

iPathwayGuide

Expression Analysis with
iPathwayGuide

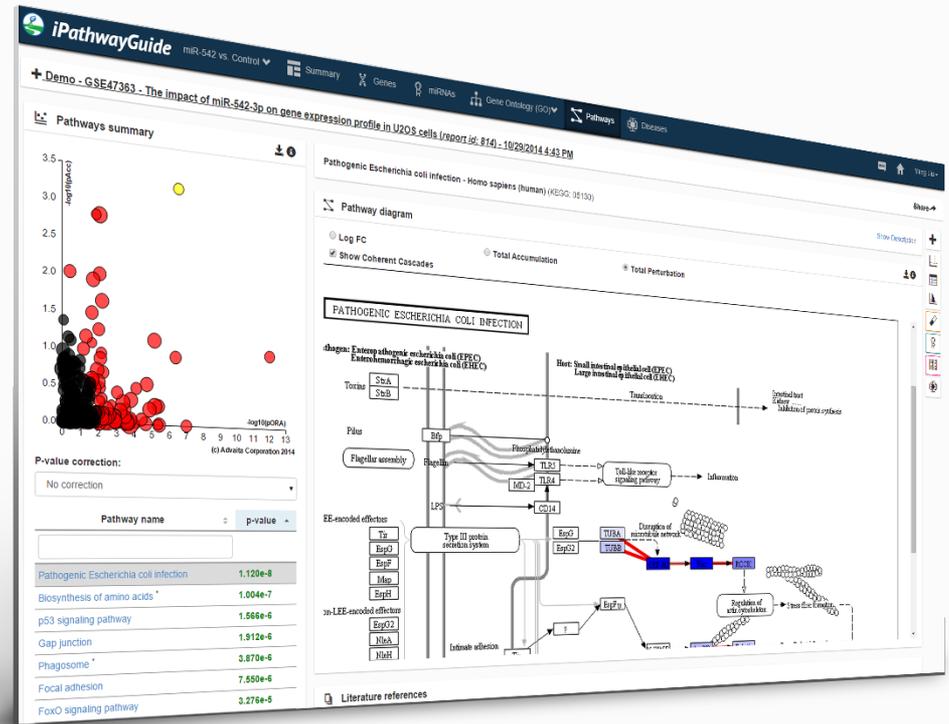


Agenda: 03. Pathway Analysis of NanoString Data

- 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

show all genes

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| 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 23899963. [Integrin \$\beta 1\$ 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 \$\beta 1\$ and \$\beta 3\$.](#)

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 \$\beta 1\$ -integrin and VEGFR2.](#)

Spring FA, et al. PloS one, 2013. PMID 23704882. [Tetraspanins CD81 and CD82 facilitate \$\alpha \beta 1\$ -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

- 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 alpha1-beta1 complex
- integrin alpha1-beta1 complex

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 ...

Drugs

miRNAs

SNPs

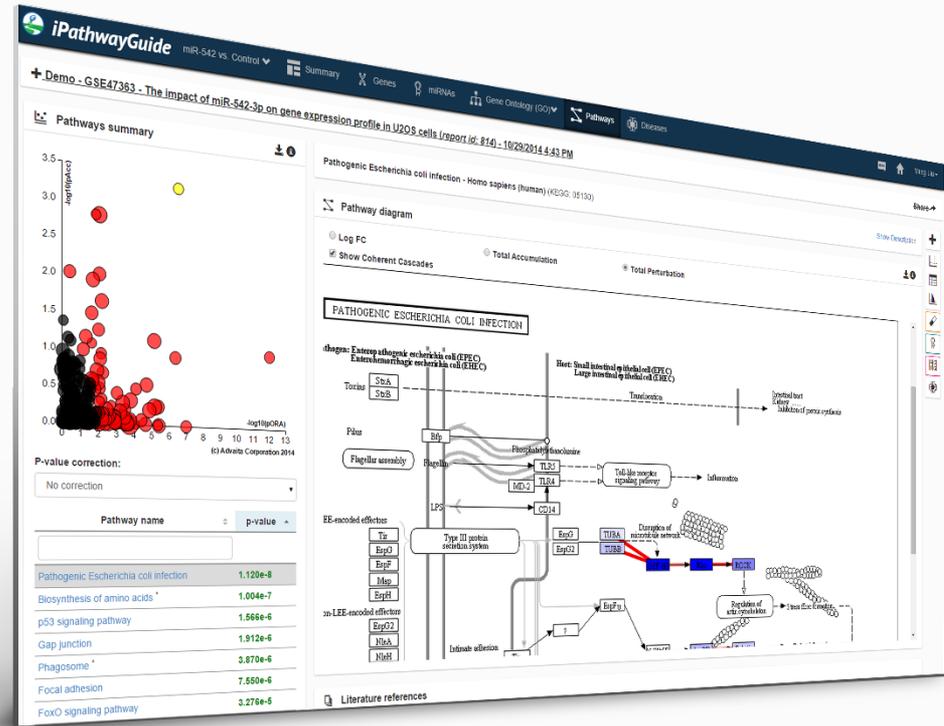
Diseases

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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

iPathwayGuide Meta-analysis ▾ Genes miRNAs Gene Ontology (GO) Pathways Diseases Andrew Olson

➤ Demo: GSE65216 - Meta analysis of 4 Breast Cancer types (report id: 2829) - 03/30/2015 04:03 PM [Share](#)

Contrast intersections by gene [Clear region filter](#)

Gene details

Title: serum deprivation response
 Identifier: [8436](#)
 Symbol: [SDPR](#)
 Aliases: [SDR](#), [CAVIN2](#), [PS-p68](#), [cavin-2](#)
 Summary: This gene encodes a calcium-independent phospholipid-binding protein whose expression increases in serum-starved cells. This protein is a substrate for protein kinase C (PKC) phosphorylation and recruits polymerase I and transcript release factor (PTRF) to caveolae. Removal of this protein causes caveolae loss and its over-expression results in caveolae deformation and membrane tubulation.[provided by RefSeq, Sep 2009]

Expression change by contrast

| Contrast | LogFC | p-value |
|-----------------------|--------|----------|
| TNBC vs. Healthy | -5.713 | 1.000e-6 |
| Her2 vs. Healthy | -4.274 | 2.310e-4 |
| Luminal A vs. Healthy | -2.504 | 0.003 |
| Luminal B vs. Healthy | -3.368 | 0.005 |

Gene symbol Entrez ID TNBC vs. Healthy LogFC p-value Her2 vs. Healthy LogFC p-value Luminal A vs. Healthy LogFC p-value Luminal B vs. Healthy LogFC p-value

| | | | | | | | | | |
|---------|--------|--------|----------|--------|----------|--------|----------|--------|----------|
| SDPR | 8436 | -5.713 | 1.000e-6 | -4.274 | 2.310e-4 | -2.504 | 0.003 | -3.368 | 0.005 |
| C2orf40 | 84417 | -7.306 | 1.000e-6 | -7.183 | 1.000e-6 | -5.358 | 2.190e-4 | -6.932 | 1.000e-6 |
| ABCA8 | 10351 | -7.498 | 1.000e-6 | -6.198 | 2.595e-6 | -3.034 | 1.999e-4 | -5.645 | 1.610e-5 |
| CIRBP | 1153 | -3.543 | 1.000e-6 | -2.617 | 3.179e-6 | -2.003 | 5.849e-4 | -2.592 | 0.003 |
| HSPB2 | 3316 | -3.128 | 1.000e-6 | -3.004 | 2.501e-6 | -2.419 | 1.824e-5 | -2.863 | 1.040e-4 |
| IGFBP6 | 3489 | -5.032 | 1.000e-6 | -3.928 | 1.000e-6 | -2.890 | 1.561e-4 | -4.001 | 4.189e-5 |
| INMT | 11185 | -3.897 | 1.000e-6 | -3.534 | 1.000e-6 | -2.242 | 0.002 | -3.069 | 1.000e-6 |
| ANP32E | 81611 | -6.047 | 1.000e-6 | -4.628 | 1.910e-4 | -3.980 | 4.644e-5 | -2.945 | 0.009 |
| SCARAS | 286133 | -6.836 | 1.000e-6 | -6.498 | 1.348e-6 | -5.750 | 3.145e-5 | -6.409 | 1.000e-6 |
| ANKRD29 | 147463 | -4.757 | 1.000e-6 | -2.895 | 9.353e-4 | -2.358 | 0.017 | -2.400 | 0.005 |

Literature references

Bai L, et al. Journal of cellular biochemistry, 2012 Jan. PMID 21913217. Down-regulation of the cavin family proteins in breast cancer. [C](#)

Balg A, et al. Proteomics, 2009 Sep. PMID 19722192. Proteomic identification of pleckstrin-associated proteins in platelets: possible interactions with actin. [C](#)

Hansen CG, et al. Nature cell biology, 2009 Jul. PMID 19525939. SDPR induces membrane curvature and functions in the formation of caveolae. [C](#)

Ogata T, et al. Molecular and cellular biology, 2008 May. PMID 18332105. MURC, a muscle-restricted coiled-coil protein that modulates the Rho/ROCK pathway, induces cardiac dysfunction and conduction disturbance. [C](#)

Barbe L, et al. Molecular & cellular proteomics : MCP, 2008 Mar. PMID 18029348. Toward a confocal subcellular atlas of the human proteome. [C](#)

