Workshop
Scripting Pathway Analysis and Data Overlays in R

# Upcoming Workshops

- **10/26-27** - Introduction to RNA-Seq Analysis
- **11/9** - Single Cell Analysis
- **11/19** - Machine Learning

Join **Gladstone Bioinformatics Core** on Slack

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | hgnc_symbol | EnsemblID | logFC | Pvalue | FDR |
| 2 | FGFBP2 | ENSG00000137441 | 4.95372148 | 2.07E-11 | 2.52E-09 |
| 3 | IGKJ4 | ENSG00000211594 | 4.57014526 | 5.80E-13 | 1.02E-10 |
| 4 | GPR3 | ENSG00000181773 | 3.07384469 | 2.37E-06 | 6.68E-05 |
| 5 | RPS20P10 | ENSG00000233971 | 3.0736699 | 1.59E-05 | 0.00033744 |
| 6 | RPS23P1 | ENSG00000243504 | 2.87680167 | 0.0004883 | 0.00566507 |
| 7 | RPS3AP44 | ENSG00000234031 | 2.87189316 | 2.02E-05 | 0.00041197 |
| 8 | RPS21 | ENSG00000171858 | 1.78201017 | 5.40E-16 | 2.76E-13 |
| 9 | ERGIC3 | ENSG00000125991 | 1.04270392 | 4.08E-07 | 1.48E-05 |
| 10 | ATP5F1E | ENSG00000124172 | 1.0419011 | 1.74E-07 | 6.97E-06 |
| 11 | OST4 | ENSG00000228474 | 1.04082316 | 8.61E-06 | 0.00020192 |
| 12 | MIR663AHG | ENSG00000227195 | 1.03864596 | 1.16E-07 | 4.96E-06 |
| 13 | BCR | ENSG00000186716 | 1.02217198 | 2.66E-08 | 1.37E-06 |
| 14 | HIST1H2AG | ENSG00000196787 | 1.02057118 | 9.38E-05 | 0.00148878 |
| 15 | SSR2 | ENSG00000163479 | 1.01915311 | 4.30E-10 | 3.79E-08 |
| 16 | EEF1A1 | ENSG00000156508 | -1.01124735 | 1.08E-07 | 4.62E-06 |
| 17 | TMEM159 | ENSG00000011638 | -1.00983307 | 0.0017788 | 0.01498506 |
| 18 | C12orf57 | ENSG00000111678 | -1.00900966 | 0.0009472 | 0.00942208 |
| 19 | ELOB | ENSG00000103363 | -1.9700433 | 1.64E-05 | 0.00034533 |
| 20 | B2M | ENSG00000166710 | -1.96259859 | 3.78E-07 | 1.37E-05 |
| 21 | EIF3K | ENSG00000178982 | -2.9604061 | 2.65E-06 | 7.30E-05 |
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| 25 | C7orf26 | ENSG00000146576 | -3.94240445 | 0.0001522 | 0.00222598 |
| 26 | JTB | ENSG00000143543 | -3.94165989 | 1.89E-06 | 5.60E-05 |
| 27 | OBSCN | ENSG00000154358 | -3.93820271 | 0.0003276 | 0.00410635 |
| 28 | TMEM14C | ENSG00000111843 | -3.93798301 | 0.0022893 | 0.01811524 |
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| 30 | COX4I1 | ENSG00000131143 | -4.91954634 | 2.37E-06 | 6.68E-05 |
| 31 | CCT3 | ENSG00000163468 | -4.91242065 | 1.55E-05 | 0.00033124 |
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| 35 | NDUFS8 | ENSG00000110717 | -5.87563243 | 0.0022648 | 0.01795672 |
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| 4 | GPR3 | ENSG00000181773 | 3.07384469 | 2.37E-06 | 6.68E-05 |
| 5 | RPS20P10 | ENSG00000233971 | 3.0736699 | 1.59E-05 | 0.00033744 |
| 6 | RPS23P1 | ENSG00000243504 | 2.87680167 | 0.0004883 | 0.00566507 |
| 7 | RPS3AP44 | ENSG00000234031 | 2.87189316 | 2.02E-05 | 0.00041197 |
| 8 | RPS21 | ENSG00000171858 | 1.78201017 | 5.40E-16 | 2.76E-13 |
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| 11 | OST4 | ENSG00000228474 | 1.04082316 | 8.61E-06 | 0.00020192 |
| 12 | MIR663AHG | ENSG00000227195 | 1.03864596 | 1.16E-07 | 4.96E-06 |
| 13 | BCR | ENSG00000186716 | 1.02217198 | 2.66E-08 | 1.37E-06 |
| 14 | HIST1H2AG | ENSG00000196787 | 1.02057118 | 9.38E-05 | 0.00148878 |
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| 17 | TMEM159 | ENSG00000011638 | -1.00983307 | 0.0017788 | 0.01498506 |
| 18 | C12orf57 | ENSG00000111678 | -1.00900966 | 0.0009472 | 0.00942208 |
| 19 | ELOB | ENSG00000103363 | -1.9700433 | 1.64E-05 | 0.00034533 |
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| 26 | JTB | ENSG00000143543 | -3.94165989 | 1.89E-06 | 5.60E-05 |
| 27 | OBSCN | ENSG00000154358 | -3.93820271 | 0.0003276 | 0.00410635 |
| 28 | TMEM14C | ENSG00000111843 | -3.93798301 | 0.0022893 | 0.01811524 |
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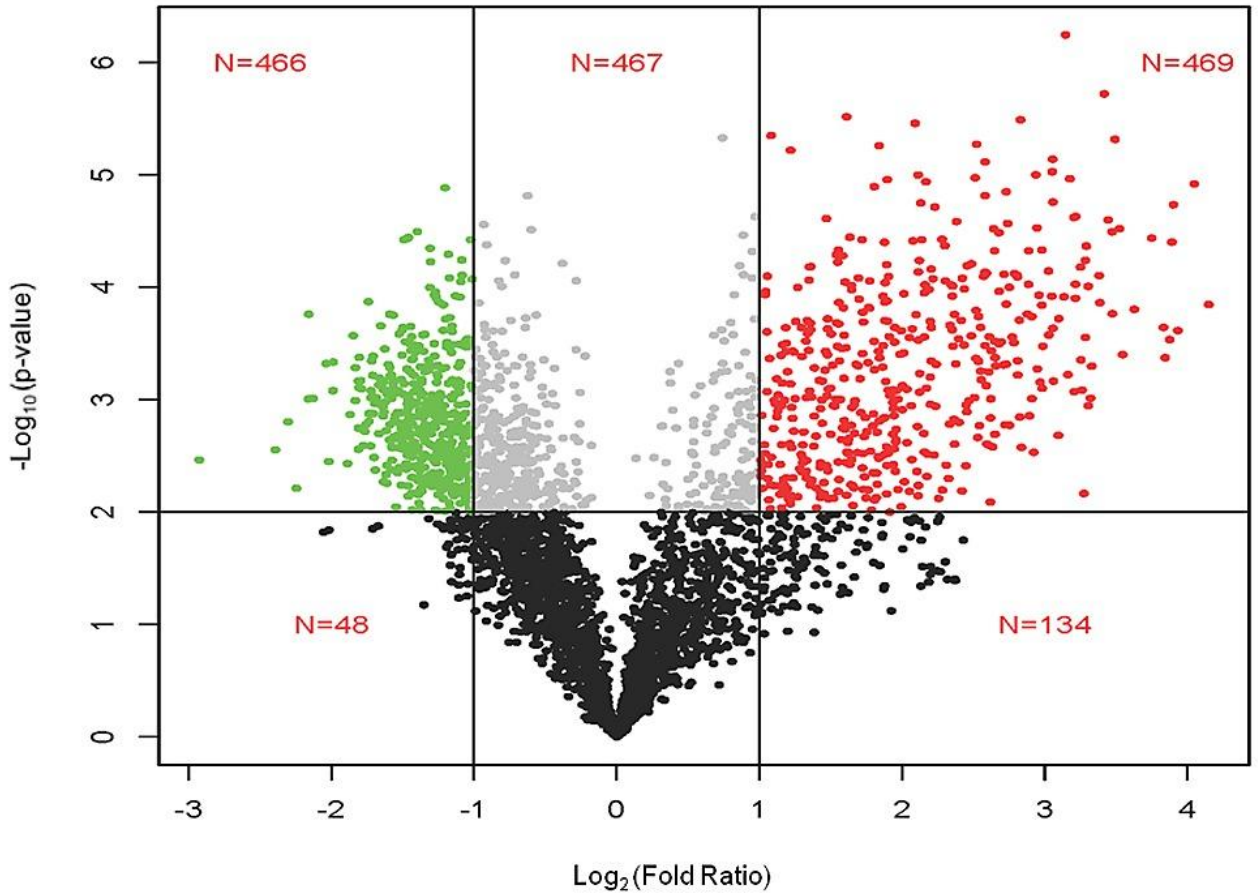
| 31 | CCT3 | ENSG00000163468 | -4.91242065 | 1.55E-05 | 0.00033124 |
| 32 | SSR4 | ENSG00000180879 | -4.9086354 | 0.0002098 | 0.00286687 |
| 33 | SNHG22 | ENSG00000267322 | -4.90693154 | 9.03E-05 | 0.00144651 |
| 34 | NDUFA4 | ENSG00000189043 | -5.879366 | 1.02E-06 | 3.31E-05 |
| 35 | NDUFS8 | ENSG00000110717 | -5.87563243 | 0.0022648 | 0.01795672 |
| 36 | ACTB | ENSG00000075624 | -5.8670115 | 4.30E-06 | 0.00011161 |

