



ROSALIND[®]

Powered by ONRAMP

ACCELERATE YOUR RESEARCH

Exploring nCounter[®] data analysis
on the ROSALIND discovery &
collaboration platform.

nanoString[®]

QUICK START GUIDE

Learn more at www.onramp.bio/nanostring

An Interactive Experience for **Analyzing** and **Collaborating** with your Genomic Datasets

Why ROSALIND?

ROSALIND is a cloud-based multi-omics discovery and collaboration platform that enhances the data analysis experience. We offer standardized pipelines for RNA-seq, scRNA-seq, ChIP-seq, ATAC-seq, smallRNA-seq, and nCounter data. Simply upload your files and receive same-day results including: quality control, differential expression, pathways, and much more.

Scientists of every skill level can utilize ROSALIND since no programming or bioinformatics expertise is required. ROSALIND instantly provides powerful downstream analysis and truly insightful visualizations on gene expression datasets. Our interactive analysis is designed to be easy to use and save valuable time. ROSALIND even offers the ability to share experiments and collaborate in real-time.

Why use NanoString nCounter for Gene Expression Analysis?

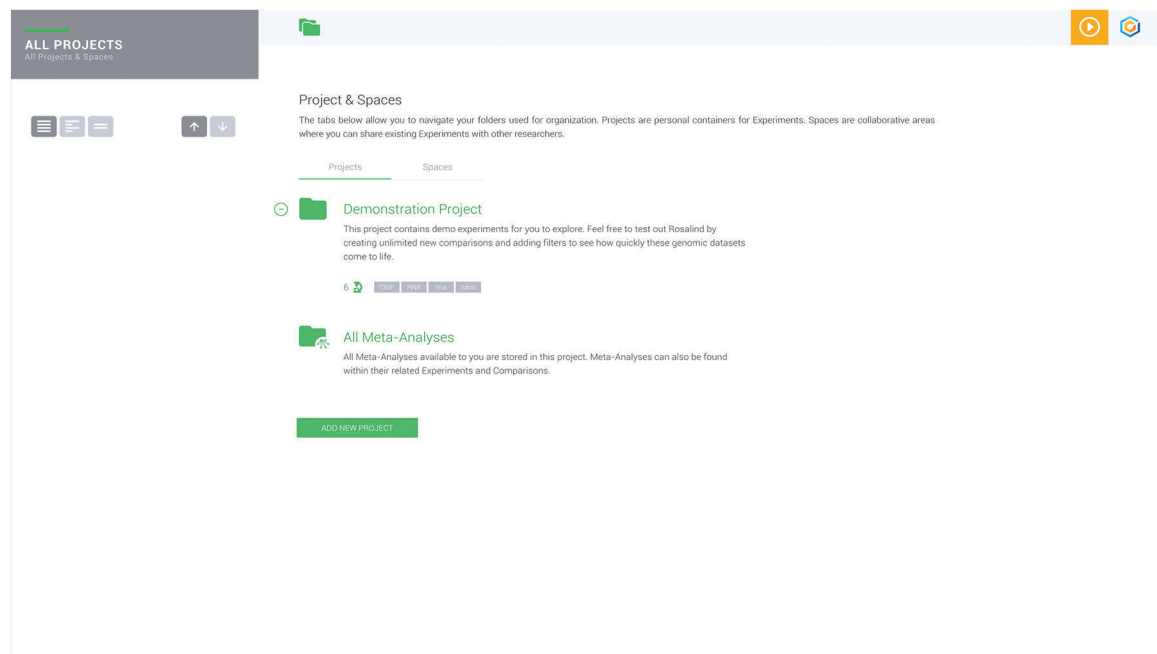
The study of gene expression provides valuable insights into the nature of diseases and the effect of treatments by quantifying the activity of RNA in a biological sample. Scientists working in Oncology, Immunology, Infectious Diseases (more recently, COVID-19), Regenerative Medicine, Drug Discovery and other areas of research often conduct experiments between healthy and disease states to identify differentially expressed genes and biological pathways to discover therapeutic targets. Comparisons between these differential patterns reveal unique gene signatures that are valuable for drug and diagnostic development.

NanoString's nCounter Analysis System utilizes a proprietary molecular barcoding technology to count RNA molecules directly without reverse transcription or amplification. This enables development of highly multiplexed gene expression assays that are simple to run and highly reproducible. Because it doesn't rely on enzymology, nCounter is highly tolerant of Formalin Fixed Paraffin Embedded (FFPE) tissue and other sample types where RNA degradation and/or effects of fixation are a concern. nCounter is often utilized in translational research studies and clinical trials as well as basic research.

NanoString offers a catalogue of pre-designed Panels spanning a wide range of biology with specific emphasis on Oncology/ImmunoOncology, Immunology, and Neuroscience. The panels consist of hundreds of highly curated and annotated genes covering a particular area of biology. These panels can be customized by adding genes specified by the end-user. NanoString also offers fully customizable designs to fit virtually any research need.

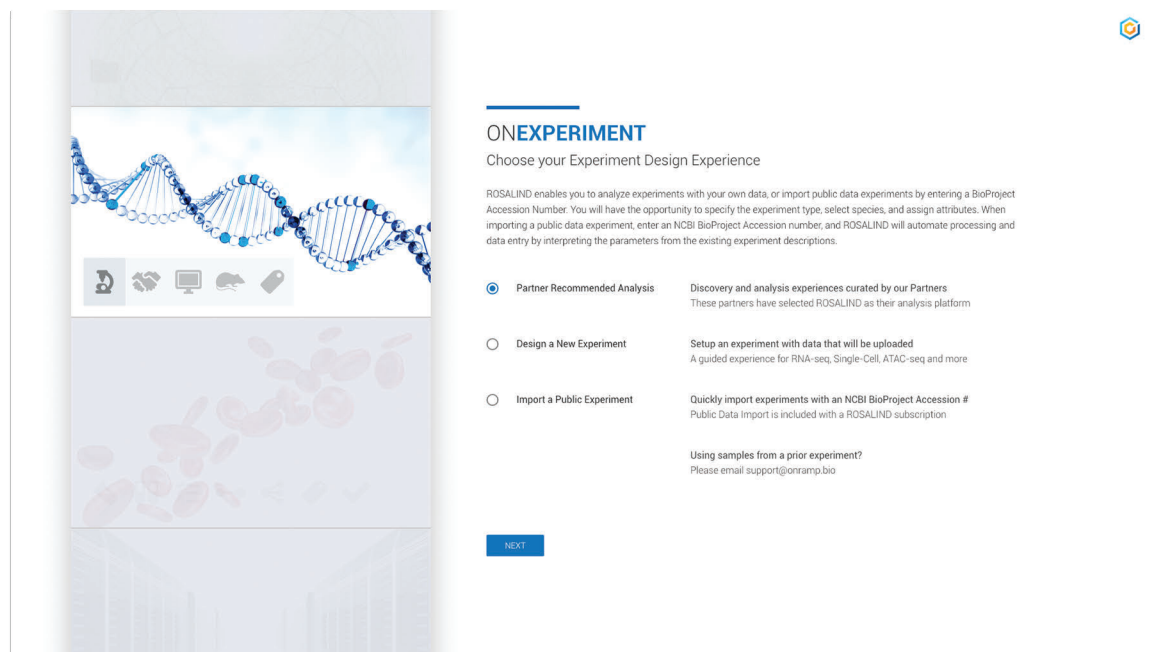
Analyze More

Setup your experiment in minutes.



Beginning your experiment design is as easy as clicking
or [ADD NEW EXPERIMENT](#) from anywhere across the platform.

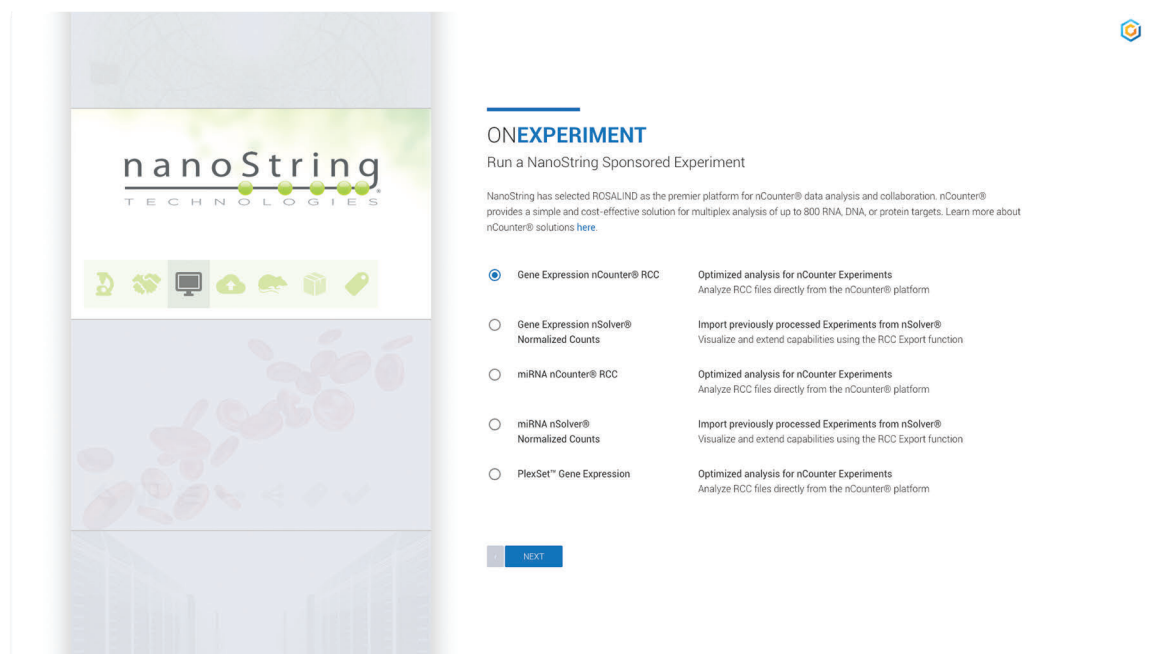
[ADD NEW PROJECT](#)



