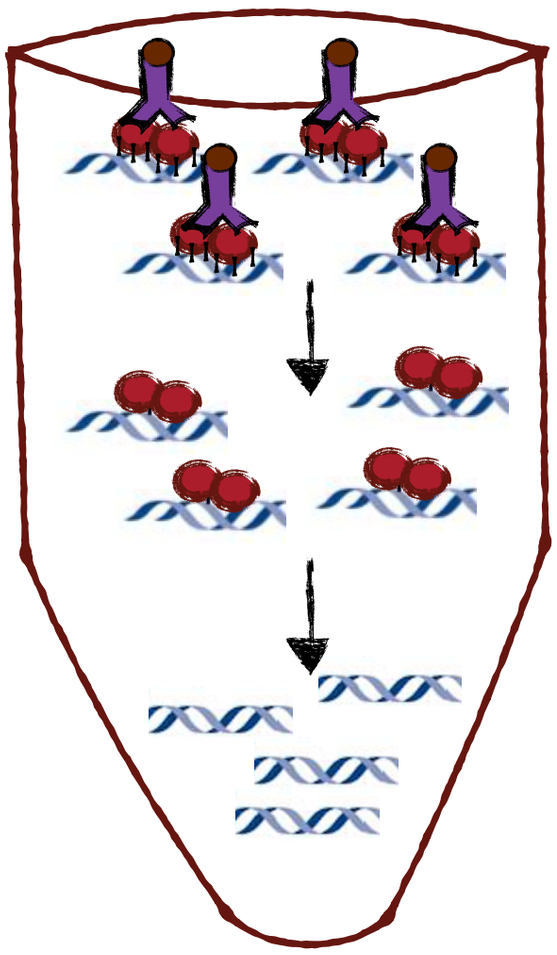
The cross-linking with formaldehyde is able to be removed through the incubation of the extract at High temperature. (65.C reverse protein-DNA; 100.C reverse protein-protein)

this step allows the detach of the protein from the DNA that is subsequently purified and analyzed

**incubation at 70 °C for 5 minutes**

Proteinase k treatment



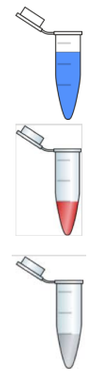
# ChIP

*(Chromatin Immunoprecipitation)*

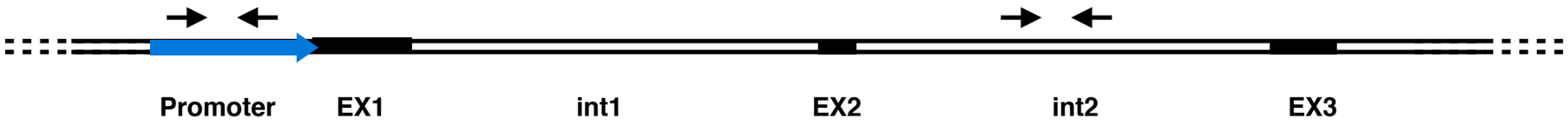
# DNA analysis

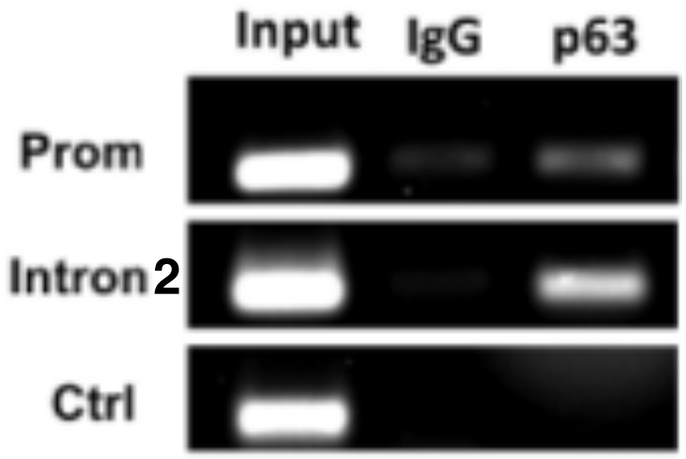
**ChIP (PCR)**

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



P63

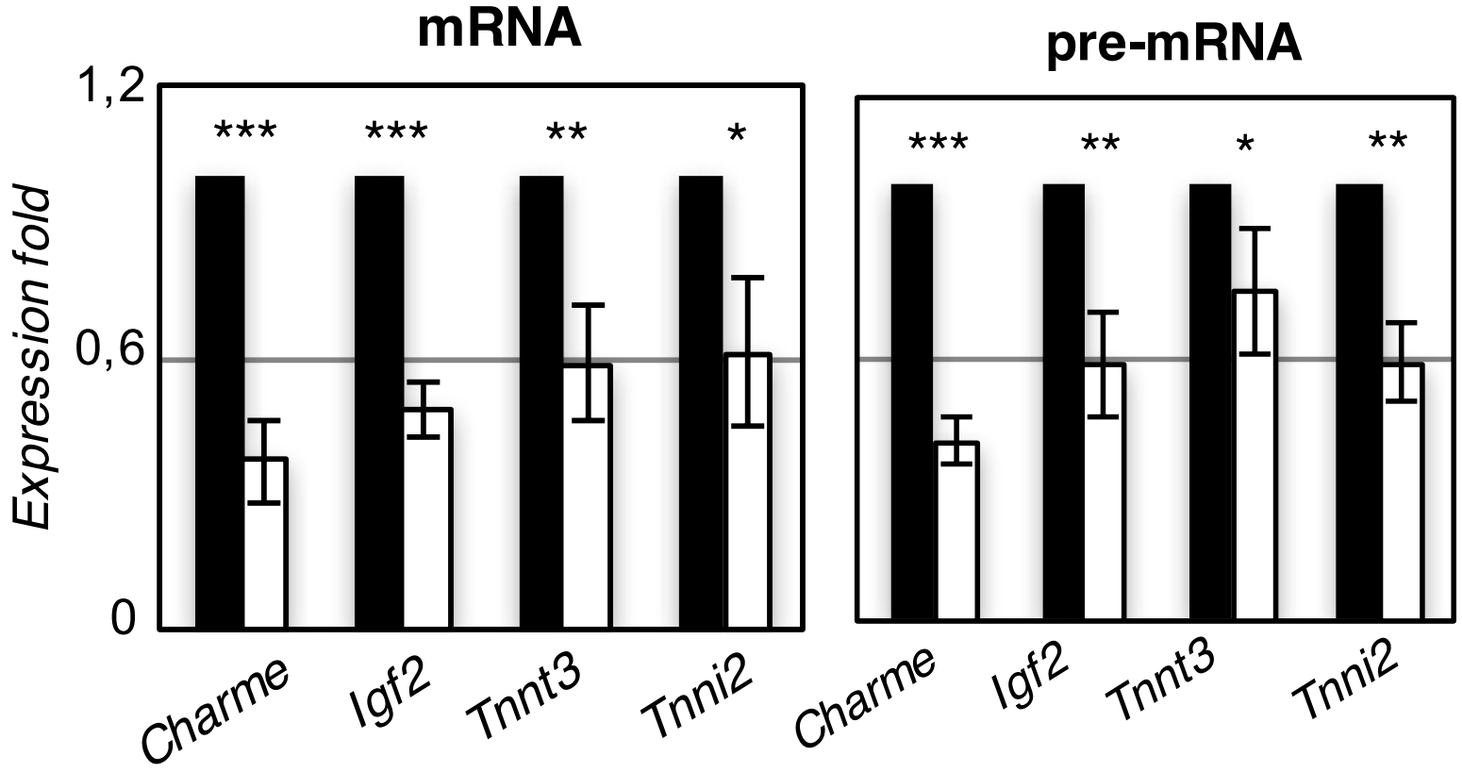


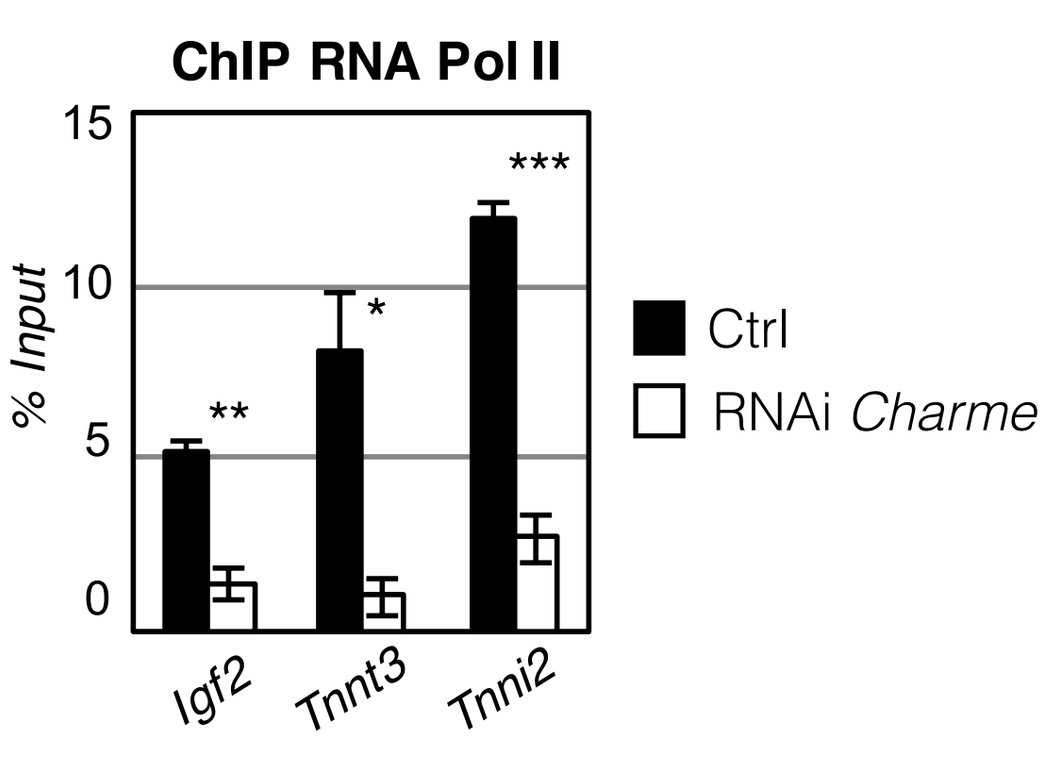
# ChIP

*(Chromatin Immunoprecipitation)*

## I want to verify if an effect on gene expression occurs at transcriptional level



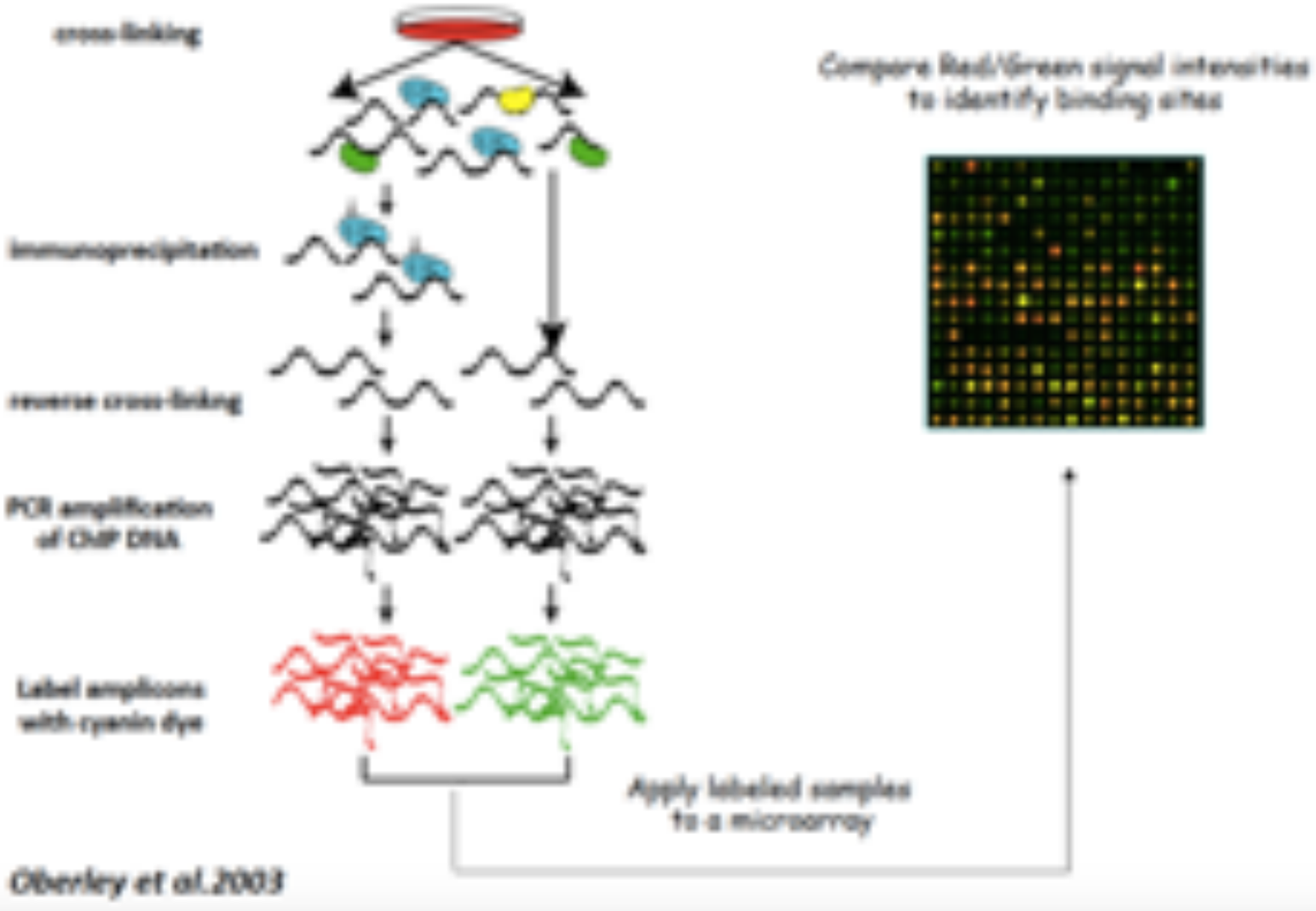

**rt-PCR**




**ChIP**

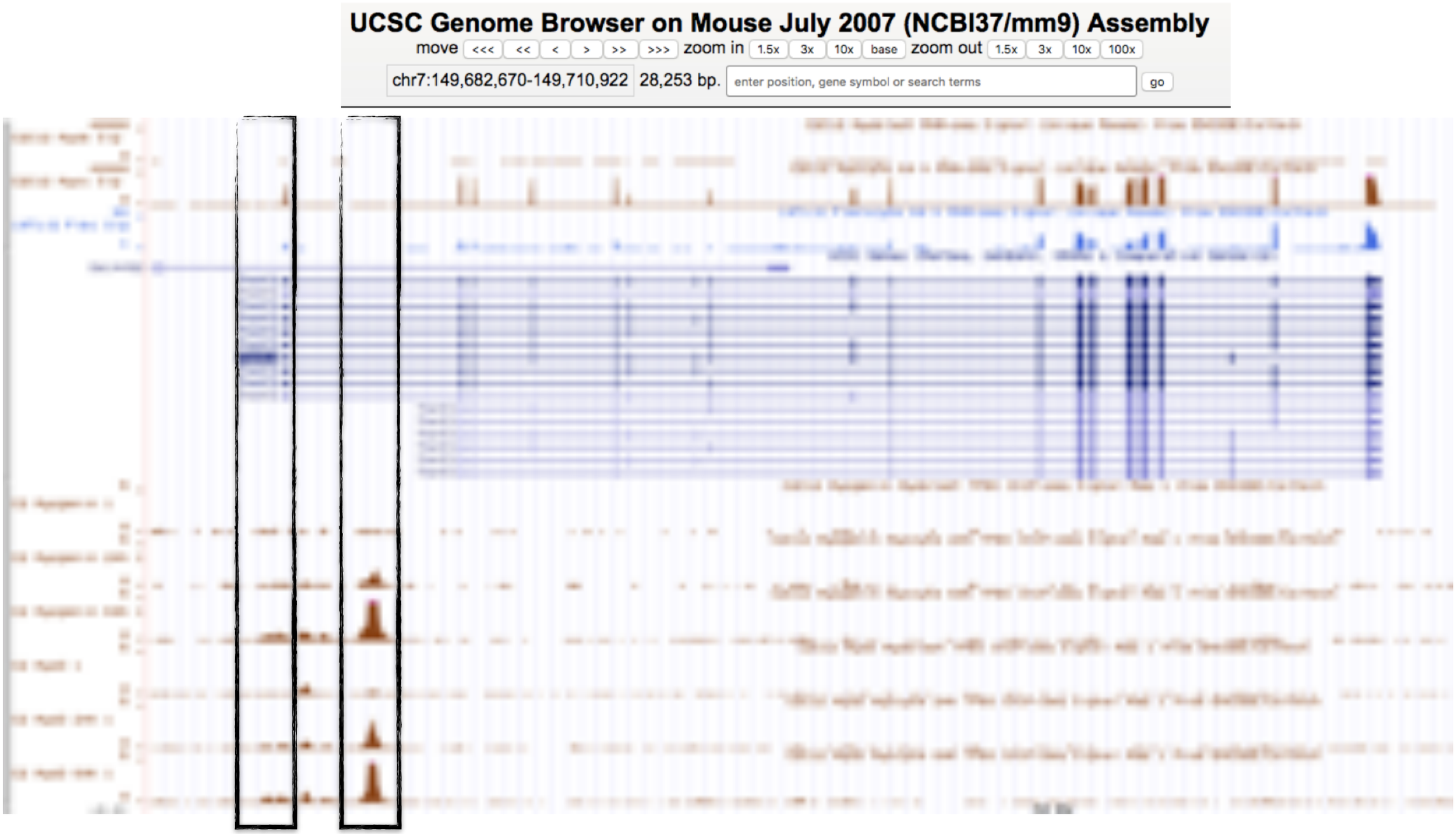
# ChIP on chip

Microarray technology allows the generation of high resolution genome-wide maps of protein/ protein modifications. DNA purified from the immunoprecipitated chromatin and input are labeled with fluorescent dyes using ligation mediated PCR.