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| 4 | GPR3 | ENSG00000181773 | 3.07384403 | 2.37E-06 | 6.68E-05 |
| 5 | RPS20P10 | ENSG00000233971 | 3.07366699 | 1.59E-05 | 0.00033744 |
| 6 | RPS23P1 | ENSG00000243504 | 2.87680167 | 0.0004883 | 0.00566507 |
| 7 | RPS3AP44 | ENSG00000234031 | 2.87189316 | 2.02E-05 | 0.00041197 |
| 8 | RPS21 | ENSG00000171858 | 1.78201017 | 5.40E-16 | 2.78E-13 |
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| 12 | MIR6G3AHG | ENSG00000227195 | 1.03864596 | 1.16E-07 | 4.96E-06 |
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| 18 | C12orf57 | ENSG00000111678 | -1.00900966 | 0.0009472 | 0.00942206 |
| 19 | ELOB | ENSG00000103363 | -1.9700433 | 1.64E-05 | 0.00034933 |
| 20 | BZM | ENSG00000166710 | -1.96259859 | 3.78E-07 | 1.37E-05 |
| 21 | EIF3K | ENSG00000178982 | -2.96040051 | 2.65E-06 | 7.30E-05 |
| 22 | FIBP | ENSG00000172500 | -2.95401909 | 0.0005135 | 0.00591282 |
| 23 | SCARNA17 | ENSG00000251992 | -2.95163973 | 1.74E-06 | 5.18E-05 |
| 24 | HLA-B | ENSG00000234745 | -2.94688319 | 6.68E-08 | 3.10E-06 |
| 25 | C7orf26 | ENSG00000146576 | -3.94240445 | 0.0001522 | 0.00222598 |
| 26 | ITB | ENSG00000143543 | -3.94165989 | 1.89E-06 | 5.60E-05 |
| 27 | OBSCN | ENSG00000154358 | -3.93832071 | 0.0003276 | 0.00410835 |
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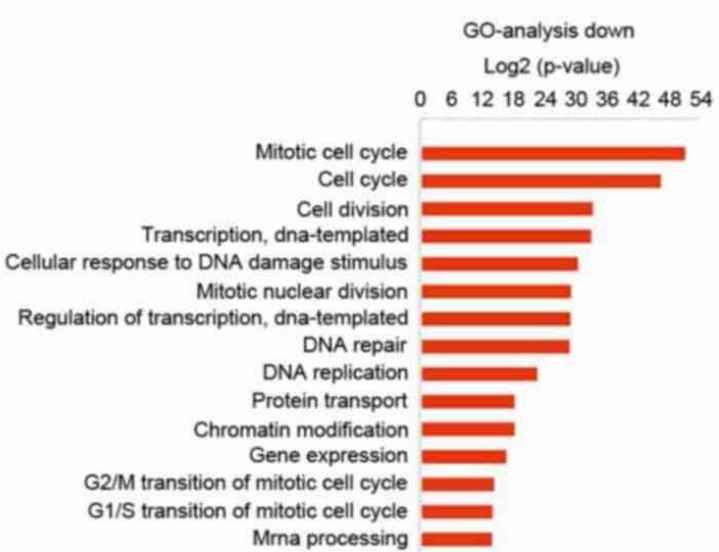
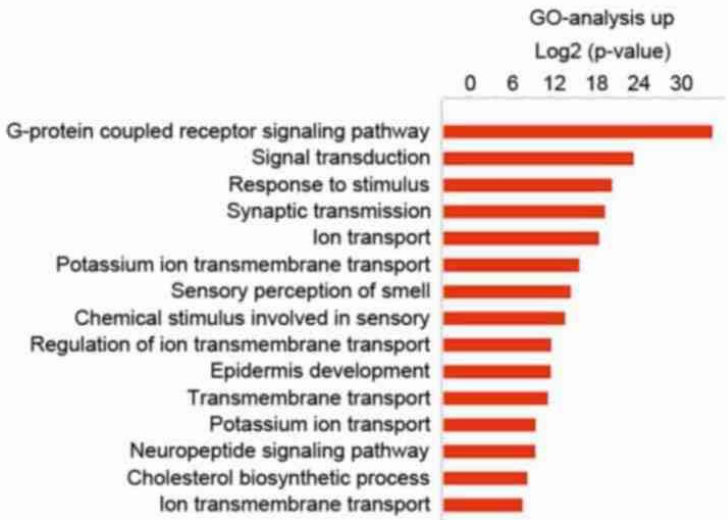
| 29 | ATP5MG | ENSG00000167283 | -3.93536083 | 0.0001484 | 0.00217768 |
| 30 | COX6I | ENSG00000131143 | -4.91954634 | 2.37E-06 | 6.68E-05 |
| 31 | CCT3 | ENSG00000163468 | -4.91243065 | 1.55E-05 | 0.00033124 |
| 32 | SSR4 | ENSG00000180879 | -4.90863054 | 0.0002098 | 0.00286687 |
| 33 | SNHG22 | ENSG00000267322 | -4.90693154 | 9.03E-05 | 0.00144851 |
| 34 | NDUFA4 | ENSG00000189043 | -5.879396 | 1.02E-06 | 3.31E-05 |
| 35 | NDUFS8 | ENSG00000110717 | -5.87363243 | 0.0022648 | 0.01795672 |
| 36 | ACTB | ENSG00000075624 | -5.86970115 | 4.30E-06 | 0.00011161 |

