# *Genome-scale pattern discovery*

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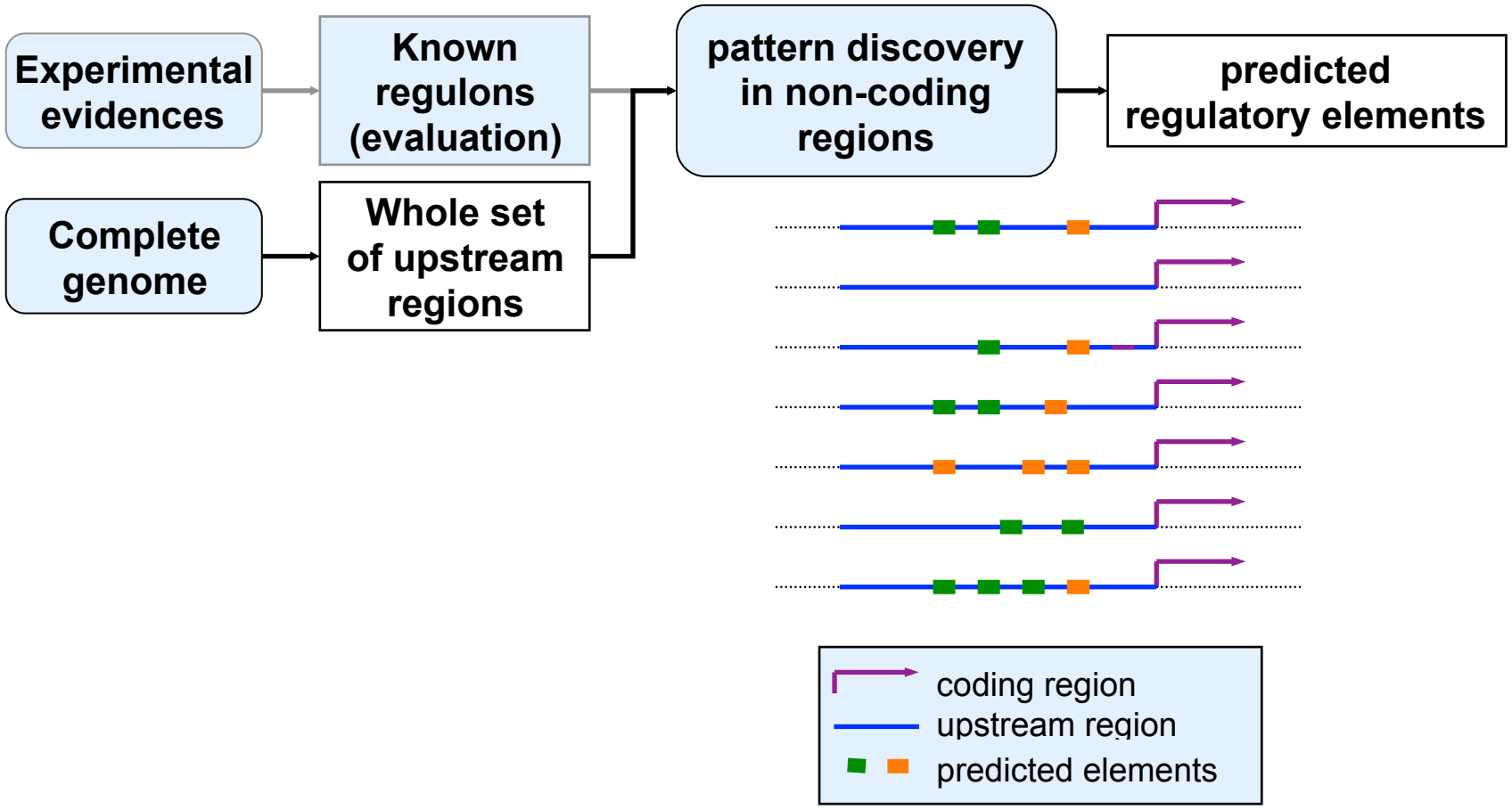
# *Genome-scale pattern discovery*



