# Whole-Exome Sequencing Assay with Boosted Clinical Content and Sensitive Deletion/Duplication Detection Has a High Diagnostic Yield

## Blueprint Genetics

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## Background

Utility of Whole Exome Sequencing (WES) in clinical diagnostics has been limited by the non-uniform sequencing coverage across exons, leaving a substantial proportion of the regions with shallow coverage that prevents accurate variant detection. We evaluated a WES assay that is specifically designed for clinical use, enables uniform sequencing coverage resembling high-coverage gene-panel based assays, and provides high sensitivity in variant detection.

### Analytical validation of IDT xGen based BpG WES assay shows high sensitivity to detect sequence variants and small del/dups

**Table 1. Analytical validation of SNV and INDEL detection in IDT xGen based BpG WES assay.**

| Performance metric | Value | Measurements |
|---|---|---|
| Accuracy (SNVs) | 0.99999 | TN: 922,349,615 |
| Sensitivity (SNVs) | 0.99653 | TP: 412,456 |
| Specificity (SNVs) | 0.99999 | FP: 9,928 |
| Positive predictive value (SNVs) | 0.97681 | FN: 1,437 |
| Sensitivity (1-10 bp INDELs) | 0.96950* | TP/FN: 17,070 / 538 |
| Sensitivity (11-20 bp INDELs) | 0.98858 | TP/FN: 791 / 9 |
| Sensitivity (21-30 bp INDELs) | 1.00000 | TP/FN: 145 / 5 |
| Sensitivity (>= 31 bp INDELs) | 1.00000 | TP/FN: 19 / 0 |
| Nucleotides with >=20x sequencing depth | 99.4% | |
| Mean sequencing depth at nt level | 174x | |
| Repeatability | 0.997 | |
| Reproducibility | 0.997 | |

*Most missing calls are in intronic homopolymer regions.

Detection of del/dups involving one or more exons was performed using WES data. Comparison of expected and observed sequencing depths at targeted genomic regions was applied to detect CNVs. Two algorithms were assessed for detection of del/dups of variable sizes (**Table 2**).

**Table 2. Analytical validation of del/dup detection in the WES assay.**

| Performance metric | Value |
|---|---|
| **Method aimed to detect larger del/dups (CNVkit)** | |
| Sensitivity ( 1 exon) | 0.44 |
| Sensitivity (5 exon) | 0.99 |
| **Method aimed to detect smaller del/dups (in-house developed)** | |
| Sensitivity (1 exon, hom) | 0.99 |
| Sensitivity (1 exon, het) | 0.93 |

### Improved coverage in clinically relevant and difficult-to-sequence regions

**Figure 3. IDT xGen based WES assay shows improved coverage in difficult regions such as *RPGR* ORF15.**
All tested seven known truncating variants in the regions were detectable from the IDT xGen data.
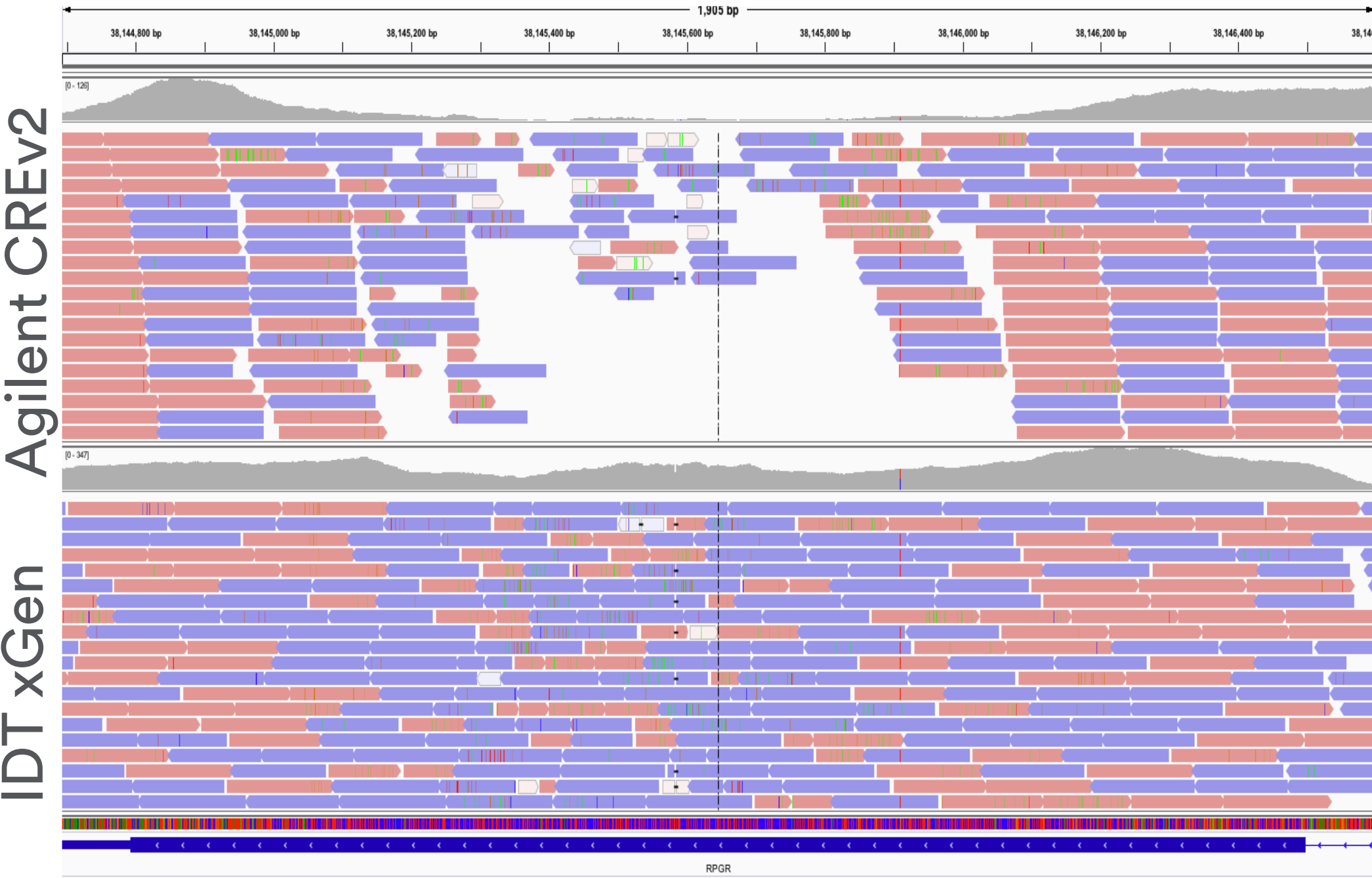


