Fast development of large customized targeted RNA fusions panels for NGS platforms

Antonio Martinez Alcantara[1], Na Li[2], Scott Myrand[3], Paul Williams[3], Santhoshi Bandla[3], John Sherlock[2], Fiona Hyland[1], Seth Sadis[3], [1]Thermo Fisher Scientific, South San Francisco, CA, 94080, [2]Thermo Fisher Scientific, Carlsbad, CA, 92008, [3]Thermo Fisher Scientific, Ann Arbor, MI 48104

Introduction

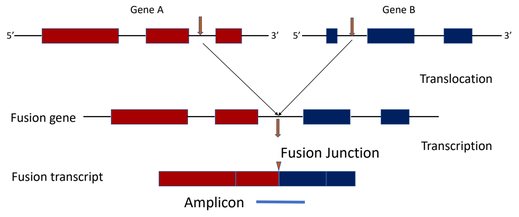
Gene fusions, are hybrid genes originated from DNA rearrangements. Gene fusions play important roles in the development and evolution of cancer and their proper identification is critical to oncology research[1]. Oncomine tumor specific panels are Ampliseq™ targeted panels comprising DNA and RNA-fusion targets for multiple tumor types, that may include more than a thousand fusion isoforms. Designing these fusion panels, including custom fusion panels, is a challenging endeavor that requires extensive curation, annotation, and storage of fusion sequence data. Consistent, correct, and comprehensive information is essential to design panels that may target thousands of gene fusions.

Methods

In order to assess the challenge of rapidly creating sizable panels for targeting gene fusions, we have developed a novel system that includes:

1. Use of a nomenclature standard that is at the same time simple, consistent and comprehensive enough to accurately capture with little or no assistance, the researcher needs. Figure 1 schematically shows the information that is captured with the purpose of design.
2. Formatted information (Table 1) allows for easy curation, annotation and posterior storage of data, that can be easily updated, searched and used in multiple projects.
3. An ad-hoc pipeline that takes the data and supports the creation of custom gene fusion panels

Figure 1. Targeted RNA-fusion assay design



Amplicons are designed targeting the fusion junction.

Using the stored information, the pipeline uses the genomic properties of the participant transcripts to correctly a) find the position of the fusion breakpoint, b) extract the gene sequences of every fusion target and c) build a design reference. Candidate amplicons are then generated against the fusion reference (Figure 1). Depending on the design requirements of pool number, conflicts among primer pair are resolved by the pipeline's pooling algorithm to eliminate interactions among primers.

Table 1. Design required information

Item	Description
Gene names	identifiers for genes A and B participating in the fusion
Transcript IDs	Transcript identifiers for the Gene A and Gene B
Exon numbers	Last exon number of gene A and first exon of gene B that participate in the fusion transcript.
Breakpoint position	Position relative to the participating exons

Figure 2 summarizes the workflow: the process starts with a customer request that uses a simple yet powerful representation of the gene fusions of interest. The data is then carefully verified to assure it is a true representation of the intended fusion targets. Once the data passes this strict QC, it takes two paths: one is to storage, so the slower process of data cleaning, verification and organization does not need to be repeated again for new requests. In the second path, the data is used as input to a specialized pipeline.

The final output of the pipeline is a set of Ampliseq™ amplicons to target the isoforms of interest using the Ion Torrent™ sequencing platforms (Figure 3).

Figure 2. RNA-fusion assay design workflow

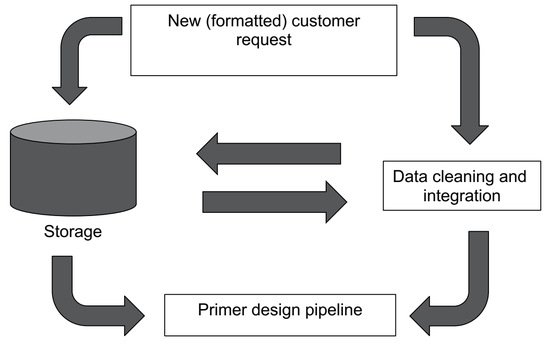


Figure 3. Ion Torrent NGS Systems

Results

Ampliseq™ Designer (https://www.ampliseq.com) offers the flexibility of creating custom fusion panels for Ion Torrent sequencing platforms. However, the number of fusions assays available is limited to a set of pre-designed amplicons. It is in this context that the flexibility of the approach here presented is relevant: an increasing number of researchers requires larger customized panels for gene fusions, even panels containing more than 1000 fusions in a single pool. Such panels can be created through the Ampliseq™ "*White Glove*" service, from Thermo Fisher Scientific, using the pipeline discussed here.

The pipeline presented here has been used in the design of multiple fusion assays, including those contained in the Oncomine™ Focus, Oncomine™ Comprehensive, Oncomine Precision Assay, and many other custom designs. The Oncomine™ Precision Assay was tested on the Ion Torrent Genexus integrated sequencer. Testing on FFPE samples with known positive fusions confirms that the expected fusions were detected with 100% accuracy.

Our system uses a simple, yet powerful standard to describe gene fusions. It allows for representation, QC, and storage of complex gene fusions data, including fusions with breakpoints at exon junctions, those with breakpoints outside exon junctions, and fusions with insertions in the sequence. Once the data has been carefully verified, cleaned and organized, it can be used multiple times to quickly create panels that may contain even thousands of gene fusions.

Conclusions

We have created an automated pipeline to generate customized multiplex RNA fusion assays for targeted next-generation sequencing. The pipeline allows for the fast creation of large panels to detect gene fusions. The approach is to QC once and reuse the data necessary to create gene fusions assays. The data that is stored, can be used directly as input to a pipeline that implements a multiplexed primer design strategy to generate Ampliseq™ panels for gene fusions. Such panels are capable of detecting thousands of isoforms using a single pool of primers with as little as 10ng of cDNA.

References

1. Parker BC, Zhang W. Fusion genes in solid tumors: an emerging target for cancer diagnosis and treatment. Chin J Cancer. 2013 Nov;32(11):594-603. doi: 10.5732/cjc.013.10178. PMID: 24206917; PMCID: PMC3845546.

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R&D team, Carlsbad, CA
R&D team, Ann Arbor, MI

Posters from scientists at Thermo Fisher Scientific:
www.thermofisher.com/

Contact information: antonio.martinezalcantara@thermofisher.com.

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