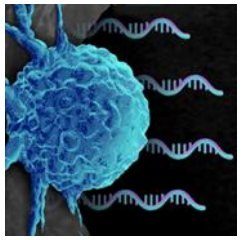
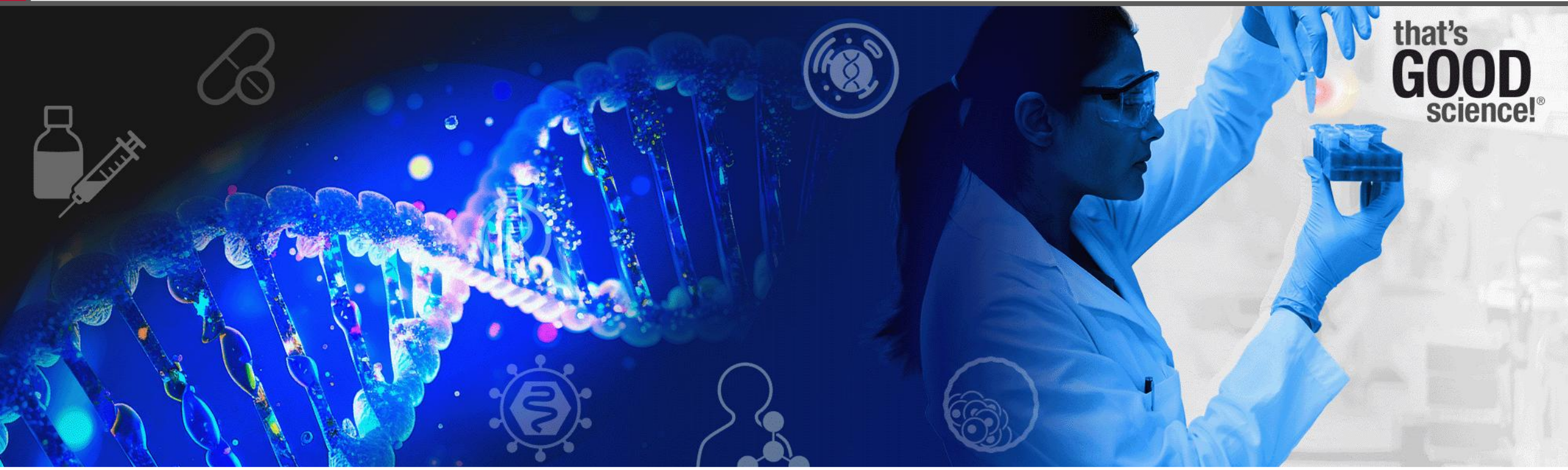


Empowering discovery with automated total RNA and WGA sequencing

Shuwen Chen, PhD, Senior Product Manager

that's GOOD science!®

Takara Bio: core capabilities



NGS

PCR, qPCR, RT-PCR

Cloning

Nucleic acid purification

Gene delivery

Functional genomics

Protein expression & purification

OEM

The power of sensitivity and full gene-body coverage

The template-switching method used in our SMART-Seq® kits allows for:

Better detection of SNVs

Log-transformed counts of SNVs were detected using different sequencing platforms.

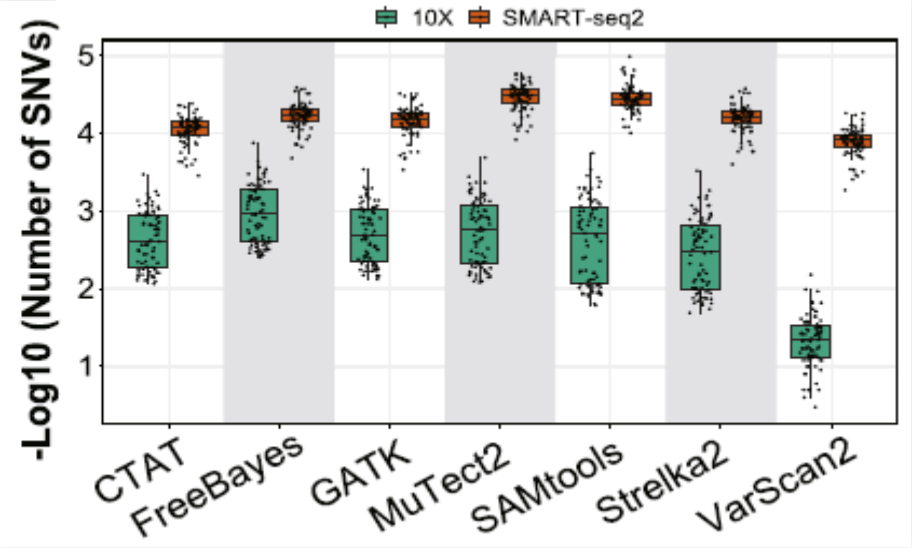


Figure adapted from ”Systematic comparative analysis of single-nucleotide variant detection methods from single-cell RNA sequencing data” (Liu et al. 2019, Genome Biol) under a CC BY 4.0 license.

Discovery of alternatively spliced isoforms

Employing SMART-Seq chemistry, the **Allen Institute for Brain Science** published a preprint paper, later published in *Nature*.

