Mammalian Ecology and Conservation Unit

Optimizing tissue sampling and extraction protocols for next-generation genomic sequencing

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Introduction

The application of genomic tools in wildlife studies allows researchers to characterize and monitor populations, as well as understand mechanisms affecting genetic variation, adaptation, and evolution. However, these tools depend on high-quality genomic DNA, which typically comes from tissues rather than noninvasive sources (e.g., scat, hair). Opportunistically collected samples (e.g., road-killed carcasses) may be an important genomic source for some species, but can result in highly variable DNA quality depending on freshness of the sample and environmental conditions. Different tissues degrade at variable rates post-mortem, with softer tissue being prone to faster degradation than harder tissue. Though softer tissue contains the highest quality and quantity of DNA, its quick rate of decay may make it a poor sampling choice for highly degraded field specimens, and harder tissue may be more suitable in these instances.[1]

Objectives

- Determine which tissue types provide the highest quality genomic DNA at different levels of decay.
- Create optimal guidelines for sample collection in the field.
- Determine the most effective and efficient extraction method to maximize DNA quality for genomic sequencing.

Methods

Sample Collection

We classified Red Fox (*Vulpes vulpes*) carcasses as either fresh, decaying (including stages of bloat, active decay, and advanced decay), or dry remains.[2] From these carcasses, we collected two different types of soft tissue (spleen, and gonads), two types of harder tissue (muscle and kidney) and three types of alternative tissue (nose, skin, and tongue).

Extraction

Each sample was extracted with both silica membrane binding (DNeasy® Blood and Tissue Kit Qiagen) and salting out precipitation (Gentra® Puregene® Qiagen) extraction methods.

Quality assessment

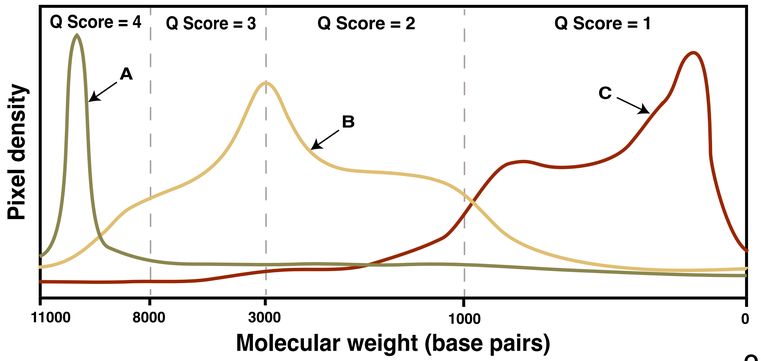
DNA fragment size was assessed using gel electrophoresis.

Analysis

We analyzed gel images with GelAnalyzer software and used pixel density to give each sample a quality score (0-4) based on the size distribution of the DNA fragments, with higher molecular weight samples corresponding to higher quality scores.[3] We assessed the difference in mean quality score (Q-Score) for each extraction method and condition using one-way ANOVA, and performed multiple pairwise comparisons between the means of groups using the Tukey Honest Significant Differences test.

	Fresh	Decay	Dry Remains
Spleen	13	-	-
Gonads	12	-	-
Kidney	13	-	-
Muscle	16	8	-
Skin	6	9	9
Nose	6	9	9
Tongue	5	6	2

Table 1. We analyzed a total of 123 tissue samples across 7 tissue types and 3 conditions. Each sample was extracted using both the DNeasy® and Gentra® Puregene® methods for a total of 246 DNA extracts.



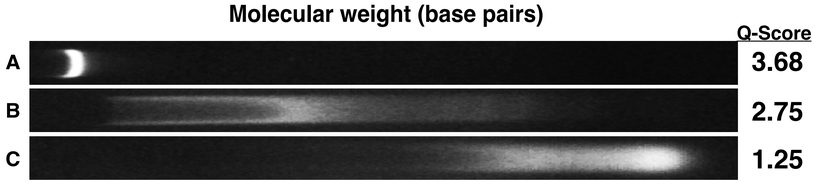


Figure 1. Using GelAnalyzer software and a 1,000 base-pair (bp) ladder (New England Biolabs) for reference, we divided each lane into four categories corresponding to molecular weight. Category 1 (0-1000 bp), Category 2 (1000-3000 bp), Category 3 (3000-8000 bp), and Category 4 (8000-11000 bp). We were then able to assign a quality score to each sample based on the percentage of pixel density present in each of the four categories. Representative samples A, B and C are shown on the graph with their corresponding gel images and final quality scores.

