

# iPathwayGuide

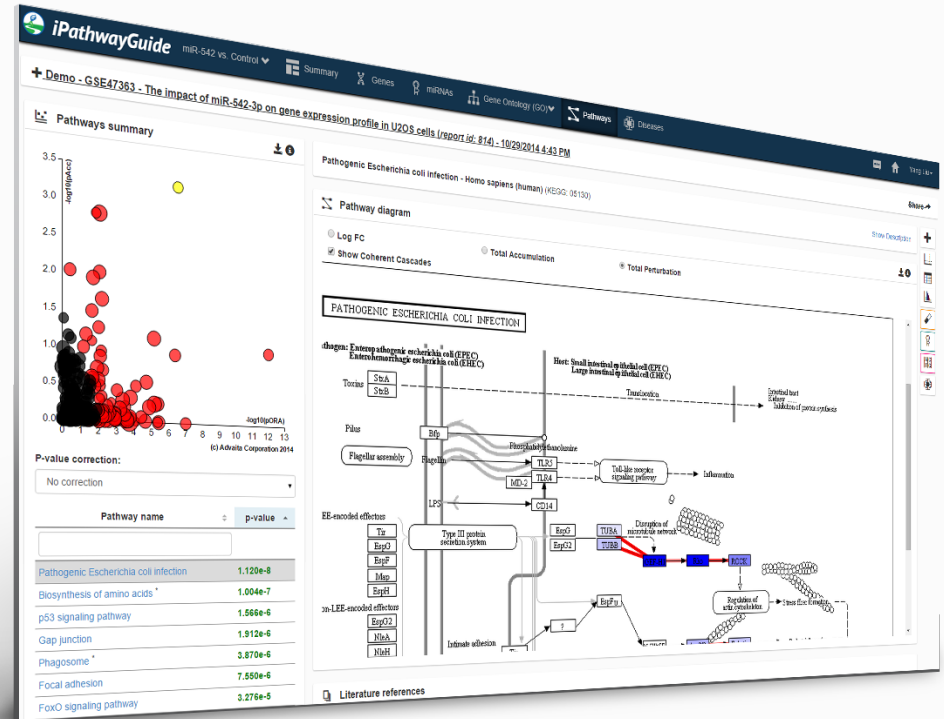
Expression Analysis with  
iPathwayGuide

# Agenda: 04. Expression Analysis after siRNA

- What is expression analysis
  - Insights from miRNA inference
  - Insights from GO terms– how we make it useful
  - Insights from Diseases
- Methodology
  - miRNA inference
  - GO Analysis: ontologies, elim & weight, additional details
  - Diseases: ontology
  - How to generate a Meta-Analysis
- TRY IT! Demonstrate how iPathwayGuide gives better insights
  - Dataset bkg
  - Step-by-step navigation: Enter from email, Summary, miRNA, GO Terms, Diseases, Printable Report
  - How to generate meta-analysis: purchase report, select contrasts, name conditions, submit
  - 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



# EXPRESSION ANALYSIS IN iPATHWAYGUIDE

## MIRNA INFERENCE

- Which miRNAs might be active in the experiment?

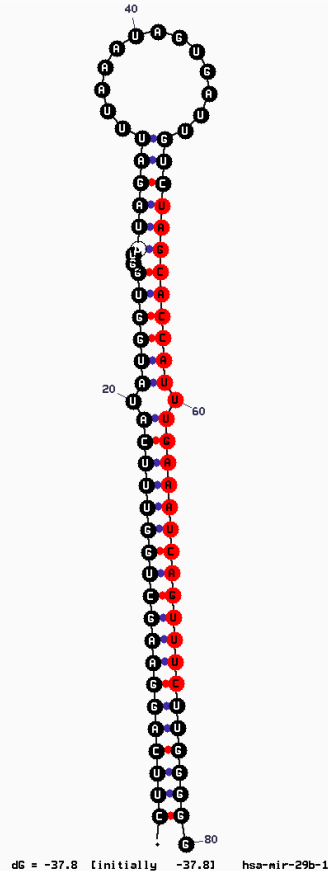
## GENE ONTOLOGY ANALYSIS

- Which biological processes, cellular components, or molecular functions are impacted in the experiment?
- What related processes are likely affected?

## RELATED DISEASES

- What human diseases are associated with similar changes in expression?

