Accurate, Reproducible and Sensitive Chimerism Monitoring with NGS-based AlloSeq HCT.

Camila Egidio, Vasant Janakiraman, Corina Marchis, Thierry Viard, Marica Grskovic
CareDx, Inc., Brisbane, USA

INTRODUCTION

Genetic chimerism testing in Hematopoietic Cell Transplant (HCT) recipients is typically done using methods with low sensitivity, high variability and limited throughput. Employment of methods with higher sensitivity improves engraftment monitoring and may predict relapse of the disease earlier (Reshef *et al*, BMT 2014). We have developed a highly sensitive chimerism test based on Next Generation Sequencing that utilizes single nucleotide polymorphisms distributed across all human autosomal chromosomes. The AlloSeq HCT streamlined workflow generates results in less than 24 hours from DNA for up to 24 samples using an automated data analysis process with a proprietary software.

METHODS & MATERIALS

The AlloSeq HCT assay utilizes the differences in single nucleotide polymorphism (SNP) loci to measure the percent DNA fraction relative to the total amount of DNA from a post-transplant sample (Figure 1). DNA panels mimicking patient samples were used in this study. The target specific amplification strategy used to build the library for sequencing is depicted in Figure 2. Libraries are sequenced on the MiSeq System (Illumina, Inc). Once sequencing is complete, the percentage DNA fraction of up to 3 genomes present in each sample is calculated using the AlloSeq HCT software (Figure 4). The DNA input into the assay is 10 ng but limited testing showed performance in line with expected at 5 ng input.

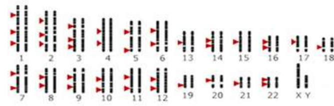

Figure 1. SNP distribution across the genome

For analytical validation of the method, four unique panels were prepared using five distinct DNA samples to mimic chimeric samples derived from HCT recipients.

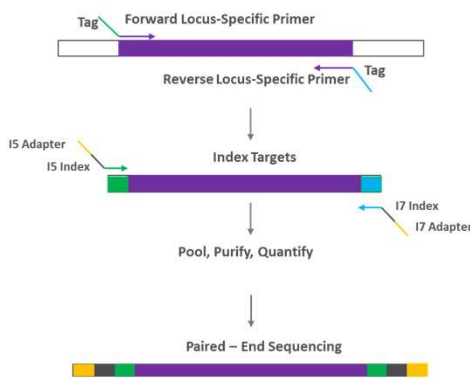

Figure 2. AlloSeq HCT library preparation workflow

Of the four panels prepared, three were obtained from mixing two distinct DNA samples (two genetic contributors) and one from three distinct DNA samples (three genetic contributors). Each panel was prepared mixing different quantities of each DNA sample to obtain different fractions as it would be obtained from series of recipient samples collected at different time points post-HCT (Table 1A and 1B).

In total, thirty-one samples designed to mimic DNA derived from HCT recipients from single and two donors were generated at fractions ranging between 0.1-99.9%. 10 ng DNA input per sample was used in 17 library preparation procedures across multiple operators, reagent lots and Illumina MiSeq instruments.

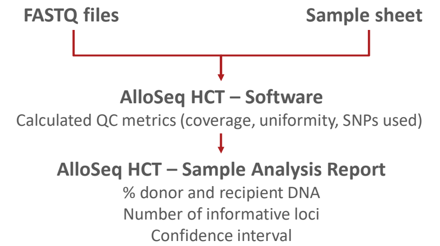

Figure 4. AlloSeq HCT – Data analysis workflow

Table 1A. Panels A, B and C with two genetic contributors

Panels	# of Replicates	Recipient gDNA %	Donor-1 gDNA %	# of Genomes
A,B,C	12	0.12	99.88	2
A,B,C	12	0.25	99.75	2
A,B,C	12	0.4	99.6	2
A,B,C	11	1	99	2
A,B,C	11	10	90	2
A,B,C	3	50	50	2
A,B,C	3	85	15	2
A,B,C	3	98	2	2

