# MetaCore:

# Common nonmutational NOTCH1 activation in chronic lymphocytic leukemia

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Manager, Solution Scientists

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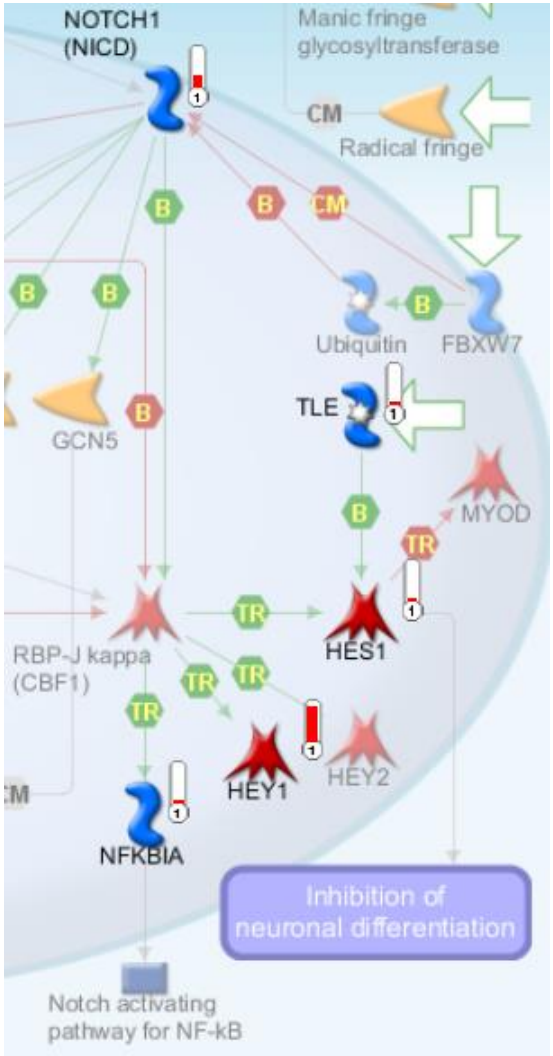
# Agenda

Introduction to MetaCore

Use case: Common nonmutational NOTCH1 activation in chronic lymphocytic leukemia

- I want to know what interactions and pathways involve NOTCH1.
- What pathways are enriched by ICN1 from RNA-seq and bound to NOTCH1 in ChIP-seq experiments?
- What transcription factors could these up regulated genes regulated by?
- How does the NOTCH1 expression profile compare with RNA-seq data from chronic lymphocytic leukemia?
- What other publicly available datasets have a similar to the NOTCH1 expression profile?

Summary

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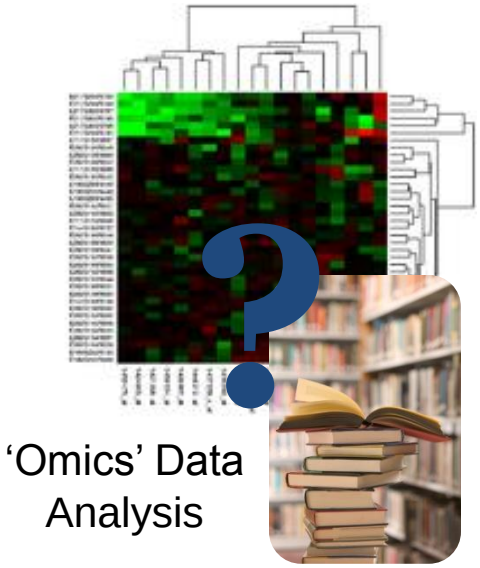
# MetaCore: Your GPS in pathway analysis



'Omics' Data Analysis

Knowledge Mining

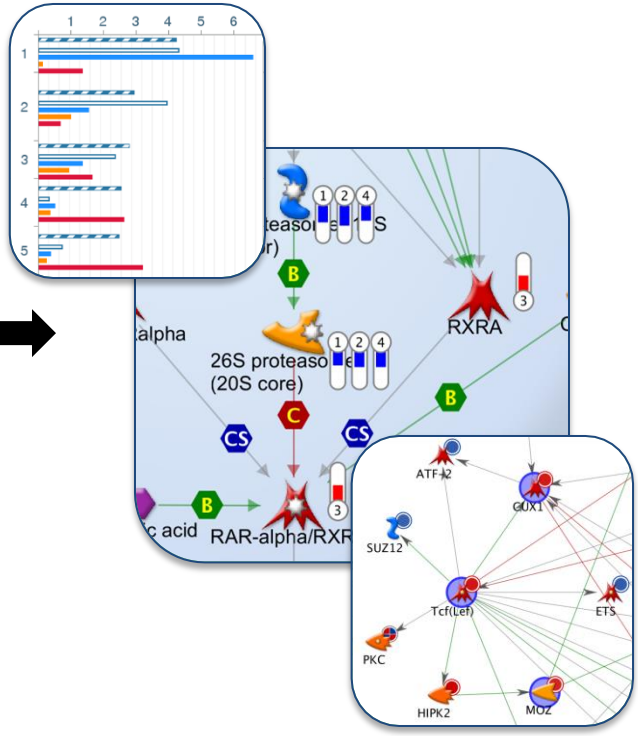
MetaCore

Pathway Analysis Platform

Upload

Search & Browse Content

Workflows & Reports




- **Gain molecular understanding of disease**
- **Analyze and understand experimental findings (Omics data) in the context of validated biological pathways.**
- **Generate and confirm hypotheses for novel biomarkers, targets, mechanisms of action**

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# MetaBase/MetaCore content overview

- **Human genes** **60,119**
- **Human SwissProt proteins** **20,115**
- **Mouse genes** **68,595**
- **Mouse SwissProt proteins** **16,829**
- **Rat genes** **47,989**
- **Rat SwissProt proteins** **7,968**
- **Compounds** **815,453**
- **Compounds with structure** **796,538**
- **Endogenous compounds** **5,405**
- **Nutritional compounds** **119**
- **Metabolites of xenobiotic** **26,761**
- **Drugs** **9,186**
  - Biologics 1,457
  - Small Molecules 7,729
  - Approved drugs 2.293
  - Withdrawn drugs 261
  - Clinical trial drugs 5,055
  - Discontinued drugs 1,189
  - Preclinical drugs 251
  - Unknown 137
  - Drug combination regimens 8,446

- **Human genes in network** **23,665**
- **Mouse genes in network** **20,673**
- **Rat genes in network** **17,534**
- **Chemical compounds** **351,887**
- **Drugs** **4,297**
- **Endogenous compounds** **3,469**
- **Metabolic reactions** **33,338**
- **Transport reactions** **4,570**
- **Processing Reactions** **1,262**
- **Pubmed journals** **3,668**
- **Pubmed records** **1,919,055**
- **Pubmed articles (unique)** **254,828**
- **Total amount of interactions** **1,743,596**
  - Protein – Protein 764,667
  - Compound – Protein 701,499
  - Compound – Compound 9,816
  - Metabolic enzyme - Reaction 43,064
  - Transporter – Reaction 4,570
  - Substrate, Product – Reaction 85,980
  - RNA – Protein 134,000

- **Pathway maps** **1,632**
  - Human genes in maps 7,370
  - Mouse genes in maps 6,723
  - Rat genes in maps 6,470
  - Interactions in maps 30,494

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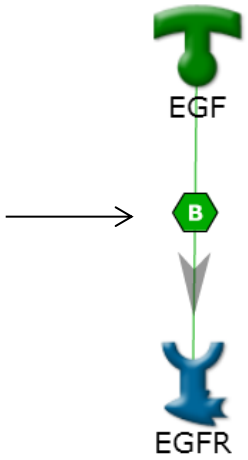
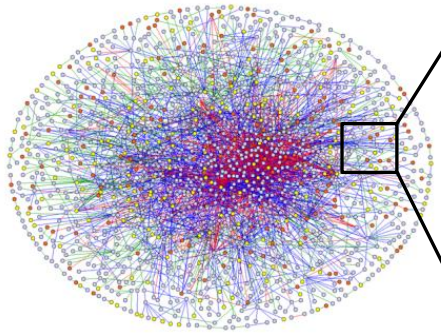
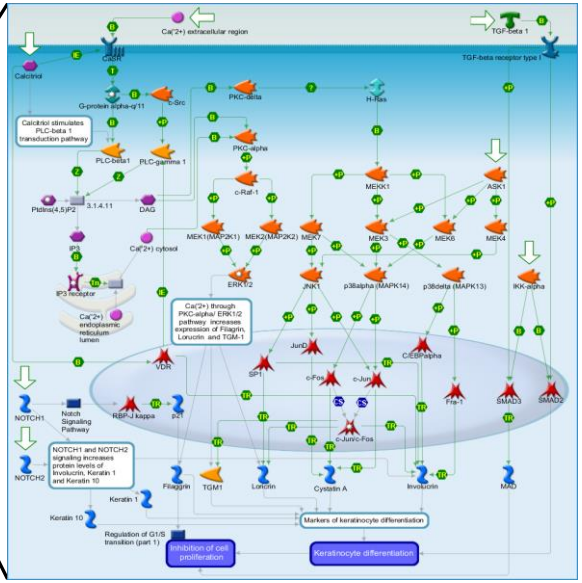
# From peer reviewed articles to signaling pathways





**PUBLICATIONS**
(209 for EGF-EGFR interaction)

**MOLECULAR INTERACTION**

**GLOBAL NETWORK:**
*~ 1,700,000 molecular interactions*

***~ 1,600 canonical and disease signaling pathways***

- Manual annotation from publications
- Team of PhDs, MDs
- More than 10 years

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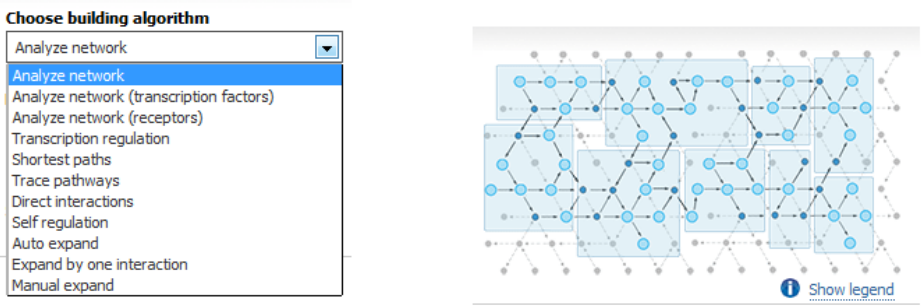
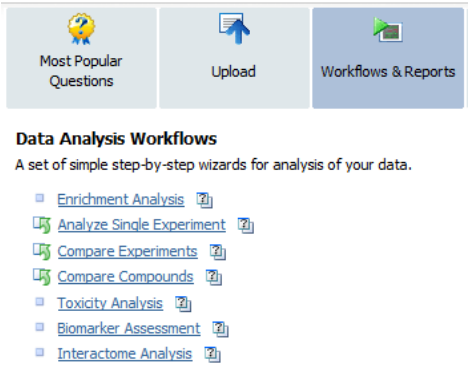
# Flexibility in data analysis tools

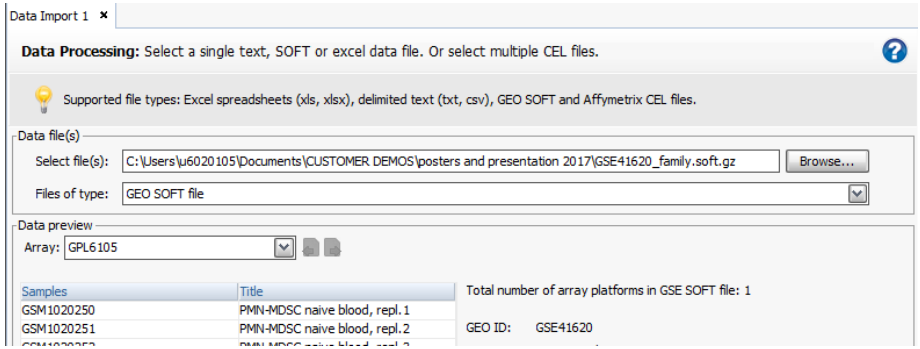
## 10 Different Network Building Algorithms, all with written and visual descriptions



