Comparison of Chimerism Determination Using a SNP-Based Chimeric ID Panel vs STR-based assays

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INTRODUCTION:

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. Here we present a comparison of the Chimeric ID panel vs the STR technology

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 the 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 5).

Conclusion:

✓ These results show the Chimeric ID Panel is a viable alternative to STR-based chimerism methods.

The associated software streamlines analysis and reduces the time required to report results.

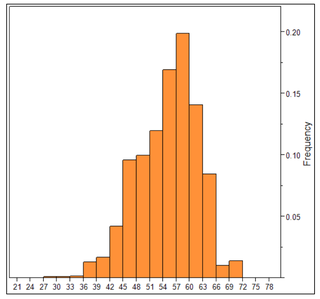

Figure 1: Experimentally Determined Number of Informative Markers for Pairwise Comparison of Unique HapMap Samples

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

METHODS

Samples Tested: Six sets of samples (N=32), each consisting of a donor, a recipient, and one or more post-transplant samples were collected and extracted. Post-transplant samples spanned peripheral blood (PBL), bone marrow (BN), CD15, or CD34 selected cells.

Procedures:

- The Chimeric ID panel (Agena Biosciences, San Diego, CA) was processed on these samples according to standard protocols (Figure 2).
- STR panel was performed according to standard protocol using the PowerPlex 16 HS kit (Promega, Madison, WI).

Data analysis:

After the Chimeric ID Panel biochemistry was completed, the Chimeric ID software was launched and a chimerism report generated.

For STR analysis, at Hackensack University Medical Center, each individual set of samples was analyzed using GeneMapper ID. Informative alleles were identified by manual comparison the STR profile between host and donor for each case. The informative alleles were then used in post-transplant specimen (peripheral blood, CD3, and CD15 sorted cells and bone marrow, CD34 sorted cells). Proportion of donor and recipient cells were calculated manually based on the area of the peak.

RESULTS

The Chimeric ID panel was highly concordant with the STR data (Figure 6) showing an r^2 of 0.98.

For the FAM003 set, the software picked up the recurrence of recipient DNA in CD3 positive cells, confirmed by the STR data (Figures 4 and 5)

Figure 2: Basic workflow for the Chimeric ID panel

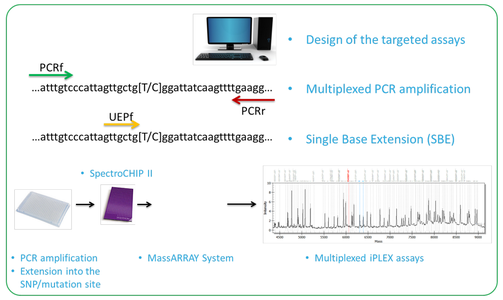

Figure 3: Chimeric ID software allows easy indication of sample type (donor, recipient or post-transplant, NTC or control). Patient Group ID is unique and is used to identify pre-existing donor and recipient data allowing the database retrieval of these data. Data can be typed into the window or uploaded using a pre-existing csv file

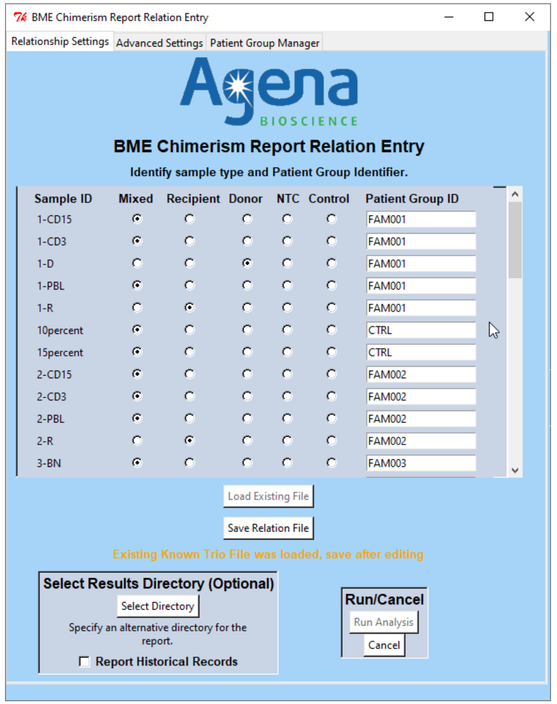

Figure 4: Using Typer spectrum overlay, clearly shows donor as C/C and recipient sample as C/T. In CD3 cells there is a relapse as the T allele (green) comes up higher. Chimeric ID software will automatically perform these analyses for each SNP assay and calculates the % recipient and Z-score.

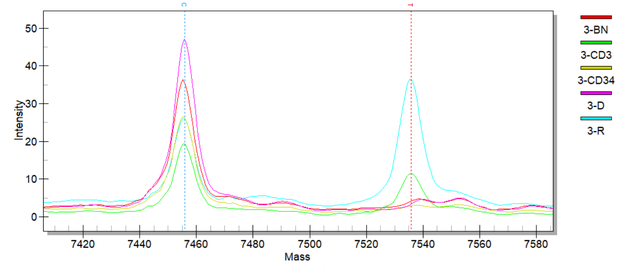

Figure 5 : Example of the report output for a specific sample set. User can indicate Patient ID and software will pull the recipient and donor data from the database for automated analysis.

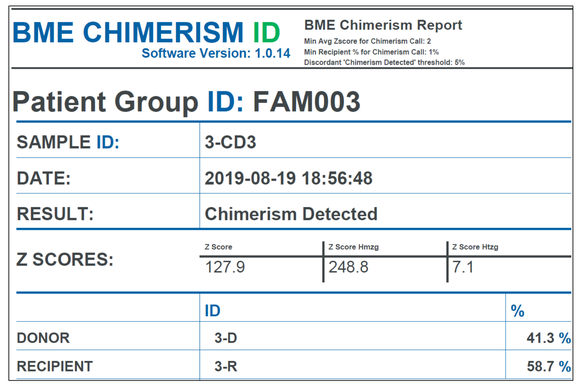

Figure 6: A comparison of STR-based analysis vs the Chimeric ID shows a high degree of similarity in outcome.