

TaqMan dPCR Liquid Biopsy Assays targeting the TERT promoter region

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ABSTRACT & INTRODUCTION

The enzyme Telomerase maintains telomeres at the ends of chromosomes. The Telomerase Reverse Transcriptase (TERT) gene codes for the enzyme's catalytic domain and is not expressed in normal somatic cells. As a consequence, normal cells acquire senescence by shortening of their telomeres during cell division and eventually undergo apoptosis. In contrast to normal somatic cells, expression of TERT is reinstated in cancer cells causing escape from senescence and apoptosis by maintaining the telomeres. It has recently been shown that mutations in the TERT promoter region play a key role in regulating and reinstating TERT expression. Up to 90% of cancers carry a mutation in the TERT promoter region. Mutations like C228T and C250T create new binding sites for the E26 transformation-specific (ETS) transcription factor that regulates TERT expression (1,2). Experimental evidence showed that the ETS factor GA-binding protein, alpha subunit (GABPA) binds to the de novo ETS motif and activates TERT transcription in cancer cells.

We undertook a project designing TaqMan[®] dPCR Liquid Biopsy Assays addressing mutations in the TERT promoter. These assays are TaqMan SNP Genotyping assays that are optimized for use in digital PCR with the Applied Biosystems QuantStudio 3D. In digital PCR, partitioning the sample into many individual reaction wells facilitates detection and quantification of rare mutant alleles. TaqMan SNP Genotyping Assays reliably discriminate mutant and wild-type allele. This will enable easy and sensitive detection of TERT promoter mutations in cancer research samples. These assays are suitable for detection in liquid biopsy applications with cell free DNA (cfDNA).

Assay design proved to be challenging due to high GC content and repetitive elements in this region of the TERT gene and required varying both the assay design and experimental cycling conditions. Template for wet-lab testing was synthetic plasmid carrying the mutation spiked into wild-type genomic DNA and wild-type genomic DNA control.

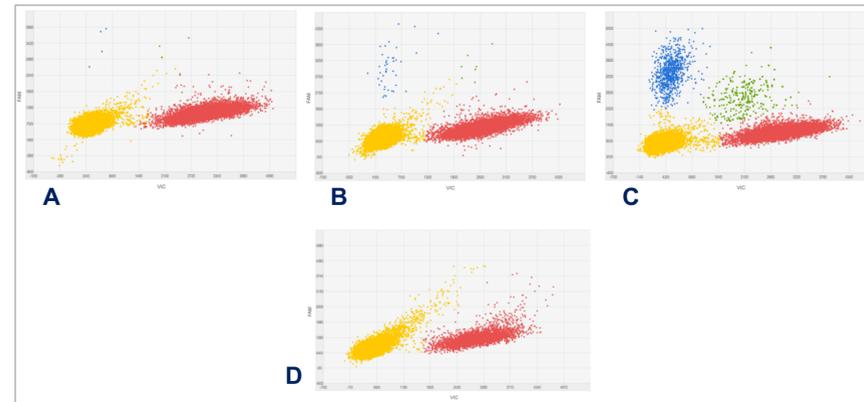
The result was two TaqMan dPCR Liquid Biopsy Assays detecting the C228T and C250T mutations in the TERT promoter region. We showed that designing TaqMan dPCR Liquid Biopsy Assays for digital PCR is feasible for the challenging TERT promoter region.

MATERIALS AND METHODS

15ng of wild-type genomic DNA containing spiked in mutant plasmid (GeneArt[®] Gene Synthesis) was used as input amount for digital PCR using the QuantStudio 3D Digital PCR System. Thermal cycling was performed on the ProFlex PCR System as shown in Figure 4.

RESULTS

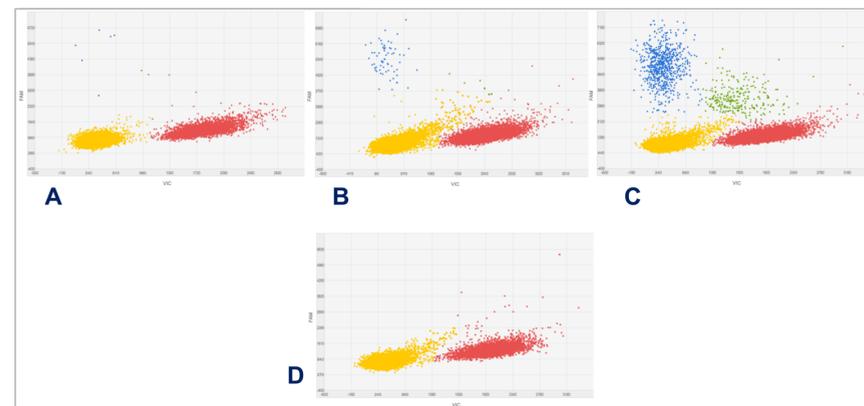
Figure 1. Performance of the TERT C228T Assay



