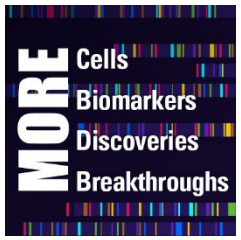
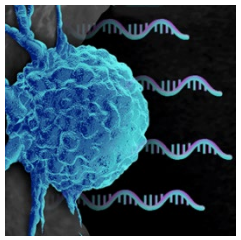


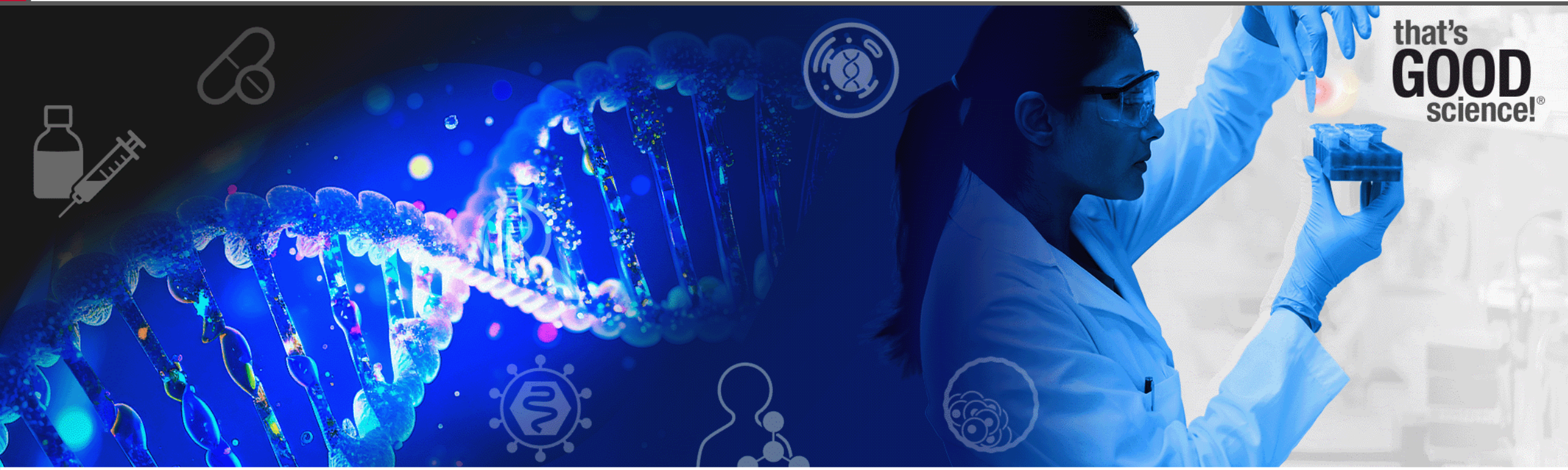


Drive biomarker discovery with a new high-throughput single-cell genome and transcriptome profiling system

