## Nextseq Platforms

The Nextseq2000 is a mid range Illumina sequencing platform. There are multiple flowcell versions which each have different data yields/ lane. The NovaSeq works well for:

* Single Cell Gene Expression
* Small Scale Whole Genome Sequencing (WGS)
* Small Scale Whole Exome Sequencing (WES)
* Shotgun Metagenomics

Please view the table below to determine the best fit for your needs. If you have issues determining the best fit, HTSF is here to help you figure out the best format for your study’s data need. There are 3 different flowcell sizes (P1, P2, P3). There is a more limited number of standard cycle set up. But the HTSF continues to run custom cycles on the NovaSeq.

A few quick view details about the Nextseq2000:

* It uses patterned flowcells and 2 color channel chemistry.
* This system replaced the HiSeq platforms with some version similar in data output size, but at a lower cost/ run.
* With a single lane, sample can be loaded as soon as the machine is available.
* To determine the best version for your study’s needs, a conference with the HTSF is required.

The below table which compares the various Nextseq loading version abilities can be found in the Forms and Guides tab, Illumina as part of [Illumina Platform Comparison and Specification Table](https://www.med.unc.edu/genomics/wp-content/uploads/sites/708/2020/07/HTSF-Illumina_Sequencing_Platform_Comparison_Chart_v3_06-2020_FINAL.pdf).

Please note, cycles can be made custom to certain library prep methods (i.e. 10x genomics, DropSeq).

### **NextSeq Standard**

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| --- | --- | --- | --- |
|  | **NextSeq2000** | | |
| **Platform** | **P1** | **P2 Reagents** | **P3 Reagents** |
| **Nickname for system** | NextSeq P1 | NextSeq P2 | NextSeq P3 |
| **Flowcells processed** | 1 | 1 | 1 |
| **Lanes/flowcell** | 1 | 1 | 1 |
| **Max PAIRED END Reads/Flowcell** | 200 M | 800 million | 2.2 billion |
| **Max SINGLE END Reads/Flowcell** | 100 M | 400 M | 1.1 B |
| **Clusters/Flowcell** | 100 M | 400 M | 1.1 B |
| **Read Type Format Available** | Paired End | Paired End | Paired End\* |
| \*some single end runs available upon request | | |
| **Read Length Available** | 150x, Custom | 50x, 100x, 150x, Custom | 50x\*, 50x, 100x, 150x, Custom |
| na | na | \*1x50 available |
| **Guaranteed Data Yield/ Flowcell** | 100M Clusters | 400M clusters | 1.1 B clusters |
| 200M Paired End Reads | 800M paired end Reads | 2.2 billion paired end Reads |
| **Key applications** | Small WGS, WES, scRNA-Seq | Small WGS, WES, scRNA-Seq | Small WGS, WES, scRNA-Seq, smallRNA/miRNA-Seq |

|  |  |  |
| --- | --- | --- |
|  | **NextSeq2000 Requirements** | |
| P2 Reagents | P3 Reagents |
| **Number of Lanes** | 1 | 1 |
| **Minimum Volume (ul)** | 15 | 15 |
| **Molarity (nM),** preferred | 5 | 5 |
| **Molarity (nM),** min to make with NO dilution in prep to run FC | 3 | 3 |
| **Number of Clusters/Flowcell** | 400M | 1.1B |
| **Number of Single End Reads Per Flowcell** | 400M | 1.1B |
|  |  |  |
|  |  |  |
|  | **Recommended Number of Samples per Pool for Each NextSeq Platform** | |
| **Small whole-genome sequencing** (300 cycles) 130 Mb genome; > 30× coverage | 30 | 82 |
| **Whole-exome sequencing** (200 cycles) 50× mean targeted coverage; 90% targeted coverage at 20× | 16 | 44 |
| **Shotgun Metagenomics** (300 cycles) 50M reads/sample | 8 | 20 |
| **Single-cell RNA-Seq** (100 cycles) 4K cells, 50K reads/cell | 2 | 5 |
| **miRNA-Seq or small RNA analysis** (50 cycles) 11M reads/sample | n/a | 96 |