NextSeq™ 1000 & 2000

Russ Carmical, PhD
Executive Sequencing Specialist
Illumina

illumina

Illumina Sequencing Instruments

iSeq™ 100

MiniSeq™

MiSeq™

NextSeq™ 550

NextSeq™ 1000 & 2000

NovaSeq™ 6000

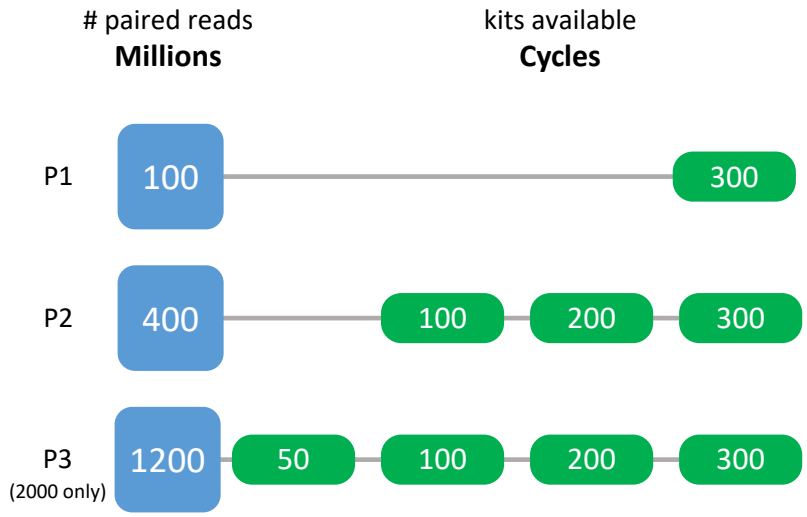
NextSeq 1000/2000

Features

- NextSeq 2000: P1/P2/P3 Flowcells
- NextSeq 1000: P1/P2 Flowcells
- 2-color SBS chemistry
- Patterned Flowcell/ExAmp
- Onboard DRAGEN™ Bio-IT Platform
- Introduced in 2020

Key Applications

- RNA seq
- Single Cell Applications
- Human Exomes
- Targeted regions
- Multiomics



Output

NovaSeq

800 (SP) — 1,650 (S1) — 4,160 (S2) — 10,000 (S4)

NextSeq

1000: 100 — 400

2000: 100 (P1) — 400 (P2) — 1200 (P3)

NextSeq 550

130 (Mid) — 400 (High)

MiSeq

1 (Nano) — 4 (Micro) — 15 (v2) — 25 (v3)

MiniSeq

8 (Mid) — 20 (Rapid) — 25 (High)

iSeq

4

● Flowcells

Clusters in **Millions**

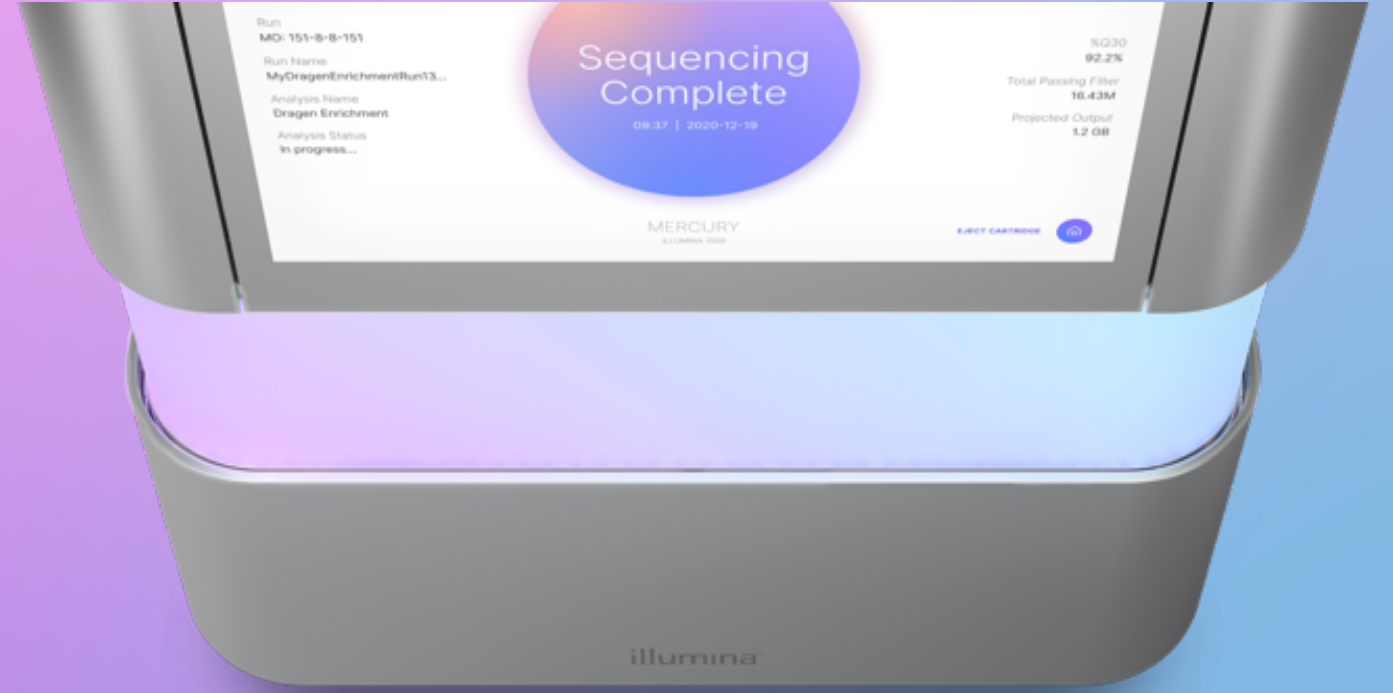
illumina®

Ultra high-density flow cells enable significant performance gains and tunable outputs

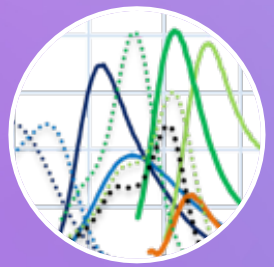
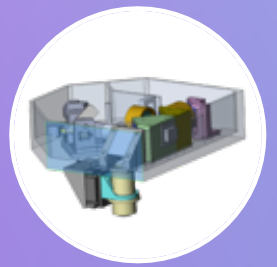
P3 Flow Cell

P3 Flow Cell		P2 Flow Cell
≥ 75% Q30	QUALITY	≥ 75% Q30
Up to 1000M	READ NUMBER	Up to 400M
40 - 300G	OUTPUT	40 - 120G
< 40 Hrs	RUN TIME	< 30 Hrs
~$20/Gb	$/Gb	~$30/Gb

