

iPathwayGuide

Expression Analysis with
iPathwayGuide



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

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

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.

Agenda: 01. Introduction

- 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



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*Vice President
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J. Banez, MA
Controller



Judes Tumuharewe, MS
Software Developer



Cordelia Ziraldo, Ph.D.
Product Manager

Over 9,000 registered users!



Bioinformatics pipeline



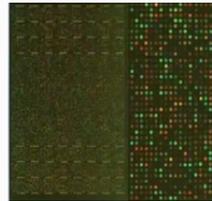
**Transcriptomics
Proteomics**



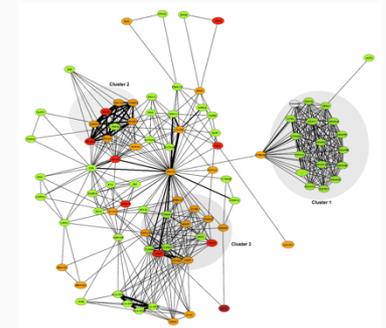
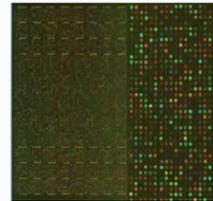
**Alignment
Annotation**



Control



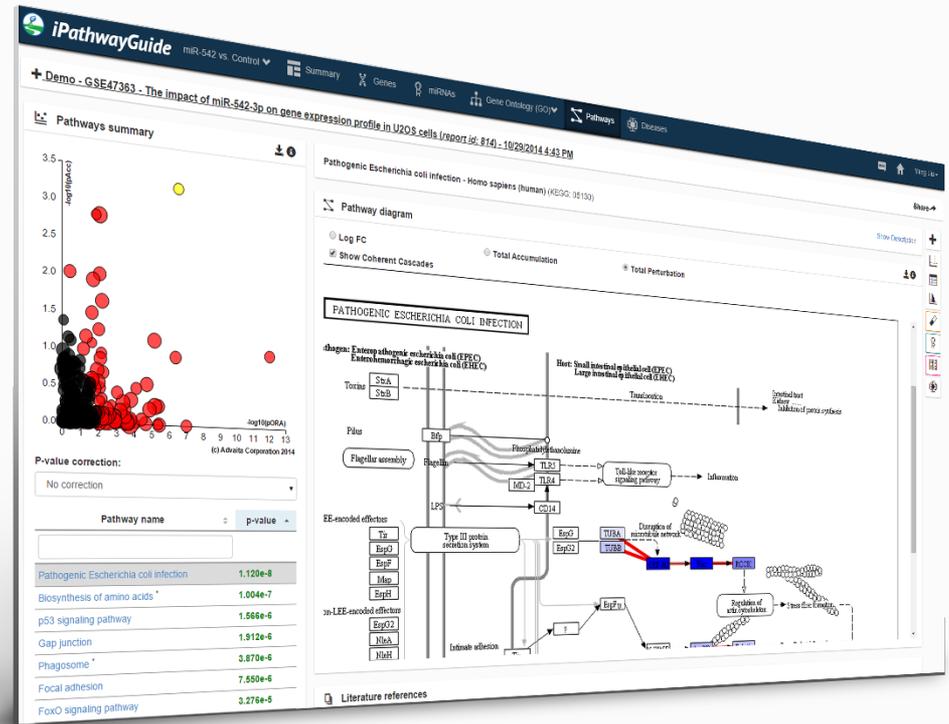
Comparison



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

- Most advanced methods
- Open licensing
- Easy to use interface
- Systems biology approach
- Meta-Analysis – Combine Omics reports
- Model miRNAs, drugs, and SNPs on pathways
- Printable report summary

BENEFITS

- Better results
- Easy Collaboration
- Shorter learning curve
- Generate hypothesis
- Identify biomarkers and druggable targets
- Elucidate molecular mechanisms
- Reduce time on reports

How to Gain Access to iPathwayGuide

The screenshot shows the 'Scientific Software' page on the NCI website. A red circle highlights the 'Advaita iPathwayGuide' link in the list of software tools. A red arrow points from this link towards the text on the right side of the slide.

NIH NATIONAL CANCER INSTITUTE Center for Cancer Research Resources Partnerships Bioinformatics Developing Technologies About STARS Request

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OSTR Office of Science and Technology Resources

Scientific Software

Home / Bioinformatics / Training and Education (BTEP) > Scientific Software

To facilitate access to the latest bioinformatics tools and resources, the OSTR has developed agreements with a variety of bioinformatics companies that provide software packages to analyze genomic and proteomic data. These tools are meant to be accessible to the majority of CCR investigators and NCI contractor employees engaged in collaborative arrangements with NCI investigators.

