Transcriptional Regulatory Elements
Detection and Evolutionary Analysis

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The Beginning

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Question:

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- "What and why am I?"

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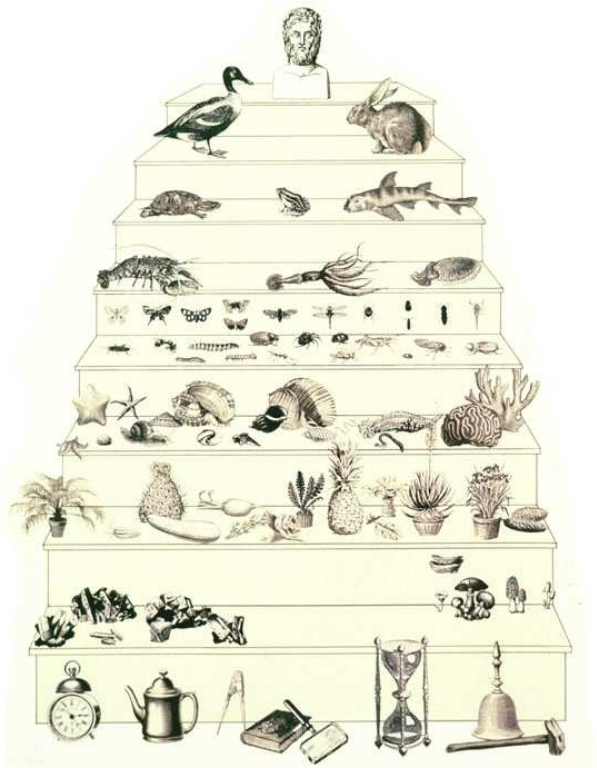
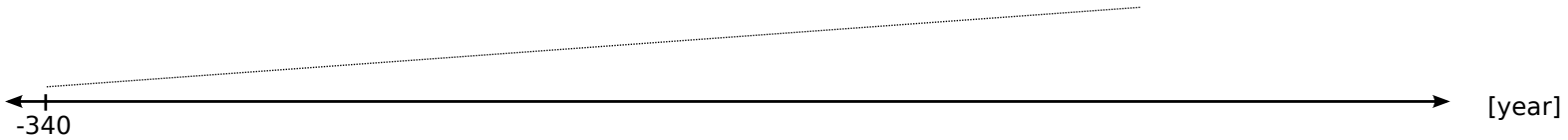
- "What and why am I?"
- or less phylosophical: "How works evolution and development?"

The Evolution of Evolution

$\sim 2,350$ years ago:

- "Scala Naturae" of Aristotle (384 BC – 322 BC)
- classification from imperfect inanimate matter to most perfect form

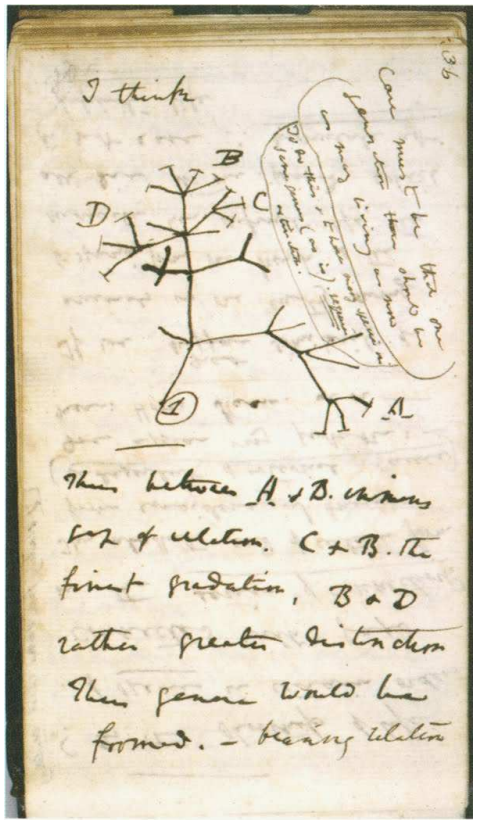


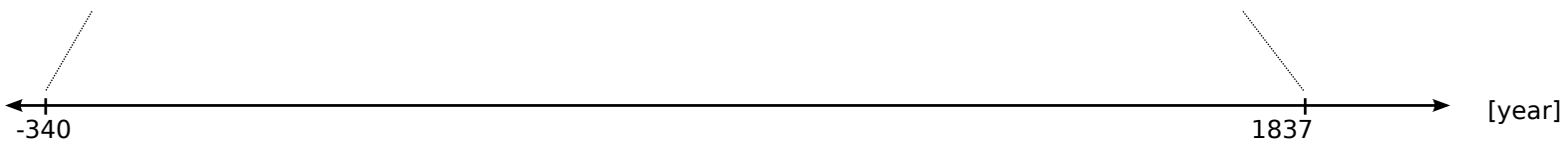


The Evolution of Evolution

174 years ago:

- "Transmutation of Species" of Charles Darwin (1809 – 1882)
- first evolutionary tree

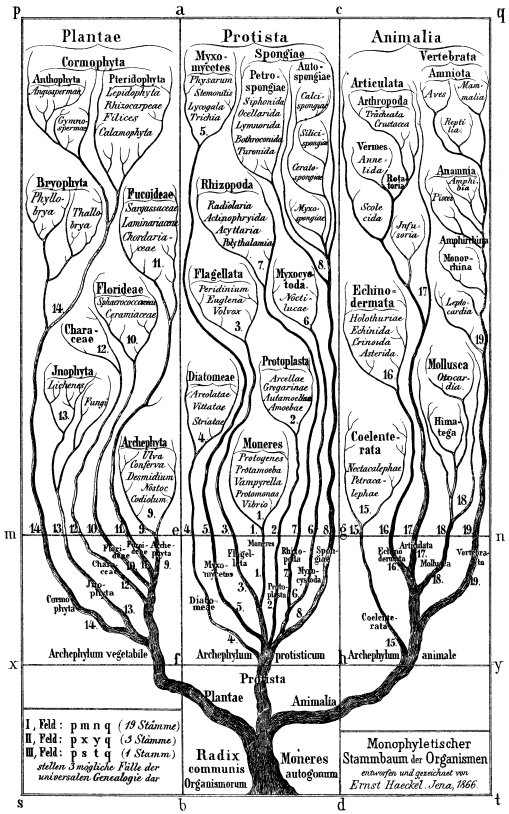


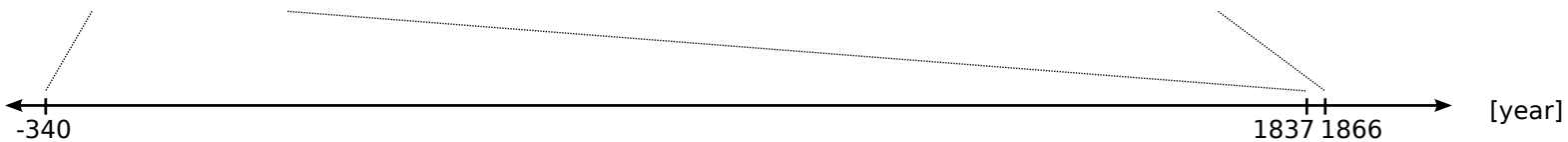


The Evolution of Evolution

145 years ago:

- "Generelle Morphologie der Organismen" of Ernst Haeckel (1834 – 1919)
- first single tree of all forms of life

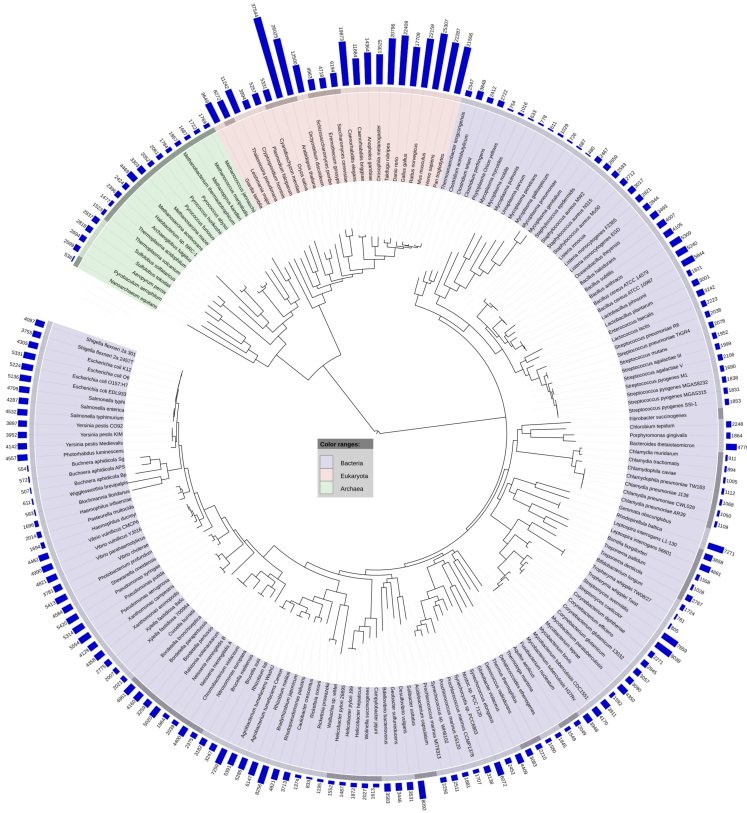


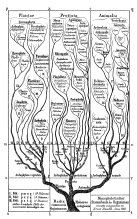


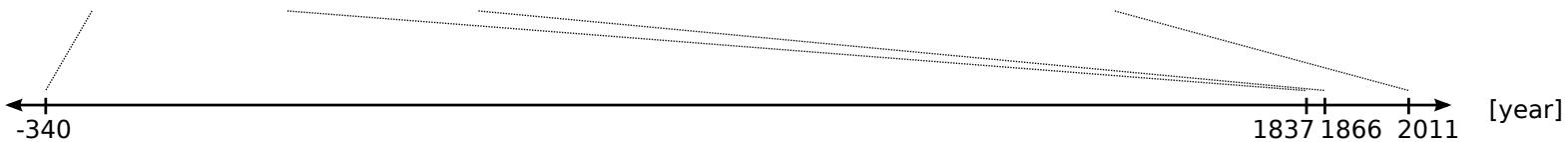
The Evolution of Evolution

today

- "Tree of Life" from European Molecular Biology Laboratory
- based on known genomes







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- end of 1950s: body plan of each life form is encoded in genomes

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- end of 1950s: body plan of each life form is encoded in genomes
- genome projects: differences between genomes are rather small
- today: time, location and amount of gene products are responsible for causal differences
- $\Rightarrow$ regulation of gene expression is basis for differentiation, morphogenesis and versatility and adaptability of any organism

Gene Expression and Regulation

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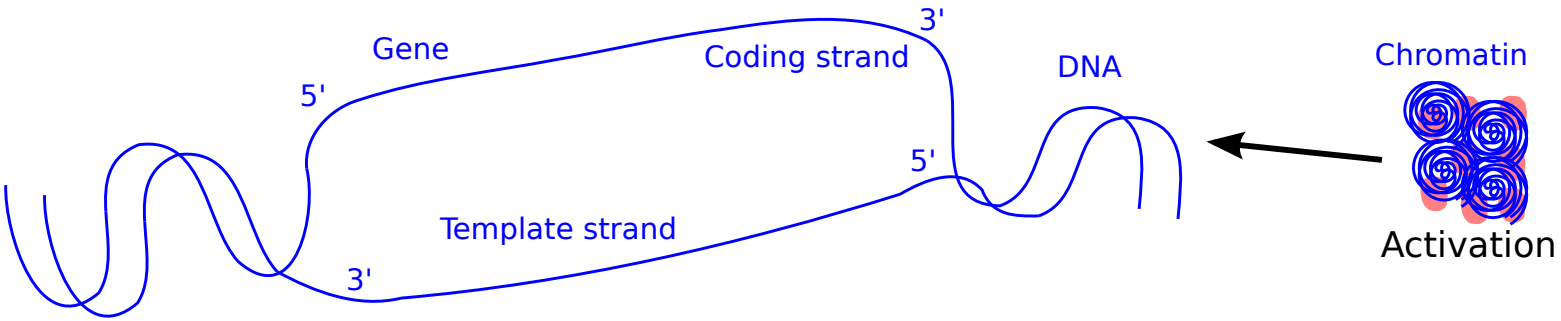

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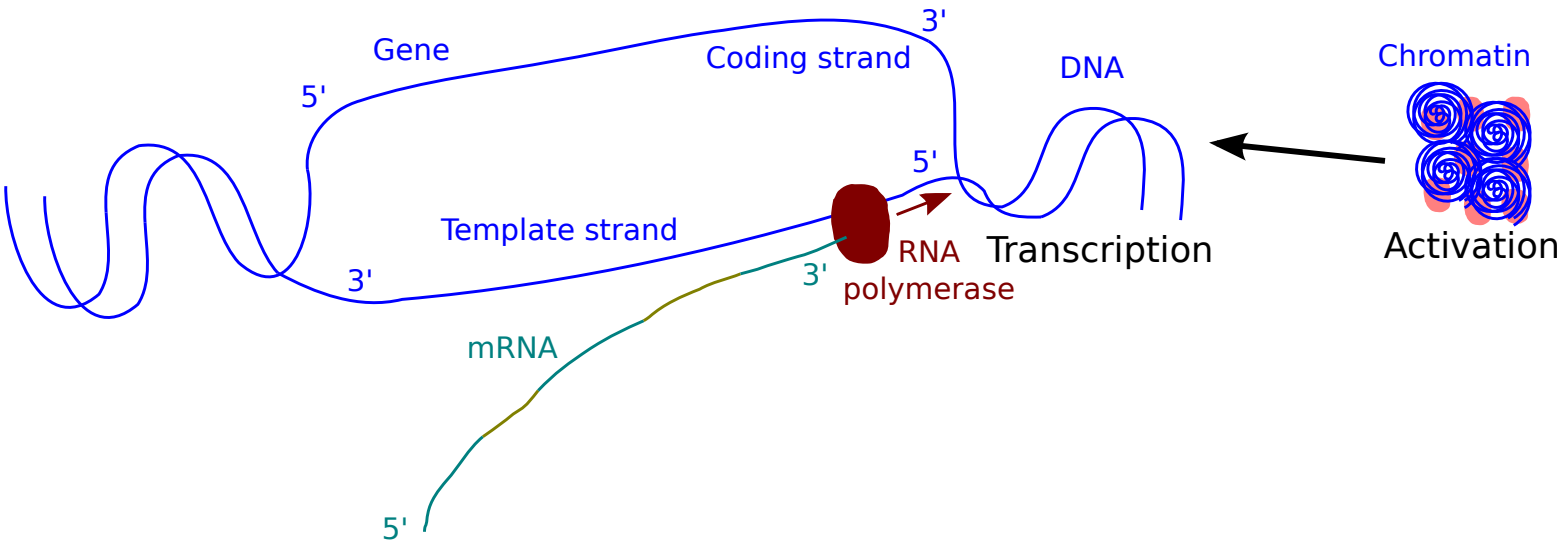

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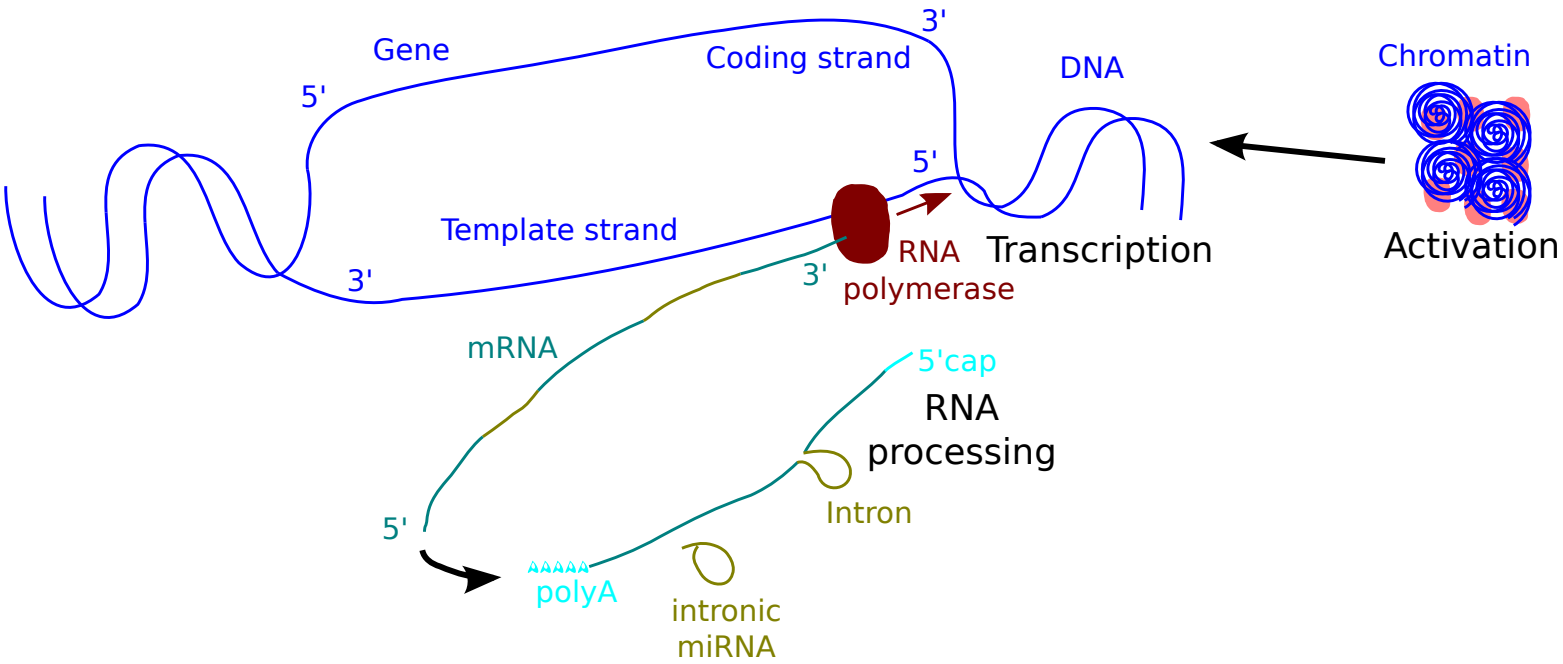

