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TECHNICAL NOTE

Production-scale whole-genome sequencing with Illumina Genomics Architecture v3

Automated DNA-to-answer framework designed for population genomics programs

Sequencing at scale

Delivers efficient WGS for population genomics research processing > 10,000 samples per year

Integrated workflow

Combines Clarity LIMS software, automated liquid handling, and data analysis tools

Reliable results every time

Ensures consistent data quality across sites with barcode tracking and reduced risk of manual error

Introduction

Precision medicine leverages advances in genomics to transform our understanding of disease and improve human health. Large-scale research efforts are underway to sequence hundreds of thousands of genomes.[1,2] These population genomics (PopGen) initiatives seek to explore genetic diversity and inform public health. Because they process large sample volumes on tight timelines, PopGen studies require streamlined next-generation sequencing (NGS) workflows for productivity, efficiency, and accuracy.[3–5] Illumina Genomics Architecture provides a complete workflow for whole-genome sequencing (WGS), designed to meet the demands of PopGen programs that process more than 10,000 samples per year. This integrated solution includes sample management, automated library prep, sequencing, data analysis, and results interpretation (Figure 1).

Illumina Genomics Architecture v2 was built for the NovaSeq™ 6000 System and allowed the SG100K project, part of the Singapore National Precision Medicine (NPM) program, to sequence over 100,000 human genomes within three years.[1–5] Illumina Genomics Architecture v3 retains the key strengths of the v2 architecture and increases sample capacity with the higher throughput of the NovaSeq X Plus System. This technical note describes the Illumina Genomics Architecture v3 framework and reports performance consistency across different sites.

The Illumina Genomics Architecture framework

Illumina Genomics Architecture v3 includes hardware and software components that automate both wet- and dry-lab workflows, enabling:

- Accelerated deployment of NGS workflows
- Rapid adoption through intuitive user-guided interfaces
- Improved workflow management and sample tracking
- Increased throughput with less user intervention
- Simplified integration of workflow components

Hardware includes an automated liquid-handling platform, the Hamilton Microlab STAR, and the NovaSeq X Plus System. Software includes Clarity LIMS™ (laboratory information management system) software, BaseSpace™ Sequence Hub, DRAGEN™ secondary analysis, and Illumina Connected Analytics (Figure 2). The workflow uses Illumina DNA PCR-Free Prep, a self-normalized, bead-based tagmentation assay, for efficient WGS library preparation. After a lab has procured all hardware, software, and appropriate reagent components, Illumina Genomics Architecture reduces the time to reach production-scale WGS workflows from approximately one year to only a few months.[3]

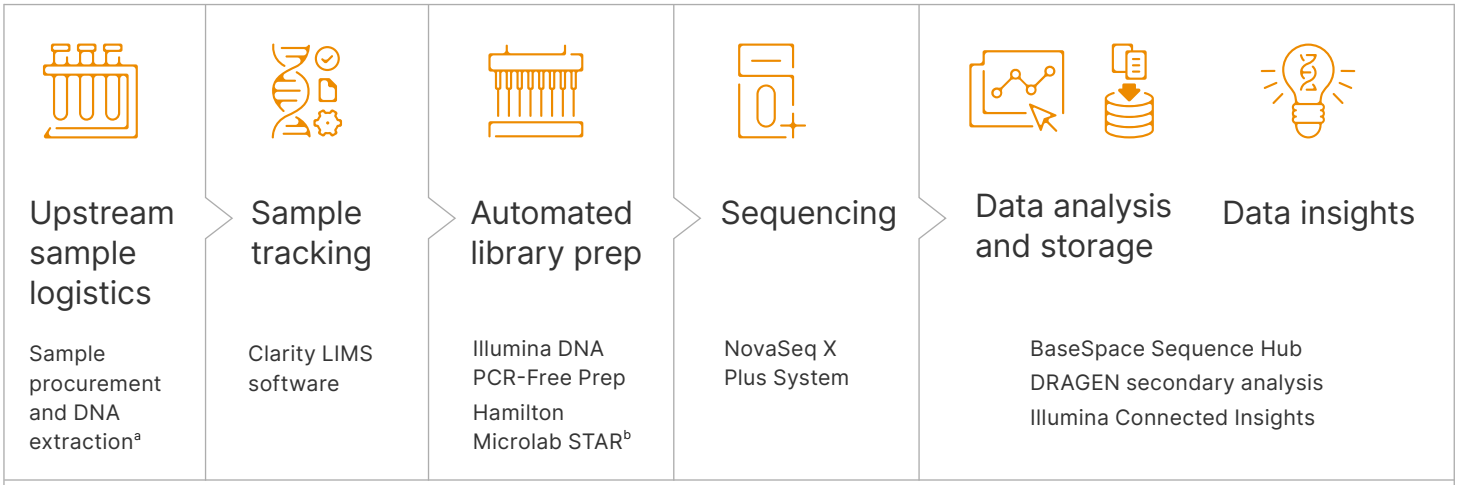

Figure 1: Illumina Genomics Architecture v3 workflow
After sample procurement and DNA extraction, Illumina Genomics Architecture v3 provides sample tracking, scripts for automated library prep, sequencing, and intuitive data analysis. The efficient WGS framework reduces workflow turnaround time while maintaining a high level of performance.

