# Feasibility of a Complete PCR-free Whole Exome Sequencing Using a New Integrated Library Preparation and Sequencing Workflow

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## Background

Over the last 15 years, whole exome sequencing (WES) has been demonstrated as a robust and cost-effective approach for research that can potentially help detect genetic variants across a broad spectrum of inherited human diseases and disorders. Despite ongoing efforts to shorten hybridization time and develop automation solutions for target capture workflows, the complex protocols, lengthy post-enrichment wash processes, and stringent temperature requirements continue to contribute to assay complexity and variability. Additionally, library preparation and PCR amplification steps limit the sensitivity of the assay due to the introduction of enzyme-based errors. Low concordance of INDELs is often seen when comparing WES and PCR-free whole genome sequencing (WGS).

We present a unique exome workflow that addresses these challenges by eliminating the need for PCR and seamlessly integrating post-hybridization steps directly into the sequencing process, removing several steps and improving the resolution of the workflow. After the enrichment step, the hybridization reaction is directly loaded onto the sequencing instrument. Applying this technology to exome enrichment with genomic reference DNA, high on-target rates (>90%), high coverage uniformity (fold-80 penalty 1.3-1.4), and a mean sample coverage depth of ~70x were observed. Eliminating post-enrichment amplification results in a 3- to 4-fold lower duplication rate relative to the traditional WES workflow.

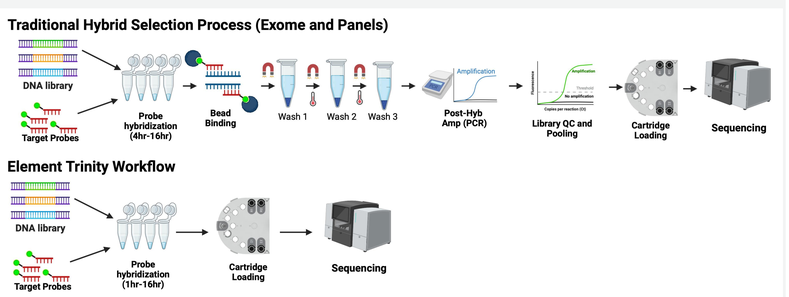


**Figure 1**: Comparison between Element Trinity workflow and traditional hybridization capture process. Trinity workflow eliminates all post-hybridization steps by directly loading the hybridization reaction into the cartridge for AVITI™ sequencing. Trinity hybridization can be as short as 1 hour, which enables the entire workflow (library prep + probe hybridization + sequencing set up) to be complete in a single day.

## Methods

**Library prep**: Element Elevate™ PCR-free libraries were prepared by using Elevate Enzymatic library prep kit coupled with Elevate long UDI adapter kit with 500 ng gDNA (Coriell DNAs: HG001, HG002, HG003, and HG004) as input for each reaction, following Elevate Enzymatic library prep user guide. Ten percent of the PCR-free libraries were utilized to prepare PCR-plus libraries following Elevate PCR-plus library prep user guide. PCR-free libraries were quantified via qPCR, while PCR-plus ones were quantified by Qubit.

**Trinity hybridization and run set up**: Trinity for Twist fast hyb was set up using 2.5-3 µg of 8-plex library pool following Trinity for Twist fast hyb user guide. Briefly, the hybridization reaction was incubated at 71ºC for 1 hour, and diluted by 150 µl Trinity fast hyb loading buffer. The library loading solution was prepared by mixing 50 µl of diluted hybridization reaction with 72 µl Trinity sequencing reagent in a final 2200 µl volume and was loaded into a Trinity sequencing cartridge. The Trinity sequencing run was initiated in AVITI™ sequencer with a Trinity flow cell at 2x150 plus indexing.

**Traditional exome library preparation and run set up**: The exome library was prepared by using PCR-plus libraries described above as the input by following Twist Target Enrichment Fast Hybridization protocol. 6 pM of the final exome library was loaded into a Cloudbreak™ Freestyle sequencing cartridge with a Cloudbreak™ Freestyle flow cell at 2x150 plus indexing.

**Data analysis**: Bases2fastq was used to generate adapter trimmed FASTQ files. Each sample was down-sampled to 80 million reads, following by alignment to the hg38 reference using Sentieon BWA-MEM. Panel metrics were calculated with the Twist Exome 2.0 bed file, and variant calling was performed using Deepvariant v1.6.0, benchmarked again the NIST v4.2.1 truth set.

## Results

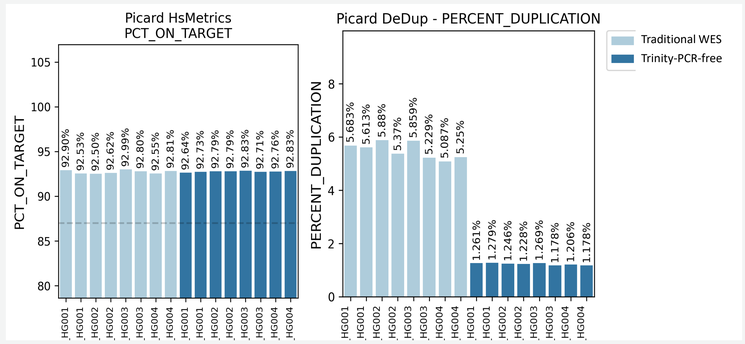


**Figure 2**. Trinity-PCR-free workflow exhibits equivalent on-target rate, with much lower duplication rate, comparing to traditional WES workflow. All samples were down-sampled to 80 M pair-ended reads for analysis.

