Identification of a new class non-coding RNA in yeast by RNA-Seq and ChIP-Seq

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Genome Analysis lab, Centre de Génétique Moléculaire

23/11/12, BioInfo Club, IJM, Paris

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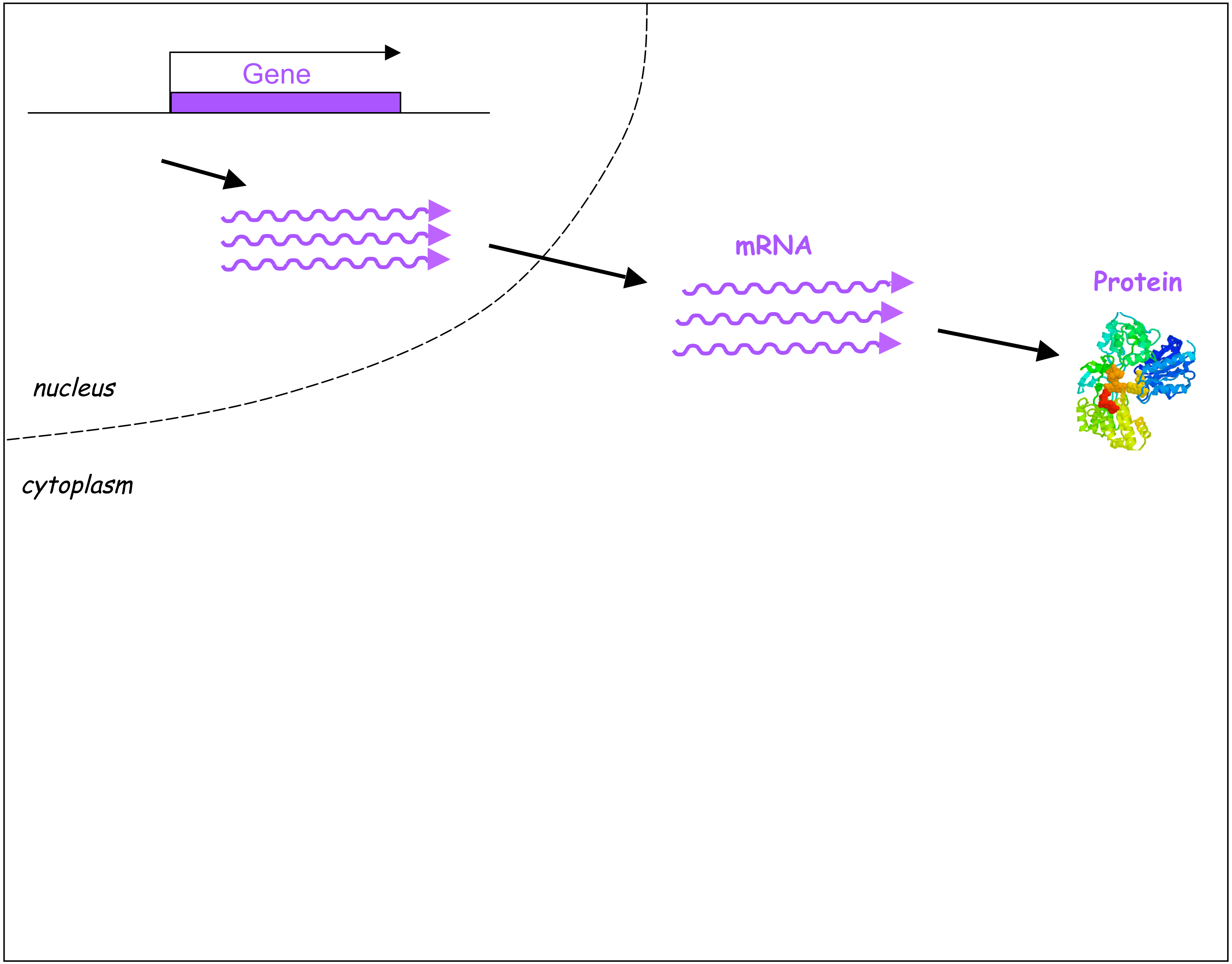
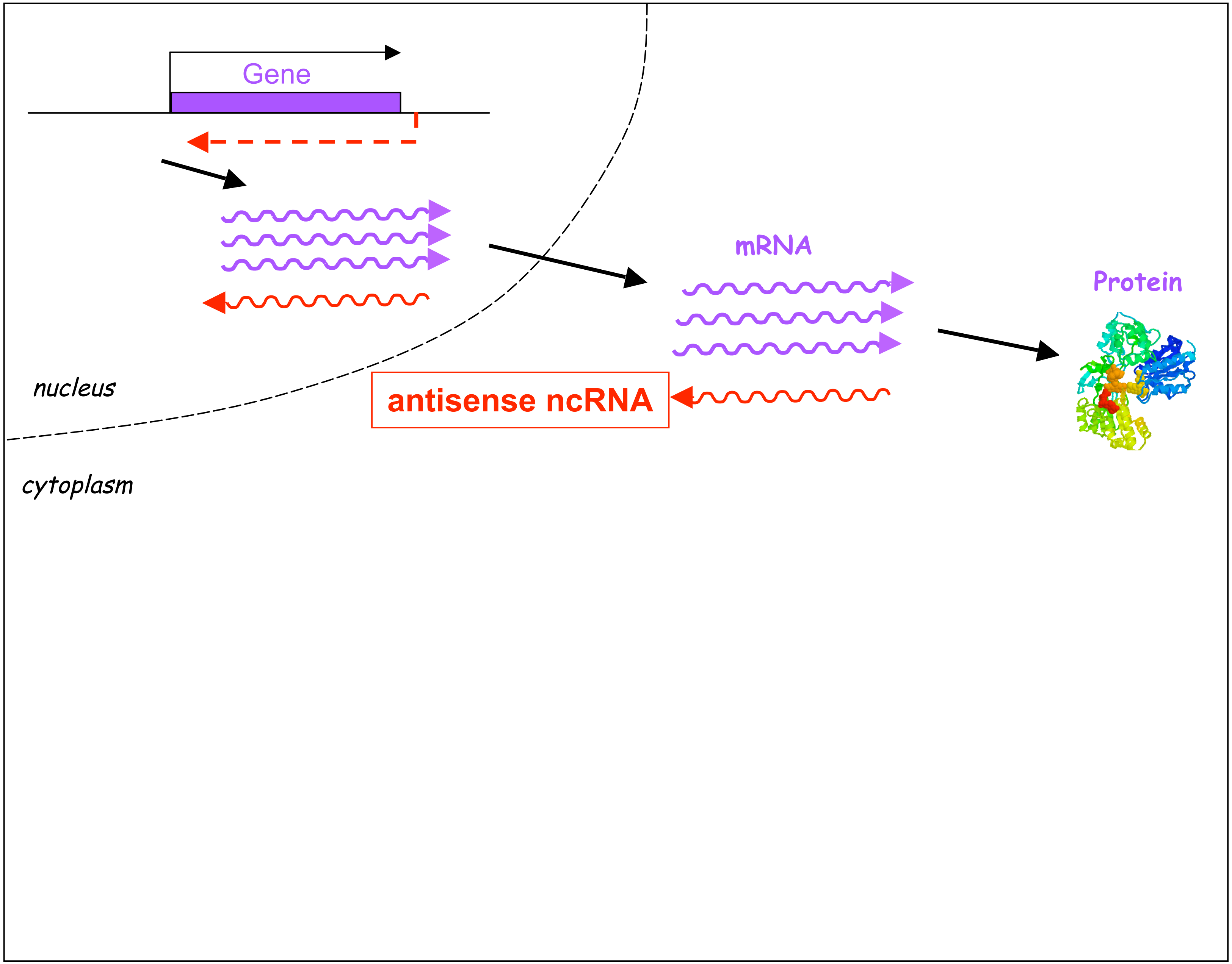
- Introduction: why need to identify new ncRNAs in yeast ?
- Technical section: how to identify new ncRNAs in yeast by NGS ?
- Conclusions and perspectives

Expression of gene : DNA ➔ mRNA ➔ Protein



Expression of gene regulated by antisense ncRNA



antisense ncRNA

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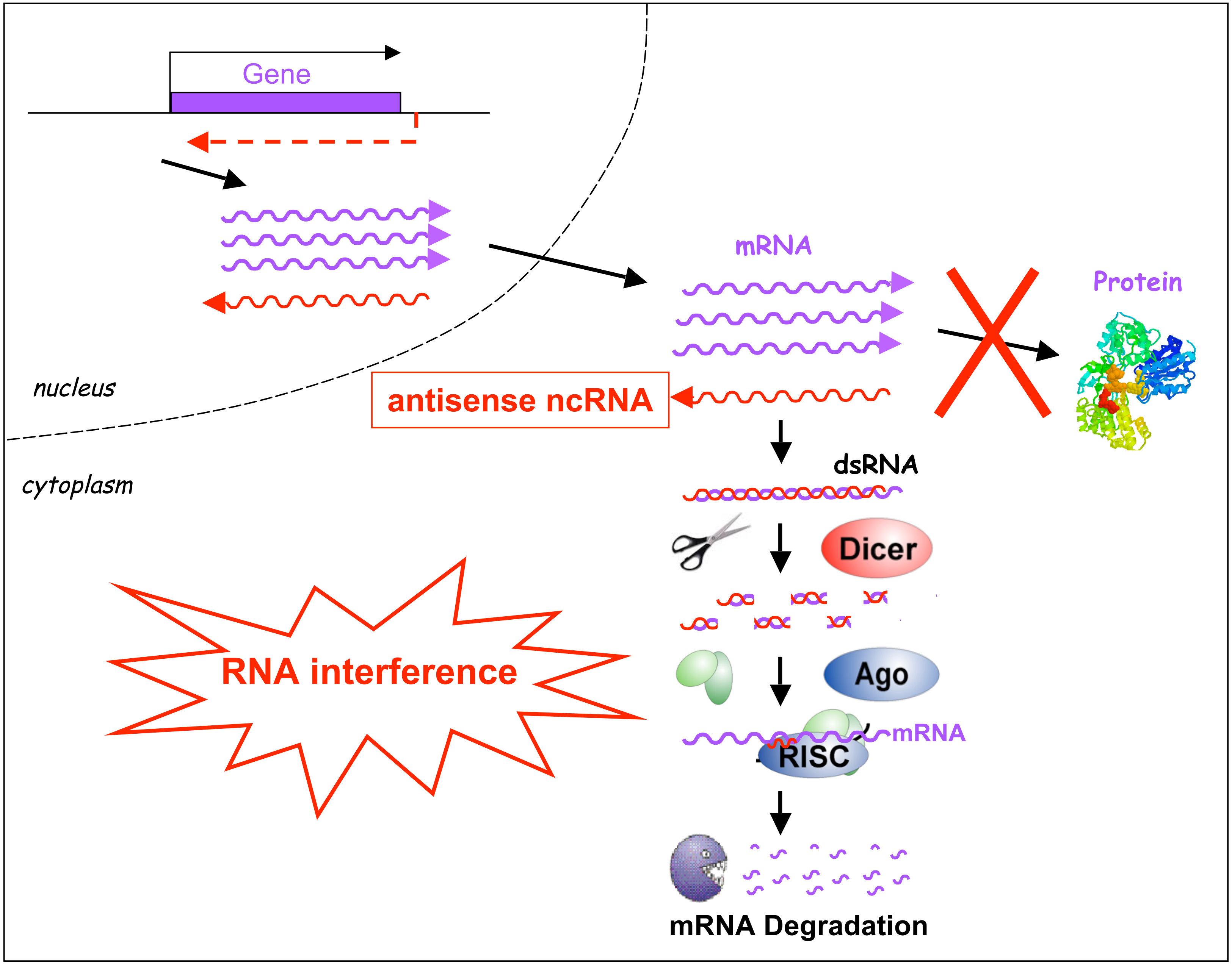
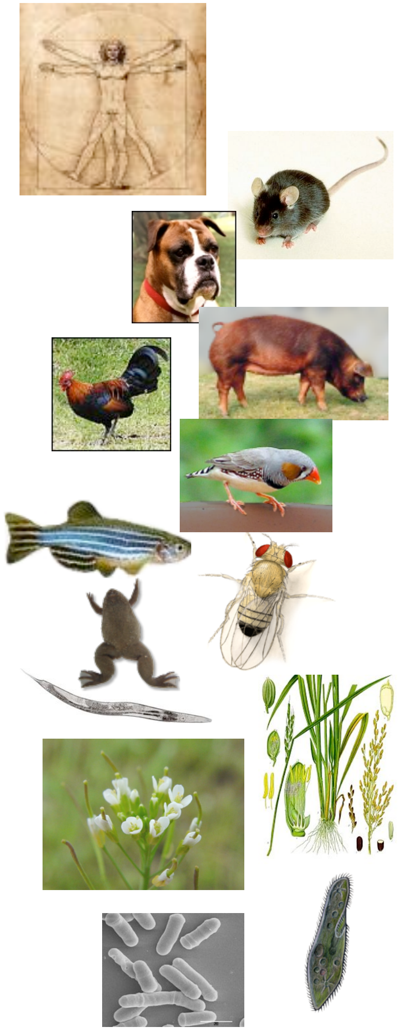
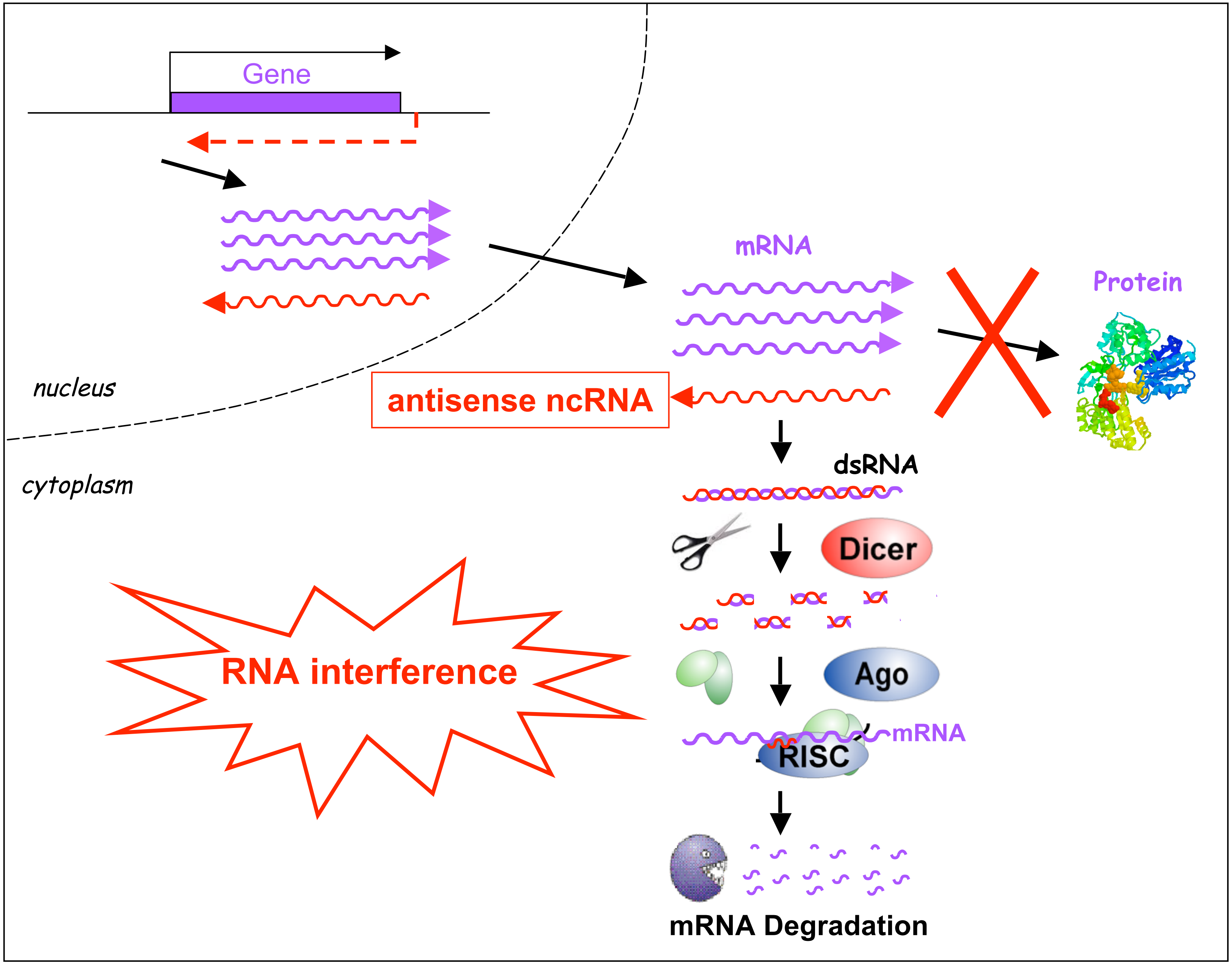


Discovered in 1998, Nobel prize 2006

In almost all eucaryotes





Gene

nucleus

cytoplasm

mRNA

Protein

antisense ncRNA

dsRNA

Dicer

Ago

mRNA

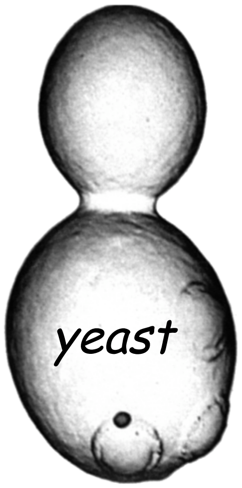
RISC

RNA interference

mRNA Degradation

Synthesize specific antisense ncRNAs to silence any targeted gene

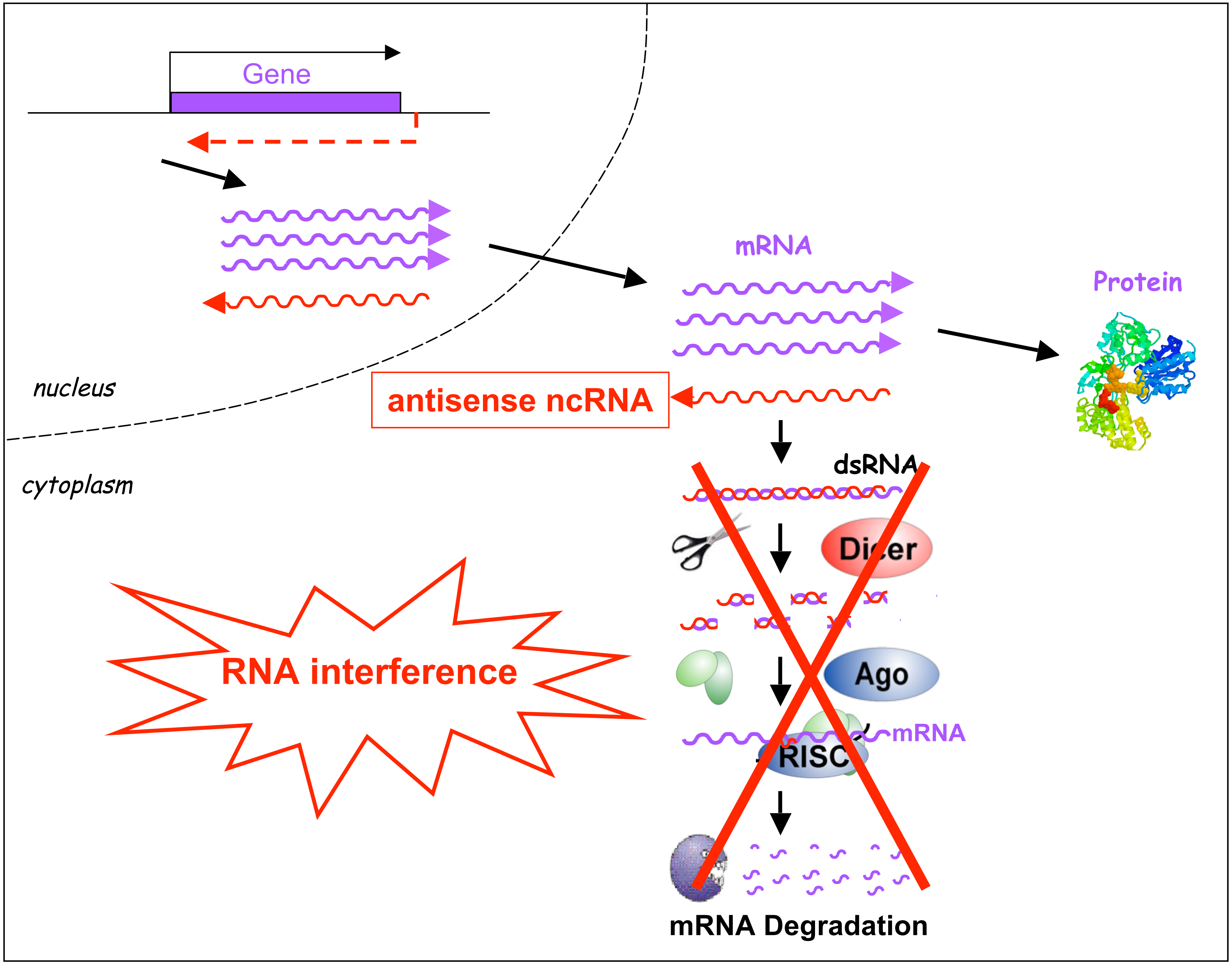
S. cerevisiae lacks RNA interference



Model organism

1st sequenced eukaryote

~13 Mpb
~6000 genes



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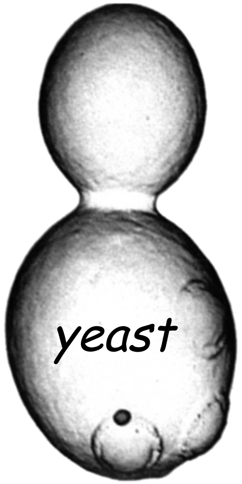

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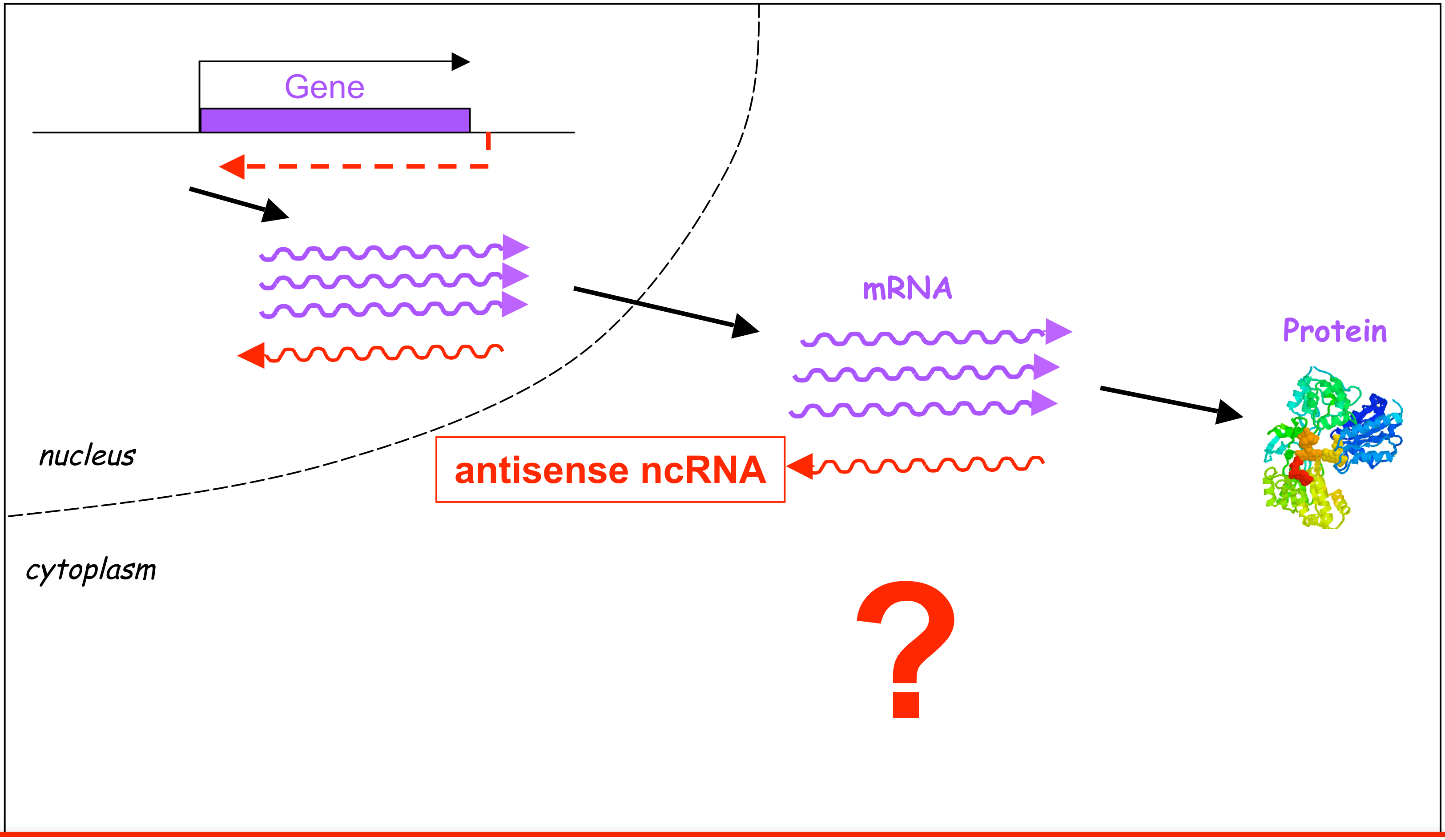
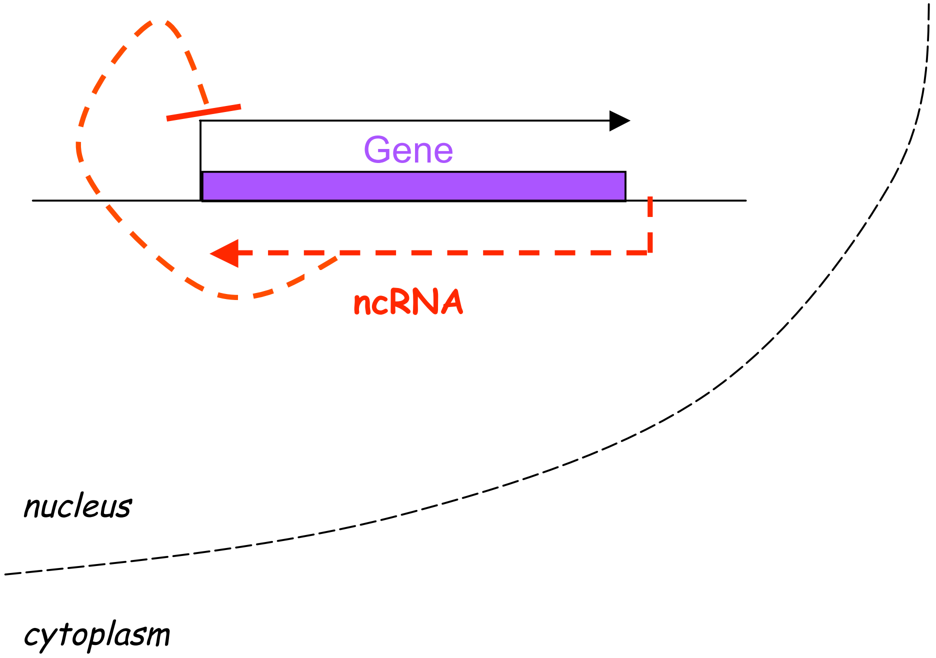


Does a mechanism of regulation of gene expression by ncRNA exist in *S. cerevisiae* , which replaces the mechanism of RNA interference?

Antisense regulatory ncRNAs in *S. cerevisiae*



3 cases have been reported :

- Ty1 Transposon
- GAL10
- PHO84

Camblong et al., Cell 2007
Berretta et al., Gen Dev 2008
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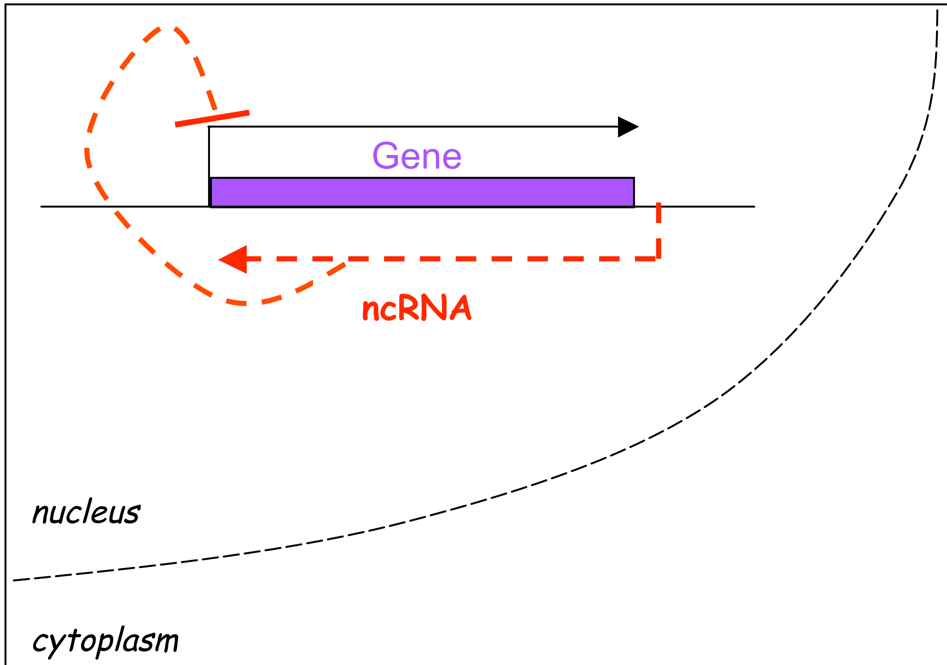

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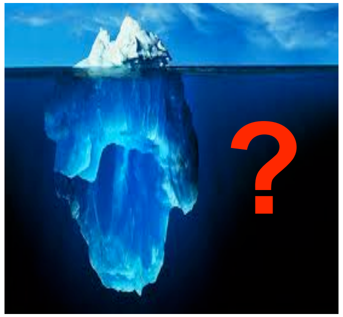

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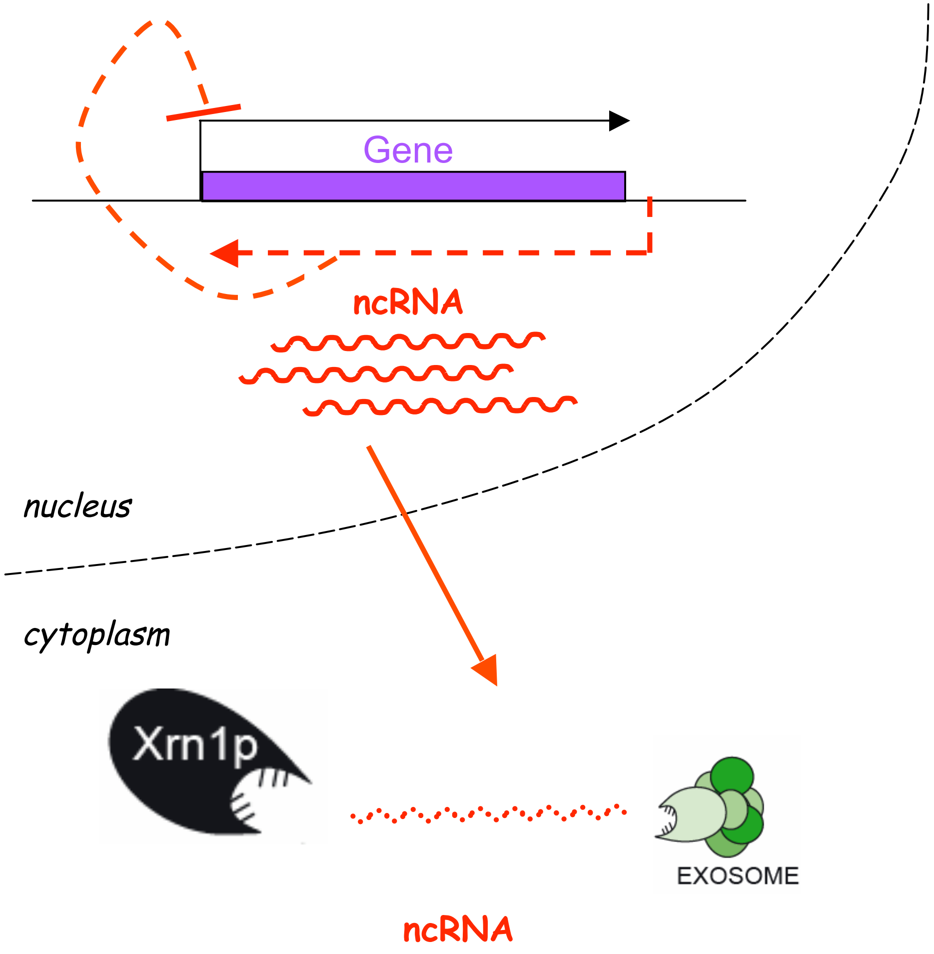
Berretta et al., Gen Dev 2008
Pinskaya et al., EMBOJ 2009

A large class of ncRNAs that regulate gene expression in the same way ?

➡ **Identify this new class of ncRNAs**

Antisense regulatory ncRNAs in *S. cerevisiae*



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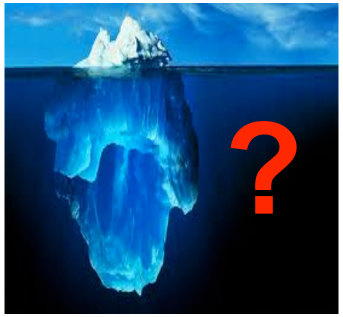

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Difficulty:

- **The half-lives of the ncRNA are short, so they are invisibles at WT.**

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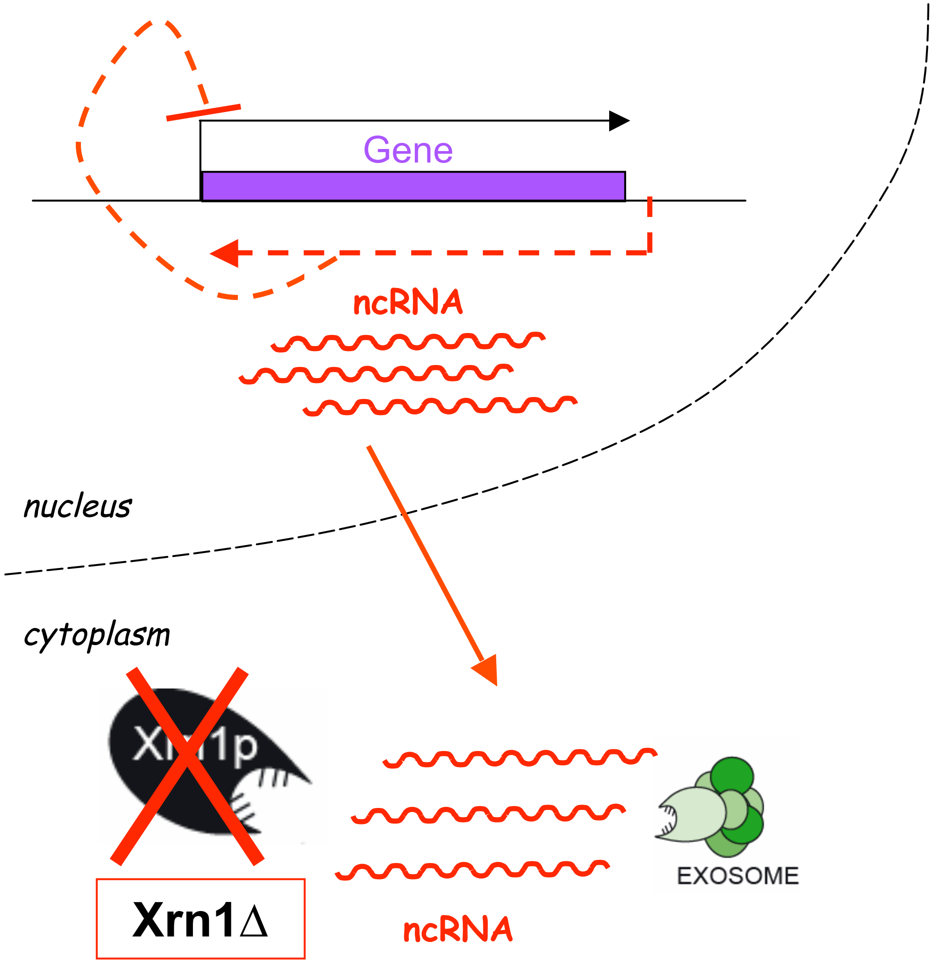

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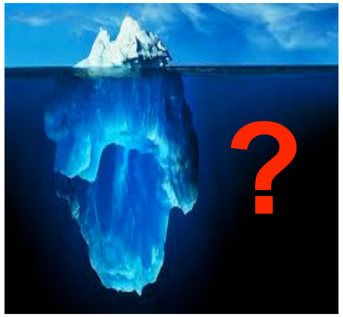

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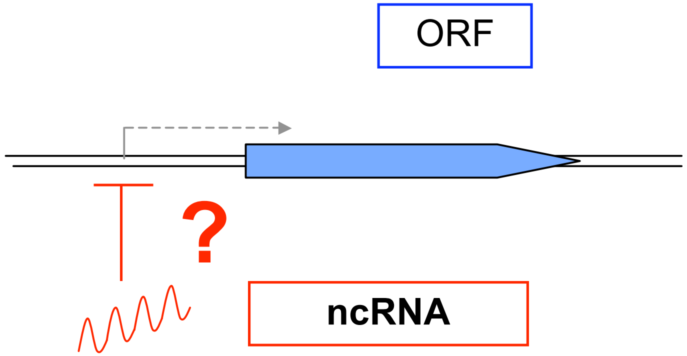
Difficulty:

- **The half-lives of the ncRNA are short, so they are invisibles at WT.**
- **But they are enriched in the mutant of an enzyme of degradation, Xrn1.**

Identification of new regulatory ncRNAs in yeast by deep sequencing

Collaboration: Antonin MORILLON (CGM/Institut Curie)

Summary



van-Dijk et al. 2011 *Nature.* 475:114

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RNA-Seq
(*xrn1*Δ, lithium, *xrn1ts*)

↓

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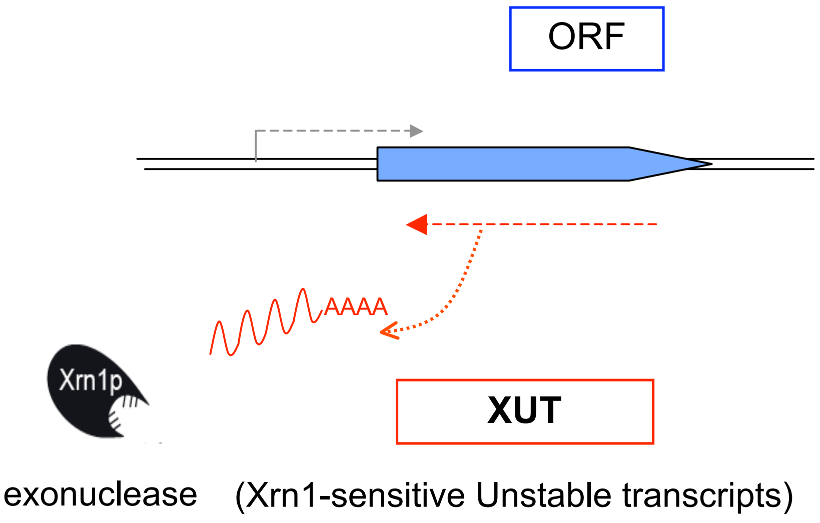
- 1,658 XUTs (Xrn1-sensitive Unstable Transcripts)
- 66% are antisense of protein-coding genes



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RNA-Seq (*xrn1*Δset1Δ)

ChIP-chip
H3K4me1/2/3
(published data)

statistical analyses:

