Bioinformatics — Lecture 2
Sequence analysis
(EG Ch. 5, 12; MM Ch. 12)

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12 XI 2024 (R35)

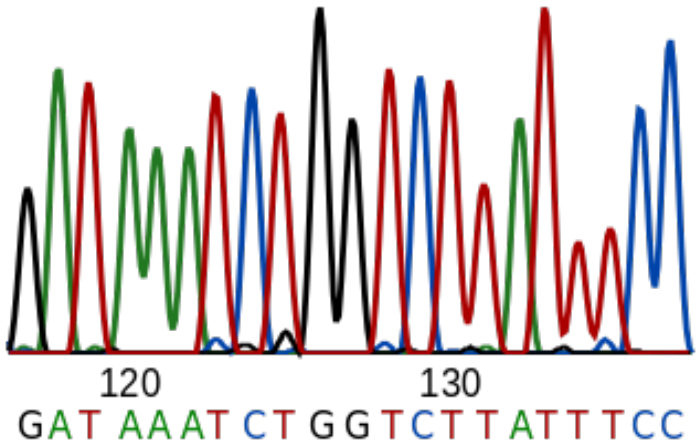
Today

- A DNA sequence
  - Human genome
  - A gene
  - TATA box
  - Nucleic acid codes
  - TATA box
  - GC content
- Sequencing
  - Timeline
  - Shotgun sequencing
  - Poisson model
  - Shortest common superstring
- Modelling DNA
  - PWM
  - Markov chains
  - HMMs

The human genome

2×23 chromosomes
3.23483 Giga–basepairs (on single chromosome copy),
6.46983 Giga–basepairs (on both chromosome copies),

$$\text{genome} \in \{A, C, G, T\}^{6.46983 \cdot 10^9}$$



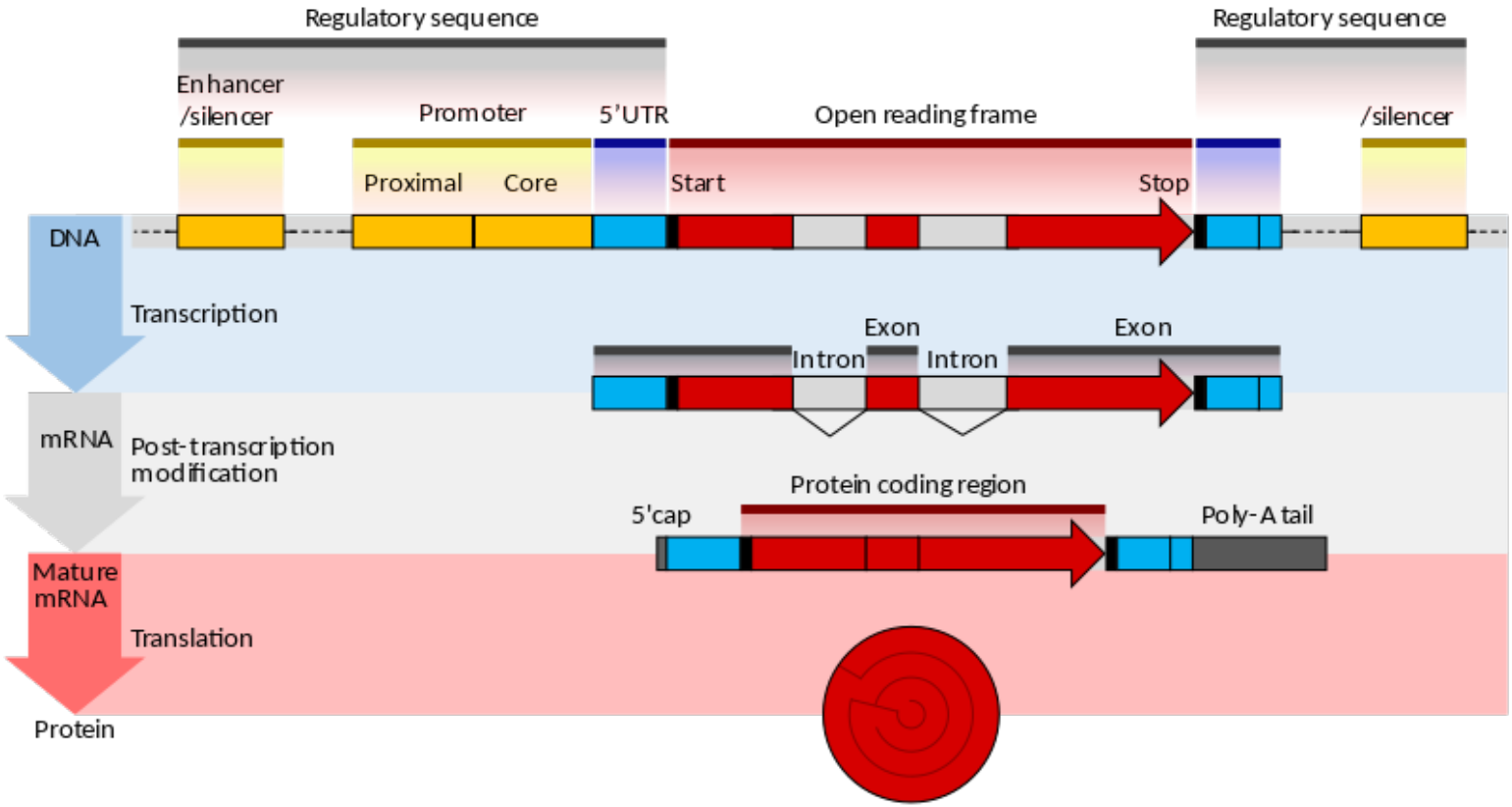
https://en.wikipedia.org/wiki/Nucleic_acid_sequence (graphic by Sjef, public domain)

The human genome https://en.wikipedia.org/wiki/Human_genome

$\approx$ 20000 protein coding genes
protein coding genes are $\approx$ 1.5% of genome
median size of protein coding gene 26288bp

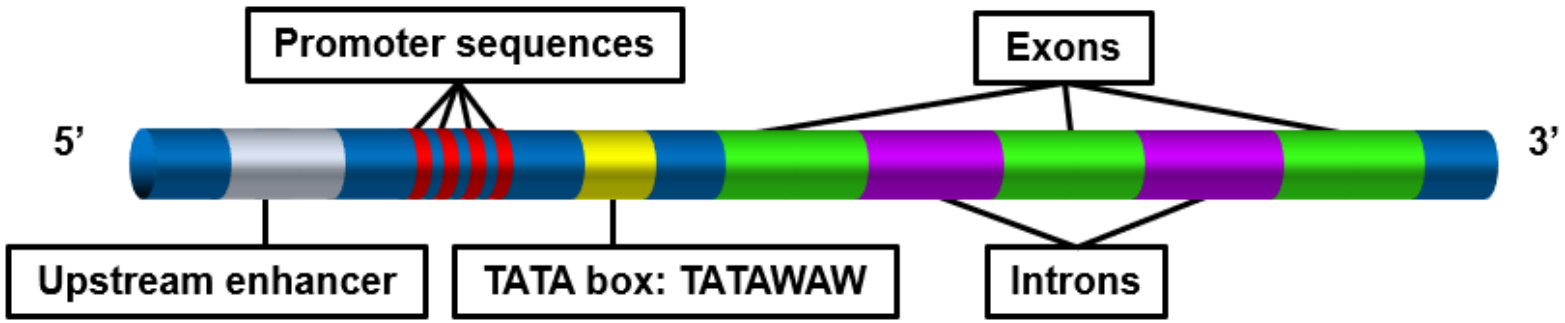
98.5%: *non–coding* RNA, *regulatory sequences*, *introns*, *retrotransposon* (DNA that is transcribed into RNA, then reverse transcribed into DNA and can be re–inserted into genome), *transposable elements* (can change position in genome)
and *unknown* sequences
pseudogenes (inactive copies of gene, often generated by gene duplication),
repetitive DNA sequences (50% of genome, DNA occurring in multiple copies)
SNPs: $\approx$ 1 in 1000bp but also CNV (copy number variation)

A gene



T. Shafee, R. Lowe (2017). "Eukaryotic and prokaryotic gene structure". WikiJournal of Medicine 4(1). DOI:10.15347/wjm/2017.002 CC BY 4.0

TATA box (description)



`https://en.wikipedia.org/wiki/TATA_box` (graphic by Luttysar, CC BY-SA 4.0)

