

# TruSight™ Whole Genome

A validated, scalable, and IVDR-compliant solution for clinical whole-genome sequencing

- Eases adoption of WGS for clinical diagnostic testing with a comprehensive, DNA-to-VCF workflow
- Leverages PCR-free library preparation chemistry for minimal bias and superior coverage uniformity
- Delivers highly accurate and precise germline variant calling for various downstream clinical applications
- Ensures optimal performance with analytical controls, including run and sample QC metrics, without the need for external batch controls



## Introduction

Traditional methods for clinical testing, including PCR and chromosomal microarray, have limited utility in their ability to detect genetic variants associated with disease. Next-generation sequencing (NGS) methods, including whole-genome sequencing (WGS), are driving breakthroughs in clinical genetic testing, accelerating diagnoses. However, single-gene tests and multigene NGS panels are targeted approaches, limited in scope and may potentially miss actionable variants. Also, these methods can be left outdated as new gene–disease associations are made, forcing clinical labs into time-consuming cycles of amending and validating new tests.

WGS provides the most comprehensive view of the human genome, and often includes regions not targeted by other methods. PCR-free WGS enables simultaneous analysis of thousands of genes with known or suspected disease associations, and discovery of novel causative variants.

For clinical labs transitioning to or incorporating a European Union (EU) *In Vitro* Diagnostic Regulation (IVDR)–compliant genomic assay, Illumina offers TruSight Whole Genome. This assay offers a comprehensive, DNA-to-variant call format (VCF) workflow for clinical WGS (Figure 1). This wet lab-to-secondary analysis solution streamlines assay validation with internal controls, automated variant calling, and analytical validation studies.

## Complete view of genomic variation

WGS overcomes the content limitations of traditional testing methods by delivering a base-by-base view of virtually any genomic alteration in a sample. TruSight Whole Genome has characterized performance for the following variant classes:

- Single nucleotide variants (SNVs)
- Insertions and deletions (indels)
- Copy number variants (CNVs)
- Runs of homozygosity (ROH)
- Short tandem repeat (STR) expansions
- Mitochondrial DNA (mtDNA) variants

## A validated, clinical WGS solution

Analytical validation of the TruSight Whole Genome assay was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines with over 450 samples over the course of > 150 sequencing runs. Quality control (QC) metrics and confidence tier annotation included in the output of TruSight Whole Genome identify poor performing samples and allow filtering of data. This reduces false-positive and false-negative calls and significantly improves performance (Table 1, Figure 2).

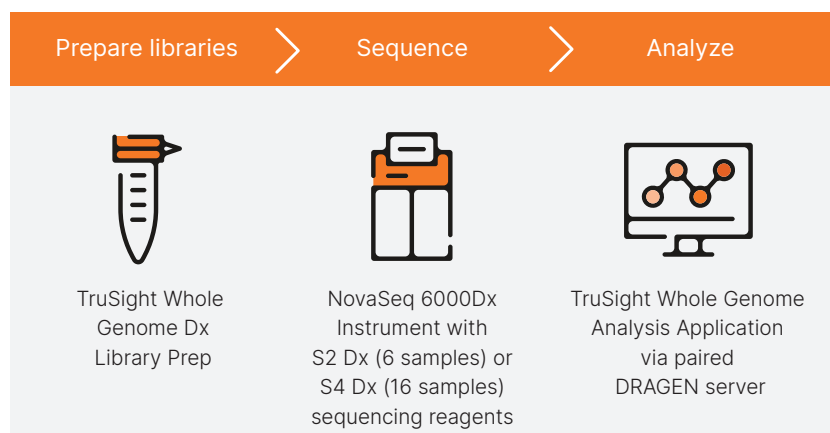


Figure 1: TruSight Whole Genome workflow—TruSight Whole Genome features a comprehensive, DNA-to-VCF workflow that includes library preparation, sequencing on the NovaSeq 6000Dx Instrument, and analysis with the TruSight Whole Genome Analysis app.

