# Détection de motifs dans les pics ChIP-seq

**M. Thomas-Chollier, M. Defrance,**

**C. Herrmann, D. Puthier**

# Goal and organisation of this session

**Goal:** introduction to motif analysis in ChIP-seq data

- **processing steps:** from reads to peaks. => see previous session
- **downstream analyses**:
  - focus on motif analyses

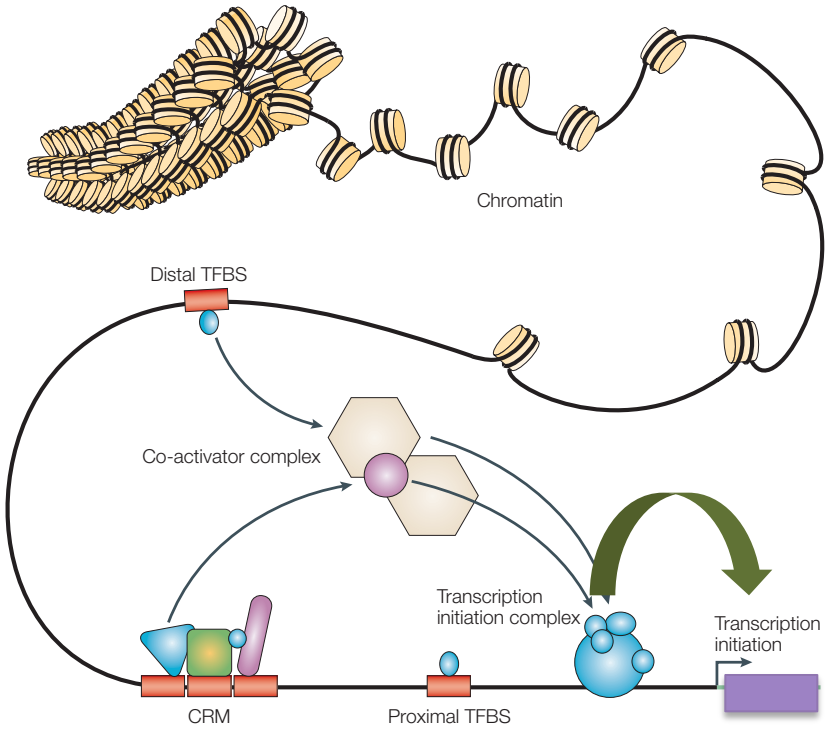
**Tools**

- **RSAT**

**Steps**

1. Retrieving **sequences** from a set of peak coordinates (*fetch-sequences*)
2. Discovering **motifs** from peak sequences (*peak-motifs*)
3. **Visualizing** the sites in the context of genome annotations (*UCSC genome browser*)

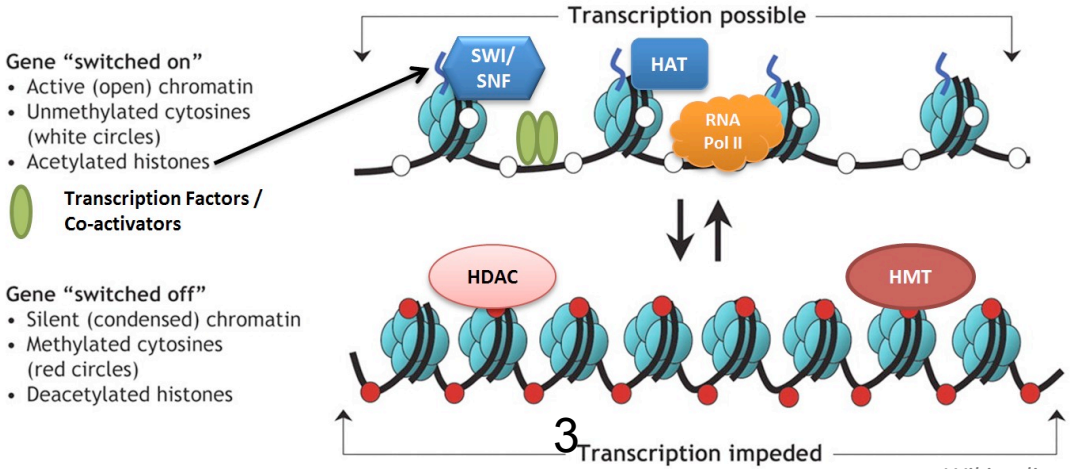
# Biological concepts of transcriptional regulation




*Wasserman et al, Nat Rev Genet, 2004*

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

**Chromatin accessibility** (open/close) and **histone modifications** (eg: acetylation) also regulate gene expression




*Wikipedia*

# *in vivo* experimental methods to identify binding sites

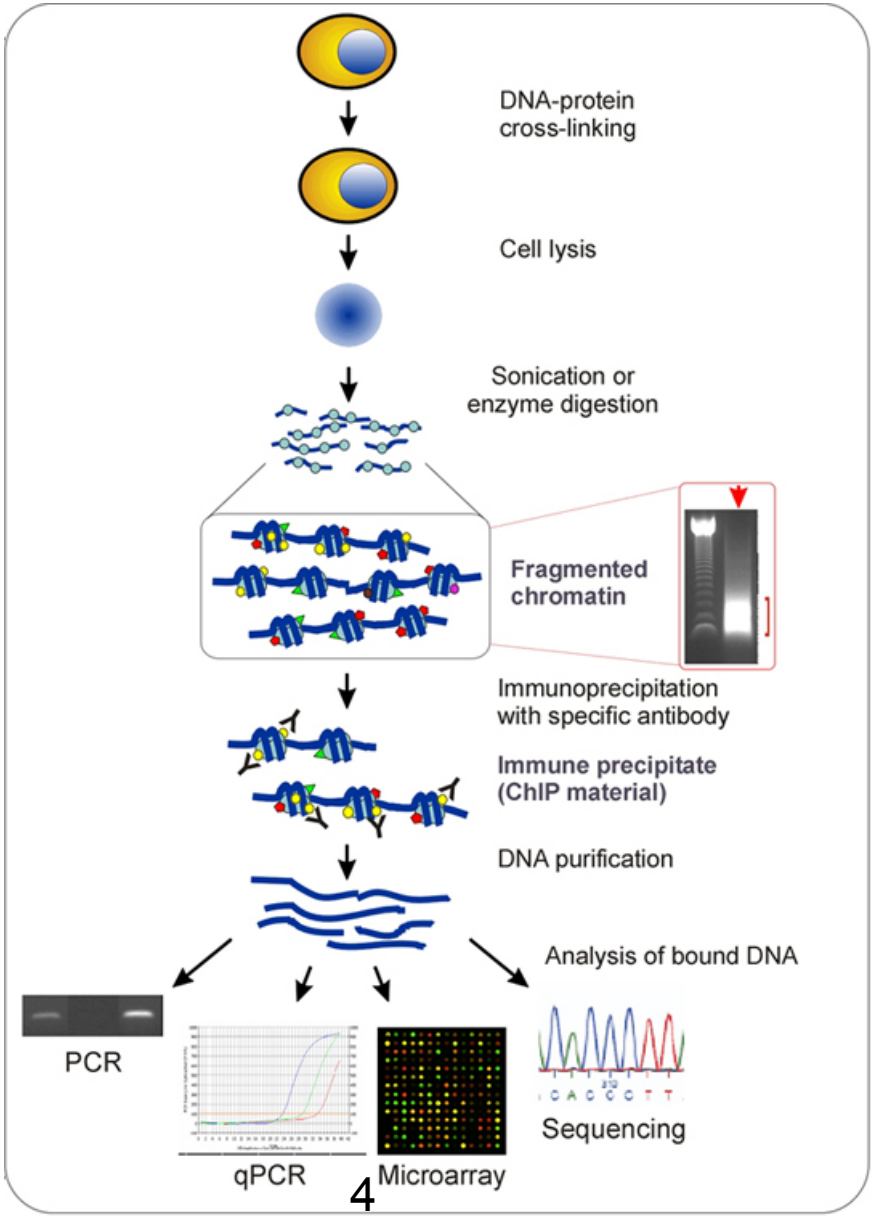
**ChIP (=Chromatin Immuno-Precipitation)**

=> differences in **methods**
**to detect** the **bound DNA**

-<u>small-scale</u>: PCR / qPCR

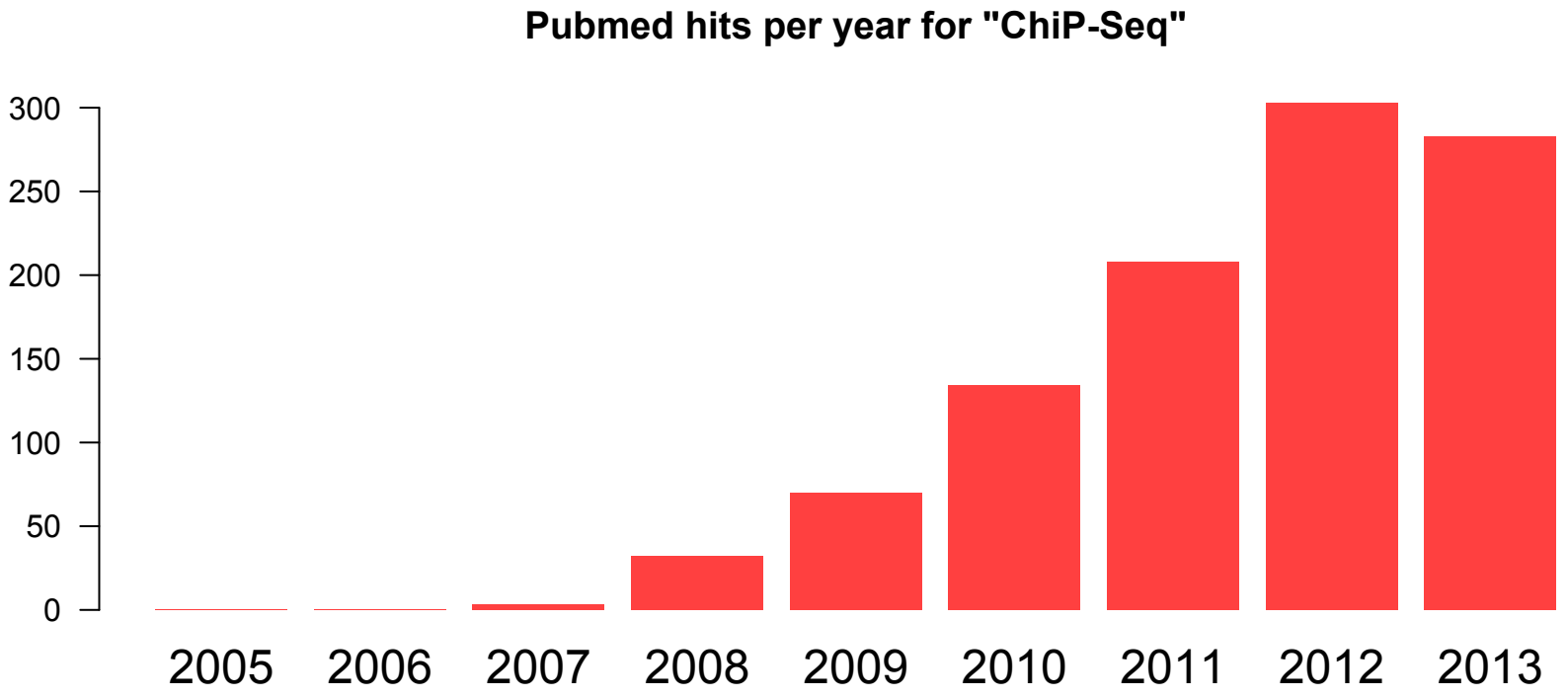
- <u>large-scale</u>:
  - microarray = **ChIP-on-chip**
  - sequencing = **ChIP-seq**

