Abstract #1927; poster session 27

High-sensitivity screening of a large number of samples for *KRAS* and *PIK3CA* mutations using digital PCR

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Introduction and methods

Colorectal cancer (CRC) is the third most common cancer in both women and men, with ~1.9 million new cases and >900,000 deaths worldwide in 2022 (1)

KRAS and _PIK3CA_ in CRC

Activating mutations in the proto-oncogene *KRAS* are among the most frequent in CRC. Of these, the *KRAS* G12 gene has 15 different point mutations (including G12S), and accounts for 6.5% of CRC cases (2). Somatic mutations in one of the PI3K catalytic subunit genes, *PIK3CA*, are also frequently found in numerous malignancies, including CRC.

There is a strong association between *KRAS* and *PIK3CA* mutations, with simultaneous mutations in *PIK3CA* exon 9 and *KRAS* exon 2 the most common (67.2%) (3). Compared with WT tumors, tumors with concomitant *KRAS* and *PIK3CA* mutations were significantly associated with aggressive clinically pathological features. For example, increased proportions of proximal colon cancer or prone to metastasize to distal organs at initial surgery (4, 5).

Methods

Here, we present a novel dPCR methodology and instrumentation to detect rare mutations, such as *PIK3CA* and *KRAS*, with consistent sensitivity, robustness and precision.

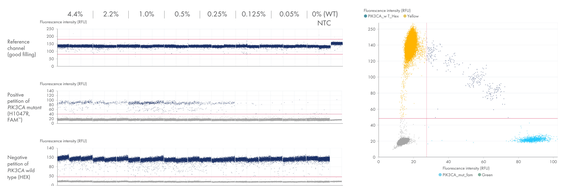
We selected two dPCR LNA Mutation Assays (QIAGEN® GeneGlobe®, cat. no. 250200; wet-lab validated) and germline cell-model systems (Horizon Discovery):

- *KRAS* G12S reference gDNA from SW48 cell line (GGT/AGT), with 50% mutation (cat. no. HD288)
- *PIK3CA* H1047R reference gDNA from PI3Ka(H1047/+) cell line, (CAT/CGT) with 50% mutation (cat. no. HD690)

These two model systems were used to demonstrate assay sensitivity and explore the minimum range of total input amount on the QIAcuity Digital PCR System (QIAcuity Four).

Results: Robust *PIK3CA* dPCR assay design and performance

Assay design and performance demonstrated here with QIAGEN *PIK3CA* (H1047R) LNA 2-plex probe assay on a QIAcuity Four instrument.

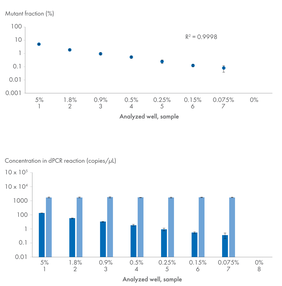


Note: A crisp, evenly spread of 1-D Scatter bands in the Reference channel indicates good sample distribution and filling.

PIK3CA (H1047R) LNA 2-plex digital PCR assay performance. **A** 1-D scatter plots: Reference channel plot demonstrated good sample distribution and sample fillings. Positive petitions of mutant (FAM™) and wild-type (HEX™) assays show good signal-to-noise (S/N) ratios demonstrating robust assay design and performance. **B** 2-D scatter plot: Good separation of mutants and negative petitions from wild-type population in this 2-plex LNA assay.

Results: Highly sensitive *PIK3CA* mutation detection using dPCR

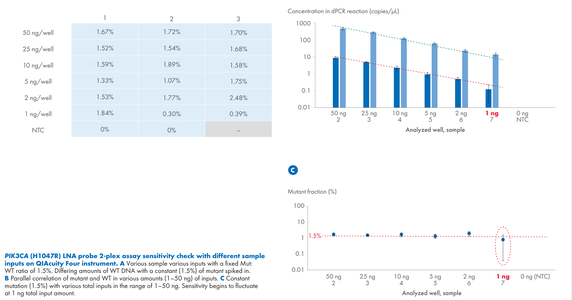
High sensitivity at 0.05% (Mut:WT) level with 100 ng total sample input. This assay used QIAGEN *PIK3CA* (H1047R; CAT/CGT) LNA Probe 2-plex assay with *PIK3CA* H1047R reference gDNA (Horizon Discovery).