Kimura K, et al. Genome research, 2006 Jan. PMID 16344560. Diversification of transcriptional

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DATASET BACKGROUND

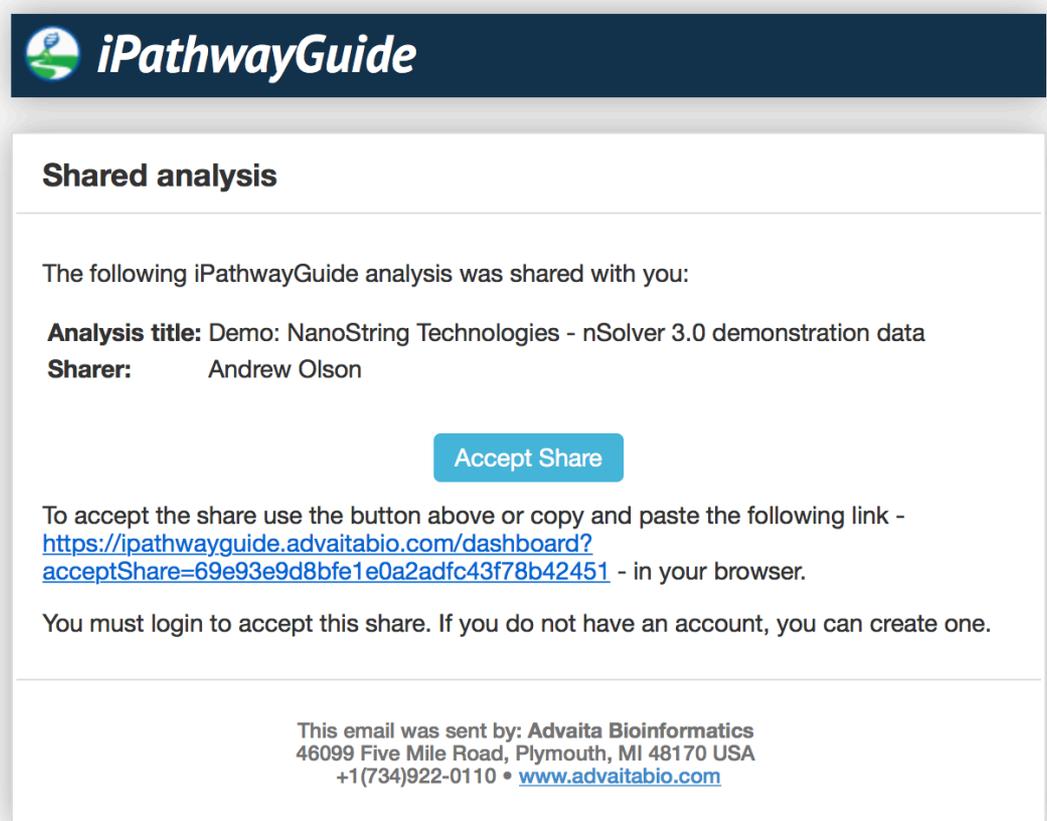
- Comes from NanoString's PanCancer Panel
- Data included with nSolver 3.0
- 4 Contrasts
 - Basal like vs. Normal
 - Her2Enriched vs. Normal
 - Luminal A vs. Normal
 - Luminal B vs. Normal

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

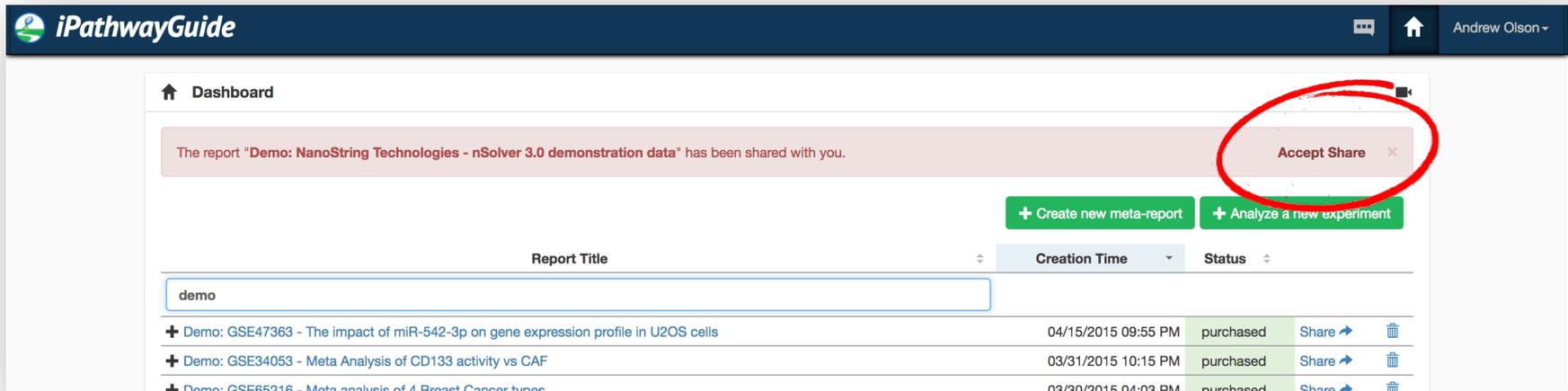
- Share notice is only good one time
- FWD a share notice will not work.
- Once you click the link, the share notice will appear in your dashboard.



The screenshot shows an email notification from iPathwayGuide. At the top is the iPathwayGuide logo, which consists of a globe icon and the text "iPathwayGuide". Below the logo is a section titled "Shared analysis". The text in this section reads: "The following iPathwayGuide analysis was shared with you:", "Analysis title: Demo: NanoString Technologies - nSolver 3.0 demonstration data", and "Sharer: Andrew Olson". Below this information is a blue button with the text "Accept Share". Underneath the button, it says: "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." The final line of text in the notification says: "You must login to accept this share. If you do not have an account, you can create one." At the bottom of the email, it provides contact information: "This email was sent by: Advaita Bioinformatics", "46099 Five Mile Road, Plymouth, MI 48170 USA", and "+1(734)922-0110 • www.advaitabio.com".

ACCEPT SHARE

- Click Accept Share

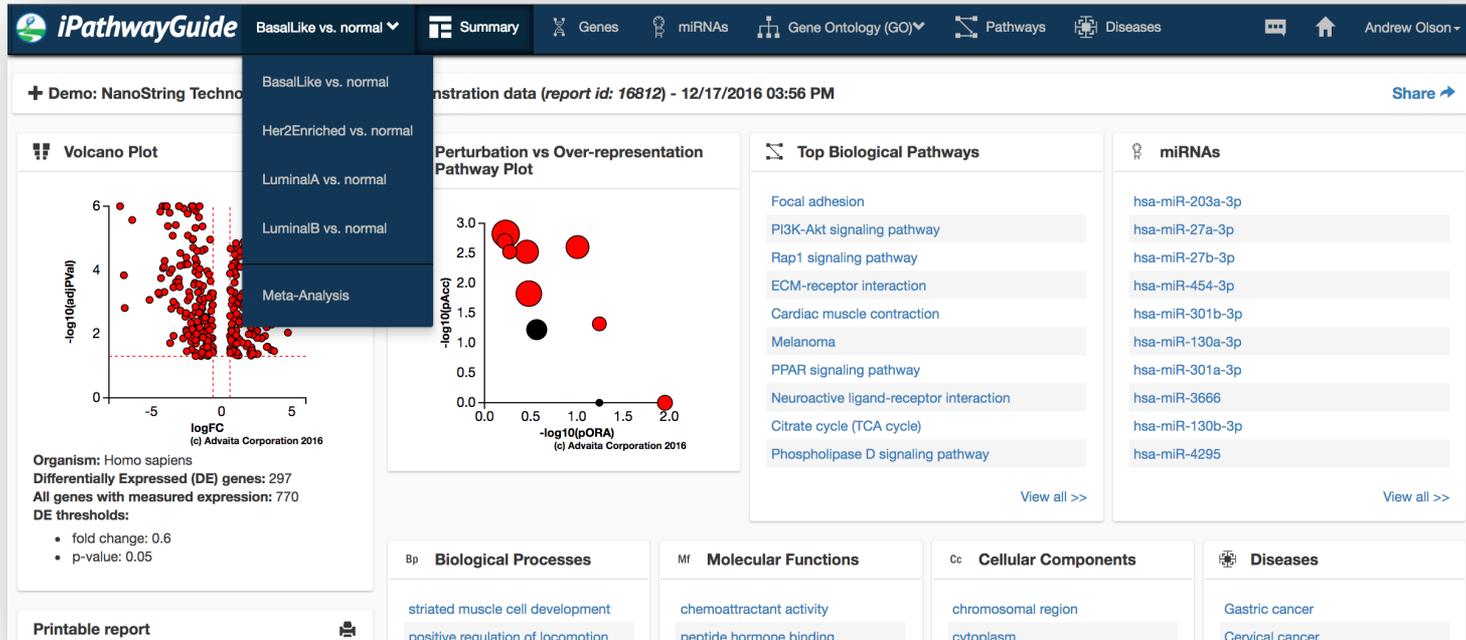


The screenshot shows the iPathwayGuide dashboard. At the top, there is a dark blue header with the iPathwayGuide logo on the left and a user profile for Andrew Olson on the right. Below the header, the main content area is titled "Dashboard". A prominent notification banner at the top of the dashboard reads: "The report 'Demo: NanoString Technologies - nSolver 3.0 demonstration data' has been shared with you." To the right of this notification is a red "Accept Share" button, which is circled in red. Below the notification, there are two green buttons: "+ Create new meta-report" and "+ Analyze a new experiment". Underneath these buttons is a search bar containing the text "demo". Below the search bar is a table with columns for "Report Title", "Creation Time", and "Status". The table contains three rows of data, each with a plus icon on the left, a report title, a creation time, a status, and a "Share" button with a right-pointing arrow.

