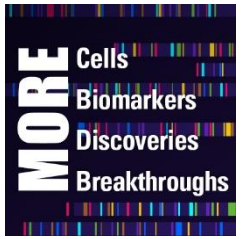
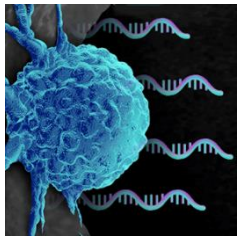




Driving biomarker discovery with high-throughput single-cell genome and transcriptome profiling

Andrew Farmer, D.Phil.
CSO/Head of R&D

that's GOOD science!®

Takara Bio: core capabilities

that's GOOD science!®

NGS

- SMARTer® and SMART-Seq® RNA-seq library preparation kits
- PicoPLEX® and ThruPLEX® DNA-seq library preparation kits

PCR, qPCR, RT-PCR

- TaKaRa Ex Premier™, LA Taq™, PrimeSTAR® GXL, SeqAmp™,Titanium® polymerases & PrimeScript™ RT
- EcoDry™ lyophilized enzymes and kits

Cloning

- In-Fusion® Snap Assembly Cloning

Nucleic acid purification

Gene delivery

- Lenti-X™, Adeno-X™, Retro-X™, and AAVpro® systems | Xfect™ Transfection Reagent
- RetroNectin® reagent

Functional genomics

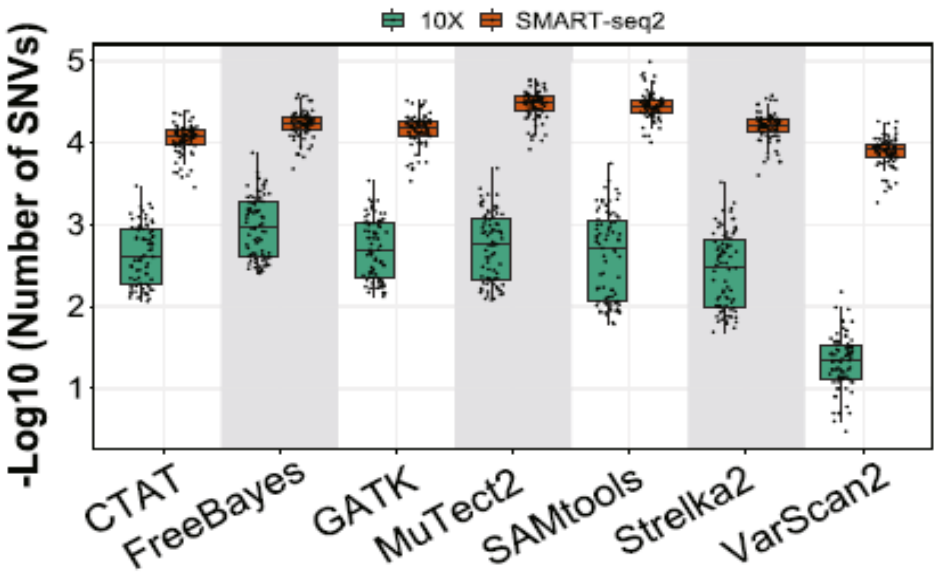
- Tet systems and iDimerize™ systems
- Guide-it™ CRISPR/Cas9 genome editing products
- Living Colors® fluorescent proteins

Protein expression & purification

- TALON® and His60 Ni protein purification

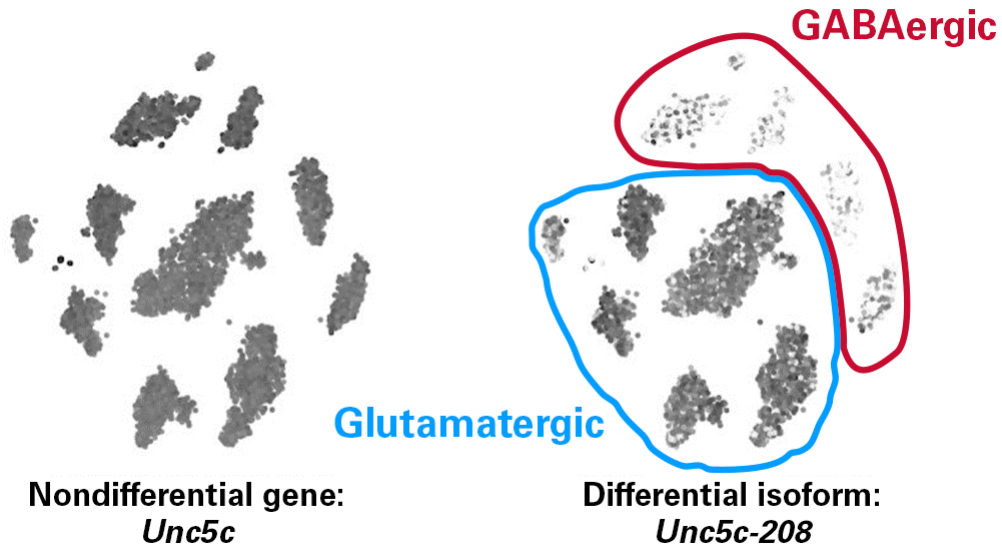
OEM

The power of sensitivity and full gene-body coverage



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 F. et al. 2019, *Genome Biol*) under a CC BY 4.0 license.

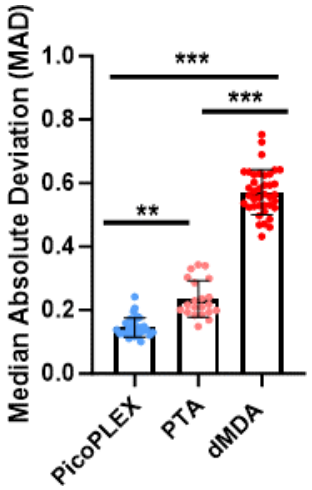


Employing SMART-Seq chemistry, the **Allen Institute for Brain Science** published a preprint paper that was later published in *Nature*.

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

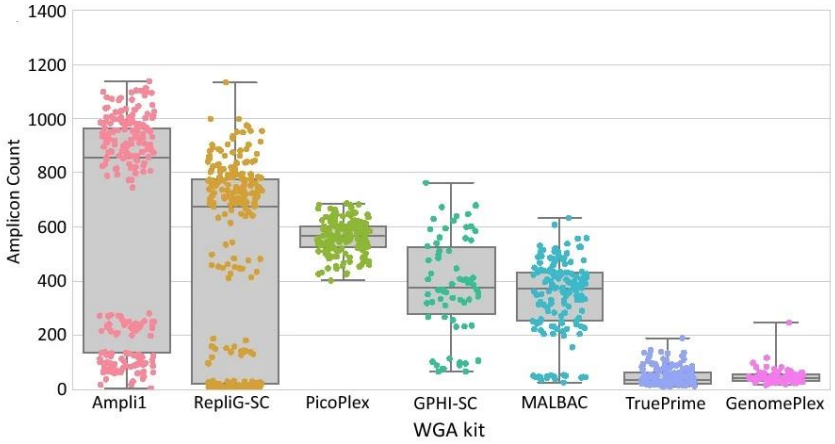
Sensitive, uniform, and reproducible whole genome amplification (WGA) with PicoPLEX technology

Most even



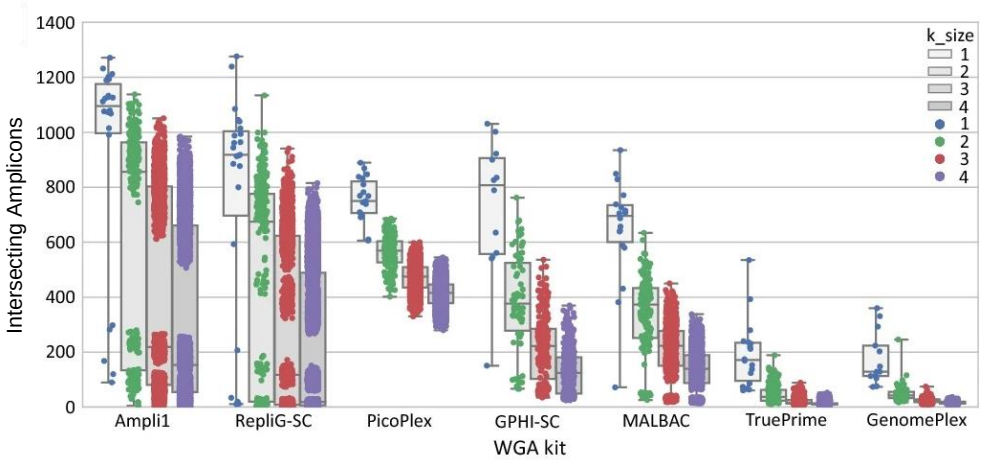
PTA provided the broadest amplification, but PicoPLEX application provided the most even amplification (2023).

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.

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.

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.

