Bruker Spatial Biology: NanoString nCounter Technology

@ Oregon Health & Science University

Bailey Longoni

nCounter Regional Account Manager

October 15th 2024

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We’re here to help!

- **NanoString Support Group**
  - Bailey Longoni – nCounter Regional Account Manager
    - Bailey.Longoni@bruker.com
  - Wes Heydeck – Field Application Scientist
    - Westley.Heydeck@bruker.com

- Free online videos and training: NanoString University (https://university.nanostring.com)
- Support: support.spatial@bruker.com

Tools for Biomarker Discovery and Translational Research

Founded: 2003
Headquartered: Seattle, WA

> 5,000 Publications To Date

nCounter® Analysis System
Launched 2008

GeoMx® Digital Spatial Profiler
Launched 2019

CosMx® Spatial Molecular Imager
Launched 2022

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NanoString nCounter

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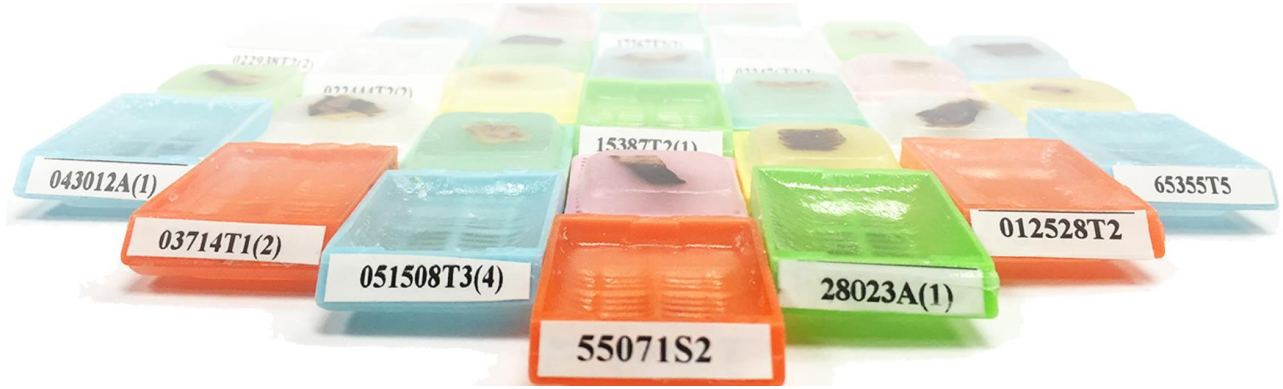
Gene expression measurement is fundamental



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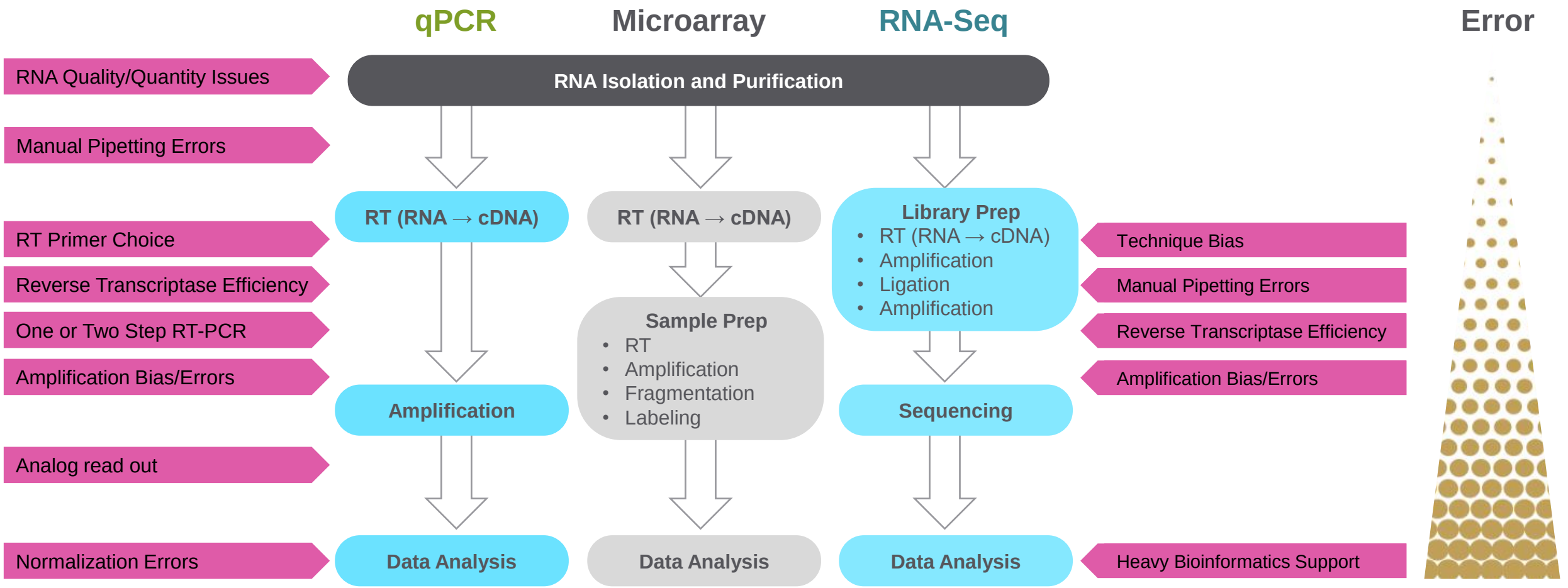
Challenges of Current Gene Expression Workflows



qPCR, microarray & RNA-Seq workflows contain numerous steps where variability can be introduced into the data

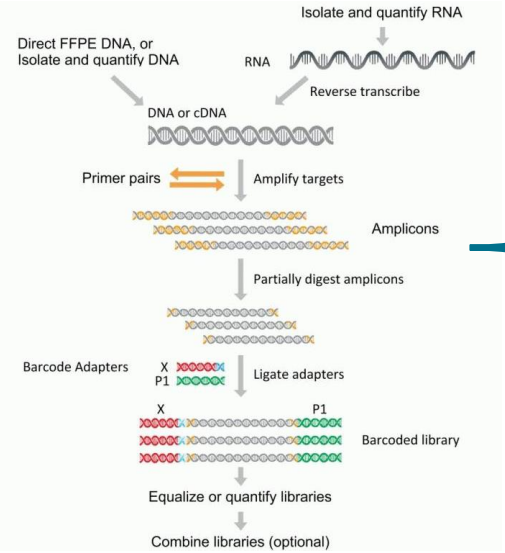
nCounter Helps RNA-Seq Users Validate Their Findings During Translational Research

Hypothesis formation using whole transcriptome discovery

Validate findings using a technology well suited for translational research

If you can't orthogonally validate your results, are they valid?

Complex Workflow



Sources of Variation

- Technique Bias
- Manual Pipetting Errors
- Reverse Transcriptase Efficiency
- Amplification Bias/Errors

RNAseq Struggles with Clinical Samples

RESEARCH ARTICLE

A Comparison of RNA-Seq Results from Paired Formalin-Fixed Paraffin-Embedded and Fresh-Frozen Glioblastoma Tissue Samples

Anna Esteve-Codina[1], Oriol Arpi[2], Maria Martinez-García[3], Estela Pineda[4], Mar Mallo[5], Marta Gut[6], Cristina Carrato[7], Anna Rovira[2], Raquel Lopez[8], Avelina Tortosa[9], Marc Dabad[1], Sonia Del Barco[10], Simon Heath[1], Silvia Bagué[11], Teresa Ribalta[12], Francesc Alameda[13], Nuria de la Iglesia[14], Carmen Balaña[15]*, on behalf of the GLIOCAT Group[¶]

In this RNA-Seq study, informative results were obtained from only 3 out of 11 matched FF& FFPE samples

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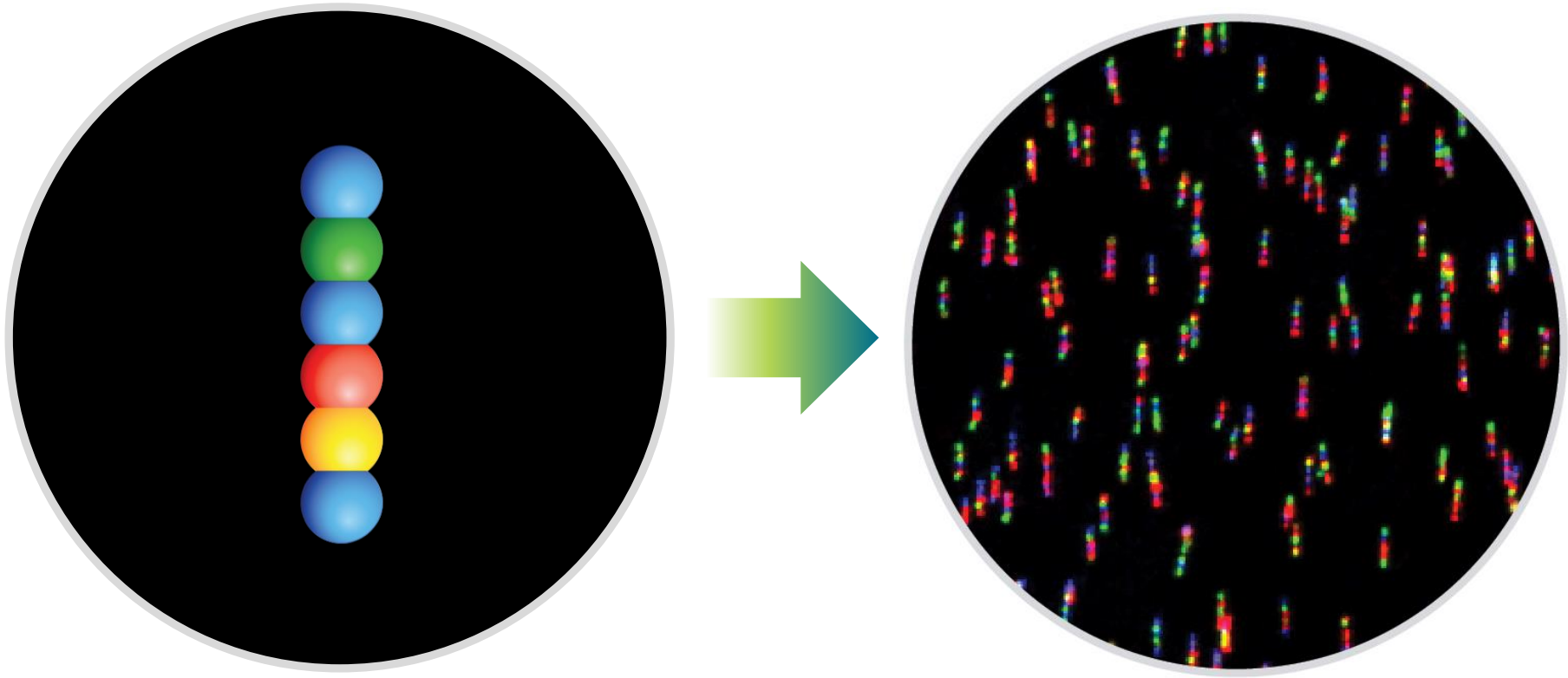
Molecules That Count®

Detection of individual nucleic acid molecules via hybridization to a fluorescent barcode



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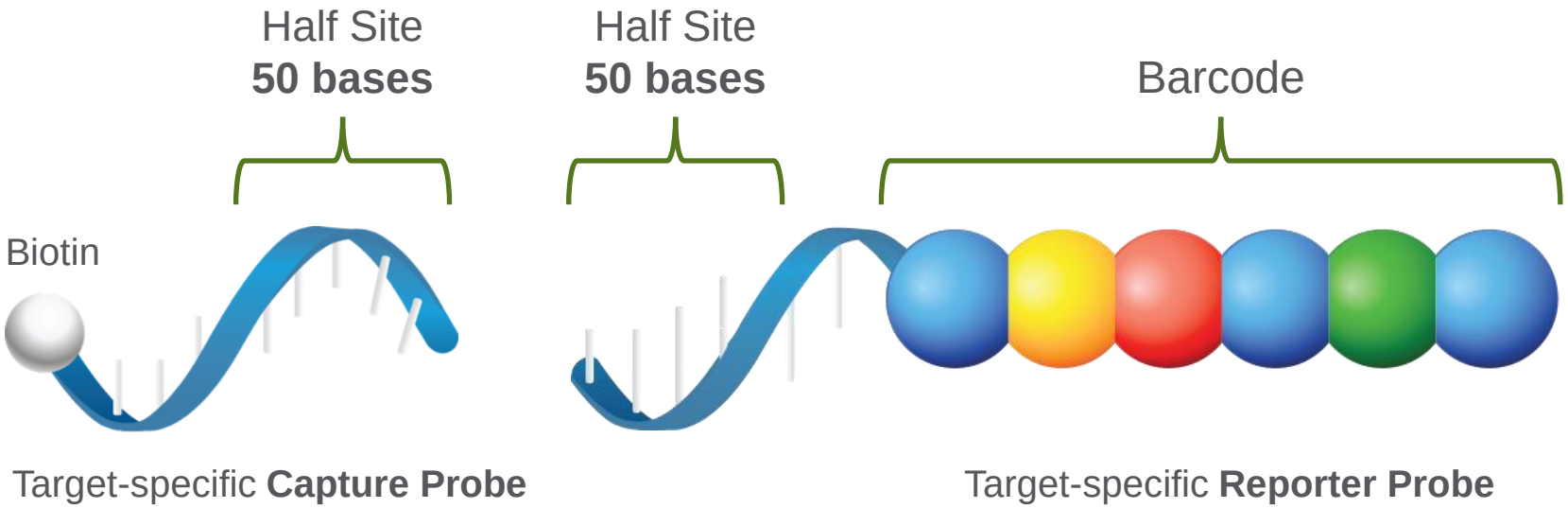
Standard nCounter barcode chemistry



Barcode	Identity
	XLSA
	FOX5
	PDCD1

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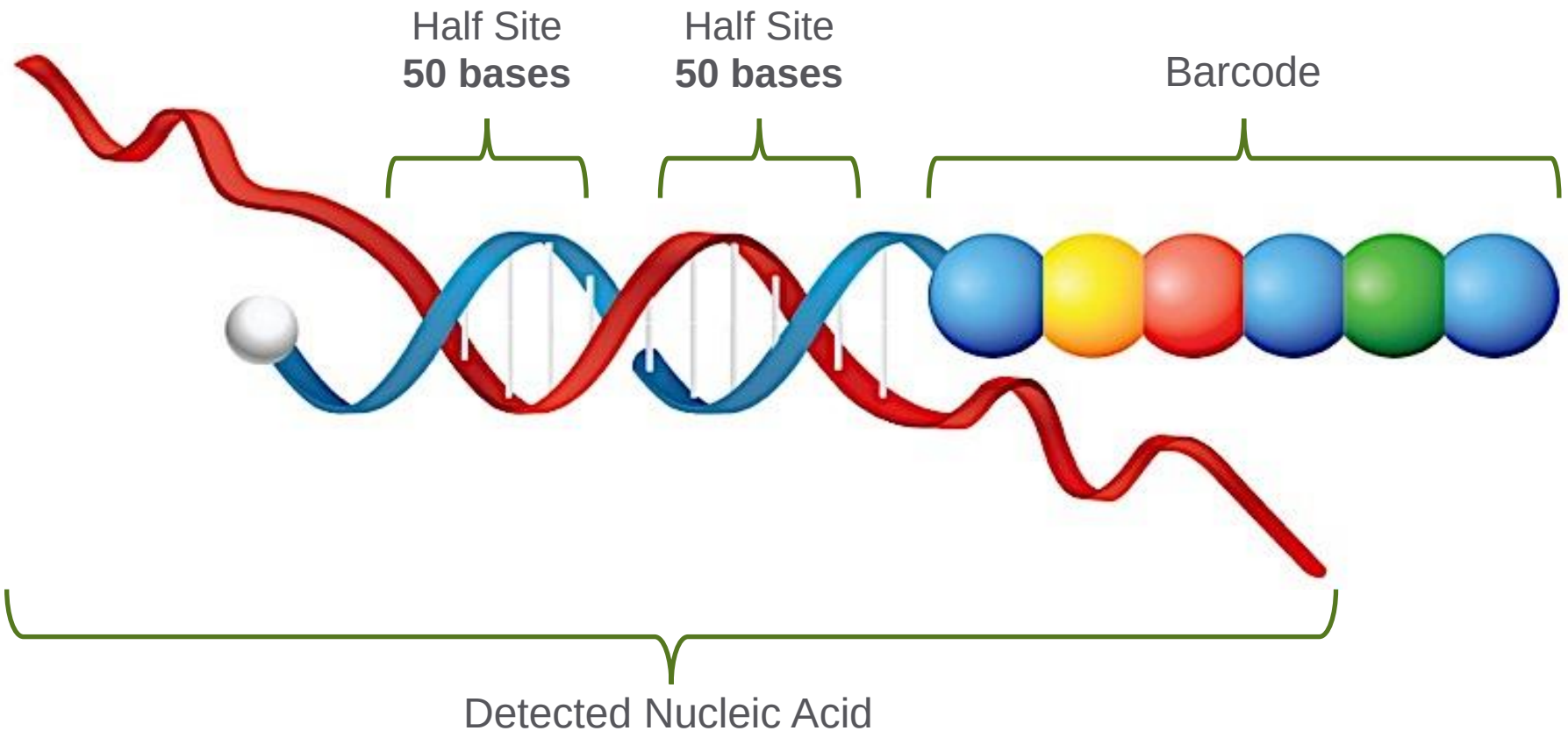
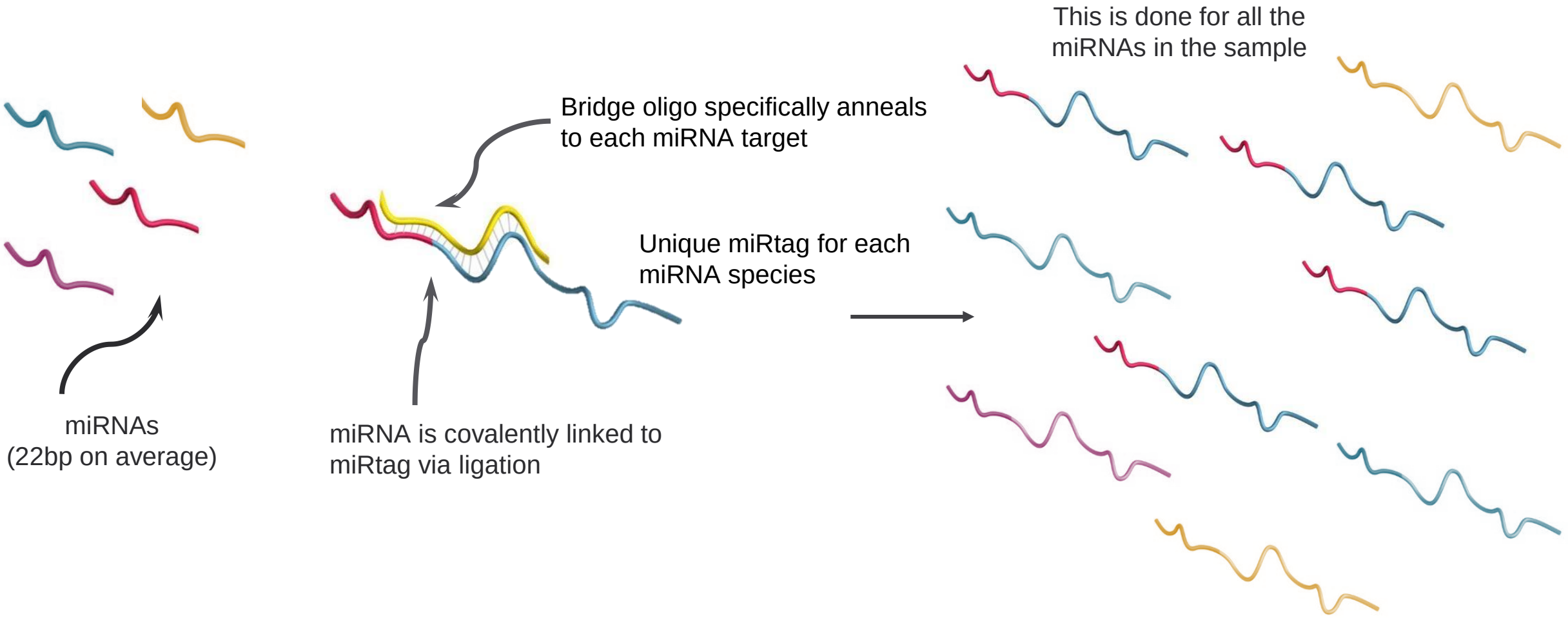
Standard nCounter barcode chemistry