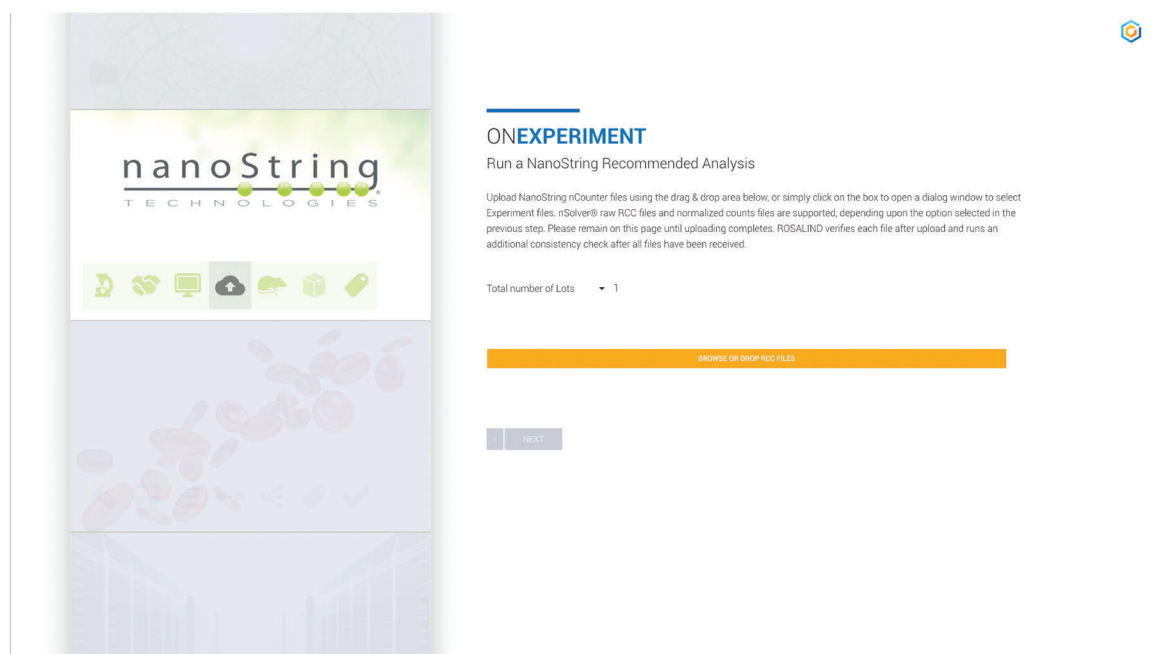
Begin your NanoString experiment by selecting the radio button:
“Partner Recommended Analysis” and clicking [NEXT](#) .

Analyze More

Setup your experiment in minutes.



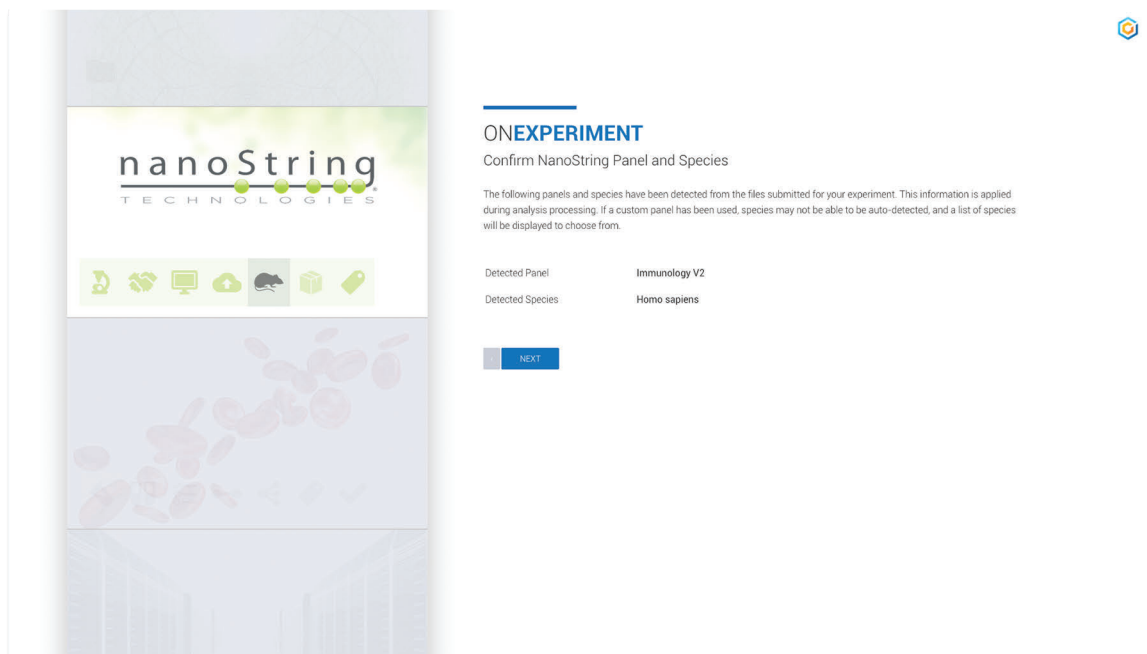
Choose one of the various nCounter® experiment types from the list provided.



Upload NanoString nCounter® files using the drag and drop box, or click on the box to open a dialog window to add the RCC files for your experiment.

Analyze More

Setup your experiment in minutes.



ONEXPERIMENT

Confirm NanoString Panel and Species

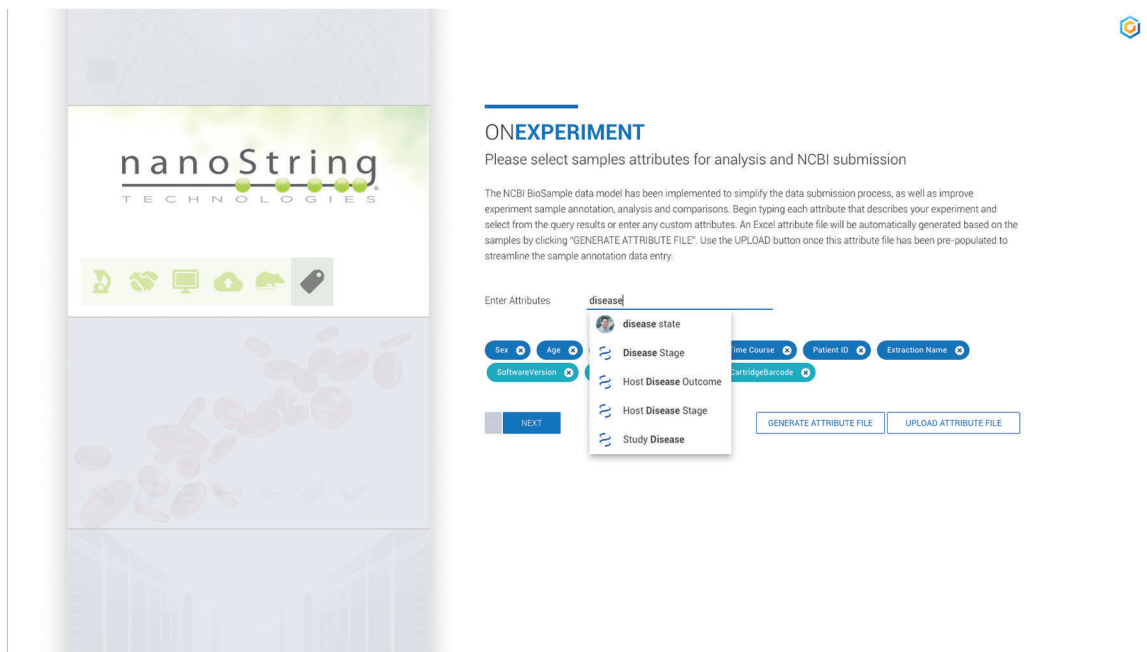
The following panels and species have been detected from the files submitted for your experiment. This information is applied during analysis processing. If a custom panel has been used, species may not be able to be auto-detected, and a list of species will be displayed to choose from.

Detected Panel: Immunology V2

Detected Species: Homo sapiens

[NEXT](#)

NanoString panels and species are usually autodetected. If not, you can also use the dropdown menu to select the desired species.



