

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

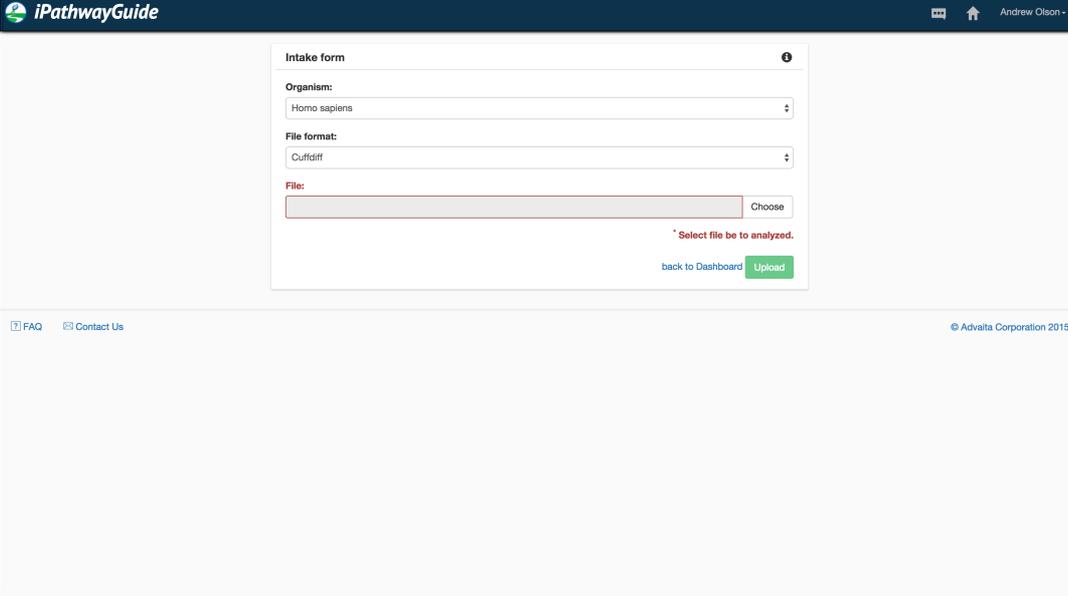
-  Custom Tab-delimited file (*.txt)

-  GEO2R/Limma

-  nSolver from NanoString

- Affy CEL file

- Sciex Protein Assembler



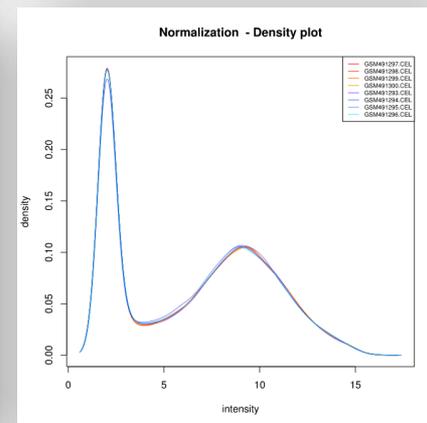
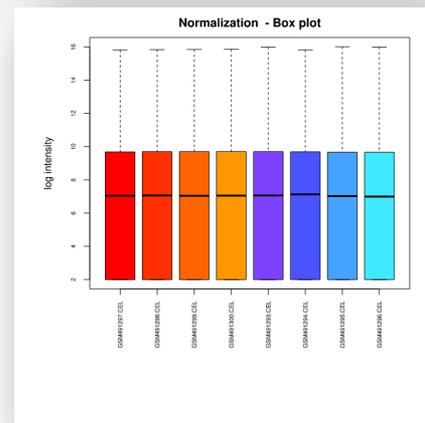
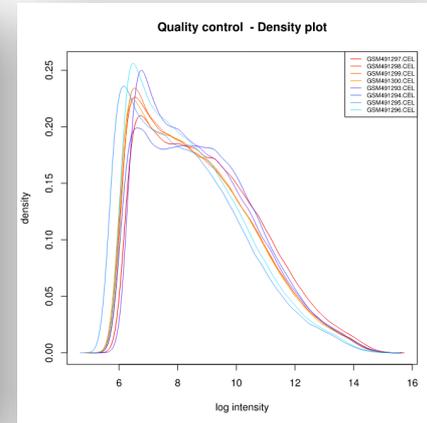
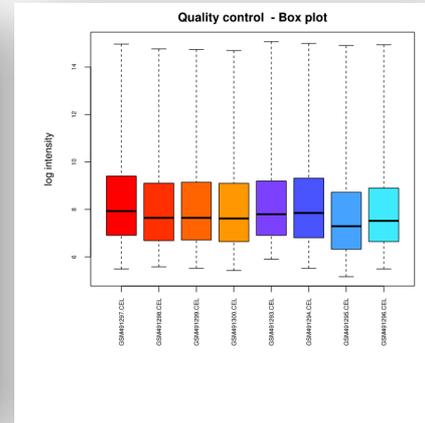
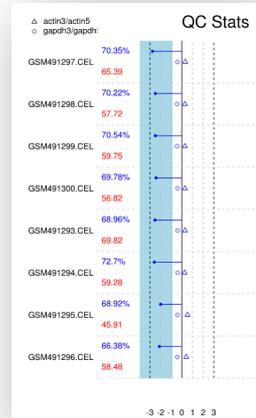
The screenshot displays the 'iPathwayGuide' Intake form. The 'Organism' dropdown is set to 'Homo sapiens' and the 'File format' dropdown is set to 'Cuffdiff'. A file upload area is present with a 'Choose' button. A red error message states '* Select file to be analyzed.' Below the form are 'back to Dashboard' and 'Upload' buttons. The page header includes the 'iPathwayGuide' logo and the user name 'Andrew Olson'. The footer contains links for 'FAQ' and 'Contact Us', and the copyright notice '© Advalita 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 Andrew Olson