# MICRO-RNA INFERENCE ANALYSIS



## miRNAs bind specific nucleotide sequences to inhibit transcription

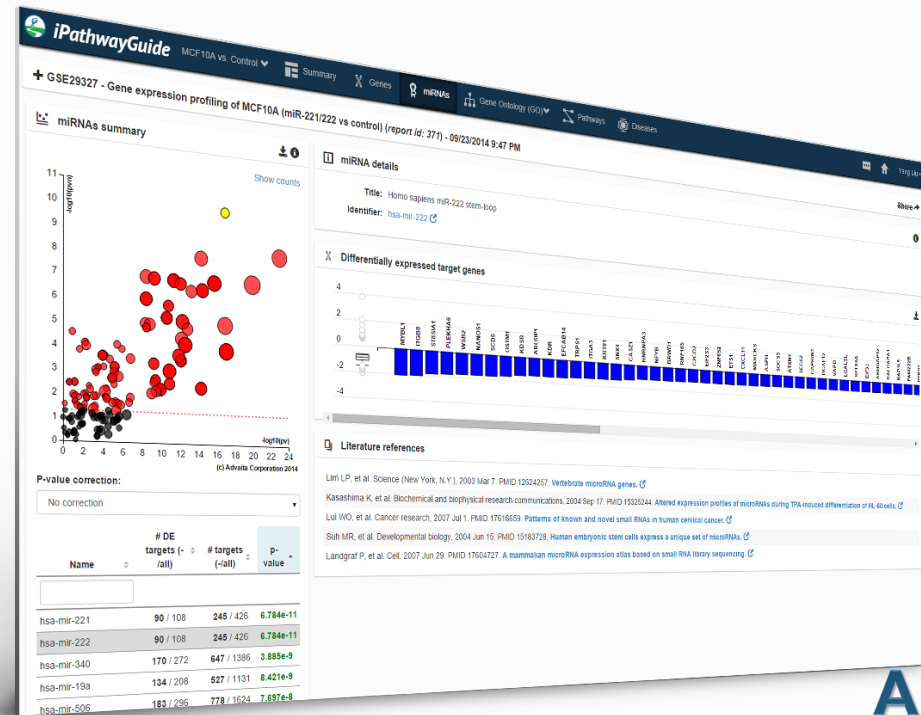
- miRNA usually have many genes (miRBase, TargetScan)
- miRNA activity can be inferred by observing how many target genes are downregulated
- Must correct for multiple comparisons

# miRNA Analysis

RESEARCH QUESTION: Which miRNAs are most likely to be active, according to the DE downregulated genes in this experiment?

## METHODOLOGY:

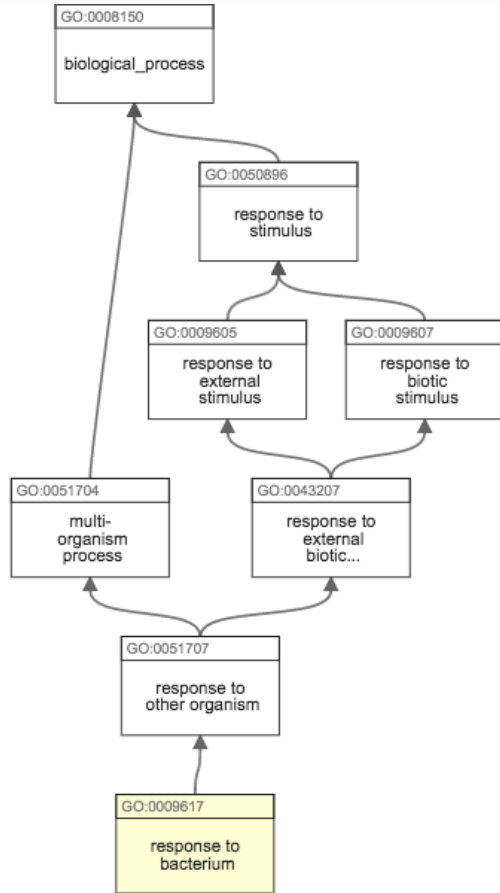
1. Identify miRNAs associated with DE genes
2. Count number of DE down vs total DE targets
3. Calculate enrichment score (pORA) for each miRNA



# GENE ONTOLOGY (GO) ANALYSIS

## AN ONTOLOGY IS A HIERARCHY OF TERMS

- Each GO TERM describes a
  - BIOLOGICAL PROCESS,
  - MOLECULAR FUNCTION, or
  - CELLULAR COMPONENT
- Associated genes are annotated to each term... and its parent(s)
- Correction factors need to account for nested terms:
  - ELIM and WEIGHT METHODS

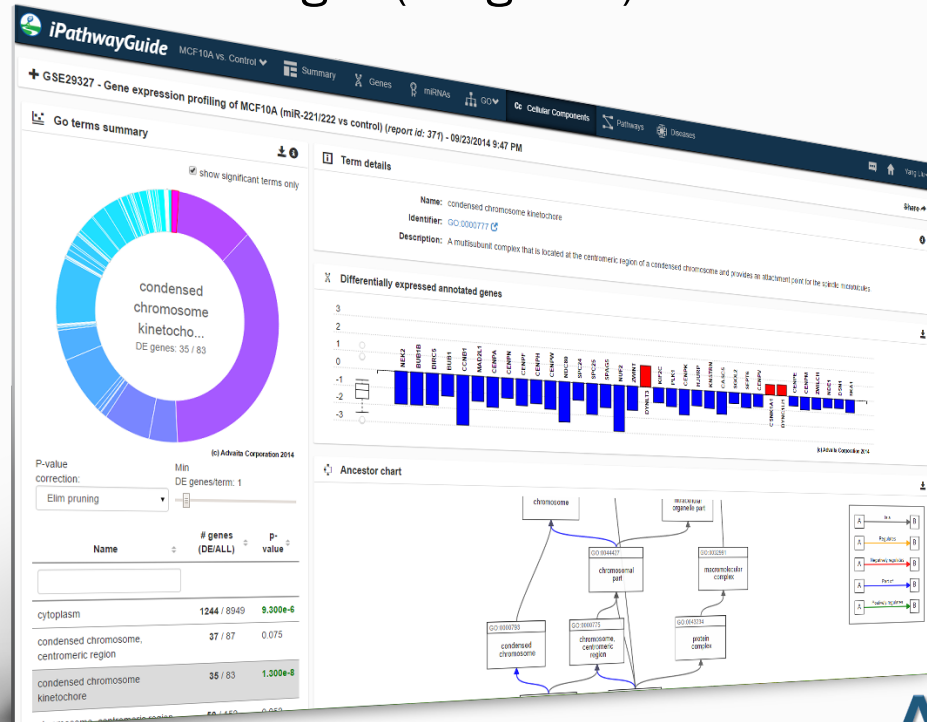


# GO Analysis

RESEARCH QUESTION: What processes/ functions/ components are most impacted by the measured expression changes (DE genes)?

