# Combining transcriptional and post-transcriptional regulation to predict somatic mutations altering the gene regulatory program in cancer cells

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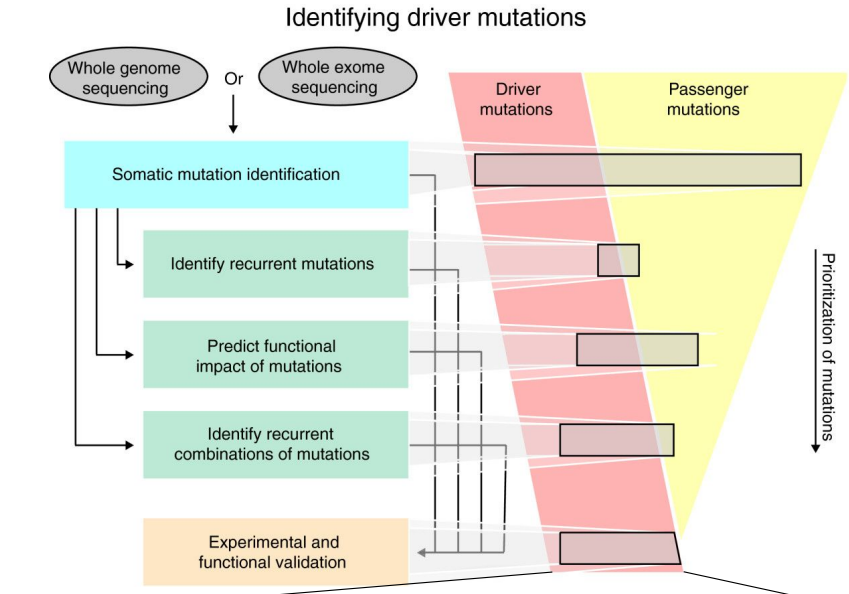
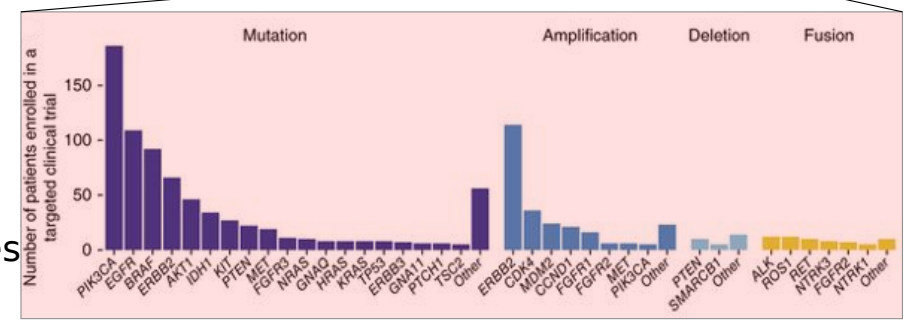
# Variant prioritization to identify cancer drivers

Driver variants identification




#patients in clinical trials identified based on actionable variants in genes




adapted from Lectures.GersteinLab.org

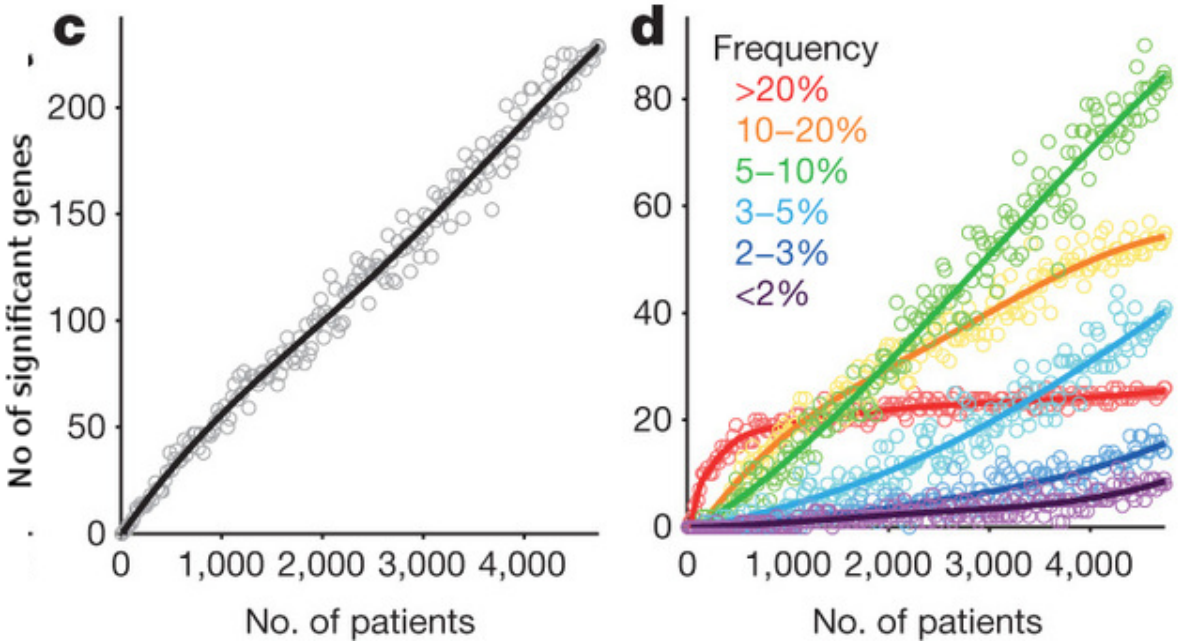
Raphael *et al.*, 2014.

Zehir *et al.*, 2017.

## Goal

Identify driver somatic events to shed light into molecular mechanisms and enable more precise diagnostics and targeted therapies.

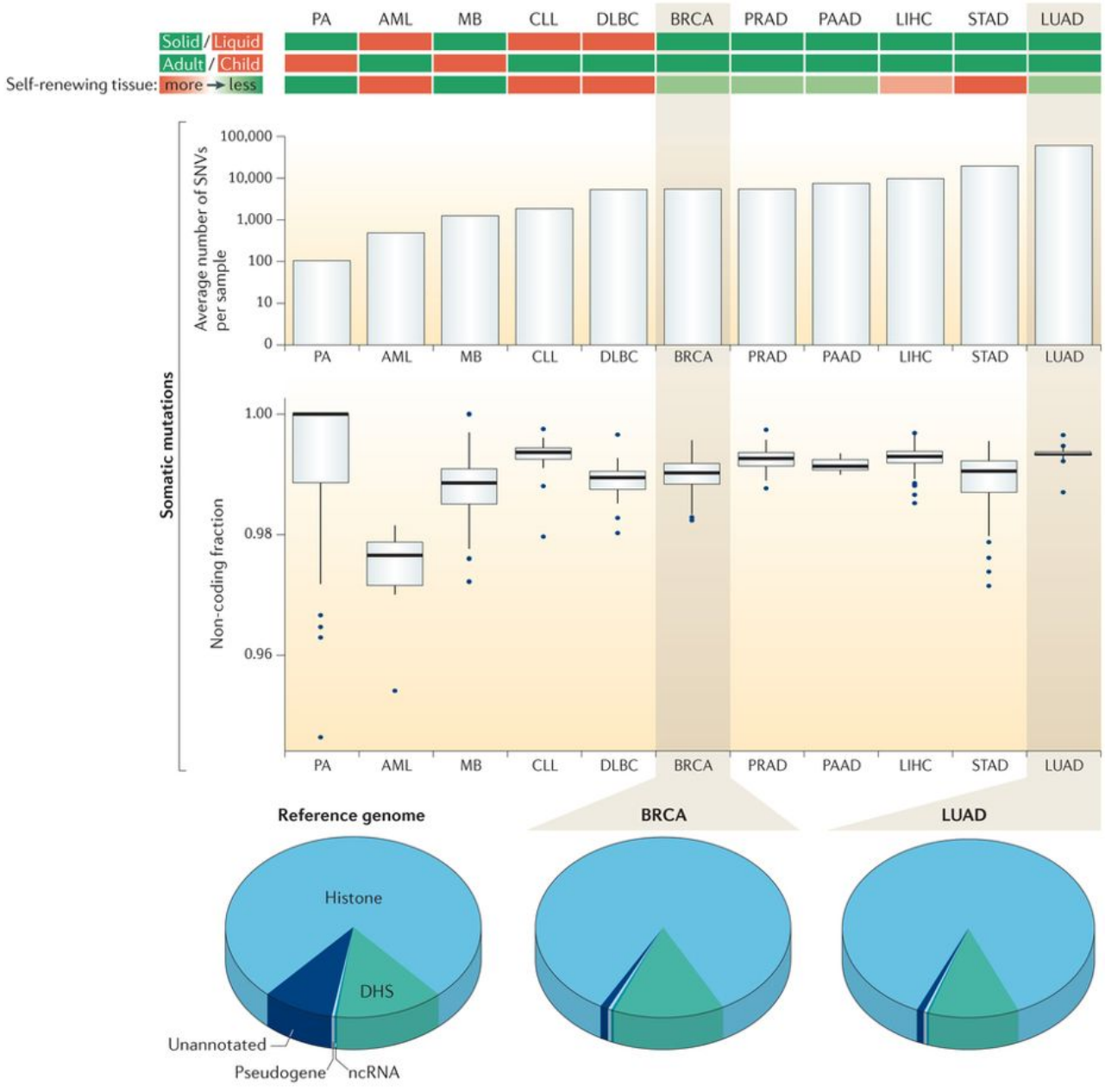
# A cancer gene discovery gap




M.S. Lawrence *et al.*, 2013.

- Highly mutated cancer genes revealed through The Cancer Genome Atlas project.
- Still a discovery gap in the search of new cancer genes.
- We assert this gap can be partially filled through the analysis of the non-coding genome.

# The vast majority of somatic mutations are non-coding



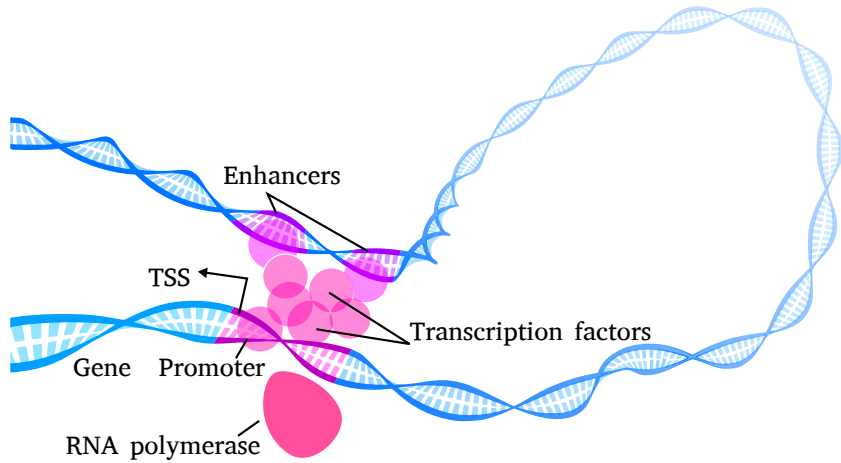

Khurana *et al.*, 2016.

Nature Reviews | Genetics

# Transcriptional regulation

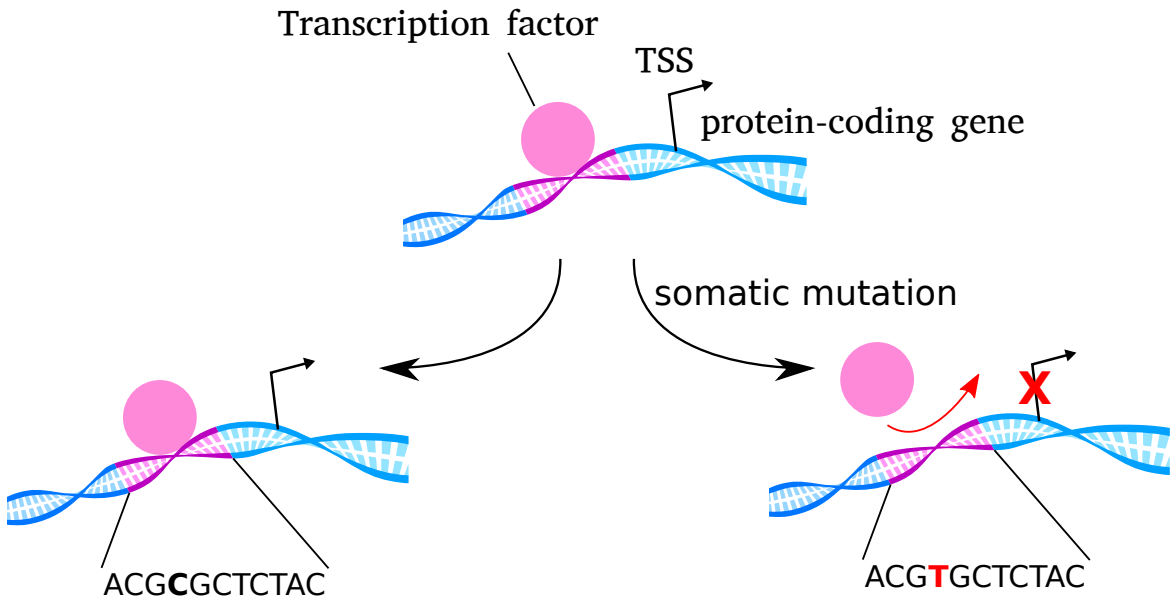
## Transcriptional regulation

Transcription factors, epigenetics, open chromatin, close chromatin, etc.

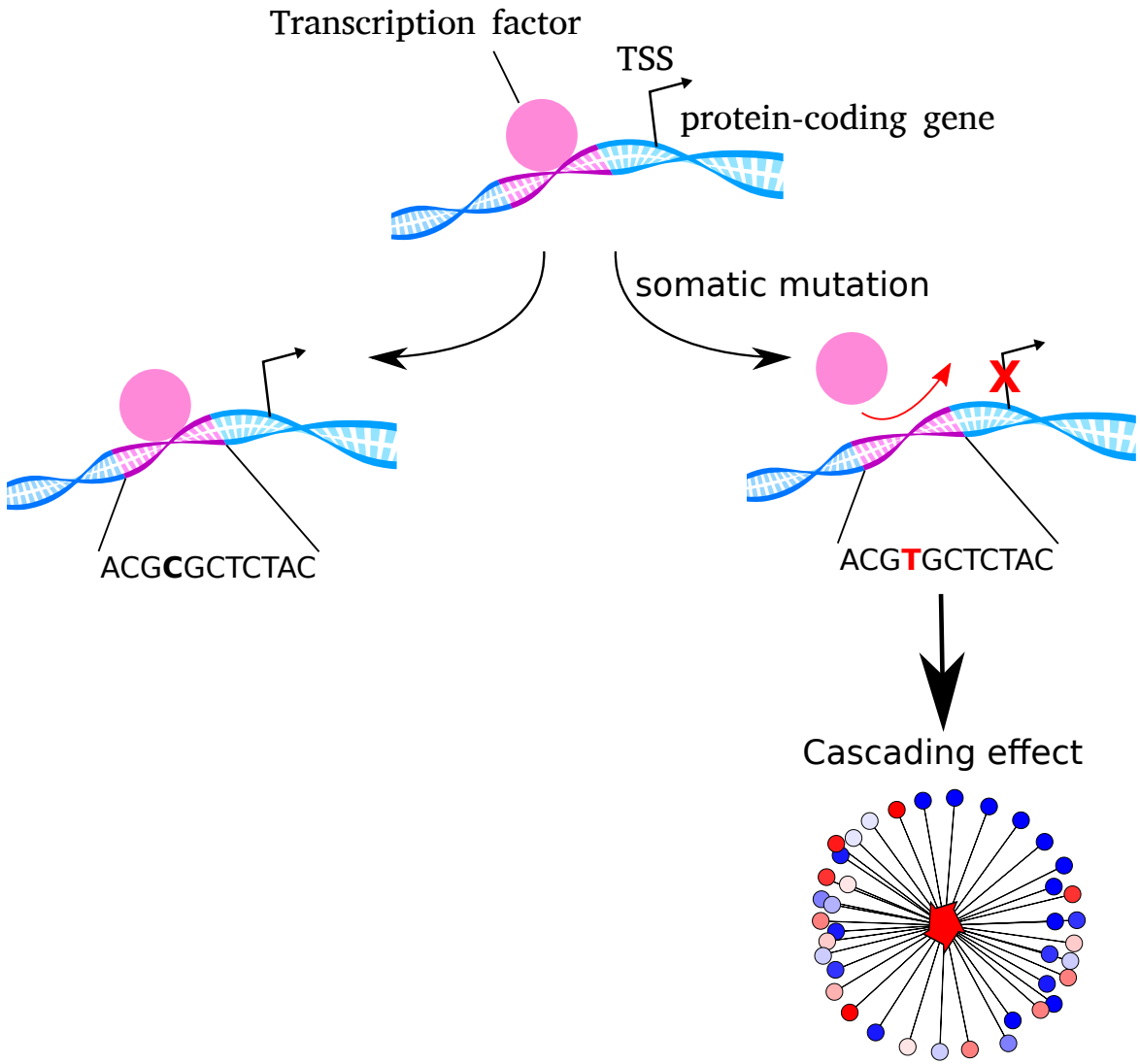



adapted from Kelvin Song's work on Wikimedia Commons

# Transcriptional deregulation and cascading effect



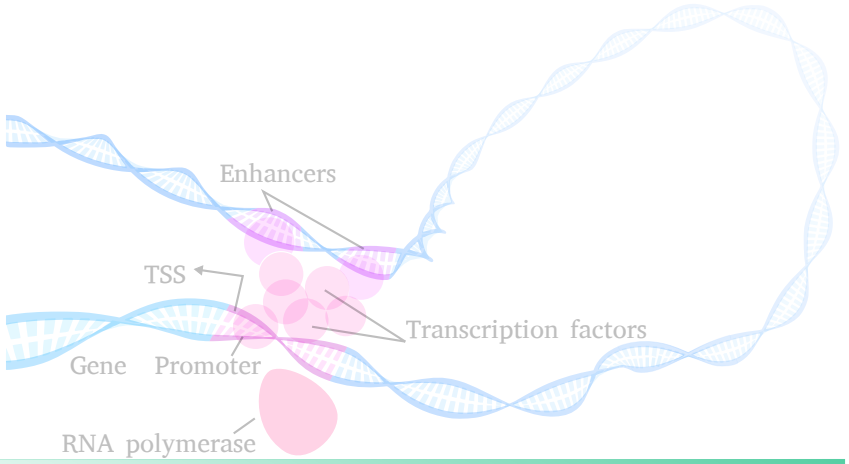
# Transcriptional deregulation and cascading effect

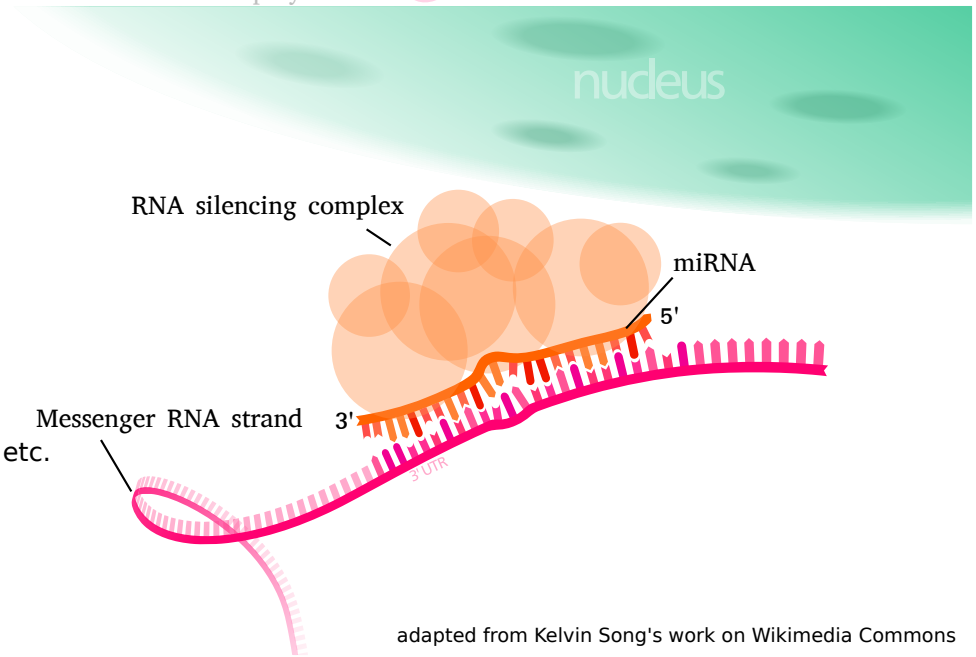


# Multiple layers of gene expression regulation

## Transcriptional regulation

Transcription factors, epigenetics,
open chromatin, close chromatin, etc.