# ChIP-seq

Direct sequencing of the DNA isolated, generates genome wide profiles. ChIP-seq combines ChIP and direct sequencing technology for genome-wide analysis of antigen distribution. Immunoprecipitated DNA is sequenced and mapped to the genome



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**Protein-RNA (Protein centric):**
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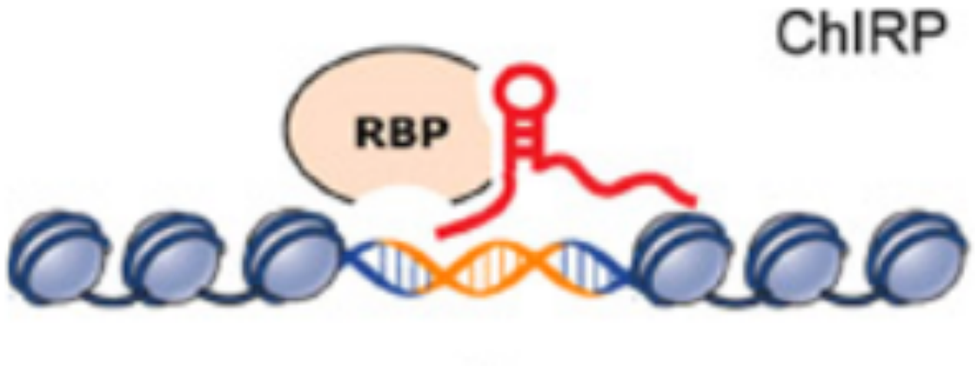
**CLIP**(Cross-linked immunoprecipitation)

# ChIRP

*(Chromatin Isolation by Rna Purification)*

**AIM: Identification of the genomic binding sites of long noncoding RNAs.**




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

# ChIRP

*(Chromatin Isolation by Rna Purification)*

## WORK FLOW

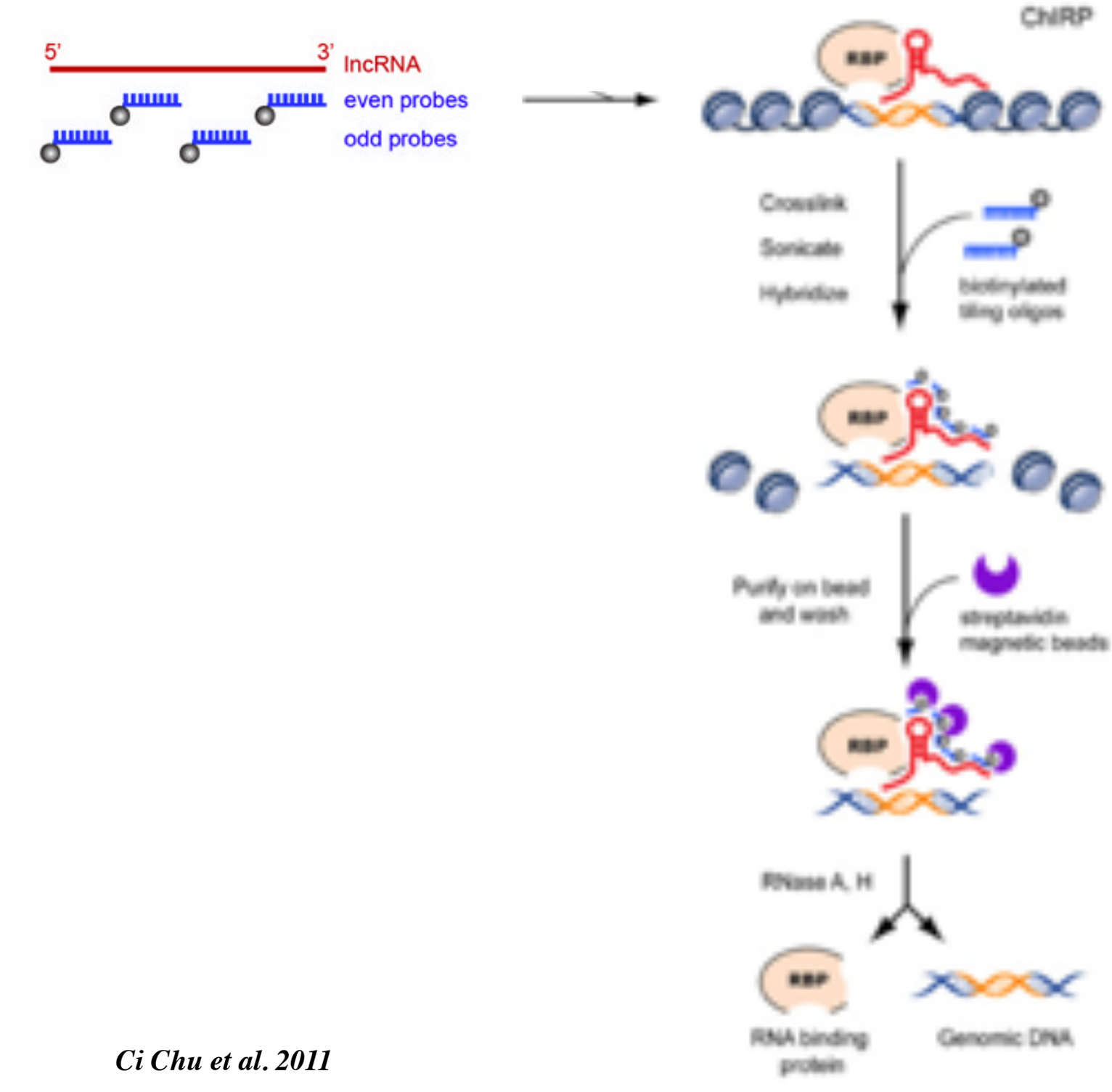
**1. Probes Design**

**2. Cross-link Cells and Collect Cell Pellet**

**3. Chromatin Sonication**

**4. Probes Hybridization and precipitation**

**5. PROTEIN and DNA Isolation**




*Ci Chu et al. 2011*

# ChIRP

*(Chromatin Isolation by Rna Purification)*

**1.Probe Design**

**2. Cross-linking**

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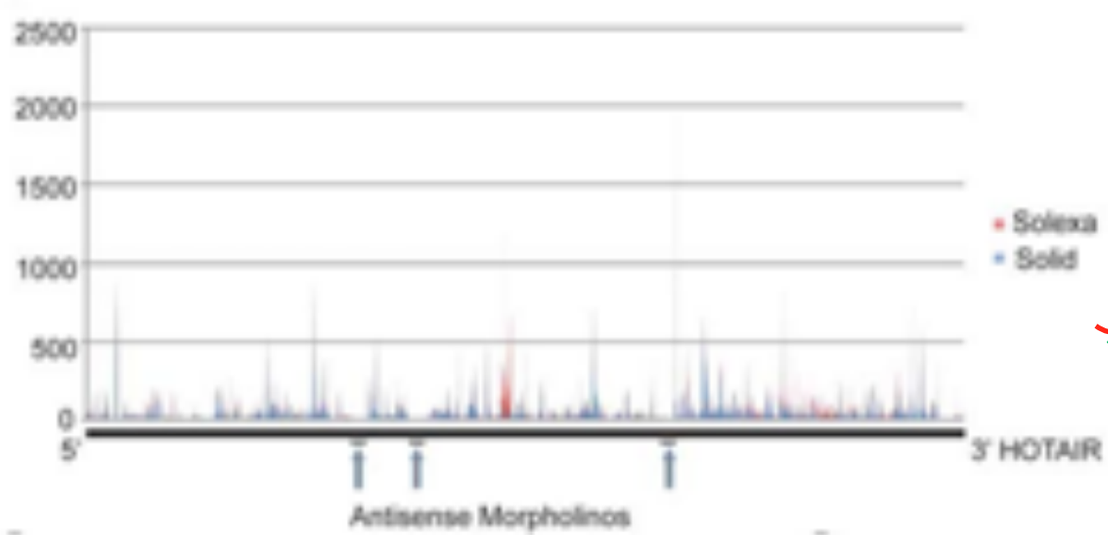
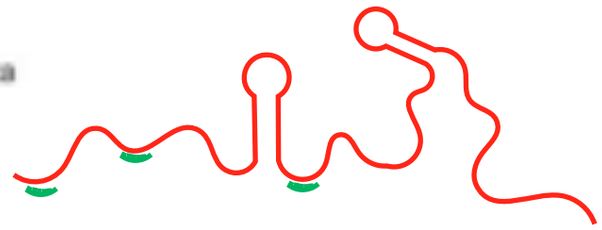
**4. Probes Hybridization and precipitation**

**5. RNA and DNA Isolation**

## SPECIFIC DESIGN

*HOTAIR ChIRP*

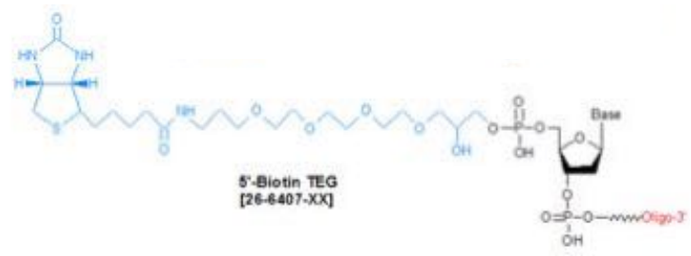


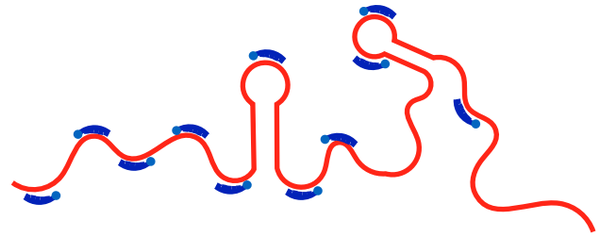



Antisense morpholinos are designed against **structurally open regions** of HOTAIR RNA. Peaks denote secondary structures previously determined by Parallel Analysis of RNA Structure (PARS) (Kertesz et al., 2010).

***Ci Chu et al. 2011***

## RANDOM DESIGN

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

# ChIRP

*(Chromatin Isolation by Rna Purification)*

*HOTAIR ChIRP*

## SPECIFIC VS RANDOM APPROACH

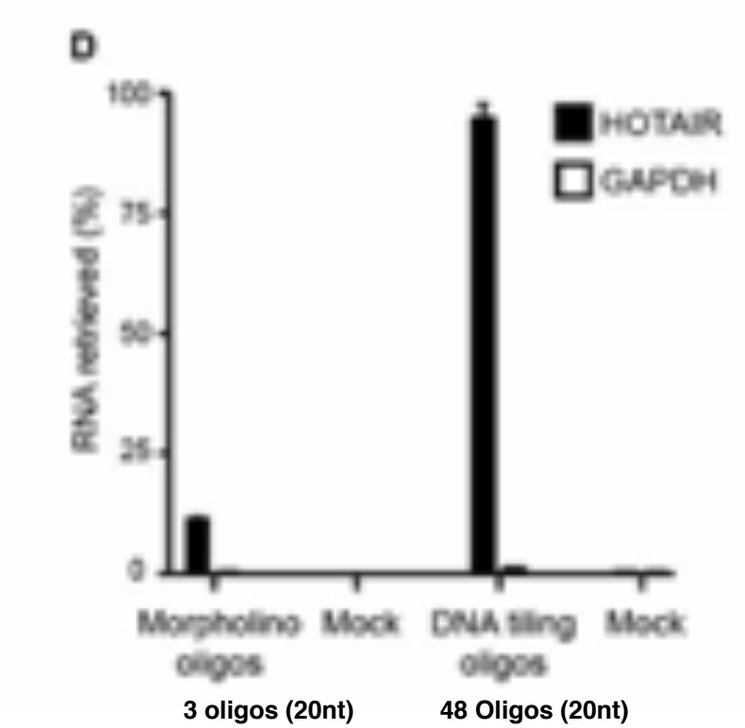
**1.Probe Design**

2. Cross-linking

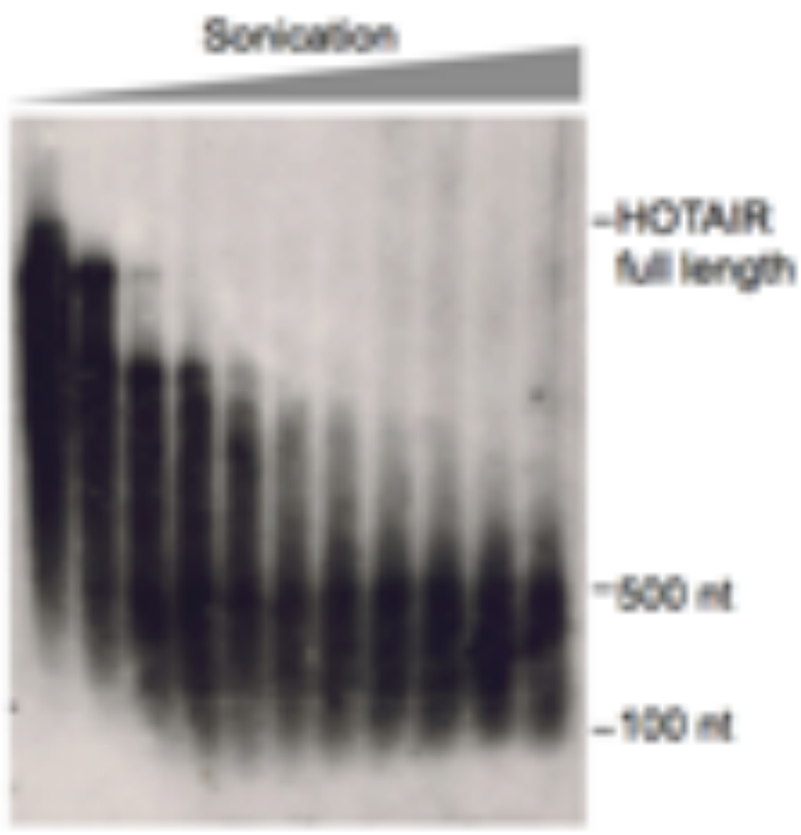
3. Chromatin Sonication

4. Probes Hybridization and precipitation

5. RNA and DNA Isolation




3 oligos (20nt) 48 Oligos (20nt)