- 297 have potential regulatory function

in *xrn1*Δ : antisense XUT ↗ and ORF ↘

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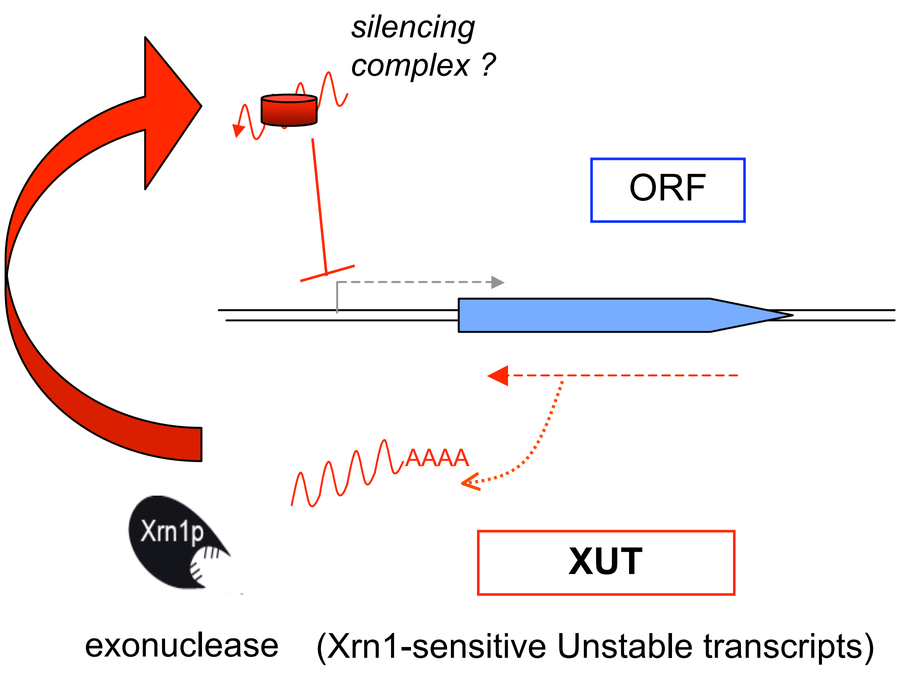

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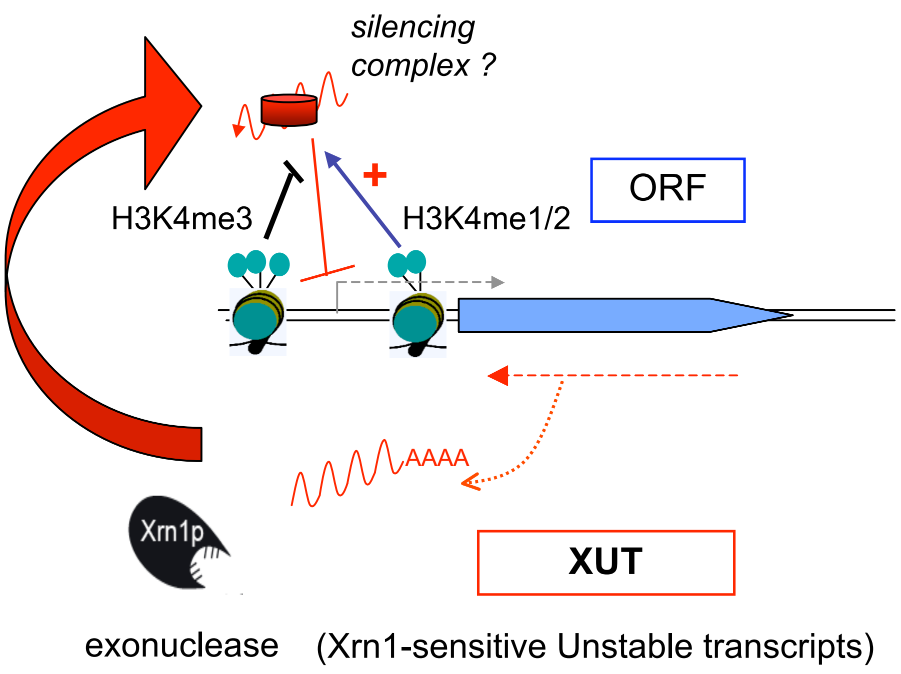

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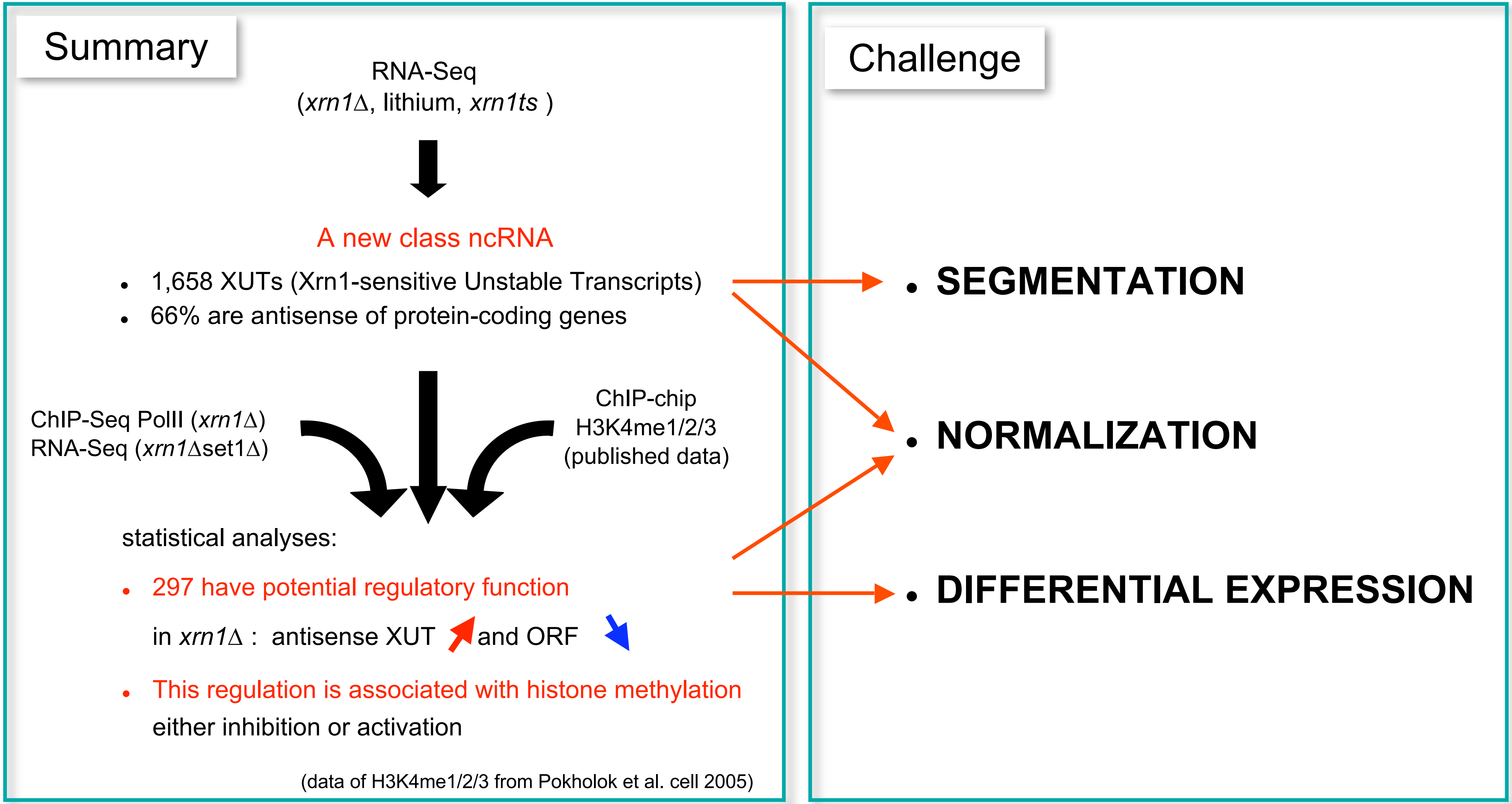

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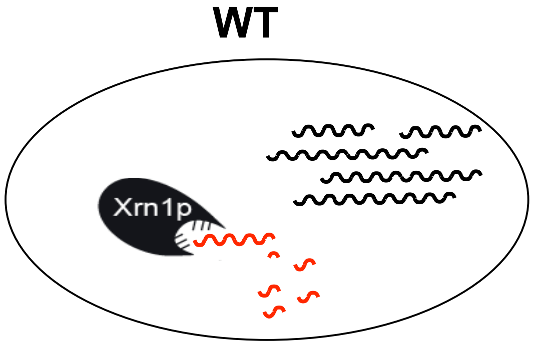
Challenge

- **SEGMENTATION**
- **NORMALIZATION**
- **DIFFERENTIAL EXPRESSION**

van-Dijk et al. 2011 *Nature.* 475:114

Identification of new ncRNAs genome-wide

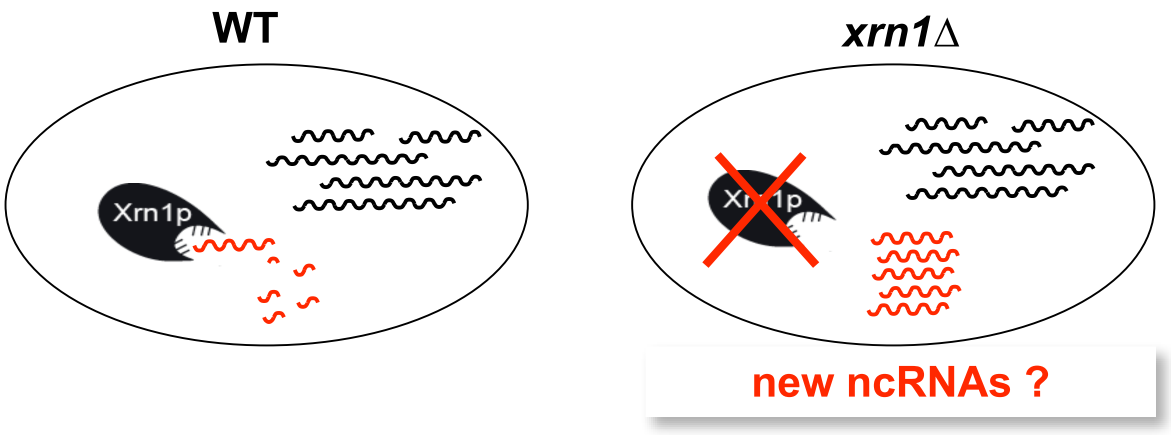


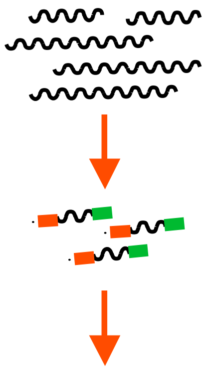
WT

*xrn1*Δ

new ncRNAs ?

Identification of new ncRNAs genome-wide





RNA extraction

Library preparation

Deep sequencing

(Illumina/SOLiD)

Identification of new ncRNAs genome-wide

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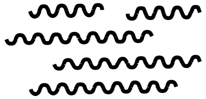

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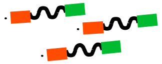
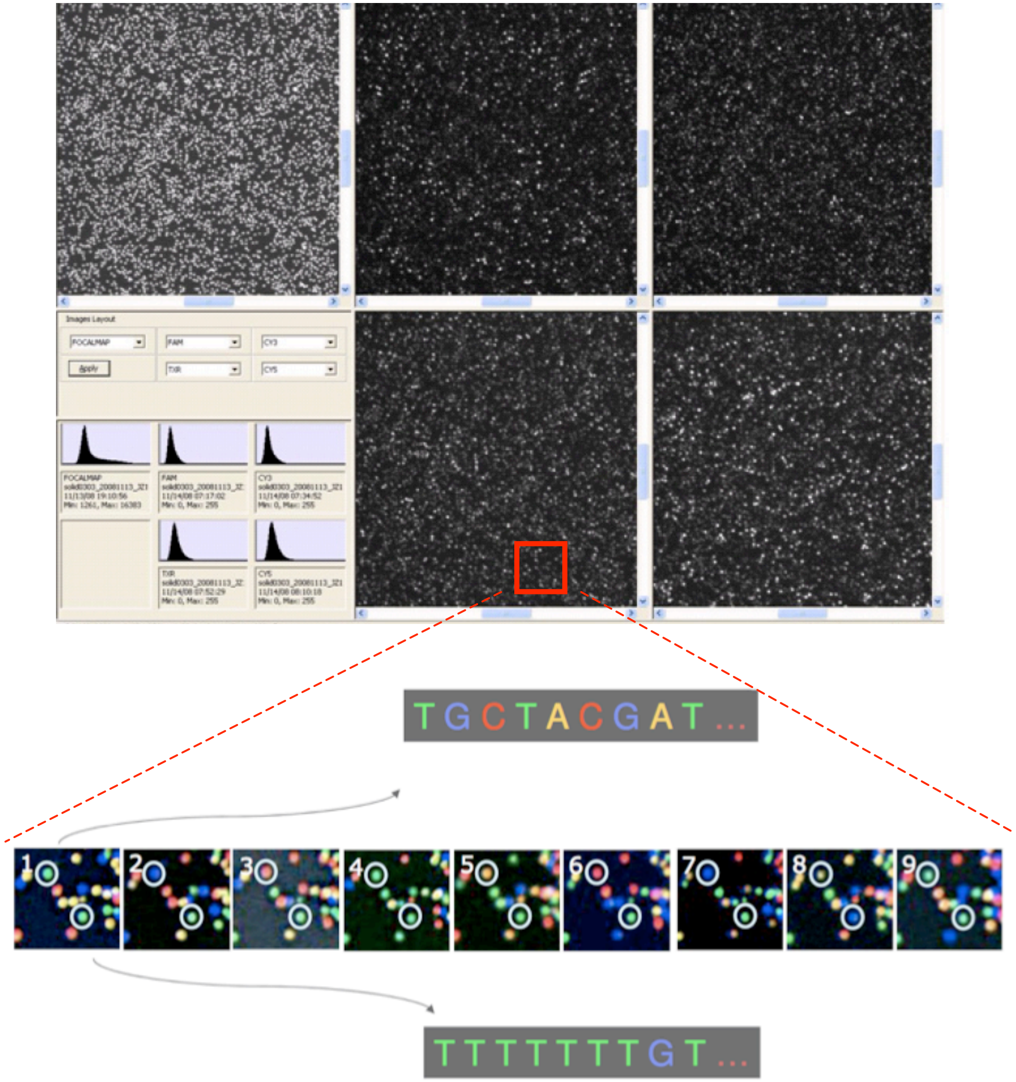


Deep sequencing

(Illumina/SOLiD)

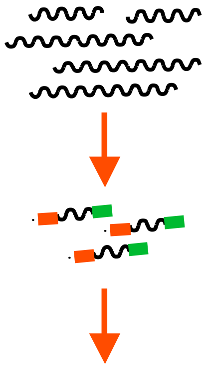
Millions of sequences in parallel



Identify the bases by images

Identification of new ncRNAs genome-wide

RNA extraction



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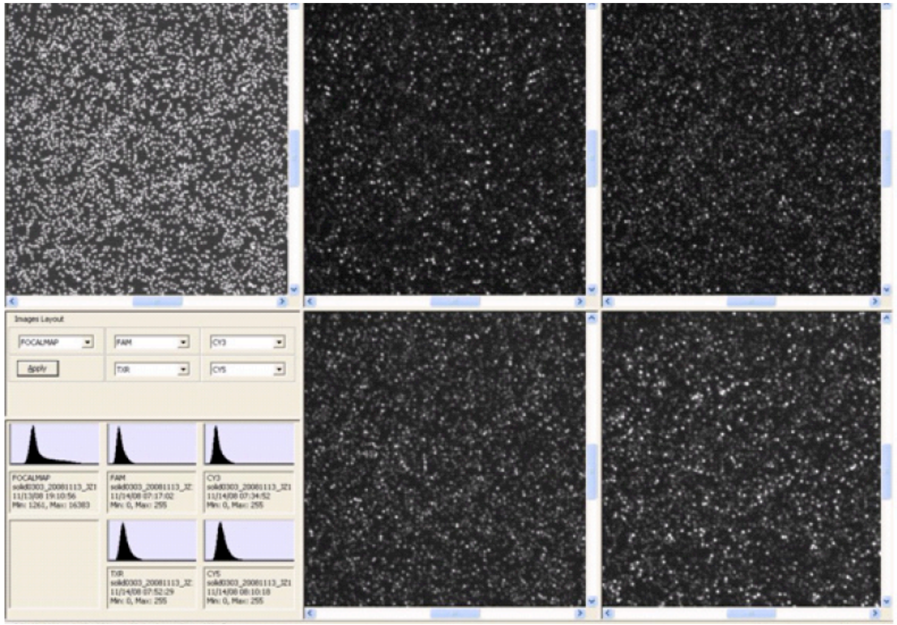

200 millions sequences of 40 nt

400X coverage of yeast genome
for each orientation

Mapping of sequence tags

ACCGTGCTTACGGGGTACATAACCGT
TAGCTGACCTGGAGGTGCGAATGCAG
TGGCACGAATGCCCCATGTATTGGCA
ACCGTGCTTACGGGGTACATAACCGT
TAGCTGACCTGGAGGTGCGAATGCAG
TGGCACGAATGCCCCATGTATTGGCA
TAGCTGACCTGGAGGTGCGAATGCAG
TGGCACGAATGCCCCATGTATTGGCA
TAGCTGACCTGGAGGTGCGAATGCAG

ACCGTGCTTACGGGGTACATAACCGTGCATTAGCATTAGCTGACCTGGAGGTGCGAATGCAG
TGGCACGAATGCCCCATGTATTGGCACGTAATCGTAAGCGACTGGACCTCCACGCTTACGTC

Mapping of sequence tags

TAGCTGACCTGGAGGTGCGAATGCAG

TAGCTGACCTGGAGGTGCGAATGCAG

ACCGTGCTTACGGGGTACATAACCGTGCATTAGCATTAGCTGACCTGGAGGTGCGAATGCAG
TGGCACGAATGCCCCATGTATTGGCACGTAATCGTAAGCGACTGGACCTCCACGCTTACGTC

Mapping of sequence tags

```
                                                GAGGTGCGAATG
                                           ACCTGGAGGTGCGAATG
                                      GCTGACCTGGAGGTGCGAATGCAG
      CTTACGGGGTACATAACCGTGCATTAG     GCTGACCTGGAGGTGCGAATGCAG
   GTGCTTACGGGGTACATAACCGTGCAT      TAGCTGACCTGGAGGTGCGAATGCAG
ACCGTGCTTACGGGGTACATAACCGTGCATTAGCATTAGCTGACCTGGAGGTGCGAATGCAG
TGGCACGAATGCCCCATGTATTGGCACGTAATCGTAAGCGACTGGACCTCCACGCTTACGTC
TGGCACGAATGCCCCATGTATTGGCA         AAGCGACTGGACCTCCACGCTTACGTC
TGGCACGAATGCCCCATGTATTGGCA
    ACGAATGCCCCATGTATTGGCACGTA
      GAATGCCCCATGTATTGGCACGTAAT
           CCCCATGTATTGGCACGTAATCGTAA
```

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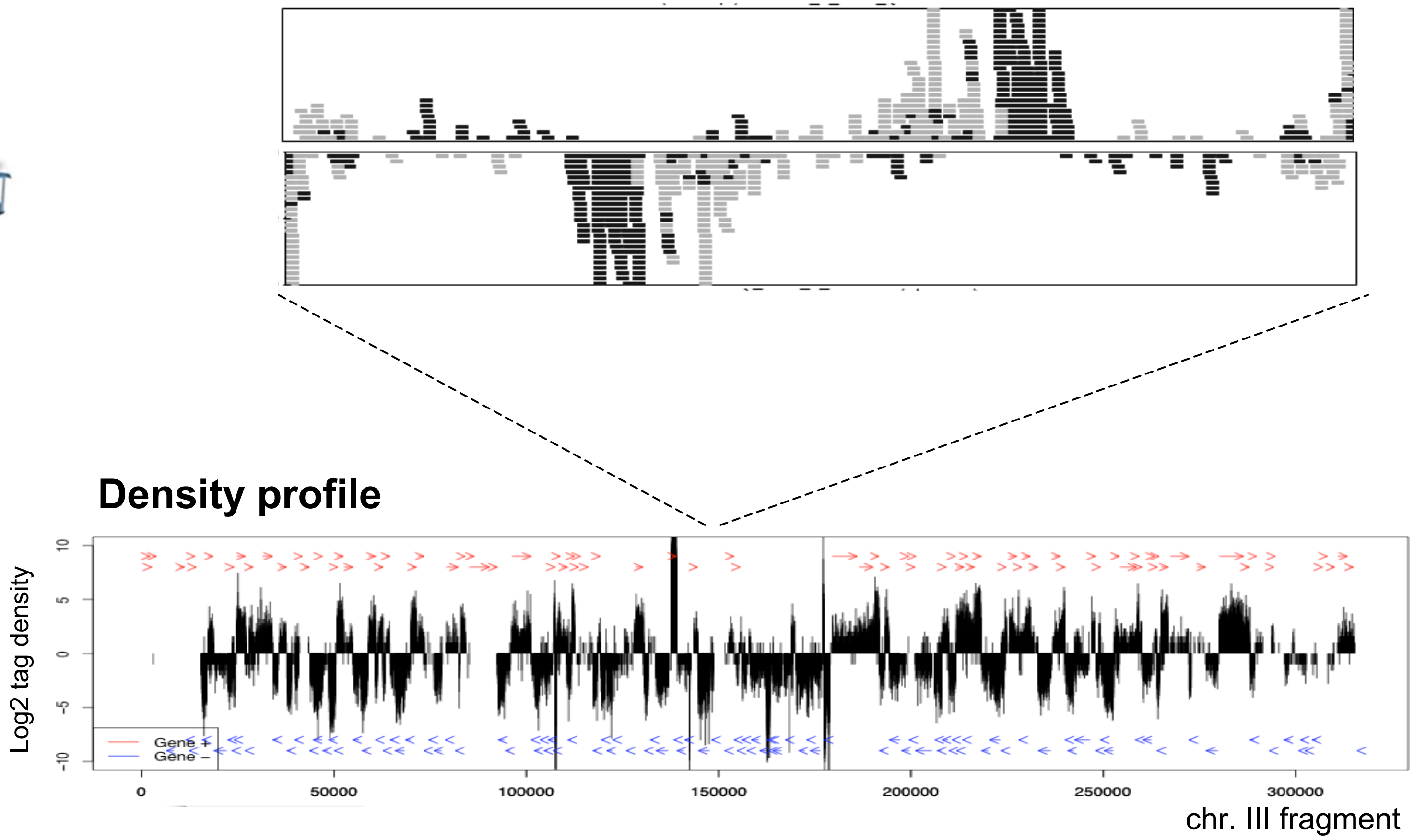
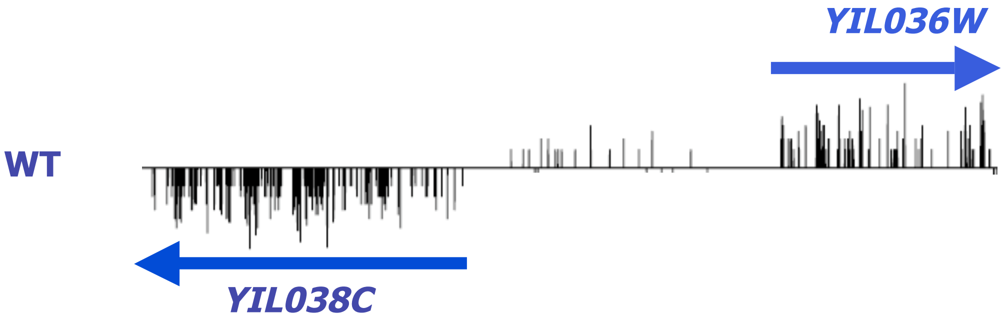


Density profile

- Vertical lines represent numbers of sequences mapped on each position along the genome.
- Disjointed blocks correspond to different genes.

Identification of new ncRNAs



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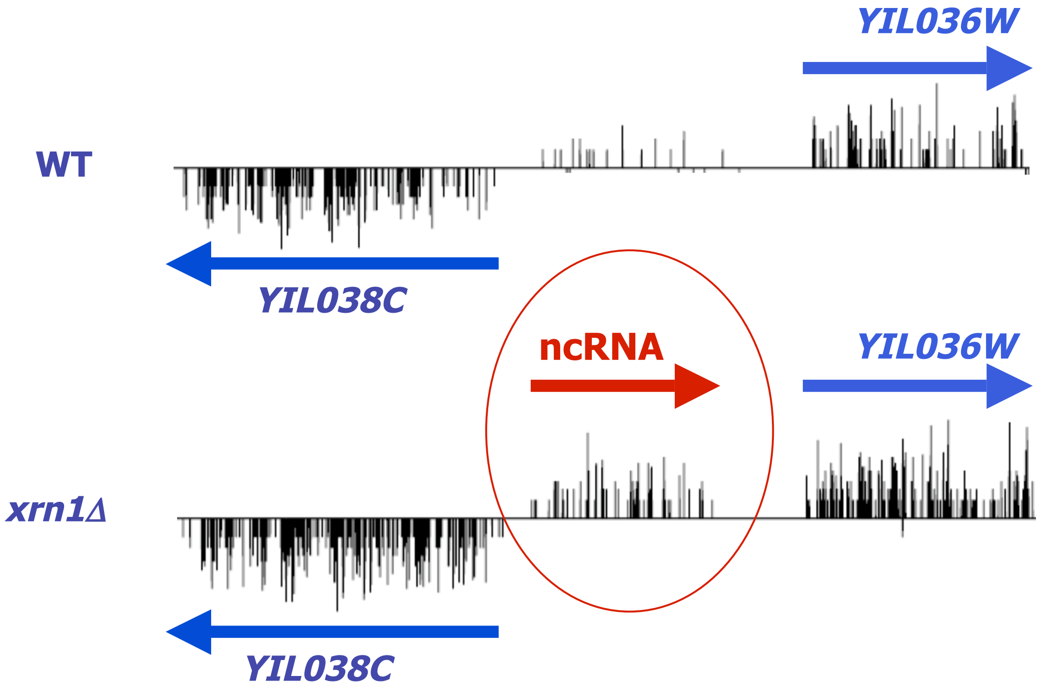

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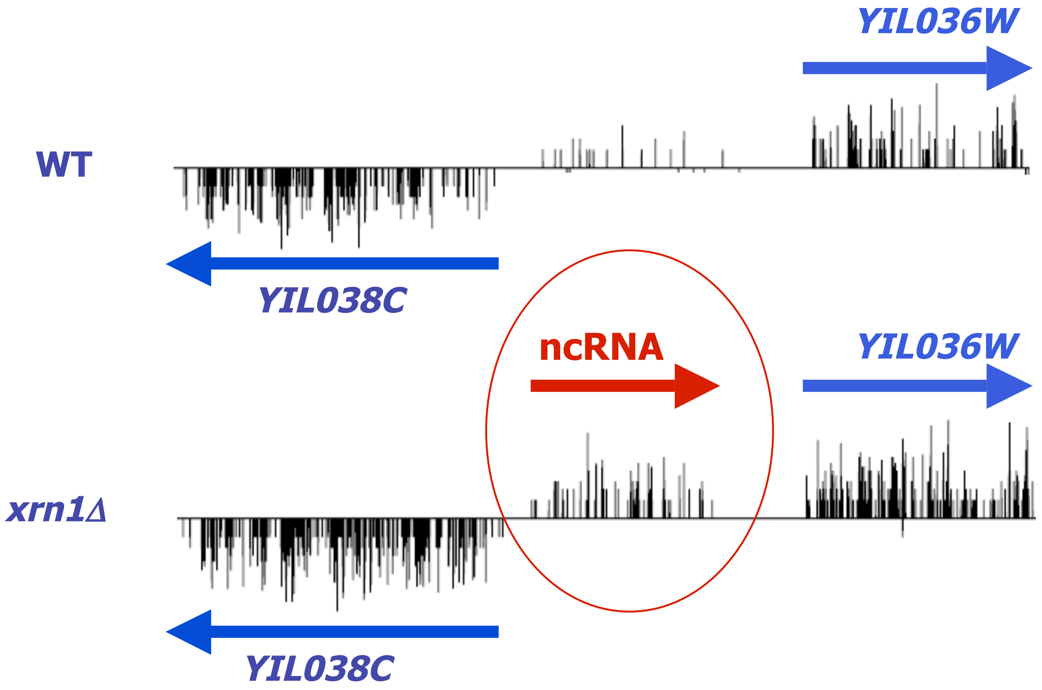
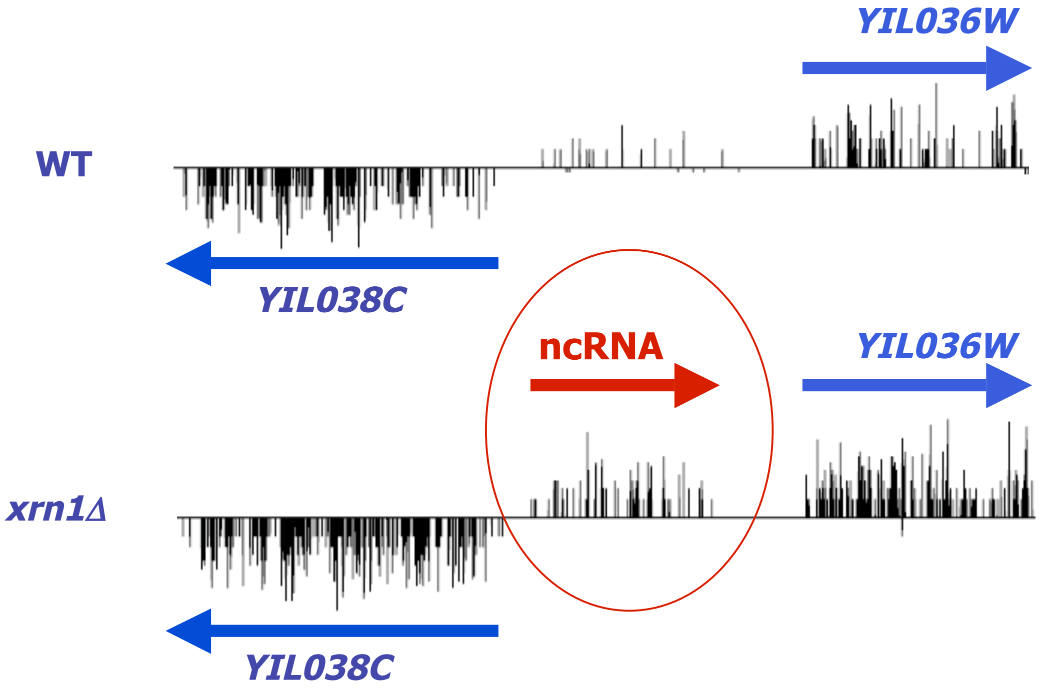
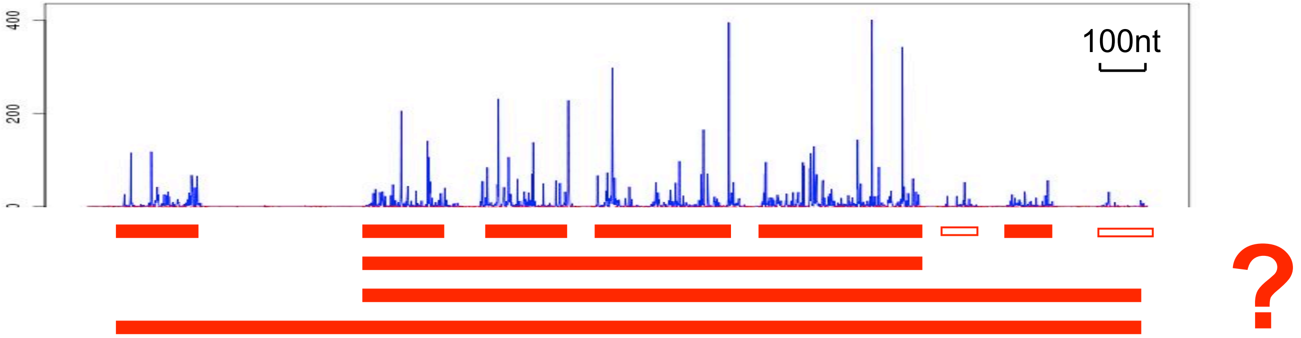


Define transcript unit
(segmentation)

Identification of new ncRNAs



Define transcript unit (segmentation)

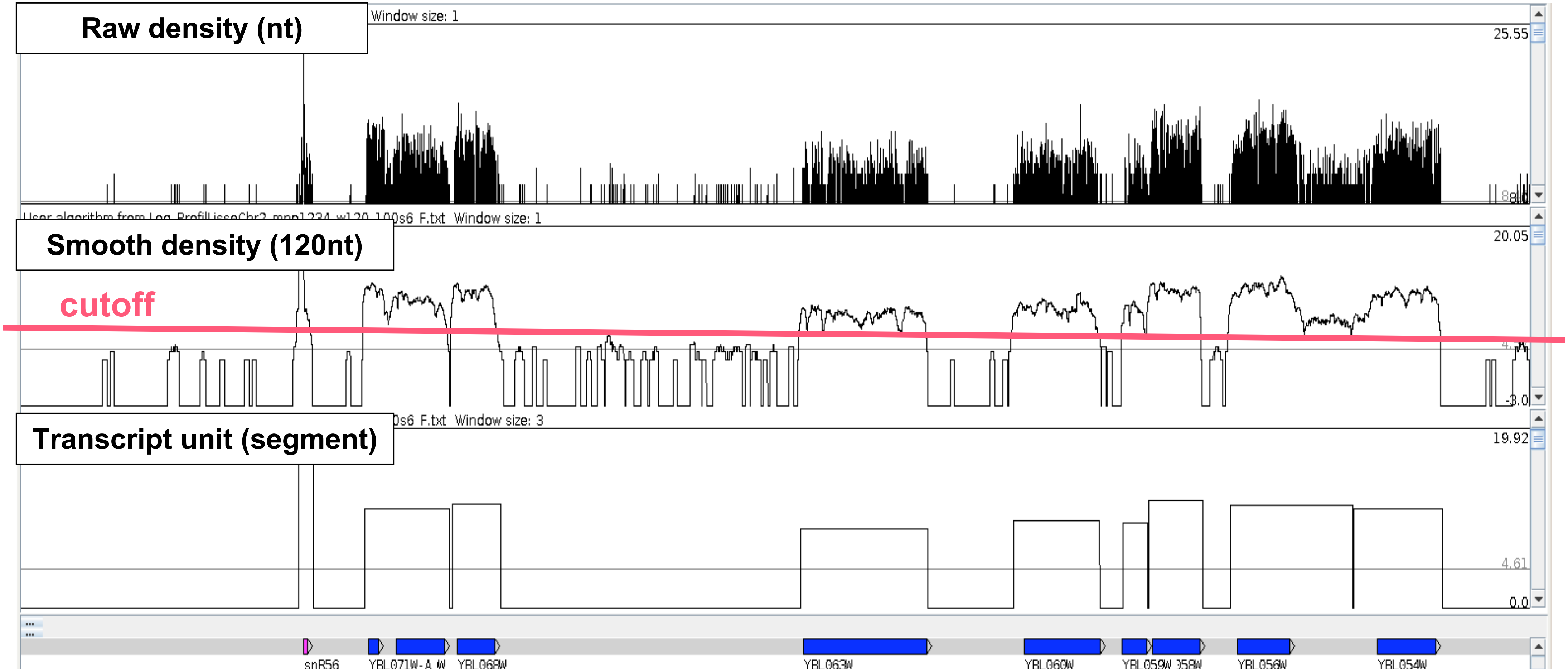


Challenge of segmentation due to data heterogeneity

Challenge 1: Define transcript unit (Segmentation)

Segmentation process

Segmentation process

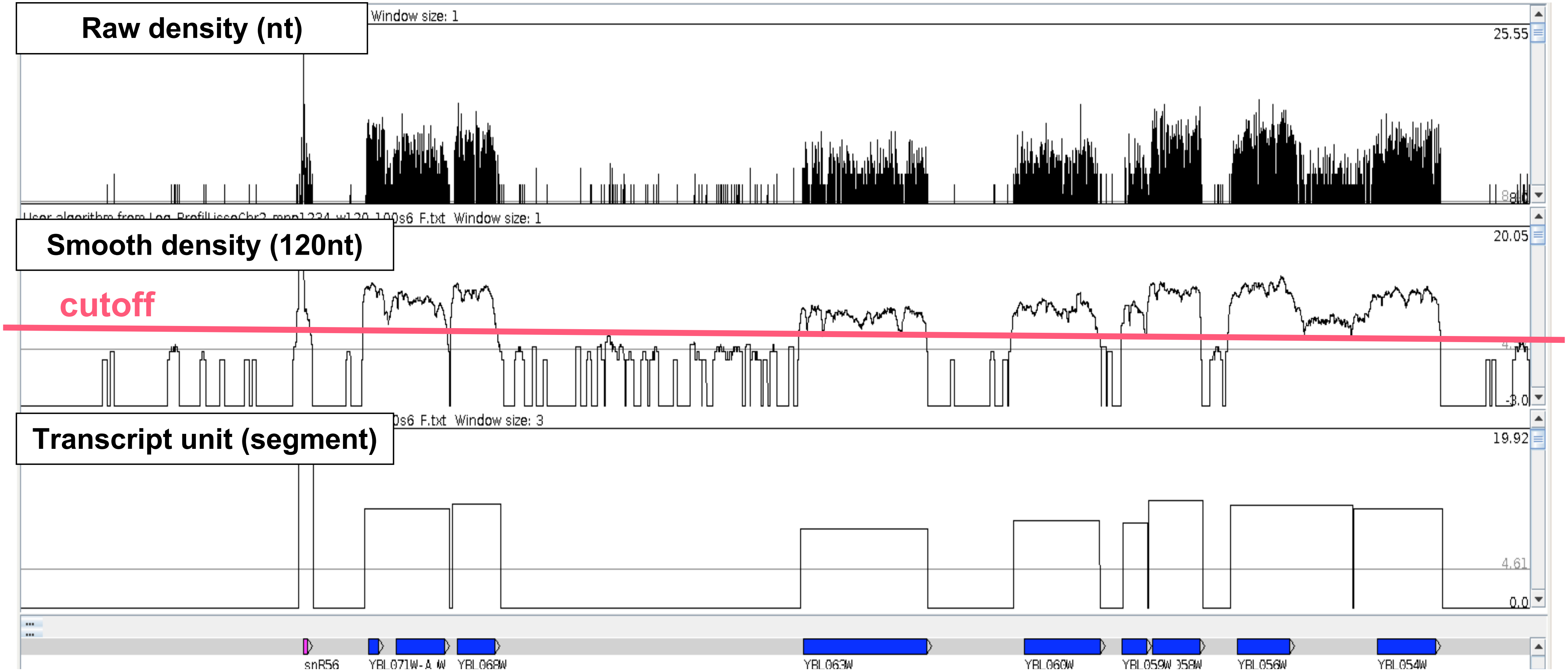


Step 1. Defining a sliding window of 120 nucleotides

Step 2. Mean of the window (smoothing) and slide +1

Step 3. Filtering (threshold) and attribute a box unit

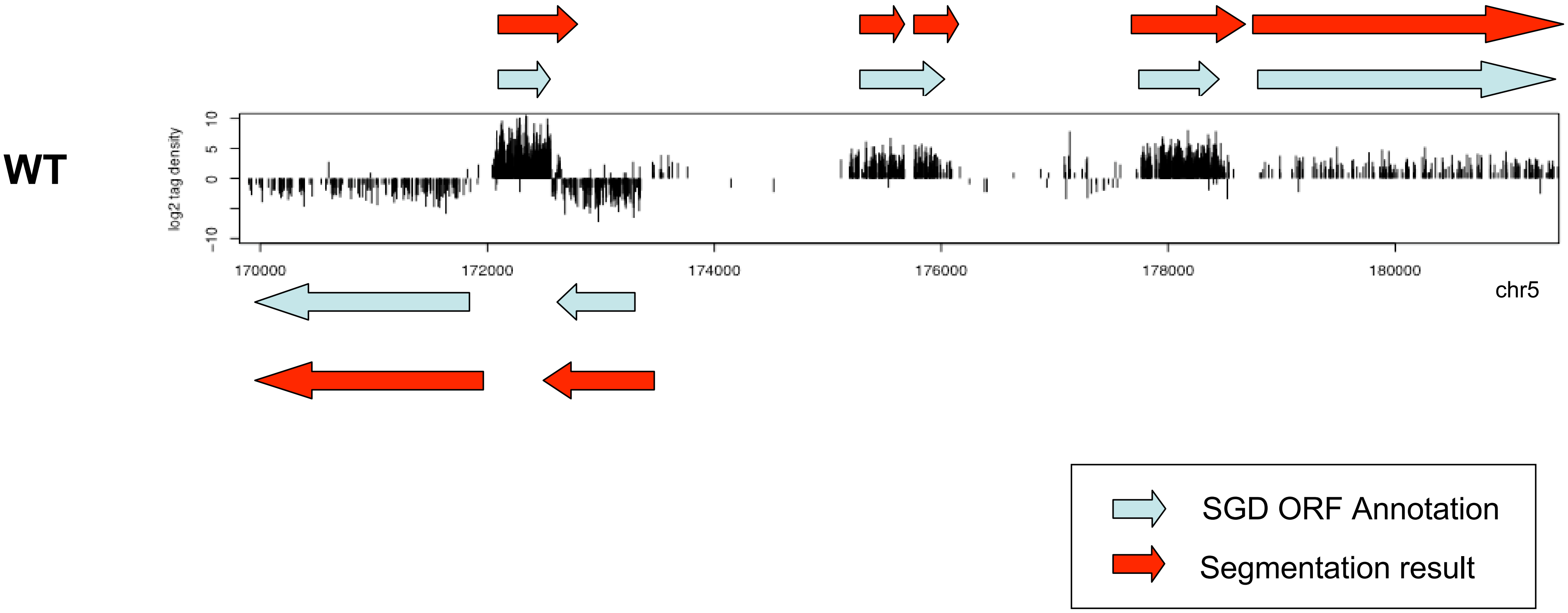
Segmentation process



Step 4. Comparison with existing features (ORFs)

