INVITAE

Detecting deletions and duplications using next-generation sequencing (NGS)

Diagnostic genetic testing requires a carefully constructed assay to thoroughly interrogate genes of medical importance. Invitae's assays comprehensively report sequence changes and deletion/duplication events in coding exons, splice sites, and other regions known to harbor pathogenic mutations.

What are deletions and duplications?

Copy-number variants (CNVs) include gross deletions (losses) or duplications (gains) of DNA material. Relatively small CNVs, ranging in size from a single exon to a full gene, are often called del/dup events to distinguish them from larger CNVs detected by traditional cytogenetics methods. Invitae's assay focuses on accurately detecting these del/dup events. While not intended to replace cytogenetics, the assay also detects the effect of larger CNVs on multiple genes when those genes are part of a test order.

Why are deletions and duplications clinically important?

Del/dup events account for a significant proportion of pathogenic changes in hereditary disorders (Table 1). Other commercially available gene panels and standard exome sequencing approaches do not always include del/dup testing or may have significant limitations, which can result in missing an important genetic change.

Table 1: Fraction of positive reports with del/dup findings

Clinical area	Fraction
Cardiology	5%
Epilepsy	15%
Hereditary Cancer	7%
Immunology	9%
Metabolic Disorders and Newborn Screening	8%
Neurology	39%
Pediatric Genetics	8%

What is Invitae's approach to deletions and duplications?

Invitae uses next-generation sequencing (NGS) to detect both del/dup events and sequence alterations. To detect del/dups, Invitae uses a custom-built, validated set of computer algorithms in conjunction with optimized biochemical methods. This combination has a number of advantages over traditional techniques:

- **Superior sensitivity:** Invitae's algorithms provide superior sensitivity for events as small as one exon and can even detect complex events invisible to traditional techniques.
- **Improved coverage:** Invitae's methods ensure that all exons of every gene, with very rare (and well documented) exceptions, are included in del/dup analysis. Traditional methods, as well as some other NGS approaches, may skip some exons and cannot always determine in which exons or genes they have adequate sensitivity.
- **Less expensive & faster turnaround:** As Invitae uses NGS itself for del/dup testing, there is no need for a separate specimen and there is never an additional cost (in contrast to traditional approaches elsewhere that require a second sample, a separate bill, and additional time). Invitae's method simultaneously identifies a broad spectrum of genetic changes, including single nucleotide variants, small indels, large indels, exonic del/dups, and certain rare copy number-neutral structural variants. All of Invitae's panel and gene tests have a turnaround time of 2 weeks on average.

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Clinically validated with 100% concordance

To validate Invitae's approach to del/dup calling, independently characterized specimens from almost 1,200 individuals were tested. These included:

- More than 1,000 patients prospectively accrued by two major cancer centers (Lincoln *et al.* J Mol Diag. 2015).
- Additional specimens known to contain del/dup events in various genes involved in cancer, cardiology, neurology, pediatric and other conditions.

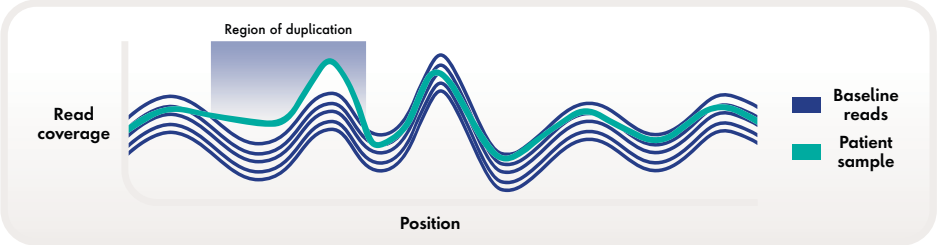
Table 2: Performance of Invitae's del/dup methods

Results		Details	Conclusions
False negatives	0	**137 of 137** previously known events were detected. This represents an effective validation population size of over 9,000 at 1.5% prevalence.	**100% sensitivity**
Detection rate	High	Over **143,000 patients** have been tested with **2,096 pathogenic del/dup** events reported in 230 distinct genes (Truty *et al. Genet Med.* 2018). Pathogenic del/dups comprise about 10% of positive findings overall, and are present in 1.9% of all patients tested.	**Prevalence fits expectations**
False positives	0	Candidate del/dup events are confirmed using a secondary assay. Very rarely, CNV calls detected with high confidence by NGS are reported with an explanation if orthogonal assays produce inconclusive results due to method-specific limitations.	**100% specificity**

Invitae's methods for detecting del/dup events

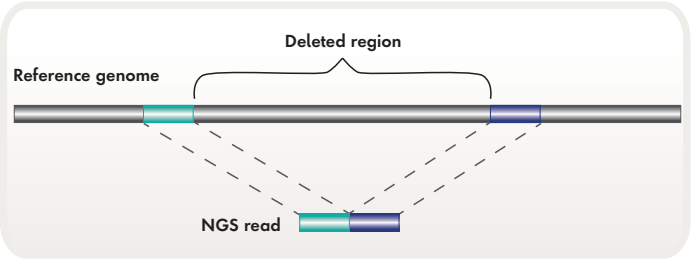
Invitae uses multiple custom approaches, each specifically designed to detect certain classes of events.