Shuwen Chen, PhD
Senior Product Manager

that's GOOD science!®

Takara Bio: core capabilities



NGS

PCR, qPCR, RT-PCR

Cloning

Nucleic acid purification

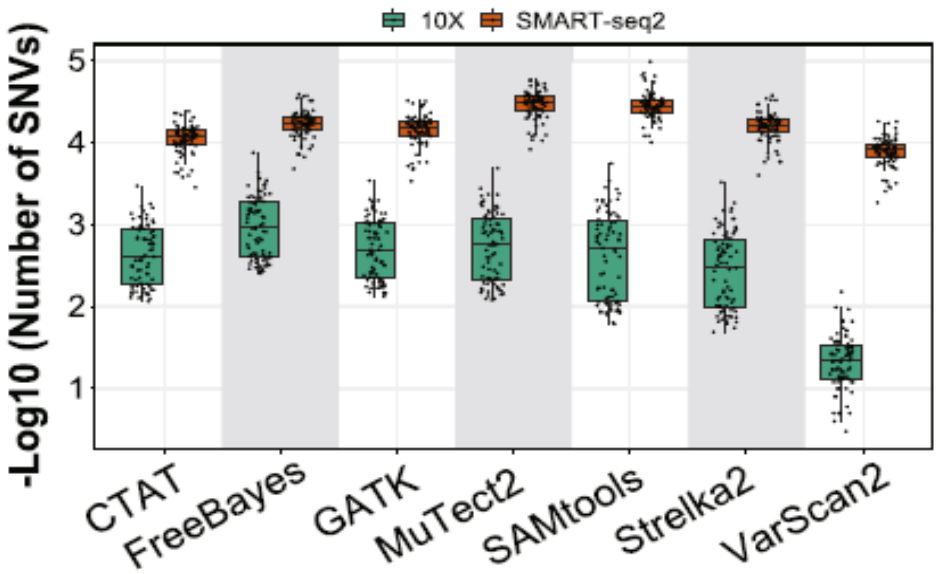
Gene delivery

Functional genomics

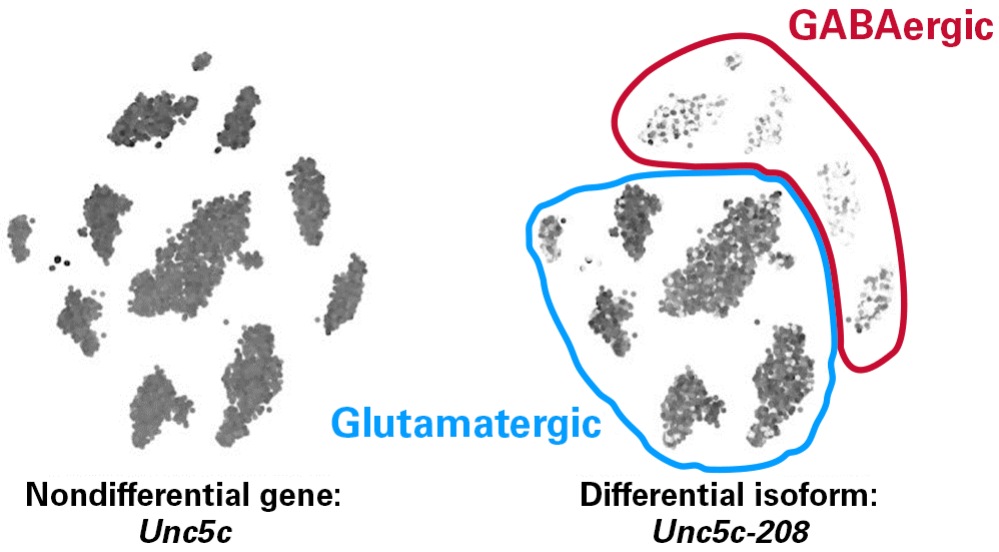
Protein expression & purification

OEM

The power of sensitivity and full gene-body coverage



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

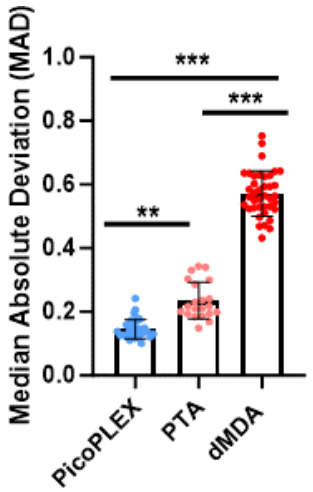


Employing SMART-Seq® chemistry, the **Allen Institute for Brain Science** published a preprint paper that was later published in Nature.

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.

Sensitive, uniform, and reproducible whole-genome amplification (WGA) with PicoPLEX® technology

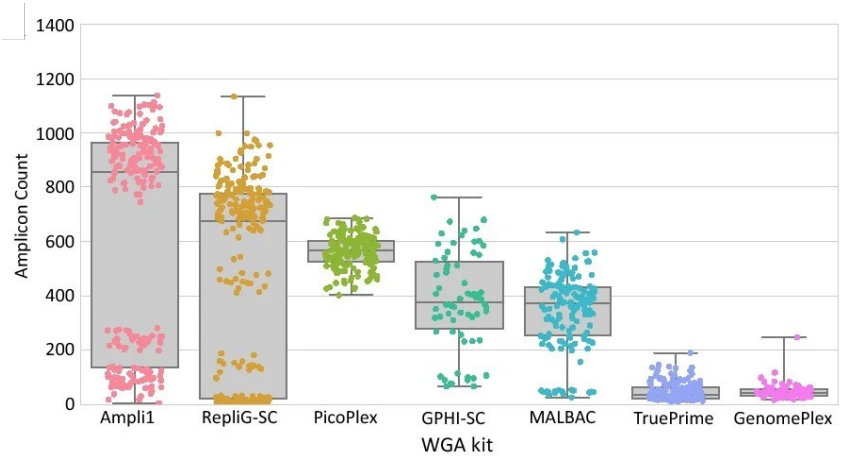
Most even



PTA provided the broadest amplification, but PicoPLEX application provided the most even amplification (2023)

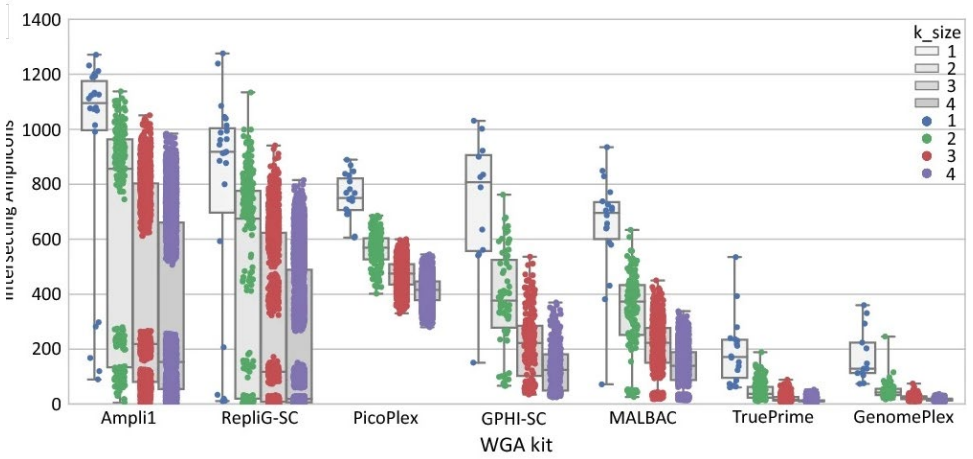
Figure adapted from "Single-cell somatic copy number variants in brain using different amplification methods and reference genomes" (Kalef-Ezra. et al. 2023, *bioRxiv)* under CC BY 4.0 license.

Most reliable



scWGA genome coverage analysis: PicoPLEX kit was the most reliable, with the tightest interquartile region (IQR) of all kits, and no failed cells.

Most reproducible



scWGA reproducibility analysis: PicoPLEX application demonstrated high reproducibility for all cells

Figures adapted from "Comparison of seven single cell whole genome amplification commercial kits using targeted sequencing" (Biezuner et al. 2021, *Sci. Rep.)* under a CC BY 4.0 license.

The scale of single-cell RNA sequencing has grown, but at the cost of data quality