P2 Flow Cell



> 75 Breakthrough Innovations deliver meaningful benefits

illumina®

3 Key technologies enable > 30× increase in data density

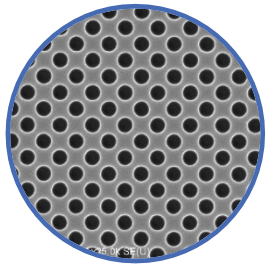
NextSeq 500

HO Flow Cell

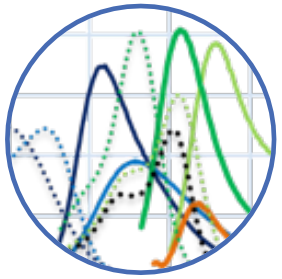
~200K

CLUSTERS/MM²

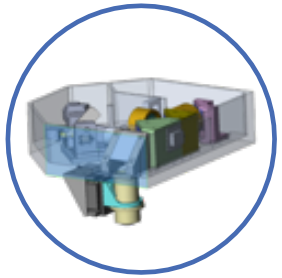
Patterned Flow Cell



Blue, 2-Channel SBS



Super-Resolution Optics



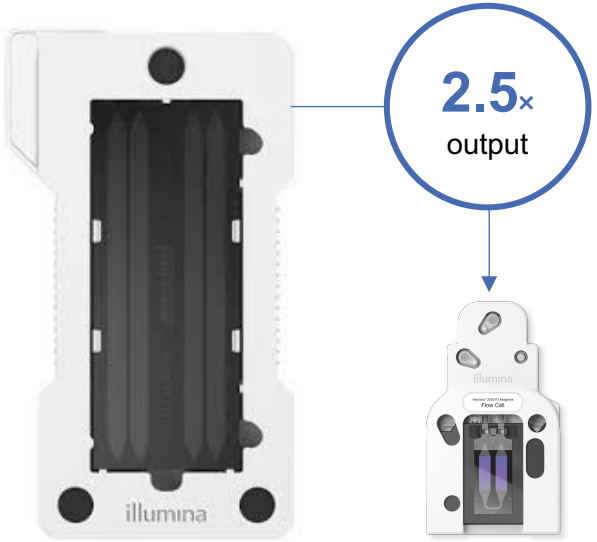
NextSeq 2000

P3 Flow Cell

~6250K

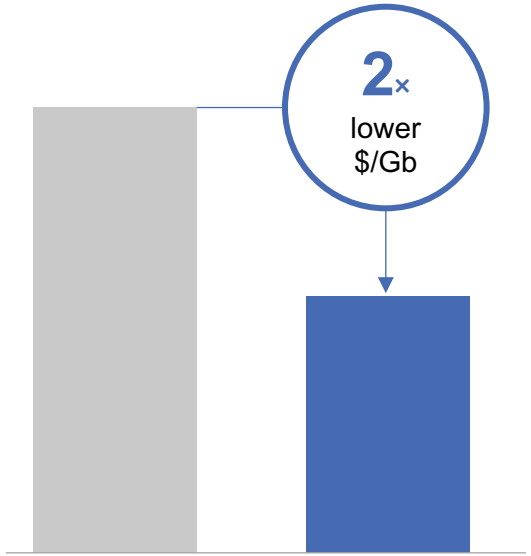
CLUSTERS/MM²

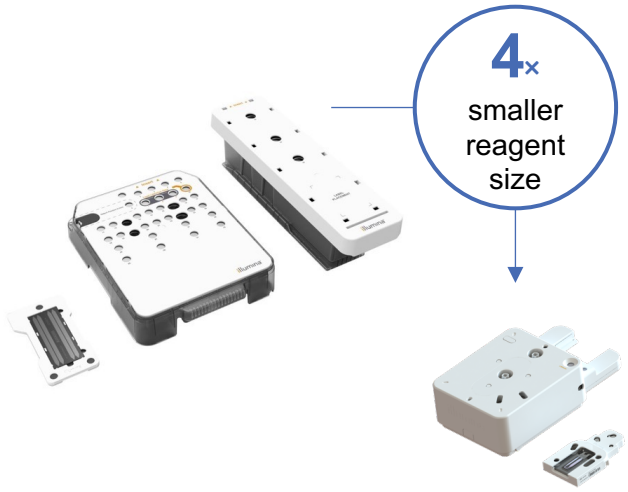
Major increase in density translates to less waste, less complexity and more value per run compared to NextSeq 550



120Gb
NextSeq 550

300Gb
NextSeq 2000





650 in^3
NextSeq 550

~160 in^3
NextSeq 2000

Major reductions in cost, storage, and waste.

Miniaturization reduces reaction volumes, which reduces cost and waste

~30% reduction in number of reagents further reduces size and simplifies design

Results in an integrated cartridge with single storage condition

Design allows recyclable parts to be separated from liquid waste

NextSeq 1000 and NextSeq 2000 are the first systems to integrate DRAGEN Bio-IT platform on board

DRAGEN Bio-IT platform:

- Fast
- Accurate
- Cost efficient
- Industry standard pipelines
- Great for both novice and expert users.

3× Reduction in touch points

6× Faster on-board secondary analysis

We’ve touched every part of the workflow to make Illumina’s most user-friendly system ever

Plan

Streamlined Run Planning

Flexible Modes of Operation

Sequence

Integrated DRAGEN

Fast Analysis in Parallel

Analyze

Automated, Turnkey Solutions

Onboard and Cloud Pipelines

DRAGEN™ is Hardware-Accelerated Secondary Analysis Dynamic Read Analysis for GENomics

DRAGEN Hardware Acceleration
Field Programable Gate Array (FPGA)

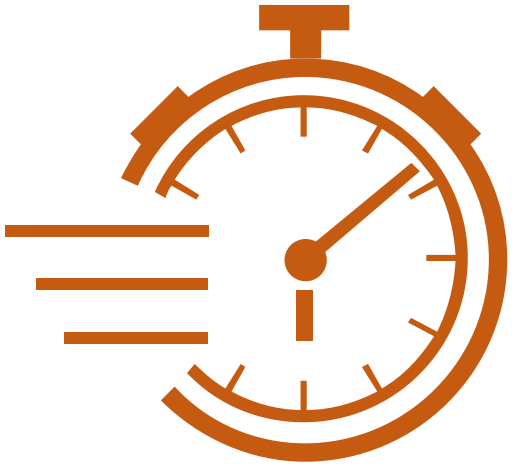
A field-programmable gate array (FPGA) is an integrated circuit designed to be configured manufacturing – hence the term "field-programmable".

FPGAs are hardware implementations of algorithms, and hardware is always **faster than** software.