The nCounter® miRNA Assay Adapts the Dual Probe System to Detect Small RNAs



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Automated digital profiling in two simple steps

5 min HANDS-ON — Day **1**

10 min HANDS-ON — Day **2** AUTOMATED

Hybridize

- Flexible sample requirements
- Only 4 pipetting steps
- No amplification
- 800 hybridizations in single tube

Purify & Count

nCounter® SPRINT

- Sensitive
- Precise
- Quantitative
- Simple

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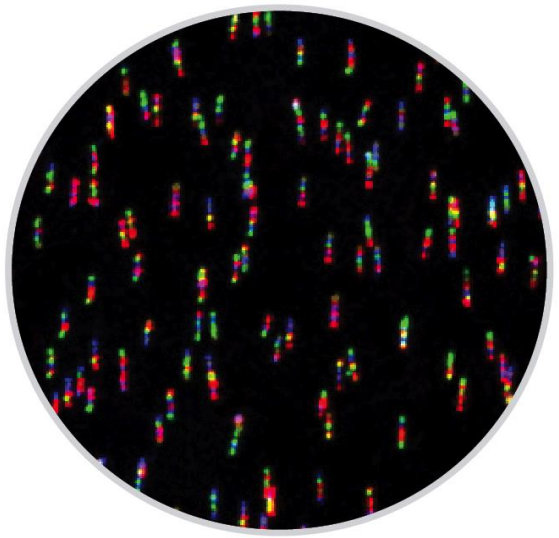
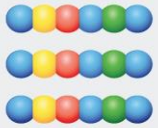
TM

Digital output: 1 molecule = 1 count



Codes are Counted and Tabulated

Barcode	Counts	Identity
	3	XLSA
	2	FOX5
	1	INSULIN

	Sample 1	Sample 2	Sample 3
Assay number	**1**	**2**	**3**
Sample ID	**PW1**	**PW2**	**PW3**
Reporter Counts			
Gene Name			
POS_A(128)	16874	16930	17013
POS_B(32)	4122	4134	4105
POS_C(8)	1144	1087	1078
POS_D(2)	379	362	357
POS_E(0.5)	82	85	55
POS_F(0.125)	36	38	28
NEG_A(0)	14	5	5
NEG_B(0)	15	17	16
NEG_C(0)	14	13	7
NEG_D(0)	23	20	31
NEG_E(0)	20	13	18
NEG_F(0)	13	7	8
NEG_G(0)	16	9	11
NEG_H(0)	27	20	9
G6PD	9958	11129	11059
GUSB	2443	2747	2843
HPRT1	3801	4311	4184
TBP	1221	1311	1359
POLR1B	1068	1124	1114

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Data Analysis

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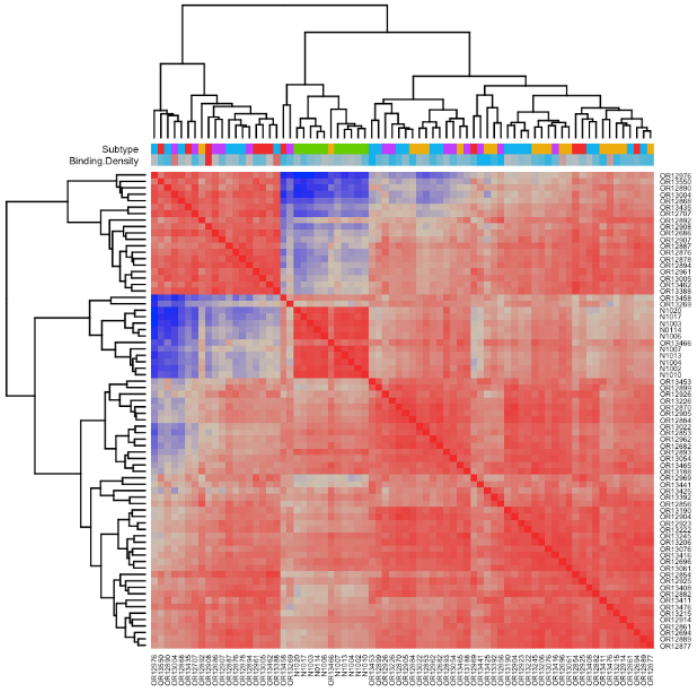
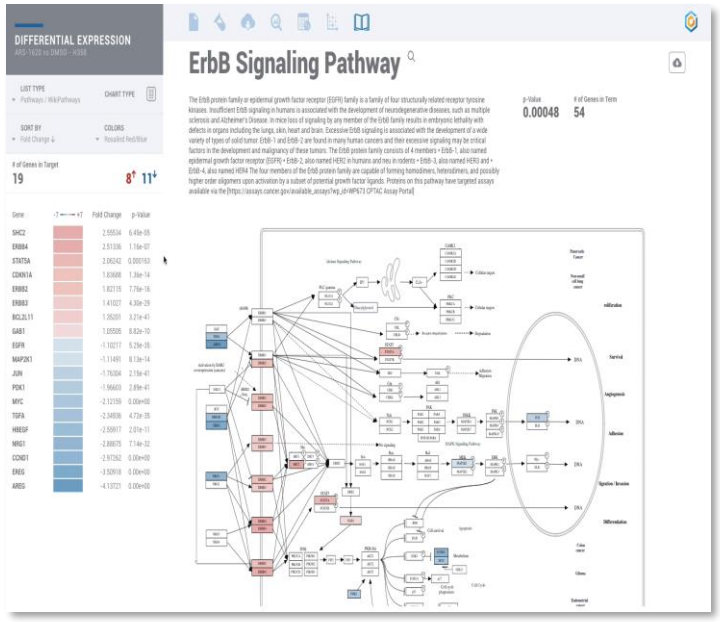
Data Analysis Analysis Tools Eliminate Time Spent Waiting for Results

nSolver



Differential expression analysis, PCA analysis, cell type profiling, pathway scoring, etc.

ROSALIND®: Cloud-Based Unified Data Analysis Platform



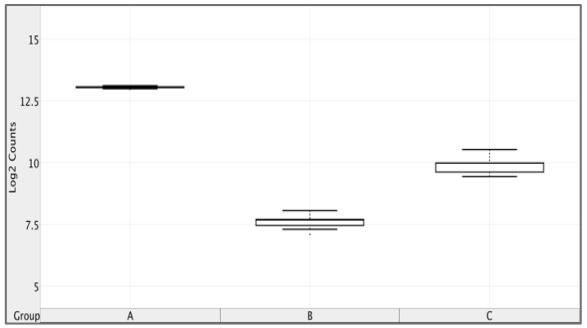
Evolving knowledge base (50+)
Shared collaboration spaces

ROSALIND

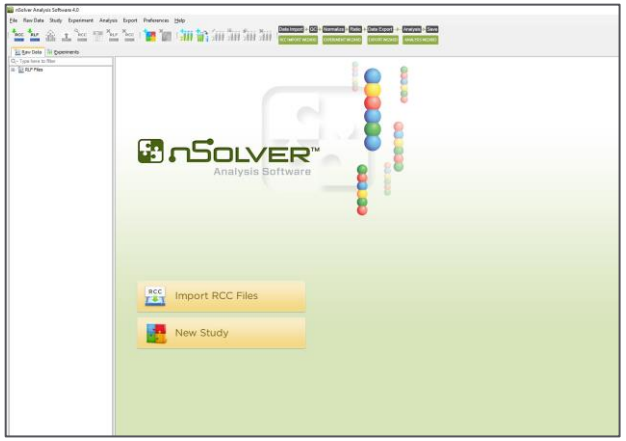
Automated Data Analysis with nSolver™ Software

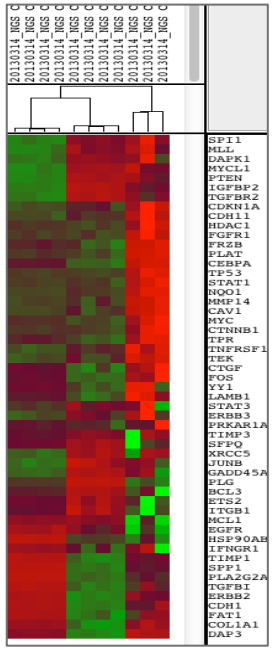
- Rapid data QC and normalization
- Advanced analysis modules look at differential expression, pathways, cell type profiling and more
- Compatible with standard analysis programs for existing workflow

Digital Data Output: Simple Visualizations via nSolver™ 4.0

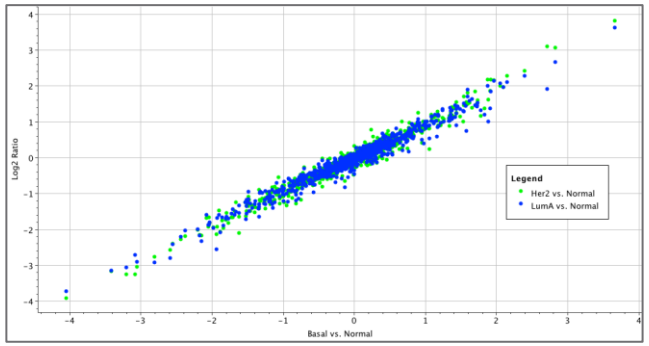


Box Plot by Treatment Group

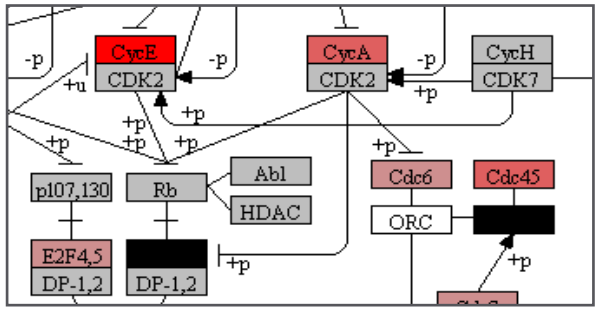




Clustering and Heat Map



Scatter Plot of All Genes



New Universal Advanced Analysis Module

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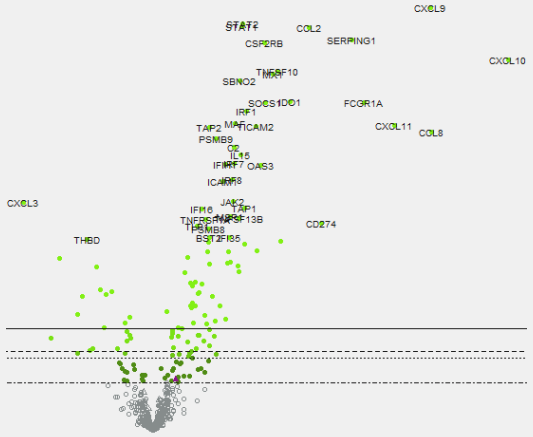
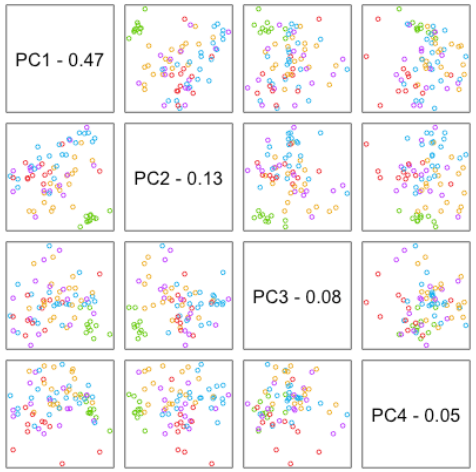
Simplified Data Analysis – nSolver™ Advanced Analysis

nCounter Advanced Analysis is a free, **easy-to-use** add-on to nSolver Basic Software for deeper data insights based on robust R statistics.

