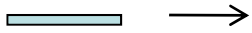
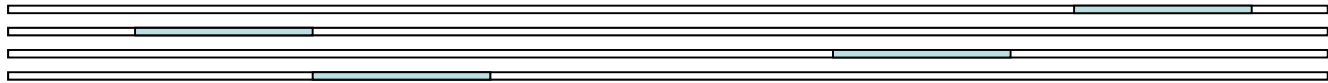


Finding Regulatory Signals in Genomes

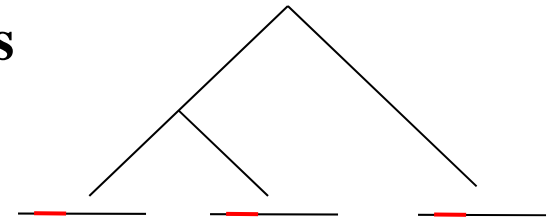
Searching for known signal in 1 sequence



Searching for unknown signal common to set of unrelated sequences

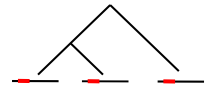


Searching for conserved segments in homologous

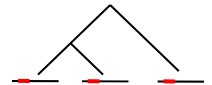


Challenges

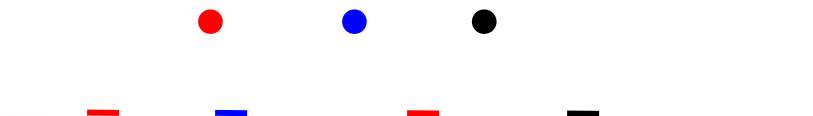
Combining homologous and non-homologous analysis



Merging Annotations



Predicting signal-regulatory protein relationships



Weight Matrices & Sequence Logos

Set of signal sequences:

$f_{b,i}$ b's in position i, $s(b)$ pseudo count.

$$\text{corrected probability: } p(b,i) = \frac{f_{b,i} + s(b)}{N + \sum_{b' \text{ nucleo}} s(b')}$$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	G	A	C	C	A	A	A	T	A	A	G	G	C	A
2	G	A	C	C	A	A	A	T	A	A	G	G	C	A
3	T	G	A	C	T	A	T	A	A	A	A	G	G	A
4	T	G	A	C	T	A	T	A	A	A	A	G	G	A
5	T	G	C	C	A	A	A	A	G	T	G	G	T	C
6	C	A	A	C	T	A	T	C	T	T	G	G	G	C
7	C	A	A	C	T	A	T	C	T	T	G	G	G	C
8	C	T	C	C	T	T	A	C	A	T	G	G	G	C

A	0	4	4	0	3	7	4	3	5	4	2	0	0	4
C	3	0	4	8	0	0	0	3	0	0	0	0	0	4
G	2	3	0	0	0	0	0	0	1	0	6	8	5	0
T	3	1	0	0	5	1	4	2	2	4	0	0	1	0

B R M C W A W H R W G G B M

A	-1.93	.79	.79	-1.93	.45	1.50	.79	.45	1.07	.79	.0	-1.93	-1.93	.79
C	.45	-1.93	.79	1.68	-1.93	-1.93	-1.93	.45	-1.93	-1.93	-1.93	-1.93	.0	.79
G	.0	.45	-1.93	-1.93	-1.93	-1.93	-1.93	-1.93	.66	-1.93	1.3	1.68	1.07	-1.93
T	.15	.66	-1.93	-1.93	1.07	.66	.79	.0	.79	-1.93	-1.93	-1.93	.66	-1.93
	1	2	3	4	5	6	7	8	9	10	11	12	13	14

T T G C A T A A G T A G T C
 .45 -.66 .79 1.66 .45 -.66 .79 .45 -.66 .79 .0 1.68 -.66 .79



Position Frequency Matrix - PFM

Consensus sequence:

Position Weight Matrix - PWM

$$PWM : W_{b,i} = \log_2 \frac{p(b,i)}{p(b)}$$

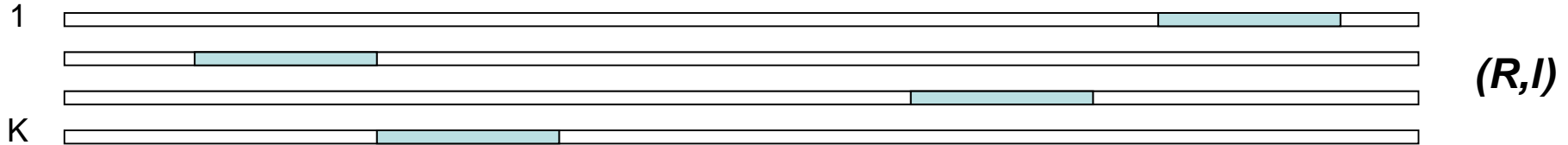
Score for New Sequence $S = \sum_{i=1}^w W_{b,i}$

Sequence Logo & Information content

$$D_i = 2 + \sum_b p_{b,i} \log_2 p_{b,i}$$

Motifs in Biological Sequences

- 1990 Lawrence & Reilly "An Expectation Maximisation (EM) Algorithm for the identification and Characterization of Common Sites in Unaligned Biopolymer Sequences Proteins 7.41-51.
 1992 Cardon and Stormo Expectation Maximisation Algorithm for Identifying Protein-binding sites with variable lengths from Unaligned DNA Fragments L.Mol.Biol. 223.159-170
 1993 Lawrence... Liu "Detecting subtle sequence signals: a Gibbs sampling strategy for multiple alignment" Science 262, 208-214.