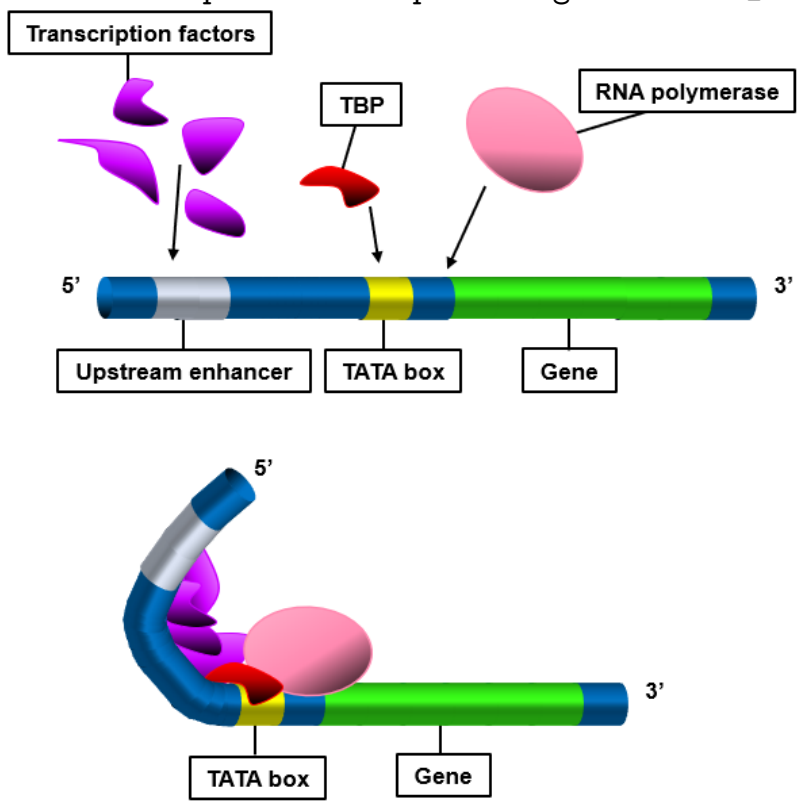
$$\mathrm{W} \in \{\mathrm{A}, \mathrm{T}\}$$

IUB/IUPAC nucleic acid codes (https://en.wikipedia.org/wiki/FASTA_format)

Nucleic Acid Code	Meaning	Mnemonic
A	A	**A**denine
C	C	**C**ytosine
G	G	**G**uanine
T	T	**T**hymine
U	U	**U**racil
R	A or G	pu**R**ine
Y	C, T or U	p**Y**rimidines
K	G, T or U	bases which are **K**etones
M	A or C	bases with a**M**ino groups
S	C or G	**S**trong interaction
W	A, T or U	**W**eak interaction
B	not A (i.e. C, G, T or U)	**B** comes after A
D	not C (i.e. A, G, T or U)	**D** comes after C
H	not G (i.e., A, C, T or U)	**H** comes after G
V	neither T nor U (i.e. A, C or G)	**V** comes after U
N	A C G T U	**N**ucleic acid
-	gap of indeterminate length	

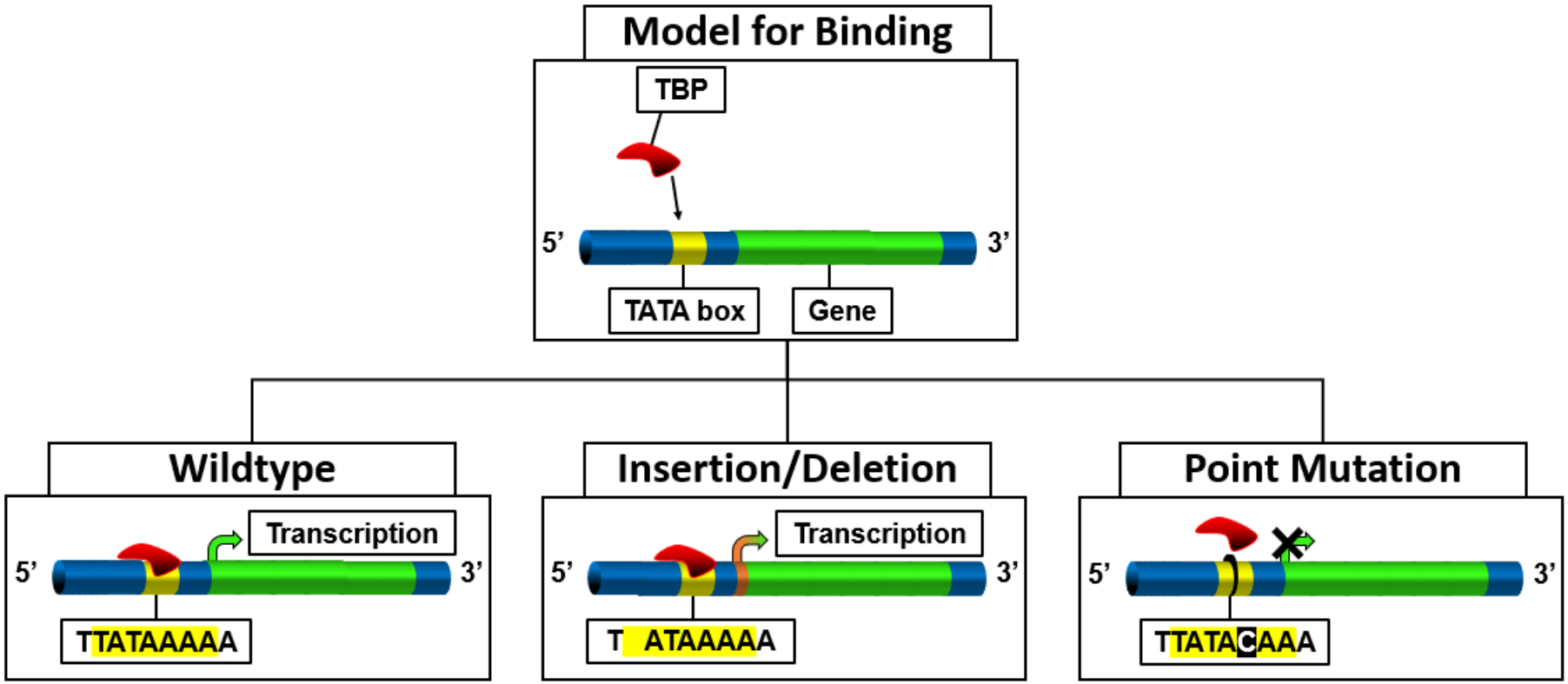
TATA box (mechanism)

https://en.wikipedia.org/wiki/TATA_box (graphic by Luttysar, CC BY-SA 4.0)



TBP: TATA binding protein
needs to bind for first step in transcription initiation

TATA box (mutation mechanism)



https://en.wikipedia.org/wiki/TATA_box (graphic by Luttysar, CC BY-SA 4.0)

CpG islands

CpG: ($5'$–*C*–*phosphate*–*G*–$3'$)
phospate (P) links any two nucleotides in DNA
Regions with "high frequency" of CpG sites
region: at least 200bp
$(\#C + \#G)/(|\text{sequence}|) > 50\%$ (GC%)and
observed–to–expected CpG ratio greater than 60

$$\text{observed} : \#CpG$$

$$\text{expected} : \frac{\#C \cdot \#G}{|\text{sequence}|} \quad \text{or} \quad \frac{((\#C + \#G)/2)^2}{|\text{sequence}|} \; (* \text{ for next slide})$$

many (vertebrate) genomes have CpG islands near start
(*promoter*) of transcribed region of genes
(esp. *housekeeping*: maintenance of basic cellular function)

https://en.wikipedia.org/wiki/CpG_site https://en.wikipedia.org/wiki/Housekeeping_gene

Human genome composition

A : 29.3%, C : 20.0%, G : 20.7%, T : 20%, GC : 40.7%
(content is chromosome specific)

https://en.wikipedia.org/wiki/Chargaff%27s_rules: $\%A = \%T$, $\%G = \%C$

rule 1: on double strand, rule 2: on both strands separately

background probability CpG$= 0.2 \cdot 0.207 = 0.0414$

Table 1. Overview of CpG distribution in the human genome

Subset	Length, Mb	GC content	Observed CpG fraction	Normalized CpG fraction
Whole genome	3.1*	0.38	0.009	0.25
1 kb upstream regions	15	0.53	0.042	0.60
1 kb downstream regions	15	0.45	0.013	0.26
Transcription units	930	0.42	0.011	0.26
Exons	45	0.50	0.028	0.45
Introns	880	0.41	0.010	0.24

Length refers to the total length of DNA examined.
*Length given in gigabases.

observed/expected(∗)

S. Saxonov, P. Berg, D. L. Brutlag (2006). "A genome–wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters". PNAS 103(5) : 1412–1417, doi:10.1073/pnas.0510310103.