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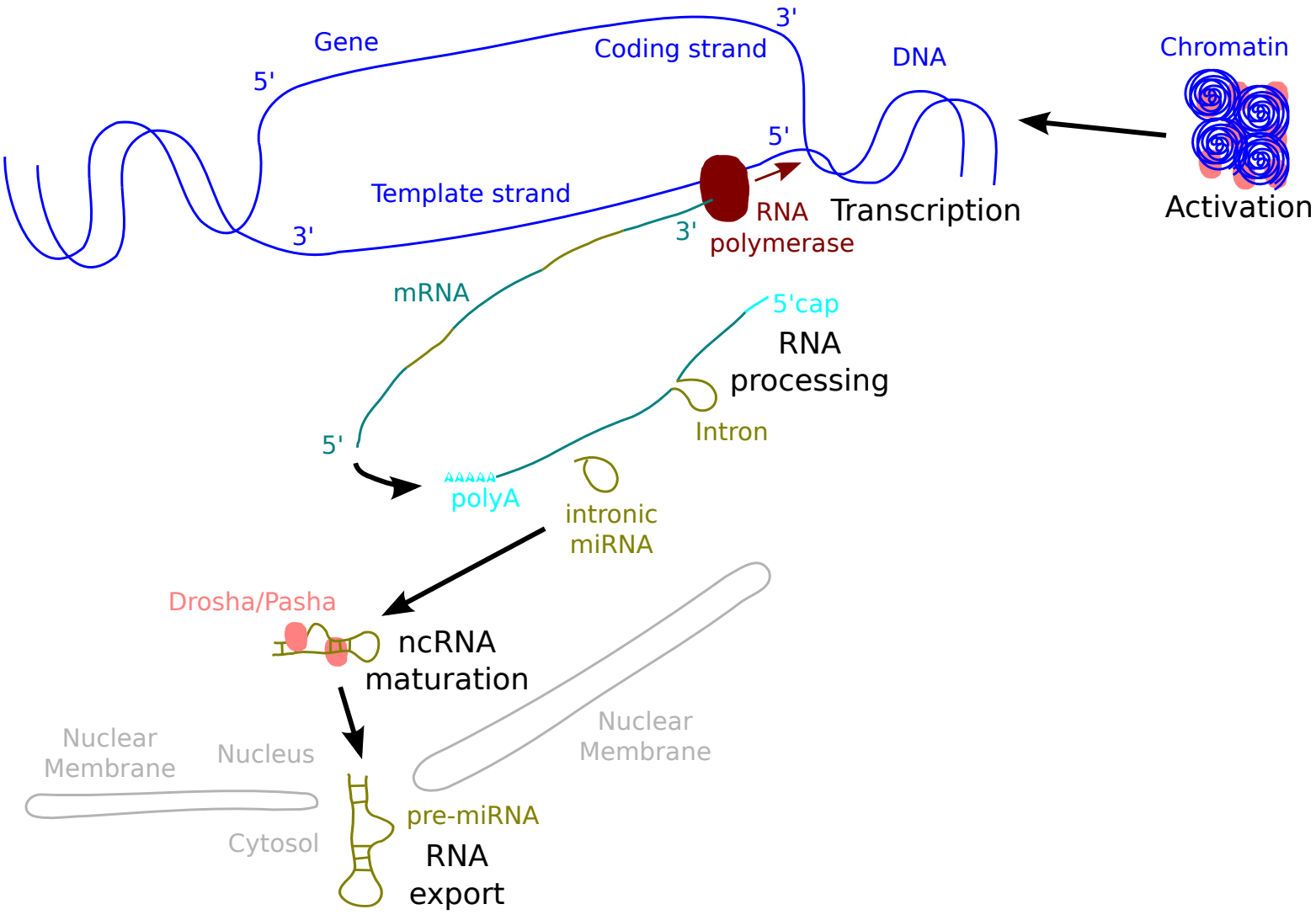

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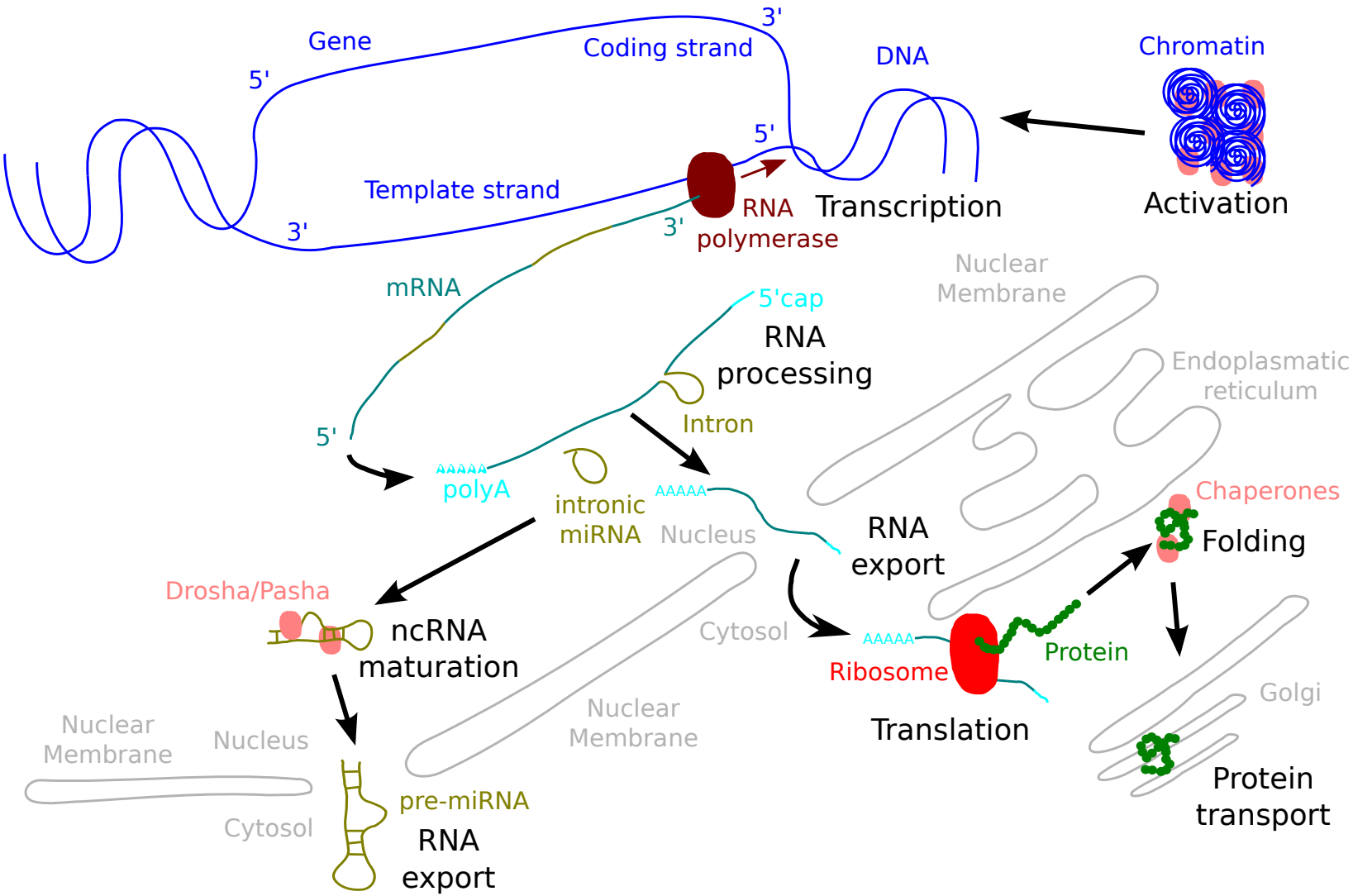

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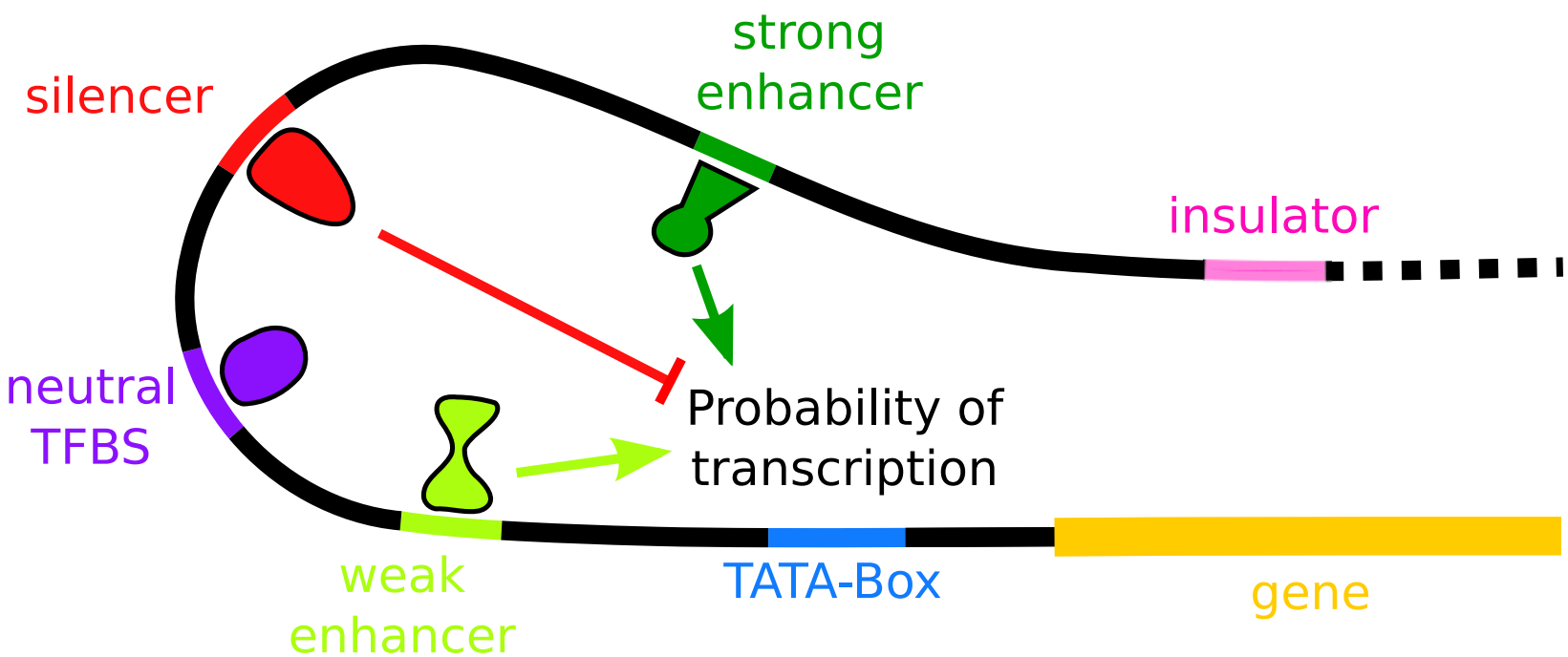

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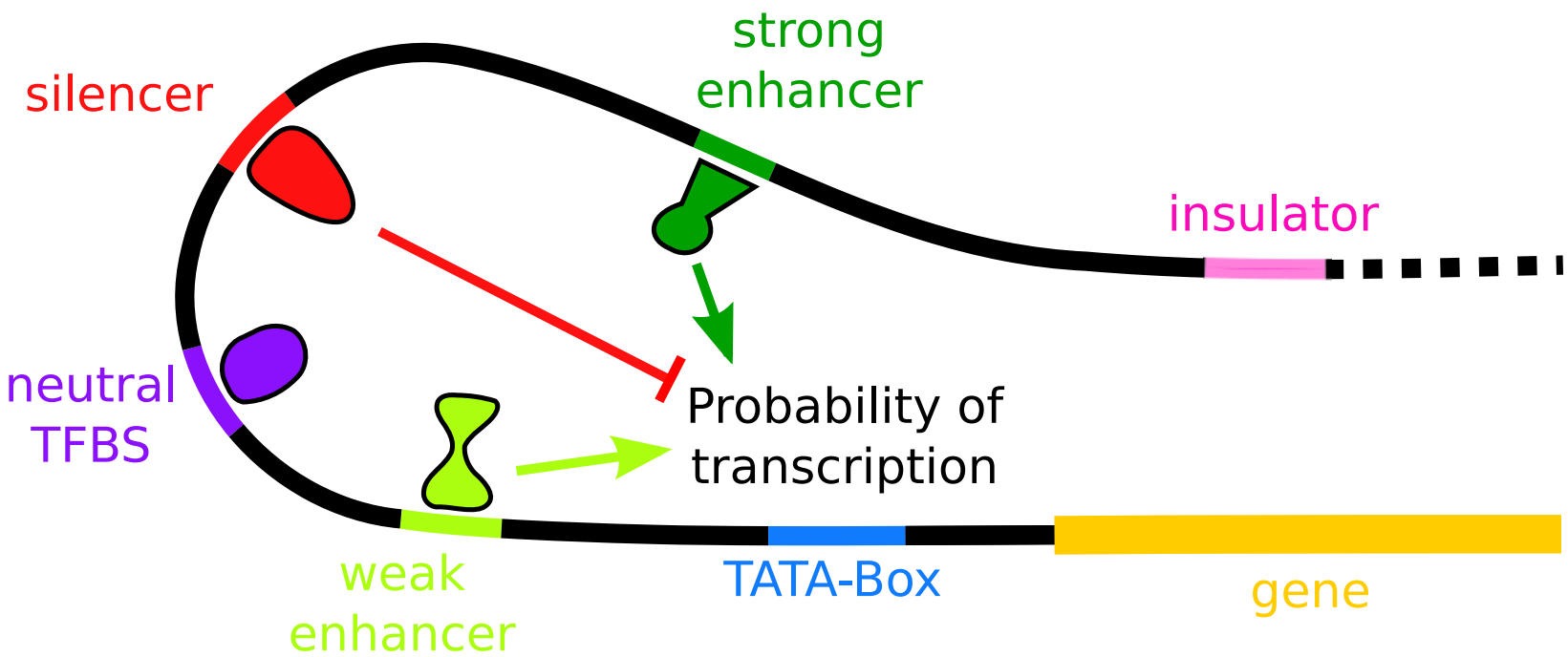

$$|\text{gene}| = f(\text{silencer}, \text{weak enhancer}, \text{strong enhancer}, \text{neutral TFBS})$$

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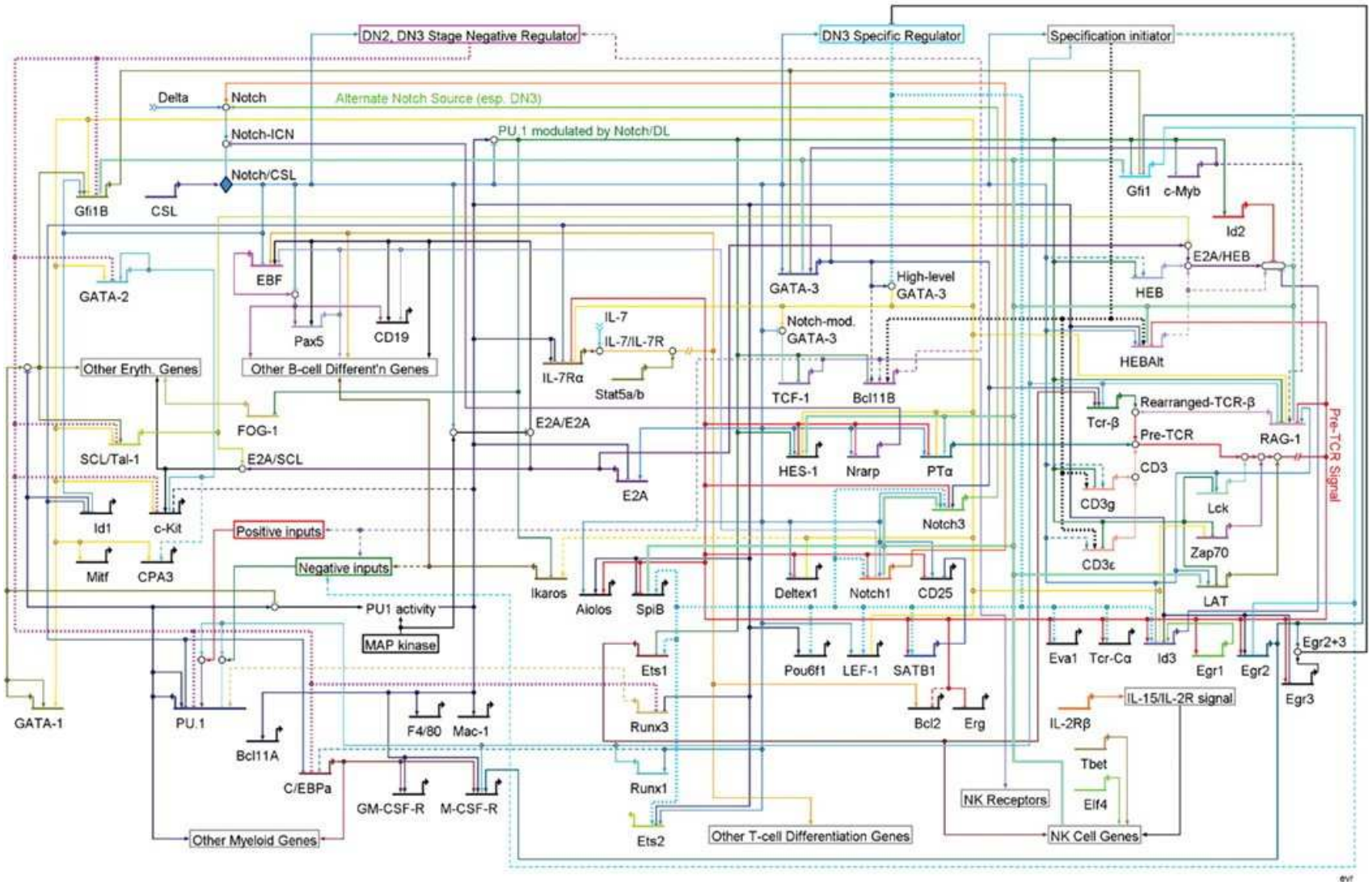
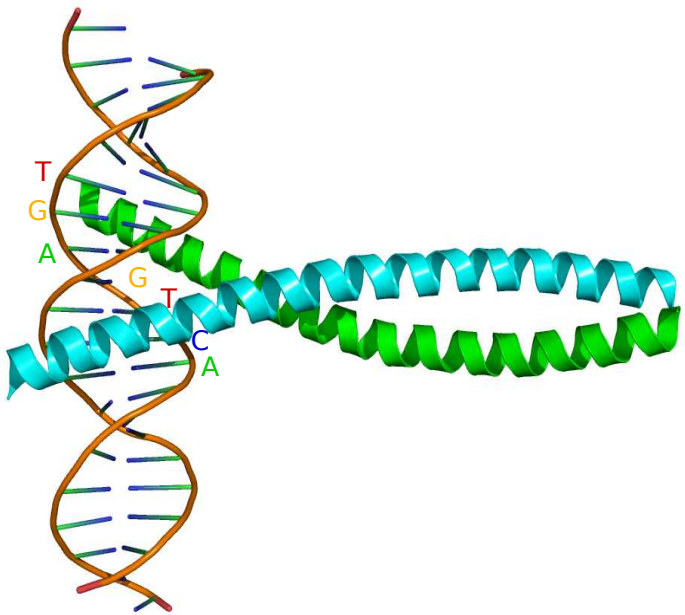


Regulatory Elements

TF / DNA Complex



Regulatory Elements

TF / DNA Complex

Binding Sites

Pos	1	2	3	4	5	6	7
Site 1	T	G	A	G	T	C	A
Site 2	T	G	A	C	T	A	A
Site 3	T	A	A	G	T	T	A
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Jundm2
Variable spacer length

TGACGTCA ✓

TGACTCA ✓
TGAGTCA ✓

ERRalpha
Position interdependence

CAAGGTCA ✓
AGGGGTCA ✓

CAGGGTCA ✗
CGGGGTCA ✗

Zfp187
Alternate recognition interfaces

ATGTACTAAT ✓

GCCCTTGTCC ✓

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- $\Rightarrow$ research of regulatory elements is one of main fields in life sciences