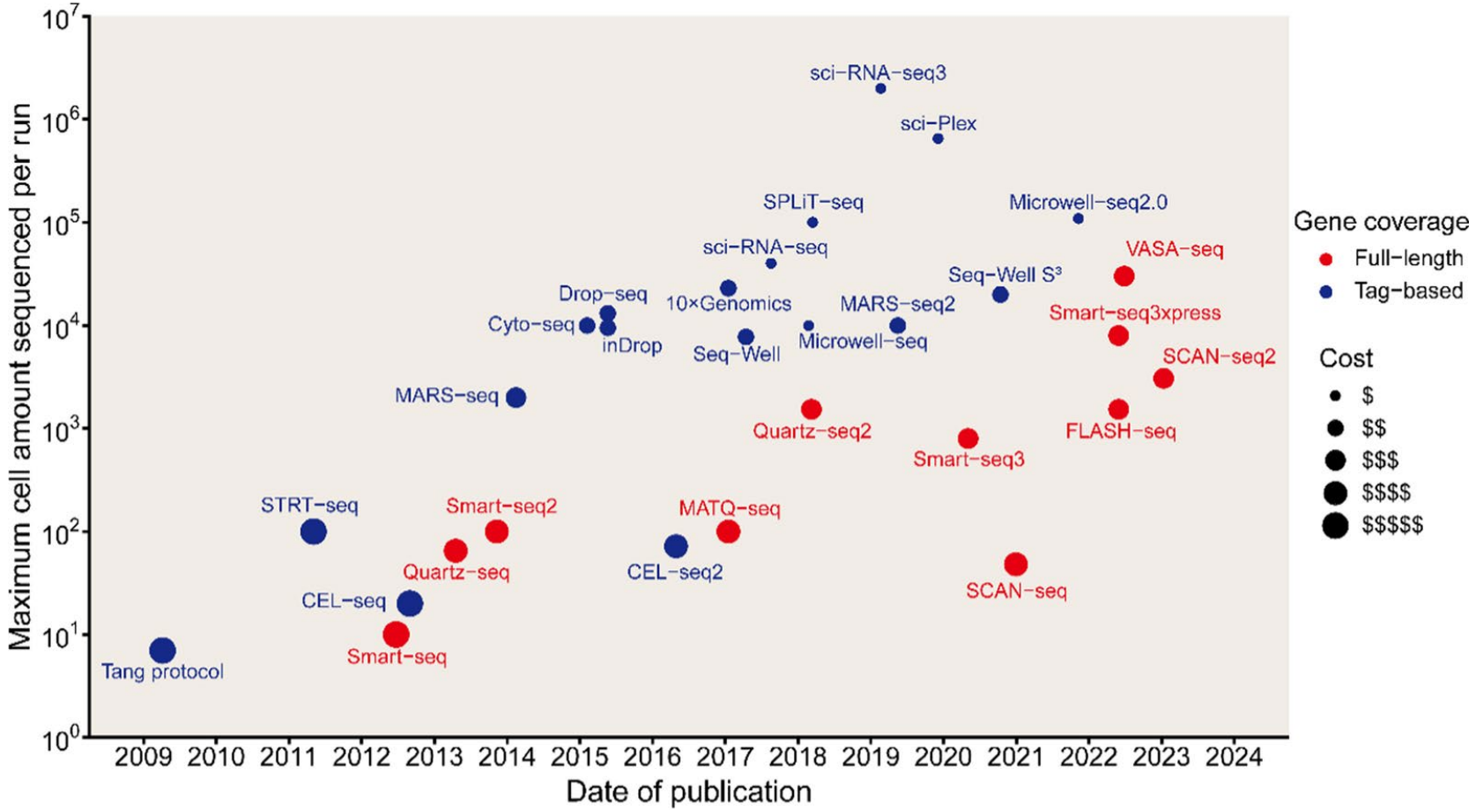
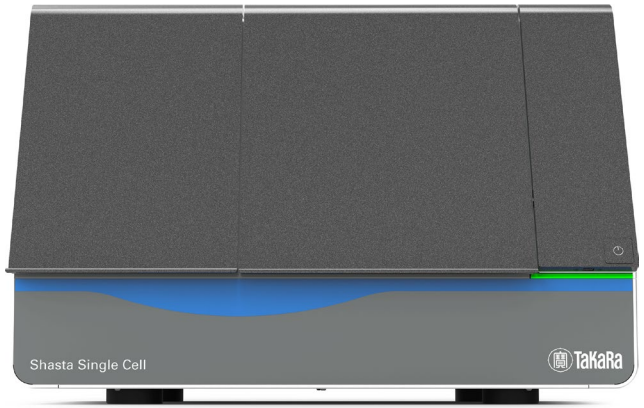


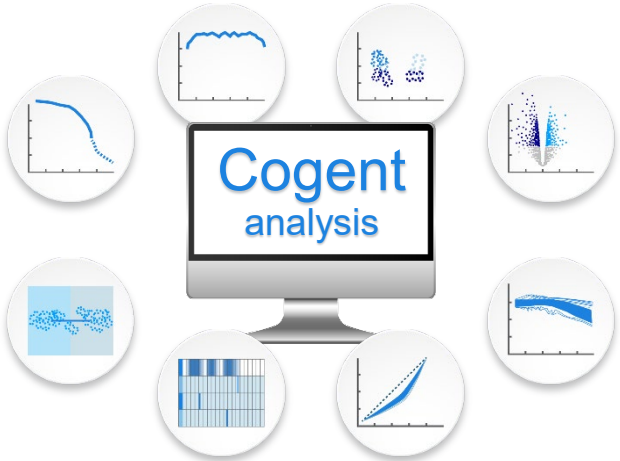
Figure adapted from "Advances in single-cell RNA sequencing and its applications in cancer research" (Huang et al. 2023, *J. Hematol. Oncol.)* under a CC BY 4.0 license.

Next-generation single-cell biomarker discovery, scaled

Shasta™ Single-Cell System



Cogent™ NGS Analysis Pipeline and Discovery Software



Shasta Total RNA-Seq Kit

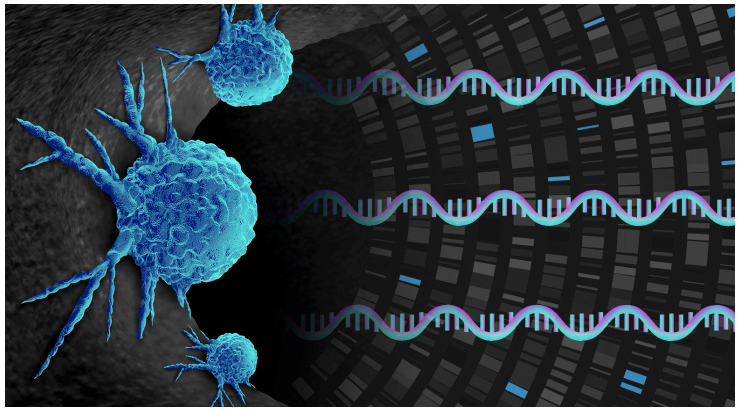
- Analyze full-length transcriptomes of up to 100,000 single cells per run with outstanding sensitivity
- Uncover multiple RNA biotypes
- Detect splicing isoforms and gene fusions
- Use bioinformatics tools to decode expression patterns of protein-coding and noncoding genes

Shasta Whole-Genome Amplification Kit

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

Shasta Total RNA-Seq: overview

Shasta Total RNA-Seq



Two-day workflow

- ✓ Random-primed total RNA-seq
- ✓ Full-length gene-body coverage

Novel indexing strategy

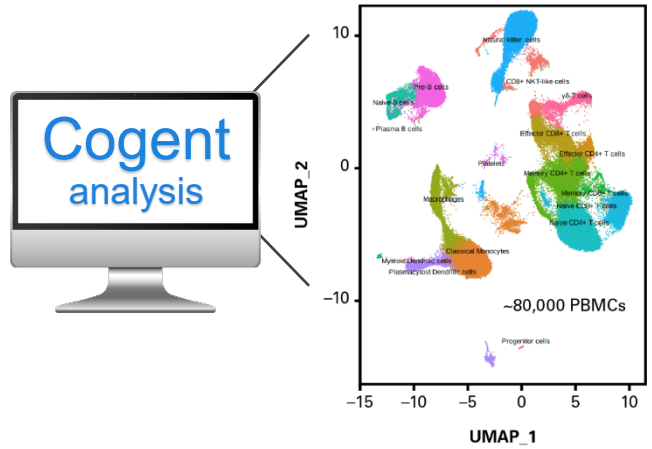
- ✓ Reduced cell loss, workflow time, and reagent costs

