CluePedia

Walk through example

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I. Download CluePedia and ClueGO plugins into Cytoscape plugin folder. *Optional*: download Cerebral plugin.
Start Cytoscape and open ClueGO/CluePedia plugins
Create a network of terms/pathways and genes

1. Open plugins.
CluePedia can take at the first run several minutes to initialize.

Keep settings

2. Open example file "Th... related genes"

3. Select Reactome

Keep settings

4. Open Advanced Settings and set 3% Genes

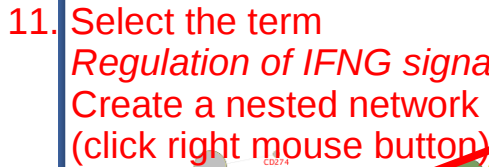*Keep settings*

5. Start analysis

ClueGO network

ClueGO+CluePedia network

Functions + associated genes

6. Select CluePedia panel

7. Show only selected genes

II. Visualize known interactions between mapped genes.
Explore a term/pathway into a nested network.
See all genes associated to this term and their known interactions.

*ClueGO+CluePedia network
showing known interactions from STRING*

9. Select all action scores

8. Select ACTIONS file

10. Update

11. Select the term
Regulation of IFNG signaling
Create a nested network
(click right mouse button)

*CluePedia
nested pathway network*

IIIa. Download example expression data (normal colon).
Calculate interactions between pathway genes.

12. Expand Show New Available Download Options and click Download

13. Select example files and click Ok

14. Select all genes (Ctrl-A)

16. Open expression data (normal colon mucosa, E-MTAB-57)

17. Set filters

18. Select the analysis type and methods

19. Name the new file

20. Start calculation

15. Expand Edge Link Files, select "Create Custom file" and click Start

If you see that the memory is too low, close some edge files or increase the Cytoscape memory (e.g. -Xmx2048m)

IIIb. Download interaction data file (colorectal tumors, Stage III). Visualize interactions between pathway genes.

21. Select "Download /Update files" and click Start

22. Select interaction file (CRCIII) and click Ok

23. Select files ACTIONS and the new created file (normalMucosa)

24. Select MIC, Pearson (0.7) and activation

25. Update

IV. Visualize normalized expression data on the network.

29. Open expression file (normal colon mucosa, E-MTAB-57)
30. Set filters
31. Normalize
32. Set max expression
33. Choose samples (1-5)
34. Check Selected nodes
35. Start

26. Select IFNGR1/2

27. Select Show expression
28. Open "CluePedia Expression Data Threshold" dialog
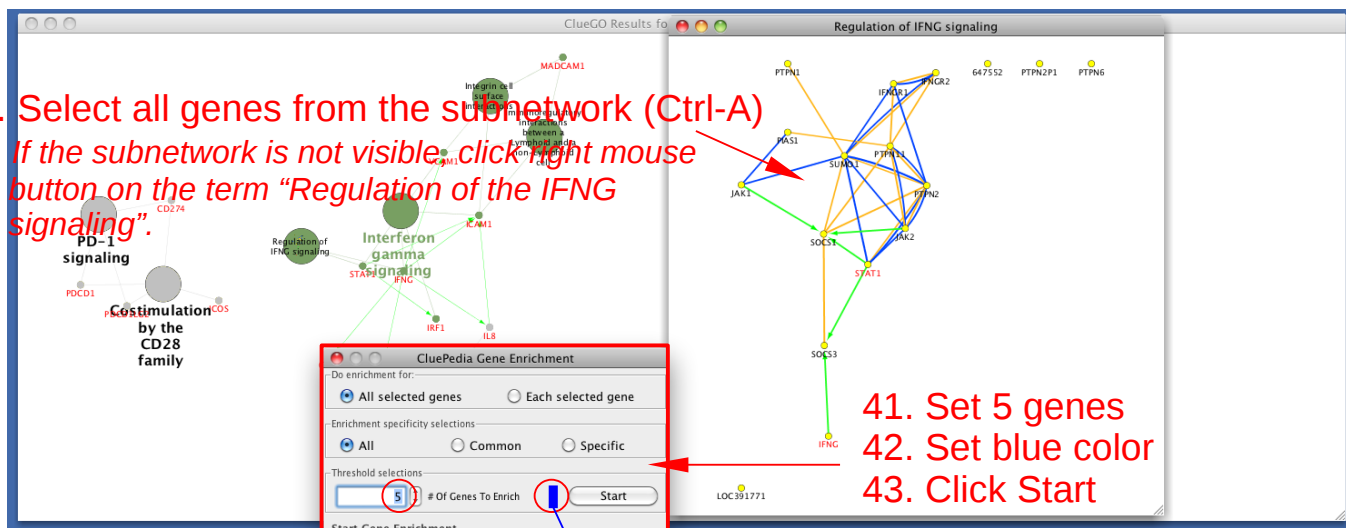
V. Visualize the network in a pathway-like view.