**Main challenge:**
-quality/specificity of the antibodies




http://www.chip-antibodies.com/

# ChIP-seq is a recently-adopted technique !

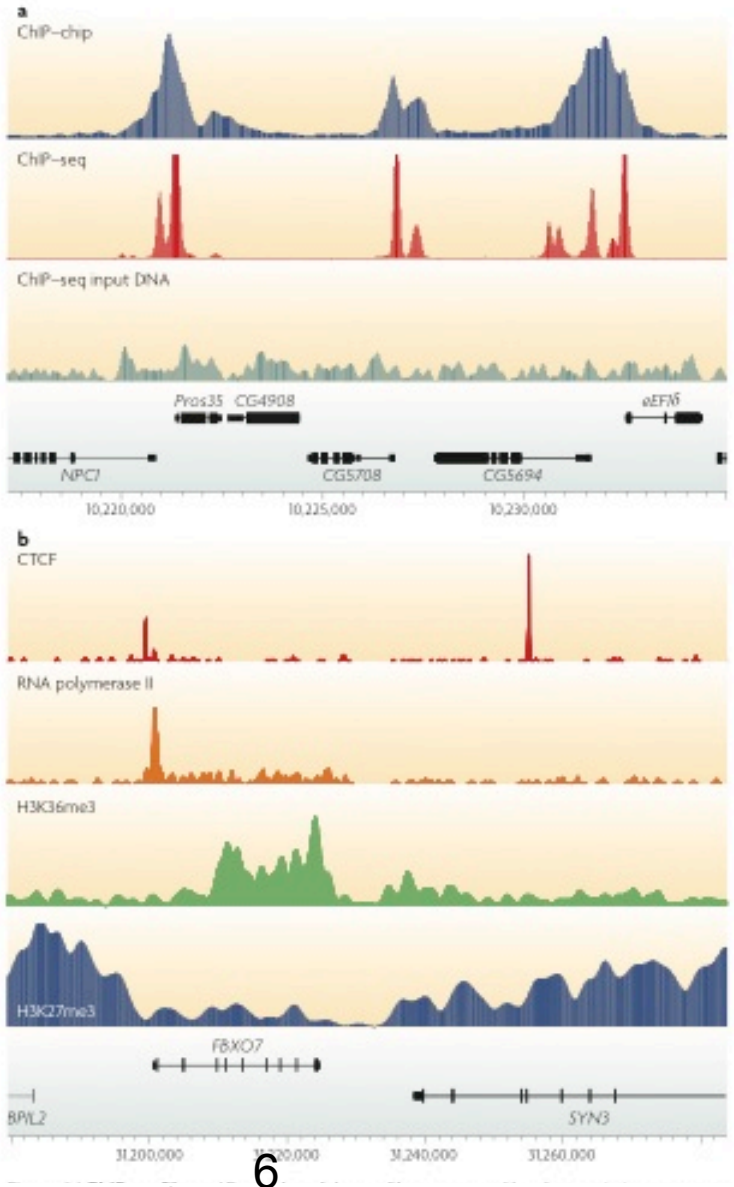


# ChIP-seq applications

- find ***all*** regions in the genome bound by
  - a specific **transcription factor**
  - **histones** bearing a specific **modification**

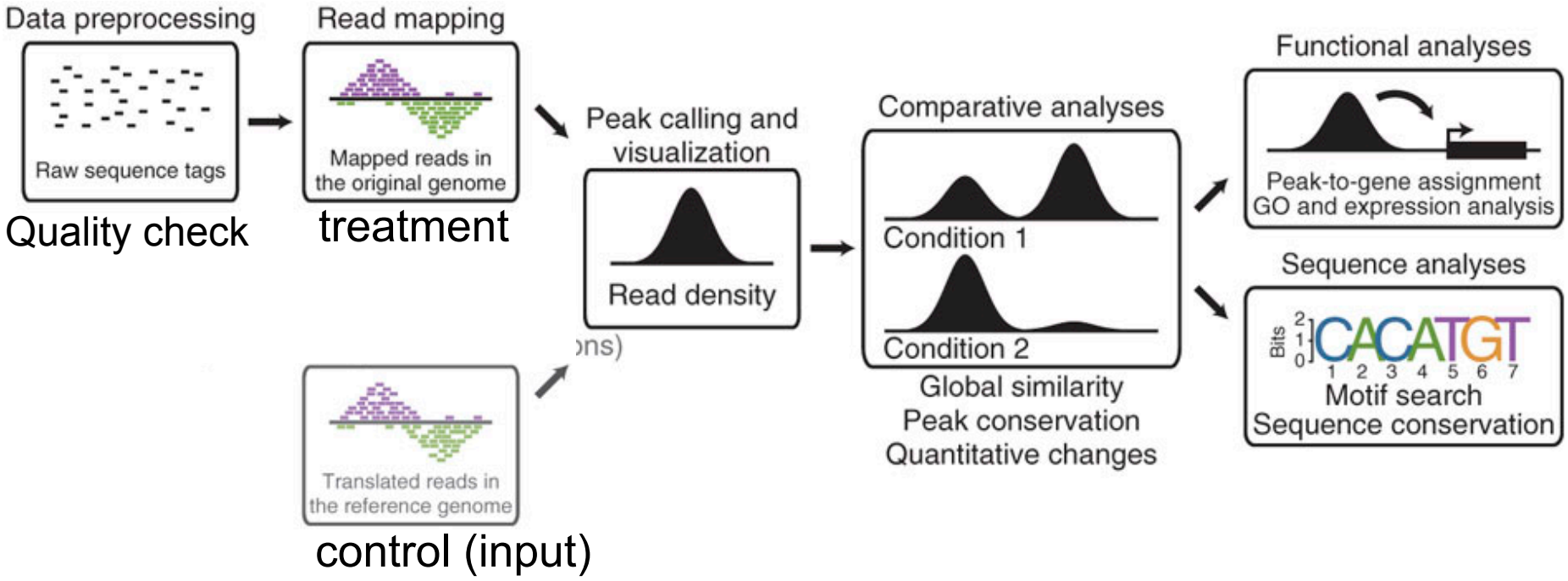
- in a given ***experimental condition*** (cell type, developmental stage,...)

The obtain ChIP-seq **profiles** have **different shapes**, depending on the targeted protein




Park, Nature reviews 2009

# ChIP-seq analysis workflow

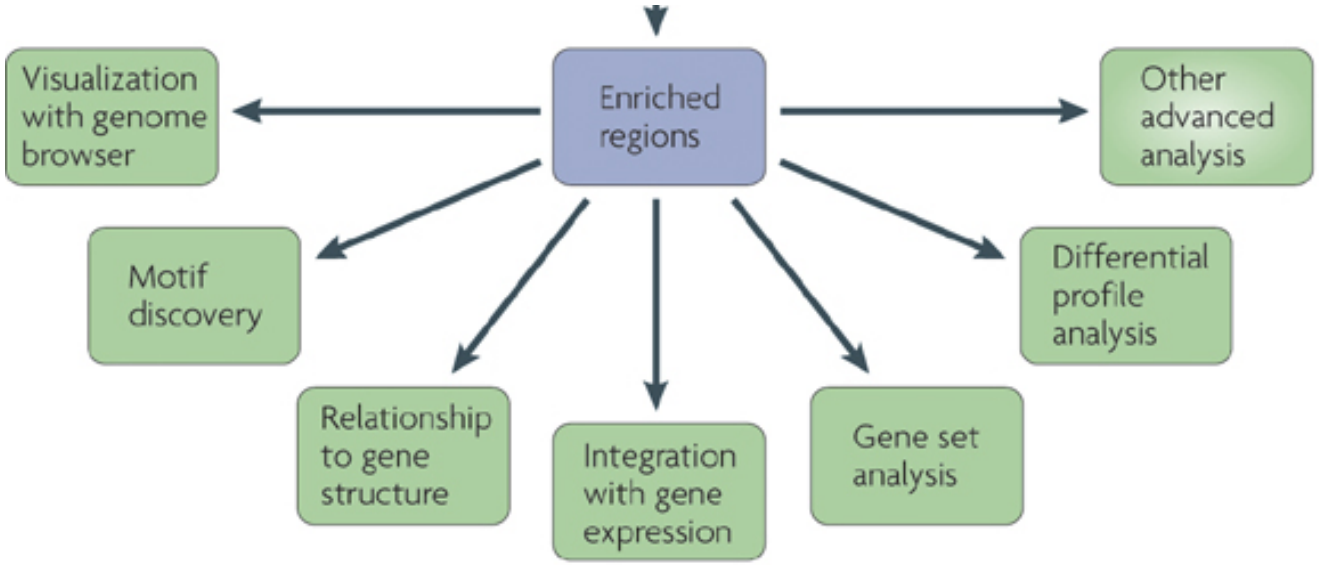




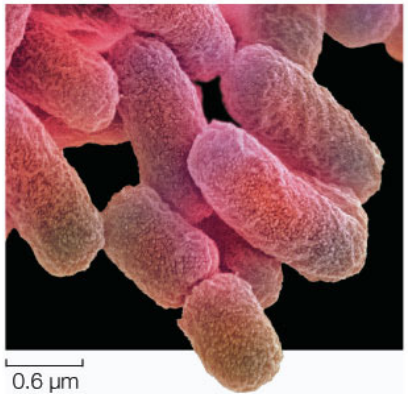


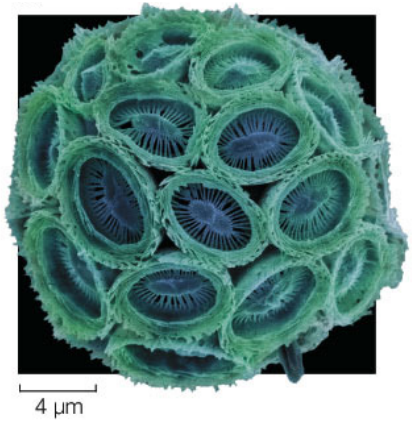

Adapted from Bardet et al, Nature Protocols, 2012

# ChIP-seq analysis workflow: downstream analyses




Park, Nature reviews 2009




# What is the biological question ?

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« see if you can find something in the data »

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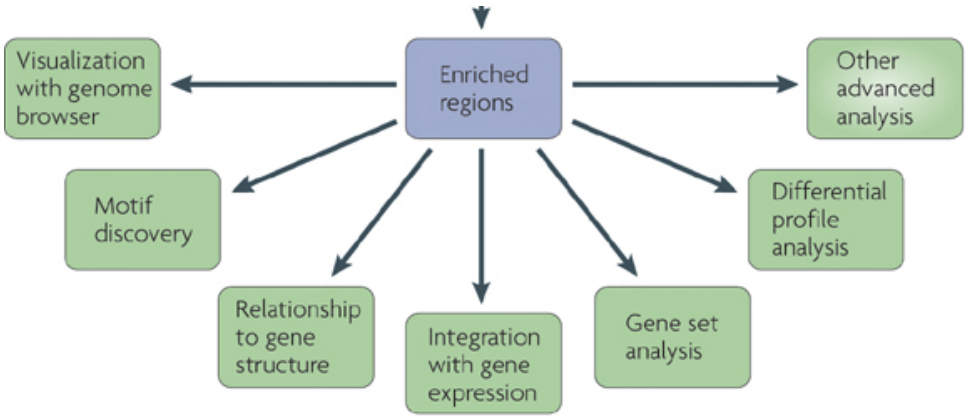
- Which **regulated genes** are directly regulated by a given TF ?

- What are the **targets** of a given TF ?

- Where are the **promoters** (PolII) and **chromatin marks** ?

## What is the biological question ?

➔ Should drive all « downstream » analyses



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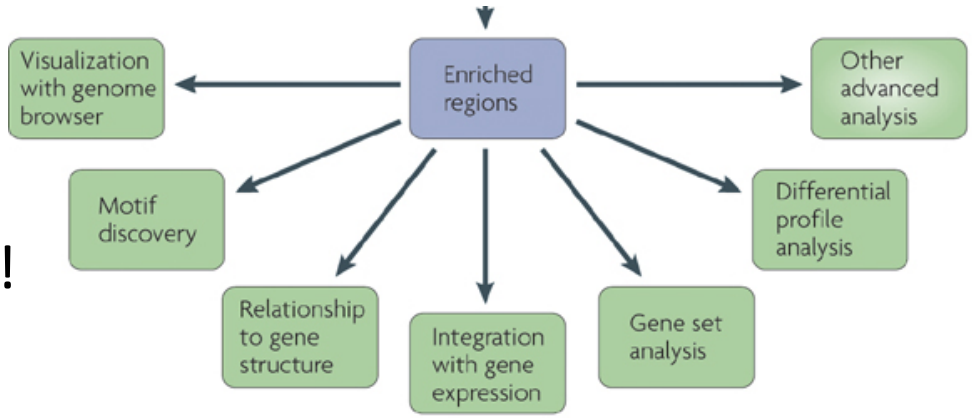


Will take time
to « do it all » !!!

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## What is the biological question ?

