AlloSeq Tx Genotyping Using DNA Extracted from Saliva

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INTRODUCTION

The AlloSeq® Tx Hybrid Capture assay is a flexible and comprehensive methodology for the typing of human leukocyte antigen (HLA) genes, and other important non-HLA transplant-associated genes, at high resolution. Although the AlloSeq® Tx assay together with AlloSeq® Assign™ software enables researchers to robustly genotype genomic DNA samples extracted from whole blood, the compatibility of the system for genotyping DNA from saliva samples has not been assessed. In order to assess the performance of this sample type, 24 genomic DNA samples extracted from saliva were tested using the AlloSeq ® Tx assay.

METHODS & MATERIALS

24 individuals provided saliva samples using Oragene DNA OG-600 (saliva) and ORAcollect DNA OCR-100 (sponge) kits (DNA genotek). Genomic DNA was subsequently extracted with a QIAamp DNA Mini Kit (Qiagen). Library construction and Enrichment were undertaken using the AlloSeq® Tx system. During this experiment, in-development Hybridisation Capture probes targeting 19 loci were utilised including HLA-A, -B, -C, -DRB1, -DQB1, -DQA1, -DPB1, -DPA1, -DRB3/4/5, -E, -F, -G, -H, MICA, MICB and two additional genes. Enriched libraries were then loaded onto a micro flowcell on the Illumina MiSeq System for Next Generation Sequencing. Finally, AlloSeq® Assign™ software was used for data analysis and allele assignments of the 19 target loci.

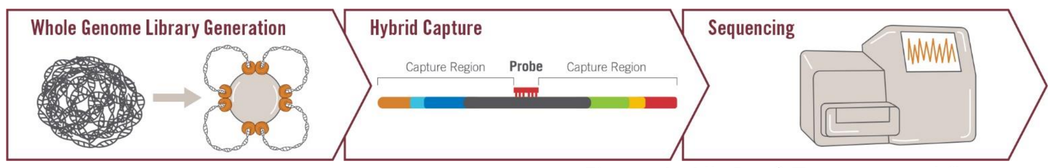


Figure 1. AlloSeq® Tx assay workflow

RESULTS

The average concentration of genomic DNA extracted from the Oragene saliva and sponge samples was 15.32 ng/µL and 6.78 ng/µL respectively, above the minimum sample input required for the AlloSeq® Tx assay. AlloSeq® Tx libraries were generated from the genomic DNA extracted from both saliva and sponge samples the with an average yield of 24.93 ng/µL and an average fragment size of 788 bp. The average enrichment pool yield was 14.05 ng/µL with an average fragment size of 728 bp. The resulting sequence data exhibited 94% of reads with an average read quality of Q30 or higher, an average read depth of 140x, and average allele balance of 42%. High resolution genotyping results were achieved with 4 field typing for Class I loci, 3 field typing results for Class II and 2 fields typing for MICA/MICB for all samples tested. All genotyping results were 100% concordant with QTYPE® genotyping results.

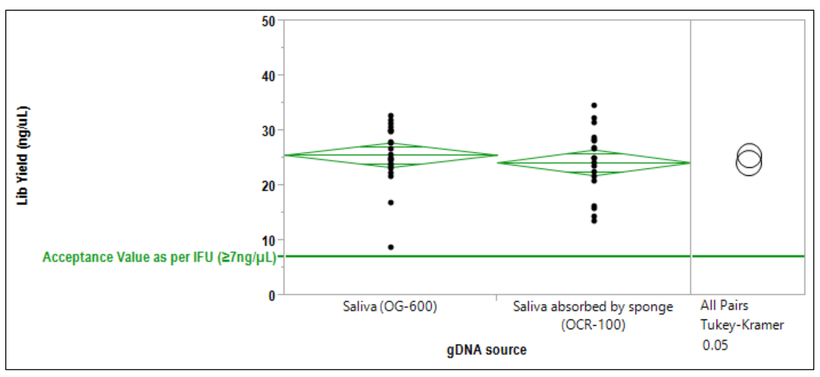


Figure 2. Library concentration yield and distribution achieved from the Oragene saliva samples compared to the yield from Oragene sponge samples. All samples were above the minimum input requirements for the AlloSeq® Tx assay.

