Motif Analysis

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Bioinformatics Resources
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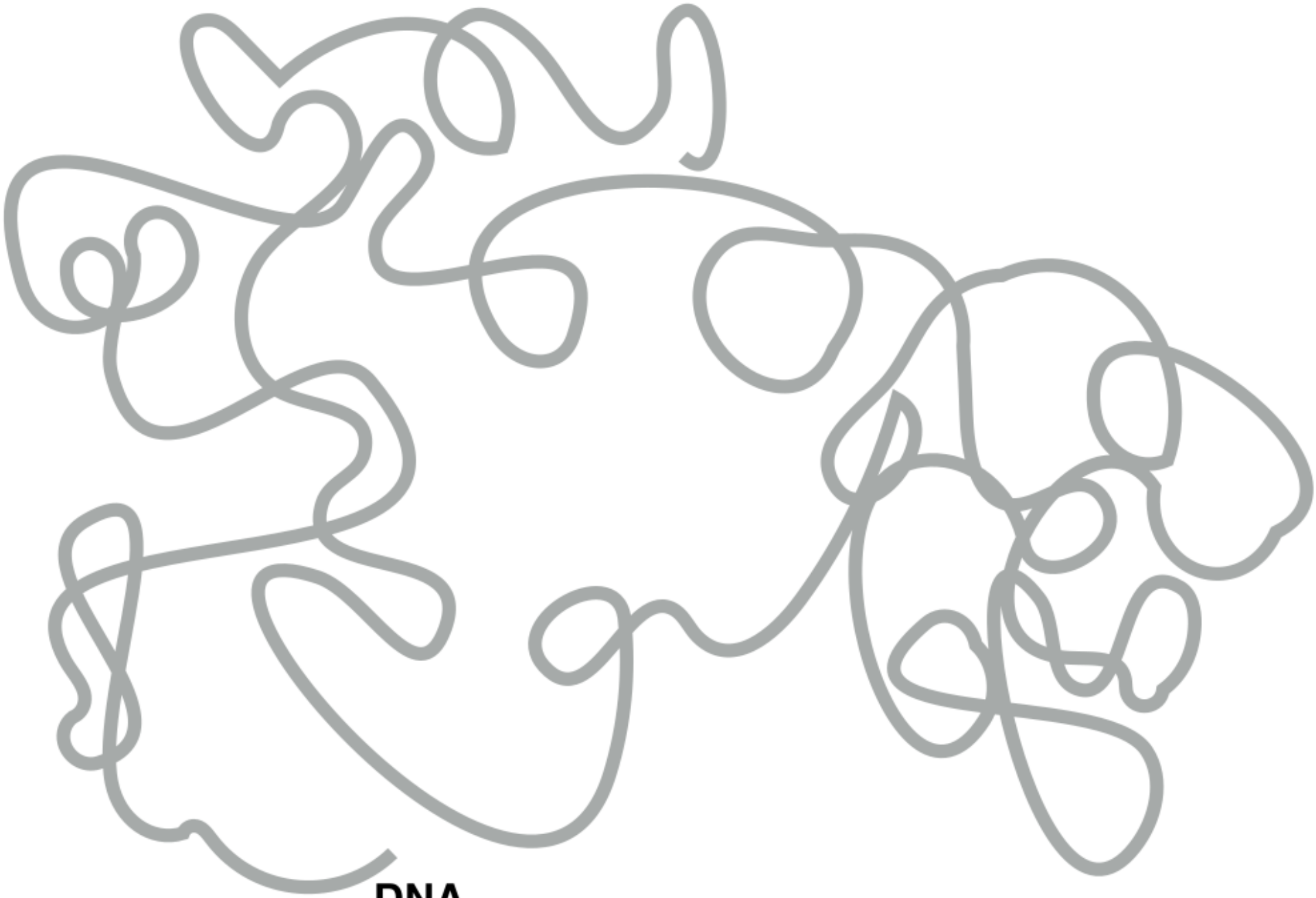
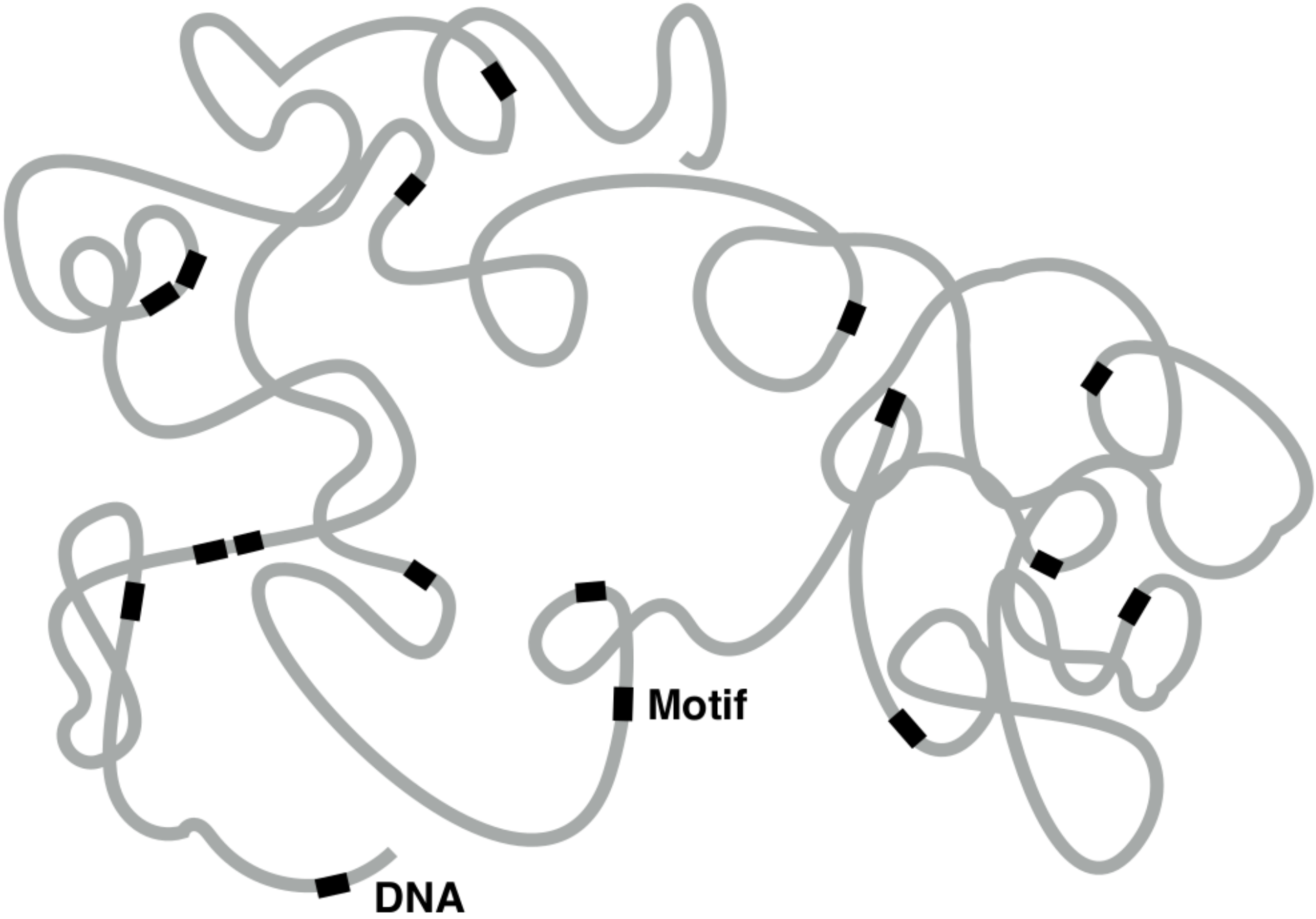
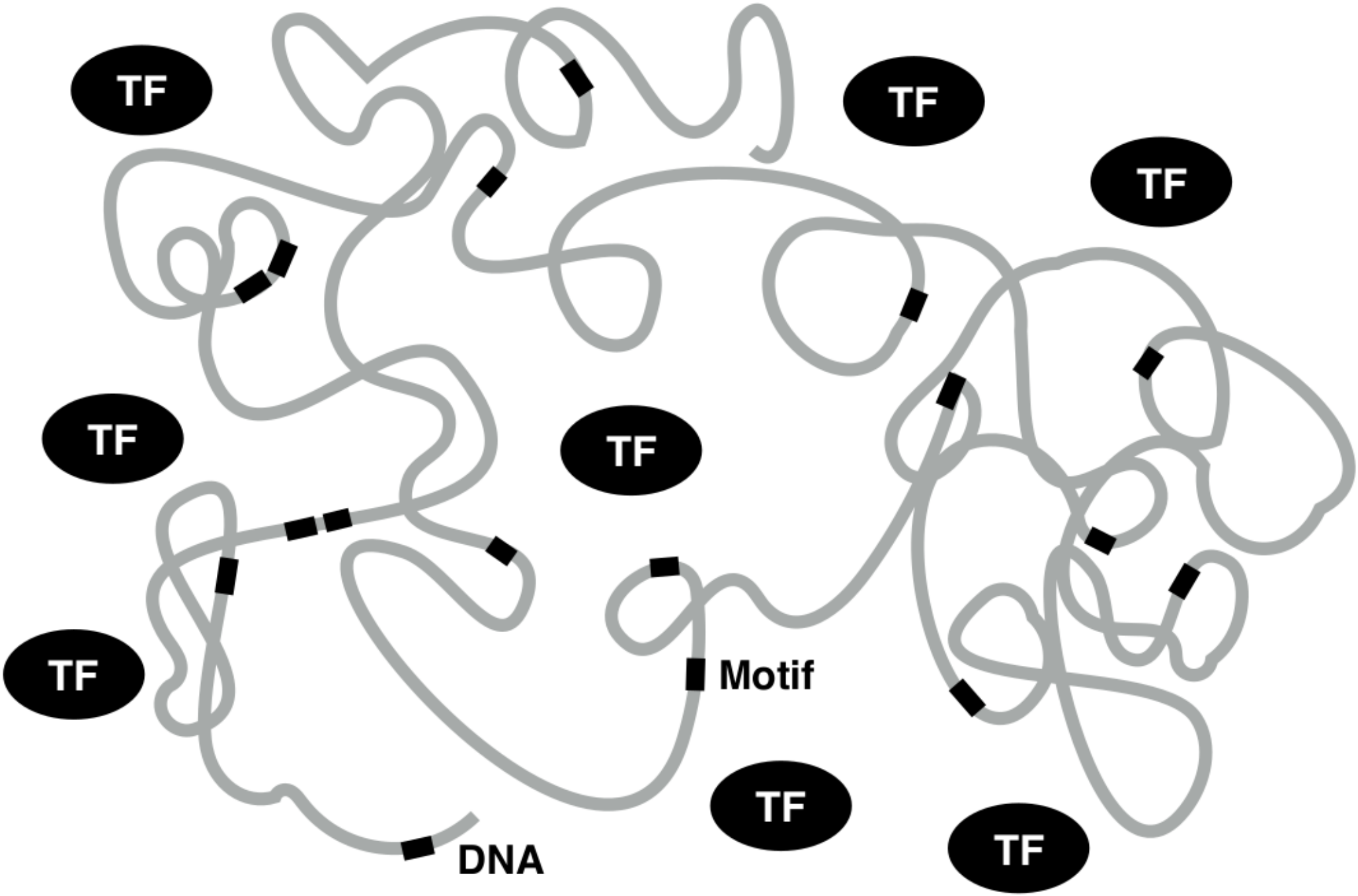


