Evaluation of microfluidic digital PCR for the detection of cancer biomarkers

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Introduction

- The emerging technique of digital PCR (dPCR) shows great promise for clinical diagnostics, including the detection of low abundance minority targets and copy number variation studies
- dPCR is achieved by partitioning samples prior to PCR amplification such that each reaction chamber contains one copy or less of target DNA. A count of reactions containing PCR product is a direct measure of the absolute nucleic acid quantity.

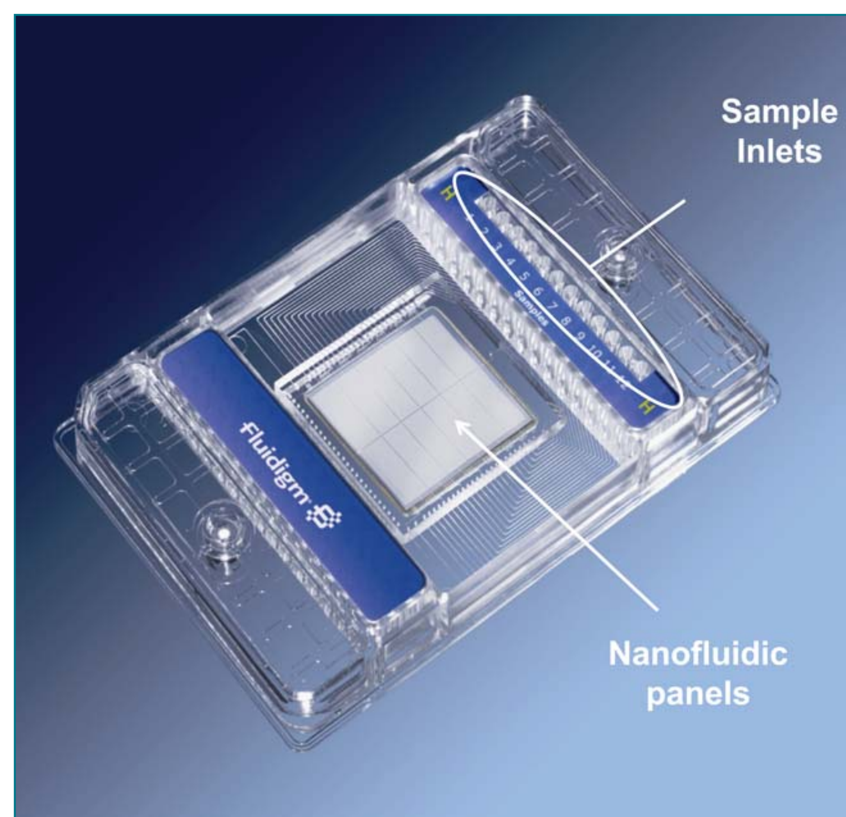
Aim

The aim of this study was to evaluate the reliability and robustness of dPCR through investigation of parameters influencing measurement bias and uncertainty.

Materials and methods

BCR-ABL standards (major breakpoint cluster region [Mbcr], Ipsogen) were spiked into a background of HCC1954BL cDNA (lymphocyte origin, negative for BCR-ABL) and used for the accuracy, precision, sensitivity and repeatability experiments. An in-house Arabidopsis alcohol dehydrogenase (Adh) assay was used for comparison of template type (plasmid versus linearised plasmid) on absolute quantification.

All experiments were performed using the Fluidigm Biomark system, 12.765 dPCR chips (shown). This enables high levels of replication (765 replicates per sample and 9180 total reactions per run), on a microfluidic platform.