Northen Blot

**More probes you have, better is**

*Ci Chu et al. 2011*

# ChIRP

*(Chromatin Isolation by Rna Purification)*

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## 2. Cross-linking

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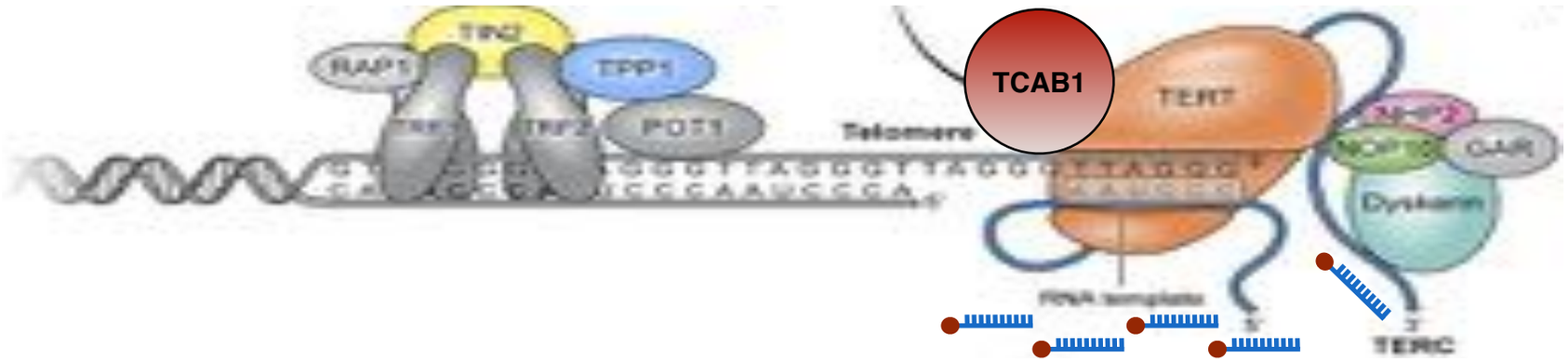
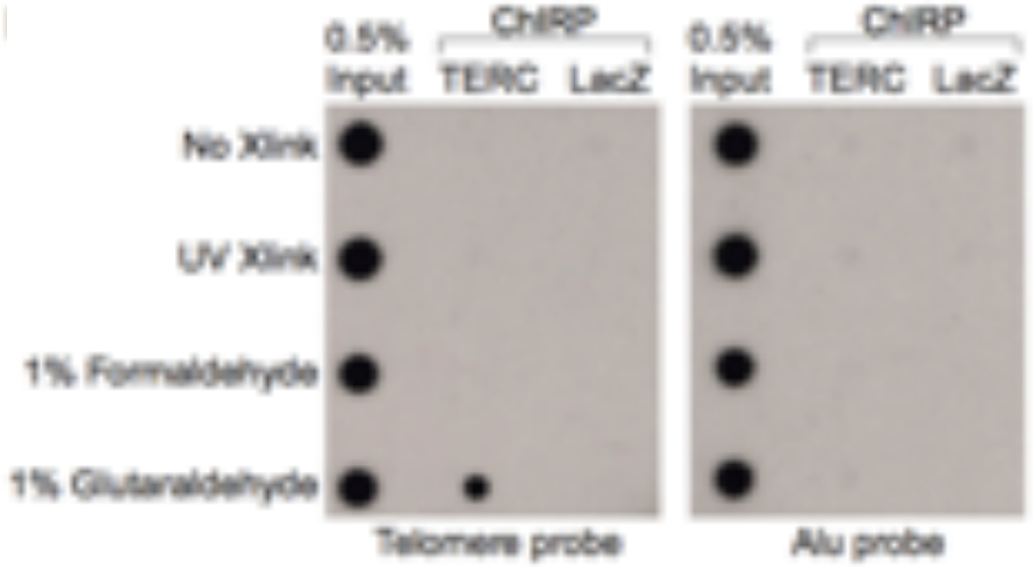
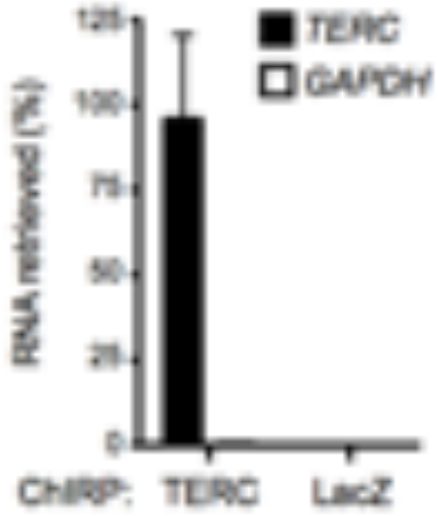