DNA


Motif
DNA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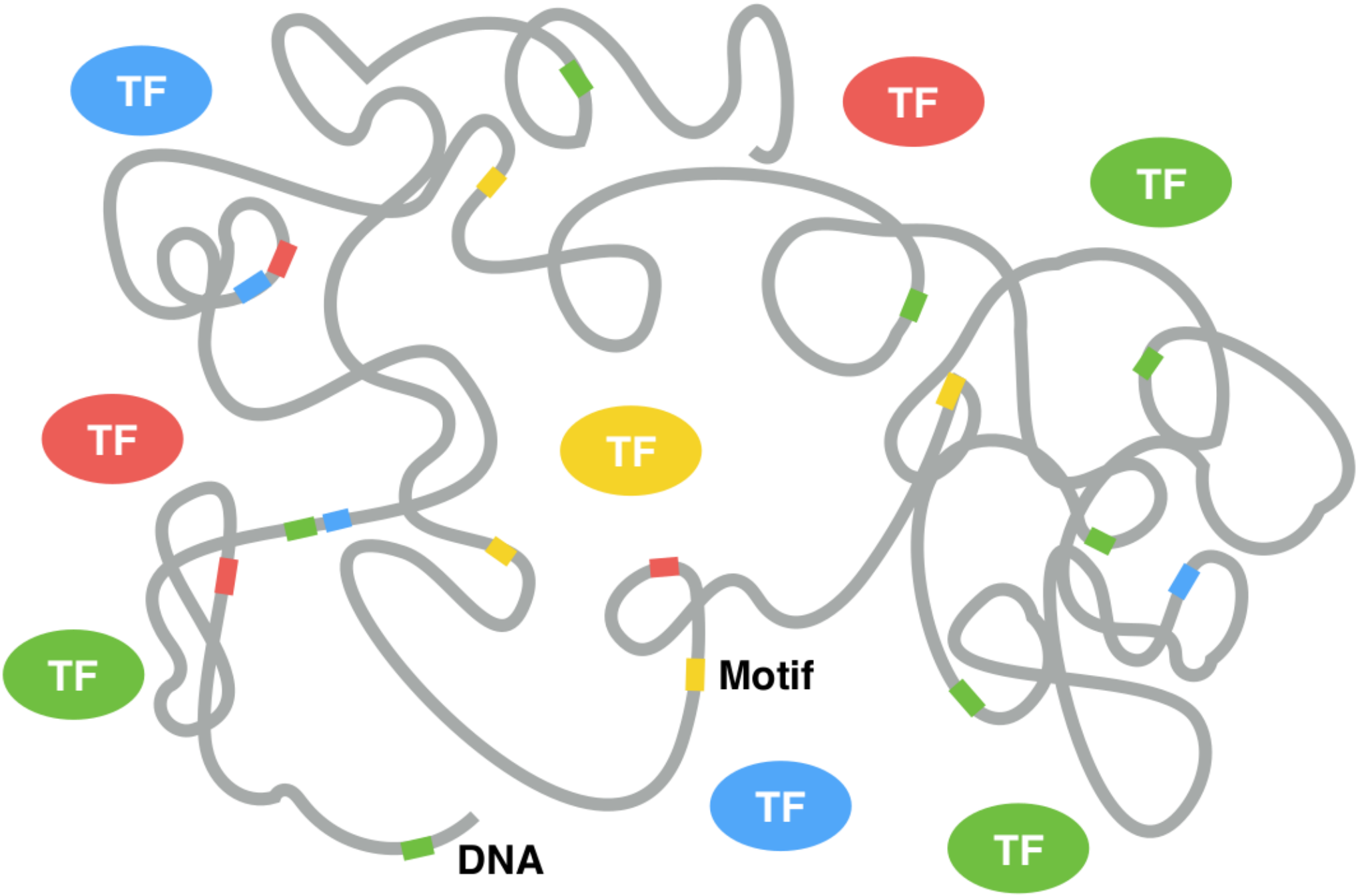
Motif
DNA


TF
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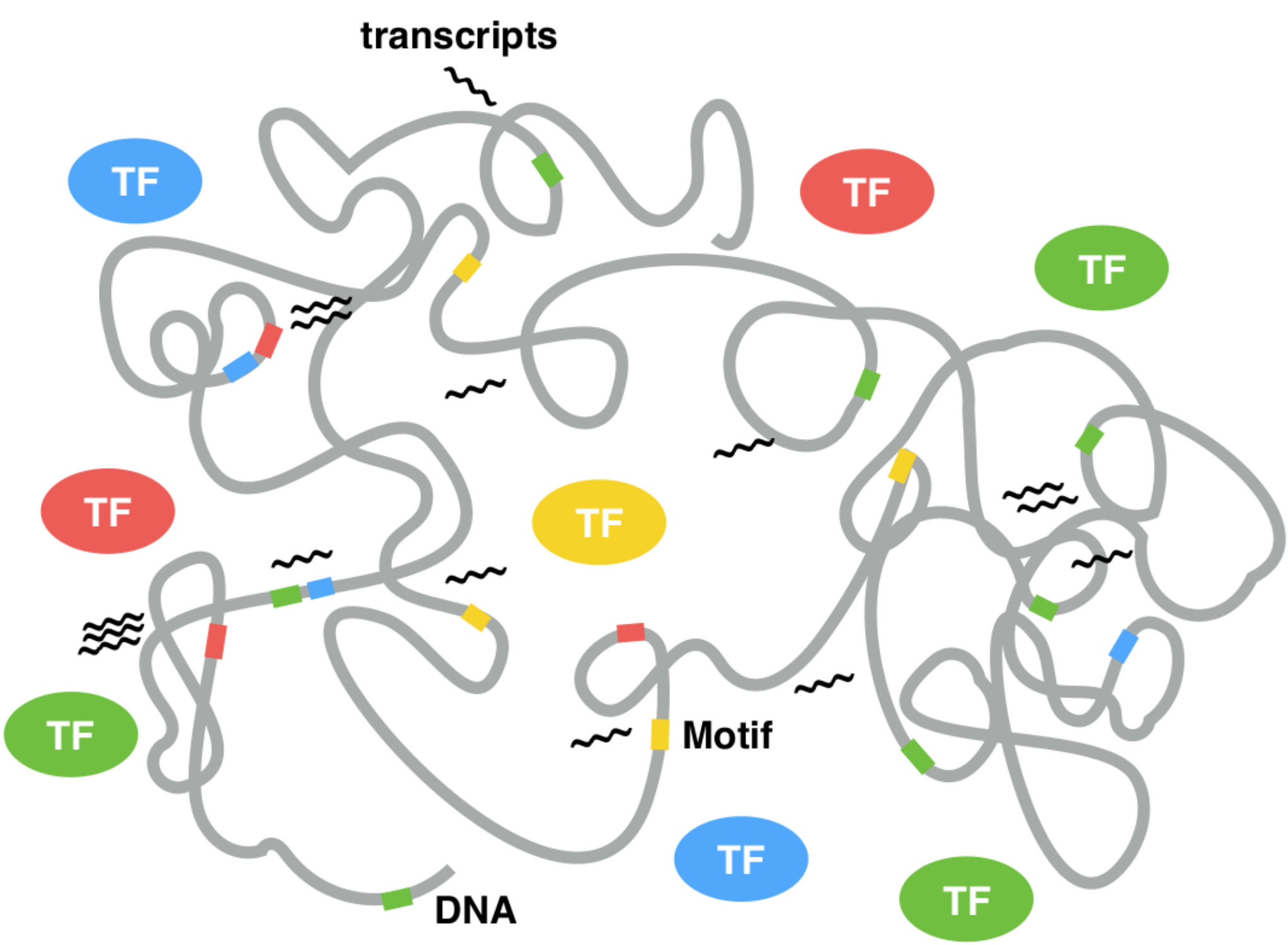
Motif
DNA


transcripts
TF
TF
TF
TF
TF
TF
TF
TF
TF
Motif
DNA

Questions...

- How to find DNA motifs?
- Relations between motifs and transcription factors (TF)?
- Given a motif, which is the corresponding TF?
- Given a set of initial sequences, which are the more represented motifs?
- Given a set of transcripts, which are the motifs (and hence the TFs) in the promoters of the corresponding genes?

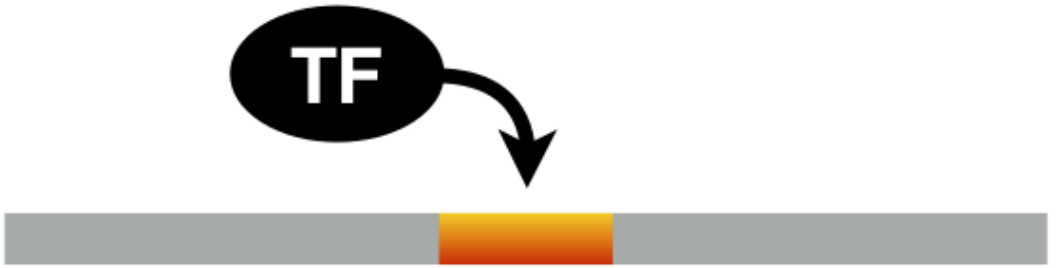
Questions...

- Given a set of motifs, which are the involved signaling pathways?
- Given a set of motifs, which are the signaling pathways that are more represented?
- ...