## METHODOLOGY:

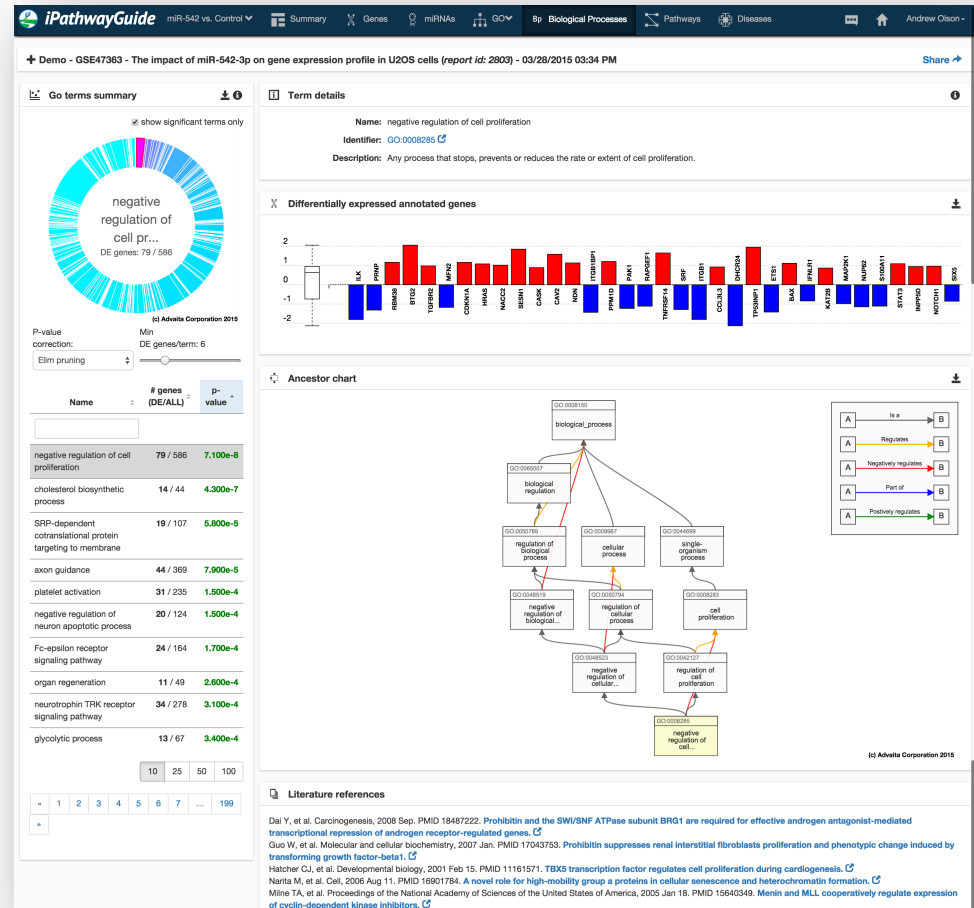
1. Identify GO terms associated with DE genes
2. Calculate enrichment score (pORA) for each term
3. Use *Elim* or *Weight* Methods to find informative terms





# iPG: GO Analysis Method

- 3 Domains:
    - Biological Processes
    - Molecular Functions
    - Cellular Components
  - Hypergeometric distribution analysis
  - 5 Correction Factors
    - Uncorrected
    - FDR
    - Bonferroni (FWER)
    - Elim\*
    - Weight\*
- \* Takes into account repeated genes in the hierarchy (Alexa et al 2006)



# DISEASE ANALYSIS

## INTERNATIONAL CLASSIFICATION OF DISEASES (ICD-10)

- A hierarchy of human diseases from the WHO, October 2015 (in US, from CMS and NCHS)
- Associated genes are annotated to each disease
  - Not necessarily nested
  - Use standard correction for multiple comparisons

