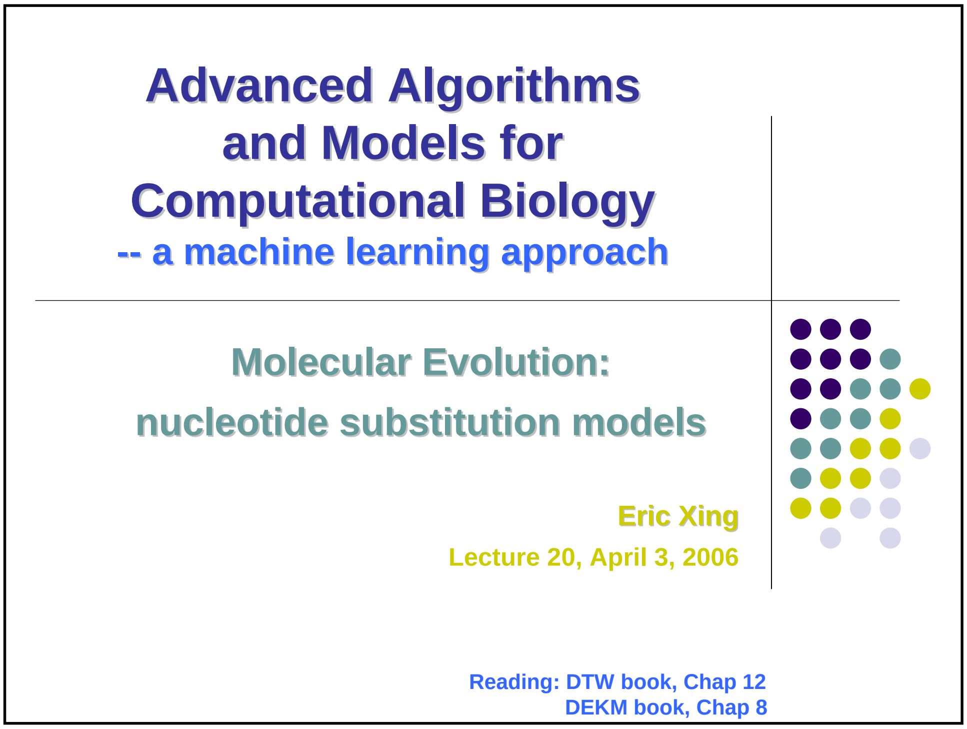


Advanced Algorithms and Models for Computational Biology

-- a machine learning approach

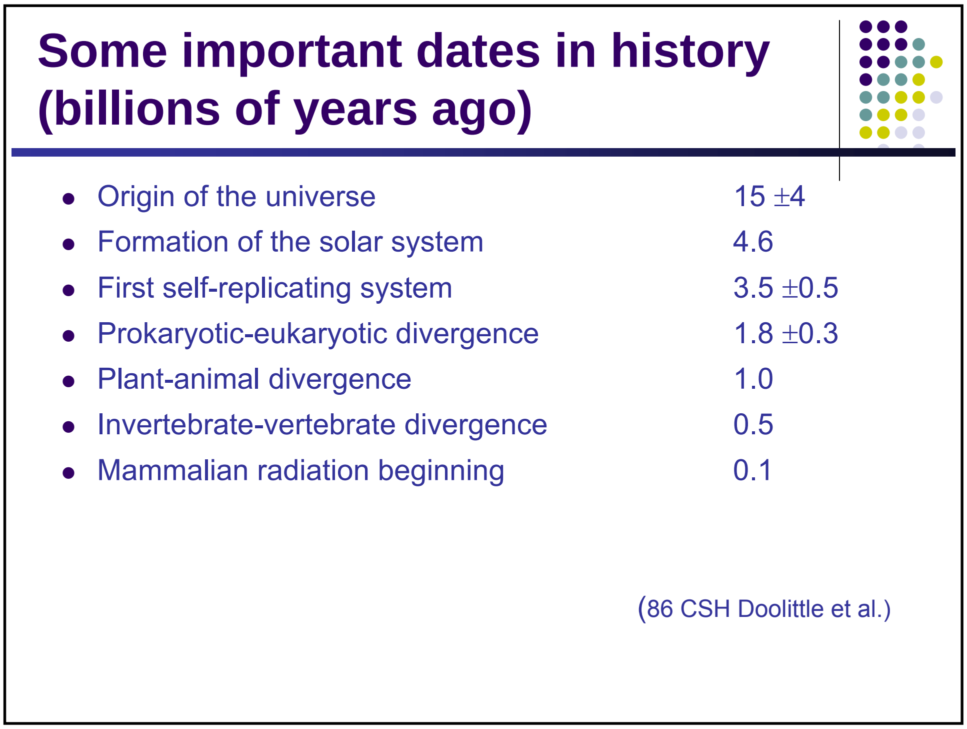
Molecular Evolution:

nucleotide substitution models

Eric Xing

Lecture 20, April 3, 2006

Reading: DTW book, Chap 12
DEKM book, Chap 8

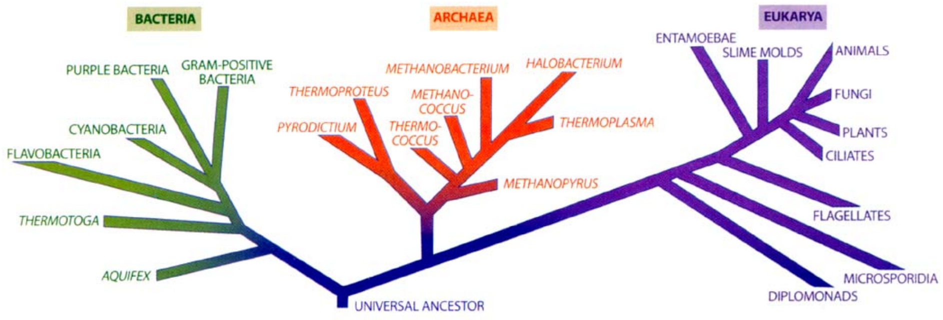


Some important dates in history (billions of years ago)

Origin of the universe	15 ±4
Formation of the solar system	4.6
First self-replicating system	3.5 ±0.5
Prokaryotic-eukaryotic divergence	1.8 ±0.3
Plant-animal divergence	1.0
Invertebrate-vertebrate divergence	0.5
Mammalian radiation beginning	0.1

(86 CSH Doolittle et al.)

The three kingdoms

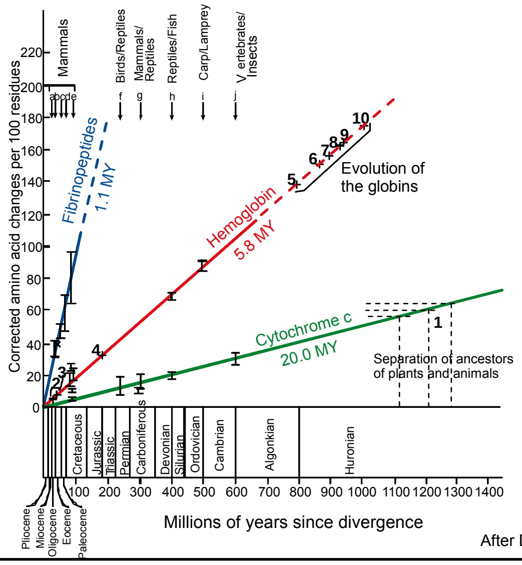


M. Madigan and B. Marrs, 1997

Two important early observations

- Different proteins evolve at different rates, and this seems more or less independent of the host organism, including its generation time.
- It is necessary to adjust the observed percent difference between two homologous proteins to get a distance more or less linearly related to the time since their common ancestor. (Later we offer a rational basis for doing this.)
- A striking early version of these observations is next.

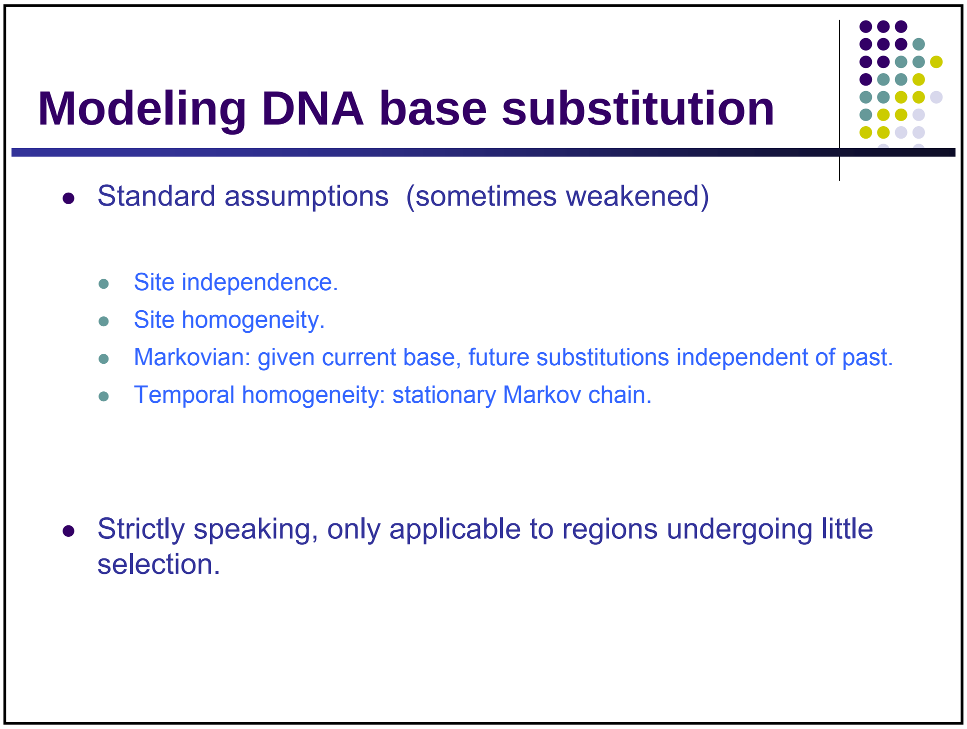
Rates of macromolecular evolution



After Dickerson (1971)

