Quality

What to consider when choosing a testing platform for your patient

Blueprint Genetics

How many genes are included in the whole exome sequencing platform?

	Blueprint Genetics	Lab A*	Lab B*	Lab C*	Lab D*
Number of genes	~20,000 genes	~20,000 genes	>18,000 genes	~20,000 genes	~6, 700 genes

* information provided on laboratory websites

How well are the genes covered? And how is this demonstrated?

	Blueprint Genetics	Lab A*	Lab B*	Lab C*	Lab D*
% coverage of target regions	99.4%-99.7% at 20x**	~95% at 10x, >98% at 1x	>99.4% at 20x	97% at >20x	~97-98% at 10x
Mean read depth	174x->244x**	Not provided	150x	>120x	100x
Validation study	Yes	Not provided	Not provided	Not provided	Not provided

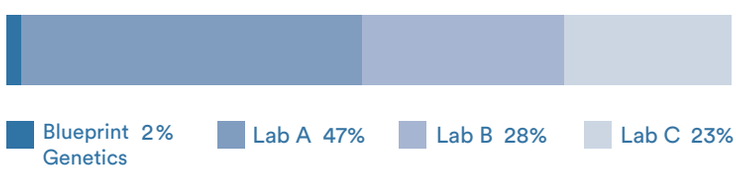
* information provided on laboratory websites
**lower value in publicly available validation samples of varying quality, higher value in patient samples

Why do these numbers matter? What do they really mean? What is the difference between 99.7% versus 95% coverage?

	Blueprint Genetics	Lab A	Lab B	Lab C
% coverage of target region	99.7% >20x	95% >10x	97% >20x	97.5% >10x
# bp covered <20x (or <10x)*	60,000 bp <20x	1,500,000 bp <10x	900,000 bp <20x	750,000 bp <10x
# exons/genes covered <20x (or <10x)*	414 exons 45 genes	10,345 exons 1,119 genes	6,207 exons 672 genes	5,100 exons 550 genes

*Estimates intended for illustrative purposes

Genes Covered Suboptimally



How does this translate to the clinic?

CASE 1:

27-year-old with polydactyly and early onset retinitis pigmentosa. Previous testing, including the Bardet-Biedl syndrome 2 (*BBS2*) gene, was negative.

Blueprint Genetics results

Sequence analysis revealed two variants in the *BBS2* gene, c.1895G>C (pathogenic) and c.534+1G>T (likely pathogenic) resulting in a diagnosis of Bardet-Biedl syndrome.

Blueprint Genetics advantage

High-quality sequencing with uniform coverage reduces the risk of false-negative results. In this case previous testing had low-coverage in some regions, resulting in a failure to detect the patient's variants.

CASE 2:

A 12-year-old male with clinical suspicion of X-linked retinitis pigmentosa due to a strong family history of maternally related affected male relatives. Testing performed at another lab was negative.

Blueprint Genetics results

A deletion was discovered in the retinitis pigmentosa GTPase regulator (*RPGR*) gene, c.2426_2427del (p.[Glu809Glyfs*25), specifically in the ORF15 region. As a result, a diagnosis of *RPGR*-related X-linked retinitis pigmentosa was made.

Blueprint Genetics advantage

Improvements to capture kit, sequencing platform, mapping quality, and bioinformatic pipeline increase the sensitivity of variant detection in genes difficult to sequence by NGS, including *RPGR*, *PKD1*, *GBA*, and others.

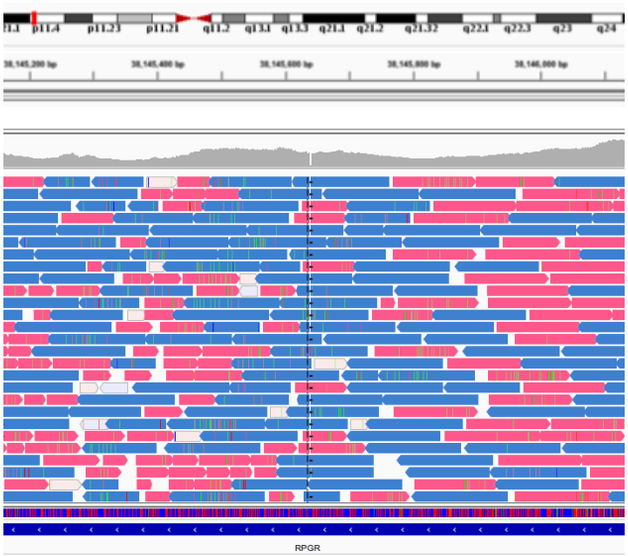


Figure 1. The new NovaSeq technology with custom oligo design shows improved coverage in the *RPGR*-ORF15 region.