Human gene content

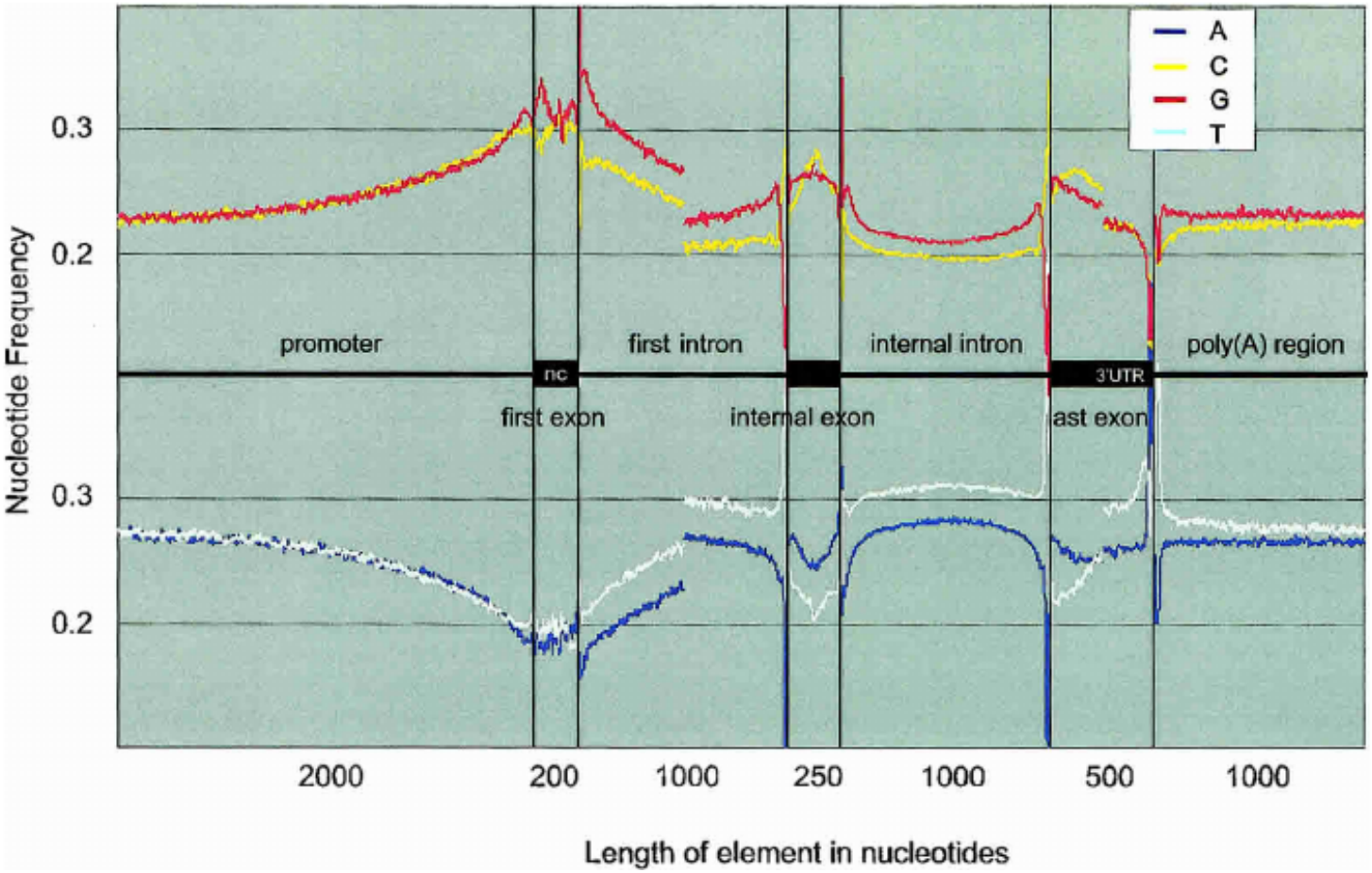


Fig. 1 in E. Louie, J. Ott, J. Majewski (2003). "Nucleotide Frequency Variation Across Human Genes". Genome Research 13 : 2594–2601. Usage permissions, CC BY-NC 4.0 .

Genome content (GC sorted)

Organism	Taxon	%A	%G	%C	%T	A / T	G / C	%GC	%AT
E. coli	*Escherichia*	24.7	26.0	25.7	23.6	1.05	1.01	51.7	48.3
Maize	*Zea*	26.8	22.8	23.2	27.2	0.99	0.98	46.1	54.0
Wheat	*Triticum*	27.3	22.7	22.8	27.1	1.01	1.00	45.5	54.4
φX174	*PhiX174*	24.0	23.3	21.5	31.2	0.77	1.08	44.8	55.2
Chicken	*Gallus*	28.0	22.0	21.6	28.4	0.99	1.02	43.7	56.4
Rat	*Rattus*	28.6	21.4	20.5	28.4	1.01	1.00	42.9	57.0
Grasshopper	Orthoptera	29.3	20.5	20.7	29.3	1.00	0.99	41.2	58.6
Human	*Homo*	29.3	20.7	20.0	30.0	0.98	1.04	40.7	59.3
Yeast	*Saccharomyces*	31.3	18.7	17.1	32.9	0.95	1.09	35.8	64.4
Octopus	*Octopus*	33.2	17.6	17.6	31.6	1.05	1.00	35.2	64.8
Sea Urchin	Echinacea	32.8	17.7	17.3	32.1	1.02	1.02	35.0	64.9

