Streamlined Dual-Omic cfDNA Analysis for Pediatric Renal Cancer with BrightSeq

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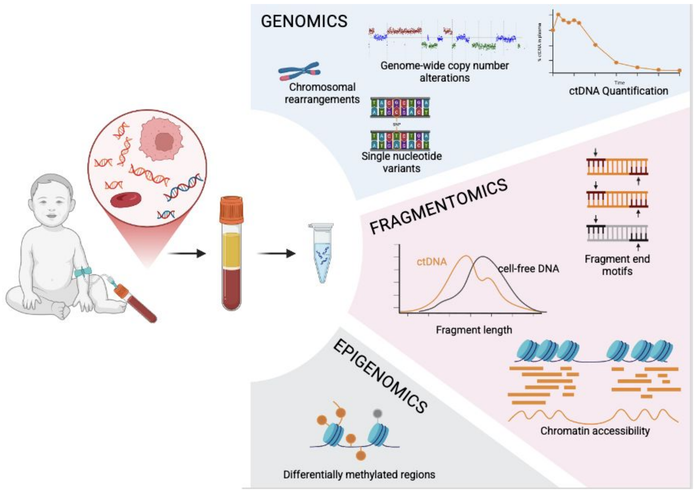
Introduction and Motivation

For decades, the diagnostic, monitoring, and treatment landscape for rare pediatric cancers has seen few transformative advances, due in part to challenges in powering genomic discovery. Pediatric tumors also harbor distinct genomic and epigenetic features that require innovative sequencing approaches to enable precision care and accelerate discovery. To address these challenges, we established *BrightSeq*, a partnership among Boston Children's Hospital, Dana-Farber Cancer Institute, and Broad Clinical Labs. BrightSeq is developing a suite of novel clinical cell-free DNA (cfDNA) and tissue-based assays to profile important events in pediatric cancers.

One priority focus is pediatric renal tumors, which comprise diverse histologies with highly variable prognoses. Current diagnostic approaches rely heavily on surgical resection as imaging and core needle biopsies have limited accuracy. Consequently, less invasive circulating-tumor DNA assays capable of distinguishing tumor types and guiding therapy are urgently needed. To explore the possibility of improving BrightSeq cfDNA assays with epigenomics features, we applied Illumina's 5-Base conversion chemistry, which enables simultaneous interrogation of genomic and methylation features from the same cfDNA sample, to a pilot cfDNA and FFPE tissue cohort.



Background Pediatric Renal Cancer: Wilms Tumor and Malignant Rhabdoid Tumor of Kidney

- Renal tumors comprise **7% of all childhood cancers**. The prognoses and therapy recommendations range broadly based on specific tumor histology.
- Although the likely tumor type can be partially predicted by patient age, presentation, and imaging a definitive diagnosis requires tumor tissue biopsy.

There are clinical management challenges without definitive diagnosis:

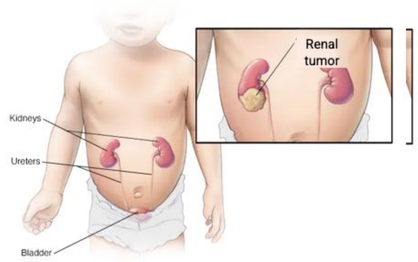
1. Missed opportunity for appropriately targeted chemotherapy prior to surgery
2. Risk of removing the kidney for a non-malignant tumor
3. Missed opportunity to study pre-therapy tumor genetics

WILMS TUMOR

~80% of cases
Less aggressive

MALIGNANT RHABDOID TUMOR OF KIDNEY

~20% of cases
More aggressive



Surgery or biopsy may not be possible for all cases

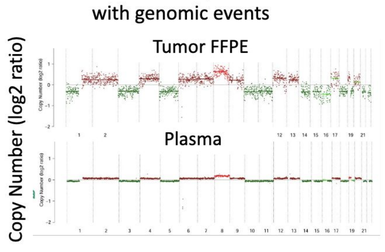
Treatment is guided by more prevalent histology (Wilms)