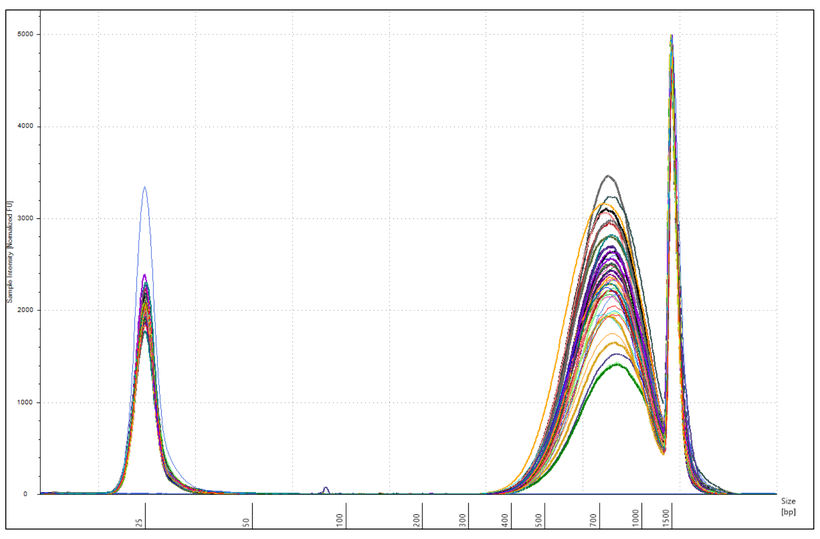
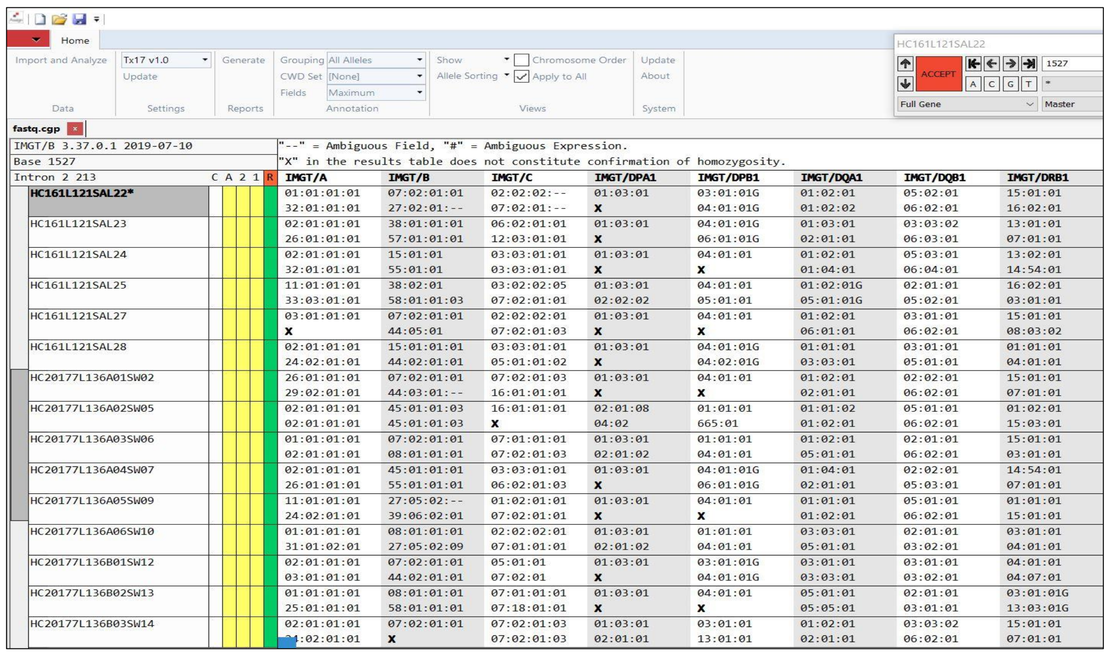


Figure 3. TapeStation trace illustrating the fragment peak size and distribution of AlloSeq® Tx libraries produced from the Oragene saliva and sponge. The average peak height is 788bp.

