Hybrid Capture Sequencing Enhances Allele Balance and Minimizes Ambiguity in Clinical HLA Typing

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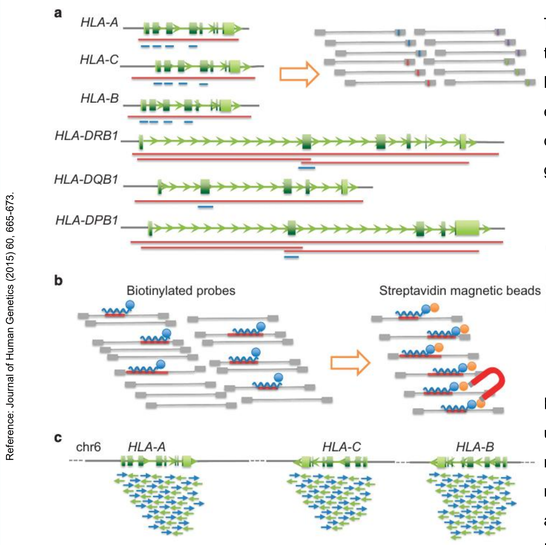
ABSTRACT

- Clinical Challenge. High resolution typing for HLA-A, -B, -C, - DRB1, -DRB43/4/5/, -DQB1, -DQA1, -DPB1, and –DPA1 remains challenging despite widely used NGS methodology due to presence of ambiguous combinations.
- Results presented in this poster, demonstrate third-field typing using Hybrid Capture with clinical samples and 100% concordance with the current clinical method.
- Hybrid Capture minimizes allele ambiguity by including intronic regions during library preparation and amplification. Allele imbalance resolution for difficult targets was also an added value for performing HLA typing using the HybriType NGS kit.
- Recent validations using Hybris Capture technology include automation using the HLA Pro and sequencing with the Illumina MiSeq i100.

INTRODUCTION

The City of Hope HLA & Immunogenetics laboratory considered adopting the Hydrid-Capture method to reduce allele ambiguity and enhance allele typing results for Recipient and Donor matching. Results presented from this validation demonstrated accuracy at 3-field typing with clinical samples.

Steps for library enrichment and Sequence alignment



Reference: Journal of Human Genetics (2015) 60, 665–673.

The first PCR-reaction targets the HLA genes. Blue lines represent targeted exons or long-range PCR can be used for the whole gene (red lines).

Hybrid Capture distinguishes itself by using biotinylated probes for enrichment of targeted sequences or HLA genes.

Enriched indexed libraries using Streptavidin magnetic beads are reconstructed by alignment to reference HLA gene sequences.

Figure 1. DNA library preparation for Hybrid Capture and NGS Sequencing

METHODS SUMMARY AND MiSeq QUALITY METRICS

- Forty-six DNA samples from blood, saliva, buccal swabs, or buccal brush were quantified using optical density or fluorescence-based (Qubit) measurements.
- Libraries were prepared with the HybriType HLA Plus Flex NGS kit.
- NGS Sequencing. Illumine MiSeq and analyzed with TSV software v3.2 using the 3.58 IPD-IMGT/HLA Database.
- Statistics. The T-test and Mann-Whitney Test evaluated statistical significance.

Table 1. NGS Quality Metrics and HLA Typing Validation Concordance

		HYBRITYPE and MiSeq Quality Metrics				
		Min Coverage	Mappability	Read Depth (All)		
Run Stats	# Samples			Min	Median	Max
01_15_26	*46*	*30 - 497*	*88 - 99*	*31*	*424*	*472*
01_28_26	*48*	*366 - 495*	*89-99*	*327*	*437*	*469*
08_26_26	48	323 -497	90 - 99	233	395	458

140 Samples were examined by Hbrid Capture; **128 of these samples showed 100%** *HLA Typing concordance* with the prior method, at the third-field resolution.

RESULTS

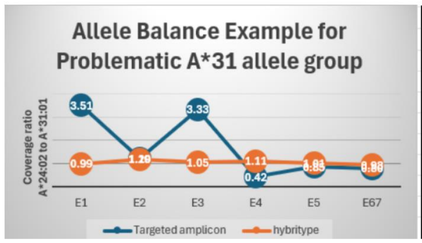
- HLA typing using the Hybrid Capture methos demonstrated **100%** concordance at **Third-Field** resolution compared with the reference method (Scisco). Table 1, shows a representative result from this analysis at the HLA-A, -B, -C, - DRB1, -DRB43/4/5/, -DQB1, -DQA1, -DPB1, and -DPA1. Importantly, the Hybrid Capture method was adopted to improve intron coverage, thus reducing allele ambiguity.

Table 2. Representative HLA typing results from validation runs

HYBRITYPE		SCISCO	
Sample ID	Final Assignment	Sample ID	Final Assignment
COH25_H	**A*02:01:01, A*68:02:01**	COH25_S	A*02:01:01, A*68:02:01
COH25_H	**B*35:01:01, B*49:01:01**	COH25_S	B*35:01:01, B*49:01:01
COH25_H	**C*07:01:02, C*16:01:01**	COH25_S	C*07:01:02, C*16:01:01
COH25_H	**DPA1*01:03:01, DPA1*02:01:08**	COH25_S	DPA1*01:03:01, DPA1*02:01:08
COH25_H	**DPB1*01:01:01, DPB1*584:01:01**	COH25_S	DPB1*01:01:01, DPB1*584:01:01
COH25_H	**DQA1*01:03:01, DQA1*05:01:01**	COH25_S	DQA1*01:03:01, DQA1*05:01:01
COH25_H	**DQB1*02:01:01, DQB1*06:03:01**	COH25_S	DQB1*02:01:01, DQB1*06:03:01
COH25_H	**DRB1*03:01:01, DRB1*13:01:01**	COH25_S	DRB1*03:01:01, DRB1*13:01:01
COH25_H	**DRB3*01:01:02, DRB3*02:02:01**	COH25_S	DRB3*01:01:02, DRB3*02:02:01

- Hybrid Capture showed improved Allele imbalance for difficult targets. Parallel analysis of Hybrid Capture vs the targeted amplicon method based on a 46-sample subset, indicated improved allele imbalance as shown in figure 2.