ONEXPERIMENT

Please select samples attributes for analysis and NCBI submission

The NCBI BioSample data model has been implemented to simplify the data submission process, as well as improve experiment sample annotation, analysis and comparisons. Begin typing each attribute that describes your experiment and select from the query results or enter any custom attributes. An Excel attribute file will be automatically generated based on the samples by clicking "GENERATE ATTRIBUTE FILE". Use the UPLOAD button once this attribute file has been pre-populated to streamline the sample annotation data entry.

Enter Attributes:

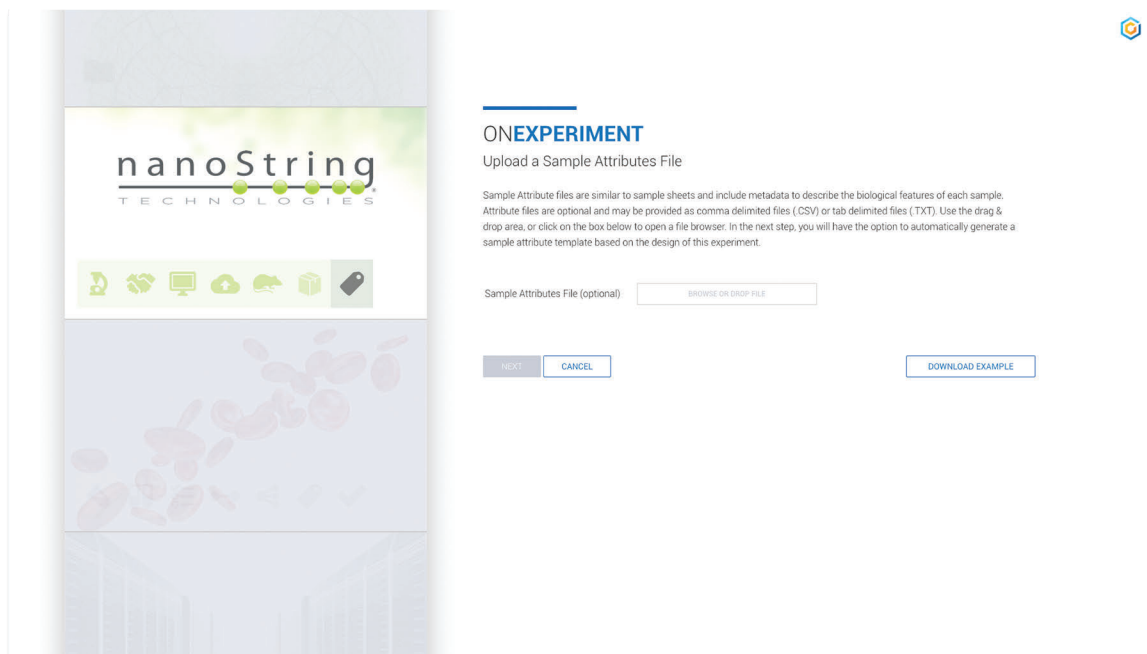
- disease state
- Disease Stage
- Host Disease Outcome
- Host Disease Stage
- Study Disease

[GENERATE ATTRIBUTE FILE](#) [UPLOAD ATTRIBUTE FILE](#)

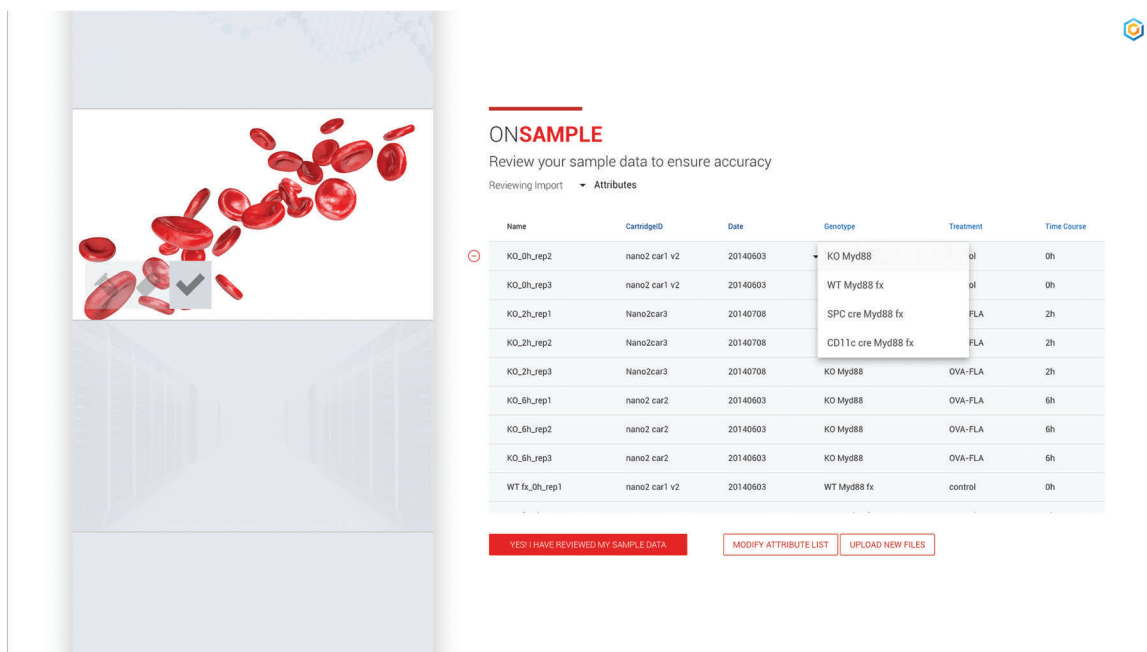
ROSALIND uses the NCBI BioProject and BioSample data model for annotating samples and to simplify GEO/SRA submissions. Click [GENERATE ATTRIBUTE FILE](#) for a custom file to edit annotation values in Excel and upload this file by clicking [UPLOAD ATTRIBUTE FILE](#).

Analyze More

Setup your experiment in minutes.



Uploading the pre-populated file can reduce the time it takes to define the metadata associated with your experiment. Additional guidance is offered by selecting [DOWNLOAD EXAMPLE](#).



ROSALIND provides a sample sheet for easy review of your experiment design before you upload your data. Use this table to remove samples, change sample names and adjust sample parameters.

Analyze More

Setup your experiment in minutes.

ONANALYSIS

Please describe the sample comparison you would like to create

To populate your comparison groups, select **Samples** or **Attributes**, choose an item class, and then drag one or more items to a group box. Note that every item added will further constrain your eligible samples within the group. You may then refine your sample selection by clicking Edit at the lower right corner of each group box. Click **Save** when you are ready to finalize this comparison.

New Comparison
KO Myd88 vs WT Myd88 fx

Select Category → Select & Drop Items →

Attributes	CartridgeID	CD11c cre Myd88 fx
Samples	Genotype	KO Myd88
	Time Course	SPC cre Myd88 fx
	Tissue	WT Myd88 fx
	Treatment	
	Date	

KO Myd88
Genotype - KO Myd88
8 single samples
EDIT

WT Myd88 fx
Genotype - WT Myd88 fx
8 single samples
EDIT

SAVE **SELECT COVARIATE** ✓

Comparisons can be set up now or after the experiment has been processed. Differential expression requires pairwise comparisons, eg treatment vs control. Drag attribute values or sample names to the condition and control boxes.

ONANALYSIS

Please describe the sample comparison you would like to create

To populate your comparison groups, select **Samples** or **Attributes**, choose an item class, and then drag one or more items to a group box. Note that every item added will further constrain your eligible samples within the group. You may then refine your sample selection by clicking Edit at the lower right corner of each group box. Click **Save** when you are ready to finalize this comparison.

Select a Covariate

Covariates will adjust fold changes and p-Values of your comparison to correct for confounding, undesirable or "extra" variables. ROSALIND will provide an uncorrected comparison in order for you to assess the effect of covariate correction. Begin by selecting the attribute to use for covariate correction.

Select Covariate: **None**
Time Course
Treatment

SAVE

KO Myd88
Genotype - KO Myd88
8 single samples
EDIT

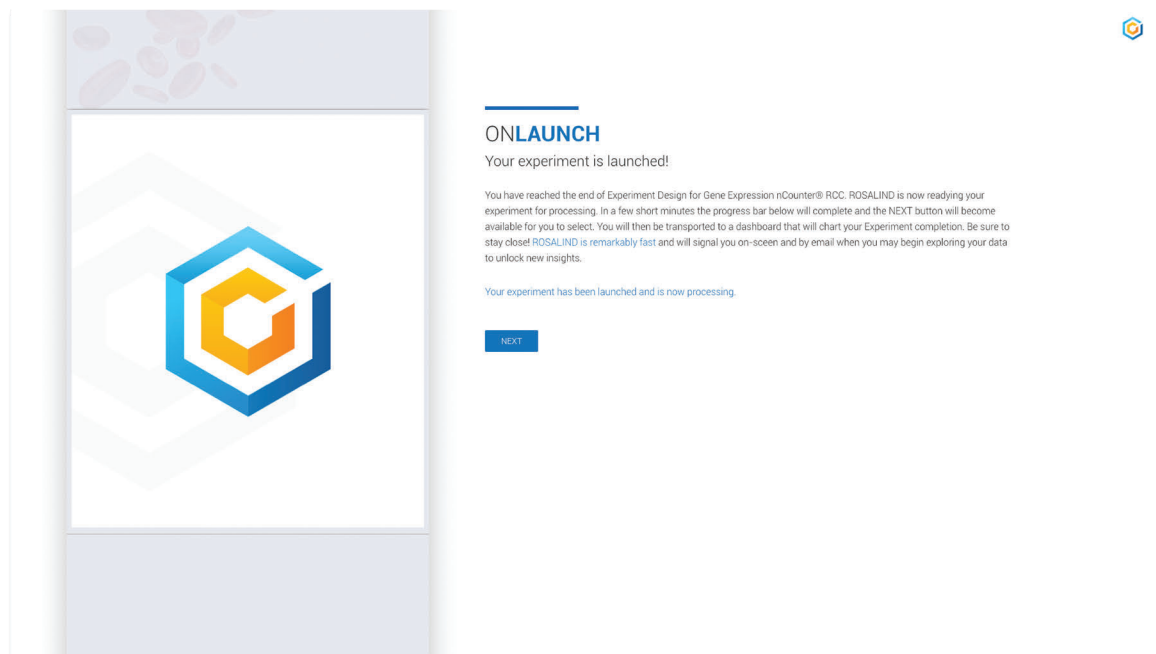
WT Myd88 fx
Genotype - WT Myd88 fx
8 single samples
EDIT