$\Theta = (\theta_{1,A}, \dots, \theta_{w,T})$ probability of different bases in the window

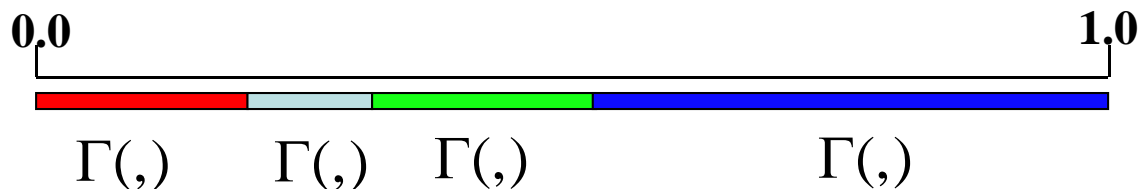
$A = (a_1, \dots, a_K)$ – positions of the windows

$\theta_0 = (\theta_A, \dots, \theta_T)$ – background frequencies of nucleotides.

$$p(R | \theta_0, \Theta, A) = \theta_0^{h(R_{\{A\}^c})} \prod_{j=1}^w \theta_j^{h(R_{A+j-1})} = \theta_0^{h(R)} \prod_{j=1}^w \left(\frac{\theta_j}{\theta_0} \right)^{h(R_{A+j-1})}$$

Priors A has uniform prior

Θ_j has Dirichlet($N_0\alpha$) prior – α base frequency in genome. N_0 is pseudocounts



Natural Extensions to Basic Model I

Multiple Pattern Occurances in the same sequences:

Liu, J. "The collapsed Gibbs sampler with applications to a gene regulation problem," *Journal of the American Statistical Association* 89 958-966.

Prior: any position i has a small probability p to start a binding site:

$$A=(a_1,\dots,a_k) \quad P(A) \approx p_0^k(1-p_0)^{N-k} \quad (\text{with nonoverlapping constraints})$$



Composite Patterns:

BioOptimizer: the Bayesian Scoring Function Approach to Motif Discovery *Bioinformatics*



Natural Extensions to Basic Model II

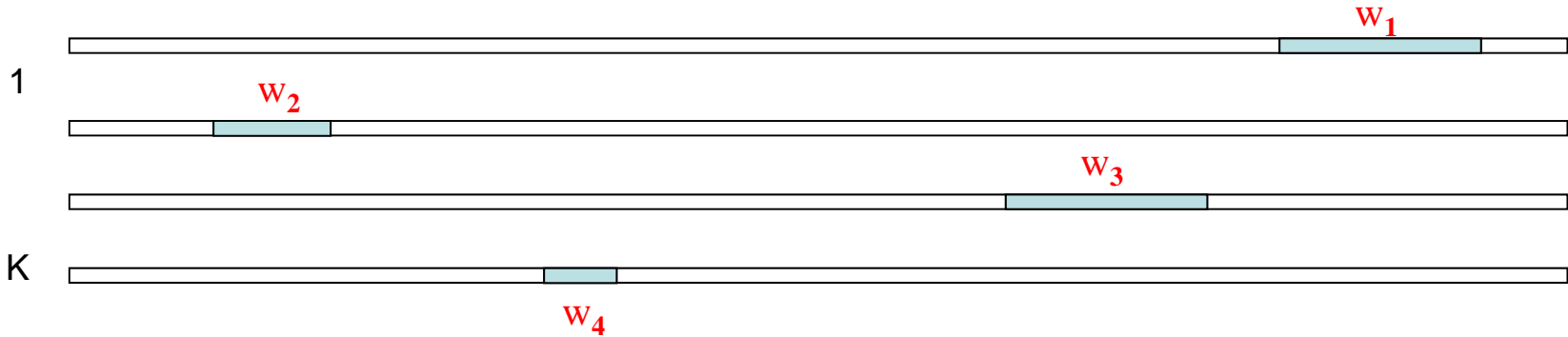
Correlated in Nucleotide Occurrence in Motif:

Modeling within-motif dependence for transcription factor binding site predictions. *Bioinformatics*, 6, 909-916.



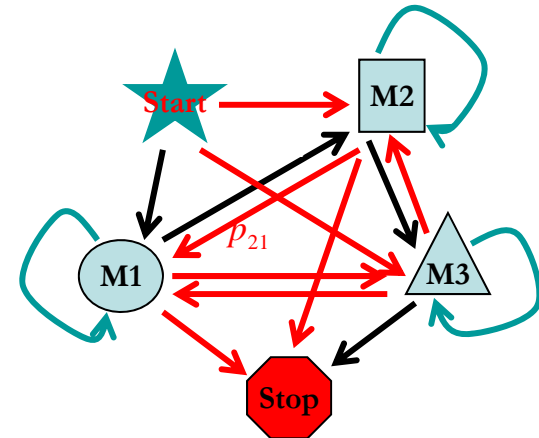
Insertion-Deletion

BALSA: Bayesian algorithm for local sequence alignment *Nucl. Acids Res.*, 30 1268-77.