| | 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 → |

VIEW REPORT

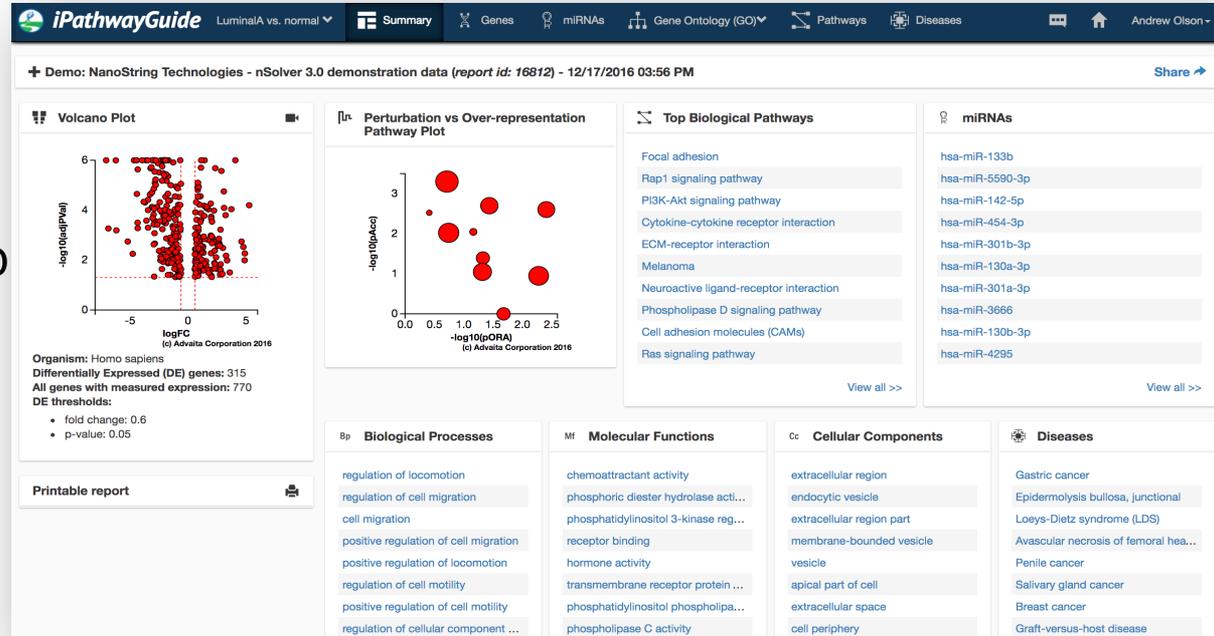
- Select a contrast to view (Luminal A)



Summary

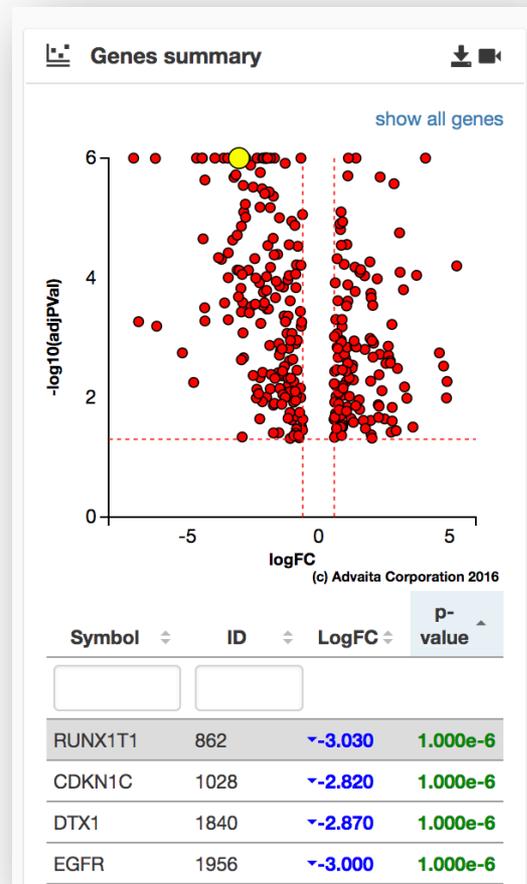
5 Key Sections

- 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 gene 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

The screenshot displays the iPathwayGuide interface for a NanoString Technologies demonstration dataset. The main navigation bar includes options for Summary, Genes, miRNAs, Gene Ontology (GO), Pathways, Diseases, and user information (Andrew Olson).

miRNAs summary: A bar chart shows the number of differentially expressed (DE) targets for various miRNAs. The x-axis represents the number of DE targets (0 to 20). The y-axis lists miRNAs: hsa-miR-133b, hsa-miR-590-3p, hsa-miR-142-5p, hsa-miR-454-3p, hsa-miR-301b-3p, hsa-miR-130a-3p, hsa-miR-301a-3p, hsa-miR-3656, hsa-miR-130b-3p, and hsa-miR-4295. The bars are color-coded: green for upregulated targets and red for downregulated targets.

miRNA details:

- Identifier:** hsa-miR-133b
- Sequence:** UUUGGUCCCUUCAACCAGCUA
- Stem-loop:** hsa-miR-133b
- Family:** miR-133
- Description:** Homo sapiens miR-133b stem-loop
- Sequence:** CCUCAGAAGAAGUAGCCCCUGUCUGGUGUCAACGGAACCAAGUCGUCUUCUGAGAGUUGUCCCUUCAACCAGCUACAGGCGCUAAGCCAGUCCAGUCCUG

Differentially expressed target genes: A bar chart showing log2 FC values for target genes. The x-axis lists genes: RIT1B, D4, RNU6T1, UBR1, LAMB3, RPL1, RPS27C, RNU6B, RPL11, CREB3, RPL17, RPL10A, RPL13A, RPL10B, RPL13B, RPL13C, RPL13D, RPL13E, RPL13F, RPL13G, RPL13H, RPL13I, RPL13J, RPL13K, RPL13L, RPL13M, RPL13N, RPL13O, RPL13P, RPL13Q, RPL13R, RPL13S, RPL13T, RPL13U, RPL13V, RPL13W, RPL13X, RPL13Y, RPL13Z, RPL13AA, RPL13AB, RPL13AC, RPL13AD, RPL13AE, RPL13AF, RPL13AG, RPL13AH, RPL13AI, RPL13AJ, RPL13AK, RPL13AL, RPL13AM, RPL13AN, RPL13AO, RPL13AP, RPL13AQ, RPL13AR, RPL13AS, RPL13AT, RPL13AU, RPL13AV, RPL13AW, RPL13AX, RPL13AY, RPL13AZ, RPL13BA, RPL13BB, RPL13BC, RPL13BD, RPL13BE, RPL13BF, RPL13BG, RPL13BH, RPL13BI, RPL13BJ, RPL13BK, RPL13BL, RPL13BM, RPL13BN, RPL13BO, RPL13BP, RPL13BQ, RPL13BR, RPL13BS, RPL13BT, RPL13BU, RPL13BV, RPL13BW, RPL13BX, RPL13BY, RPL13BZ, RPL13CA, RPL13CB, RPL13CC, RPL13CD, RPL13CE, RPL13CF, RPL13CG, RPL13CH, RPL13CI, RPL13CJ, RPL13CK, RPL13CL, RPL13CM, RPL13CN, RPL13CO, RPL13CP, RPL13CQ, RPL13CR, RPL13CS, RPL13CT, RPL13CU, RPL13CV, RPL13CW, 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RPL13YI, RPL13YJ, RPL13YK, RPL13YL, RPL13YM, RPL13YN, RPL13YO, RPL13YP, RPL13YQ, RPL13YR, RPL13YS, RPL13YT, RPL13YU, RPL13YV, RPL13YW, RPL13YX, RPL13YY, RPL13YZ, RPL13ZA, RPL13ZB, RPL13ZC, RPL13ZD, RPL13ZE, RPL13ZF, RPL13ZG, RPL13ZH, RPL13ZI, RPL13ZJ, RPL13ZK, RPL13ZL, RPL13ZM, RPL13ZN, RPL13ZO, RPL13ZP, RPL13ZQ, RPL13ZR, RPL13ZS, RPL13ZT, RPL13ZU, RPL13ZV, RPL13ZW, RPL13ZX, RPL13ZY, RPL13ZZ.

Literature references:

- Landgraf P, et al., Cell. 129:1401-1414(2007), PMID:17604727 A mammalian microRNA expression atlas based on small RNA library sequencing.
- Lim LP, et al., Science. 299:1540(2003), PMID:12624257 Vertebrate microRNA genes.

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.