Advances in single-cell RNA-seq scale over time

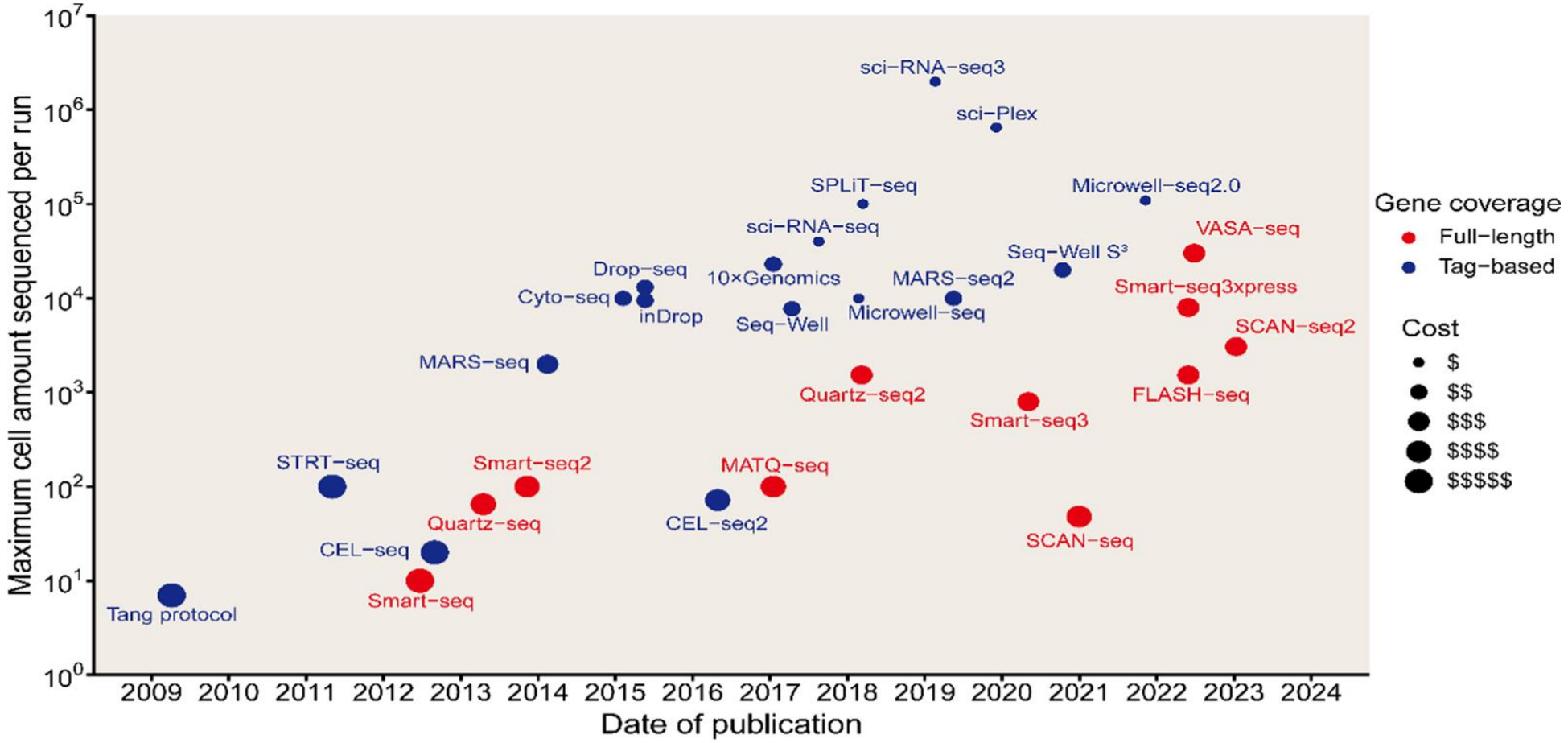
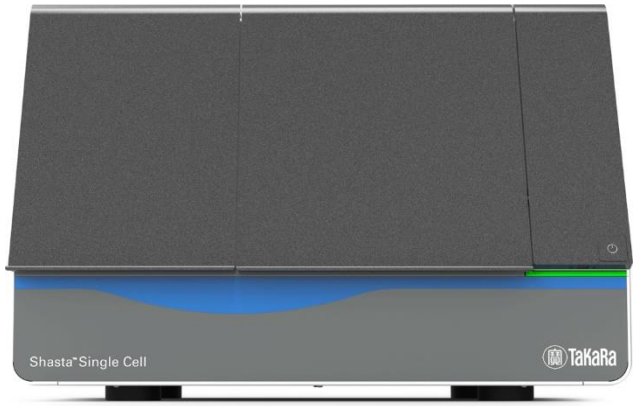


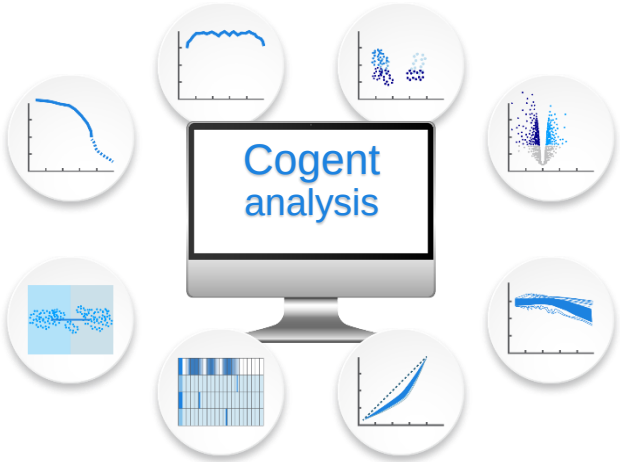
Figure adapted from "Advances in single-cell RNA sequencing and its applications in cancer research" (Huang et al. 2023, *J. Hematol. Oncol.)* under a CC BY 4.0 license.

Scaled next-generation single-cell biomarker discovery

Shasta™ Single-Cell System



Cogent™ NGS Analysis Pipeline and Discovery Software



Shasta Total RNA-Seq Kit

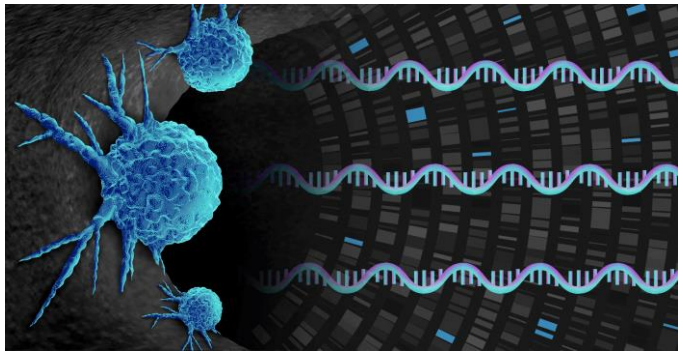
- Analyze up to 100,000 single cells per run with outstanding sensitivity and full gene body coverage
- Uncover multiple RNA biotypes
- Detect splicing isoforms and gene fusions
- Use bioinformatics tools to decode expression patterns of protein-coding and noncoding genes