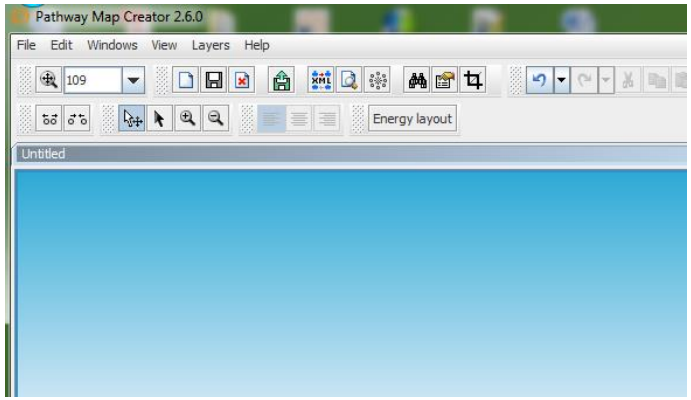

## Multiple automated Workflows to save, share and export




## Data Annotation & Processing Tool calculate differential expression and upload




## Pathway Map Creator customize and build maps

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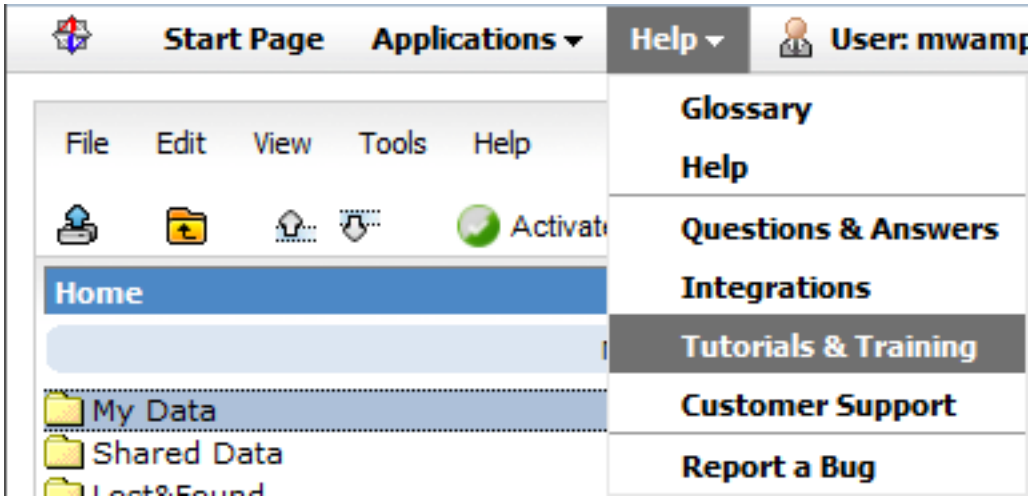
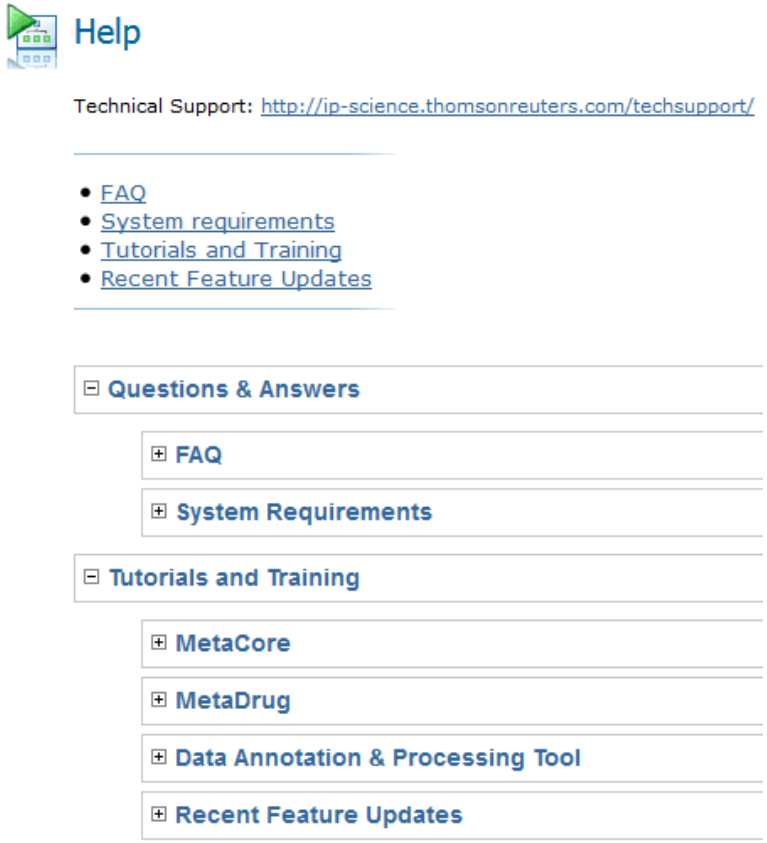
# Getting support

Technical Support & questions:
sbsupport@thomsonreuters.com

Training webinar recordings available at:
lsresearch.thomsonreuters.com
(in the Knowledge section)

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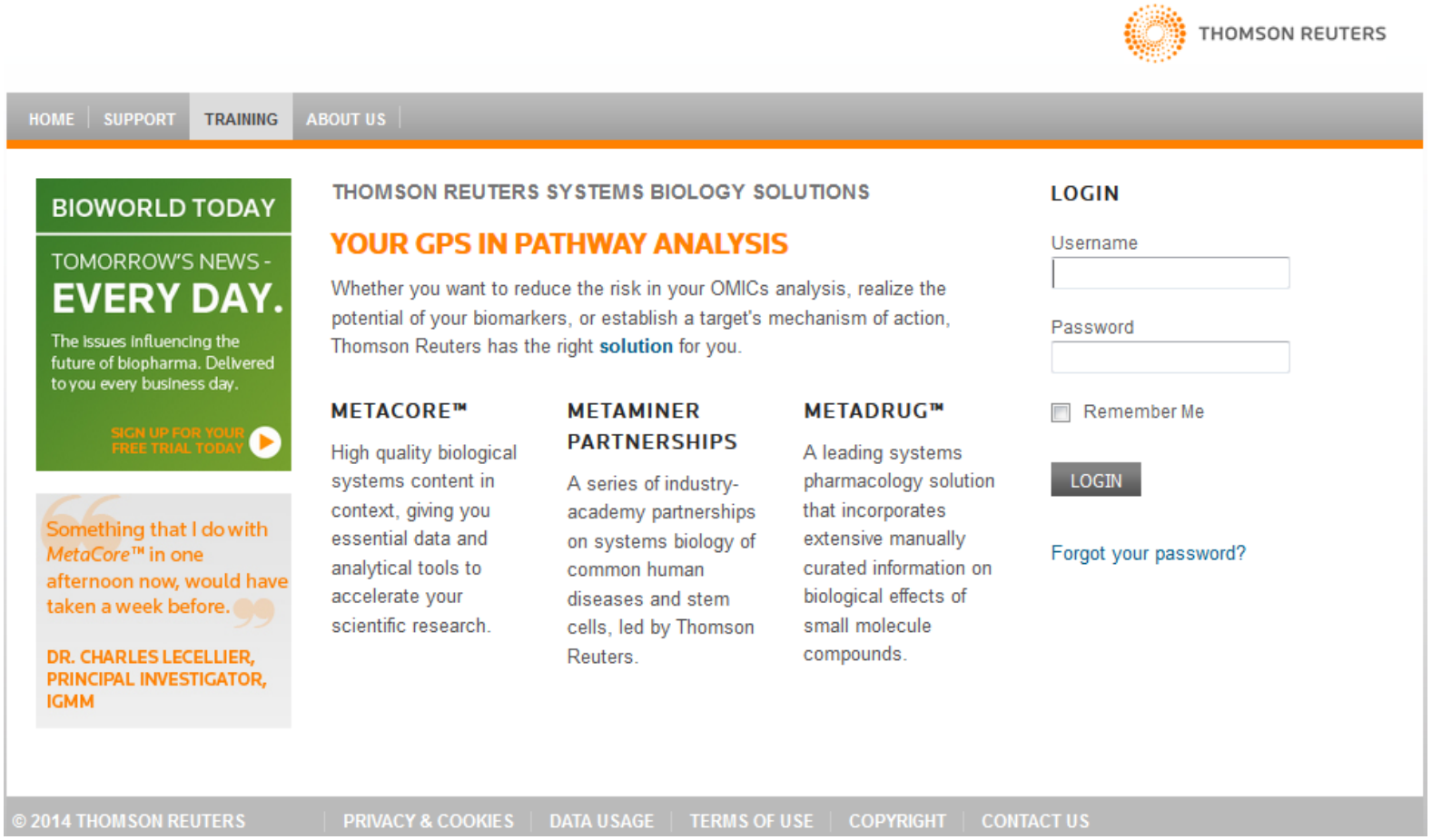
# Login to MetaCore

Go to portal.genego.com

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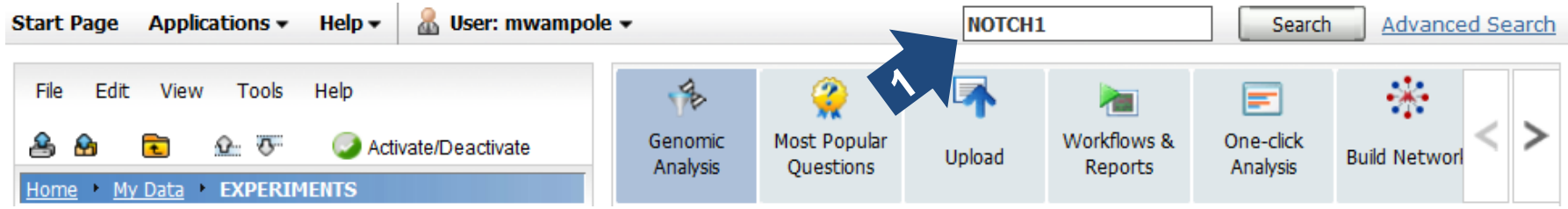
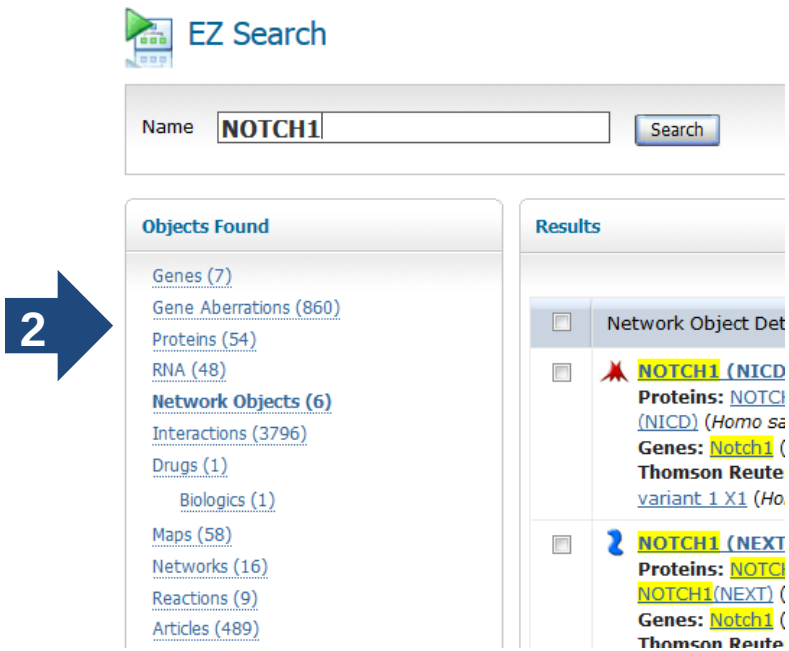
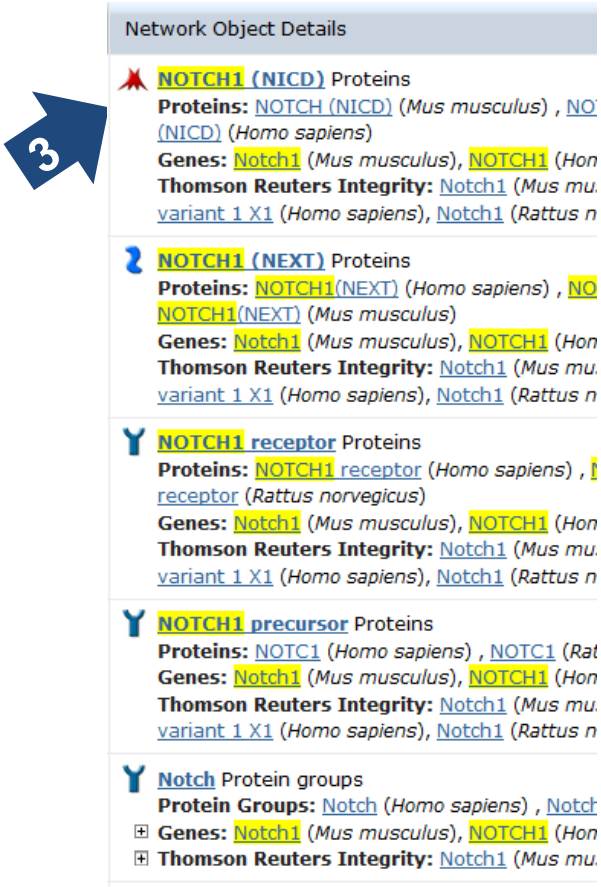
# Use EZ search to look up NOTCH1

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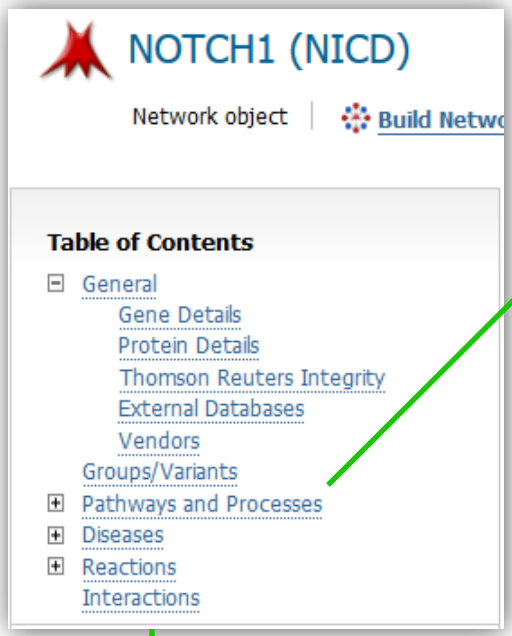
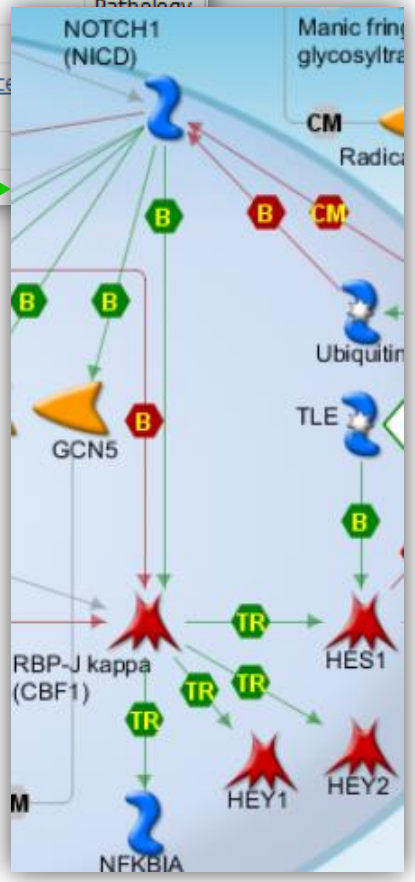
# I want to know what interactions and pathways involve NOTCH1




| # | Name | Map Type |
|---|---|---|
| 1 | Aberrant B-Raf signaling in melanoma progression | Pathology |
| 2 | Aberrant production of IL-2 and IL-17 in SLE T cells | Pathology |
| 3 | Activation of Notch signaling in breast cancer | |
| 4 | Canonical Notch signaling pathway in colorectal cancer | |
| 5 | Development_Epigenetic and transcriptional regulation of oligodendrocyte precursor ce differentiation and myelination | |
| 6 | Development_Keratinocyte differentiation | |
| 7 | Development_Notch Signaling Pathway | |