Good parameters (e.g. cutoff) ⟷ High Sensitivity & Specificity

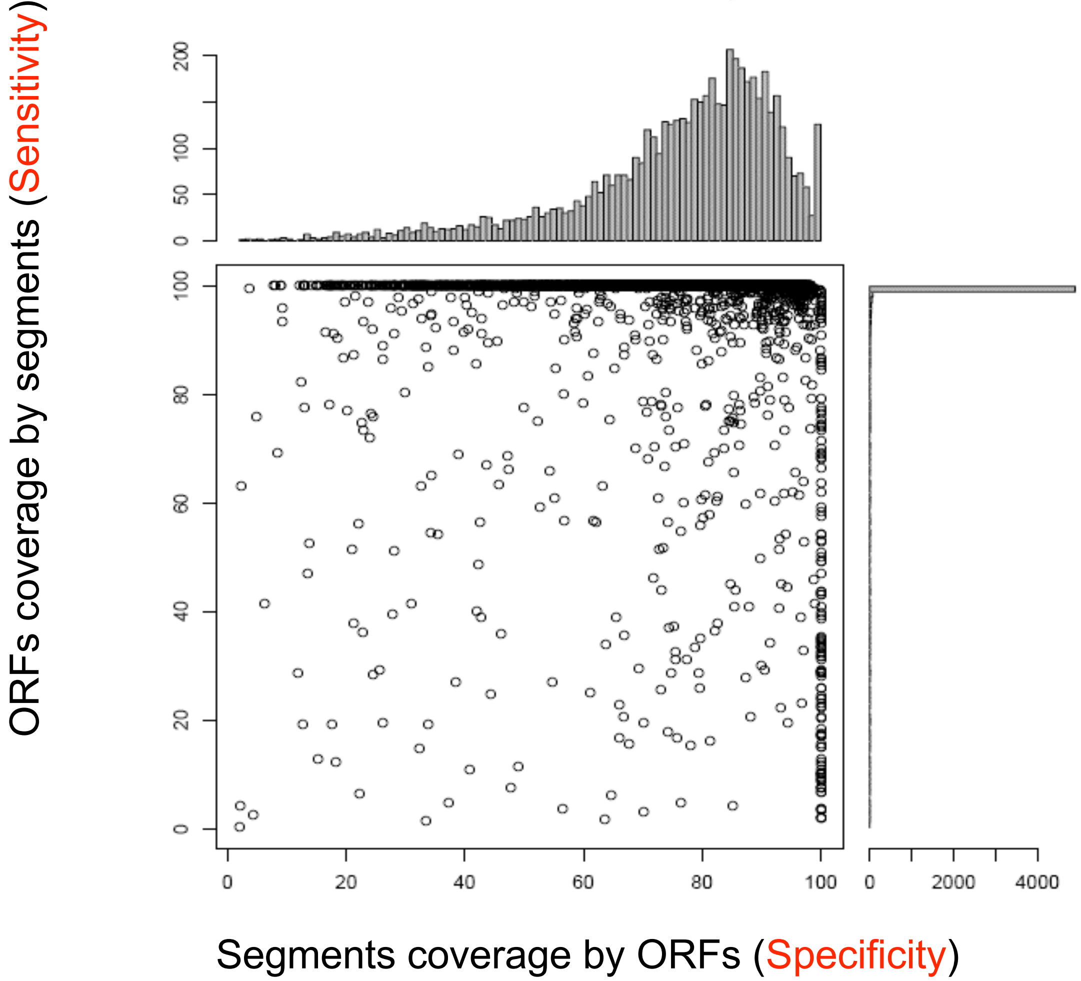
Segmentation process

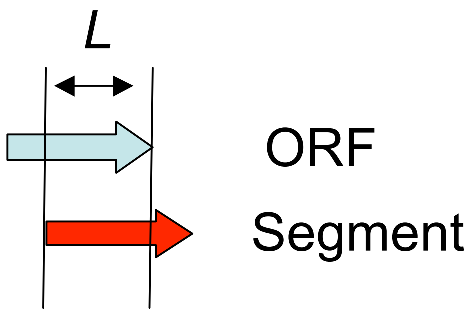


Step 4. Comparison with existing features (ORFs)

Validation of the segmentation process

ORF coverage



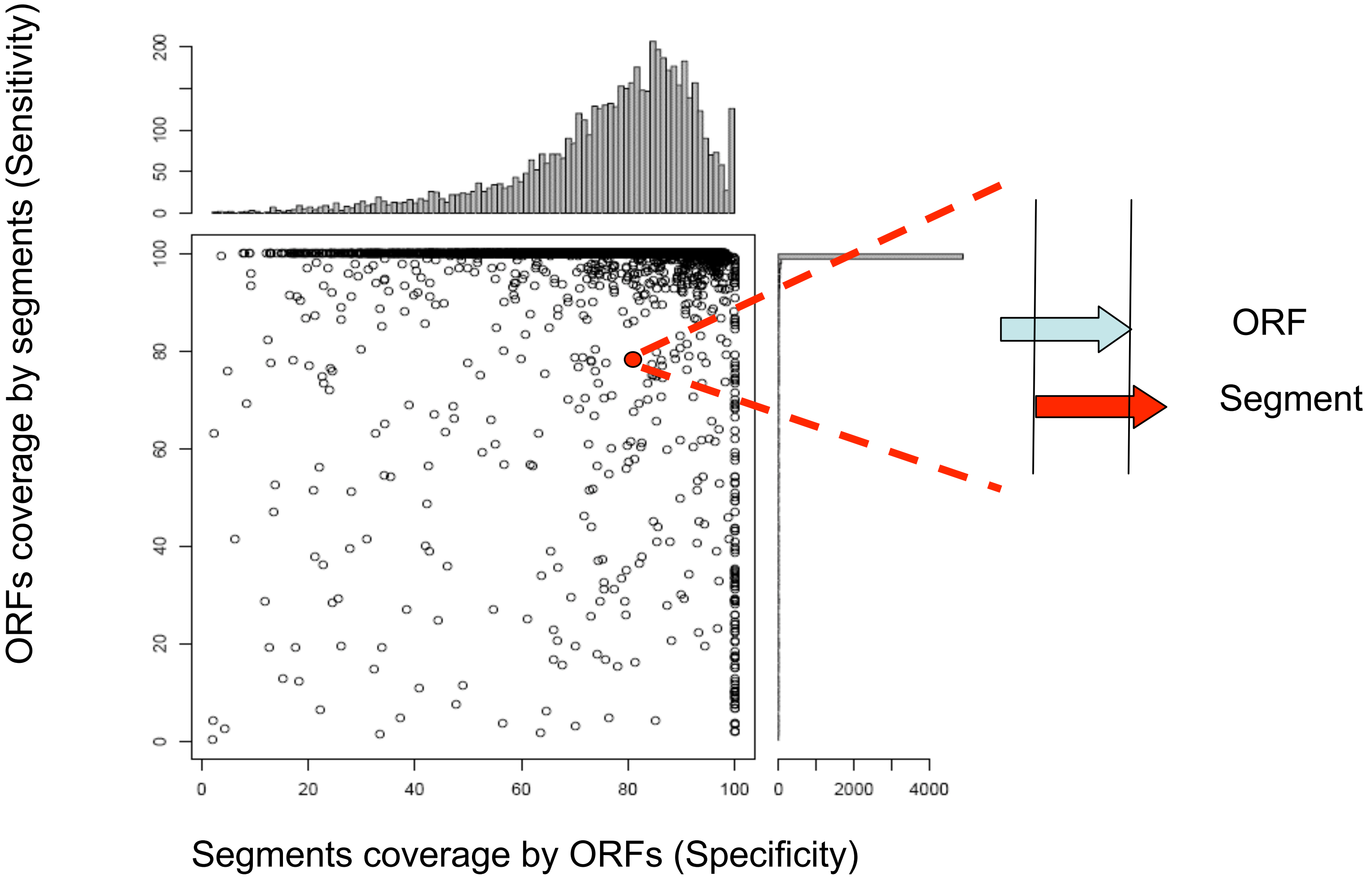


Sn (Sensitivity) : ORFs coverage by segments = L/L_{ORF}

Sp (Specificity) : segments coverage by ORF = $L/Lseg$

Validation of the segmentation process

ORF coverage



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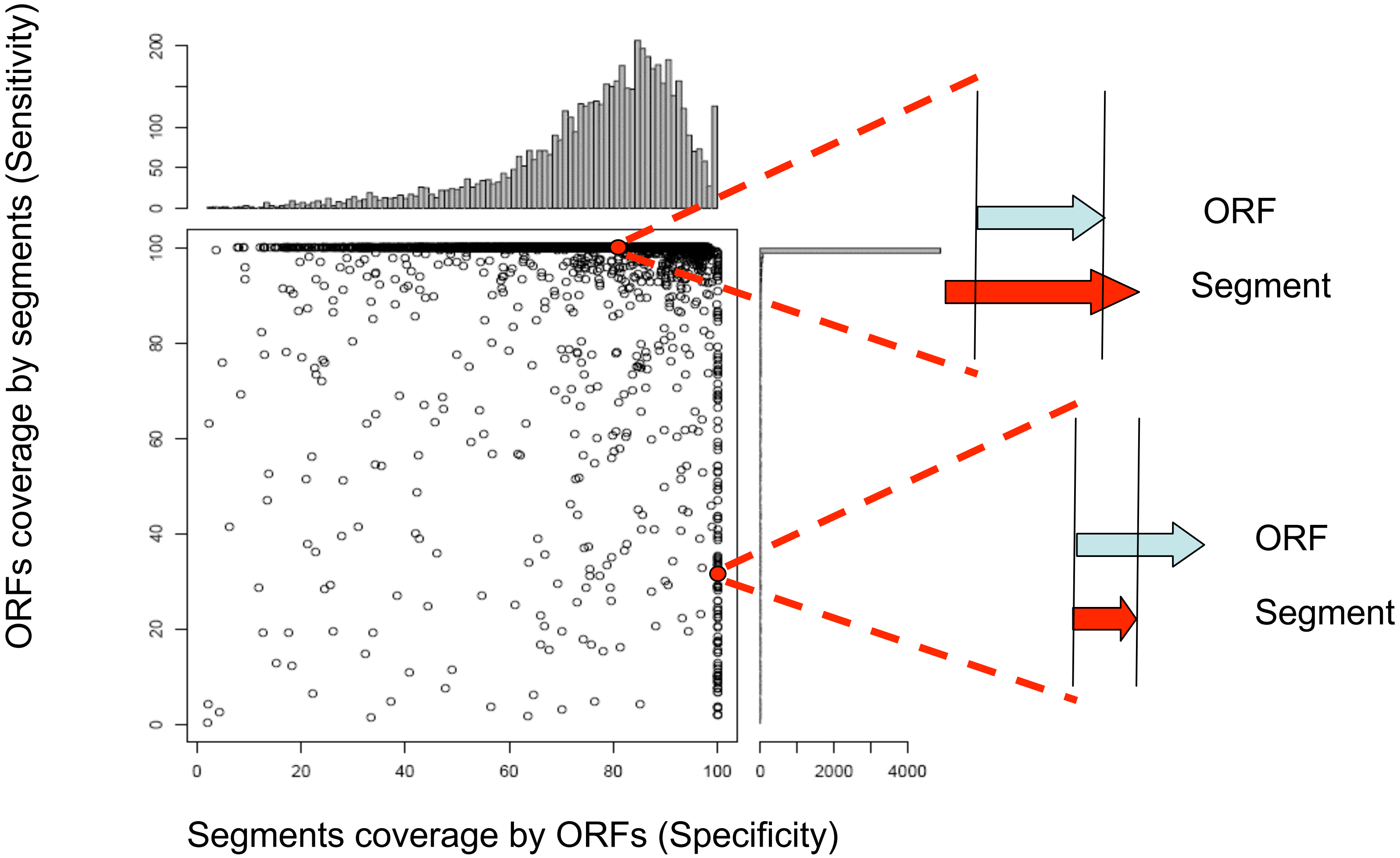

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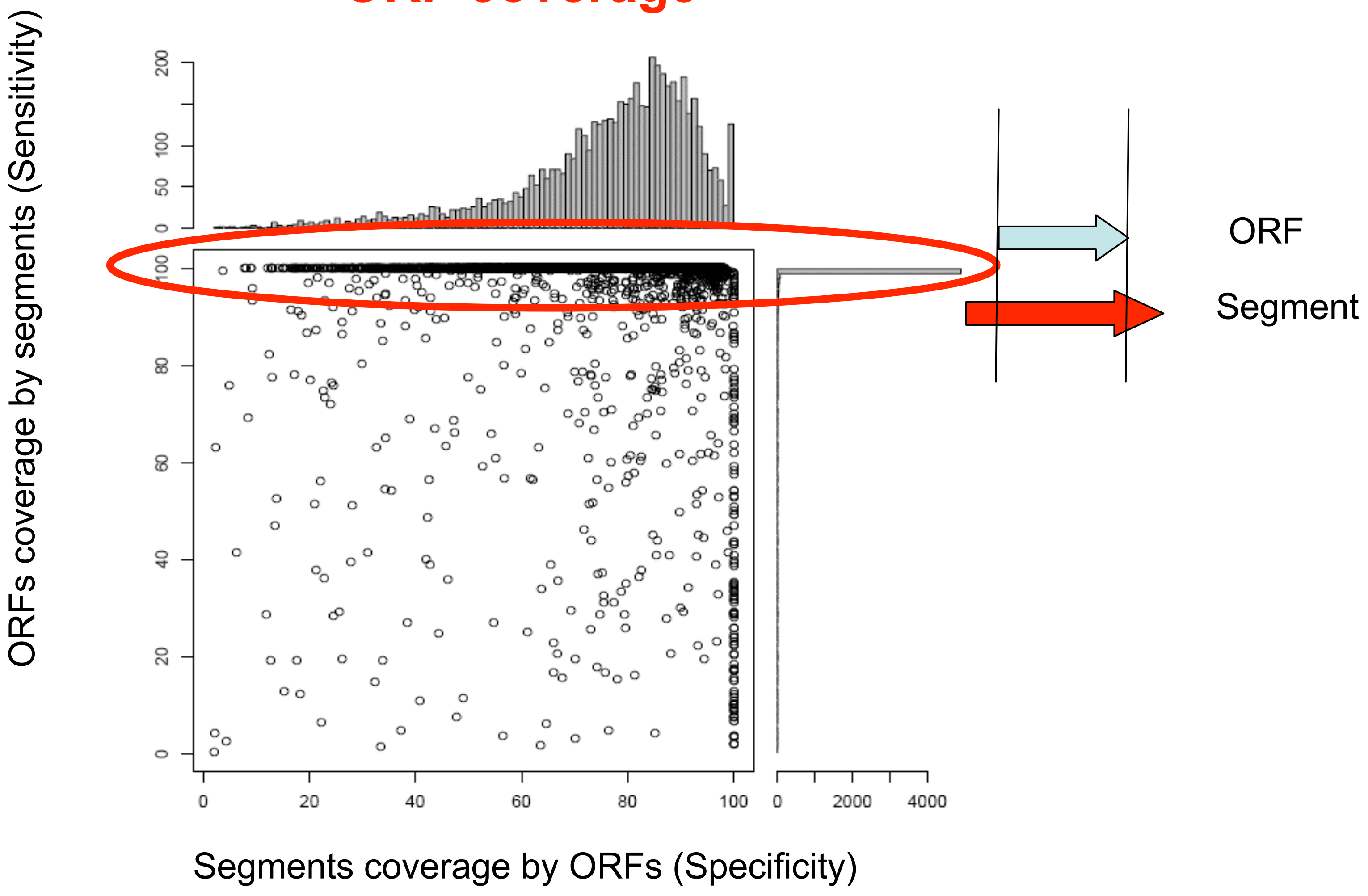

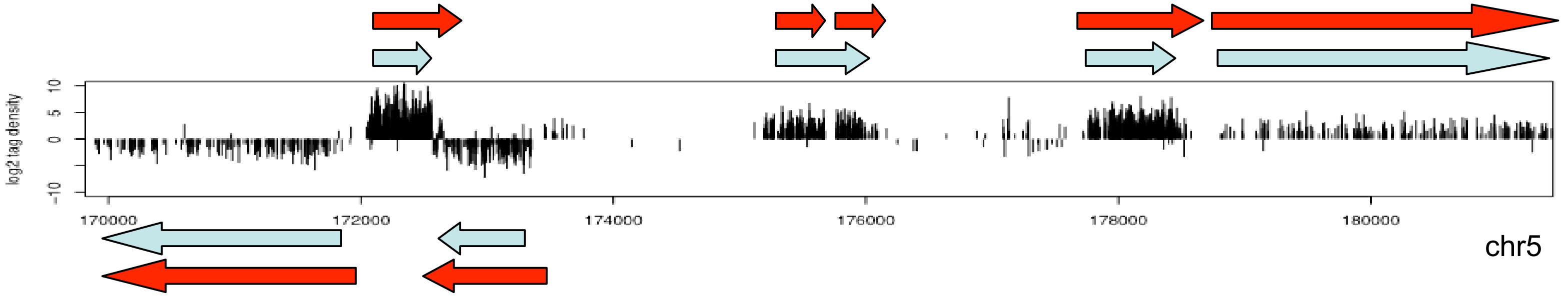

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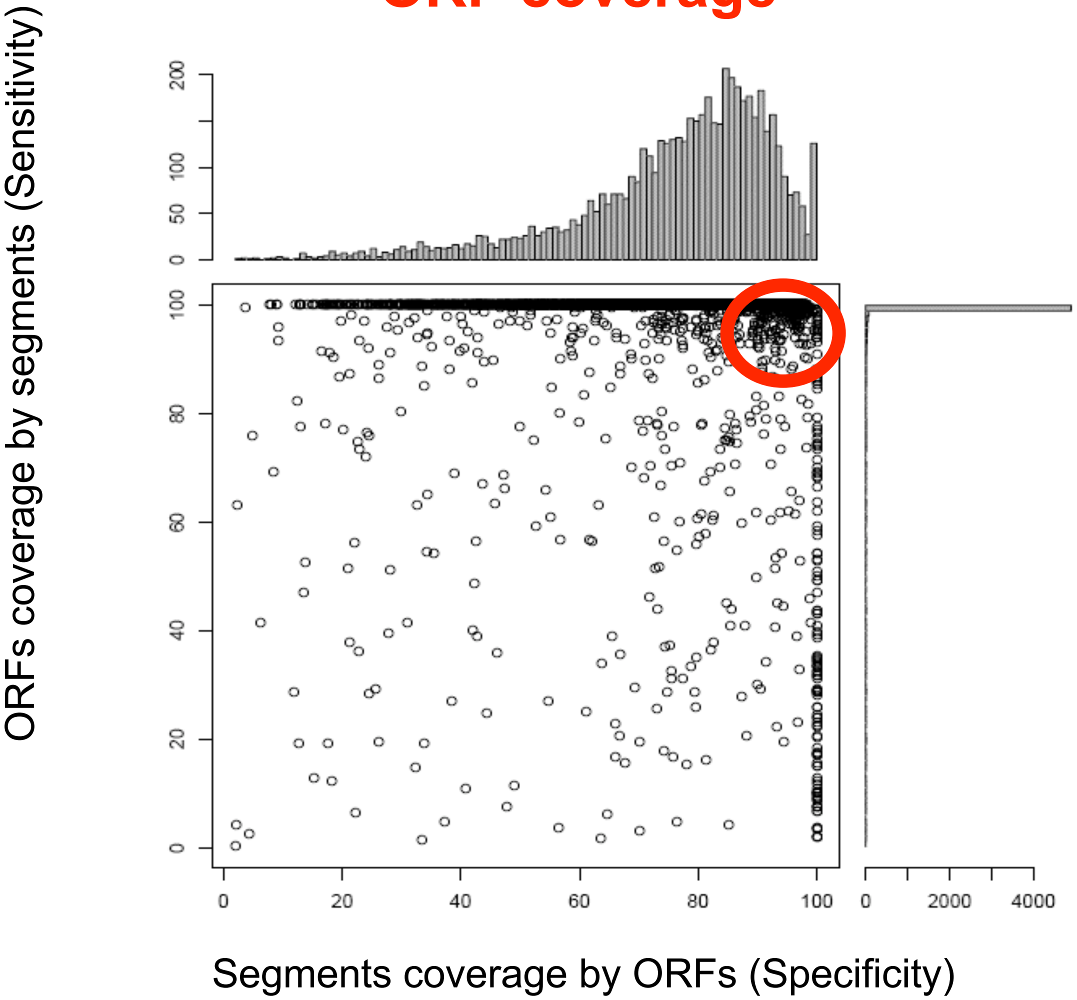
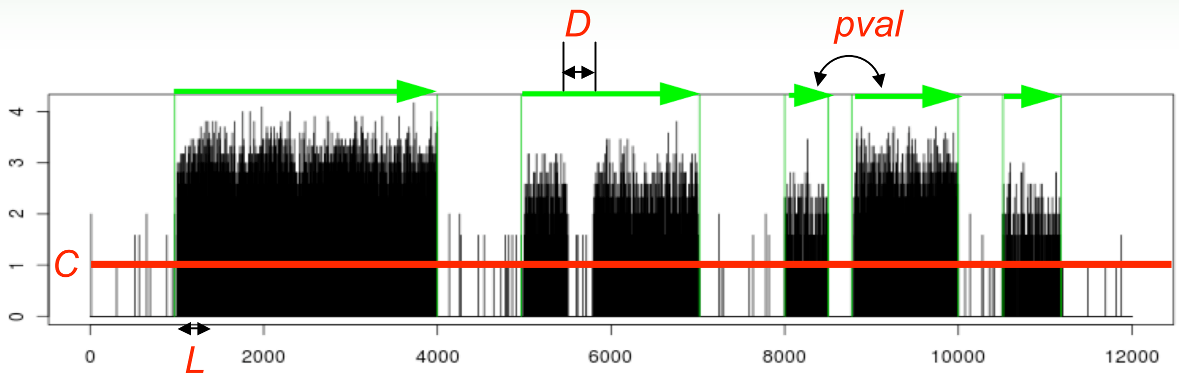


Good assembly ⟷ High Sensitivity & Specificity

Validation of the segmentation process

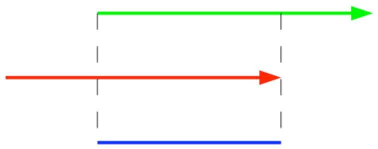


Parameters :

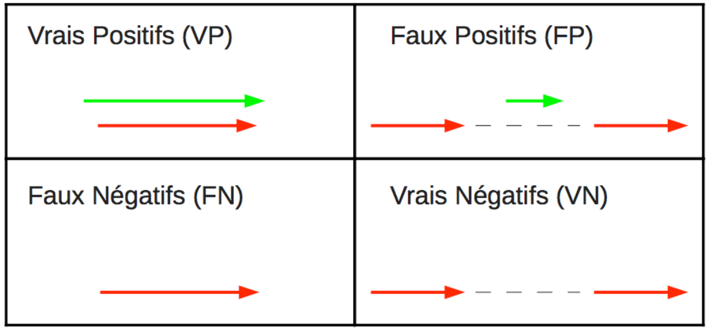
- *L* : Length of sliding window
- *C* : Cut-off of coverage threshold
- *D* : Distance max between adjacent transcripts
- *pval* : p-value cut-off of likelihood test

Optimisation

- Transcrit correctement détecté :

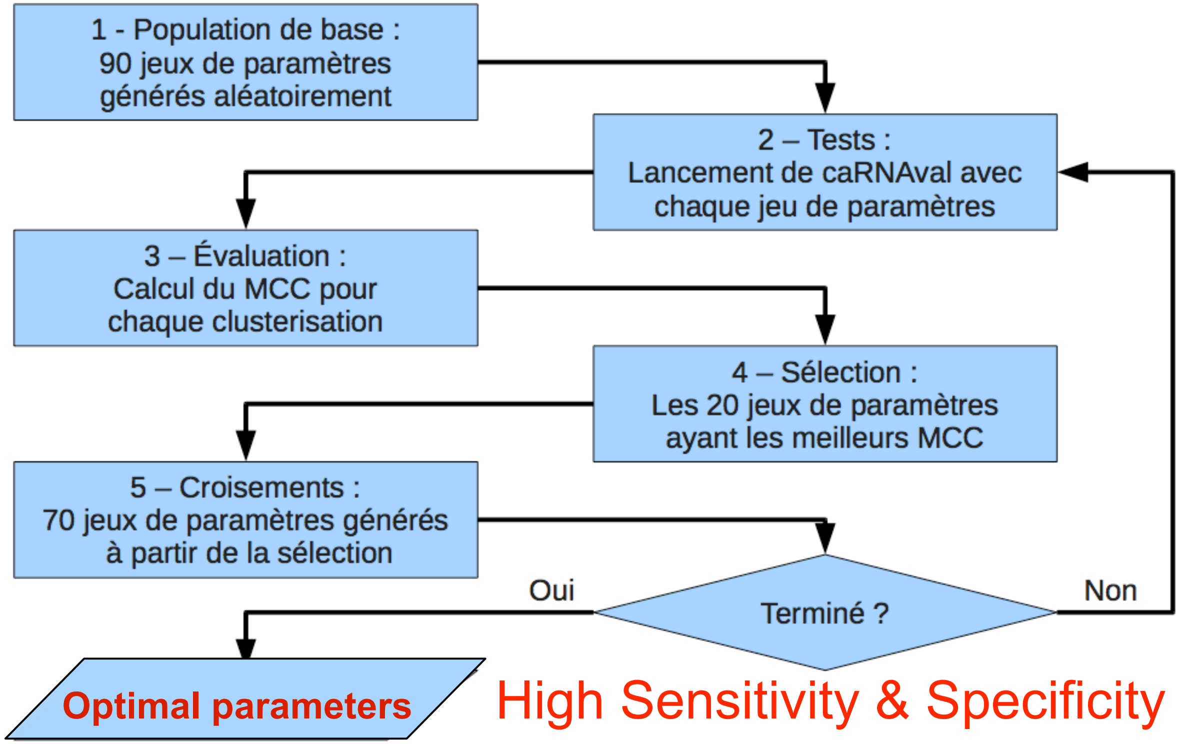


Intersection / transcrit ≥ 75%
ET
Intersection / segment ≥ 75%



Matthews Correlation Coefficient (MCC)

$$MCC = \frac{VP \times VN - FP \times FN}{\sqrt{(VP+FP)(VP+FN)(VN+FP)(VN+FN)}}$$



1 - Population de base :
90 jeux de paramètres
générés aléatoirement

2 – Tests :
Lancement de caRNAval avec
chaque jeu de paramètres

3 – Évaluation :
Calcul du MCC pour
chaque clusterisation

4 – Sélection :
Les 20 jeux de paramètres
ayant les meilleurs MCC

5 – Croisements :
70 jeux de paramètres générés
à partir de la sélection

Terminé ?

Oui

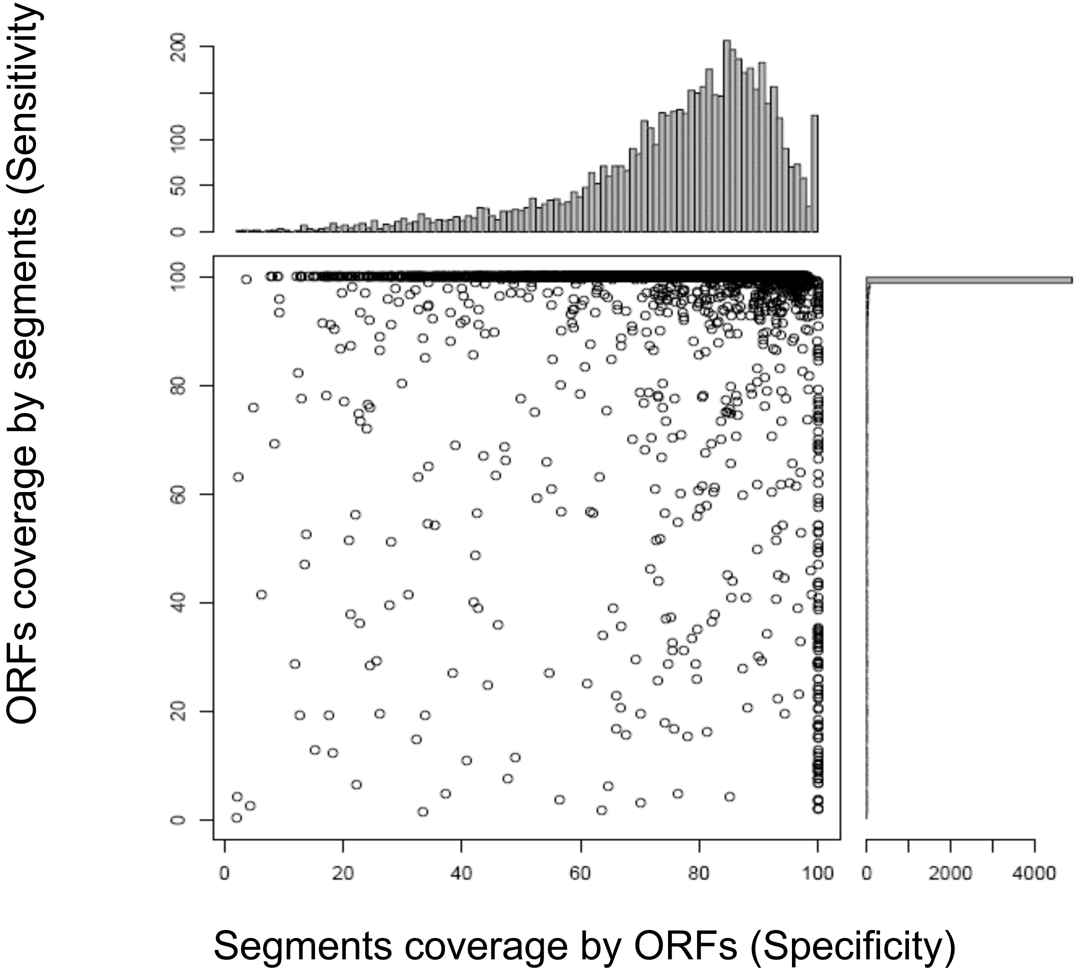
Non

Optimal parameters

High Sensitivity & Specificity

Validation of the segmentation process

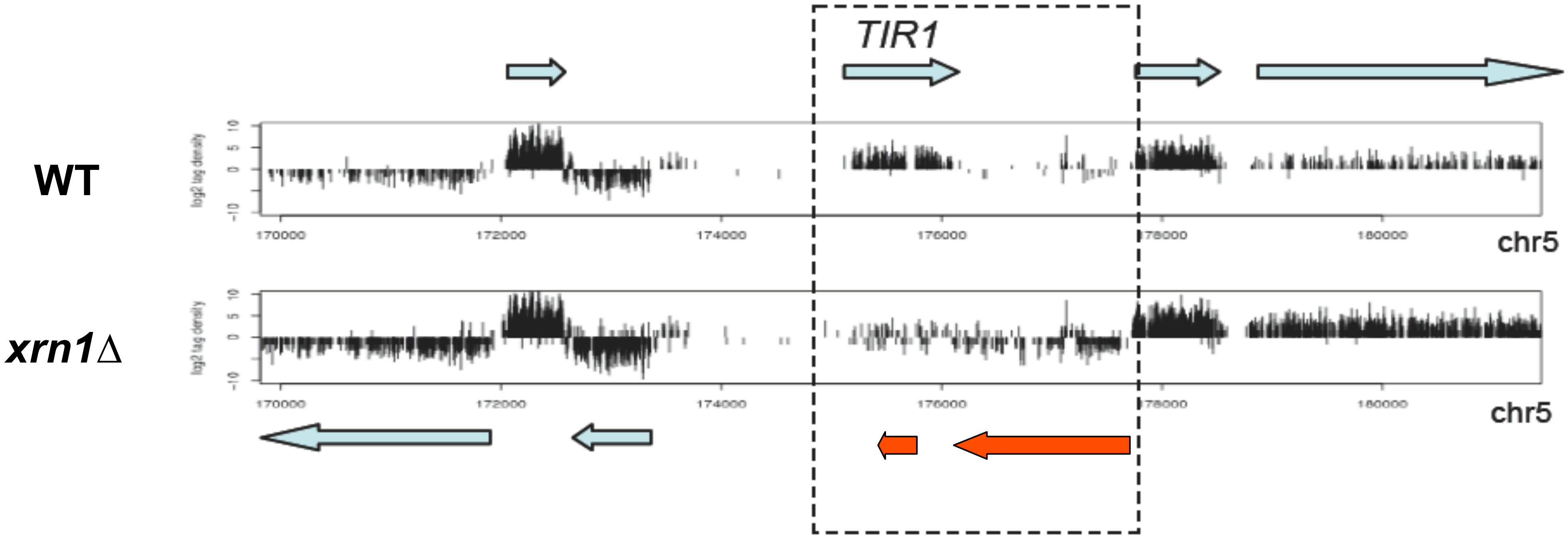
ORF coverage



In average 85% of the segment is covered by an ORF

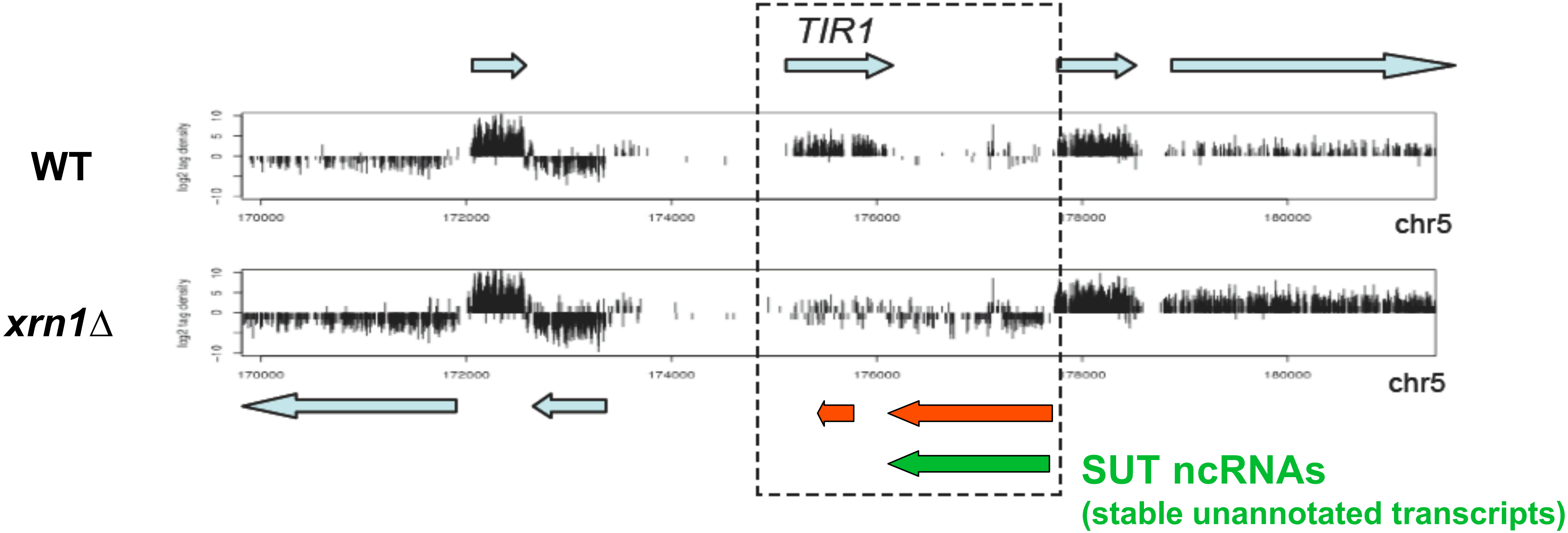
In average 95% of the ORF is covered by a segment

Segmentation process



1747 ncRNA transcripts (with length>250nt) were identified by segmentation

Segmentation process



SUT ncRNAs
(stable unannotated transcripts)

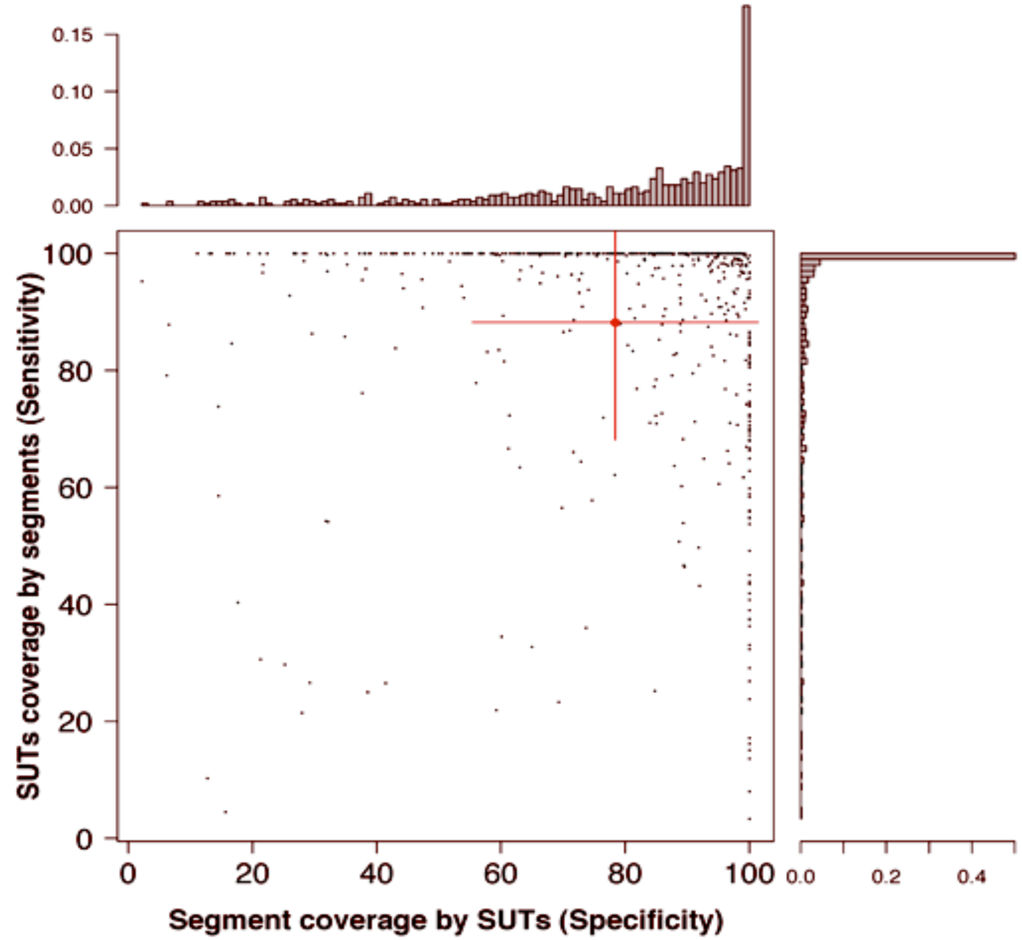
Xu. et al. 2009 *Nature* 457:1033

1747 ncRNA transcripts (with length>250nt) were identified by segmentation

Step 4. Comparison with existing features (**SUT**)

