# iPathwayGuide

Expression Analysis with iPathwayGuide

**ADVAITA**

a bioinformatics company

# Today's Goals

- Introduce alternative method for GX/PX data

- How to use iPathwayGuide

  – Uploading data

  – Select for DEGs/DEPs

  – Pathway Analysis

  – miRNA Prediction

  – GO Analysis

  – Disease Analysis

  – Meta Analysis

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# Today's Agenda

| | |
|---|---|
| 9:30a | Introduction to iPathwayGuide |
| 10:15a | Uploading Data |
| | *10 min break* |
| 11:05a | Demonstration: Pathway Analysis |
| | *30 min lunch* |
| 1:00p | Guided Analysis: Expression Analysis |
| | *10 min break* |
| 2:30p | On Your Own: Actionable Insights |

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# Housekeeping

- Cell phones to silent

- Sign-in Sheet with name & NIH email

- Ensure you have activated subscription

- Check email for demo datasets and shares

- We will not be submitting LIVE data today, but we will be sharing the results.

- About Advaita
- iPG Benefits
- HOW TO REGISTER WITH OSTR
- GETTING HELP
- Overview of Advaita's iPathwayGuide
- Introduce topology-based pathway analysis
- Q & A
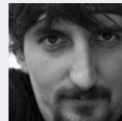
# Who is Advaita Bioinformatics

## Team Members



Sorin Draghici, Ph.D.
*President and CEO*

Andrew Olson, MBA
*Vice President
Business Development*

Calin Voichita, Ph.D.
*Director R&D*

Radu Vanciu, Ph.D.
*Software Developer*

Yang Liu, MS & MA
*UX Designer*

Sidra Ahsan, Ph.D.
*Product Manager*

Anukiran Solur, MSc
*Data Visualizations*

J. Banez, MA
*Controller*

Judes Tumuhairewe, MS
*Software Developer*

Cordelia Ziraldo, Ph.D.
*Product Manager*
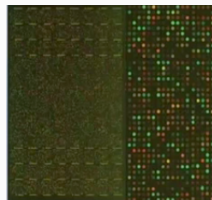
## Over 9,000 registered users!

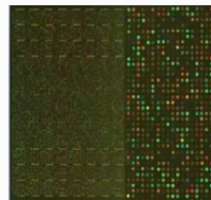# Bioinformatics pipeline



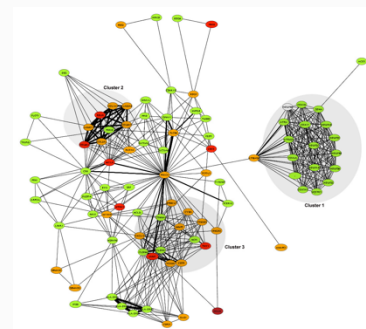**Transcriptomics Proteomics**

**Alignment Annotation**
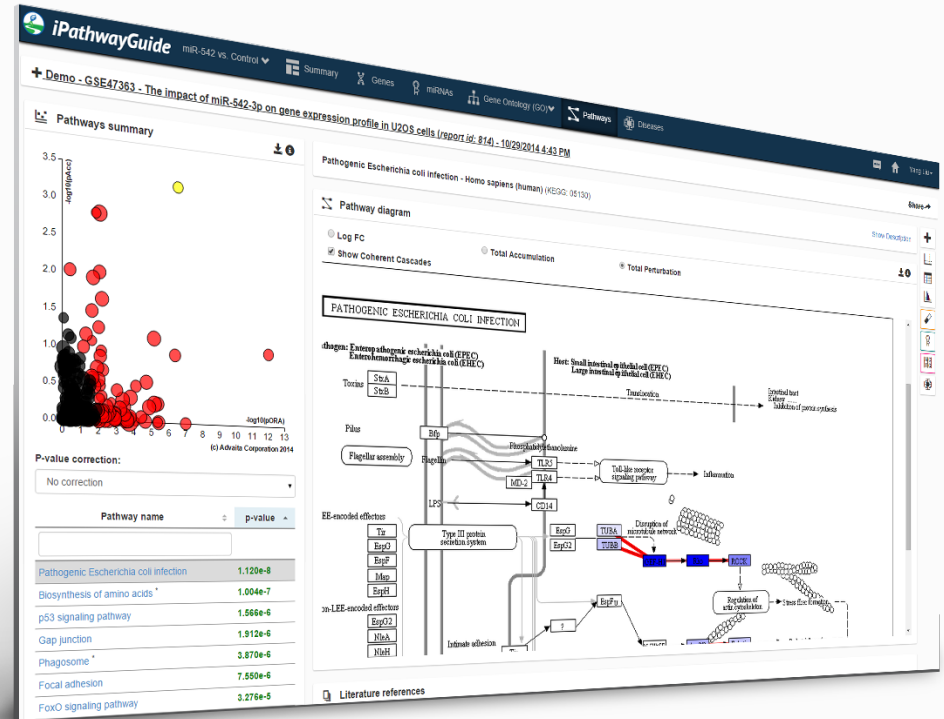
Control  Comparison

**Differential Expression**

**Pathway Analysis**

# 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
- Generate Hypotheses

# What can iPathwayGuide do for you?

| FEATURES | BENEFITS |
|---|---|
| • Most advanced methods | • Better results |
| • Open licensing | • Easy Collaboration |
| • Easy to use interface | • Shorter learning curve |
| • Systems biology approach | • Generate hypothesis |
| • Meta-Analysis -- Combine Omics reports | • Identify biomarkers and druggable targets |
| • Model miRNAs, drugs, and SNPs on pathways | • Elucidate molecular mechanisms |
| • Printable report summary | • Reduce time on reports |

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# How to Gain Access to iPathwayGuide



- Access Information: OSTR/Software

- You must submit a request through "NCI at Your Service" to obtain the access code before visiting the Advaita Bioinformatics website to register for access.

- iPathwayGuide is available to all researchers affiliated with the NCI. Once you have obtain the access code, please use the registration link below to initiate your user account (NCI affiliated e-mail address required).

