Sensitive somatic mutation detection in cfDNA with mutation PCR array coupled with preamplification

Samuel Long and Eric Lader

QIAGEN Center of Excellence in Biological Content, Frederick, MD, USA

Abstract

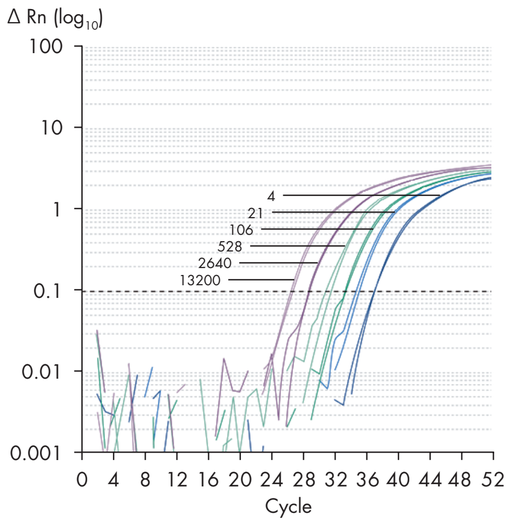
Cell-free circulating DNA (cfDNA) as a non-invasive, liquid biopsy has recently been exploited for use in a range of cancer applications. Compared to static sampling of DNA from an archival/FFPE tumor, cfDNA provides real-time mutational information of primary and metastatic tumors, which can be used to facilitate treatment decisions and monitoring. cfDNA mutation analysis also allows interrogation of intra-patient mutation heterogeneity.

Challenges for cfDNA mutation analysis include the low concentration of cfDNA in plasma/serum and the potentially low level of mutations in cfDNA. In addition, the fragmented nature of some cfDNA samples can further reduce the effective input in PCR-based detection applications. QIAGEN has developed and optimized a PCR-based, targeted preamplification solution that enriches the target regions of interest in cfDNA by 4000-fold, dramatically increasing the effective template input for mutation analysis using qBiomarker somatic mutation assays or arrays. Preamplification can be performed in singleplex PCR for single mutation detection, or in a multiplex PCR (up to 400plex), to allow profiling of potentially up to 1217 frequently occurring cancer hotspot mutations in 163 genes.

Here, targeted preamplification paired with qBiomarker mutation PCR array was used to detect serum or plasma cfDNA mutations present in lung or colon cancer primary tumor tissues. In 3 of 5 cases, the same mutations characterized in the solid tumors were present in cfDNA. In the 2 non-concordant cases, the mutations appeared absent from the cfDNA or below the mutation assay detection limit. qBiomarker mutation PCR array screening also identified 2 additional somatic mutations from the cfDNA. These mutations were previously not examined in the solid tumors. Analysis of an additional 15 cfDNA from previously un-genotyped cancer serum samples detected 6 somatic mutations.

The pairing of targeted preamplification and qBiomarker mutation PCR arrays/assays provides a cost-effective translational research tool to detect cancer mutations earlier and more sensitively in cfDNA.

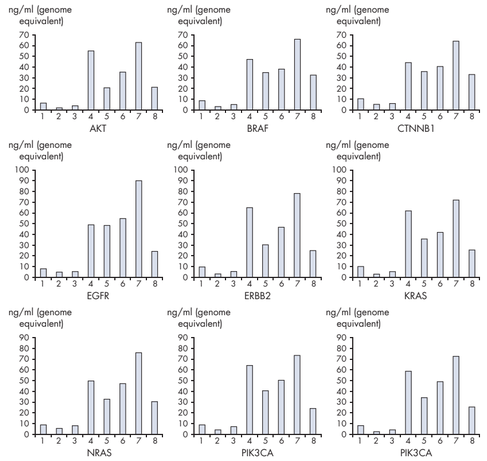
qBiomarker somatic mutation assay detection sensitivity



qBiomarker Somatic Mutation PCR Assay performance. Assay sensitivity test for p53 R280K qBiomarker Somatic Mutation PCR Assay. A series of 10 ng genomic DNA samples, which contain 4, 21, 106, 528, 2640, and 13,200 copies of mutant DNA template respectively, from MDA-MB-231 cell line (mutant harboring the p53 R280K mutation) mixed with genomic DNA from the Coriell GM00131 cell line (wild type; WT), were tested with the p53 R280K mutation assay.

Amplification plots for duplicate reactions are shown. Mutation detection limit for this assay is determined to be 0.03% (4 copies of mutant template out of a total of 13,200 copies of DNA template).

