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TECHNICAL NOTE

TruPath™ Genome performance with samples of varying type and quality

Compatible with a range of sample types, including blood, isolated cells, saliva, dried blood spots, and buccal swabs

High-quality results from a range of sample qualities, including DNA from standard and high-molecular-weight extraction kits

Robust performance with 175 ng to 550 ng DNA input

Introduction

Short-read sequencing offers a flexible, reliable method for performing high-accuracy whole-genome sequencing (WGS). However, a small proportion of the human genome remains difficult to map (eg, regions with high sequence homology or repetitive sequences and some variant types like structural variants (eg, inversions, translocations, insertions and deletions, and complex rearrangements)). Long-read sequencing methods can help resolve these regions and variant types but are hindered by the need for high DNA input amounts, strict input quality requirements, complex workflows, and variable results.[1–4]

Illumina TruPath Genome revolutionizes the next-generation sequencing (NGS) workflow, providing comprehensive whole-genome sequencing with unprecedented simplicity.[5] Powered by proximity mapped read technology, TruPath Genome uses a highly simplified workflow that essentially eliminates the traditional library preparation step and delivers a streamlined workflow that goes from purified DNA to loading the sequencing system in ~10 minutes (Figure 1).

In addition to changing the WGS workflow paradigm, TruPath Genome uses advanced informatics to combine high-accuracy short-read data with nanowell proximity information from DNA templates on the flow cell. This proximity information allows labs to generate long-distance genomic insights for sequences separated by up to millions of bases. Combining these long-distance genomic insights with the strengths of short-read sequencing allows TruPath Genome to make comprehensive genomes accessible.

This technical note demonstrates the highly robust capabilities of TruPath Genome to resolve previously difficult-to-map regions of the genome and perform comprehensive variant detection with various sample types, DNA quality, and DNA inputs.

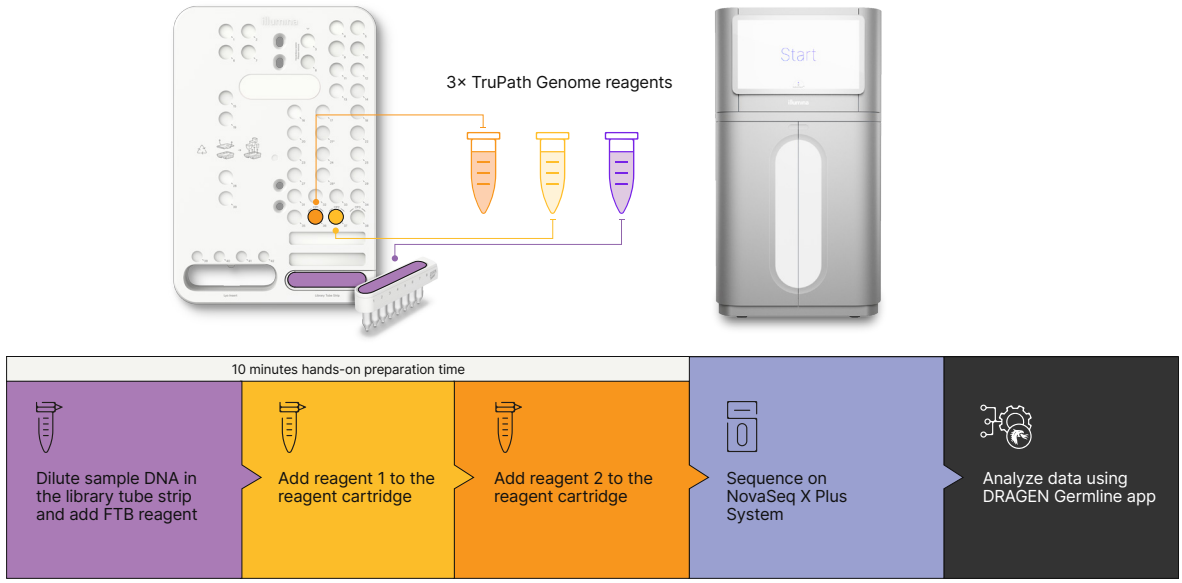

Figure 1: Streamlined TruPath Genome workflow
Purified DNA is added directly to the library tube strip along with FTB reagent and water to desired concentration. The tube strip and remaining TruPath reagents are added to the sequencing cartridge. Next, the cartridge and flow cell are loaded into the NovaSeq X Series and the run is started. Hands-on time to set up the assay is 10 minutes.

