Optimization of mixed pools of buccal and peripheral DNA for routine testing with AlloSeq™ Tx 17 hybrid capture.

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Introduction

Increasingly, laboratories are presented with a range of diverse sample types for diagnostic testing. Buccal DNA (BD) preparations are a less invasive, economical alternative to peripheral blood (PB) DNA extractions. Genomic DNA yield and integrity for BD is often reduced, compared to that of PB samples, which may lead to an under-representation of BD in mixed sample pools. This study investigated how combinations of BD and PB DNA preparations may be routinely assayed together, without sample-type bias for genetic matching in transplantation.

Methods and Materials

AlloSeq™ Tx 17® (24) hybrid capture was used to prepare indexed libraries for 20x-BD and 20x-PB samples. When equal library volume for 20x-BD and 4x-PB DNA samples were pooled and analyzed, we demonstrated a 3 to 4-fold bias, in both index representation (%) and mean locus coverage (read depth (x)), for PB over BD. Adjusting library input volumes by sample-type (4:1 and 3:1 ratios) we re-tested a pool of 20x BD and 4x PB DNA samples, and the resulting data demonstrated comparable index allocation percentage and mean coverage for both sample types. Now applying a volume input ratio of 3:1 (BD:PB), we investigated differing BD/PB sample number combinations by running 12/12 and 4/20 separately to assess whether sample-type plexity had an adverse effect on results.

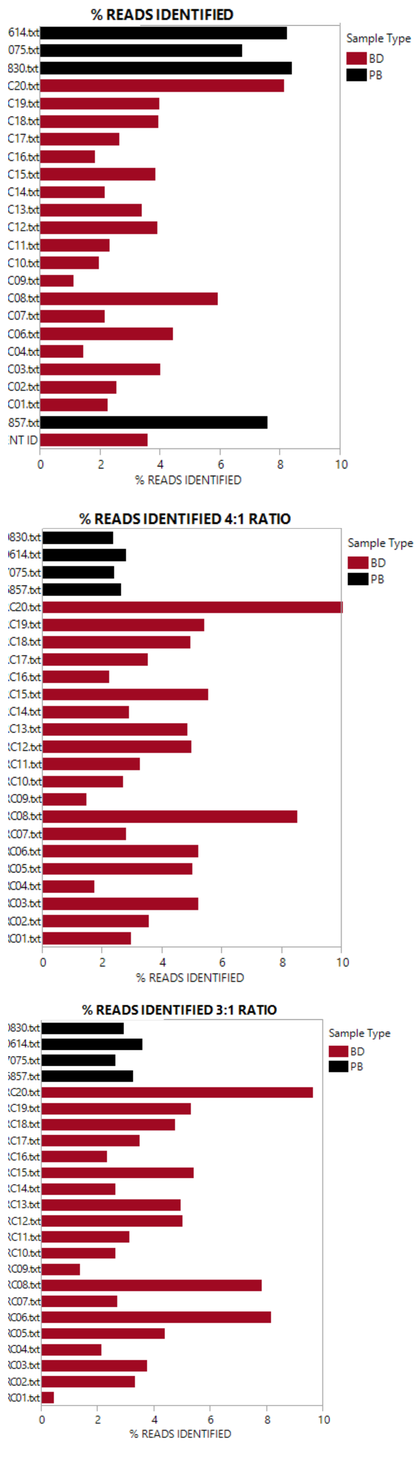

Figure 1: Control.
24 –plex (20-BD and 4-PB).
Equal volume of BD and PB samples reveals a bias towards PB samples (black) (HC20267).

Figure 2: 4:1 library ratio (BD:PB).
24 –plex (20-BD and 4-PB).
Over-representation of BD (red) compared to PB (black) (HC20276).

Figure 3: 3:1 library ratio (BD:PB).
24 –plex (20-BD and 4-PB).
Improved representation of both BD and PB (*note user error on sample A01) (HC20279).

Figure 4: 3:1 library ratio (BD:PB).
24–plex (12-BD and 12-PB).
Increased BD to PB, however adequate percentage of reads for all samples (HC20282).

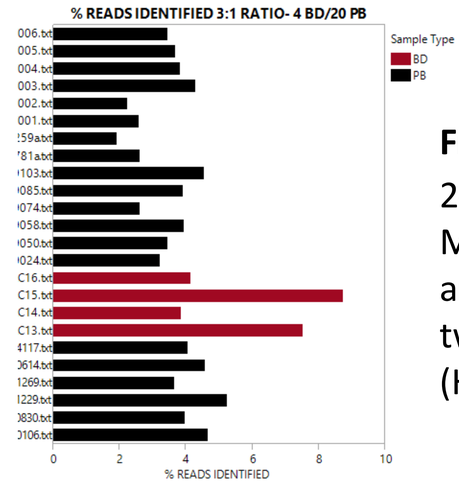

Figure 5: 3:1 library ratio (BD:PB).
24–plex (4-BD and 20-PB).
Majority equal representation among BD and PB except for the two BD samples exceeding 6% (HC20283).