Performance of assay Hs000000092_rm detecting the TERT C228T (COSM1716558) mutation in the promoter region of the TERT gene. The mutation is located at -124 base pairs upstream of the ATG start codon. The assay was tested on wild-type genomic DNA with mutant plasmid spiked in.

A. 0.1% mutation B. 1% mutation C. 10% mutation D. wild-type only control

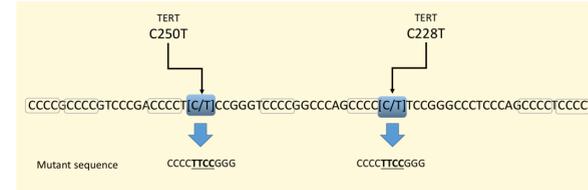
Figure 2. Performance of the TERT C250T Assay



Performance of assay Hs000000093_rm detecting the TERT C250T mutation (COSM1716559) in the promoter region of the TERT gene. The mutation is located at -146 base pairs upstream of the ATG start codon. The assay was tested on wild-type genomic DNA with mutant plasmid spiked in.

A. 0.1% mutation B. 1% mutation C. 10% mutation D. wild-type only control

Figure 3. Challenging Context Sequence



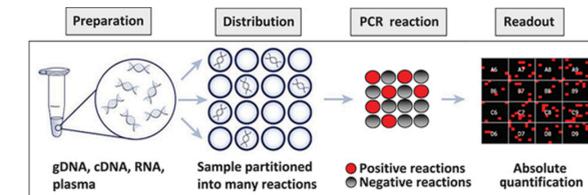
The sequence context in the TERT promoter region is highly GC-rich and contains repetitive motifs. This presented a challenge for assay design. Initial assay designs showed no cluster separation, indicating that the probes may have annealed non-specifically at multiple sites. Both mutations generate an identical 11bp sequence containing the ETS motif GGAA on the reverse strand (2).

Figure 4. Experimental Conditions

Stage 1	Stage 2		Stage 3	
96.0°C	55.0°C	98.0°C	55.0°C	10.0°C
1.6°C/sec	1.6°C/sec	1.6°C/sec	1.6°C/sec	1.6°C/sec
0:10:00	0:02:00	0:00:30	0:02:00	∞
1x (Hold)	54x (Cycles)		1x (Hold)	

Final thermo-cycling conditions for both TERT C228T and TERT C250T assays. Cycling was performed on the ProFlex PCR System. This protocol has been adjusted with lower annealing temperature and increased cycle number according to the standard protocol for TaqMan dPCR Liquid Biopsy Assays.

Figure 5. Digital PCR



Digital PCR using the QuantStudio 3D was the approach to detect rare mutations. The sample is partitioned into 20,000 individual compartments on the digital chip. This partitioning enables the detection of low frequency somatic mutations since they are not competing against an overwhelming number of wild-type alleles.

Figure 6. TaqMan Rare Mutation Analysis



Wet-lab tested TaqMan dPCR Liquid Biopsy Assays for key somatic mutations plus the power of digital PCR on the QuantStudio 3D Digital PCR combined with AnalysisSuite Cloud Software are an optimal workflow solution to routinely quantify cancer mutations at a frequency as low as 0.1%.

CONCLUSIONS

We successfully designed and optimized TaqMan dPCR Liquid Biopsy Assays for TERT C228T and TERT C250T mutations for use in digital PCR using the QuantStudio 3D. Assay design and optimization of thermo-cycling conditions were key in developing these assays. Digital PCR allows detection of both mutations at a 0.1% mutation rate.

REFERENCES

Huang FW et al. 2013. Highly Recurrent TERT Promoter Mutations in Human Melanoma. SCIENCE Vol 339, No 22: 957-959

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TRADEMARKS/LICENSING

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