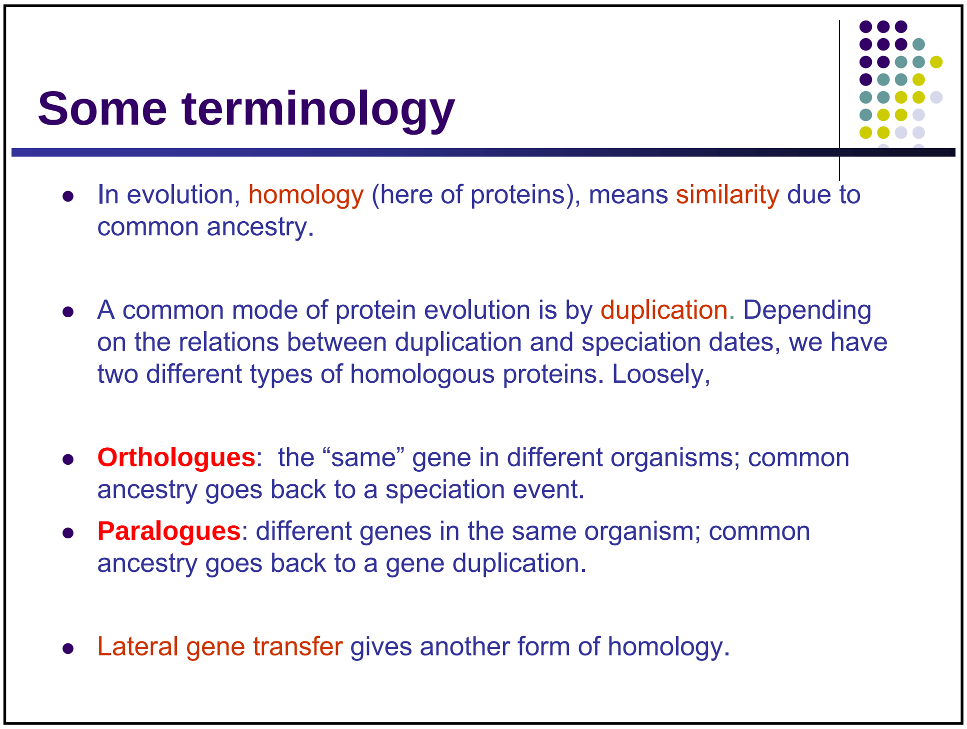
How does sequence variation arise?

- **Mutation**:
  - (a) Inherent: DNA replication errors are not always corrected.
  - (b) External: exposure to chemicals and radiation.
- **Selection**: Deleterious mutations are removed quickly. Neutral and rarely, advantageous mutations, are tolerated and stick around.
- **Fixation**: It takes time for a new variant to be established (having a stable frequency) in a population.



Modeling DNA base substitution

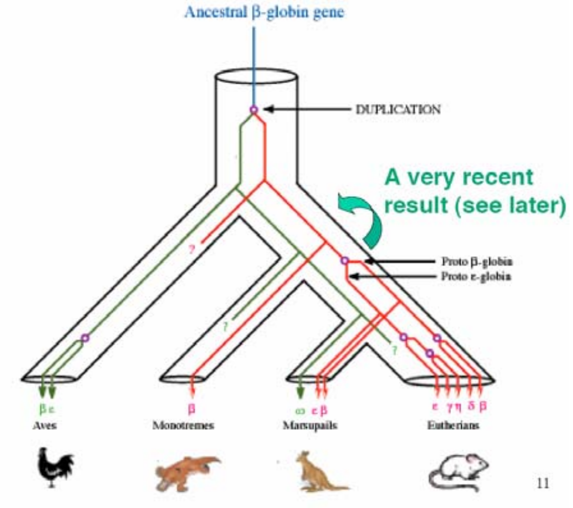
- Standard assumptions (sometimes weakened)
  - Site independence.
  - Site homogeneity.
  - Markovian: given current base, future substitutions independent of past.
  - Temporal homogeneity: stationary Markov chain.
- Strictly speaking, only applicable to regions undergoing little selection.



Some terminology

- In evolution, homology (here of proteins), means similarity due to common ancestry.
- A common mode of protein evolution is by duplication. Depending on the relations between duplication and speciation dates, we have two different types of homologous proteins. Loosely,
- **Orthologues**: the “same” gene in different organisms; common ancestry goes back to a speciation event.
- **Paralogues**: different genes in the same organism; common ancestry goes back to a gene duplication.
- Lateral gene transfer gives another form of homology.

Speciation vs. duplication



Beta-globins (orthologues)

```
                               10                  20                  30                  40
BG-human      M V H L T P E E K S A V T A L W G K V N V D E V G G E A L G R L L V V Y P W T Q
BG-macaque    - . . . . . . . . N . . . T . . . . . . . . . . . . . . . . . . . . . . . . . .
BG-bovine     - - M . . A . . . A . . . . F . . . . K . . . . . . . . . . . . . . . . . . . .
BG-platypus   - . . . . S G G . . . . . N . . . . . . I N . L . . . . . . . . . . . . . . . .
BG-chicken    . . . W . A . . . Q L I . G . . . . . . . A . C . A . . . A . . . I . . . . . .
BG-shark      - . . W S E V . L H E I . T T . K S I D K H S L . A K . . A . M F I . . . . . T

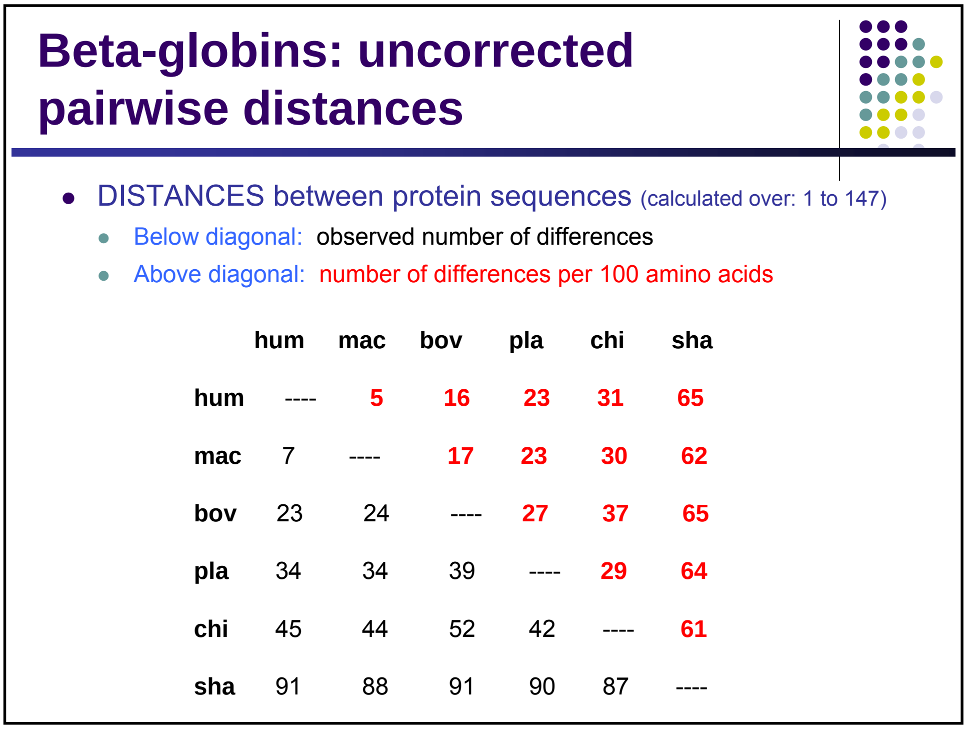
                               50                  60                  70                  80
BG-human      R F F E S F G D L S T P D A V M G N P K V K A H G K K V L G A F S D G L A H L D
BG-macaque    . . . . . . . . . . S . . . . . . . . . . . . . . . . . . . . . . . . . N . . .
BG-bovine     . . . . . . . . . . . A . . . . N . . . . . . . . . . . . D S . . N . M K . . .
BG-platypus   . . . . A . . . . . S A G . . . . . . . . . . . . A . . . T S . G . A . K N . .
BG-chicken    . . . A . . . N . . S . T . I L . . . M . R . . . . . . . T S . G . A V K N . .
BG-shark      . Y . G N L K E F T A C S Y G - - - - - . . E . A . . . T . . L G V A V T . . G

                               90                  100                 110                 120
BG-human      N L K G T F A T L S E L H C D K L H V D P E N F R L L G N V L V C V L A H H F G
BG-macaque    . . . . . . . Q . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . .
BG-bovine     D . . . . . . A . . . . . . . . . . . . . . . . K . . . . . . . V . . . R N . .
BG-platypus   D . . . . . . K . . . . . . . . . . . . . . . . N R . . . . . I V . . . R . . S
BG-chicken    . I . N . . S Q . . . . . . . . . . . . . . . . . . . . D I . I I . . . A . . S
BG-shark      D V . S Q . T D . . K K . A E E . . . . V . S . K . . A K C F . V E . G I L L K

                               130                 140
BG-human      K E F T P P V Q A A Y Q K V V A G V A N A L A H K Y H
BG-macaque    . . . . . Q . . . . . . . . . . . . . . . . . . . . .
BG-bovine     . . . . . V L . . D F . . . . . . . . . . . . . R . .
BG-platypus   . D . S . E . . . . W . . L . S . . . H . . G . . . .
BG-chicken    . D . . . E C . . . W . . L . R V . . H . . . R . . .
BG-shark      D K . A . Q T . . I W E . Y F G V . V D . I S K E . .
```

