



LAB RESULTS

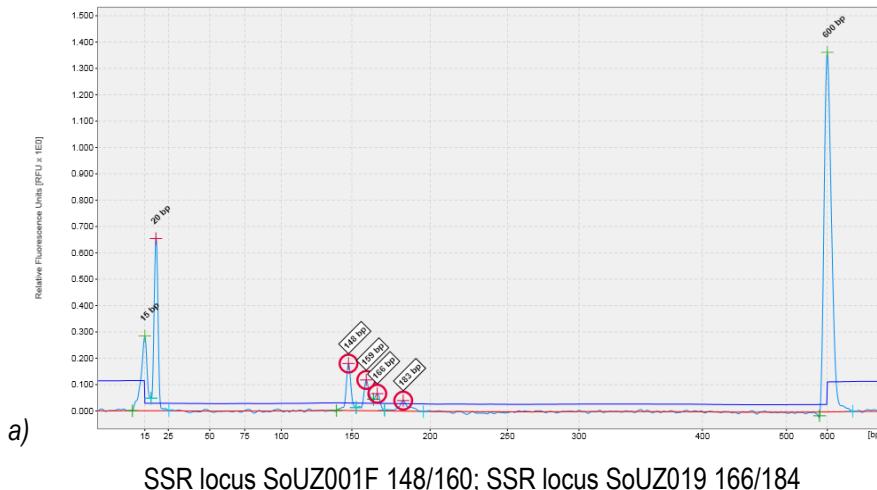
PART 1. ISOLATION OF DNA FROM HERBARIUM LEAF TISSUE AND BALKAN CHAMOIS BONE REMAINS

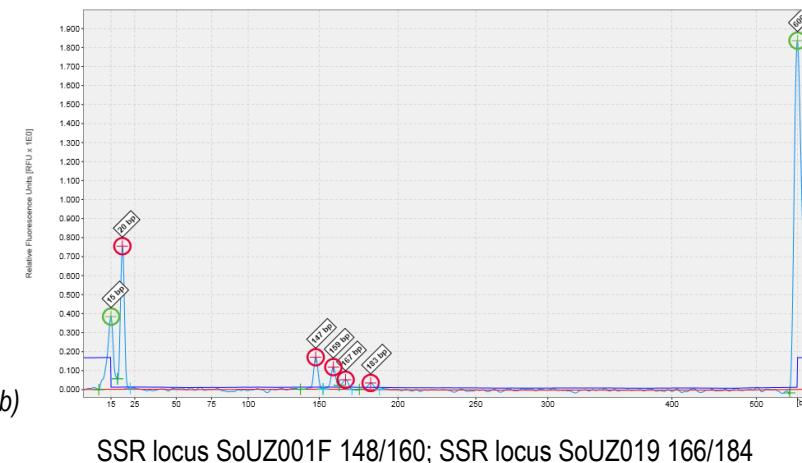
➤ DNA samples:

- 1) Fresh leaf of plant grown in Zagreb Botanical Garden
 - a) $c = 18,3 \text{ ng}/\mu\text{l}$ $260/280 = 1,738$
 - b) $c = 37,5 \text{ ng}/\mu\text{l}$ $260/280 = 1,786$
 - 2) Leaf from Herbarium Croaticum (ZA) collected on the Island of Biševo (Cro) in 1970
 $c = 13,0 \text{ ng}/\mu\text{l}$ $260/280 = 1,733$
 - 3) Leaf from Herbarium Croaticum (ZA) collected near city of Šibenik (Cro) in 1995
 $c = 3,5 \text{ ng}/\mu\text{l}$ $260/280 = 1,750$
 - 4) Leaf from Herbarium Croaticum (ZA) collected on the Island of Krk (Cro) in 1981
 $c = 5,0 \text{ ng}/\mu\text{l}$ $260/280 = 1,333$
 - 5) Leaf from Herbarium Croaticum (ZA) collected on the Island of Šipan (Cro) in 1979
 $c = 13,8 \text{ ng}/\mu\text{l}$ $260/280 = 1,618$
 - 6) Balkan chamois molar remains (Velebit (Cro) 1893)
 $c = 52,3 \text{ ng}/\mu\text{l}$ $260/280 = 1,802$
 - 7) Balkan chamois maxilla remains (location and date unknown)
 $c = 35,8 \text{ ng}/\mu\text{l}$ $260/280 = 1,879$
- 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.

