Element Biosciences

Technical Note

Trinity™ Workflow with IDT xGen™ Exome Kit and 1-2 hour Fast Hybridization

Introduction

Unlike traditional hybrid capture workflows, the Trinity sequencing workflow eliminates manual post-hybridization steps, saving hours of hands-on time prior to sequencing. While an overnight 16-hour hybridization is considered part of the standard workflow, those seeking an even faster sample preparation time can do so with Trinity enabled fast-hybridization.

Here, we successfully demonstrate 1-2 hour hybridization reactions for the Trinity workflow with the IDT xGen Exome Kit, allowing pre-sequencing hands-on time to be completed within 8 hours (Figure 1) with minimal impact to final metrics.

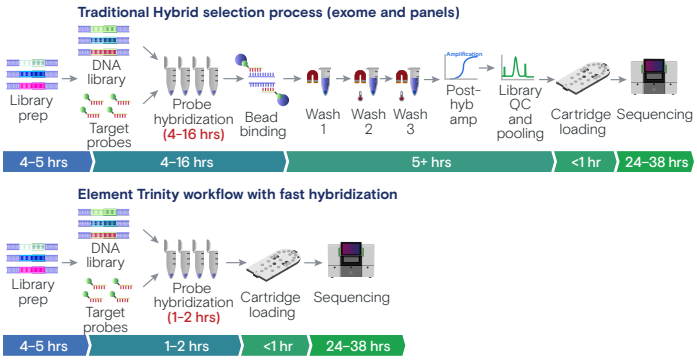

Figure 1. With the fast hybridization protocol, library prep, hybridization, and sequencing run set-up can be completed in a single day with the Trinity workflow.

Methods

Library preparation

Two sets of 24 libraries were prepared using the xGen™ Exome Sequencing Kit Trinity for Element AVITI™ System. For the first set of libraries, xGen Stubby Adapters and xGen Indexing Primers for Element (10017037) were appended to the sheared gDNA with seven cycles of PCR. After library preparation, this set of libraries was end-polished with terminal primers and an additional five cycles of PCR prior to library QC. For the second set of libraries, Element Elevate™ v2 full-length adapters (830-00010) were appended to the sheared DNA with seven cycles of PCR. Libraries in the full-length adapter set underwent an additional 1.0x post-ligation SPRI clean-up (after the existing 0.8x post-ligation SPRI clean-up in the protocol) prior to library QC.

Library Set	Hybridization Input	Loading
1-hr (stubby)	15 µg	100 µL
1-hr (full)	12 µg	150 µL
2-hr (stubby)	12 µg	70 µL
2-hr (full)	12 µg	90 µL

Table 1. Hybridization parameters for 1 and 2 hour fast hybridization. "Hybridization Input" refers to total input into the hybridization reaction, while "Loading" refers to total loading of the post-hybridization dilution onto the sequencer.

Hybridization and sequencing

Libraries were pooled by 24-plex for hybridization reactions (a summary of input conditions and instrument loading amount is shown in Table 1). After pooling, Human Cot DNA and Trinity Binding Reagent were added to each reaction prior to being dried down as per the xGen Exome Hybridization & Trinity Run Setup protocol. After reactions were dried down, some minor deviations were made from the standard 16 hour hybridization workflow: the pellets were resuspended in 8.5 µL of xGen 2x Hybridization Buffer, 4 µL of xGen Exome v2 Panel, and 4.5µL of nuclease-free water (omitting the xGen Hybridization Buffer Enhancer). These reactions were incubated at room temperature for 5-10 minutes, then sealed and transferred to a thermal cycler, where hybridization would occur at a higher temperature than the standard 16-hour hybridization protocol. The thermal cycler conditions are listed below in Table 2.

Step	Temperature (°C)	Duration
Initialization	95°C	5 minutes
Hybridization	80°C	1-2 hours
Hold	80°C	HOLD

Table 2. Thermal cycling parameters for 1-2 hour hybridization reaction. Lid temperature should be set to 100°C.

After hybridization was complete, the reactions were removed from the thermal cycler and Trinity run setup proceeded as per the xGen Exome Hybridization & Trinity Run Setup protocol. The quantity of each diluted hybridization reaction used for final loading was adjusted depending on the library type (stubby or full-length adapter libraries) and hybridization time (1-2 hours) (Table 1). Reactions were sequenced on AVITI with a 2x75 Trinity Sequencing Kit. After downsampling to 30 M paired-end reads (30 M polonies, 60 M total reads/sample), data was analyzed using Picard, Sentieon, DeepVariant, and internal pipelines.

Equivalent performance of fast hybridization

The Trinity workflow fast hybridization method demonstrates comparable performance to a standard overnight hybridization while enabling all hands-on work to be completed in a single day (Figure 1). Libraries prepared with the fast hybridization method require a few minor changes to the workflow, including changes to the total library input required per hybridization reaction and certain reagent volumes. In addition, the use of Element Elevate v2 full length adapters or an end polish of stubby adapter libraries is recommended during library preparation to reduce input requirements (see Methods for details). In this study, libraries prepared with either full-length or stubby adapters and a 1 hour or 2 hour hybridization reaction produce equivalent performance metrics that meet all product specifications, with the 2 hour hybridization also providing a small boost in polony density (Table 3).

The on-target rate for libraries prepared with the fast hybridization protocol ranges from 86.6-89.9%, and fold-80 base penalty ranges from 1.4-1.43. The percent of target bases at 20x and 30x coverage is also comparable between all sequencing runs (Table 3). Both the 1 hour and 2 hour configurations produce uniform target coverage depth, and the 2 hour hybridization reactions show improved performance for on-target rate and uniformity metrics, like fold-80.

Metric	1 hr (stubby)	1 hr (full)	2 hr (stubby)	2 hr (full)	16 hr (ref.)
Total PF Polonies	820.8 M	924.6 M	942.5 M	975.6 M	898.7 M
% Q30	92.2	95.68	94.17	95.51	92.79
On-Target Rate	86.84	86.64	89.9	89.76	92.34
Fold-80	1.43	1.43	1.4	1.4	1.39
Mean Target Coverage	55.49	55.33	57.44	57.97	60.94
% Targets at 30x Cov.	91.77	91.55	93.2	93.06	93.17
% Targets at 20x Cov.	96.81	96.74	97.04	96.94	96.5
% Duplication	1.27%	1.26%	1.05%	1.27%	0.91%
AT Dropout	1.97	1.81	2.07	1.68	1.76
GC Dropout	2.9	2.65	2.46	2.81	3.95
Zero-Target Cov.	1.13%	1.12%	1.14%	1.14%	1.19%
SNP-F1	0.9906	0.9907	0.9905	0.9906	0.989
Indel-F1	0.9806	0.9796	0.9766	0.978	0.966

Table 3. Analysis metrics for 1-2 hour hybridization with both stubby adapter and full-length adapter libraries prepared with the IDT xGen library prep and xGen Exome Trinity kits and sequenced with the Trinity workflow, compared to a 16 hour hybridization for reference. Sequencing data was downsampled to 30 M paired-end polonies for this comparison.

Conclusion

Using a fast hybridization method with the Trinity workflow and IDT xGen Exome kit enables library prep, hybridization, and sequencing run set-up to be completed in a single day, with only minor changes to the hybridization protocol required and without compromising secondary analysis metrics. This drastically reduces the turnaround time for exome sequencing.

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