# GETTING HELP

- QUICK-TIP VIDEOS (Instant learning)

- FEEDBACK BUTTON (24hour response)

- WEBSITE
  (http://www.advaitabio.com/support-ipathwayguide.html)

- CALL US (734-922-0110  -  9AM-5PM ET)

# Features of iPathwayGuide

- Summary

- Genes View

- Predicted miRNAs

- GO Analysis
  - Biological processes
  - Molecular functions
  - Cellular components

- **Pathway Analysis**
  (Drugs, SNPs, miRNAs)

- Diseases
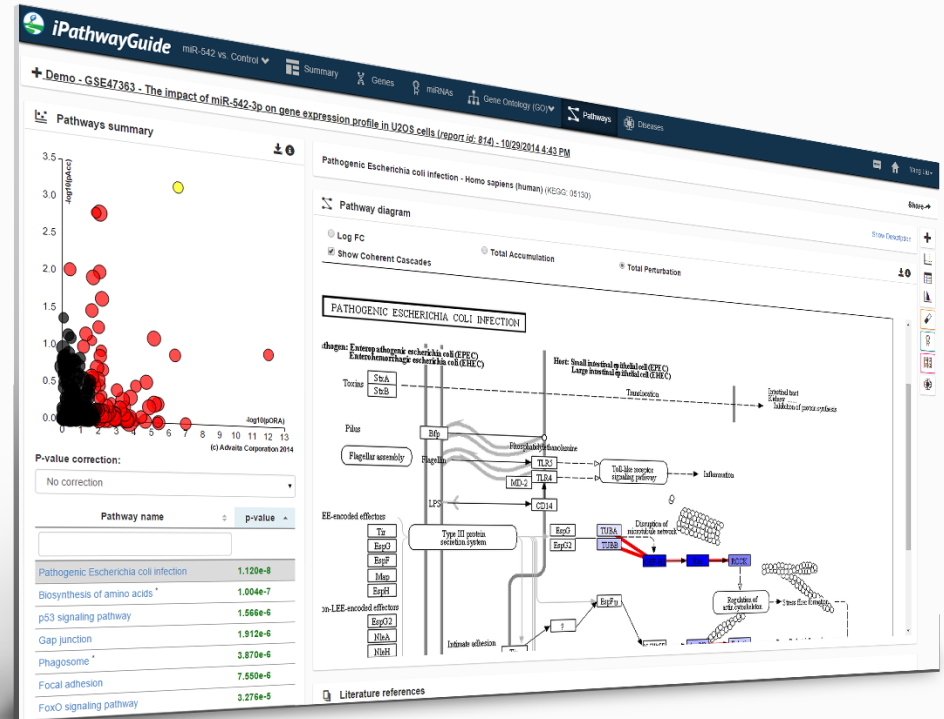
# DE Genes Summary

- DE genes identified using user-supplied thresholds

- log2FC & p-value

- Annotations for each genes to GO terms, pathways, miRNAs, SNPs, Drugs
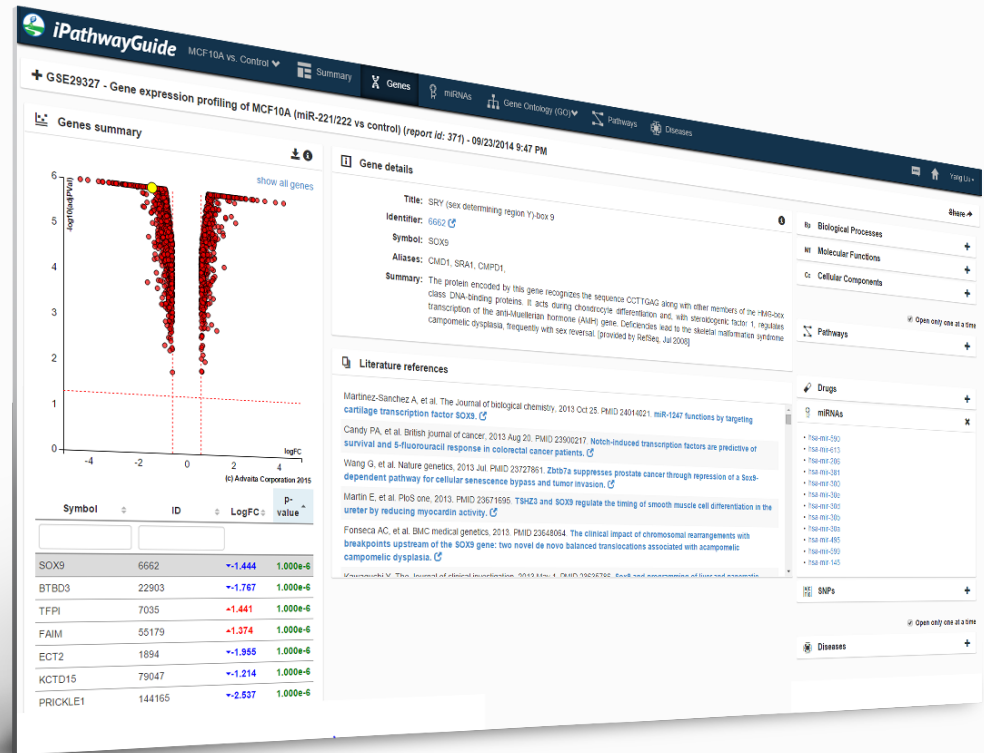
# Pathway Analysis

