Bringing the AVITI to the MSU Genomics Core

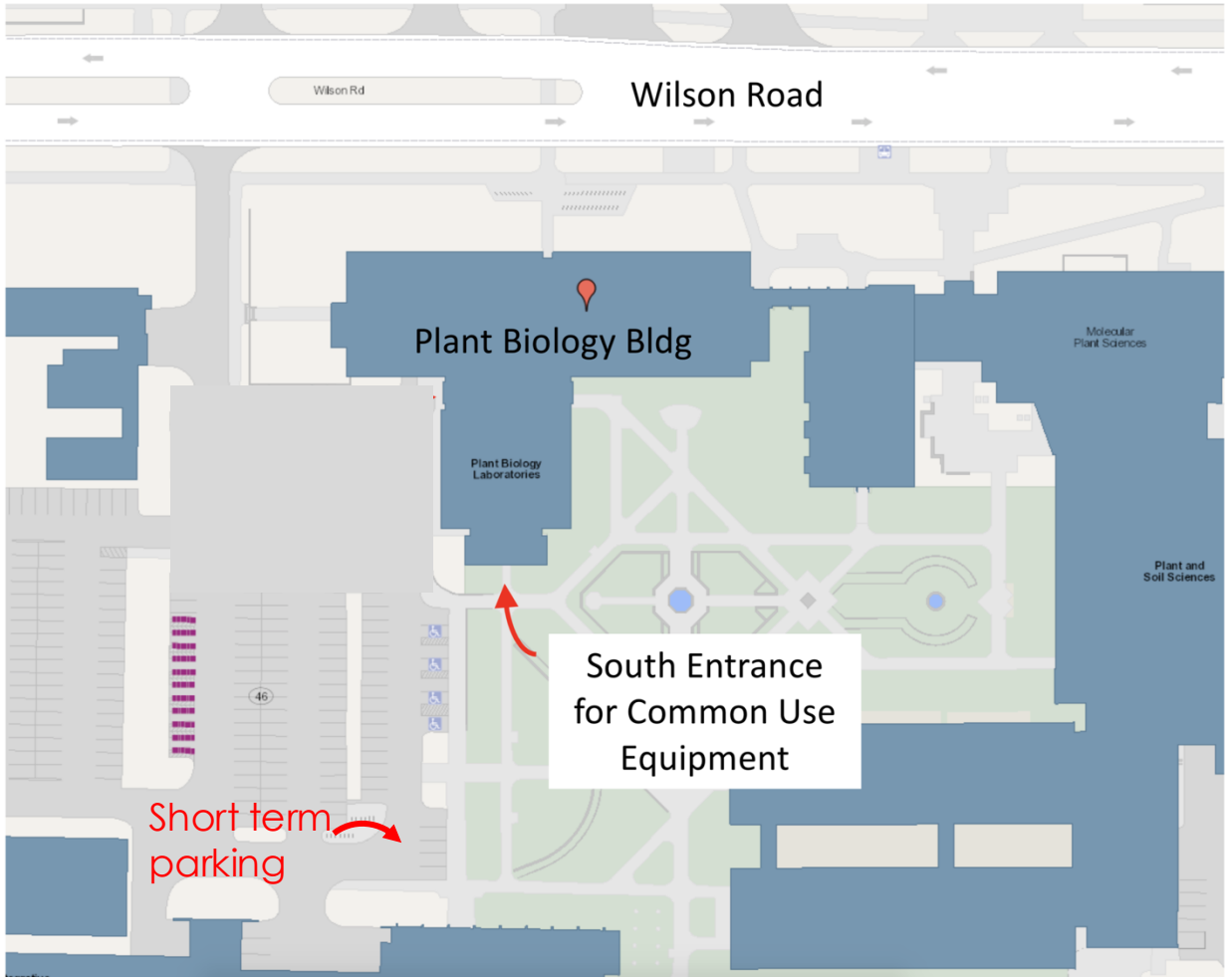
Kevin Childs
Director MSU Genomics Core
Assistant Professor Fixed Term – Plant Biology
9 October 2024

For research use only

Genomics Core – Where are we?

Plant Biology Building
S18 and S20
(in the basement)

Sample drop off in the refrigerator in the hallway outside the lab



Genomics Core – Who we are?

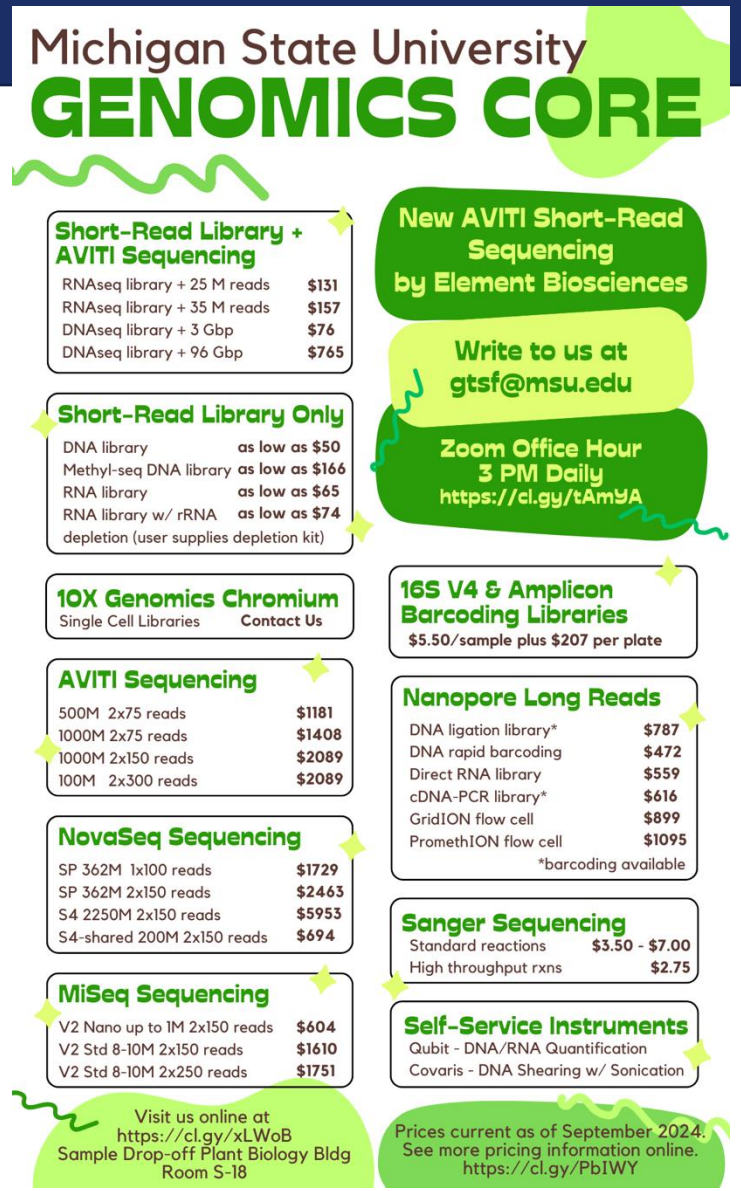
Operations Manager
- Emily Crisovan

Research Assistants
- Shari Tjugum-Holland
- Colleen Curry
- Krystle Wiegert-Rininger
- Justin Nummer
- Mercy Kitavi, Ph.D.

Data Management Specialist
- Kevin Carr

Services Overview

- Short-read sequencing libraries
  - DNA-seq, RNA-seq
  - 16S V4 & custom amplicons
  - Single cell RNA-seq
  - Researcher-prepared libraries
- Nanopore long-read sequencing
- Sanger sequencing



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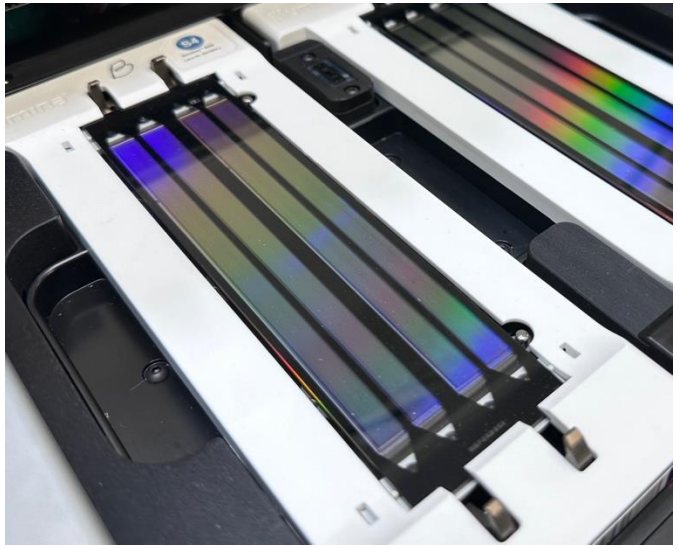
Past Illumina Sequencing Instruments in the Genomics Core

- Genome Analyzer
- Genome Analyzer II
- MiSeqs
- HiSeq 2500
- HiSeq 4000 & NextSeq 500
- NovaSeq 6000
- NovaSeq X



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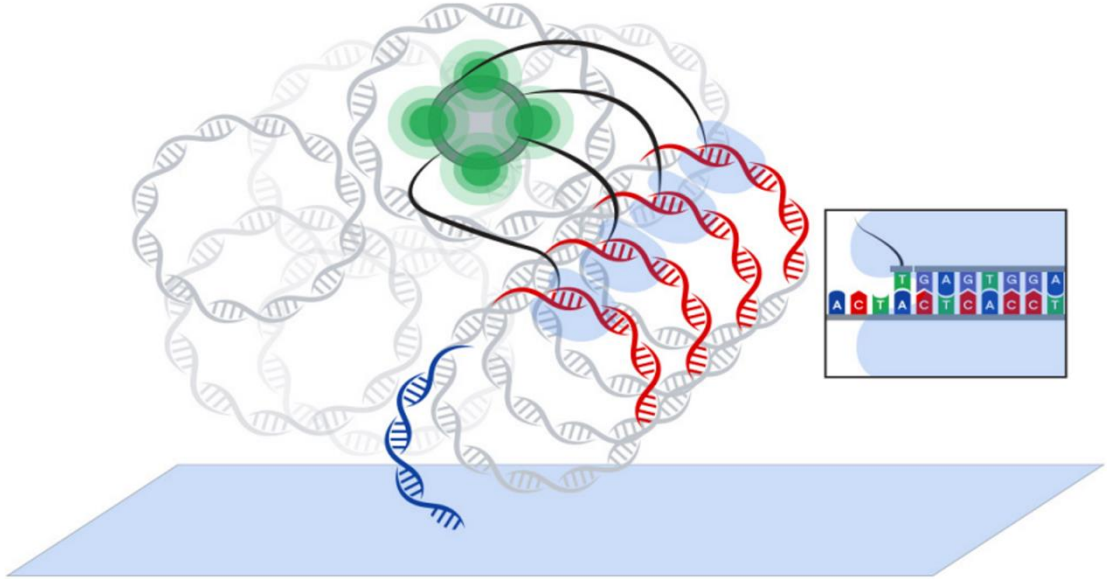
Rationale for an AVITI

- Illumina-style libraries
- Fastq output files
- Regular analysis pipelines
- Flow cell pricing
- Smaller flow cells
- Very low duplication rate
- Increased accuracy
- Instrument & service contract costs



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AVITI Flow Cells

Read Format	Capacity	Read Pairs	Gbp	Cost	Cost per Gbp
2 x 75 bp	Medium	500 M	75	$1,181	
2 x 75 bp	High	1,000 M	150	$1,408	
2 x 150 bp	Low	250 M	75	$1,181	
2 x 150 bp	Medium	500 M	150	$1,408	
2 x 150 bp	High	1,000 M	300	$2,089	
2 x 300 bp	Medium	100 M	60	$2,089	
2 x 300 bp	High	300 M	180	$2,950	

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AVITI Flow Cells

Read Format	Capacity	Read Pairs	Gbp	Cost	Cost per Gbp
2 x 75 bp	Medium	500 M	75	$1,181	$15.75
2 x 75 bp	High	1,000 M	150	$1,408	$9.39
2 x 150 bp	Low	250 M	75	$1,181	$15.75
2 x 150 bp	Medium	500 M	150	$1,408	$9.39
2 x 150 bp	High	1,000 M	300	$2,089	$6.96
2 x 300 bp	Medium	100 M	60	$2,089	$34.82
2 x 300 bp	High	300 M	180	$2,950	$16.39

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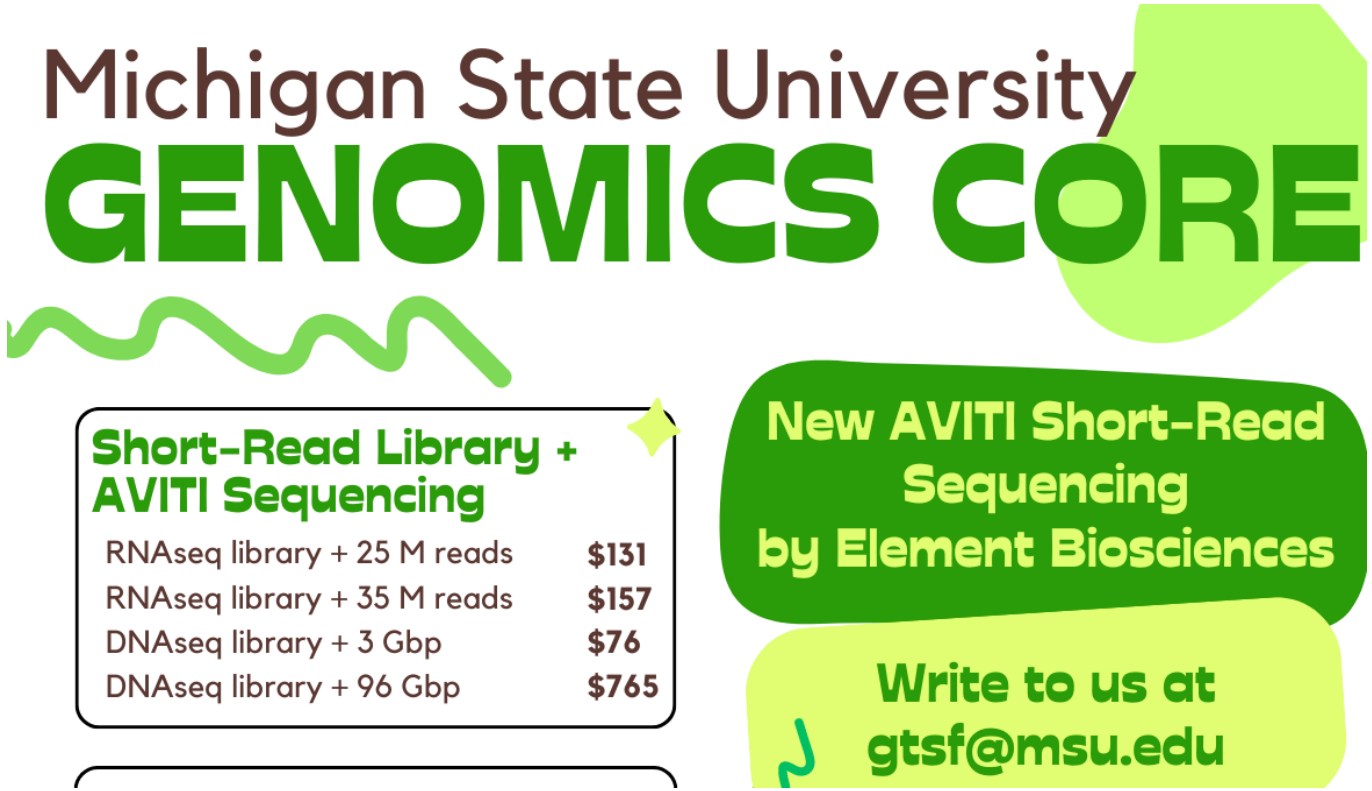
Easy-Peasy Pricing

- Bundled flat-rate pricing
- Library + fixed amount of sequencing
- Run on shared high-output 2 x 150 bp lane
- Similar to shared NovaSeq S4 lane but cheaper



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AVITI Flow Cell Grant

- Abstract about your proposed sequencing project (250 words)
- One high output 2 x 150 bp flow cell or one high output 2 x 300 bp flow cell
- Does not include library preparation
- Two winners
- Submit before November 8, 2024

https://go.elementbiosciences.com/MSUgrant

Element Biosciences

Novel Developments in NGS; Unlocking Further Insights with AVITI

Anthony Popkie, Ph.D.
Executive Account Manager
anthony.popkie@elembio.com

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Our journey to empower science

Element is founded by Molly He, Michael Previte and Matt Kellinger, three scientists seeking to disrupt genomics by reinventing every "element" of sequencing

JULY 2017

Company announces Series A financing led by Foresite Capital and Venrock

JUNE 2019

Loop Genomics

Element closes acquisition of Loop Genomics, enabling both short and long-read sequencing on the same desktop machine

FEBRUARY 2022

Element opens new headquarters of Alexandria Tech Center in San Diego

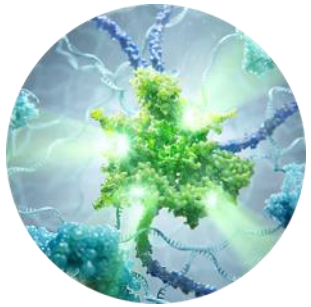
MAY 2022

Element announces first set of global distributors, showing strong demand for AVITI around the world

APRIL 2023

MAY 2019

Element invents Avidity Sequencing, enabling high accuracy with low reagent consumption



JUNE 2021

Element closes $276M Series C financing to deliver on the promise of disruptive sequencing

MARCH 2022

Element launches AVITI, making affordable, high-quality sequencing a reality for labs of all sizes

JANUARY 2023

Element announces the $200 genome on AVITI, delivering the highest quality sequencing at an unprecedented low cost

JUNE 2023

Element launches new Cloudbreak chemistry, advancing Avidity with even faster runtimes

Re-imagining what is possible on a benchtop sequencing platform

Where We Started:
Best in Class Sequencing

Quality	**Q30** ➡ **Q50**
Affordability	**$200 genome** **on a benchtop**
Reliability	**MTBF > 160 days** *Mean time between failure

300+ Installs

30+ Countries

10k+ Runs/Year (and counting!)

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Element's innovation vision

Where We Started:
Best in Class Sequencing

Quality	**Q30** ➡ **Q50**
Affordability	**$200 genome** on a benchtop
Reliability	**MTBF > 150 days** *Mean time between failure

2024 and Beyond:
Innovation Pillars

Simplified Workflows	**Onboard enrichment**
Integrated Multiomics	**Multiomic CytoProfiling**
Scale	**More experiments, less time**

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Element AVITI™ fit

Empowering more researchers with flexible genomic solutions

AVITI

Unparalleled performance with throughput from 100 million to 2 billion reads

Quality, affordability, and flexibility, on your timeline

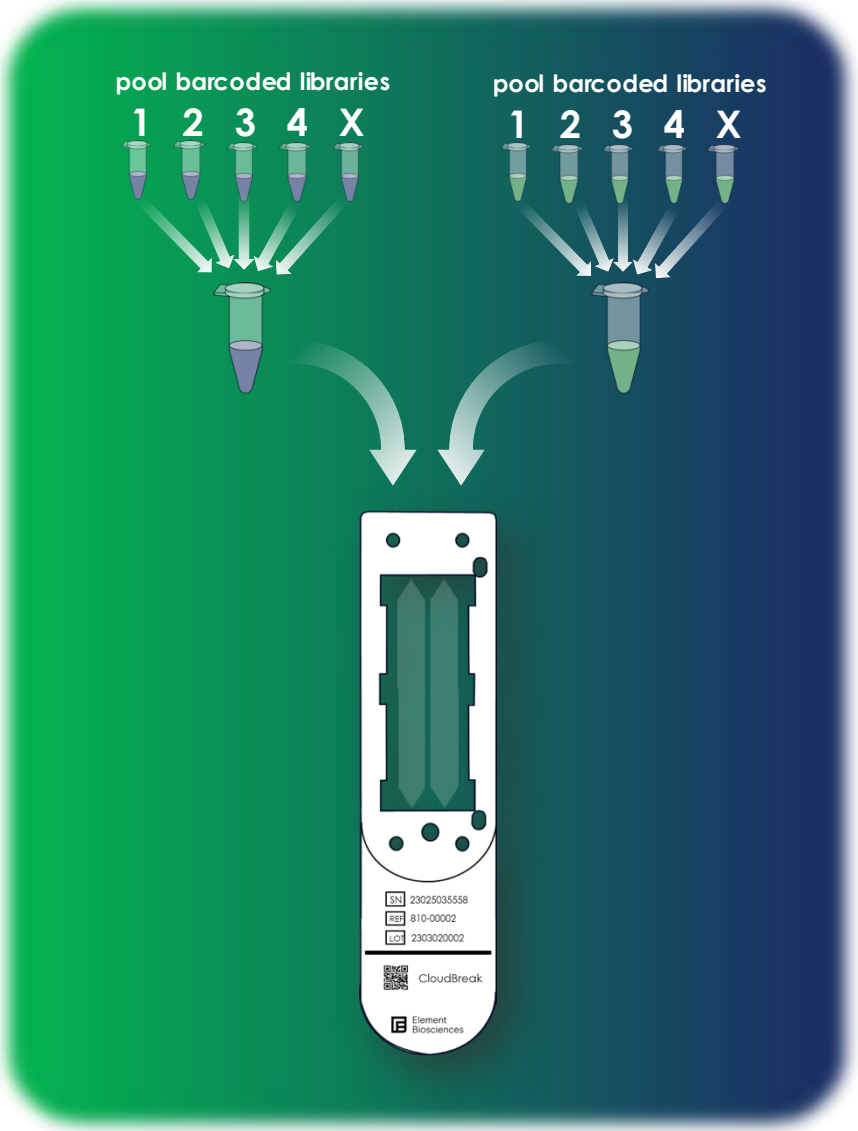
- >90% Q30 and >70% Q50 data with UltraQ
- 300 Gb / 1 B reads per flow cell
- Two fully independent flow cells
- Two addressable lanes per flow cells
- Guaranteed reagent costs
- Ecosystem compatibility
- Negligible index hopping and duplication
- Low-diversity samples (<5% PhiX)

AVITI

Individually addressable lanes

More flexibility when you need it

- Optional upgrade to enable separate libraries/pools to be loaded in each of two lanes in a single flow cell
- Increased multiplexing capabilities
- Greater flexibility for variable sample volume throughput
- Increased control of read distribution within pools
- Available for both the AVITI and AVITI LT systems on 2x75 and 2x150 flow cells



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Our ecosystem partnerships make end-to-end application workflows accessible

Prepare | Sequence | Analyze

Agilent
10x GENOMICS
Roche
QIAGEN
IDT INTEGRATED DNA TECHNOLOGIES
NEW ENGLAND BioLabs Inc.
TWIST BIOSCIENCE

and many more…

WGS Trios, lpWGS, WES,
RNA sequencing,
Single cell genomics,
Epigenetics

Google DeepVariant
FABRIC GENOMICS
Sentieon
GENOOX
gencove

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Element Biosciences

Element's AVITI™ Sequencer: Next Level Sequencing Solution for Multiomic NGS Applications

Solomon Hailu, Ph.D., M.Sc.
Sr. Applications Technical Specialist
Solomon.Hailu@ElemBio.com

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Outline

- Flexibility, Higher Quality and Low Cost
- Superior Performance
- Applications
- Upcoming Innovations

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Element's AVITI™ - Empowering researchers with flexible genomic solutions

Features

- Dual independent flow cells
- Two addressable lanes per flow cells
- Q30 >90%, no PCR error propagations
- Negligible index hopping
- Low AT/GC bias and duplicate rates,
- Low-diversity samples (<5% PhiX)
- Simultaneous Single Cell Multiomics + Morphology*
- Long insert and flexible Library Preparation