## What can be the following experimental work ?

- ➔ cell biology (eg: luciferase assay) ?
- ➔ in vitro assays (eg: EMSA) ?
- ➔ Proteomic (eg: mass spectrometry) ?
- ➔ Transgenics ?
- ➔ Will depend on
  - ✓ the organism
  - ✓ available infrastructure

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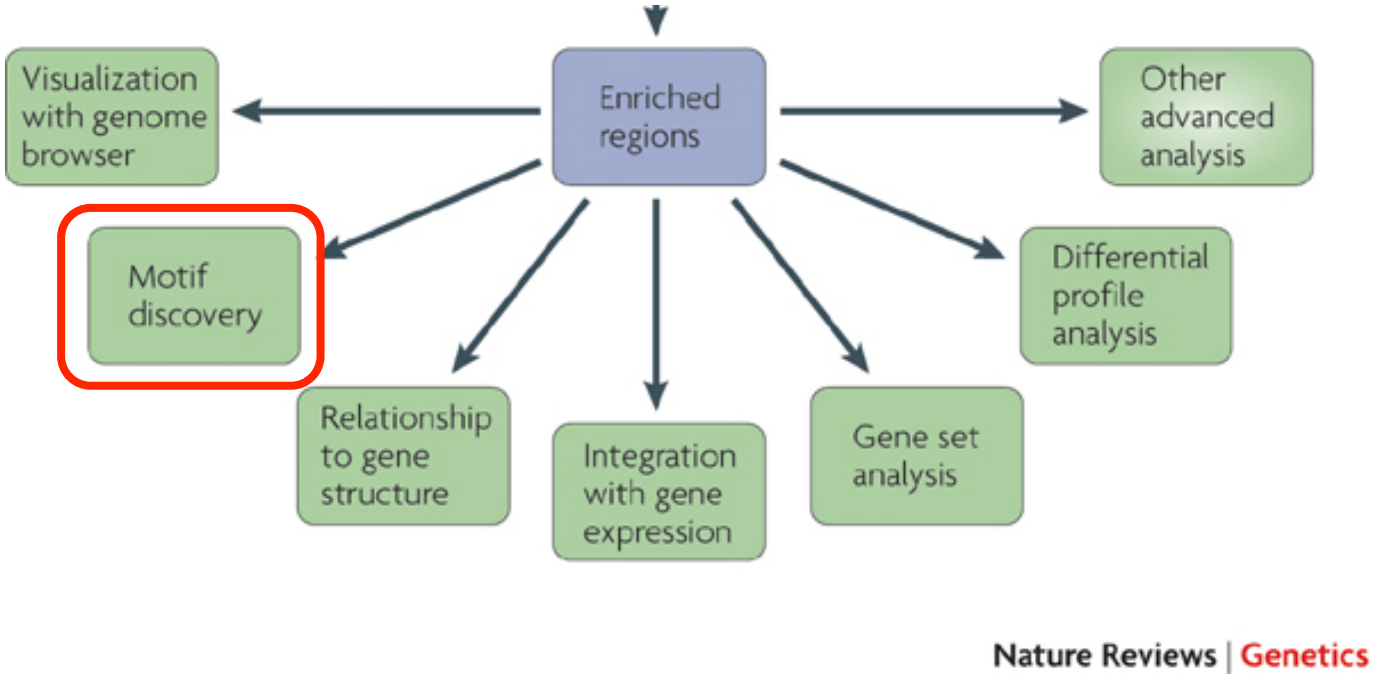
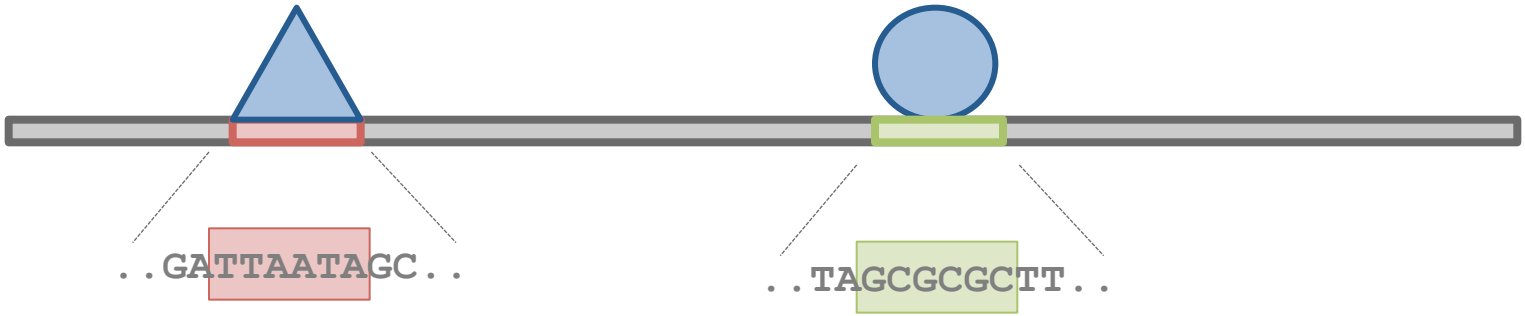
# ChIP-seq analysis workflow: downstream analyses




Park, Nature reviews 2009

# Transcription factor specificity

*How do TF « know » where to bind DNA ?*

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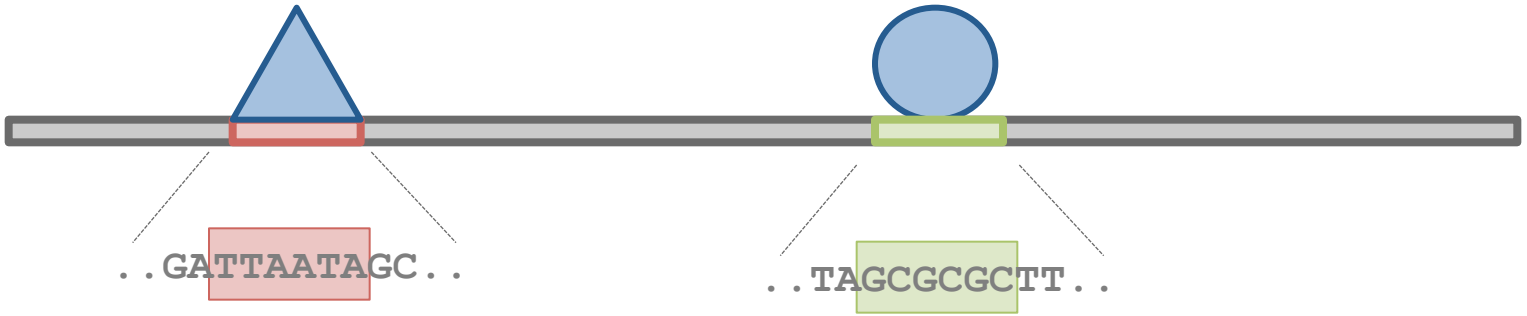


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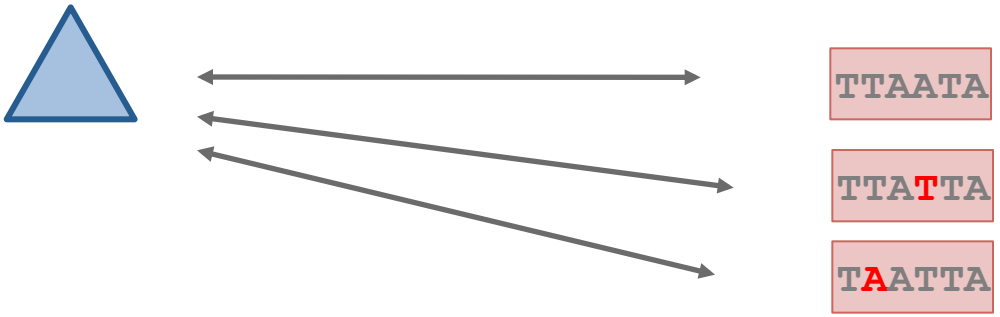
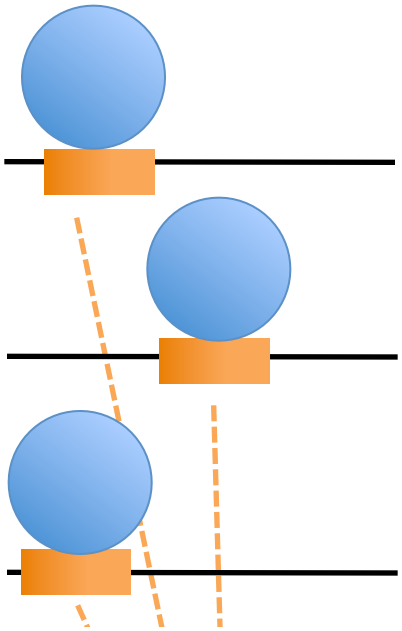


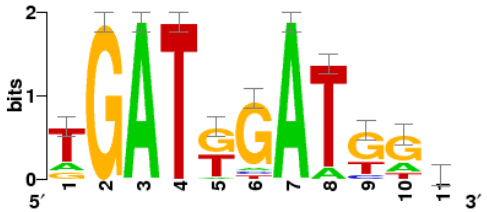

TFBSs are *degenerate*:
a given TF is able to bind DNA on TFBSs with different sequences

# Binding specificity of a given TF

transcription factor

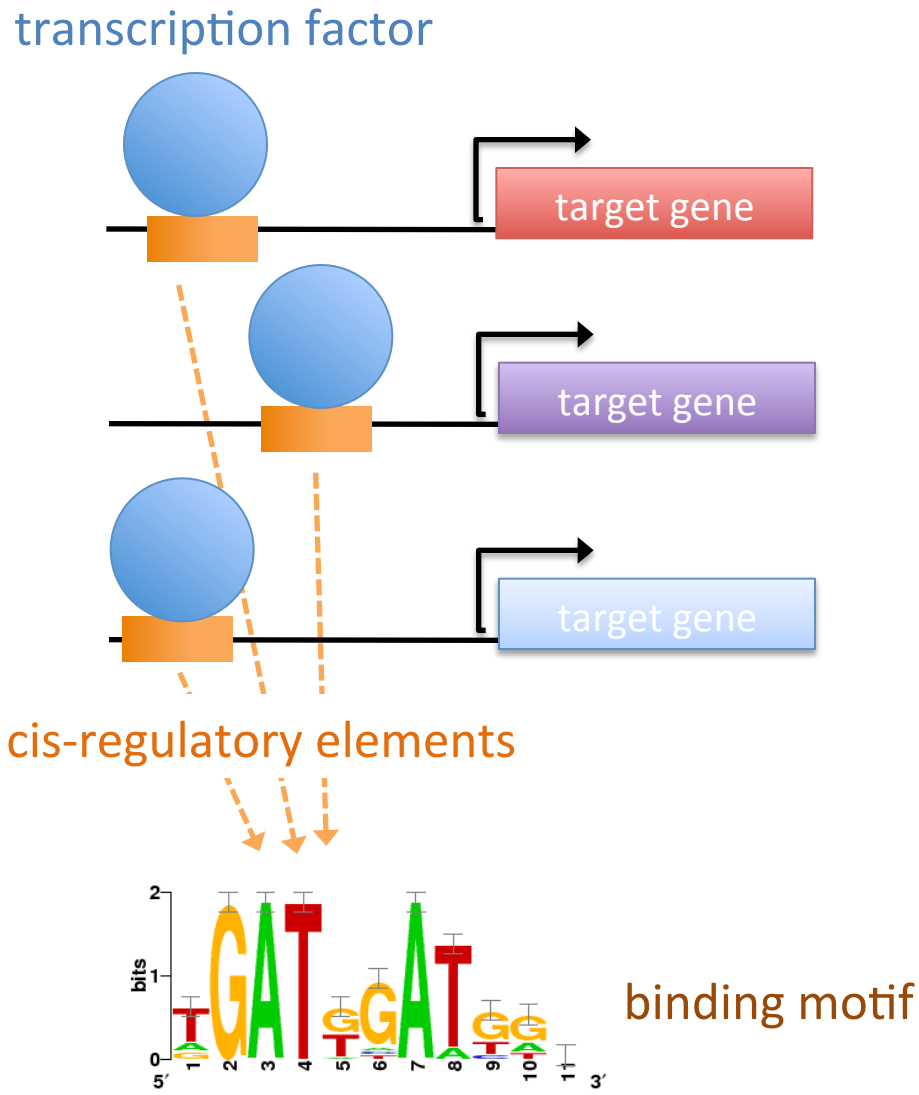


cis-regulatory elements




binding motif (represented as a sequence logo)

# *de novo* motif discovery




*Problem :*
*How can we model/describe the binding specificity of a given TF ?*

*If there is a common regulating factor, can we discover its motif only using these sequences ?*

# *de novo* motif discovery

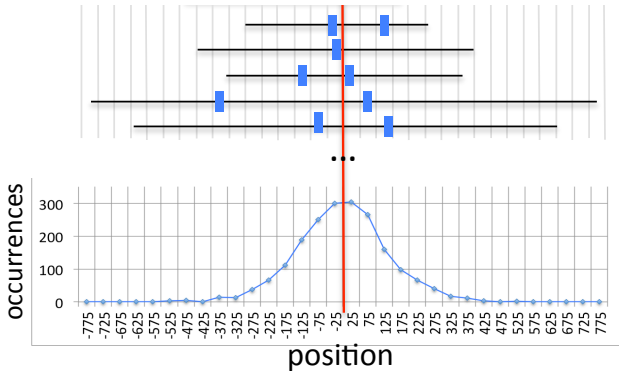
- Find exceptional motifs based on the sequence only

*(A priori* no knowledge of the motif to look for)

- Criteria of exceptionality:

  – higher/lower frequency than expected by chance (**over-/under-representation**)

  – concentration at specific positions relative to some reference coordinate (**positional bias**)



# *de novo* motif discovery

- Tools already exist for a long time !
  - MEME (1994)
  - RSAT oligo-analysis (1998)
  - AlignACE (2000)
  - Weeder (2001)
  - MotifSampler (2001)

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# Regulatory Sequence Analysis Tools (RSAT)

- **Since 1998 (15 years !)**
- **Initiated in Cuernavaca, Mexico**
- **yeast cis-regulatory elements**