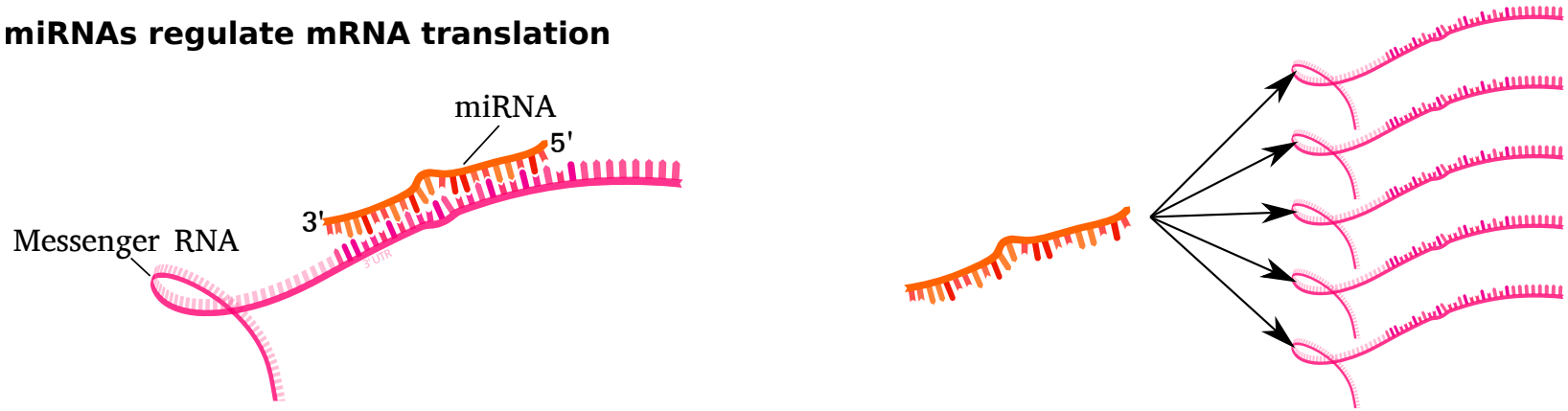




## Post-transcriptional regulation

miRNAs, mRNA localization,
RNA-binding proteins, splicing, etc.

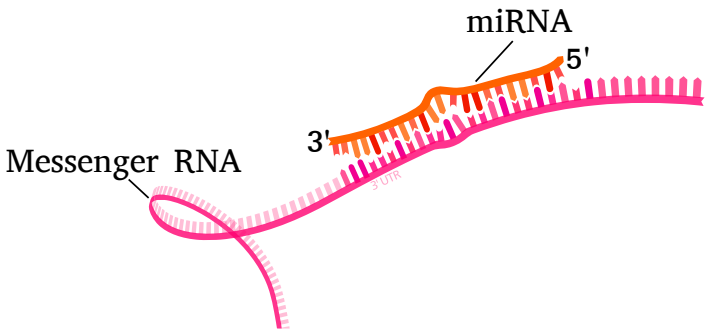
adapted from Kelvin Song's work on Wikimedia Commons

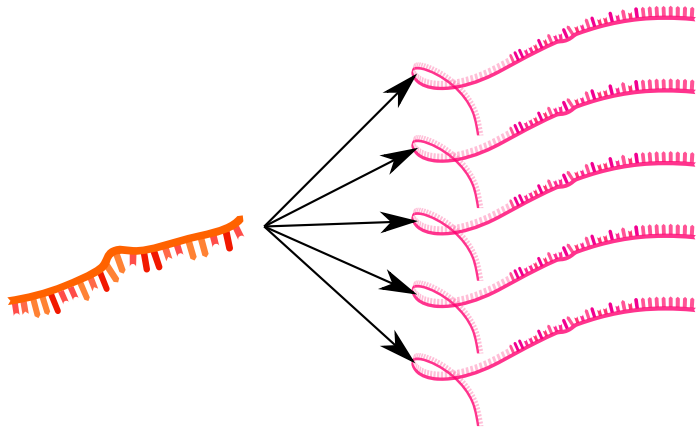
# Transcriptional and post-transcriptional deregulation



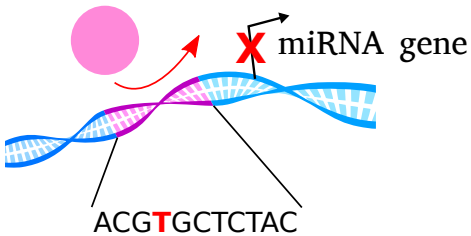
# Transcriptional and post-transcriptional deregulation

**miRNAs regulate mRNA translation**

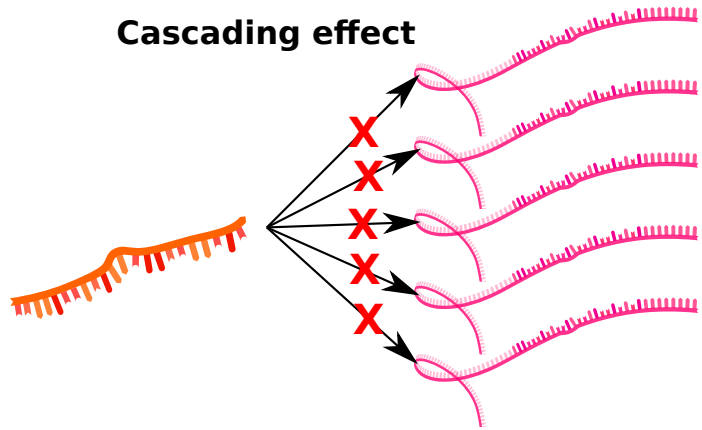


**miRNA transcription must be accurately controlled**

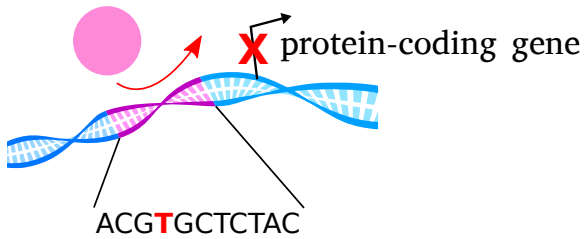


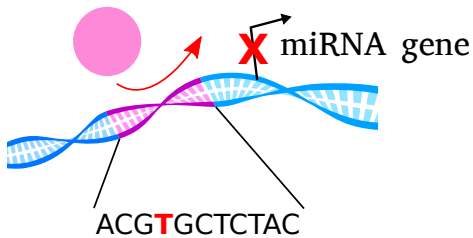

**Cascading effect**



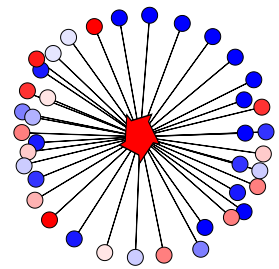
# Predicting cis-regulatory mutations altering the regulatory program in cancer cells

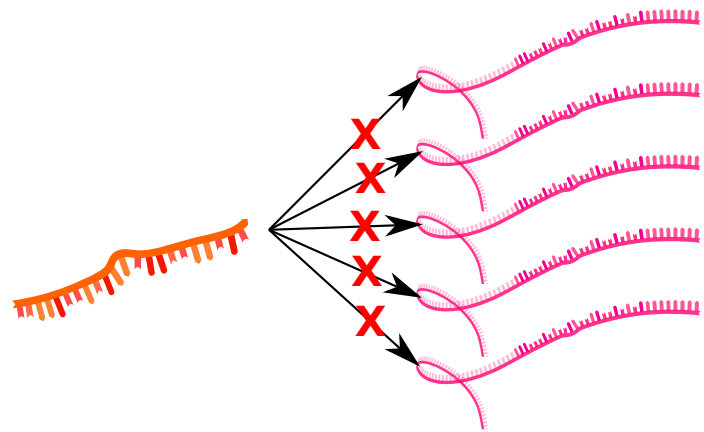
**Transcriptional dysregulation**




**Cascading effect**





# Predicting cis-regulatory mutations altering the regulatory program in cancer cells

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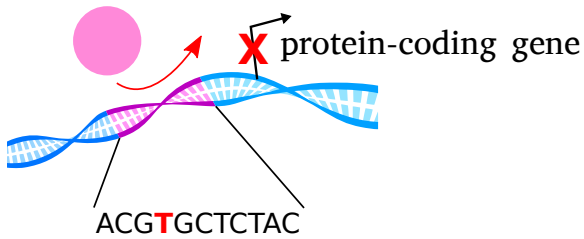
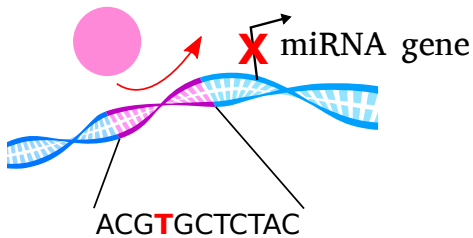
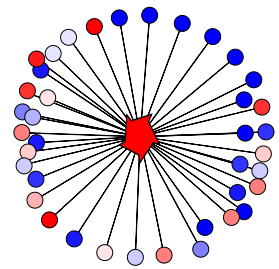
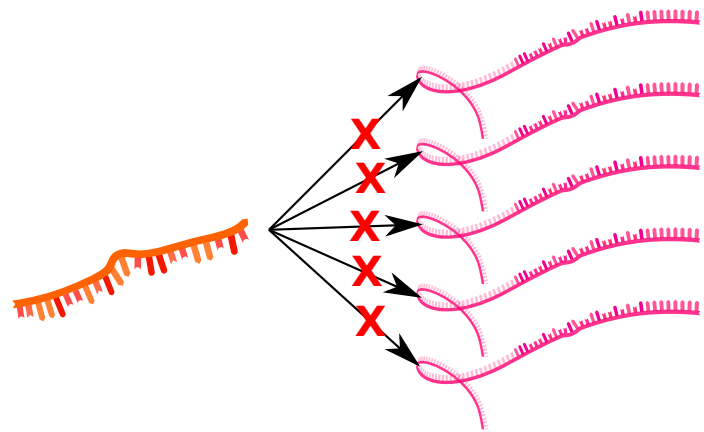



**Cascading effect**





One needs to accurately locate TFBSs to identify and characterize the regulatory sequences controlling specific genes transcription.

# Outline

1. Improving our capacity to predict transcription factor binding events

2. Combining transcriptional and post-transcriptional regulation to predict mutations altering the gene regulatory program in cancer cells

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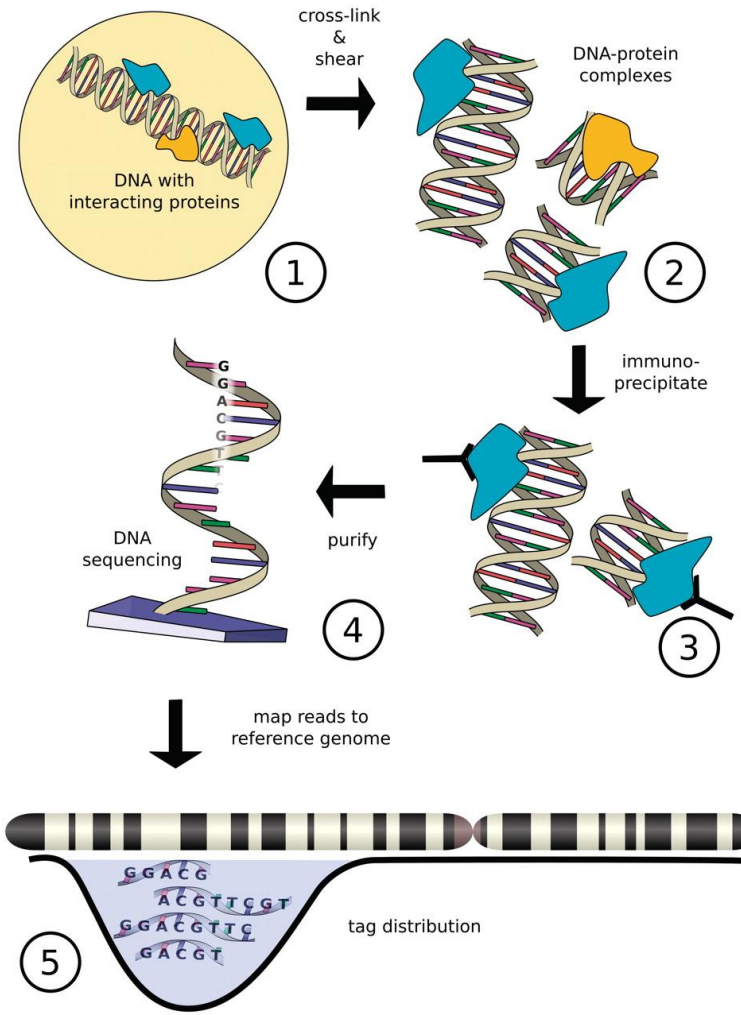
# Genome-scale data capturing TFBSs: ChIP-seq




```
>Seq 1
AGTTCAAAGTTCAAGTTCAAA
GTTCAAGTTCAAAGTTCAAGT
TCAAAGTTCA
>Seq 2
AGTCCAAAGTTCAAGTCCAAA
GTTCAAGTCCAAA
        ...
>Seq 33554
CTACCGGGGACCGGGGTGGA
ACCGGGG
>Seq 33555
ACCGGGGACCGGGGACCGGG
GACCGGGGGGCAAGGTTCATA
```

adapted from

A.M. Szalkowski and C.D. Schmid, 2010.

# You do not always ChIP what you expect

## Highly expressed loci are vulnerable to misleading ChIP localization of multiple unrelated proteins

Leonid Teytelman[a,b,1], Deborah M. Thurtle[c,1], Jasper Rine[c,2], and Alexander van Oudenaarden[a,b,d,2]

Nucleic Acids Research Advance Access published June 27, 2015

*Nucleic Acids Research, 2015* **1**
*doi: 10.1093/nar/gkv637*

## Active promoters give rise to false positive 'Phantom Peaks' in ChIP-seq experiments

**Dhawal Jain, Sandro Baldi, Angelika Zabel, Tobias Straub and Peter B. Becker***

Worsley Hunt and Wasserman *Genome Biology* 2014, **15**:412
http://genomebiology.com/2014/15/7/412

Genome Biology

RESEARCH Open Access

## Non-targeted transcription factors motifs are a systemic component of ChIP-seq datasets

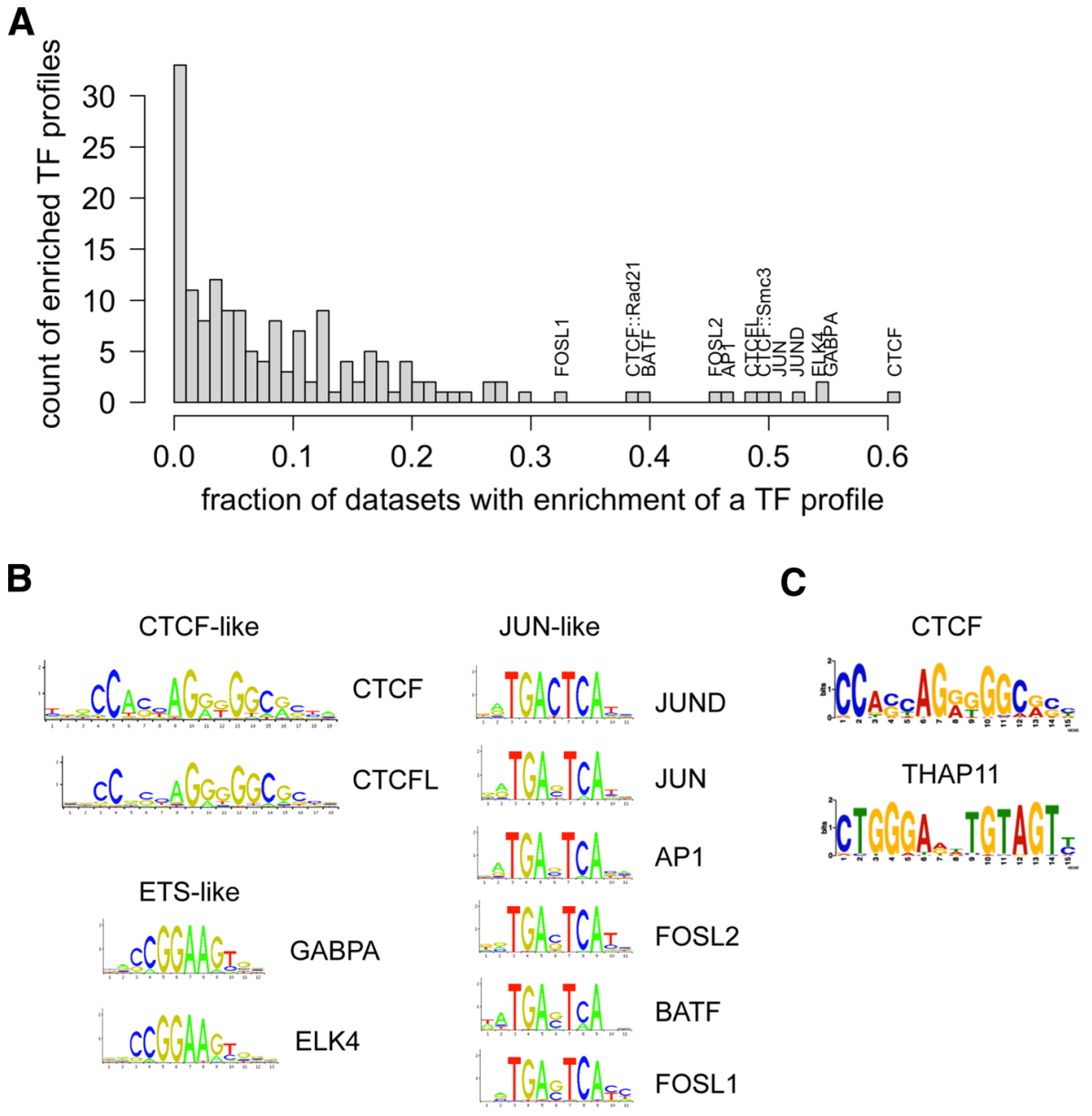
Rebecca Worsley Hunt[1,2] and Wyeth W Wasserman[1,3*]



## HOT or not: examining the basis of high-occupancy target regions

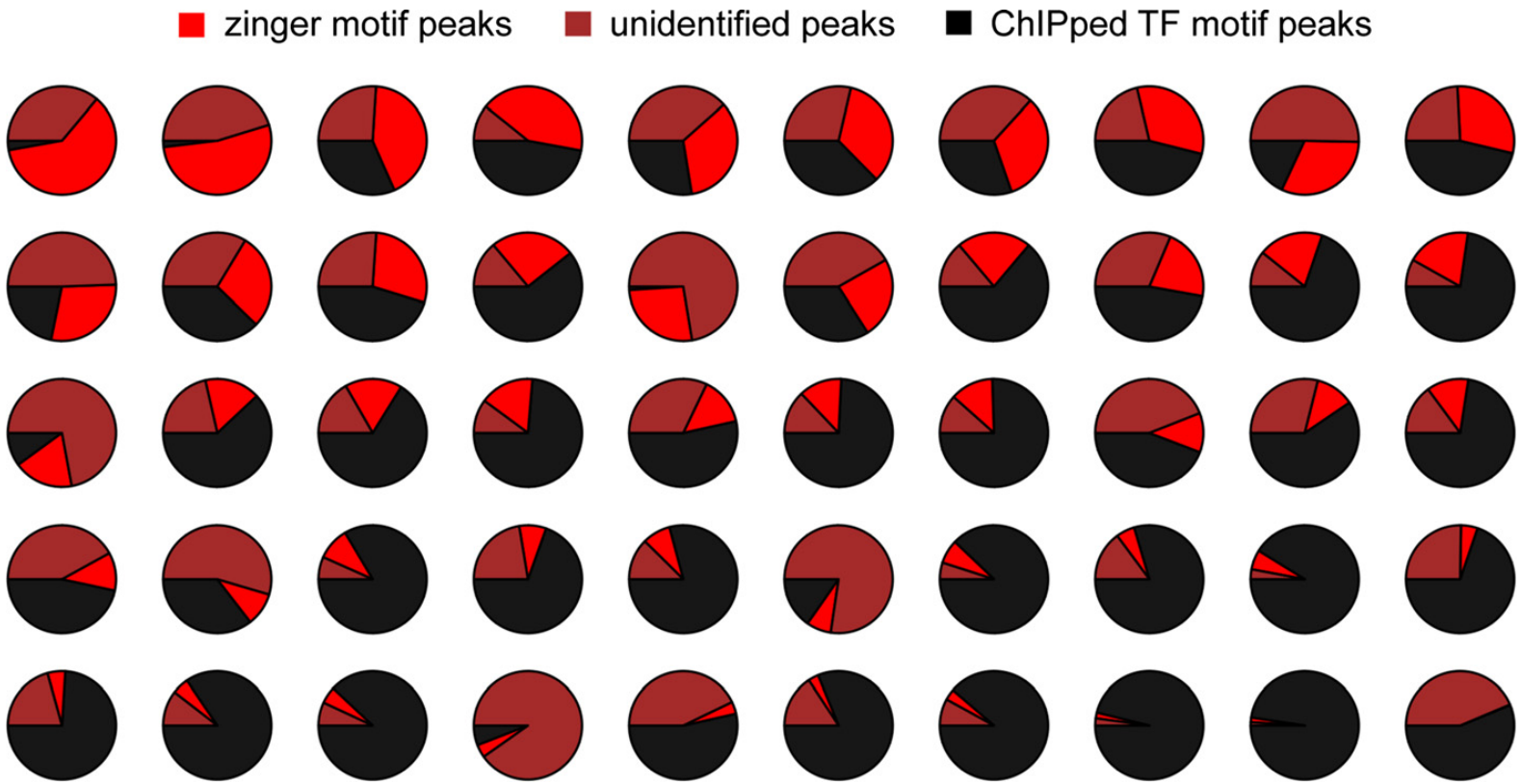
Katarzyna Wreczycka[1*], Vedran Franke[1*], Bora Uyar[1], Ricardo Wurmus[1], Altuna Akalin[1#]

# ChIP-seq peaks are enriched for zingers




Worsley-Hunt and Wasserman, 2014.

# ChIP-seq peaks are enriched for zingers




Worsley-Hunt and Wasserman, 2014.