PIK3CA assay sensitivity with 100 ng total input on QIAcuity Four instrument. **A** The assay demonstrated good mutant titration correlation ($R^2 = 0.9998$) under constant 25 ng WT total input. 2-plex digital PCR assay conditions: 95°C 2 min; 95°C 15 s/60°C 1 min (40X). Imaging conditions: 500 ms/Gain 6 (FAM/HEX). **B** *PIK3CA* (H1047R) mutation percentage. LNA mutation (FAM) and WT (HEX) 2-plex assay is run against *PIK3CA* (H1047R) mutant titration under constant 100 ng of WT background with varying percentages of mutant in the mixture; 3 replicates.

Results: High *PIK3CA* dPCR assay sensitivity

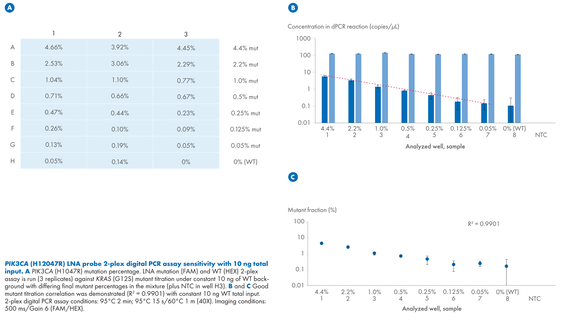
The QIAcuity dPCR System is a robust system for detecting *PIK3CA* mutants with high sensitivity. The system tolerates low sample inputs, down to as little as 1–2 ng while maintaining consistent detection sensitivity at 1.5% (Mut:WT) level.



PIK3CA (H1047R) LNA probe 2-plex assay sensitivity check with different sample inputs on QIAcuity Four instrument. **A** Various sample various inputs with a fixed Mut: WT ratio of 1.5%. Differing amounts of WT DNA with a constant (1.5%) of mutant spiked in. **B** Parallel correlation of mutant and WT in various amounts (1–50 ng) of inputs. **C** Constant mutation (1.5%) with various total inputs in the range of 1–50 ng. Sensitivity begins to fluctuate at 1 ng total input amount.

Results: Low sample input for *PIK3CA* mutation detection with dPCR

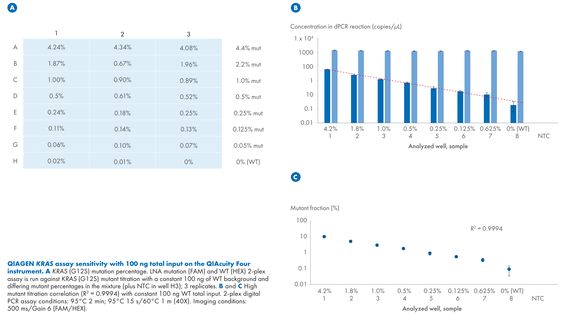
Moderate-to-low sample input requirement at 10 ng while maintaining excellent mutation detection sensitivity at 0.1% on the QIAcuity Four dPCR instrument run in 8-plex.



PIK3CA (H1047R) LNA probe 2-plex digital PCR assay sensitivity with 10 ng total input. **A** *PIK3CA* (H1047R) mutation percentage. LNA mutation (FAM) and WT (HEX) 2-plex assay is run (3 replicates) against *KRAS* (G12S) mutant titration under constant 10 ng of WT background with differing final mutant percentages in the mixture (plus NTC in well H3). **B** and **C** Good mutant titration correlation was demonstrated ($R^2 = 0.9901$) with constant 10 ng WT total input. 2-plex digital PCR assay conditions: 95°C 2 min; 95°C 15 s/60°C 1 m (40X). Imaging conditions: 500 ms/Gain 6 (FAM/HEX).

