Accurate and Efficient Chimerism Determination Using a SNP-Based Chimeric ID Panel

Keith Jackson, Sara Hummel, Aleksey Nakorchevsky, Theresa Kelly, Robin E. Everts, Darryl Irwin

Agena Bioscience, 4755 Eastgate Mall, San Diego, CA 92121, USA

INTRODUCTION:

Recipients of allogeneic hematopoietic stem cell transplants (HSCTs) require clinical monitoring to allow for early diagnosis of post-transplant adverse events such as rejection, graft vs. host disease or malignancy relapse. Triaging of transplant recipients in a clinical setting is commonly achieved either by Minimal Residual Disease (MRD) monitoring or via testing and performing chimerism analysis on post-transplant specimens to determine the genetic contribution from the transplant recipient and the donor. While MRD monitoring involves detection of malignancy-specific markers, measuring the chimerism can be achieved via general PCR-based techniques. The most commonly used methods for monitoring chimerism in post-transplant samples are based on analysis of short tandem repeats (STRs). However, assay setup and data analysis remain complicated and time-consuming processes.

METHODS

Assay Design: The Chimeric ID panel is a highly multiplexed SNP-based chimerism determination panel developed by Agena Bioscience. The panel leverages the iPLEX Pro chemistry and is processed using the MassARRAY system. The panel consists of 92 independent (absence of linkage disequilibrium) SNPs with minor allele frequency (MAF) of 0.45-0.5 across major HapMap populations including ASW, CEU, CHB, GIH, JPN, and MEX. The 92 SNPs are multiplexed into 8 wells. The panel includes only A<>T and C<>T transitions as these result in the highest mass differences and highest quality data. The informative SNPs will vary for different donor/recipient combinations. 92 SNP markers with high MAF provides the panel with sufficient power to compare related and unrelated individuals. (Figure 1).

Software Design: The Chimeric ID Panel is accompanied by a reporting software that automatically analyzes recipient/donor pre-transplant profiles, determines which SNPs are informative, stores the profile for future reference and leverages the archived profile to calculate percent recipient/donor contribution in post-transplant follow-up specimens. By detecting peak height at each informative SNP, the algorithm calculates the composition of the sample and assigns a Z-score value which represents the confidence level in the call. These values are analyzed, and a final result is displayed in an easy to interpret report (Figure 7).

Summary of key software features:

- Automatic analysis of recipient/donor pre-transplant profiles to identify informative SNPs
- Archive functionality saves pre-transplant profiles, so they only need to be run once
- Recipient/donor contribution in post-transplant follow-up specimens is calculated in seconds
- All results displayed in easy to interpret reports
- Historic results for a given recipient can be easily recalled and displayed in an intuitive report
- Multiple donor analysis

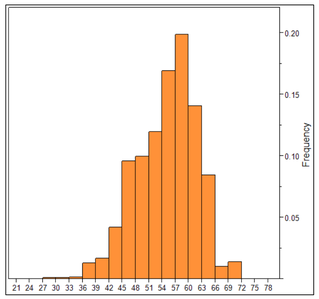

Figure 1: Experimentally Determined Number of Informative Markers for Pairwise Comparison of Unique HapMap Samples

METHODS

Samples Tested: Analytical validation studies were performed across a wide range of contribution levels using contrived samples. These samples were created by extracting gDNA from whole blood drawn from male and female donors. Unmixed male and female DNA pairs were used to generate pre-transplant profiles. Mixtures were created to mimic post-transplant follow-up specimens at various contribution levels. Natural variability in extracted DNA concentration, fragmentation and dilution accuracy made it difficult to accurately create mixtures at the intended target contribution levels. To ensure that chimerism results were being compared to an accurate representation of the mixture composition, ddPCR X/Y chromosome analysis was performed to verify the percent recipient and donor contribution in each dilution.

Experiments: Experiments were done to determine the Chimeric ID Panel's accuracy, reproducibility, limit of detection, tolerance to DNA input levels and performance compared to STR-based chimerism methods.

- ✓ **Accuracy** – How close are the Chimeric ID results to the experimentally determined "truth"?
- ✓ **Reproducibility** – Does the Chimeric ID Panel return similar results each time a sample is analyzed?
- ✓ **Limit of detection** – At which minor contribution levels is the Chimeric ID panel able to reliably distinguish between "pure" unmixed DNA and low-level contribution from the recipient/donor? See Figure 2
- ✓ **DNA input tolerance** – What is the panel's optimal DNA input range? See Figure 5
- ✓ **Comparison to STR** – Does the panel give similar results to STR-based methods? See Figure 6

Conclusion: These results paired with streamlined data analysis and assay setup efficiencies make the Chimeric ID Panel a viable alternative to STR-based chimerism methods.

