Assignment set 2 / Biometry and bioinformatics II / 2013

- Reports to be submitted to course Moodle Tuesday 15.10
- You will not get personal response about your answers, but we work out the solutions during our last session Wednesday 16.10. Time for this session is 12.00 – 15.30. We agreed on this change during the lecture 3.10 because many students have an overlap (16.00 -> an exam, Algorithms for bioinformatics).

The goal of assignment set 1 is to familiarize with some general molecular evolutionary concepts, nucleotide substitution modelling, composition, codon usage, synonymous, non-synonymous substitutions

You can use either MEGA5-software, which you have been using for assignment set 1, (http://www.megasoftware.net/) or DnaSP5 (http://www.ub.edu/dnasp/, installed in C128) which was introduced in session 3.10.

Assignment set 2.1

Modelling nucleotide substitutions is an elementary part of (for example) phylogeny reconstructions (with the exception of parsimony methods). Jukes-Cantor model assumes that all substitutions occur with equal probabilities. Derivation is given in lecture slides and, for convenience, here anew.

Derive the two-parameter model including separate parameters for transitions and transversions. In case you consider this as too demanding, it is enough that you construct the strating scheme and the first equations, i.e. the part corresponding equations (1) – (3) in Jukes-Cantor one-parameter model.

We shall work out the whole derivation during our last session 16.10.

Assumption: all nucleotide substitutions occur with equal probabilites, $\boldsymbol{\alpha}$, Jukes-Cantor model (1969)

- The rate of substitution for each nucleotide is 3α per unit time

	A	T	C	G
A		$\boldsymbol{\alpha}$	$\boldsymbol{\alpha}$	$\boldsymbol{\alpha}$
T	$\boldsymbol{\alpha}$		$\boldsymbol{\alpha}$	$\boldsymbol{\alpha}$
C	$\boldsymbol{\alpha}$	$\boldsymbol{\alpha}$		$\boldsymbol{\alpha}$
G	$\boldsymbol{\alpha}$	$\boldsymbol{\alpha}$	$\boldsymbol{\alpha}$	

- At time 0: Assumption that at a certain nucleotide site there is A, $P_{\mathrm{A}(0)} = 1$
- Question: probability that this site is occupied by A at time t , $P_{\mathrm{A}(t)}$?
- At time 1, probability of still having A at this site is

$$P_{\mathrm{A}(1)} = 1 - 3\alpha \qquad (1)$$

- 3α is the probability of A changing to T, C, or G
- The probability of the site having A at time 2 is

$$P_{\mathrm{A}(2)} = (1 - 3\alpha\ )\mathrm{P}_{\mathrm{A}(1)} + \alpha\ [1 - P_{\mathrm{A}(1)}] \qquad (2)$$

This includes two possible courses of events from time points t=0 --> t = 1 --> t = 2

t = 0		t = 1		t = 2
A	no substitution	A	no substitution	A
A	substitution	T or C or G	substitution	A

- The following recurrence equation holds for any t

$$P_{\mathrm{A}(t+1)} = (1 - 3\alpha\ )P_{\mathrm{A}(t)} + \alpha[1 - P_{\mathrm{A}(t)}\ ] \qquad (3)$$

Note that this holds also for t = 0, because $P_{\mathrm{A}(0)} = 1$ and thus

$$P_{\mathrm{A}(0+1)} = (1 - 3\alpha)\ P_{\mathrm{A}(0)} + \alpha\ [1 - P_{\mathrm{A}(0)}\ ] = 1 - 3\alpha$$

which is identical with equation (1).