36. Select "Use Cerebral Layout"

VI. Enrich the network with new genes using normal mucosa interaction file.

38. Select all genes from the subnetwork (Ctrl-A)
If the subnetwork is not visible, click right mouse button on the term "Regulation of the IFNG signaling".
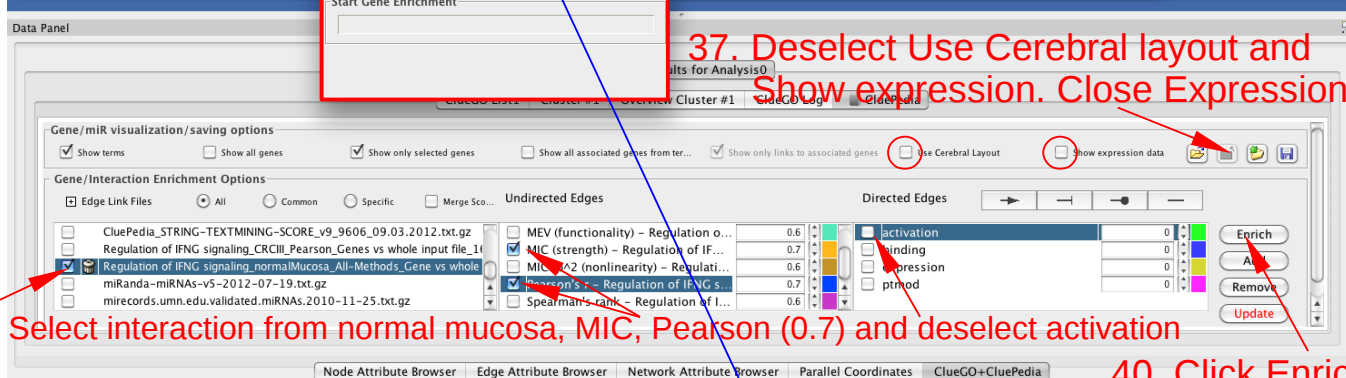
41. Set 5 genes
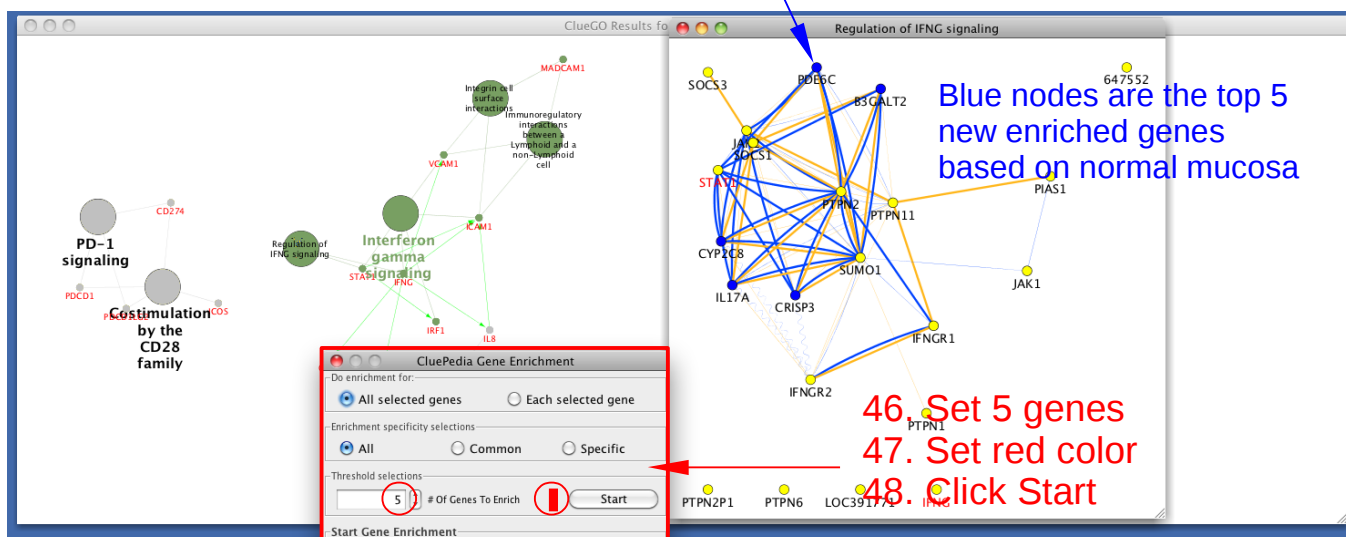**42. Set blue color**
43. Click Start