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

miRNAs summary

Show counts

P-value correction: Bonferroni

| Name | # DE targets (- / all) | # targets (- / all) | P-value |
|------------------|------------------------|---------------------|----------------|
| hsa-miR-542-3p | 45 / 47 | 186 / 279 | 3.97e-4 |
| hsa-miR-548n | 61 / 84 | 422 / 891 | 0.001 |
| hsa-miR-4496 | 32 / 38 | 283 / 551 | 0.020 |
| hsa-miR-3180-5p | 47 / 64 | 378 / 770 | 0.046 |
| hsa-miR-4692 | 53 / 71 | 331 / 635 | 0.049 |
| hsa-miR-4514 | 53 / 71 | 331 / 635 | 0.049 |
| hsa-miR-539-5p | 43 / 56 | 355 / 696 | 0.081 |
| hsa-miR-4524a-5p | 43 / 57 | 281 / 552 | 0.086 |
| hsa-miR-137 | 74 / 107 | 575 / 1119 | 0.100 |
| hsa-miR-3182 | 26 / 32 | 170 / 347 | 0.131 |

10 25 50 100

1 2 3 4 5 6 7 ... 145

miRNA details

Identifier: hsa-miR-542-3p
Sequence: UGUGACAGAUUGAUUCUGAAA

Stem-loop: hsa-mi-542
Family: mi-542

Description: Homo sapiens miR-542 stem-loop

```

-----caga      auc  GG  UCA      -A  c
ucucagac  UCGG  AUCA  UGUCACGAG  uac  a
          |||||  |||  |||  |||||  |||
agggucug  AGUC  UAGU  ACAGUGUuc  gus  g
acuucucacacgg  gAA  AA  UAG      ac  u
    
```

Sequence: CAGAUUCAGACAUCUCGGGGCAUCAGUACACAGAUACCAGUGUGCAUCUUGUGACAGAUUGAUUCUGAAAAGGUCUGGAGCCACUCAUCUCCA

Differentially expressed target genes

Literature references

Sewer A, et al., BMC Bioinformatics. 6:267(2005), PMID:16274478 Identification of clustered microRNAs using an ab initio prediction method.

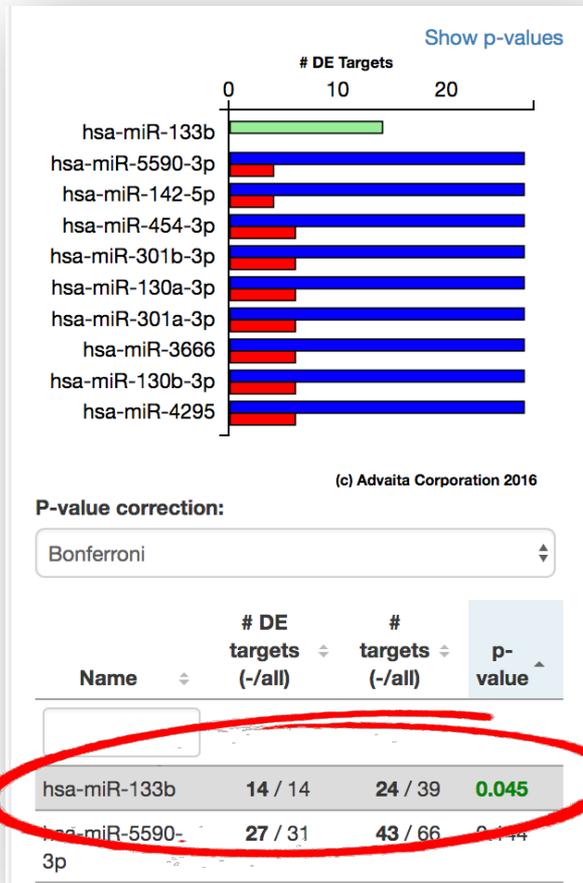
Landgraf P, et al., Cell. 129:1401-1414(2007), PMID:17604727 A mammalian microRNA expression atlas based on small RNA library sequencing.

Lui WO, et al., Cancer Res. 67:6031-6043(2007), PMID:17619659 Patterns of known and novel small RNAs in human cervical cancer.

FAQ Contact Us

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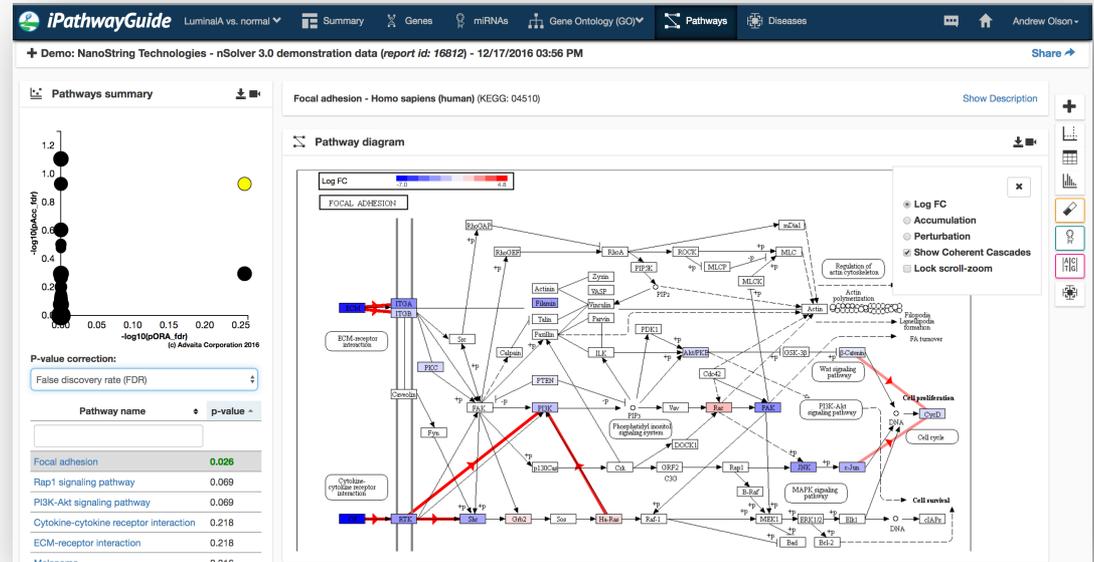
miRNA PREDICTION



