CleanPlex® Technology: Target-Enriched NGS Libraries with Low Background and High Variant Detection Accuracy using a Fast, Single-Tube Workflow

Lucie Lee, Lily Liu, Jeffrey Liu, Guoying Liu, Jacey Zhang, Zhitong Liu | Paragon Genomics, Inc, 3521 Investment Blvd Suite 1, Hayward, CA 94545, USA

Introduction

Next- generation sequencing (NGS) technologies have accelerated research efforts in the fields of biomedical sciences, clinical diagnostics, environmental genomics, and agrigenomics. Especially with multiplex PCR (mPCR), scientists are capable of utilizing NGS to detect and analyze gene sequences in humans, viruses, and bacteria for variant identification, species identification, and de novo sequencing. Amplicon- based library preparation methods, utilizing mPCR, are preferred by many as the more time and cost-efficient method over hybrid-capture methods.

However, multiplex PCR has inherent difficulties such as high background and poor uniformity. To address and resolve these drawbacks, we developed a high-performing, 2.5hr, one tube, amplicon-based library preparation solution for NGS. The Paragon Genomic CleanPlex® system includes primer pool(s) designed with proprietary algorithm, an mPCR mix formulated to minimize GC bias and improve uniformity, a background removing Digestion Reagents to selectively remove nonspecific products, and a second PCR mix to produce a clean and robust library ready for any NGS application.

Workflow

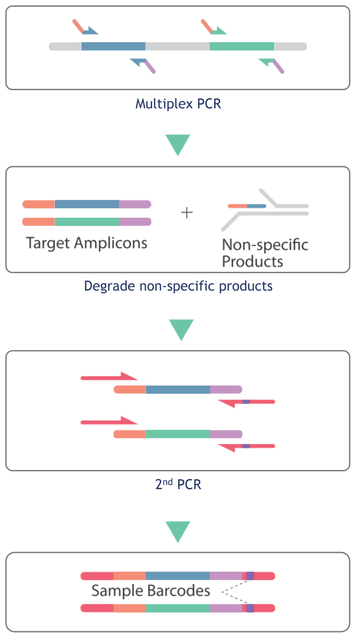

Figure 1: Target sequences are amplified through multiplex PCR using oligonucleotides containing a partial adaptor sequence. Primer-dimers, heteroduplexes, and other side products are then degraded and removed from the reaction solution using the CleanPlex® Digestion Reagent. Finally, Illumina adapter sequences and sample barcodes are added to the library through a second round of PCR, which anneals to the partial adapter sequence.

Background Cleanup

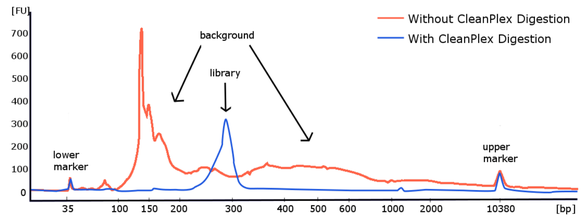

Figure 2: Comparison of Agilent BioAnalyzer traces of samples treated (blue plot) and not treated (red plot) with CleanPlex® Digestion Reagent. The proprietary CleanPlex® Digestion Reagent is essential for removing undesired side products formed during the mPCR amplification of target sequences.

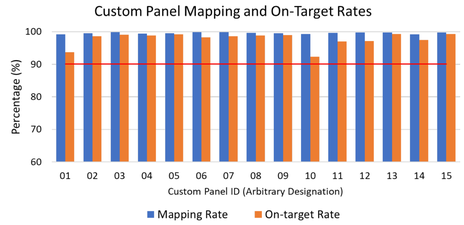

Figure 3: Examples of mapping and on-target rate for custom panels designed by Paragon Genomics showing the consistency in performance in various custom panels.

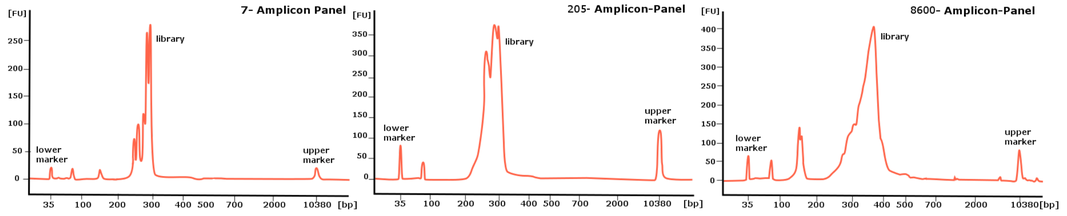

Figure 4: Examples of Agilent BioAnalyzer traces of 7-8600 amplicon panels designed by the Paragon Genomics Bioinformatic team. Clean libraries are effectively obtained using CleanPlex® technology for panels covering a large range of target sequences.

