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.

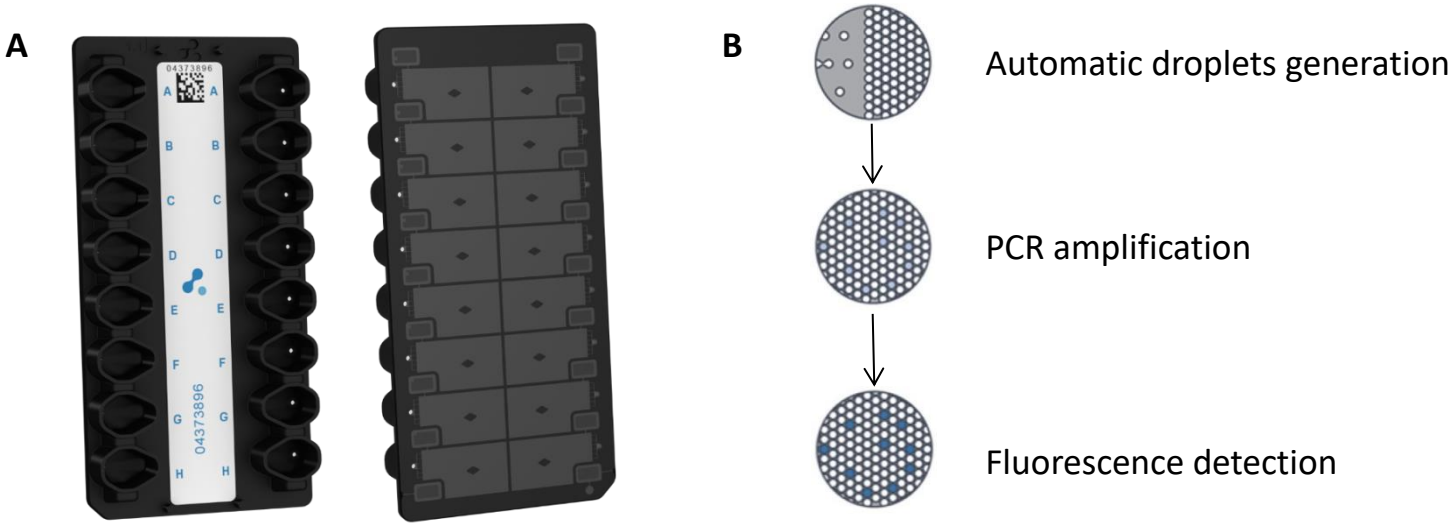

Figure 1: Opal™ dPCR microfluidic chip (Stilla). A. The larger unit of the crystal dPCR Naica™ system allows to perform up to 48 dPCR reactions simultaneously (16 per chip). **B. Crystal dPCR process.**

[1]Gourri et al. DGHO Annual conference 2016.
[2]Madic et al. Biomol Detect Quantif. 2016.

Results 1/2

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. We tested different amounts of input DNA per reaction and as foreseen, we observed an increasing amount of measured copies/µl with increasing amounts of input DNA. With our routine experimental setup (20 ng input DNA) around 650 copies/µl is expected and used as an internal quality control (Figure 2).

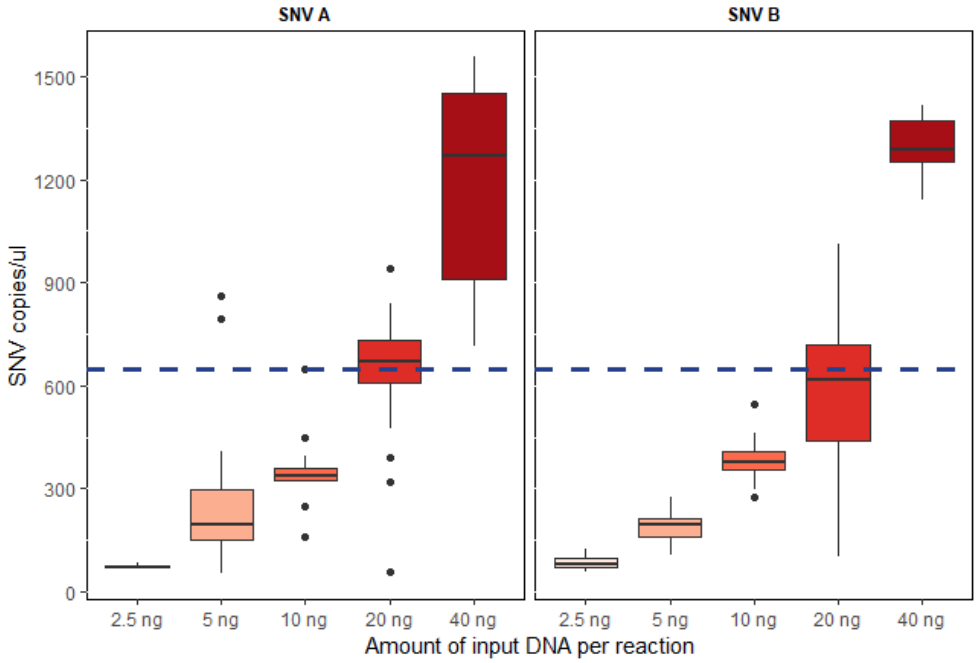

Figure 2: Total measured copies/µl per reaction with increasing amount of DNA per reaction. Taqman™ assays specific for 2 different SNVs were used in 16 replicates. As expected, we observed an increasing amount of detected copies/µl by increasing the amount of input DNA. Around 650 copies/µl are expected with our routine experimental setup of 20 ng input DNA per reaction.