DNA motif (definition)

- Pattern of nucleotide sequences
- Usually they are associated to DNA-protein binding sites (regulatory regions)
- Small pattern (5-30bp) that can recur many times in the genome and many times for the same gene
- Standard motifs, palindromes and gapped

DNA motifs (definition)



Standard

Palindrome
(e.g. CACGTG)

Gapped

DNA motifs (functions)

- Sequence specific binding sites
  - TF, Nuclease, Ribosome
- mRNA processing
  - Splicing: Exonic Splicing Enhancer (ESE)
  - Editing: Protospacer adjacent motif (PAM), DNA sequence that immediately follow the target DNA sequence of Cas9 nuclease in the CRISPR system
  - Polyadenilation
  - Transcription termination

Motifs and TFs

- Motifs in regulatory regions are often similar but variable
- Transcription factors are often pleiotropic (1:N)
  - Regulate a lot of genes but need to be expressed at different levels
- An effect of degenerate motifs is the non-specific binding
  - A protein can bind in genomic positions that are different with respect to the one corresponding to the expected functional sites

Motif search (objectives)

- Identify over-represented motifs in the genome
- Identify motifs that are conserved in ortholog sequences
- Identify sequences that can be a candidate for TF binding

How to represent motifs

- Consensus sequences
- Profiles
  - Positional matrix
  - Hidden Markov Model (HMM)

Represent motifs

- TF binding sites are often represented a consensus binding sites
- Consensus sequence
  - Represents the result of multiple sequence alignments with the goal of finding recurrent motifs across the sequences
  - Potentially different from all input sequences
  - Presents only the most conserved sequences for each position

Consensus sequence

T	A	C	G	A	T	A	A	G
T	A	T	A	A	T	A	G	G
T	A	T	A	A	T	A	A	C
T	A	T	A	C	T	A	A	C
T	A	T	G	A	T	A	A	A
T	A	T	G	T	T	A	A	T

Consensus sequence

T	A	C	G	A	T	A	A	G
T	A	T	A	A	T	A	G	G
T	A	T	A	A	T	A	A	C
T	A	T	A	C	T	A	A	C
T	A	T	G	A	T	A	A	A
T	A	T	G	T	T	A	A	T
T	**A**	**T**	**G**	**A**	**T**	**A**	**A**	**G**

Remember IUPAC notation

Symbol[2]	Description	Bases represented				
A	**A**denine	A				1
C	**C**ytosine		C			
G	**G**uanine			G		
T	**T**hymine				T	
U	**U**racil				U	
W	**W**eak	A			T	2
S	**S**trong		C	G		
M	a**M**ino	A	C			
K	**K**eto			G	T	
R	pu**R**ine	A		G		
Y	p**Y**rimidine		C		T	
B	not A (**B** comes after A)		C	G	T	3
D	not C (**D** comes after C)	A		G	T	
H	not G (**H** comes after G)	A	C		T	
V	not T (**V** comes after T and U)	A	C	G		
N or **-**	any **N**ucleotide (not a gap)	A	C	G	T	4

Consensus sequence

T	A	C	G	A	T	A	A	G
T	A	T	A	A	T	A	G	G
T	A	T	A	A	T	A	A	C
T	A	T	A	C	T	A	A	C
T	A	T	G	A	T	A	A	A
T	A	T	G	T	T	A	A	T
T	**A**	**T**	**G**	**A**	**T**	**A**	**A**	**G**

Sequenza consenso IUPAC?

Symbol[2]	Description	Bases represented				
A	**A**denine	A				1
C	**C**ytosine		C			
G	**G**uanine			G		
T	**T**hymine				T	
U	**U**racil				U	
W	**W**eak	A			T	2
S	**S**trong		C	G		
M	a**M**ino	A	C			
K	**K**eto			G	T	
R	pu**R**ine	A		G		
Y	p**Y**rimidine		C		T	
B	not A (**B** comes after A)		C	G	T	3
D	not C (**D** comes after C)	A		G	T	
H	not G (**H** comes after G)	A	C		T	
V	not T (**V** comes after T and U)	A	C	G		
N or **-**	any **N**ucleotide (not a gap)	A	C	G	T	4

https://en.wikipedia.org/wiki/Nucleic_acid_notation

Consensus sequence

T	A	C	G	A	T	A	A	G
T	A	T	A	A	T	A	G	G
T	A	T	A	A	T	A	A	C
T	A	T	A	C	T	A	A	C
T	A	T	G	A	T	A	A	A
T	A	T	G	T	T	A	A	T
T	**A**	**T**	**G**	**A**	**T**	**A**	**A**	**G**
T	**A**	**Y**	**R**	**H**	**T**	**A**	**R**	**N**

Symbol[2]	Description	Bases represented				
A	**A**denine	A				1
C	**C**ytosine		C			
G	**G**uanine			G		
T	**T**hymine				T	
U	**U**racil				U	
W	**W**eak	A			T	2
S	**S**trong		C	G		
M	a**M**ino	A	C			
K	**K**eto			G	T	
R	pu**R**ine	A		G		
Y	p**Y**rimidine		C		T	
B	not A (**B** comes after A)		C	G	T	3
D	not C (**D** comes after C)	A		G	T	
H	not G (**H** comes after G)	A	C		T	
V	not T (**V** comes after T and U)	A	C	G		
N or **-**	any **N**ucleotide (not a gap)	A	C	G	T	4

https://en.wikipedia.org/wiki/Nucleic_acid_notation

Positional matrix

- Alternative to consensus
- The elements in the matrix represent all possible bases at each position

- Position Frequency Matrix (PFM) (PSWM)
- Position Probability Matrix (PPM) (PFM)
- Position Weight Matrix (PWM) (PSSM)

PFM matrix

T	A	C	G	A	T	A	A	G
T	A	T	A	A	T	A	G	G
T	A	T	A	A	T	A	A	C
T	A	T	A	C	T	A	A	C
T	A	T	G	A	T	A	A	A
T	A	T	G	T	T	A	A	T

	1	2	3	4	5	6	7	8	9
A	0								
C	0								
G	0								
T	6								

PFM matrix

T	A	C	G	A	T	A	A	G
T	A	T	A	A	T	A	G	G
T	A	T	A	A	T	A	A	C
T	A	T	A	C	T	A	A	C
T	A	T	G	A	T	A	A	A
T	A	T	G	T	T	A	A	T

	1	2	3	4	5	6	7	8	9
A	0	6	0	3	4	0	6	5	1
C	0	0	1	0	1	0	0	0	2
G	0	0	0	3	0	0	0	1	2
T	6	0	5	0	1	6	0	0	1

Matrix

PFM

	1	2	3	4	5	6	7	8	9
A	0	6	0	3	4	0	6	5	1
C	0	0	1	0	1	0	0	0	2
G	0	0	0	3	0	0	0	1	2
T	6	0	5	0	1	6	0	0	1

Matrix

PFM

	1	2	3	4	5	6	7	8	9
A	0	6	0	3	4	0	6	5	1
C	0	0	1	0	1	0	0	0	2
G	0	0	0	3	0	0	0	1	2
T	6	0	5	0	1	6	0	0	1

PPM

	1	2	3	4	5	6	7	8	9
A	0								
C	0								
G	0								
T	1								

Matrix

PFM

	1	2	3	4	5	6	7	8	9
A	0	6	0	3	4	0	6	5	1
C	0	0	1	0	1	0	0	0	2
G	0	0	0	3	0	0	0	1	2
T	6	0	5	0	1	6	0	0	1

PPM

	1	2	3	4	5	6	7	8	9
A	0	1	0	0.5	0.67	0	1	0.83	0.17
C	0	0	0.17	0	0.17	0	0	0	0.34
G	0	0	0	0.5	0	0	0	0.17	0.34
T	1	0	0.83	0	0.17	1	0	0	0.17

Matrix

PFM

	1	2	3	4	5	6	7	8	9
A	0	6	0	3	4	0	6	5	1
C	0	0	1	0	1	0	0	0	2
G	0	0	0	3	0	0	0	1	2
T	6	0	5	0	1	6	0	0	1

PPM

	1	2	3	4	5	6	7	8	9
A	0	1	0	0.5	0.67	0	1	0.83	0.17
C	0	0	0.17	0	0.17	0	0	0	0.34
G	0	0	0	0.5	0	0	0	0.17	0.34
T	1	0	0.83	0	0.17	1	0	0	0.17

PWM

	1	2	3	4	5	6	7	8	9
A	-inf	1.38	-inf	0.69	0.99	-inf	1.38	1.20	-0.39
C	-inf	-inf	-0.39	-inf	-0.39	-inf	-inf	-inf	0.31
G	-inf	-inf	-inf	0.69	-inf	-inf	-inf	-0.39	0.31
T	1.38	-inf	1.20	-inf	-0.39	1.38	-inf	-inf	-0.39

PPM matrix

	1	2	3	4	5	6	7	8	9
A	0	1	0	0.5	0.67	0	1	0.83	0.17
C	0	0	0.17	0	0.17	0	0	0	0.34
G	0	0	0	0.5	0	0	0	0.17	0.34
T	1	0	0.83	0	0.17	1	0	0	0.17

$$M_{k,j} = \frac{1}{N} \sum_{i=1}^{N} I(X_{i,j} = k)$$

- *k* is the set of all symbols in the alphabet (A,C,G,T)
- *N* is the number of aligned sequences
- *I* is an indicator function (1 if Xi,j=k, 0 otherwise)
- *j* ranges from the 1 to the length of the sequences

PPM matrix

	1	2	3	4	5	6	7	8	9
A	0	1	0	0.5	0.67	0	1	0.83	0.17
C	0	0	0.17	0	0.17	0	0	0	0.34
G	0	0	0	0.5	0	0	0	0.17	0.34
T	1	0	0.83	0	0.17	1	0	0	0.17

- Probabilities are calculated for each position independently
  - ?

PPM matrix

	1	2	3	4	5	6	7	8	9
A	0	1	0	0.5	0.67	0	1	0.83	0.17
C	0	0	0.17	0	0.17	0	0	0	0.34
G	0	0	0	0.5	0	0	0	0.17	0.34
T	1	0	0.83	0	0.17	1	0	0	0.17

- Probabilities are calculated for each position independently
  - We assume there is no statistical dependence between position in the pattern

PPM matrix

	1	2	3	4	5	6	7	8	9
A	0	1	0	0.5	0.67	0	1	0.83	0.17
C	0	0	0.17	0	0.17	0	0	0	0.34
G	0	0	0	0.5	0	0	0	0.17	0.34
T	1	0	0.83	0	0.17	1	0	0	0.17

- Given an input sequence, which is the probability to *belong* to a PPM?

  S: TACACTAGT

  P(S|M) = ?

PPM matrix

	1	2	3	4	5	6	7	8	9
A	0	1	0	0.5	0.67	0	1	0.83	0.17
C	0	0	0.17	0	0.17	0	0	0	0.34
G	0	0	0	0.5	0	0	0	0.17	0.34
T	1	0	0.83	0	0.17	1	0	0	0.17

- Given an input sequence, which is the probability to *belong* to a PPM?