Shasta Whole-Genome Amplification Kit

- Analyze over 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



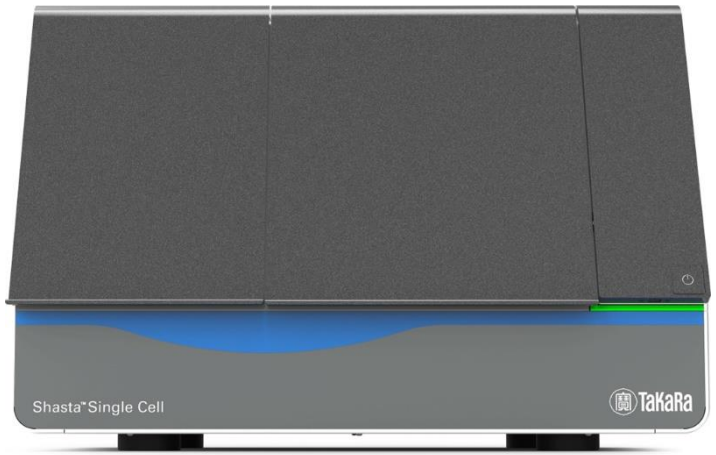
Two-day workflow

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

Novel indexing strategy

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

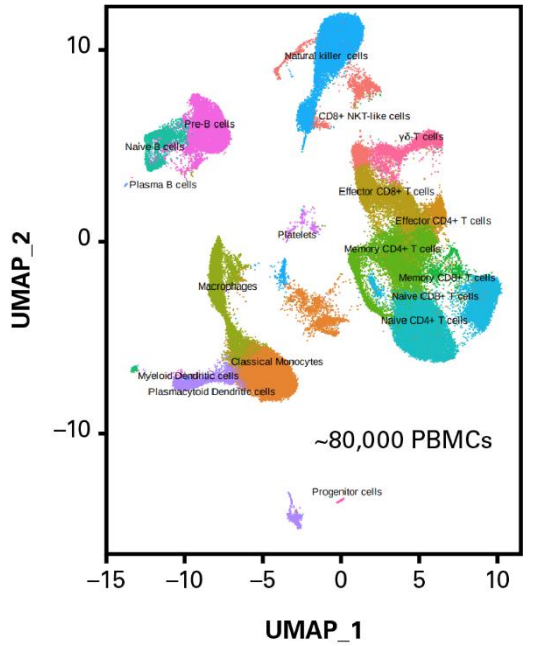
Shasta Single-Cell System



High-throughput automation

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

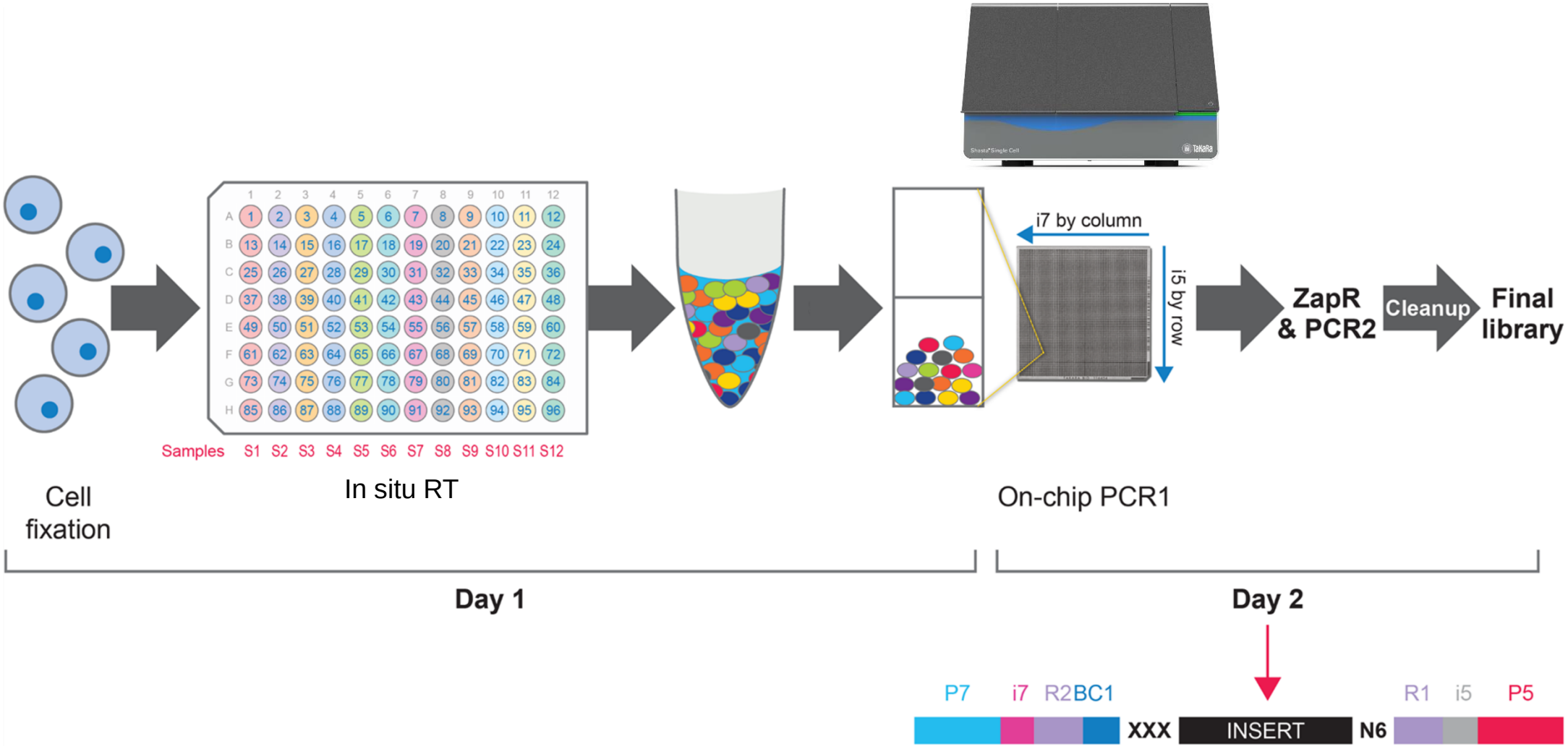
Cogent NGS pipeline



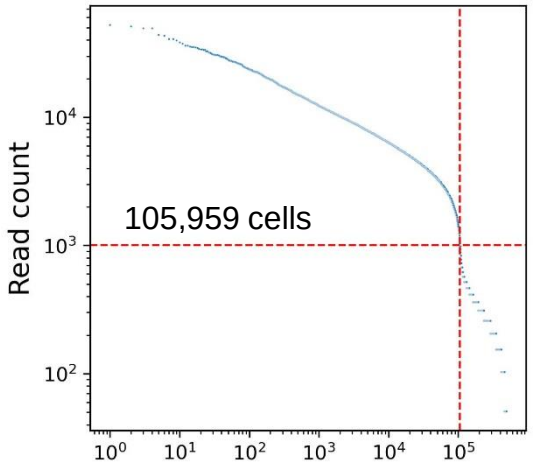
Free analysis tools

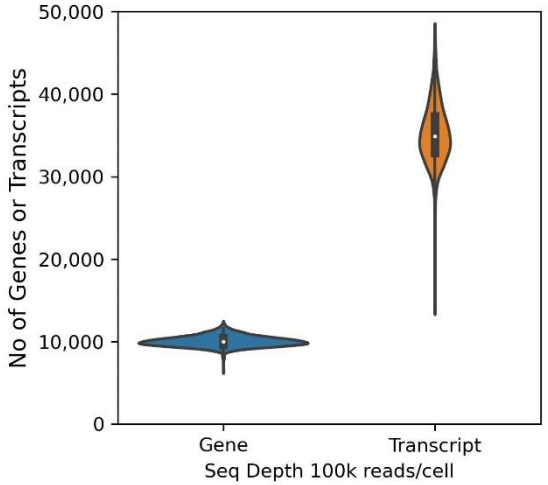
- ✓ Protein-coding and noncoding gene pipelines
- ✓ Publication-quality figures

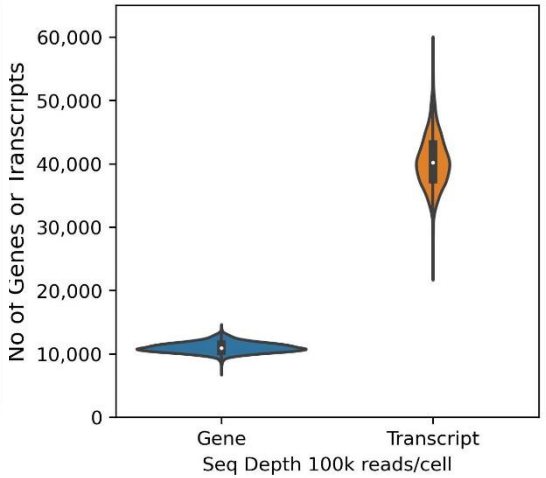
Shasta Total RNA-Seq workflow

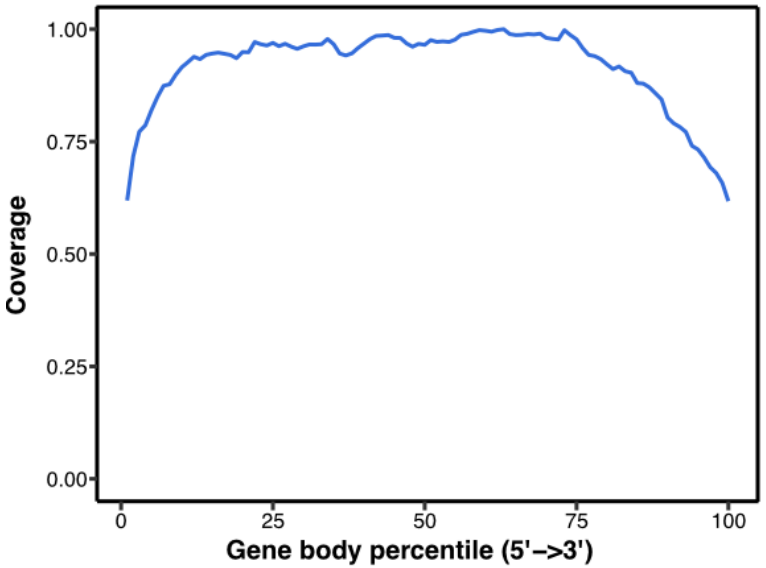


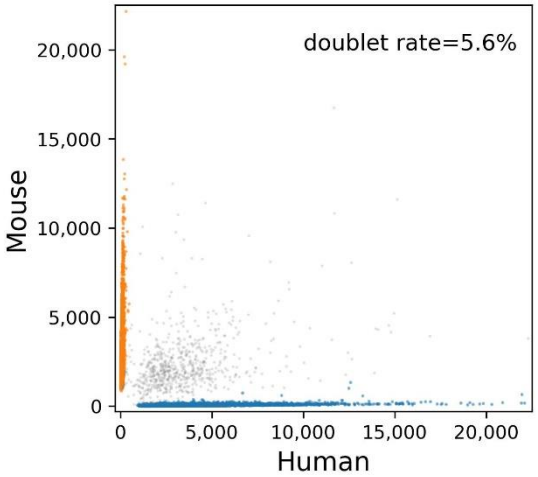
Achieve outstanding gene sensitivity, low doublet rates, and full gene-body coverage

