# Workflow Improvements of AlloSeq™ Tx 17 Hybrid Capture Assay – A single day workflow.

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## Introduction

The AlloSeq Tx 17 Hybrid Capture assay facilitates interrogation of 17 transplant relevant human leukocyte antigen (HLA) and non-HLA gene loci (MICA and MICB). Although the existing workflow is flexible and robust, hybrid capture workflows can appear prolonged when compared with PCR-based Next-Generation Sequencing (NGS) alternatives. Review and optimisation of key protocol steps demonstrate that it is possible to perform the AlloSeq Tx hybrid capture assay workflow, from DNA samples to sequencer, within a standard workday (<8 hours), without impacting sequencing yield or data quality.

## Methods and Materials

A single day workflow was created by earlier sample pooling and reducing hybridization. The modifications to the assay were then applied to test a total of 15,617 alleles across 288 library preparations and 9 hybridisation experiments (504 samples in total, including replicates). AlloSeq™ Assign® software was used for data analysis. The hybrid capture pools were compared for 24-, 48- and 96-plexity to assess impacts of the changes made to the protocol. Finally, data generated from the improved workflow was compared against the AlloSeq Tx 17 standards that displayed in figure 2-4 as upper and lower control limits (UCL and LCL).

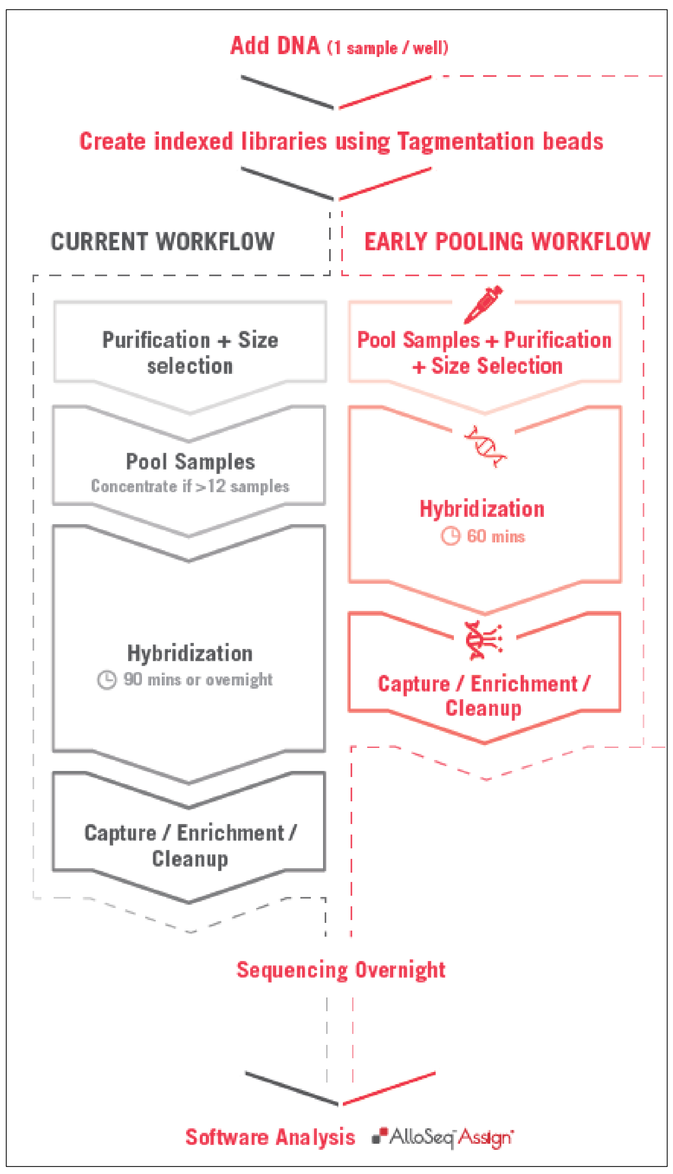


**Figure 1. An overview of current versus early pooling (improved) workflow.**

## Results

All sequencing metrics complied with Illumina NGS expectations (%Q30 and yield) for both iSeq and MiSeq systems. Mean locus read coverage for target loci was 200x (144-217x, S.D. 20.72). Mean heterozygous allele balance was 44.81% (40.48-48.67%, S.D. 1.09%). Overall genotyping accuracy for expected allele typing (exact allele syntax resolution match) was 99.86%. In 0.14% of allele call instances, a difference in allele ambiguity or resolution was obtained which could be attributed to sample-specific variation in resolution.