Regulatory Modules:

De novo cis-regulatory module elicitation for eukaryotic genomes. *Proc Nat'l Acad Sci USA*, 102, 7079-84



Combining Signals and other Data

Expression and Motif Regression:

Integrating Motif Discovery and Expression Analysis Proc.Natl.Acad.Sci. 100.3339-44



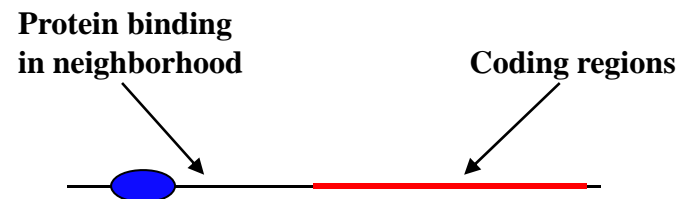
1. Rank genes by $E = \log_2(\text{expression fold change})$
2. Find “many” (hundreds) candidate motifs
3. For each motif pattern m , compute the vector S_m of matching scores for genes with the pattern

4. Regress E on S_m
$$Y_g = \alpha + \beta_m S_{mg} + \epsilon_g$$



ChIP-on-chip - 1-2 kb information on protein/DNA interaction:

An Algorithm for Finding Protein-DNA Interaction Sites with Applications to Chromatin Immunoprecipitation Microarray Experiments *Nature Biotechnology*, 20, 835-39



Phylogenetic Footprinting (homologous detection)

Term originated in 1988 in Tagle et al. **Blanchette et al.:** For unaligned sequences related by phylogenetic tree, find all segments of length **k** with a history costing less than **d**. Motif loss an option.

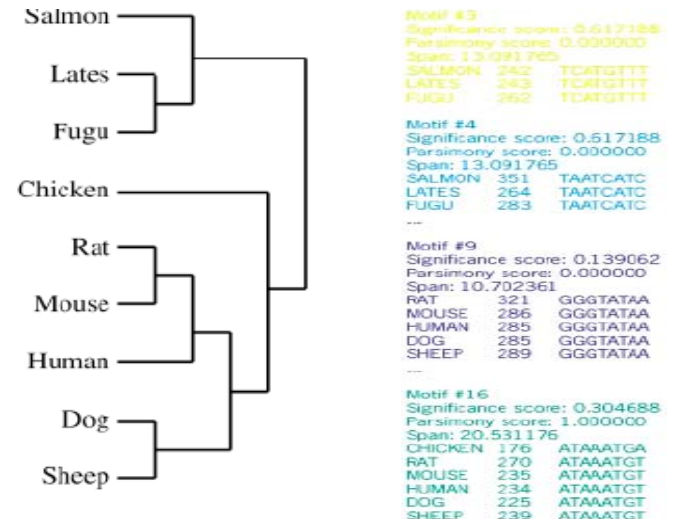
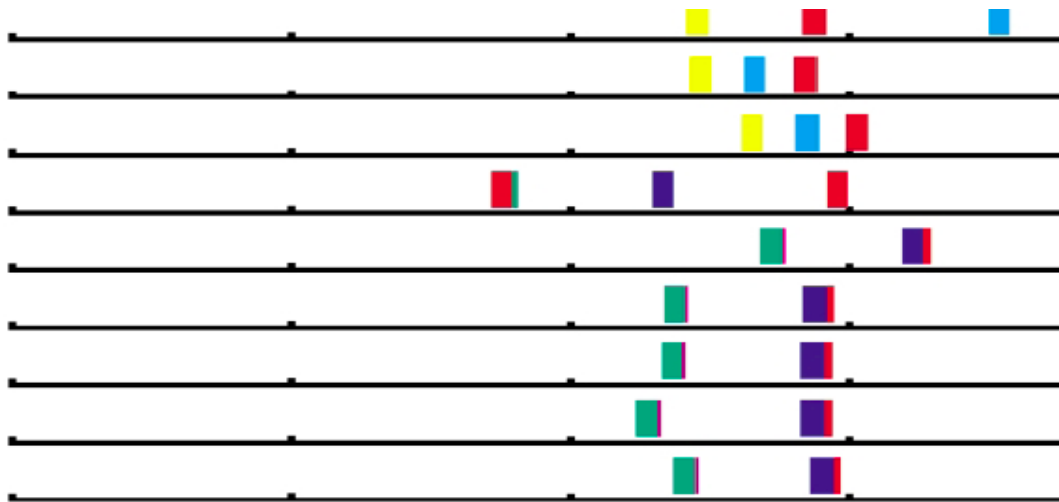
$$D_i^{begin} = \min\{ D_{i,\Delta}^{begin} + d(i,\Delta) \}$$

$$D_i^{signal,1} = \min\{ D_{i,\Delta}^{begin} + d(i,\Delta) \}$$

$$D_i^{signal,j} = \min\{ D_{i,\Delta}^{signal,j-1} + d(i,\Delta) \}$$

...

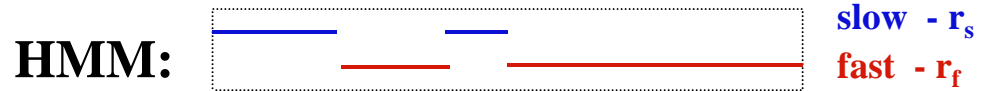
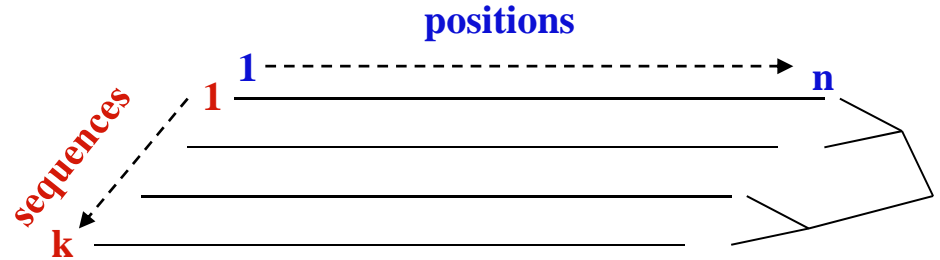
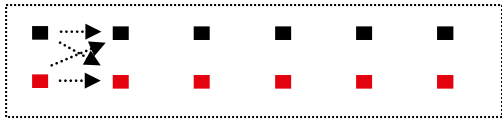
$$D_i^{end} = \min\{ D_{i,\Delta}^{end} + d(i,\Delta) \}$$



