POSTER NOTE
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COMPARISON OF WHOLE-EXOME SEQUENCING WORKFLOWS USING SHORT HYBRIDIZATION TIMES

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INTRODUCTION

The rapid growth of targeted next-generation sequencing (NGS) applications has increased the demand for workflows with short turnaround times. However, most hybridization-based target enrichment workflows include an overnight hybridization step, presenting a major obstacle in the development of single-day protocols for whole-exome sequencing (WES) and other targeted sequencing applications.

The KAPA HyperCap Workflow v3 is a high-performance, streamlined target enrichment solution that combines high-efficiency KAPA DNA library preparation kits with high-performance KAPA Target Enrichment probes. This portfolio includes the new comprehensive-yet-compact ~43 KAPA HyperExome panel, which covers exonic regions defined by the CCDS, RefSeq, Ensembl, GENCODE, and ClinVar databases, including medically relevant variants.

Towards the development of a single-day target enrichment workflow, we have tested the effectiveness of the KAPA HyperCap Workflow v3 with hybridization times as short as 15 minutes using KAPA HyperExome, and compared the results to data produced using the recommended overnight (~16 h) hybridization step. In addition, we compared the results to a market-equivalent WES workflow using the same range of hybridization times.

EXPERIMENTAL DESIGN

The KAPA HyperCap Workflow v3 comprises the following steps **(Figure 1)**: library preparation using the KAPA HyperPlus Kit (enzymatic DNA fragmentation) or the KAPA HyperPrep Kit (mechanical fragmentation); hybridization of biotinylated probes to the regions of interest; capture of targeted fragments with streptavidin beads; and post-capture amplification by ligation-mediated PCR. The quality and quantity of enriched libraries is assessed prior to sequencing.

In this study, library preparation and target enrichment recommendations (including DNA input amounts) were followed according to supplied documentation for both the KAPA HyperCap Workflow v3 (using **KAPA HyperExome probes**) and a market-equivalent kit for WES. The only variable changed in this study was the duration of the hybridization step. Triplicate libraries were prepared for each kit, for each hybridization time point tested.

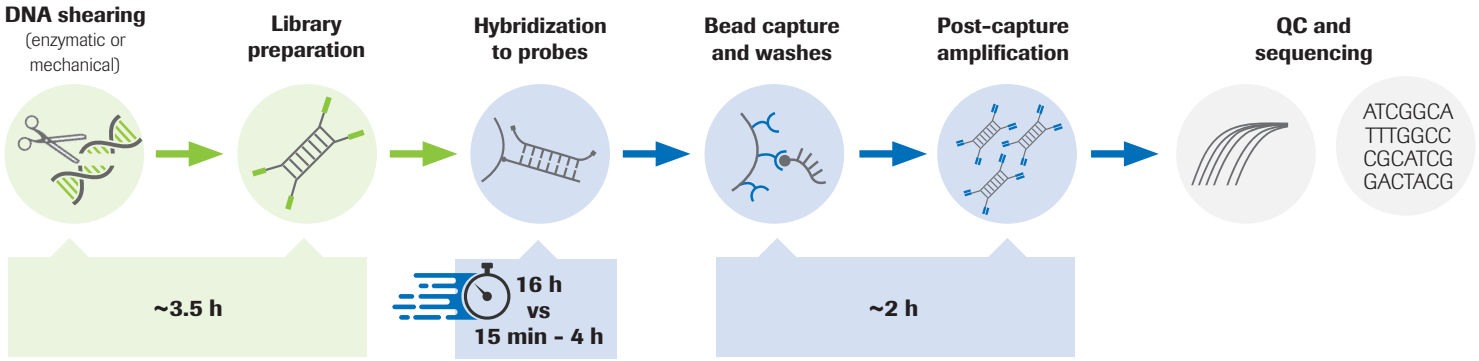

Figure 1: Overview of the KAPA HyperCap Workflow v3. The estimated processing time for each step is outlined. The hybridization to probes was performed for a recommended 16 hours, and shorter protocols were performed with 15 minutes to 4 hours hybridization (see **Table 1**).