Applications

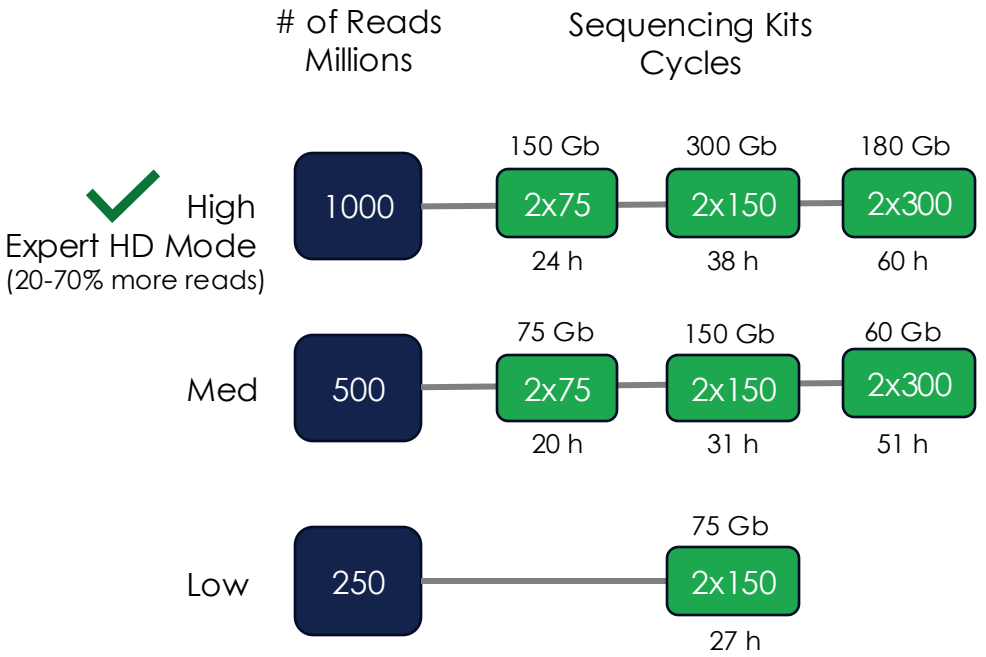
- Single cell and bulk RNA sequencing
- WGS / Trios / lpWGS / liquid biopsy
- Exomes and panels
- Epigenetics: Hi-C, ATAC-Seq, ChIP-Seq (CUT&RUN / CUT&Tag)
- Low diversity and targeted sequencing
- Metagenomics, HLA typing and immune profiling
- Long-read sequencing - 6 Kb with LoopSeq

100M to 1B reads per side

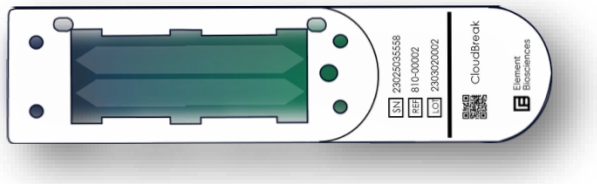
100M to 500M reads per side

*AVITI + Cellular Profiling

* Coming H2



AVITI LT - Med and Low flow cells
High 600 cycle (300M reads)
Med 600 cycle (100M reads)



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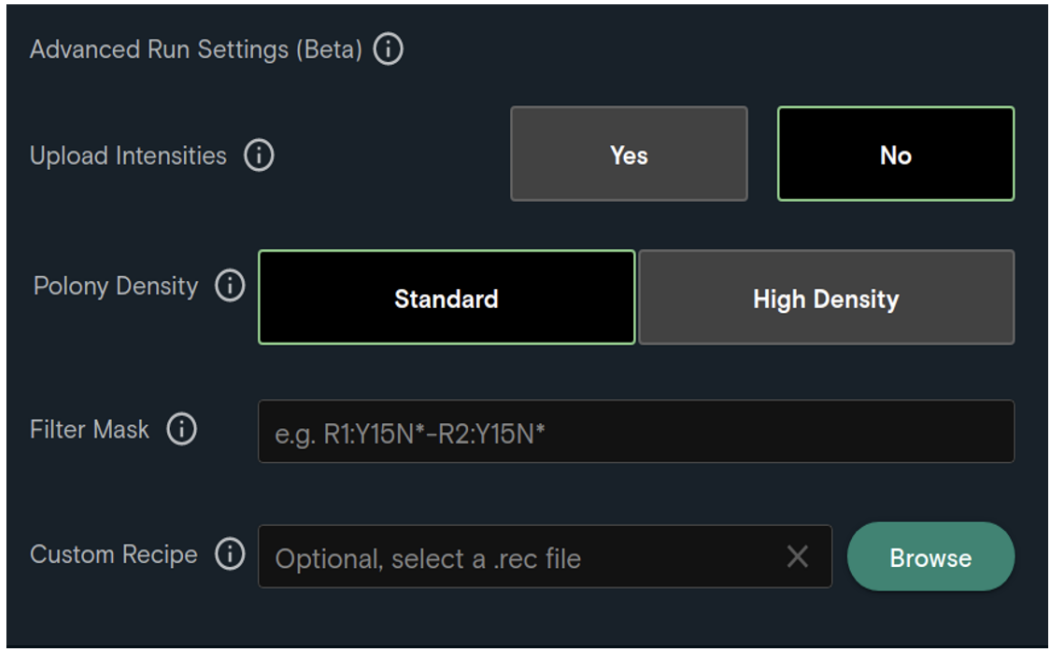
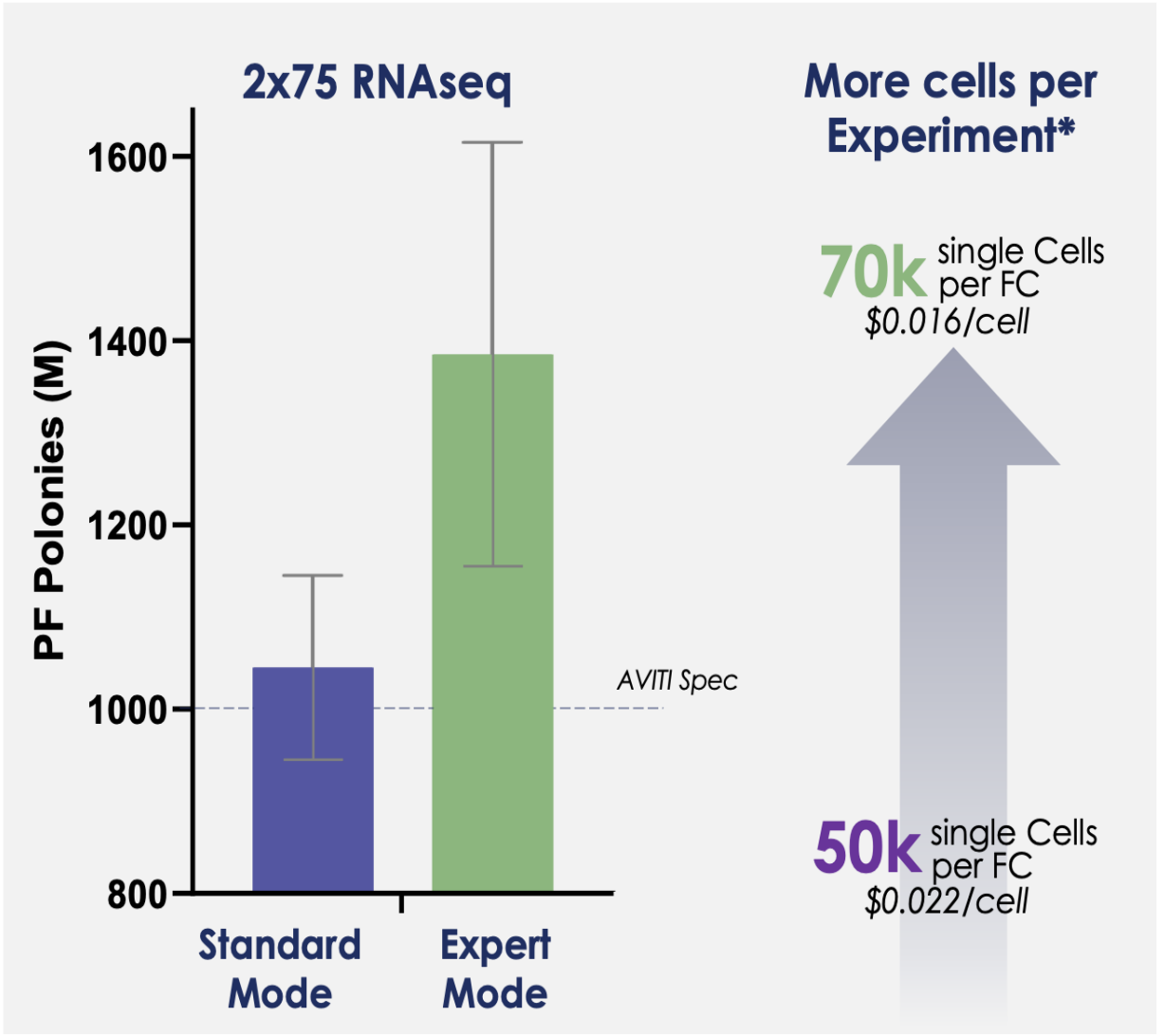
Element's AVITI™ - Empowering researchers with flexible genomic solutions



Advanced settings allow additional customization options for expert users

High Density Mode
Custom Recipes for Unique Applications



*Assuming 20k reads/cell using 2x75 HO kit, actual results may differ based on lab-specific factors.

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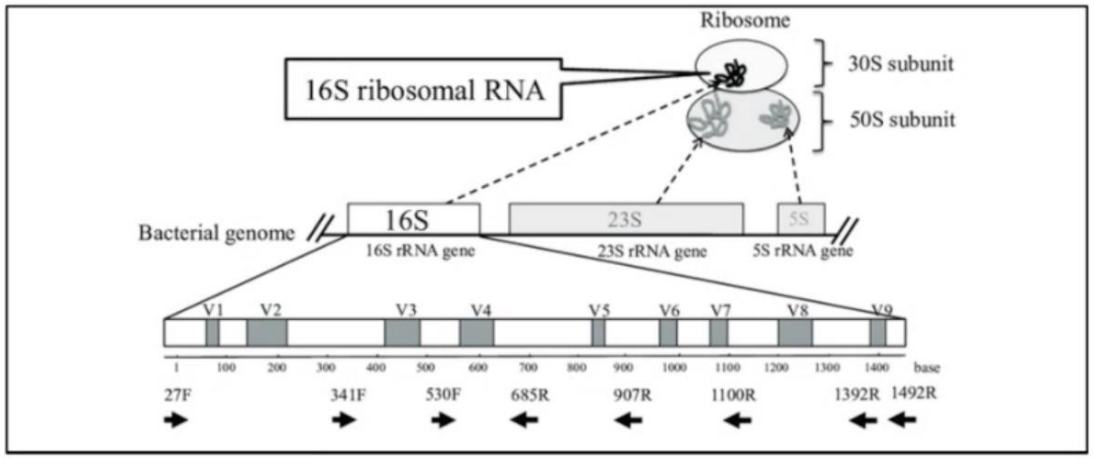
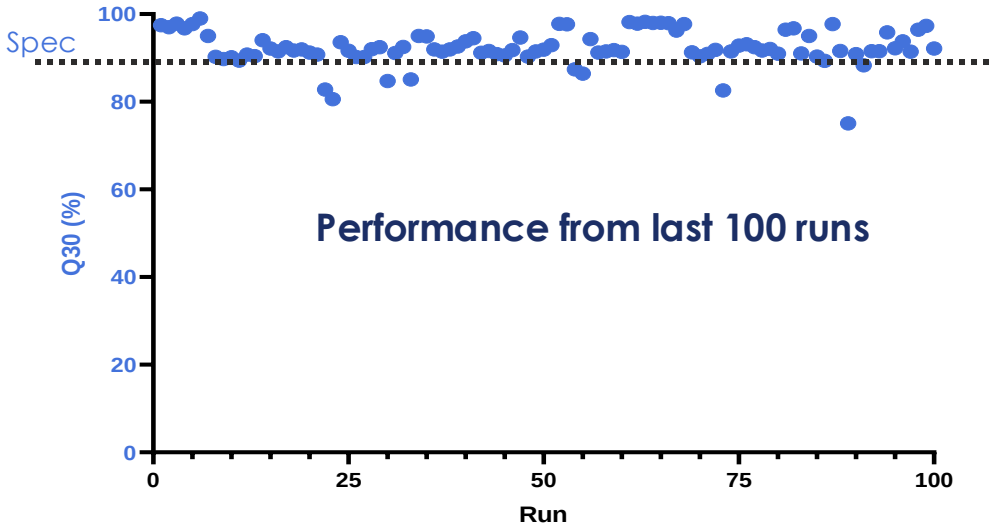
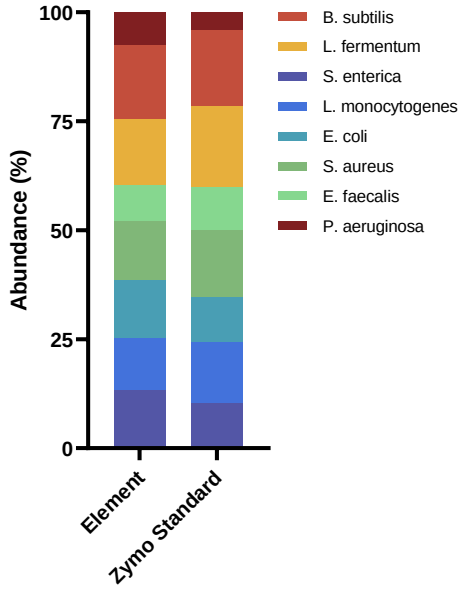
AVITI 2 x 300 Kits provides leading data quality and throughput at affordable price



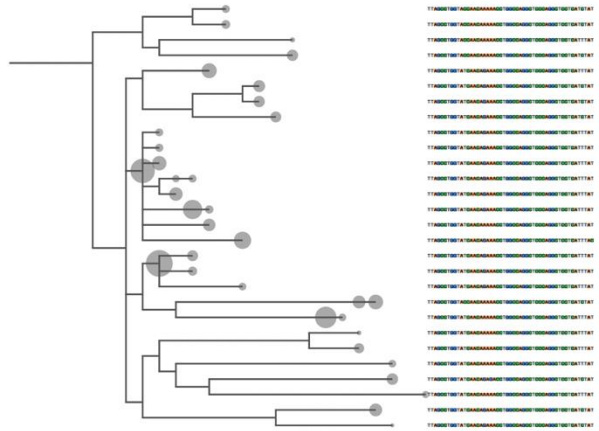


16S Microbiome Sequencing

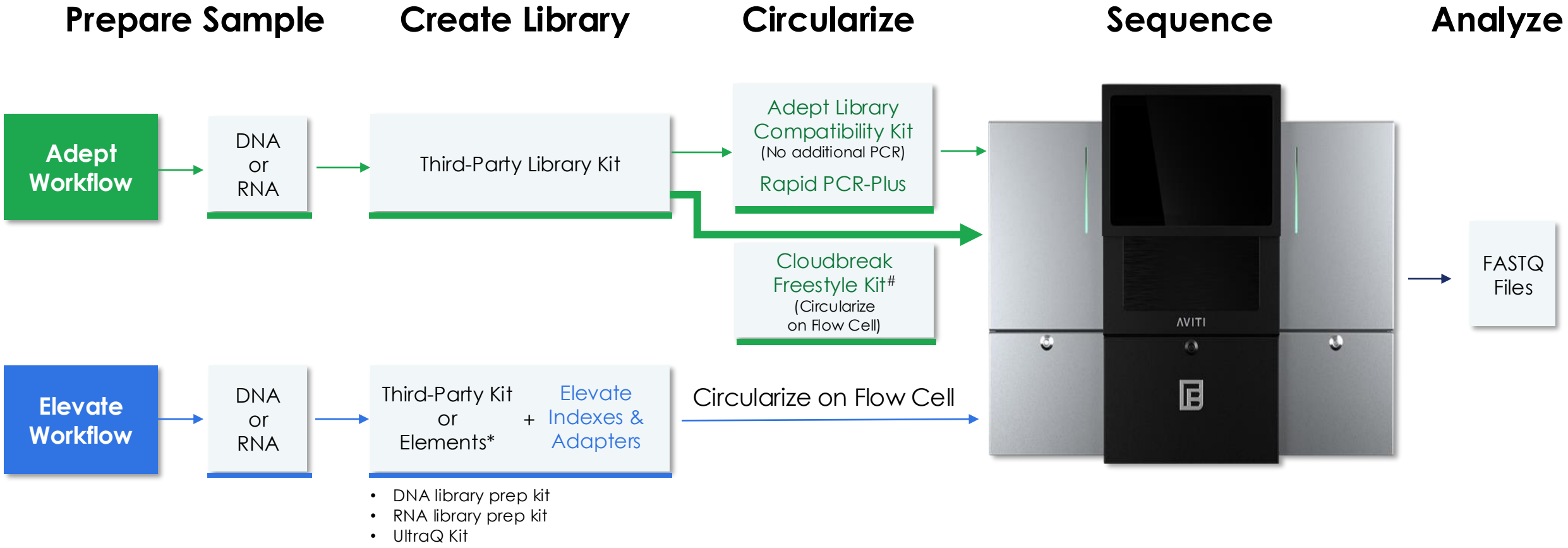


Output	Compared to MiSeq
300M	12x throughput @ ~10x lower cost
100M	4x throughput @ ~5x lower cost
25M - MiSeq	-

BCR Repertoire Sequencing



Elevate library prep simplifies your workflow with onboard circularization

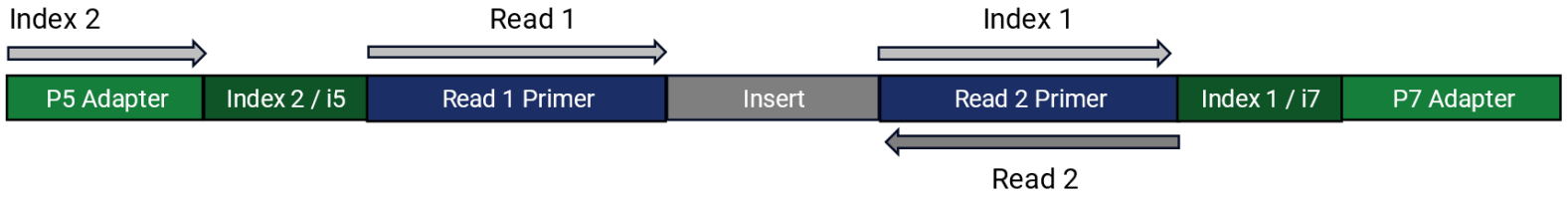


The Adept workflow enables adaption of existing libraries without any changes to the library prep

Library structure

*Cloudbreak **Freestyle** Chemistry*

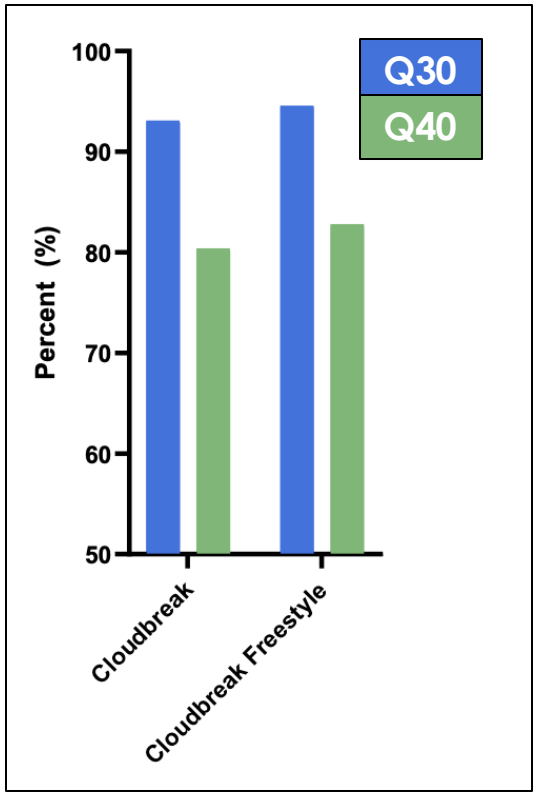
Third-Party Library



Elevate Library

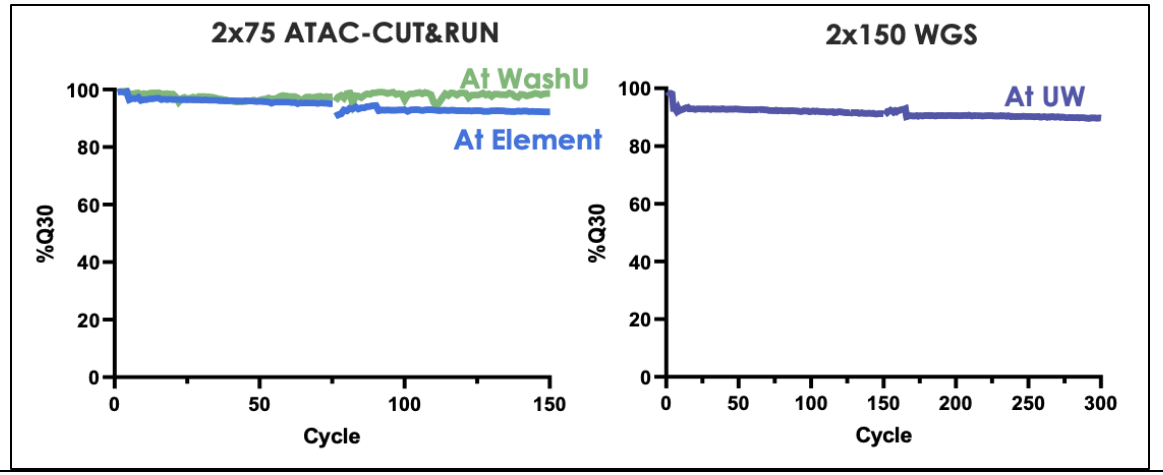
Adept Library

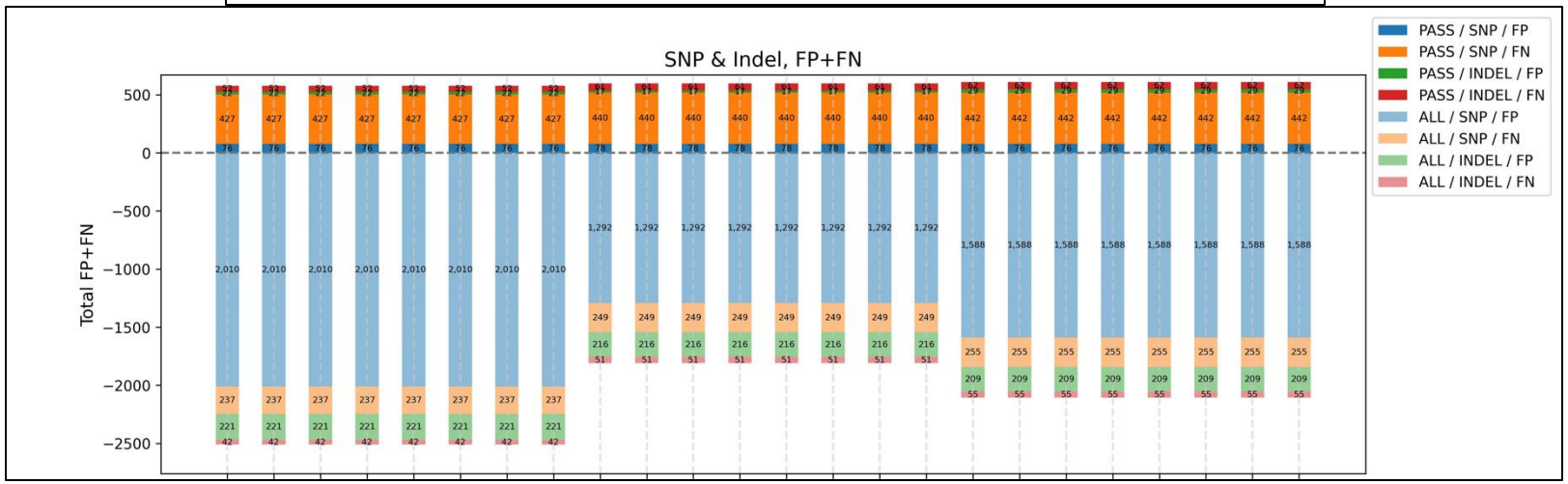
Cloudbreak freestyle delivers same high data quality with easier workflow



