Validating and Optimizing NGS-Based Diagnostics to Improve Diagnostic Yield and Clinical Utility

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Background

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

Methods

WES capture experiments were performed 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. The data was downsampled to 100M reads. Reference samples with high-quality sequence variant calls, or samples with known clinically relevant del/dups were used to assess the performance of the assay.

Results

Analytical validation of IDT xGen based BpG WES assay demonstrates a 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 Mean sequencing depth at nt level	99.4% 174x	
Repeatability	0.997	
Reproducibility	0.997	

*Most missing calls are in intronic homopolymer regions.

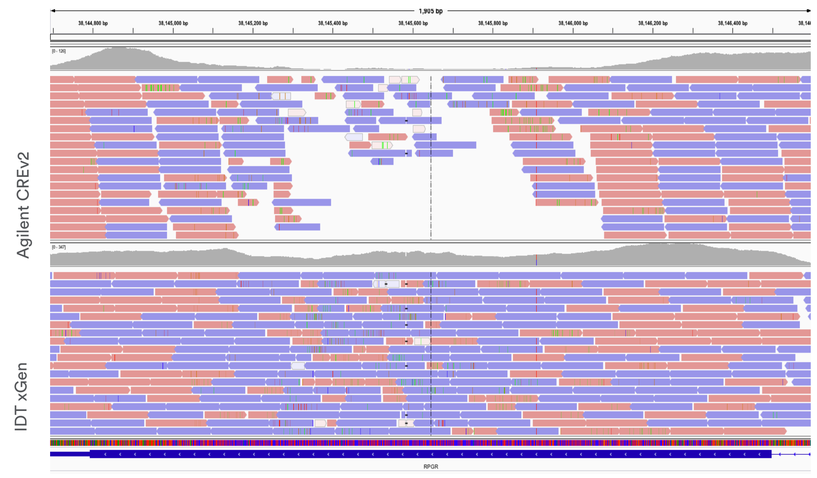
Del/dup detection involving one or more exons was performed using WES data. Comparison of expected and observed sequencing depths at targeted genomic regions was applied to detect CNVs. Two algorithms were assessed for detection of del/dups of variable sizes (**Table 2**).

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

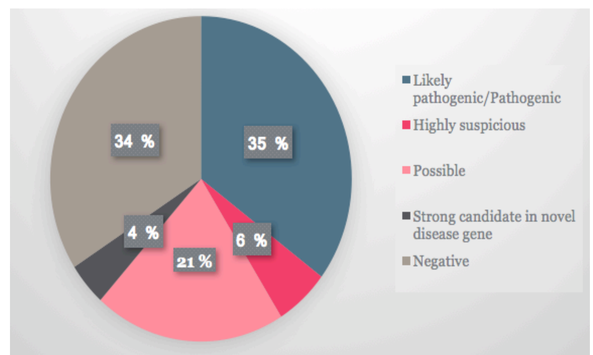
Figure 3. IDT xGen based WES assay shows improved coverage in difficult regions such as RPGR-ORF15.



All tested seven known truncating variants in the regions were detectable from the IDT xGen data.

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

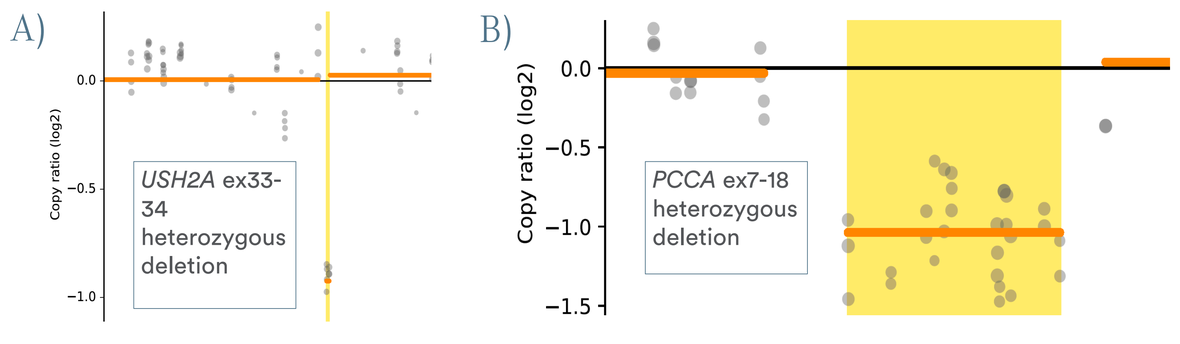
Figure 1. Diagnostic yield of BpG WES (n=258).



Categories with definite or highly suspicious findings in known disease genes, or strong candidates in novel disease genes account 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) B) Patients with propionic acidemia are compound heterozygous for a known pathogenic synonymous variant and a 12-exon heterozygous deletion in ***PCCA.***



Summary

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

Conflict of interest statement: All authors are employed by Blueprint Genetics.