Development of a digital PCR-based chimerism monitoring assay

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Introduction

- Monitoring white blood cell chimerism after stem cell transplantation provides valuable insight into engraftment success.
- We developed a dPCR-based assay for chimerism monitoring, which offers various advantages over established techniques (Table 1).
- The assay is based on GenDx KMR, comprising the same 45 markers in 15 mixes, each containing three markers in different dyes (Figure 1).
- Quantification is calibrated on an internal reference marker in the Orange (TAMRA) channel.
- The assay performance was assessed in a proof-of-principle study by testing linearity and precision on two dPCR systems (QIAcuity and Absolute Q).

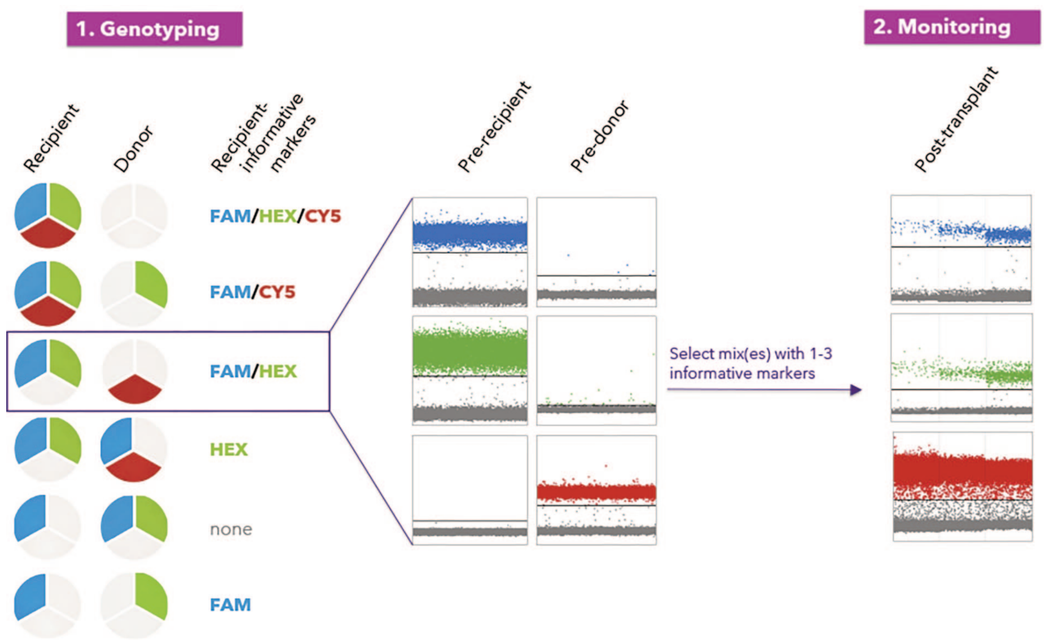

Figure 1. dPCR genotyping and monitoring. Multiple mixes are used for genotyping of donor and recipient to identify recipient-informative markers. At least one mix is selected for subsequent monitoring.

Table 1. Comparison of chimerism monitoring techniques

	dPCR	qPCR (GenDx KMR)	NGS	STR
Genotyping & monitoring	Single kit	Separate kits	Single kit	Single kit
Sensitivity	++	++	+	-
Quantification method	Absolute	Requires pre - transplant reference material	Relative	Relative
TAT	<3 hours	<3 hours	~1 day	Variable
Hands-on time	~20 minutes	~30 minutes	~75 min	Variable
Flexibility	++	++	-	Variable
Throughput	+	+	++	Variable

Methods

- The dPCR chimerism assay was performed by running the dPCR plate loaded with Master mix, Assay mix, DNA (150 ng/reaction) and – for the QIAcuity – XbaI restriction enzyme.
- To mimic chimerism samples, one single set of two cell-line samples was mixed at different percentages and measured using Mix1, Mix3, Mix5, Mix6, Mix10 and Mix13 on both systems. All informative markers were included in the analysis.