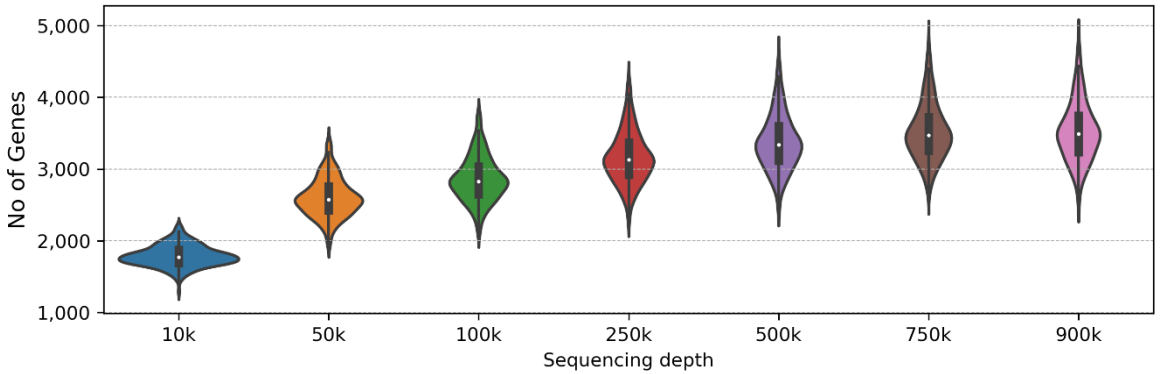












Case study: discovering biomarkers regulated by p53 and epitherapy treatment

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

WT

P53-WT

WT_T

P53-WT + epitherapy treatment

KO

P53-KO

KO_T

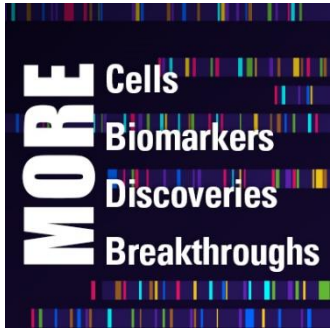
P53-KO + epitherapy treatment

Shasta Total RNA-Seq Kit

Cogent NGS Analysis Pipeline

- ✓ Standard analysis
- ✓ lncRNA analysis

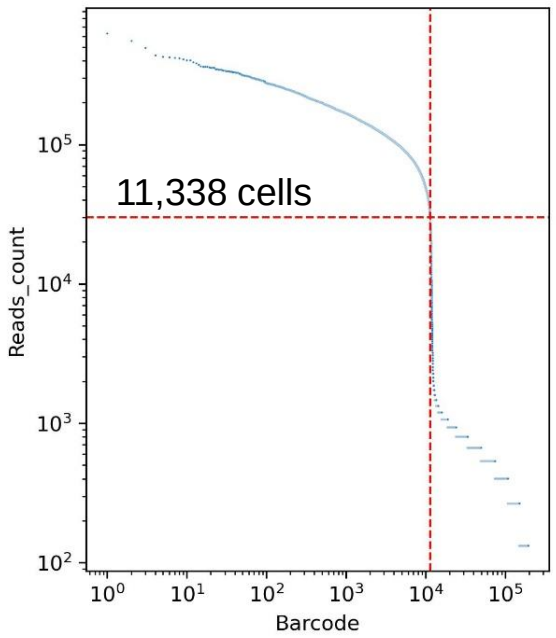
Biomarker discovery

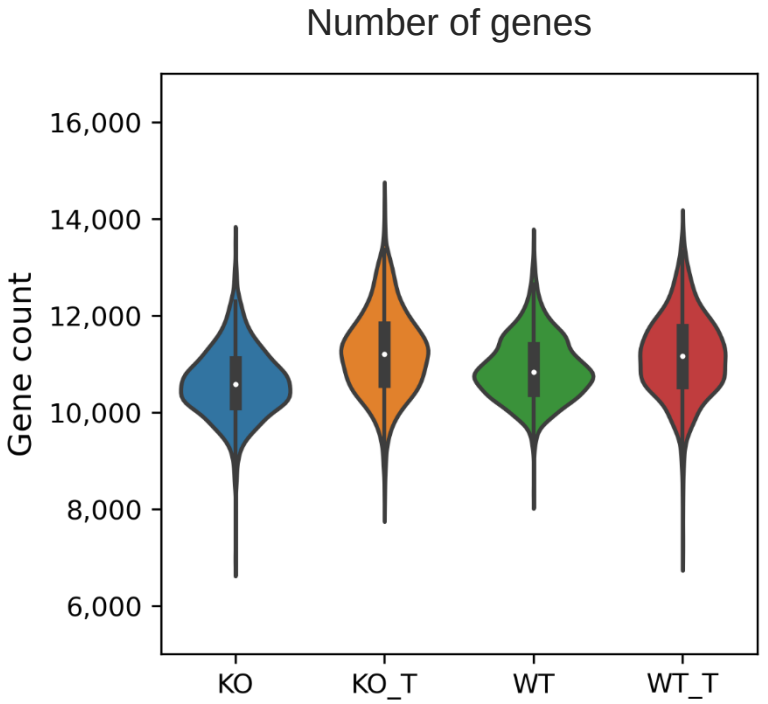


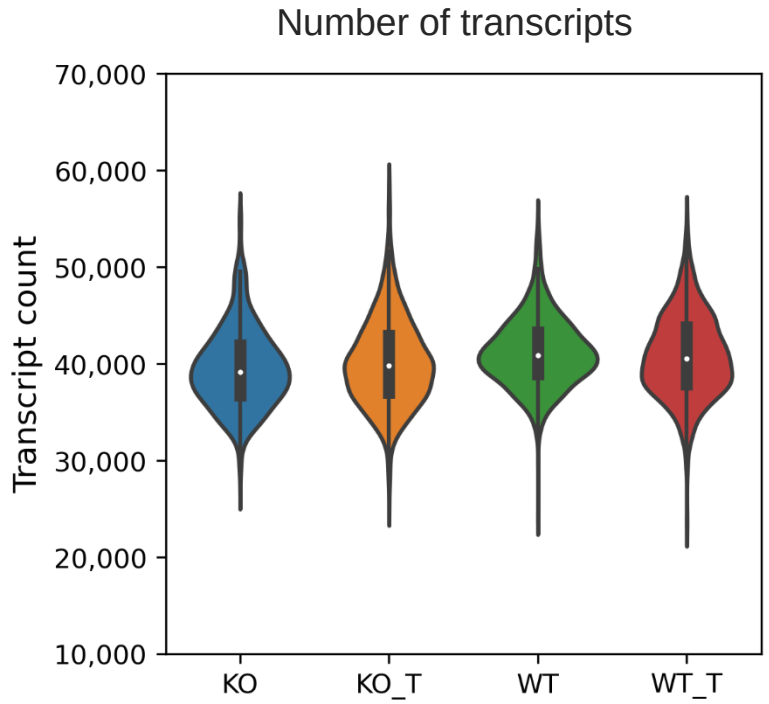
Created with BioRender.com.

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

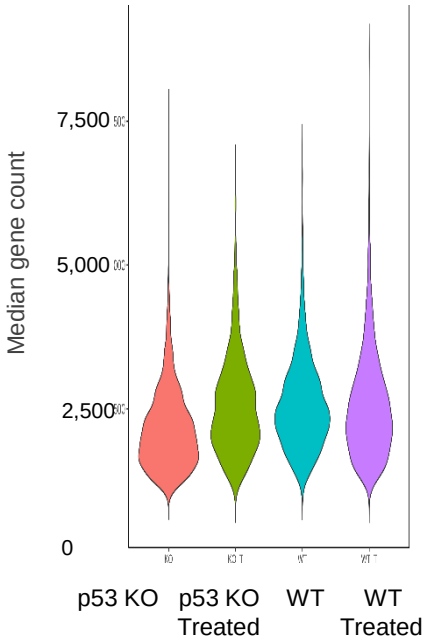




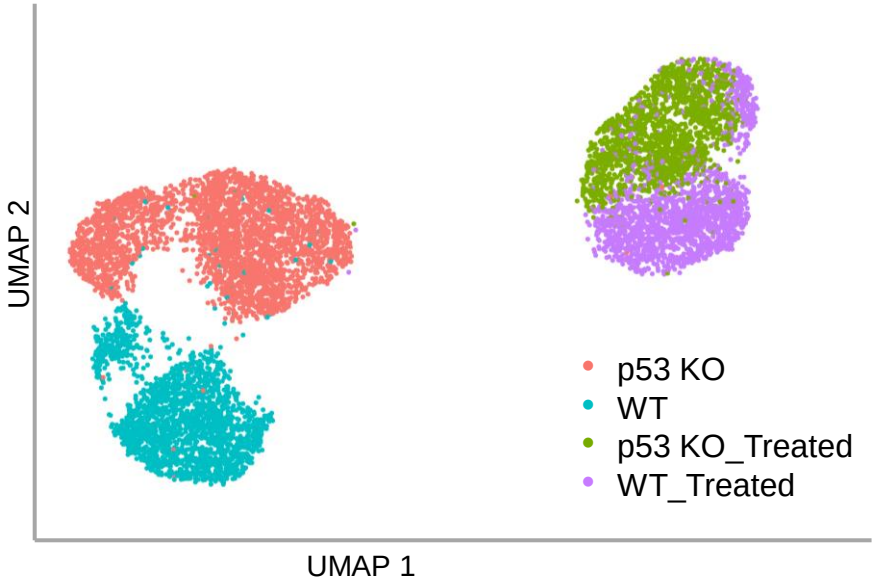


Cellular phenotype associated with differentially expressed lncRNAs

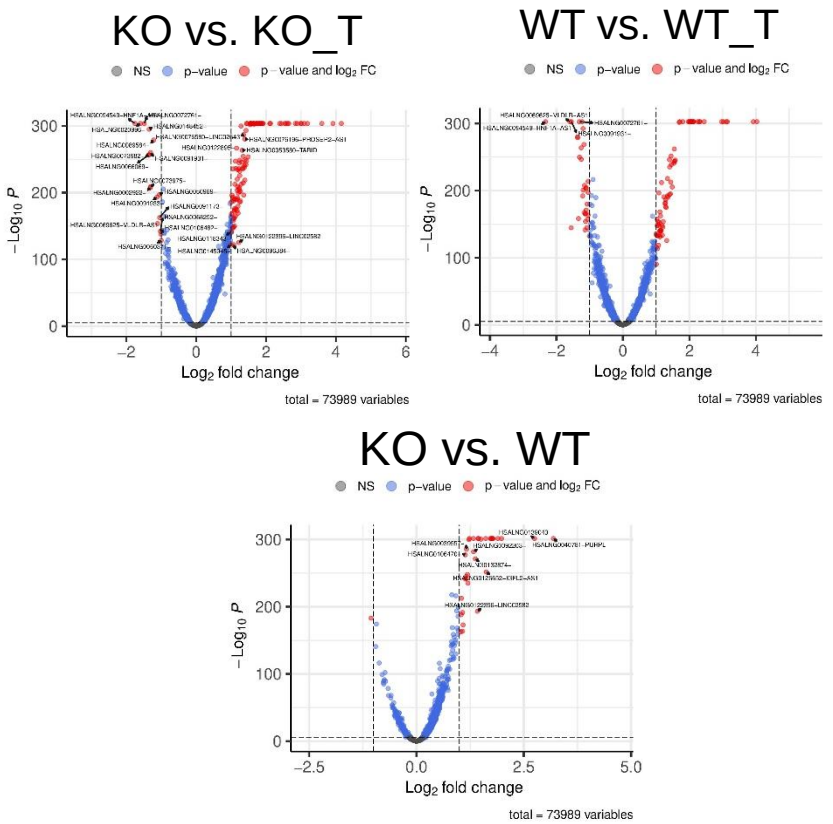
Sensitivity
lncRNA detection



UMAP



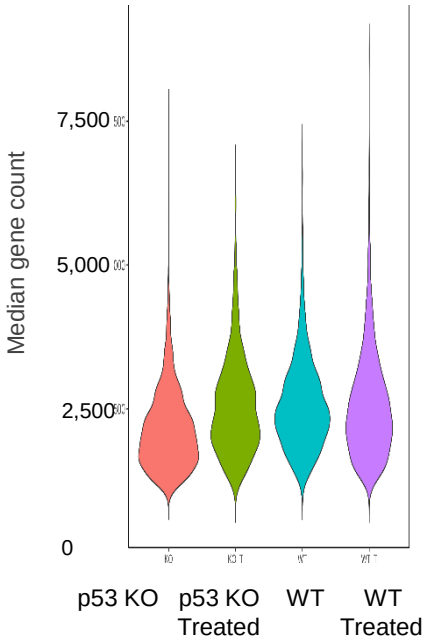
Identify differentially expressed lncRNAs



