

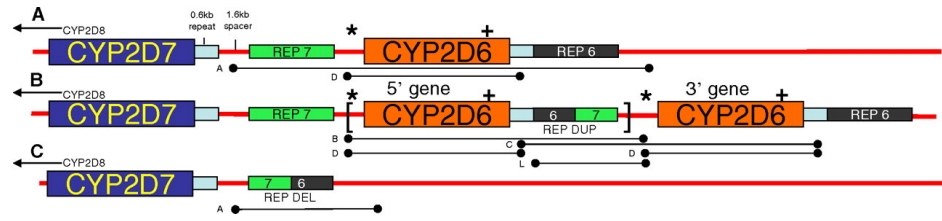


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Full-Length Sequencing of *CYP2D6* Locus with HiFi Reads Increases Genotyping Accuracy

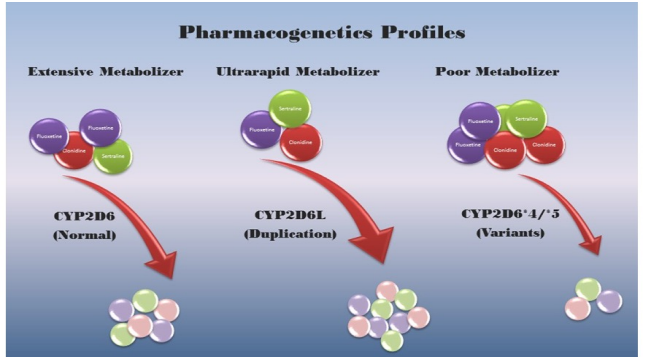
Lei Zhu, John Harting, Aaron Wenger, Zev Kronenberg, Josiah Wilcots, Jonas Korlach, Primo Baybayan

HIGHLY VARIABLE CYP2D6 IMPACTS DRUG METABOLISM



- **CYP2D6**: cytochrome P450 oxidase family
Affects drug metabolism in 25% of most prescribed drugs
- **CYP2D6** is highly variable in individuals
Valuable for personalized medicine

Black et al., 2012; Butler, 2018; Gates et al., 2006



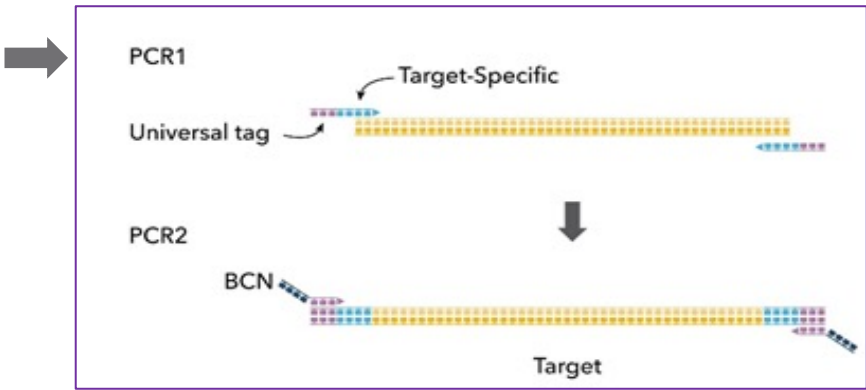
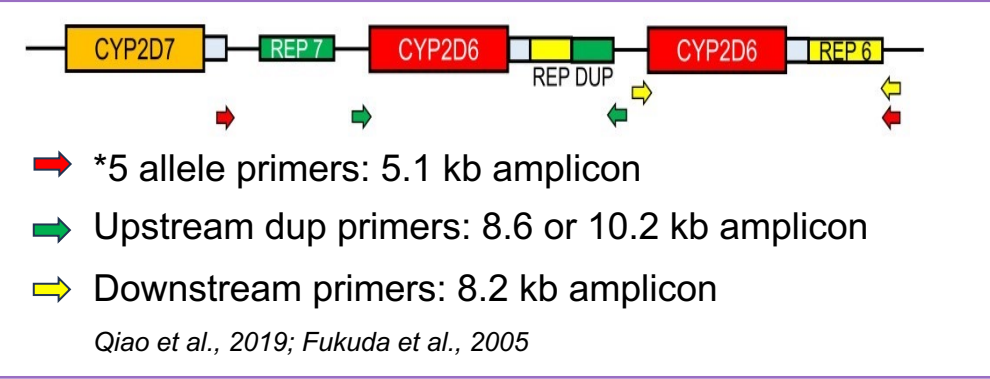
Pharmacogenetics Profiles	Extensive Metabolizer	Ultrarapid Metabolizer	Poor Metabolizer
Cardioactive drugs	CYP2D6 (Normal)	CYP2D6L (Duplication)	CYP2D6*4/*5 (Variants)
Antidepressants			
Antipsychotic			
Beta-blockers			
Antihypercholesterolemic			
Analgesics			

