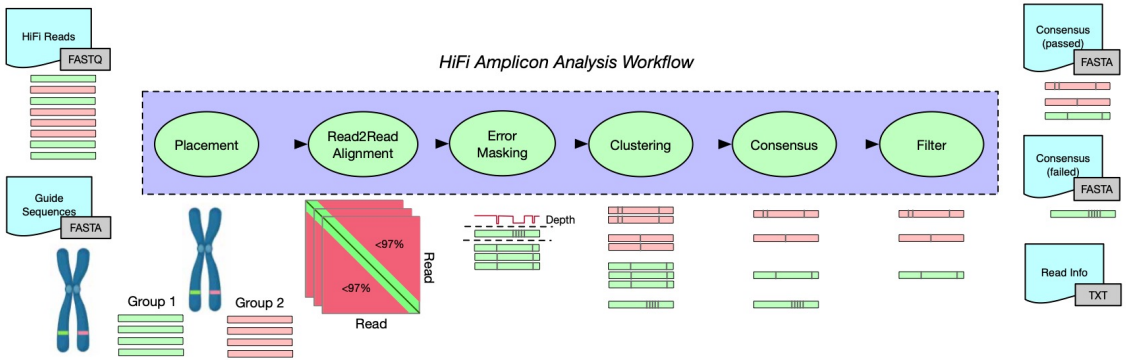
Resolving complex pathogenic alleles using HiFi long-range amplicon data and a new clustering algorithm

J. Harting[1], C. Heiner[1], I. McLaughlin[1], Z. Kronenberg[1]

[1]Pacific Biosciences, Menlo Park, CA, USA

Introduction

Genetic diseases caused by pathogenic variants in genes with highly homologous pseudogenes commonly include structural rearrangements and distantly separated heterozygous SNV that are difficult to call.



We leverage highly accurate, long-range single-molecule HiFi reads to accurately call a wide range of complex disease-causing variants in two gene-pseudogene systems, CYP21A2 and GBA, by applying a single multiplexed long-range paired assay to each target.

https://github.com/PacificBiosciences/pbAA

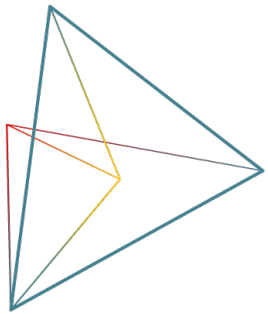
TARGETS AND ANALYSIS METHODS

Targets

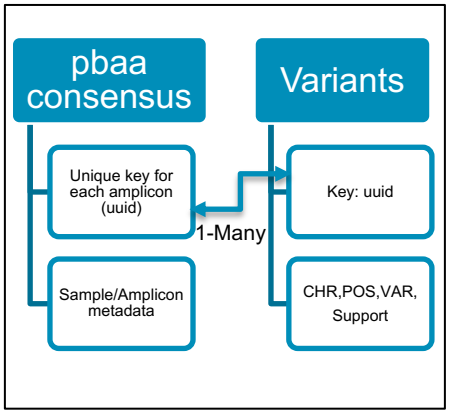
- Co-Amplified Targets
  - *7 samples: CYP21A2* (10kb)/*CYP21A1P* (8kb)
  - *13 samples: GBA* (12kb)/*GBAP1* (15kb)
- Samples with known difficult genotypes from Coriell
- Primers designed to include full-length genes, pseudogenes, and flanking regions for comprehensive variant detection.
- Sequencing on PacBio Sequel Systems.
- See **P15.015.C** - ***Targeting clinically significant dark regions of the human genome with high-accuracy, long-read sequencing*** for details on experimental design and sequencing.

Analysis

- Demultiplex and identify primers
- Deconvolute and generate consensus with pbaa
- Call variants from consensus
- Import to annotation database
  - Gene features (e.g. gene and exons coordinates)
  - ClinVar annotations
  - Call large SV
- Export Report and/or VCF



