

Methods

The following steps were performed during the analysis:

- 1) Mafft - multiple sequence alignment
- 2) MEGA - construction of phylogenetic tree using Neighbor Joining approach
- 3) MEGA - calculation of real pairwise distance
- 4) Nucleotide bp length – mean of length of 2 selected sequences
- 5) Age calculation:

$$\frac{\text{number of differences}}{\text{mutation rate per nucleotide per year} * \text{mean sequence length}}$$

- 6) Mutation rate was considered to be as much as $4 * 10^{-8}$ (based on mtDNA literature data obtained from trio sequencing ([reference](#)))

1. Mitochondrial Eve

In order to estimate the age of Mitochondrial Eve, max pairwise distance between samples was found.

Max difference corresponds to the following samples:

KX440262.1_Homo_sapiens_isolate_JT76_haplogroup_J2a2d1_mitochondrion_complete_genome

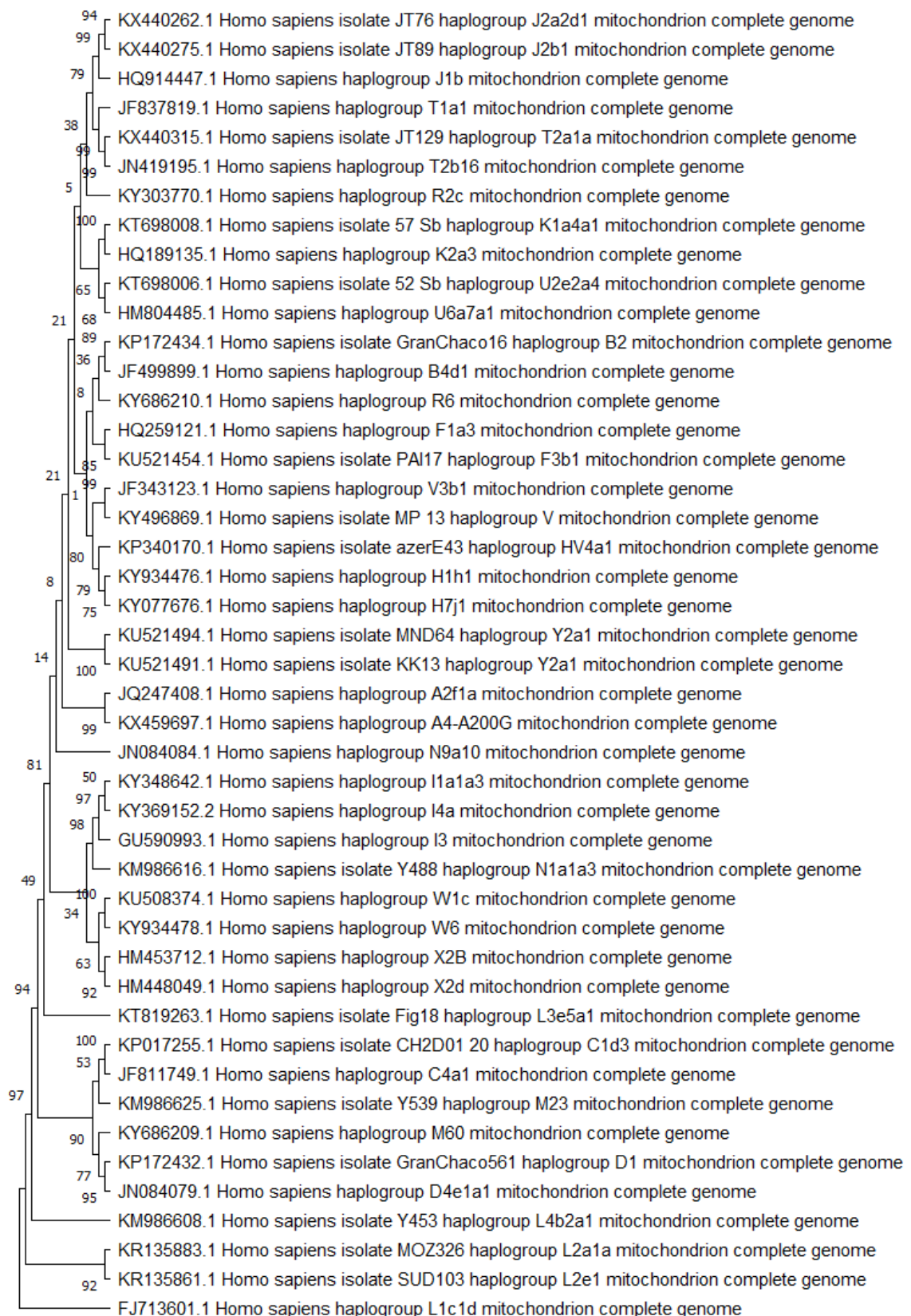
&

FJ713601.1_Homo_sapiens_haplogroup_L1c1d_mitochondrion_complete_genome

And equals to **95**

The age of Mitochondrial Eve:

$$(95/16567) / 4 * 10^{-8} = \mathbf{143,357}$$



2. Most recent ancestor of all non-Africans

In order to estimate the age of most recent ancestor of all non-Africans, we should find max distance for non-African populations.

It corresponds to these samples:

JQ247408.1_Homo_sapiens_haplogroup_A2f1a_mitochondrion_complete_genome (Nat American)

JF811749.1_Homo_sapiens_haplogroup_C4a1_mitochondrion_complete_genome (Turkish)

and equals to 58.