FPGA latencies are an order of magnitude less **than** that of **GPUs** – hundreds of nanoseconds vs. single-digit microseconds.

Record-Breaking Analysis Speed

Guinness World Records®
Fastest Genetic Diagnosis



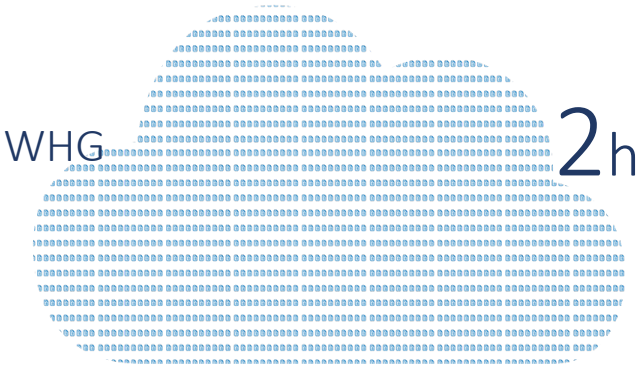
Sample > Answer

19.5 Hours

In 2018, Rady Children's Institute for Genomic Medicine set the Guinness World Records® for Fastest Genetic Diagnosis leveraging the Illumina DRAGEN Bio-IT Platform.

Guinness World Records®
Fastest Analysis of 1,000 Genomes

1000WHG 2hr25min



In 2017, **Children's Hospital of Philadelphia (CHOP)** set the Guinness World Records® for Fastest Analysis of 1,000 Genomes using the Illumina DRAGEN Bio-IT Platform in the cloud.

Accurate Data

Detects small variants, copy number variants and structural variants with **high analytical sensitivity** and **specificity**

precisionFDA

DRAGEN identified all 50 hidden variants and ranked 1st in the following categories*

Hidden Variants	Indel Precision	Indel Recall	Indel F-Score	SNP F-score	SNP Recall
50/50	1st	1st	1st	1st	1st

* Amongst entries that identified all hidden variants

Translating Sequencing Data into Insights

Unlocking the Power of the Genome through Secondary Analysis

Library Prep

Sequencing

Secondary Analysis

Processing raw sequencing data:

- **Demultiplexing** (BCL > FASTQ)
- **Read Mapping & Alignment** (FASTQ>BAM)
- **Variant Calling** (BAM > VCF)

Tertiary Analysis

Key DRAGEN Applications

	DRAGEN On-Premise Server	BaseSpace	Illumina Connected Analytics	NextSeq 1000/2000
Demultiplexing (BCL Convert)	✔	✔	✔	✔
DRAGEN ORA lossless genomic compression	✔			✔
Map & Align	✔	✔	✔	✔
Whole genome (germline & somatic)	✔	✔	✔	Germline only
Exome enrichment (germline & somatic)	✔	✔	✔	✔
COVIDSeq	✔	✔		
RNA Pathogen Detection		✔		
RNA-Seq (gene fusion & quantification)	✔	✔	✔	✔
Single-Cell RNA	✔		✔	✔

NextSeq 1000 & 2000 provide customers significant reduction in running costs

Kit	List	$/G	$/M Read	$/G Reduction vs NSQ 550
P2 100 cycles (40G)	$1420	$35.50	$3.55	34%
P2 200 cycles (80G)	$2670	$33.38	$6.68	35%
P2 300 cycles (120G)	$3540	$29.50	$8.85	29%
P3 100 cycles (100G)	$3250	$32.50	$3.25	40%
P3 200 cycles (200G)	$5750	$28.75	$5.75	44%
P3 300 cycles (300G)	$6000	$20.00	$6.00	52%

Reduction in Running Costs

- Enabled by new, exclusive NextSeq 1000 and NextSeq 2000 technologies
- Enables benefits for current and emerging applications
- Additional efficiencies afforded when scaling from NextSeq 1000/2000 P2 Reagents and NextSeq 2000 P3 Reagents
- Clear ROI path for majority of existing NextSeq customers
- Standard sales discounting to apply

Key Applications

iSeq

MiniSeq

MiSeq

NextSeq 550

NextSeq 1000/2000

NovaSeq 6000

Targeted regions

Small genomes (Bacteria, virus)