Sample type testing

Methods

Samples

A range of sample types, including blood, isolated cells, saliva, buccal swabs, and dried blood spots (DBS), were used to assess TruPath Genome performance (Table 1). DNA extraction was performed using multiple purification methods (eg, silica spin-column, magnetic beads, alcohol precipitation) and included high molecular weight (HMW) and standard extraction methods (Table 2).

DNA quantity was measured using the Qubit dsDNA High Sensitivity Assay on the Qubit 4 Fluorometer (Thermo Fisher Scientific, Catalog no. Q32851). DNA quality was assessed with a 4200 TapeStation System (Agilent, Catalog no. G2991BA) (Figure 2).

Table 1: Samples used in the TruPath Genome assay

Sample type	Source
Whole blood	Blood samples stored in K_2EDTA from healthy donors were purchased from Research Donors (London, UK)
Cell pellets	Multiple cultures of lymphoblastoid or fibroblasts cell lines ordered from Coriell Institute for Medical Research HMW and standard DNA samples, including the reference samples from the Genome in a Bottle Consortium (GIAB) HG001, HG002, HG003, HG004, HG005, HG006, and HG007 (Corriell Institute for Medical Research; NJ, USA)
Saliva	Saliva samples from healthy donors were purchased from Research Donors (London, UK)
Buccal swabs	Buccal swabs samples from healthy donors were purchased from Research Donors (London, UK)
DBS	DBS were prepared on Whatman 903 Protein Saver Cards using 50 µl of < 3-day-old K_2EDTA whole blood from Research Donors (London UK)

Table 2: DNA extraction kits used in the TruPath Genome assay

Sample type: collection method	Extraction kits
Blood: K_2EDTA	Monarch HMW DNA Extraction Kit for Cells & Blood (NEB, Catalog no. T3050S)
	Wizard HMW DNA Extraction Kit (Promega, Catalog no. A2920)
	MagAttract HMW DNA Kit (48) (Qiagen, Catalog no. 67563)
	chemagic Prepito DNA Universal Kit, (Revvity, Catalog no. CMG-2034)
	Mag-Bind Blood & Tissue DNA HDQ 96 Kit (OmegaBiotek, Catalog no. M6399)
	QIAamp DNA Blood Mini Kit (50)(Qiagen, Catalog no. 51104)
Cells: dry pellet	Monarch HMW DNA Extraction Kit for Cells & Blood (NEB, Catalog no. T3050S)
	QIAamp DNA Blood Mini Kit (50)(Qiagen, Catalog no. 51104)
DBS: Whatman 903	chemagic Prepito DNA Universal Kit, (Revvity, Catalog no. CMG-2034)
	sparQ Lysis Kit (Quantabio, Catalog no. 95220)
	MagMAX DNA Multi-Sample Ultra 2.0 Kit (Thermo Fisher Scientific Catalog no. A36570)
Saliva: GFX-02	GeneFix Saliva-Prep 2 DNA Isolation Kit (Isohelix, Catalog no. GSPN)
	chemagic Prepito DNA Universal Kit, (Revvity, Catalog no. CMG-2034)
Saliva: OGD-600	prepIT•L2P DNA extraction reagent (DNAGenotek, Catalog no. PT-L2P)
	chemagic Prepito DNA Universal Kit, (Revvity, Catalog no. CMG-2034)
	MagMAX Saliva gDNA Isolation Kit (Thermo Fisher Scientific, Catalog no. A39059)
Swabs: OCR-100	prepIT•L2P DNA extraction reagent (DNAGenotek, Catalog no. PT-L2P)
	chemagic Prepito DNA Universal Kit, (Revvity, Catalog no. CMG-2034)