a. A separate DNA extraction kit is required.
b. Requires Illumina Genomics Architecture deck layout.

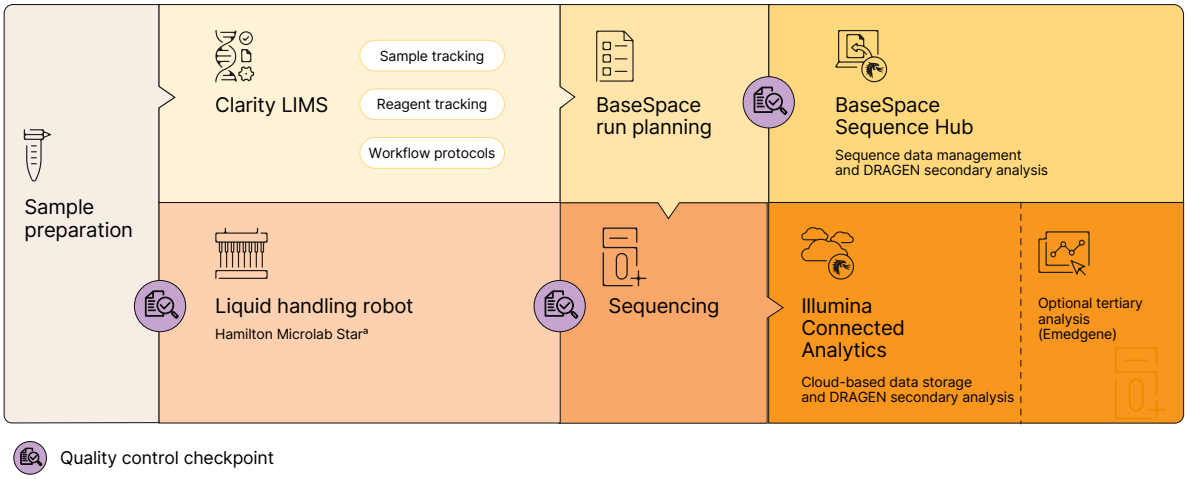

Figure 2: Illumina Genomics Architecture v3 framework

Illumina Genomics Architecture features dedicated robot scripts for library preparation and software integration to automate and streamline sequencing and data analysis. The framework consists of hardware, including a liquid-handling platform and the NovaSeq X Plus System, and software, including Clarity LIMS, BaseSpace Sequence Hub, DRAGEN secondary analysis, and Illumina Connected Analytics. These components coordinate to provide a DNA-to-data workflow for production-scale WGS. Quality control checkpoints are included at key steps to help prevent errors, enable monitoring, and facilitate recovery.

a. Requires Illumina Genomics Architecture deck layout.

Workflow management and sample tracking

Clarity LIMS software guides the workflow and coordinates the liquid handler, sequencing system, and data analysis tools. Preconfigured workflows in Clarity LIMS software, combined with instrument integration, help reduce turnaround times, minimize manual steps, and improve sample throughput while lowering the risk of error. Unique barcode verification enables a full audit trail.

Automated library prep

Illumina Genomics Architecture v3 uses four dedicated robot automation scripts for Illumina DNA PCR-Free Prep (Illumina, Catalog no. 20041794), executed on the Hamilton Microlab STAR liquid-handling platform with the Illumina Genomics Architecture deck layout. These scripts enable fully automated, end-to-end library preparation with minimal manual intervention and support high-throughput, reliable workflows for production-scale WGS. The wet-lab automation reduces human error and fatigue, improves well-to-well reproducibility, and enhances reagent transfer accuracy. Barcode verification of reagents and consumables throughout the workflow supports full traceability and prevents positional errors when loading items onto the robot deck.