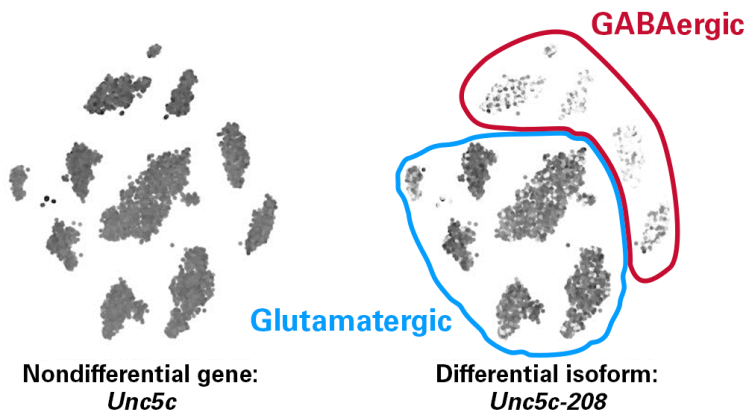
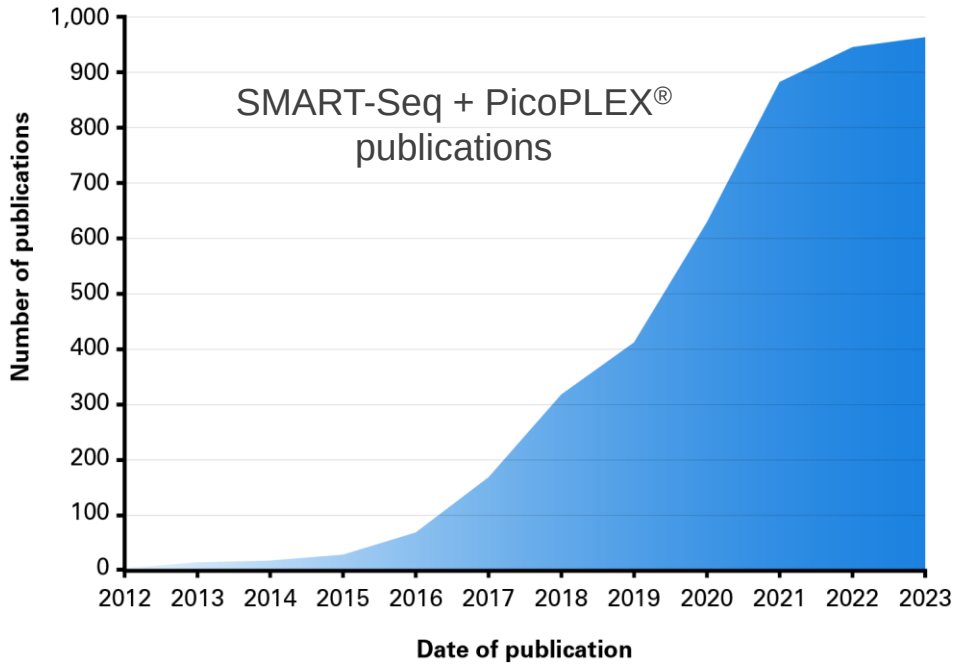


Figure adapted from “Isoform cell type specificity in the mouse primary motor cortex” (Booeshaghi et al. 2020, bioRxiv) under a CC BY 4.0 license.

The desire for scale has grown

Takara Bio single-cell solutions maintain sensitivity and have increased in popularity



Advances in scale of scRNA-seq over the years sacrifice sensitivity and detection of biomarkers

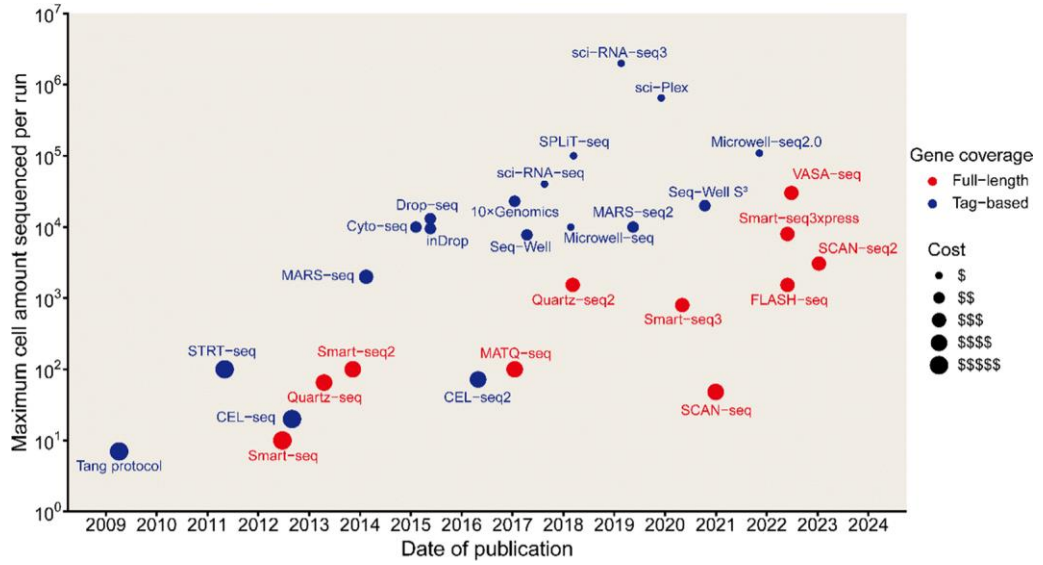
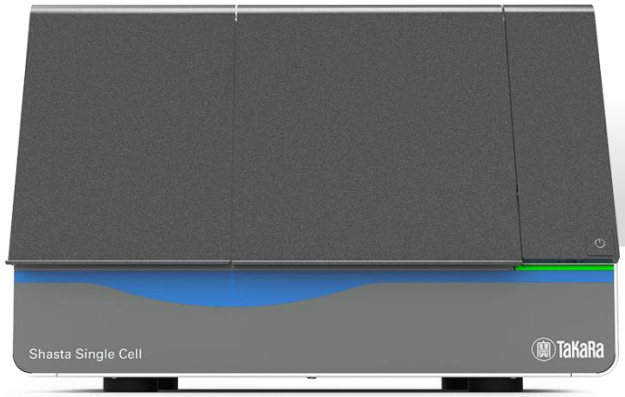


Figure adapted from "Advances in single-cell RNA sequencing and its applications in cancer research" (Huang et al. 2023, *J. Hematol. Oncol.)* under a CC BY 4.0 license.

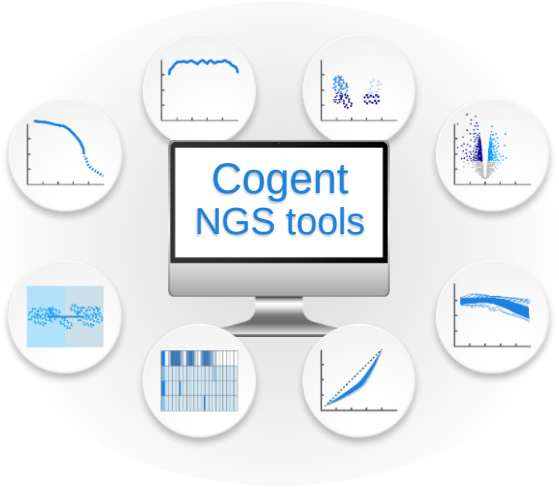
Next-generation single-cell biomarker discovery, scaled