`https://en.wikipedia.org/wiki/Chargaff%27s_rules`

Sequencing projects historical timeline

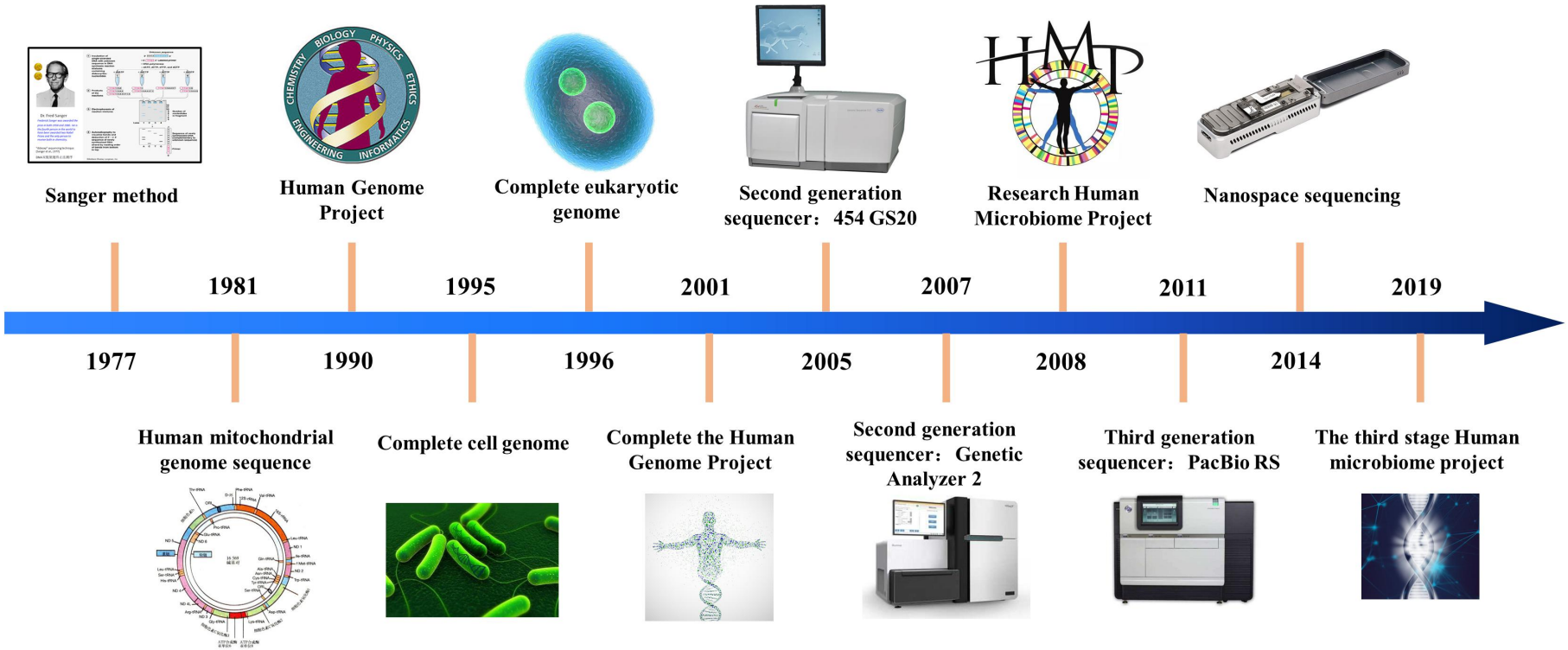
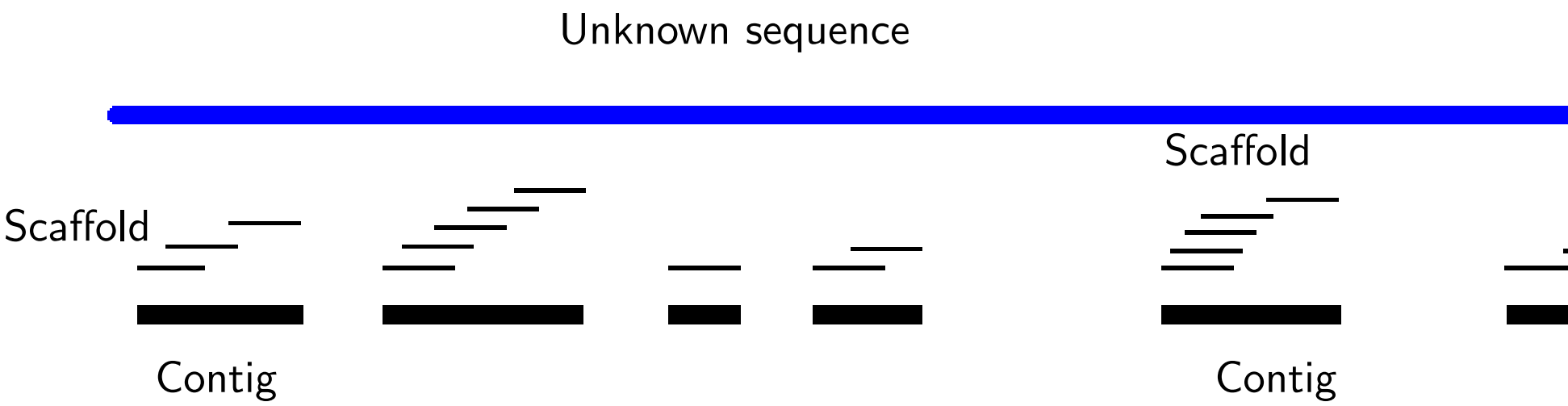


Fig. 1 in A. Yang, W. Zhang, J. Wang, K. Yang, Y. Han and L. Zhang (2020) Review on the Application of Machine Learning Algorithms in the Sequence Data Mining of DNA. Front. Bioeng. Biotechnol. 8. doi:10.3389/fbioe.2020.01032 CC BY

see also Fig. 1 in International Human Genome Sequencing Consortium (2001). Initial sequencing and analysis of the human genome. Nature 409 : 860–921. doi:10.1038/35057062

Shotgun sequencing (EG Ch. 5.1): Contigs



see also EG Ch 5.1

In reality *locations* and *orientation* of contigs is unknown.
How does one assemble then?

Poisson model

N fragments of length L, from genome of length $G \gg L$
(end effects ignored)

Coverage: $a := NL/G$

fragments taken at random:
left–hand ends i.i.d. $X \sim \mathrm{Unif}[0, G]$ (continuous approximation)

$$P(X \in (x, x+h)) = h/G$$

$$\#X \in (x, x+h) \sim \mathrm{Binomial}(N, hG) \Rightarrow \mathsf{E}\,[\#X] = Nh/G$$

N large, h small $\Rightarrow \mathrm{Binomial}(N, hG) \approx \mathrm{Poisson}(\mathrm{mean} = Nh/G)$

Poisson model

$Y \sim \text{Poisson}(a)$: # of fragments with left–hand end in a given interval of length L

$$Prob(Y = 0) = e^{-a}$$

Mean proportion of genome covered: $1 - e^{-a}$
point chosen at random is covered by at least one fragment:
at least one fragment has its left–hand end in the L–interval immediately to the left of this point

mean number of contigs: $Ne^{-a} = Ne^{-NL/G}$
N times probability that a fragment is the rightmost member of a contig, i.e. no other fragment has its left–hand end inside it

Exercises (EG Ch. 5.1)

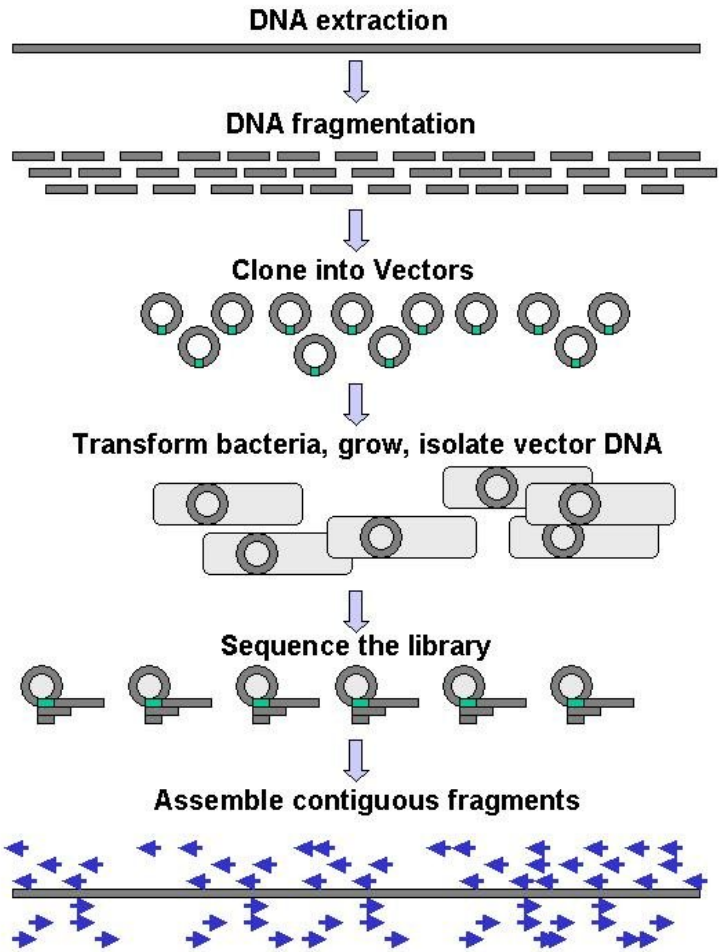
Exercise: study $1 - e^{-a}$ is there a point beyond which increasing a is pointless?

Exercise: Plot Ne^{-a} as a function of N, explain

Read: What is the mean contig size?

Read: What is the mean number of fragments covering a point (base)?

