ChIP-seq technology and applications

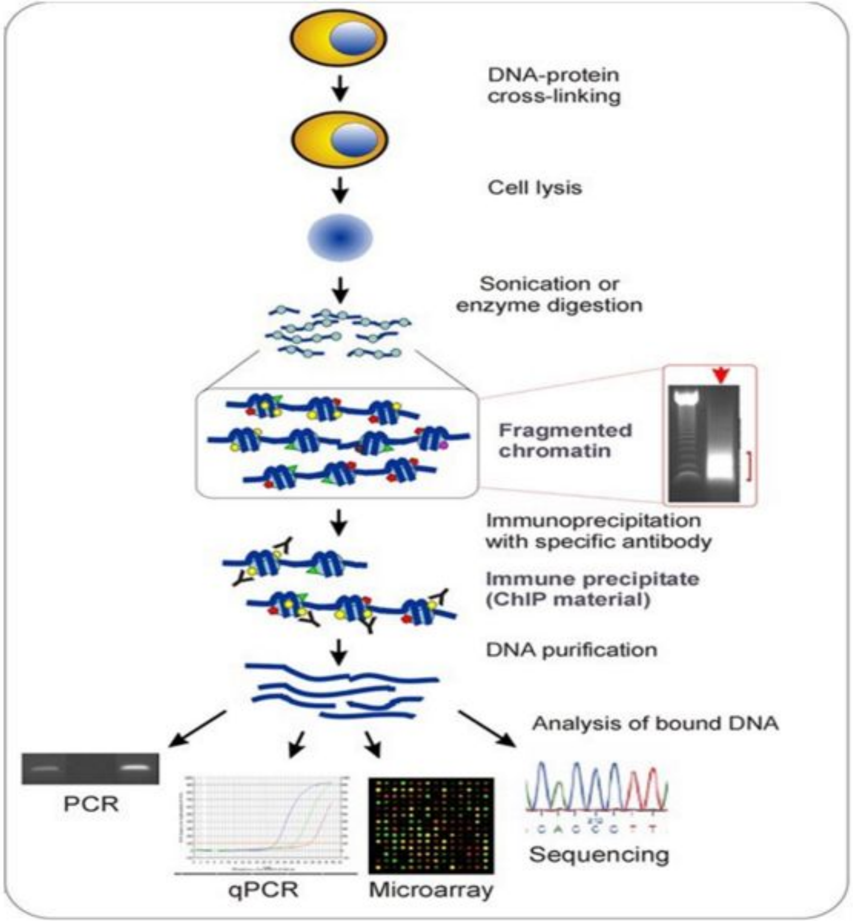
D. Puthier, C. Rioualen, J. van Helden

A compilation of slides recycled from the workshop on NGS organized in Cuernavaca in 2017

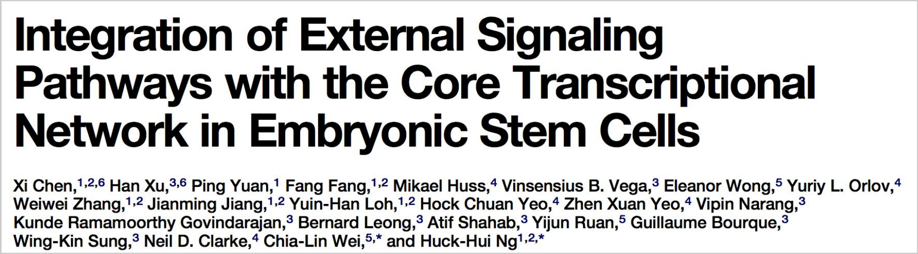
ChIP-seq technology

ChIP-Seq principle

- Used to analyze, at the level of whole genomes:
  - transcription factor binding locations
  - histone modifications

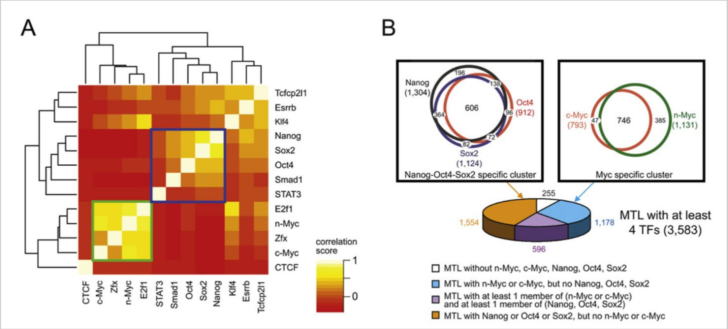

DNA-protein cross-linking
Cell lysis
Sonication or enzyme digestion
Fragmented chromatin
Immunoprecipitation with specific antibody
Immune precipitate (ChIP material)
DNA purification
Analysis of bound DNA
PCR
qPCR
Microarray
Sequencing

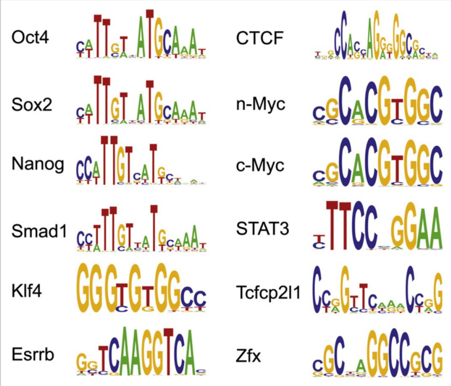
ChIP-seq for 13 TFs in mouse ES cells

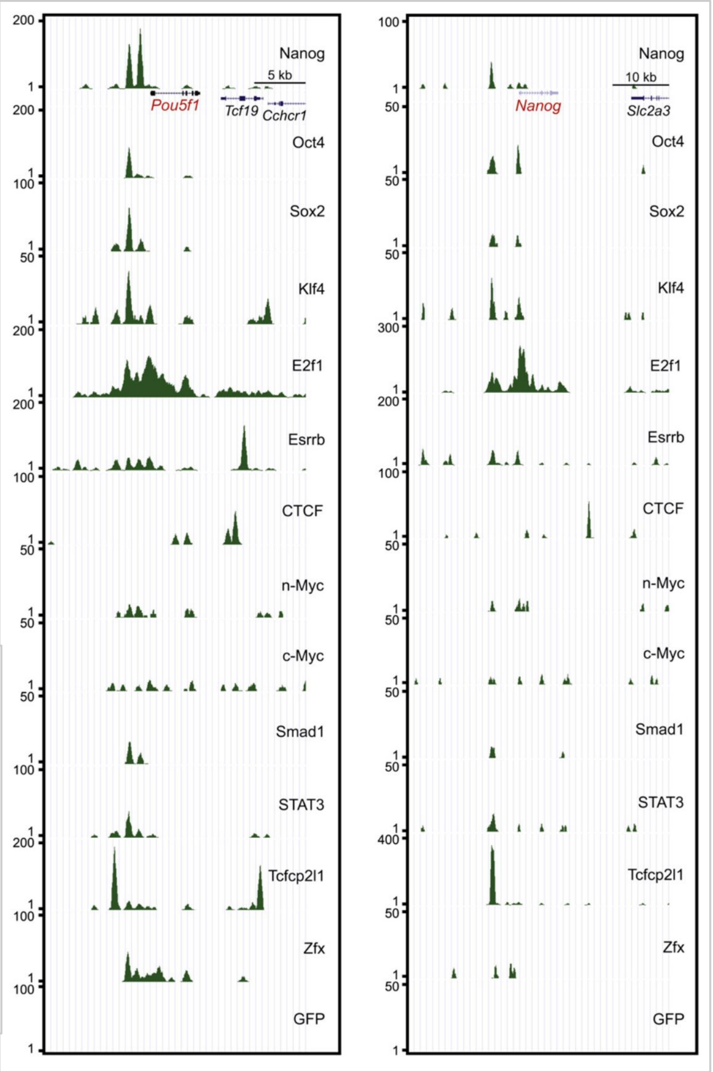


Integration of External Signaling Pathways with the Core Transcriptional Network in Embryonic Stem Cells

Xi Chen,[1,2,6] Han Xu,[3,6] Ping Yuan,[1] Fang Fang,[1,2] Mikael Huss,[4] Vinsensius B. Vega,[3] Eleanor Wong,[5] Yuriy L. Orlov,[4] Weiwei Zhang,[1,2] Jianming Jiang,[1,2] Yuin-Han Loh,[1,2] Hock Chuan Yeo,[4] Zhen Xuan Yeo,[4] Vipin Narang,[3] Kunde Ramamoorthy Govindarajan,[3] Bernard Leong,[3] Atif Shahab,[3] Yijun Ruan,[5] Guillaume Bourque,[3] Wing-Kin Sung,[3] Neil D. Clarke,[4] Chia-Lin Wei,[5,*] and Huck-Hui Ng[1,2,*]

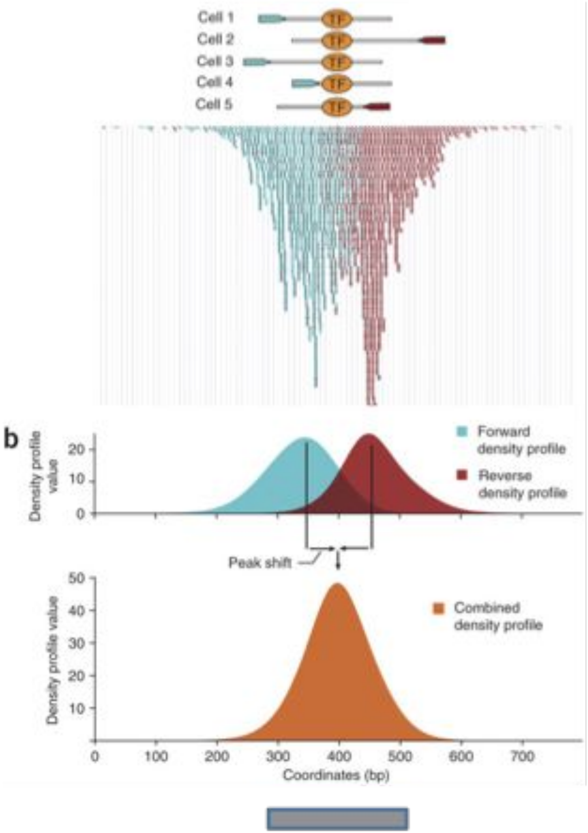






ChIP-Seq analysis in brief

- Fragments (typically ~300bp) cover the region of interest + some pieces on both side.
- We only sequence a short read on one or both extremities
- **The binding site is thus generally not in our reads !**
- Solutions
    - Bioinfo read extension
    - Bioinfo: read shifting
    - Experiment: Exo-ChIP (digest flanks between sequencing).



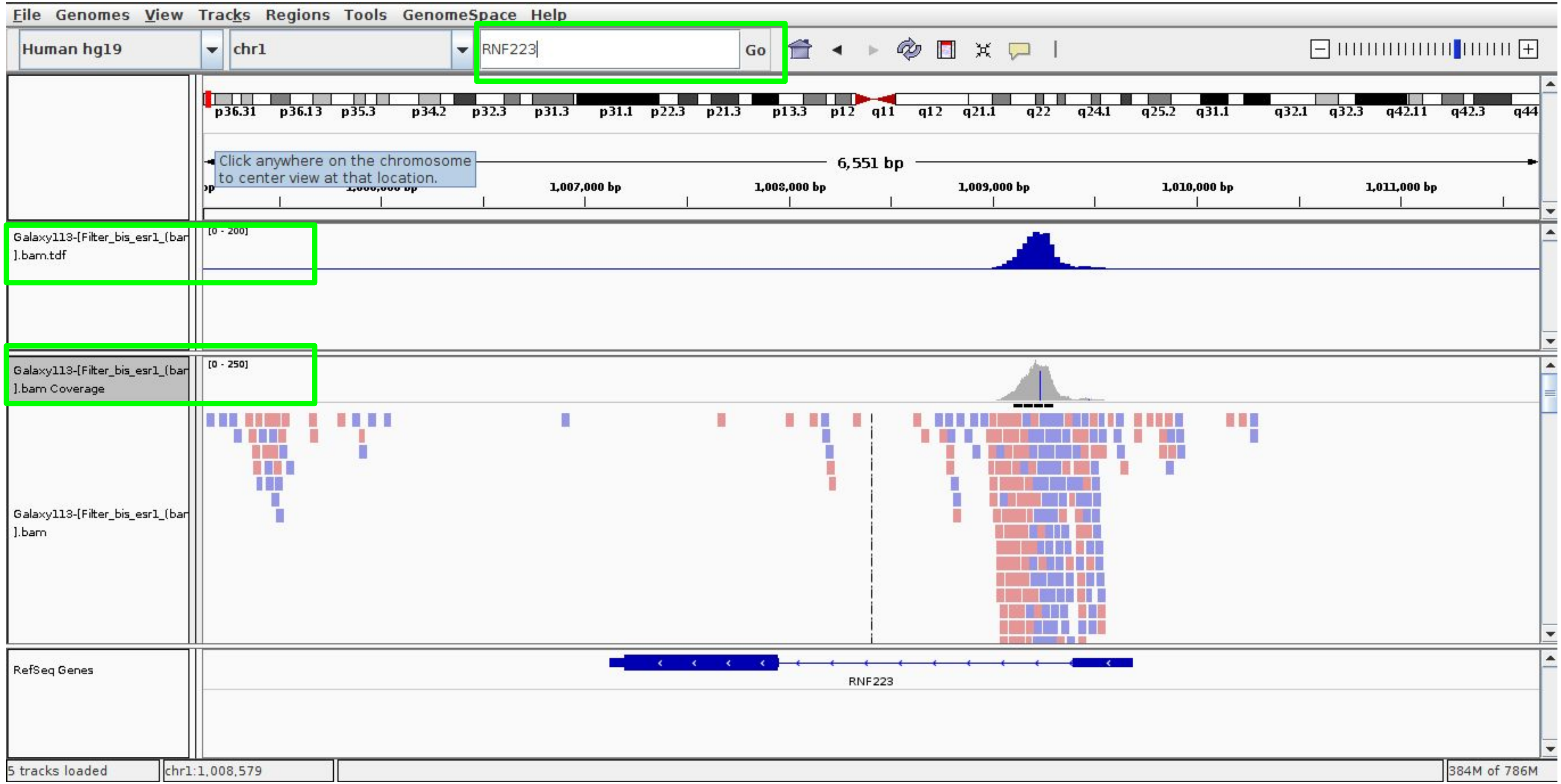
Aligned reads

Binding profile

Binding Peak

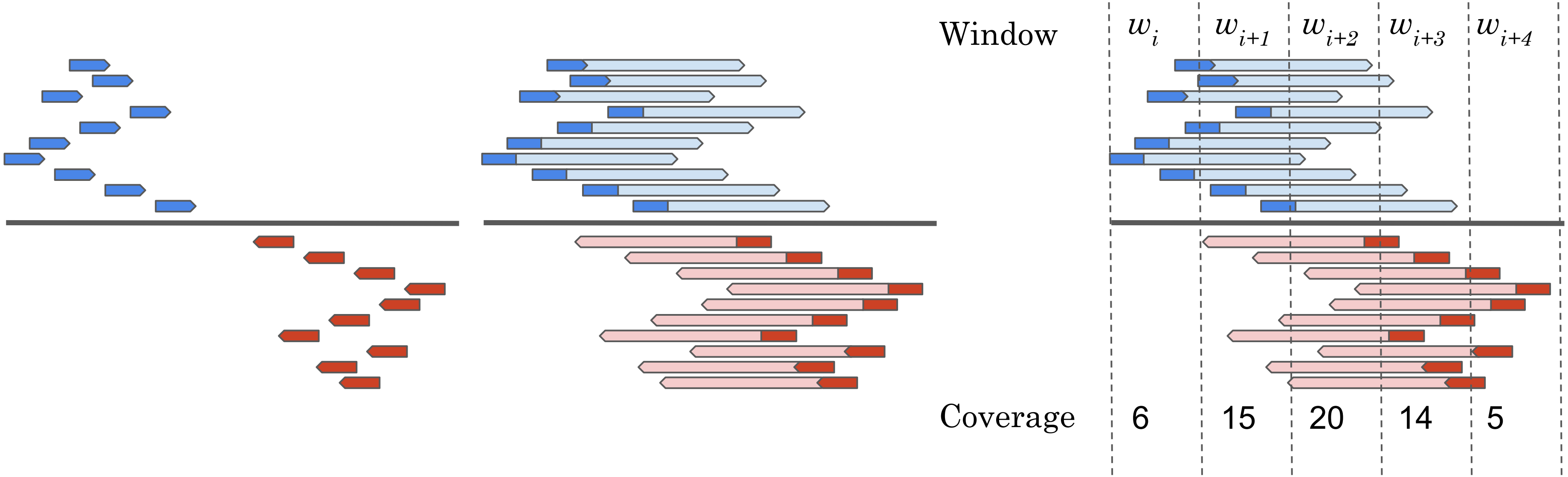
Identifying peaks from ChIP-seq reads

Example of read mapping


File Genomes View Tracks Regions Tools GenomeSpace Help
Human hg19
chr1
RNF223
Go
p36.31 p36.13 p35.3 p34.2 p32.3 p31.3 p31.1 p22.3 p21.3 p13.3 p12 q11 q12 q21.1 q22 q24.1 q25.2 q31.1 q32.1 q32.3 q42.11 q42.3 q44
Click anywhere on the chromosome to center view at that location.
6,551 bp
1,006,000 bp
1,007,000 bp
1,008,000 bp
1,009,000 bp
1,010,000 bp
1,011,000 bp
Galaxy113-[Filter_bis_esr1_(bar].bam.tdf
[0 - 200]
Galaxy113-[Filter_bis_esr1_(bar].bam Coverage
[0 - 250]
Galaxy113-[Filter_bis_esr1_(bar].bam
RefSeq Genes
RNF223
5 tracks loaded
chr1:1,008,579
384M of 786M

Coverage file and read extension

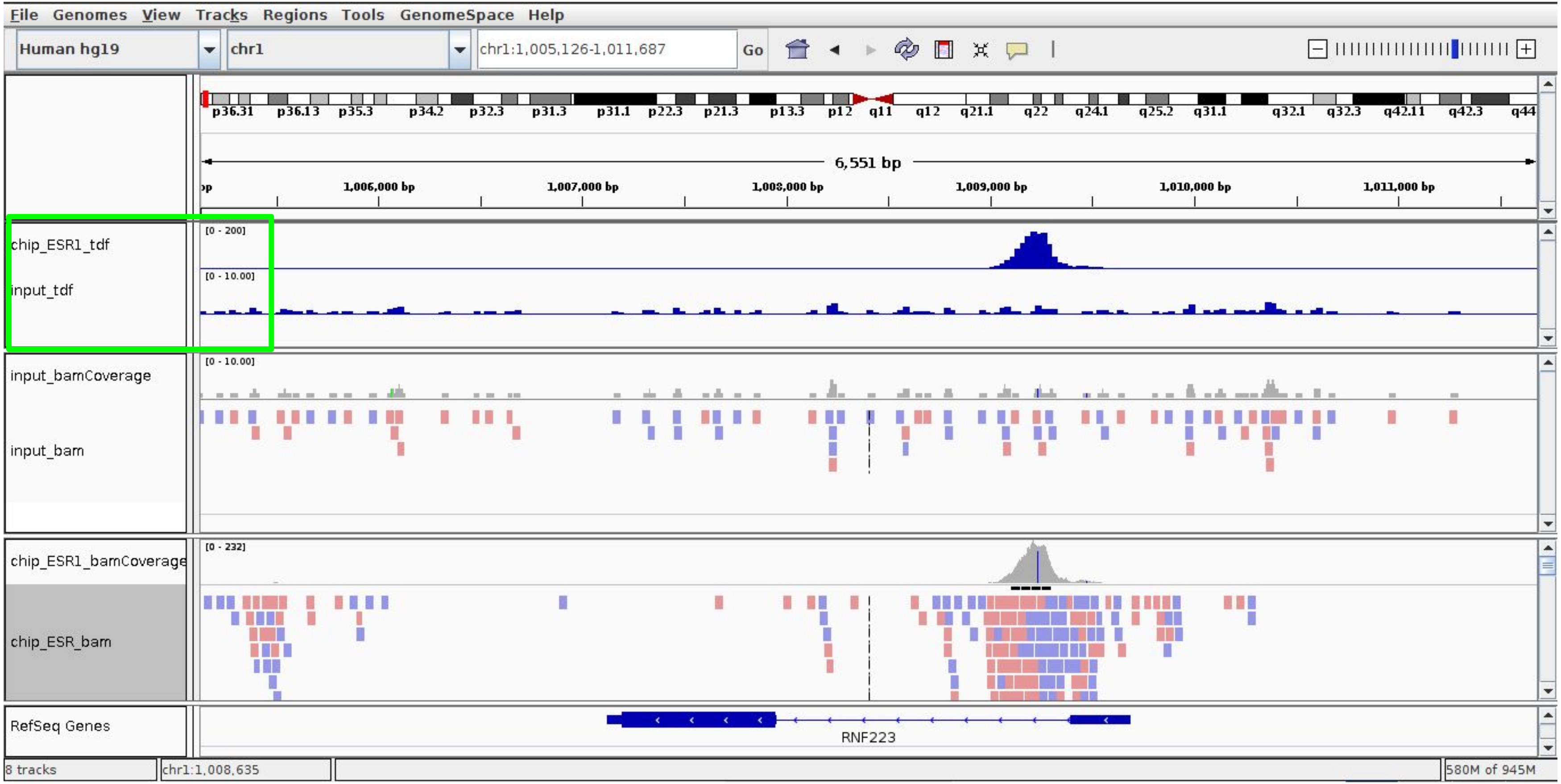
- BAM files **do not contain fragment location** but read location
- We need to extend reads to compute fragments coordinates before coverage analysis
- Not required for PE



