# Genetic Characterization of *Canis* Samples

**Submitted by Joseph Butera**

Report Prepared for Joseph Butera
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## Purpose:

Provide a genetic characterization of a single *Canis* sample submitted by Joseph Butera.

## Executive Summary:

The sample submitted yielded sufficient DNA to allow for genetic assignment among Canadian *Canis* categories in context of an in-house reference database. The sample was of an admixed nature predominated by Great Lakes wolf ancestry.

## Items Receipt:

One 15 mL tube was received by the Natural Resources DNA Profiling and Forensic Centre on March 22nd, 2022. The tube contained a tongue sample cut into two pieces, suspended in ethanol. The tube was labelled as follows:

Sample: 85-pound canid's tongue for DNA testing
Collected: 1/15/2022, New York State
By: Joseph Butera
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The sample was given the laboratory ID "CAN_T" and stored in a refrigerator upon arrival.

## Results Summary:

A CAN_T mtDNA sequence was assigned a *C. lycaon* haplotype and inferred ancestral origins according to Wilson et al. 2003 (**Table 1**). We identified the sample as male based on Zfx/Zfy amplification for sex identification (**Table 1**). We obtained a genotype for the sample at 12 microsatellite loci (**Table 2**) and conducted an ancestry proportion test in *STRUCTURE* (**Table 1**).

## Conclusion:

| **Laboratory ID** | **Genetic assignment based on microsatellite data and ancestry Q-value > 80% in context of current *Canis* database** |
|---|---|
| CAN_T | admixed; predominated by Great Lakes wolf ancestry |

**Table 1. Summary of sexing, mtDNA haplotype, and nuDNA microsatellite results**

| Laboratory ID | Type | Sex (Zfx/Zfy) | mtDNA Haplotype and Inferred Ancestral Lineage | | Microsatellite Loci (of 12) | Microsatellite Inferred Ancestry | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Dog | SK Coyote | Eastern Coyote | NT Grey Wolf | Great Lakes Wolf | Eastern Wolf |
| CAN_T | Tissue | Male | CRL5_C3 | *C. lycaon* | 12 | 0.007 | 0.009 | 0.003 | 0.345 | 0.526 | 0.109 |

**Table 2. Microsatellite genotyping data**

| Laboratory ID | Microsatellites locus (cxx#) | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 225 | | 200 | | 123 | | 377 | | 250 | | 204 | | 172 | | 109 | | 253 | | 442 | | 410 | | 147 | |
| CAN_T | 163 | 167 | 211 | 217 | 147 | 153 | 154 | 156 | 131 | 131 | 200 | 200 | 156 | 156 | 149 | 149 | 109 | 109 | 166 | 166 | 114 | 118 | 172 | 174 |

## Methods:

### *DNA Extraction*

A small portion of tissue was taken using a scalpel and digested in 0.5 mL of tissue lysis buffer plus 40 µL of Proteinase K. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following manufacturer protocols. The sample was processed in duplicate.

### *DNA Amplification*

The mitochondrial DNA (mtDNA) control region was amplified and sequenced with primers AB13279 and AB13280. PCR and sequencing conditions used as described in Wheeldon et al. (2010) with the following adjustments in amplification conditions: an initial denaturing at 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 2 minutes. Amplified products were separated on an agarose gel stained with ethidium bromide and visualized under UV light. Amplified products were cleaned with ExoSAPIT (Thermofisher) and sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (Thermofisher).

For sex amplification, DNA was amplified at a region of the Zfx/Zfy genes on the X and Y-chromosomes and a region of the Sry gene on the Y-chromosome (P1-5EZ and P2-3EZ: Aasen and Medrano 1991; Y53-3C and Y53- 3D: Fain and LeMay 1995). Sex-specific amplified products were visualized under UV light.

12 autosomal microsatellite loci (cxx225, cxx200, cxx123, cxx377, cxx250, cxx204, cxx172, cxx109, cxx253, cxx442, cxx410, and cxx147) were amplified as three multiplex reactions for each sample in a total reaction volume of 15 µL with 1X Qiagen Multiplex Mastermix (Qiagen), 0.12 µM of primers, and 2 µL of DNA. Products were amplified under the following conditions: 94°C for 15 minutes, 32 cycles of 94°C for 30 seconds, 56-58°C for 90 seconds, 72°C for 1 minute, with a final extension at 60°C for 45 minutes.

### *Sequenced Analysis*

The mtDNA control region was sequenced using an ABI 3730 genetic analyzer (Applied Biosystems). Sequences were edited and aligned in the software package MEGA v11 and consensus sequences were aligned with sequences of known haplotypes from the NRDPFC *Canis* database consisting of published haplotypes from Wilson et al 2003, Rutledge et al 2010, and Wheeldon et al. 2010 Additionally, sequences were compared to the National Center for Biotechnology Information (NCBI) database with the Basic Local Alignment Search Tool (BLAST). Haplotypes were assigned based on the NRDPFC database, and in reference to any identical sequences in the NCBI database. Putative species of origin were assigned to each sample based on the mtDNA sequences in context of published haplotypes and putative ancestry origin according to Wilson et al. (2003).

### *Microsatellite Genotyping*

All autosomal microsatellite amplifications were analyzed on an ABI 3730 (Applied Biosystems) genetic analyzer.

### *Inferring Ancestry Proportion using* STRUCTURE

To infer *Canis* ancestry proportions of CAN_T, we used an in-house database consisting of 350 reference genotypes from samples assigned to Ontario (ON) *Canis* groups (60 Great Lakes wolf, 60 eastern wolf, 60 eastern coyote), 60 ON domestic dog, 60 Saskatchewan (SK) coyote (unhybridized *C. latrans*), and 50 Northwest Territories (NT) grey wolf (unhybridized *C. lupus*). We estimated population clustering and ancestry proportions using *STRUCTURE* v2.3.4 (Pritchard et al. 2000, Falush et al. 2003, 2007) with default settings. We implemented the F-model (i.e., the correlated allele frequencies model) and ran the admixture model 50 times at K = 1 through K = 10 for 5.0E+5 iterations following an initial burn-in of 2.5E+5 iterations. Results were evaluated using the online tool CLUMPAK vbeta (Kopelman et al. 2015), where we retrieved *STRUCTURE* plots and admixture proportions (Q-values) across the runs. We assigned the individual to populations based on a Q-value threshold of 0.800 (note, this value is a commonly used threshold in the literature, but other values could be applied); otherwise, we considered the individual to be admixed.

## References:


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