	HyperCap v3 with KAPA HyperPrep Kit	KAPA HyperCap v3 with KAPA HyperPlus Kit	Supplier X	
gDNA input mass (Coriell DNA NA12878)	100 ng	100 ng	50 ng	
DNA fragmentation method (mode size)	Mechanical (200 bp)	Enzymatic (200 bp)	Enzymatic (200 bp)	
Adapter type	Universal	Universal	Universal	
Pre-capture PCR primers	Unique Dual-Indexed	Unique Dual-Indexed	Unique Dual-Indexed	
Exome panel capture size	43 Mb	43 Mb	41.2 Mb	
DNA sample library input into capture	1000 ng (singleplex)	1000 ng (singleplex)	500 ng (singleplex)	
Concentration of pre-hybridization libraries	Magnetic beads	Magnetic beads	Vacuum concentrator	
Hybridization and wash kit	KAPA HyperCapture	KAPA HyperCapture	Fast kit	Regular kit
Hybridization duration (recommended)	**16 to 20 h**	**16 to 20 h**	**15 min to 4 h**	**16 h**
Hybridization durations tested in this study	**15 min, 1 h, 4 h, 16 h**	**15 min, 1.5 h, 4 h, 16 h**	**15 min, 1.5 h, 4 h**	**16 h**
Hybridization temperature	55 °C	55 °C	60 °C	70 °C
Wash temperature	55 °C	55 °C	70 °C and 48 °C	48 °C
Sequencing platform	Illumina® NextSeq® 500 (2 x 75 bp)	Illumina NextSeq 500 (2 x 75 bp)	Illumina NextSeq 500 (2 x 75 bp)	Illumina NextSeq 500 (2 x 75 bp)

Table 1: Experimental design. Experiments were performed according to manufacturer instructions, except for hybridization times, which varied as noted.

HYBRIDIZATION TIMES AS SHORT AS 15 MINUTES YIELD HIGH-QUALITY RESULTS WITH THE KAPA HYPERCAP WORKFLOW V3 TO THE ORIGINAL

SHORT HYBRIDIZATION USING ENZYMATICALLY FRAGMENTED DNA HAS MINIMAL IMPACT ON SEQUENCING METRICS

To assess the performance of the KAPA HyperCap Workflow v3 with reduced hybridization times using enzymatically fragmented input DNA, libraries were prepared with the KAPA HyperPlus Kit and enriched using KAPA HyperExome probes; hybridization times ranged from 15 minutes to the standard 16 hours.

Similar sequencing coverage was obtained across all hybridization time points, with a slight reduction for the 15-minute samples. Shorter hybridization times had minimal impact on capture efficiency–described by the percent of on-target reads and the fold-80 base penalty–and only a small effect on library uniformity across the GC% spectrum. On the other hand, the percent of PCR duplicates was highly impacted by the shortest hybridization time, with shorter protocols generating less complex libraries. However, applications that do not require high sensitivity for rare allele detection can tolerate higher duplication rate.