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 2620 0.3 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
SNRNPB	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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Thresholds & DEGs Selection

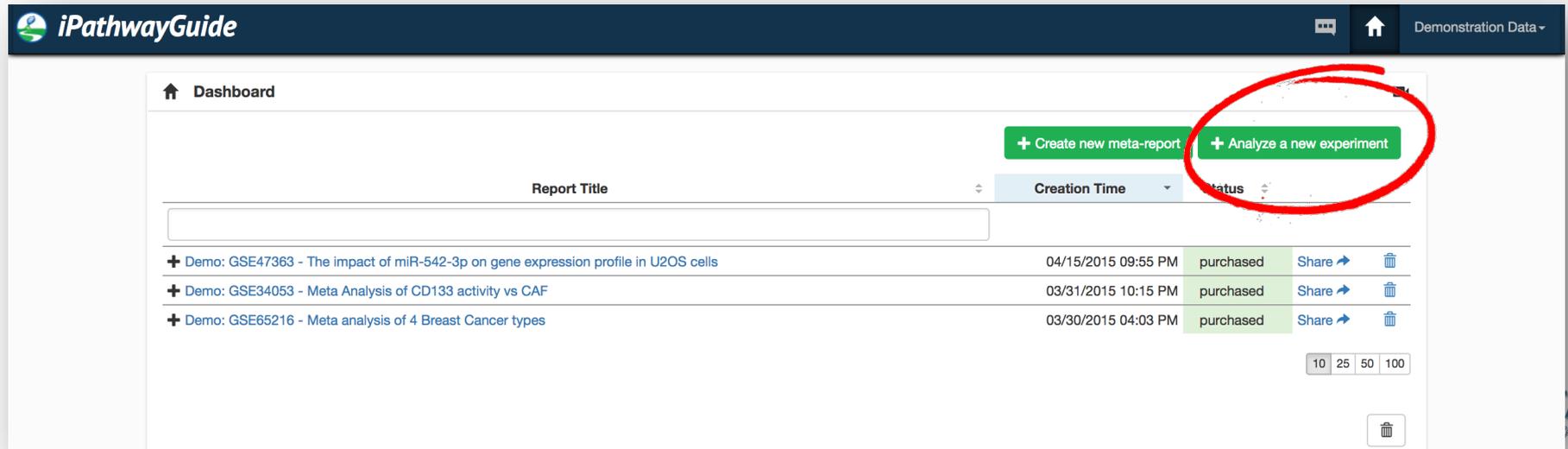
Analyze Contrast	Contrast		All genes	DE threshold		Selected DE genes	
	Condition	Control		Fold change (log)	Adjusted p-value		
<input checked="" type="checkbox"/>	Condition	Control	2620	0.6	0.05	<input type="button" value="View/Apply"/>	23

- 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 left is the iPathwayGuide logo. At the top right, there are icons for a home page and a user profile, with the text 'Demonstration Data' next to the user profile icon. Below the navigation bar, there is a 'Dashboard' section with a home icon. In the main content area, 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 buttons is a table with columns for 'Report Title', 'Creation Time', and 'Status'. The table contains three rows of demo data. At the bottom right of the table, there are pagination controls showing '10', '25', '50', and '100' items per page.

Report Title	Creation Time	Status	Share	Trash
+ 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 →	🗑️

NAVIGATION: DASHBOARD -> INTAKE -> UL

- Choose: HUMAN
- Select: Custom Tab delimited *.txt
- Choose file: Blastomere_ProteinData_Sample.txt

The screenshot shows the iPathwayGuide web application interface. At the top left is the logo and text "iPathwayGuide". At the top right are icons for chat, home, and a dropdown menu labeled "Demonstration Data". The main content area is titled "Intake form" and contains a form with the following elements:

- Organism:** A dropdown menu currently showing "Homo sapiens".
- File Type Selection:** A list of file formats with checkboxes. The "Custom tab-delimited *.txt" option is selected and highlighted in blue. The list includes:
 - Affymetrix CEL files
 - ✓ Cuffdiff
 - DESeq(2)
 - EdgeR
 - GEO2R / Limma (R/Biconductor)
 - JMP Genomics
 - nSolver (NanoString Technologies)
 - Sciex SWATH Proteomics data
 - Custom tab-delimited *.txt
- Navigation:** At the bottom right 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
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
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"/>	Condition	Control	2620	0.6	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**