# *Genome-scale pattern discovery*

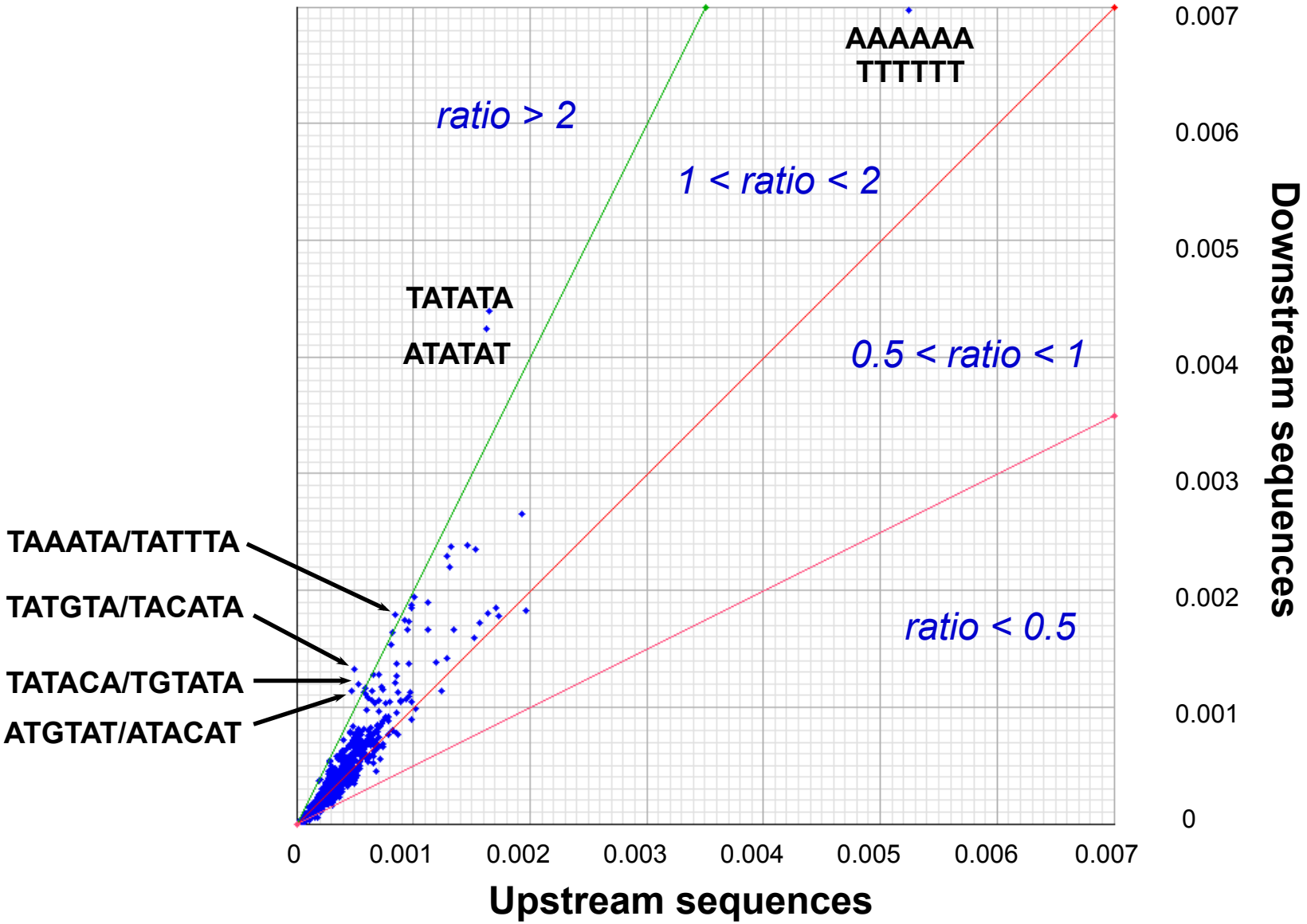
- Goal : extraction of functional signals involved in general mechanisms :
  - 5'-end signals (initiation of transcription)
  - 3'-end signals (termination of transcription, RNA cleavage and maturation)
- 3' end signal analysis
  - 6217 downstream sequences
  - 200 bp from the stop codon
- Problem: how to estimate expected word frequencies ?
  - The family now includes all yeast genes

# *Expected frequencies: external reference*

- Downstream sequences vs whole genome frequencies
  - problem of interpretation
    - may reflect merely differences between non-coding and coding sequences, which represent 73% of the genome
- Downstream versus upstream sequences
  - problem of interpretation:
    a word may be significant because
    - over-represented in downstream sequences
    - under-represented in upstream sequences

# *Analysis of downstream sequences reveals signals of transcriptional termination, RNA cleavage and poly-adenylation*

# Downstream vs upstream sequences





- van Helden, J., del Olmo, M. and Pérez-Ortín, J.E. (2000) Statistical analysis of yeast genomic downstream sequences reveals putative polyadenylation signals. *Nucleic Acids Res*, **28**, 1000–1010.

# *Estimation of expected frequencies with Markov models*

- Estimation of expected word frequencies
  - On basis of the input sequences themselves
  - Markov chain models: the expected frequency of each k-letter word is estimated on basis of sub-word frequencies.

- Example
  - Estimation of hexanucleotide frequencies with a 4th order Markov chain mode.

$$\text{e.g.: } \exp\{GATAAG\} = \frac{\text{obs}\{GATAA\} \times \text{obs}\{ATAAG\}}{\text{obs}\{ATAA\}}$$

# Oligo-analysis with Markov chain models

- Analysis of a set of 6217 downstream sequences, 200bp each
- Detection of over-represented words, and grouping by sequence similarity

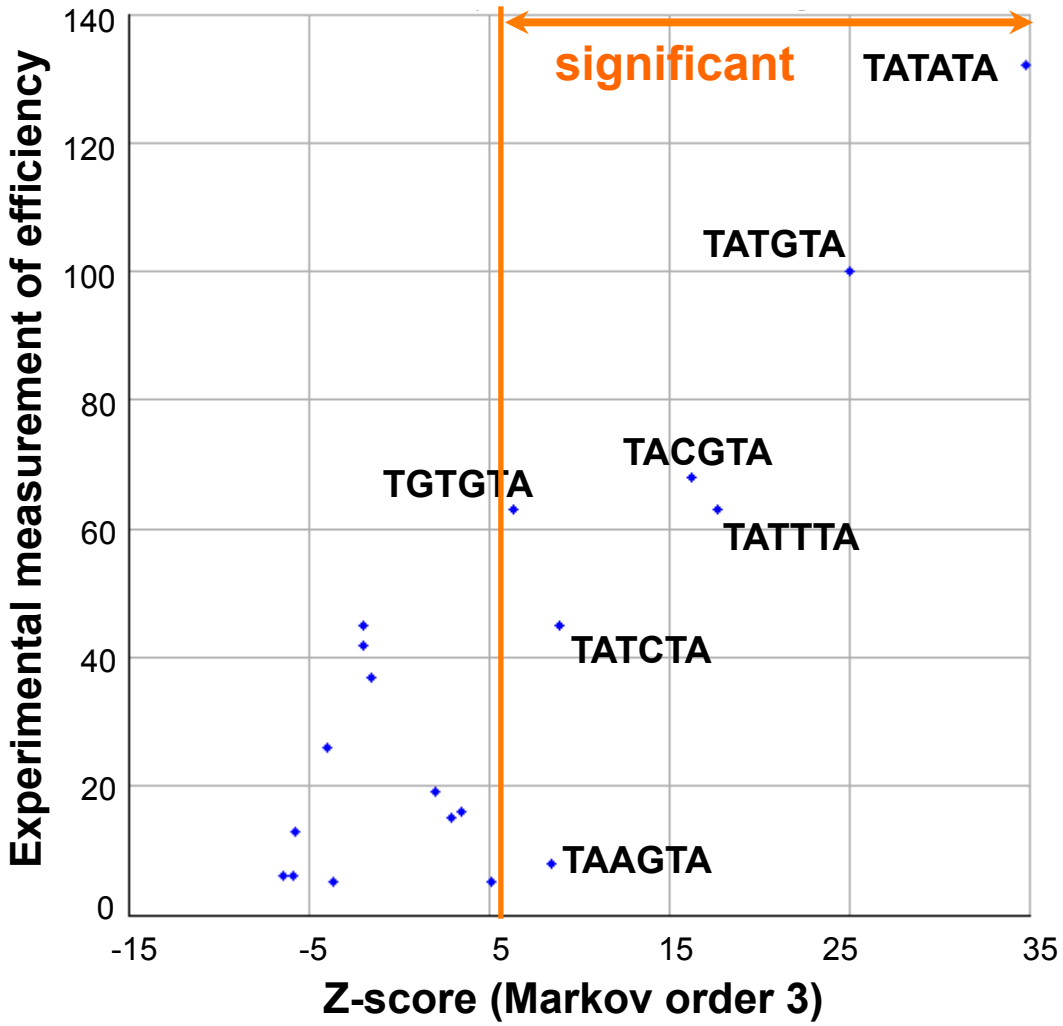
| | |
|---|---|
| ATATAT. | 27.0 |
| ATACAT. | 15.5 |
| ATGTAT. | 11.9 |
| ATAAAT. | 9.9 |
| ATAGAT. | 9.9 |
| ATTTAT. | 9.8 |
| GTATAT. | 8.2 |
| ATATGT. | 7.8 |
| ACATAT. | 7.7 |
| ATATAC. | 7.4 |
| .TATATA | 34.9 |
| .TACATA | 27.7 |
| .TATGTA | 25.0 |
| .TAAATA | 22.0 |
| .TATTTA | 17.7 |
| .TAGATA | 11.9 |
| .TGTATA | 8.6 |
| .TATACA | 7.3 |
| .CATATA | 3.5 |