. means same as reference sequence

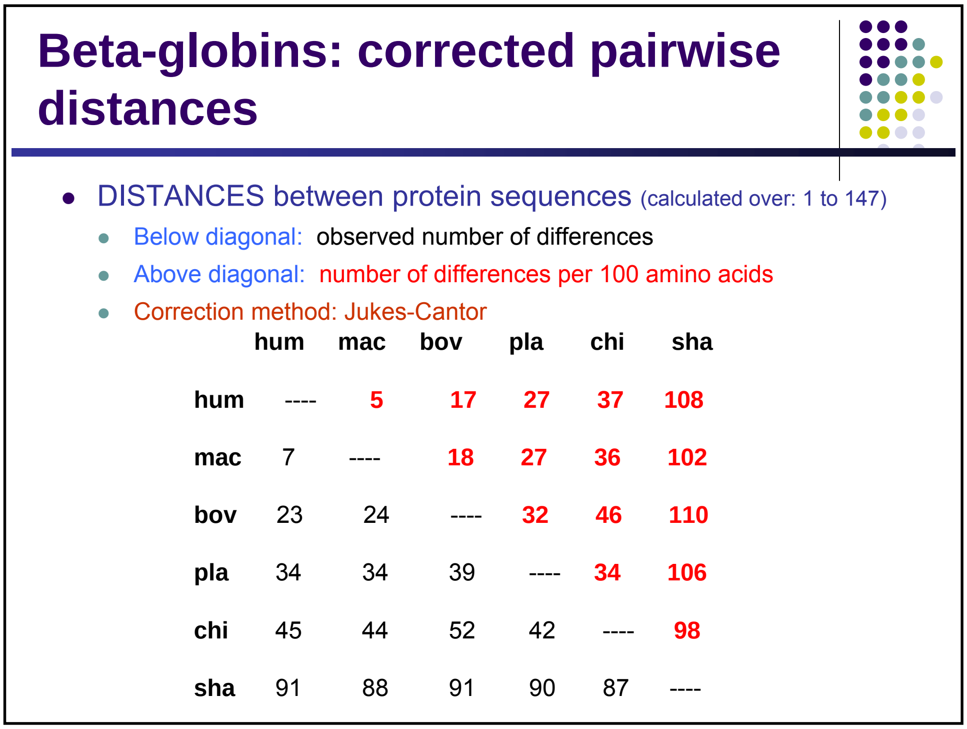
- means deletion



Beta-globins: uncorrected pairwise distances

- DISTANCES between protein sequences (calculated over: 1 to 147)
  - Below diagonal: observed number of differences
  - Above diagonal: number of differences per 100 amino acids

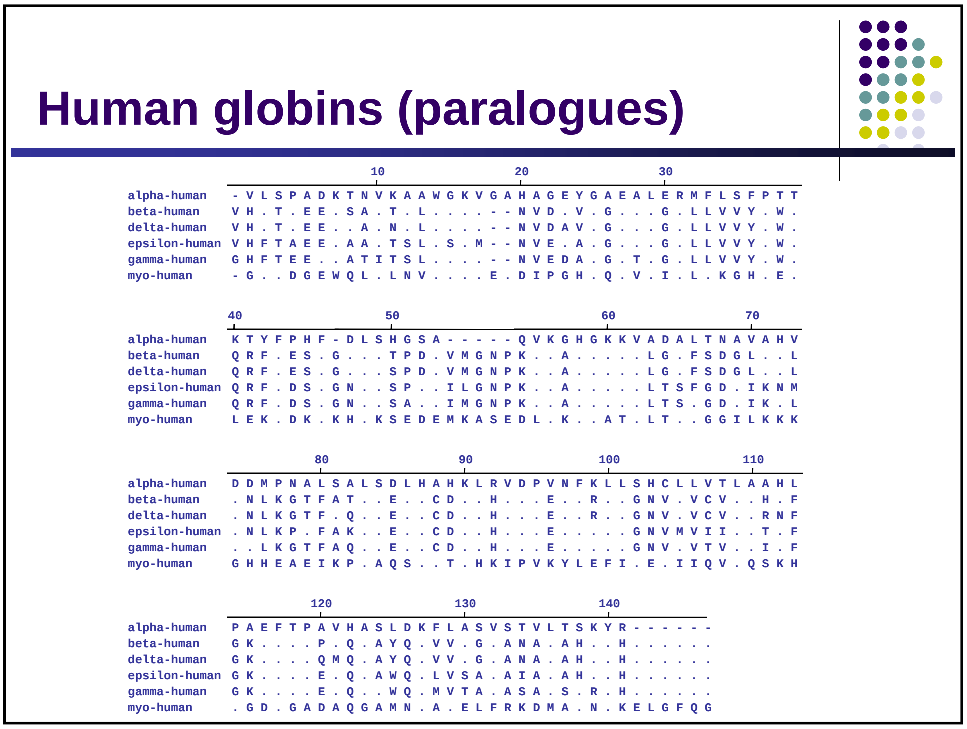
	hum	mac	bov	pla	chi	sha
hum	----	**5**	**16**	**23**	**31**	**65**
mac	7	----	**17**	**23**	**30**	**62**
bov	23	24	----	**27**	**37**	**65**
pla	34	34	39	----	**29**	**64**
chi	45	44	52	42	----	**61**
sha	91	88	91	90	87	----



Beta-globins: corrected pairwise distances

- DISTANCES between protein sequences (calculated over: 1 to 147)
  - Below diagonal: observed number of differences
  - Above diagonal: number of differences per 100 amino acids
  - Correction method: Jukes-Cantor

	hum	mac	bov	pla	chi	sha
hum	----	**5**	**17**	**27**	**37**	**108**
mac	7	----	**18**	**27**	**36**	**102**
bov	23	24	----	**32**	**46**	**110**
pla	34	34	39	----	**34**	**106**
chi	45	44	52	42	----	**98**
sha	91	88	91	90	87	----