RESULTS (cont'd)



	IMGT/A	IMGT/B	IMGT/C	IMGT/DPA1	IMGT/DPB1	IMGT/DQA1	IMGT/DQB1	IMGT/DRB1
HC161L121SAL22*	01:01:01:01	07:02:01:01	02:02:02:--	01:03:01	03:01:01G	01:02:01	05:02:01	15:01:01
	32:01:01:01	27:02:01:--	07:02:01:--	X	04:01:01G	01:02:02	06:02:01	16:02:01
HC161L121SAL23	02:01:01:01	38:01:01:01	06:02:01:01	01:03:01	04:01:01G	01:03:01	03:03:02	13:01:01
	26:01:01:01	57:01:01:01	12:03:01:01	X	06:01:01G	02:01:01	06:03:01	07:01:01
HC161L121SAL24	02:01:01:01	15:01:01	03:03:01:01	01:03:01	04:01:01	01:02:01	05:03:01	13:02:01
	32:01:01:01	55:01:01	03:03:01:01	X	X	01:04:01	06:04:01	14:54:01
HC161L121SAL25	11:01:01:01	38:02:01	03:02:02:05	01:03:01	04:01:01	01:02:01G	02:01:01	16:02:01
	33:03:01:01	58:01:01:03	07:02:01:01	02:02:02	05:01:01	05:01:01G	05:02:01	03:01:01
HC161L121SAL27	03:01:01:01	07:02:01:01	02:02:02:01	01:03:01	04:01:01	01:02:01	03:01:01	15:01:01
	X	44:05:01	07:02:01:03	X	X	06:01:01	06:02:01	08:03:02
HC161L121SAL28	02:01:01:01	15:01:01:01	03:03:01:01	01:03:01	04:01:01G	01:01:01	03:01:01	01:01:01
	24:02:01:01	44:02:01:01	05:01:01:02	X	04:02:01G	03:03:01	05:01:01	04:01:01
HC20177L136A01SW02	26:01:01:01	07:02:01:01	07:02:01:03	01:03:01	04:01:01	01:02:01	02:02:01	15:01:01
	29:02:01:01	44:03:01:--	16:01:01:01	X	X	02:01:01	06:02:01	07:01:01
HC20177L136A02SW05	02:01:01:01	45:01:01:03	16:01:01:01	02:01:08	01:01:01	01:01:02	05:01:01	01:02:01
	02:01:01:01	45:01:01:03	X	04:02	665:01	01:02:01	06:02:01	15:03:01
HC20177L136A03SW06	01:01:01:01	07:02:01:01	07:01:01:01	01:03:01	01:01:01	01:02:01	02:01:01	15:01:01
	02:01:01:01	08:01:01:01	07:02:01:03	02:01:02	04:01:01	05:01:01	06:02:01	03:01:01
HC20177L136A04SW07	02:01:01:01	45:01:01:01	03:03:01:01	01:03:01	04:01:01G	01:04:01	02:02:01	14:54:01
	26:01:01:01	55:01:01:01	06:02:01:03	X	06:01:01G	02:01:01	05:03:01	07:01:01
HC20177L136A05SW09	11:01:01:01	27:05:02:--	01:02:01:01	01:03:01	04:01:01	01:01:01	05:01:01	01:01:01
	24:02:01:01	39:06:02:01	07:02:01:01	X	X	01:02:01	06:02:01	15:01:01
HC20177L136A06SW10	01:01:01:01	08:01:01:01	02:02:02:01	01:03:01	01:01:01	03:03:01	02:01:01	03:01:01
	31:01:02:01	27:05:02:09	07:01:01:01	02:01:02	04:01:01	05:01:01	03:02:01	04:01:01
HC20177L136B01SW12	02:01:01:01	07:02:01:01	05:01:01	01:03:01	03:01:01G	03:01:01	03:01:01	04:01:01
	03:01:01:01	44:02:01:01	07:02:01	X	04:01:01G	03:03:01	03:02:01	04:07:01
HC20177L136B02SW13	01:01:01:01	08:01:01:01	07:01:01:01	01:03:01	04:01:01	05:01:01	02:01:01	03:01:01G
	25:01:01:01	58:01:01:01	07:18:01:01	X	X	05:05:01	03:01:01	13:03:01G
HC20177L136B03SW14	02:01:01:01	07:02:01:01	07:02:01:03	01:03:01	03:01:01	01:02:01	03:03:02	15:01:01
	24:02:01:01	X	07:02:01:03	02:01:01	13:01:01	02:01:01	06:02:01	07:01:01

Figure 4. Screenshot of the summary view from the AlloSeq® Assign™ software displaying 4 fields genotypes for Class I loci, and 3 fields for Class II loci. A number of novel alleles with variants in non-coding and/or UTR regions of class I loci were identified, which were reported to 3 fields.

