

# iPathwayGuide

Expression Analysis with  
iPathwayGuide

# Agenda: 02. Uploading Data

- What is differential expression
  - Where does the data come from
  - iPG supported files, organisms, & platforms
- What's in the data
  - Custom tab files
  - What if I have more than 2 conditions
- TRY IT! Demonstrate simplicity & flexibility of upload
  - Dataset
  - Step-by-step navigation
  - Cautions
  - Submitting analysis
- Q & A

# Input Data

- Organism

- Human
  - Mouse
  - Rat

- File Type

- CuffDiff
  - DESeq(2)
  - EdgeR
  - JMP Genomics

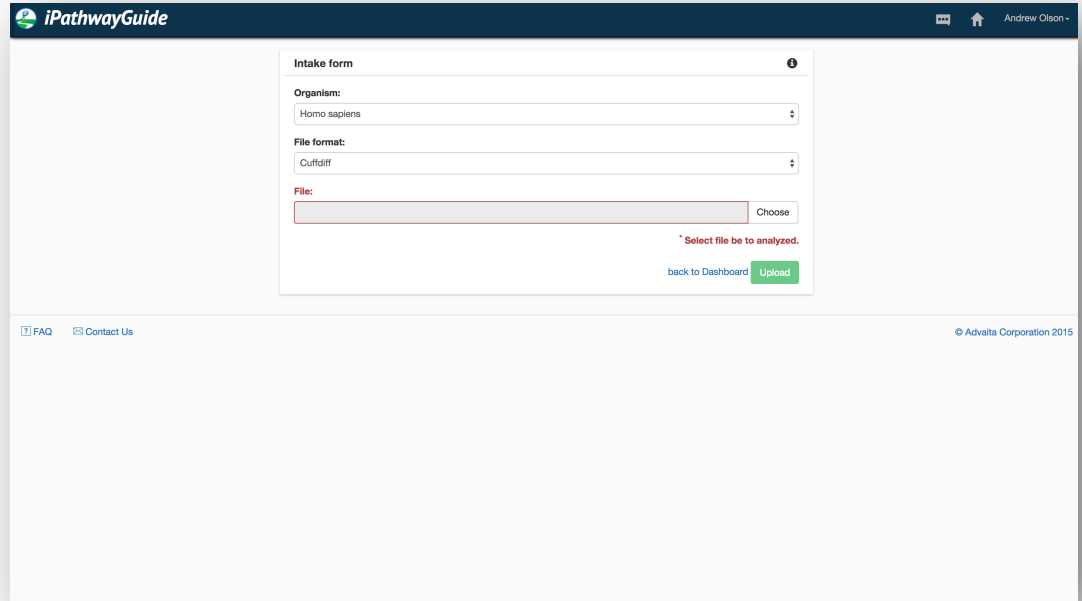
- Custom Tab-delimited file (\*.txt)

