

## Delineation of Complex Genomic Alterations via Iso-Seq in a Comprehensive Genomic Profiling Protocol for Pediatric Cancer

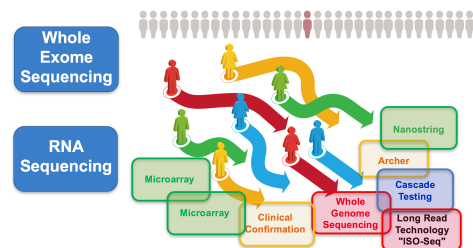
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### Introduction

The Institute for Genomic Medicine developed a translational protocol to evaluate the genomic landscape of cancer and hematologic disease amid our patient population. This comprehensive approach allows for detection of germline and somatic single nucleotide variants, small indels, copy number alterations, structural variation and expression. Additional methodologies to further delineate complex genomic alterations are employed including long-read technologies. To date, we have enrolled 63 subjects and have sequenced 55 cases using Illumina short-read chemistries.

Following protocol enrollment, genomic profiling methodologies are employed using an **N-of-1** approach tailored to the patient and disease status.



**Figure 1.** Our N-of-1 sequencing approach uses Whole Exome and RNA Sequencing for an initial genomic evaluation. Additional genomic profiling is performed on case-by-case basis including long read sequencing technologies.

### Enhanced Whole Exome Sequencing :

Disease and comparator normal samples target 250X average coverage depth. The "enhanced exome" includes the IDT xGen exome plus two additional IDT probe panels: the IDT xGen CNV backbone panel (9385 probes) to help identify copy number variants, and >2400 probes among clinically relevant cancer loci implicated in copy number alteration and mutation.

### RNA-Sequencing:

Whole transcriptome profiling by short read RNA-Seq. Total RNA is processed using the NEBNext Ultra II Directional library prep kit for Illumina and targeting 80-100 million paired-end reads per library. The RNA analysis pipeline uses an ensemble approach for fusion detection. Current tools in the fusion pipeline include JAFFA, FusionMap, MapSplice, SOAPfusion, FusionCatcher, StarFusion, and TopHat-Fusion.

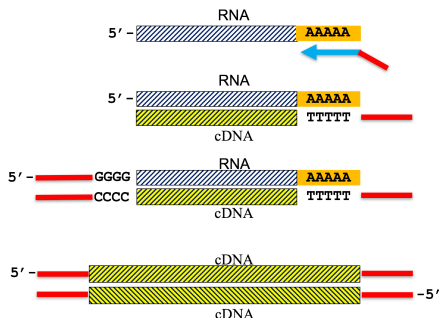
Somatic events of diagnostic, prognostic, or therapeutic relevance included inter or intra-genic structural variation in 16 patient samples. A subset of these were evaluated by Pacific Biosciences Iso-Seq to characterize novel isoforms and resolve complex rearrangements (Table 1).

**Table 1.** Somatic fusions detected by RNA-Seq

Diagnosis	Fusion	Junction
Alveolar rhabdoid sarcoma	PAX3-FOXO1	CTCTGGGAGAGATTTACCAATGATT@ctgag gtgagagcattgcaatgag
Ependymoma	C11orf95-RELA	TCTCTCTGAGCTACCCAGCTCCACACAG@aacgtg ccctccatctccagagatg
Large cell lymphoma	NPM1-ALK	CTTGACAGCTCTGCTGCTCCGGCGGTACAG@ctactac atctctctctctctctctctct
Spindle cell lesion	COL3A1-PLAG1	TTTCTGCTGGTGGGTAACTGATAAC@ctgttcca acatctctctctctctctctct
Infantile fibrosarcoma	RBPM5-MET	AACTCTTGAATGATTTAGGAAGTATG@atggtc atggtcagctgagctgagctg
Meningeal sarcoma	PDGFRB	ΔGATGGAAGGTGATTG
Spindle cell neoplasm	PDGFRB	Complex

### Pacific Biosciences Iso-Seq

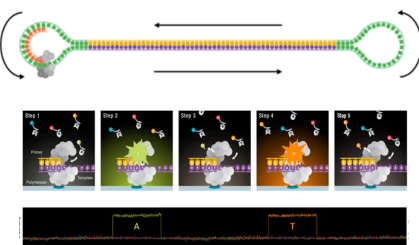
Iso-Seq is an RNA-Seq protocol aiming to represent full-length RNA transcripts using the Takara SMARTer cDNA library prep (Figure 2). We are incorporating Iso-Seq into our translational protocol to identify expressed RNA isoforms and further characterize structural variation inferred by short read sequencing technologies.