Human globins (paralogues)

```
                                 10                  20                  30
alpha-human   - V L S P A D K T N V K A A W G K V G A H A G E Y G A E A L E R M F L S F P T T
beta-human    V H . T . E E . S A . T . L . . . . - - N V D . V . G . . . G . L L V V Y . W .
delta-human   V H . T . E E . . A . N . L . . . . - - N V D A V . G . . . G . L L V V Y . W .
epsilon-human V H F T A E E . A A . T S L . S . M - - N V E . A . G . . . G . L L V V Y . W .
gamma-human   G H F T E E . . A T I T S L . . . . - - N V E D A . G . T . G . L L V V Y . W .
myo-human     - G . . D G E W Q L . L N V . . . . E . D I P G H . Q . V . I . L . K G H . E .

              40                   50                            60                  70
alpha-human   K T Y F P H F - D L S H G S A - - - - - Q V K G H G K K V A D A L T N A V A H V
beta-human    Q R F . E S . G . . . T P D . V M G N P K . . A . . . . . L G . F S D G L . . L
delta-human   Q R F . E S . G . . . S P D . V M G N P K . . A . . . . . L G . F S D G L . . L
epsilon-human Q R F . D S . G N . . S P . . I L G N P K . . A . . . . . L T S F G D . I K N M
gamma-human   Q R F . D S . G N . . S A . . I M G N P K . . A . . . . . L T S . G D . I K . L
myo-human     L E K . D K . K H . K S E D E M K A S E D L . K . . A T . L T . . G G I L K K K

                         80                  90                  100                 110
alpha-human   D D M P N A L S A L S D L H A H K L R V D P V N F K L L S H C L L V T L A A H L
beta-human    . N L K G T F A T . . E . . C D . . H . . . E . . R . . G N V . V C V . . H . F
delta-human   . N L K G T F . Q . . E . . C D . . H . . . E . . R . . G N V . V C V . . R N F
epsilon-human . N L K P . F A K . . E . . C D . . H . . . E . . . . . G N V M V I I . . T . F
gamma-human   . . L K G T F A Q . . E . . C D . . H . . . E . . . . . G N V . V T V . . I . F
myo-human     G H H E A E I K P . A Q S . . T . H K I P V K Y L E F I . E . I I Q V . Q S K H

                         120                 130                 140
alpha-human   P A E F T P A V H A S L D K F L A S V S T V L T S K Y R - - - - - -
beta-human    G K . . . . P . Q . A Y Q . V V . G . A N A . A H . . H . . . . . .
delta-human   G K . . . . Q M Q . A Y Q . V V . G . A N A . A H . . H . . . . . .
epsilon-human G K . . . . E . Q . A W Q . L V S A . A I A . A H . . H . . . . . .
gamma-human   G K . . . . E . Q . . W Q . M V T A . A S A . S . R . H . . . . . .
myo-human     . G D . G A D A Q G A M N . A . E L F R K D M A . N . K E L G F Q G
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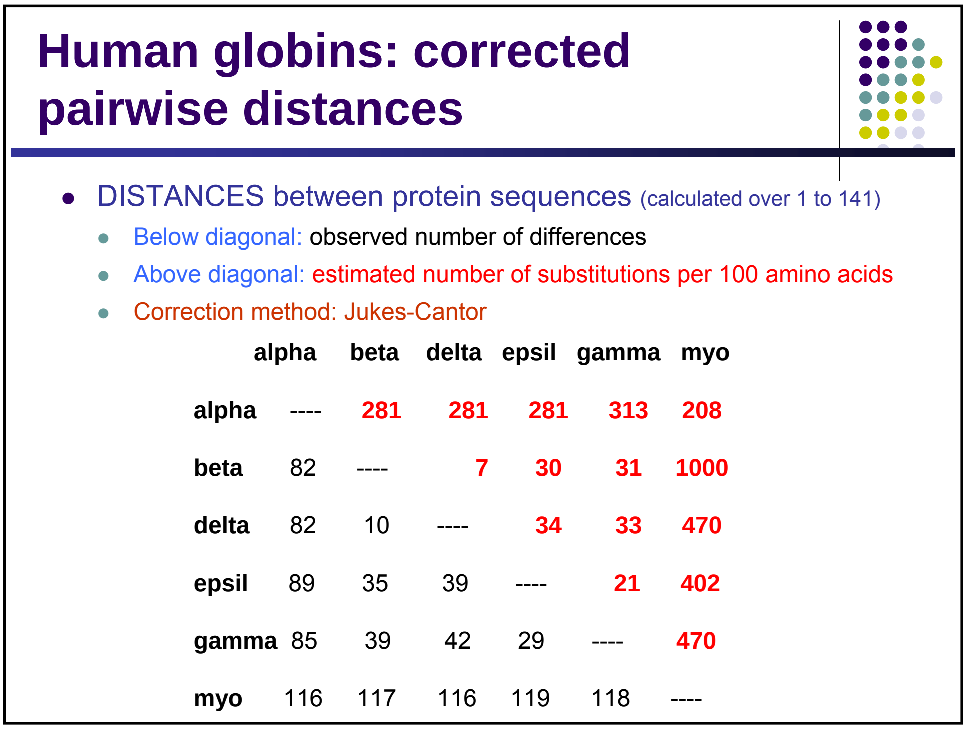


Human globins: corrected pairwise distances

- DISTANCES between protein sequences (calculated over 1 to 141)
  - Below diagonal: observed number of differences
  - Above diagonal: estimated number of substitutions per 100 amino acids
  - Correction method: Jukes-Cantor

	alpha	**beta**	**delta**	**epsil**	**gamma**	**myo**
alpha	----	**281**	**281**	**281**	**313**	**208**
beta	82	----	**7**	**30**	**31**	**1000**
delta	82	10	----	**34**	**33**	**470**
epsil	89	35	39	----	**21**	**402**
gamma	85	39	42	29	----	**470**
myo	116	117	116	119	118	----

Correcting distances between DNA and protein sequences

- Why it is necessary to adjust observed percent differences to get a distance measure which scales linearly with time?
- This is because we can have multiple and back substitutions at a given position along a lineage.
- All of the correction methods (with names like Jukes-Cantor, 2-parameter Kimura, etc) are justified by simple probabilistic arguments involving Markov chains whose basis is worth mastering.