CCR Bioinformatics/Bioinformatics Toolbox - Access to software for the analysis of gene expression, promoter analysis, array CGH, genetics, signal transduction pathways, and genomics analysis:

- Partek Genomics Suite
- Ingenuity Pathway Analysis (IPA)
- **Advaita iPathwayGuide**
- Genomatix Suite and the GGA/GMS platform for Next Generation Sequencing data analysis
- GeneSpring Microarray and arrayCGH software
- Geneious Pro

Publications Facilitated by OSTR

September 28th, 2016
MET signaling in keratinocytes activates EGFR and initiates squamous carcinogenesis: Yuspa Lab

August 12th, 2016
Capillary nano-immunoassays: advancing quantitative proteomics analysis, biomarker assessment, and molecular diagnostics: Jessie Chen, Ph.D. (Collaborative Proteomics Technology Resource)

June 18th, 2015
An Apella RNA-Containing Negative Feedback Loop Regulates p53-Mediated Apoptosis in Embryonic Stem Cells: J. Huang Lab

Calendar of Events

No upcoming events
View the Full Calendar

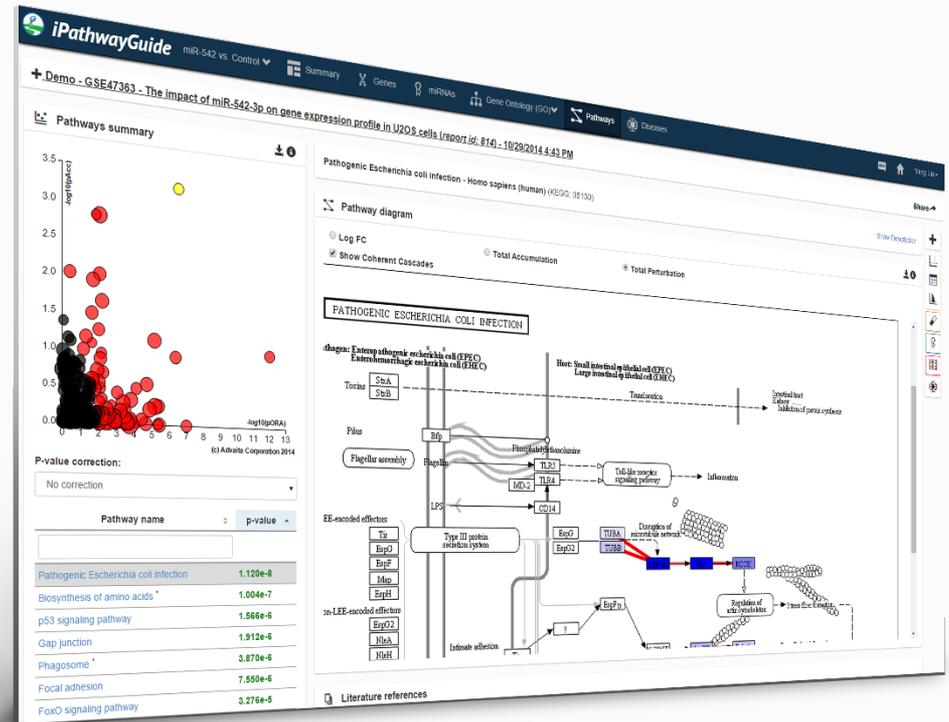
- Access Information: OSTR/Software
- You must submit a request through "**NCI at Your Service**" to obtain the access code before visiting the Advaita Bioinformatics website to register for access.
- iPathwayGuide is available to all researchers affiliated with the NCI. Once you have obtained the access 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)