Results 2/3

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 3).

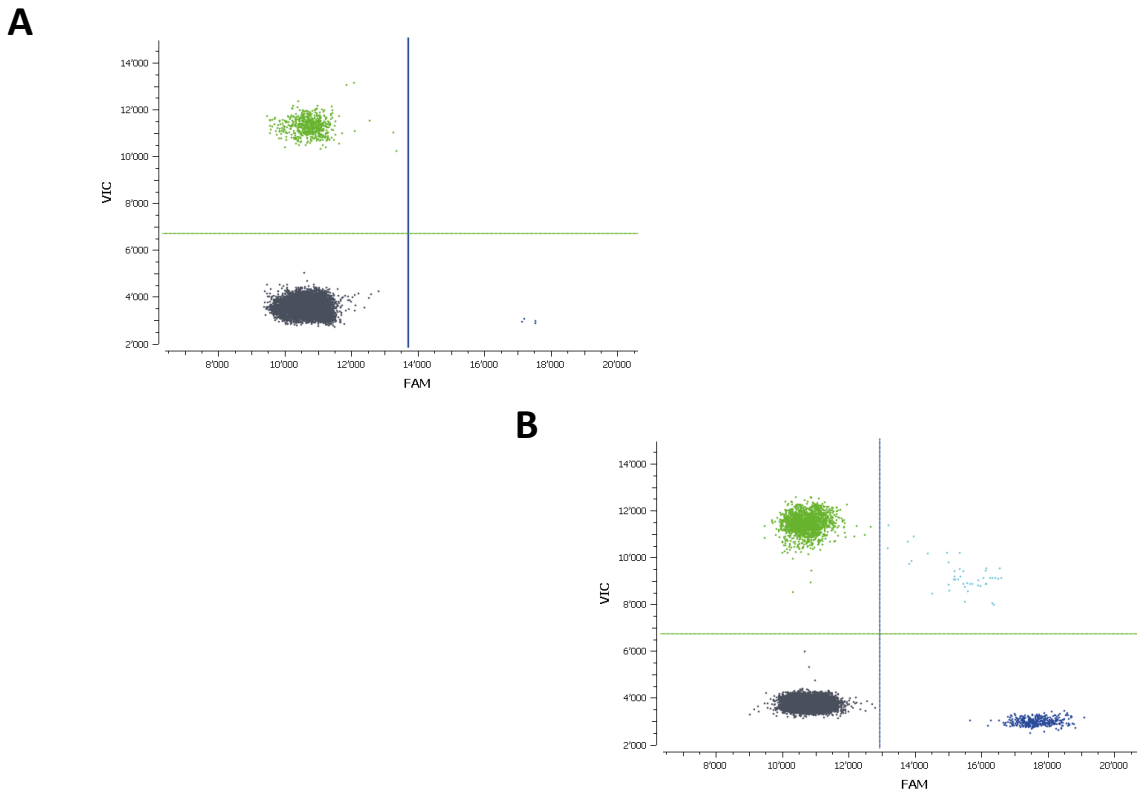

Figure 3: Crystal dPCR 2D-Plots for artificial DNA mixes. A. Analysis of 5ng of an artificial DNA mix: DNA A, homozygous for the allele G of the SNV rs1058396 (FAM-labelled in the specific Taqman™ assay, Thermofisher), was mixed with DNA B, homozygous for the allele A (VIC-labelled). The crystal dPCR system allowed 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 3/3

In addition, the reliability of the crystal dPCR platform was confirmed on previously monitored patient's samples and several external proficiency testing (EQA) samples (Table 1).

Sample	Expected Value	1s-range	Crystal dPCR Result
61 a	**0.00%**	0.00%-0.00%	**0.03%***
62 a	**3.96%**	3.47%-4.46%	**3.96%**
63 a	**6.86%**	5.92%-7.80%	**7.12%**
65 a	**57.00%**	54.21%-59.79%	**57.41%**

Sample	Expected Value	1s-range	Crystal dPCR Result
61 b	**2.85%**	1.64%-4.06%	**2.45%**
62 b	**4.77%**	3.16%-6.38%	**4.12%**
63 b	**9.22%**	6.59%-11.85%	**9.22%**
65 b	**78.10%**	67.60%-88.60%	**75.39%**

Table 1: Results of 2 EQAs from Instand e.V. Samples from 2 independant chimerism EQAs (artificial blood mixes) from 2017 (a) and 2018 (b) were analyzed in duplicate with 2 Taqman assays targeting SNVs. Results are given in percent of "receiver". Results within the 1s-range are awarded with the maximum grade. *0.03% corresponds to a negative control and would be reported as "absence of chimerism".

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. DGHO Annual conference 2016.

[2]Madic et al. Three-color crystal dPCR. Biomol Detect Quantif. 2016 Dec; 10: 34–46.

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