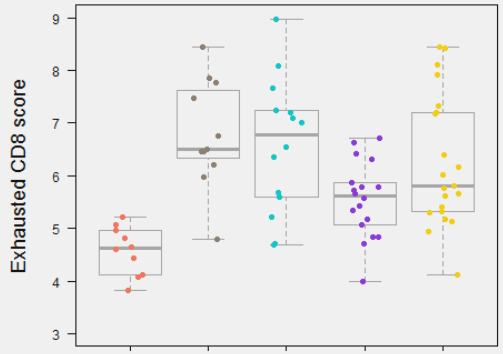
Differential Expression Analysis



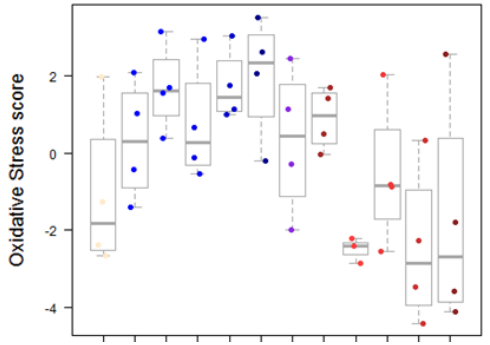
PCA Analysis



Cell Type Profiling



Pathway Scoring



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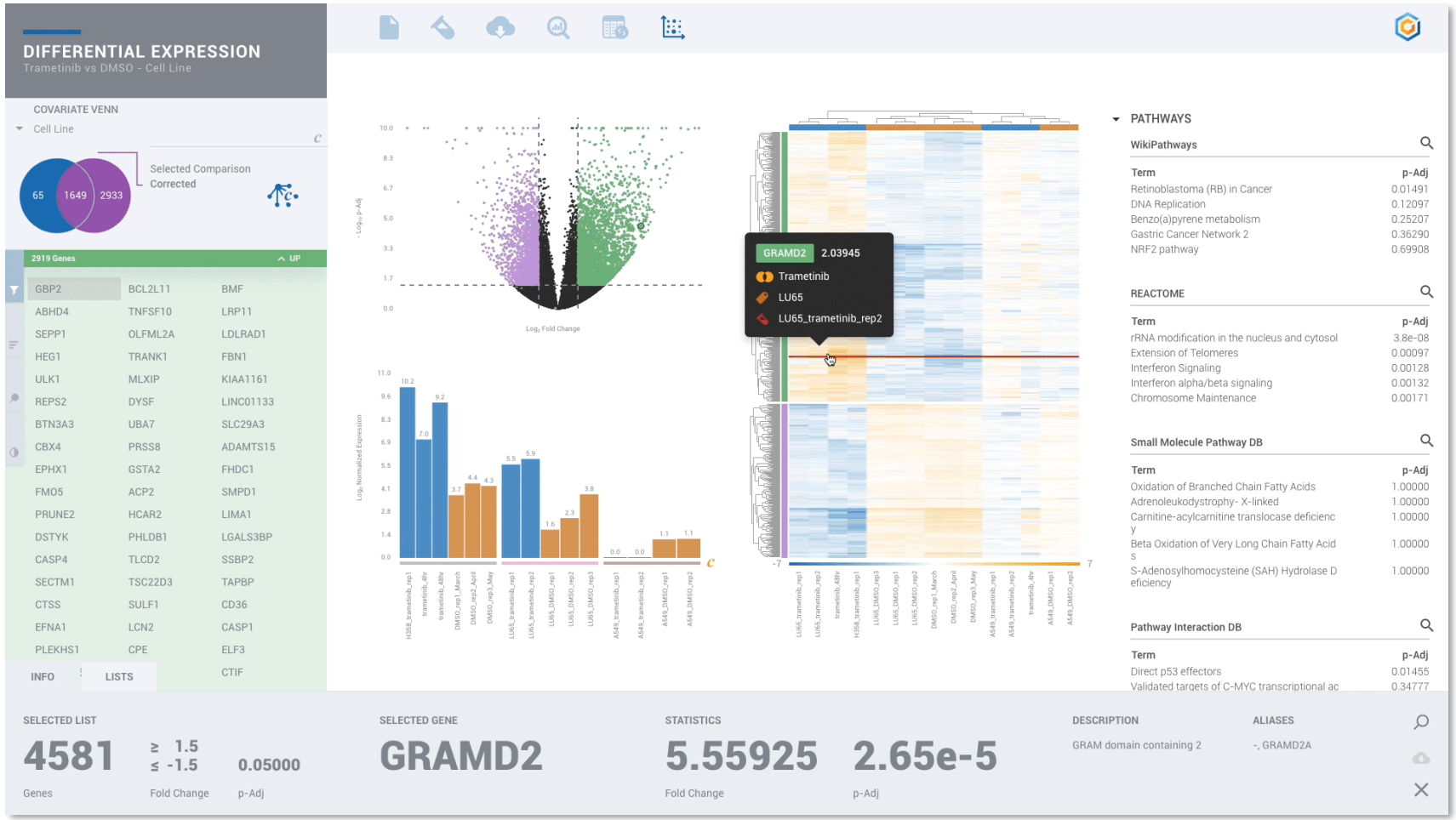
INTERACTIVE VISUALIZATIONS
INTERPRET YOUR DATA LIKE A BIOINFORMATICIAN



Empower researchers to:

- ✓ Reduce the time required to get from sequencing to data interpretation
- ✓ Enjoy an engaging user experience to interactively explore results and pathways
- ✓ Setup experiments and explore interactive results the very same day
- ✓ Create new comparisons and change cut-offs in minutes
- ✓ No programming expertise required

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COLLABORATE EFFECTIVELY
AGGREGATE AND ANALYZE SILOED DATASETS

- Breakdown Barriers to Collaboration
  - ✓ Collaborate in real-time, any where in the world, with consistent access to shared experiments for all participants
  - ✓ No need to download data or transfer data
  - ✓ Collaborate with an engaging user experience, not offline CSV files

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Performance

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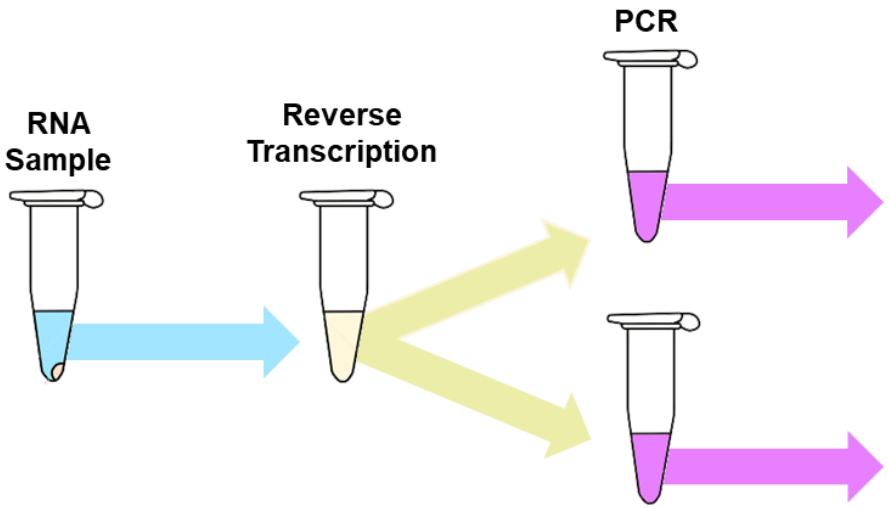
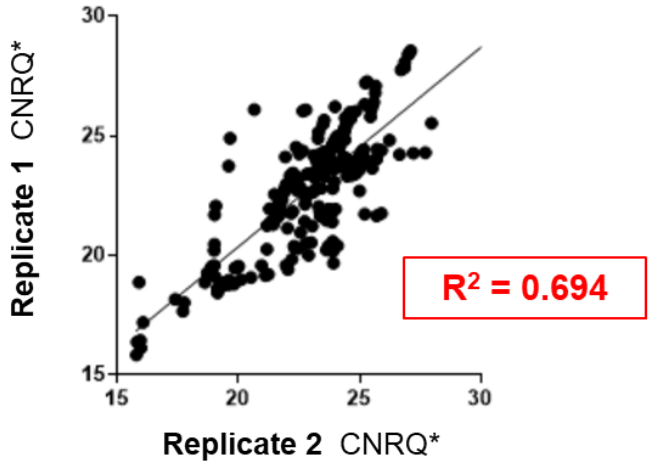
Technical reproducibility

mitobridge

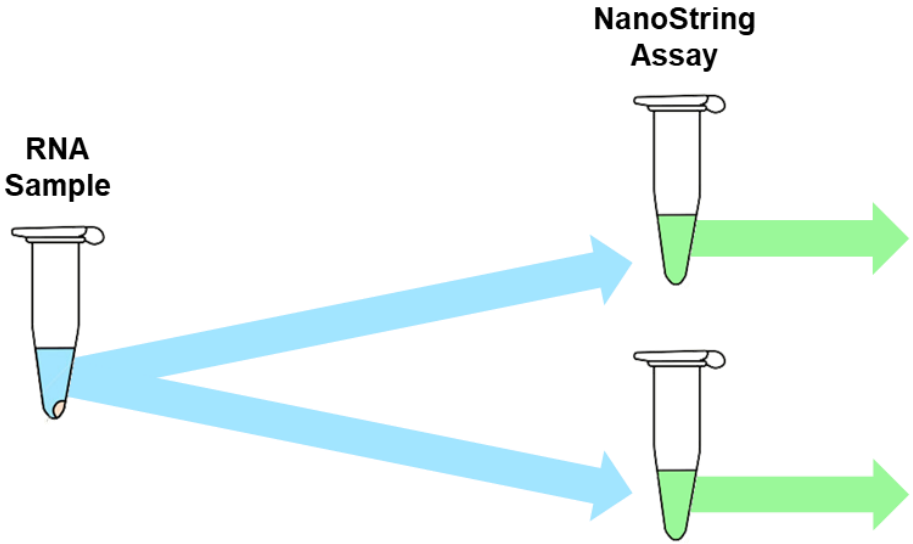
qPCR

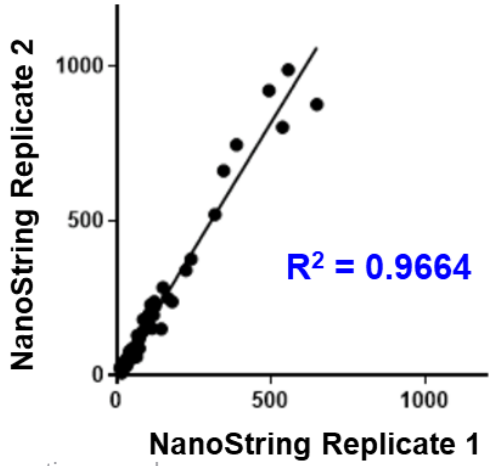




*CNRQ=Calibrated Normalized Relative Quantity (qbase+ software)
Data shown in this experiment are representative of many runs

nCounter





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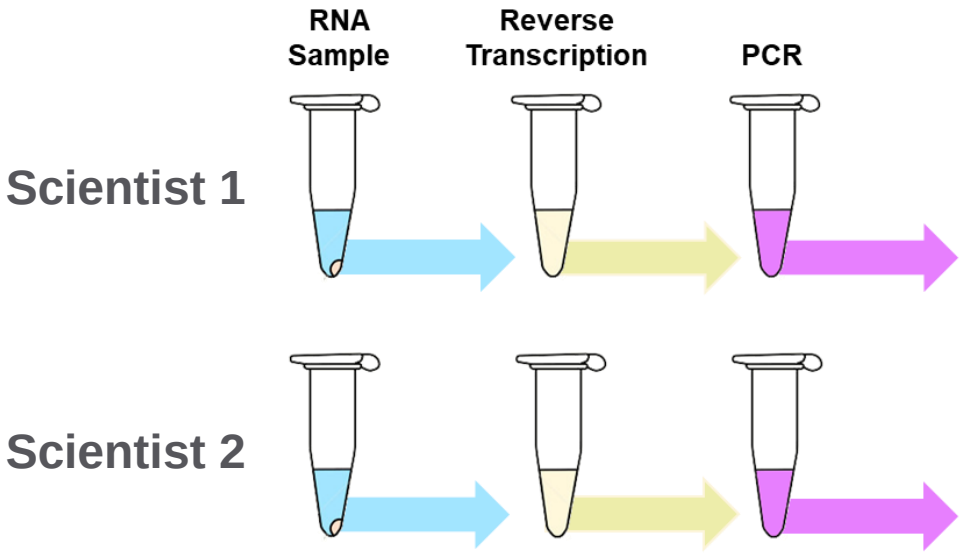
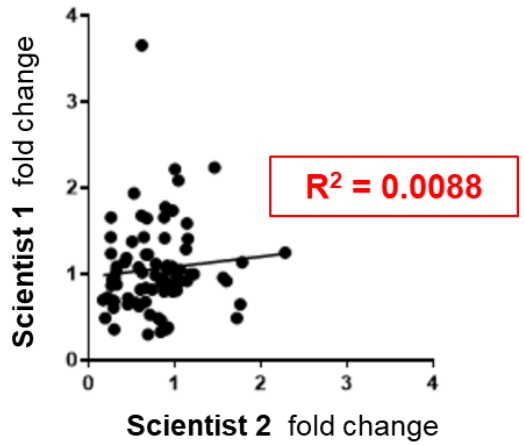
Reproducibility between researchers

qPCR





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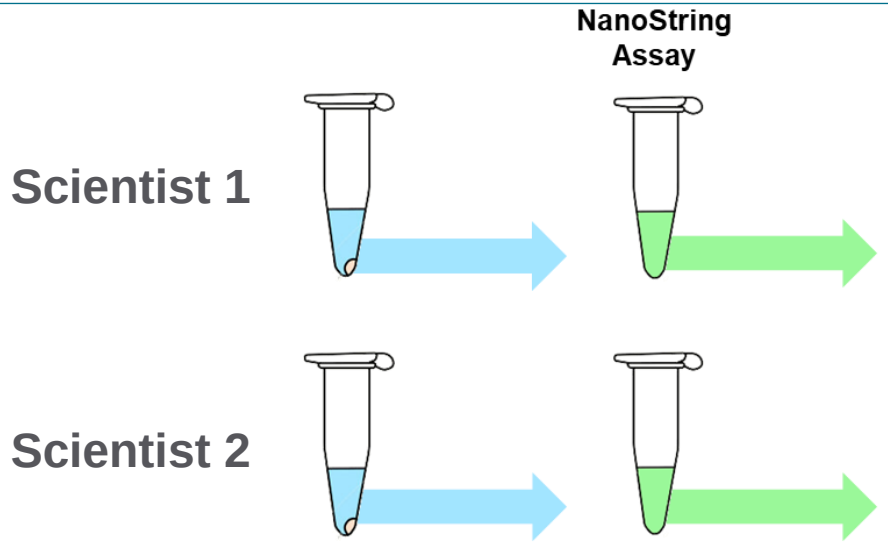

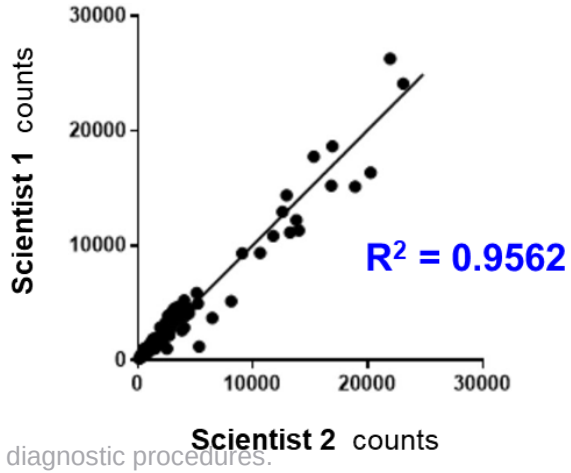

Reproducibility between researchers

qPCR

No need for technical replicates!

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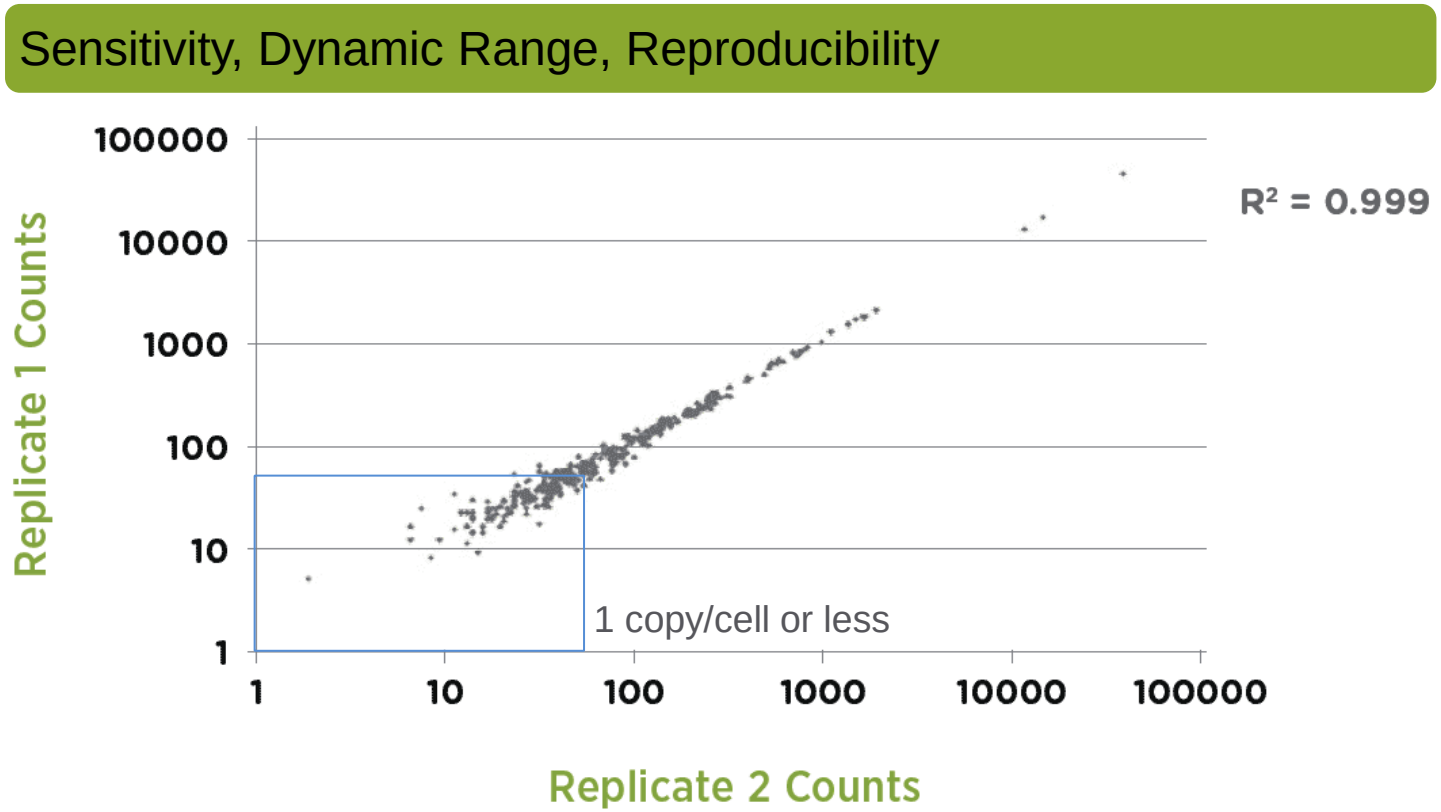
High sensitivity and dynamic range



- Reproducible and linear over a wide dynamic range (5-6 logs)
- Note the reproducibility even at very low expression levels

