iPathwayGuide

Expression Analysis with iPathwayGuide

ADVAITA

a bioinformatics company

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

- **<u>Pathway Analysis</u>**
  (Drugs, miRNAs, SNPs)

- Diseases

- Meta analysis
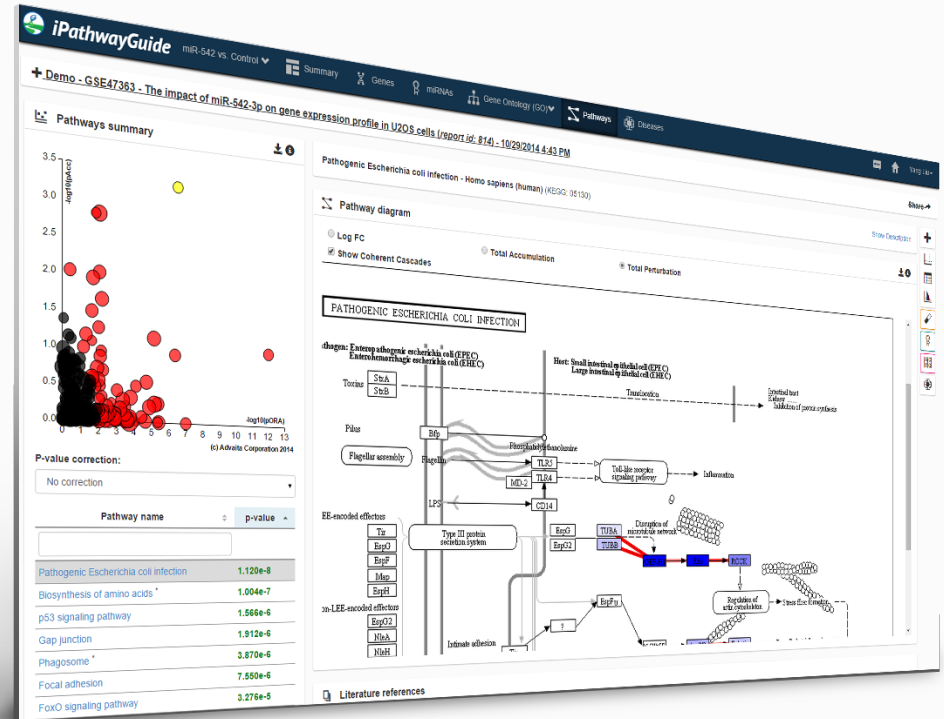
Research challenges

- 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 genes to GO terms, pathways, miRNAs, SNPs, Drugs, References

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
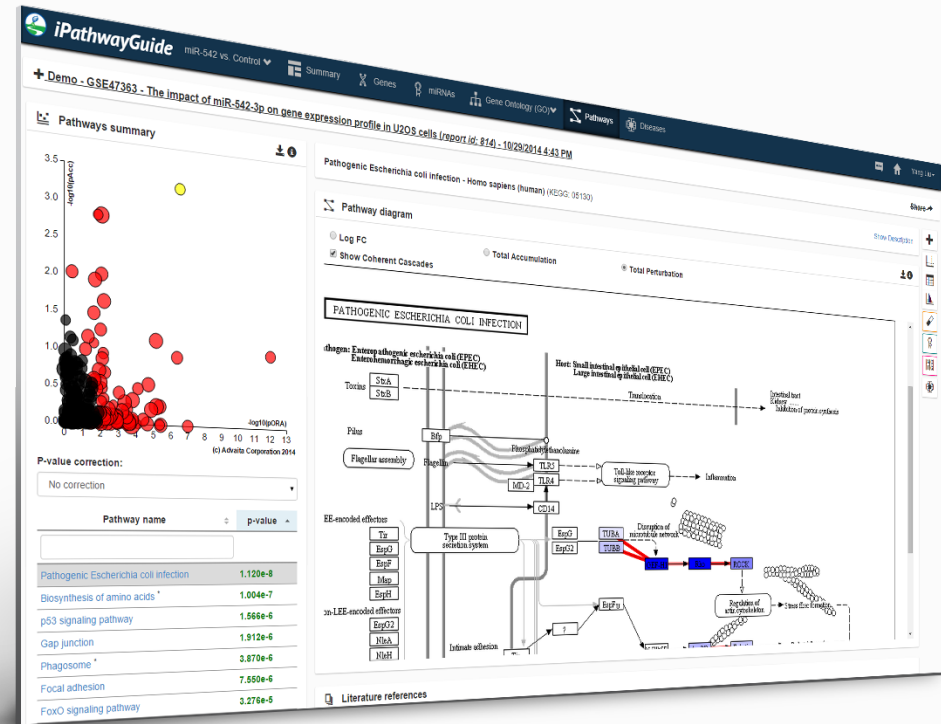
iPG: Meta Analysis – Premium Feature

- Build and compare up to 5 datasets

- Combine proteomic and transcriptomic analyses

- Quickly identify common or unique traits
  - Venn Diagram
  - Sortable columns
  - Correction factors
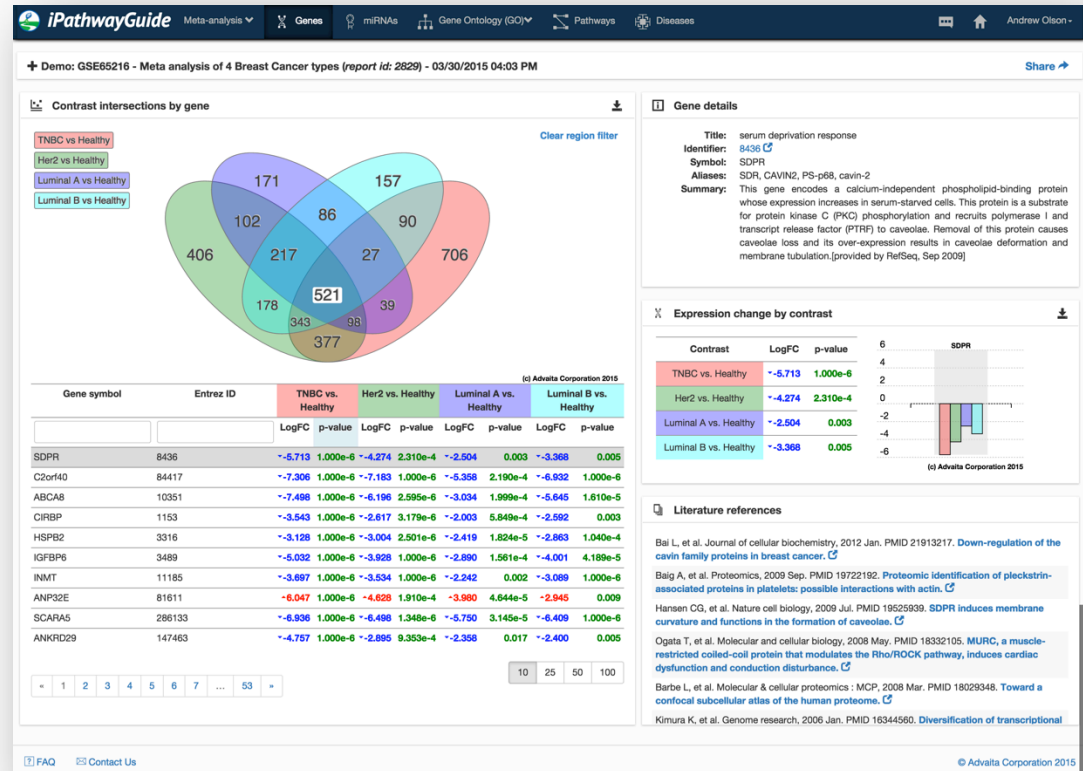
DATASET BACKGROUND

- 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
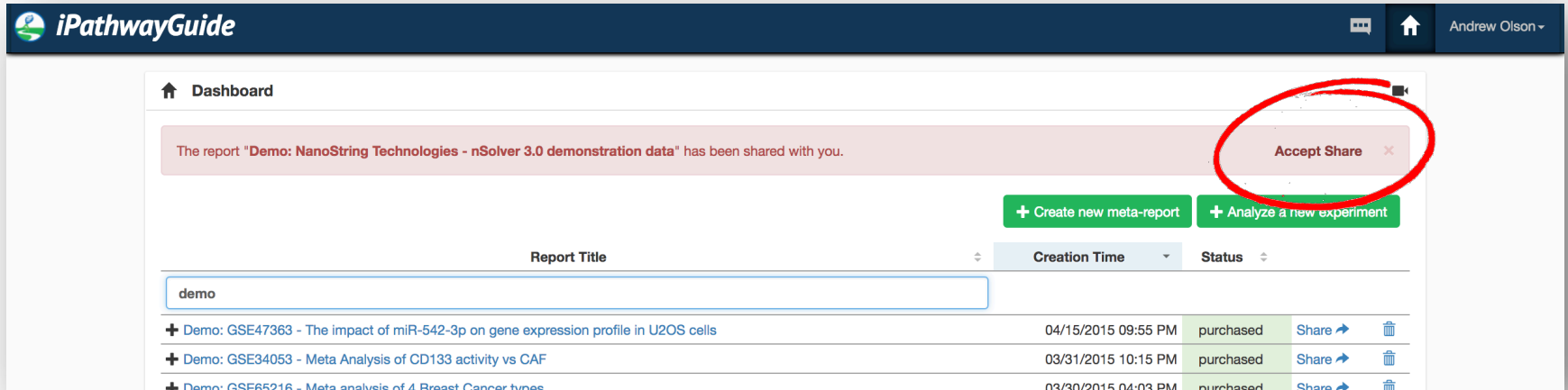**Sharer:**   Andrew Olson

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.

