TECHNICAL NOTE

# Single-cell and spatial sequencing on NextSeq™ 1000 and NextSeq 2000 XLEAP-SBS™ flow cells

Perform high-resolution genomics on a market-leading benchtop system

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# Introduction

Complex biological systems are determined by the coordinated functions of individual cells in organized tissues. Conventional methods that look at dissociated samples in bulk can mask the cellular heterogeneity and spatial relationships that drive this complexity. Single-cell and spatial sequencing are next-generation sequencing (NGS) methods that provide a high-resolution view of cell-to-cell variation and organization.

10x Genomics, an Illumina partner, has commercialized multiple single-cell and spatial biology solutions. These widely adopted library preparation and analysis products rely on the accuracy and simplicity of Illumina sequencing systems. The NextSeq 1000 and NextSeq 2000 Systems with XLEAP-SBS chemistry are the ideal benchtop systems for single-cell and spatial studies, offering flexibility, scalability, and high data quality to match the sensitivity of these assays.

## Single-cell and spatial RNA sequencing

Profiling gene expression at the single-cell level or with preserved spatial context increases discovery power to understand the mechanisms of development and disease more deeply and precisely.

Single-cell RNA sequencing (scRNA-Seq) uses cell partitioning and oligonucleotide barcodes to examine the transcriptomes of hundreds to tens of thousands of individual cells. By providing a detailed view of cell-to-cell variation, scRNA-Seq facilitates the identification of novel biomarkers and rare cell types that would otherwise be missed with bulk RNA-Seq.[1,2]

Spatial transcriptomics combines high-throughput imaging and sequencing technologies to show mRNA expression at the cellular level in structurally preserved tissues. Visualizing tissue morphology overlaid with gene activity can help researchers understand the spatial relationship between cells within normal and diseased tissues.

Both single-cell and spatial sequencing approaches can be applied to modalities beyond RNA, including DNA, epigenome, or protein. For example, 10x Genomics offers multiomic solutions that pair scRNA-Seq with assays for chromatin accessibility or protein expression. Spatial solutions can measure RNA and protein expression together as well.

## NextSeq 1000 and NextSeq 2000 Systems

The NextSeq 1000 and NextSeq 2000 Systems are powered by XLEAP-SBS chemistry, the fastest, highest quality, and most robust Illumina sequencing by synthesis (SBS) chemistry to date. Built upon the proven foundation of standard Illumina SBS chemistry, XLEAP-SBS chemistry delivers improved reagent stability with two-fold faster incorporation times.[3]

The NextSeq 1000 and NextSeq 2000 Systems offer scalability to accommodate a wide range of single-cell or spatial project needs, allowing adjustment of cells per sample, reads per cell, and samples per experiment (Table 1, Table 2).* Whether researchers want to sequence deeper to access lower abundance transcripts or sequence more cells or samples, the NextSeq 1000 and NextSeq 2000 Systems offer an accessible solution for sequencing and primary analysis on a benchtop system. With four available flow cell types, researchers have flexibility to use multiple methods of NGS analysis and accommodate various experimental designs.

This technical note demonstrates the synergy of using the NextSeq 1000 and NextSeq 2000 Systems for 10x Genomics single-cell and spatial solutions, across multiple library types and flow cell outputs. We also compare performance with XLEAP-SBS chemistry vs standard SBS chemistry and provide guidance on run setup, expected sequencing performance, and example analysis metrics.

# Methods

## scRNA-Seq assays

Chromium Single Cell Gene Expression assays from 10x Genomics offer a streamlined solution for scRNA-Seq. GEM-X technology improves performance and boosts the workflow efficiency of Chromium assays, while providing single-cell transcriptome 3' gene expression and multiomic capabilities. The Chromium Gene Expression Flex assay allows gene expression profiling for thousands to hundreds of thousand of fixed cells or nuclei with a sensitive probe-based method. A typical scRNA-Seq experiment with

* For spatial biology assays, projects are measured in terms of tissue spots and capture areas instead of cells.

Chromium single-cell assays follows a workflow of sample preparation, cell partitioning and barcoding, library prep, sequencing, and analysis (Figure 1).