Shasta Single-Cell System

High-throughput automation

- ✓ ~100,000 cells with low doublet rate
- ✓ Up to 12 samples per experiment

Cogent NGS tools



Free analysis software

- ✓ Protein-coding and noncoding gene pipelines
- ✓ Publication-quality figures

Case study: discovering biomarkers regulated by p53 and epitherapy treatment

Four A549 samples
from Dr. Ting Wang's lab
at Washington University School
of Medicine in St. Louis

WT

P53-WT

WT_T

P53-WT + epitherapy treatment

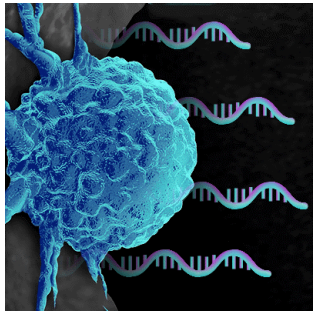
KO

P53-KO

KO_T

P53-KO + epitherapy treatment

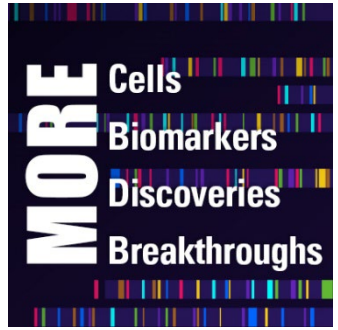
Shasta Total RNA-Seq Kit



Cogent NGS Analysis Pipeline

- ✓ Standard analysis
- ✓ lncRNA analysis

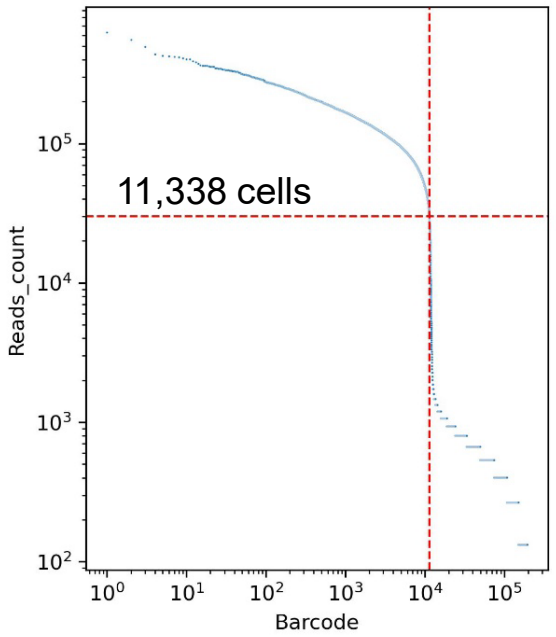
Biomarker discovery

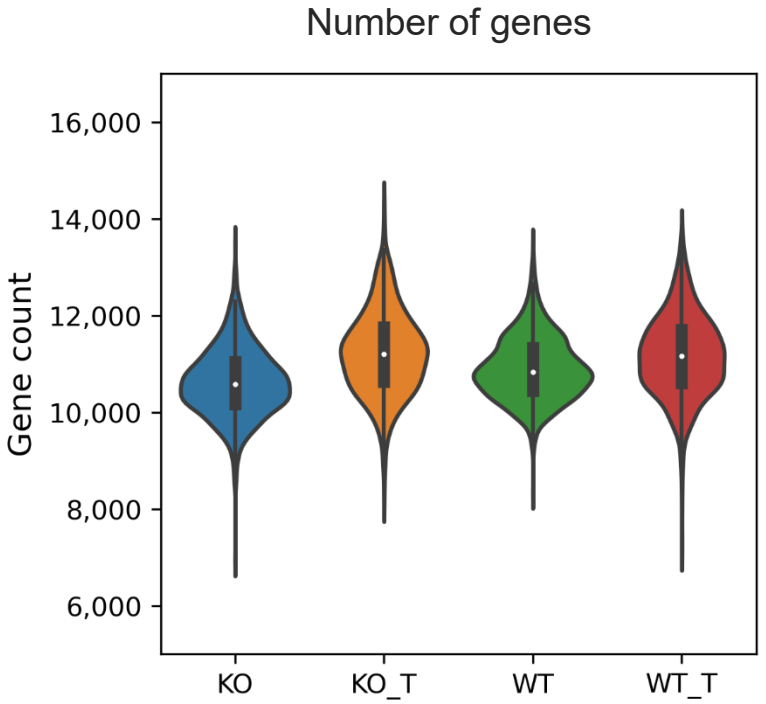


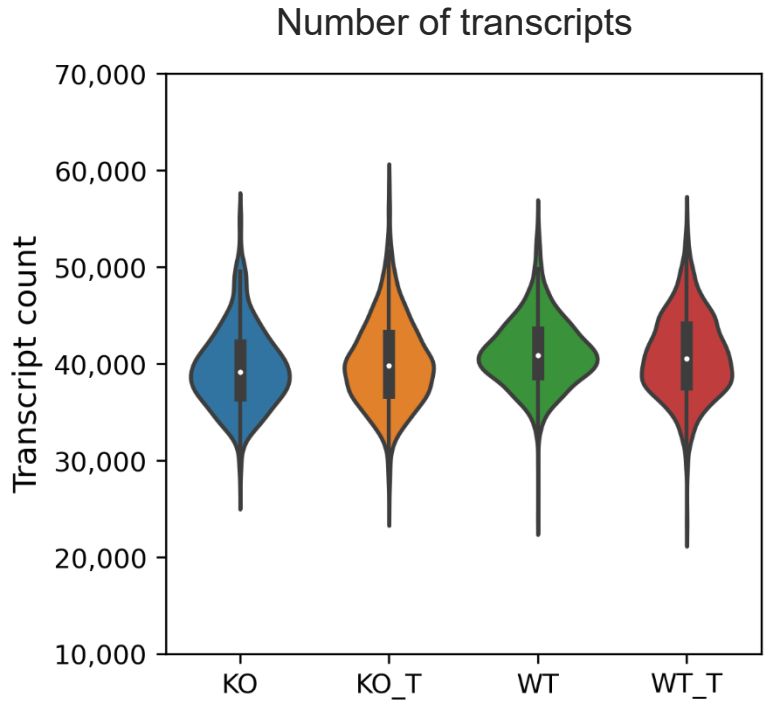
Created with BioRender.com.