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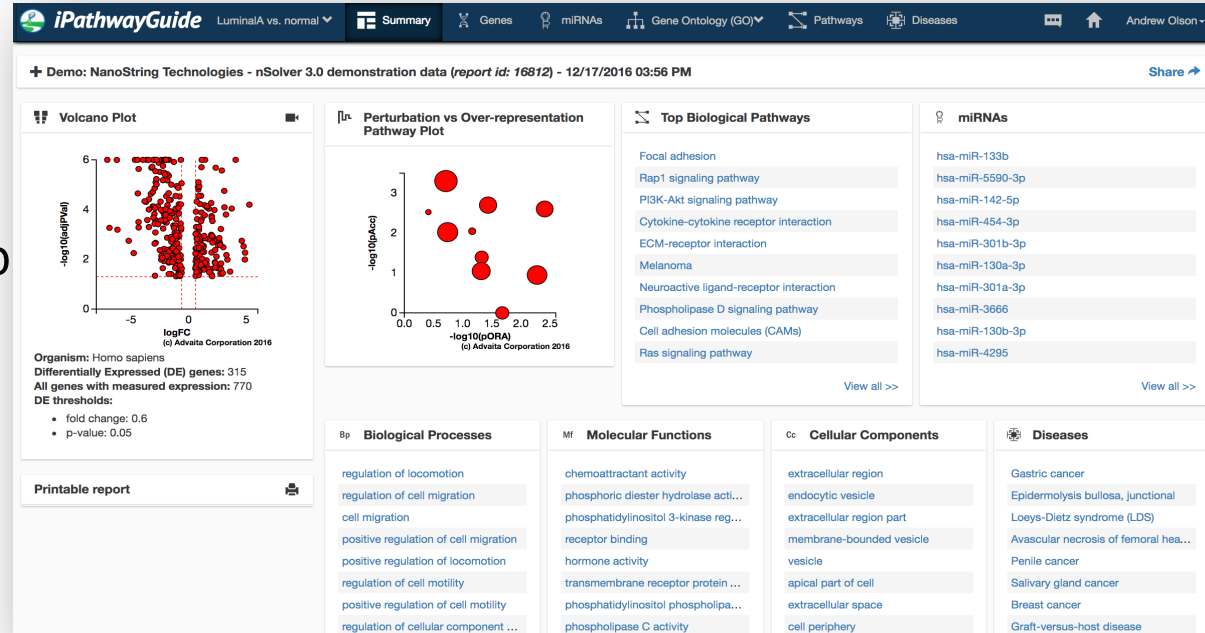
VIEW REPORT

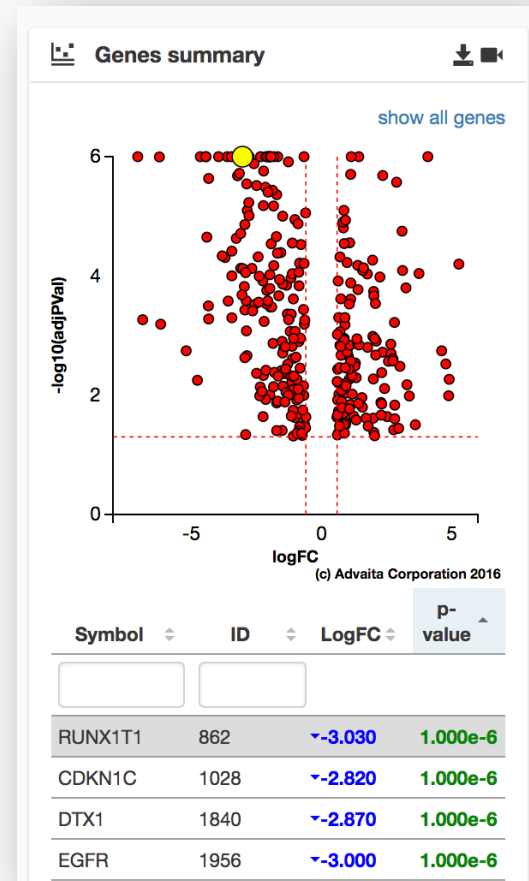- ## Select a contrast to view (Luminal A)

Summary

5 Key Sections

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

- <u>Printable Report Summary</u>

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

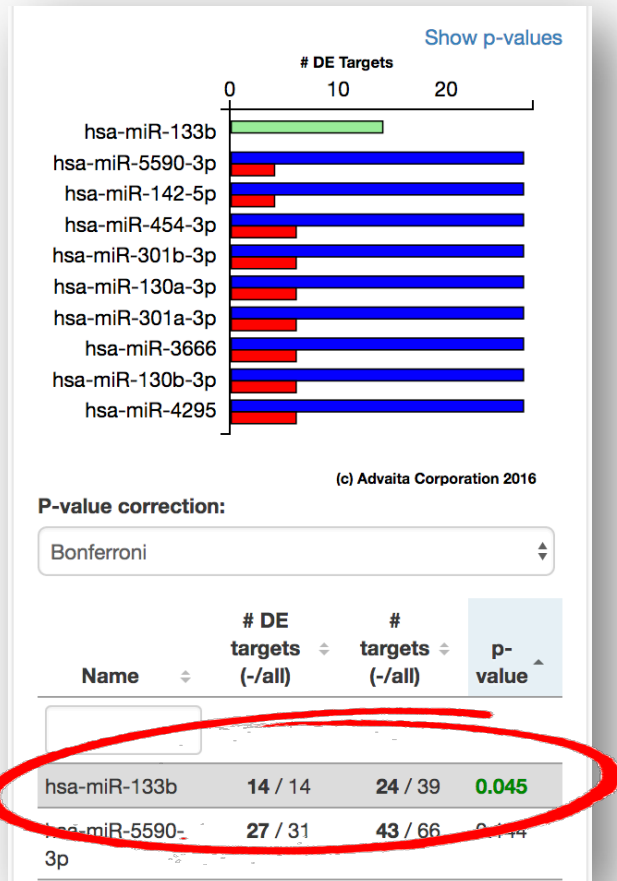- Clicking on any gene will navigate to genes pages
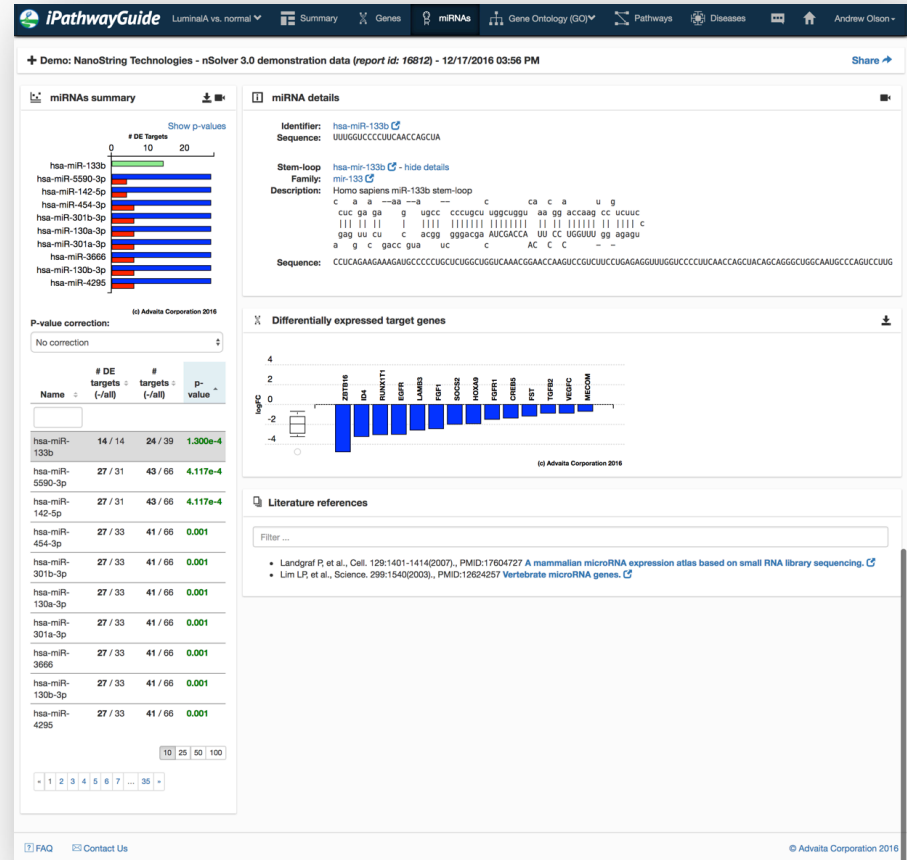
iPG: Predicted miRNA (alt view)