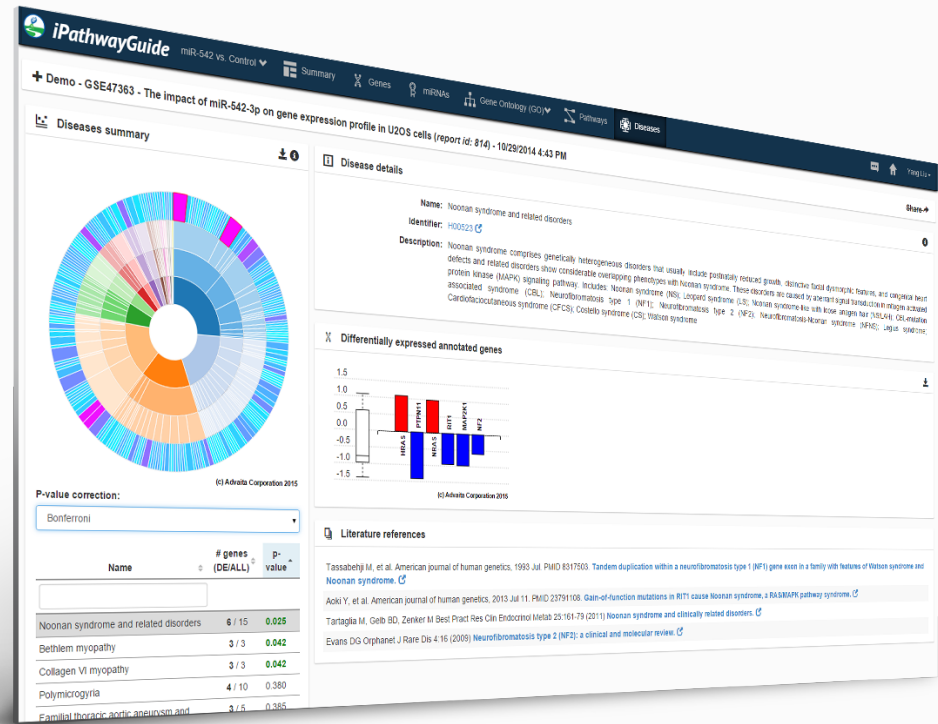


# Disease Analysis

RESEARCH QUESTION: What diseases are most impacted by the measured DE Genes?

## METHODOLOGY:

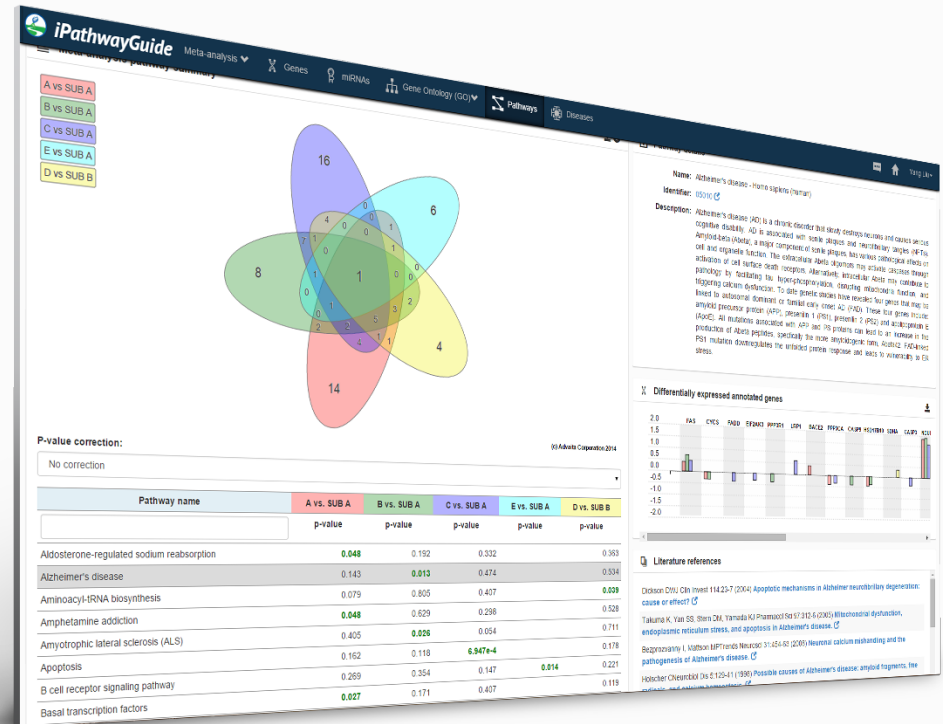
1. Identify ICD terms associated with DE genes
2. Calculate enrichment score (pORA) for each term
3. Use correction factors to eliminate false positives
4. Navigate hierarchy to explore results



# Meta Analysis

RESEARCH QUESTION: How do differential expression profiles vary across experimental conditions?

- Compare up to 5
  - time points
  - doses
  - disease subtypes, etc.
- Combine proteomic and transcriptomic analyses
- Quickly identify common or unique patterns
  - Genes
  - miRNAs
  - GO terms
  - Pathways
  - Diseases



# DATASET BACKGROUND

www.nature.com/scientificreports

## SCIENTIFIC REPORTS

**OPEN** **AML1/ETO accelerates cell migration and impairs cell-to-cell adhesion and homing of hematopoietic stem/progenitor cells**

Received: 22 March 2016  
Accepted: 20 September 2016  
Published: 07 October 2016

Marco Saia<sup>1,\*</sup>, Alberto Termanini<sup>2</sup>, Nicoletta Rizzi<sup>2</sup>, Massimiliano Mazza<sup>3</sup>, Elisa Barbieri<sup>1,4</sup>, Debora Valli<sup>5</sup>, Paolo Ciana<sup>3</sup>, Alicja M. Gruszka<sup>1,4,\*</sup> & Myriam Alcalay<sup>1,3,\*</sup>

The AML1/ETO fusion protein found in acute myeloid leukemias functions as a transcriptional regulator by recruiting co-repressor complexes to its DNA binding site. In order to extend the understanding of its role in preleukemia, we expressed AML1/ETO in a murine immortalized pluripotent hematopoietic stem/progenitor cell line, EML C1, and found that genes involved in functions such as cell-to-cell adhesion and cell motility were among the most significantly regulated as determined by RNA sequencing. In functional assays, AML1/ETO-expressing cells showed a decrease in adhesion to stromal cells, an increase of cell migration rate *in vitro*, and displayed an impairment in homing and engraftment *in vivo* upon transplantation into recipient mice. Our results suggest that AML1/ETO expression determines a more mobile and less adherent phenotype in preleukemic cells, therefore altering the interaction with the hematopoietic niche, potentially leading to the migration across the bone marrow barrier and to disease progression.

