Searching For Alternative Splice Sites In FFPE Samples

Does Tissue Preservation Method Matter?

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Introduction

In addition to normal cellular function, alternative splicing (AS) is known to be associated with various cancers and metastatic processes. Robust identification of AS events can therefore be used to better understand mechanisms of disease, establish diagnostic biomarkers, and drive development of novel cancer treatments.

A large proportion of available tumor samples are preserved as formalin-fixed, paraffin-embedded samples due to the relative ease and affordability of doing so (no need for long-term cryogenic storage). However, this preservation method introduces random artifacts and fragmentation into the RNA sequence, potentially confounding analysis.

To date, much of the work done to compare FFPE samples to Fresh Frozen (FF) samples has only considered differential gene expression. We began an exploratory analysis comparing AS events between FF and FFPE samples, utilizing a unique dataset from the Trans Cancer Genome Atlas (TCGA) involving 35 patients with paired, time-matched samples.

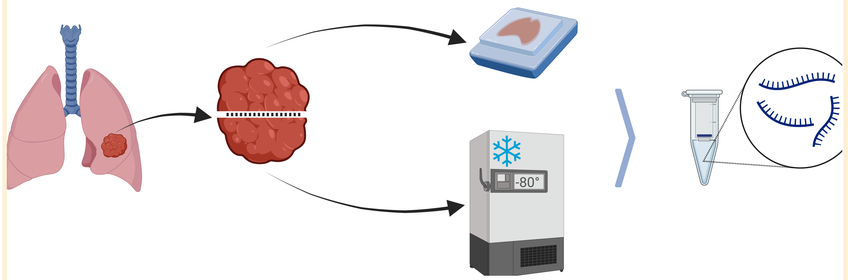
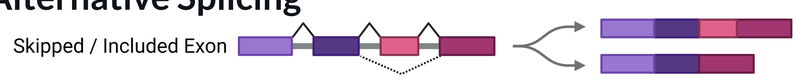


Figure 1.: TCGA Pilot Study Design. Samples were collected from patients [enrolled in protocol…?] undergoing clinical resection of tumors . Tumors were immediately bisected , with one half preserved as FF and the other as FFPE samples. RiboZero Total RNA sequencing was performed on all paired sets.

Alternative Splicing



Key Questions

1. **How do AS events between FF and FFPE samples compare?**
   - Raw counts
   - Shared Events (intersection)
2. **What is driving any differences between the two?**
   - RNA quality
   - Read mapping
   - Gene expression
3. **How does event support vary with preservation method?**
   - SJC, IJC
   - JRS

Results