INTRODUCTION
Growing interest in cancer classification and progression has accelerated the rate of novel gene fusion discovery with increasing recognition of their roles as biomarkers. RNA-Seq is an attractive method for expressed fusion discovery and detection because of its ability to provide unbiased fusion sequencing information. The ability to detect low expression fusion transcripts, however, requires high sequencing depth and represents a significant financial barrier and identification of clinically relevant fusion sequences from a large data set can be a bioinformatics challenge.

To address these challenges we have tested the Ovation® Fusion Panel Target Enrichment System V2, a targeted RNA-sequencing method using the Single Primer Enrichment Technology (SPIA), with a number of control and clinical samples. Initial studies were performed using a comprehensive target enrichment panel targeting 502 genes with three samples from Horizon DX containing known fusions. Target enriched libraries were constructed with 10 ng and 100 ng input DNA and the data was analyzed using the NuFuseD pipeline (available as a point-and-click BaseSpace application or downloadable Linux package) which has been optimized for fusion analysis from this data. Expected fusions were identified at both input levels, even when down sampled to 50K reads, with fewer fusion calls compared to other publicly available fusion detection software (Chimerica and SOAPfusions), suggesting a lower false positive rate. NuFuseD fusion calls are provided with a P-value to help prioritize the identified fusions for subsequent validation. Additionally, NuFuseD detected novel fusions in the clinical samples demonstrating the advantage of a comprehensive panel compared to more restricted panels. We further validated the target panel using control RNA (SHH and Human Brain) and fresh or FFPE cell lines (NCI-H2228, HCC1937) to further demonstrate our ability to identify known fusions.

Finally, the system was evaluated at an external site using patient FFPE samples. These samples (N=5) were from a set of breast, liver and ovarian cancer, containing a unique fusion in 4 of the samples based on DNA barcoding sequencing. Only 1 of the 4 expected fusions were identified using whole transcriptome data (100 million reads) while all 4 of the 4 fusions were detected with this assay (10 million reads) demonstrating its ability to generate targeted RNA-sequencing libraries with increased sensitivity of gene fusion detection and reduced sequencing costs compared to standard RNA-Seq methods. Integrated workflow with Ovation® Fusion Panel and NuFuseD was validated for use with FFPE sample clinical screening.

METHODS

PROBE DESIGN FOR FUSION DETECTION

DETECTION OF KNOWN AND NOVEL GENE FUSIONS

Figure 1: A comprehensive target enrichment panel for fusion detection

Figure 2: Schematic of probe design for fusion detection. A. Probes are designed to all exons in a targeted transcript. Multiple probes are designed to larger exons providing full coverage of the exon and the ability to detect potential fusion events that occur within the loop region. B. Multiple probes located adjacent to the fusion site (red arrows) or probes targeting near-by exons generate芝麻 to provide evidence of a fusion event. Novel fusion events can be detected as long as one of the genes involved is targeted.

Figure 3: Complete, optimized method for fusion detection with less false positives

Figure 4: The Ovation Fusion Panel Target Enrichment System enables targeted detection of known and novel fusion events. A. Using 100 ng of UHR total RNA, the known fusions in this sample (BCL3-FAM84B) were easily detected as well as two novel fusions (FGFR3-TACC3 (F) and MLLT10-PICALM). B. The novel fusions were detected using a targeted enrichment panel (~2 M reads) as opposed to whole transcriptome sequencing (~2 M reads). C. The generic fusion detection system was designed to detect fusions as well as investigating the difference in expression level using a targeted enrichment panel.

Figure 5: Validation of the Ovation Fusion Panel Target Enrichment System V2. A. Total DNA and RNA were isolated from clinical FFPE samples and were analyzed for genomic rearrangements and fusions using different methods. B. Representative results are shown. The whole transcriptome analysis of sample CCD212 identified multiple fusions, including the known EML4-ALK fusion. Genetic rearrangements involving these genes were not identified using the commercial panel, potentially due to exclusion criteria. The targeted enrichment panel on the other hand, identified the EML4-ALK fusion in addition to several other fusions, indicating genomic rearrangement analysis in parallel with targeted fusion detection is necessary to identify additional fusion events. C. The targeted enrichment panel of samples CCDC212 and CCD211 revealed concordance between the fusion-identified events of the whole transcriptome and targeted panes. D. The expected fusion was also detected with the targeted enrichment method further validating the method as a alternative whole transcriptome fusion detection.

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CONCLUSIONS

• Integrated workflow with Ovation® Fusion Panel and NuFuseD was validated for use with FFPE sample clinical screening.
• Targeted sequencing provides similar results as whole transcriptome sequencing without the need for high sequencing depth.
•Panel targeting all exons from 502 genes known to be involved in gene fusion provides a comprehensive method for detection and discovery of known and novel gene fusions.