

iPathwayGuide

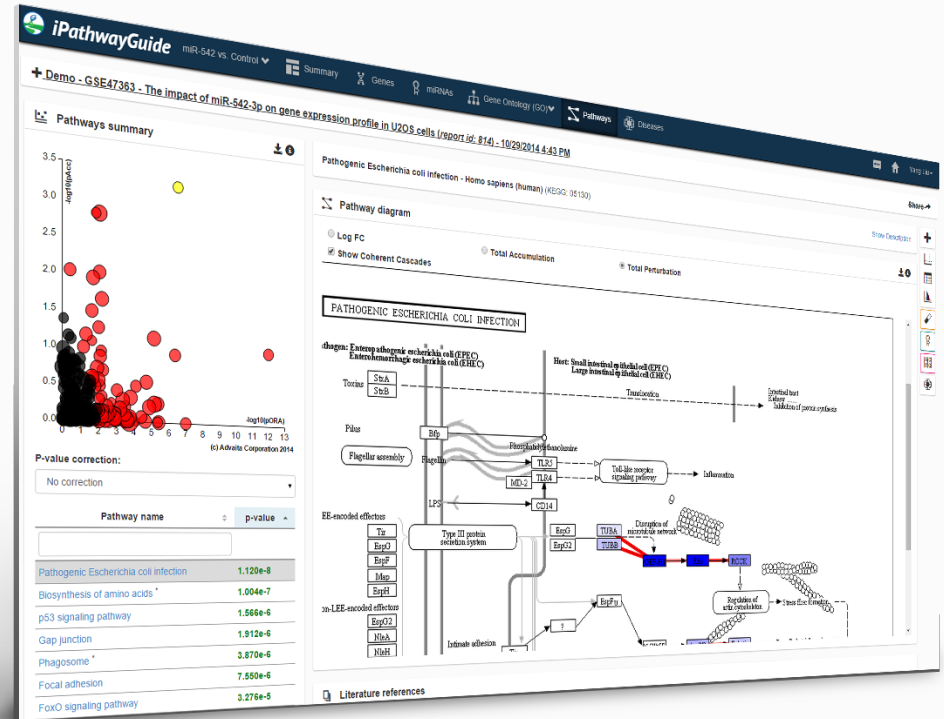
Expression Analysis with
iPathwayGuide

Agenda: 03. Pathway Analysis of NanoString Data

- What is expression analysis
 - Insights from DE genes
 - Insights from pathways – how IA is superior
- Components of Pathway Analysis in iPG
 - Pathway plot
 - Reading the diagram
 - Additional details
 - Meta-Analysis
- TRY IT! Demonstrate how IA gives better pathway results
 - Dataset bkg
 - Step-by-step navigation: Share In, Summary, Genes, Pathways, Share Out
 - Meta-Analysis: identify biomarkers (select regions, apply corr fac, view table & rank, explore genes, export)
- Q & A

iPathwayGuide Core Functions

- DE Genes
- Predicted miRNAs
- GO Analysis
 - Biological processes
 - Molecular functions
 - Cellular components
- **Pathway Analysis**
(Drugs, miRNAs, SNPs)
- Diseases
- Meta analysis



Research challenges

- Identify significantly impacted pathways
- Prioritize a list of given genes of interest
 - Unbiased
 - Based on observed data
- Offer alternative genes of interest

PATHWAY ANALYSIS IN IPATHWAYGUIDE

DIFFERENTIAL EXPRESSION

- What proportion of measured genes are DE?
- What is already known about the DE genes?
- What do the DE genes have in common?

IMPACT ANALYSIS

- What pathways are truly impacted between contrasts?
- What mechanisms are activated/ deactivated?
- What drugs, SNPs, miRNAs, etc. could affect the observed changes?

META ANALYSIS

- How do results differ across disease subtypes? Treatment modalities? Doses? Time points? Omics measurements? Animal models?
- Which DE genes (biomarkers) can differentiate between experiments?

iPG: Differentially Expressed Genes

- See relationships for genes that are being considered
- Non-significant genes also viewable by clicking “Show all genes”
- Annotations for each gene to GO terms, pathways, miRNAs, SNPs, Drugs, References

iPathwayGuide miR-542 vs. Control Summary Genes miRNAs Gene Ontology (GO) Pathways Diseases Andrew Olson

+ Demo - GSE47363 - The impact of miR-542-3p on gene expression profile in U2OS cells (report id: 2803) - 03/28/2015 03:34 PM Share

Genes summary

(c) Advalita Corporation 2015

Symbol	ID	LogFC	p-value
ITGB1	3688	-1.801	1.000e-6
PSTPIP2	9050	-1.586	1.000e-6
CTSB	1508	-1.161	1.000e-6
CTTN	2017	-1.198	1.000e-6
STAT3	6774	-1.090	1.000e-6
RAB21	23011	-1.007	1.000e-6
CSRFP1	1465	-0.841	1.000e-6
DHCR7	1717	-1.333	1.000e-6
S100A11	6282	-1.109	1.000e-6
GLA	2717	-0.929	1.000e-6

10 25 50 100

« 1 2 3 4 5 6 7 ... 117 »

Gene details

Title: Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)

Identifier: 3688

Symbol: ITGB1

Aliases: CD29, FNBR, MDF2, VLAB, GPIIA, MSK12, VLA-BETA

Summary: Integrins are heterodimeric proteins made up of alpha and beta subunits. At least 18 alpha and 8 beta subunits have been described in mammals. Integrin family members are membrane receptors involved in cell adhesion and recognition in a variety of processes including embryogenesis, hemostasis, tissue repair, immune response and metastatic diffusion of tumor cells. This gene encodes a beta subunit. Multiple alternatively spliced transcript variants which encode different protein isoforms have been found for this gene. [provided by RefSeq, Jul 2008]

Literature references

Okada H, et al. The Journal of clinical investigation, 2013 Oct 1. PMID 24091324. [Integrins protect cardiomyocytes from ischemia/reperfusion injury.](#)

Lee S, et al. Surgery, 2013 Aug. PMID 23889963. [Integrin β1 is critical for gastrin-releasing peptide receptor-mediated neuroblastoma cell migration and invasion.](#)

Shen Z, et al. Journal of surgical oncology, 2013 Aug. PMID 23857544. [Novel focal adhesion protein kindlin-2 promotes the invasion of gastric cancer cells through phosphorylation of integrin β1 and β3.](#)

Larusch GA, et al. American journal of physiology. Heart and circulatory physiology, 2013 Aug 1. PMID 23709605. [Domain 2 of uPAR regulates single-chain urokinase-mediated angiogenesis through β1-integrin and VEGFR2.](#)

Spring FA, et al. PloS one, 2013. PMID 23704882. [Tetraspanins CD81 and CD82 facilitate αβ1-mediated adhesion of human erythroblasts to vascular cell adhesion molecule-1.](#)

Wood E, et al. Journal of bacteriology, 2013 Aug. PMID 23687274. [BB0172, a Borrelia burgdorferi outer membrane](#)

Biological Processes

Mf Molecular Functions

Cc Cellular Components

- acrosomal vesicle
- basement membrane
- cell surface
- cleavage furrow
- cytoplasm
- external side of plasma membrane
- extracellular vesicular exosome
- filopodium
- focal adhesion
- hemidesmosome
- integrin alpha1-beta1 complex
- integrin alpha10-beta1 complex
- integrin alpha11-beta1 complex

☒ Open only one at a time

Pathways

- Arrhythmic right ventricular cardiomyopathy (ARVC)
- Axon guidance
- Bacterial invasion of epithelial cells
- Cell adhesion molecules (CAMs)
- Dilated cardiomyopathy
- ECM-receptor interaction
- Focal adhesion
- Hypertrophic cardiomyopathy (HCM)
- Leishmaniasis
- Leukocyte transendothelial migration
- Pathogenic Escherichia coli infection
- Pathogenesis of HIV