Shortest common superstring (NPc)



https://en.wikipedia.org/wiki/DNA_sequencing
(graphic by Abizar Lakdawalla, public domain)

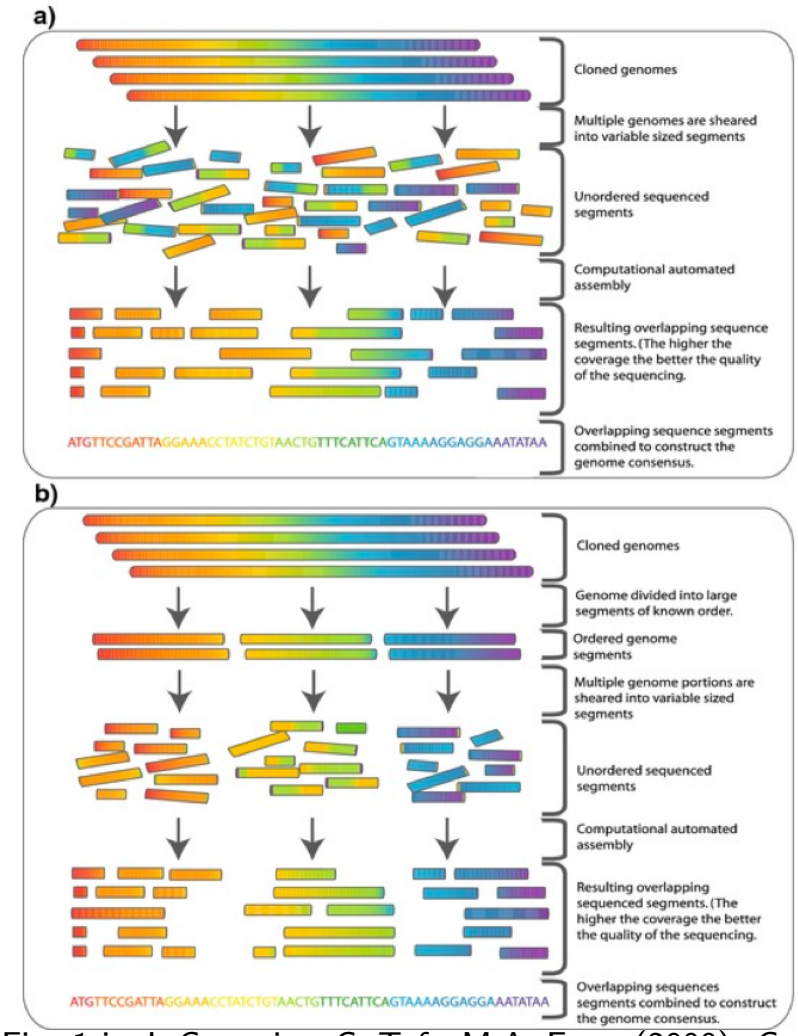
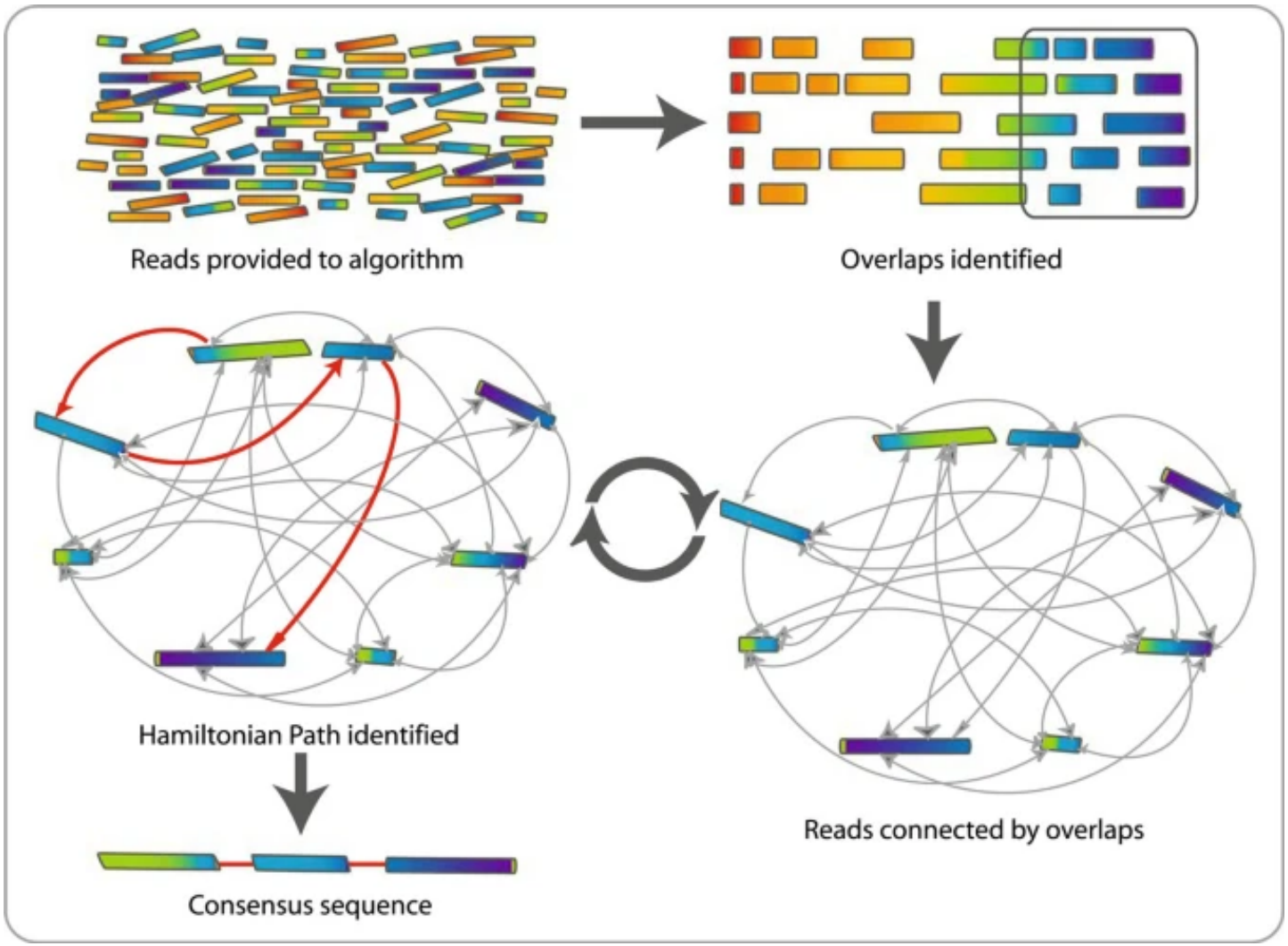


Fig. 1 in J. Commins, C. Toft, M.A. Fares (2009). Computational Biology Methods and Their Application to the Comparative Genomics of Endocellular Symbiotic Bacteria of Insects. Biol. Proc. Online 11:52-78. doi:10.1007/s12575-009-9004-1. CC BY 2.0

Shortest common superstring (NPc)



Overlap should be

end to end

not in the middle

Fig. 2 in J. Commins, C. Toft, M.A. Fares (2009). Computational Biology Methods and Their Application to the Comparative Genomics of Endocellular Symbiotic Bacteria of Insects. Biol. Proc. Online 11:52-78. doi:10.1007/s12575-009-9004-1. CC BY 2.0

Weighted set cover problem (NPc)

We are given
a universe $\mathcal{U}$
family of sets $\mathcal{S} \ni s \subseteq \mathcal{U}$
with weights $w(s)$,

AIM: Find a *cover* $\mathcal{C} \subseteq \mathcal{S}$ with minimal weight
i.e.
minimize $\sum_{c \in \mathcal{C}} w(c)$ over all covers of $\mathcal{U}$ contained in $\mathcal{S}$