The automated workflow includes the following key steps:

- **Preparing input DNA using a standard of 350 ng per well, processed in batches of 24 or 96 samples:** this batching approach supports consistent well-to-well performance and leverages the self-normalizing chemistry to streamline input normalization
- **Constructing libraries using a ~155 minute Illumina DNA PCR-Free Prep method optimized for automation and reliable execution of critical steps, including purification using Illumina Purification Beads:** these refinements help meet the insert size specifications.[6] The final elution volume of 30 µl per library supports pooling for up to two sequencing runs, enabling re-pooling if needed or use of a second pool as a backup

- **Pooling libraries with index correction factors applied by Clarity LIMS to balance representation across libraries:** pooling volumes are automatically adjusted based on batch size, flow cell type, and target loading concentrations to ensure optimal sequencing performance
- **Denaturing and transferring libraries on-deck into strip tubes for reagent cartridges at the desired loading concentration:** this fully integrated step minimizes handling errors and ensures precise loading, resulting in pooled libraries ready to be sequenced on the NovaSeq X Plus system

Sequencing

Prepared libraries are sequenced on the NovaSeq X Plus System (Illumina, Catalog no. 20084804) with a run configuration of 2 × 151 bp. Each dual flow cell can sequence up to 128 human genomes at 30× coverage, with 24 samples per 10B flow cell (Illumina, Catalog no. 20085594) or 64 samples per 25B flow cell (Illumina, Catalog no. 20104706). As part of the automated workflow with Illumina Genomics Architecture, Clarity LIMS software directs the liquid-handling platform to perform bulk pooling, denaturation, and library loading into a strip tube. The user then transfers the strip tube to the NovaSeq X Plus System. Clarity LIMS automatically sends run-planning information to BaseSpace, simplifying run setup and downstream data analysis in Illumina Connected Analytics.

Data analysis

Illumina Connected Analytics is a comprehensive cloud-based platform for data storage, management, and analysis, built with a security-first approach to provide data privacy and compliance. It interfaces directly with DRAGEN secondary analysis to deliver rapid, automated primary (BCL to FASTQ conversion) and secondary (mapping, alignment, and variant calling) analyses. With Illumina Connected Analytics, users have access to powerful tools and machine-learning models to support variant prioritization and interpretation. Once data are available, autolaunch capabilities initiate analysis automatically, streamlining the process.

Verified workflow ensures consistent WGS performance

Illumina Genomics Architecture v3 for the 10B and 25B flow cells on the NovaSeq X Plus System meets the proven standards of Illumina Genomics Architecture v2 (Table 1, Table 2, Figure 3).[1,4,5] Analysis of variance (ANOVA) shows that the Illumina Genomics Architecture v3 workflow delivers consistent results across different sites, robots, and operators when using the same automation scripts and standard Illumina Genomics Architecture workflow protocols (Figure 4).

Table 1: Illumina Genomics Architecture v3 run metrics and passing criteria

Metric	25B flow cell[a]	10B flow cell[b]
Mean yield per run	9.68 Tb	3.81 Tb
Passing filter	74.6%	76.7%
Mean yield per sample	130 Gb	140 Gb
Mean Q30 bases[c]	110 Gb	120 Gb
Mean no. of reads per sample	864M	900M
Median index CV (range)	14.4% (13.4%–16.8%)	11.70% (10.5%–12.9%)
Average autosomal coverage	39×	41×
Median insert length	479 bp	479 bp
Mean bases > Q30[d] (mean)	90.8%	89.5%
Duplicate reads	8.30%	6.85%
Mean genome coverage (15×)[e]	93.23%	93.48%

a. For 25B flow cells 256 samples were tested with 64 samples per flow cell.
b. For 10B flow cells 168 samples were tested with 24 samples per flow cell.
c. Excludes duplicate reads and clipped bases.
d. Excludes index read data.
e. Indicates the percentage of the genome covered at or above 15× sequencing depth

Table 2: Illumina Genomics Architecture v3 variant calling precision and recall

	Single nucleotide variants (SNV)			Insertion-deletions (Indels)		
Sample[a]	Precision	Recall	F1	Precision	Recall	F1
NA12878	99.928%	99.956%	99.945%	99.623%	99.426%	99.525%
NA24385	99.929%	99.953%	99.944%	99.624%	99.426%	99.526%

a. Data are from 17 replicates of each reference cell line obtained from the Coriell Institute for Medical Research.