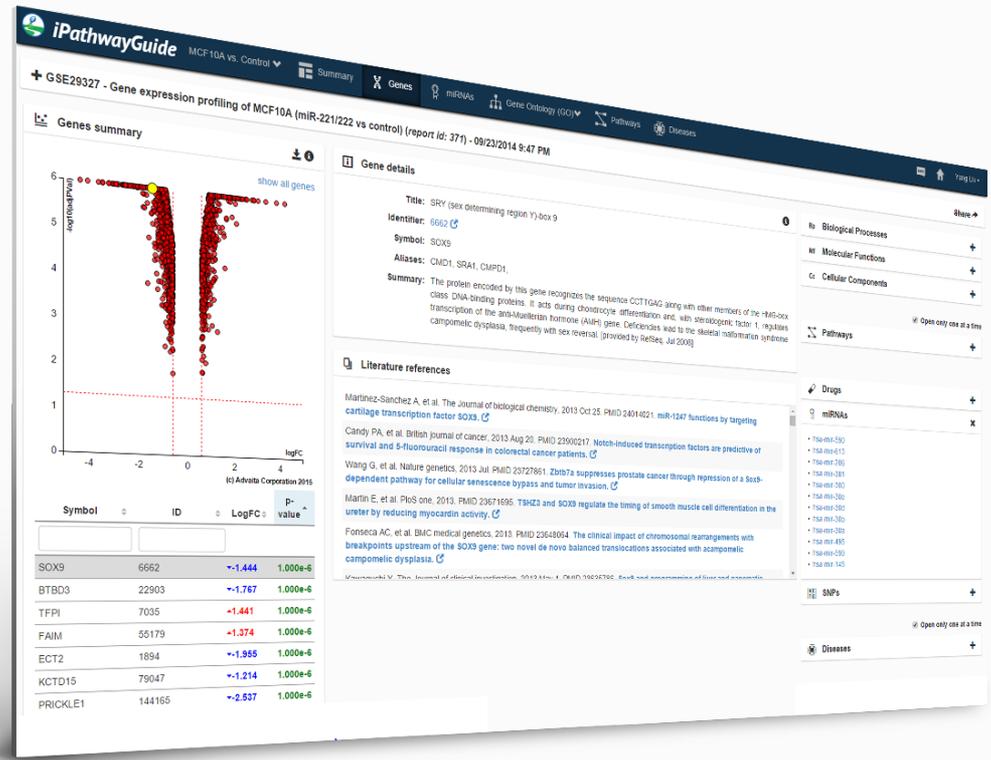
Features of iPathwayGuide

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



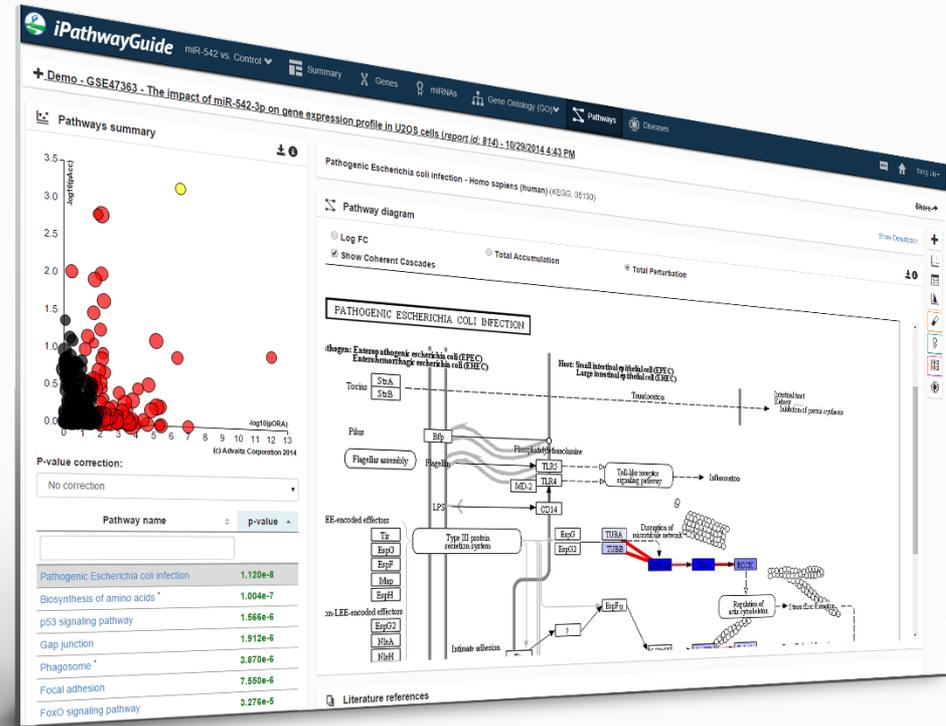
DE Genes Summary

- DE genes identified using user-supplied thresholds
- log2FC & p-value
- Annotations for each genes to GO terms, pathways, miRNAs, SNPs, Drugs