# Modeling TFBSs

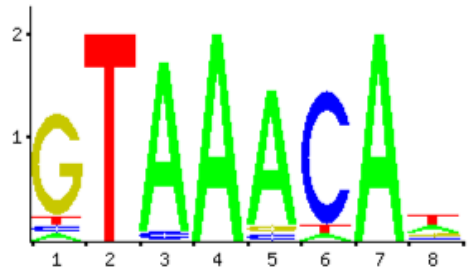
Known binding sites:

GTAACAAT
GTAAACAT
GTAAACAA
GTAAACAA
GTAAACAT
GTAAACAA
GTAAACAC
GTCAACAG
GTAAACAT
GTAAACAA
GTAAACAT
TTAAGTAA
ATAAACAA
CTAAACAG
GTAAACAT
GTAAACAA
GTAAACAT
GTAAACAC
GTAAACAT
GTAAACAG



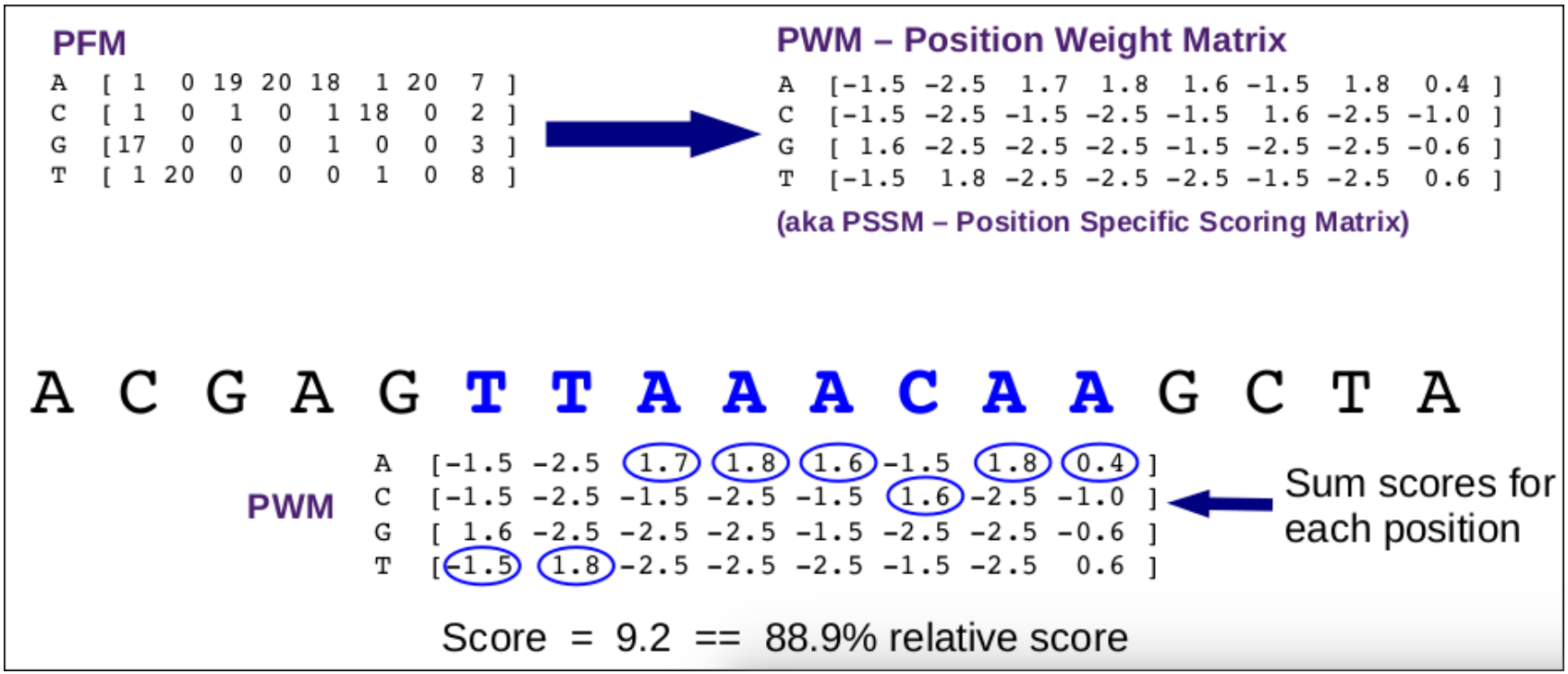
Position Frequency Matrix:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | [ 1 | 0 | 19 | 20 | 18 | 1 | 20 | 7] |
| C | [ 1 | 0 | 1 | 0 | 1 | 18 | 0 | 2] |
| G | [17 | 0 | 0 | 0 | 1 | 0 | 0 | 3] |
| T | [ 1 | 20 | 0 | 0 | 0 | 1 | 0 | 8] |

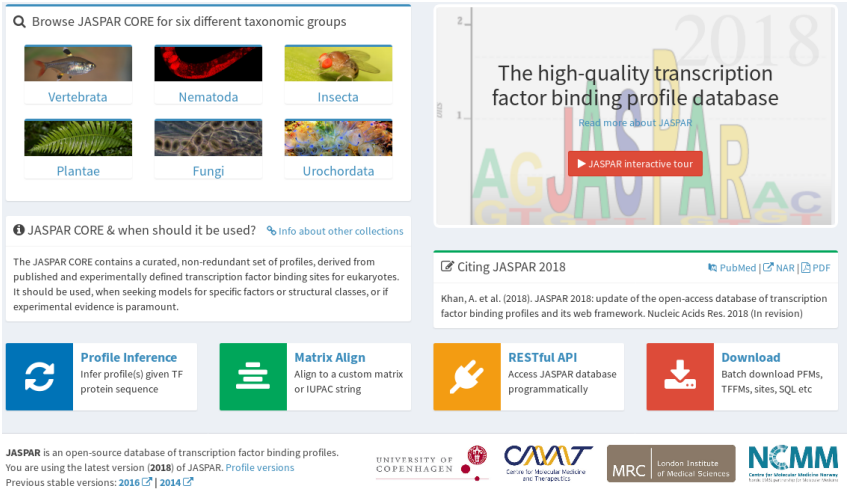


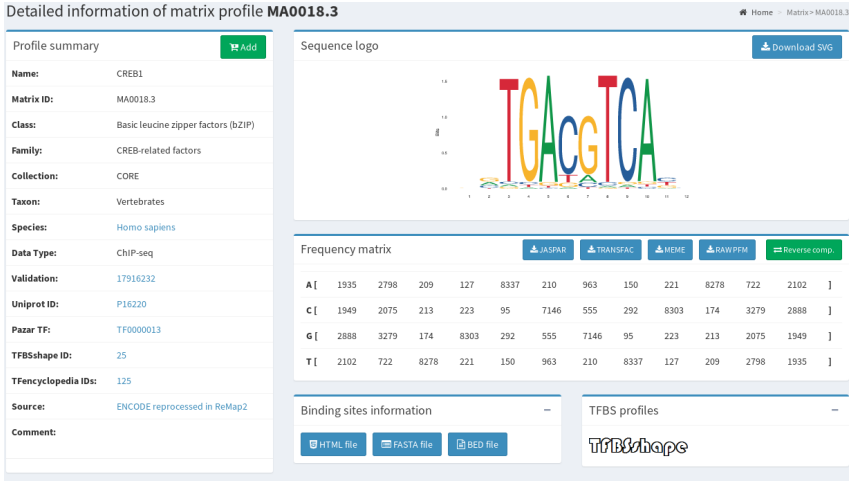

PFMs reflect the preferred binding motifs associated to TFs.

# Scoring potential TFBSs



# JASPAR



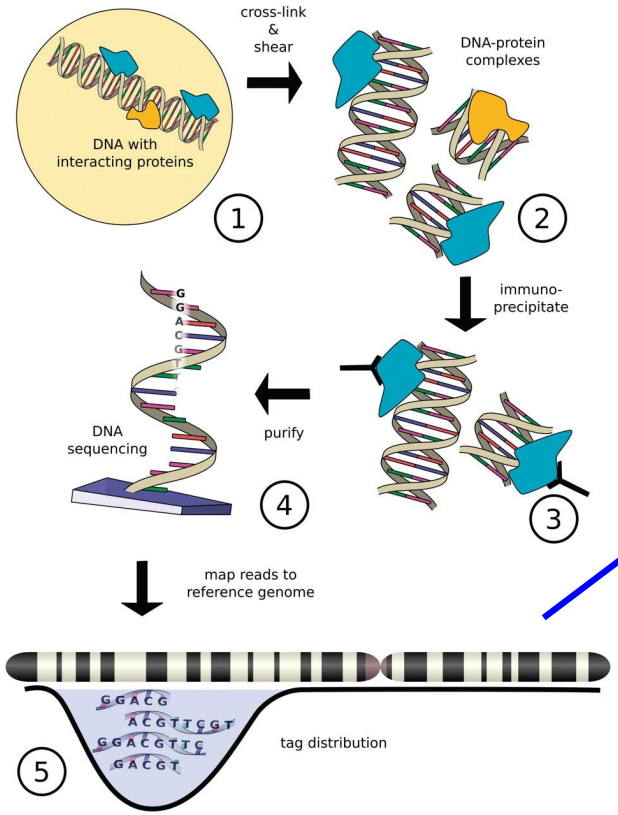




**Largest open-access database of manually curated TF binding profiles.**

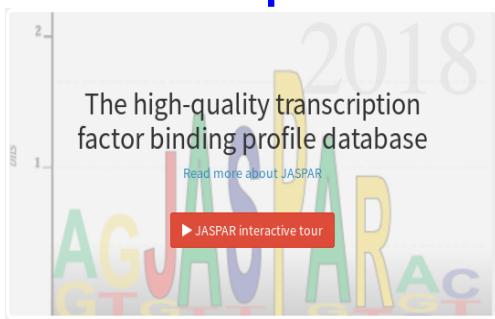
Khan, Fornes, *et al.*, 2018.

| Subset | # TF binding profiles |
|---|---|
| **Vertebrates** | **579** |
| Plants | 489 |
| Insects | 133 |
| Nematodes | 26 |
| Fungi | 176 |
| Urochordata | 1 |
| **Total** | **1404** |

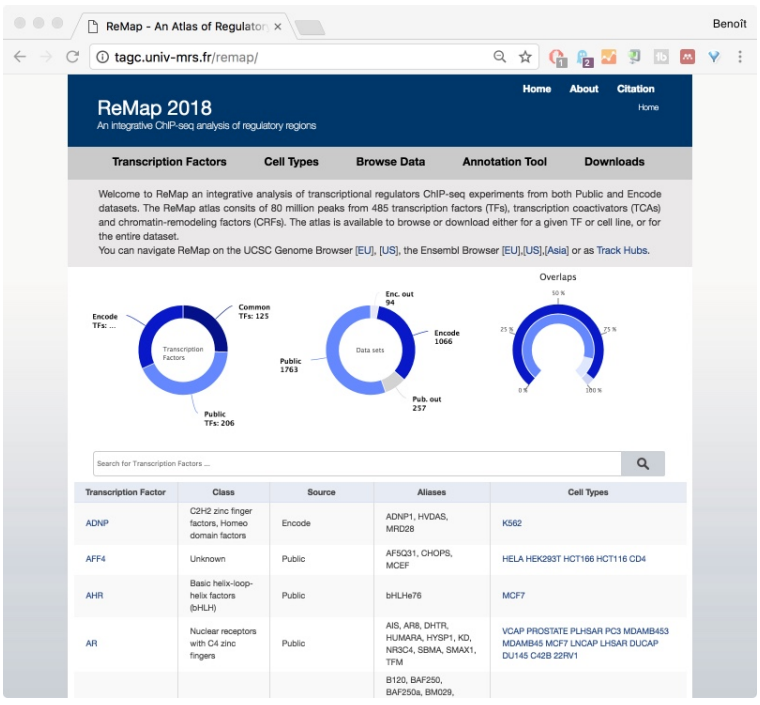
# Combining ChIP-seq peaks with JASPAR TF binding profiles



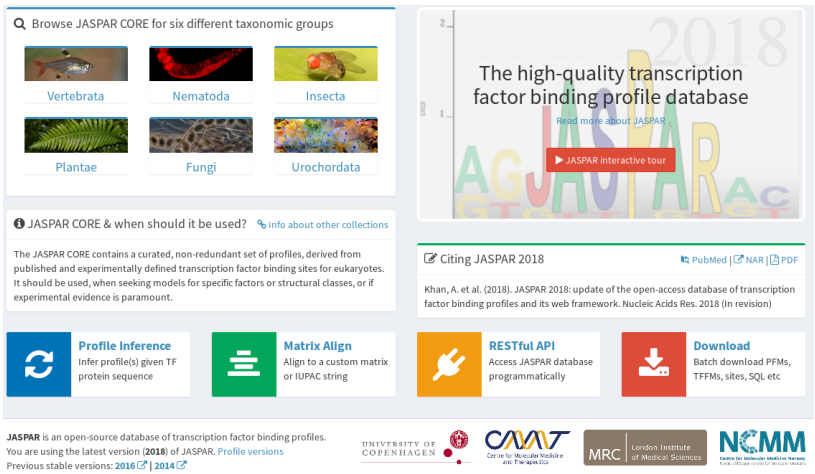

...gctaa**GTAACAAT**gagc...
...ctaaa**GTAAACAT**gcga...
...ccaat**GTAAACAA**acgg...



# Combining ChIP-seq peaks and TF-binding profiles

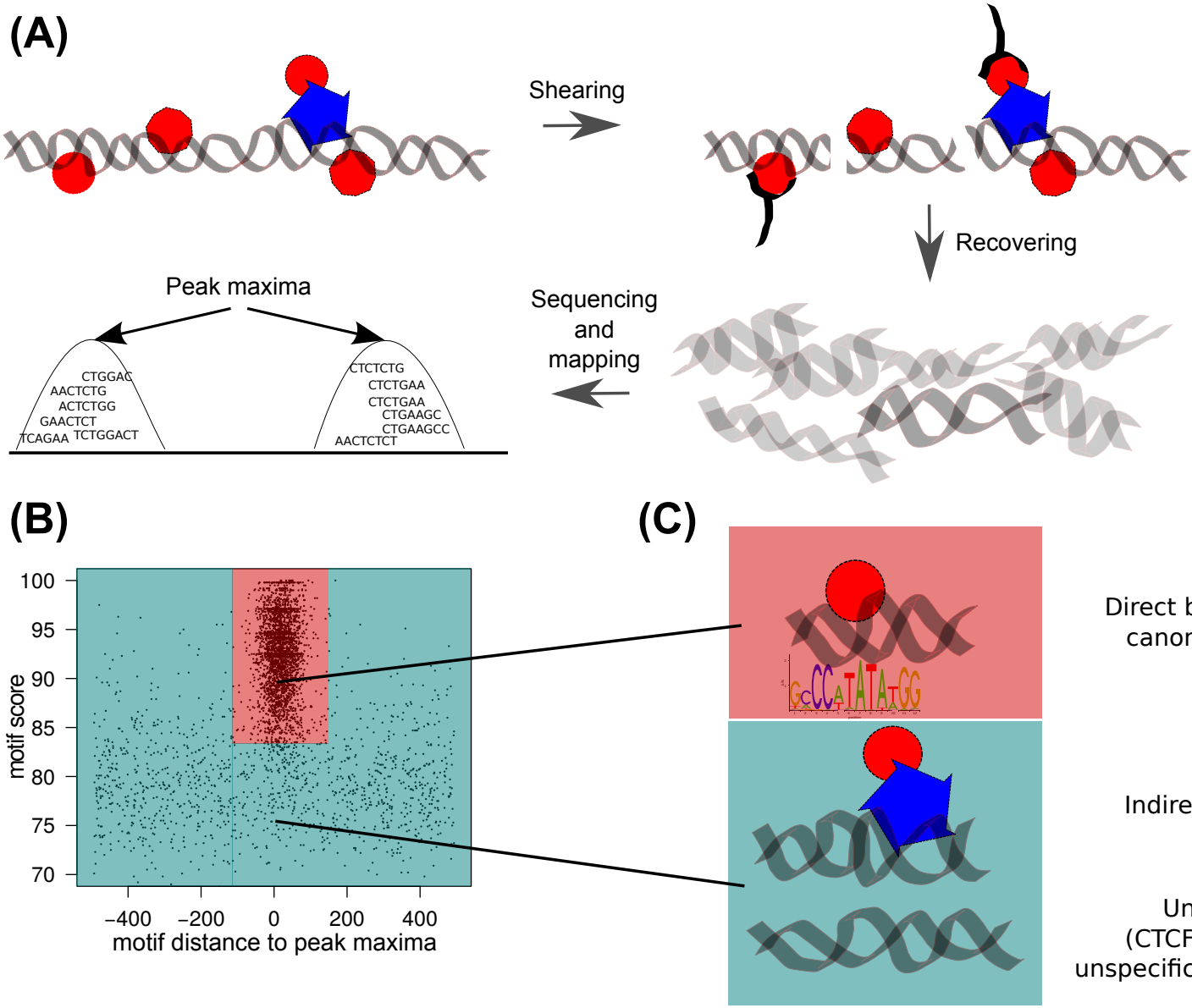



Chèneby *et al.*, 2018.



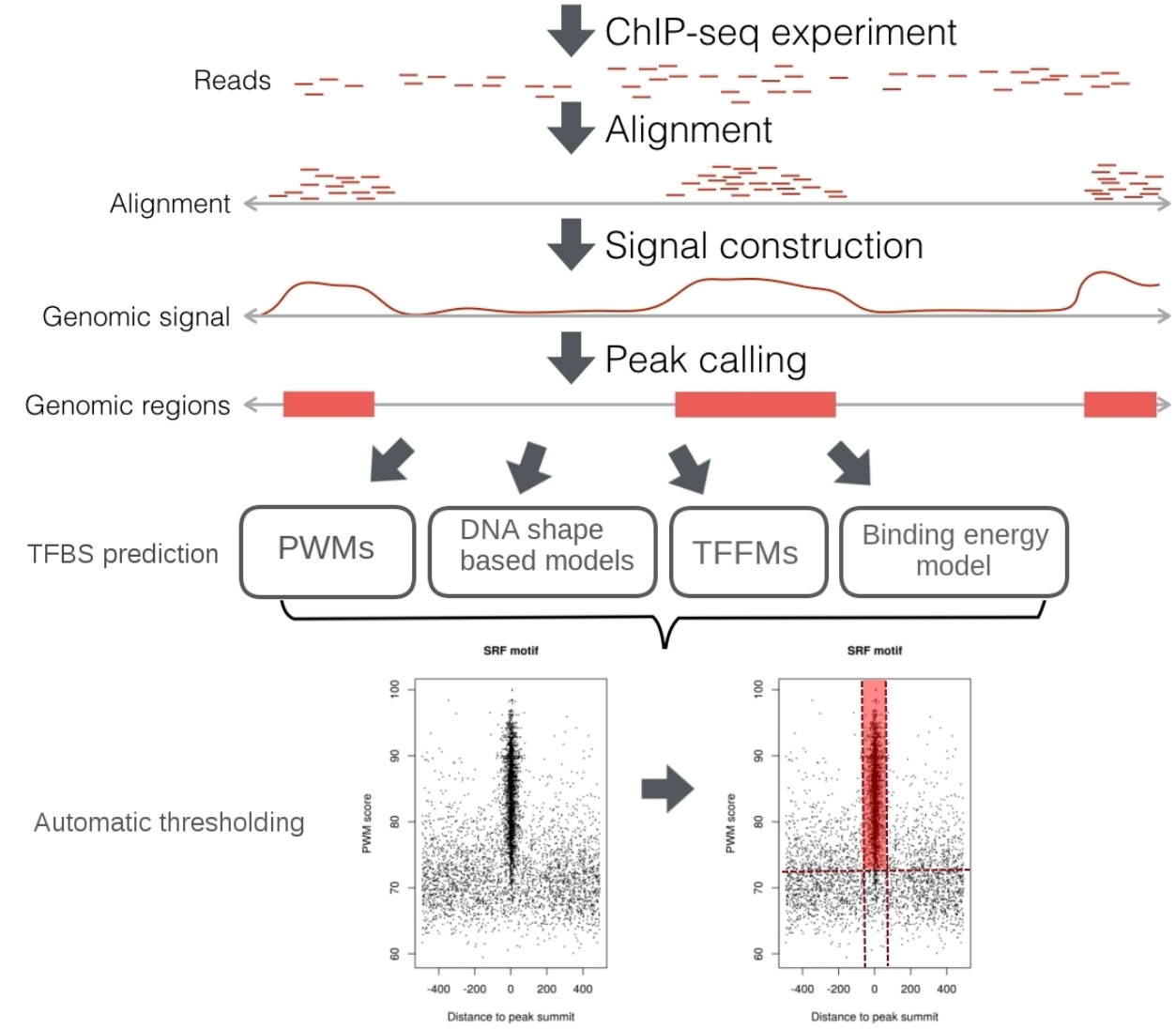

Khan, Fornes, *et al.*, 2018.

# What to expect when you are ChIP'ing



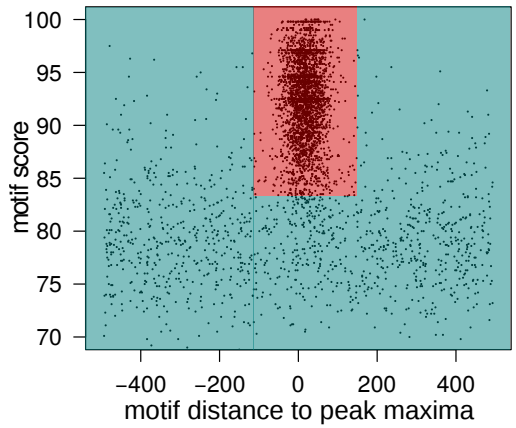

Mathelier, Shi, and Wasserman, 2015.