RNA seq

Human Exomes

Human Genomes

Enabling advances in science; Key applications

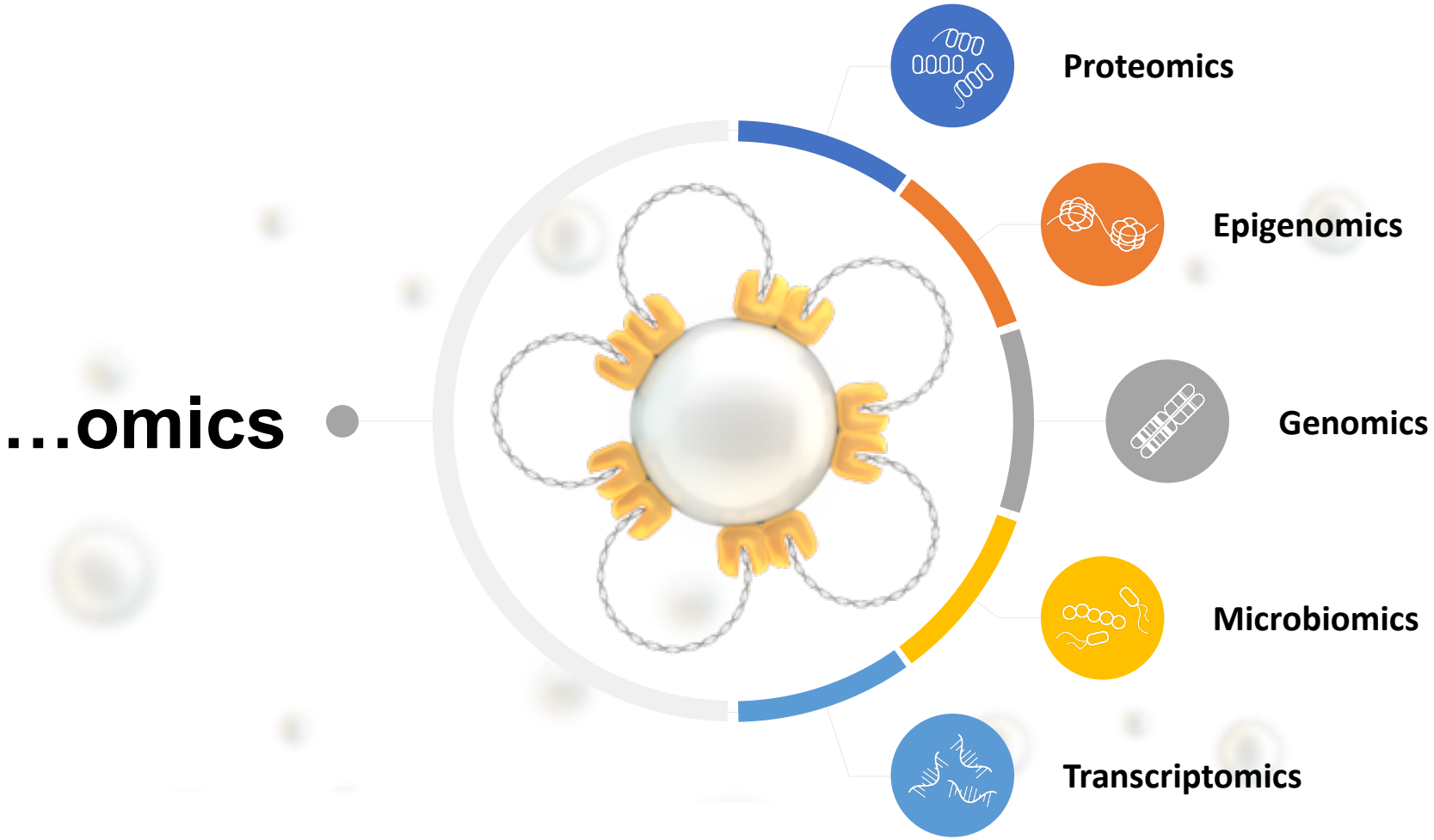
Single Cell applications

Multi-omic approaches

Spacial Genomics

Multi-Omics Approaches

Answering more complex questions



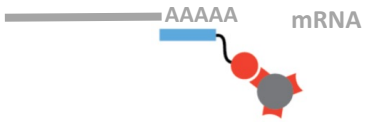
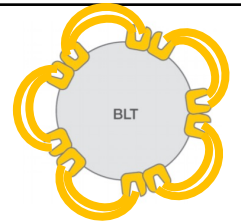
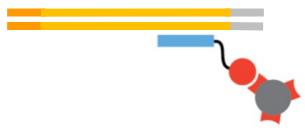
RNA-Seq

Total RNA Seq - Analyze coding plus multiple forms of noncoding RNA for a comprehensive picture of the transcriptome.

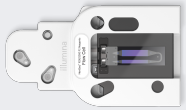
mRNA-Seq -Quantify gene expression of both known and novel transcript isoforms.

Targeted RNA Seq - Select and sequence specific transcripts of interest for gene expression profiling studies.

RNA-Seq Workflows: Technology Overview

	Total	mRNA	Enrichment
RNA Selection / Depletion	rRNA Depletion by RNaseH 	Poly-A Capture 	None
Library Prep	Y-adapter Ligation 	Y-adapter Ligation 	BLT 
Enrichment	None	None	1-Hyb 
Stranded	Yes	Yes	No
Indexes	192/384 UDI	384 UDI	384 UDI

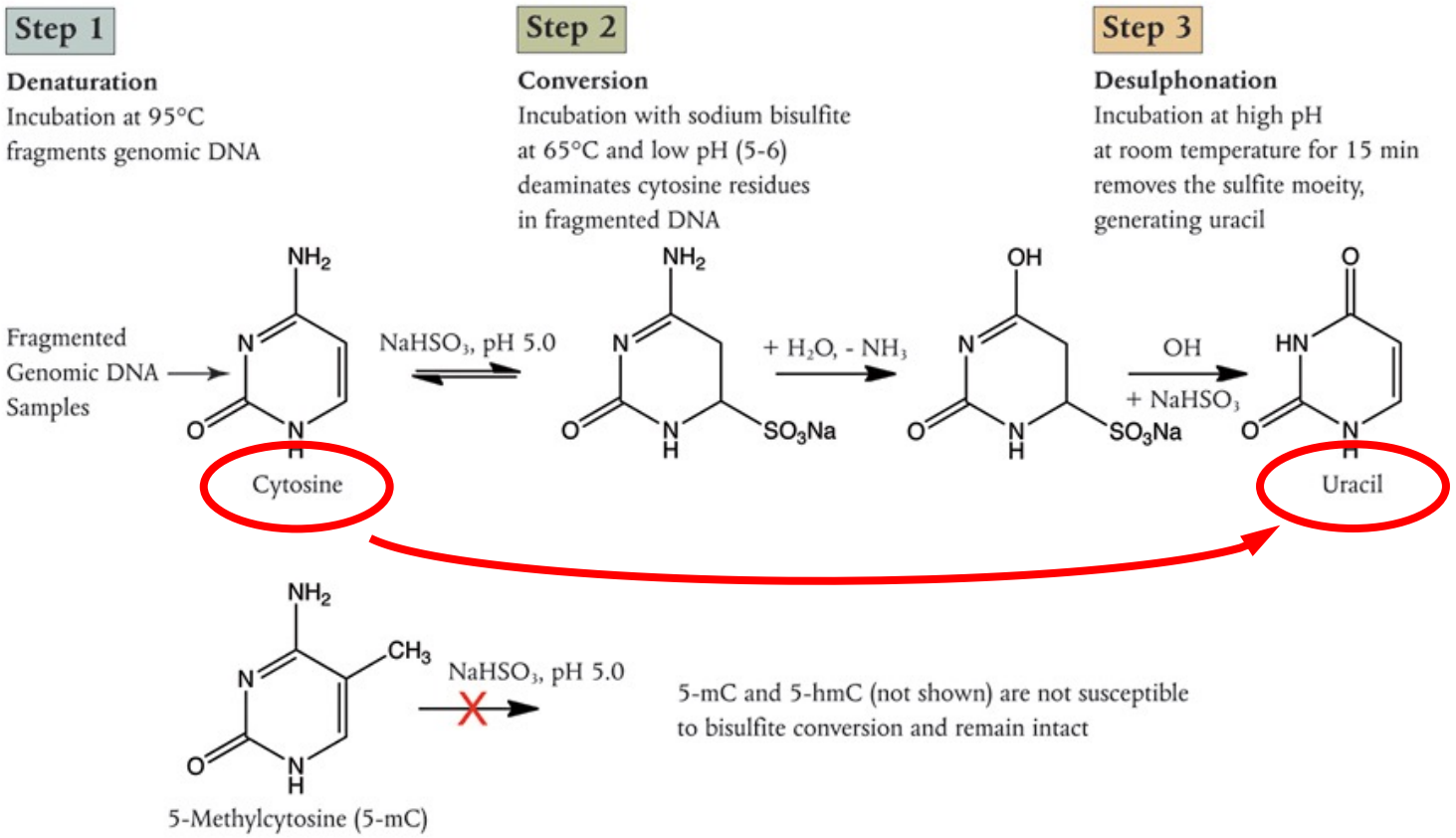
RNA-seq on NextSeq™ 2000 System: Access multiple applications

