Blueprint Genetics

Validation of OS-Seq panels for clinical diagnostics of inherited disorders

Samuel Myllykangas, Pertteli Salmenperä, Massimiliano Gentile, Siina Bruce, Miko Valori, Mikko Muona, Antti-Pekka Sarin, Juha Koskenvuo
Blueprint Genetics, Biomedicum 2U, Tukholmankatu 8, Helsinki, 00290, Finland

OS-Seq Panels for Inherited Disorders

Blueprint Genetics utilizes proprietary targeted sequencing technology, Oligonucleotide-Selective Sequencing (OS-Seq) *(1)* and custom data analysis pipelines for identification of single nucleotide variants (SNVs), Insertions and Deletions (INDELs) and Deletions and Duplications (Del/Dups) in all inherited disorders (**Table 1**).

Table 1 Blueprint Genetics' gene panels for inherited disorders.

Category	Number of panels	Number of genes	Target size (bp)
Cardiology	8	287	613 878
Dermatology	16	119	300 229
Ear-Nose-Throat	23	222	610 222
Endocrinology	10	161	466 458
Gastroenterology	22	389	724 439
Hematology	10	103	272 647
Hereditary Cancer	7	82	235 524
Immunology	22	242	779 856
Malformations	12	156	350 938
Metabolic Disorders	35	429	1217 522
Nephrology	16	175	417 173
Neurology	22	299	806 175
Ophthalmology	25	595	1 557 606
Pulmonology	14	165	501 495
TOTAL	212	2 051	5 059 870

Analytic validation of SNVs, INDELs and Del/Dups

Blueprint Genetics applies independent, publicly available sample materials and data sets as the reference materials in all validation studies to ensure full traceability of the validation results. 37 reference samples with confirmed SNVs and INDELs, 283 reference samples with confirmed Del/Dups and the golden standard reference sample (NA12878) were applied in the validation of SNVs and INDELs (**Table 3**) and Del/Dups (**Table 4**).

Table 3 Analytic validation of SNV and INDEL detection.

Performance metric	Value	Measurements
Accuracy (SNVs) Sensitivity (SNVs) Specificity (SNVs) Positive predictive value (SNVs)	0.999 0.993 0.999 0.993	TN: 17 387 846 TP: 10 393 FP: 70 FN: 74
Sensitivity (1-10 bp INDELs)	0.961	TP/FN: 1 494 / 61
Sensitivity (11-20 bp INDELs)	0.885	TP/FN: 904 / 118
Sensitivity (21-30 bp INDELs)	0.668	TP/FN: 245 / 122
Sensitivity (31-46 bp INDELs)	0.198	TP/FN: 41 / 166
Nucleotides with >15x sequencing depth Mean sequencing depth at nucleotide level	99.60% 234x	
Reportable range (SNVs) Reportable range (INDELs)	Hom, Het 0-46 bp	
Repeatability	0.994	
Reproducibility	0.998	

In-process quality control

A well-characterized golden-standard reference sample (NA12878) is added into each sample processing, sequencing and data analysis batch to control for quality of the testing and to assess the sensitivity of the performed assays (**Table 5**). For each clinical sample and in-process quality control sample, a coverage value (**Figure 1**) and SNV and INDEL sensitivity values (**Figure 2**) are measured that ensures that each analysis fulfills the criteria derived from the analytic validation studies.

Table 5 Quality metrics and acceptance criteria for clinical testing.

Quality metrics	Acceptance criteria	Actual*
Test sample coverage (nucleotides with >15x sequencing depth)	>98%	99.4%
In-process reference sample sensitivity	>0.98	0.993
Test sample sensitivity (5 exon)	>0.98	0.993

* 3-month average.

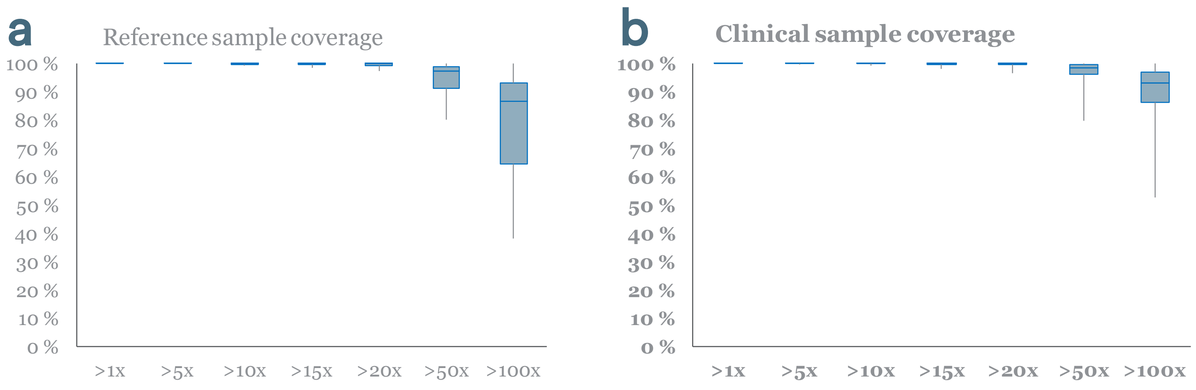

Figure 1 Box plot of target region coverage in **a** reference samples and **b** clinical samples during a 3-month period.

Blueprint Genetics performs analytic validation of all laboratory and data analysis assays per ACMG guidelines *(2)*. Metrics included in the analytic validation of Blueprint Genetics diagnostics assays are described in **Table 2**.