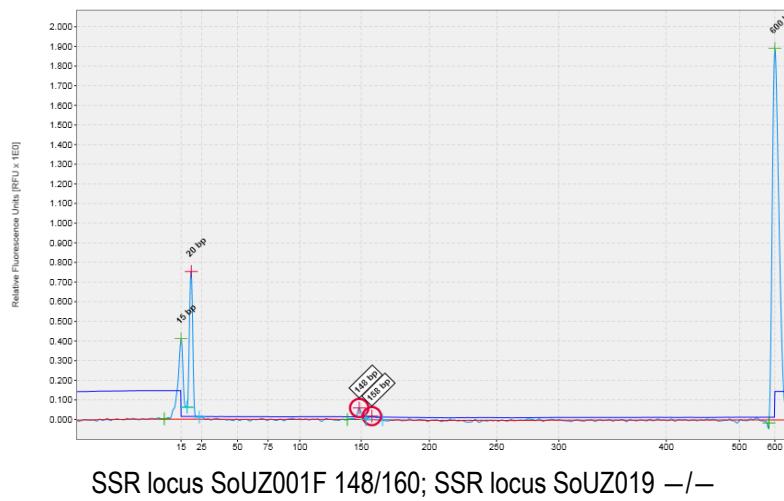
PART 2. DETECTION OF DALMATIAN SAGE SSR ALLELES

- 1) Fresh leaf of plant grown in Zagreb Botanical Garden

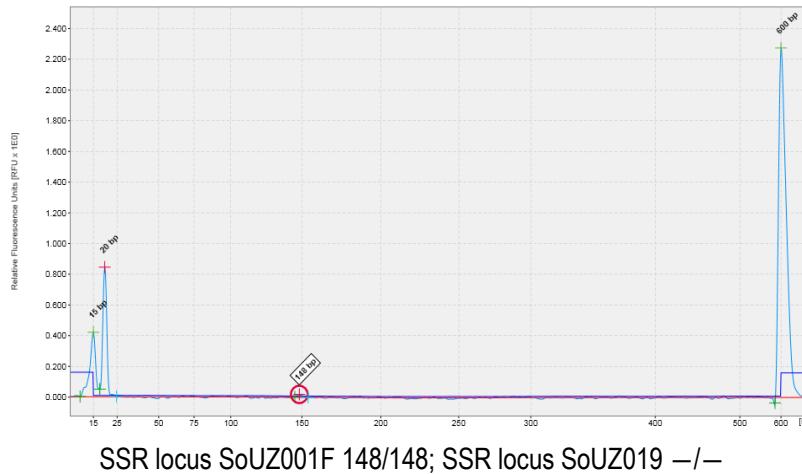




2) Leaf from Herbarium Croaticum (ZA) collected on the Island of Biševo (Cro) in 1970

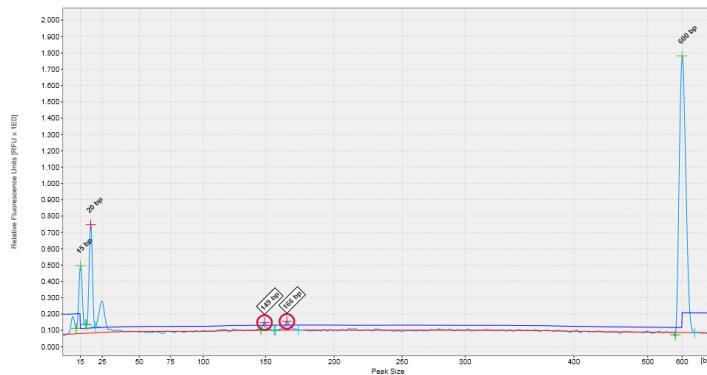


3) Leaf from Herbarium Croaticum (ZA) collected near city of Šibenik (Cro) in 1995



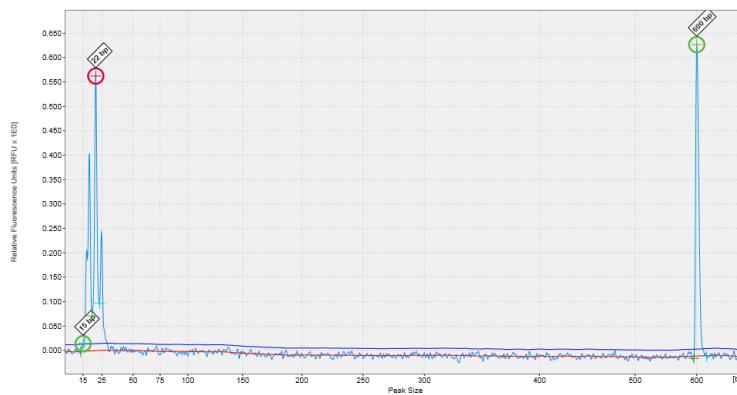


4) Leaf from Herbarium Croaticum (ZA) collected on the Island of Krk (Cro) in 1981