Approximately 12–15% of cases with adult acute myeloid leukemia (AML) carry the (8:21) translocation, which fuses the *AML1* (also known as *RUNX1*) and *ETO* (otherwise *RUNX1T1* or *MTG8*) genes and results in expression of the AML1/ETO chimeric protein<sup>1</sup>. The published expression data demonstrate that AML1/ETO expression induces a distinct gene expression profile that involves the regulation of haematopoietic transcription and

- AML1/ETO fusion is common in AML; necessary but not sufficient to cause AML in mice
- Expressed AML1/ETO in HSC, used cytokines to induce myeloid differentiation
  - EV (empty vector)
  - A22 (transfected cells)
  - D0 (day 0)
  - D3 (day 3 differentiation)

# LET'S TRY IT!

- STEPS:
  - ACCEPT SHARE
  - GENERATE META-REPORT
  - GO TERMS
  - DISEASES
  - PRINTABLE REPORT
  - META-ANALYSIS

# HOW TO GENERATE A NEW META-ANALYSIS

- Dashboard> +Create new meta-report

+ Create new meta-report

+ Analyze a new experiment

- Find reports to include
  - Missing? Make sure it's purchased
  - Use green arrow to include a contrast (names can be edited later)
  - Repeat for up to 5 contrasts

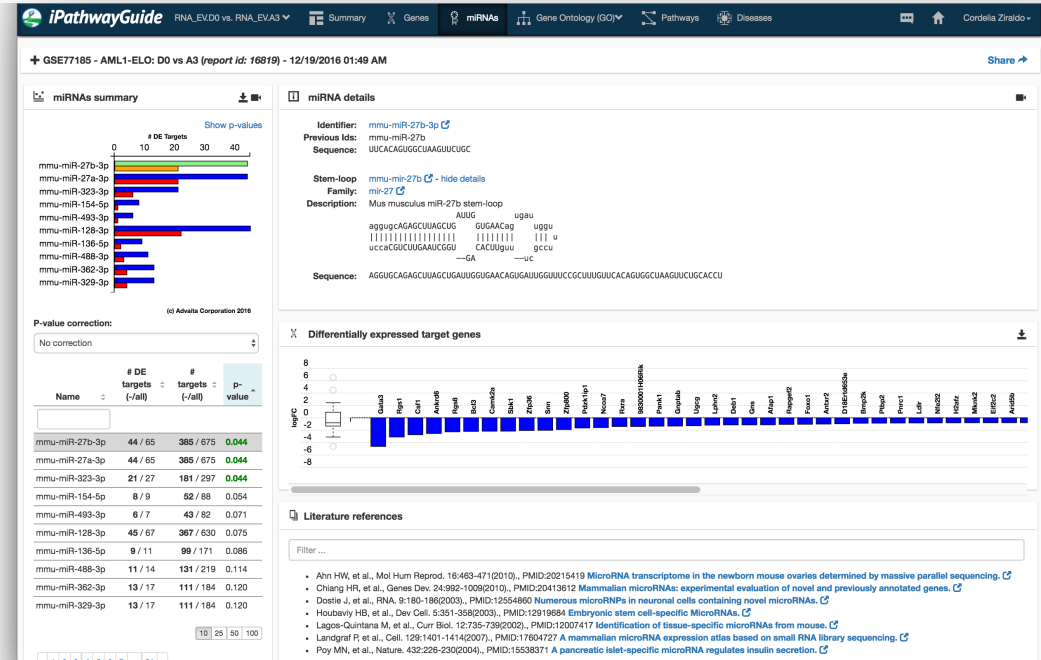
Project Description	Creation Time	
+ GSE48018 - Trivalent Influenza Vaccine - day 3	08/17/2016 03:42 PM	
+ GSE77185 - AML1-ELO	12/19/2016 02:20 AM	
+ GSE77185 - AML1-ELO: D0 vs A3	12/19/2016 01:49 AM	
+ GSE77185 - AML1-ELO: EV vs AE22	12/19/2016 01:43 AM	
✖ GSE77185 - AML1/ELO	12/09/2016 04:54 PM	
RNA_EV.D0 vs. RNA_EV.A3		
RNA_EV.D0 vs. RNA_AE22.D0		
RNA_EV.D0 vs. RNA_AE22.A3		>
RNA_EVA3 vs. RNA_AE22.A3		
RNA_AE22.D0 vs. RNA_AE22.A3		

Selected contrasts:	
RNA_EV.D0 vs. RNA_EV.A3	<
RNA_EV.D0 vs. RNA_AE22.D0	<
RNA_EVA3 vs. RNA_AE22.A3	<
RNA_AE22.D0 vs. RNA_AE22.A3	<