- 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.
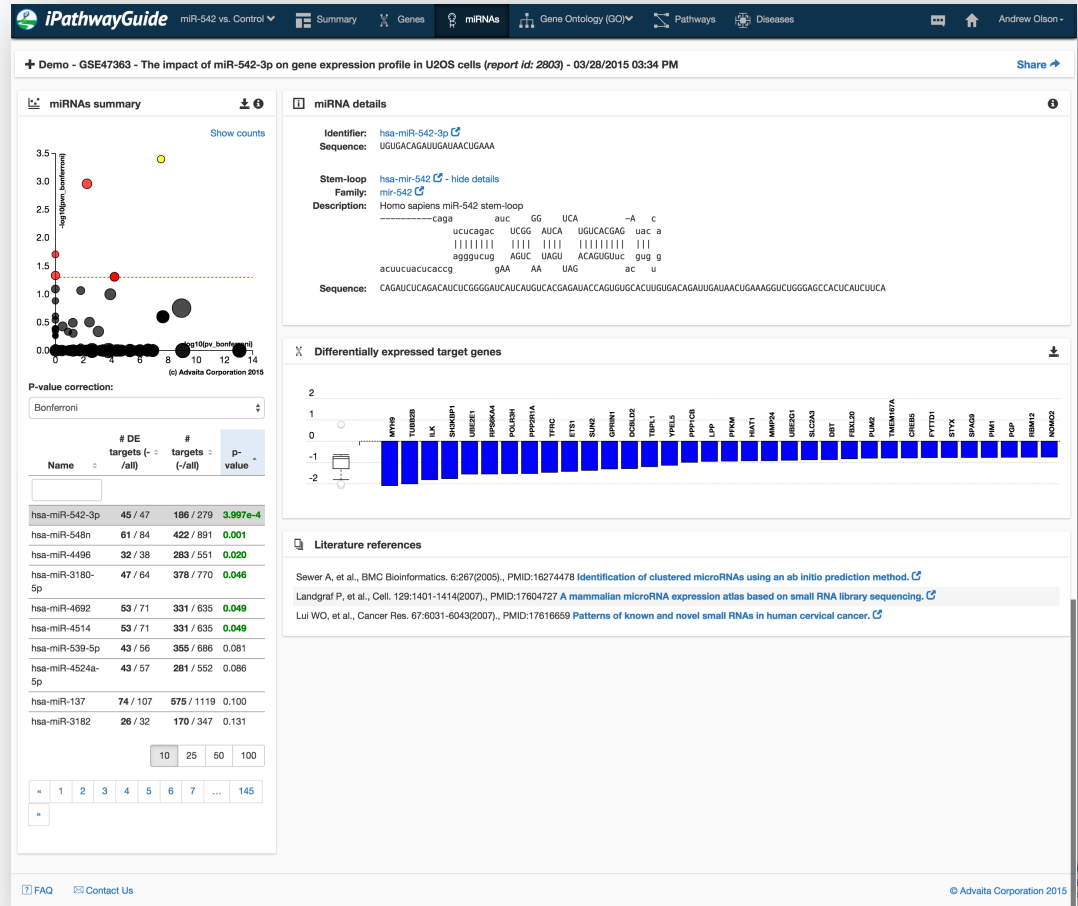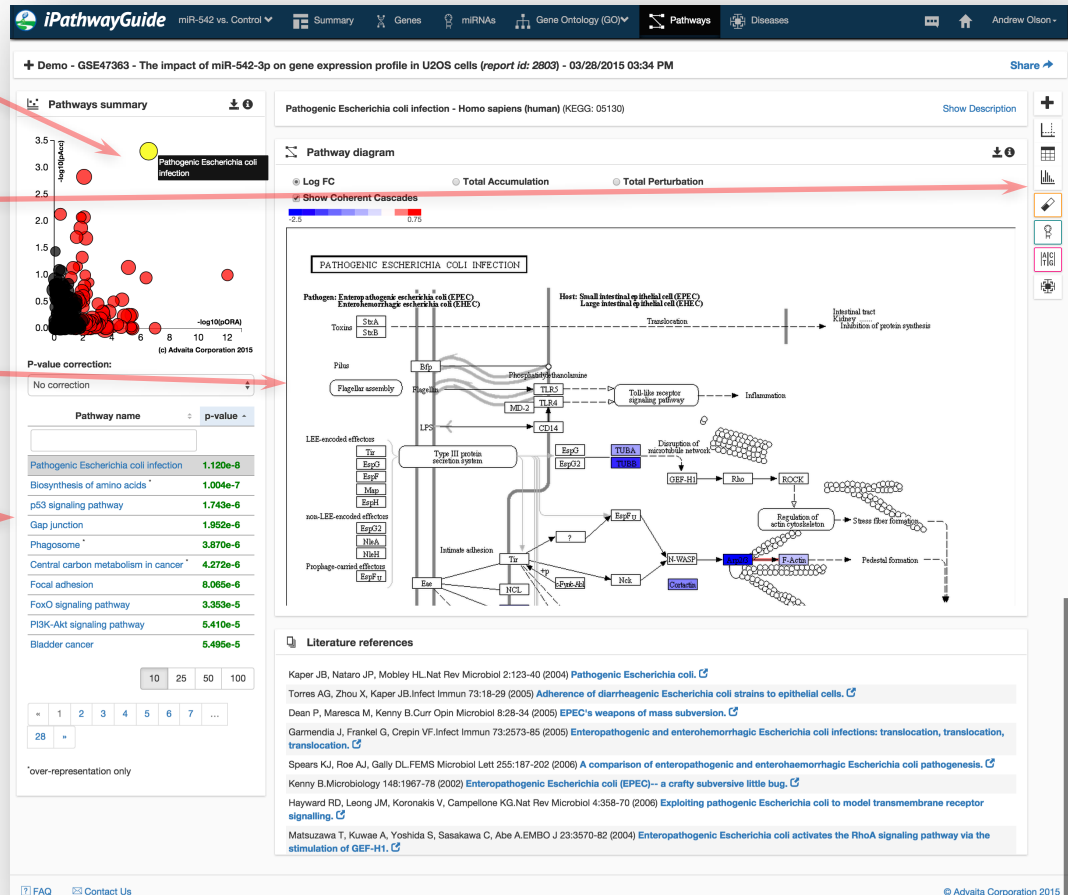
miRNA PREDICTION