- NOTCH1 (NICD) represents cleaved intracellular portion of NOTCH1 after ligand binding.
- This region is important in the regulation of a number of genes.
- Aberrations in NOTCH1 have been associated with many diseases including chronic lymphocytic leukemia

| # | From | | To | Direction | Effect | Mechanism | Link info |
|---|---|---|---|---|---|---|---|
| 1 | NOTCH1 (NICD) | TR | c-IAP2 | Outgoing | Activation | Transcription regulation | |
| 2 | NOTCH1 (NICD) | TR | CD133 | Outgoing | Activation | Transcription regulation | |
| 3 | NOTCH1 (NICD) | TR | CDK4 | Outgoing | Activation | Transcription regulation | |
| 4 | NOTCH1 (NICD) | TR | CDK6 | Outgoing | Activation | Transcription regulation | |

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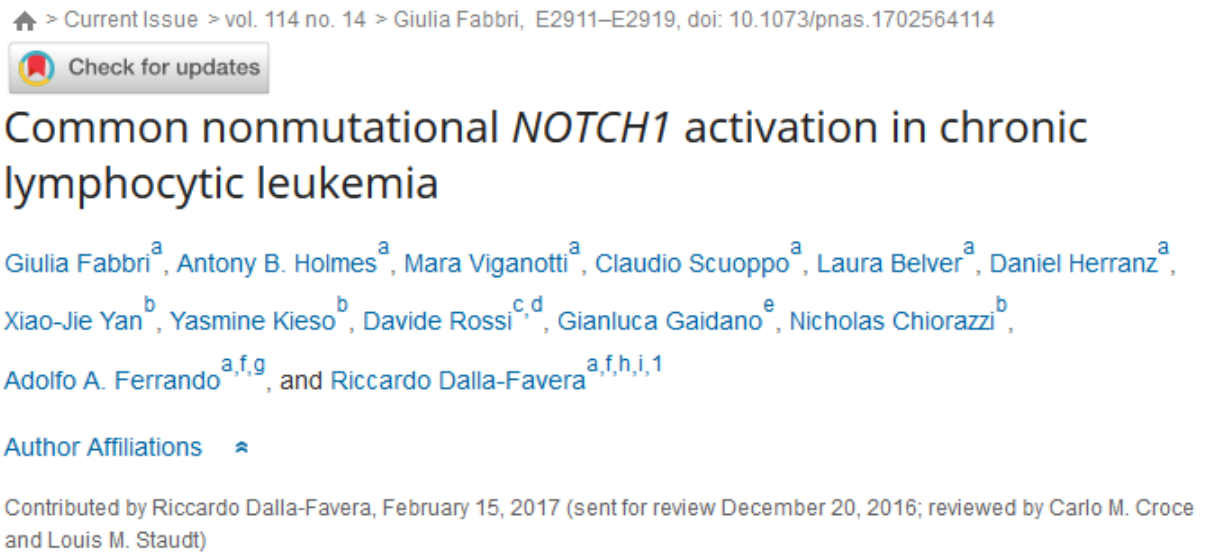
# Live Demo



> Current Issue > vol. 114 no. 14 > Giulia Fabbri, E2911–E2919, doi: 10.1073/pnas.1702564114

Check for updates

## Common nonmutational *NOTCH1* activation in chronic lymphocytic leukemia

Giulia Fabbri[a], Antony B. Holmes[a], Mara Viganotti[a], Claudio Scuoppo[a], Laura Belver[a], Daniel Herranz[a], Xiao-Jie Yan[b], Yasmine Kieso[b], Davide Rossi[c,d], Gianluca Gaidano[e], Nicholas Chiorazzi[b], Adolfo A. Ferrando[a,f,g], and Riccardo Dalla-Favera[a,f,h,i,1]

Author Affiliations

Contributed by Riccardo Dalla-Favera, February 15, 2017 (sent for review December 20, 2016; reviewed by Carlo M. Croce and Louis M. Staudt)

GSE92626 – Identification of genes induced by NOTCH1 in a chronic lymphocytic leukemia (CLL) cell line and tracking of these genes in primary CLL patients

- Illumina HiSeq 2000 – RNA-seq expression analysis

GSE92701 – Identification of NOTCH1 bound genes in chronic lymphocytic leukaemia (CLL) cells

- Illumina HiSeq 2000 – ChIP-seq expression analysis

Gene Expression Omnibus

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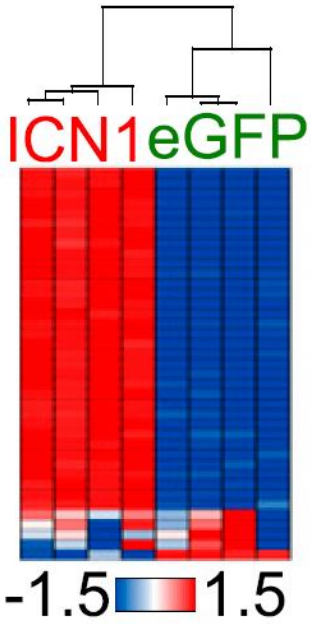
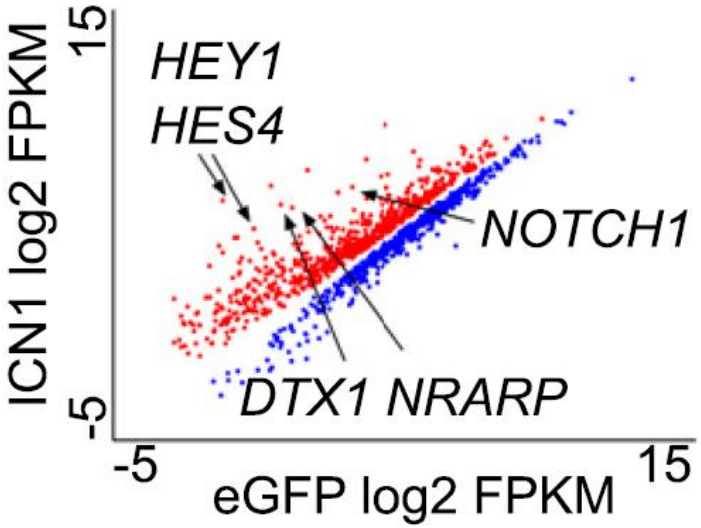
# RNA-seq data

- CLL cell line (MO1043) was used for NOTCH1 signaling induction
- MO1043 was either induced with a lentiviral system expressing either:
  - NOTCH1 intracellular region (ICN1)
  - Control eGFP
- Over 700 up-regulated transcripts were identified
  - Median fold change 1.7
  - False Discover Rate < 0.001




Hierarchical clustering of RNA-Seq profiles of MO1043 cells induced with NOTCH1 (ICN1) or eGFP




Scatter plot of log2-transformed RNA-Seq FPKM values of differentially expressed genes between MO1043 cells induced with ICN1 and -eGFP control CLL cells (FDR < 0.001).

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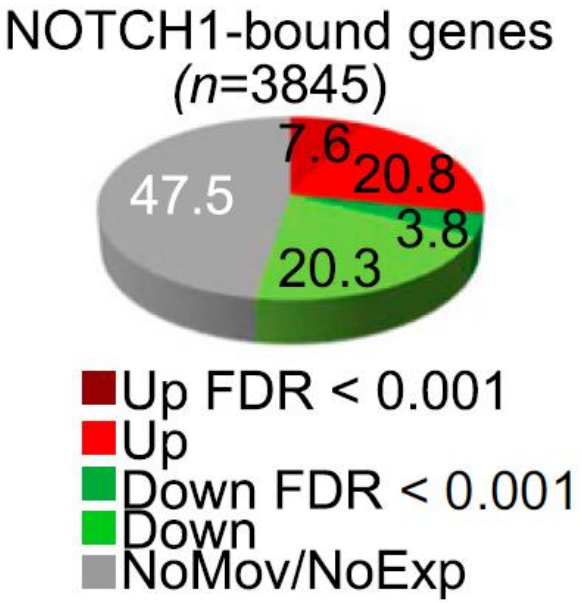
# ChIP-seq data and intersection with RNA-seq data

- ChIP-seq identified 4,737 NOTCH1 binding sites.
- Intersection of RNA-seq and ChIP-seq data was used to create ICN1 induced signature
    - Genes bound by NOTCH1 in ChIP-seq data
    - Genes upregulated via ICN1 induction with a FDR < 0.001




Intersection between RNA-Seq and ChIP-Seq data obtained in ICN1 induced MO1043 CLL cells

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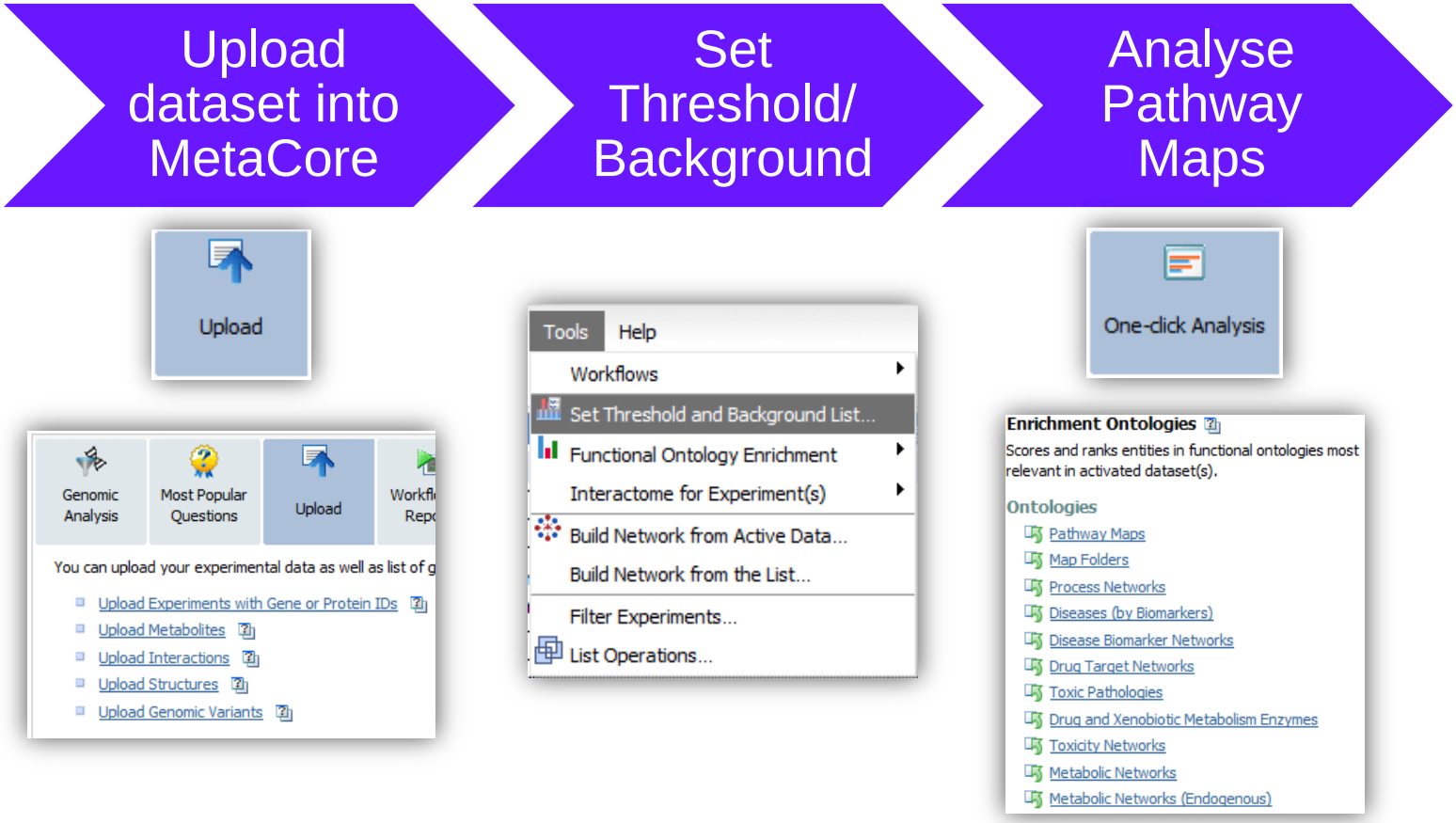
# Upload data and run a pathway enrichment




Question:

- What pathways are enriched by ICN1 from RNA-seq and bound to NOTCH1 in ChIP-seq experiments?