- The same molecular evolutionary models can be used in scoring sequence alignments.

Markov chain

- State space = {A,C,G,T}.

  p(i,j) = pr(next state S_j | current state S_i)

- Markov assumption:

  p(next state S_j | current state S_i & any configuration of states before this) = p(i,j)

  Only the *present* state, not previous states, affects the probs of moving to next states.

The multiplication rule

pr(state after next is S_k | current state is S_i)

= $\sum_j$ *pr*(state after next is S_k, next state is S_j | current state is S_i) [*addition rule*]

= $\sum_j$ *pr*(next state is S_j| current state is S_i) *x* *pr*(state after next is S_k | current state is S_i, next state is S_j) [*multiplication rule*]

= $\sum_j p_{i,j} \; x \; p_{j,k}$ [*Markov assumption*]

= (*i*,*k*)-element of P^2, where $P=(p_{i,j})$.

More generally,

pr(state t steps from now is S_k | current state is S_i) = *i*,*k* element of P^t

Continuous-time version

- For any (*s*, *t*):
  - Let $p_{ij}(t) = pr(S_j$ *at time t+s* | S_i *at time s*) denote the stationary (time-homogeneous) transition probabilities.
- Let $P(t) = (p_{ij}(t))$ denote the matrix of $p_{ij}(t)$'s.
  - Then for any (*t*, *u*): $P(t+u) = P(t)\,P(u)$.
- It follows that $P(t) = exp(Qt)$, where $Q = P'(0)$ (the derivative of *P(t)* at $t = 0$).
- *Q* is called the infinitesimal matrix (transition rate matrix) of *P*(*t*), and satisfies

$$P'(t) = QP(t) = P(t)Q.$$

- Important approximation: when t is small,

$$P(t) \approx I + Qt.$$

Interpretation of Q

- Roughly, q_{ij} is the **rate** of transitions of *i* to *j*, while $q_{ii} = -\Sigma_{j\neq i}\, q_{ij}$, so each row sum is *0* (Why?).
- Now we have the short-time approximation:

$$p_{i\neq j}(t+h) = q_{ij}h + o(h) \qquad p_{i=j}(t+h) = 1 + q_{ii}h + o(h)$$

where $p_{ij}(t+h)$ is the probability of transitioning from i at time t to j at time $t+h$

- Now consider the Chapman-Kolmogorov relation: (assuming we have a continuous-time Markov chain, and let $p_j(t) = pr(S_j \text{ at time } t)$)

$$\begin{aligned} p_j(t+h) &= \sum_i pr(S_i \text{ at } t, S_j \text{ at } t+h) \\ &= \sum_i pr(S_i \text{ at } t)\, pr(S_i \text{ at } t+h \mid S_j \text{ at } t) \\ &= p_j(t)\times(1+q_{jj}h) + \sum_{i\neq j} p_i(t)\times hq_{ij} \end{aligned}$$

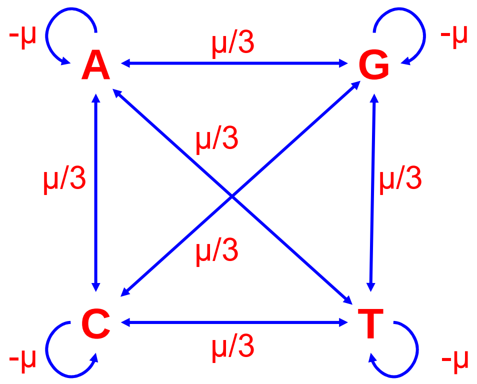
i.e., $h^{-1}\left(p_j(t+h) - p_j(t)\right) = p_j(t)q_{jj} + \sum_{i\neq j} p_i(t)q_{ij}$, which becomes: $P' = QP$ as $h\downarrow 0$.

Probabilistic models for DNA changes

Orc:	ACAGTGACGCCCCAAACGT
Elf:	ACAGTGACGCTACAAACGT
Dwarf:	CCTGTGACGTAACAAACGA
Hobbit:	CCTGTGACGTAGCAAACGA
Human:	CCTGTGACGTAGCAAACGA

The Jukes-Cantor model (1969)

- **Substitution rate:**



the simplest symmetrical model for DNA evolution

Transition probabilities under the Jukes-Cantor model

- IID assumption:
  - All sites change independently
  - All sites have the same stochastic process working at them
- Equiprobablity assumption:
  - Make up a fictional kind of event, such that when it happens the site changes to one of the 4 bases chosen at random equiprobably
- Equilibrium condition:
  - No matter how many of these fictional events occur, provided it is not zero, the chance of ending up at a particular base is 1/4 .
- Solving differentially equation system *P’ = QP*

Transition probabilities under the Jukes-Cantor model (cont.)

- **Prob transition matrix:**

$$P(t) = \begin{array}{c|cccc} & A & C & G & T \\ \hline A & r(t) & s(t) & s(t) & s(t) \\ C & s(t) & r(t) & s(t) & s(t) \\ G & s(t) & s(t) & r(t) & s(t) \\ T & s(t) & s(t) & s(t) & r(t) \end{array}$$