- GEO2R/Limma

- nSolver from NanoString

- Affy CEL file

- Sciex Protein Assembler



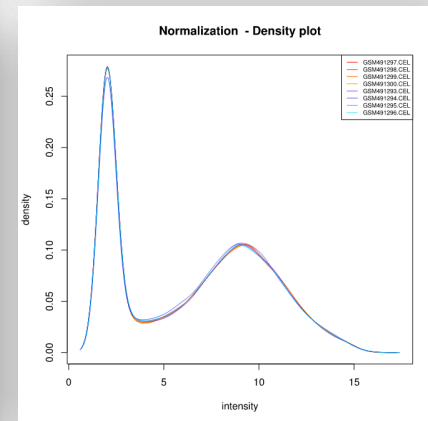
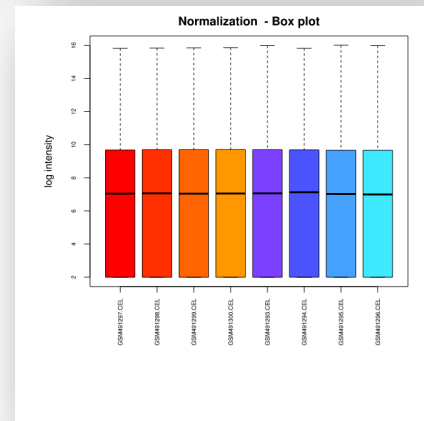
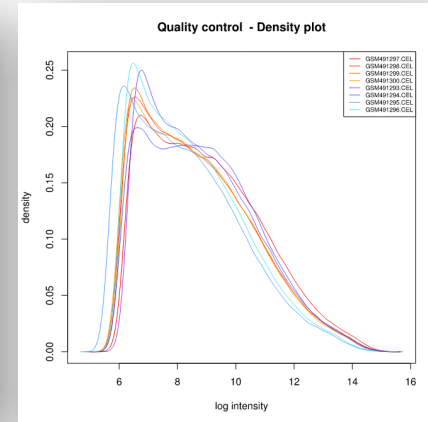
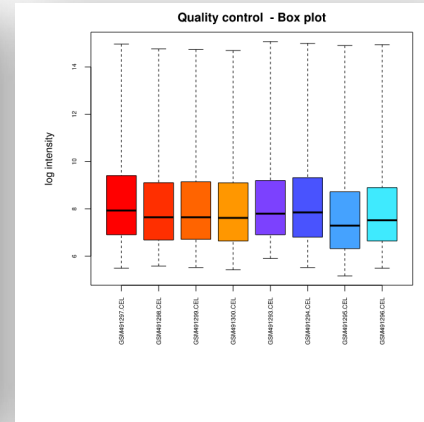
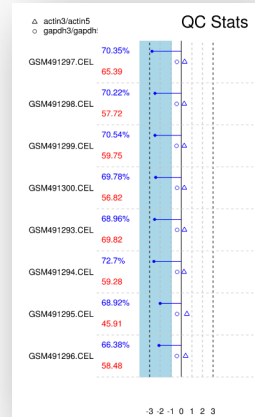
The screenshot shows the iPathwayGuide website's intake form. The header includes the iPathwayGuide logo, a home icon, a user profile icon, and the name "Andrew Olson". The form itself is titled "Intake form" and contains three dropdown menus: "Organism" (set to "Homo sapiens"), "File format" (set to "Cuffdiff"), and "File" (with a "Choose" button). Below the "File" field is a red error message: "\* Select file to be analyzed." At the bottom of the form are two buttons: "back to Dashboard" and "Upload". The footer of the page includes links for "FAQ" and "Contact Us", and a copyright notice: "© Advaita Corporation 2015".

# Multiple Contrast Files

- These files support multiple contrasts in a single input file
  - CuffDiff
  - DESeq(2)
  - EdgeR
  - JMP Genomics
  - nSolver from NanoString
  - Sciex Protein Assembler

# Affymetrix CEL file QC/Normalization

- Affymetrix CEL files require QC & Normalization because input data is sample-based
- Takes between 2-8 minutes (depending on # of files)
- Be sure to download and save QC and Normalization data before submitting for analysis



# Contrasts Page

- Project title
- Project description
- Select which contrasts to include (max 5)
- Preview data

iPathwayGuide

Report intake form (file: Blastomere\_ProteinData\_Sample.txt)

Report title

Report description

Analyze Contrast

Condition

Control

All genes

2620

DE threshold

Fold change (log)

0.3

Adjusted p-value

0.05

View/Apply

153

Report title is missing or has invalid characters.

Report description is missing.

Cancel Analyze data

show DE genes only

Gene Symbol

Entrez gene ID

Fold change (log)