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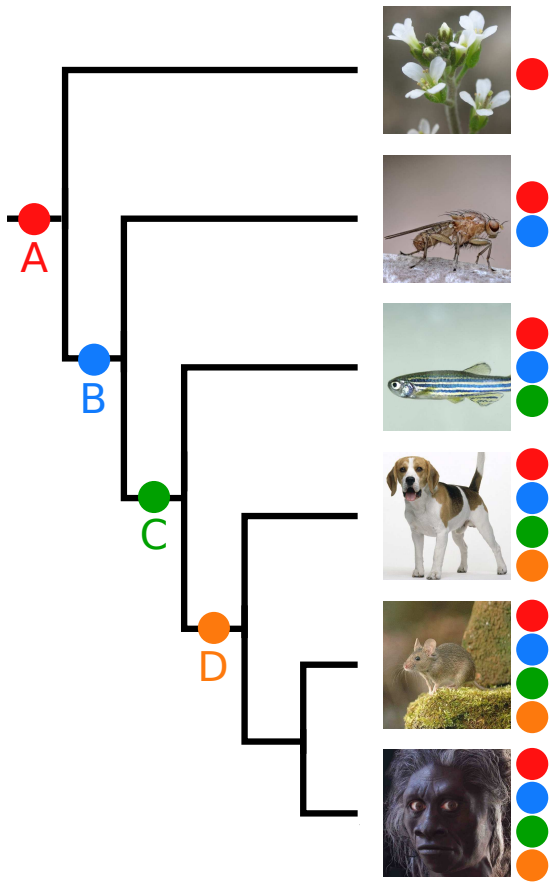

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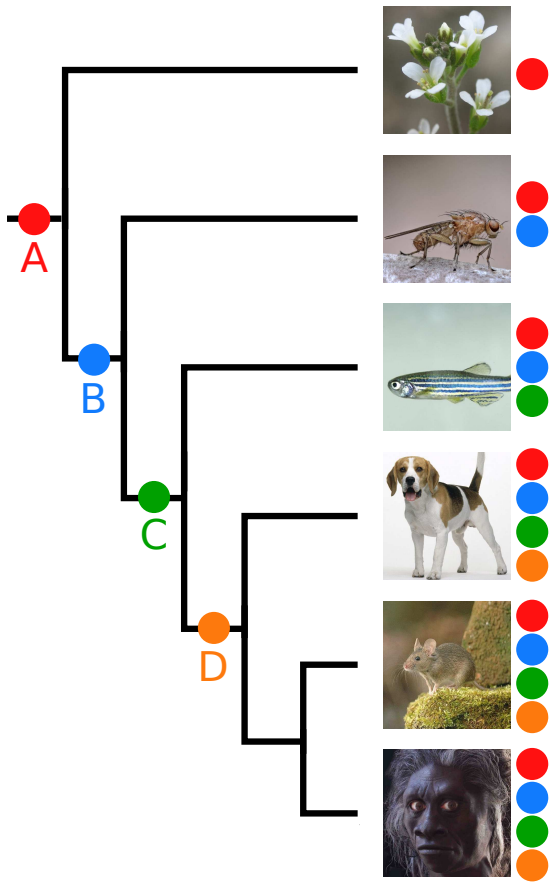
- detectable by comparative sequence analysis $\Rightarrow$ phylogenetic footprinting



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- aim: determining alignments between evolutionary related areas

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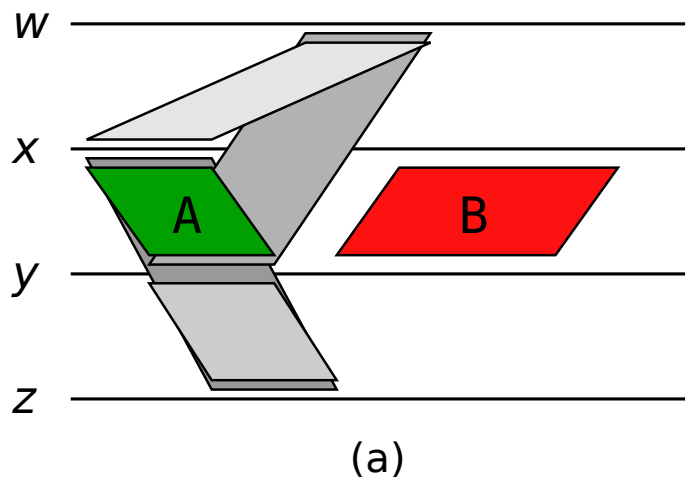

(a)

Evolutionary Information

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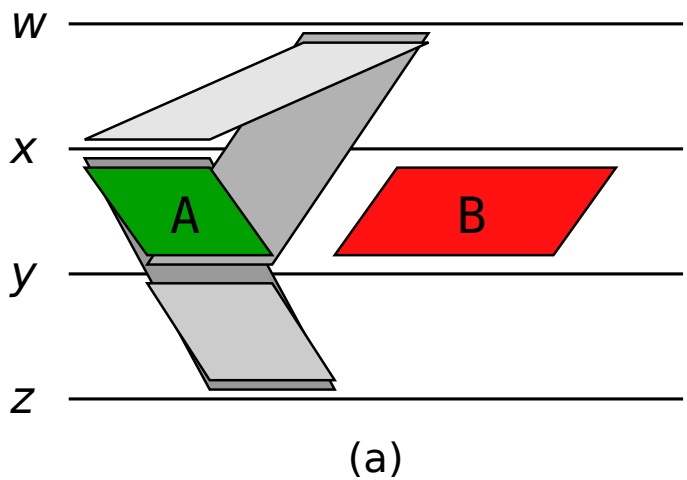
- (b) order of motifs defines windows for new motifs $\Rightarrow$ consistence



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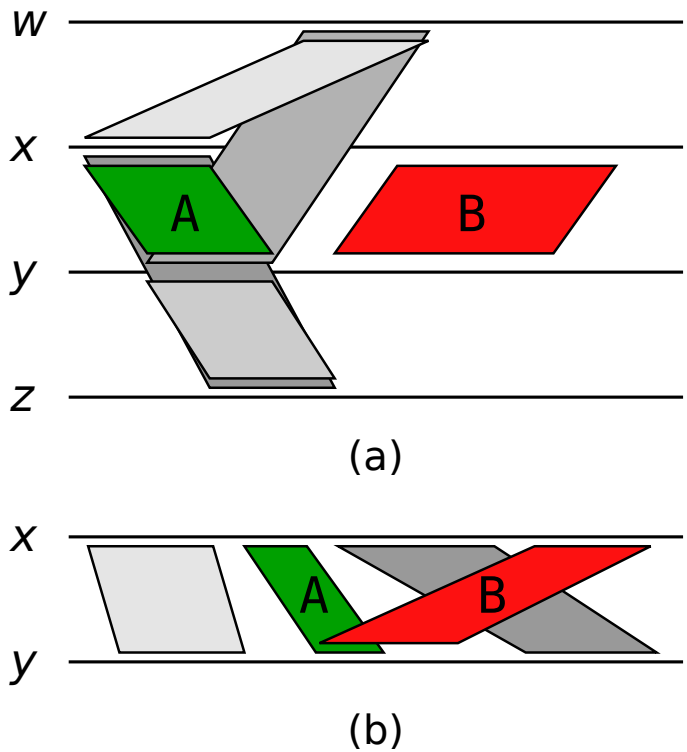

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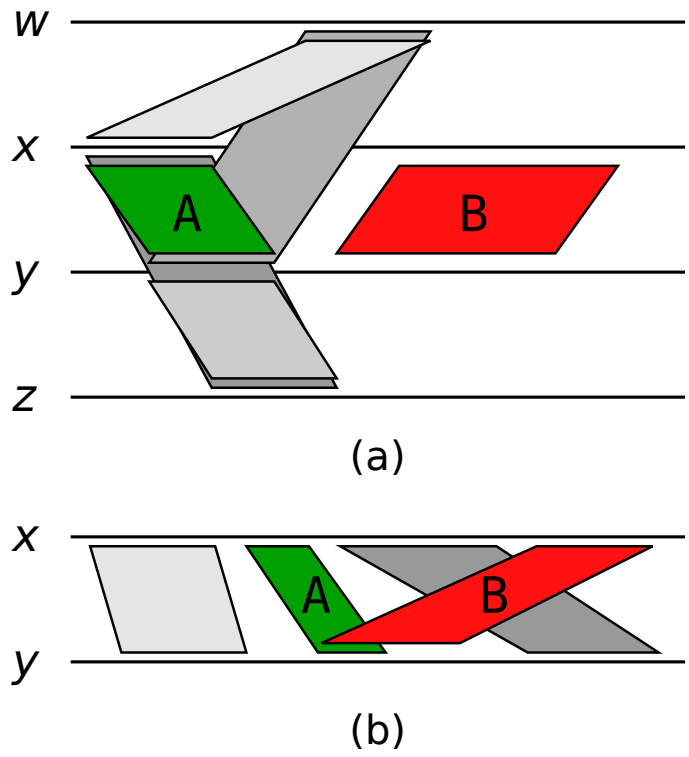
- idea: calculate pairwise local alignments with low stringency, determine maximal consistent subsets based on support



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S_1	A	C	G	A
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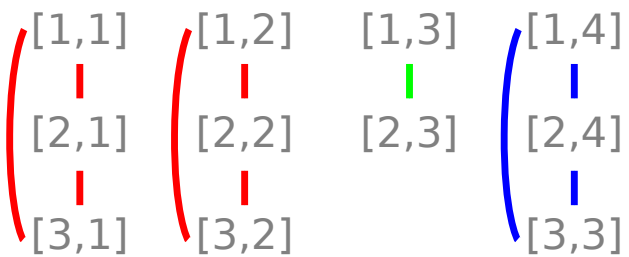
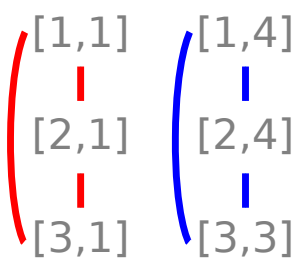
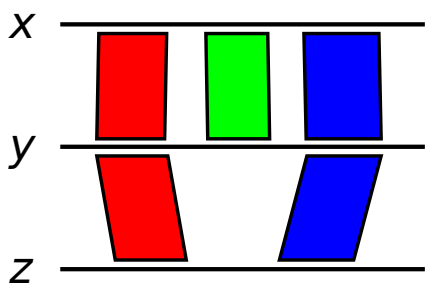
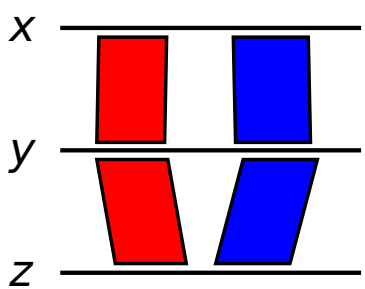
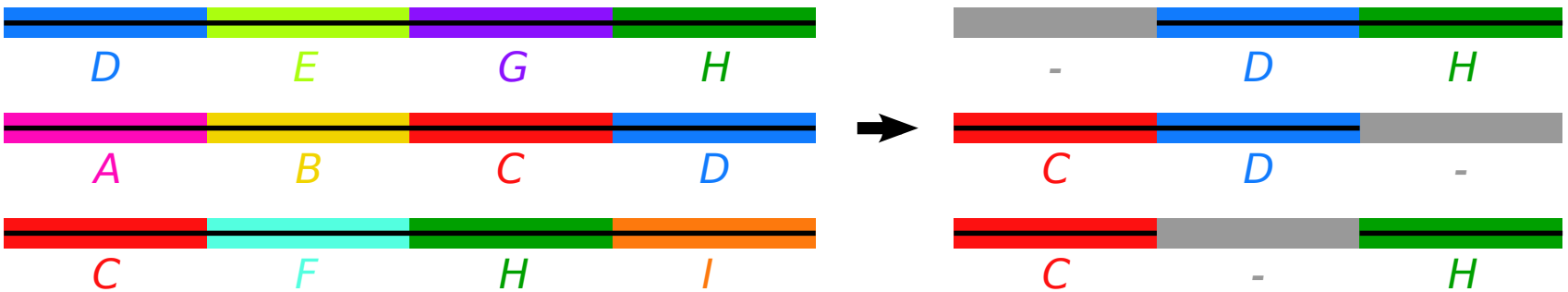








Motif Alignment



Consistent Alignments

Definition (Consistency)

An alignment collection $\mathcal{A} = \{A_1, \ldots, A_n\}$ over sequences $\mathcal{S} = \{S_1, \ldots, S_m\}$ is *consistent* $\Leftrightarrow$ it exists a multiple alignment M over $\mathcal{S}$ so that all pairs of nucleotides aligned by alignments in $\mathcal{A}$ are also aligned in M.

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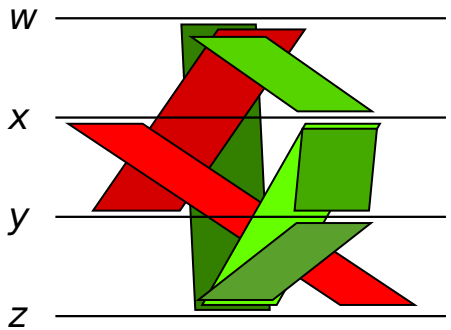

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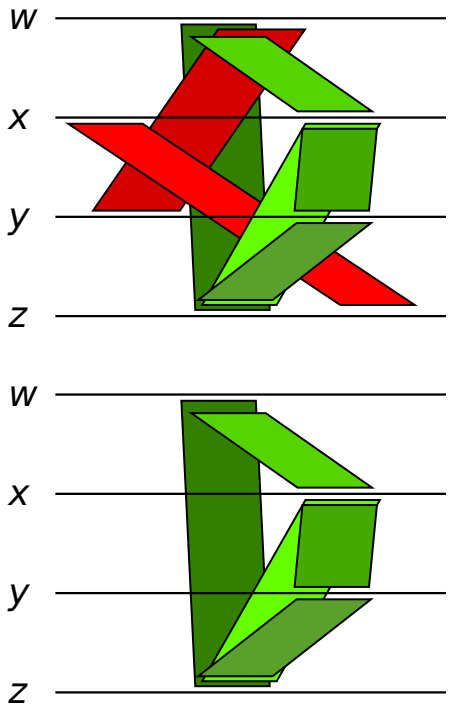

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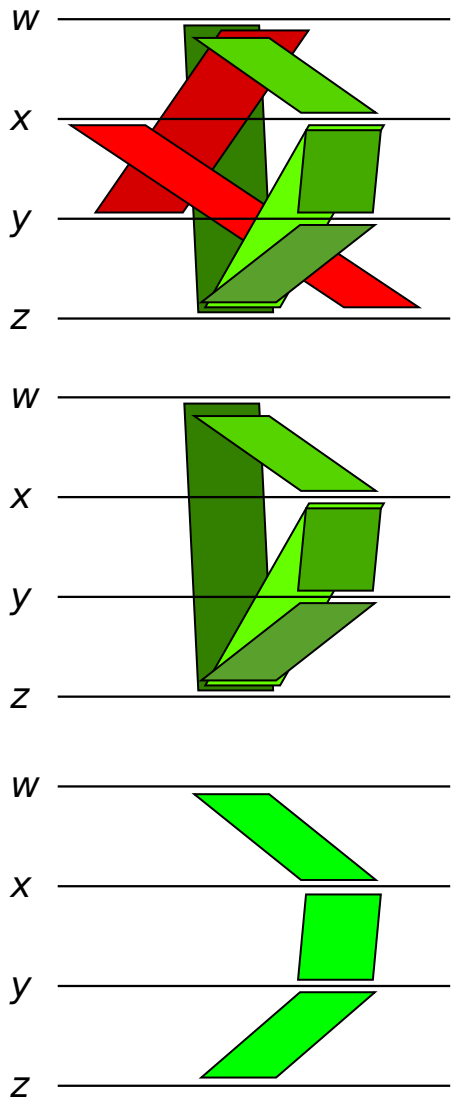

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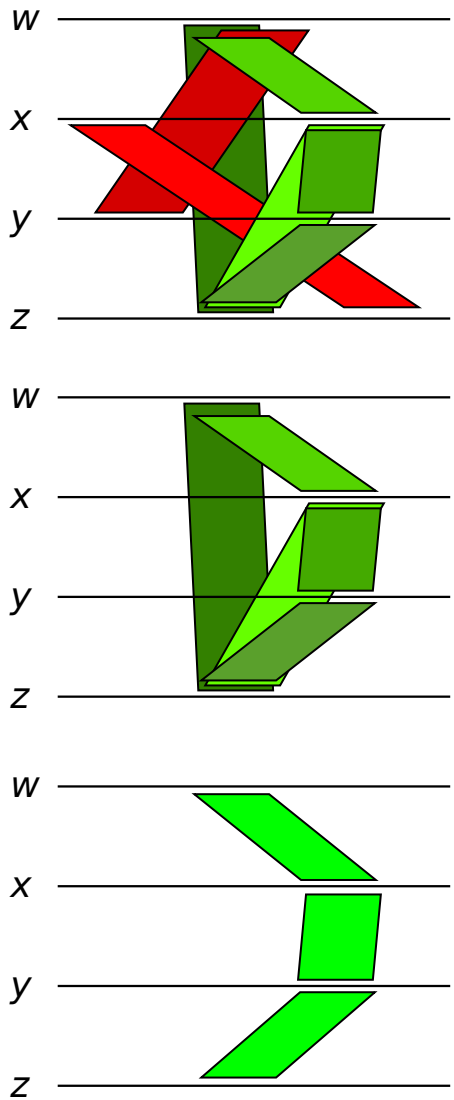

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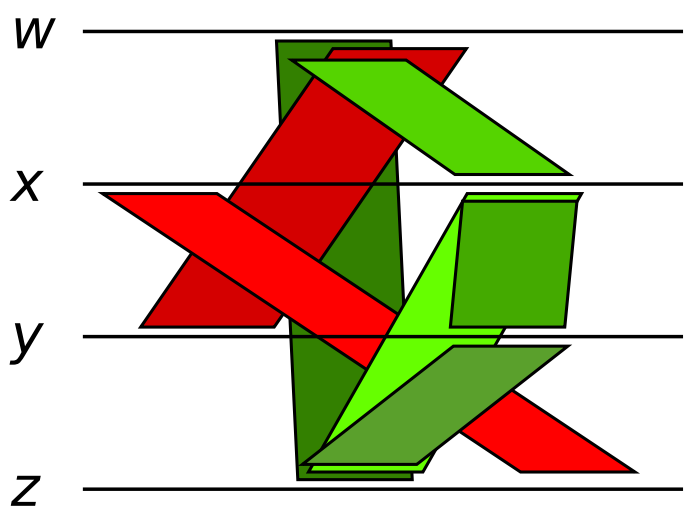

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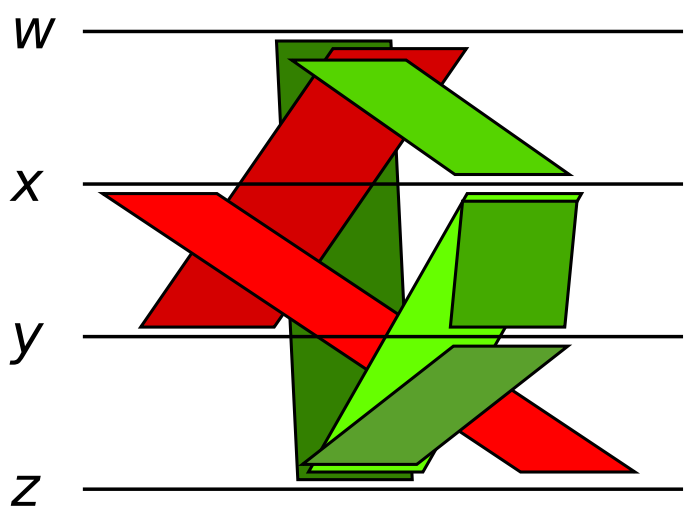