Cover: $\bigcup_{c \in \mathcal{C}} c = \mathcal{U}$

Shortest common superstring reduction

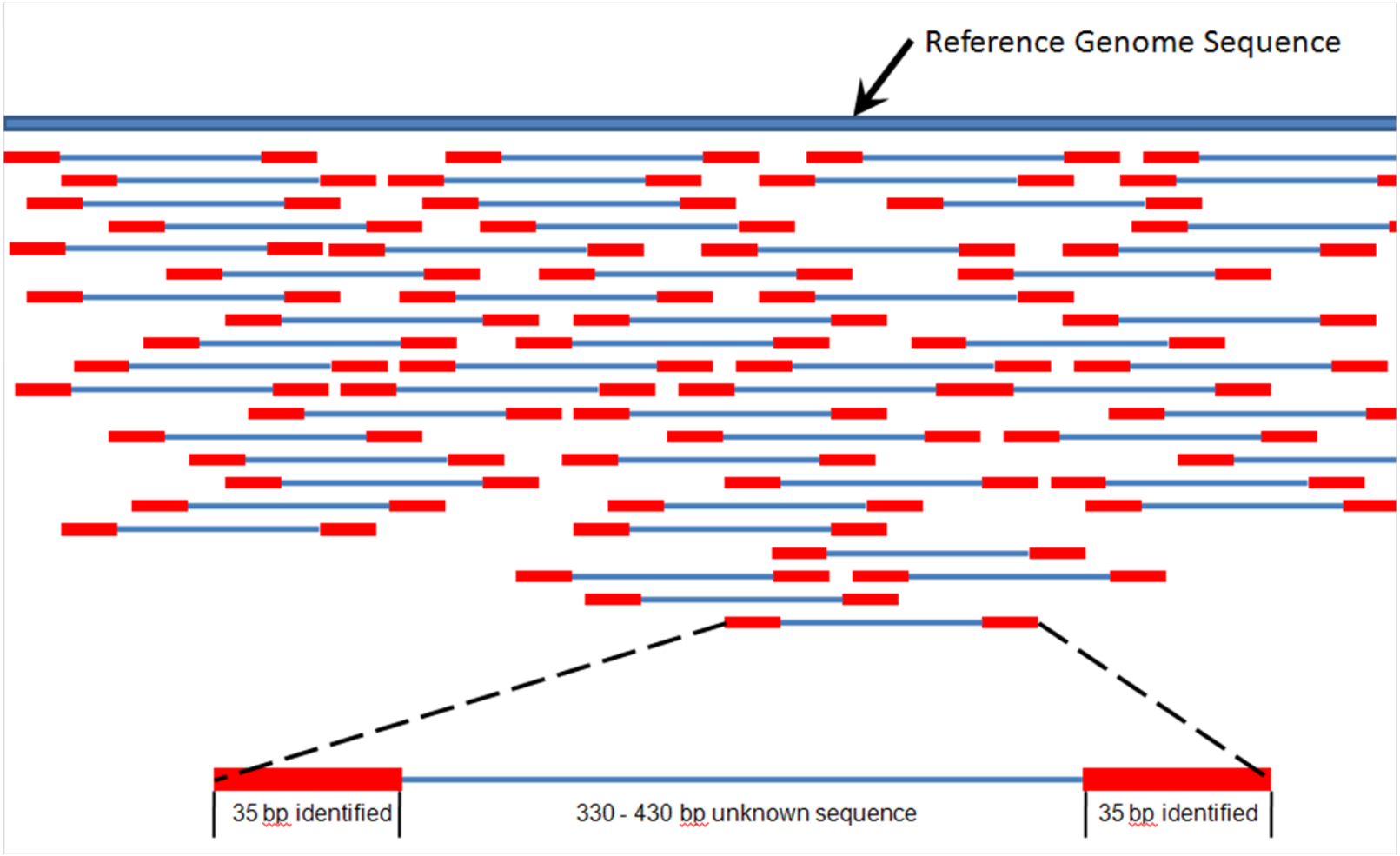
$S = \{s_1, \ldots, s_n\}$ (collection of strings for SCS)

$M = \emptyset$ and then "for each pair of strings s_i and s_j, if the last k symbols of s_i are the same as the first k symbols of s_j, then add a string to M that consists of the concatenation with maximal overlap of s_i with s_j"
$\mathcal{U} = S$
$\mathcal{S} = \{P(x) : x \in S \cup M\}$ ($P(x)$: set of all substrings of x)
$w(P(x)) = |x|$

solve by weighted set cover algorithm and SCS is arbitrary concatenation of xs from chosen $P(x)$ sets
Set–cover is NP–complete but has $O(\log(n))$–approximation algorithm, $w \leq \log(n) w_{opt}$

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

Reference genome (alignment, BLAST)



https://en.wikipedia.org/wiki/DNA_sequencing (graphic by Suspencewl, public domain)

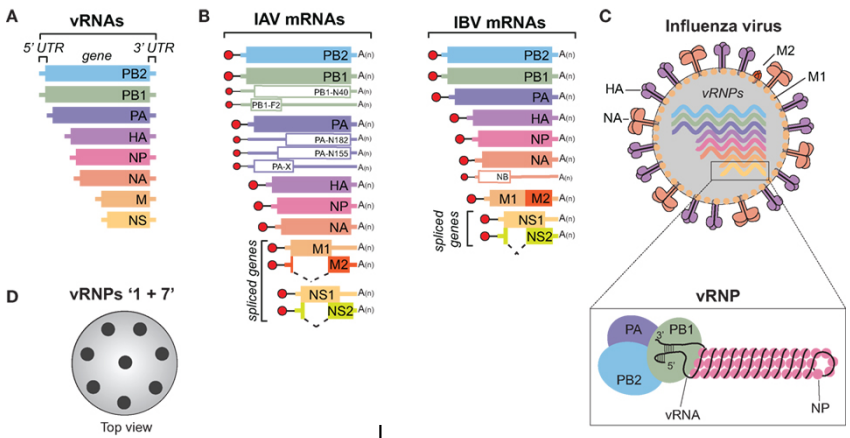
Genome variability

`https://en.wikipedia.org/wiki/Position_weight_matrix` (graphic by Gnomehacker, CC BY-SA 3.0)

Position weight matrix (PWM, EG Ch. 5.3.2)

Positions assumed independent

	position				
	1	2	3	4	...
A	p_{A1}	p_{A2}	p_{A3}	p_{A4}	...
C	p_{C1}	p_{C2}	p_{C3}	p_{C4}	...
G	p_{G1}	p_{G2}	p_{G3}	p_{G4}	...
T	p_{T1}	p_{T2}	p_{T3}	p_{T4}	...
Total	1	1	1	1	...



Avian flu: PA gene segment

GIPL amino acids (synonymous changes)

Sequence	# strains
GGT ATA CCG TTA	6
GGG ATA CCG CTG	19
GGT ATA CCG CTA	27
GGG ATA CCG CTA	1
	14

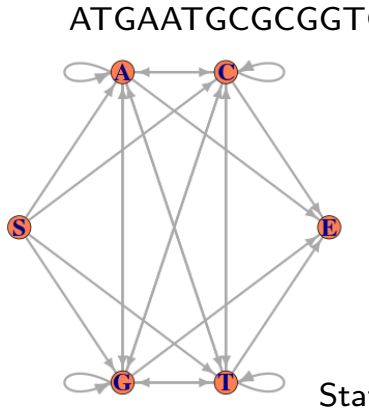
K. Bartoszek, Bayesian Variable Selection Applied to the Assessment of Pathogenicity of Avian Flu, 2008, MPHil Dissertation, Cambridge Univ.

Fig. 1 in D. Dou, R. Revol, H. Östbye, H. Wang, R. Daniels, 2018. Influenza A Virus Cell Entry, Replication, Virion Assembly and Movement. Front. Immunol. 9:1581. doi: 10.3389/fimmu.2018.01581. CC BY

	position											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0.79	0	0.79	0	0	0	0	0	0.51
C	0	0	0	0	0	0	0.79	0.79	0	0.7	0	0
G	0.79	0.79	0.3	0	0	0	0	0	0.79	0	0	0.28
T	0	0	0.49	0	0.79	0	0	0	0	0.09	0.79	0
—	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Total	1	1	1	1	1	1	1	1	1	1	1	1

Markov chains (EG Ch. 4.5–4.10, MM Ch. 12)

$S = \{A, C, G, T\} \ni X_n$ nucleotide at position n of chromosome/genome/DNA fragment/e.t.c.



State diagram

transition probability:

$$p_{ij} = P(X_{n+1} = j | X_n = i) = P(X_{n+1} = j | X_n = i, \ldots, X_0 = i_0)$$

Markov chain of order k:

$$P(X_{n+1} = j | X_n = i, \ldots, X_0 = i_0) = P(X_{n+1} = j | X_n = i, \ldots, X_{n-k+1} = i_{n-k+1})$$

Remember: 3 nucleotides per amino acid

Markov chains

transition matrix: $P = [p_{ij}] \in [0,1]^{|S|\times|S|}$

2*–step transition probability*: $p_{ij}^{(2)} = \sum_{k\in S} p_{ik}p_{kj}$, i.e. P^2

n–step transition probability: P^n

absorbing state: i s.t. $p_{ii} = 1$ (or $p_{ij} = 0,\ i \neq j$)
if $X_0 \sim \phi_0$ then $X_1 \sim \phi_1^T = \phi_0^T P$

stationary distribution: $\phi^T = \phi^T P$

Markov chains

	position				
	A	C	G	T	Total
A	p_{AA}	p_{AC}	p_{AG}	p_{AT}	1
C	p_{CA}	p_{CC}	p_{CG}	p_{CT}	1
G	p_{GA}	p_{GC}	p_{GG}	p_{GT}	1
T	p_{TA}	p_{TC}	p_{TG}	p_{TT}	1
Total	$p_{\to A}$	$p_{\to C}$	$p_{\to G}$	$p_{\to T}$	

stochastic matrix

$p_{\to A} = p_{\to C} = p_{\to G} = p_{\to T} = 1$

doubly stochastic matrix

Higher order: transition matrices large, parameter rich
(data can be insufficient)

Maximal dependence decomposition for group of *aligned* sequences

Ch 5.3.4 and T.–Y. Lee et. al. (2011). "Exploiting maximal dependence decomposition to identify conserved motifs from a group of aligned signal sequences". Bioinformatics 27(13) : 1780–1787.