| | |
|---|---|
| AAAAAA | 18.28 |
| AAATAA | 16.65 |
| AATAAA | 14.09 |
| AAGAAA | 9.27 |
| AACAAA | 9.02 |
| AAAGAA | 8.17 |
| AAACAA | 7.69 |

| | |
|---|---|
| TTTTTT | 16.87 |
| TTATTT | 16.74 |
| TTTATT | 13.25 |
| TTTCTT | 9.42 |
| TTTGTT | 8.72 |
| TTCTTT | 8.46 |

| | |
|---|---|
| ACATAC. | 12.21 |
| ACACAC. | 11.15 |
| .CACACA | 13.00 |
| .CATACA | 8.81 |

# Comparison with experimental values

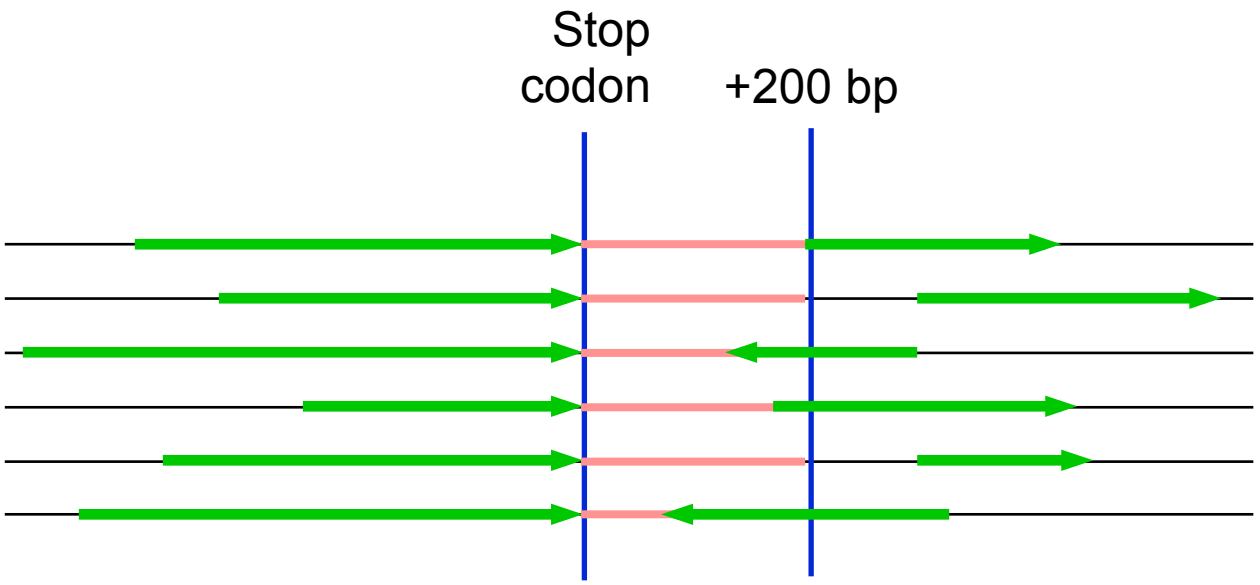



- Irniger and Braus (1994) performed a saturation mutagenesis and measured the the efficiency of all single-base mutants of TATGTA.
- High Z-score values from Markov 4 model correlate pretty well with experimental efficiency


- van Helden, J., del Olmo, M. and Pérez-Ortín, J.E. (2000) Statistical analysis of yeast genomic downstream sequences reveals putative polyadenylation signals. *Nucleic Acids Res*, **28**, 1000–1010.