Report intake form (file: Blastomere_ProteinData_Sample.txt)

Report title
Test Protein Data

Report description
Test

Analyze Contrast

Condition	Contrast	All genes	DE threshold	Selected DE genes			
<input checked="" type="checkbox"/>	Condition	Control	2620	0.3	0.05	View/Apply	153

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
SNRNPB	6628	-0.414	1.000e-6
UGP2	7360	-0.512	1.000e-6
FASN	2194	-0.539	1.000e-6
PAICS	10606	-0.617	1.000e-6
EEF2	1938	-0.687	1.620e-6
IDH1	3417	-0.536	2.370e-5
FLNA	2316	-0.368	2.950e-5
ENO1	2023	-1.000	3.180e-5

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FAQ Contact Us

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DASHBOARD UPDATE

- ETA
- Email when ready

The screenshot shows the iPathwayGuide dashboard interface. At the top, there is a dark blue header with the iPathwayGuide logo on the left and a user profile for Andrew Olson on the right. Below the header, the main content area is titled "Dashboard". A light blue notification bar at the top of the dashboard states: "The estimated completion time for your report is 12/17/2016 12:59 PM." Below this notification, there are two green buttons: "+ Create new meta-report" and "+ Analyze a new experiment". Underneath these buttons is a table with columns for "Report Title", "Creation Time", and "Status". The first row of the table contains the text "Blastomere Protein Data", "12/17/2016 12:44 PM", and "pending". To the right of the "pending" status, there are "Share" and "trash" icons.

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 shows the iPathwayGuide dashboard interface. At the top, there is a navigation bar with the iPathwayGuide logo, a user profile for Andrew Olson, and two main action buttons: '+ Create new meta-report' and '+ Analyze a new experiment'. Below this is a 'Dashboard' section with a search box and a table of experiments. The table has columns for 'Project Description', 'Creation Time', and 'Status'. The first experiment is 'Demo: GSE47363 - The impact of miR-542-3p on gene expression profile in U2OS cells', which is 'purchased' and has a 'Share' link. Below it is a detailed description: '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.' Other experiments listed include 'Meta Analysis of CD133 activity vs CAF', 'Meta analysis of 4 Breast Cancer types', and 'NanoString PanCancer Panel sample data report'. A pagination control shows 7 items per page. Below the first table is a 'Trash / Restore' section with another table listing various experiments like 'TNBC vs Healthy default cutoff', 'meta test', 'Rat test', and 'CD133 neg vs CAF'. This table also has 'Creation Time' and 'Status' columns, with 'Restore' links. A second pagination control shows 5 items per page. At the bottom, there are links for 'FAQ' and 'Contact Us', and a copyright notice for Advaita Corporation 2015.

Multi-Contrast File

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

Report intake form

Report title
Sample Report

Report description
This is a demonstration of a multi-contrast report

Analyze Contrast	Condition	Contrast	All genes	DE threshold	Selected DE genes		
<input checked="" type="checkbox"/>	A	Normal	770	0.6	0.05	View/Apply	247
<input checked="" type="checkbox"/>	C	Normal	770	0.6	0.05	View/Apply	311
<input checked="" type="checkbox"/>	B	Normal	770	0.6	0.05	View/Apply	313
<input checked="" type="checkbox"/>	D	Normal	770	0.6	0.05	View/Apply	306

show DE genes only

Gene Symbol	Entrez gene ID	Fold change (log)	Adjusted p-value
CDMP	1311	-5.320	1.000e-6
COL11A1	1501	-4.330	1.000e-6
BMPRI1B	658	-3.620	1.000e-6
INHBA	3624	-3.300	1.000e-6
MMP9	4318	-3.200	1.000e-6
SPP1	6696	-3.030	1.000e-6
FN1	2335	-2.690	1.000e-6
PPP2R2C	5522	-2.410	1.000e-6
BMP8A	253500	-2.280	1.000e-6
LEF1	51176	-2.150	1.000e-6

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.

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 checked="" 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 checked="" type="checkbox"/>	LuminalA	normal	770	0.6	0.05	View/Apply	315
<input checked="" type="checkbox"/>	LuminalB	normal	770	0.6	0.05	View/Apply	297

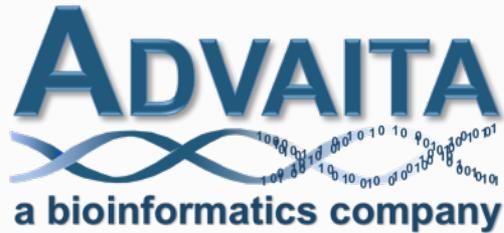
- Report title is missing or has invalid characters.
- Report description is missing.

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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