

Optimizing clinical next-generation sequencing workflows through evidence-based expansion of statistical confirmation criteria

Authors:

Jared Evans (Ambry Genetics), Rebecca Mar-Heyming (Ambry Genetics), Marcus Mahar (Ambry Genetics), Katherine Shortt (Ambry Genetics)

Abstract:

Variant confirmation by statistics is an established method to optimize clinical next-generation sequencing (NGS) workflows, but criteria are often conservatively defined. Strategically routing a subset of variants for orthogonal confirmation allows the upstream primary variant calling pipeline to remain highly sensitive and aggressive. We evaluated clinical confirmation data and ground truth samples from cancer multi-gene panels, exomes, and whole-genome sequencing (WGS) from a credentialed diagnostic laboratory to build upon our existing validation framework and determine the accuracy of expanding statistical thresholds across various variant types and quality metrics.

Variant quality metrics including read depth, genotype quality, variant allele frequency, mapping quality, and strand bias were extracted from clinical pipelines. Machine learning models (XGBoost) were trained to differentiate high-confidence variant calls from those requiring orthogonal confirmation, prioritizing a recall of 1.0 to maintain strict clinical safety standards while maximizing workflow efficiency.

Our analysis demonstrated that refining multiple confirmation criteria safely and significantly reduces the Sanger sequencing burden. For cancer panels, increasing the insertion and deletion (indel) length threshold eligible for statistical confirmation from 3 to 50 base pairs (bp) safely eliminated Sanger follow-ups for 31% of indels (~ 8,700 variants). Additionally, identifying and adding top recurring false-positive artifacts in genes like RUNX1, SMAD4, and MUTYH to an updated artifacts list provided a 10% reduction, yielding a total 41% decrease in confirmatory testing. In exome pipelines, expanding the eligible indel length threshold to 50 bp and optimizing allele balance windows achieved a 30% reduction in total Sanger volume. For WGS, combining stringent read depth, genotype quality, and allele frequency thresholds delivered a variant precision exceeding 0.999.

Strict exceptions were maintained for high-risk variants, such as those in PMS2, due to pseudogene interference. These findings demonstrate that data-driven expansion of statistical confirmation criteria -

spanning longer indels, optimized quality metrics, and artifact filtering - safely minimizes unnecessary Sanger testing, significantly improving clinical laboratory efficiency without compromising analytical accuracy.