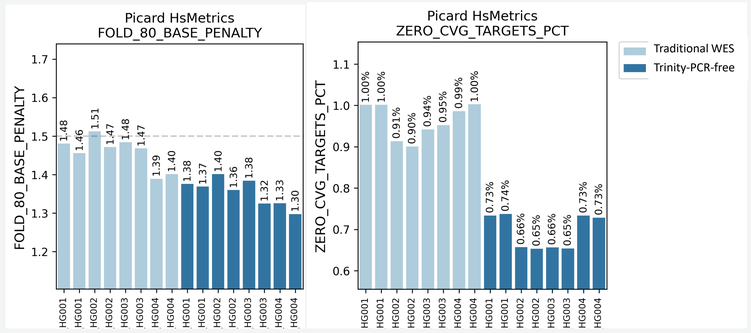


**Figure 3**. Trinity-PCR-free workflow exhibits a more uniform and better coverage.

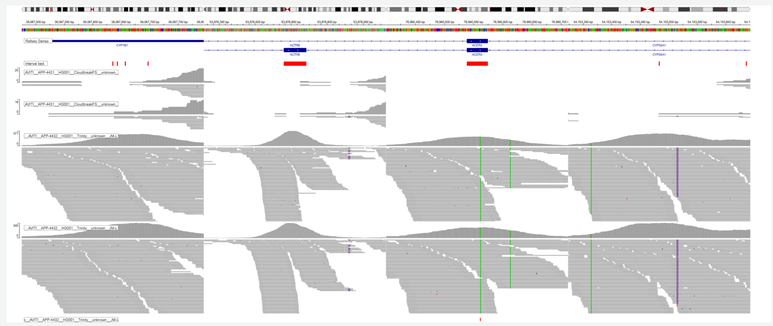

**Figure 4**. IGV snapshot of regions covered by Trinity-PCR-free but not by the traditional WES workflow. The top tracks display coverage for the traditional WES samples, while the bottom tracks show coverage for the Trinity-PCR-free samples.

## Results continue

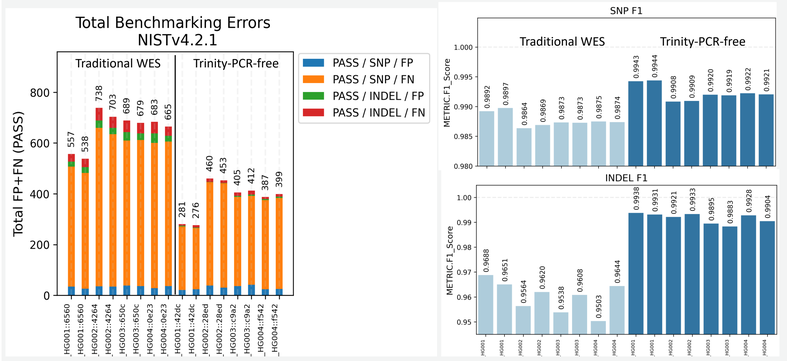


**Figure 5**. Trinity-PCR-free workflow showed much better benchmarking comparing to traditional WES workflow. Total Benchmarking errors are much lower, and both SNP and INDEL F1 scores are higher in the Trinity-PCR-free run.

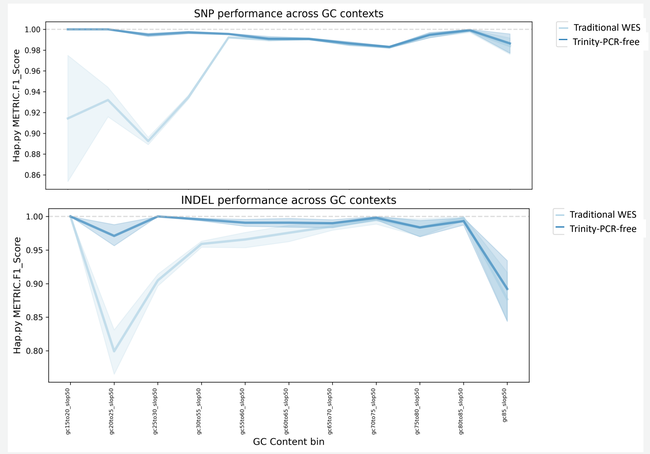


**Figure 6**. SNP and INDEL performance across different GC content bins. Trinity-PCR-free workflow exhibits more uniform and higher SNP and INDEL F1 scores in a broad range of GC bins.

## Conclusions

- This study demonstrates an improved workflow and performance for hybridization-based sequencing approaches, by using Trinity in AVITI™ sequencer.
- This study also demonstrates a feasibility of a complete PCR-free WES workflow using Trinity product in AVITI sequencer.
- Significant improvements were observed with the PCR-free WES Trinity workflow comparing to the traditional hybridization capture process, including but not limited to more uniform coverage, better coverage, lower duplication rate, and much better benchmarking results.

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