digitalTRACE™
dPCR Chimerism Monitoring

dPCR CHIMERISM ANALYSIS

TECHNICAL SUPPLEMENT

DIGITAL PCR USES SAMPLE PARTITIONING FOR EXCEPTIONAL SENSITIVITY

The methodology we call digital PCR (dPCR) today was first described in early 1990´s in an attempt to quantify DNA targets using limiting dilution *(Simmonds et al., 1990, Sykes et al., 1992)*. In 1999, Vogelstein and Kinzler suggested the term "digital PCR" for transforming the exponential (analog) PCR signal into a linear (digital) readout using fluorescence. The crucial step of dPCR consists in **subdividing the sample into thousands of subnanoliter-sized partitions**, allowing the PCR reaction and absolute quantification to be carried out in each partition individually. In this way, dPCR can detect low-abundant templates in the background of major DNA component with an exceptional sensitivity.

THE PARTITIONING OF THE SAMPLE PRIOR TO dPCR ENABLES HIGHLY ACCURATE, PRECISE, AND SENSITIVE MEASUREMENTS

THE POWER OF POISSON STATISTICS FOR AN ACCURATE QUANTIFICATION

S.D.POISSON

To quantify the amount of target molecules in the partitioned sample, Poisson statistics are applied to correct for the possible presence of multiple targets in one partition.

The number of molecules per unit partition is estimated from the fraction of negative partitions. This estimate is then divided by partition volume to obtain a measure of the concentration.

DIGITAL PCR AS AN EXCELLENT TOOL FOR CHIMERISM ANALYSIS

Accurate and sensitive quantification of chimerism after HSCT is a key to an effective therapy strategy and dPCR is a valuable tool for chimerism analysis, combining high sensitivity, accuracy and precision. Multiple studies have shown the usefullness of dPCR chimerism monitoring *(George et al., 2013; Stahl et al., 2015; Mika et al., 2019)*. While the sensitivity of classical approaches such as STR-PCR is limited to 1%-5%, dPCR allows to quantify DNA mixtures **down to 0.01% minor component** with the potential to go even lower. Absolute quantification using dPCR does not require a reference sample, and other advantages of dPCR chimerism analysis are lower DNA sample input or the possibility of multiplexing. More importantly, it has been shown that dPCR can predict relapse on average **17.5 days earlier** when compared to quantitative real-time PCR *(Valero-Garcia et al., 2019)* and this substantial amount of time can play a role in making major therapeutic decisions.

To avoid the (rare) possibility of false negative results due to genomic instability of the leukemic cells, a minimum of two markers located on different chromosomes should be used for a reliable and accurate dPCR chimerism quantification.

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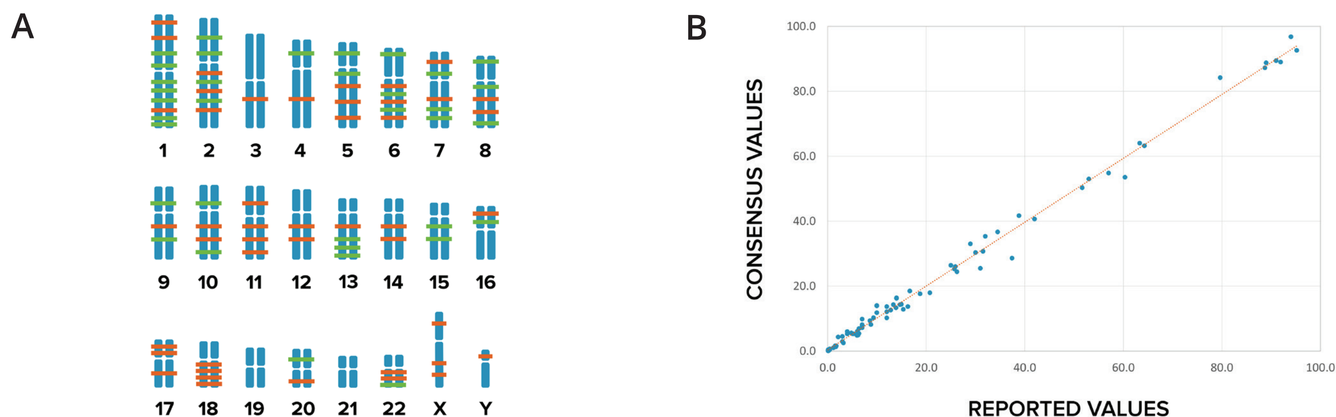
DigitalTRACE™ ASSAYS – SENSITIVE AND ACCURATE CHIMERISM QUANTIFICATION

The **DigitalTRACE™ Assays** combine high accuracy and outstanding sensitivity in dPCR chimerism analysis. The whole panel comprises 80 dPCR INDEL Assays and this allows for an easy selection of informative markers even in case of closely related donor/recipient couples.



A) THE CHROMOSOMAL LOCATION OF THE 80 DigitalTRACE™ MARKERS (ORANGE-STANDARD PANEL; GREEN-EXTENDED PANEL).
(B) THE CHART DISPLAYING REPORTED VS. CONSENSUS VALUES (N=82) IN EXTERNAL PROFICIENCY TESTING (EPT) TRIALS 2020-2022, INCLUDING UK NEQAS, ASHI AND SFHI CHIMERISM EPT, DOCUMENTS THE HIGH PERFORMANCE OF THE DigitalTRACE™ ASSAYS.

The DigitalTRACE™ Assays show excellent correlation with quantification results obtained by qPCR, but with an outstanding precision and accuracy over a broad range of target DNA quantities.

The workflow is flexible and enables the choice from both qPCR and dPCR techniques to determine the genotype of the DNA samples. The quantification step is performed using dPCR. All assays are formulated with a Reference Gene (RNase P) Assay which allows for the absolute quantification of the target DNA. The DigitalTRACE™ Assays are validated for the major dPCR platforms.

A range of **DigitalTRACE™ HLA Assays** is available for the monitoring of the HLA loss events by dPCR.

TRACE Analysis™ Software is designed for performing analysis of chimerism through an intuitive user interface. The software guides the user through assay setup, performs data analysis, generates reports and stores the data collected for samples over time.

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