	NextSeq 1000/2000 with P2 Flow Cell 400M Reads	NextSeq 2000 with P3 Flow Cell 1000M Reads
		
Gene Expression Profiling	TruSeq™ mRNA Stranded 40 SAMPLES 10m READS	TruSeq™ mRNA Stranded 100 SAMPLES 10m READS
Coding RNA Discovery	TruSeq™ RNA Access 16 SAMPLES 25m READS	TruSeq™ RNA Access 40 SAMPLES 25m READS
Coding and Non-Coding Discovery	TruSeq™ Stranded Total RNA 8 SAMPLES 50m READS	TruSeq™ Stranded Total RNA 20 SAMPLES 50m READS

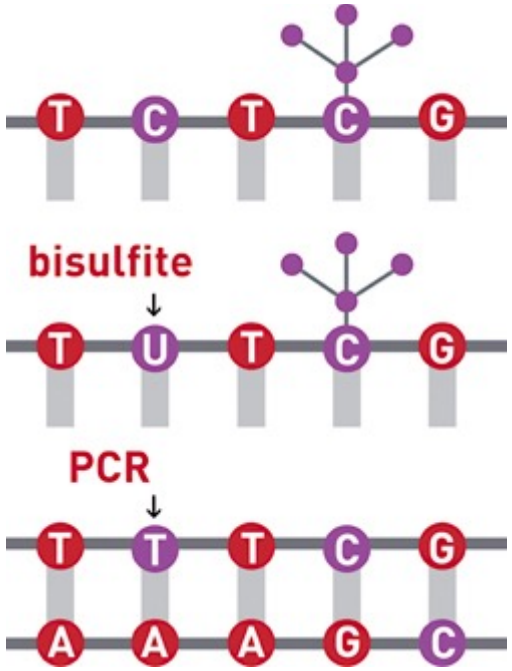
Methyl-Seq

Methylation sequencing is a powerful tool for understanding genome-wide methylation with single nucleotide resolution and is considered a gold standard.

Methyl-Seq: : Technology Overview



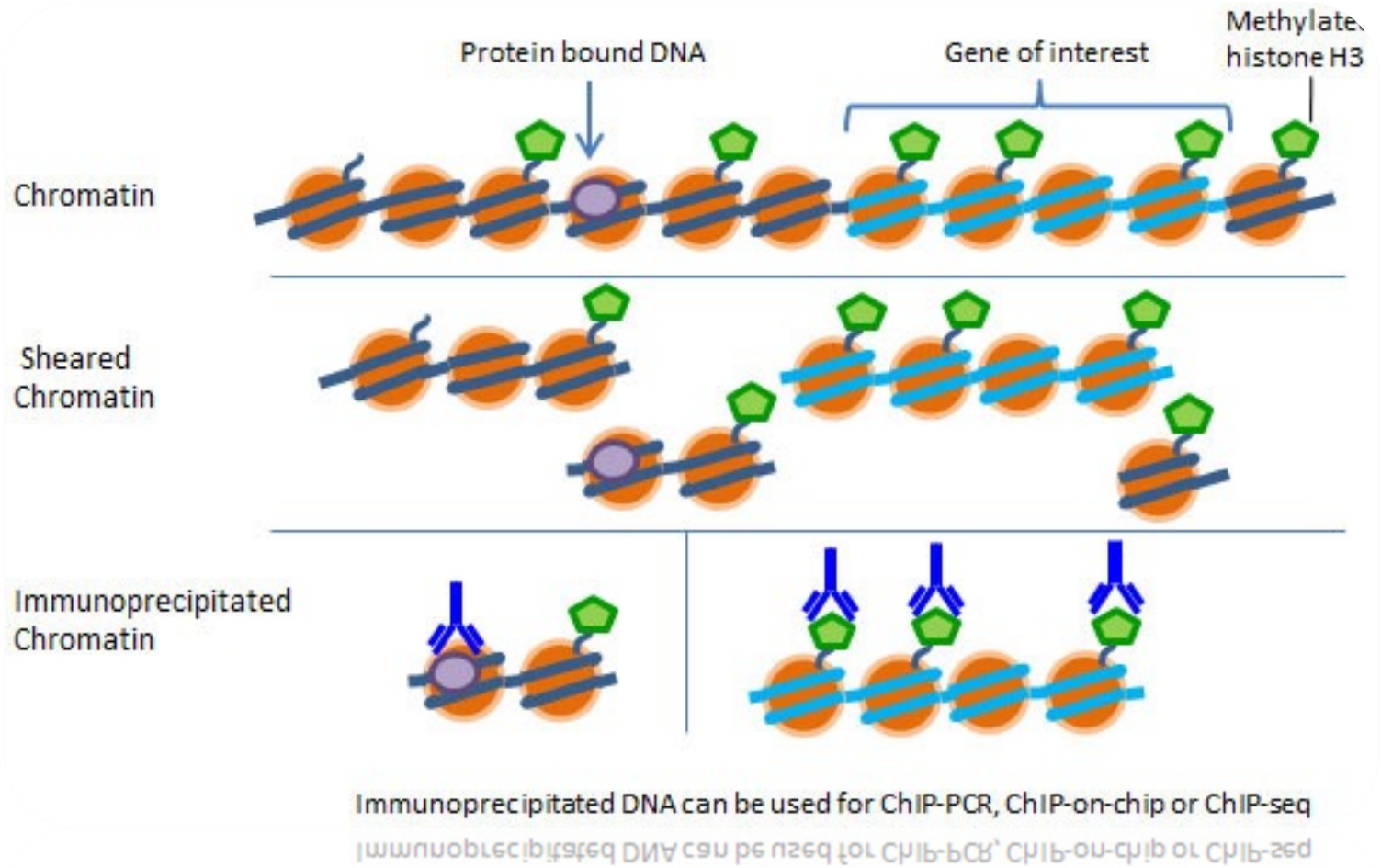
Methyl-Seq: : Technology Overview



ChIP-Seq

Chromatin immunoprecipitation sequencing (ChIP-Seq) is a powerful method for analyzing DNA-protein interactions and performing genome-wide surveys of gene regulation.