☒ Open only one at a time

Drugs

miRNAs

SNPs

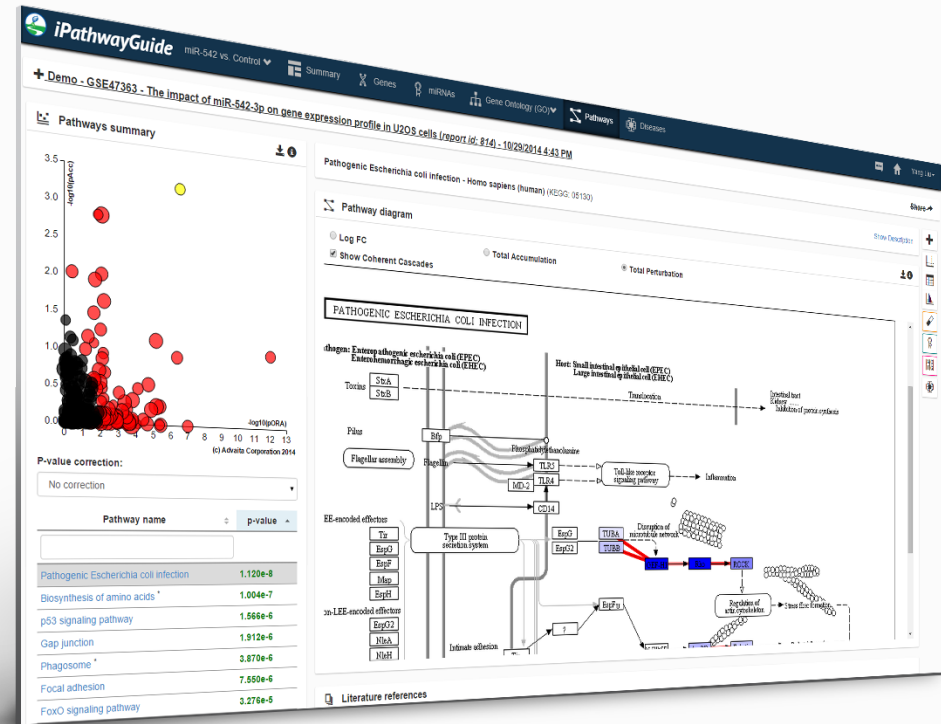
Diseases

FAQ Contact Us

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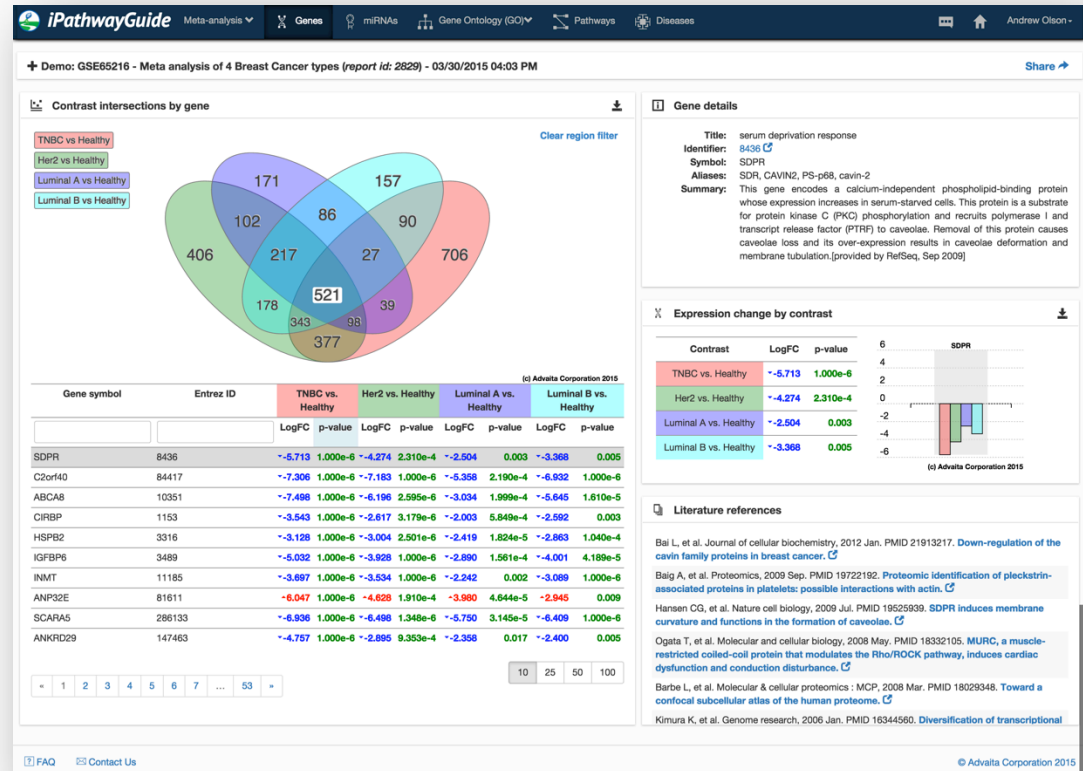
Pathway Analysis

- Uses Impact Analysis to score pathways using two evidences
 - Enrichment (x-axis)
 - Accumulated perturbation (y-axis)
- Leverages pathway topology
- Minimizes false positive results
- Patented methodology



iPG: Meta Analysis – Premium Feature

- Build and compare up to 5 datasets
- Combine proteomic and transcriptomic analyses
- Quickly identify common or unique traits
 - Venn Diagram
 - Sortable columns
 - Correction factors



DATASET BACKGROUND


- Comes from NanoString's PanCancer Panel
- Data included with nSolver 3.0
- 4 Contrasts
 - Basal like vs. Normal
 - Her2Enriched vs. Normal
 - Luminal A vs. Normal
 - Luminal B vs. Normal

LET'S TRY IT! (NANOSTRING)

- STEPS:
 - CLICK LINK IN EMAIL
 - LOG IN
 - ACCEPT SHARE
 - ENTER REPORT
 - SUMMARY
 - DE GENES
 - PATHWAYS
 - META-ANALYSIS

EMAIL SHARE NOTICE

- Share notice is only good one time
- FWD a share notice will not work.
- Once you click the link, the share notice will appear in your dashboard.

 **iPathwayGuide**

Shared analysis

The following iPathwayGuide analysis was shared with you:

Analysis title: Demo: NanoString Technologies - nSolver 3.0 demonstration data
Sharer: Andrew Olson

Accept Share

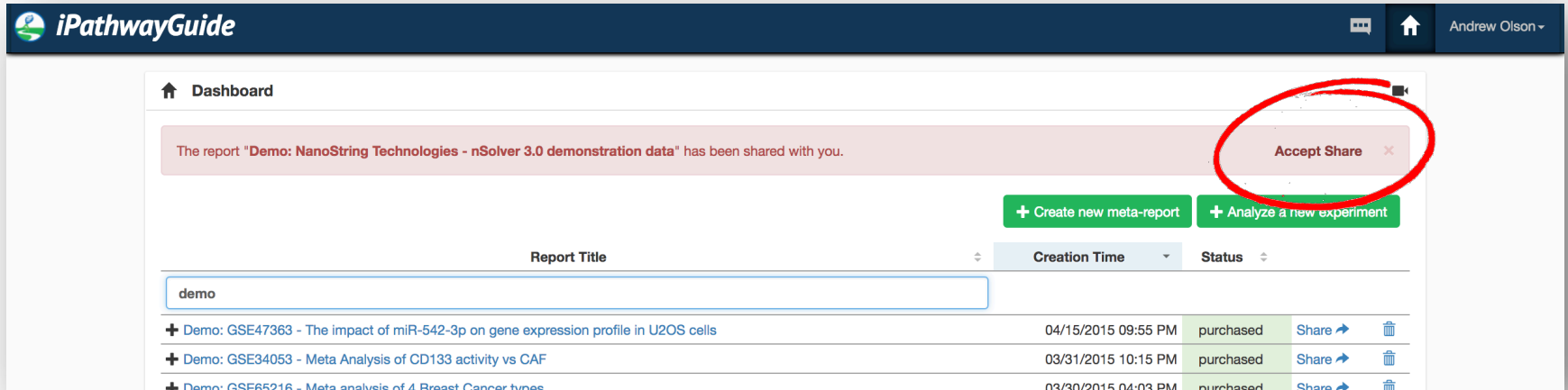
To accept the share use the button above or copy and paste the following link - <https://ipathwayguide.advaitabio.com/dashboard?acceptShare=69e93e9d8bfe1e0a2adfc43f78b42451> - in your browser.

You must login to accept this share. If you do not have an account, you can create one.

This email was sent by: **Advaita Bioinformatics**
46099 Five Mile Road, Plymouth, MI 48170 USA
+1(734)922-0110 • www.advaitabio.com

ACCEPT SHARE

- Click Accept Share



The screenshot shows the iPathwayGuide dashboard. At the top, there's a dark blue header with the iPathwayGuide logo on the left and a user profile 'Andrew Olson' on the right. Below the header, a 'Dashboard' section contains a notification bar that says 'The report "Demo: NanoString Technologies - nSolver 3.0 demonstration data" has been shared with you.' To the right of this bar is an 'Accept Share' button, which is circled in red. Below the notification bar are two green buttons: '+ Create new meta-report' and '+ Analyze a new experiment'. Underneath these is a table with columns for 'Report Title', 'Creation Time', and 'Status'. The 'Report Title' column has a search box containing the word 'demo'. The table lists three demo reports, each with a 'Share' link and a trash icon.

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
+ Demo: GSE34053 - Meta Analysis of CD133 activity vs CAF	03/31/2015 10:15 PM	purchased
+ Demo: GSE65216 - Meta analysis of 4 Breast Cancer types	03/30/2015 04:03 PM	purchased

VIEW REPORT

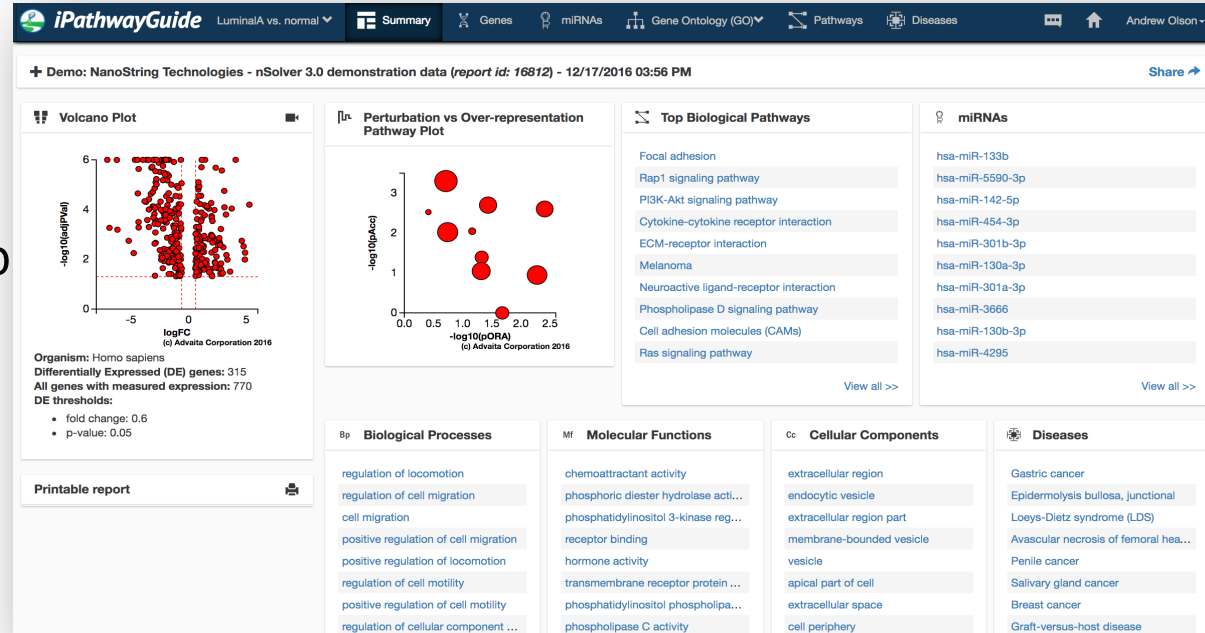
- Select a contrast to view (Luminal A)