Whatman 903, Protein saver cards (Millipore Sigma, Catalog no. WHA10534612); GFX-02, GeneFix Saliva DNA/RNA Collector GFX-02 (Isohelix, Catalog no. GFX-02); Oragene•Dx saliva collection device (DNA Genotek, Catalog no. OGD-600); OCR100, ORAcollect•DNA (DNA Genotek, Catalog no. OCR-100)

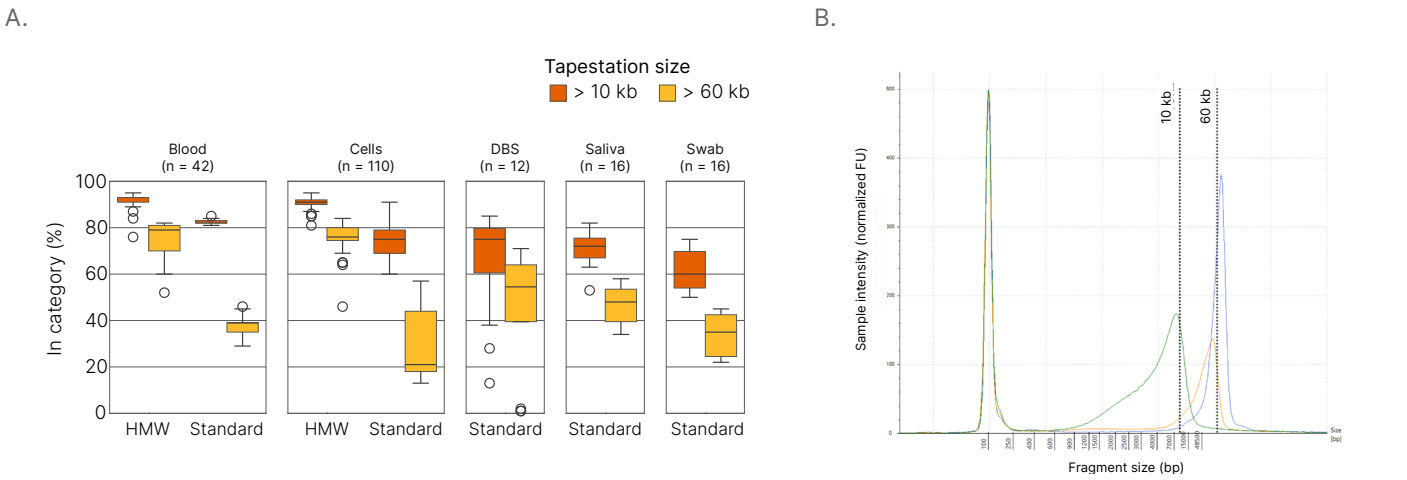

Figure 2: DNA quality by sample type.
(A) A range of DNA quality is observed across different sample types and extraction kits. DNA was extracted from blood and cell samples using both HMW and standard commercially available kits. DNA was extracted from DBS, saliva, and buccal swabs using standard commercially available kits. Analysis of DNA quality was performed using genomic DNA ScreenTape assay (Agilent, Catalog no.5067-5366 and 5067–5365) on the Agilent 4200 TapeStation by assessing the fragment percent greater than 10 kb and 60 kb. (B) Analysis of DNA quality using genomic DNA ScreenTape assay (Agilent, Catalog no. 5067-5366 and 5067–5365) on the Agilent 4200 TapeStation for cells reveals varying quality by sample type. Blood HMW (blue), swabs (orange), and DBS (green) samples.

Run setup and sequencing

DNA was added to a sample library tube strip along with TruPath Genome reagents (Illumina, Catalog no. 20157406) according to the manufacturer's instructions. The standard input amount was 350 ng DNA, however DBS samples used the entire extracted amount. The minimum DNA input was 175 ng. The library tube strip, TruPath Genome reagents, and a NovaSeq™ X C8 flow cell were loaded on to the NovaSeq X Plus System (Illumina, Catalog no. 20084804) for sequencing according to the user manual.