Adjusted p-value

HIST2H3D 653604 -0.458 1.000e-6

HIST1H1D 3007 -0.435 1.000e-6

SNRPB 6628 -0.414 1.000e-6

GNB2 2783 0.281 1.000e-6

UBQLN1 29979 0.026 1.000e-6

TSR2 90121 0.000 1.000e-6

GDF5 8200 0.000 1.000e-6

HDHD3 81932 0.000 1.000e-6

C21orf33 8209 0.000 1.000e-6

CACUL1 143384 0.000 1.000e-6

10 25 50 100

1 2 3 4 5 6 7 ... 262

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ITA

a bioinformatics company

# Thresholds & DEGs Selection

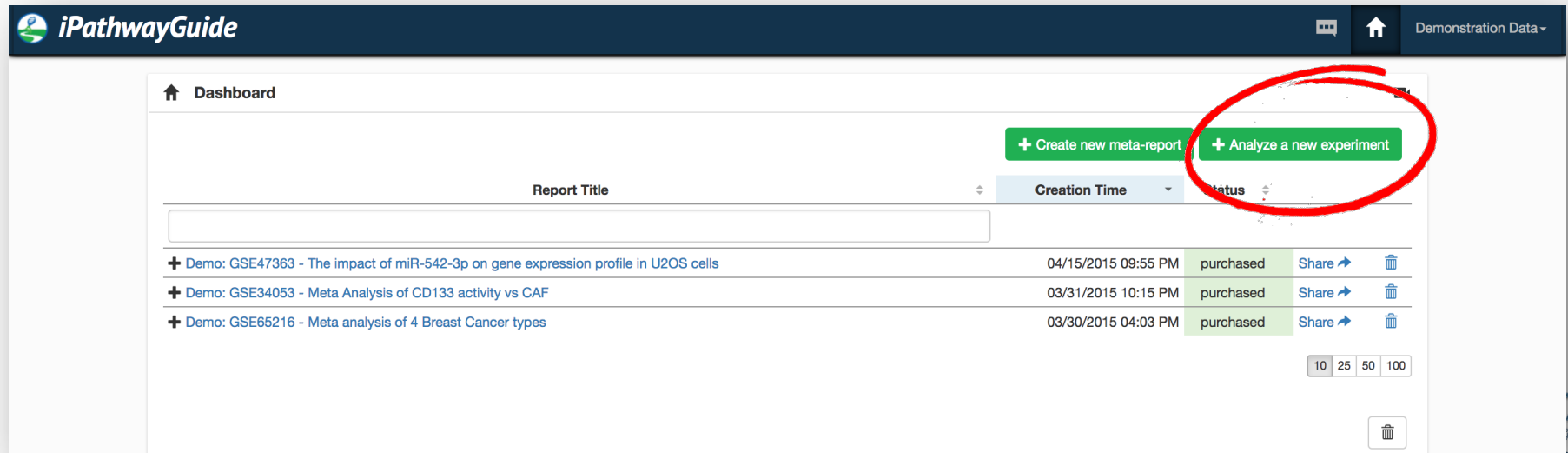
Analyze Contrast	Contrast		All genes	DE threshold		Selected DE genes	
	Condition	Control		Fold change (log)	Adjusted p-value		
<input checked="" type="checkbox"/>	<input type="text" value="Condition"/>	<input type="text" value="Control"/>	2620	<input type="text" value="0.6"/>	<input type="text" value="0.05"/>	<input type="button" value="View/Apply"/>	23

- Ideally, you want 5%-10% DEGs
- If less than 5%, change FC=0
- P-value = 0.05 should yield 5% DEG by chance alone.
  - If DEGs < 5%, then data may not be good quality.
  - If DEGs > 5%, then adjust FC until you have 5%-10% DEGs

# LET'S TRY IT! (CUSTOM PROTEIN DATA)

## BLASTOMERE DATASET

- Log into your iPathwayGuide.com account
- Choose “Analyze a new experiment”



The screenshot shows the iPathwayGuide dashboard. At the top, there is a dark blue header with the iPathwayGuide logo on the left and a 'Demonstration Data' dropdown on the right. Below the header, the main content area has a 'Dashboard' section with a home icon. In the center, there is a table with columns for 'Report Title', 'Creation Time', and 'Status'. Above the table, there are two green buttons: '+ Create new meta-report' and '+ Analyze a new experiment'. The '+ Analyze a new experiment' button is circled in red. Below the table, there are three demo entries, each with a plus icon, a title, a creation time, a status, and a share icon. At the bottom right, there is a pagination control showing '10', '25', '50', and '100'.

iPathwayGuide

Dashboard

+ Create new meta-report + Analyze a new experiment

Report Title	Creation Time	Status
+ Demo: GSE47363 - The impact of miR-542-3p on gene expression profile in U2OS cells	04/15/2015 09:55 PM	purchased Share
+ Demo: GSE34053 - Meta Analysis of CD133 activity vs CAF	03/31/2015 10:15 PM	purchased Share
+ Demo: GSE65216 - Meta analysis of 4 Breast Cancer types	03/30/2015 04:03 PM	purchased Share