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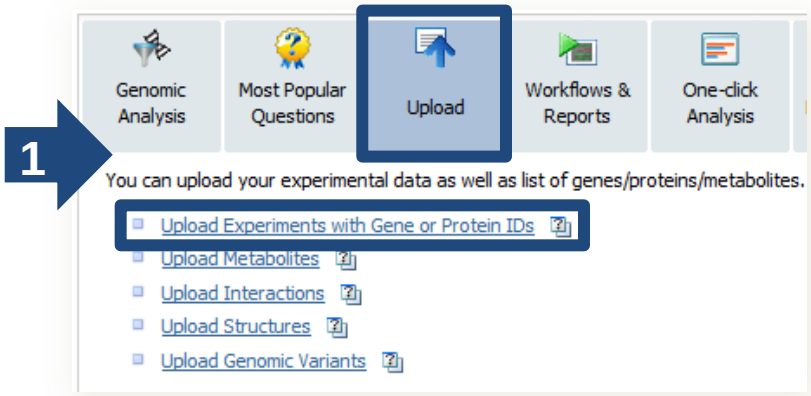
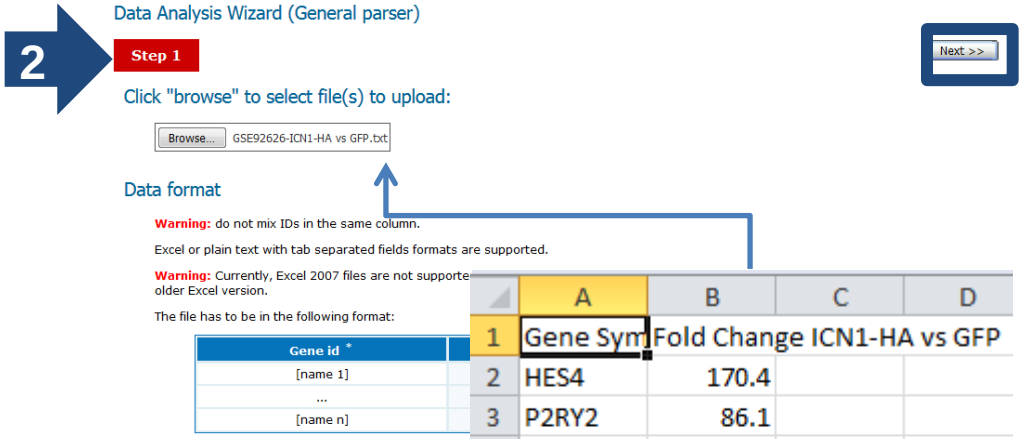
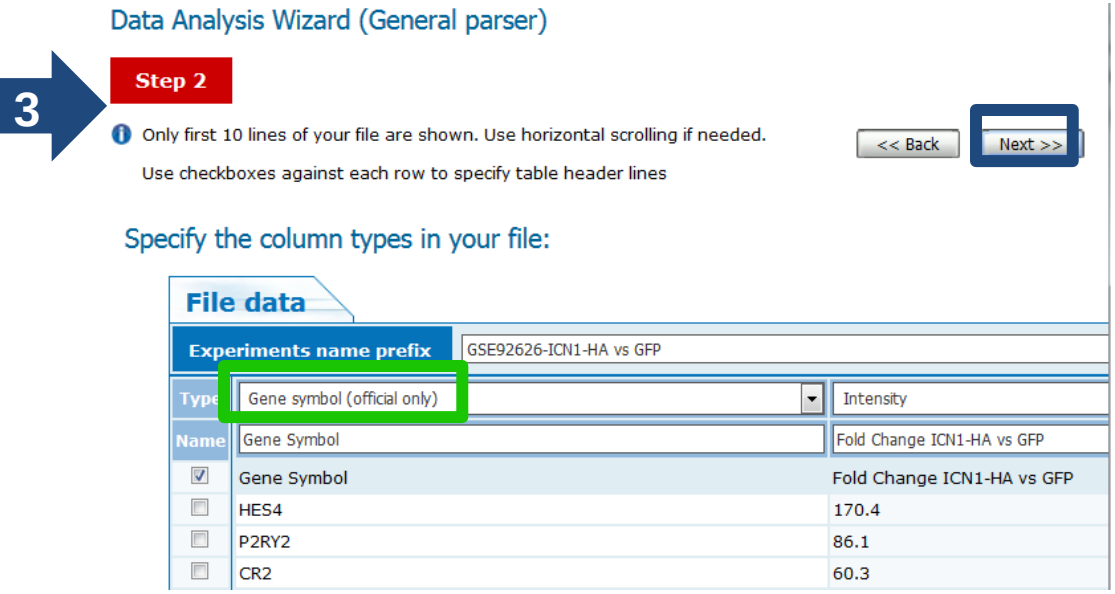
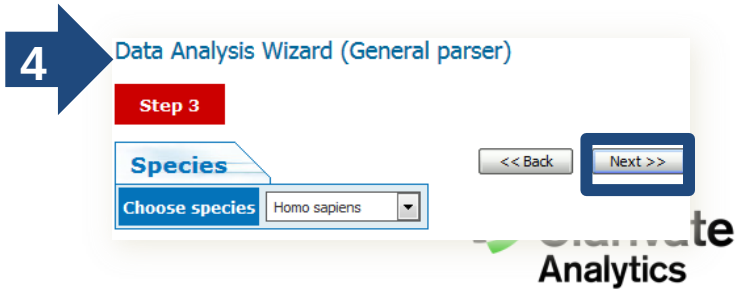
# Upload RNA-seq expression data into MetaCore