37. Deselect Use Cerebral layout and Show expression. Close Expression file

39. Select interaction from normal mucosa, MIC, Pearson (0.7) and deselect activation

40. Click Enrich

VII. Enrich the network with new genes using tumor (CRCIII) interaction file.

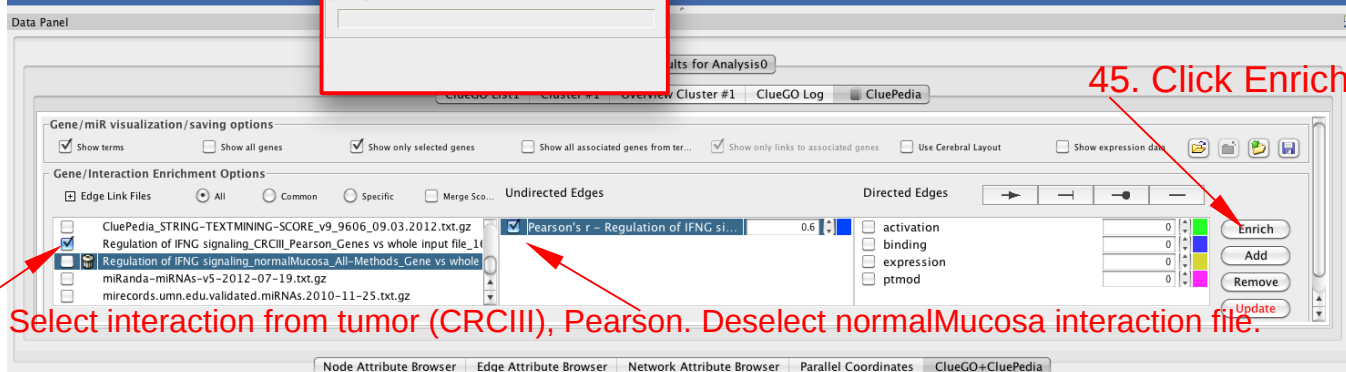Blue nodes are the top 5 new enriched genes based on normal mucosa

46. Set 5 genes
47. Set red color
48. Click Start

45. Click Enrich

44. Select interaction from tumor (CRCIII), Pearson. Deselect normalMucosa interaction file.

VIII. Enrich the network with miRNAs using miRanda prediction file.

Red nodes are the top 5 new enriched genes based on tumor (CRCIII).