10 25 50 100



# NAVIGATION: DASHBOARD -> INTAKE -> UL

- Choose: HUMAN
- Select: Custom Tab delimited \*.txt
- Choose file: Blastomere\_ProteinData\_Sample.txt

The screenshot shows the iPathwayGuide web interface. At the top is a dark blue header with the iPathwayGuide logo on the left and a home icon with the text "Demonstration Data" on the right. Below the header is a white "Intake form" box. Inside the form, the "Organism:" dropdown menu is open, showing a list of options: Affymetrix CEL files, Cuffdiff (checked with a blue checkmark), DESeq(2), EdgeR, GEO2R / Limma (R/Biconductor), JMP Genomics, nSolver (NanoString Technologies), Sciex SWATH Proteomics data, and Custom tab-delimited \*.txt (highlighted in blue). At the bottom of the form are two buttons: "back to Dashboard" and "Upload".

# CHOOSE COLUMNS & UPLOAD

- Type of ID and Column
- LogFC
- pValue

Hit “Upload”

**Intake form**

**Organism:**  
Homo sapiens

**File format:**  
Custom tab-delimited \*.txt

**File:**  
Blastomere\_ProteinData\_Sample.txt Choose

☐ Gene Symbol ☒ Protein ID

**Protein ID**  
AccessionID

**Fold change**  
logFC


**p-value (adjusted p-value recommended)**  
PVal



**File content preview**


N	Unused	Total	%Cov	%Cov(50)	%Cov(95)	AccessionID	Name
Species	Peptides(95%)	FC	logFC	PVal			
1	173.09	173.09	50.20999908	45.21999955	37.02000082		
sp P21333 FLNA_HUMAN			Filamin-A	OS=Homo sapiens	GN=FLNA	PE=1	SV=4 HUMAN
137	0.774689376	-0.368310139	2.95E-05				
2	143.59	142.79	46.32000029	38.83000016	28.15999985		

[back to Dashboard](#) Upload


# REPORT INTAKE FORM

 **iPathwayGuide**

  **Demonstration Data**

Report intake form (file: Blastomere\_ProteinData\_Sample.txt) 

