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Application Note

Trinity™ Workflow for IDT xGen™ Pan-Cancer Panel

Highlights

- The IDT xGen Pan-Cancer Hyb Panel is fully compatible with the Trinity targeted sequencing workflow.
- Analysis metrics are improved or comparable when using the Trinity workflow compared to conventional target capture controls.

Introduction

The Trinity sequencing workflow changes the way targeted sequencing is performed, eliminating many of the manual steps of target capture, saving hours of hands-on time, and removing the need for certain specialized equipment. With the Trinity workflow, prepared DNA library is hybridized to the target probes of interest before being directly loaded onto the AVITI™ system. In contrast, conventional target capture workflows require additional hands-on steps after hybridization, including on-bead capture, a series of temperature-regulated washes, post-wash amplification, and additional library QC. By removing post-hybridization steps, including post-capture amplification, Trinity can provide performance benefits, such as reduced per-sample duplication rates and increased library complexity.

While initially established for exome sequencing, the Trinity workflow can also be applied to other hybrid capture based targeted sequencing panels such as the IDT xGen Pan-Cancer Hybridization Panel. This panel spans approximately 800 kb of the human genome and consists of 7,816 probes. The Pan-Cancer Hyb Panel was developed based on the research findings of The Cancer Genome Atlas network to target significantly mutated genes across twelve tumor tissue types.

Here, we evaluate the compatibility and performance of the IDT xGen Pan-Cancer Hyb Panel with the Trinity workflow and provide guidance for input and sample multiplexing.

Methods

Library Preparation

Sets of 24 libraries were prepared using the IDT xGen DNA Library Prep EZ Kit and 100 ng NA12878 genomic DNA (gDNA) input into the library prep. During library preparation, genomic DNA was sheared enzymatically and xGen Stubby Adapters and xGen UDI Primers for Element (10017037) were appended using 7 cycles of PCR. After library preparation, 100 ng of each library was end-polished with terminal primers and an additional 5-7 cycles of PCR. A set of 12 additional libraries were prepared by the same method but without the end-polish PCR for use in the conventional target capture workflow and sequencing on the AVITI with Cloudbreak Freestyle™ sequencing kits.

Trinity Workflow and Sequencing

For the Trinity workflow, the xGen™ Exome Sequencing Kit Trinity for Element AVITI™ System guide was consulted, with some modifications made for use with the Pan-Cancer Hyb Panel. A pool of 24 libraries was created at 24 µg total input (1 µg per library) into the hybridization reaction for this panel. While this experiment was completed with a 24-plex pool, higher plexity pools would reduce input requirements per individual library while the total hybridization input is held constant.

After pooling, the hybridization reaction was set up according to the xGen Exome Hybridization & Trinity Run Setup protocol, but with 4 µL of the IDT Pan-Cancer Hyb Panel used as the capture panel in the reaction. The reaction was incubated at room temperature for 5-10 minutes, then sealed and transferred to a thermal cycler for hybridization. After hybridization, the reaction was removed from the thermal cycler and diluted as per the Trinity Run Setup protocol. The entire 200 µL diluted hybridization reaction was used for loading onto the sequencer with Trinity Sequencing Reagent and Library Loading Buffer, then sequenced on AVITI with a 2x150 Trinity Sequencing Kit.

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Control Library Hybridization and Sequencing

For the conventional workflow, a pool of 12 libraries was created at 6 µg total (500 ng per library) for input into the hybridization reaction. All protocol defaults for the IDT xGen Hybridization Capture of DNA Libraries workflow with the Pan-Cancer Hyb Panel were followed for hybridization, capture, washing, and post-capture amplification. After library QC, the final linear library pool was diluted to 7 pM and loaded onto AVITI with a spike-in of PhiX Control Library at 0.1 pM. The library was sequenced with a 2x150 Cloudbreak Freestyle (FS) High Output kit.

Analysis

After sequencing, Bases2Fastq software demultiplexed the AVITI-generated bases files and output FASTQ files. All data were then downsampled to 4 million reads per sample for comparison across runs and conditions. Additionally, downsampling at 2 million, 5 million, 8 million, and 20 million reads per sample was completed to compare mean target coverage and other metrics. Data analysis was conducted via Picard, Sentieon, DeepVariant, and internal pipelines.