### Chromium GEM-X Single Cell 3′ Gene Expression v4

Samples for scRNA-Seq were prepared from a cryopreserved human peripheral blood mononuclear cells (PBMCs). Single-cell libraries were prepared using the Chromium GEM-X Single Cell 3′ Reagent Kits v4 (10x Genomics, Catalog no. PN-1000691) following the protocol in the user guide (10x Genomics, Document no. CG000731, Rev A).[4] Sequencing was performed on the NextSeq 2000 System with NextSeq 2000 XLEAP-SBS P4 reagent kits (100 cycles) (Illumina, Catalog no. 20100994). For comparison, the same libraries were also sequenced on the NextSeq 2000 System with the standard SBS NextSeq 2000 P3 reagent kit (100 cycles) (Illumina, Catalog no. 20040559). Run configurations were set up according to the parameters provided by 10x Genomics: 28-cycle read 1, 10-cycle i7 and i5 index reads, and 90-cycle read 2. Loading concentration for both XLEAP-SBS and standard SBS reagents was 650 pM and 1% PhiX was spiked in. Data analysis was performed using the Cell Ranger pipeline v8.0.0 (10x Genomics).

### Chromium Single Cell Gene Expression Flex

Samples were prepared from cryopreserved human PBMCs. Cells were fixed using the Chromium Next GEM Single Cell Fixed RNA Sample Preparation Kit (10x Genomics, Catalog no. PN-1000414) following the demonstrated protocol (10x Genomics, Document no. CG000478, Rev D).[5] Libraries were prepared using the Chromium Fixed RNA Kit, Human Transcriptome (10x Genomics, Catalog no. PN-1000476) following the protocol in the user guide (10x Genomics, Document CG000527 Rev F).[6] Sequencing was performed on the NextSeq 2000

Table 1: Example sample throughput for Chromium single-cell assays on NextSeq 1000 and NextSeq 2000 Systems

| Product | Minimum read pairs per cell[a] | Cells per sample | No. of samples per run[b] for NextSeq 1000 and NextSeq 2000 flow cells | | | |
|---|---|---|---|---|---|---|
| | | | P1[c] | P2 | P3[d] | P4[d] |
| Chromium GEM-X Single Cell 3′ Gene Expression v4 | 20K | 5K | 1 | 4 | 12 | 18 |
| Chromium Single Cell Gene Expression Flex – Multiplex | 10K | 5K | 2 | 8 | 24 | 36 |

a. Minimum read recommendations provided courtesy of 10x Genomics. Adjust sequencing depth for the required performance or application. The sequencing saturation metric and curve in the Cell Ranger run summary can be used to optimize sequencing depth for specific sample types.
b. The number of single-cell samples per sequencing run is based on an Illumina PhiX control library at supported cluster densities and loading concentration. Actual performance parameters may vary based on sample type, sample quality, and clusters passing filter.
c. P1 flow cells are a good option for single-cell quality control experiments.
d. P3 and P4 flow cells are only available on the NextSeq 2000 System.

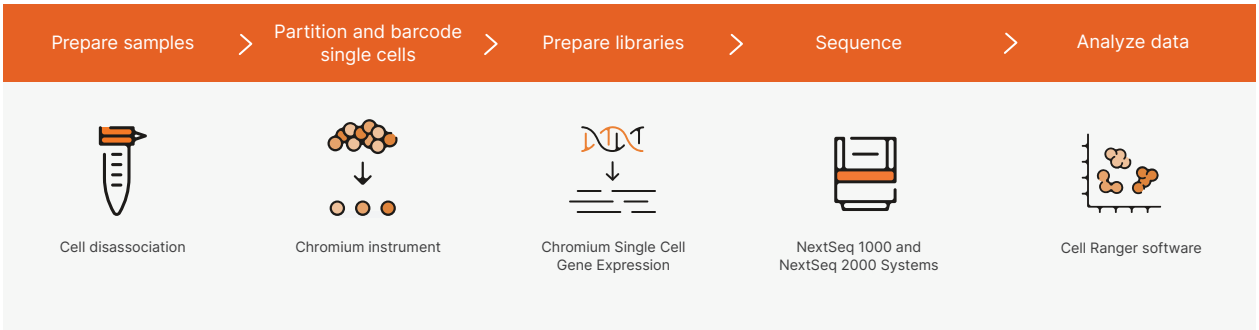


Figure 1: Chromium Single Cell Gene Expression workflow—A typical scRNA-Seq experiment follows a workflow of sample preparation, cell partitioning and barcoding, library preparation, sequencing, and data analysis.