Shasta™ Single Cell System



Automated dispensing

+



Cogent™ NGS Analysis Pipeline and Discovery Software

New chemistries

Shasta Total RNA-Seq Kit

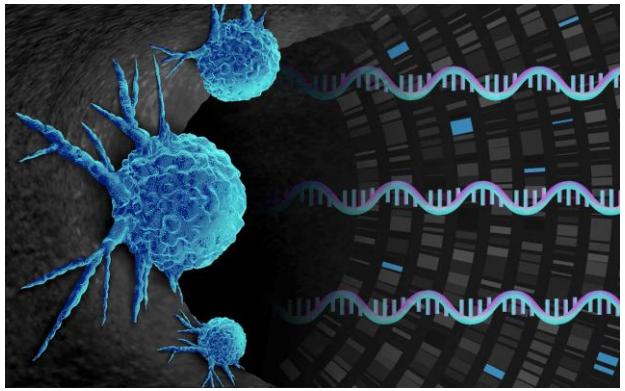
- Analyze up to 100,000 single cells per run
- Achieve outstanding sensitivity and full gene-body coverage
- Uncover multiple RNA biotypes
- Detect splicing isoforms and gene fusions

Shasta Whole-Genome Amplification (WGA) Kit

- Analyze up to 1,500 single cells per run
- Profile copy number variation (CNV) and single-nucleotide variation (SNV) data, including chromosomal aneuploidies
- Resolve tumor heterogeneity and track clonal evolution with user-friendly bioinformatics tools

Shasta Total RNA-Seq: overview

Shasta Total RNA-Seq Kit



Two-day workflow
Reduced hands-on time and reagent costs

Full gene-body coverage
Detect gene fusions and splicing isoforms

Random-priming-based RT

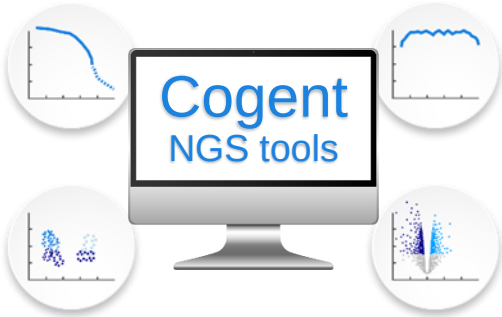
Shasta Single Cell System

High-throughput automation
Approximately 100,000 cells per run

Low doublet rate for more accurate data analysis

Up to 12 different samples per experiment

Cogent NGS tools



Free analysis tools
Protein-coding and noncoding gene pipelines

Publication-quality figures

Case study: discovering biomarkers regulated by p53 and epitherapy treatment

Four cell samples

From **Dr. Ting Wang's lab** at Washington University School of Medicine in St. Louis

p53 wild type (WT)

p53 wild type + epitherapy treatment (WT_T)

p53 knockout (KO)

p53 knockout + epitherapy treatment (KO_T)

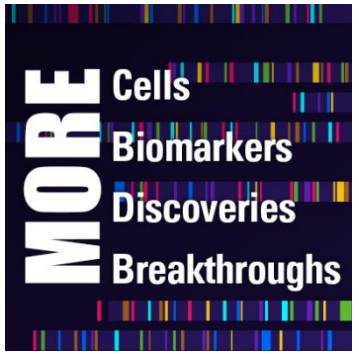
Created with BioRender.com

Shasta Total RNA-Seq Kit

Cogent NGS tools

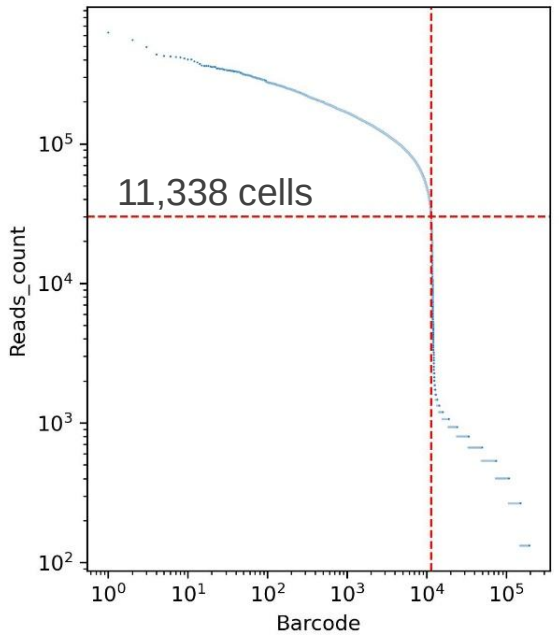
Standard analysis
lncRNA analysis

Biomarker discovery

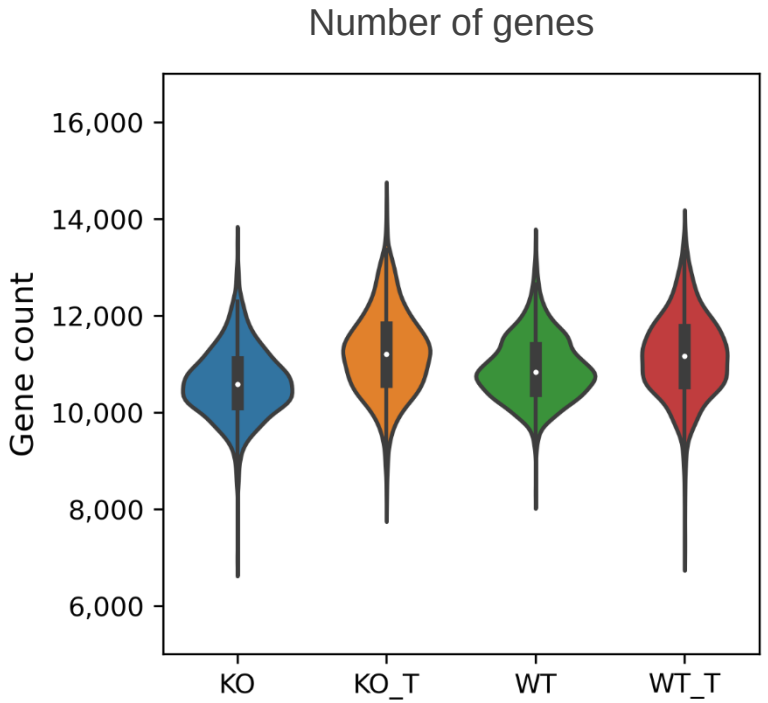


Achieved outstanding sensitivity for both genes and transcripts

Analyzed ~11,000 cells

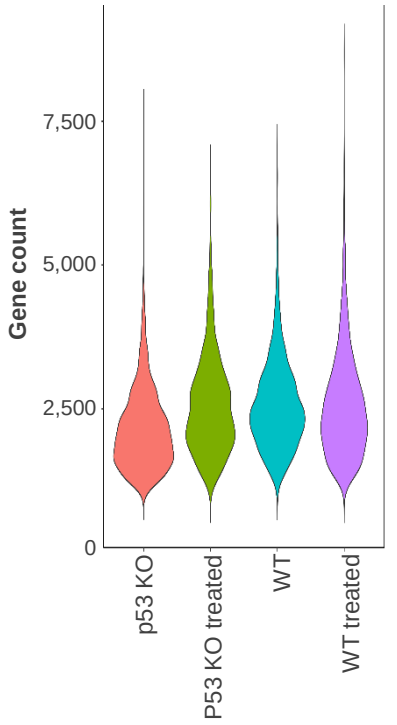


Detected ~10,000 genes and ~40,000 transcripts per sample at a sequencing depth of 100,000 reads/cell