- The amount of change in $P_{A(t)}$ per unit time, rewriting equation (3):

$$\Delta P_{A(t)} = P_{A(t+1)} - P_{A(t)} = - 3\alpha P_{A(t)} + \alpha[1 - P_{A(t)}\ ] = - 4\alpha P_{A(t)} + \alpha \qquad (4)$$

- Approximating the previous discrete-time model by a continuous-time model, by regarding $\Delta \mathrm{P}_{\mathrm{A(t)}}$ as the rate of change at time t. With this approximation equation (4) is rewritten as

$$\mathrm{d}P_{A(t)}\ /\ \mathrm{d}t = - 4\alpha P_{A(t)} + \alpha \qquad (5)$$

- The solution of this first-order linear differential equation is

$$P_{A(t)} = ¼ + (P_{A(0)} - ¼)e^{-4\alpha t} \qquad (6)$$

- The starting condition was A at the given site, $P_{A(0)} = 1$, consequently

$$P_{A(t)} = ¼ + ¾ e^{-4\alpha t} \qquad (7)$$

- Equation (6) holds regardless of the initial conditions, for example if the initial nucleotide is not A, then $P_{A(0)} = 0$, and the probability of having A at time t

$$P_{A(t)} = ¼ + ¼ e^{-4\alpha t} \qquad (8)$$

- Equations (7) and (8) describe the substitution process. If the initial nucleotide is A, then $P_{A(t)}$ decreases exponentially from 1 to ¼ . If the initial nucleotide is not A, then $P_{A(t)}$ will increase monotonically from 0 to ¼ .

- Under this simple model, after reaching equilibrium, $P_{A(t)}=P_{T(t)}=P_{C(t)}=P_{G(t)}$ for all subsequent times.

- Equation (7) can be rewritten in a more explicit form to take into account that the initial nucleotide is A and the nucleotide at time t is also A

$$P_{AA(t)} = ¼ + ¾ e^{-4\alpha t} \qquad (9)$$

- If the initial nucleotide is G instead of A, from equation (8)

$$P_{GA(t)} = ¼ + ¼ e^{-4\alpha t} \qquad (10)$$

Since all the nucleotides are equivalent under the Jukes-Cantor model, the general probability, $P_{ij(t)}$, that a nucleotide will become *j* at time *t*, given that it was *i* at time 0, equations (9) and (10) give the general probabilities $P_{ii(t)}$ and $P_{ij(t)}$, where $i \neq j$.

$$P_{ii(t)} = ¼ + ¾ e^{-4\alpha t} \quad \text{and} \quad P_{ij(t)} = ¼ + ¼ e^{-4\alpha t} \qquad (11)$$

Number of substitutions, *nucleotide divergence*, between two sequences

- We assume that all sites in sequence evolve at the same rate and follow the same substitution scheme. The number of sites compared between two sequences is denoted by *L.*

- Consider the probability that a nucleotide at a given site at time *t* is the same in both sequences. Suppose that the nucleotide at a given site was A at time point 0. At time *t*, the probability that a descendant sequence will have A at this site is $P_{AA(t)}$, and consequently the probability that two descendant sequences have A at this site is $P^2_{AA(t)}$. Similarly, the probabilities that both sequences have T, C or G at this site are $P^2_{AT(t)}$, $P^2_{AC(t)}$, and $P^2_{AG(t)}$

- The probability that the nucleotide at a given site at time *t* is the same in both sequences is

$$I_{(t)} = P^2_{AA(t)} + P^2_{AT(t)} + P^2_{AC(t)} + P^2_{AG(t)} \qquad (12)$$

- From equations (11) we obtain

$$I_{(t)} = ¼ + ¾ e^{-8\alpha t} \qquad (13)$$

- Equation (13) also holds for T, C or G. Therefore, regardless of the initial nucleotide at a given site, $I_{(t)}$ represents the proportion of *identical* nucleotides between two sequences that diverged *t* time units ago. The probability that the two sequences are *different* at a site at time *t* is $p = 1 - I_{(t)}$. Thus

$$p = ¾\,(1 - e^{-8\alpha t}) \qquad \text{or} \qquad 8\alpha t = \ln(1 - (4/3)\,p) \qquad (14)$$

- The time of divergence between two sequences is usually not known, and thus estimation of α is not possible. Instead, it possible to calculate ***K*, which is the number of substitutions per site since the time of divergence between the two sequences**. In the case of the one-parameter model, $K = 2(3\,\alpha t)$, where $3\,\alpha t$ is the number of substitutions per site in a single lineage.