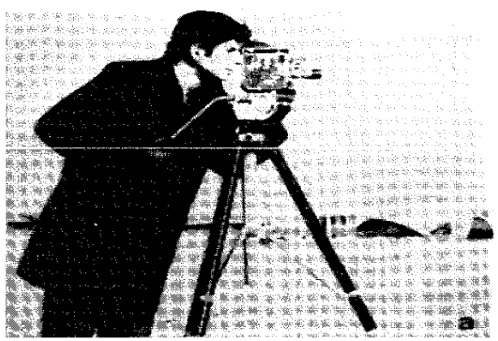
# ChIP-eat: from raw reads to high quality TFBSs

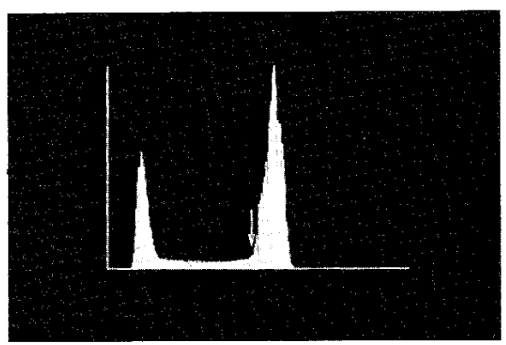



Gheorghe *et al.*, 2018.

# Entropy to automatically define TFBS enrichment zones

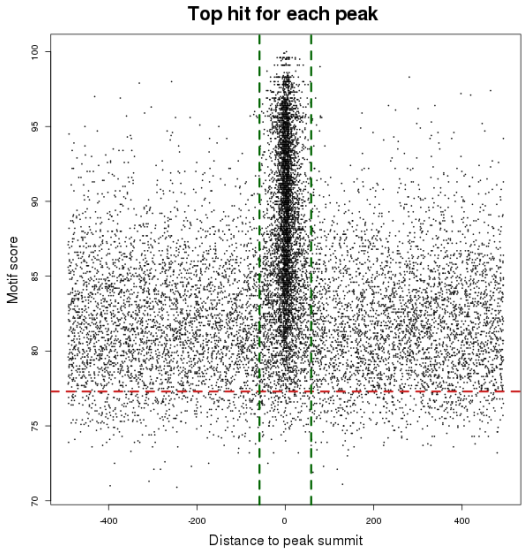


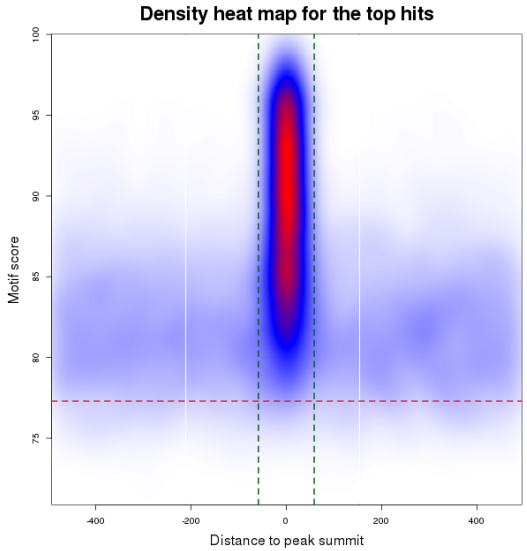



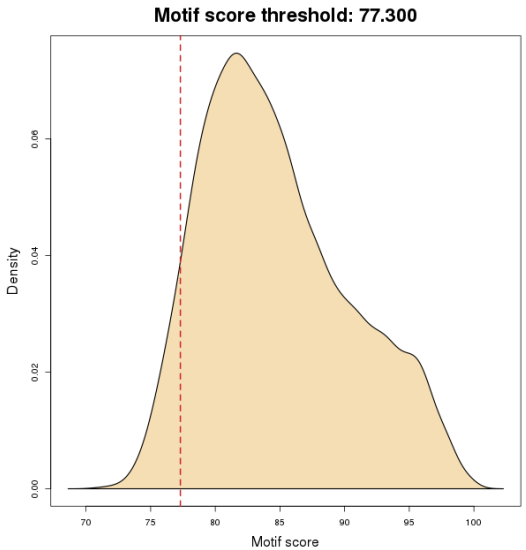


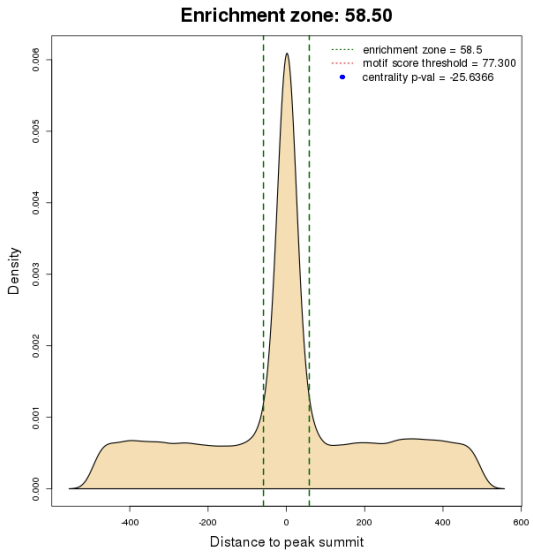
Kapur *et al.*, 1985.

# A map of TF-DNA interactions in the human genome



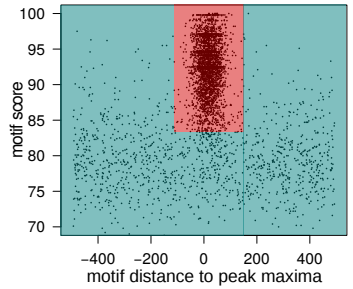



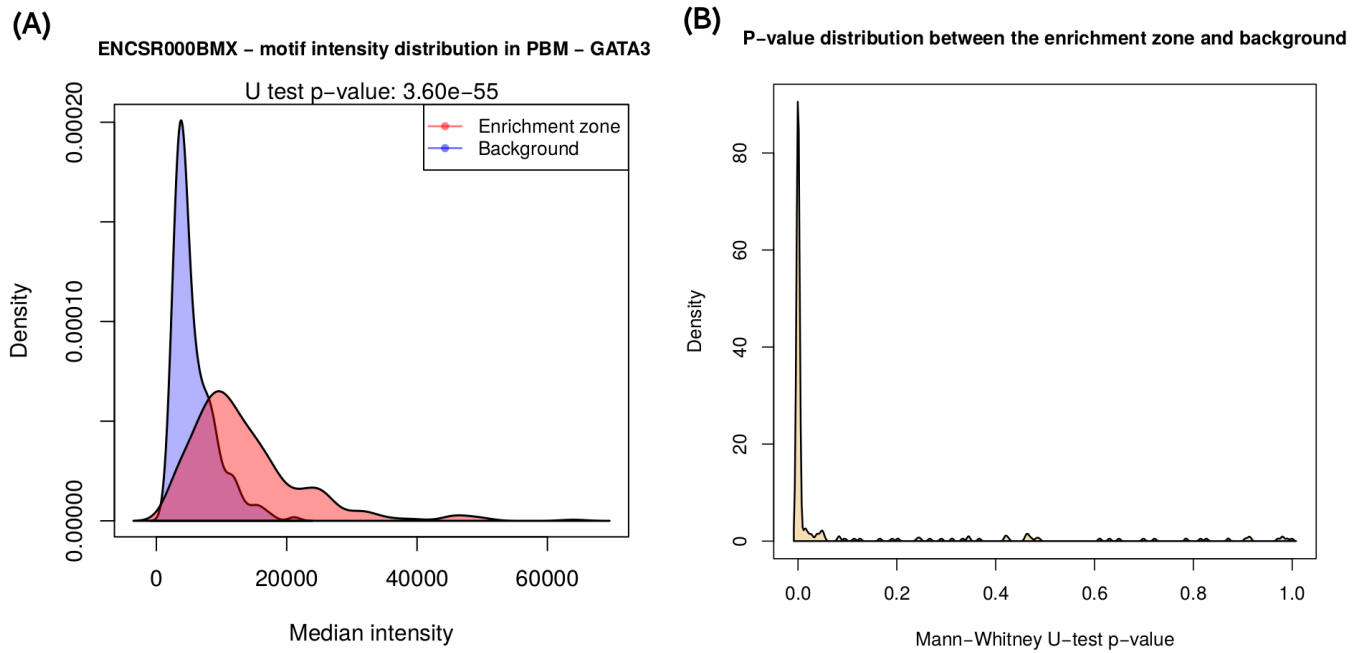




Gheorghe *et al.*, 2018.

We predict **direct TF-DNA interactions** covering $>$ **2%** of the human genome from 1,982 ChIP-seq data sets for 231 TFs.

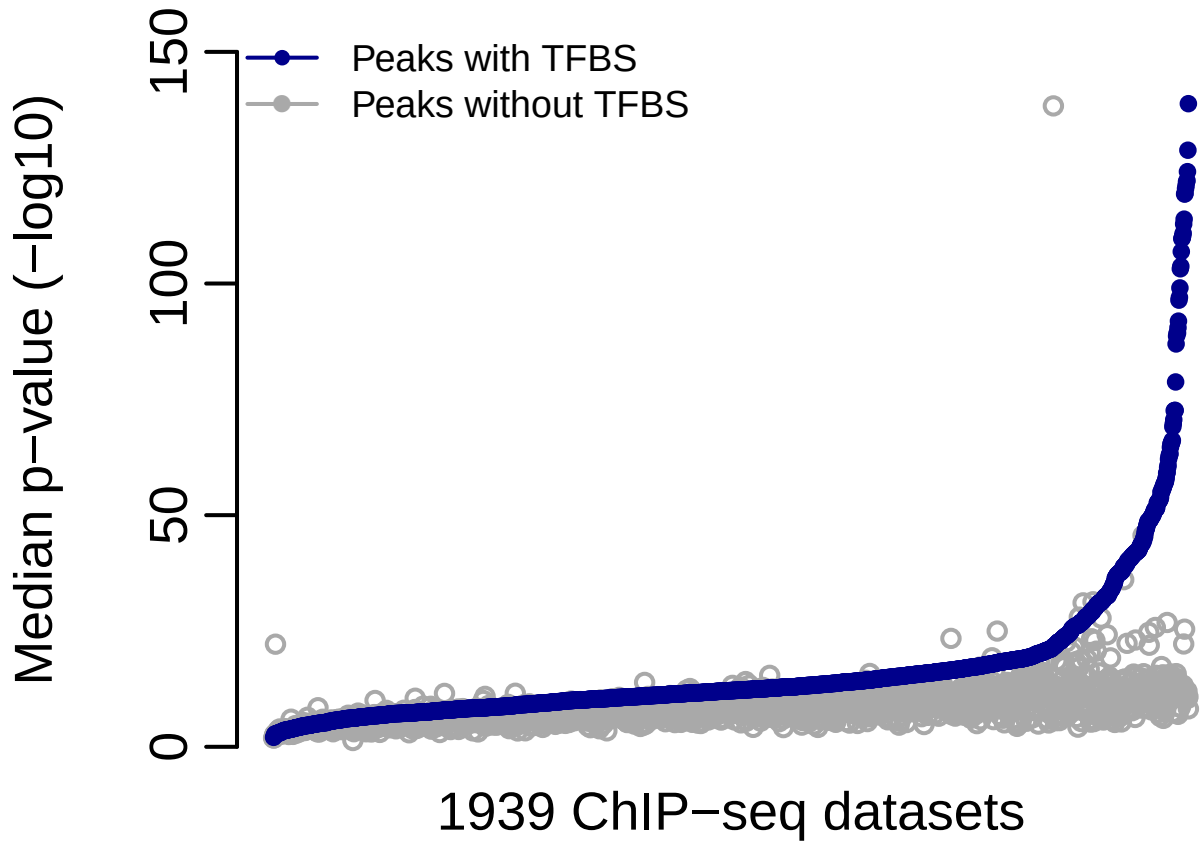
# The TFBS enrichment zone highlights higher binding affinity



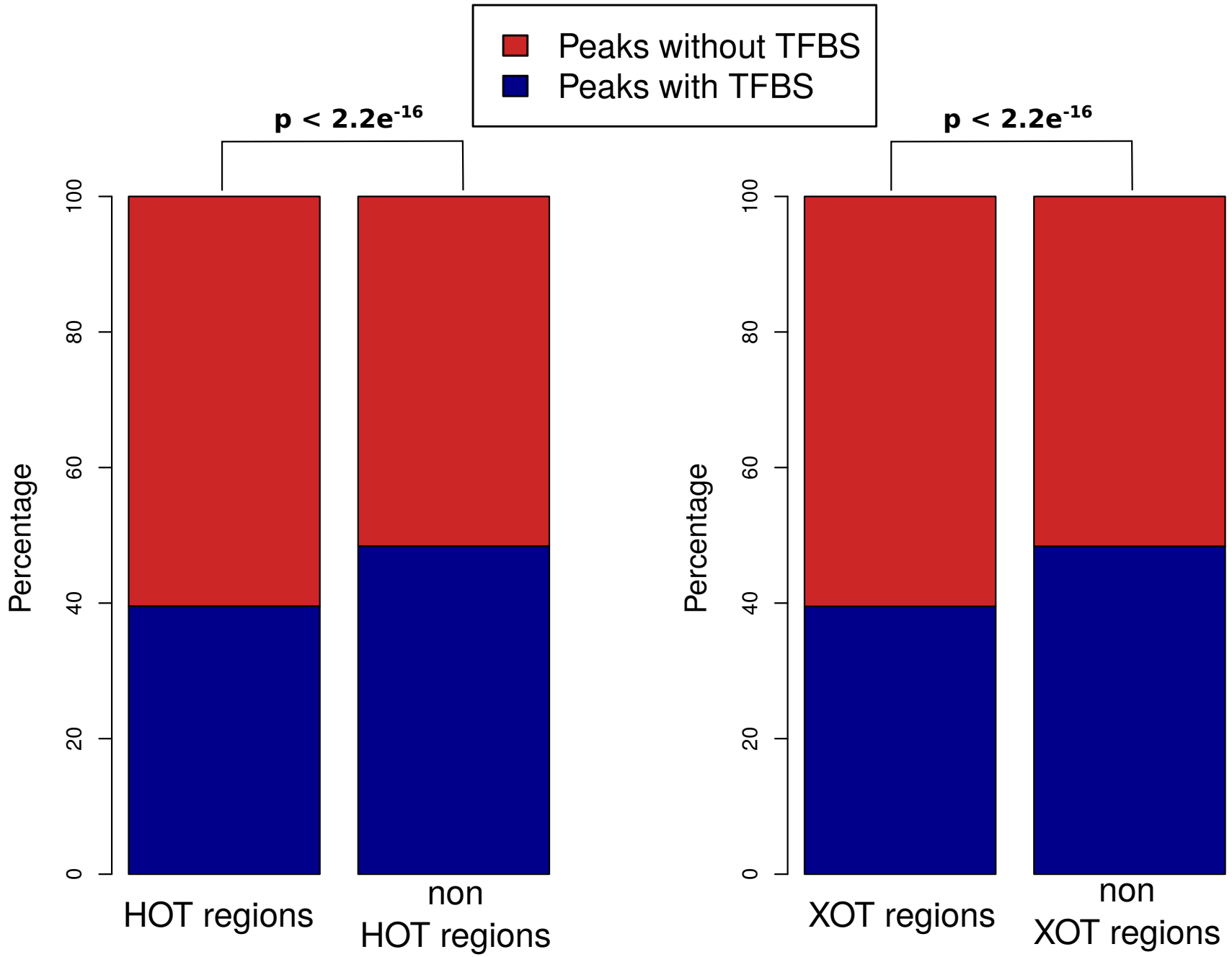




TFBSs in enrichment zones show higher PBM binding affinity than hits outside.

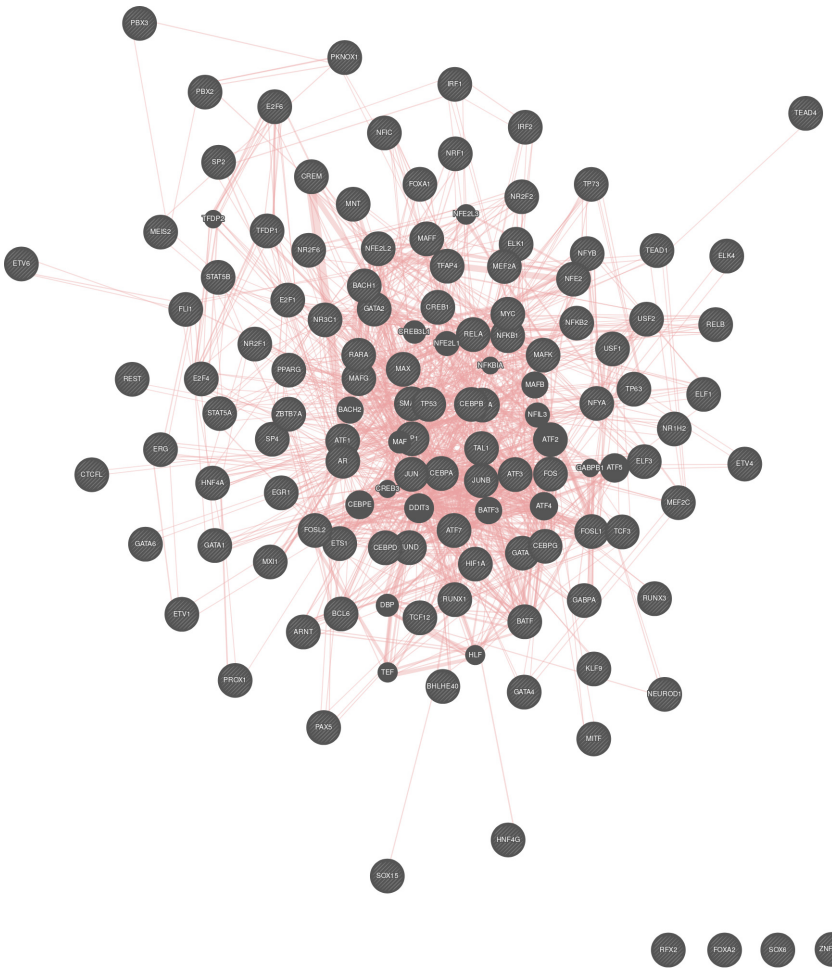
# Direct TF-DNA interactions are found in high confidence peaks



# HOT regions are depleted of TFBSs in enrichment zones

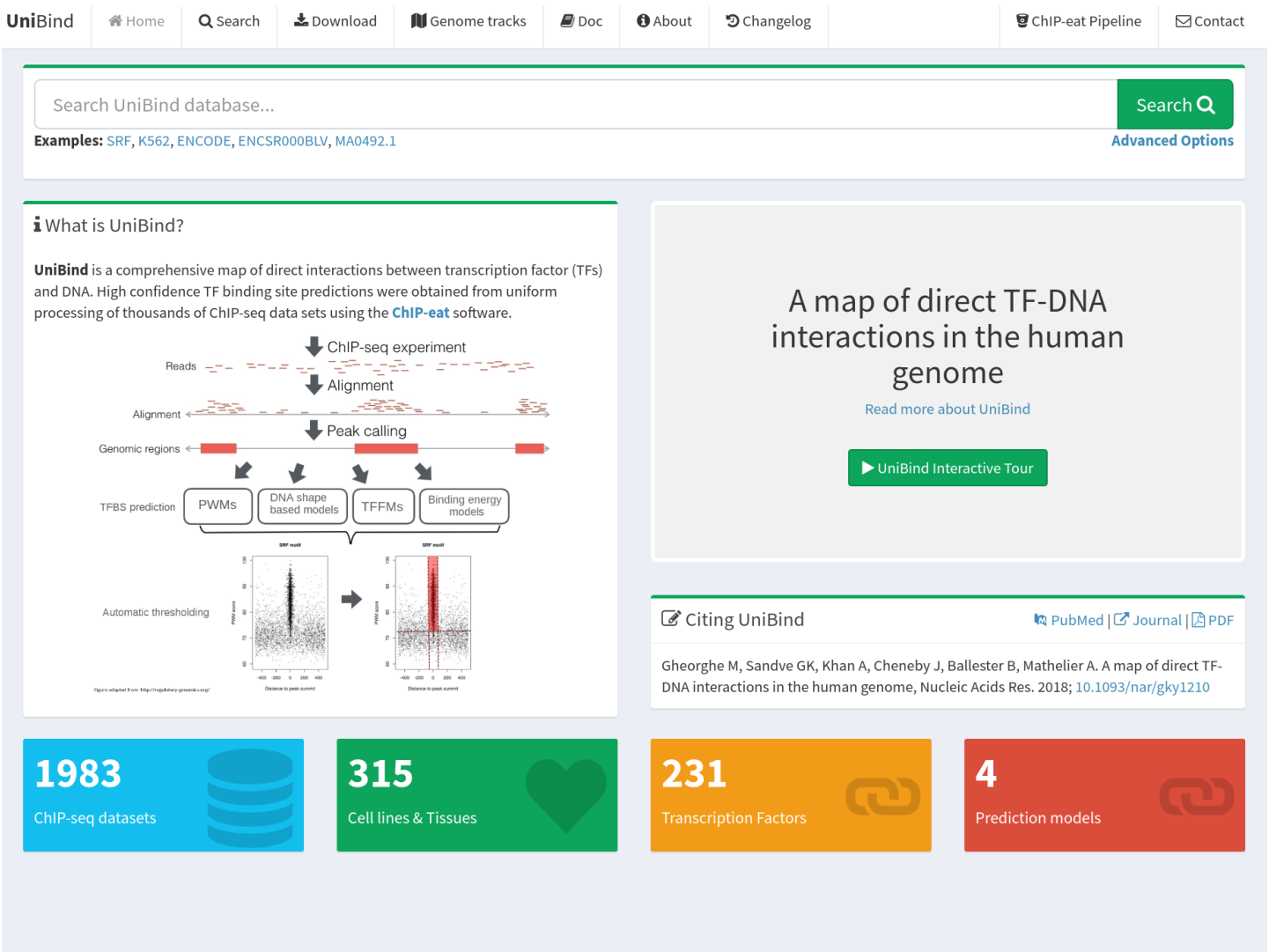


# Direct TF-DNA interactions reveal co-localizing TFs



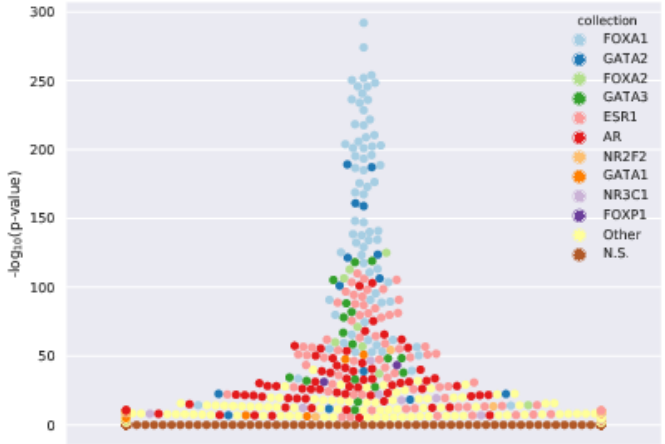

Out of 231 available TFs (26,796 pairs tested), 150 pairs of co-binding (112) TFs are predicted, 82% of which known in PPI database.