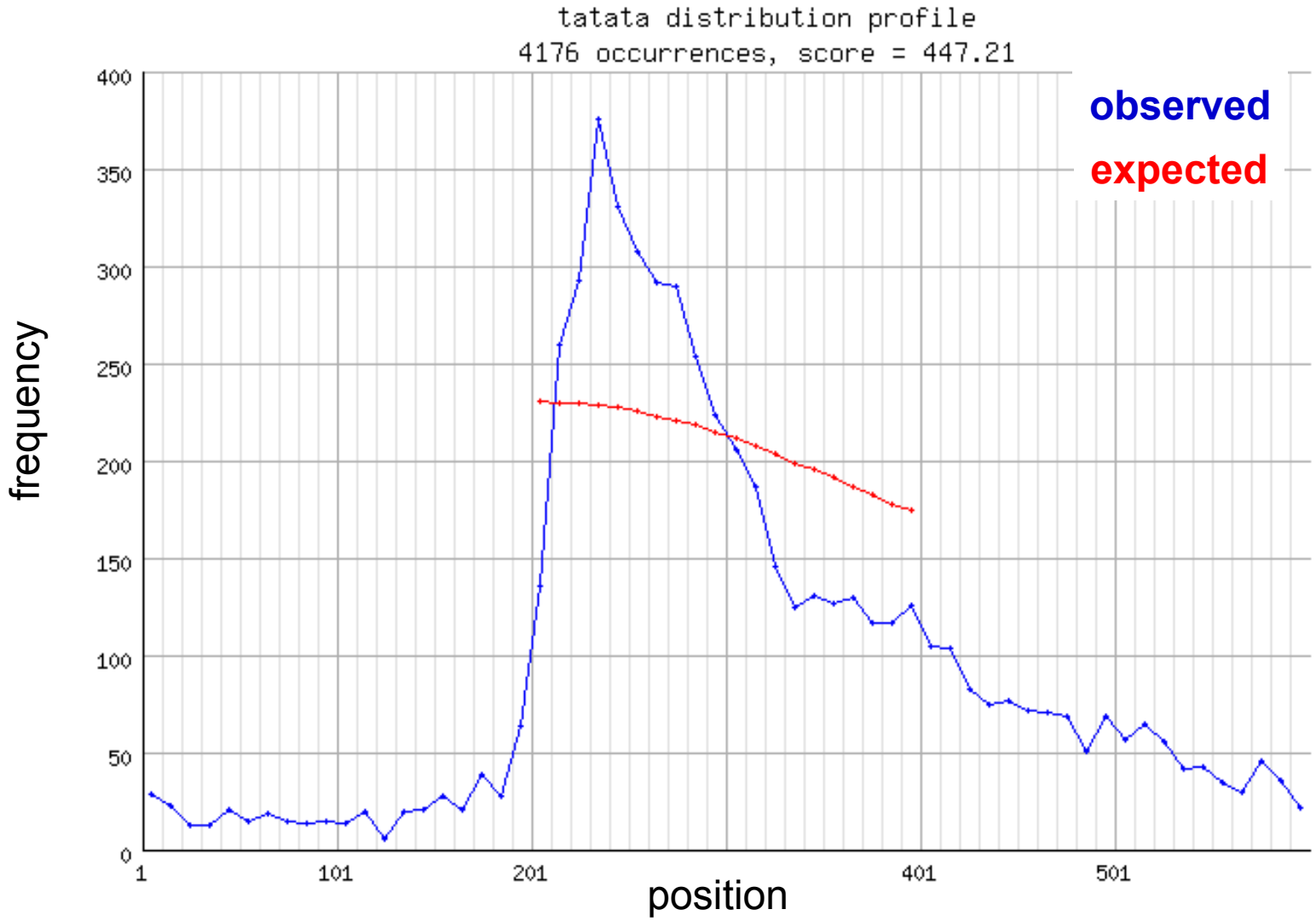
# *Position analysis*

- Measure the positional distribution of each word
- Perform a test of homogeneity and select all words with a significant bias
- Significance of the non-homogeneity is estimated with a $\chi 2$ test
- Note : in our case, homogeneous is not flat, because sequences are clipped when there is a downstream ORF closer than 200 bp