SAVE **SELECT COVARIATE** ✓

For an advanced analysis with covariate correction, click **SELECT COVARIATE** and choose any one of the valid covariate variables. Once all comparisons are defined, click **SAVE** and then **NEXT** to launch the experiment for analysis.

Analyze More

Setup your experiment in minutes.



Once complete, you will receive an email to notify you that your experiment is ready for exploration.

EXPERIMENT
Nanostripping of whole lung RNA from cell...

33% Standard Queue

Workflow Start: Apr 27, 2020 07:15 AM
Total Samples: 35

Nanostripping of whole lung RNA from cell-specific MyD88 KO at 0h 2h and 6h after in vivo sensitization with OVA-standard flagellin

Allergic asthma is a chronic disease of the airways characterized by eosinophilic and neutrophilic inflammation. MYD88, the adaptor molecule for TLR and IL-1 family member signaling, is required for allergic sensitization through the airway in animal models of allergic asthma. We generated conditionally mutant mice separately lacking MyD88 in airway epithelial cells (ECs) or dendritic cells (DCs) and alveolar macrophages (AMs) to define the contribution of MyD88 expression in each of these cell types. To examine crosstalk between ECs and CD11c-expressing cells in vivo, we examined transcriptional profiles from whole lung RNA at baseline, or following 2h or 6h in vivo lung allergic sensitization through the airways from WT MyD88 f/f, SPC cre+ MyD88 f/f (EC-MYD88 KO), CD11c cre+ MyD88 f/f (DC-MYD88 KO), and full MyD88 KO mice. We observed immune-specific transcriptional changes in whole lung RNA that were altered based on EC- or CD11c-specific deletion of MYD88. We also observed transcriptional (linked data set) and epigenetic changes in chromatin conformation in cDCs by ATAC-seq (linked data set) as well as changes in immune-specific sorted EC RNA, sorted AM RNA, and sorted cDC RNA, by Nanostring nCounter Immunology Codeset Analysis (additional linked files).

Method (Technology)	nanoString	Number of Samples	35
Experiment Type	Gene Expression nCounter® RCC	Attributes	CartridgeID, Date, Genotype, Treatment, Time Course, Tissue
Species	Mus musculus	Replicates	
Genome Build	mm10		
Panel	Immunology		

Rosalind™ Nanostring Gene Expression Methods

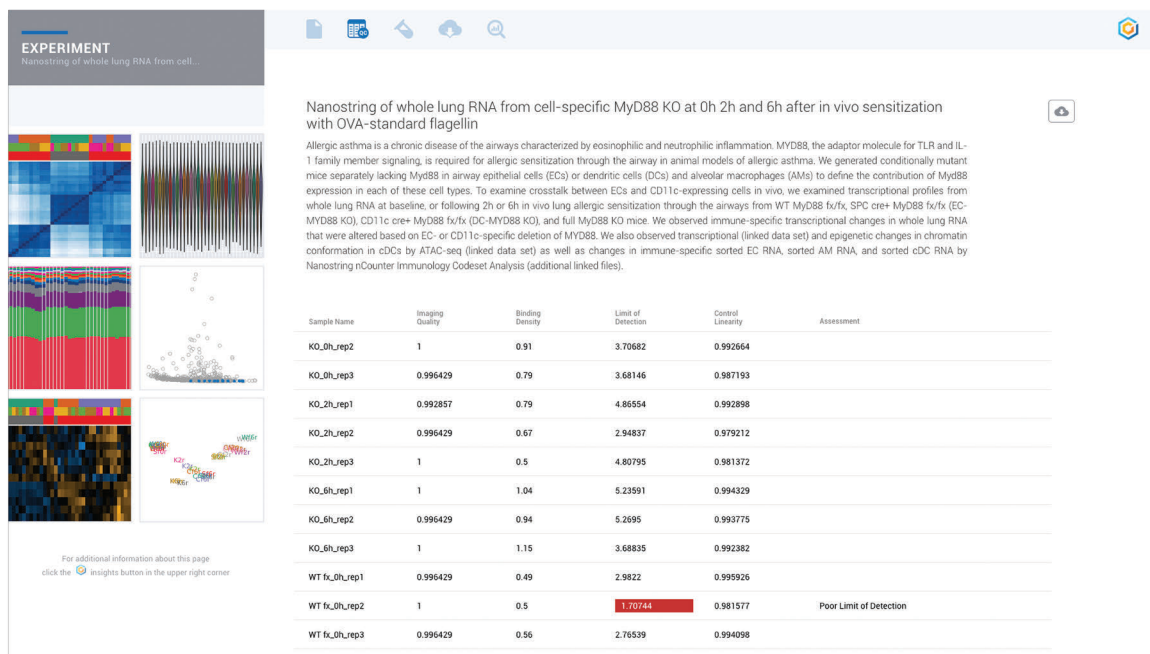
Data was analyzed by Rosalind (<https://rosalind.onramp.bio/>) with a HyperScale architecture developed by OnRamp Bioinformatics, Inc. (San Diego, CA). Read Distribution percentages, violin plots, identity heatmaps, and sample MDS plots were generated as part of the QC step. Normalization is done by dividing counts within a lane by the geometric mean of the normalizer probes from the same lane. The NormqPCR R library¹ was used to select normalizer probes using the getnorm algorithm. Fold changes and p-values were calculated using criteria provided by [Nanostring](#). Clustering of genes for the final heatmap of differentially expressed genes was done using the PAM (Partitioning Around Medoids) method using the fpc R library² that takes into consideration the direction and type of all signals on a pathway, the position, role and type of every gene, etc. Hypergeometric distribution was used to analyze the enrichment of pathways, gene ontology, domain structure, and other ontologies. The topGO R library³ was used to determine local similarities and dependencies between GO terms in order to perform Elim pruning correction. Several database sources were referenced for enrichment analysis, including Interpro⁴, NCBI⁴, KEGG^{4,5}, MSigDB^{6,7}, REACTOME⁸, WikiPathways⁹. Enrichment was calculated relative to a set of background genes relevant for the experiment.

- Perkins, J.R. et al. ReadqPCR and NormqPCR: R packages for the reading, quality checking and normalisation of RT-qPCR quantification cycle (Cq) data. *BMC Genomics* **13**: 286+ (2012).
- Hennig, C. Cran-package fpc: <https://cran.r-project.org/web/packages/fpc/index.html>
- Alexa A, Rahnenfuhrer J. topGO: Enrichment Analysis for Gene Ontology. *R* package version 1.38.1 (2019).
- Mitchell, A. et al. InterPro in 2019: improving coverage, classification and access to protein sequence annotations. *Nucleic Acids Research* **47**, D351–D360 (2019).

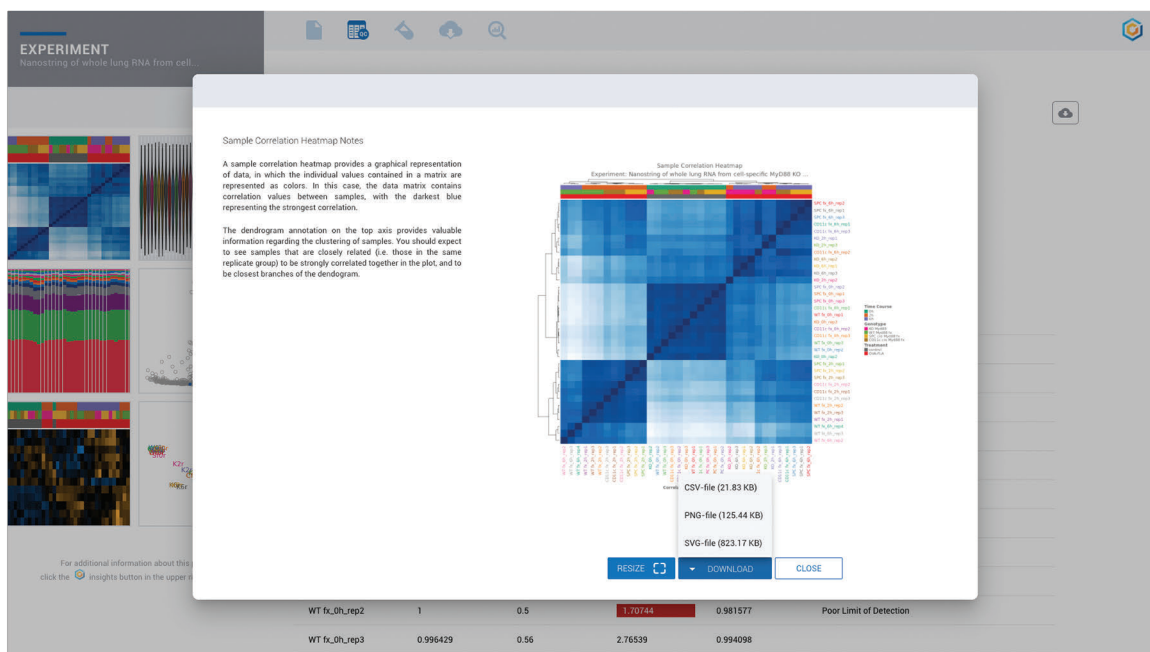
ROSALIND offers a way to monitor the experiment progress and important parameters while the analysis is underway.