**Report title**

Report title 

**Report description**

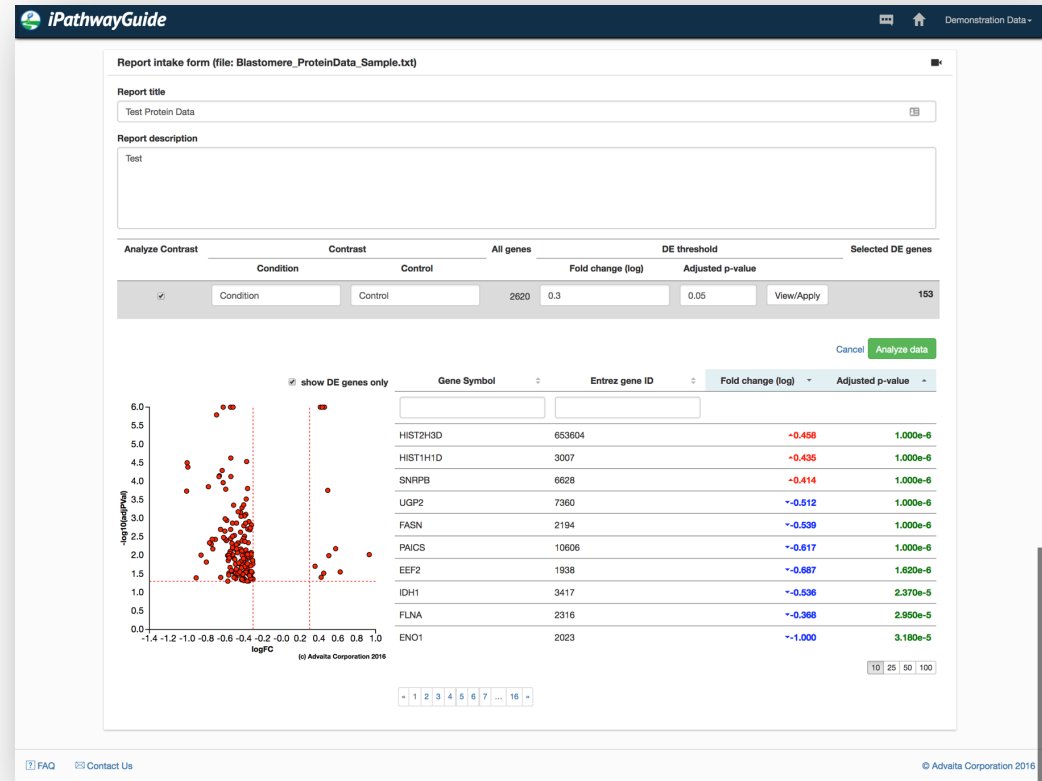
Report description

Analyze Contrast	Contrast		All genes	DE threshold		Selected DE genes
	Condition	Control		Fold change (log)	Adjusted p-value	
<input checked="" type="checkbox"/>	<input type="text" value="Condition"/>	<input type="text" value="Control"/>	2620	<input type="text" value="0.6"/>	<input type="text" value="0.05"/>	<input type="button" value="View/Apply"/> 23

- Report title is missing or has invalid characters.
- Report description is missing.

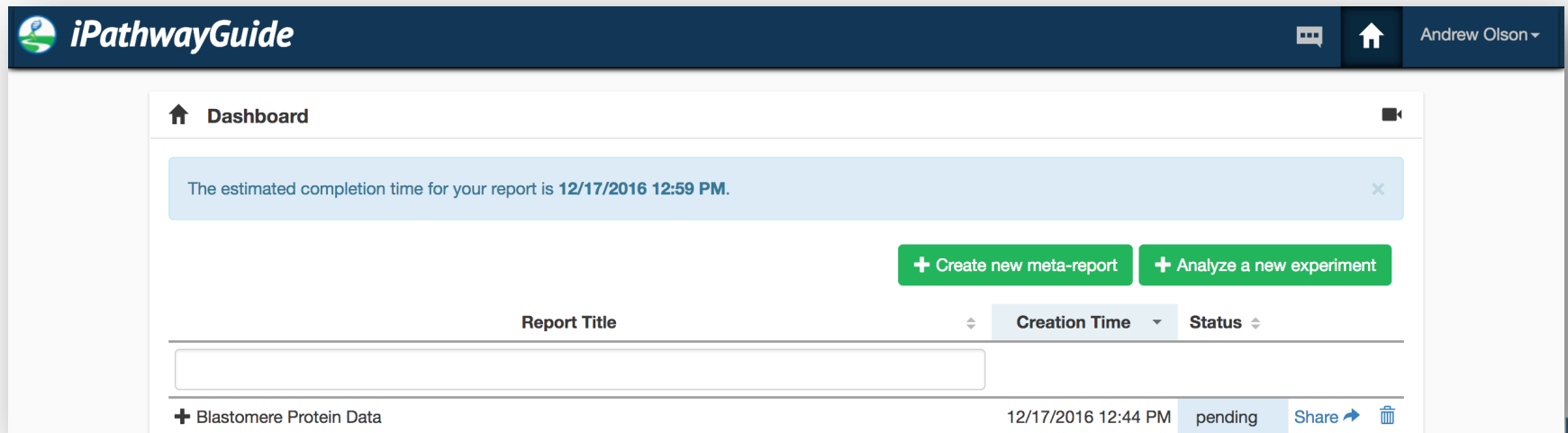
# ADJUST THRESHOLDS & ADD LABELS

- Adjust FC to 0 to assess quality
- Adjust contrast labels (if needed)
- Give Title and Description
- **PLEASE DO NOT SUBMIT DATA**



# DASHBOARD UPDATE

- ETA
- Email when ready



The screenshot displays the iPathwayGuide dashboard. At the top, a dark blue header contains the iPathwayGuide logo, a home icon, and the user name "Andrew Olson". Below the header, a "Dashboard" section is visible. A light blue notification bar states: "The estimated completion time for your report is 12/17/2016 12:59 PM." Below this, there are two green buttons: "+ Create new meta-report" and "+ Analyze a new experiment". A table with the following headers is shown: "Report Title", "Creation Time", and "Status". The first row of the table contains the text "+ Blastomere Protein Data", the date and time "12/17/2016 12:44 PM", and the status "pending". To the right of the status are links for "Share" and a trash icon.

