LAB RESULTS

PART 1. ISOLATION OF DNA FROM HERBARIUM LEAF TISSUE AND BALKAN CHAMOIS BONE REMAINS

- DNA samples:

1) Fresh leaf of plant grown in Zagreb Botanical Garden

a) c= 18,3 ng/µl *260/280 = 1,738*

b) c = 37,5 ng/µl *260/280 = 1,786*

2) Leaf from Herbarium Croaticum (ZA) collected on the Island of Biševo (Cro) in 1970

c = 13,0 ng/µl *260/280 = 1,733*

3) Leaf from Herbarium Croaticum (ZA) collected near city of Šibenik (Cro) in 1995

c = 3,5 ng/µl *260/280 = 1,750*

4) Leaf from Herbarium Croaticum (ZA) collected on the Island of Krk (Cro) in 1981

c = 5,0 ng/µl *260/280 = 1,333*

5) Leaf from Herbarium Croaticum (ZA) collected on the Island of Šipan (Cro) in 1979

c = 13,8 ng/µl *260/280 = 1,618*

6) Balkan chamois molar remains (Velebit (Cro) 1893)

c = 52,3 ng/µl *260/280 = 1,802*

7) Balkan chamois maxilla remains (location and date unknown)

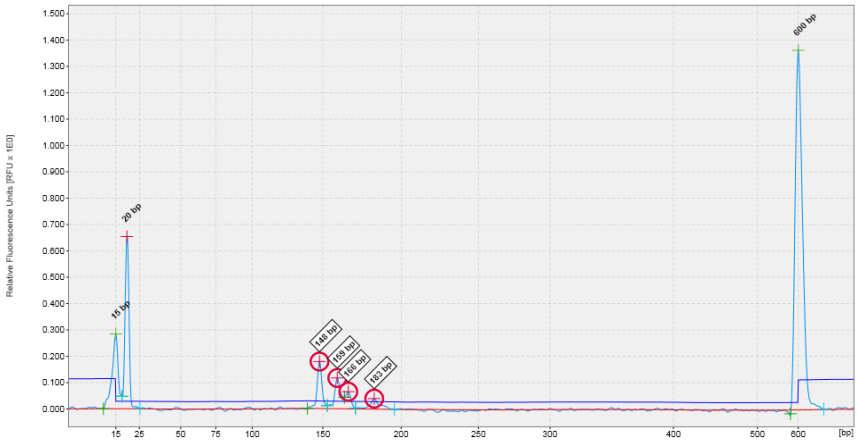
c = 35,8 ng/µl *260/280 = 1,879*

- Nucleic acids have absorbance maxima at 260 nm. It is common for nucleic acid samples to be contaminated with proteins, organic compounds etc. The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A 260/280 nm ratio of >1.7 is generally accepted as "pure" for DNA.

PART 2. DETECTION OF DALMATIAN SAGE SSR ALLELES

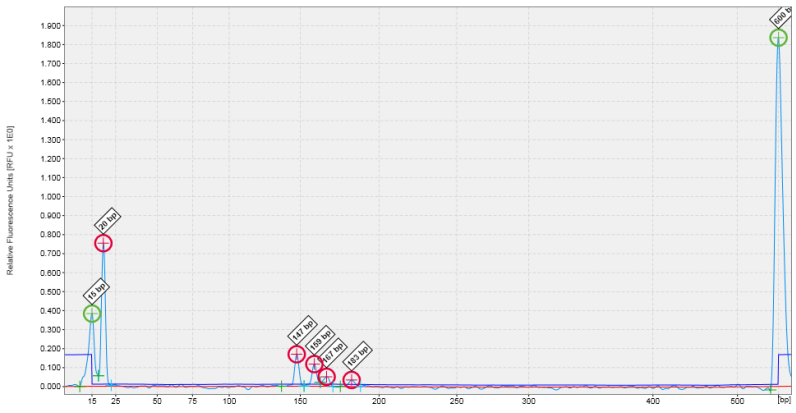
1) Fresh leaf of plant grown in Zagreb Botanical Garden

a)