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*

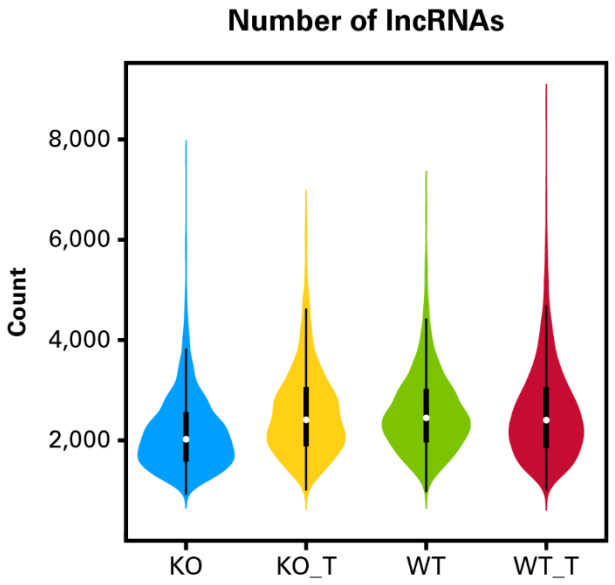




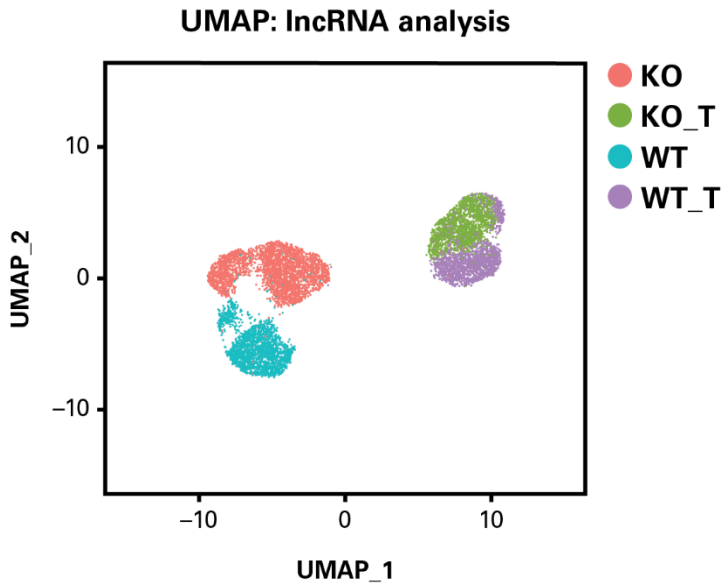


Found novel biomarkers by detecting differentially expressed lncRNAs

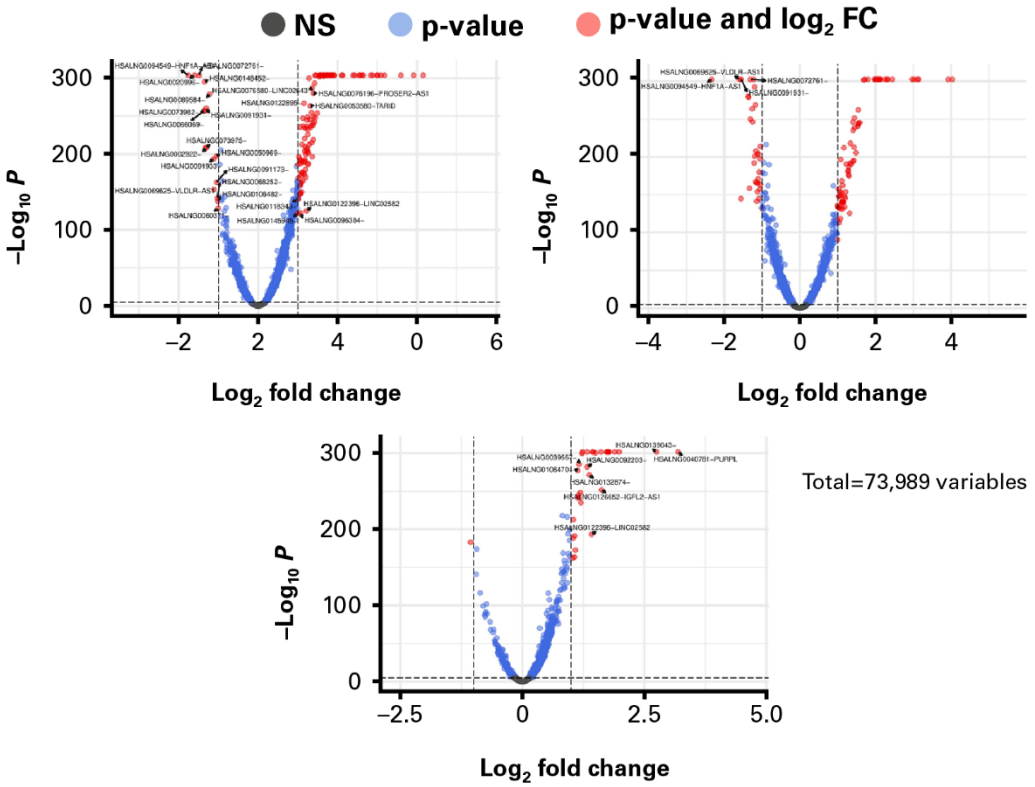
Sensitivity and lncRNA detection



UMAP



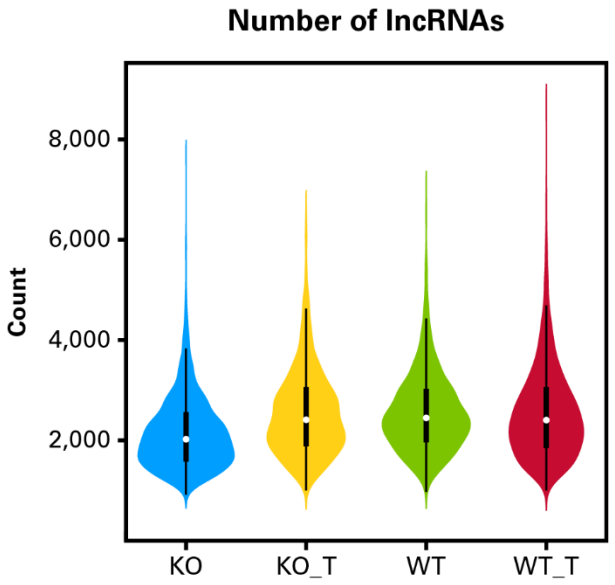
Identification of differentially expressed lncRNAs