Washington University

University of Washington





Cloudbreak | Freestyle 1 | Freestyle 2

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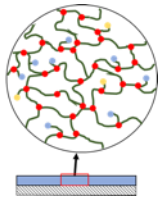
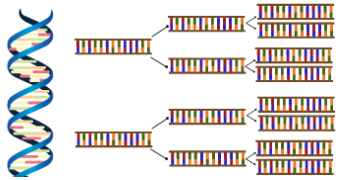
Every aspect of the DNA sequencing process redesigned to fit customer needs

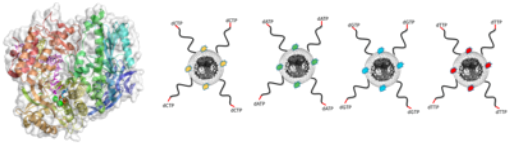
Accelerating Science Through Innovation



Flow Cell / Surface
Reagent Cost, Accuracy

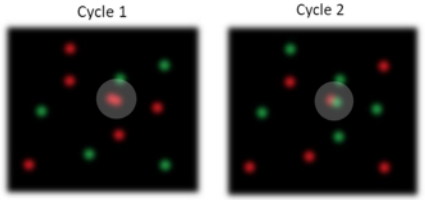


DNA Amplification
Ease of Use, Reagent Cost, Accuracy



Avidity Base Chemistry
Reagent Cost, Accuracy, Turnaround

Instrumentation
Throughput, COGS Reduction



Base Calling
Accuracy, Throughput

Workflow
Ease of Use, Paired End, Low Sample Input

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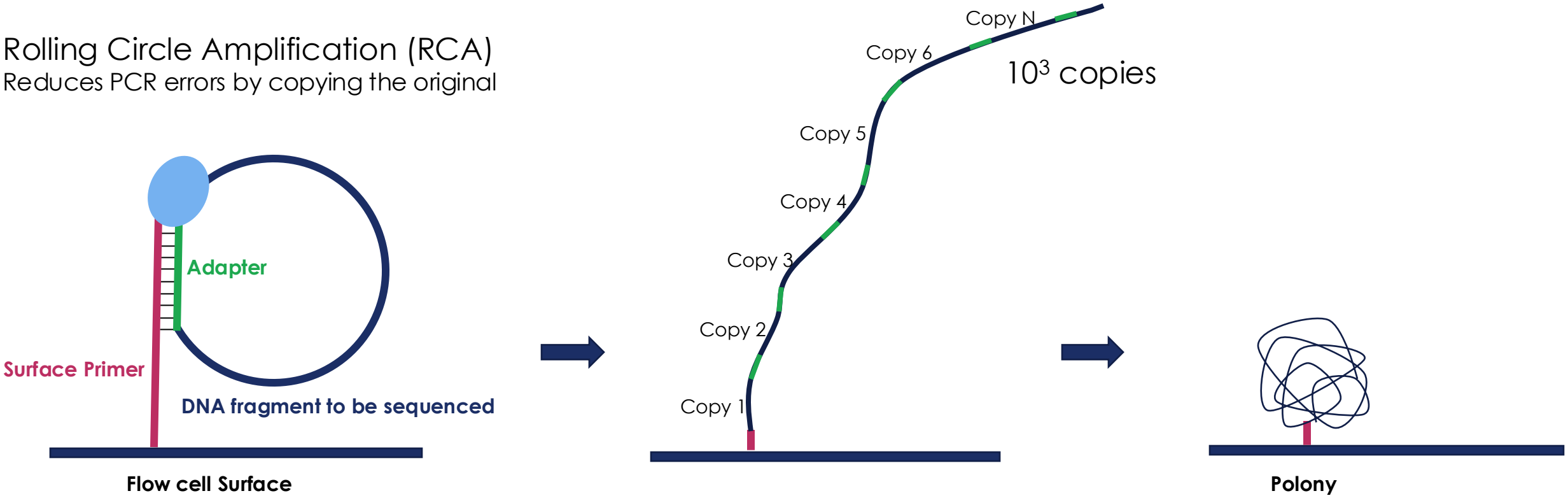
Element's ABC workflow – Rolling circle amplification brings accuracy benefits

Rolling Circle Amplification (RCA)

Reduces PCR errors by copying the original

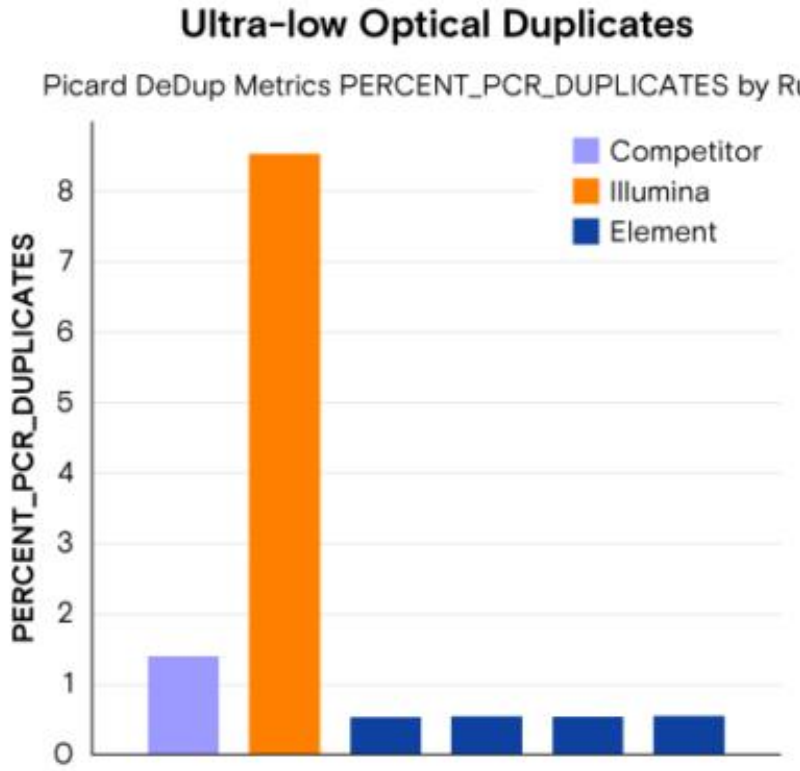


Primary sources of error removed

- ✓ No on-instrument PCR = less errors
- ✓ Reduced optical duplicates
- ✓ Index hopping undetectable

Nature Biotechnology Publication

Negligible index hopping and duplication rate with ABC Sequencing



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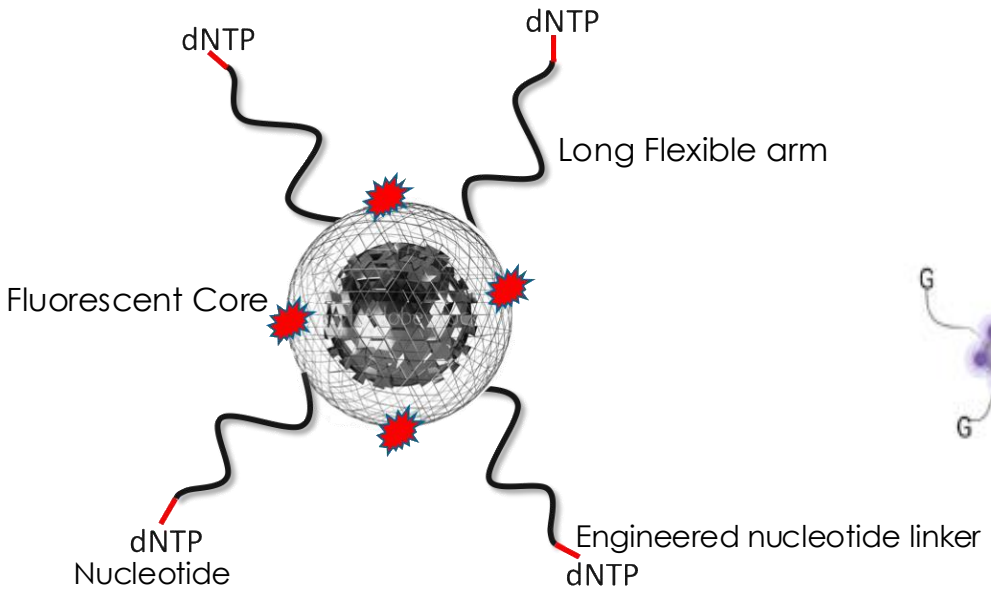
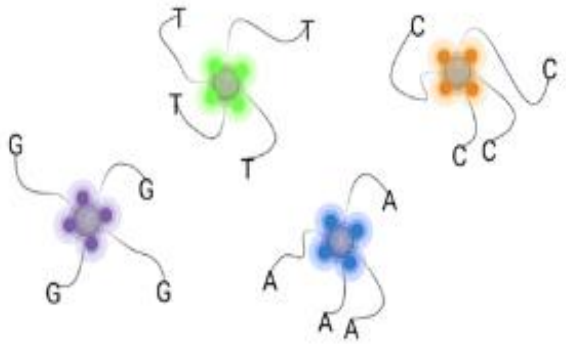
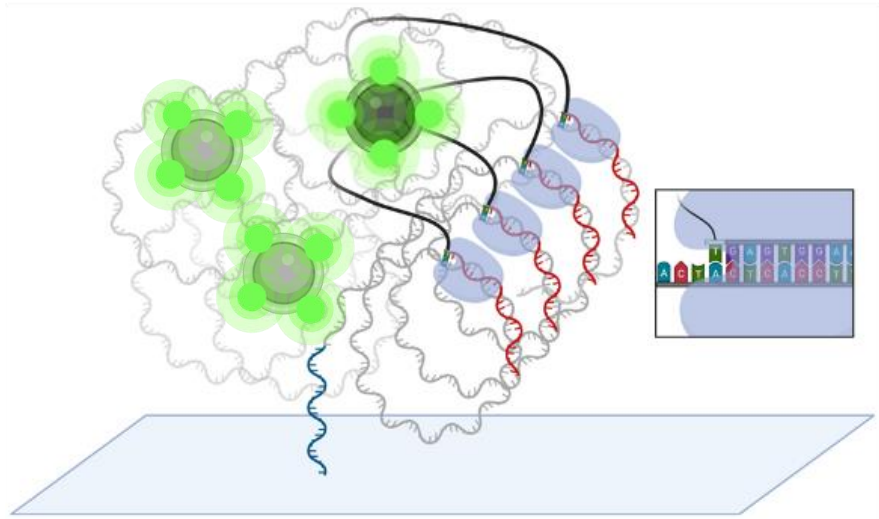
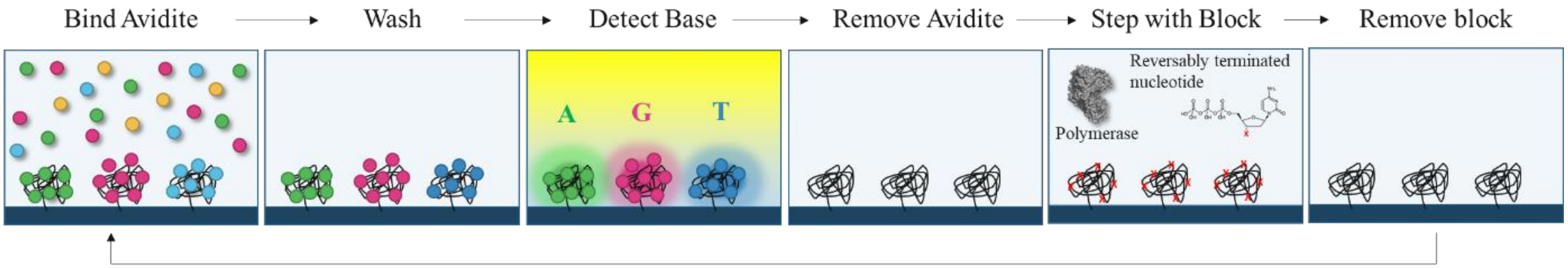
ABC leverages multivalent binding increasing data quality and reducing cost









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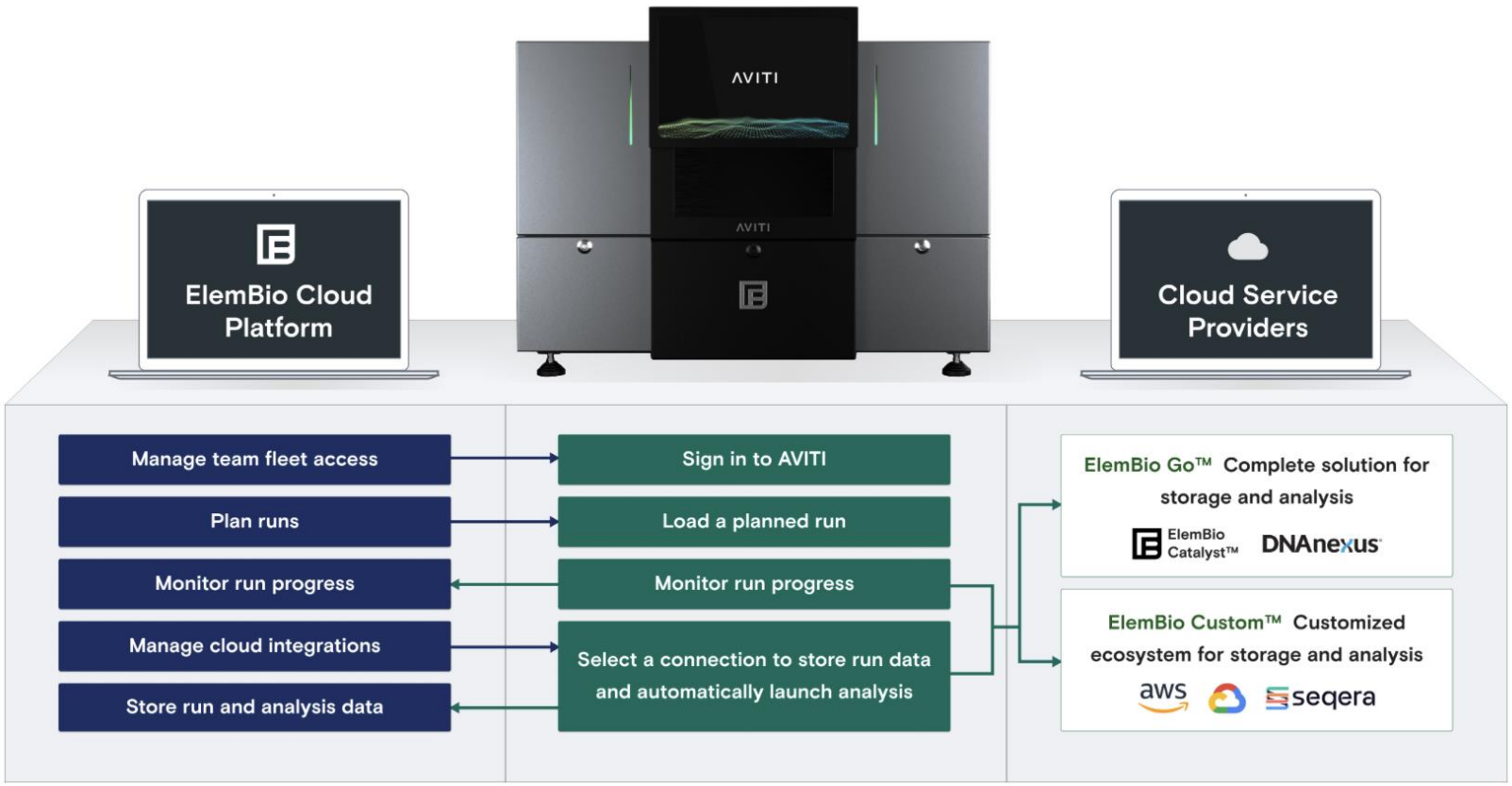
ElemBio Cloud: a transparent solution that enables end-to-end NGS workflow



Integrated solution for:

Remote Run Setup
Run Monitoring Visualizations
Connection to your cloud storage
Workflows for data analysis

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Outline

- Flexibility, Higher Quality and Low Cost
- Superior Performance
- Applications
- Upcoming Innovations

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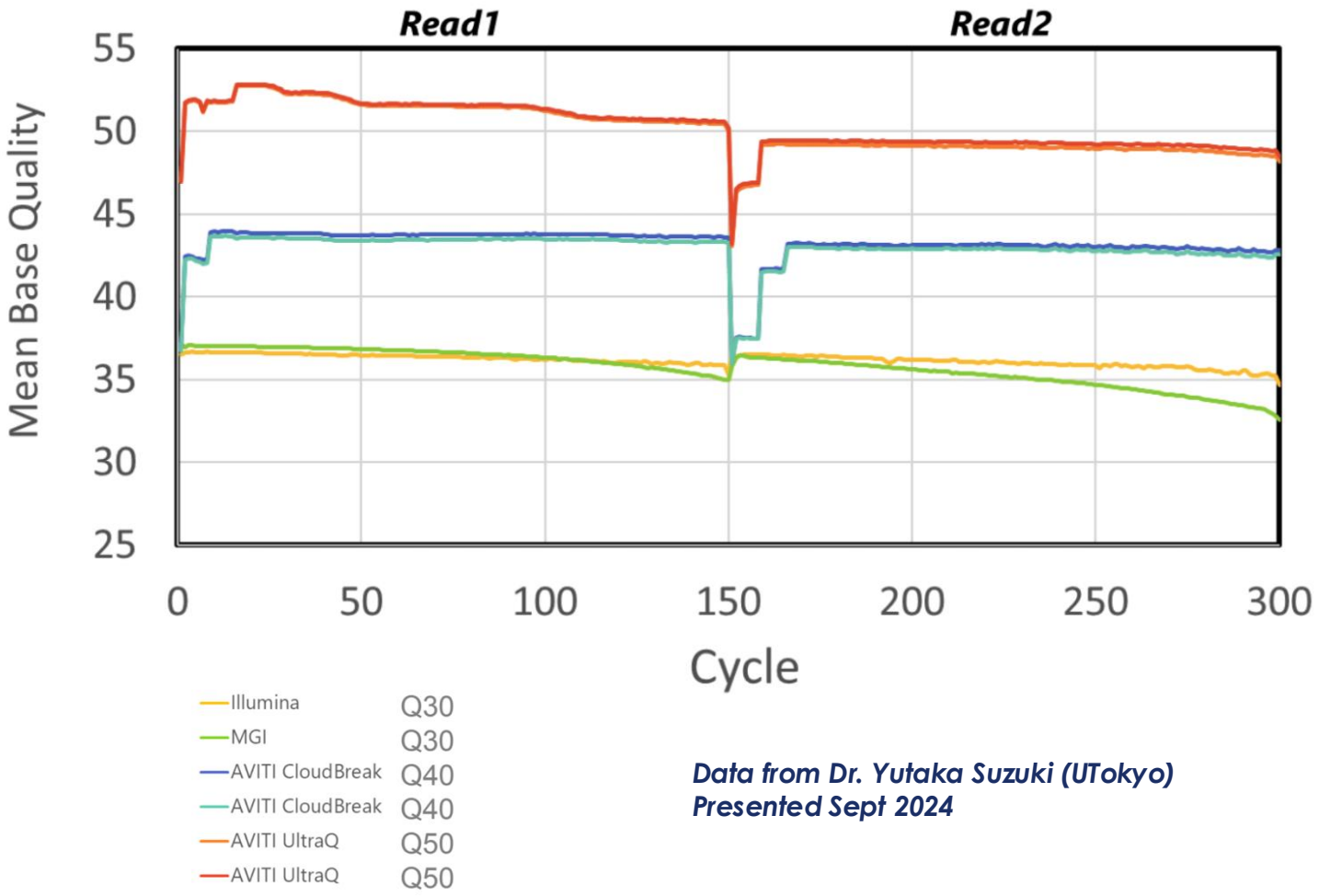
AVITI sets a new standard for sequencing quality

Q score or Phred score ($Q = -10 \log_{10} e$, where e is the base calling error probability)



Data from Dr. Yutaka Suzuki (UTokyo)
Presented Sept 2024

With UltraQ, >70% of reads at Q50

Quality Score	Interpretation
Q10	1 error in 10 bases
Q20	1 error in 100 bases
Q30	1 error in 1,000 bases
Q40	**1 error in 10,000 bases**
Q50	1 error in 100,000 bases

If a collection of bases is **Q40**, then we expect **1 in 10,000 of those bases to be incorrect**

bioRχiv
THE PREPRINT SERVER FOR BIOLOGY

Characterizing and addressing error modes to improve sequencing accuracy

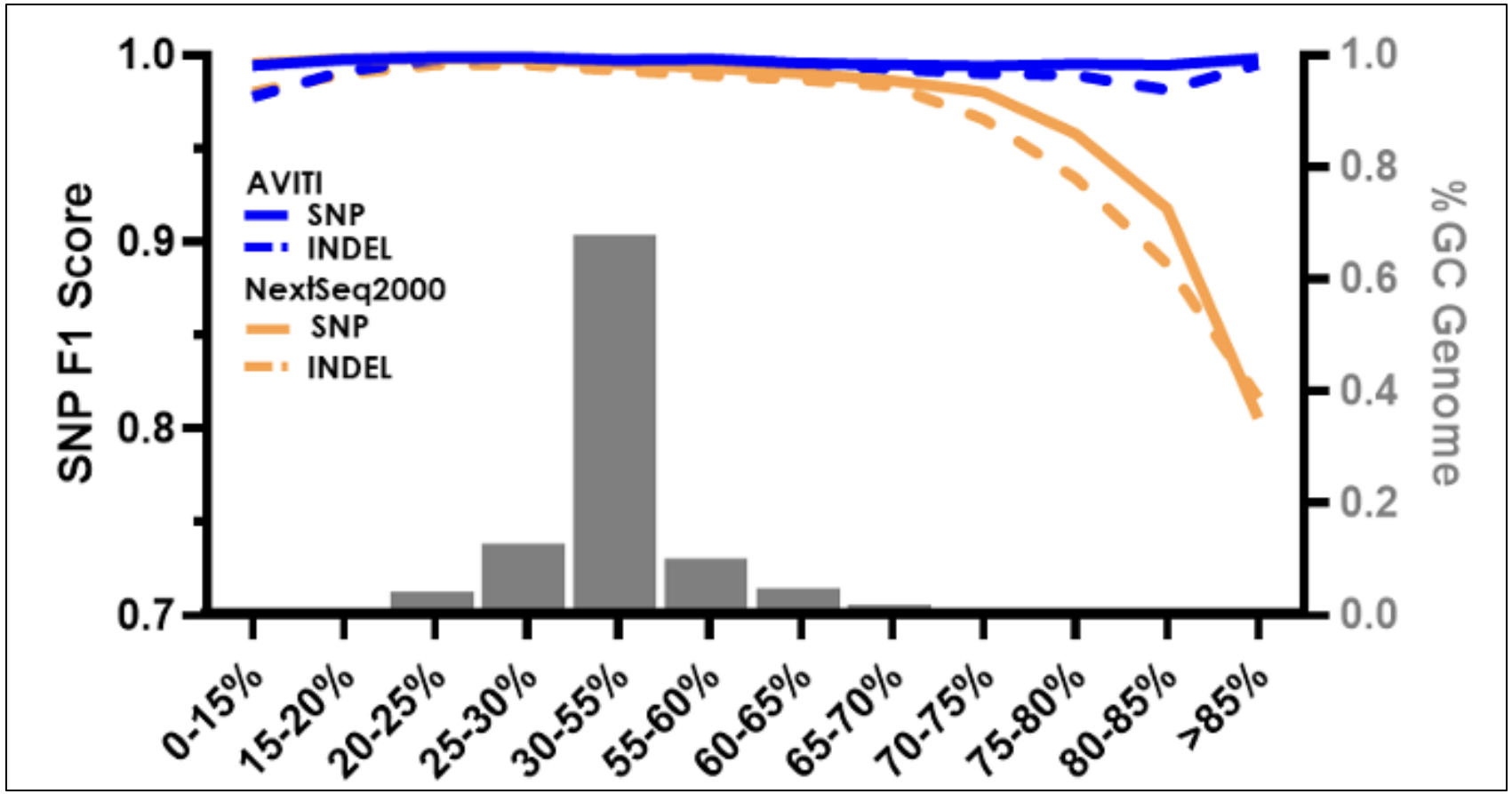
Semyon Kruglyak[1], Andrew Altomare[1], Mark Ambroso[1], Vivian Dien[1], Bryan Lajoie[1], Kelly N. Wiseman[1], Shawn Levy[1], and Matthew Kellinger[1]
[1]Element Biosciences, San Diego, CA