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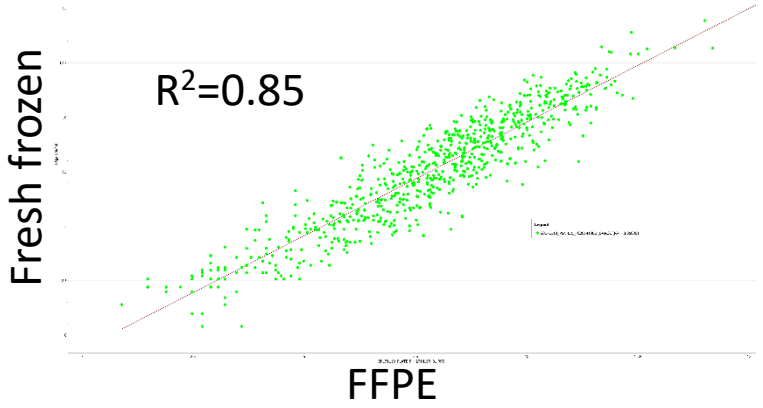
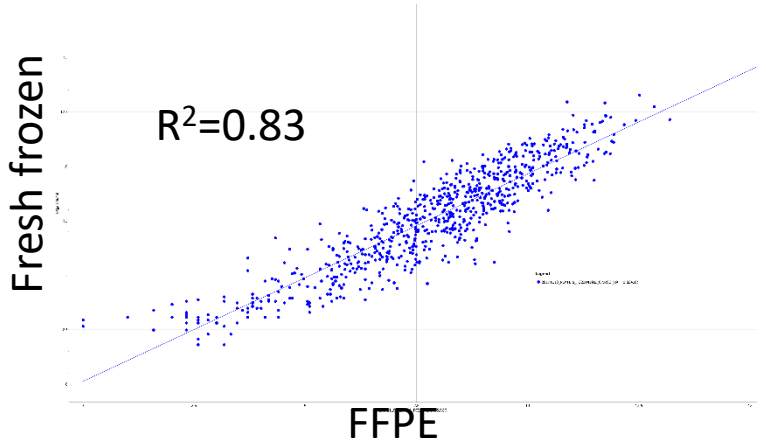
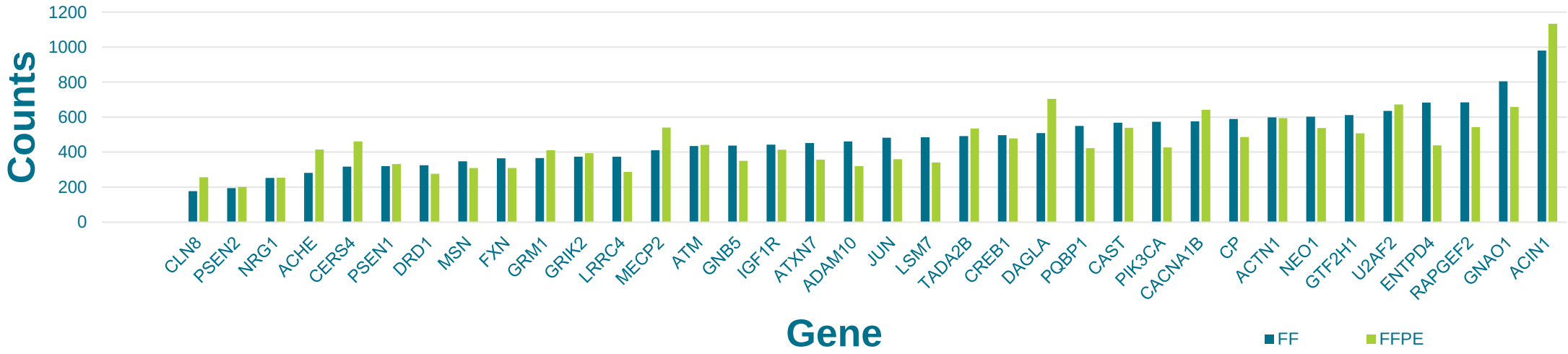
Consistent performance with varying sample preparation







Sample Inputs

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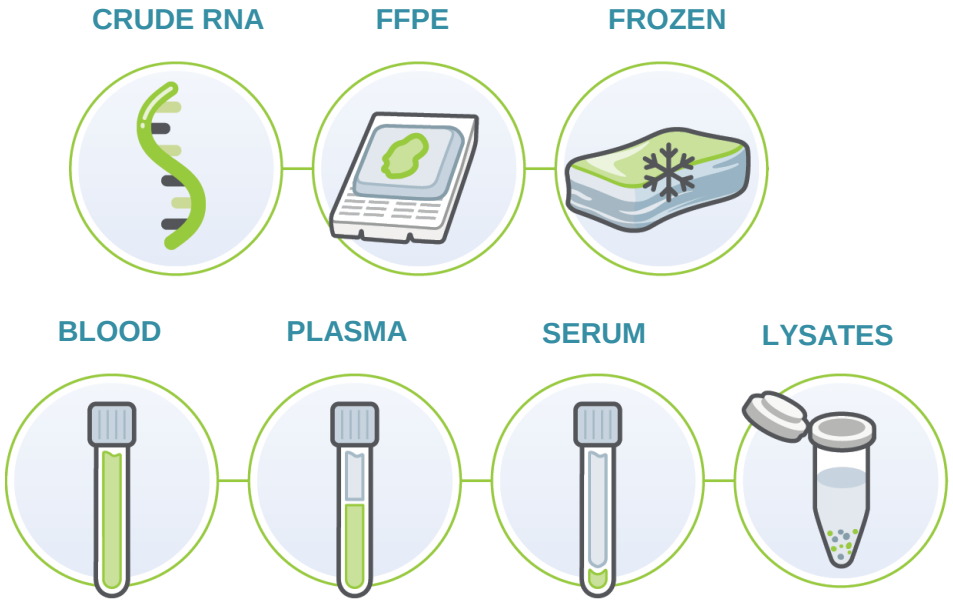
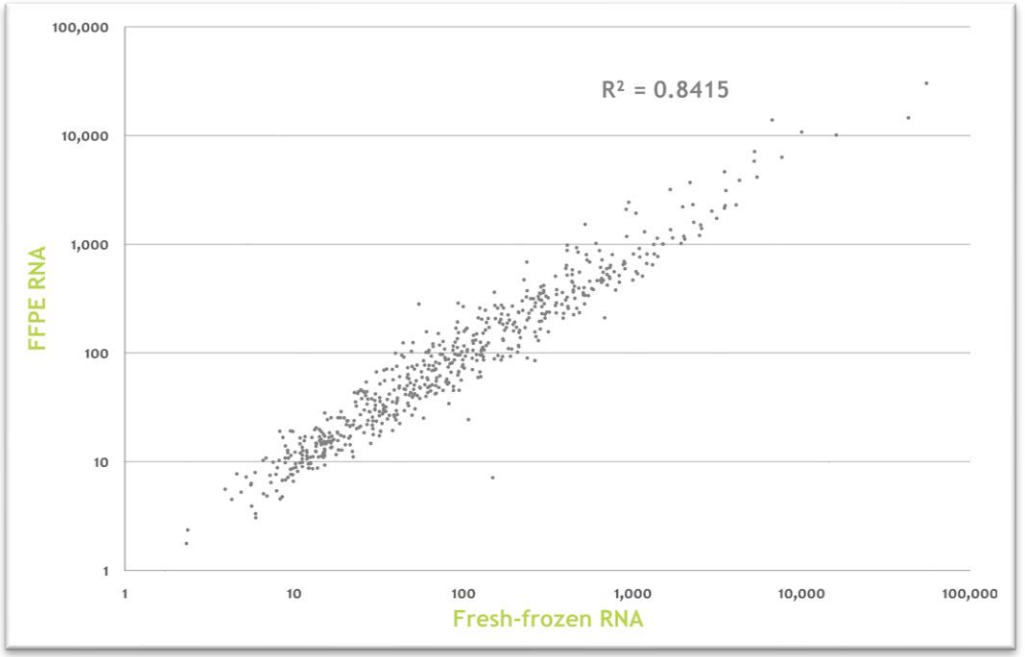
Challenging Sample Types Are No Challenge for nCounter

Broad Sample Compatibility



Even Low-Quality Samples Succeed



Decades Old FFPE Directly Correlates with FF

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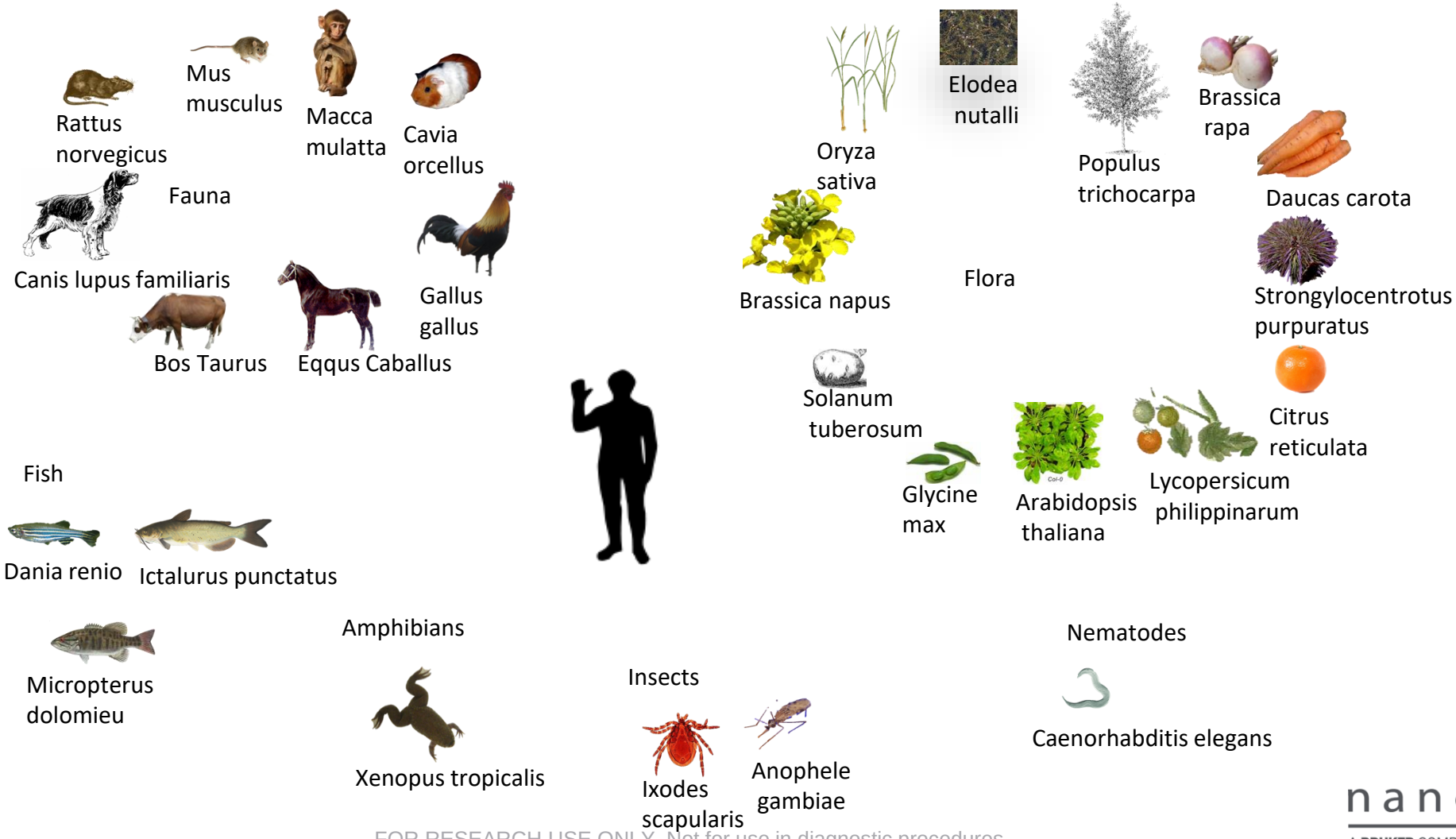
General guidelines for NanoString nCounter samples

- Good quality RNA
  - A_{260}/A_{280} ratios ~ 2.0
  - A_{260}/A_{230} ratios ~ 2.0
  - DV200 = 50%
- Typical inputs for gene expression assays from high quality samples
  - SPRINT system: 50 ng (25 - 150 ng)
- Keep in mind only ~5% of total RNA is mRNA!

RNA is RNA is RNA…It's not just human



Gene Expression Panels

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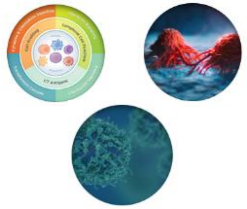
Panels – Ready to use – or make your own!

Oncology

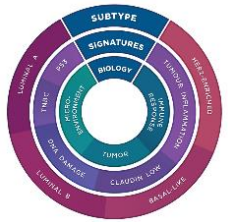
PanCancer
pathways, immune, & progression



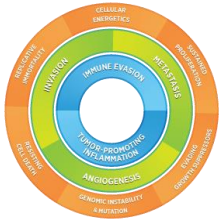
Canine IO

IO360

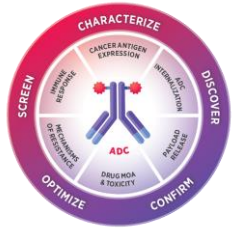
BC360



TS360



ADC Development

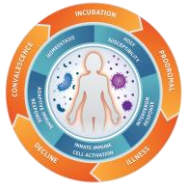


Immunology

Generic Immunology and Inflammation Panels

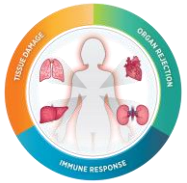
Autoimmune Profiling

Host Response

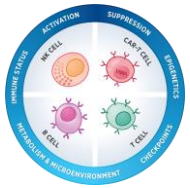


Fibrosis

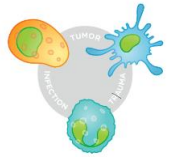
Human Organ Transplant



Immune Exhaustion

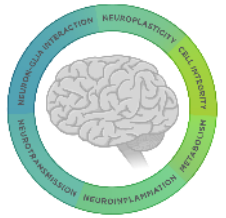


Myeloid Innate Immunity

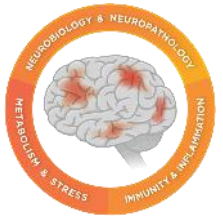


Neuroscience

Neuropathology



Neuroinflammation



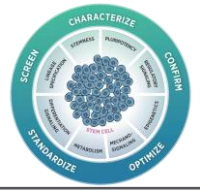
Glial Profiling

Add your own targets to a panel!

Spike in up to 55 additional targets

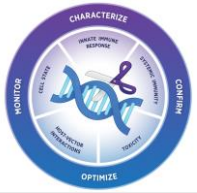
Multi-Market

Stem Cell Characterization

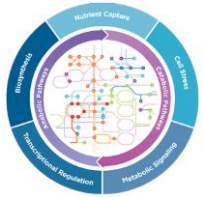


CAR-T Characterization

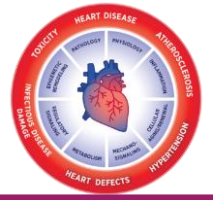
Gene Therapy Optimization



Metabolic Pathways



CVD Pathophysiology



And more!

miRNA

Custom

Elements™

PlexSet™

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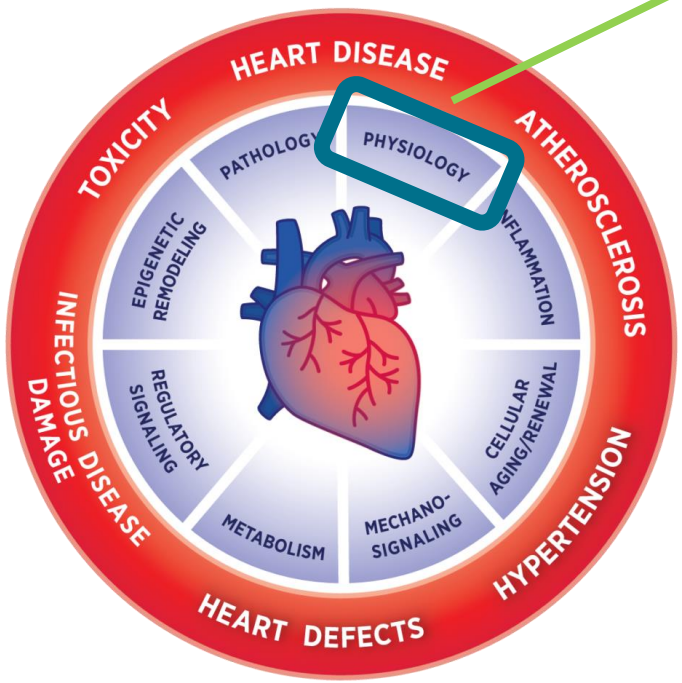
Cardiovascular Disease Pathophysiology Panel