## Methods

We performed WES capture experiments using an assay with boosted clinical content, namely IDT xGen Exome Research Panel assay that was spiked-in with custom designed clinical content including baits for >1,400 clinically relevant non-coding variants. Sequencing was performed at Blueprint Genetics (BpG) using an Illumina NovaSeq sequencing system and data was downsampled to 100M reads. Performance of the assay was assessed by using reference samples with high-quality sequence variant calls, or samples with known clinically relevant del/dups.

### BpG WES has a high diagnostic yield with diagnoses involving non-coding variants, small del/dups, and likely diagnostic candidate variants in novel disease genes

**Figure 1. Diagnostic yield of BpG WES (n=258).**
Categories with definite or highly suspicious findings in known disease genes, or strong candidates in novel disease genes account together for 45% of all cases. Definite diagnoses in known disease genes occur more often in Family WES than in proband only WES (38% vs 33%).
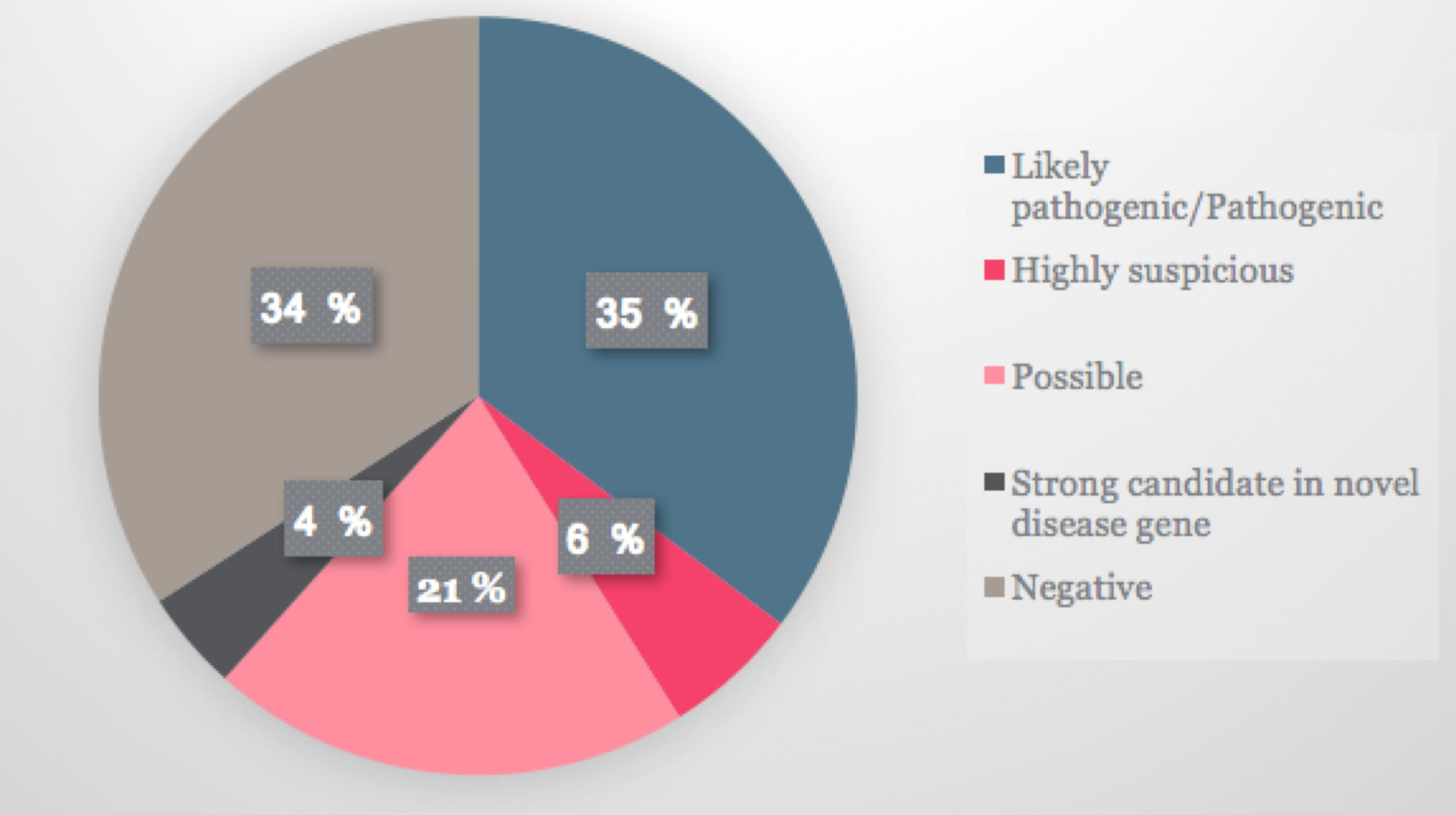