- Add Title & Description, then Create Report

## iPG: Predicted miRNAs

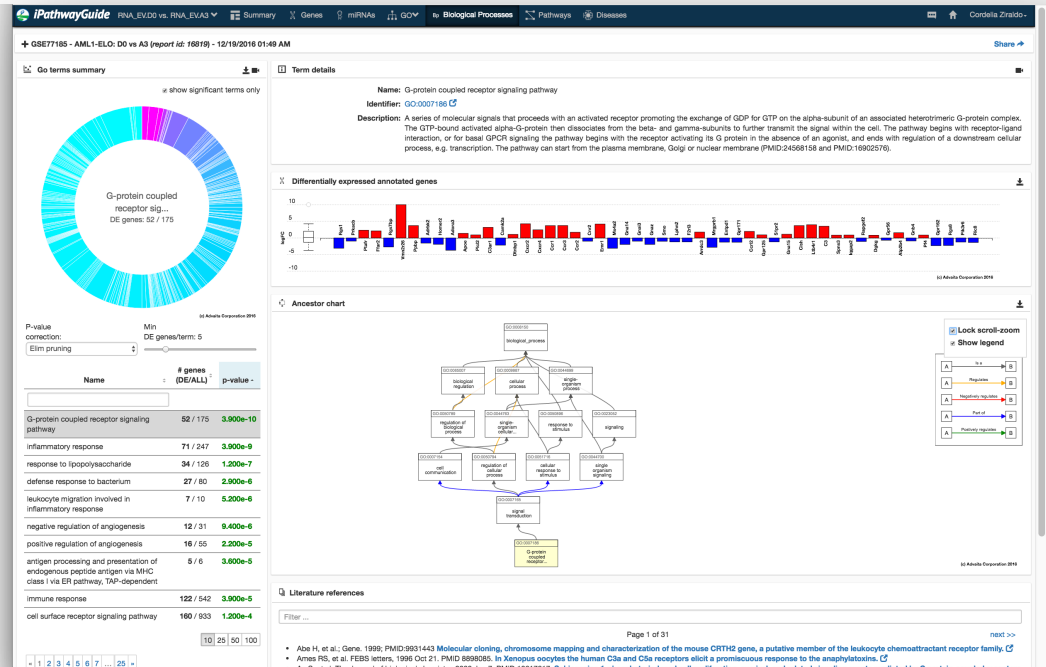
- Bar plot displays count of downregulated targets vs upregulated targets
- Gene's plot lists target DE genes from max -FC to max +FC
- Clicking on any gene will navigate to genes pages





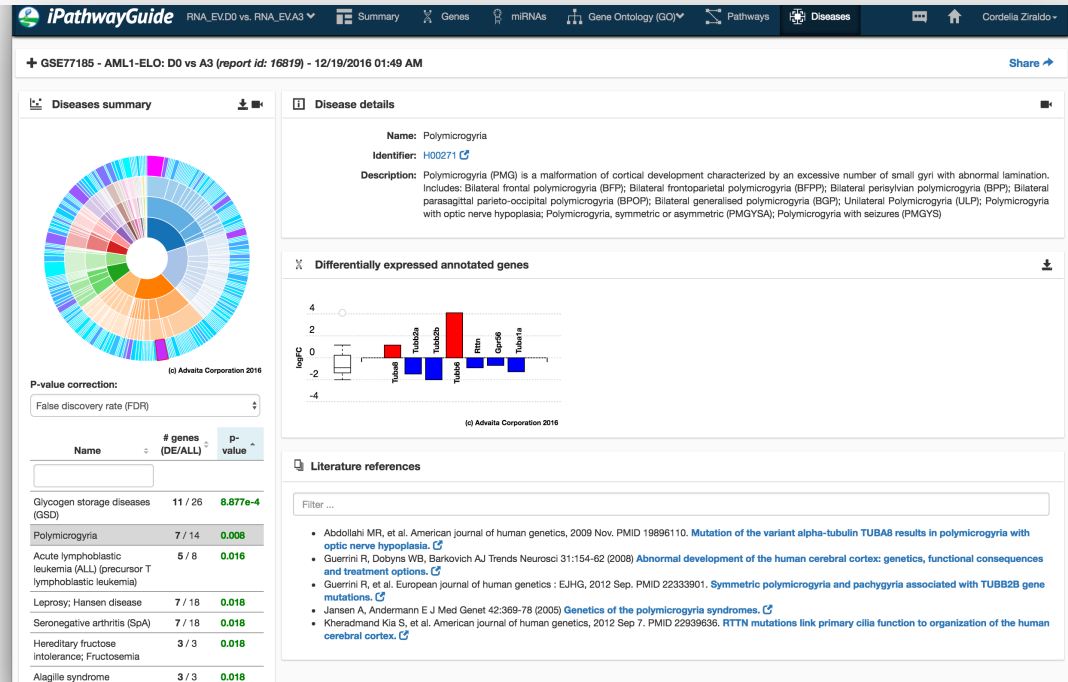
# iPG: GO Analysis

- Graph shows relative significance  
Cyan = less significant  
Magenta – most significant
- Can filter by # of DE Genes per term
- Genes plot shows directly annotated genes to GO Term
- GO tree shows hierarchy (interactive)