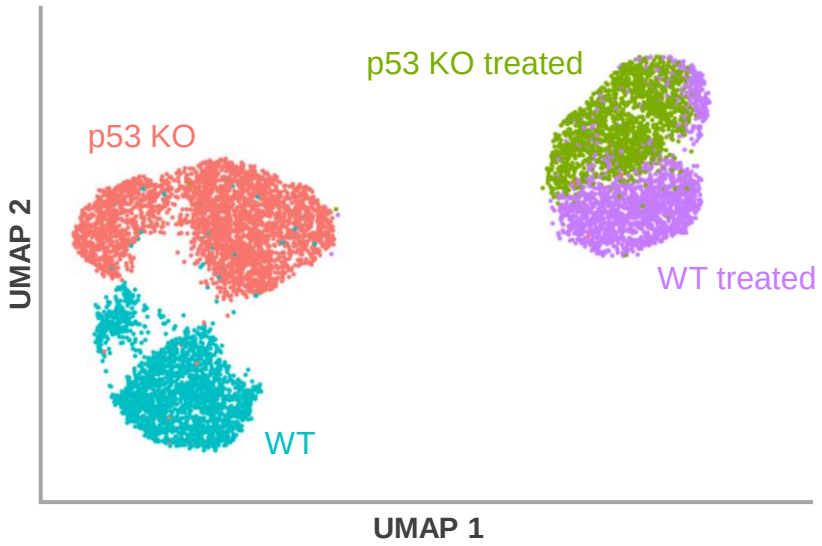


Cellular phenotype associated with differentially expressed lncRNAs

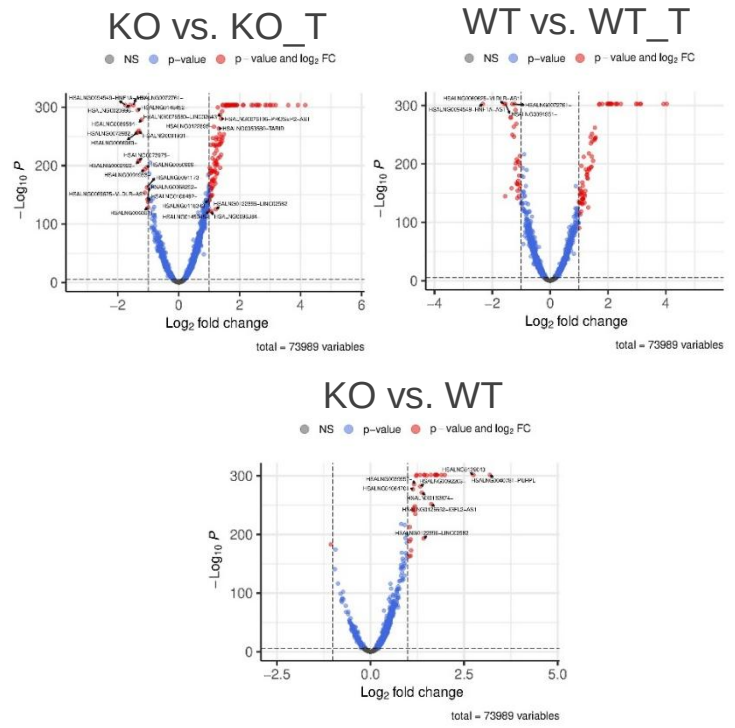
Detect lncRNA with high sensitivity



Map and group cells by lncRNA expression

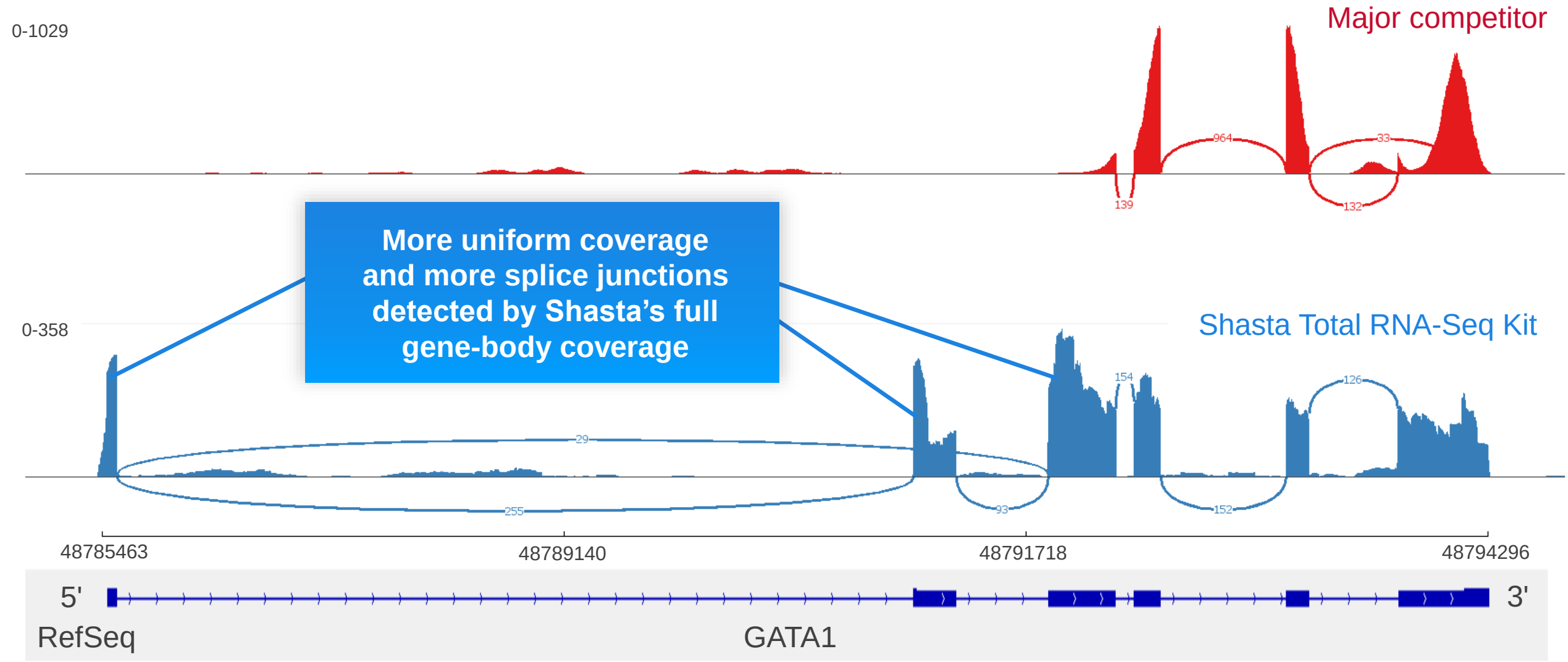


Identify differentially expressed lncRNAs



lncRNA: analysis done using Cogent NGS Analysis Pipeline (AP)

Capture all GATA1 splice junctions with full gene-body coverage by Shasta Total RNA-Seq Kit



Full gene-body coverage is more advantageous than end-counting methods for analyzing splice junctions