Lost time for optimal treatment

Wilms tumor can often but not always be detected with conventional genomics approaches in cfDNA (WGS + iChor Tumor fraction estimation). MTRK cancers have exceptionally stable genomes with the exception of mutations or focal events in the SMARCB1 gene (Chr 22) that can be difficult to detect at low allele fraction in cfDNA. These tumors do however have tumor specific methylation signal so we are exploring the 5-Base approach for tumor detection and subtyping in these cancers.

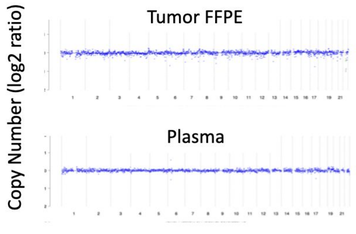
WILMS TUMOR

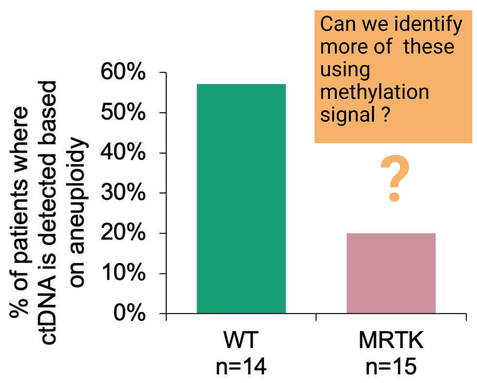
Often but not always detectable with genomic events



MALIGNANT RHABDOID TUMOR OF KIDNEY

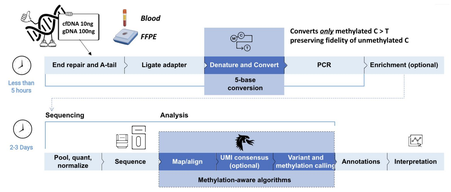
Relatively stable genomes





5-Base Dual-Omic Sequencing Pilot Data Generation

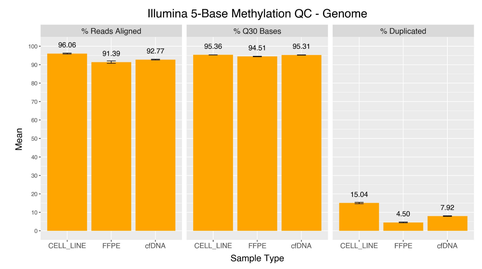
5-Base Workflow:

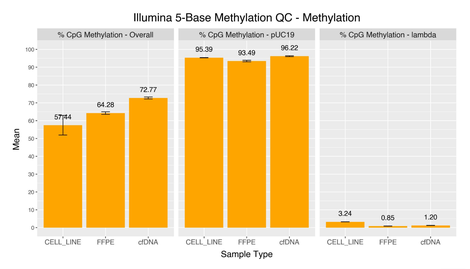


Samples Tested:

COHORT	# 5-BASE GENOMES	COVERAGE TARGET
Controls (HG001, 2, Zymo +/-)	5	50x
Cancer Cell Lines	7	50x
Healthy Donors cfDNA	9	30x
Pediatric Renal cfDNA	47	15x - 30x
Pediatric Renal FFPE DNA	28	15x - 30x
Neuroblastoma cfDNA	12 Pan-Cancer 396 Gene Targeted Enrichment	

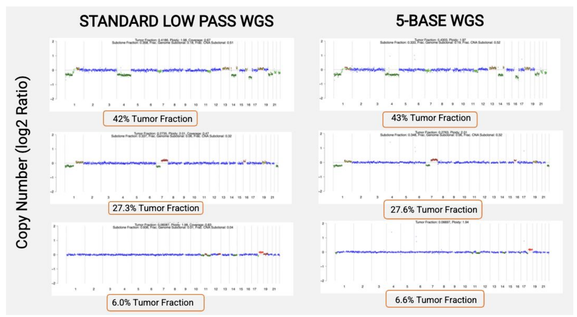
5-Base Quality Metrics by Sample Type:



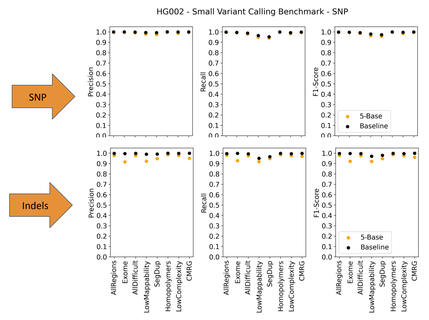


Genomics Analysis with 5-Base

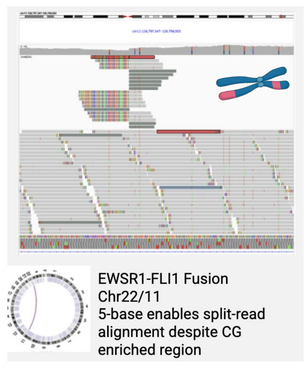
Tumor fraction estimation using WGS vs 5-Base Dual-Omic approach with iChor is comparable



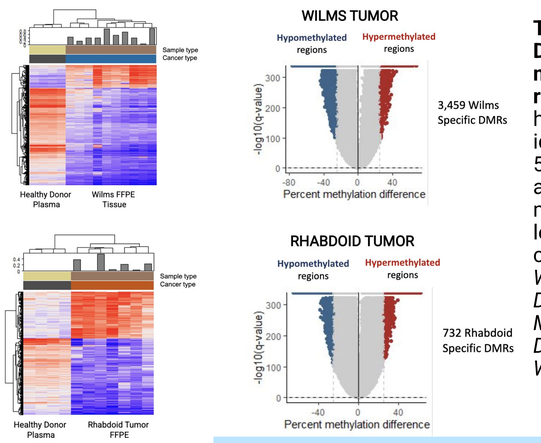
Variant calling benchmarking: WGS vs Dual-Omic 5-Base with HG002



Structural Variants are often driver events of pediatric cancer. 5-Base enables detection of EWSR1-FLI1 event despite GC enriched sequence context of this loci.

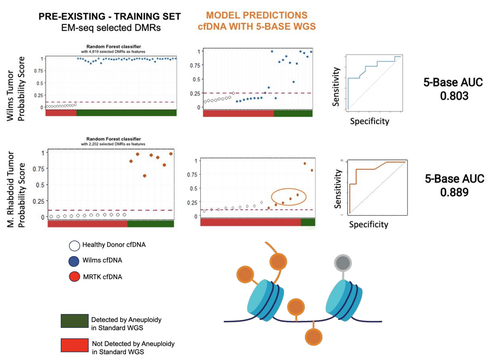


Epigenomics Analysis with 5-Base



Tumor Specific Differentially methylatated regions (DMRs) have been identified using 5-Base which are used to train machine learning classifiers for *Wilms vs Healthy Donors* and *MRTK vs Healthy Donors* and also *Wilms vs MRTK*.

We applied 5-Base WGS sequenced cfDNA samples from known cases of Wilms or MRTK to pre-existing tumor specific DMR based random forest classifier models. As expected few additional cases of Wilms were predicted that were not also called with conventional WGS but several additional MRTK cases were identified.



Conclusions & Next Steps

1. 5-Base technology produces adequate data for high quality somatic profiling of FFPE and cfDNA
2. Preliminary data suggests that using epigenomics based classifiers may help to detect and distinguish molecularly quiet tumors and will add to translational research efforts for rare cancers.
3. Training *5-Base specific* models with additional FFPE and healthy donor specimens is likely to improve classification results and is the focus of our current work.

Contact us for more info:
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