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

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

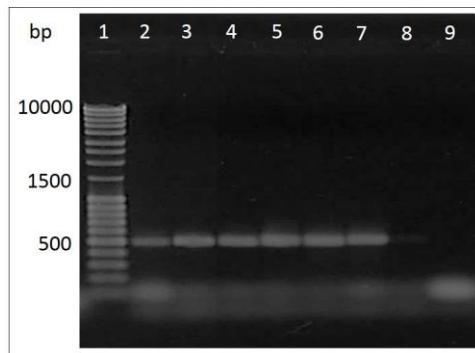


SSR locus SoUZ001F —/—; SSR locus SoUZ019 —/—

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.



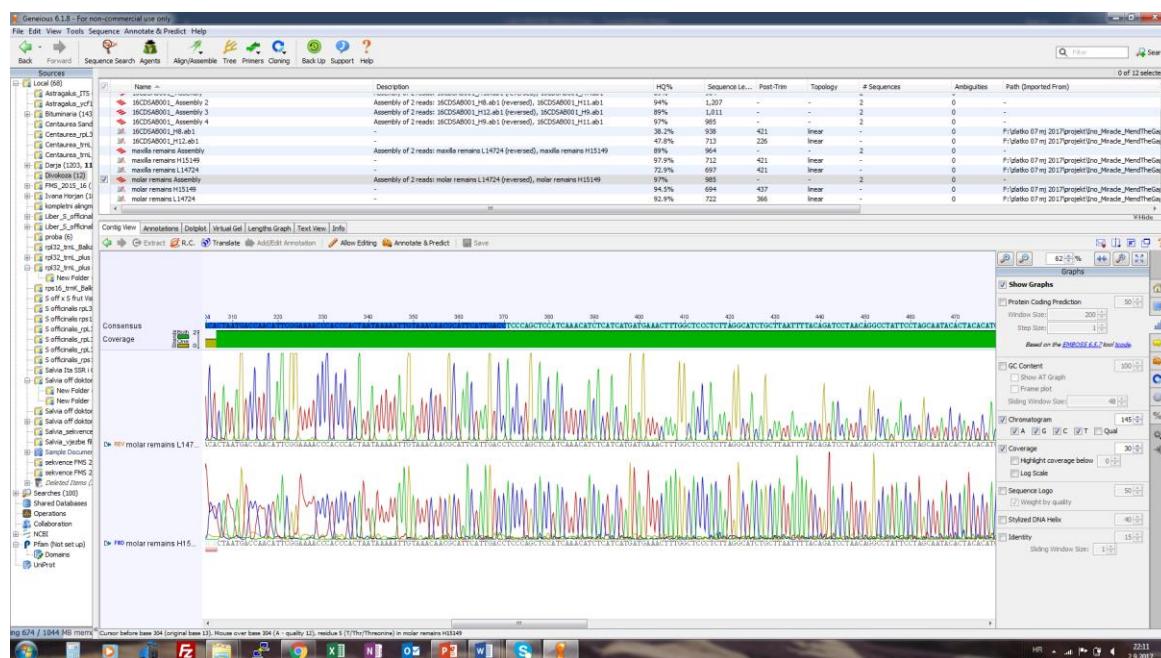
PART 3. AGAROSE-GEL ELECTROPHORESIS OF PCR AMPLIFIED MITOCHONDRIAL REGION OF BALKAN CHAMOIS



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'attcaactacaagaactaatgtaccaacattcgaaaaaccaccttaataaaaatgtaaacaacgcattcatgtacccagctccatcaaacatctcatatgtatggaaacttggctcc
ctcttaggcattctgctaattttacagatcataacaggcatttccttagcaatacacatcacatccgatacagcaacagcatttcctctgtatacccacatgtccgagatgtaaactacggctaatca



tccgatacatacatgcaaatggagcataaatttcatctgcatttatacatgttaggacggccatataccgatatactttctagaaacatgaaacatcgaggataatccctactcaca
acaatagccacagcgttatggctacgtcctaccatgaggacaatatcattctgagg 3'

BLAST Results

Job title: Nucleotide Sequence (445 letters)

RID UP4UNG0H014 (Expires on 09-04 04:23 am)