Window w_i w_{i+1} w_{i+2} w_{i+3} w_{i+4}

Coverage 6 15 20 14 5

Comparison between the input and the chip samples



Human hg19

chr1

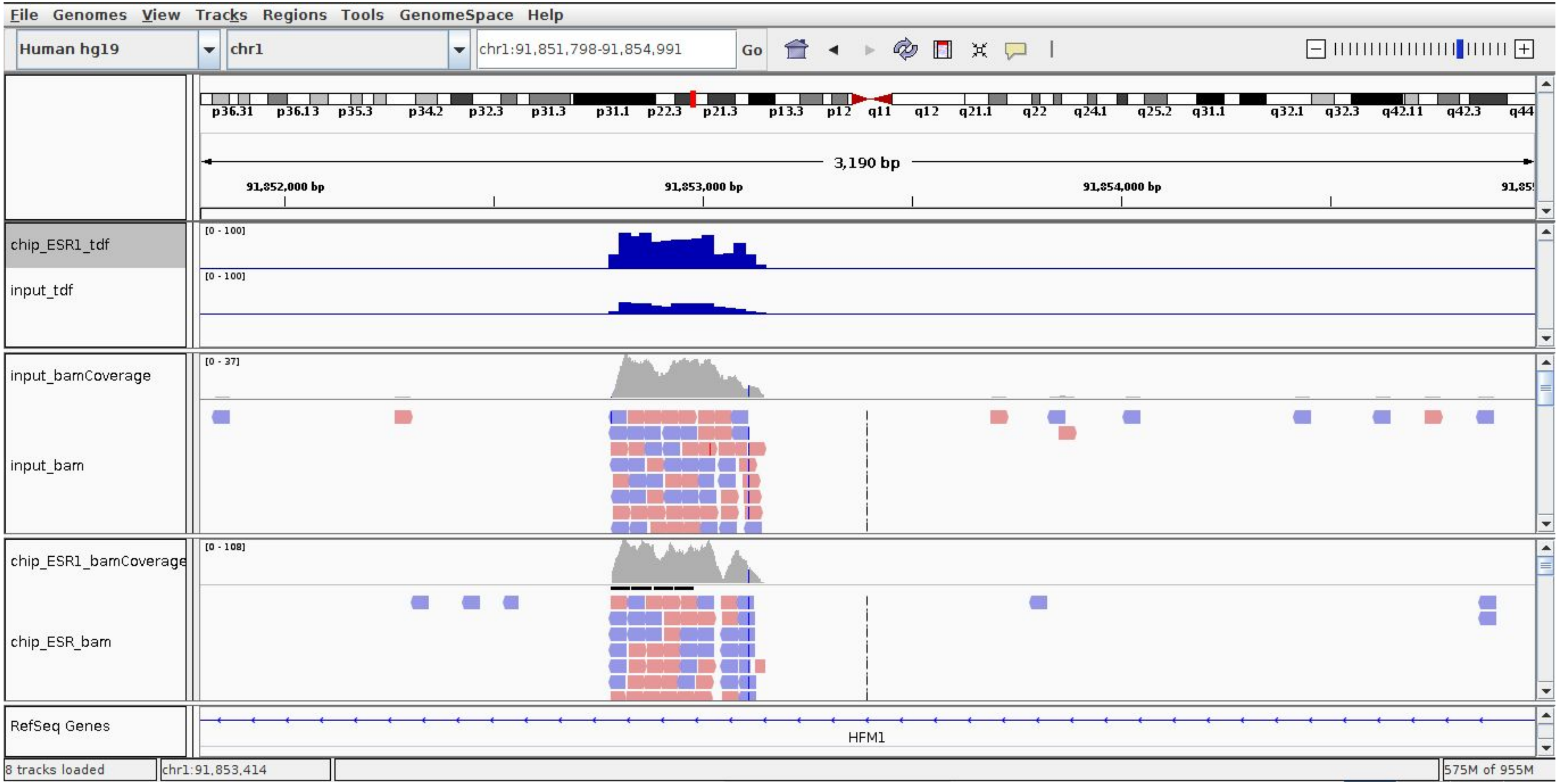
chr1:1,005,126-1,011,687

8 tracks

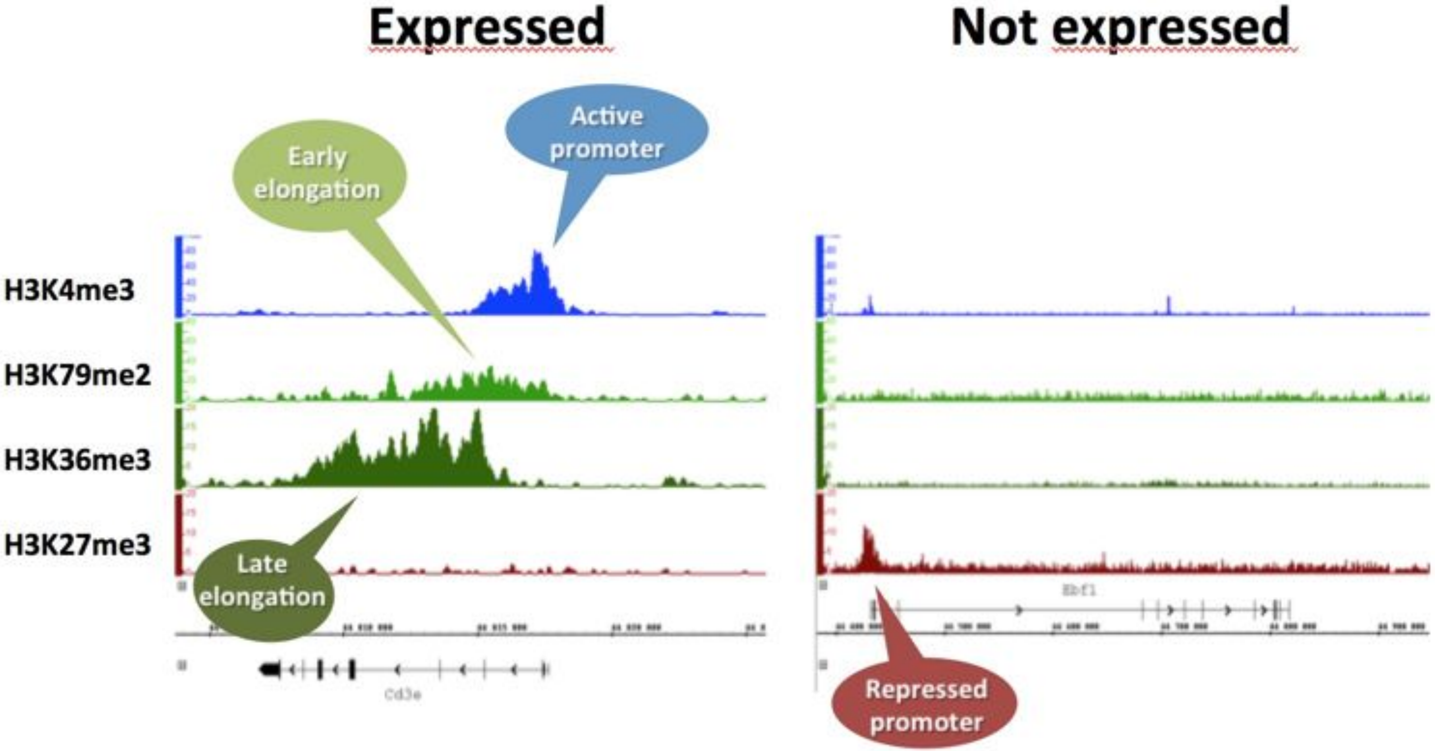
chr1:1,008,635

580M of 945M

Why we use an input…



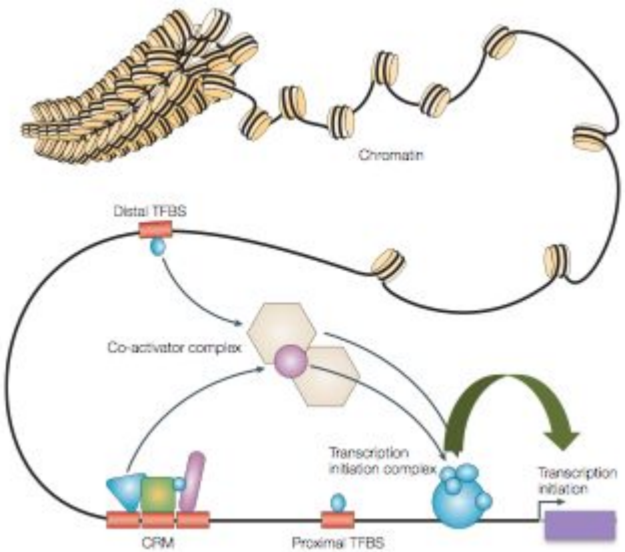
Epigenetic modifications of histones



Discovering motifs in the peaks

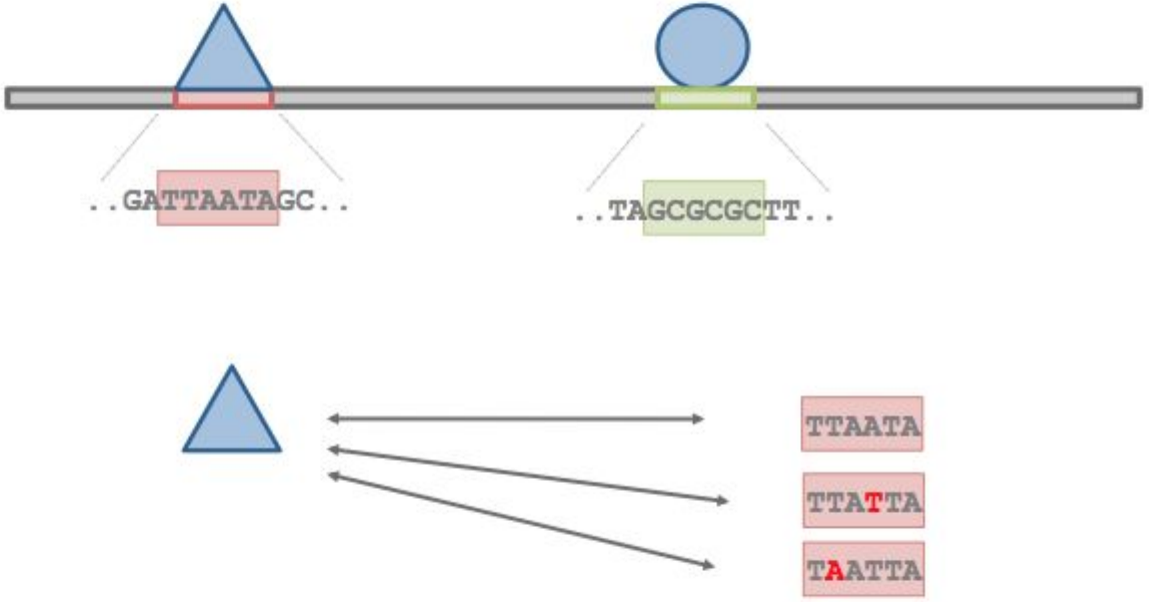
Biological concepts of transcriptional regulation

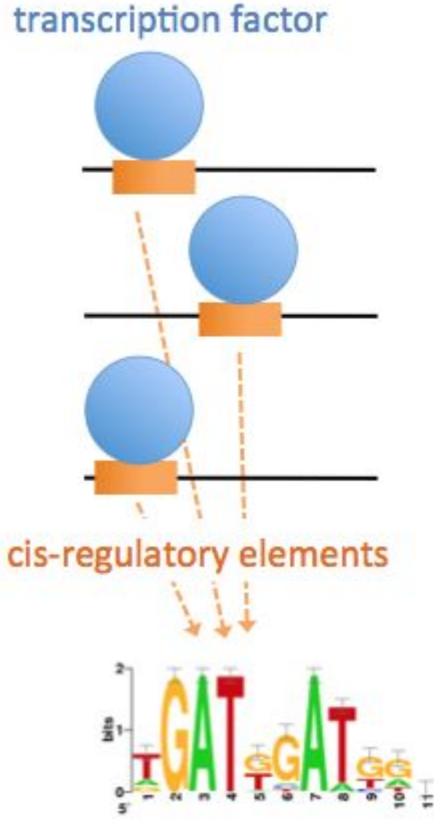
Transcription factors are proteins that modulate (activate/repress) the expression of **target genes** through the binding on **DNA cis-regulatory elements**



Wasserman et al, Nat Rev Genet, 2004

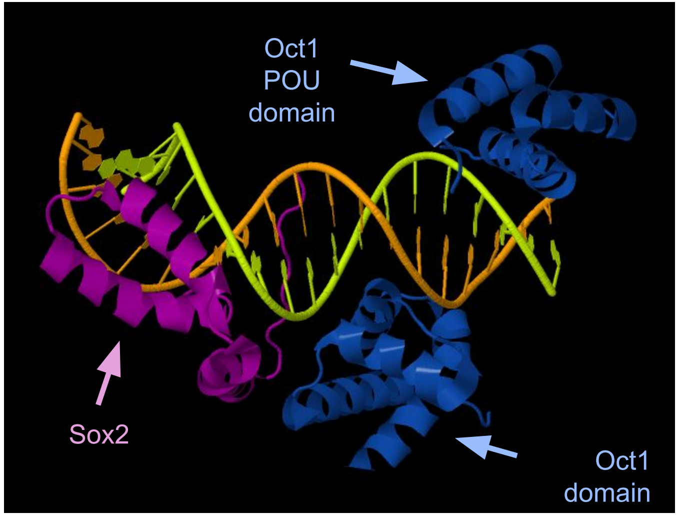
Transcription factor specificity





Sox2/Oct4 cooperative binding

- The Sox2 and Oct4 transcription factors recognize specific DNA motifs.
- Cooperative binding: Sox2 and Oct4 closely interact to bind DNA.
- The pair of transcription factors recognizes a composite motif called the « SOCT » motif (SOx+OCT).



http://www.pdb.org/pdb/explore/explore.do?structureId=1O4X

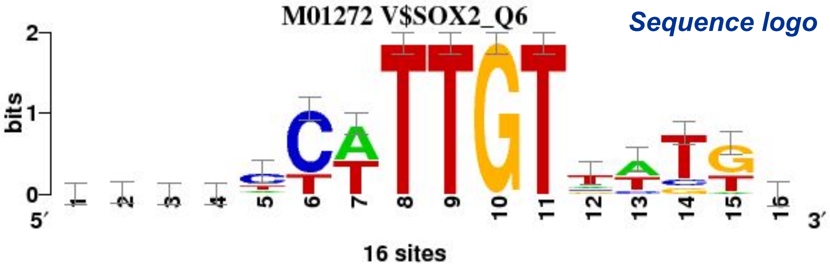
Sox2 : from binding sites to binding motif

Collection of binding sites
used to build the Sox2 matrix
(TRANSFAC M01272)

R15133	GCCCTCATTGTTATGC
R15201	AAACTCTTTGTTTGGA
R15231	TTCACCATTGTTCTAG
R15267	GACTCTATTGTCTCTG
R16367	GATATCTTTGTTTCTT
R17099	TGCACCTTTGTTATGC
R19276	AATTCCATTGTTATGA
R19367	AAACTCTTTGTTTGGA
R19510	ATGGACATTGTAATGC
R22342	AGGCCTTTTGTCCTGG
R22344	TGTGCTTTTGTNNNNN
R22359	CTCAACTTTGTAATTT
R22961	GCAGCCATTGTGATGC
R23679	CACCCCTTTGTTATGC
R25928	TTTTCTATTGTTTTTA
R27428	AAAGGCATTGTGTTTC

Position-specific scoring matrix (PSSM)

A	6	7	4	4	2	0	**8**	0	0	0	0	2	**7**	0	1	4
C	2	2	6	5	**9**	**12**	0	0	0	0	0	2	2	2	0	6
G	4	3	2	4	1	0	0	0	0	**16**	0	2	0	2	**9**	3
T	4	4	4	3	**4**	**4**	**8**	**16**	**16**	0	**16**	**9**	**6**	**11**	**5**	2



Sequence logo

“Family” binding motifs (FBM)

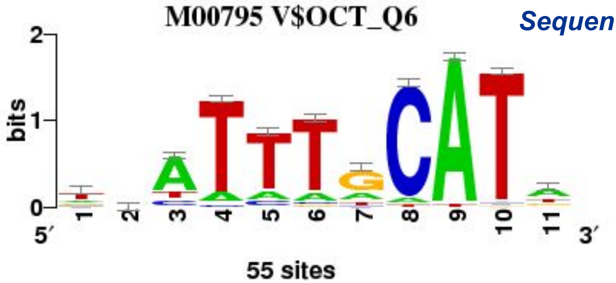
- In addition to TF-specific matrices, TRANSFAC contains matrices representing the “consensus” of the binding specificity for several transcription factors belonging to the OCT family.
- This matrix was built from 55 sites, collected from different organisms (mouse, human, cat, xenopus, ...).

