XNA Increases Assay Sensitivity in Sanger Sequencing, qPCR, NGS and CRISPR Mutant Screening

Wei Liu, Robert Brown, Andrew Fu, Shuo Shen, Larry Pastor, Michael Sha, and Aiguo Zhang
DiaCarta, Inc., 4385 Hoppyard Road, Suite 100, Pleasanton, California 94588 • Correspondence should be addressed to msha@diacarta.com
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INTRODUCTION

XNA \(^1\) is a small DNA oligo analog that binds to wildtype sequence with 100% sequence match. Due to the high-affinity binding nature, the XNA binding to the wildtype sequence blocks its amplification in PCR. In contrast, the mutant sequence with one or more mismatch to the XNA sequence is able to be amplified and therefore gets enriched. The enrichment of mutant sequence allows mutation detection with much better sensitivity for Sanger sequencing, qPCR, NGS, and CRISPR screening qPCR assay.

RESULTS

XNA increases Sanger sequencing sensitivity from 15-20% to 0.04% VAF.

Sanger sequencing sensitivity is low - thus preventing its use in companion diagnostics. XNA-based Sanger sequencing improves sensitivity >100-fold.\(^2\)

XNA increases JAK2 qPCR sensitivity

XNA added to JAK2 qPCR reaction allows the mutation to be detected at 0.039% (1.2 copies), while regular qPCR has the assay sensitivity at 1%.

XNA allows mutations detected earlier in NGS, reducing sequencing depth

Low-frequency somatic mutations are harder to be detected in targeted NGS panels with 50 to 100x depth. Deep sequencing is necessary to find these mutations. Addition of XNA into NGS panels allows discovery of the mutations with less sequencing depth compared to NGS without XNA.

CONCLUSION

• XNA can increase genomic assay sensitivities including Sanger sequencing, qPCR and NGS. Low detection limit is achieved as one copy allows XNA to be a potential tool for MRD assay development.

• XNA-based Sanger sequencing can detect low-frequency mutation detection otherwise missed by regular Sanger sequencing.

• XNA-based NGS panel allows low-frequency mutation detection without deep sequencing, saving cost for CDx MRD detection.

REFERENCE


METHODS

Sanger Sequencing

BRAF wildtype or V600E fragment was amplified by PCR in the presence of BRAF XNA, and Sanger sequencing was performed by Sequetech (Mountain View, CA).

qPCR Assay

JAK2 qPCR assay that detects the JAK2 V617F in the absence or presence of XNA were performed at different concentrations of V617F variant (variant allele frequency, VAF) from 0.039% to 5%. The qPCR reactions were performed on either Bio-Rad CFX 384, Thermo Fisher QuantStudio 5, or Roche 480 II. The qPCR products were sent for Sanger sequencing by Sequetech.

NGS Assay

Important somatic mutation targets (OptiSeq\(^\text{TM}\) Dual Cancer Mini-MRD kit) designed in the NGS panel were tested in the presence or absence of the target XNAs. The targeted NGS was performed according to the kit instruction manual.