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| 6 | RPS23P1 | ENSG00000243504 | 2.87680167 | 0.00004883 | 0.00566507 |
| 7 | RPS3AP44 | ENSG00000234031 | 2.87189316 | 2.02E-05 | 0.00041197 |
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| 12 | MIR6G3AHG | ENSG00000227195 | 1.03864596 | 1.16E-07 | 4.96E-06 |
| 13 | BCR | ENSG00000186716 | 1.02217138 | 2.66E-08 | 1.37E-06 |
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| 22 | FIBP | ENSG00000172500 | -2.95401909 | 0.00001135 | 0.00591282 |
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| 25 | C7orf26 | ENSG00000146576 | -3.94240445 | 0.00011522 | 0.00222598 |
| 26 | JTB | ENSG00000143543 | -3.94165989 | 1.89E-06 | 5.60E-05 |
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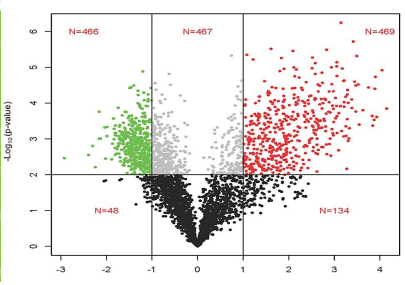
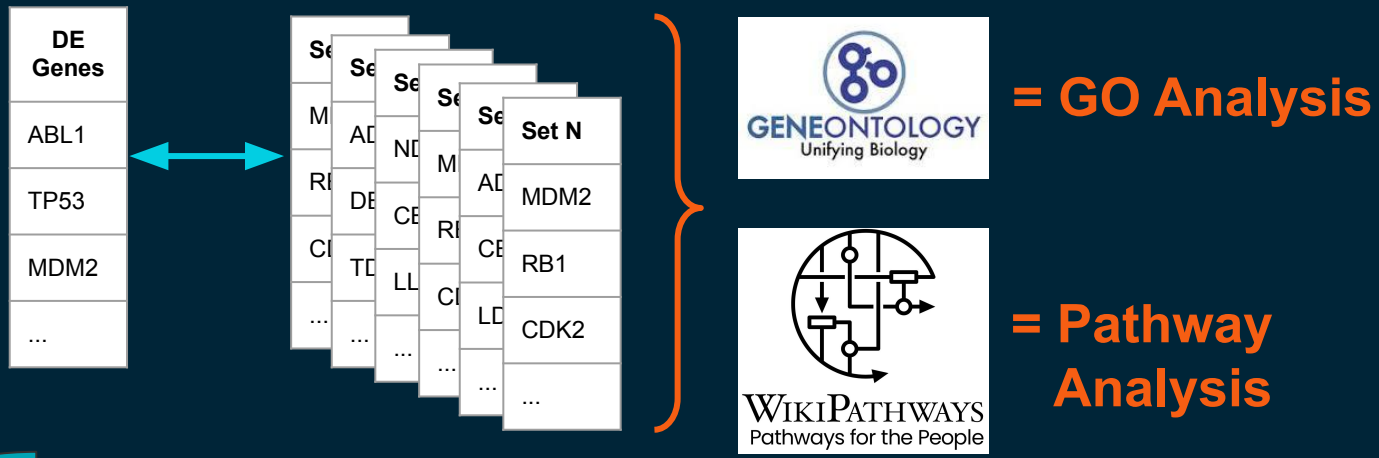


# Functional Enrichment Analysis



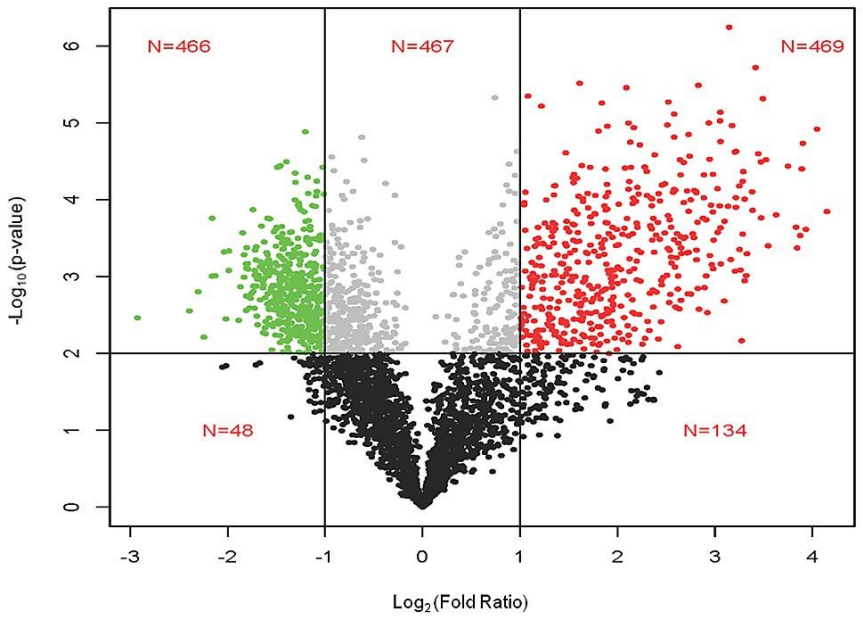

| GO Term or Pathway | Ratio | P-Value | Z-Score | List of Genes |
|---|---|---|---|---|
| Cell Cycle | 9/45 | 4.2E-12 | 2.5 | MDM2, ABL1, RB1, ... |
| DNA Repair | 9/52 | 2.6E-10 | 3.2 | MDM2, ABL1, RB1, ... |
| TNF Signaling | 8/40 | 1.1E-10 | 2.4 | CDK2, TNFA1, ABL1, ... |

# Functional Enrichment Analysis

1. **Overrepresentation Analysis (ORA)**
   - Pick cutoffs
   - Apply Fisher's Exact Test
     or Hypergeometric Test

2. **Gene Set Enrichment Analysis (GSEA)**
   - Rank all genes
   - Apply GSEA method

# 1. Overrepresentation Analysis (ORA)




**Pick cutoffs**

- Log2FC
- Adjusted p-value

- Upregulated
- Downregulated
- Significantly changed

# 1. Overrepresentation Analysis (ORA)

## Apply statistical test

- Fisher's exact test or Hypergeometric test
- See detailed descriptions and comparisons