  S: TACACTAGT

  P(S|M) = 1*1*0.17*0.5*0.17*1*1*0.17*0.17

  = 0.000417605

PPM matrix

	1	2	3	4	5	6	7	8	9
A	0	1	0	0.5	0.67	0	1	0.83	0.17
C	0	0	0.17	0	0.17	0	0	0	0.34
G	0	0	0	0.5	0	0	0	0.17	0.34
T	1	0	0.83	0	0.17	1	0	0	0.17

- Given an input sequence, which is the probability to *belong* to a PPM?

  S: TAC**C**CTAGT

  P(S|M) = ?

PPM matrix

	1	2	3	4	5	6	7	8	9
A	0	1	0	0.5	0.67	0	1	0.83	0.17
C	0	0	0.17	0	0.17	0	0	0	0.34
G	0	0	0	0.5	0	0	0	0.17	0.34
T	1	0	0.83	0	0.17	1	0	0	0.17

- Given an input sequence, which is the probability to *belong* to a PPM?

  S: TAC**C**CTAGT

  P(S|M) = ?

Laplace smoothing (pseudocounts): Allows to estimate probabilities in case of few observations

Pseudocounts

- A *pseudocount* is an amount (integer or double) added to the number of observed cases in order to change the expected probability
  - When values not known to be 0

$$p_i, \text{ empirical} = \frac{x_i}{N} \qquad p_i, \alpha\text{-smoothed} = \frac{x_i + \alpha}{N + \alpha d}$$

d is the number of observations

Pseudocounts

	1	2	3	4	5	6	7	8	9
A	0	6	0	3	4	0	6	5	1
C	0	0	1	0	1	0	0	0	2
G	0	0	0	3	0	0	0	1	2
T	6	0	5	0	1	6	0	0	1

S: TACACTAGT
P(S|M) = ?

S: TACCCTAGT
P(S|M) = ?

	1	2	3	4	5	6	7	8	9
A	1	7	1	4	5	1	7	6	2
C	1	1	2	1	2	1	1	1	3
G	1	1	1	4	1	1	1	2	3
T	7	1	6	1	2	7	1	1	2

PWM matrix

M

	1	2	3	4	5	6	7	8	9
A	-inf	1.38	-inf	0.69	0.99	-inf	1.38	1.20	-0.39
C	-inf	-inf	-0.39	-inf	-0.39	-inf	-inf	-inf	0.31
G	-inf	-inf	-inf	0.69	-inf	-inf	-inf	-0.39	0.31
T	1.38	-inf	1.20	-inf	-0.39	1.38	-inf	-inf	-0.39

$$M_{k,j} = \log_2 \left(M_{k,j} / b_k\right)$$

b represents a background model

$$b_k = 1/|k|$$

- b=0.25 for nucleotides (n=4) and 0.05 for amino acids (n=20)
- b can vary across nucleotide for organisms with high GC content

PWM matrix

M

	1	2	3	4	5	6	7	8	9
A	-inf	1.38	-inf	0.69	0.99	-inf	1.38	1.20	-0.39
C	-inf	-inf	**-0.39**	-inf	-0.39	-inf	-inf	-inf	0.31
G	-inf	-inf	-inf	0.69	-inf	**-inf**	-inf	-0.39	0.31
T	**1.38**	-inf	1.20	-inf	-0.39	1.38	-inf	-inf	-0.39

$$M_{T,1} = \ln(1/0.25) = 1.38$$

PWM matrix

M

	1	2	3	4	5	6	7	8	9
A	-inf	1.38	-inf	0.69	0.99	-inf	1.38	1.20	-0.39
C	-inf	-inf	**-0.39**	-inf	-0.39	-inf	-inf	-inf	0.31
G	-inf	-inf	-inf	0.69	-inf	**-inf**	-inf	-0.39	0.31
T	**1.38**	-inf	1.20	-inf	-0.39	1.38	-inf	-inf	-0.39

$$M_{T,1} = \ln (1/0.25) = 1.38$$

$$M_{C,3} = \ln (0.17/0.25) = -0.39$$

PWM matrix

M

	1	2	3	4	5	6	7	8	9
A	-inf	1.38	-inf	0.69	0.99	-inf	1.38	1.20	-0.39
C	-inf	-inf	**-0.39**	-inf	-0.39	-inf	-inf	-inf	0.31
G	-inf	-inf	-inf	0.69	-inf	**-inf**	-inf	-0.39	0.31
T	**1.38**	-inf	1.20	-inf	-0.39	1.38	-inf	-inf	-0.39

$$M_{T,1} = \ln (1/0.25) = 1.38$$

$$M_{C,3} = \ln (0.17/0.25) = -0.39$$

$$M_{G,6} = \ln (0/0.25) = -\inf$$

PWM matrix

M

	1	2	3	4	5	6	7	8	9
A	-inf	1.38	-inf	0.69	0.99	-inf	1.38	1.20	-0.39
C	-inf	-inf	-0.39	-inf	-0.39	-inf	-inf	-inf	0.31
G	-inf	-inf	-inf	0.69	-inf	-inf	-inf	-0.39	0.31
T	1.38	-inf	1.20	-inf	-0.39	1.38	-inf	-inf	-0.39

- Given an input sequence, which is the probability to *belong* to a PPM?
  S: TACACTAGT
  Score = ?

PWM matrix

M

	1	2	3	4	5	6	7	8	9
A	-inf	1.38	-inf	0.69	0.99	-inf	1.38	1.20	-0.39
C	-inf	-inf	-0.39	-inf	-0.39	-inf	-inf	-inf	0.31
G	-inf	-inf	-inf	0.69	-inf	-inf	-inf	-0.39	0.31
T	1.38	-inf	1.20	-inf	-0.39	1.38	-inf	-inf	-0.39

- Given an input sequence, which is the probability to *belong* to a PPM?
  S: TACACTAGT
  Score = 1.38+1.38-0.39+0.69-0.39+1.38+1.38-0.39-0.39
  = 4.65
- The score indicates how much the sequence is different from a random sequence

PWM matrix

M

	1	2	3	4	5	6	7	8	9
A	-inf	1.38	-inf	0.69	0.99	-inf	1.38	1.20	-0.39
C	-inf	-inf	-0.39	-inf	-0.39	-inf	-inf	-inf	0.31
G	-inf	-inf	-inf	0.69	-inf	-inf	-inf	-0.39	0.31
T	1.38	-inf	1.20	-inf	-0.39	1.38	-inf	-inf	-0.39

- Given an input sequence, which is the probability to *belong* to a PPM?
  S: TAC***C***CTAGT
  Score = ?

PWM matrix

M

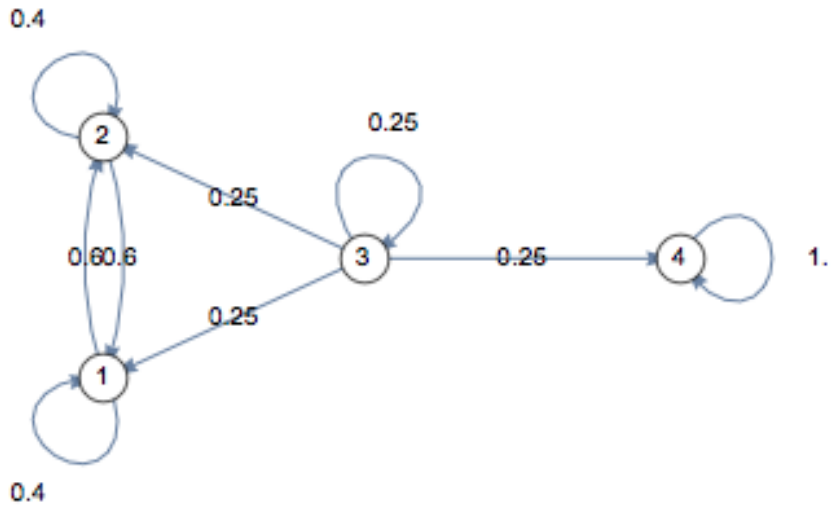
	1	2	3	4	5	6	7	8	9
A	-inf	1.38	-inf	0.69	0.99	-inf	1.38	1.20	-0.39
C	-inf	-inf	-0.39	-inf	-0.39	-inf	-inf	-inf	0.31
G	-inf	-inf	-inf	0.69	-inf	-inf	-inf	-0.39	0.31
T	1.38	-inf	1.20	-inf	-0.39	1.38	-inf	-inf	-0.39

- Given an input sequence, which is the probability to *belong* to a PPM?
  S: TAC***C***CTAGT
  Score = ?

  PSEUDOCOUNTS!!!!

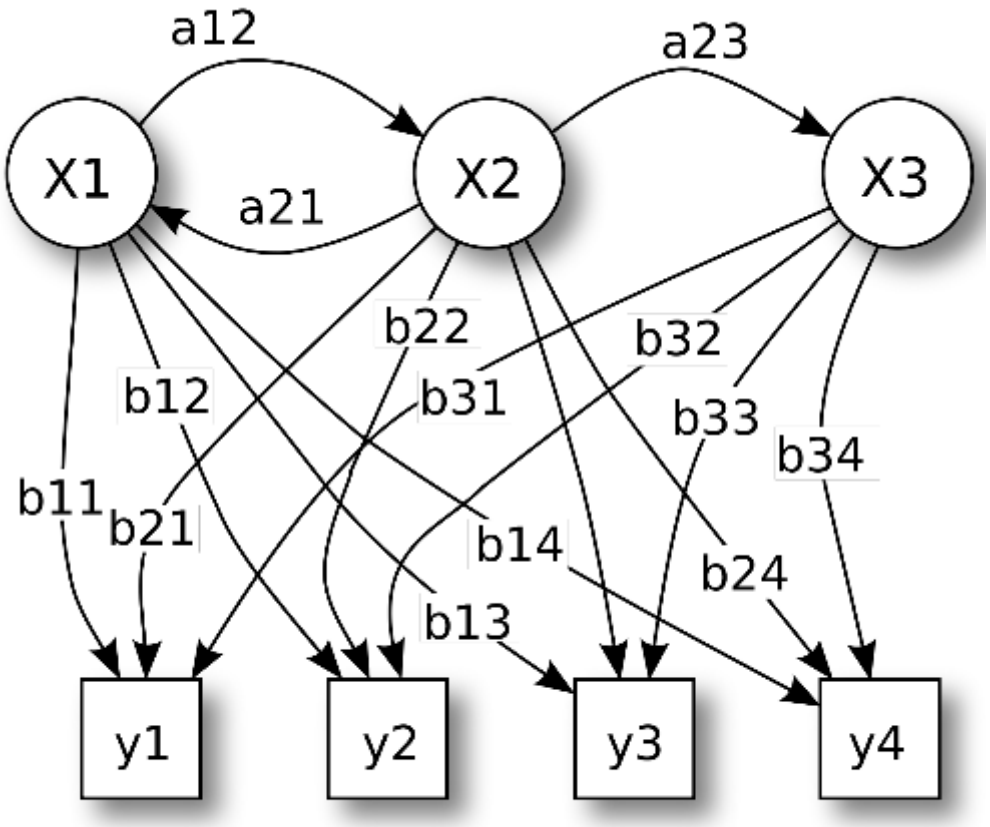
HMM profile

- HMM: Hidden Markov Model
- A Markov chain is a mathematical system that experiences transitions from one state to another according to certain probabilistic rules
  - no matter how the process arrived at its present state, the possible future states are fixed



