

Διάλεξη 1

Μοριακές Τεχνικές στην Οικολογία

(Κ. Μαθιόπουλος)

Τι είναι Μοριακή Οικολογία;

-Η εφαρμογή μεθόδων μοριακής γενετικής σε προβλήματα οικολογίας

-Ποια προβλήματα μπορεί να αντιμετωπίσει;

- Η κατανόηση της εμφάνισης της μοριακής οικολογίας βρίσκεται μέσα στο εξελικτικό πλαίσιο της βιολογίας.
- Η μοριακή οικολογία είναι ριζωμένη στη συστηματική, δηλαδή στην προσπάθεια διαχωρισμού και ταξινόμησης της πολυπλοκότητας στη φύση και των εξελικτικών διαδικασιών που την υποστηρίζουν.

Μοριακοί δείκτες

- Έχουν τη δυνατότητα να απαντούν σε ερωτήσεις που δύσκολα αντιμετωπίζονται με άλλους τρόπους.
- Μπορούν να ποσοτικοποιηθούν με μεγαλύτερη ακρίβεια =>
- Παρέχουν καλύτερα δεδομένα για ποσοτικές αναλύσεις.

	Single locus	Codominant	PCR assay	Overall variability
Mitochondrial and chloroplast DNA				
RFLP	Yes	Haplotypes	Yes	Low/medium
Sequence	Yes	Haplotypes	Yes	Low-high
Nuclear multilocus				
Minisatellite and microsatellite fingerprints	No	No	No	High
RAPD	No	No	Yes	High
AFLP	No	No	Yes	High
Ribosomal DNA*	No	No	Yes	Medium/high
Nuclear single locus				
Allozymes	Yes	Yes	No	Low/medium
Minisatellites	Yes	Yes	Not usual	High
Microsatellites	Yes	Yes	Yes	High
Sequence	Yes	Yes	Yes	Low-high

Notes: Codominant markers are those in which homozygotes and heterozygotes can be distinguished.
 RFLP, restriction fragment length polymorphism; RAPD, randomly amplified polymorphic DNA; AFLP, amplified fragment length polymorphism.
 * Mostly used in microbial molecular ecology.

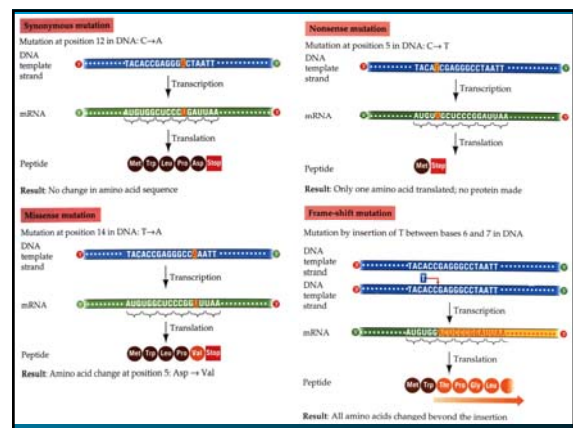
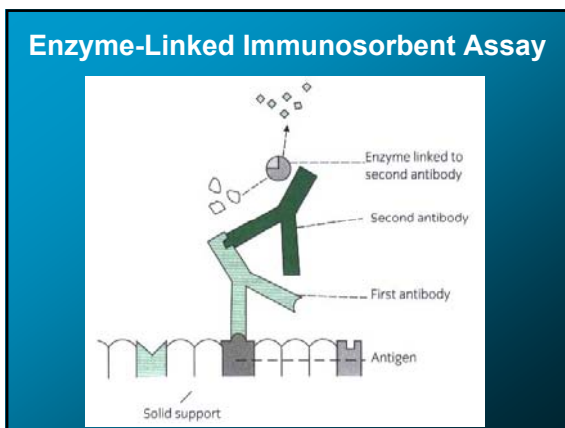
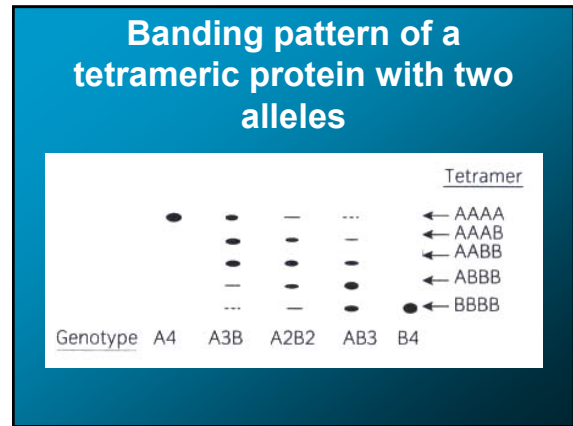
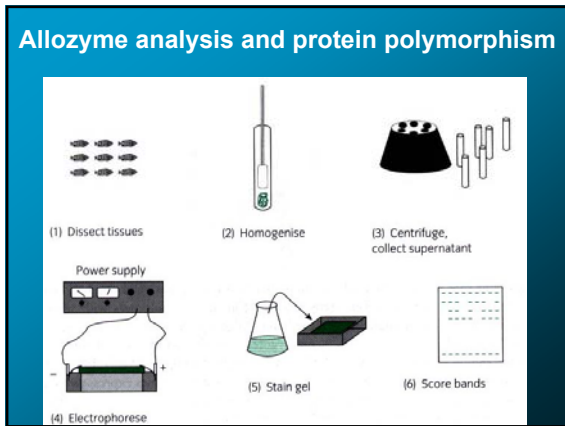
Μοριακοί δείκτες που χρησιμοποιούνται στη Μοριακή Οικολογία