Summary

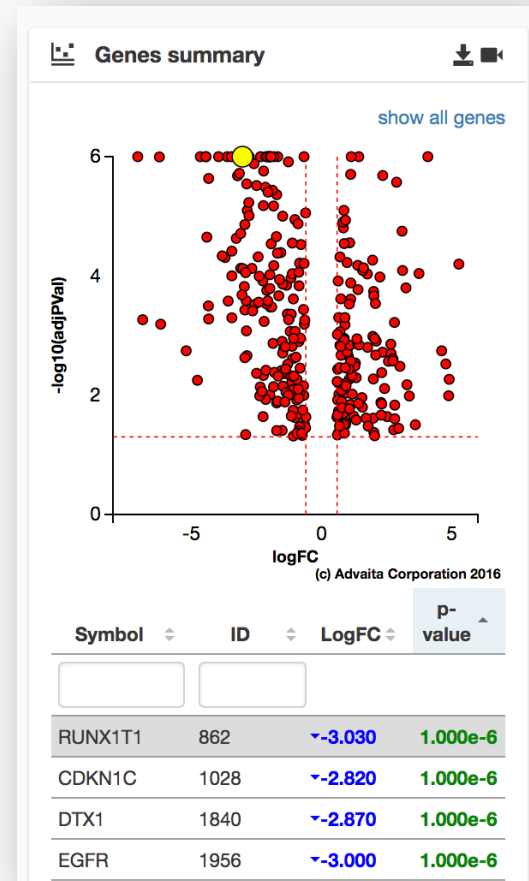
5 Key Sections

- DE Genes
- Pathways (Top 10)
- Predicted miRNAs (Top 10)
- GO Terms (Top 10)
- Diseases (Top 10)
- Printable Report Summary



DE GENES

- Search for gene
- Show all genes
- Re-order table
- Annotations for each genes to GO terms, pathways, miRNAs, SNPs, Drugs

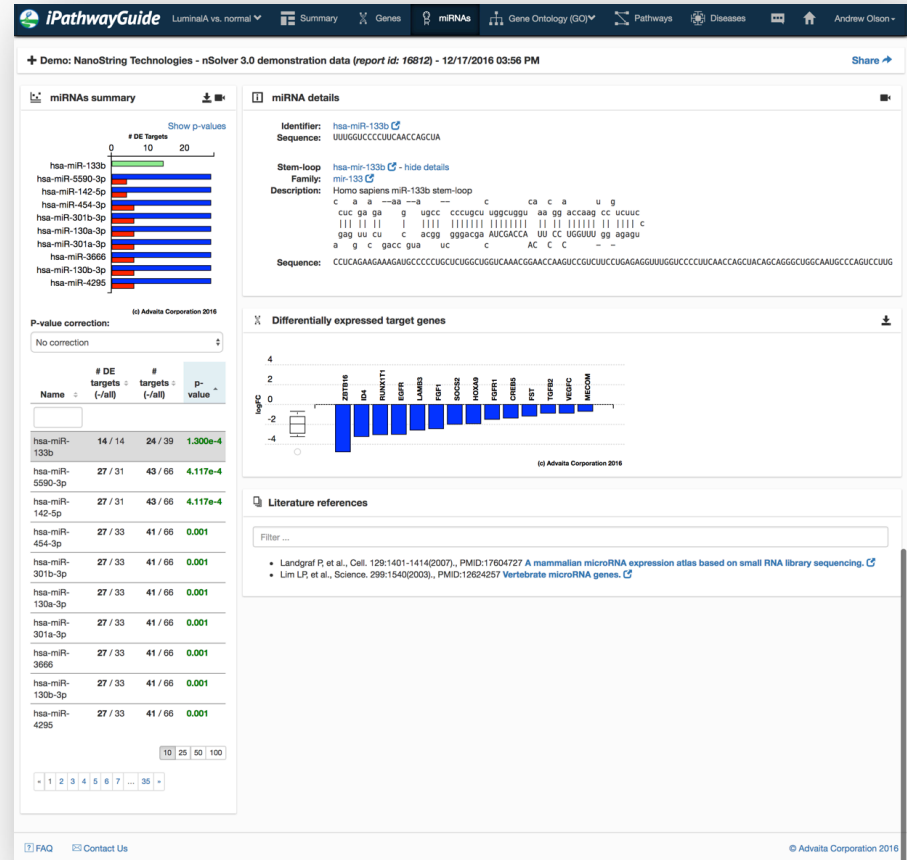


iPG: Predicted miRNAs - Method

- Prediction of active miRNAs (Friedman et al., 2009; Lewis et al., 2005) is based on enrichment of differentially downregulated target genes.
- Because miRNAs are generally considered to have an inhibitory effect, the method computes the ratio between the number of significant differentially expressed downregulated targets and all differentially expressed targets, and compares it to the ratio of all downwardly expressed targets to all targets.
- iPathwayGuide calculates the probability of observing a greater number of differentially downregulated target genes just by chance.
- This p-value is computed using the hypergeometric distribution method.

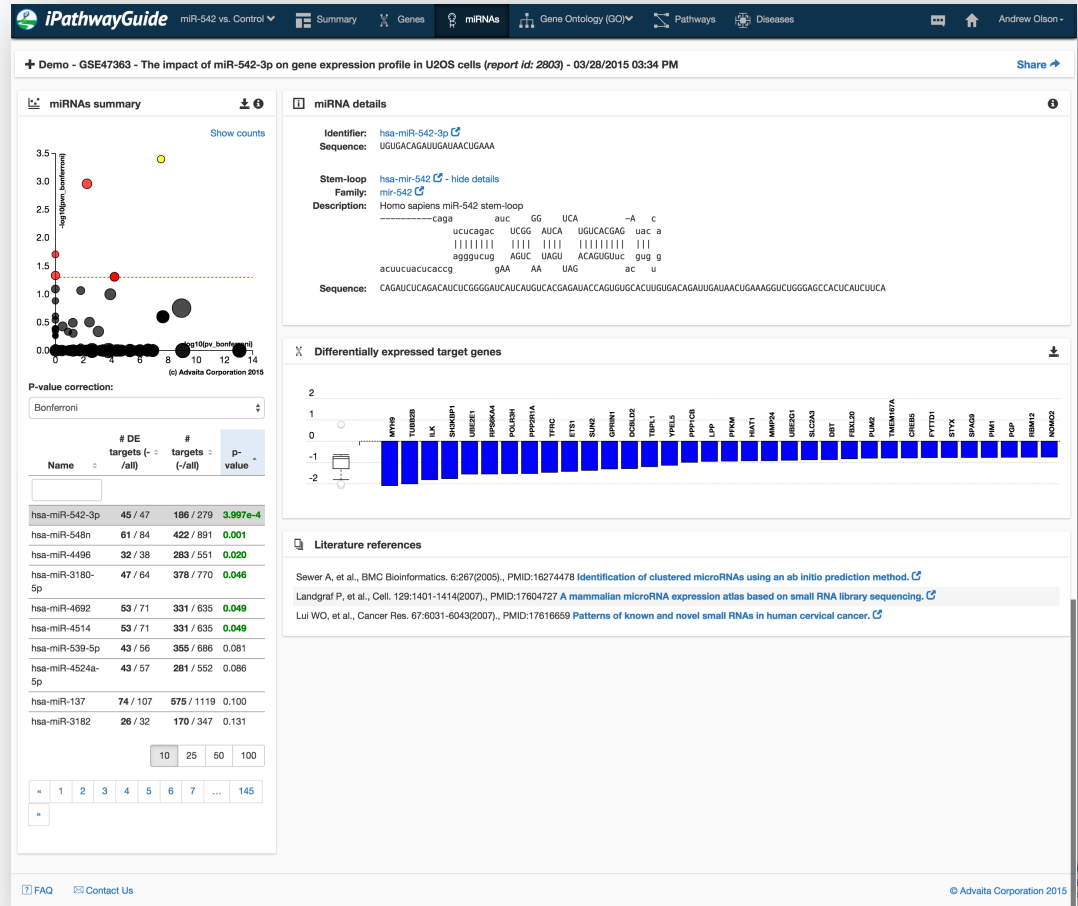
iPG: Predicted miRNAs

- Bar plot displays count of downregulated targets vs upregulated targets
- Gene's plot lists target DE genes from max -FC to max +FC
- Clicking on any gene will navigate to genes pages

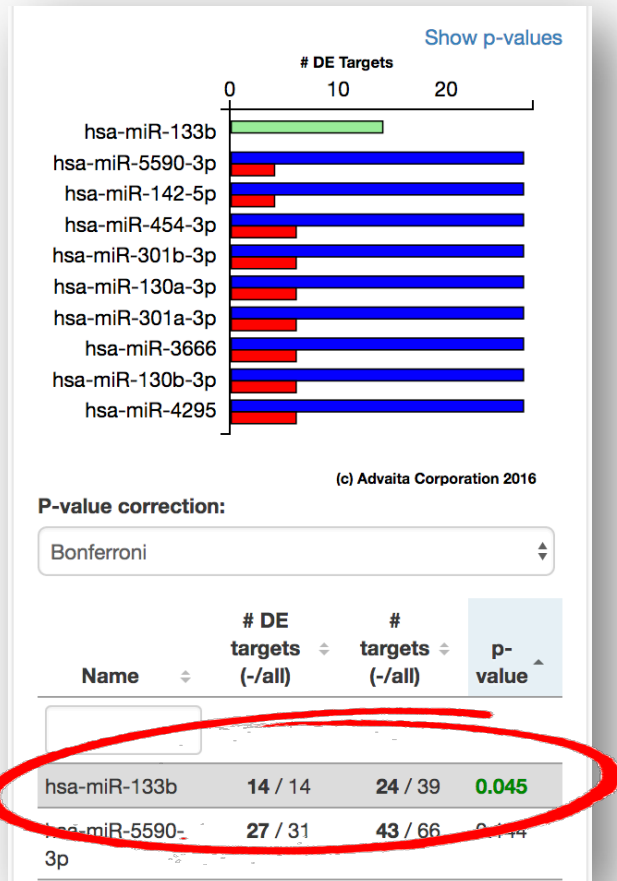


iPG: Predicted miRNA (alt view)

- Click on show p-values shows 2-way plot.
- X-axis = p-value based the total number of DE target genes versus the total number of target genes. (Not used)
- Y-axis = p-value based on the number of downwardly expressed DE targets versus the total number of DE targets.