- Using Bonferroni, 1 miRNA is significant

- We can hypothesize that miR - 133b may be active in Luminal A
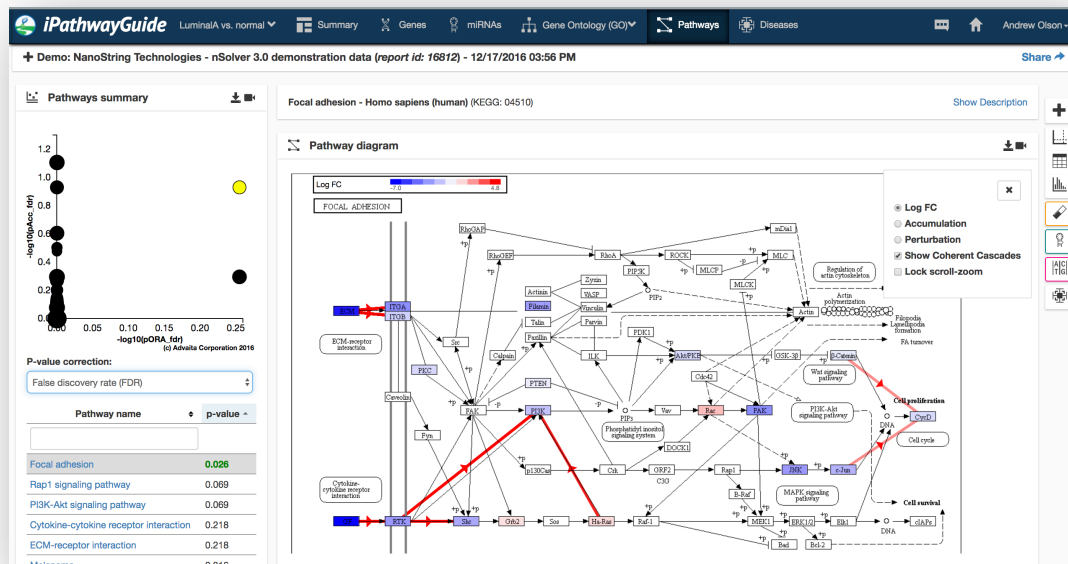
Pathways (Overview)

- Impact Analysis plot

- Pathway Details

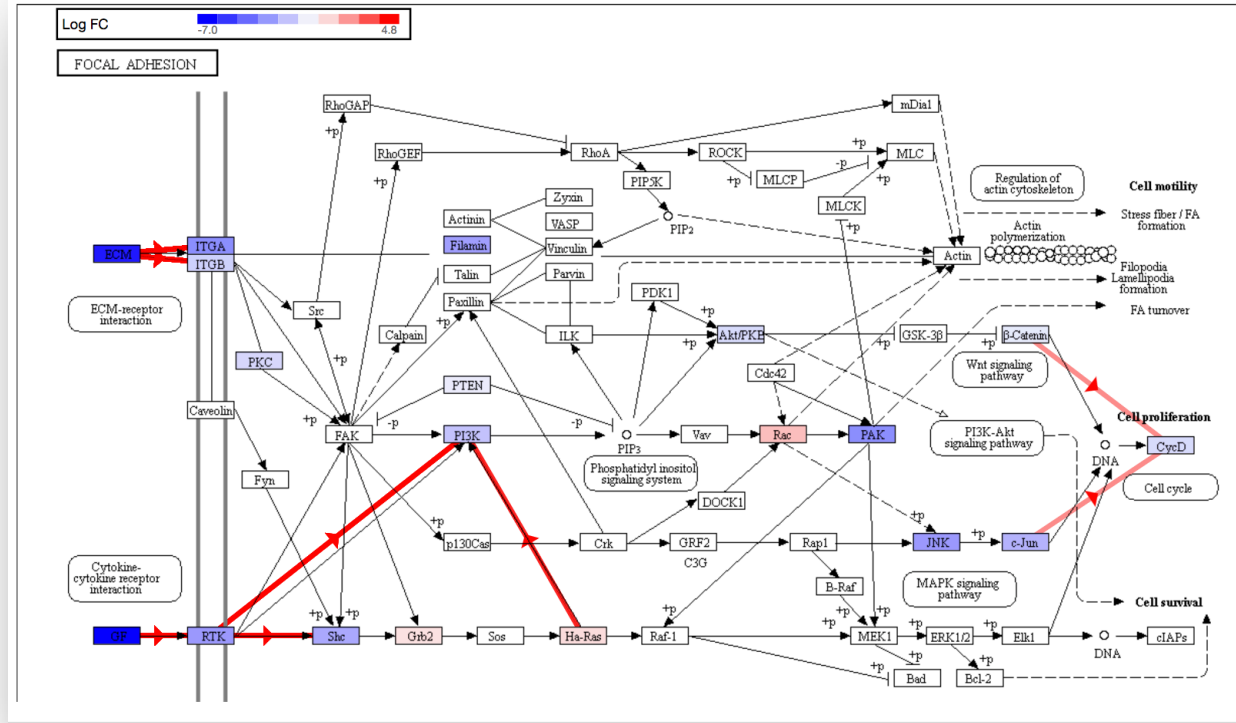- Pathway diagram

- 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

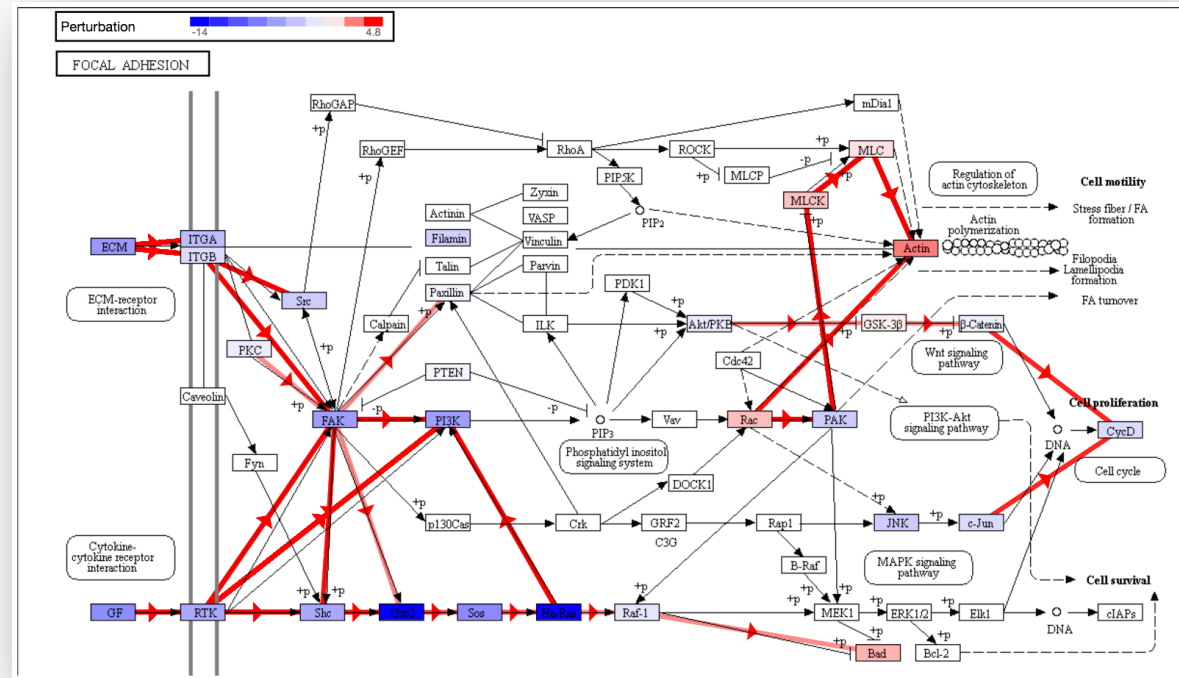- Look for mechanisms using "Coherent Cascades"

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
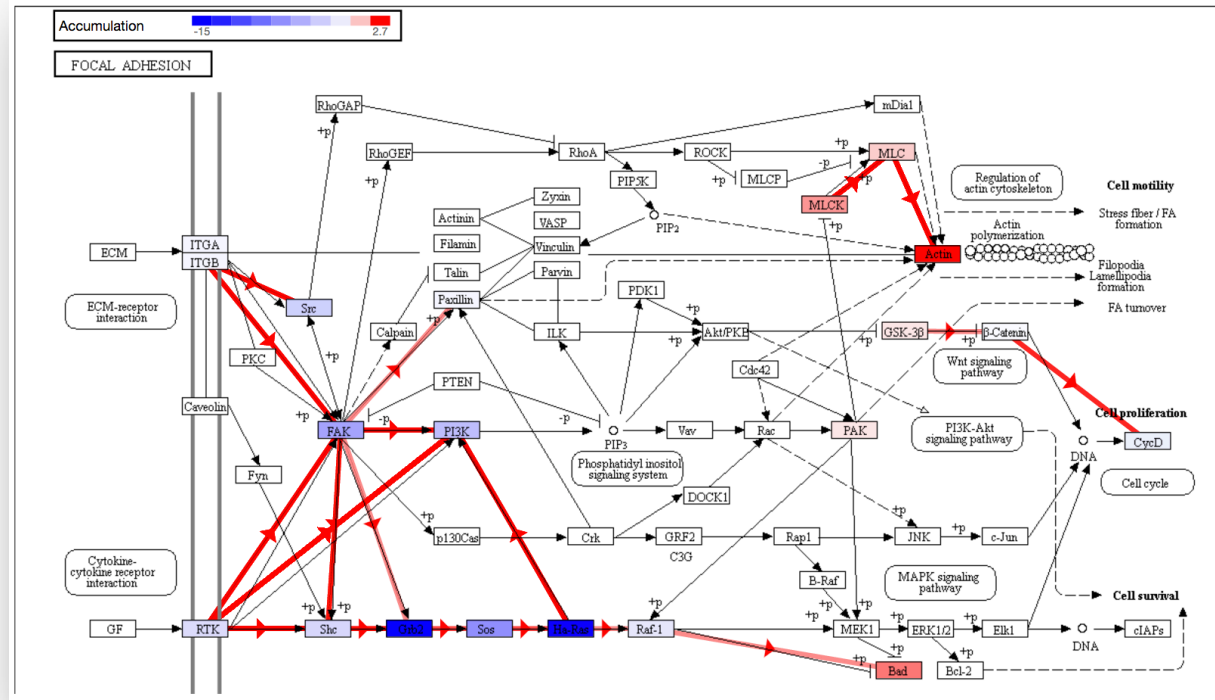
Reading Pathway Diagrams