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

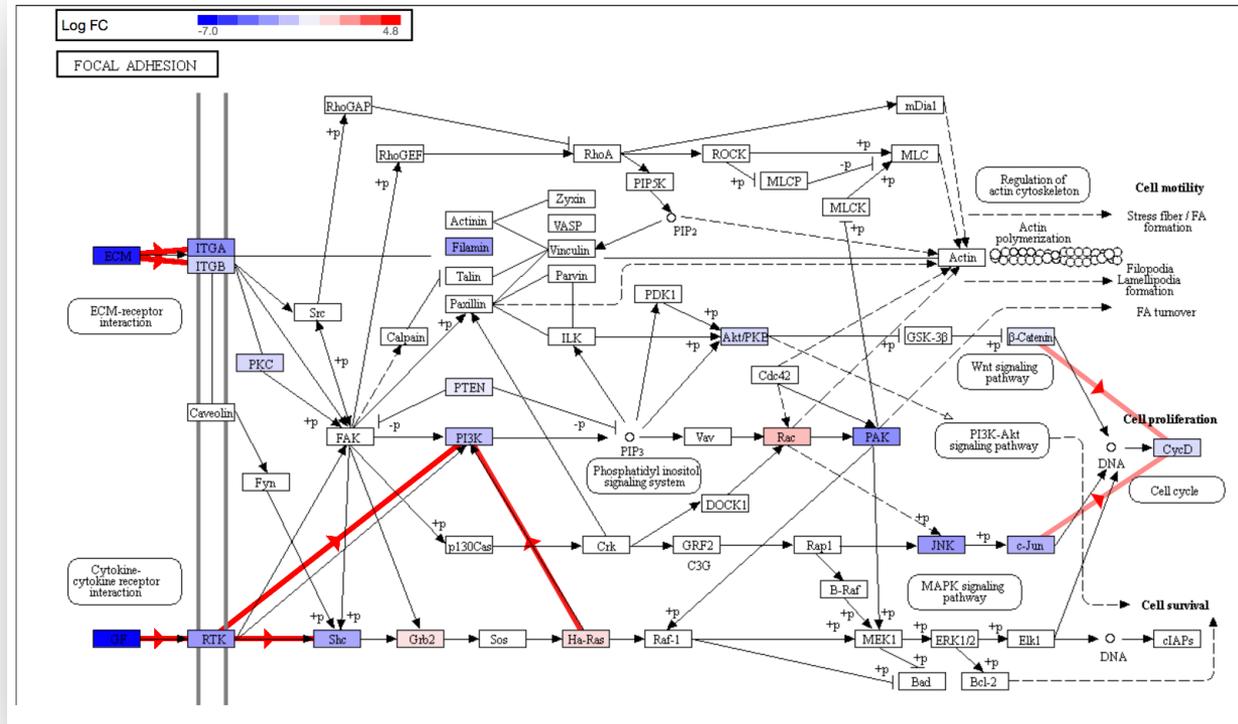
PATHWAYS

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



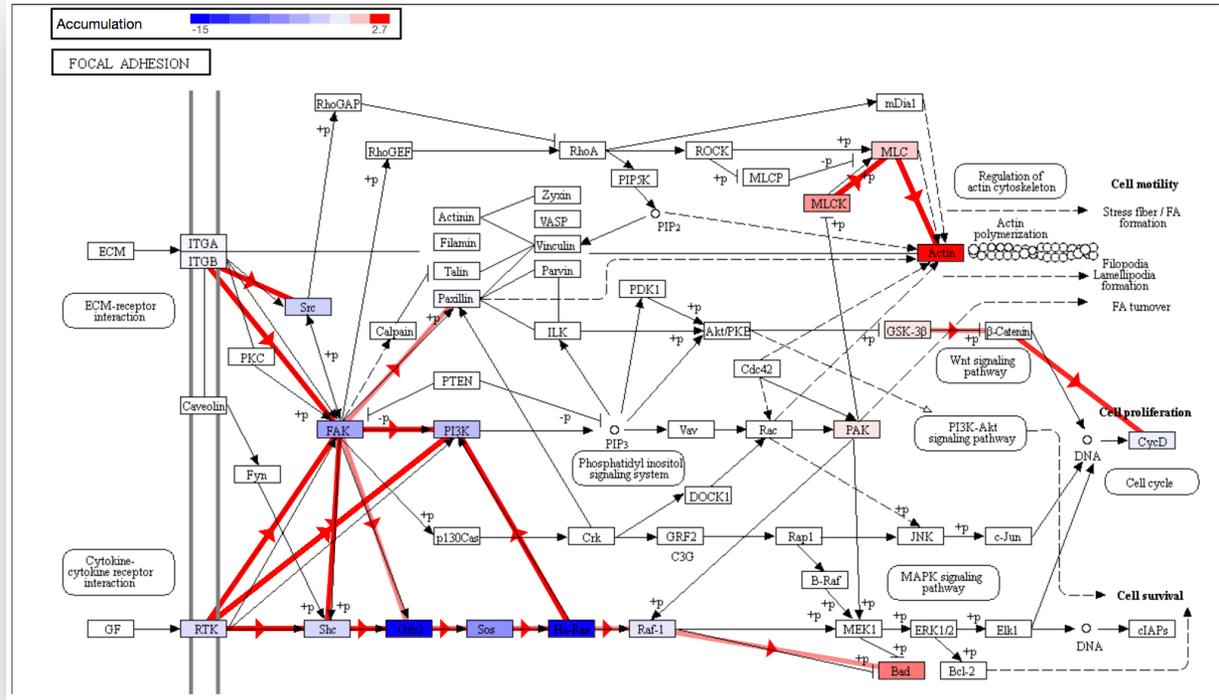
Reading Pathway Diagrams

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



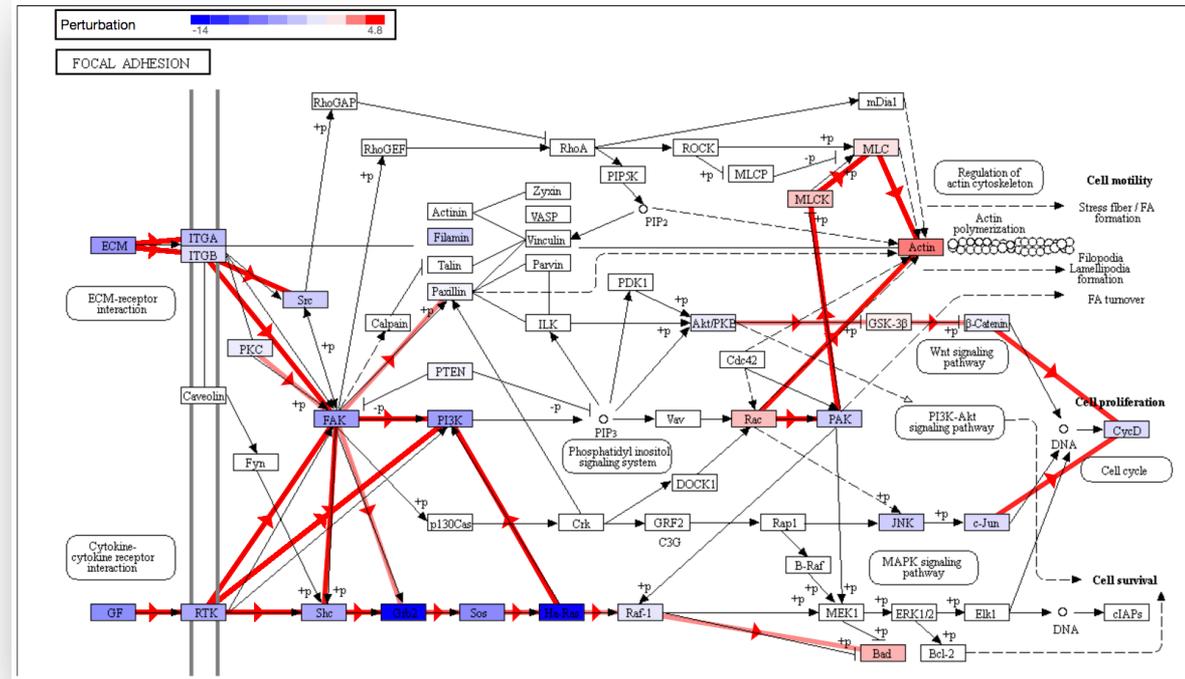
Reading Pathway Diagrams

- 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



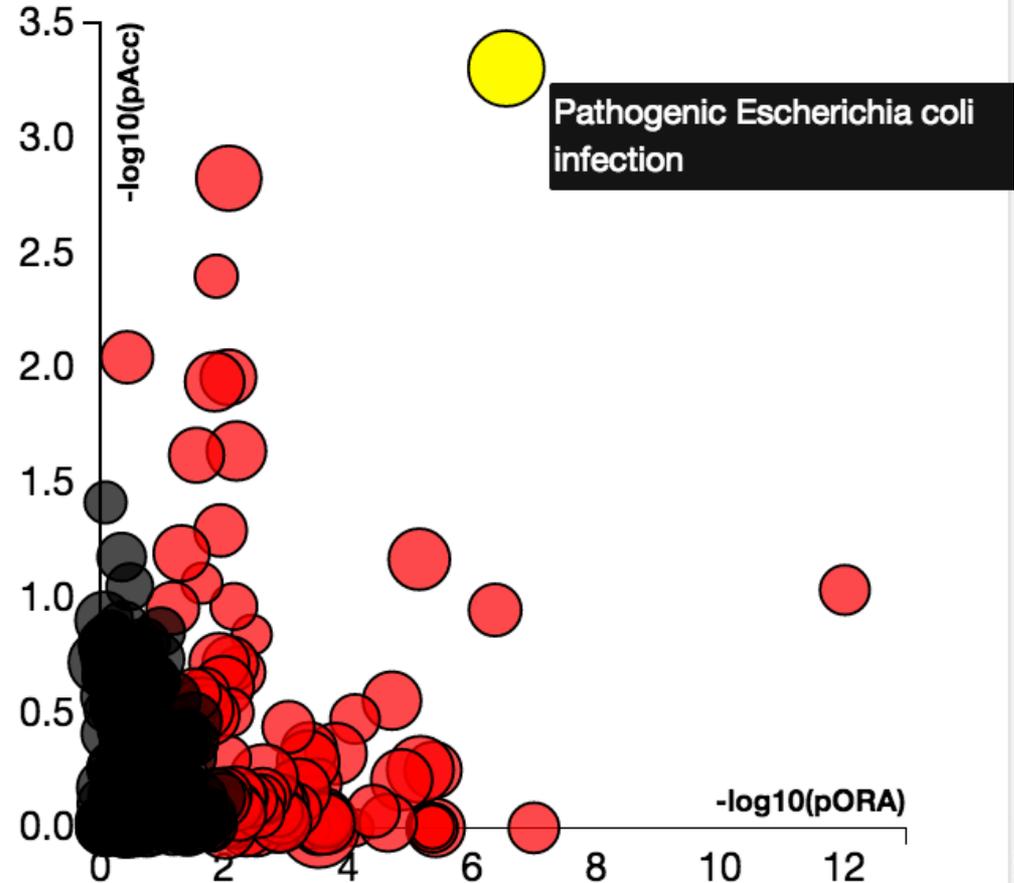
Reading Pathway Diagrams

- 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



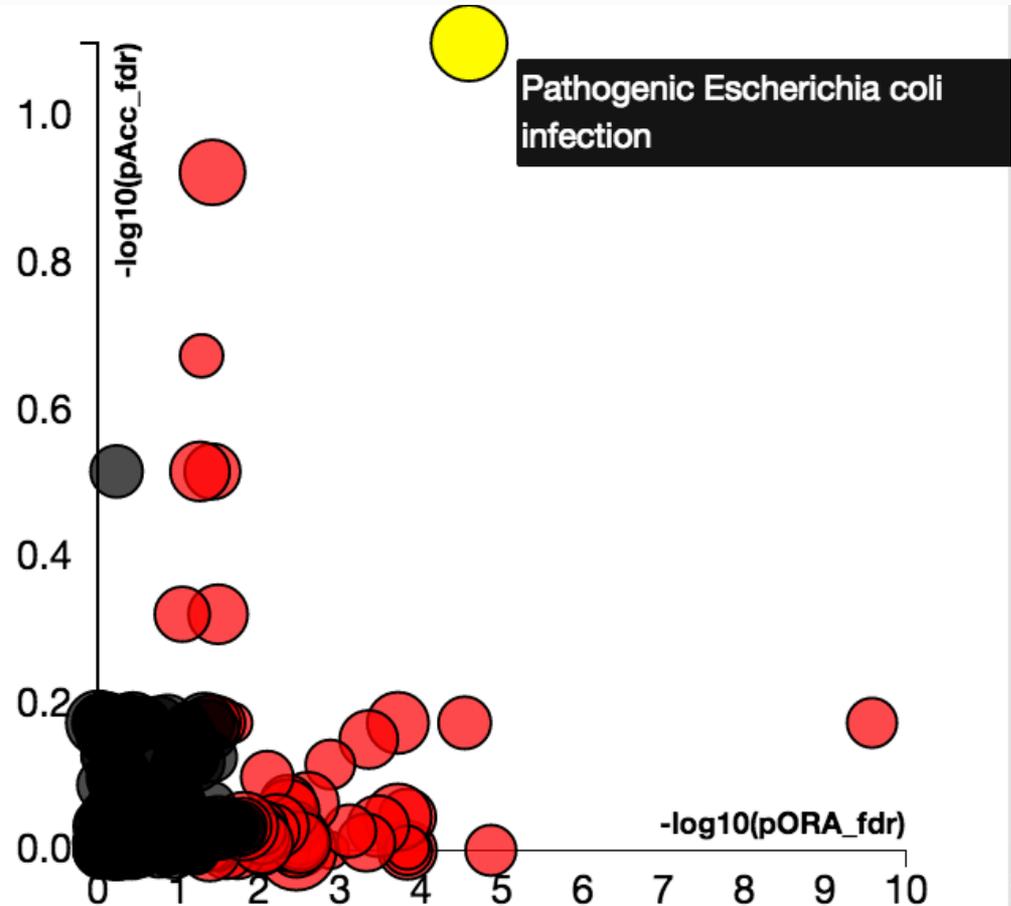
Correction factors

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



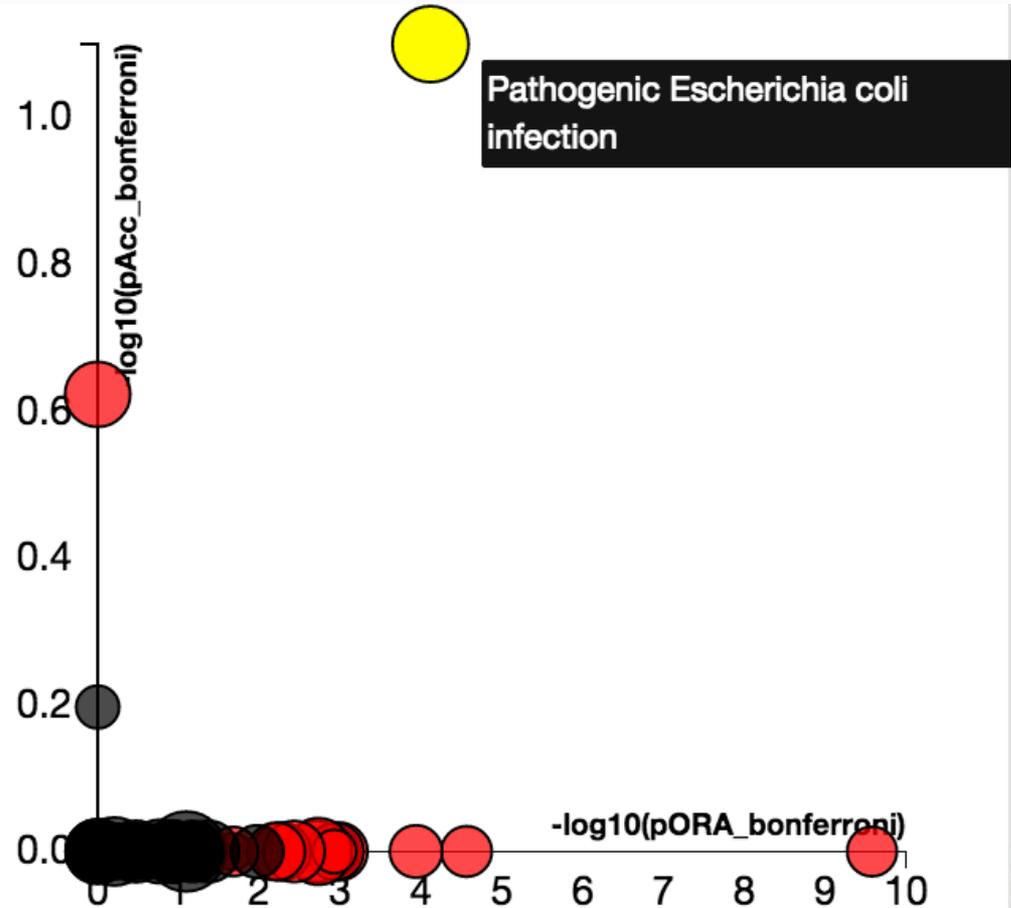
Correction factors: FDR