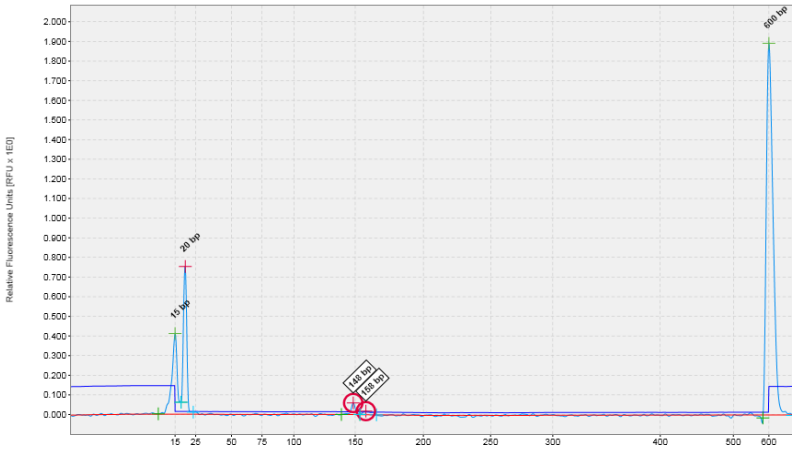
SSR locus SoUZ001F 148/160; SSR locus SoUZ019 166/184

b)



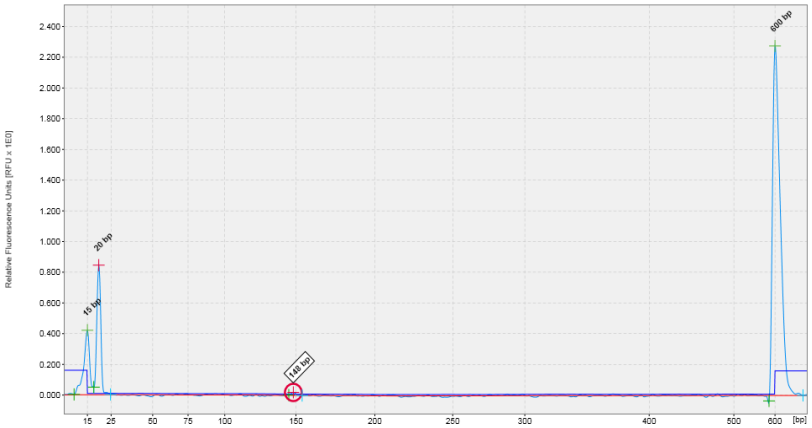
SSR locus SoUZ001F 148/160; SSR locus SoUZ019 166/184

2) Leaf from Herbarium Croaticum (ZA) collected on the Island of Biševo (Cro) in 1970



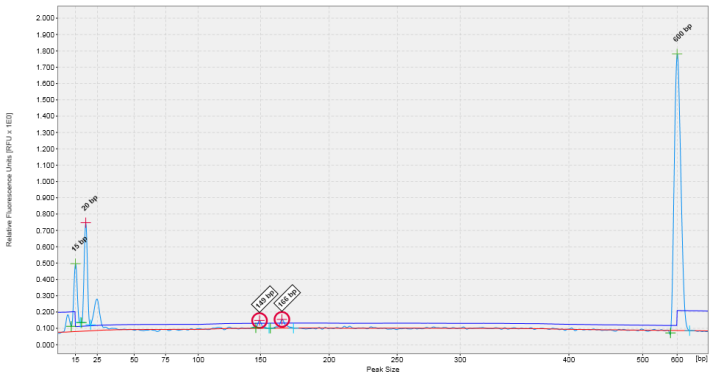
SSR locus SoUZ001F 148/160; SSR locus SoUZ019 —/—

3) Leaf from Herbarium Croaticum (ZA) collected near city of Šibenik (Cro) in 1995



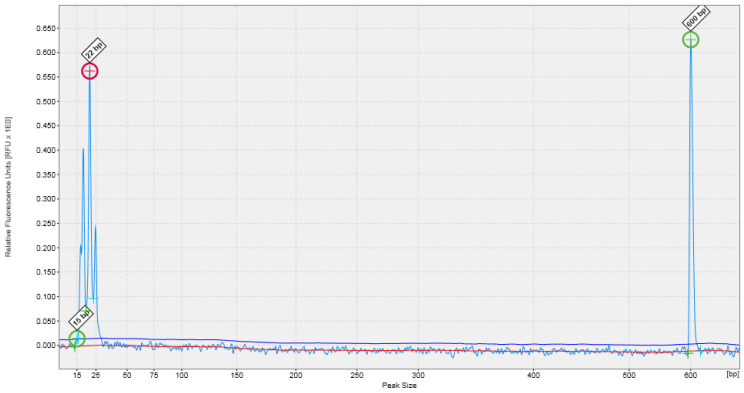
SSR locus SoUZ001F 148/148; SSR locus SoUZ019 —/—

4) Leaf from Herbarium Croaticum (ZA) collected on the Island of Krk (Cro) in 1981