Cardiovascular Pathology	Cardiovascular Physiology	Vascular Inflammation	Cellular Aging & Renewal	Metabolism	Mechano Signaling	Regulatory Signaling	Epigenetic Remodeling
Atherosclerosis	Angiotensin System	eNOS Activation	Apoptosis	Fatty Acid Metabolism	ECM Remodeling	Calcium Signaling	Histone Modifications
Cardiac Hypertrophy	Cardiac Muscle Contraction	IL-1 Signaling	Autophagy	Glucose Metabolism	Hippo Signaling	EGFR Signaling	Acetyl Transferases
Cardiomyopathy	Cardiac Electrophysiology	IL-6 Signaling	Cell Cycle	Hypoxia Response	Integrin Signaling	MAPK Signaling	Deacetylases
Foam Cell Formation	GABAergic Signaling	Other Cytokine Signaling	Senescence & Quiescence	Lipid Metabolism	Rho ROCK Signaling	Notch Signaling	Methyl Transferases
Ischemia	Vascular Smooth Muscle Contraction	Immune Cell Infiltration	Telomere Maintenance	Cholesterol Metabolism		TGF-beta Signaling	
Myocarditis	Vasopressin System	JAK-STAT Signaling		Lipoprotein Clearance		VEGF Signaling	
Thrombosis	Cardiac Morphogenesis	mTOR Signaling		Oxidative Stress Response		Wnt Signaling	
Hemostasis	ER Stress	NF-kappaB Signaling					
Endocarditis		PI3K-AKT Signaling					
Pericarditis		PPAR Signaling					
Hypertension		TLR Signaling					
		TNF Signaling					
		Checkpoint Signaling					

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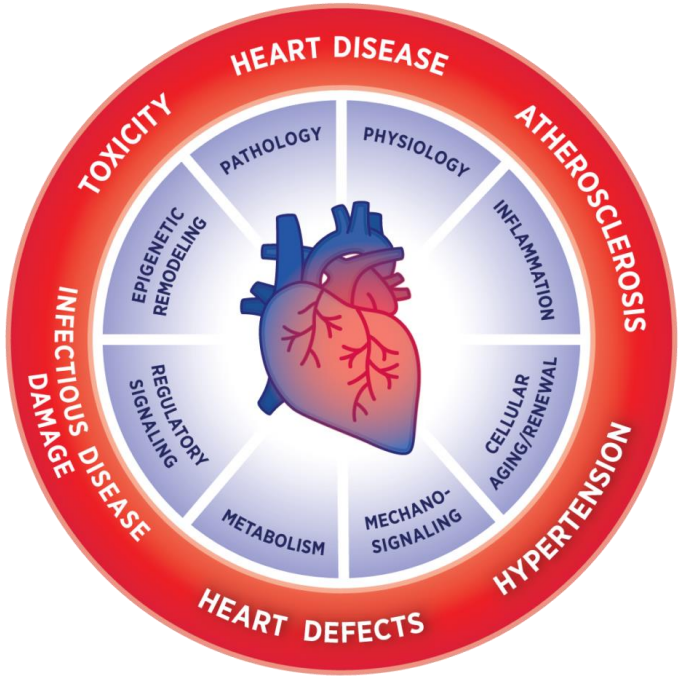
Cardiovascular Disease Pathophysiology Panel



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Thrombosis	Cardiac Morphogenesis	mTOR Signaling		Oxidative Stress Response		Wnt Signaling	
Hemostasis	ER Stress	NF-kappaB Signaling					
Endocarditis		PI3K-AKT Signaling					
Pericarditis		PPAR Signaling					
Hypertension		TLR Signaling					
		TNF Signaling					
		Checkpoint Signaling					

Gene	Cell Type	Internal Reference Gene	Angiotensin System	Apoptosis	Atherosclerosis
CSK		-	-	-	-
CSNK2B		-	-	-	-
CST3		-	-	-	-
CTF1		-	-	-	-
CTLA4		-	-	-	-
CTNNB1		-	-	-	-
CTNND1		-	-	-	-
CTSA		-	+	-	-
CTSD		-	+	+	-
CTSG		-	+	-	-
CTSW	Cytotoxic Cel	-	-	+	-
CTSZ		-	+	+	-
CX3CL1		-	-	-	-
CXCL12		-	-	-	-
CXCL8		-	-	-	+
CXXC1		-	-	-	-
CYBA		-	-	-	+
CYC1		-	-	-	-
CYCS		-	-	+	+
CYGB		-	-	-	-
CYP27A1		-	-	-	-
CYP2U1		-	-	-	-
CYSLTR2		-	-	-	-
DCN	Fibroblasts	-	-	-	-
DCXR		-	-	-	-
DDAH1		-	-	-	-
DDAH2		-	-	-	-

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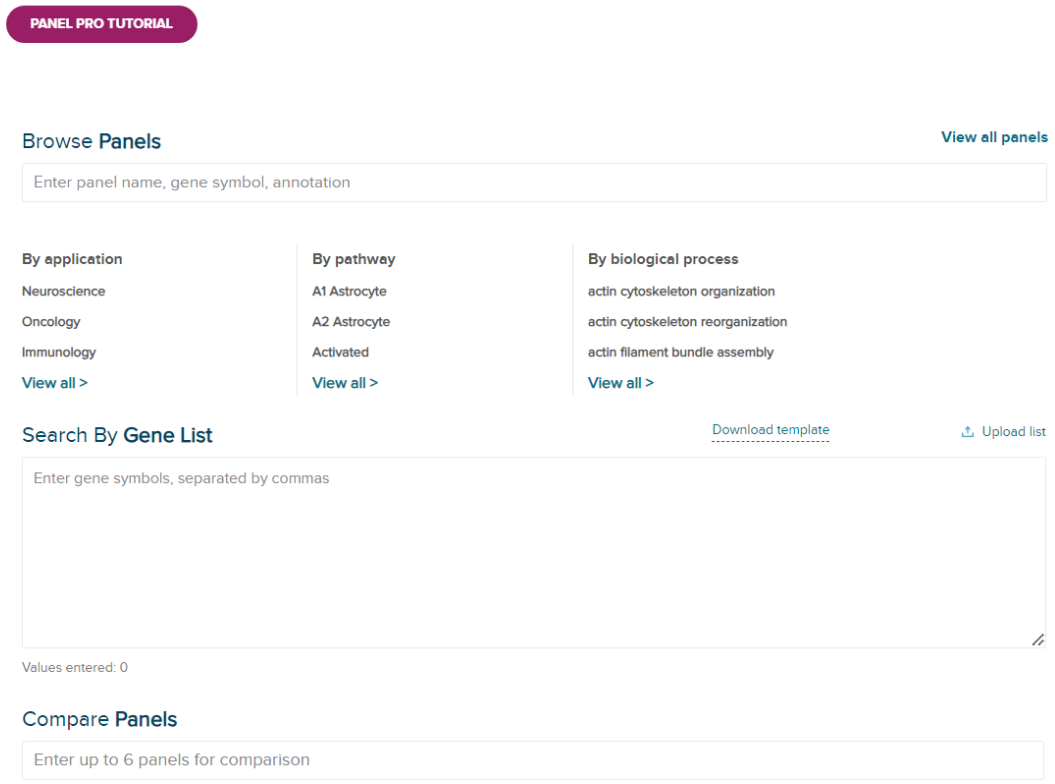
nCounter Panel Selection Tool



Panel Selection Tool

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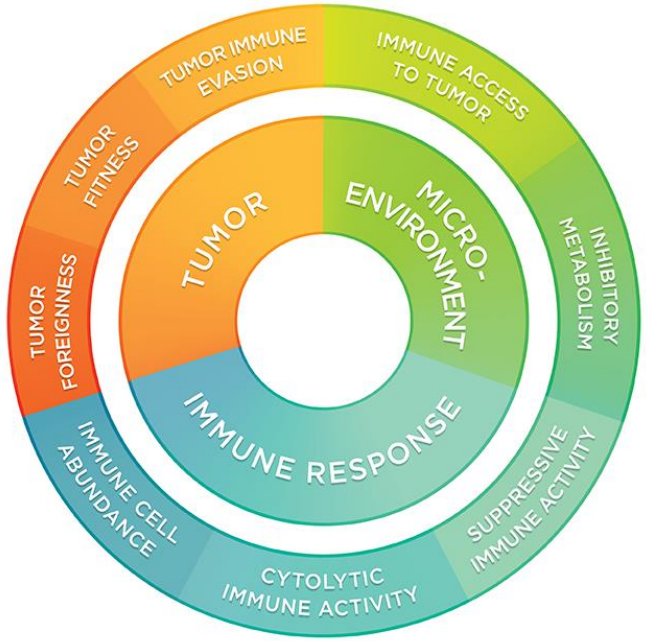
Customize an existing panel or create a new one

Panel Plus:
Customizable Add-on to a Panel

OR

Build Content that is Customized for Any Gene, Any Organism

PanCancer IO 360™ Gene Expression Panel + **Up to 55 User-defined Targets**



Custom CodeSets

Turnkey Solution for Any Project

- Maximum target number: 800
- Study novel and non-model organisms
- Address transcript variants and transgenes

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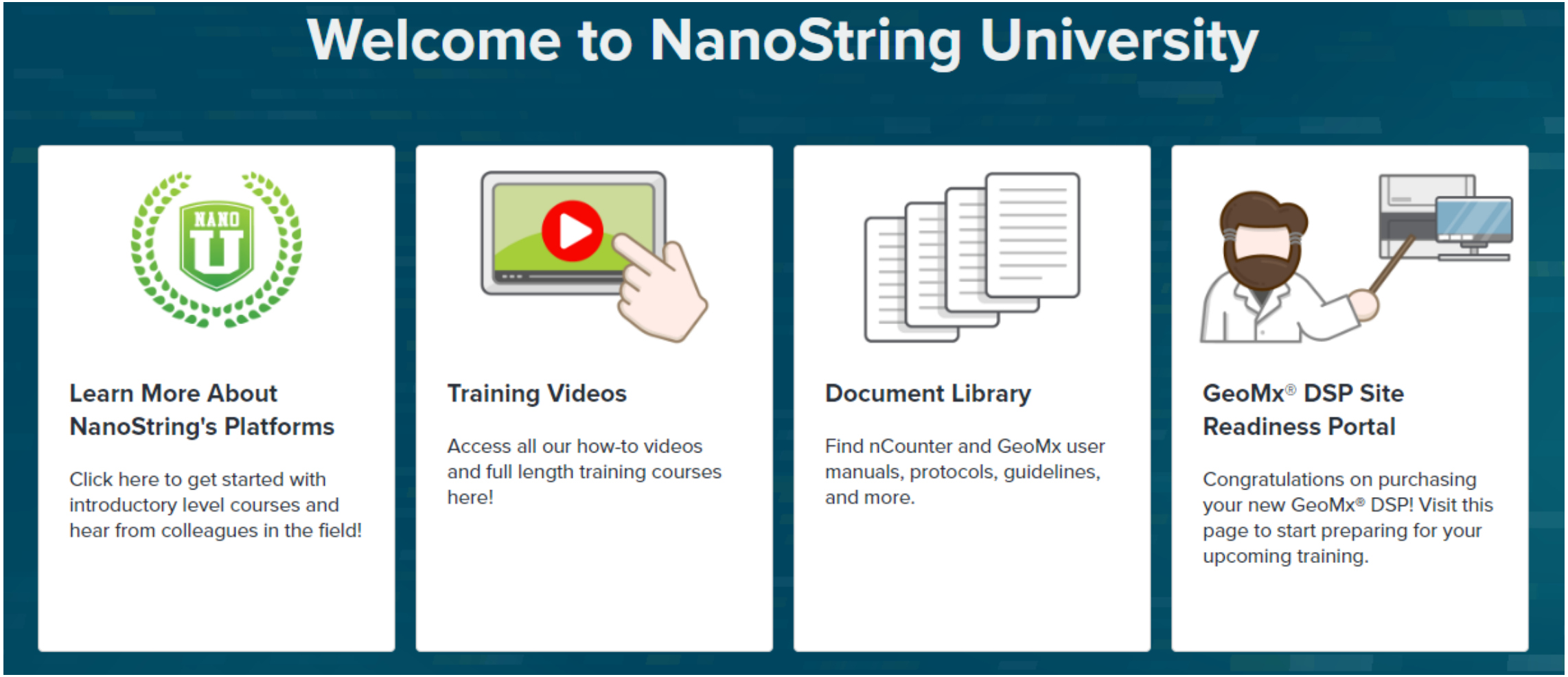
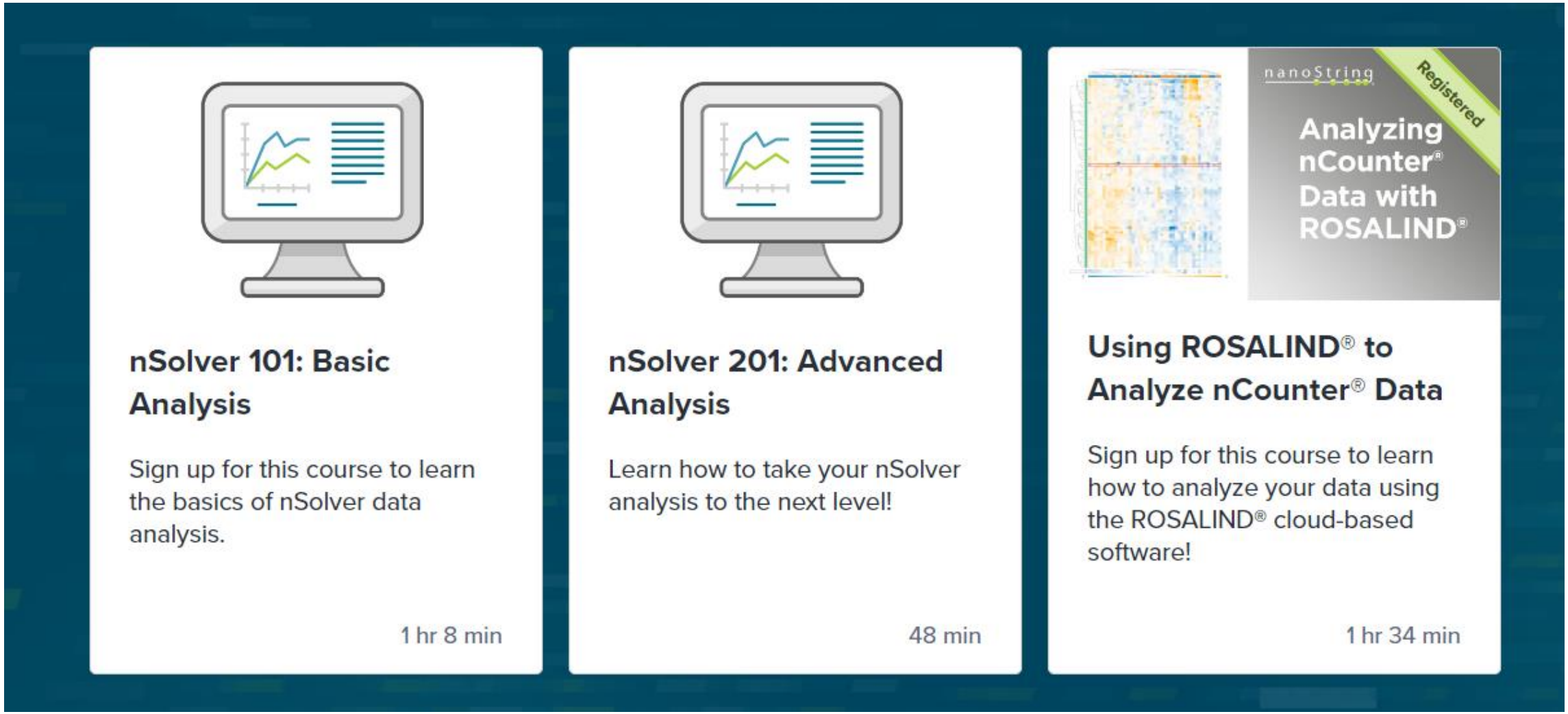
Support



Support for Data Analysis on NanoString University



Thank You!

