Characterisation of allele variants at canine FH2054 microsatellite locus

Jakob Lavrencic, Jernej Ogorevc and Peter DOVC

Department of Animal Science

Biotechnical Faculty, University of Ljubljana, Slovenia

Background:

- In the DCT 2016 were identified some issues regarding the genotyping of microsatellite locus FH2054
- The rate of mistakes was high (81 from 1360 genotypes)
- The locus was considered to be tetranucleotide repeat and therefore the most laboratories genotyped alele 148, however the „problem animal“ DCT-05 was genoyped by several labs as 147/176.
- SC members decided that sequencing of allele variants for this particular locus could solve the problem and the sequencing task was assigned to our lab.

Methodology

- PCR amplification of samples from 2016 DCT, some saples from 2014 DCT and some samples from the local Karst Shepherd dogs
- Isolation of PCR products from agarose gel
- Cloning of PCR products using pGEM®-T Cloning kit (Promega)
- Sequencing recombinant plasmids using Sanger chemistry on ABI 3100xl.

Successfully amplified and sequenced alleles

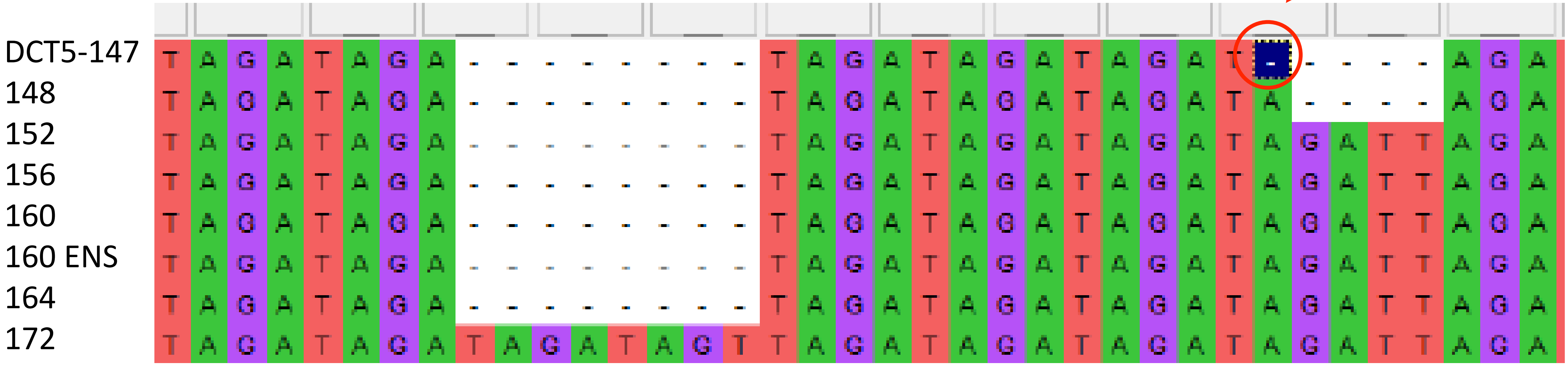
- FH2054 147 (individual DCT-5)
- FH2054 148
- FH2054 152
- FH2054 156
- FH2054 160 (comparison with sequence from Ensembl Database)
- FH2054 164
- FH2054 168 (not cloned yet)
- FH2054 172
- FH2054 176

Summary of the sequence in the MS region

- allele_{147}TCTTCTA(GATA)$_3$ GAT (GATA)$_8$ ______GATA ATAGCTCCAAAGTT*
- allele_{148}TCTTCTA(GATA)$_3$ GAT (GATA)$_8$ _____AGATA ATAGCTCCAAAGTT
- allele_{152}TCTTCTA(GATA)$_3$ GAT (GATA)$_8$ GATTAGATA ATAGCTCCAAAGTT
- allele_{156}TCTTCTA(GATA)$_3$ GAT (GATA)$_9$ GATTAGATA ATAGCTCCAAAGTT
- allele_{160}TCTTCTA(GATA)$_3$ GAT (GATA)$_{10}$GATTAGATA ATAGCTCCAAAGTT
- ENS_{160} TCTTCTA(GATA)$_3$ GAT (GATA)$_{10}$GATTAGATA ATAGCTCCAAAGTT
- allele_{164}TCTTCTA(GATA)$_3$ GAT (GATA)$_{11}$GATTAGATA ATAGCTCCAAAGTT
- allele_{172}TCTTCTA(GATA)$_3$ GAT (GATA)$_{13}$GATTAGATA ATAGCTCCAAAGTT
- allele_{176}TCTTCTA(GATA)$_3$ GAT (GATA)$_{14}$GATTAGATA ATAGCTCCAAAGTT