pbAA



Variant Calling

Annotation

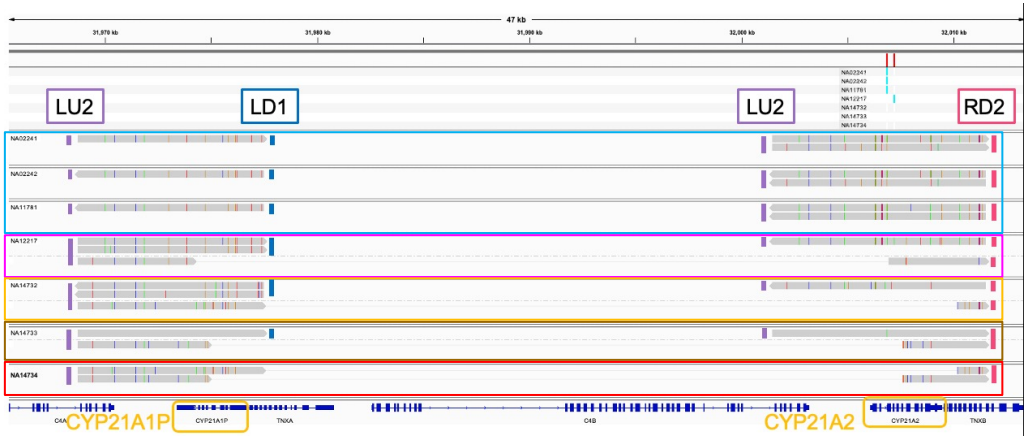
Report

VCF

RESULTS: *CYP21A2 / CYP21A1P*

Sample	Status	Correctly typed	CYP21A2 Alleles	Pathogenic Variant Calls	CYP21A1P Alleles
NA02241	Affected	✓	2	Homozygous SNV	1
NA02242	Affected	✓	2	Homozygous SNV	1
NA11781	Affected	✓	2	Homozygous SNV	1
NA12217	Affected	✓	1	Homo SNV & Fusion	3
NA14732	Carrier	✓	1	CYP21A2 Deleted	3
NA14733	Carrier	✓	1	Gene Fusion	2
NA14734	Affected	✓	0	Deletion & Fusion	2

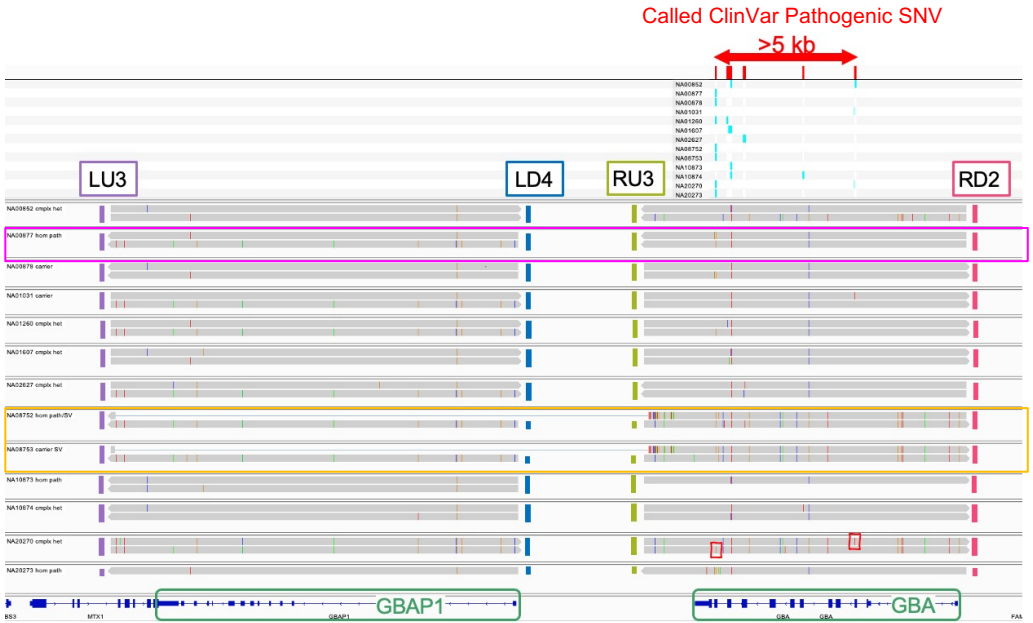
trio



- Common large (~30 kb) deletions occur in as many as 30% of some populations.
- 3 unique deletions identified in NA12217, NA14732, NA14733; 2 were confirmed in proband sample NA14734
- Copy number variation (CNV) for both gene and pseudogene are common.
- CNV is identified for *CYP21A1P* in NA12217 and NA14732
- Long-range amplicons confirm 2 unique alleles for homozygous calls in NA02241, NA02242, NA11781

RESULTS: *GBA / GBAP1*

Sample	Status	Correctly typed	GBA Alleles	Pathogenic Variant Calls	Variant Separation	GBAP1 Alleles
NA00852	Affected	✓	2	Complex Hetero SNV	4817 bp	2
NA00877	Affected	✓	2	Homozygous SNV	-	2
NA00878	Carrier	✓	2	Heterozygous SNV	-	2
NA01031	Carrier	✓	2	Heterozygous SNV	-	2
NA01260	Affected	✓	2	Complex Hetero SNV	456 bp	2
NA01607	Affected	✓	2	Complex Hetero SNV	71 bp	2
NA02627	Affected	✓	2	Complex Hetero SNV	51 bp	2
NA08752	Affected	✓	2	Homo SNV & Fusion	-	1
NA08753	Carrier	✓	2	Heterozygous SNV	-	1
NA10873	Affected	✓	1	Homozygous SNV	-	2
NA10874	Affected	✓	2	Complex Hetero SNV	2786 bp	2
NA20270	Affected	✓	2	Complex Hetero SNV	5377 bp	2
NA20273	Affected	✓	1	Homozygous SNV	-	1



- Phased complex heterozygous calls span genomic distances > 5 kb
- Homozygous SNV in NA00877 confirmed with 2 unique long-range alleles
- Homozygous SNV in NA08752 linked to deletion of *GBAP1* from the mother, NA08753. Paternal allele separately identified.

CONCLUSION

PacBio HiFi reads, with the features shown here, have the potential to greatly increase resolution in difficult regions of the genome for all types of genetic variation:

- Comprehensive variant detection
- SNV and multi-kb structural variants
- Accurate and reproducible
- Phased results
- Uniquely map-able to gene or pseudogene
- Single targeted assay

HiFi reads and pbaa capture 3 unique SV in a single family, including a duplicate pseudogene that differs by a single SNV in an 8kb amplicon