HMM profile

- In a Markov chain, the state is directly visible to the observer
  - state transition probabilities are the only parameters
- In a HMM the state is not directly visible, but the output, dependent on the state, is visible



https://en.wikipedia.org/wiki/Hidden_Markov_model

HMM profile

- A HMM of the first order is defined as:
  - A finite set of states S
  - A discrete alphabet of symbols
  - A matrix of transition probabilities

    T = P(i|j)

    probability of transition from state j to i
  - A matrix of emission probabilities

    T = P(X|i)

    probability of X emission in state i

HMM profile

A C A C A A

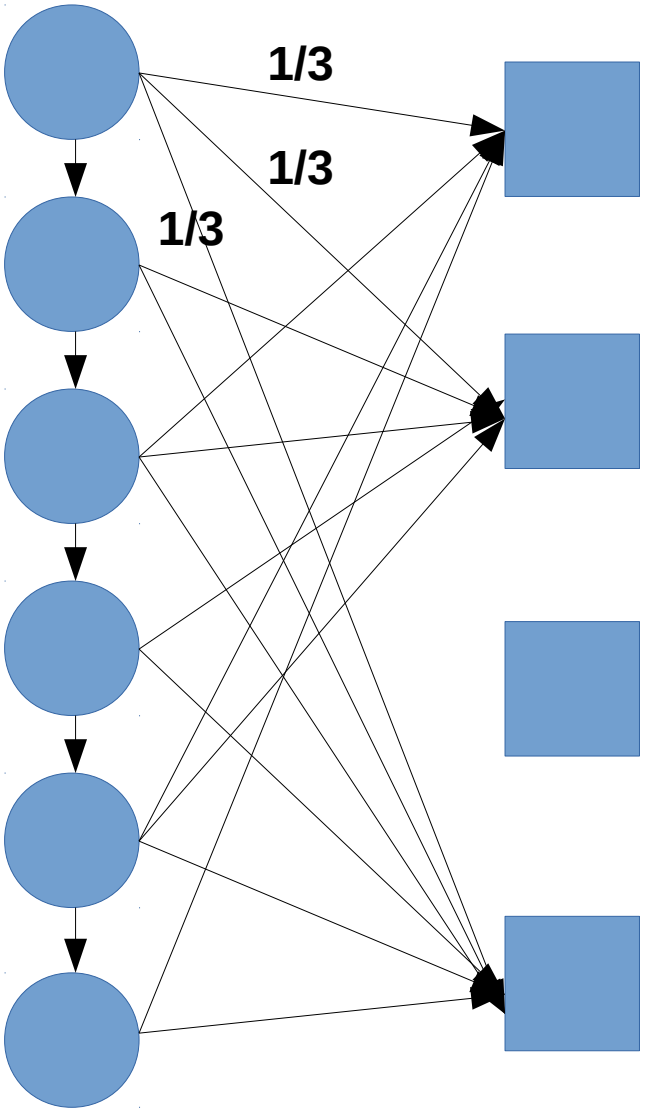
A T A C A A

T T T C T G

T T T C T G

C T C C C G

C T C T C G



HMM profile

A C A C A A

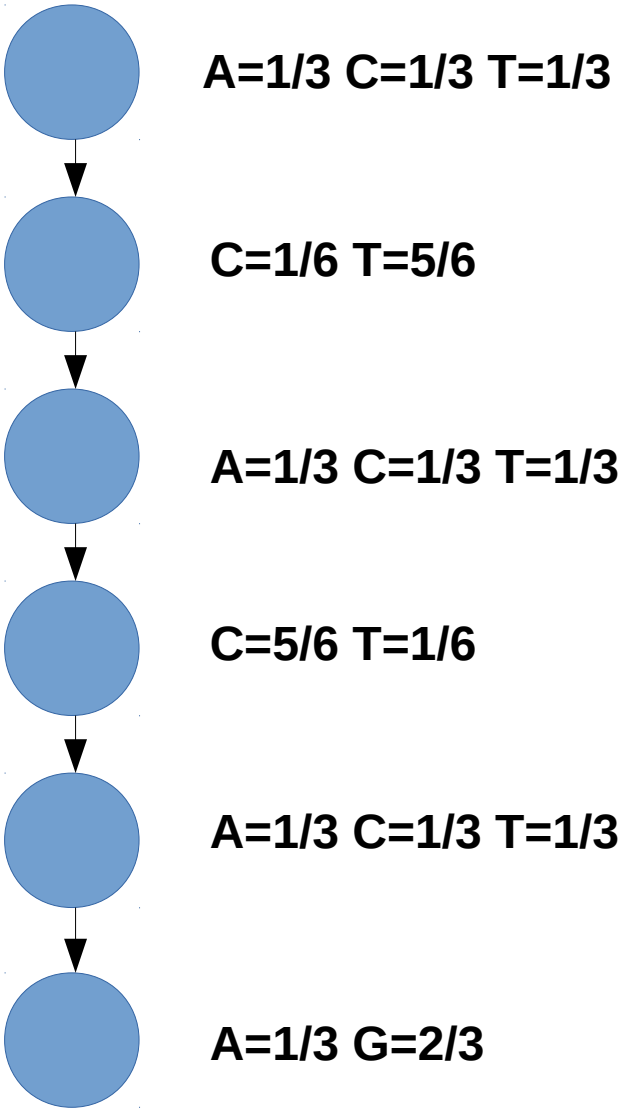
A T A C A A

T T T C T G

T T T C T G

C T C C C G

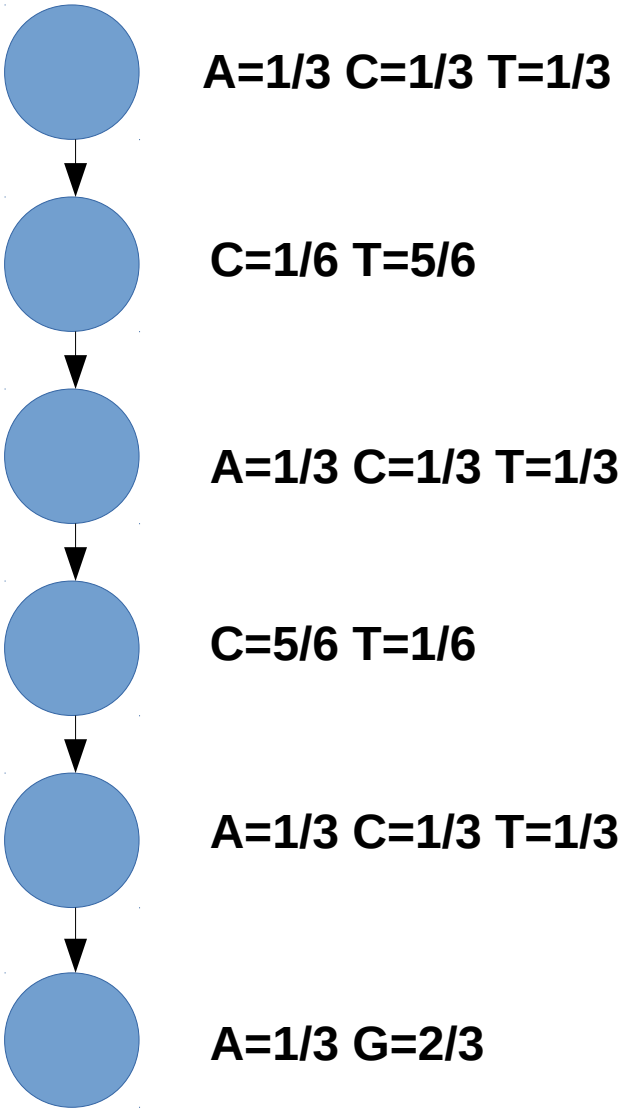
C T C T C G



HMM profile

S = CCATAA

P(S|M) = ?



HMM profile

S = CCATAA

P(S|M)