SSR locus SoUZ001F 148/166; SSR locus SoUZ019 —/—

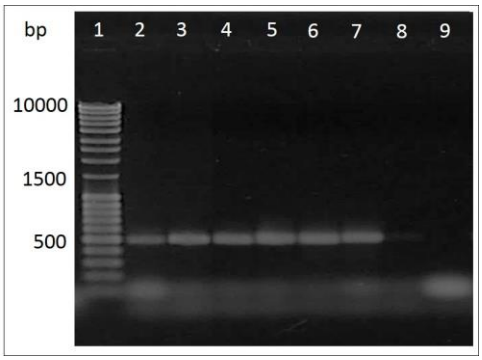
5) Leaf from Herbarium Croaticum (ZA) collected on the Island of Šipan (Cro) in 1979



SSR locus SoUZ001F —/—; SSR locus SoUZ019 —/—

Conclusion: Both SSR loci were detected in DNAs isolated from fresh leaf tissue. On the other hand, from DNA samples isolated from herbarium material only shorter SSR locus, probably because of DNA degradation, has been amplified. From DNA sample isolated from herbarium material from the island of Šipan none of two SSR loci has been amplified. This DNA isolate showed low DNA concentration and the lowest 260/280 ratio.

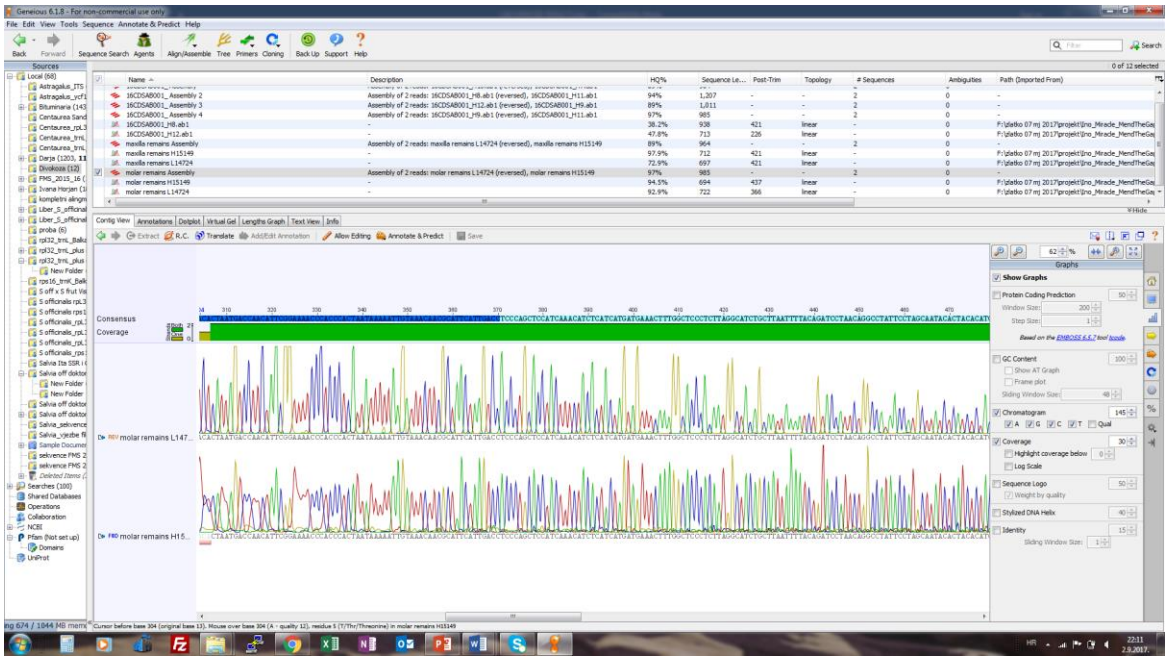
PART 3. AGAROSE-GEL ELECTROPHORESIS OF PCR AMPLIFIED MITOCHONDRIAL REGION OF BALKAN CHAMOIS



1. Massruler DNA ladder mix (Thermo™ Scientific), 2–5. Balkan chamois molar remains (Velebit (Cro) 1893) /DNA = 2,5; 5; 25 and 50 ng/20 µl PCR reaction/, 6-9. Balkan chamois maxilla remains (location and date unknown) /DNA = 2,5; 5; 25 and 50 ng/20 µl PCR reaction/.