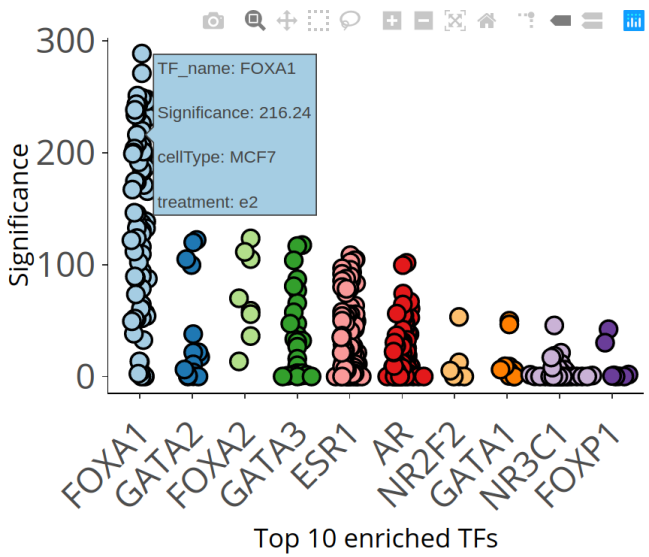
# UniBind

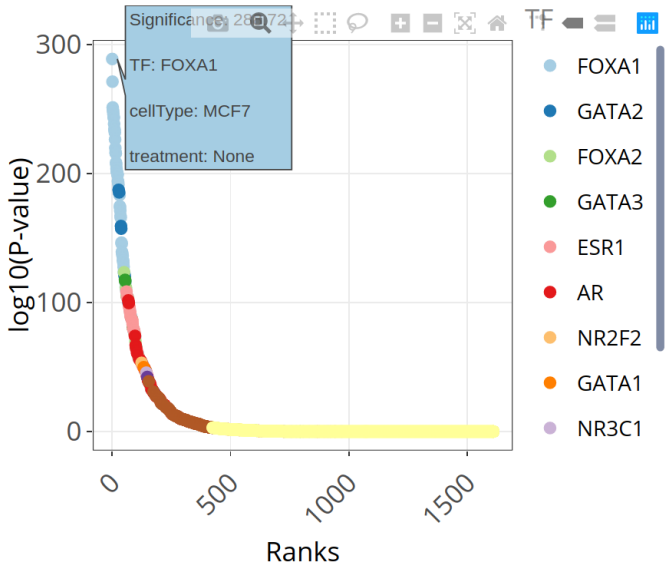



http://unibind.uio.no

# TFBS sets enrichment analyses




You can query UniBind to compute enrichment against your genomic regions.

# Summary

- We provide a genome-wide map of direct TF-DNA interactions by combining both experimental and computational evidences.
- TFBSs predicted in the enrichment zones cover $> 2\%$ of the human genome (1,983 ChIP-seq data sets - 231 TFs).
- TFBSs in enrichment zones show high PBM binding affinity and are found in high quality peaks.
- Direct TF-DNA interactions reveal co-binding TFs.
- *cis*-regulatory modules derived from TFBSs are enriched for disease- and trait-associated SNPs.

Gheorghe *et al.*. 2018.

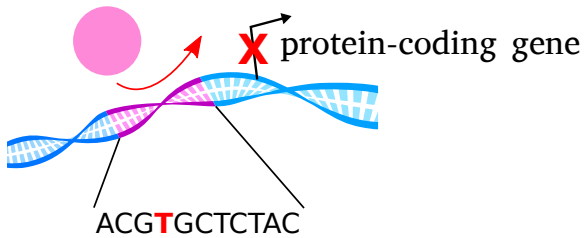
# Outline

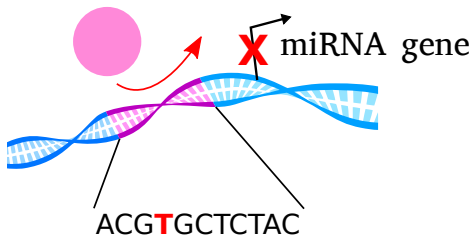
1. Improving our capacity to predict transcription factor binding events

2. Combining transcriptional and post-transcriptional regulation to predict mutations altering the gene regulatory program in cancer cells

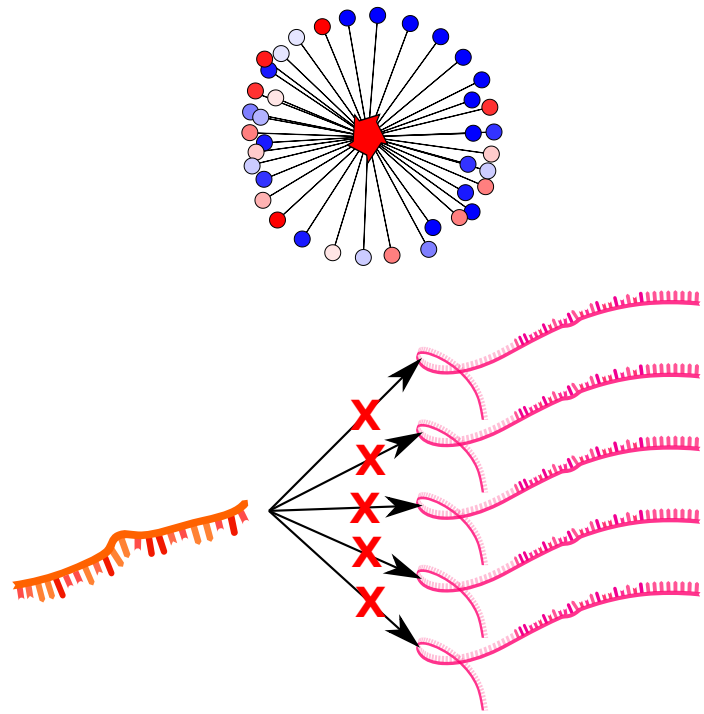
# Predicting somatic mutations altering the gene regulatory program in cancer cells

**Transcriptional dysregulation**



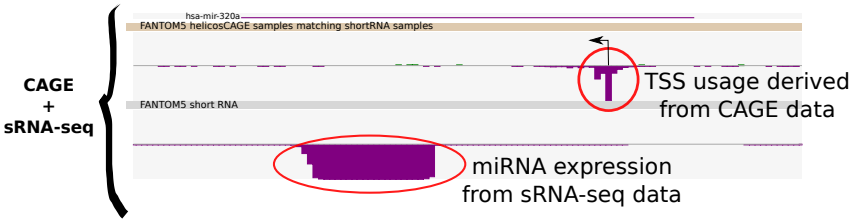




**Cascading effect**



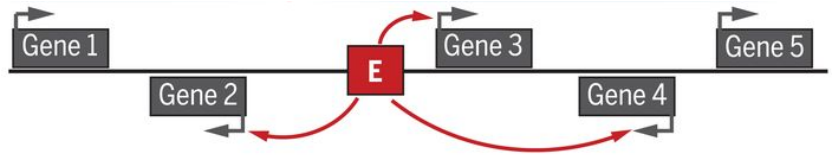
# Data to analyze transcriptional regulation of miRNAs and protein-coding genes

## miRNA TSSs




De Rie *et al.*, 2017.

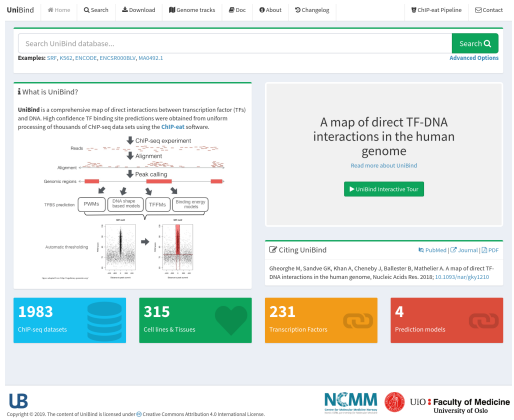
## Enhancer-TSS associations




Furlong *et al.*, 2018.

Fishilevich *et al.*, 2017.

## TFBSs from UniBind




Gheorghe *et al.*, 2018.

## miRNA - target networks


Agarwal *et al.*, 2015.

# Available cancer data cohorts

- Data requirements:
  - WGS (tumor and normal) to call SNVs and indels
  - RNA-seq
  - Copy number alterations

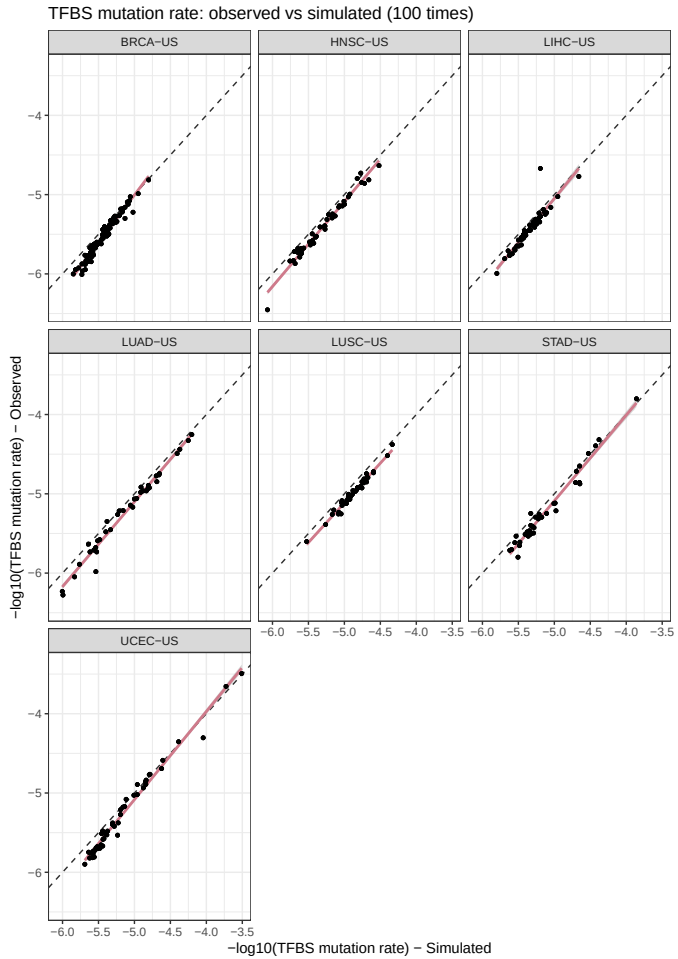
- Cohorts:
  - TCGA: 343 samples (7 cancer types)

| | | |
|---|---|---|
| BRCA | Breast cancer | 91 |
| LIHC | Liver Hepatocellular Carcinoma | 50 |
| UCUC | Uterine Corpus Endometrial Carcinoma | 48 |
| HNSC | Head and Neck Squamous Cell Carcinoma | 43 |
| LUSC | Lung Squamous Cell Carcinoma | 42 |
| LUAD | Lung Adenocarcinoma | 37 |
| STAD | Gastric Adenocarcinoma | 35 |

  - BASIS: 296 breast cancer samples

# TFBSs are less mutated than expected by chance

13,107,508 mutations in TCGA (from 211,421 to 2,141,178 per cohort).

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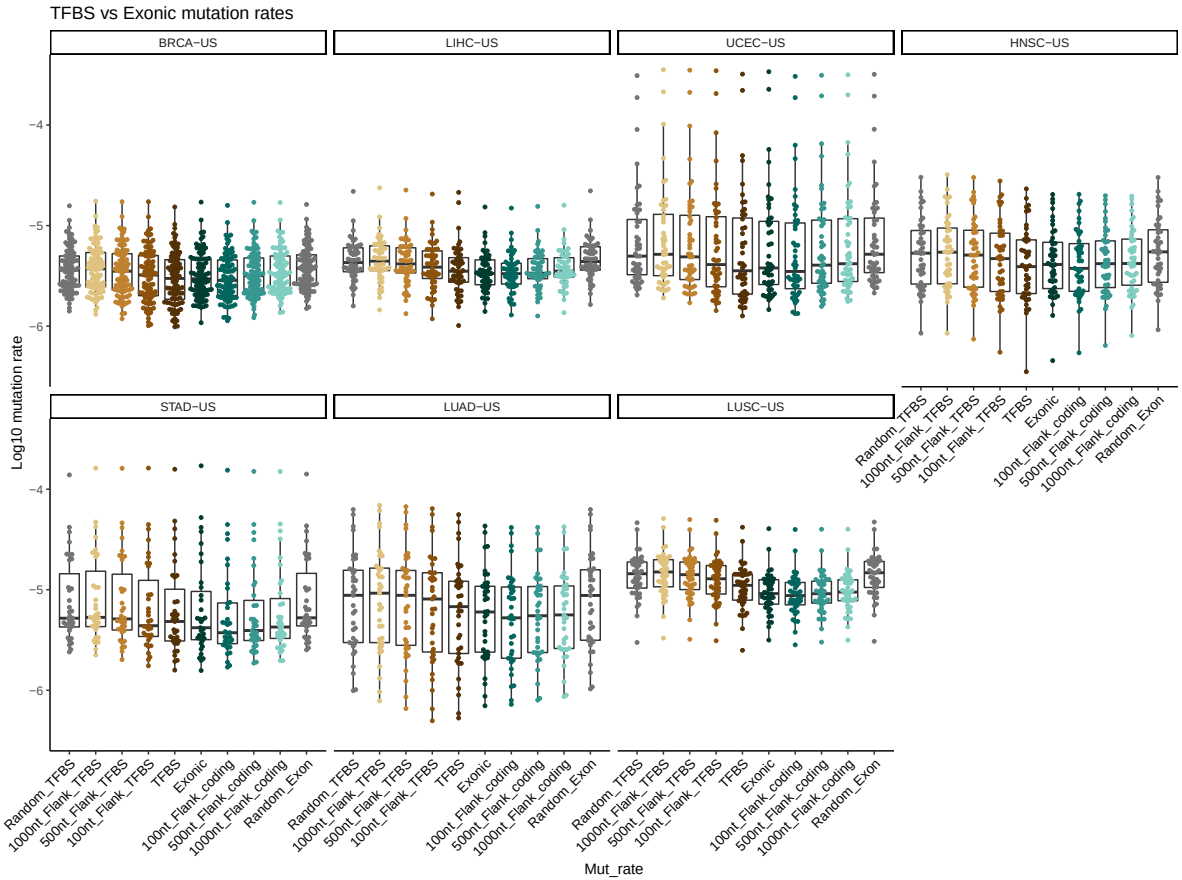


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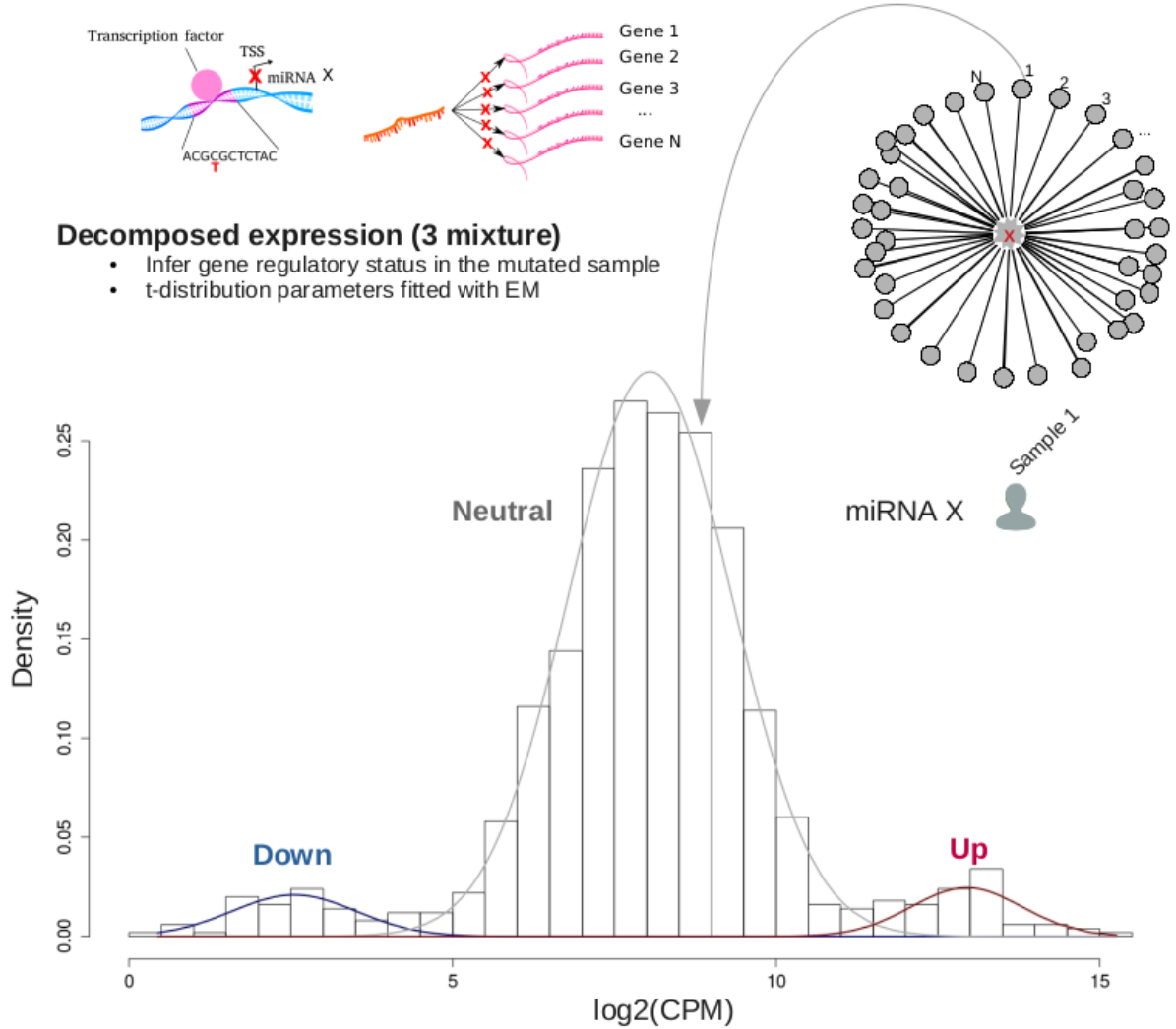
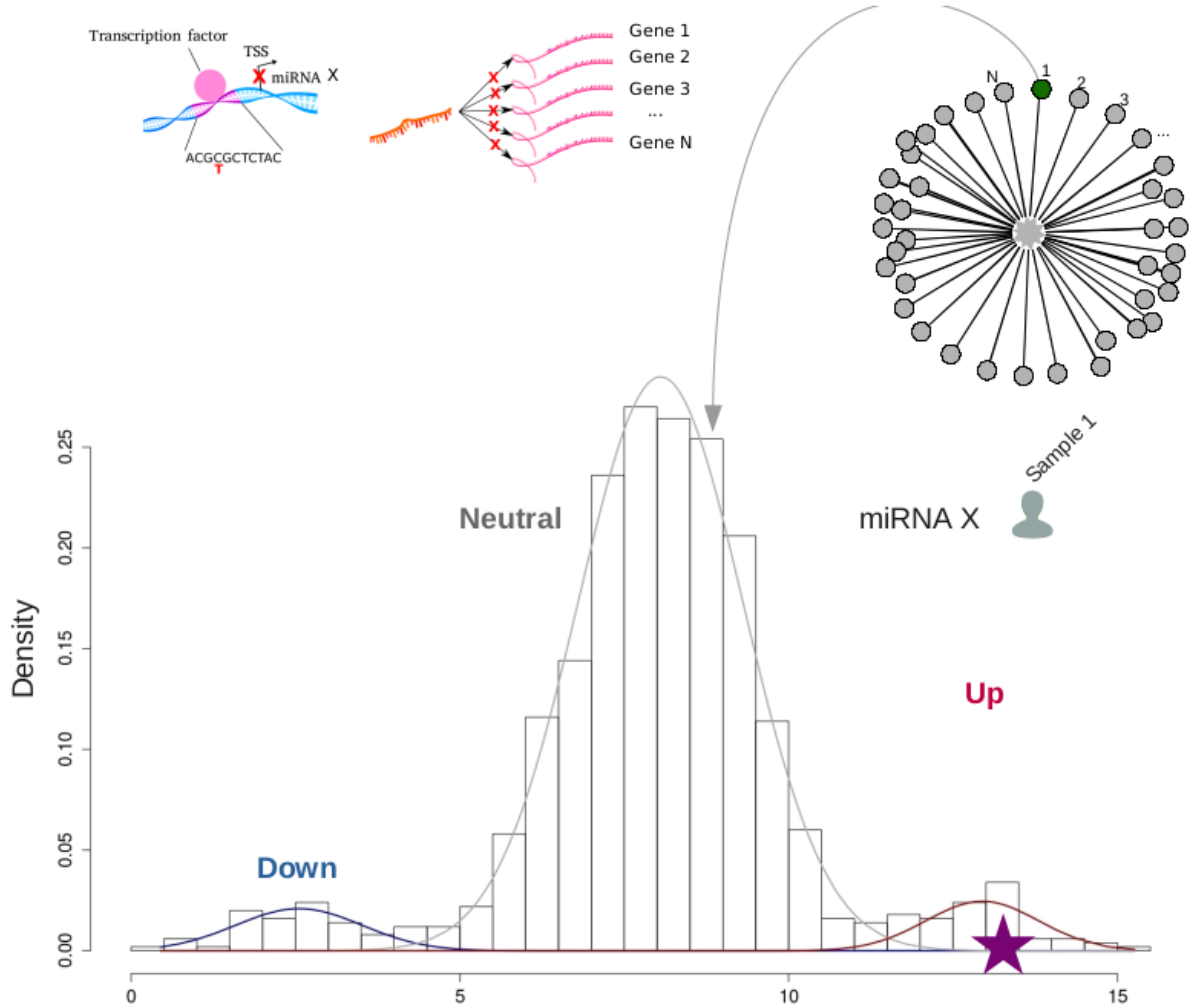
# Prediction of gene expression dysregulation