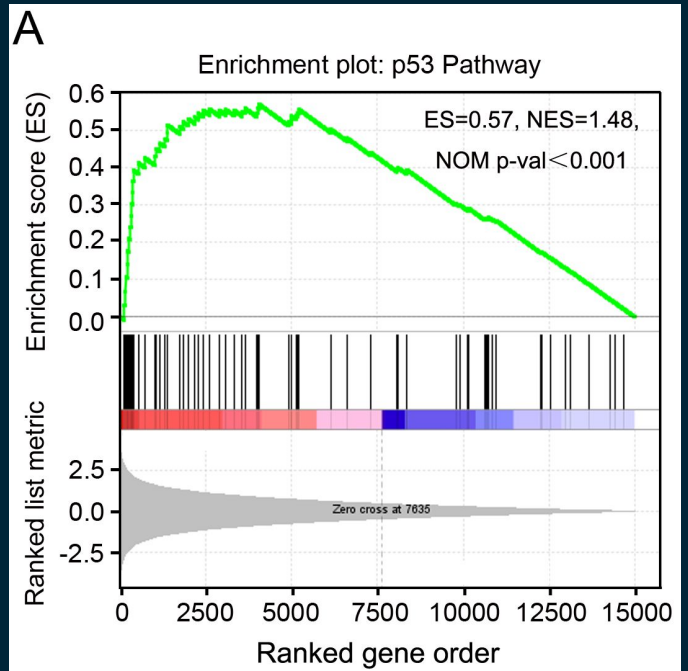
## Important factors

- Source of ontology and pathways sets
- Pre-filtering
- Post-filtering
- Use of background or reference set

# 2. Gene Set Enrichment Analysis (GSEA)

## Rank all genes

- sign(log2FC) * -log(Pvalue)



# 2. Gene Set Enrichment Analysis (GSEA)

## Appy statistical test

- Subramanian, et al. PNAS 2005

## Important factors

- Source of ontology and pathways sets
- Pre-filtering
- Post-filtering

## Advantages

- No arbitrary cutoffs
- Background is built into ranked gene set

*Which method should I use?*

**Both!**

# Survey of Tools

- ***Enrichr*** - simple test of 154 resources (ORA only)
  - Paste in human or mouse symbols
  - Browse and export results
  - Can’t provide background set
  - See documentation
- ***WebGestalt*** - advanced test on select resources
  - Paste in symbols or identifiers ~15 species
  - Specify background set
  - Advanced options; advanced data visualizations
  - See Wang, et al. NAR 2017

# Survey of Tools

- ***clusterProfiler*** - R package
  - ORA and GSEA
  - Advanced input and configuration options
  - Built-in GO analysis; expansion options
  - Build-in data visualization; expansion options
  - See documentation

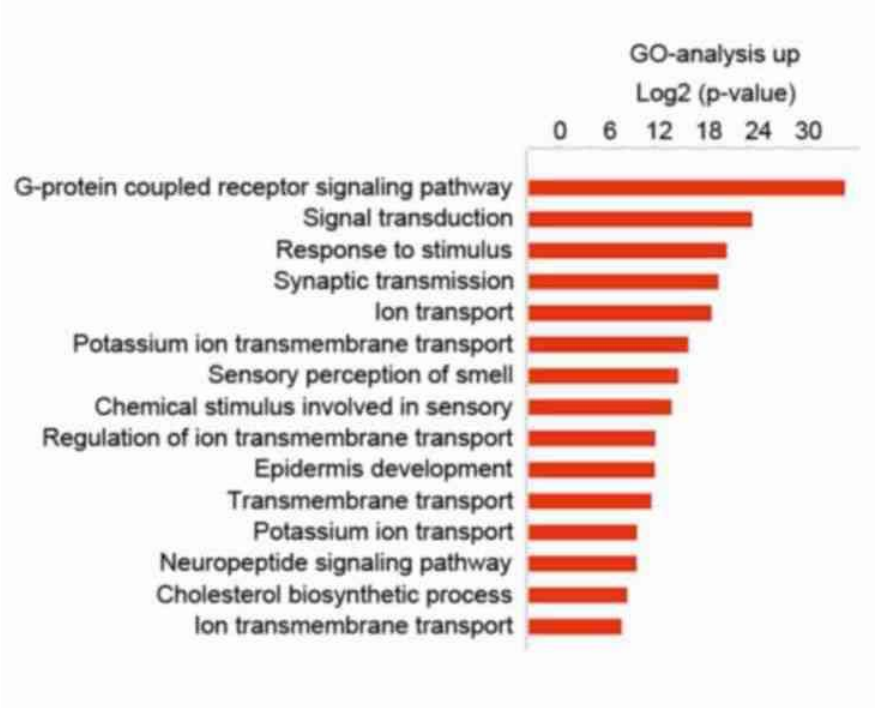
*Which tool should I use?*

**Multiple!**

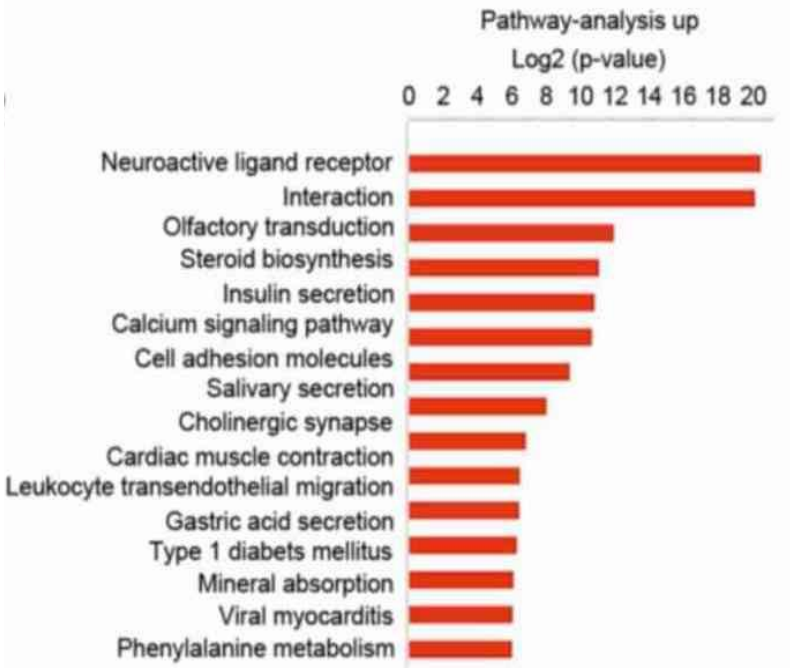
# Additional Reading

- Huang 2009
- Tipney & Hunter 2010
- Kelder, Pico, et al. 2010
- Nguyen, et al. 2019