Uniformity and GC Bias

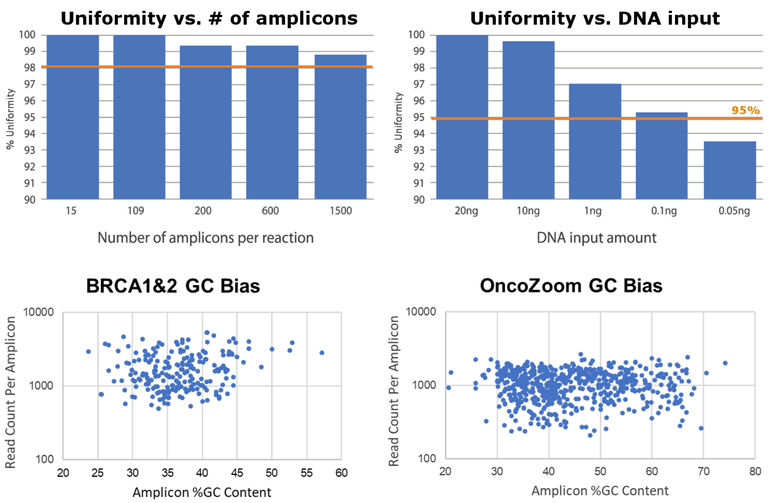

Figure 5: (Left) Uniformity of >98% above 0.2X mean reads is maintained in Paragon Genomics's designed libraries of 15 to 1500 amplicons. (Right) With a 600 amplicon panel, CleanPlex® technology also produces >95% uniformity at 0.2X means reads using as low as 0.1ng of input genomic DNA.

Figure 6: Coverage Depth vs. Amplicon %GC Content of libraries made with the Cleanplex® BRCA1&2 Panel (left) and the Cleanplex® OncoZoom Panel (right). Both libraries demonstrate even coverage of amplicons with ~ 25-45% GC content and ~20-75% GC content respectively, with no evidence of GC bias.

Limit of Detection and Variant Call Concordance

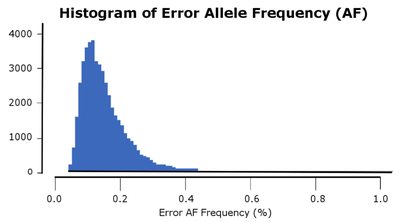

Figure 7: A histogram of the frequency of random errors from sequencing a Paragon Genomics OncoZoom library made with 10 ng of Horizon Discovery's cfDNA Reference Standard (HD780) at an average read depth of 8500 shows that the CleanPlex® Library Kit Reagents yield random errors well below a frequency of 1%.

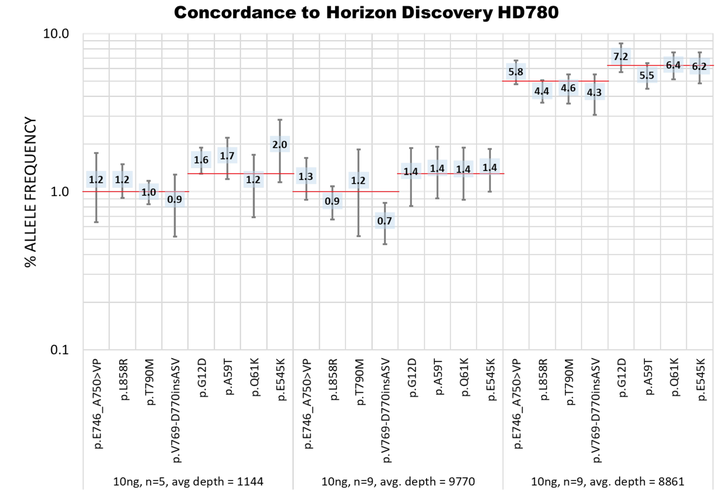

Figure 8: Libraries prepared with Paragon Genomics's cancer hotspot Oncozoom Panel using 10 ng of Horizon Discovery HD780 cfDNA Reference Standard Set were sequenced at average depths ranging from around 1100-9800 reads per amplicon. The red lines represent the known allele frequencies of eight mutations, and numbers are the averages of the detected frequencies for each allele with standard deviation error bars.

Summary

Paragon Genomics developed a new NGS library preparation chemistry system that reduces the workflow of creating high quality libraries for sequencing.

- CleanPlex® Digestion Reagent significantly reduces the background products, such as adaptor-dimers, prior to sequencing.
- CleanPlex® panels produce high mapping and on-target rates over a wide range of target numbers and input DNA amounts.
- Proprietary multiplex PCR reagents and expert primer design result in >95% uniformity and no observed GC bias.
- Libraries prepared using CleanPlex® technology demonstrated accurate variant calling in concordance experiments, and limit of detection below 1%.
- There are no specialized equipment requirements other than a thermocycler.

Paragon Genomics, Inc. | www.paragongenomics.com | contact@paragongenomics.com | 1-510-363-9918