

Enhanced sequencing capabilities with the NovaSeq™ 6000 v1.5 Reagent Kit

Achieve the same high data quality as with v1 reagents with better economics, support for more applications, and increased flexibility on the NovaSeq 6000 System.

Introduction

The NovaSeq 6000 System has expanded next-generation sequencing (NGS), enabling researchers to ask and answer increasingly difficult biological questions and uncover more about the genome than ever before. The throughput, speed, and flexibility of the NovaSeq 6000 System supports a wide variety of applications, including whole-genome sequencing (WGS), ultradeep exome sequencing, and single-cell sequencing. Extending these features even further enables more labs to benefit from the capabilities of the NovaSeq 6000 System and empowers researchers to make bigger discoveries.

To help researchers achieve these goals, Illumina offers the NovaSeq 6000 v1.5 Reagent Kit (Figure 1). This kit provides improved economics, more flexible sequencing workflows, and extended reagent shelf-life. The NovaSeq 6000 v1.5 Reagent Kit allows labs to elevate their NGS capabilities, while maintaining the same high data quality previously obtained with v1.0 reagents. This technical note provides an overview of the advanced capabilities of the NovaSeq 6000 v1.5 Reagent Kit and reviews the concordance in quality scores across the reagent kits.

More cost-efficient sequencing

With the NovaSeq 6000 v1.5 Reagent Kit, Illumina aims to make WGS more accessible and affordable for labs of all sizes with the introduction of the \$600 genome. This brings data-rich applications such as combining WGS with RNA sequencing (RNA-Seq), transitioning from bulk to single-cell sequencing, and performing deeper sequencing for increased discovery power within closer reach of researchers, helping them unlock the full story of genetic variation and answer their biggest biological questions.

Support for more applications

The NovaSeq 6000 v1.5 Reagent Kit includes 38 extra cycles of reagents to support unique dual indexes (UDIs) and unique molecular identifiers (UMIs). By providing sufficient reagents to sequence UDIs and UMIs without truncated reads, the kit delivers optimal specificity and sensitivity required for adopting advanced applications, including liquid biopsy, somatic variant discovery, and microbial detection.

In addition to enhanced support for UDIs and UMIs, the NovaSeq 6000 v1.5 Reagent Kit includes sequencing primers needed to run Illumina DNA PCR-Free Prep, Tagmentation. This innovative library prep kit uses On-Bead Tagmentation to generate sequencing-ready libraries in < 1.5 hours. Combining Illumina DNA PCR-Free Prep, Tagmentation with v1.5 reagents avoids introducing PCR bias into WGS studies, which can lead to uneven coverage across regions of the genome.



Figure 1: NovaSeq 6000 v1.5 Reagent Kit—The NovaSeq 6000 v1.5 Reagent Kit provides improved economics, more flexible sequencing workflows, and extended reagent shelf-life.

The NovaSeq 6000 v1.5 Reagent Kit also includes a 35-cycle kit option, providing the optimal read length needed to implement smaller cycle applications, such as gene expression profiling and gene regulation studies, including the Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-Seq) and chromatin immunoprecipitation sequencing (ChIP-Seq). The 35-cycle option offers a 14-hour turnaround time with output up to 350 Gb, produces mean Q30 scores $\geq 90\%$, and includes the extra 38 cycles of reagents needed to support UDIs and UMIs.

Improved flexibility for lab operations

Extension of reagent shelf life

Improvements to the NovaSeq 6000 v1.5 Reagent Kit have extended the shelf life of the kit from three months to six months. This enables labs to purchase large quantities of reagents at one time, supports efficient planning for future research, and provides greater flexibility of reagent use.

Adoption of the reverse complement workflow

Dual-indexed libraries are important for library multiplexing to support increased sample throughputs. Illumina sequencing systems use two different workflows for dual-indexed sequencing. The key difference between the workflows is when the second Index Read occurs. The NovaSeq 6000 v1.5 Reagent Kit switches from the forward strand workflow (used by v1.0 reagents) to the reverse complement workflow (Figure 2). Importantly, the change to the reverse complement workflow with v1.5 reagents is consistent with other Illumina sequencing systems, including the iSeq™ 100, MiniSeq™, NextSeq™ 550, HiSeq™ 3000, HiSeq 4000, and HiSeq X Systems. This allows users to sequence the same sample across different platforms using the same sample sheet to streamline operations and scale studies as needed. Furthermore, the reverse complement workflow enables better compatibility with third-party indexing strategies, providing more flexibility for sequencing applications.

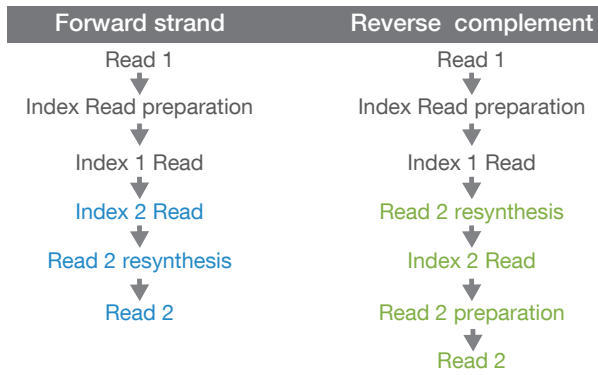


Figure 2: Comparison of dual-indexed sequencing workflows—The NovaSeq 6000 v1.5 Reagent Kit switches from the forward strand to the reverse complement workflow, consistent with many other Illumina sequencing systems. Read the [Indexed Sequencing Overview Guide](#) to learn more.

