A low-cost, flexible diagnostic test for hundreds of inherited conditions

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Abstract

Background: Historically, diagnostic testing for hereditary genetic disorders has been limited to testing small sets of genes in patients with specific clinical indications. The rationale for this strategy has been the high cost of standard assays (Sanger sequencing, MLPA, etc.) and the challenges clinically interpreting genetic results. However, this testing strategy can lead to underdiagnosis of genetic disorders or to expensive and time-consuming reflex testing to obtain a genetic diagnosis.

Methods: Invitae is a CLIA-certified laboratory with an in-house infrastructure for Next Generation Sequencing (NGS) assay development, validation, and operation. We have a hybrid calling and data QC pipeline employing GATK, Freebayes, and custom algorithms for different variant classes. Preliminary reports for known and novel SNVs, indels, and CNVs are automatically generated for review, and a team of medical specialists then classifies variants according to ACMG guidelines given the patient's indication and signs out final clinical reports. These processes are highly scalable, allowing the assay to grow to report on the vast majority of genetic conditions with high accuracy.

Results: 712 individuals have been tested to date in three validation studies. Over this combined set, in direct comparison with the "gold-standard" data, 100% analytic sensitivity and 100% analytic specificity have been observed for all pathogenic DNA variations within the analytic range of the Invitae test. Both small sequence changes as well as larger deletion/duplication events are included in this result. We note that this level of sensitivity and specificity was achieved from the primary sequencing data alone without either gap-filling or confirmation of pathogenic variants using an orthogonal technology. Nevertheless, Invitae's current standard testing procedures utilize both gap-filling and confirmation on patient samples to further ensure completeness and accuracy.

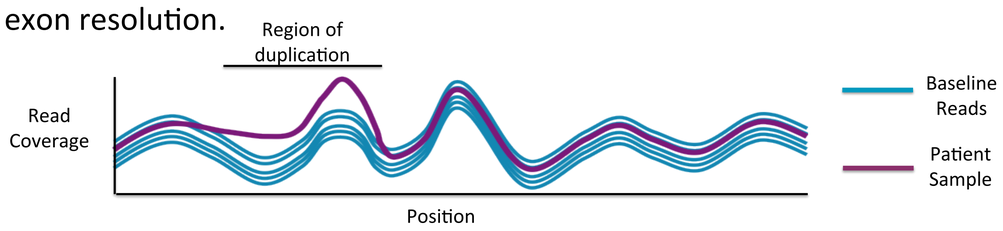
Conclusions: Our assay aggregates many genetic tests into a single low-cost assay. The clinical report is customized to the ordering specification, so that only the information sought by the clinician is reported. However, additional genetic information can easily be obtained by re-querying the assay's raw data. Thus, results on all the hereditary cancer genes can be reported at once or in a reflex fashion beginning with the highest index-of-suspicion genes. This process is achieved for less than one-third of the cost of current routine clinical testing for hereditary cancer.

Assay Design & Variant Classification

Assay Design: Invitae is a CLIA-certified clinical diagnostic laboratory performing full gene sequencing and deletion duplication analysis using next-generation sequencing technology.

Our sequencing analysis covers clinically-important regions of each gene including coding exons, at least +/- 10 base pairs of intronic sequence, and known pathogenic variants in non-coding regions.

Our deletion/duplication analysis detects most intragenic deletions and duplications at single exon resolution.



Variant Classification: Our variant interpretation adheres closely to the ACMG guidelines for variant classification. Each variant is evaluated based on:

- Consequences for mRNA splicing and on the encoded protein's physical structure.
- Frequency at which the variant is observed in the general population relative to the incidence of its associated disease.
- Evidence in the clinical literature regarding case histories, the pattern of variant segregation with disease in family pedigrees, and case-control studies.
- Evidence in scientific literature regarding functional studies of the variant's impact on protein function at the molecular and organismal levels.
- Information from Invitae's database regarding frequency and segregation of the variant with disease.
- Indirect evidence about the consequences of the sequence change, including the mutational spectrum associated with the gene and computational predictors.
- Indirect evidence about the the variant's position, such as evolutionary conservation.