Collection of binding sites *used to build the motif of the OCT* ***family*** *(TRANSFAC M00795)*

```
R00306TAATTAGCATA
R00551ATATTTGCATT
R00662TTATTTGCATA
R00664TCATTTGCATA
R00666ACATTTGCATA
R00814TCGTTAGCATG
R00815CGCATGGCATC
R00820GGAATTCCATT
R00824CGTATCTCATT
R00834TTATTTGCATA
R00842GGATTTGCATA
R00855GTATTTGCATA
R00872TAATTTGCATT
R00888CGATTTGCATA
R00893TGATTTGCATA
... 40 other sites
```

Position-specific scoring matrix (PSSM)

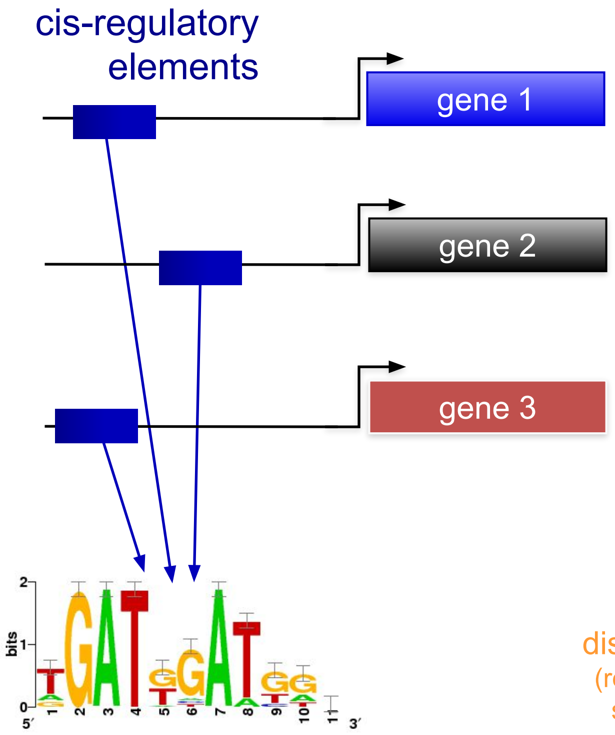
A	10	14	**37**	6	7	6	**11**	3	**53**	1	**27**
C	7	12	7	2	5	2	3	**50**	0	1	4
G	10	15	2	0	1	2	**34**	0	0	1	10
T	**28**	14	9	**47**	**42**	**45**	7	2	2	**52**	14



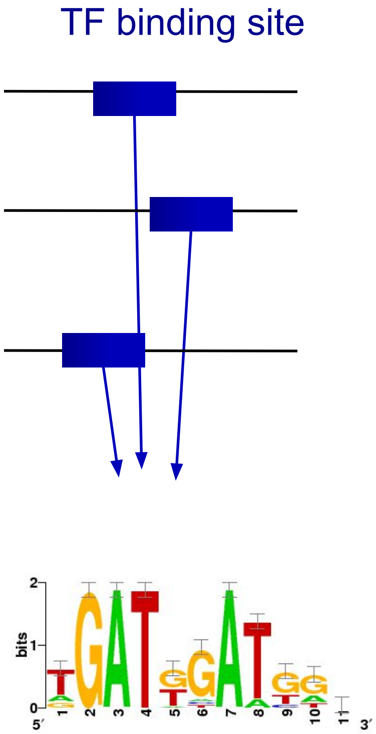
Sequence logo

De novo motif discovery

Case 1: promoters of co-expressed genes



Case 2: ChIP-seq peaks



discovered motif
(represented as a
sequence logo)

De novo motif discovery

- Find exceptional motifs based on the sequence only
- (No prior knowledge of the motif to look for)
- Criteria of exceptionality:
    - ***Over-/under-representation:*** higher/lower frequency than expected by chance
    - ***Position bias:*** concentration at specific positions relative to some reference coordinates (e.g. TSS, peak center, …).

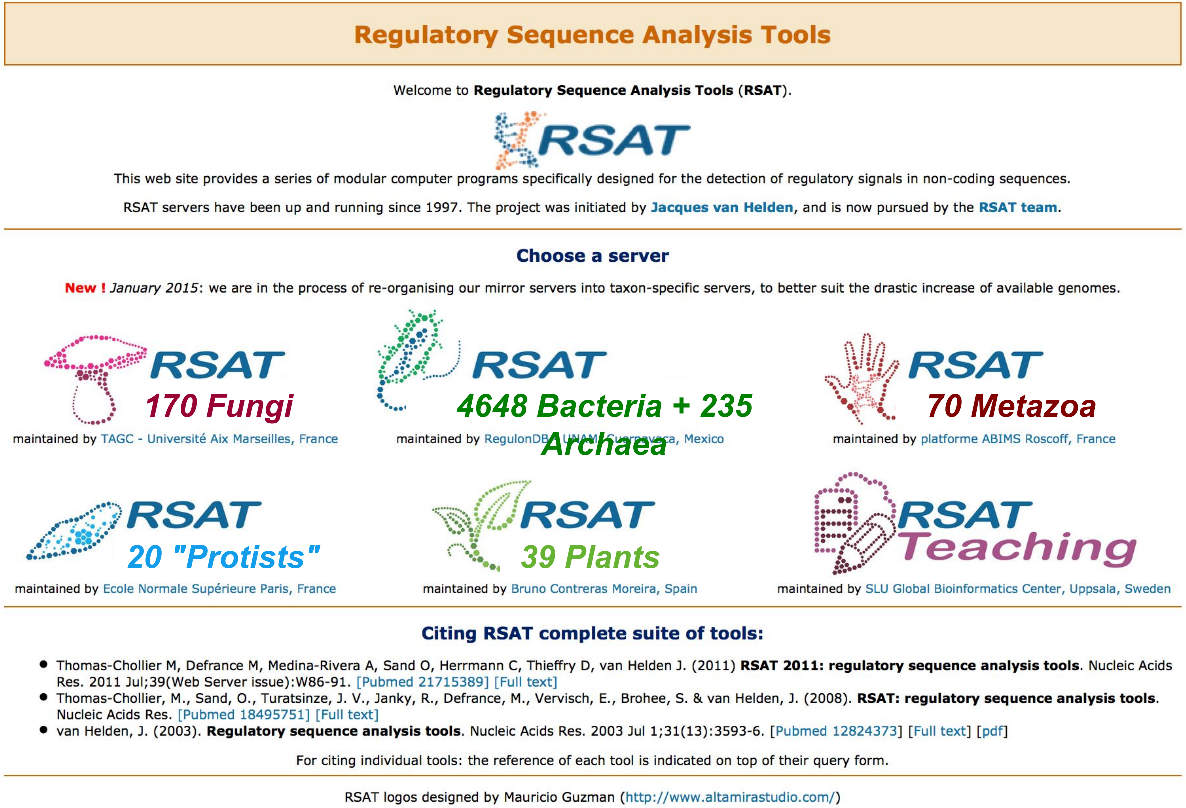
Some motif discovery tools

- MEME (Bailey et al., 1994)
- **RSAT oligo-analysis (van Helden et al., 1998)**
- AlignACE (Roth et al. 1998)
- **RSAT position-analysis (van Helden et al., 2000)**
- Weeder (Pavesi et al. 2001)
- MotifSampler (Thijs et al., 2001)
- … many others

Motif analysis on ChIP-seq peaks

- ***Motif discovery*** from peak sequences, without a priori (*"de novo" analysis*).
  - Check if the ***expected motif*** (ChIP-ped factor) can be discovered from the peaks.
    - If not, evaluate if the experiment and bioinformatics treatment was OK (e.g. functional enrichment).
  - ***Improve annotated motifs***
    - Obtain a well-documented motifs (built from thousands of sites), supposedly more reliable than "classical" motifs build from individual experiments (e.g. 10 sites from footprints and EMSA).
    - Main annotation path for recent motif database releases (JASPAR, TRANSFAC, …).
  - Discover ***partner transcription factors***.
- ***Differential motif discovery***
  - Discover differentially represented motifs between a peak set of interest (*test*) compared to another one (*control*).
- ***Peak scanning***
  - Goal: identify binding sites within the peaks.
  - Typical ChIP-seq peak: ~100 to 1000bp   Actual binding site: 6 to 10 bp.
- ***Peak enrichment*** for known motifs
  - Scan sequences to identify putative binding sites for TFs known to interact.
  - Compare observed/expected number of sites.

Regulatory sequence Analysis Tools (http://rsat.eu/)



Regulatory Sequence Analysis Tools

Welcome to **Regulatory Sequence Analysis Tools** (**RSAT**).

This web site provides a series of modular computer programs specifically designed for the detection of regulatory signals in non-coding sequences.

RSAT servers have been up and running since 1997. The project was initiated by **Jacques van Helden**, and is now pursued by the **RSAT team**.

Choose a server

New ! *January 2015*: we are in the process of re-organising our mirror servers into taxon-specific servers, to better suit the drastic increase of available genomes.

170 Fungi
maintained by TAGC - Université Aix Marseilles, France

4648 Bacteria + 235 Archaea
maintained by RegulonDB, UNAM Cuernavaca, Mexico

70 Metazoa
maintained by platforme ABIMS Roscoff, France

20 "Protists"
maintained by Ecole Normale Supérieure Paris, France

39 Plants
maintained by Bruno Contreras Moreira, Spain

RSAT Teaching
maintained by SLU Global Bioinformatics Center, Uppsala, Sweden

Citing RSAT complete suite of tools:

- Thomas-Chollier M, Defrance M, Medina-Rivera A, Sand O, Herrmann C, Thieffry D, van Helden J. (2011) **RSAT 2011: regulatory sequence analysis tools**. Nucleic Acids Res. 2011 Jul;39(Web Server issue):W86-91. [Pubmed 21715389] [Full text]
- Thomas-Chollier, M., Sand, O., Turatsinze, J. V., Janky, R., Defrance, M., Vervisch, E., Brohee, S. & van Helden, J. (2008). **RSAT: regulatory sequence analysis tools**. Nucleic Acids Res. [Pubmed 18495751] [Full text]
- van Helden, J. (2003). **Regulatory sequence analysis tools**. Nucleic Acids Res. 2003 Jul 1;31(13):3593-6. [Pubmed 12824373] [Full text] [pdf]

For citing individual tools: the reference of each tool is indicated on top of their query form.

RSAT logos designed by Mauricio Guzman (http://www.altamirastudio.com/)

Contributors From ULB

B!GRe
Bioinformatique des
Génomes et Réseaux

Sylvain Brohée
Postdoc

Nicolas Simonis
Postdoc

Didier Croes
Postddoc

Didier Gonze
Premier assistant

Myriam Loubriat
Secretary

Ariane Toussaint
Professor Emeritus

Jacques van Helden
Professor

Leon Juvenal
HajingaboE
PhD Student

Maud Vidick
PhD Student (co-direction)

Elodie Darbo
PhD Student
co-direction Marseille

Alejandra Medina
PhD Student
co-direction Mexico

Morgane
Thomas-Chollier
PhD student+postdoc

Matthieu Defrance
Postdoc

Olivier Sand
Postdoc

Jean Valéry
Turatsinze
PhD student

Collaborators

Bruno André
(ULB, Bruxelles, Belgium)

Initiation of the RSAT project.
Conception of oligo-analysis.
Analysis of yeast regulation.

ULB

Julio Collado-Vides
(CCG, Cuernavaca - Mexico)

Initiation of the RSAT project

Analysis [illegible]on in bacterial genomes

CCG Centro de Ciencias Genómicas

Bruno Contreras
(CSIC, Saragossa, Spain)

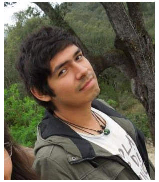
Denis Thieffry
(ENS, Paris, France)

ChIP-seq tools + regulatory networks.

Alejandra Medina-Rivera
(CCG, Cuernavaca - Mexico)

Evaluation of matrix quality.
Phylogenetic footprints in

CCG Centro de Ciencias Genómicas



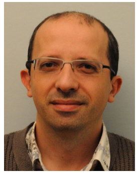
Jaime Castro-Mondragon
(PhD at TAGC, Marseille, France)

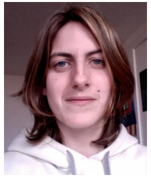
Carl Herrmann
(TAGC, Marseille, France)

ChIP-seq analysis (peak-motifs, compare-matrices).

Lionel Spinelli
(TAGC, Marseille, France)

Development of peak-footprints.





Elodie Darbo
(TAGC, Marseille, France)

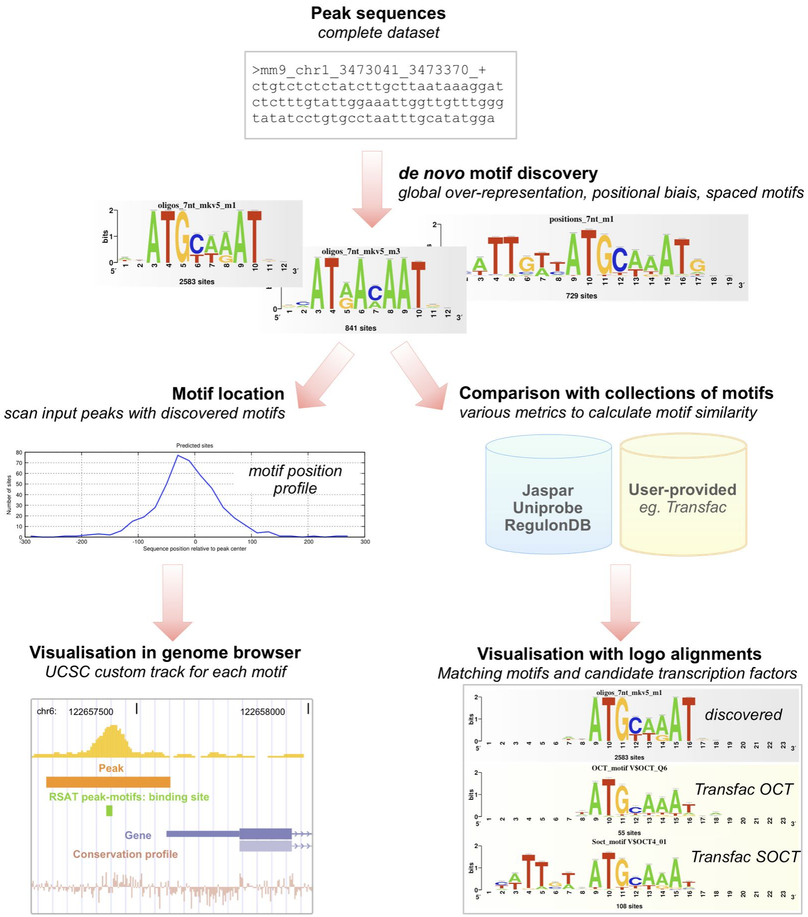
Analysis of co-expression clusters + ChIP-seq data (transcription factors, chromatin marks).

Cei Abreu-Goodger
(Sanger Institute, Hinxton, UK)

Evaluation of matrix quality on bacterial regulons.

Peak-motifs

- A workflow enabling to discover motifs in large sequence sets (tens of Mb) resulting from ChIP-seq experiments.
- ***Complementary pattern discovery criteria***
  - Global over-representation
  - Positional biases
  - Local over-representation
- Links ***from motifs to putative binding factors***
  - motif databases
  - user-specified reference motifs
- ***Prediction of binding sites*** within the peaks.
  - Inspect distribution around peak centers
  - Can be loaded as UCSC track
- ***Interfaces***
  - Web interface
  - Stand-alone (Unix command-line)
  - Web services (SOAP/WSDL)
  - Virtual Machine for VirtualBox
  - Virtual machine at the IFB cloud
  - *Soon: Debian package*
  - *Soon: Docker container*



Peak sequences
complete dataset

```
>mm9_chr1_3473041_3473370_+
ctgtctctctatcttgcttaataaaggat
ctctttgtattggaaattggttgtttggg
tatatcctgtgcctaatttgcatatgga
```

de novo **motif discovery**
global over-representation, positional biais, spaced motifs

Motif location
scan input peaks with discovered motifs

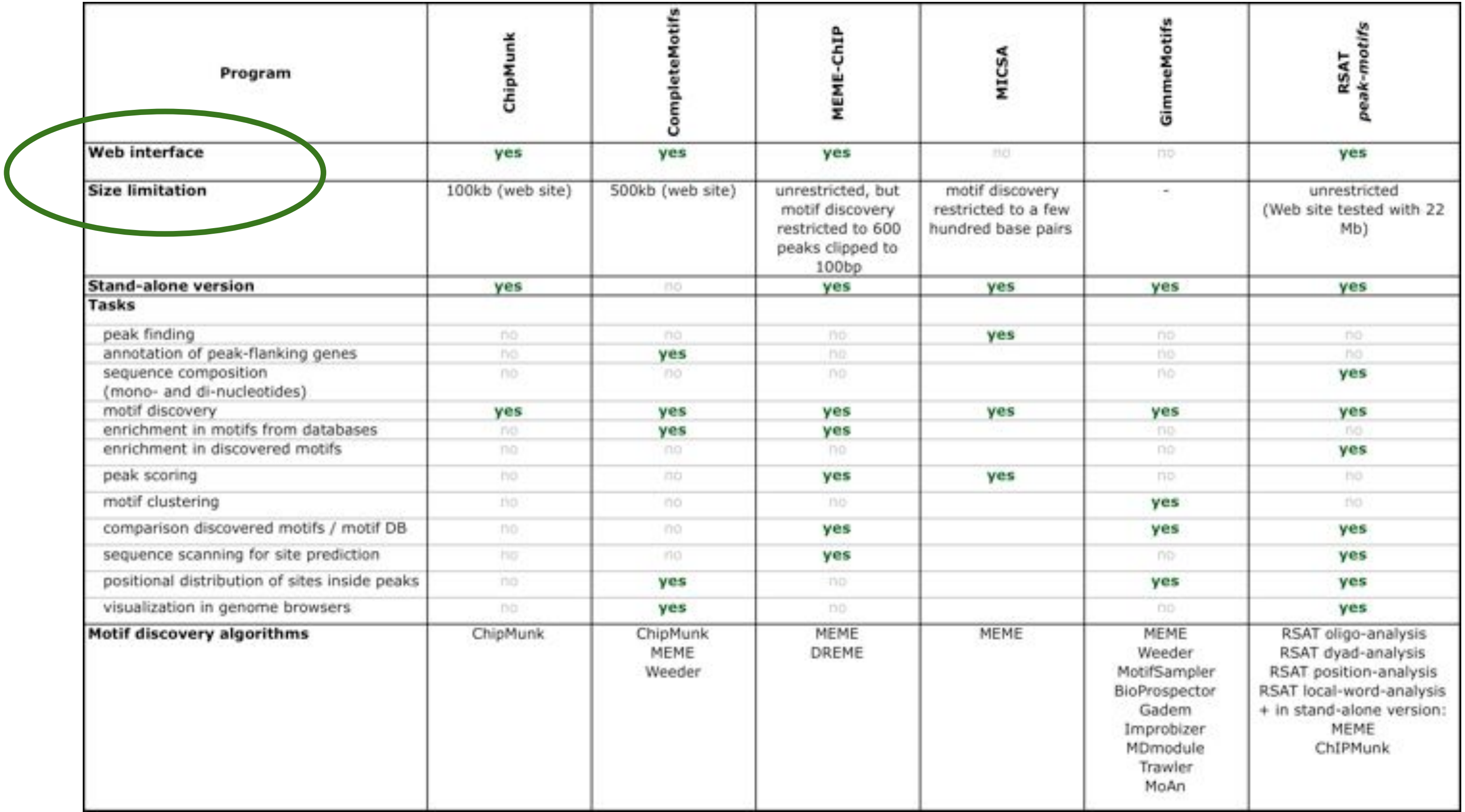
Comparison with collections of motifs
various metrics to calculate motif similarity

Visualisation in genome browser
UCSC custom track for each motif

Visualisation with logo alignments
Matching motifs and candidate transcription factors

1. Thomas-Chollier M, Herrmann C, Defrance M, Sand O, Thieffry D, van Helden J. 2012. RSAT peak-motifs: motif analysis in full-size ChIP-seq datasets. Nucleic Acids Res 40(4): e31.
2. Thomas-Chollier,M., Darbo,E., Herrmann,C., Defrance,M., Thieffry,D. and van Helden,J. (2012). A complete workflow for the analysis of full-size ChIP-seq (and similar) data sets using peak-motifs. *Nature Protocols*, **7**, 1551–1568.

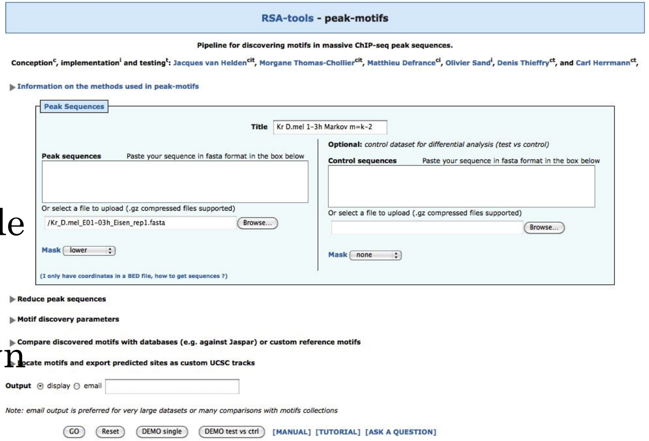
Peak-motifs: why providing yet another tool?



