

Analytical performance of targeted long-read HiFi sequencing to detect pharmacogenomic *HLA-A* and *-B* alleles implicated in drug-induced hypersensitivity reactions

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Aim: Drug-induced hypersensitivity reactions are life-threatening adverse events that are strongly associated with specific human leucocyte antigen (HLA) alleles, including *HLA-A*31:01* (carbamazepine/oxcarbazepine), *HLA-B*15:02* (phenytoin/fosphenytoin), *HLA-B*57:01* (abacavir), and *HLA-B*58:01* (allopurinol). To enable clinical pharmacogenomic (PGx) HLA screening, many genotyping assays only interrogate single nucleotide polymorphisms (SNPs) in linkage disequilibrium with the PGx HLA risk alleles; however, these proxy-SNPs have inadequate sensitivity and specificity across diverse populations. Given the recent advantages of long-read HiFi sequencing for variant calling and phasing, we aimed to evaluate this platform for clinical *HLA-A* and *-B* screening as part of a larger germline PGx panel.

Methods: Both Genetic Testing Reference Material (GeT-RM) (n=14) and blinded previously tested clinical samples (n=33) with known allelic resolution HLA typing (positive or negative for the four PGx alleles) were included in this analytical evaluation. Targeted long-read HiFi sequencing was performed using the 2 Mb Twist Alliance 'Long-Read PGx Panel' hybrid capture with sequencing on the Sequel IIe (PacBio). The GeT-RM and clinical samples were sequenced at 14-17 samples per SMRTcell, which resulted in a mean sequencing depth of 245X across all target loci and average read lengths of ~4800 bp. Variant calling and haplotype phasing were accomplished using pbmm2, DeepVariant and WhatsHap, with up to four-field HLA allele calling using StarPhase.

Table 1: CPIC Guidelines for Drug-Gene Interactions

Gene	Medication	PMID
<i>HLA-A*31:01</i>	Carbamazepine/Oxcarbazepine	23695185 29392710
<i>HLA-B*15:02</i>	Phenytoin/Fosphenytoin	25099164 32779747
<i>HLA-B*57:01</i>	Abacavir	24561393 22378157
<i>HLA-B*58:01</i>	Allopurinol	23232549 26094938

Results: Among the 94 total *HLA-A* and *-B* alleles interrogated, 100% were correctly detected as positive/negative for the four PGx alleles to the second field, resulting in an overall accuracy of >99.9% (95%CI: 99.6 – 100%). In three samples that were negative for PGx alleles, allele drop-out at the *HLA-A* locus was detected; however, in two of the three samples, the correct second allele was detected upon repeat testing.

Conclusions: These results indicate that targeted long-read HiFi sequencing is highly accurate in detecting the clinically significant PGx *HLA-A* and *-B* alleles. An expanded validation study is ongoing to further assess non-PGx *HLA* allele drop-out and its potential implications in PGx panel reporting.

Figure 1: Stanford Medicine Clinical Genomics Long-Read HiFi Sequencing Workflow

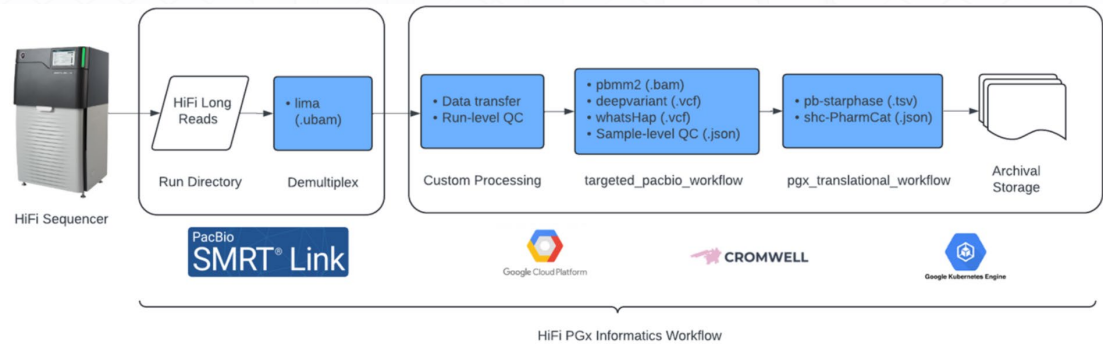


Figure 2: Illustration of Integrative Genomics Viewer

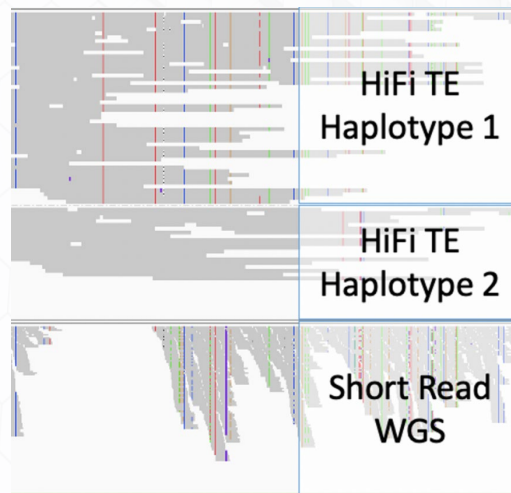


Table 2: Genetic Testing Reference Material List

Sample	<i>HLA-A</i>	<i>HLA-B</i>
NA02016	*30:02:01:01/*02:01:01:01	*58:01:01:01/*53:01:01:01
NA17019	*11:01:01:01/*02:01:01:01	*15:02:01:01/*15:11:01
NA17039	*30:01:01:01/*31:01:02:01	*42:02:01:02/*35:08:01:01
NA17229	*31:01:02:01/*24:02:01:01	*35:01:01:05/*35:20:01
NA17235	*03:01:01:01/*03:01:01:01	*07:02:01:01/*40:01:02:04
NA17240	*30:02:01:01/*03:01:01:01	*57:01:01:01/*07:02:01:01
NA17247	*01:01:01:01/*24:03:01:01	*57:01:01:01/*44:03:01:19
NA17277	*31:01:02:01/*24:02:01:01	*56:01:01:04/*27:05:02:05
NA17281	*31:01:02:01/*02:01:01:01	*56:01:01:04/*39:06:02:01
NA17288	*01:01:01:01/*02:01:01:01	*57:01:01:01/*44:02:01:01
NA17290	*11:01:01:01/*26:01:01:01	*57:01:01:01/*07:02:01:01
NA23090	*11:01:01:01/*11:01:01:01	*51:01:01:01/*15:02:01:01
NA23093	*11:01:01:01/*11:01:01:01	*15:02:01:01/*15:02:01:01
NA17244	*30:02:01:01/*02:01:01:01	*13:02:01:01/*51:01:01:03

Table 3: Analytical Performance Characteristics of *HLA-A* and *-B* alleles by Hifi Sequencing

Allele	TP	FN	TN	FP	Accuracy	Sensitivity	Specificity
<i>HLA-A*31:01</i>	13	0	81	0	>99.9%	>99.9%	>99.9%
<i>HLA-B*15:02</i>	9	0	85	0	>99.9%	>99.9%	>99.9%
<i>HLA-B*57:01</i>	10	0	84	0	>99.9%	>99.9%	>99.9%
<i>HLA-B*58:01</i>	8	0	86	0	>99.9%	>99.9%	>99.9%
All	40	0	54	0	>99.9%	>99.9%	>99.9%

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