Detection of rare events using the QIAcuity® Digital PCR System

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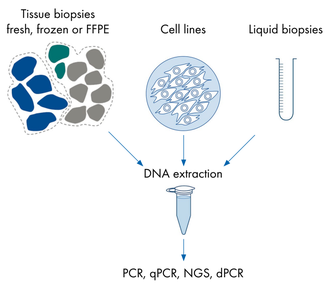
Finding the needle in the haystack

For detection of rare events in the samples, a highly sensitive detection of the target DNA molecules are required. The sample source and its heterogeneity, as well as the suboptimal DNA extraction methods might pose additional challenges for detection of rare events.

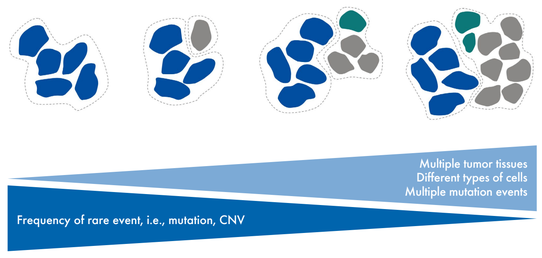
Sample source

- Highly variable sample quality
- Limited sample amounts
- Presence of contaminants, inhibitors
- High fragmentation
- Suboptimal DNA extraction of samples



Typical sample sources.

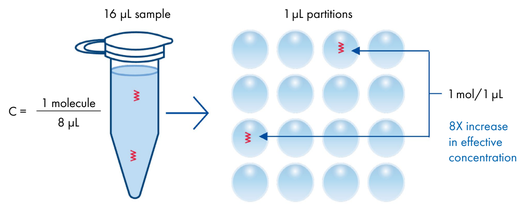
Sample heterogeneity



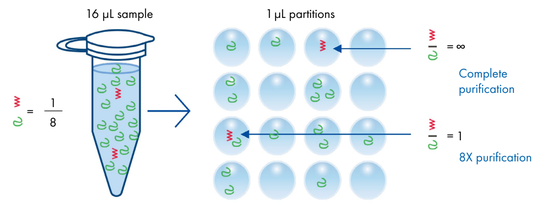
Sample heterogeneity. The frequency of a rare event within the sample drastically decreases with the increased heterogeneity of the sample. The rare event is most likely limited to a fraction of cells which are diluted out among thousands of wildtype background cells.

Digital PCR increases accuracy and sensitivity of detection

Through partitioning of bulk samples, individual target molecules can be effectively isolated from interfering molecules and detected at much higher sensitivity using digital PCR.



Increase of effective concentration.

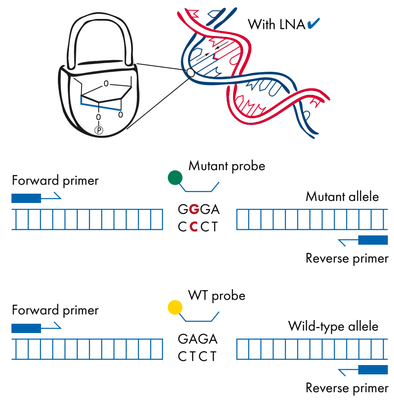


Decrease of interfering molecules.

dPCR LNA Mutation Assays: Highly sensitive and specific detection of mutations related to cancer and oncogenesis

Features

- Duplex assay design detects mutated and wild-type sequences
- LNA-enhanced primers and probes increase assay specificity and sensitivity
- Wet-lab tested dPCR assays with sensitivity down to 0.1% mutation frequency in a single well
- Portfolio covers ~200 assays targeting cancer genes or pathways
- Suitable for use with circulating tumor DNA, liquid biopsy, FFPE and other tissue samples
- Multiplexing option with FAM™ + HEX™ and ATTO 550 + ROX™ fluorescent dye combinations
- Intended for use with QIAcuity Probe PCR Kits
- Can be used in combination with QIAcuity 26k or 8.5k nanoplates

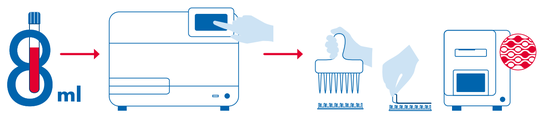


LNA principle. The assay, provided in a single-tube format, contains a primer pair and two probes – a mutant probe and a wild-type (WT) probe – for detecting both mutant and wild-type alleles in the same reaction.

Load more, see more: Find the needle in the haystack

Using QIAcuity dPCR enables a high template input amount to be used in a mutation analysis workflow with 26k nanoplates.

A higher number of mutation copies can be detected with the combination of sample processing on EZ2 Connect and mutational analysis by QIAcuity compared to other platforms.



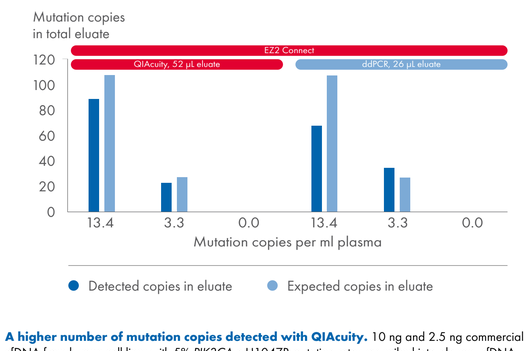
EZ2 Connect QIAcuity workflow. Up to 8 mL plasma can be processed on the EZ2 Connect combined with high eluate loading (up to 26 µL) on the QIAcuity.