Repeats (EG Ch. 5.4, MM p. 223)

Does a given nucleotide (say A) repeat itself more often than expected by chance in a *long* DNA sequence of length N?

p: probability of observing A,
Y: number of repeats of A, $P(Y = y) = (1 - p)p^y$

n such sequences
$Y_{\max}$ longest: $P(Y_{\max} \geq y) = 1 - (1 - p^y)^n$

a success (A) has to be preceded by a failure,
$\approx (1 - p)N$ failures implying $\approx (1 - p)N$ successes
take $n \approx (1 - p)N$

Repeats

$Y_{\max}$ test statistic with p–value (EG Eq. 5.15):

$$1 - (1 - p^{y_{\max}})^{(1-p)N} \approx 1 - \exp(-(1-p)Np^{y_{\max}})$$

N large
$(1-p)Np^{y_{\max}} < 1$
remember: $(1 + x/n)^n \to e^x$.

Is $(1-p)N$ a good approximation for number of failures?
Average of binomial with parameters N and $1-p$.

Patterns (EG Ch. 5.6–5.8)

Counting specific repeats: e.g. *GCGC*,

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

2 or **3** ?

Different models and results depending if *overlaps* counted.

Motifs (EG Ch. 5.9)

Many short sequences serve specific functions and do not tolerate many mutations.
transcription factor binding sites, *splice junction signals*

Motif: collection of m different (uncontained) sequences, similar and of same length

Example:
$M = \{GATGGTGG, GCTGGTGG, GGTGGTGG, GTTGGTGG\}$
(crossover hotspot initiator in *Hemophilus influenzae*, bacteria)

Motifs (EG Ch. 5.9)

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

A motif can be represented as a PWM

$$M = \begin{matrix} A \\ C \\ G \\ T \end{matrix} \begin{bmatrix} 0.3 & 0.6 & 0.1 & 0.0 & 0.0 & 0.6 & 0.7 & 0.2 & 0.1 \\ 0.2 & 0.2 & 0.1 & 0.0 & 0.0 & 0.2 & 0.1 & 0.1 & 0.2 \\ 0.1 & 0.1 & 0.7 & 1.0 & 0.0 & 0.1 & 0.1 & 0.5 & 0.1 \\ 0.4 & 0.1 & 0.1 & 0.0 & 1.0 & 0.1 & 0.1 & 0.2 & 0.6 \end{bmatrix}$$

GAGGTAAAC
TCCGTAAGT
CAGGTTGGA
ACAGTCAGT
TAGGTCATT
TAGGTACTG
ATGGTAACT
CAGGTATAC
TGTGTGAGT
AAGGTAAGT

Probability of observing a motif

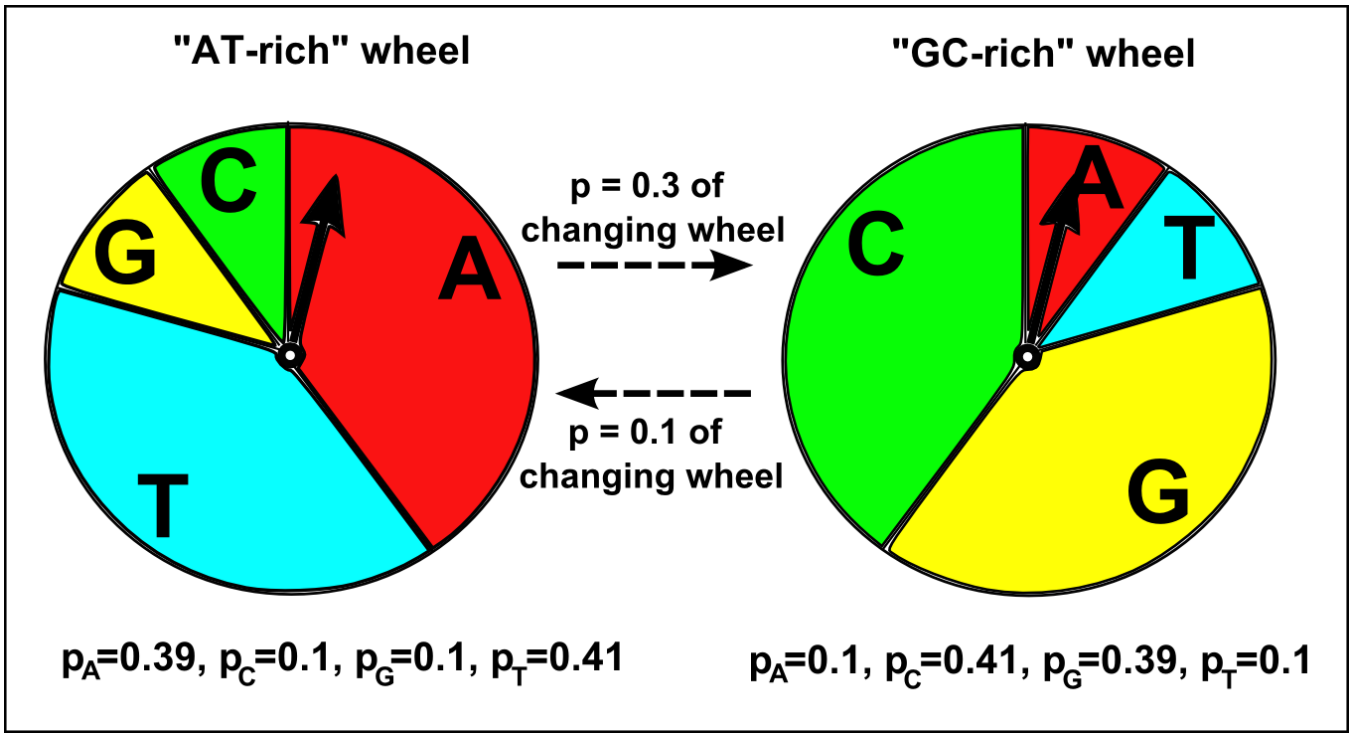
$$P(M) = \sum_{u \in M} P(u)$$

$P(u)$: independent positions, product over them,

$$P(GAGGTAAAC) = 0.1 \cdot 0.6 \cdot 0.7 \cdot 1 \cdot 1 \cdot 0.6 \cdot 0.7 \cdot 0.2 \cdot 0.2$$

N: sequence length
mean number of motif occurrences: $(N - |M| + 1)P(M)$
variance with or without overlaps, Eq. (5.91) or (5.92)
test if occurs as often as expected

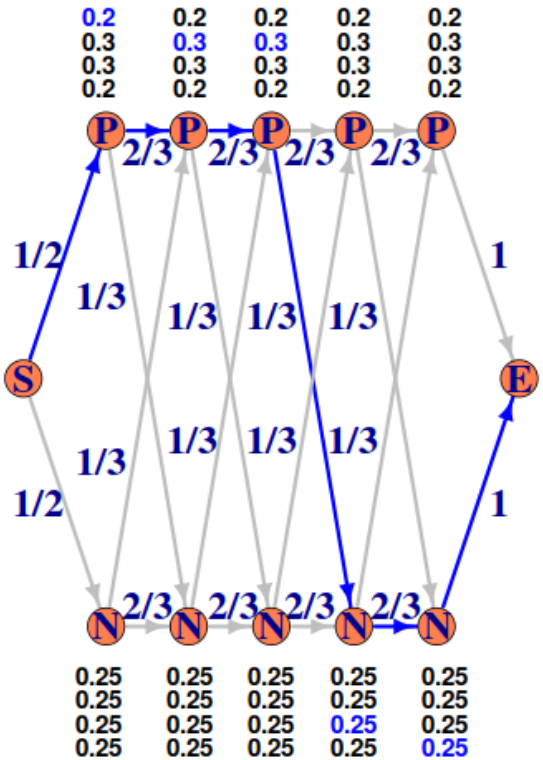
Hidden Markov Models (EG Ch. 12, MM Ch. 12)



