Mentype® **DigitalScreen/DigitalQuant**

Assay Performance Data for Absolute Quantification of Chimerism Samples

Bio type® Diagnostic GmbH

Accurate and sensitive determination of the chimerism status is essential to detect engraftment failure, secondary graft rejection or disease relapse. Digital PCR (dPCR) combines excellent sensitivity with exact quantification and high reproducibility over a very wide measurement range[1]. In addition, dPCR is easy to perform and requires only small DNA amounts (50 - 100 ng) for routine chimerism monitoring. Based on Insertion/Deletion polymorphisms (herein called DIPs) a complete, easy-to-use dPCR system for highly sensitive and precise assessment of hematopoietic chimerism was established.

Mentype® **DigitalScreen** mediates the donor/recipient screening for informative loci. Mentype® **DigitalQuant** describing allele-specific duplex-assays allow highly sensitive and accurate chimerism monitoring. These duplex assays represent 29 FAM-labelled DIP-marker that are combined with either a HEX-labelled reference gene (DIP/REF) or a HEX-labelled Y-chromosome specific locus (DIP/SRY). Additionally, the combination SRY/REF was composed.

Performance and Sensitivity

All Mentype® **DigitalQuant** assays were optimized towards high specificity and sensitivity. Robustness against fluctuations of DNA input, as well as, PCR conditions, PCR cycles and assay performance on various PCR thermocycler were successfully improved. After this technical validation, dPCR assays uniformly showed accurate cluster separation together with high sensitivity (Fig.1).

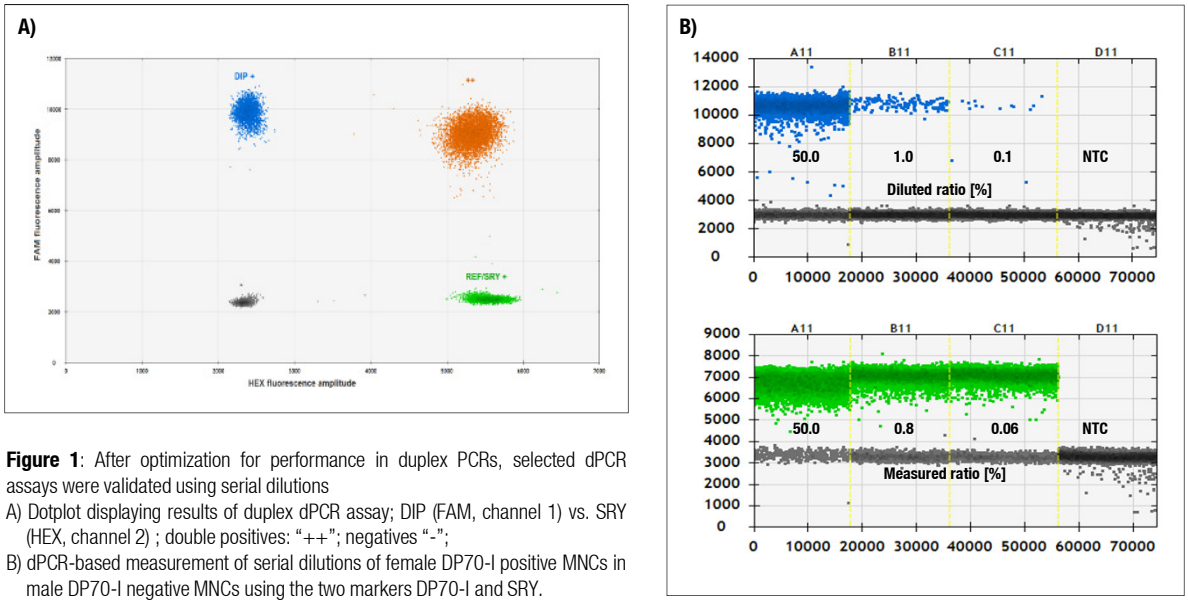

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Figure 1: After optimization for performance in duplex PCRs, selected dPCR assays were validated using serial dilutions

A) Dotplot displaying results of duplex dPCR assay; DIP (FAM, channel 1) vs. SRY (HEX, channel 2) ; double positives: "++"; negatives "-";

B) dPCR-based measurement of serial dilutions of female DP70-I positive MNCs in male DP70-I negative MNCs using the two markers DP70-I and SRY.

Reproducibility and Precision

Reproducibility and precision of the dPCR Mentype® **DigitalQuant** assays were tested in a parallel analyses (Fig.2). Irrespective of the used DIP marker Mentype® **DigitalQuant** assays showed an excellent performance over a broad range of concentrations. Importantly, the mixed chimerism status was accurately determined (Fig.2 and Fig.4).

Serial dilution [%]	Ratio with different marker [%]												Mean	Standard deviation
	70-D/R	70-D/Y	88-I/Y	114-I/R	114-I/Y	128-D/R	128-D/Y	131-I/R	131-I/Y	133-I/R	133-I/Y	152-D/Y		
0.1	0,14	0,16	0,12	0,1	0,12	0,12	0,16	0,15	0,14	0,12	0,19	0,13	0,14	0,025
1	1,2	1,2	1,2	1,2	1,1	1,3	1,1	1	1,1	1,2	1,2	1,2	1,2	0,078
50	50,7	52,7	51,5	50,6	45,5	51,3	52,2	46	49,7	47,2	52,9	50	50,0	2,514

Firgure 2: Parallel analyses of chimerism for one and the same DNA-sample using different dPCR Mentype® **DigitalQuant** assays; "D" means deletion; "I" means insertion; "R" means reference (REF), "Y" means SRY;

[1] Stahl et al., Digital PCR to assess hematopoietic chimerism after allogeneic stem cell transplantation; Experimental Hematology 2015;43:462–468

Comparative Analysis to Determine the Clinical Value of dPCR in Chimerism Analysis

Various techniques have been developed for chimerism analysis. However, while the classical PCR-based short tandem repeat (STR) approach has sensitivities ≥1%, the quantitative PCR (qPCR)-based technique combines its high sensitivity with limited resolution power in the state of mixed chimerism due to methodological peculiarities. Digital PCR overcomes this limitation through technological advantages. Fig. 3 depicts the accuracy of dPCR-based Mentype® **DigitalQuant** assays particularly for the important situation of mixed chimerism.