Ορόσημα της Μοριακής Οικολογίας

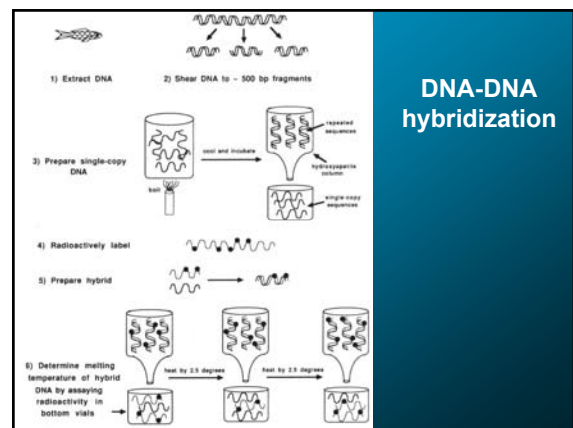
- Τα πρώτα χρόνια
- Ηλεκτροφόρηση αλλοενζύμων
- RFLPs
- Minisatellite DNA fingerprinting
- PCR
- PCR-based molecular markers
- DNA sequencing

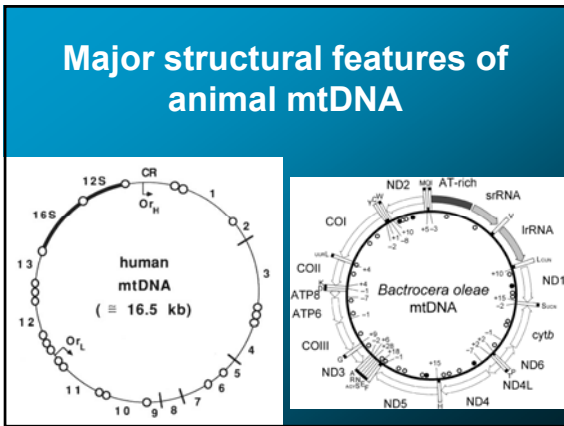
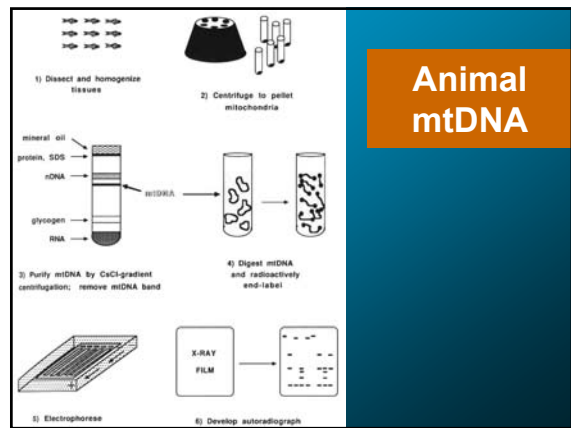
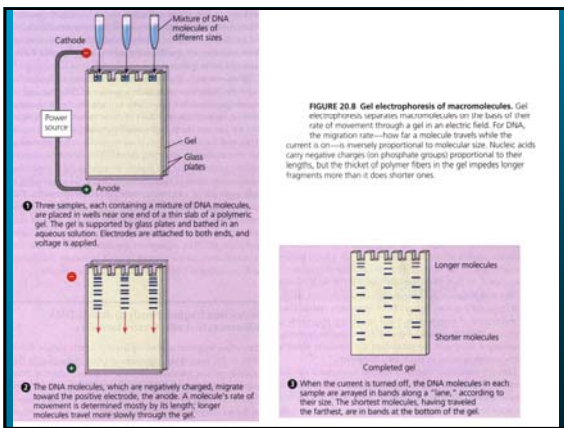
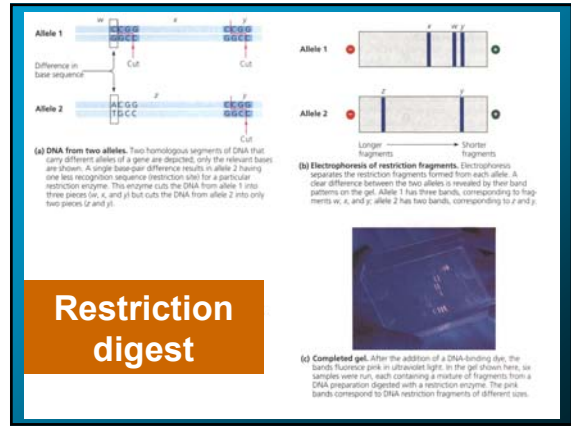
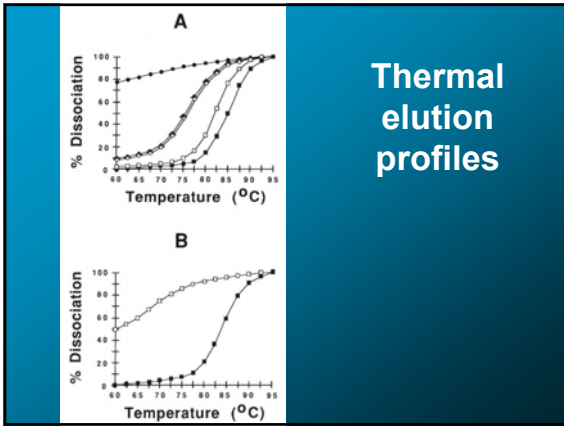
Protein-based methods

- Allozyme analysis and protein polymorphism
- Protein profiling
- Immunological methods



- ### DNA-based methods
- DNA-DNA hybridization
 - Restriction analyses
 - PCR based analyses
 - Multilocus minisatellite DNA fingerprinting
 - Microsatellite analysis
 - DNA sequencing
 - Single strand conformation polymorphism
 - DNA microarrays

