Enrichment tools to better understand the different types of circulating nucleosomes and their associated genome patterns in the plasma of dogs with lymphoma

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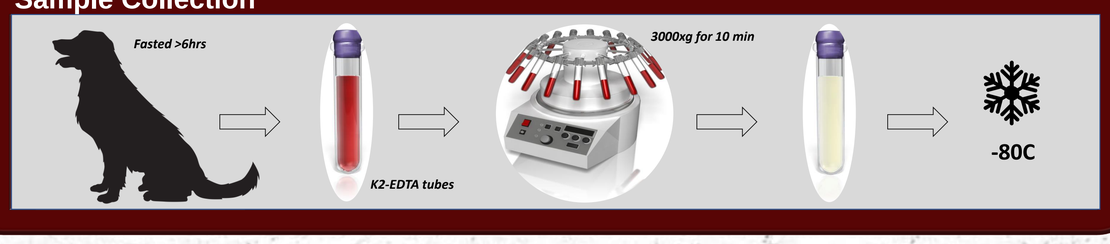
Introduction

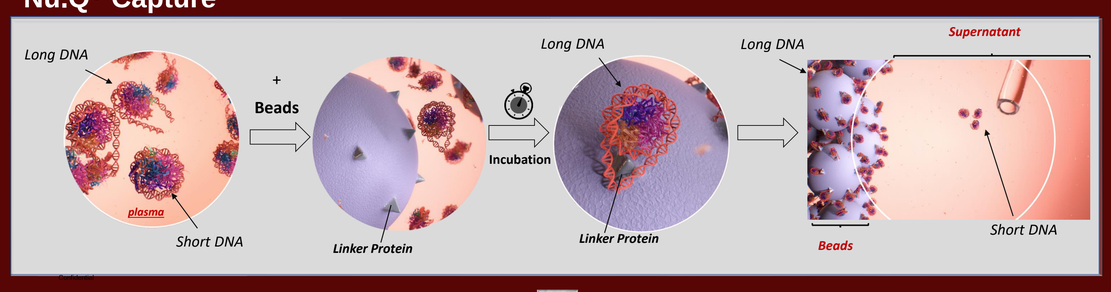
- Nucleosomes are the most basic structural components of DNA packaging, consisting of an eight-histone core around which ~146 base pairs of DNA are wrapped (1).
- Circulating nucleosomes are derived from dying/activated white blood cells, apoptotic cells, and tumor cells (2).
- Circulating nucleosome levels are elevated in disease and have been shown to have prognostic significance in humans with sepsis, severe burns, immune-mediated diseases, and cancer (2-3).
- An ELISA targeting nucleosomes containing histone H3.1, one of the histones comprising the protein core, and has been previously validated for measuring circulating nucleosomes in dogs with lymphoma and elevations in cancer-associated nucleosomes have been documented in this patient population (3-4).
- Due to the loss of linker DNA, circulating cancer-associated nucleosomal DNA is shorter (147bp) than nucleosomes derived from normal cellular turnover (165bp) (5).
- H1 Nu.Q® Capture is an enrichment methodology utilizing tosyl-activated magnetic beads coated with H1 protein to efficiently bind linker DNA retained by non-cancer derived (i.e., normal) nucleosomes from plasma, thereby enriching shorter, cancer-derived nucleosomes (5).
- To date, there are no studies describing enrichment of cancer-associated nucleosomes by Nu.Q® Capture methodology in dogs with multicentric lymphoma.
- The **aim** of this study was to isolate and sequence cancer-derived nucleosomes from dogs diagnosed with lymphoma.

Methods

Sample Collection



Nu.Q® Capture



Sequencing Analysis

Whole genome sequencing	Align to CanFam4	Variant calling	Variant annotation
Illumina 2x150bp Average Depth 40X	Bwa-mem2	GATK 4.1.10 HaplotypeCaller Mutect2	SNPeff