Results

Libraries hybridized with the xGen Pan-Cancer Hyb Panel and sequenced with the Trinity workflow show comparable sequencing quality and secondary metrics to the control libraries prepared using the same panel with the conventional IDT xGen Hybridization Capture workflow (Table 1).

Metric	Conventional	Trinity
Total Library Input (Hyb. Rxn.)	6 µg	24 µg
Density (Total PF Polonies)	1038 M	1073 M
Quality (% Q30)	95.06%	91.63%
On-Target Rate	85.43%	88.88%
Mean Target Coverage	553.93	608.26
% Targets at 30x Cov.	99.62%	99.59%
Zero-Target Cov.	0.04%	0.04%
% Duplication	6.69%	5.99%

Table 1. Libraries hybridized with the IDT xGen Pan-Cancer Panel and sequenced with the Trinity workflow show comparable performance to control libraries prepared with the conventional target capture workflow. Data was downsampled to 4 million reads per sample prior to analysis.

An input of 24 µg total library into the hybridization reaction resulted in over one billion total pass-filter reads for libraries sequenced with the Pan-Cancer Hyb Panel and Trinity workflow.

Sequencing run quality remained high for the Pan-Cancer Hyb Panel with both the conventional and Trinity workflows, with %Q30 for the Trinity sequencing run averaging 91% (Table 1).

NGS metrics for assessing coverage and uniformity were comparable or better for libraries sequenced with the Trinity workflow versus conventional capture workflow. For example, the on-target rate for libraries sequenced with the Trinity workflow is 85-89%, while similar libraries prepared with the conventional workflow achieve an on-target rate of 85% (Figure 1).

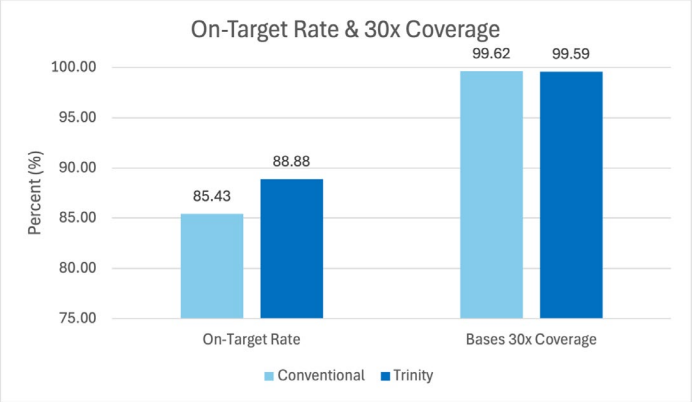

Figure 1. On-target rate and percent of bases with 30x coverage (averaged across samples for each run) for IDT xGen Pan-Cancer Hyb Panel libraries sequenced with the Trinity vs. conventional workflow. Data was downsampled to 4 million reads per sample.

In an analysis of four million reads per sample, Trinity libraries achieve 99.59% of target bases with 30x coverage on average, which is comparable with conventional libraries (Figure 1). In all samples, over 99% of targets achieve greater than 30x coverage, and libraries sequenced with the Trinity workflow achieve slightly higher percentages of target bases at various coverage depths than those sequenced with the conventional workflow (Figure 2).

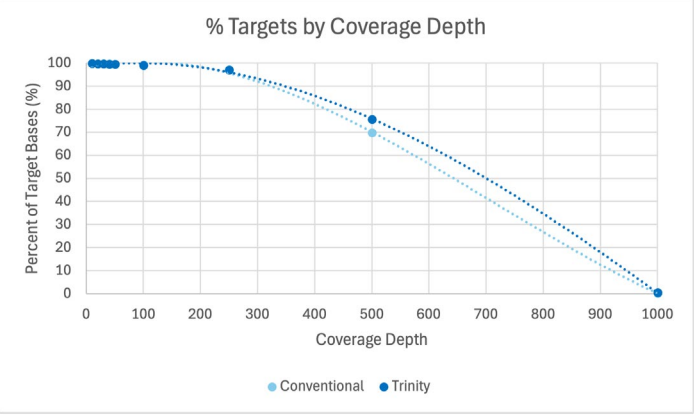

Figure 2. Percent of target bases by coverage depth for IDT xGen Pan-Cancer Hyb Panel libraries sequenced with the Trinity vs. conventional workflow. Data was downsampled to 4 million reads per sample.

