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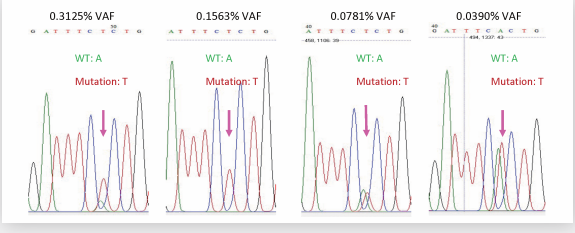
BRAF V600 Mutation-Enriching Sanger Sequencing as a Lab Developed Test

Why a Mutation-Enriching Sanger Sequencing Assay?

Sanger sequencing as a gold standard is the most commonly used method. Although Next Generation Sequencing (NGS) is gaining traction in different applications, Sanger Sequencing is still used in areas where NGS is more costly, time-consuming, or more complicated in result interpretation due to its lack of standardization or known clinical significance of certain mutations. However, Sanger Sequencing has disadvantages compared to NGS, such as low-throughput and low-sensitivities (15 to 20% variant allele frequencies, VAF). Low-sensitivity has hampered Sanger Sequencing for being used as an accurate companion diagnostics (CDx) assays for targeted therapies against mutations that respond to specific inhibitors, such as certain mutations in EGFR, BRAF and KRAS. These CDx assays require high-sensitivity to identify the low-frequency mutations in FFPE or liquid biopsy samples.

Improvement of Sanger Sequencing provides the potential for the technique to be used as a CDx tool. We have developed a mutation-enriching Sanger Sequencing assay using our proprietary XNA technology for detection of BRAF V600, the most common BRAF mutation in multiple cancers. **The BRAF V600 Mutation Enrichment Assay** enriches the mutation in a PCR template preparation reaction, and significantly increases the downstream Sanger Sequencing sensitivity from 15 to 20% VAF to 0.04%. With such a high-sensitivity of mutation-enriching Sanger Sequencing, CDx assays against BRAF V600 mutation detection can be developed and validated in clinical labs as laboratory developed tests (LDT) for low-cost, high-accuracy mutation analysis. The mutation-enriching Sanger Sequencing can be applied to detection of important cancer mutations for targeted therapies.



The limit of detection of the BRAF V600 in the presence of 500 nM XNA using templates with different VAF. The reverse primer was used for Sanger sequencing and the reverse/-complement strand was read. The c1799 BRAF V600E mutation, the T to A mutation, was read as A to T mutation in sequencing results. A as the wildtype and T as the mutant.

Current Companion Diagnostics Assays for BRAF V600 Approved by the FDA

Different companion diagnostic assays have been approved by the FDA for detection of BRAF V600 mutations. These assays can detect the BRAF V600 with high sensitivity to meet the needs of CDx for targeted cancer therapies.

CDx Approved by FDA	Type of Cancer	Inhibitors or Drugs for Target Therapy	Technology
Cobas 4800 BRAF V600 Mutation Test by Roche Molecular Systems	Melanoma	Zelboraf (vemurafenib), or Cotellic (cobimetinib) - in combination with Zelboraf (vemurafenib)	qPCR
THXID BRAF Kit by bioMérieux	Melanoma	Braftovi (encorafenib) in combination with Mektovi (binimetinib), or Mekinist (tramatenib), or Tafinlar (dabrafenib)	ARMS-Real-Time PCR
Therascreen BRAF V600E RGQ PCR Kit by Qiagen GmbH	Colorectal Cancer	BRAFTOVI (encorafenib) in combination with Erbitux (cetuximab)	ARMS-Real-Time PCR
Oncomine™ Dx Target Test by Thermo Fisher Scientific	Metastatic Non-small Cell Lung Cancer (NSCLC)	Combination of dabrafenib and trametinib	NGS

The Advantages of the Mutation-Enriching Sanger Sequencing

The approved CDx assays use sensitive qPCR or its derivative techniques to detect the mutation. Recently, NGS assays have also been approved for BRAF V600 detection. However, the qPCR technique only detects the mutation but does not directly tell the mutation nature. NGS assays are more economical when they are applied to multiple mutation detection, for instance, in non-small cell lung cancer (NSCLC).

Mutation-enriching Sanger sequencing, however, provides comparable or even better sensitivity than traditional qPCR and NGS assays and can be used as a potential CDx technique. Since the CDx usually targets a single or a few mutations, mutation-enriching Sanger Sequencing would be a great fit for the mutation detection with low cost and availability in most molecular diagnostic labs.