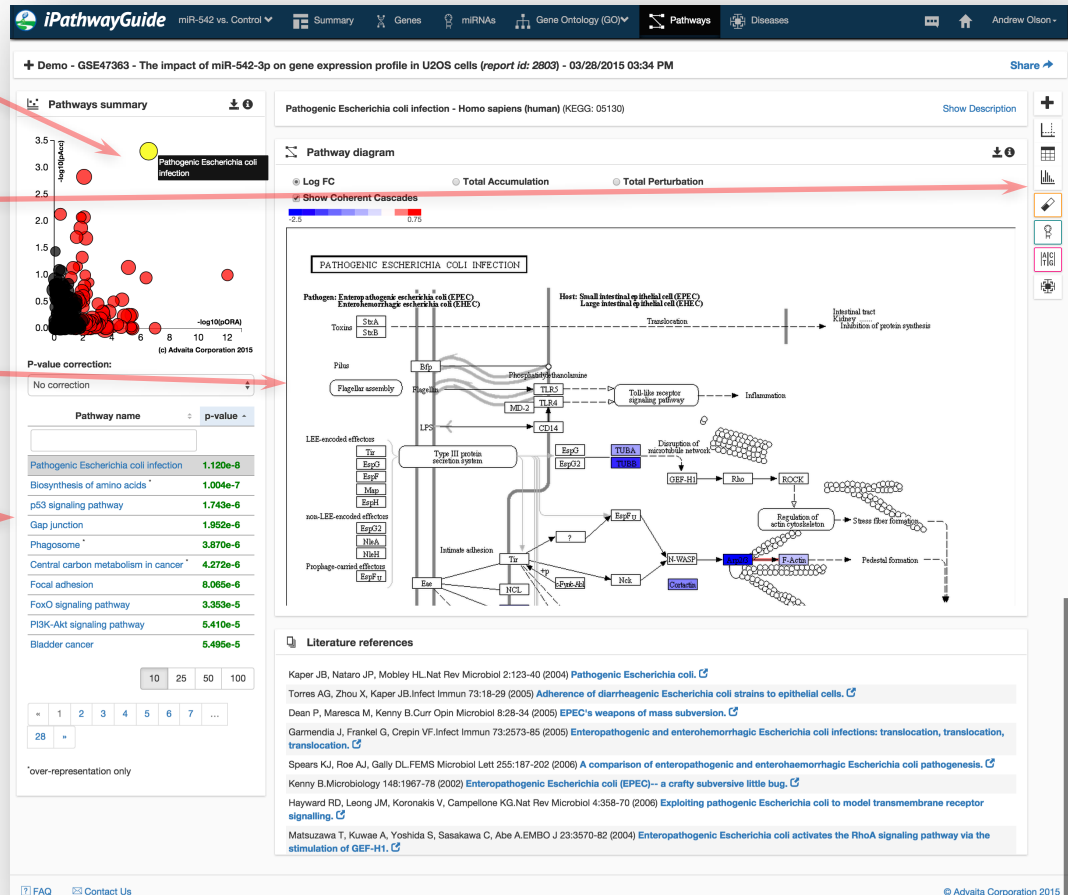
miRNA PREDICTION



- Using Bonferroni, 1 miRNA is significant
- We can hypothesize that miR - 133b may be active in Luminal A

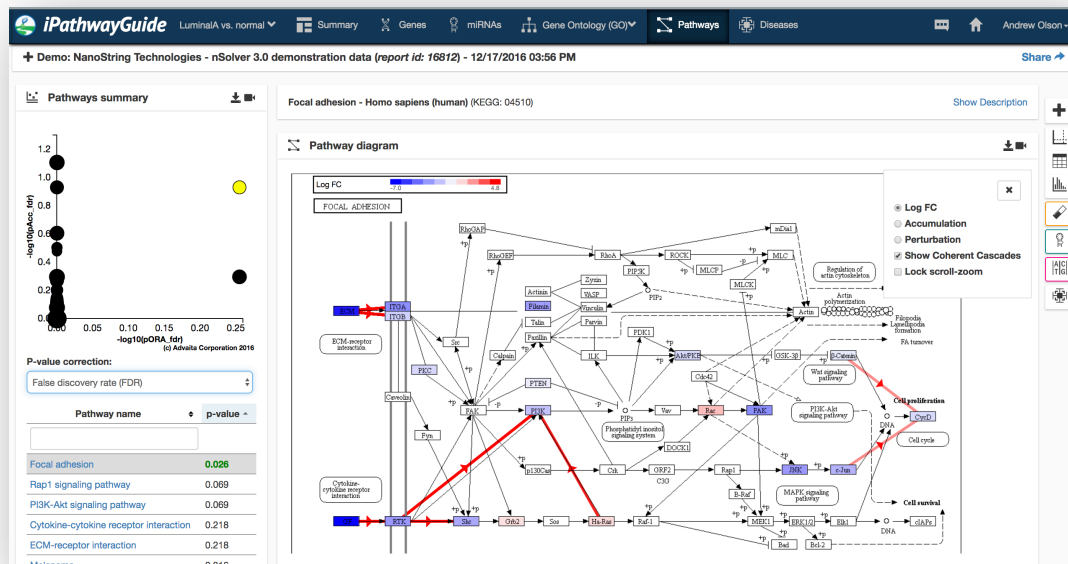
Pathways (Overview)

- Impact Analysis plot
- Pathway Details
- Pathway diagram
- Results table



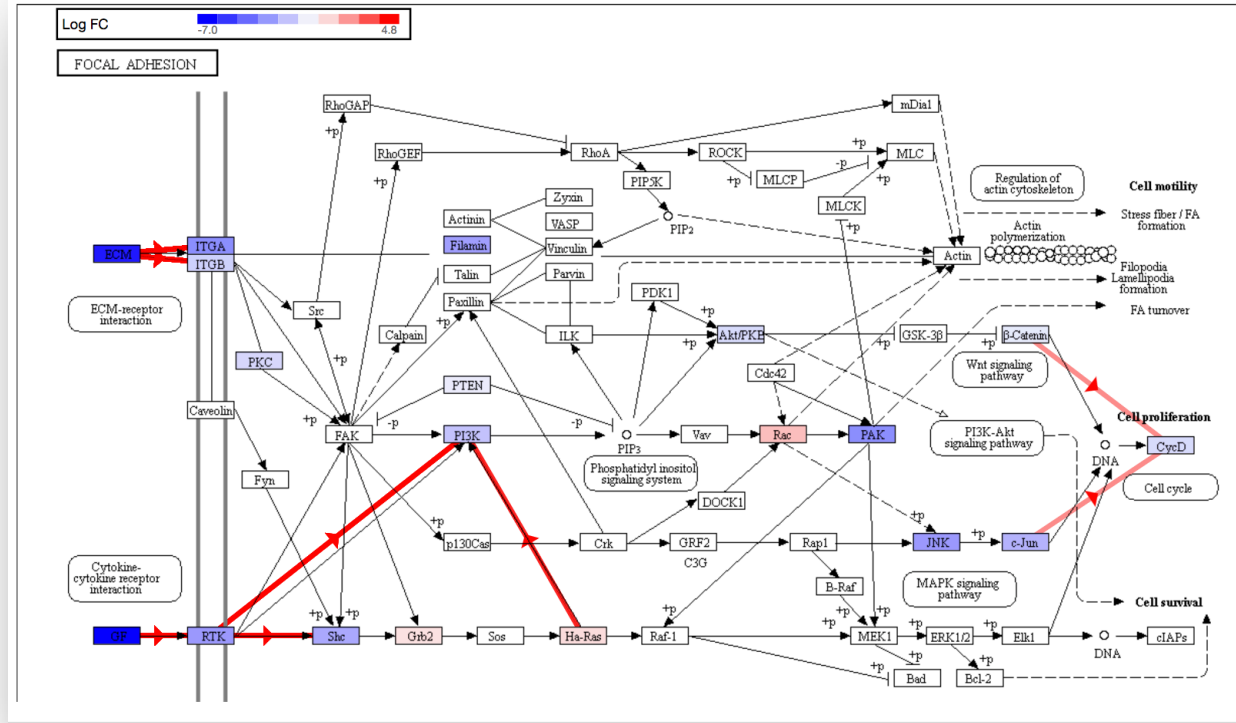
PATHWAYS

- Apply FDR Correction
- Only 1 Pathway is still significant
(Focal Adhesion)
- Combined p-Value is <0.05



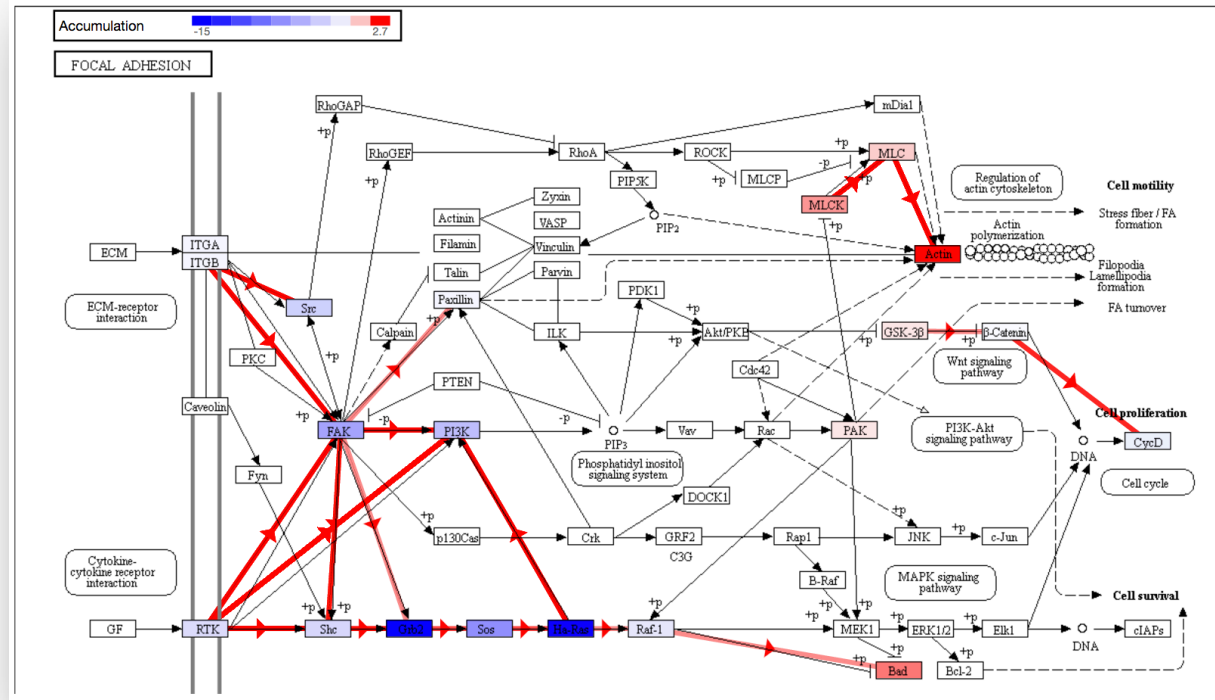
Reading Pathway Diagrams

- Start with measured Fold Change
- Identifies which genes were measured and their relative fold changes
- Look for mechanisms using “Coherent Cascades”



