A novel generation of digital PCR allows fast, convenient and accurate chimerism monitoring after hematopoietic stem cell transplantation

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Introduction

Monitoring of chimerism in patients after stem-cell transplantation is part of the surveillance of impending clinical relapse. Briefly, it consists of measuring the amount of stem-cell donor DNA in the blood of the receiver following transplantation. We previously established a method for chimerism monitoring based on detection of bi-allelic SNVs by Taqman™ assays on a chip-digital PCR (chdPCR) platform (Thermofisher)[1]. This method enabled reliable monitoring on nearly 2,000 clinical samples. However, substantial hands-on-time renders this platform less suitable for high sample throughput. We aimed to establish an alternative system to our approved chdPCR platform providing higher sample throughput and minimal hands-on-time.

Methods

The Opal™ dPCR chip (Figure 1A) represents the larger unit of the Naica™ crystal dPCR system[2] (Stilla) and allows SNV detection in approximately 20,000 droplets in each of the 16 reaction chambers of the chip. Crystal dPCR combines automated droplets formation (Figure 1B) and thermocycling on a single device (Naica™ Geode). Fluorescence signal of each droplet is measured in the Naica™ Prism instrument. With up to 3 chips or 48 reactions per run, the system represents a fast and economical alternative to other dPCR platforms.

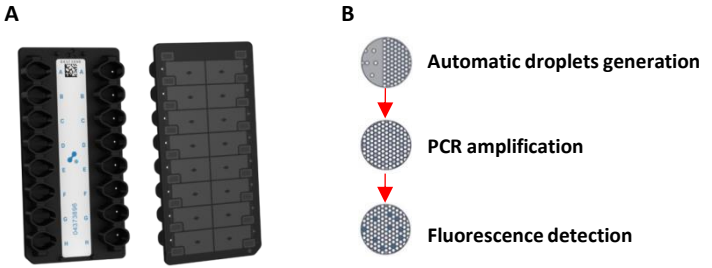

Figure 1: Opal™ dPCR microfluidic chip (Stilla). A. The larger unit of the crystal dPCR Naica™ system allows to perform up to 48 dPCR reactions simultaneously (16 per chip). **B. Crystal dPCR process.**

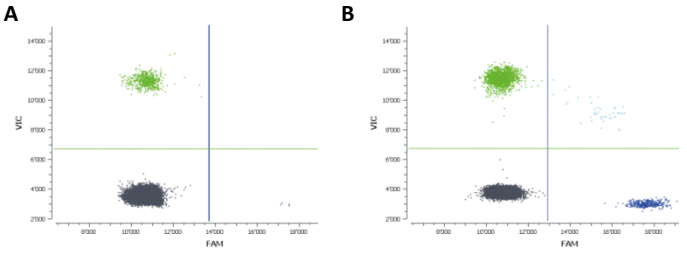

Figure 2: Crystal dPCR 2D-Plots for artificial DNA mixes. A. Analysis of 5ng of an artificial DNA mix: DNA A, homozygous for the allele G of the SNV rs1058396 (FAM-labelled in the specific Taqman™ assay, Thermofisher), was mixed with DNA B, homozygous for the allele A (VIC-labelled). The crystal dPCR system allowed for accurate quantification of 0.5% DNA A in DNA B. **B.** Analysis of 20 ng of artificial DNA mix with 16% DNA A in 84% DNA B.

Results

From the number of positive droplets and the total number of droplets, the crystal dPCR analysis software (Crystal Miner™) allows the calculation of the target (SNV) concentration in copies/µl thanks to Poisson statistics. With our routine experimental setup (20 ng input DNA) around 650 copies/µl is expected.

The analysis of artificial DNA mixes provided reproducible quantification of the minor allele, even below 0.25%. Notably, the clinically required 0.5% minor allele sensitivity was achieved with only 5 ng of input DNA per reaction (Figure 2).

In addition, the reliability of the crystal dPCR platform was confirmed on previously monitored patient's samples and several external proficiency testing (EQA) samples (Instand e.V.).

Conclusion

This new generation of dPCR is fully compatible with our established SNV-based chimerism monitoring and has been proven to be fast, convenient and highly accurate. In addition to minimal hands-on-time, and consumable use, the low amount of input DNA per reaction should be emphasized, opening the option of lineage specific chimerism monitoring.

References

[1]Gourri et al. Chip-based dPCR: an accurate and sensitive method for routine chimerism monitoring after HSCT. DGTI/DGI Annual conference 2016. [2]Madic et al. Three-color crystal dPCR. Biomol Detect Quantif. 2016 Dec; 10: 34–46.