Rare event scenario: 10 target copies (cp) in 70 µL eluate

	QIAcuity Nanoplate 26k	Supplier T	Supplier B*
dPCR reaction volume	40 µL	9 µL	20 µL
Maximum eluate volume	26 µL†	5.85 µL	13 µL
Copies analyzed and seen	1.85 cp	0.76 cp	1 cp

* Assuming 16K droplets.
† Based on 4x mastermix and 10x assay.

Data obtained in experiments conducted by QIAGEN R&D, Hilden, Germany.



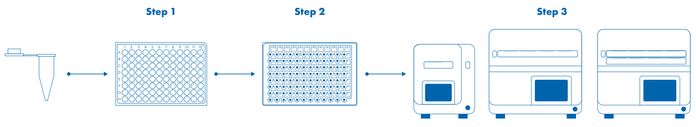
A higher number of mutation copies detected with QIAcuity. 10 ng and 2.5 ng commercial cfDNA from human cell lines with 5% *PIK3CA* p.H1047R mutation rate was spiked into plasma. cfDNA was prepared from plasma using an 8 mL sample volume on EZ2 Connect with the EZ1&2™ ccfDNA Kit. dPCR was run using a dPCR Mutation Assay *PIK3CA* 775 Human and standard protocols were used for both the QIAcuity and the digital PCR platform from another supplier.

dPCR CNV Probe Assays: Focused copy number profiling

dPCR CNV Probe Assays.

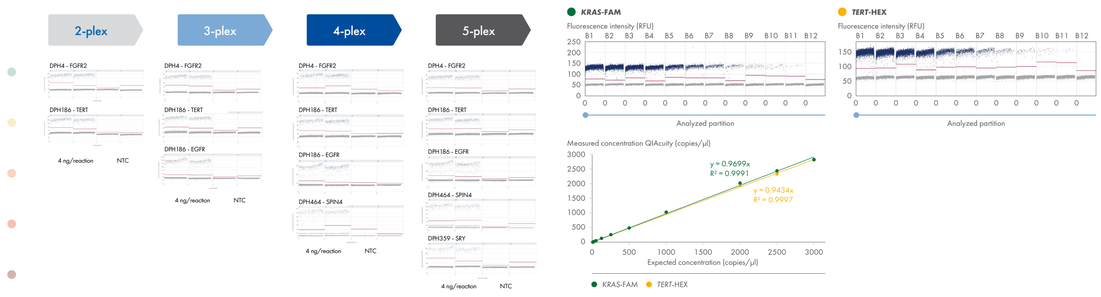
dPCR CNV Probe Assays features:

- Wet-bench validated assays for more than 200 targets ensure outstanding performance
- Assays target widely studied cancer genes and pathways
- LNA-enhanced primers and probes ensure assay specificity and sensitivity
- Dye selection enables multiplexing of up to 5 targets per reaction
- Gene of interest, reference and centromeric reference assays available
- Ready-to-use assays have been validated for a range of samples such as FFPE, cell lines and gDNA
- Simple and fast dPCR workflow on the QIAcuity
- Convenient copy number data analysis using the QIAcuity Software Suite
- Can be used in combination with QIAcuity 26k or 8.5k nanoplates



dPCR CNV Probe Assays workflow.

dPCR CNV Probe Assays: Flexible, precise detection with all template amounts

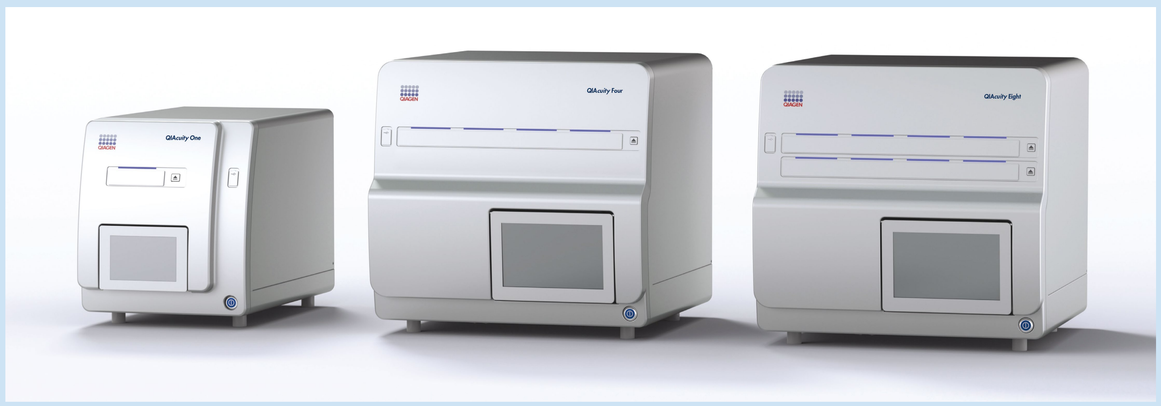


Multiplexing of up to 5-channels. gDNA was loaded at 4 ng/reaction in duplicate reactions including no-template controls (NTCs). The standard dPCR CNV Probe Assay protocol was performed using 8.5k Nanoplates.

Accurate detection at high and low template loading amounts. gDNA loaded at 5–3000 copies/µL. The standard dPCR CNV Probe Assay protocol was performed using 8.5k Nanoplates.

Summary

- The QIAcuity Digital PCR System provides highly accurate and precise absolute quantification of target molecules.
- Due to partitioning and end-point PCR, amplification efficiency bias is eliminated, increasing the analytical sensitivity of detection for rare events and low abundance targets.
- High template loading volumes increase the likelihood of loading rare target molecules into the PCR reaction, and hence their detection.
- QIAcuity LNA Mutation Detection and Probe CNV Assay portfolios provide highly sensitive and specific LNA-enhanced assays, targeting commonly studied cancer genes and related pathways.



QIAcuity dPCR instruments.

Sample to Insight