ChIP-Seq: : Technology Overview

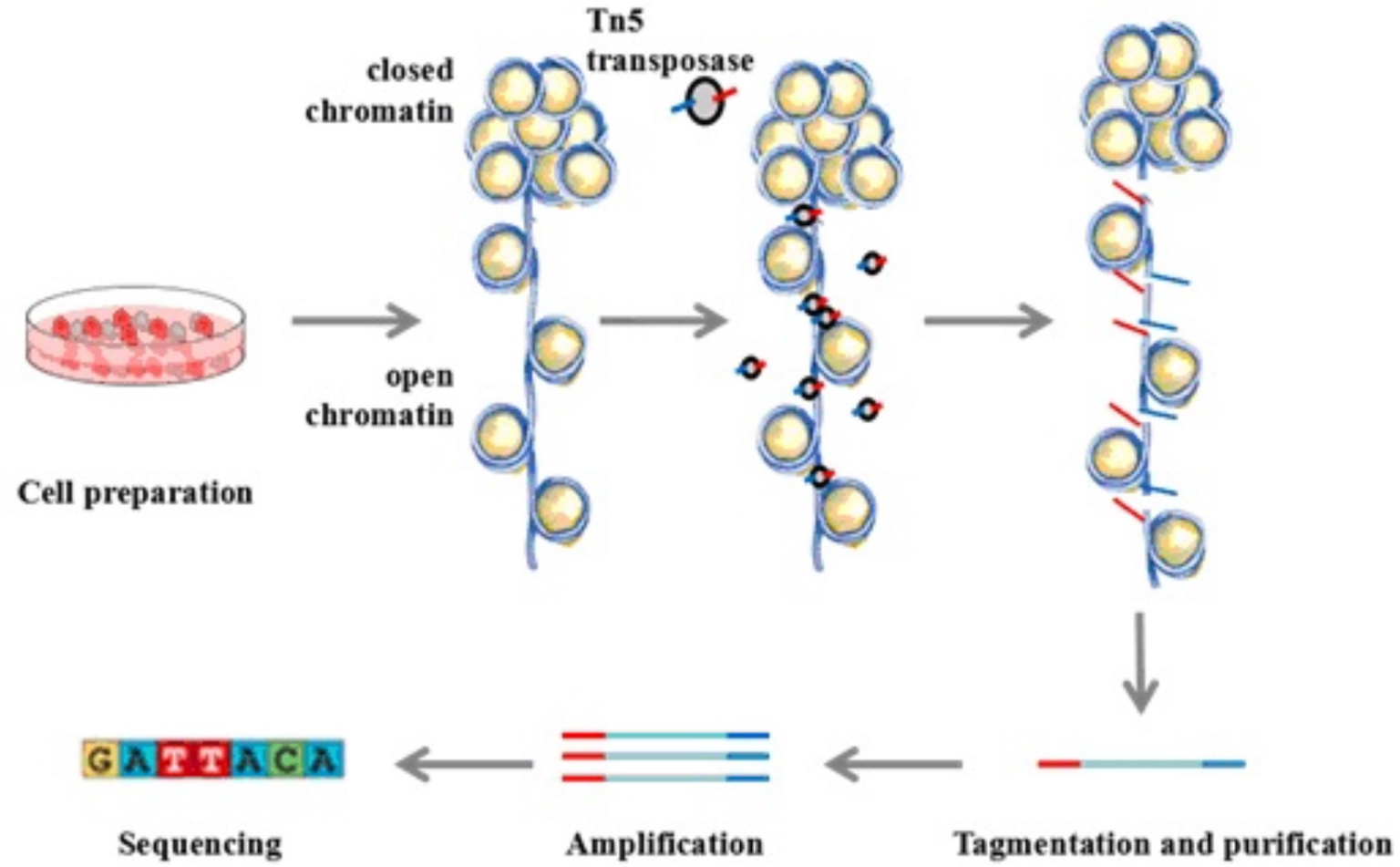


For Research Use Only. Not for use in diagnostic procedures.

ATAC-Seq

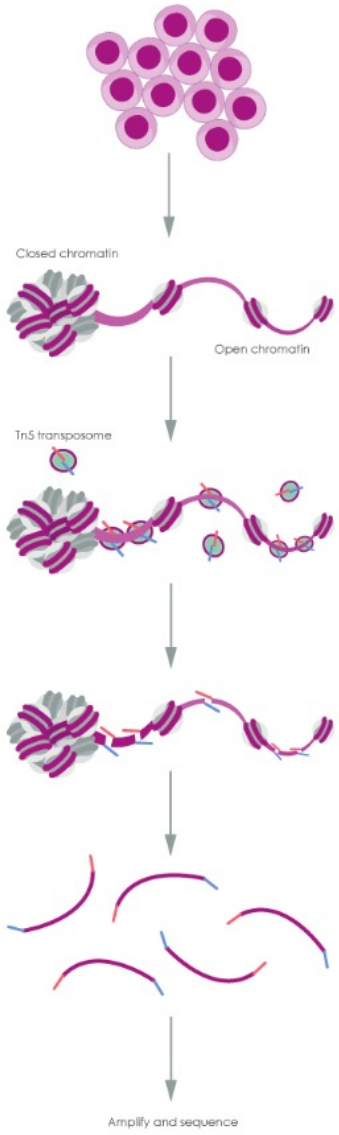
The assay for transposase-accessible chromatin with sequencing (ATAC-Seq) is a popular method for determining chromatin accessibility across the genome.

ATAC-Seq: : Technology Overview



ATAC-Seq: : Technology Overview

- Easy, 3 hour workflow
- Requires least amount of cells compared to other methods
- Number of reads for a region correlates with how open that chromatin is at a single nucleotide resolution.



Harvest cells. An accurate cell number is key to the success of the experiment.

Lyse cells to generate a crude nuclei preparation.

Tn5 tagmentation

Purify the fragmented and tagged DNA.

PCR amplify and purify the amplified DNA.

Sequence

For Research Use Only. Not for use in diagnostic procedures.

How many ATAC-seq samples can you pool in a run?