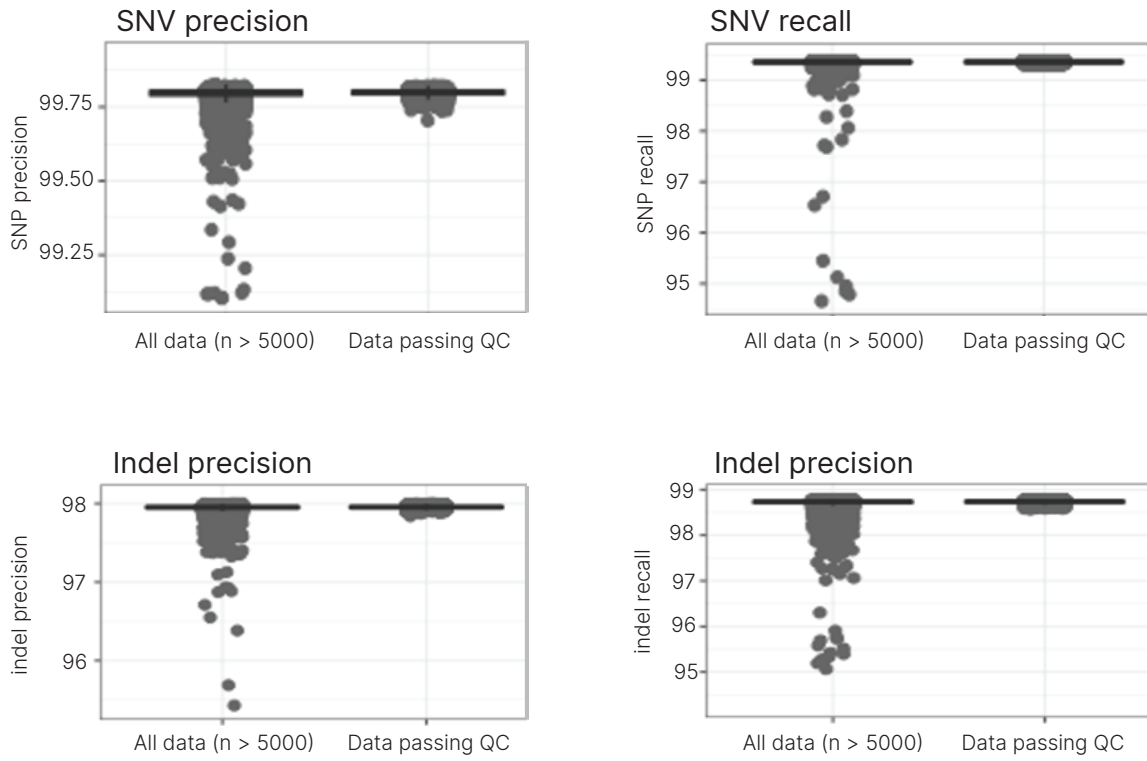


Figure 2: Built-in analytical QC metrics ensure performance—QC based on run and sample performance specifications, metrics, including coverage, uniformity, and base quality, fails poor performing samples to ensure optimal analytical performance without the need for external batch controls.

Table 1: Filtering for variants of high and intermediate confidence improves performance

	Unfiltered	High confidence	Intermediate confidence	Low confidence
SNVs	99.79%	99.99%	99.85%	94.36%
Insertions ≤ 5 bp	99.87%	99.99%	99.93%	99.05%
Deletions ≤ 5 bp	99.84%	99.97%	99.91%	99.12%
Insertions ≤ 15 bp	99.75%	—	99.96%	99.43%
Deletions ≤ 15 bp	99.70%	—	99.93%	99.34%

## Flexible, scalable workflow

TruSight Whole Genome offers a flexible and reliable workflow (Table 2), enabling up to 24 libraries to be prepared in as little as 2.5 hours.

Table 2: TruSight Whole Genome at a glance

Feature	Description
Sequencing system	NovaSeq 6000Dx Instrument
Sample types	gDNA extracted from whole blood
DNA input requirement	280 ng
Sample throughput <sup>a</sup>	6 samples per S2 Dx flow cell and 16 samples per S4 Dx flow cell
Variant types detected	SNVs, indels, CNVs, ROH, STR expansions, mtDNA variants
Total assay time	< 3 days
Library prep time	~ 2.5–4 hrs
Sequencing run time	~ 44 hrs
Sequencing cycles	2 × 150 bp

a. Dual flow cells can be run simultaneously to double throughput.

## Proven Illumina sequencing

Prepared libraries are run on the NovaSeq™ 6000Dx Instrument, using either S2 Dx or S4 Dx flow cells and reagents. The NovaSeq 6000Dx Instrument is a CE-marked, *in vitro* diagnostic (IVD) instrument that enables clinical laboratories to develop and perform NGS-based IVD assays.

## Automated data analysis

Analysis is performed automatically in the TruSight Whole Genome Analysis App. Using DRAGEN™ secondary analysis, the app provides highly accurate variant calling, and annotation. The output is a genome VCF (gVCF) file, suitable for any interpretation platform. With Original Read Archive (ORA) compression technology included, storage of WGS FASTQ.ORA files require 5× less space when compared to traditional FASTQ.GZ format.