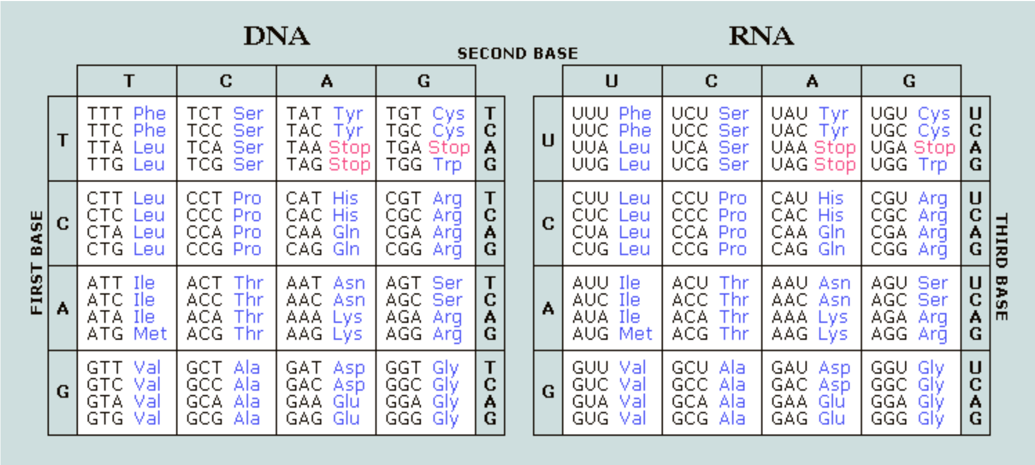
$$K = 6\,\alpha t = -\,¾\,\ln(1 - (4/3)\,p) \qquad (15)$$

where *p* is the observed proportion of different nucleotides between the two sequences.

An example. Page 3 (book chapter page 143) in *Phylogeny methods based on distance matrices* (see course webpage, week 1) shows how Jukes-Cantor model serves like a *correction* to sequence diverge calculation.

Assignment set 2.2

Background



DNA

SECOND BASE

FIRST BASE	T	C	A	G	THIRD BASE
T	TTT Phe TTC Phe TTA Leu TTG Leu	TCT Ser TCC Ser TCA Ser TCG Ser	TAT Tyr TAC Tyr TAA Stop TAG Stop	TGT Cys TGC Cys TGA Stop TGG Trp	T C A G
C	CTT Leu CTC Leu CTA Leu CTG Leu	CCT Pro CCC Pro CCA Pro CCG Pro	CAT His CAC His CAA Gln CAG Gln	CGT Arg CGC Arg CGA Arg CGG Arg	T C A G
A	ATT Ile ATC Ile ATA Ile ATG Met	ACT Thr ACC Thr ACA Thr ACG Thr	AAT Asn AAC Asn AAA Lys AAG Lys	AGT Ser AGC Ser AGA Arg AGG Arg	T C A G
G	GTT Val GTC Val GTA Val GTG Val	GCT Ala GCC Ala GCA Ala GCG Ala	GAT Asp GAC Asp GAA Glu GAG Glu	GGT Gly GGC Gly GGA Gly GGG Gly	T C A G

RNA

FIRST BASE	U	C	A	G	THIRD BASE
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

- Each amino acid is coded by a “triplet of nucleotides”, a codon, having three “sites”, first, second and third site or position.

- Nucleotide sites (positions) are classified into nondegenerate, twofold degenerate, and fourfold degenerate sites:

- A site is nondegenerate if all possible changes at this site are non-synonymous: nucleotide change => amino acid change, twofold degenerate if one of the three possible changes is synonymous (nucleotide change => no amino acid change), and fourfold degenerate if all possible changes at the site are synonymous

- For example, the first two positions at the codon TTT (Phe) are nondegenerate, while the third position is twofold degenerate. The third position at the codon GTT (Val) is fourfold degenerate.