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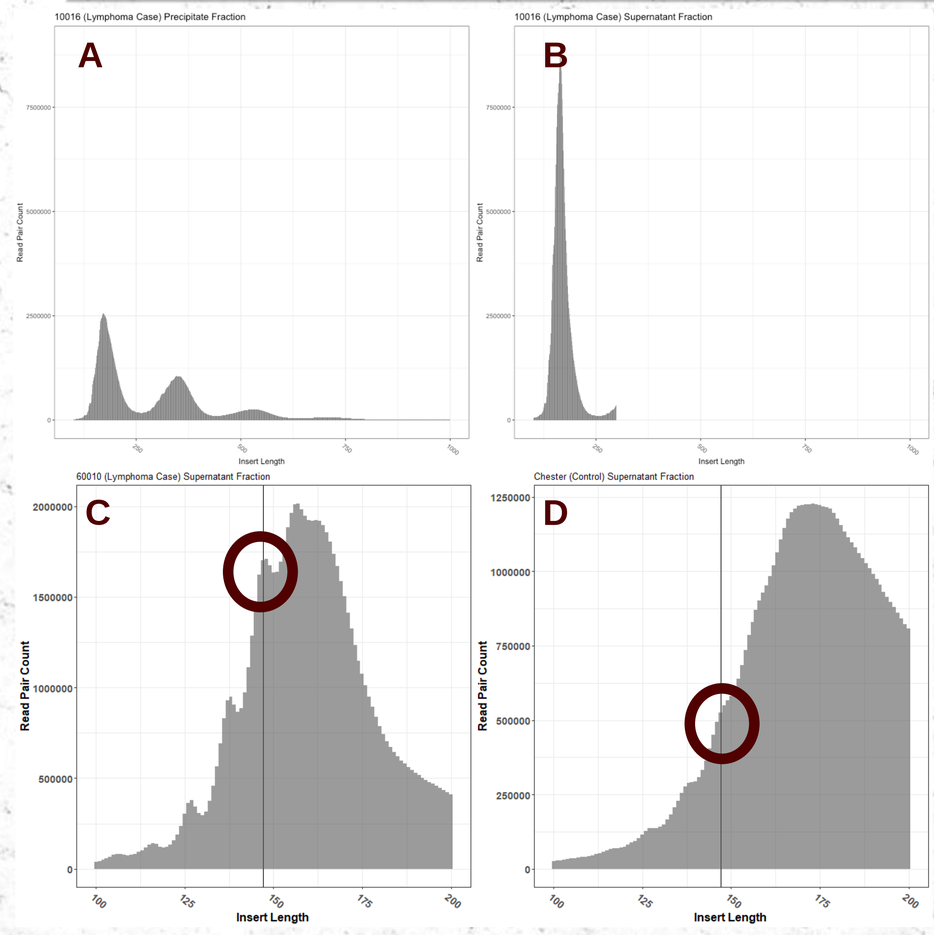
Results



Patient ID	Nucleosome Content (ng/mL)
Chester (c)	24.4
LSA (10016)	>2500
LSA (60010)	>2500

Figure 1: Plasma-derived nucleosome sequencing histograms. A: Sequencing histogram of lymphoma patient 10016 immuno-precipitate. B: Sequencing histogram of lymphoma-patient 10016 supernatant. C: Sequencing histogram of supernatant fraction highlighting enrichment of 147bp insert size. D: Sequencing histogram of control (healthy) patient supernatant fraction.

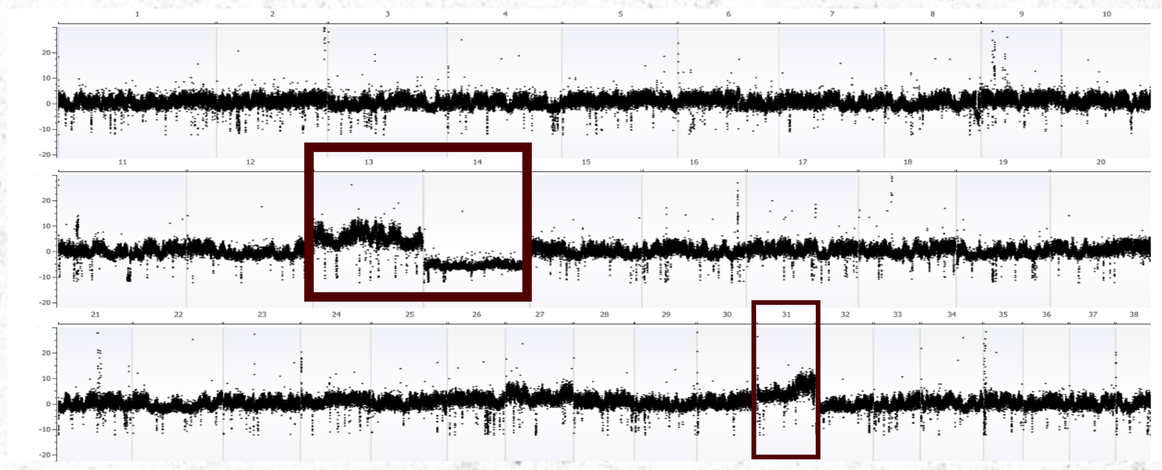


Figure 2: Aneuploidy and copy number variation. Depicting gain of chromosome 13, loss of chromosome 14, and potential gain of the q arm of chromosome 31 (highlighted panels). Other potential copy number variants exist across chromosomes.

Conclusions

- Canine lymphoma patients have circulating nucleosomes lacking linker DNA (i.e., shorter nucleosomes) that are not detected in plasma from healthy canines.
- The Nu.Q® Capture is capable of enriching canine cancer-associated nucleosomes in plasma of naïve multicentric lymphoma patients.
- These shorter, canine lymphoma-associated, nucleosomes demonstrate rare genetic variants, most notably gain of chromosome 13, loss of chromosome 14, and potential aberrations on chromosome 31.

Future Directions

- Optimize Nu.Q® Capture assay for enhanced cancer-associated nucleosome enrichment.
- Deeper analysis of candidate genes and known drivers of canine lymphoma with the goal of identifying diagnostic and prognostic markers in a wider variety of lymphoma cases.
- Expansion of the Nu.Q® methodology and sequencing pipelines utilized in this study to additional canine cancers, including hemangiosarcoma and osteosarcoma.
- Combine H1 Nu.Q® Capture with additional assays to better understand cancer derived circulating nucleosomes

References

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