# *Word position distribution*

- Positions relative to the stop codon

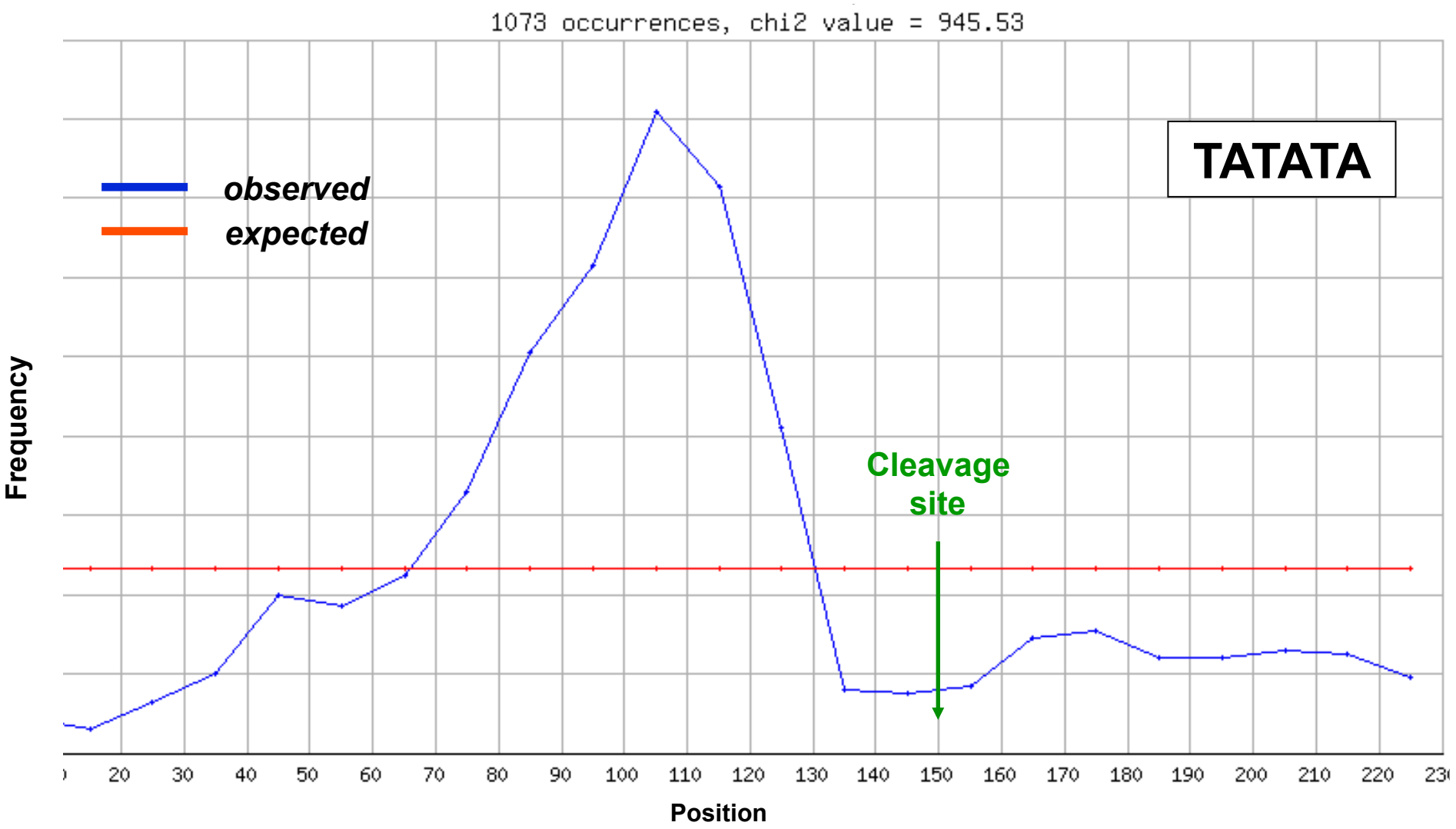




- van Helden, J., del Olmo, M. and Pérez-Ortín, J.E. (2000) Statistical analysis of yeast genomic downstream sequences reveals putative polyadenylation signals. *Nucleic Acids Res*, **28**, 1000–1010.

# Position analysis : profiles of word distribution

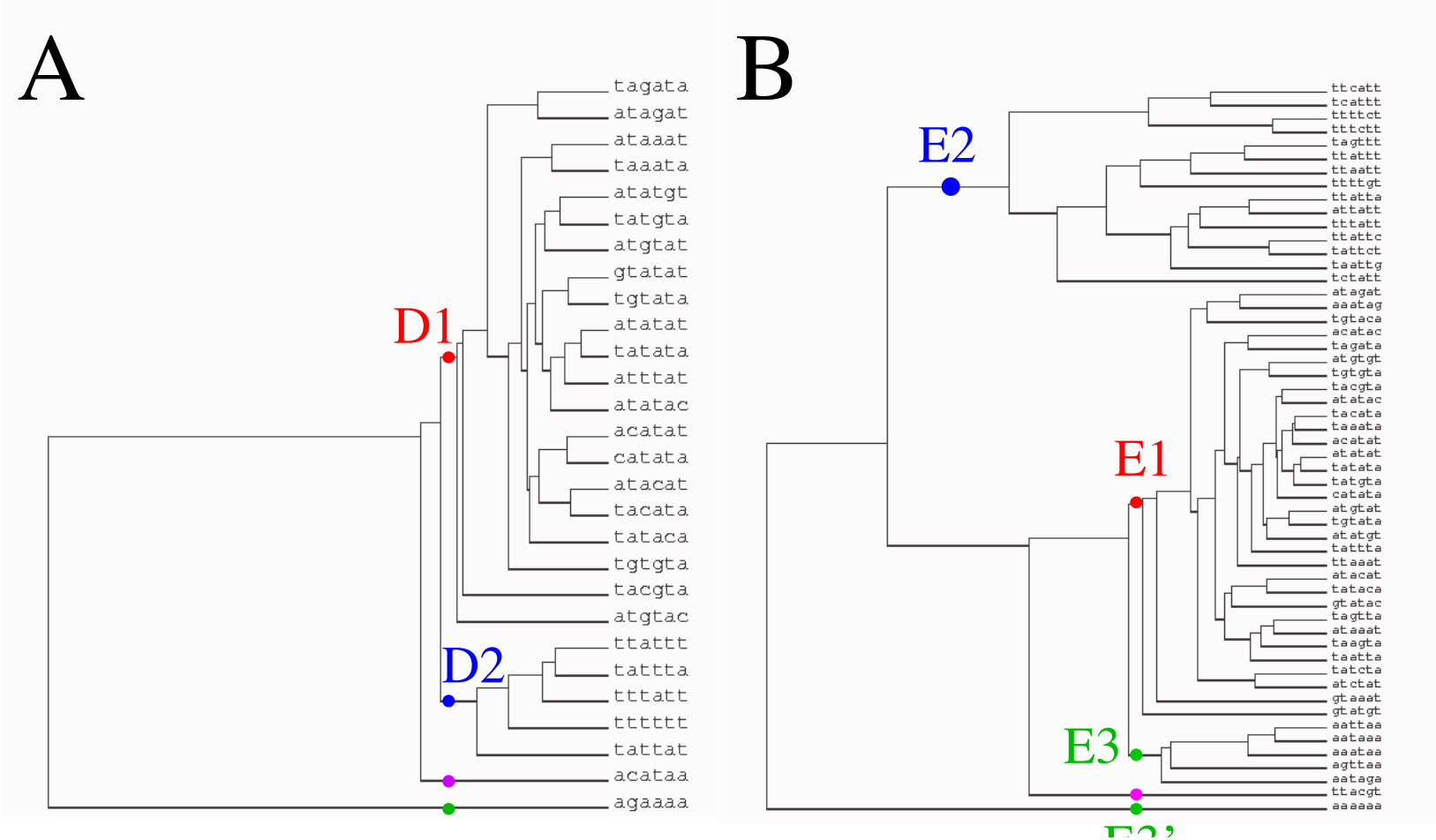
- Positions relative to the cleavage site





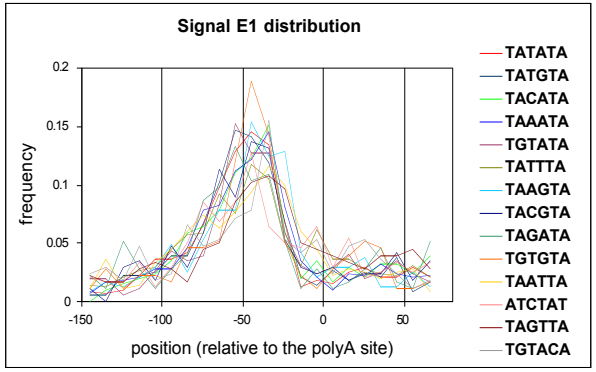
- van Helden, J., del Olmo, M. and Pérez-Ortín, J.E. (2000) Statistical analysis of yeast genomic downstream sequences reveals putative polyadenylation signals. *Nucleic Acids Res*, **28**, 1000–1010.