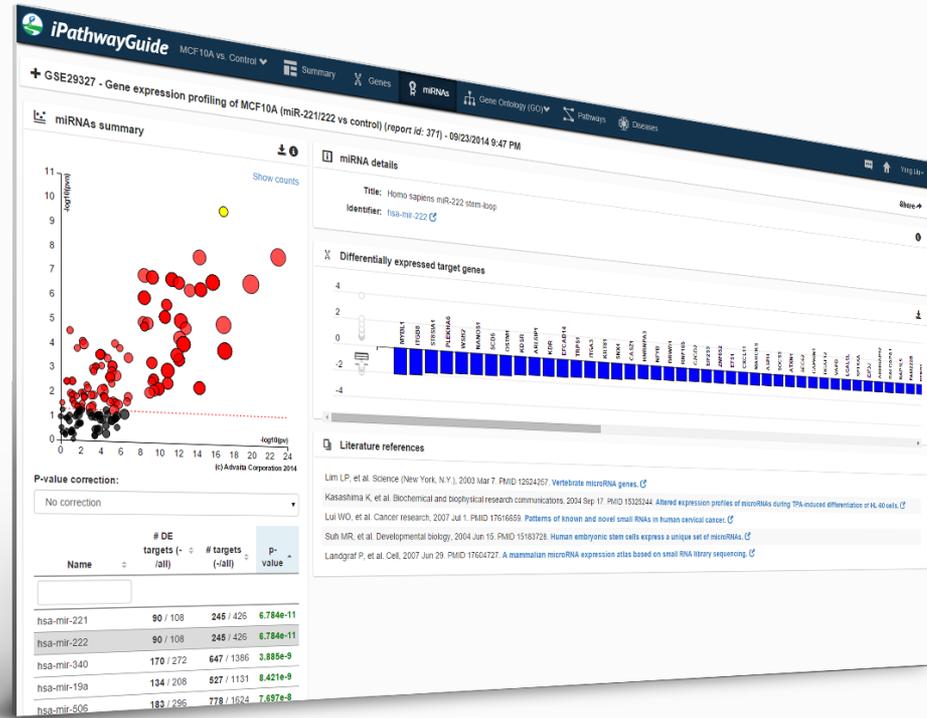
Pathway Analysis

- 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



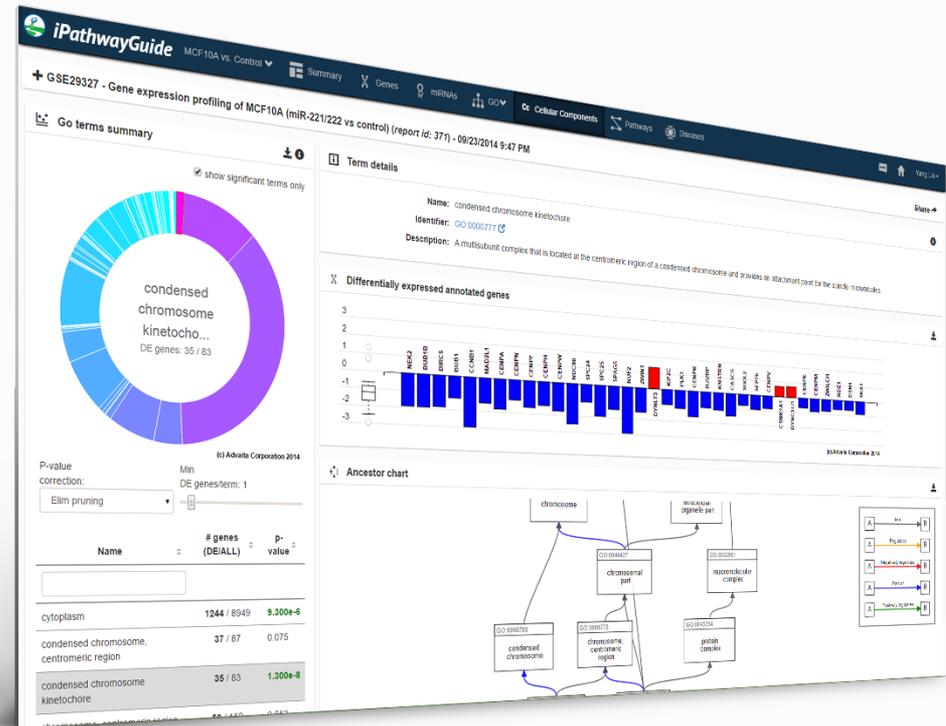
miRNA Analysis

- Predict active miRNA base on expression signature
- Identify target genes and relevant expression profiles
- Generate hypotheses



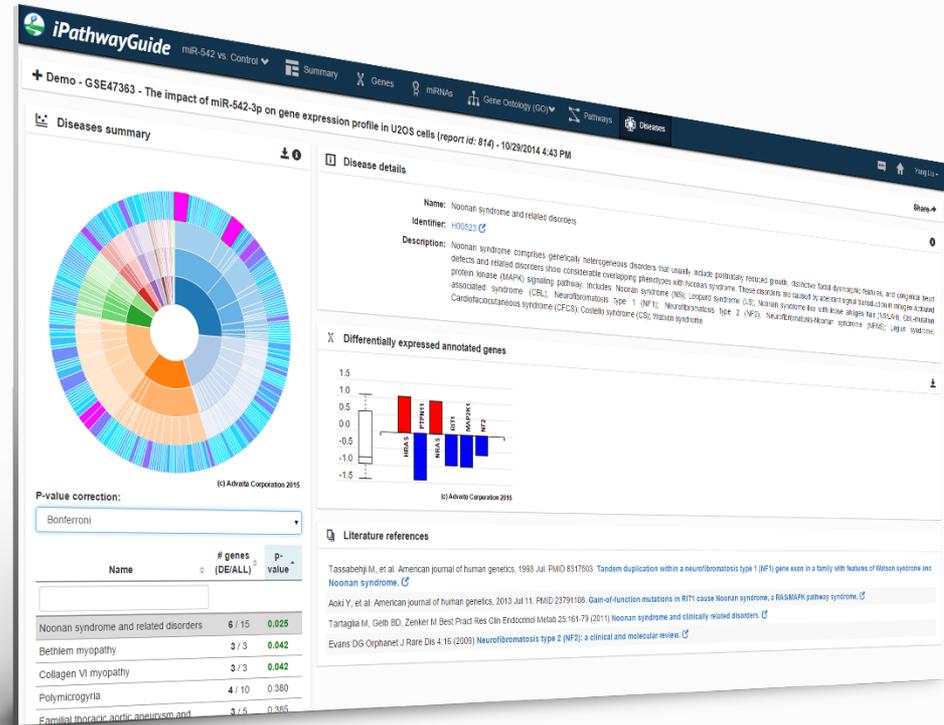
GO Analysis

- 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

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

Legend: TNBC vs Healthy, Her2 vs Healthy, Luminal A vs Healthy, Luminal B vs Healthy

Clear region filter

Gene symbol	Entrez ID	TNBC vs. Healthy		Her2 vs. Healthy		Luminal A vs. Healthy		Luminal B vs. Healthy	
		LogFC	p-value	LogFC	p-value	LogFC	p-value	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	-3.089	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

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Gene details

Title: serum deprivation response
 Identifier: 8436
 Symbol: SDPR
 Aliases: SDPR, 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

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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