Reliable performance

To demonstrate the performance of the v1.5 reagents, sequencing metrics were compared between the NovaSeq 6000 v1.0 and v1.5 Reagent Kits. The NovaSeq 6000 v1.5 Reagent Kit exhibited highly concordant primary (Table 1) and secondary (Figure 3) metrics with v1.0 reagents for WGS. Comparison of metrics from bulk total RNA-Seq and single-cell RNA-Seq (scRNA-Seq) libraries also showed high data quality and concordance between the two reagent kits (Table 2). Publicly available data sets described in this technical note can be accessed through [BaseSpace™ Sequence Hub](#) (Table 3).

Table 1: Comparison of primary sequencing metrics

Metric	v1.0 reagents	v1.5 reagents	Std. deviation
Error rate R1	0.15	0.14	0
Error rate R2	0.20	0.20	0
% ≥ Q30 R1	93.49	94.17	0.1
% ≥ Q30 R2	90.36	91.25	0.1

Sequencing run on S4 flow cell.

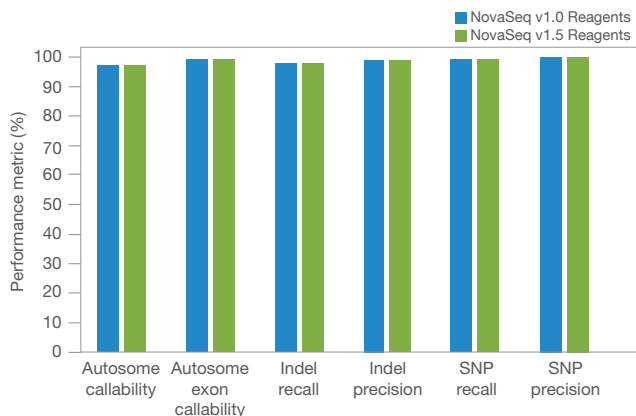


Figure 3: Comparison of secondary sequencing metrics—The NovaSeq 6000 v1.5 Reagent Kit produces highly concordant data with v1.0 reagents.

Table 2: Comparison of secondary sequencing metrics

Metric	v1.0 reagents	v1.5 reagents
Bulk total RNA-Seq		
% aligned reads	98%	98%
% stranded	99%	99%
% abundant	6%	6%
No. of genes detected	18,319	18,357
scRNA-Seq		
No. of cells	1284	1215
% sequencing saturation	92%	93%
% Q30 in barcode	96%	96%
% Q30 bases in RNA reads	94%	94%
Fraction of reads in cells	94%	95%

Table 3: Data sets available in BaseSpace Sequence Hub

NovaSeq S4: Illumina DNA PCR-Free (v1.5 chemistry)
NovaSeq S4: NFE Exome with IDT Panel (v1.5 chemistry)
NovaSeq S4: TruSeq PCR-Free WGS (v1.5 chemistry)
NovaSeq SP: Illumina Stranded mRNA (v1.5 chemistry)
NovaSeq SP: Single-Cell RNA (v1.5 chemistry)

Summary

The NovaSeq 6000 v1.5 Reagent Kit maintains the same high data quality as v1.0 reagents, while offering several advantages. These include more cost-efficient sequencing, innovations to reagent and cycle chemistry, extension of reagent shelf life, and more.

Learn more

To learn more about the NovaSeq 6000 v1.5 Reagent Kit, visit www.illumina.com/products/by-type/sequencing-kits/cluster-gen-sequencing-reagents/novaseq-reagent-kits.html

Appendix: NovaSeq 6000 v1.5 Reagent kits

Flow cell	Reagent kit	Catalog no.
S4	NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles)	20028312
	NovaSeq 6000 S4 Reagent Kit v1.5 (200 cycles)	20028313
	NovaSeq 6000 S4 Reagent Kit v1.5 (35 cycles)	20044417
S2	NovaSeq 6000 S2 Reagent Kit v1.5 (300 cycles)	20028314
	NovaSeq 6000 S2 Reagent Kit v1.5 (200 cycles)	20028315
	NovaSeq 6000 S2 Reagent Kit v1.5 (100 cycles)	20028316
S1	NovaSeq 6000 S1 Reagent Kit v1.5 (300 cycles)	20028317
	NovaSeq 6000 S1 Reagent Kit v1.5 (200 cycles)	20028318
	NovaSeq 6000 S1 Reagent Kit v1.5 (100 cycles)	20028319
SP	NovaSeq 6000 SP Reagent Kit v1.5 (500 cycles)	20028402
	NovaSeq 6000 SP Reagent Kit v1.5 (300 cycles)	20028400
	NovaSeq 6000 SP Reagent Kit v1.5 (200 cycles)	20040719
Xp	NovaSeq 6000 SP Reagent Kit v1.5 (100 cycles)	20028401
	NovaSeq Xp 4-Lane Kit v1.5	20043131
	NovaSeq Xp 2-Lane Kit v1.5	20043130