**Jacques van Helden**

Aix*Marseille université

# RSAT developers/contributors over the years


Sylvain Brohée
Postdoc


Nicolas Simonis
Postdoc


Didier Croes
Postddoc


Didier Gonze
Premier assistant

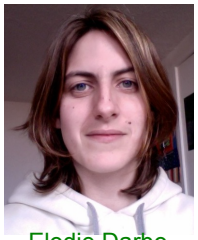

Carl Herrman
MCU


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


Raphaël Leplae
Postdoc


Gipsi Lima
PhD + Postdoc


Karoline Faust
PhD student


Rekin’s Janky
PhD student


Eric Vervisch
Research fellow


Guy Bottu
Postddoc


Lionel Spinelli
Engineer

- **Conception, implementation, evaluation and application of bioinformatics methods for the analysis of genomes and biomolecular networks.**
- **Regulatory sequences**
  - Motif analysis algorithms (*Olivier Sand , Matthieu Defrance , Maud Vidick, Alejandra Medina-Rivera)*
  - Evolution of cis-acting elements in Bacteria (*Rekin’s Janky, Alejandra Medina-Rivera*)
  - Regulation of development in Drosophila (*Jean Valéry Turatsinze, Elodie Darbo*)
  - Hox regulation in Vertebrates (*Morgane Thomas-Chollier)*
  - Work flows on transcriptional regulation (*Olivier Sand, Eric Vervisch)*
- **Biomolecular networks**
  - Network analysis tools (*Sylvain Brohée)*
  - Inference of metabolic pathways (*Karoline Faust, Didier Croes* )
  - Host-virus interaction networks (*Nicolas Simonis, Leon Juvénal Hagingambo)*
  - Analysis of regulatory networks (*Sylvain Brohée, Rekin’s Janky*)
- **Mobile genetic elements in prokaryotes** *(Raphaël Leplae, Gipsi Lima, Ariane Toussaint)*
- **Modelling of dynamical systems** *(Didier Gonze)*
- **e-Learning for bioinformatics** *(Guy Bottu)*

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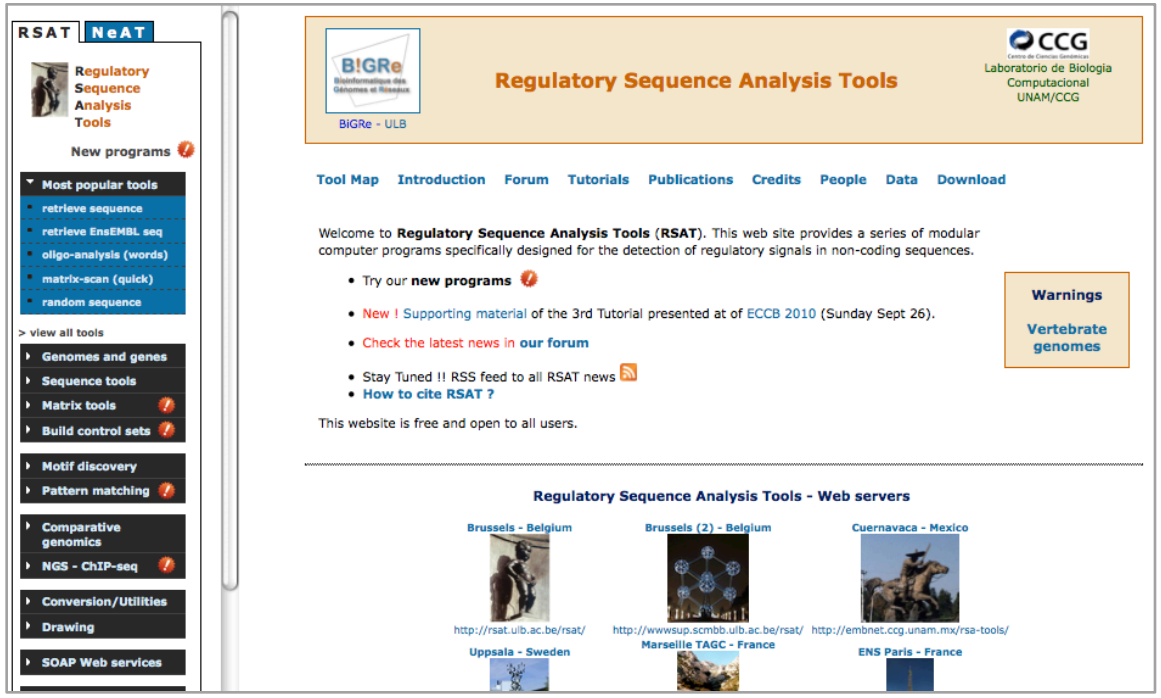
# RSAT improvements over the years

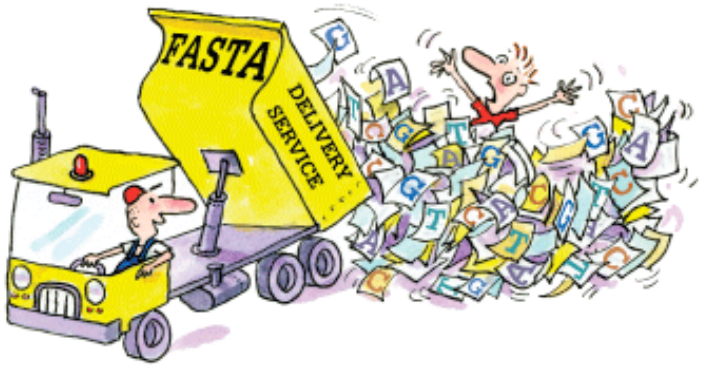



RSAT

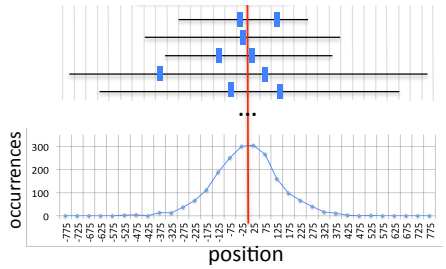
www.rsat.eu


*Thomas-Chollier, Darbo, Herrmann, Defrance , Thieffry, van Helden* ***Nature Protocols****, 2012*
*Thomas-Chollier Defrance, Medina-Rivera, Sand, Herrmann, Thieffry, van Helden* ***Nucleic Acids Research****, 2012*
*Medina-Rivera, Abreu-Goodger, Thomas-Chollier, Salgado, Collado-Vides, van Helden* ***Nucleic Acids Research****, 2011*
*Sand, Thomas-Chollier, van Helden* ***Bioinformatics****, 2009*
*Thomas-Chollier*, Sand*, Turatsinze, Janky, Defrance, Vervisch, van Helden* ***Nucleic Acids Research****, 2008*
*Sand, Thomas-Chollier, Vervisch, van Helden* ***Nature Protocols****, 2008*
*Thomas-Chollier*, Turatsinze*, Defrance, van Helden* ***Nature Protocols****, 2008*
*van Helden,* ***Nucleic Acids Research****, 2003*
*van Helden, André, Collado-Vides* ***Yeast****, 2000*

# New approaches for ChIP-seq datasets



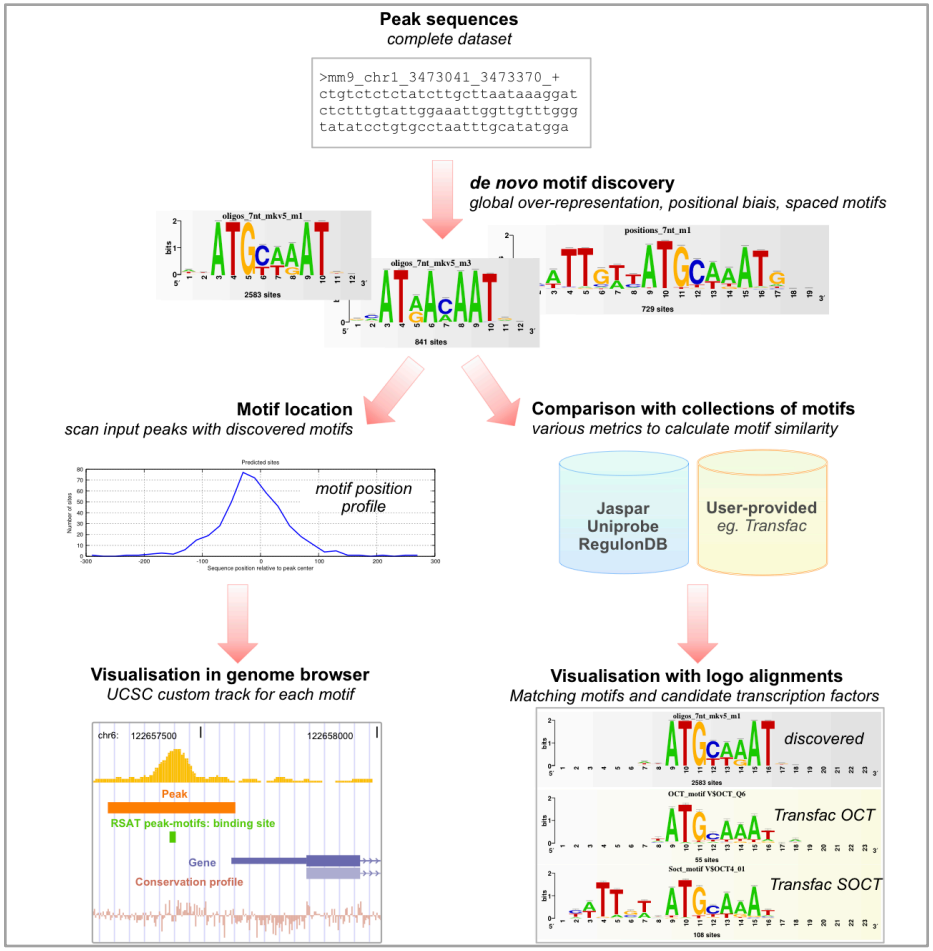

- **Size, size, size**
  - limited numbers of promoters and enhancers
  - dozens of thousands of peaks !!!!!!
- **the problem is slightly different**
  - promoters: 200-2000bp from co-regulated genes
  - peaks: 300bp, positional bias




- **motif analysis: not just for specialists anymore !**
  - complete user-friendly workflows

# New approaches for ChIP-seq datasets

- ***de novo* motif discovery (*peak-motifs* in RSAT)**




*Thomas-Chollier et al Nucleic Acids Research, 2012*

# Peaks coordinates

**BED**

```
chr1    3002142 3002195
chr1    3002804 3002853
```

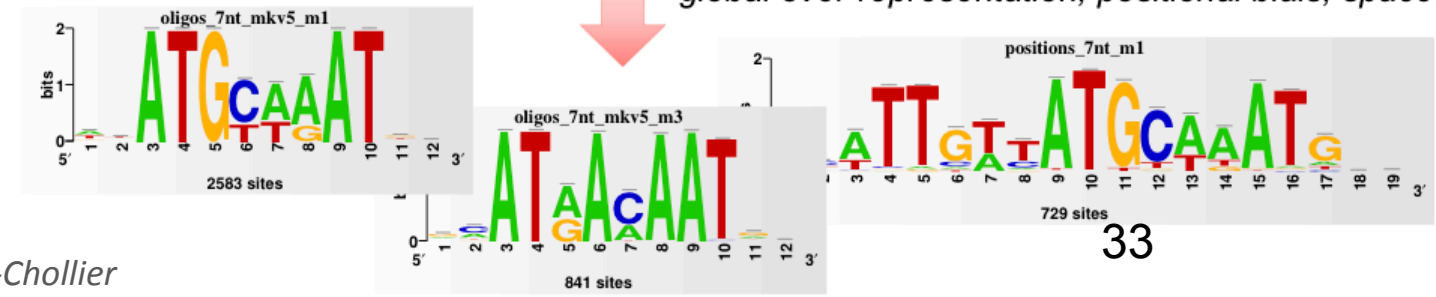
## Peak sequences

*complete dataset*

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

***de novo* motif discovery**

*global over-representation, positional biais, spaced motifs*



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Extract corresponding sequences

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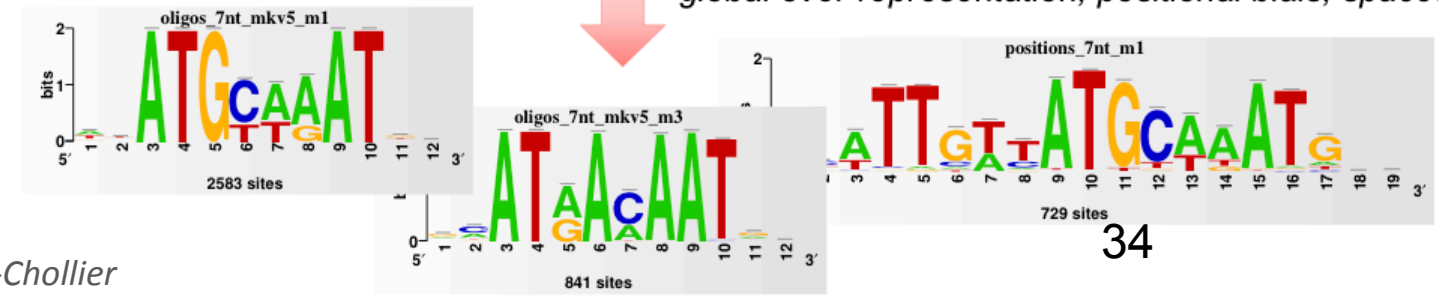
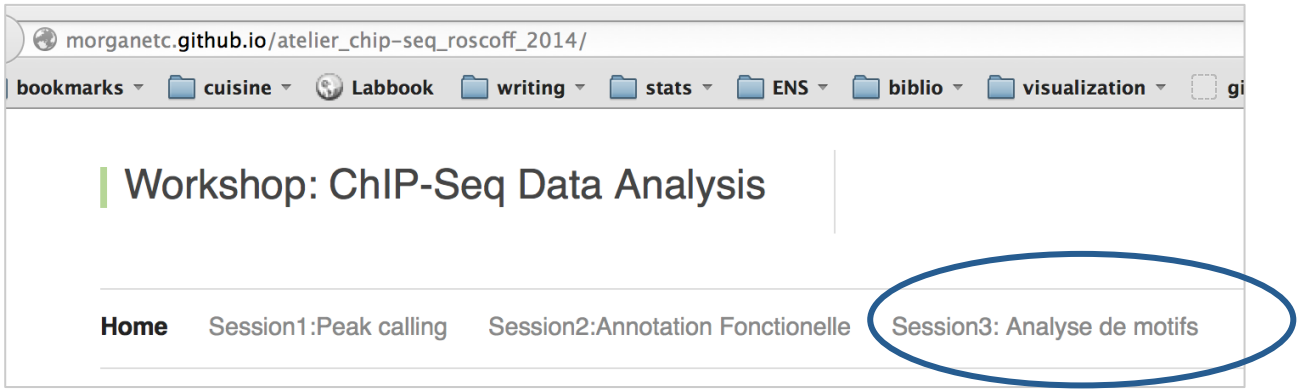