Results

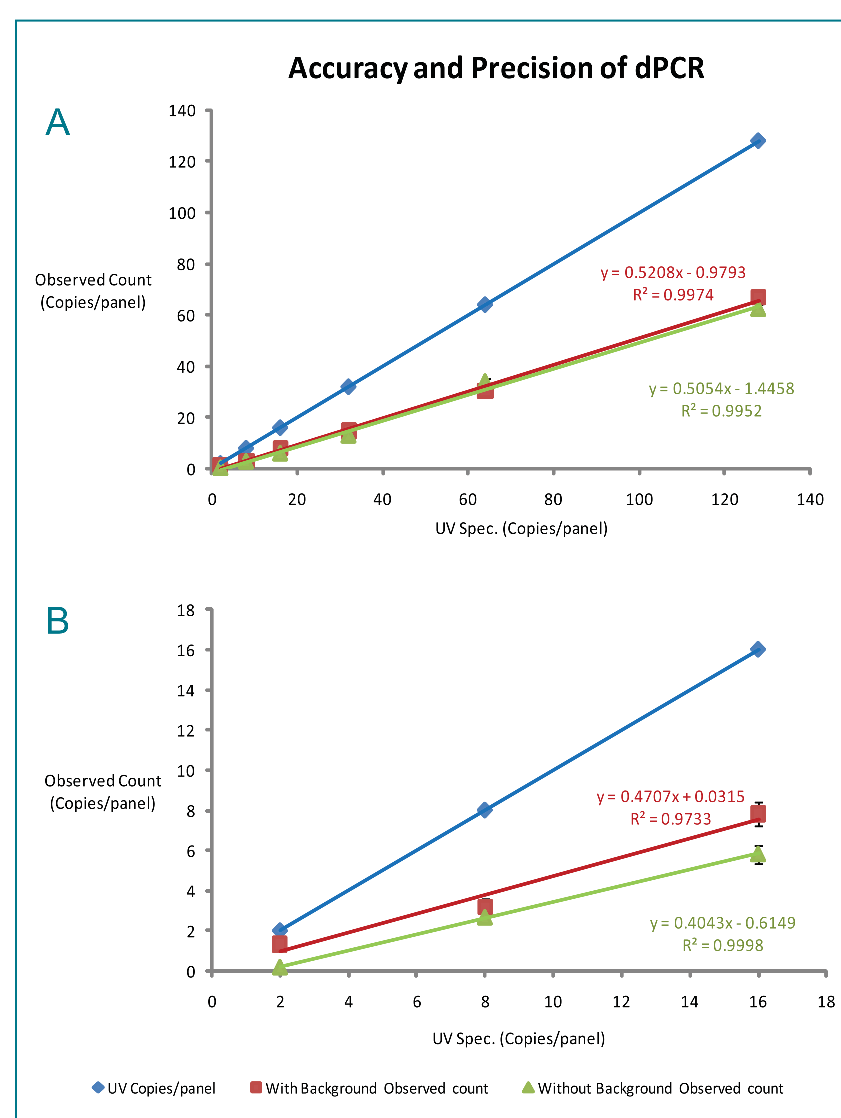
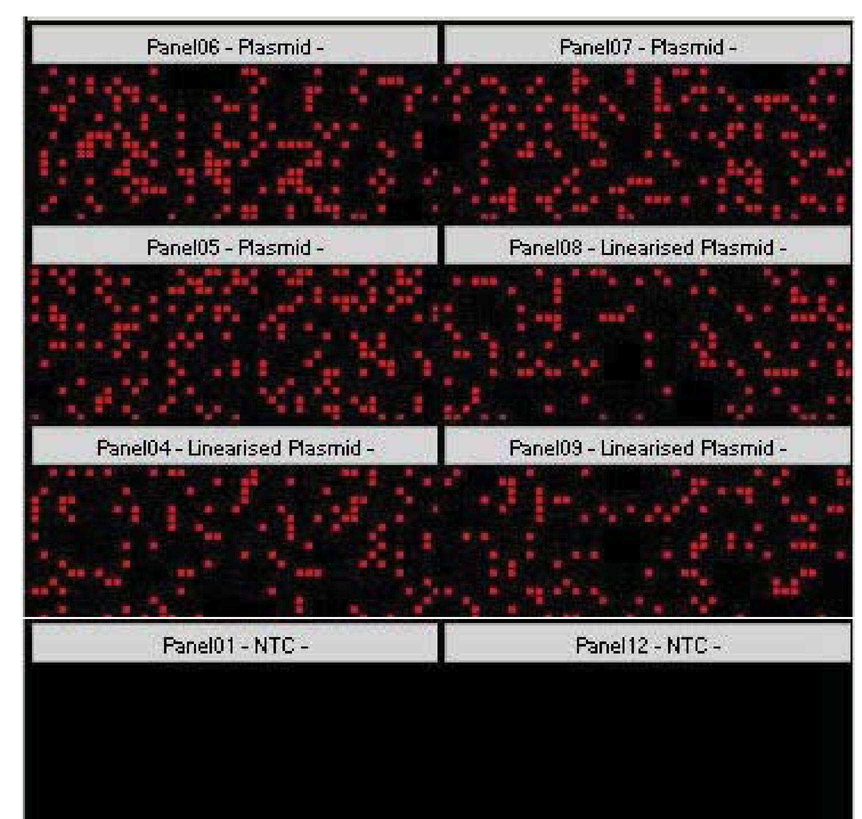


Figure 1. BCR-ABL standards were diluted to give approximately 2, 8, 16, 32, 64 or 128 copies per assay (based on Ipsogen's quoted quantification). Samples were spiked into a background of cDNA or water, and analysed by the Fluidigm Biomark platform. (A) all samples, (B) extended lower range, 2-16 copies. Data is plotted against expected copy number from UV spec. Error given as standard error of the mean (SEM), n=3.

- dPCR detected trace levels of target DNA (Fig 1)
- dPCR measurements were very precise using different sample dilutions and is reproducible at all copy number levels
- There was no significant difference, at the 95% confidence level, in absolute copy number quantification when standards were analysed alone or when spiked into a cDNA background (average P value = 0.3). However, there was a trend for increased sensitivity at the lowest copy number in samples with cDNA background. This may suggest that the background is acting as a carrier for low copy number targets
- dPCR absolute copy number quantification was typically 50% lower than UV spectroscopy-based estimations
- DNA template type has an effect on absolute dPCR measurements (Fig 2), cut plasmid having a lower count than uncut.

- Data shows variation (Ct spread) in individual PCR cycle threshold values when using cut and uncut plasmid templates, suggesting low PCR efficiency, Ct=31+/-5 cycles (data not shown). However, dPCR measurements are independent of PCR efficiency as only positive amplification is counted, not relative Ct values.



Plasmid

UV Spec. Copies/panel	Observed Copies/panel
250	181.33

Linearised Plasmid

UV Spec. Copies/panel	Observed Copies/panel
250	123.33

Positive Amplification   No Amplification

Figure 2. Heat map view generated by the Biomark dPCR analysis software. The 8 panels represent 8 samples: Adh whole (uncut) plasmid; Adh linearised (cut) plasmid, each in triplicate, and no template control (NTC) in duplicate. Each sample consisted of 250 copies/panel according to UV quantification. The red spots indicate amplification has occurred, i.e. target DNA is present.

Conclusions

- dPCR measurements using the Biomark instrument showed good precision, sensitivity and reproducibility
- Interestingly, dPCR absolute copy number measurements were discordant with UV-based DNA measurements. This could be due to PCR failures. However, it is equally possible that dPCR may actually provide a more accurate count of amplifiable sequences present in a sample, with UV spec. providing an over estimation. Further investigation is warranted
- The nature of DNA template affects absolute quantification and should be considered in comparative studies.

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