- 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

- Uncorrected p-values do not take into consideration that multiple comparisons are being made
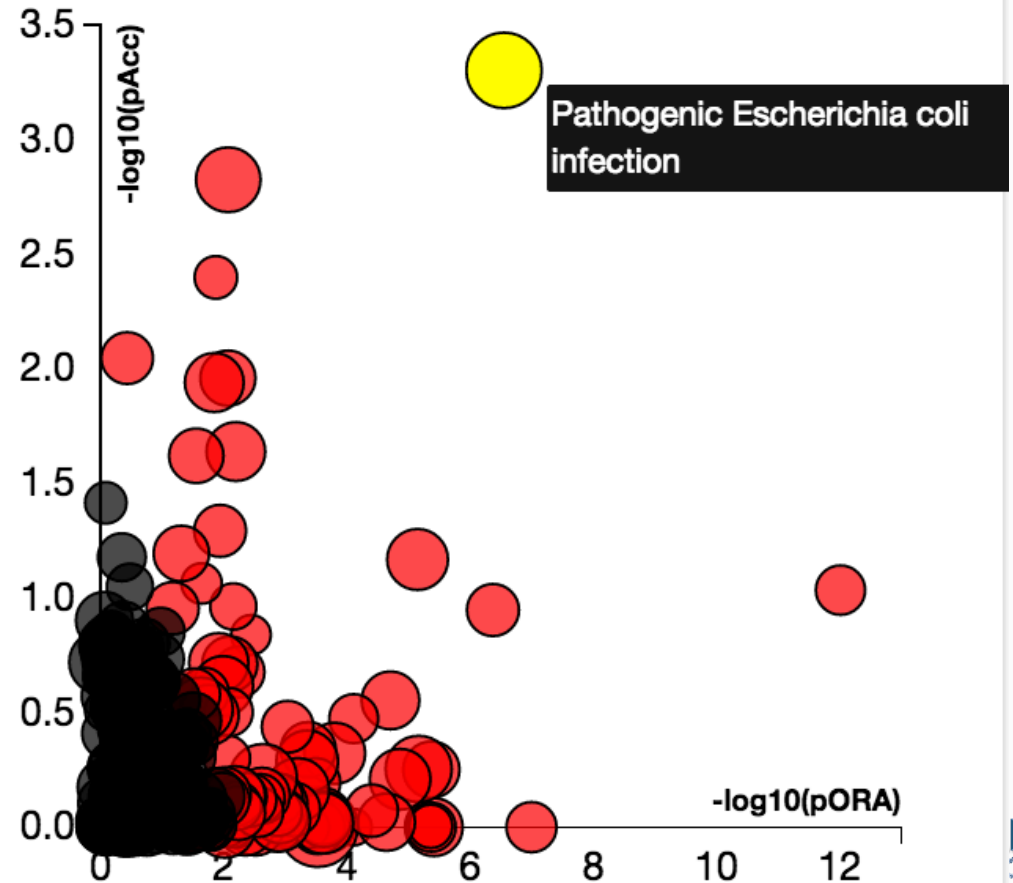
Correction factors: FDR

- 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.

Pathogenic Escherichia coli infection
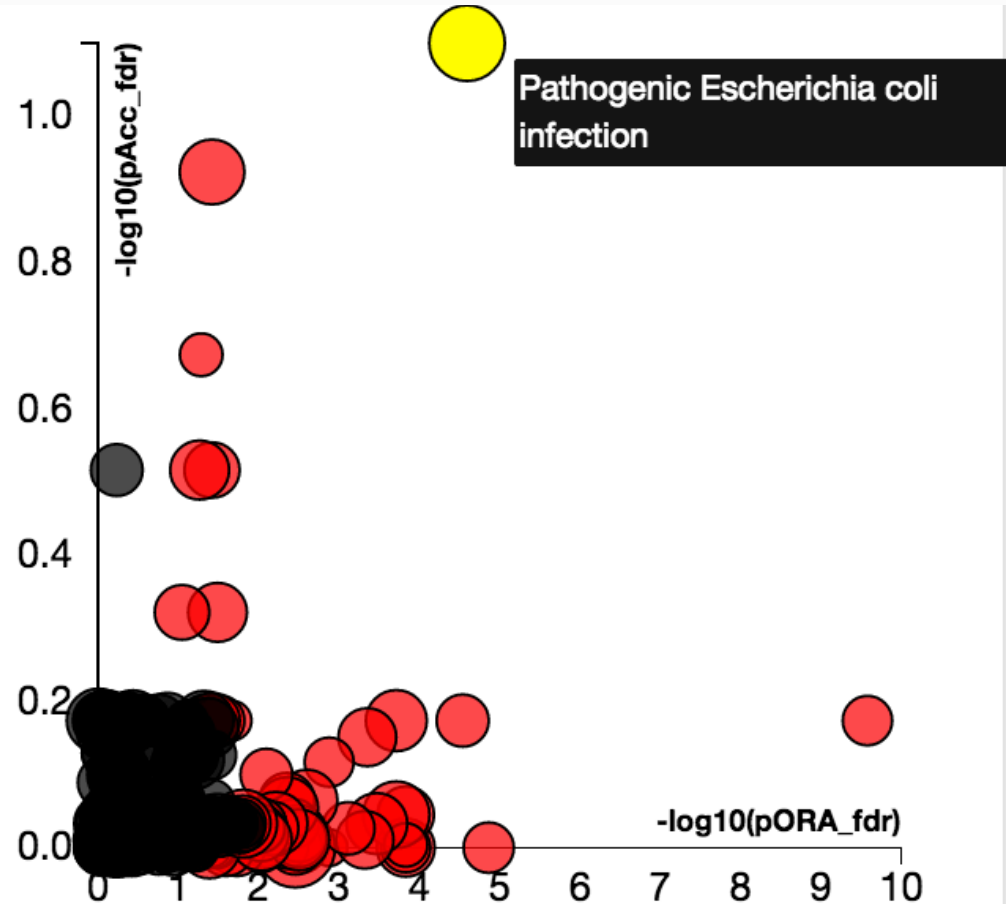
Correction factors: Bonferroni