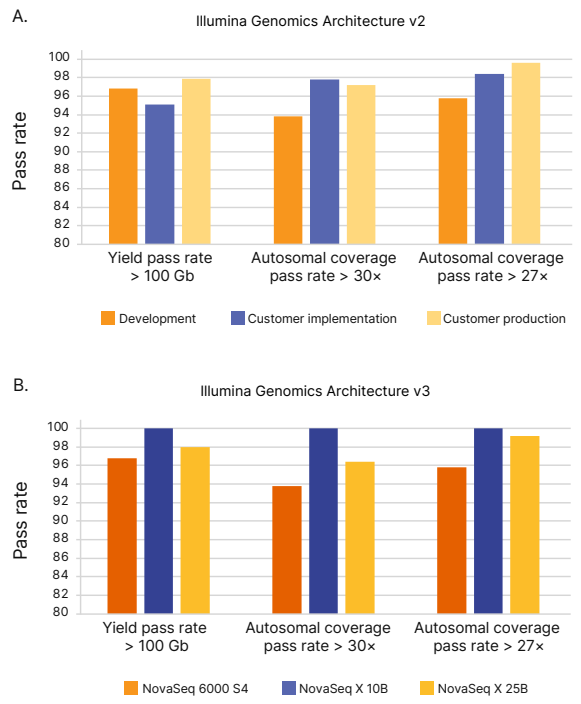

Figure 3: Example performance metrics over ramp up to production-scale WGS

Sequencing yield pass rate and autosomal coverage pass rates at 30× and 27× for (A) Illumina Genomics Architecture v2, from development through customer implementation and into production-scale throughput; and (B) Illumina Genomics Architecture v3 for NovaSeq 6000 S4, NovaSeq X 10B, and NovaSeq X 25B flow cells.

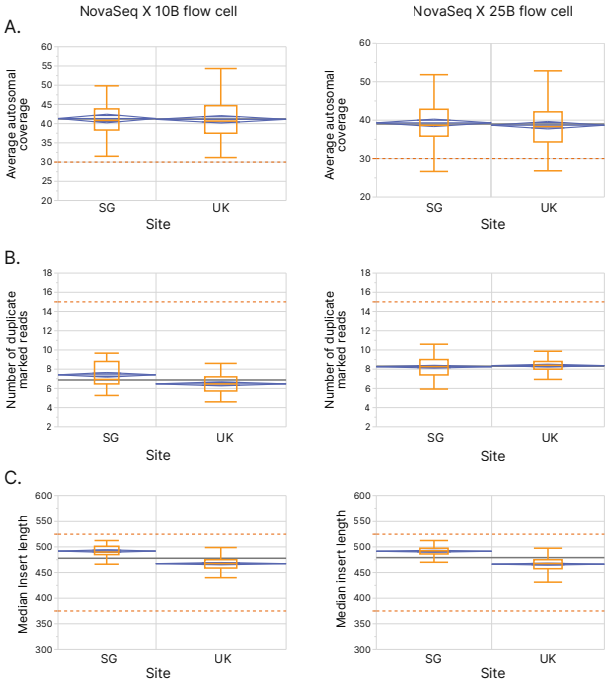

Figure 4: Data performance and consistency with independent operators across different sites

Illumina Genomics Architecture v3 supported WGS operations at development sites in Singapore (SG) and the United Kingdom (UK). ANOVA of sequencing data across the two sites showed highly consistent performance as indicated by (A) average autosomal coverage, (B) number of dupulicate marked reads, and (C) median insert length. WGS was performed with 24 commercial samples obtained from the Coriell Institute for Medical Research. JMP software was used for statistical analysis and to generate graphs.

Summary

Illumina Genomics Architecture v3 provides an integrated, end-to-end framework for streamlined, production-scale WGS using the NovaSeq X Plus System. The streamlined, scalable workflow and integrated software deliver a consistent, site-ready sequencing solution. Implementing Illumina Genomics Architecture v3 reduces manual effort and turnaround time, lowering the risk of error and enabling more efficient WGS at scale.

Contact your Illumina support team to begin implementing Illumina Genomics Architecture.

Learn more →

Illumina Genomics Architecture

Accelerating NGS workflows with Illumina Genomics Architecture

Datasets available on BaseSpace Sequence Hub

Customer spotlight

Clarity LIMS software

Illumina DNA PCR-Free Prep

NovaSeq X Series

Illumina Connected Analytics

Ordering information

Product	Catalog no.	Vendor
Hamilton MicroLab STAR (Illumina Genomics Architecture deck layout and accessories[a])	173027	Hamilton
Illumina DNA PCR-Free Prep (24/96 samples)	20041794/20041795	Illumina
Illumina DNA/RNA UD Indexes	20091654, 20091656, 20091658, 20091660	Illumina
NovaSeq X Plus System	20084804	Illumina
NovaSeq X 10B/25B 300 cycles	20104706/20085594	Illumina
Clarity LIMS cloud Professional/Enterprise annual subscription	20042028/20042029	Illumina
Illumina Connected Analytics Professional/Enterprise annual subscription	20044976/20038994	Illumina

a. Contact Illumina Support for more information

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