ENHANCED MIXED DONOR CHIMERISM ANALYSIS WITH DIGITAL PCR

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INTRODUCTION

With the need for early detection of relapse following allogeneic stem cell transplantation (allo-HCT), achieving heightened sensitivity in chimerism testing is paramount. While short tandem repeat (STR) amplification is currently the "gold standard" for chimerism assessment due to its reliable accuracy, its limited sensitivity (≥1%) poses a challenge. Digital PCR (dPCR) can offer high reproducibility across a wide dynamic range and increased sensitivity (≤0.1%). The aim of this study was to evaluate the accuracy and sensitivity of the dPCR platform-based chimerism assay in comparison to STR.

METHODS

Donor chimerism was evaluated using the dPCR platform QTRACE assay (JETA/Omixon), which was performed on the QIAcuity dPCR System (Qiagen). The effectiveness of QTRACE in determining the percentage of donor was evaluated using genomic DNA collected from recipients of allo-HCT and in admixtures. Chimerism results obtained from QTRACE was compared with those from conventional STR analysis (Promega).

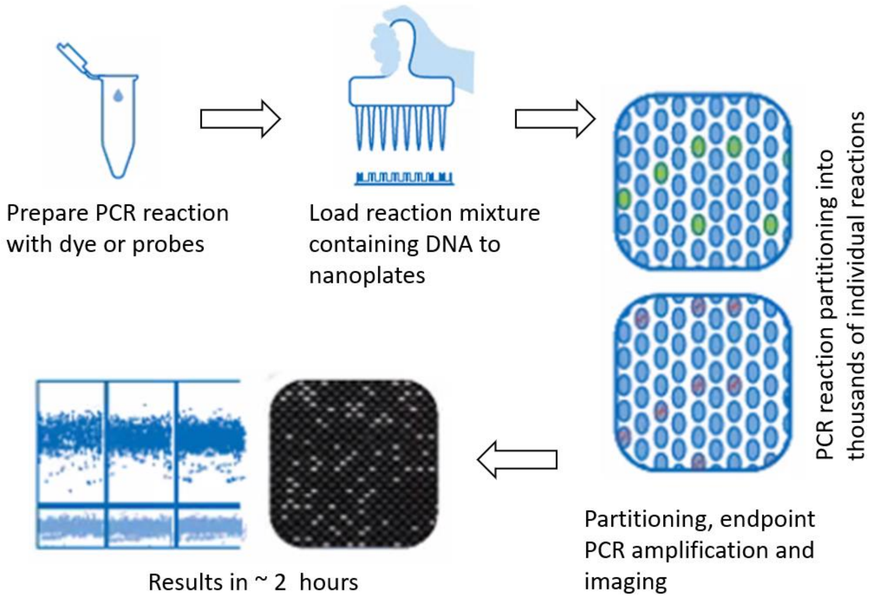

Figure 1. Principle and basic workflow for Qiagen dPCR platform. Nanoplates play a critical role in the dPCR platform. There are two main types of nanoplates: the 8.5K partition nanoplate and the 26K partition nanoplate, offering flexible resolution and throughput.

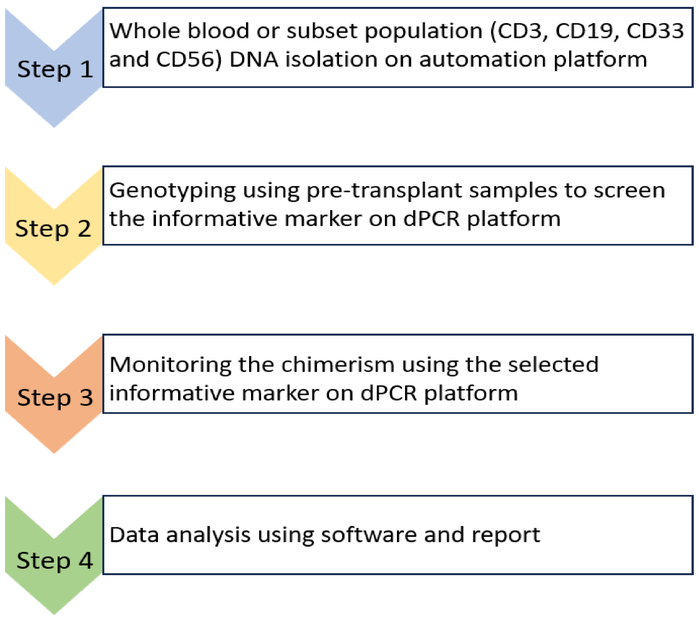

Figure 2. Workflow for dPCR-based chimerism detection assay. The assay includes chimerism markers located throughout the entire human genome. It is optimized for the Qiagen dPCR platform. Genotyping involves detecting pre-transplant samples using all markers, followed by selecting the informative markers. Monitoring involves using the informative markers to evaluate the percentage of donor cells.