Where we can derive:

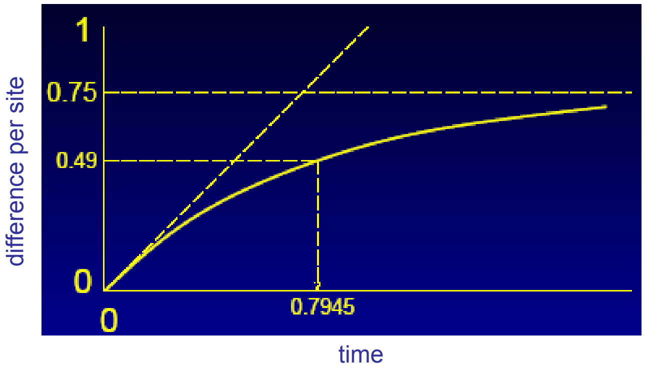
$$r(t) = \frac{1}{4}\left(1 + 3e^{-\frac{4}{3}\mu t}\right)$$

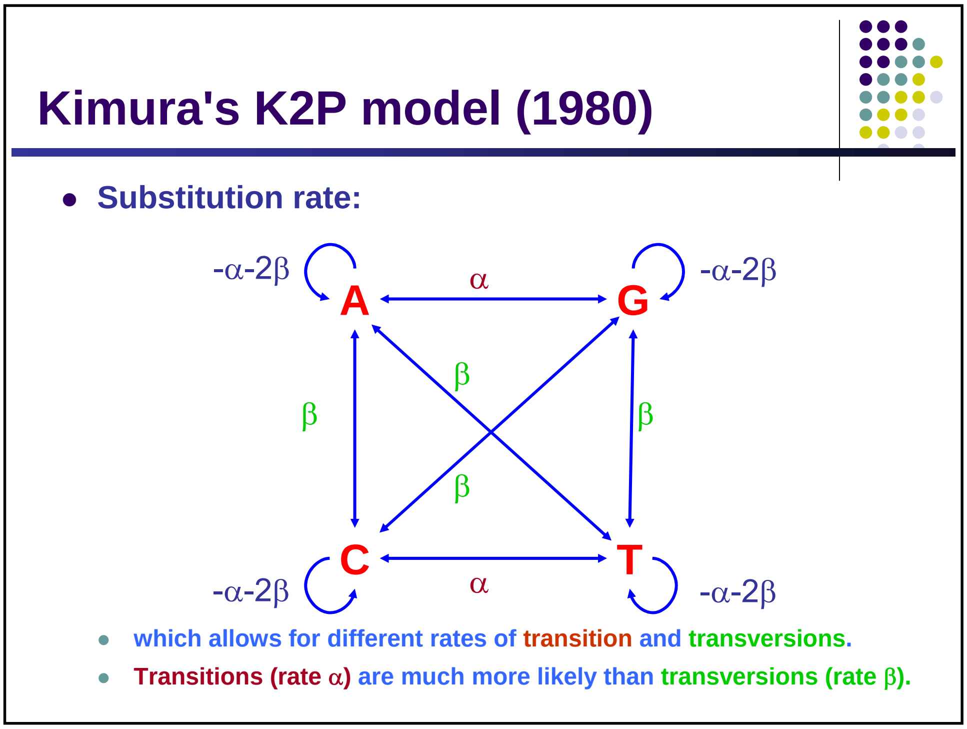
$$s(t) = \frac{1}{4}\left(1 - e^{-\frac{4}{3}\mu t}\right)$$

Homework!

Jukes-Cantor (cont.)

- **Fraction of sites differences**



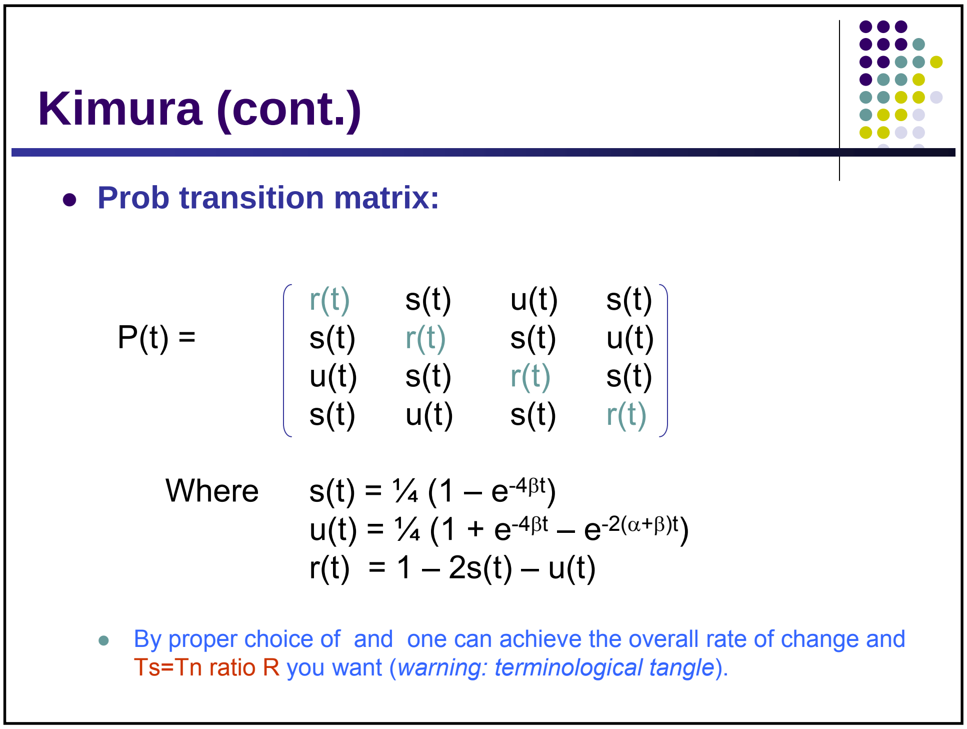


Kimura's K2P model (1980)

- Substitution rate:

-α-2β
α
-α-2β
A
G
β
β
β
β
C
T
-α-2β
α
-α-2β

- which allows for different rates of transition and transversions.
- Transitions (rate α) are much more likely than transversions (rate β).



Kimura (cont.)

- Prob transition matrix:

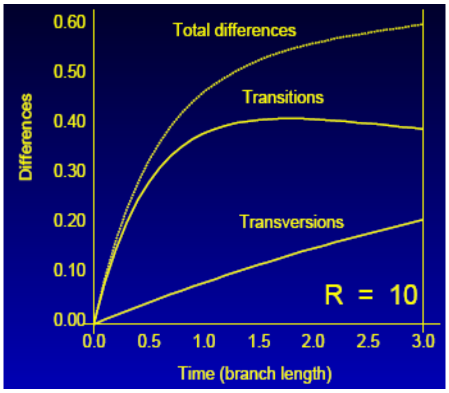
$$P(t) = \begin{pmatrix} r(t) & s(t) & u(t) & s(t) \\ s(t) & r(t) & s(t) & u(t) \\ u(t) & s(t) & r(t) & s(t) \\ s(t) & u(t) & s(t) & r(t) \end{pmatrix}$$

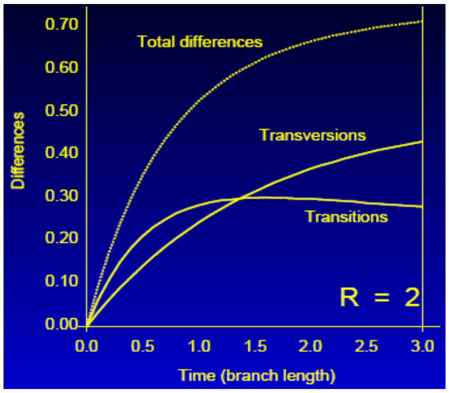
Where
$$s(t) = \tfrac{1}{4}(1 - e^{-4\beta t})$$
$$u(t) = \tfrac{1}{4}(1 + e^{-4\beta t} - e^{-2(\alpha+\beta)t})$$
$$r(t) = 1 - 2s(t) - u(t)$$

- By proper choice of and one can achieve the overall rate of change and Ts=Tn ratio R you want (*warning: terminological tangle*).

Kimura (cont.)

- Transitions, transversions expected under different R:

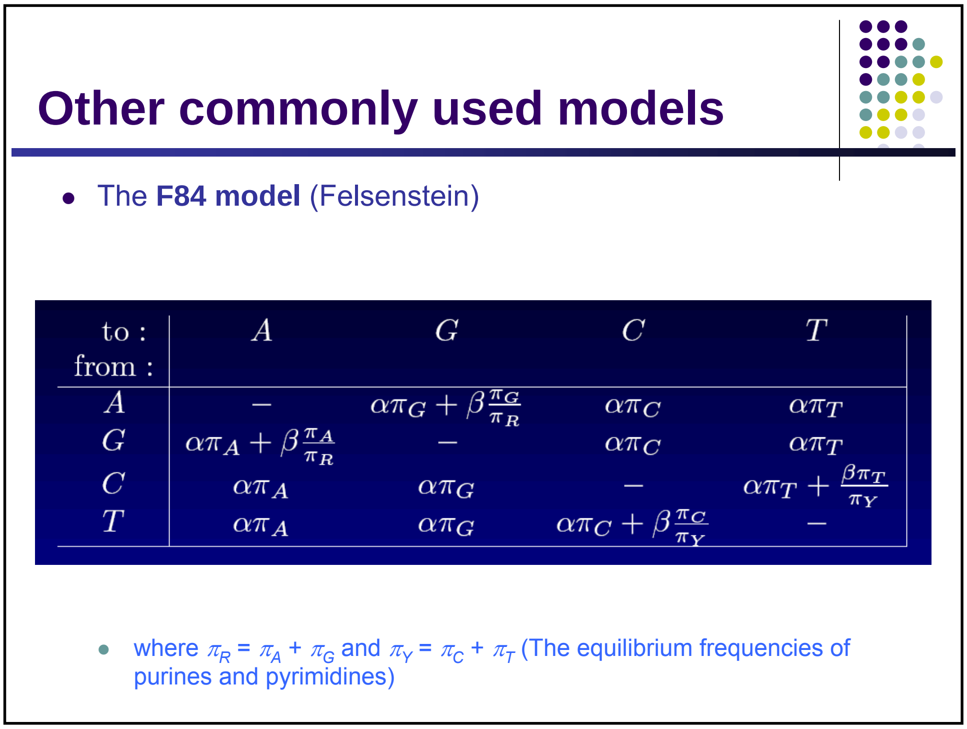




Other commonly used models

- Two models that specify the equilibrium base frequencies (you provide the frequencies A; C; G; T and they are set up to have an equilibrium which achieves them), and also let you control the transition/transversion ratio:
- The **Hasegawa-Kishino-Yano (1985) model**:

to : from :	A	G	C	T
A	$-$	$\alpha\pi_G + \beta\pi_G$	$\alpha\pi_C$	$\alpha\pi_T$
G	$\alpha\pi_A + \beta\pi_A$	$-$	$\alpha\pi_C$	$\alpha\pi_T$
C	$\alpha\pi_A$	$\alpha\pi_G$	$-$	$\alpha\pi_T + \beta\pi_T$
T	$\alpha\pi_A$	$\alpha\pi_G$	$\alpha\pi_C + \beta\pi_C$	$-$

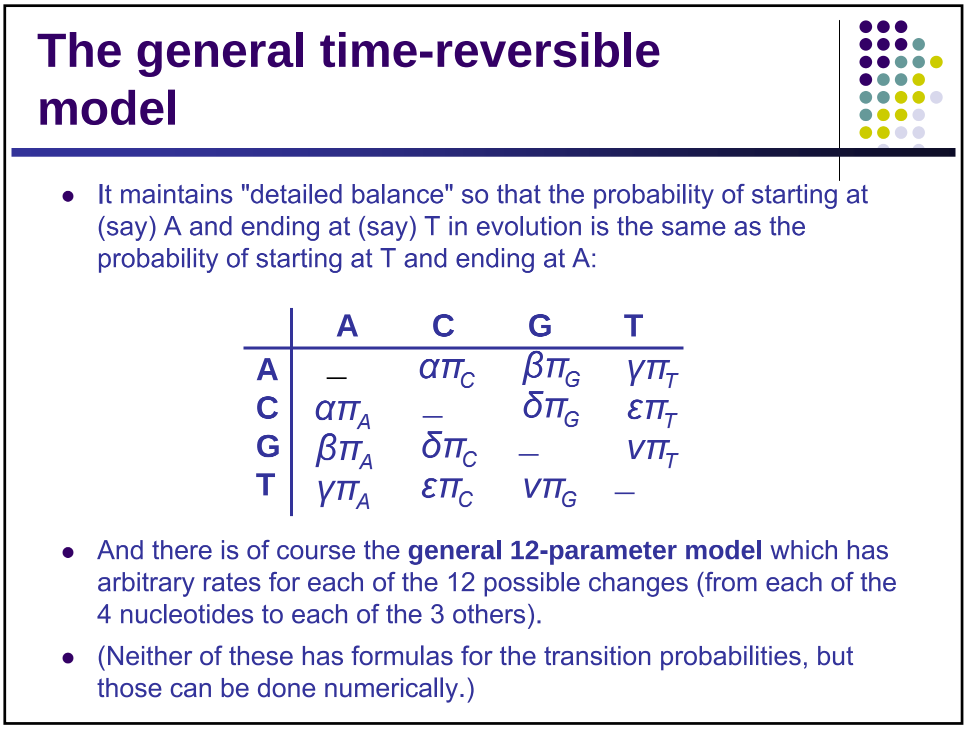


Other commonly used models

- The **F84 model** (Felsenstein)

to : from :	A	G	C	T
A	$-$	$\alpha\pi_G + \beta\frac{\pi_G}{\pi_R}$	$\alpha\pi_C$	$\alpha\pi_T$
G	$\alpha\pi_A + \beta\frac{\pi_A}{\pi_R}$	$-$	$\alpha\pi_C$	$\alpha\pi_T$
C	$\alpha\pi_A$	$\alpha\pi_G$	$-$	$\alpha\pi_T + \frac{\beta\pi_T}{\pi_Y}$
T	$\alpha\pi_A$	$\alpha\pi_G$	$\alpha\pi_C + \beta\frac{\pi_C}{\pi_Y}$	$-$

  - where $\pi_R = \pi_A + \pi_G$ and $\pi_Y = \pi_C + \pi_T$ (The equilibrium frequencies of purines and pyrimidines)



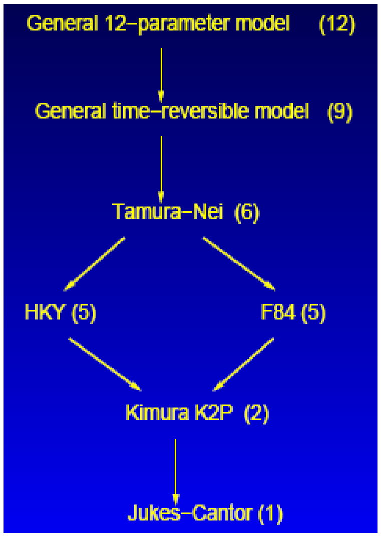
The general time-reversible model

- It maintains "detailed balance" so that the probability of starting at (say) A and ending at (say) T in evolution is the same as the probability of starting at T and ending at A:

	A	C	G	T
A	–	$\alpha\pi_C$	$\beta\pi_G$	$\gamma\pi_T$
C	$\alpha\pi_A$	–	$\delta\pi_G$	$\varepsilon\pi_T$
G	$\beta\pi_A$	$\delta\pi_C$	–	$\nu\pi_T$
T	$\gamma\pi_A$	$\varepsilon\pi_C$	$\nu\pi_G$	–

- And there is of course the **general 12-parameter model** which has arbitrary rates for each of the 12 possible changes (from each of the 4 nucleotides to each of the 3 others).
- (Neither of these has formulas for the transition probabilities, but those can be done numerically.)

Relation between models


General 12–parameter model (12)

General time–reversible model (9)

Tamura–Nei (6)

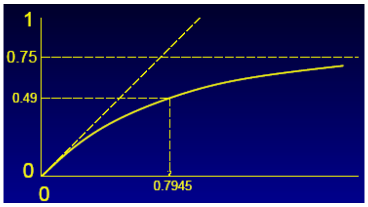
HKY (5)

F84 (5)

Kimura K2P (2)

Jukes–Cantor (1)

Adjusting evolutionary distance using base-substitution model



The Jukes-Cantor model

Common ancestor of human and orang

t time unit

Human (now)