- 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
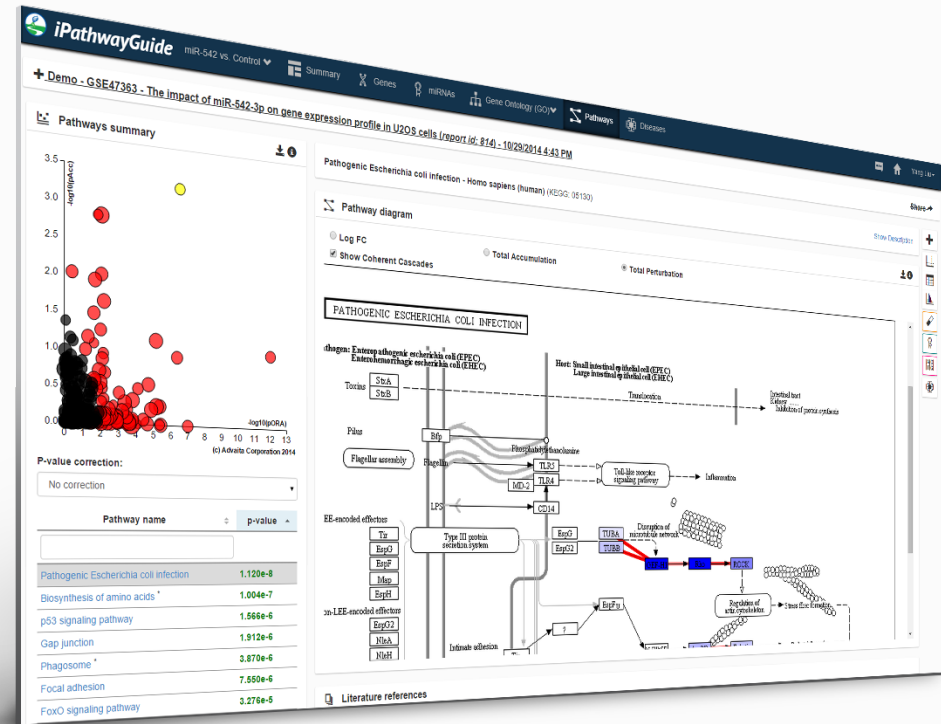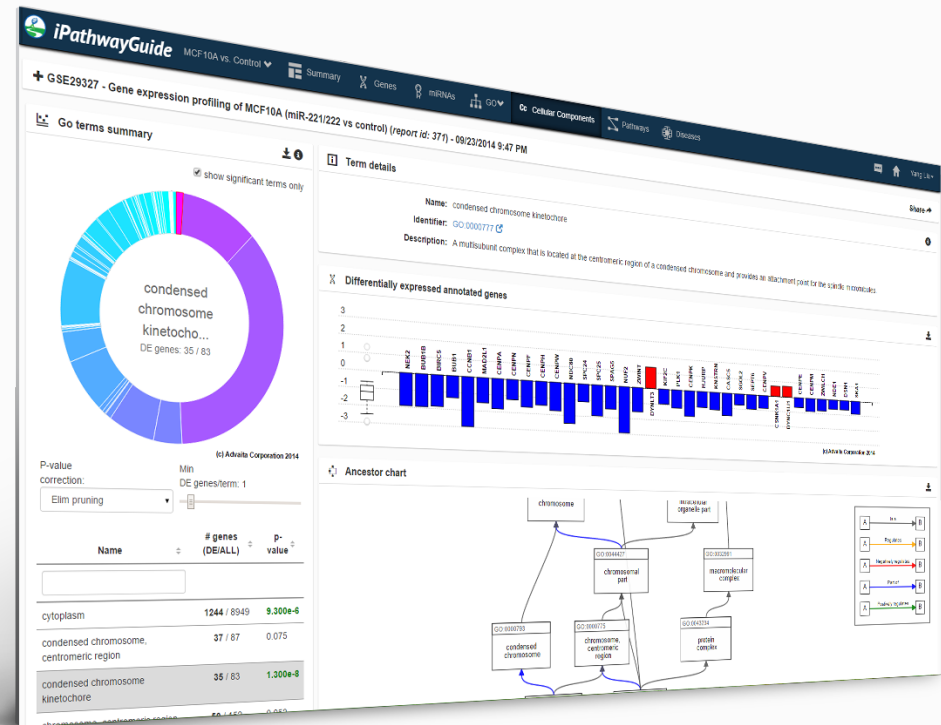
# miRNA Analysis

- Predict active miRNA base on expression signature

- Identify target genes and relevant expression profiles

- Generate hypotheses
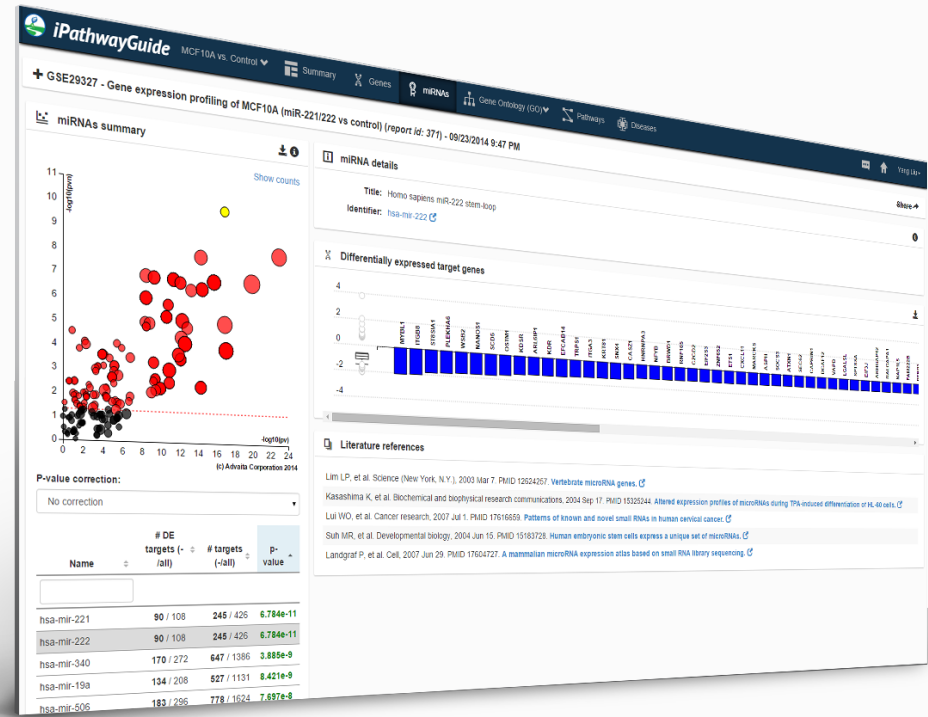
# GO Analysis

- Identify significant GO terms for:
    - Biological Processes
    - Molecular Functions
    - Cellular Components
- Identify target genes and relevant expression profiles
- Employ advanced correction factors like Elim and Weight
- Generate hypotheses