51. Set 5 genes
52. Set beige color
53. Click Start

50. Click Enrich

49. Select miRNA prediction file and miRanda SCORE. Deselect tumor interaction file.

IX. Enrich the network with validated miRNAs using mirecords file.

Beige nodes are the top 5 new enriched miRNAs based on miRANDA score.
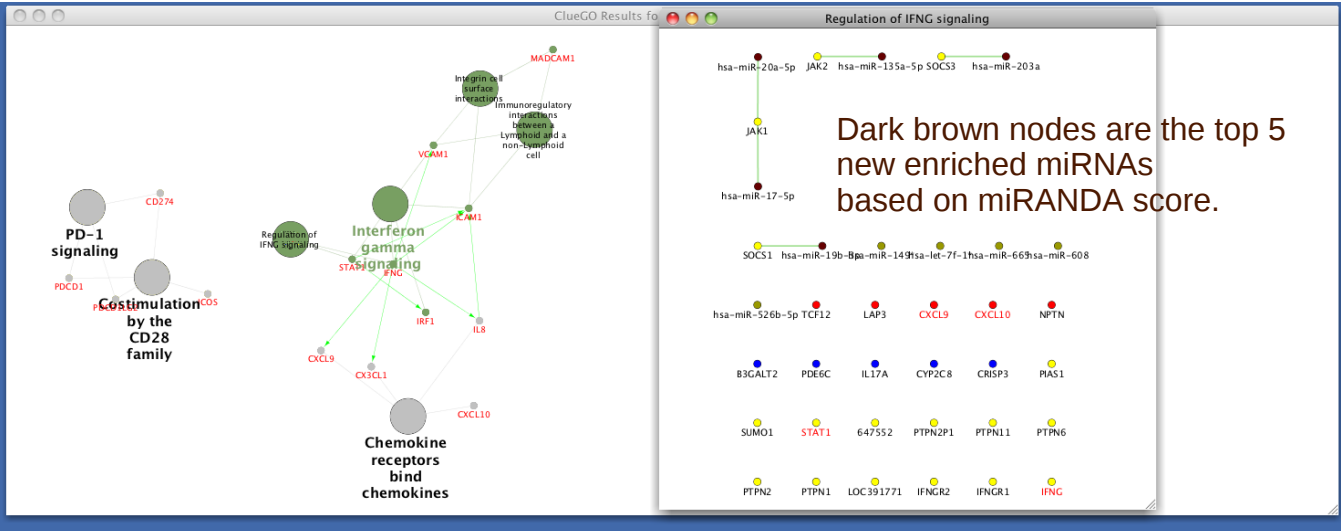
56. Set 5 genes
57. Set dark brown color
58. Click Start

55. Click Enrich

54. Select mirecords validated file and validated miRNA. Deselect miRanda file.

X. Create final network.

Dark brown nodes are the top 5 new enriched miRNAs based on miRANDA score.

59. Select one by one the files used for enrichments, their scores and fit the color to the corresponding enrichment. Select activation.

60. Click Update
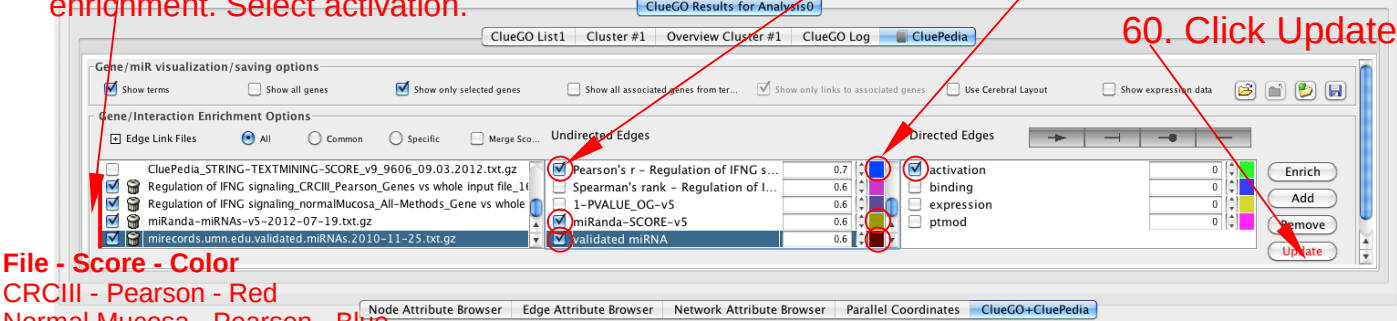
File - Score - Color
CRCIII - Pearson - Red
Normal Mucosa - Pearson - Blue
Predicted miRNAs - miRanda score - Beige
Validated miRNAs - validated miRNA - Brown