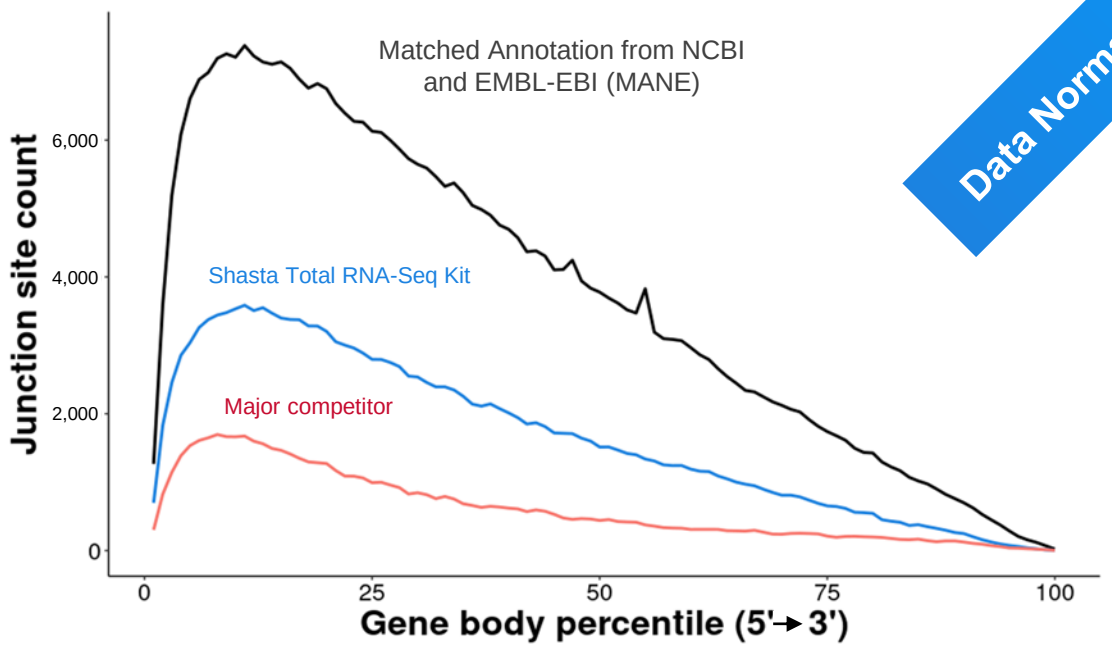
Experimental conditions

K562 cells

~20 x 10^6 read pairs of input data

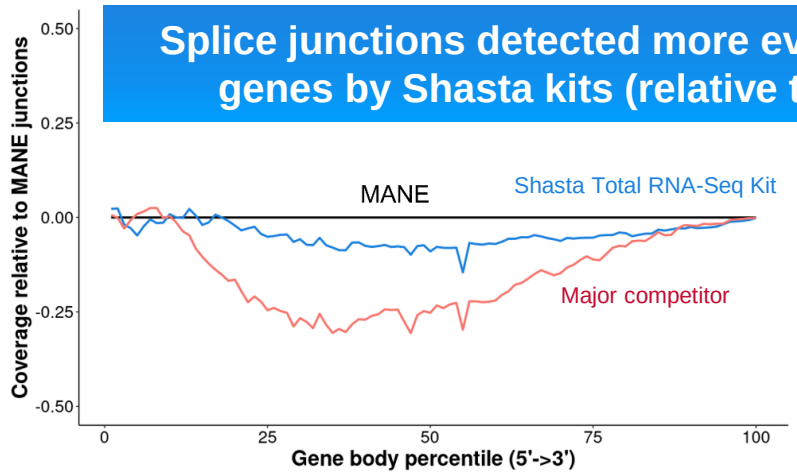
- Shasta Total RNA-Seq Kit: 1,300 cells, using full gene-body coverage
- Major Competitor: 1,500 cells, using end-counting methods

Pseudo-bulk (to avoid cell-to-cell variability effects)

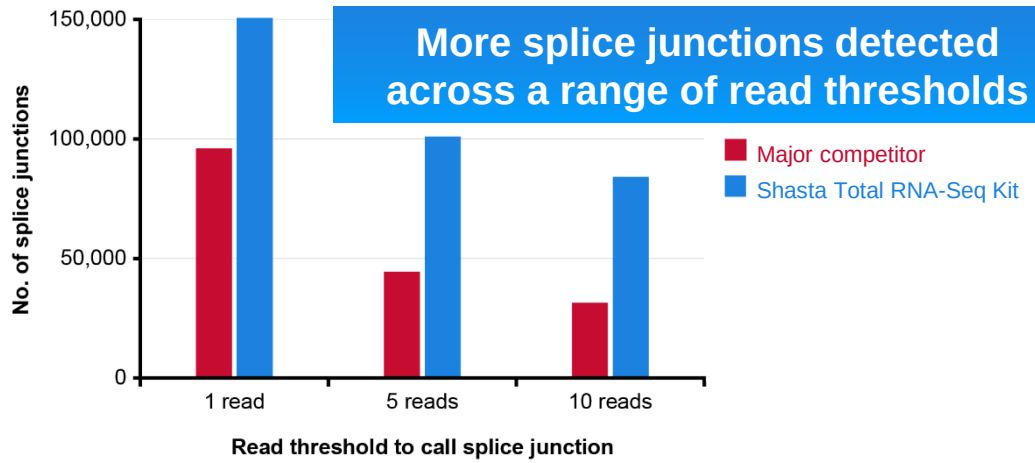


Data Normalization

Splice junctions detected more evenly across genes by Shasta kits (relative to MANE)