Application	# Reads/Sample	P2	P3
Nucleosome mapping	50 million	4.0	20
Inferring differences in open chromatin (human)	>50 million	<4.0	<20
Transcription factor foot-printing	200 million	2.0	5.0

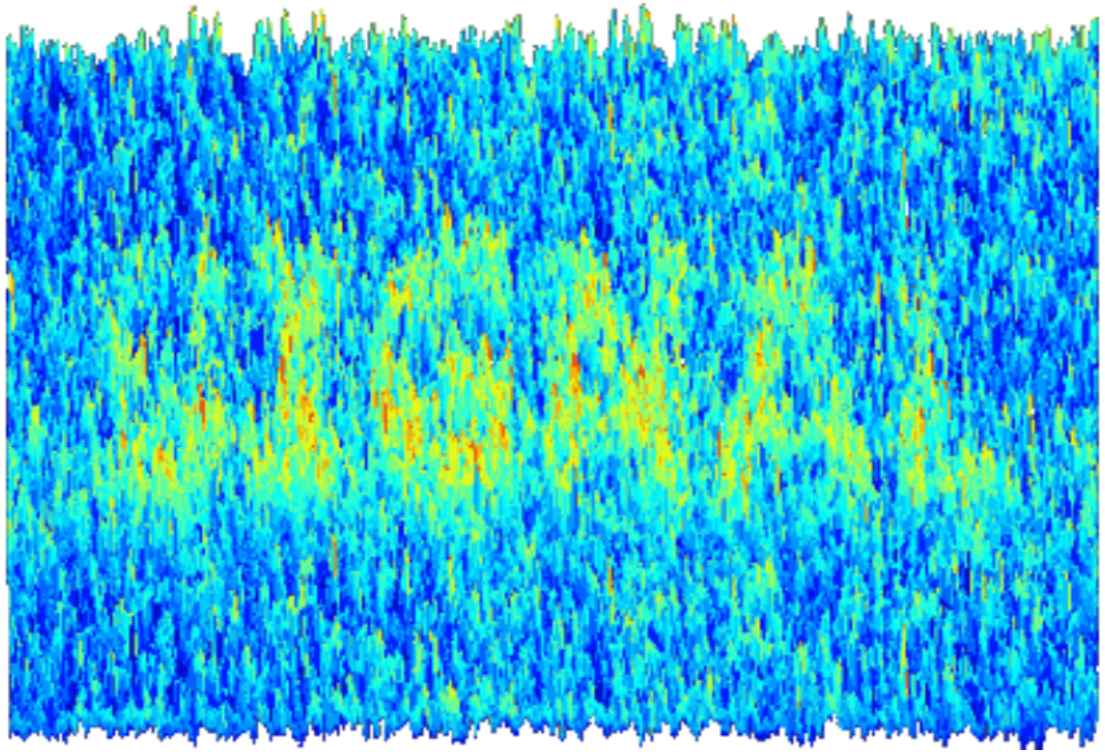
For Research Use Only. Not for use in diagnostic procedures.

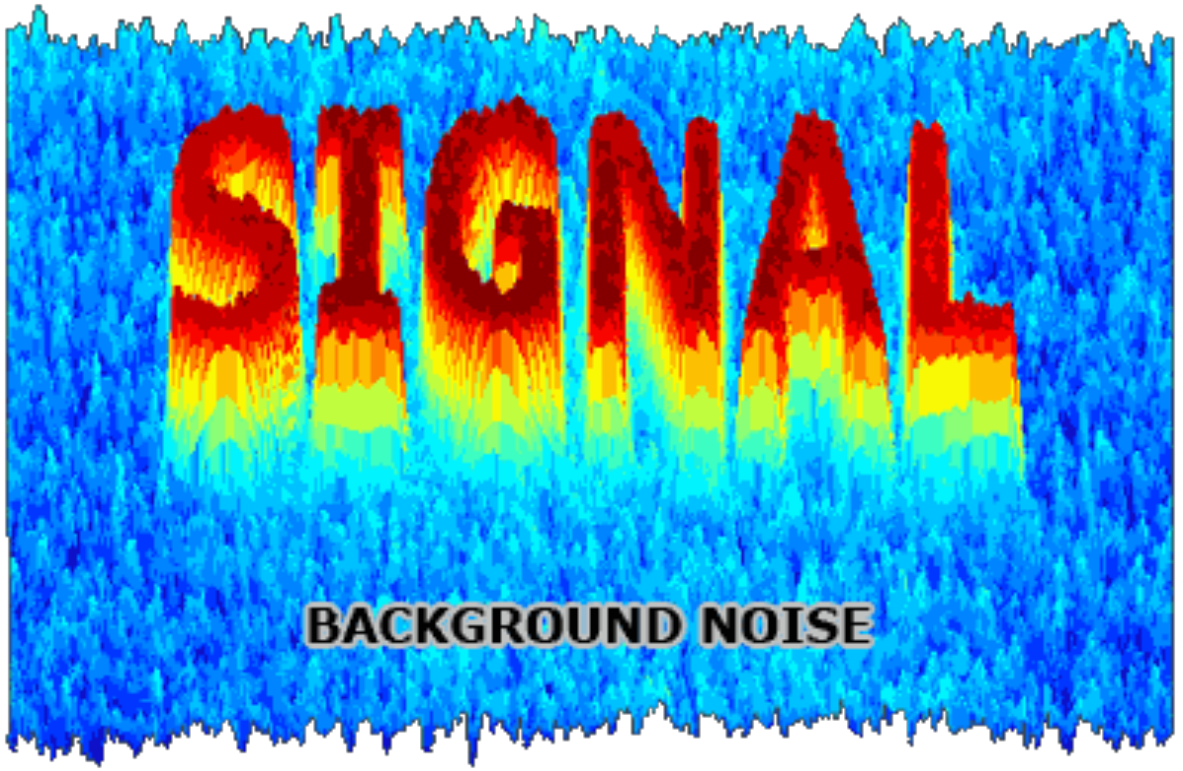
Single Cell Sequencing

scRNA-Seq - provides transcriptional profiling across thousands of cells within a heterogeneous sample.

scATAC-Seq - identifies areas of open chromatin at single cell resolution such that when examining a heterogenous cell population

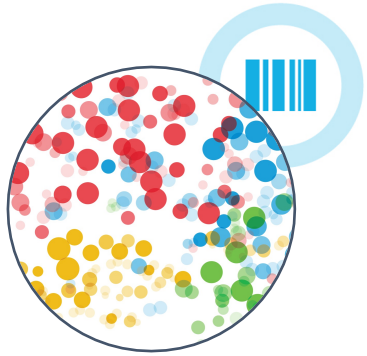
Purpose of Single Cell Experiment?





10x Genomics Solutions

Gene Expression
3’ RNAseq

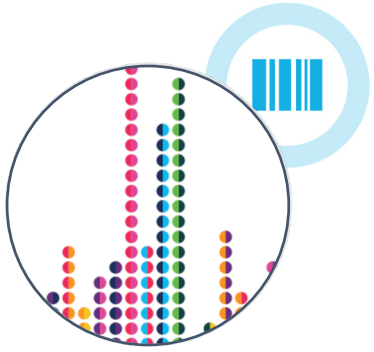


Single Cell
Gene Expression
VERSION 3

Epigenomics

Single Cell ATAC

Immunology
5’ V(D)J



Single Cell Immune
Profiling

Single Cell on NextSeq™ 2000 System: Access multiple applications

	NextSeq 1000/2000 with P2 Flow Cell 400M Reads			NextSeq 2000 with P3 Flow Cell 1000M Reads		
						
5’ TCR/BCR	25 SAMPLES	3k CELLS	5k READS/CALLS	65 SAMPLES	3k CELLS	5k READS/CALLS
3’ Gene Expression Profiling	7 SAMPLES	3k CELLS	20k READS/CALLS	17 SAMPLES	3k CELLS	20k READS/CALLS
scATAC	3 SAMPLES	3k CELLS	50k READS/CALLS	7 SAMPLES	3k CELLS	50k READS/CALLS

Spacial Genomics

Spatial transcriptomics - a molecular profiling method that allows scientists to measure all the gene activity in a tissue sample and map where the activity is occurring.