Analysis

After sequencing, the DRAGEN™ Germline pipeline was used to combine short-read sequencing data with nanowell proximity information. The GRCh38 reference genome was used for variant calling from phased reads.

Results

TruPath Genome delivers high-quality results from samples of variable quality

Standard whole genome metrics, including autosomal coverage and base call accuracy, achieved with TruPath Genome were unaffected by DNA quality and robust performance was observed for all sample types (Figure 3). The average autosomal coverage was ~64× and the average Q30 was 92%. For some saliva and swab samples, the reduction in coverage was due to the natural presence of bacterial reads in the samples which reduced human genome mapped read percent.

DNA sample quality was strongly associated with TruPath Genome proximity metrics (Figure 4). For most sample types, the percentage of DNA fragments over 10 kb was a strong predictor of the proximity rate performance, with a higher fragment percentage over 10 kb producing a higher Q25 proximity rate (ie, the percentage of reads that have at least one other read in close proximity with a quality score above Q25*).

* The proximity quality score is the Phred-scaled likelihood that two reads from the same region of the genome landed within the same flow cell neighborhood by chance as calculated by the DRAGEN proximity model. A higher proximity quality score indicates higher confidence that two reads that are nearby in the genome and on the flow cell originated from the same input DNA molecule.

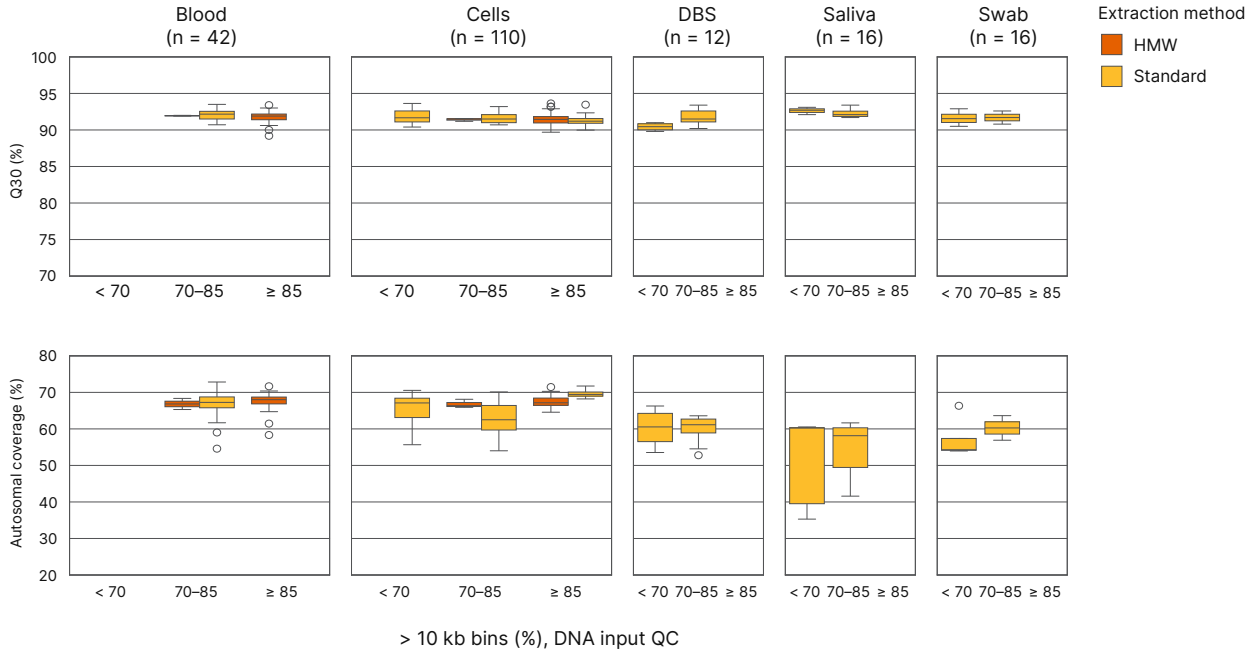

Figure 3: Autosomal coverage and average percent Q30 are not impacted by DNA quality across different sample types

DNA was extracted from blood and cells using HMW and standard methods. DNA was extracted from DBS, saliva, and buccal swabs using standard methods. Strong performance for coverage and quality scores was observed with all samples, regardless of DNA purification method. DNA intactness was measured on the Agilent 4200 TapeStation with a regional analysis as fragment percent > 10 kb binned along the X-axis.

