Point processes in biological sequence analysis

Statistical modeling

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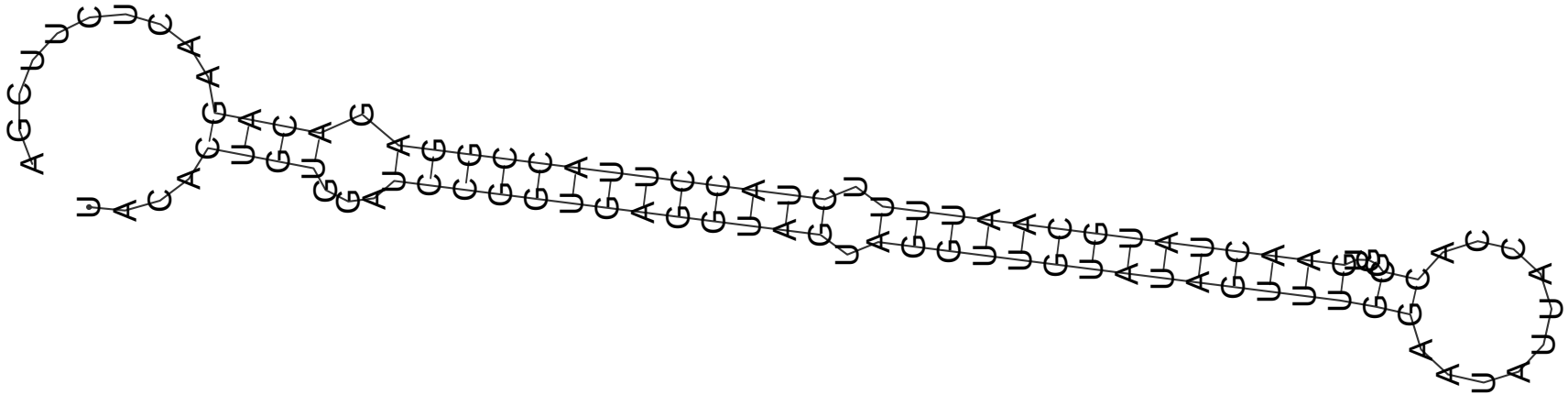
RNA molecular structure

Let-7 (pre-cursor) from C. Elegans.

UACACUGUGGAUCCGGUGAGGUAGUAGGUUGUAUAGUUUGGAAUAUUACCACCGGUGAACUAUGCAAUUUUCUACCUUACCGGAGACAGAACUCUUCGA

Member of the family of micro RNAs that terminate or inhibit the translation of mRNA to protein. The pre-cursor is embedded as a gene in the DNA – we want to find genes with similar structure.



StemSearch

StemSearch is an implementation of a search algorithm for general stem-loop motifs.