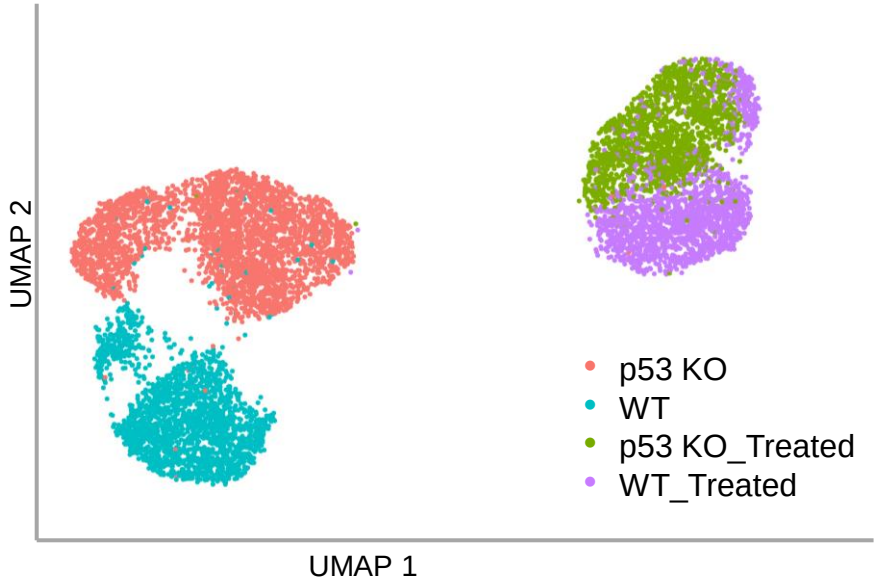
lncRNA: analysis done using Cogent AP with LncBook v2 reference containing only ncRNAs.

Cellular phenotype associated with differentially expressed lncRNAs

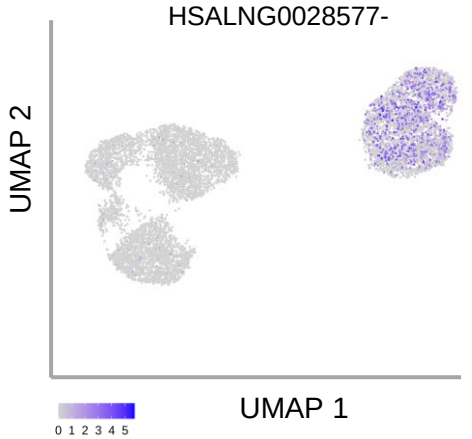
Sensitivity
lncRNA detection



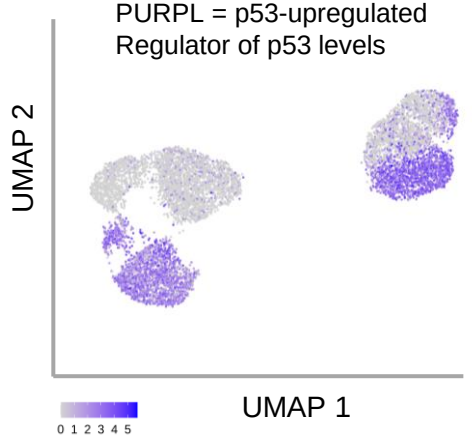
UMAP



Differentially expressed lncRNAs



PURPL = p53-upregulated
Regulator of p53 levels



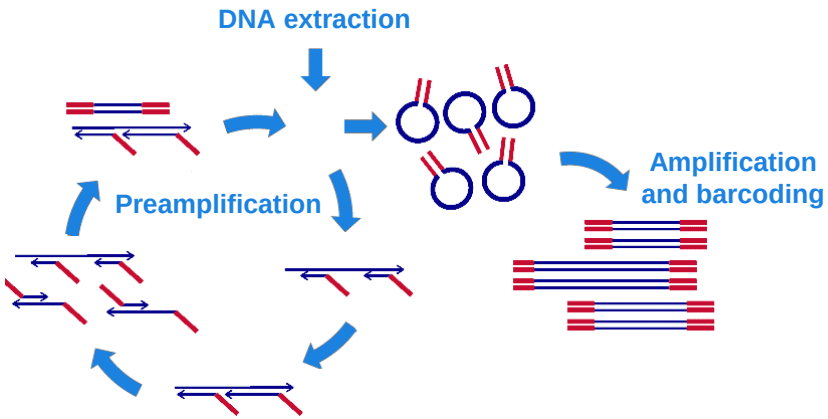
lncRNA: analysis done using Cogent AP with LncBook v2 reference containing only ncRNAs.

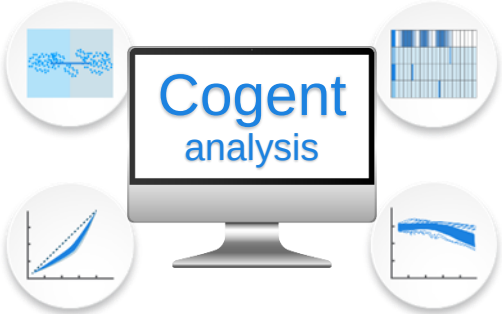
Automated, high-throughput solution for single-cell WGA

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

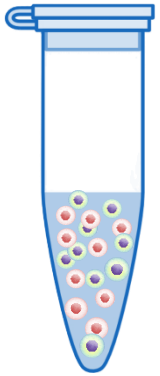


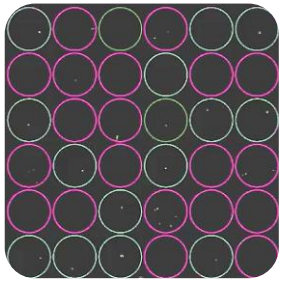


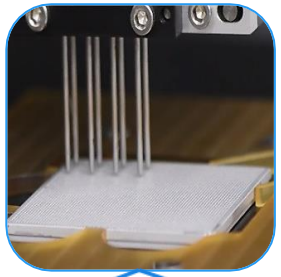
Shasta WGA workflow

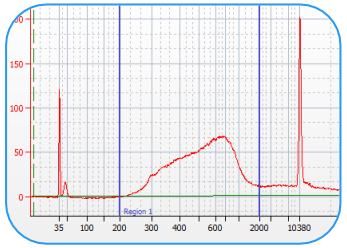
DAY 1: Laborious pipetting replaced by automatic dispensing

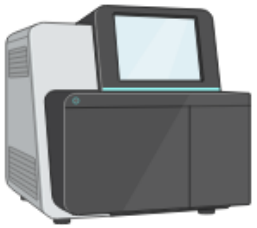
Stained cell suspension → Cell dispense & selection → Library generation → Cleanup & quantification → Sequencing → Analysis & visualization