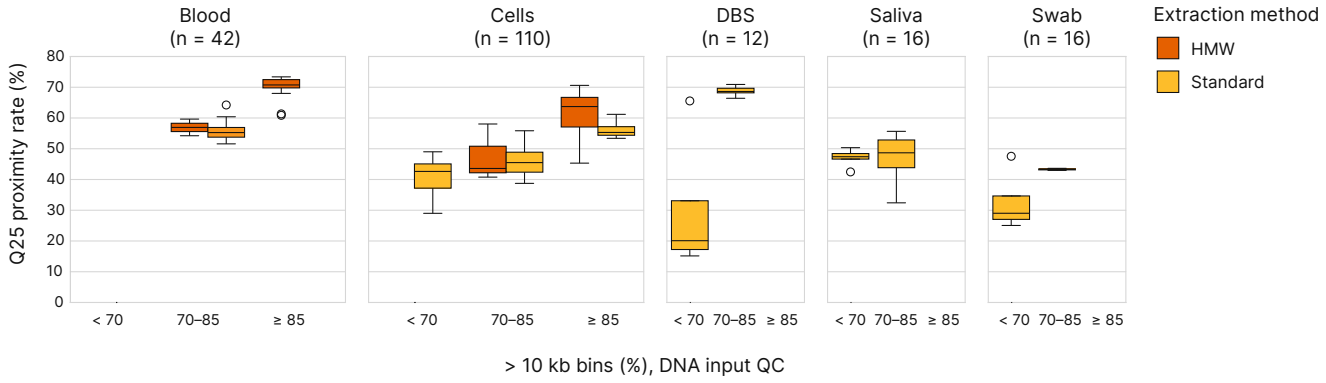

Figure 4: Impact of DNA quality on Q25 proximity rate

DNA was extracted from blood and cells using HMW and standard methods. DNA was extracted from DBS, saliva, and buccal swabs using standard methods. Q25 proximity rates are strongly associated with DNA quality, in particular with the DNA fragment % > 10 kb measured on the Agilent 4200 TapeStation and binned along the X-axis.

TruPath Genome supports phased sequencing

The presence of larger DNA fragments is a strong predictor of phasing performance—the higher the DNA fragment percentage over 60 kb, the larger the size of the phasing block NG50† and the higher small variant calling performance. TruPath Genome enables the study of full-length DNA templates, making it well suited for human genome phasing studies (Figure 5).

TruPath Genome performs best with high-quality, fresh samples

To assess the impact of sample age on TruPath Genome performance, DNA was extracted from blood and DBS samples at two specific time points. For blood samples, DNA was extracted within three days of blood collection and after seven days of storage at 4ºC. DBS samples were stored either for one month or one year at room temperature.

For both blood and DBS samples, the size of the phasing block NG50 changed in relation to the percentage of DNA fragments over 60 kb (Figure 6). Both metrics were lower for the aged samples compared with fresh samples. For blood samples, over 40% of fragments > 60 kb were obtained even for samples where DNA was isolated after one week when DNA was extracted using a HMW method.

TruPath Genome performs robustly across a range of DNA input amounts

TruPath Genome assay performance was evaluated across a range of DNA input amounts: 175, 200, 350, and 550 ng. Sequencing results with the 175 ng input demonstrated high-quality standard whole genome metrics (eg, autosomal coverage and percent > Q30) and proximity metrics (eg, Q25 proximity rate and phasing block NG50). While the recommended input is 350 ng, lower inputs can be used (Figure 7).

† Phase block NG50 is the length of the phase block once 50% of the target region (genome or other) has been phased.

