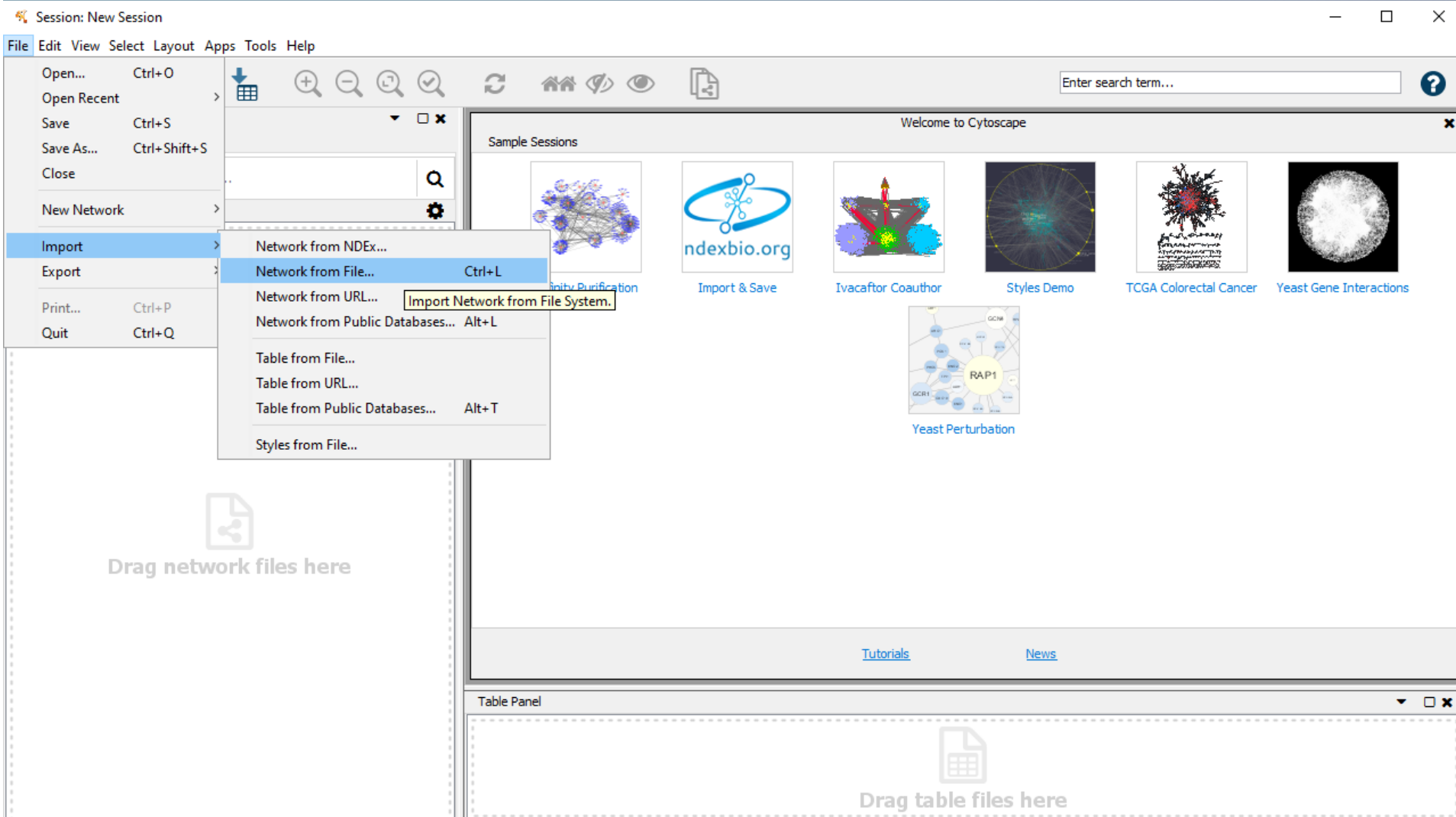


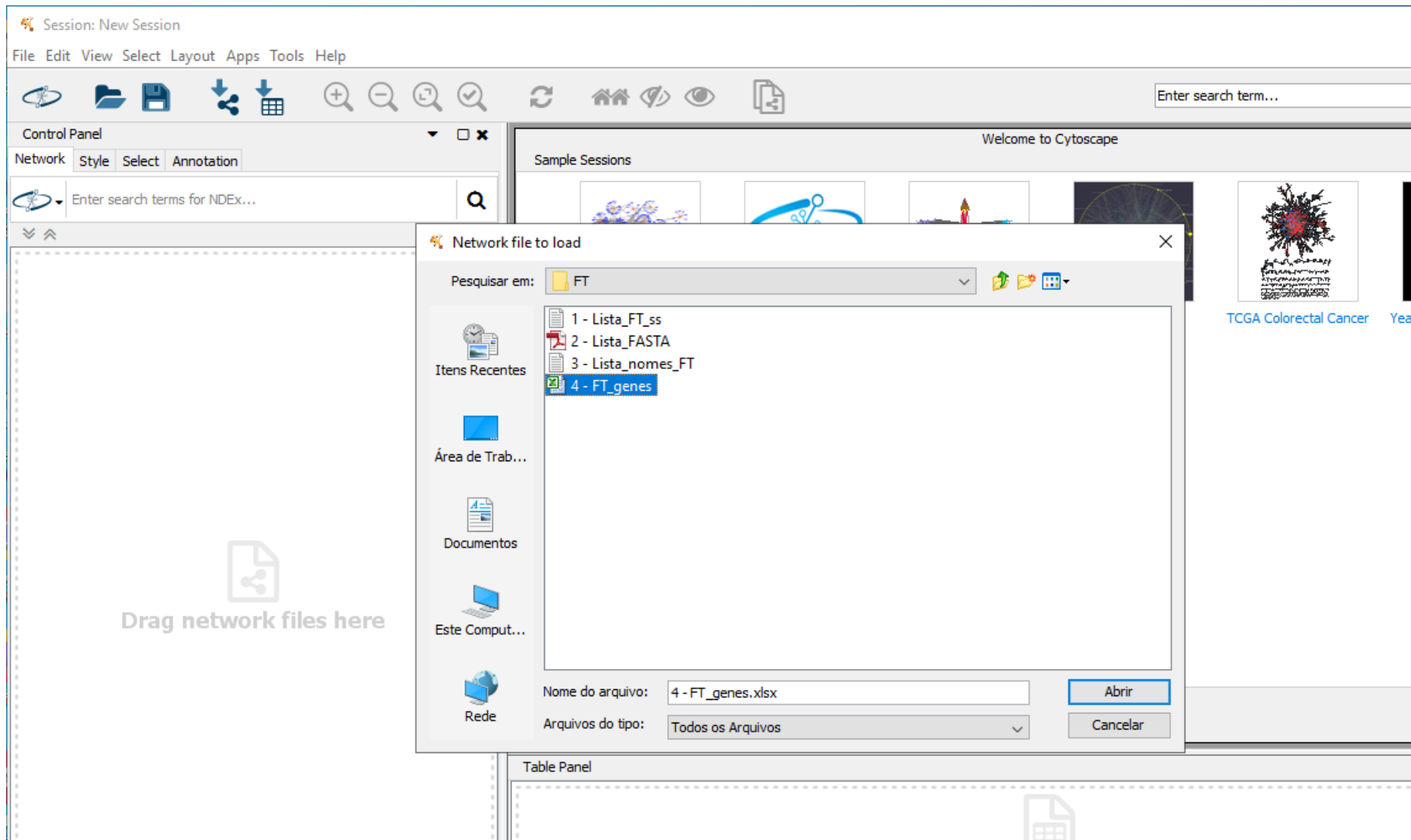
# **NETWORK ANALYZER**

Com o programa aberto, clicar em File → Import → Network from File...



Pesquisar a planilha que foi salva no 9º passo (agora com genes e FT mais relevantes – após revisão bibliográfica)