Program	ChipMunk	CompleteMotifs	MEME-ChIP	MICSA	GimmeMotifs	RSAT *peak-motifs*
Web interface	yes	yes	yes	no	no	yes
Size limitation	100kb (web site)	500kb (web site)	unrestricted, but motif discovery restricted to 600 peaks clipped to 100bp	motif discovery restricted to a few hundred base pairs	-	unrestricted (Web site tested with 22 Mb)
Stand-alone version	yes	no	yes	yes	yes	yes
Tasks						
peak finding	no	no	no	yes	no	no
annotation of peak-flanking genes	no	yes	no		no	no
sequence composition (mono- and di-nucleotides)	no	no	no		no	yes
motif discovery	yes	yes	yes	yes	yes	yes
enrichment in motifs from databases	no	yes	yes		no	no
enrichment in discovered motifs	no	no	no		no	yes
peak scoring	no	no	yes	yes	no	no
motif clustering	no	no	no		yes	no
comparison discovered motifs / motif DB	no	no	yes		yes	yes
sequence scanning for site prediction	no	no	yes		no	yes
positional distribution of sites inside peaks	no	yes	no		yes	yes
visualization in genome browsers	no	yes	no		no	yes
Motif discovery algorithms	ChipMunk	ChipMunk MEME Weeder	MEME DREME	MEME	MEME Weeder MotifSampler BioProspector Gadem Improbizer MDmodule Trawler MoAn	RSAT oligo-analysis RSAT dyad-analysis RSAT position-analysis RSAT local-word-analysis + in stand-alone version: MEME ChIPMunk

Peak-motifs: why providing yet another tool?

- **Fast and scalable**
- **Treat full-size datasets**
- **Complete pipeline**
  - Peak properties (nucleotide, dinucleotide composition, lengths)
  - Motif discovery
  - Comparison with known motifs
  - Peak scanning
- **Accessible to non-specialists**
  - Demo buttons
  - Tutorials & Protocols
  - Human-readable HTML report with links to all result files.



RSA-tools - peak-motifs

Pipeline for discovering motifs in massive ChIP-seq peak sequences.

Conception[c], implementation[i] and testing[t]: Jacques van Helden[cit], Morgane Thomas-Chollier[cit], Matthieu Defrance[ci], Olivier Sand[i], Denis Thieffry[ct], and Carl Herrmann[ct],

▶ Information on the methods used in peak-motifs

Peak Sequences

Title Kr D.mel 1-3h Markov m=k-2

Peak sequences Paste your sequence in fasta format in the box below

Or select a file to upload (.gz compressed files supported)

/Kr_D.mel_E01-03h_Eisen_rep1.fasta Browse...

Mask lower

(I only have coordinates in a BED file, how to get sequences ?)

Optional: *control dataset for differential analysis (test vs control)*

Control sequences Paste your sequence in fasta format in the box below

Or select a file to upload (.gz compressed files supported)

Browse...

Mask none

▶ Reduce peak sequences

▶ Motif discovery parameters

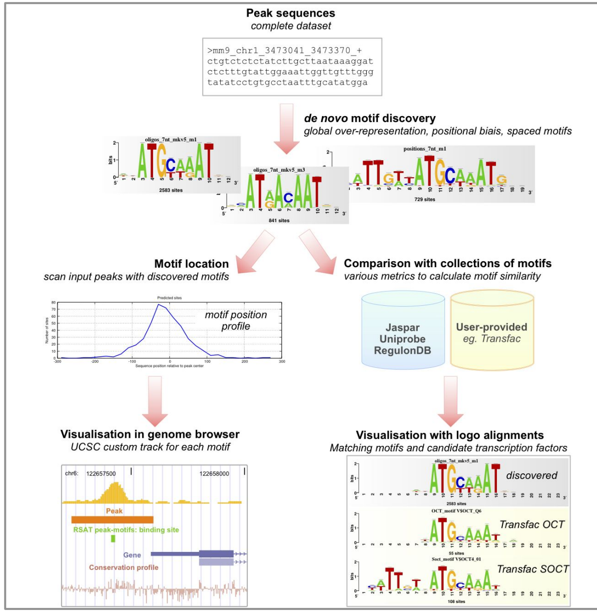
▶ Compare discovered motifs with databases (e.g. against Jaspar) or custom reference motifs

▶ Locate motifs and export predicted sites as custom UCSC tracks

Output ● display ○ email

Note: email output is preferred for very large datasets or many comparisons with motifs collections

GO · Reset · DEMO single · DEMO test vs ctrl · [MANUAL] [TUTORIAL] [ASK A QUESTION]



Peak sequences
complete dataset

```
>mm9_chr1_3473041_3473370_+
ctgtctctctatcttgcttaataaaggat
ctctttgtattggaaattggttgtttggg
tatatcctgtgcctaatttgcatatgga
```

***de novo* motif discovery**
global over-representation, positional biais, spaced motifs

Motif location
scan input peaks with discovered motifs

Comparison with collections of motifs
various metrics to calculate motif similarity

Visualisation in genome browser
UCSC custom track for each motif

Visualisation with logo alignments
Matching motifs and candidate transcription factors

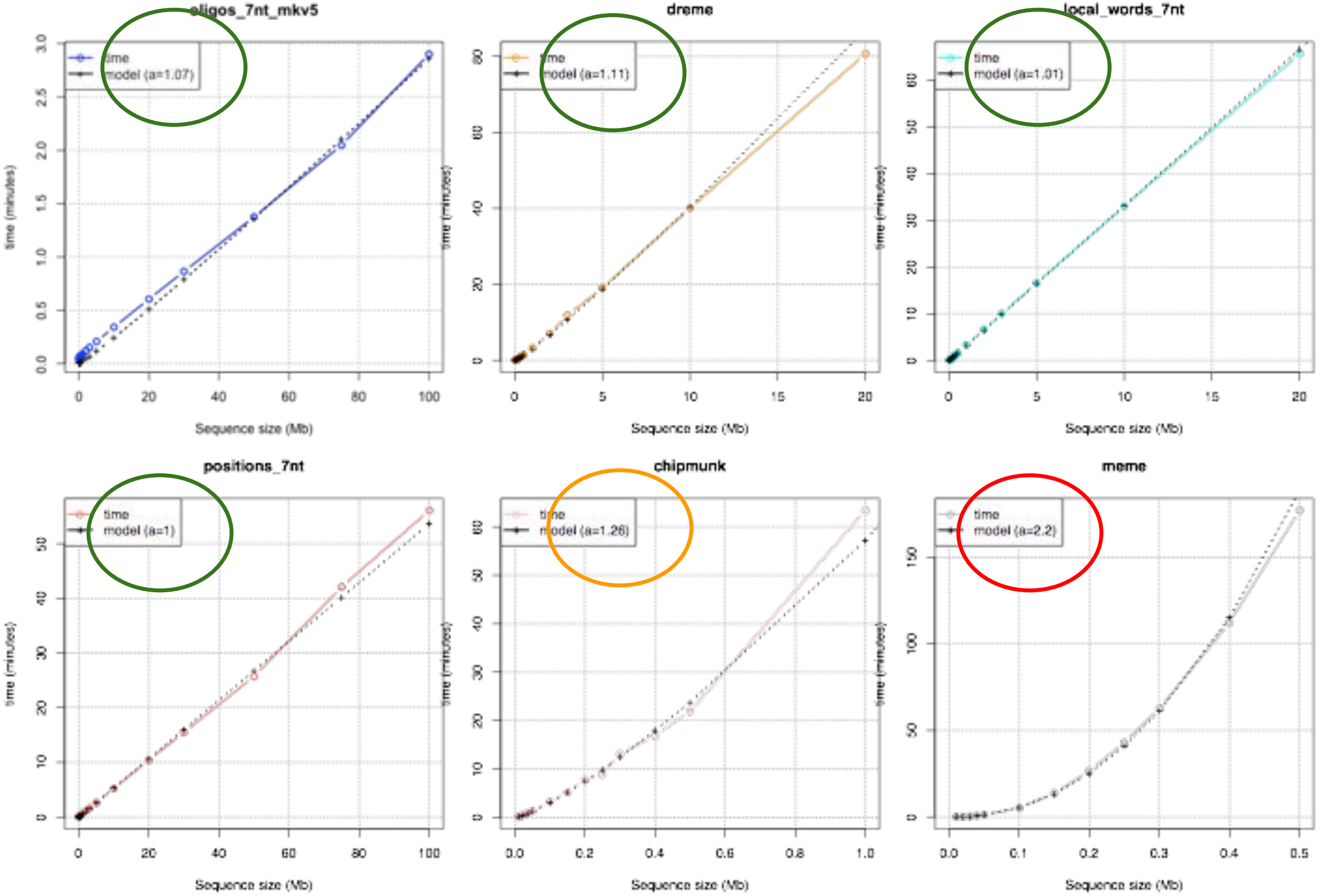
Thomas-Chollier, Herrmann, Defrance, Sand, Thieffry, van Helden **Nucleic Acids Research**, 2012

Time complexity of motif discovery algorithms

Linear

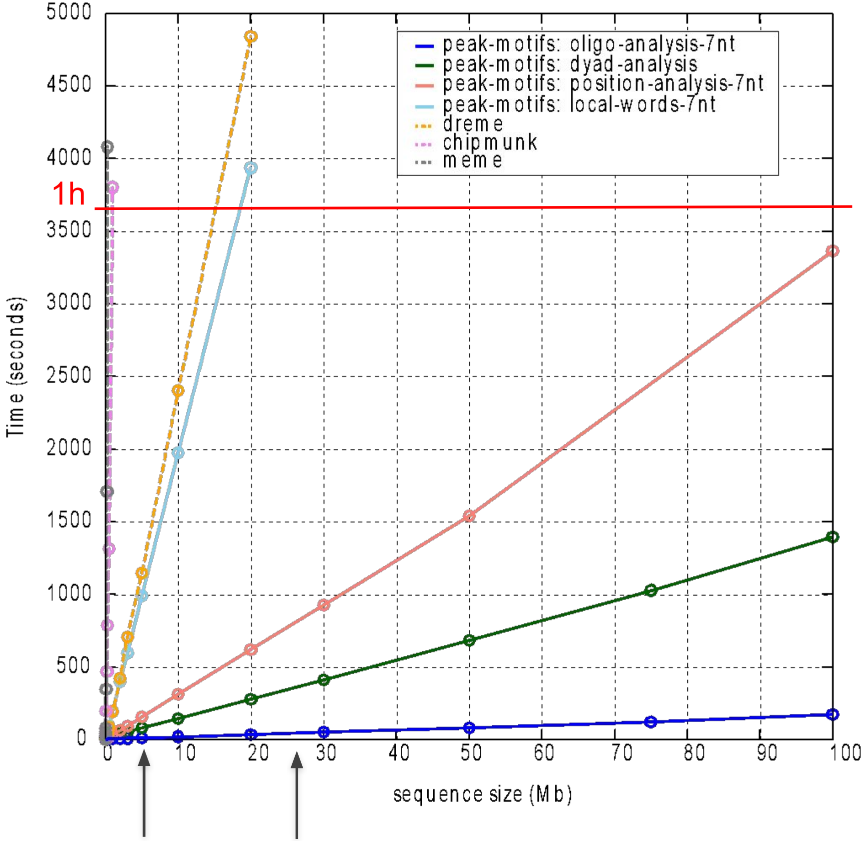
> linear

> quadratic



Peak-motifs: scalability

- **Fast and scalable**
- **Treat full-size datasets**
- **Using 4 complementary algorithms**
  - **Global over-representation**
    - **oligo-analysis**
    - **dyad-analysis (spaced motifs)**
  - **Positional bias**
    - **position-analysis**
    - **local-words**

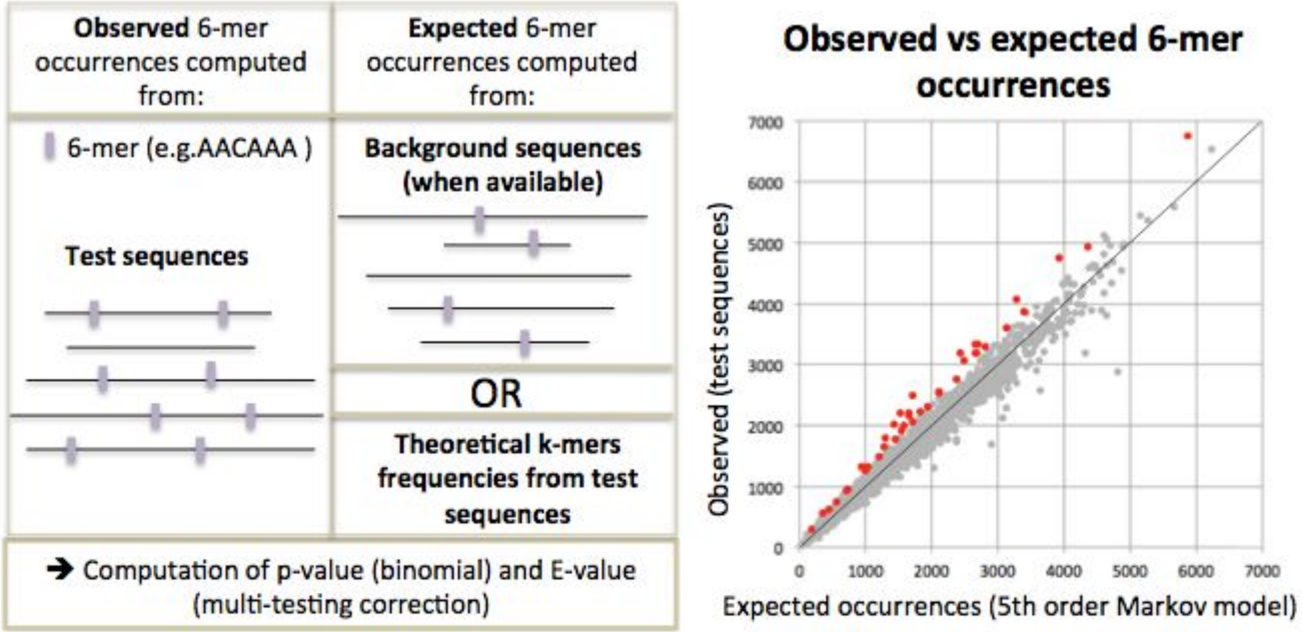


size limit of other websites

typical ChIP-seq dataset

Thomas-Chollier, Herrmann, Defrance, Sand, Thieffry, van Helden **Nucleic Acids Research**, 2012

Motif discovery: k-mer over-representation



Observed 6-mer occurrences computed from:

▮ 6-mer (e.g.AACAAA)

Test sequences

Expected 6-mer occurrences computed from:

Background sequences (when available)

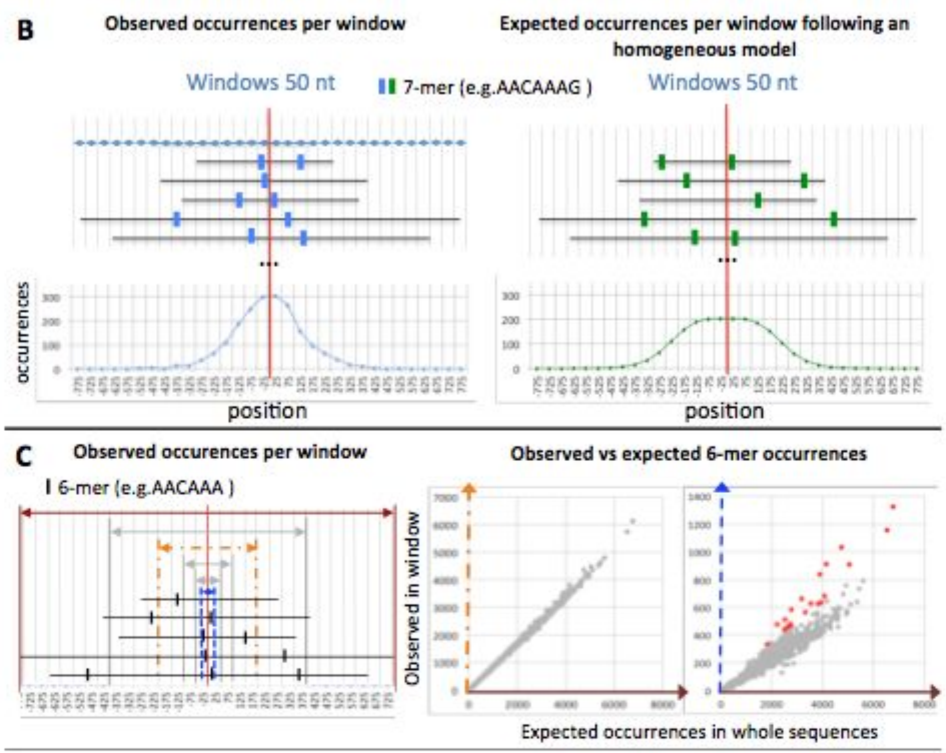
OR

Theoretical k-mers frequencies from test sequences

➔ Computation of p-value (binomial) and E-value (multi-testing correction)

Observed vs expected 6-mer occurrences

Motif discovery: k-mer position biases

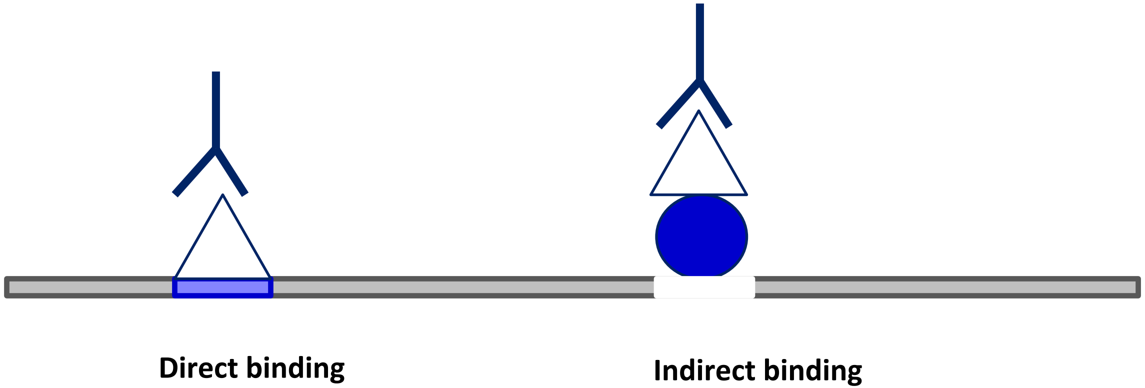


position-analysis

local-words

Direct versus indirect binding

- ChIP-seq does not necessarily reveal **direct binding:** The motif of the targeted TF is not always found in peaks!

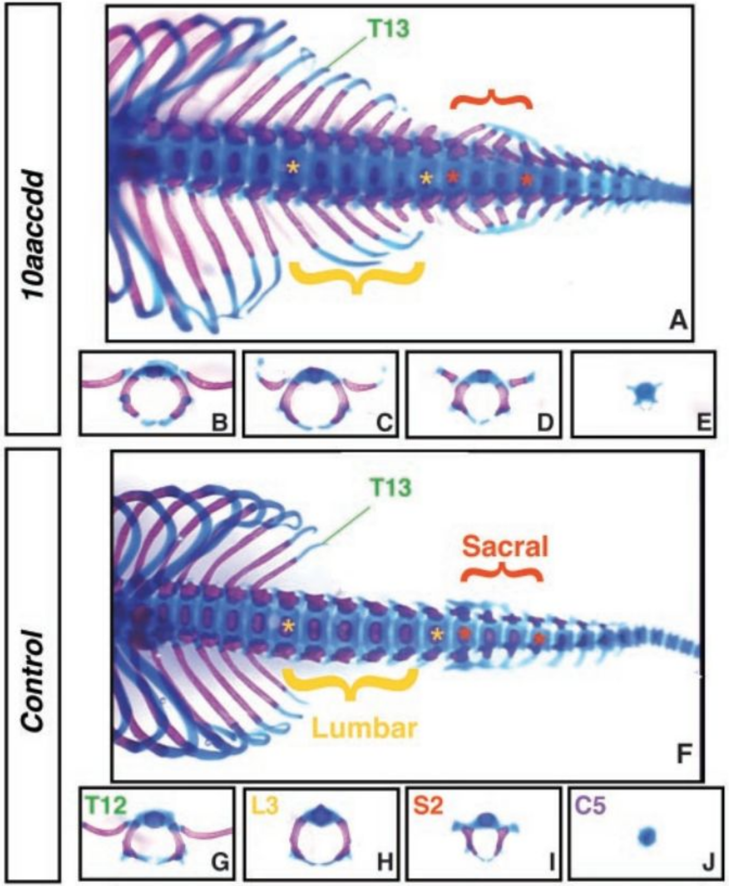


Negative Controls

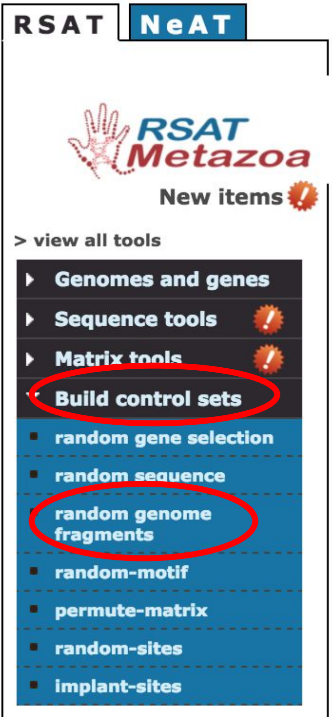
Negative Controls in biology

One example from a multitude: Wellik and Mario R Capecchi, Science, 2003.

