# IDT rhAmp *EGFR* p.T790M rare allele quantification on the Absolute Q

## Highlights

- **Demonstration of rhAmp genotyping assay system on a digital PCR (dPCR) system**
- **Rare molecule detection down to 5 copies among high non-target background (0.1% MAF)**
- **EGFR p.T790M is a potential application for Liquid Biopsy assays**

## Introduction

Precise and sensitive detection of mutation bearing DNA molecules can be critical to drug selection in cancer treatment. For instance, EGFR is an important monitoring target in the treatment of Non-small Cell Lung Carcinoma (NSCLC). Specifically, the presence of EGFR p.T790M mutation indicates tumor resistance to treatment with tyrosine kinase inhibitors (TKIs).[1]

Integrated DNA Technologies' rhAmp SNP Genotyping System utilizes RNase H2-depended PCT (rhPCR), a two-enzyme PCR chemistry, which enables highly precise interrogation of SNPs within challenging genomic regions.[2] The COMBiNATi Absolute Q digital PCR (dPCR) system utilizes micro-molded plastic picoliter partitions (Figure 1) instead of oil/water emulsions, thus enabling flexibility to accommodate the rhAmp chemistry. For the first time, the IDT rhAmp assay performance was demonstrated on a micro-chamber array based digital PCR platform.

## Workflow and Methods

To demonstrate performance and specificity of the IDT rhAmp *EGFR* p.T790M assay on the COMBiNATi Absolute Q a limit of detection test was conducted.

### Materials Used

Wild-type and *EGFR* p.T790M containing gblocks were obtained from IDT and mixed to create samples with mutation allele fractions (MAF) of 10%, 1% 0.1% and 0%. Each mixture contained between 500 and 5 total copies of the *EGFR* p.T790M mutation per reaction in a background of 5000 wild-type copies. These low copy numbers simulate the expected low MAF expected in NSCLC patient samples .

### Assay Preparation and Absolute Q Parameters

The standard protocol for rhAmp assay preparation was adapted for optimal performance on the Absolute Q with an addition of Tween-20 at a final PCR concentration of 0.5%. Digitization, thermal cycling and scanning were performed on the Absolute Q according to Table 2. As an

| rhAmp PCR Mix Preparation | | |
|---|---|---|
| **Reagent** | **Final Conc.** | **10µL** |
| 2X rhAmp Genotyping MasterMix + reporters | 1X | 5.25 µL |
| Passive reference dye (100X) | 1X | 0.1 µL |
| rhAmp Assay (20X)* | 1X | 0.5 µL |
| gBlock Mixture (2500cp/µL) | 500 cp/µL | 2.0 µL |
| Tween 20 (1%) | 0.05% | 0.5 uL |
| Water | N/A | 1.65 µL |

Table 1. **PCR Mix Preparation**

| Temperature | Duration | Number of Cycles |
|---|---|---|
| 95°C | 1:00 | 1 |
| 95°C | 0:05 | 40 |
| 60°C | 0:02 | |
| 68°C | 0:03 | |