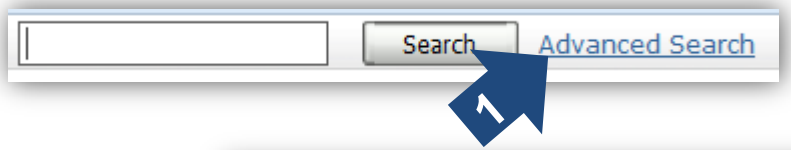



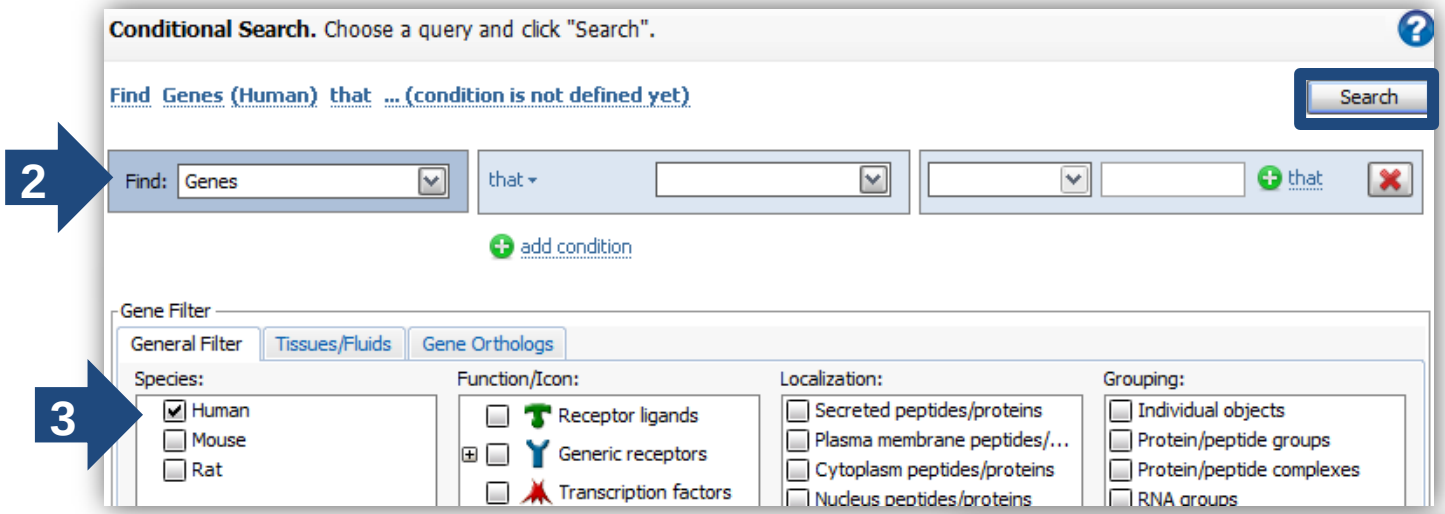



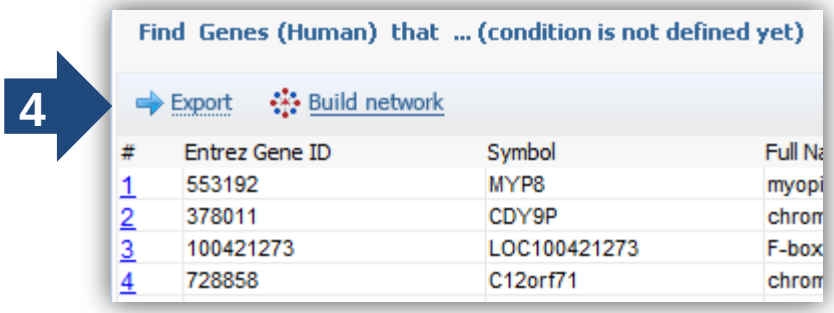



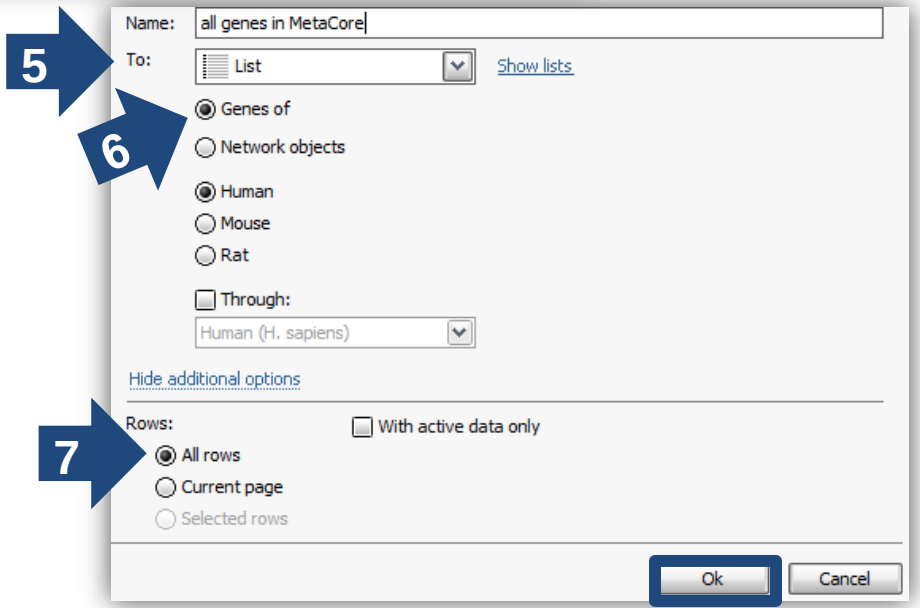

Note: use OFFICIAL gene symbols

# Create background list for RNA-seq

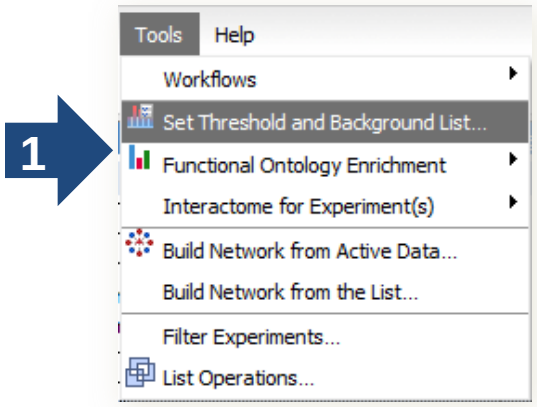


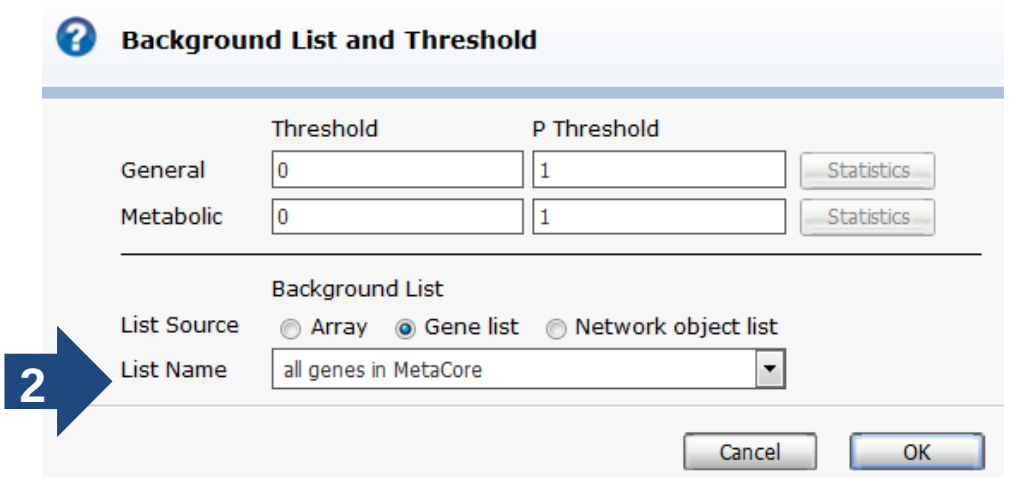



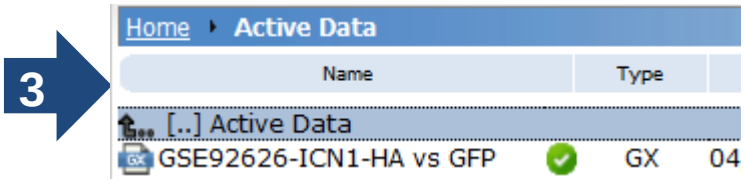



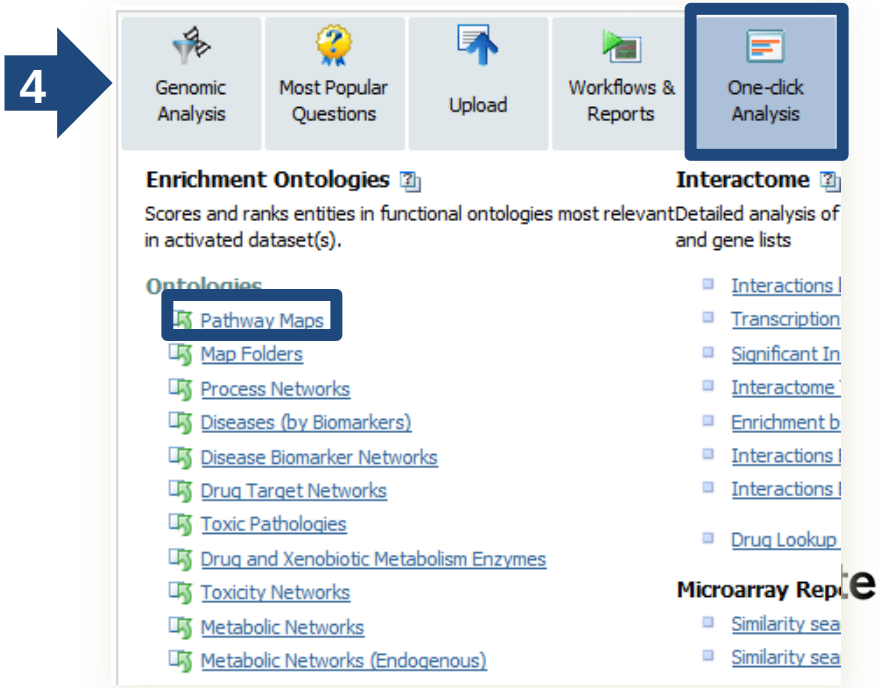



# Set background list and run pathway map enrichment



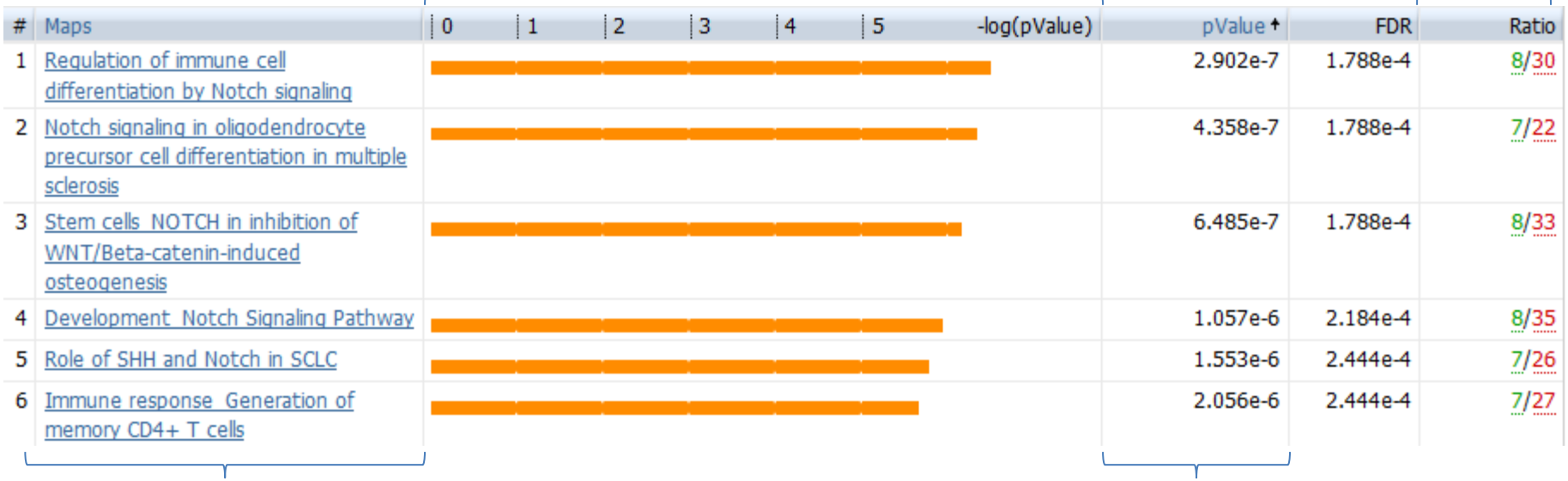



# What pathways are enriched by NOTCH1 induced up-regulation signature?

Ratio of differentially expressed genes from dataset (**GREEN**) over all network objects in folder (**RED**)

Graphical representation of –log(pValue)



| # | Maps | 0 1 2 3 4 5 -log(pValue) | pValue | FDR | Ratio |
|---|---|---|---|---|---|
| 1 | Regulation of immune cell differentiation by Notch signaling | | 2.902e-7 | 1.788e-4 | 8/30 |
| 2 | Notch signaling in oligodendrocyte precursor cell differentiation in multiple sclerosis | | 4.358e-7 | 1.788e-4 | 7/22 |
| 3 | Stem cells_NOTCH in inhibition of WNT/Beta-catenin-induced osteogenesis | | 6.485e-7 | 1.788e-4 | 8/33 |
| 4 | Development_Notch Signaling Pathway | | 1.057e-6 | 2.184e-4 | 8/35 |
| 5 | Role of SHH and Notch in SCLC | | 1.553e-6 | 2.444e-4 | 7/26 |
| 6 | Immune response_Generation of memory CD4+ T cells | | 2.056e-6 | 2.444e-4 | 7/27 |

Map name

Significance of overlap of differentially expressed genes in the folder

- Notch related signaling pathways are disrupted by this signature
- Could impact many processes related to immune cell processes in diseases ranging from multiple sclerosis to cancer.

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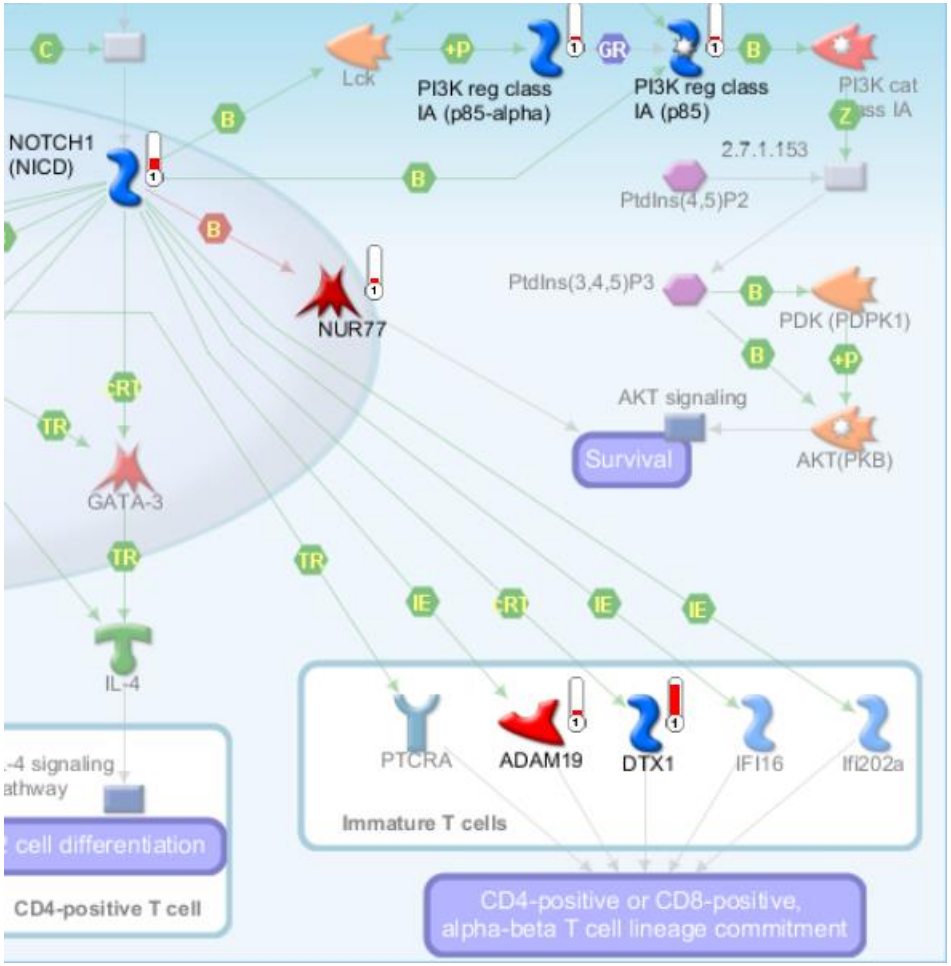
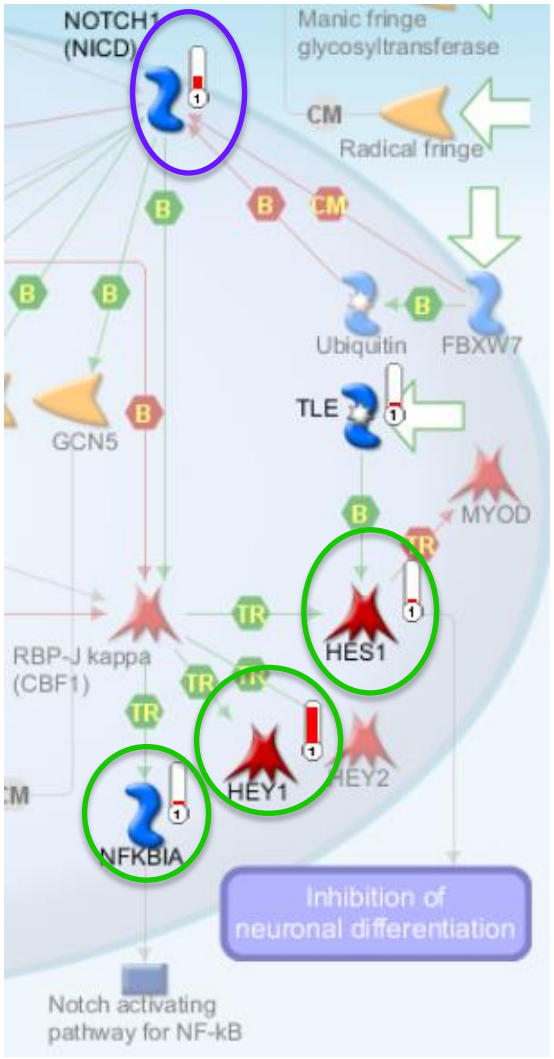
# How could this signature be impacting cellular processes?




Regulation of immune cell differentiation by Notch signaling




Development_Notch Signaling Pathway

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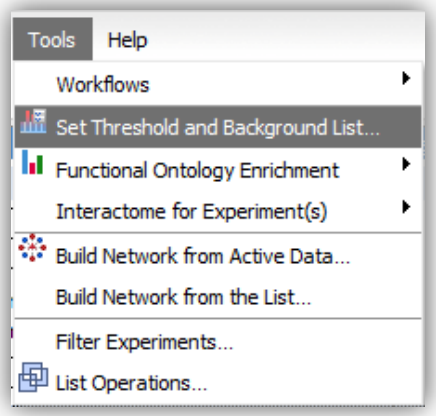
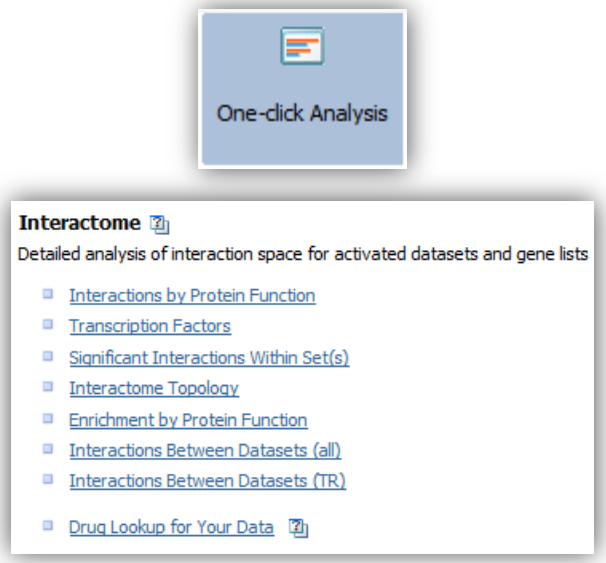
# How to find transcription factors potentially regulating expression data

Set Threshold/ Background

Interactome Analysis (Transcription Factors)



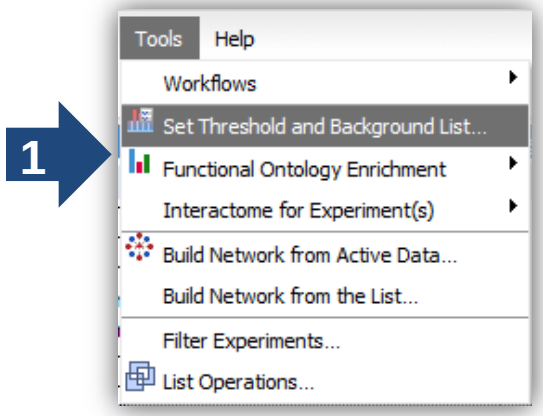



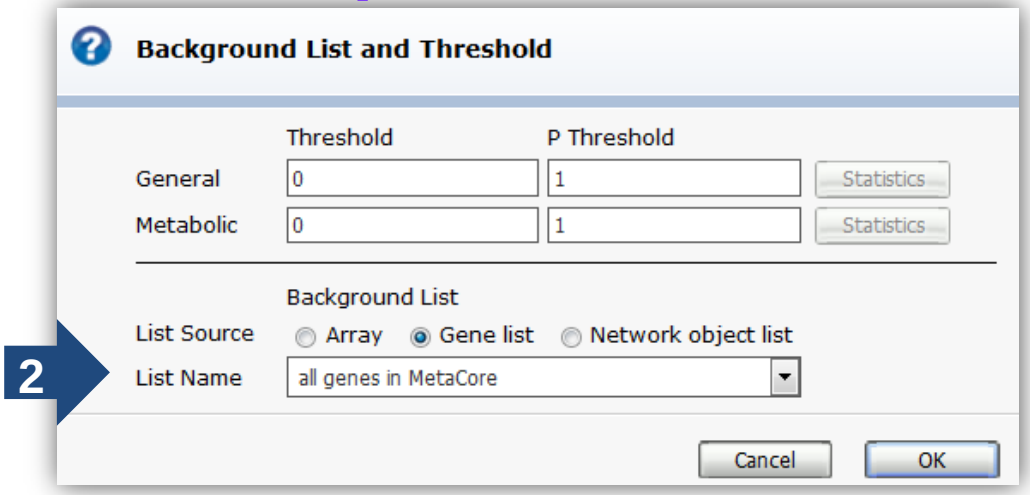

Question:

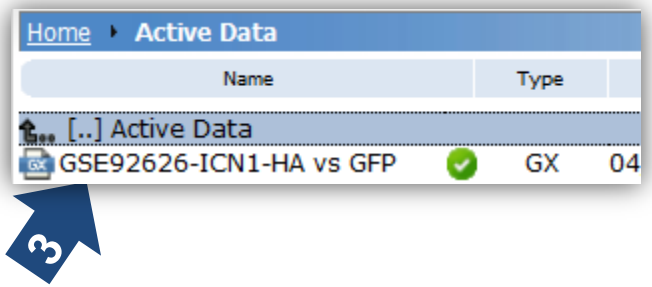
- What transcription factors could these up regulated genes regulated by?