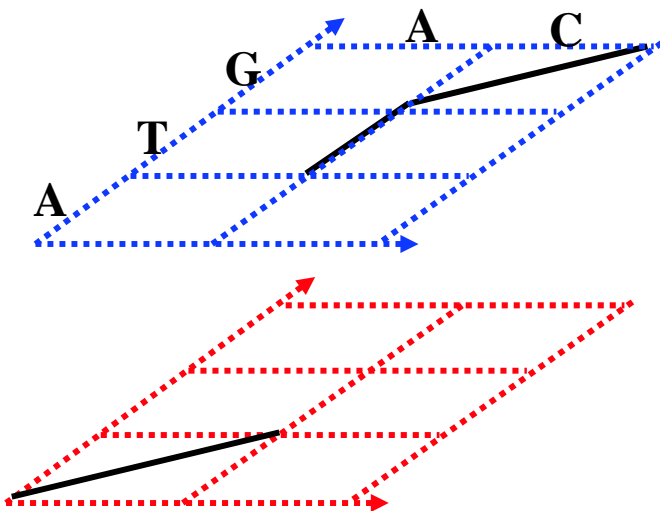
The Basics of Footprinting

- Many aligned sequences related by a known phylogeny:

HMM:



- Two un-aligned sequences:



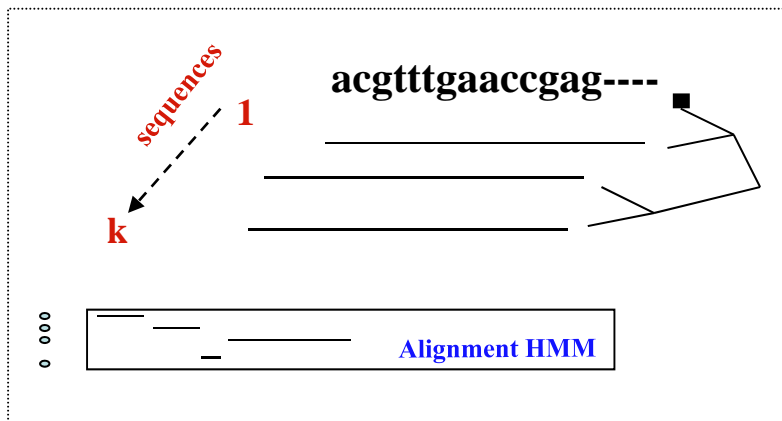
ATG

A-C

Statistical Alignment and Footprinting.

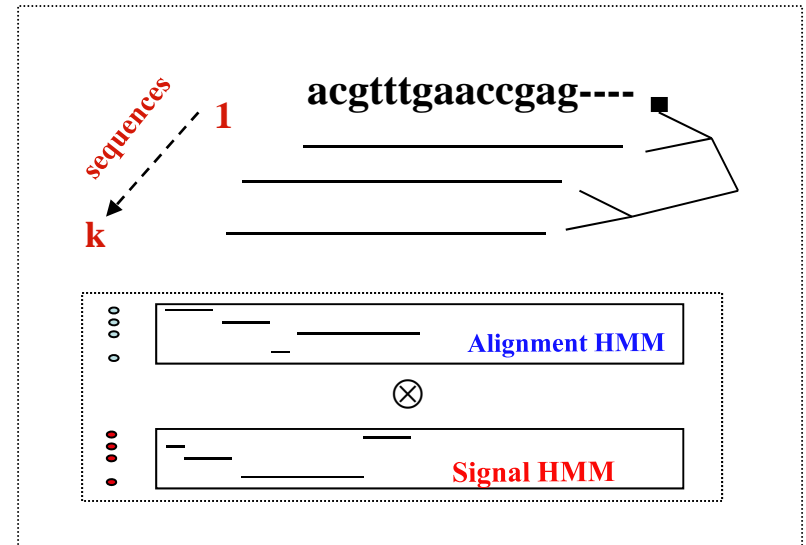
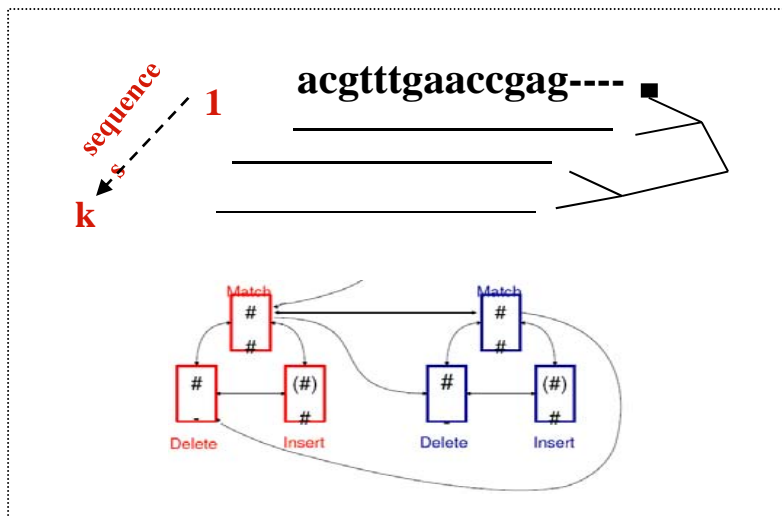
• Many un-aligned sequences related by a known phylogeny:

- Conceptually simple, computationally hard
- Dependent on a single alignment/no measure of uncertainty

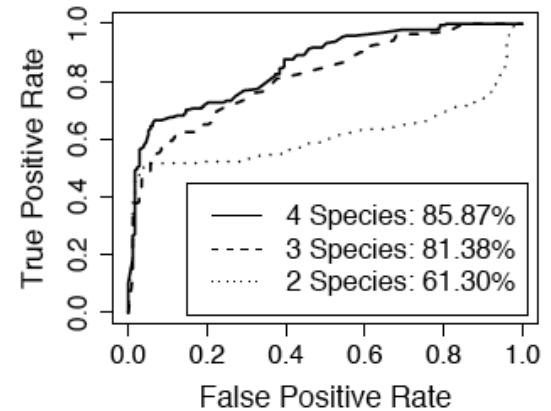
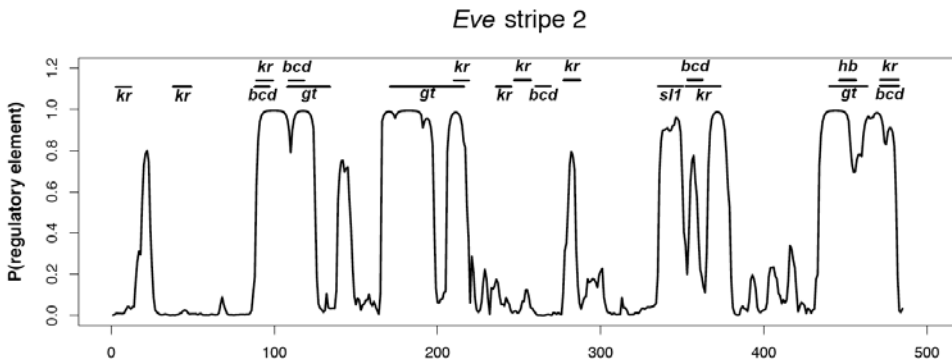
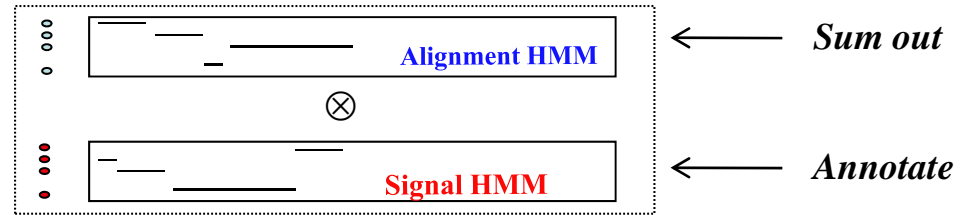
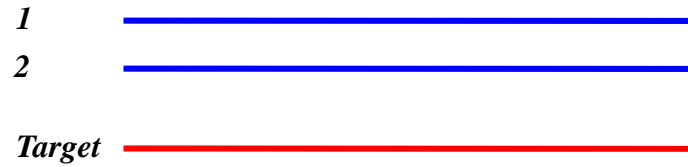
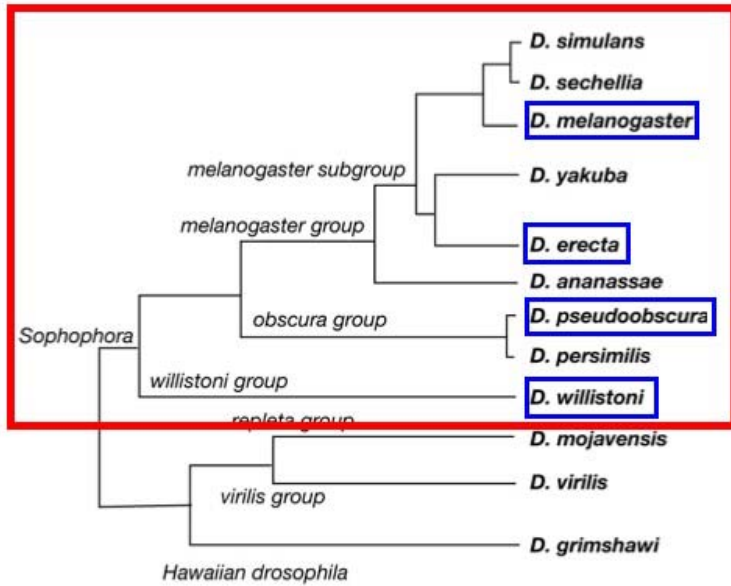


Solution:

Cartesian Product of HMMs



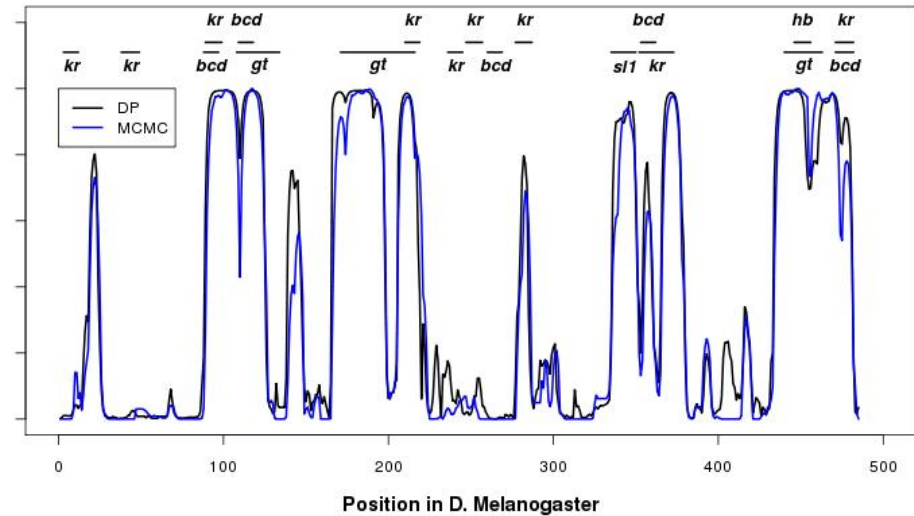
SAPF - Statistical Alignment and Phylogenetic Footprinting



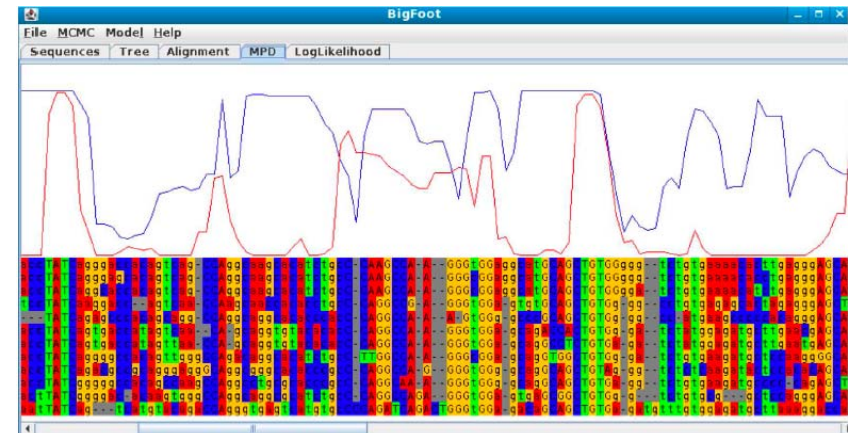
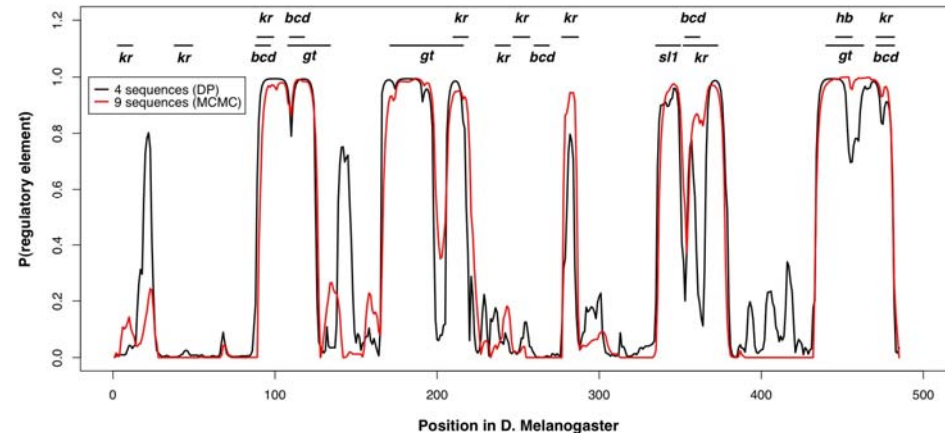
BigFoot

- *Dynamical programming is too slow for more than 4-6 sequences*
- *MCMC integration is used instead – works until 10-15 sequences*
- *For more sequences other methods are needed.*

Eve stripe 2



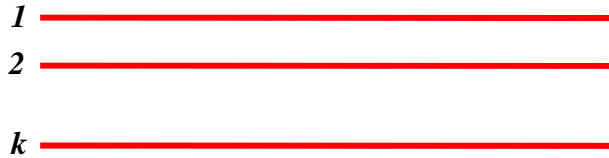
Eve stripe 2



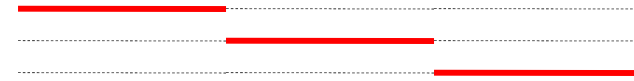
FSA - Fast Statistical Alignment

Pachter, Holmes & Co

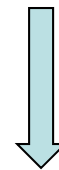
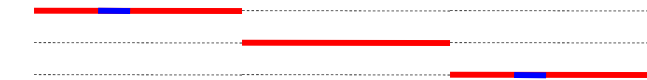
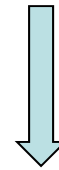
Data – k genomes/sequences:



Iterative addition of homology statements to shrinking alignment:



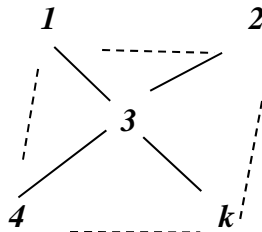
Add most certain homology statement from pairwise alignment compatible with present multiple alignment



- i. Conflicting homology statements cannot be added
- ii. Some scoring on multiple sequence homology statements is used.