# Disease Analysis

- Identify diseases based on enrich annotated DE genes
- Identify target genes and relevant expression profiles
- Generate hypotheses
- Identify literature references
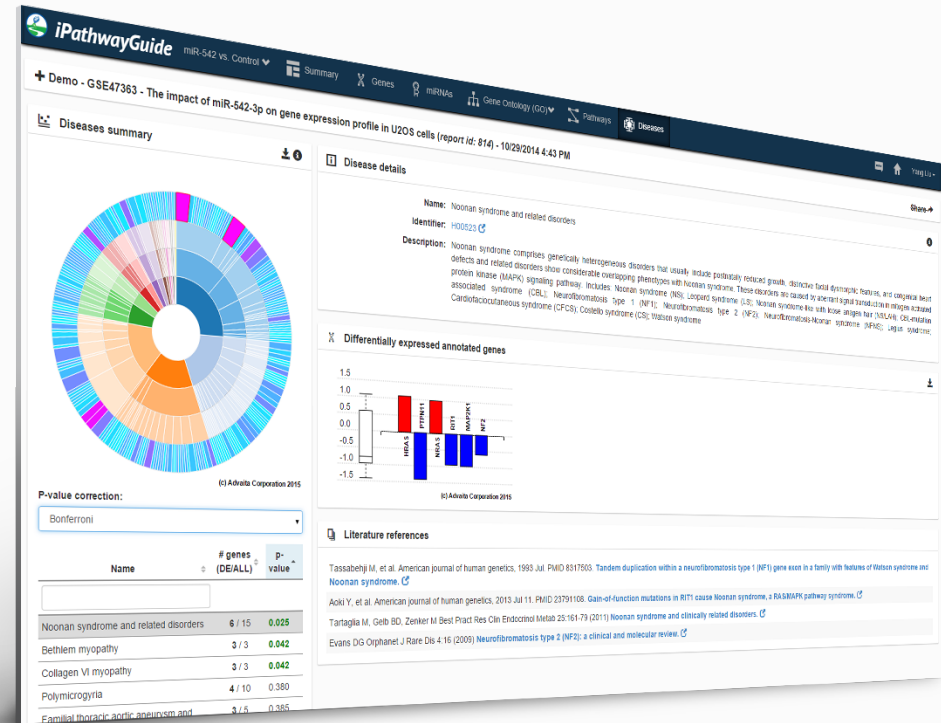
# iPG: Meta Analysis – Premium Feature

- Build and compare up to 5 datasets

- Combine proteomic and transcriptomic analyses

- Quickly identify common or unique traits

  - Venn Diagram

  - Sortable columns

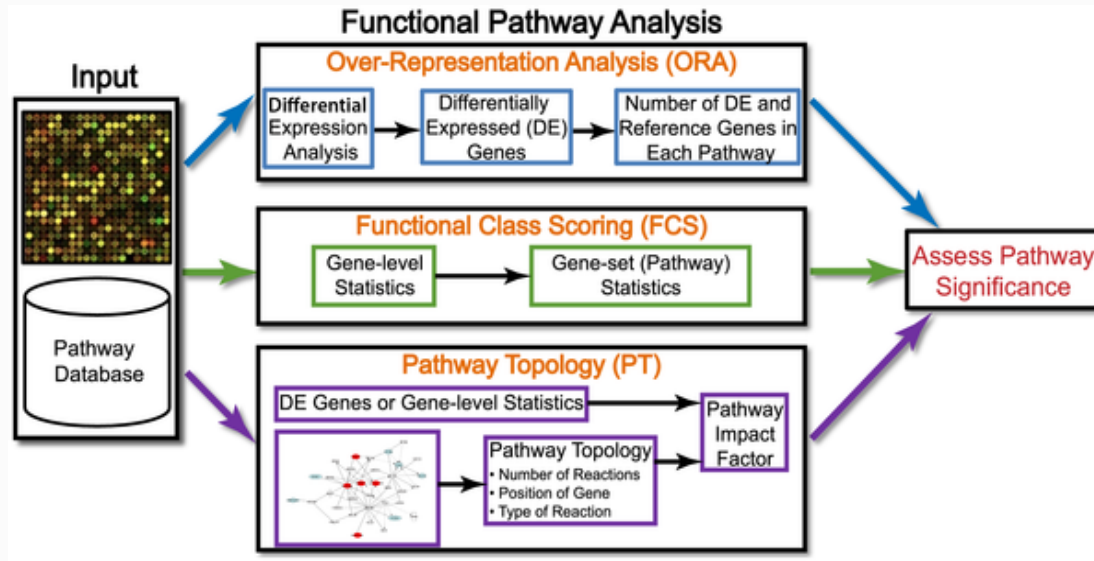  - Ranked table

  - Correction factors
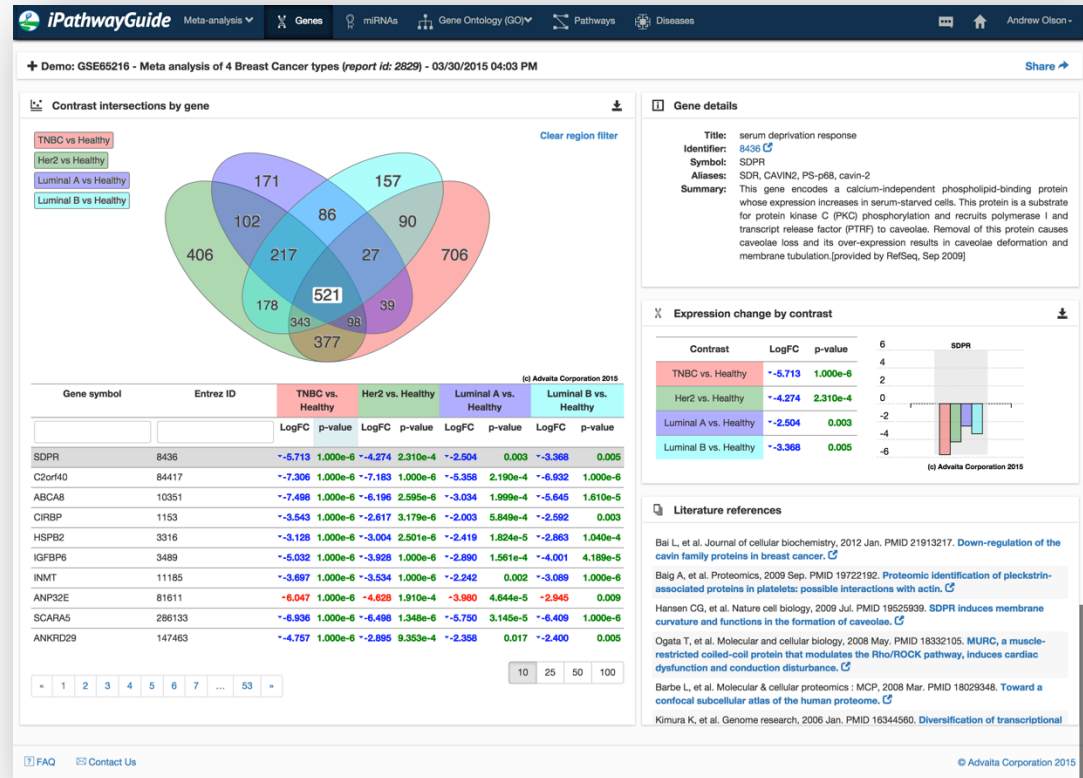
# iPG: Printable Summary – Premium Feature

