The Modi Operandi of the VideoScan Platform for the Detection and Analysis of Nucleic Acids

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Introduction

In medical routine diagnostics is a high necessity to deliver patient-related information (e.g., infections, personalized medicine) both at a high multiplex level and accuracy. Many assay platforms for nucleic acids have been developed which meet specific tasks. The performance of multiplex PCRs in conventional real-time PCR technologies is limited by available probe colors and/or the ability to perform melting temperature analysis. We expanded the power of nucleic acid analysis by building a multi-purpose tool for multiparameter analytics based on image analysis.

Material and Methods

Here we show that our platform, designated VideoScan, provides the basis for various use case scenarios. VideoScan is a highly versatile real-time image analysis platform and optimal for the quantification of thousands of microscopic objects within a single sample [1].

Results

VideoScan offers different levels for analysis and quantification of nucleic acids. This includes the ability to perform highly multiplex end-point and real-time quantification methods under precise temperature controlled conditions (Fig. 1). We developed heterogeneous multiplex microbead assays as a high-throughput technology (8 – 11 targets / cavity in 96-well plates). This technology was recently used for the comparison of Escherichia coli from human and domestic and wild animals in our in-house developed Mutiplex-PCR Microbead Assay (MPMA) [2] (Fig. 2). We designed novel microbead nucleic acid-based probe systems for homogenous assays that use Fluorescence Resonance Energy Transfer (FRET). The LoopTag real-time PCR probe system [3] (Attomol GmbH) was adapted as part of an upcoming homogenous microbead probe system for increased sample throughput and in-deep target discrimination by melting curve analysis (Fig. 3). Moreover, we implemented dual-hybridization probes for the quantification of nucleic acids and used this approach for melting curve analysis and the detection of Single Nucleotide Polymorphisms [4] (Fig. 4).

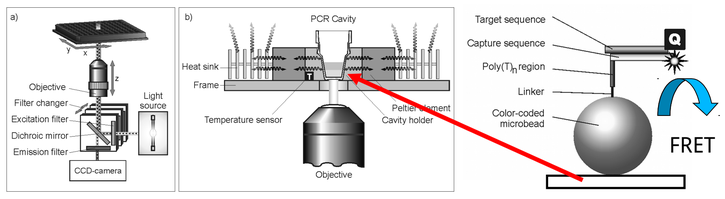

Figure 1. Scheme of the VideoScan hardware components and the heating-cooling unit (HCU) for standard PCRs, qPCRs and melting curve analysis. a) The main hardware components are a motorized fluorescence microscope, a stage, and a CCD camera controlled by a personal computer. b) Scheme of a single HCU for dynamic heating of a single well of standard PCR modules. Changes of fluorescence intensity can be monitored in a broad temperature range in different channels for both solutions and microbeads.

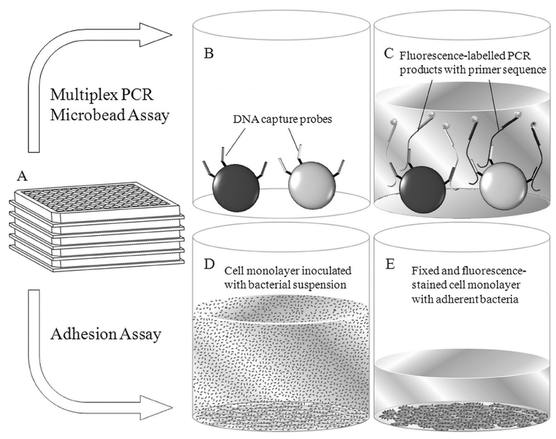

Figure 2. Principle of MPMA and the 96-well plate adhesion assay. DNA capture probes were coupled to different microbead populations. Microbeads were fixed to the bottom of a plate (A & B). *E. coli* gene fragments were amplified from bacterial lysates in a multiplex format (3 - 9 plex mPCR) in the presence of microbeads (C). During amplification, PCR products were labeled by one Cy5-labeled primer. PCR fluorescence-labeled amplicons hybridized to gene-specific capture probes. Reagents and nonhybridized PCR products were removed by washing. The hybridized PCR products were quantified by measuring the fluorescent corona around a microbead. Microbeads were analyzed using the VideoScan system. Adhesion assay (D & E). A monolayer epithelial cells was inoculated with a bacterial suspension. The supernatant with nonadherent bacteria was removed (E). After washing with 1x PBS, adherent *E. coli* cells were fixed with 4% paraformaldehyde and fluorescence stained with propidium iodide. Adherent bacteria were examined by a special data-imaging-processing algorithm of VideoScan.