System with XLEAP-SBS chemistry on P4 100-cycle flow cells and standard SBS chemistry on P3 100-cycle flow cells with the following read length: 28-cycle read 1, 10-cycle i7 and i5 index reads, and 90-cycle read 2. Loading concentration was 650 pM and 5% PhiX was spiked in. Data analysis was performed using the Cell Ranger pipeline v7.1.0 (10x Genomics).

## Spatial RNA-Seq assays

Visium Spatial Gene Expression assays from 10x Genomics map the whole transcriptome with morphological context in formalin-fixed, paraffin-embedded (FFPE) and fresh-frozen tissues. Visium v1 and v2 tissue slide capture areas have an array of ~5000 barcoded spots, 55 µm in diameter, spaced 100 µm apart. By contrast, high-definition Visium HD capture areas contain a continuous grid of 2 × 2 µm barcoded squares, ~11.2 million in total. A typical spatial RNA-Seq experiment with Visium spatial assays follows a workflow of tissue sample preparation, imaging, library prep, sequencing, and analysis (Figure 2).

### Visium CytAssist Spatial Gene Expression v2

A mouse brain FFPE block was sectioned as described in the Visium CytAssist Spatial Gene Expression for FFPE tissue preparation demonstrated protocol (10x Genomics, Document no. CG000518, RevD).[7] Deparaffinization, H&E staining, imaging, and decrosslinking followed that demonstrated protocol (10x Genomics, Document no.

Table 2: Example sample throughput for Visium spatial assays on NextSeq 1000 and NextSeq 2000 Systems

| Product | Sample type | Read pairs per tissue spot[c] | Reads per tissue section[c] | No. of capture areas per run[a,b] for NextSeq 1000 and NextSeq 2000 flow cells | | |
|---|---|---|---|---|---|---|
| | | | | P2 | P3[d] | P4[d] |
| Visium CytAssist Spatial Gene Expression v2[a] | FFPE tissue | 25K | 125M | 3 | 9 | 14 |
| | Fresh-frozen tissue | 50K | 250M | 1 | 4 | 7 |
| Visium HD Spatial Gene Expression[b] | FFPE tissue | – | 275M | 1 | 4 | 6 |
| | Fresh-frozen tissue | – | 700M | – | 1 | 2 |

a. Minimum read recommendations provided courtesy of 10x Genomics. Sample throughput calculated based on recommended read pairs per tissue spot, 5000 tissue spots per capture area, and average 50% capture area covered by tissue section. Visium Spatial Gene Expression slides for fresh-frozen tissue have four capture areas for running up to four tissue sections per slide. Visium Spatial Gene Expression slides for FFPE tissue have two capture areas for running up to two tissue sections per slide.
b. Sample throughput calculated based on 275M reads (for FFPE tissue) or 700M reads (for fresh-frozen tissue) per capture area fully covered by tissue section (or in proportion to the capture area covered by the tissue section). Visium HD Spatial Gene Expression slides contain a continuous grid of 2 × 2 µm barcoded squares.
c. Minimum read recommendations provided courtesy of 10x Genomics. Read depth guidance for Visium HD for fresh-frozen tissue is for reference only and not yet validated on the NextSeq 2000 System with XLEAP-SBS chemistry.
d. P3 and P4 flow cells are only available on the NextSeq 2000 System.