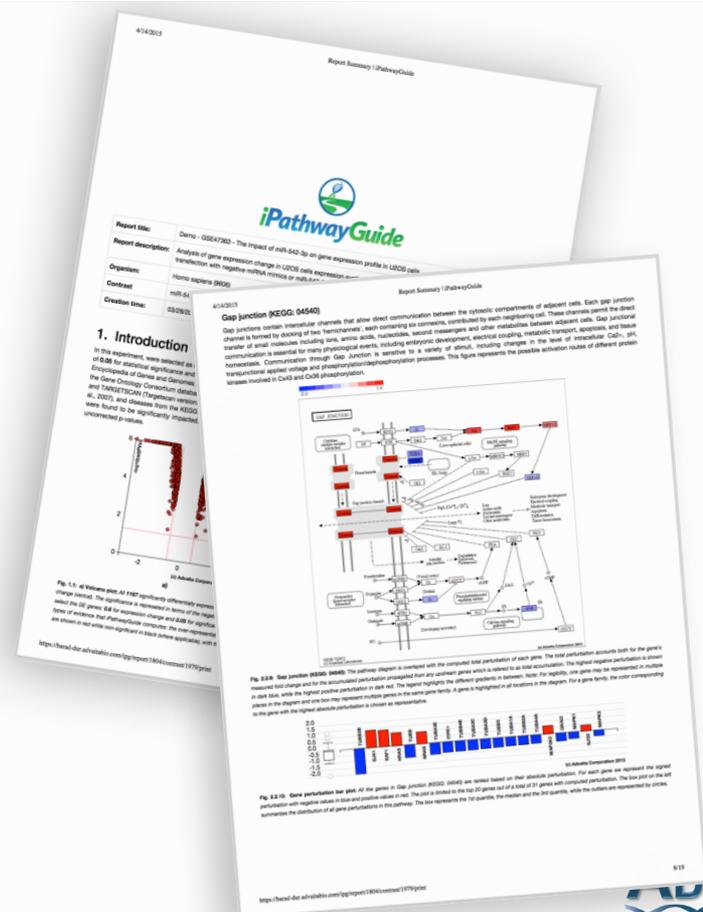
FAQ | Contact Us

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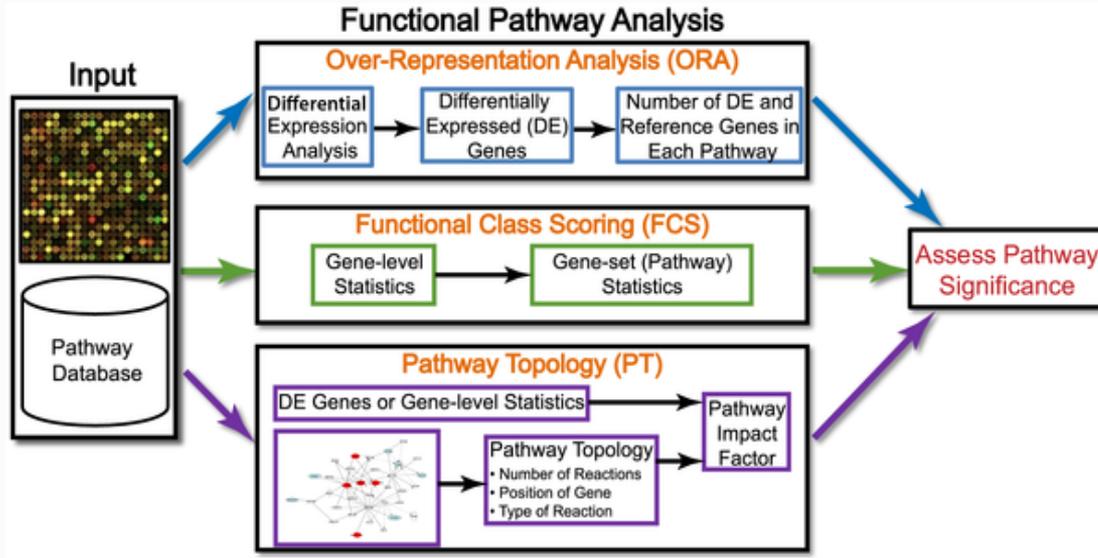
iPG: Printable Summary – Premium Feature

Printable report summary provides descriptive report with:

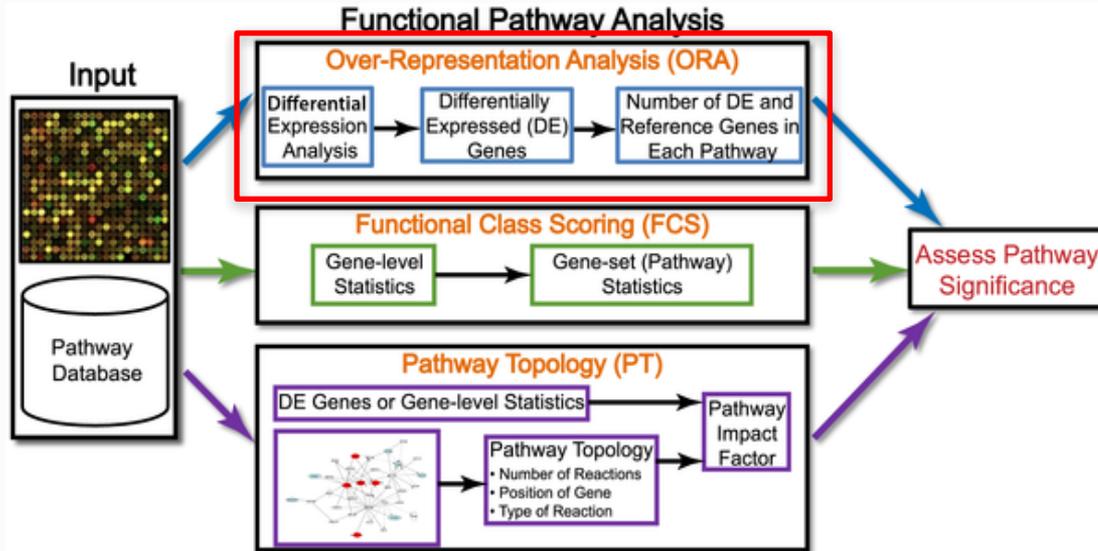
- Top results for each category (based on p -value)
- Top genes for each analysis
- Detailed methods
- References