Access here

Blog post

No on-instrument PCR limits AT/GC bias, providing more even coverage

AVITI 2 x 300 variant-calling accuracy vs GC content



GC Content

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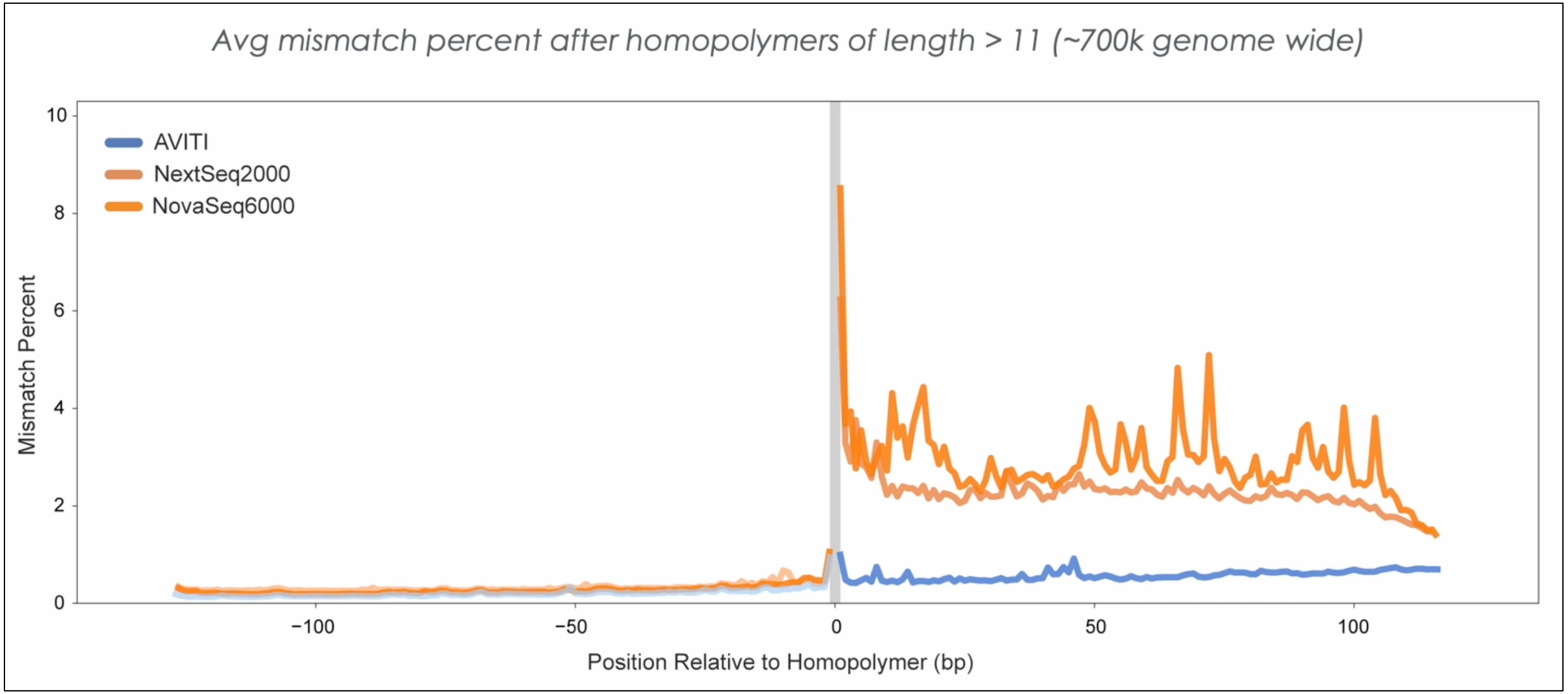
Exceptional performance across homopolymer regions



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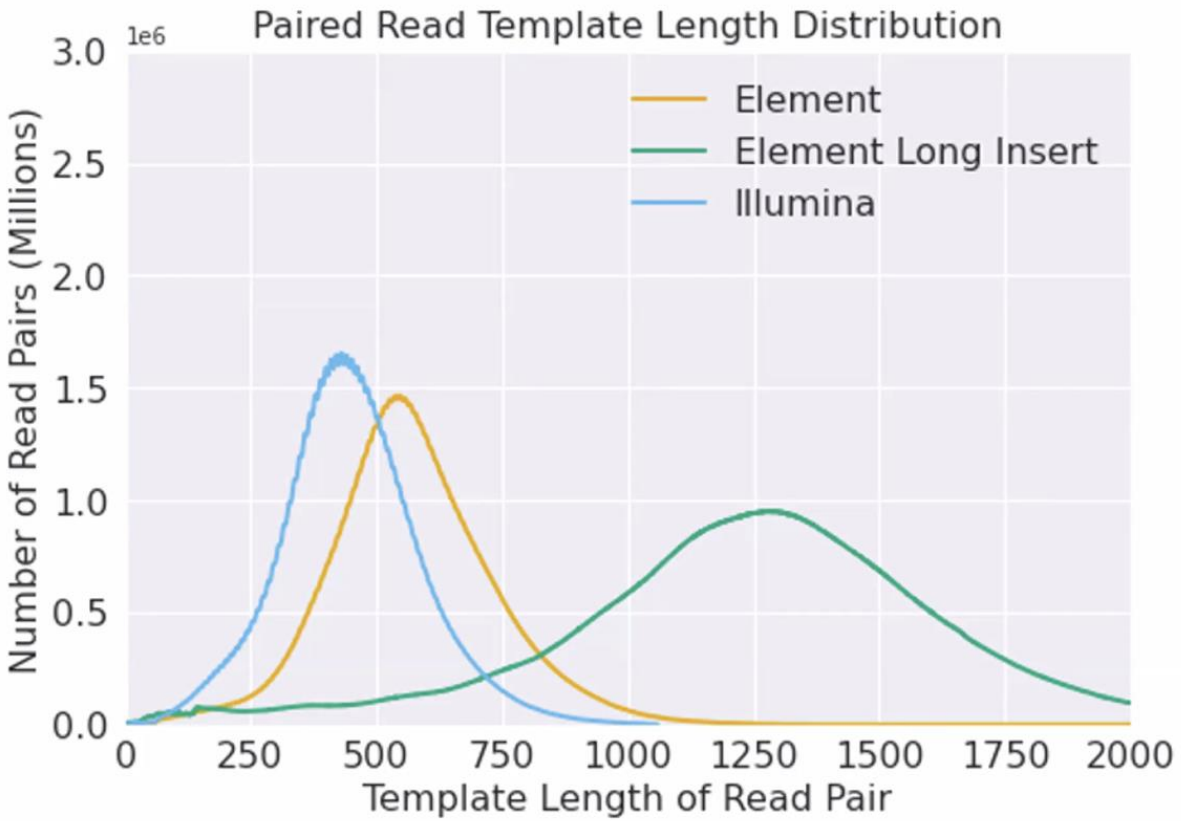
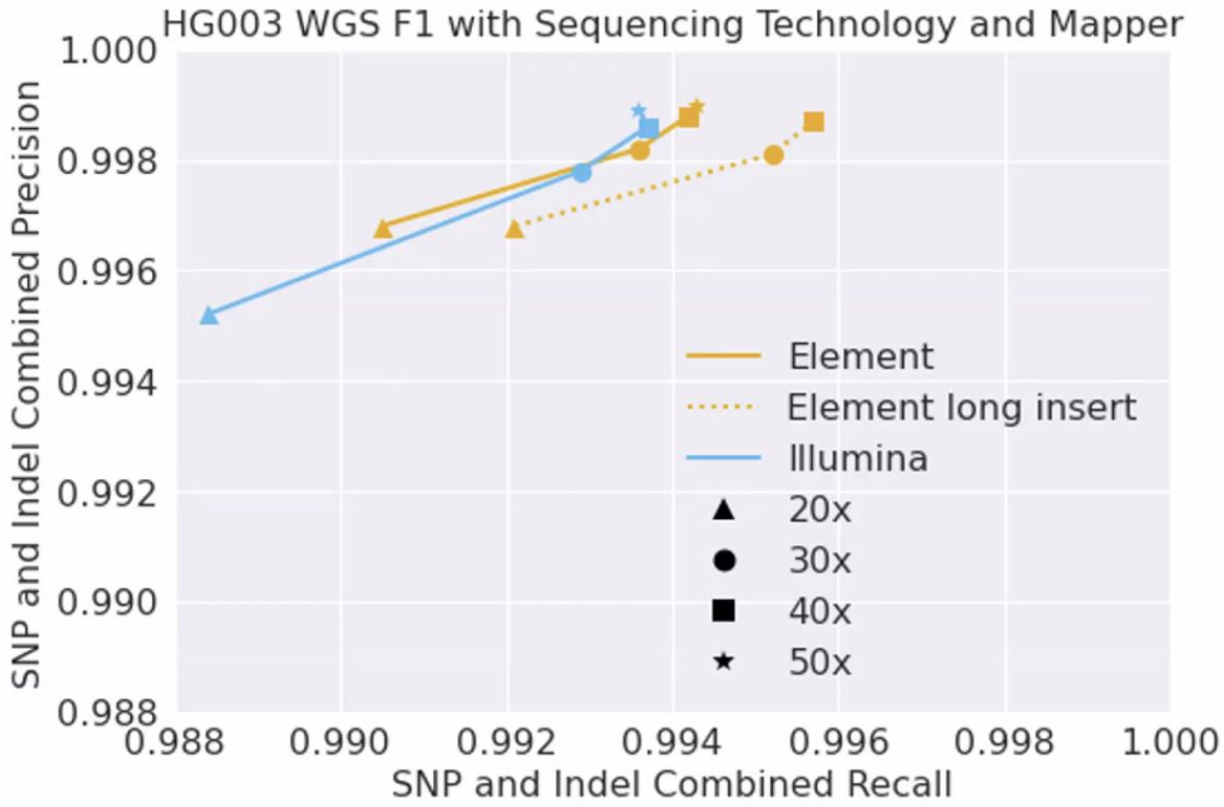
Advantages of insert size on mapping and variant calling accuracy





Accurate human genome analysis with Element Avidity sequencing

Andrew Carroll[1] , Alexey Kolesnikov[1] , Daniel E. Cook[1] , Lucas Brambrink[1] , Kelly N. Wiseman[2] , Sophie M. Billings[2] , Semyon Kruglyak[2] , Bryan R. Lajoie[2] , June Zhao[2] , Shawn E. Levy[2] , Cory Y. McLean[1] , Kishwar Shafin[1] , Maria Nattestad[1] , Pi-Chuan Chang[1]

1. Google LLC, Mountain View CA, USA
2. Element Biosciences, San Diego, CA, USA

Outline

- Flexibility, Higher Quality and Low Cost
- Superior Performance
- Applications
- Upcoming Innovations

For research use only

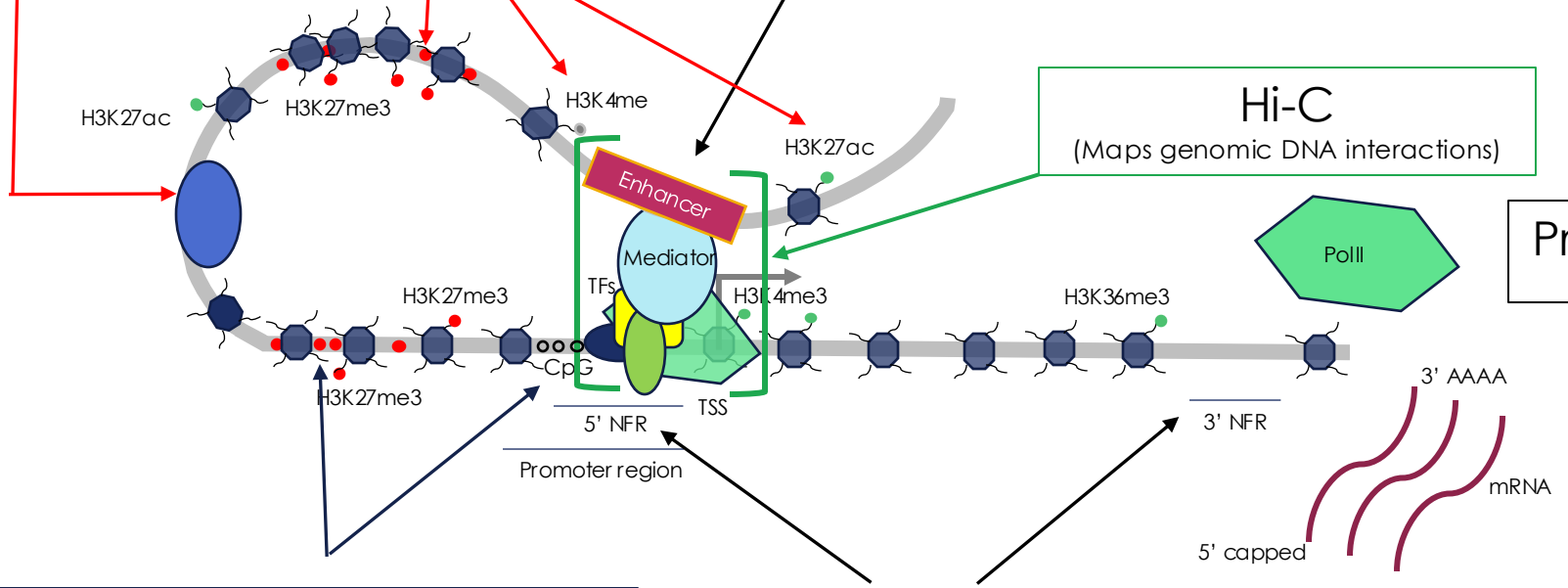
Approaches to investigate mechanisms of gene regulation

ChIP-Exo/ChIP-Seq (CUT&RUN and CUT&Tag)
(Assesses the location of proteins, DNA and histone modifications)

ATAC-Seq
(Identify open regions of the genome)

Hi-C
(Maps genomic DNA interactions)

Proteins (+modifications)
Aptamer/antibody based



DNA Methylation
(Identify DNA modifications – 5mc and 5hmC)

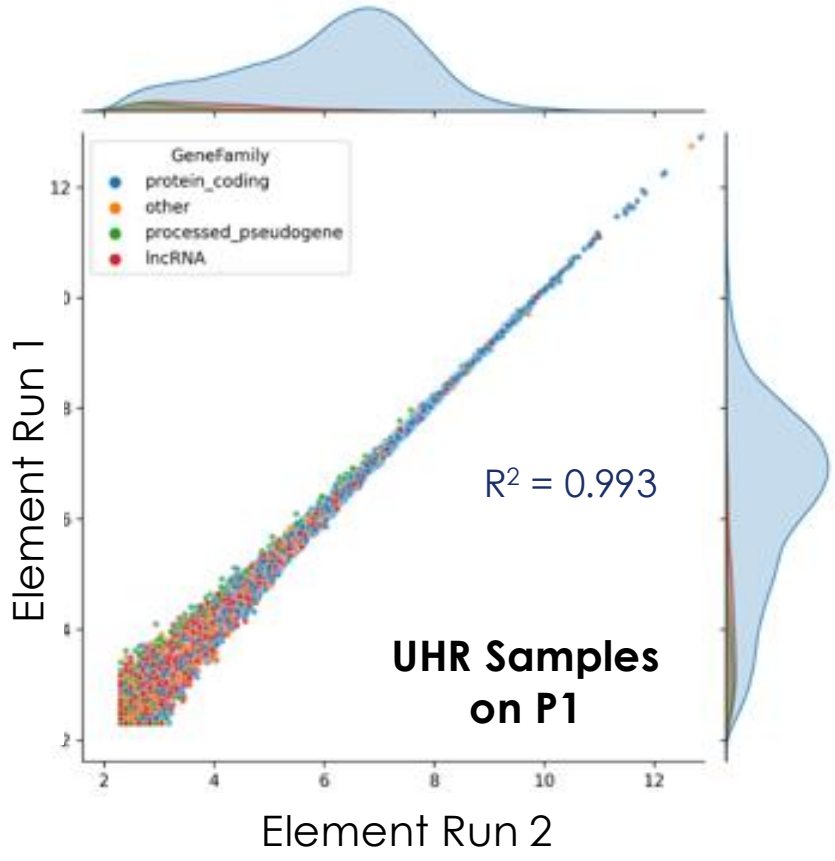
ATAC-Seq

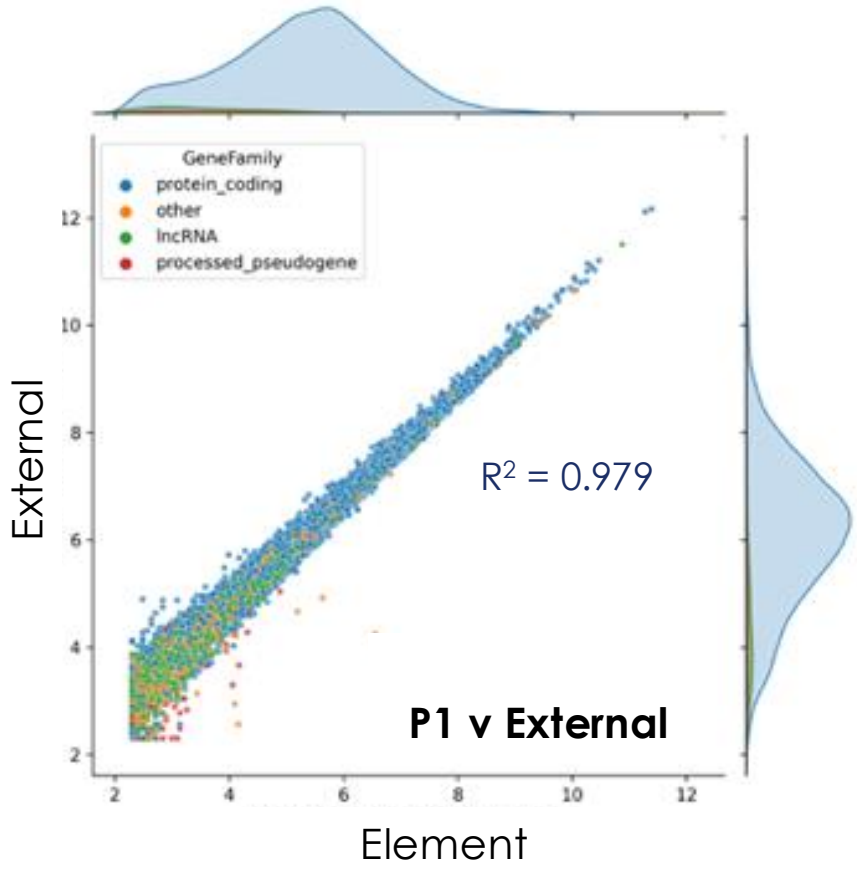
mRNA, RNA modifications, ncRNA

Combined with WGS and Immune profiling, can lead to better understanding of disease mechanisms

For research use only

Element bulk RNA sequencing produces data that is replicable and consistent





Sample-to-sample:
target R^2 > 98%
actual R^2 > **99.3%**,

Platform-to-platform:
target R^2 > 95%
actual R^2 > **97.9%**

For research use only

Element bulk RNA sequencing produces better data

VIB Nucleomics Core – Leuven, Belgium

Experimental design

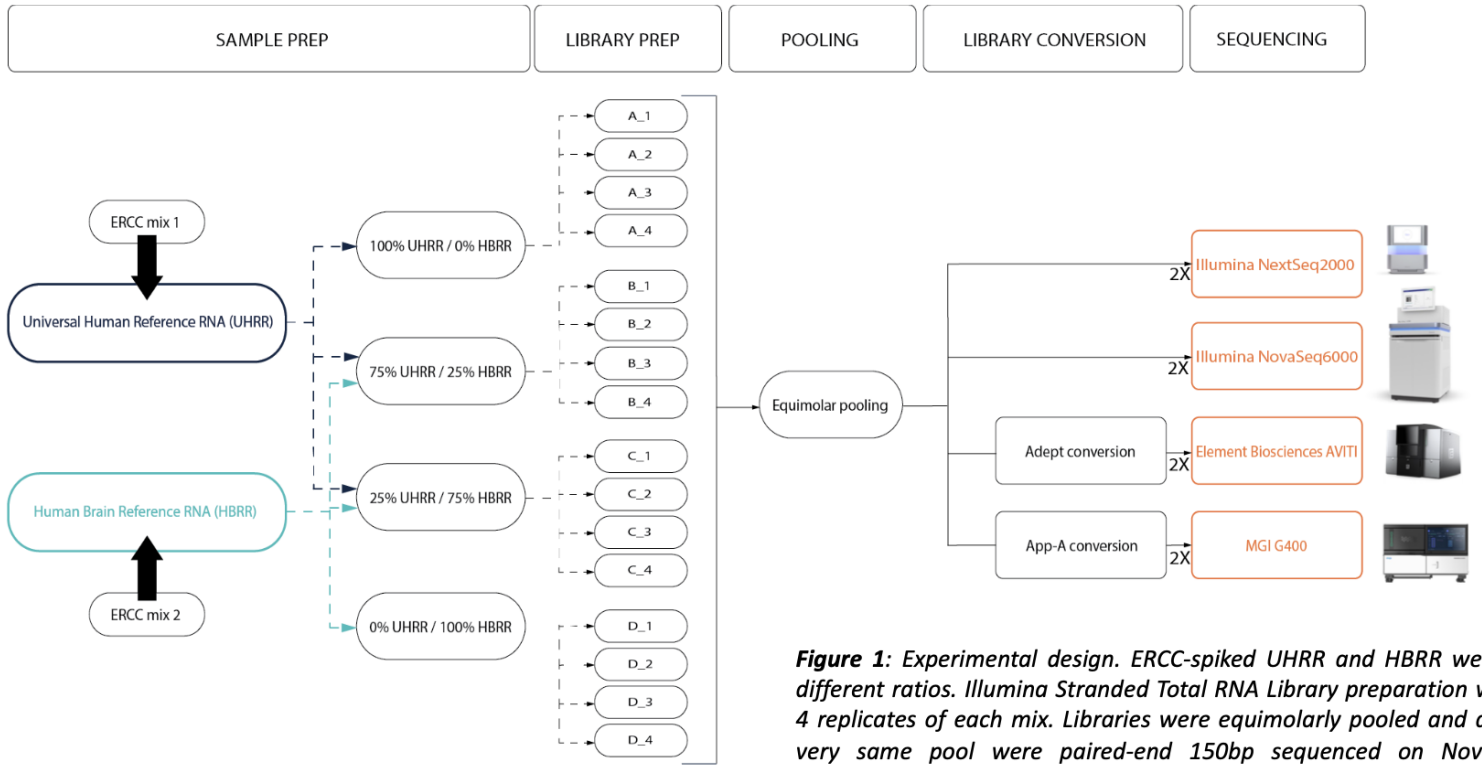


***Figure 1**: Experimental design. ERCC-spiked UHRR and HBRR were mixed at 4 different ratios. Illumina Stranded Total RNA Library preparation was applied to 4 replicates of each mix. Libraries were equimolarly pooled and aliquots of the very same pool were paired-end 150bp sequenced on NovaSeq6000 SP, NextSeq2000 P3, AVITI, and G400 FCL. Conversion steps were introduced following instructions by the providers to make the pool compatible with AVITI and G400 sequencing.*

