# Two Detection Methods of Clinical KRAS G12C Mutation Detection for Companion Diagnostics

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## INTRODUCTION

KRAS G12C is a common mutation in approximately 13% of lung adenocarcinoma, 3% of colorectal cancer and 2% of other solid tumors. The FDA approved sotorasib, a KRAS G12C inhibitor, for targeted treatment of patients with KRAS G12C-mutated locally or metastatic non-small cell lung cancer (NSCLC). It is essential to detect the KRAS G12C mutation with a companion diagnostic before the drug is administered. We have developed two KRAS G12C mutation detection assays for qPCR or Sanger sequencing. Both QClamp® assays use our proprietary xeno nucleic acid (XNA) technology which specifically enhances the amplification of the target mutant DNA sequence while blocking amplification of the wildtype sequence, thus significantly improving the sensitivity of the assays for qPCR (~1%) and Sanger sequencing (~15 to 20%) to 0.1% variant allele frequency. These simple and sensitive assays can be used for clinical research or validated in CLIA labs for KRAS G12C mutation detection for the purpose of companion diagnostic use. Although NGS methods are gaining traction in clinical settings, our methods are suitable for standard molecular diagnostics laboratory settings and do not add any additional steps to the qPCR or Sanger sequencing protocols. Our methods are especially valuable for labs that appreciate simple, economical, yet sensitive companion diagnostics assays for KRAS G12C mutation. QClamp® assays can be easily adapted to detect other single gene mutations with high sensitivity for companion diagnostics use or other mutation detection applications.

## METHOD

### KRAS c.12 qPCR Assay

The KRAS c.12 qPCR assay was performed in the presence or absence of G12 XNA at 8 different concentrations of the G12C variant (variant allele frequency, VAF) from 0.039% to 5%, with negative and no-template controls. The qPCR reactions were performed in triplicate on the Bio-Rad CFX96 instrument (Fig. 1-3), and the qPCR products were sent for Sanger sequencing by Sequetech (Mountain View, CA) for confirmation. Analysis was performed in Geneious Prime® 2023.0.4. The KRAS c.12 assay was performed on QuantStudio 5.

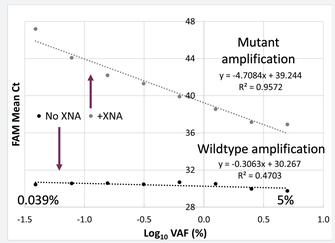
### KRAS G12C Mutation Enrichment and Sanger Sequencing

The KRAS G12 wildtype or G12C mutant target sequences at 0.01, 0.1, 0.25, 0.5, 1.25, and 5% VAF were amplified by PCR in duplicate on the ABI ProFlex™ in the presence or absence of XNA and Sanger sequencing was performed by Sequetech.

## RESULTS

### XNA increases KRAS c.12 qPCR sensitivity by inhibiting G12 wildtype amplification

KRAS G12 XNA specifically binds the wildtype G12 sequence preventing amplification by DNA polymerase, therefore allowing preferential amplification of the G12C mutant target sequence in a linear dose-dependent manner. Amplification from the wild-type G12 sequence masks the mutant signal in the absence of XNA, and the G12C mutant is undetectable at 5% VAF. The qPCR samples were subsequently analyzed by Sanger sequencing.




| VAF (%) | FAM Mean Ct | |
|---|---|---|
| | No XNA | + XNA |
| 5 | 29.76 | 36.91 |
| 2.5 | 29.98 | 37.15 |
| 1.25 | 30.52 | 38.55 |
| 0.625 | 30.69 | 39.91 |
| 0.313 | 30.46 | 41.32 |
| 0.156 | 30.58 | 42.19 |
| 0.078 | 30.58 | 44.10 |
| 0.039 | 30.44 | 47.19 |
| 0 | 30.42 | 48.72 |

**Figure 1. KRAS G12C standard curves with and without XNA.** G12C mutant DNA template was serially diluted two-fold from 5% to 0.039% variant allele frequency (VAF) in 10 ng fragmented wildtype DNA. The G12C mutant could be detected at 0.039% VAF (estimated ~1 copy) in the presence of G12 XNA. The table shows the mean data for each template dilution with and without XNA plotted in the graph.