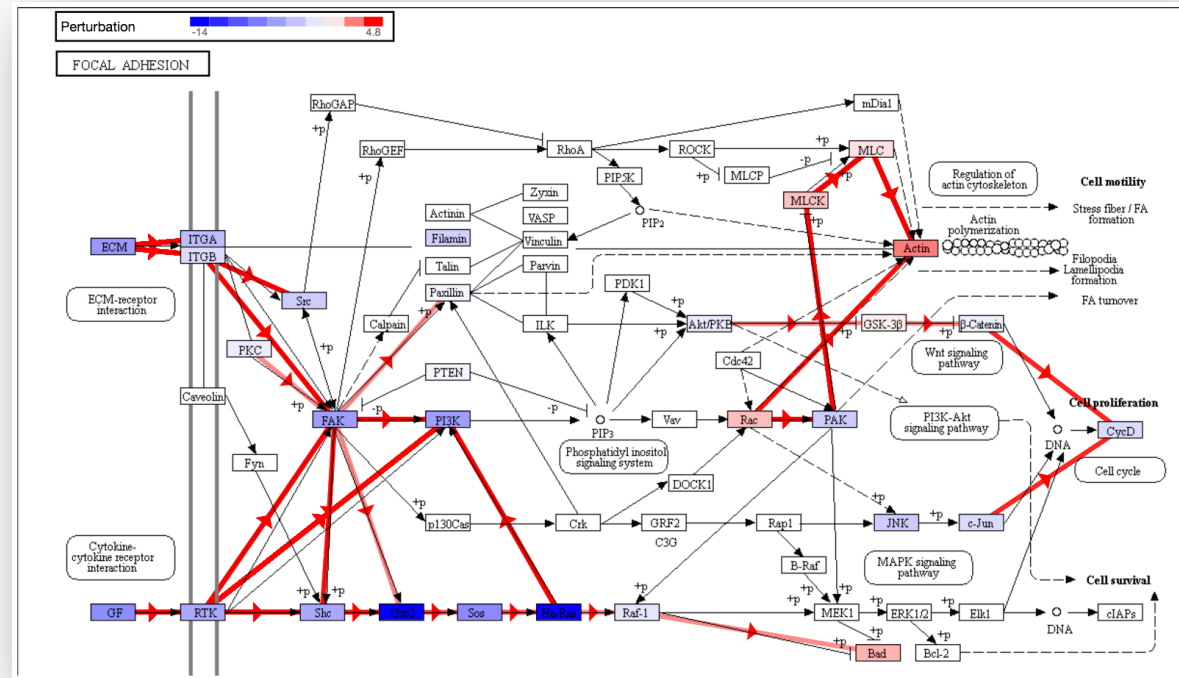
Reading Pathway Diagrams

- Next, look at Accumulation
- These are the genes that have accumulated perturbation from upstream genes
- We know this because of how the pathway describes the relationships between genes
- Can be thought of as an inferred fold change



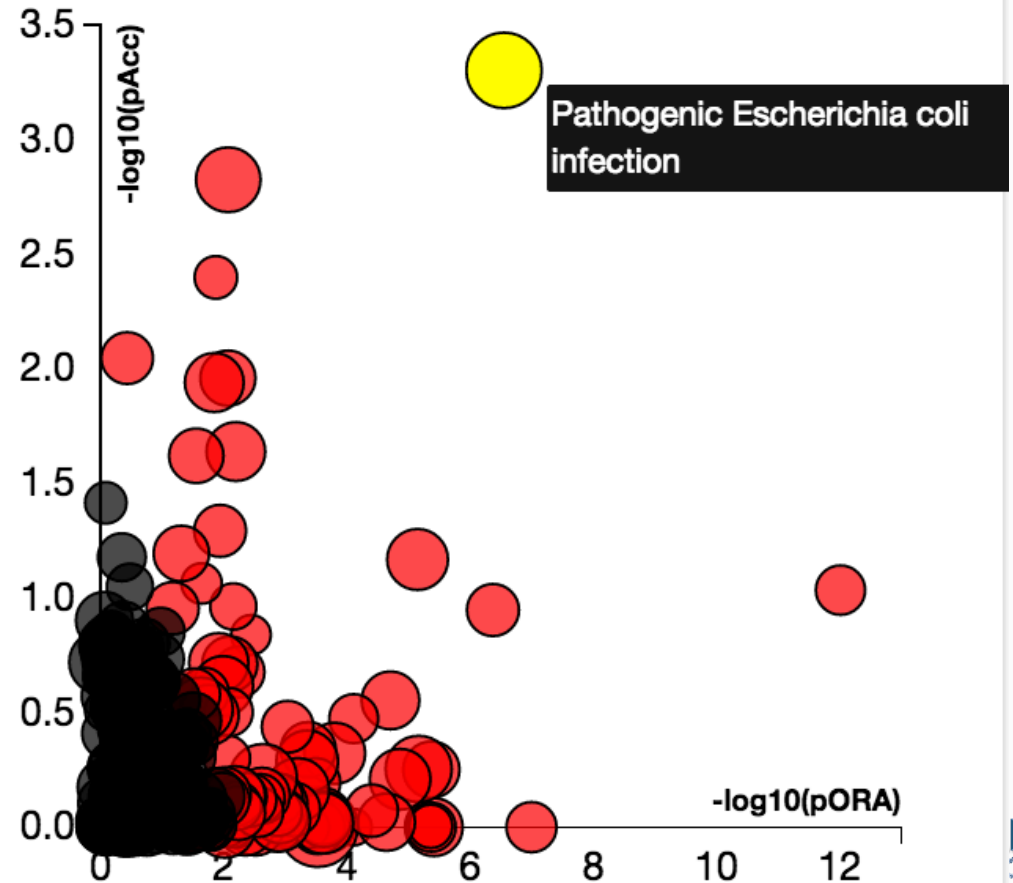
Reading Pathway Diagrams

- Finally, look at (Total) Perturbation
- This combines measured fold change plus accumulated perturbation
- Red lines identify coherence between the data and the pathway diagrams
- Serve as putative mechanisms



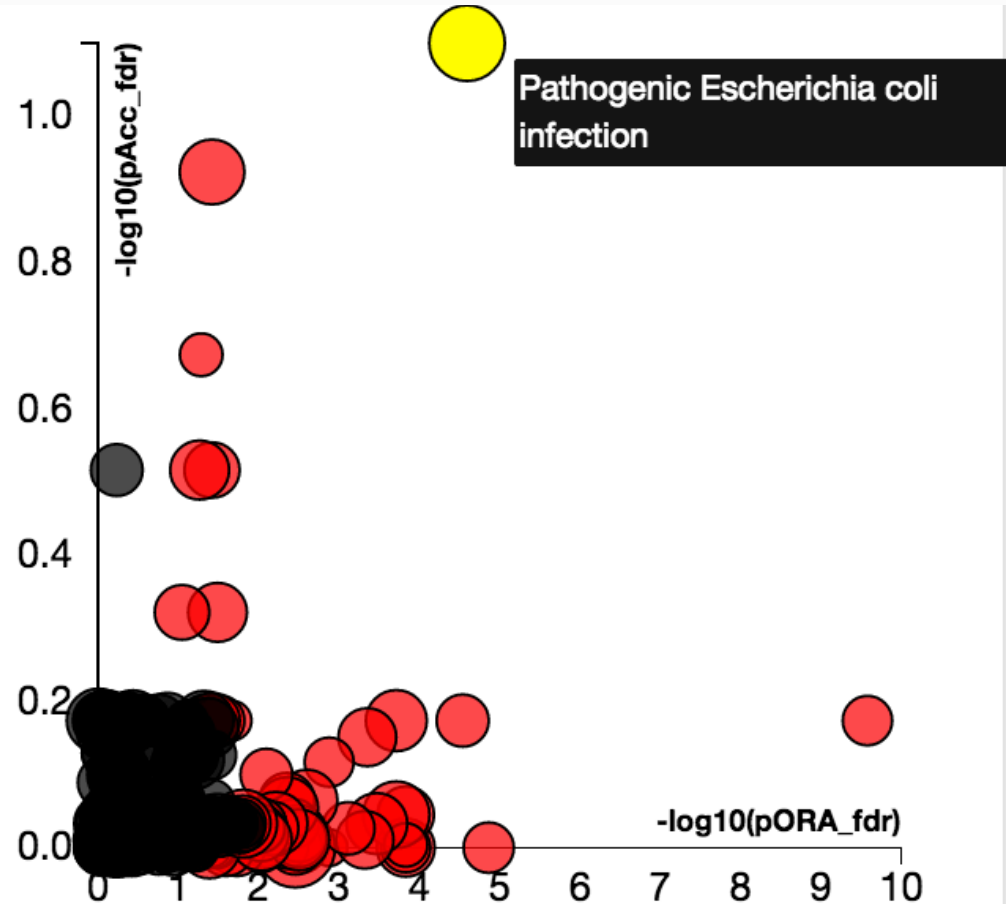
Correction factors

- Uncorrected p-values do not take into consideration that multiple comparisons are being made