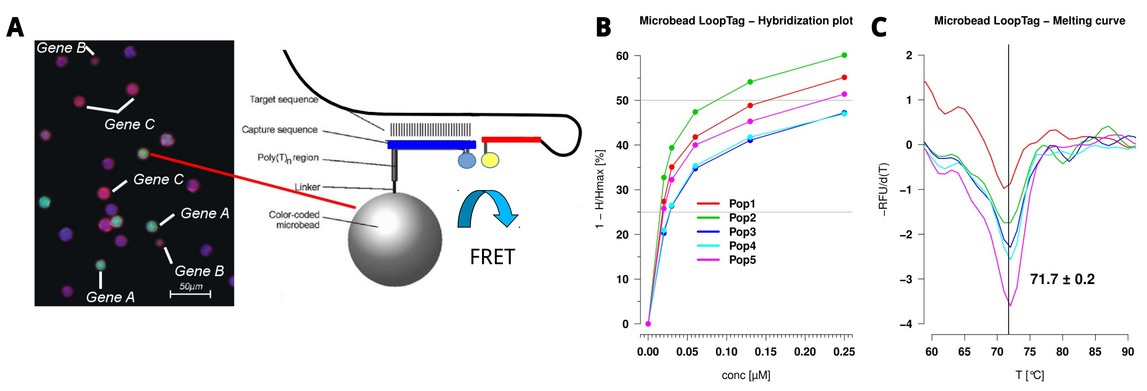

Figure 3. LoopTag probe system microbead real-time PCR. LoopTag formation: Hybridization of one part of one LoopTag primer amplified strand to the microbead bound LoopTag capture probe. The FRET signal is monitored in real-time (A). Dose-dependent detection of DNA by hybridization analysis (B) and differentiation by melting curve analysis (C) is possible.

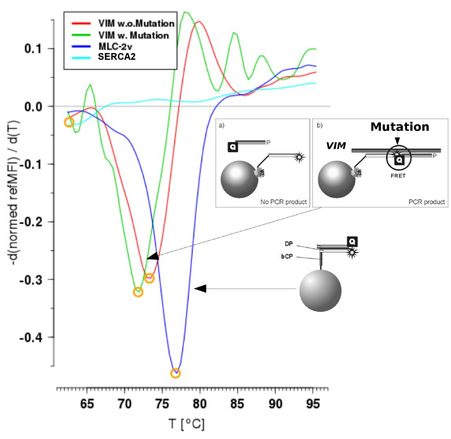

Figure 4. Surface melting curve analysis to detect single base mutations. VideoScan technology in combination with the precise temperature control of the HCU can be used to identify single base mutations. In our example T_M differences of Vimentin and *ventricular myosin light chain 2* (*MLC-2v*) were investigated. High throughput is achievable by using different capture probe microbead combinations. The HCU can be applied for genotyping applications to identify samples that contain their mutation of interest from wild type.

The rising star in nucleic acid analysis for diagnostic applications is digital PCR (dPCR). Recently we contributed the open source "dpcR" R package for the analysis of digital PCR experiments to the scientific community [5]. We are currently testing the VideoScan platform as a potential readout device for the novel droplet digital PCR solution developed by Stilla Technologies (France), which is based on an innovative on-chip sample partitioning microfluidic technique [6]. Digital PCR is carried out in 2D-droplet arrays within the chip chambers, which can be imaged using the VideoScan system (Fig. 5). Image processing algorithms for droplets in chambers are currently developed for the VideoScan platform.

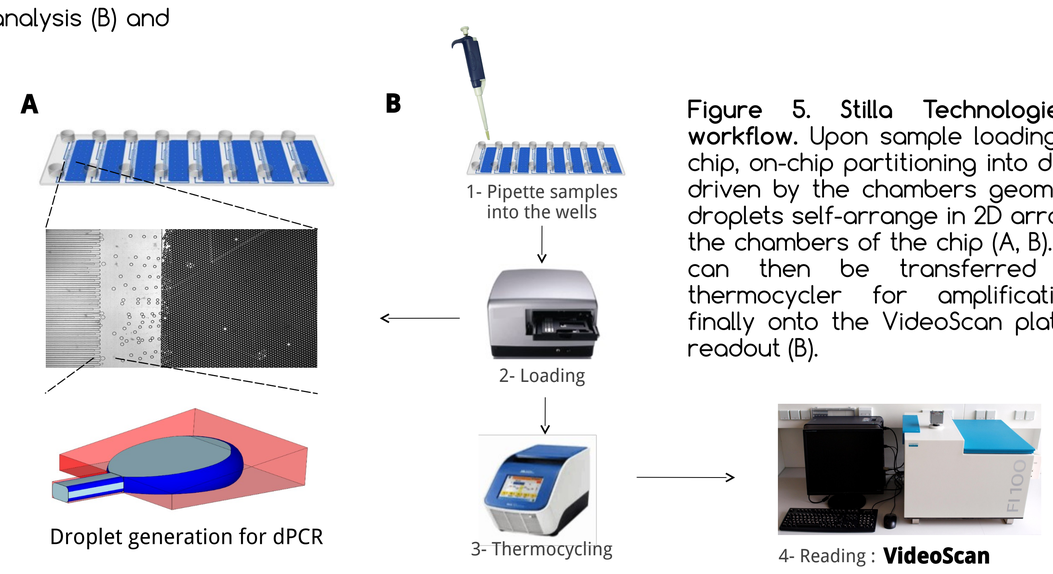

Figure 5. Stilla Technologies dPCR workflow. Upon sample loading into the chip, on-chip partitioning into droplets is driven by the chambers geometry. The droplets self-arrange in 2D arrays within the chambers of the chip (A, B). The chip can then be transferred onto a thermocycler for amplification, and finally onto the VideoScan platform for readout (B).

Discussion and Conclusion

In conclusion the VideoScan technology is a useful platform for various assays and can be used to build novel customized multiplex on a single platform.

Acknowledgments

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