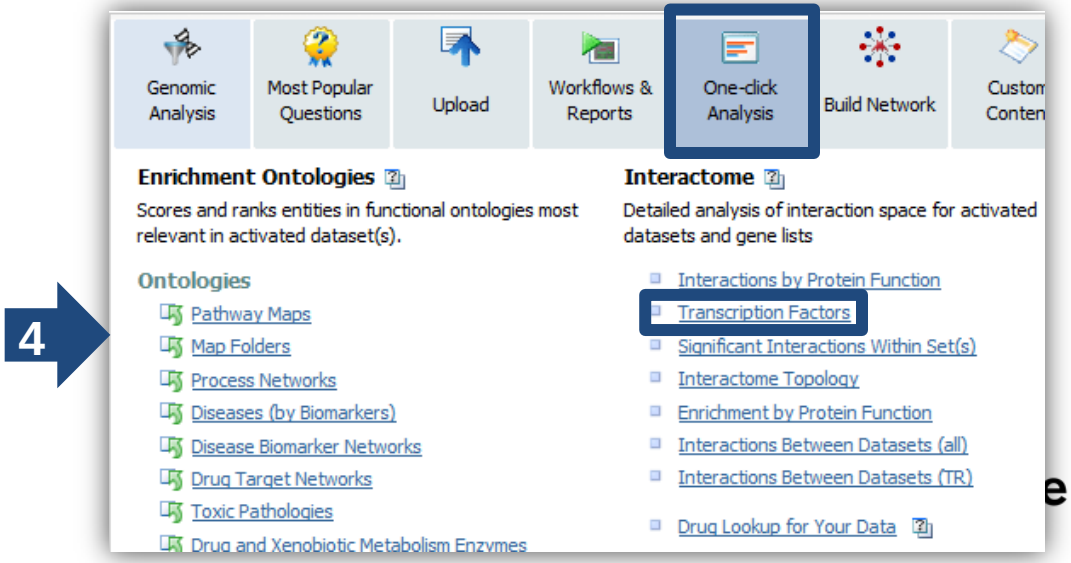
Clarivate Analytics

# Set threshold/background and run interactome analysis (Transcription Factors)



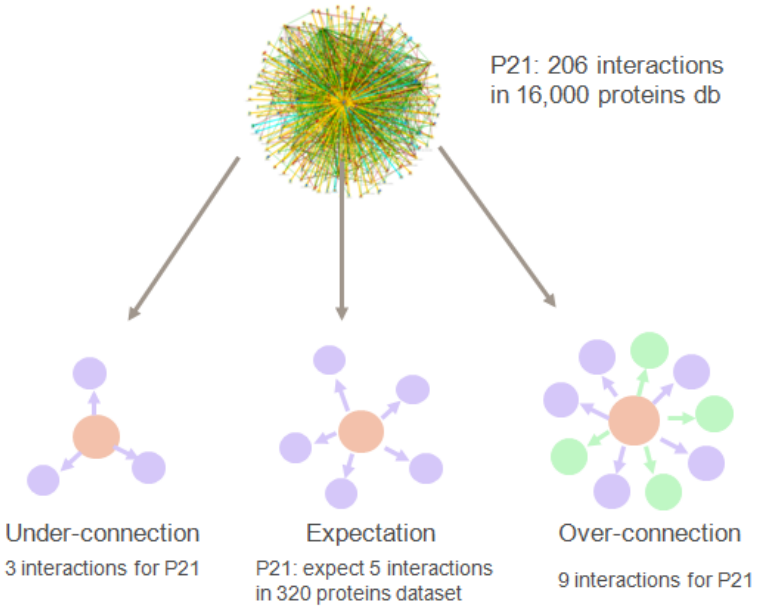
# How does interactome analysis work?

Export

Active data set only | Show all columns

| IDs in active data set | Object name | Actual | n | R | N | Expected | Ratio | p-value | z-score |
|---|---|---|---|---|---|---|---|---|---|
| 202531_at; 238725_at | IRF1 | 45 | 371 | 199 | 19137 | 3.858 | 11.66 | 4.462e-35 | 21.26 |

**N** – Total number of genes in the database (or in array background list)
**R** – Total number of genes being regulated by the TRANSCRIPTION FACTOR
**n** – Total number of genes in the dataset
**ACTUAL** – genes in dataset being regulated by this transcription factor
**EXPECTED** – (**R** / **N**) * **n**
**RATIO** – **ACTUAL** / **EXPECTED**

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# More then just RBP-J are affect by NOTCH1

| IDs in active data set | Object name | Actual | n | R | N | Expected | Ratio | p-value | z-score |
|---|---|---|---|---|---|---|---|---|---|
| | CREB1 | 111 | 289 | 5126 | 38450 | 38.53 | 2.881 | 8.74e-27 | 12.59 |
| | c-Myc | 72 | 289 | 2522 | 38450 | 18.96 | 3.798 | 2.85e-23 | 12.65 |
| | RelA (p65 NF-kB subunit) | 42 | 289 | 939 | 38450 | 7.058 | 5.951 | 1.829e-20 | 13.37 |
| | p53 | 47 | 289 | 1283 | 38450 | 9.643 | 4.874 | 2.506e-19 | 12.28 |
| | ESR1 (nuclear) | 40 | 289 | 1289 | 38450 | 9.688 | 4.129 | 3.671e-14 | 9.943 |
| | SP1 | 45 | 289 | 1648 | 38450 | 12.39 | 3.633 | 7.311e-14 | 9.507 |
| | STAT3 | 29 | 289 | 708 | 38450 | 5.322 | 5.45 | 1.918e-13 | 10.4 |
| IRF8 | IRF8 | 18 | 289 | 258 | 38450 | 1.939 | 9.282 | 1.497e-12 | 11.62 |
| | HIF1A | 28 | 289 | 726 | 38450 | 5.457 | 5.131 | 2.149e-12 | 9.78 |
| | IRF4 | 21 | 289 | 434 | 38450 | 3.262 | 6.438 | 2.188e-11 | 9.914 |
| | PU.1 | 20 | 289 | 399 | 38450 | 2.999 | 6.669 | 3.551e-11 | 9.906 |
| | FOXP3 | 17 | 289 | 282 | 38450 | 2.12 | 8.02 | 6.348e-11 | 10.3 |
| | EBF | 12 | 289 | 126 | 38450 | 0.947 | 12.67 | 2.352e-10 | 11.42 |
| | SRF | 19 | 289 | 413 | 38450 | 3.104 | 6.121 | 4.692e-10 | 9.105 |
| | AHR | 16 | 289 | 286 | 38450 | 2.15 | 7.443 | 6.964e-10 | 9.518 |
| | PAX5 | 12 | 289 | 145 | 38450 | 1.09 | 11.01 | 1.203e-09 | 10.51 |
| | c-Jun | 26 | 289 | 835 | 38450 | 6.276 | 4.143 | 1.338e-09 | 7.99 |
| | Ikaros | 9 | 289 | 71 | 38450 | 0.5337 | 16.86 | 3.349e-09 | 11.64 |
| | NF-kB1 (p50) | 19 | 289 | 473 | 38450 | 3.555 | 5.344 | 4.358e-09 | 8.273 |
| | EGR1 | 18 | 289 | 454 | 38450 | 3.412 | 5.275 | 1.371e-08 | 7.974 |
| | RBP-J kappa (CBF1) | 11 | 289 | 145 | 38450 | 1.09 | 10.09 | 1.484e-08 | 9.547 |
| | STAT1 | 16 | 289 | 361 | 38450 | 2.713 | 5.897 | 1.914e-08 | 8.135 |
| | SMAD3 | 14 | 289 | 268 | 38450 | 2.014 | 6.95 | 1.955e-08 | 8.506 |
| NR3C1 | GCR | 18 | 289 | 488 | 38450 | 3.668 | 4.907 | 4.103e-08 | 7.56 |
| | p63 | 17 | 289 | 445 | 38450 | 3.345 | 5.083 | 5.955e-08 | 7.538 |

Highlights of top 3 Transcription Factors the authors found via Transfac enrichment

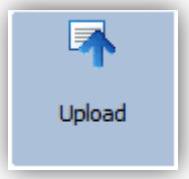
Clarivate Analytics

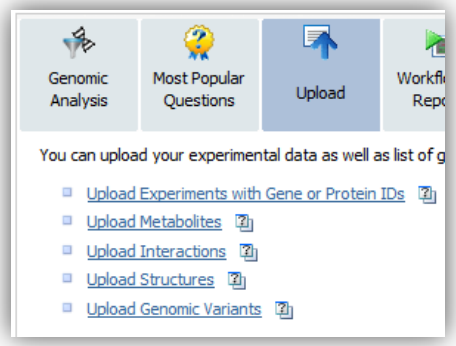
# Upload data and run enrichment analysis

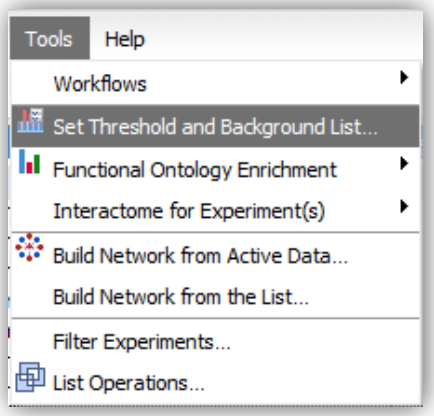
Upload dataset into MetaCore

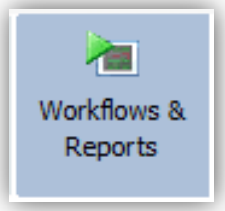
Set Threshold/ Background

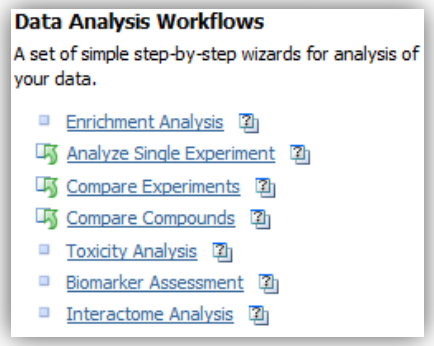
Enrichment Analysis



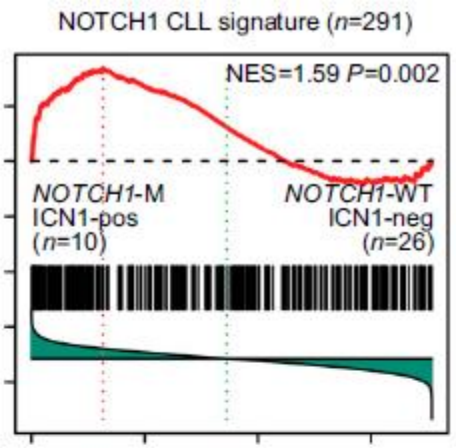




Question:

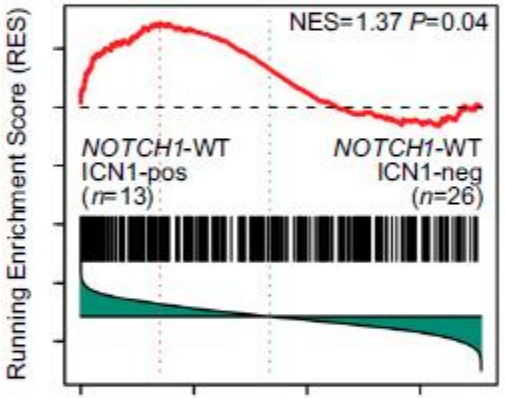
- How does the NOTCH1 expression profile compare with RNA-seq data from chronic lymphocytic leukemia?

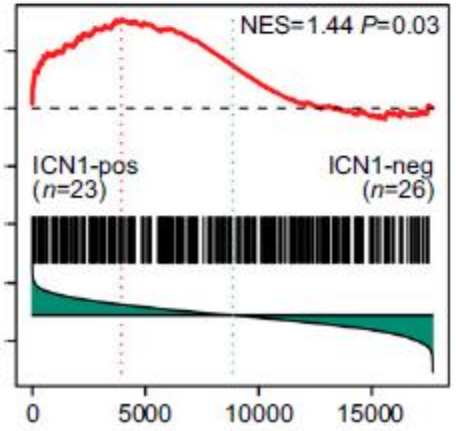
Clarivate Analytics

# RNA-seq expression from peripheral blood of chronic lymphocytic leukemia patients

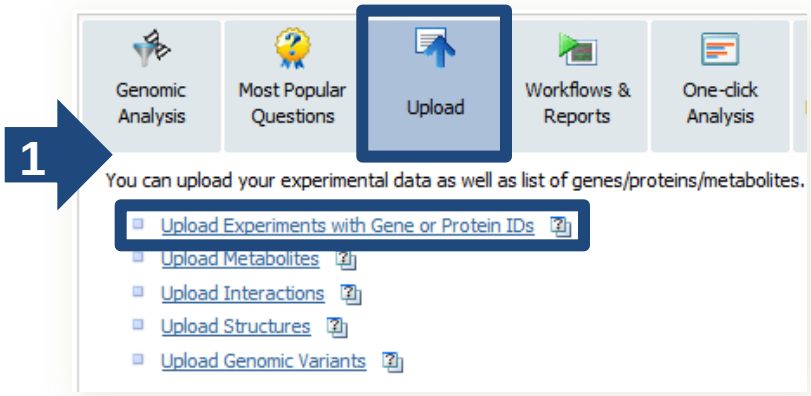


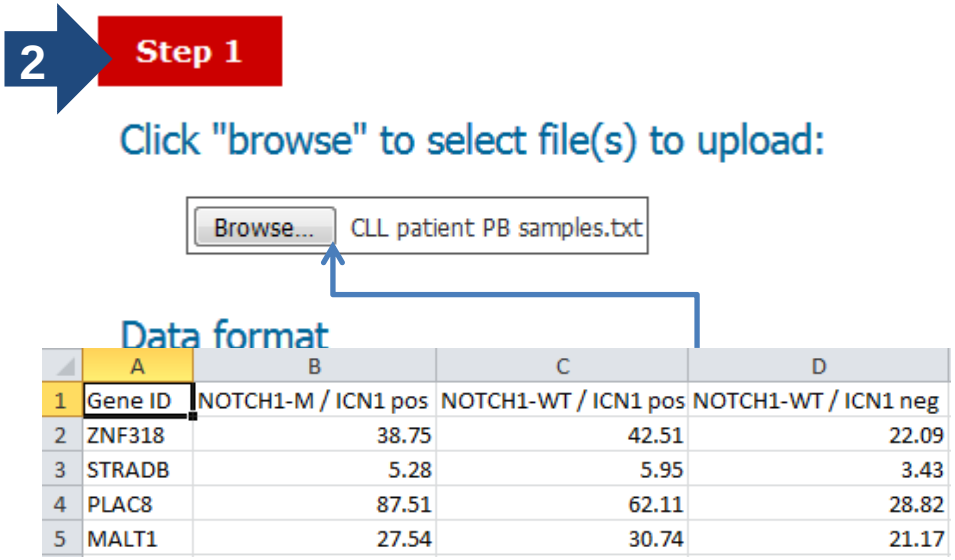

- RNA-seq expression data from 49 samples characterized with NOTCH1 mutational status and ICN1 expression
  - 10 cases mutated NOTCH1 with ICN1 expression
  - 13 cases wildtype NOTCH1 with ICN1 expression
  - 26 cases wildtype NOTCH1 without ICN1 expression
- GSEA analysis found significant enrichment of NOTCH1 CLL signature in both NOTCH1 mutated and NOTCH1 wildtype expression ICN1 as compared to NOTCH1 wildtype not expressing ICN1.
- Data uploaded into MetaCore are the 90 leading edge genes for each group with mean FMPK values



