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

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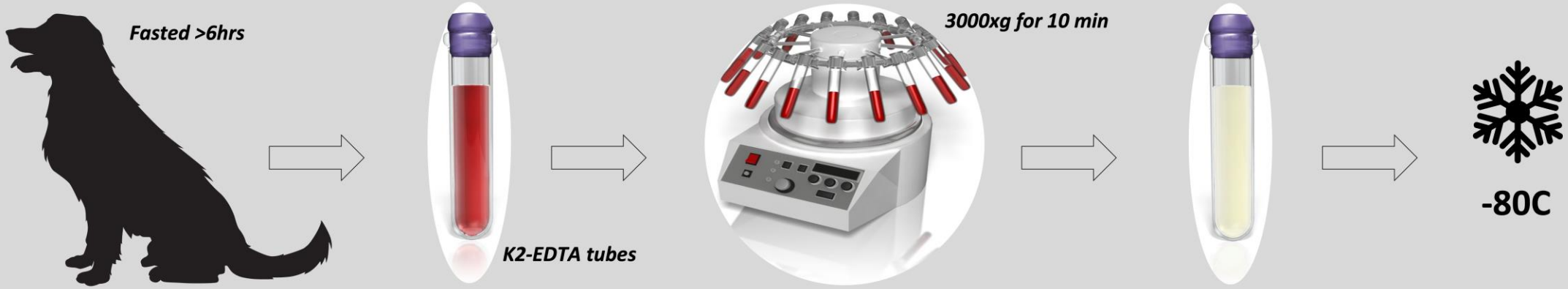
## Introduction

- Nucleosomes = most basic structural components of DNA
- Methods by which circulating nucleosomes are produced
  - Dying/activated WBCs
  - Apoptotic cells
  - Tumor cell secretion
- Elevated nucleosome levels in sepsis, severe burns, immune-mediated diseases, cancer
- H3.1 ELISA validated for measurement of nucleosome levels in canine lymphoma (LSA) patients
- H1 Nu.Q® Capture

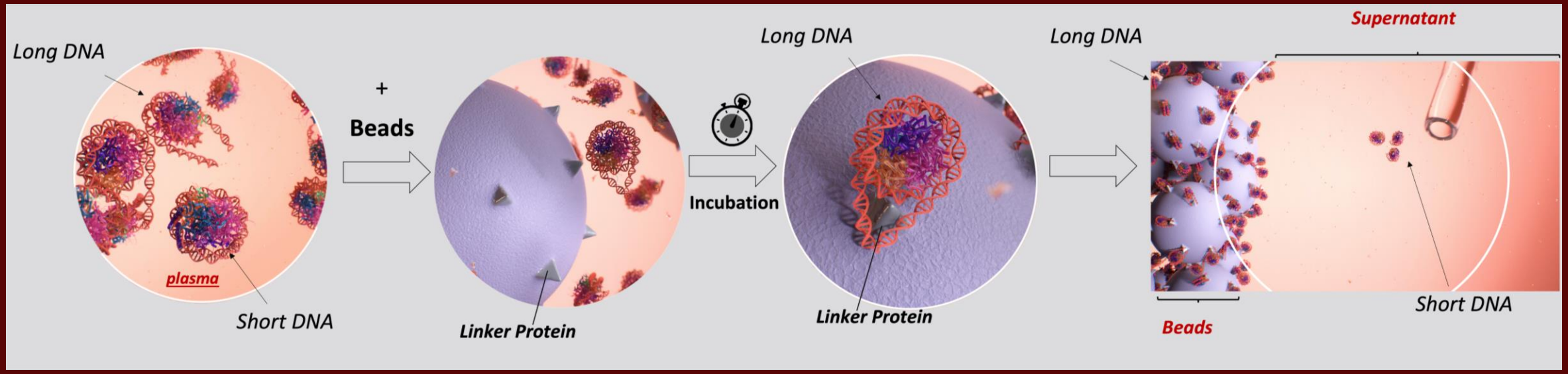
**AIM:** To isolate and sequence cancer-derived nucleosomes from dogs diagnosed with LSA