Spanning tree

Additional edges



An edge – a pairwise alignment



————— 1,3 2,3 3,4 3,k

----- 12 2,k 1,4 4,k

Rate of Molecular Evolution versus estimated Selective Deceleration

Neutral Process

	A	C	G	T
A	-	$q_{A,C}$	$q_{A,G}$	$q_{A,T}$
C	$q_{C,A}$	-	$q_{C,G}$	$q_{C,T}$
G	$q_{G,A}$	$q_{G,C}$	-	$q_{G,T}$
T	$q_{T,A}$	$q_{T,C}$	$q_{T,G}$	-

How much selection?

Selection => deceleration

Selected Process

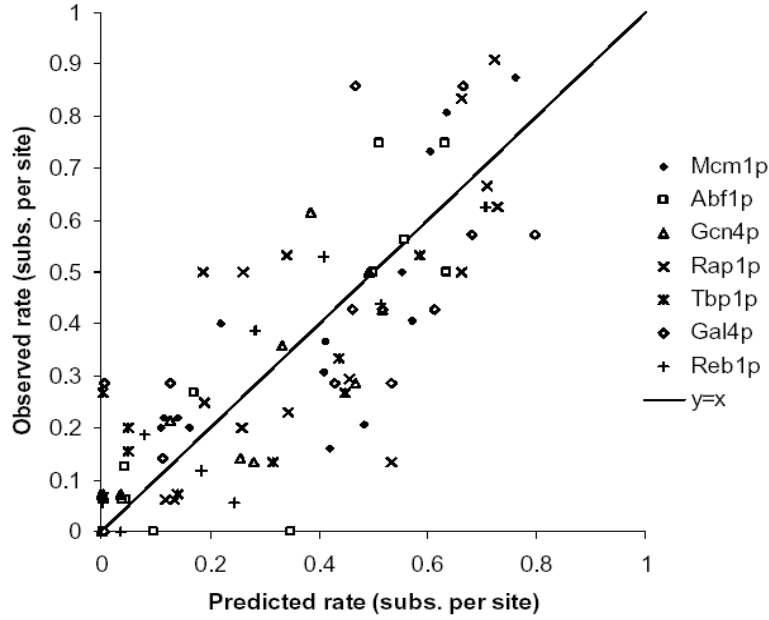
	A	C	G	T
A	-	$q'_{A,C}$	$q'_{A,G}$	$q'_{A,T}$
C	$q'_{C,A}$	-	$q'_{C,G}$	$q'_{C,T}$
G	$q'_{G,A}$	$q'_{G,C}$	-	$q'_{G,T}$
T	$q'_{T,A}$	$q'_{T,C}$	$q'_{T,G}$	-

Neutral Equilibrium

$$(\pi_A, \pi_C, \pi_G, \pi_T)$$

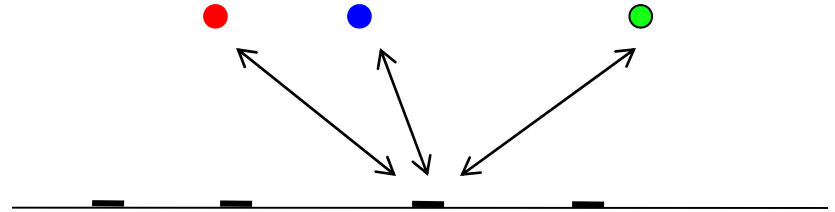
Observed Equilibrium

$$(\pi'_A, \pi'_C, \pi'_G, \pi'_T)$$



Signal Factor Prediction

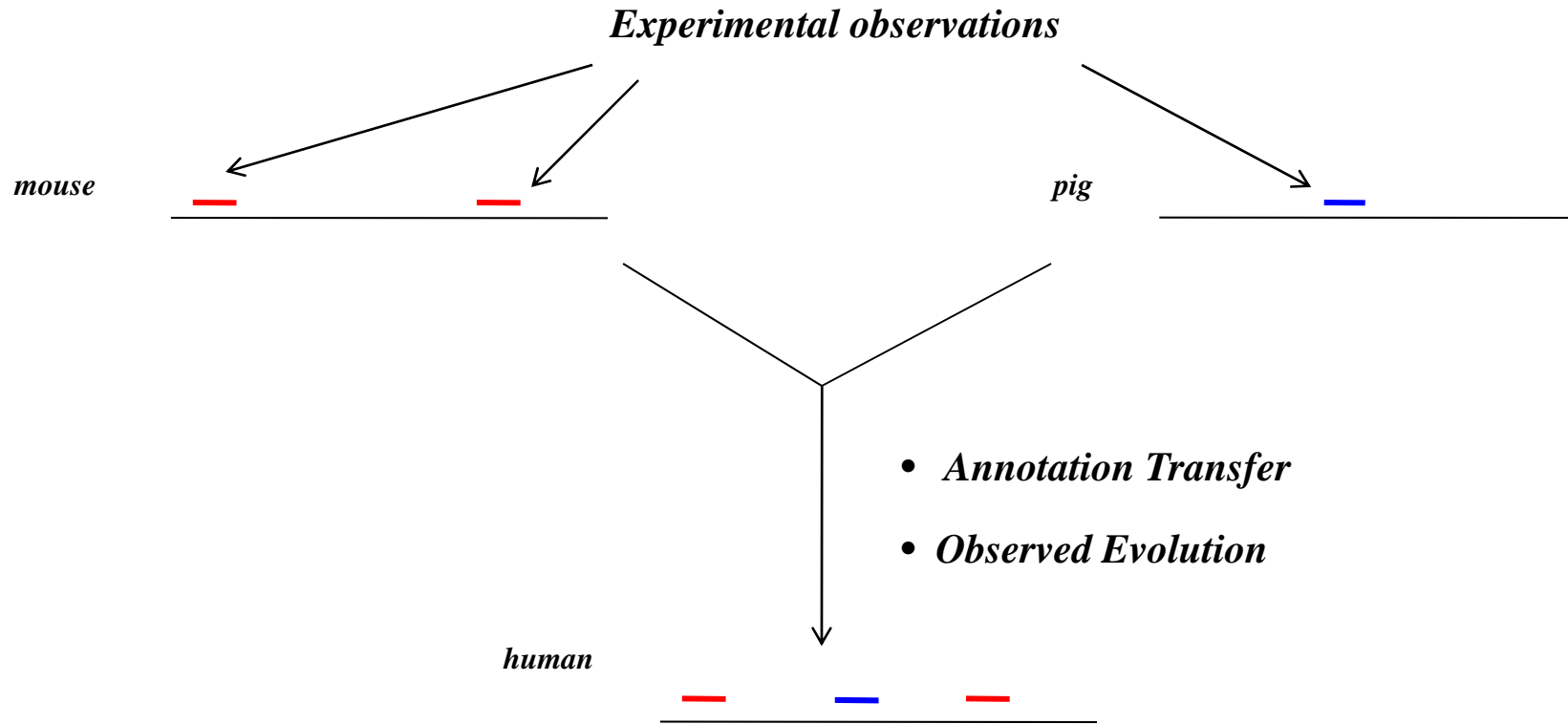
- *Given set of homologous sequences and set of transcription factors (TFs), find signals and which TFs they bind to.*