PART 4. DNA-SEQUENCING RESULTS AND GEN BANK COMPARISON OF PCR AMPLIFIED MITOCHONDRIAL REGION OF BALKAN CHAMOIS

- Balkan chamois molar remains (Velebit (Cro) 1893)



Assembling of two strands of the same DNA sequence in Geneious® software

Consensus sequence:

5'attcaactacaagaacactaatgaccaacattcggaaaacccacccactaataaaaattgtaaacaacgcattcattgacctcccagctccatcaaacatctcatcatgatgaaactttggctcc
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tccgatacatacatgcaaatggagcatcaatatttttcatctgcctatttatacatgtaggacgaggcctatattacggatcatatacttttctagaaacatgaaacatcggagtaatcctcctactcaca
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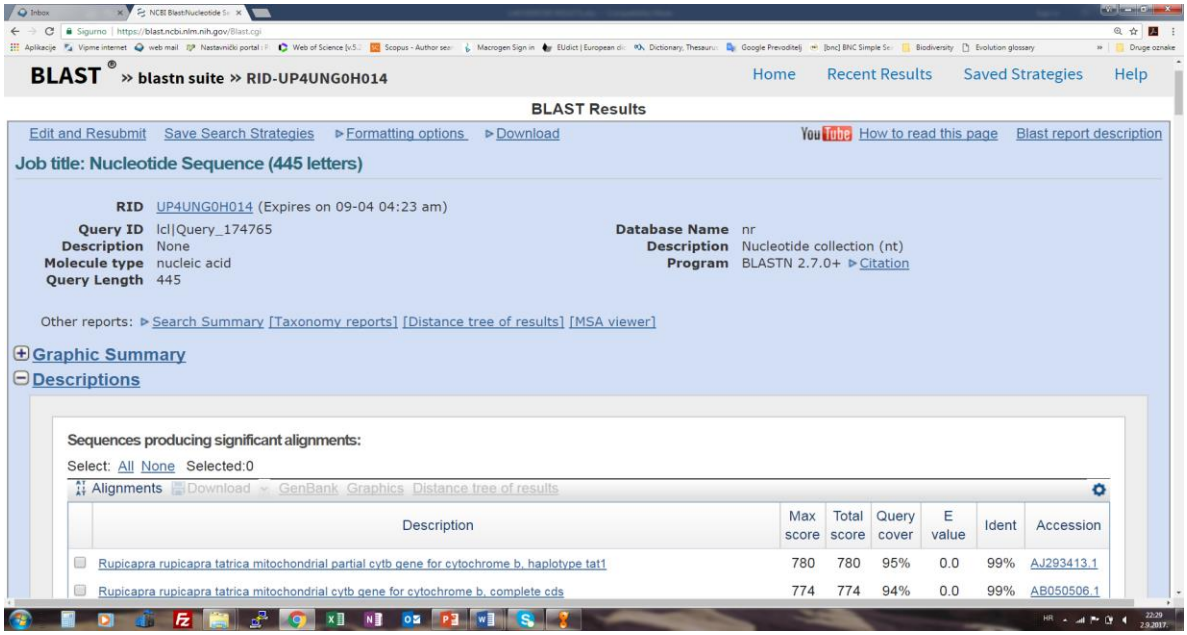
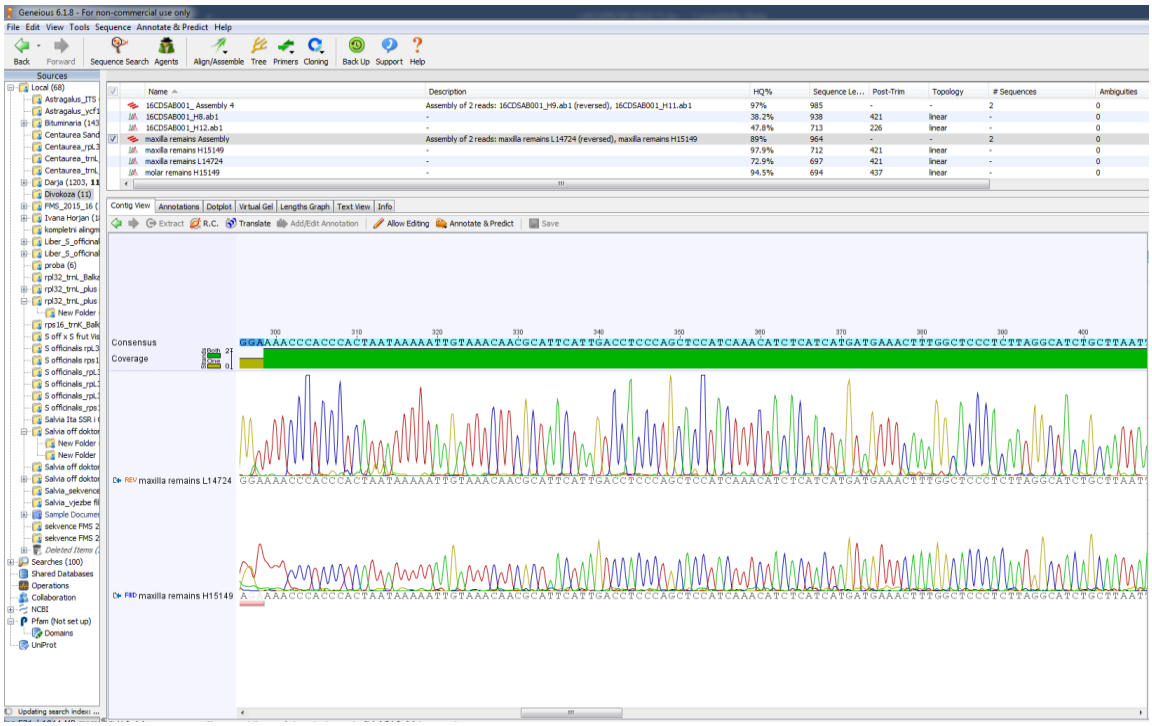