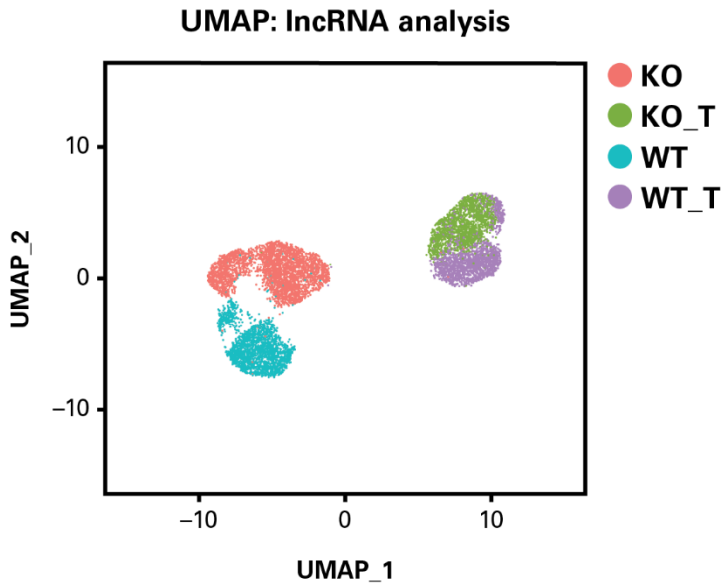
lncRNA: analyzed using CogentAP with RNAcentral reference containing only ncRNAs.

Found novel biomarkers by detecting differentially expressed lncRNAs

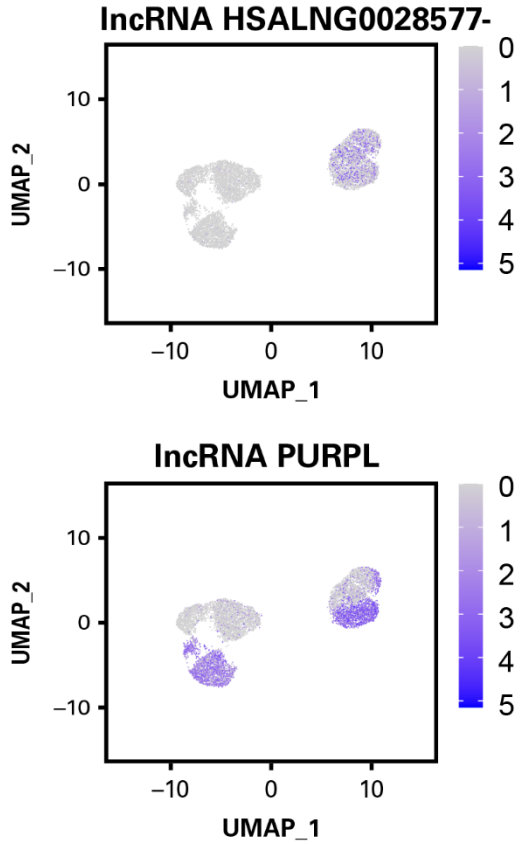
Sensitivity and lncRNA detection



UMAP



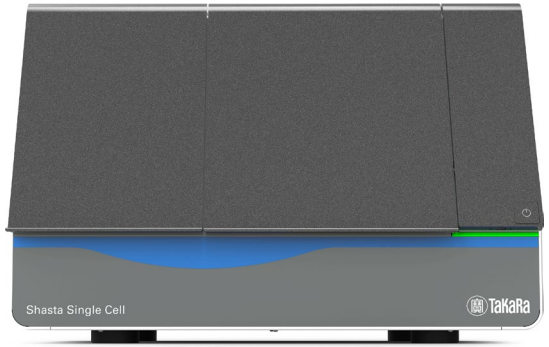
Identification of differentially expressed lncRNAs



lncRNA: analyzed using current CogentAP with LncBook v2 reference containing only ncRNAs.

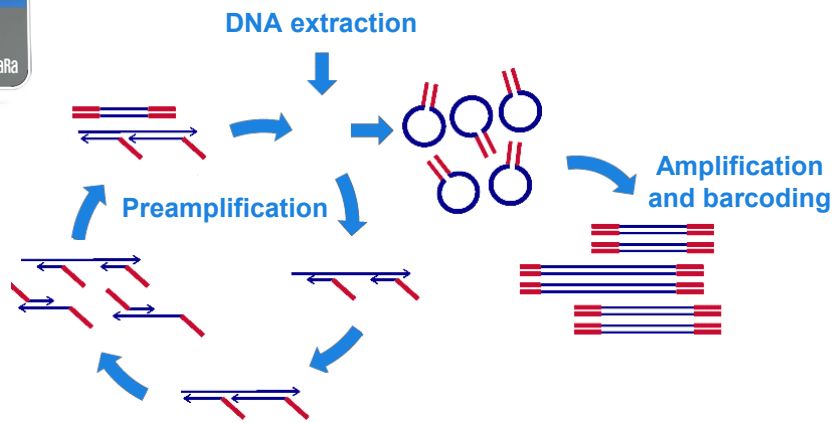
Automated, high-throughput solution for single-cell WGA