Validation of the segmentation process

SUT coverage

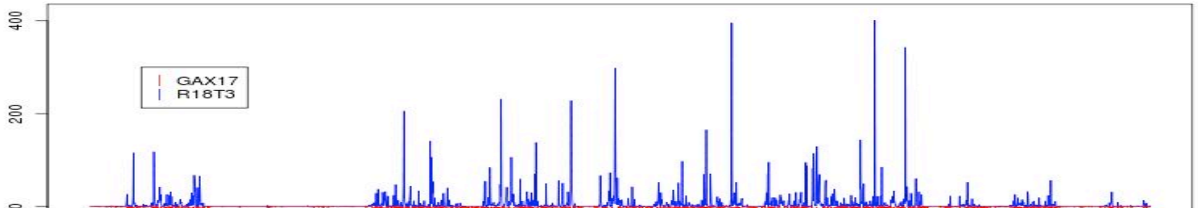


In average 80% of the segment is covered by a SUT

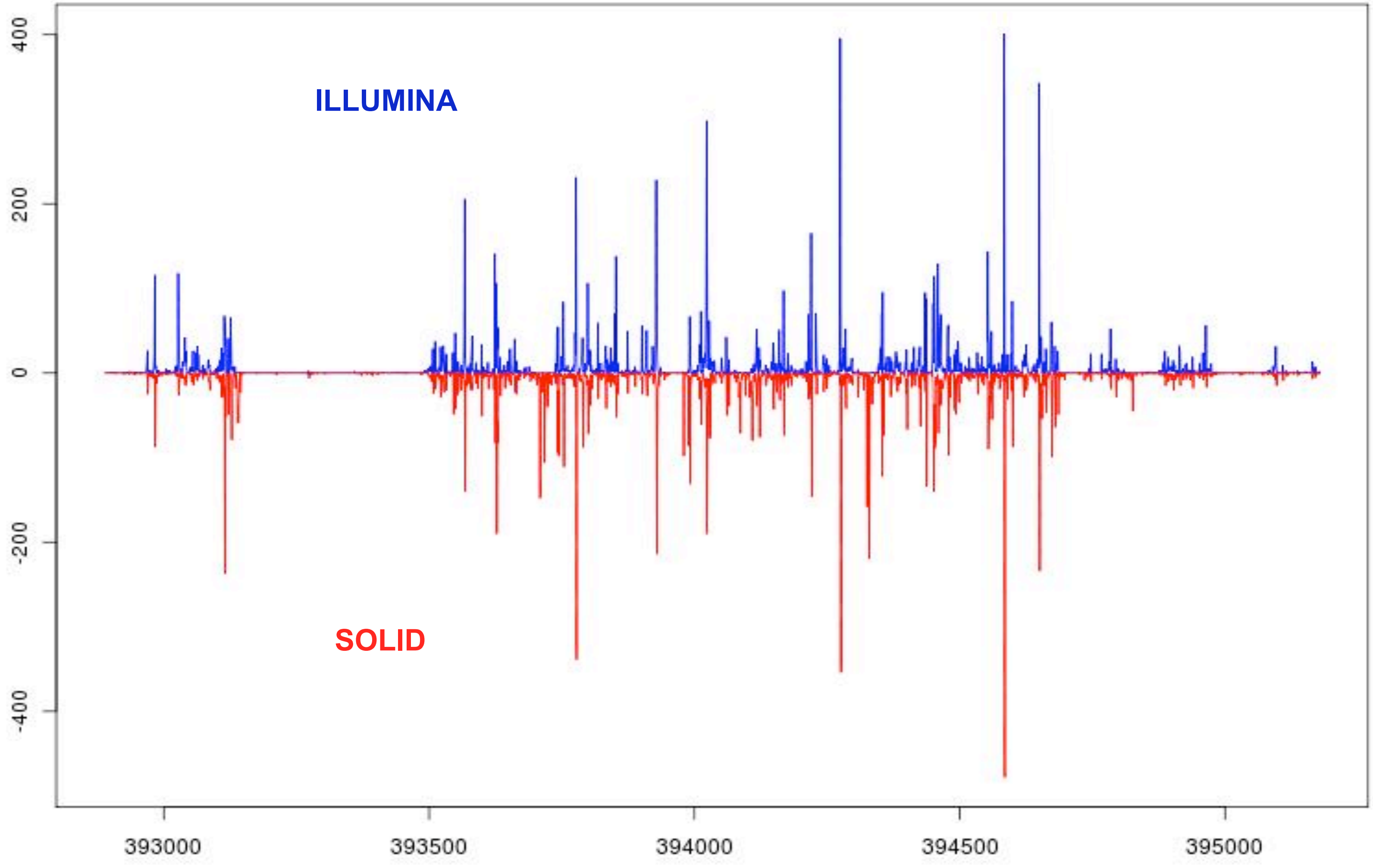
In average 87% of the SUT is covered by a segment

→ 90% of the SUTs are covered by a unique detected segment

How to reduce the heterogeneity?

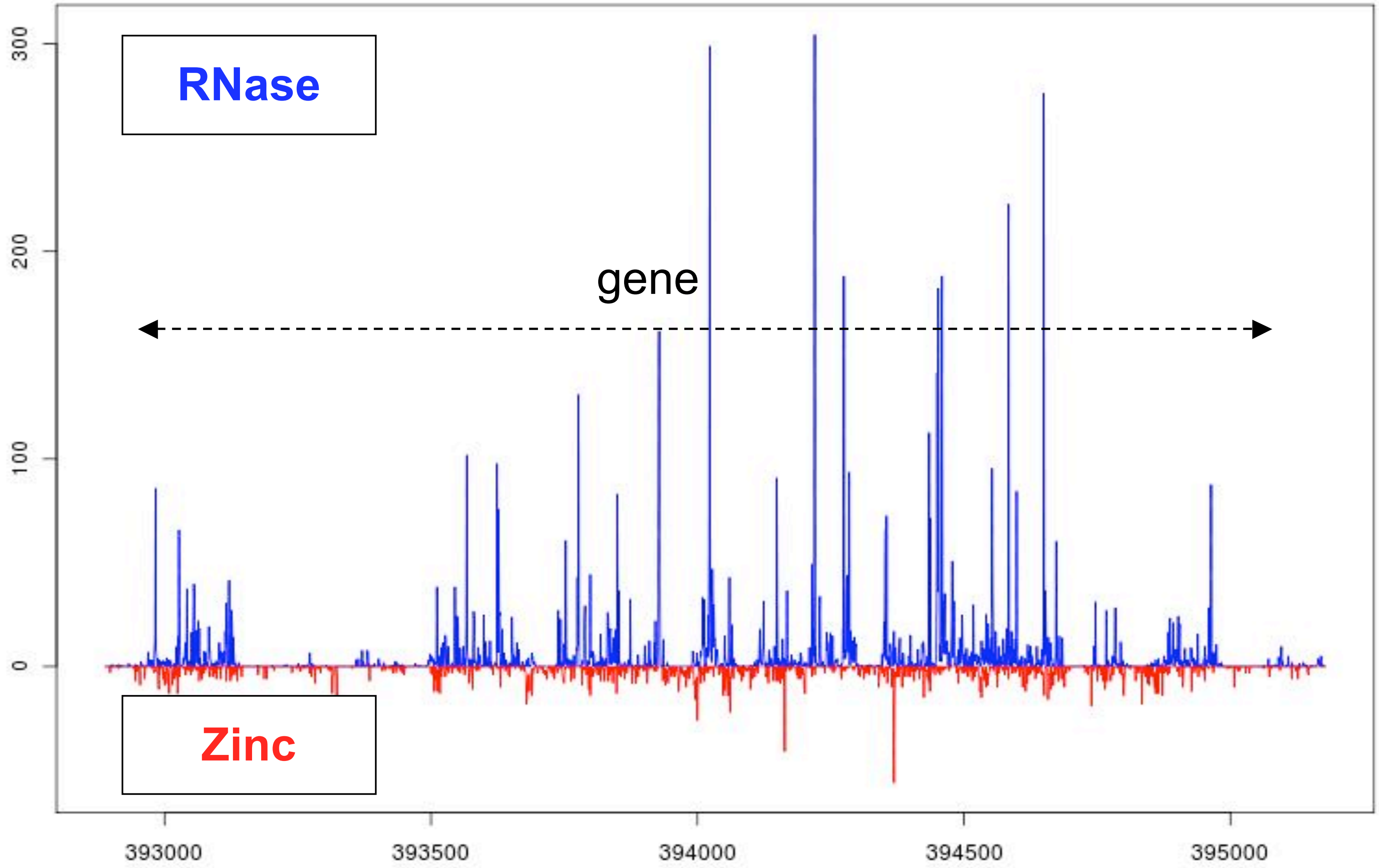


Data heterogeneity: similar effect on different sequencing devices



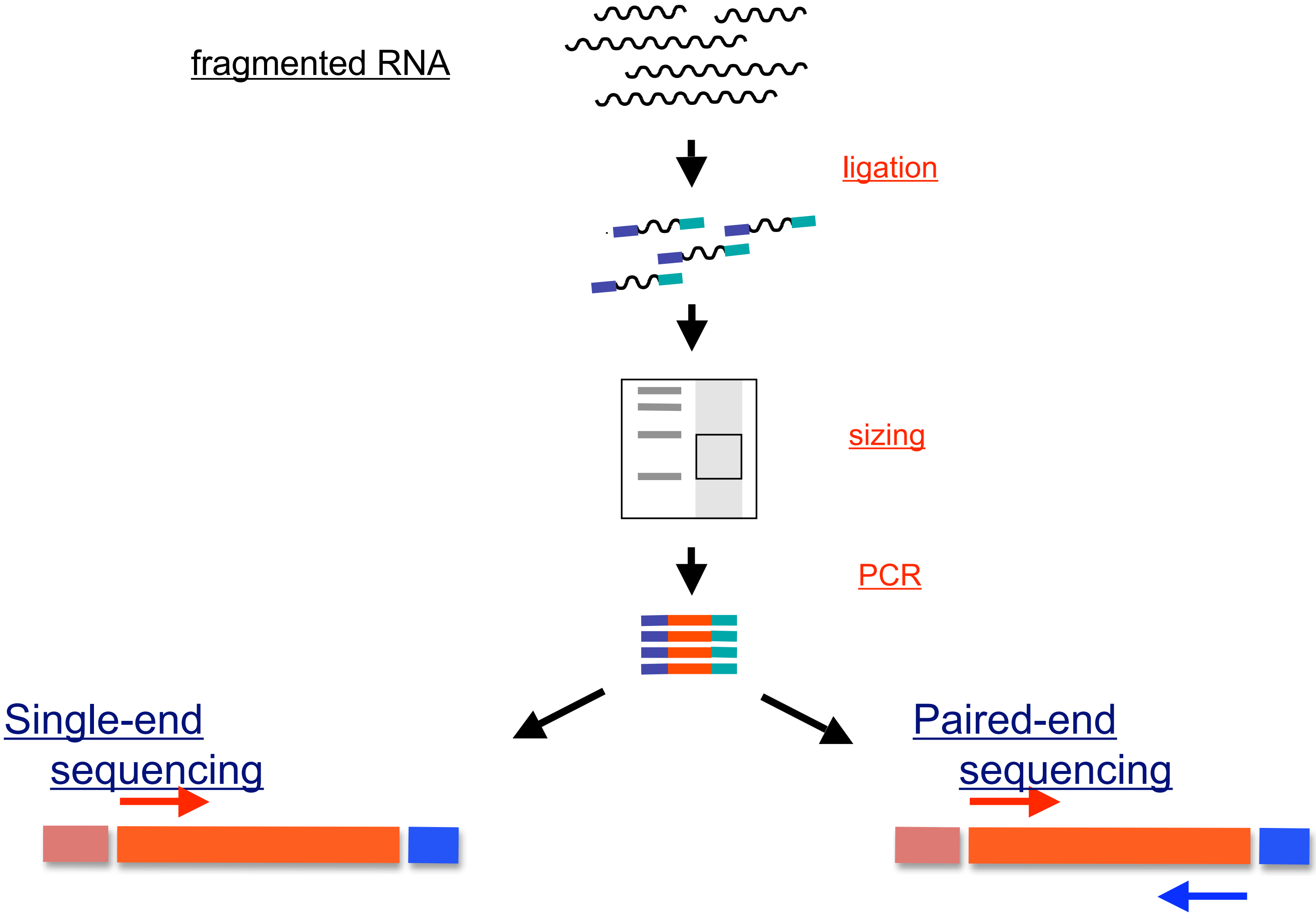
The same library sequenced on two different devices

Data heterogeneity: problem resulting from library preparation



Wery et al. in preparation

Oriented paired-end RNA-seq can help to improve the segmentation



fragmented RNA

ligation

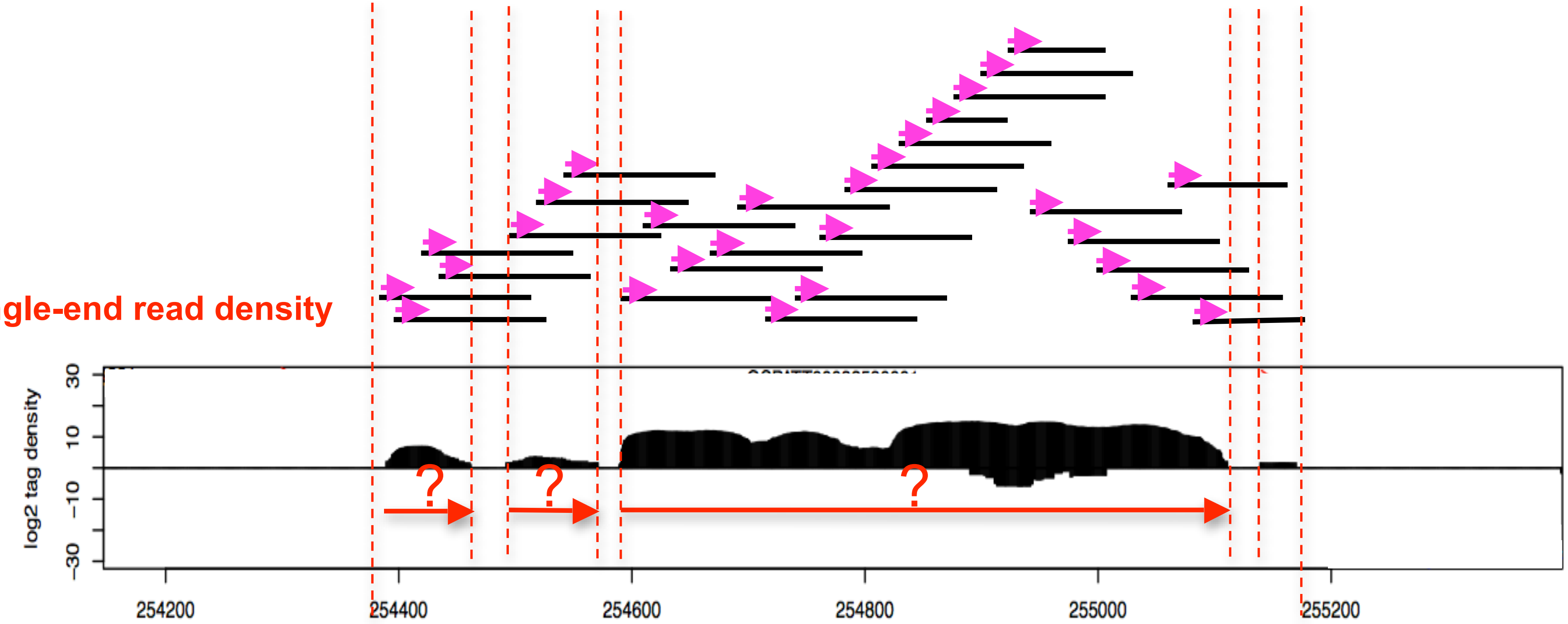
sizing

PCR

Single-end sequencing

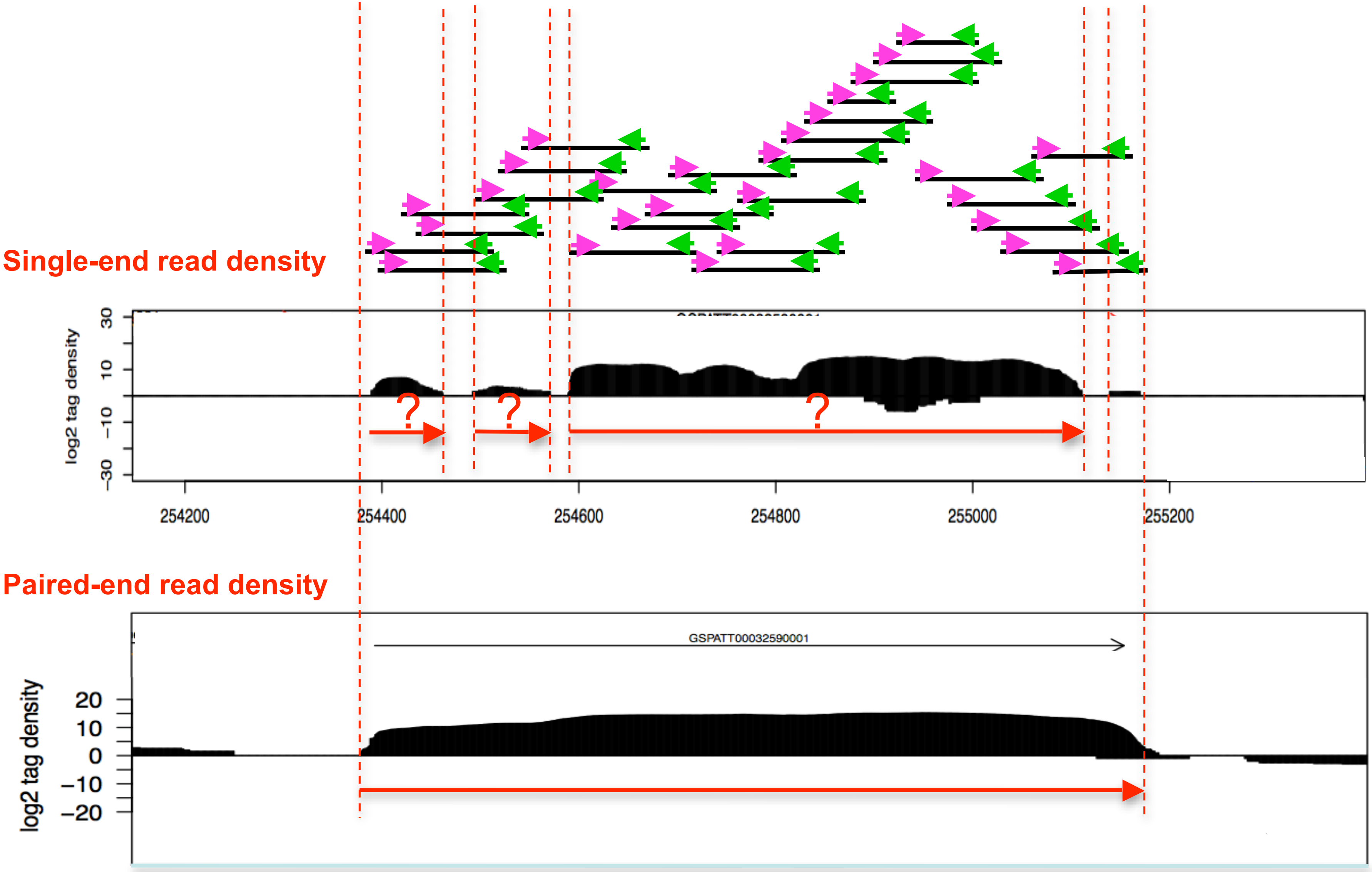
Paired-end sequencing

Oriented paired-end RNA-seq can help to improve the segmentation

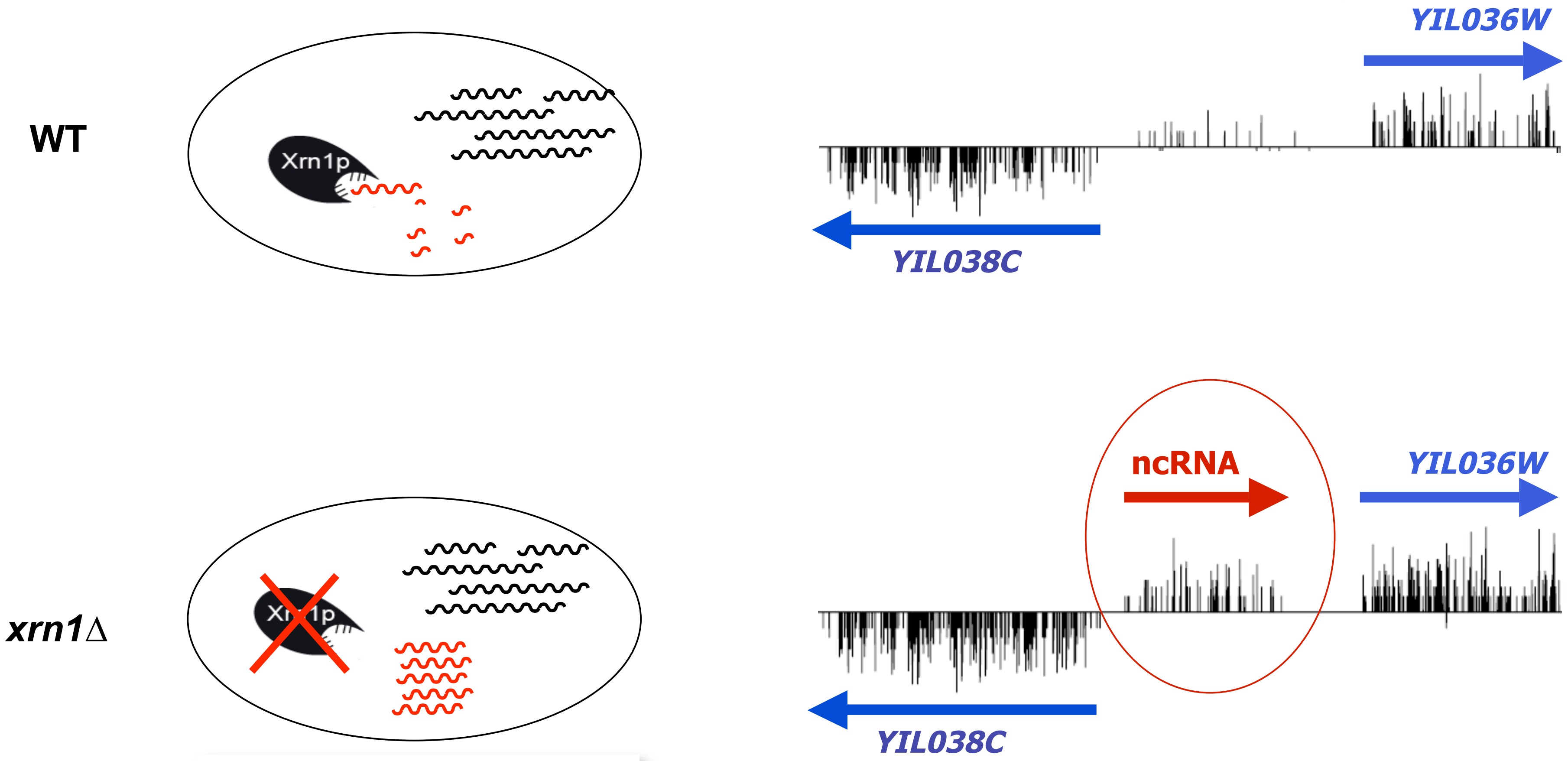


Single-end read density

Oriented paired-end RNA-seq can help to improve the segmentation



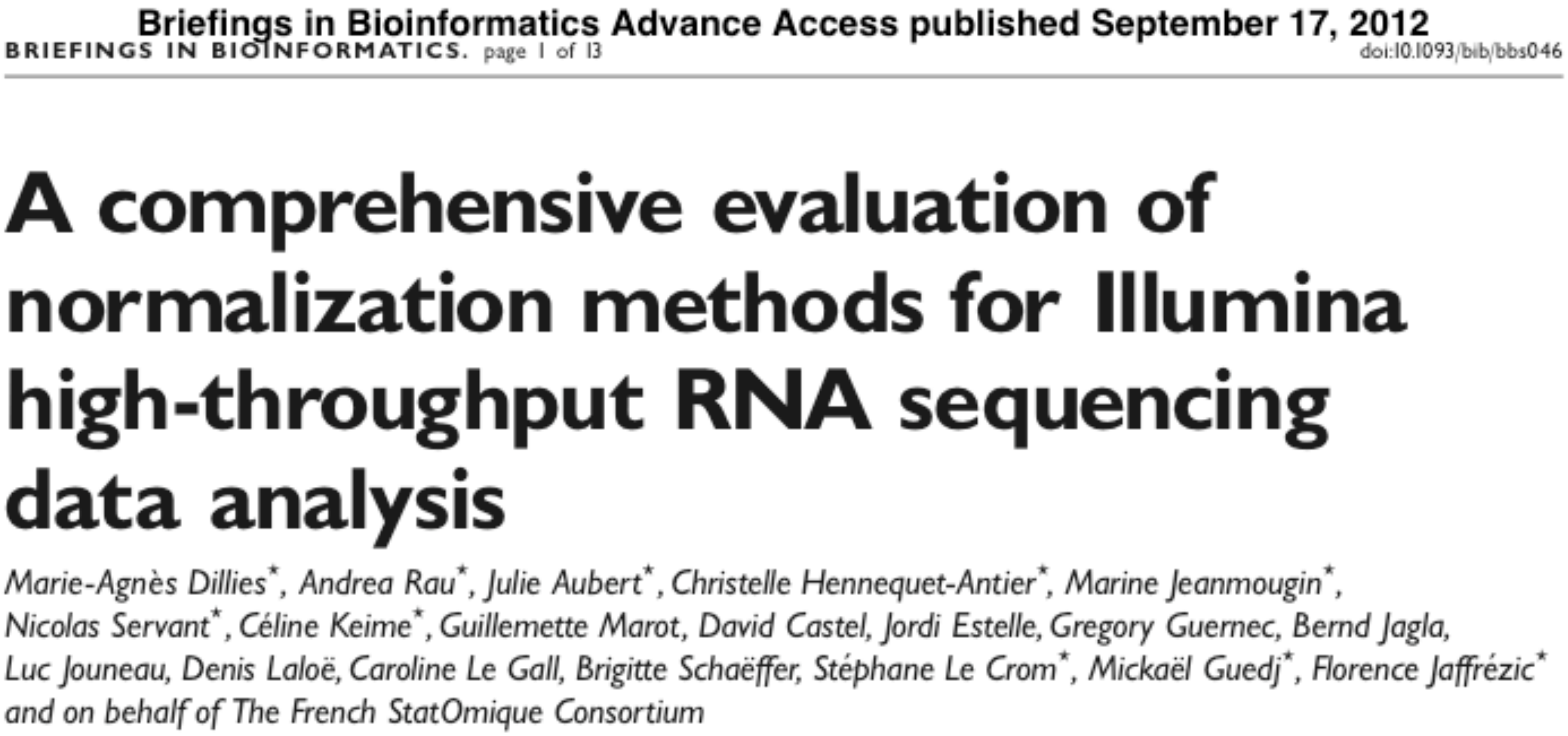
Define transcripts sensitive to Xrn1 (XUTs)



new ncRNAs ?

Challenge 2: Data normalization

Challenge 2: Data normalization



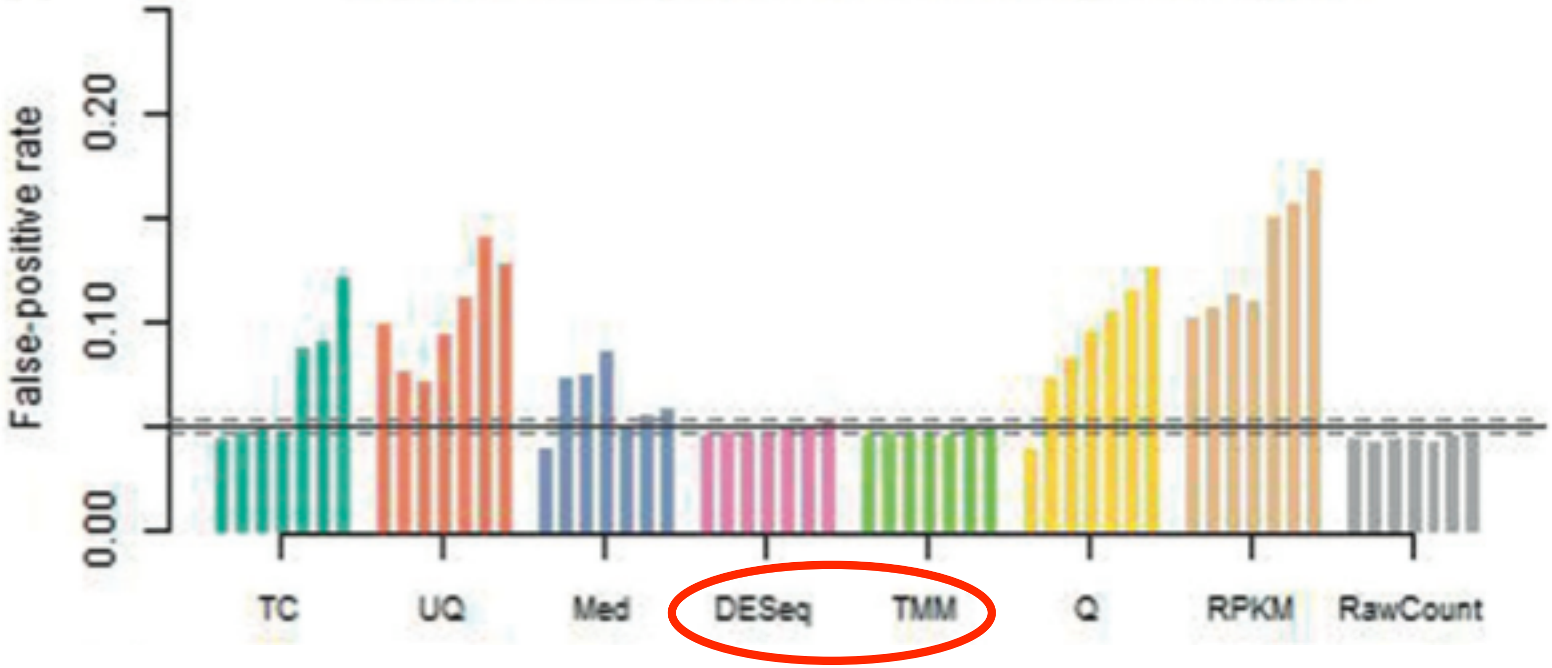
Briefings in Bioinformatics Advance Access published September 17, 2012
BRIEFINGS IN BIOINFORMATICS. page 1 of 13
doi:10.1093/bib/bbs046

A comprehensive evaluation of normalization methods for Illumina high-throughput RNA sequencing data analysis

Marie-Agnès Dillies, Andrea Rau*, Julie Aubert*, Christelle Hennequet-Antier*, Marine Jeanmougin*, Nicolas Servant*, Céline Keime*, Guillemette Marot, David Castel, Jordi Estelle, Gregory Guernec, Bernd Jagla, Luc Jouneau, Denis Laloë, Caroline Le Gall, Brigitte Schaëffer, Stéphane Le Crom*, Mickaël Guedj*, Florence Jaffrézic* and on behalf of The French StatOmique Consortium*

The French StatOmique Consortium. *Brief Bioinform.* 2012

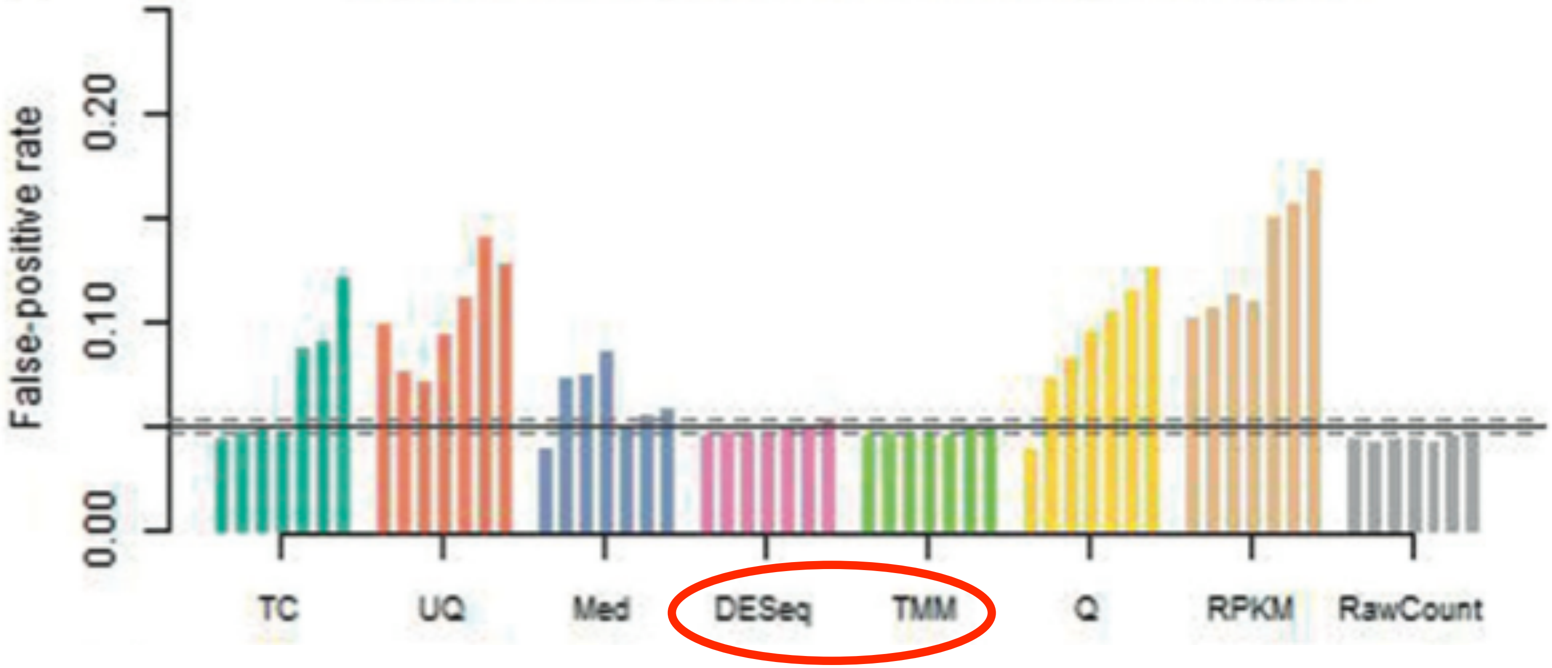
Challenge 2: Data normalization



Hypothesis : “the majority of genes under consideration are assumed to be non-differentially expressed between conditions.”

The French StatOmique Consortium. *Brief Bioinform.* 2012

Challenge 2: Data normalization



Hypothesis : “the majority of genes under consideration are assumed to be non-differentially expressed between conditions.”