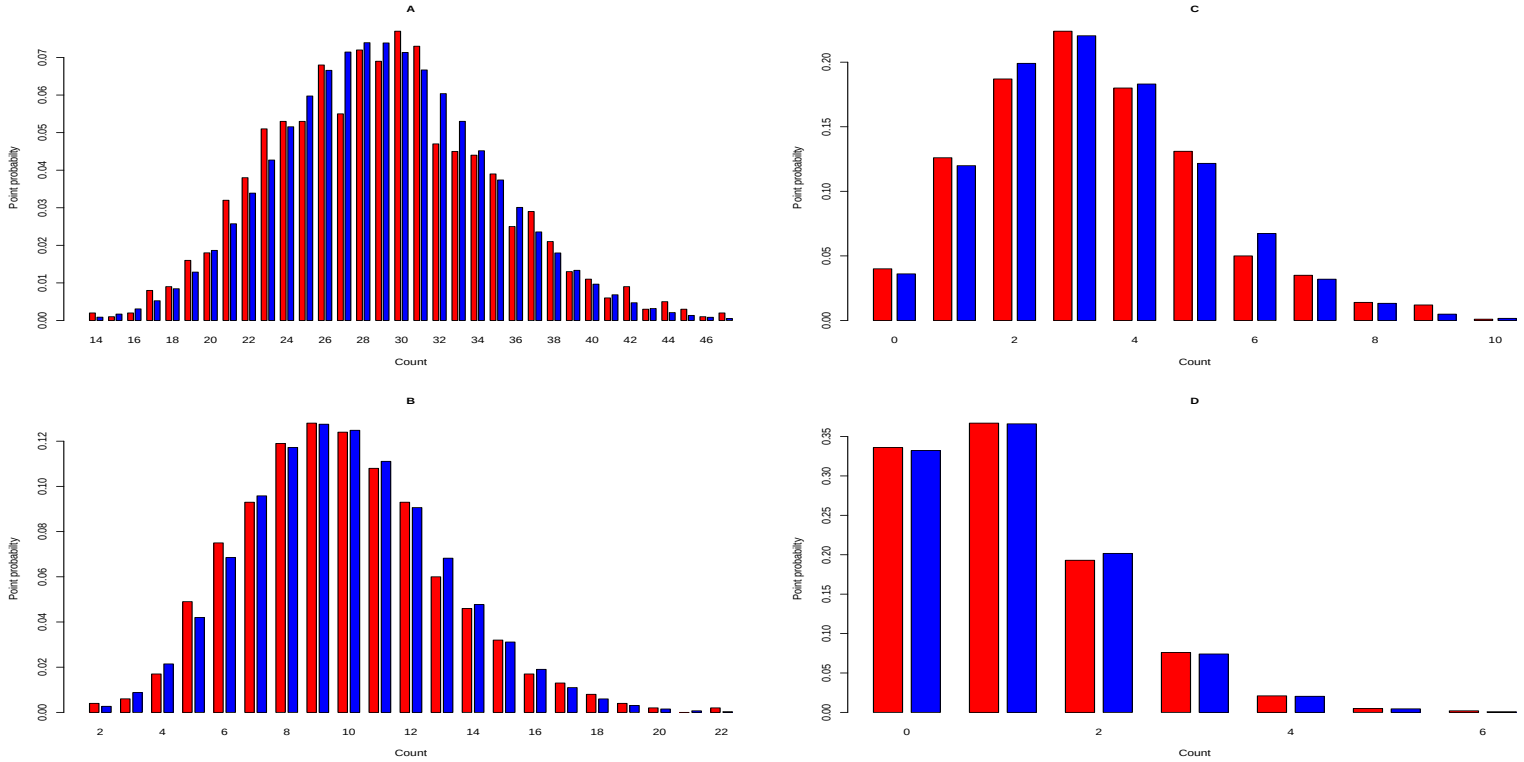
For a fixed threshold t and a sequence of length n we are given the N_t (declumped) findings with a score exceeding t. The statistical null model states the N_t is Poisson distributed with

$$\mathbb{E}(N_t) = nK \exp(-\lambda t)$$

and the excesses are iid exponentially distributed with parameter λ.

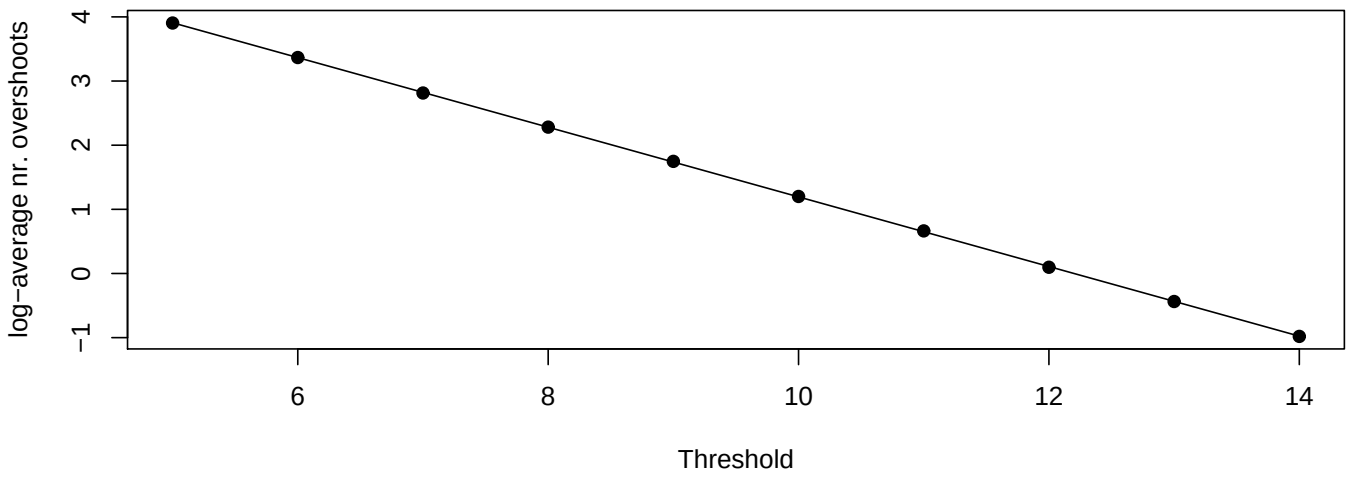
The model is only valid for t sufficiently large. [4]

