Concordance during technology upgrades: Psomagen customer case study illustrating Orchid Health's conversion to the NovaSeq X Plus

Introduction

Psomagen, Inc. provides multiomic next-generation sequencing services on many cutting-edge instruments, including the Illumina NovaSeq X Plus and NovaSeq 6000. In May of this year, Psomagen became one of three NovaSeq X Plus launch partners in the US. The groundbreaking NovaSeq X Plus technology uses XLEAP-SBS chemistry to deliver the highest level of output, data accuracy, and performance.

With NovaSeq X Plus instruments, Psomagen supports a wide range of applications including whole genome sequencing, whole genome bisulfite sequencing, whole exome sequencing, bulk and single-cell RNA transcriptomics, applications like the Olink PEA biomarker assay, and many more.

In this paper, we share results from Psomagen client Orchid Health as they compared NovaSeq 6000 and NovaSeq X Plus performance on their preimplantation genetic testing (PGT) whole genome assay, designed to profile variants in amplified embryo biopsies. Orchid Health's mission is to help reveal how genetics influence a child's health during the IVF process, by quantifying the level of genetic risk parent(s) can pass on to their children. This concordance test focused on the genomic coverage, accuracy, precision, sensitivity, and specificity of both Illumina instruments.

Concordance Analysis

Samples

The same pool of DNA libraries prepared from 5 Genome in a Bottle (GIAB) cell lines were sequenced using whole genome sequencing on the NovaSeq 6000 and NovaSeq X Plus at 30x coverage. Notably, each DNA sample prior to library construction was diluted to a picogram followed by a whole genome amplification to mimic the amount of DNA extracted during PGT.

Analysis

Psomagen delivered sequencing results in FASTQ files. Orchid analyzed the data from the NovaSeq 6000 and X Plus through their clinically validated pipeline without further modifications. Comparative analysis was performed with an emphasis on the genomic coverage, accuracy, precision, sensitivity and limit of detection. All of the above parameters were critical evaluating whether the new sequencer is clinically satisfying test requirements. No GIAB benchmark regions were excluded.

Results

Coverage. The average of mean coverage and percentage of genomic coverage on the NovaSeq X Plus was 39.1x and 98.6%, compared to 36.1x and 98.6% on the NovaSeq 6000. According to Dhruva Chandramohan, Orchid Health Lead Bioinformatics Scientist, "After running both GIAB datasets through our current pipeline and applying our standard variant filters, we evaluated accuracy, sensitivity, and specificity of the callsets against the matching benchmark calls for each sample."

Chandramohan continued, "What we observed is that the NovaX achieved on average higher accuracy, specificity, sensitivity, and comparable precision to the Nova 6000."

Depth. On average, NovaSeq X Plus provided an additional 3x reads compared to the NovaSeq 6000.

The table below shows the performance summary of GIAB samples in NovaSeq 6000 vs. NovaSeq X Plus. Statistics are reported as the mean over the samples using Orchid's internal variant filters.

Sequencer	Coverage	Accuracy	Sensitivity	Precision	Specificity
NovaSeq X Plus	99%	99.99%	97.94%	99.39%	99.99%
NovaSeq 6000	99%	99.99%	97.58%	99.55%	99.99%

Table 1. Calling performance of NovaSeq 6000 and X Plus on Orchid Amplified GIAB ≥5 Samples.
Callers were filtered using Orchid's internal filters. Percentages are out of all calls in each group.

Limit of detection at depth. The same limit of detection was obtained.

The concordance of the NovaSeq 6000 and NovaSeq X Plus was reported as well. A 98% concordance in variants was observed with ~0.3-0.5% being false positives but most of them can be resolved by Sanger confirmation.

Summary

After conducting these runs, Orchid Health scientists concluded both NovaSeq 6000 and NovaSeq X Plus are performing equally well on data quality and reproducibility satisfying clinical need, and that they would be able to maintain their level of performance while transitioning their workflows to the NovaSeq X Plus with minimum changes to the current wet lab and dry lab workflow.

At a depth of 30x, the NovaSeq X Plus achieves high accuracy, sensitivity, specificity, and comparable precision using Orchid Health's pipeline. The technology was able to consistently call the variants necessary for Orchid's applications.

For additional information about Psomagen's services on the NovaSeq X Plus, please visit landing.psomagen.com/novaseq-x-plus.