Report Title	Creation Time	Status
+ Blastomere Protein Data	12/17/2016 12:44 PM	pending

# iPG: Dashboard

- Information about most recent experiments
- Search box
- Start new analysis
- Trash / Restore
- Share
- Purchase

The screenshot displays the iPathwayGuide dashboard. At the top, there's a navigation bar with the iPathwayGuide logo, a user profile 'Andrew Olson', and buttons for 'Create new meta-report' and 'Analyze a new experiment'. Below this is a 'Dashboard' section with a search bar and filters for 'Project Description', 'Creation Time', and 'Status'. A table lists various experiments, including 'Demo: GSE47363 - The impact of miR-542-3p on gene expression profile in U2OS cells' and 'Demo: GSE34053 - Meta Analysis of CD133 activity vs CAF'. Each entry shows its creation time, status (e.g., 'purchased', 'expired'), and options to 'Share' or 'Trash'. A pagination bar at the bottom shows 10, 25, 50, and 100 items per page.

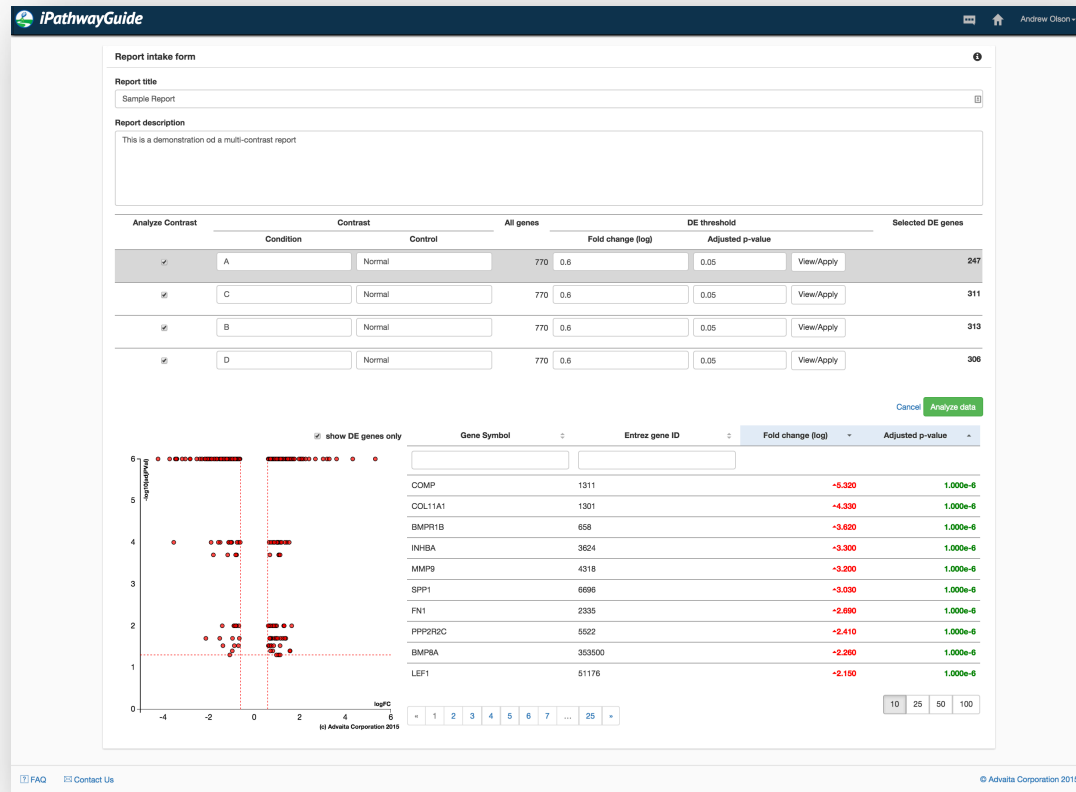
Project Description	Creation Time	Status	Share	Trash
<p>✖ Demo: GSE47363 - The impact of miR-542-3p on gene expression profile in U2OS cells</p> <p>Analysis of gene expression change in U2OS cells expression synthetic miR-542-3p mimics. Forty-eight hours after transfection with negative miRNA mimics or miR-542-3p mimics, U2OS cells were subjected to RNA isolation.</p>	04/15/2015 09:55 PM	purchased	Share	Trash
✚ Demo: GSE34053 - Meta Analysis of CD133 activity vs CAF	03/31/2015 10:15 PM	purchased	Share	Trash
✚ Demo: GSE65216 - Meta analysis of 4 Breast Cancer types	03/30/2015 04:03 PM	purchased	Share	Trash
✚ Demo - NanoString PanCancer Panel sample data report	03/28/2015 04:16 PM	expired	Share	Trash
✚ GSE65216 - TNBC vs Healthy	03/28/2015 03:54 PM	purchased	Share	Trash
✚ Demo - GSE47363 - The impact of miR-542-3p on gene expression profile in U2OS cells	03/28/2015 03:34 PM	purchased	Share	Trash
✚ GSE8859 - Human SMA1 vs SMA3 Default thresholds - 80 DEGs	03/17/2015 03:02 PM	purchased	Share	Trash
✚ GSE19674: Gene Expression Analysis of spinal cord obtained from a mouse model of severe Spinal Muscular Atrophy	03/02/2015 04:20 PM	purchased	Share	Trash
✚ Mouse SMA Meta Report	03/02/2015 02:26 PM	purchased	Share	Trash
✚ GSE56284 - Mouse SMA - standard thresholds	02/28/2015 03:24 PM	purchased	Share	Trash