- 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?**
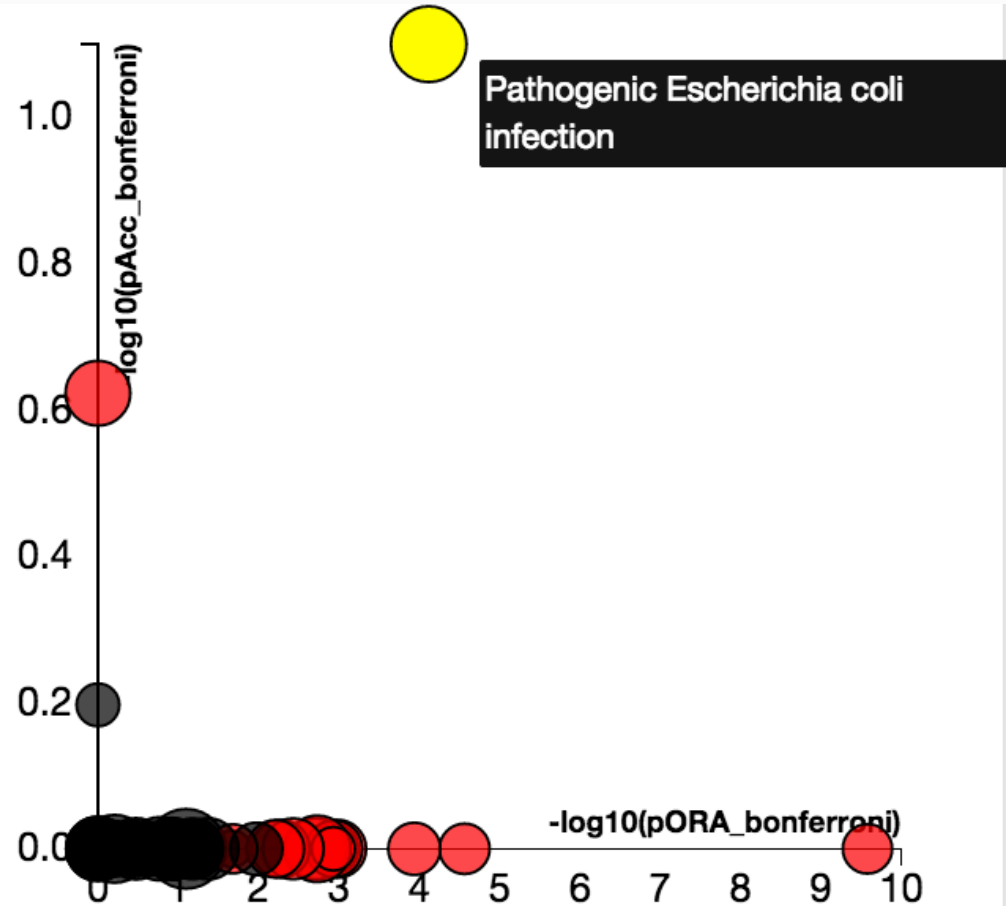
- Gene table lists all genes for the selected pathway

- Can be sorted and filtered

- Clicking on a row will highlight gene in pathway

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
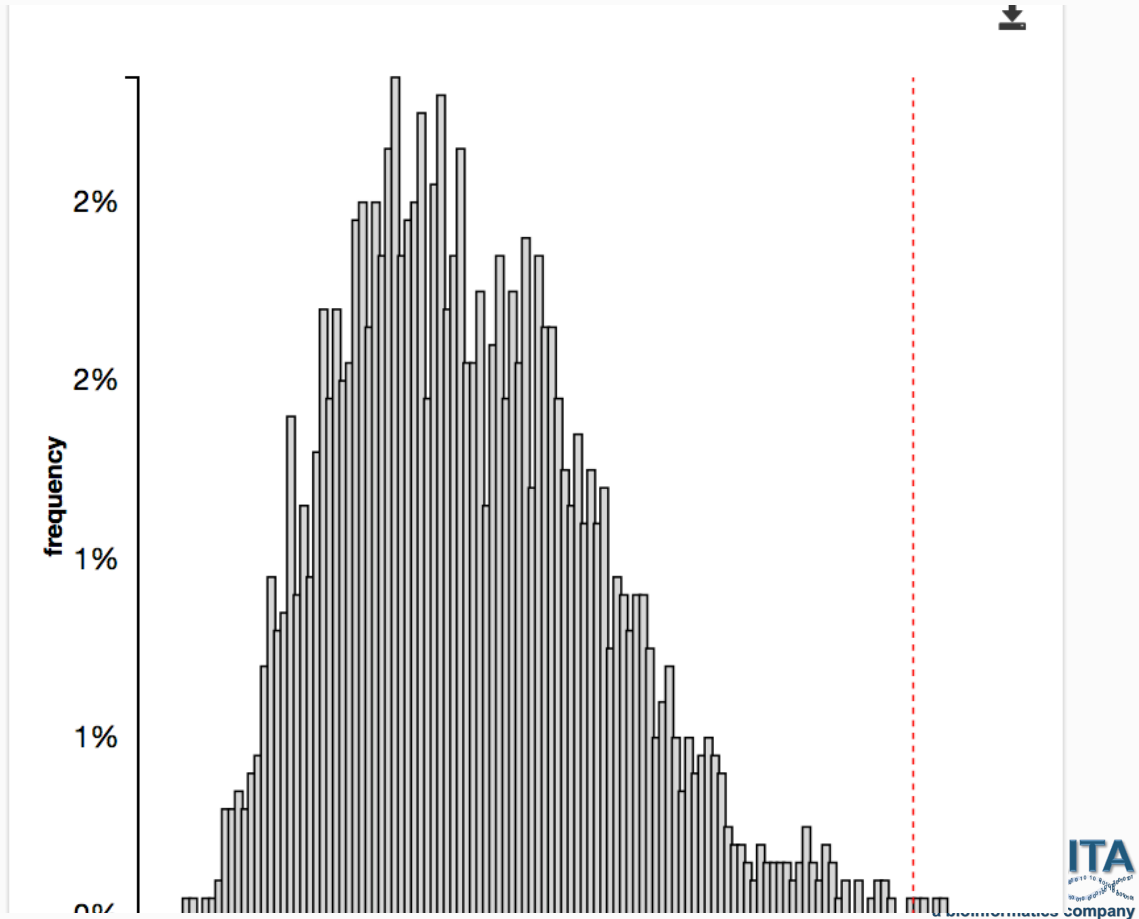
iPG: Pathway Details – Two-way Plot

- 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
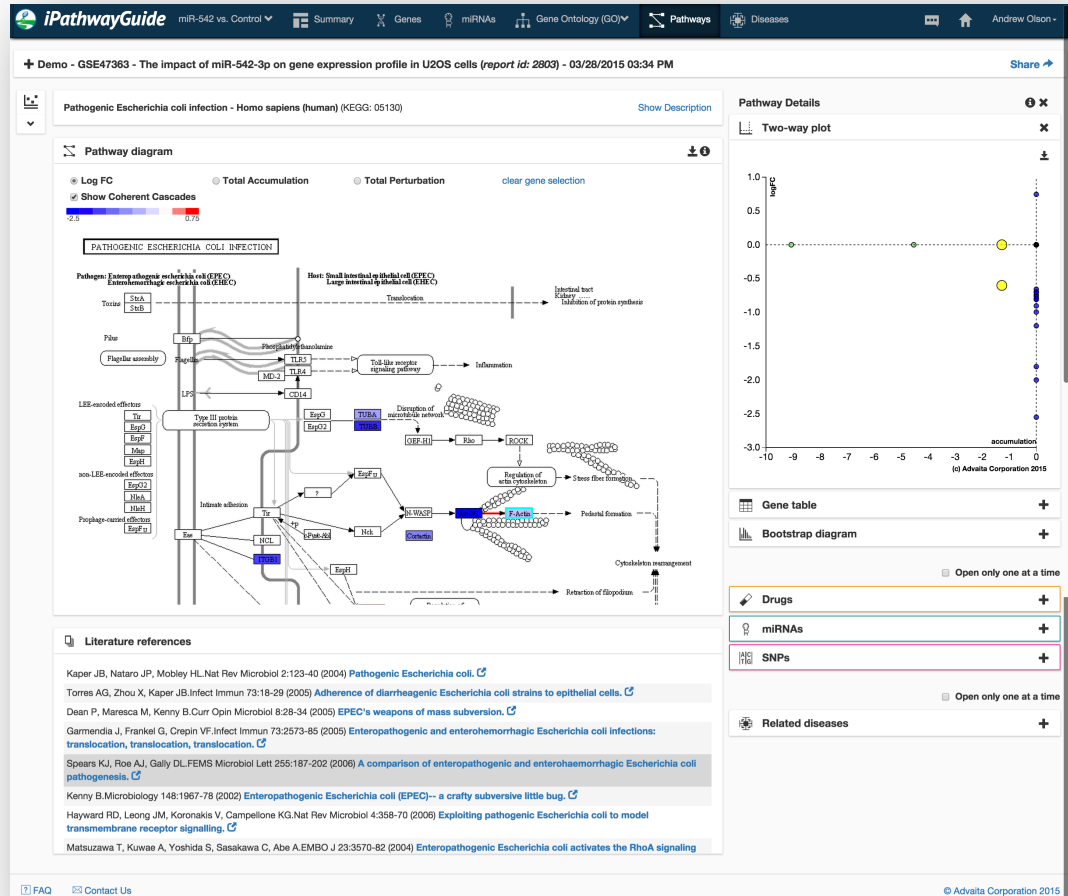
Pathway Details – Model miRNAs, SNPs, Drugs