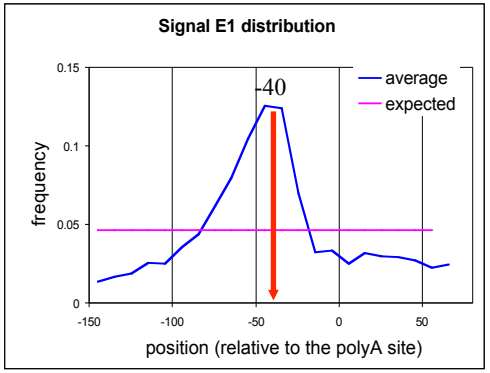
# Word clustering according to position profiles

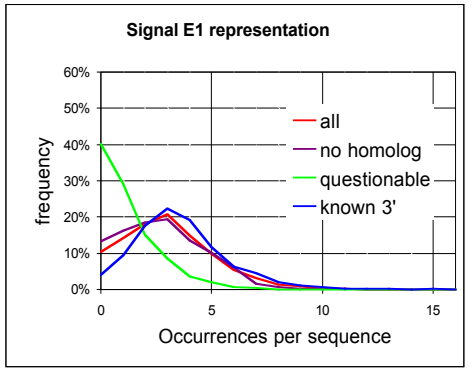


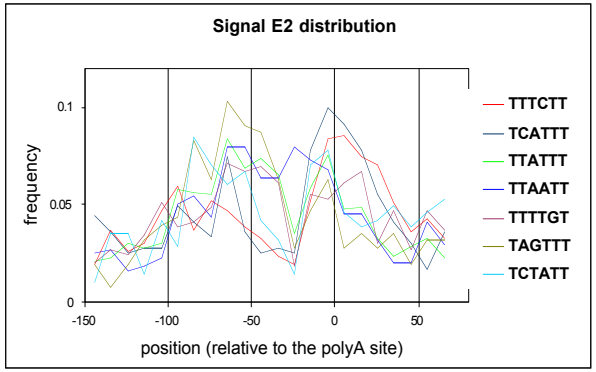

- van Helden, J., del Olmo, M. and Pérez-Ortín, J.E. (2000) Statistical analysis of yeast genomic downstream sequences reveals putative polyadenylation signals. *Nucleic Acids Res*, **28**, 1000–1010.

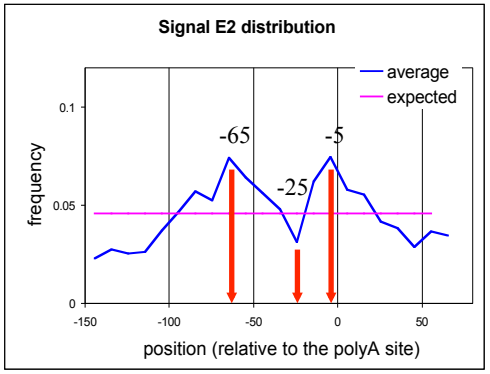
# Signal distribution and representation