Instant **Quality Control**

Optimized QC for NanoString experiments.



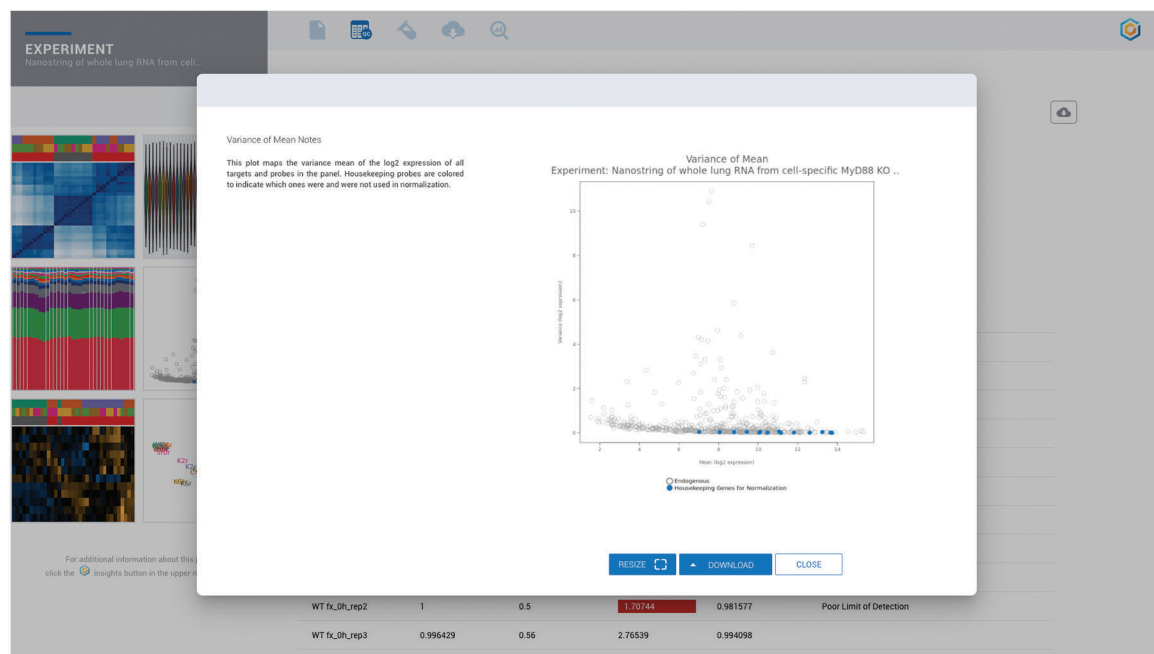
ROSALIND Quality Control has been optimized for NanoString Experiments to provide detailed plots for verification and validation of samples before diving into your results.



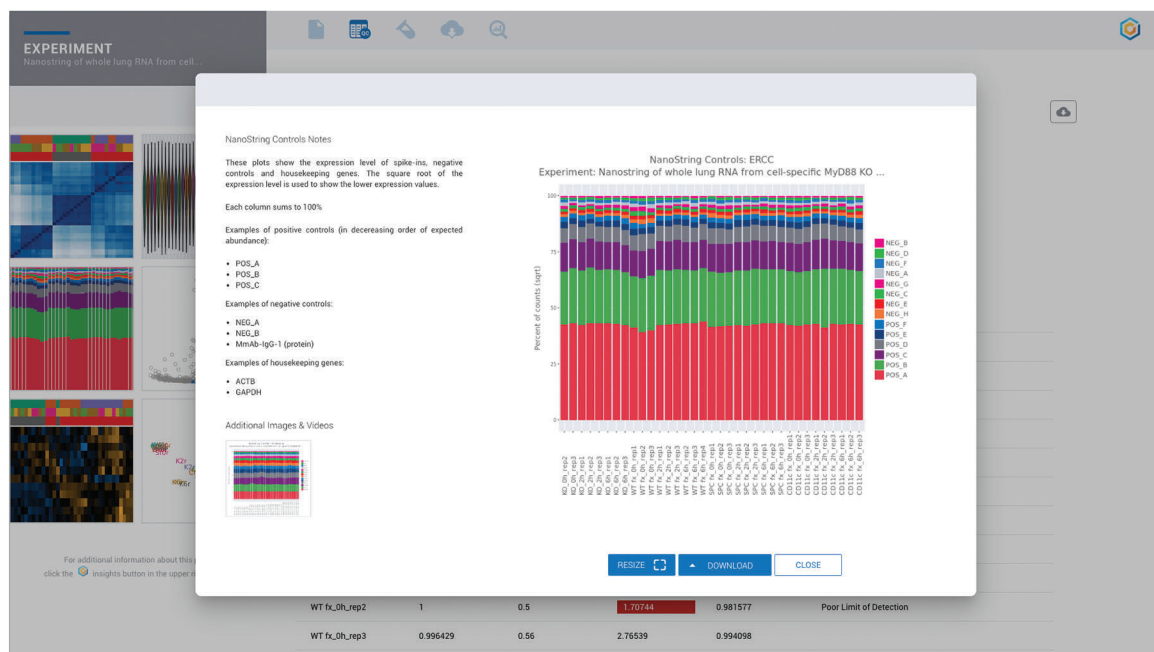
The Sample Correlation Heatmap provides a quick snapshot of each sample and their correlation. All figures are publication ready and made available to download in CSV, SVG, and PNG file formats.

Instant **Quality Control**

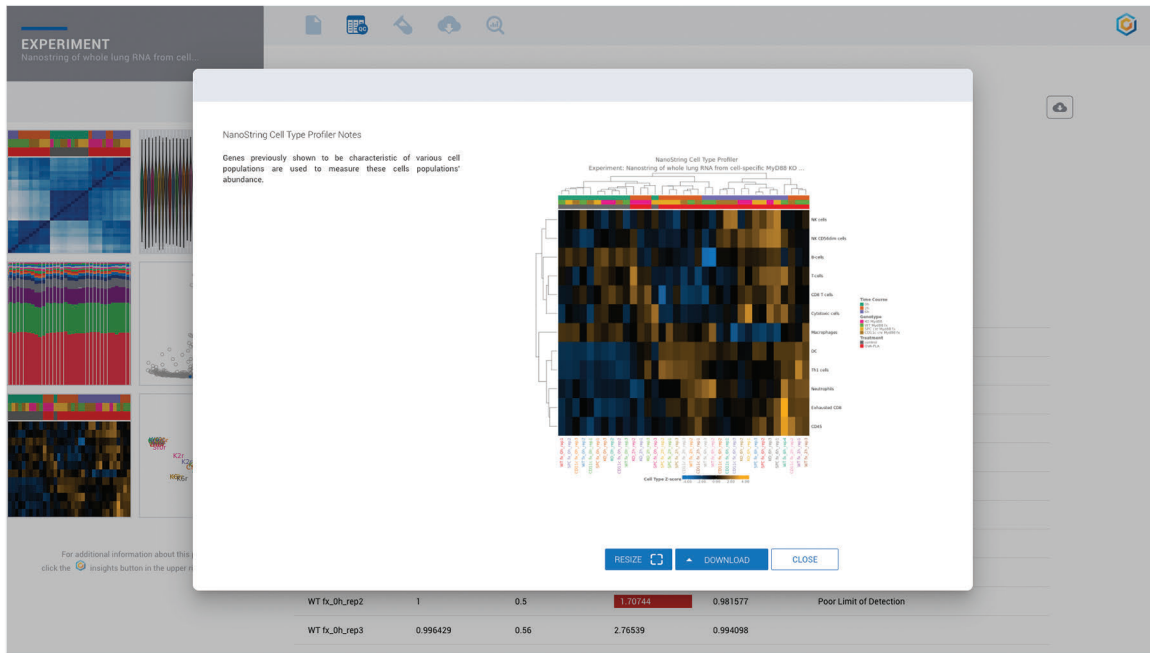
Optimized QC for NanoString experiments.