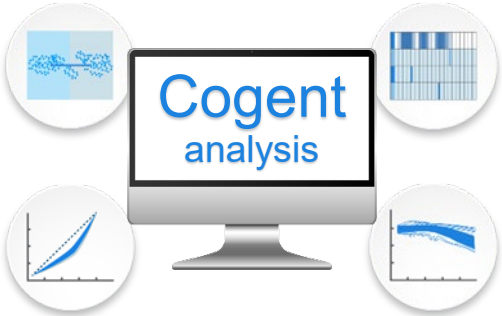
Shasta WGA Kit



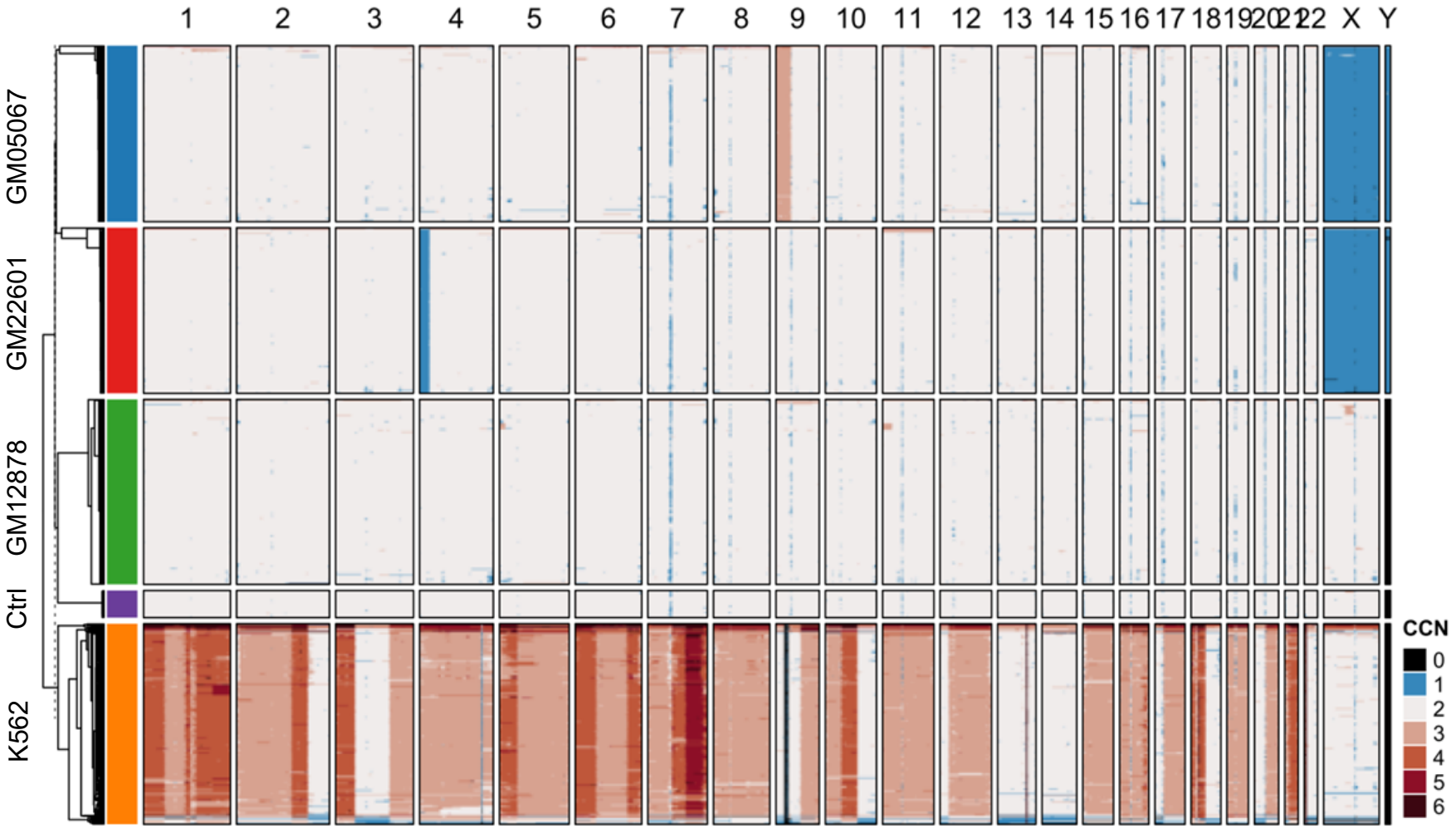
- ✓ High-throughput WGA
  Process up to 1,500 cells per run
- ✓ Lower sequencing cost
  Analyze CNV, SNV, and structural variation at low depth
- ✓ Automated workflow on the Shasta instrument
  Obtain library in one day
- ✓ Leading chemistry for uniformity and reproducibility
  Take advantage of PicoPLEX WGA chemistry
- ✓ End-to-end solution
  Use free Cogent bioinformatics tools