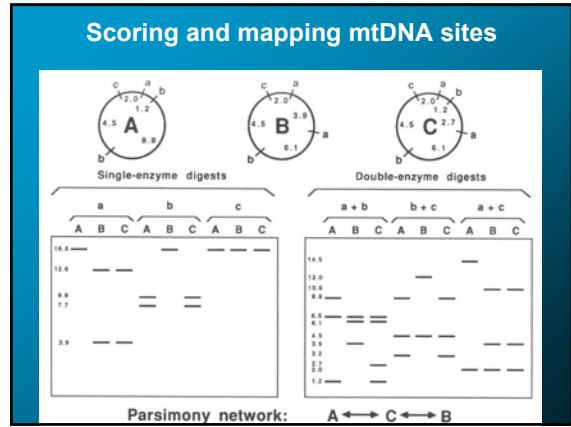
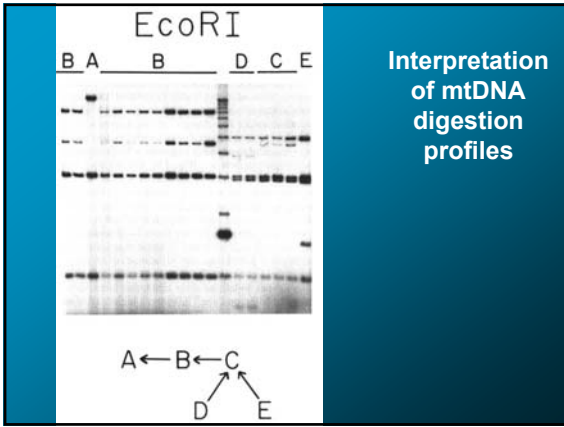




Box 3.3. Examples of Conserved Primer Sequences Within Various Mitochondrial Genes That Have Proved Useful for PCR Amplifications.

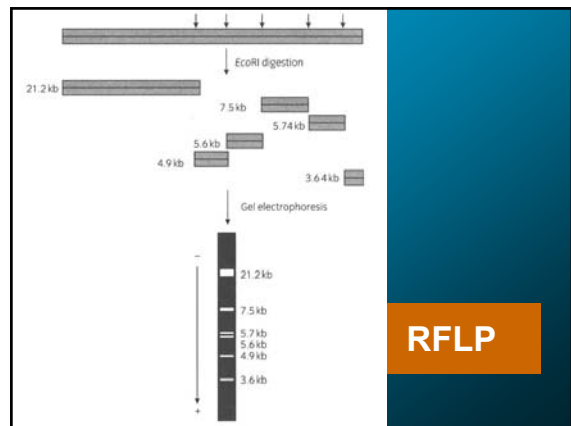
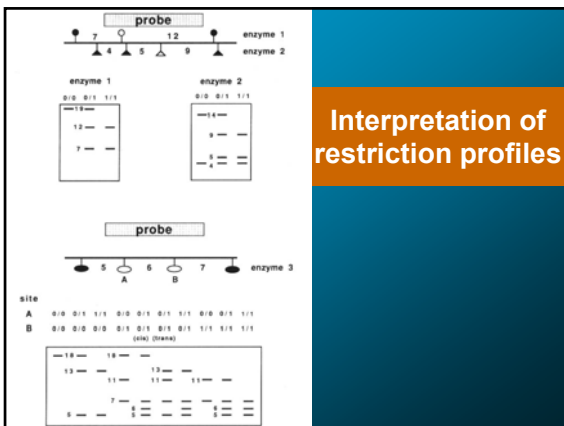
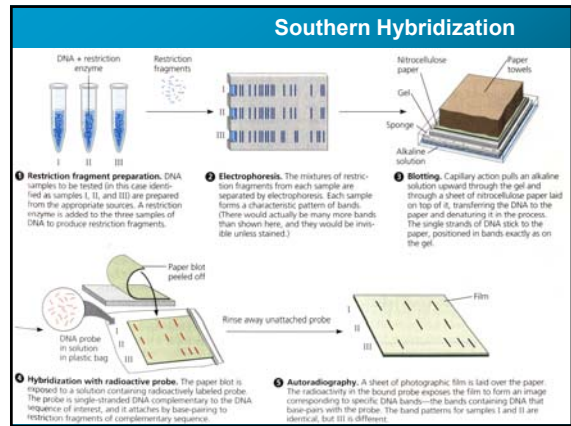
Sequences are given in the 5' to 3' direction; Y indicates either a cytosine (C) or thymine (T); R either an adenine (A) or guanine (G); and W indicates an A or T. Several of these primers were developed for fishes, but in many cases they successfully amplify DNA from other animals as well.