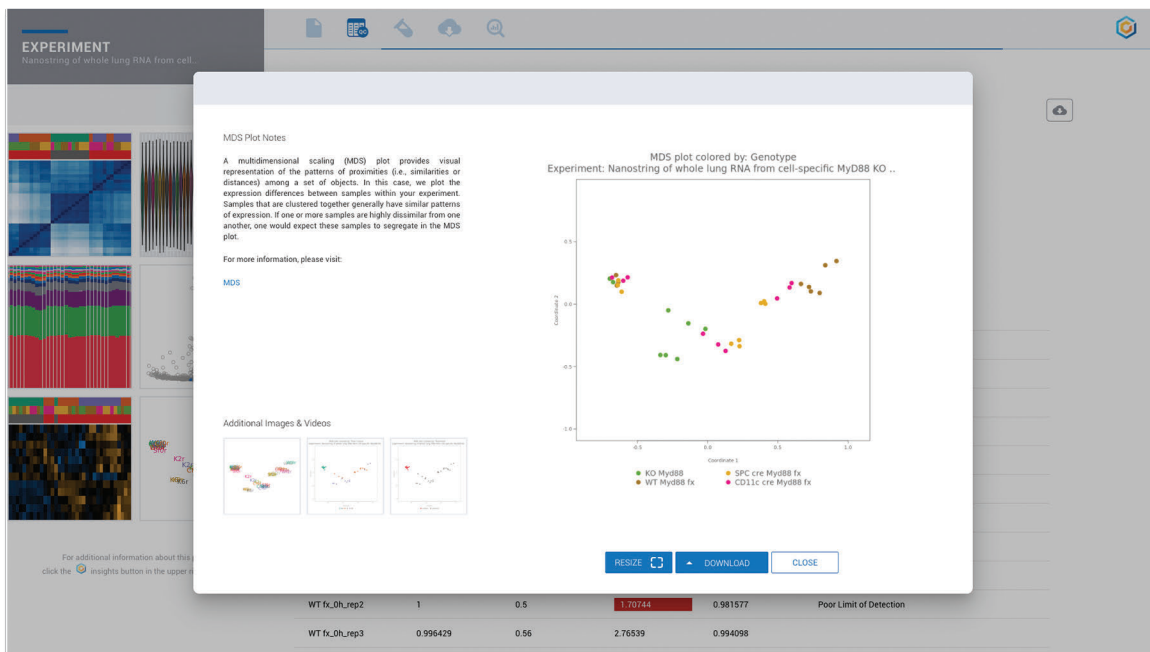
The Variance Plot shows the expression variance of all targets and highlights which ones were used for housekeeping normalization.



Review expression levels of NanoString controls and click the thumbnail on the lower left to view levels for the housekeeping genes.



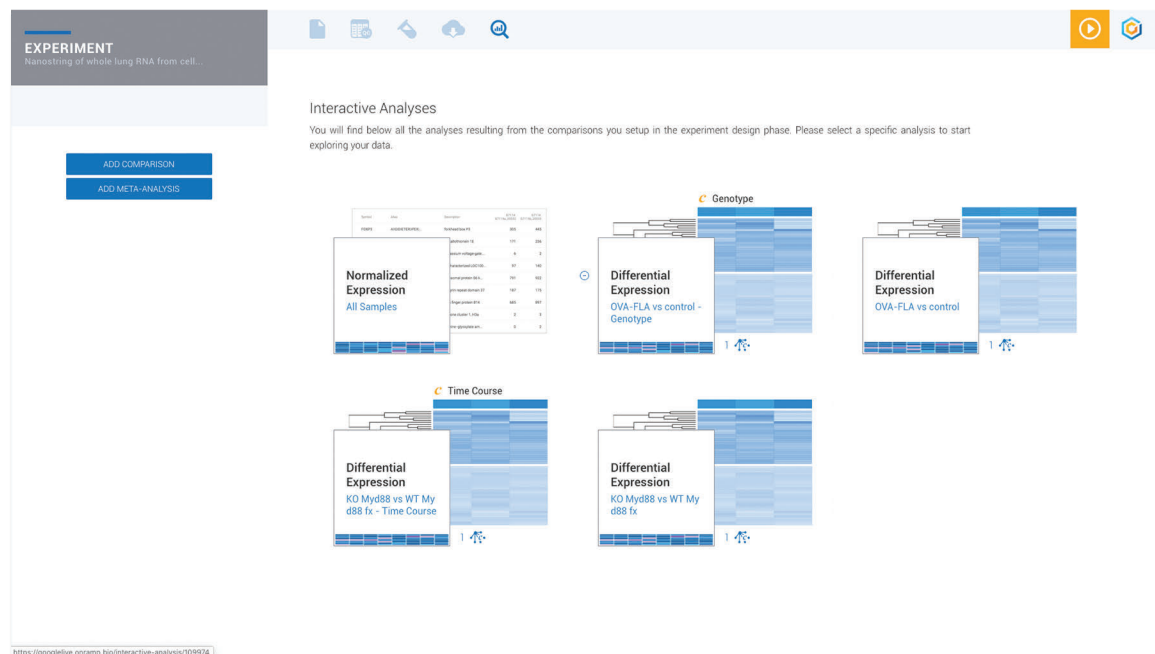
NanoString Cell Type Profiling analysis provides insights into the abundance of individual cell types by quantifying the expression of marker genes. Click [▶ DOWNLOAD](#) to access the complete Cell Type Profiling analysis.



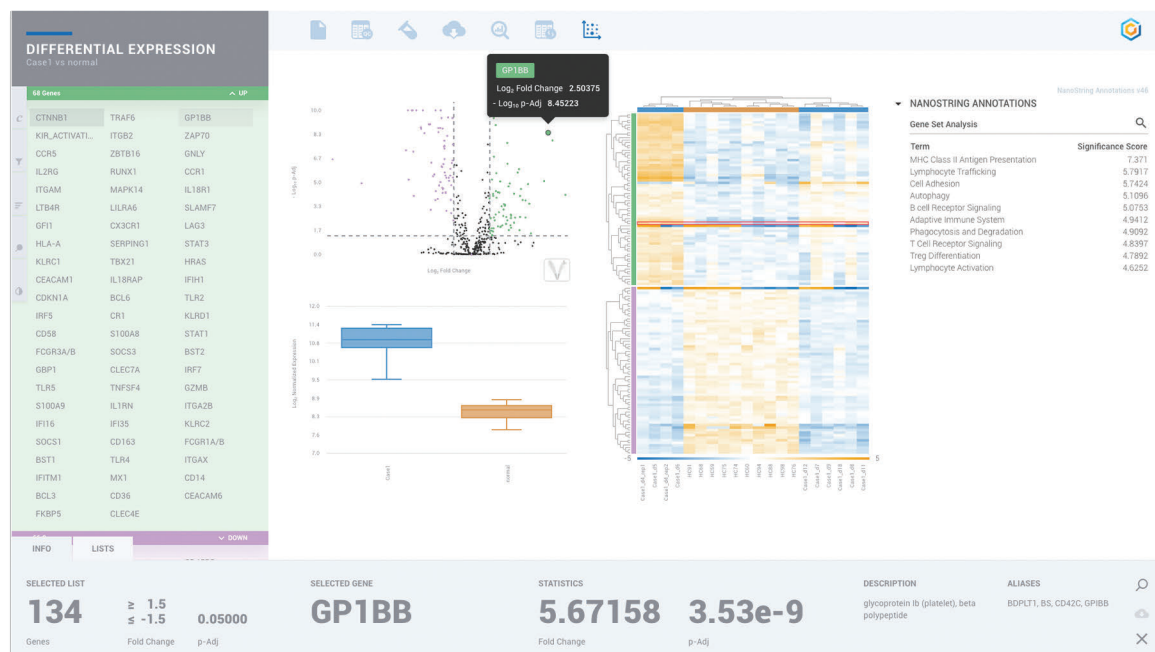
Verify the separation and grouping of samples with multidimensional scaling plots. **ROSALIND** provides an MDS plot for every attribute in the experiment.

Interpret More

Interactive experiences allow deeper exploration.