Fig. 1. Axial skeletons of *Hox10* and *Hox11* triple mutants at embryonic day 18.5 (E18.5). Ventral views of the axial skeleton from the lower thoracic region through the early caudal region of a *Hox10* triple mutant (**A**), a control (**F**), and a *Hox11* triple mutant (**K**) are shown. Yellow asterisks indicate lumbar vertebrae; red asterisks indicate sacral vertebrae. A five-allele mutant from the *Hox10* and *Hox11* paralogous mutant group is shown in (**P**) and (**Q**), respectively (red arrows indicate sacral wing formation). Analogous vertebrae were dissected from the control and from each triple mutant to compare single vertebral identities. The 19th vertebral element, normally T12, is shown in (**B**), (**G**), and (**L**). The 23rd element, normally L3, is shown in (**C**), (**H**), and (**M**). The 28th element, normally S2, is



Negative and positive controls in bioinformatics



RSAT NeAT

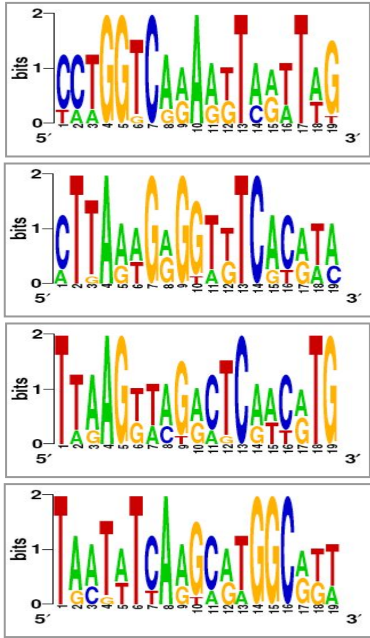
RSAT Metazoa

New items

> view all tools

- Genomes and genes
- Sequence tools
- Matrix tools
- Build control sets
  - random gene selection
  - random sequence
  - random genome fragments
  - random-motif
  - permute-matrix
  - random-sites
  - implant-sites

- **Negative control**: quantify the capability of the program to return a negative answer when there are no regulatory elements.
  - Artificial sequences
    - RSAT ***random-sequences*** (Markov models to mimic k-mer frequencies of the organism)
  - Biological sequences without common regulation
    - RSAT ***random-genes*** (negative control for expression clusters)
    - RSAT ***random-genome-fragments*** (negative controls for ChIP-seq)
  - Randomized motifs: column permutations preserve nucleotide frequencies and information content
    - RSAT ***permute-matrix***
- **Positive control**: quantify the capability of the program to detect known regulatory elements
  - Annotated sites (e.g. sites from TRANSFAC) in their original context (promoter sequences).
  - Annotated sites implanted in other context
    - Biological sequences (random selection).
    - Artificial sequences.
  - Artificial sites implanted in artificial sequences.
    - RSAT ***random-motif***
    - RSAT ***random-sites***
    - RSAT ***implant-sites***

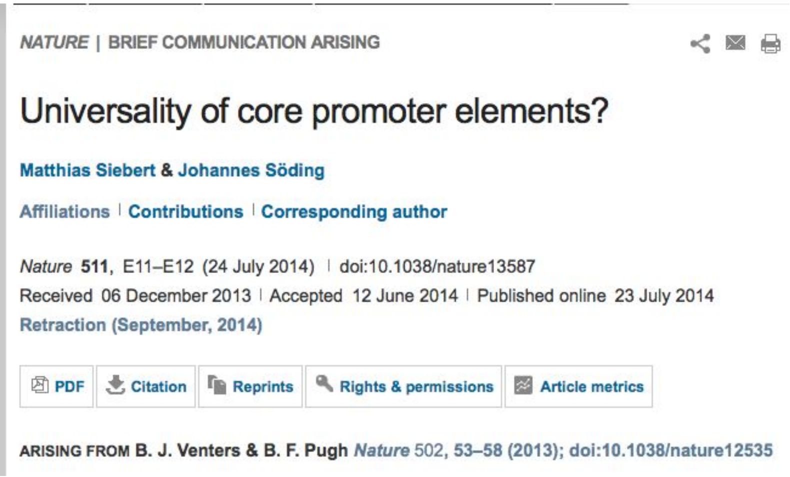


RSAT random-genome-fragments

- Select a set of fragments with random positions in a given genome, and return their coordinates and/or sequences
- Adapted to chip-seq ?
  - Yes: same number of peaks + same size
  - No: composition of the sequences (nucleotides, k-mers) may change depends on genomic regions
  -
- Complexify the control
  - Make sure no peak is covered
  - Take regions close / far from the peaks
  - Maintain same composition
  - Maintain same dataset size
  - …

Why is it important ?

To prevent this ….



NATURE | BRIEF COMMUNICATION ARISING

Universality of core promoter elements?

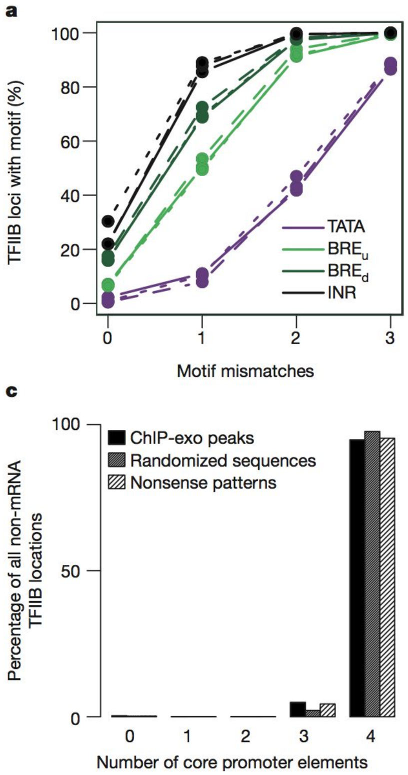
Matthias Siebert & Johannes Söding

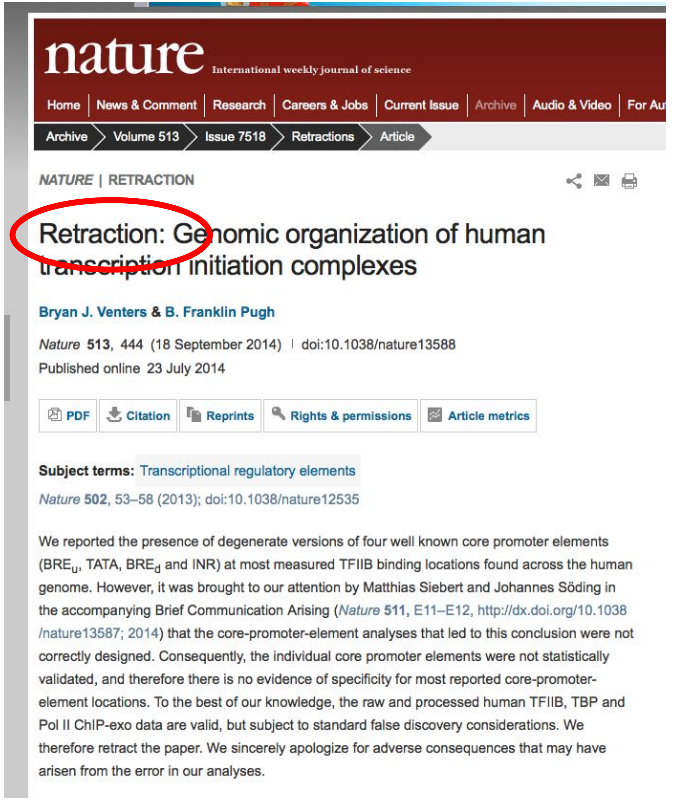
Affiliations | Contributions | Corresponding author

Nature **511**, E11–E12 (24 July 2014) | doi:10.1038/nature13587
Received 06 December 2013 | Accepted 12 June 2014 | Published online 23 July 2014
Retraction (September, 2014)

ARISING FROM B. J. Venters & B. F. Pugh *Nature* 502, 53–58 (2013); doi:10.1038/nature12535

We show that the claimed universality of CPEs is explained by the low specificities of the patterns used and that the same match frequencies are obtained with two negative controls (randomized sequences and scrambled patterns).
Our analyses also cast doubt on the biological significance of most of the 150,753 non-messenger-RNA-associated ChIP-exo peaks, 72% of which lie within repetitive regions.





NATURE | RETRACTION

Retraction: Genomic organization of human transcription initiation complexes

Bryan J. Venters & B. Franklin Pugh

Nature **513**, 444 (18 September 2014) | doi:10.1038/nature13588
Published online 23 July 2014

Subject terms: Transcriptional regulatory elements

Nature **502**, 53–58 (2013); doi:10.1038/nature12535

We reported the presence of degenerate versions of four well known core promoter elements (BRE$_u$, TATA, BRE$_d$ and INR) at most measured TFIIB binding locations found across the human genome. However, it was brought to our attention by Matthias Siebert and Johannes Söding in the accompanying Brief Communication Arising (*Nature* **511**, E11–E12, http://dx.doi.org/10.1038/nature13587; 2014) that the core-promoter-element analyses that led to this conclusion were not correctly designed. Consequently, the individual core promoter elements were not statistically validated, and therefore there is no evidence of specificity for most reported core-promoter-element locations. To the best of our knowledge, the raw and processed human TFIIB, TBP and Pol II ChIP-exo data are valid, but subject to standard false discovery considerations. We therefore retract the paper. We sincerely apologize for adverse consequences that may have arisen from the error in our analyses.

Supplementary information

To go further

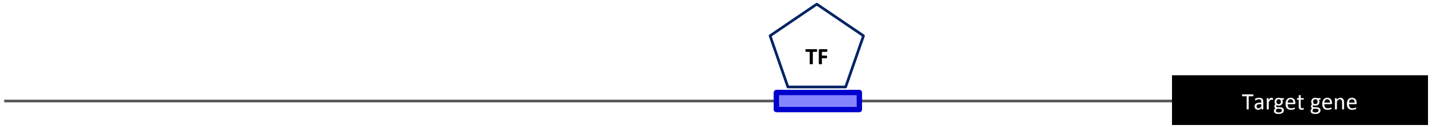
- The next slides explain step by step the algorithm behind oligo-analysis
- Peak-motifs : follow this protocol to grasp the detailed tweaking of parameters (send us an email to have free access to the PDF if necessary)
    - Thomas-Chollier et al. A complete workflow for the analysis of full-size ChIP-seq (and similar) data sets using peak-motifs. Nature Protocols 7, 1551–1568 (2012).
- Description and evaluation of peak-motifs
    - Matrix-quality : RSAT program that can be used to evaluate the enrichment of motifs in peaks
- Description of the RSAT software suite
    - Medina-Rivera A, Abreu-Goodger C, Thomas-Chollier M, Salgado H, Collado-Vides J, van Helden J.Theoretical and empirical quality assessment of transcription factor-binding motifs.Nucleic Acids Res. 2011 Feb;39(3):808-24. doi: 10.1093/nar/gkq710. Epub 2010 Oct 4.
- Tutorial for ECCB 2014 : http://rsat.ulb.ac.be/eccb14/

More info: RSAT descriptions + protocols

1. Medina-Rivera,A., Defrance,M., Sand,O., Herrmann,C., Castro-Mondragon,J.A., Delerce,J., Jaeger,S., Blanchet,C., Vincens,P., Caron,C., et al. (2015) RSAT 2015: Regulatory Sequence Analysis Tools. Nucleic Acids Res, 43, W50–6.
2. Thomas-Chollier,M., Darbo,E., Herrmann,C., Defrance,M., Thieffry,D. and van Helden,J. (2012) A complete workflow for the analysis of full-size ChIP-seq (and similar) data sets using peak-motifs. Nature Protocols, 7, 1551–1568.
3. Thomas-Chollier,M., Herrmann,C., Defrance,M., Sand,O., Thieffry,D. and van Helden,J. (2012) RSAT peak-motifs: motif analysis in full-size ChIP-seq datasets. Nucleic Acids Res, 40, e31–e31.
4. Thomas-Chollier,M., Defrance,M., Medina-Rivera,A., Sand,O., Herrmann,C., Thieffry,D. and van Helden,J. (2011) RSAT 2011: regulatory sequence analysis tools. Nucleic Acids Res, 39, W86–91.
5. Thomas-Chollier,M., Sand,O., Turatsinze,J.-V., Janky,R., Defrance,M., Vervisch,E., Brohée,S. and van Helden,J. (2008) RSAT: regulatory sequence analysis tools. Nucleic Acids Res, 36, W119–27.
6. Sand,O., Thomas-Chollier,M., Vervisch,E. and van Helden,J. (2008) Analyzing multiple data sets by interconnecting RSAT programs via SOAP Web services: an example with ChIP-chip data. Nature Protocols, 3, 1604–1615.
7. Turatsinze,J.-V., Thomas-Chollier,M., Defrance,M. and van Helden,J. (2008) Using RSAT to scan genome sequences for transcription factor binding sites and cis-regulatory modules. Nature Protocols, 3, 1578–1588.
8. Defrance,M., Janky,R., Sand,O. and van Helden,J. (2008) Using RSAT oligo-analysis and dyad-analysis tools to discover regulatory signals in nucleic sequences. Nature Protocols, 3, 1589–1603.

Principle: detect unexpected patterns

- Binding sites are represented as “words” = “oligonucleotides”=“k-mer”
  - e.g. **acgtga** is a 6-mer
- Signal is likely to be more frequent in the upstream regions of the co-regulated genes than in a random selection of genes
- We will thus detect over-represented words (k-mers, oligonucleotides).



5’- TCTCTCTCCACGGCTAATTAGGTGATCATGAAAAAATGAAAAATTCATGAG**AAAAGAGTCA**GACATCGAAACATACAT ***...HIS7***

5’- ATGGCAGAATCACTTTAAAACGTGGCCCCACCCGCTGCACCCTGTGCATTTTGTACGTTACTGCG**AAATGACTCA**ACG ***...ARO4***

5’- CACATCCAACGAATCACCTCACCGTTATCG**TGACTCACTT**TCTTTCGCATCGCCGAAGTGCCATAAAAAATATTTTTT ***...ILV6***

5’- TGCGAAC**AAAAGAGTCA**TTACAACGAGGAAATAGAAGAAAATGAAAAATTTTCGACAAAATGTATAGTCATTTCTATC ***...THR4***

5’- ACAAAGGTACCTTCCTGGCCAATCTCACAGATTTAATATAGTAAATTGTCATGCATA**TGACTCATCC**CGAACATGAAA ***...ARO1***

5’- ATTGAT**TGACTCATTT**TCCTCTGACTACTACCAGTTCAAAATGTTAGAGAAAAATAGAAAAGCAGAAAAAATAAATAA ***...HOM2***

5’- GGCGCCACAGTCCGCGTTTGGTTATCCGGC**TGACTCATTCTGACTCTTTT**TTGGAAAGTGTGGCATGTGCTTCACACA ***...PRO3***

Motif discovery using word counting

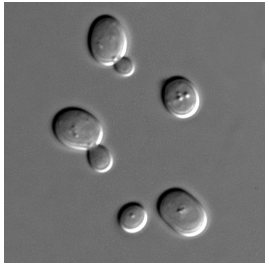
Idea:

motifs corresponding to binding sites are generally repeated in the dataset
→ capture this statistical signal

- **Algorithm**
  - count occurrences of **all k-mers** in a set of related sequences (promoters of co-expressed genes, in ChIP bound regions,...)

Let's take an example (yeast Saccharomyces cerevisiae)

- NIT
  - 7 genes expressed under low nitrogen conditions
- MET
  - 10 genes expressed in absence of methionine
- PHO
  - 5 genes expressed under phosphate stress



		PHO
aaaaaa	tttttt	51
aaaaag	cttttt	15
aagaaa	tttctt	14
gaaaaa	tttttc	13
tgccaa	ttggca	12
aaaaat	attttt	12
aaatta	taattt	12
agaaaa	ttttct	11
caagaa	ttcttg	11
aaacgt	acgttt	11
aaagaa	ttcttt	11
acgtgc	**gcacgt**	**10**
aataat	attatt	10
aagaag	cttctt	10
atataa	ttatat	10

		MET
aaaaaa	tttttt	105
atatat	atatat	41
gaaaaa	tttttc	40
tatata	tatata	40
aaaaat	attttt	35
aagaaa	tttctt	29
agaaaa	ttttct	28
aaaata	tatttt	26
aaaaag	cttttt	25
agaaat	atttct	24
aaataa	ttattt	22
taaaaa	ttttta	21
tgaaaa	ttttca	21
ataata	tattat	20
atataa	ttatat	20

		NIT
aaaaaa	tttttt	80
cttatc	**gataag**	**26**
tatata	tatata	22
ataaga	tcttat	20
aagaaa	tttctt	20
gaaaaa	tttttc	19
atatat	atatat	19
agataa	ttatct	17
agaaaa	ttttct	17
aaagaa	ttcttt	16
aaaaca	tgtttt	16
aaaaag	cttttt	15
agaaga	tcttct	14
tgataa	ttatca	14
atataa	ttatat	14

The most frequent oligonucleotides are not informative

- A (too) simple approach would consist in **detecting the most frequent oligonucleotides** (for example hexanucleotides) for each group of upstream sequences.
- This would however lead to deceiving results.
    - In all the sequence sets, the same kind of patterns are selected: **AT-rich hexanucleotides**.

		PHO
aaaaaa	tttttt	51
aaaaag	cttttt	15
aagaaa	tttctt	14
gaaaaa	tttttc	13
tgccaa	ttggca	12
aaaaat	attttt	12
aaatta	taattt	12
agaaaa	ttttct	11
caagaa	ttcttg	11
aaacgt	acgttt	11
aaagaa	ttcttt	11
acgtgc	gcacgt	10
aataat	attatt	10
aagaag	cttctt	10
atataa	ttatat	10

		MET
aaaaaa	tttttt	105
atatat	atatat	41
gaaaaa	tttttc	40
tatata	tatata	40
aaaaat	attttt	35
aagaaa	tttctt	29
agaaaa	ttttct	28
aaaata	tatttt	26
aaaaag	cttttt	25
agaaat	atttct	24
aaataa	ttattt	22
taaaaa	tttttta	21
tgaaaa	ttttca	21
ataata	tattat	20
atataa	ttatat	20

		NIT
aaaaaa	tttttt	80
cttatc	gataag	26
tatata	tatata	22
ataaga	tcttat	20
aagaaa	tttctt	20
gaaaaa	tttttc	19
atatat	atatat	19
agataa	ttatct	17
agaaaa	ttttct	17
aaagaa	ttcttt	16
aaaaca	tgtttt	16
aaaaag	cttttt	15
agaaga	tcttct	14
tgataa	ttatca	14
atataa	ttatat	14

A more relevant criterion for over-representation

- The most frequent patterns do not reveal the motifs specifically bound by specific transcription factors.

- They merely **reflect the compositional biases** of upstream sequences.

- A more relevant criterion for over-representation is to detect patterns which **are more frequent** in the upstream sequences of the selected genes (co-regulated) **than the random expectation**.