- 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 pathway "Focal adhesion - Homo sapiens (human) (KEGG: 04510)". The main area shows a complex signaling pathway diagram with various proteins and their interactions. A color scale for Log FC is visible at the top of the diagram.

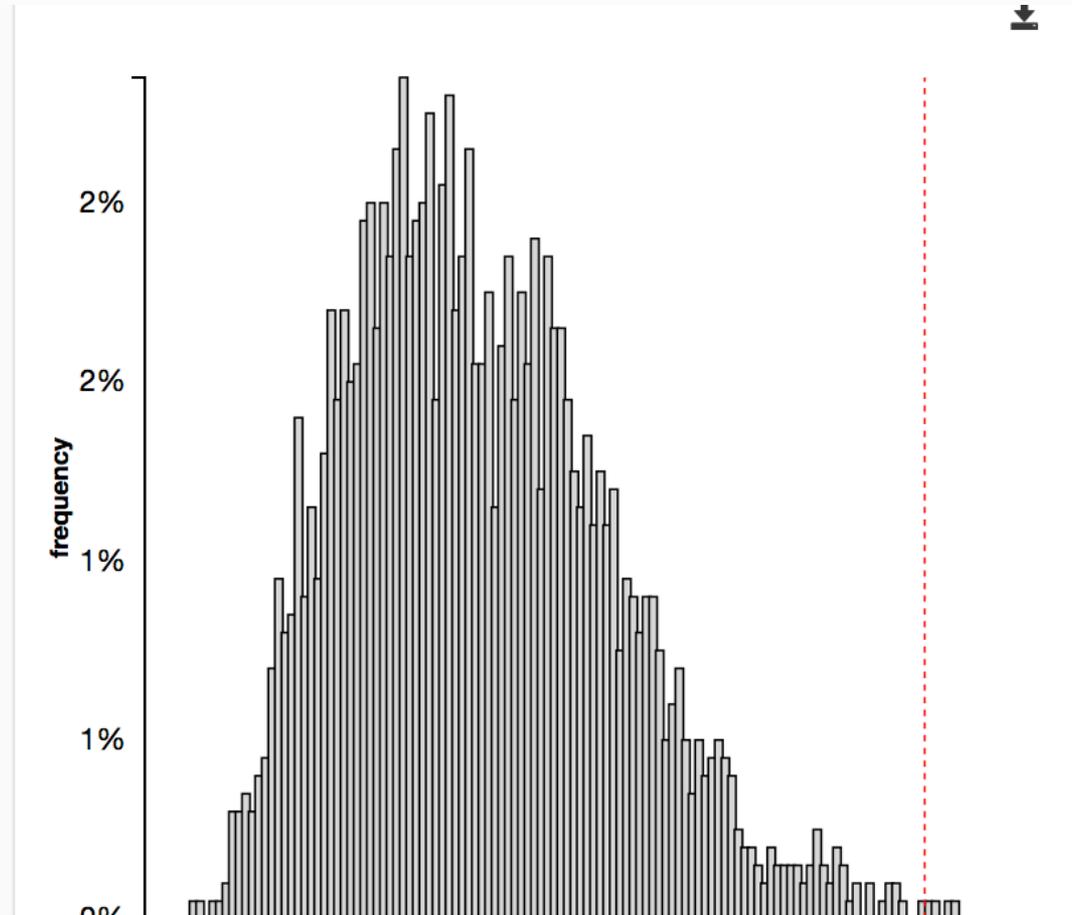
On the right side, the "Pathway Details" panel is open, showing a "Gene table" with the following data:

| 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 |

Below the gene table, there are sections for "Differentially expressed pathway genes" (a bar chart), "Literature references" (a list of citations), and "Related diseases" (a list of conditions like Alport syndrome).