BLAST nucleotide databases search using a nucleotide query

➢ Balkan chamois maxilla remains (location and age unknown)



Assembling of two strands of the same DNA sequence in Geneious® software

Consensus sequence:

5’attcaactacaagaacactaatgaccaacattcggaaaacccacccactaataaaaattgtaaacaacgcattcattgacctcccagctccatcaaacatctcatcatgatgaaactttggct
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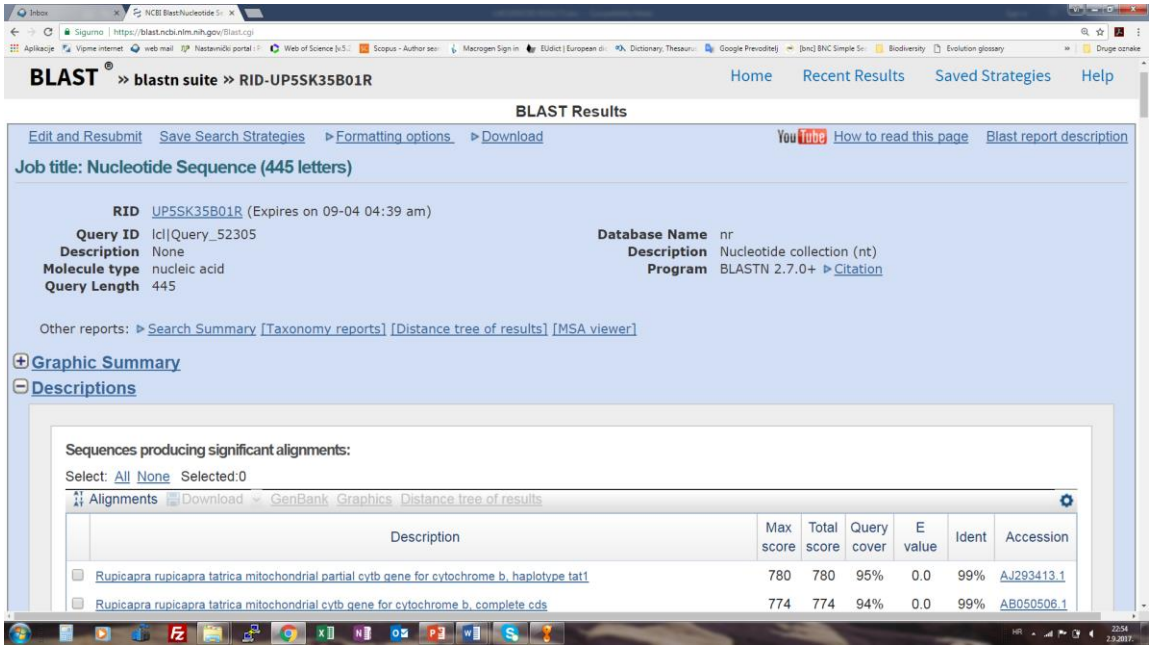

BLAST nucleotide databases search using a nucleotide query

Conclusion: This result confirmed the success of applied DNA isolation method for isolation of DNA from old bone remains. Since PCR primers are not selective and can be also used for amplification of human mitochondrial DNA, this result also indicates that there was no DNA contamination with modern DNA during DNA isolation procedure.