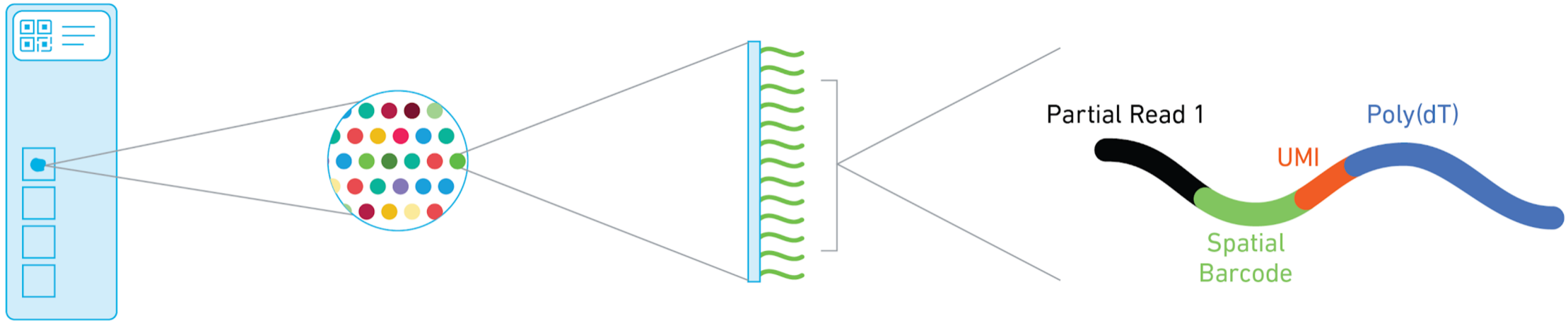
Spatial Transcriptomics: : Technology Overview

Utilizing Poly A Capture and Unique Spatial Barcodes

Visium Spatial Gene Expression Slide

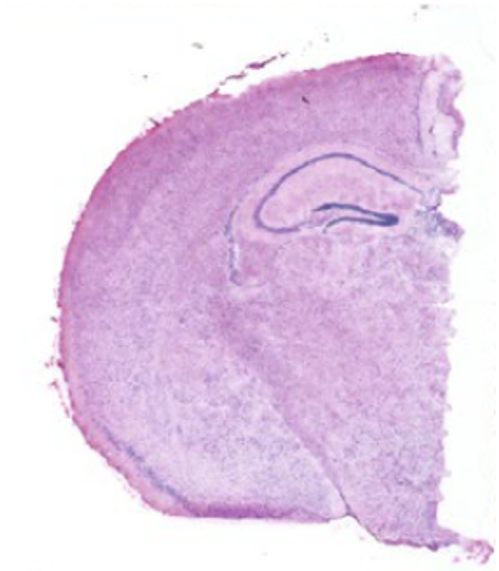
Capture Area with 5000 Barcoded Spots

Visium Gene Expression Barcoded Spots

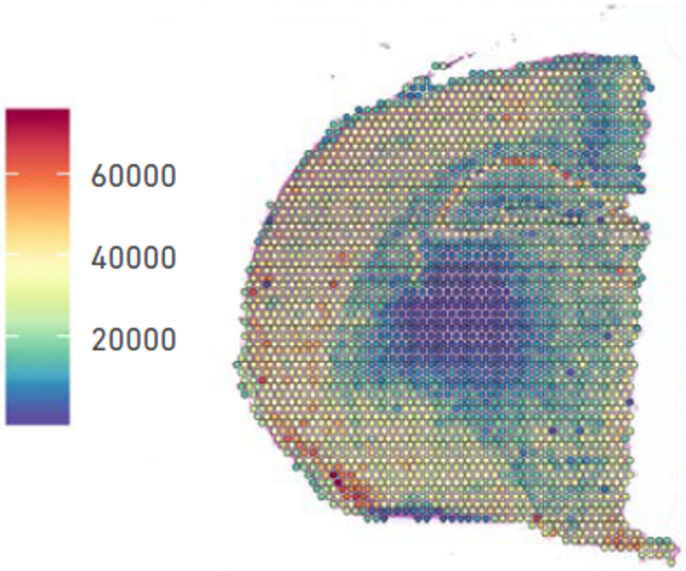


Spatial Transcriptomics: : Technology Overview

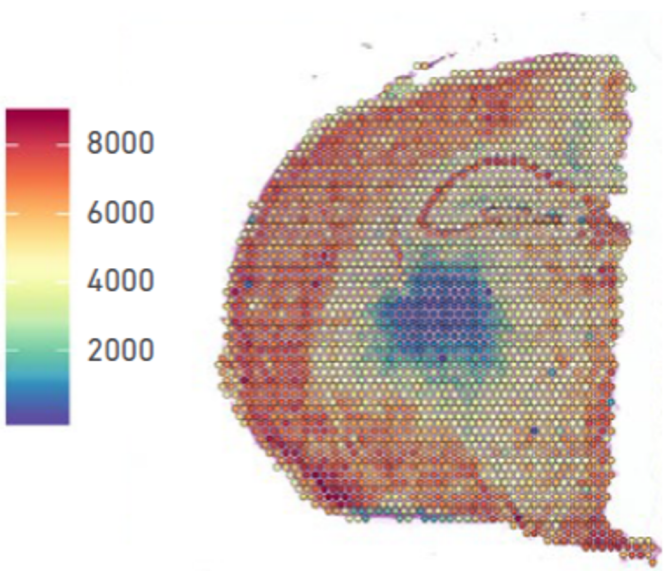
H&E Stain



UMI Counts



Gene Counts



Determining Sequencing Depth

Visium Spatial Gene Expression Libraries

- Starting point: 50K reads/clusters per tissue covered spot
  - Total sequencing depth = (Coverage Area x total spots on the Capture Area) x 50,000 read pairs/spot
  - **Example calculation for 60% tissue coverage**: (0.60 x 5,000 total spots) x 50,000 read pairs/spot = 150 million total read pairs for that sample
  - Note: calculation is only 1 of the 4 capture areas on the Visium slide