Quality scores

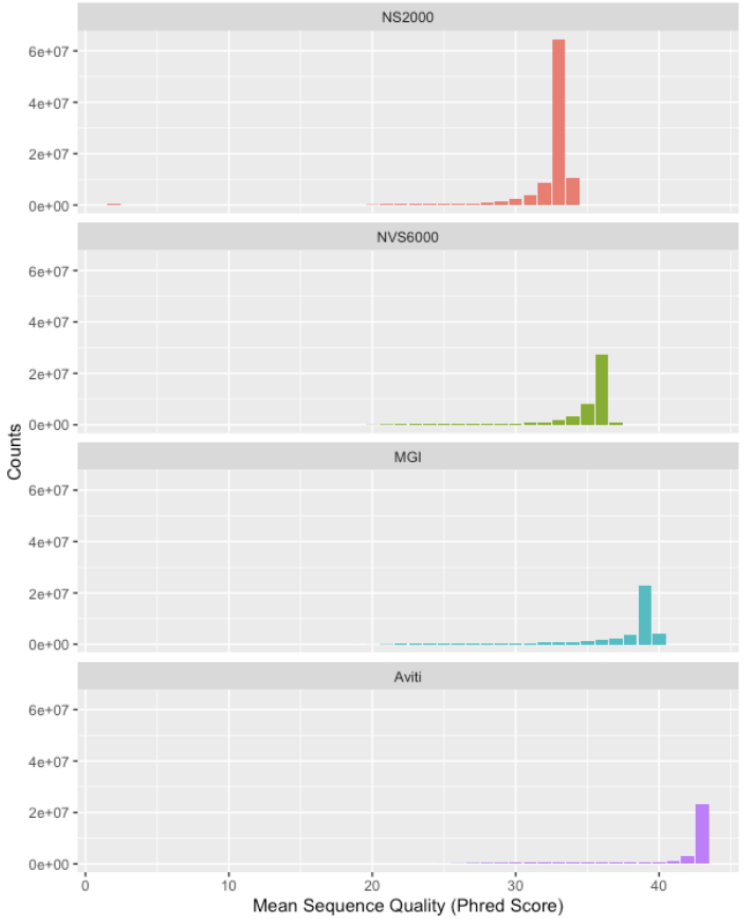


***Figure 4**: Read count versus mean Q-score of platforms*

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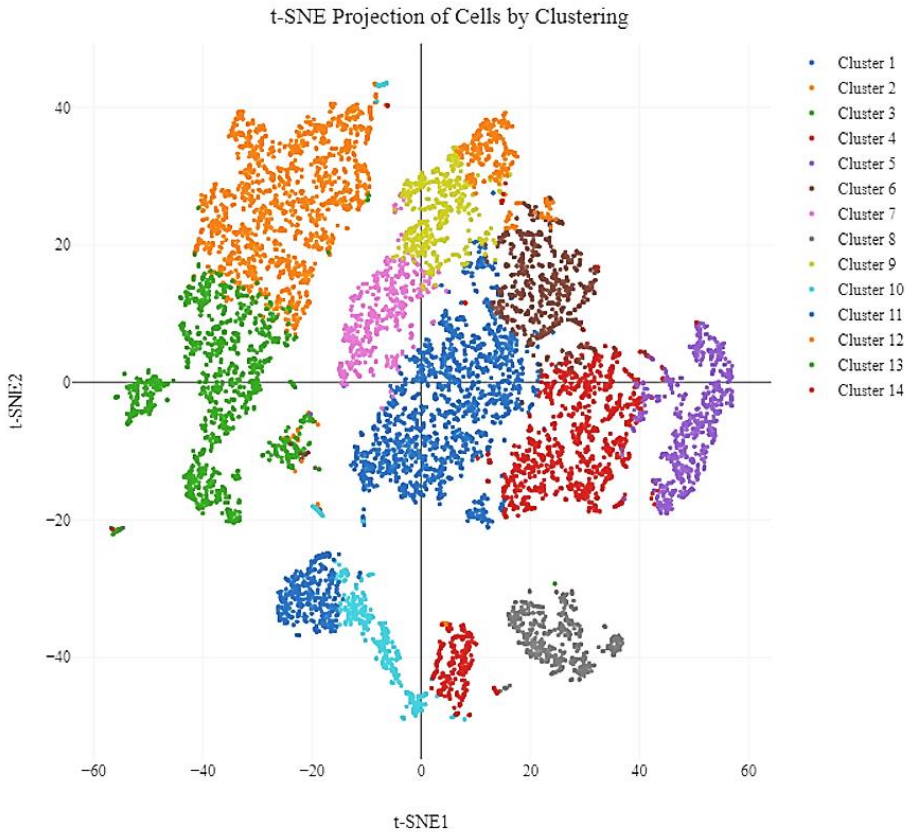
Element is a certified 10X genomics compatible partner

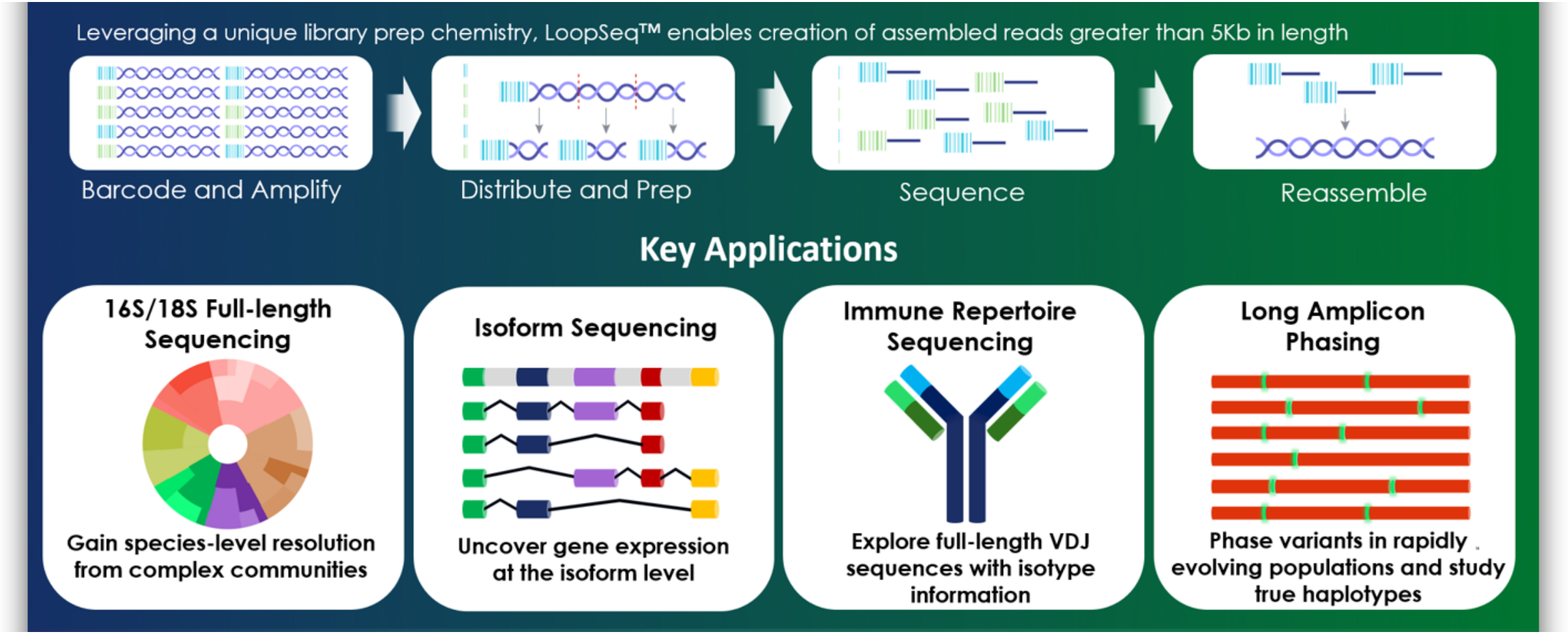
10K and 1K Human PBMC Reference from 10x Genomics

10X Cellranger Count Metrics (v7.0)	CPP Expectation	AVITI 10k cells	AVITI 1k cells
Valid Barcodes	>90%	97.5%	97.5%
Reads mapped confidently to Exonic Regions	>50%	53.0%	53.8%
Reads mapped confidently to Transcriptome	>40%	74.7%	77.8%
Fraction Reads in cells	>80%	95.5%	92.6%
Q30 bases in barcode	>85%	99.5%	99.5%
Q30 bases in RNA read	>75%	98.6%	98.8%
Mean Reads per cell	>50,000	61,326	68,766
Median Genes per cell	>1700	2,910	2,951
Estimated number of cells	+/- 20%	8,513	922



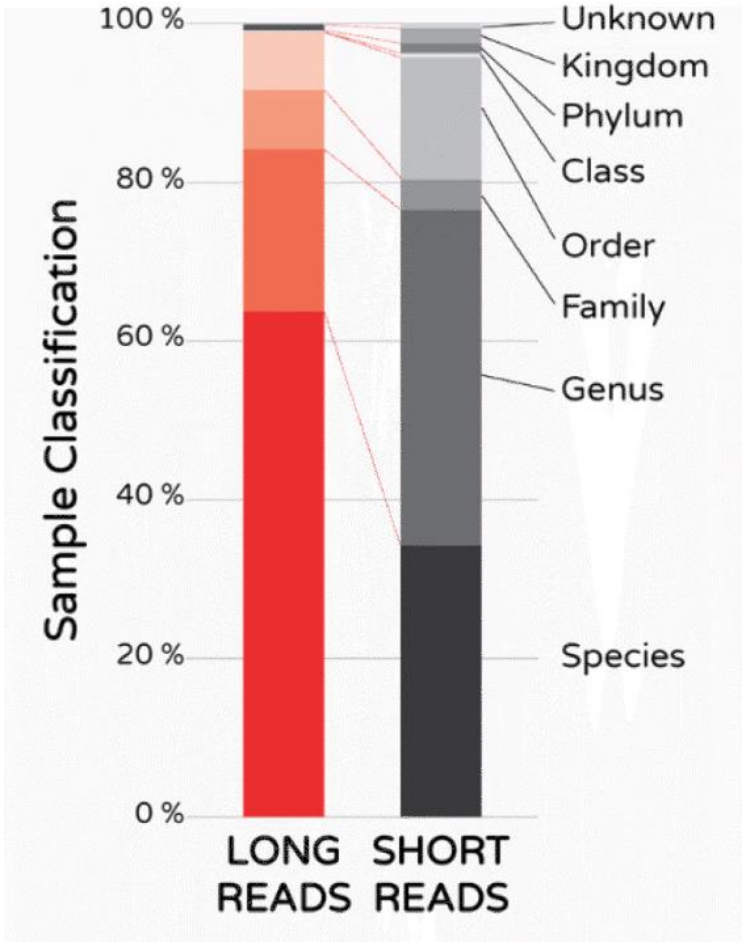
Sequencing data available for download on our website: www.elementbiosciences.com

LoopSeq™: On-demand access to long reads with AVITI

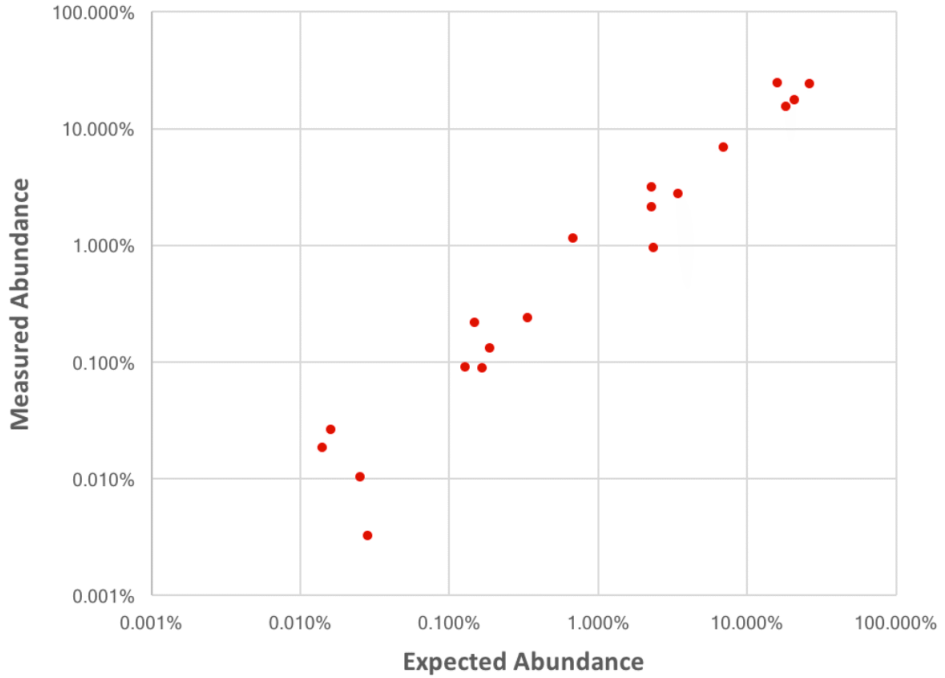


Targeted Sequencing for ACMG/PGx/CMRG genes, off-target assessment of CRISPR, RNA isoform discovery

LoopSeq 16S sequencing improves both species resolution and quantification

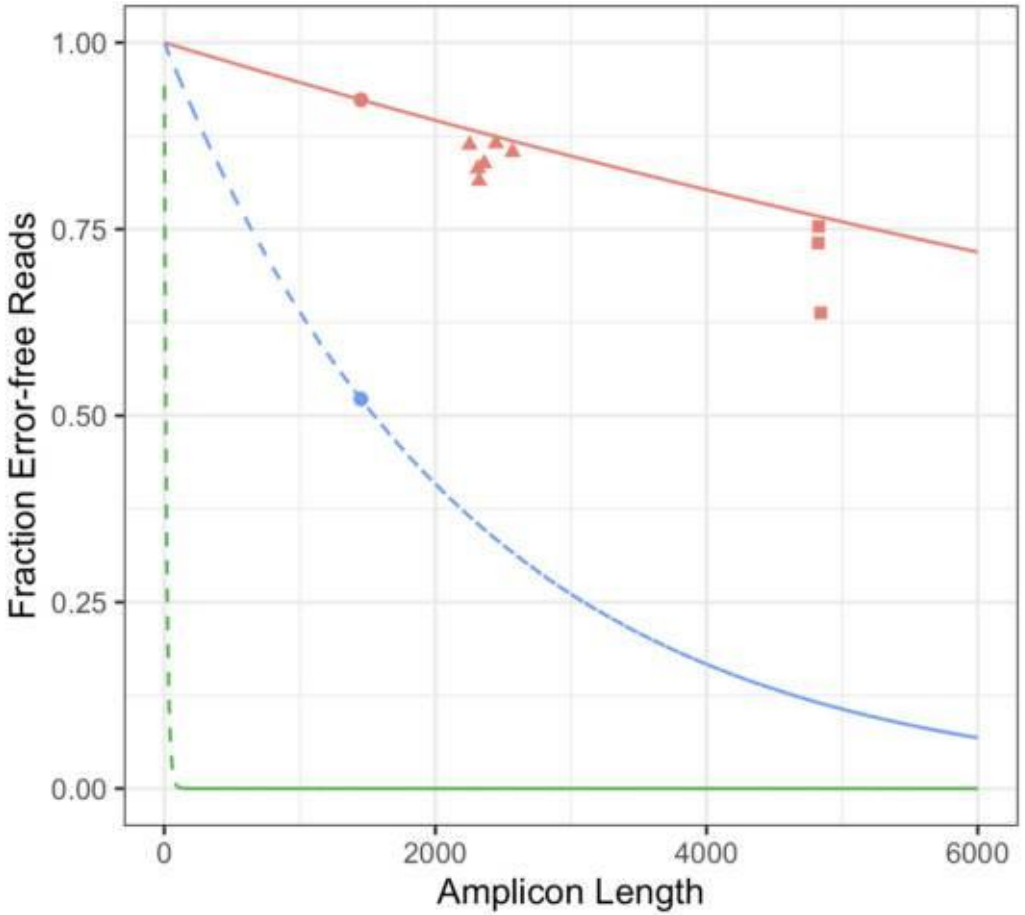


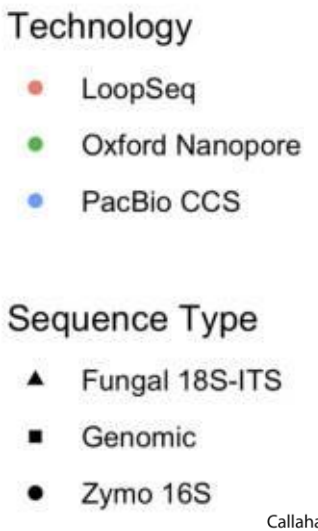
- Complex soil sample using short-read sequencing versus LoopSeq full-length 16S
- Unlike short reads, LoopSeq Microbiome method classified >99% of unique 16S molecules down to species or genus level
- LoopSeq enhances accurate quantification by leveraging UMIs to remove PCR duplicates

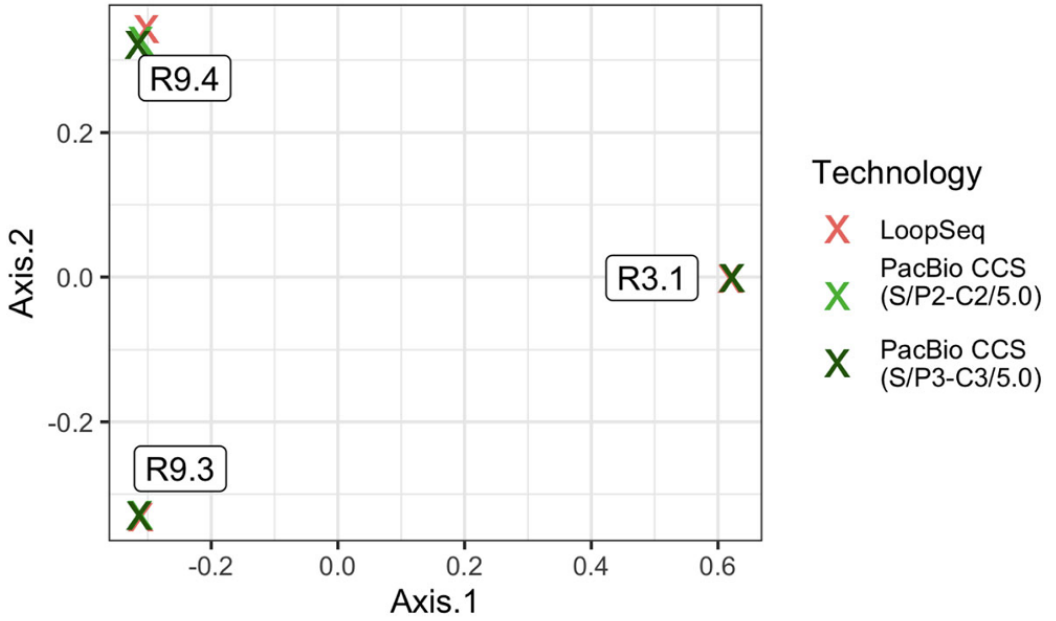


For research use only

LoopSeq delivers higher fraction of error free reads than alternate long read technologies







Callahan *et al. Microbiome* (2021) 9:130
https://doi.org/10.1186/s40168-021-01072-3

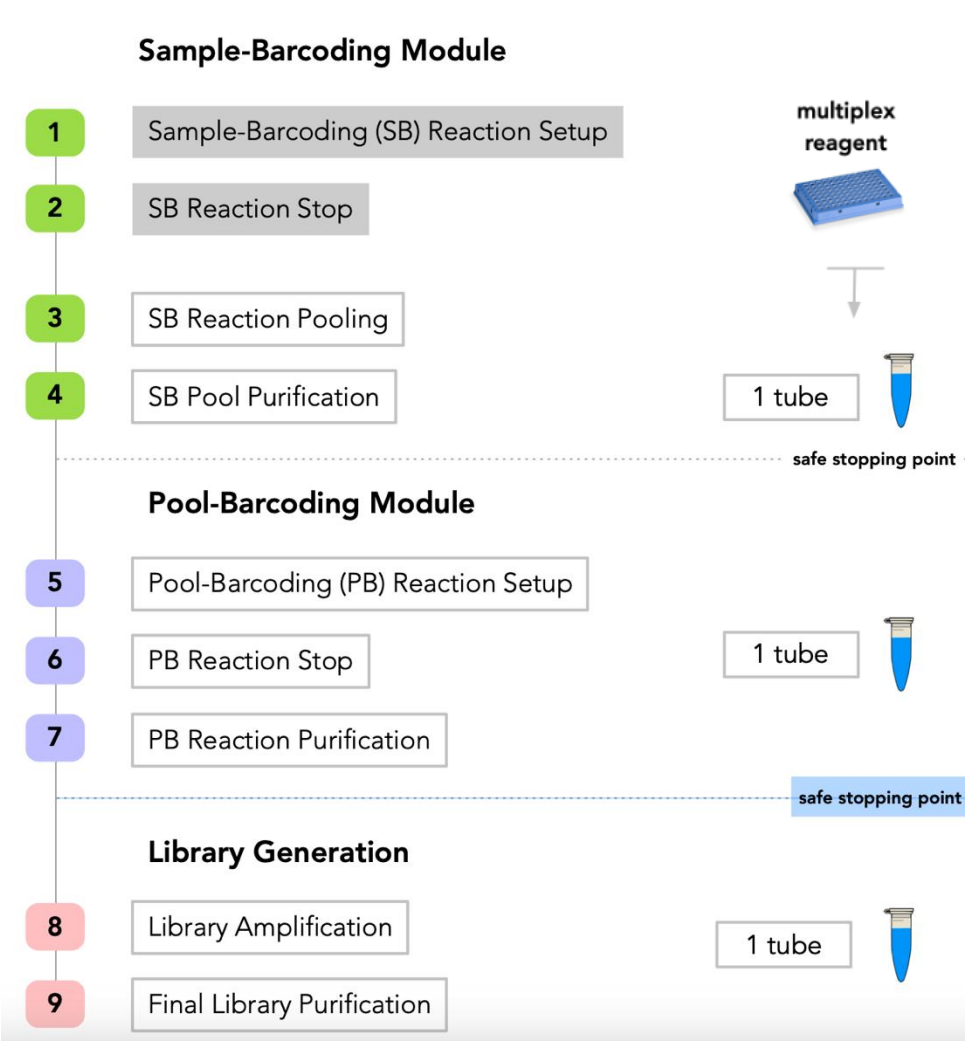
Microbiome

RESEARCH Open Access

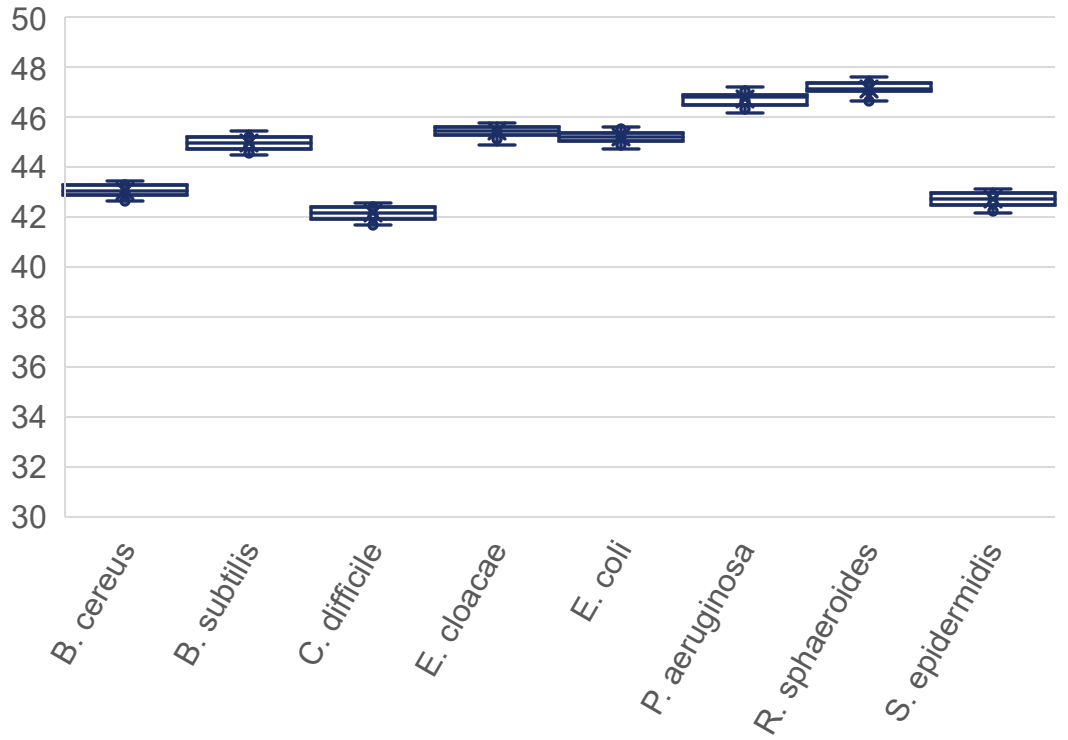
Ultra-accurate microbial amplicon sequencing with synthetic long reads