- The **random expectation** is calculated by counting the frequency of each pattern in the complete set of upstream sequences (all genes of the genome).
  => **“Background”**

Motif discovery using word counting

Idea:

motifs corresponding to binding sites are generally repeated in the dataset

→ capture this statistical signal

- theoretical background model (Markov Models)

Estimation of word expected frequencies from background sequences

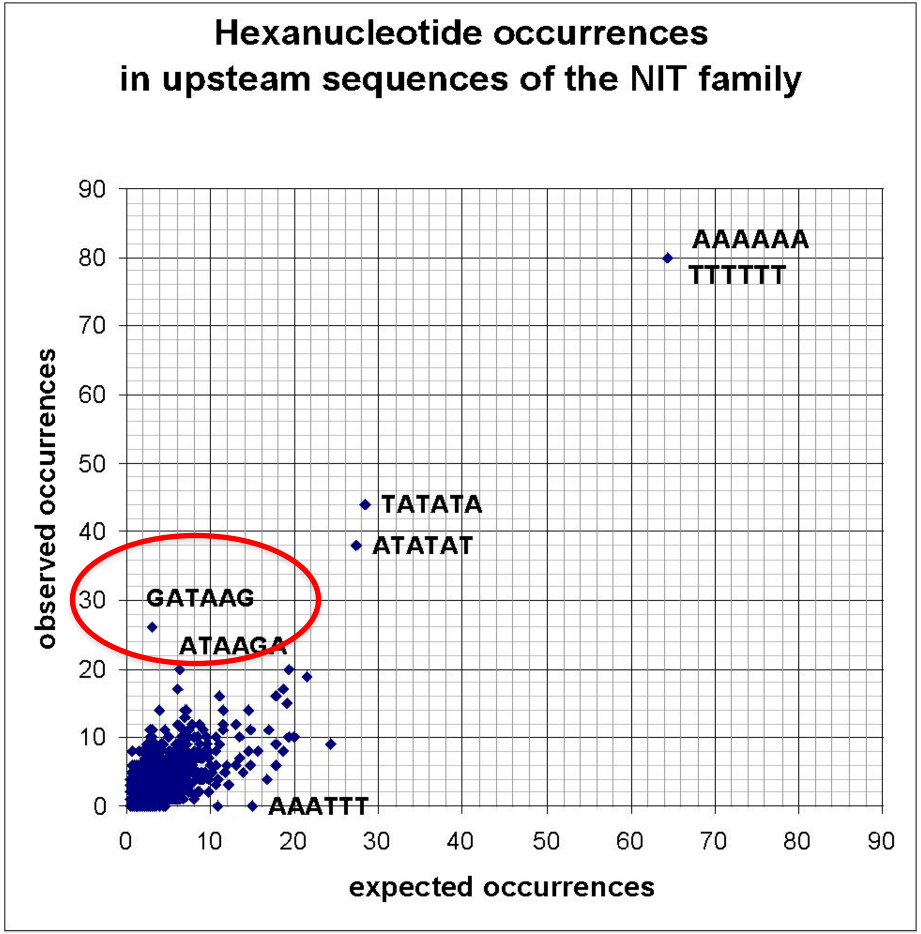
Example:

6nt frequencies in the whole set of 6000 yeast **upstream** sequences

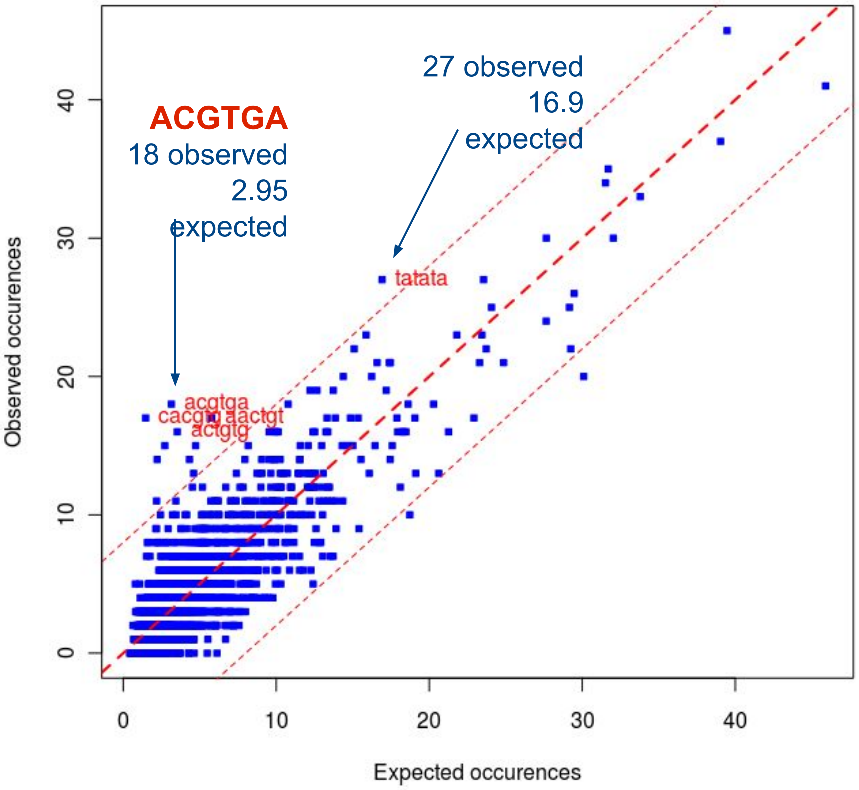
;seq	identifier	observed_freq	occ
aaaaaa	aaaaaa|tttttt	0,00510699	14555
aaaaac	aaaaac|gtttt	0,00207402	5911
aaaaag	aaaaag|ctttt	0,00375191	10693
aaaaat	aaaaat|atttt	0,00423577	12072
aaaaca	aaaaca|tgttt	0,0019828	5651
aaaacc	aaaacc|ggttt	0,00088526	2523
aaaacg	aaaacg|cgttt	0,00090105	2568
aaaact	aaaact|agttt	0,0014621	4167
aaaaga	aaaaga|tcttt	0,00323016	9206
aaaagc	aaaagc|gcttt	0,00135824	3871
aaaagg	aaaagg|ccttt	0,0017849	5087
aaaagt	aaaagt|acttt	0,0019035	5425
aaaata	aaaata|tattt	0,00336805	9599
aaaatc	aaaatc|gattt	0,00131368	3744
aaaatg	aaaatg|cattt	0,00185648	5291
aaaatt	aaaatt|aattt	0,00269156	7671
aaacaa	aaacaa|ttgtt	0,00209999	5985
aaacac	aaacac|gtgtt	0,00071684	2043
aaacag	aaacag|ctgtt	0,00096491	2750
aaacat	aaacat|atgtt	0,00108982	3106
aaacca	aaacca|tggtt	0,00074421	2121

Hexanucleotide occurrences in yeast Nitrogen-responding genes

	NIT
aaaaaa|tttttt	80
cttatc|gataag	26
tatata|tatata	22
ataaga|tcttat	20
aagaaa|tttctt	20
gaaaaa|tttttc	19
atatat|atatat	19
agataa|ttatct	17
agaaaa|ttttct	17
aaagaa|ttcttt	16
aaaaca|tgtttt	16
aaaaag|cttttt	15
agaaga|tcttct	14
tgataa|ttatca	14
atataa|ttatat	14



Motif discovery using word counting



How to evaluate expected number of occurrences ?

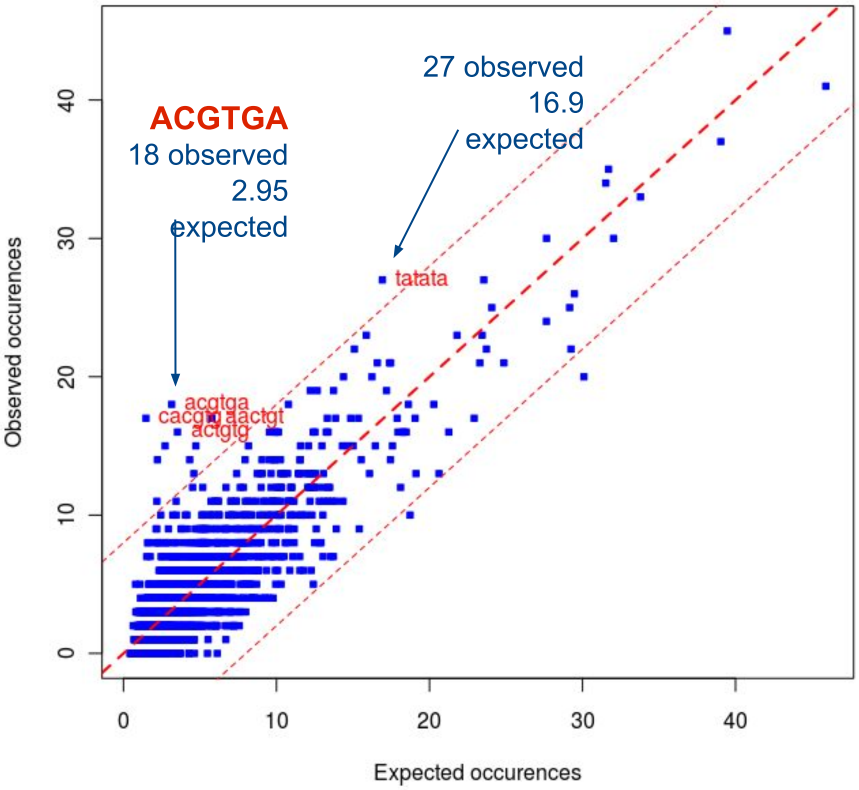
Motif discovery using word counting

Idea:

motifs corresponding to binding sites are generally repeated in the dataset
⟶ capture this statistical signal

- **Algorithm**
  - count occurrences of **all k-mers** in a set of related sequences (promoters of co-expressed genes, in ChIP bound regions,...)
  - estimate the **expected number of occurrences** from a background model
    - empirical based on observed k-mer frequencies
    - theoretical background model (Markov Models)
  - **statistical evaluation of the deviation observed** (P-value/E-value)

Statistical significance



ACGTGA
18 observed
2.95
expected

27 observed
16.9
expected

How « big » is the surprise to observe 18 occurrences when we expect 2.95 ?

Statistical significance

How « big » is the surprise to observe 18 occurrences when expecting 2.95 ?

- at each position in the sequence, there is a **probability *p*** that the word starting at this position is ACGTGA
- we consider ***n*** positions
- what is the probability that ***k*** of these ***n*** positions correspond to ACGTGA ?
- **Application** : *p* = 3.4e-4 (intergenic frequencies)
  n = 9000 position
  x = 18 observed occurrences

$$\boxed{P(X \geq x) = \sum_{i=x}^{T} \frac{n!}{i!(n-i)!} p^{i}(1-p)^{n-i}}$$

Binomial distribution to measure the exceptionality of the occurrences

Sequencing

- Sequencer : Illumina HiSeq 4000
- No. of reads per run, per sample :
  - 1st run on the GAIIx : 10-20 millions of reads per lane
  - (HiSeq 2500) 4 samples per lane :~41 millions per sample
  - (HiSeq 4000) 8 samples per lane :~43 millions per sample
- Length of DNA fragment : ~200bp
- No. of cycle per run : 50

Single end or paired end?

- Single end (most of the time)

- Paired-end sequencing

  ○ Improve identification of duplicated reads

  🙂 Better estimation of the fragment size distribution

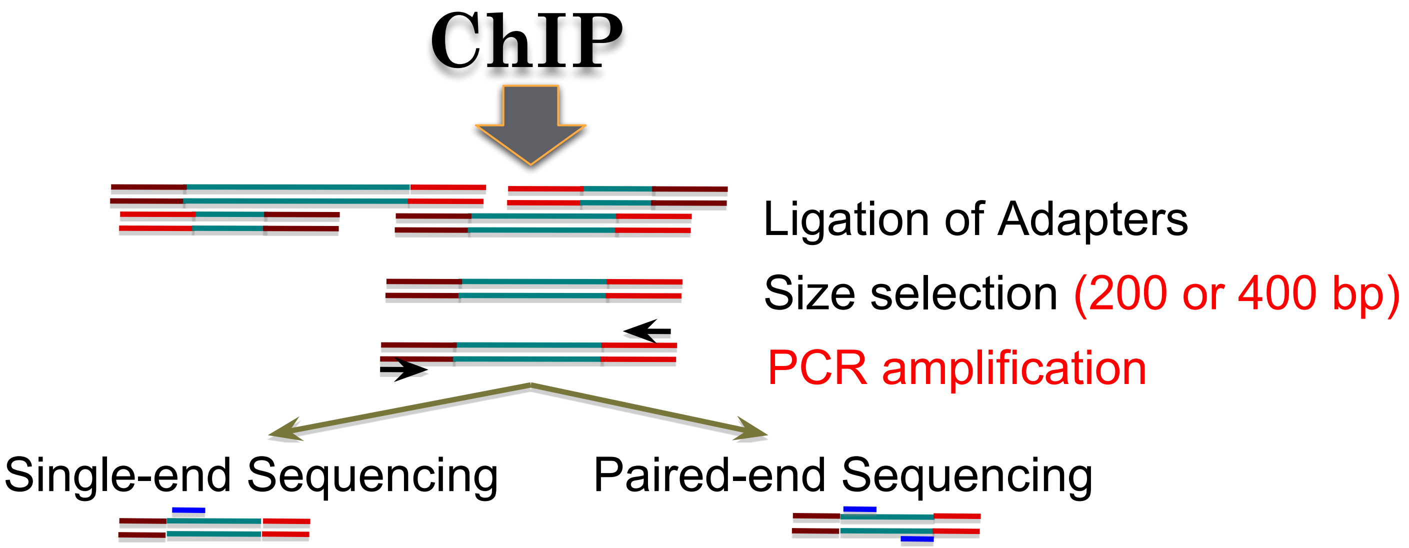
  🙂 Increase the mapping efficiency to **repeat regions**

  🙂 The price!

  🙁

Library prep

- Step between ChIP and sequencing.
- The goal is to prepare DNA for the sequencing.
- Starting material: ChIP sample (1-10ng of sheared DNA).



ChIP

Ligation of Adapters

Size selection (200 or 400 bp)

PCR amplification

Single-end Sequencing

Paired-end Sequencing

Considerations on ChIP

- Antibody
  - Antibody quality varies, even between independently prepared batches of the same antibody (Egelhofer, T. A. *et al*. 2011).
- Number of cells
  - Large numbers of cells are required for a ChIP experiment (limitation for small organisms).
- Shearing of DNA (Mnase I, sonication, Covaris): trying to narrow down the size distribution of DNA fragments

→ **Complexity in DNA fragments**

Controls

- Used mostly to filter out false positives (high level of noise)
  - Idea: potential false positive will be enriched in both treatment and control.
- A control will fail to filter out false positives if its enrichment profile is very different from the enrichment profile of false positive regions in the treatment sample.
- 3 types of controls are commonly used :
  - ***'Input' DNA***: a portion of DNA sample removed prior to IP
  - ***DNA from non specific IP***: DNA obtained from IP with an antibody not known to be involved in DNA binding or chromatin modification, such as IgG.
  - ***Mock IP DNA***: DNA obtained from IP without antibodies.
- 'Input' most generally prefered.

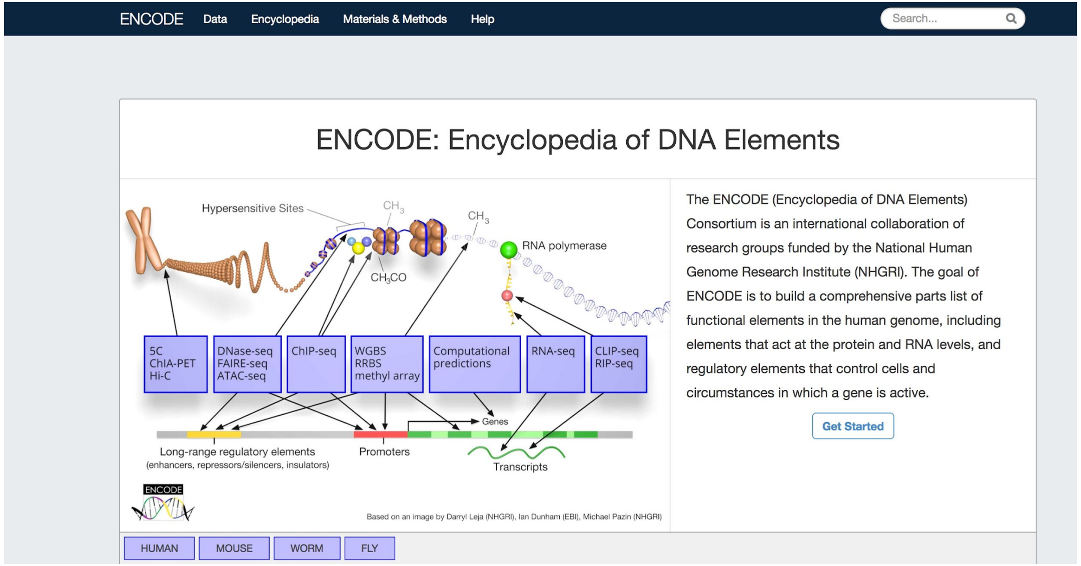
Replicates

- A **minimum** of two replicates should be carried out per experiment.
- Get ***biological replicates*** rather than technical replicates
  - i.e. taken from an independent cell culture, embryo pool or tissue sample.

ENCODE

See: https://www.encodeproject.org/

- The **ENCyclopedia Of DNA Elements** (ENCODE) consortium has carried out hundreds of ChIP-seq experiments and has used this experience to develop a set of working standards and guidelines.



ENCODE: Encyclopedia of DNA Elements

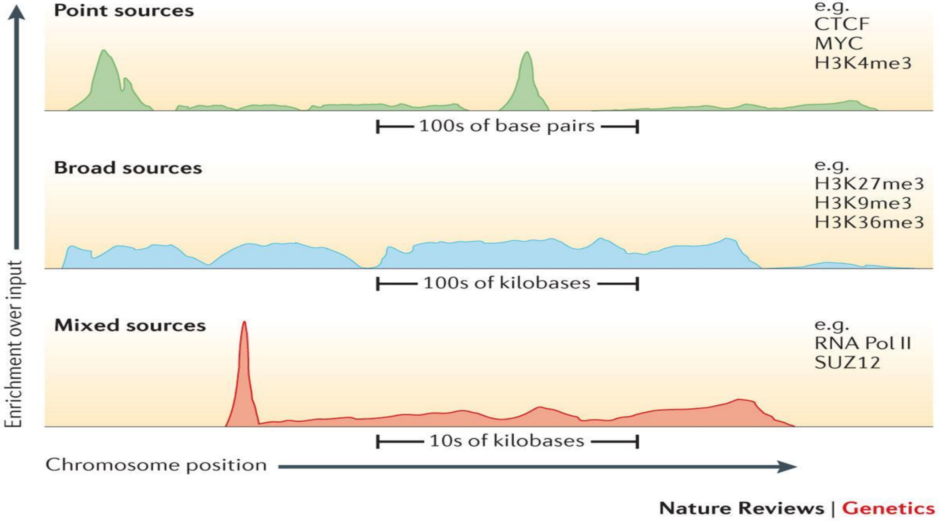
Based on an image by Darryl Leja (NHGRI), Ian Dunham (EBI), Michael Pazin (NHGRI)

The ENCODE (Encyclopedia of DNA Elements) Consortium is an international collaboration of research groups funded by the National Human Genome Research Institute (NHGRI). The goal of ENCODE is to build a comprehensive parts list of functional elements in the human genome, including elements that act at the protein and RNA levels, and regulatory elements that control cells and circumstances in which a gene is active.

https://www.encodeproject.org/

Sequencing depth

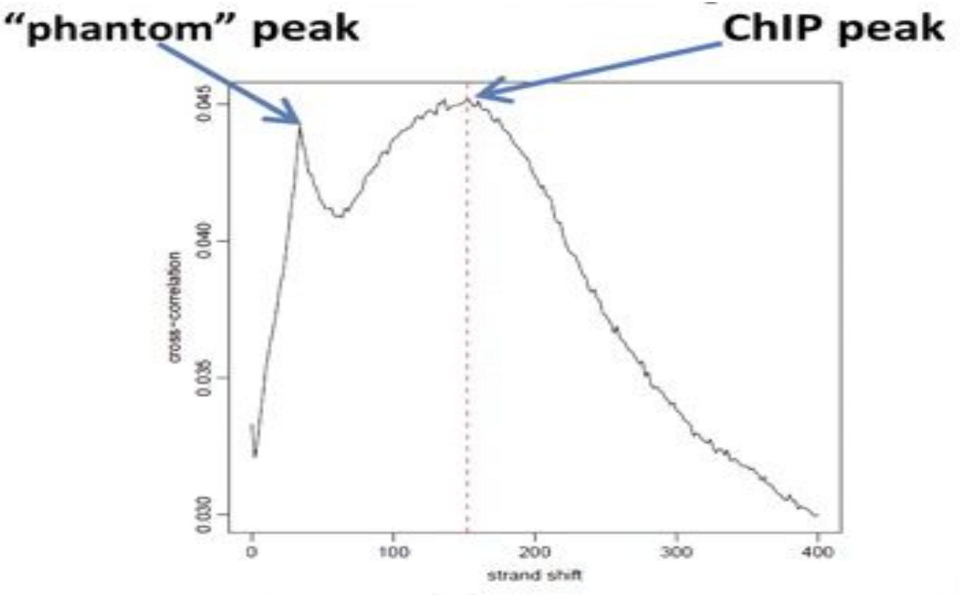
- Estimate the required depth depending on:
  - ChIP-ped protein
  - Expected profile type
  - Expected number of binding sites
  - Genome size
- Examples
  - For human genome
    - 20 million uniquely mapped read sequences for point-source peaks.
    - 40 million for broad-source peaks.
  - For fly genome: 8 million reads.
  - For worm genome: 10 million reads.



