Analytic Validation of a Next Generation Sequencing Based Primary Immunodeficiency Panel

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INTRODUCTION

Genetics diagnostics of patients with suspicion of immunodeficiency has been affected by poorly validated genetic tests, lack of transparency, and testing solutions that are not optimized for maximal diagnostic yield. Our goal was to develop a high quality next generation sequencing (NGS) platform, transparently and comprehensively validate its quality and performance, and report our experiences with hundreds of patients suspected with primary immunodeficiency (PID).

METHODS

We developed and validated a 274 gene Primary immunodeficiency panel, with traceable sample sets, for detecting single-nucleotide variants (SNVs), insertions and deletions (INDELs) and copy number variants (CNVs). Our assay targeted the coding exons, the splice regions, and selected deep intronic variants. We also utilized our platform to diagnose hundreds of patients during 2016-17. We report here our experiences with our previous 230 gene PID panel.

RESULTS

On average, we demonstrate 0.997 sensitivity and 0.999 specificity for detecting SNVs and 0.969 ,0.989, and 0.999 sensitivity for detecting INDELs of 1-10, 11-20, and 21-30 bases, respectively. Repeatability and reproducibility of the assays were reported at 0.997 and 0.997. 99.6% of the target regions were covered with over 20x sequencing depth and >Q20 mapping quality for our validation samples. We showed that the assays had a 0.923 sensitivity to detect single-exon deletions and 0.99 sensitivity to detect copy number aberrations covering two exons. Using ACMG guidelines for variant classification, a diagnosis was established in 13% of PID suspicious patients (the cohort did not include SCID patients). Clinically significant findings were found in 44 different genes.

CONCLUSIONS

Our results demonstrate the analytic validity of the developed tests and show that the technology is well-suited for clinical diagnostics of immune disorders. It also demonstrated a cost-effective diagnostic tool to simultaneously diagnose various types of mutations from SNVs to copy number variations.

Table 1. Characteristics of the Primary Immunodeficiency Panel

Primary Immunodeficiency Panel	
Genes	274
Exons	6985
Bases	800733
Mean Depth of Coverage (validation samples)	186x
Mean Depth of Coverage (production samples)	>200x
Base Pair Coverage at minimum 20x (validation samples)	99.6%
Base Pair Coverage at minimum 20x (production samples)	99.6%
Sensitivity (41-50 INDELs)	1
Non coding variants covered	>120
CNV analysis	Yes

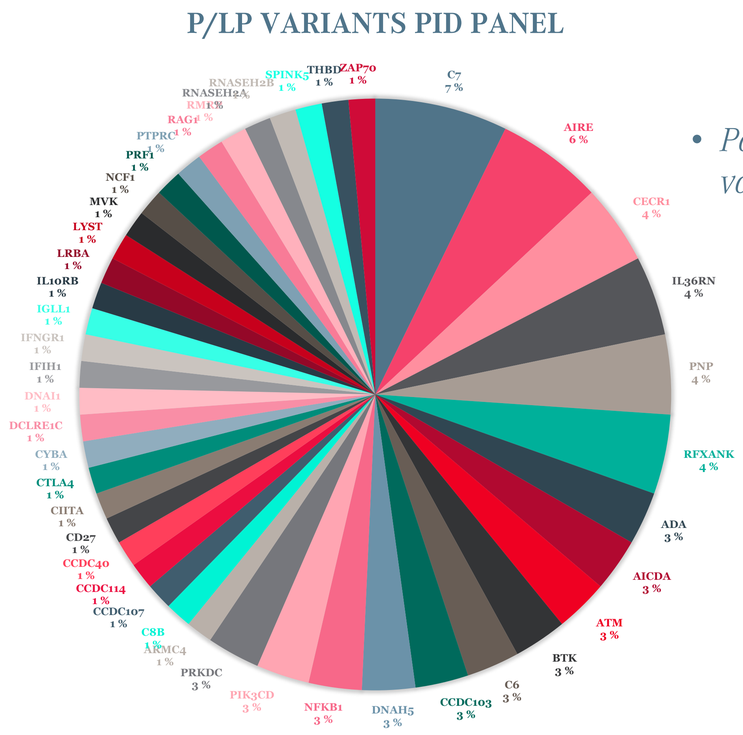
Table 2. Analytic Validation of Copy Number Variants

Performance Metric	Value	Approach
Clinical sensitivity* (1 exon)	92.3%	24/26 detected
Clinical sensitivity* (2 exon)	100.0%	11/11 detected
Clinical sensitivity* (3-7 exon)	93.3%	14/15 detected
Clinical sensitivity** (Microdeletion syndromes, 0.10-47 Mb)	100.0%	37/37 detected