Query ID Icl|Query_174765

Description None

Molecule type nucleic acid

Query Length 445

Database Name nr

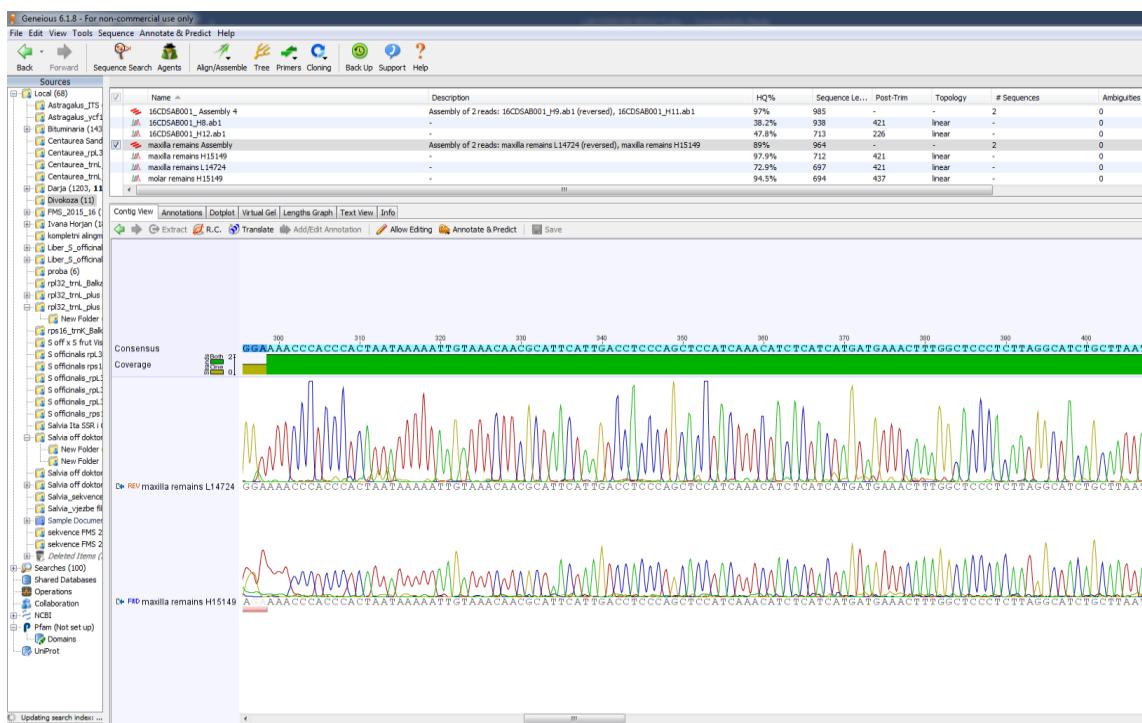
Description Nucleotide collection (nt)

Program BLASTN 2.7.0+ ►Citation

Description	Max score	Total score	Query cover	E value	Ident	Accession
Rupicapra rupicapra tatra mitochondrial partial cytb gene for cytochrome b, haplotype tat1	780	780	95%	0.0	99%	AJ293413.1
Rupicapra rupicapra tatra mitochondrial cytb gene for cytochrome b, complete cds	774	774	94%	0.0	99%	AB050506.1

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'attcaactacaagaacactaatgaccaaacattcgaaaaacccaccactaataaaaattgtaaacaacgcattcattgacctcccagctccatcaaacatctcatcatgtgaaaacttggctccctttaggcattgtcttaattttacagatcctaacaggcatttccttagcaatacacatcacatccgatcacagaacacgcatttcctctgttaaccacatttgccgagatgtaaactacggotgaatcatccgatatacatatcgaaatgggcatcaataattttcatctgcatttatacatgttaggacgaggcattatacggatcatatactttctagaaaacatgaaacatcgagtaatccctactcacaacaatagccacagcgttatggctacgttaccatgaggagacaatatacatctgagg 3'

The screenshot shows a web browser window with the URL <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. The page title is "BLAST Results". The search parameters are listed as follows:

Query ID	Icl Query_52305
Description	None
Molecule type	nucleic acid
Query Length	445

The database used is "nr" (Nucleotide collection (nt)) with the program "BLASTN 2.7.0+". The search results table shows two significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Rupicapra rupicapra tatraica mitochondrial partial cytb gene for cytochrome b, haplotype tat1	780	780	95%	0.0	99%	AJ293413.1
Rupicapra rupicapra tatraica mitochondrial cytb gene for cytochrome b, complete cds	774	774	94%	0.0	99%	AB050506.1

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.