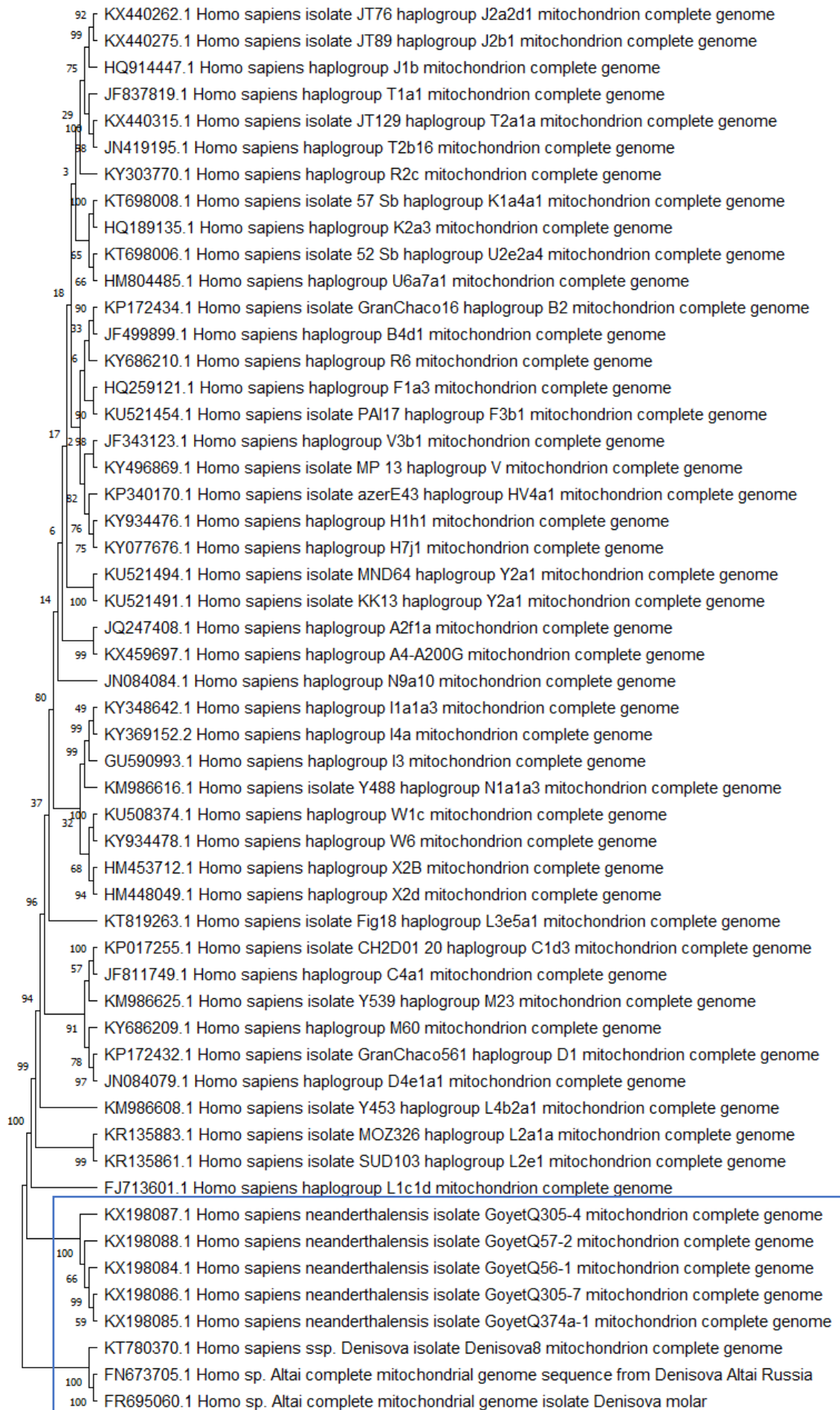
The age of most recent ancestor of all non-Africans:

$$(58/16569) / 4 * 10^{-8} = \mathbf{87,512}$$

3. Neanderthal and Denisovan samples

3.1 The most recent Denisova-modern human ancestor

Based on obtained tree topology, Denisovan (blue) and Neanderthal (green) samples comprise monophyletic groups.



Max difference between Denisova and modern human samples equals **401** and corresponds to the following samples:

FR695060.1_Homo_sp._Altai_complete_mitochondrial_genome_isolate_Denisova_molar

KR135861.1_Homo_sapiens_isolate_SUD103_haplogroup_L2e1_mitochondrion_complete_genome

The age of most recent ancestor of Denisovans and modern humans:

$$401/(16570 * 4 * 10^{-8}) = 605,009$$

4. The most recent Neanderthal-modern human ancestor

Max difference between Neanderthal and modern human samples equals **220** and corresponds to the following samples:

KX440262.1_Homo_sapiens_isolate_JT76_haplogroup_J2a2d1_mitochondrion_complete_genome

KX198086.1_Homo_sapiens_neanderthalensis_isolate_GoyetQ305-7_mitochondrion_complete_genome

The age of most recent ancestor of Neanderthals and modern humans:

$$220/(16565 * 4 * 10^{-8}) = 332,025$$

5. Pan mtDNA tree topology

Based on obtained tree, tree topology has not changed as Pan mtDNA samples comprise separate branch.