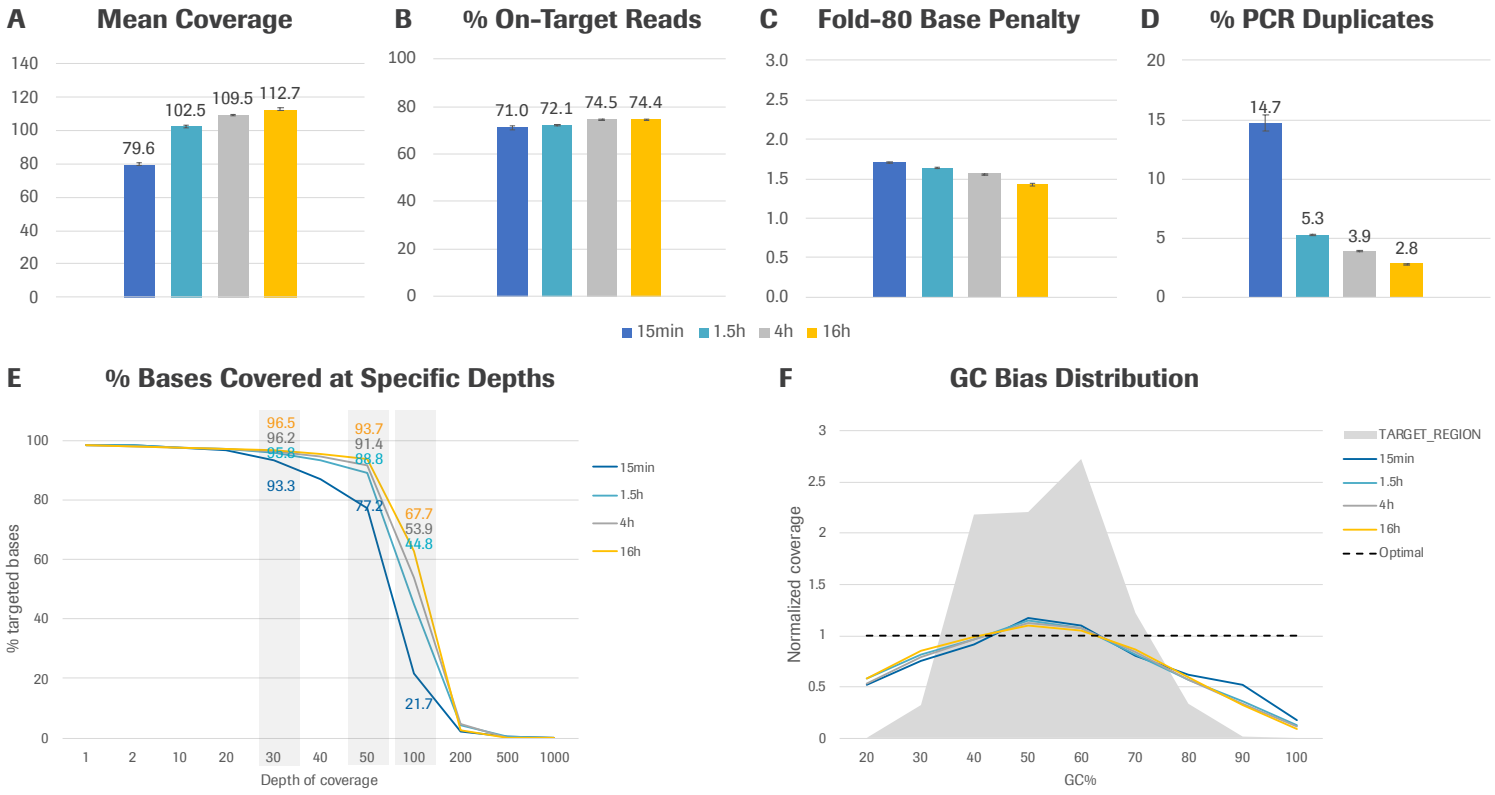

Figure 2: Performance of the KAPA HyperCap Workflow v3 with the KAPA HyperPlus Kit (enzymatic DNA fragmentation) and KAPA HyperExome probes using a range of hybridization times. Sequencing reads were downsampled to 135.5 M raw reads per sample. The percent of on-target reads refers to the percent of mapped, non-duplicate reads overlapping a target region by at least 1 base. Bars represent the mean for triplicate libraries and error bars indicate the standard deviation. In the GC bias distribution chart, each line represents the average of normalized coverage across GC% bins for triplicate libraries; the horizontal line represents the optimal normalized coverage; the shaded area is the GC% distribution of 100 bp windows in the target region.

SHORT HYBRIDIZATION USING MECHANICALLY FRAGMENTED DNA HAS MINIMAL IMPACT ON SEQUENCING METRICS

To assess the performance of the KAPA HyperCap Workflow v3 with reduced hybridization times using mechanically fragmented input DNA, libraries were prepared with the KAPA HyperPrep Kit using Covaris®-sheared DNA and then enriched using KAPA HyperExome probes; hybridization times ranged from 15 minutes to the standard 16 hours.

The overall sequencing performance of libraries derived from mechanically sheared DNA using shorter hybridization was concordant with the enzymatic fragmentation data. Consistent coverage, on-target rate, and uniformity were obtained across all hybridization times, while the PCR duplicates rate was the most affected sequencing metric–especially with the shortest hybridization time.