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Trinity libraries demonstrate a higher mean target coverage than their conventional counterparts, reaching ~608x coverage on average, while the average for the conventional libraries was ~554x. In addition, a comparable or larger percentage of Trinity libraries reach 500x coverage in a variety of downsampling schemes, from 2 million reads per sample to 20 million reads per sample (Figure 3).

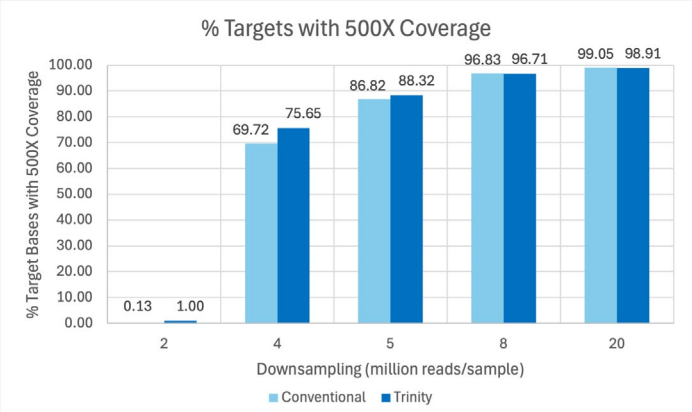

Figure 3. Percent of target bases with 500x coverage (averaged across samples for each run analysis) across various downsampling schemes for IDT xGen Pan-Cancer Hyb Panel libraries sequenced with the Trinity vs. conventional workflow. Data was downsampled to 2 million, 4 million, 5 million, 8 million, and 20 million reads per sample.

Trinity libraries also generally exhibit lower duplicates than libraries prepared with the conventional workflow, since the Trinity workflow does not include a post-capture amplification step. On average, libraries sequenced with Trinity exhibited slightly lower duplicates (5.99%) than libraries prepared with the conventional target capture workflow (6.69%). Trinity libraries also exhibit comparable performance to conventional libraries for variant calling recall and precision (Figure 4).

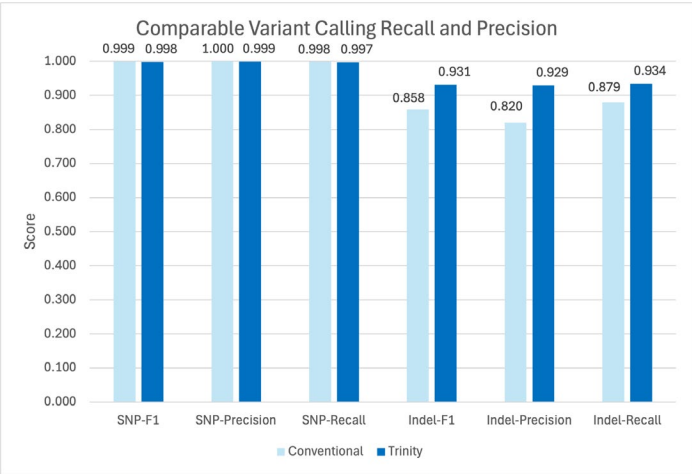

Figure 4. Scores for SNP-F1, Precision, and Recall and Indel-F1, Precision and Recall for IDT xGen Pan-Cancer Panel libraries sequenced with the Trinity vs. conventional workflow. Data was downsampled to 4 million reads per sample.

Conclusion

The IDT xGen Pan-Cancer Hybridization Panel performs well with the Trinity workflow, with all metrics meeting or exceeding those achieved with a conventional in-solution target capture workflow. While total input per hybridization reaction remains constant for this panel (24 µg), pool plexity can be adjusted accordingly based on per-sample read requirements. This work with theIDT xGen Pan-Cancer Hyb Panel demonstrates the compatibility of a small capture panel with the Trinity sequencing workflow, with only minor adjustments needed to achieve full throughput of the instrument.

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