GENEONTOLOGY
Unifying Biology



WikiPathways
Pathways for the People



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| 8 | C12orf57 | ENSG00000111678 | -1.00900966 | 0.0009472 | 0.0094226 |
| 9 | ELOB | ENSG00000103363 | -1.9700433 | 1.64E-05 | 0.0003455 |
| 0 | B2M | ENSG00000166710 | -1.96259859 | 3.78E-07 | 1.37E-0 |
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| 2 | FIBP | ENSG00000172500 | -2.95401909 | 0.0000135 | 0.0059128 |
| 3 | SCARNA17 | ENSG00000251992 | -2.95163973 | 1.74E-06 | 5.18E-0 |
| 4 | HLA-B | ENSG00000234745 | -2.94688319 | 6.68E-08 | 3.10E-0 |
| 5 | C7orf26 | ENSG00000146576 | -3.94240445 | 0.0001522 | 0.0022255 |
| 6 | JTB | ENSG00000143543 | -3.94165989 | 1.89E-06 | 5.60E-0 |
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| 8 | TMEM14C | ENSG00000111843 | -3.93798301 | 0.0022893 | 0.0181151 |
| 9 | ATP5MG | ENSG00000167283 | -3.93536083 | 0.0001484 | 0.0021775 |
| 0 | COX4I1 | ENSG00000131143 | -4.91954634 | 2.37E-06 | 6.68E-0 |
| 1 | CCT3 | ENSG00000163468 | -4.91242065 | 1.55E-05 | 0.0003312 |
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| 3 | SNHG22 | ENSG00000267322 | -4.90693154 | 9.03E-05 | 0.0014465 |
| 4 | NDUFA4 | ENSG00000189043 | -5.879366 | 1.02E-06 | 3.31E-0 |
| 5 | NDUFS8 | ENSG00000110717 | -5.87563243 | 0.0022648 | 0.0179561 |
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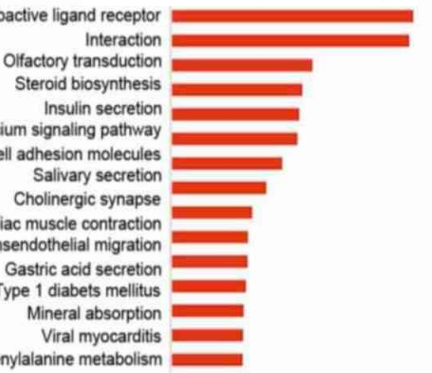


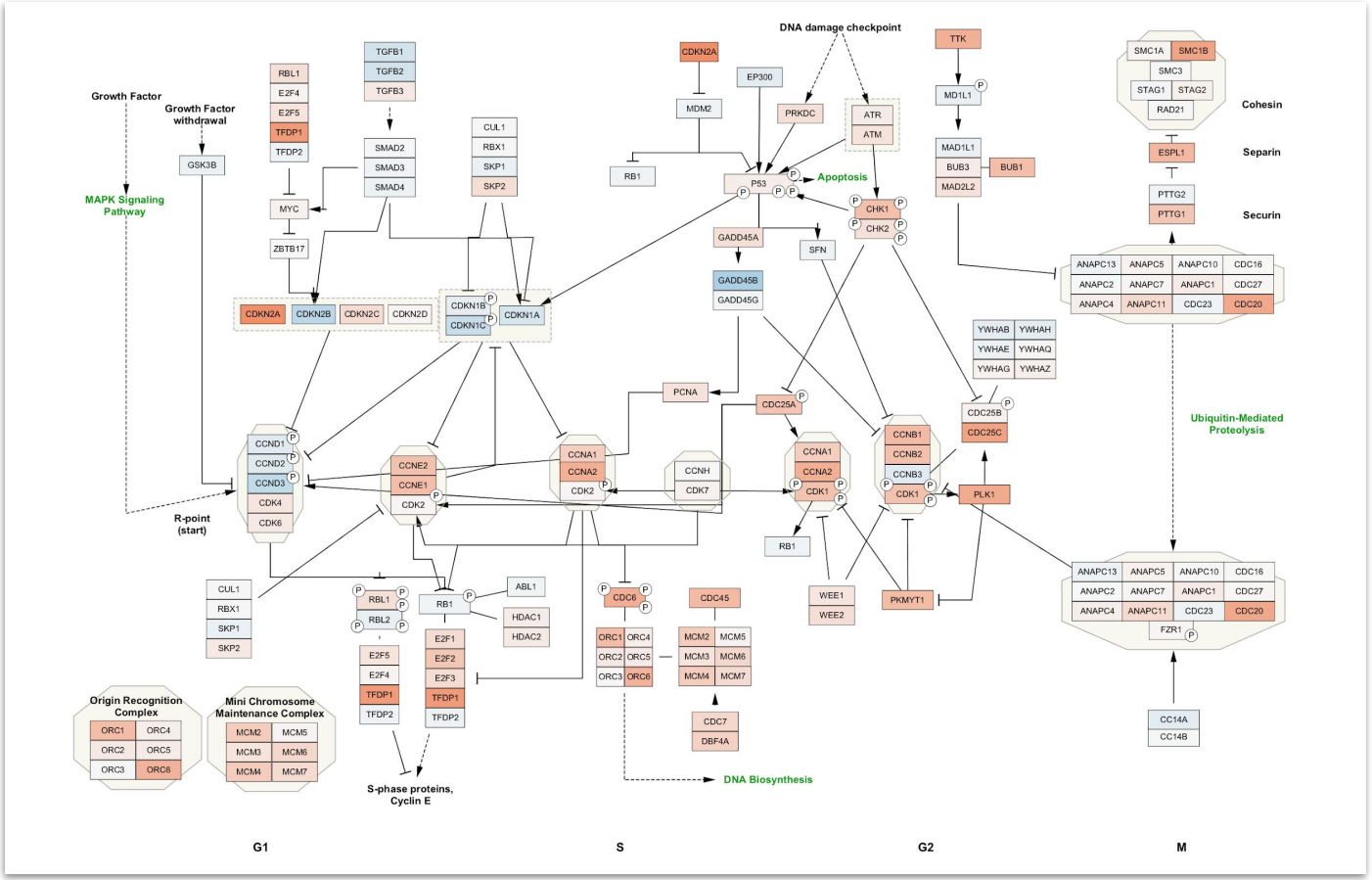

| | A | B | C | D | E |
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| | GPR3 | ENSG00000181773 | 3.07384469 | 2.37E-06 | 6.68E-5 |
| | RPS20P10 | ENSG00000233971 | 3.0736699 | 1.59E-05 | 0.0003374 |
| | RPS23P1 | ENSG00000243504 | 2.87680167 | 0.0004883 | 0.0056656 |
| | RPS3AP44 | ENSG00000234031 | 2.87189316 | 2.02E-05 | 0.0004115 |
| | RPS21 | ENSG00000171858 | 1.78201017 | 5.40E-16 | 2.76E-1 |
| | ERGIC3 | ENSG00000125991 | 1.04270392 | 4.08E-07 | 1.48E-5 |
| 0 | ATP5F1E | ENSG00000124172 | 1.0419011 | 1.74E-07 | 6.97E-5 |
| 1 | QST4 | ENSG00000228474 | 1.04082316 | 8.61E-06 | 0.0002011 |
| 2 | MIR663AHG | ENSG00000227195 | 1.03864596 | 1.16E-07 | 4.96E-5 |
| 3 | BCR | ENSG00000186716 | 1.02217198 | 2.66E-08 | 1.37E-5 |
| 4 | HIST1H2AG | ENSG00000196787 | 1.02057118 | 9.38E-05 | 0.0014883 |
| 5 | SSR2 | ENSG00000163479 | 1.01915311 | 4.30E-10 | 3.79E-6 |
| 6 | EEF1A1 | ENSG00000156508 | -1.01124735 | 1.08E-07 | 4.62E-5 |
| 7 | TMEM159 | ENSG00000011638 | -1.00983307 | 0.0017788 | 0.0149858 |
| 8 | C12orf57 | ENSG00000111678 | -1.00900966 | 0.0009472 | 0.0094226 |
| 9 | ELOB | ENSG00000103363 | -1.9700433 | 1.64E-05 | 0.0003455 |
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| 4 | HLA-B | ENSG00000234745 | -2.94688319 | 6.68E-08 | 3.10E-5 |
| 5 | C7orf26 | ENSG00000146576 | -3.94240445 | 0.0001522 | 0.0022255 |
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| 7 | OBSCN | ENSG00000154358 | -3.93820273 | 0.0003276 | 0.0041061 |
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| 0 | COX4I1 | ENSG00000131143 | -4.91954634 | 2.37E-06 | 6.68E-5 |
| 1 | CCT3 | ENSG00000163468 | -4.91242065 | 1.55E-05 | 0.0003311 |
| 2 | SSR4 | ENSG00000180879 | -4.9086354 | 0.0002098 | 0.0028668 |
| 3 | SNHG22 | ENSG00000267322 | -4.90693154 | 9.03E-05 | 0.0014465 |
| 4 | NDUFA4 | ENSG00000189043 | -5.879366 | 1.02E-06 | 3.31E-5 |
| 5 | NDUFS8 | ENSG00000110717 | -5.87563243 | 0.0022648 | 0.0179561 |
| 6 | ACTB | ENSG00000075624 | -5.8670115 | 4.30E-06 | 0.0001114 |