Results

Data from each of the 3:1 ratio hybrid capture experiments demonstrates comparable mean locus coverage (BD 181x, PB 171x) and allele balance (BD 44.0% and PB 43.5%) result for both sample types. Allele calls were obtained for the AlloSeq Tx 17 gene loci for all samples in each capture experiment and combination. No distinct difference was observed between sample-type plexity.

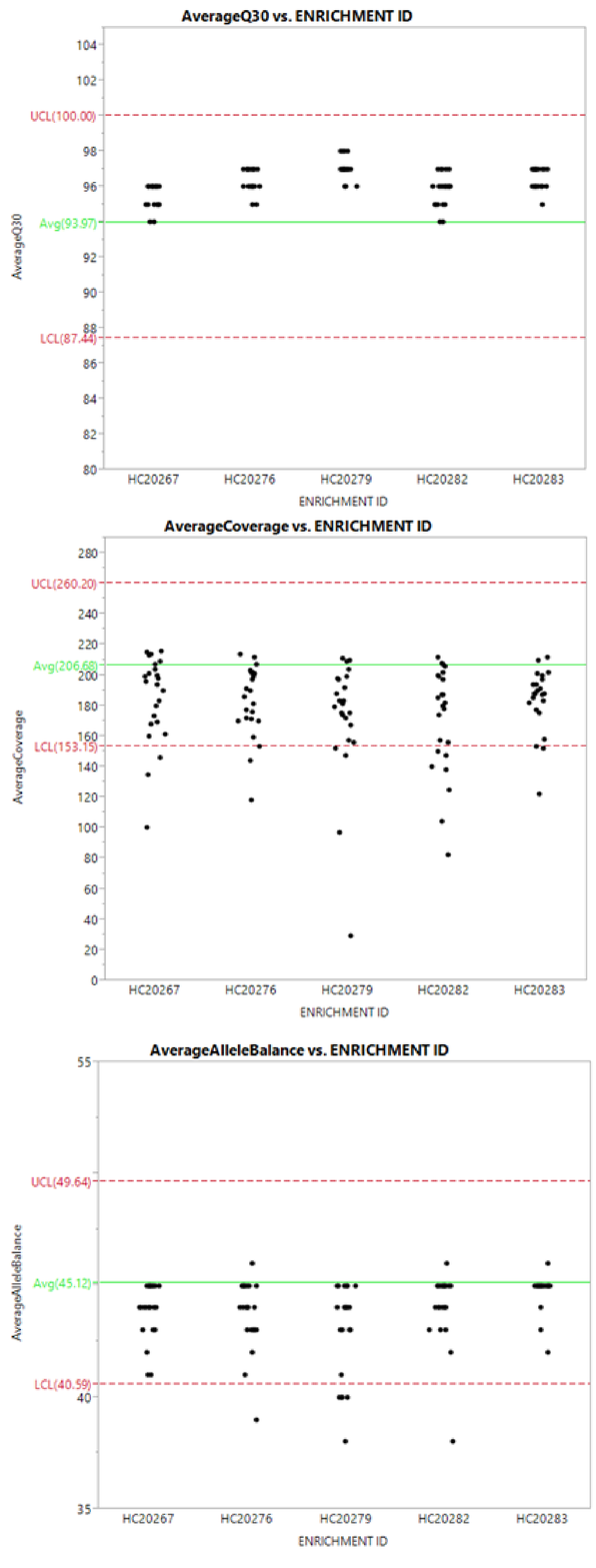

Figure 6. Average Q30 (quality score) for all samples within our expected performance limits.

Figure 7. The average coverage varied by sample, however all produced genotypes using Assign software (outlier from HC20279 is a poor quality sample).

Figure 8. Allele balance was below the LCL for a few samples across HC20276, HC20279, and HC20282 with everything else within expectations.

Conclusions

This study demonstrates that buccal and peripheral blood DNA samples can successfully be typed from the same pool. Using a similar verification approach and AlloSeq® Tx 17 hybrid capture, other laboratories may determine an optimal library input ratio, and process buccal and peripheral blood DNA samples together without the need for additional concentration steps.

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