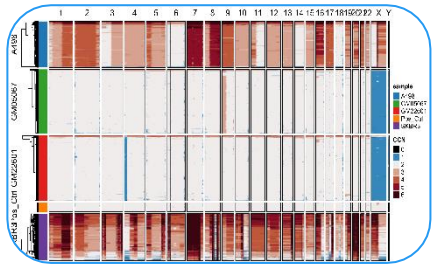


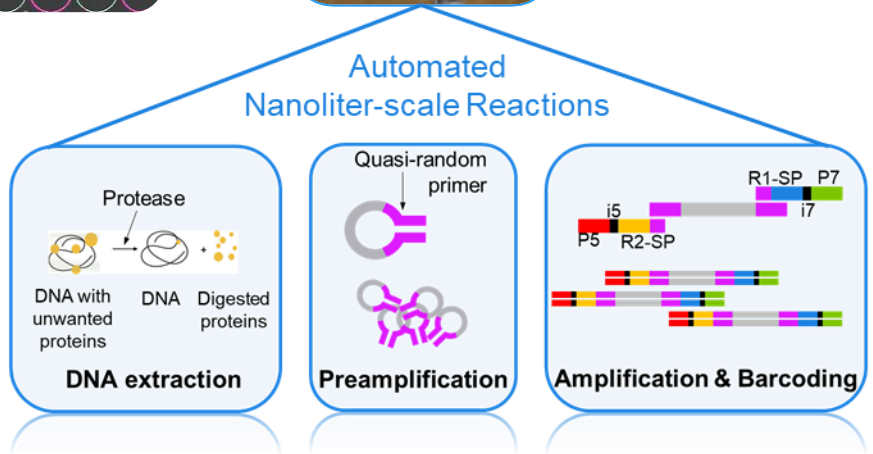






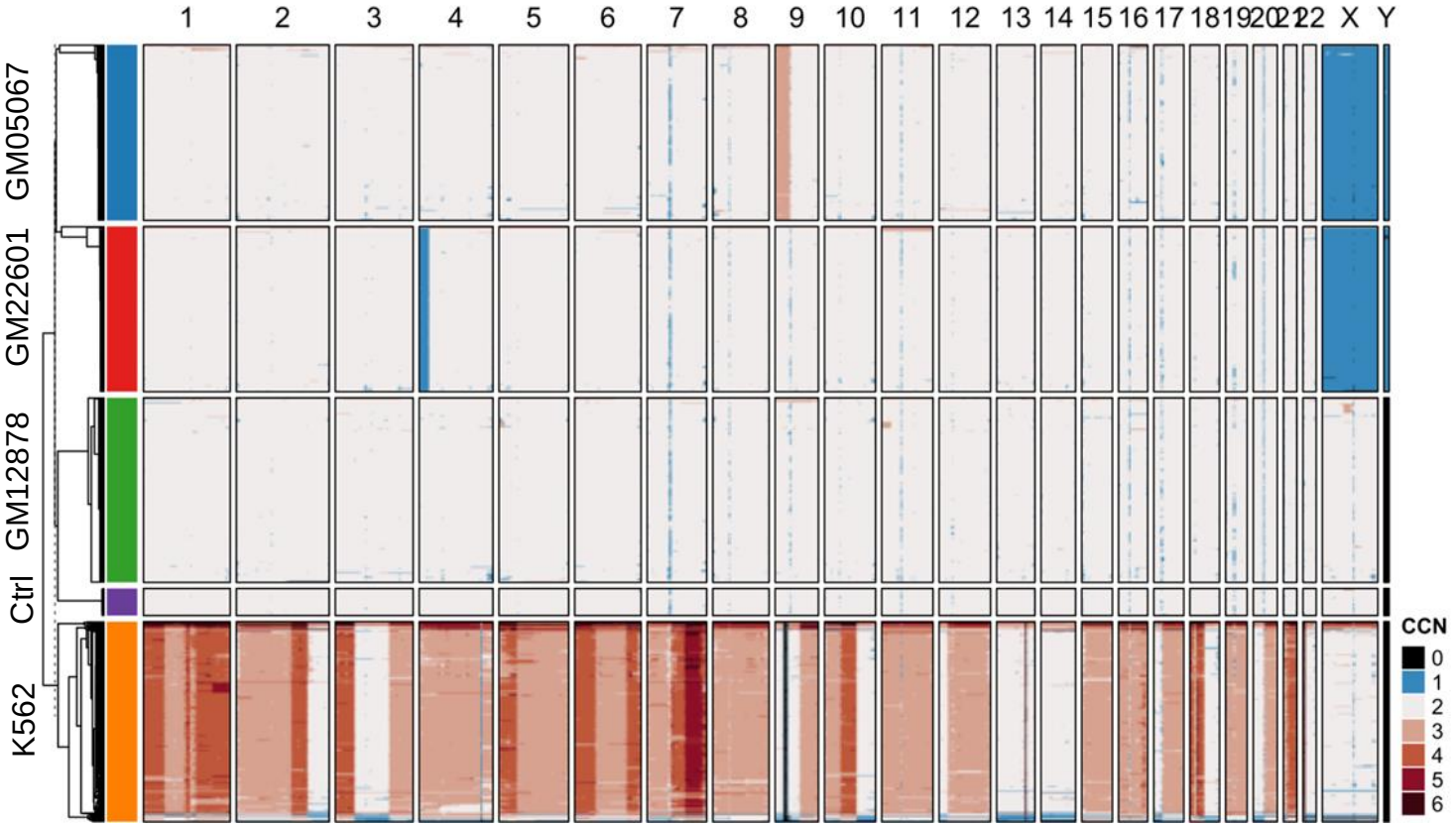






Created with BioRender.com

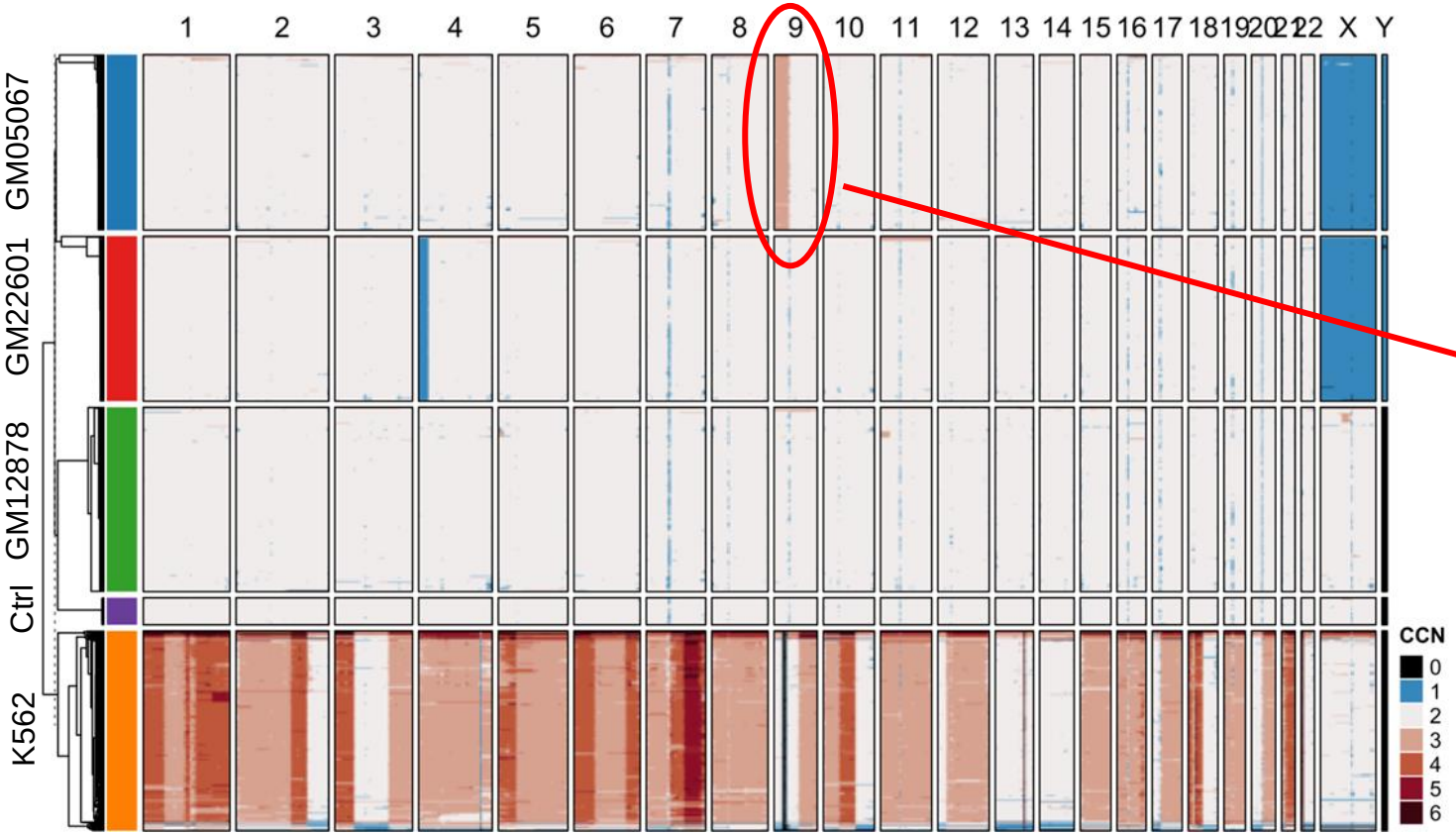
Get CNV profiles of >1,000 cells in one run



- 1,124 single cells
- 4 cell lines;
  - GM05067
  - GM22601
  - GM12878
  - K562

250,000 reads/cell, 2 x 75 bp, 1Mb average bin size

Get CNV profiles of >1,000 cells in one run



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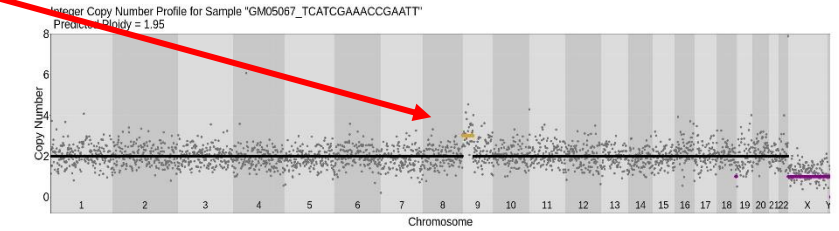

250,000 reads/cell, 2 x 75 bp, 1Mb average bin size

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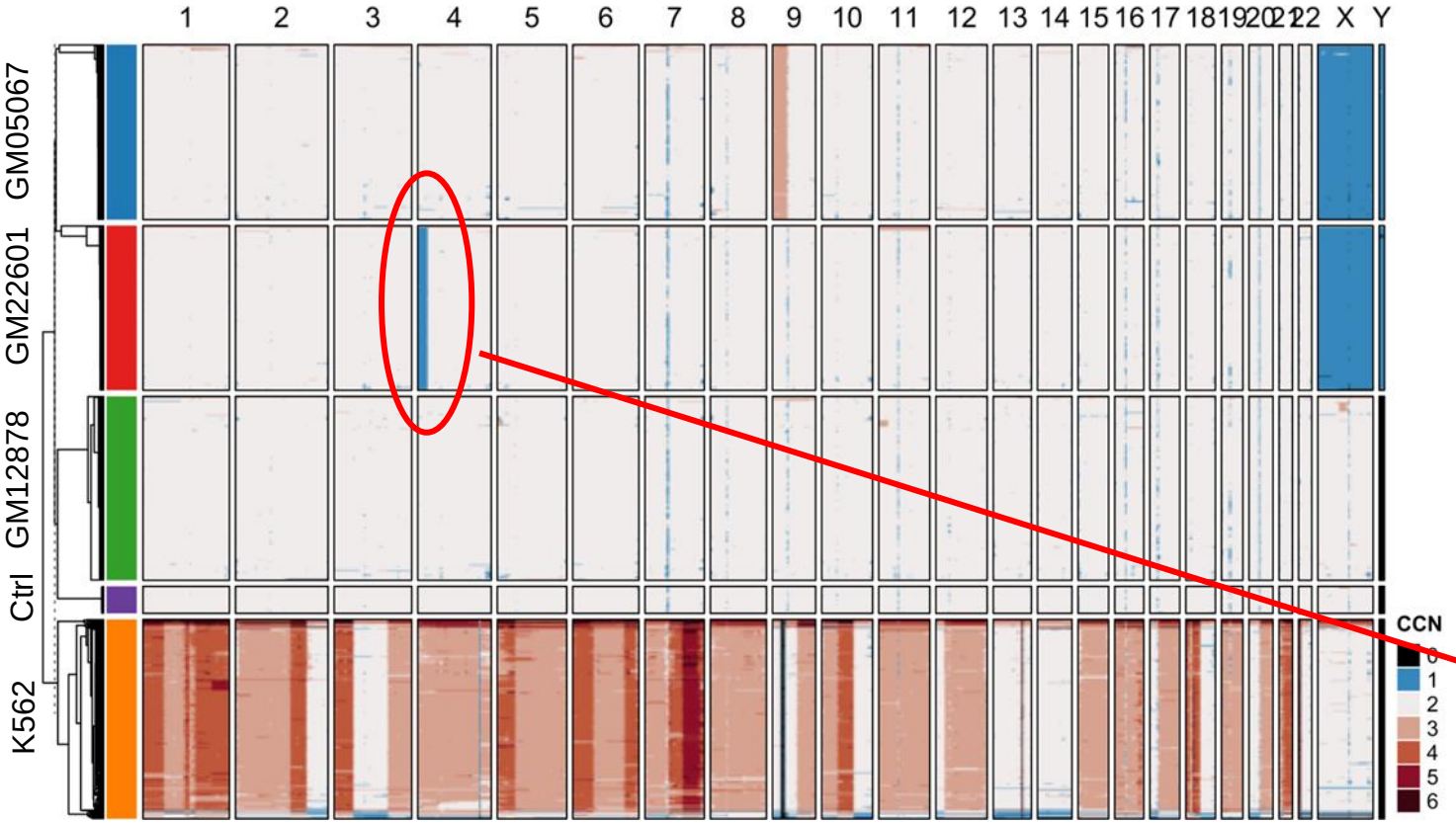