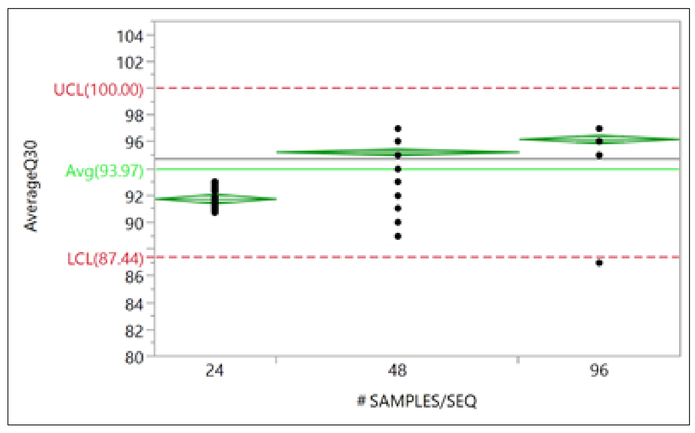


**Figure 2. Quality Score (%Q30) -** Mean sample percent Q30 by sample plexity rests within our acceptance criteria. 1/504 samples fell below 87.44% lower control limit (87%) but above the Illumina 82% acceptance threshold.

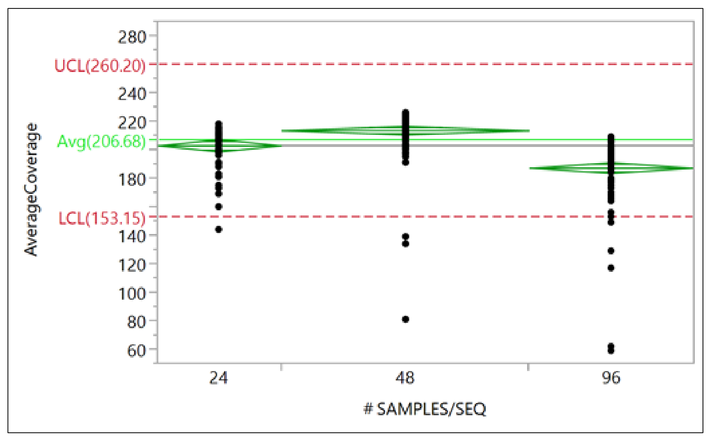


**Figure 3. Coverage -** The read depth averages observed were 24-plex (203x), 48-plex (213x), and 96-plex (193x) pools, respectively. The study suggested that 97.8% of data points was above the coverage threshold of 100x. Only one sample was below the minimum threshold of 30x coverage, which was due to the absence of DNA detected in the stock DNA tube.

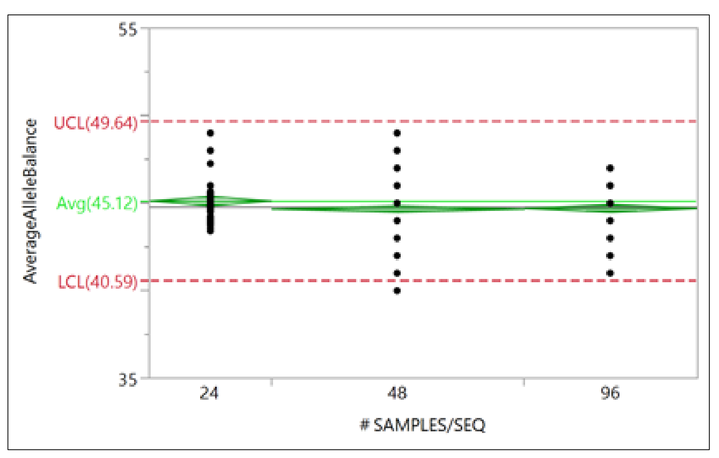


**Figure 4. Allele balance** - Mean allele balance by pool plexity. 99.80% of data points displayed greater than 40.59% allele balance whereas 1/504 samples fell below LCL (40%).

## Conclusions

This streamlined workflow combines earlier pooling of indexed samples with truncation of selected incubation and hybridisation steps. One hundred percent of samples genotyped produced the same allele, or allele groups, to the expected result and no marked difference was observed between results from the original and the early pooling workflows.

Furthermore, the improvements applied to the workflow not only allow operators to achieve the AlloSeq Tx 17 Hybrid Capture assay in a single day, but also highlight a user-friendly feature as a result of an elimination of individual sample transfer steps, which reducing user error.

Finally, the improved workflow is proven to be more cost-effective due to the fact that the library size selection, purification, and concentration can be completed in a single tube.

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