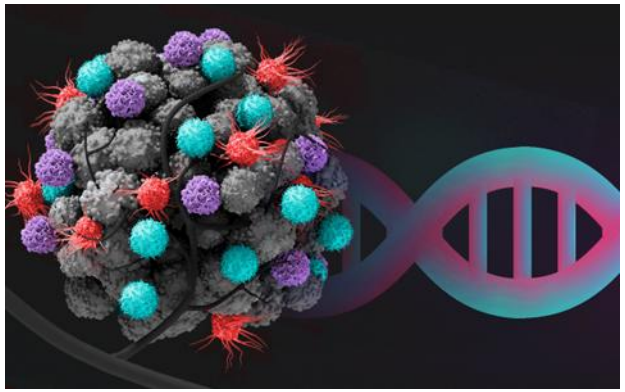


More splice junctions detected across a range of read thresholds



Shasta WGA: overview

Shasta WGA Kit



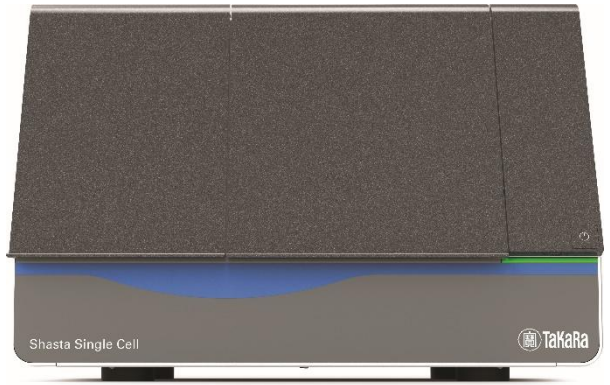
Leading chemistry for uniformity and reproducibility

Incorporates PicoPLEX WGA chemistry

Lower sequencing cost

Analyzes CNVs and SNVs at low sequencing depth

Shasta Single Cell System



Automated workflow on the Shasta instrument

Obtain library in one day

High-throughput WGA

Process up to 1,500 cells per run

Cogent NGS tools

Free analysis tools

End-to-end solution

Publication-grade figures

TaKaRa

Case study: reveal distinct copy number profiles at the single-cell level for leukemia samples

Four cell samples

From **Dr. Ilaria Iacobucci** from St. Jude Children's Research Hospital

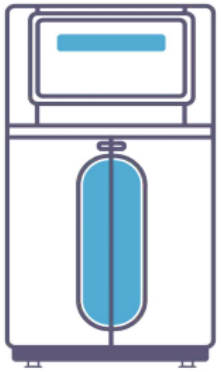
FACS

CD3+	CD45+
Normal T-cells	Leukemic blasts
S1 CD3+	S1 CD45+
S2 CD3+	S2 CD45+

Frozen bone marrow mononuclear cells from two patients (S1 and S2)

Shasta WGA Kit

Sequencing



Average ~450,000 paired-end (PE) reads/cell 2 x 76 bp

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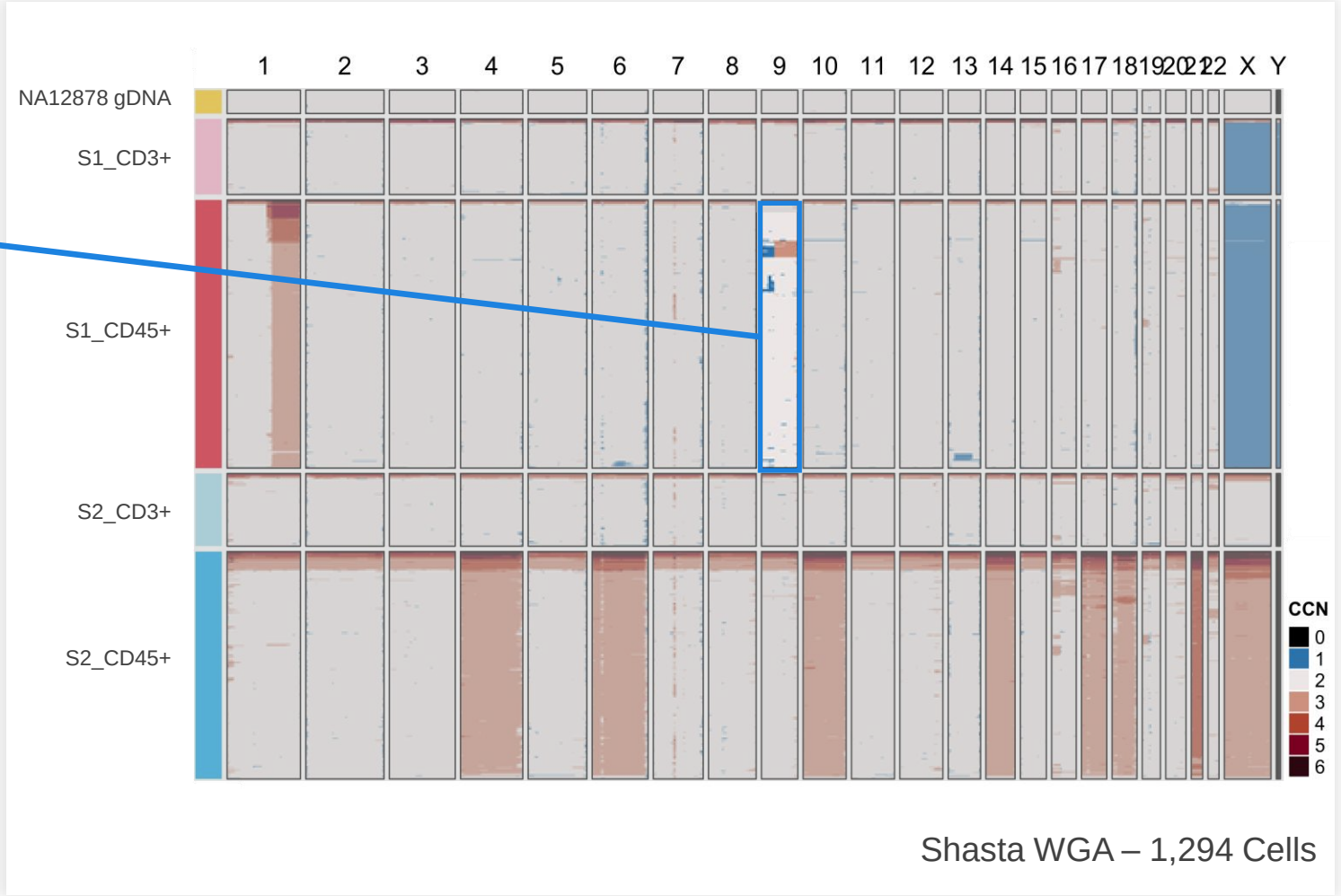
Cogent NGS tools