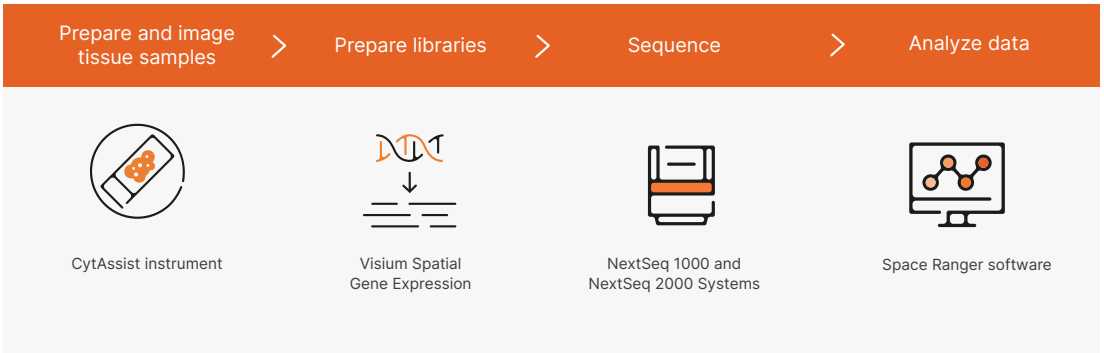


Figure 2: Visum Spatial Gene Expression workflow—A typical spatial RNA-Seq experiment follows a workflow of tissue sample preparation, imaging, library preparation, sequencing, and data analysis.

CG000520, Rev C)[8] and tissue was transferred to a Visium CytAssist Spatial Gene Expression slide using the CytAssist instrument. Libraries were prepared using Visium CytAssist Spatial Gene Expression for FFPE (10x Genomics, Catalog no. PN-1000521) following the protocol in the user guide (10x Genomics, Document no. CG000495, Rev F).[9] Sequencing was performed on the NextSeq 2000 System with XLEAP-SBS chemistry on P4 100-cycle flow cells and standard SBS chemistry on P3 100-cycle flow cells with the following read length: 28-cycle read 1, 10-cycle i7 and i5 index reads, and 90-cycle read 2. Loading concentration was 650 pM and 1% PhiX was spiked in. Data analysis was performed using the Space Ranger pipeline v2.0 (10x Genomics).

#### Visium HD Spatial Gene Expression

A mouse embryo FFPE block was sectioned, deparaffinized, H&E stained, and imaged following the Visium HD FFPE tissue preparation handbook (10x Genomics, Document no. CG000684, Rev A). Probe hybridization, probe ligation, slide preparation, probe release, extension, and library construction used Visium HD Reagent Kit (10x Genomics, Catalog no. PN-1000668) following the protocol in the user guide (10x Genomics, Document no. CG000685, Rev A).[10] Sequencing was performed on the NextSeq 2000 System with XLEAP-SBS chemistry on P4 100-cycle flow cells and standard SBS chemistry on P3 100-cycle flow cells with the following read length: 43-cycle read 1, 10-cycle i7 and i5 index reads, and 50-cycle read 2. Loading concentration was 650 pM and 1% PhiX was spiked in. Data analysis was performed using the Space Ranger pipeline v3.0 (10x Genomics).

## Results

Sequencing of Chromium single-cell libraries or Visium spatial libraries using high-performance XLEAP-SBS chemistry on the NextSeq 1000 and NextSeq 2000 Systems delivered results that are comparable to standard SBS chemistry, while enabling higher data output and faster run times (Table 3).

### scRNA-Seq assays

#### Chromium GEM-X Single Cell 3′ Gene Expression v4

Results demonstrate high data concordance between XLEAP-SBS chemistry and standard SBS chemistry for Chromium scRNA-Seq libraries. For primary metrics, there is approximately a 2% improvement in percent clusters passing filter (% PF), and Q30 scores (Table 4). For sequencing metrics, there is a 2% improvement in Q30 bases in the barcode and RNA reads and a 4% improvement in Q30 bases in unique molecular identifiers (UMI) (Table 5). Visualization of cell type classification by t-SNE plot shows comparable results for both data sets (Figure 3A).

Table 3: Loading guidance and expected outcome of run metrics for NextSeq 2000 P4 XLEAP-SBS flow cells vs NextSeq 2000 P3 standard SBS flow cells

| Product | Sequencing configuration (R1, i7, i5, R2) | Library loading concentration | Percent PhiX input | Percent PhiX aligned[a] | Cluster % PF P4 XLEAP-SBS[b] | Cluster % PF P3 standard SBS[b] |
|---|---|---|---|---|---|---|
| Chromium GEM-X Single Cell 3′ Gene Expression v4 | 28, 10, 10, 90 | 650 pM | 1% | 0.4% | 77.4% | 75.8% |
| Chromium Single Cell Gene Expression Flex – Multiplex | 28, 10, 10, 90 | 650 pM | 5% | 2.7% | 88.9% | 84.4% |
| Visium CytAssist Spatial Gene Expression v2 | 28, 10, 10, 90 | 650 pM | 1% | 0.9% | 87.8% | 78.3% |
| Visium HD Spatial Gene Expression | 43, 10, 10, 50 | 650 pM | 1% | 0.5% | 89.2% | 72.2% |

a. Percent PhiX aligned values shown for NextSeq 2000 P4 XLEAP-SBS flow cells. See Tables 4, 6, 8, and 10 for NextSeq 2000 P3 standard SBS flow cell values for percent PhiX aligned. Variation in percent PhiX aligned is within normal run-to-run variation.
b. % PF, percent clusters passing filter.