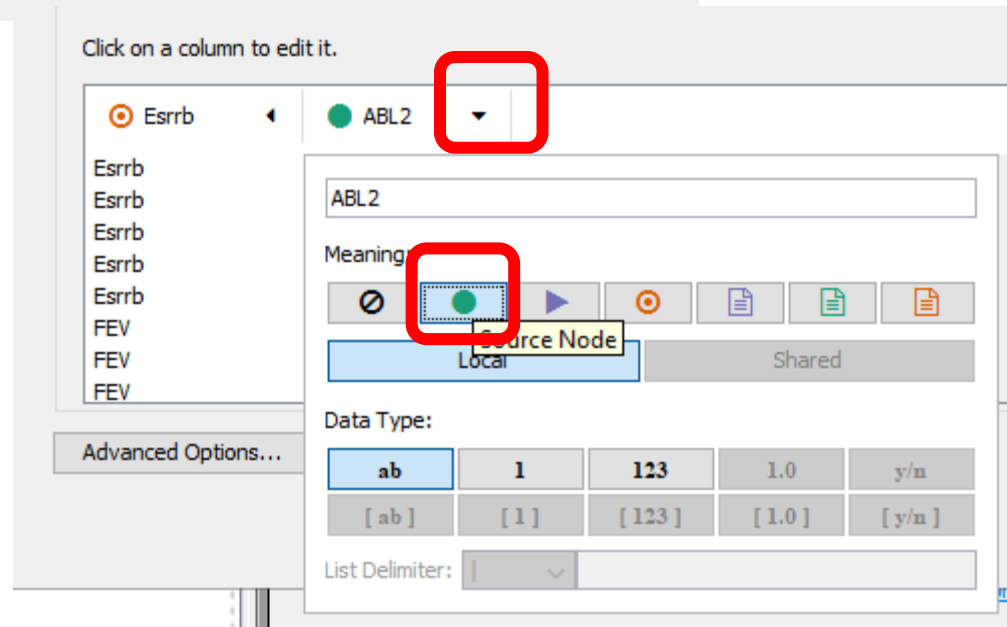
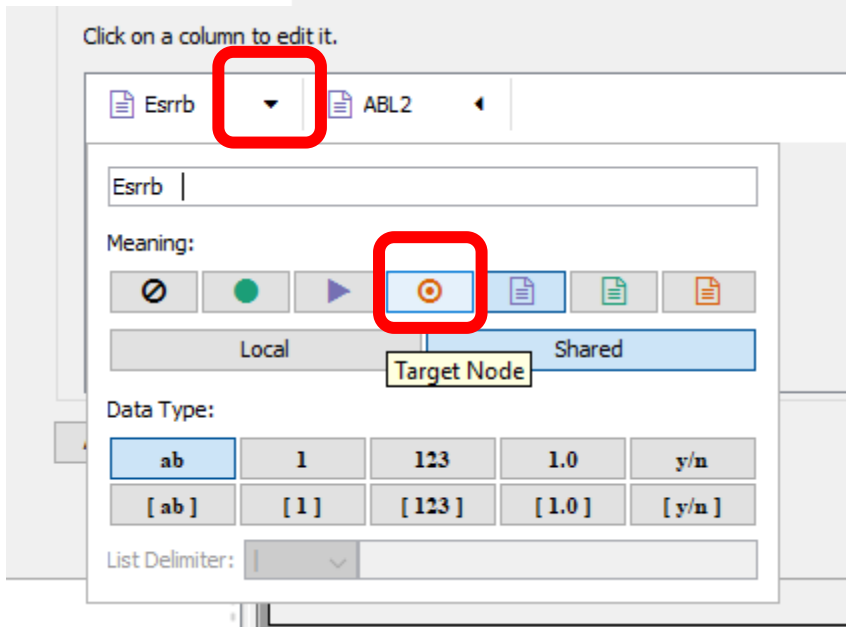
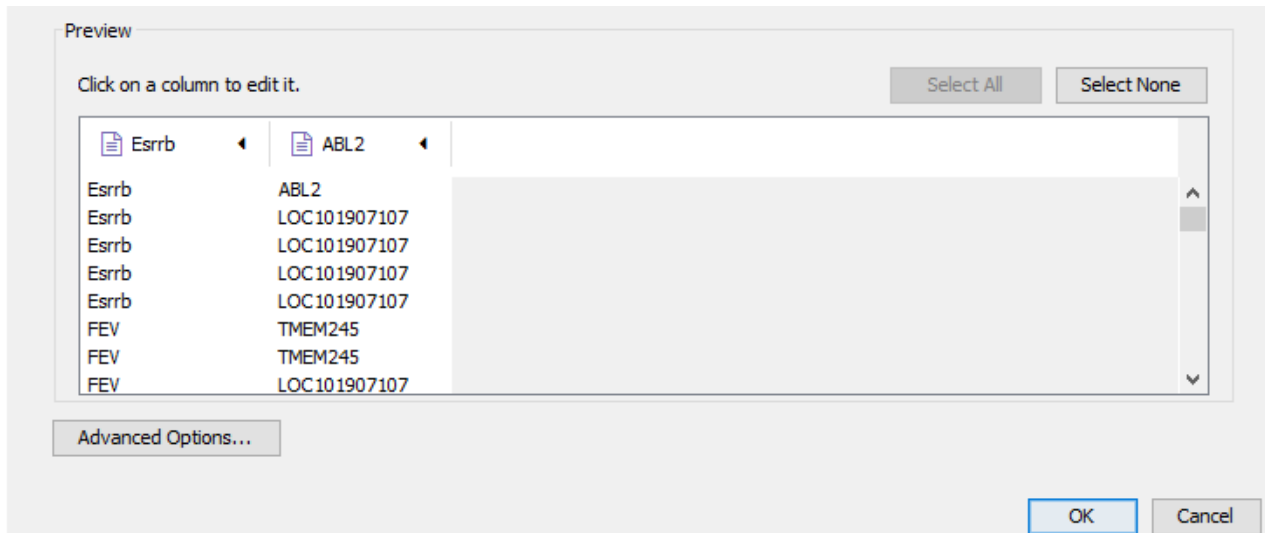
Selecionar o arquivo e clicar em abrir