Classical analysis approaches



Classical analysis approaches



ORA/Enrichment

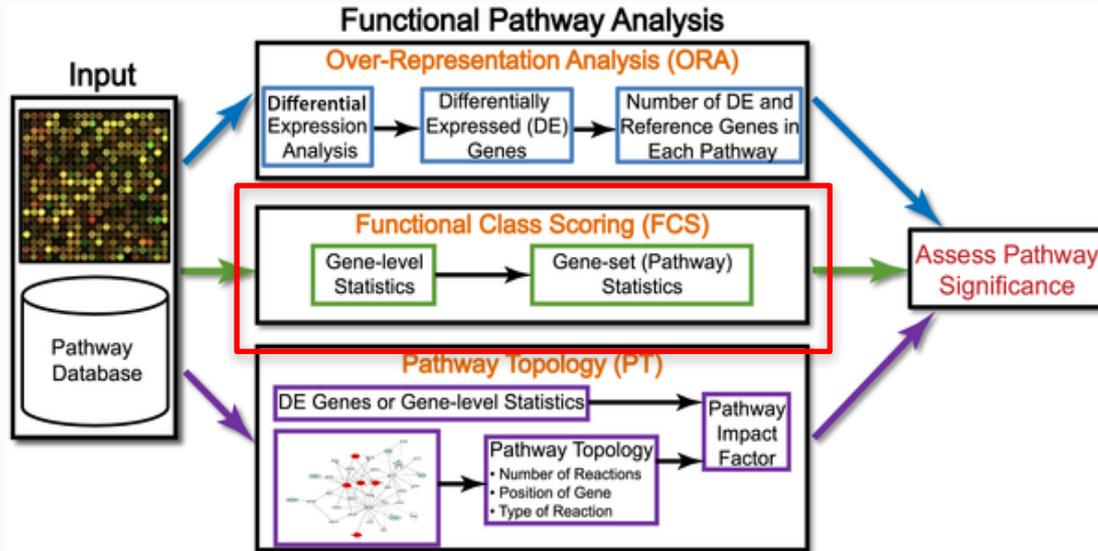
Pros

- Simple
- Requires less input data

Cons

- Assumes all genes are independent
- Uses only significant genes, providing a “snapshot” view
- Many false positives

Classical analysis approaches



Functional Class Scoring

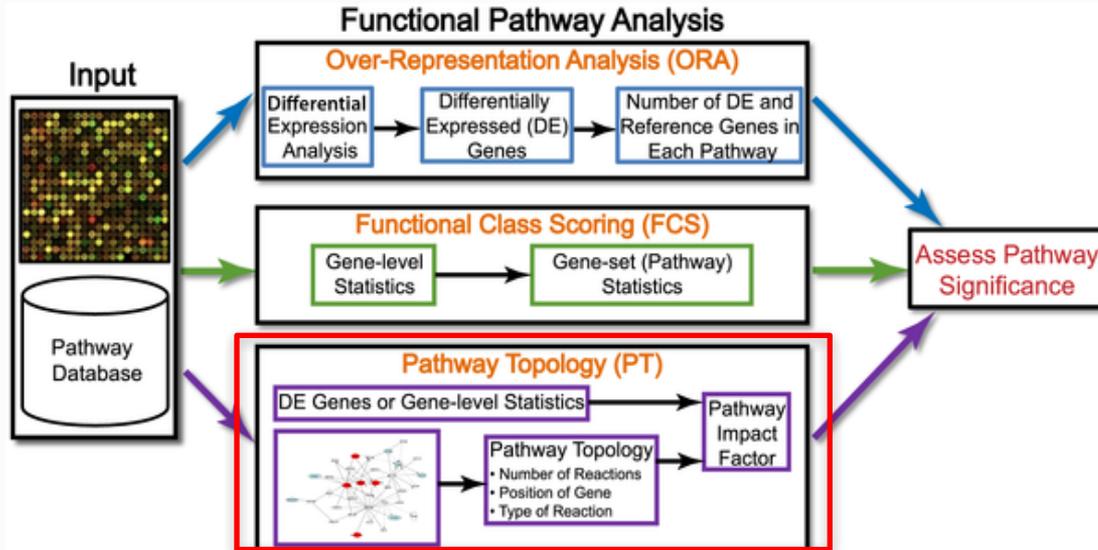
Pros

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

Cons

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

Classical analysis approaches



Pathway Topology

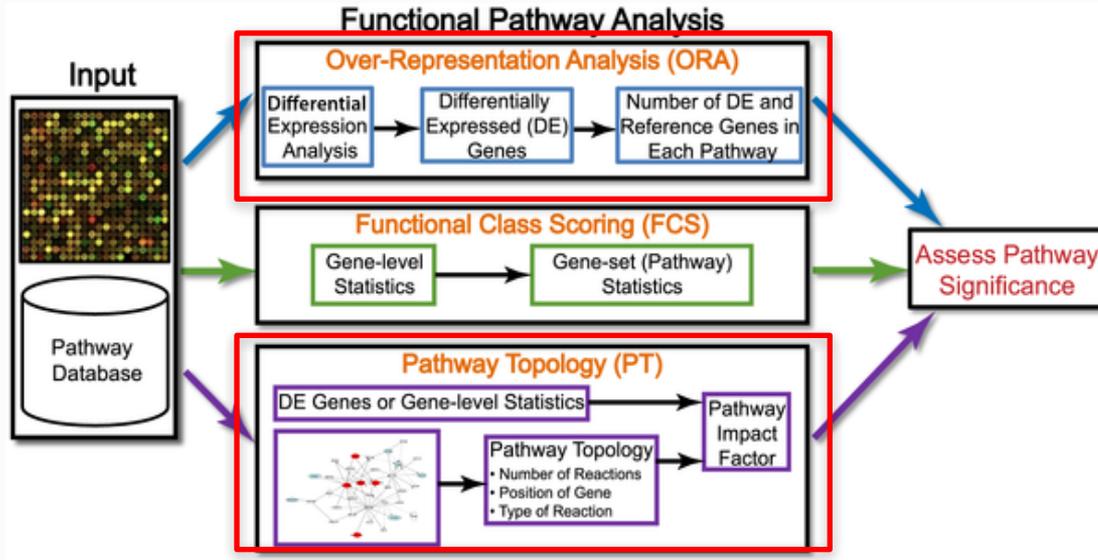
Pros

- Considers role and position
- Able to “interpolate”