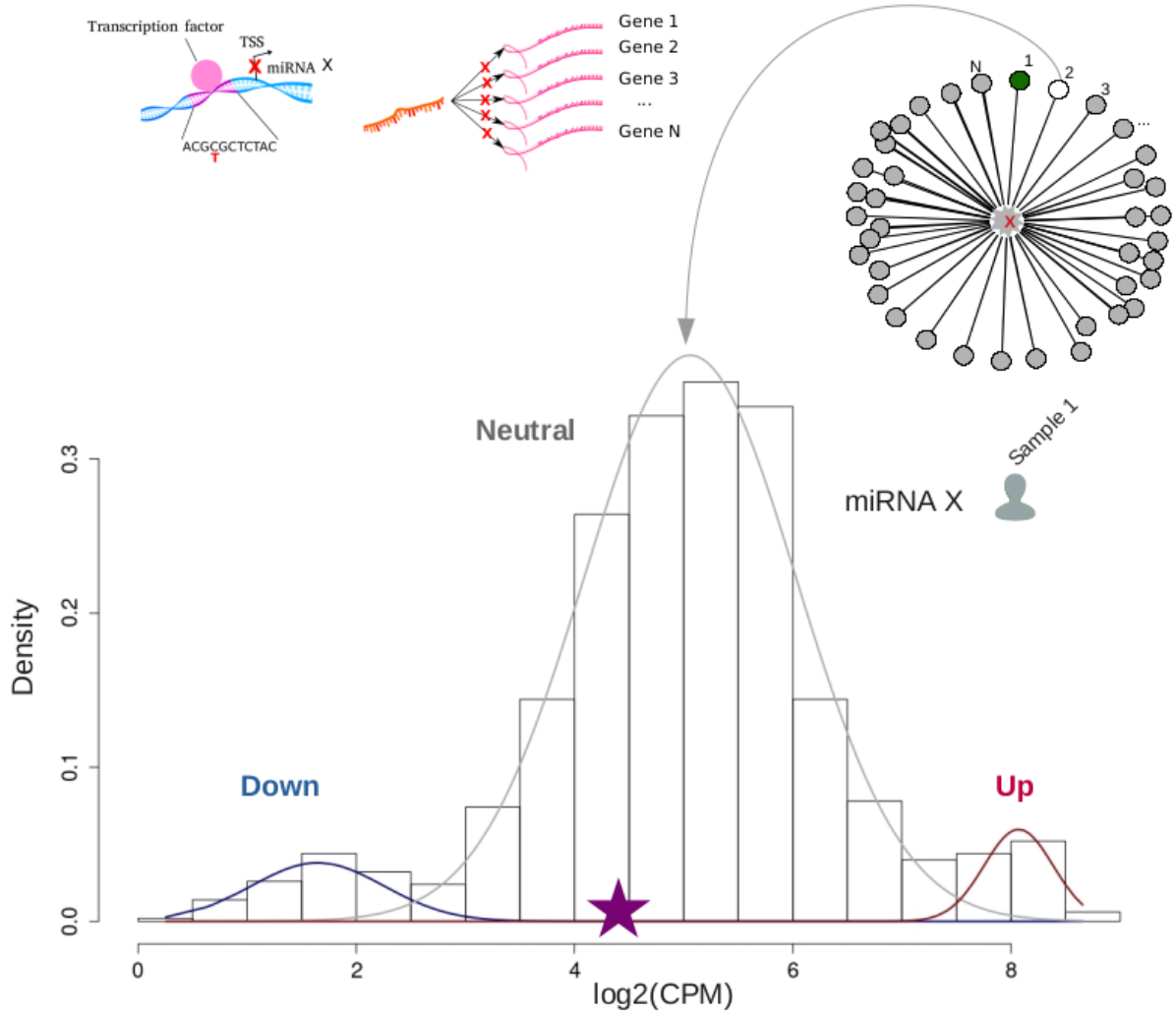

1. Ding et al. (2015) Systematic analysis of somatic mutations impacting gene expression in 12 tumour types. Nat Comms

# Prediction of gene expression dysregulation




1. Ding et al. (2015) Systematic analysis of somatic mutations impacting gene expression in 12 tumour types. Nat Comms

# Prediction of gene expression dysregulation

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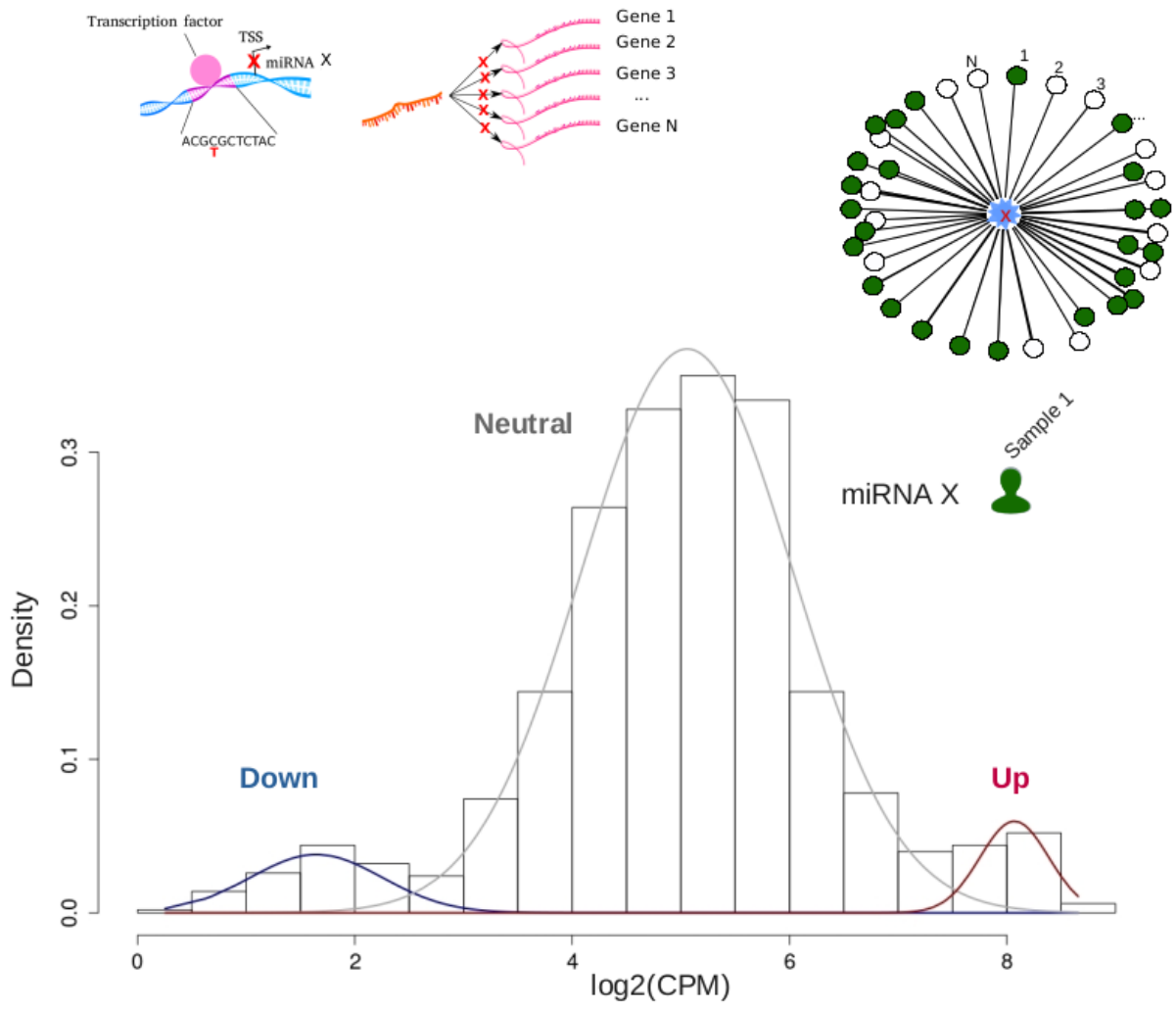
# Prediction of gene expression dysregulation

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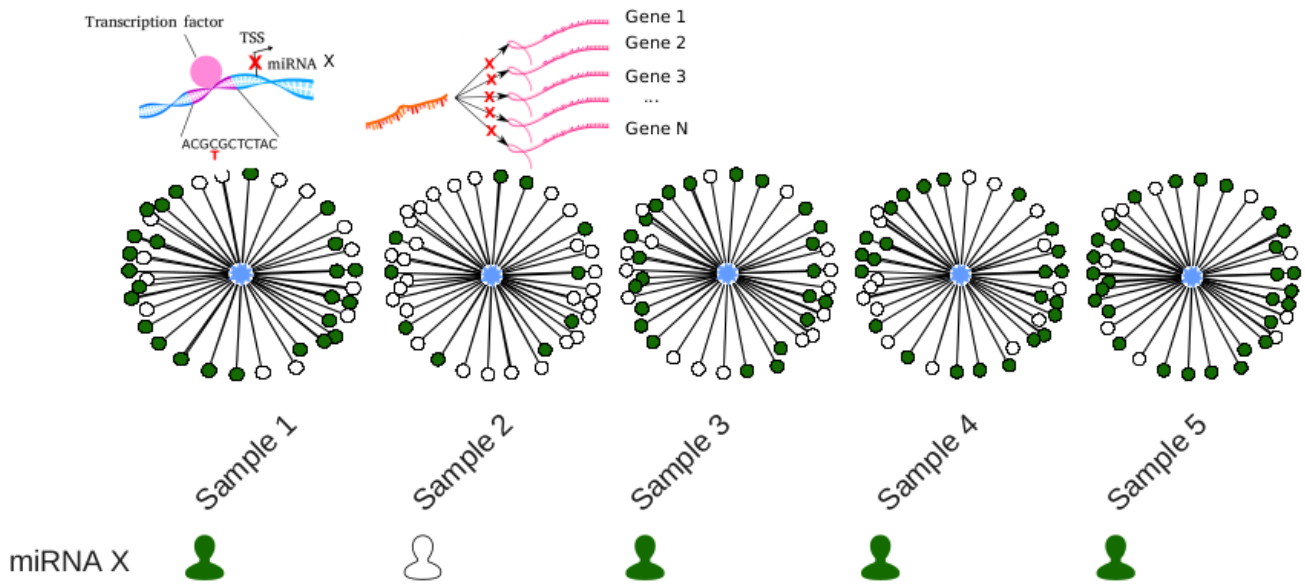


1. Ding et al. (2015) Systematic analysis of somatic mutations impacting gene expression in 12 tumour types. Nat Comms

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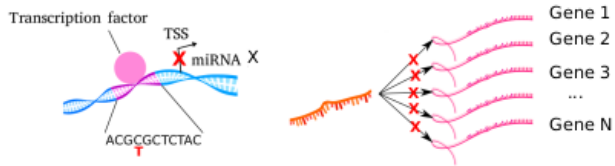


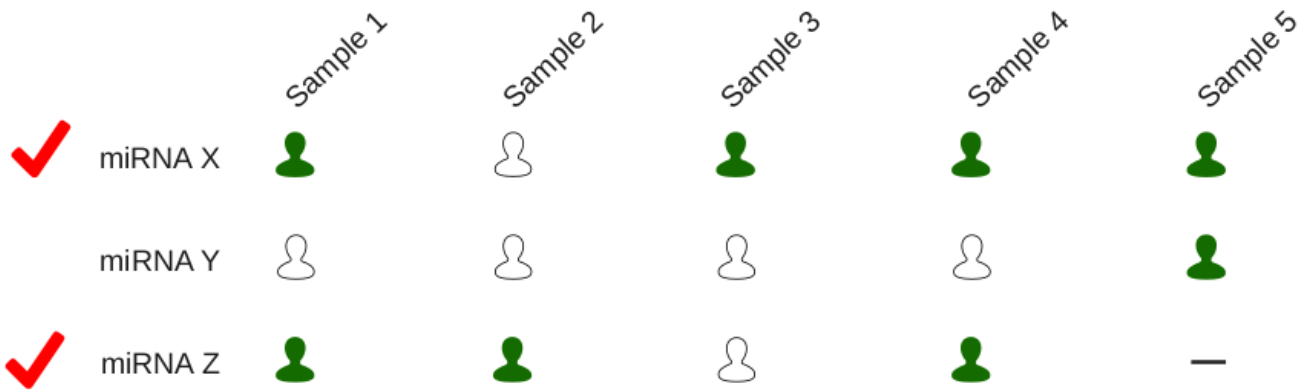


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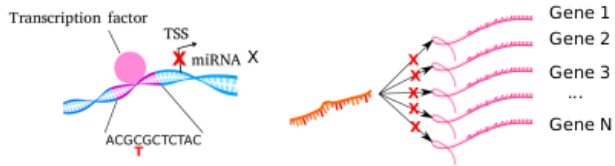
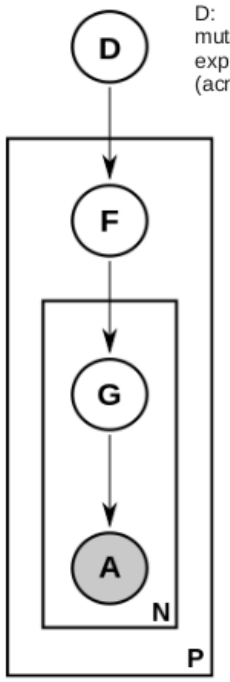
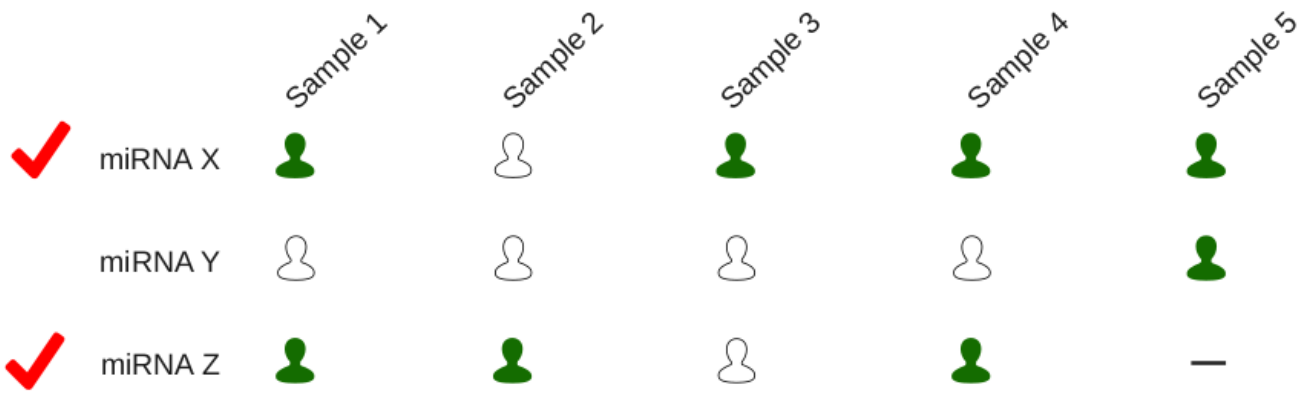
# Prediction of gene expression dysregulation




**Bayesian network**



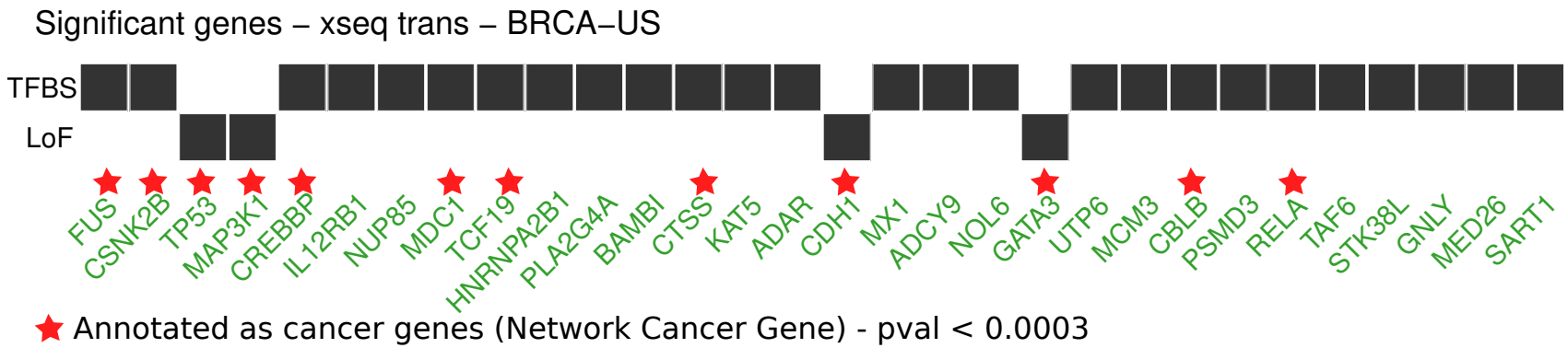

D: likely association between mutations and target gene expression dysregulation (across the cohort).