Benjamin J. Callahan[1,2*], Dmitry Grinevich[1], Siddhartha Thakur[1], Michael A. Balamotis[3] and Tuval Ben Yehezkel[3]

Microbial WGS is accurate and affordable on the Element AVITI system



seqWell



- 384 multiplexed samples were aligned against the ATCC reference for each sample
- All replicates had mean quality score >40

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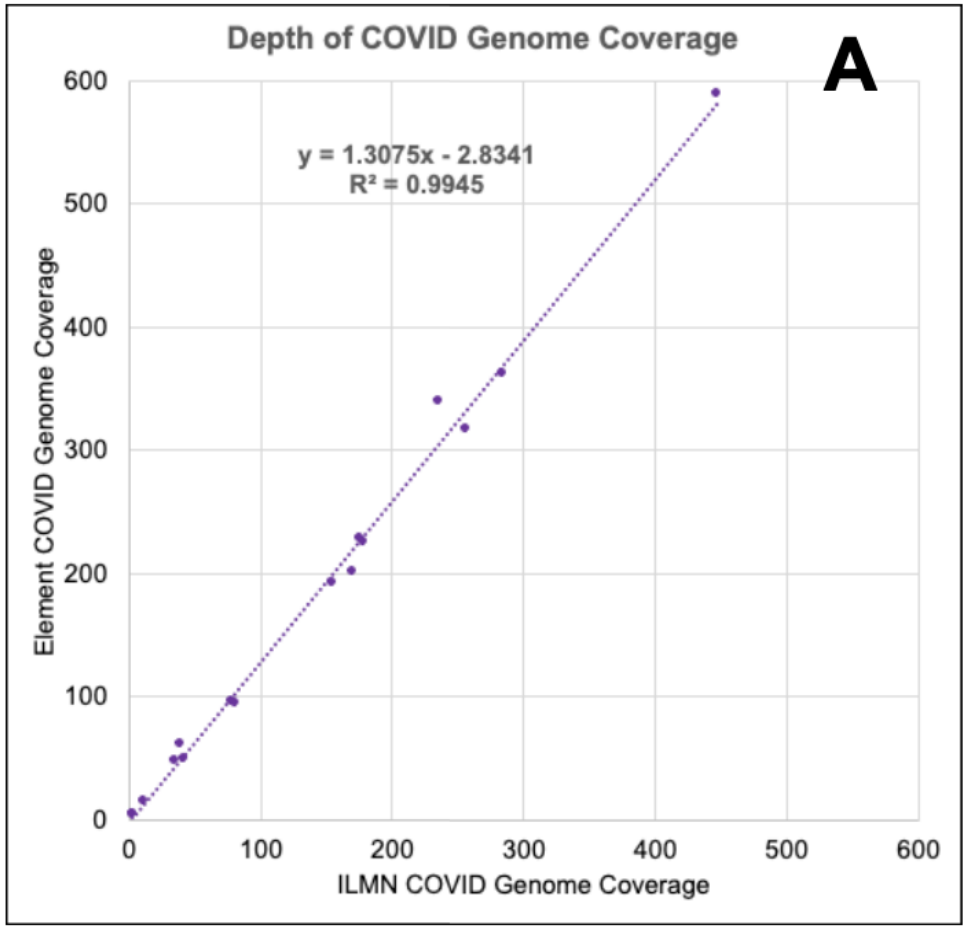
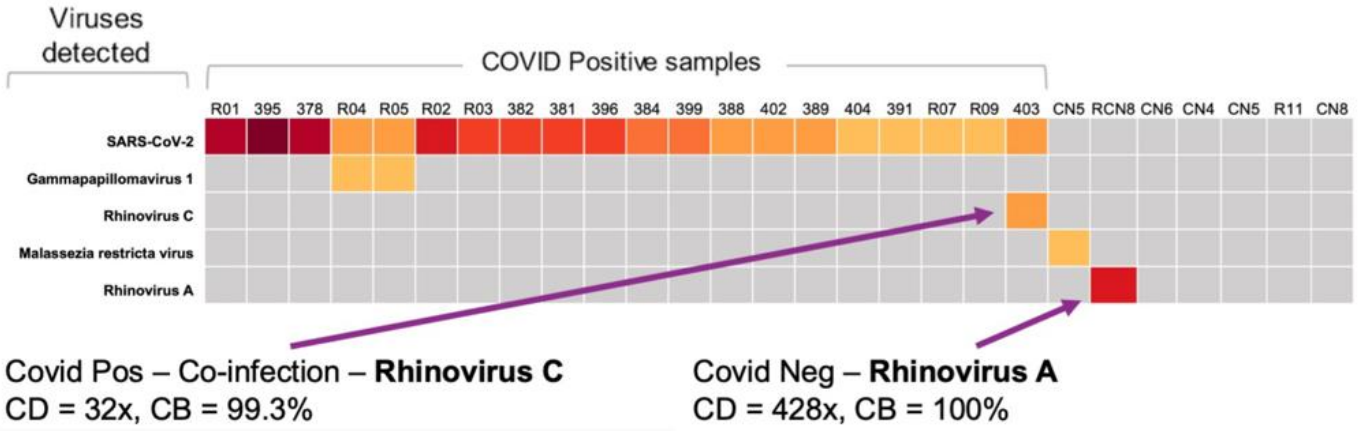
Beyond COVID surveillance: Shotgun meta-transcriptomics as a method for a universal infectious disease surveillance assay



- Samples processed with JumpCode CRISPRclean Plus
  - ➢ deplete high abundance host and bacteria rRNA
- STAR/GATK for alignment against the SARS-CoV-2 reference
- In several cases, the method was able to detect co-infection with additional viruses



DNA methylation analysis

Methyl-Seq on AVITI using enzymatically converted DNA

Lambda DNA
(0% methylation)

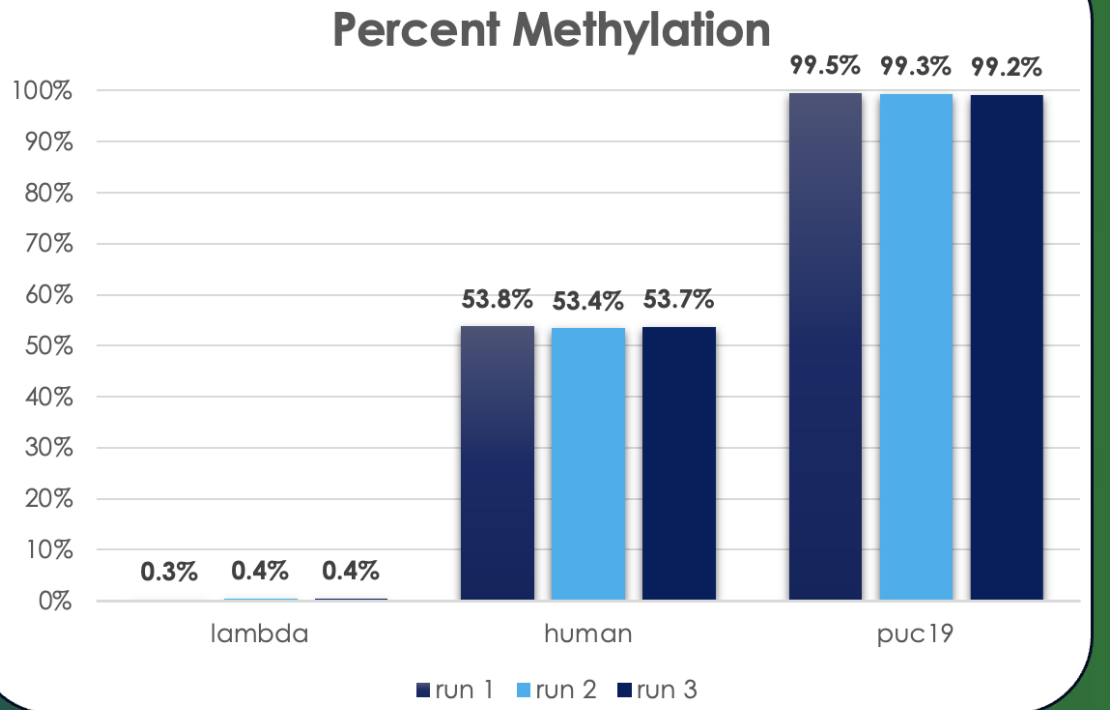
human DNA
(53.4% methylation)

Puc19 DNA
(100% methylation)

NEBNext® Enzymatic Methyl-seq Kit

2x150 Sequencing on AVITI

Bismark Bisulfite Read Mapper and Methylation Caller



Whole genome methylation sequencing on AVITI produces highly accurate results relative to truth data

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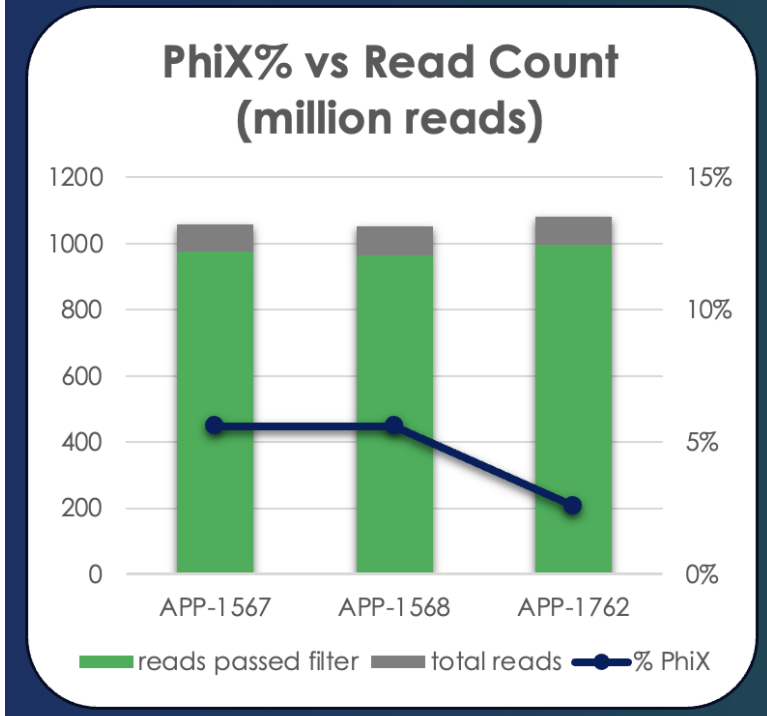
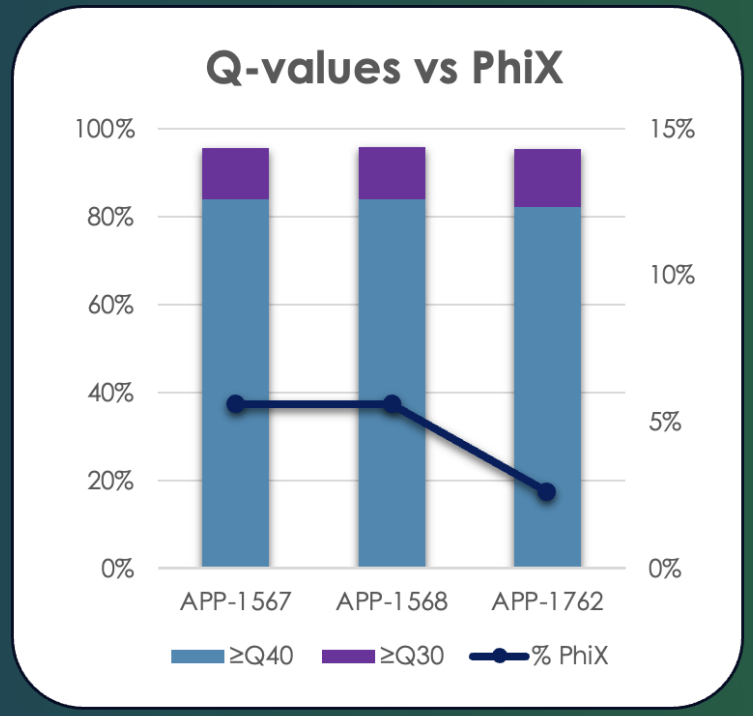
DNA methylation analysis

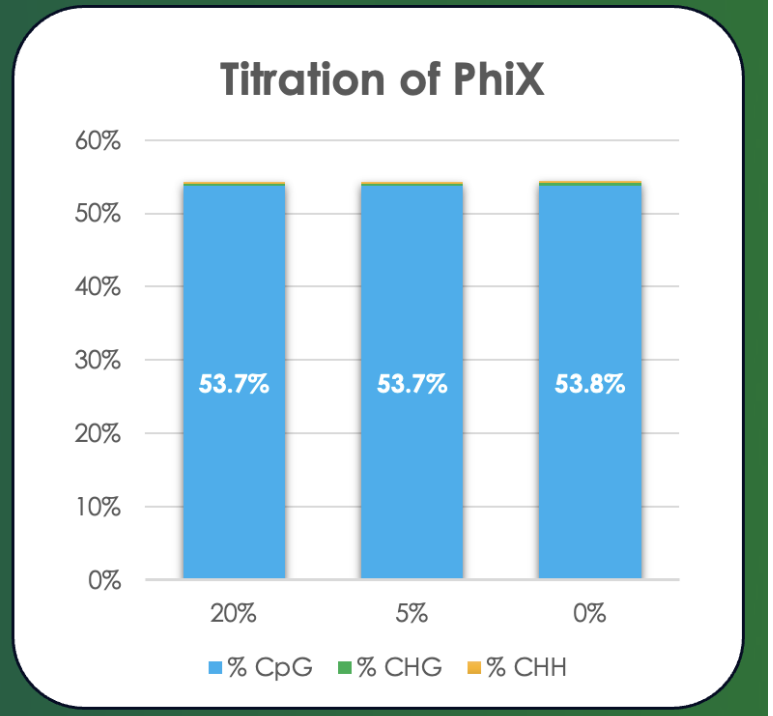
Robustness in polony-finding and base calling with low-complexity libraries



Impact of total usable reads with reduced PhiX to 2%



Minimal impact on quality scores with reduced PhiX to 2%



Comparison of methylation analysis results with PhiX titrated to 0%

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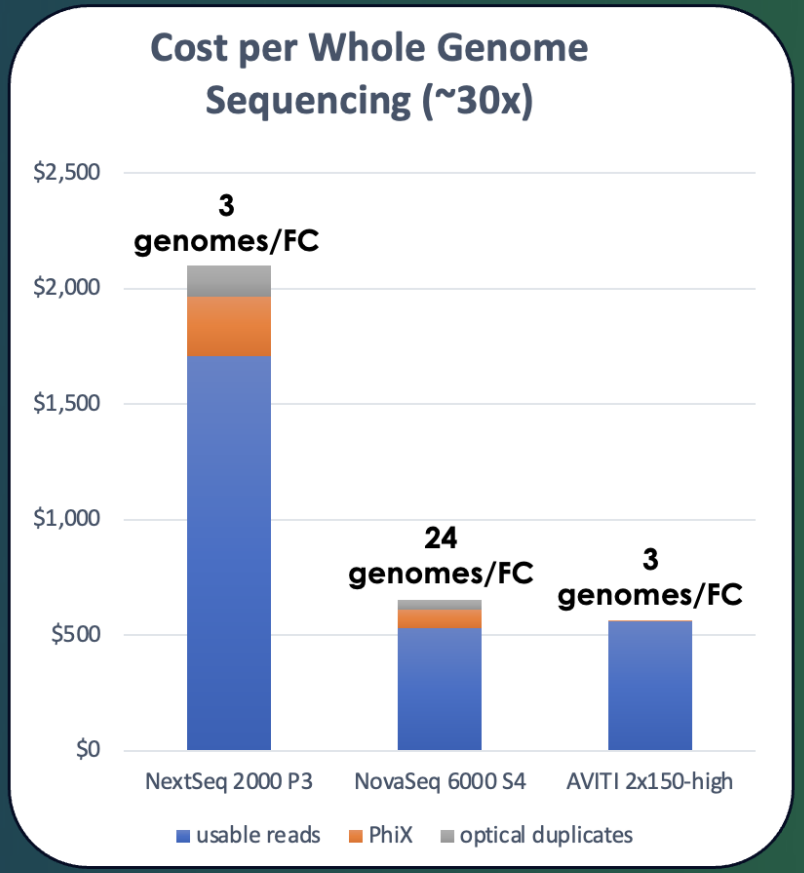
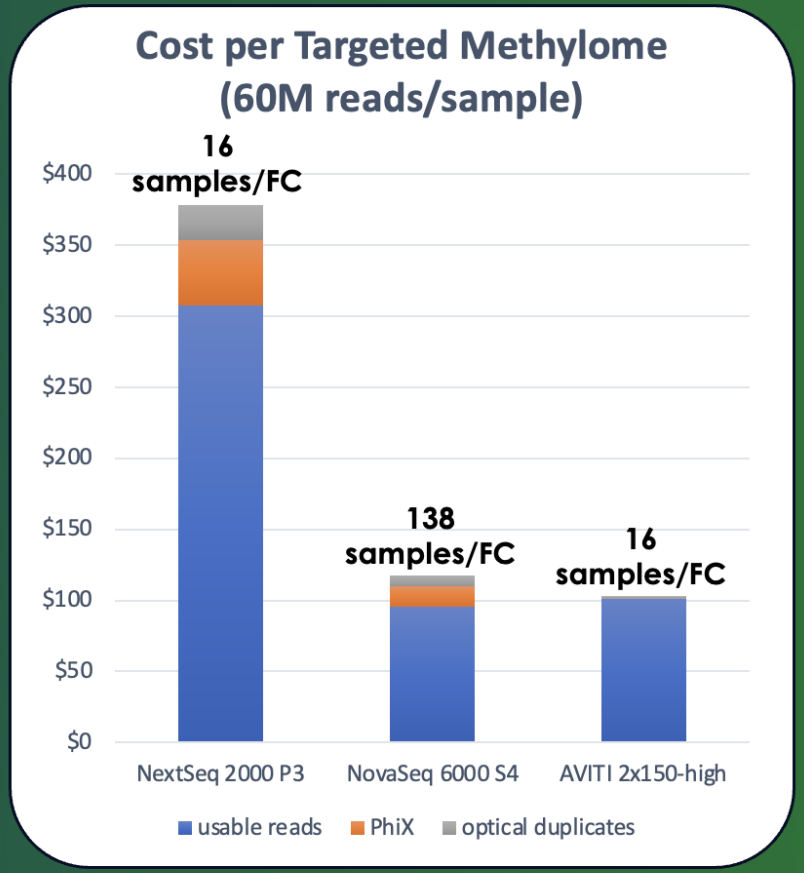
DNA methylation analysis

Translating technical benefits into cost savings

Reducing read allowance to exogenous controls adds to cost advantage over Illumina platforms without the need for high sample plexity





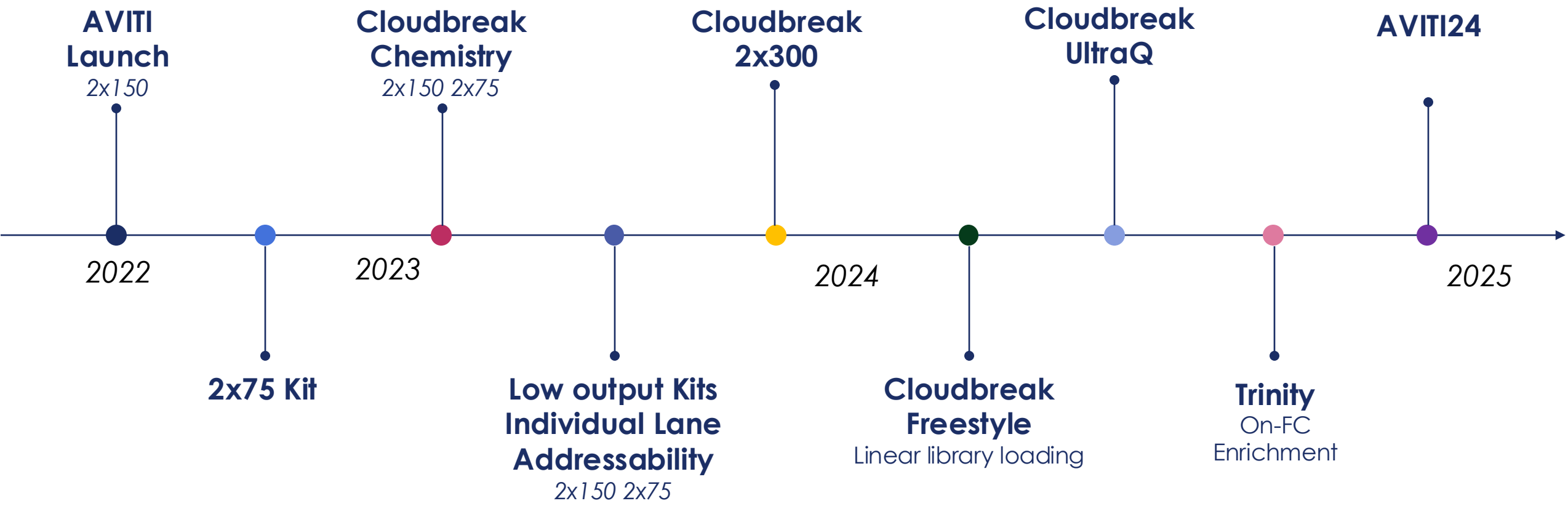
Estimating 15% PhiX required for Illumina platforms and 8% loss of reads due to optical duplicates

Outline

- Flexibility, Higher Quality and Low Cost
- Superior Performance
- Applications
- Upcoming Innovations

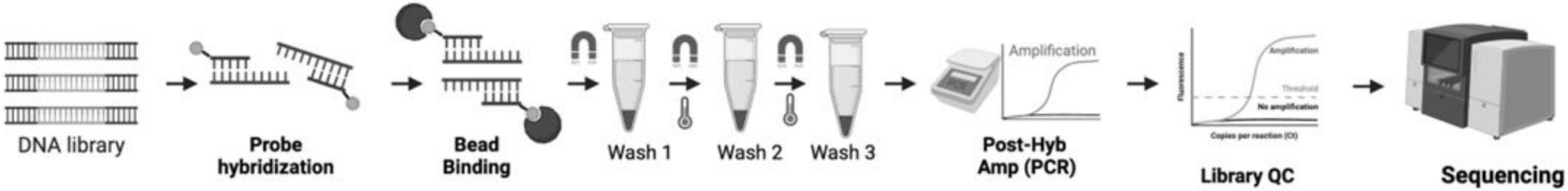
For research use only

AVITI past, present, future



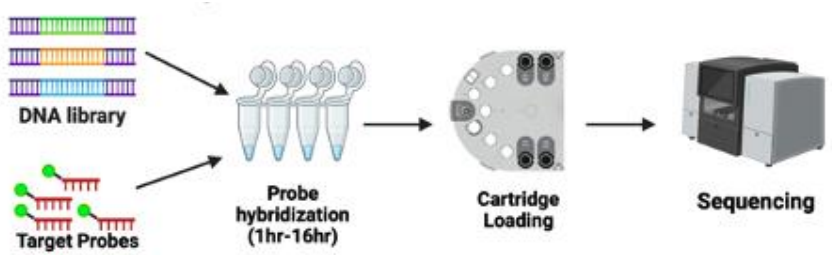
Trinity: On-flow cell targeted sequencing

