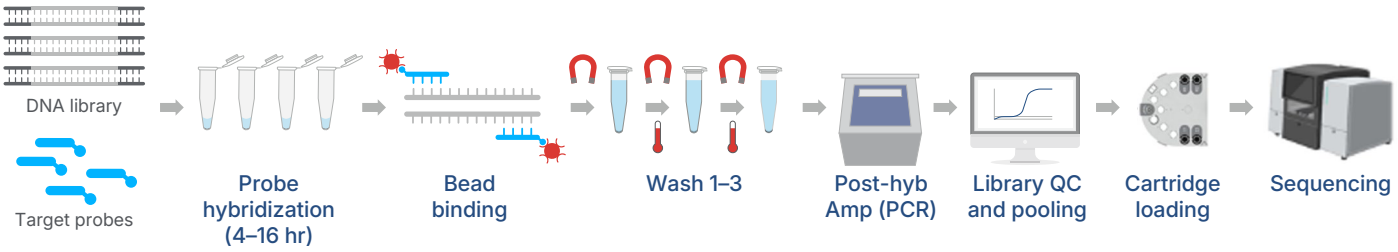
xGen™ Exome Sequencing Kit Trinity™ for Element

Target enrichment research reagents for the Trinity™ workflow on the Element Biosciences AVITI™ sequencing platform

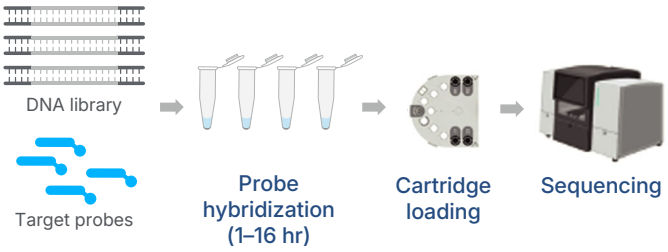
The xGen Exome Sequencing Kit Trinity for Element Biosciences offers a complete whole exome sequencing (WES) workflow streamlined through on-sequencer target enrichment providing labs with a seamless transition from hybridization capture to sequencing. The Trinity™ workflow reduces hands on time by up to 4 hours compared to traditional hybridization capture (hyb cap), without sacrificing quality.

Trinity workflow compared to traditional hybridization capture workflow

Traditional hybrid selection process (exome and panels)



Element Trinity workflow



The pairing of IDT's xGen technology with the Element Bioscience's Trinity™ workflow for the AVITI™ platform offers labs exceptional specificity across samples as well as time saving benefits by reducing hands on time by up to 4 hours when compared to standard hyb cap workflows.

To support labs prioritizing turnaround time, IDT evaluated our xGen technology with the Trinity workflow using a 1-hour hybridization capture protocol developed by Element Biosciences. This approach uses an optimized hybridization temperature and loading volume, dramatically shortening the hyb cap step in a WES workflow. The protocol is available upon request or can be accessed via our **demonstrated protocol**.

Performance data using IDT standard overnight hyb cap protocol vs 1-hour hyb cap workflow

Although both approaches consistently generated acceptable performance metrics, the IDT recommended protocol (overnight hybridization capture) produced the highest sequencing performance metrics, while the shortened hyb cap workflow generated slightly lower performance metrics (**Figure 1**, **Table 1**).

