APPLICATION NOTE

CUSTOM ENRICHMENT FOR GERMLINE TARGETED SEQUENCING WITH LoopCap™ DNA TARGET CAPTURE TECHNOLOGY

HIGH PERFORMANCE TARGETED CAPTURE FOR INHERITED DISEASE RESEARCH

Summary

The LoopCap Target Capture technology is ideally suited to a wide range of germline genomics research applications, including genetic testing for inherited cancer and other heritable diseases. Content is fully customizable, with no upper or lower limit on panel size. In this application note, we present typical performance with LoopCap DNA Target Capture Kits with custom panels ranging from 60 kb to 700 kb in size.

molecular loop

Materials and Methods

Custom panels ranging in size from 60 kb to 700 kb were designed according to customer specifications from .bed files provided (Table 1).

Performance was evaluated with the LoopCap™ DNA Target Capture workflow (Figure 1) using 150 ng of human genomic DNA as input, and 4 – 8 replicates per probe pool, depending on target size. Replicates were pooled and purified with a 0.8X SPRI bead cleanup using KAPA Pure Beads (Roche). Libraries were quantified using the Agilent Bioanalyzer DNA 1000 assay. Sequencing (PE150) was performed on the Illumina MiniSeq system. Sequence data analysis was performed using a modified Picard/samtools pipeline. UMI-tools was used for deduplication.

Results and Discussion

The Bioanalyzer electropherogram for each pooled sequencing library is shown in Figure 2. We obtained mean and median coverage of 671X and 647X, 608X and 584X, and 379X and 267X for the 60 kb, 320 kb, and 700 kb panels, respectively. Sequencing data for key quality metrics are shown in Figures 3 to 6. We observed excellent specificity as evidenced by consistently high rates of disarming/alignment and on-target/on-bait (Figure 3). Target coverage was >98% complete, with 2% or less of zero coverage targets (Figure 4). Coverage uniformity was consistently high, with >95% of bases within 0.2X of the mean, and fold-80 base penalties of <1.8 (Figure 5). Low duplication rates (<6%) were observed with all three panels (Figure 6).

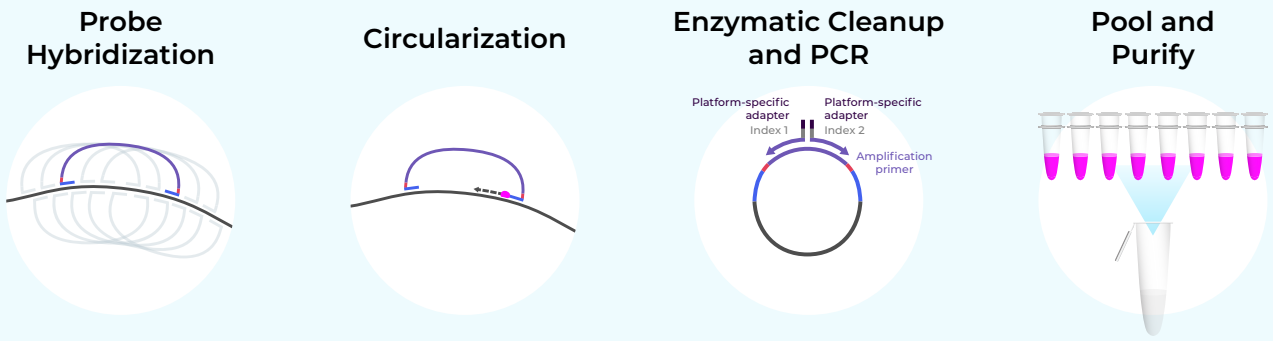

Figure 1. LoopCap DNA Target Capture workflow. Probes are hybridized to purified DNA in a 4 – 16 hour hybridization. The next step is an enzymatic circularization followed by digestion of non-circular DNA and PCR. The PCR adds platform-specific, unique dual-indexed barcodes and amplifies the library. Next, samples are pooled and a SPRI bead purification is performed on the library pool, generating a sequencing-ready library.

Table 1. LoopCap custom panels used in this study.