Final CluePedia Network

Predicted miRNAs   Validated miRNAs
Known activation
Tumor CRC stageIII
Normal mucosa
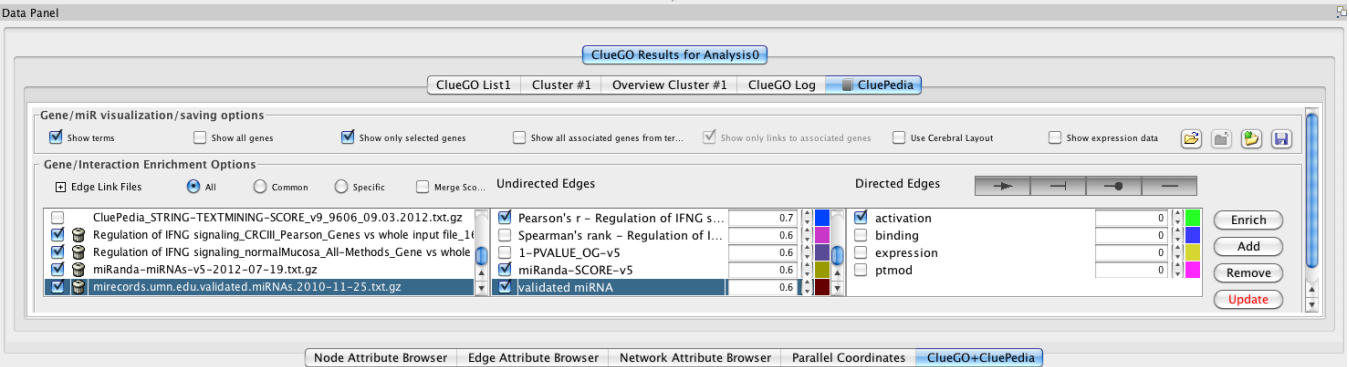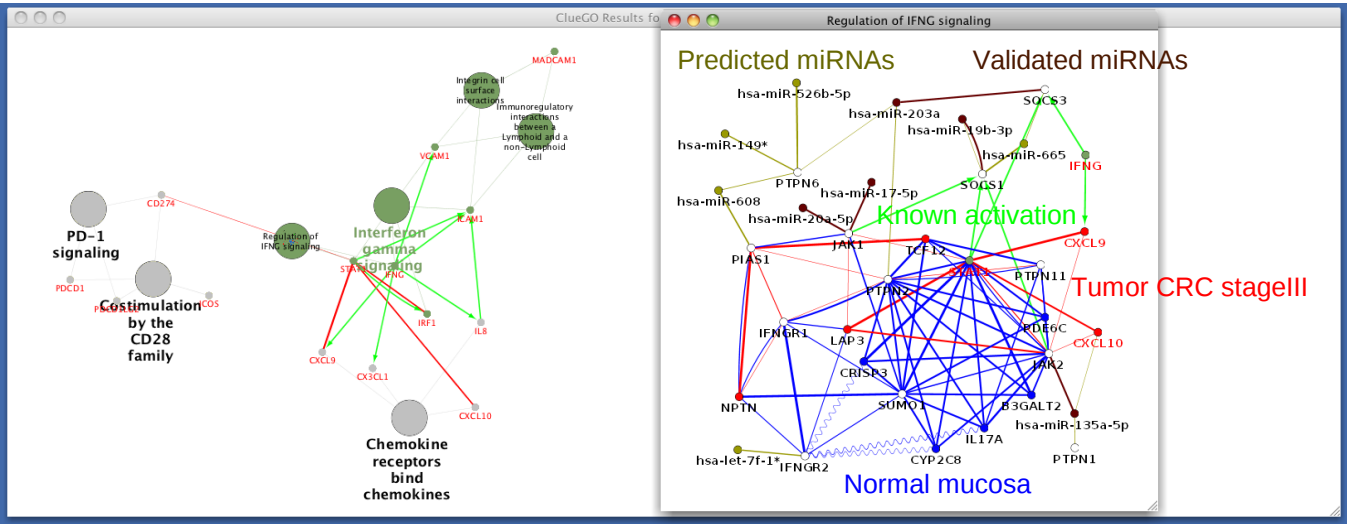
I **Create a ClueGO+CluePedia network of terms/pathways and genes.**

- Download CluePedia and ClueGO plugins in the Cytoscape plugin folder.
  Optional Download Cerebral plugin.