Figure 2. Coverage in the *RPGR*-ORF15 region using the previous NGS technology.

	Blueprint Genetics	Lab A*	Lab B*	Lab C*	Lab D*
Deep intronic variants	20 bps from exon-intron boundary + >1,500 disease causing deep intronic variants included	Not provided	Not provided	Not provided	Not provided

* information provided on laboratory websites

CASE 3:

A 4-year-old with bilateral choanal atresia, bilateral lacrimal duct obstruction, abnormal eyelids, and moderate unilateral conductive hearing loss. Sequence analysis at another lab revealed the genetic variant *TXNL4A* c.88_110del23 (likely pathogenic) which is associated with Burn-McKeown syndrome, but insufficient for an autosomal recessive disease diagnosis.

Blueprint Genetics results

Sequence analysis revealed the previously described *TXNL4A* c.88_110del23 variant as well as c.-222_-189del (pathogenic), a previously described 34 bp deletion in the promoter. As a result, the Burn-McKeown syndrome diagnosis was confirmed, and another relevant variant was identified.

Blueprint Genetics advantage

More than 1,500 previously described disease-causing deep intronic variants are

included in our panels and whole exome sequencing.

	Blueprint Genetics	Lab A*	Lab B*	Lab C*	Lab D*
SNV detection	99.7%	Not provided	Not provided	Not provided	~93.2%
Indel detection	1–10 bp 96.9% 11–20 bp 98.9% 21–30 bp 100% 31–40 bp 100%	Not provided	<50 bp reliably detected	Not provided	Not provided
CNV detection	1 exon del 92.3% 2 exon del 100% 3 exon del 93.3% Microdeletion syndromes 100%	May detect CNV 3 exons or larger.	Reliable detection of CNVs 4 exons or larger with high confidence. Not intended to detect large CNVs.	1, 2 and 3 exon CNVs ~ 70%; 4 or more exon CNVs >95%	Not provided

* information provided on laboratory websites
SNV, single nucleotide variant; CNV, copy number variant.

CASE 4:

An 11-month-old baby with abnormal soft tissue calcification at joints, mild global developmental delay, and failure to thrive. Parents are consanguineous and chromosomal microarray (CMA) testing was normal.

Blueprint Genetics results

Genetic testing showed that the patient was homozygous for a one exon (~273 bp) deletion in the *ENPP1* gene, c.1091+1_1092-1_1164+1_1165-1 (likely pathogenic), while the parents are both heterozygous. The resulting diagnosis was generalized arterial calcification of infancy.

Blueprint Genetics advantage

NGS-based CNV analysis able to detect CNV missed by CMA.

CASE 5:

A 4-month-old with clinical and laboratory features consistent with propionic acidemia.

Blueprint Genetics results

Sequencing analysis identified *PCCA* c.1746G>A (pathogenic). CNV analysis revealed a deletion of exons 7-18 in the *PCCA* gene. These variants, confirmed to be in trans, are consistent with a diagnosis of propionic acidemia.

Blueprint Genetics advantage

The combination of SNV and CNV detection in one test decreases the need to resort to non-NGS deletion/duplication assays when only one SNV is identified.

A quick and easy checklist for quality testing platforms

- ☑ High-quality sequencing platform with >20X coverage across >99.4% of targets
- ☑ Publicly available analytic validation that demonstrates sensitivity to detect SNVs, indels, and CNVs across all genes
- ☑ Inclusion of disease-causing deep intronic variants
- ☑ High-quality bioinformatics pipeline and rigorous variant interpretation
- ☑ Clinical statement that includes all data and evidence used to evaluate variants
- ☑ Competitive turnaround time and price