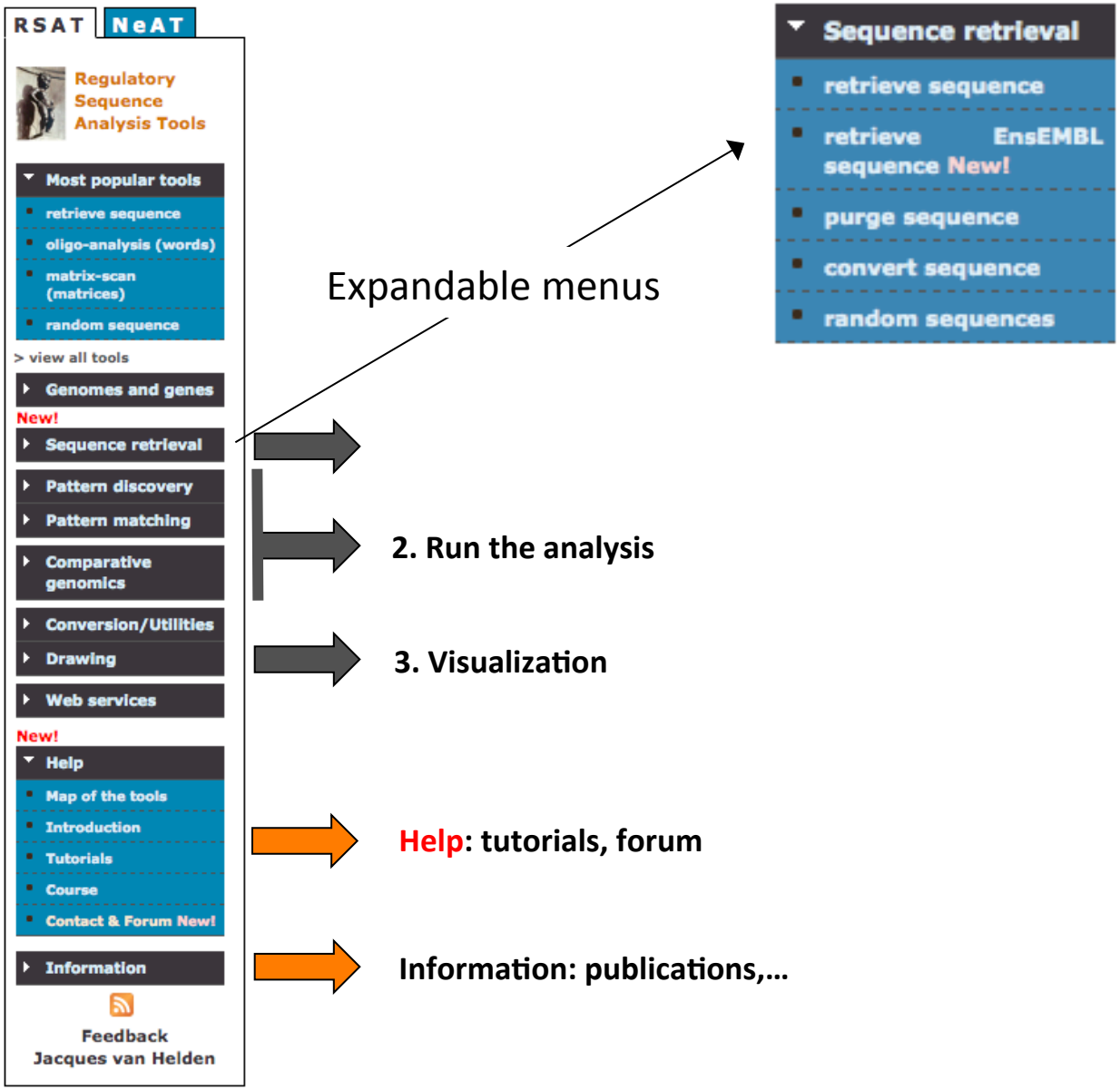
# Hands on !

- Go to the companion website
  - http://ecole-bioinfo-aviesan.sb-roscoff.fr/
  - Section “chip-seq”

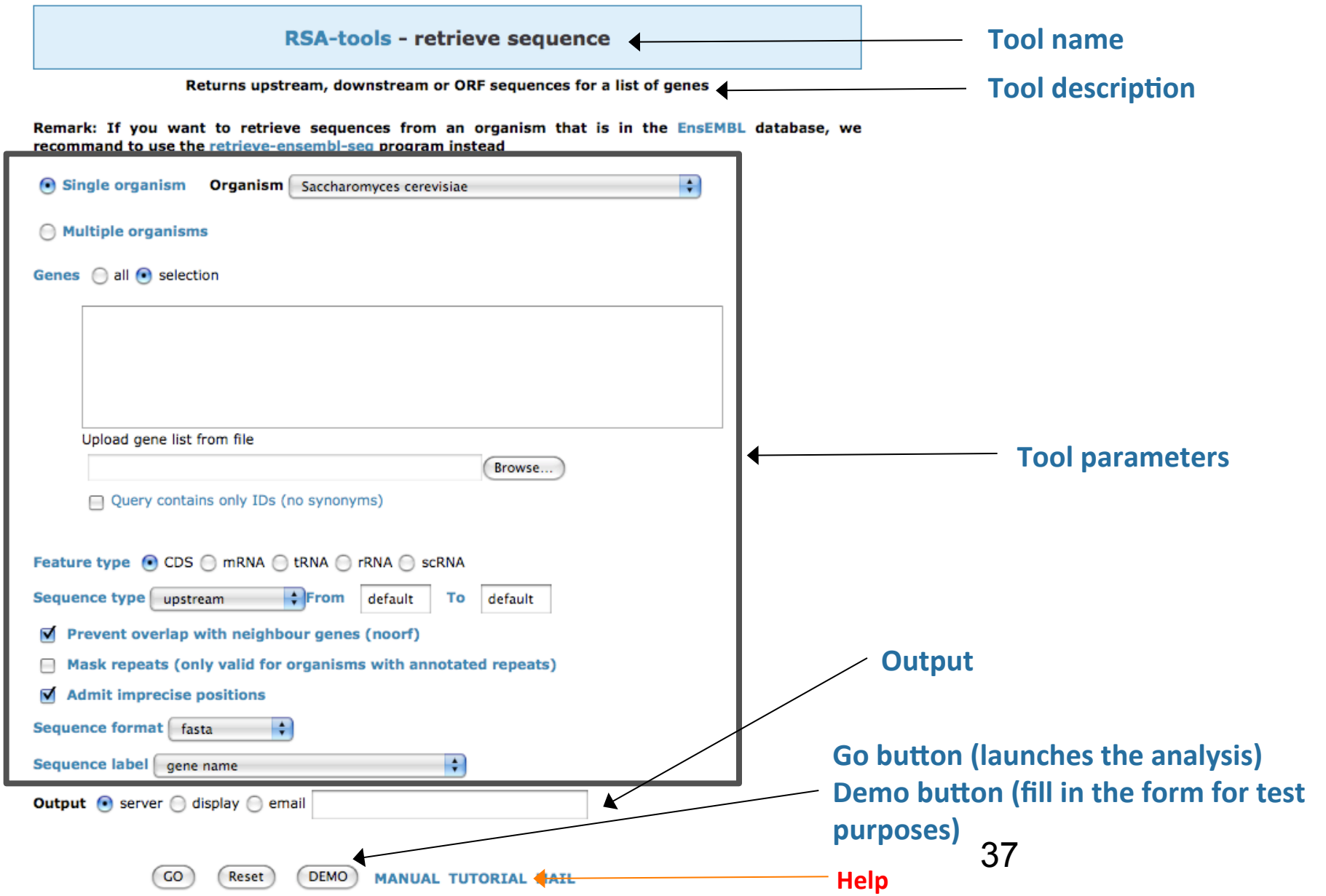



- Follow all steps of **Retrieving sequences from your peaks**

# Using RSAT


RSAT
NeAT
Regulatory Sequence Analysis Tools
Most popular tools
retrieve sequence
oligo-analysis (words)
matrix-scan (matrices)
random sequence
> view all tools
Genomes and genes
New!
Sequence retrieval
Pattern discovery
Pattern matching
Comparative genomics
Conversion/Utilities
Drawing
Web services
New!
Help
Map of the tools
Introduction
Tutorials
Course
Contact & Forum New!
Information
Feedback
Jacques van Helden
Sequence retrieval
retrieve sequence
retrieve EnsEMBL sequence New!
purge sequence
convert sequence
random sequences
Expandable menus
2. Run the analysis
3. Visualization
Help: tutorials, forum
Information: publications,...

# RSAT Web forms



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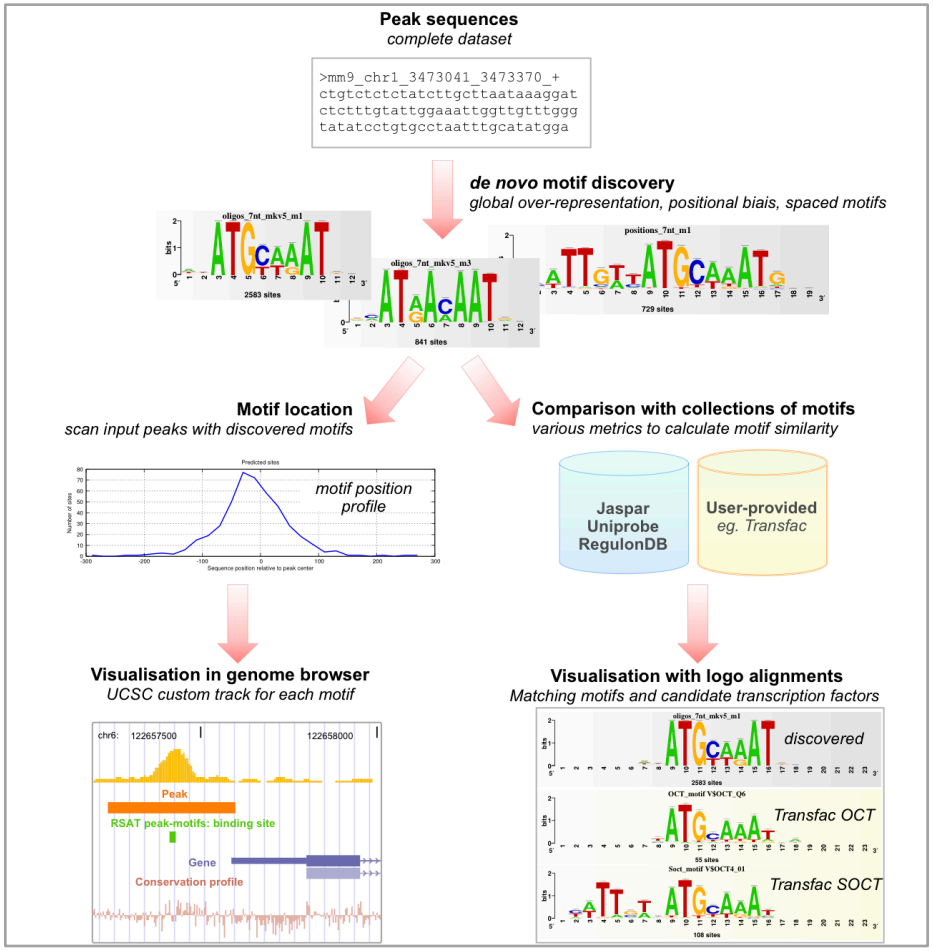