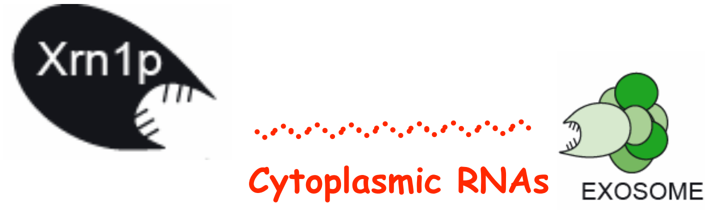
Not the case in our study

Xrn1p preferentially destabilizes de-adenylated RNA

RNA-seq

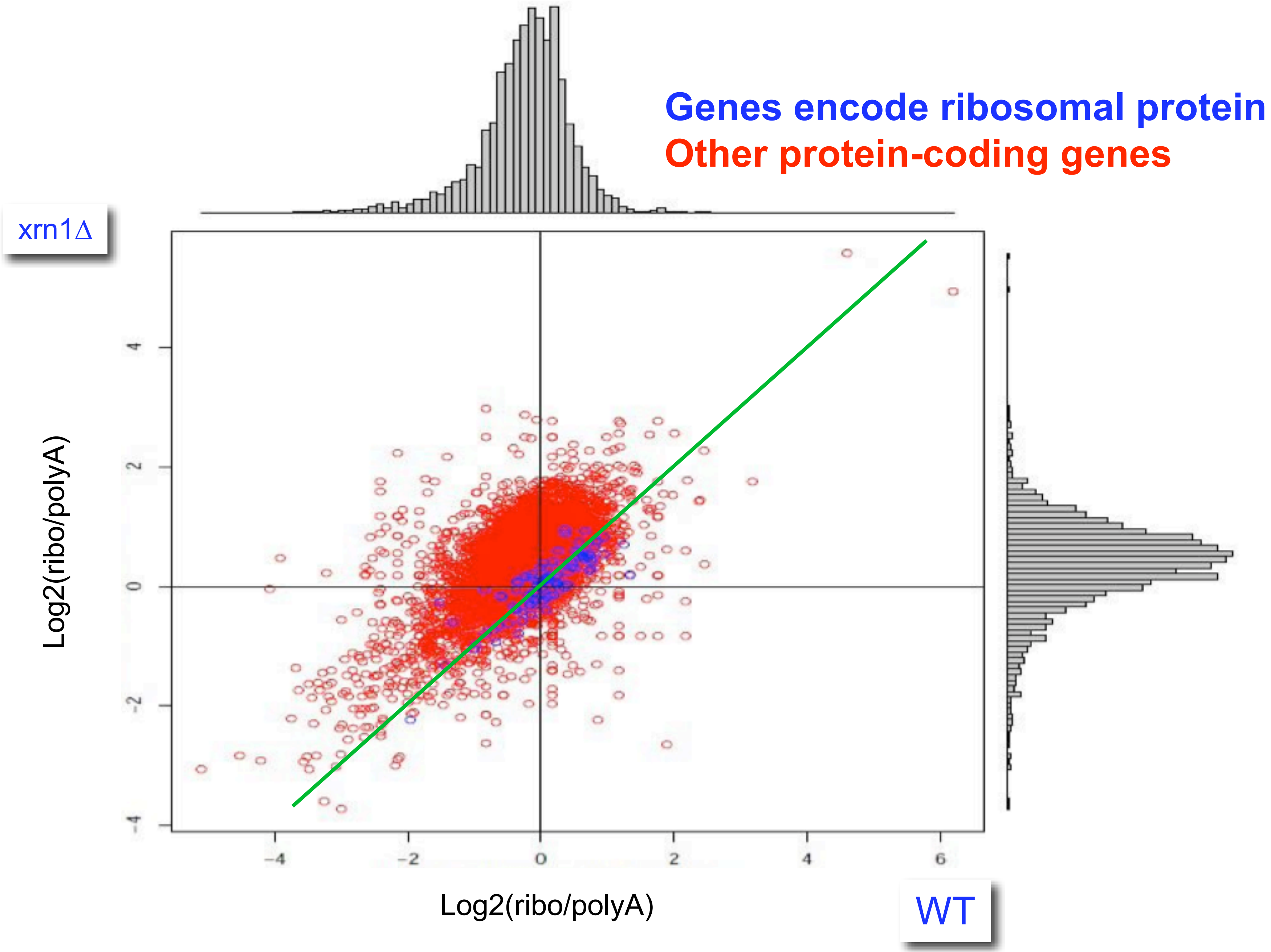
	Sample	Total reads	Mapped	Unique
Ribo$^-$	WT	320 680 804	117 678 134	**69 432 376**
	xrn1Δ	234 617 538	79 411 831	**48 427 785**
polyA$^+$	WT	51 559 241	24 153 108	**11 895 794**
	xrn1Δ	56 017 358	20 684 510	**11 933 068**

(Solid: 50 nucleotides long- 6 mismatches)



Cytoplasmic RNAs

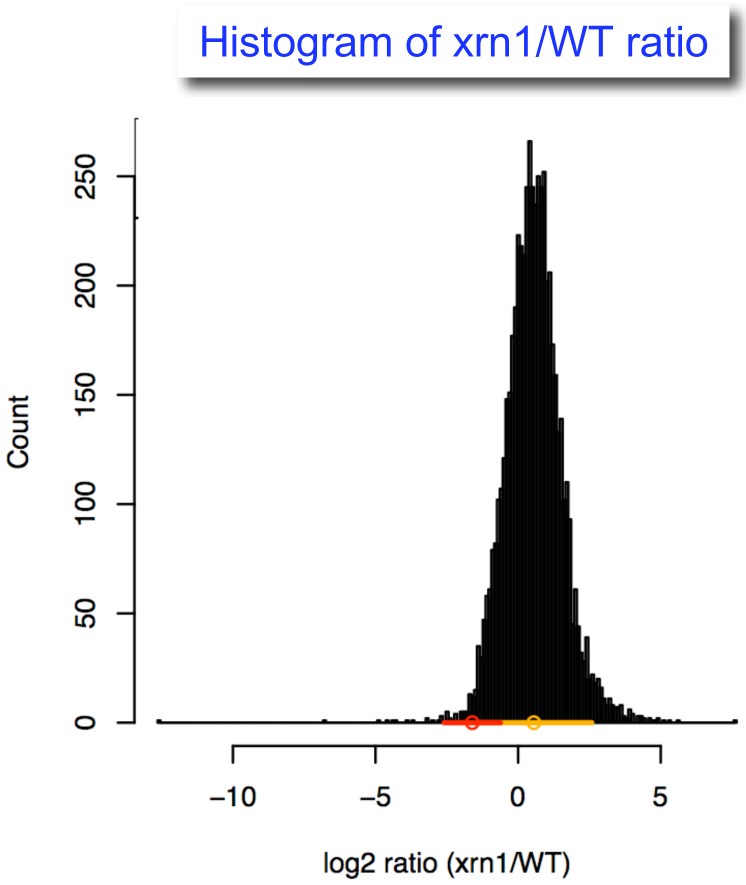
Xrn1p preferentially destabilizes de-adenylated RNA



Genes encode ribosomal protein

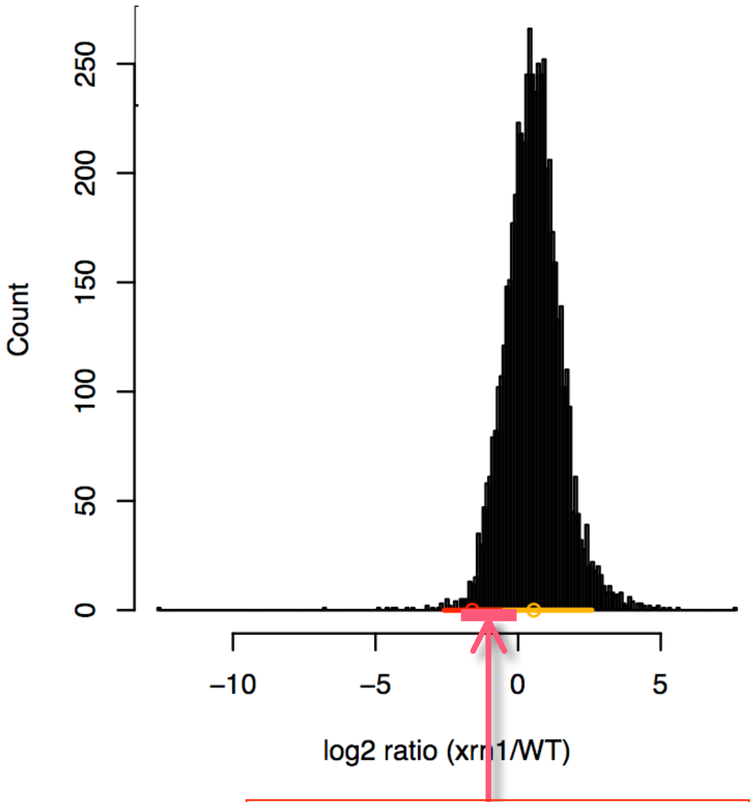
Other protein-coding genes

Search for transcripts less affected by Xrn1p



Search for transcripts less affected by Xrn1p

Histogram of xrn1/WT ratio

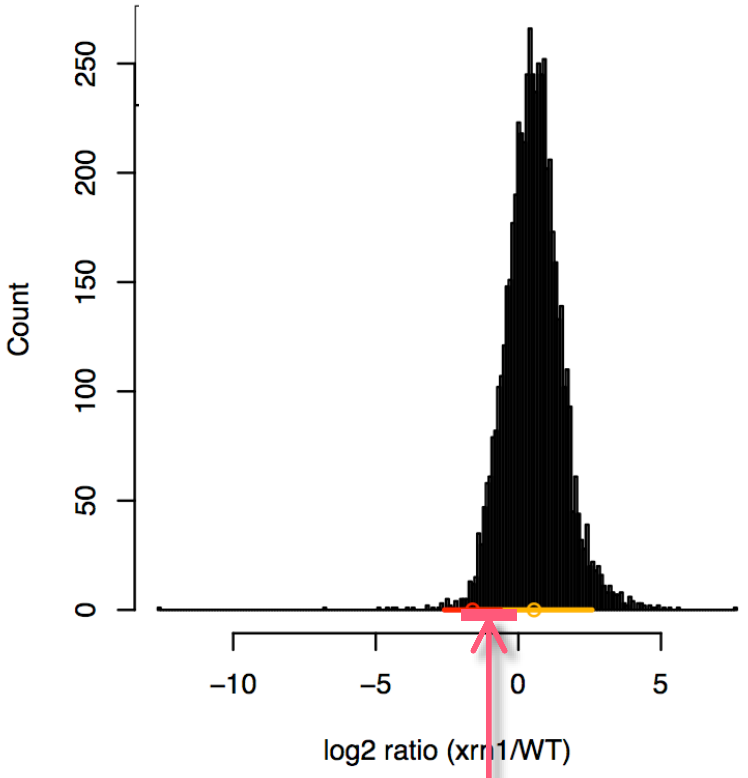


Ribosomal transcripts

Ratio = -0.9

Search for transcripts less affected by Xrn1p

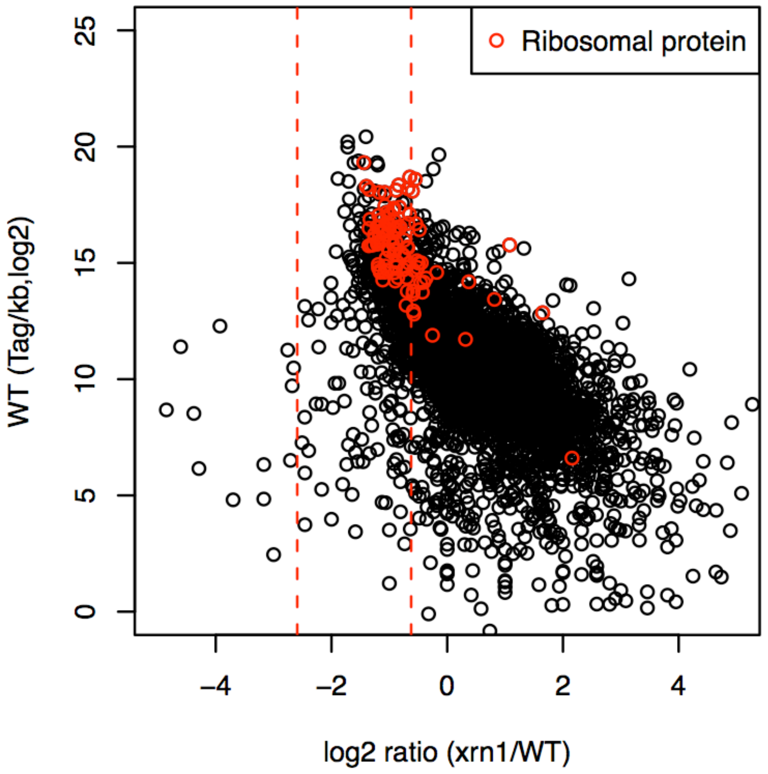
Histogram of xrn1/WT ratio



Ribosomal transcripts

Ratio = -0.9

Read count Vs xrn1/WT ratio

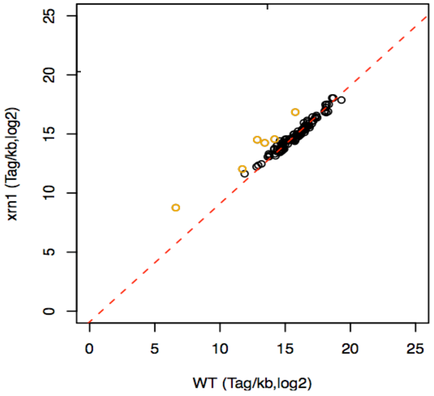


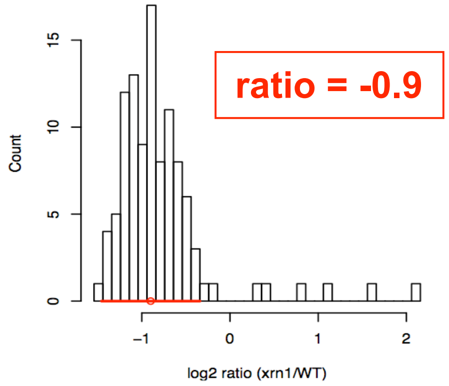
Higher abundance, smaller ratio

WT Vs xrn1 abundance

Histogram of xrn1/WT ratio

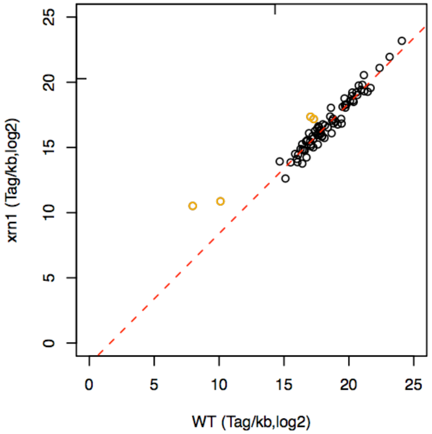
Ribosomal transcripts

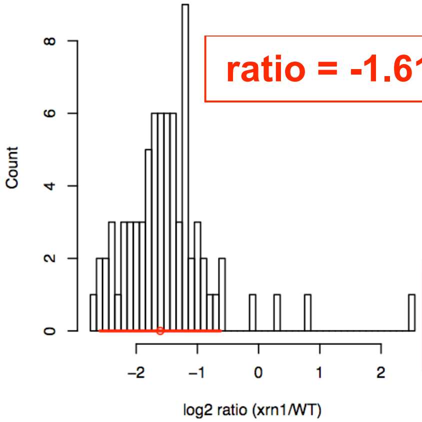




ratio = -0.9

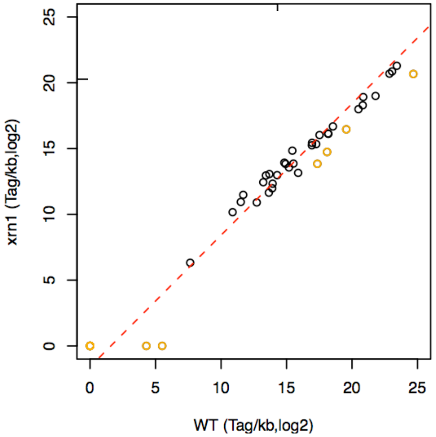
snoRNA

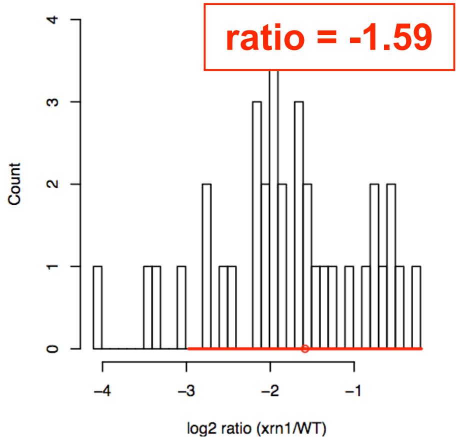




ratio = -1.61

tRNA

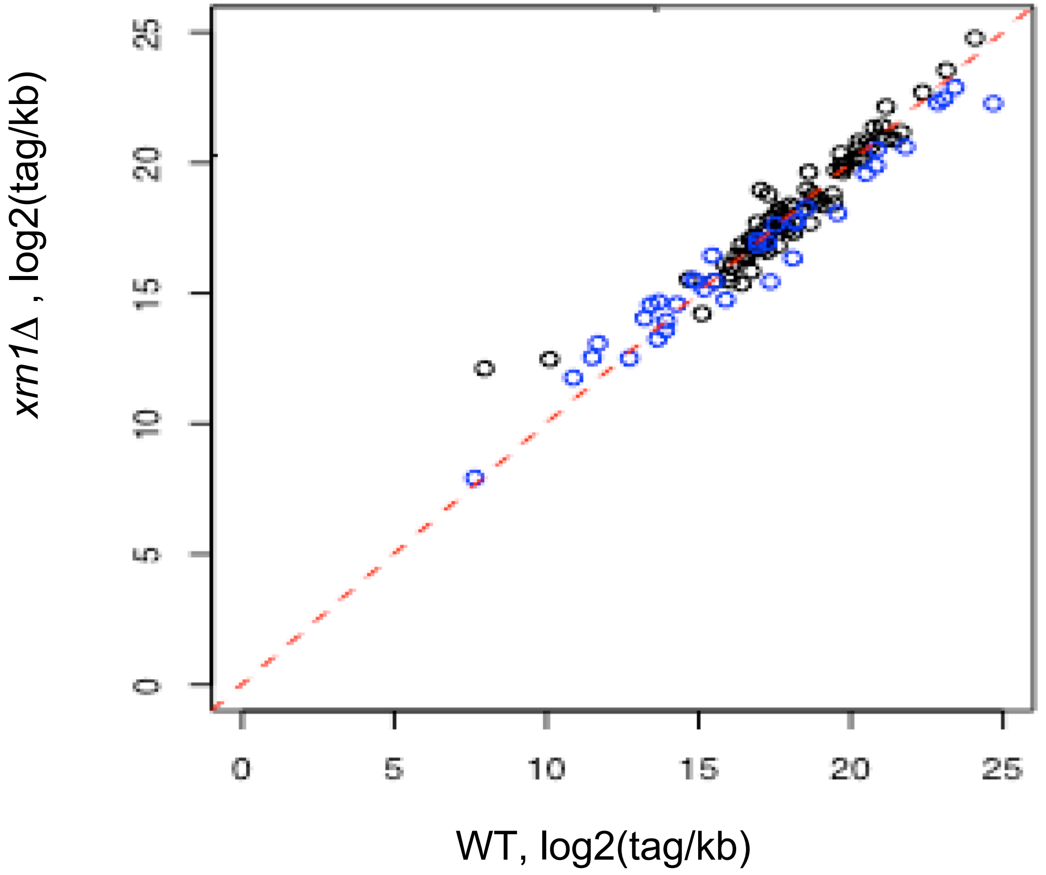




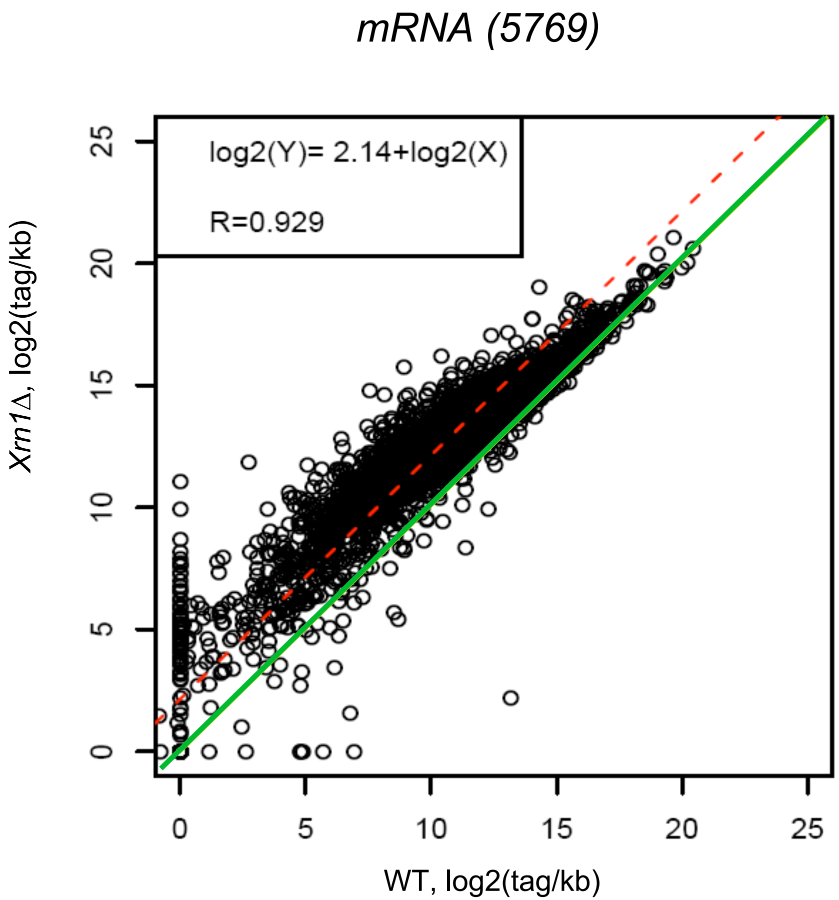
ratio = -1.59

snoRNAs and tRNAs
Highest abundance, smallest ratio

snoRNA, tRNA



snoRNAs and tRNAs used for normalization

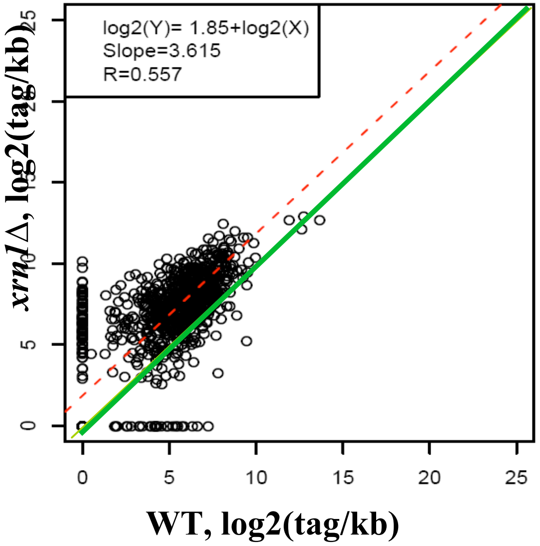


4.4-fold increase

➡ Xrn1p is the major exonuclease responsible for mRNAs turnover

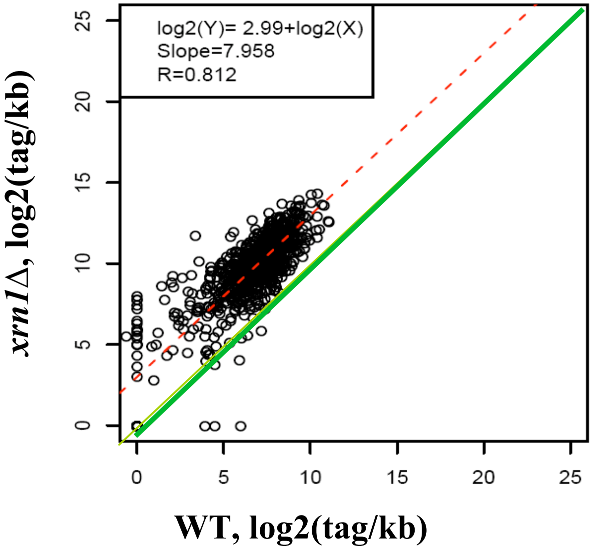
Non-coding Xrn1-sensitive Unstable Transcripts

CUTs (925)



3.6-fold increase

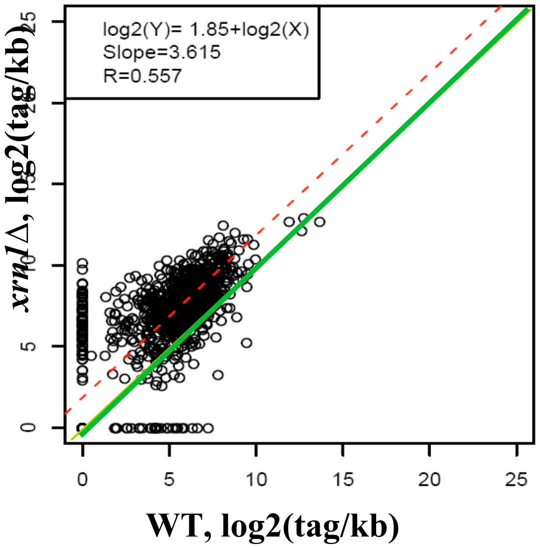
SUTs (847)



7.95-fold increase

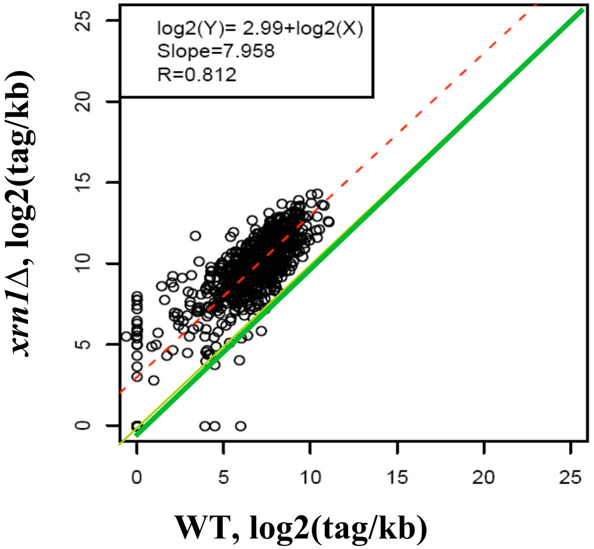
Non-coding Xrn1-sensitive Unstable Transcripts

CUTs (925)



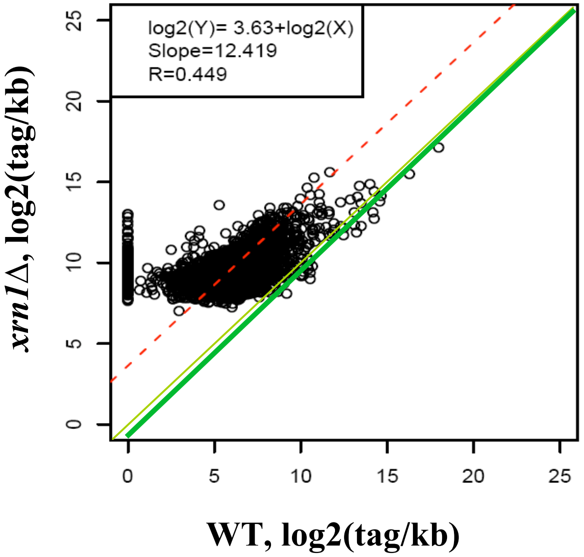
3.6-fold increase

SUTs (847)



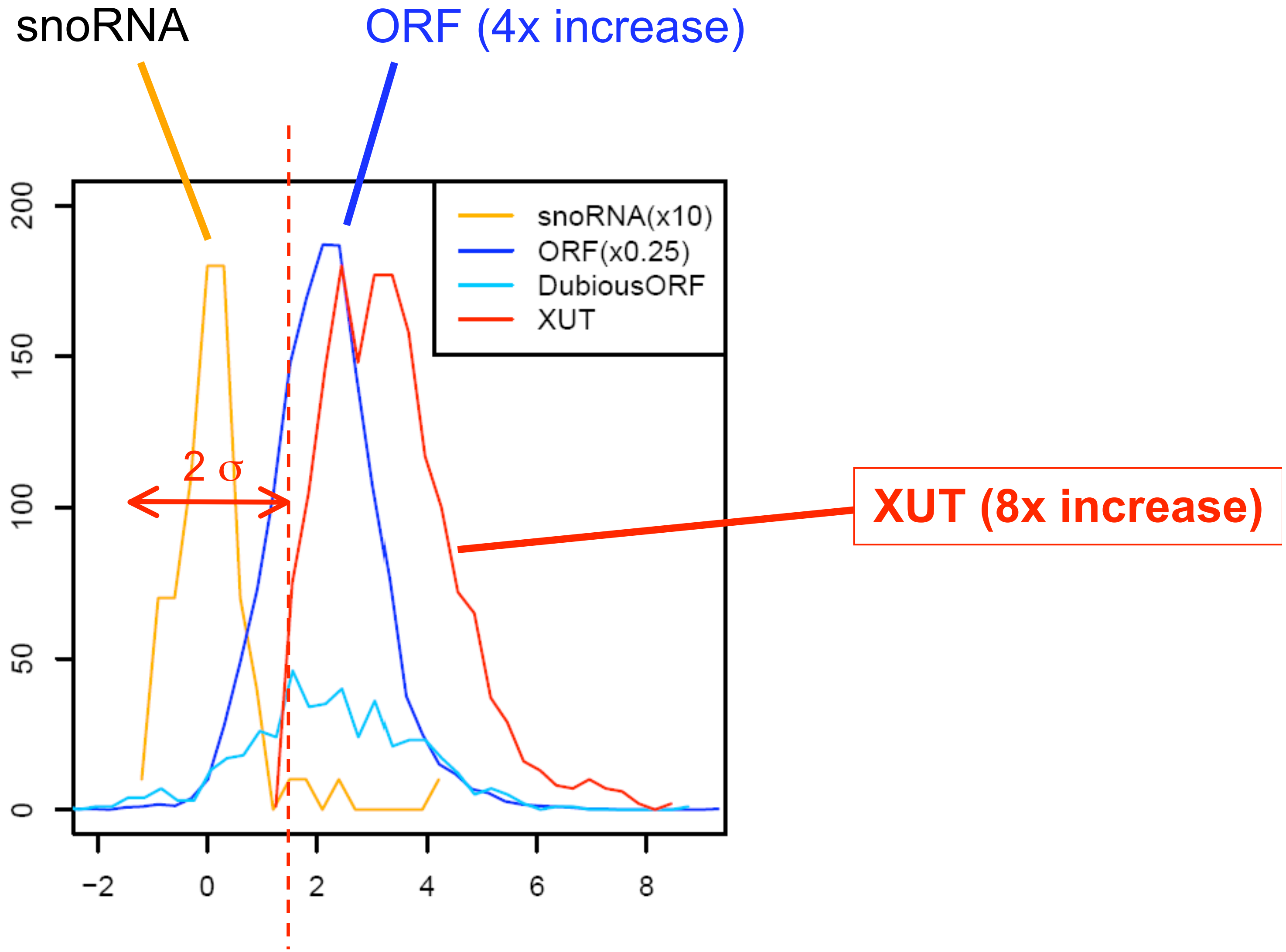
7.95-fold increase

New (932)

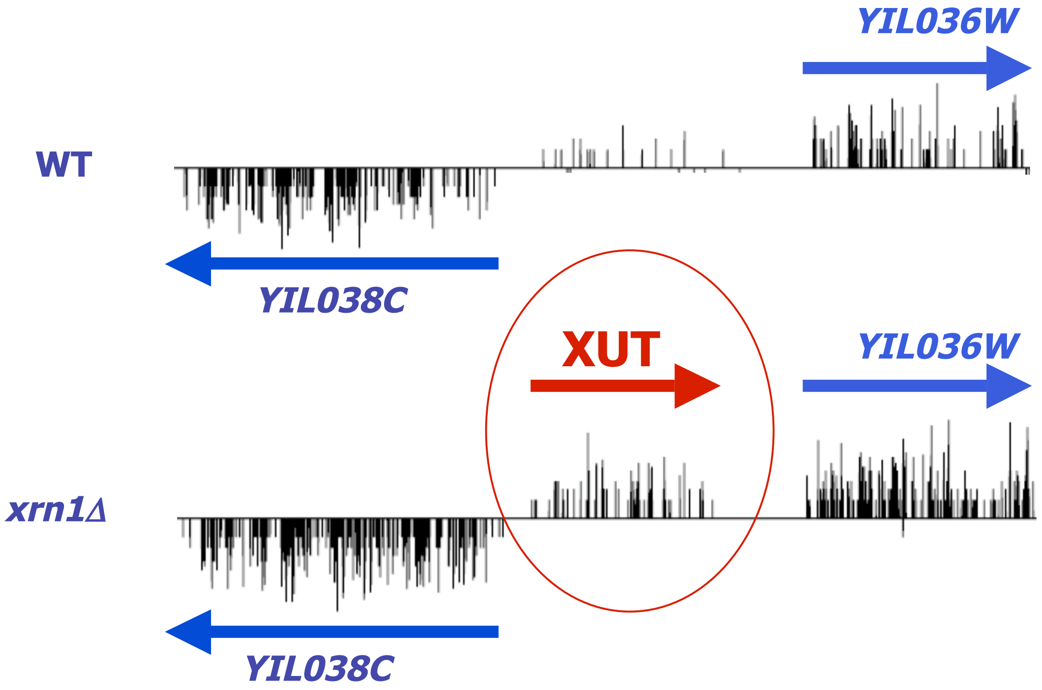


10.8-fold increase

Non-coding Xrn1-sensitive Unstable Transcripts



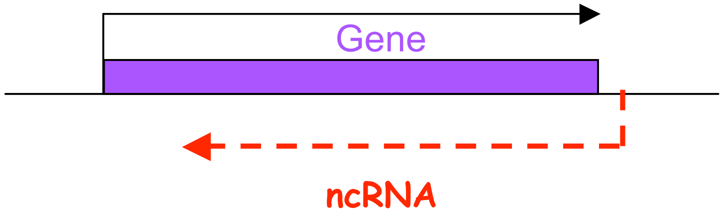
Identification of new ncRNAs



1658 new transcripts : XUT (Xrn1-sensitive Unstable Transcript)

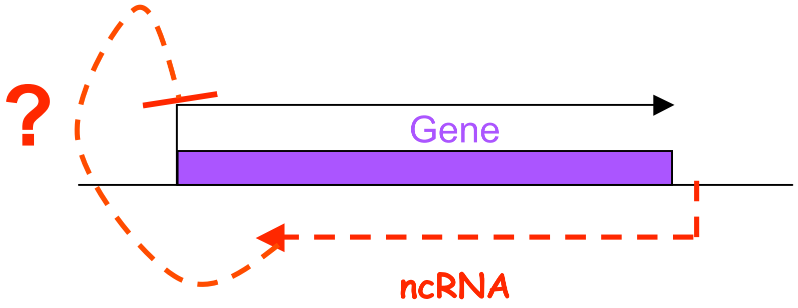
increase ~30% of the gene number in yeast

XUT locates mostly on antisense of protein-coding genes



1094 of 1658 (66%)

XUT locates mostly on antisense of protein-coding genes



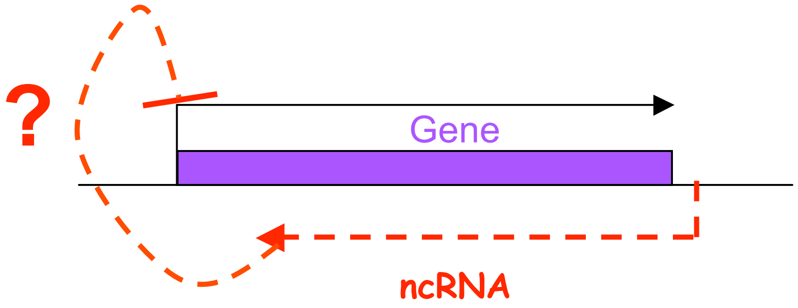
1094 of 1658 (66%)

Do they regulate gene expression ???

Challenge 3: Regulatory function?

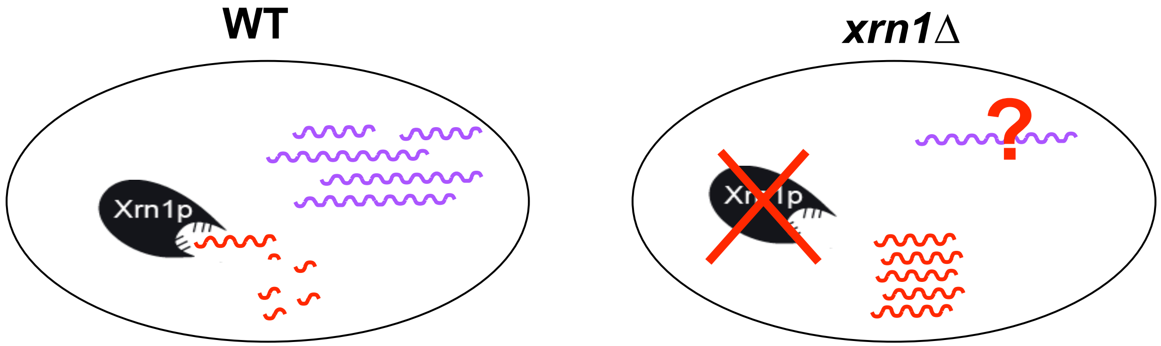
Differential expression

Do they regulate gene expression ???



1094 of 1658 (66%)

Principle

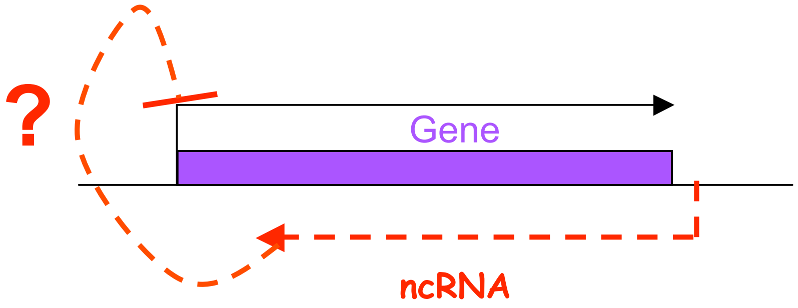


ncRNA accumulate in the mutant of xrn1

⬇

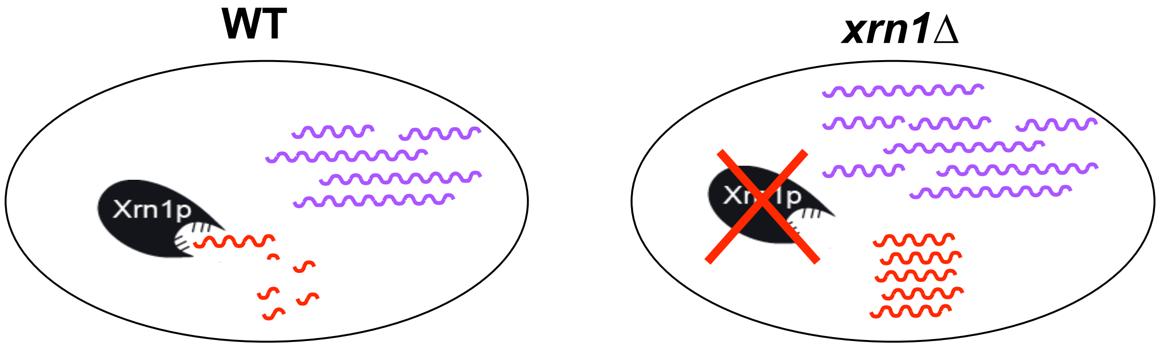
Decrease of the correspond mRNA level ?

Do they regulate gene expression ???