- *Use PWM and Bruno-Halpern (BH) method to make TF specific evolutionary models*
- *Drawback BH only uses rates and equilibrium distribution*

- *Superior method: Infer TF Specific Position Specific evolutionary model*
- *Drawback: cannot be done without large scale data on TF-signal binding.*

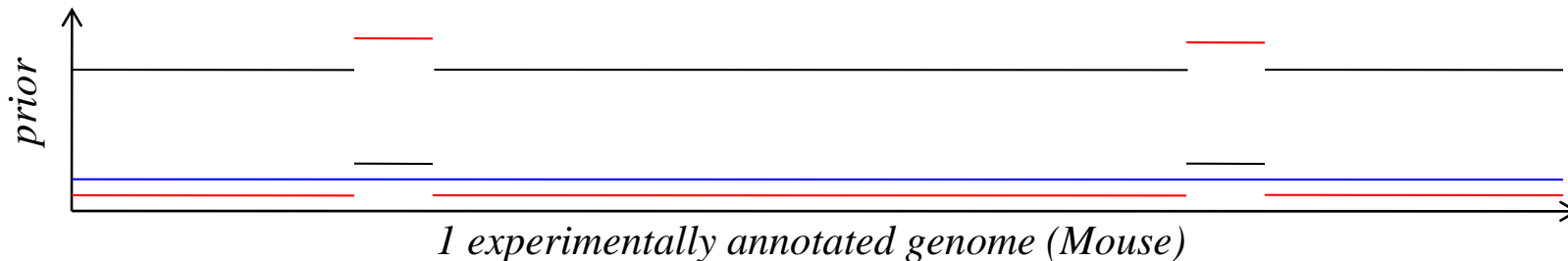
Knowledge Transfer and Combining Annotations



Must be solvable by Bayesian Priors

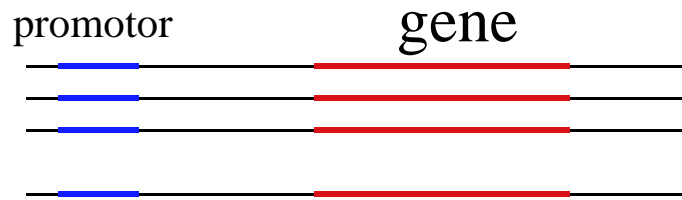
Each position p_i probability of being j 'th position in k 'th TFBS

If no experiment, low probability for being in TFBS

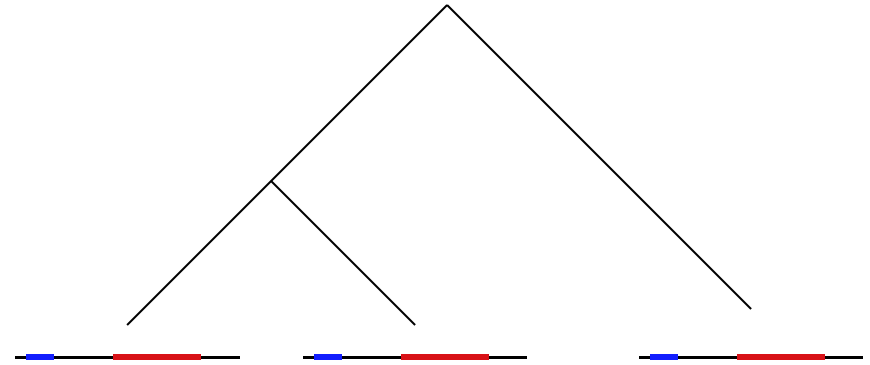


(Homologous + Non-homologous) detection

Unrelated genes - similar expression



Related genes - similar expression



Combine above approaches