CNV calling for each barcode and each sample

Distinct copy number profiles revealed at the single-cell level for two leukemia samples

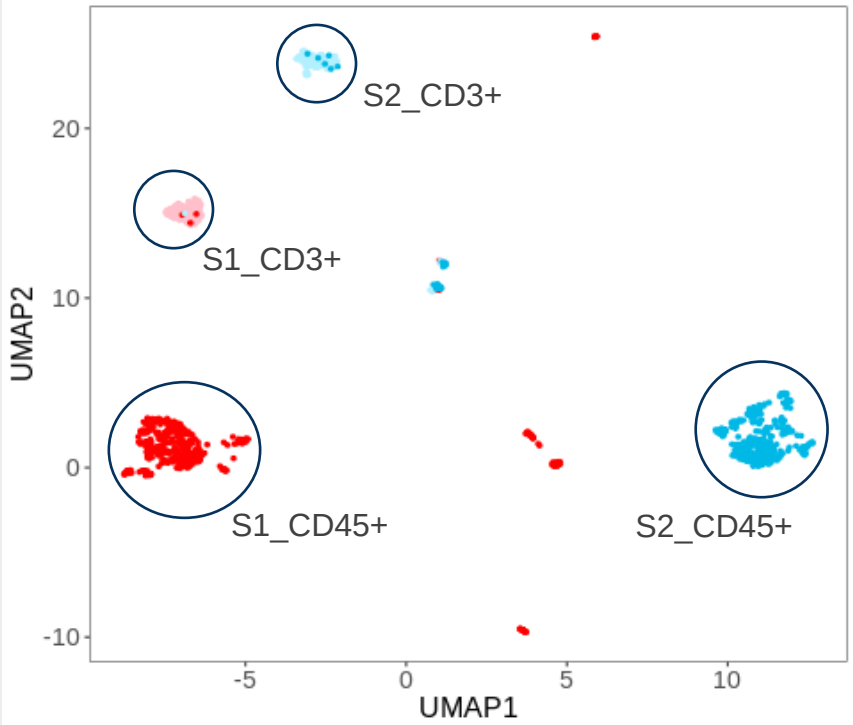
Detected a biologically interesting sub-group in S1_CD45+ with −9p and +9q

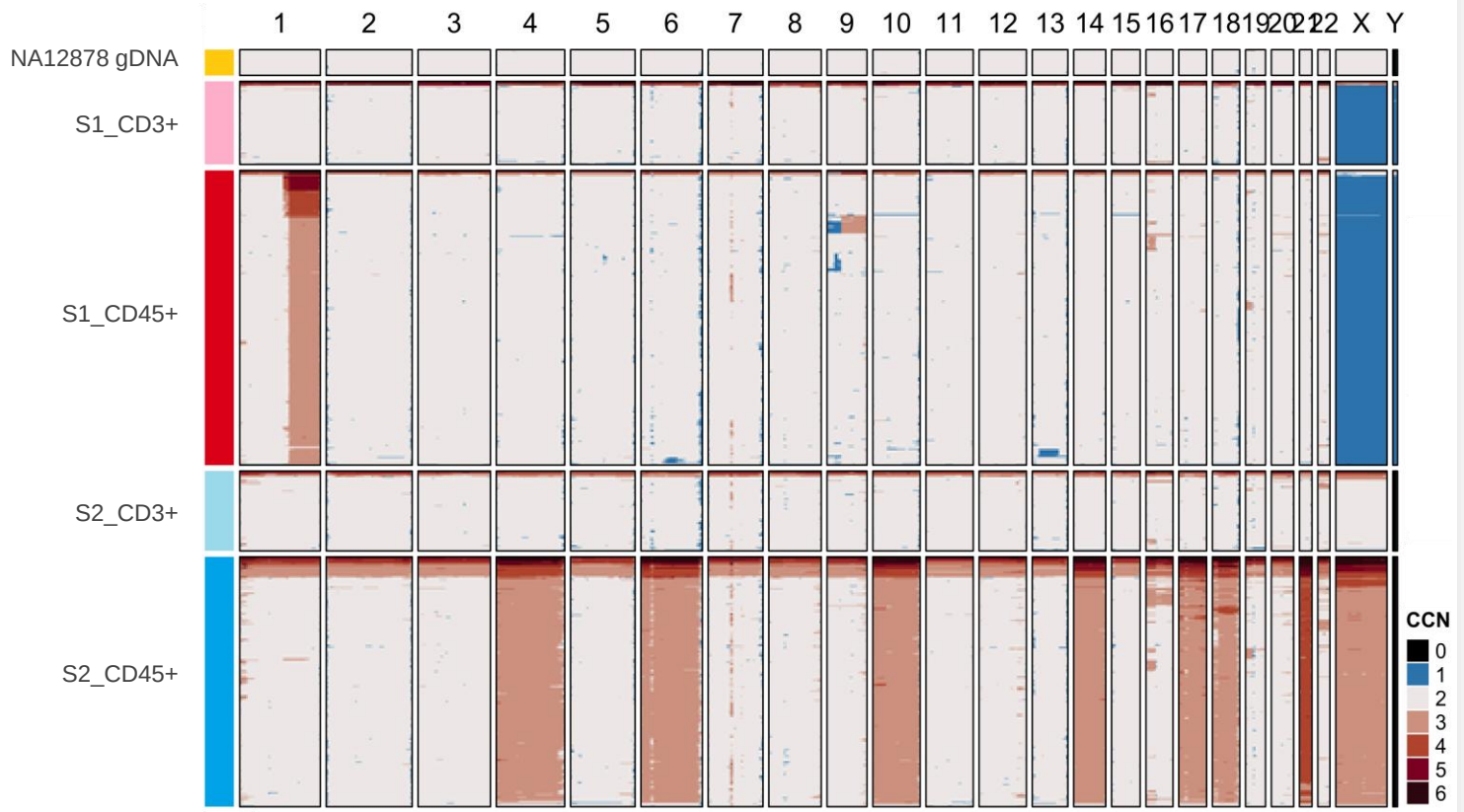


Shasta WGA – 1,294 Cells

Distinct copy number profiles revealed at the single-cell level for two leukemia samples