- Start Cytoscape. Open ClueGO+CluePedia (Plugins menu).

- *Keep standard settings*: Analysis Mode (Function), Cluster Analysis Type (Single), Organism (Homo Sapiens), Automatic recognition of ids.

- Open example file CluePediaExampleFile_Th1 related genes.txt.
  Genes: PDCD1LG1, IRF1, GNLY, PDCD1LG2, PDCD1, TBX21, IFNG, TNFRSF6B, ICOS, TNF, CXCL9, CXCL10, IL8, STAT1, MADCAM1, ICAM1, VCAM1, CX3CL1

- Select Ontology: Reactome (version 10.07.2012)

- Select Show Advanced Settings.
  Set Gene percentage to 3%.
  Keep all the other advanced settings.

- Start the analysis
  => ClueGO network of functions

- Select CluePedia panel

- Select Show only selected genes
  => ClueGO+CluePedia network of functions + associated genes.

II **Visualize known interactions between mapped genes.**
  Explore a term/pathway of interest into a nested network.
  See all genes associated to this term and their known interactions.

- Select ACTIONS interaction file (CluePedia_STRING-ACTIONS_v9_9606_09.03.2012).

- Select Action types: activation, binding, expression, ptmode (all scores)

- Update the network
  => ClueGO+CluePedia network of terms and genes. Terms are interconnected based on kappa score and genes based on known interactions from STRING.

- Select the term: Regulation of IFNG signaling

- Create ClueGO Nested Network (Right mouse click)

  => CluePedia nested pathway network with all genes associated to this pathway inter-connected based on known interactions from STRING (interconnection score applies to both, initial and nested network).

III **Calculate and visualize interactions between pathway genes using expression data.**

Expression data from normal colon mucosa and colorectal tumors was used to calculate interrelations between genes associated with *Regulation of IFNG signaling*. The initial data can be downloaded and used for calculation. Files with calculated scores are provided as well. Data sources are presented in CluePedia documentation.

(a) **Download example expression data (normal colon). (ClueGO+CluePedia selection panel, Analysis mode: Function)**

- Expand Show New Available Download Options

- Select Example Data sets

- Click Download => Selection Dialog

- Select expression data from normal colon
  (Homo Sapiens-E-MTAB-57_normalColonMucosa.txt)

- Click OK => The file will be saved in ClueGOExampleFiles folder.

Calculate interactions between pathway genes.

- Select all the genes from the nested network

- Expand Edge link files

- Select Create Custom File

- Click Start => Selection Dialog

- Open expression data (normal colon mucosa)

- Set filters:

  - Experiments to pass: 12

- – Expression Upper threshold: 3.

- – Expression should be beyond the defined interval.

- Select all correlation methods

- Choose Selected Nodes vs whole input file

- Name the new file (e.g. Regulation of IFNG signaling_normalMucosa)

- Click Start

  => Interrelations between genes involved in regulation of IFNG signaling and all other tested genes (Affymetrix U133A) will be calculated.

- The analysis time is approx. 5 minutes (Intel Core 2 Quad 525W 2.66GHz, 1066MHz FSB, 8GB RAM)

- If you see that the memory is too low, close some edge files or increase the Cytoscape memory (e.g. -Xmx2048m, Cytoscape.vmoptions file, in the Cytoscape folder)
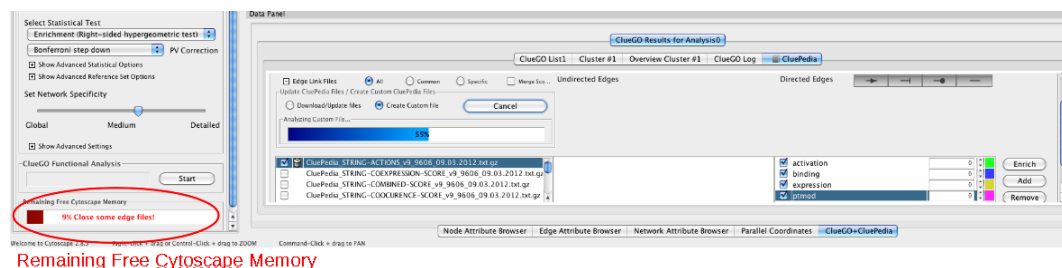

Figure 1: *CluePedia: too less memory for calculation*

(b) **Download interaction data file, colorectal tumors Stage III (CluePedia panel)**

- Expand Edge Link Files

- Select Download/Update Files

- Click Start => Selection Dialog

- Select colorectal tumor data CRCIII

  (Regulation of IFNG signaling_CRCIII_Pearson_Genes vs whole input file_16.07.2012.txt.gz)

- Click Ok => The files will be saved in ClueGOSourceFiles folder.

