Single Primer Enrichment Technology (SPET) for targeted RNA sequencing

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ABSTRACT

Recently, we have developed an innovative targeted RNA sequencing assay based on Single Primer Enrichment Technology (SPET) that provides an inexpensive and easy to use workflow for identifying gene fusion events in an RNA sample. The SPET technology for targeted RNA sequencing is flexible enough to target any gene in any organism for gene fusion detection or gene expression analysis, thus representing an important step toward affordable RNA-Seq based cancer diagnostic and prognostic tests.

The fully customizable assay is also offered as a standard gene panel targeting all possible fusion events involving any of 500 genes implicated with fusion events in various cancers as compiled by the Wellcome Trust COSMIC database, ChimerDB 2.0 and TCGA .

We have used the 500-gene panel to identify gene fusion events in cultured and primary FFPE tumor samples.

Fig. 1

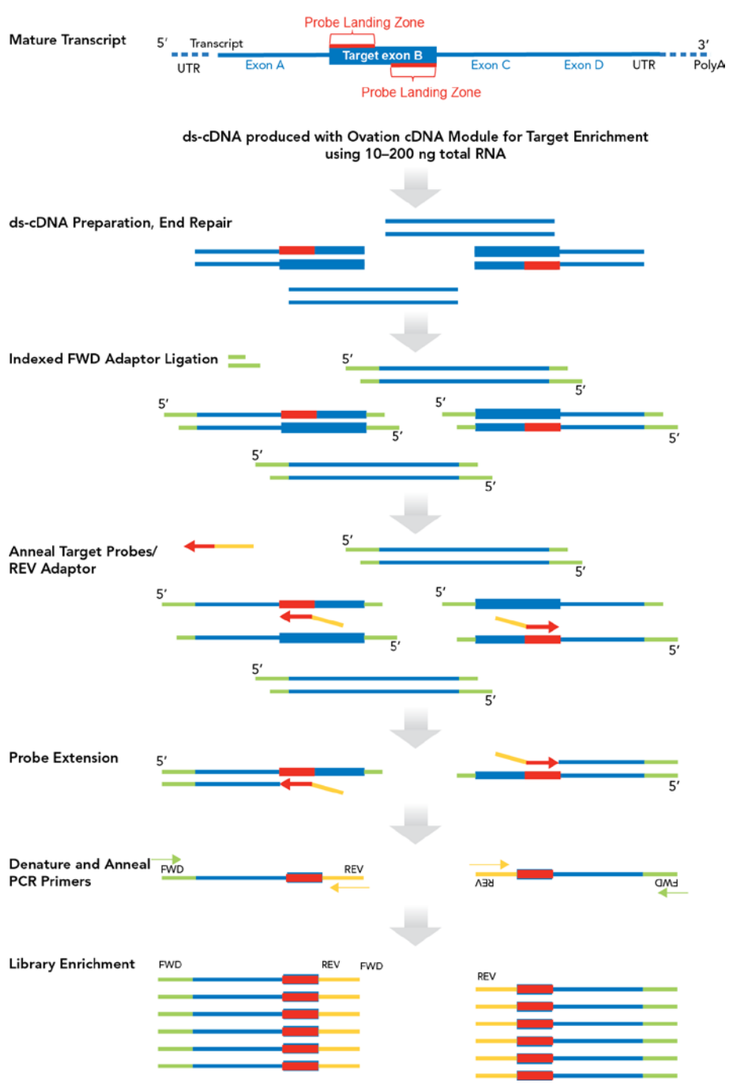

Figure 1: Schematic of the RNA Target Enrichment procedure. Probes have been designed at Exon-Exon borders facing outwards to generate reads from the neighboring regions. This allows for the detection of gene fusions with known partners and/or breakpoints, as well as the detection of novel fusion events.

Fig. 2

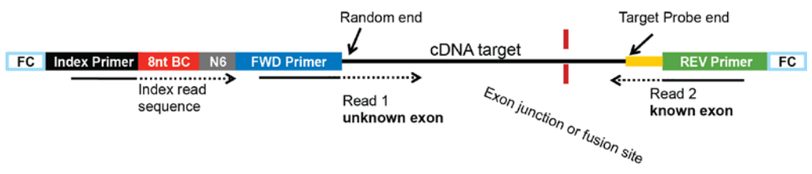

Figure 2: Schematic of the novel forward sequencing adaptor structure. Relocating the index to the forward adaptor allows for the unambiguous tagging of individual samples prior to multiplex probe hybridization and extension. The index is an 8-base edit-3 distance index followed by six random nucleotides (N6). The purpose of the N6 sequence is to identify duplicate reads generated by PCR.