Table 4: Primary metrics for Chromium GEM-X Single Cell 3′ Gene Expression v4

| | P4 XLEAP-SBS chemistry | P3 standard SBS chemistry |
|---|---|---|
| Run configuration | 28, 10, 10, 90 | 28, 10, 10, 90 |
| Yield | 244.89 Gb | 172.07 Gb |
| Loading concentration | 650 pM | 650 pM |
| % PF | 77.4% | 75.8% |
| % PhiX aligned | 0.4% | 1.9% |
| Read 1 bases ≥ Q30 | 96.3% | 93.7% |
| Read 2 bases ≥ Q30 | 94.2% | 92.3% |
| Read 1 error rate | 0.07% | 0.07% |
| Read 2 error rate | 0.16% | 0.16% |
| Estimated no. of cells per sample | 1077 | 1072 |
| No. of genes detected | 26,825 | 26,312 |
| Median UMI counts per cell | 22,683 | 21,174 |

Table 5: Sequencing run metrics for Chromium GEM-X Single Cell 3′ Gene Expression v4

| | P4 XLEAP-SBS chemistry | P3 standard SBS chemistry |
|---|---|---|
| Valid barcodes | 95.7% | 95.7% |
| Reads mapped confidently to exonic regions | 57.2% | 57.0% |
| Reads mapped confidently to transcriptome | 70.7% | 70.6% |
| Fraction reads in cells | 96.9% | 96.9% |
| Q30 bases in barcode | 96.2% | 93.9% |
| Q30 bases in RNA read | 95.8% | 93.6% |
| Q30 bases in UMI | 96.9% | 93.0% |

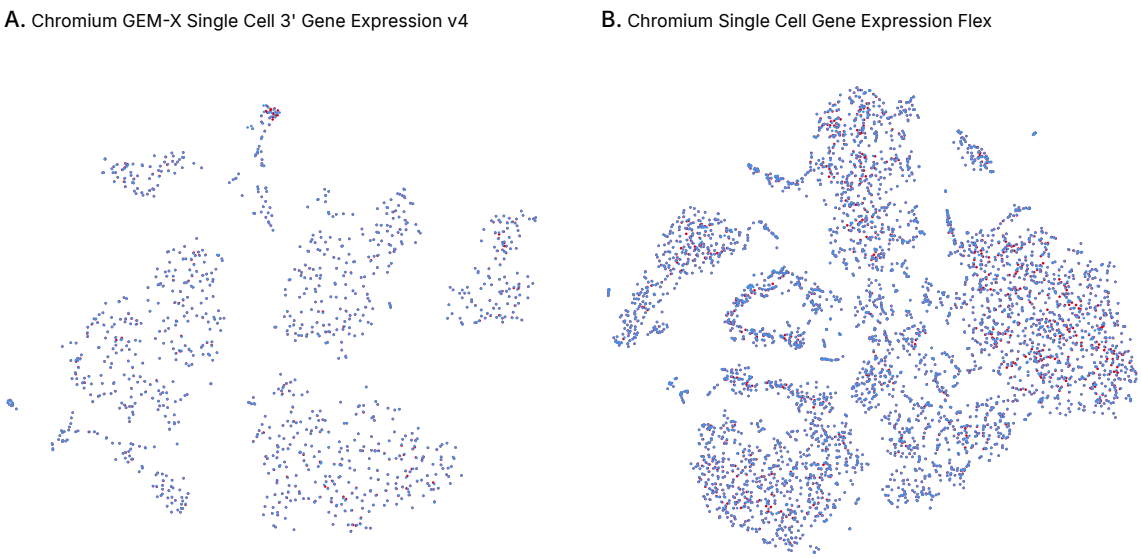


Figure 3: Chromium Single Cell Gene Expression on the NextSeq 2000 System—Visualization of cell type classification with t-distributed stochastic neighbor embedding (t-SNE) plots for (A) Chromium GEM-X Single Cell 3′ Gene Expression v4 and (B) Chromium Single Cell Gene Expression Flex, sequenced on the NextSeq 2000 System with the NextSeq 2000 P4 flow cell with XLEAP-SBS chemistry (blue) and NextSeq 2000 P3 flow cell with standard SBS chemistry (orange). Note that blue and orange dots may be hard to distiguish because of the data overlap.