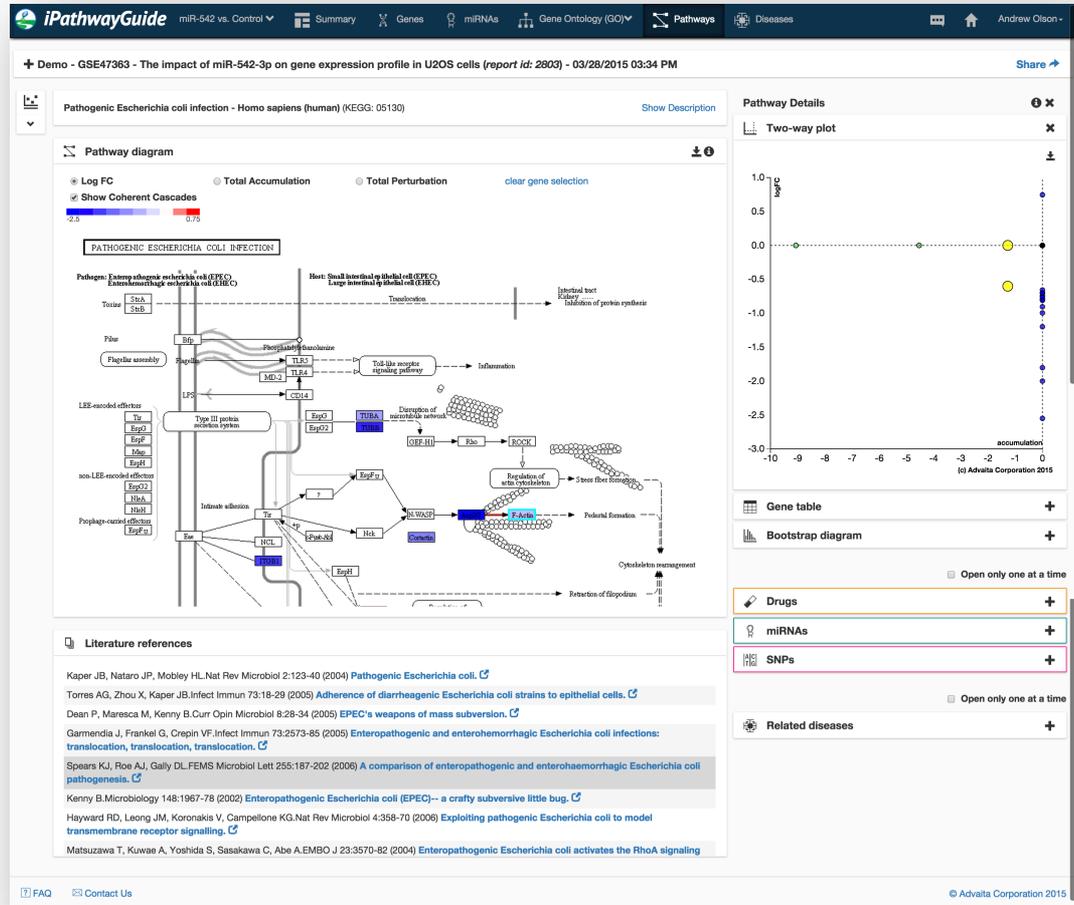
Pathway Details - Perturbation

- 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



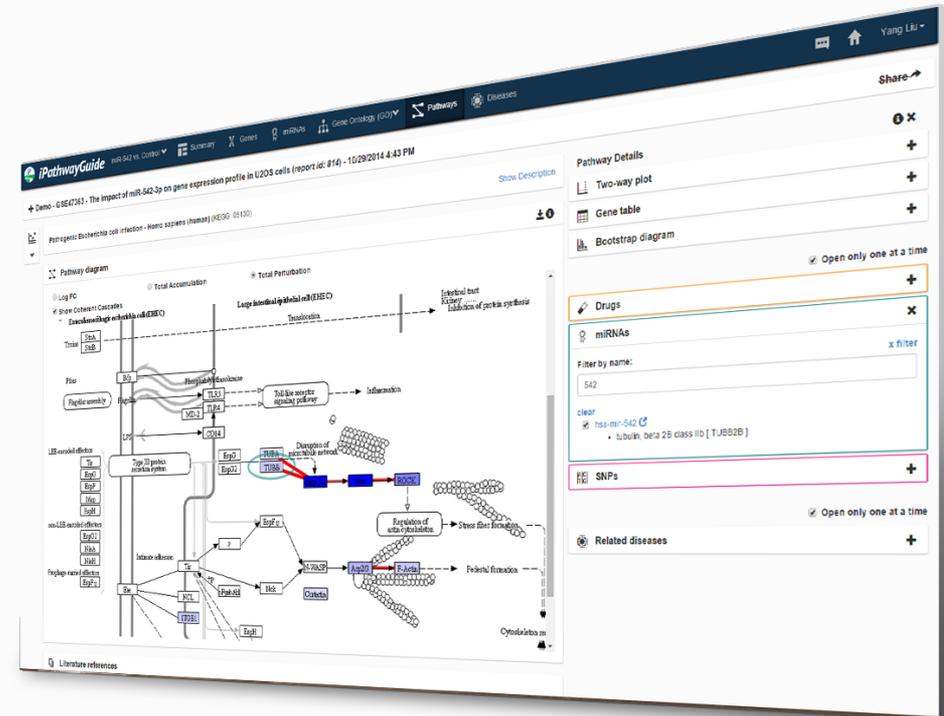
iPG: Pathway Details – Two-way Plot

- 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



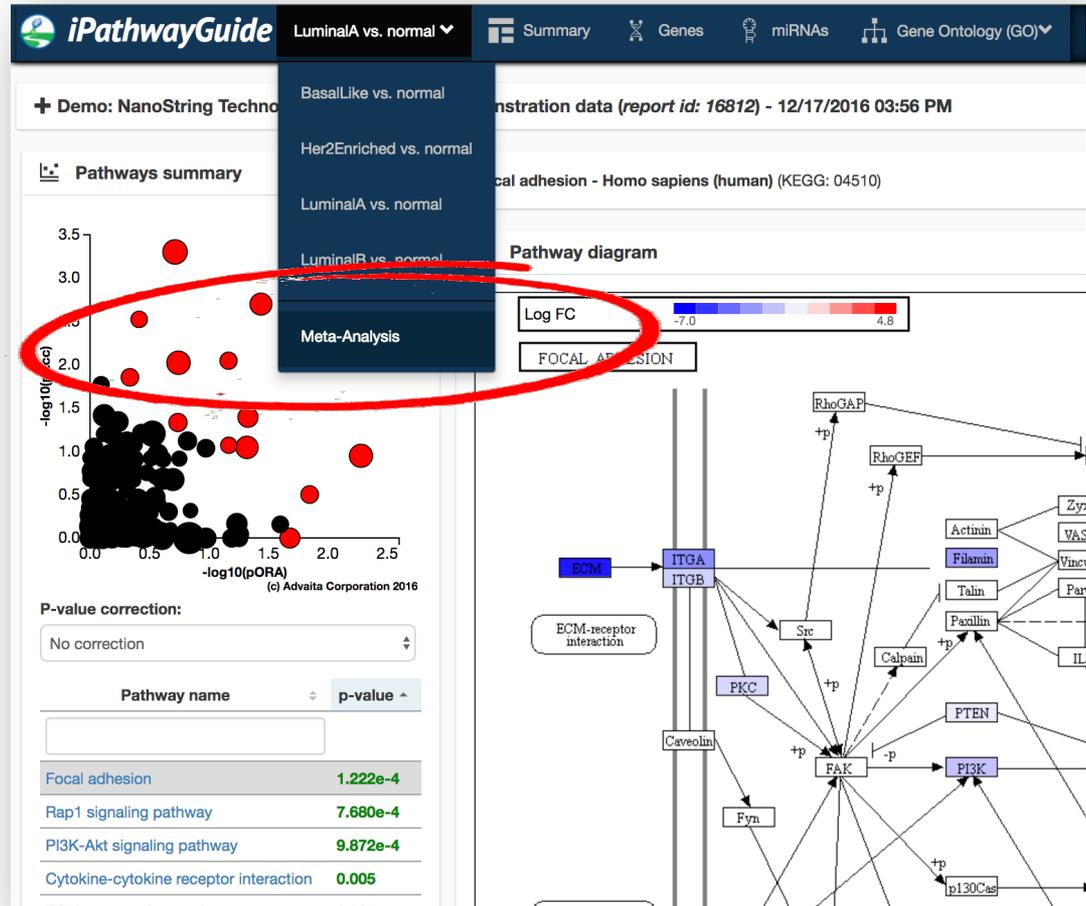
Pathway Details – Model miRNAs, SNPs, Drugs