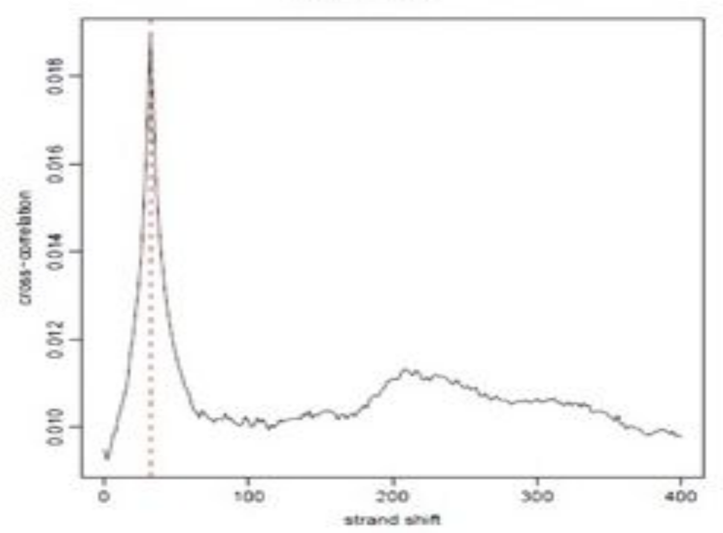
Nature Reviews | Genetics

QC: Strand cross-correlation

Successful



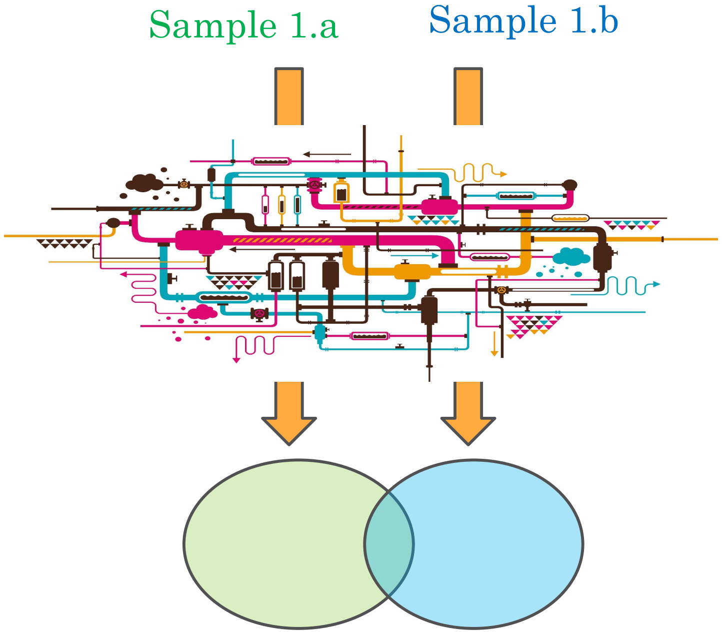
Failed



How to deal with replicates?

How to deal with replicates

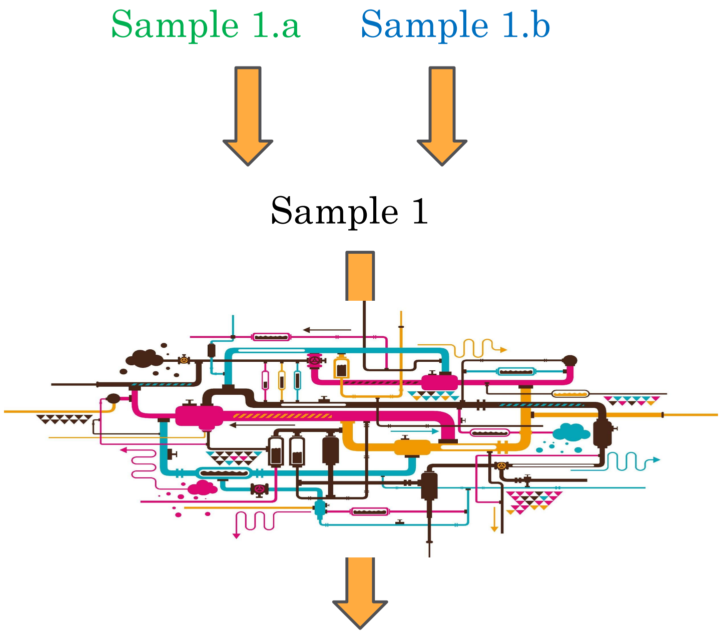
Analyze samples separately and take union or intersection of resulting peaks



Sample 1.a

Sample 1.b

Merge samples prior to the peak calling (e.g recommended by MACS)



Sample 1.a

Sample 1.b

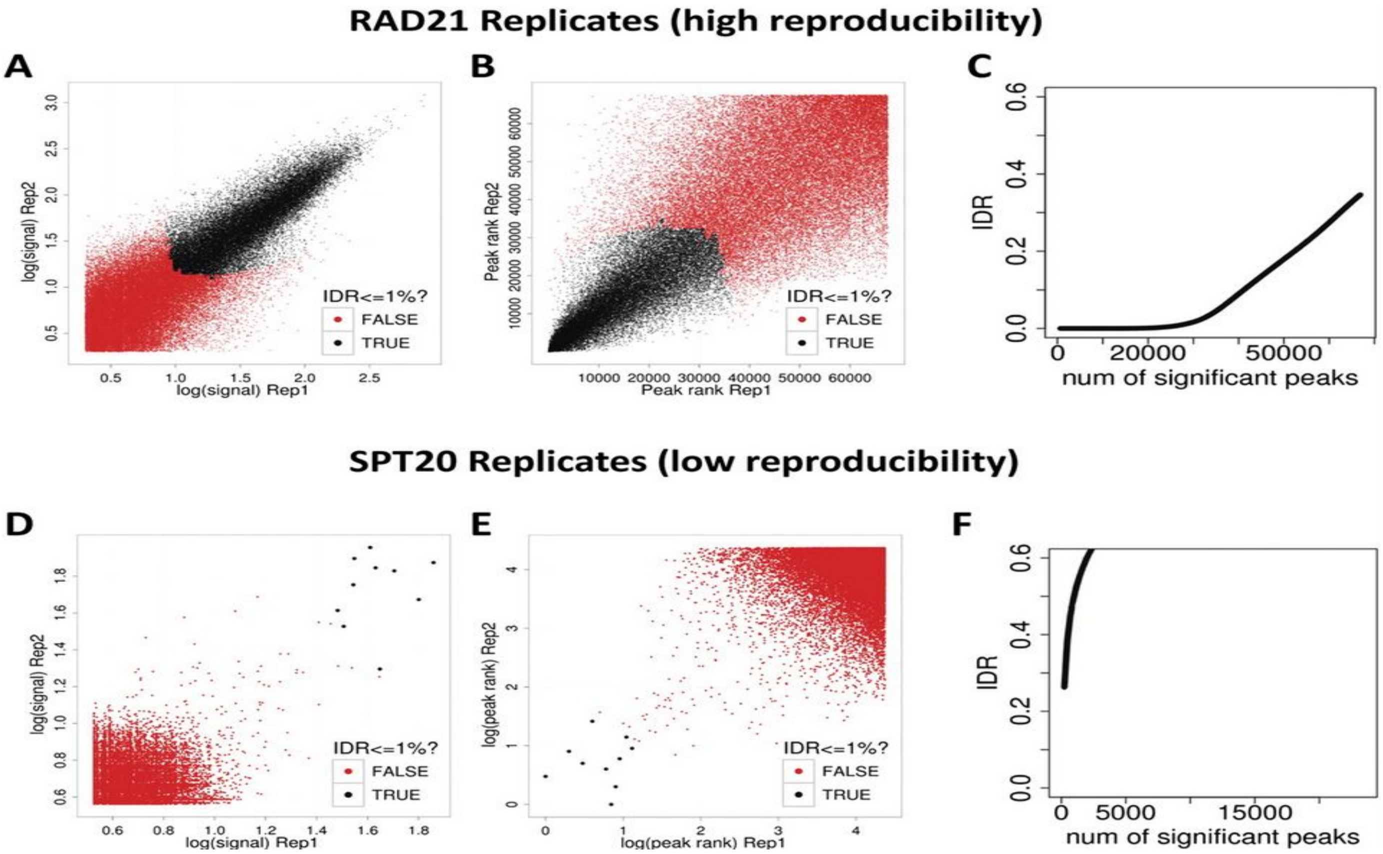
Sample 1

IDR

- IDR = Irreproducible Discovery Rate.
- Measures (in)consistency between replicates.
- Uses reproducibility between score rankings of peaks in the respective replicates to determine an optimal cutoff for significance.
- Idea:
    - The most significant peaks are expected to have high consistency between replicates.
    - The peaks with low significance are expected to have low consistency.

https://sites.google.com/site/anshulkundaje/projects/idr

IDR



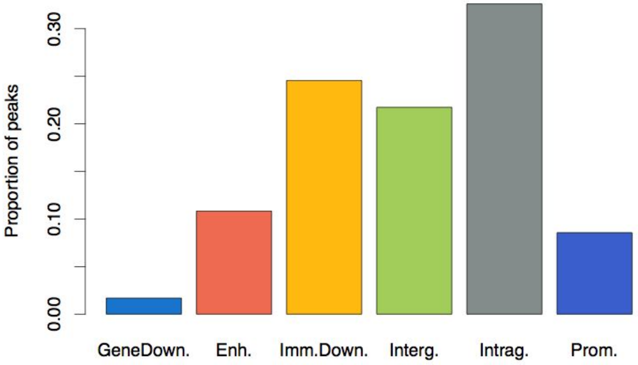
RAD21 Replicates (high reproducibility)

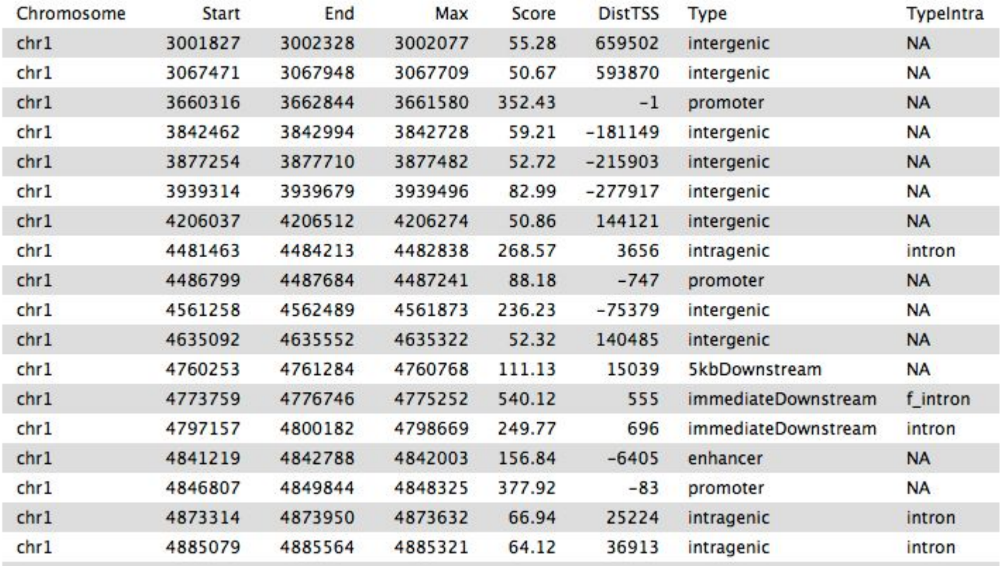
SPT20 Replicates (low reproducibility)

(!) IDR doesn’t work on broad source data!

Galaxy: Annotate peaks

- Input
  - bed file with peaks
- Output
  - Fraction of peaks per genomic elements and annotated peaks

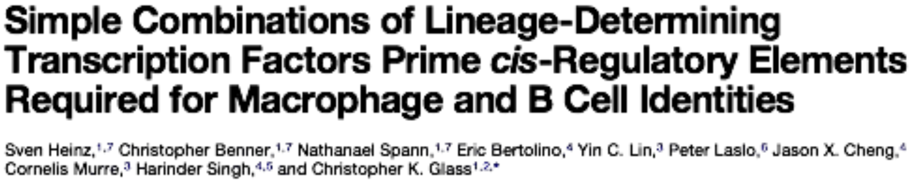




Chromosome	Start	End	Max	Score	DistTSS	Type	TypeIntra
chr1	3001827	3002328	3002077	55.28	659502	intergenic	NA
chr1	3067471	3067948	3067709	50.67	593870	intergenic	NA
chr1	3660316	3662844	3661580	352.43	-1	promoter	NA
chr1	3842462	3842994	3842728	59.21	-181149	intergenic	NA
chr1	3877254	3877710	3877482	52.72	-215903	intergenic	NA
chr1	3939314	3939679	3939496	82.99	-277917	intergenic	NA
chr1	4206037	4206512	4206274	50.86	144121	intergenic	NA
chr1	4481463	4484213	4482838	268.57	3656	intragenic	intron
chr1	4486799	4487684	4487241	88.18	-747	promoter	NA
chr1	4561258	4562489	4561873	236.23	-75379	intergenic	NA
chr1	4635092	4635552	4635322	52.32	140485	intergenic	NA
chr1	4760253	4761284	4760768	111.13	15039	5kbDownstream	NA
chr1	4773759	4776746	4775252	540.12	555	immediateDownstream	f_intron
chr1	4797157	4800182	4798669	249.77	696	immediateDownstream	intron
chr1	4841219	4842788	4842003	156.84	-6405	enhancer	NA
chr1	4846807	4849844	4848325	377.92	-83	promoter	NA
chr1	4873314	4873950	4873632	66.94	25224	intragenic	intron
chr1	4885079	4885564	4885321	64.12	36913	intragenic	intron

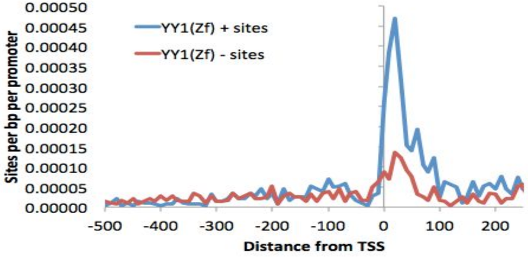
HOMER

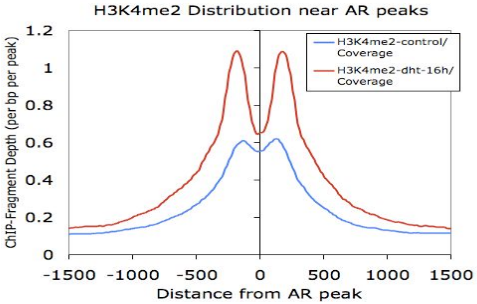
Motif discovery and NGS data analysis

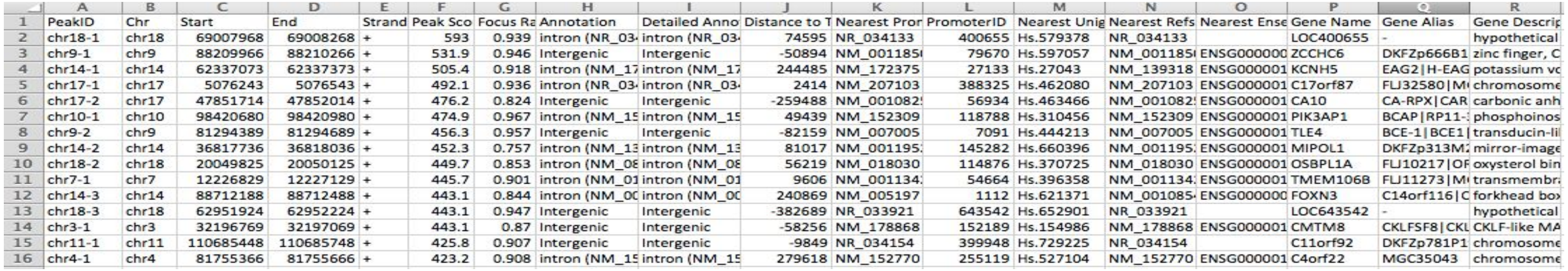


Simple Combinations of Lineage-Determining Transcription Factors Prime *cis*-Regulatory Elements Required for Macrophage and B Cell Identities

Sven Heinz,[1,7] Christopher Benner,[1,7] Nathanael Spann,[1,7] Eric Bertolino,[4] Yin C. Lin,[3] Peter Laslo,[6] Jason X. Cheng,[4] Cornelis Murre,[3] Harinder Singh,[4,5] and Christopher K. Glass[1,2,*]