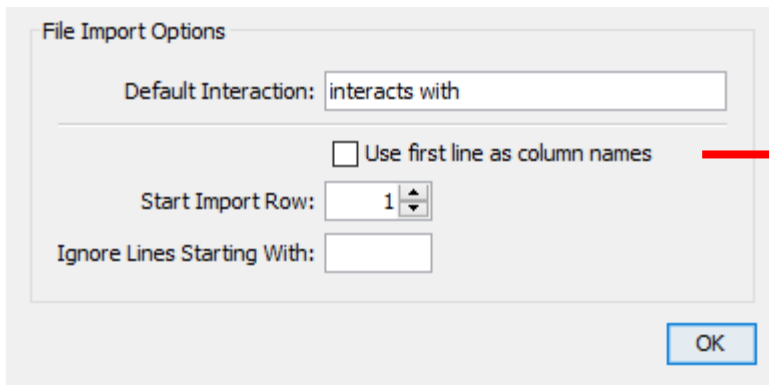
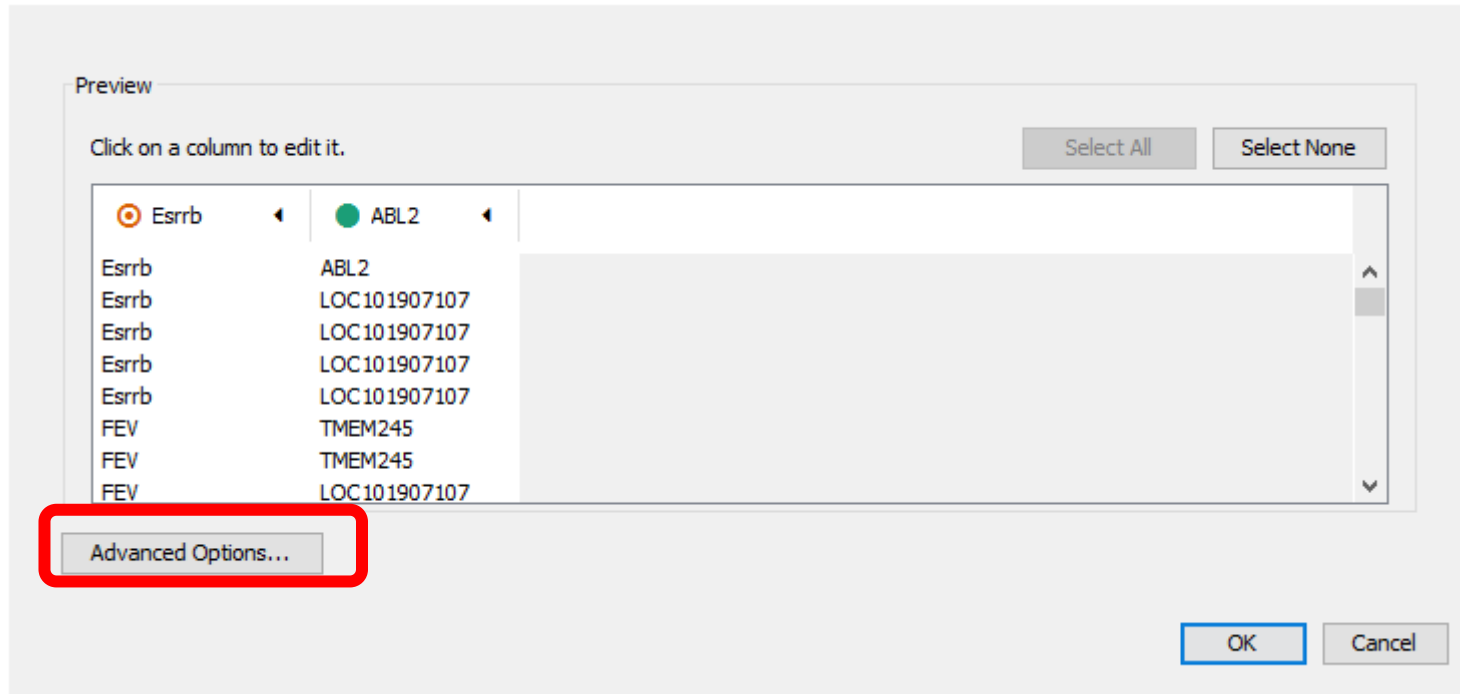
Vai aparecer a seguinte tela:

Na primeira coluna, clicar na setinha e clicar em Target Node (Alvo)

Na segunda coluna, clicar na setinha e clicar em Source Node (gene original)



Clicar em Advanced Options...



- Se esta opção estiver selecionada, remover a seleção, pois os dados da planilha não tem cabeçalho
- Se tiver deixado cabeçalho na planilha no 9º passo, pode deixar essa opção marcada

Depois dar OK nas duas caixas abertas

# Vai aparecer a seguinte tela com as interações entre genes e FTs

The screenshot displays a network visualization software interface. The main window shows a complex network of nodes and edges. The nodes are labeled with gene and FT names: FEV, ELK4, PEAK1, TMEM245, LOC101907107, FOXO3, Esrrb, and ABL2. The edges represent interactions between these entities. A search bar at the top right contains the text "Enter search term...".

On the left side, there is a "Control Panel" with tabs for "Network", "Style", "Select", and "Annotation". Below these tabs, there is a search bar for "NDEx..." and a list of networks. The current network is "Plan1", which is selected and shows 8 nodes and 50 edges.

At the bottom, there is a "Table Panel" with a table showing the shared name and name of the selected nodes. The table has two columns: "shared name" and "name".

shared name	name
ABL2	ABL2
Esrrb	Esrrb

Below the table, there are tabs for "Node Table", "Edge Table", and "Network Table".

No próximo passo:  
Clicar em Tools → Merge → Networks...



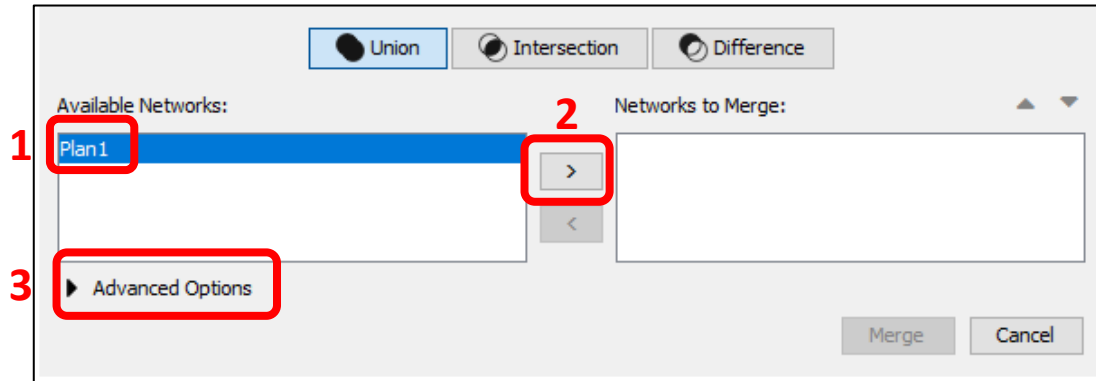
The screenshot shows the software interface with the 'Tools' menu open. The 'Merge' option is selected, and its sub-menu is visible, showing 'Networks...' and 'Tables...'. The main window displays a network diagram with nodes labeled FEV, ELK4, PEAK1, TMEM245, FOXO3, ABL2, and Esrrb. A search bar at the top right contains the text 'Enter search term...'. The bottom panel shows a 'Table Panel' with the following data:

shared name	name
ABL2	ABL2
Esrrb	Esrrb

No próximo passo:

1 – Clicar na opção disponível na caixa ‘Available Networks’