15% discount on all off-the-shelf nCounter Panel orders through December 13th, 2024

Schedule a project consultation through bailey.longoni@bruker.com

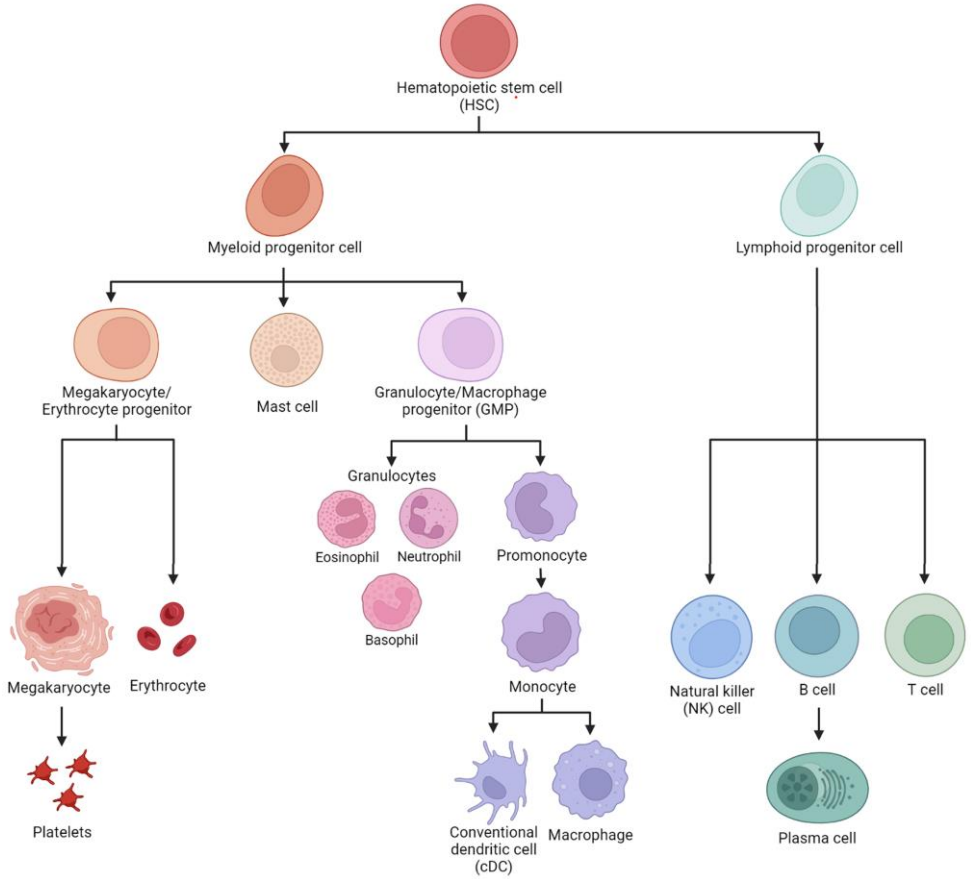
Classifying AML Tumor Cell State to Identify Responsiveness to Venetoclax-Based Therapy

Steve Kurtz
Chris Eide
Alexandria Miller
Sud Anand
Dan Bottomly
Jeff Tyner

KNIGHT CANCER *Institute*

10/15/2024

Acute Myeloid Leukemia (AML) results from the expansion of immature white blood cells in the bone marrow and peripheral blood



Hematopoietic stem cells are developmentally blocked and proliferate as immature myeloid cells (blasts)

Genetically heterogeneous: numerous mutations and translocations

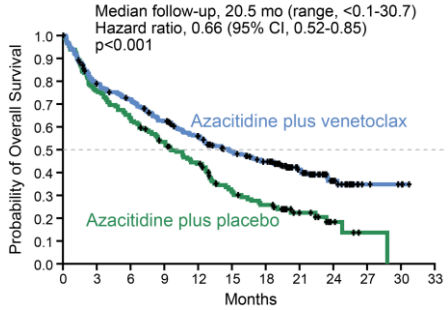
Conventional treatment: Intensive chemotherapy of cytarabine+daunorubicin (7+3)
More recently: Hypomethylating agents (azacytidine, decatibine), Bcl2 inhibitor (venetoclax)

Survival remains poor as patients relapse

Advances and limitations of venetoclax-based therapy for AML

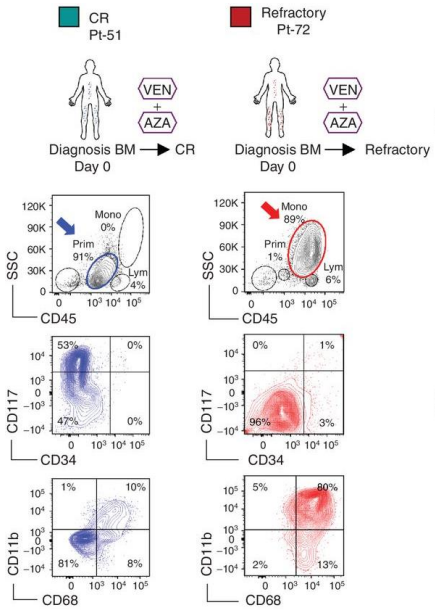
Azacytidine plus the BCL2 inhibitor venetoclax (Ven) is standard-of-care for elderly or unfit patients with AML

DiNardo et al., NEJM 2020



Ven+Azacytidine improves response rates with certain genetic mutations (e.g. *IDH1/2*, *NPM1*), with poorer responses in *TP53* and *RAS* mutated AML

Stahl et al., Blood Adv 2021
DiNardo et al., Blood 2020



Differential sensitivity to Ven +/- Azacytidine associates with primitive but not differentiated AML tumor cell state

Pei et al., Cancer Discov 2020
Zhang et al., Nat Cancer 2020
Kuusanmäki et al., Haematologica 2020

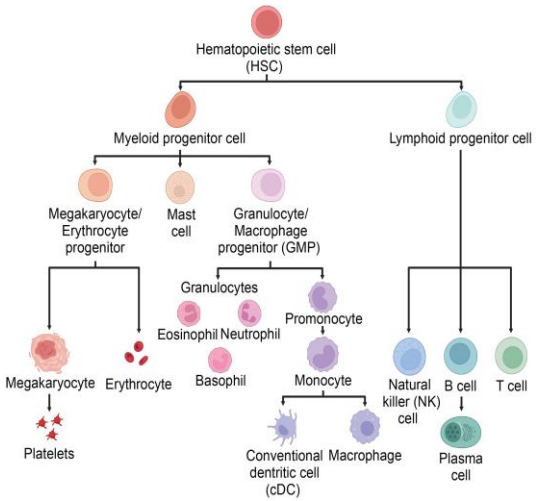
Classifying AML by tumor cell state

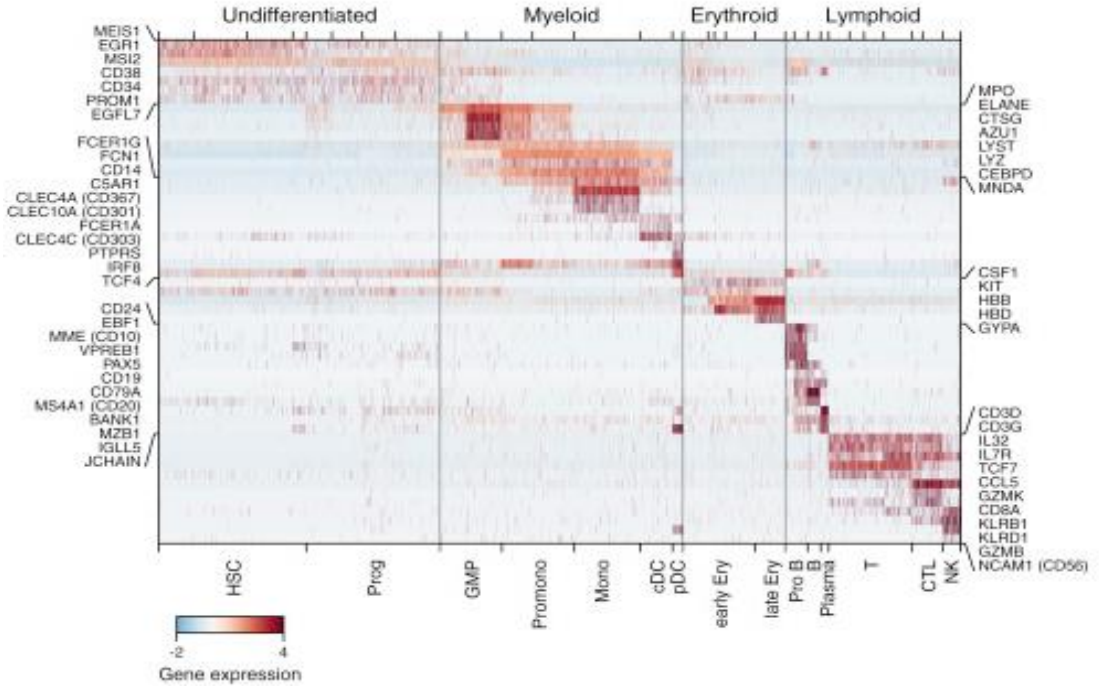
Single-cell RNAseq and genotyping methodology identified gene sets that distinguish cell hierarchy lineages and differentiation

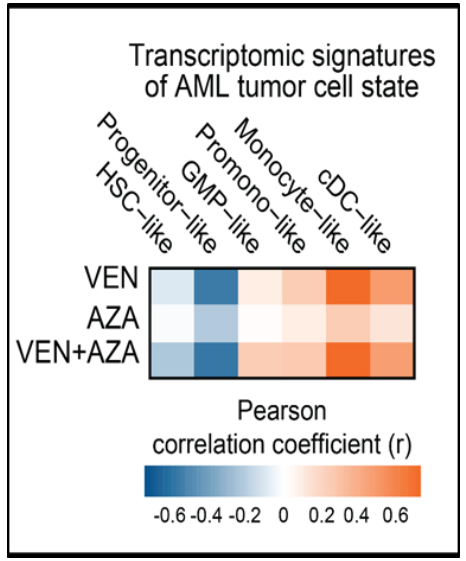
van Galen et al., Cell 2019

Application of these signatures to AML patient samples determined tumor cell state (e.g. primitive or monocytic) associations with Ven ex vivo sensitivity

Bottomly et al., Cancer Cell 2022
Zeng et al., Nature Medicine 2022
Eide and Kurtz et al., Blood Cancer Discov 2023







RNAseq data

Translating the findings: Assays to rapidly profile tumor cell state

~50-60% of newly diagnosed AML patients are elderly or unfit for intensive chemotherapy; these patients receive Ven-based treatment

Latagliata et al., Annals of Onc 2006
Oyogoa et al., Cancers (Basel) 2023

~30% of elderly or unfit AML patients treated with frontline Aza+Ven do not achieve complete response

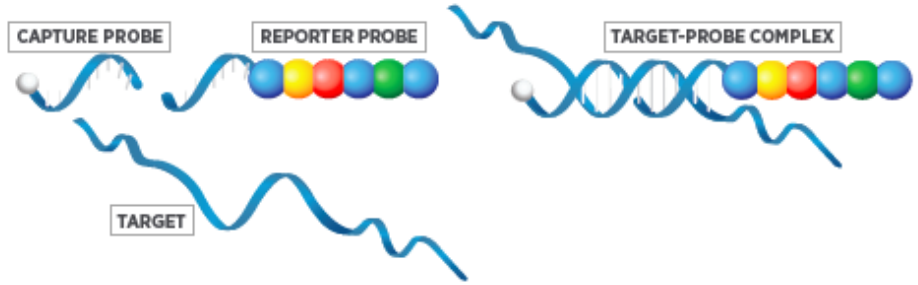
DiNardo et al., NEJM 2020

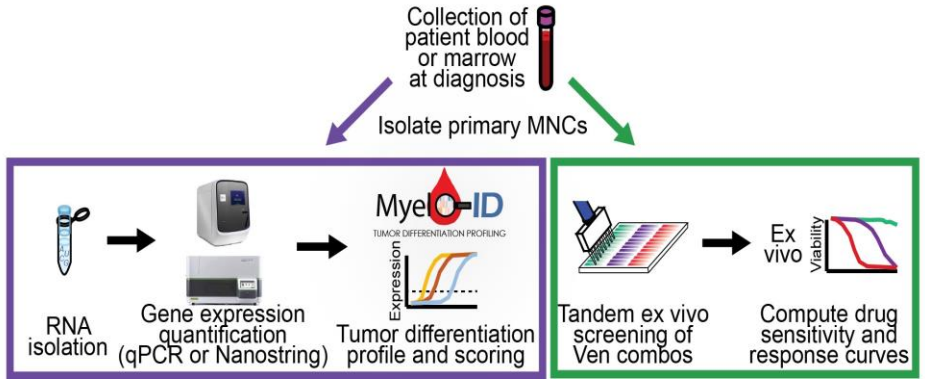
RNAseq is gold standard for transcriptomic profiling, but analysis time/cost effectiveness of prospective single sample runs not compatible with clinical decision-making timelines in AML

Goal: Develop a rapid assay to profile cell state gene expression signatures in AML patients and align with prediction of Aza+Ven sensitivity

Technology for Rapid Determination of Cell State

Nanostring Technology





Designed custom Nanostring panel targeting:

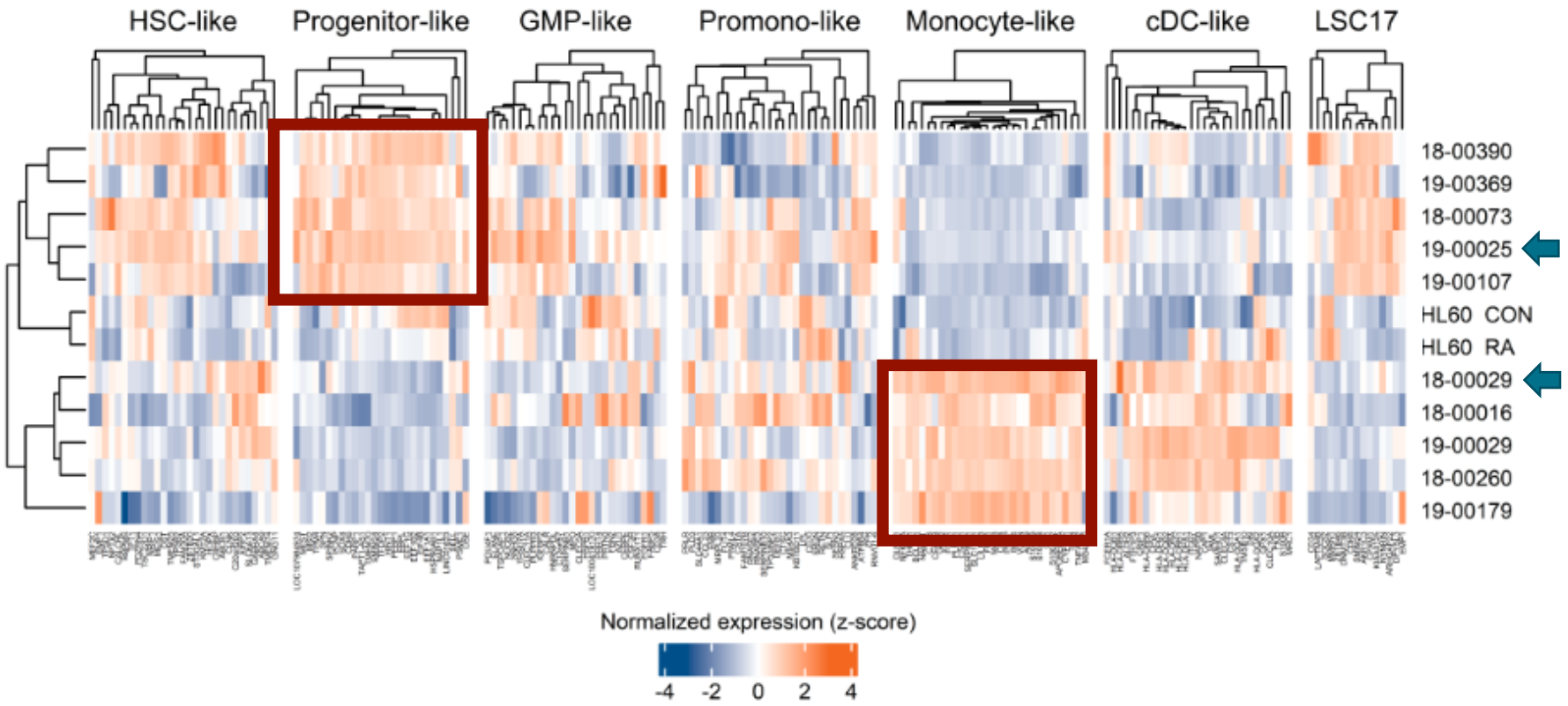
AML hematopoietic cell state signatures (6 sets of 30 genes/set from van Galen et al., *Cell* 2019)

AML LSC17 “stemness” signature (17-gene set from Ng et al., *Nature* 2016)

Control genes (n=7)

Classification of AML Tumors by Cell State Profiling

Pilot study: RNAs from 5 samples previously defined as Progenitor-like and 5 samples previously defined as Monocytic-like by deconvolution from bulk RNAseq



Cartridges run 12 samples each

Selected 2 control samples to run on every cartridge

Samples are clustered in rows and genes are clustered, grouped by gene set. Shading reflects the z-scores for each sample for a given gene with increased (orange) or decreased (blue) color normalized gene expression.

Design Considerations and Performance

Sample Expression Distribution is consistent across independent batch runs

QC criteria were determined based on Nanostring recommendations, existing CLIA lab protocols, and collaborator input.