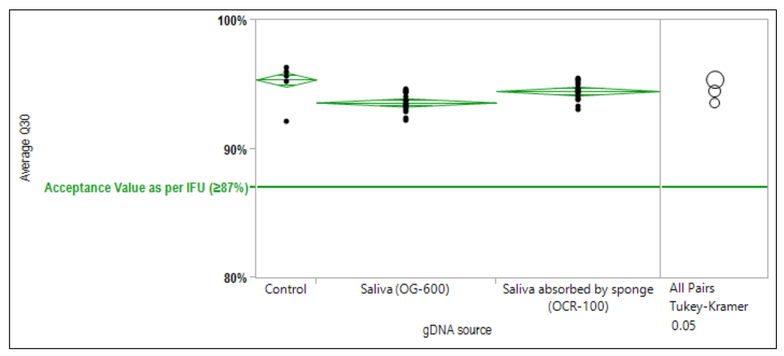


Figure 5. Comparison of the %Q30 score observed for samples extracted from Oragene saliva and sponge samples compared with HLA Reference Standards (IHWG) controls processed via AlloSeq® Tx system. All samples' Q-score pass illumina's run specifications.

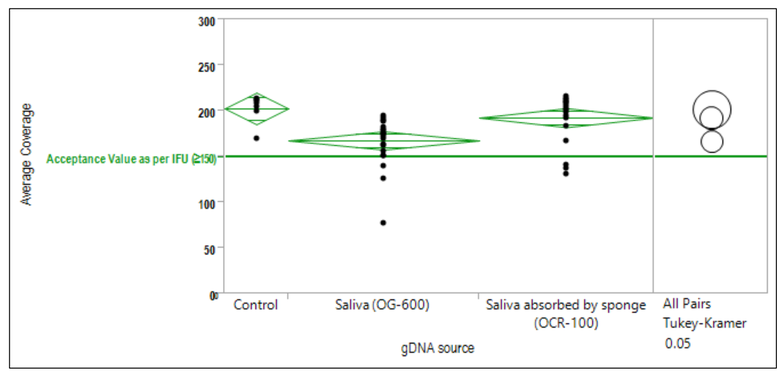


Figure 6. Comparison of the average coverage (or read depth) observed for samples extracted from Oragene saliva and sponge samples compared with HLA Reference Standards (IHWG) controls processed via AlloSeq® Tx system. A single outlier sample was observed with an average coverage of less than 100x, which still genotyped successfully.

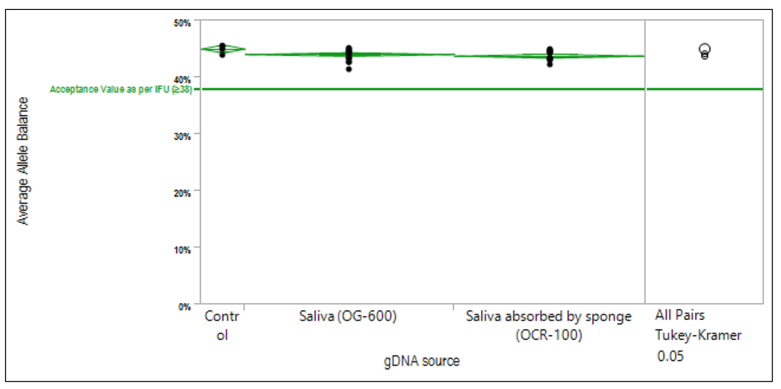


Figure 7. Comparison of the average allele balance observed for samples extracted from Oragene saliva and sponge samples compared with HLA Reference Standards (IHWG) controls processed via AlloSeq® Tx system.

CONCLUSIONS

This study has demonstrated successful high-resolution genotyping and high-quality data achieved from genomic DNA extracted from Oragene saliva and sponge samples with the AlloSeq® Tx assay. Performance with this sample type enables wider research application of the AlloSeq® Tx assay.

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