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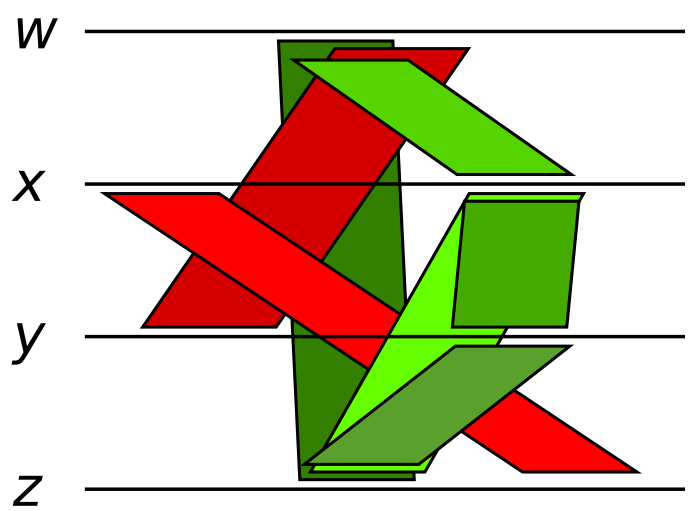

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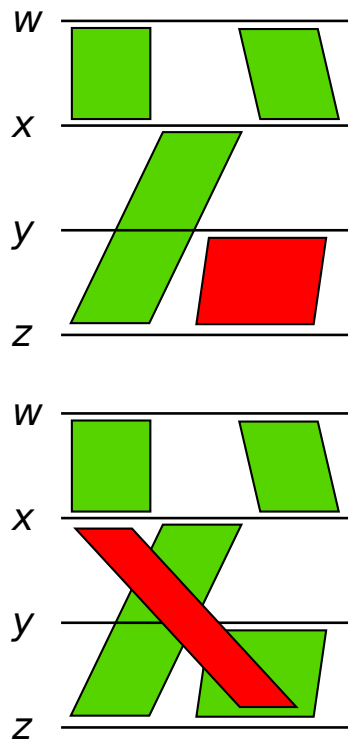

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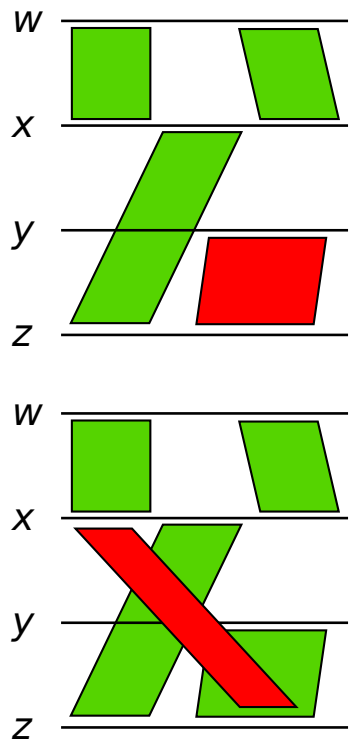

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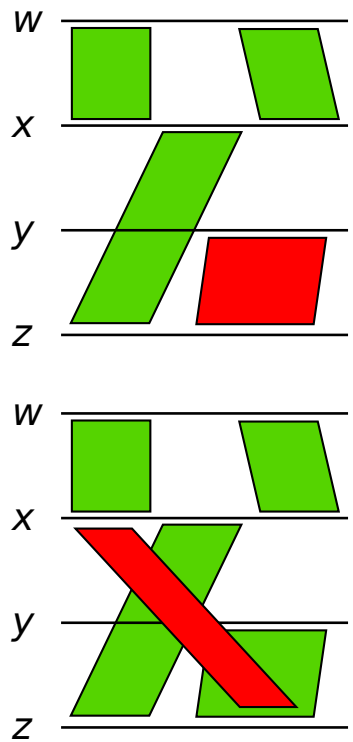

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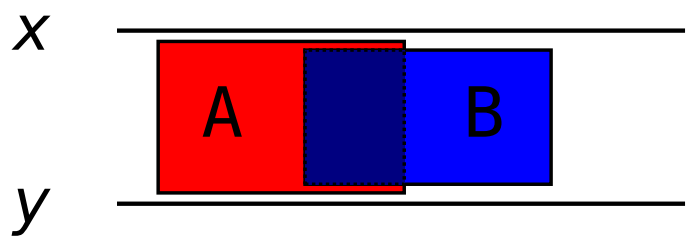

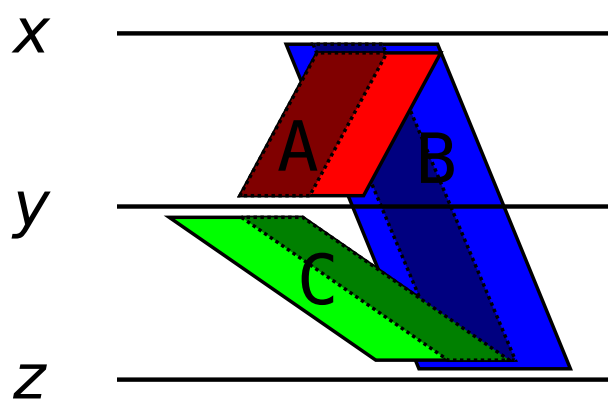

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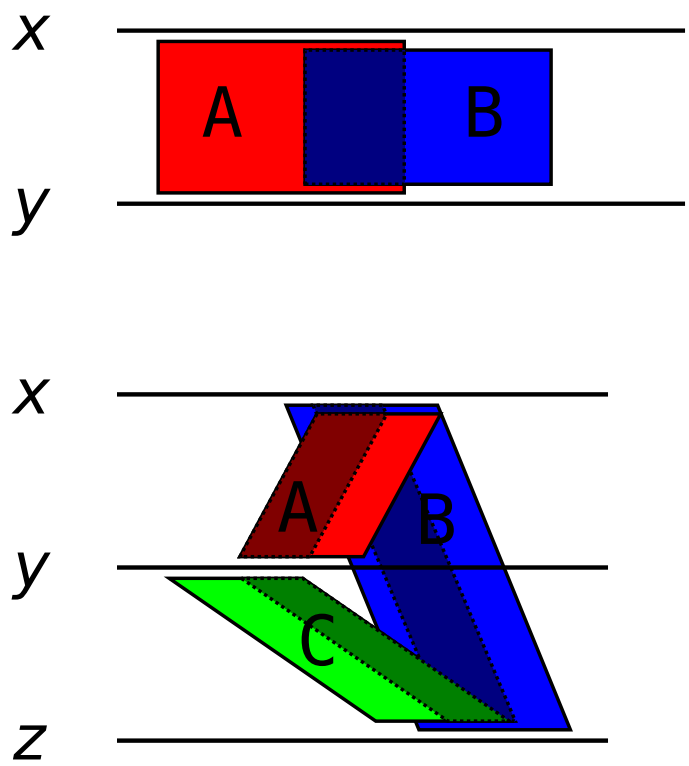

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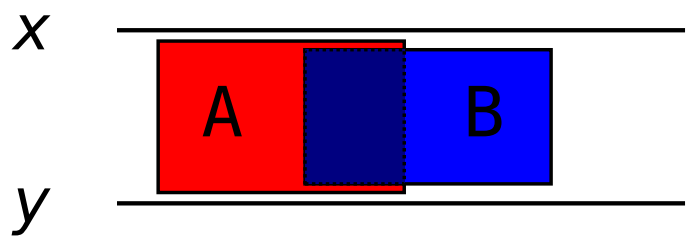

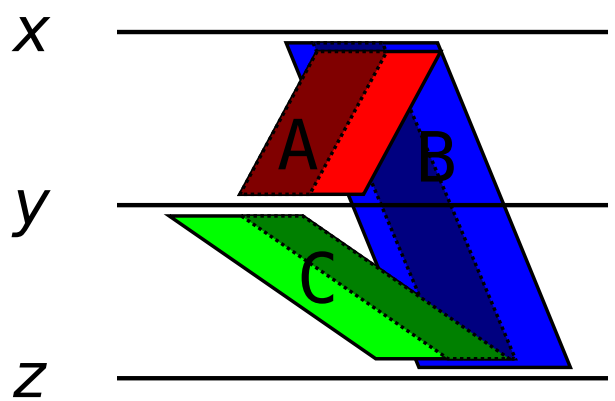

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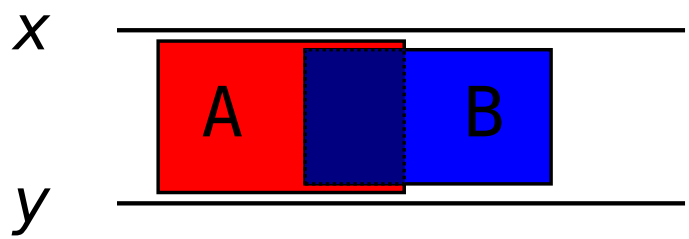

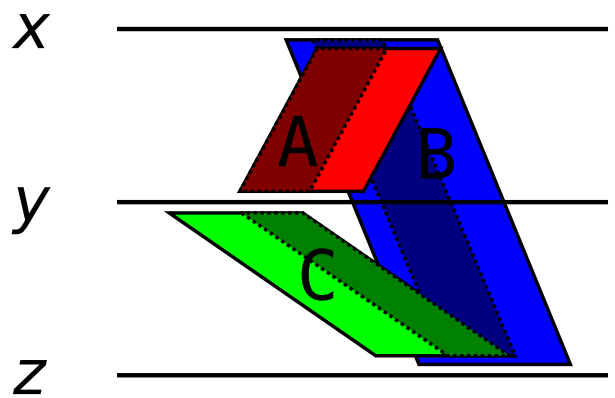

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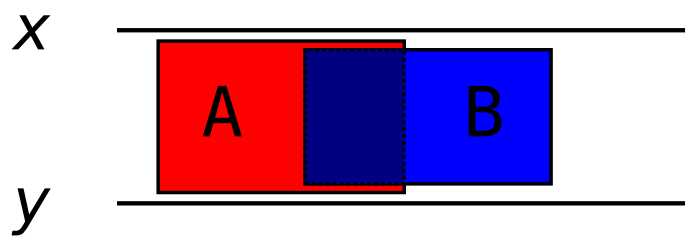

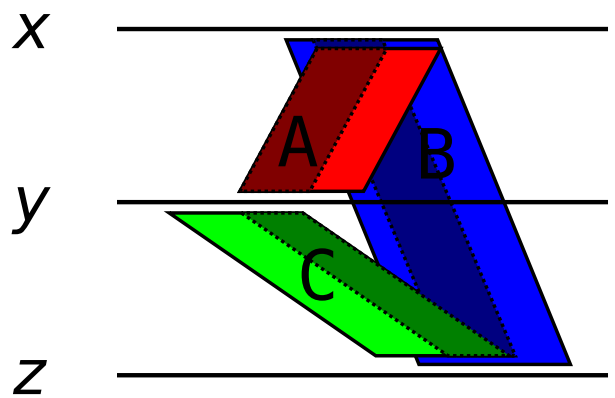

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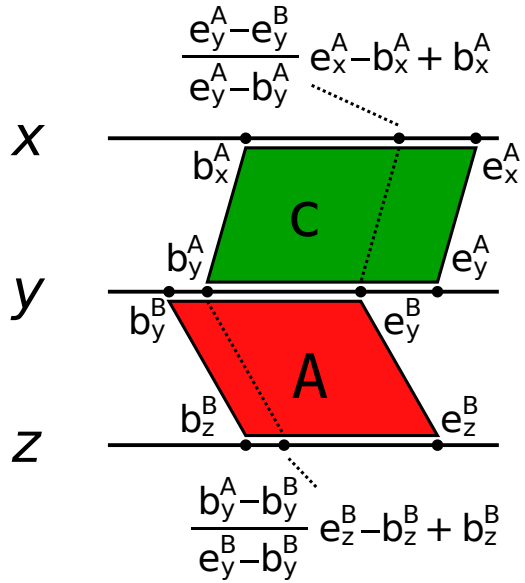

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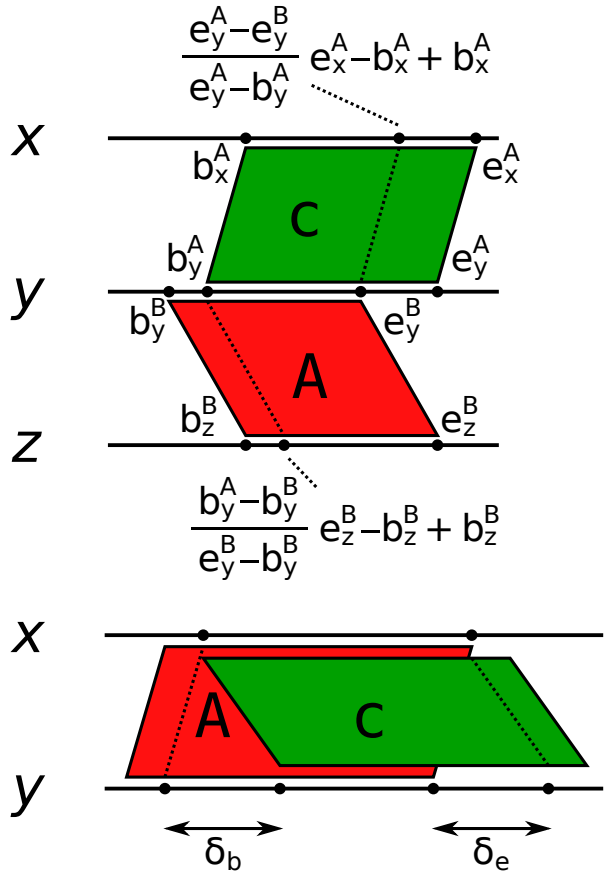

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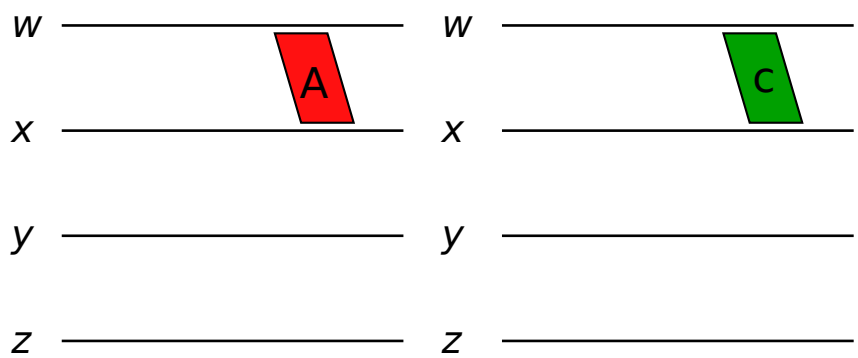

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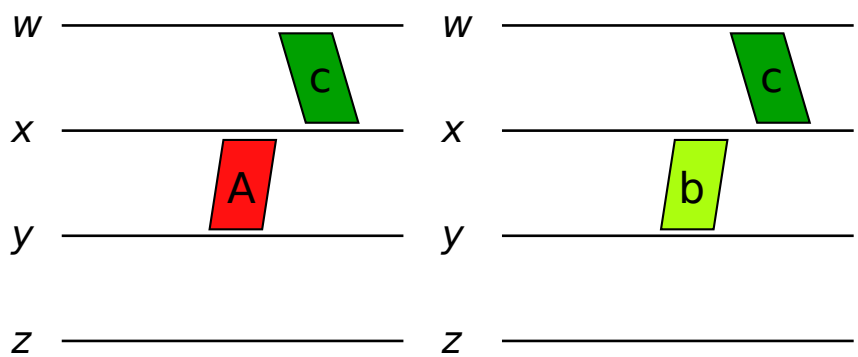

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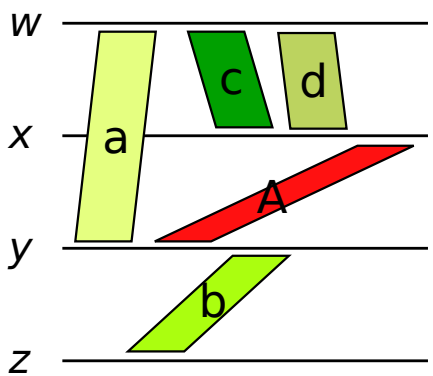

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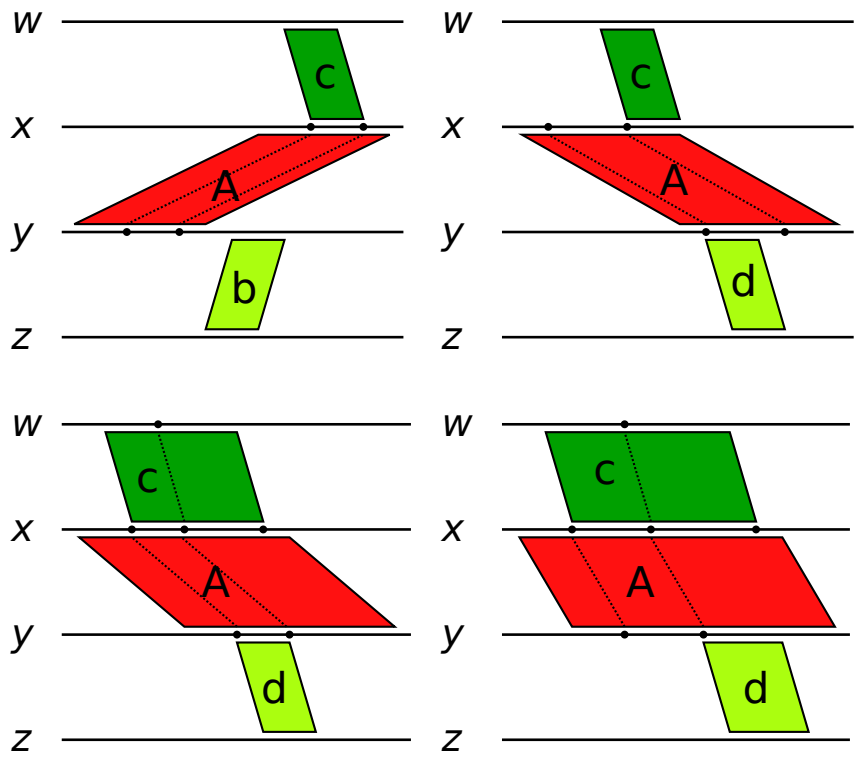