Analytic Validation Summary

Analytic Validation Summary: To demonstrate the high-quality results of Invitae's testing, we collaborated with two major medical centers to test more than 700 patient samples. In direct comparison with the "gold- standard" data, 100% analytic sensitivity and 100% analytic specificity have been observed for all pathogenic DNA variations within the analytic range of the Invitae test.

Positive Events	Event Type
122	Single nucleotide changes
124	Sequence insertions, deletions, and del-ins (≤ 10bp)
6	Sequence insertions and deletions (≥ 10bp and < 1 exon)
22	Deletion or duplication (≥ 1 exon and ≤ 1 targeted gene)
22	Cytogenetic scale copy number changes
22	Events in/near specific homopolymer regions

Results	# of Events
Clinical Variants in Previous Data	318
True Positives: Previous variants also detected by Invitae.	316
Additional True Positives: Variants detected by Invitae, but not by previous lab. Invitae positive result confirmed by third-party lab	7
True Negatives: Invitae data negative but previous lab positive. Invitae negative result confirmed by third-party lab.	2
Total Confirmed True Positives	323
False Positives: Invitae positive, previous data negative; previous lab's result confirmed by third-party lab.	0
False Negatives: Invitae negative, previous data positive; previous lab's result confirmed by third-party lab.	0

Invitae's data shows a high level of concordance with the "gold-standard" data. Moreover, in the (very few) discordant cases, the Invitae result was always confirmed by send-out to the third-party laboratory. In more detail: 100% sensitivity for the Invitae test has been observed to date: Every known variant in the previous data was detected by Invitae, with the only exception being 2 sites which also were negative according to the third-party lab when sent out for resolution. This result is true across all sequence changes and also all deletion/duplication events. Similarly 100% specificity has also been observed. All together, 1192 individual gene tests were performed (counting all gene x individual combinations where data were available from both Invitae and from the previous lab for comparison). Among the 1192 individual gene tests, no false positives were present in the Invitae data: Every Invitae finding was either matched in the "gold-standard" or confirmed by the third-party lab.

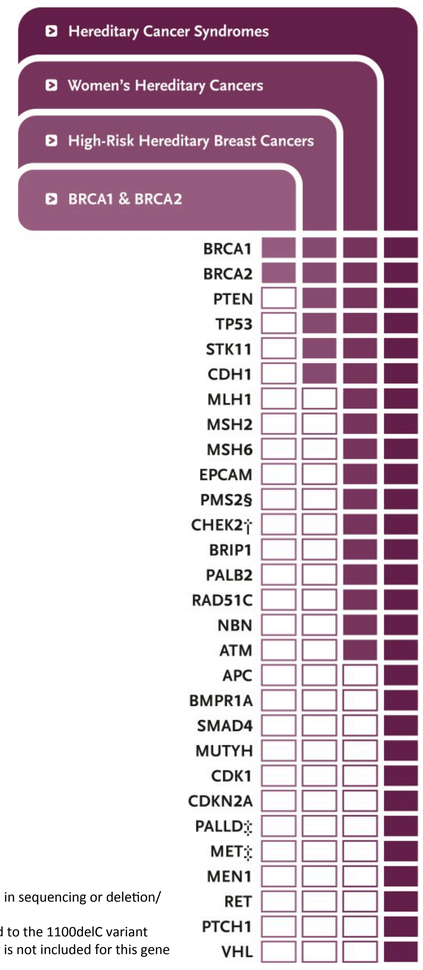
Low-cost, Flexible Genetic Testing Options

BRCA Testing Options:
Invitae offers clinicians the option to specifically order BRCA1 and BRCA2 testing or to select a broader multi-gene panel for the same low price.

One advantage of our next-generation sequencing (NGS) assay is aggregating many genes into a single low cost assay. When a clinician orders a test, he or she can specify which genes will be clinically interpreted by our sign-out team. This allows the clinician to control how much information is initially reported.

For instance, a clinician can order only BRCA1 and BRCA2 testing, or select any of the 6 Invitae curated cancer panels (3 are shown here), or order any combinations of the 29 cancer related genes offered by Invitae.

If the initial test report is negative or inconclusive, we offer the option to re-requisition additional genes for a broader analysis of the patient's indication for testing without additional cost to either the patient or the physician.



§Exons 12-15 are not included in sequencing or deletion/duplication analysis
†Sequencing analysis is limited to the 1100delC variant
‡Deletion/duplication analysis is not included for this gene