StemSearch



Empirical (red) and theoretical Poisson point probabilities (blue) using *StemSearch* with $n = 5000$, $b = 200$, $v = (-4, -1, -1)$ and *C.Elegans* genome first order Markov transition probabilities. Here 1000 sequences with thresholds $t = 6$ (A), $t = 8$ (B), $t = 10$ (C) and $t = 12$ (D). The variance-to-mean ratio for the empirical counts are 1.117 (A), 1.034 (B), 1.052 (C) and 1.037 (D).

StemSearch



The log-average number of overshoots as a function of the threshold for a simulation study using *StemSearch* with $n = 5000$, $b = 200$ and $v = (-4, -1, -1)$ on sequences generated by a first order Markov chain with *C.Elegans* genome transition probabilities. The line is the least squares fit to the points with slope -0.54 and intercept 6.62.

StemSearch

We use the standard Hill estimator from extreme value statistics;

$$\hat{\lambda} = \left(\frac{1}{N} \sum_{i}^{N} S_{(m-i+1):m} - S_{(m-N):m} \right)^{-1}$$

of λ, where

$$S_{1:m} < \ldots < S_{m:m}$$

denote the ordered m overshoots of a (suitable) threshold t.

$$\hat{K} = \exp(\hat{\lambda} S_{(m-N):m}) \frac{N}{n}.$$

Poisson process limits

With $(X_k)_{k\geq 1}$ a sequence of random variables we can often associate a random measure

$$\mu_n = \sum_i \delta_{(t_i, m_i)} \in \mathcal{M}([0,1] \times E)$$

which places motif m_i at position t_i.
With restrictions of the following type:

- Stationarity or asymptotic stationarity of $(X_k)_{k\geq 1}$.
- Rare motifs – $\mathbb{E}(\mu_n([0,1] \times E)) \simeq \lambda$ for large n and rare motifs.
- Motifs are declumped.
- Weak – or moderate – dependence in $(X_k)_{k\geq 1}$.

Then $\mu_n(\cdot \times E)$ converges weakly to an homogeneous Poisson random measure (Poisson process) on $[0,1]$ for $n \to \infty$.

Motifs in genomes

The DNA-alphabet is $E = \{\mathsf{A}, \mathsf{C}, \mathsf{G}, \mathsf{T}\}$.

- Words are finite strings; ACGTTA, GTAACA, AGA, ...
- A collection of words is a Motif.
- Regular expressions; A.*[CG]TT., G.[AG][AG]C., ...
- Weight matrices; $W = \{W_{x,i}\}_{x \in E, i=1,\ldots,k}$.
  A word $w = x_1 \ldots x_k$ receives the score

$$S_w = \sum_{i=1}^{k} W_{x_i,i}.$$

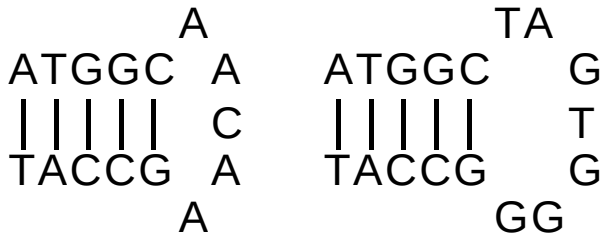
A motif is specified as $\{w | S_w > t\}$.
See [2] for a probabilistic treatment.