$$Q = \begin{bmatrix} -3\alpha & \alpha & \alpha & \alpha \\ \alpha & -3\alpha & \alpha & \alpha \\ \alpha & \alpha & -3\alpha & \alpha \\ \alpha & \alpha & \alpha & -3\alpha \end{bmatrix}$$

$$P = \begin{bmatrix} r & s & s & s \\ s & r & s & s \\ s & s & r & s \\ s & s & s & r \end{bmatrix}$$

Consider e.g. the 2nd position in a-globin2 Alu1.

$r = (1+3e^{-4\alpha t})/4, \quad s = (1-e^{-4\alpha t})/4.$

Definition of PAM

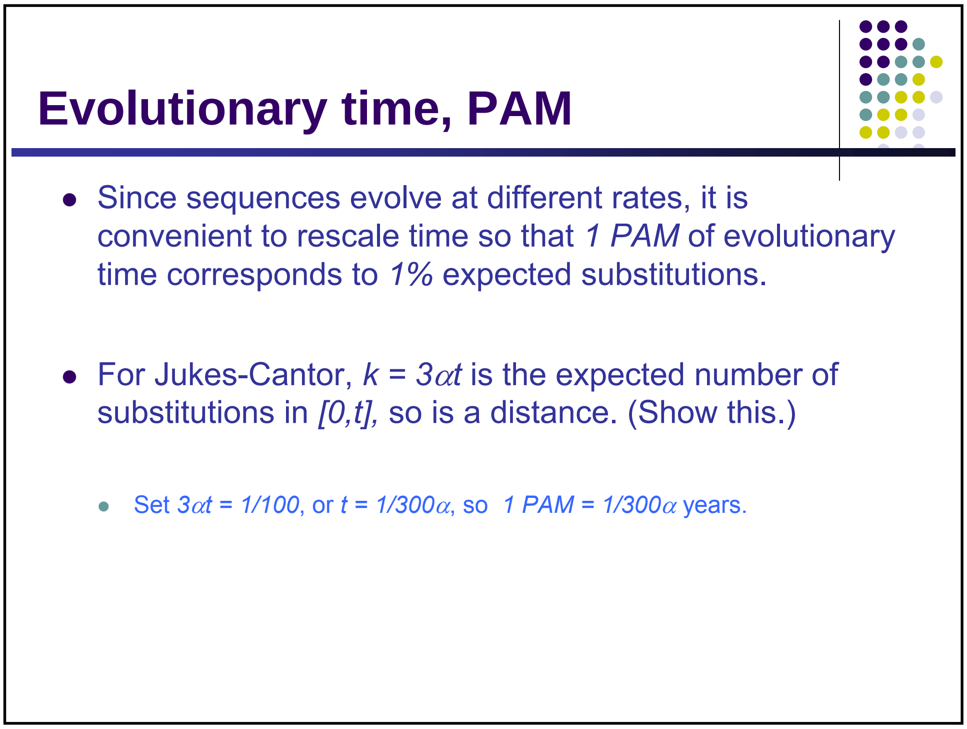
- Let $P(t) = exp(Qt)$. Then the A,G element of $P(t)$ is

$$pr(G\ now \mid A\ then) = (1 - e{-}4\alpha t)/4.$$

  - Same for all pairs of different nucleotides.
  - Overall rate of change $k = 3\alpha t$.

- ***PAM* = accepted point mutation**
  - When $k = .01$, described as *1 PAM*
  - Put $t = .01/3\alpha = 1/300\alpha$. Then the resulting $P = P(1/300\alpha)$ is called the *PAM(1)* matrix.

- Why use *PAMs?*

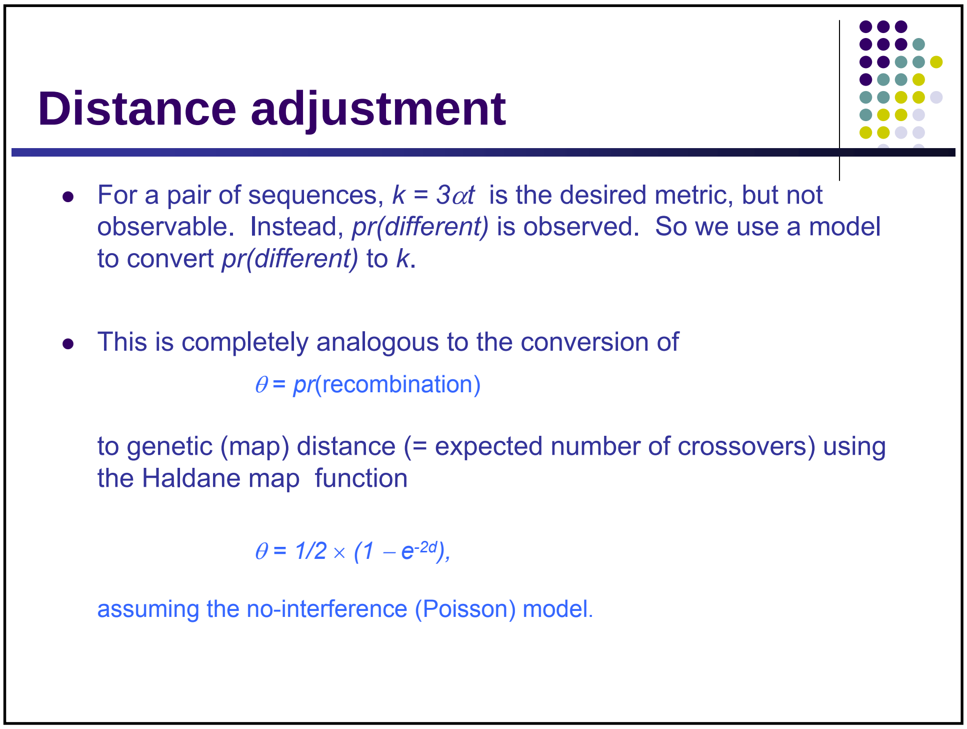


Evolutionary time, PAM

- Since sequences evolve at different rates, it is convenient to rescale time so that *1 PAM* of evolutionary time corresponds to *1%* expected substitutions.

- For Jukes-Cantor, $k = 3\alpha t$ is the expected number of substitutions in $[0,t]$, so is a distance. (Show this.)

  - Set $3\alpha t = 1/100$, or $t = 1/300\alpha$, so $1 \text{ PAM} = 1/300\alpha$ years.



Distance adjustment

- For a pair of sequences, $k = 3\alpha t$ is the desired metric, but not observable. Instead, *pr(different)* is observed. So we use a model to convert *pr(different)* to *k*.

- This is completely analogous to the conversion of

  $$\theta = pr(\text{recombination})$$

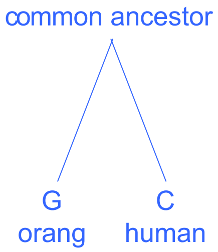
  to genetic (map) distance (= expected number of crossovers) using the Haldane map function

  $$\theta = 1/2 \times (1 - e^{-2d}),$$

  assuming the no-interference (Poisson) model.

Towards Jukes-Cantor adjustment

- E.g., 2nd position in a-globin Alu 1
- Assume that the common ancestor has A, G, C or T with probability 1/4.



common ancestor

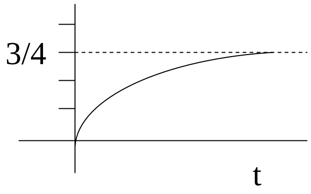
G
orang

C
human

- Then the chance of the nt differing

  $p_{\neq} = 3/4 \times (1 - e^{-8\alpha t})$

  $= 3/4 \times (1 - e^{-4k/3})$, since $k = 2 \times 3\alpha t$



Jukes-Cantor adjustment

- If we suppose all nucleotide positions behave identically and independently, and $n_{\neq}$ differ out of n, we can invert this, obtaining

$$\hat{k} = -\frac{3}{4} \times \log\left(1 - \frac{4}{3} n_{\neq} / n\right)$$

- This is the corrected or adjusted fraction of differences (under this simple model). × 100 to get PAMs
- The analogous simple model for amino acid sequences has

$$\hat{k} = -\frac{19}{20} \times \log\left(1 - \frac{20}{19} n_{\neq} / n\right)$$

× 100 for PAM.

Illustration

1. Human and bovine beta-globins are aligned with no deletions at 145 out of 147 sites. They differ at 23 of these sites. Thus $n_{\neq}/n = 23/145$, and the corrected distance using the Jukes-Cantor formula is (natural logs)

   $– 19/20 \times \log(1 – 20/19 \times 23/145) = 17.3 \times 10^{-2}$.

2. The human and gorilla sequences are aligned without gaps across all 300 bp, and differ at 14 sites. Thus $n_{\neq}/n = 14/300$, and the corrected distance using the Jukes-Cantor formula is

   $– 3/4 \times \log(1 – 4/3 \times 14/300) = 4.8 \times 10^{-2}$.

Correspondence between observed a.a. differences and the evolutionary distance (Dayhoff et al., 1978)

Observed Percent Difference	Evolutionary Distance in PAMs
1	1
5	5
10	11
15	17
20	23
25	30
30	38
35	47
40	56
45	67
50	80
55	94
60	112
65	133
70	159
75	195
80	246
85	328

Scoring matrices for alignment

How scoring matrices work