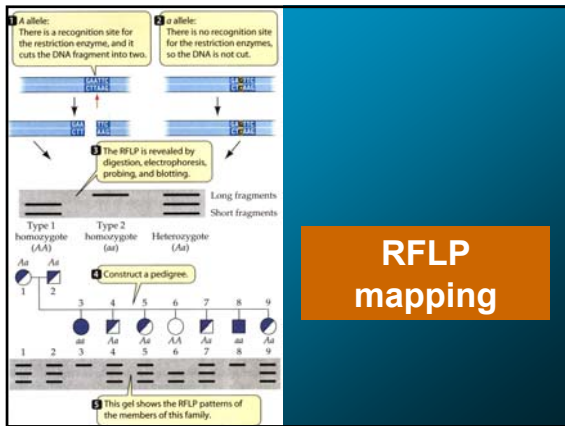
Gene	Primer	Primer Sequence	Reference
Control region	L1926	TCAAAGCTTCACAGCTCTGT-TAAACC	Kocher et al., 1989
	H1408	CCTGAAGTAGGAACCAAGTG	Meyer et al., 1990
	L16518	CATCTGGTCTCTTCTCAGG-CCAT	Meyer, 1993
12S rRNA	H1109	GTOGGGTATCTAATCCAGTT	Meyer, 1993
	L1091	AAAAGCTTCACCTGGGAT-AGATACCCCAT	Kocher et al., 1989
Cytb	H1478	TGACTCGAGAGGGTAC-GGGGCGGTGT	Kocher et al., 1989
	L5950	ACAATACAAAGAYATYGG	Nomark et al., 1991
ATPase 6	H8517	GGGRACCTTTCAGTGGTACT	Meyer, 1993
	L8580	AGCCCCACATACTAGGTA-TCCC	Meyer, 1993
	H8907	GGGTCTCTCAGGCAAT-AAATG	Meyer, 1993
Cytb	L9225	CACCAAGCACACOCATACCA-CAT	Meyer, 1993
	H9407	AAAGTCTCTGTGTGTG-CGGGG	Meyer, 1993
Cytb	L14841	AAAAGCTTCATCAA-CATCTACAGATATAAAA	Kocher et al., 1989
	L15020	GCTAAGGCC-ATCT-TTATT	Meyer, 1993
	H15149	AACTCGACGCCCTCAGAA-TGATATTGCTCA	Kocher et al., 1989