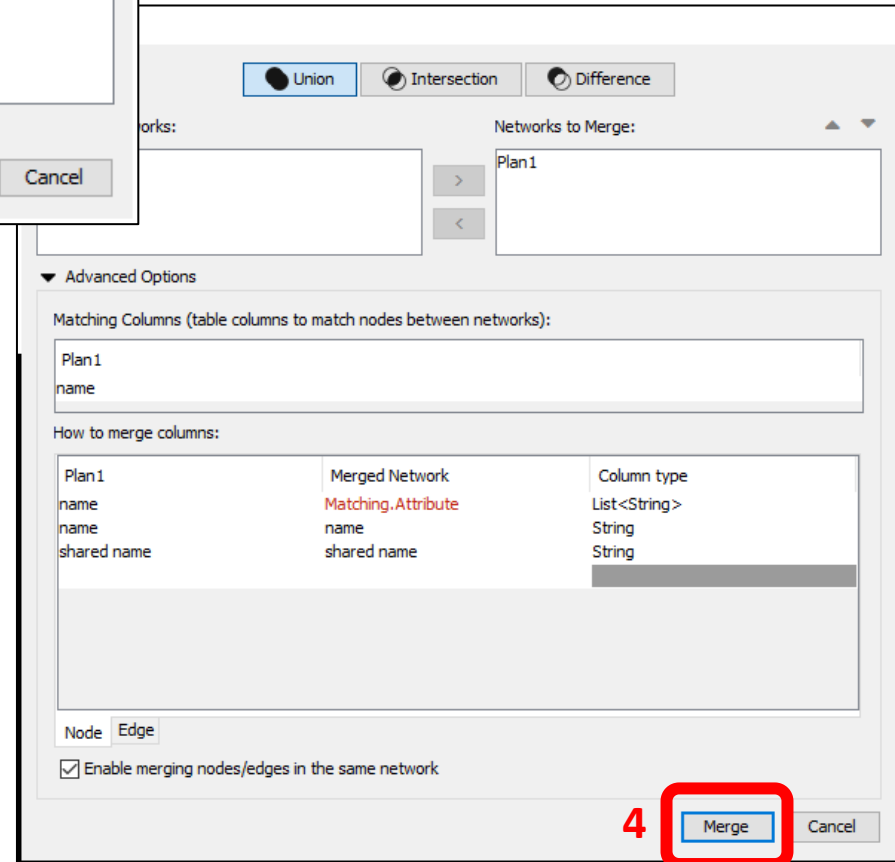
2 – Clicar na setinha para a direita para passar para ‘Networks to Merge’



3 – Clicar em Advanced Options

Não alterar mais nenhum parâmetro

4 – Clicar em Merge



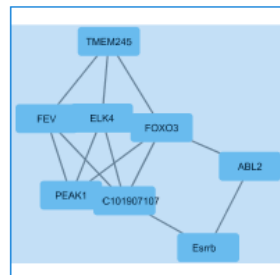
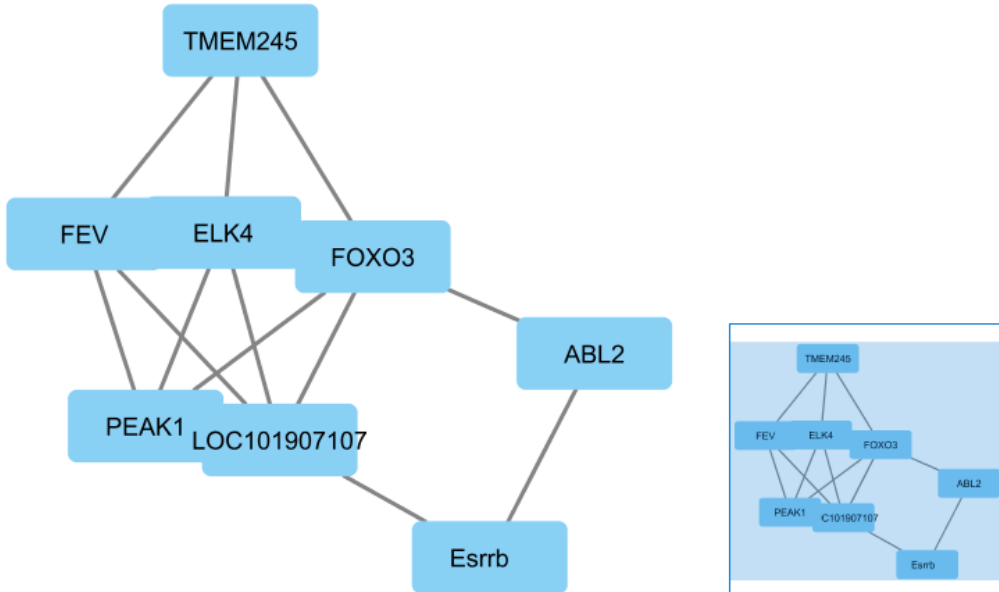




Enter search term... ?

