Methods

The following steps were performed during the analysis:

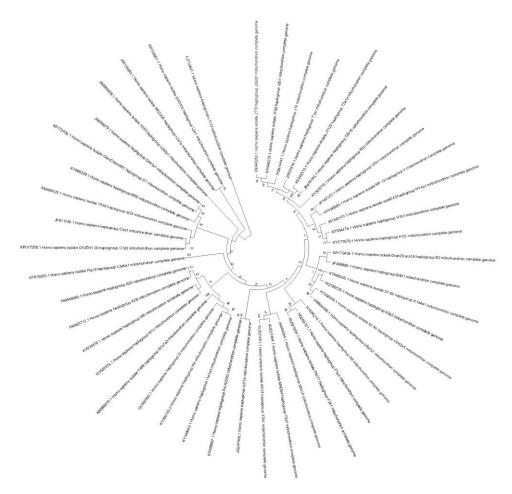
- 1) Mafft multiple sequence alignment
- 2) MEGA construction of phylogenetic tree using Neighbor Joining approach
- 3) MEGA calculation of real pairwise distance
- 4) Nucleotide bp length mean of length of 2 selected sequences
- 5) Age calculation:

number of differences

mutation rate per nucleotide per year * 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 (<u>reference</u>))

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_c omplete_genome

&

FJ713601.1_Homo_sapiens_haplogroup_L1c1d_mitochondrion_complete_genom e

And equals to 95

The age of Mitochondrial Eve:

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

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_genom e (Nat American)

JF811749.1_Homo_sapiens_haplogroup_C4a1_mitochondrion_complete_genome (Turkish)

and equals to 58.

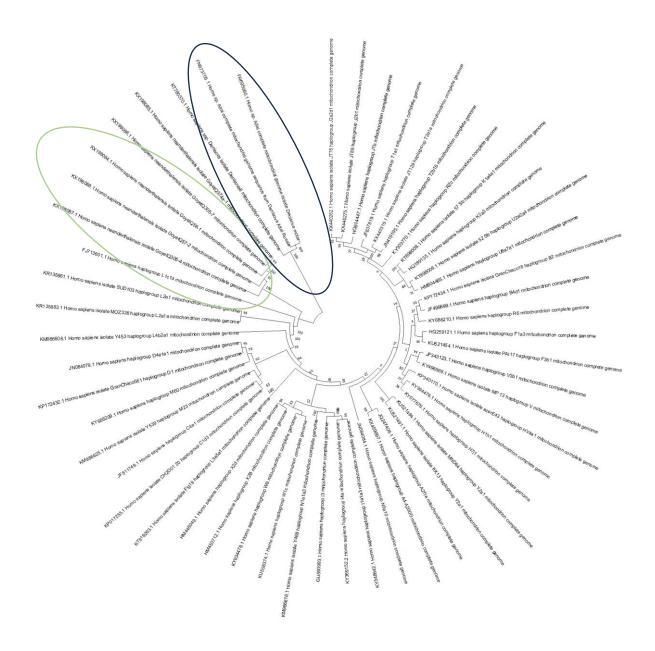
The age of most recent ancestor of all non-Africans:

$$(58/16569) / 4 * 10^{-8} =$$
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_c omplete 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.