```
134 LQQGELDLVMTSDILPRSELHYSPMFDFEVRLVLAPDHPLASKTQITPEDLASETLLI
    |     |||         |       |        ||||||    |    || ||
137 LDSNSVDLVLMGVPPRNVEVEAEAFMDNPLVVIAPPDHPLAGERAISLARLAEETFVM
```

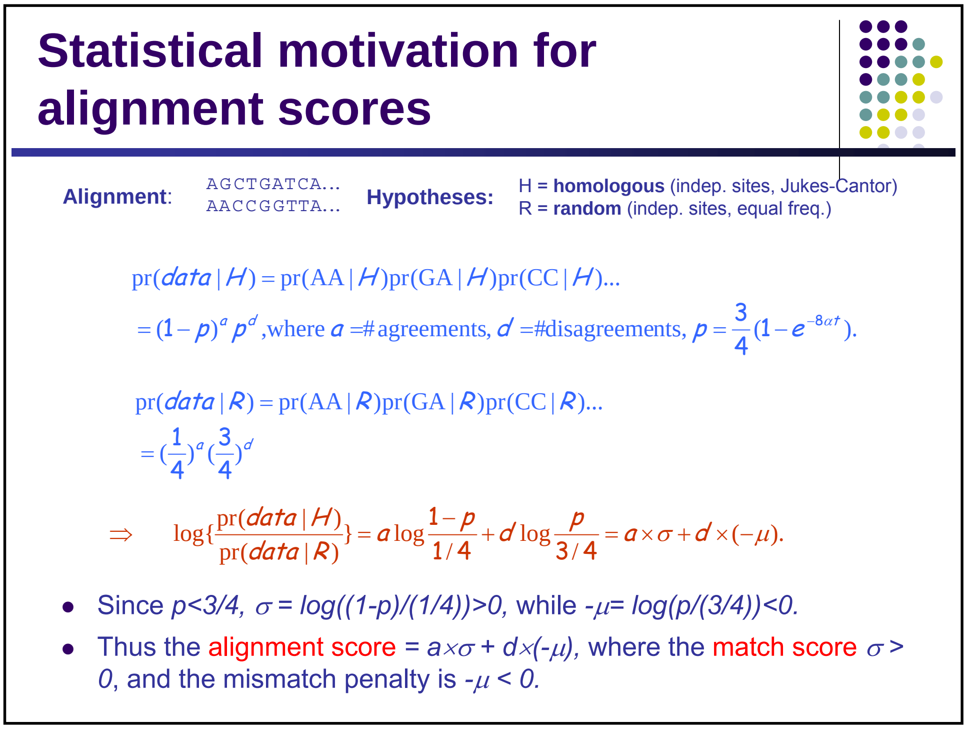
D:D = +6

D:R = -2

BLOSUM62

C	9																			
S	-1	4																		
T	-1	1	5																	
P	-3	-1	-1	7																
A	0	1	0	-1	4															
G	-3	0	-2	-2	0	6														
N	-3	1	0	-2	-2	0	6													
D	-3	0	-1	-1	-2	-1	1	6												
E	-4	0	-1	-1	-1	-2	0	2	5											
Q	-3	0	-1	-1	-1	-2	0	0	2	5										
H	-3	-1	-2	-2	-2	-2	1	-1	0	0	8									
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	5								
K	-3	0	-1	-1	-1	-2	0	-1	1	1	-1	2	5							
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	5						
I	-1	-2	-1	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	4					
L	-1	-2	-1	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	4				
V	-1	-2	0	-2	0	-3	-3	-3	-2	-2	-3	-3	-2	1	3	1	4			
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	6		
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	7	
W	-2	-3	-2	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	11
	C	**S**	**T**	**P**	**A**	**G**	**N**	**D**	**E**	**Q**	**H**	**R**	**K**	**M**	**I**	**L**	**V**	**F**	**Y**	**W**

From Henikoff 1996



Statistical motivation for alignment scores

Alignment: AGCTGATCA... AACCGGTTA... **Hypotheses:** H = **homologous** (indep. sites, Jukes-Cantor) R = **random** (indep. sites, equal freq.)

$$\mathrm{pr}(data \mid H) = \mathrm{pr}(\mathrm{AA} \mid H)\mathrm{pr}(\mathrm{GA} \mid H)\mathrm{pr}(\mathrm{CC} \mid H)\ldots$$

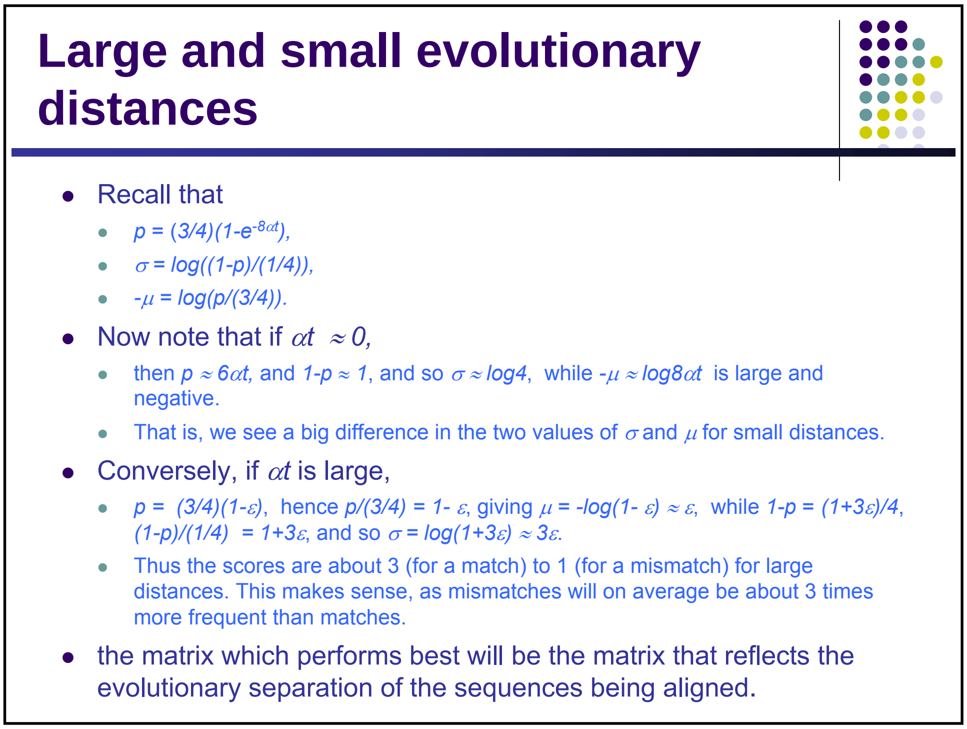
$$= (1-p)^a p^d \text{, where } a = \#\text{agreements, } d = \#\text{disagreements, } p = \frac{3}{4}(1-e^{-8\alpha t}).$$

$$\mathrm{pr}(data \mid R) = \mathrm{pr}(\mathrm{AA} \mid R)\mathrm{pr}(\mathrm{GA} \mid R)\mathrm{pr}(\mathrm{CC} \mid R)\ldots$$

$$= (\frac{1}{4})^a (\frac{3}{4})^d$$

$$\Rightarrow \quad \log\{\frac{\mathrm{pr}(data \mid H)}{\mathrm{pr}(data \mid R)}\} = a \log \frac{1-p}{1/4} + d \log \frac{p}{3/4} = a \times \sigma + d \times (-\mu).$$

- Since $p<3/4$, $\sigma = log((1\text{-}p)/(1/4))>0$, while $-\mu = log(p/(3/4))<0$.
- Thus the alignment score = $a \times \sigma + d \times (-\mu)$, where the match score $\sigma > 0$, and the mismatch penalty is $-\mu < 0$.

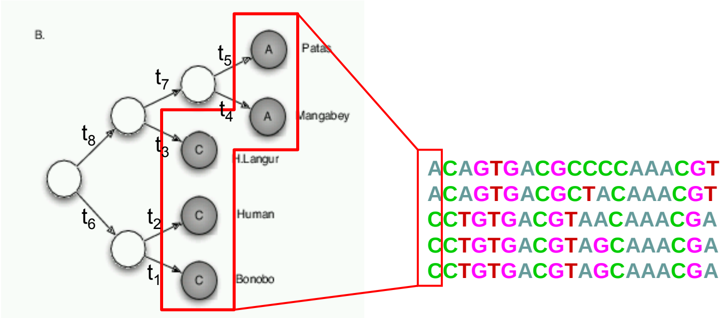


Large and small evolutionary distances

- Recall that
  - $p = (3/4)(1\text{-}e^{-8\alpha t})$,
  - $\sigma = log((1\text{-}p)/(1/4))$,
  - $-\mu = log(p/(3/4))$.
- Now note that if $\alpha t \approx 0$,
  - then $p \approx 6\alpha t$, and $1\text{-}p \approx 1$, and so $\sigma \approx log4$, while $-\mu \approx log8\alpha t$ is large and negative.
  - That is, we see a big difference in the two values of σ and μ for small distances.
- Conversely, if αt is large,
  - $p = (3/4)(1\text{-}\varepsilon)$, hence $p/(3/4) = 1\text{-}\ \varepsilon$, giving $\mu = -log(1\text{-}\ \varepsilon) \approx \varepsilon$, while $1\text{-}p = (1+3\varepsilon)/4$, $(1\text{-}p)/(1/4) = 1+3\varepsilon$, and so $\sigma = log(1+3\varepsilon) \approx 3\varepsilon$.
  - Thus the scores are about 3 (for a match) to 1 (for a mismatch) for large distances. This makes sense, as mismatches will on average be about 3 times more frequent than matches.
- the matrix which performs best will be the matrix that reflects the evolutionary separation of the sequences being aligned.

What about multiple alignment

- Phylogenetic methods: a tree, with branch lengths, and the data at a single site.



ACAGTGACGCCCCAAACGT
ACAGTGACGCTACAAACGT
CCTGTGACGTAACAAACGA
CCTGTGACGTAGCAAACGA
CCTGTGACGTAGCAAACGA

- See next lecture for how to compute likelihood under this hypothesis

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