Before

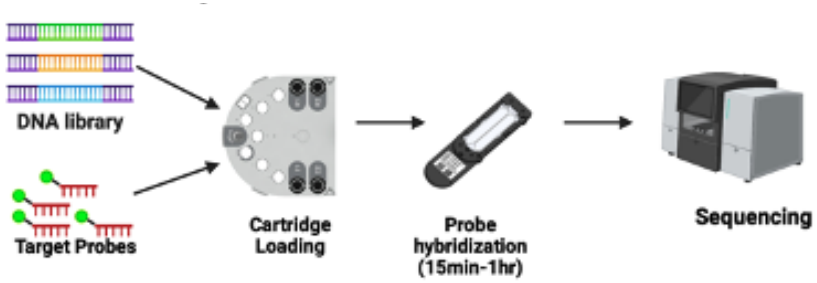


After

Trinity



Trinity Plus



- Lower sample input
- Lower bait requirements and improved performance
- Minimal hand-on time (No bead handling and post-hybridization PCR)
- Coming H2 ‘2024

B73

B73xMo17

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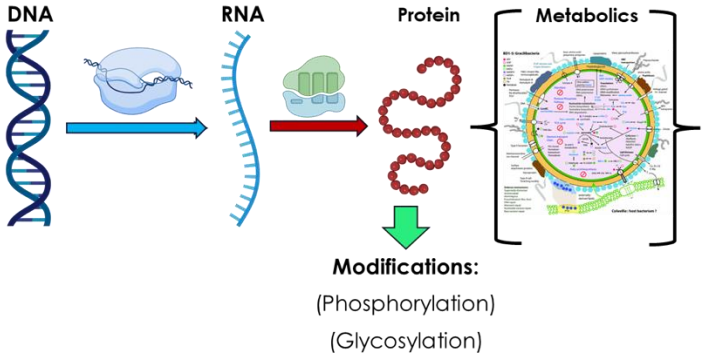
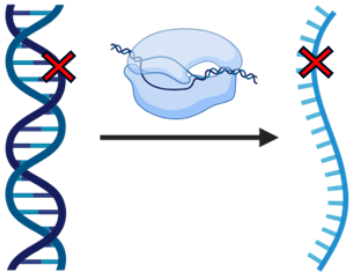
Critical Need: understand the rules that govern biology's circuits

Systems Biology assays must identity, quantity, and map multiple biological pieces at any given time

A single cell has
30B + functional molecules



19% of cancers have a
RAS mutation*

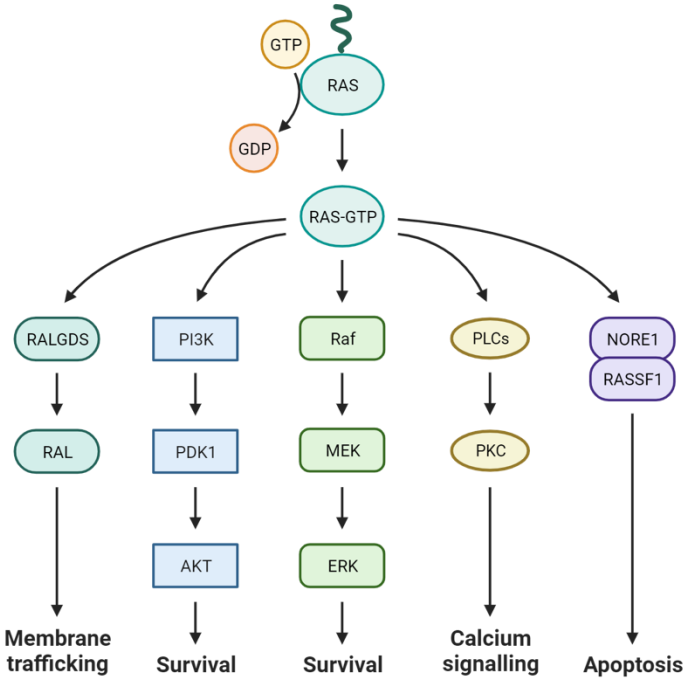


RAS expression can vary 100-fold*

Too much → senescence / cell death**

Too little → tumor formation**

Biology's complex circuits



*Prior, IA, et al., *Cancer Res* (2020) 80 (14): 2969–2974.

**Sarkisian, CJ et al, *Nat. Cell. Biol*, (2007) 9(50), 493.

AVITI24 Teton workflow enables sample to answer in 24 hours

<1 hour

24 hours

Cell Preparation	Sample Preparation	Sequencing + Primary Analysis	Data Visualization

Culture or deposit cells directly on Teton flow cell

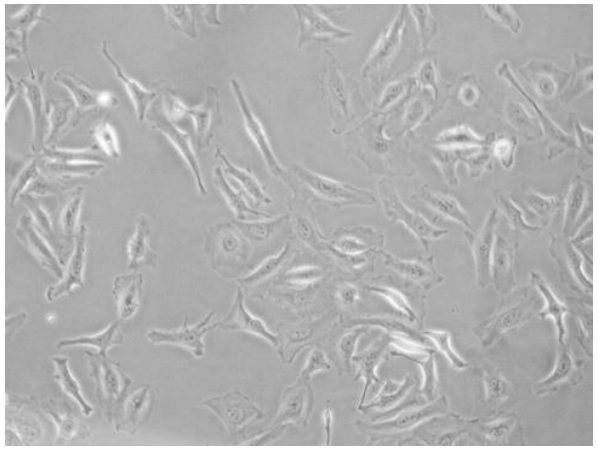
50k cells/well (12-well)
1M cells/well (1-well)

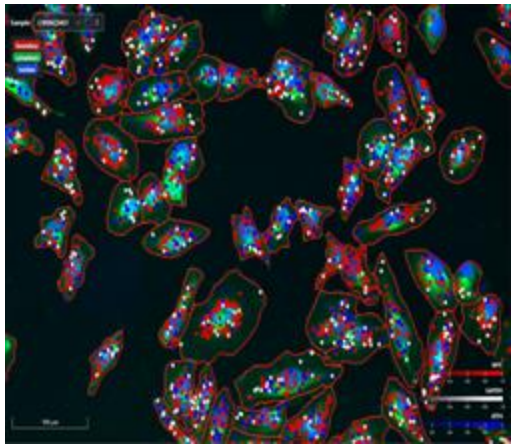
Wash, fix, and permeabilize cells

Assemble Teton flow cell

AVITI24: compatible with adherent, suspension, and targeted cell capture

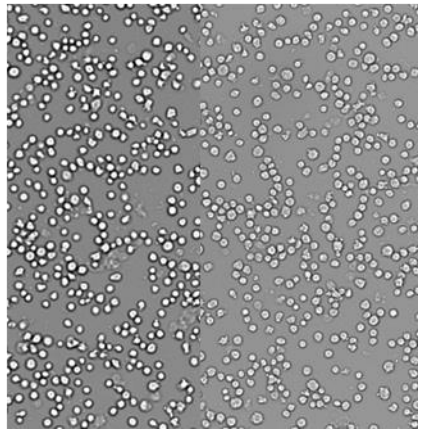
Adherent Cells



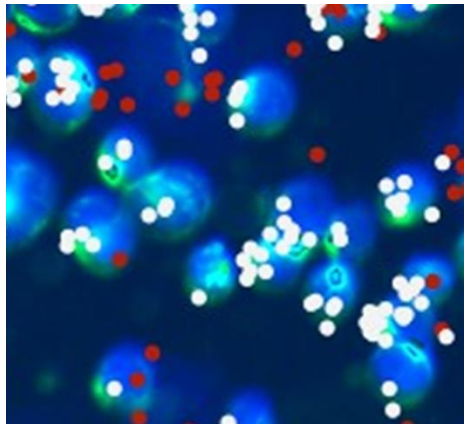


Suspension Cells

Before Wash After Wash

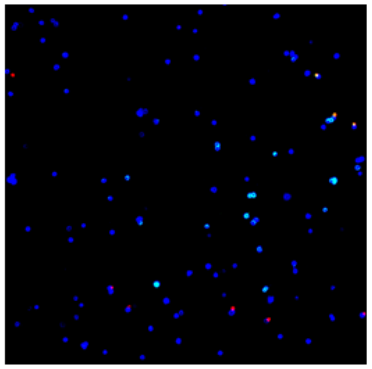


Sequencing in Suspension Cells

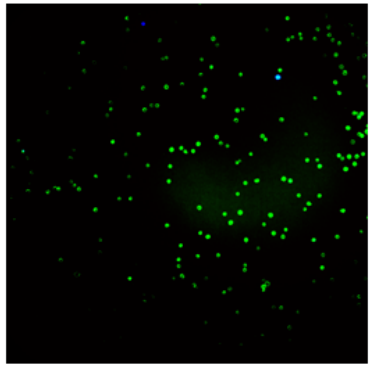


Targeted Cell Capture from PBMCs

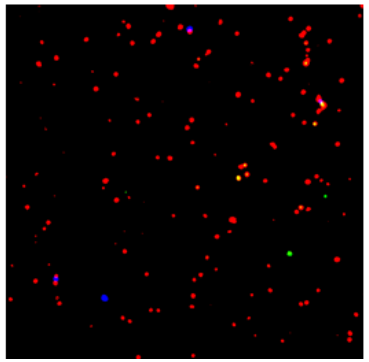
Colon Cancer Cells



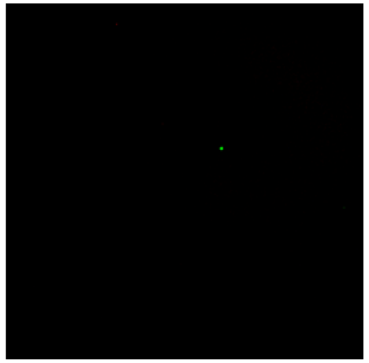
Leukemia Blast Cells



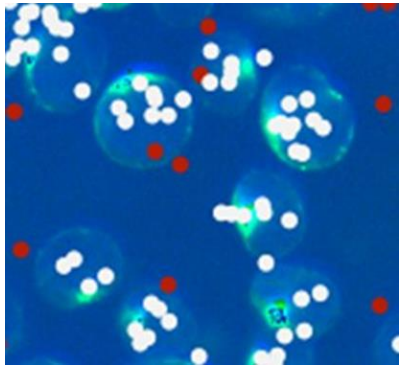
Leukemia T Cells



Negative Control

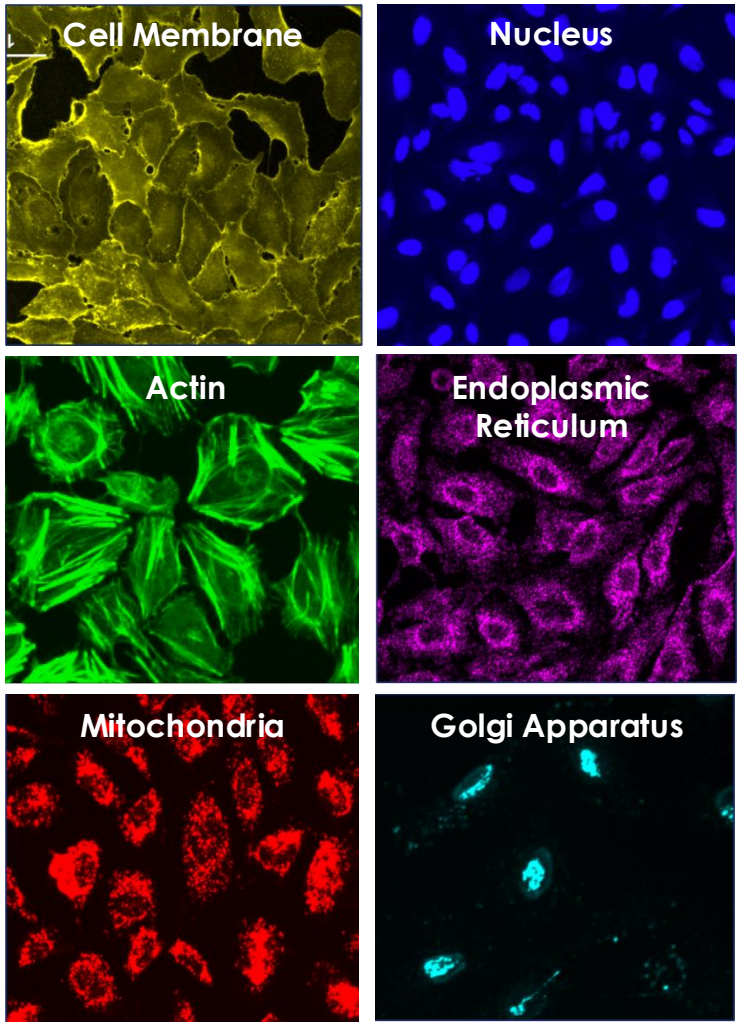


Detection in Cells

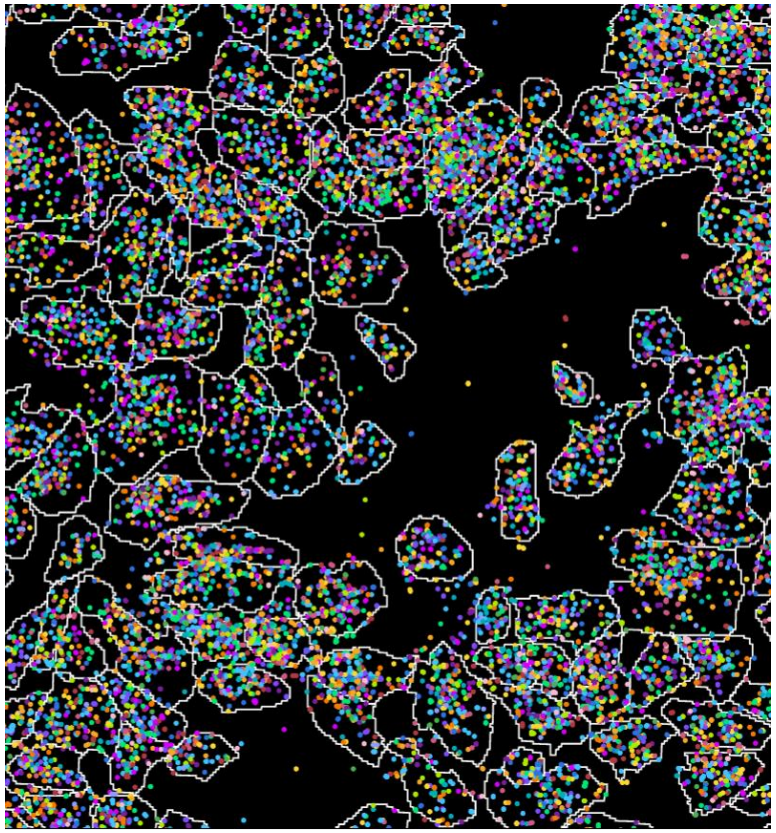


Unlocking morphology and multiomic read-outs in <24h with a single assay

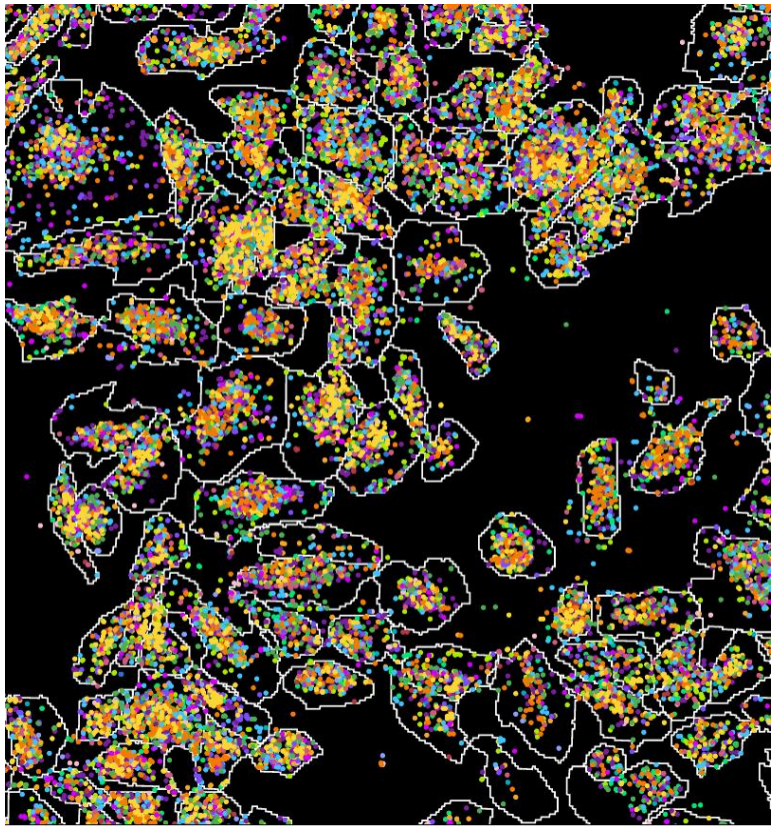
Cell morphology
6 Targets



RNA Detection
350+ Targets



Protein Detection
50+ Surface, Intracellular, & Phosphorylated Proteins



Available at Launch: MAPK Cell Cycle + Apoptosis Panels

Coming in 2025: Immunology, Neuro, Custom

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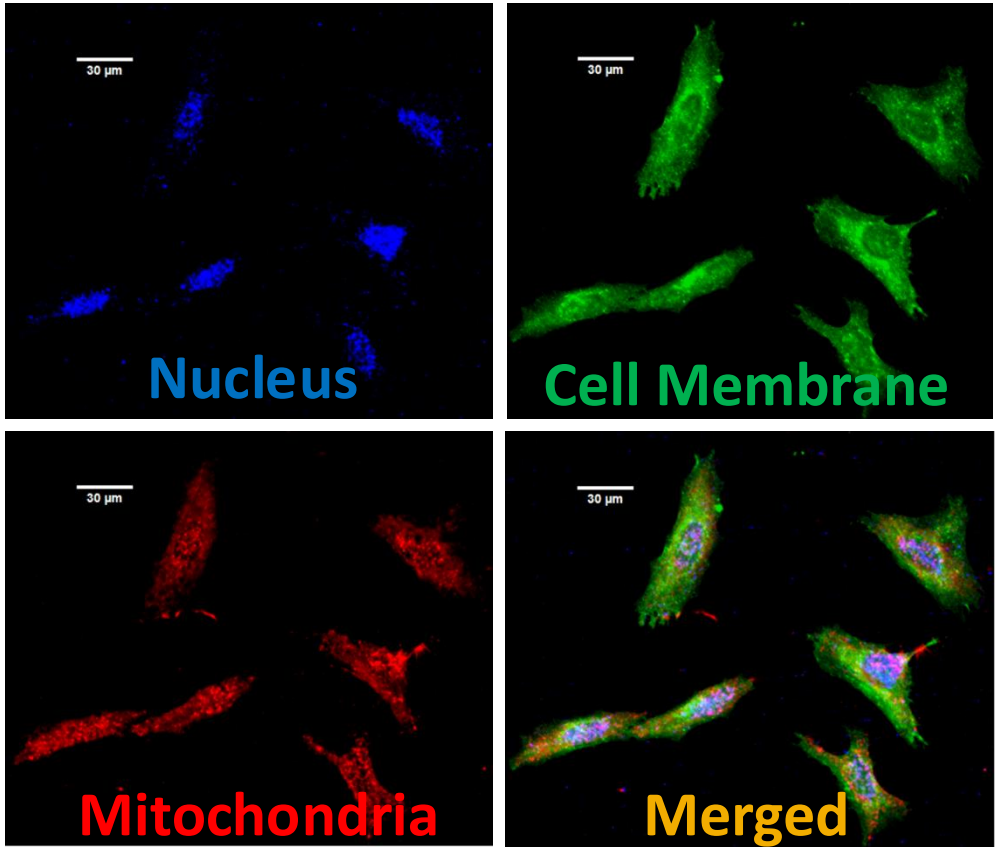
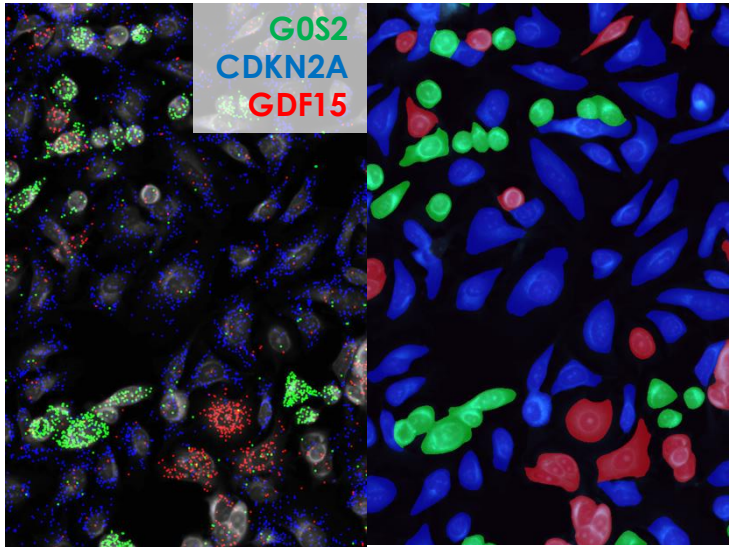
AVITI24: proprietary morphology detection with Element ABC cell paint

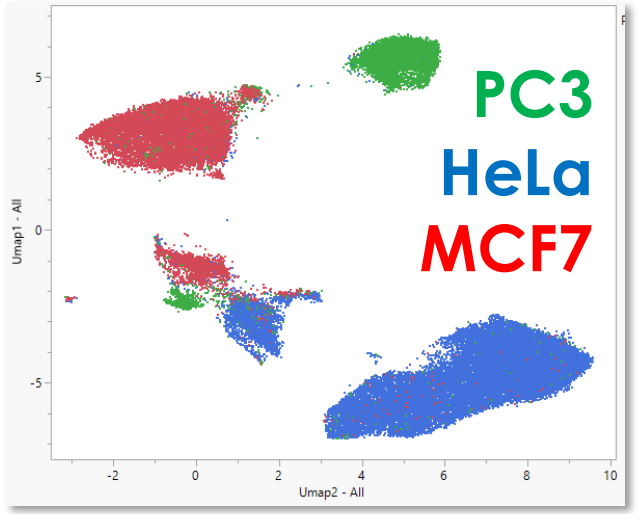
Element Proprietary Cell Paint on AVITI24