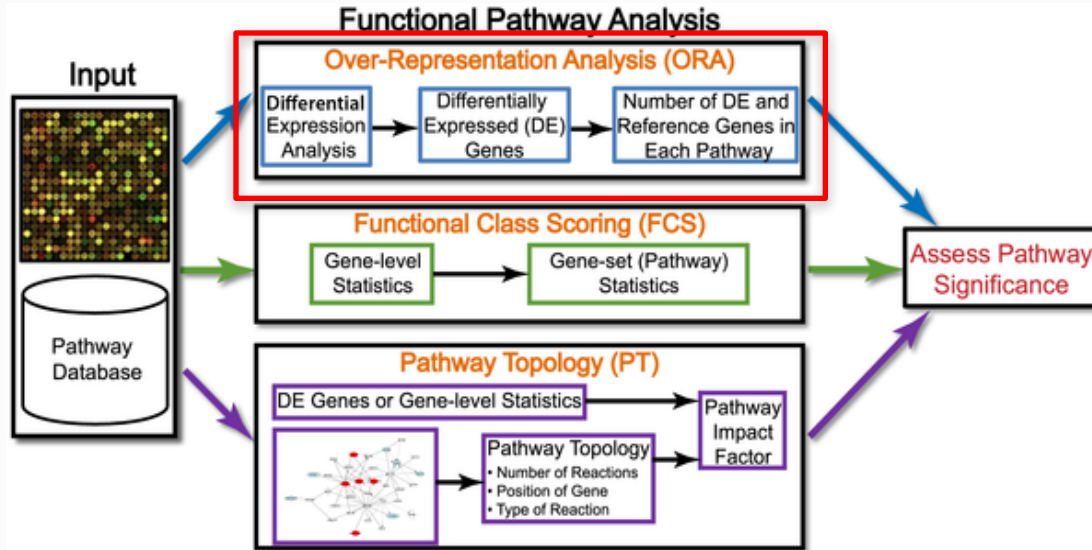Printable report summary provides descriptive report with:

- Top results for each category (based on *p*-value)

- Top genes for each analysis

- Detailed methods

- References

# Classical analysis approaches



*Khatri P et al., Plos Comp Biol, 2012*
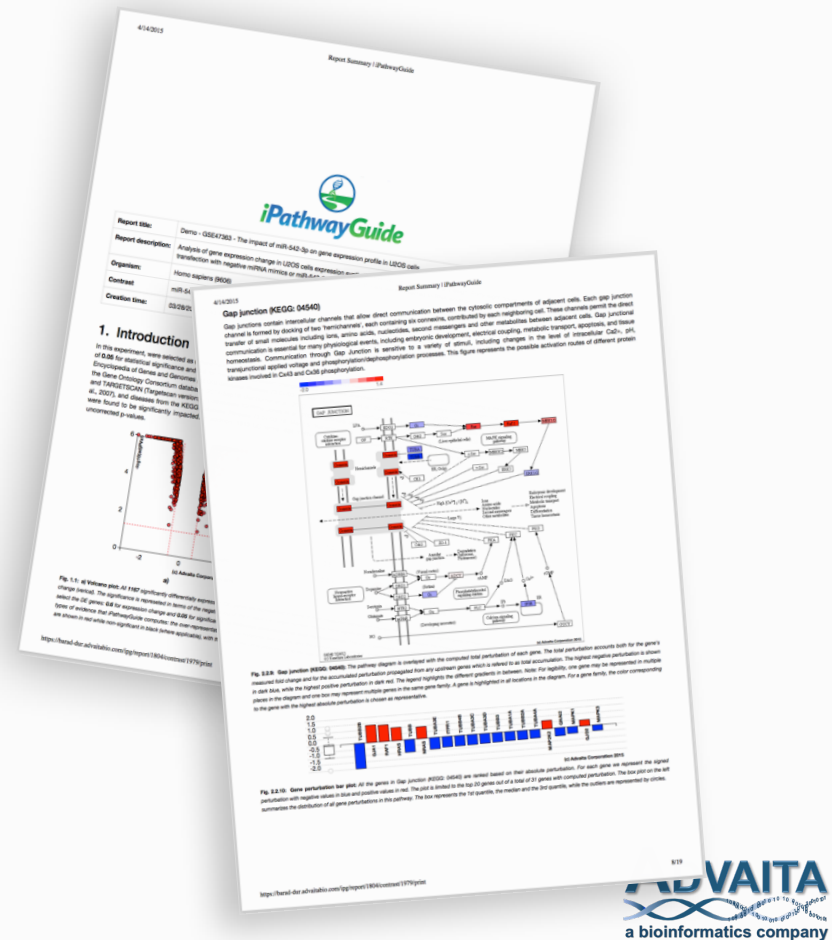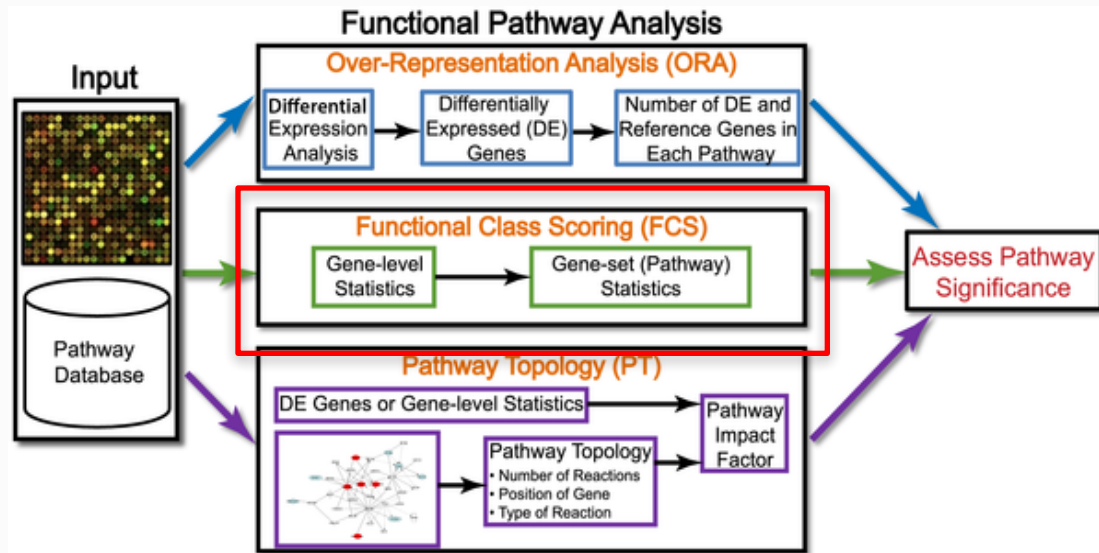
# Classical analysis approaches