**N:** genes connected to gene A.
**P:** samples with mutations in gene A

1. Ding et al. (2015) Systematic analysis of somatic mutations impacting gene expression in 12 tumour types. Nat Comms

# Prediction results on protein-coding genes in breast cancer

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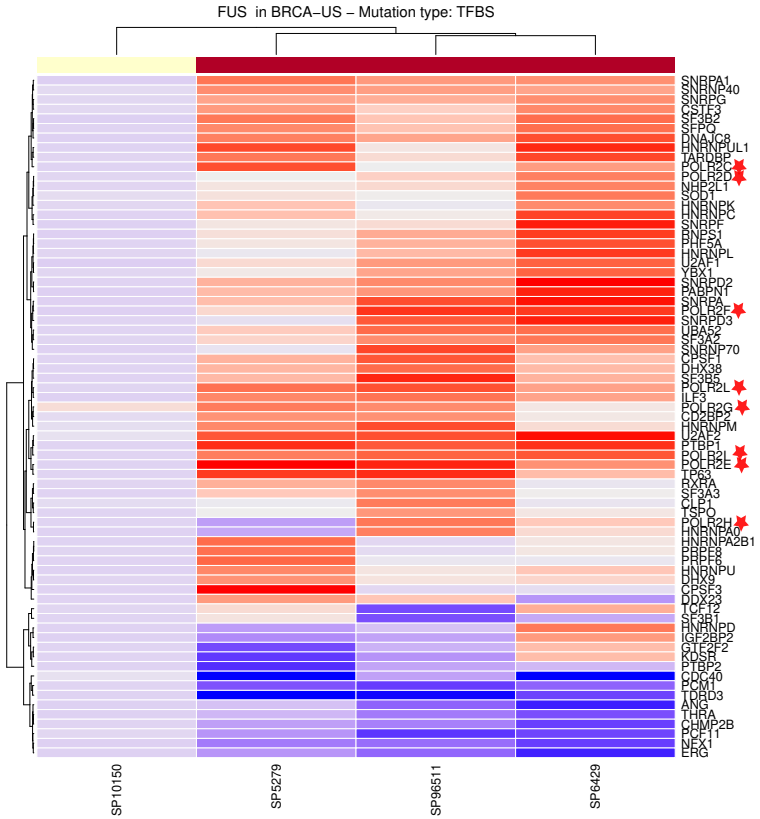
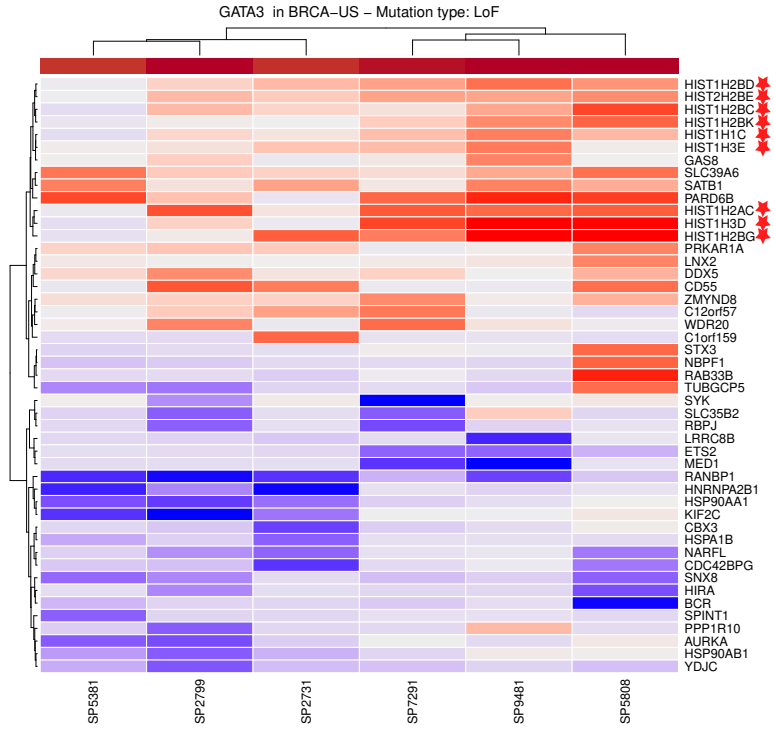
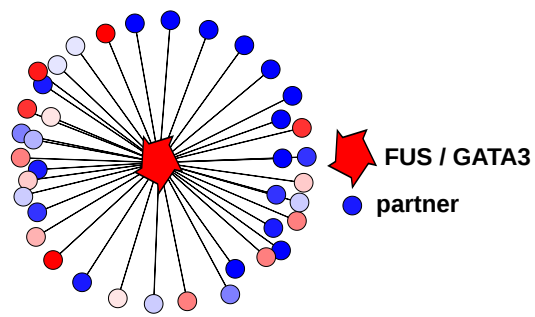
# Visualization of the regulatory network alteration

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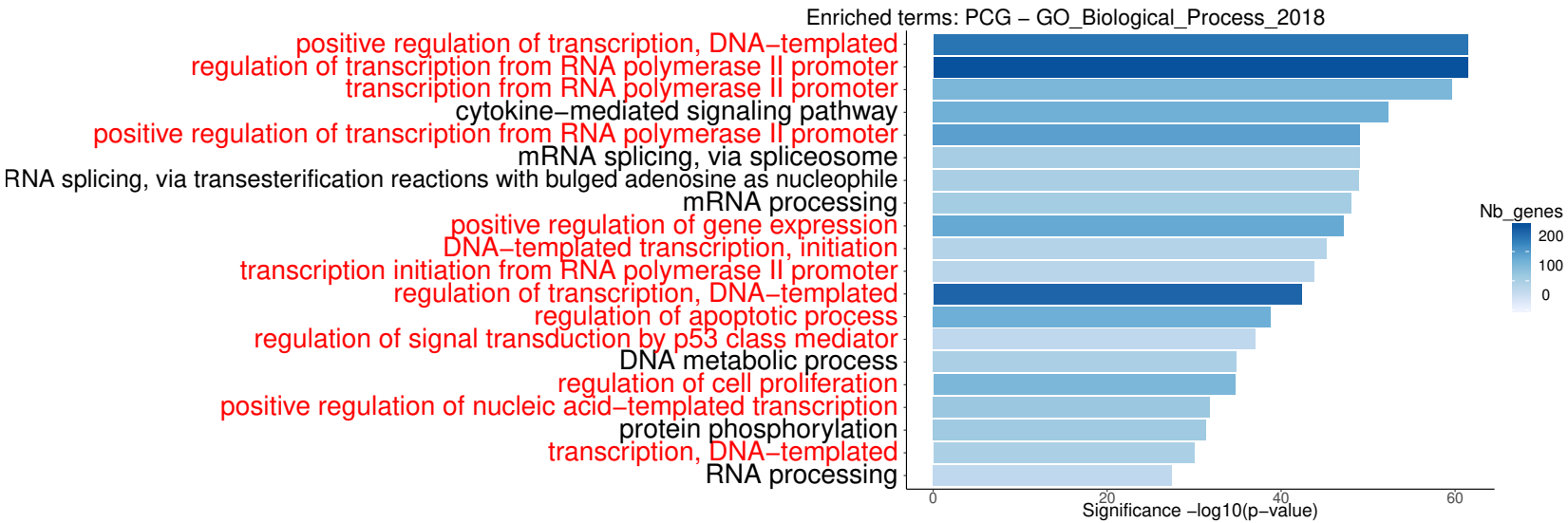
# Dysregulated networks for PCGs highlight key pathways

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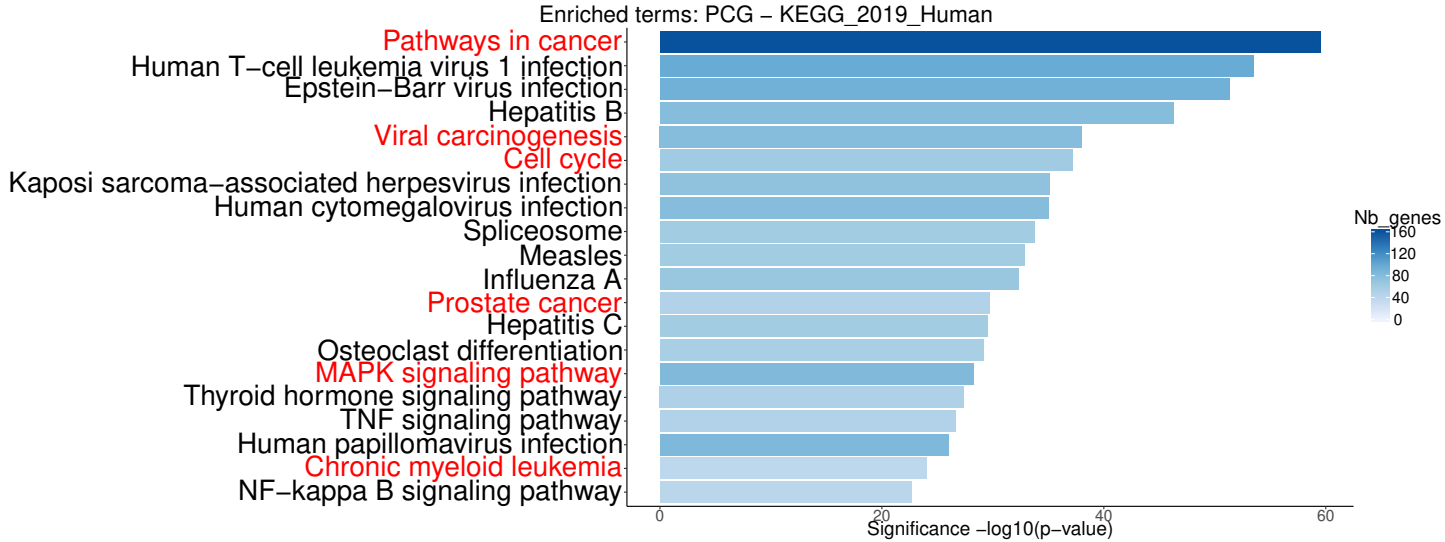


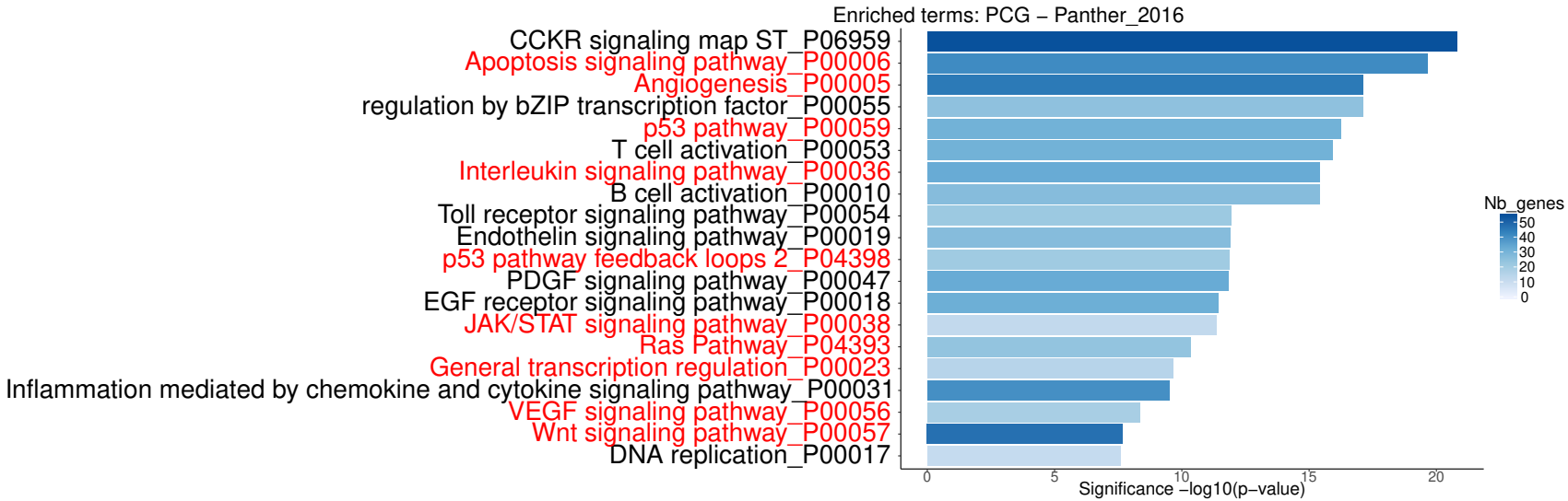


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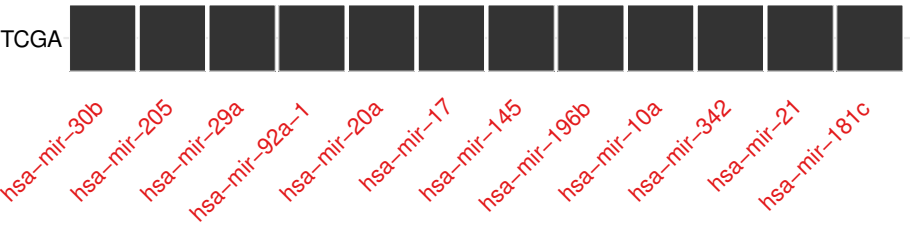
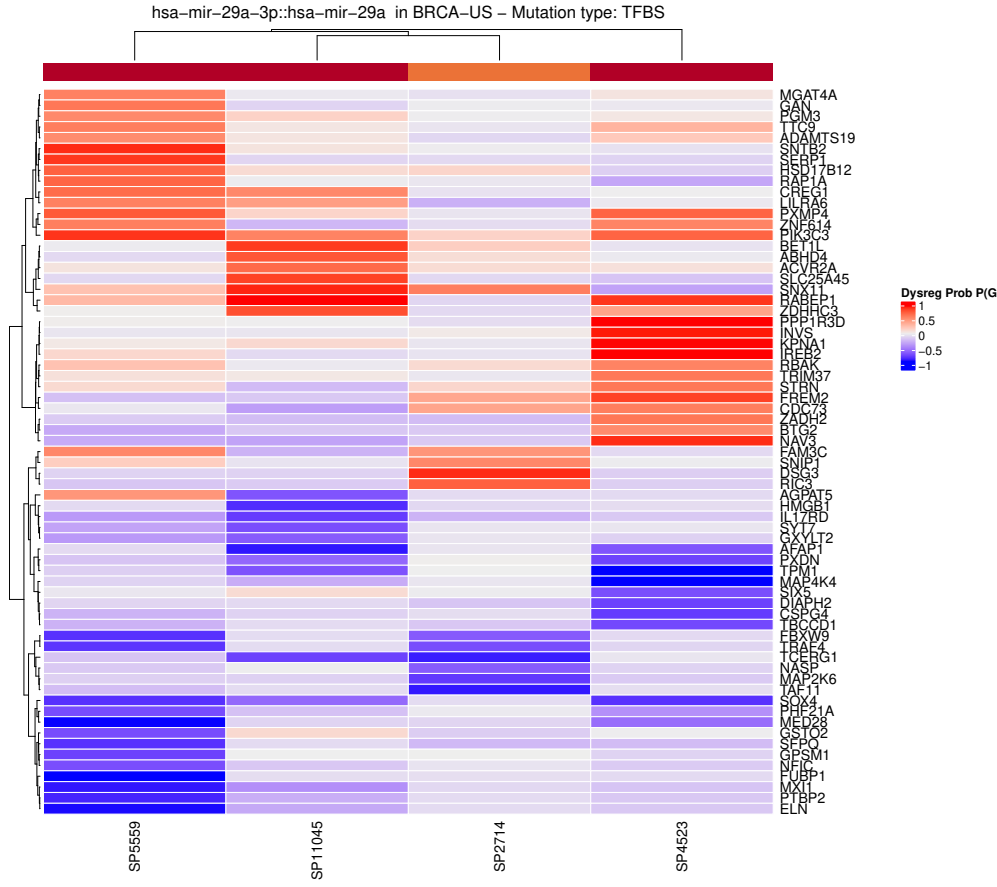
# Predictions on miRNA genes in breast cancer

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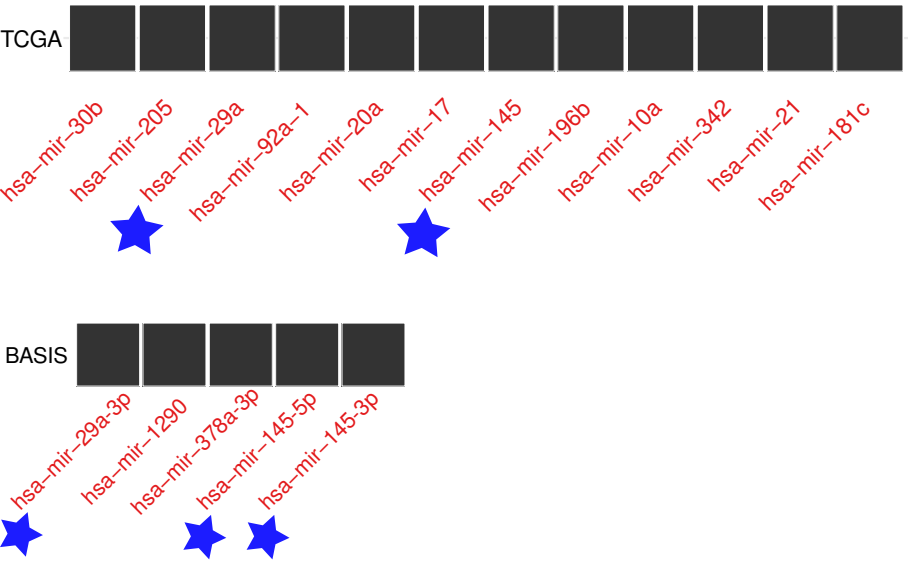


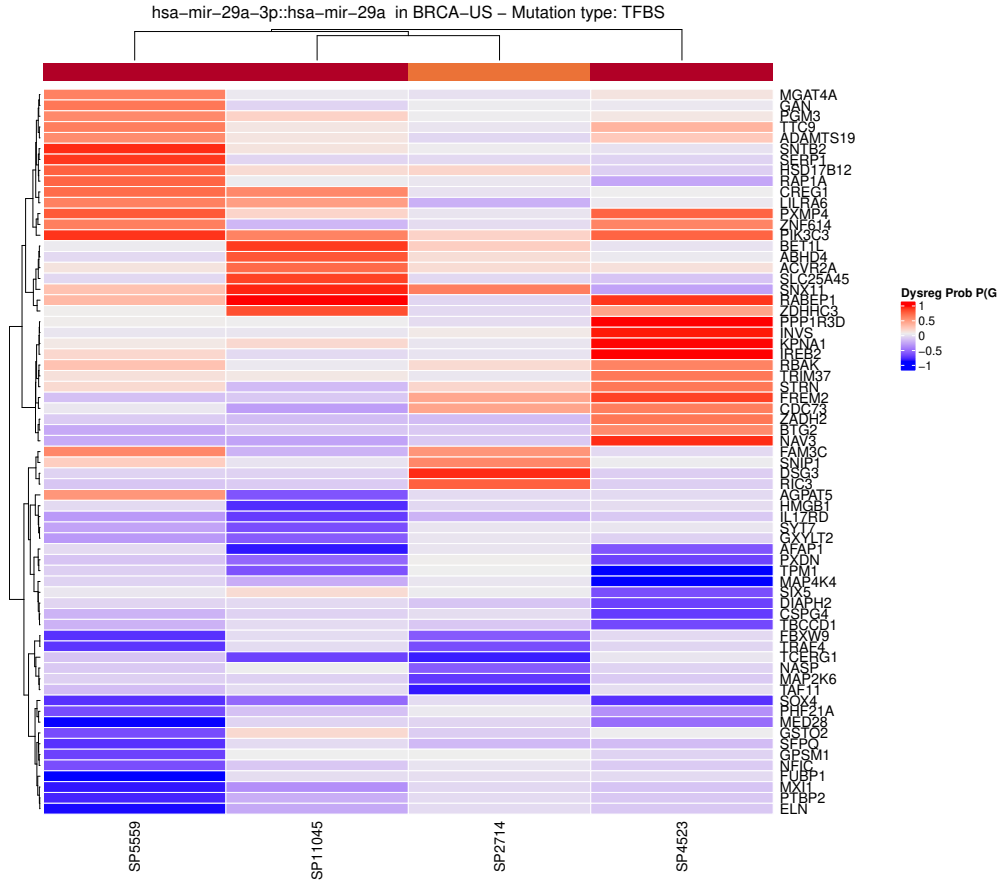


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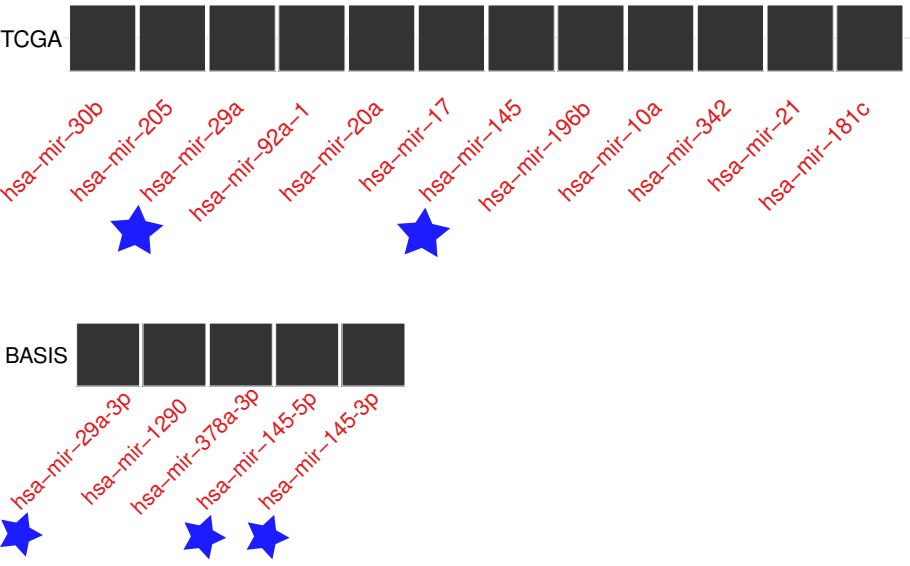