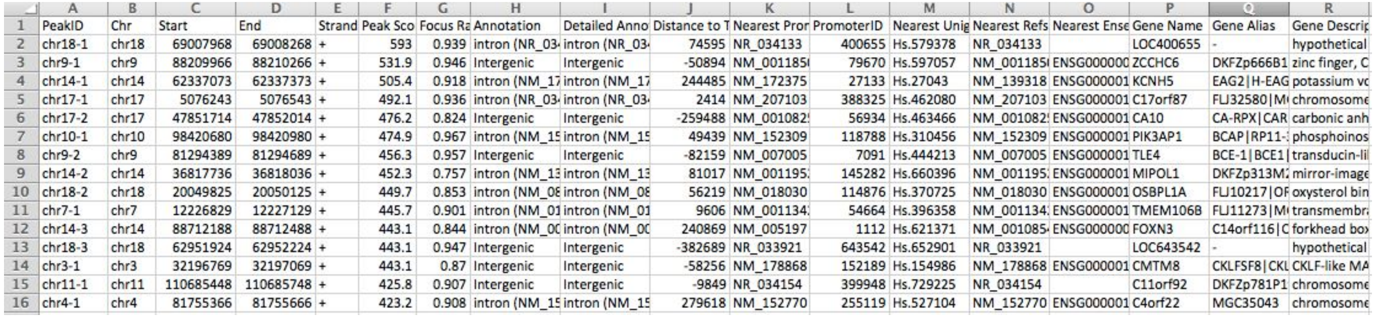




	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	PeakID	Chr	Start	End	Strand	Peak Sco	Focus Ra	Annotation	Detailed Anno	Distance to T	Nearest Pror	PromoterID	Nearest Unig	Nearest Refs	Nearest Ense	Gene Name	Gene Alias	Gene Descrip
2	chr18-1	chr18	69007968	69008268	+	593	0.939	intron (NR_03	intron (NR_03	74595	NR_034133	400655	Hs.579378	NR_034133		LOC400655	-	hypothetical
3	chr9-1	chr9	88209966	88210266	+	531.9	0.946	Intergenic	Intergenic	-50894	NM_001185	79670	Hs.597057	NM_001185	ENSG000000	ZCCHC6	DKFZp666B1	zinc finger, C
4	chr14-1	chr14	62337073	62337373	+	505.4	0.918	intron (NM_17	intron (NM_17	244485	NM_172375	27133	Hs.27043	NM_139318	ENSG000001	KCNH5	EAG2|H-EAG	potassium vc
5	chr17-1	chr17	5076243	5076543	+	492.1	0.936	intron (NR_03	intron (NR_03	2414	NM_207103	388325	Hs.462080	NM_207103	ENSG000001	C17orf87	FLJ32580|M	chromosome
6	chr17-2	chr17	47851714	47852014	+	476.2	0.824	Intergenic	Intergenic	-259488	NM_001082	56934	Hs.463466	NM_001082	ENSG000001	CA10	CA-RPX|CAR	carbonic anh
7	chr10-1	chr10	98420680	98420980	+	474.9	0.967	intron (NM_15	intron (NM_15	49439	NM_152309	118788	Hs.310456	NM_152309	ENSG000001	PIK3AP1	BCAP|RP11-	phosphoinos
8	chr9-2	chr9	81294389	81294689	+	456.3	0.957	Intergenic	Intergenic	-82159	NM_007005	7091	Hs.444213	NM_007005	ENSG000001	TLE4	BCE-1|BCE1|	transducin-li
9	chr14-2	chr14	36817736	36818036	+	452.3	0.757	intron (NM_13	intron (NM_13	81017	NM_001195	145282	Hs.660396	NM_001195	ENSG000001	MIPOL1	DKFZp313M	mirror-image
10	chr18-2	chr18	20049825	20050125	+	449.7	0.853	intron (NM_08	intron (NM_08	56219	NM_018030	114876	Hs.370725	NM_018030	ENSG000001	OSBPL1A	FLJ10217|OF	oxysterol bin
11	chr7-1	chr7	12226829	12227129	+	445.7	0.901	intron (NM_01	intron (NM_01	9606	NM_001134	54664	Hs.396358	NM_001134	ENSG000001	TMEM106B	FLJ11273|M	transmembr
12	chr14-3	chr14	88712188	88712488	+	443.1	0.844	intron (NM_00	intron (NM_00	240869	NM_005197	1112	Hs.621371	NM_001085	ENSG000000	FOXN3	C14orf116|C	forkhead box
13	chr18-3	chr18	62951924	62952224	+	443.1	0.947	Intergenic	Intergenic	-382689	NR_033921	643542	Hs.652901	NR_033921		LOC643542	-	hypothetical
14	chr3-1	chr3	32196769	32197069	+	443.1	0.87	Intergenic	Intergenic	-58256	NM_178868	152189	Hs.154986	NM_178868	ENSG000001	CMTM8	CKLFSF8|CKL	CKLF-like MA
15	chr11-1	chr11	110685448	110685748	+	425.8	0.907	Intergenic	Intergenic	-9849	NR_034154	399948	Hs.729225	NR_034154		C11orf92	DKFZp781P1	chromosome
16	chr4-1	chr4	81755366	81755666	+	423.2	0.908	intron (NM_15	intron (NM_15	279618	NM_152770	255119	Hs.527104	NM_152770	ENSG000001	C4orf22	MGC35043	chromosome

http://homer.salk.edu/homer/

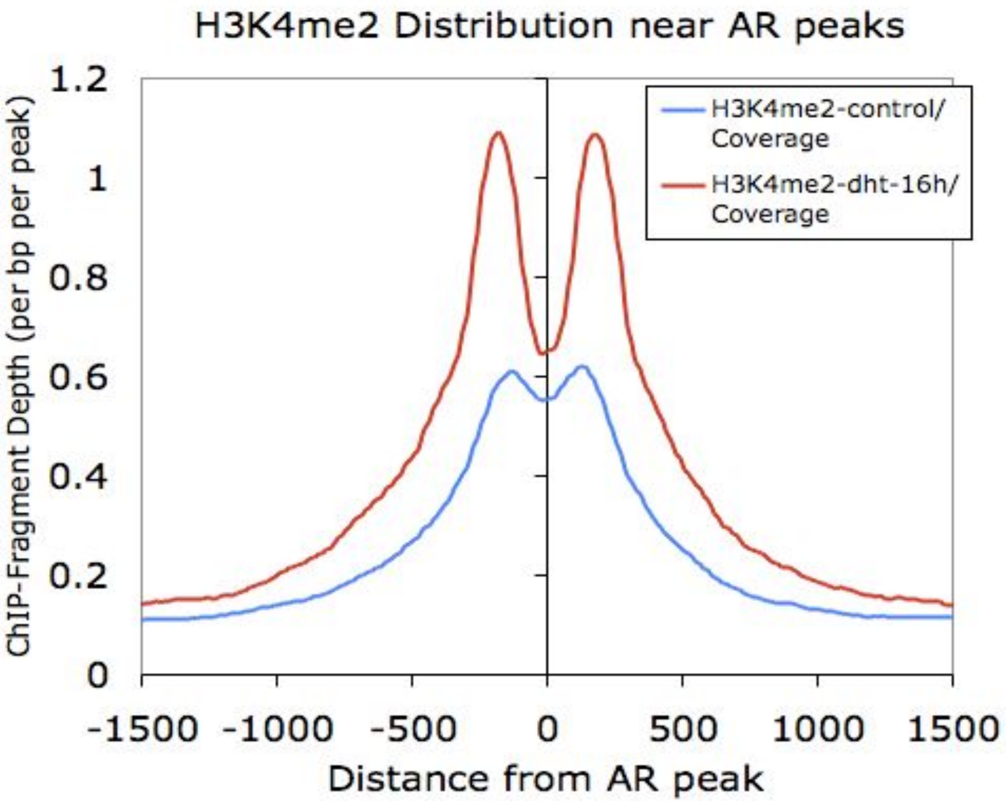
HOMER: annotate peaks



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	PeakID	Chr	Start	End	Strand	Peak Sco	Focus Ra	Annotation	Detailed Anno	Distance to T	Nearest Pror	PromoterID	Nearest Unig	Nearest Refs	Nearest Ense	Gene Name	Gene Alias	Gene Descrip
2	chr18-1	chr18	69007968	69008268	+	593	0.939	intron (NR_03	intron (NR_03	74595	NR_034133	400655	Hs.579378	NR_034133		LOC400655	-	hypothetical
3	chr9-1	chr9	88209966	88210266	+	531.9	0.946	Intergenic	Intergenic	-50894	NM_001185	79670	Hs.597057	NM_001185	ENSG000000	ZCCHC6	DKFZp666B1	zinc finger, C
4	chr14-1	chr14	62337073	62337373	+	505.4	0.918	intron (NM_17	intron (NM_17	244485	NM_172375	27133	Hs.27043	NM_139318	ENSG000001	KCNH5	EAG2|H-EAG	potassium vc
5	chr17-1	chr17	5076243	5076543	+	492.1	0.936	intron (NR_03	intron (NR_03	2414	NM_207103	388325	Hs.462080	NM_207103	ENSG000001	C17orf87	FLJ32580|M	chromosome
6	chr17-2	chr17	47851714	47852014	+	476.2	0.824	Intergenic	Intergenic	-259488	NM_001082	56934	Hs.463466	NM_001082	ENSG000001	CA10	CA-RPX|CAR	carbonic anh
7	chr10-1	chr10	98420680	98420980	+	474.9	0.967	intron (NM_15	intron (NM_15	49439	NM_152309	118788	Hs.310456	NM_152309	ENSG000001	PIK3AP1	BCAP|RP11-	phosphoinos
8	chr9-2	chr9	81294389	81294689	+	456.3	0.957	Intergenic	Intergenic	-82159	NM_007005	7091	Hs.444213	NM_007005	ENSG000001	TLE4	BCE-1|BCE1|	transducin-li
9	chr14-2	chr14	36817736	36818036	+	452.3	0.757	intron (NM_13	intron (NM_13	81017	NM_001195	145282	Hs.660396	NM_001195	ENSG000001	MIPOL1	DKFZp313M	mirror-image
10	chr18-2	chr18	20049825	20050125	+	449.7	0.853	intron (NM_08	intron (NM_08	56219	NM_018030	114876	Hs.370725	NM_018030	ENSG000001	OSBPL1A	FLJ10217|OF	oxysterol bin
11	chr7-1	chr7	12226829	12227129	+	445.7	0.901	intron (NM_01	intron (NM_01	9606	NM_001134	54664	Hs.396358	NM_001134	ENSG000001	TMEM106B	FLJ11273|M	transmembr
12	chr14-3	chr14	88712188	88712488	+	443.1	0.844	intron (NM_00	intron (NM_00	240869	NM_005197	1112	Hs.621371	NM_001085	ENSG000000	FOXN3	C14orf116|C	forkhead box
13	chr18-3	chr18	62951924	62952224	+	443.1	0.947	Intergenic	Intergenic	-382689	NR_033921	643542	Hs.652901	NR_033921		LOC643542	-	hypothetical
14	chr3-1	chr3	32196769	32197069	+	443.1	0.87	Intergenic	Intergenic	-58256	NM_178868	152189	Hs.154986	NM_178868	ENSG000001	CMTM8	CKLFSF8|CKL	CKLF-like MA
15	chr11-1	chr11	110685448	110685748	+	425.8	0.907	Intergenic	Intergenic	-9849	NR_034154	399948	Hs.729225	NR_034154		C11orf92	DKFZp781P1	chromosome
16	chr4-1	chr4	81755366	81755666	+	423.2	0.908	intron (NM_15	intron (NM_15	279618	NM_152770	255119	Hs.527104	NM_152770	ENSG000001	C4orf22	MGC35043	chromosome

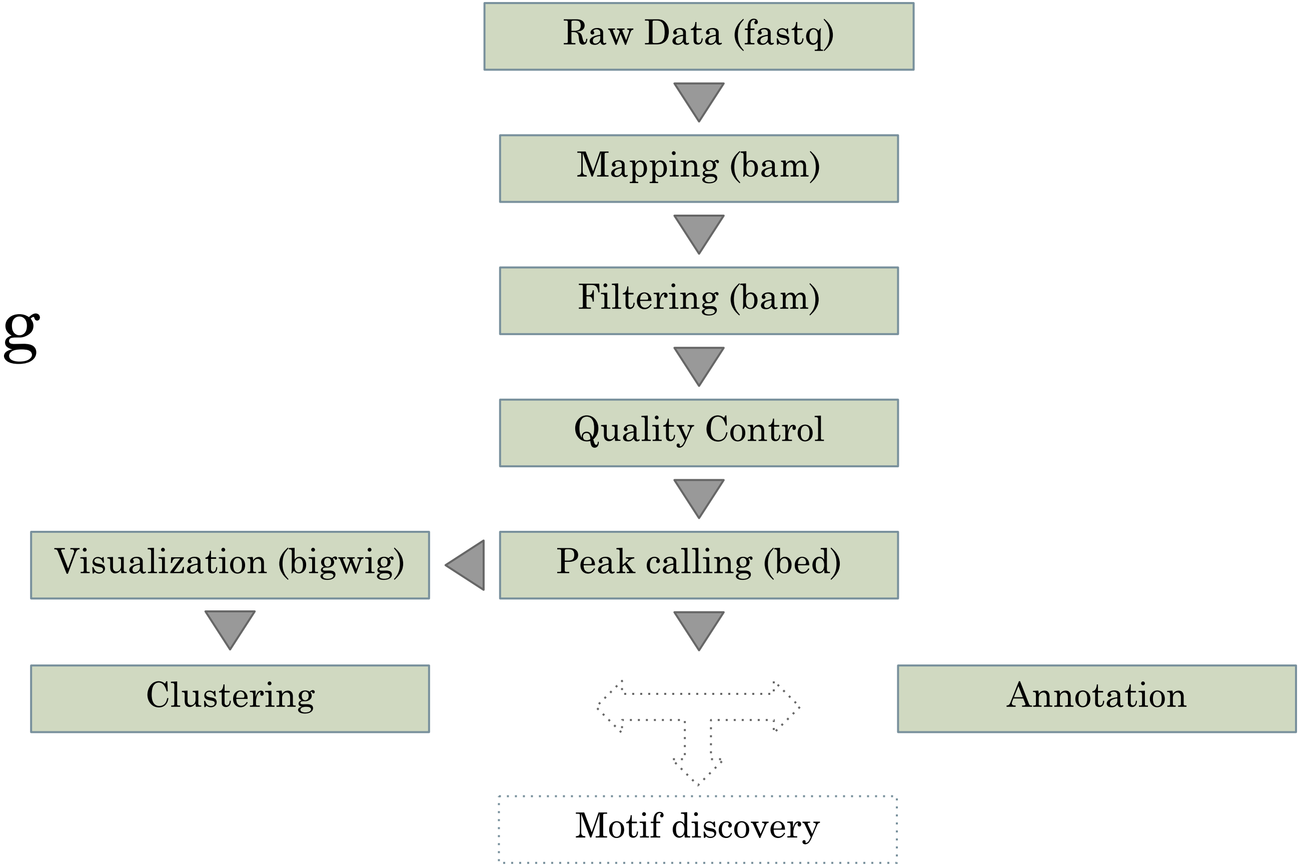
1 Peak ID
2 Chromosome
3 Peak start position
4 Peak end position
5 Strand
6 Peak Score
7 FDR/Peak Focus Ratio/Region Size
8 Annotation (i.e. Exon, Intron, ...)
9 Detailed Annotation (Exon, Intron etc. + CpG Islands, repeats, etc.)
10 Distance to nearest RefSeq TSS
11 Nearest TSS: Native ID of annotation file
12 Nearest TSS: Entrez Gene ID
13 Nearest TSS: Unigene ID
14 Nearest TSS: RefSeq ID
15 Nearest TSS: Ensembl ID
16 Nearest TSS: Gene Symbol
17 Nearest TSS: Gene Aliases
18 Nearest TSS: Gene description
19 Additional columns depend on options selected when running the program.

HOMER: compare peaks



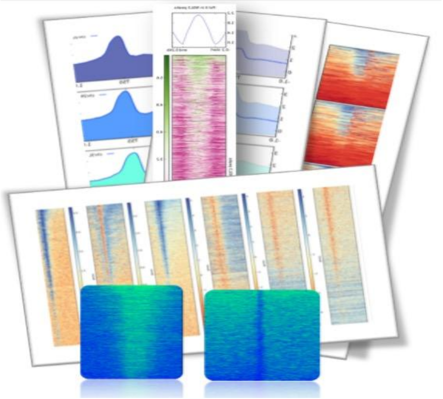
Peak co-occurrence statistics
Co-bound peaks
Differentially bound peaks

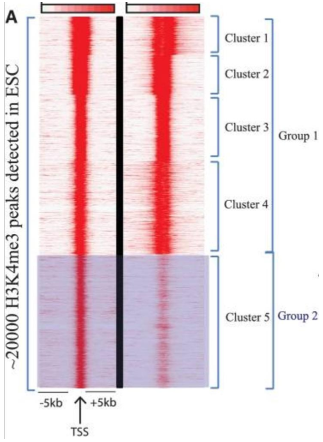
Clustering


Raw Data (fastq)
Mapping (bam)
Filtering (bam)
Quality Control
Visualization (bigwig)
Peak calling (bed)
Clustering
Annotation
Motif discovery

Based on signal distribution, are there any classes of genomic regions?

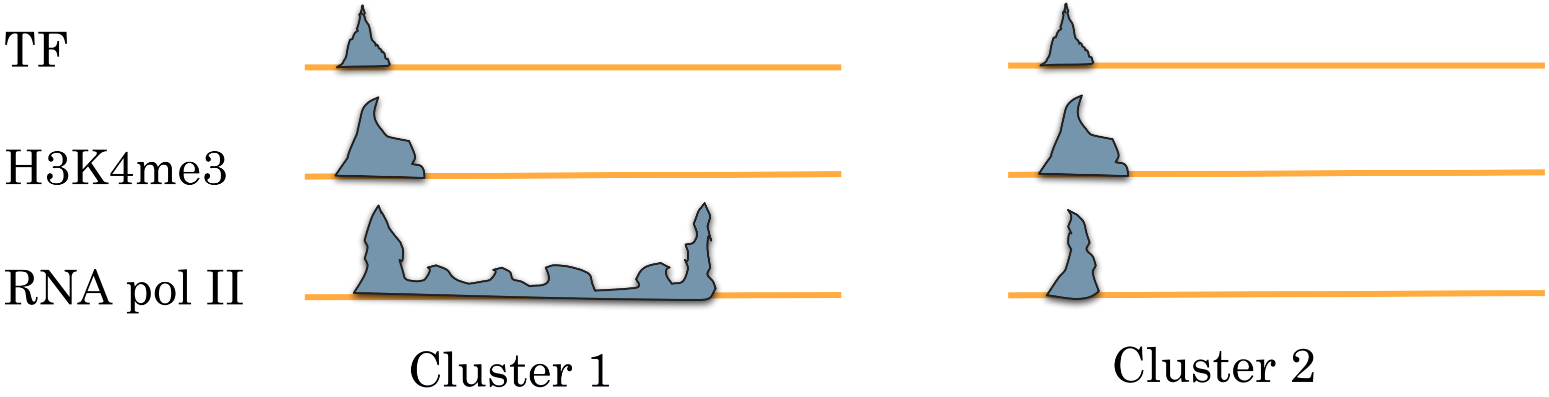
- How does the signal (read counts) distribute around or inside:
  - Transcriptional start sites (TSS)
  - Transcriptional termination sites (TTS)
  - Gene bodies, exons, introns
- Tools:
  - Deeptools (heatmapper)
  - seqMINER
- Unsupervised clustering methods (e.g k-means)
  - Discover some underlying classes of genomic regions





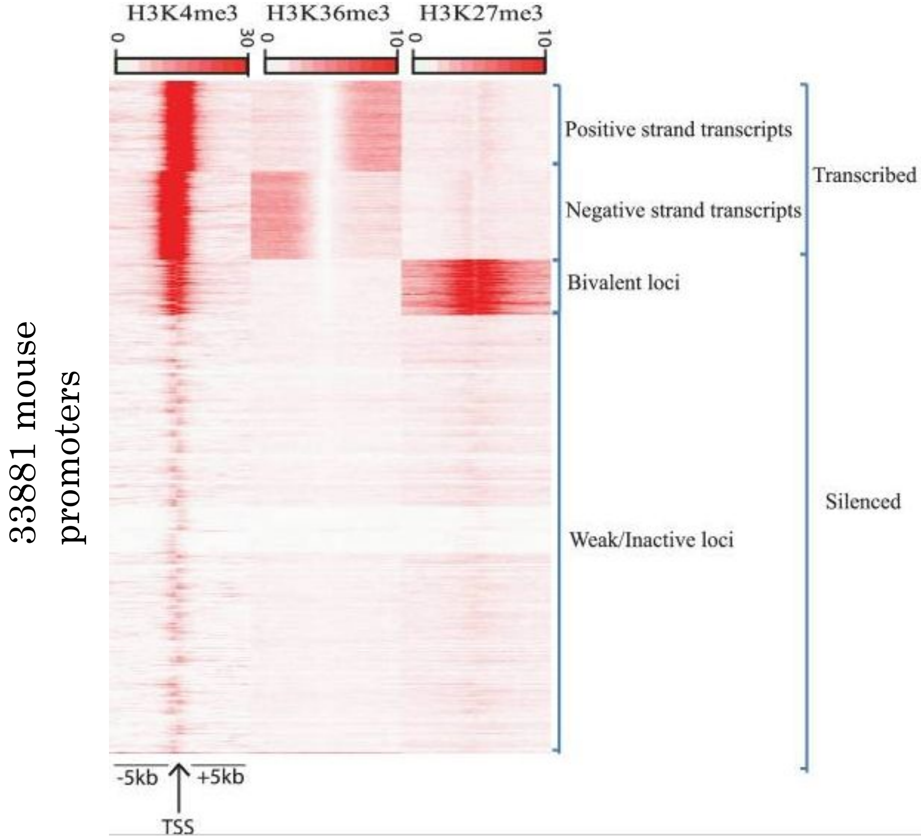
Clustering

- Group together genomic regions with similar enrichments
- In a single sample or multiple samples
- E.g:



Clustering

- **seqMINER**
  - User friendly interactive interface with multiple graphical representations
  - Multiple dataset comparison
  - Java, multi-platform


H3K4me3
H3K36me3
H3K27me3
33881 mouse promoters
Positive strand transcripts
Negative strand transcripts
Bivalent loci
Weak/Inactive loci
Transcribed
Silenced
-5kb
+5kb
TSS