**ORA/Enrichment**

**Pros**
- Simple
- Requires less input data

**Cons**
- Assumes all genes are independent
- Uses only significant genes, providing a "snapshot" view
- Many false positives

*Khatri P et al., Plos Comp Biol, 2012*

# Classical analysis approaches



**Functional Pathway Analysis**

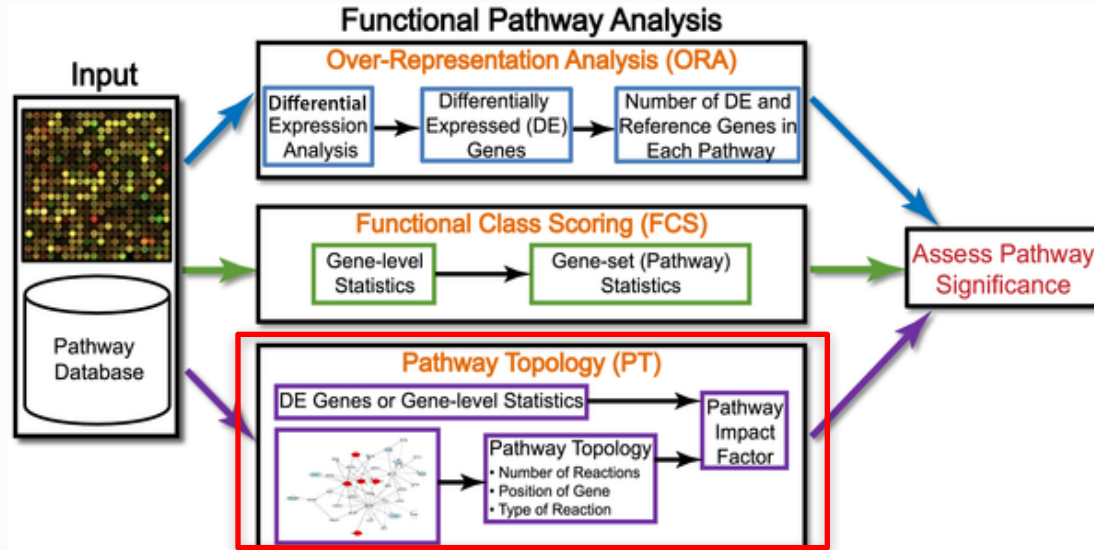**Functional Class Scoring**

**Pros**
- More accurate than ORA
- Uses entire list of genes measured

**Cons**
- Assumes all genes are independent
- Analyzes each pathway independently
- Many false positives

*Khatri P et al., Plos Comp Biol, 2012*
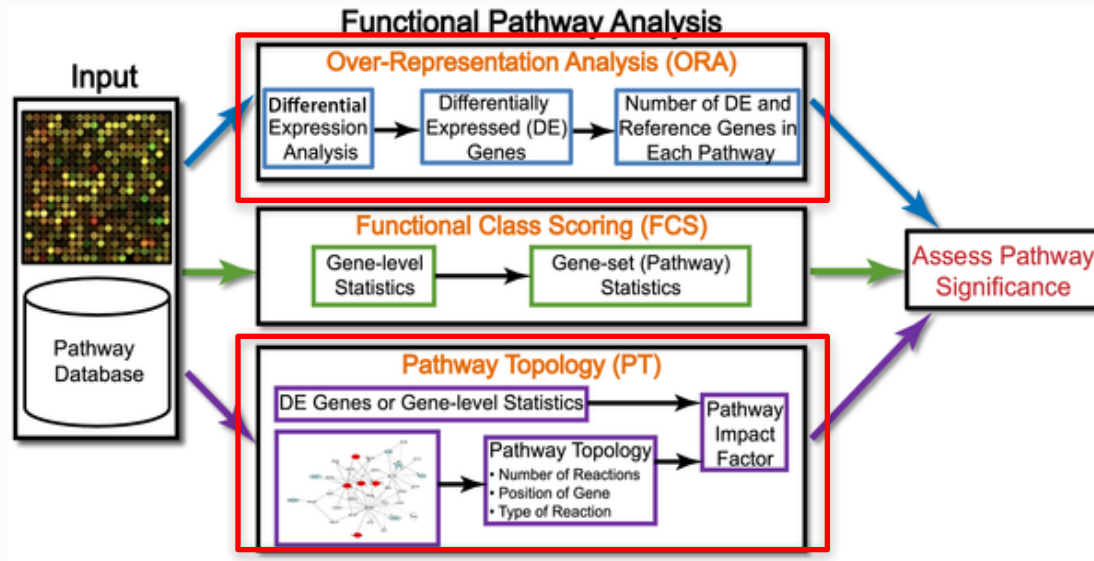
# Classical analysis approaches