Amiodarone, encainide, flecainide, lidocaine, mexiletine, propafenone,
Amitriptyline, clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, nortriptyline, paroxetine, trazodone, venlafaxine
Chlorpromazine, haloperidol, perphenazine, quetiapine, risperidone, thioridazine,
Alprenolol, carvedilol, labetalol, metoprolol, penbutolol, pindolol, propafenone, propranolol, timolol
Simvastatin
Codeine, fentanyl, meperidine, oxycodone, propoxyphene, tramadol

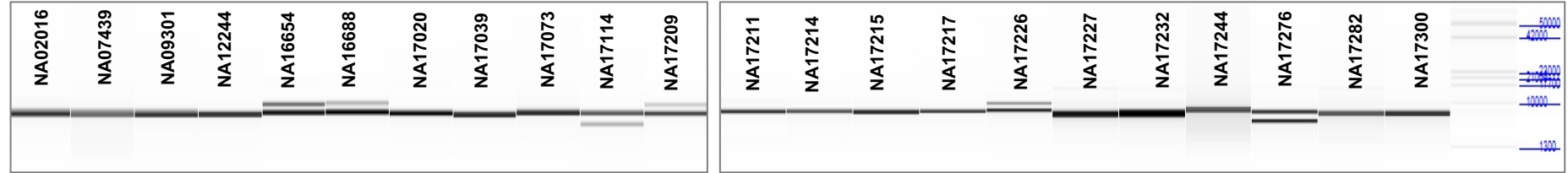
THREE-AMPLICON APPROACH TO AMPLIFY CYP2D6

Gene-Specific Primers in PCR1 for the Amplification of Upstream, Downstream, and *5 Allele, Respectively

2-Step Barcoded PCR Strategy for Multiplexing

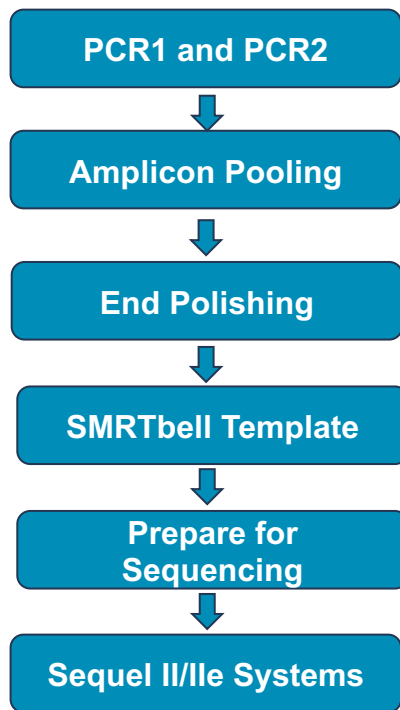


Barcoded PCR2 Products from CYP2D6 Gene Locus



CYP2D6 allele sequences of 22 Coriell reference samples were amplified from the three-amplicon approach.