- 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



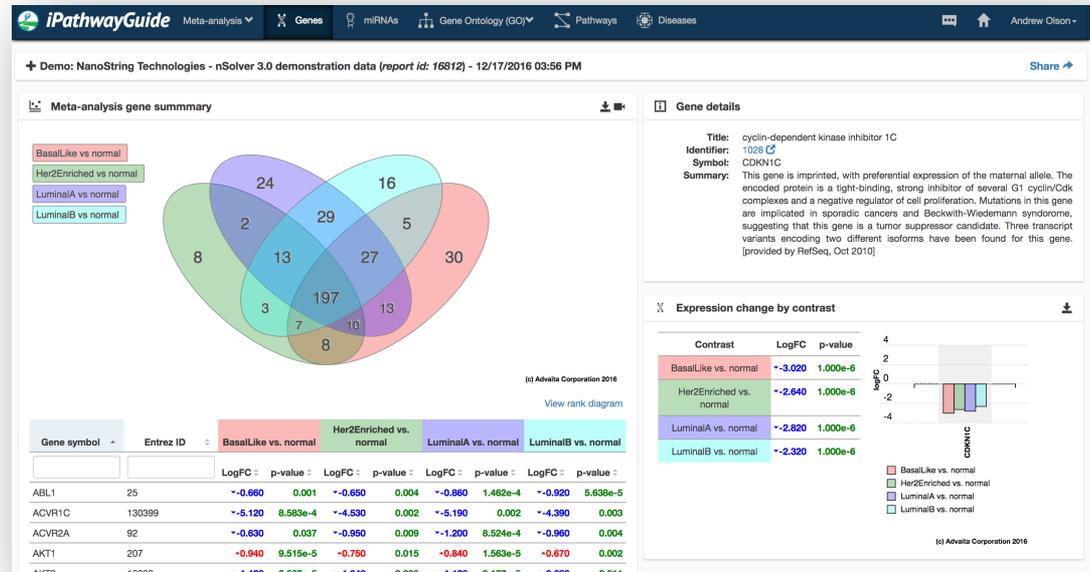
META-ANALYSIS OVERVIEW

- 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



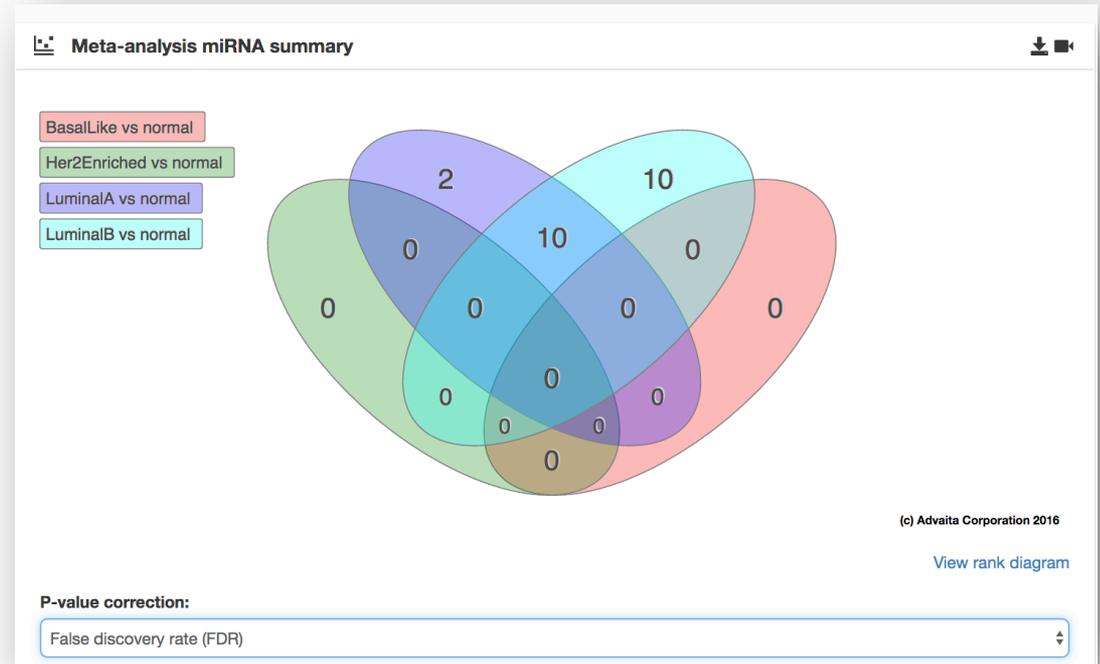
META-ANALYSIS OVERVIEW

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



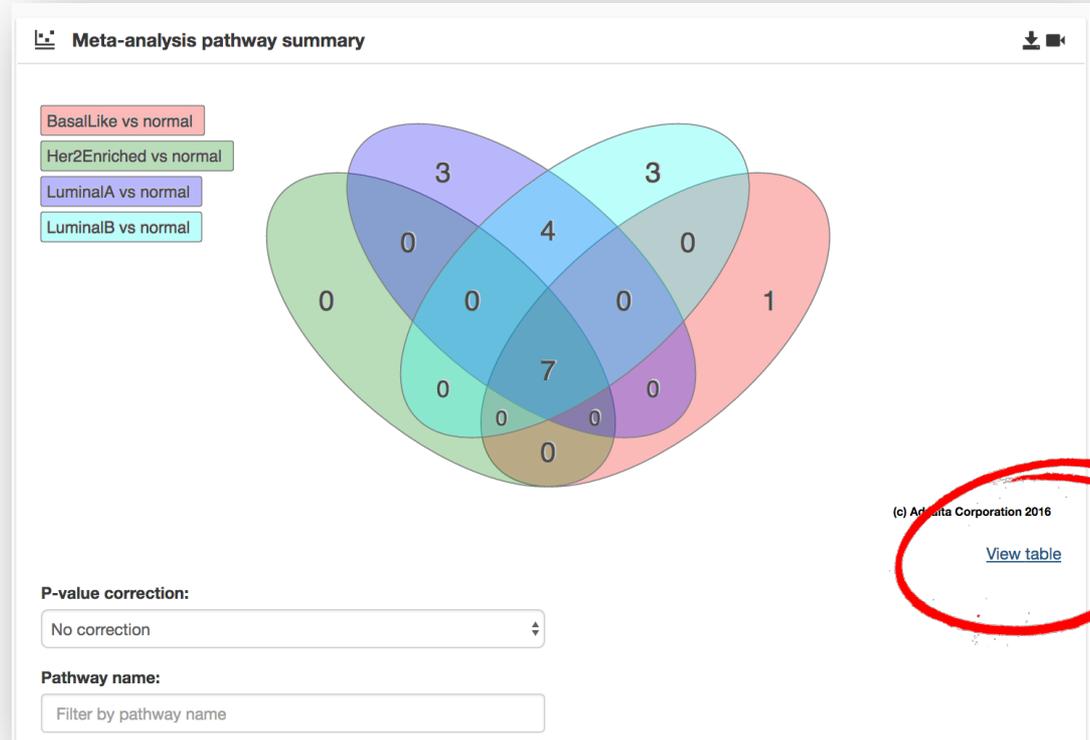
MIRNA INFERENCE (VIA META ANALYSIS)

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



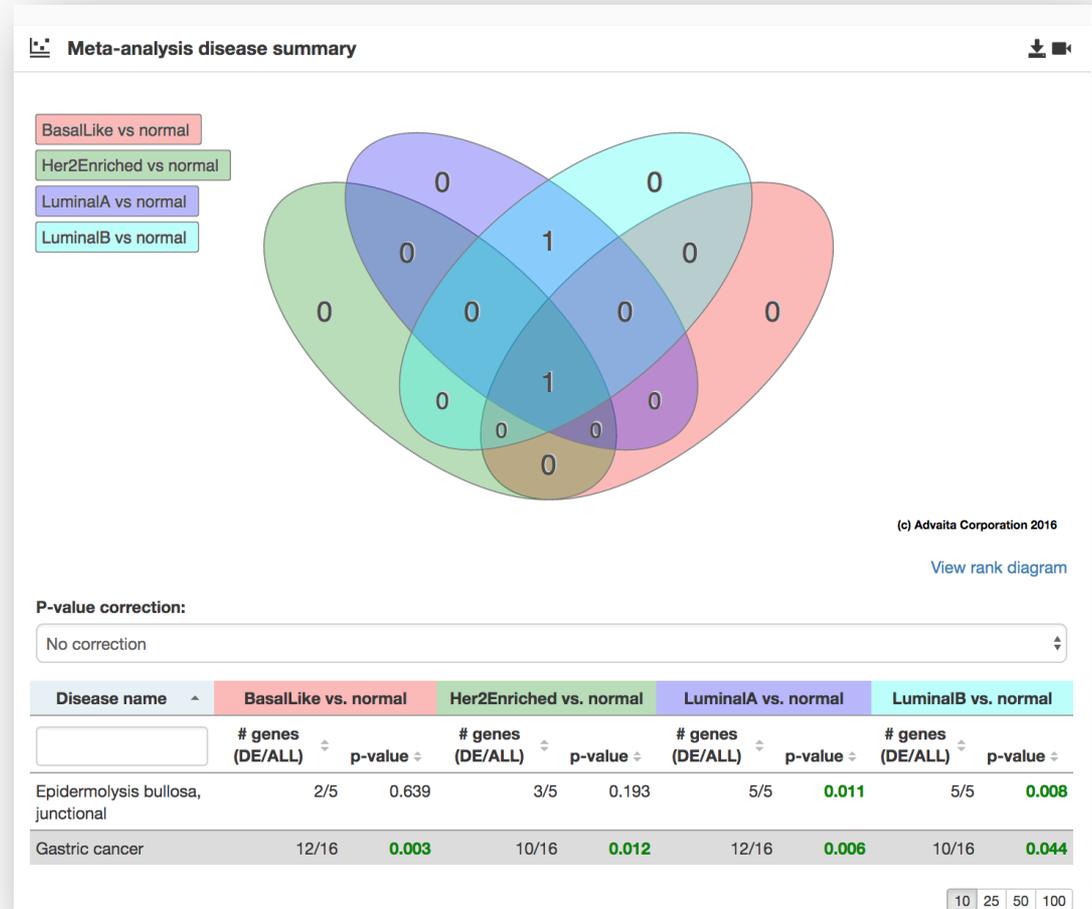
PATHWAYS (VIA META ANALYSIS)

- 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

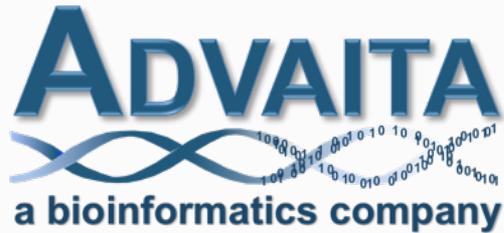


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- Type in email (doesn't matter which email)
- If no account, they will be prompted to sign up

Stop and Questions

Be back at 1:00 PM



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