Rapid detection of somatic mutations in cancer genes

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Introduction

· Recent studies have demonstrated that DNA sequencing is one of the least sensitive methods. Sequencing can only characterize mutation with only 10-20% of mutated DNA detected

· Consequently, tumor driver mutations may not be detectable using standard DNA sequencing methods, especially in liquid biopsy applications like whole blood. Digital PCR still has issues to eliminate the wild-type DNA in the background, same issue as sequencing has.

· To reduce the wild-type background and improve sensitivity, a molecular clamp has been designed to hybridize selectively to wild-type template DNA and block its amplification. This molecular clamp consists of a synthetic, sequence-specific Xeno-nucleic acid (XNA) probe. It is called QClamp™.

Technology & Methods

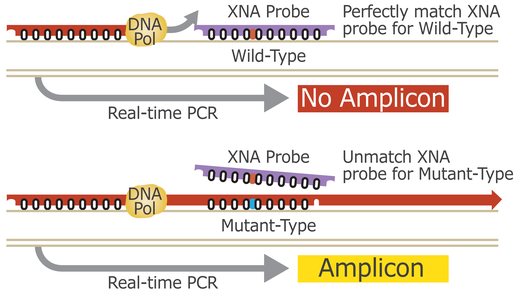

Figure 1. QClamp Technology : QClamp Probe Suppresses Wild-type DNA, only Amplifies and Detects Mutants

The modified DNA oligo probe binds-or clamps-to wild-type DNA and blocks further wild-type DNA amplification. This, or XNA "clamps" does not bind to mutated DNA, allowing it to be amplified and detected.

Results

· **Mutated DNA was spiked in samples with wild-type gDNA at concentrations of 50%, 20%, 5%, 2% and 0.1% and 0%. This level of sensitivity enables detection of gene mutations in the oncology therapeutic clinical setting utilizing patient biosy, surgical tissue, or FFPE tissue.**

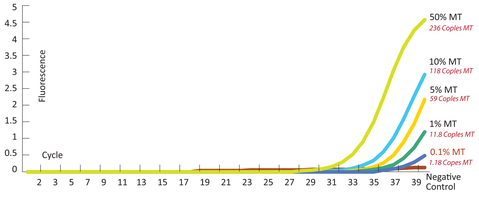

Figure 2. QClamp detects below 0.1% mutated DNA

· **Study performed at clinical diagnostic laboratory in Beijing. Patient diagnosed with myeloproliferative disorder (MPD) V617F mutation positive by sequencing. 50uL of patients whole blood subjected to QZol treatment and QClamp qPCR**

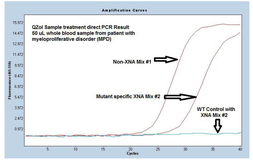

Figure3. V617F mutation detected within 2 hours

Supported by HRM Data

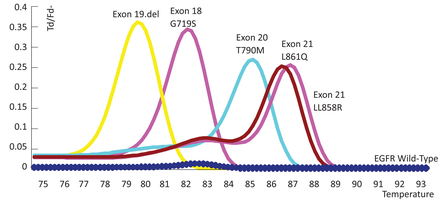

Figure4. High-Resolution Melting (HRM) profiles of clinically relevant EGFR mutations generated by the QClamp EGFR Test

QClamp successfully detected all the clinically relevant EGFR mutations, including T790M, directly from FFPE samples without DNA extraction, which was further demonstrated by high-resolution melting curves. See Figure 4. Since the wild-type DNA was fully blocked from the PCR reaction, the high resolution melting curve gave the best separations between each mutation.

Comparision

· ***QClamp v.s. NGS***

QClamp™ has superior sensitivity over Next Generation Sequencing (NGS)

NGS: Typically this takes somewhere between 4 and 8 hours for one sample. It takes 3 days to finish the assay.
QClamp: No DNA extraction and takes less than 2 hours from start to finish for the report

Mutation Frequency in Sample	*% Detected by NGS*	*% Detected by QClamp*
10-20%	*100%*	*100%*
5-9%	*18%*	*100%*
1-5%	*13%*	*100%*
Below 0.1%	*0%*	*100%*

· ***QClamp vs Taqman qPCR Assay***

EGFR T790M Taqman hydrolysis probes show non-specific binding to wild-type EGFR and consequently are unable to detect T790M mutations:

QClamp EGFR T790M assay does not show this problem. QClamp EGFR mutation assay is highly specific for T790M mutant and wild-type EGFR is completely clamped by the XNA clamp probe during the PCR reaction. Only T790M mutant containing templates are amplified

Summary

The results demonstrated that QClamp™ technology can:

·Detect below 0.1% mutated DNA directly from whole blood without DNA purification.
·It strongly suggested that QClamp can be a tool for liquid biopsy applications, which will contribute to a better understanding and clinical management of drug resistance in patients with cancer.

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