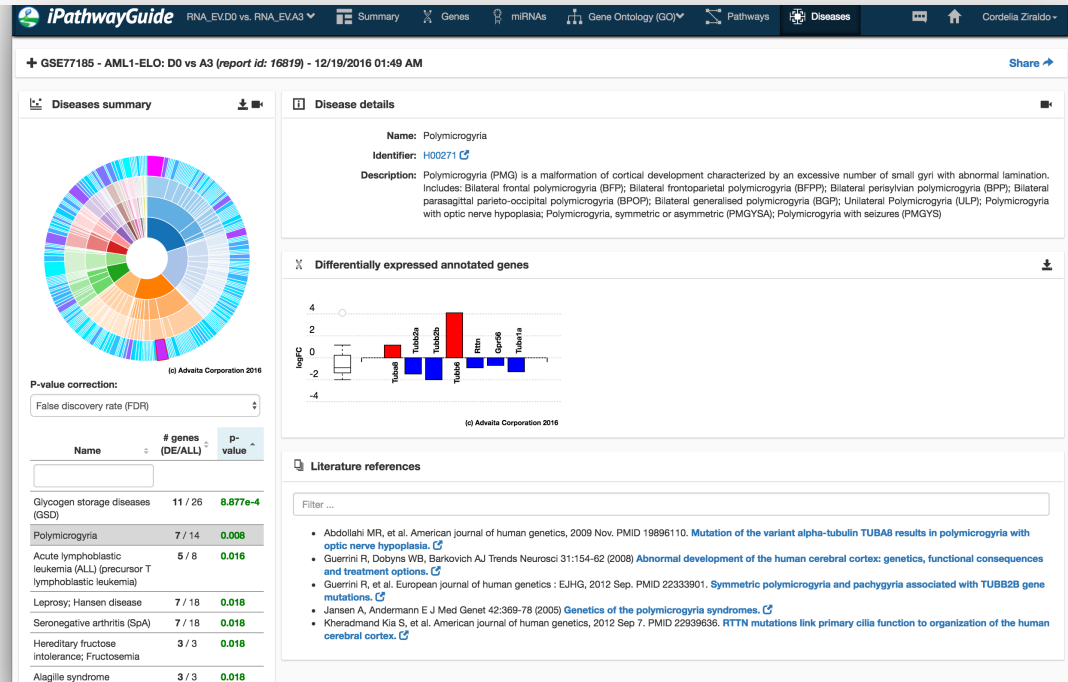
# iPG: Diseases

- Graph shows relative significance  
Cyan = less significant  
Magenta – most significant
- Can filter diseases by classification
- Genes plot shows directly annotated genes to disease



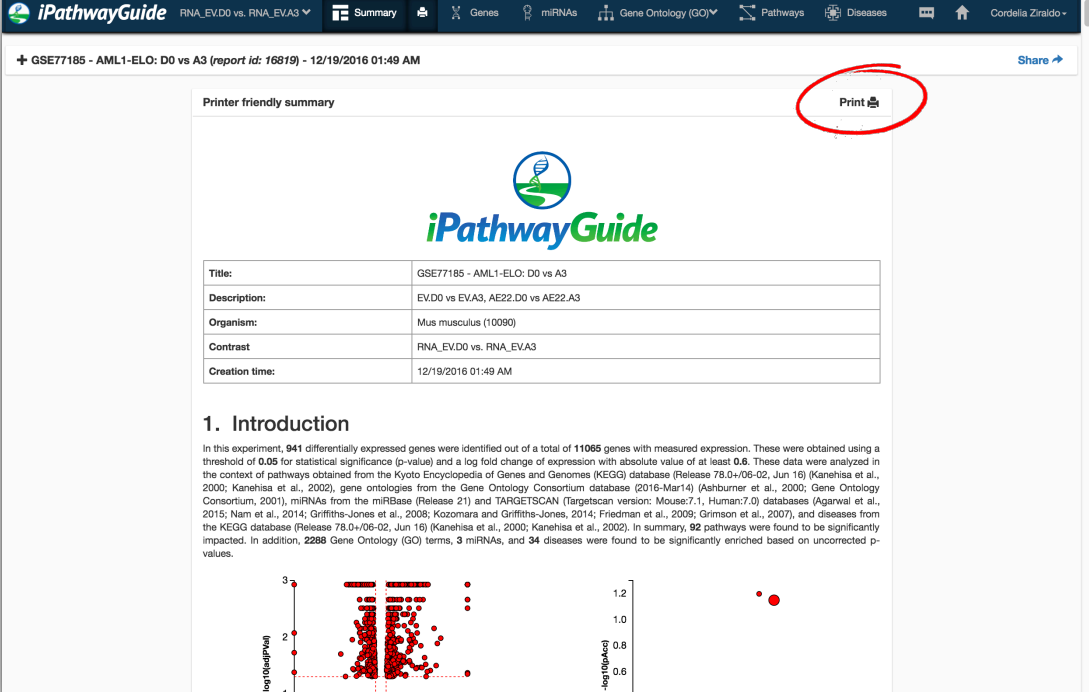
# iPG: Diseases

- Graph shows relative significance  
Cyan = less significant  
Magenta – most significant
- Can filter diseases by classification
- Genes plot shows directly annotated genes to disease