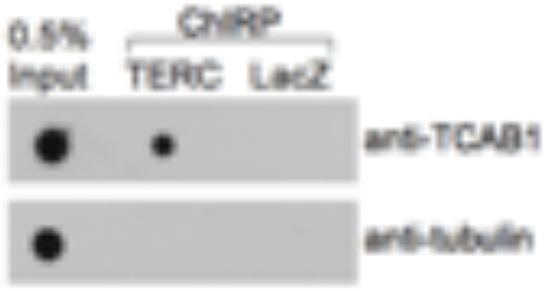

**Southern Blot**




**RT-PCR**




**DOT BLOT**




**1% of glutaraldehyde**

*Ci Chu et al. 2011*

# ChIRP

*(Chromatin Isolation by Rna Purification)*

1. Probe Design

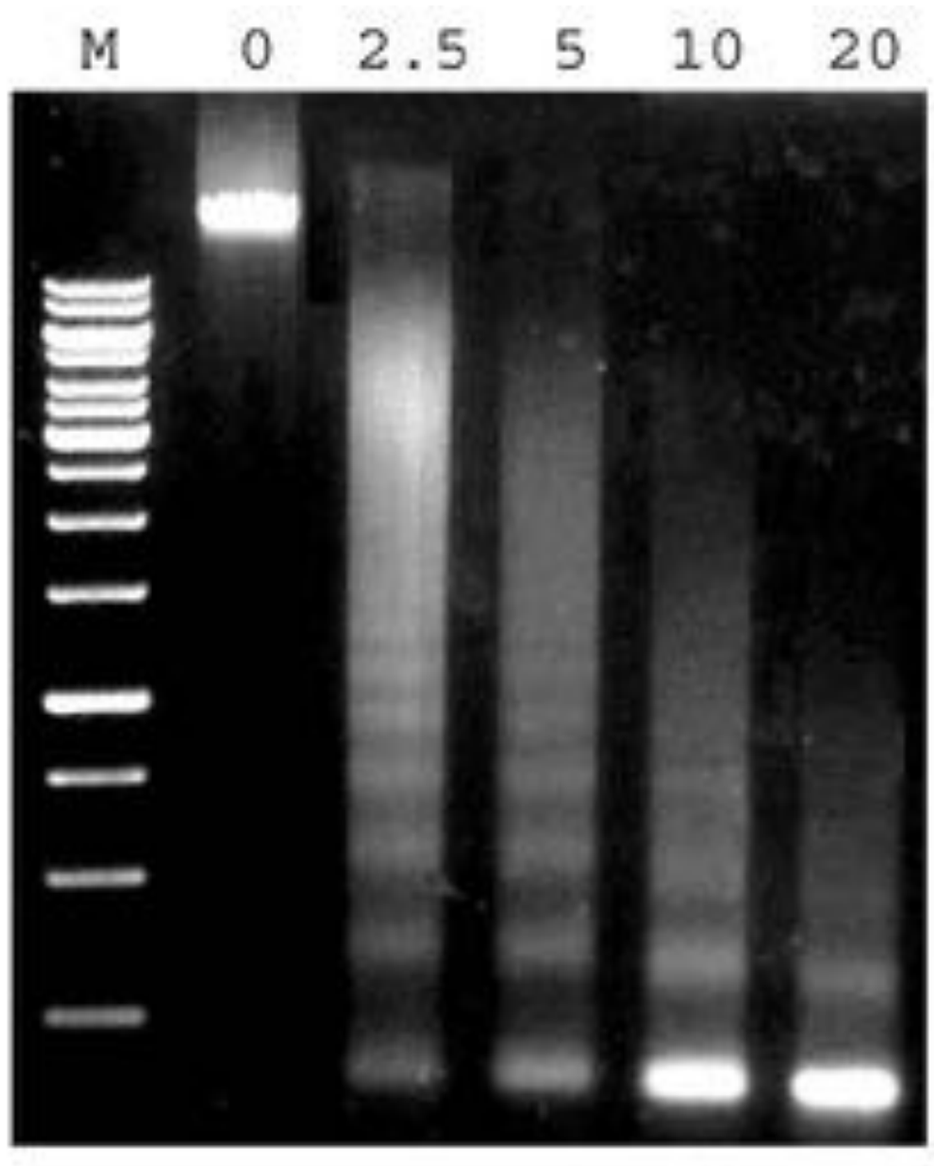
2. 2. Cross-linking

**3. Chromatin Sonication**

4. Probes Hybridization and precipitation

5. RNA and DNA Isolation

## Bioruptor




size range: 100-500 bp

# ChIRP

*(Chromatin Isolation by Rna Purification)*

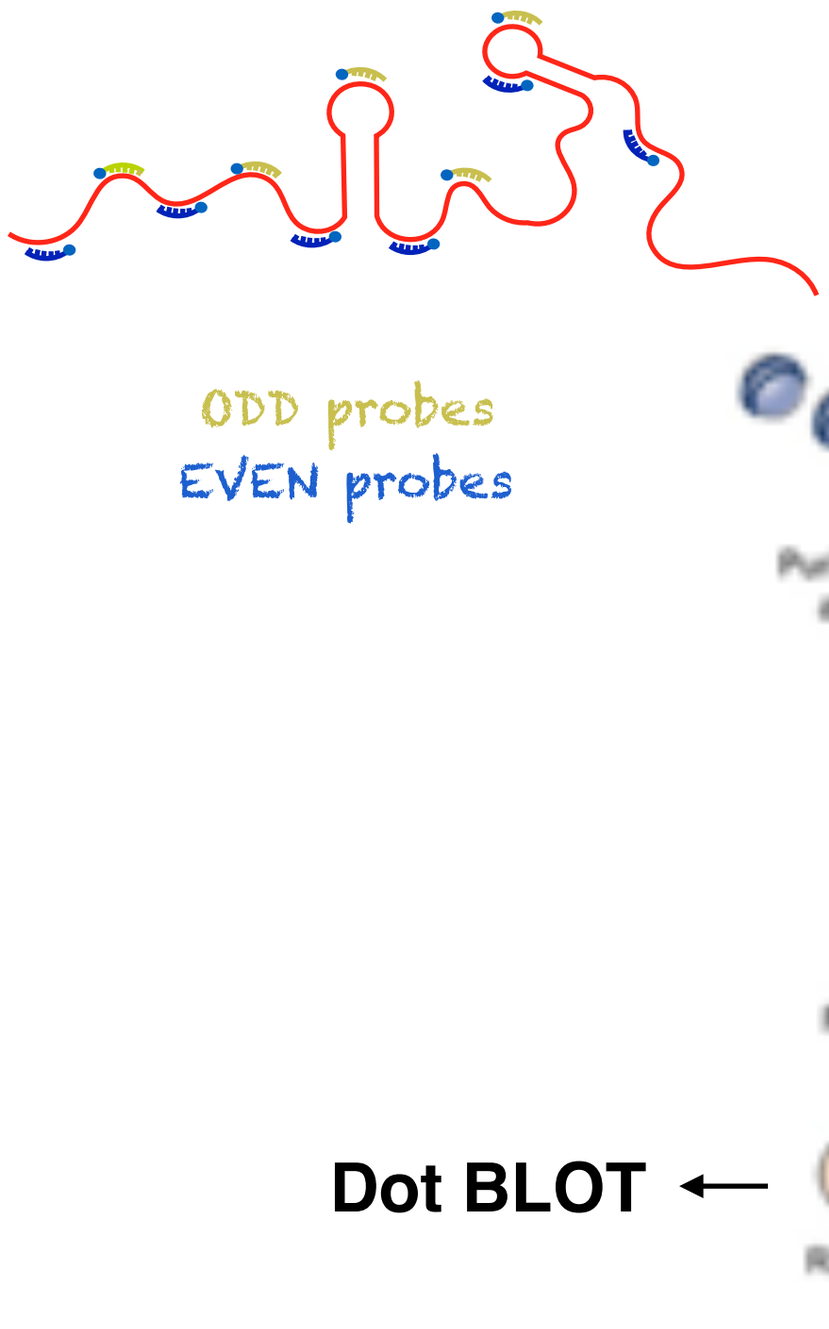
1. Probe Design

**2. Cross-linking**

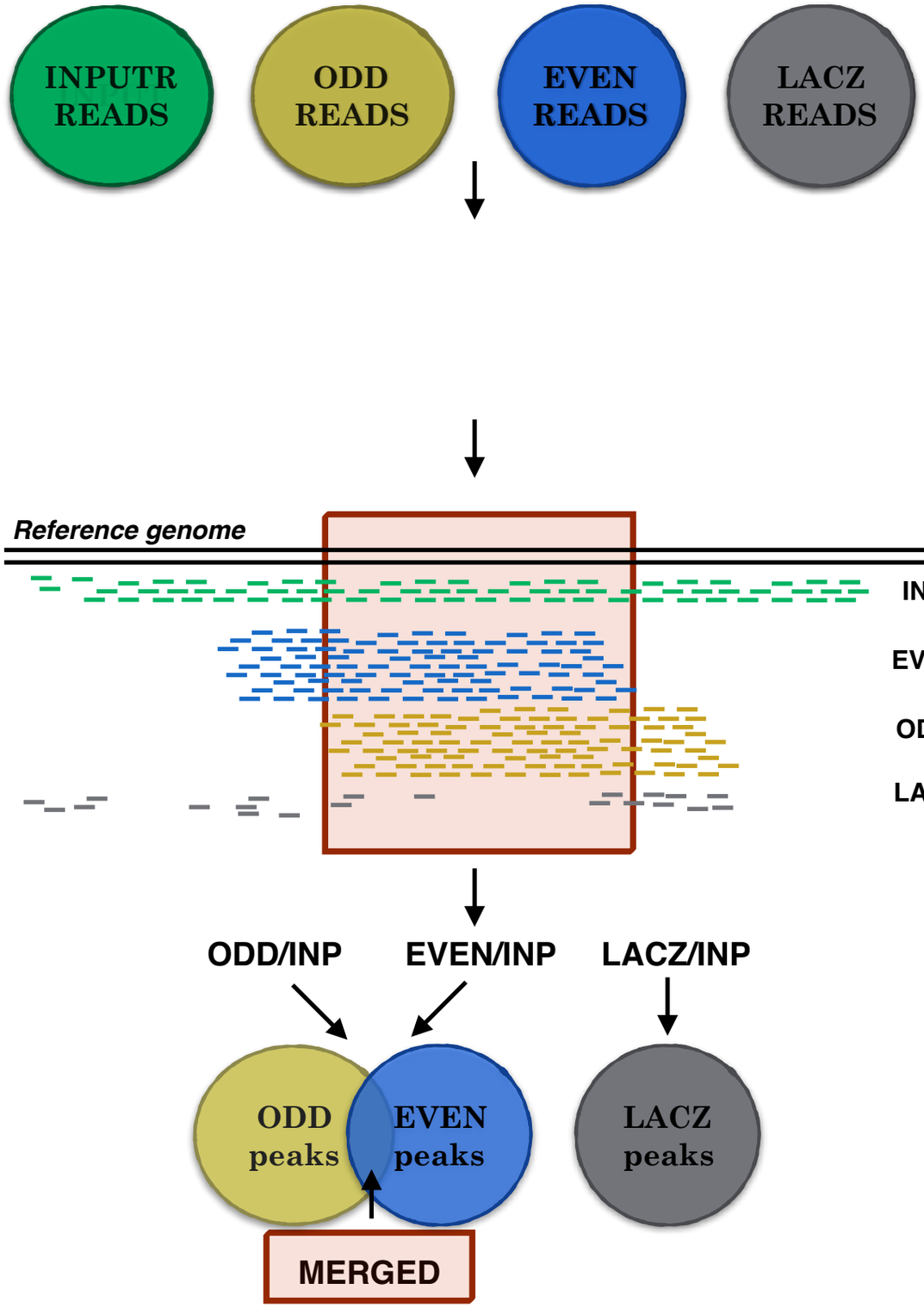
3. Chromatin Sonication

4. Probes Hybridization and precipitation

5. RNA and DNA Isolation

# ChIRP-seq




## PIPELINE

**Total reads**

**Trimming**

**Alignment**

**Peak calling**

**Merged vs Lacz**

# EXAMPLE: HOTAIR STORY

## Functional data

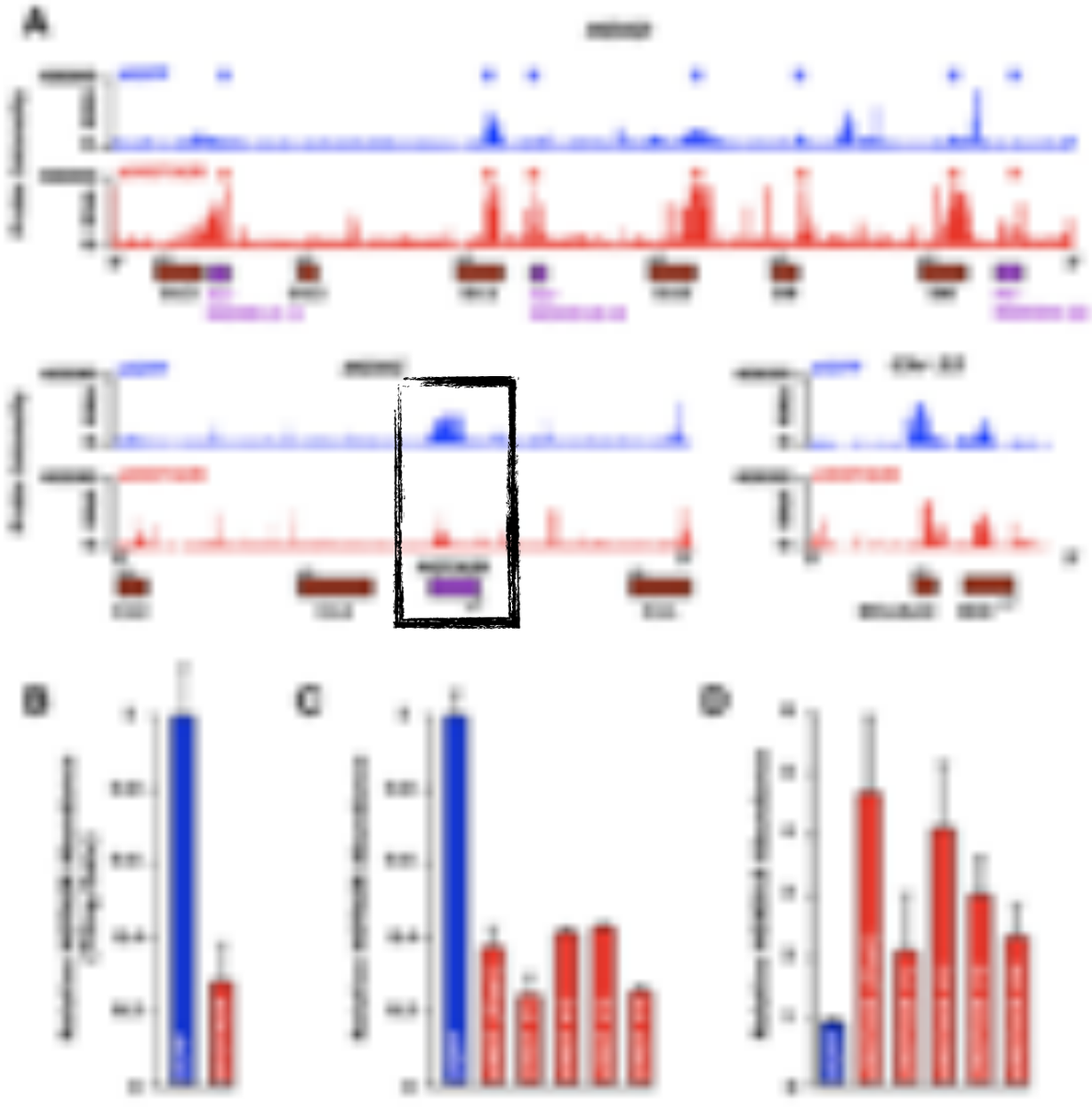



Figure 5. Loss of HOTAIR Results in Transcriptional Induction of HOXD Locus

(A) RNA expression profiles at HOXD locus (top), HOXC locus surrounding HOTAIR (bottom left), and a control region on chromosome 11 (bottom right) following transfection of siRNA targeting GFP (siGFP) or a pool of four siRNAs targeting HOTAIR (siHOTAIR). Intensities of RNA hybridized to the tiling array from the siGFP and the siHOTAIR transfections are plotted on a linear scale in blue and red, respectively. * indicates genes with significant increased transcription.

(B) qRT-PCR measuring the relative abundance of the HOTAIR transcript in the primary foreskin samples shown in (A). Mean ± standard deviation are shown.

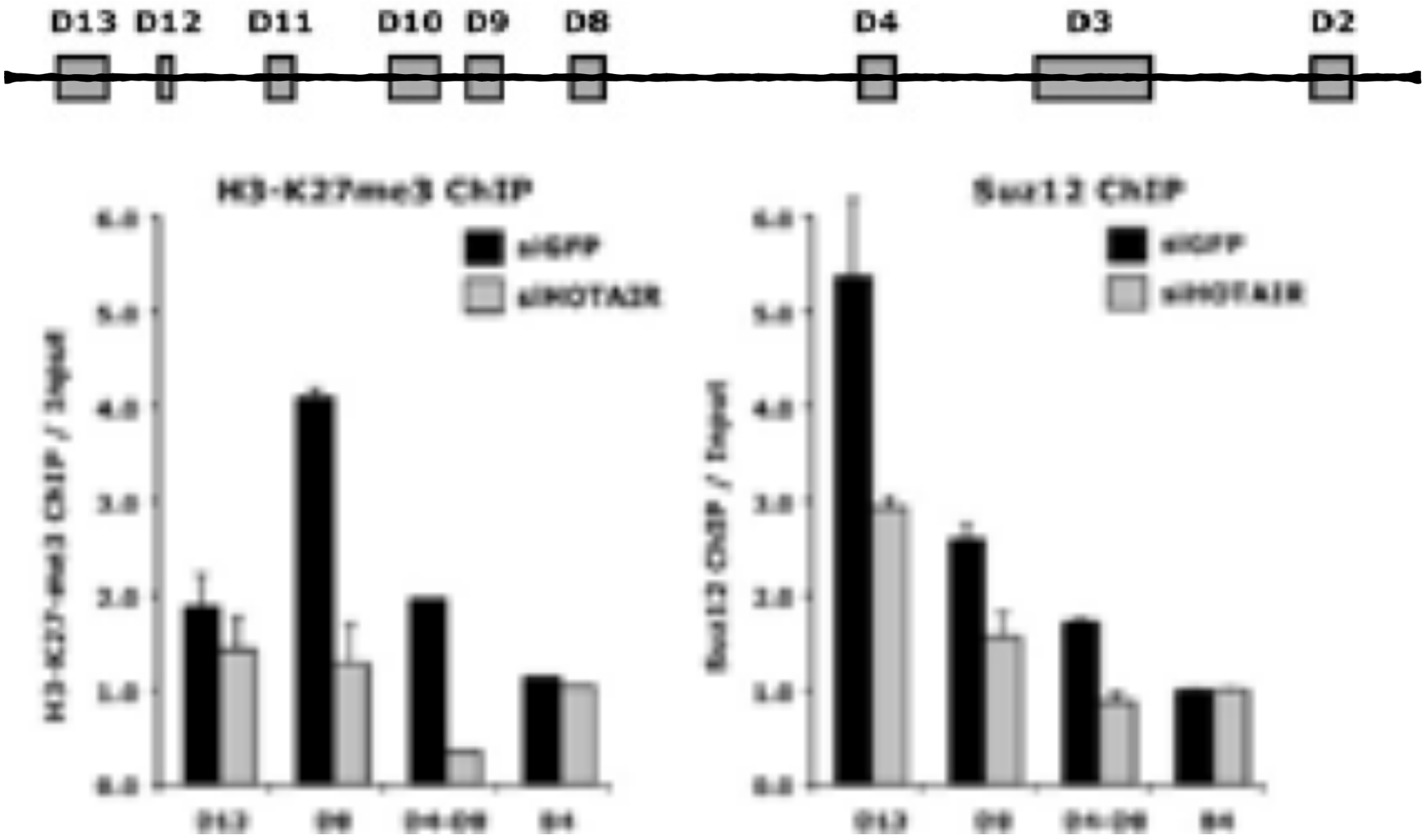
(C and D) qRT-PCR measuring the relative abundance of the HOTAIR (C) and HOXD10 (D) transcripts after depletion of four individual siRNAs to HOTAIR and the pool. Mean ± standard deviation are shown.

***Rinn et al. 2007***

# EXAMPLE: HOTAIR STORY

## HOX D GENOMIC LOCUS

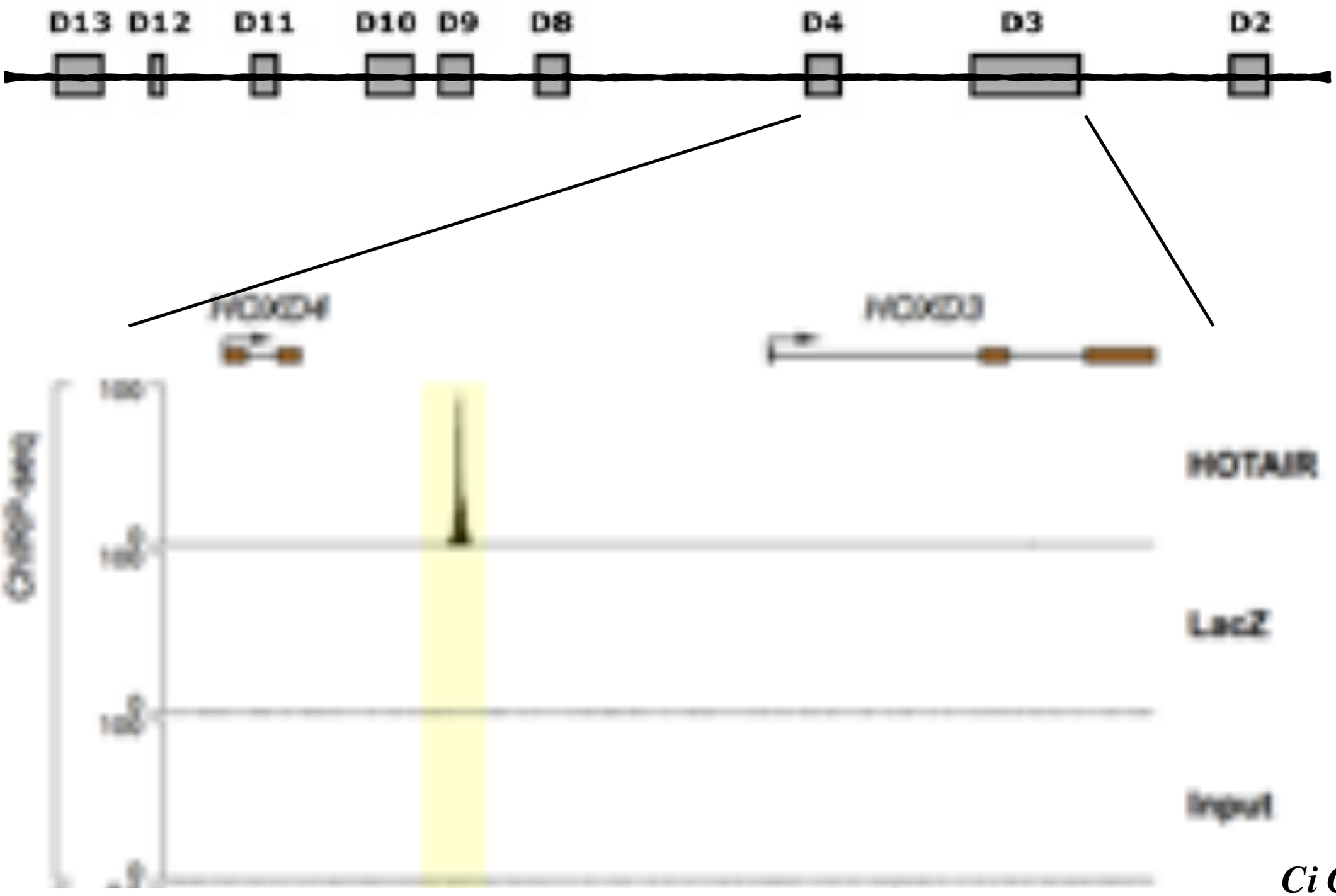
***ChIP** Suz12 and H3-K27me3*




***Rinn et al. 2007***

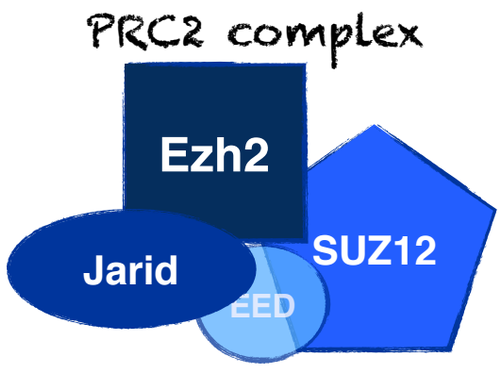
# EXAMPLE: HOTAIR STORY

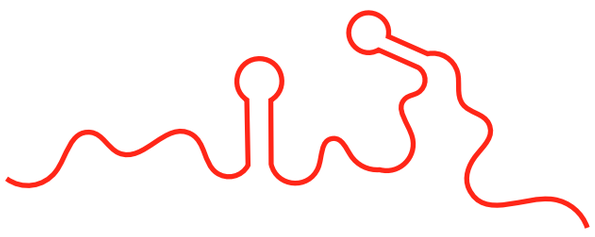
*HOTAIR* ***ChIRP***

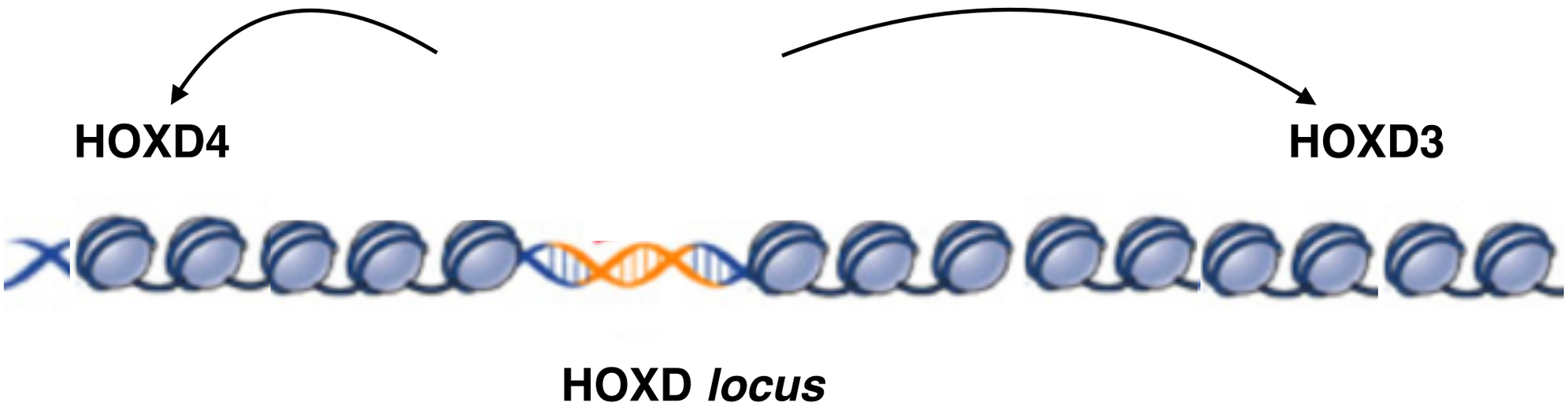



***Ci Chu et al. 2011***

# Model proposed






**Physical association between SUZ12 and HOTAIR?**

# Molecular techniques for the study of the interaction between:

**DNA-protein:**

**ChIP** (Chromatin immunoprecipitation)

**RNA-DNA:**

**ChIRP** (Chromatin isolation by RNA purification)

**RNA-Protein (RNA centric):**

**Exogenous RNA pulldown,**

**Endogenous RNA pulldown**

**RAP (**RNA antisense purification)

**Protein-RNA (Protein centric):**

**RIP,** (RNA immunoprecipitation)

**CLIP**(Cross-linked immunoprecipitation)

# RNA-centric techniques

**AIM: Identification of the protein interactors of an RNA.**

## Precipitation of the RNA and PROTEINS checking

Total Cytoplamic or Nuclear extract

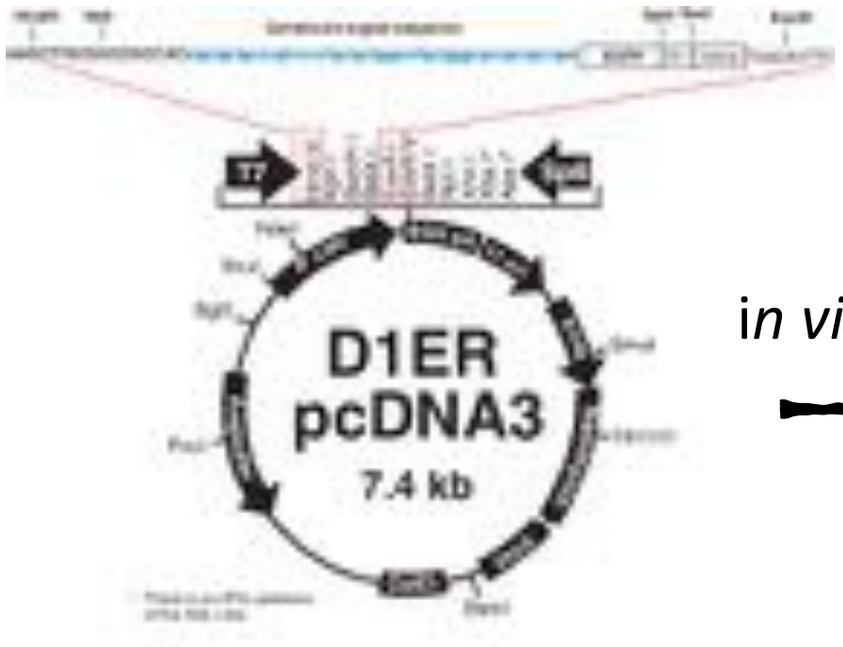
Huge amount of cellular extract is needed

