Intra-Laboratory Evaluation of a Next-Generation Sequencing-Based Solution for Chimerism Monitoring.

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Introduction

Chimerism testing is a critical tool for monitoring engraftment and diagnosing disease relapse in hematopoietic cell transplant recipients. However, the most widely utilized testing method (short tandem repeat analysis) suffers from a low level of sensitivity, high variability, and cumbersome analysis. These shortcomings have hindered progress towards standardizing the approach to chimerism testing as well as clinical interpretation of results.

In this study, two external test sites evaluated well characterized DNA sample mixes with the AlloSeq HCT assay, a low input next generation sequencing (NGS) chimerism test that analyzes 202 single nucleotide polymorphisms distributed across all autosomal chromosomes via a highly streamline workflow. Results were rapidly generated (sample to answer in less than 24 hours) via an automated analysis pipeline and evaluated for accuracy, sensitivity, and precision.

Methods and Materials

Chimerism Quantification Assay

The concept of the CareDx AlloSeq HCT assay is centered around a single multiplex PCR followed by sequencing to determine the fraction of donor-specific nucleotides at 202 selected SNP loci, allowing the relative quantification of donor's DNA relative to recipient's DNA (figure 1).

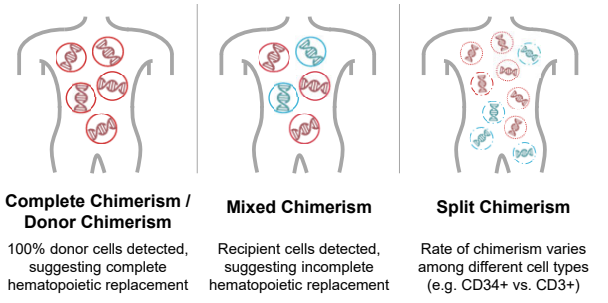
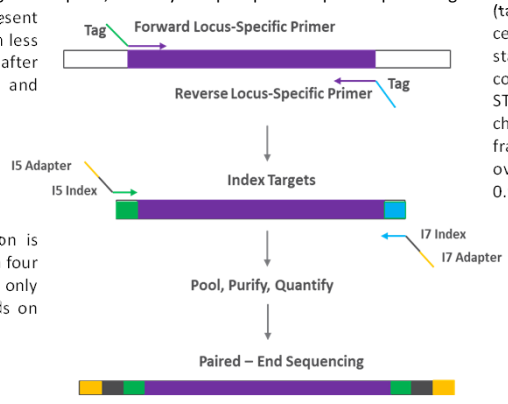


Figure 1. typical graft immune cell fraction compositions

The kit includes a locus-specific oligonucleotide primer pool to amplify the targeted regions of interest. In combination with index adapters, a unique cycling protocol amplifies the locus-specific regions and indexes the libraries concurrently. Indexed samples are subsequently pooled and purified together to prepare for sequencing.

After sequencing is complete, an analysis report provides precise percentage of chimerism present in each sample, in less than 24 hours after sample receipt and DNA extraction.



Library preparation is completed within four hours of which only one hour is hands on (figure 2).

Figure 2. Library preparation workflow

Analysis Software

Results are generated automatically by AlloSeq HCT software from the sequencer-generated FastQ file. Multiple recipients can be analyzed in parallel, but the AlloSeq HCT Windows software design also allows monitoring Chimerism levels in a same recipient over time. During monitoring, one or more samples are taken post-transplant. The software calculates Chimerism levels from these post-transplant samples and displays the results in a report to compare levels from different samples to support building longitudinal studies.

Results

In this study across two independent laboratories, evaluation was initially conducted on 6 artificial gDNA blends to mimic gDNA samples isolated from whole blood from patients who received single or double Cord Blood transplants. Samples were tested at 10ng input per sample for library preparation.
Results showed a high correlation between the chimerism determined by both laboratories, with less than 0.05% difference observed between measurements (Table 1).

Table 1.