= 1/3*1*

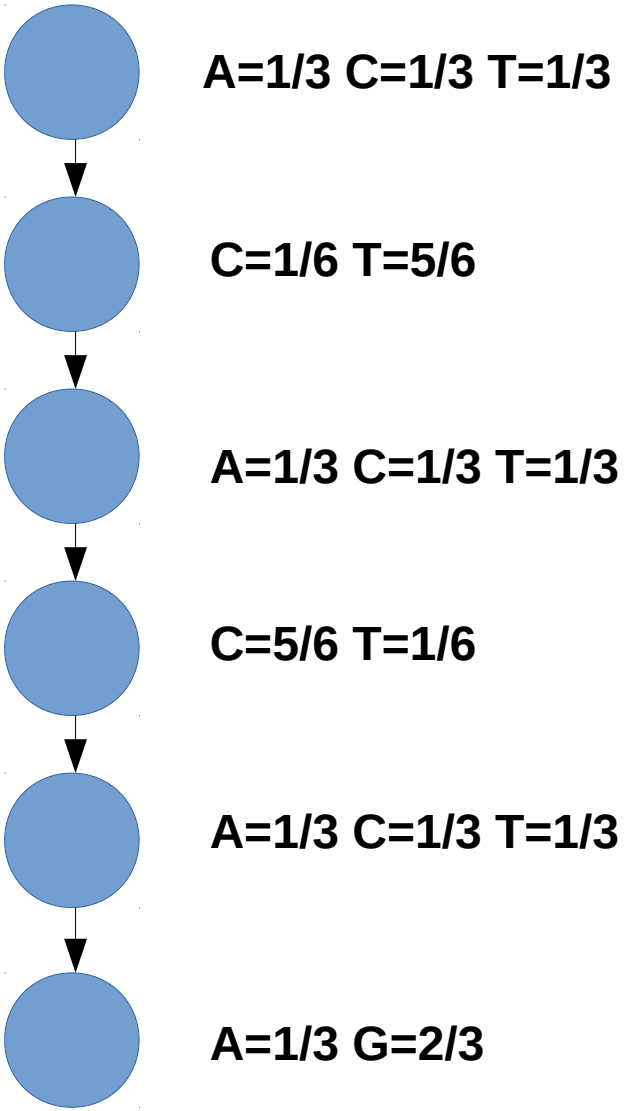
1/6*1*

1/3*1*

1/6*1*

1/3*1*

1/3*1



HMM and score

A C A C A A

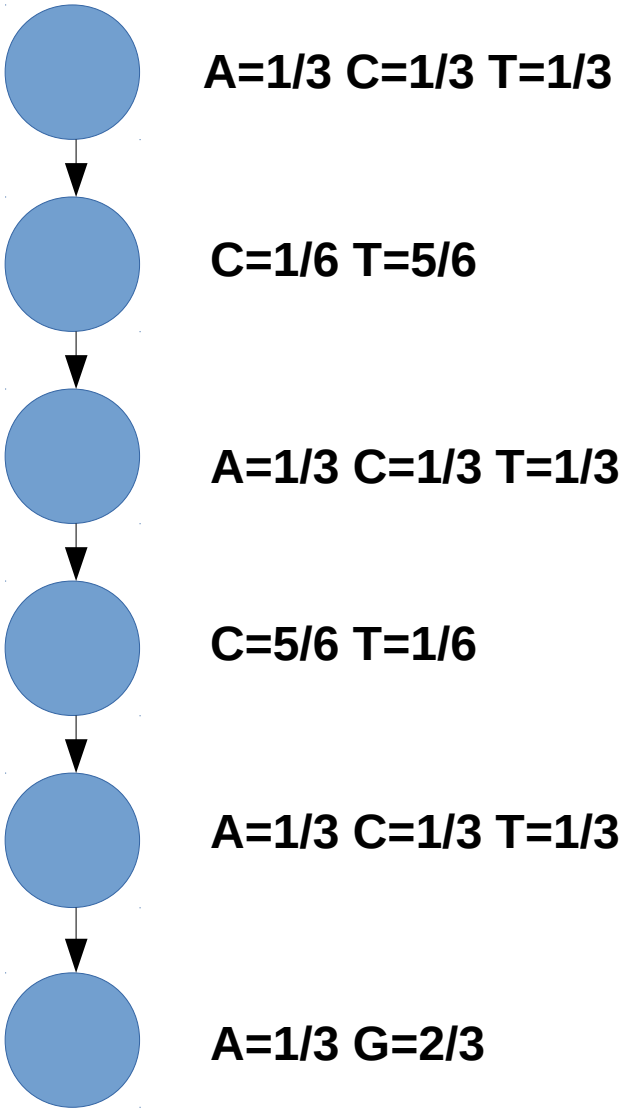
A T A C A A

T T T C T G

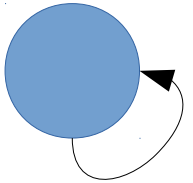
T T T C T G

C T C C C G

C T C T C G



Background model



A=1/4 C=1/4
G=1/4 T=1/4

HMM and score

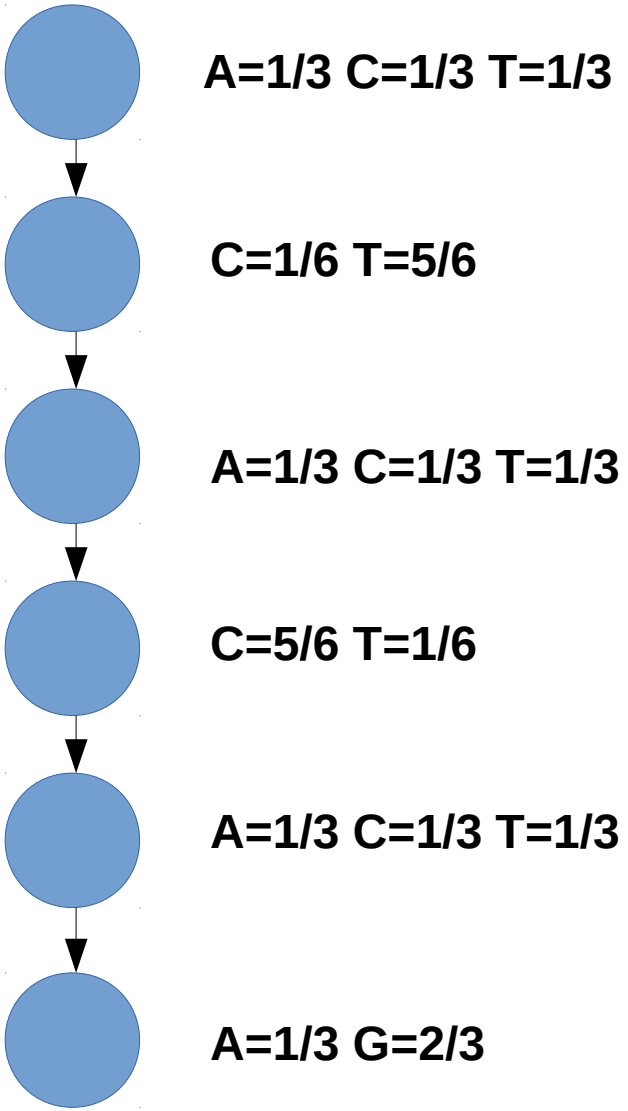
S = CCATAA

Score(S) =

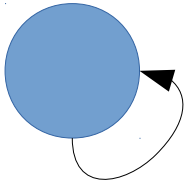
log((1/3)/(1/4))+

log((1/6)/(1/4))+

….



Background model



A=1/4 C=1/4
G=1/4 T=1/4

HMM profile (insertions/deletions)

A C A C A A

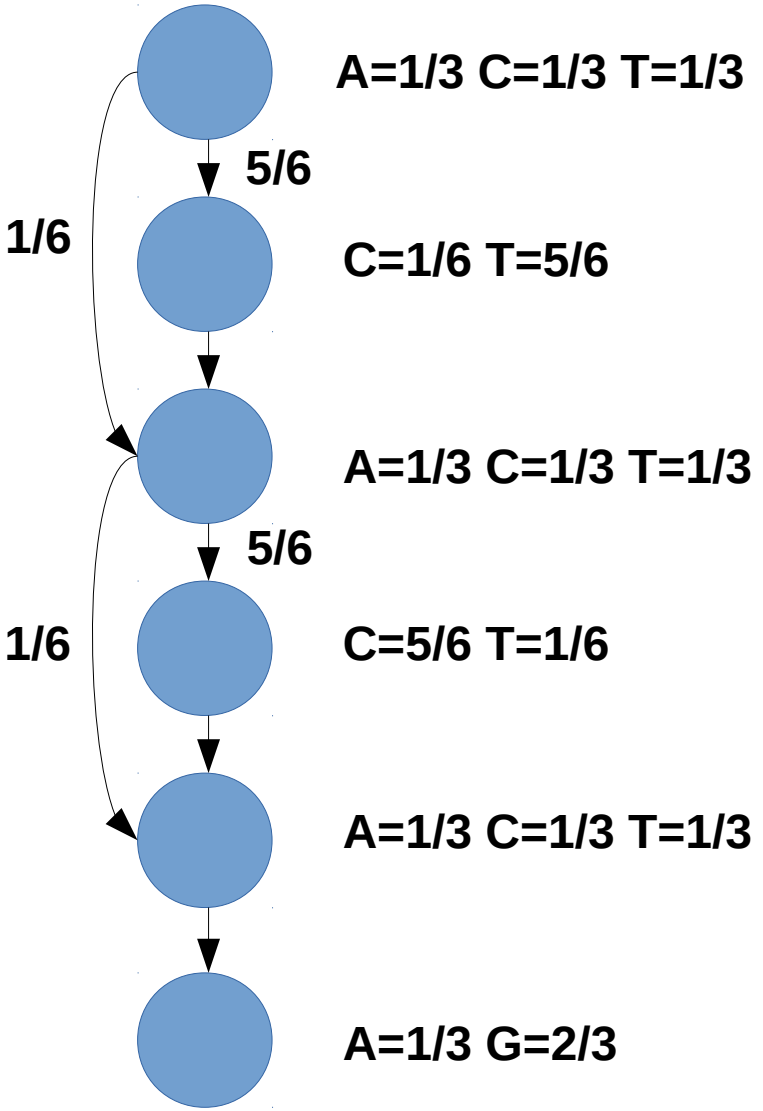
A T A - A A

T T T C T G

T T T C T G

C T C C C G

C - C T C G



HMM profile (insertions/deletions)