Table 3. Analytic Validation of SNVs and INDELs

Performance Metric	Value	Approach
Accuracy (SNVs) Sensitivity (SNVs) Specificity (SNVs)	99.9 % 99.7% 99.9%	TN: 922, 349, 615 TP: 412, 456 FP: 9,928 FN: 1,437
Sensitivity (1-10 bp INDELs)	96.9%	TP/FN: 17,070/538
Sensitivity (11-20 bp INDELs)	98.9%	TP/FN: 791/9
Sensitivity (21-30 bp INDELs)	100.0%	TP/FN: 145/0
Sensitivity (31-40 bp INDELs)	100.0%	TP/FN:19/0
Sequencing (nucleotides with >20x sequencing depth)	99.4%	
Mean sequencing depth at nucleotide level (CCDS genes and boosted content)	174x	
Reportable range (SNVs) Reportable range (insertions) Reportable range (deletions)	Hom, Het 1-221 bp 1-210 bp	
Repeatability	99.7%	
Reproducibility	99.7%	
Intended use (blood samples with >98% of nucleotides with >20x sequencing depth)	100%	
Intended use (saliva samples with >98% of nucleotides with >20x sequencing depth)	100%	

Key features of the Primary Immunodeficiency Panel



- *Pathogenic or likely pathogenic sequence variant(s) identified in 44 different genes*
- *Over 40 different P/LP sequence variants identified*
- *2 sequence variants identified in at ≥5 patients*
- *34/42 (81%) sequence variants identified were unique (only in one patient)*

274 genes are covered by the Primary Immunodeficiency Panel

ACD, ACP5, ACTB, ADA, ADAM17, ADAR, AICDA, AIRE, AK2, AP3B1, ARPC1B, ATM, BACH2, BCL10, BCL11B, BLM, BLNK, BTK, C1QA, C1QB, C1QC, C1S, C2, C3, CARD9, CARD11, CARD14, CASP8, CASP10, CD3D, CD3E, CD3G, CD8A, CD19, CD27, CD40, CD40LG, CD46, CD55, CD59, CD70, CD79A, CD79B, CD81, CD247, CDCA7, CEBPE, CECR1, CFB, CFD, CFH, CFI, CFP, CFTR, CHD7, CIITA, CLCN7, CLPB, COLEC11, COPA, CORO1A, CR2, CSF2RA, CSF2RB, CSF3R, CTC1, CTLA4, CTPS1, CTSC, CXCR4, CYBA, CYBB, DCLRE1C, DDX58, DGKE, DKC1, DNAJC21, DNMT3B, DOCK2, DOCK8, ELANE, EPG5, ERCC6L2, EXTL3, FADD, FAS, FASLG, FERMT3, FOXN1, FOXP3, G6PC3, G6PD, GATA2, GFI1, GINS1, HAX1, HELLS, HYOU1, ICOS, IFIH1, IFNAR2, IFNGR1, IFNGR2, IGLL1, IKBKB, IKZF1, IL1RN, IL2RA, IL2RG, IL7R, IL10, IL10RA, IL10RB, IL12B, IL12RB1, IL17RA, IL17RC, IL21, IL21R, IL36RN, IRAK4, IRF2BP2, IRF8, ISG15, ITGB2, ITK, JAGN1, JAK1, JAK3, KRAS, LAMTOR2, LAT, LCK, LIG4, LPIN2, LRBA, LYST, MAGT1, MALT1, MAP3K14, MASP1, MEFV, MKL1, MOGS, MRE11A, MSN, MTHFD1, MVK, MYD88, MYO5A, NBN, NCF1, NCF2, NCF4, NCSTN, NFKB1, NFKB2, NFKBIA, NHEJ1, NHP2, NLRC4, NLRP1, NLRP3, NLRP12, NOD2, NOP10, NRAS, NSMCE3, OFD1, ORAI1, OTULIN, PARN, PEPD, PGM3, PIGA, PIK3CD, PIK3R1, PLCG2, PMS2, PNP, POLE, POLE2, PRF1, PRKCD, PRKDC, PSENEN, PSMB8, PSTPIP1, PTPRC, RAB27A, RAC2, RAG1, RAG2, RASGRP1, RBCK1, RECQL4, RFX5, RFXANK, RFXAP, RHOH, RLTPR, RMRP, RNASEH2A, RNASEH2B, RNF31, RNF168, RNU4ATAC, RORC, RPSA, RTEL1, SAMD9, SAMD9L, SAMHD1, SBDS, SERPING1, SH2D1A, SLC7A7, SLC29A3, SLC35C1, SLC37A4, SLC46A1, SMARCAL1, SMARCD2, SP110, SPINK5, SRP72, STAT1, STAT2, STAT3, STAT5B, STIM1, STK4, STX11, STXBP2, TAP1, TAP2, TAPBP, TBX1, TCF3, TCN2, TERC, TERT, TFRC, THBD, TINF2, TMC6, TMC8, TMEM173, TNFAIP3, TNFRSF1A, TNFRSF4, TNFRSF13B, TRAF3IP2, TREX1, TRNT1, TTC7A, TYK2, UNC13D, UNC93B1, UNC119, UNG, USB1, USP18, VPS13B, VPS45, WAS, WDR1, WIPF1, WRAP53, XIAP, ZAP70, ZBTB24, ZNF341

KEY FINDINGS

- We have demonstrated the analytic validation of one of the most comprehensive PID panels in the market
- The panel demonstrates high resolution detection for CNVs and indels of various sizes
- The panel offers comprehensive coverage for clinically relevant non-coding variants which are typically missed by standard NGS platforms