Stem-loop motifs

The regular expression:

$$\texttt{ATGGC.}\{5,7\}\texttt{GCCAT}$$

corresponds to stem-loop structures



with 5-7 letters in the loop.

Reinert and Schbath [5] investigate Poisson approximations focusing on exact error bounds for motifs in homogeneous Markov chains – including stem-loop motifs as the above.

Problems

The homogeneous Poisson process model suffer from some problems - even as a null model:

- Non-Markov nature of genomic sequences.
- Heterogeneity of genomic sequences:
  - Heterogeneous nucleotide frequencies.
  - Low-complexity and repeat patterns (fixed by repeat masker?).
  - Heterogeneous distribution of larger motifs.
- Dependence structures of biologically relevant motifs.

How to get beyond the null model?

Example

Is there an over-representation of simultaneous occurrence of the two words $w_1 =$ AACCTGG and $w_2 =$ ATGCCAT in the sequences $x_1, \ldots, x_m$ ($x_i = x_{i1} \ldots x_{in(i)}$)?

Null model: The words occur as independent Poisson processes in each sequence (intensities λ_1^i and λ_2^i), and the sequences are independent.

$$R = \sum_{i=1}^{m} 1(w_1 \in x_i, w_2 \in x_2) \overset{\text{approx}}{\sim} \text{Poi}(\xi)$$

with

$$\xi = \sum_{i=1}^{m} (1 - e^{-\lambda_1^i})(1 - e^{-\lambda_2^i}).$$

A theoretical foundation is given in Reinert and Schbath [5].

Example - continued

In a concrete application, Marc Riemer Friedländer investigated in his Master's Thesis the co-occurrence of miRNA target sites (7 letter words) in the 3'UTR of mRNA taking

$$\log(\lambda_w^i) = \beta_w + \beta_w(0)\log n(i) + \beta_w(\mathsf{A})\log f_{\mathsf{A}}(i) + \ldots \beta_w(\mathsf{T})\log f_{\mathsf{T}}(i)$$

with $f_{\mathsf{A}}(i), \ldots, f_{\mathsf{T}}(i)$ the relative frequency of nucleotides in sequence i.

Parameters were estimated using Poisson regression with a much better model fit than the iid sequence model where $\beta_w(0) = 1$, $\beta_w = 0$ and