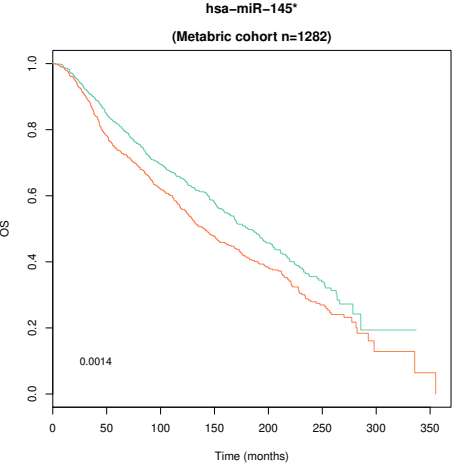


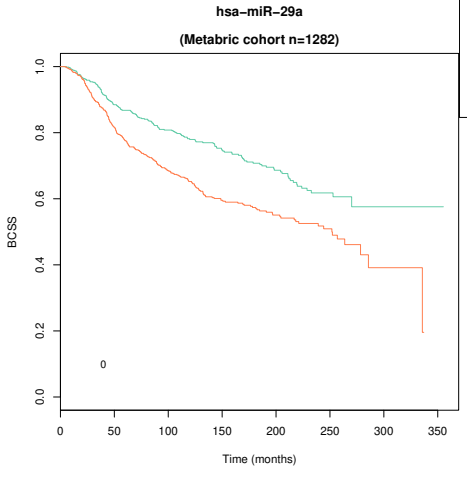


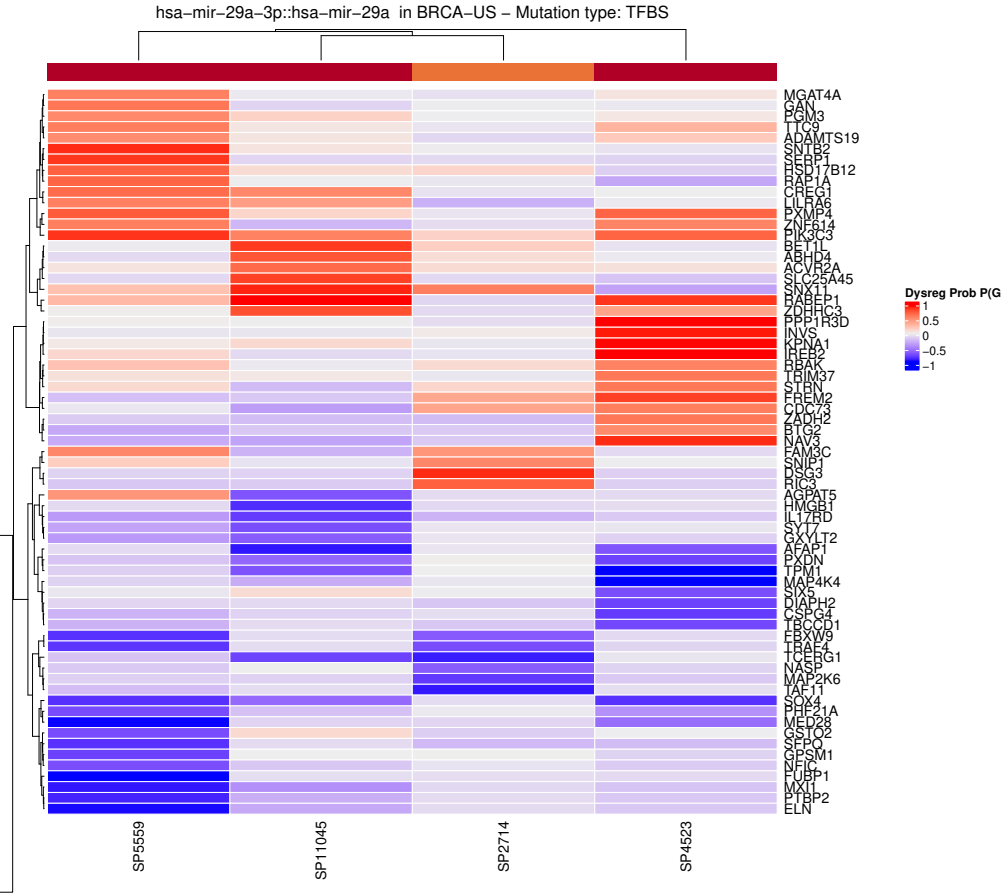


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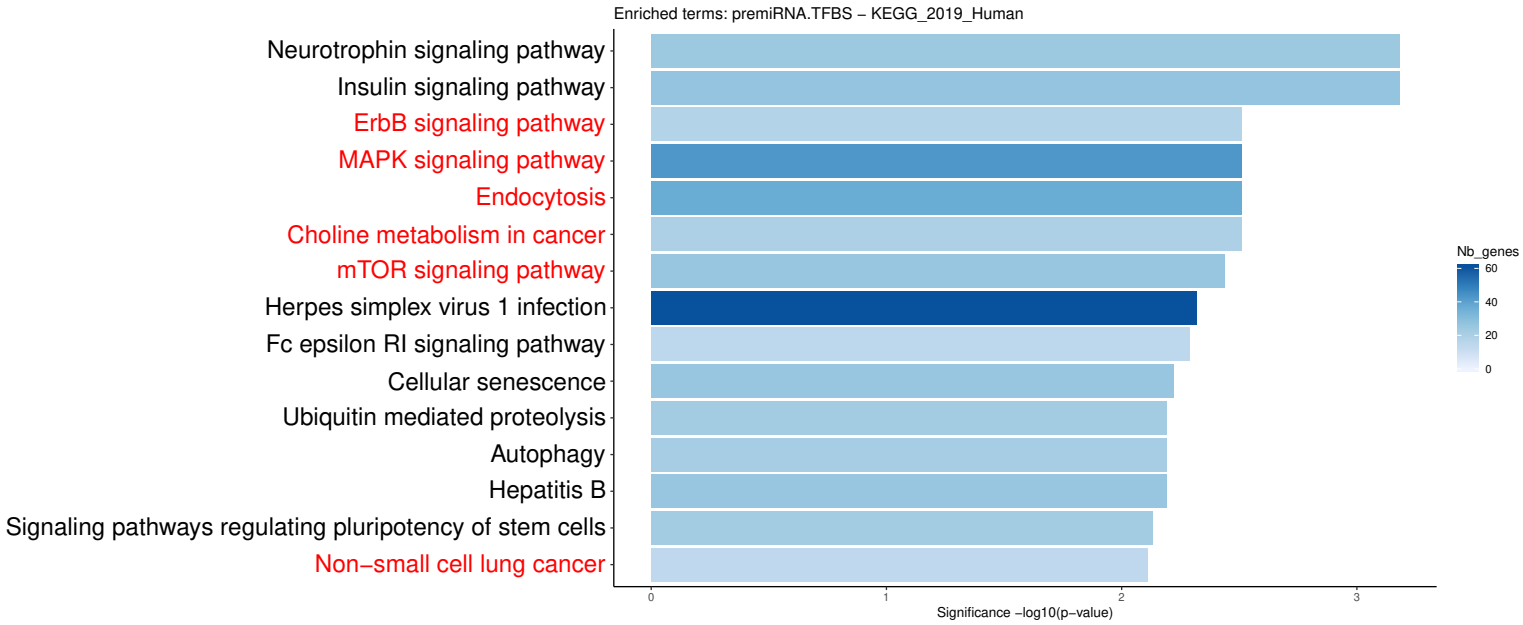
# Dysregulated networks for miRNAs highlight key pathways

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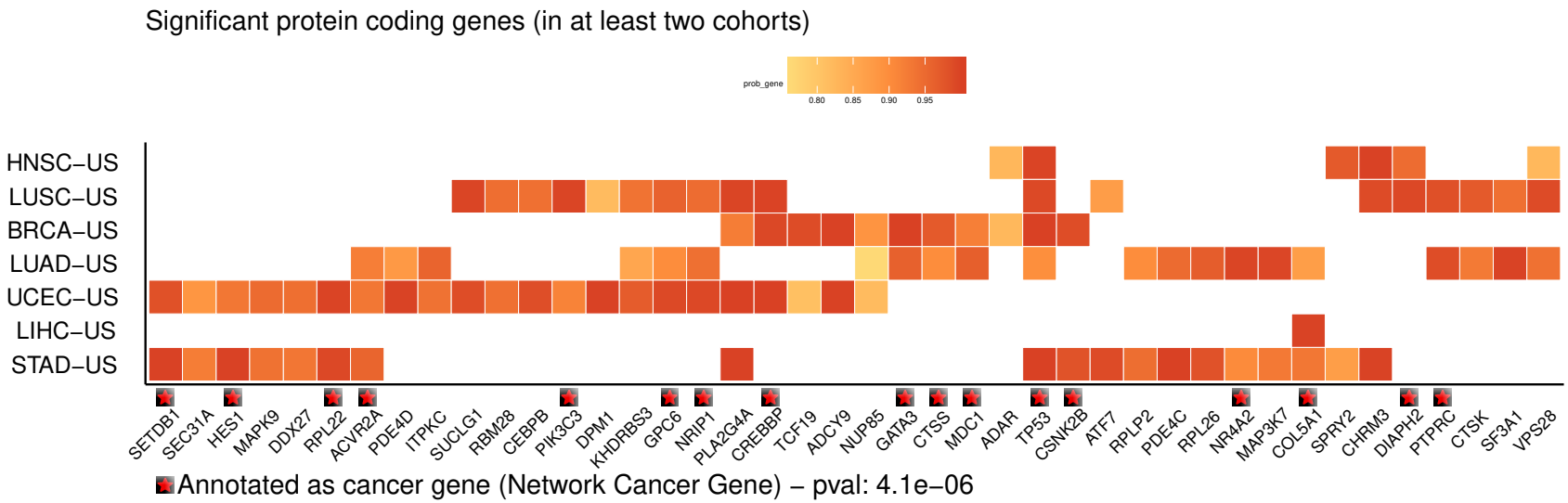
# Pan-cancer analysis of PCG networks dysregulation

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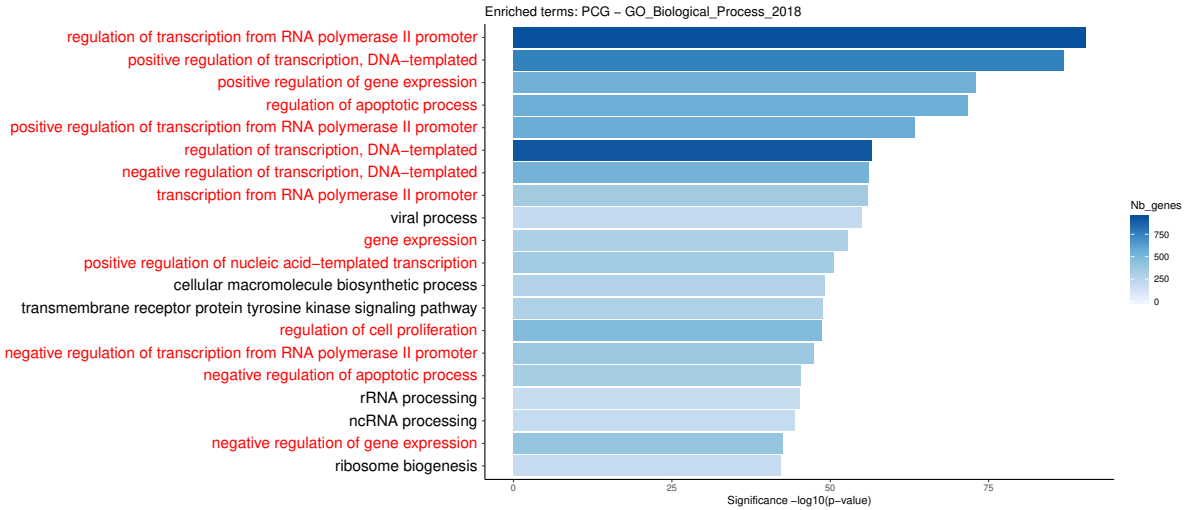


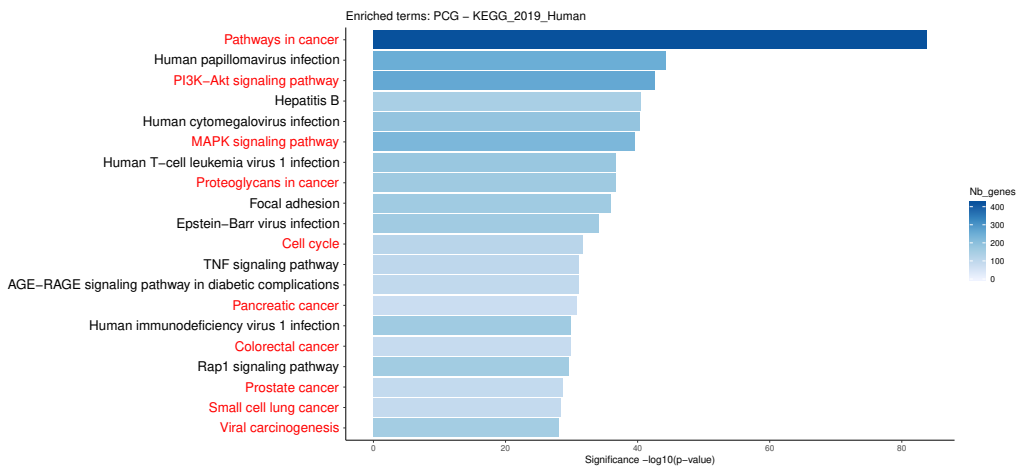


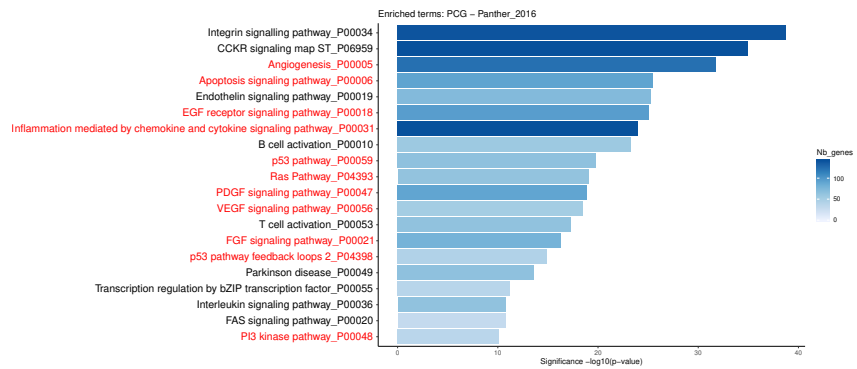


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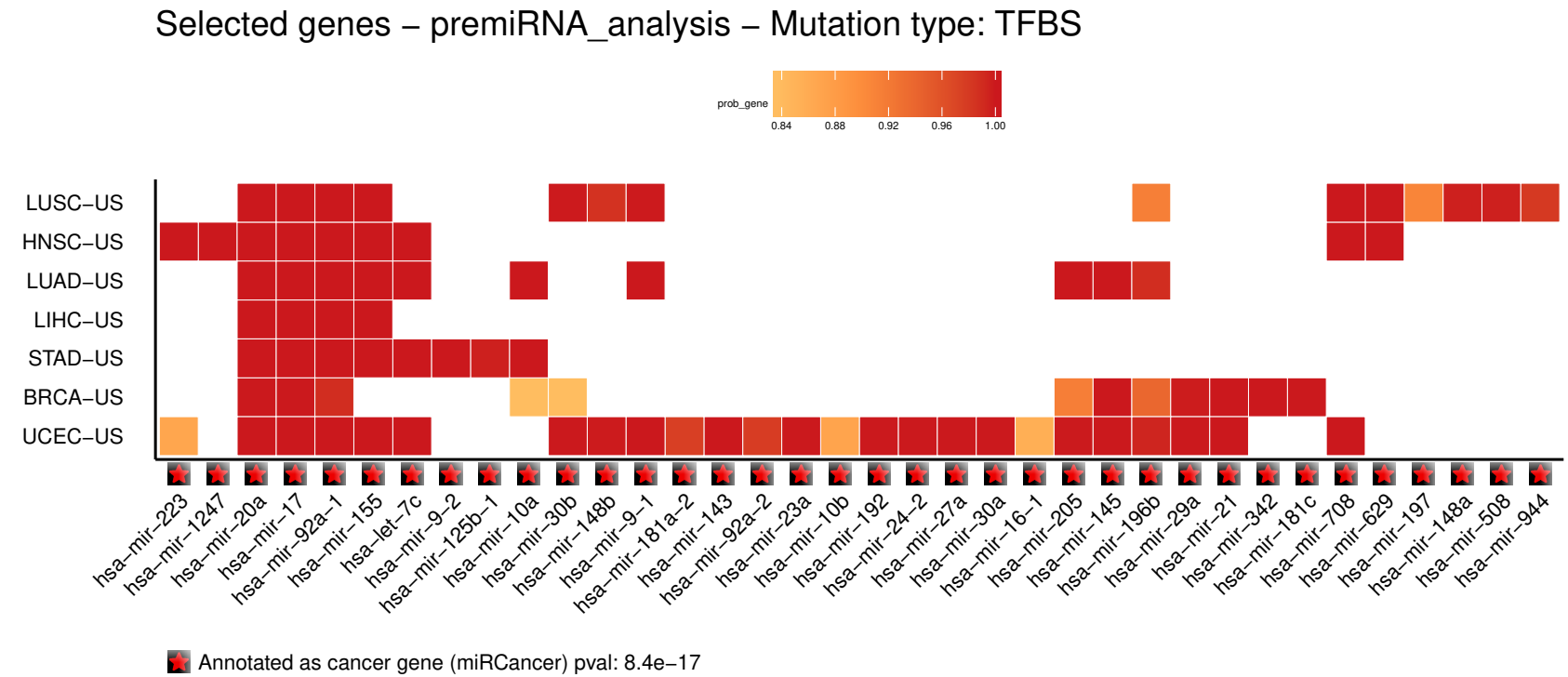


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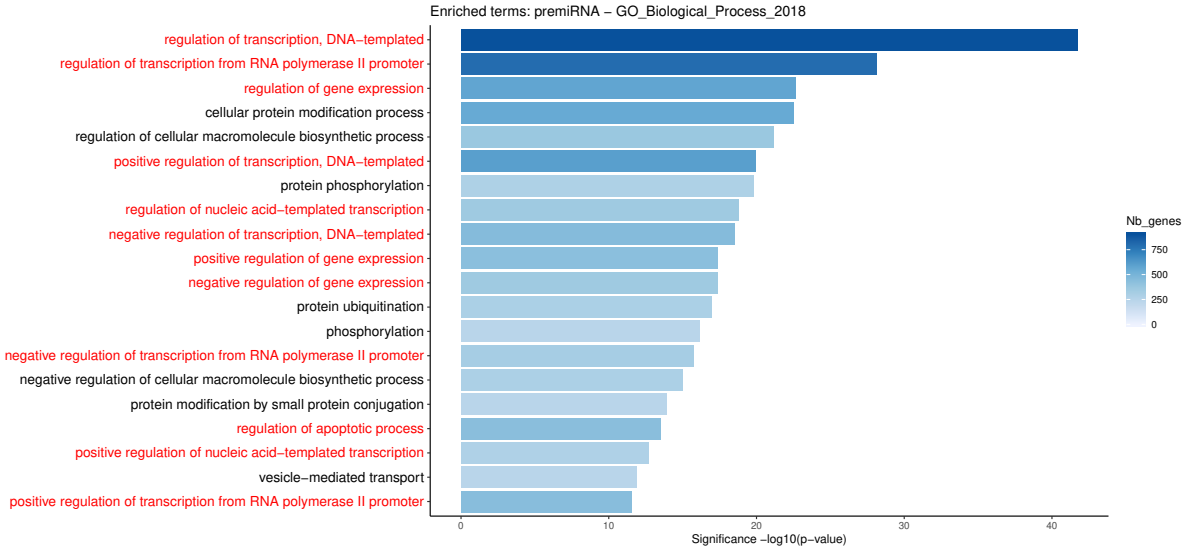


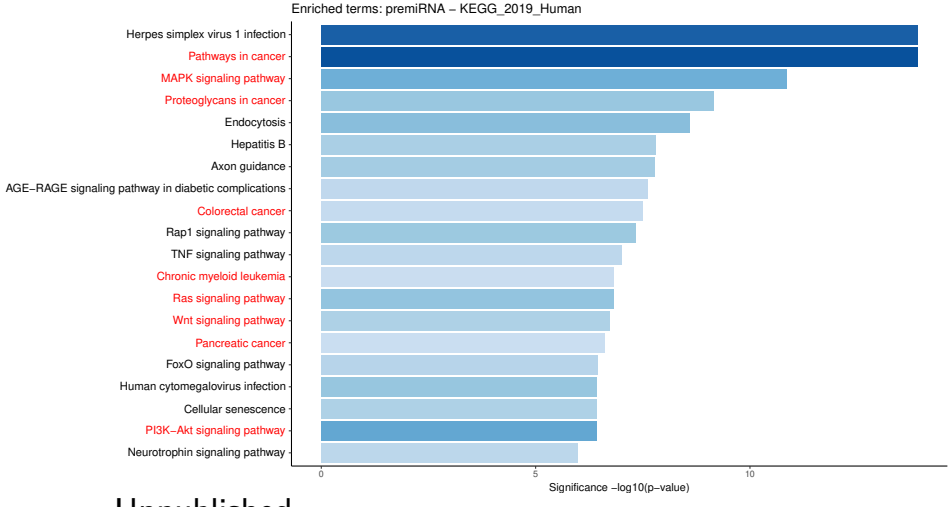


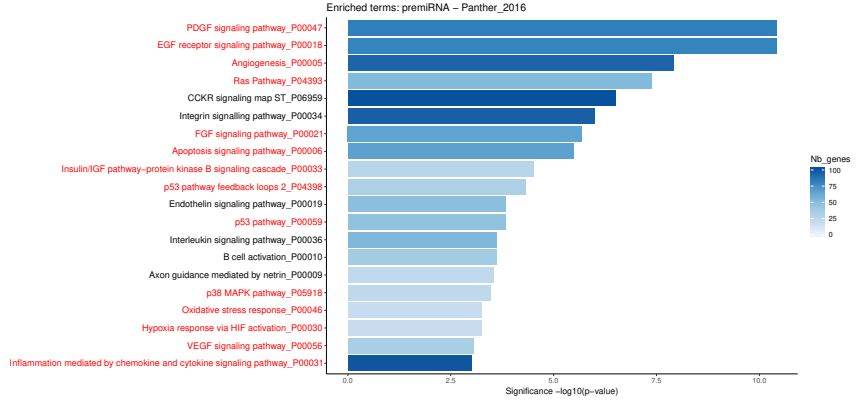


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# Summary

- Combining LoF mutations with mutations in TFBSs highlights candidate driver PCGs pan-cancer

- dysmiR predicts candidate driver miRNAs that are dysregulated through cis-regulatory mutations with cascading effects on the gene expression regulation program.

- We highlight candidate regulatory-disrupting variations dysregulating the gene expression regulatory program in cancer pathways

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# Acknowledgements

**Marius Gheorghe**
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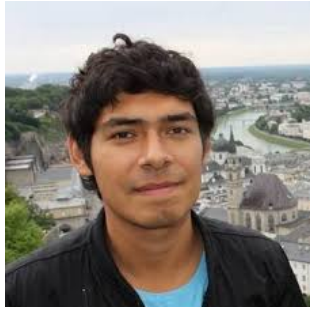
JASPAR - ReMap



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JASPAR

**Miriam Ragle-Aure**
Cancer deregulation

**Aziz Khan**
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UniBind

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- Arnaud Stigliani
- Robin van der Lee
- Adrien Bessy
- Jeanne Cheneby
- Shubhada Kulkarni
- Ge Tan
- Damir Baranasic
- David Arenillas
- Albin Sandeling
- Klaas Vandepoele
- Boris Lenhard
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- Wyeth Wasserman
- Francois Parcy

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- Jeanne Cheneby
- Marie Artufel
- Benoit Ballester

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- Anne-Lise Borresen-Dale
- Anita Langerod
- BASIS consortium

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HELSE SØR-ØST