1094 of 1658 (66%)

Difficulty

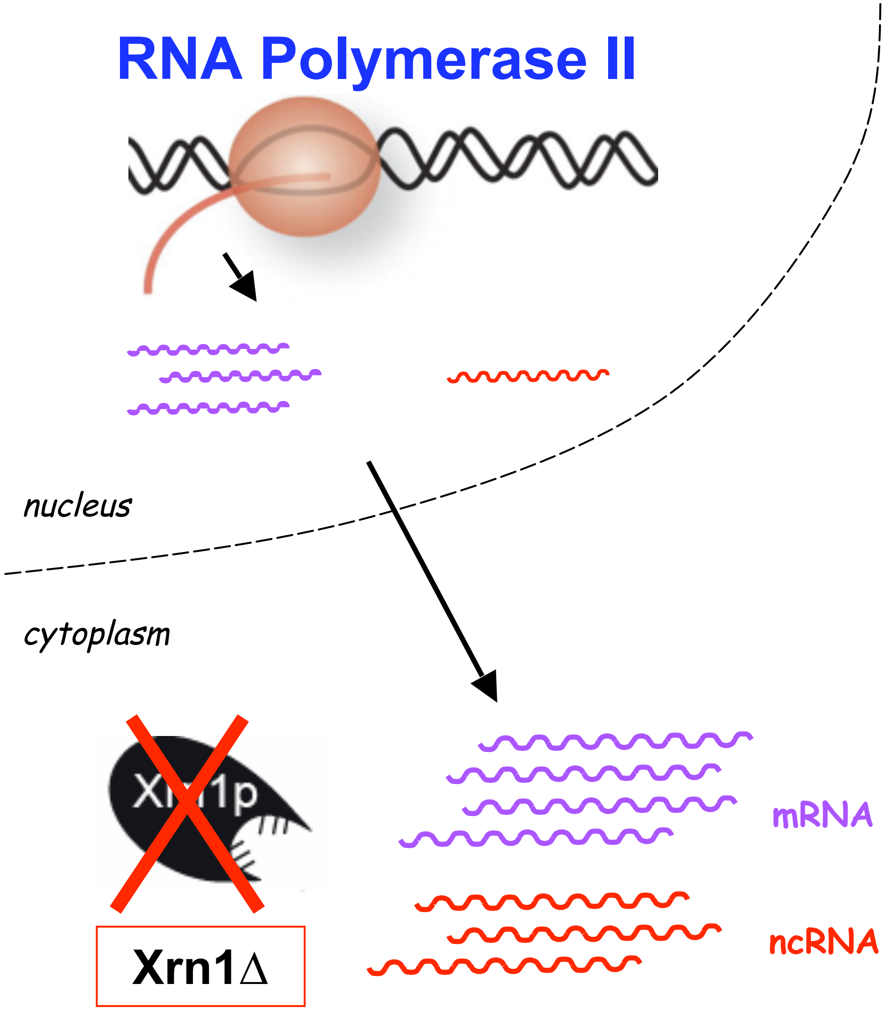


In xrn1 mutant, mRNA also accumulate

⬇

Hence, the mRNA level is not the best indicator for gene expression level

Measure directly gene transcription level within nucleus



RNA Polymerase II

nucleus

cytoplasm

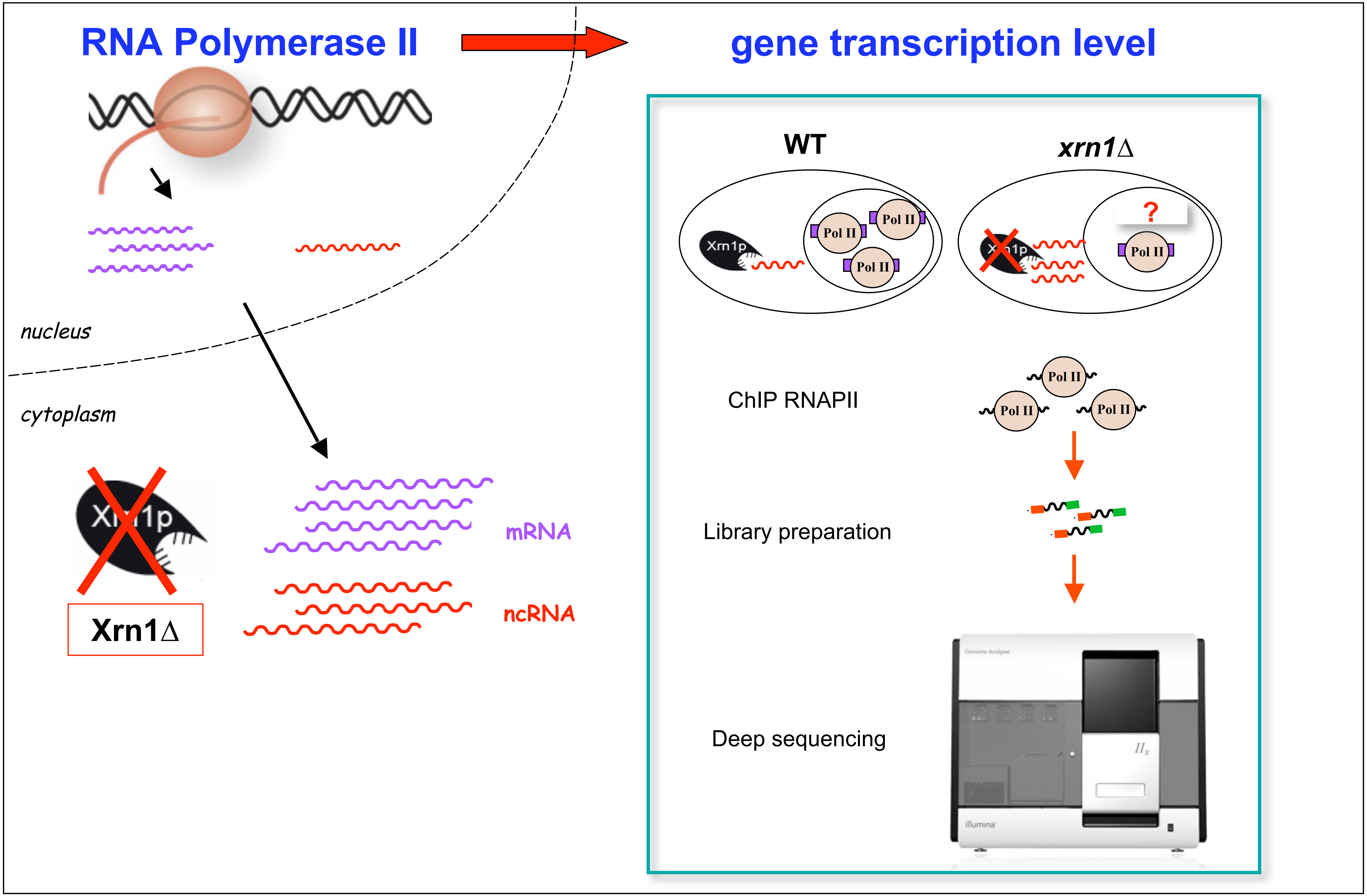
Xrn1p

mRNA

ncRNA

Xrn1Δ

Measure directly gene transcription level within nucleus



RNA Polymerase II → **gene transcription level**

nucleus

cytoplasm

Xrn1∆

WT

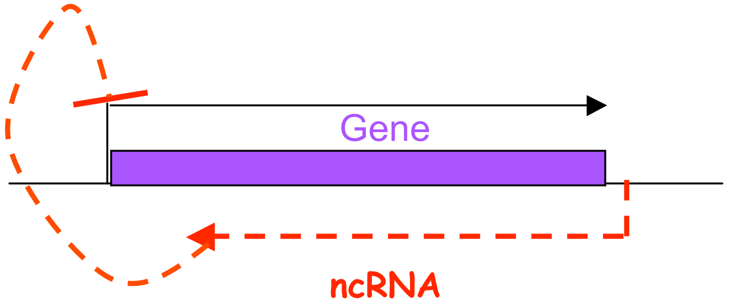
xrn1∆

ChIP RNAPII

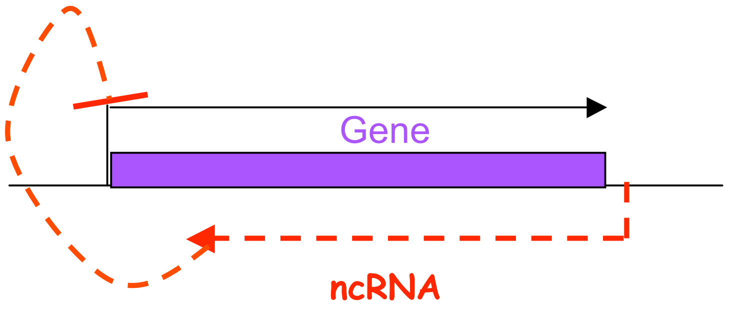
Library preparation

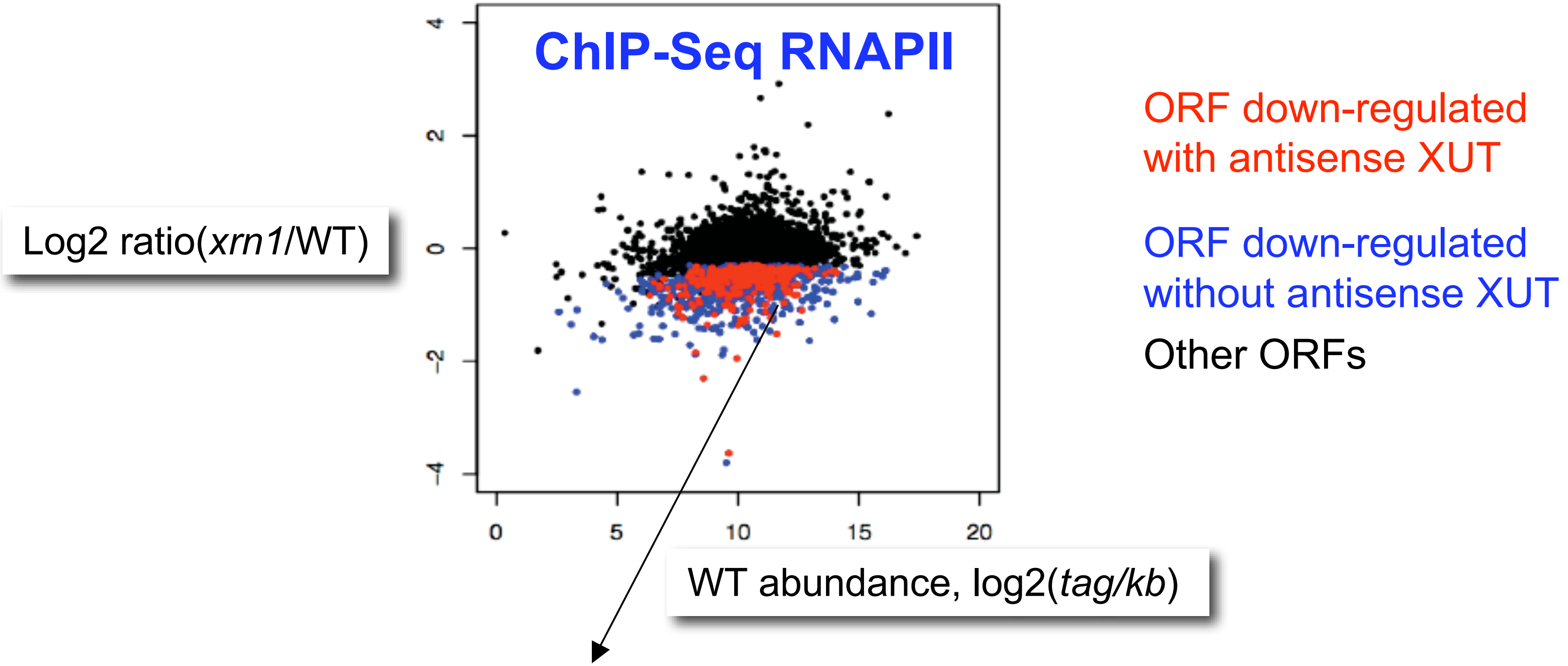
Deep sequencing

273 genes with antisense XUT show a significant decrease in transcription level



273 genes with antisense XUT show a significant decrease in transcription level





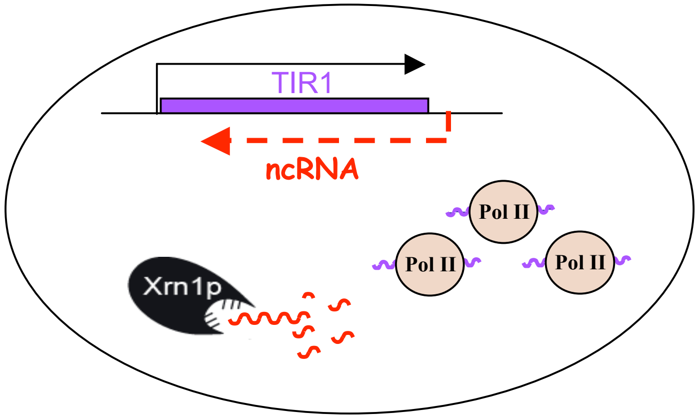
273/986 (30%) present antisense XUT,

Significantly larger than the ORFs that do not present reduced RNAPII levels, only 15% of which associates with antisense XUTs.

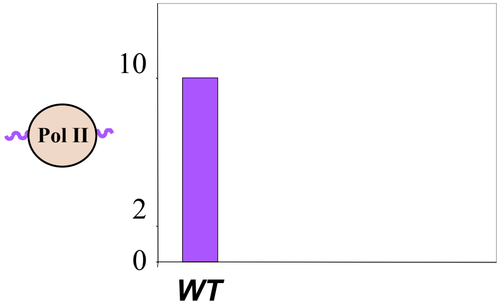
273 genes with antisense XUT show a significant decrease in transcription level

Example of gene TIR1

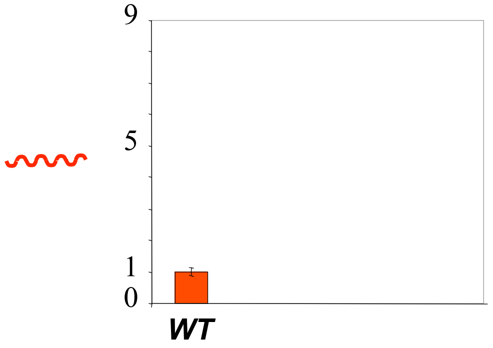
WT



Gene

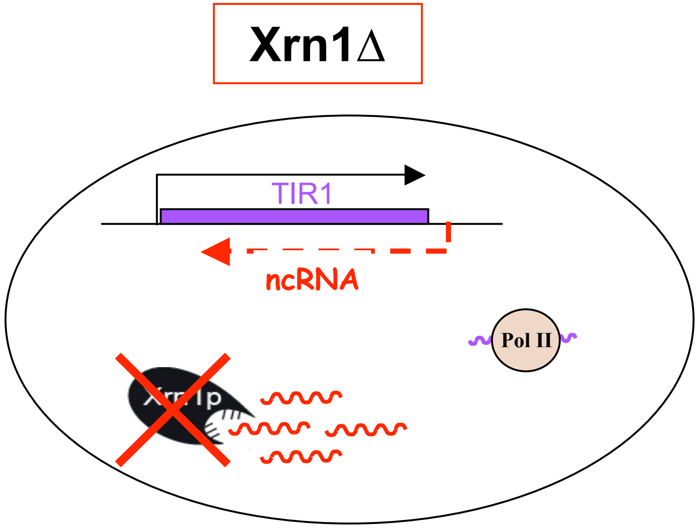


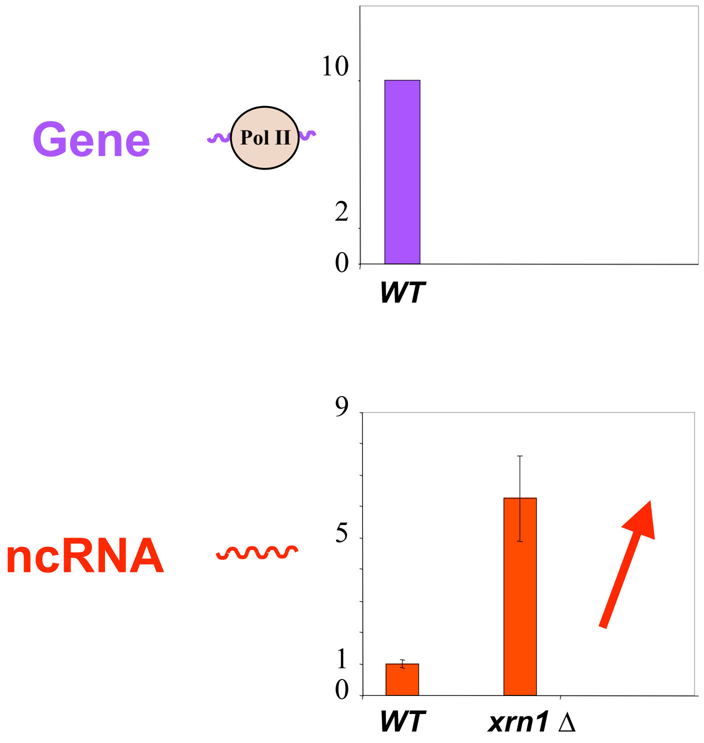
ncRNA



273 genes with antisense XUT show a significant decrease in transcription level

Example of gene TIR1

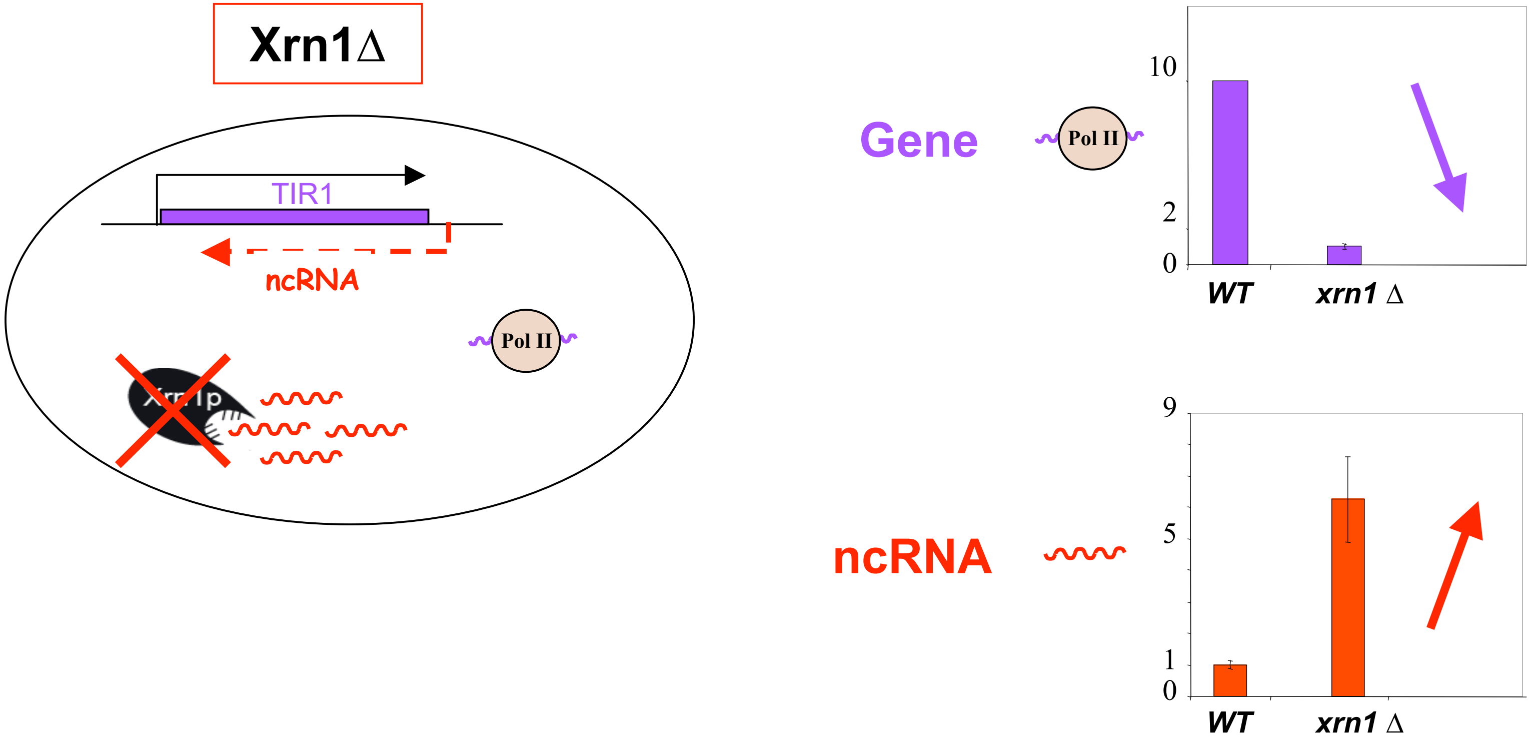




Increase of ncRNA

273 genes with antisense XUT show a significant decrease in transcription level

Example of gene TIR1

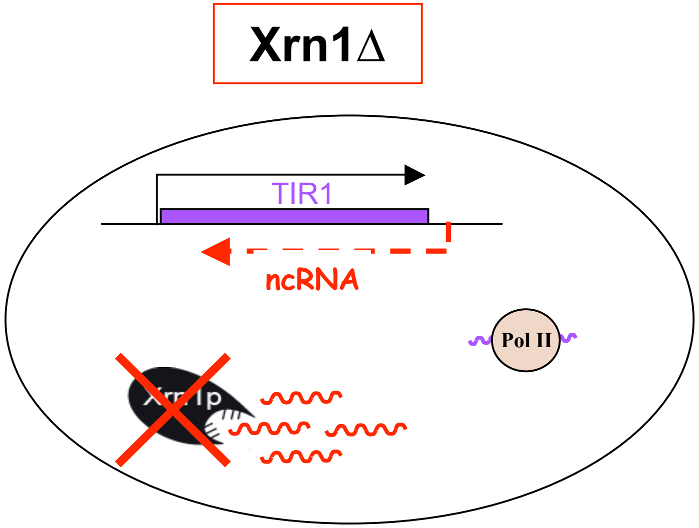


Increase of ncRNA

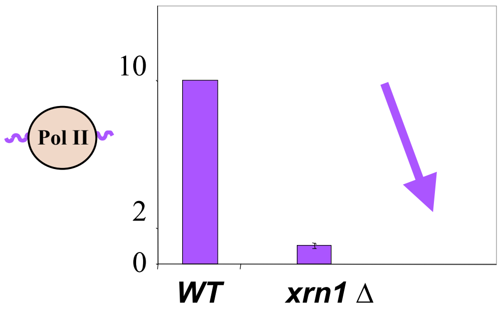
Repression of gene

273 genes with antisense XUT show a significant decrease in transcription level

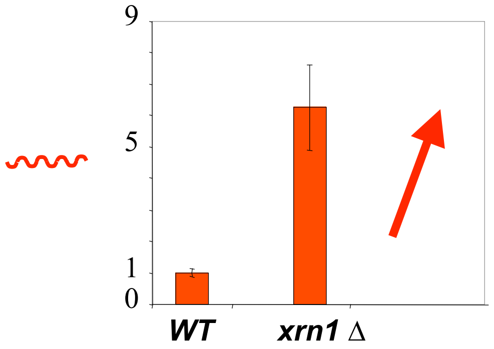
Example of gene TIR1



Gene



ncRNA

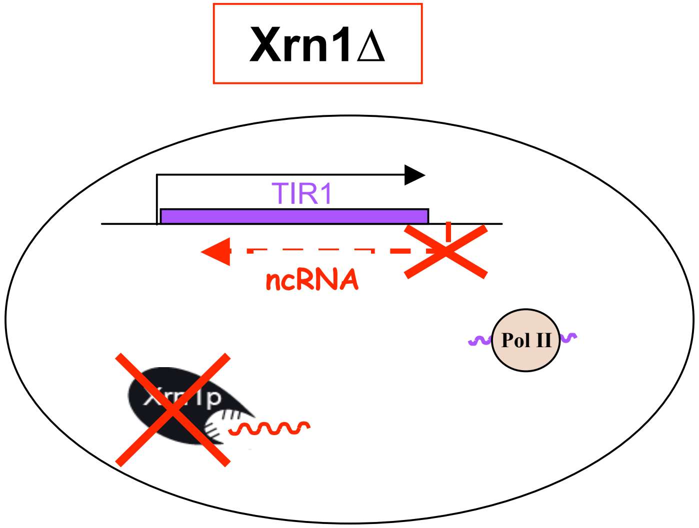


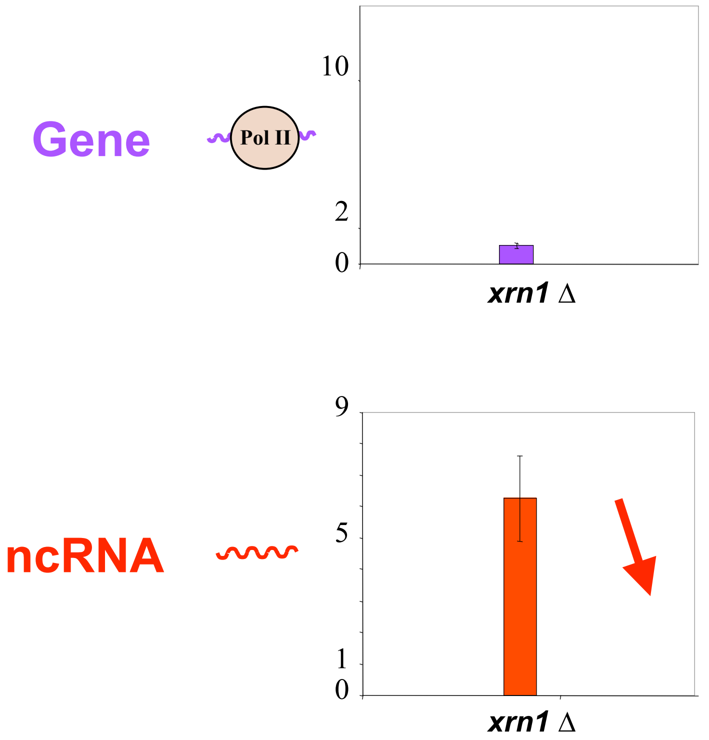
Increase of ncRNA ? ⟷ **Repression of gene**

Direct effect

273 genes with antisense XUT show a significant decrease in transcription level

Example of gene TIR1

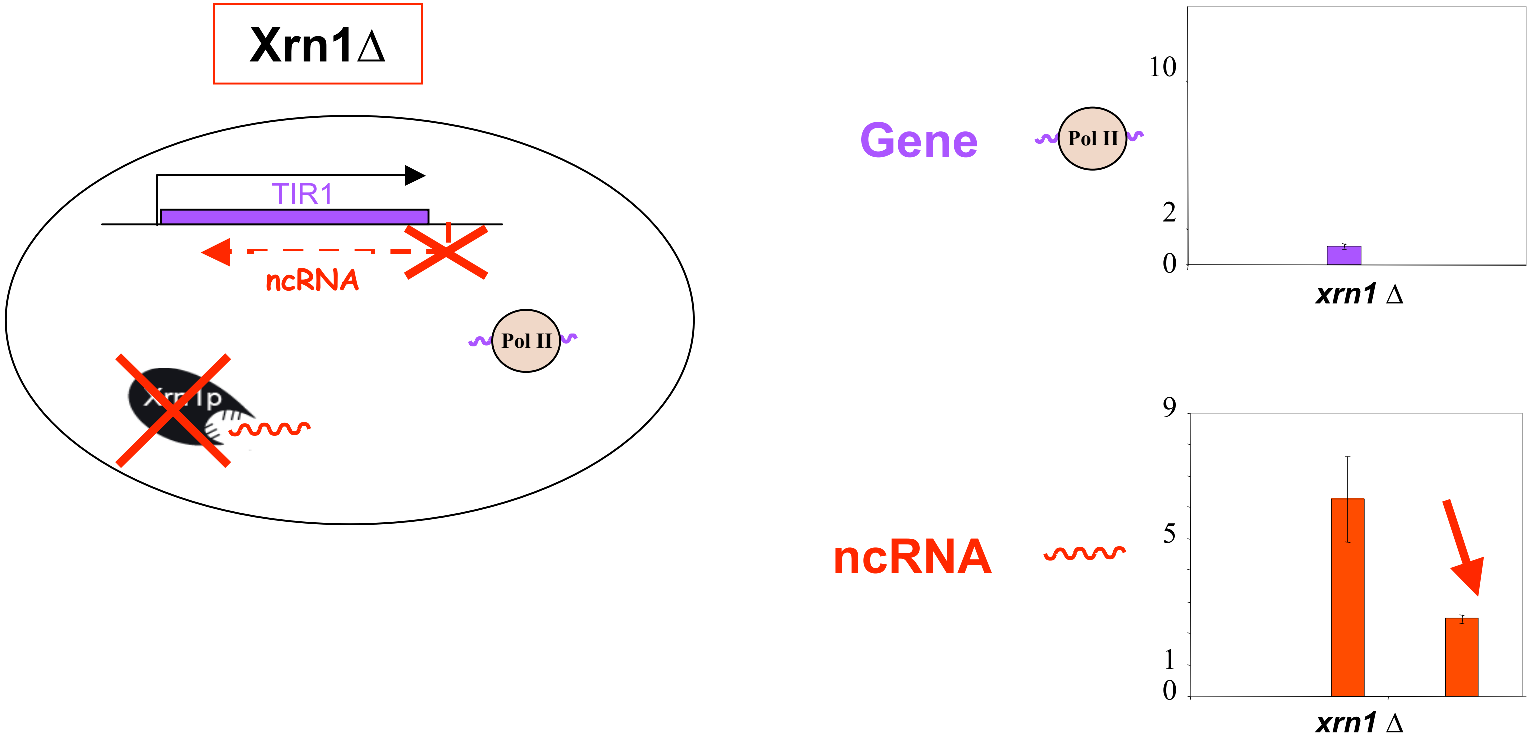




Suppression of ncRNA

273 genes with antisense XUT show a significant decrease in transcription level

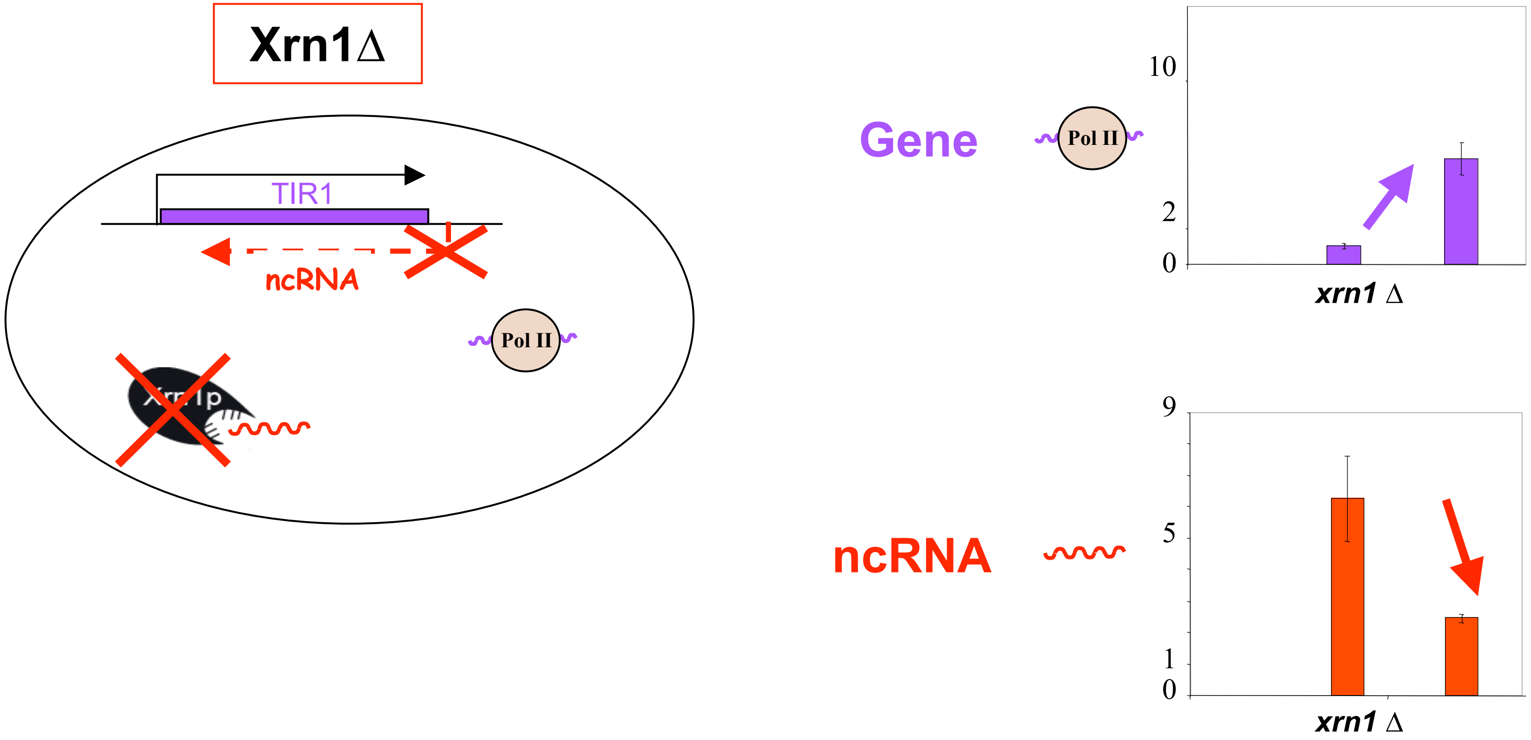
Example of gene TIR1



Suppression of ncRNA

273 genes with antisense XUT show a significant decrease in transcription level

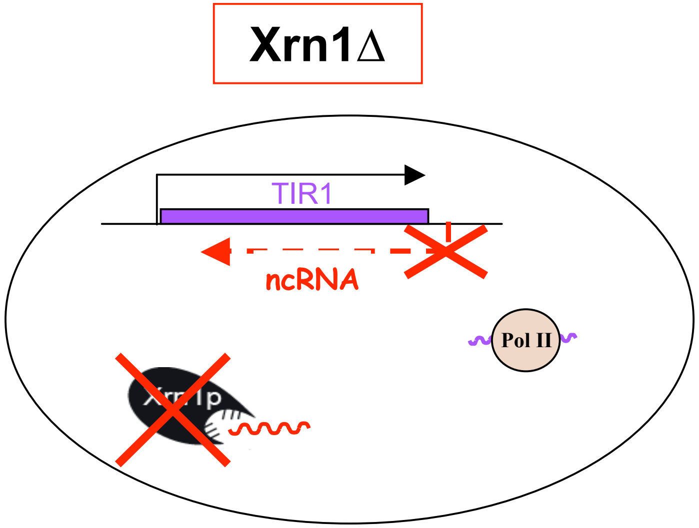
Example of gene TIR1

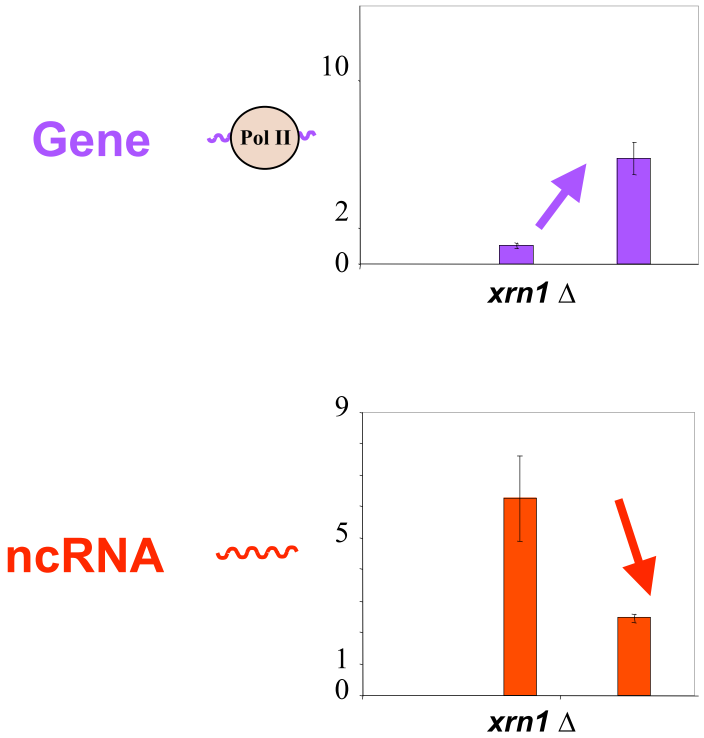


Suppression of ncRNA → Increase gene transcription

273 genes with antisense XUT show a significant decrease in transcription level

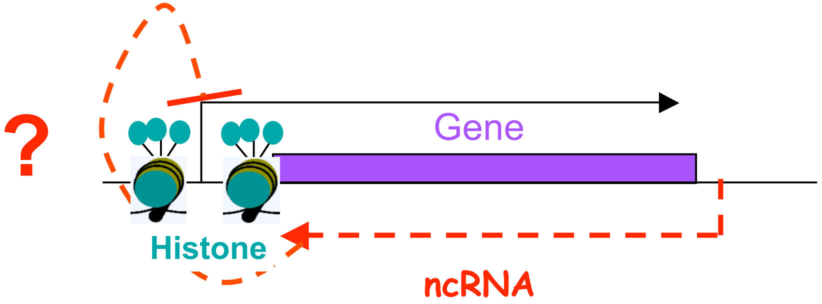
Example of gene TIR1





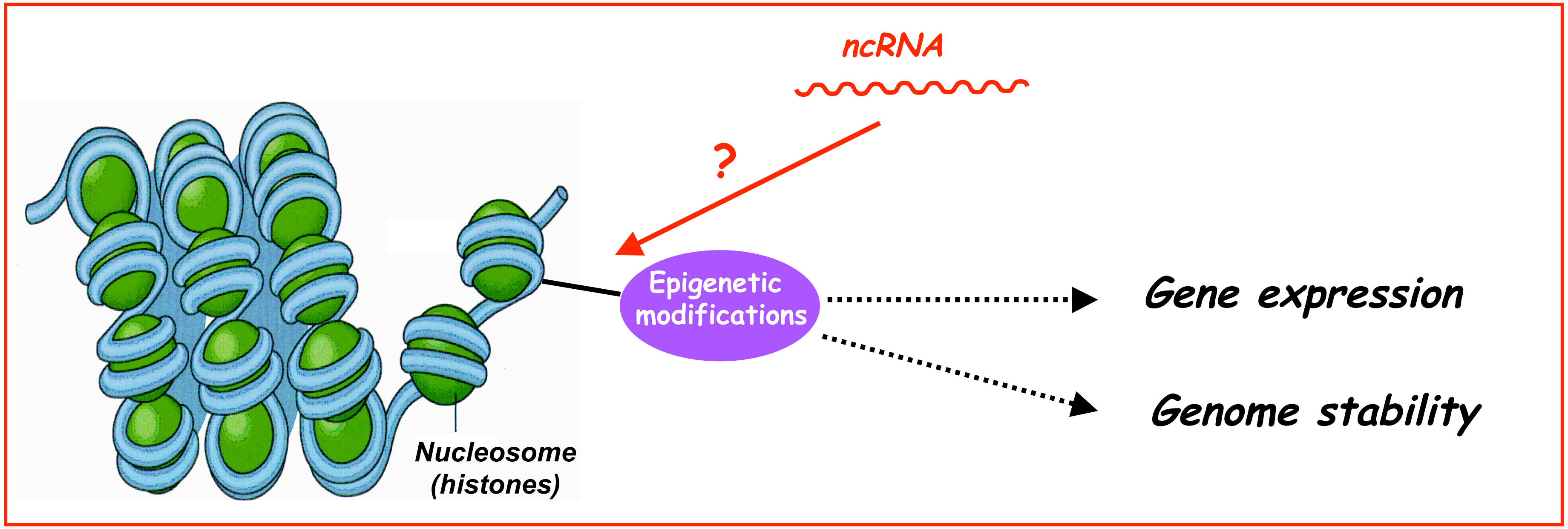
Transcriptional gene silencing is associated with antisense ncRNA

How does it work ?



Camblong et al., Cell 2007
Berretta et al., Gen Dev 2008
Pinskaya et al., EMBOJ 2009

- **Why some genes are regulated by antisense ncRNA but the others are not?**
- **Is it associated with epigenetic modification?**


ncRNA

?