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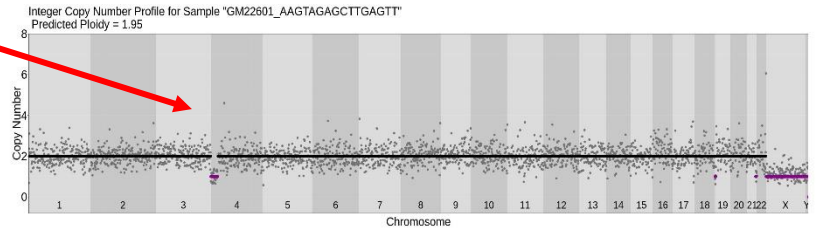
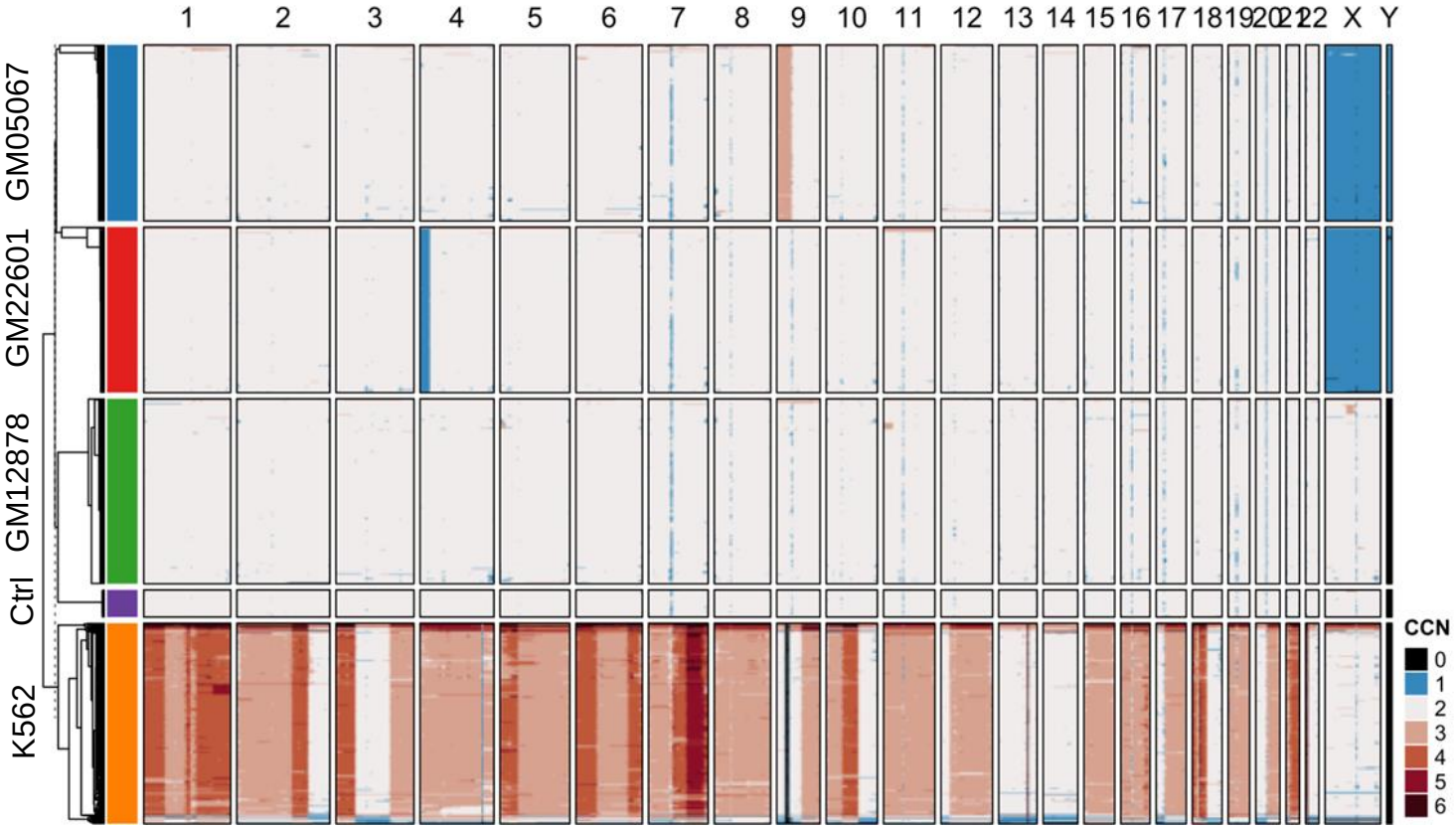
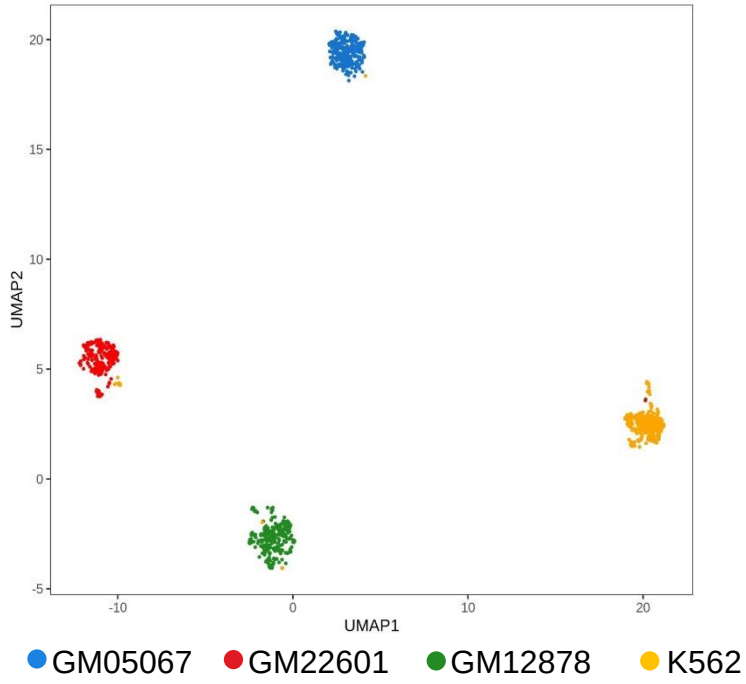
  - GM12878
  - K562



250,000 reads/cell, 2 x 75 bp, 1Mb average bin size

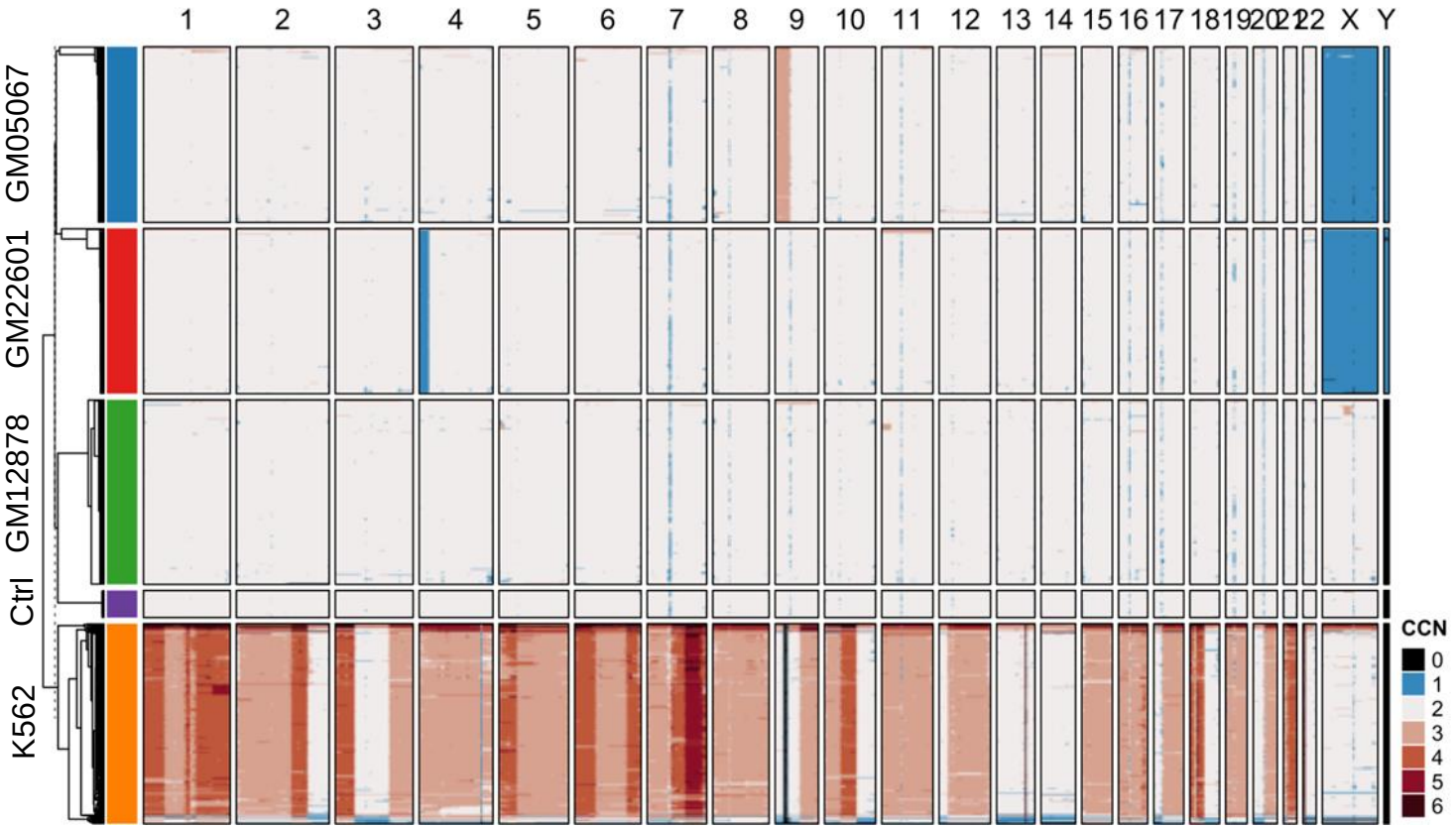
Cells can be clustered based on their CNV profiles



