New Technologies for Target Enrichment from Low Input and FFPE Damaged Samples

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Abstract

The current frontier in Next-Generation Sequencing (NGS) is to improve sequencing from low input and formalin-fixed samples to increase the number of clinical samples that can be analyzed, particularly for low frequency somatic mutations. Technologies that require higher inputs limit the number of analyzable formalin-fixed, paraffin-embedded (FFPE) clinical samples, which contain limited and damaged DNA. Many clinical samples are unable to be utilized for sequencing due to damaged or low amounts of DNA. Herein, we introduce targeted enrichment techniques utilizing multiplexed amplicon sequencing and hybridization capture for inputs in the 1-100 ng range that are capable of efficiently facilitating library generation from damaged DNA. We have developed the Accel-Amplicon™ 56G Oncology Panel, which covers 56 oncology-related genes using a combination of hotspot loci and overlapping amplicons with contiguous gene coverage in a single tube. Smaller amplicon panels have also been developed for the TP53 gene and the EGFR pathway. These panels show effective variant calling with damaged DNA and are able to detect a 1-5% allele frequency with 10 ng input, depending on sample quality. Accel-Amplicon panels for germline mutations using low inputs are also in development, including CPTF and ADME panels, as well as a new screened panel.

Hybridization capture is another important tool for exploring exomes and other subsets of the genome in a cost-effective manner, but often requires over 100 ng input DNA. We have developed a library preparation technology that is capable of producing comprehensive genome coverage from as low as 1 ng input when combined with existing hybridization capture panels. Hybridization capture with 1 ng Coriol NA12878 yielded high quality variant calling with 98.72% sensitivity and 99.53% precision when compared to the NIST GIAB truth list at 99.89% concordance for the Medecome panel. This workflow is compatible with FFPE damaged samples and produces negligible loss in library complexity and coverage uniformity compared to high quality genomic DNA samples. Both the amplicon sequencing and hybridization capture techniques can be used to advance oncology research by enabling analysis of clinical samples consisting of low input quantities of damaged DNA.

Accel-NGS® 2S Hyb

- Simple with-bead protocol
- Broad input range: 10 pg-1μg
- Sequential repeat steps enable use of damaged DNA
- Compatible with ctDNA and FFPE samples
- Increased library complexity
- Balanced coverage of AT/GC-rich regions

Figure 1. Accel-NGS 2S Hyb Library Kit workflow. The Accel-NGS 2S Hyb Kit has 5 steps: T7 RNA polymerase converts the DNA, Repair II repairs and polishes DNA ends, Ligature II adds the P7 adapter to the 5′ terminus, and PCR amplifies the library for hybridization capture.

Small Sample Limitations

- 1 ng of DNA = 30 chromosomal copies of any locus
- Achieving 1% detection of a mutation — 3 chromosomal copies

Conversion Rates

Figure 2. Genomic considerations of low input. Conversion rates measure the amount of input DNA converted to functional NSG library molecules. Factors influencing library molecule loss include the degree of DNA damage, the source of this damage, and size selection. Measuring conversion rates can be problematic as accurately quantifying the DNA as it changes during the NGS workflow is difficult.

Limit of Detection

Figure 3. Limit of detection schematic. To assess the limit of detection of the Accel-NGS 2S Hyb Kit, DNA samples from two individuals with different ethnic backgrounds were used to prepare libraries. 100 ng of DNA from one individual with a 0.5% or 1% spike-in of the DNA from the second individual was used as the input DNA. Once libraries were prepared, they were hybridized to xGen Pan-Cancer probes and SNPs were detected within this panel.

Table 1. Accel-NGS 2S Hyb performance metrics using the xGen Pan-Cancer Panel for hybridization capture with FFPE DNA. The Accel-NGS 2S Hyb Kit was used to make libraries of two different inputs from the FFPE lung tumor sample. Many thanks to QI Lab Solutions for generation and sequencing of libraries.

Table 2. Accel-NGS 2S Hyb performance metrics using the Agilent SureSelect® QP Labs Solutions Comprehensive Cancer Panel for hybridization capture with FFPE DNA. The Accel-NGS 2S Hyb Kit was used to make libraries of two different inputs from the FFPE lung tumor sample. Many thanks to QI Lab Solutions for generation and sequencing of libraries.

Figure 4. A) Accel-Amplicon workflow. Accel-Amplicon Panels feature a single-tube workflow with a 90-minute target enrichment amplification step and a 20-minute adapter ligation step, yielding a 2 hour start-to-finish procedure. B) 56G Panel genes and number of amplicons per gene. Hotspot loci (white), contiguous, overlapping amplicons (blue), and comprehensive coding exon coverage for TP53 (darker blue). C) The coverage uniformity, as the percentage of the bases covered at least 0.2x, 0.3x, 0.4x or 0.5x of the average depth, determined across four sample types. The percentage of reads on target was greater than 95% for all sample types. D) Coverage of all coding regions of the TP53 gene. Represented here in a Sashimi plot (SVG, Broad Institute).

Table 4. Allele frequency detected using the Accel-Amplicon 56G Oncology Panel. Mutants were detected in FFPE tumor tissue and ctDNA. The 56 gene amplicon panel identified point mutations in both FFPE tumor samples and ctDNA. Mutation detection was done as low as 1% in ctDNA samples. Concordance was observed between corresponding ctDNA and FFPE tumors, when mutations were detected.

Table 5. Comparative performance metrics between Accel-NGS 2S Hyb and Kapa using SeqCap EZ MedExome hybridization capture libraries. Libraries were made using HapMap DNA NA12878 (Coriell) with both the Swift Accel-NGS 2S Hyb Kit and the Kapa Library Preparation Kit, followed by the NimbleGen SeqCap EZ MedExome Panel.

In this study, we have demonstrated that:
- The Accel-NGS 2S Hyb DNA Library Kit produces high complexity library with few PCR duplicates, even at low input.
- The Accel-NGS 2S Hyb Kit produces library with much higher complexity than the competition, particularly at low input.
- The Accel-NGS 2S Hyb Kit is capable of making high quality library even from damaged FFPE samples.
- The Accel-NGS 2S Hyb Kit enables detection of variants even at 1%.
- The Accel-Amplicon 56G Panel successfully produces NGS libraries from high quality, FFPE samples.

The Accel-Amplicon 56G Panel can detect low (~1-5%) allele frequencies with low input and damaged DNA.

We acknowledge Q2 Lab Solutions for Agilent SureSelect™ library preparation and sequencing.

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