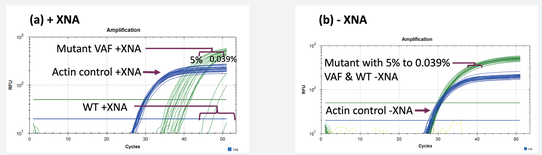



**Figure 2. KRAS G12C qPCR amplification plots with and without XNA.** Amplification curves with XNA (a) for the triplicates of each positive control increase in Ct value stoichiometrically by ~1 Ct from 5% to 0.039% VAF for the G12C mutant, whereas without XNA the triplicates of each positive control (5% to 0.039% VAF) and the negative control have consistent Ct values for the G12C mutant. Amplification from the internal β-actin control is consistent for all samples independent of XNA.

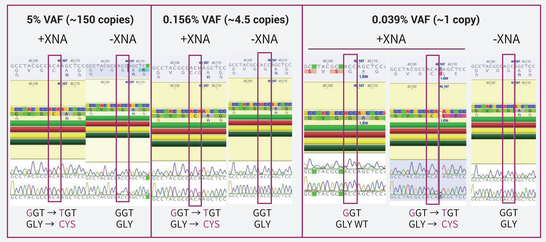



**Figure 3. Sanger Sequencing of KRAS c.12 qPCR assays with XNA can detect the G12C mutant as low as 0.039% VAF.** In assays with the KRAS G12 XNA, the mutant cysteine codon (TGT) is identified in all the chromatograms for the 5%, 2.5%, 1.25%, 0.313% VAF positive control triplicates. Whereas for 0.625% and 0.156% VAF the mutant TGT codon was identified in 2 of 3 replicates, and for 0.078% and 0.039% VAF the TGT codon was identified in 1 of 3 replicates. By our estimates 0.039% VAF is equivalent to approximately one copy.

| VAF(%) | G12C Positive (%) | |
|---|---|---|
| | No XNA | + XNA |
| 5 | 0 | 100 |
| 2.5 | 0 | 100 |
| 1.25 | 0 | 100 |
| 0.625 | 0 | 66 |
| 0.313 | 0 | 100 |
| 0.156 | 0 | 66 |
| 0.078 | 0 | 33 |
| 0.039 | 0 | 33 |
| 0 | 0 | 0 |

The glycine codon (GGT) is identified in all the replicates for the positive controls in assays without XNA, and in all the replicates for the negative control with or without XNA. The KRAS gene is on the minus strand, so the sequence is read in reverse in the figure, therefore GGT is ACC and TGT is ACA.

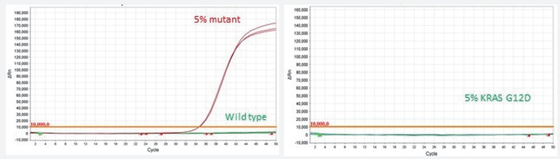



**Figure 4. KRAS G12C qPCR assay with XNA and an allele specific forward primer is specific to G12C mutant with no cross-reactivity.** Amplification curves for G12C multiplex assay with triplicate results for 5% VAF of the reference standards (Horizon Discovery) G12C (a) and G12D (b). Additional results with G12A, G12R, G12S, G12V, and G13D reference standards (Horizon Discovery) confirmed the analytical specificity of the assay. XNA improved assay performance by clamping to the wildtype G12 sequence to prevent a low level of off-target amplification

### QClamp® PCR Mutation Enrichment of KRAS G12C Mutant

KRAS G12C PCR with XNA inhibits wildtype amplification and Sanger sequencing identify the G12C mutant codon in positive controls.

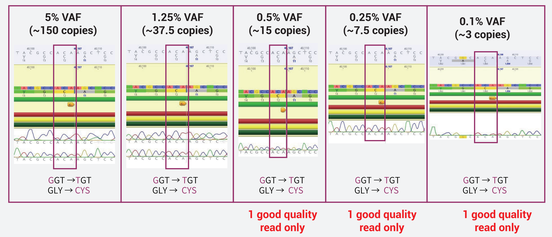



**Figure 5. Sanger Sequencing of KRAS G12 PCR assays with XNA can detect the G12C mutant as low as 0.1% VAF.** The G12C mutant codon (TGT) is identified in the duplicate samples for 5%, 1.25%, 0.5%, and 0.25% VAF, and one of two replicates for 0.1% VAF positive control.

## CONCLUSIONS

KRAS G12 QClamp® qPCR and mutation enrichment for Sanger sequencing assays increase sensitivity for the low copy G12C mutant variant by inhibiting amplification of the wildtype with the G12 XNA, thus allowing the preferential amplification of the G12C mutant, hence increasing the signal-to-noise ratio. The KRAS G12 QClamp® CE and RUO assays are simple, reliable, and economical kits for mutation analysis in clinical specimens.

## REFERENCE

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