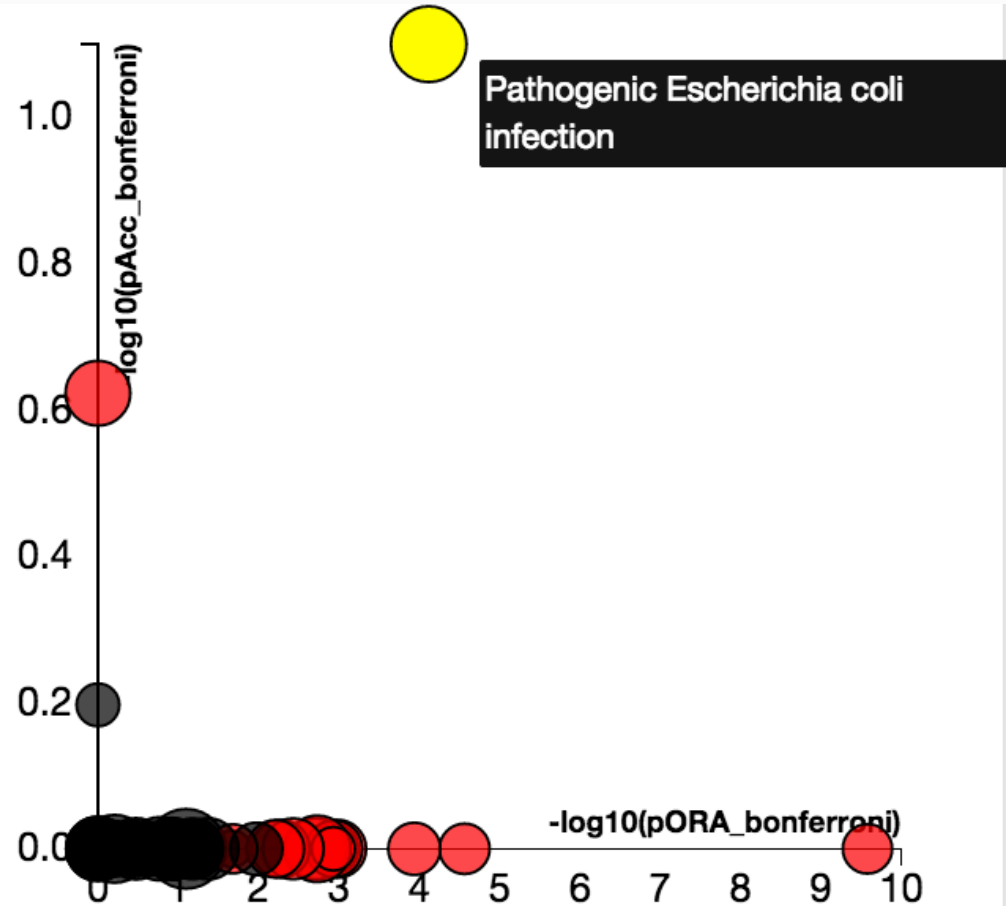
Correction factors: FDR

- FDR correction controls the proportion of false positive calls in all positive calls at the desired significance level (0.05)
- Shifts focus to predicted positives and accepts that some will be wrong. An FDR of 0.05 means out of 100 predicted positives, 5 are wrong.



Correction factors: Bonferroni

- Bonferroni (FWER) correction controls the probability of making **any** false positive call at the desired significance level (0.05)
- Bonferroni is most stringent of the correction factors and guards against any false positives
- Notice how many dots have fallen to the origin?



iPG: Pathway Details – Gene Table

- Gene table lists all genes for the selected pathway
- Can be sorted and filtered
- Clicking on a row will highlight gene in pathway

The screenshot displays the iPathwayGuide interface for the 'Focal adhesion' pathway (KEGG: 04510). The main panel shows a detailed pathway diagram with various proteins and their interactions. A color scale for Log FC is provided. To the right, the 'Pathway Details' panel is open, showing the 'Gene table' tab. The table lists genes associated with the pathway, sorted by Log FC. Below the table, there are sections for 'Differentially expressed pathway genes' (a bar chart), 'Literature references', and 'Related diseases'.

Gene	ID	LogFC	Accum.	Part.
ACTB	60	0.000	2.683	2.683
ACTG1	71	0.000	2.683	2.683
ACTN1	87	0.000	0.000	0.000
ACTN2	88	0.000	0.000	0.000
ACTN3	89	0.000	0.000	0.000
ACTN4	81	0.000	0.000	0.000
AKT1	207	0.840	0.000	0.840
AKT2	208	0.000	0.000	0.000
AKT3	10000	-1.120	0.000	-1.120
ARHGAP35	2909	0.000	0.000	0.000

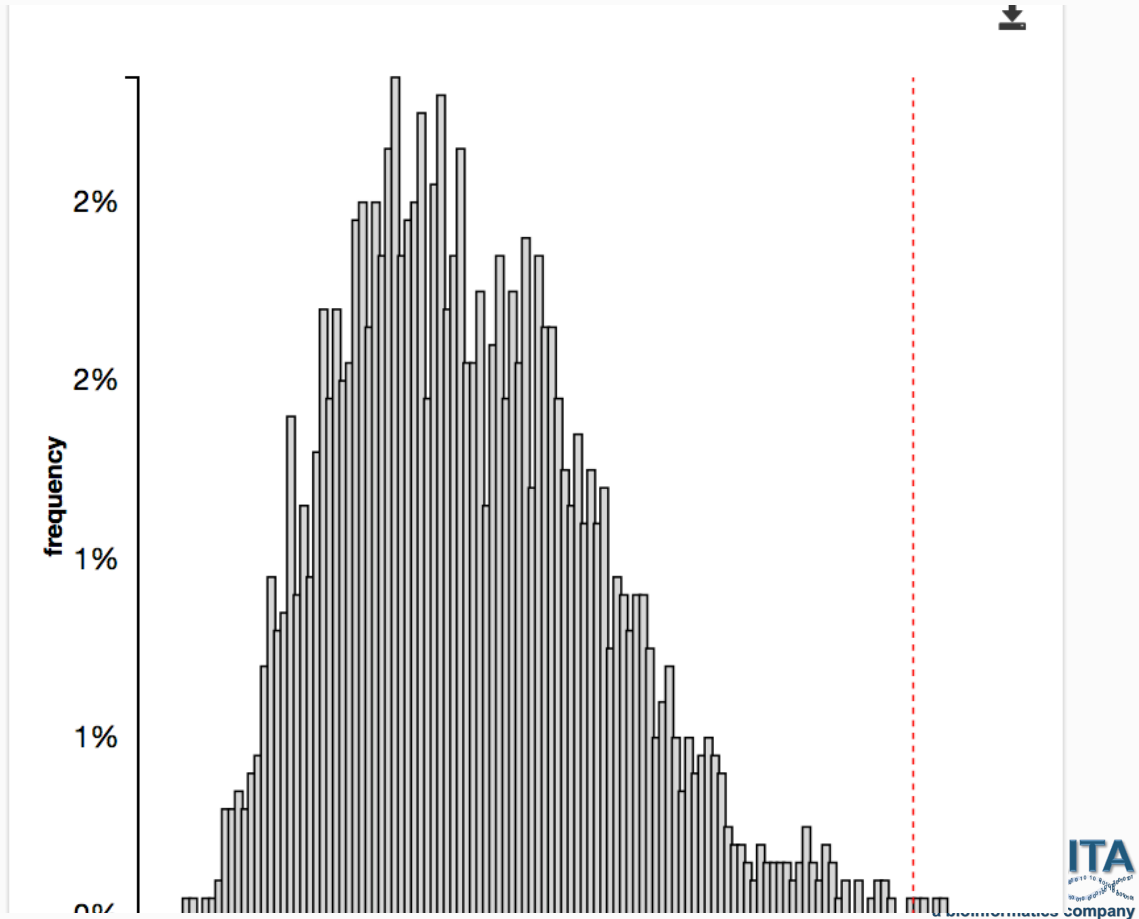
Differentially expressed pathway genes

Literature references

- Berman AE, Kozlov NI, Morozovitch GE. Biochemistry (Moscow) 68:1284-99 (2003) Integrins: structure and signaling.
- Burdge K, Wennerberg K. Cell 116:167-79 (2004) Rho and Rac take center stage.
- Corrogiu PM, Boccassini C, Traupe L. Curr Opin Cell Biol 15:565-71 (2003) Interactions between growth factor receptors and adhesion molecules: breaking the rules.
- Danon SH, Yamaoka KM, J. Cell Physiol 188:1-13 (2001) Fibronectin, integrins, and growth control.
- Guo W, Giampini F. Nat Rev Mol Cell Biol 5:516-26 (2004) Integrin signaling during tumour progression.
- Hobbs A, Krollova J, Bartek J, Lukas J, Kolar Z. Blommed Pap Med Fac Univ Palacky Olomouc Czech Repub 147:19-25 (2003) The mechanism of action of the tumour suppressor gene PTEN.
- Lee JW, Juliano R. Mol Cells 17:188-202 (2004) Mitogenic signal transduction by integrin- and growth factor receptor-mediated pathways.
- Mitra SK, Hanson DA, Schaefer DD. Nat Rev Mol Cell Biol 6:56-68 (2005) Focal adhesion kinase: in command and control of cell motility.
- Nicholson KM, Anderson NG. Cell Signal 14:381-96 (2002) The protein kinase B/Akt signaling pathway in human malignancy.
- Oloini A, McPhee T, Dedhar S. Biochim Biophys Acta 1691:1-15 (2004) Regulation of E-cadherin expression and beta-catenin/Tcf transcriptional activity by the integrin-linked kinase.