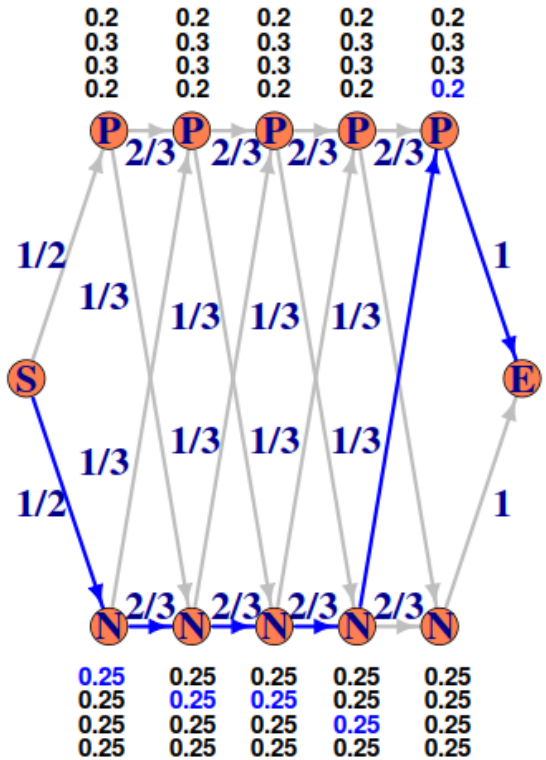
A. Coghlan, (2011) A Little Book of R For Bioinformatics
`https://a-little-book-of-r-for-bioinformatics.readthedocs.io/en/latest/` CC BY

Hidden Markov Models

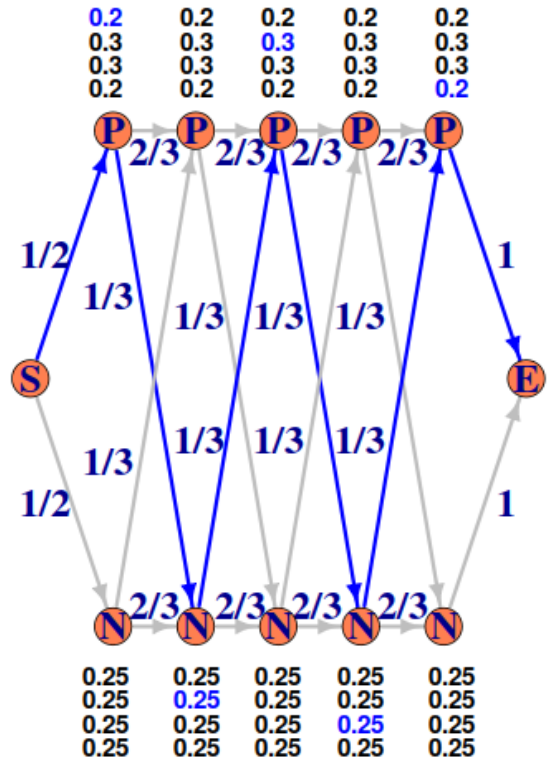
Sequence: **ACCGT**, P: protein coding, N: non–coding



$\frac{1}{2}\frac{1}{5}\frac{2}{3}\frac{3}{10}\frac{2}{3}\frac{3}{10}\frac{1}{3}\frac{1}{4}\frac{2}{3}\frac{1}{4} \approx 0.000192$



$\frac{1}{2}\frac{1}{4}\frac{2}{3}\frac{1}{4}\frac{2}{3}\frac{1}{4}\frac{2}{3}\frac{1}{4}\frac{1}{3}\frac{1}{5} \approx 3.858 \cdot 10^{-5}$



$\frac{1}{2}\frac{1}{5}\frac{1}{3}\frac{1}{4}\frac{1}{3}\frac{3}{10}\frac{1}{3}\frac{1}{4}\frac{1}{3}\frac{1}{5} \approx 4.63 \cdot 10^{-6}$

Hidden Markov Models

Observed outputs: $\mathcal{O} = \mathcal{O}_1, \ldots, \mathcal{O}_T$

Hidden sequence: $Q = q_1, \ldots, q_T$

Values of hidden states: $S_1, \ldots, S_N$

Model parameters: λ

Chain homogeneous in time, i.e. in $1, \ldots, T$

Notation:

$\pi_i = P(q_1 = S_i)$
$b_i(a) = P(S_i \text{ emits } a)$
p_{ij}: transition probability of hidden chain

Hidden Markov Models

Aims:

1. find $P(\mathcal{O}|\lambda)$
2. find hidden sequence Q, i.e.

$$\arg\max_{Q} P(Q|\mathcal{O})$$

3. find λ, *heuristic*: Baum–Welch algorithm

$$\arg\max_{\lambda} P(\mathcal{O}|\lambda)$$

Number of possible paths make exact algorithm computationally impossible.

The likelihood, forward algorithm $O(TN^2)$, (EG p. 411)

$$\alpha(t,i) := P(\mathcal{O}_1,\ldots,\mathcal{O}_t, q_t = S_i)$$

$$P(\mathcal{O}) = \sum_{i=1}^{N} \alpha(T,i)$$

initialization: $\alpha(1,i) = P(q_1 = S_i)P(S_i \text{ emits } \mathcal{O}_1) = \pi_i b_i(\mathcal{O}_1)$
induction:

$$\alpha(t+1,i) = \sum_{j=1}^{N} \alpha(t,j) p_{ji} b_i(\mathcal{O}_{t+1})$$

because

$$\alpha(t+1,i) = \sum_{j=1}^{N} P(\mathcal{O}_1,\ldots,\mathcal{O}_{t+1}, q_{t+1} = S_i, q_t = S_j)$$

remember: $p_{ji} = P(S_j \to S_i)$

The likelihood, backward algorithm, (EG p. 412)

Aim: calculate

$$\beta(t,i) := P(\mathcal{O}_{t+1},\ldots,\mathcal{O}_T|q_t = S_i)$$

initialization: $\beta(T,j) \equiv 1$ for all j

recursion:

$$\beta(t-1,i) = \sum_{j=1}^{N} p_{ij} b_j(\mathcal{O}_t)\beta(t,j)$$

Viterbi algorithm $O(TN^2)$, (EG p. 413)

$$\delta_t(i) = \max_{q_1,\ldots,q_{t-1}} P(q_1, q_2, \ldots, q_{t-1}, q_t = S_i, \mathcal{O}_1, \ldots, \mathcal{O}_t)$$

$\delta_t(i)$: max. probability to end in state S_i and time t with observations $\mathcal{O}$

initialization: $\delta_1(i) = \pi_i b_i(\mathcal{O}_1)$
induction: for $2 \leq t \leq T$, $1 \leq j \leq N$

$$\delta_t(j) = \max_{1\leq i\leq N} \delta_{t-1}(i) p_{ij} b_j(\mathcal{O}_t)$$

Recover hidden states:

$$\psi_T := \arg\max_{1\leq i\leq N} \delta_T(i), \text{ put } q_T = S_{\psi_T}$$

and for $t \leq T-1$

$$\psi_t := \arg\max_{1\leq i\leq N} \delta_t(i) p_{i\psi_{t+1}} \text{ and put } q_t = S_{\psi_t}$$

Baum–Welch algorithm, (EG p. 414, MM p. 324)

Data: multiple sequences $\{\mathcal{O}^{(d)}\} = \{\mathcal{O}^{(1)}, \ldots, \mathcal{O}^{(n)}\}$

Initialize: π_i, p_{jk}, $b_i(a)$ at some value

Update

$\bar{\pi}_i$: as expected proportion of times $q_1 = S_i$ given $\{\mathcal{O}^{(d)}\}$

$\bar{p}_{jk} = \mathrm{E}\left[N_{jk}|\{\mathcal{O}^{(d)}\}\right] / \mathrm{E}\left[N_j|\{\mathcal{O}^{(d)}\}\right]$

$\bar{b}_i(a) = \mathrm{E}\left[N_i(a)|\{\mathcal{O}^{(d)}\}\right] / \mathrm{E}\left[N_i|\{\mathcal{O}^{(d)}\}\right]$

where

N_{jk}: (random) number of transitions $q_\cdot = S_j$ to $q_{\cdot+1} = S_k$

N_i: (random) number of $q_\cdot = S_i$

$N_i(a)$: (random) number of emissions of a by S_i

for a **single random** sequence

Iterate until convergence

Exact computational details: EG p. 415, 416

Questions?