$$\beta_w(\alpha) = \text{number of times } \alpha \text{ occurs in word } w$$

ENCODE

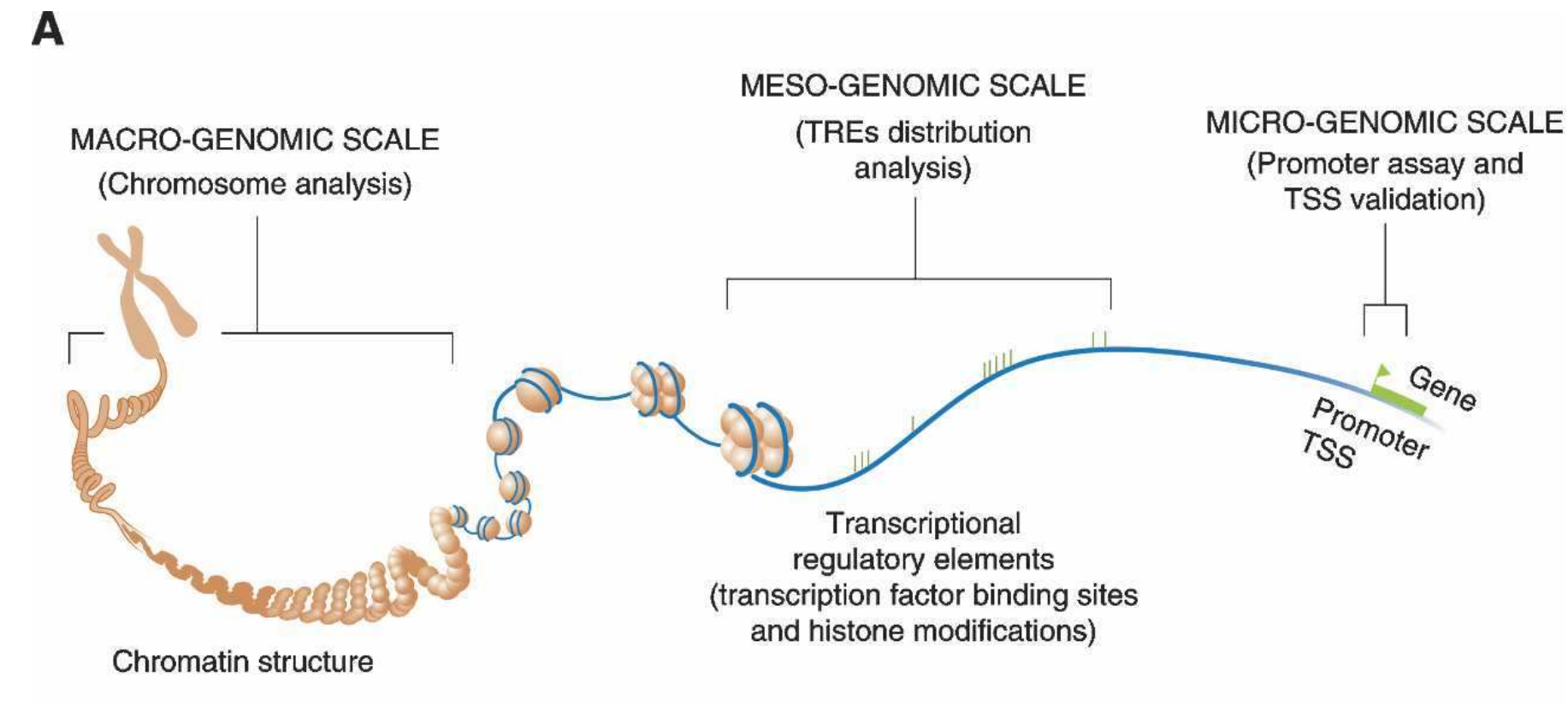


Illustration from [6] – a statistical analysis of regulatory elements in the ENCODE regions.

Transcription Factor Binding Sites

- Protein binding sites on DNA serve a central role in the regulatory mechanisms for gene transcription.
- Typically hard to locate computationally – computational predictions are noisy.
- Better experimental data is becomming available (ChIP-chip), which provides actual binding sites of proteins (e.g. ENCODE data).

Intensity based modeling

For a multivariate point-process $(N_1(t), \ldots, N_k(t))$ with filtration $(\mathcal{F}_t)_{t \geq 0}$ and adapted intensity process $\lambda(t) = (\lambda_1(t), \ldots, \lambda_k(t))$ we have

$$\mathbb{P}(N_i(t+\epsilon) - N_i(t) > 0 | \mathcal{F}_t) \simeq \lambda_i(t)\epsilon$$

We also have the log-likelihood process

$$\sum_{i=1}^{k} \left[\int_0^t \log \lambda_i(t) N_i(\mathrm{d}t) - \int_0^t \lambda_i(t) \mathrm{d}t \right].$$

A statistical modeling approach using Hawkes processes was first attempted by Gaëlle Gusto and Sophie Schbath in [3].

Hawkes processes

- Multivariate point-process $(N_1, \ldots, N_k)$ with intensity

$$\lambda_i(t) = \phi\left(\sum_{j=1}^{k} \int_0^t h_{ij}(t-s) N_j(\mathrm{d}s)\right).$$

- Lisbeth Carstensen (Ph.D.-student, Copenhagen) has an implementation fitting two-dimensional Hawkes processes with spline-based expansions of h_{ij} – including additional local sequence covariates.
- Ongoing projects: More then two dimensions, inclusion of a Cox-process component, superpositions, model selection and test-statistics for $h_{ij} = 0$.

Concluding remarks

- Actually proving an asymptotic Poisson result can be an arbitrary hard mathematical challenge.
- The iid or homogeneous Markov chain models for sequences attempt modeling on a microscopic scale.
- I believe that biologically relevant questions are better addressed with statistical models directly at the mesoscopic scale.
- Thanks for your time, thanks to Richard Gill for inviting me, and thanks to Lisbeth and Mark and the entire Bioinformatics Centre in Copenhagen.

References

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