Visualize interrelations between pathway genes

- Select ACTIONS file and the newly created interaction file (normal Mucosa)

- Select MIC (strength) and Pearson correlation

- Set r >0.7 for both

- Select activation

- Click Update

  => CluePedia nested network showing gene interrelations based on calculated MIC and Pearson scores and on known activation score.

IV Visualize normalized expression data on the network.

- Select IFNGR1 and IFNGR2

- Select Show expression

- Open "CluePedia Expression Data Threshold" dialog
  => Selection Dialog

- Open expression file (normal colon mucosa data)

- Set filters:

  - Experiments to pass: 12

  - Expression Upper threshold: 3.

  - Expression should be beyond the defined interval.

- Check Normalize

- Set Max Expression Level: 1.5

- Select 5 samples to display

- Check Selected Nodes Only

- Click Start => Normalized expression data will be shown for selected genes. If not needed anymore, expression data should be closed.

V See the network in a pathway-like view

- Select "Use Cerebral Layout". =>Genes from the network will be automatically displayed on cellular locations defined within CluePedia.

VI **Enrich the network with new genes using normal mucosa interaction file.**

- Select all genes from the subnetwork

- Select the file containing interactions from normal mucosa

- Select MIC and Pearson's r (edge score 0.7)

- Deselect activation

- Click Enrich/(Right mouse click on the subnetwork) => CluePedia Enrichment panel

- *Keep standard settings*: Enrichment for All selected genes and specificity: All

- Set: 5 genes

- Set color: blue

- Click Start => The top 5 new enriched genes based on normal colon mucosa will be added to the network as blue nodes.

VII **Enrich the network with new genes using colorectal tumor interaction file.**

- Select the file containing interactions from colorectal tumors (CRCIII)

- Deselect normal mucosa interaction file

- Select Pearson's r (edge score 0.7)

- Click Enrich/(Right mouse click on the subnetwork) => CluePedia Enrichment panel

- *Keep standard settings*: Enrichment for All selected genes and specificity: All

- Set: 5 genes

- Set color: red

- Click Start => The top 5 new enriched genes based on CRCIII will be added to the network as red nodes.

VIII **Enrich the network with miRNAs using miRanda prediction file.**

- Select the file containing miRanda predictions

- Deselect CRCIII interaction file

- Select miRanda score (edge score 0.6)

- Click Enrich/(Right mouse click on the subnetwork) => CluePedia Enrichment panel

- *Keep standard settings*: Enrichment for All selected genes and specificity: All

- Set: 5 miRNAs

- Set color: beige

- Click Start => The top 5 new enriched miRNAs based on miRanda predictions will be added to the network as beige nodes.

IX **Enrich the network with validated miRNAs using mirecords file.**

- Select the file containing mirecords validations

- Deselect miRanda prediction file

- Select validated miRNA score (edge score 0.6)

- Click Enrich/(Right mouse click on the subnetwork) => CluePedia Enrichment panel

- *Keep standard settings*: Enrichment for All selected genes and specificity: All

- Set: 5 miRNAs

- Set color: brown

- Click Start => The top 5 new enriched validated miRNAs based on mirecords will be added to the network as brown nodes.

X **Create final network.**

- Select all four files used for enrichment

- Check the corresponding scores

- Fit the color of the edge to the corresponsing enrichment

- Select activation

14

- Click Update

  => Final CluePedia network for the pathway *Regulation of IFNG regulation*. The network includes pathway genes, enriched genes from normal mucosa and from CRCIII as well as predicted and validated miRNAs. Known activation scores and calculated interrelations are shown.