Sample	Donor(s)	% Recipient	% Donor 1	% Donor 2	Avg % recipient measured		
					Geneva	Rush	Delta
Alpha	1	0.34%	99.66%	NA	0.43%	0.44%	0.01%
Beta	1	0.21%	99.79%	NA	0.22%	0.22%	0.00%
Gamma	1	0.33%	99.59%	NA	0.34%	0.29%	0.05%
Delta	1	0.35%	99.65%	NA	0.39%	0.35%	0.04%
Epsilon	2	0.53%	9.17%	90.30%	0.54%	0.59%	0.05%
Zeta	2	0.46%	0.83%	98.71%	0.52%	0.55%	0.03%

A second set of 8 post HCT samples from peripheral blood were taken at the same time point for cellular subfraction and isolation of CD15+, CD33+, granulocytes (GR) and monocytes (MN) cells prior to genomic DNA (gDNA) extraction. For each recipient, donor gDNA was input as a reference sample. QC and data analysis for the evaluation of percent chimerism (median ± CI 95%) was performed automatically by the provided software. Results below limit of detection (LOD of 0.32% for NGS and 3% for STR) were excluded in the Mann-Whitney and Spearman correlation analyses. For inter-assay variation 3 subfraction samples with results along the dynamic range were repeated 2-3 times and the coefficient of variation (CV) was calculated.
CD3+ cells dominated graft cellular composition with a chimerism of 31.6% (10.4 to 44.3%), followed by MN cells at 10.0% (1.4 to 18.6%) and GR cells at 7.3% (0.56 to 13.9%), and CD15+ was the smallest fraction with 2.9% (-4.0 to 9.8%) (table 2). Median levels of chimerism in each cellular subfraction of the grafts were not statistically significant different when comparing the NGS solution AlloSeq HCT and STR (p>0.5) (figures 3.A and 3.B) and chimerism results obtained for each cell fraction using both methods exhibited an overall correlation of r=0.96 (CI 95%: 0.88 to 0.98) (figure 3.C).

Table 2.

	AlloSeq HCT	STR	Mann-Whitney
CD3+	31.6% (10.4 to 44.3%)	26.7% (13.8 to 39.5%)	n.s
MN	10.0% (1.4 to 18.6%)	11.1% (1.3 to 21.0%)	n.s
GR	7.3% (0.6 to 13.9%)	8.8% (2.6 to 15.1%)	n.s
CD15+	2.9% (-4.0 to 9.8%)	6.8% (-3.0 to 16.7%)	n.s

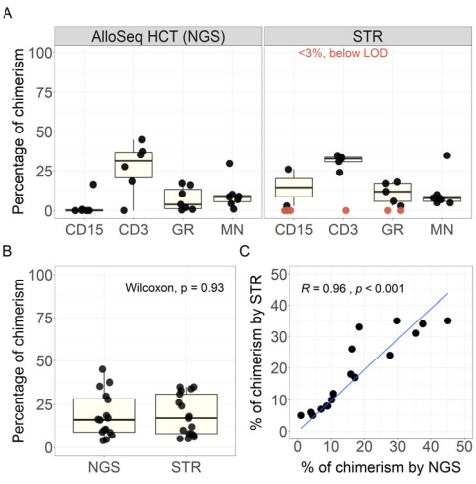


Figure 3.

LOD results were reported in 5 cases with the NGS solution compared to 9 with STR out of the total 26 subfraction tested (19% vs 35%, figure 4).

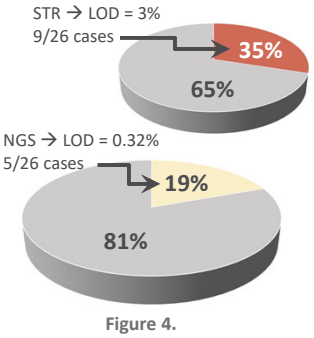


Figure 4.

The inter-run CV was 1.7, 14.5 and 1.6 with a means percent chimerism of 0.8, 17.0 and 14.3, respectively.

Conclusions

The data generated yielded high levels of accuracy, excellent intra-lab reproducibility, and a larger dynamic range compared with STR, as well as increased sensitivity by reporting 4 cases where percent chimerism was undetectable by STR. NGS-based chimerism provides an optimal method for routine chimerism testing in clinical labs and achieves a level of sensitivity that potentially enables early detection of disease relapse.

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