UMAP clustering by whole-genome CNPs

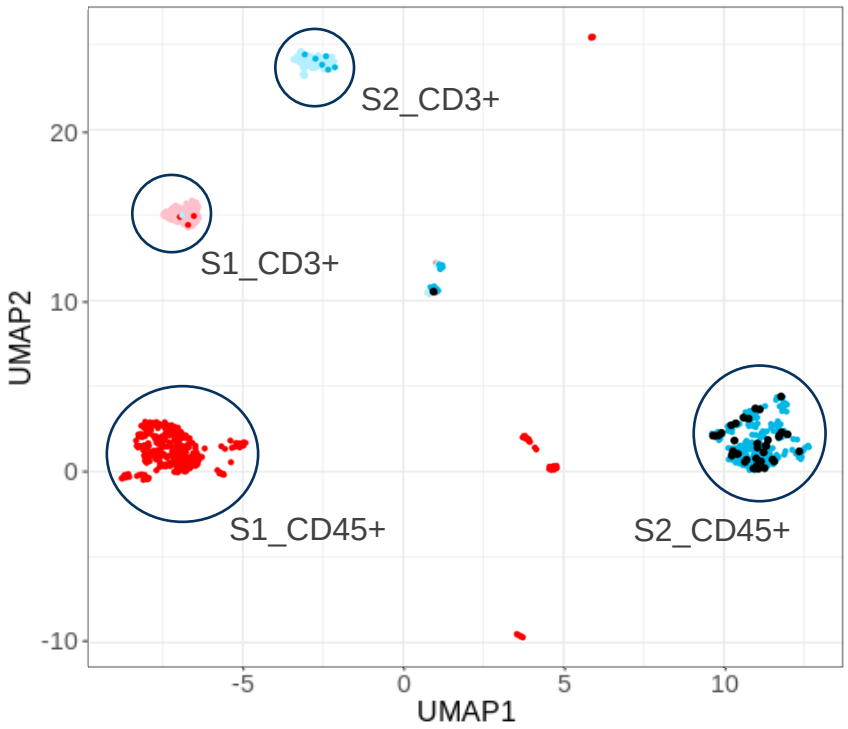




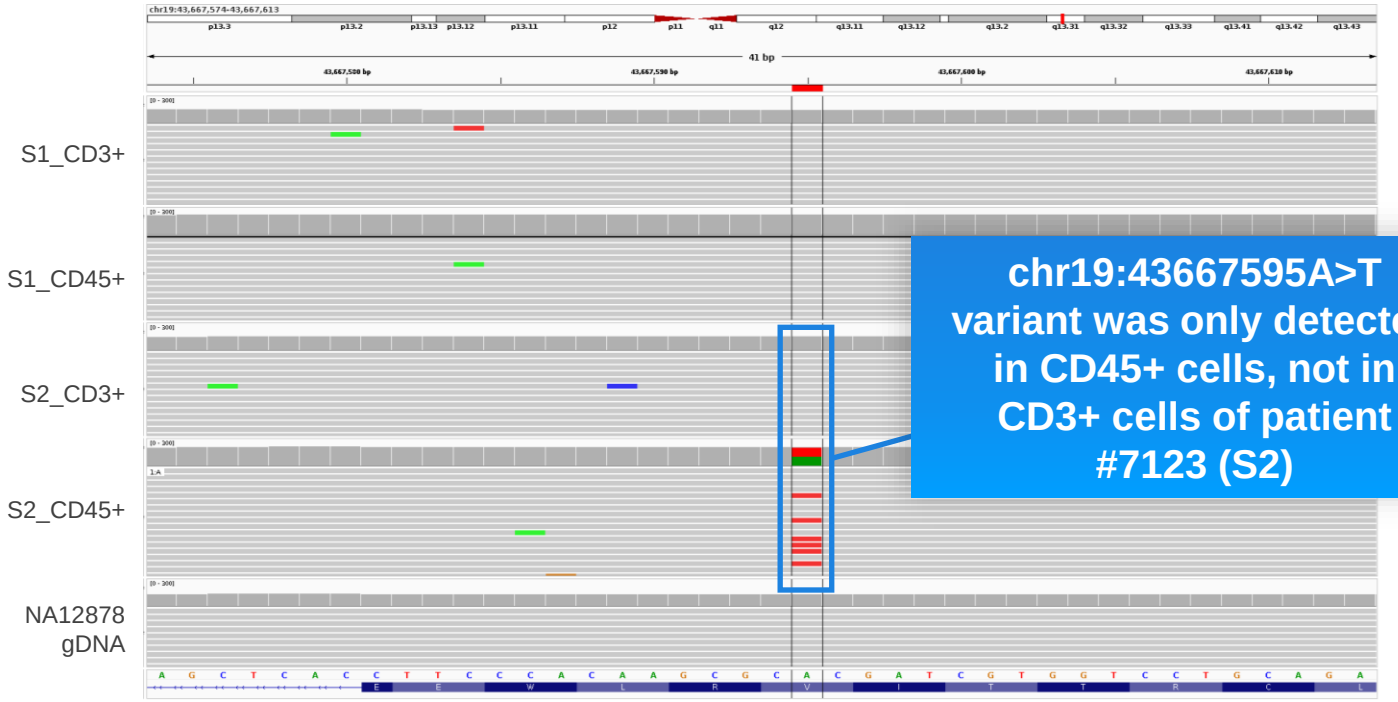
Shasta WGA – 1,294 Cells

Achieve sufficient coverage for pseudo-bulk SNV analysis

Chr 19: chr19:43667595A>T
A variant within the *PLAUR* gene



Integrative Genomics Viewer (IGV) view of chr19:43667595



chr19:43667595A>T variant was only detected in CD45+ cells, not in CD3+ cells of patient #7123 (S2)

Shasta WGA – 1,294 Cells

Generating meaningful biological discoveries

Shasta Single-Cell System

- **First-to-market high-throughput WGA and high-throughput total RNA-seq**
- **Retain coverage and sensitivity at scale without compromise**
- **Integrate automation, chemistries, and bioinformatics solutions**
- **Discover the biomarkers you are missing**

TaKaRa

that's
GOOD
science!®