## Exceptional performance

### Highly accurate and repeatable variant calling

Analytical validation studies show that TruSight Whole Genome provides accurate and repeatable variant calling performance for all variant types and subclassifications covered by the assay (Table 3). Variance component analysis conducted as part of the within-laboratory precision study attributed on average only minimal variance to reagent lot (< 1.8%), sequencing instrument (1.2%), and sequencing kit lot (9.7%).

The accuracy study showed a low incidence of failure (1.4%) when tested with 496 samples, 40 preps, 59 runs, 6 library prep lots, 4 sequencing consumable lots, 7 sequencing systems, and 8 operators.

Table 3: TruSight Whole Genome variant calling performance

Variant type	Sub-classification	Analytical accuracy			Within-laboratory precision	
		PPA	TPPV	NPA	APA	ANA
SNVs	High confidence	99.4	99.9	99.9	99.9	> 99.9
	Intermediate confidence	94.1	97.7	97.7	98.8	98.8
Indels 1-5 bp	High confidence	98.6/98.3	99.6/99.5	N/A	99.9/99.6	N/A
	Intermediate confidence	96.0/98.4	96.5/98.5	N/A	98.8/98.8	N/A
Indels 6-15 bp	Intermediate confidence	97.8/97.7	97.9/97.9	N/A	99.2/98.1	N/A
Indels 16-31 bp	Intermediate confidence	98.1/96.0	94.9/91.5	N/A	96.8/94.6	N/A
CNVs	Gains > 10 kbp	86.6	88.7	> 99.9	95.2	> 99.9
	Losses > 10 kbp	93.3	91.0	> 99.9	95.6	> 99.9
ROH	> 500 kbp	> 99.9	85.5	N/A	98.3	N/A
mtDNA SNVs		> 99.99	99.91	99.24	97.2 <sup>a</sup>	99.9 <sup>a,b</sup>
STR exp	<i>AR</i>	> 99.99	> 99.99	> 99.99	N/A	> 99.99 <sup>b</sup>
	<i>ATN1</i>	> 99.99	> 99.99	> 99.99	N/A	> 99.99 <sup>b</sup>
	<i>ATXN1</i>		> 99.99	> 99.99	N/A	> 99.99 <sup>b</sup>
	<i>C9ORF72</i>	> 99.99	> 99.99	> 99.99	N/A	> 99.99 <sup>b</sup>
	<i>DMPK</i>	> 99.99	> 99.99	> 99.99	N/A	> 99.99 <sup>b</sup>
	<i>FMR1</i>	> 99.99	> 99.99	> 99.99	> 99.99 <sup>b</sup>	> 99.99 <sup>b</sup>
	<i>HTT</i>	> 99.99	99.49	83.33	> 99.99 <sup>b</sup>	99.8 <sup>b</sup>
<i>SMN1</i>	c. 840C negative	> 99.99	> 99.99	> 99.99	> 99.99 <sup>b</sup>	> 99.99 <sup>b</sup>

a. Results based on contrived mixture targeting 1 × LoD (4.75% VAF) for 32-40 sites.

b. Results are reported as PPC and PNC instead of APA and ANA when comparison is made to ground truth, as opposed to a characterization run.

PPA, positive percent agreement; PPC, percent positive calls; TPPV, technical positive predicative value; NPA, negative percent agreement; PNC, percent negative calls; APA, average positive agreement; ANA, average negative agreement; exp, expansions; VAF, variant allele frequency

For more information on initial characterization of variant types used to measure accuracy, see TruSight Whole Genome Package Insert.

## Summary

TruSight Whole Genome is a validated clinical WGS workflow that provides accurate and repeatable detection of germline variants. This DNA-to-data solution facilitates adoption by streamlining the process of developing controls, bioinformatic pipelines, and performing costly and time-consuming analytical validation studies. The filtered and annotated genome.vcf files generated by the system are suitable for use in various downstream germline clinical testing application.

## Learn more

[TruSight Whole Genome](#)

[NovaSeq 6000Dx Instrument](#)

[DRAGEN secondary analysis](#)

## Ordering information

Product	Catalog no.
TruSight Whole Genome Dx Library Prep with UD Indexes, 24 sample	20093209

## Intended use statement

TruSight Whole Genome is a qualitative *in vitro* diagnostic device intended for whole-genome sequencing and detection of single nucleotide variants, insertion/deletions, copy number variants, runs of homozygosity, short tandem repeat expansions, and mitochondrial variations in human genomic DNA extracted from blood.

TruSight Whole Genome includes the TruSight Whole Genome Dx Library Prep with UD Indexes and the TruSight Whole Genome Analysis Application Software. The device is intended to be used with compatible downstream germline applications to develop *in vitro* diagnostic assays, and by qualified laboratory personnel and assay developers.

TruSight Whole Genome is intended to be used on the NovaSeq 6000Dx Instrument.



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