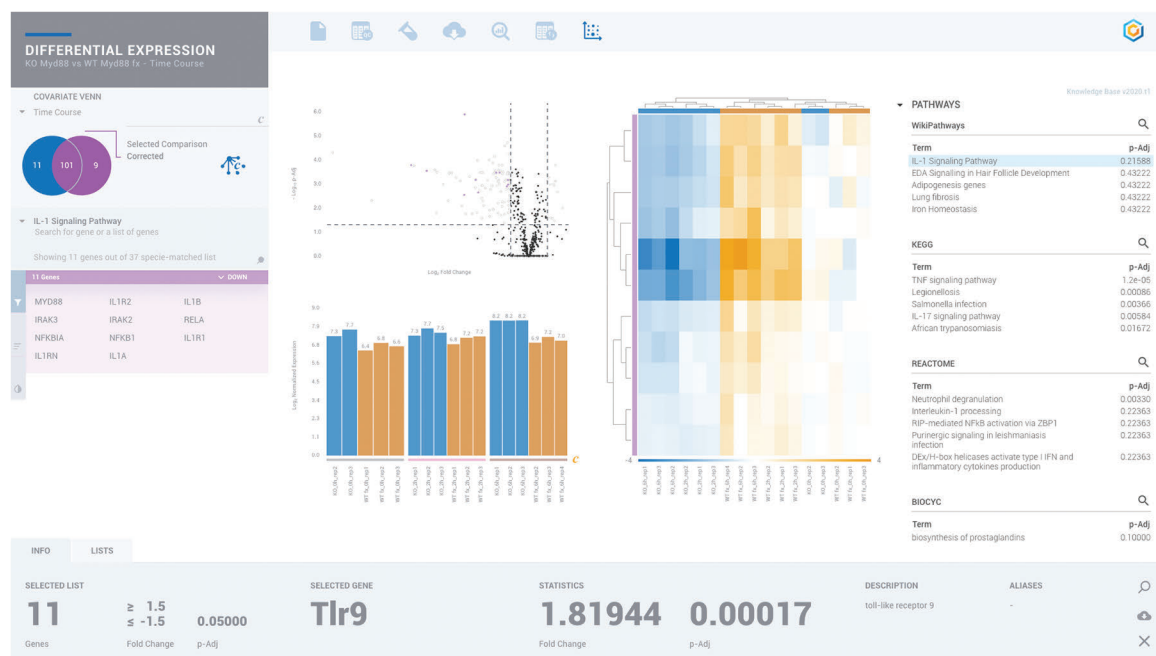
Explore differential expression results in each comparison, add new comparisons, meta-analyses, or investigate all gene expression levels within normalized expression.



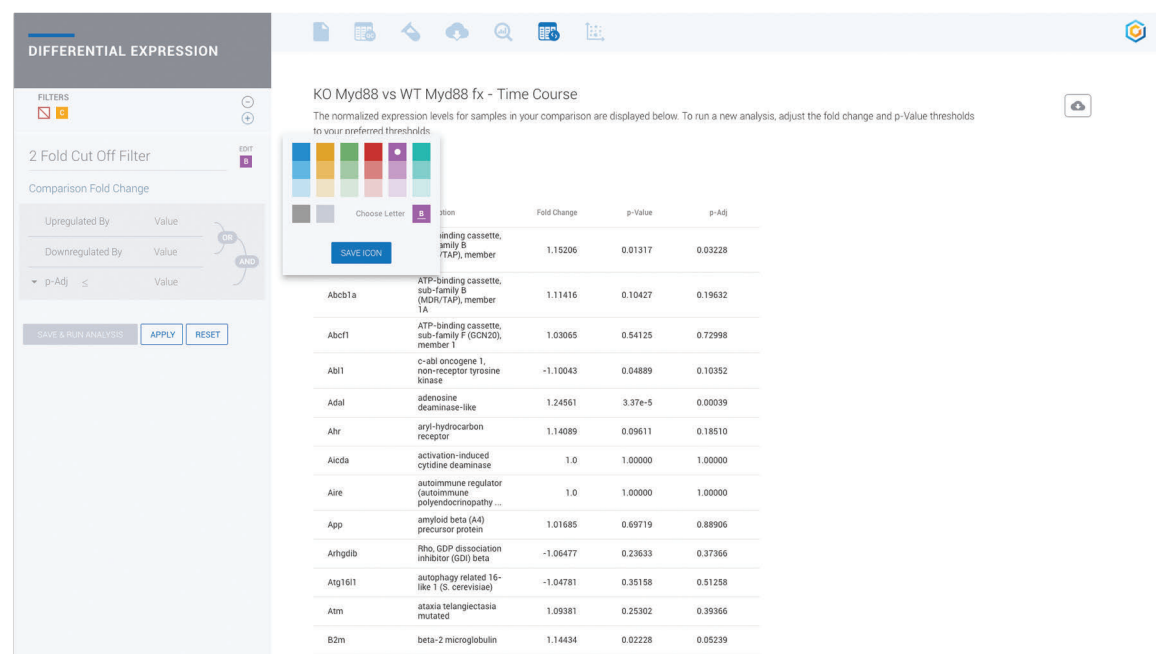
An interactive discovery experience provides dynamic charts of differentially expressed genes with deep interpretation from over 50 knowledge bases, including NanoString panel annotations, pathways, oncology, diseases, and more.

Interpret More

Interactive experiences allow deeper exploration.



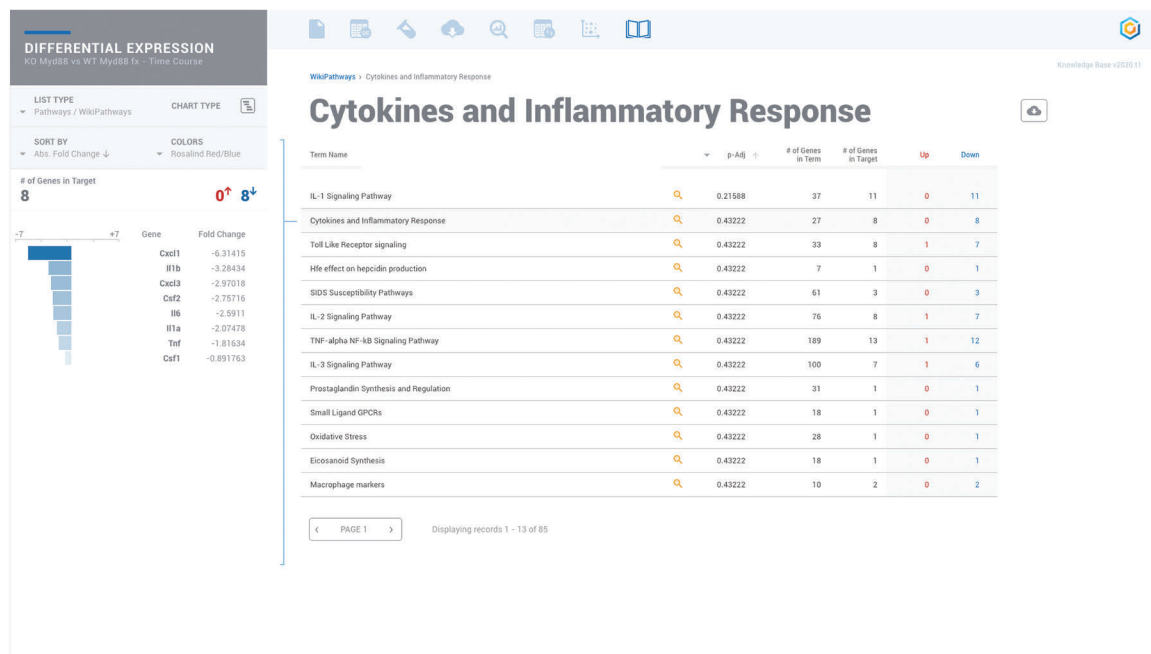
Select top pathways, gene lists or signatures for a focused experience showing only the results that are relevant to the area of interest.



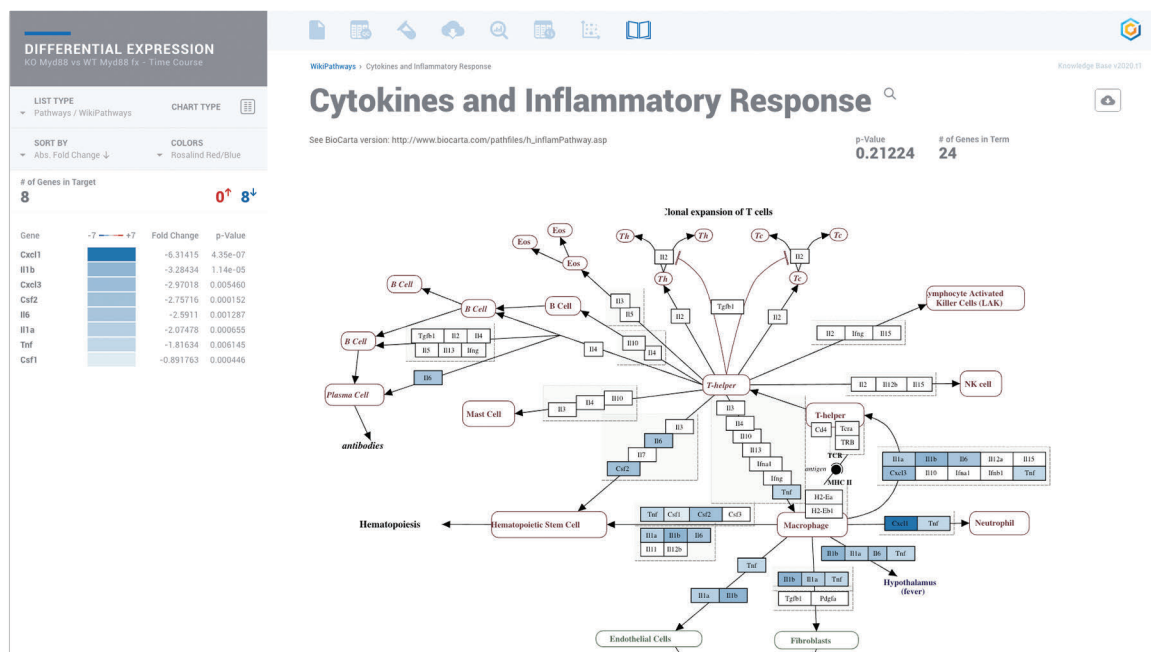
Adjust cut-offs by clicking the **+** button in the Filter Control to create a new filter and update the interactive graphs and pathway interpretation.