● By using the data 2 (see the data description in assignment set 1) calculate

- ● Codon usage of the bacteria in the data.
- ● The so called GC-content of the bacteria in the data.

● The concepts and, in many instances, practical "tools", *codon usage* and *GC-content* will be introduced in more detail during the lecture 8.10. Now we get familiar with these by working out the facts: codons are not used at random, GC-content is not evenly distributed. During the session 3.10 we had a look at these (by using another data) by using DnaSP5.

NOTE !!!!

You get a table of GC-content of all sequences (i.e. one table). Write about differences you can observe.

But codon usage: each sequence item has it´s own table. You are not supposed to start inspecting tens of such tables!

> Just pick up some tables (not many !) and explain some part of the results you notice. You are not supposed to write an extensive report commenting all codon – amino acid issues. Pick up, for example, some amino acids and inspect them, and write about your observations.

Hint: When you have worked with this data for assignment set 1, you have got a clustering structure of the bacteria, i.e. different species and different serotypes of one species (*Streptococcus pneumoniae*). *Use your clustering structure as framework for selecting some items (sequences, which are different species or different within-species serotypes). This means: pick up sequence items from clearly different clusters.*

Assignment set 2.3

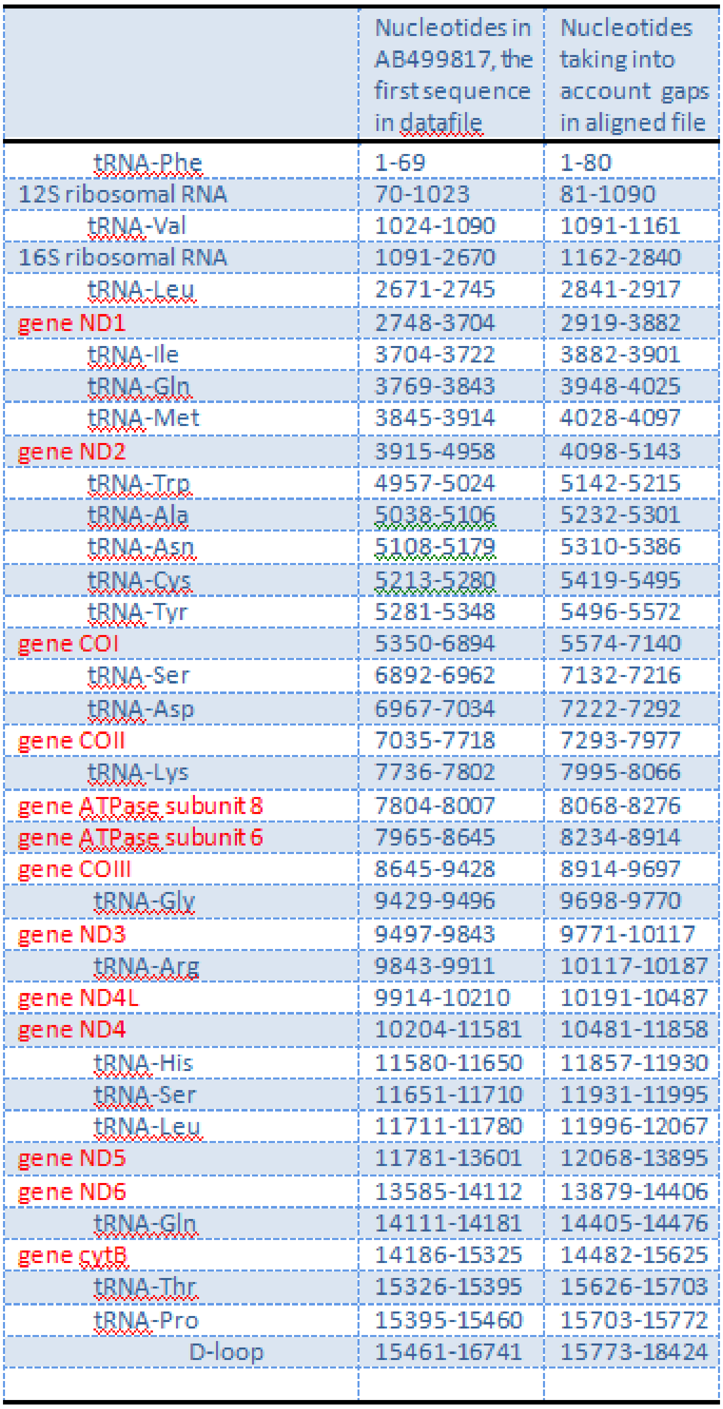
The initial question in 3.10 version (synonymous and non-synonymous in data_4, see assignment set 1) is now retracted.