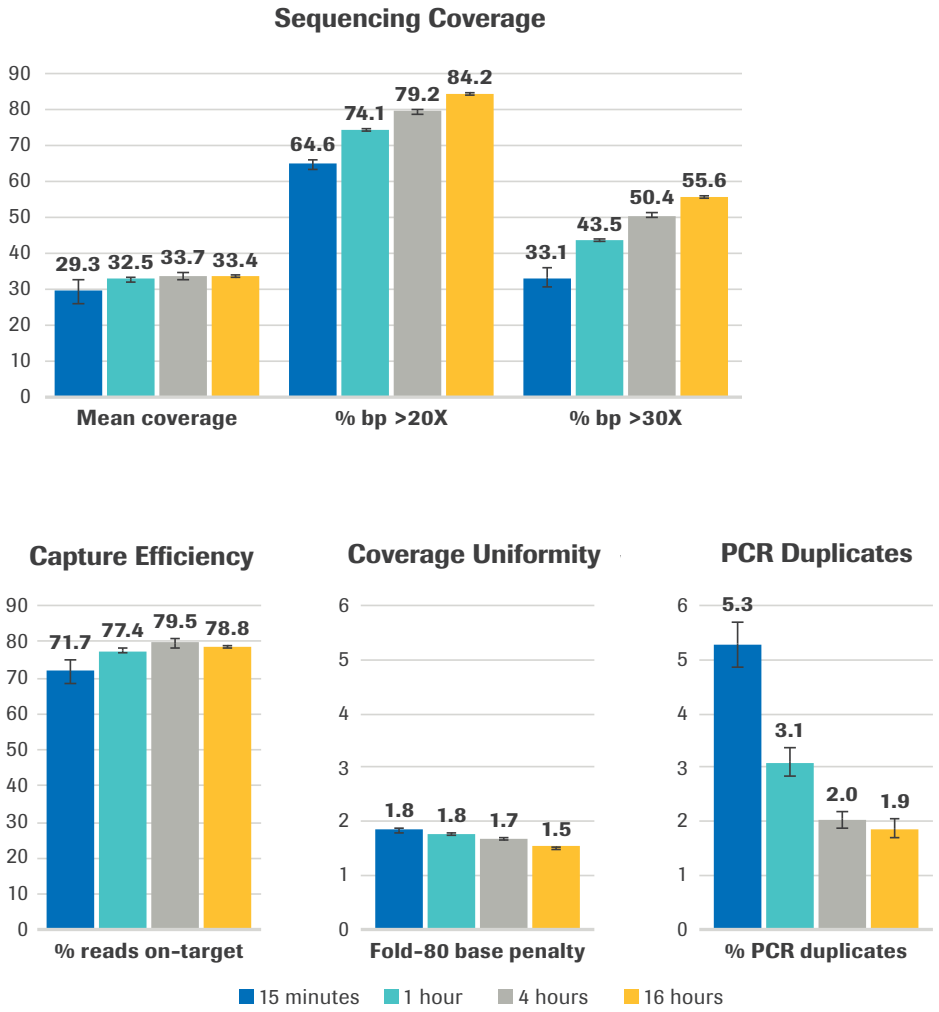

Figure 3: Performance of the KAPA HyperCap Workflow v3 with the KAPA HyperPrep Kit (mechanical DNA fragmentation) and KAPA HyperExome probes using a range of hybridization times. Sequencing data was downsampled to 50X raw coverage. Bars represent the mean for triplicate libraries and error bars indicate the standard deviation.

KAPA HYPEREXOME OUTPERFORMS A MARKET-EQUIVALENT SHORT-HYBRIDIZATION KIT

The KAPA HyperCap Workflow v3 (with KAPA HyperPlus and KAPA HyperExome) was compared to the Supplier X workflow for WES, using overnight and shorter hybridization times (see **Table 1** for workflow details). Both workflows utilize enzymatic fragmentation of DNA. In the KAPA HyperCap workflow, the KAPA HyperCapture Kit was used to hybridize libraries for all time points. The Supplier X offers two hybridization and wash kits: the fast kit, recommended for 15 minutes to 4 hours hybridization, and the regular kit, for 16-hour hybridization.

KAPA HyperCap Workflow v3 with KAPA HyperExome outperformed the Supplier X workflow across several key sequencing metrics. For example, following the standard 16-hour hybridization, KAPA HyperCap yielded equivalent coverage and uniformity, higher on-target rate, and lower duplication rate than the Supplier X regular kit.

