Introduction

While many assays and technologies exist for germline pharmacogenomic testing, several clinically-relevant pharmacogenomic loci remain challenging to characterize due to low sequence complexity and/or the presence of highly homologous pseudogenes. Long and accurate PacBio HiFi sequencing enables:

- Comprehensive detection of genetic variation, including SNPs, indels and structural variants
- Unambiguous haplotyping resolution through direct phasing, without the need for imputation
- Ancestry-agnostic capture of novel and rare variants

Targeted sequencing allows for high-resolution characterization of gene panels at a scale and cost that is more accessible than whole genome sequencing. We describe a method to leverage Twist Bioscience’s double-stranded DNA probes that can be individually tuned to enrich target regions with exceptional uniformity and fully capture a panel of 20+ pharmacogenes, reducing the overall cost of sequencing.

Panel design

A pharmacogenomics research panel was developed through the Twist Bioscience custom panel design process. Probes were optimized using a proprietary algorithm to enable balanced capture of complex regions. Probes were designed to cover a 2 Mb target region of interest with sparse ligation density at 0.1x.

Sample preparation, capture, and sequencing

We used HG002 and 23 Coriell Get-RM samples1 to evaluate the gene panel. Laboratory methods are described below (Fig. 1):

- Shear DNA
- End-repair / A-tailing
- Adapter ligation
- Library pooling
- Hybridization with probes
- Capture & wash
- Post-capture PCR
- SMRTbell library
- Repairs

Results

24 GeT-RM Coriell samples were sequenced on 1 SMRT Cell 8M on the Sequel IIe system (Table 2). Samples had on average 117 k HiFi reads, with a mean on-target read length of ~6.5 kb (Fig. 3). The percent of targeted regions covered was fairly uniform except for two samples, NA18518 and NA18868 (Fig. 4). Across all samples, 94% of target regions exceeded 30x coverage.

Data analysis workflow

SMRT Link was used to generate HiFi reads, remove PCR duplicates, and demultiplex, and a PacBio WGS pipeline was used to call variants for individual samples (Fig. 2).

Table 1. Targets included in the pharmacogenomics panel.

Table 2. HiFi sequencing metrics.

Conclusions

We demonstrate a long-read capture method using Twist Bioscience enrichment probes to accurately and efficiently capture a research panel of 23 pharmacogenomic targets. This approach may be applied broadly to other custom gene panels, allowing access to the benefits of long-read HiFi sequencing in a targeted, high-throughput, and cost-effective manner.

References


References