Introduction

- Genetic instability is a hallmark in tumor prevalence, resulting in aneuploidy and copy-neutral loss of heterozygosity (cn-LOH) events.\(^1\)
- The samples used in this pilot study were stored in formalin-fixed and paraffin-embedded (FFPE). Use of FFPE involves cross-linking, which causes DNA damage.\(^2\)
- In addition, normal cell contamination and degradation in nucleic acid can make it difficult to identify mutations within a tumor sample due to dilution of the signal within the cells.\(^1\)
- The hapLOH method was developed to assist in the detection of copy-number events. When adding the restoration step, the DNA concentration is further reduced.

Materials and Methods

- The hapLOH method was used to determine the occurrence of subtle allelic imbalance (AI) in prostate samples.
- The best methodology (non-restored vs. restored) was determined.
- 24 samples were run twice -- with/without restoration -- for a total of 48 samples.
- Analyzed distribution of call rates and the specific genotype calls at single nucleotide polymorphisms (SNPs) between restored vs non-restored.

Results

Histograms and hapLOH plots were analyzed for the 24 paired samples. Figures 1 and 2 show examples of the analyses that was conducted for two of the patients.

![ZZ464 Sample](image1)

**Figure 1.** Histogram showing the genotype counts for sample ZZ464. Fewer missing data was seen when using the restored method.

![ZZ607 Sample](image2)

**Figure 2A.** hapLOH plots for the non-restored and restored methods for sample ZZ607. A greater number of mCAs were detected using the restored method.

**Figure 2B.** Histogram showing the genotype counts for sample ZZ607. Fewer missing data was seen when using the restored method.

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<thead>
<tr>
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<th>Non-restored</th>
<th>Restored</th>
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<td>Number of Detected mCAs</td>
<td>40</td>
<td>53</td>
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**Table 1.** A greater number of mosaic chromosomal alterations (mCAs) were detected when undergoing the restoration step.

Conclusions

The restored method was determined to be the best and most accurate method because:
- It uses a modest amount of DNA, while detecting approximately 14 percent more mCAs.
- It yielded fewer missing data.

A drawback of the restored method is that it is three times more costly for the specific array used here.

Future Directions

- The study team plans to conduct a much larger study.
- The large-scale study will include approximately 1,000 patients with advanced prostate cancer and 1,000 patients without advanced prostate cancer.

References