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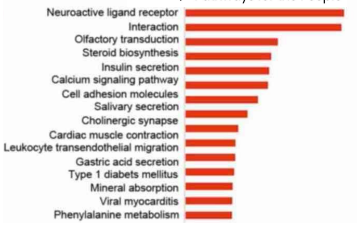
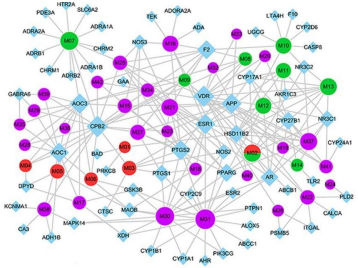
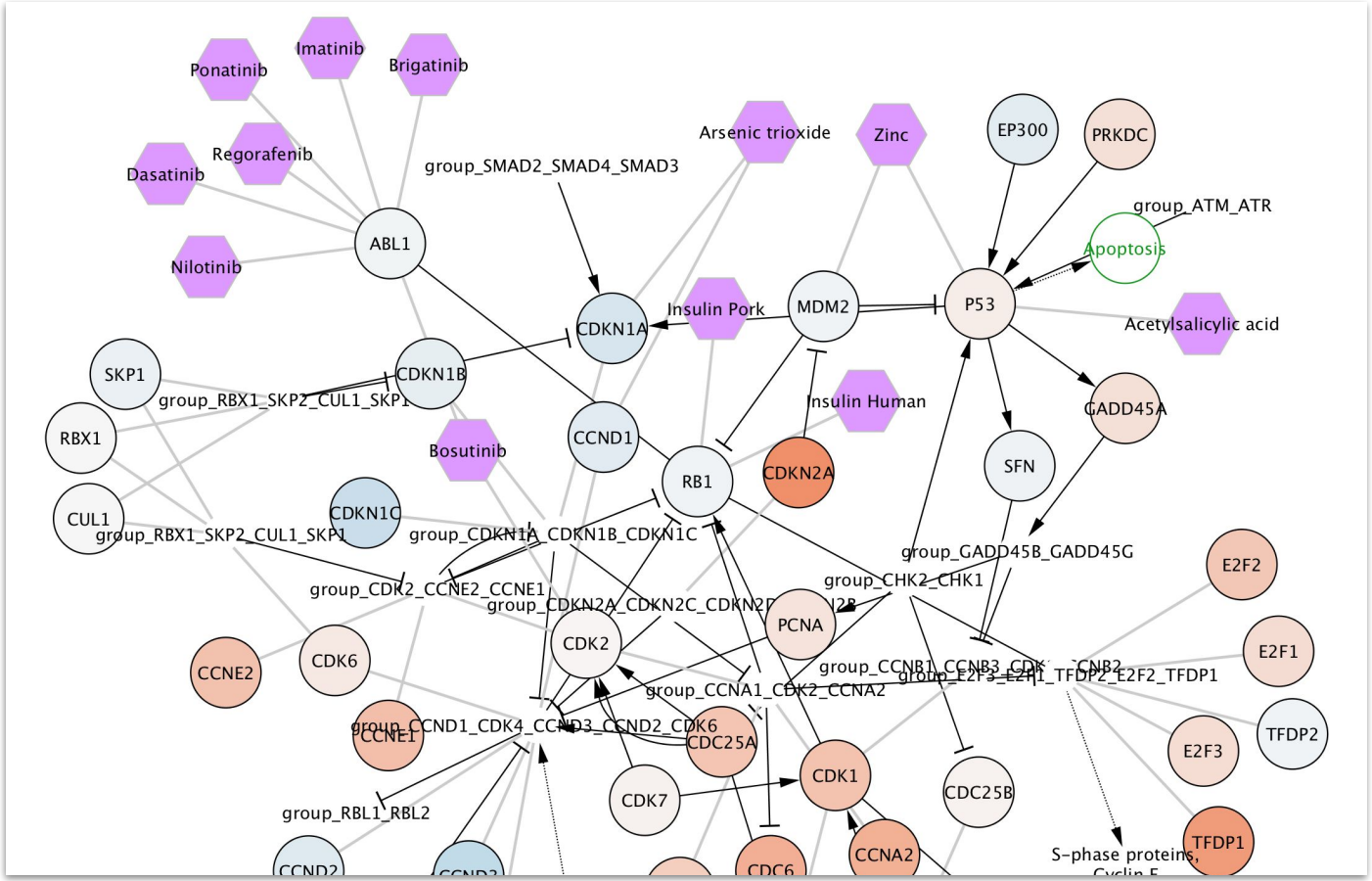