Table 1B. Panel E with three genetic contributors

Panels	# of Replicates	Recipient gDNA %	Donor-1 gDNA %	Donor-2 gDNA %	# of Genomes
E	12	0.5	10	89.5	3
E	12	1	10	89	3
E	3	5	10	85	3
E	3	20	10	70	3
E	3	40	10	50	3
E	12	0.2	1	98.8	3
E	12	0.5	1	98.5	3

RESULTS

The results showed a high correlation between expected and observed chimerism levels with a correlation of ≥0.99. Lower limit of quantification was 0.3% and 0.4% for samples containing a single or two donors, respectively. DNA input of 5 ng was also tested and showed similar results. All data generated showed high reproducibility with a coefficient of variability within and between runs <1.8%.

AlloSeq HCT assay detected percent recipient or donor DNA in the range between 0.1 – 99.88% in the panels with up to two (Figure 5) or three genetic contributors (Figure 6). The sensitivity of the test (Limit of Quantification) was 0.32% for single donor samples and 0.43% for two-donor samples. The variability of the assay was evaluated across multiple operators, different reagent lots and multiple sequencing runs (figure 8).

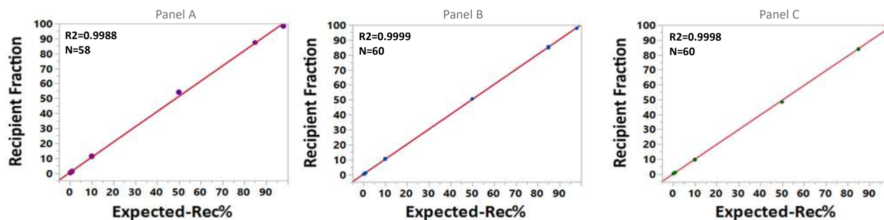

Figure 5. Percent DNA Recipient fractions in samples with two unrelated (A,B) and related (C) genetic contributors. Signal linearity with the expected %DNA fraction was 0.99 across all single donor panels.

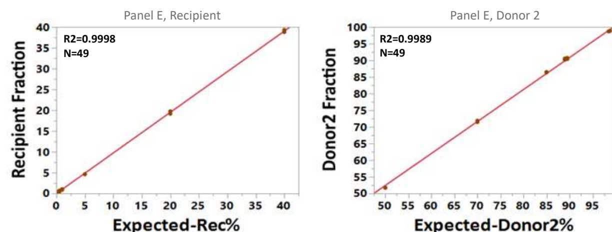

Figure 6. Percent DNA Donor/Recipient fractions in samples with three unrelated genetic contributors (E). Signal linearity with the expected % DNA fraction was 0.99 across all two donor panels.

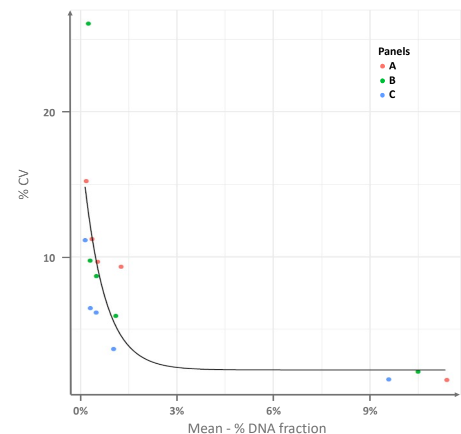

Figure 8. Coefficient of variation of AlloSeq HCT libraries across different reagent kit lots, sequencing runs and instruments, measured for each spike-in percent DNA (0.12%, 0.25%, 0.4%, 1%and 10%). CV of replicate measurements plotted against the mean %DNA fraction of the replicates. The best-fit nonlinear curve plotted indicated a decrease in CV with the increase in mean %DNA.

CONCLUSIONS

The AlloSeq HCT assay includes a simple workflow with automated data analysis and minimal hands-on time. Combined with the accuracy, reproducibility and sensitivity of the data generated, it is the optimal method for routine chimerism research in clinical labs.

REFERENCE *Methodology used for calculations: "Protocols for Determination of Limits of Detection and Limits of Quantitation", CLSI Oct 2004

Disclaimer For Research Use Only

CareDx® Your Partner in Transplant Care