Fig. 3

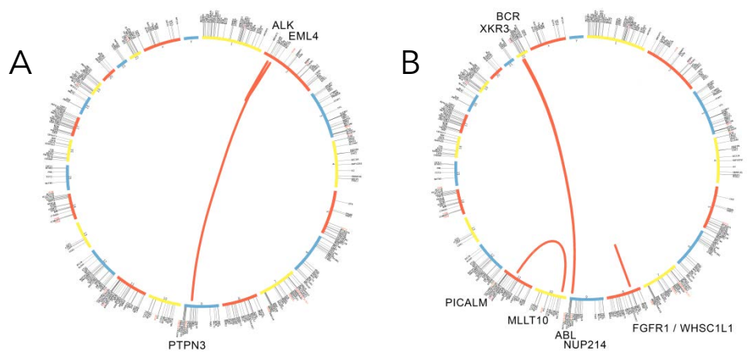

Figure 3: Detected gene fusions in a H2228 Lung Adenocarcinoma cell line (Panel A) and Universal Human Reference (UHR) RNA (Panel B). The fusions detected in the H2228 cell line were as previously reported. The UHR RNA is a mix of 10 different cancer cell lines. This means each fusion event is a representation of 10% of the original input. In the UHR sample two known fusions (BCR-ABL and NUP214-XKR3) as well as two novel fusions (FGF1R-WHSC1L1 and MLLT10-PICALM) were detected. The novel fusions were confirmed by RT-PCR and Sanger sequencing.

Table 1

Input	Reads	Aligned to Genome	On Target
1ng	1.6M	90%	92%
10ng	5.5M	89%	93%
100ng	4.0M	92%	92%

Table 1: Overview of the alignment and on target rate with different inputs of total RNA from the H2228 Lung Carcinoma cell line, using 2x75 bp paired-end reads. This system typically shows more than 85% of the probes on target.

Fig. 4

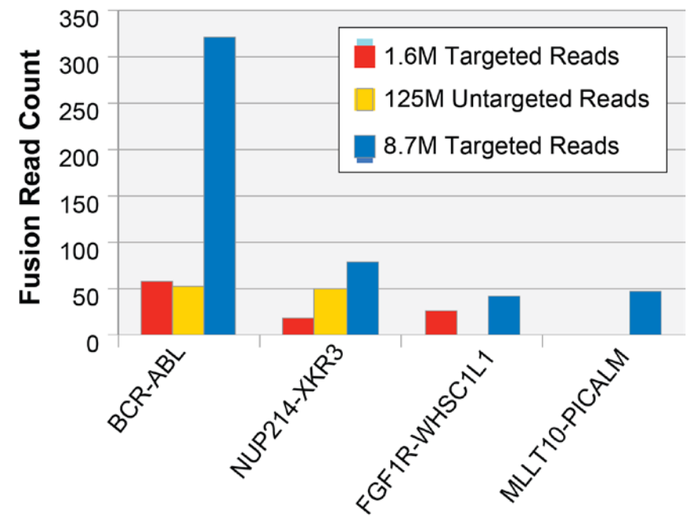

Figure 4: Superior detection of fusion events using target enrichment. Even with 1.6 M reads more fusion gene events can be detected in an enriched panel than in an untargeted 125 M read experiment. Unreported fusions were confirmed by RT-PCR and Sanger sequencing.

METHODS & RESULTS

An input of 100 ng of total RNA from different sources was used in the Ovation Fusion Panel Target Enrichment System which allows detection of fusion events on a total of 500 genes. The resulting library was sequenced on the Illumina MiSeq using 2x75 bp paired-end reads. Fusion gene analysis was done using SOAPFuse. All novel fusions were confirmed by RT-PCR and Sanger sequencing.

CONCLUSIONS

NuGEN has developed a powerful and customizable RNA target enrichment system that provides:

- Detection of both known and unknown fusions and fusion breakpoints
- Enriched libraries exhibiting high % alignment, high % bases on target and even coverage
- Detection of more gene fusions than standard RNA-Seq

PRODUCT FEATURES

- Low input - create a library from as little as 10 ng of total RNA
- Ability to handle poor sample quality – analyze both high quality as well as degraded samples (such as RNA derived from FFPE material)
- Custom probe design with low design dropout and low experimental dropout
- Flexibility to choose:
  - Catalog panel of 500 cancer genes
  - Custom panels available in three weeks from design approval

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