*Thomas-Chollier et al Nucleic Acids Research, 2012*

# Comparison of tools for ChIP-seq

| Program | peak-motifs | ChipMunk | CompleteMotifs | MEME-ChIP | MICSA | GimmeMotifs |
|---|---|---|---|---|---|---|
| **Web interface** | yes | yes | yes | yes | no | no |
| **Size limitation** | unrestricted (Web site tested with 22 Mb) | 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 | - |
| **Stand-alone version** | yes | yes | no | yes | yes | yes |
| **Tasks** | | | | | | |
| peak finding | no | no | no | no | yes | no |
| annotation of peak-flanking genes | no | no | yes | no | | no |
| sequence composition (mono- and di-nucleotides) | yes | no | no | no | | no |
| motif discovery | yes | yes | yes | yes | yes | yes |
| enrichment in motifs from databases | no | no | yes | yes | | no |
| enrichment in discovered motifs | yes | no | no | no | | no |
| peak scoring | no | no | no | yes | yes | no |
| motif clustering | no | no | no | no | | yes |
| comparison discovered motifs / motif DB | yes | no | no | yes | | yes |
| sequence scanning for site prediction | yes | no | no | yes | | no |
| positional distribution of sites inside peaks | yes | no | yes | no | | yes |
| visualization in genome browsers | yes | no | yes | no | | no |
| **Motif discovery algorithms** | RSAT oligo-analysis<br>RSAT dyad-analysis<br>RSAT position-analysis<br>RSAT local-word-analysis<br>+ in stand-alone version:<br>MEME<br>ChIPMunk | ChipMunk | ChipMunk<br>MEME<br>Weeder | MEME<br>DREME | MEME | MEME<br>Weeder<br>MotifSampler<br>BioProspector<br>Gadem<br>Improbizer<br>MDmodule<br>Trawler<br>MoAn |
| **Pattern matching algorithms** | RSAT matrix-scan-quick | no | patser | MAST + AME (enrichment) | | no |
| **Motif comparison algorithm** | RSAT compare-motifs | no | STAMP | TOMTOM | | STAMP |
| **Motif clustering algorithm** | | | | | | STAMP |
| **Comparison between discovered motifs** | yes | no | yes | no | | yes |
| **Motif database comparisons** | JASPAR<br>UNIPROBE<br>DMMPMM<br>RegulonDB<br>upload your own database | no | JASPAR<br>TRANSFAC | JASPAR<br>TRANSFAC<br>UNIPROBE<br>FLYREG<br>DPINTERACT<br>SCPD<br>DMMPMM<br>and many others | | no |
| **Motif sizes** | variable (multiple word assembly) | user-specified | <=25 for MEME<br><=12 for Weeder<br><=13 for ChipMunk | | | predefined ranges (small, medium, large, extra-large) |
| **Multiple motifs** | yes | no | yes | yes | | yes |
| **Ref (PMID)** | This article | 20736340 | 21183585 | 21486936 | 20375099 | 21081511 |

# Comparison of tools for ChIP-seq

| Program | peak-motifs | ChipMunk | CompleteMotifs | MEME-ChIP | MICSA | GimmeMotifs |
|---|---|---|---|---|---|---|
| **Web interface** | yes | yes | yes | yes | no | no |
| **Size limitation** | unrestricted (Web site tested with 22 Mb) | 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 | - |
| peak finding | no | no | no | no | yes | no |
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*Thomas-Chollier, Herrmann, Defrance, Sand, Thieffry, van Helden* ***Nucleic Acids***

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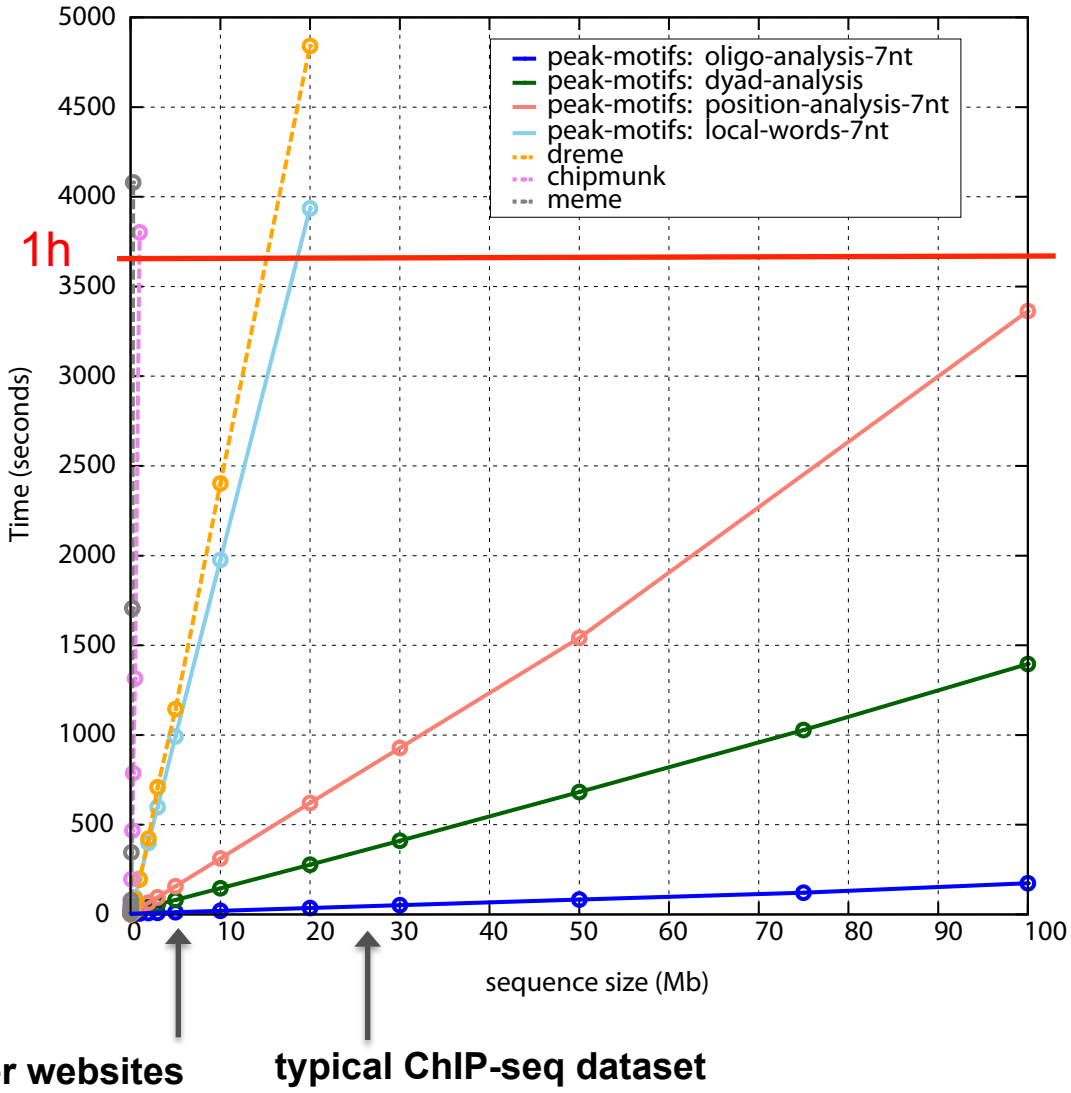
*Thomas-Chollier, Herrmann, Defrance, Sand, Thieffry, van Helden* ***Nucleic Acids***

# Peak-motifs: why providing yet another tool ?

- **fast and scalable**
- **treat full-size datasets**

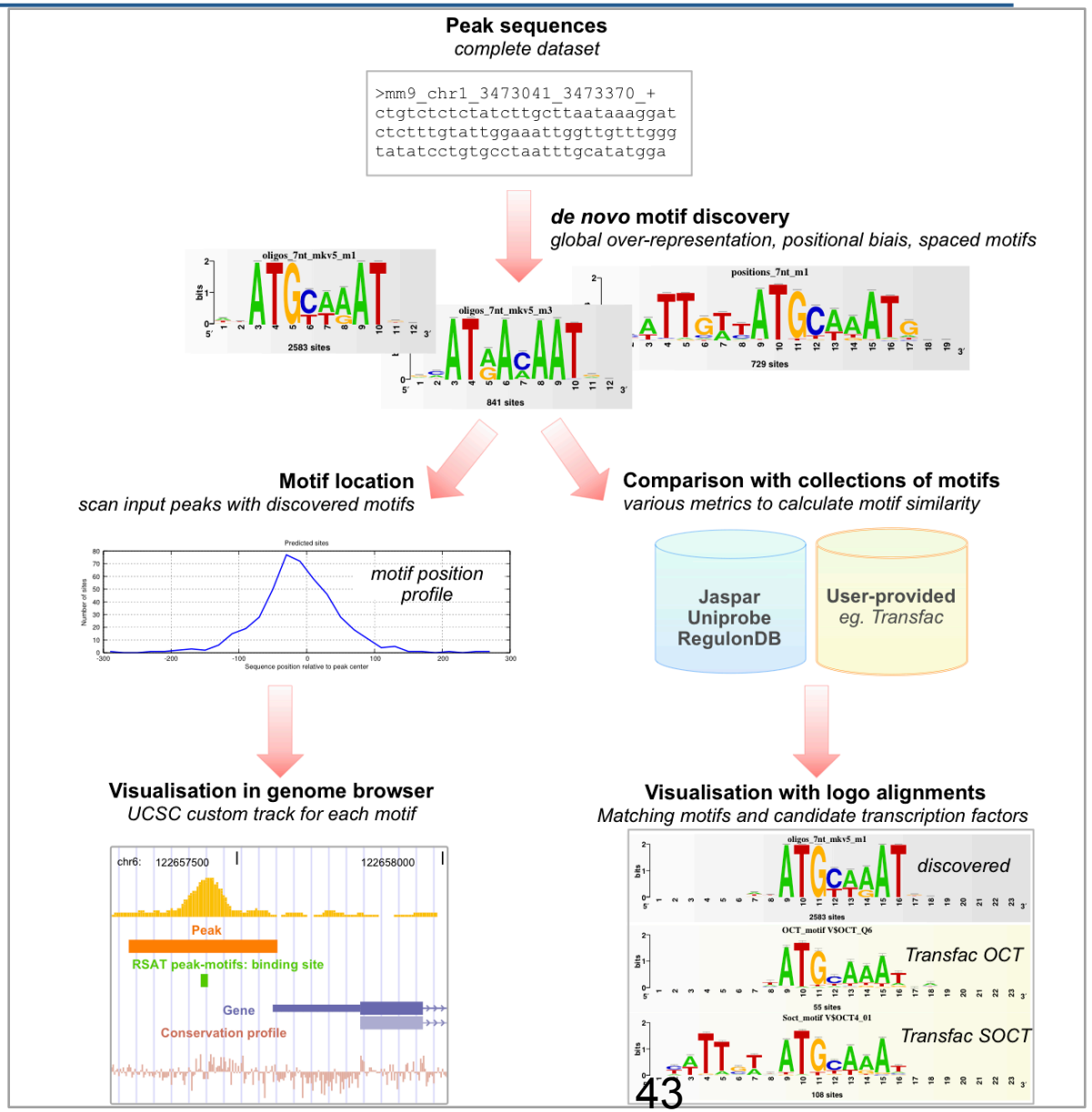





*Thomas-Chollier, Herrmann, Defrance, Sand, Thieffry, van Helden* ***Nucleic Acids Research****, 2012*

# Peak-motifs: why providing yet another tool ?

- fast and scalable
- treat full-size datasets
- **complete pipeline**

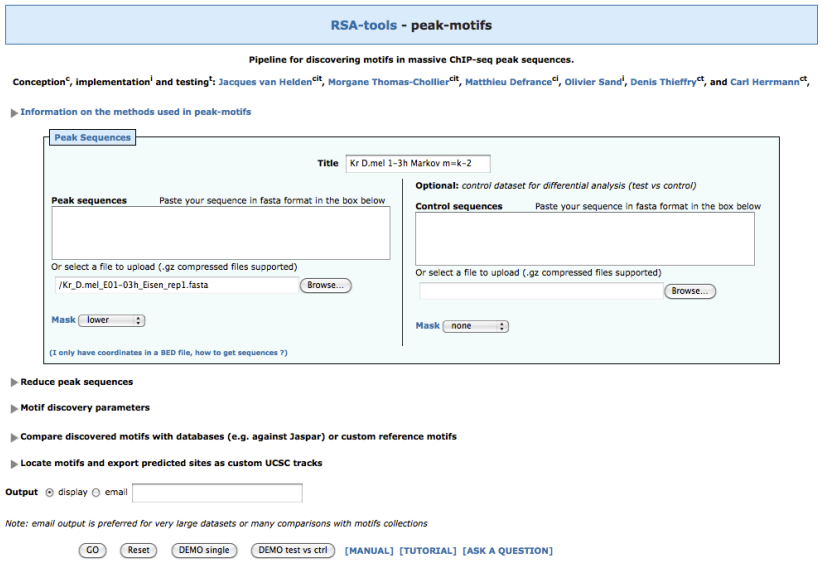





*Thomas-Chollier, Herrmann, Defrance, Sand, Thieffry, van Helden* ***Nucleic Acids***

# Peak-motifs: why providing yet another tool ?

- **fast and scalable**
- **treat full-size datasets**
- **complete pipeline**
- **web interface**