RESULTS

Results: The panel was tested on samples with a range of chimerism levels and showed excellent accuracy (0.8% average variance from truth) and reproducibility (0.65% Standard Deviation) at wide gDNA input levels. In addition to its performance for single donor samples, the Chimeric ID panel can also monitor multiple donors per transplant.

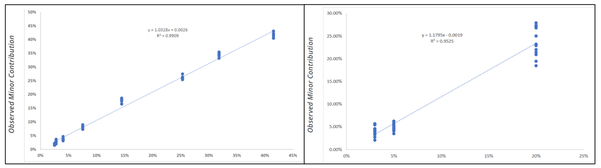

Figure 2: Single Site (L) and multiple site (R) Accuracy Study showed overall very high concordance to expected dilutions. The single site (2 dilutions, each 8 times) showed an r^2 of 0.99, and the multi-site comparison (3 labs and 3 dilution series) showed an r^2 of 0.95.

Sample	Replicates	No Chimerism Detected	Chimerism Detected	% Identified as Mixed
1%	12	10	2	16.7%
2%	12	2	10	83.3%
3%	13	0	13	100%
5%	13	0	13	100%
"Pure"	48	48	0	0%

Figure 3: Limit of Detection showed all samples were detected down to 3% contribution levels.

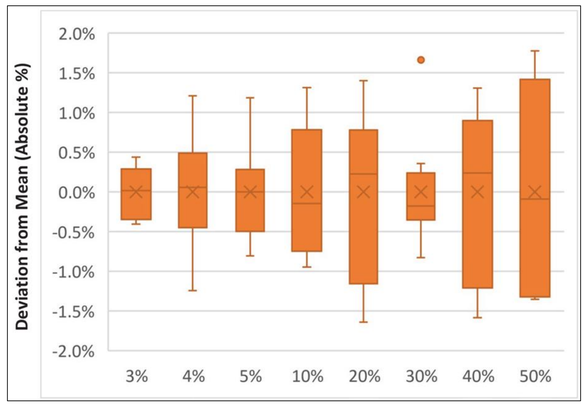

Figure 4: Eight independent runs of two different dilution series each showed excellent reproducibility with a variance from the truth of 1.3%.

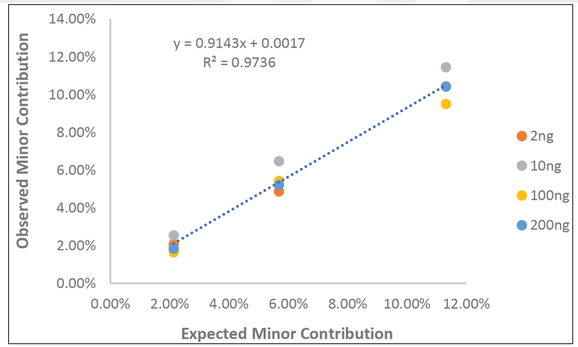

Figure 5: Single Site Accuracy Study Results using different amounts of input DNA per reaction shows overall high concordance to expected results (r^2 = 0.97).

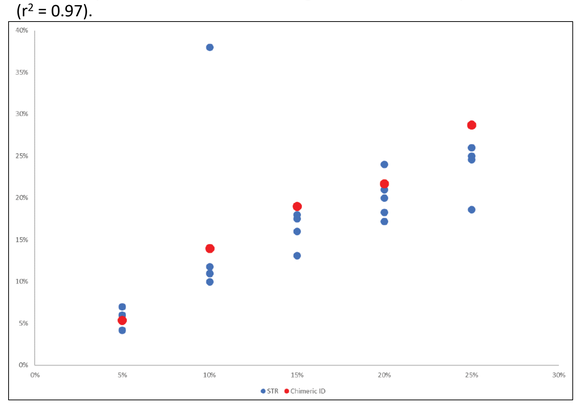

Figure 6: DNA samples were sent out to 4 different labs for STR analysis. As can be seen STR and Chimeric ID show very similar outcome.

BME CHIMERISM ID
Software Version: 1.0.14

BME Chimerism Report
Min Avg Zscore for Chimerism Call: 2
Min Recipient % for Chimerism Call: 1%
Discordant 'Chimerism Detected' threshold: 5%

Patient Group ID: FAM003

SAMPLE ID:	3-CD3	
DATE:	2019-08-19 18:56:48	
RESULT:	Chimerism Detected	
Z SCORES:	Z Score: 127.9 / Z Score Hmzg: 248.8 / Z Score Htzg: 7.1	
	ID	%
DONOR	3-D	41.3 %
RECIPIENT	3-R	58.7 %

Figure 7: Example of a report output for a specific sample set. User can indicate Patient ID and software will pull the recipient and donor data from the database for automated analysis.