### Chromium Single Cell Gene Expression Flex

Results demonstrate high data concordance between XLEAP-SBS chemistry and standard SBS chemistry for Chromium Flex scRNA-Seq libraries. For primary metrics, there is approximately a 5% improvement in % PF (Table 6). Increase in % PF means a higher number of bases passed the filter, thereby increasing output of the flow cell. Sequencing metrics and t-SNE plots show comparable results between data sets (Table 7, Figure 3B).

## Spatial RNA-Seq assays

### Visium CytAssist Spatial Gene Expression v2

Results demonstrate high data concordance between XLEAP-SBS chemistry and standard SBS chemistry for Visium Spatial RNA-Seq libraries. For primary metrics, there is approximately a 12% improvement in % PF (Table 8). Sequencing metrics and spatial gene expression visualization show comparable results between data sets (Table 9, Figure 4).

### Visium HD Spatial Gene Expression

Results demonstrate high data concordance between XLEAP-SBS chemistry and standard SBS chemistry for Visium HD Spatial RNA-Seq libraries. For primary metrics, there is approximately a 23% improvement in % PF (Table 10). For sequencing metrics, there is a 2% improvement in Q30 bases in barcode and RNA read, and Q30 bases in UMI (Table 11). Visualization of spatial gene expression shows higher UMI counts for data generated with XLEAP-SBS chemistry (Figure 5).

Table 6: Primary metrics for Chromium Single Cell Gene Expression Flex

| | P4 XLEAP-SBS chemistry | P3 standard SBS chemistry |
|---|---|---|
| Run configuration | 28, 10, 10, 90 | 28, 10, 10, 90 |
| Yield | 281.25 Gb | 191.85 Gb |
| Loading concentration | 650 pM | 650 pM |
| % PF | 88.9% | 84.4% |
| % PhiX aligned | 2.7% | 1.8% |
| Read 1 bases ≥ Q30 | 97.3% | 95.9% |
| Read 2 bases ≥ Q30 | 73.8% | 74.5% |
| Read 1 error rate | 0.06% | 0.05% |
| Read 2 error rate | 12.02% | 12.24% |
| Estimated no. of cells per sample | 4033 | 4015 |
| No. of genes detected | 13,717 | 13,686 |
| Median UMI counts per cell | 5809 | 5472 |

Table 7: Sequencing run metrics for Chromium Single Cell Gene Expression Flex

| | P4 XLEAP-SBS chemistry | P3 standard SBS chemistry |
|---|---|---|
| Valid barcodes | 97.0% | 95.3% |
| Confidently mapped reads in cells | 96.3% | 96.2% |
| Fraction reads confidently mapped to the filtered probe set | 94.9% | 94.9% |
| Q30 bases in barcode | 95.7% | 94.8% |
| Q30 bases in probe read | 91.9% | 92.0% |
| Q30 bases in UMI | 98.0% | 96.4% |

Table 8: Primary metrics for Visium CytAssist Spatial Gene Expression v2

| | P4 XLEAP-SBS chemistry | P3 standard SBS chemistry |
|---|---|---|
| Run configuration | 28, 10, 10, 90 | 28, 10, 10, 90 |
| Yield | 277.89 Gb | 177.56 Gb |
| Loading concentration | 650 pM | 650 pM |
| % PF | 87.8% | 78.3% |
| % PhiX aligned | 0.9% | 1.4% |
| Read 1 bases ≥ Q30 | 97.2% | 96.3% |
| Read 2 bases ≥ Q30 | 75.0% | 70.1% |
| Read 1 error rate | 0.06% | 0.04% |
| Read 2 error rate | 3.99% | 11.72% |
| No. of genes detected | 19,358 | 19,354 |
| Median UMI counts per spot | 180,374 | 119,010 |