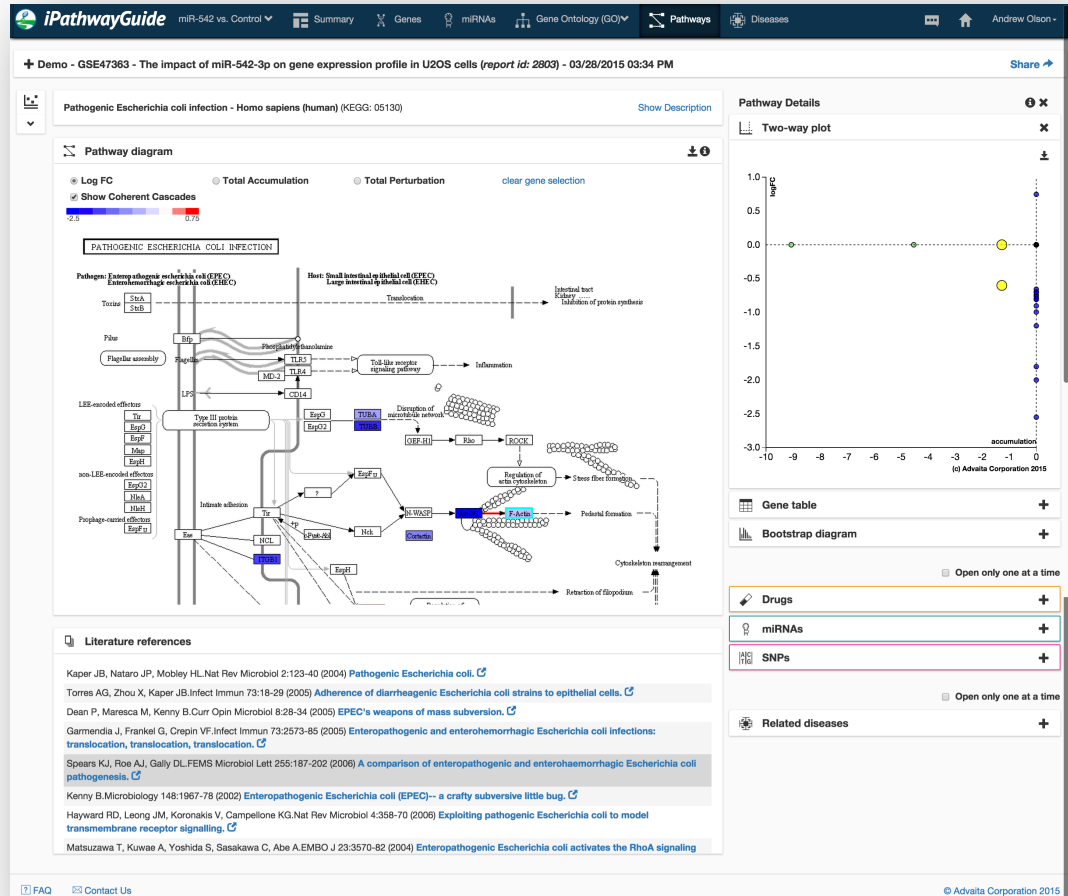
Pathway Details - Perturbation

- Perturbation calculated for each pathway based on DE genes
- System of equations bootstrapped and solved
- Visualize expected downstream perturbation distribution for each pathway
- Entry points to pathway have greater effect than downstream genes



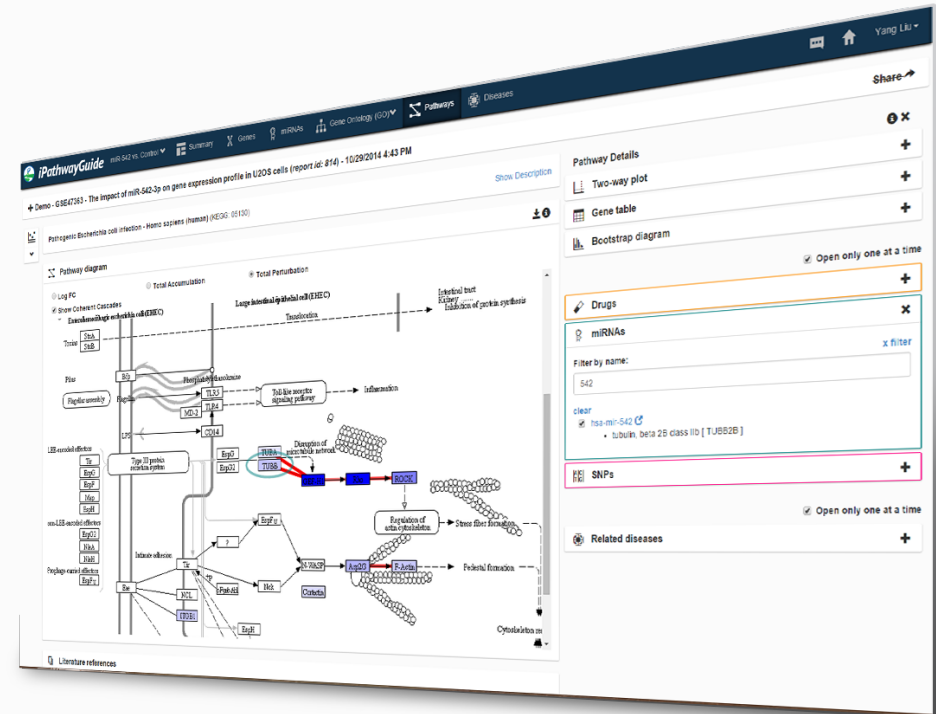
iPG: Pathway Details – Two-way Plot

- The Two-way plot allows users to see logFC vs Accumulation
- = only logFC
- = only Accumulation
- = both logFC & Accum
- = not perturbed
- = current selection
- If you highlight a genes on the pathway diagram, it will light up on two-way plot



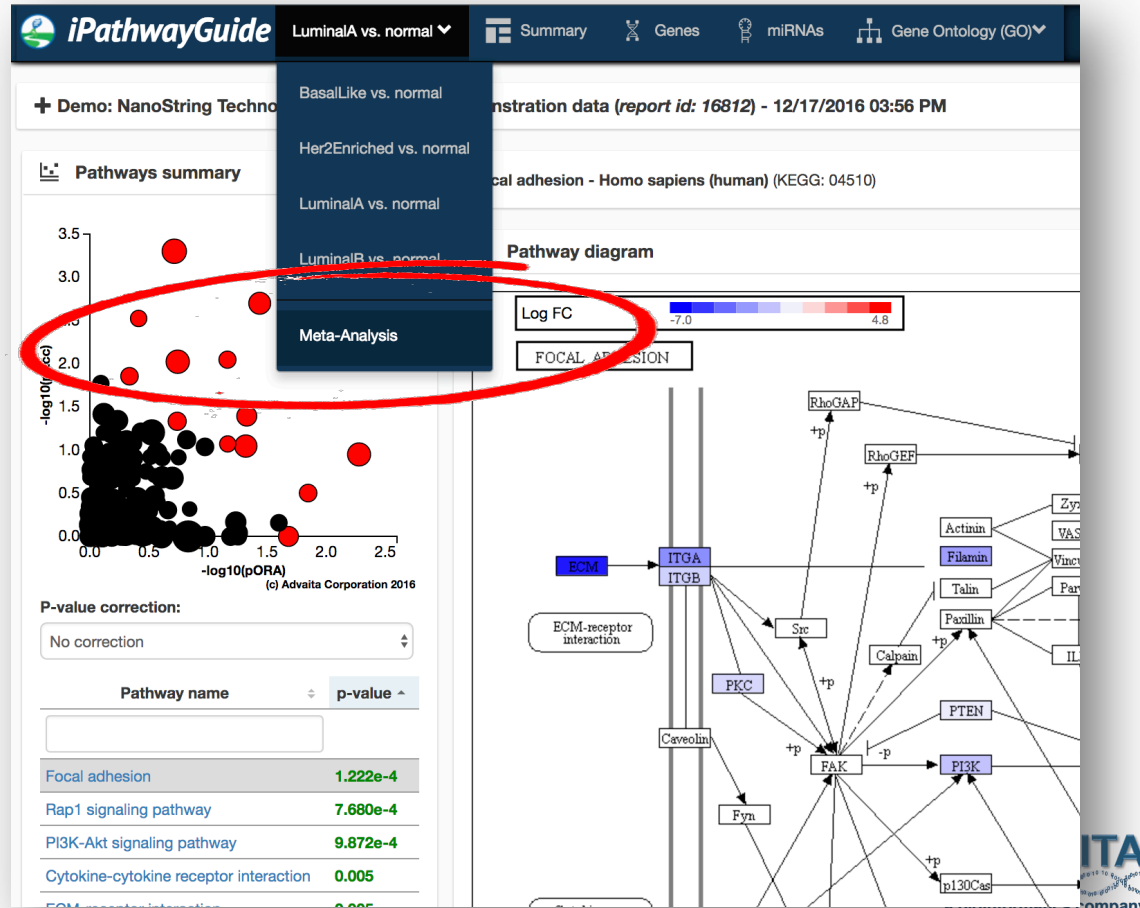
Pathway Details – Model miRNAs, SNPs, Drugs

- Model gene influencers directly on pathway
 - Drugs
 - miRNAs
 - SNPs
- Identify drug targets and see their interactions or potential side-effects
- Generate additional hypotheses for influencers on each system