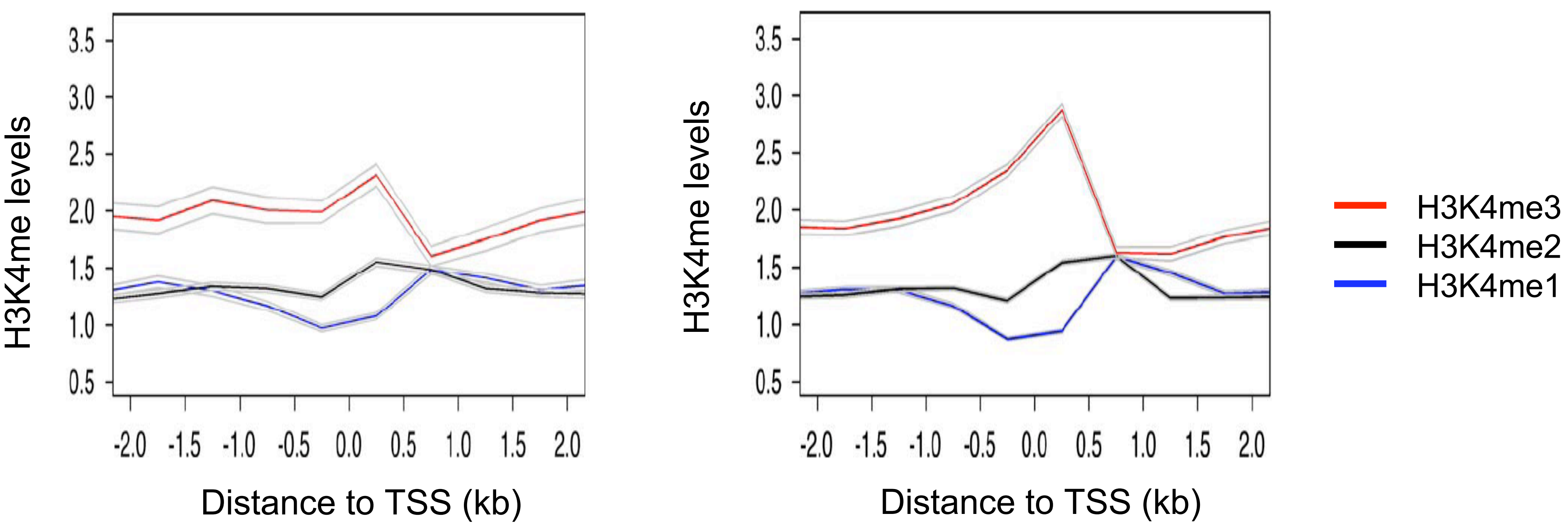
Epigenetic modifications

Gene expression

Genome stability

Nucleosome (histones)

Transcriptional gene silencing depends on histone modification

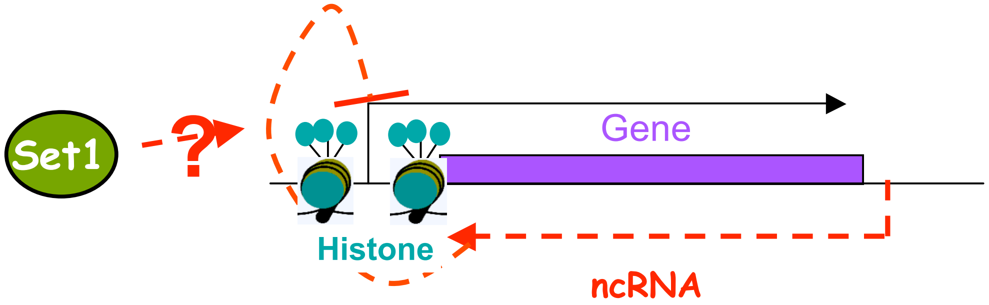


Down-regulated ORF
with antisense XUT

Other ORF
With antisense XUT

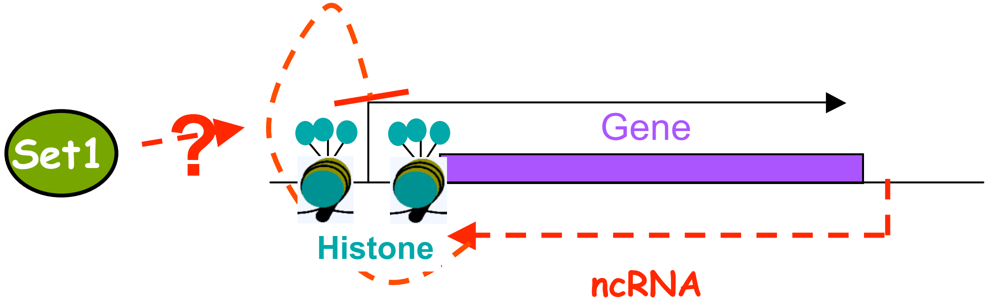
The down-regulated genes with antisense XUT present low H3k4me3 level

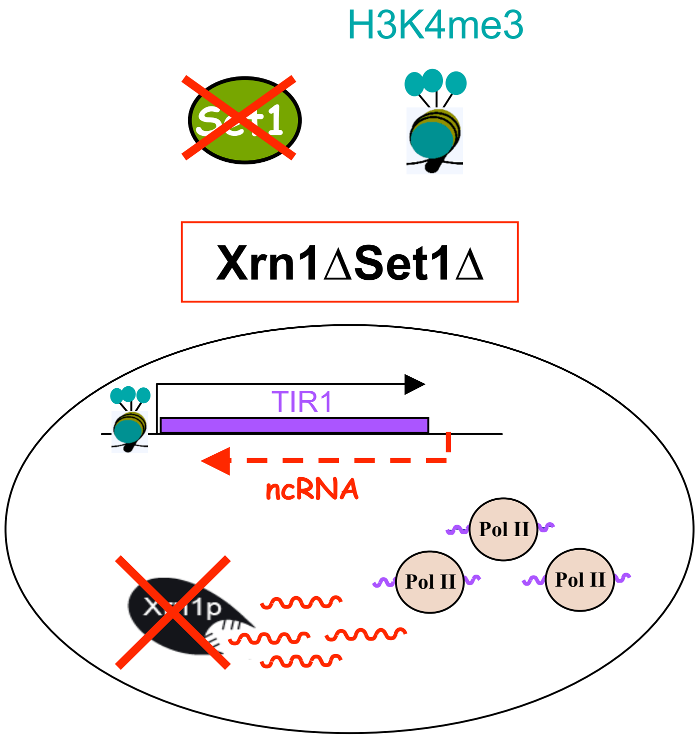
(ChIP-chip data from Pokholok et al. 2005 *Cell.* 122:517)



Set1: methylation of histone H3K4

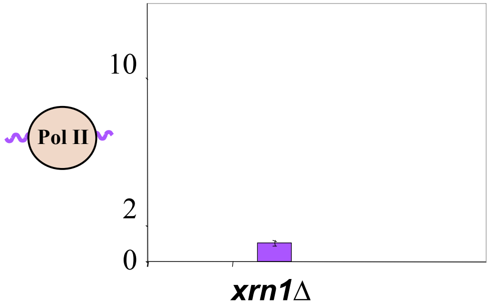
Does it contribute to regulation of gene expression?



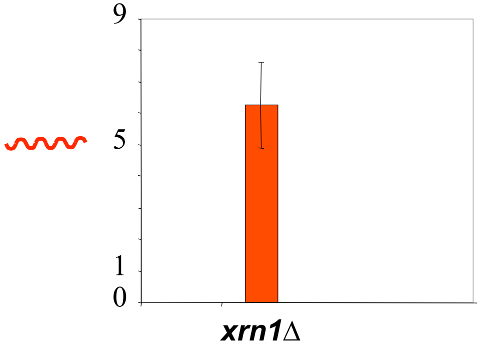


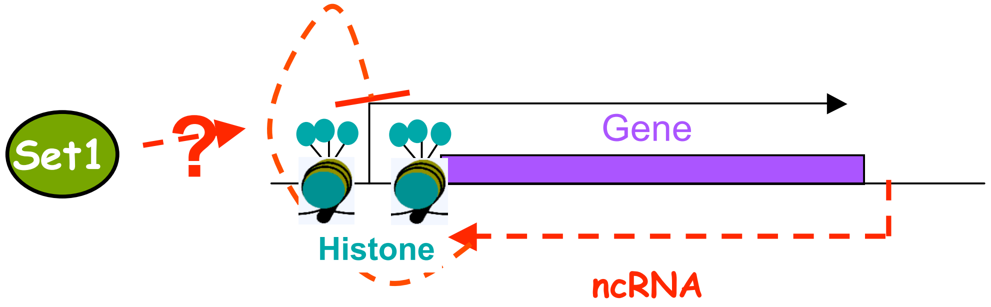
Xrn1ΔSet1Δ

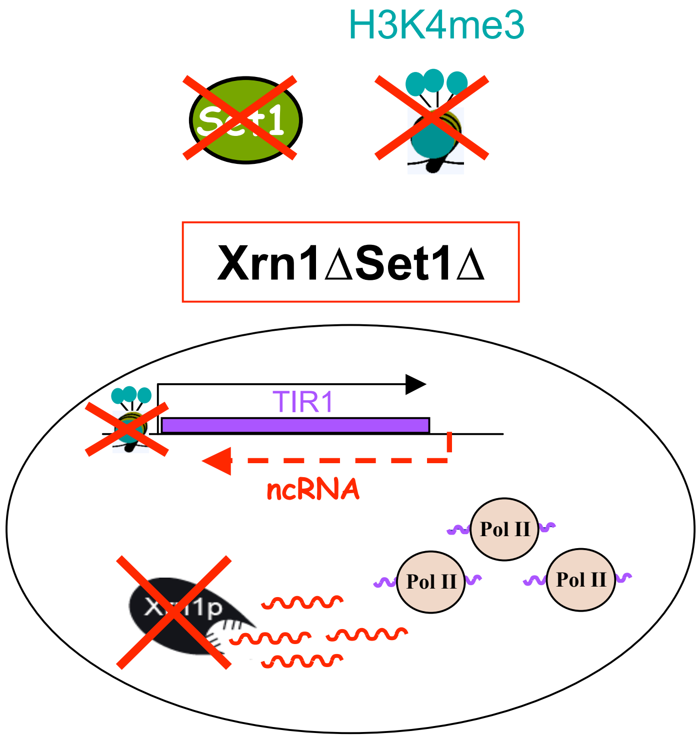
Gene

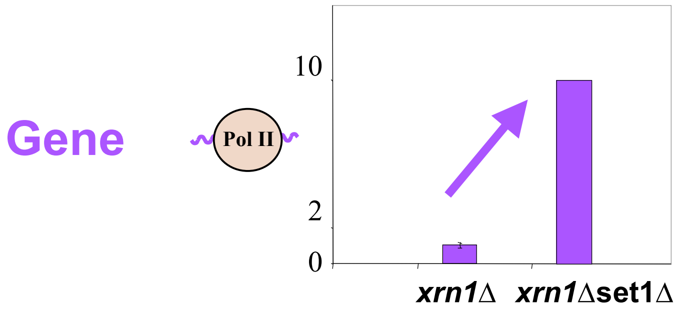


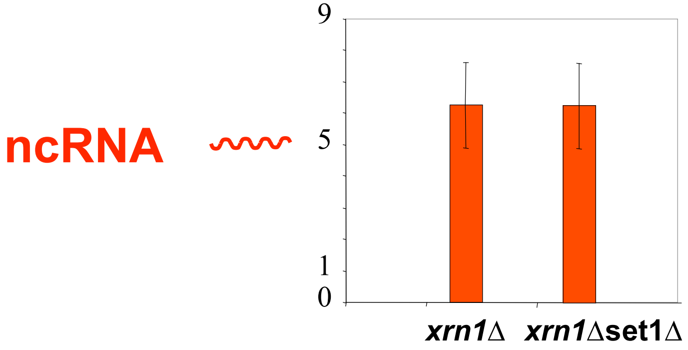
ncRNA

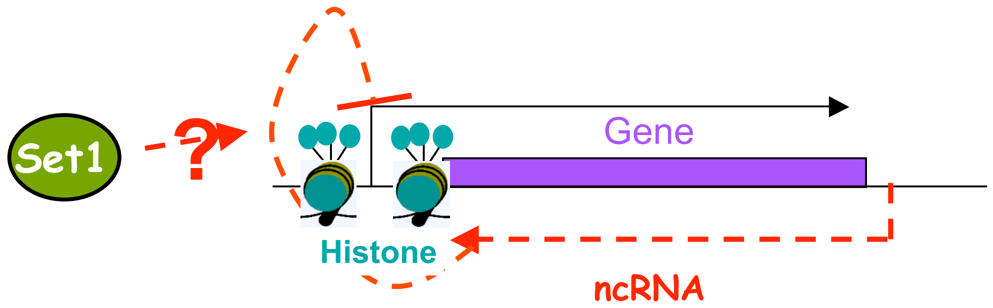



Set1
?
Gene
Histone
ncRNA

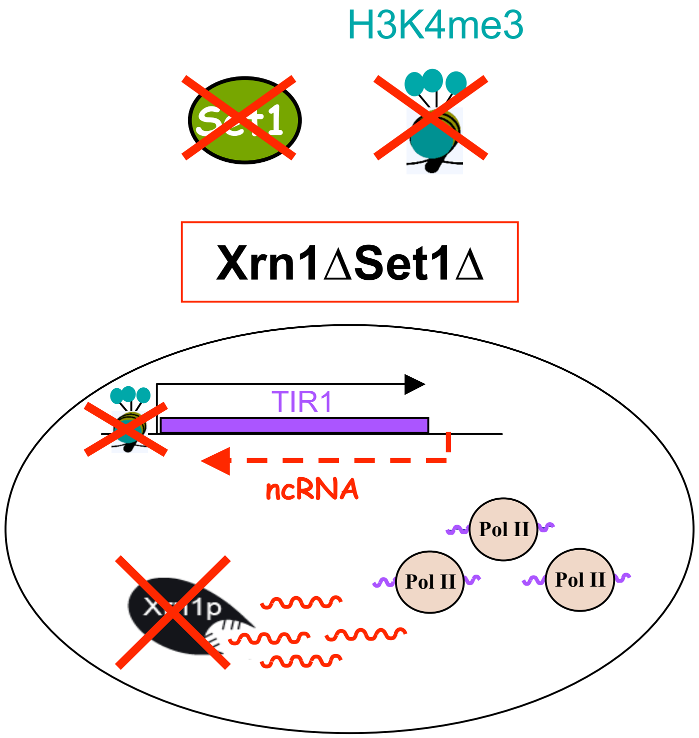

H3K4me3
Set1
Xrn1ΔSet1Δ
TIR1
ncRNA
Pol II
Pol II
Pol II
Xrn1p

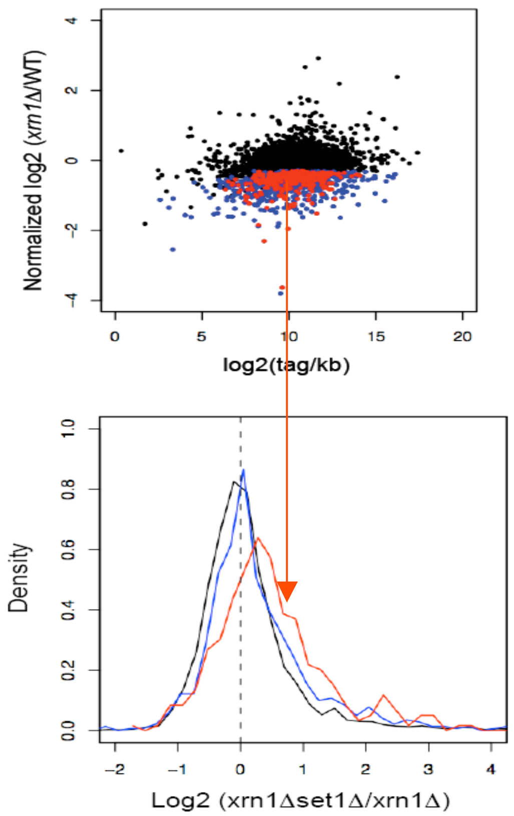

Gene
Pol II
10
2
0
*xrn1*Δ
*xrn1*Δset1Δ


ncRNA
9
5
1
0
*xrn1*Δ
*xrn1*Δset1Δ



Set1 silences the 273 genes



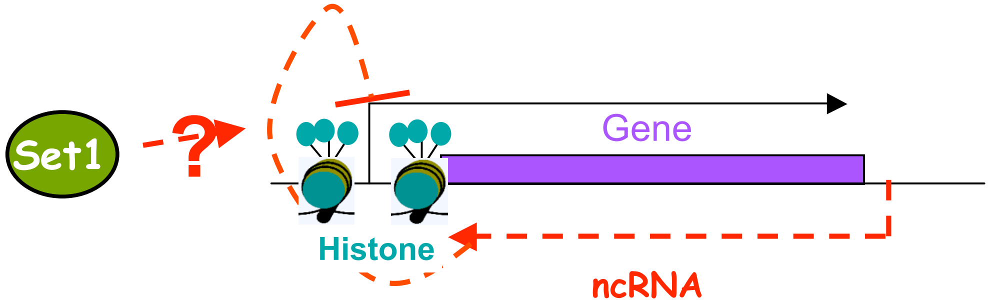


ORF down-regulated with antisense XUT

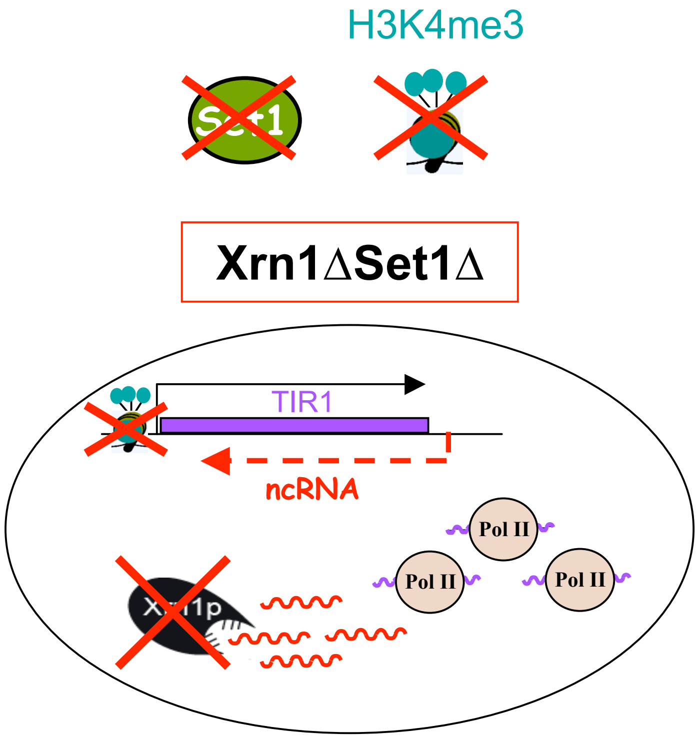
ORF down-regulated without antisense XUT

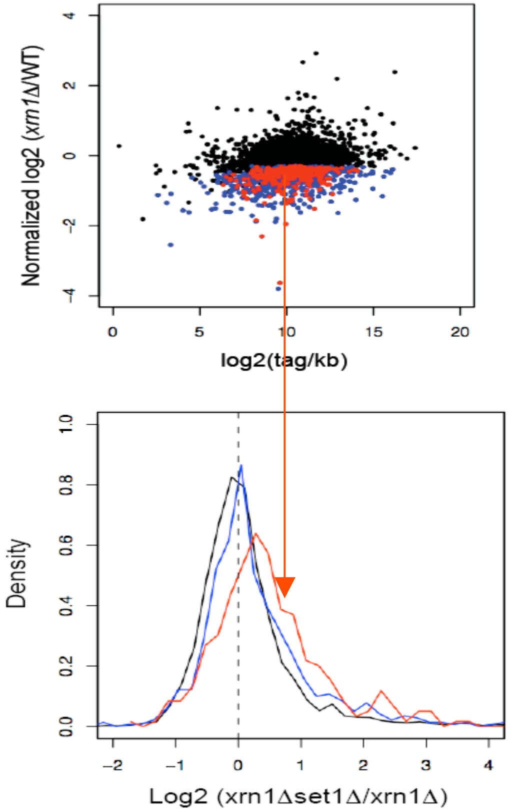
Other ORFs

Transcription level of genes with antisense XUT increase in the Xrn1ΔSet1Δ compare with the Xrn1Δ, but the others do not.



Set1 silences the 273 genes





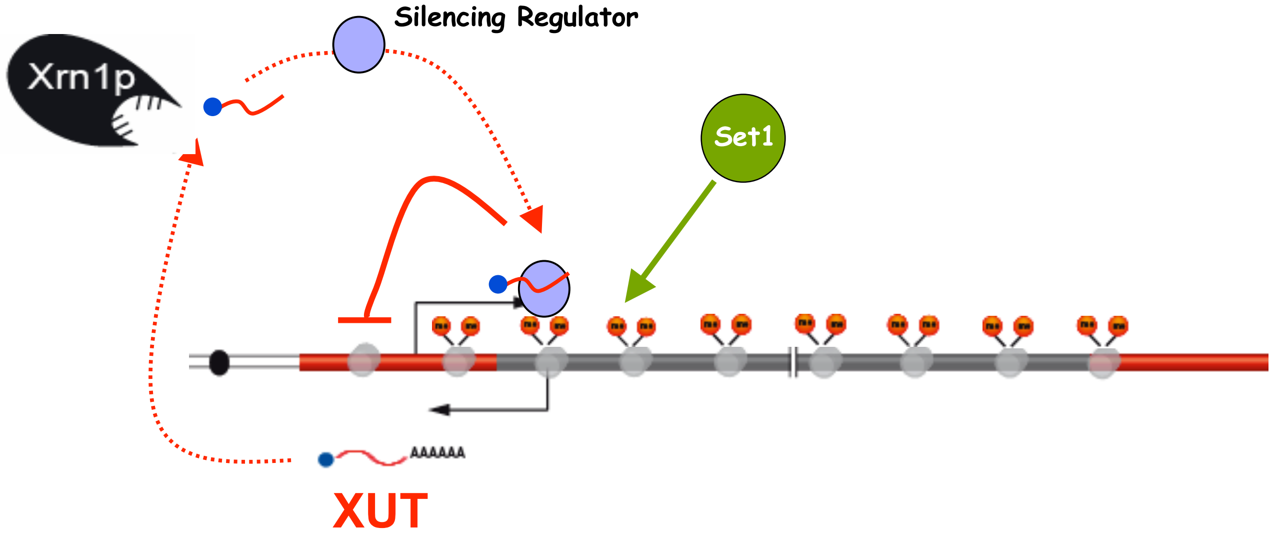
ORF down-regulated with antisense XUT

ORF down-regulated without antisense XUT

Other ORFs

Transcriptional gene silencing depends on H3K4 methylation

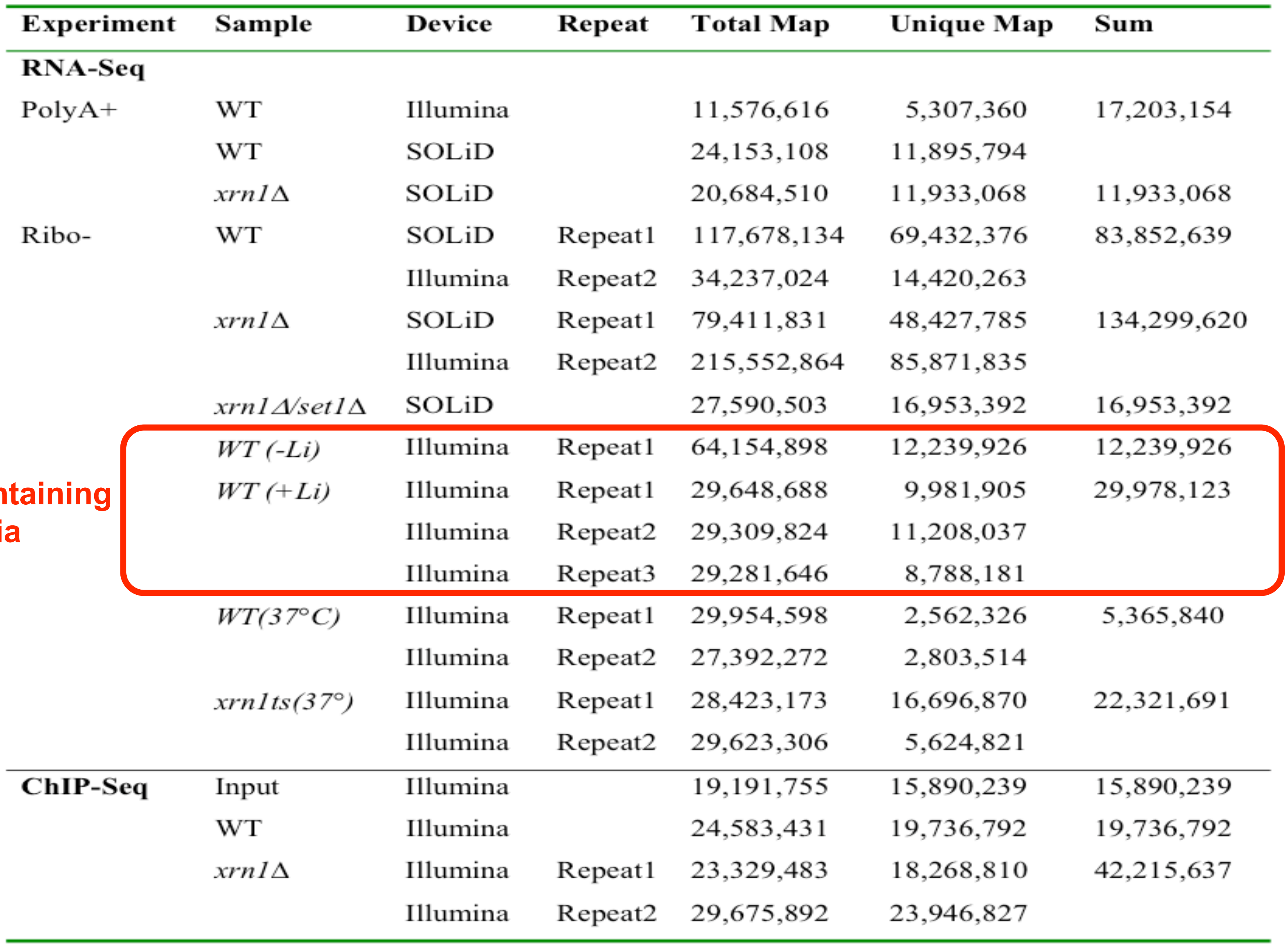
Conclusions



- Identification of 1658 ncRNA sensitive to Xrn1 (XUT)
- Antisense XUT contribute to transcriptional gene silencing
- This transcriptional gene silencing is associated with epigenetic modification
- XUT are naturally observed in some stress conditions

van-Dijk et al. 2011 *Nature.* 475:114

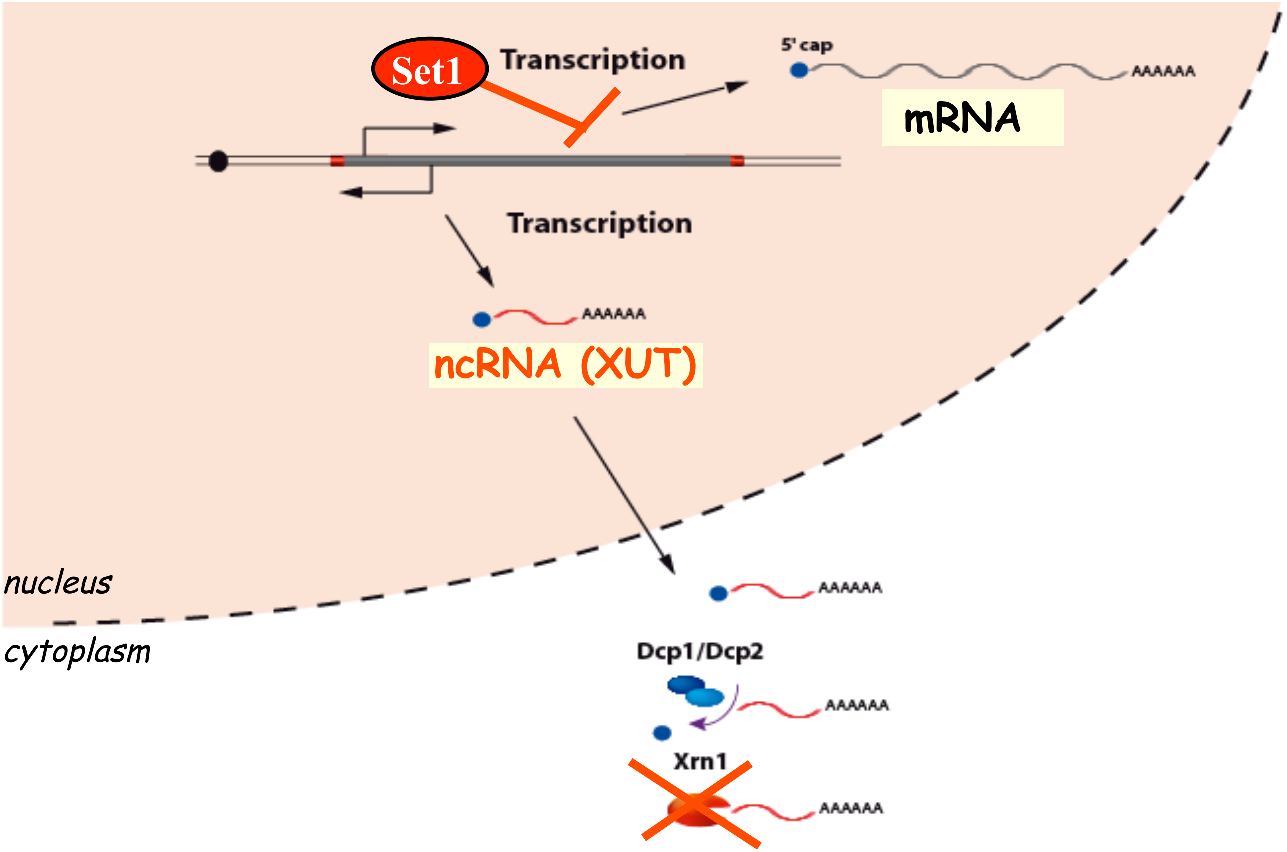
All sequencing data generated by this study



Experiment	Sample	Device	Repeat	Total Map	Unique Map	Sum
RNA-Seq						
PolyA+	WT	Illumina		11,576,616	5,307,360	17,203,154
	WT	SOLiD		24,153,108	11,895,794	
	xrn1Δ	SOLiD		20,684,510	11,933,068	11,933,068
Ribo-	WT	SOLiD	Repeat1	117,678,134	69,432,376	83,852,639
		Illumina	Repeat2	34,237,024	14,420,263	
	xrn1Δ	SOLiD	Repeat1	79,411,831	48,427,785	134,299,620
		Illumina	Repeat2	215,552,864	85,871,835	
	xrn1Δ/set1Δ	SOLiD		27,590,503	16,953,392	16,953,392
	WT (-Li)	Illumina	Repeat1	64,154,898	12,239,926	12,239,926
	WT (+Li)	Illumina	Repeat1	29,648,688	9,981,905	29,978,123
		Illumina	Repeat2	29,309,824	11,208,037	
		Illumina	Repeat3	29,281,646	8,788,181	
	WT(37°C)	Illumina	Repeat1	29,954,598	2,562,326	5,365,840
		Illumina	Repeat2	27,392,272	2,803,514	
	xrn1ts(37°)	Illumina	Repeat1	28,423,173	16,696,870	22,321,691
		Illumina	Repeat2	29,623,306	5,624,821	
ChIP-Seq	Input	Illumina		19,191,755	15,890,239	15,890,239
	WT	Illumina		24,583,431	19,736,792	19,736,792
	xrn1Δ	Illumina	Repeat1	23,329,483	18,268,810	42,215,637
		Illumina	Repeat2	29,675,892	23,946,827	

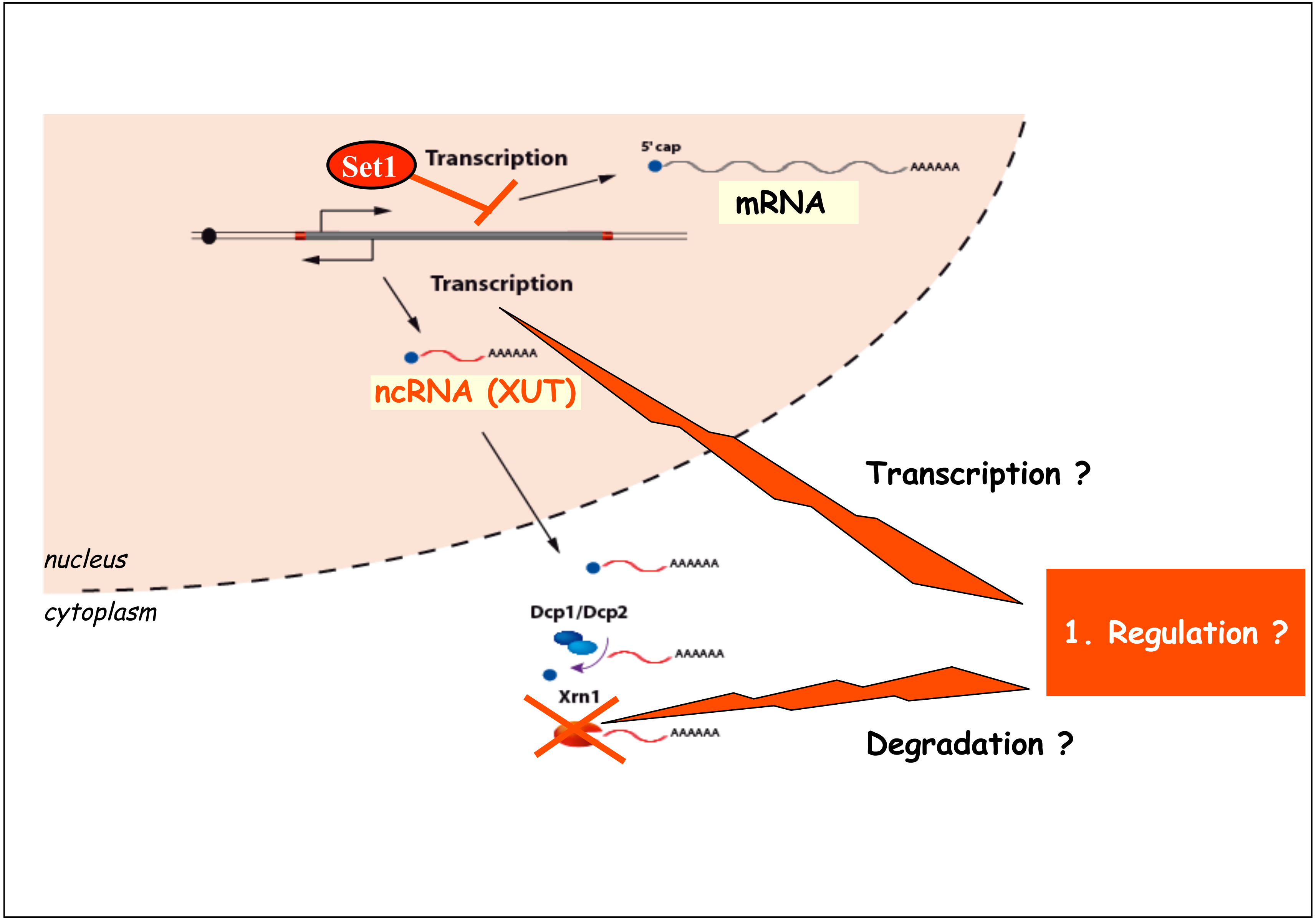
lithium-containing media

Model and perspectives

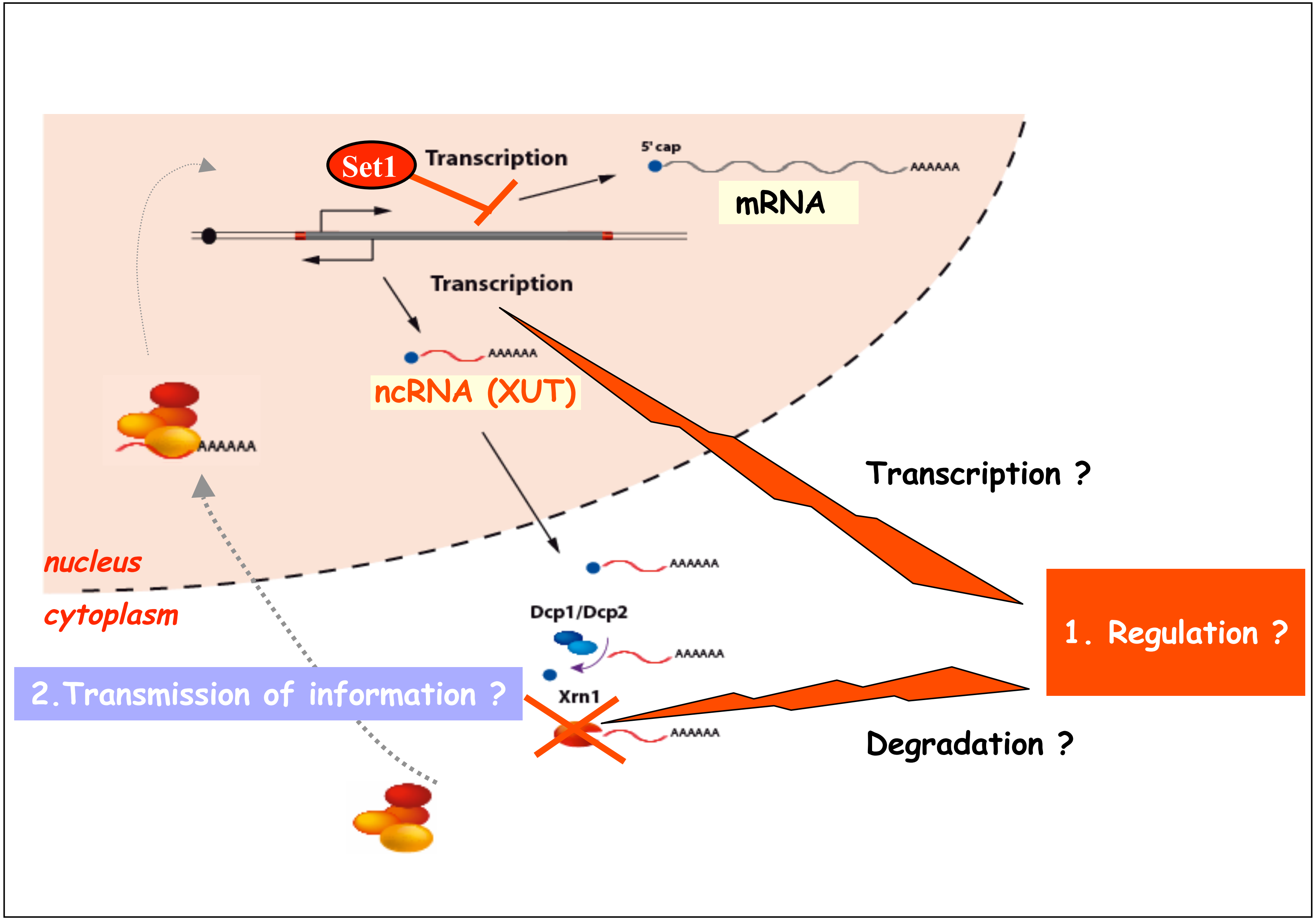


1. Regulation ?

Model and perspectives



Model and perspectives



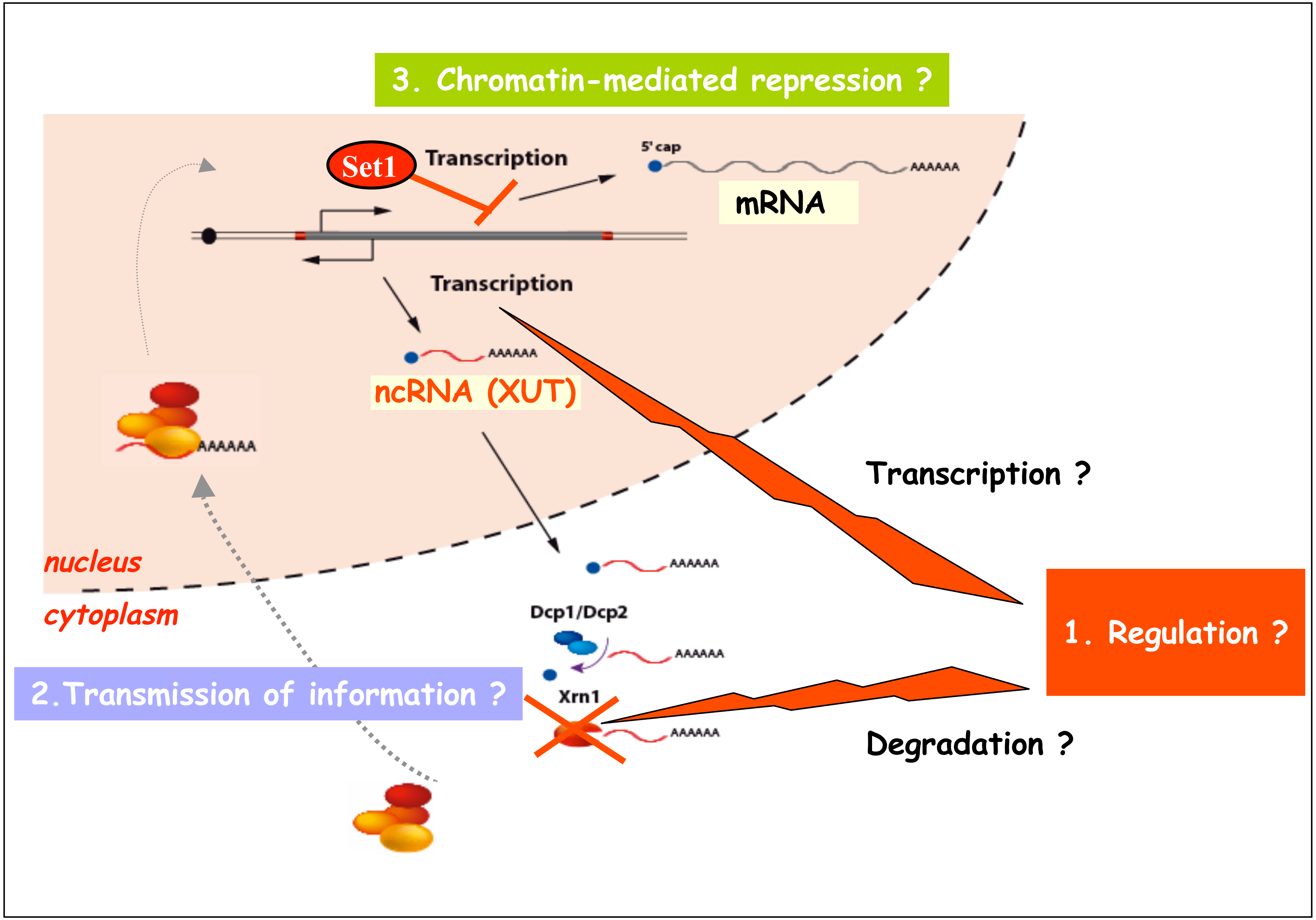
Transcription ?