The efficiency depends on the abundance of the RNA

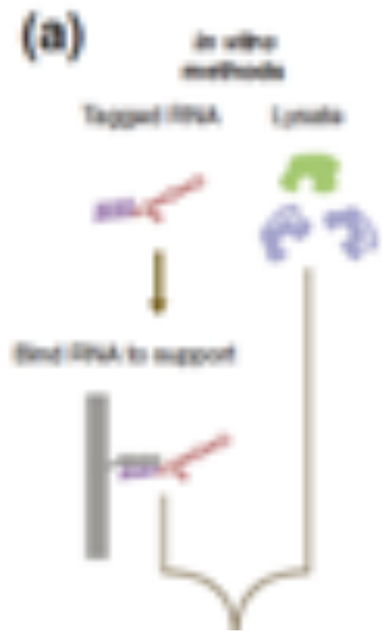
# Exogenous RNA pull down

(in vitro method)

## RNA affinity capture methods




i*n vitro* transcription



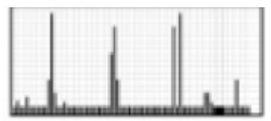

**Capture protein from lysate**

**Washing**

**Boil with SDS**

**Western Blot**

**Mass spectrometry**



### Different Tags

**MS2 viral protein** —> Loop stem loop

**Cy4** —> RNA aptamer

**STREPTAVIDIN** —> S1 aptamer

# Exogenous RNA pull down

## (in vitro method)

## WORK FLOW

1. RNA tagging

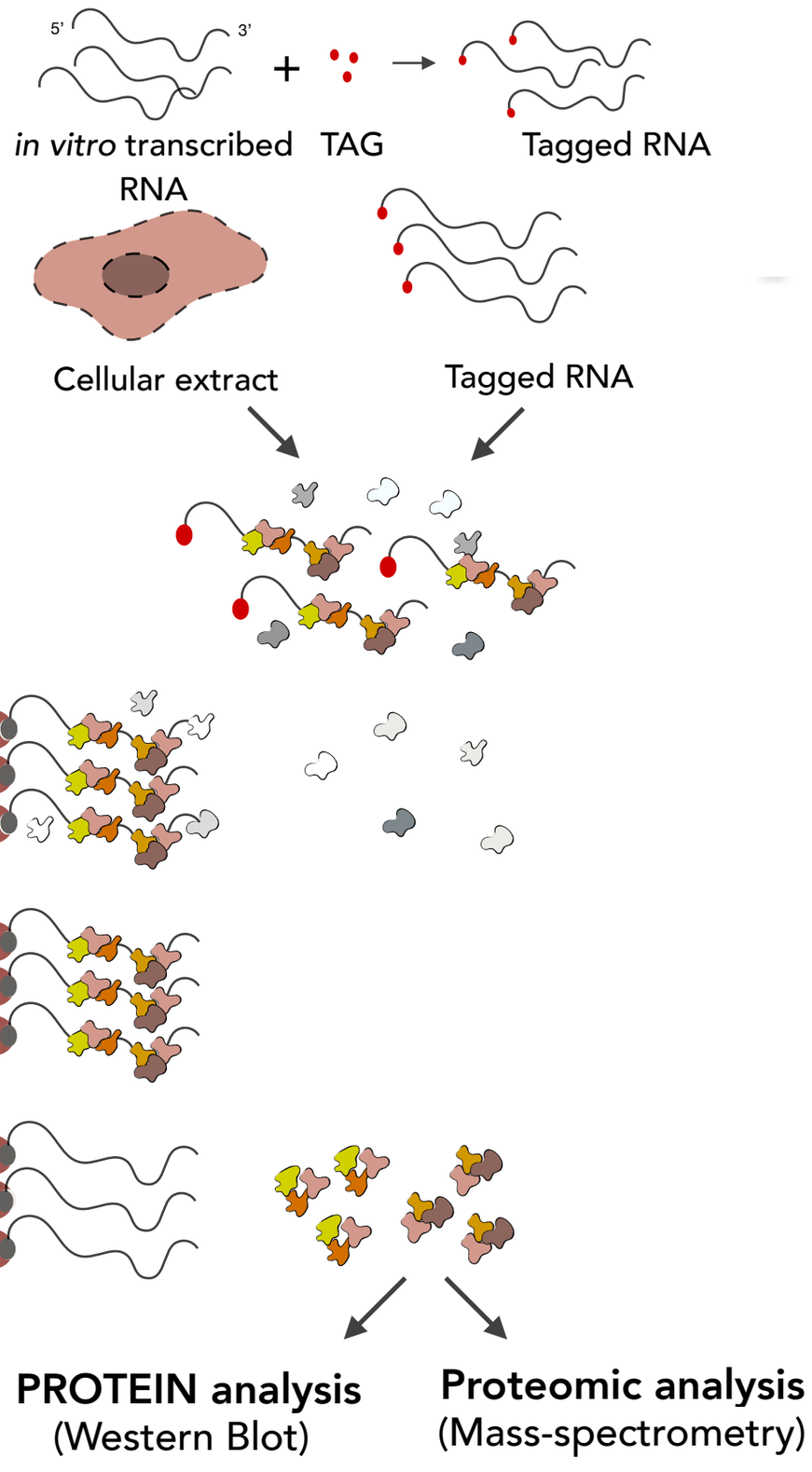
2. Cell lysis

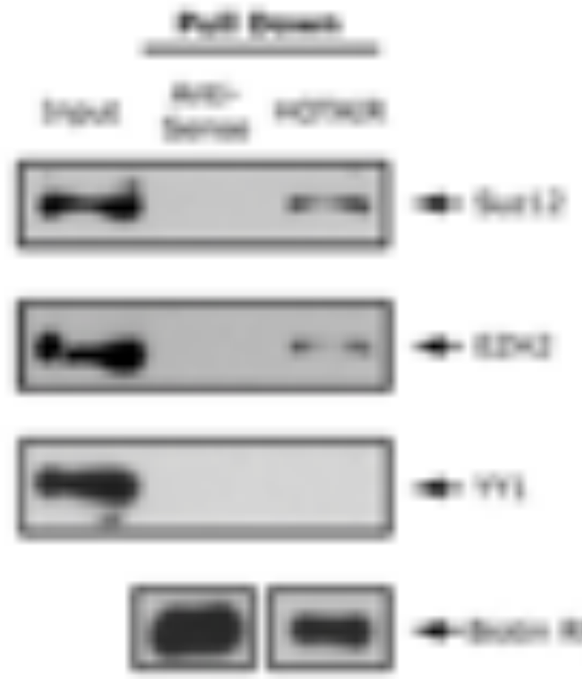
3. Cell extract/tagged RNA incubation

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 pull down
(in vitro method)

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 CONTROLL**

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

# Exogenous RNA pull down (EPIC FAIL)

Article

## 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], Iaysin Vassiliev[1,2], Michel Wassef[1,2], Nicolas Servant[3,4], Bruno Sarguet[1] & 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 the causal mode of regulation remains scarce. 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 transcriptome 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*, three 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).

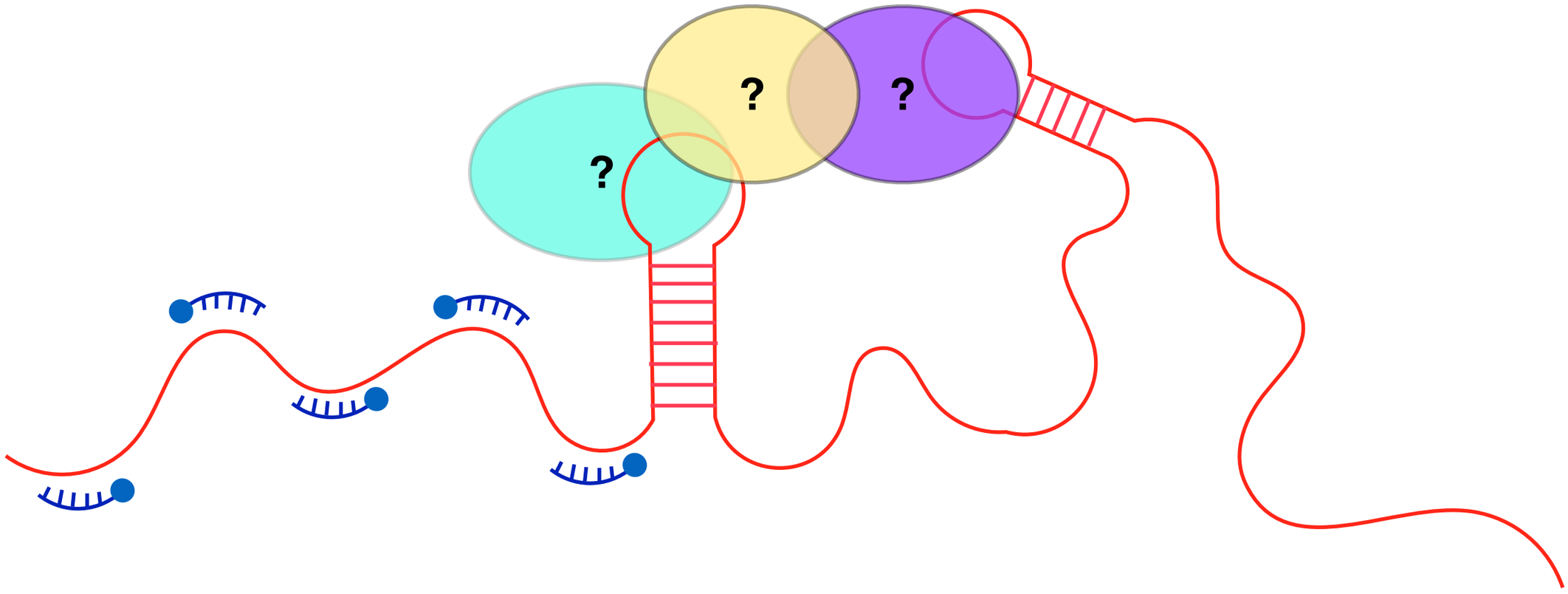
Although our understanding of how PRC2 contacts chromatin has improved, how it is specifically recruited to defined genomic loci is still only partially understood. The core PRC2 has no known sequence-specific DNA-binding domain. In *Drosophila*, DNA sequences known as Polycomb response elements (PREs) 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 on PRC2 genome-wide recruitment. 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 none apply this concept account for the specificity and dynamics of

# in vivo or in vitro, this is the question

The biochemical interactions that are established *in vitro* not necessarly represent the real interaction established *in vivo*

# Endogenous RNA pull down (in vivo and native)

Precipitation of endogenous RNA together with its protein interactors using biotinilated oligos



# Endogenous RNA pull down (in vivo and native)

## WORK FLOW

**1. Probe Design**

**2 Cell lysis**

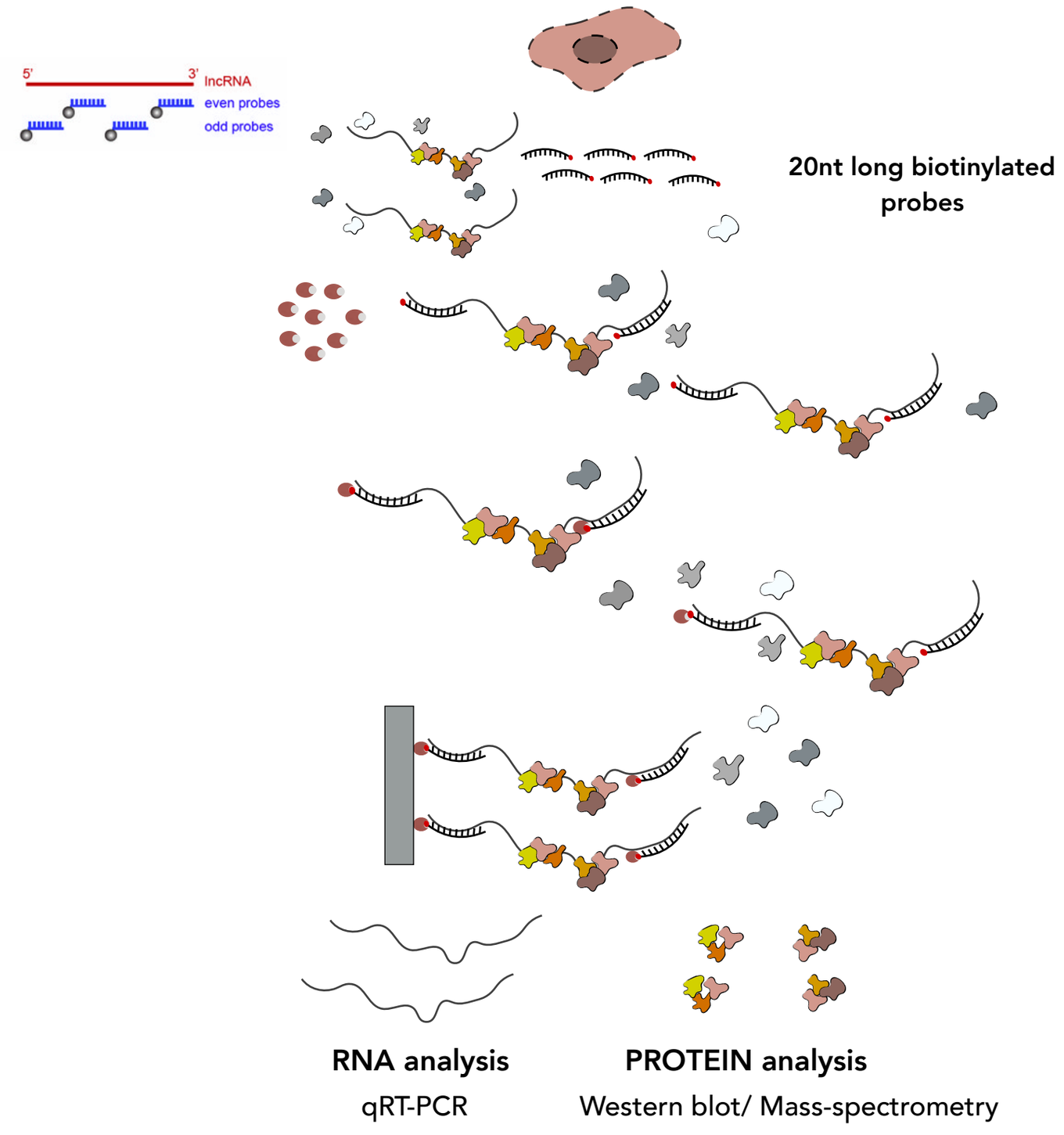
**3. Cell extract/probes incubation**

**3. Binding step**

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

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

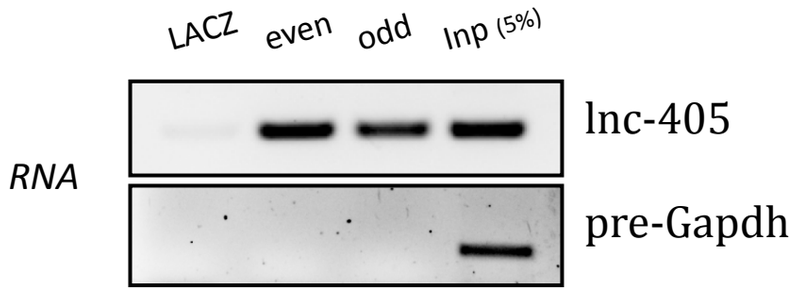
**6. Protein and RNA elution and analysis**