Drug-Target Interactions





| | A | B | C | D | E |
|---|---|---|---|---|---|
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| 3 | IGKJ4 | ENSG00000211594 | 4.57014526 | 5.80E-13 | 1.02E-1 |
| 4 | GPR3 | ENSG00000181773 | 3.07384469 | 2.37E-06 | 6.68E-0 |
| 5 | RPS20P10 | ENSG00000233971 | 3.0736699 | 1.59E-05 | 0.0003374 |
| 6 | RPS23P1 | ENSG00000243504 | 2.87680167 | 0.0004883 | 0.0056656 |
| 7 | RPS3AP44 | ENSG00000234031 | 2.87189316 | 2.02E-05 | 0.0004131 |
| 8 | RPS21 | ENSG00000171858 | 1.78201017 | 5.40E-16 | 2.76E-1 |
| 9 | ERGIC3 | ENSG00000125991 | 1.04270392 | 4.08E-07 | 1.48E-0 |
| 10 | ATP5F1E | ENSG00000124172 | 1.0419011 | 1.74E-07 | 6.97E-0 |
| 11 | OST4 | ENSG00000228474 | 1.04082316 | 8.61E-06 | 0.0002015 |
| 12 | MIR663AHG | ENSG00000227195 | 1.03864596 | 1.16E-07 | 4.96E-0 |
| 13 | BCR | ENSG00000186716 | 1.02217198 | 2.66E-08 | 1.37E-0 |
| 14 | HIST1H2AG | ENSG00000196787 | 1.02057118 | 9.38E-05 | 0.0014887 |
| 15 | SSR2 | ENSG00000163479 | 1.01915311 | 4.30E-10 | 3.79E-0 |
| 16 | EEF1A1 | ENSG00000156508 | -1.01124735 | 1.08E-07 | 4.62E-0 |
| 17 | TMEM159 | ENSG00000011638 | -1.00983307 | 0.0017788 | 0.0149856 |
| 18 | C12orf57 | ENSG00000111678 | -1.00900966 | 0.0009472 | 0.0094220 |
| 19 | ELOB | ENSG00000103363 | -1.9700433 | 1.64E-05 | 0.0003453 |
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| 23 | SCARNA17 | ENSG00000251992 | -2.95163973 | 1.74E-06 | 5.18E-0 |
| 24 | HLA-B | ENSG00000234745 | -2.94688319 | 6.68E-08 | 3.10E-0 |
| 25 | C7orf26 | ENSG00000146576 | -3.94240445 | 0.0001522 | 0.0022255 |
| 26 | JTB | ENSG00000143543 | -3.94165989 | 1.89E-06 | 5.60E-0 |
| 27 | OBSCN | ENSG00000154358 | -3.93820271 | 0.0003276 | 0.0041061 |
| 28 | TMEM14C | ENSG00000111843 | -3.93798301 | 0.0022893 | 0.0181152 |
| 29 | ATP5MG | ENSG00000167283 | -3.93536083 | 0.0001484 | 0.0021775 |
| 30 | COX4I1 | ENSG00000131143 | -4.91954634 | 2.37E-06 | 6.68E-0 |
| 31 | CCT3 | ENSG00000163468 | -4.91242065 | 1.55E-05 | 0.0003312 |
| 32 | SSR4 | ENSG00000180879 | -4.9086354 | 0.0002098 | 0.0028668 |
| 33 | SNHG22 | ENSG00000267322 | -4.90693154 | 9.03E-05 | 0.0014465 |
| 34 | NDUFA4 | ENSG00000189043 | -5.879366 | 1.02E-06 | 3.31E-0 |
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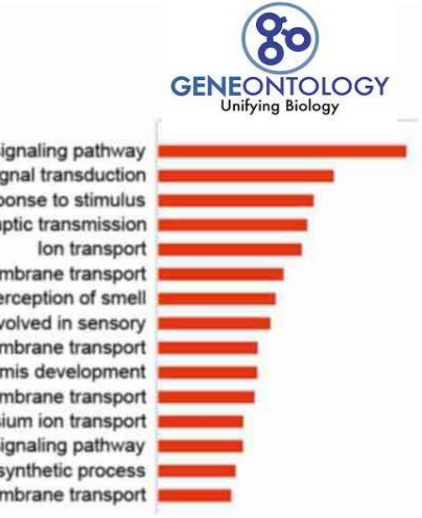