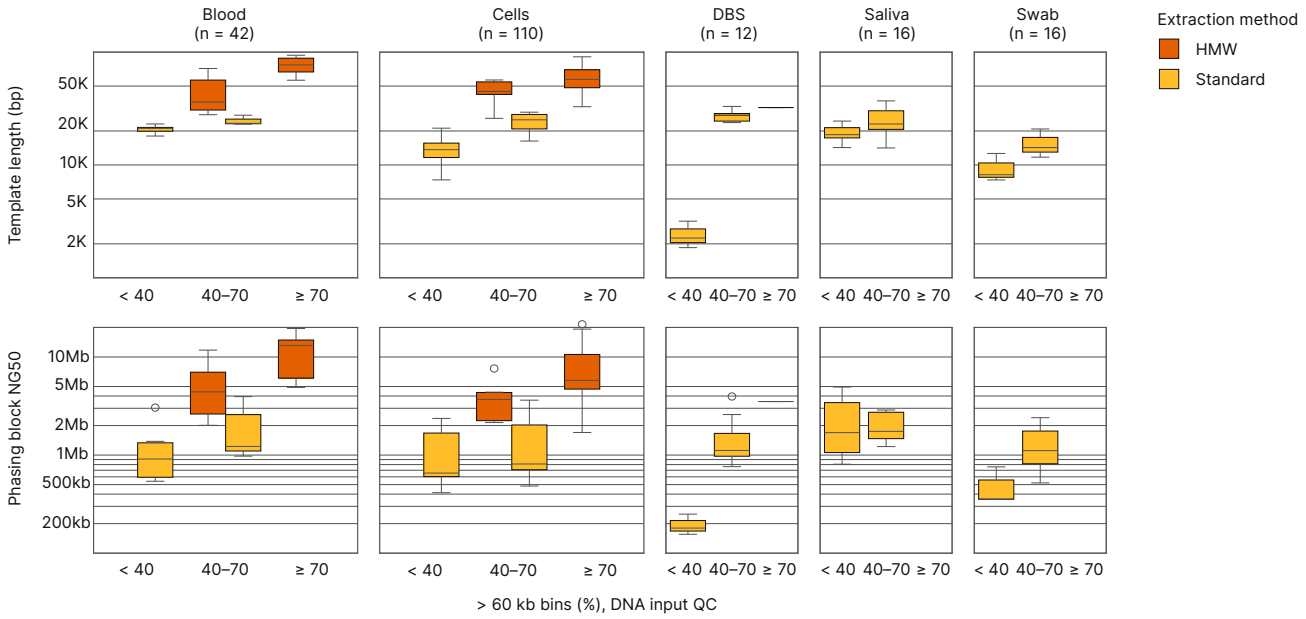

Figure 5: TruPath Genome phasing performance is highly robust with variable sample types
DNA was extracted from blood and cells using HMW and standard methods. DNA was extracted from DBS, saliva, and buccal swabs using standard methods. DNA intactness was measured on the Agilent 4200 TapeStation with a regional analysis and fragment percent > 60 kb binned along the X-axis. Template Length on the Y-axis represents the 75th percentile template molecule size. Increased DNA quality, with larger percent > 60Kb, correlates with increased template length and phasing block NG50.