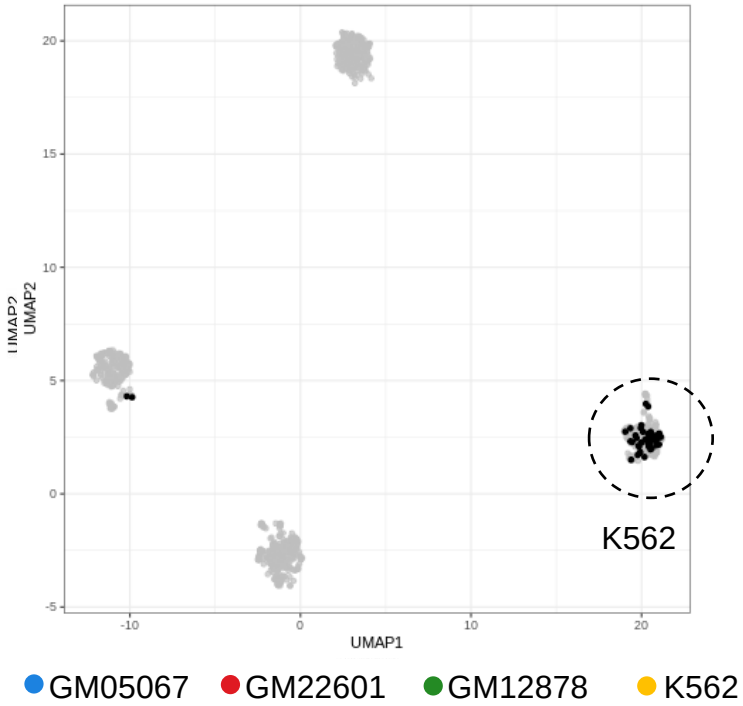


Dou, J., et al. Single-nucleotide variant calling in single-cell sequencing data with Monopogen. *Nat Biotechnol* 42, (2024). https://doi.org/10.1038/s41587-023-01873-x

Pseudobulk analysis identifies putative SNVs in single cells



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



Identification of putative SNV for each single cell based on pseudobulk SNV analysis using Monopogen.

Dou, J., et al. Single-nucleotide variant calling in single-cell sequencing data with Monopogen. Nat Biotechnol 42, (2024). https://doi.org/10.1038/s41587-023-01873-x

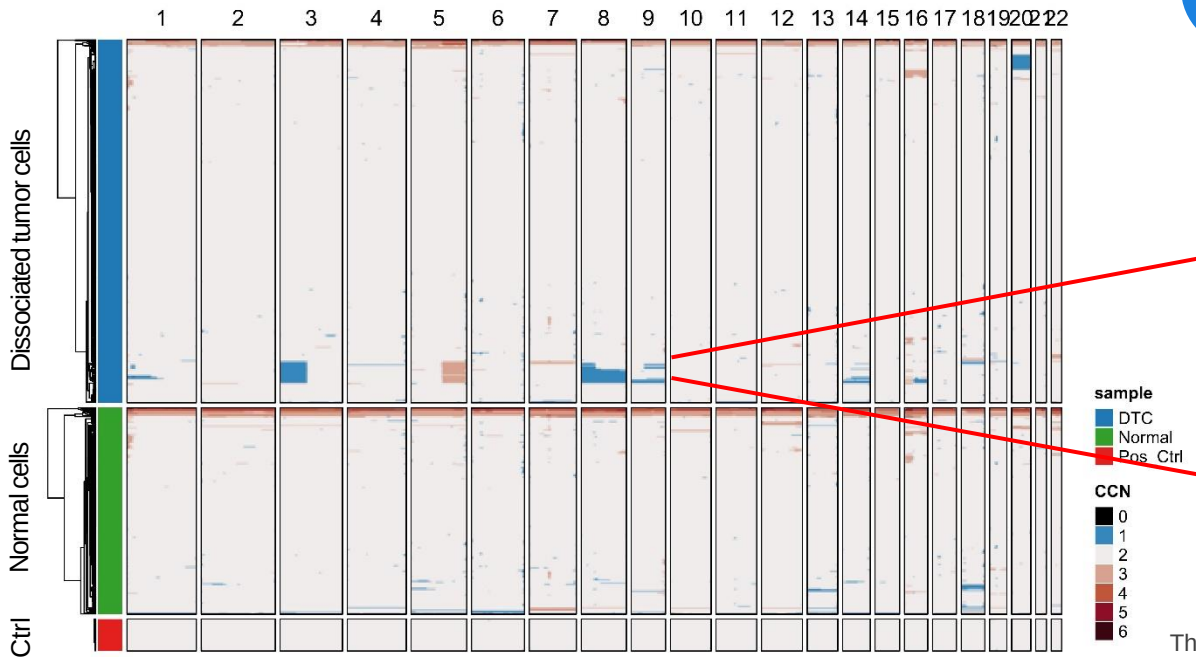
Find CNV events in small subclones in a heterogeneous tumor sample

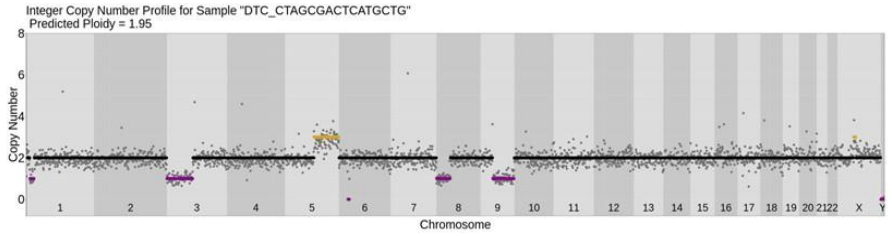
- Dissociated cells from Stage III (858 cells) clear-cell renal-cell carcinoma (ccRCC) tumor and adjacent normal tissue
- ~370,000 reads/cell, 2 x 75 bp

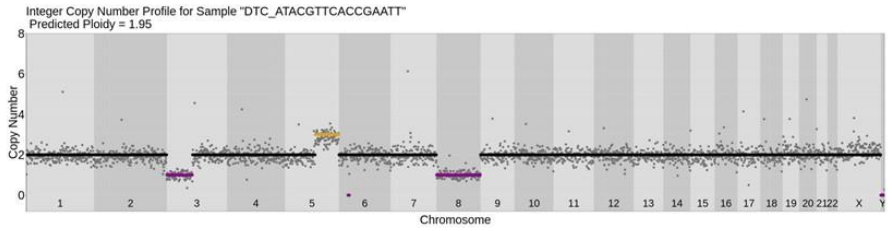
−3p: a cytogenic hallmark of ccRCC, encompassing four commonly mutated genes: *VHL*, *PBRM1*, *BAP1*, and *SETD2* (Creighton et al. 2013).

+5q: associated with better patient survival (Creighton et al. 2013).

Partial or complete loss of chr. 8: associated with *TCEB1* mutations (Sato et al. 2013).







The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature* 499, (2013). https://doi.org/10.1038/nature12222

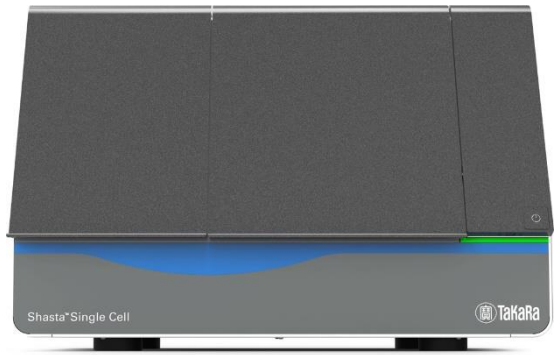
Sato, Y., et al. Integrated molecular analysis of clear-cell renal cell carcinoma. *Nat Genet* 45, (2013). https://doi.org/10.1038/ng.2699

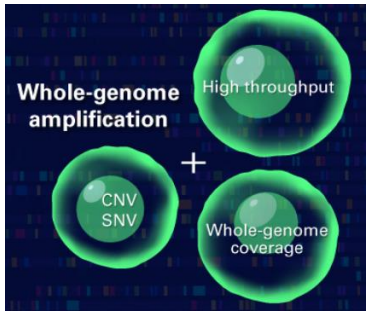
Summary

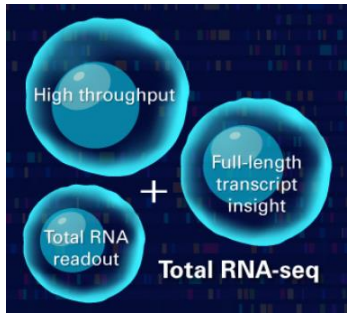
More cells. More biomarkers. More discoveries. More breakthroughs!

- 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

that's GOOD science!®







TaKaRa

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GOOD
science!®