Results: Highly sensitive *KRAS* mutation detection using dPCR

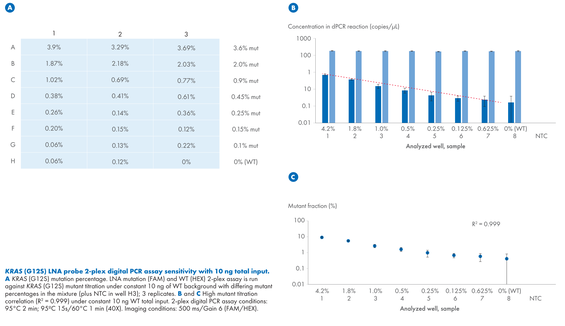
High sensitivity at 0.05% (Mut:WT) level with 100 ng total sample input. This assay used QIAGEN *KRAS* (G12S; GGT/AGT) LNA Probe 2-plex assay; with KRAS G12S reference gDNA from SW48 cell line (Horizon Discovery).



QIAGEN _KRAS_ assay sensitivity with 100 ng total input on the QIAcuity Four instrument. **A** *KRAS* (G12S) mutation percentage. LNA mutation (FAM) and WT (HEX) 2-plex assay is run against KRAS (G12S) mutant titration with a constant 100 ng of WT background and differing mutant percentages in the mixture (plus NTC in well H3); 3 replicates. **B** and **C** High mutant titration correlation ($R^2 = 0.9994$) with constant 100 ng WT total input. 2-plex digital PCR assay conditions: 95°C 2 min; 95°C 15 s/60°C 1 m (40X). Imaging conditions: 500 ms/Gain 6 (FAM/HEX).

Results: Low sample input for *KRAS* mutation detection with dPCR

Moderate-to-low sample input requirement at 10 ng while maintaining excellent mutation detection sensitivity at 0.1% on QIAcuity Four instrument.



KRAS (G12S) LNA probe 2-plex digital PCR assay sensitivity with 10 ng total input. **A** *KRAS* (G12S) mutation percentage. LNA mutation (FAM) and WT (HEX) 2-plex assay is run against *KRAS* (G12S) mutant titration under constant 10 ng of WT background with differing mutant percentages in the mixture (plus NTC in well H3); 3 replicates. **B** and **C** High mutant titration correlation ($R^2 = 0.999$) under constant 10 ng WT total input. 2-plex digital PCR assay conditions: 95°C 2 min; 95°C 15s/60°C 1 min (40X). Imaging conditions: 500 ms/Gain 6 (FAM/HEX).

Conclusions

- The QIAcuity Digital PCR System is highly consistent and sensitive, requiring no standards. The system can detect as low as 0.05–0.1% of mutation level reliably and consistently from 100–10 ng of total sample input volumes from two germline cell models:
  - *KRAS* (G12S/+) SW48 cell line (Horizon Discovery, cat. no. HD288)
  - *PIK3CA* (H1047/+) cell line (Horizon Discovery, cat. no. HD690).
- We have demonstrated the QIAcuity Four instrument and related kits are capable of mutation detection to the sub-0.1% level, making it highly suited for use in clinical cancer research with samples that are often scarce and/or poor quality (e.g., FFPE tumor samples and cell-free DNA)
- QIAcuity dPCR is easy to adapt from quantitative PCR assays, making the migration of laboratory-developed or commercial qPCR to dPCR assays robust and seamless.
- Mutational LNA probe assays from QIAGEN GeneGlobe database are wet-lab validated. This study demonstrates that assay performance is robust, sensitive and applicable for the detection of cancer targets in multiplexing.
- The sensitivity, robustness and low sample requirement of dPCR assays may be well suited for translational research topics, such as disease progression monitoring.

References

1. WHO International Agency for Research on Cancer. https://www.iarc.who.int/cancer-type/colorectal-cancer/. Accessed: March 17, 2025. **2.** Meng M, et al. *Biomed Pharmacother.* 2021;140:111717.**3.** Li H-T, et al. *Oncol Rep.* 2011;25(6):1691-7. **4.** Rosty C, et al. *PLoS One.* 2013;8(6):e65479. **5.** Luo Q, et al. *Transl Oncol.* 2020;13(12):10087.