#	Application	Target size	Bait size	Number of Probes	Probes per base	Content
1	Carrier screening	60 kb	162 kb	~2,900	5X	Full genes
2	Inherited cancer	320 kb	730 kb	~13,000	5X	Combination of full genes, exons, and 5' UTRs
3	Carrier screening	700 kb	1.93 Mb	~42,000	4X – 9X	Exons, selected 5' UTRs, and 96 sample tracking SNPs

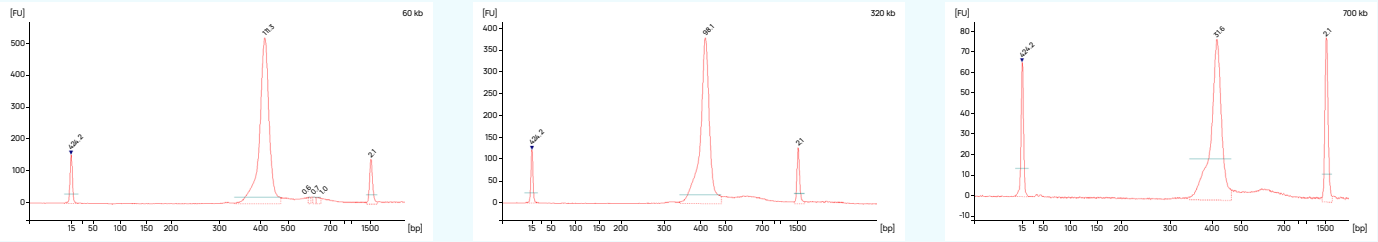

Figure 2. Bioanalyzer results for QC library pools for three custom panels. For each panel, we observed a sharp peak of the expected size (~410 bp for a 225 bp captured region), with no non-specific peaks present.

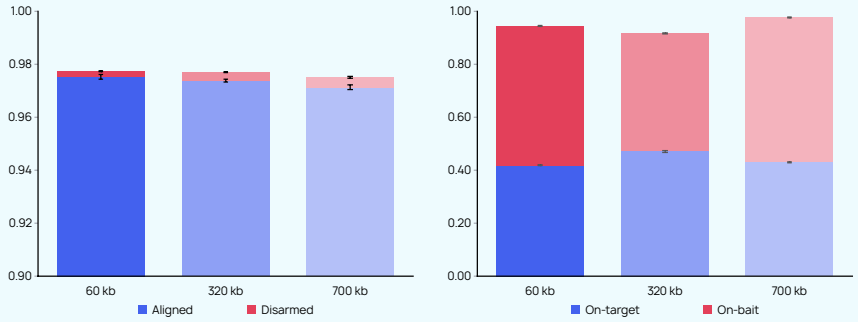

Figure 3. Fraction of disarmed and aligned reads (left) and fraction of on-target and on-bait bases (right)

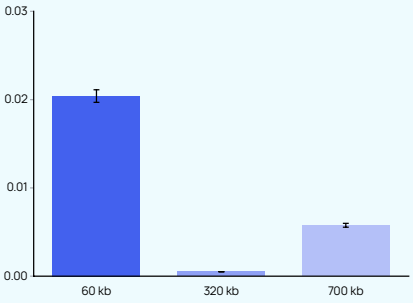

Figure 4. Fraction of zero coverage targets

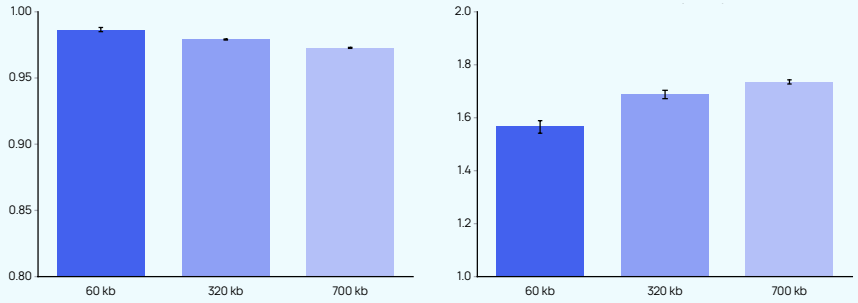

Figure 5. Fraction of bases within 0.2X of the mean (left) and fold-80 base penalty (right)

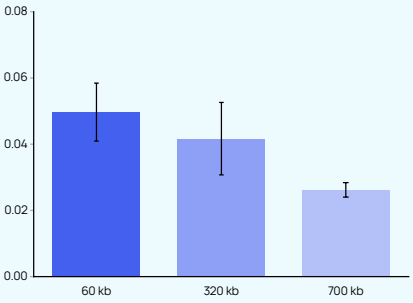

Figure 6. Duplication rates

Conclusion

LoopCap™ DNA Target Capture technology combines a simple and efficient 4-step addition-only workflow, with precision-designed panels. The outcome is exceptional data, regardless of panel size or contents, and a solution ideally suited for carrier screening and hereditary oncology research applications.

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