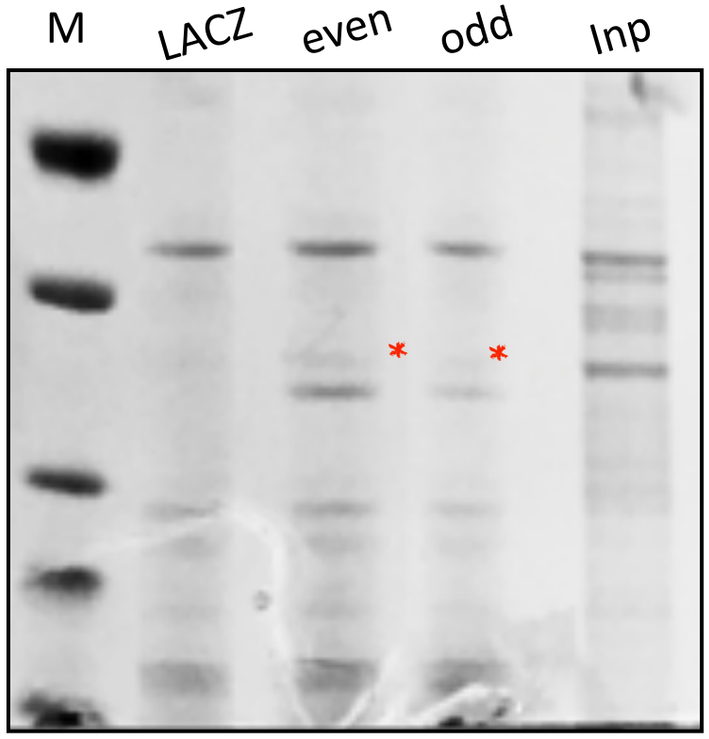

***Cipriano and Ballarino, FMB 2018***

# Endogenous RNA pull down (in vivo and native)

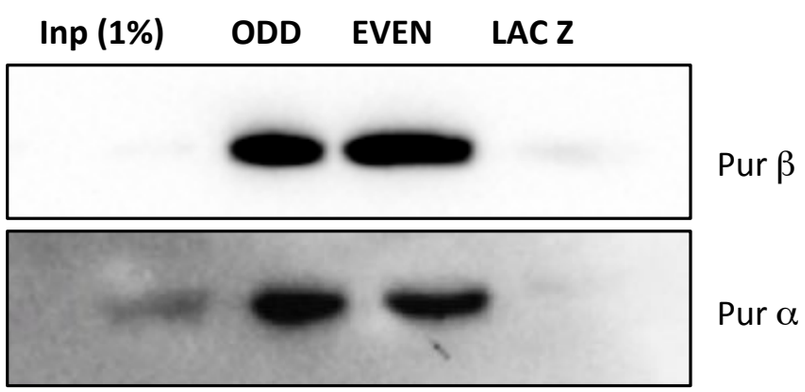
*Lnc-405 endogenous pulldown*




***Semiq RT-PCR***



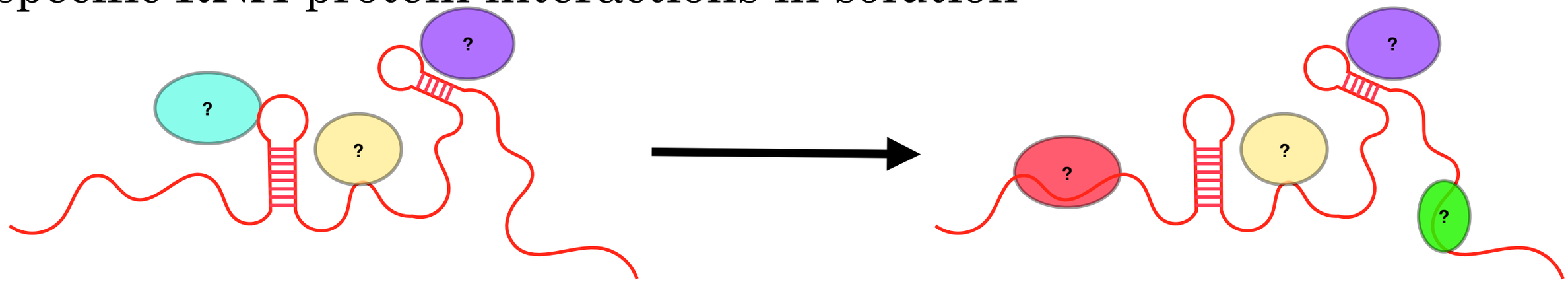

*WB*

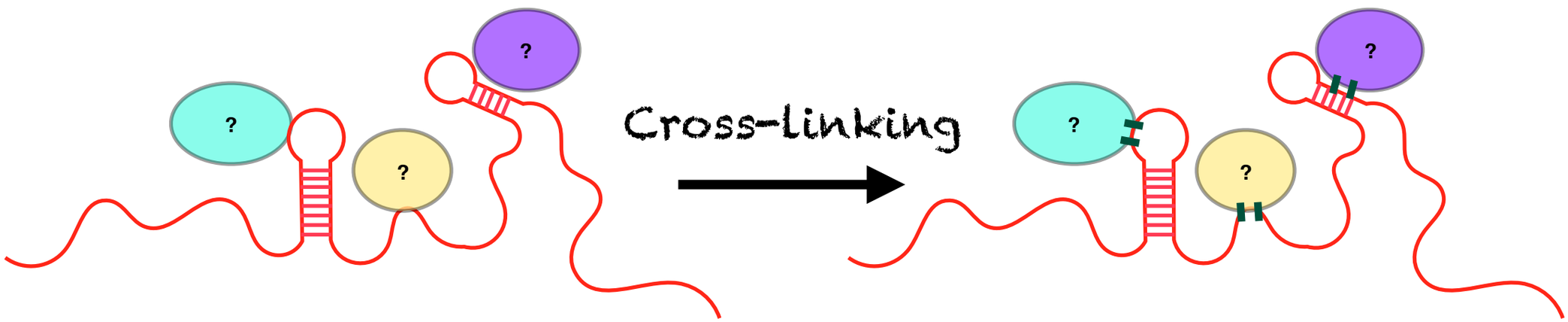



| Accession | Description |
|---|---|
| Q9QXS1 | Plectin OS=Mus musculus GN=Plec PE=1 SV=3 - [PLEC_MOUSE] |
| Q8VDD5 | Myosin-9 OS=Mus musculus GN=Myh9 PE=1 SV=4 - [MYH9_MOUSE] |
| P62843 | 40S ribosomal protein S15 OS=Mus musculus GN=Rps15 PE=1 SV=2 - [RS15_MOUSE] |
| P42669 | Transcriptional activator 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] |

# native or cross-linking strategy?

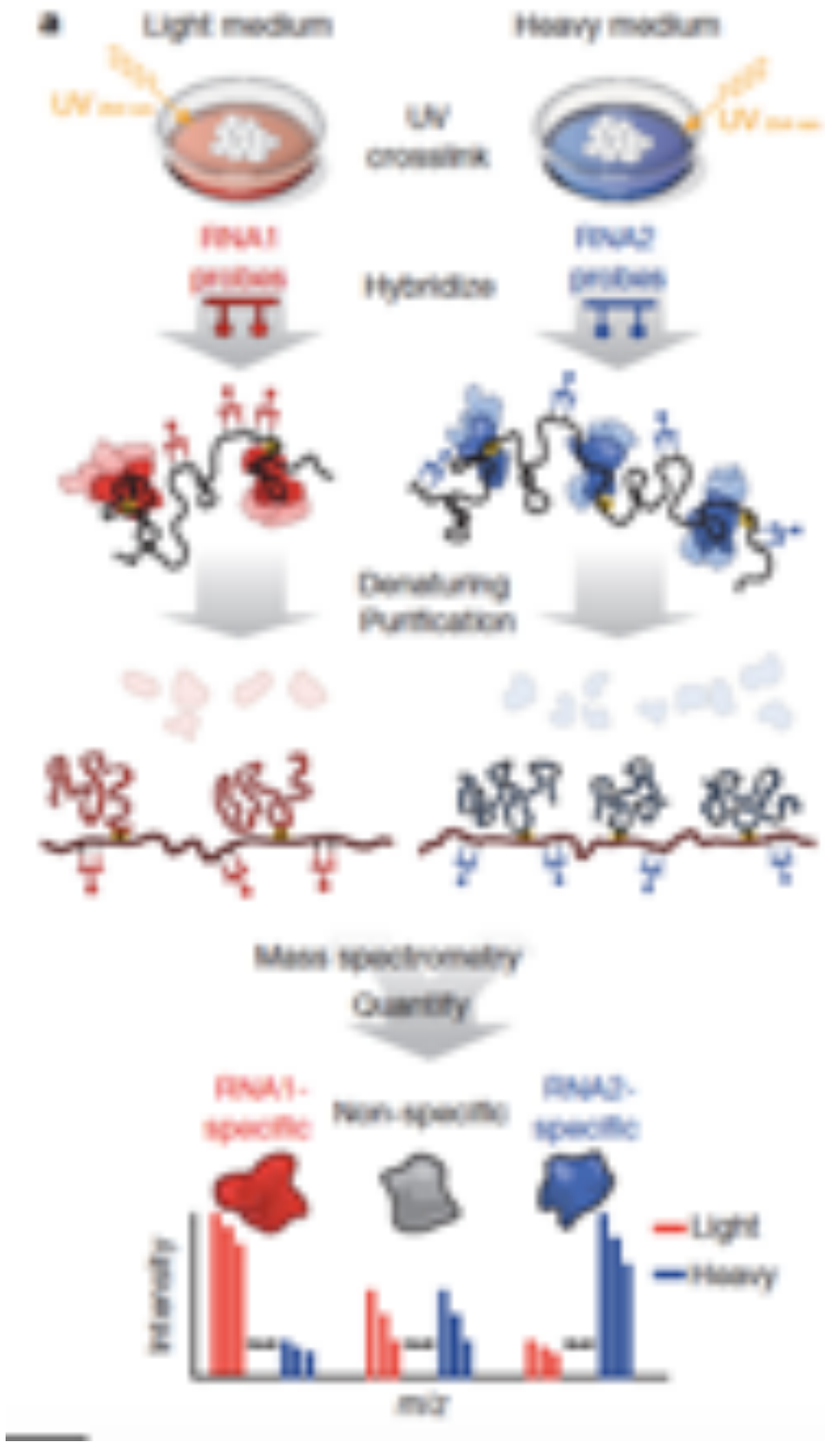
Similar to the exogenous pulldown, purification of endogenous RNA under native conditions can led to re-association or formation of non-specific RNA-protein interactions in solution




# RAP (RNA Antisense Purification)

(in vivo and cross-linked)




## *In vivo* UV crosslinking

Longer probes (90nt)

High stringency binding conditions

High stringency wash conditions

# RAP (RNA Antisense Purification)

(in vivo and cross-linked)

## WORK FLOW

**UV cross-linking**

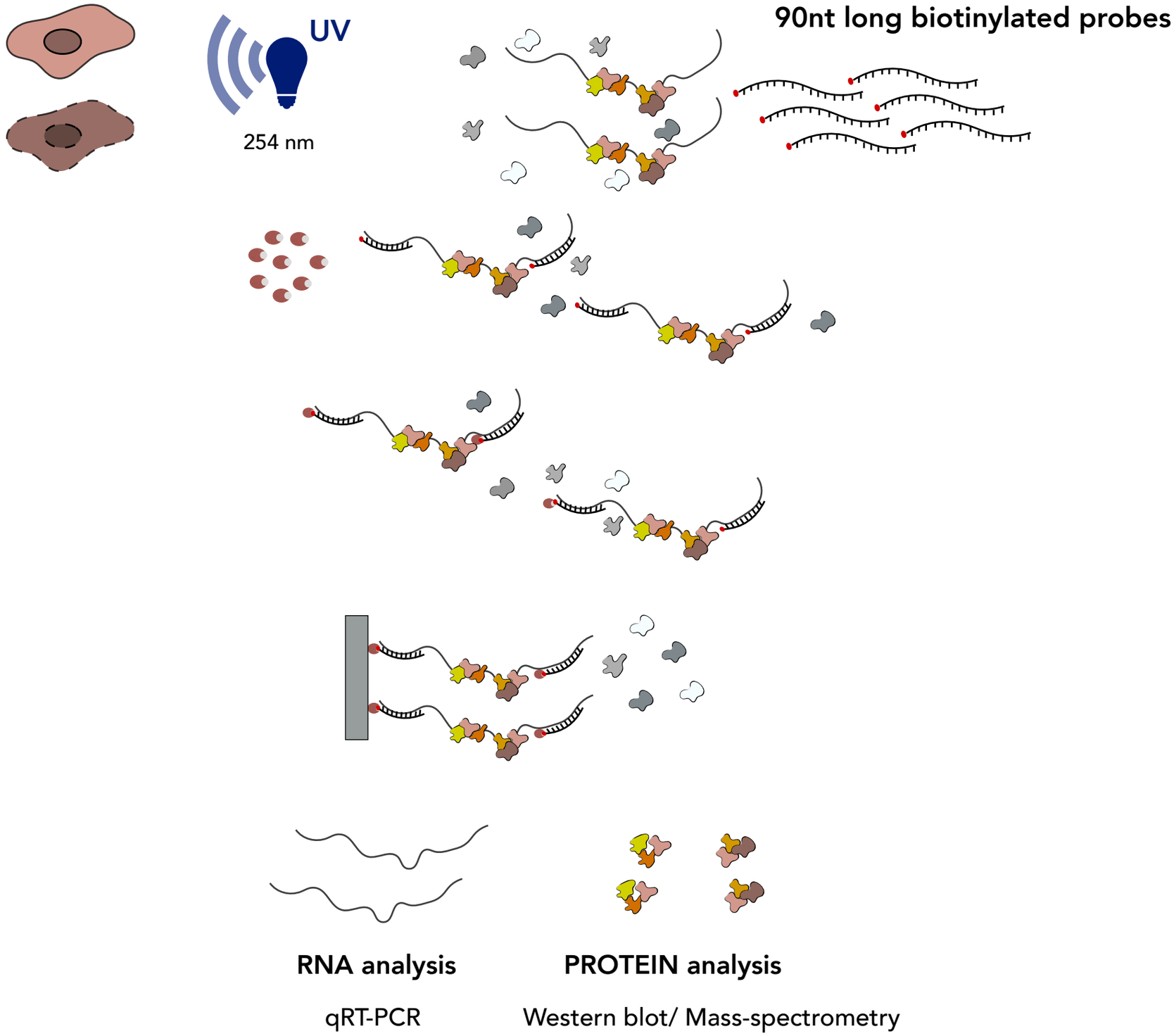
**Cell lysis**

**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)**

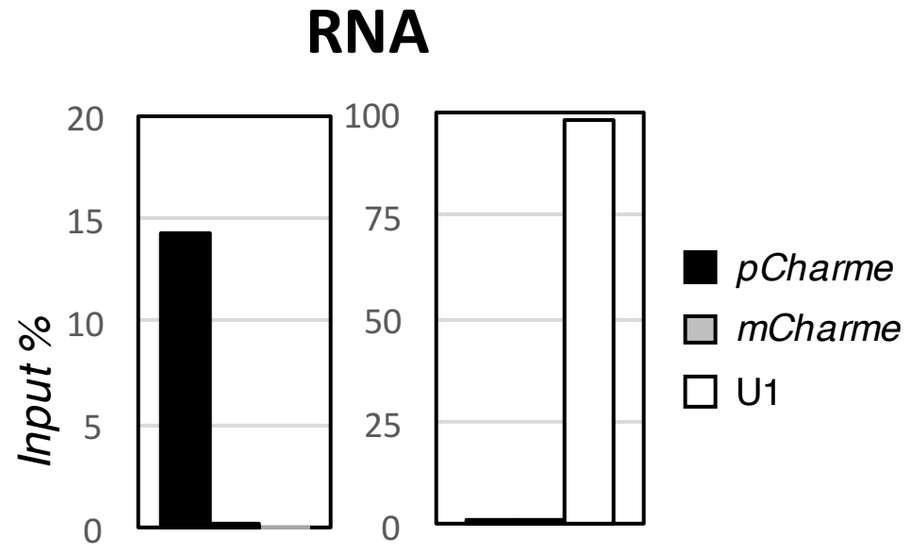
**RNA and PROTEIN elution**




***Cipriano and Ballarino, FMB 2018***

# RAP (RNA Antisense Purification)

(in vivo and cross-linked)


pCharme PD

snRNAU1 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 |

# Molecular techniques for the study of the interaction between:

**DNA-protein:**
**ChIP** (Chromatin immunoprecipitation)

**RNA-DNA:**
**ChIRP** (Chromatin isolation by RNA purification)

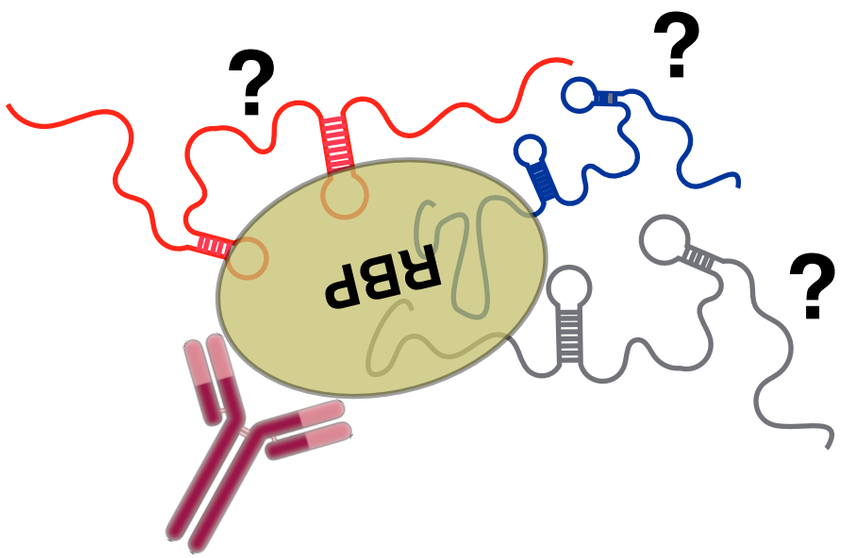
**RNA-Protein (RNA centric):**
**Exogenous RNA pulldown,**
**Endogenous RNA pulldown**
**RAP (**RNA antisense purification)