**Figure 2. Examples of patients where a comprehensive assay is required for the genetic diagnosis.**
A) Patient with Usher syndrome is compound heterozygous for a known pathogenic missense variant and a 2-exon heterozygous deletion in *USH2A*. B) Patient with propionic acidemia is compound heterozygous for a known pathogenic synonymous variant and a 12-exon heterozygous deletion in *PCCA*.
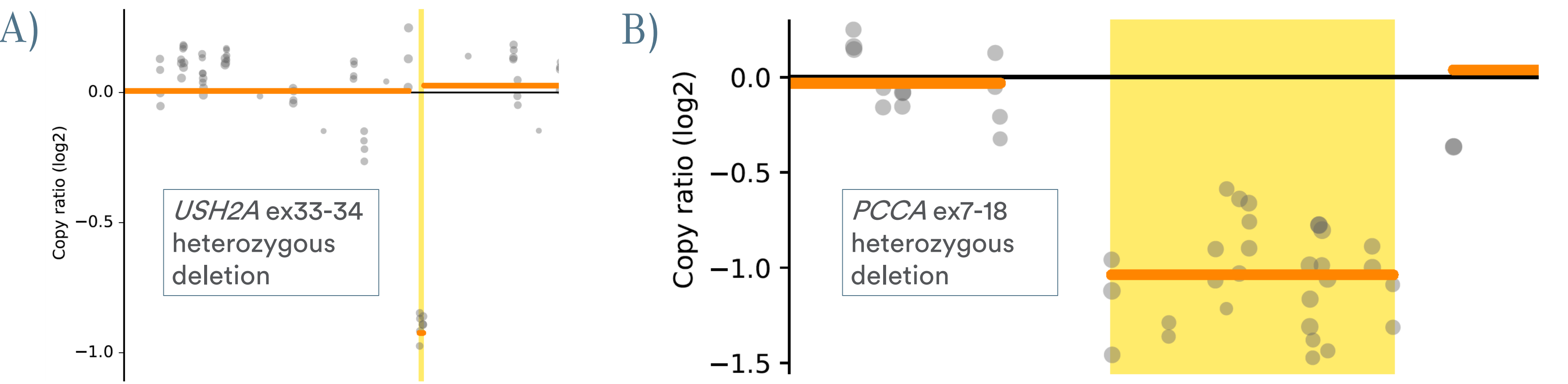


### Summary

- IDT xGen based BpG WES assay provides high and uniform sequencing coverage allowing sensitive detection of both sequence variants and small del/dups.
- BpG WES assay is boosted with baits for >1,400 clinically relevant non-coding variants.
- BpG WES results in a likely diagnosis in known disease genes for 41% of cases, supplemented by strong candidate variant findings in 4%.