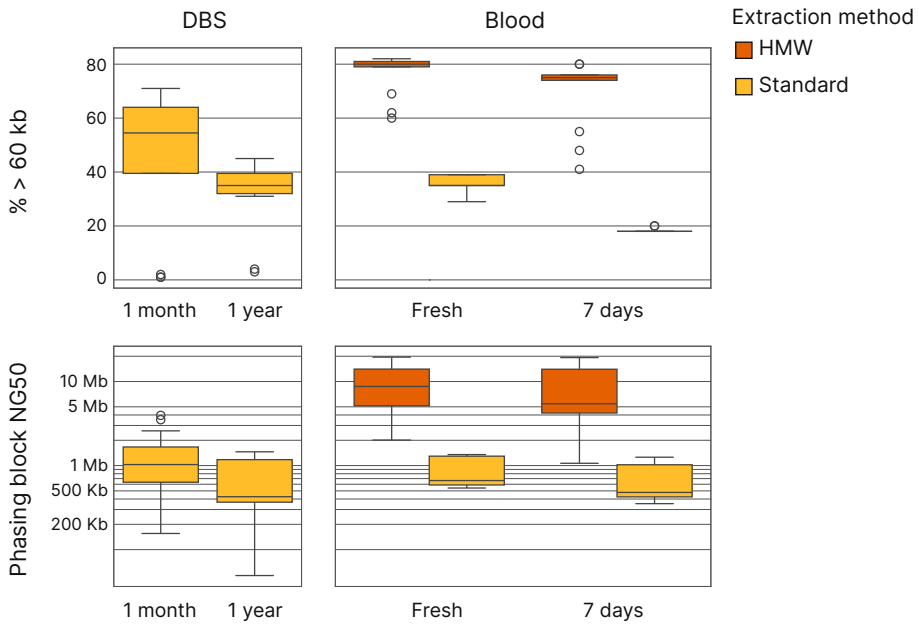

Figure 6: Impact of blood sample storage on DNA quality and phasing block NG50

Blood samples were stored at 4°C for < 3 days (fresh) or 7 days. DBS samples were stored at room temperature for < 30 days or ~1 year. DNA was extracted from blood using HMW and standard methods. DNA was extracted from DBS samples using standard methods. Impact of primary sample age on DNA quality (percent > 60 kb) and phasing block NG50. Percent > 60 kb is measured using genomic DNA in the ScreenTape assay on the Agilent 4200 TapeStation. For both sample types, the percentage of fragments higher than 60 kb and the phasing block NG50 size are lower for the samples that have been stored longer prior to extraction.

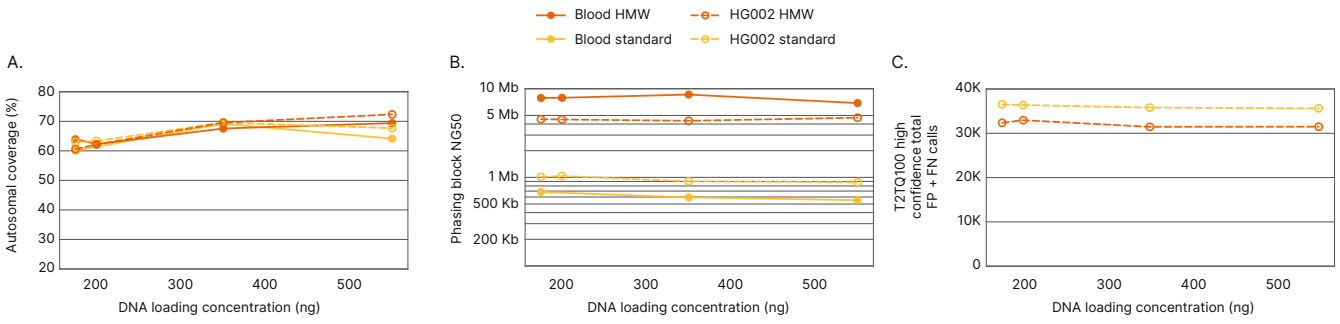

Figure 7: TruPath Genome performs well with a range of input amounts

Sequencing results for TruPath Genome prepared using 175, 200, 350, and 550 ng inputs generate similar data quality for standard whole genome and TruPath Genome proximity metrics; including (A) autosomal coverage, (B) phasing block NG50, and (C) small variant calling performance (total FP+FN calls). DNA was extracted from blood and cells using HMW and standard methods. Small variant calling performance (SNP + Indel) was benchmarked against T2T-Q100 V1.1 V0.019 truth set.

Summary

TruPath Genome uses proximity mapped read technology to provide a comprehensive whole-genome sequencing solution with unprecedented simplicity. The unique workflow applies the benefits of short-read sequencing methods combined with proximity information on the flow cell to unlock long-distance insights. This technical note demonstrates the high-quality, robust performance achieved with TruPath Genome from samples of varying types, quantity, quality, and storage conditions.

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