**Protein-RNA (Protein centric):**
**RIP,** (RNA immunoprecipitation)
**CLIP**(Cross-linked immunoprecipitation)

# RIP

(in vivo and native)

## AIM: Identification of the RNAs bound to a known Protein




- 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)

# RIP

(in vivo and native)

## WORK FLOW

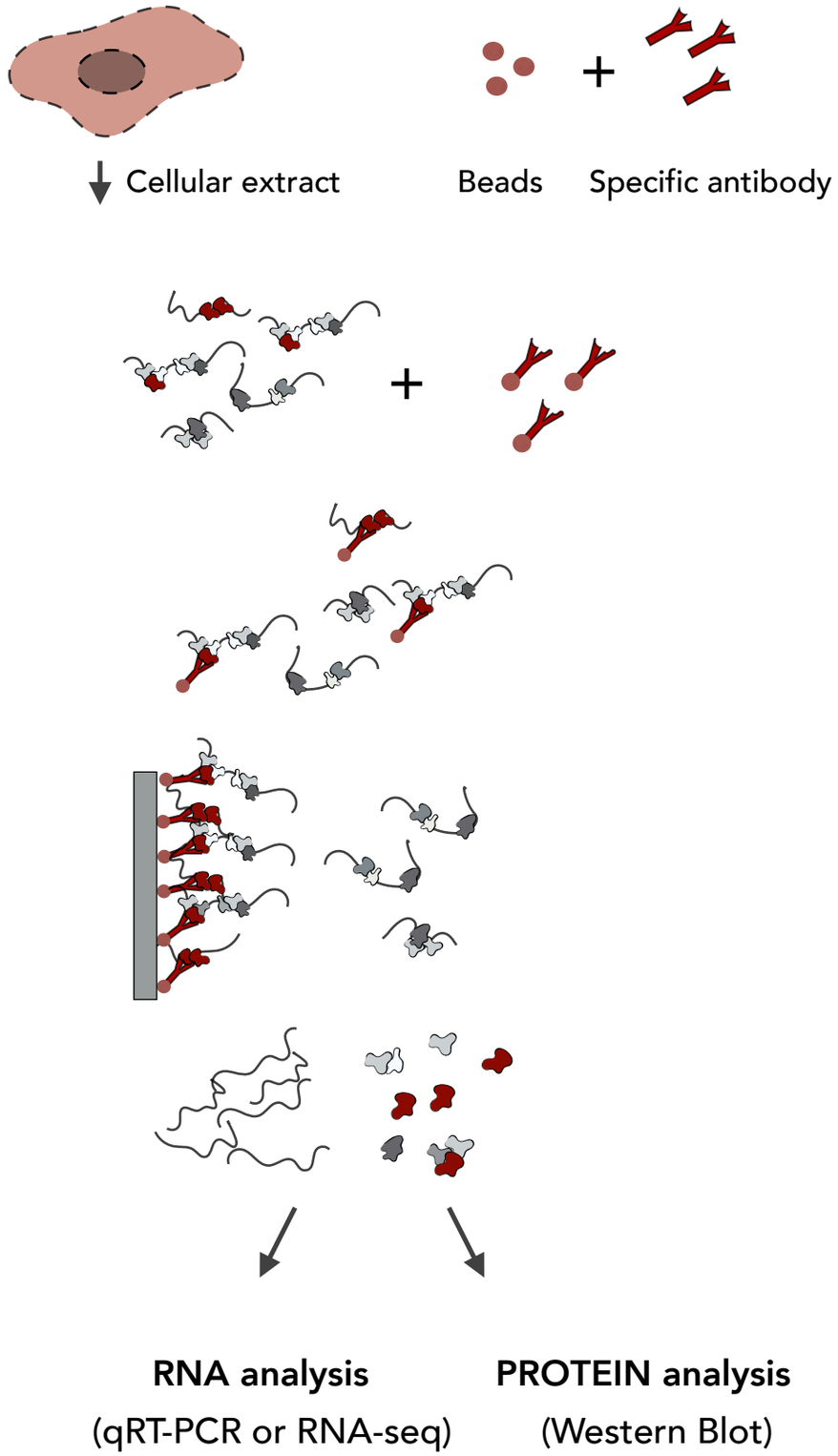
**1. Lysis Cells and collect cell extract**

**2 Prebinding between AntiBody and Beads**

**3. Introduction of cellular extract (Binding step)**

**4. Wash and Purification of RNA-protein complexes**

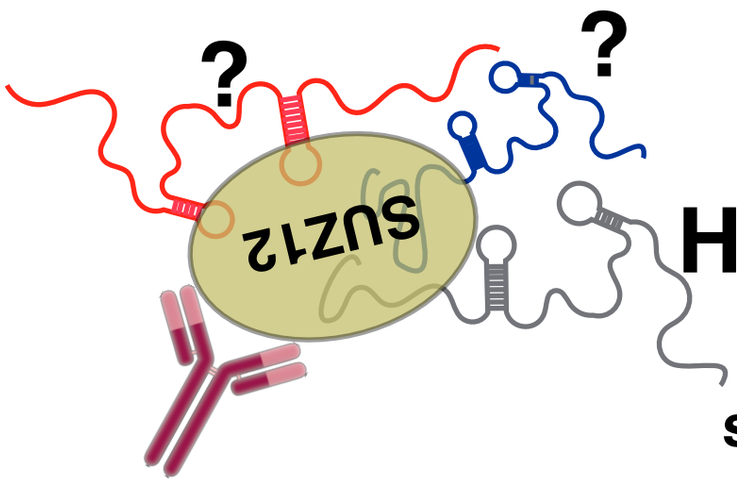
**5. Protein and RNA elution**




***Cipriano and Ballarino, FMB 2018***

# RIP

(in vivo and native)



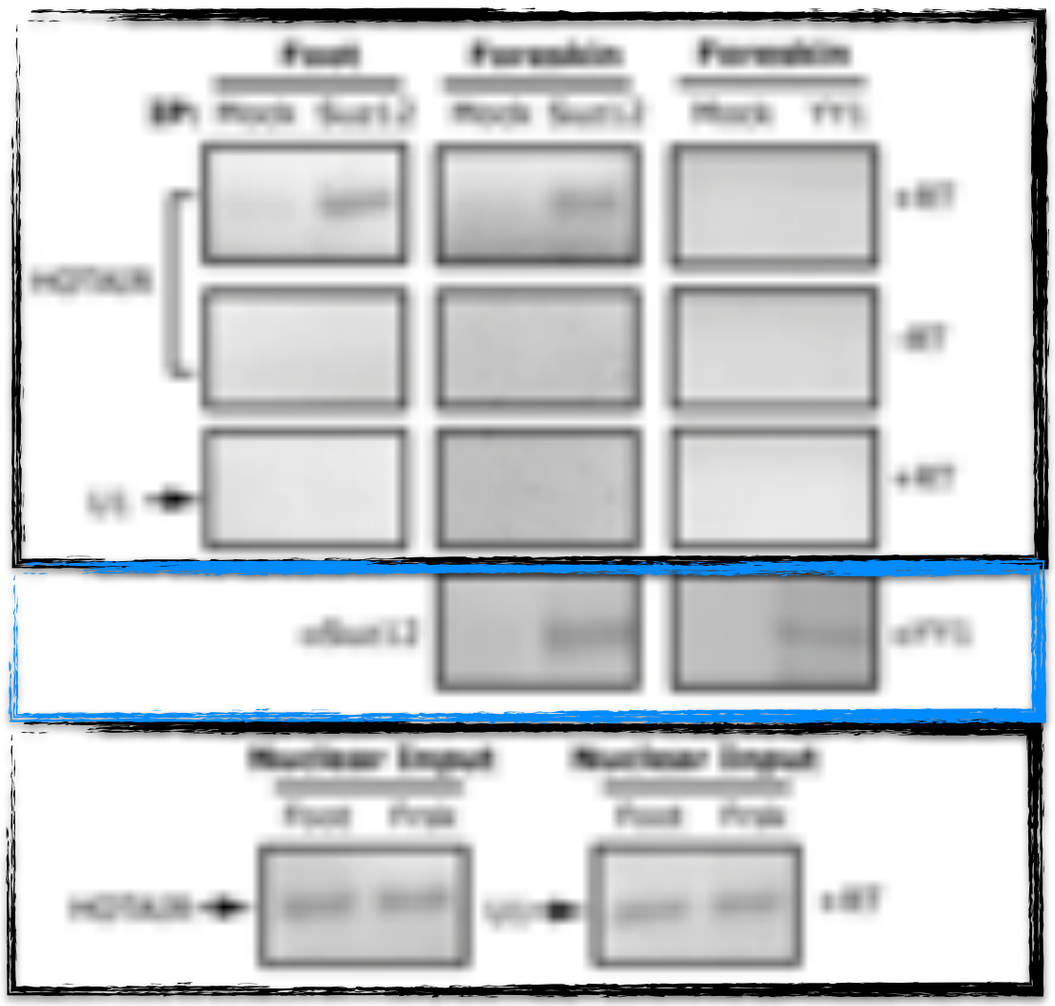

**HOTAIR**

**semiq-rtPCR**

**Western Blot**

**semiq-rtPCR**

*SUZ12 RIP*



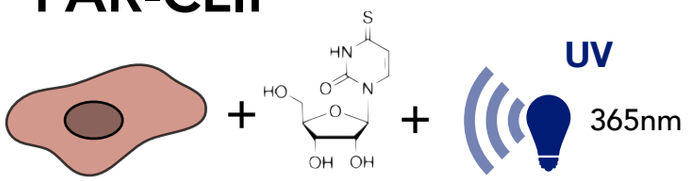
*Rinn et al., Cell 2007*

# CLIP

## (in vivo and cross-linked)

### HITS-CLIP

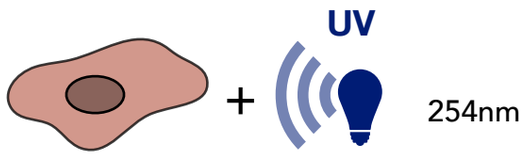
### PAR-CLIP




① UV crosslinking

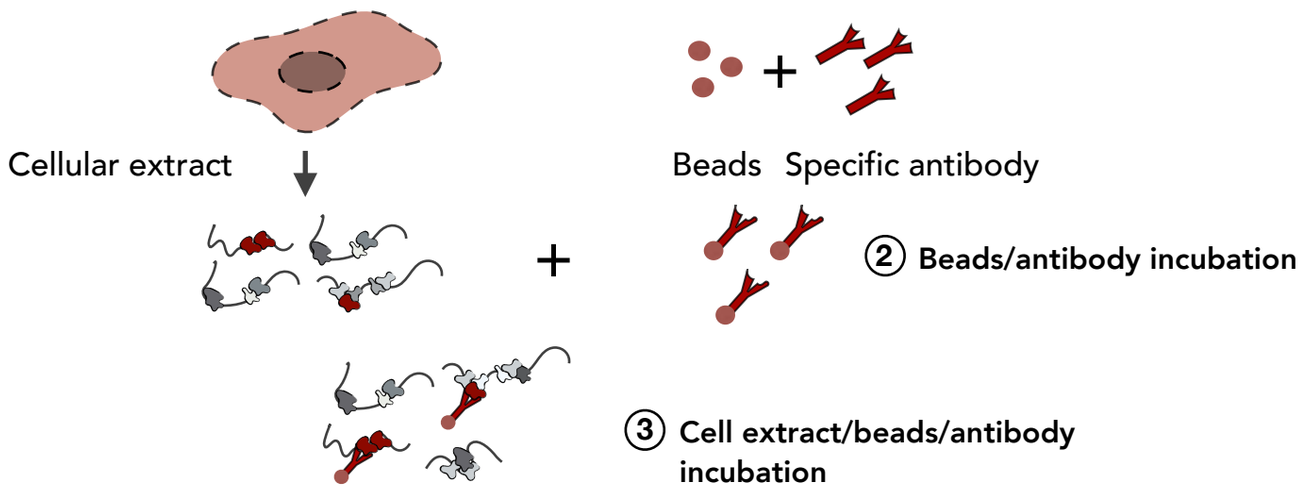
### iCLIP/eCLIP

### CRAC



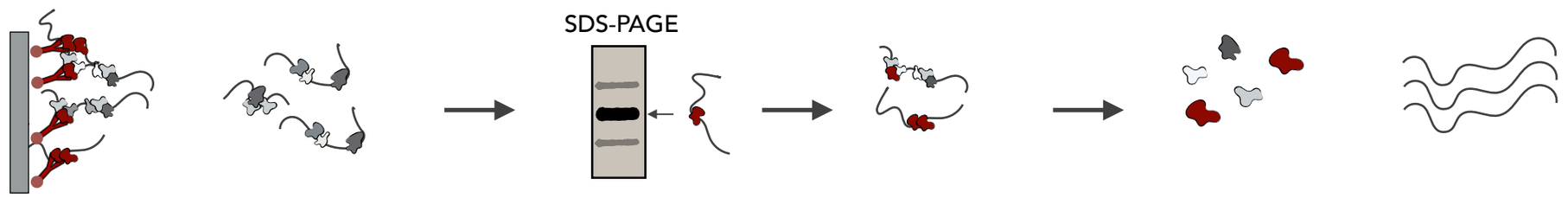

① UV crosslinking

### CLIP




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

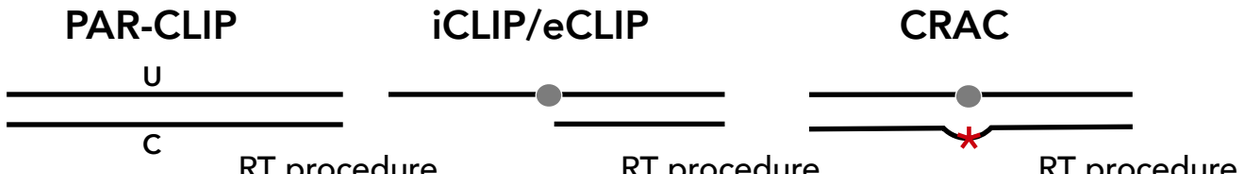
⑥ PROTEIN/RNA complexes purification

⑦ PROTEIN analysis (Western Blot)