Gene copy number assays on mutation PCR array quantify cfDNA oncogene and TS gene amount



Quantification of oncogenes and tumor suppressor (TS) gene. cfDNA was isolated from 200–500 µl serum from 5 lung cancer (#4–8) and 3 controls (#1–3) using the QIAamp® Circulating Nucleic Acid Kit. Each purified cfDNA or 20 ng WT human gDNA (Promega) was preamplified using the human lung cancer preamp primer pool. Preamp products were then analyzed using the qBiomarker Somatic Mutation PCR Array for lung cancer. Genomic equivalent amount of each cancer gene was calculated using ΔC_T method in reference to the gene amount in the WT human DNA.

Mutation status correlation in cfDNA and tumor tissue samples

Tissue	Sample ID	cfDNA source	Mutation detected in tumor tissue	AA change	Detected by preamp + qBiomarker array	Mutation (%)	Mutation assay detection limit	Additional mutation identified by preamp + qBiomarker array	Mutation (%)	Mutation assay detection limit
Lung	40	Serum	KRAS	G12C	Yes	0.62%	0.02%	KRAS Q61H	0.03%	0.01%
Lung	41	Serum	KRAS	G12D	Yes	0.18%	0.18%	TP53 Q192*	0.11%	0.08%
Lung	42	Serum	KRAS	G12D	Yes	0.48%	0.18%	–	–	–
Colon	43	Serum	BRAF	V600E	No	–	0.06%	–	–	–
Colon	53	Plasma	BRAF	V600E	No	–	0.06%	–	–	–

cfDNA was isolated from 250 µl each of 5 serum or plasma samples from lung or colon cancer. The mutation status of the corresponding primary tumor tissues was previously determined (Asterand). Each purified cfDNA was preamplified using human lung or colon cancer preamp primer pool. Preamp products were then analyzed on qBiomarker Somatic Mutation PCR Arrays for lung/colon cancer. Mutation call in cfDNA was calculated based on $\Delta\Delta C_T$ method. Mutant sample mutation percentage was determined based on the ΔC_T value at the relevant mutation locus. Mutation assay detection limit was based on the ΔC_T value at the relevant mutation locus from at least 3 samples that are wild type at the locus.

In summary, 5 somatic mutations were detected in 3 cfDNA samples (samples 40, 41, and 42). The BRAF mutant level in samples 43 and 53 was below the assay detection limit (0.06%).

Mutation profiling in cfDNA with qBiomarker mutation PCR arrays

Cancer	Sample ID	Mutated gene	COSMIC ID	nt change	AA change	Mutation (%)	Mutation assay detection limit
Lung	7	EGFR	6213	c.2582T>A	p.L861Q	0.06%	0.05%
Lung	7	P53	43629	c.745A>T	p.R249W	0.20%	0.01%
Endometrial	17	KRAS	522	c.35G>C	p.G12A	0.14%	0.04%
Breast	14	AKT1	33765	c.49G>A	p.E17K	0.17%	0.01%
Breast	9	AKT1	33765	c.49G>A	p.E17K	0.43%	0.01%
Colon	12	P53	10659	c.817C>T	p.R273C	0.44%	0.18%

cfDNA was isolated from 200–500 µl each of 15 serum samples from lung (5), colon (3), breast (5) and endometrial (2) cancer samples. The mutation status of the corresponding primary tumor tissues was previously not characterized. Each purified cfDNA was preamplified using a human cancer preamp primer pool that matched the serum sample cancer type. Preamp products were then analyzed on matching qBiomarker cancer mutation PCR arrays. Mutation call in cfDNA was calculated based on $\Delta\Delta C_T$ method. Mutant sample mutation percentage was determined based on the ΔC_T value at the relevant mutation locus. Mutation assay detection limit was based on the ΔC_T value at the relevant mutation locus from at least 3 samples that are wild type at the locus.

Conclusion

- qBiomarker somatic mutation assays have detection sensitivity as high as 0.01%.
- The PCR-based, targeted preamplification solution enriches the target regions of interest in cfDNA by 4000-fold, therefore dramatically increasing the effective template input for downstream mutation analysis.
- The pairing of targeted preamplification and qBiomarker mutation PCR arrays/assays provides a cost-effective translational research tool to detect cancer mutations earlier and more sensitively in cfDNA.
- This method can be used to detect, quantify, and monitor potentially 1217 mutations in 163 genes from the same cfDNA sample.

The applications presented here are for research use only. Not for use in diagnostic procedures.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

1082817 06/2014