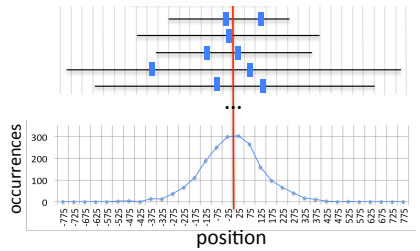

- **accessible to non-specialists**
  - Demo buttons
  - Tutorials & Protocols

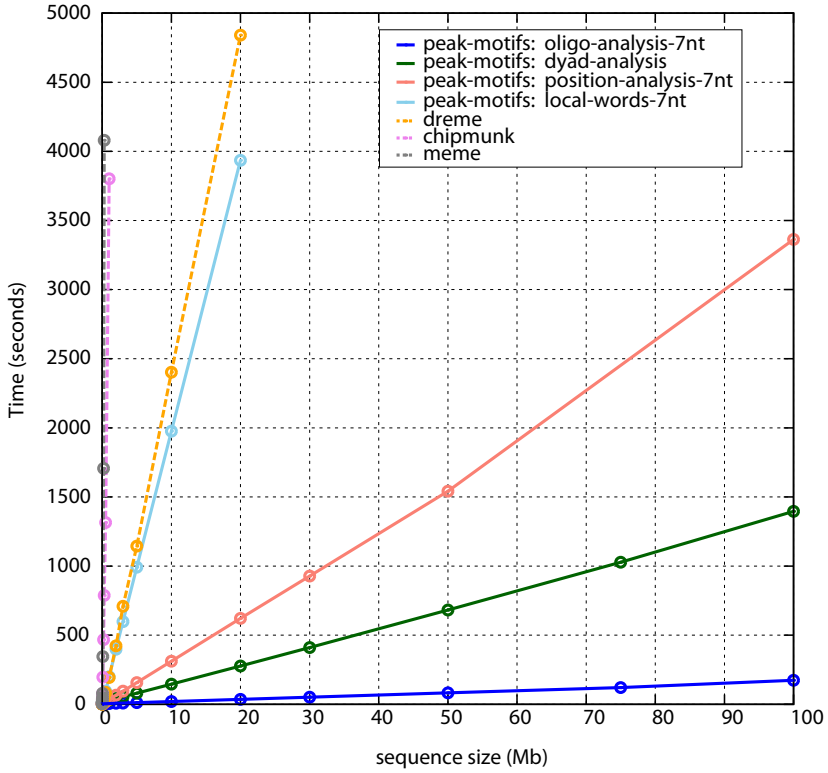
  *Thomas-Chollier, Darbo, Herrmann, Defrance, Thieffry, van Helden* ***Nature Protocols****,2012*

  - HTML report

# Peak-motifs: why providing yet another tool ?

- fast and scalable
- treat full-size datasets
- complete pipeline
- web interface
- accessible to non-specialists
- **using 4 complementary algorithms**
  - Global over-representation
    - oligo-analysis
    - dyad-analysis (spaced motifs)
  - Positional bias
    - position-analysis
    - local-words

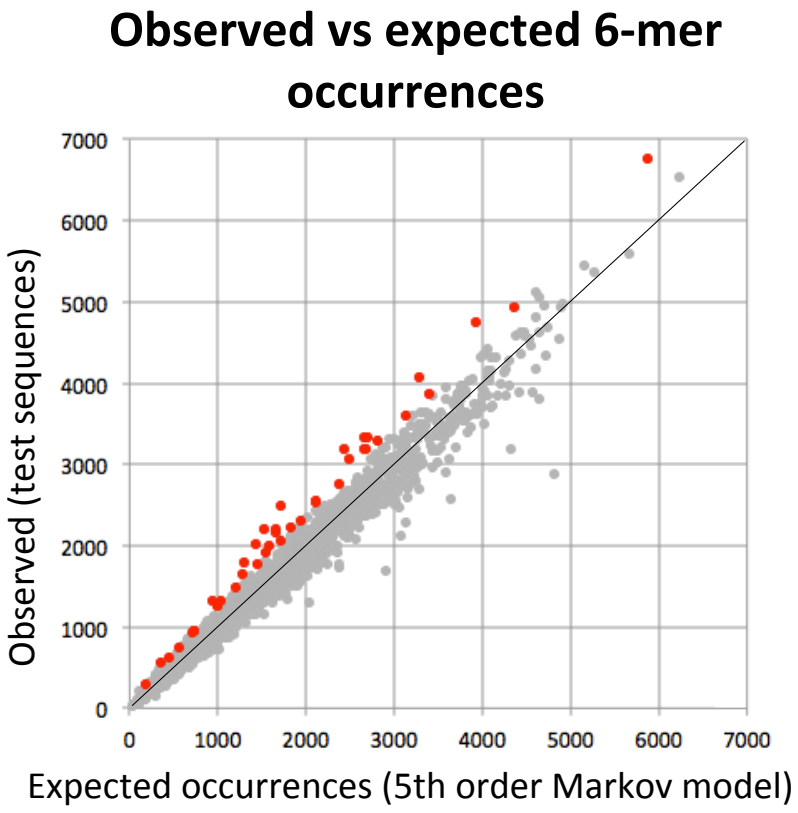


# Motif discovery methods: frequency

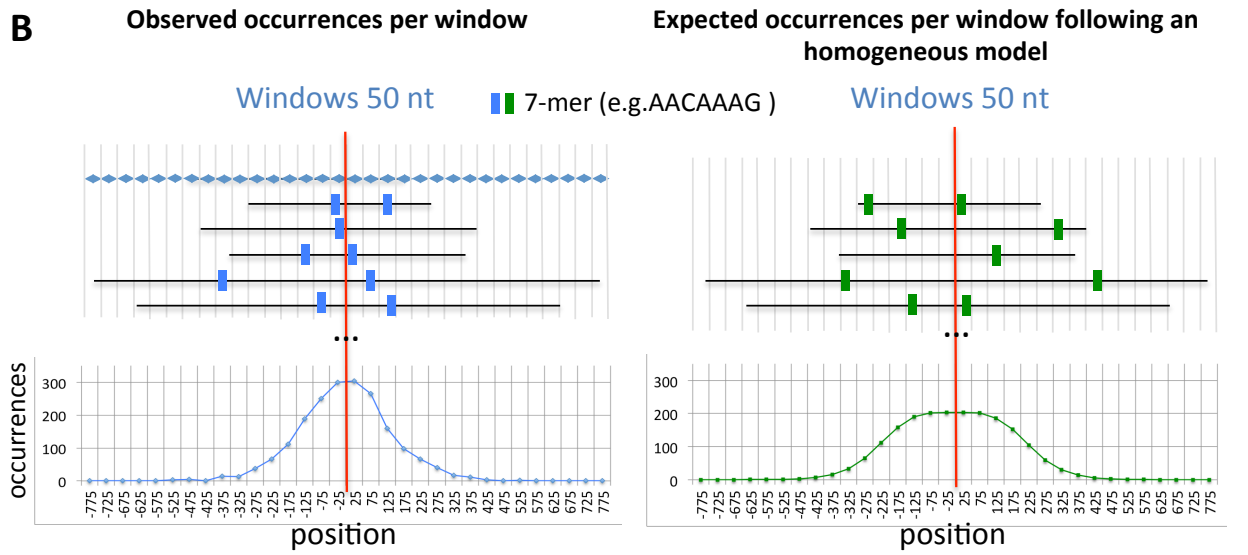
| **Observed** 6-mer occurrences computed from: | **Expected** 6-mer occurrences computed from: |
|---|---|
| 6-mer (e.g.AACAAA )<br>**Test sequences** | **Background sequences (when available)** |
| | OR |
| | **Theoretical k-mers frequencies from test sequences** |

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

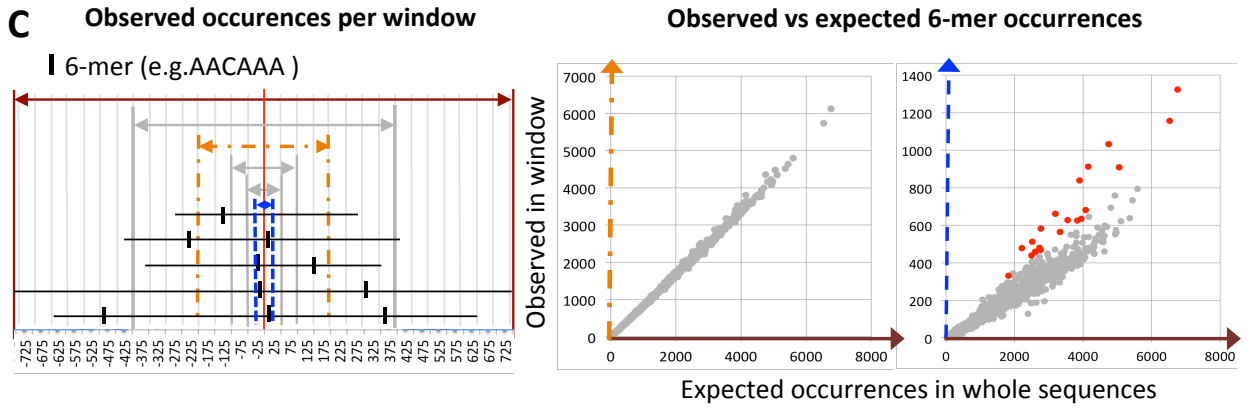



**oligo-analysis**
**dyad-analysis (spaced motifs)**

# Motif discovery methods: positional bias




position-analysis




local-words

# Hands on !

- Go to the companion website
- Follow **all steps** of **Discovering motifs from peak sequences**

# Hands on !

- Go to the companion website
- Follow all steps of **Visualizing the sites in the context of genome annotations**

# Hands on !

- Go to the companion website
- Follow all steps of **Visualizing the sites in the context of genome annotations**

# Hands on !

- Go to the companion website
- Follow all steps of **Motif analysis with MEME-chip**

# 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).
- **Matrix-quality** : RSAT program that can be used to evaluate the enrichment of motifs in peaks

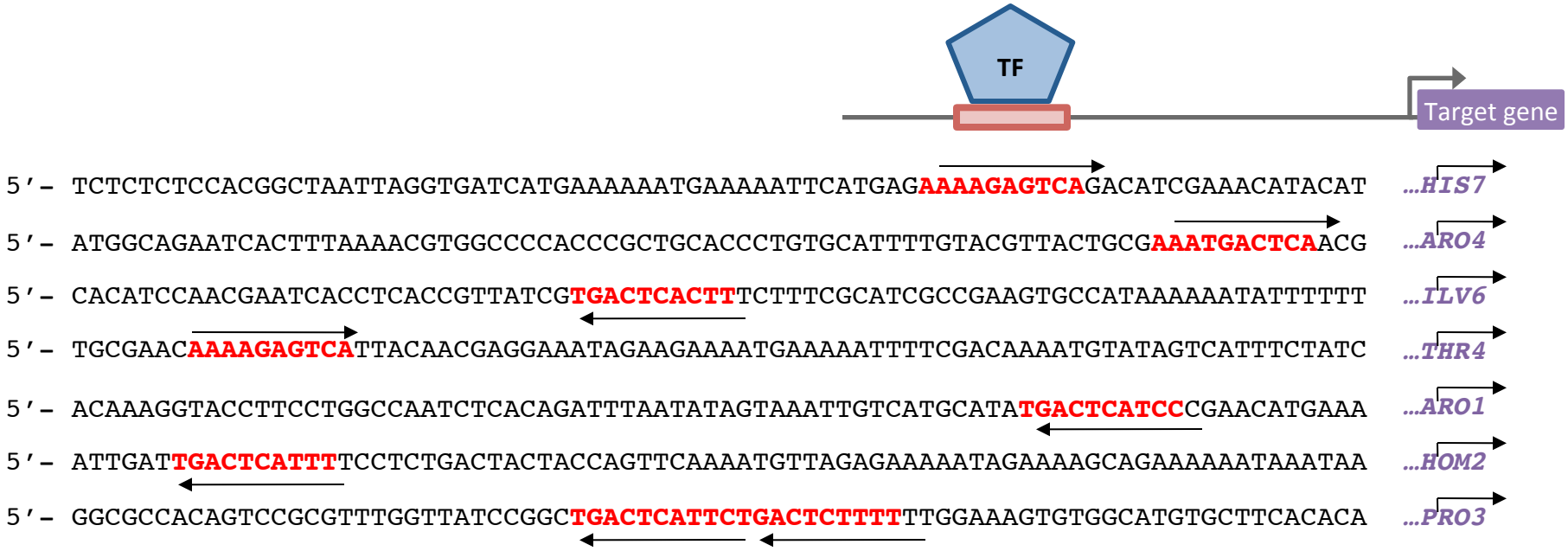
  Medina-Rivera A, Abreu-Goodger C, Thomas-Chollier M, Salgado H, Collado-Vides J, van Helden J.Theoretical and empirical quality assessment of transcription factor-binding motifs.Nucleic Acids Res. 2011 Feb;39(3):808-24. doi: 10.1093/nar/gkq710. Epub 2010 Oct 4.