Linearity

- The three different dyes used in our dPCR-based assay are compatible for monitoring chimerism. (Figure 2).
- The different mixes show high mean linearity between expected and measured chimerism (Figure 3).
- Sensitivity of the assay was at least 0.04%, where 0.02% showed reduction in linearity.
- Linearity was shown for all tested markers on both systems, showing compatibility of our assay on QIAcuity and Absolute Q.

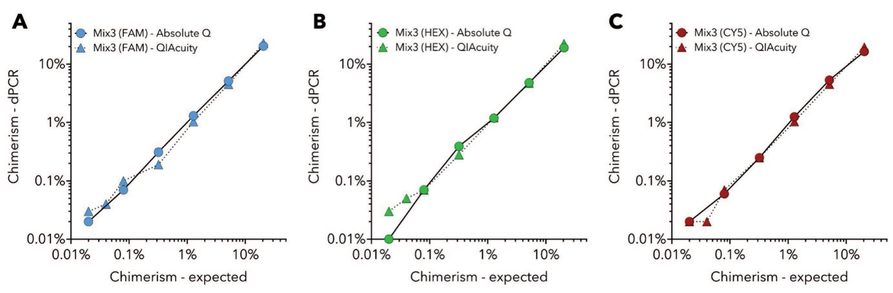

Figure 2. Linearity of (A) FAM, (B) HEX and (C) CY5 markers. Multiplex measurement of three markers shows linear relationship between measured chimerism and mixing percentage on the Absolute Q and QIAcuity. Mix3 is a representative example of all six mixes tested.

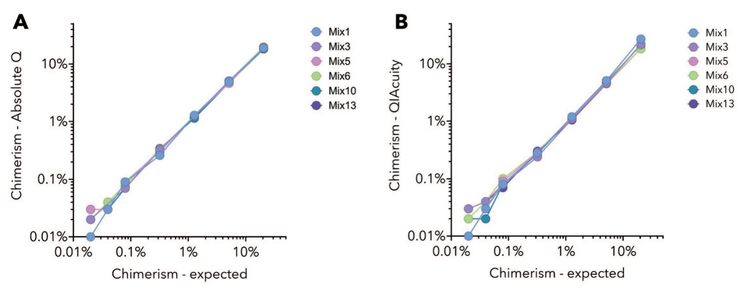

Figure 3. Linearity of assay mixes on (A) Absolute Q and (B) QIAcuity. For each Mix tested, a linear relationship is shown between the mean chimerism measured for the informative markers and the mixing percentage.

Precision

- High intra-marker precision was observed for all tested markers on both systems (Figure 4), showing marker reliability.
- The inter-marker variation is slightly higher than the intra-marker variation and will be optimized in downstream developments of the assay.

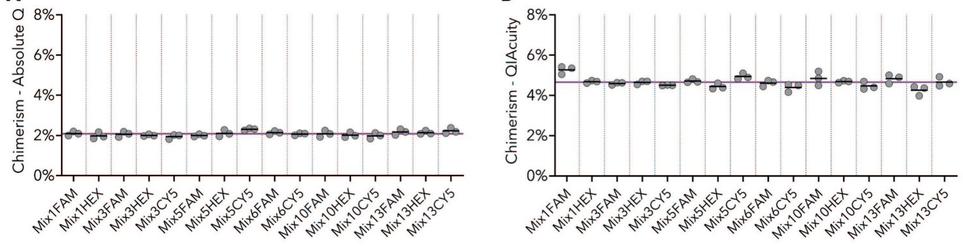

Figure 4. Precision on Absolute Q of a 2% sample (A) and of a 5% sample on QIAcuity (B). All measurements were performed in triplicate. The mean chimerism of all markers is shown as a purple line.

Conclusion

- In this proof-of-principle study dPCR-based chimerism monitoring was shown to enable fast and reliable quantification of three markers within a single mix.
- Accurate chimerism monitoring can be performed on the Absolute Q and QIAcuity systems down to chimerism levels of at least 0.04%.
- Further developments will focus on improving sensitivity and inter-marker precision.

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