# Methods

## Sample Collection




## Nu.Q® Capture



# Methods (cont.)

## Whole genome sequencing

- Illumina 2x150bp
- Average Depth 40x

## Align to CanFam4

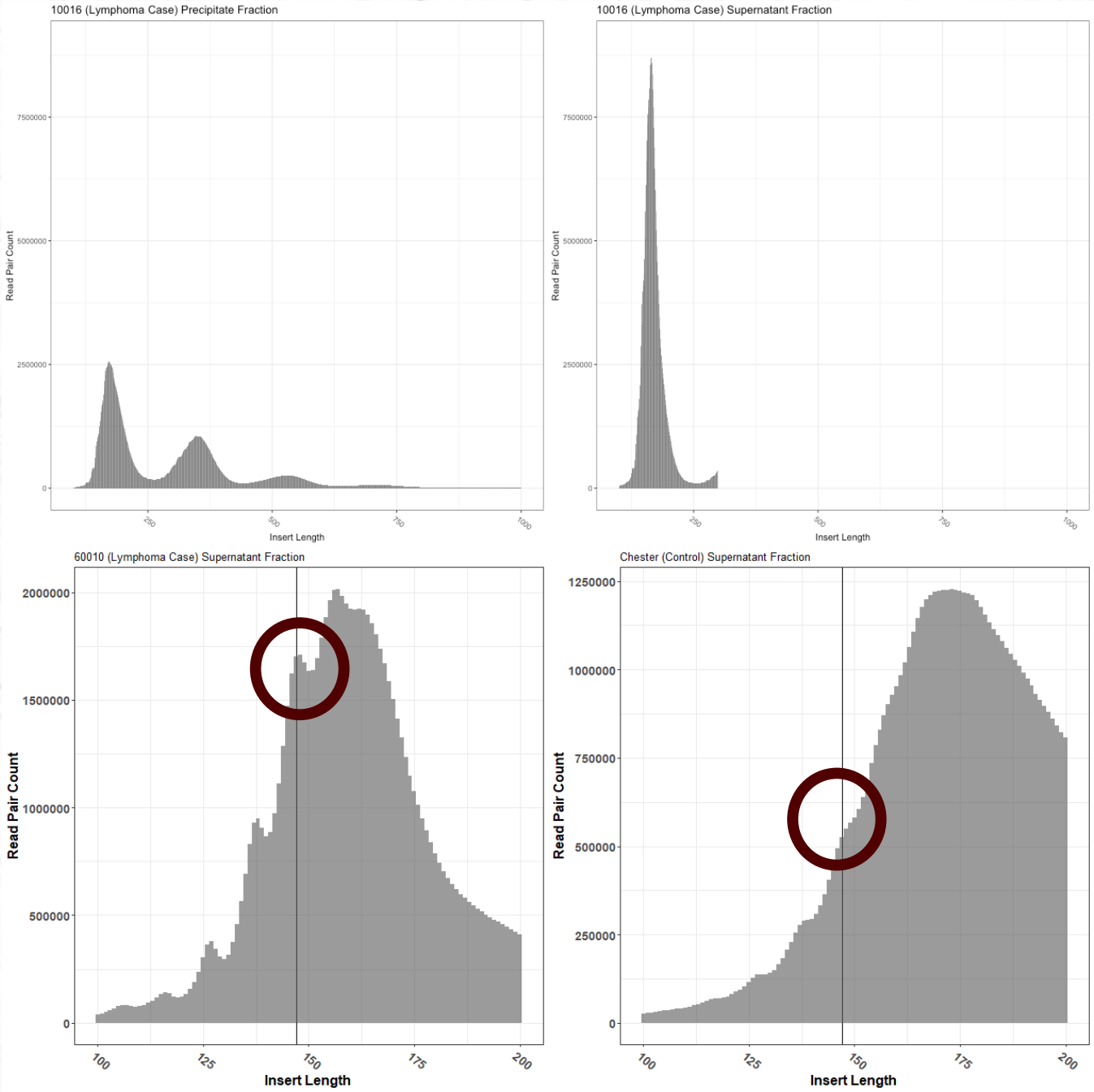
- Bwa-mem2

## Variant calling

- GATK 4.1.10
- HaplotypeCaller
- Mutect2

## Variant annotation

- SNPeff