# iPG: Printable Report

- Generate Report from Summary Page
- Click Print to Save or Print
- Top 5 Pathways, top GO Terms, miRNAs, Diseases, all included with accompanying diagrams
- Complete Methods & References



**iPathwayGuide** RNA\_EV.D0 vs. RNA\_EVA3 Summary Genes miRNAs Gene Ontology (GO) Pathways Diseases Cordelia Ziraldo

+ GSE77185 - AML1-ELO: D0 vs A3 (report id: 16819) - 12/19/2016 01:49 AM [Share](#)

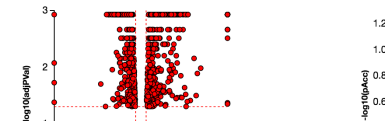
Printer friendly summary **Print**

**iPathwayGuide**

Title:	GSE77185 - AML1-ELO: D0 vs A3
Description:	EV.D0 vs EVA3, AE22.D0 vs AE22.A3
Organism:	Mus musculus (10090)
Contrast:	RNA_EV.D0 vs. RNA_EVA3
Creation time:	12/19/2016 01:49 AM

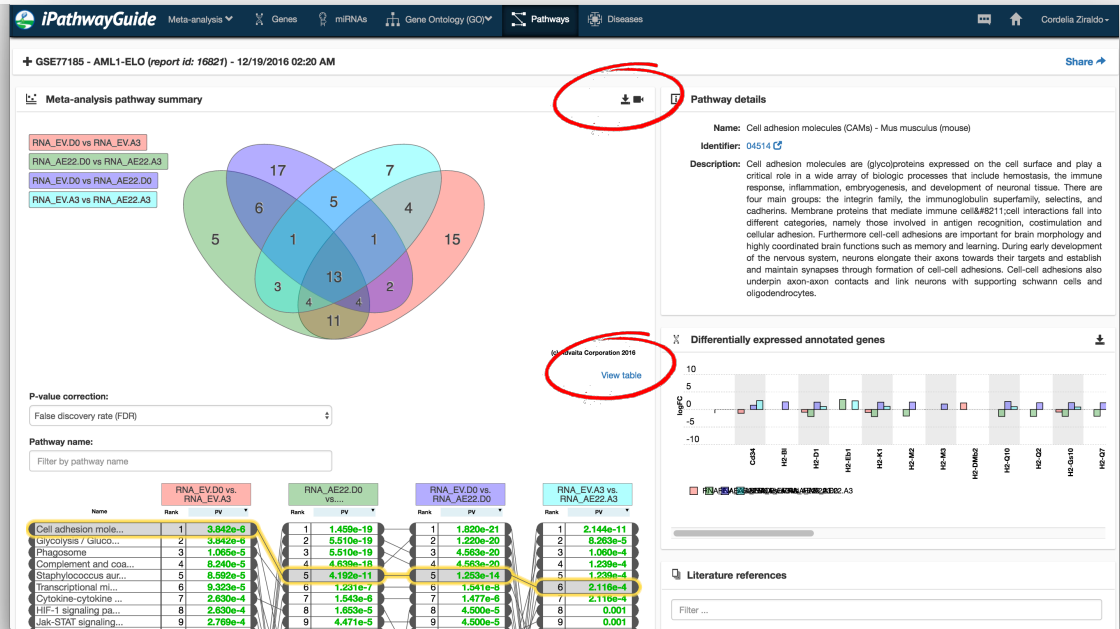
### 1. Introduction

In this experiment, **941** differentially expressed genes were identified out of a total of **11065** genes with measured expression. These were obtained using a threshold of **0.05** for statistical significance (p-value) and a log fold change of expression with absolute value of at least **0.6**. These data were analyzed in the context of pathways obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Release 78.0+/06-02, Jun 16) (Kanehisa et al., 2000; Kanehisa et al., 2002), gene ontologies from the Gene Ontology Consortium database (2016-Mar14) (Ashburner et al., 2000; Gene Ontology Consortium, 2001), miRNAs from the miRBase (Release 21) and TARGETSCAN (TargetsCan version: Mouse:7.1, Human:7.0) databases (Agarwal et al., 2015; Nam et al., 2014; Griffiths-Jones et al., 2008; Kozomara and Griffiths-Jones, 2014; Friedman et al., 2008; Grimson et al., 2007), and diseases from the KEGG database (Release 78.0+/06-02, Jun 16) (Kanehisa et al., 2000; Kanehisa et al., 2002). In summary, **92** pathways were found to be significantly impacted. In addition, **2288** Gene Ontology (GO) terms, **3** miRNAs, and **34** diseases were found to be significantly enriched based on uncorrected p-values.

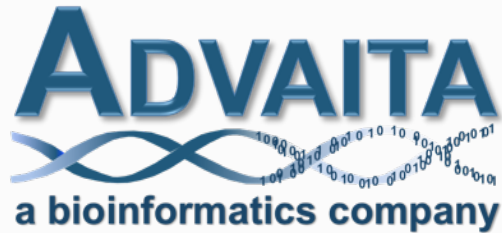


# iPG: Meta Analysis

- View comparisons across any module
- Use Gene Bar Plot to identify potential biomarkers
- Download all figures and tables
- View Significant entities as a table or rank diagram



# Stop Point Questions



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