Interpret More

Interactive experiences allow deeper exploration.



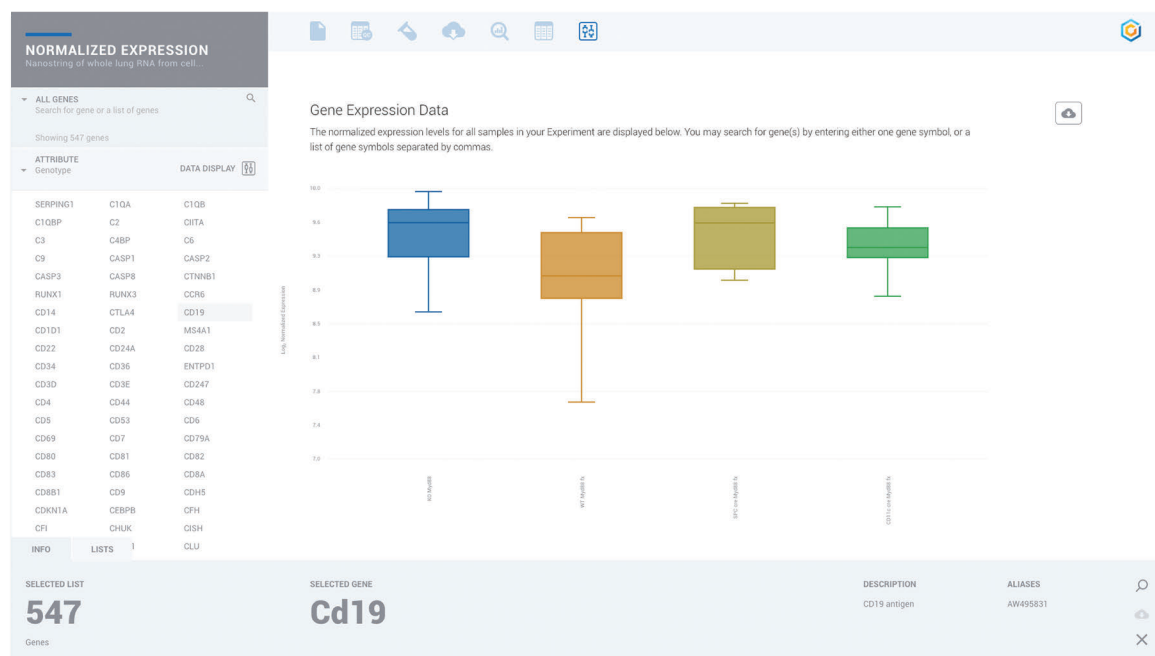
Seamlessly explore more than 50 knowledge bases and discover the relationships between differentially expressed genes and each associated pathway, gene ontology, protein interaction and more.



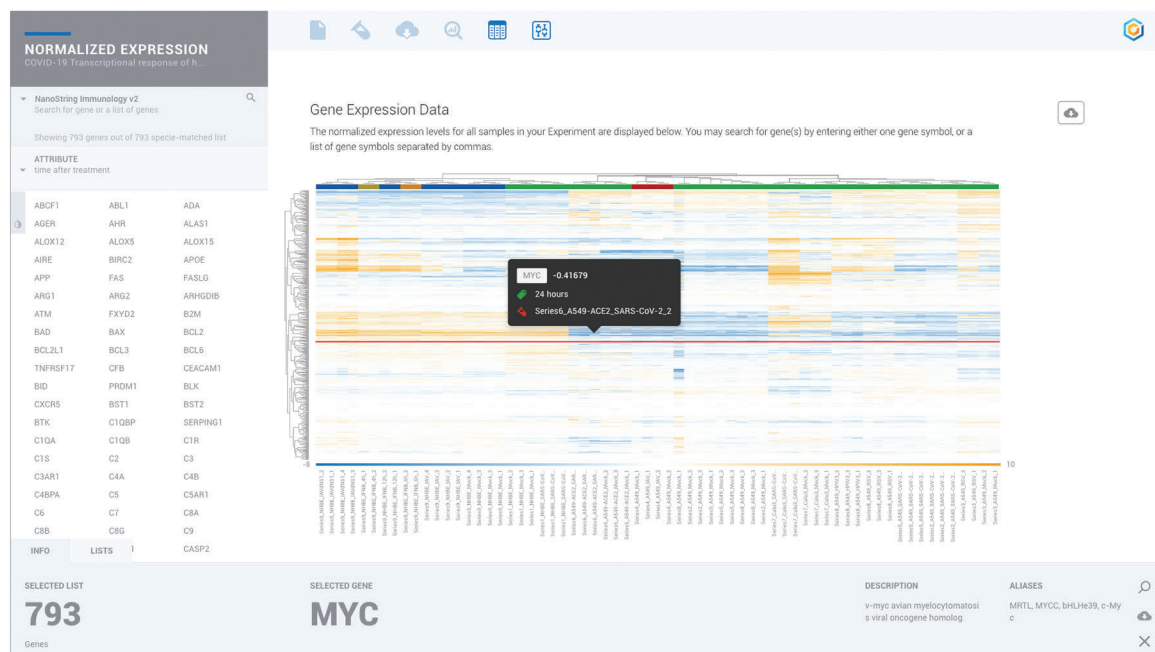
Fully interactive pathway diagrams and heatmaps enable rapid observation of gene expression and gene regulation effects.

Interpret More

Interactive experiences allow deeper exploration.



Select Normalized Expression to discover trends and patterns in expression levels across all samples and genes.



Custom heatmaps, box plots and bar plots show values across attributes and samples.

Collaborate Effortlessly

Share experiments without transferring or downloading data.

The screenshot displays the 'Collaborative Training Space' interface. On the left, a sidebar shows 'Participants' (8) and 'Experiments' (10). The main area has tabs for 'Experiments', 'Meta-Analyses', and 'Participants'. The 'Participants' tab is active, showing a list of users with their profiles, company/institution, title, team, email, and phone. A green 'ADD PARTICIPANTS' button is at the bottom.

Profile	Company/Institution	Title	Team	Email	Phone
Jeremy Davis-Turak	OnRamp Bioinformatics	VP of Bioinformatics	---	jeremy@onramp.bio	---
Jean Lezack	OnRamp Bioinformatics	Chief Science Officer	Exec Team	jean@onramp.bio	(619) 269-4900
Tim Wesselman	Onramp Bioinformatics, Inc.	CEO	Exec Team	tim@rosalind.bio	(858) 705-1356
Tim Wesselman	onrampcorp.com	Director Discovery Science	ROSALIND	tim@onrampcorp.com	---
Timothy Wesselman (Nano)	ROSALIND	CEO	Executive	tim@geneexpression.bio	(858) 705-1356
Arizona Milotich	Onramp Bioinformatics, Inc.	Research Success Advisor	Sales	arizona@rosalind.bio	(760) 208-7005
Arizona Milotich	Onramp	---	Stuff	arizona@geneexpression.bio	(760) 208-7005
Sam Kim	OnRamp Bioinformatics	---	Onramp	sam@onramp.bio	(310) 592-7036

Easily create a space and invite colleagues or collaborators to work alongside you on your experiments.

The screenshot displays the 'Collaborative Training Space' interface with the 'Experiments' tab active. It shows a list of experiments with their titles, descriptions, and engagement metrics (likes, comments, shares). The experiments listed are 'IO360 Multi-Lot NanoString nCounter Data Analysis', 'Using PlexSet for comprehensive drug screening assays', and 'A Dynamic Immune Response Shapes COVID-19 Progression'.

Experiment Title	Description	Engagement
IO360 Multi-Lot NanoString nCounter Data Analysis	Trended analysis of clinical trial patients with the IO360 Panel.	24 likes, 2 comments, 1 share
Using PlexSet for comprehensive drug screening assays	A broad analysis of transcriptional responses to drug concentrations and compounds on potential targets.	96 likes, 2 comments, 1 share
A Dynamic Immune Response Shapes COVID-19 Progression	Through daily transcriptomic profiling of whole blood from SARS-CoV-2 patients, Ong et al. reveal that the early immune response is highly dynamic in COVID-19 patients.	33 likes, 3 comments, 1 share

Collaborate, explore and analyze the same data simultaneously without having to download, transfer or install anything.

About ROSALIND

Based in the Genomics Capital of San Diego, OnRamp.Bio provides ROSALIND®, the first-ever genomics analysis platform specifically designed for life science researchers to analyze and interpret datasets, without any prior bioinformatics skills.

Named in honor of pioneering researcher Rosalind Franklin, who made a major contribution to the discovery of the double-helix structure of DNA with her famous photograph 51, the ROSALIND® platform aims to simplify the practice of genomic data interpretation, so biologists, researchers and drug developers can harness the potential of genomic information from DNA sequencing to microarrays and mass spec, while reducing costs and increasing productivity.

ROSALIND® puts the researcher in the driver's seat of data analysis, and helps to free up valuable time for Bioinformatics Cores to offload standard analyses and focus precious resources on more complex challenges. ROSALIND® brings bioinformatics analyses to the bench by broadly expanding access to genomic and proteomic technologies for cancer research and precision medicine.

Learn More: www.onramp.bio

Register for Free: www.onramp.bio/nanostring



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