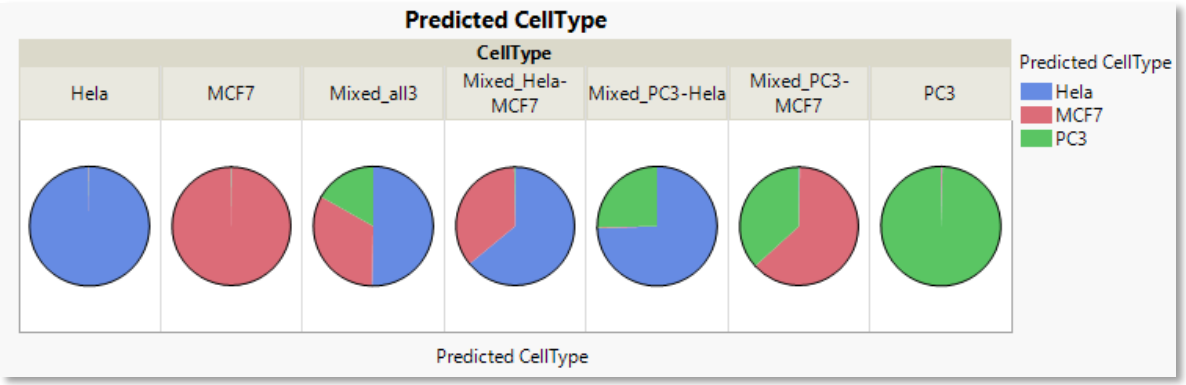


Up to 20 features simultaneously

Cell Paint powered sorting from cocultures* on AVITI24







*Cocultured 3 different cell types and AVITI[24] separated cell types

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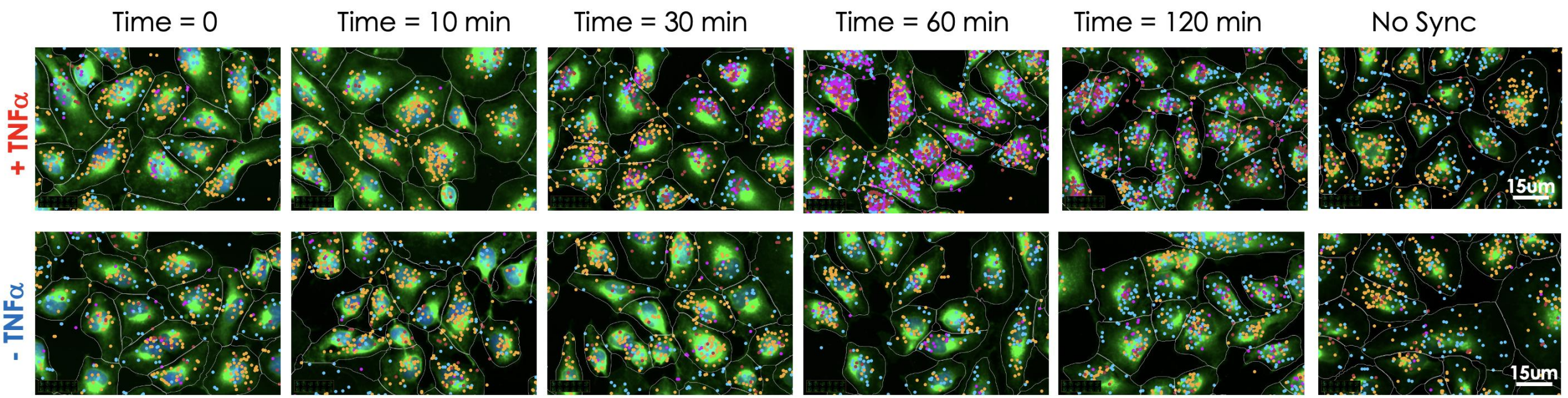
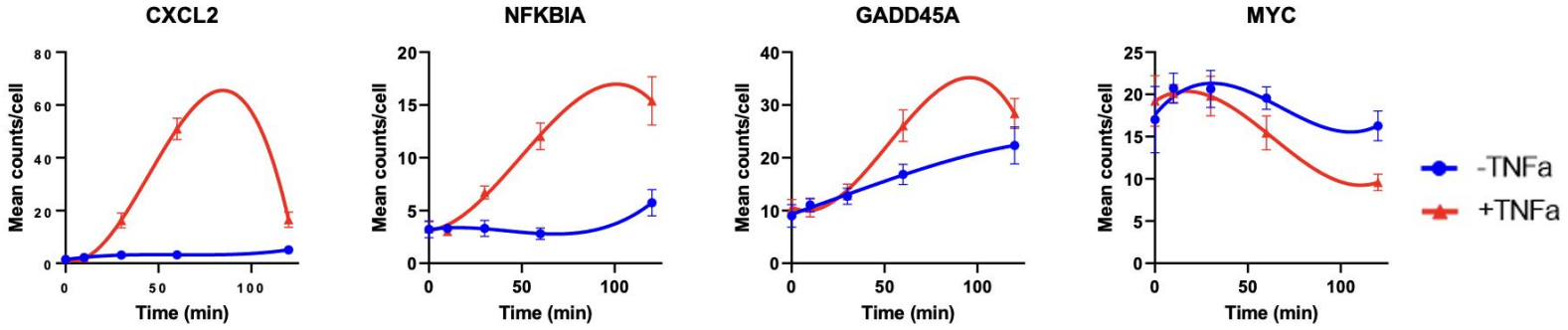
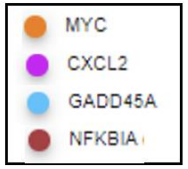
RNA detection results: cell synchronization assay







MYC
CXCL2
GADD45A
NFKBIA

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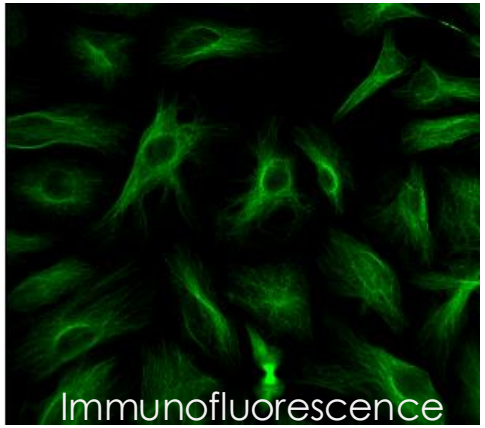
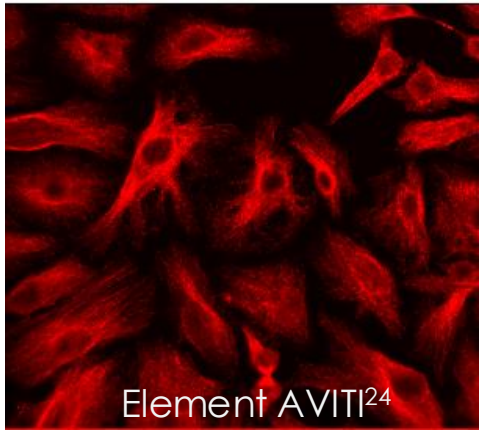
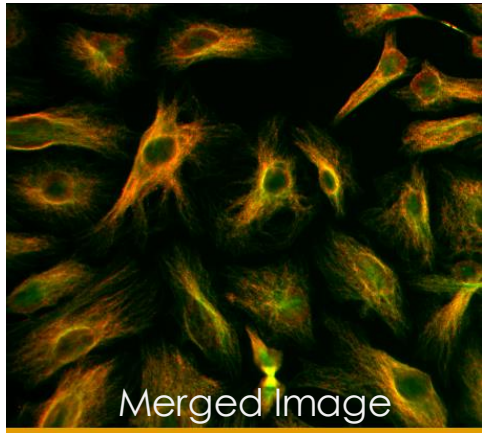
Teton measures 50+ cell surface, intracellular, and phosphorylated proteins

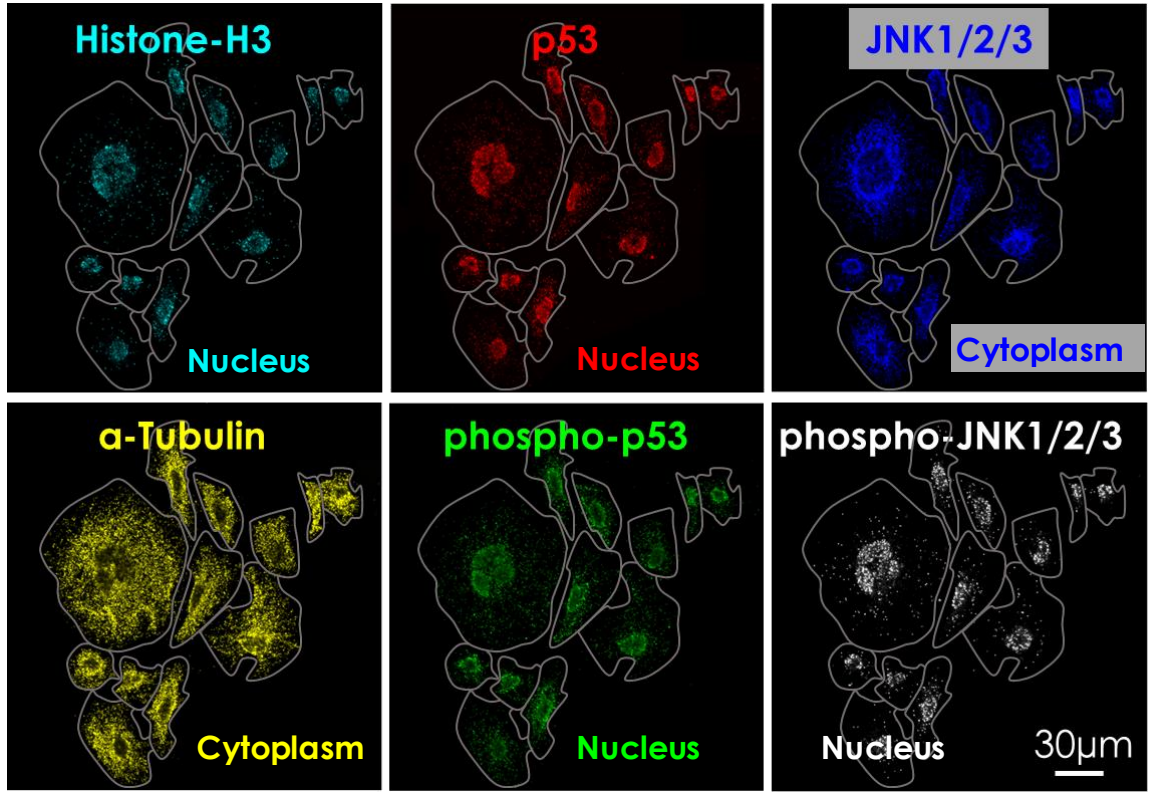
Immunofluorescence vs. AVITI24







Protein Localization (AVITI24)



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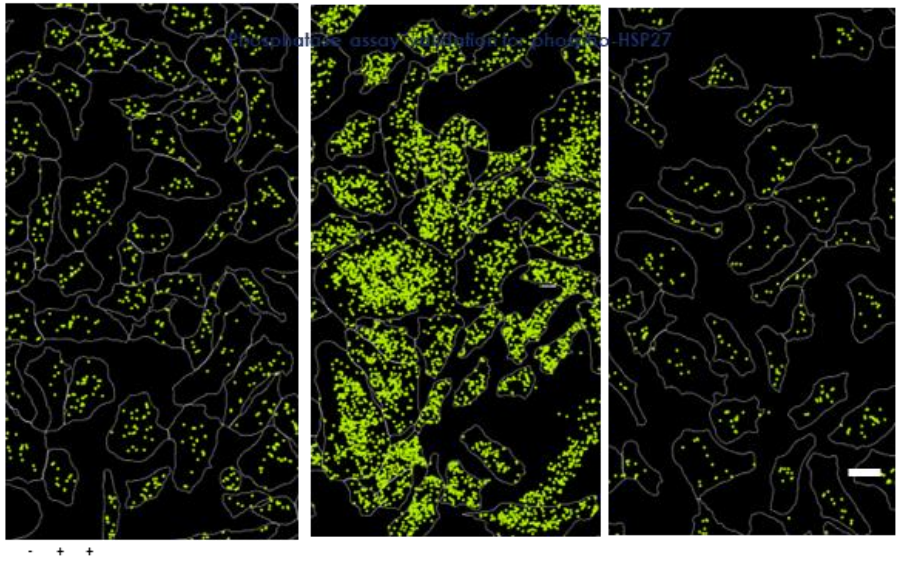
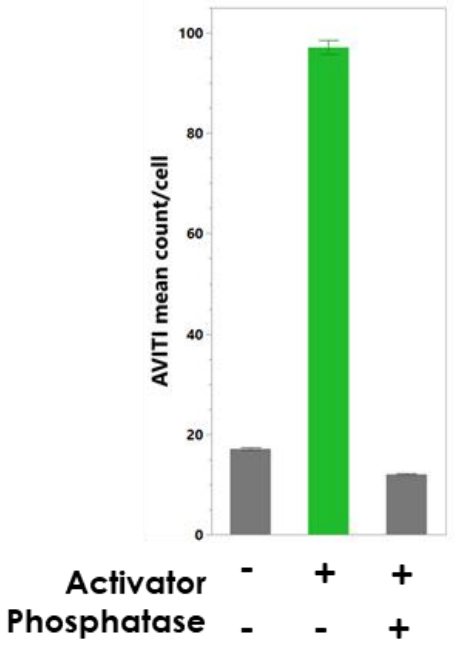
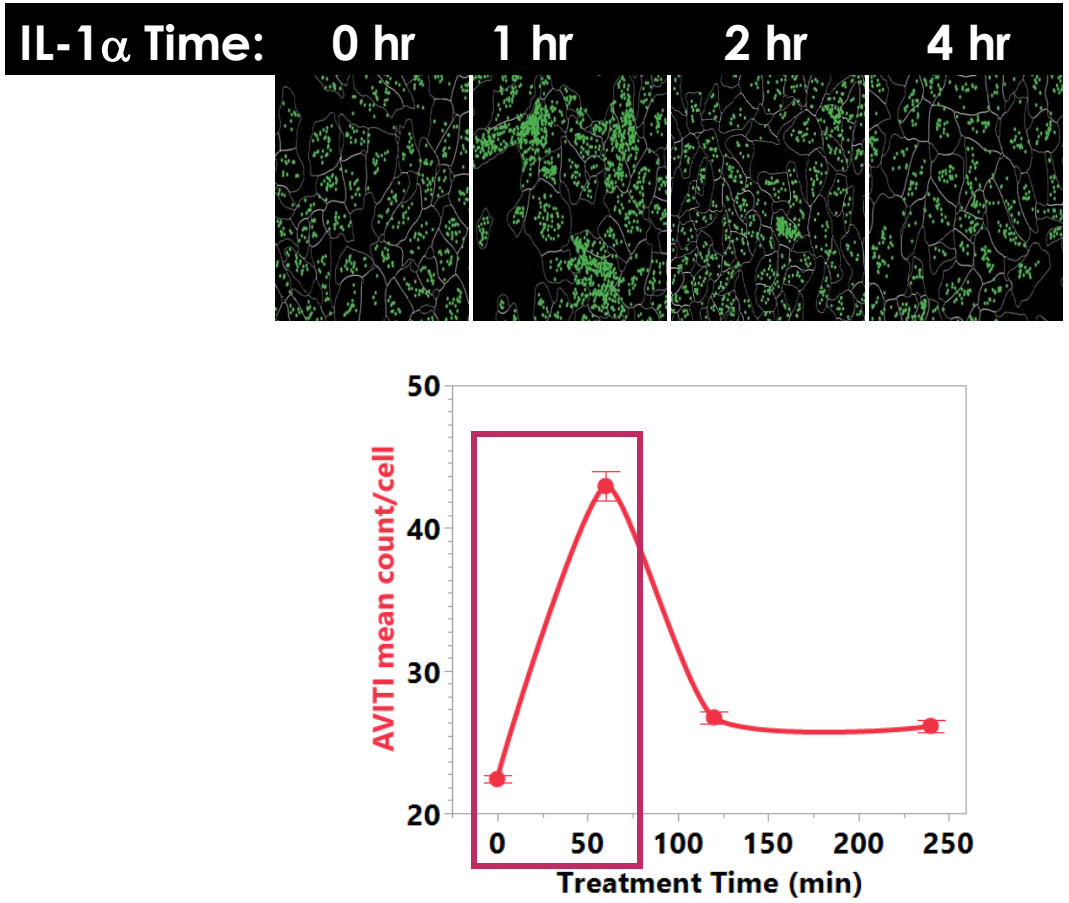
Phosphorylated protein detection at biologically meaningful timescales



Wild Type HeLa | Activation by anisomycin | Activation + phosphatase

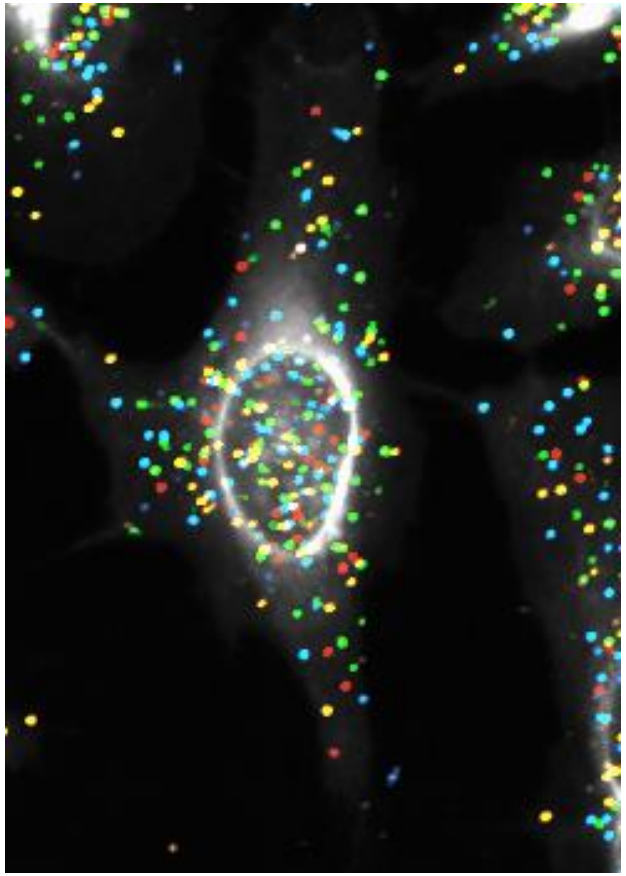




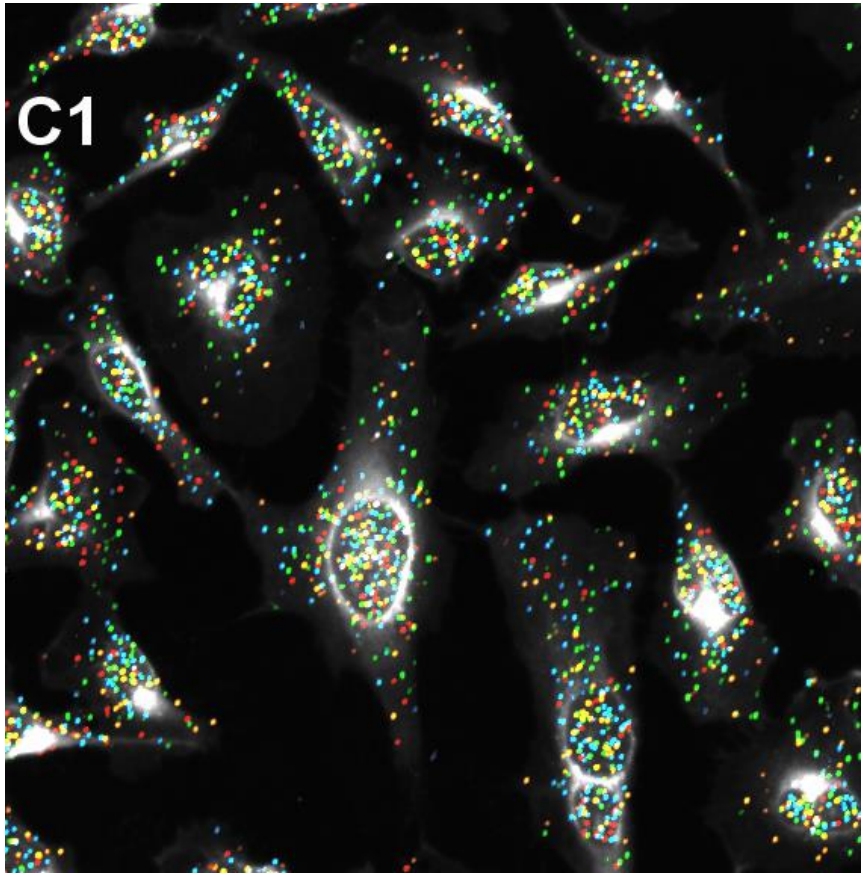
In Vitro 3D ABC Sequencing (2025)

100 cycle zoomed in region of targeted sequencing region

Single Cycle 3D Stack



Zoomed in 2D Projection 3D Images
(100 cycles)



Applications:

- Untargeted Transcriptome
- Optical Pooled Screens (guide RNA-seq)
- TCR/BCR-seq in situ
- Expressed SNP detection
- and more!

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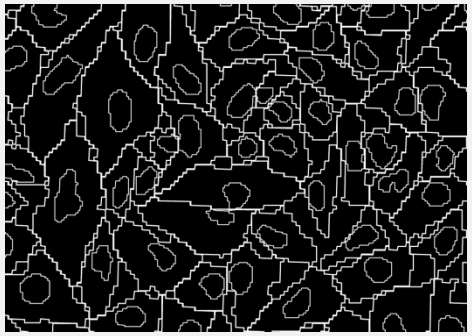
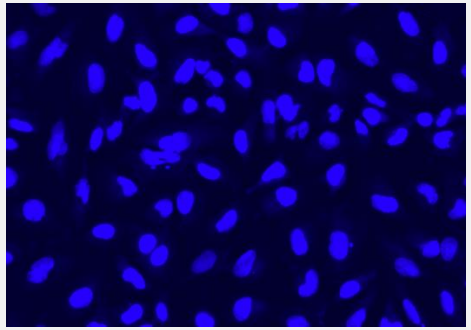
AVITI24 Teton software accelerates the data analysis journey

Onboard Primary Analysis

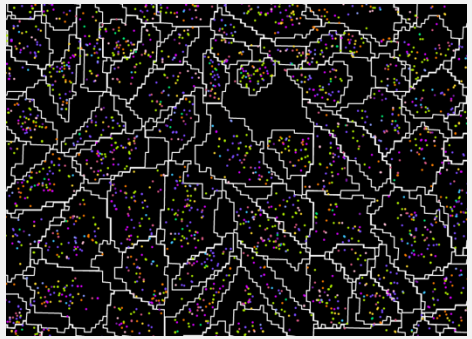
Real-time processing for ready-to-analyze data in 24h



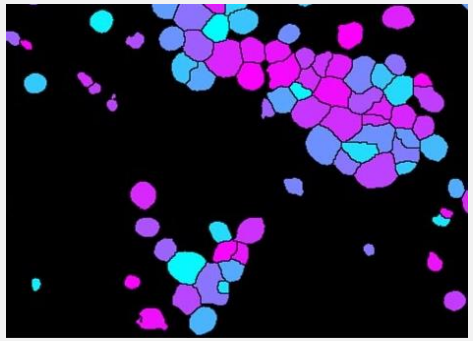
Cell Segmentation



Intensity Quantification (cell paint)



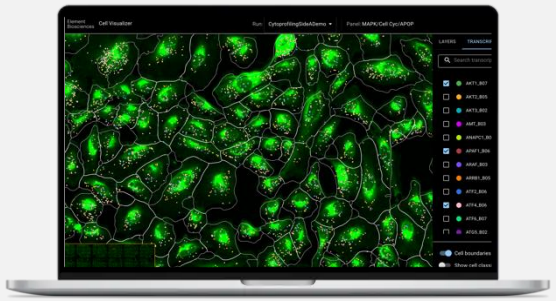
Cell Assignment (transcripts/proteins)



Morphology Feature Extraction

Offboard Analysis

Interactive data visualization and analysis



Element CytoCanvas Visualization Software

	gene1	gene2	...	geneN
Cell1	3	1	.	13
Cell2	2	14	.	18
...	.	.	.	.
CellN	25	0	.	2

Open data format for easy integration into community tools

AVITI24: an integrated platform for NGS and CytoProfiling

Core Sequencing:

- **Accurate:** reach Q50 data with Cloudbreak UltraQ kit
- **Flexible:** dual independent flow cells with two lanes
- **Compatible:** direct loading of linear libraries with Cloudbreak Freestyle
- **Simplified:** on-flow cell enrichment with Trinity
- **Affordable:** 20-70% more reads per run at no extra cost with Expert Mode HD

AVITI24™

Teton CytoProfiling:

- **Multiomic:** RNA, protein, 300bp seq, morphology
- **Subcellular:** 200nm res.; multi-feature cell segmentation
- **Sensitive & Specific:** with Avidity Base Chemistry (ABC)
- **High Throughput:** up to 1 million cells with 10cm2 usable area in <24 h; 2 concurrent runs (20cm2 total)
- **Versatile Inputs:** on-flow cell cultured cells or fixed cell suspensions

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AVITI24 TAP: acceptance criteria and what is supported

TAP Program Requirements

- Be **registered** on TAP website and willing to accept our **terms and conditions**
- Be willing to **pay for TAP** → processing of **one12-well** Teton Flow cell run
- Submit **adherent human cells**
- Use Element **MAPK panels**; no customization
- Be able to perform **own data analysis**
- Be tied to an **instrument upgrade/purchase decision**

Project Criteria

Adherent Human Cell Lines

- HeLa
- HEK293
- HUVEC
- PC3
- HepG2
- MCF7

Internally tested

Slide surface

- Poly-L-Lysine coated (PLL)

Supported format/density

- 1, 12-well FC (0.5cm²/well)
- Up to 50,000 cells

Plexity and targets supported

- RNA – 350 targets
- Protein – 50 targets
- Cell Paint – 6 targets

Quotable PN	TAP Service	List Price
992-00100	TAP Service: Teton Human MAPK & Cell Cycle, PLL, 12 wells	$9,980
992-00102	TAP Service: Teton Human MAPK & Apoptosis, PLL, 12 wells	$9,980

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