PicoPLEX WGA





Copy number profiles of 1,124 single cells



Four cell lines

- GM05067
- GM22601
- GM12878
- K562

250,000 reads/cell

2 x 75 bp

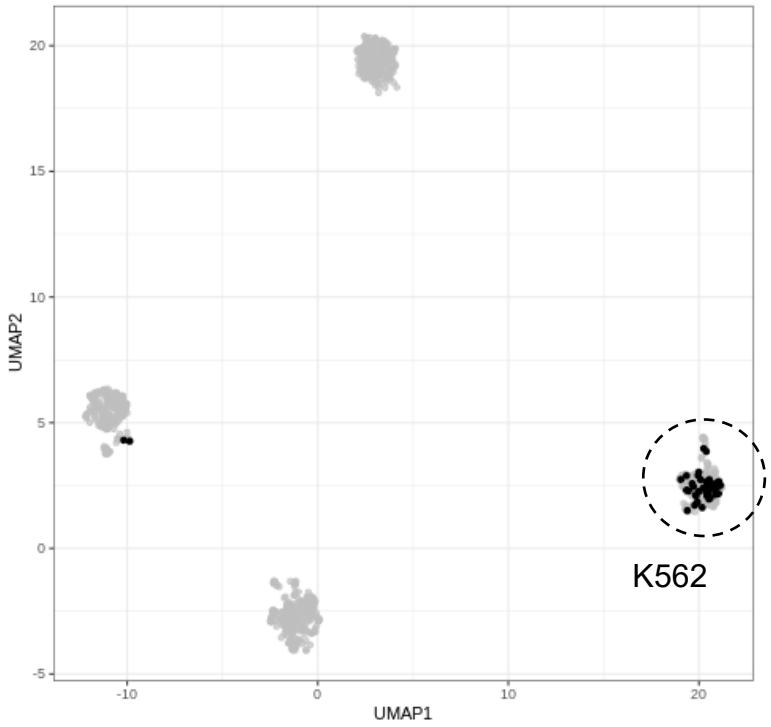
1 Mb average bin size

Pair sensitivity and sequencing affordability

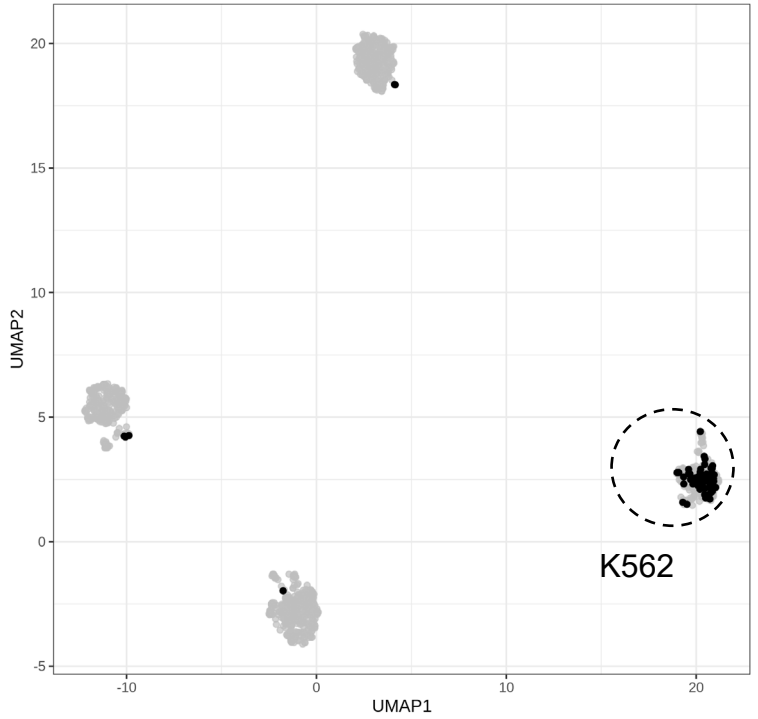
Pseudo-bulk SNV analysis for cell clusters

- Clustered single cells by their CNV profiles (~100 million reads)
- Performed pseudo-bulk SNV analysis (Monopogen [Dou et al. 2023])
- Called germline variants for each cluster and putative somatic variants for each single cell

Chr. 7: 55034534: A:C
A variant within the *EGFR* gene



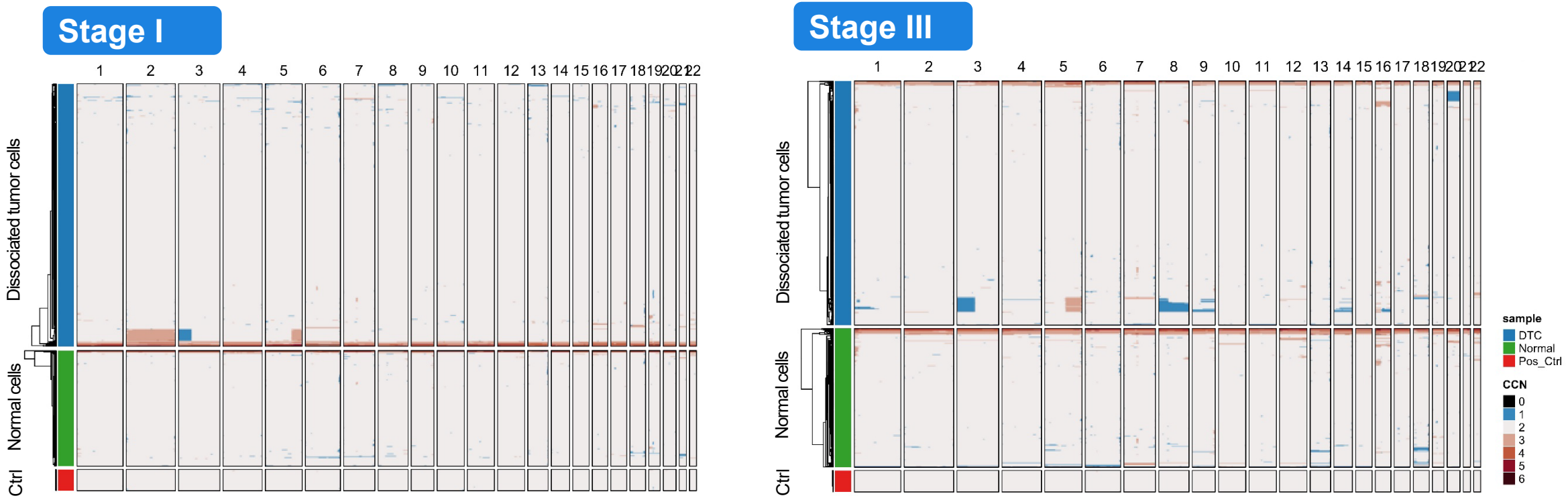
Chr. 4: 105200804: G:A
A variant within the *TET2* gene



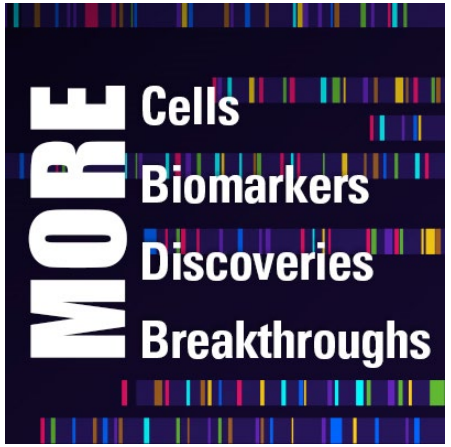
Dou, et al. “Single-nucleotide variant calling in single-cell sequencing data with Monopogen.” *Nat. Biotechnol.* (2023)

Find CNV events in small subclones amongst a heterogeneous tumor sample

- Dissociated cells from tumor tissues of Stage I (815 cells) and Stage III (858 cells) clear-cell renal-cell carcinoma (ccRCC) and adjacent normal tissue
- ~370,000 reads/cell, 2 x 75 bp



Generating meaningful biological discoveries



Visit us at Booth 89!

The Shasta single-cell solution

- Three novel, validated applications:

  Shasta Total RNA-Seq Kit
  Go beyond mRNA to discover multiple RNA biotypes

  Shasta Whole-Genome Amplification Kit
  Resolve tumor heterogeneity and track clonal evolution

  Shasta mRNA-Seq Kit
  Detect low-expressed biomarkers

- Free, easy-to-use Cogent bioinformatics software
- Intuitive user interface and easy maintenance

TaKaRa

that's
GOOD
science!®