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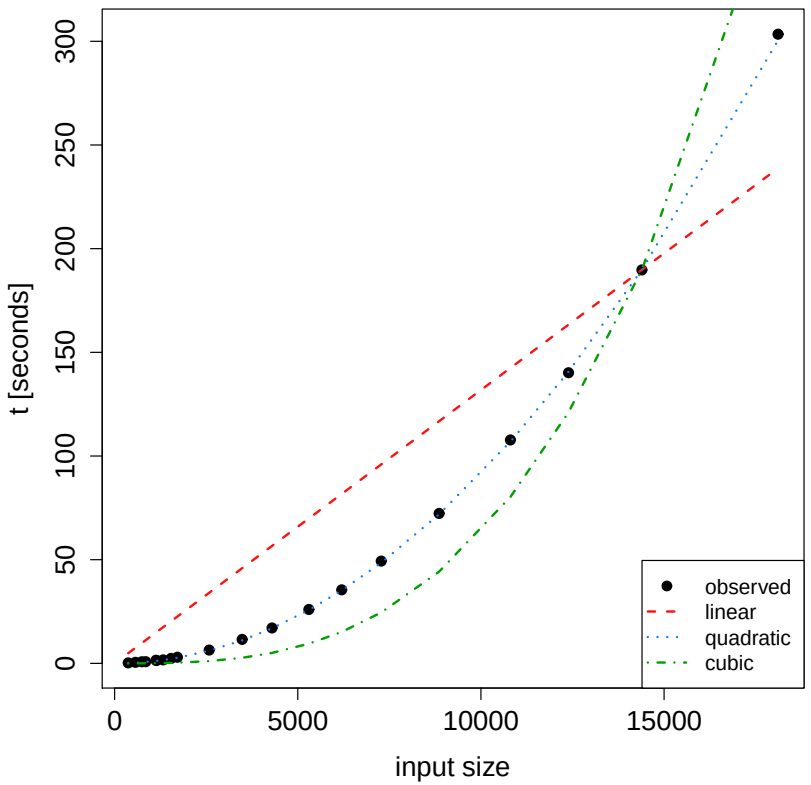

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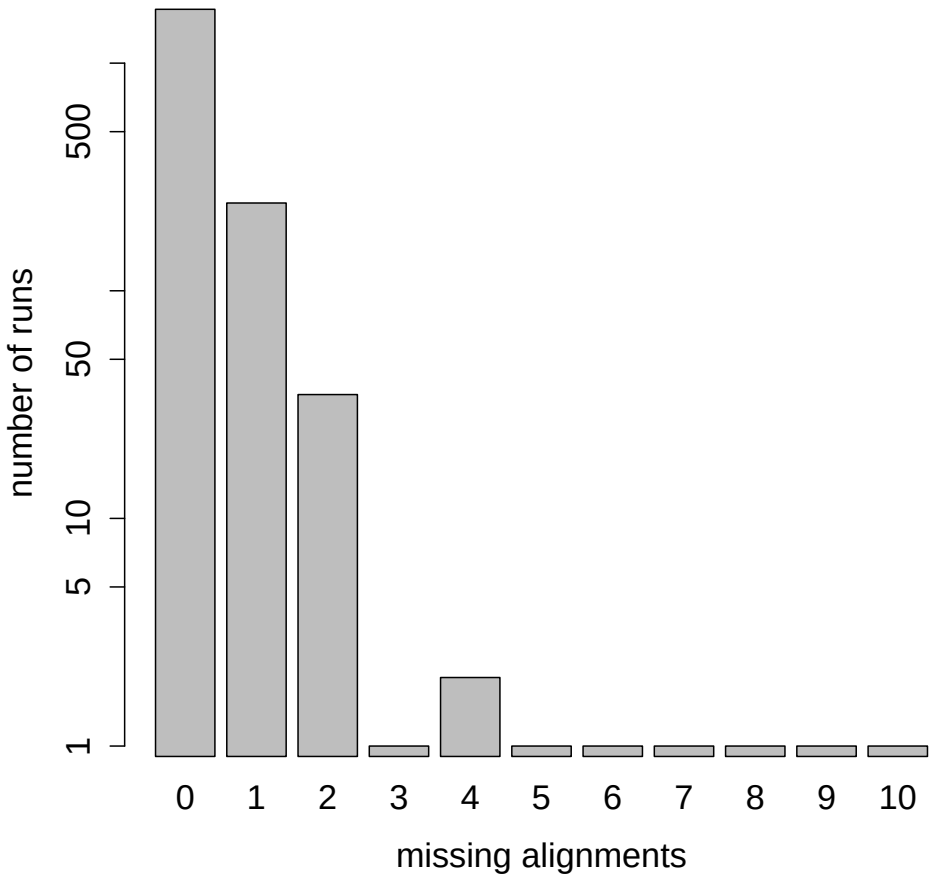
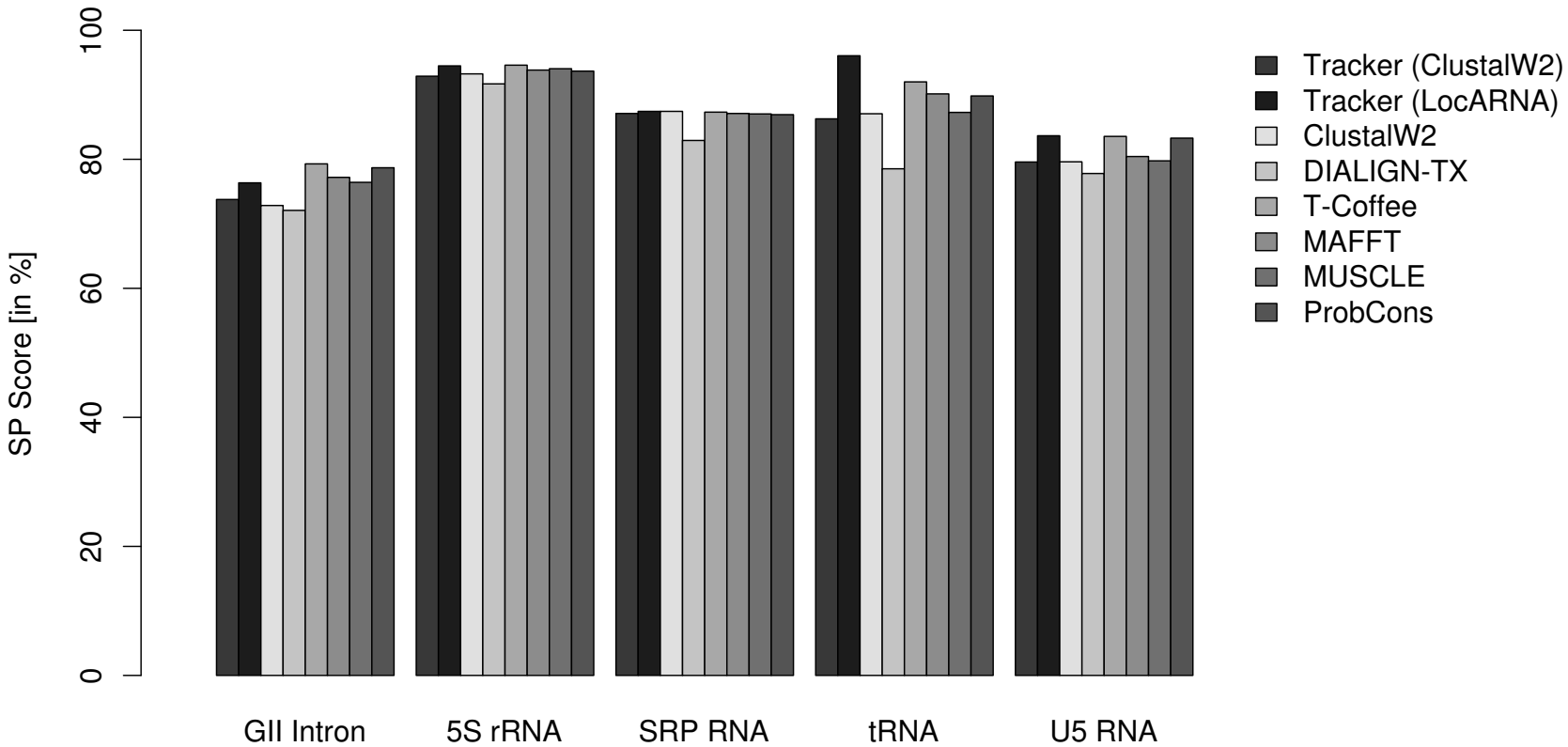


Results: Alignment Calculation on BRaliBase II



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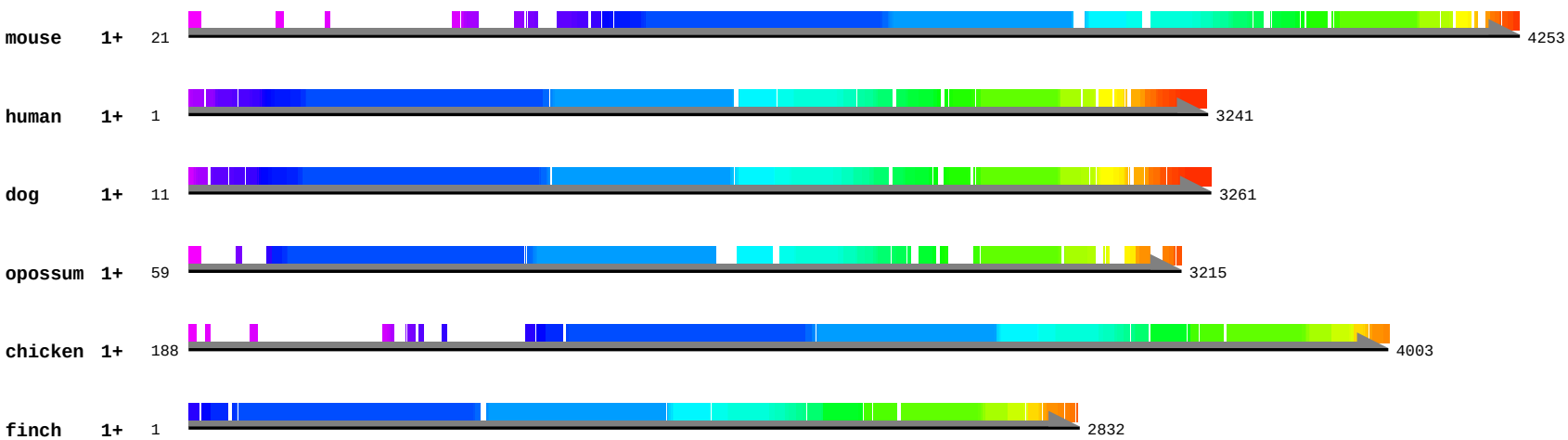
Tracker: CSB and Digit Indentity in Birds



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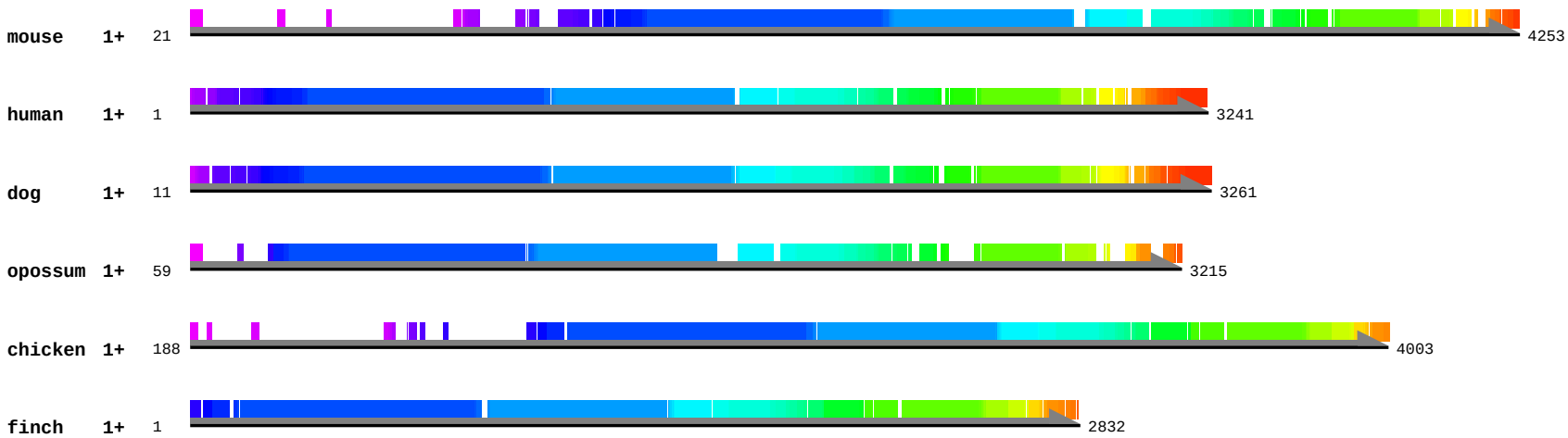
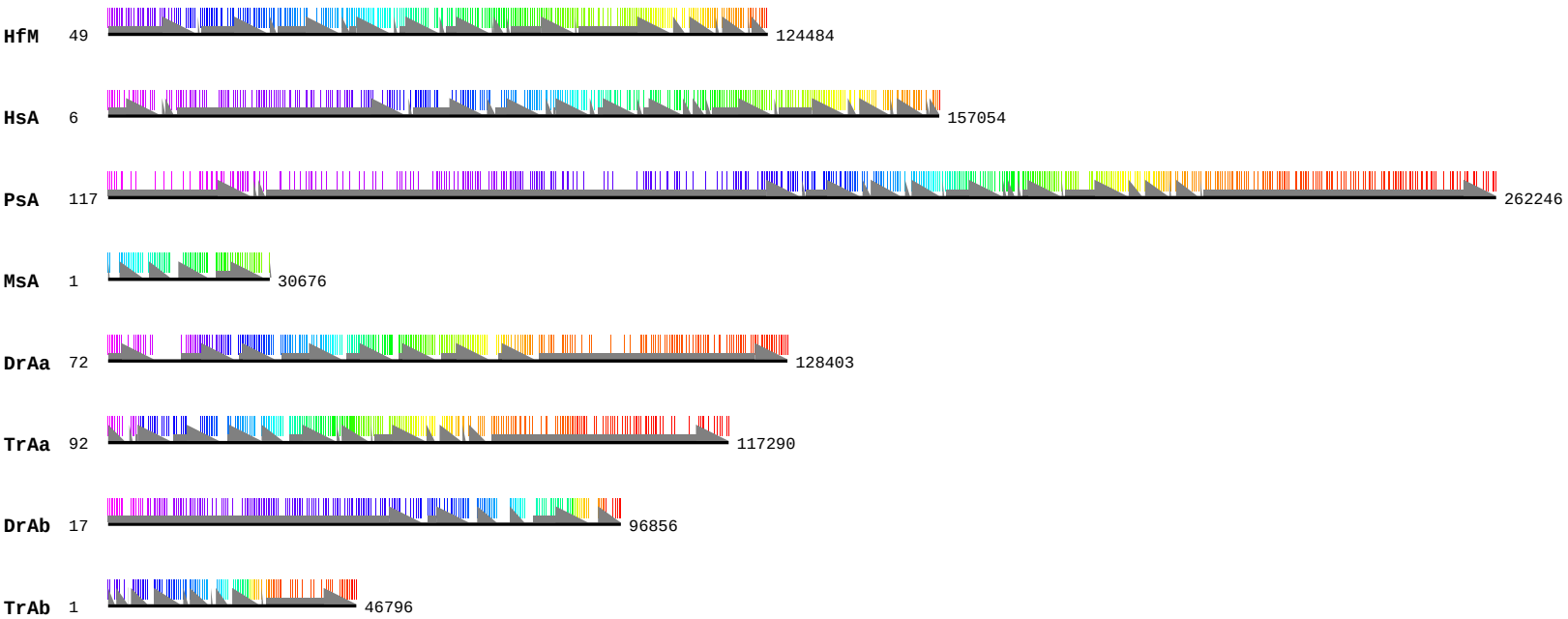



```
mouse:    0|+|3404-3435|32  74 (0)  TCATTACCT-TTTTGGAAAAACACTTCTCTCCC  (27) 77
human:    0|+|2251-2282|32  72 (16) TCATTACTT-TTTCAGAAAAGCACTTTTTTCCC  (0)  76
dog:      0|+|2251-2282|32  72 (16) TCATTACCT-TTTCGGAAAAGCACTTTTTTGCC  (0)  76
opossum:  0|+|2312-2342|31  74 (0)  TCATTGCCTCTTTTGAAAGAGCA--TGTTTCCC  (0)  76
chicken:                            --------------------------------
finch:                              --------------------------------
                                    ***** * * ***   ** * **  * * * **

mouse:                                   -------------------------
human:                                   -------------------------
dog:                                     -------------------------
opossum:                                 -------------------------
chicken:  0|+|3376-3400|25  82 (0)  AAAAGGAGGTAATACTTAAAGGAAA  (1) 93
finch:    0|+|2145-2169|25  82 (0)  ATAAGGAGGCAATACTTAAAGTGAA  (7) 93
                                    * ******* ***********  **
```

Tracker: HoxA-Clusters in Vertebrates



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- todo: usage of phylogenetic information, check for motif overrepresentation in footprints

Evolutionary Analysis of Regulatory Elements

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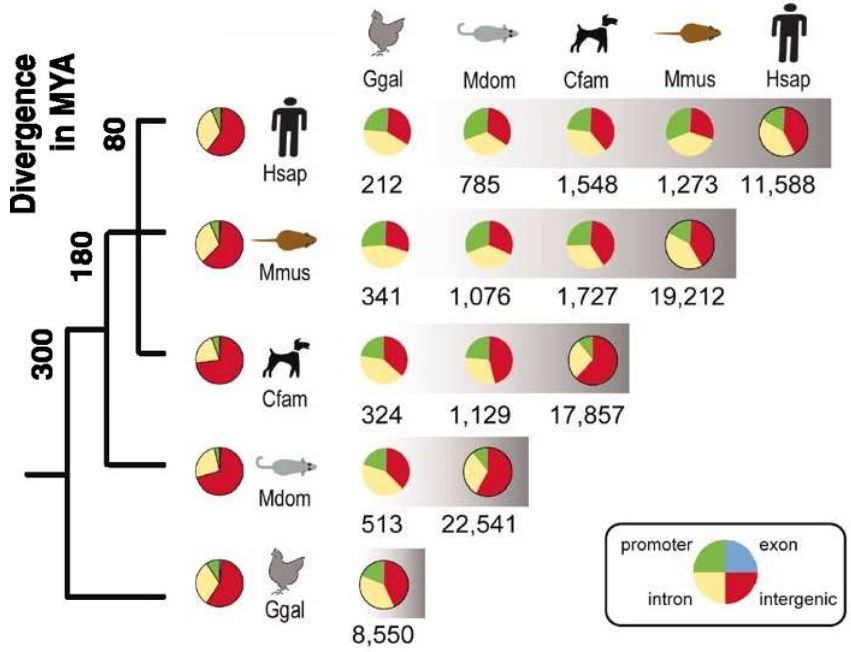
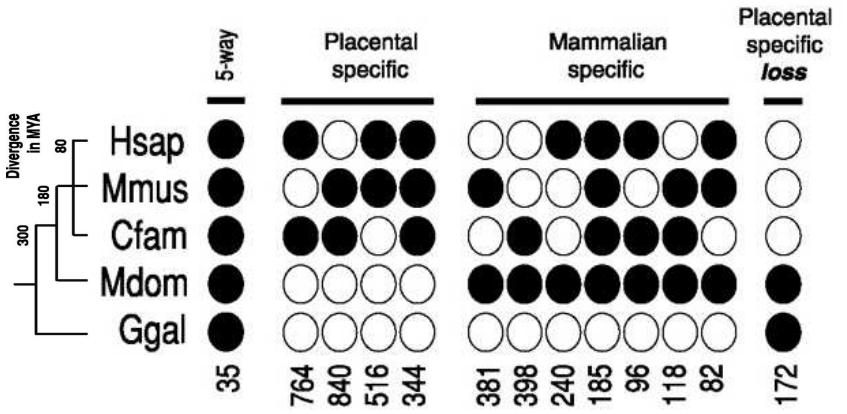
Schmidt *et al.*, Science, May 2010:

- determination of genome-wide occupancy for CCAAT/enhancer-binding protein alpha (CEBPA) in five vertebrates
- CEBPA: regulation of leptin and growth arrest in cultured cells





Binding Site Turnover

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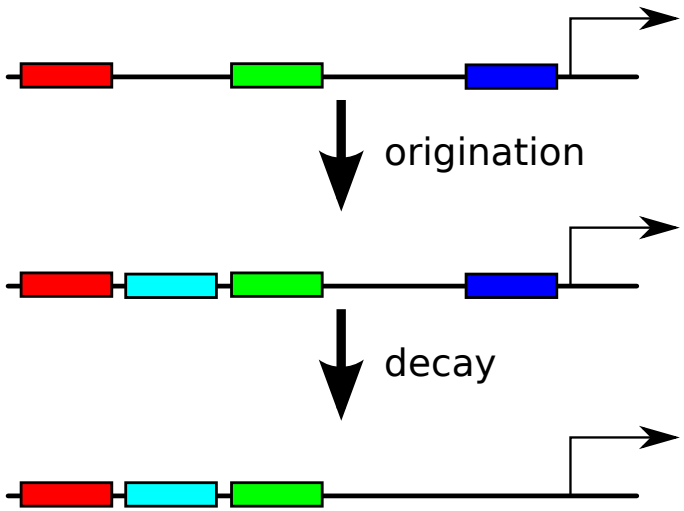

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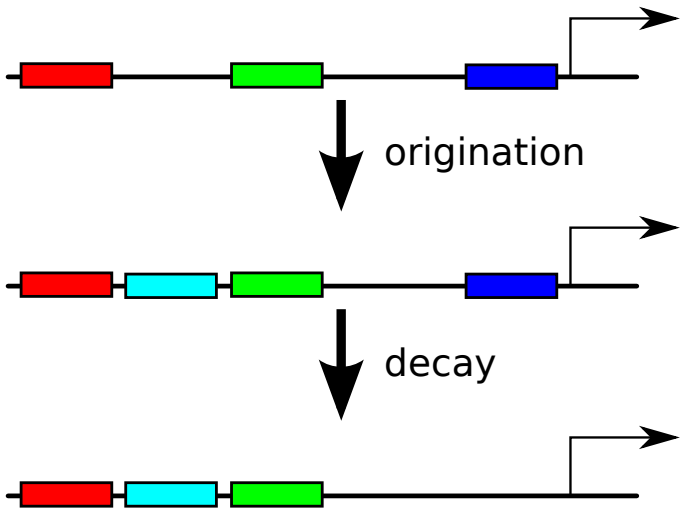

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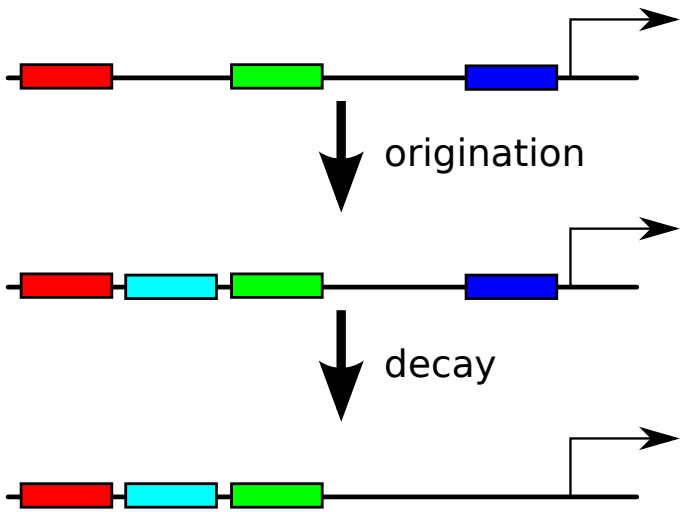

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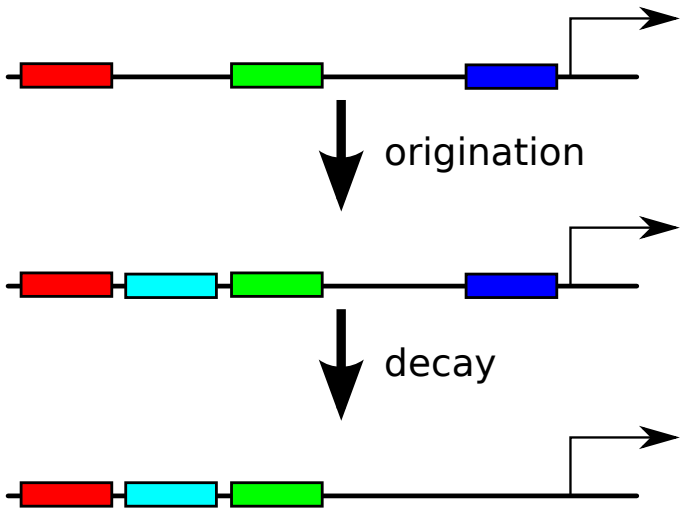
- arrival of new TFBS with origination rate and retention with decay rate



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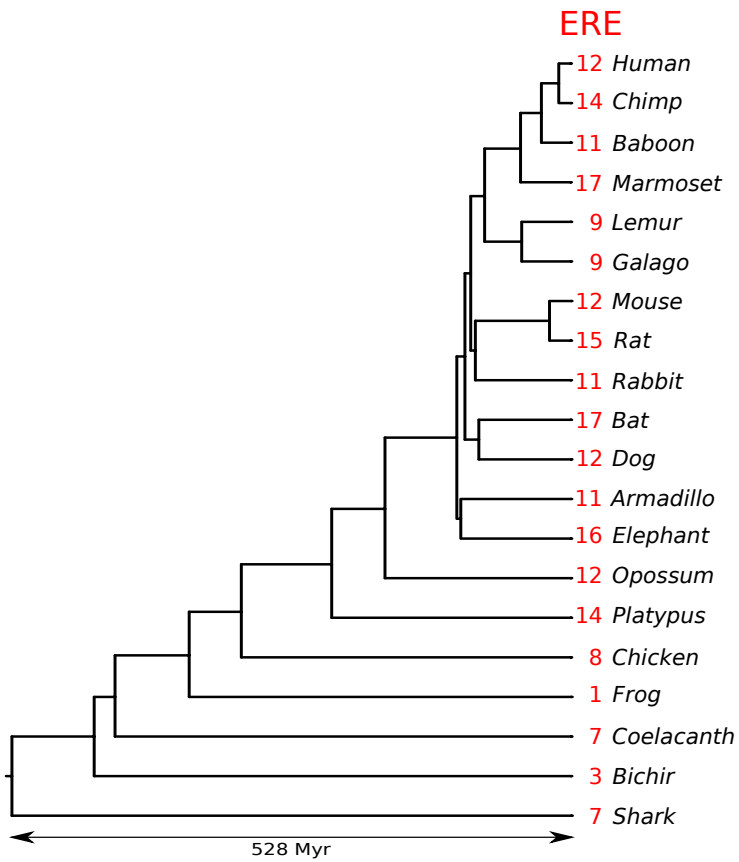
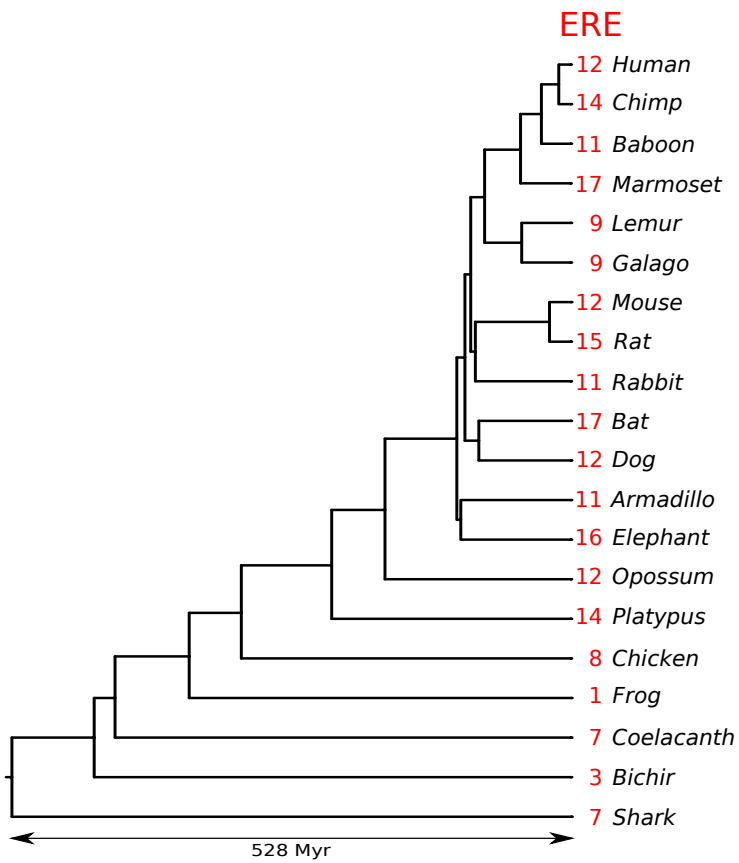


Model for Binding Site Turnover

- what are the most likely rates, how likely is given tree in respect to these rates?



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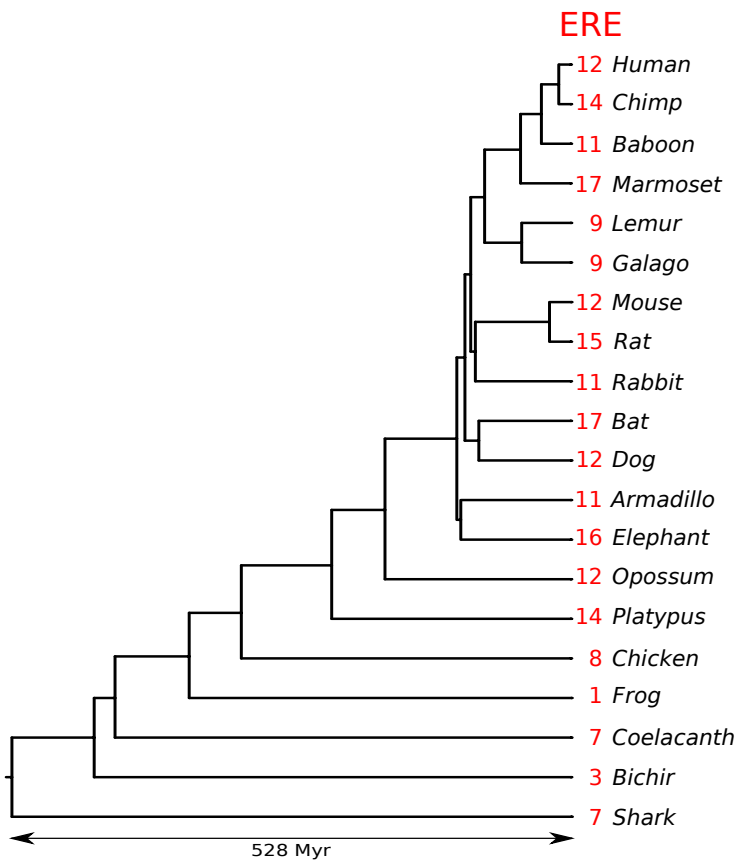

Model for Binding Site Turnover

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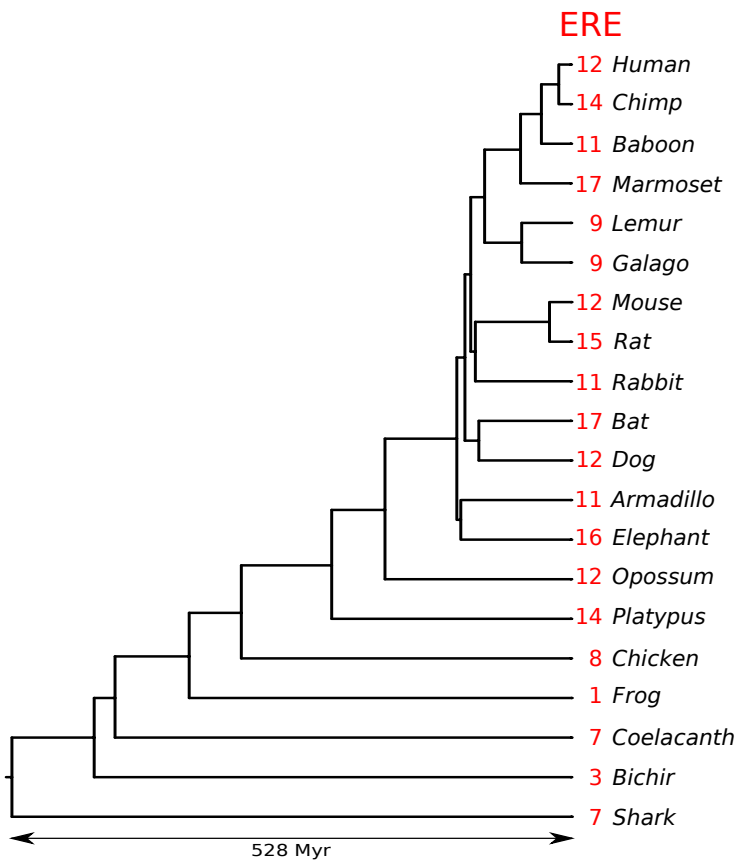
- what is the probability of having a specific binding site number, given start number, time and evolutionary rates?



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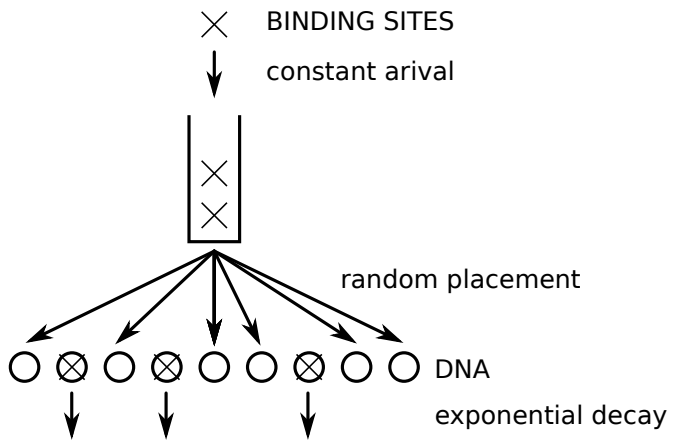

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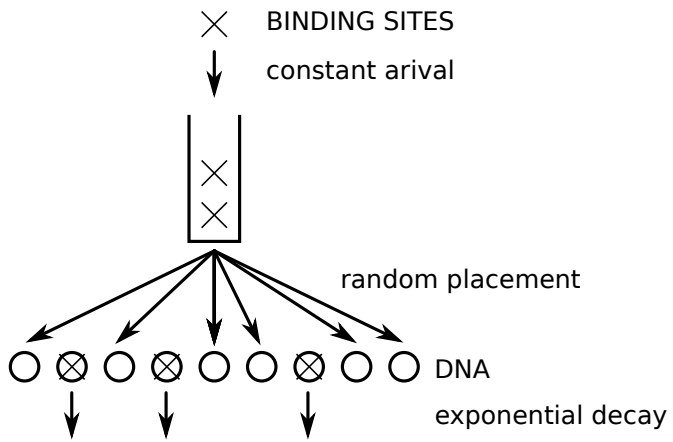

$\Rightarrow$ Kolmogorov forward equation:

$$\dot{p}_0(t) = -\lambda p_0(t) + \mu p_1(t) \quad (1)$$

$$\dot{p}_n(t) = \lambda p_{n-1}(t) - (\lambda + \mu n) p_n(t) + (n+1)\mu p_{n+1}(t) \quad (2)$$

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- use characteristic equations to solve PDE
- expected binding site number:

$$E[n(t)] = \frac{\lambda}{\mu}(1-e^{-\mu t}) + E[n(t=0)]e^{-\mu t} \qquad (4)$$

Model: Transient probability distribution

- solution:

$$p_n(t) = \frac{1}{n!} e^{-(\lambda/\mu)(1-e^{-\mu t})} \sum_{k=0}^{min(n_0,n)} k! \binom{n}{k} \binom{n_0}{k} \left(\frac{\lambda}{\mu}\right)^{n-k} \times (e^{-\mu t})^k (1-e^{-\mu t})^{n+n_0-2k} \quad (5)$$

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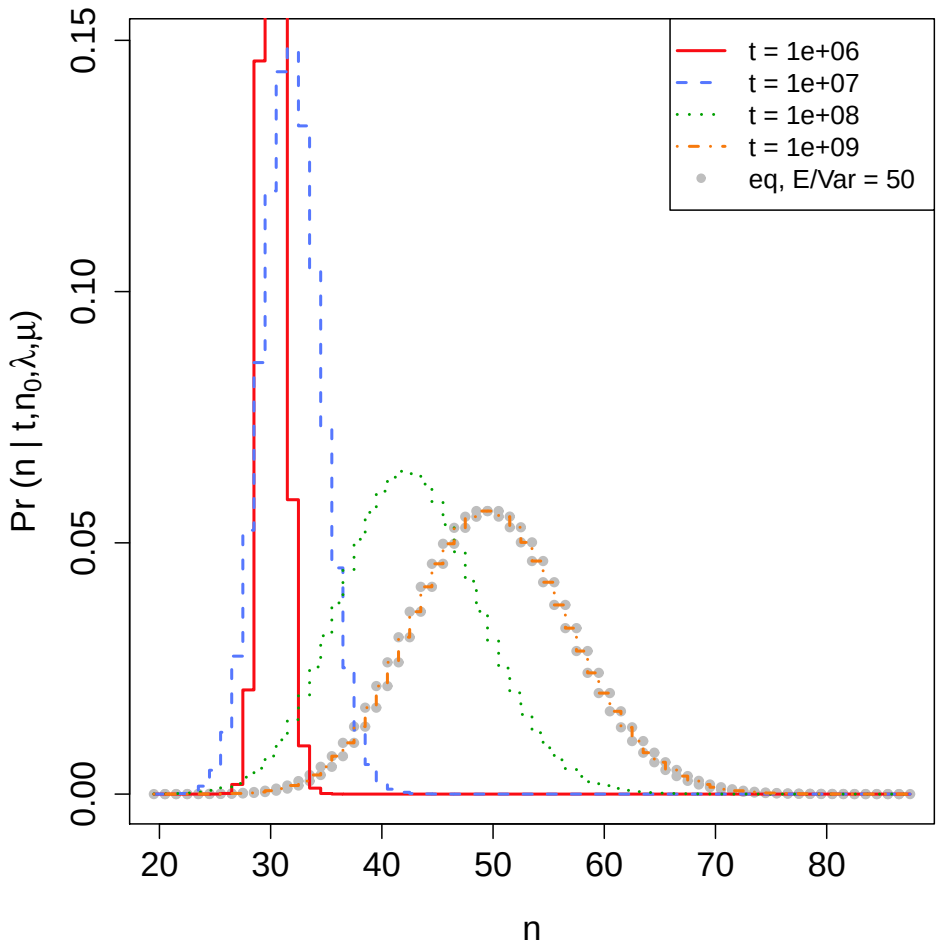
- for $t \to \infty$ follows stationary Poisson distribution:

$$\hat{p}_n = \left(\frac{1}{n!}\right) \left(\frac{\lambda}{\mu}\right)^n e^{-\lambda/\mu} \quad (6)$$

Characteristics

- probability for binding site numbers at different times
  - start binding site number for $t = 0$: $n_0 = 30$
  - origination rate: $\lambda = 5 \times 10^{-7}$
  - decay rate: $\mu = 1 \times 10^{-8}$
  - ratio: $\lambda/\mu = 50$.