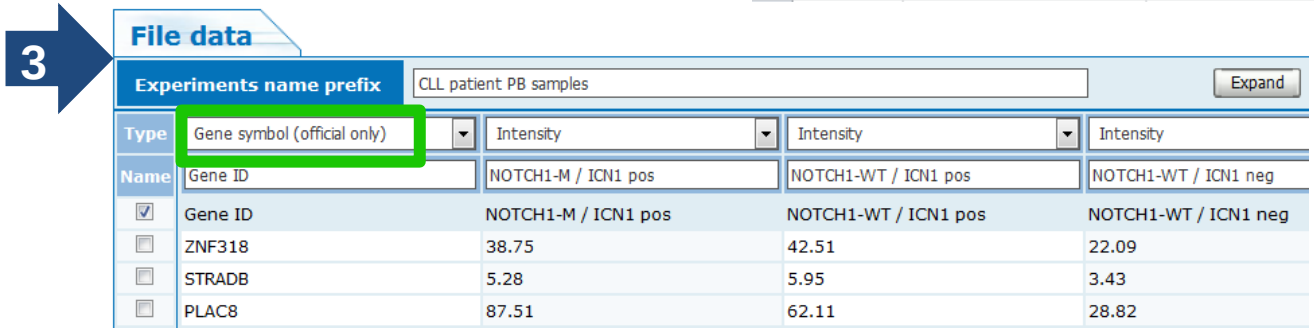



# Upload RNA-seq expression data into MetaCore




| | A | B | C | D |
|---|---|---|---|---|
| 1 | Gene ID | NOTCH1-M / ICN1 pos | NOTCH1-WT / ICN1 pos | NOTCH1-WT / ICN1 neg |
| 2 | ZNF318 | 38.75 | 42.51 | 22.09 |
| 3 | STRADB | 5.28 | 5.95 | 3.43 |
| 4 | PLAC8 | 87.51 | 62.11 | 28.82 |
| 5 | MALT1 | 27.54 | 30.74 | 21.17 |

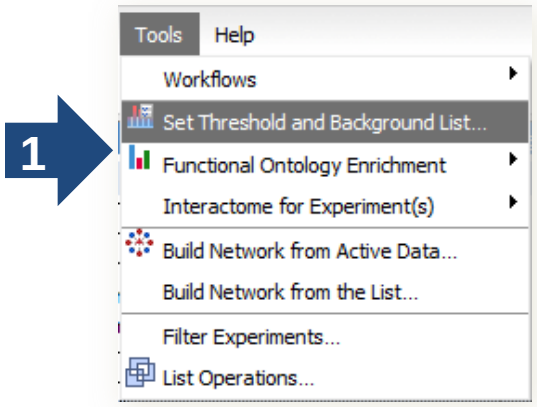


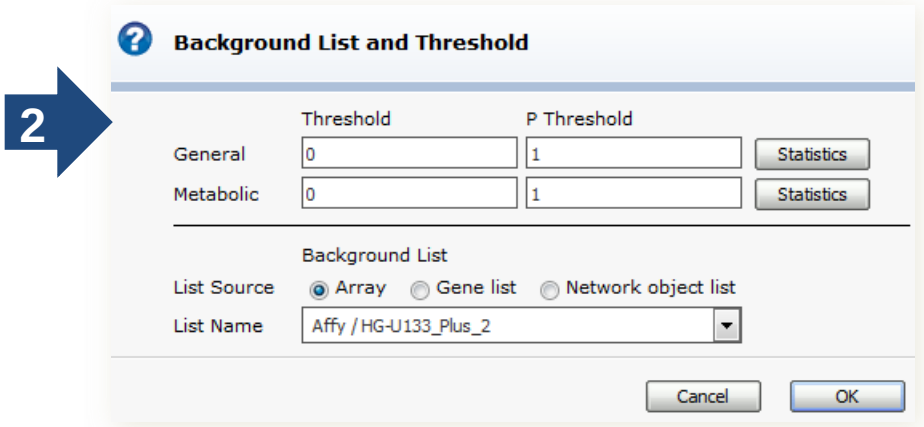

| | | | | |
|---|---|---|---|---|
| ☑ | Gene ID | NOTCH1-M / ICN1 pos | NOTCH1-WT / ICN1 pos | NOTCH1-WT / ICN1 neg |
| ☐ | ZNF318 | 38.75 | 42.51 | 22.09 |
| ☐ | STRADB | 5.28 | 5.95 | 3.43 |
| ☐ | PLAC8 | 87.51 | 62.11 | 28.82 |

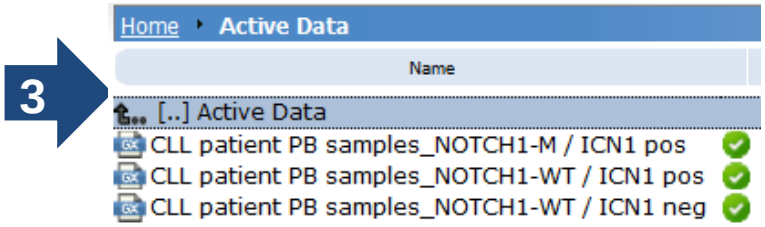
Note: use OFFICIAL gene symbols

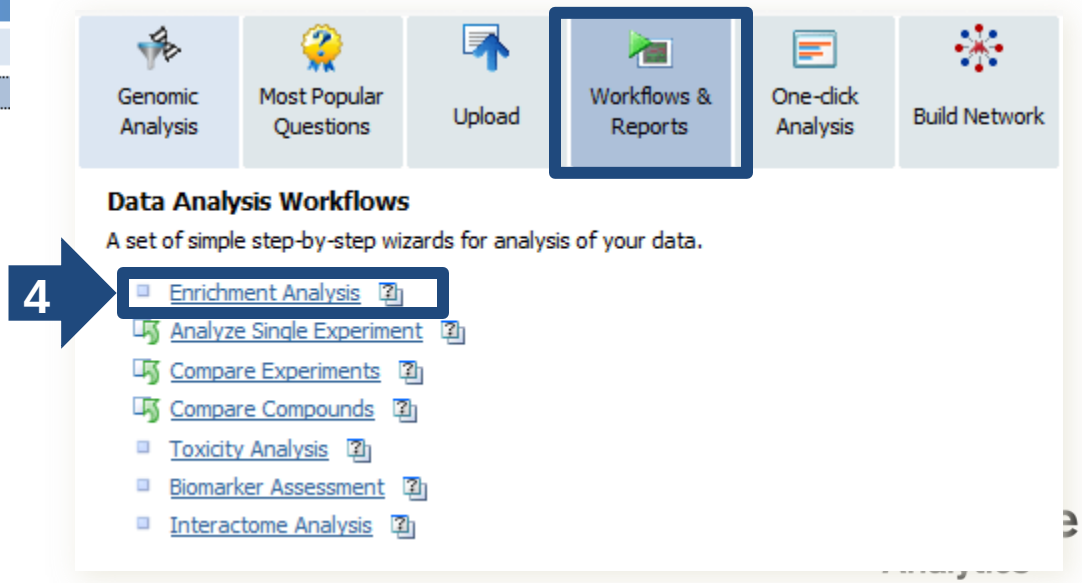
Clarivate Analytics

# Set threshold/background and run ‘Compare Experiments’ workflow

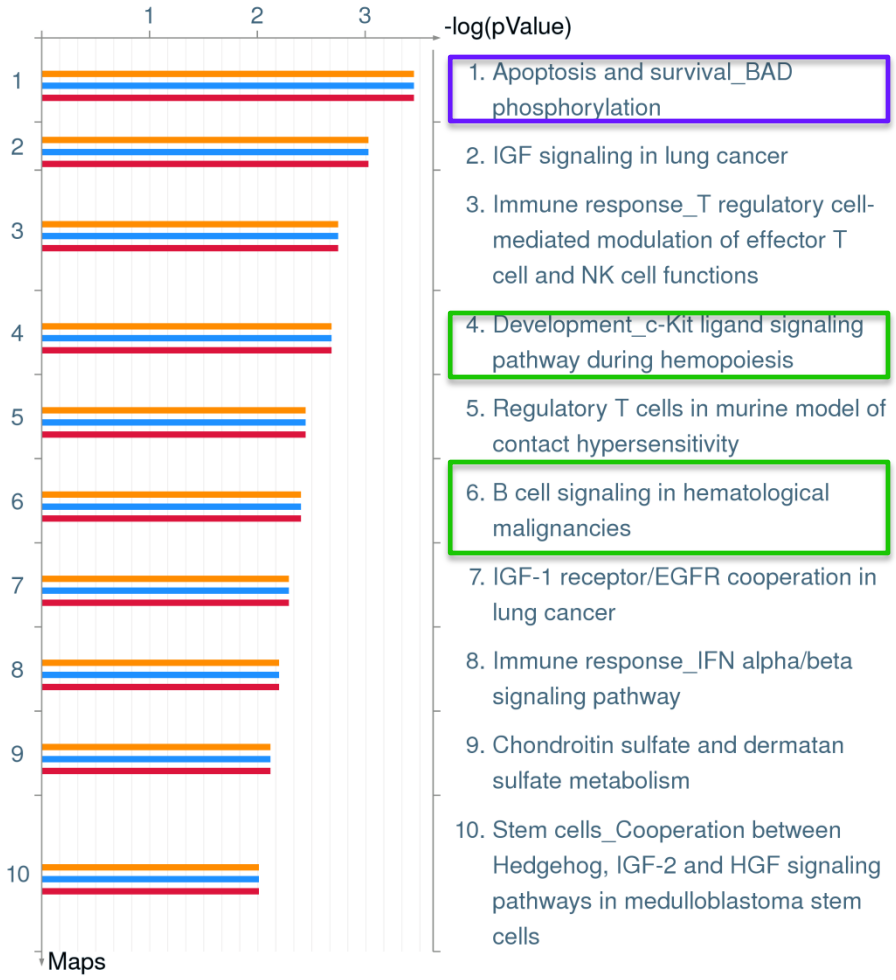


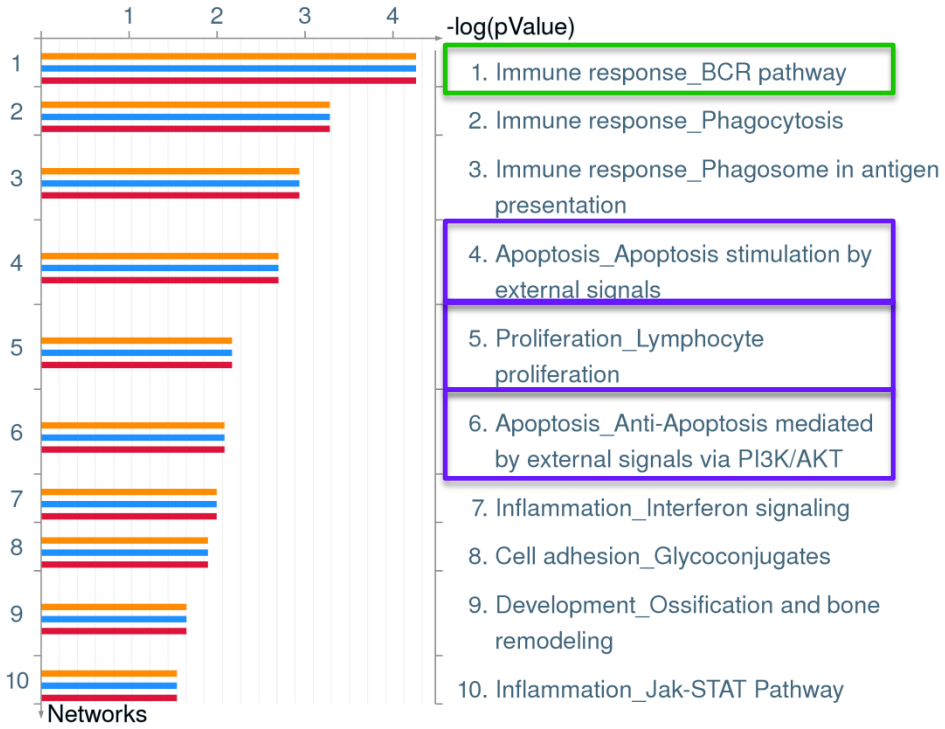



# What pathways are affected by genes unique to patients with a low count of infiltrating T-cells?



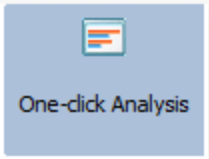



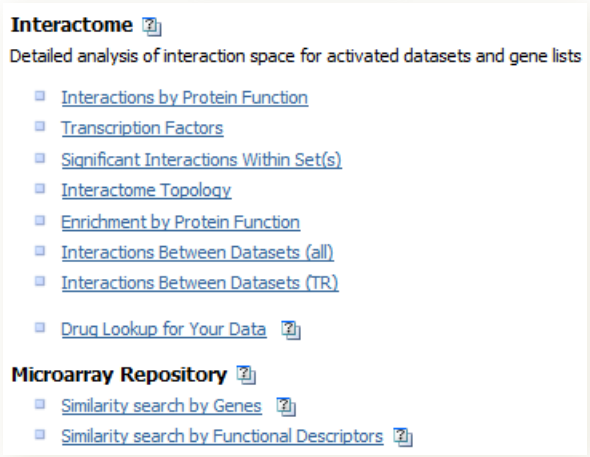

The leading edge genes pull a number of maps and processes related to **B cell signaling** and **apoptotic** processes

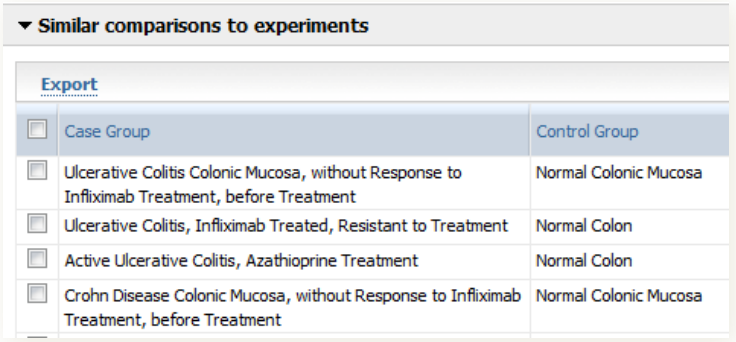
Clarivate Analytics

# Use similarity search by genes to find datasets with similar gene signatures

Similarity Search by Genes → Export Similar Comparisons



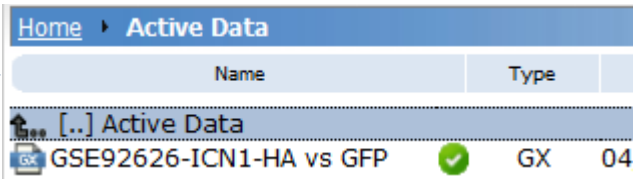

Question:

- What other publicly available datasets have a similar to the NOTCH1 expression profile?