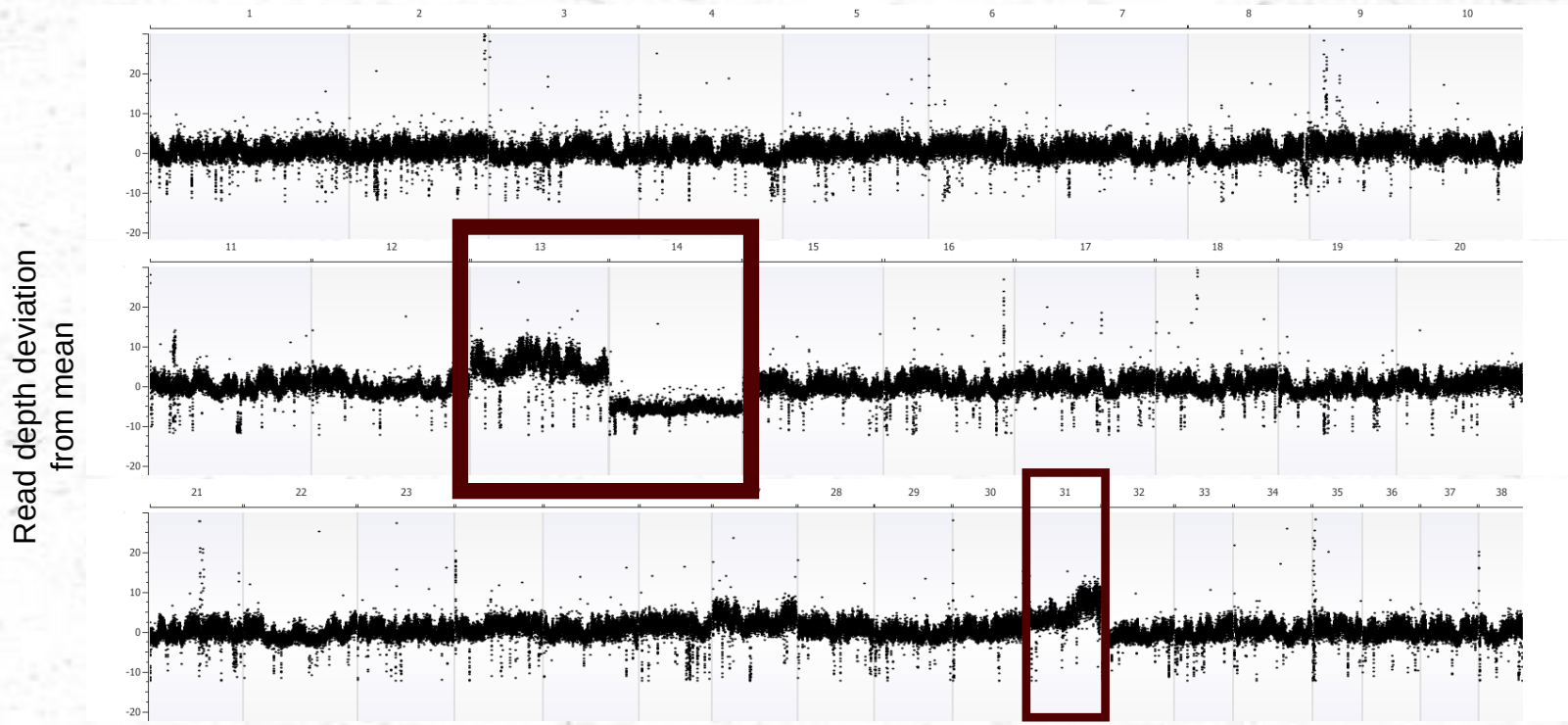



# Results

| 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 immunoprecipitated. 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 lacking the peak at 147bp.

**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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