With the shorter protocols, the KAPA HyperExome libraries showed overall better targeted coverage, higher target enrichment efficiency, greater library complexity, and significantly higher library uniformity than the Supplier X fast kit across the range of hybridization times tested.

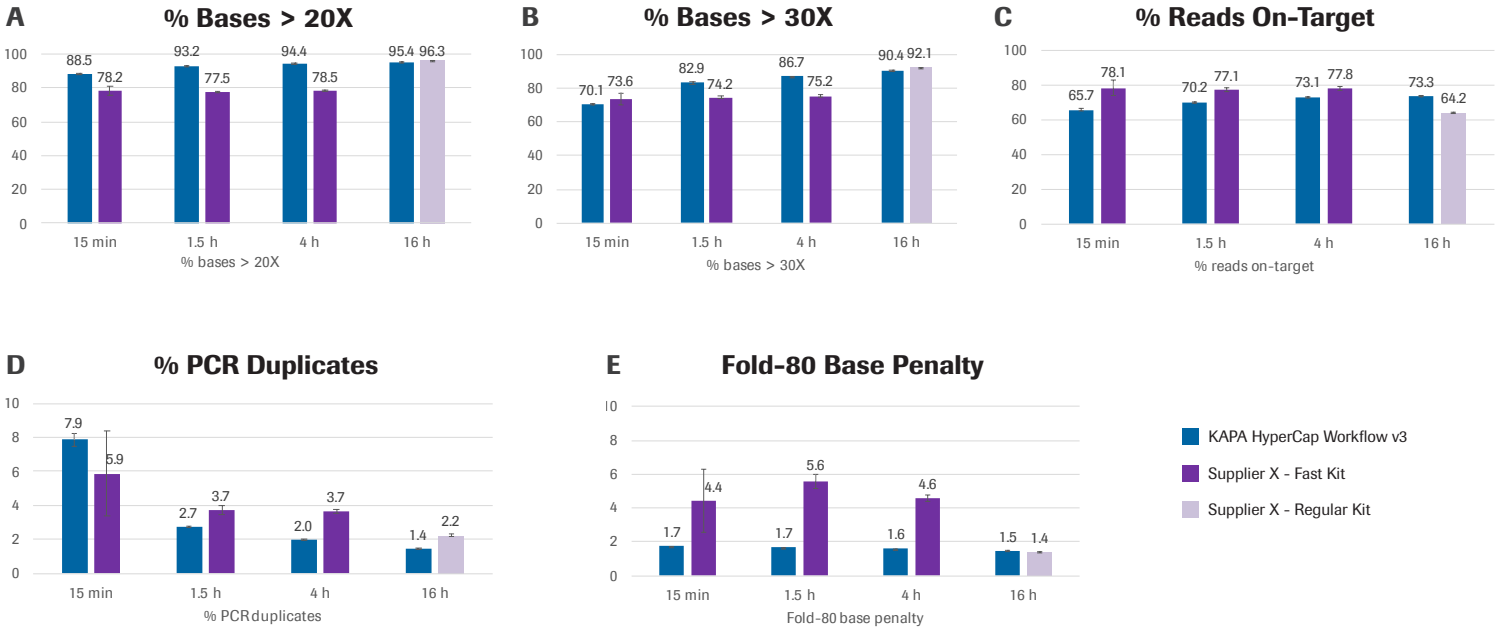

Figure 4: KAPA HyperCap Workflow v3 with KAPA HyperExome compared to a market-equivalent kit (Supplier X) using short hybridization protocols. Sequencing data was downsampled to 142X raw coverage per sample. Bars represent the mean from triplicate libraries and error bars indicate the standard deviation.

CONCLUSIONS

In summary, the results presented here show that:

- Enzymatically and mechanically fragmented input DNA yielded similar sequencing metrics following target enrichment with shorter hybridization times, and both input types performed similarly when compared to the respective 16-hour hybridization workflows.
- KAPA HyperCap Workflow v3 with KAPA HyperExome outperformed a market-equivalent short-hybridization kit according to multiple metrics without any additional modification to the recommended instructions (except for hybridization times).
- Thus, the KAPA HyperCap Workflow v3 offers a robust, single-day solution for target-enriched NGS.

Disclaimer: Although the results of this study are promising, this workflow is still in development and has not been fully validated by Roche.

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