Clarivate Analytics

# Run similarity search by gene analysis and choose filtering thresholds

1

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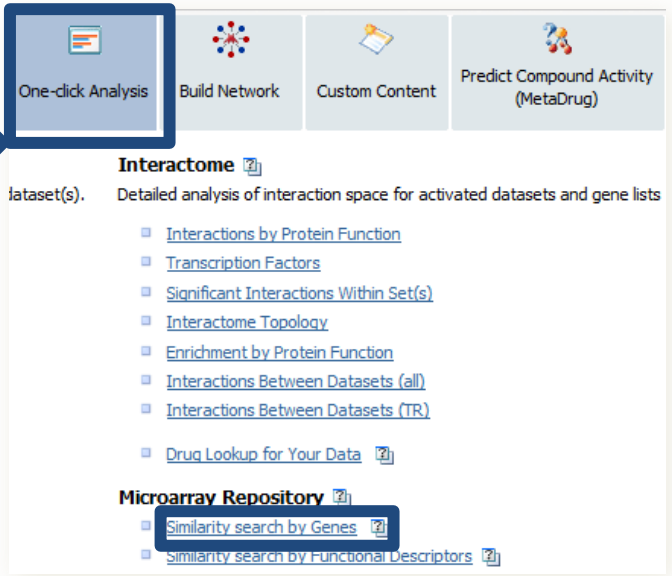
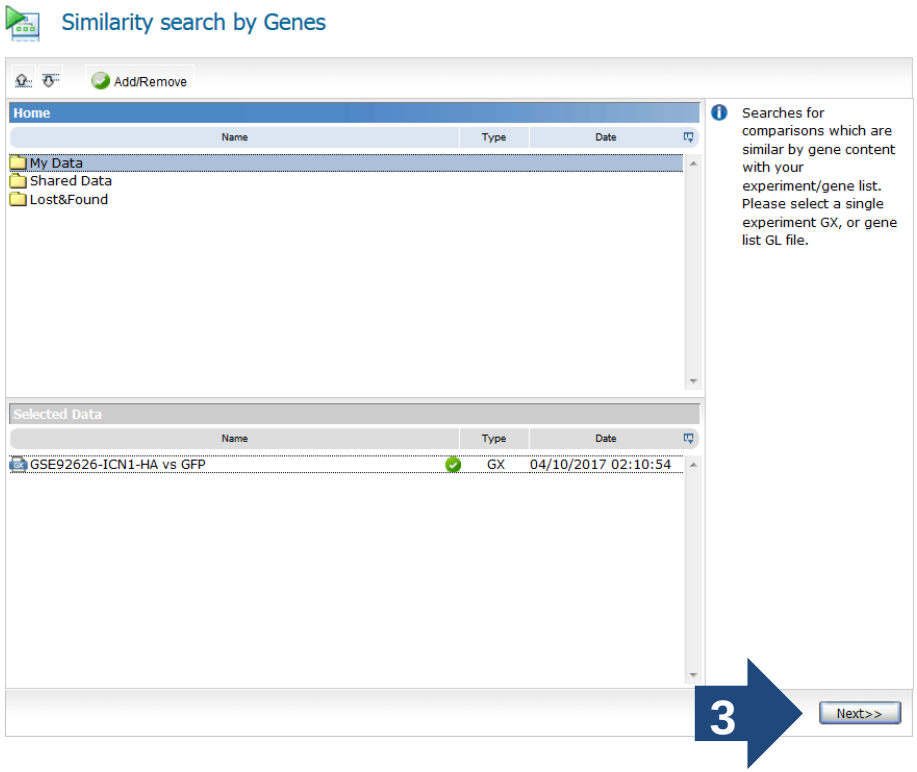
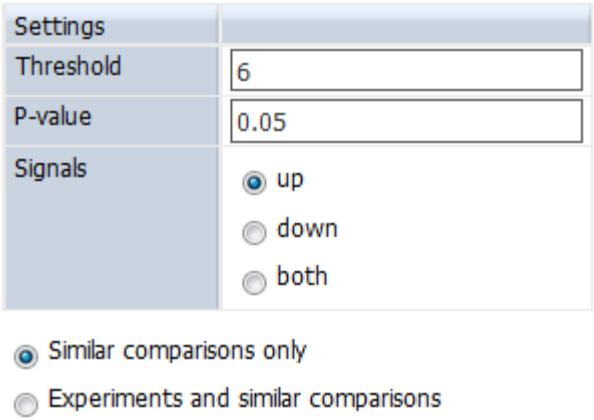



3

4

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# Other public data with a similar signature to the NOTCH1 induced gene signature

| Case Group | Control Group | Species | Gene Overlap | p-Value | View |
|---|---|---|---|---|---|
| Colorectal Cancer Leukocytes (CD45+ EpCAM-) | Colorectal Cancer Stromal Cells (CD45- EpCAM-) | Homo sapiens | 52/1038 | 7.556e-37 | |
| Choroideremia CD14+ Monocytes | Choroideremia Cultured Fibroblasts | Homo sapiens | 39/562 | 5.322e-33 | |
| Severe Asthma CD8+ T-Lymphocytes | Non-Severe Asthma CD8+ T-Lymphocytes | Homo sapiens | 40/733 | 8.386e-30 | |
| Severe Asthma CD4+ T-Lymphocytes | Non-Severe Asthma CD4+ T-Lymphocytes | Homo sapiens | 33/521 | 9.224e-27 | |
| Cultured B-Lymphocytes from B-Cell Chronic Lymphocytic Leukemia under Dimethyl Sulfoxide (DMSO) Control | Normal Cultured T-Lymphocytes under Dimethyl Sulfoxide (DMSO) Control | Homo sapiens | 26/334 | 1.443e-23 | |
| Normal Monocytes, 1.5 Hours IFNA2 Stimulated | Normal Monocytes without Any Incubation | Homo sapiens | 23/243 | 6.396e-23 | |
| Normal B-Lymphocytes | Normal Plasma Cells | Homo sapiens | 23/263 | 3.943e-22 | |
| Psoriasis, Normal Adjascent Skin (Non-Lesional), Interferon gamma-1b Treated | Psoriasis, Normal Adjacent Skin (Non-Lesional), Untreated | Homo sapiens | 21/232 | 1.268e-20 | |
| Anaplastic Thyroid Carcinoma | Follicular Thyroid Carcinoma | Homo sapiens | 24/403 | 3.976e-19 | |
| Cultured B-Lymphocytes from B-Cell Chronic Lymphocytic Leukemia Rolipram (20 uM) Treated | Normal Cultured T-Lymphocytes, Rolipram (20 uM) Treated | Homo sapiens | 22/334 | 1.468e-18 | |

B cell related dataset come up, as well as other interesting datasets related to Asthma, colorectal cancer and interferon stimulated cells.

(interferon signaling came up in the leading edge analysis)

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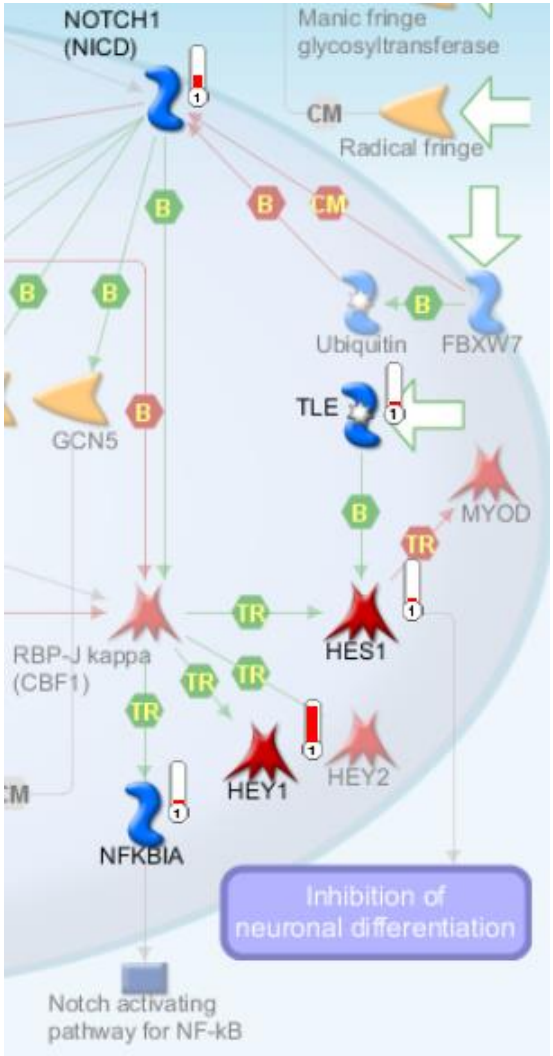
# Summary

The NOTCH1 induced gene signature can impact many different pathways.

NOTCH1 signaling could potentially regulate a wide range of transcription factors including STATs and IRFs.

The 90 leading edge genes from CLL patients that overlap with the NOTCH1 signature point towards disruptions in apoptosis and IFN signaling processes.

Comparing the NOTCH1 induced signature with against other public datasets, pulled up similar signature in other B-lymphocyte comparisons as well as similarities with IFN induced signatures, colorectal cancer, asthma, and other diseases.

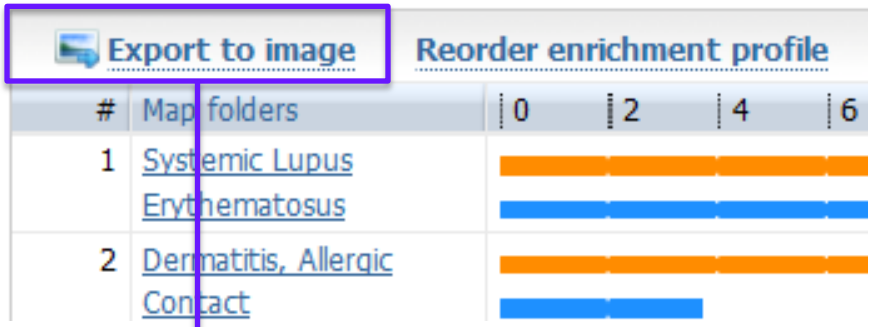



Clarivate Analytics

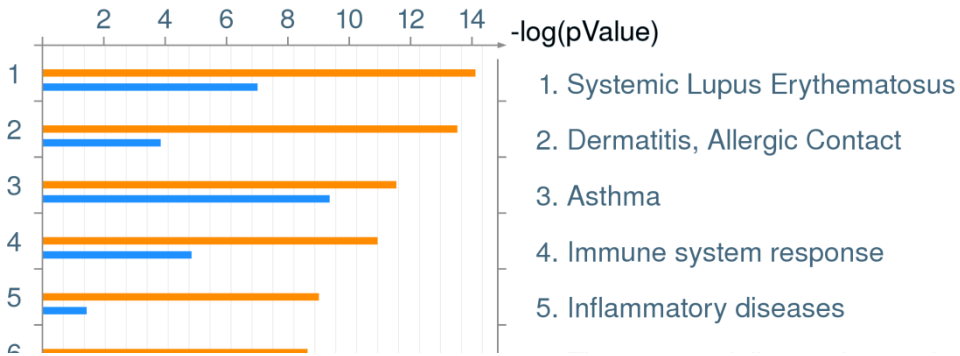
# Tips on exporting visuals and citing MetaCore

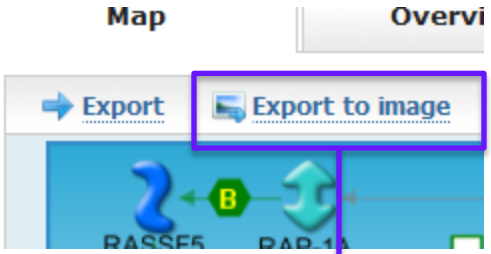
MetaCore+MetaDrug™ version 6.30 build 68780

Cite that you used MetaCore and the version number when you ran the analysis

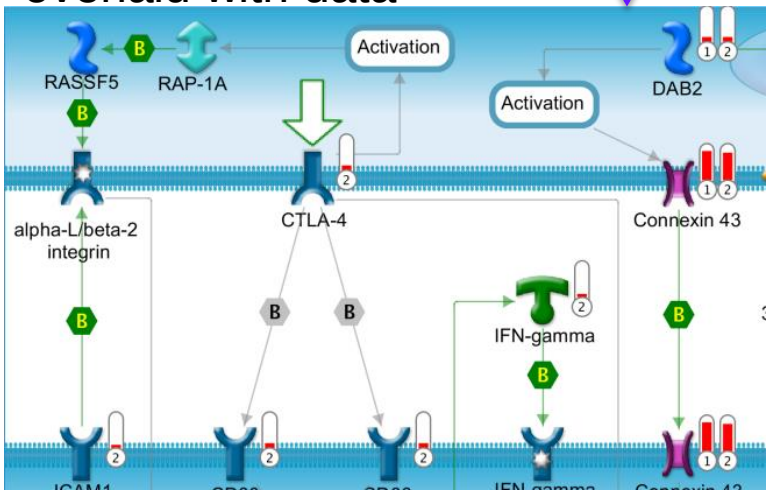



Export to image will create a png file of your enrichment results




Can also export images of pathway maps overlaid with data

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