INTRODUCTION

Next-generation sequencing (NGS) is widely used to detect sequence variations and an array of genetic markers for oncological diagnostic research and, in combination with bioinformatics, is increasingly used to analyze multiple biomarkers in a low-cost, time-effective manner[1]. However, one of the challenges in detecting cancer variants with standard NGS analysis is the low-frequency of these mutant alleles in cancer cells amongst a background of wild-type alleles in healthy cells.

Xenonucleic Acid (XNA) Molecular Clamp is an innovative nucleic acid molecular oligomers that hybridize by Watson-Crick base pairing to target DNA sequences, which are used during polymerase chain reaction (PCR) to selectively suppress amplification of DNA with wild-type alleles and amplify DNA containing mutant alleles. Mutants with low allele frequency will be easily detectable without deep sequence enrichment by adding XNA in PCR[2,3]. Here, we introduce a highly sensitive OptiSeq™ NGS cancer Nano Panel V2 powered by the proprietary XNA technology to detect low-frequency variants in reference human tumor samples. This NGS diagnostic platform significantly improves the detection sensitivity of variants for diagnosis of cancer mutants even at ultra-low allele frequency. Upon XNA blocking of wild-type alleles, variant allele frequency (VAF) of mutants can be enriched by ~30 fold from 10 ng of gDNA samples containing mutants as low as 0.25% VAF.

RESULTS

1. Effects of XNAs Mix on Enrichment of Mutants in Human Tumor gDNA Samples by Using OptiSeq™ Nano Panel V2

XNA mix was spiked into the PCR reaction containing OptiSeq™ Nano Panel V2 primer mix and reference human tumor control samples containing mutants at different abundance levels. The estimated VAFs of 17 hotspots in human tumor samples were at 0.00%, 0.25%, and 1.25%. The detected VAFs by sequencing are shown in Figure 3. The mutant detection powered by the XNA mix was dramatically boosted. There were, on average, 30 and 19 folds of increase in VAF for tumor samples with 0.25% and 1.25% mutants, respectively. On samples originally with 1.25% of mutants, in 14 of 17 hotspots, observed VAFs were more than 10% after XNA enrichment. This result suggested that XNA can be used to enrich mutant alleles and make high confidence calls. It is also noticeable that some hotspots were enriched less efficiently than others. These results suggested that the design of XNA and/or experimental condition may be further improved.

Figure 2. Experiment and data analysis workflows for study of XNA effects on enrichment of variant alleles

2. Effects of XNAs Mix on Sequencing Coverage of Mutants Using OptiSeq™ Nano Panel V2

Sufficient sequencing coverage is necessary to get reliable results for mutant detection. One of the concerns of using XNA for blocking wild-type DNA amplification is that it may also eliminate amplicons for mutant detection. The total coverage of all loci after XNA enrichment PCR is displayed in Figure 4. Average coverage per hotspot in tumor samples containing 0.25% and 1.25% of mutants upon XNA enrichment were 1177X and 1353X, respectively. In some hotspots, such as “NRAS G12V” and “NRAS G13D”, coverages at these hotspots were too low. Although the coverage was relatively low, there were 31 and 21 mutant reads in “NRAS G12V” and “NRAS G13D”, respectively. Hence, there is enough confidence to identify mutants even at these hotspots with low coverage.

Figure 3. Effects of XNA mix on variant Allelic Frequency (VAF) using OptiSeq™ Nano Panel V2 (10ng DNA Input)

Figure 4. Effects of XNA mix on total coverage using OptiSeq™ Nano Panel V2 (10ng DNA Input)

CONCLUSION

XNA molecular clamp technology in combination with NGS have a great potential for cancer molecular diagnosis of cancer mutations in ultra-low allele frequency. OptiSeq™ Nano Panel V2 powered by XNAs is able to report mutants from 10 ng of input gDNA with allele frequency as low as 0.25%. XNAs mix is able to achieve average 30 times improvement in detected allele frequency for 17 hotspots in samples with original mutant VAF of 0.25%. The total coverage was 1177 for each region containing cancer mutation hotspots, which was sufficient to obtain reliable results for calling mutants. Significant progress has been made in characterizing and optimizing the use of XNA in conjunction with OptiSeq™ oncology NGS panel, which provides a promising solution to detect mutants with low-frequency with improved sensitivity and confidence.

REFERENCES:

1. Tao Zhang, Wenjing Feng, Michael J. Powell, Ping Wu, Tim Tran, Ke Zhan and Michael Sha (2020) Novel XNA Molecular Clamp Application in NGS Diagnostic Platform OptiSeq™ Cancer Panels. Diacarta, Inc., 2600 Hilltop Drive, Richmond, California 94806. USA

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