**Pathway Topology**

**Pros**
- Considers role and position
- Able to "interpolate"

**Cons**
- Cross-talk between pathways
- Requires more data
- Takes slightly longer

*Khatri P et al., Plos Comp Biol, 2012*

# Classical analysis approach
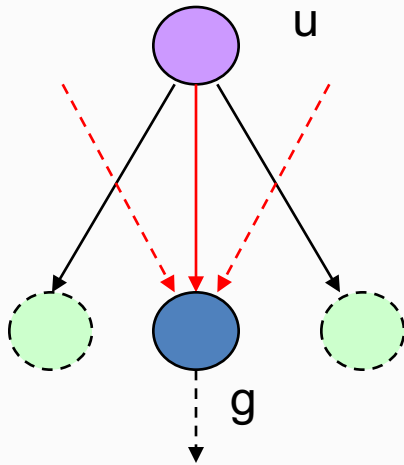


iPathwayGuide combines **both** methods.

Other analytical tools only use **one** form of evidence (ORA/FCS).

*Khatri P et al., Plos Comp Biol, 2012*

# iPG Approach: Impact Analysis

## Two forms of evidence

✓ Classical enrichment

✓ Perturbation Analysis

$$PF(g) = \alpha(g) \cdot \Delta E(g) + \sum_{u \in US_g} \beta_{ug} \frac{PF(u)}{N_{ds}(u)}$$

$PF(g)$ – perturbation factor of gene g
$\alpha$ - a priori type of impact expected from that gene
$\Delta E(g)$ – change in expression level of gene g
$US_g$ – set of genes directly upstream of g
$N_{ds}(u)$ - number of genes directly downstream of u
$\beta_{ug}$ - efficiency of the connection between u and g

Genome Res. 2007; 17(10): 1537-1545

- Impact Analysis™
- Combines two types of evidence:
  - Perturbation ($P_{Acc}$)
  - Enrichment ($P_{ORA}$)
- Combines *p*-values using Fisher's method
- Values to upper right of diagonal line are significant
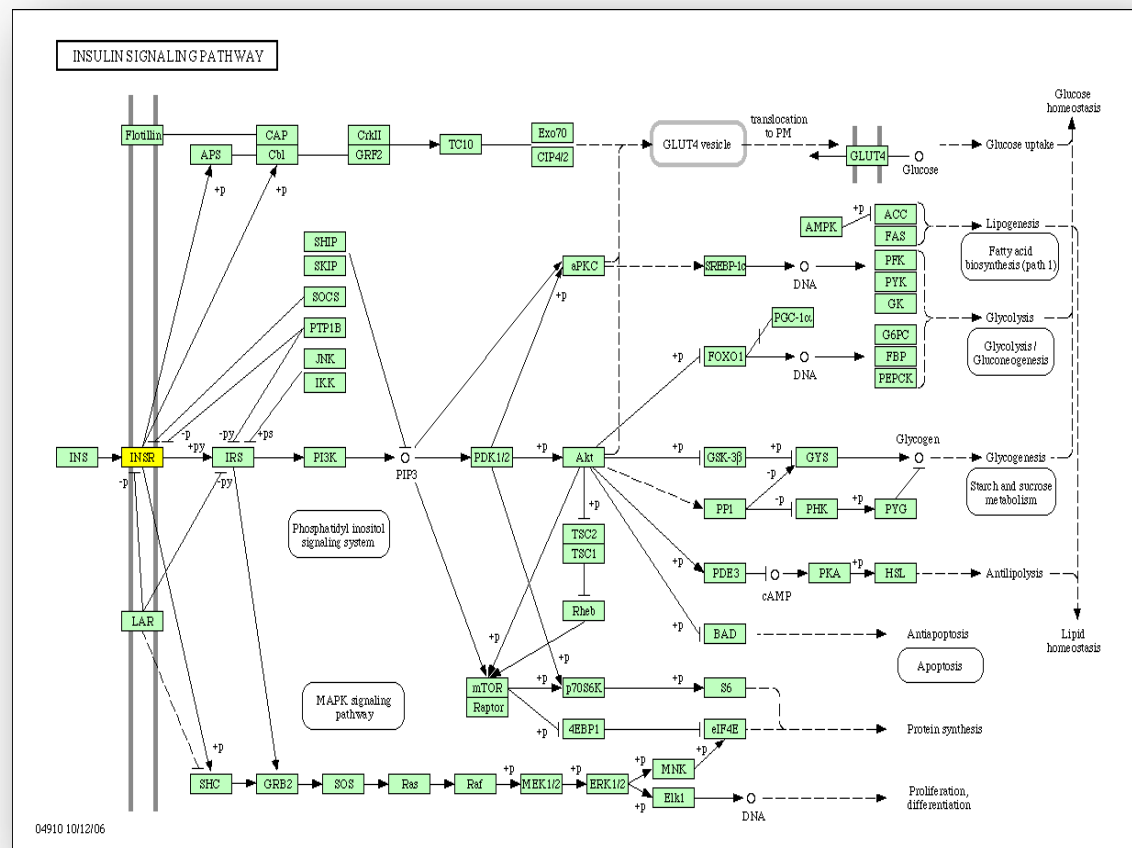