Field of view (FOV) > 75%

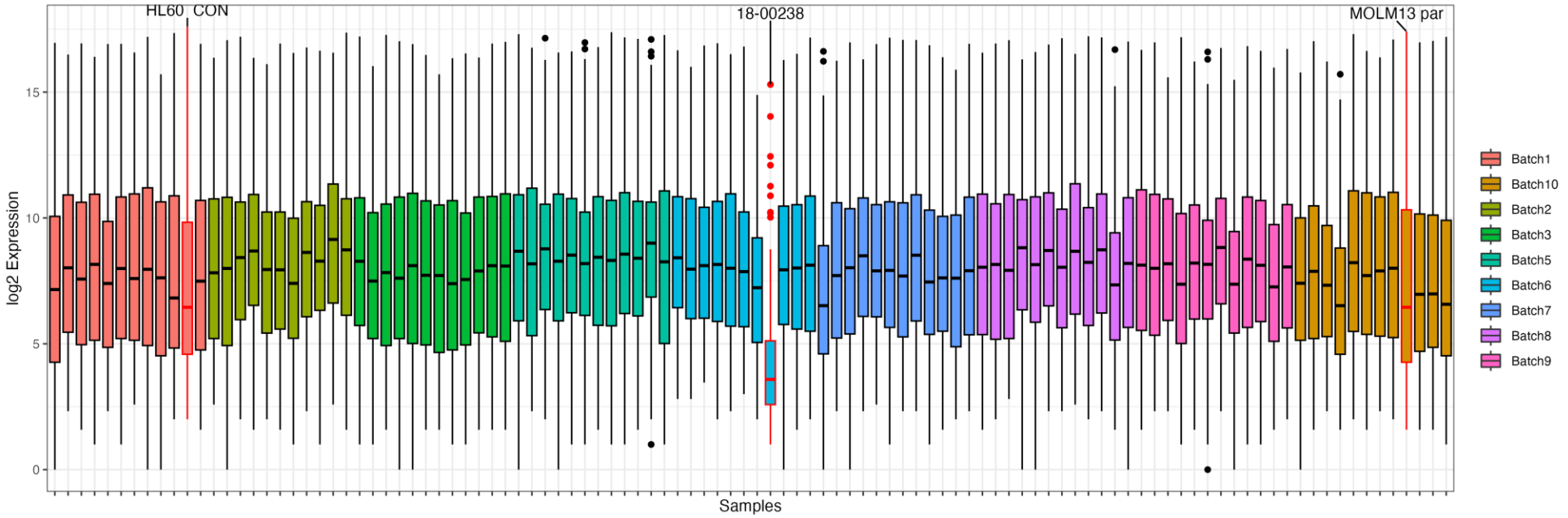
Binding density > 0.5

Positive control linearity R2 values at least 0.95

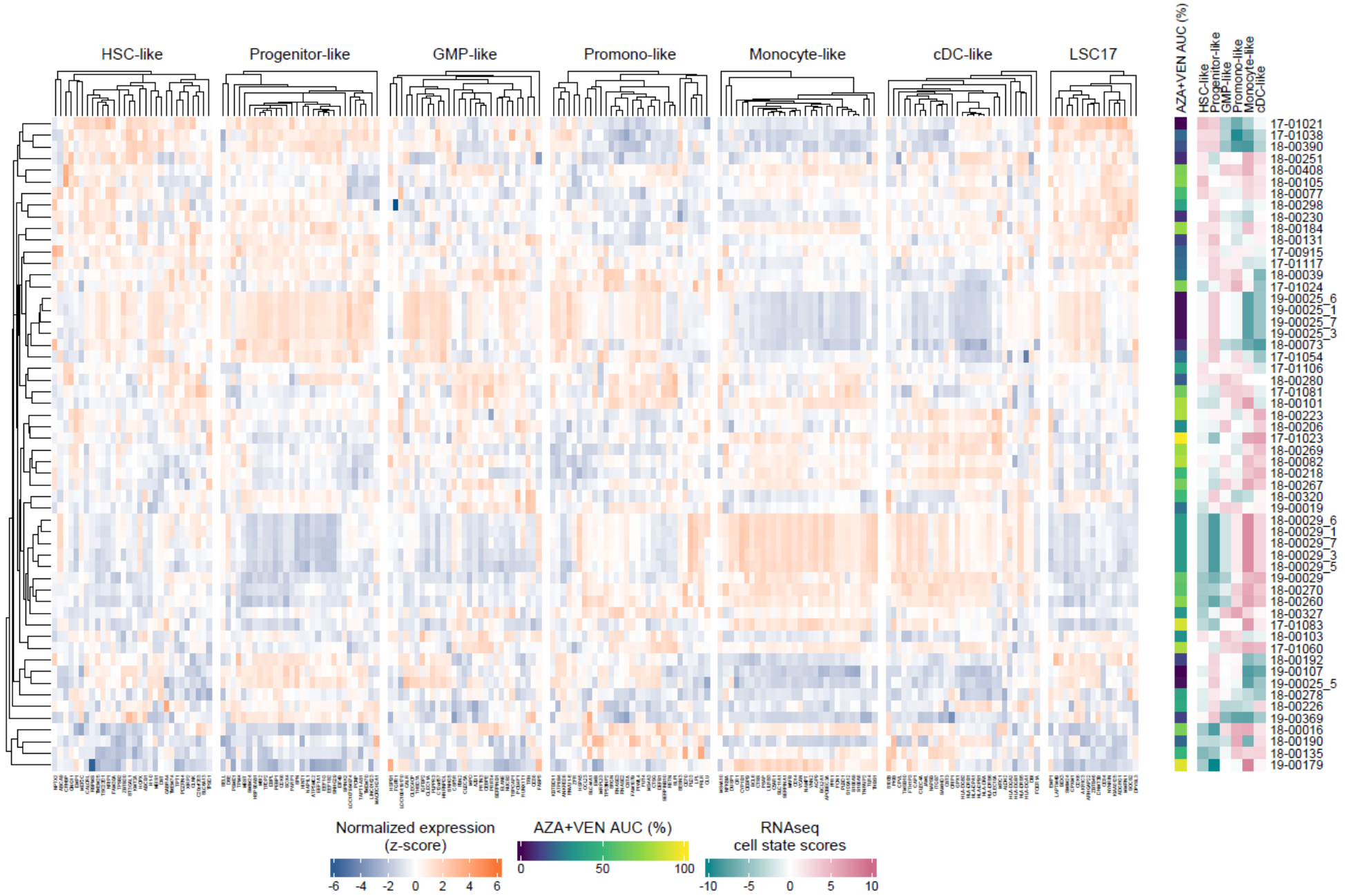
Lower limit of detection (LOD) threshold value is 2 SD above the geometric mean of the negative controls

The lowest abundance positive control probe (0.5 fM) > LOD threshold value

Distribution of Raw Counts Across Batches



Nanostring Profiling on 49 RNAs with matching RNAseq data



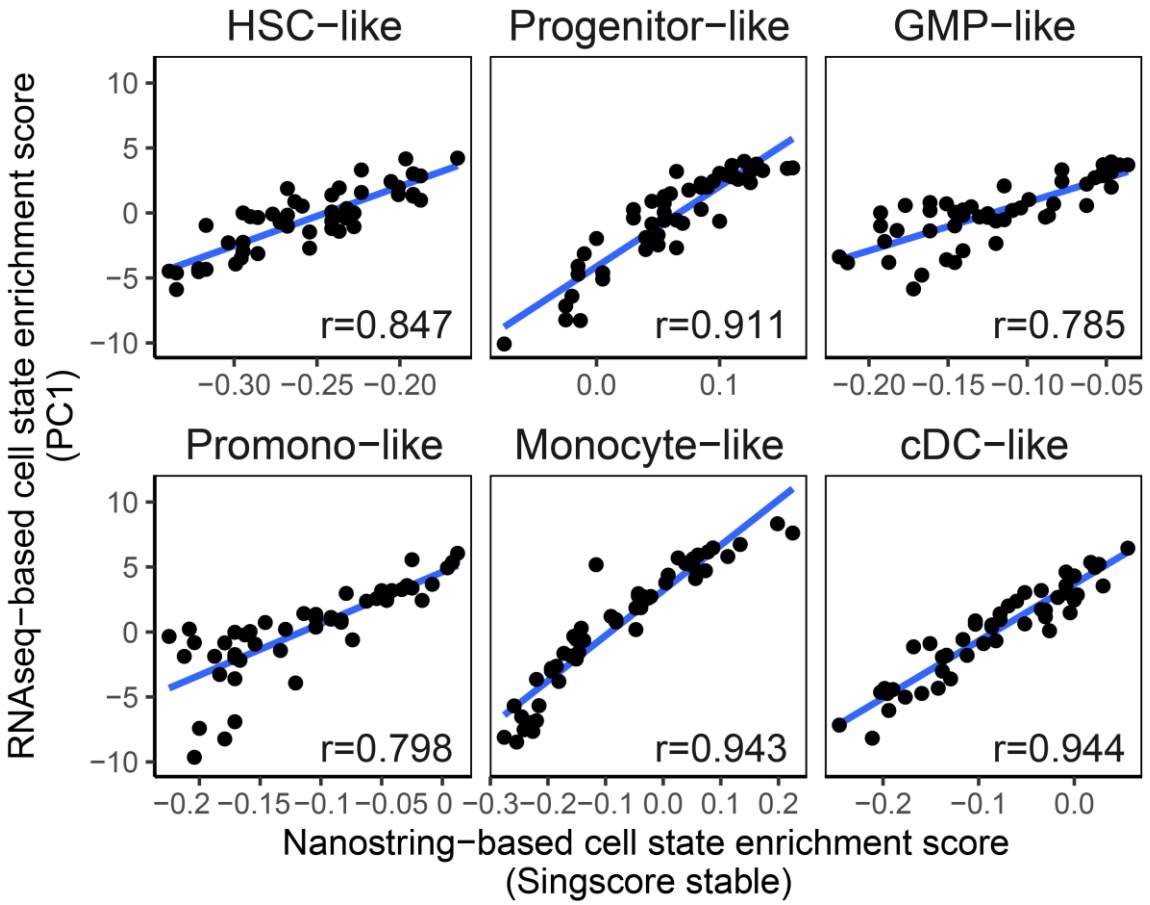
Nanostring Assay concordance with RNAseq

Gene set enrichment was calculated using a modified single-sample method (Singscore)

Foroutan et al., *BMC Bioinformatics* 2018

Nanostring-based results correlate with cell state scores determined from deconvolution of bulk RNAseq data

Bottomly et al., *Cancer Cell* 2022

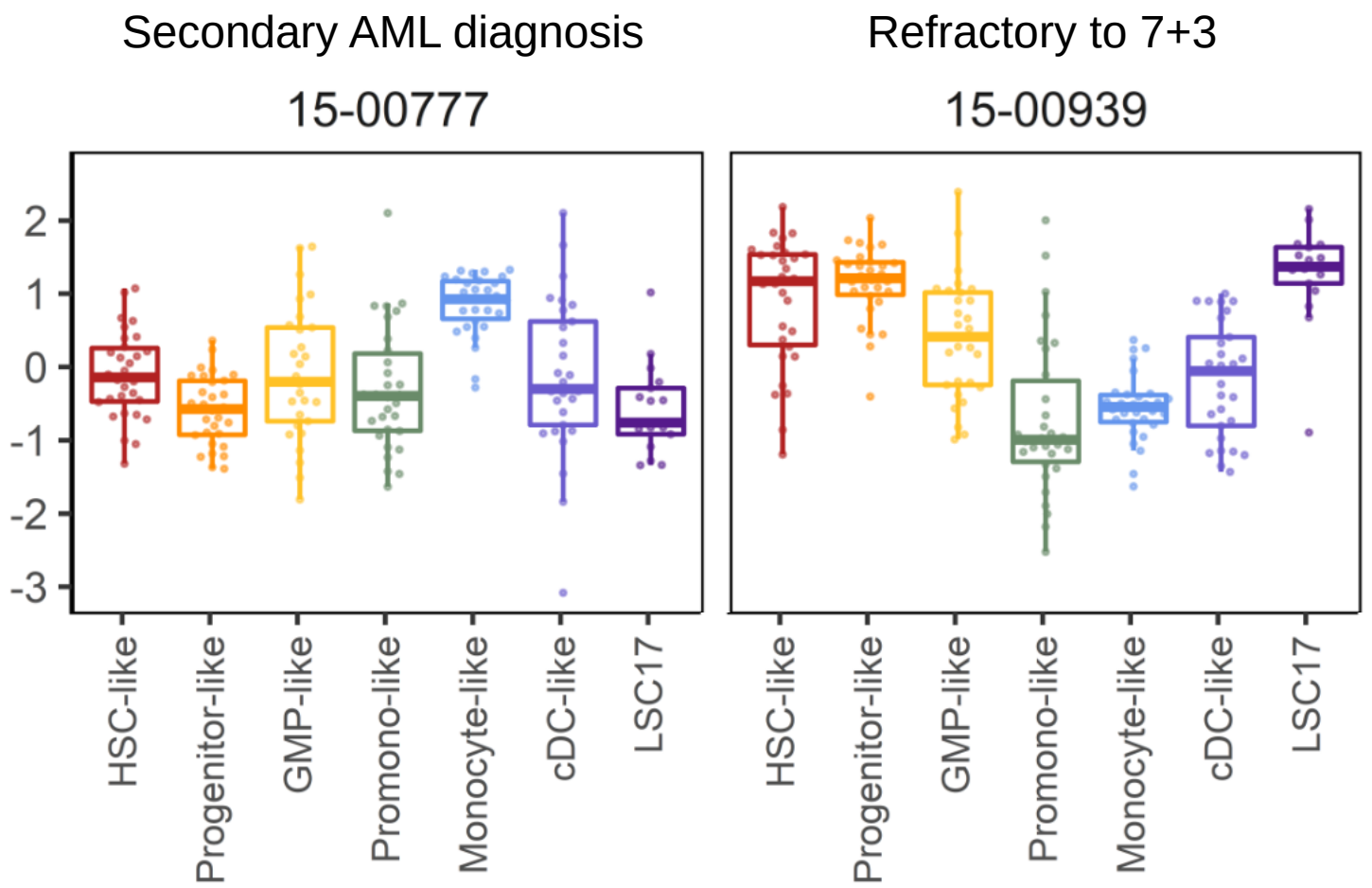


Changes in Tumor cell state profile on Intensive Chemotherapy: Patient 2314

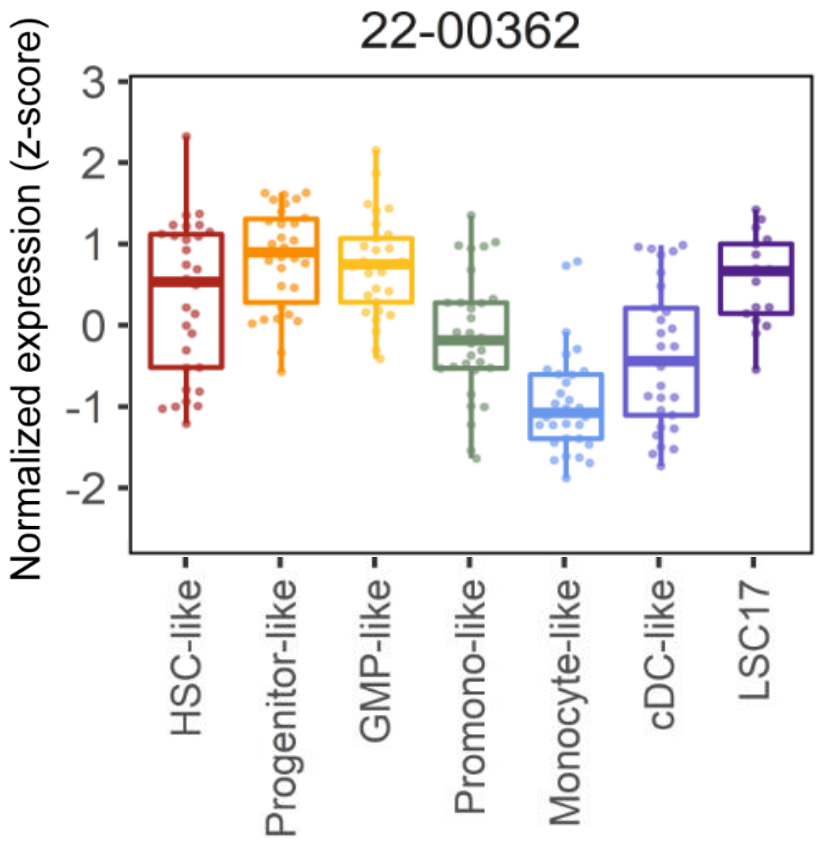
At diagnosis, patient with MDS, treated with azacytidine, but transformed to AML with monocytic differentiation.

Treated with 7+3 induction, did not achieve a complete response.

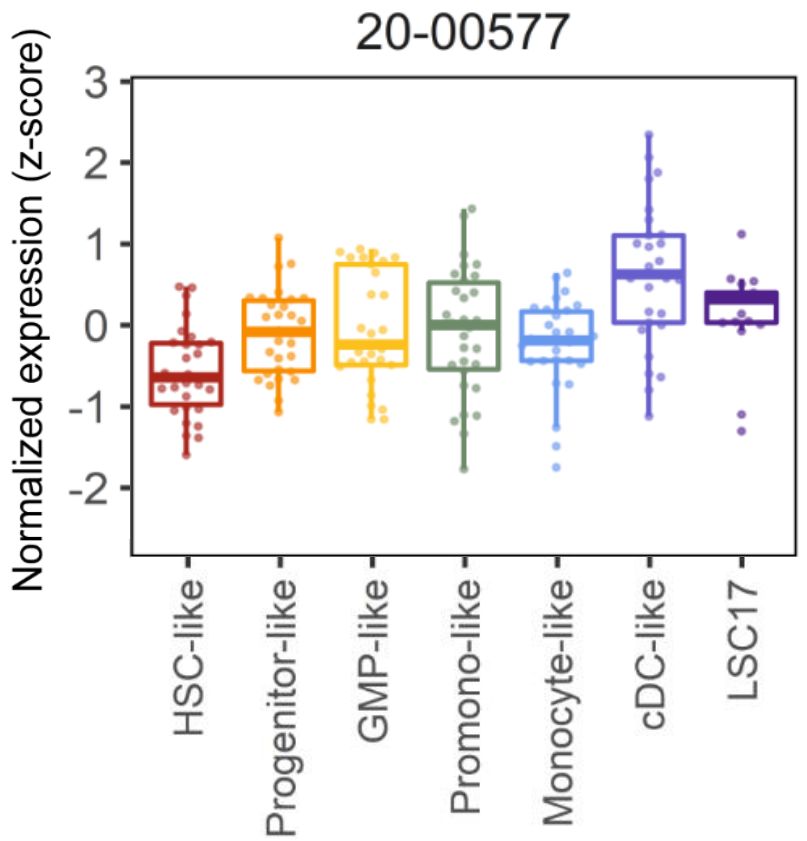
Tumor profile shifted to primitive cell state following treatment.