LIBRARY PREP WORKFLOW TO GENERATE FULL-LENGTH *CYP2D6* HIFI READS



20-Hour Sequencing



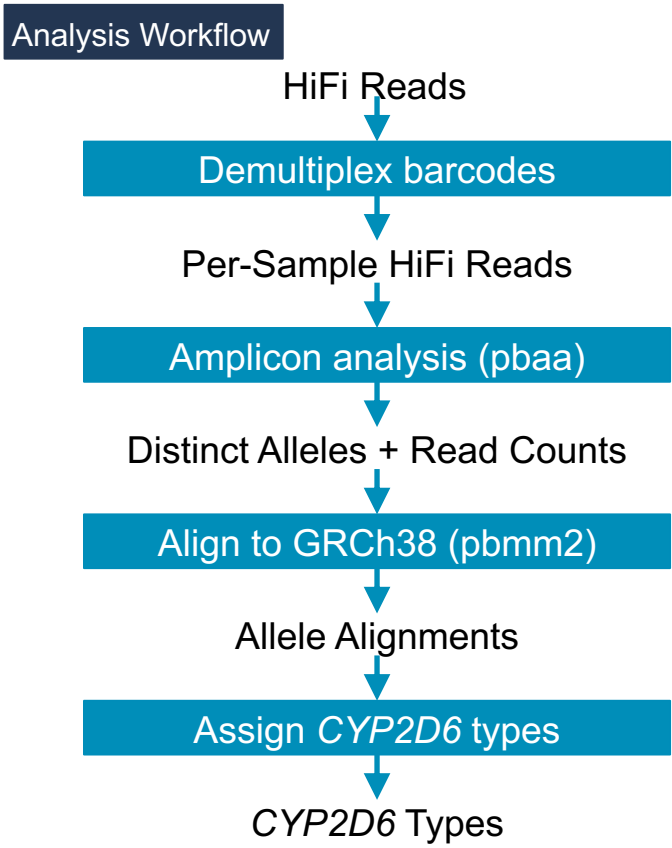
SMRT Link v10.1

HiFi Reads Available on SMRT Link

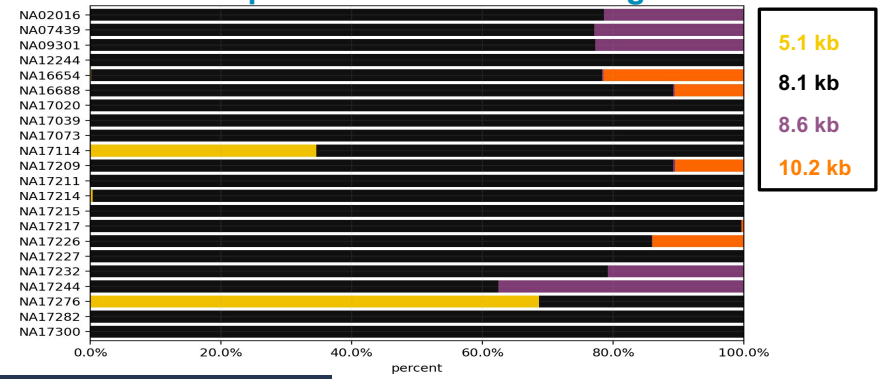
Analysis Metric	HiFi Data per SMRT Cell
HiFi Reads	1,639,369
HiFi Yield (bp)	12,219,494,057
HiFi Read Length (mean, bp)	7,453
HiFi Read Quality (median)	Q31
HiFi Number of Passes (mean)	12

From PCR Products to SMRTbell Library

DATA ANALYSIS WITH PBAA*



99% of Demultiplexed Reads Are On-Target to *CYP2D6*



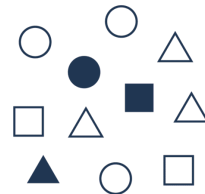
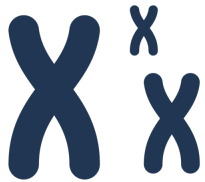
CYP2D6 Allele Type Calling from pbaa

Sample	<i>CYP2D6</i> Reference Alleles	HiFi + pbaa Calling	Sample	<i>CYP2D6</i> Reference Alleles	HiFi + pbaa Calling
NA02016	*2×N/*17	*2×2/*17	NA17211	*2/*4	*2/*4
NA07439	*4×N/*41	*4×2/*41	NA17214	*2/*2	*2/*2
NA09301	Duplication	*1/*2×2	NA17215	*4/*41	*4/*41
NA12244	*35/*41	*35/*41	NA17217	*1/*41	*33/*41
NA16654	*10/*10	*10 + *36	NA17226	*4/*4	*4 + *36
NA16688	*2/*10	*2/*10 + *36	NA17227	*1/*9	*1/*9
NA17020	*1/*10	*1/*10	NA17232	*2/*2×N	*2×2/*35
NA17039	*2/*17	*2/*17	NA17244	DUP *4/*2A	*2×2/*4
NA17073	*1/*17	*1/*17	NA17276	*2/*5	*2/*5
NA17114	*1/*5	*1/*5	NA17282	*41/*41	*41/*41
NA17209	*1/*4	*1/*4 + *36	NA17300	*1/*6	*1/*6

* PBAA, PacBio Amplicon Analysis, <https://github.com/PacificBiosciences/pbAA>

CONCLUSIONS

- Three primer sets used for this study generate specific amplicons for downstream, upstream dup and *5 allele across all the samples tested.
- The 2-step barcoded PCR strategy with three-amplicon approach produces very reliable and high-quality results.
- Nearly all (>99%) demultiplexed reads were on target to *CYP2D6*.
- HiFi long-read sequencing provides base-to-base sequences throughout the targeted region.
- Full-length *CYP2D6* sequences from PacBio Sequencing allows for accurate detection of the polymorphic gene locus.





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