Instead, with data_4 answer the question posed above in 2.2: nucleotide composition and codon usage bias. And, also for some other gene from data_3 (the mitochondrial genome, see assignment set 1): nucleotide composition and codon usage bias.

See the Note above in 2.2: codon usage biases only from some animals, not from the whole data. Take, for example, dog, horse and cow. And the same animals from both genes.

Data_4 –file is the cytochrome-gene from the mitochondrial genome (cut from the data in data_3, which is now given in a bit modified version: data 3_aligned_IUPACedited (see http://www.dnabaser.com/articles/IUPAC%20ambiguity%20codes.html; The reason: DnaSP does not recognize other than A,C,T,G´s and N – all Y´s and W´s and R´s, i.e. the "not clear bases" have been replaced by N.)
Your task is to cut also another gene. How to cut: (was shown during the session 3.10) One way is to use Clustal, define the coordinates to be included, and save that file as a new

fasta-file. In DnaSP: there is the window which allows definition (by using coordinates) of a region to be analysed. The same is true for MEGA5, in which you can also delete the regions which you don´t want to be included. The data_3 coordinate table is, for convenience, given here anew.



	Nucleotides in AB499817, the first sequence in datafile	Nucleotides taking into account gaps in aligned file
tRNA-Phe	1-69	1-80
12S ribosomal RNA	70-1023	81-1090
tRNA-Val	1024-1090	1091-1161
16S ribosomal RNA	1091-2670	1162-2840
tRNA-Leu	2671-2745	2841-2917
gene ND1	2748-3704	2919-3882
tRNA-Ile	3704-3722	3882-3901
tRNA-Gln	3769-3843	3948-4025
tRNA-Met	3845-3914	4028-4097
gene ND2	3915-4958	4098-5143
tRNA-Trp	4957-5024	5142-5215
tRNA-Ala	5038-5106	5232-5301
tRNA-Asn	5108-5179	5310-5386
tRNA-Cys	5213-5280	5419-5495
tRNA-Tyr	5281-5348	5496-5572
gene COI	5350-6894	5574-7140
tRNA-Ser	6892-6962	7132-7216
tRNA-Asp	6967-7034	7222-7292
gene COII	7035-7718	7293-7977
tRNA-Lys	7736-7802	7995-8066
gene ATPase subunit 8	7804-8007	8068-8276
gene ATPase subunit 6	7965-8645	8234-8914
gene COIII	8645-9428	8914-9697
tRNA-Gly	9429-9496	9698-9770
gene ND3	9497-9843	9771-10117
tRNA-Arg	9843-9911	10117-10187
gene ND4L	9914-10210	10191-10487
gene ND4	10204-11581	10481-11858
tRNA-His	11580-11650	11857-11930
tRNA-Ser	11651-11710	11931-11995
tRNA-Leu	11711-11780	11996-12067
gene ND5	11781-13601	12068-13895
gene ND6	13585-14112	13879-14406
tRNA-Gln	14111-14181	14405-14476
gene cytB	14186-15325	14482-15625
tRNA-Thr	15326-15395	15626-15703
tRNA-Pro	15395-15460	15703-15772
D-loop	15461-16741	15773-18424

Assignment set 2.4

The file data HLA_gene contains human alleles at one *human leukocyte* gene.

- Synonymous and non-synonymous ?

- Nondegenerate, two-fold degenerate, four-fold degenarte ?
  (note that MEGA5 has buttons-to-be-clicked to get these).