A C A C A - A

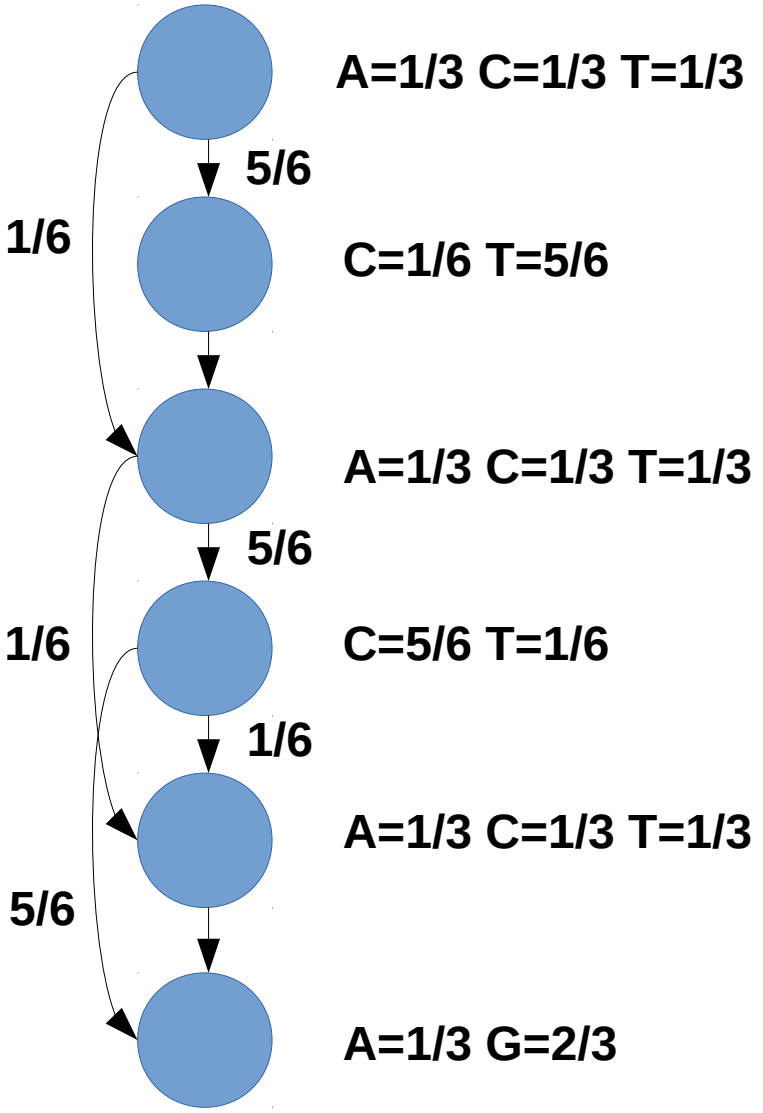
A T A - A - A

T T T C T - G

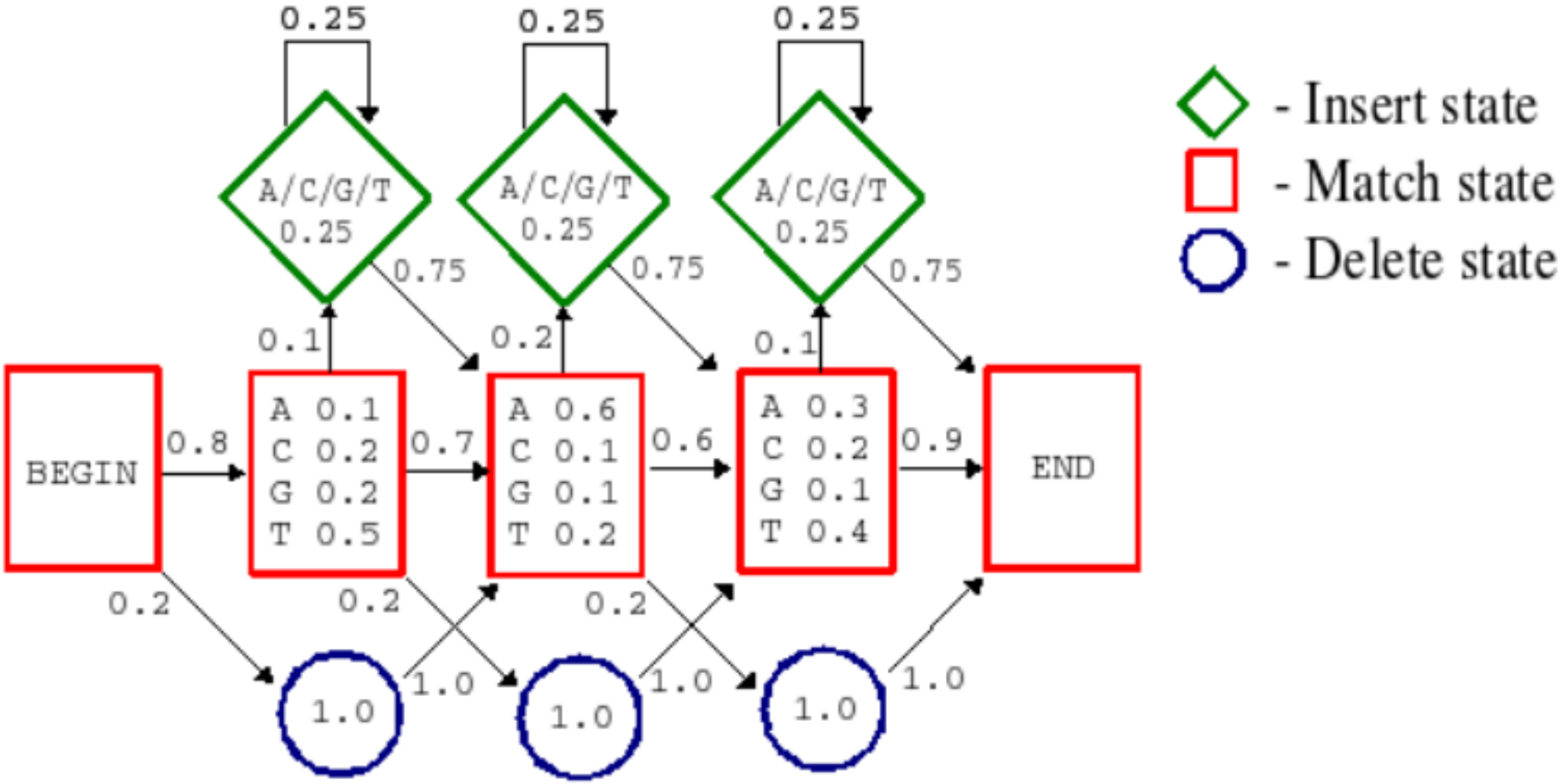
T T T C T - G

C T C C C - G

C - C T C C G

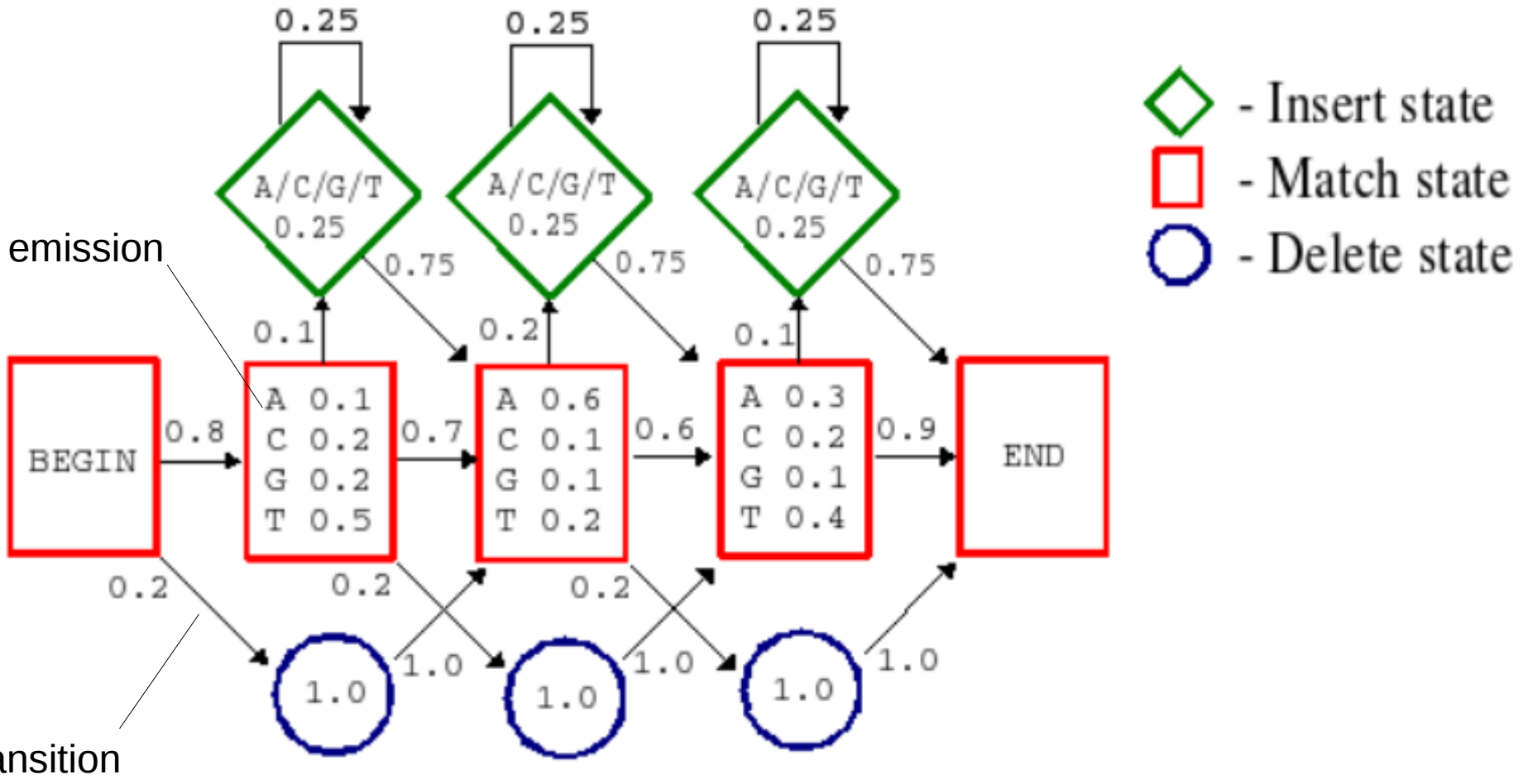


HMM profile



http://www.bioinfo.ifm.liu.se/edu/TFTB29/HT2013/assignment3.html

HMM profile

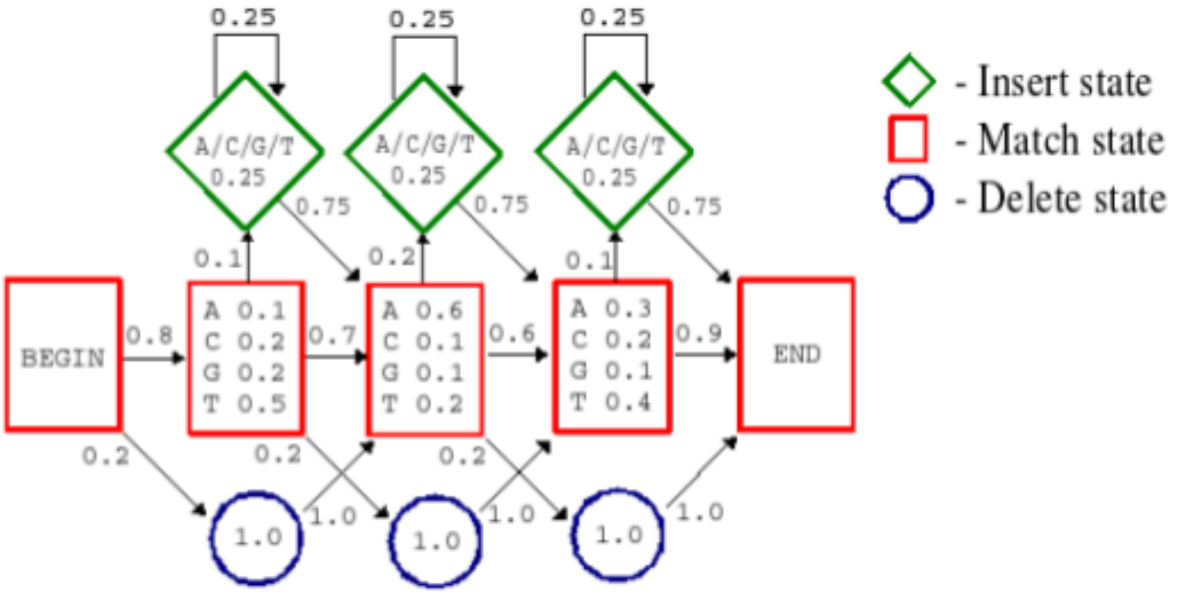


http://www.bioinfo.ifm.liu.se/edu/TFTB29/HT2013/assignment3.html

HMM profile

- Match a sequence to the HMM profile

Model

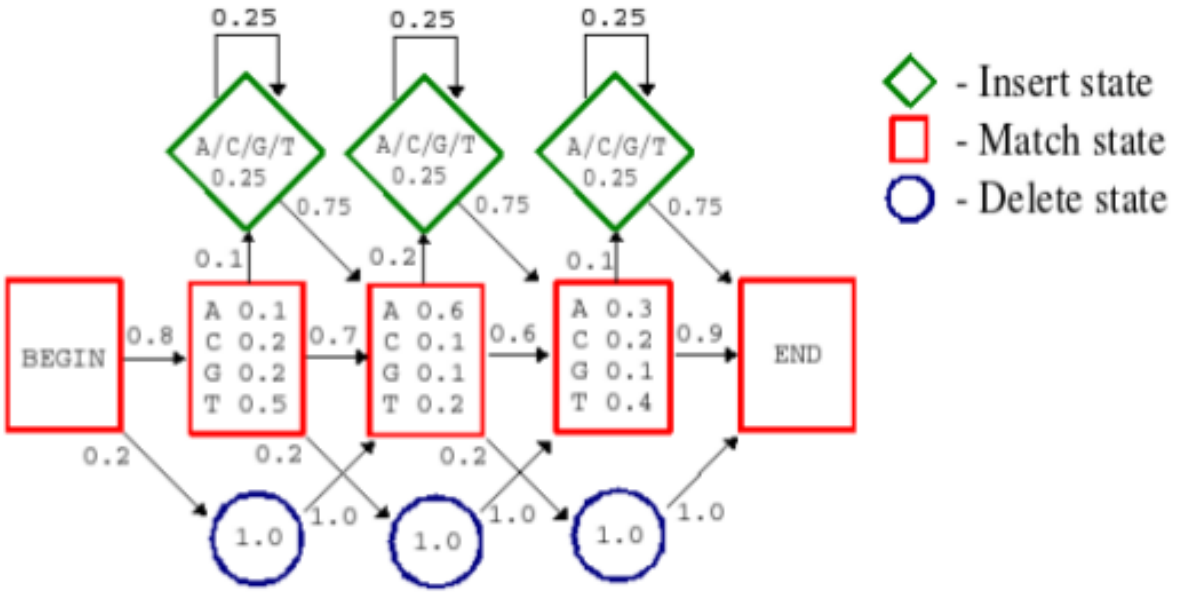


S: ATG → P(S | Model)=?

HMM profile

- Match a sequence to the HMM profile

Model

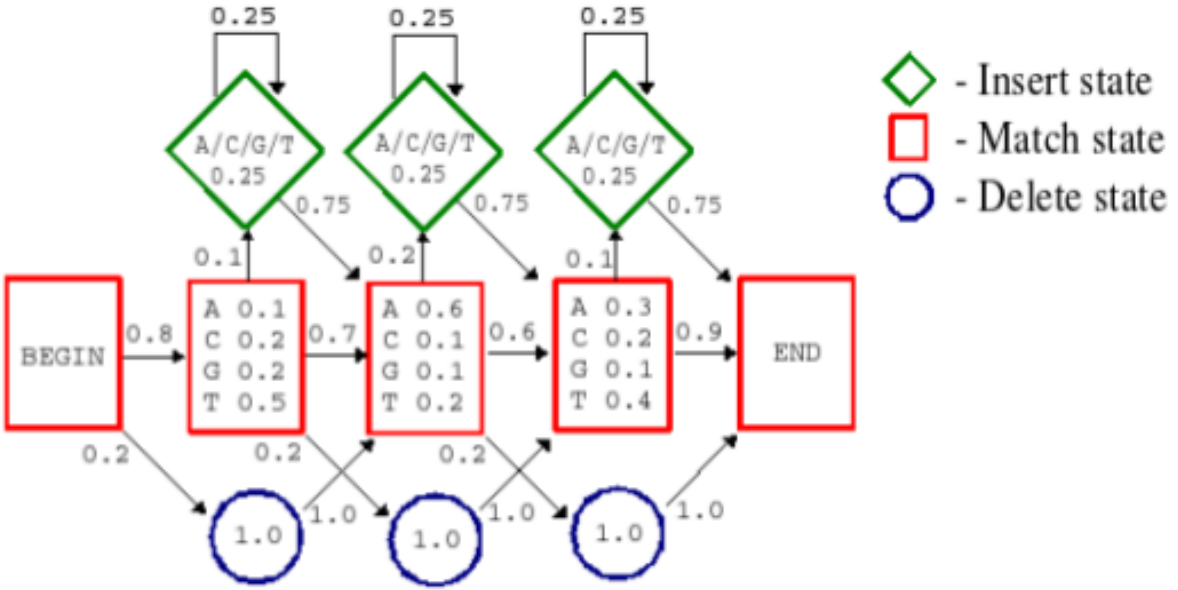


S: ATG → P(S | Model)=0.8*0.1*0.7*0.2*0.6*0.1*0.9 = 0.0006048 BMMME

HMM profile

- Match a sequence to the HMM profile

Model



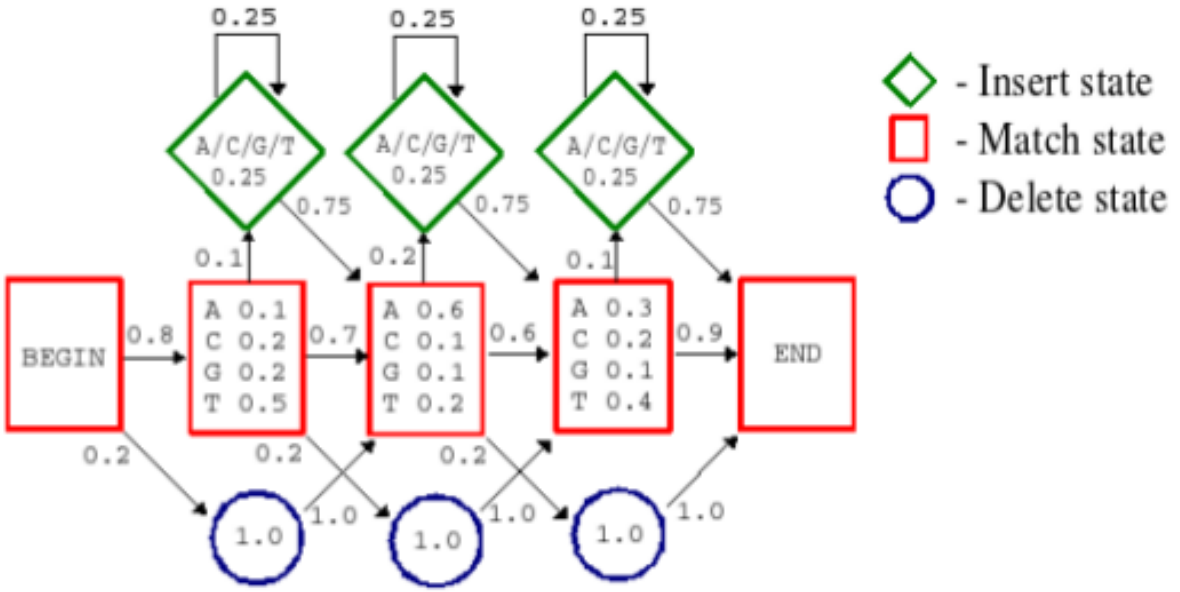
S: ATG → P(S | Model)=0.8*0.1*0.7*0.2*0.6*0.1*0.9 = 0.0006048 BMMME