- 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
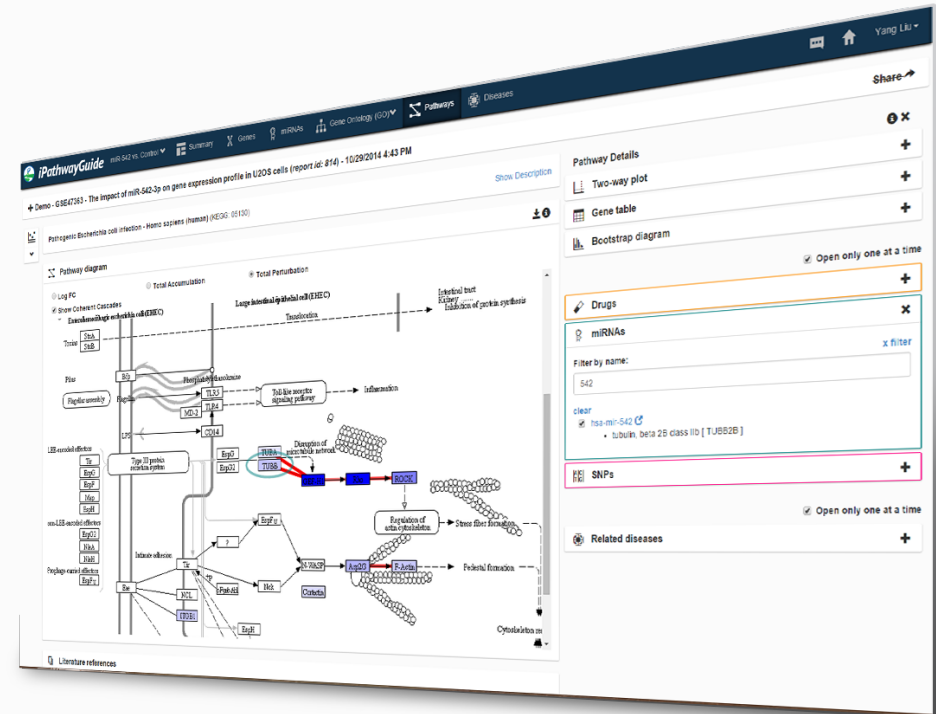
META-ANALYSIS OVERVIEW

- 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
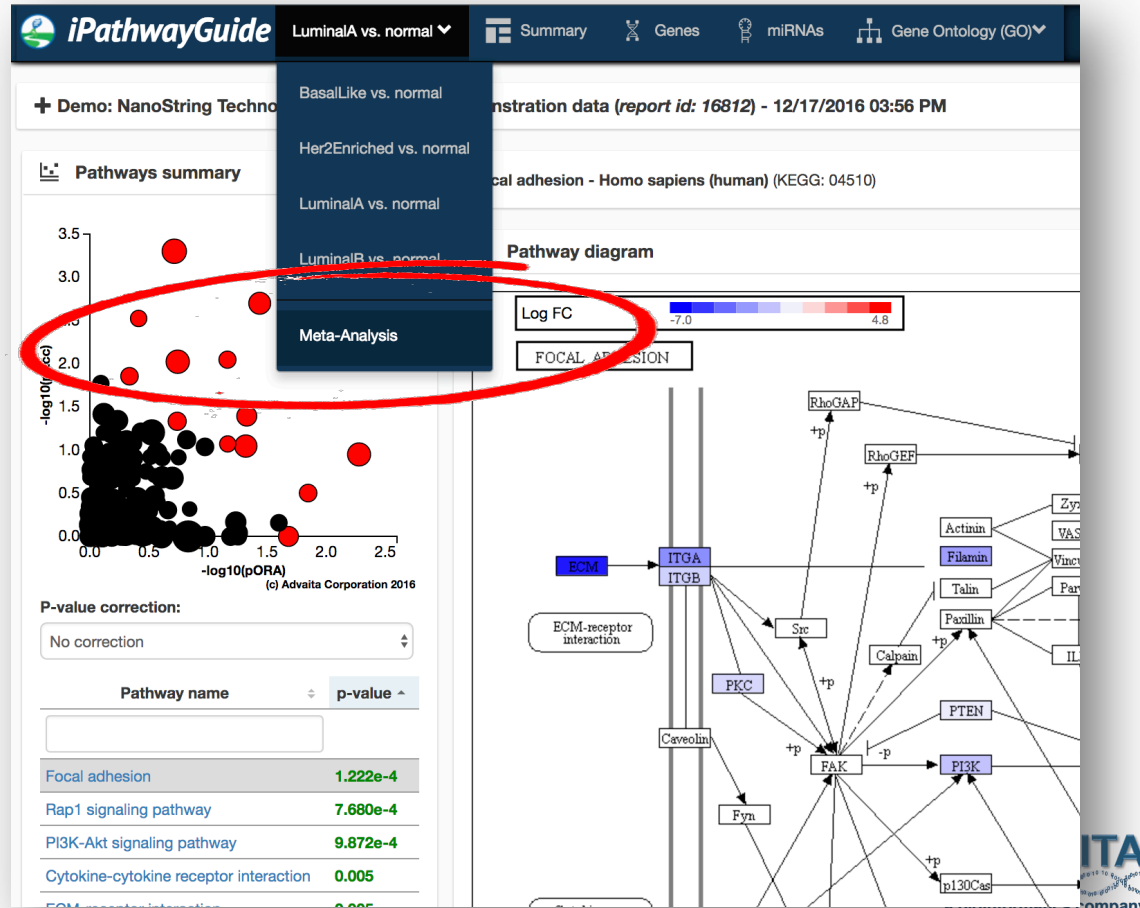
META-ANALYSIS OVERVIEW

- Quickly identify regions that interest you

- Build up a custom table

- See changes in expression by sorting
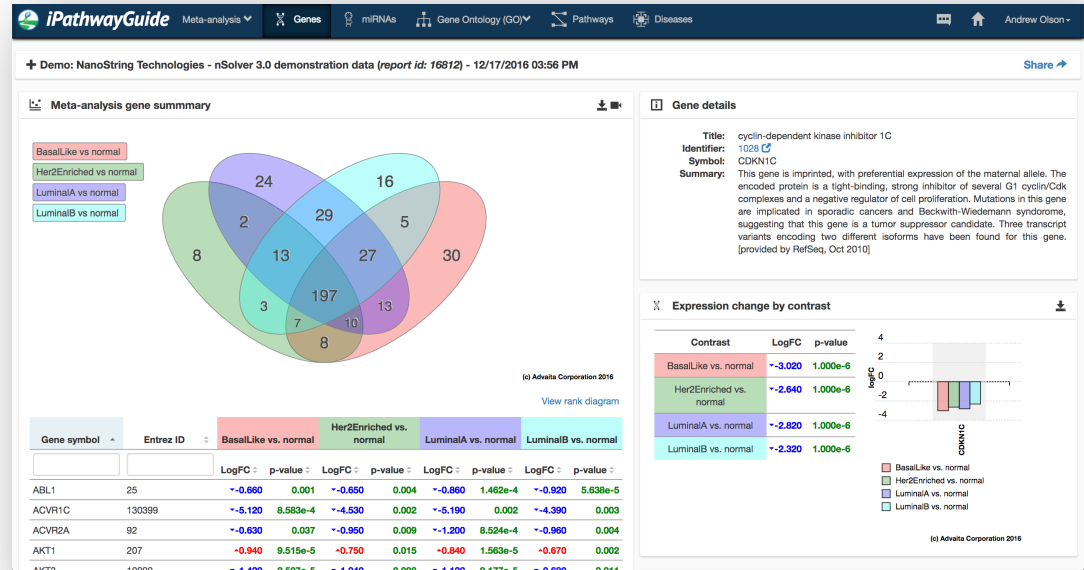
MIRNA INFERENCE (VIA META ANALYSIS)