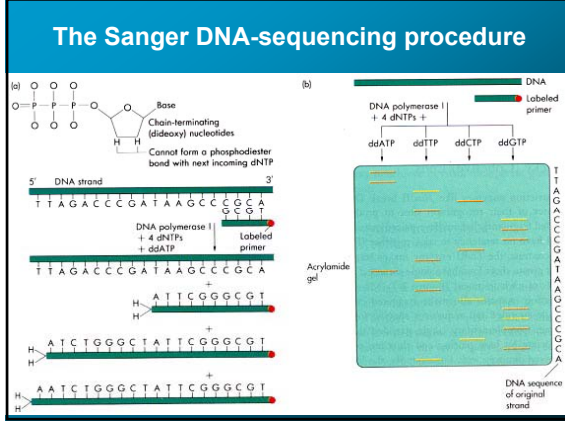
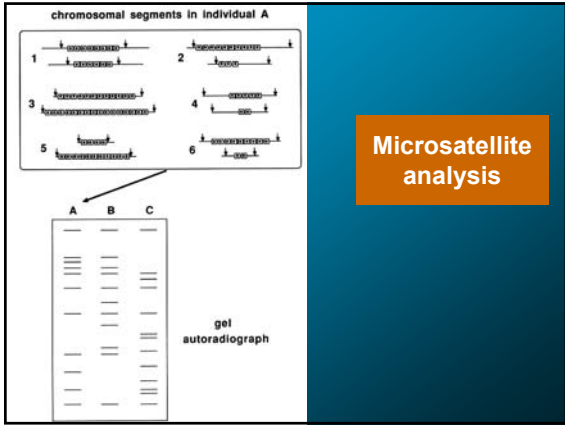
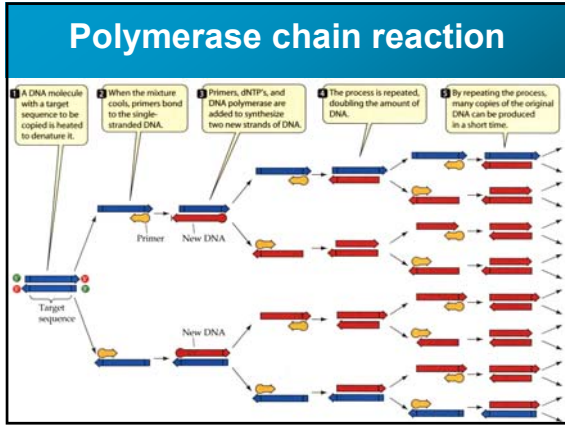
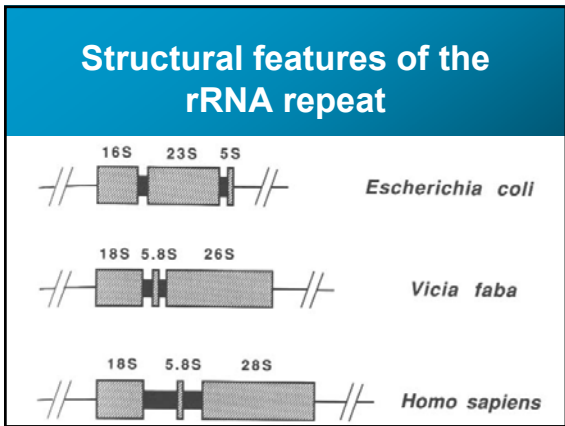
Box 3.2. Presence (+) Versus Absence (0) Restriction Site Matrix. This example involves 96 restriction sites representing 20 different mtDNA haplotypes (a-t) observed among 107 sharp-tailed sparrows, *Ammodramus caudacutus* (data from Rising and Avise, 1993).

a00.0.....0000.....00.0.....0.0.0.....
b00.2.....0.....0000.....00.0.....0.0.0.....
c00.0.....0.....0000.....00.0.....0.0.0.....
d00.0.....0.....0000.....00.0.....0.0.0.....
e00.0.....0.....0000.....00.0.....0.0.0.....
f00.0.....0.....0000.....00.0.....0.0.0.....
g00.0.....0.....0000.....00.0.....0.0.0.....
h00.0.....0.....0000.....00.0.....0.0.0.....
i00.0.....0.....0000.....00.0.....0.0.0.....
j00.0.....0.....0000.....00.0.....0.0.0.....
k00.0.....0.....0000.....00.0.....0.0.0.....
l00.0.....0.....0000.....00.0.....0.0.0.....
m00.0.....0.....0000.....00.0.....0.0.0.....
n00.0.....0.....0000.....00.0.....0.0.0.....
o00.0.....0.....0000.....00.0.....0.0.0.....
p00.0.....0.....0000.....00.0.....0.0.0.....
q00.0.....0.....0000.....00.0.....0.0.0.....
r00.0.....0.....0000.....00.0.....0.0.0.....
s00.0.....0.....0000.....00.0.....0.0.0.....
t00.0.....0.....0000.....00.0.....0.0.0.....





RFLP mapping



Box 3.4. Nucleotide Sequences in the mtDNA Cytochrome b Gene of Marine Turtles (Taxa a-n)
(after Brown et al., 1993a).

The matrix data set involved more than 500 nucleotide positions per sample (only 61 of which are shown here), which is typical for many sequencing studies.

1. Nucleotide positions. Each dash indicates a base identical to that of the reference species, "a."

Reference sequence

Position	a	ACC	GGA	ATC	TTC	TTG	GCA	ATA	CAC	TAT	TCA	CCA	GAT	ATC	CTG	GCA	TTC	TCA	TCA	ATC	ATC
1	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
54	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
56	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
61	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

2. Some sites involved in protein ("") amino positions ("")

3. Some sites involved in amino acid sequences (the reference is the mitochondrial gene code)