Combined Significance Threshold
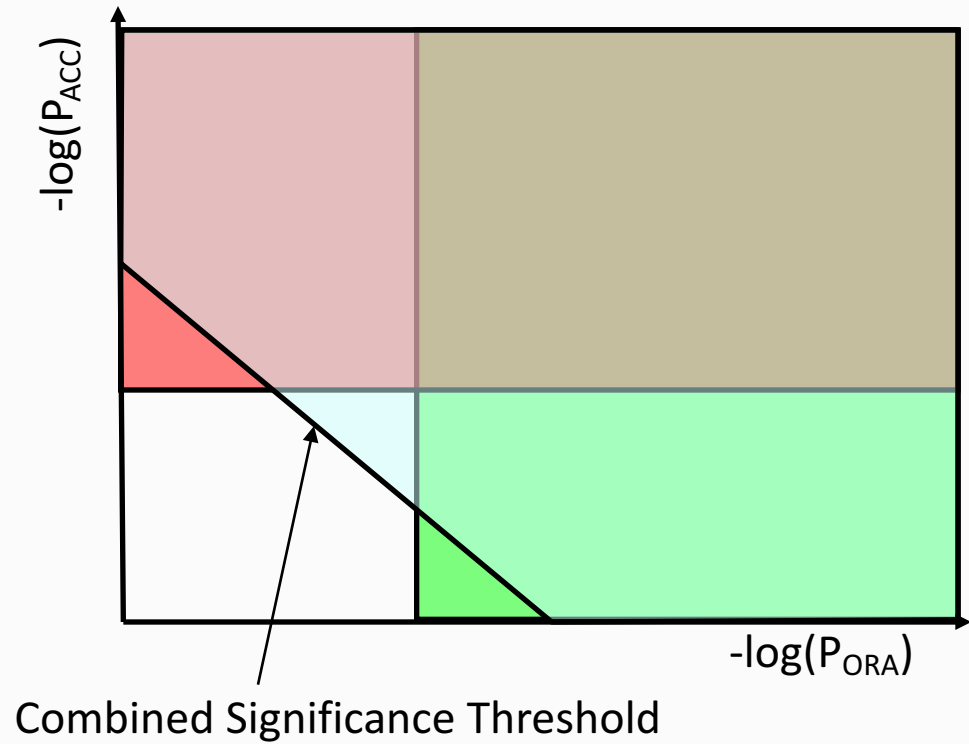
# Enrichment vs. Impact Analysis

INSR (Insulin signaling receptor) is key gene in this pathway (Insulin signaling)

Enrichment does not consider the role of this key gene on the pathway

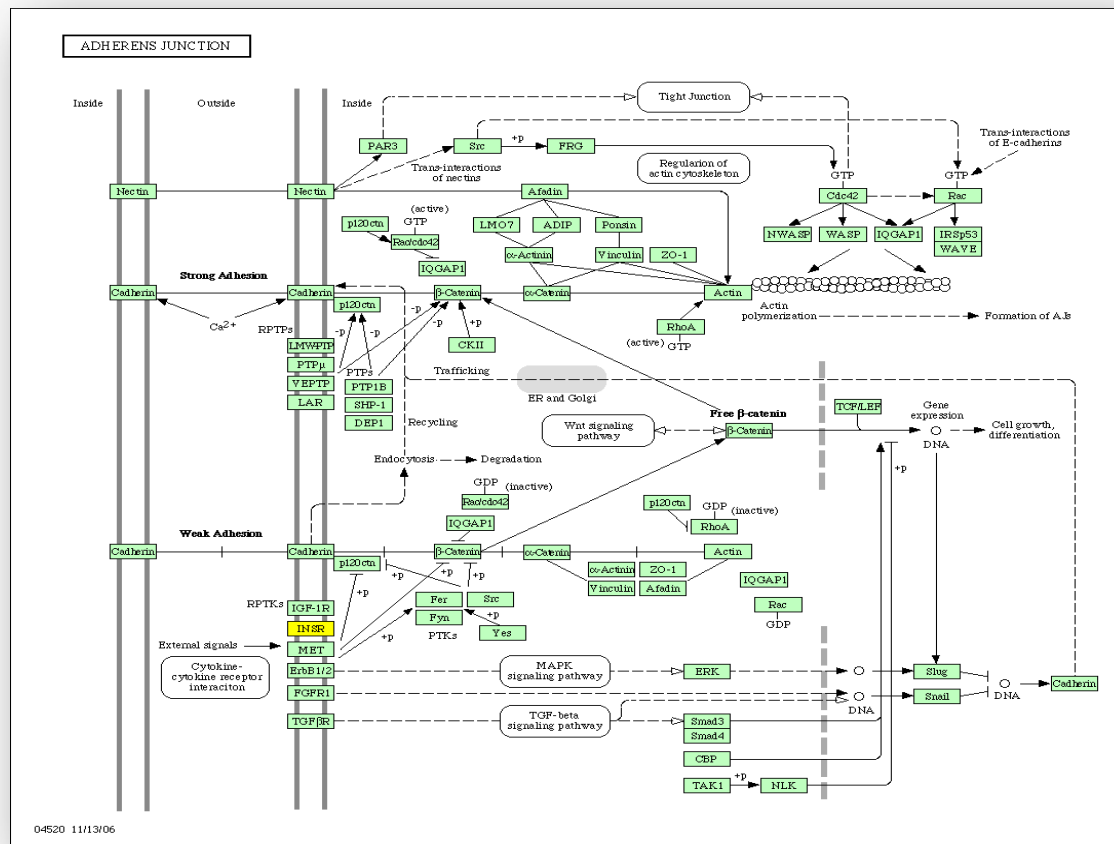Impact Analysis will identify this as the key entry point to the system

# Enrichment vs. Impact Analysis

INSR is one of many tyrosine kinase receptors on this pathway

Enrichment will score this pathway the same as Insulin Signaling pathway (1 DE-gene)

Impact Analysis will recognize this gene has less of a role on this pathway



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# iPathwayGuide Data Requirements

## User Input:

- <u>Entire</u> list of genes (NOT just DEGs)

- Log fold change

- *p*-values (corrected values recommended)

**Organisms:**
- ✓ Human
- ✓ Mouse
- ✓ Rat

**Supported file formats:**
- ✓ Affy CEL files
- ✓ CuffDiff
- ✓ DE Seq(2)
- ✓ EdgeR
- ✓ JMP Genomics
- ✓ GEO2R/Limma
- ✓ nSolver (NanoString)
- ✓ Sciex Protein Assembler
- ✓ Custom Tab delimited file (*.txt)
- ✓ Protein ID's Coming Soon

# Stop Point
# Questions

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