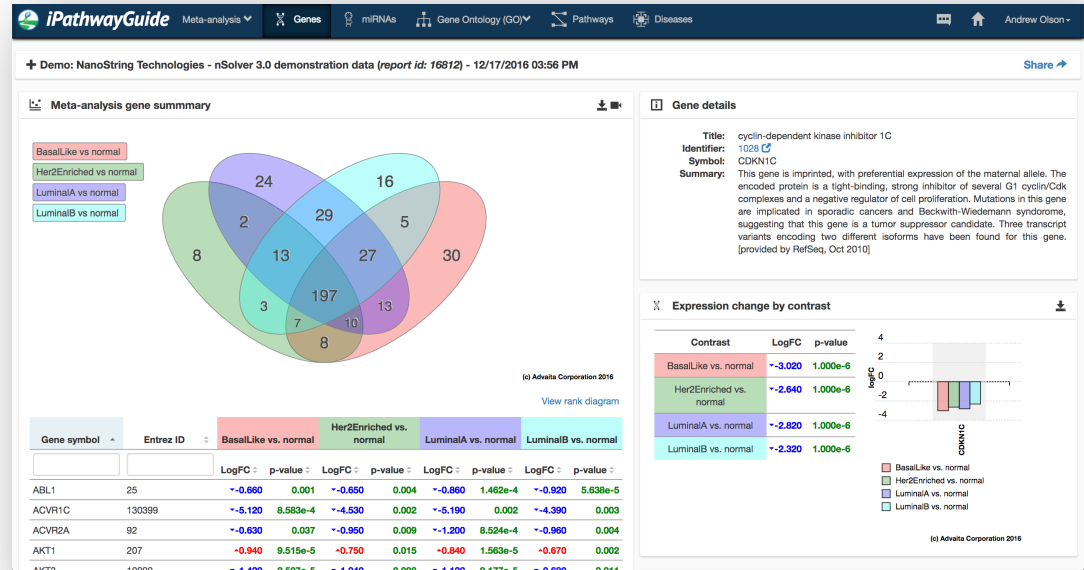
META-ANALYSIS OVERVIEW

- Choose “Meta-Analysis” from dropdown list of available contrasts
- Same analyses, but cuts across each contrast
- Identify unique or common traits and features
- Identify progression



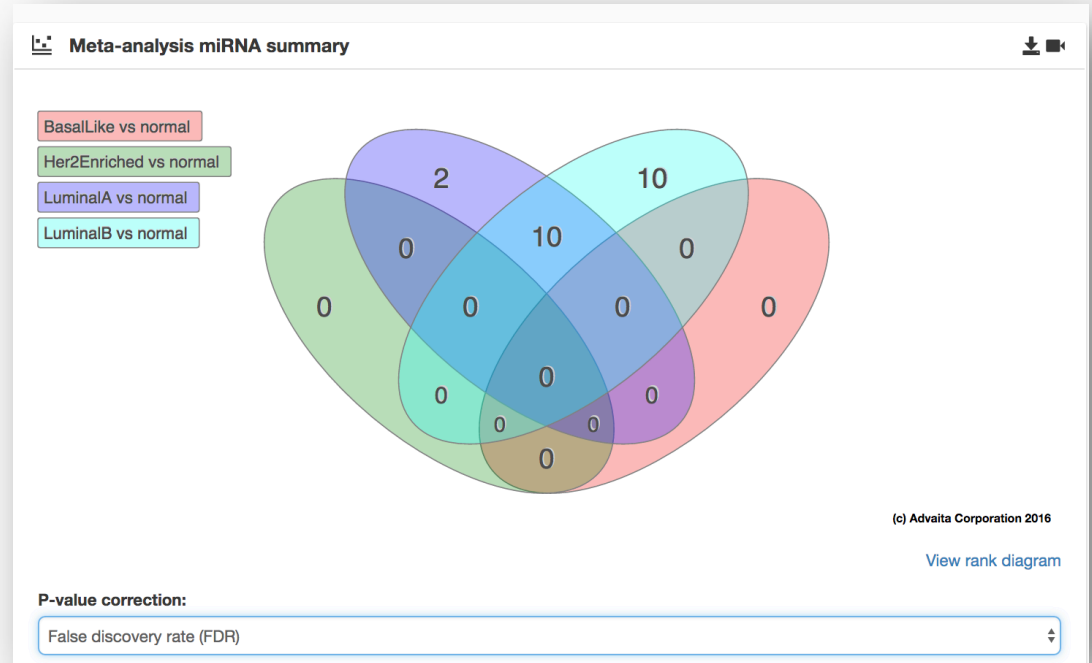
META-ANALYSIS OVERVIEW

- Quickly identify regions that interest you
- Build up a custom table
- See changes in expression by sorting



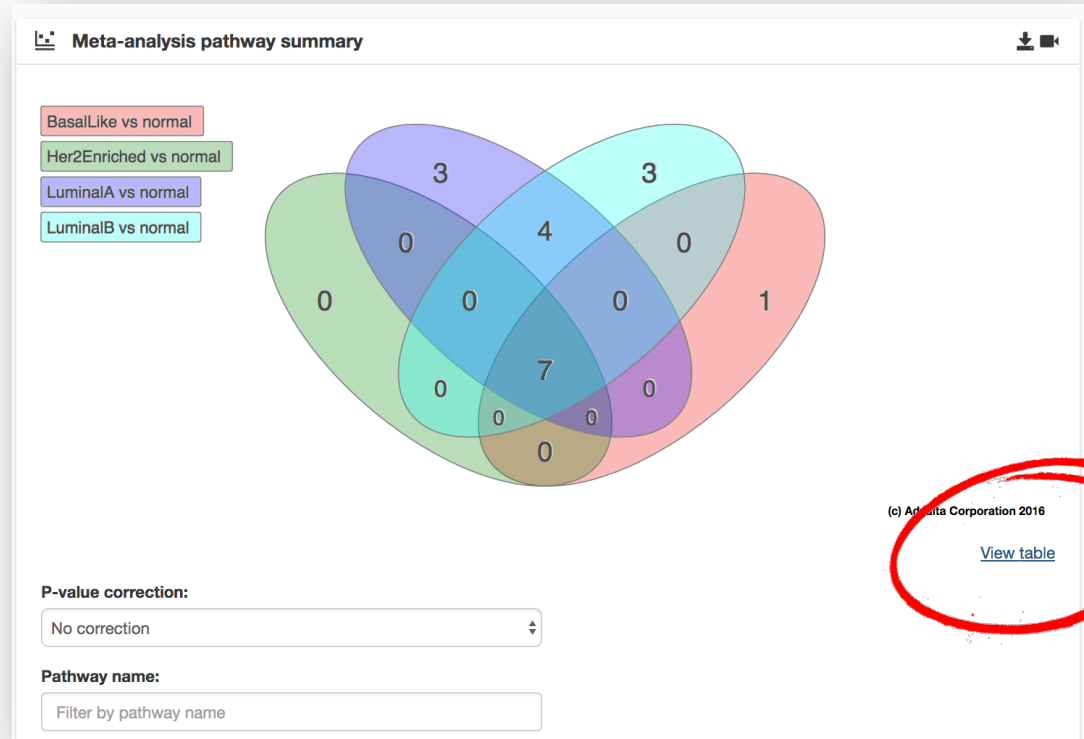
MIRNA INFERENCE (VIA META ANALYSIS)

- 22 miRNAs identified after FDR
- 1 after Bonferroni
- Only in Luminal A & B



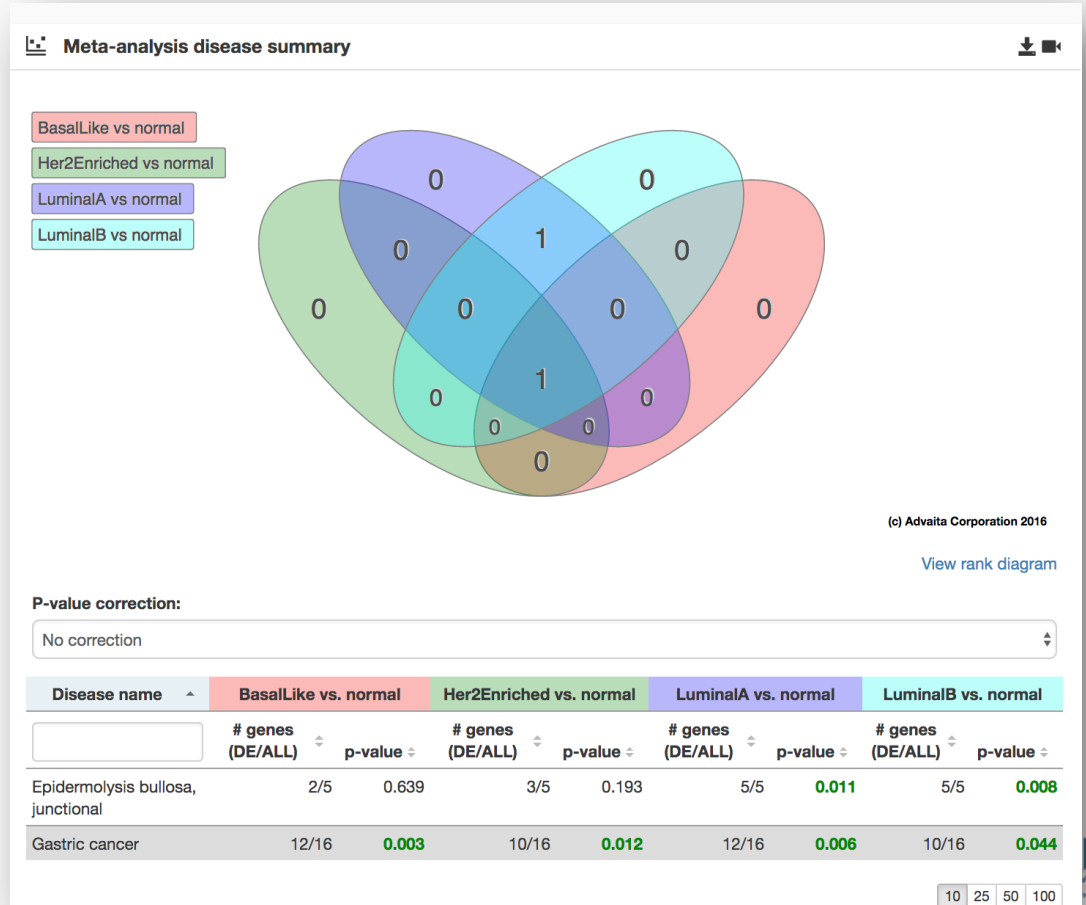
PATHWAYS (VIA META ANALYSIS)

- Only 1 significant pathway using FDR
- Can be an issue with smaller datasets like this
- Nominal p-value may still provide clues



DISEASES (VIA META ANALYSIS)

- Only two significant Diseases
- Likely due to small dataset



SHARING REPORTS IN IPG

- Click “SHARE”
- Type in email (doesn't matter which email)
- If no account, they will be prompted to sign up

Stop and Questions

Be back at 1:00 PM



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