Validation by Sequence Evolution

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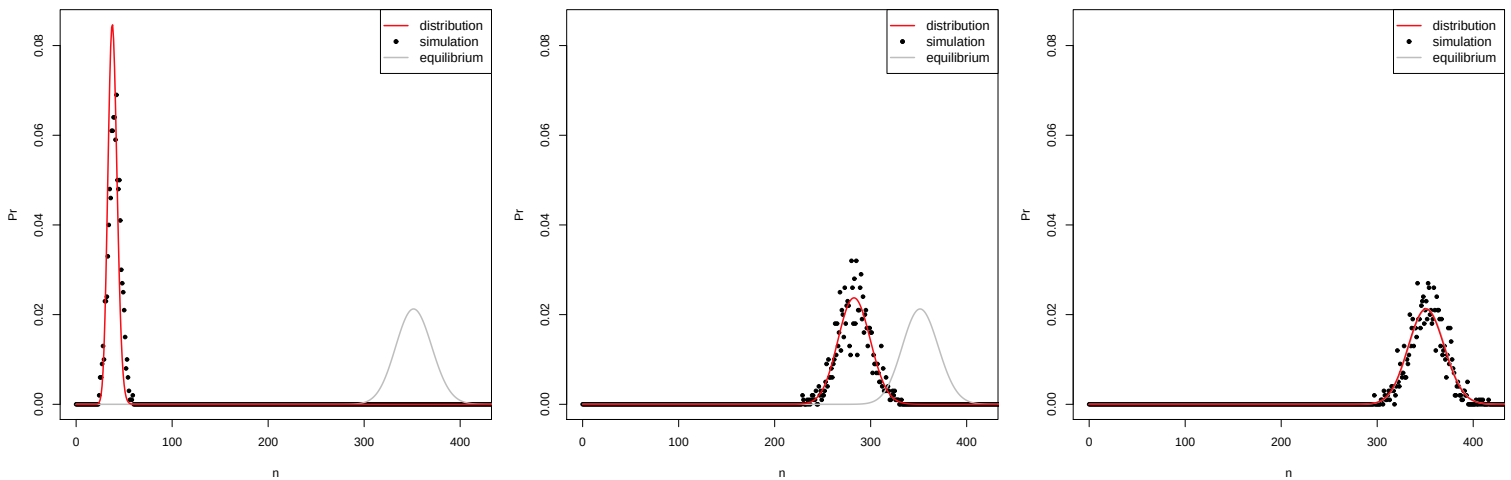

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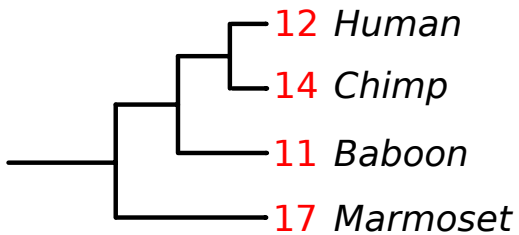

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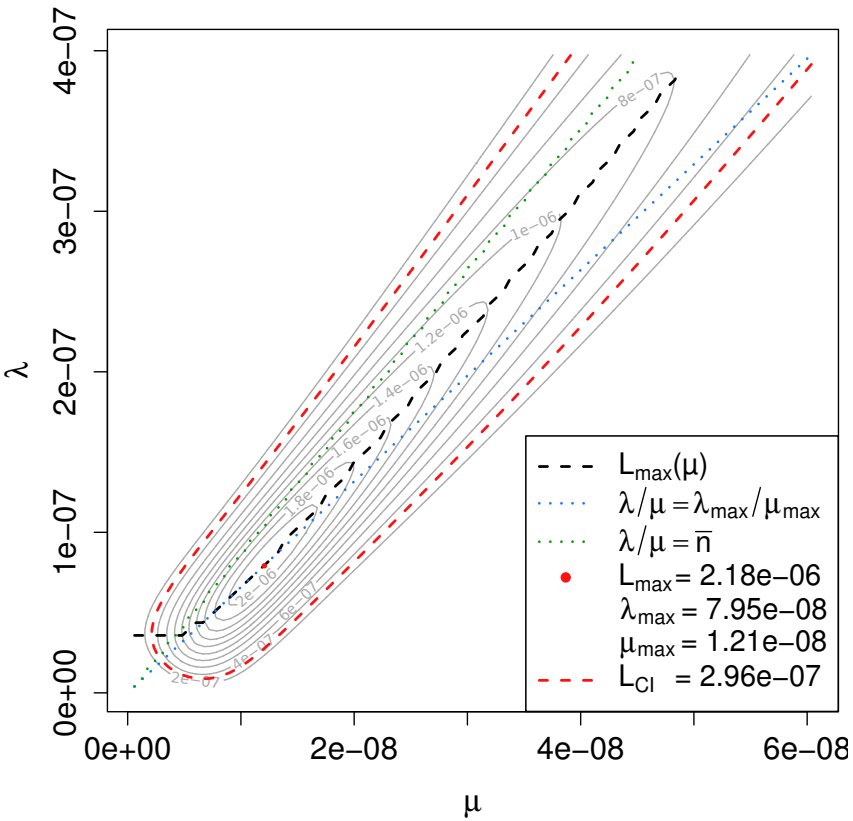
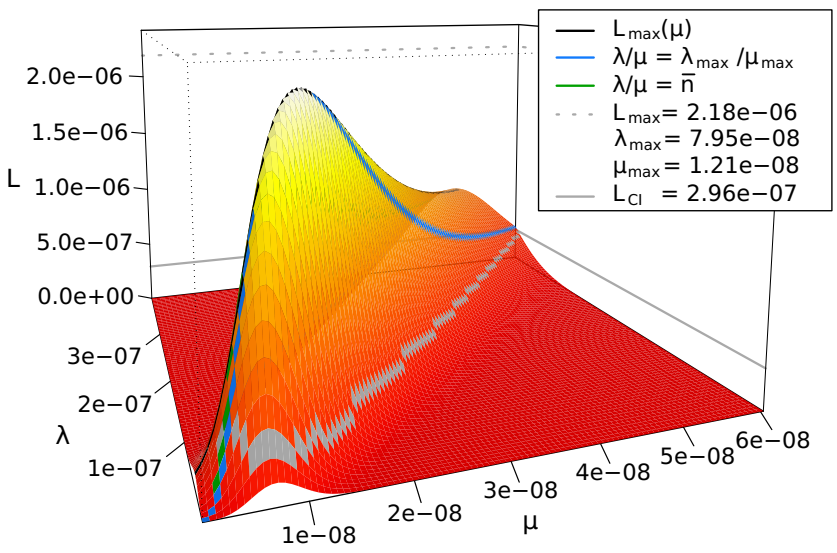
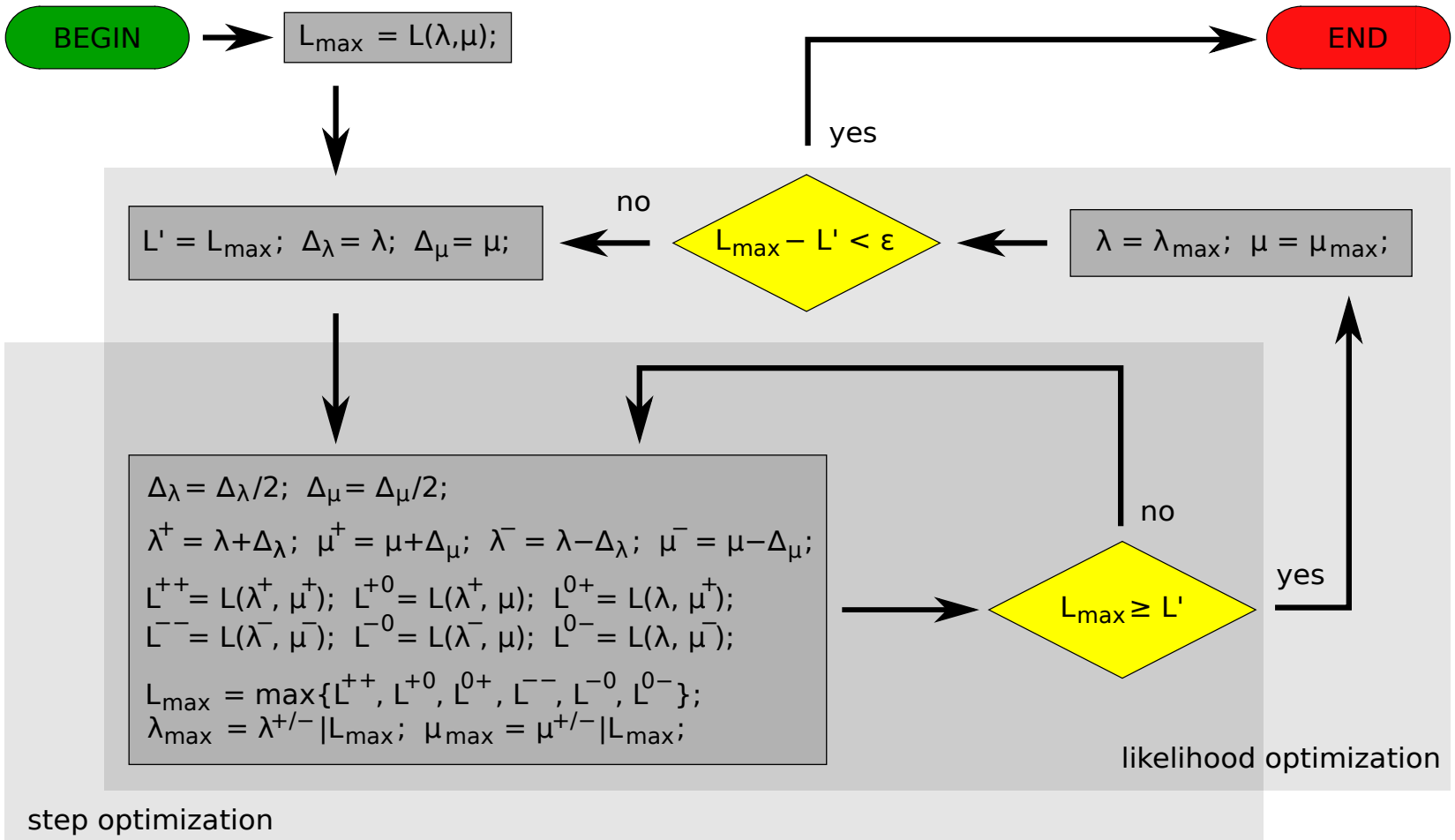
Likelihood Landscape



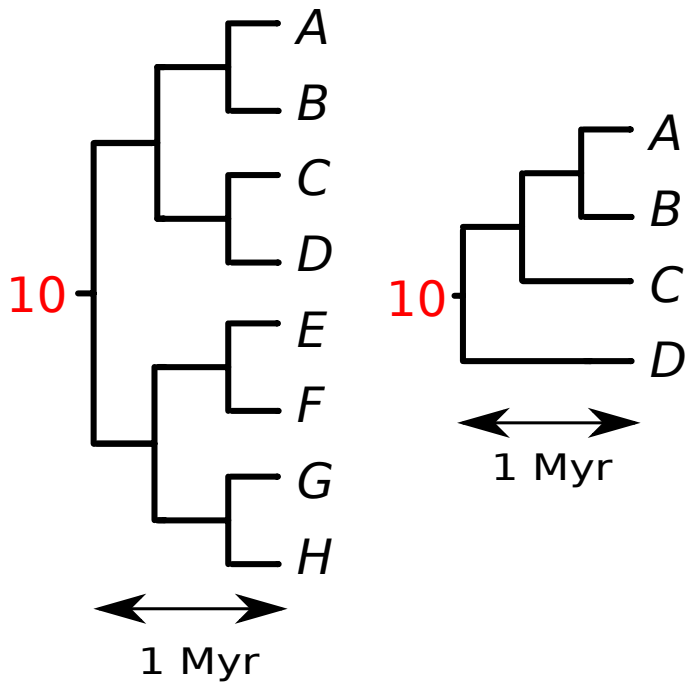


Optimization: Hill Climbing



Results: Simulation of Evolution

- simulation on linear and binary trees with different taxa number



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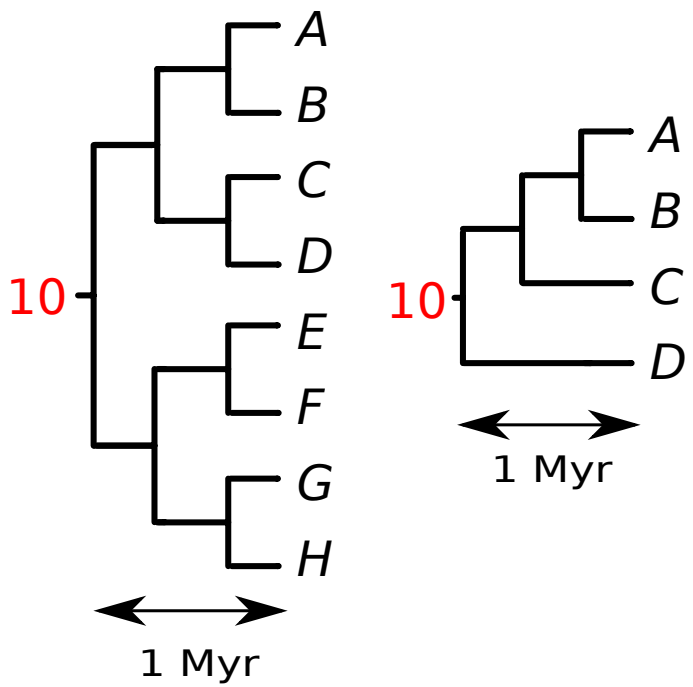

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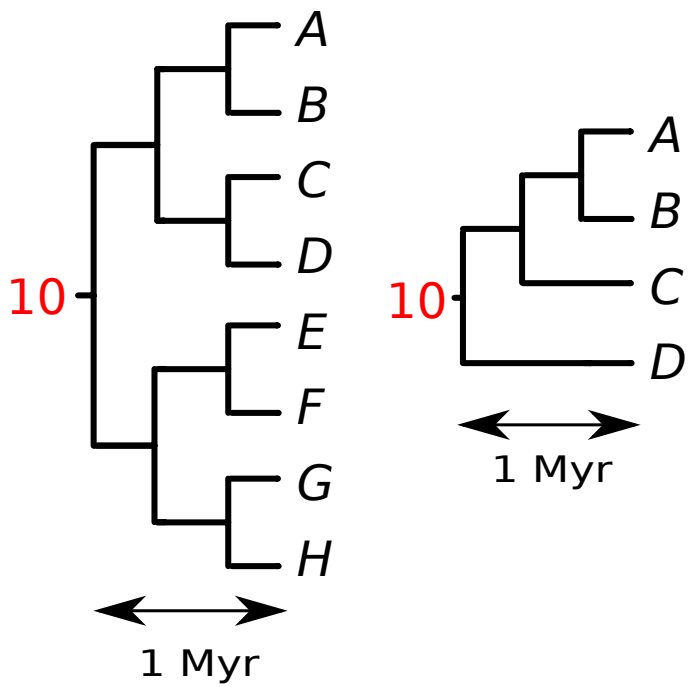

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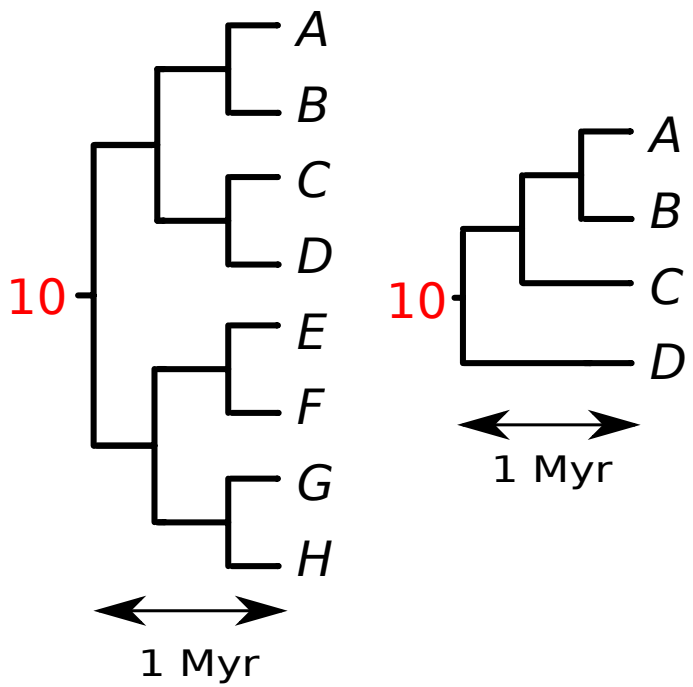