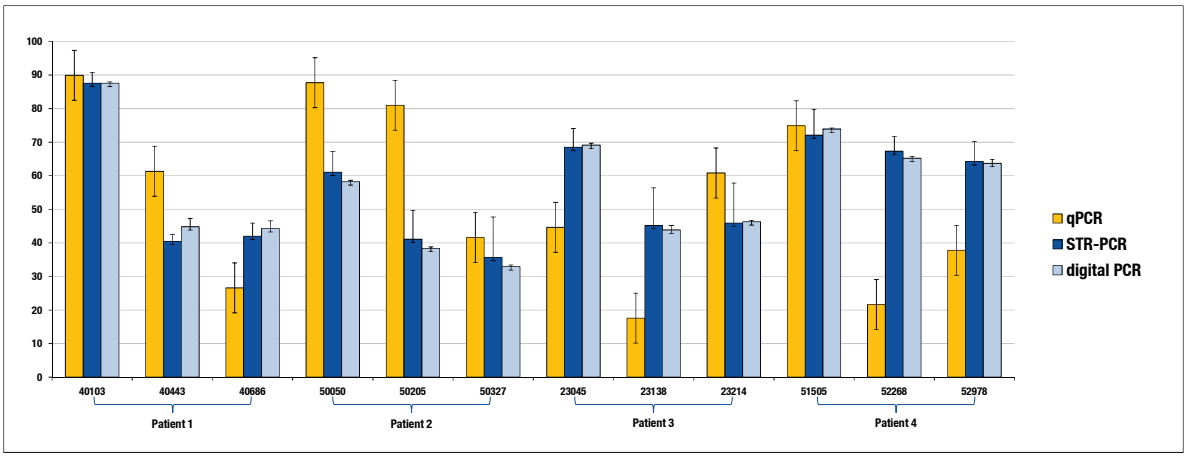

Figure 3: Data from 3 monitoring samples (post-Tx) of four patients were analyzed with STR-PCR, qPCR and dPCR. Donor fractions were calculated. Comparison of the three methods shows derivations in cases of mixed chimerism between qPCR and the results of dPCR and STR-PCR.

dPCR Performance in Chimerism over a Wide Range of Values

The most frequently used method to asses chimerism is the mulitplex-STR analysis. This method provides a high power of discrimination and analyse multiple loci simultaneously. To asses the clinical value of the newly developed Mentype® **DigitalQuant** assays 148 monitoring samples (post allo-SCT samples of 21 patients) were analysed with dPCR duplex-assays and compared with data obtained from STR analysis (Mentype® **Chimera**®). Data were analyzed for linear regression of % donor chimerism. A high degree of correlation (r^2=0,98) was observed (Fig. 4) . Further, investigating 176 patients for informative loci with 98,9 % the assays showed an excellent power of discrimination.

Conclusion

Mentype® **DigitalQuant** assays combine reliable performance with a very good sensitivity. The 29 DIP-Loci either run in a duplex-reaction with SRY or a reference gen. Addressed DIP-loci are distributed over 15 chromosomes.

Notably, dPCR enables accurate absolute quantification without the need of standard curves and replicate measurements. All assays were optimized for uniform conditions with high specificity and good assay performance.

Mentype® **DigitalScreen** allows initial genotyping and easy DIP assessment for every-day diagnostics.

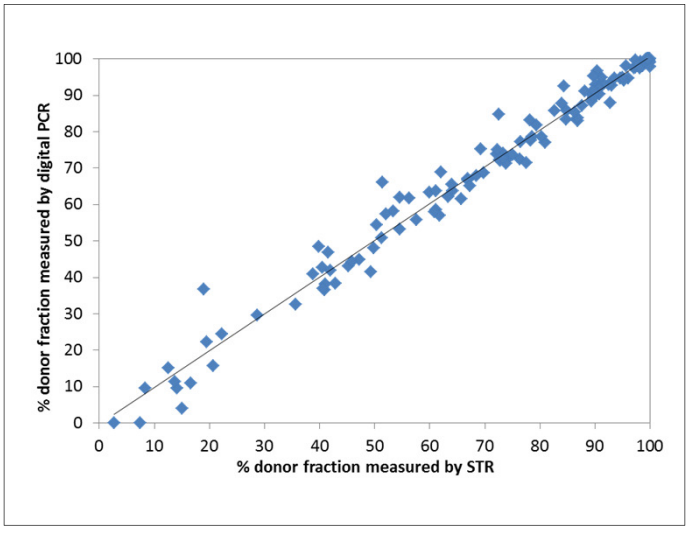

Figure 4: Correlation of chimerism data as obtained on clinical samples by dPCR vs. STR analyses. Chimerism was assessed for 148 monitoring samples post HSCT by both dPCR and STR analyses.

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