Table 2 Metrics applied in the analytic validation of Blueprint Genetics' diagnostic panels.

Validation metric
PRECISION is a statistical measure of the performance of the assay to generate correct test result. For estimation of the precision, true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) are measured and sensitivity, specificity, positive predictive value and accuracy are calculated.
CLINICAL SENSITIVITY provides a statistical measure of the assays clinical performance. It reflects the assay's ability to provide a diagnosis in specific clinical use cases. Clinical sensitivity is measured by calculating the extent in which all clinical diagnosis scenarios are met.
REPORTABLE RANGE is the functional range of an assay over which the analyte can be analyzed.
REPEATABILITY is the technical variation in measurements taken by a single person on the same instrument, on the same experiment, under the same conditions, and in a short time.
REPRODUCIBILITY is the ability of a test result to be duplicated under all variable conditions (different users, between reagent lots, using different instruments and different testing times).

Table 4 Analytic validation of Del/Dup detection.

Performance metric	Value	Measurements
Sensitivity (1 exon) Specificity (1 exon)	0.715 1.000	TP/FN: 89 692 / 35 769 TN/FP: 1 782 470 / 545
Sensitivity (2 exon) Specificity (2 exon)	0.952 1.000	TP/FN: 59 837 / 2998 TN/FP: 1 782 672 / 343
Sensitivity (3 exon) Specificity (3 exon)	0.990 1.000	TP/FN: 41 544 / 431 TN/FP: 1 782 751 / 264
Sensitivity (4 exon) Specificity (4 exon)	0.999 1.000	TP/FN: 31 430 / 34 TN/FP: 1 782 823 /192
Sensitivity (5 exon) Specificity (5 exon)	0.999 1.000	TP/FN: 25 225 / 28 TN/FP: 1 782 866 / 149
Clinical sensitivity (% pathogenic Del/Dups detected*)	92.42%	
Clinical sensitivity (% target genes covered**)	97.01%	29 992 / 30 916 exons
Reportable range (deletions)	317 bp and larger	
Reportable range (duplications)	545 bp and larger	
Repeatability	0.997	
Reproducibility	0.993	

* Estimated proportion of patients with Del/Dups that will get a diagnosis with our assay. Analysis has been performed based on sensitivity to detect Del/Dups of different sizes and estimated proportion of different sized pathogenic Del/Dups in LDLR, FBN1, BRCA1 and DMD based on literature review and HGMD.

** Human genome contains regions that are affected by pseudogenes, repeats and extreme GC-content that are not reproducibly analyzed using short read sequencing.

Blueprint Genetics diagnostic panels are effective tools to detect pathogenic SNV, INDEL and Del/Dup variants that underlie inherited disorders (**Figure 3**).

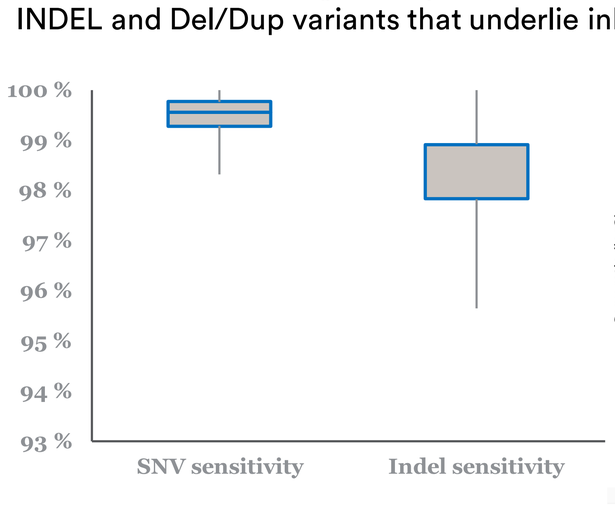

Figure 2 Box plot showing **a** SNV detection and **b** INDEL detection sensitivity for in-process reference samples during a 3-month period.

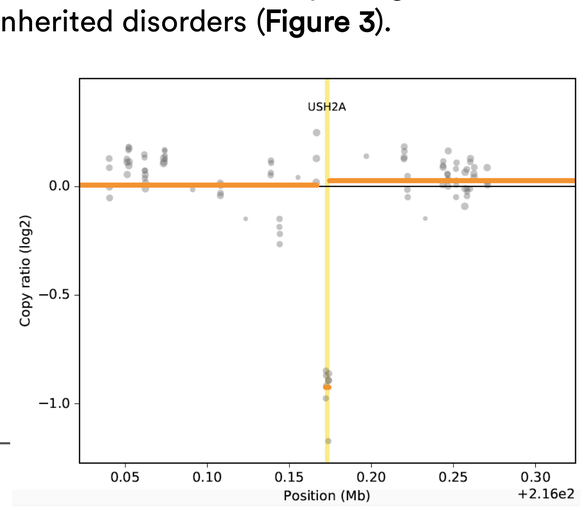

Figure 3 A deletion encompassing 2 exons of *USH2A*. The patient had a clinical diagnosis of Retinitis Pigmentosa and is also heterozygous for a pathogenic *USH2A* missense variant.

References

(1) Myllykangas S, Buenrostro JD, Natsoulis G, Bell JM, Ji HP. Efficient targeted resequencing of human germline and cancer genomes by oligonucleotide-selective sequencing. Nat Biotechnol. 2011 Oct 23;29(11):1024-7.

(2) Rehm HL, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, Friez MJ, Funke BH, Hegde MR, Lyon E; Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Commitee. ACMG clinical laboratory standards for next-generation sequencing. Genet Med. 2013 Sep;15(9):733-47