Table 2. **Absolute Q thermal cycling parameters**

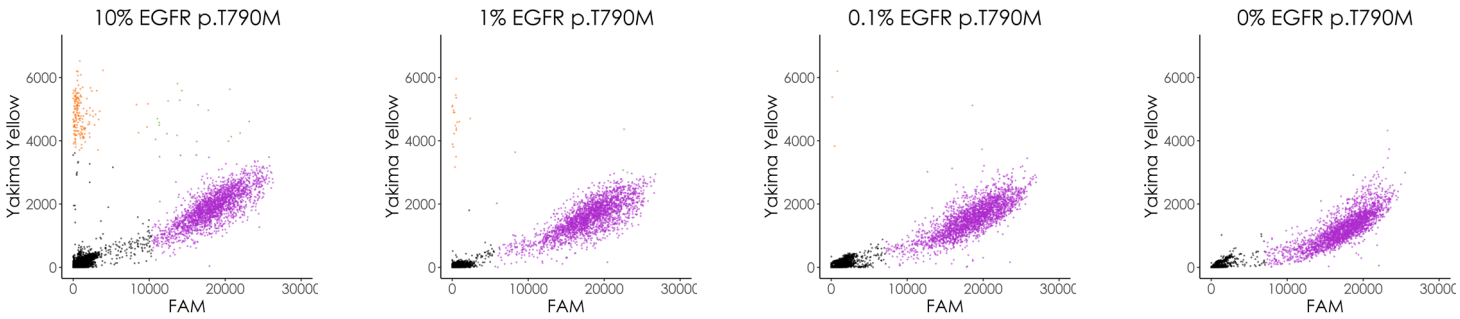



**Figure 1.** Mutation allele fraction (MAF) dynamic range evaluation of *EGFR* p.T790M molecules spiked into a high background of wild-type molecules, ranging from 10% to 0.1% of total. Images of one representative assay unit. Mutation probe contained Yakima Yellow and reference probe contained FAM. Three replicates were tested for each point.

orthogonal demonstration of rare molecule detection on the Absolute Q, the same prepared dilution series material was used as input for an IDT Affinity Plus *EGFR* p.T790M multiplex assay according to the standard protocol.

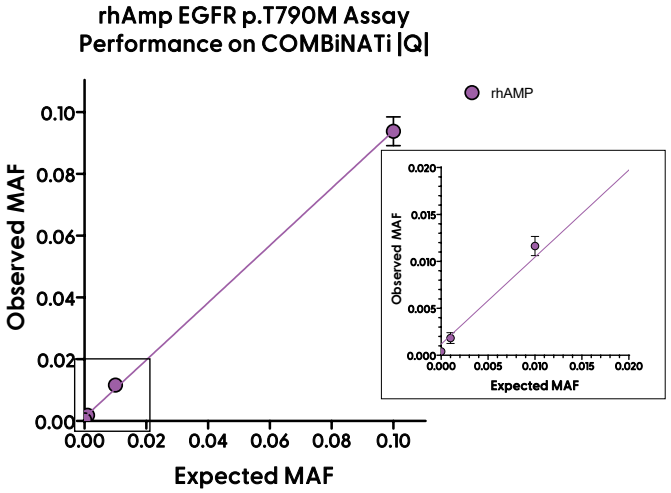
## Performance Data

Two-dimensional scatter-plots of dPCR data demonstrate the sequentially reduced signal of the mutation target across the dilution series. Separation between the mutant and wild-type can be clear observed as shown in Figure 1.

Across 3 technical replicates, the quantification of each MAF point across the serial dilution was accurate and overall demonstrated a high correlation with the expected MAF (Figure 2).

## Summary

The rhAmp Genotyping system is a highly efficient method of accurate PCR-based genotyping. Over 10 million assays have been pre-designed for human SNPs. The Combinati Absolute Q's uniquely designed Microfluidic Array Partitioning (MAP) plate enable adaptation of this chemistry to a dPCR platform, and for the first time demonstrate the capability for highly precise rare molecule detection using rhPCR based assays.




| Sample | Average Percent Mutation (±STDEV) |
|---|---|
| *EGFR* p.T790M 10% | 8.15% (±0.50%) |
| *EGFR* p.T790M 1% | 0.70% (±0.08%) |
| *EGFR* p.T790M 0.1% | 0.18% (±0.05%) |
| *EGFR* p.T790M 0% | 0.00% (±0.00%) |
| **Pearson Correlation Coefficient:** | **$R^2$: 1.0 (p=0.0002)** |

**Figure 2.** Mutation allele fraction (MAF) observations versus expectations between rhAmp and Affinity plus multiplex EGFR p.T790M assays. Average percent mutation molecules are reported with the associated standard deviation, three replicates were run per point.

## References

1. Moran C. (2011). Importance of molecular features of non-small cell lung cancer for choice of treatment. The American journal of pathology, 178(5), 1940–1948. https://doi.org/10.1016/j.ajpath.2010.12.057

2. rhAmp SNP Genotyping System. https://www.idtdna.com/pages/products/qpcr-and-pcr/genotyping/rhamp-snp-genotyping