**Figure 2.** Total RNA is reverse transcribed with a tailed polyA primer (Blue/Red arrow). The RT reaction generates first strand cDNA (yellow hatch //) and an extended RNA template (red bar GGGG). Template switching synthesizes the extended template into first strand cDNA (red bar CCCC). PCR of tailed cDNA templates generates double-stranded cDNA.

### Iso-Seq Single Molecule Real Time (SMRT) bell libraries

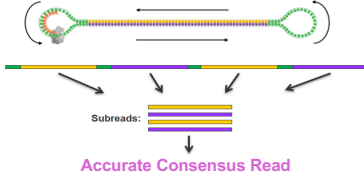
One microgram of double-stranded cDNA is size selected between 500bp and 2kbp and greater than 2kbp. Size-fractionated cDNAs are ligated with PacBio hair-pin adapters generating a SMRTbell configuration. Libraries are primed and complexed with DNA polymerase and loaded into zero mode wave guides (ZMWs).



### Iso-Seq SMRT Sequencing

Within the ZMW, DNA replication occurs producing nucleotide-specific fluorescent signals. Circular consensus sequencing (ccs) allows the polymerase to replicate the circularized SMRTbell template for up to 20 hours producing long reads (N50 = 15kb).

Post sequencing, the SMRTbell adapters are trimmed and the single-molecule fragments are aligned generating an accurate consensus read from a single molecule with multiple sequencing passes.



### Iso-Seq Results

Identification of C11orf95-RELA fusion isoforms in two patients diagnosed with ependymoma allowed for refinement in diagnosis enabling classification as a genetically defined ependymoma subtype: Ependymoma, RELA fusion-positive (2016 WHO CNS guidelines).

#### Ependymoma 1: C11orf95-RELA

Transcript 18927  
R11 R12 R3 R8 R7 R6 R5 R4 R3 R2 C3 C2 c11orf95 Intron 1 307bp

Transcript 33967  
R11 R12 R3 R8 R7 R6 R5 R4 R3 R2 C3 C2 c11orf95 Intron 2 138bp

#### Ependymoma 2: C11orf95-RELA

Transcript 18953  
R11 R12 R3 R8 R7 R6 R5 R4 R3 R2 RELA Intron 1 417bp c11orf95 Intron 1 209bp

Transcript 33967  
R11 R12 R3 R8 R7 R6 R5 R4 R3 R2 RELA Intron 1 417bp c11orf95 Intron 2 209bp

### A complex PDGFRB genomic alteration

An exon 11-15 intragenic rearrangement was identified by RNA-Seq in an infant with a spindle cell lesion. Iso-Seq identified two expressed isoforms with a complex rearrangement. We observed one isoform harboring an exon 2 deletion, while both isoforms carried a 154bp repeat of exon 15 sequence inserted between exon 11 and an altered exon 12 sequence (with a 50bp deletion). This alteration results in an in-frame insertion/deletion altering the amino acid sequence of the juxtamembrane domain. Identification of this PDGFRB alteration, predicted to be activating in nature, allows for refinement in diagnosis (now best classified as infantile myofibromatosis) and enables potential for use of targeted therapeutics.

#### PDGFRB - Iso-Seq Transcript 355

R12 R13 R14 R15 R16 R17 R18 R19 R20 R21 R22 R23 R24 R25 R26 R27 R28 R29 R30 R31 R32 R33 R34 R35 R36 R37 R38 R39 R40 R41 R42 R43 R44 R45 R46 R47 R48 R49 R50 R51 R52 R53 R54 R55 R56 R57 R58 R59 R60 R61 R62 R63 R64 R65 R66 R67 R68 R69 R70 R71 R72 R73 R74 R75 R76 R77 R78 R79 R80 R81 R82 R83 R84 R85 R86 R87 R88 R89 R90 R91 R92 R93 R94 R95 R96 R97 R98 R99 R100 R101 R102 R103 R104 R105 R106 R107 R108 R109 R110 R111 R112 R113 R114 R115 R116 R117 R118 R119 R120 R121 R122 R123 R124 R125 R126 R127 R128 R129 R130 R131 R132 R133 R134 R135 R136 R137 R138 R139 R140 R141 R142 R143 R144 R145 R146 R147 R148 R149 R150 R151 R152 R153 R154 R155 R156 R157 R158 R159 R160 R161 R162 R163 R164 R165 R166 R167 R168 R169 R170 R171 R172 R173 R174 R175 R176 R177 R178 R179 R180 R181 R182 R183 R184 R185 R186 R187 R188 R189 R190 R191 R192 R193 R194 R195 R196 R197 R198 R199 R200 R201 R202 R203 R204 R205 R206 R207 R208 R209 R210 R211 R212 R213 R214 R215 R216 R217 R218 R219 R220 R221 R222 R223 R224 R225 R226 R227 R228 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