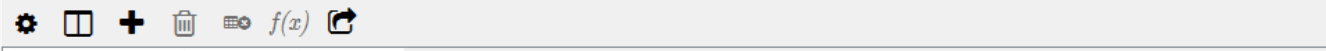
Control Panel  
 Network Style Select Annotation

- Enter search terms for NDEx... Q
- 1 of 2 Networks selected ⚙
- Plan1 1
  - Plan1 8 50
  - Merged Network 1
  - Merged Network 8 12**



Merged Network 0 0 0 0

Table Panel ⌵ □ ×



shared name	name	Matching
ELK4	ELK4	[ELK4]
FOXO3	FOXO3	[FOXO3]

Node Table Edge Table Network Table



Memory

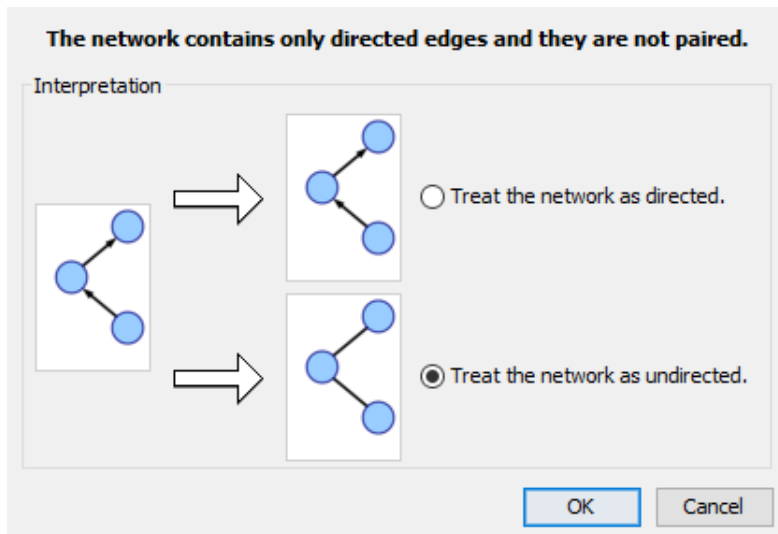
No próximo passo:

- Clicar em Tools → NetworkAnalyzer → Network Analysis → Analyze Network...

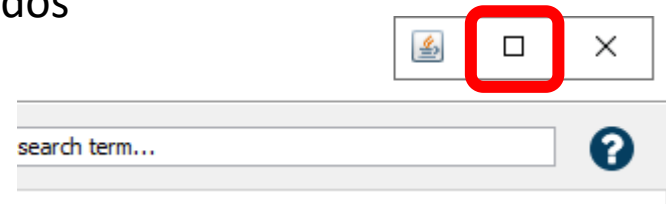
The screenshot shows the Cytoscape software interface. A red arrow points to the 'Tools' menu. The 'Tools' menu is open, showing 'NetworkAnalyzer' as the selected option. The 'NetworkAnalyzer' sub-menu is also open, showing 'Network Analysis' as the selected option. The 'Network Analysis' sub-menu is open, showing 'Analyze Network...' as the selected option. The main window displays a network diagram with nodes labeled TMEM245, FEV, ELK4, FOXO3, ABL2, PEAK1, LOC101907107, and Esrb. The 'Table Panel' at the bottom shows a table with columns for 'shared name', 'name', and 'Matching'.

shared name	name	Matching
ELK4	ELK4	[ELK4]
FOXO3	FOXO3	[FOXO3]

# 1 – Selecionar ‘Treat the network as undirected’



2 - Depois de clicar em OK, vai aparecer uns ícones pequenos no canto superior direito. Clicar no quadradinho para ampliar a tela de resultados