P(S | Model)=0.8*0.1*0.2*0.4*0.25*0.75 = 0.0012 BMDMIE

HMM profile

- Match a sequence to the HMM profile

Model



S: ATG → P(S | Model)=0.8*0.1*0.7*0.2*0.6*0.1*0.9 = 0.0006048 BMMME

P(S | Model)=0.8*0.1*0.2*0.4*0.25*0.75 = 0.0012 BMDMIE

P(S | Model)=0.2*0.6*0.2*0.25*0.75*0.1*0.9 = 0.000405 BDMIME

HMM profile

- What is the probability that a given sequence S was generated by the HMM?

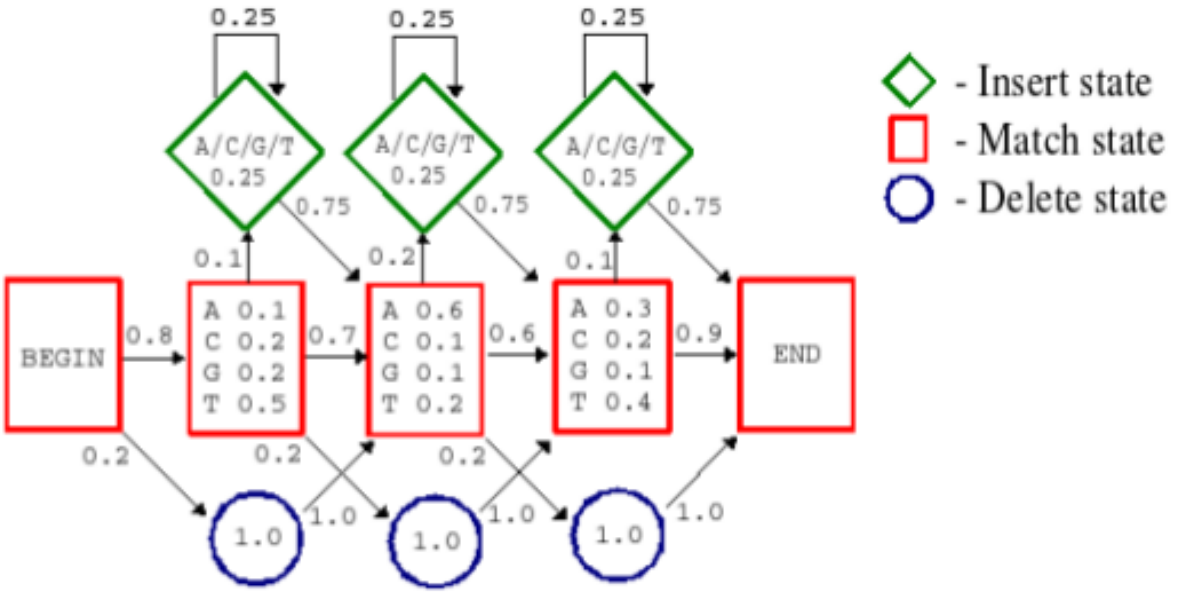
$$P(S \mid w) = \sum_{\pi} P(S, \pi \mid w)$$

  - S = sequence
  - w = parameters (probabilities)
  - π = all possible paths
- Computationally inefficient
  - There are efficient algorithms
  - Forward-Backwad and Viterbi

HMM profile

- Match a sequence to the HMM profile

Model



S: ATG → P(S | Model)=?

Finding the path with highest probability means to find the best alignment to the HMM profile