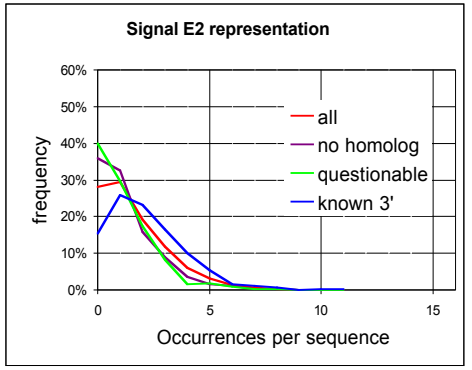


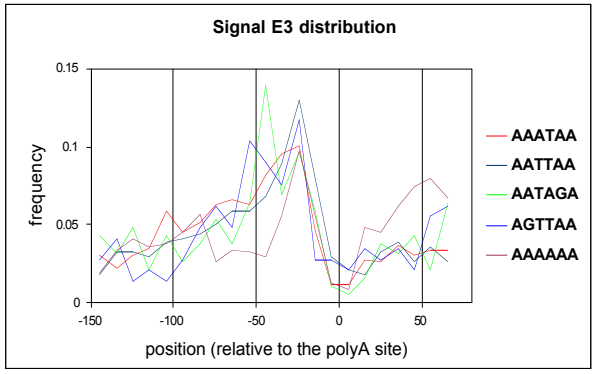



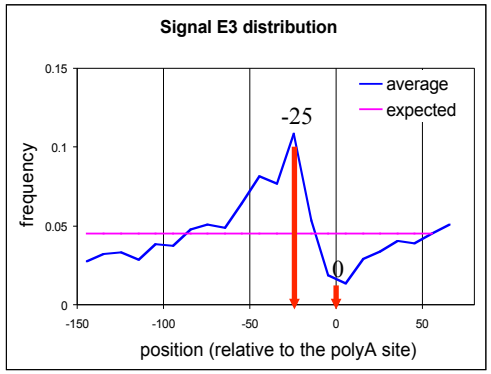



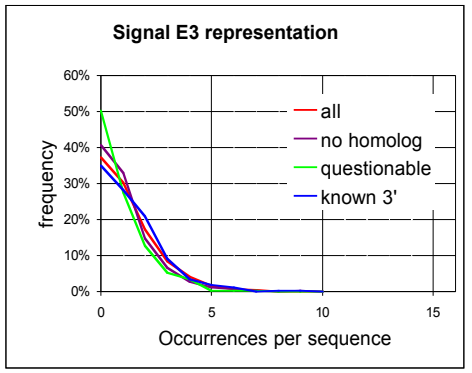





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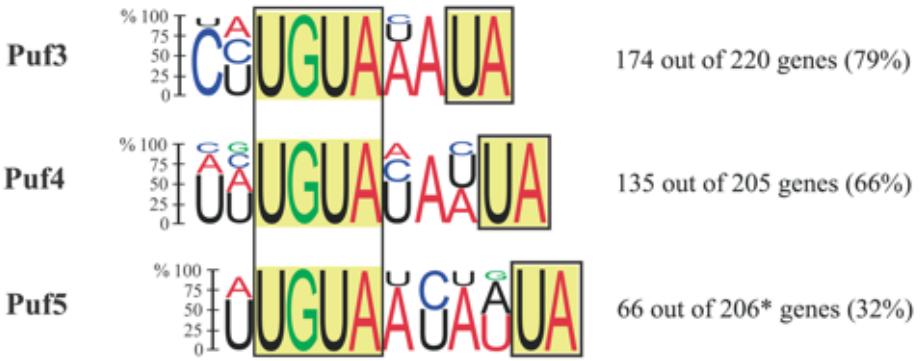
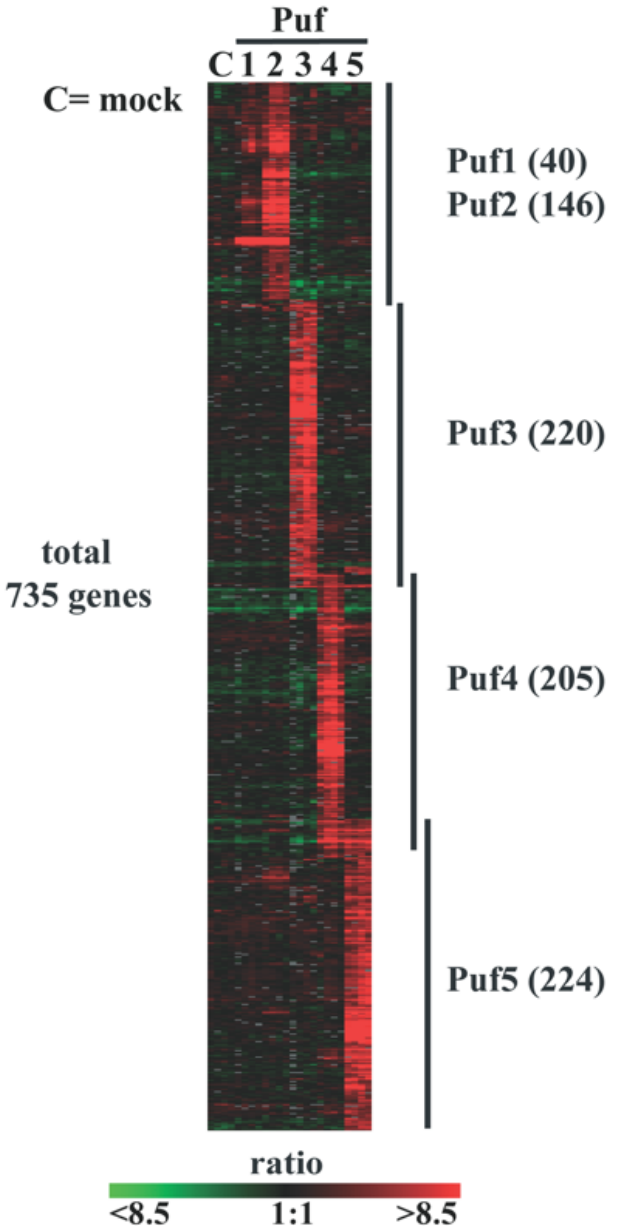
# RNA signals recognized by specific RNA-binding proteins

- The untranslated regions of the mRNA (3’ and 5’ UTRs) represent an important fraction of the mRNA size.
- These UTRs are involved in RNA maturation, regulation of translation initiation, RNA decay, RNA targeting.
- Those processes are mediated by specific protein/RNA recognition.
- Gerber et al. (2004) identifier groups of mRNAs that are specifically bound by some proteins of the PUF family: Puf1, Puf2, Puf3, Puf4 and Puf5, respectively.
- They identified over-represented signals in the mRNAs of Puf3, Puf4 and Puf5 but not in Puf1 or Puf2.