1. Regulation ?

Degradation ?

2.Transmission of information ?

Model and perspectives


3. Chromatin-mediated repression ?

ncRNA (XUT)

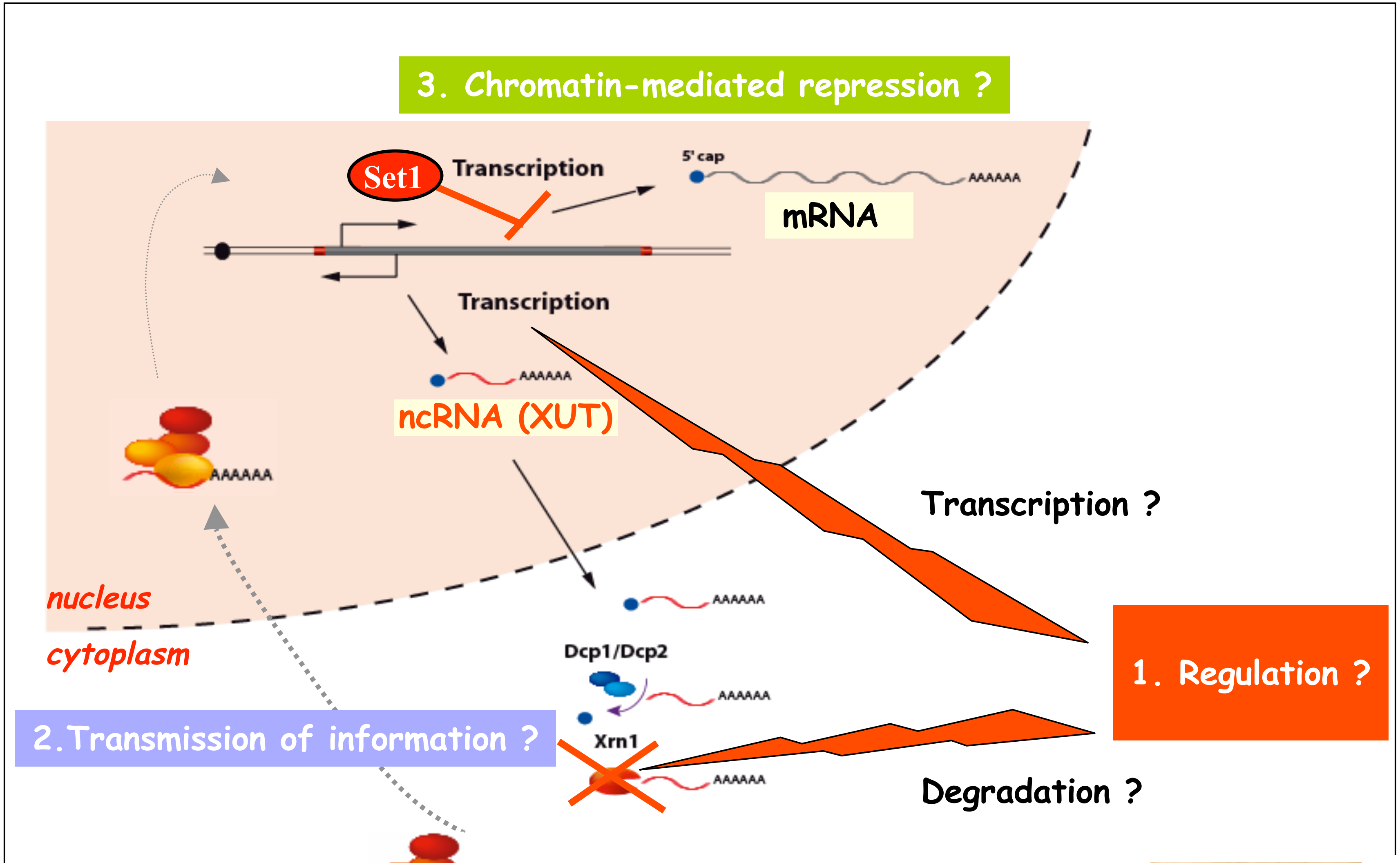
Transcription ?

1. Regulation ?

Degradation ?

2.Transmission of information ?

Model and perspectives



3. Chromatin-mediated repression ?

Transcription ?

1. Regulation ?

2.Transmission of information ?

Degradation ?

?

4. In others eukaryotes ?

Acknowledgments and collaborations

C.L. Chen
Y. d'Aubenton-Carafa
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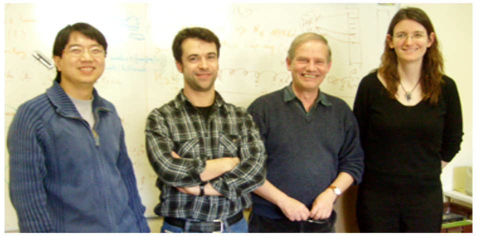
M. Descrimes
C. Thermes
(CGM, Gif-sur-Yvette)



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(Institut Curie)

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A. Aubert
(Plateforme IMAGIF, Gif)

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P. Legoix-Né
A. Nicolas
(Plateforme SOLiD, Institut Curie)

CGM
Centre de Génétique Moléculaire
CNRS - UPR 2167 - Gif-sur-Yvette

institut Curie
Ensemble, prenons le cancer de vitesse.

imagif
La plateforme du vivant

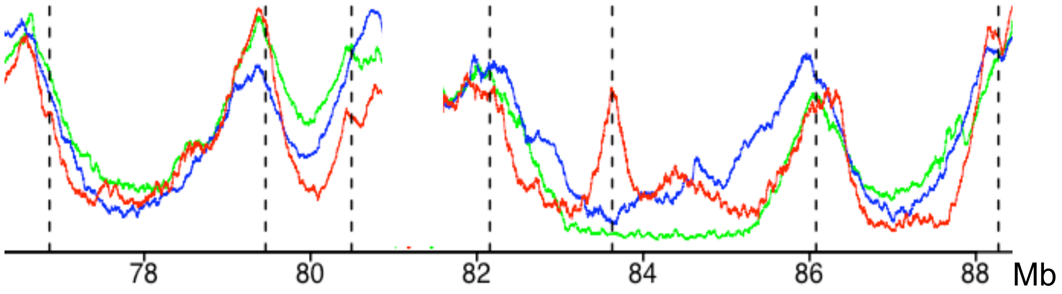
cnrs
dépasser les frontières

Agence Nationale de la Recherche
ANR

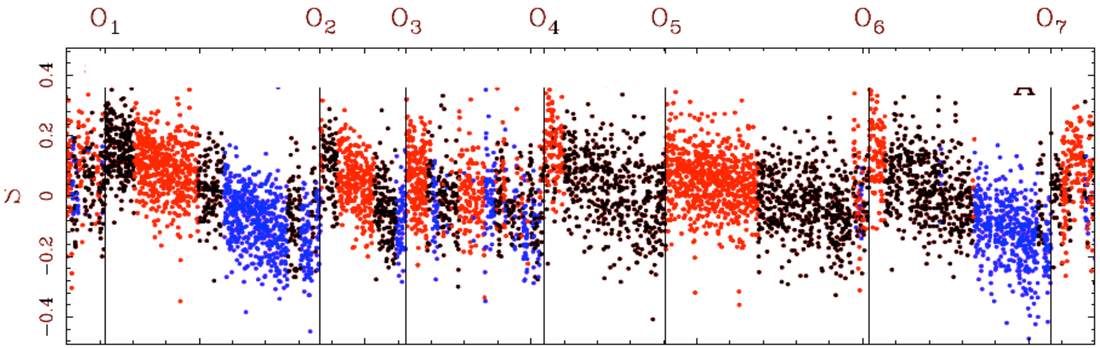
Merci!

Human replication timing program : determination by deep sequencing & role in genome evolution

Replication timing



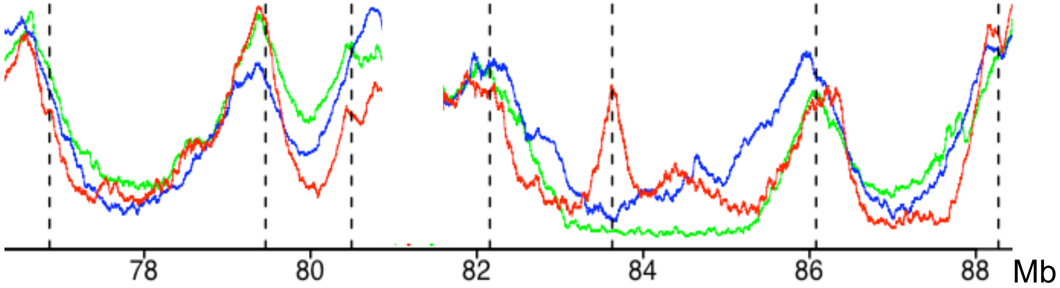
Nucleotide compositional skew



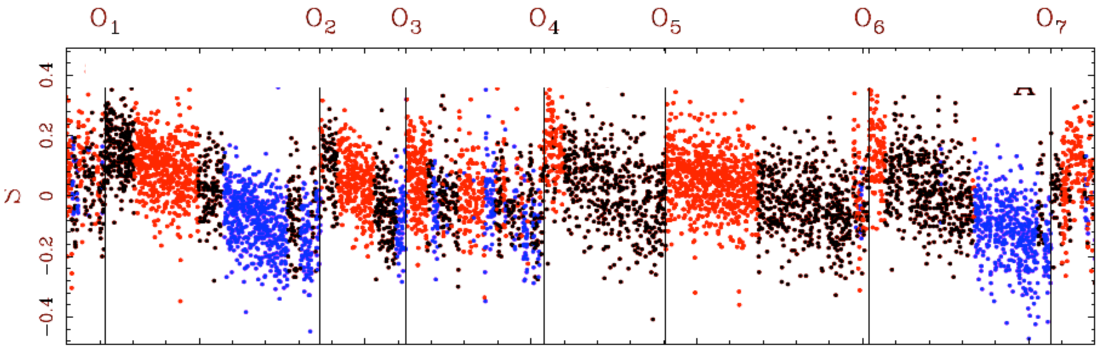
- **What's these beautiful profiles?**
- **What can we learn from these profiles?**

Human replication timing program : determination by deep sequencing & role in genome evolution

Replication timing



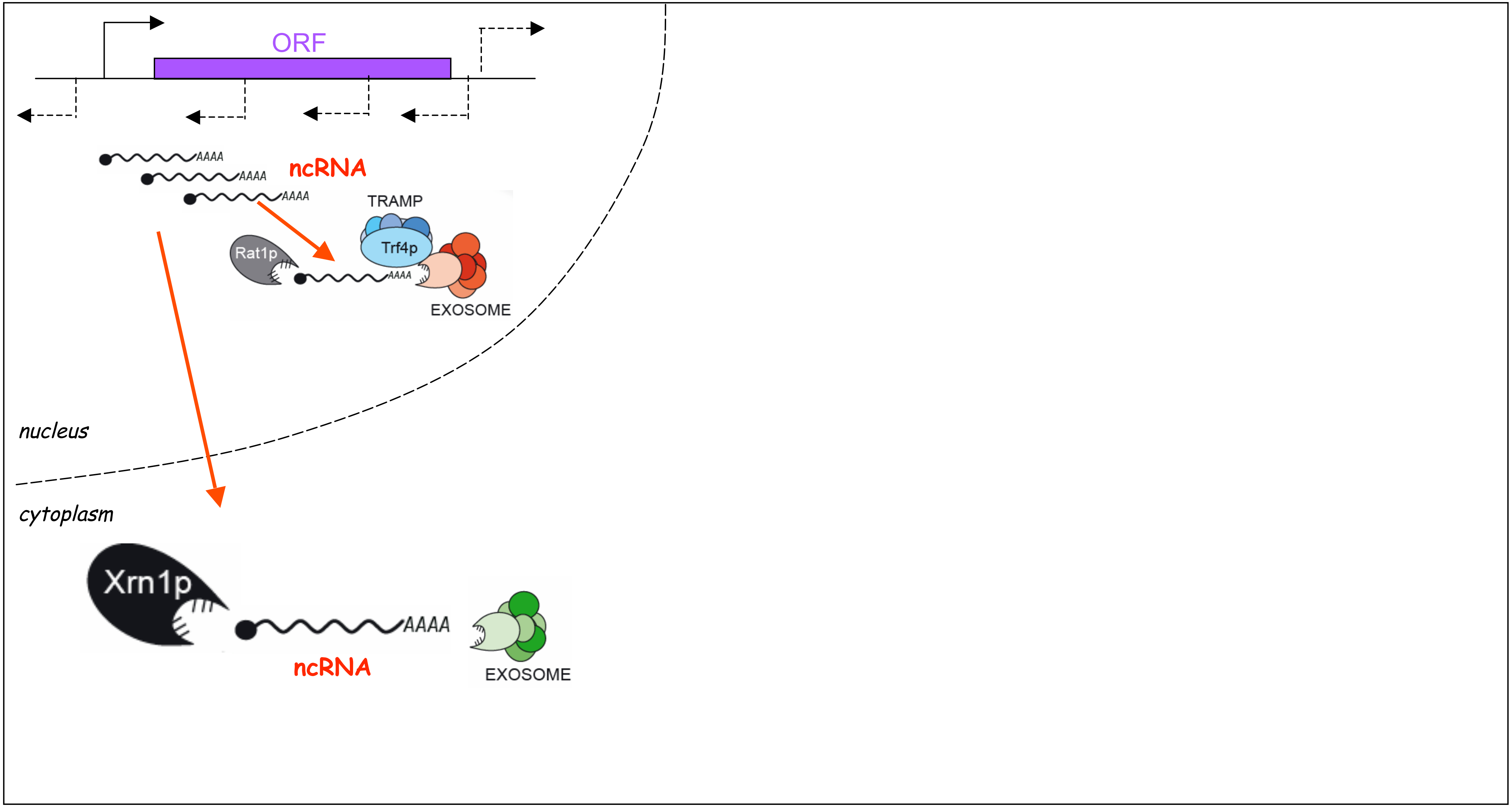
Nucleotide compositional skew



See you next time!

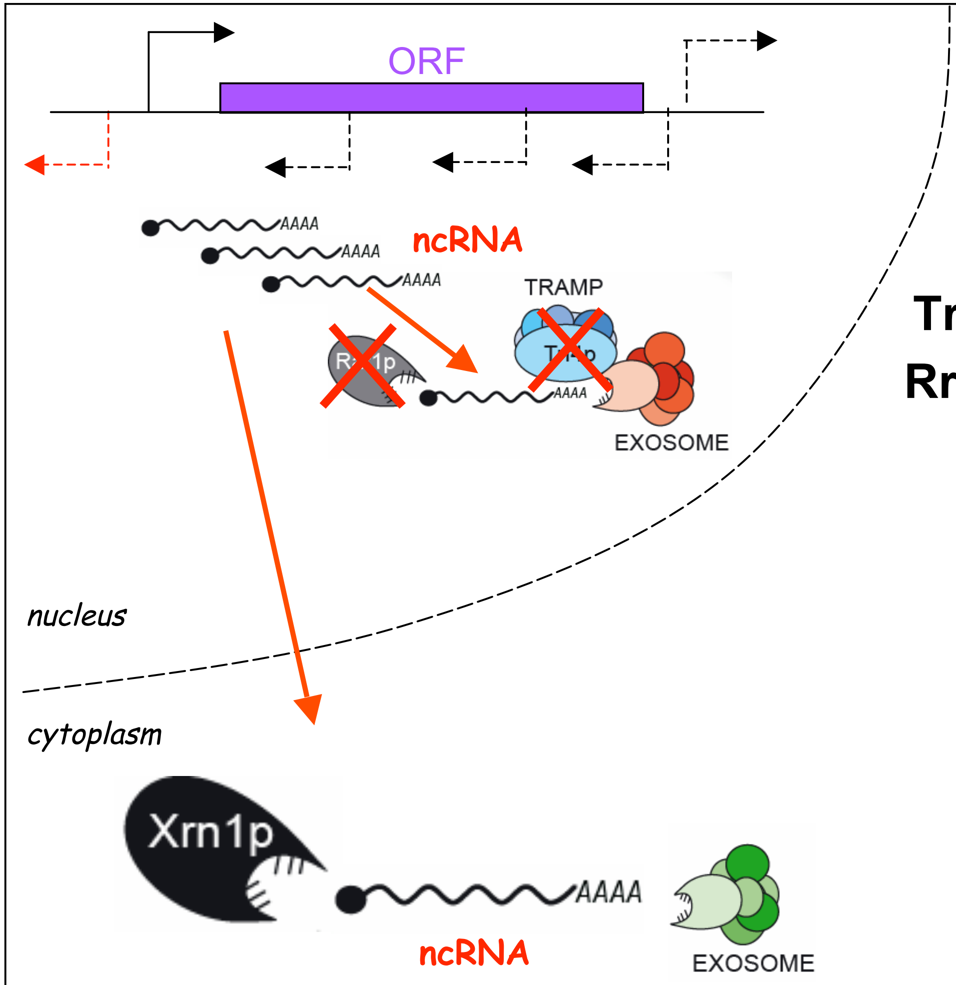
07/12/12, BioInfo Club, IJM, Paris

Cryptic transcripts in yeast



Short half-lives, unvisible at WT but enriched in mutant defective in RNA turnover

Cryptic transcripts in yeast



Wyers et al 2005 *Cell* 121:725
Neil et al 2009 *Nature* 457:1038
Xu. et al. 2009 *Nature* 457:1033

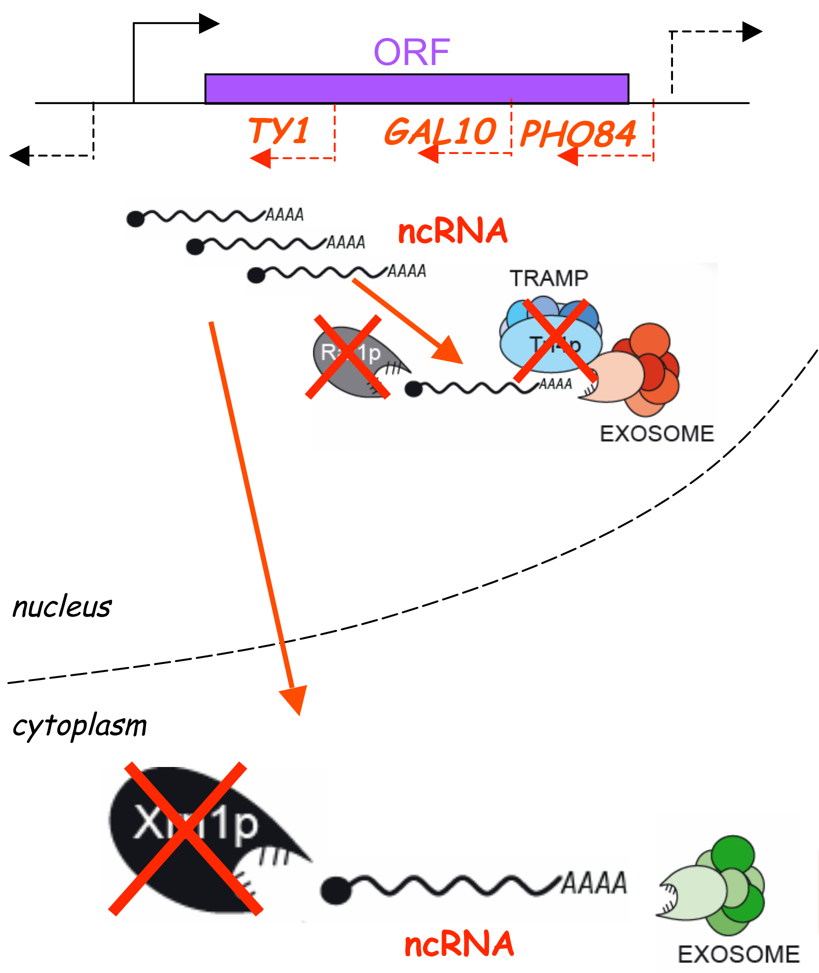
Trf4Δ
Rrp6Δ → **CUT (Cryptic Unstable Transcripts)**

64% divergent

SUT (Stable Unannotated Transcripts)

Short half-lives, unvisible at WT but enriched in mutant defective in RNA turnover

Cryptic transcripts in yeast



Wyers et al 2005 *Cell* 121:725
Neil et al 2009 *Nature* 457:1038
Xu. et al. 2009 *Nature* 457:1033

Trf4Δ
Rrp6Δ → **CUT (Cryptic Unstable Transcripts)**

SUT (Stable Unannotated Transcripts)

Xrn1Δ → **XUT**
(Xrn1-sensitive Unstable Transcripts)

Berretta et al 2008 *Gene Dev.* 22:615
Pinskaya. et al. 2009 *EMBO* 28:1697
van-Dijk et al. 2011 *Nature.* 475:114

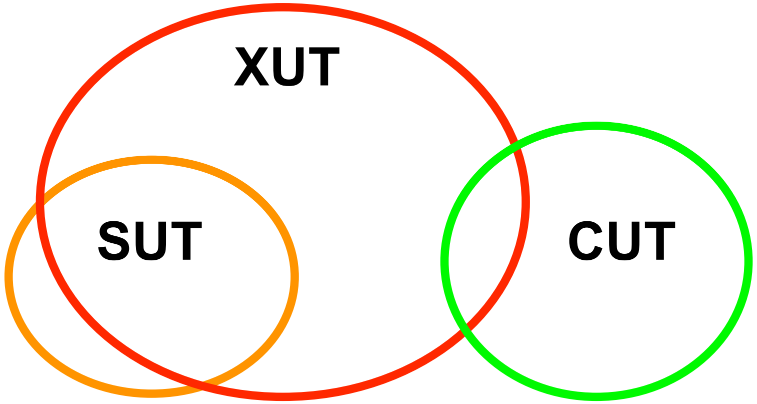
Short half-lives, unvisible at WT but enriched in mutant defective in RNA turnover

1658 Non-coding Xrn1-sensitive Unstable Transcripts (XUT)

1658 XUTs

-183 CUTs (8 fold, 20%)
-531 SUTs (9.6 fold, 75%)

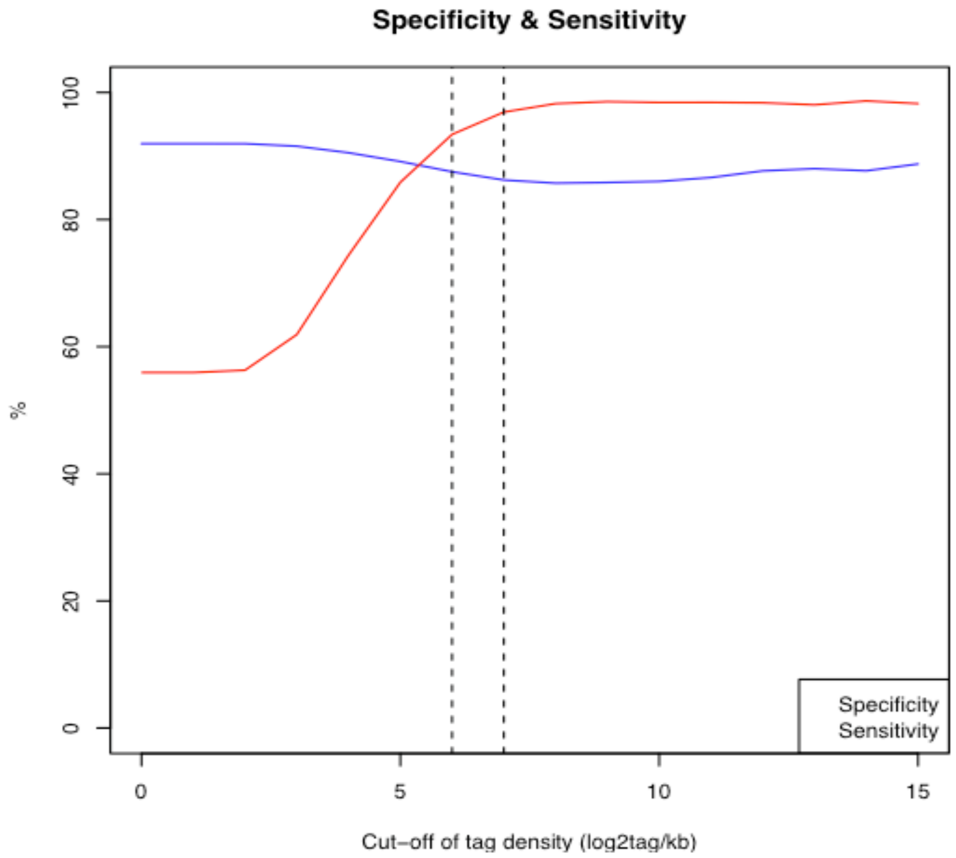
-932 new ncRNAs (10.8 fold)

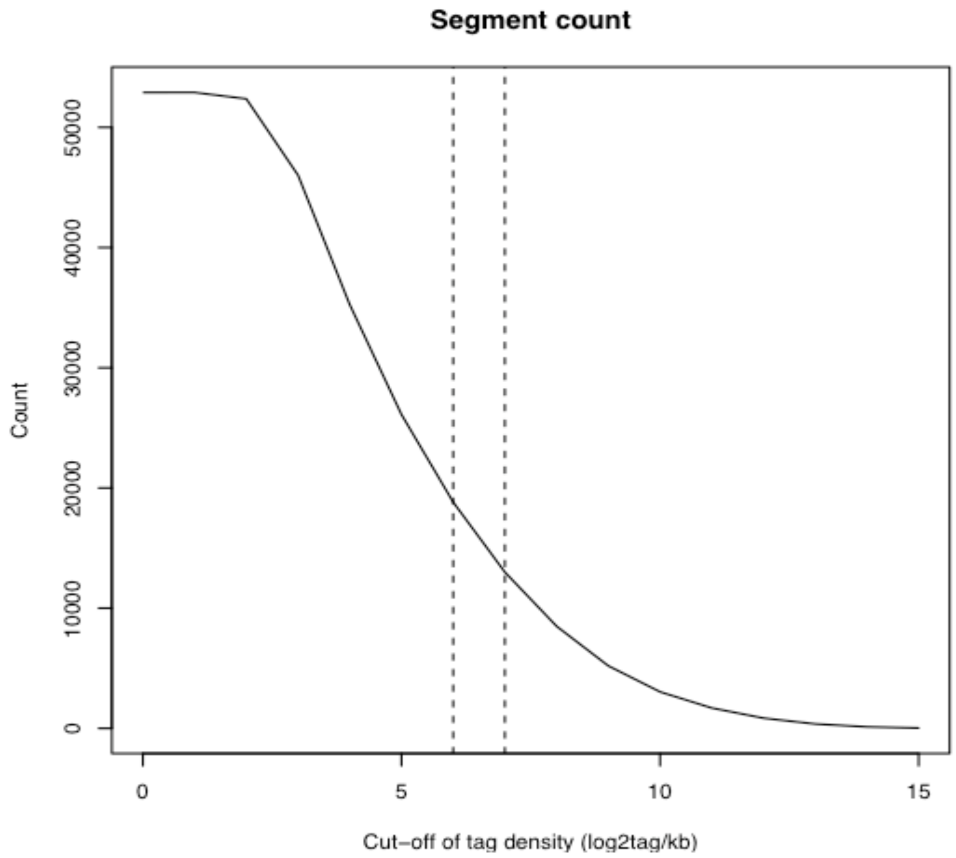


CUT (Cryptic Unstable Transcripts)
SUT (stable unannotated transcripts)

Wyers et al 2005 *Cell* 121:725
Neil et al 2009 *Nature* 457:1038
Xu. et al. 2009 *Nature* 457:1033

Specificity & Sensitivity in function of transcription level



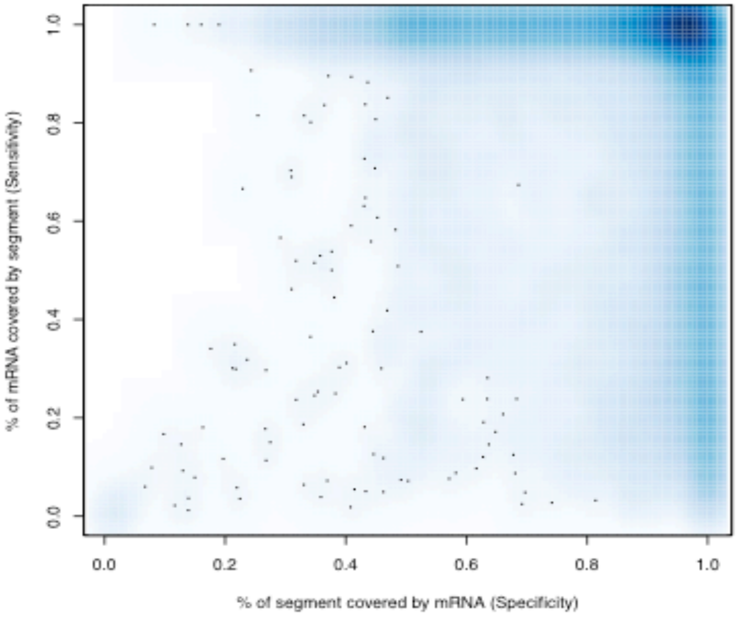


— Specificity

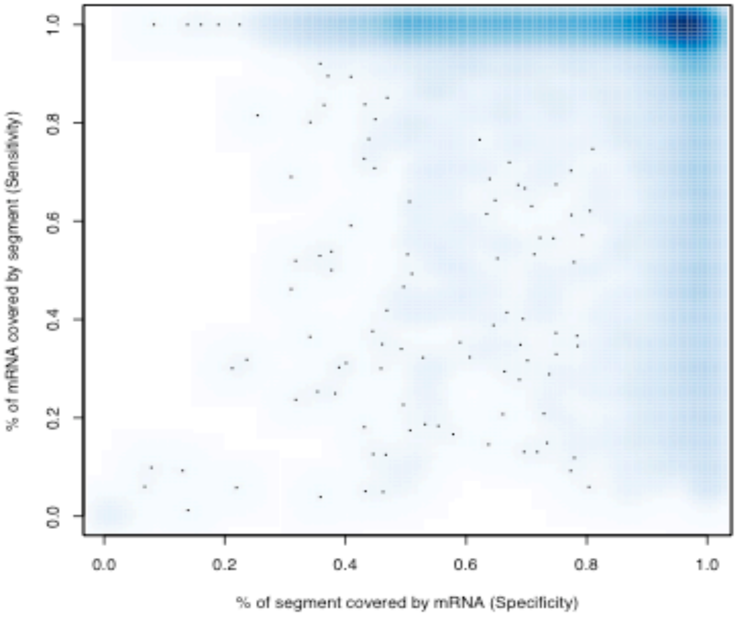
— Sensitivity

Specificity & Sensitivity in function of transcription level

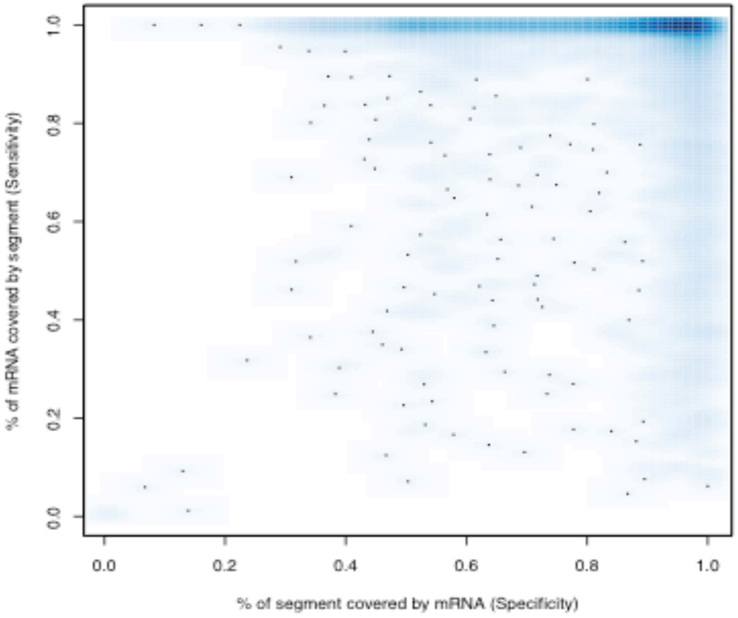
Cutoff = 5



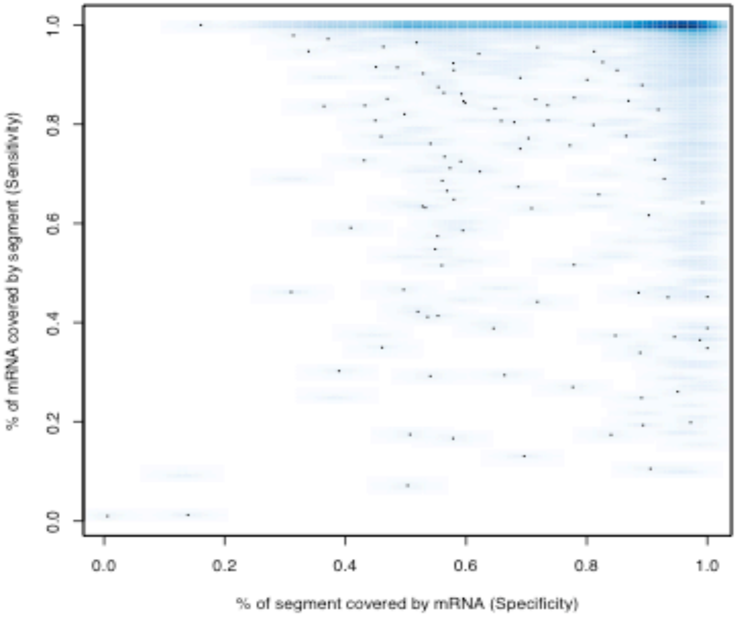
Cutoff = 6



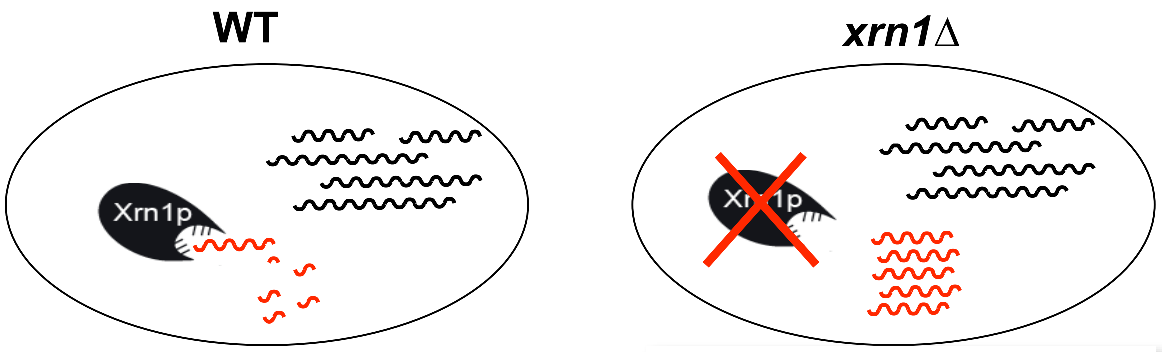
Cutoff = 7



Cutoff = 8



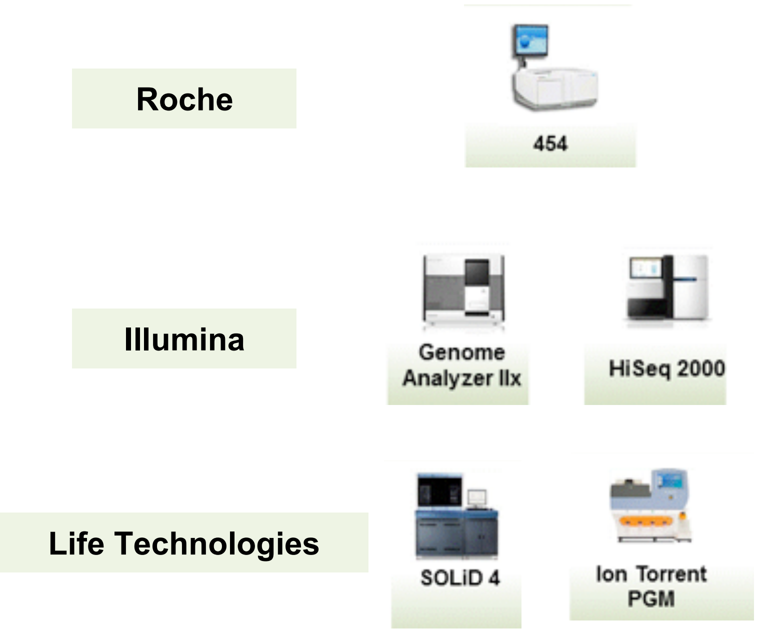
Identification of new ncRNAs genome-wide



new ncRNAs ?

By using deep sequencing : a revolution in genomics

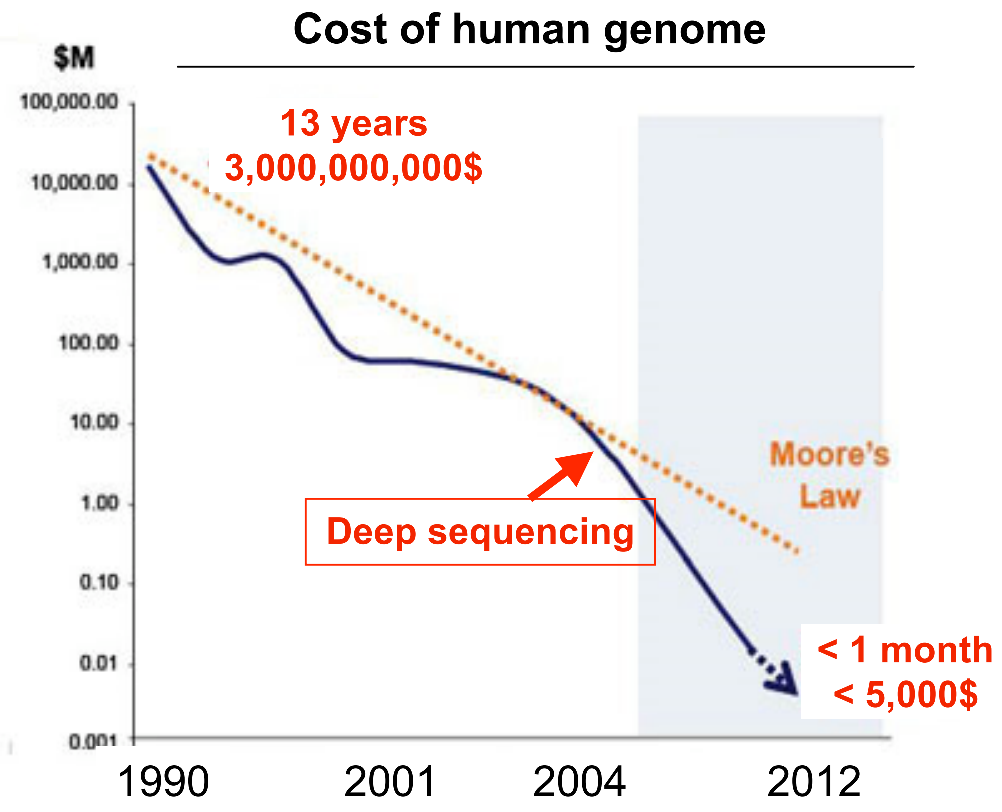
Millions of sequences in parallel



Roche
454

Illumina
Genome Analyzer IIx
HiSeq 2000

Life Technologies
SOLiD 4
Ion Torrent PGM



13 years
3,000,000,000$

Deep sequencing

< 1 month
< 5,000$