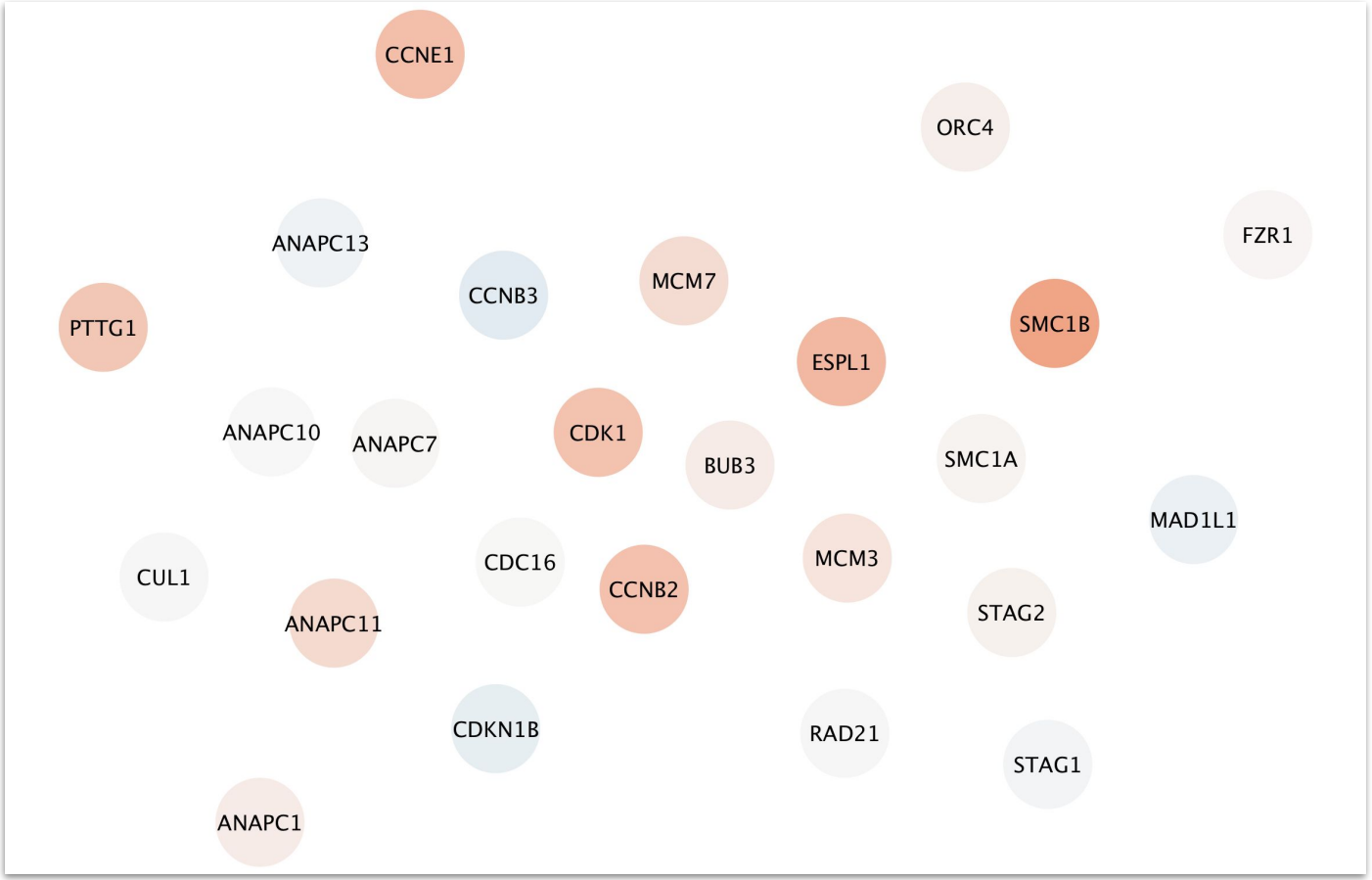

| | A | B | C | D | E |
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| 1 | IGKJ4 | ENSG00000211594 | 4.57014526 | 5.80E-13 | 1.02E-1 |
| 1 | GPR3 | ENSG00000181773 | 3.07384469 | 2.37E-06 | 6.68E-0 |
| 1 | RPS20P10 | ENSG00000233971 | 3.0736699 | 1.59E-05 | 0.0003374 |
| 1 | RPS23P1 | ENSG00000243504 | 2.87680167 | 0.0004883 | 0.0056656 |
| 1 | RPS3AP44 | ENSG00000234031 | 2.87189316 | 2.02E-05 | 0.0004131 |
| 1 | RPS21 | ENSG00000171858 | 1.78201017 | 5.40E-16 | 2.76E-1 |
| 1 | ERGIC3 | ENSG00000125991 | 1.04270392 | 4.08E-07 | 1.48E-0 |
| 10 | ATP5F1E | ENSG00000124172 | 1.0419011 | 1.74E-07 | 6.97E-0 |
| 11 | OST4 | ENSG00000228474 | 1.04082316 | 8.61E-06 | 0.0002015 |
| 12 | MIR663AHG | ENSG00000227195 | 1.03864596 | 1.16E-07 | 4.96E-0 |
| 13 | BCR | ENSG00000186716 | 1.02217198 | 2.66E-08 | 1.37E-0 |
| 14 | HIST1H2AG | ENSG00000196787 | 1.02057118 | 9.38E-05 | 0.0014887 |
| 15 | SSR2 | ENSG00000163479 | 1.01915311 | 4.30E-10 | 3.79E-0 |
| 16 | EEF1A1 | ENSG00000156508 | -1.01124735 | 1.08E-07 | 4.62E-0 |
| 17 | TMEM159 | ENSG00000011638 | -1.00983307 | 0.0017788 | 0.0149856 |
| 18 | C12orf57 | ENSG00000111678 | -1.00900966 | 0.0009472 | 0.0094220 |
| 19 | ELOB | ENSG00000103363 | -1.9700433 | 1.64E-05 | 0.0003453 |
| 20 | B2M | ENSG00000166710 | -1.96259859 | 3.78E-07 | 1.37E-0 |
| 21 | EIF3K | ENSG00000178982 | -2.9604061 | 2.65E-06 | 7.30E-0 |
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| 29 | ATP5MG | ENSG00000167283 | -3.93536083 | 0.0001484 | 0.0021775 |
| 30 | COX4I1 | ENSG00000131143 | -4.91954634 | 2.37E-06 | 6.68E-0 |
| 31 | CCT3 | ENSG00000163468 | -4.91242065 | 1.55E-05 | 0.0003312 |
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| 34 | NDUFA4 | ENSG00000189043 | -5.879366 | 1.02E-06 | 3.31E-0 |
| 35 | NDUFS8 | ENSG00000110717 | -5.87563243 | 0.0022648 | 0.0179567 |
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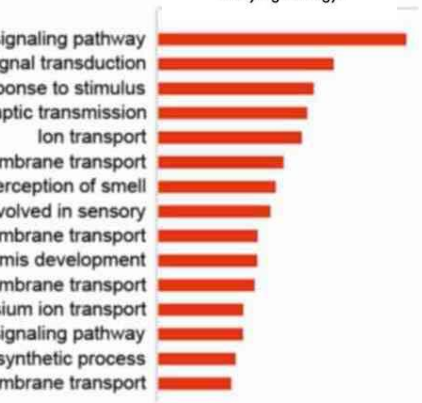


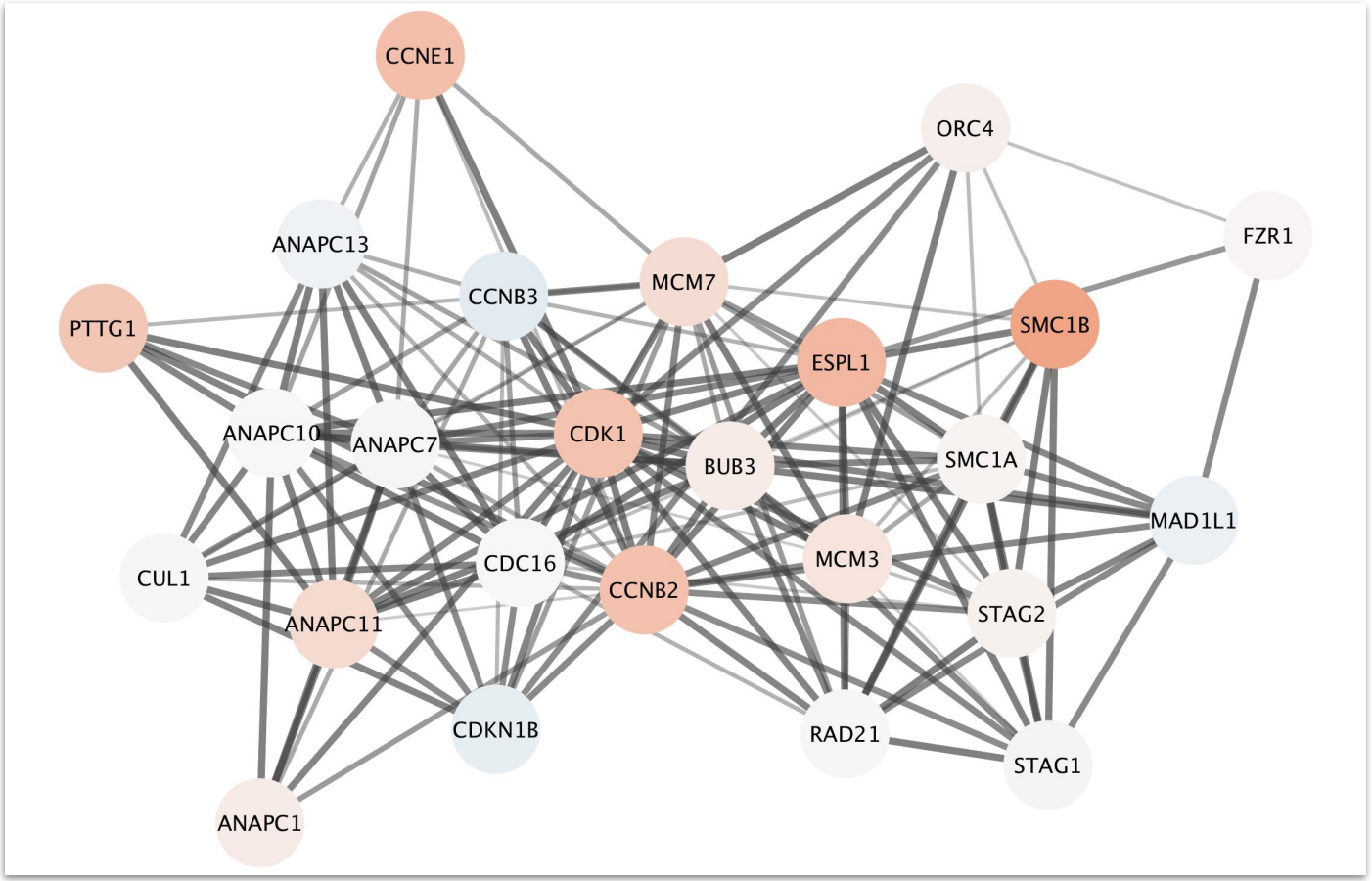

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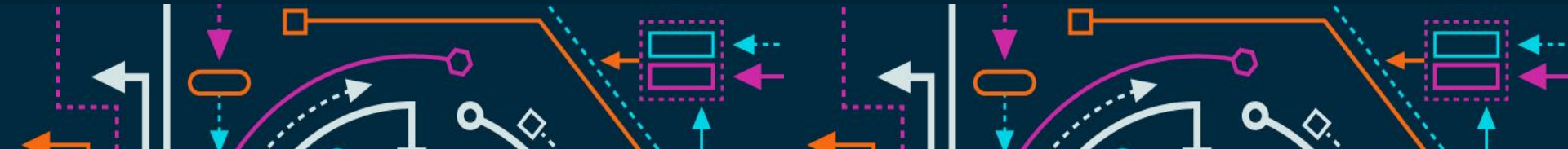
1. Launch RStudio
2. Open Pathway Analysis Vignette
   a. Download .Rmd