Method optimization for the detection of chimerism by real-time PCR and droplet digital PCR

Ioana Lambrescu[1,2*], Victor Ionescu[1*], Gisela Gaina[1,3], Alexandra Popa[1], Cristina Niculite[1,2], Valeriu Cismasiu[1]
1. Cell Biology, Neurosciences and Experimental Myology Laboratory, Victor Babes Institute of Pathology, 050096 Bucharest, Romania
2. Department of Cellular and Molecular Biology and Histology, Carol Davila University of Medicine and Pharmacy, 050474 Bucharest, Romania
3. Department of Biochemistry and Molecular Biology, University of Bucharest, 030018 Bucharest, Romania

*Equal contribution

Background

Objective: The selection of combinations of primers for genetic polymorphisms (INDELS) used in the analysis of chimerism associated with post-transplant hematopoietic regeneration as well as to test them under droplet digital PCR (ddPCR) and real-time PCR conditions.

Methods

Allele-specific assays were designed based on the Ensembl Variation database. Multiple rounds of optimisation were applied in order to obtain the lowest attainable analytical limits, confirmed by standard curves.

Results

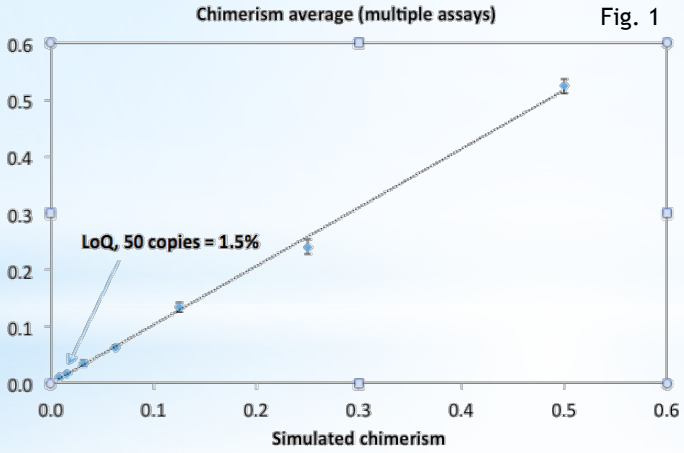


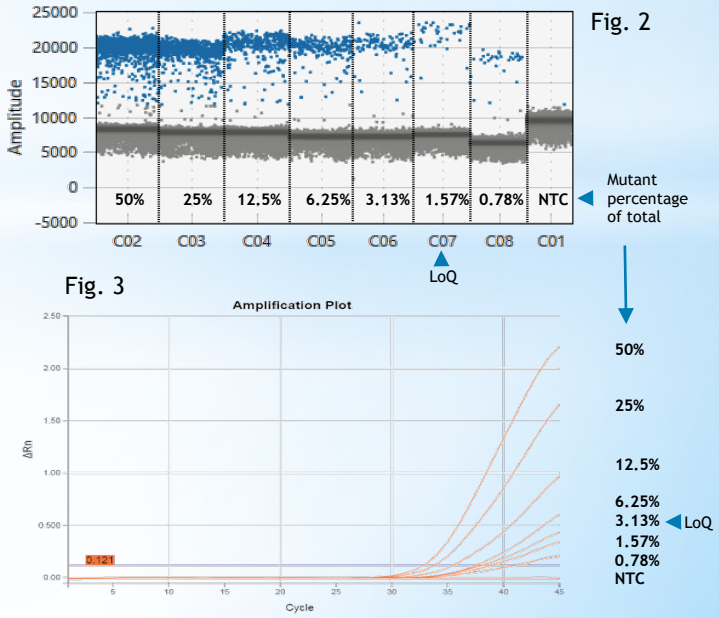
Fig. 1

Fig. 1. Simulated and obtained chimerism levels showed good correlation between 50% and 1.5% (R^2=0.998), LoQ 50 copies.

Conclusions

The differences between positive and negative samples are more clearly determined in the case of ddPCR, which confirms that this method is more accurate than the qPCR method.

Fig. 2. For ddPCR increasing primer concentration determines the increase of the amplitude difference between the negative and the positive droplets without affecting the allele specificity. Fluorescence plot for a standard curve in ddPCR.
Fig. 3. Amplification plot showing qPCR serial dilution performance.



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