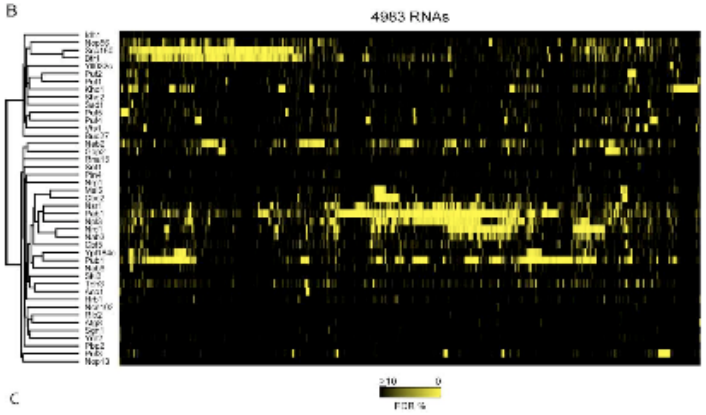


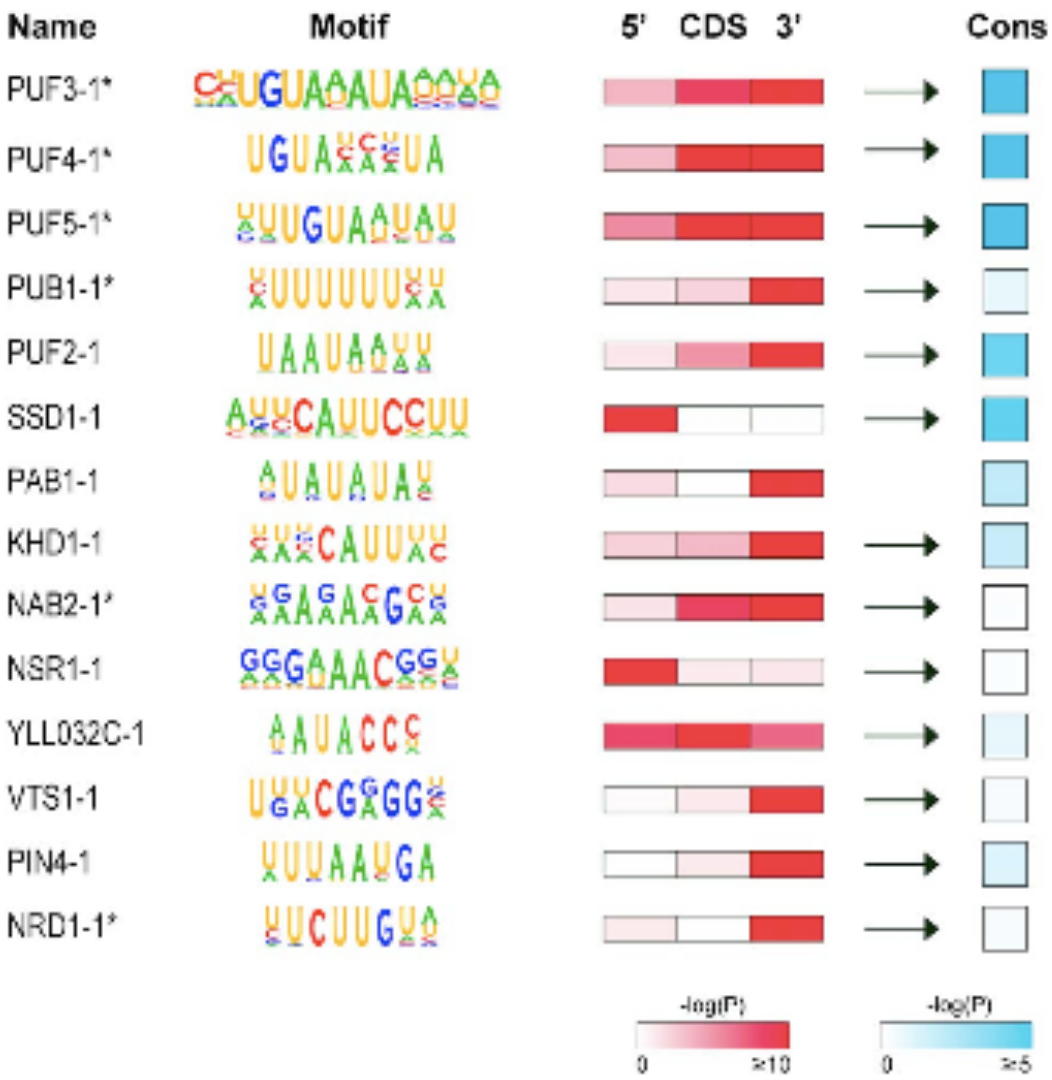




Gerber, A. P., Herschlag, D. and Brown, P. O. (2004). Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast. PLoS Biol 2, E79.

# RNA signals recognized by specific RNA-binding proteins

- Hogan et al. (2008) extended this anaysis to 40 RNA-binding proteins of the yeast Saccharomyces cerevisiae.
- They combined a word-counting algorithm and MEME to extract RNA signals that would be spevifically associated to each RNA-binding protein.
- They analyzed the combinations of sites found in the RNAs.



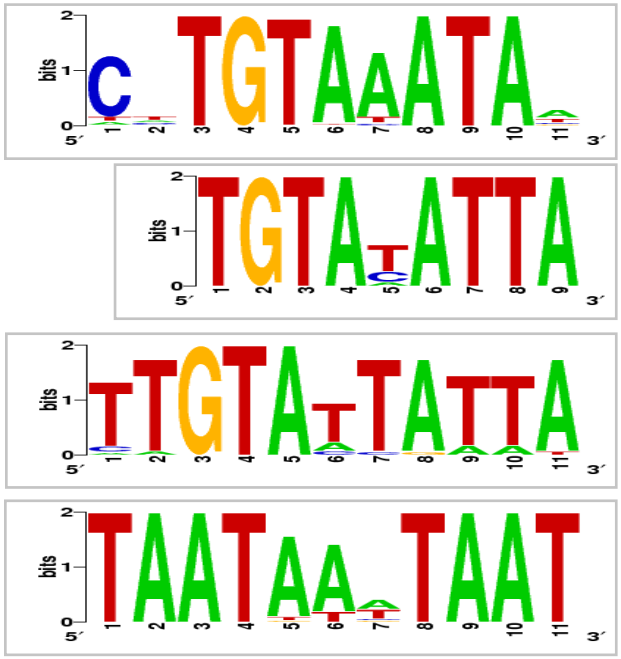




Hogan, D. J., Riordan, D. P., Gerber, A. P., Herschlag, D. and Brown, P. O. (2008). Diverse RNA-binding proteins interact with functionally related sets of RNAs, suggesting an extensive regulatory system. PLoS Biol 6, e255.

# *Discoverying specific RNA motifs with dyad-analysis*

- Patrice Godard (PhD thesis 2006) made a quick test to evaluate the capability of dyad-analysis to discover specific signals in the set sof mRNAs specifically recognized by Puf1, Puf2n Puf3, Puf4, Puf5, respectively.
- Significant dyads are detected in the 3' UTR of each group (with a weak significance for Puf1).
- Those signals correspond to those reported by Hogan et al. (2008).

**Dyad-analysis + matrix-from-patterns**




**Hogan et al. (2008)**



Hogan, D. J., Riordan, D. P., Gerber, A. P., Herschlag, D. and Brown, P. O. (2008). Diverse RNA-binding proteins interact with functionally related sets of RNAs, suggesting an extensive regulatory system. PLoS Biol 6, e255.

Patrice Godard (2006). Analyse systématique de l'influence de la source d'azote sur le transcriptome de la levure *Saccharomyces cerevisiae*. PhD thesis, ULB. http://theses.ulb.ac.be/ETD-db/collection/available/ULBetd-07032006-160950/

# *Genome-scale pattern discovery - references*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- Brazma, A., Jonassen, I., Vilo, J. & Ukkonen, E. Predicting gene regulatory elements in silico on a genomic scale. Genome Res 8, 1202-15 (1998).