Table 9: Sequencing run metrics for Visium CytAssist Spatial Gene Expression v2

| | P4 XLEAP-SBS chemistry | P3 standard SBS chemistry |
|---|---|---|
| Valid barcodes | 99.1% | 99.1% |
| Valid UMIs | 99.5% | 100.0% |
| Fraction reads in spots under tissue | 91.4% | 91.4% |
| Fraction reads confidently mapped to the filtered probe set | 92.8% | 92.6% |
| Q30 bases in barcode | 97.3% | 96.5% |
| Q30 bases in RNA read | 97.3% | 94.6% |
| Q30 bases in UMI | 97.7% | 96.6% |

**A.** NextSeq 2000 P4 flow cell with XLEAP-SBS chemistry

**B.** NextSeq 2000 P3 flow cell with standard SBS chemistry

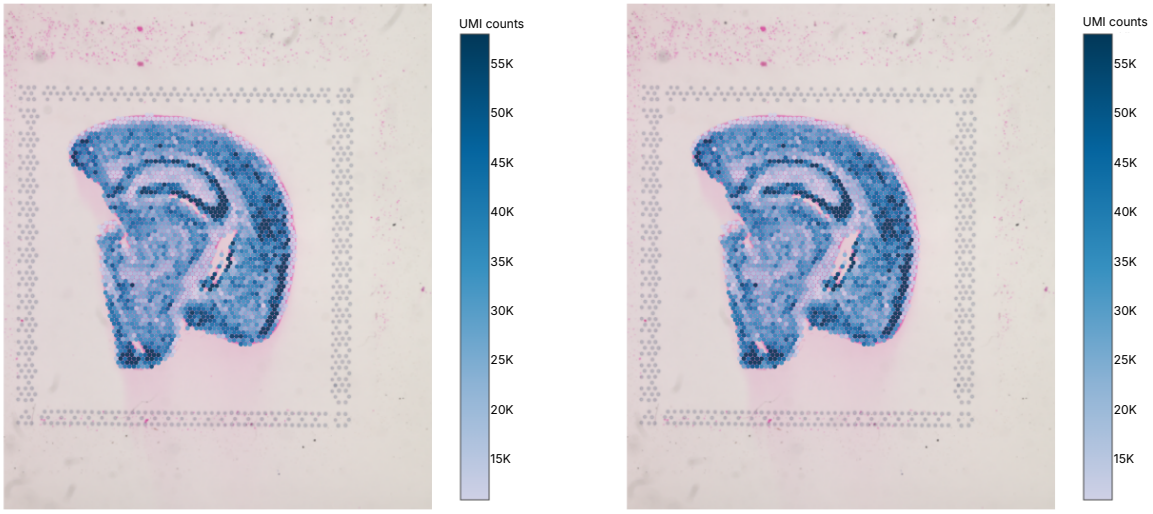


Figure 4: Visium Spatial Gene Expression for FFPE on the NextSeq 2000 System—Visualization of gene expression in context of tissue architecture for mouse brain FFPE tissue sections using Visium CytAssist Spatial Gene Expression v2, sequenced on the NextSeq 2000 System with (A) NextSeq 2000 P4 flow cell with XLEAP-SBS chemistry and (B) NextSeq 2000 P3 flow cell with standard SBS chemistry. Tissue plots are colored by UMI count. The P4 flow cell and XLEAP-SBS chemistry enable comparable data with higher UMI counts.

Table 10: Primary metrics for Visium HD Spatial Gene Expression

| | P4 XLEAP-SBS chemistry | P3 standard SBS chemistry |
|---|---|---|
| Run configuration | 43, 10, 10, 50 | 43, 10, 10, 50 |
| Yield | 228.07 Gbp | 133.24 Gbp |
| Loading concentration | 650 pM | 650 pM |
| % PF | 89.2% | 72.2% |
| % PhiX aligned | 0.5% | 1.9% |
| Read 1 bases ≥ Q30 | 97.3% | 95.6% |
| Read 2 bases ≥ Q30 | 96.7% | 94.4% |
| Read 1 error rate | 0.09% | 0.06% |
| Read 2 error rate | 0.10% | 0.14% |
| No. of genes detected | 19,038 | 19,036 |
| Median UMI counts per 8 µm bin | 522.4 | 424.4 |