⑧ RNA analysis

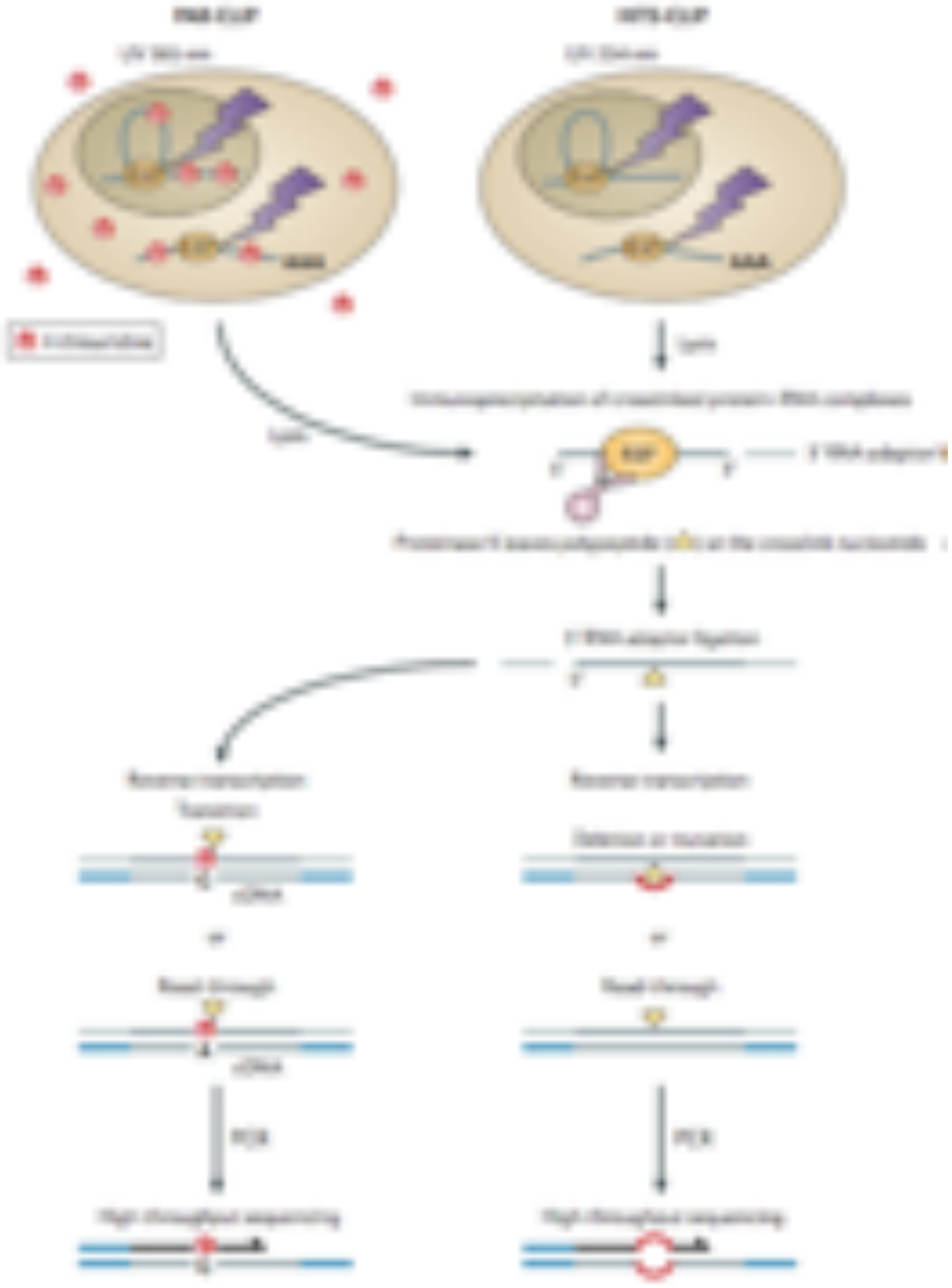
Library preparation and sequencing

qRT-PCR




*Cipriano and Ballarino, FMB 2018*

# PAR-CLIP and i-CLIP



*Julian König et al., Nature Review vol.13, February 2012*

# PAR-CLIP and i-CLIP

## WORK FLOW

**0. Adding of 4-thiouridin in cell medium**

**1. Collect cells**

**2. UV Crosslinking (365 nm)**

**3. Immunoprecipitate RBP and phosphorylate RNA 5' end**

**4. Ligate the 5' RNA adapter**

**5. Dephosphorylate RNA 3' end**

**6. Purify RBP-RNA on SDS-PAGE**

**7. Digest the RBP**

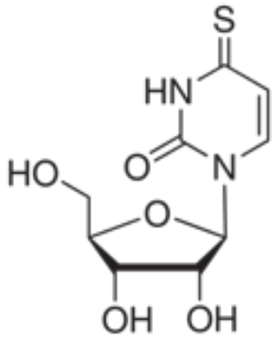
**8. Ligate the 3' RNA adapter**

**9. Purify RNA on urea-TBE gel**

**10. Reverse transcription**

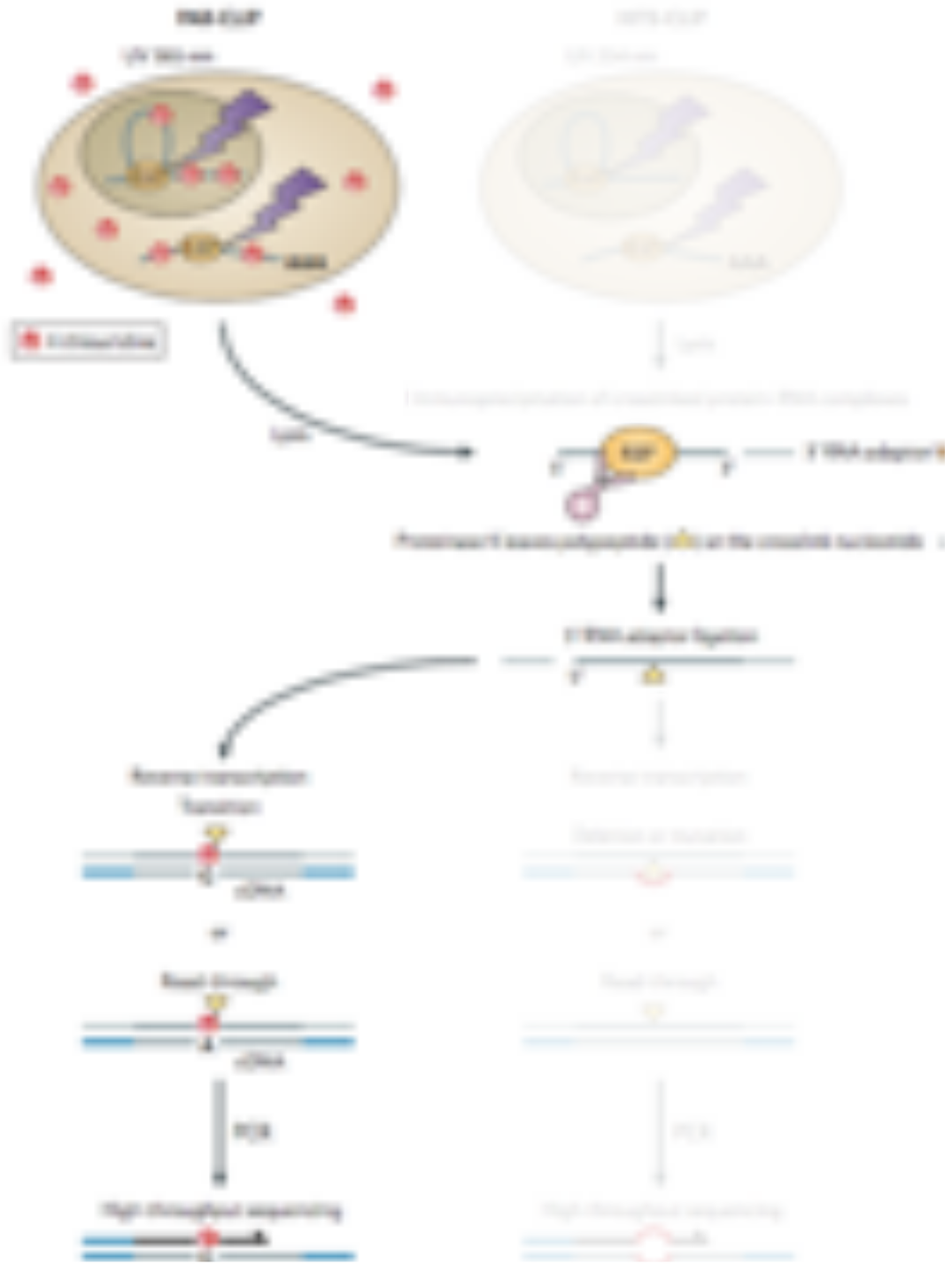
**11. PCR**

**12. Illumina paired-end sequencing**




U 4-thiouridine

U → C



*Julian König et al., Nature Review vol.13, February 2012*

# PAR-CLIP and i-CLIP

## WORK FLOW

**1. Collect cells**

**2. UV Crosslinking (254 nm)**

**3. Immunoprecipitate RBP and phosphorylate RNA 5' end**

**4. Ligate the 5' RNA adapter**

**5. Dephosphorylate RNA 3' end**

**6. Purify RBP-RNA on SDS-PAGE**

**7. Digest the RBP**

**8. Ligate the 3' RNA adapter**

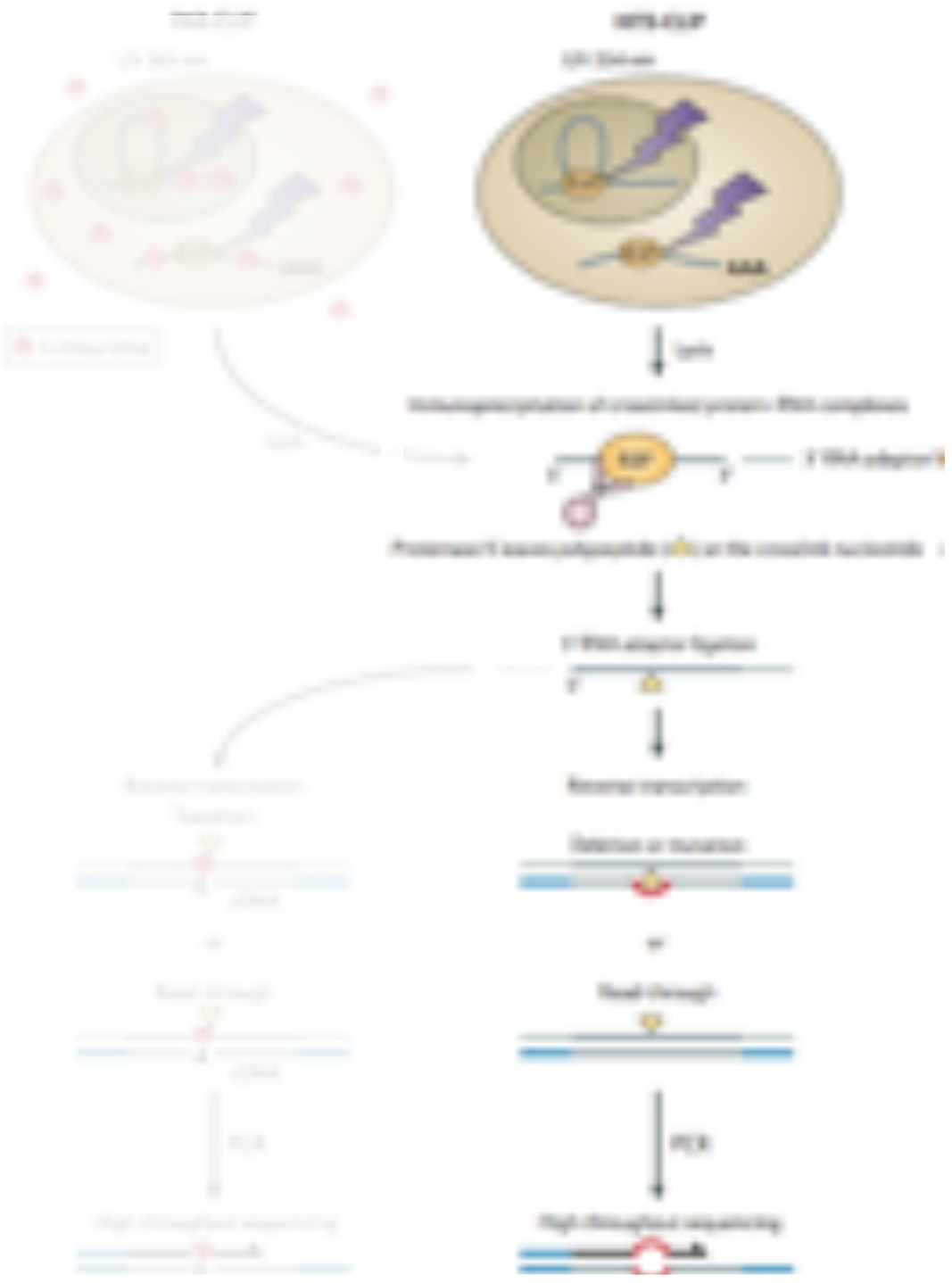
**9. Purify RNA on urea-TBE gel**

**10. Reverse transcription**

**11. PCR**

**12. Illumina paired-end sequencing**

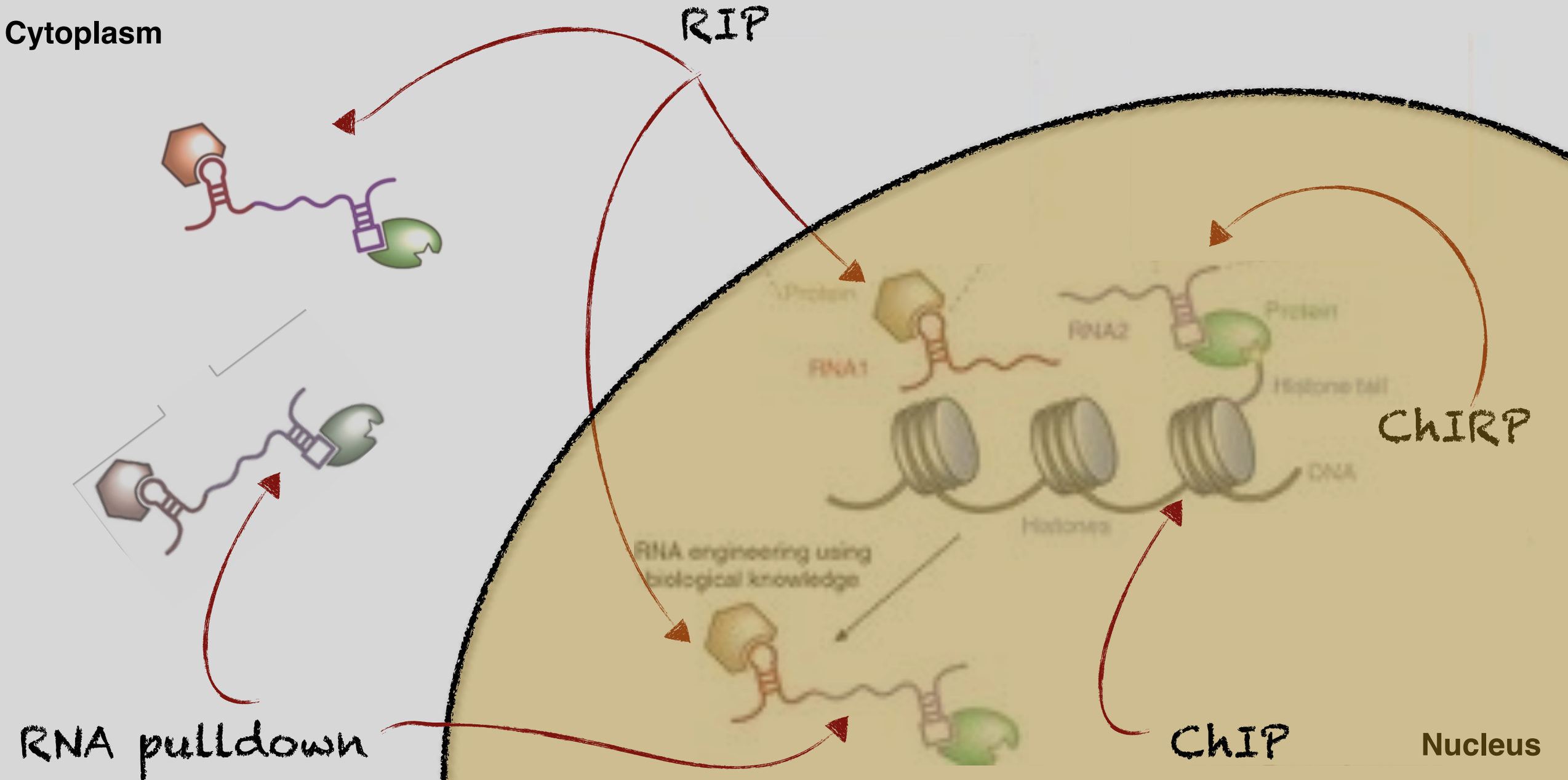


*Julian König et al., Nature Review vol.13, February 2012*

# Summary

| | PRECIPRITATION | CHECK FOR |
|---|---|---|
| ChIRP | RNA | DNA/PROTEIN |
| RNA pulldown | RNA | RNA/PROTEIN |
| RIP | PROTEIN | RNA/PROTEIN |
| ChIP | PROTEIN | DNA |



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