Chromosomal rearrangements bring together coding sequences or regulatory elements of genes that are normally separated. Many of the resulting fusion genes have been key drivers of tumor growth in cancer, and the chimeric protein products may serve as specific therapeutic targets. Traditional approaches for identifying whether a gene may be involved in a fusion can be challenging to perform or difficult to interpret. Furthermore, methods such as reverse transcription PCR (RT-PCR) rely on prior knowledge of both genes involved in the fusion, while techniques like fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) are unable to identify the fusion partner of the gene of interest. While whole transcriptome RNA sequencing (RNA-seq) has proven to be a powerful tool in discovering new fusions, several challenges remain, stemming from both the complex nature of such large-scale sequencing and the variable quality of samples and the RNA isolated from them. The large dynamic range of the transcriptions often means that a few highly abundant transcripts account for the majority of sequencing reads while low-abundance transcripts account for only a small percentage of sequencing reads. These rare transcripts therefore require high sequencing depth to be reliably detected. Additionally, clinical samples may have RNA of unknown quality, necessitating methods that can amplify both high-quality and severely degraded RNA, such as that from FFPE (formalin-fixed paraffin-embedded) tissue. Targeted RNA-seq enables the capture of low-frequency transcripts that are present in limited quantities but would require a much greater number of sequencing reads to be detected, including chimeric gene fusions, transcript splice variants, and variants.

We leveraged SMARTer® 5'-RACE technology for direct amplification of the 5' ends of transcripts of interest to develop a target-specific protocol with high sensitivity and low background starting from total RNA of any quality. The resulting gene-specific priming method demonstrated considerable enrichment from 10 ng to 1 µg of a variety of degraded total RNA inputs. The protocol enables the detection of gene fusions and other structural variation in expressed RNAs without knowledge of features 5' of the gene of interest from degraded total RNA. We leveraged SMARTer® 5'-RACE technology for direct amplification of the 5' ends of transcripts of interest to develop a target-specific protocol with high sensitivity and low background starting from total RNA of any quality. The resulting gene-specific priming method demonstrated considerable enrichment from 10 ng to 1 µg of a variety of degraded total RNA inputs. The protocol enables the detection of gene fusions and other structural variation in expressed RNAs without knowledge of features 5' of the gene of interest from degraded total RNA. SMART erRNA Fusion Kit amplifies all fusion events, including rare retention of intron 19 of ALK gene. Image: https://www.mycancergenome.org/content/disease/lung-cancer/alk/ (2014). The ALK gene has a common breakpoint at the end of intron 19. The resulting breakpoint is present in a variety of tumors, including a small subset of non-small cell lung cancers (NSCLC). Fusions in cancer are complex, and targeting resulted in a significant increase in the number of reads mapping to regions of the ALK gene. Unmapped reads were visualized using the Ensembl Genome Browser (www.ensembl.org). This approach is a sensitive tool for identification of structural variation in transcripts of interest from degraded total RNA.

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High on-target performance and identification of known fusions

% Reads to ALK

<table>
<thead>
<tr>
<th>FFPE1 ALK+</th>
<th>FFPE2 ALK+</th>
<th>FFPE3 ALK+</th>
</tr>
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<tbody>
<tr>
<td>98%</td>
<td>97%</td>
<td>64%</td>
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</tbody>
</table>

Targeted SMARTer fusion RNA protocol

- FFPE1 ALK+: 98%
- FFPE2 ALK+: 97%
- FFPE3 ALK+: 64%

Non-targeted (random-primed WTA protocol)

- Percent reads: 0.012%
- Other sequences: 0.015%
- Other: 0.019%

Identification of a rare subtype of an ALK fusion event

- Expected detection of reads into intron 19 of ALK
- Unexpected detection of reads into intron 19 of ALK

Conclusions

Library generation without adapter ligation or known sequences

- SMARTer technology in combination with a 5’-RACE-based approach allows unbiased capture of all structural variation upstream of a location of interest

Discovery of fusions in cancer research samples

- Rare fusion events identified in degraded RNA and RNA purified from FFPE tissue without ribosomal RNA removal

Confirmation of fusions in a control fusion sample

- This approach identifies all targeted fusions in a reference material for fusion RNA

References

- My Cancer Genome. Available at: https://www.myccg.org.
- Takara Bio USA, Inc. Available at: www.takarabio.com.

For more information, please visit takarabio.com/nextgen-dx-smarter-fusion.