Table 1. Comparison between dPCR and conventional STR assays

Mothed	Sensitivity	Marker type	Workflow	DNA input	Assay time	Cost
STR	1-5%	STR	informative marker selection; need sequencing setup; data analysis time-consuming	2.5-5 ng	~5 hours	$
dPCR	0.05% (8500 partitions); 0.01% (20,000 partitions)	SNP	informative marker selection; dPCR setup is relatively complex; data analysis easy	300 ng	~4 hours	$$

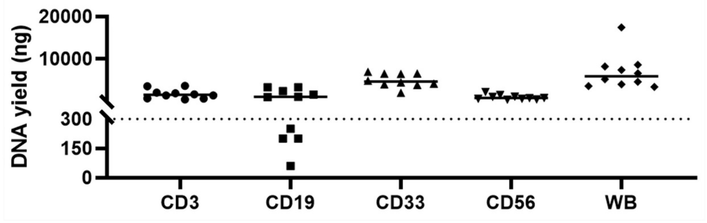

Figure 3. DNA yield from whole blood (WB) and subset population: CD3, CD19, CD33, and CD56.

RESULTS

The comparison between dPCR and STR platforms indicates that dPCR demonstrates promising ultra-sensitivity (0.01%) and straightforward software analysis (Figure 1, 2, and Table 1). However, it presents challenges such as requiring a higher input of DNA quantity, which is particularly problematic for the CD19 subset population (Figure 3), and entails higher costs compared to STR.

Nine samples, enriched for DNA isolated from CD3, CD19, CD33, and CD56 cells, underwent testing on both the STR and dPCR platforms. The results revealed strong concordance between the dPCR and STR methodologies (R= 0.99, Figure 4). Furthermore, the tests for ultra-sensitivity revealed the dPCR's reproducibility and precision for mixed chimerism analysis within the 0.01% to 1% range. These findings indicate a strong correlation (R=0.99, Figure 4) between the anticipated and observed chimerism levels, confirming the dependability and appropriateness of the dPCR platform for such assessments.

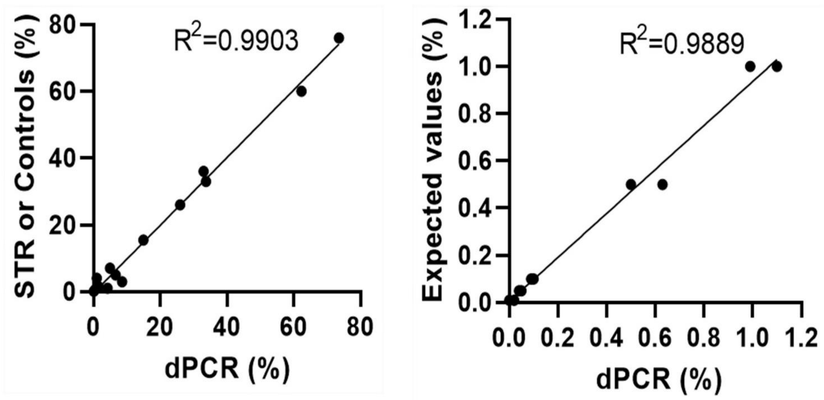

Figure 4. Ultra-low concentration artificial DNA mixture for dPCR detection. The study evaluated the performance of the dPCR platform in analyzing mixed chimerism using two sets of artificial mixes spanning concentrations (0.01%, 0.05%, 0.10%, 0.50% and 1.00%). These mixes were generated from DNA samples extracted from peripheral blood of both pre-transplant recipients and donors.

DISCUSSION

- Our results indicate that the dPCR based assay can provide comparable results to STR with higher sensitivity for chimerism testing.
- The ability to detect engraftment at increased sensitivity would benefit patients in early detection of relapse.