* Underlined are MS flanking sequences

Sequence alignment illustrating alelle 147



```
DCT5-147  TAGATAGA--------TAGATAGATAGAT-----AGA
148       TAGATAGA--------TAGATAGATAGATA----AGA
152       TAGATAGA--------TAGATAGATAGATAGATTAGA
156       TAGATAGA--------TAGATAGATAGATAGATTAGA
160       TAGATAGA--------TAGATAGATAGATAGATTAGA
160 ENS   TAGATAGA--------TAGATAGATAGATAGATTAGA
164       TAGATAGA--------TAGATAGATAGATAGATTAGA
172       TAGATAGATAGATAGTTAGATAGATAGATAGATTAGA
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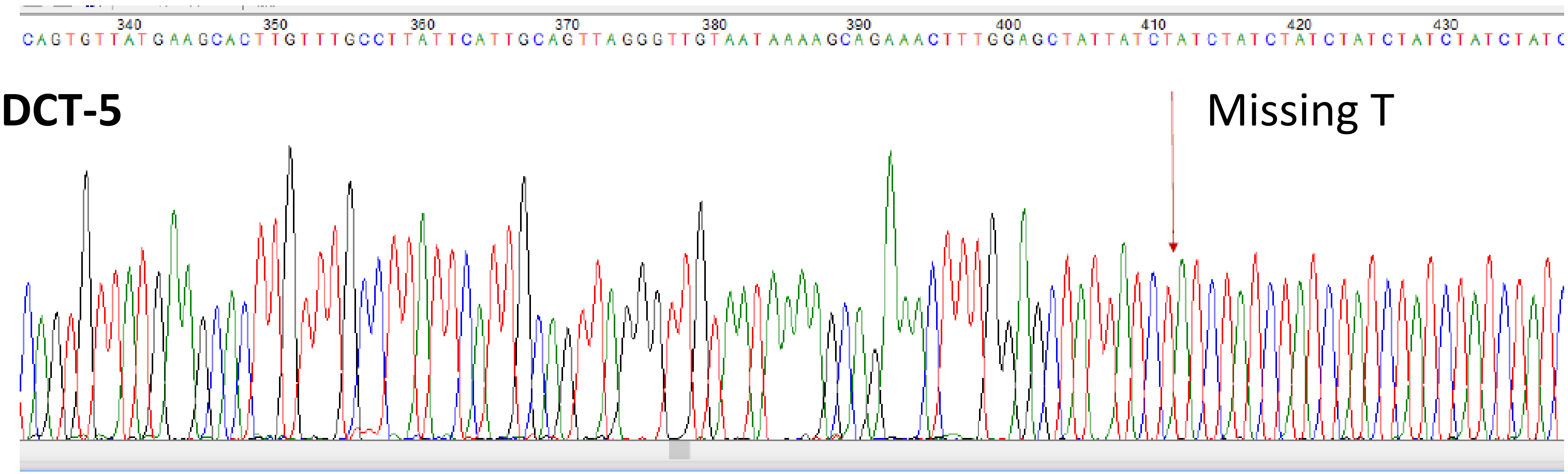
Missing A in DCT-5

Difference between 164 and 172

Sequence of the DCT-5 individual



DCT-5

Missing T

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340 350 360 370 380 390 400 410 420 430
CAGTGTTATGAAGCACTTGTTTGCCTTATTCATTGCAGTTAGGGTTGTAATAAAAGCAGAAACTTTGGAGCTATTATCTATCTATCTATCTATCTATCTATCTAT
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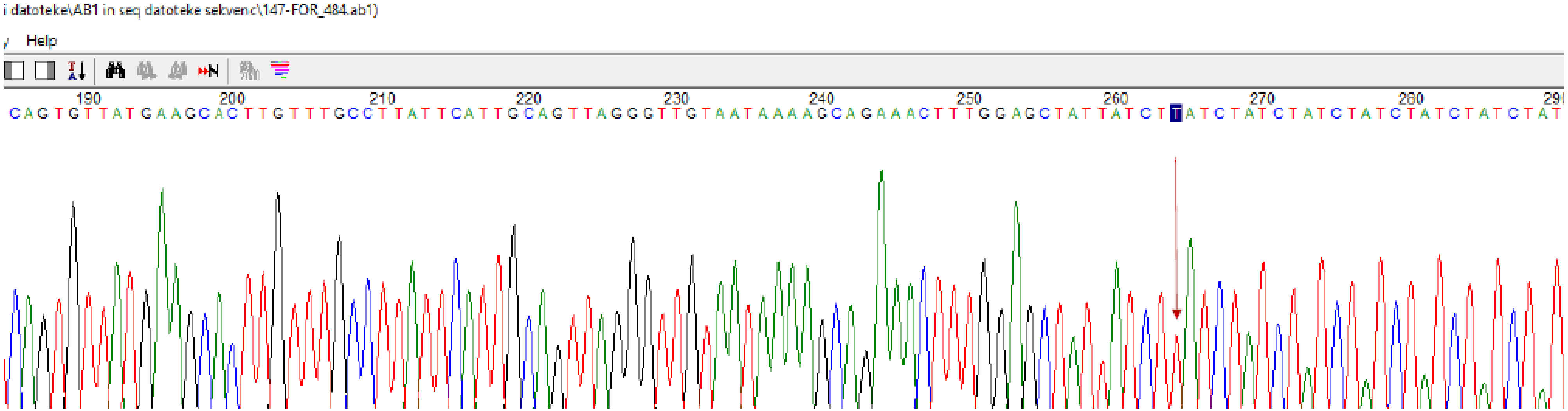

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190 200 210 220 230 240 250 260 270 280
CAGTGTTATGAAGCACTTGTTTGCCTTATTCATTGCAGTTAGGGTTGTAATAAAAGCAGAAACTTTGGAGCTATTATCTTATCTATCTATCTATCTATCTATCTAT
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Summary

- MS FH2054 is a tetranucleotide microsatellite with alleles 148, 152, 156, 160, 164, 168, 172 and 176.
- The repeat region is composed of GATA repeats with several irregularities (composed MS containing also GAT and GATT motifs)
- One animal from DCT 2016, DCT-5, showed a „shorter allele“ (147), which is due to the deletion of A, which is in all other animals inserted in front of the last GATA motif. This reduces the fragment length from 148 to 147 and was the reason for different genotyping results in DCT 2016.