Project Description	Creation Time	Status	Restore
TNBC vs Healthy default cutoff	04/15/2015 06:36 PM	trial	Restore
meta test	04/15/2015 12:03 PM	trial	Restore
Rat test	04/03/2015 12:28 PM	expired	Restore
CD133 neg vs CAF	03/31/2015 08:14 PM	purchased	Restore
CD133pos vs CAF fc3 pv0.01	03/31/2015 07:16 PM	purchased	Restore
CD133pos vs CAF	03/31/2015 06:15 PM	purchased	Restore
GSE34053 - CD133- vs CFA	03/31/2015 05:02 PM	purchased	Restore
GSE34053 - CD133 pos. vs CAF - Colon Cancer	03/31/2015 04:12 PM	purchased	Restore
GSE65216 - Luminal B vs Healthy	03/30/2015 10:49 AM	purchased	Restore
GSE65216 - Luminal A vs Healthy	03/30/2015 10:00 AM	purchased	Restore

# Multi-Contrast File

- iPathwayGuide will display up to 20 contrasts
- Select which contrasts to include (max 5)
- Preview data



# LET'S TRY IT! (NanoString nSolver)

NanoString nSolver dataset:

- Log into your iPathwayGuide.com account
- Choose “Analyze a new experiment”
- Upload “nSolver 3.0 Human data.txt”



# Multi-Contrast Files

Note that we now see more than one contrast.

**iPathwayGuide** Andrew Olson

Report intake form (file: nSolver 3.0 Human data.txt)

**Report title**  
Report title

**Report description**  
Report description

Analyze Contrast	Contrast		All genes	DE threshold		Selected DE genes
	Condition	Control		Fold change (log)	Adjusted p-value	
<input type="checkbox"/>	BasalLike	normal	770	0.6	0.05	View/Apply 297
<input checked="" type="checkbox"/>	Her2Enriched	normal	770	0.6	0.05	View/Apply 248
<input type="checkbox"/>	LuminalA	normal	770	0.6	0.05	View/Apply 315
<input type="checkbox"/>	LuminalB	normal	770	0.6	0.05	View/Apply 297

- Report title is missing or has invalid characters.
- Report description is missing.

[Cancel](#) [Analyze data](#)

# ORDER OF CONTRASTS: CHECK GEO2R!

- DON'T HAVE A GOOD DATA SET FOR THIS
- Should only do this if we have time.

# Questions & Break



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