Cons

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

Classical analysis approach



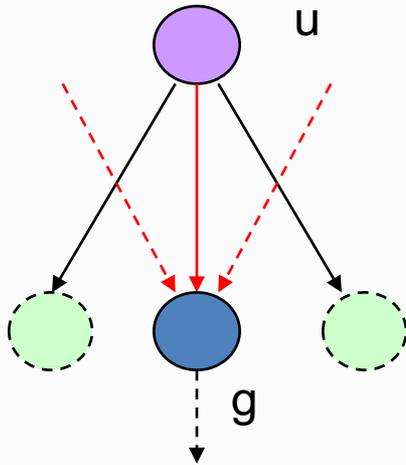
iPathwayGuide combines **both** methods.

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

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

α - 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

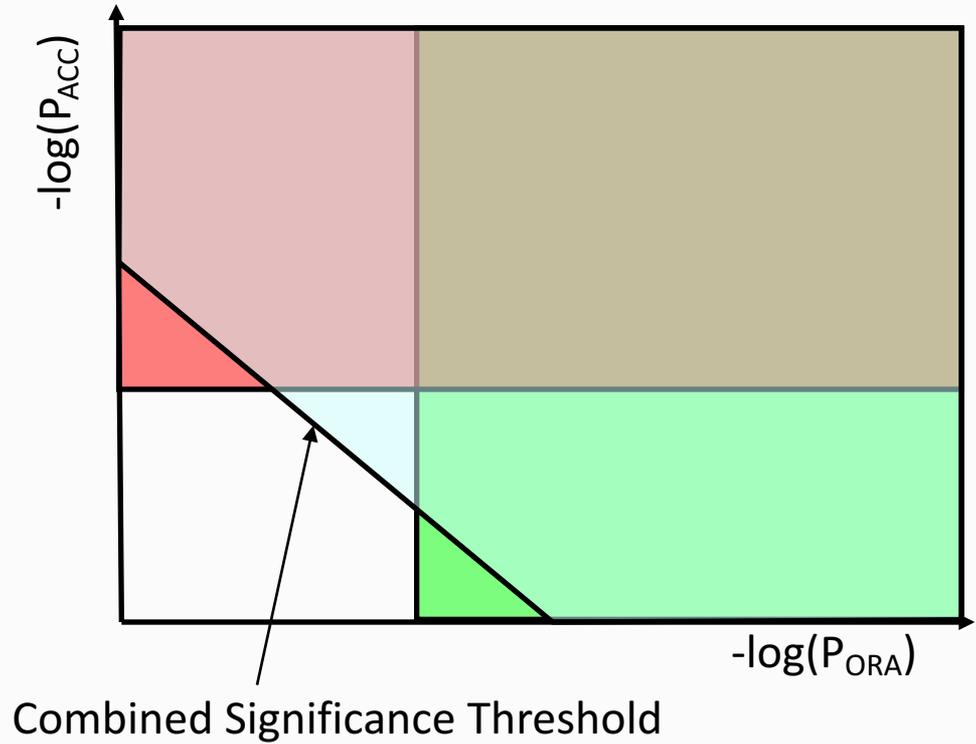
$N_{ds}(u)$ - number of genes directly downstream of u