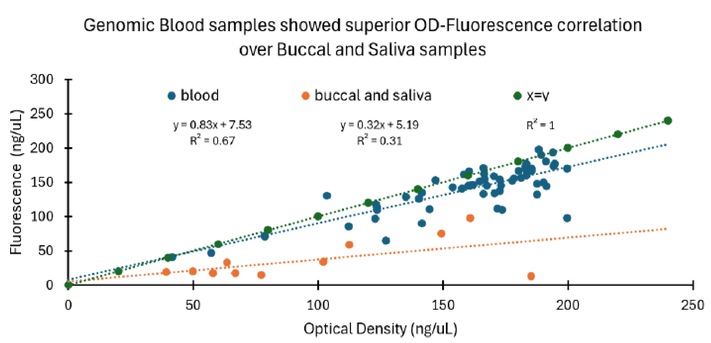
Figure 2. Hybrid-Capture resolves allele Imbalance in areas most impacted by targeted amplicon NGS



Targeted Amplicon Rate of Imbalance > 3:1 (N = 121)				
Locus	Exon 1	Exon 2	Exon 3	Exon 4
A	12%	19%	13%	0%
B	0%	7%	23%	0%
C	3%	0%	7%	5%
DPA1	0%	1%	0%	0%
DPB1	0%	0%	0%	0%
DQA1	0%	17%	0%	0%
DQB1	2%	2%	0%	0%
DRB1	12%	0%	0%	8%
DRB345	9%	7%	0%	3%

- Because the COH HLA laboratory determines DNA concentration by optical density (OD); part of the validation evaluated the risk for using OD values instead of the values obtained by using the recommended fluorescence-based method (Qubit). A 14-sample subset containing challenging nuclei acid material arising from saliva and buccal swaps, demonstrated that OD values can be safely used for NGS runs using Hybrid Capture workflow.

Figure 3. Buccal and Saliva DNA samples concentration from OD performed similarly to Qubit quantitation by Hybrid-Capture NGS*



*Saliva and buccal swabs, OD mean values (114.1 ng/µL) exceeded Qubit values (52 ng/µL; pairwise-T test $p < 0.0001$), yet sequencing performance was unaffected: coverage was comparable for class I (median value for Minimum Coverage from OD was 450 reads vs Qubit 430 reads; $p = 0.83$) and class II alleles (420 vs 450 reads; $p = 0.85$, respectively)

Validation with 96-External Samples

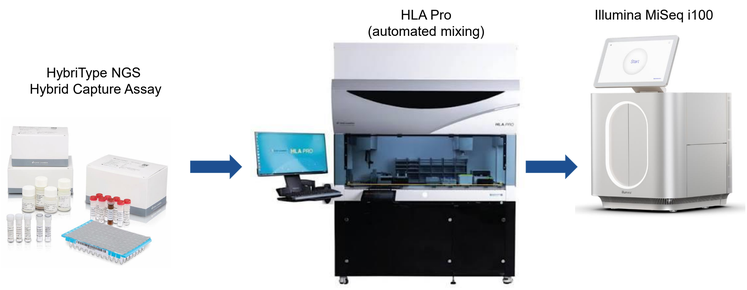
The 19th International HLA & Immunogenetics Workshop

May 19–24, 2026 | Numazu, Japan

The City of Hope HLA & Immunogenetics laboratory participated in the 19th International workshop. The Hybrid Capture method was used for genotype assessment of 96-samples derived from reference cell lines. NGS information and final genotypes were submitted prior to the Meeting in Numazu, Japan. Consensus HLA typing results are pending for additional validation of NGS by using Hybrid Capture .

High-Resolution HLA typing Recent Upgrades

- A 47-sample set with 44 unique samples was used for HLA Pro (One Lambda, Thermo Scientific) validation using the **Hybrid Capture method**.
- The **HLA Pro** or automated approach produced equivalent HLA typing results across the11-loci (HLA-A, -B, -C, - DRB1, -DRB43/4/5/, -DQB1, -DQA1, -DPB1, and -DPA1).
- **Benefits**. The HLA Pro automated pipetting (mixing) system reduces operator interaction by approximately *three-hours* and removes uncertainty usually seen in manual procedures.
- Implementation of the Illumina **MiSeq i100** platform was also recently adopted and is under validation for clinical testing.
- **Benefits**: HLA typing for the 11-loci with the MiSeq i100 produces twice the total read depth in *seven-hours* compared to the classic MiSeq which requires about 24 hours).



SUMMARY

- HLA typing at the third-field provides higher confidence in matching recipient and potential donors. Improved HLA typing supports better outcomes for patients in need of hematopoietic stem cell transplantation for blood malignancies and a variety of immunodeficiency disorders.
- Hybrid Capture provides accurate HLA typing results across the 11-loci (HLA-A, -B, -C, - DRB1, -DRB43/4/5/, -DQB1, -DQA1, -DPB1, and -DPA1).
- In depth analysis revealed improved allele imbalance using the hybrid capture method in contrast to the targeted amplicon-based method.
- HLA typing with the Hybrid Capture method, including automation with the HLA Pro, and sequencing with the MiSeq i100 demonstrated accuracy, reliability, and reduced time in sample preparation and sequencing.

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