Results

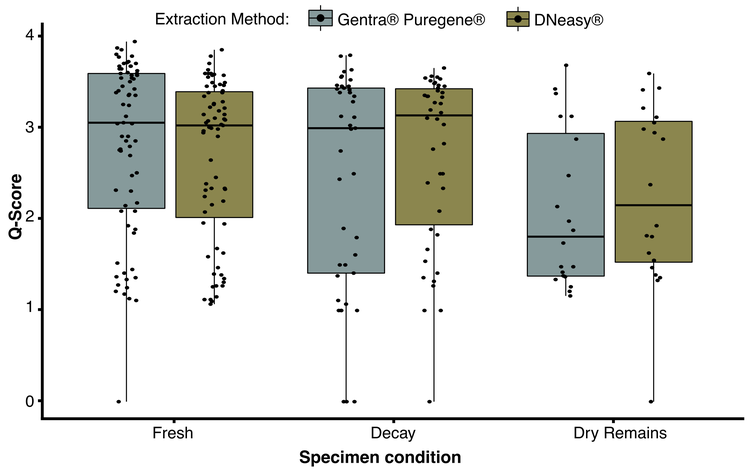


Figure 2. Boxplot showing Q-Scores of all tissue samples grouped by specimen condition and extraction method. One-way ANOVA results showed no significant difference between extraction methods.

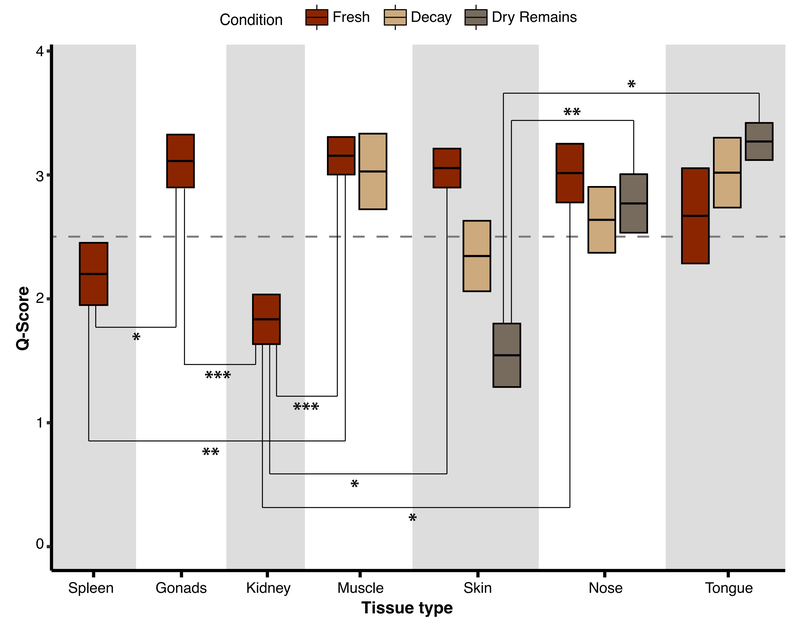


Figure 3. Plot showing the means and standard errors of quality scores from DNeasy® extracts grouped by tissue type and specimen condition. Significant results from the multiple pairwise comparison of means are indicated as follows: p<0.05 = (*), p<0.01=(**), p<0.001=(***). Samples above the dashed line (Q-score >2.5) are suitable for use in genomic studies.

Conclusions

- There was no significant difference in quality scores between extraction method across all decay levels and tissue types, however we found that Gentra® Puregene® is both more costly and more time consuming.
- We found that muscle and gonads produced the highest quality DNA in fresh samples.
- Nose, tongue, and skin are the only tissues that were reliably available under all specimen conditions, and while skin produces high quality DNA in fresh samples, nose and tongue perform more consistently across conditions, and remain suitable for genomic sequencing in all stages.
- Assuming the results of this experiment are transferrable to other vertebrates, we recommend using the DNeasy® Blood and Tissue Kit, and sampling the following tissue for each carcass condition:
  - Fresh - muscle, gonads, or skin
  - Decay - nose, tongue, or muscle
  - Dry Remains - nose or tongue

If consistent sampling is desired across all decay levels, we recommend sampling nose or tongue as it was a reliable source of genomic quality DNA under all conditions.

References

1) Wong, P. B., Wiley, E. O., Johnson, W. E., Ryder, O. A., O'Brien, S. J., Haussler, D., et. al. (2012). Tissue sampling methods and standards for vertebrate genomics. *GigaScience*, *1*(1), 8.
2) Payne, J.A. (1965). "A summer carrion study of the baby pig sus scrofa Linnaeus." *Ecology*. 46 (5):592-602.2
3) GelAnalyzer Software version 2010a