β_{ug} - efficiency of the connection between u and g

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

iPG: Impact Analysis™

- 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

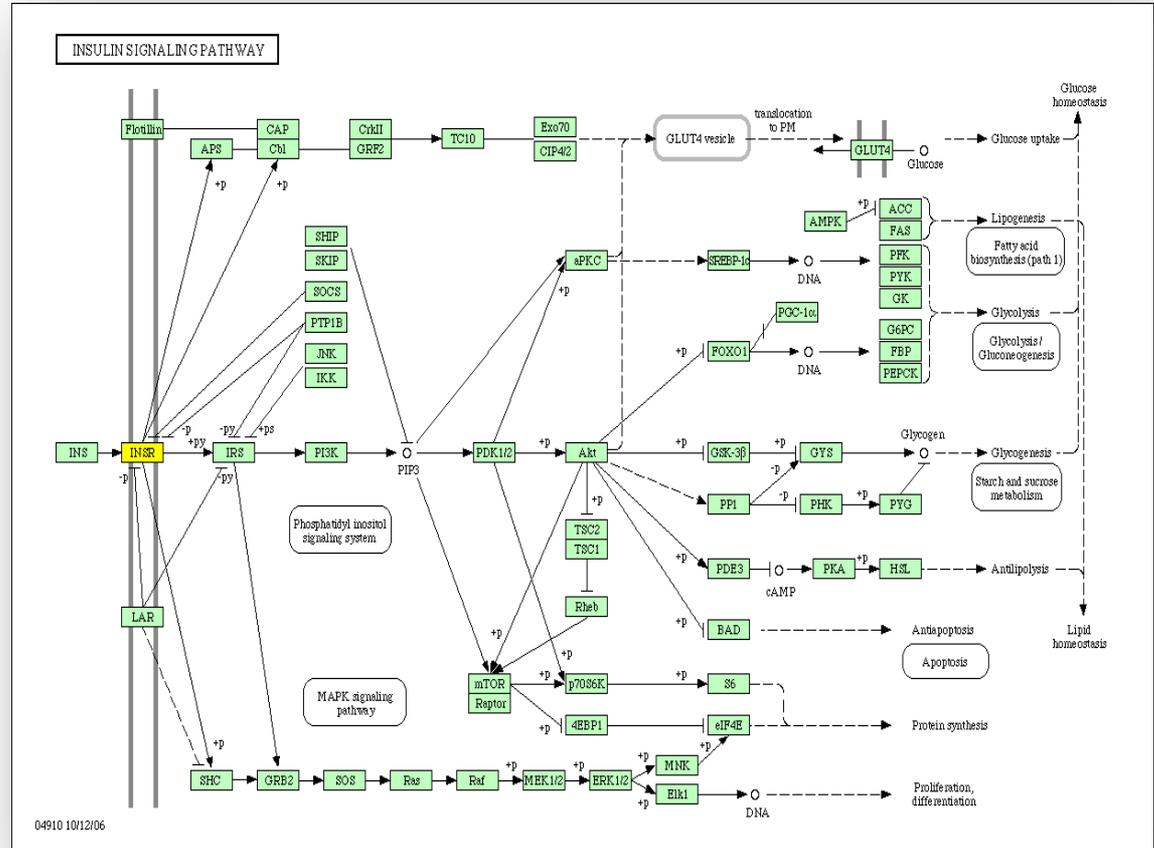


Enrichment vs. Impact Analysis

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

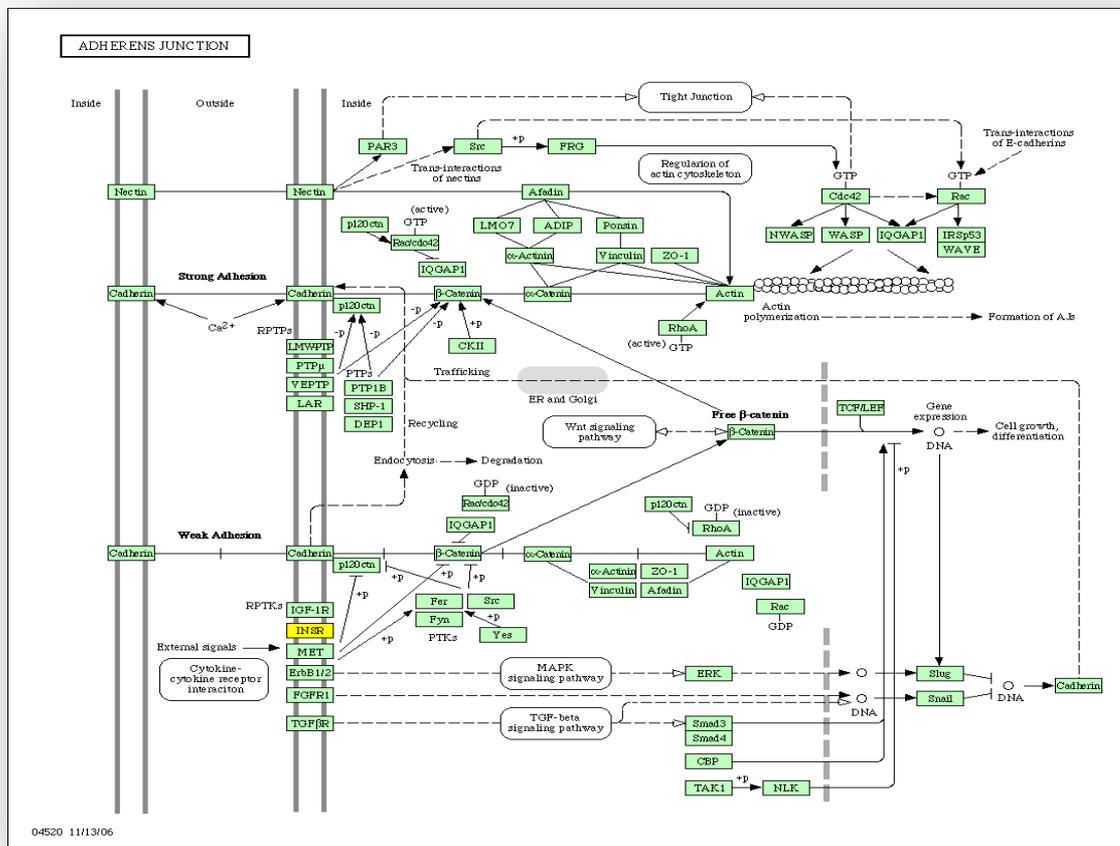


Enrichment vs. Impact Analysis

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



iPathwayGuide Data Requirements

User Input:

- Entire list of genes (NOT just DEGs)
- Log fold change
- *p*-values (corrected values recommended)

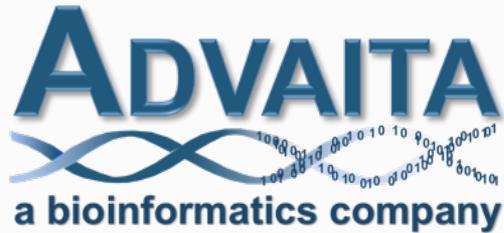
Organisms:

- ✓ 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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