Figure 1: Read-depth approach to deletion/duplication analysis by NGS. Example of a duplication.



One approach based on NGS **read depth** calls events by calculating the statistical likelihood of each possible copy number by comparing the depth of sequence coverage to a calibrated set of baseline specimens (Figure 1). Invitae's biochemical methods ensure high reproducibility of coverage profiles across specimens, allowing robust detection of events as small as one exon. This approach is optimized for sensitivity, as reportable events are then confirmed with an independent technology (thus providing specificity).

Figure 2: Split-read approach to deletion/duplication analysis by NGS.



An additional set of methods based on **split-read analysis** detect other classes of events, particularly those with breakpoints in or near an exon, which often are missed by traditional approaches (Figure 2). Finally, specialized methods are used for del/dup analysis of particularly challenging regions, such as the highly homologous *SMN1* and *SMN2* genes, and the homologous pseudogene portion (exons 12–15) of *PMS2*.

Case study

A woman with breast cancer in her 30s and a strong family history was tested by Invitae. A duplication of exons 5–11 in *BRCA2* was detected using read-depth analysis (Figure 3). Breakpoints were identified using split-read analysis, showing the event to be a novel tandem duplication disrupting exon 11 (Figure 4). This finding was confirmed by Sanger sequencing. Other methods, such as qPCR, MLPA, or microarrays, would not have provided information about the breakpoints and would have made it challenging to determine if the event is pathogenic or not.

Figure 3: CNVitae read-depth plot showing detection of *BRCA2* exon 5–11 duplication

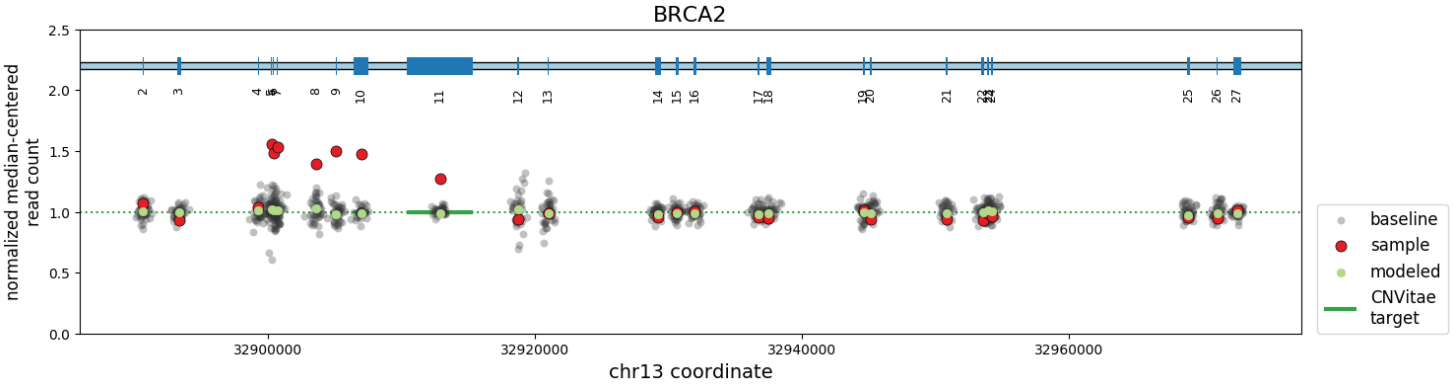
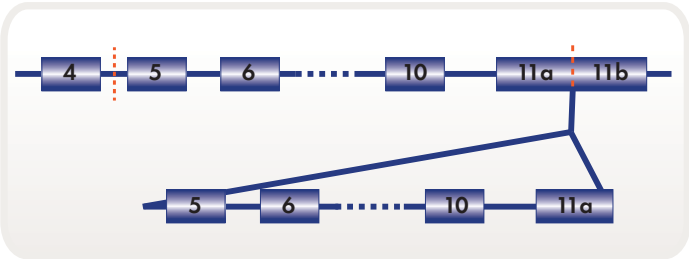


Figure 4: *BRCA2* exons 5–11 showing tandem duplication inserted into exon 11.



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