Table 11: Sequencing run metrics for Visium HD Spatial Gene Expression

| | P4 XLEAP-SBS chemistry | P3 standard SBS chemistry |
|---|---|---|
| Valid barcodes | 92.8% | 93.3% |
| Valid UMIs | 100.0% | 99.7% |
| Fraction reads confidently mapped to the filtered probe set | 98.9% | 98.7% |
| Q30 bases in barcode | 97.6% | 95.5% |
| Q30 bases in RNA read | 97.1% | 94.9% |
| Q30 bases in UMI | 97.6% | 95.9% |

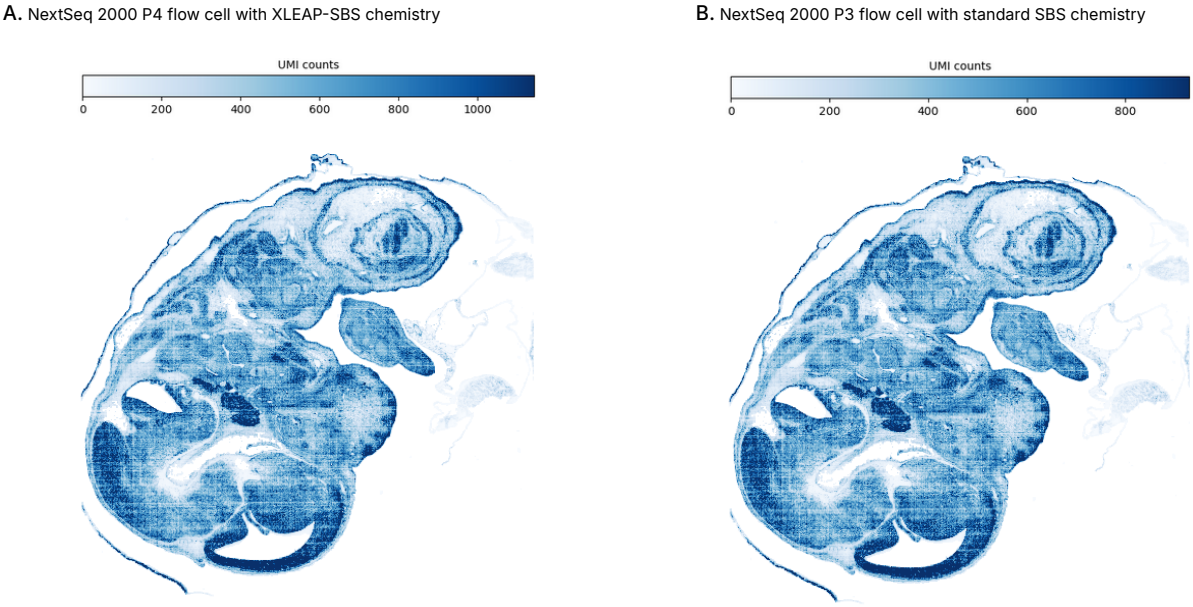


Figure 5: Visum HD Spatial Gene Expression on the NextSeq 2000 System—Visualization of gene expression in context of tissue architecture for mouse embryo FFPE tissue sections using Visium HD Spatial Gene Expression, sequenced on the NextSeq 2000 System with (A) NextSeq 2000 P4 flow cell with XLEAP-SBS chemistry and (B) NextSeq 2000 P3 flow cell with standard SBS chemistry. Tissue plots are colored by UMI count. The P4 flow cell and XLEAP-SBS chemistry enable comparable data with higher UMI counts.

## Summary

Single-cell and spatial sequencing methods can help researchers unlock a deeper understanding of complex cell populations and tissues. The NextSeq 1000 and NextSeq 2000 Systems, in synergy with 10x Genomics single-cell and spatial library prep and analysis solutions, make these high-resolution NGS methods accessible for more labs. High-performance XLEAP-SBS chemistry on the NextSeq 1000 and NextSeq 2000 Systems offers results comparable to standard SBS chemistry, while enabling higher data output and faster run times for single-cell and spatial gene expression assays.

## Learn more

NextSeq 1000 and NextSeq 2000 Systems

Chromium Single Cell Gene Expression

Visium Spatial Gene Expression

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