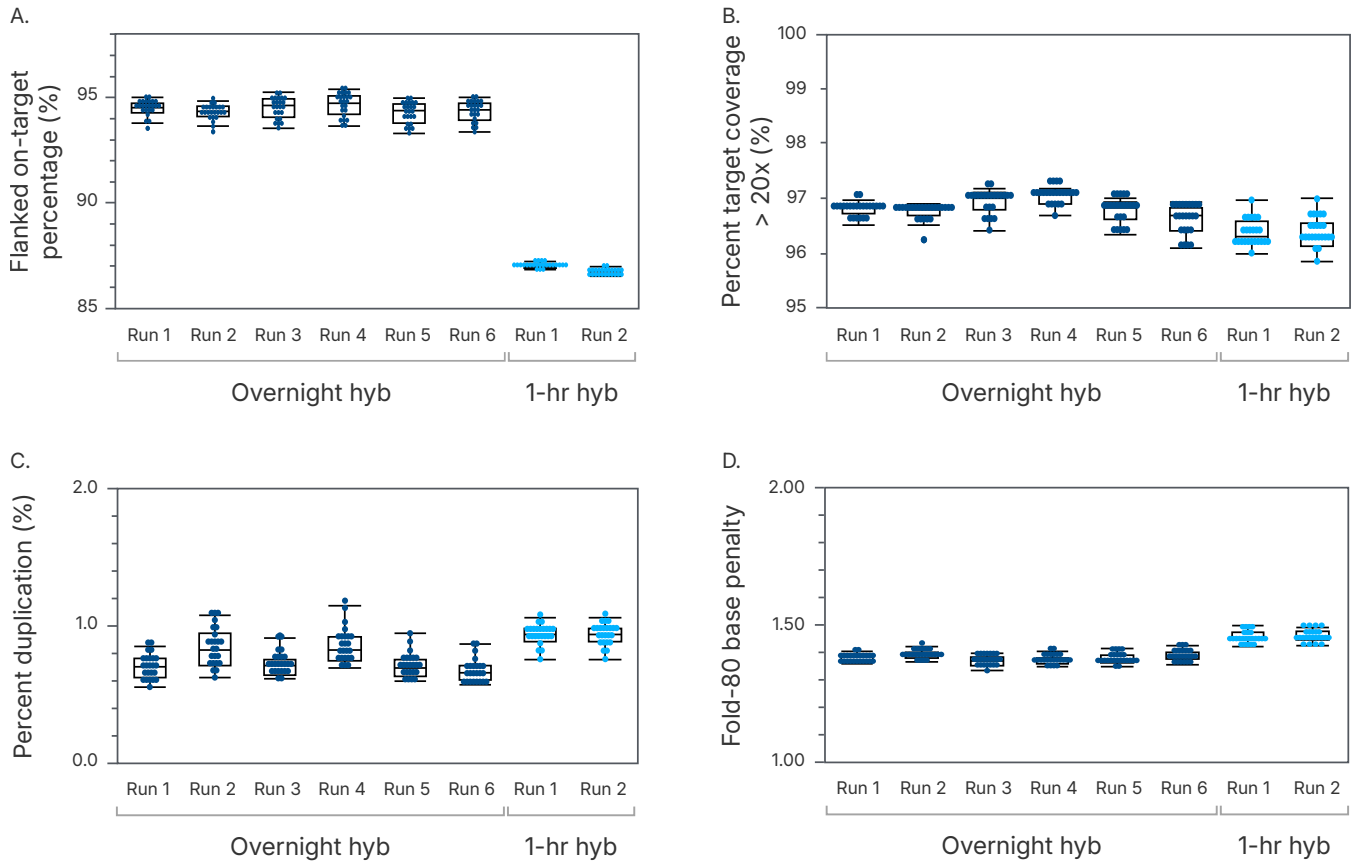


Figure 1. Consistent sequencing metrics across samples and runs. Libraries generated using the xGen Exome Sequencing Kit Trinity™ for Element were consistent in regard to flanked on-target percentage (**A**), percent target base coverage > 20X (**B**), percent duplication (**C**), and in fold-80 base penalty (**D**). Libraries used in both hybridization reactions were constructed from 100 ng input of Coriell NA12878 genomic DNA (gDNA) using the xGen DNA EZ UNI Library Prep Kit per the manufacturer's instructions. Libraries used in 1-hour hybridization reactions underwent end-polishing using the xGen Library Amplification Primer Mix for Element and five cycles of PCR. Both end-polished (1-hour hybridization) and non-end polished (overnight hybridization) libraries were then hybridized using an xGen Exome v2 Hybridization Panel following the xGen Exome Sequencing Kit Trinity™ for Element AVITI™ System Protocol. Hybridized libraries were sequenced using a Trinity 2 × 150 Sequencing Kit on an Element Biosciences AVITI™ Sequencing System. Following sequencing, samples were downsampled to 40 M reads before analysis using Picard (1-hour hybridized libraries: *n* = 24 per run x 2 runs; overnight hybridization libraries: *n* = 24 per run x 6 runs).

Table 1. Comparative performance across key functional NGS-metrics when using 1-hour vs overnight hybridization reaction times.

	Overnight hybridization (*n* = 6 runs)		1-hour hybridization (*n* = 2 runs)	
Sequencing metric	**Observed average**	**Observed range**	**Observed average**	**Observed range**
Flanked On-target %	94.41%	93.31–95.38%	86.85%	86.54–87.24%
Percent Selected Bases	93.27%	92.23–94.16%	85.73%	85.42–86.02%
% Target Base Coverage at 20X	96.82%	96.11–97.17%	96.35%	95.85–96.99%
% Target Base Coverage at 30X	93.26%	90.68–94.65%	89.01%	87.33–90.88%
% Duplication	0.74%	0.55–1.15%	0.92%	0.75–1.06%
Fold-80 Base Penalty	1.38	1.34–1.43	1.46	1.42–1.50

Adaptable hybridization capture workflows

The choice between an overnight hybridization step and a shortened hybridization step depends on several factors. Labs requiring high-flanked on-target reads may prefer an overnight hybridization, while others may need to prioritize faster turnaround times. By comparing the results for both workflows, labs have the necessary data to determine which workflow best fits their needs (**Table 2**). The data presented above illustrates that the xGen Exome Sequencing Kit Trinity™ for Element Biosciences when paired with the AVITI™ platform can be adapted to support both overnight hybridization and 1-hour hybridization workflows.

Table 2. Overnight hybridization and 1-hour hybridization reaction comparison.

Overnight hybridization	1-hour hybridization
+ High flanked on-target % (≥ 93.31%) with consistent performance among replicates (0.49% CV)	+ Fast turnaround time from finished library to sequencing start (≤ 3-hours)
+ Low Fold-80 base penalty (≤ 1.43) with consistent performance among replicates (1.32% CV)	+ Acceptable and consistent flanked on-target % ≥ 86.54%, 0.13% CV)
– Increased turn-around time to generate whole exome sequencing data	– Compared to overnight hybridization, lower flanked on-target % (≥ 86.54%) and higher fold-80 base penalty (≤ 1.50)

If you would like assistance determining which protocol is ideal for you, IDT experts are available to discuss your specific needs and the available data of both workflows. Request a Consultation at **idtdna.com/xGen-Exome-AVITI**.

Additional information on the Trinity Workflows visit **idtdna.com/xGenTrinity**