Alloseq HCT: A Wide Range Chimerism Testing Solution with High Sensitivity, Accuracy and Precision for Routine Post Hematopoietic Cell Transplant Monitoring

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INTRODUCTION

Hematopoietic stem cells transplantation (HCT) provides healthy stem cells that improved the immunity in patients with leukemia, multiple myeloma or lymphoma. Genetic chimerism testing in hematopoietic cell transplant recipients is typically done to monitor the success of donor cell engraftment. The most common method used is based on Short Tandem Repeats (STR). More sensitive chimerism detection methods may aid in engraftment monitoring and potentially predict early relapse of the disease. We have developed and analytically validated a highly sensitive chimerism test based on Next Generation Sequencing. The test combines a clinical laboratory compatible protocol with streamlined workflow and a fully automated analysis software to accurately quantify the level of chimerism. The entire workflow from extracted DNA to sample report takes 24 hours.

MATERIALS

Four unique panels were prepared using five distinct DNA samples to mimic chimeric samples derived from HCT recipients. 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).

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

METHODS

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 2). 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 3. 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.

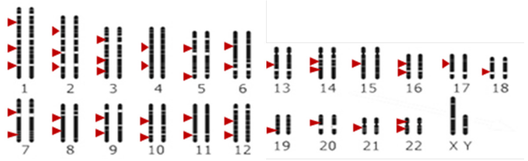

Figure 2. SNP distribution across the genome

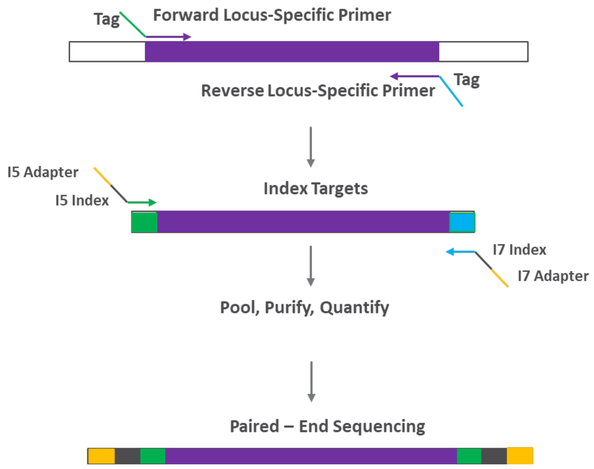

Figure 3. AlloSeq HCT library preparation workflow

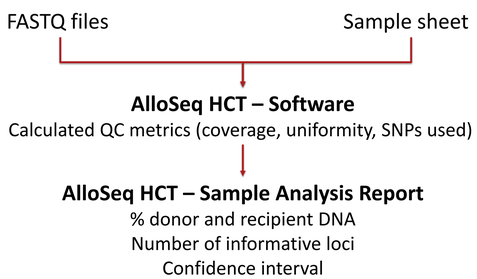

Figure 4. AlloSeq HCT – Data analysis workflow

RESULTS

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.

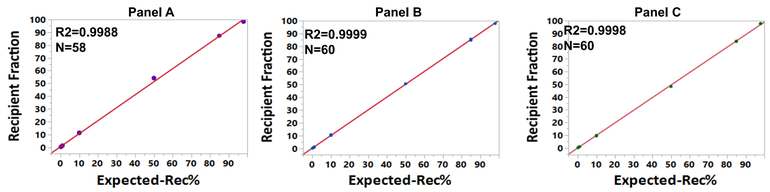

Figure 5. Percent DNA Recipient fractions in samples with two unrelated (A,B) and related (C) genetic contributors. Signal linearity with the expected %DNA fraction was 0.99 across all single donor panels.

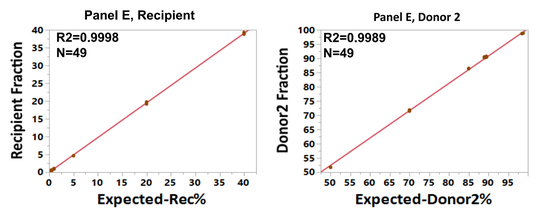

Figure 6. Percent DNA Donor/Recipient fractions in samples with three unrelated genetic contributors (E). Signal linearity with the expected % DNA fraction was 0.99 across all two donor panels.

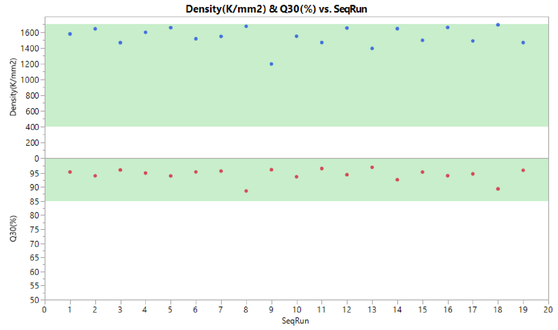

Figure 7. Sequencing QC Metrics - Cluster density and Q30 scores. All libraries produced QC in line with Illumina's recommendation for cluster density and Q30 scores when loaded at 30pM for 10ng input. Acceptable Cluster Density and Q30 scores shaded in green.

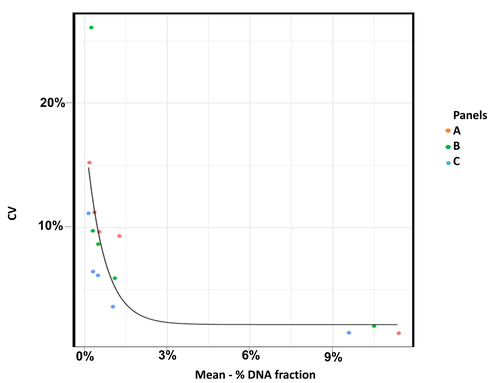

Figure 8. Coefficient of variation of AlloSeq HCT libraries across different reagent kit lots, sequencing runs and instruments, measured for each spike-in percent DNA (0.12%, 0.25%, 0.4%, 1%and 10%). CV of replicate measurements plotted against the mean %DNA fraction of the replicates. The best-fit nonlinear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

CONCLUSIONS

AlloSeq HCT test accurately and reproducibly quantifies the level of chimerism when two to three related/unrelated genetic contributors present in samples mimicking post-transplant patient DNA samples. Combined with automated data analysis, ease of use, 24-hour workflow and minimal hands-on time, AlloSeq HCT is an optimal method for routine chimerism testing.

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

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