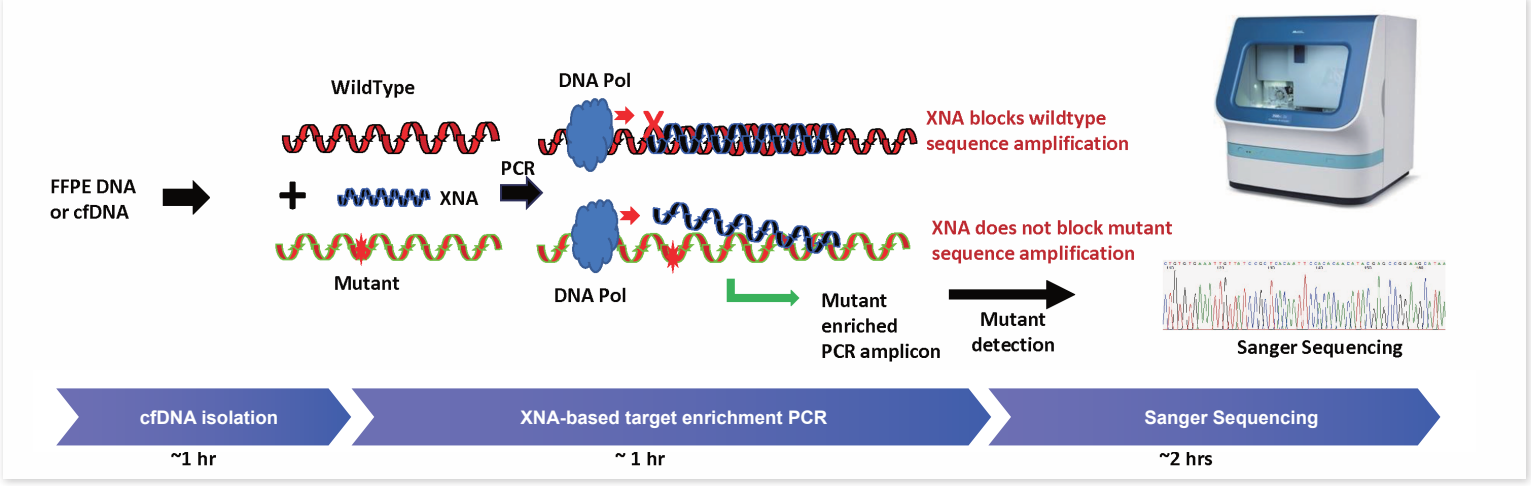
How to Use the QClamps® BRAF V600 Mutation Enrichment Kit for Sanger Sequencing?

The QClamps® BRAF V600 Mutation Enrichment Kit, just like a traditional PCR reaction mix, is used to prepare the PCR template for downstream Sanger sequencing. Different from the traditional PCR reaction mix, the enrichment kit contains specific BRAF V600 XNA that enriches the mutation during the PCR reaction. The PCR product with enriched BRAF V600 is then purified and sequenced by commercially available Sanger sequencing reagents.

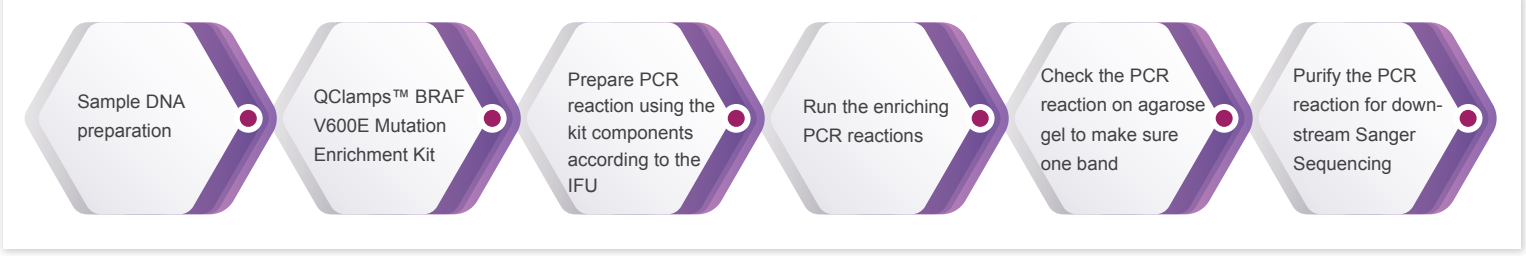
Clinical laboratories can validate the BRAF V600E mutation-enriching Sanger sequencing assay in their own labs using our user manual. Research labs can also use this technique to sequence or confirm the BRAF V600 mutations in their samples.

BRAF V600 Mutation-Enriching Sanger Sequencing Workflow

A: Mutation-enriching Sanger sequencing workflow



B: Detailed Mutation Enrichment workflow



Product Name	Pack Size	Intended Use	Catalog Number
QClamp® BRAF V600 Mutation Enrichment Kit	24 Reactions	Research-Use-Only (RUO)	DC-10-0198
	96 Reactions	Research-Use-Only (RUO)	DC-10-0199