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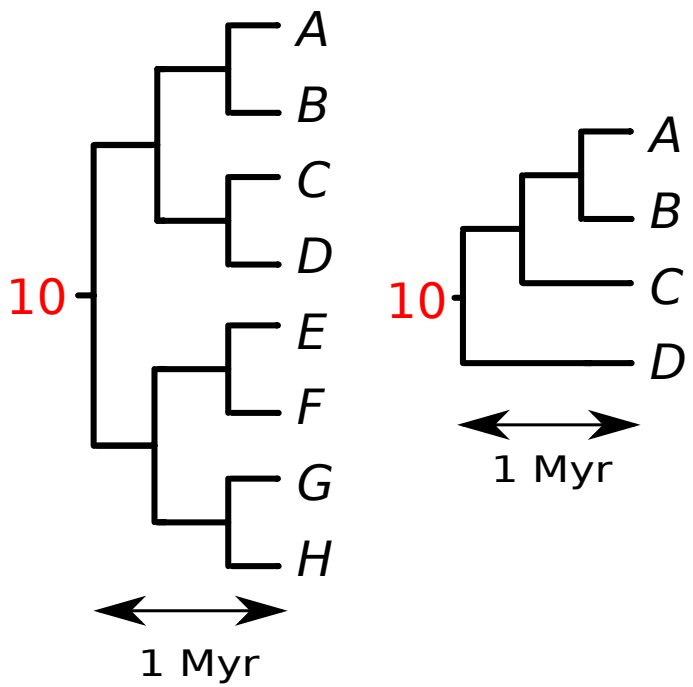
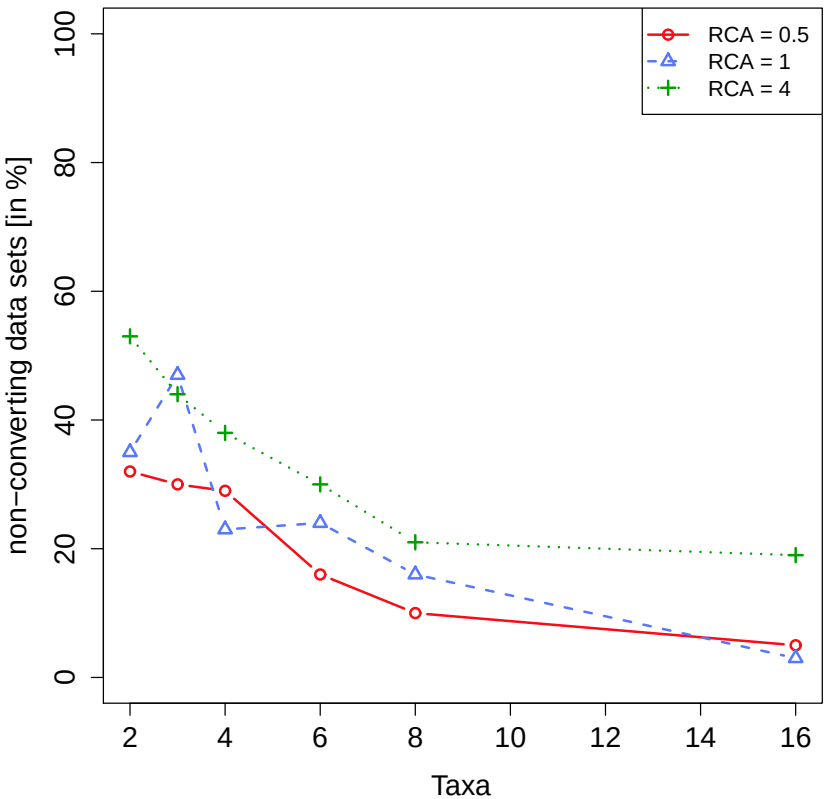
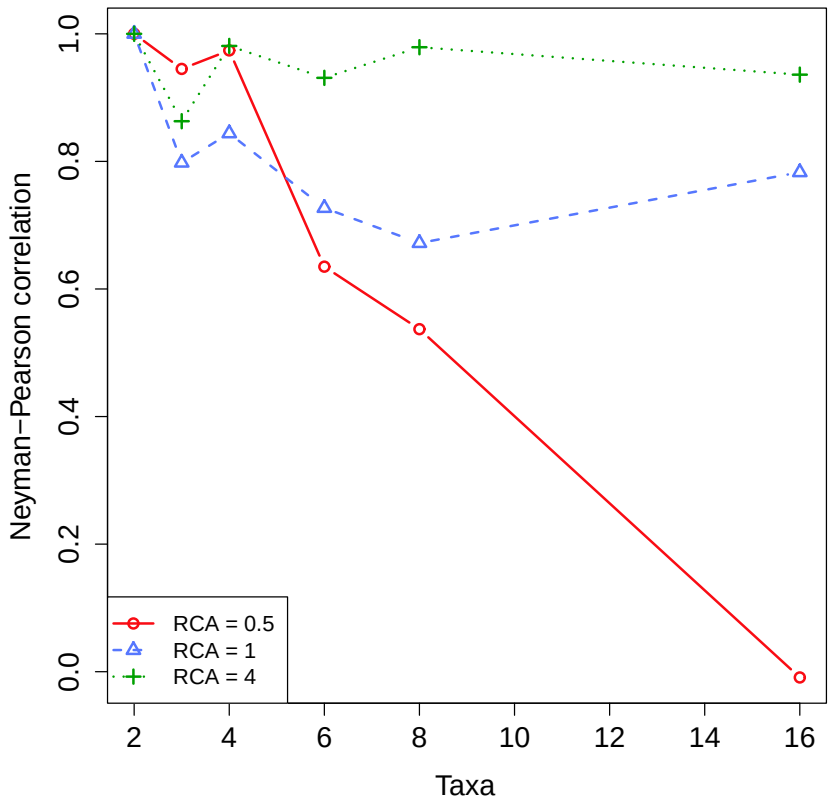
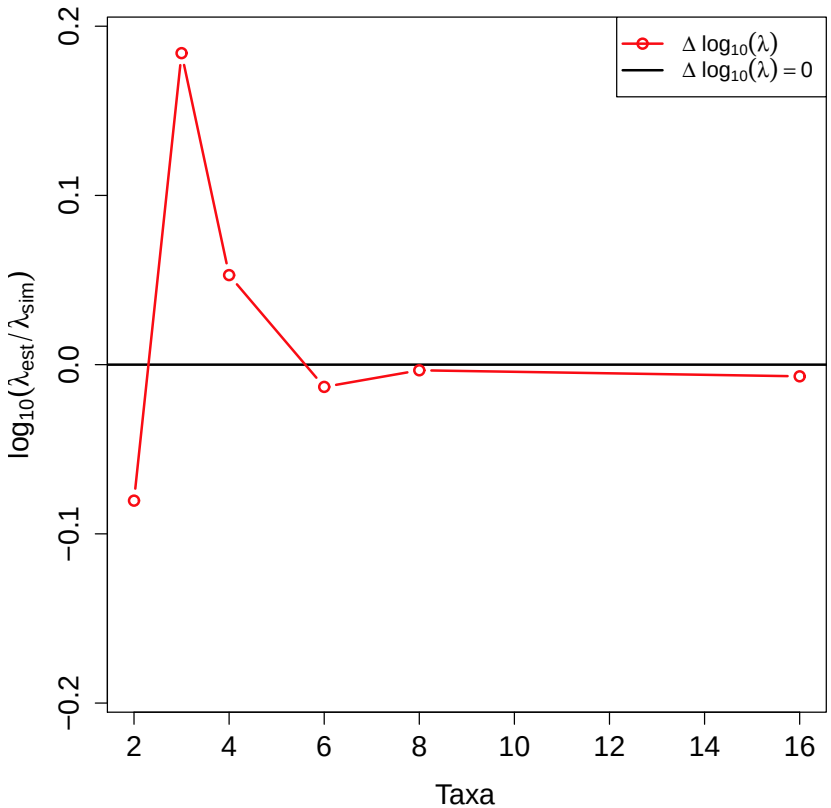


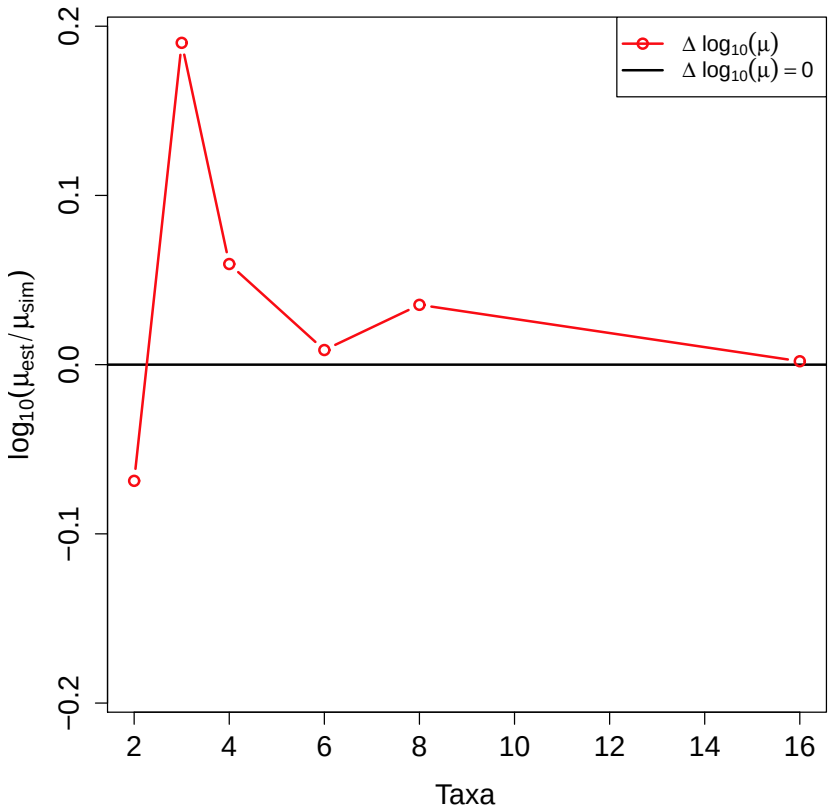
Results: Conversion and λ/μ–$\bar{n}$–Correlation



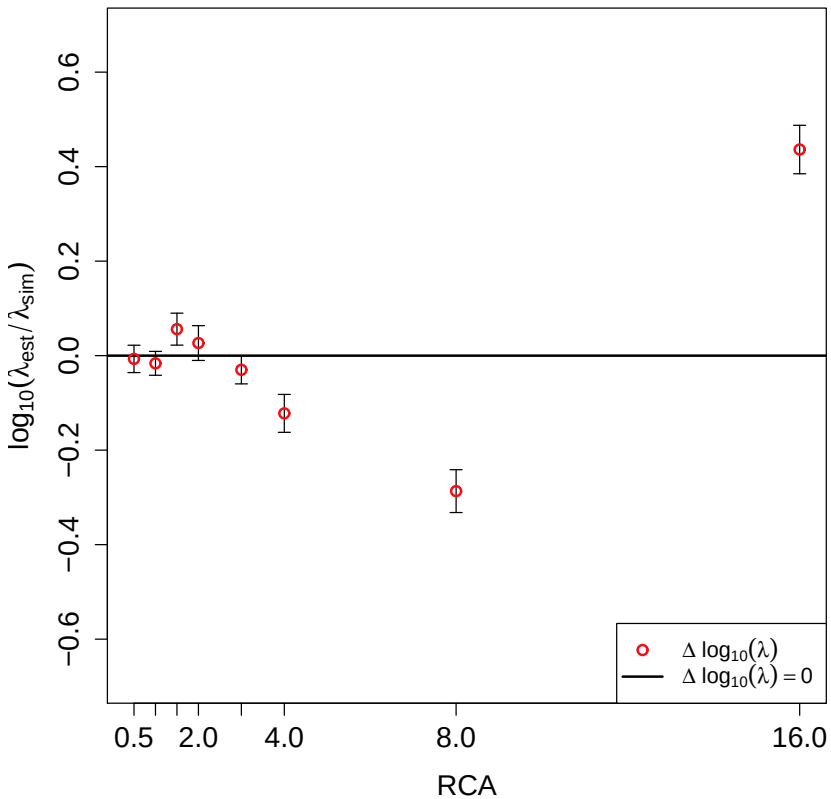


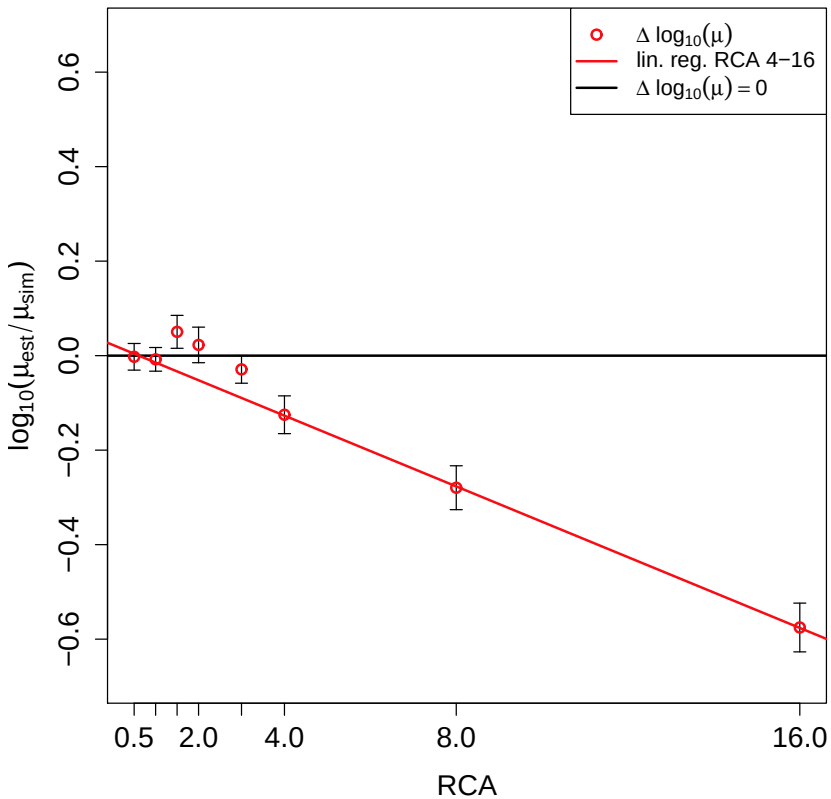
Results: Accuracy in Dependence of Taxa



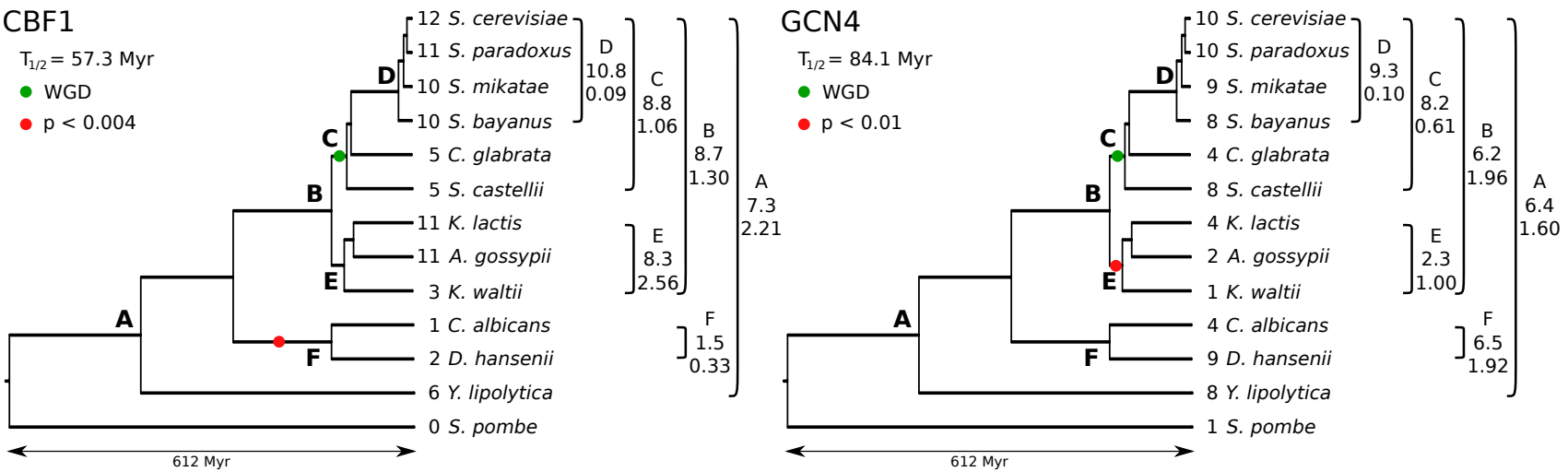


Results: Accuracy in Dependence of RCA

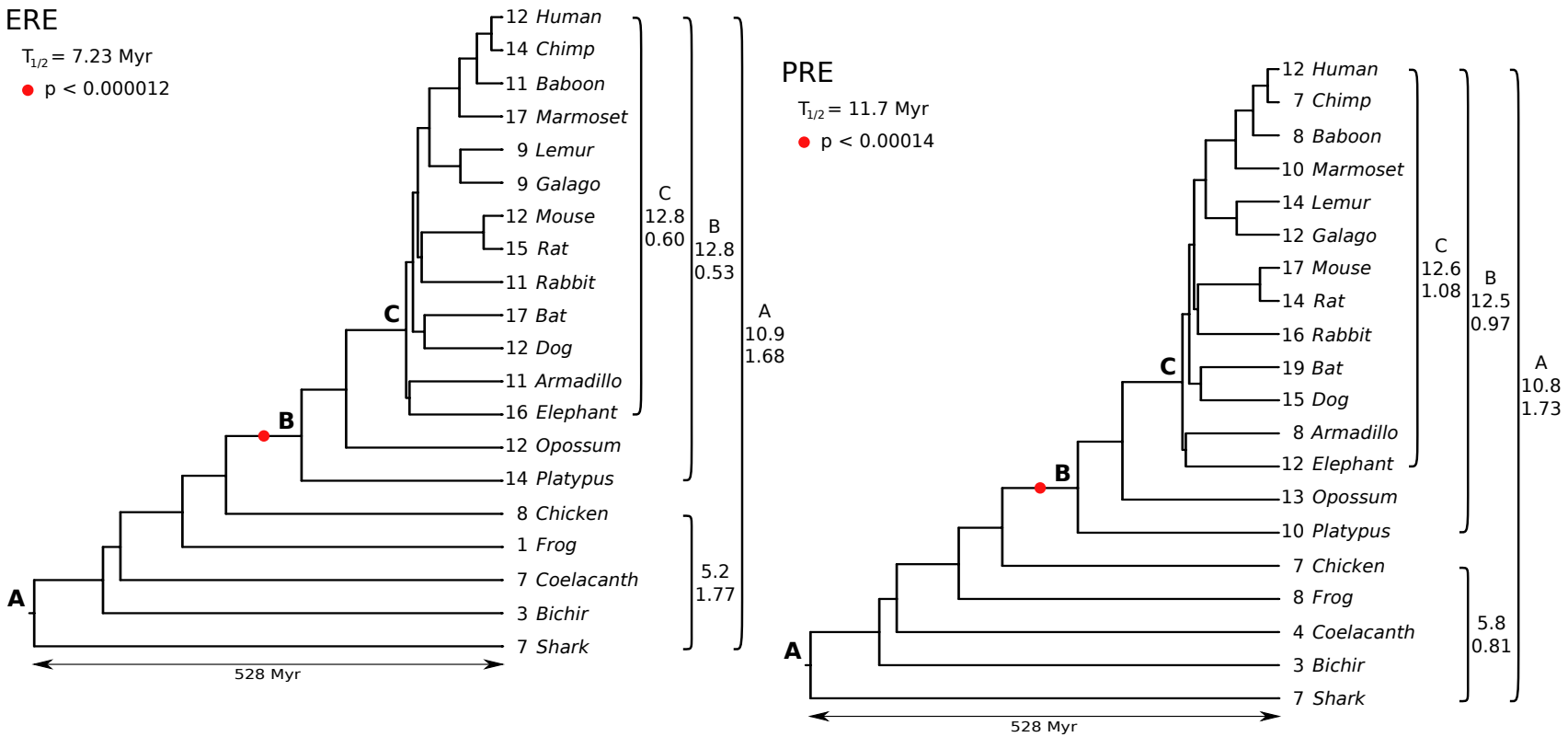




Results: Evolution of Methionine Pathway in Yeast



Results: Evolution of Vertebratee HoxA Clusters



Summary: Evolutionary Analysis of Regulatory Elements

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- simple, mathematically non-trivial, phenomenological model for binding site number evolution at a genomic locus
- allows detection of heterogeneity in rate of origination/decay between different lineages/clades $\Rightarrow$ hints for functionally important changes in the evolution of regulation

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- My PhD-time was supported by:
  - International Max Planck Research School 'Mathematics in the Sciences'
  - Konrad–Adenauer–Foundation

Thank You

Then the Dean repeated the mantra that has had such a marked effect on the progress of knowledge throughout the ages.

"Why don't we just mix up absolutely everything and see what happens?" he said.

And Ridcully responded with the traditional response.

"It's got to be worth a try." he said.

Terry Pratchett, Hogfather