# To go further

- Tutorial for ECCB 2014 : http://rsat.ulb.ac.be/eccb14/

- Master classes in analysis of cis-regulatory regions (over one week) at Ecole Normale Supérieure every september (contact : mthomas@biologie.ens.fr)

# Principle: detect unexpected patterns




- Binding sites are represented as “words” = “string”=“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

# Motif discovery using word counting

**Idea:**

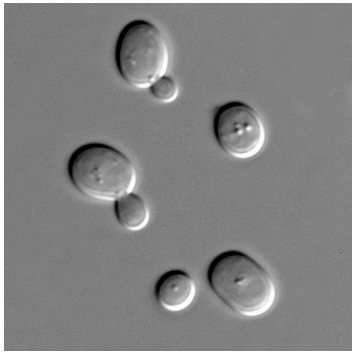
motifs corresponding to binding sites are generally repeated in the dataset

→ 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\|ctttttt | 15 |
| aagaaa\|tttctt | 14 |
| gaaaaa\|ttttttc | 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\|ctttttt | 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\|ttttttc | 19 |
| atatat\|atatat | 19 |
| agataa\|ttatct | 17 |
| agaaaa\|ttttct | 17 |
| aaagaa\|ttcttt | 16 |
| aaaaca\|tgtttt | 16 |
| aaaaag\|ctttttt | 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 | ctttttt | 15 |
| aagaaa | tttctt | 14 |
| gaaaaa | ttttttc | 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 | ctttttt | 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 | ttttttc | 19 |
| atatat | atatat | 19 |
| agataa | ttatct | 17 |
| agaaaa | ttttct | 17 |
| aaagaa | ttcttt | 16 |
| aaaaca | tgtttt | 16 |
| aaaaag | ctttttt | 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

- **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)

# 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\|ttttt | 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 |

| NIT | | |
|---|---|---|
| aaaaaa | tttttt | 80 |
| **cttatc** | **gataag** | **26** |
| tatata | tatata | 22 |
| **ataaga** | **tcttat** | **20** |
| aagaaa | tttctt | 20 |
| gaaaaa | ttttttc | 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 |
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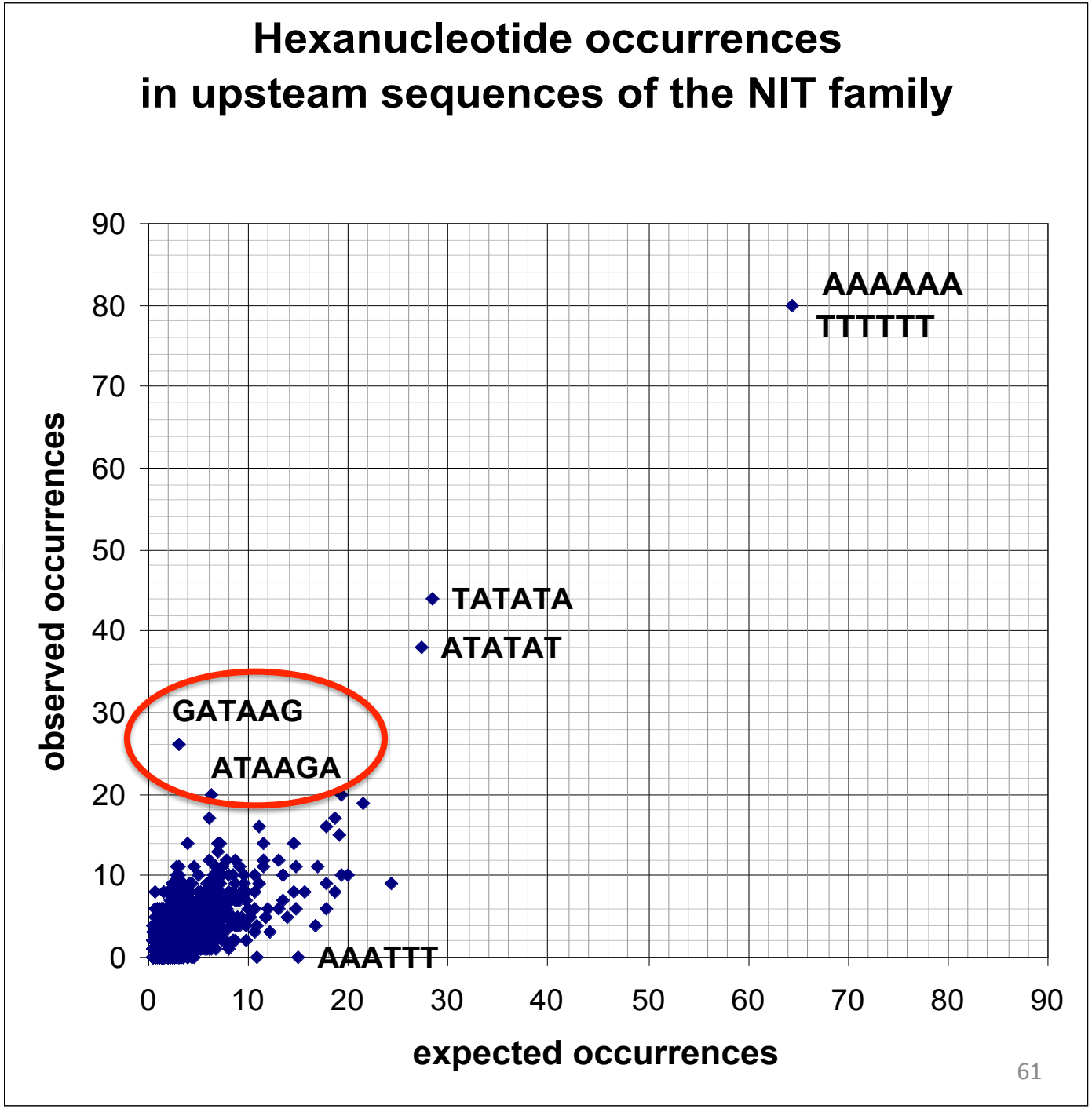
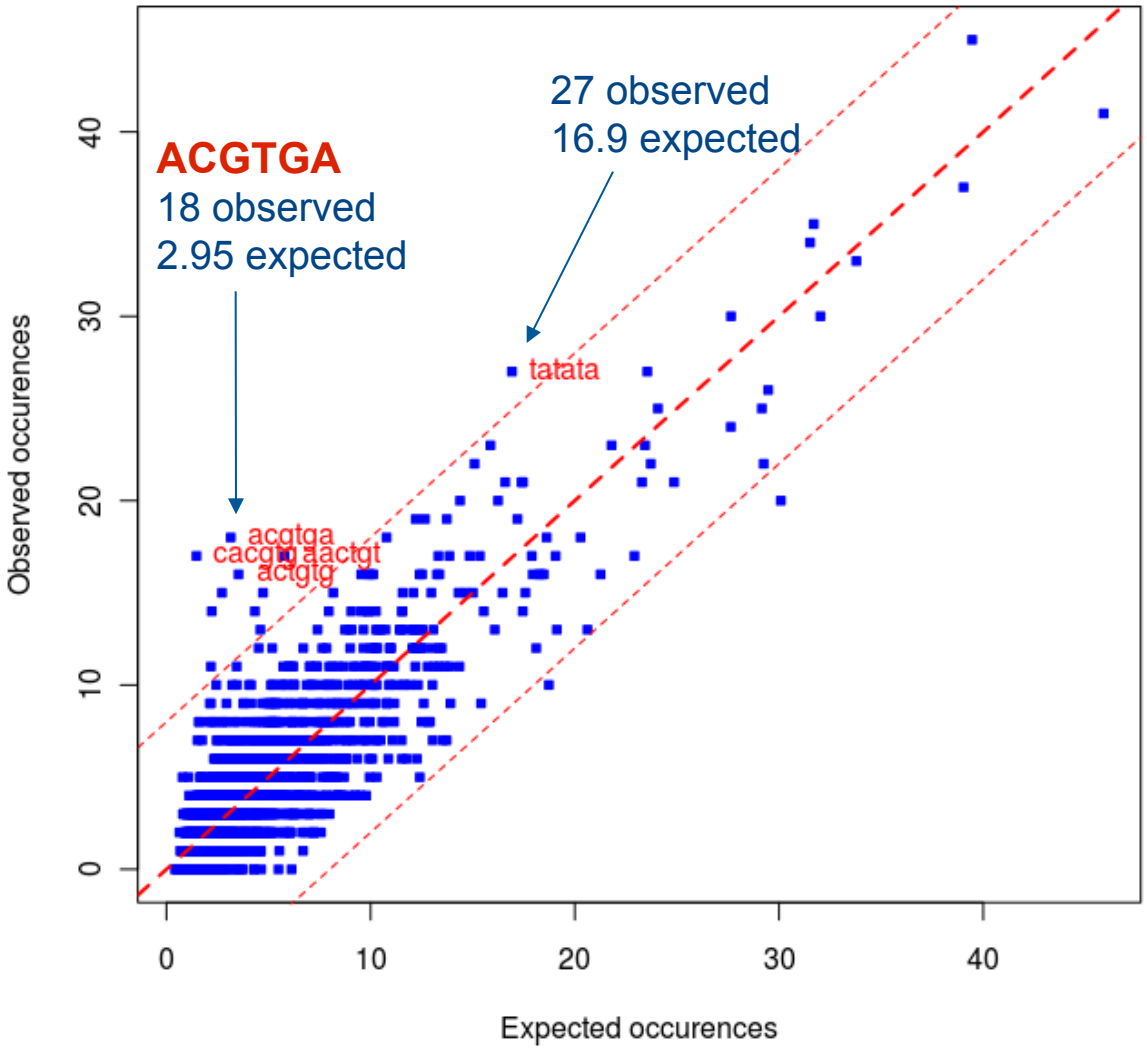


# Motif discovery using word counting




*How to evaluate expected number of occurrences ?*

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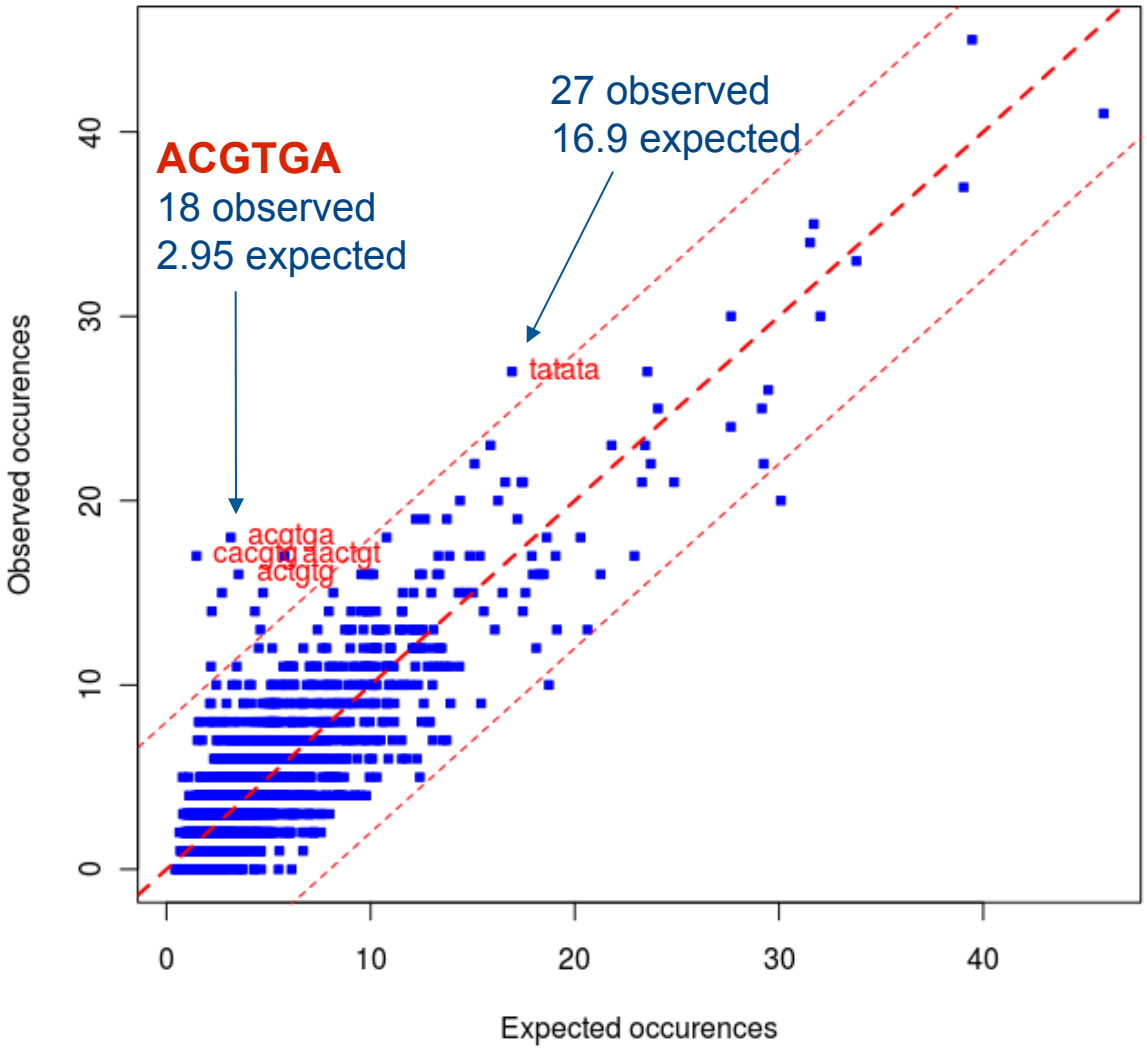
  - estimate the **expected number of occurrences** from a background model
    - empirical based on observed k-mer frequencies
    - theoretical background model (Markov Models)
  - **statistical evaluation of the deviation observed** (P-value/E-value)

# Statistical evaluation




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

## Statistical evaluation

*How « big » is the surprise to observe 18 occurrences when we expect 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 occurences

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

**Binomial distribution** to measure the "surprise"