Classifying cell state at AML diagnosis provides context for anticipating response to Aza+Ven

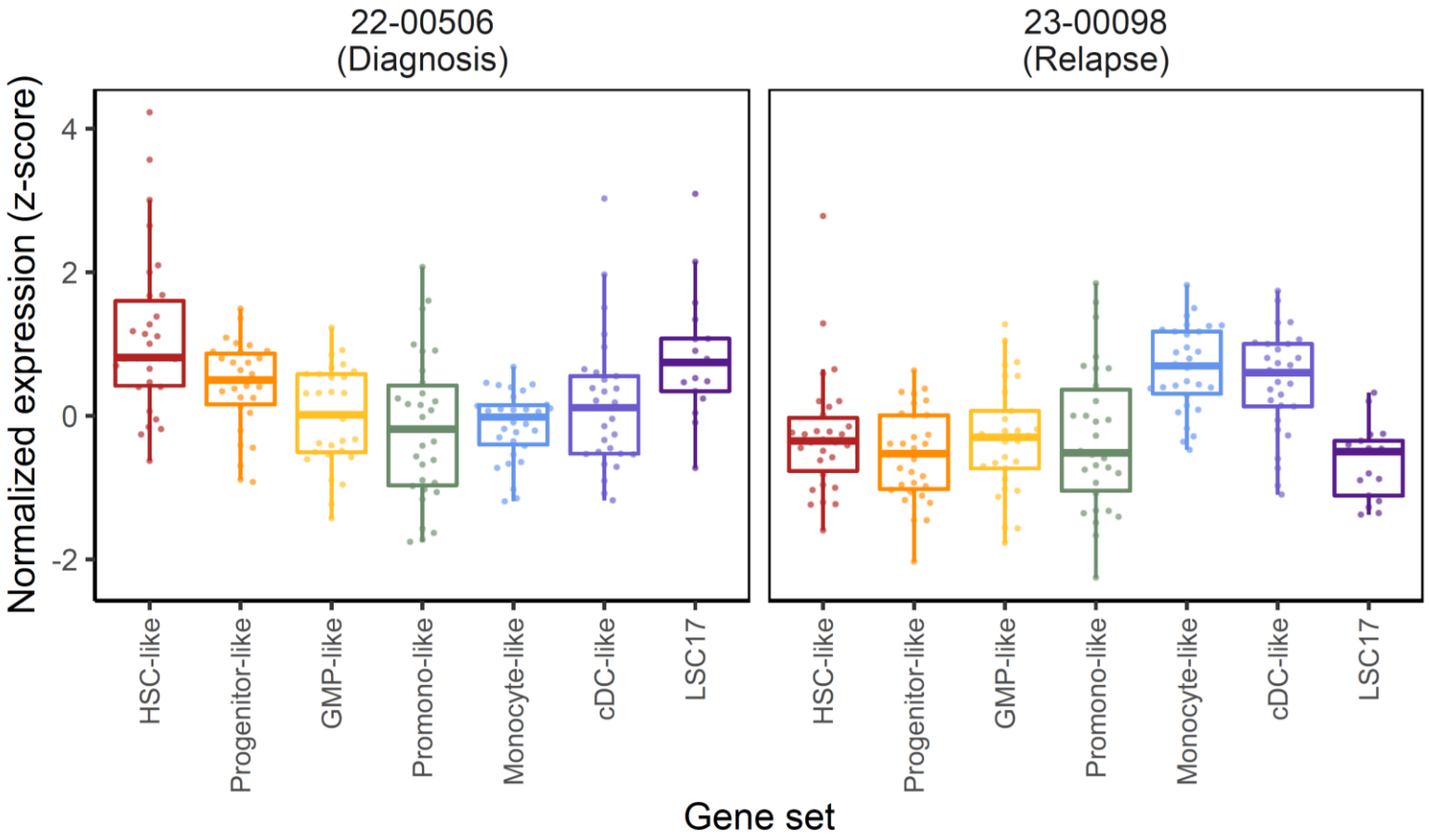


Clinical AZA+VEN outcome:
Complete Response



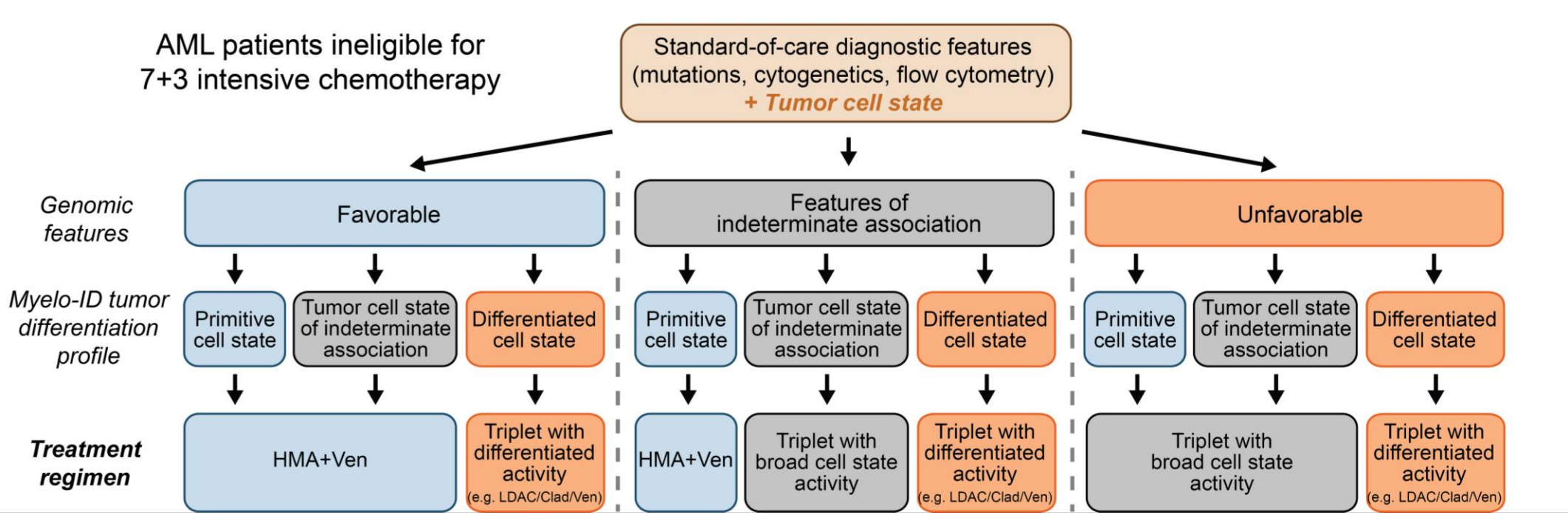
Clinical AZA+VEN outcome:
Refractory

Changes in Tumor cell state profile on AZA+VEN : Patient 6282



Tumor profile shifted from primitive to monocytic cell state following treatment.

Translational Goal



OREGON HEALTH & SCIENCE UNIVERSITY

University Shared Resources

NanoString Services

RNA & DNA Services of the Integrated Genomics Laboratory (IGL)

Jinah Kim, PhD
Core Scientist
Oct, 15th, 2024

Advantages of NanoString Technology

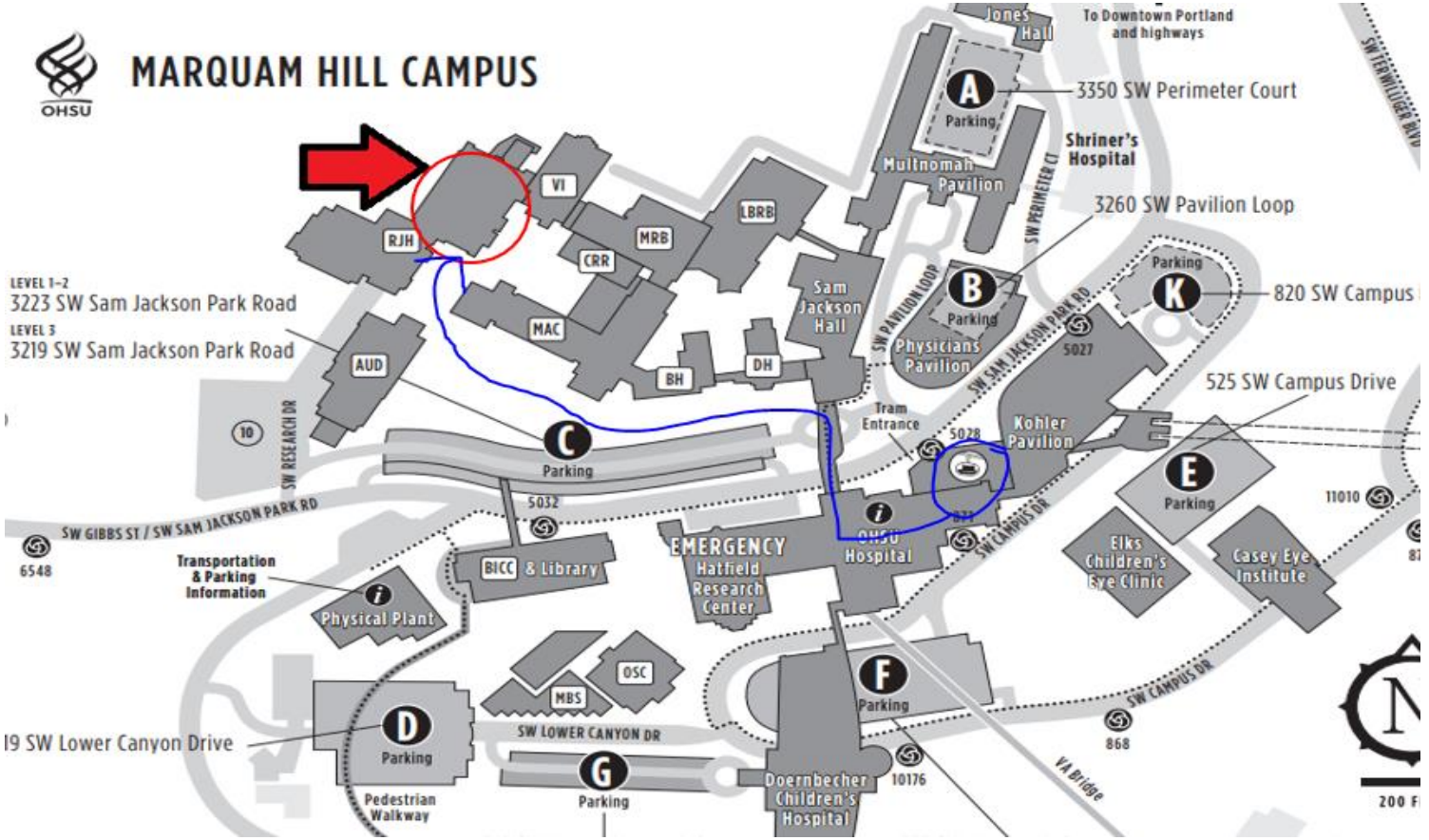
- Direct quantification of RNA and miRNA using molecular barcode without the need for amplification

- Each multiplex panel can profile up to 770 genes, with the option to customize by adding up to 55 additional unique targets

- Faster than NGS (in terms of setup and processing), more sensitive than targeted RNAseq (especially for genes with low expression levels), and simpler than qPCR (as it requires no amplification and uses a direct hybridization method).

We are located in Richard T. Jones Hall (RJH), 5th floor, Room 5330.

RJH



Integrated Genomics Laboratory

OREGON HEALTH & SCIENCE UNIVERSITY

NanoString nCounter SPRINT Workstation (RJH, 5330)

Day1: CodeSet Hybridization using the thermocycler **(16 hours)**

Day 2: Sample loading into the Cartridge and running the nCounter SPRINT Profiler **(6 hours)**

Training is required

- **Training includes:**
  - Technical Information and Background Reading
  - Virtual Meeting (45 minutes)
  - Hands-on, in-person training (45 minutes)

- **Current Training Fee:**
  - $150 (Internal)
  - $180 (External Academic)
  - Max 2 people per lab per training session
  - Slated to increase in FY25

Post Training Support - For your first NanoString run, IGL staff will shadow trainees to provide support with CodeSet hybridization, cartridge loading, and the instrument operation. Staff remain available during subsequent runs to answer questions.

Reservation

- **Bookings through iLab**, must be made **24 hours in advance** on workdays.
  - **8 hours:** Cartridge loading and SPRINT Run only
  - **24 hours**: Total RNA CodeSet hybridization, cartridge loading, and SPRINT run
  - **24 hours + miRNA Sample Prep**: Contact Jinah about booking extra bench time for the miRNA Sample Prep protocol.

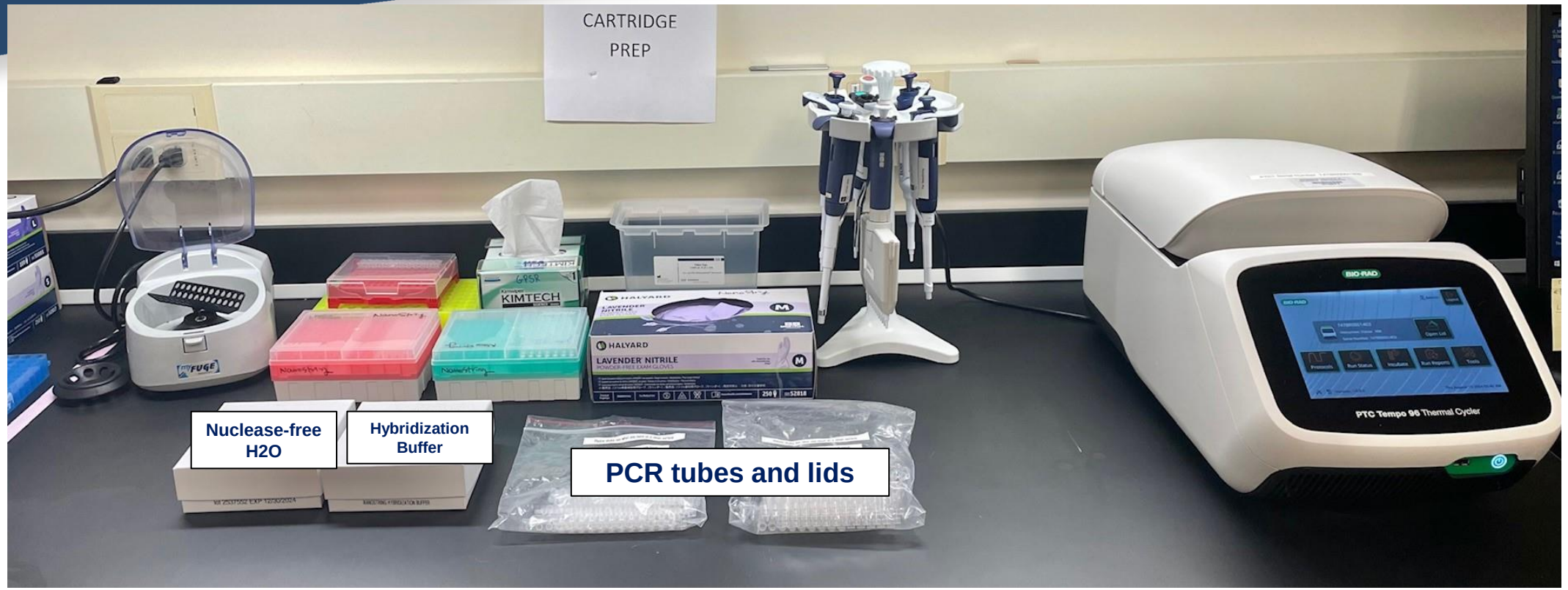
- **Current Run Fee:**
  - $285 OHSU Internal
  - $328 External Academic
  - Slated to increase in FY25

- **NanoString Instrument can only be used by <u>trained users</u>.**

We provide following Support:

- Direct client support for questions and liaison with Nanostring customer service.
- Monitor the instrument during your NanoString run.
- Email Data to the Users.
- Maintain the instrument through the annual service contract provided by NanoString (~$15,000/year).
- Provide the basic supplies such as calibrated pipets and consumables.

Support items provided by IGL RNA/DNA Services



- -20 °C storage

Not provided, must be supplied by user:

- ✓ Samples
- ✓ NanoString CodeSet Panel
- ✓ Sample Prep Kits (if applicable)
- ✓ NanoString SPRINT kit Cartridge
- ✓ Report Library File (RLF) USB drive including your CodeSet
- ✓ -80 °C storage

Other available Services at IGL to support nCounter SPRINT run

- ❑ Manual and Automated Nucleic Acid Isolations
- ❑ RNA quality assessment with Bioanalyzer
- ❑ DNA quality assessment with Tapestation
- ❑ RNA & DNA UV quantification with NanoDrop
- ❑ RNA & DNA fluorometric quantification with Qubit

Contact information

IGL members

- Jinah Kim (kimjinah@ohsu.edu)
- Britt Daughtry (daughtry@ohsu.edu)
- Robert Searles (searlesr@ohsu.edu)

NanoString support

- support.spatial@bruker.com
- Westley Heydeck (Westly.Heydeck@bruker.com): Sr. Field Application Scientist
- Bailey Longoni (Bailey.Longoni@bruker.com): nCounter Regional Account Manager