- 22 miRNAs identified after FDR

- 1 after Bonferroni

- Only in Luminal A & B
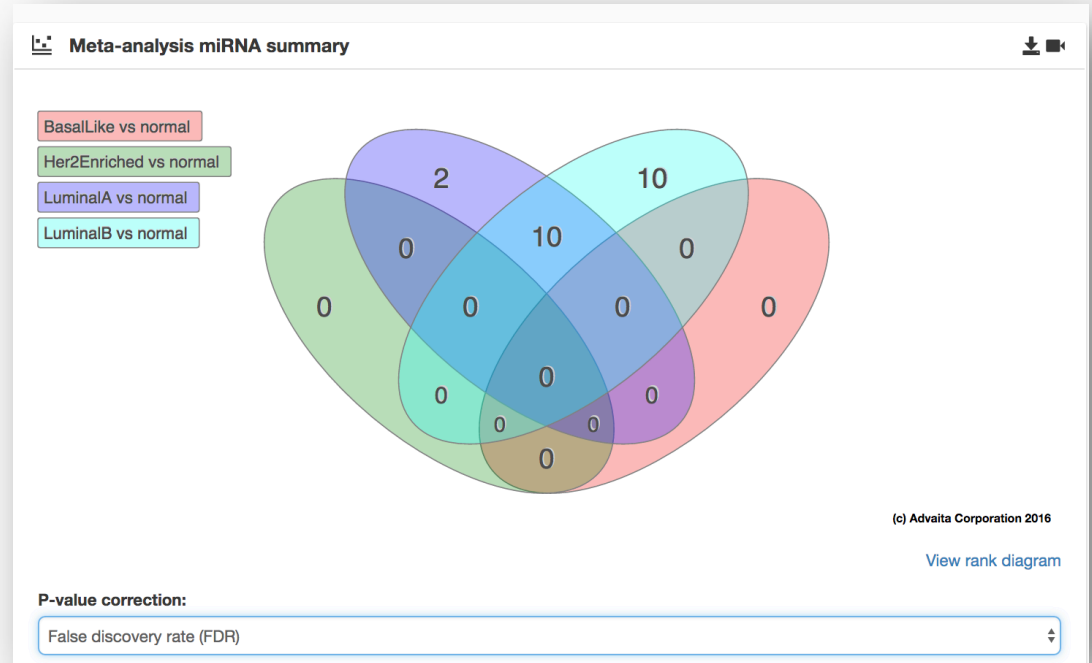
PATHWAYS (VIA META ANALYSIS)

- 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

Meta-analysis disease summary

BasalLike vs normal
Her2Enriched vs normal
LuminalA vs normal
LuminalB vs normal

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View rank diagram

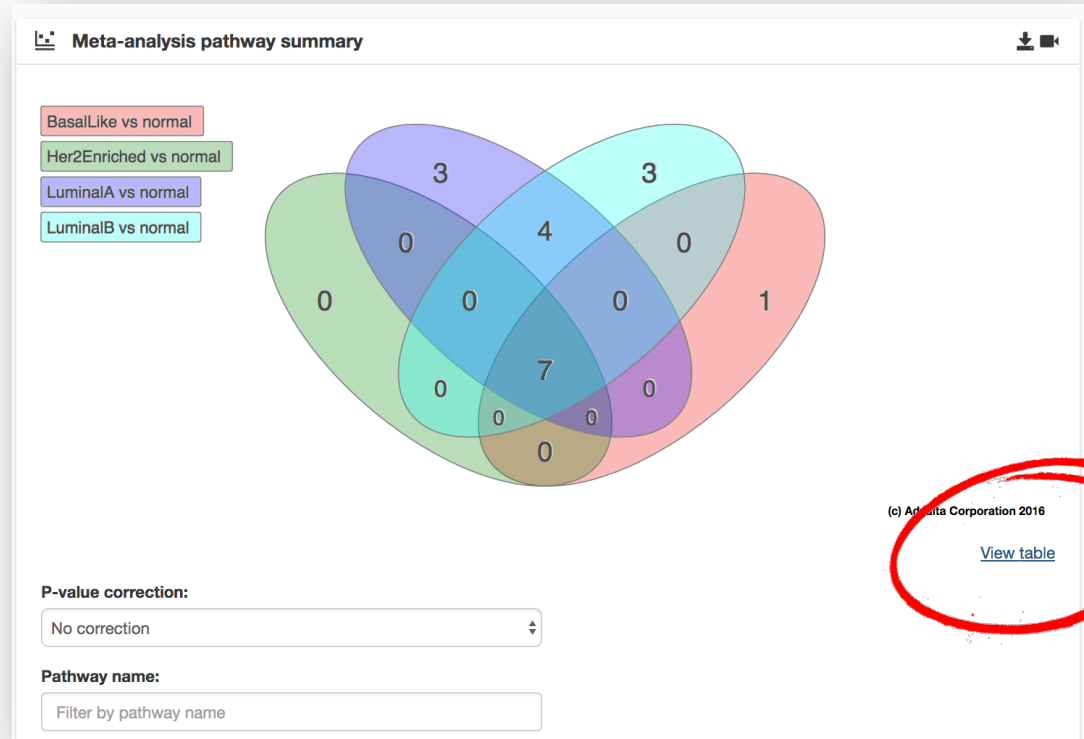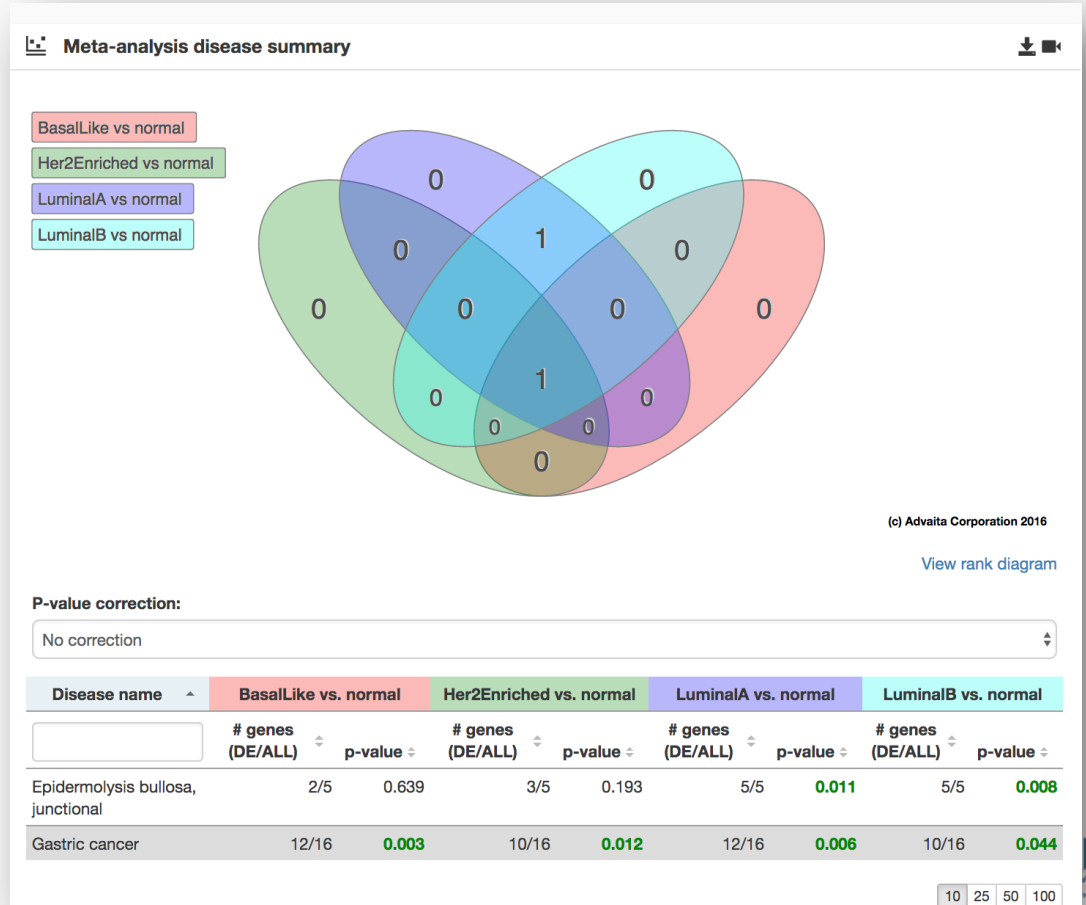**P-value correction:**

No correction

Disease name	BasalLike vs. normal		Her2Enriched vs. normal		LuminalA vs. normal		LuminalB vs. normal	
	# genes (DE/ALL)	p-value	# genes (DE/ALL)	p-value	# genes (DE/ALL)	p-value	# genes (DE/ALL)	p-value
Epidermolysis bullosa, junctional	2/5	0.639	3/5	0.193	5/5	0.011	5/5	0.008
Gastric cancer	12/16	0.003	10/16	0.012	12/16	0.006	10/16	0.044

10 25 50 100

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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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