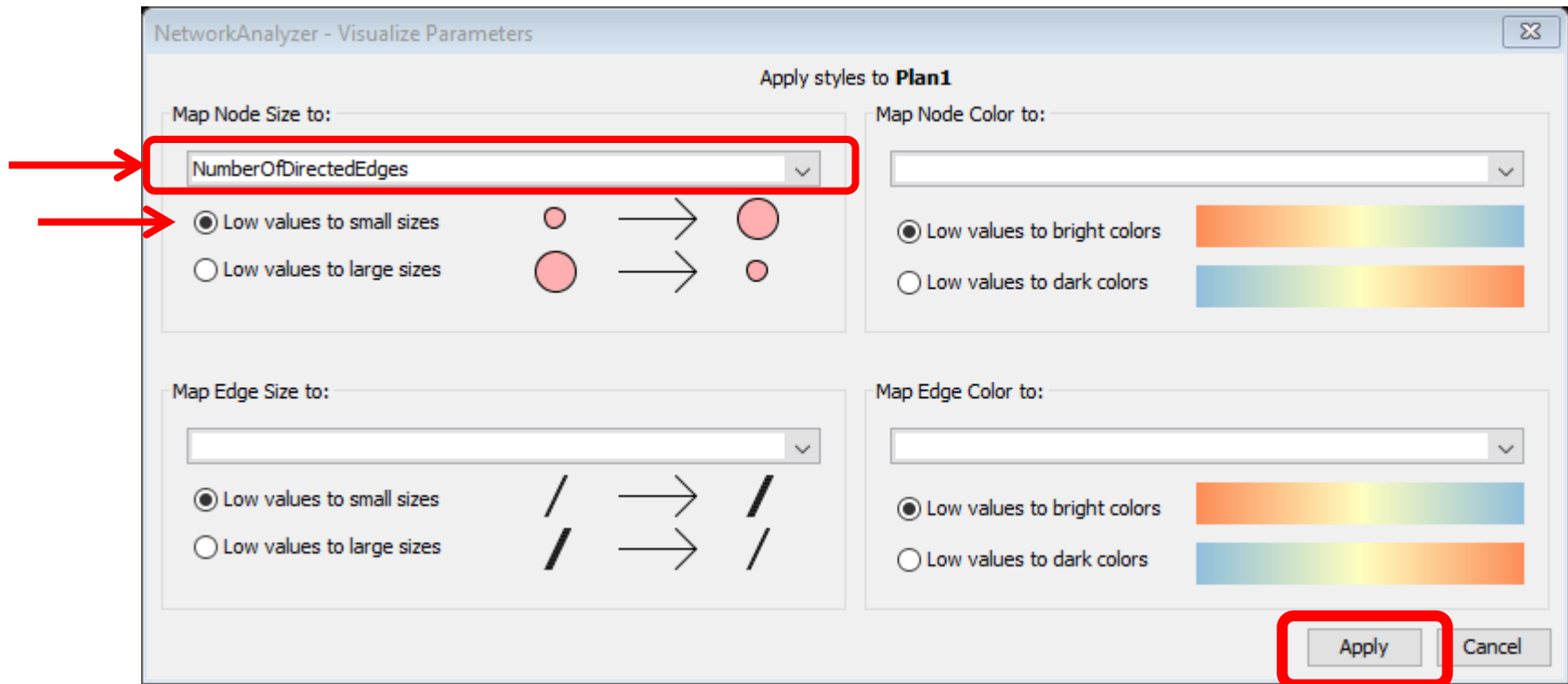
Network Statistics of Plan1 (undirected)

Shortest Path Length Distribution	Shared Neighbors Distribution	Neighborhood Connectivity Distribution	Betweenness Centrality	Closeness Centrality	Stress Centrality Distribution
Simple Parameters	Node Degree Distribution		Avg. Clustering Coefficient Distribution		Topological Coefficients
Clustering coefficient : <b>0.0</b>		Number of nodes : <b>8</b>			
Connected components : <b>1</b>		Network density : <b>0.429</b>			
Network diameter : <b>3</b>		Network heterogeneity : <b>0.236</b>			
Network radius : <b>2</b>		Isolated nodes : <b>0</b>			
Network centralization : <b>0.190</b>		Number of self-loops : <b>0</b>			
Shortest paths : <b>56 (100%)</b>		Multi-edge node pairs : <b>11</b>			
Characteristic path length : <b>1.714</b>		Analysis time (sec) : <b>0.032</b>			
Avg. number of neighbors : <b>3.0</b>					

Save Statistics Visualize Parameters Close Tab

3 - Clicar em Visualize Parameters

- Na parte 'Map Node Size to:'
- Escolher a opção: 'NumberofDirectedEdges'
- Selecionar 'Low values do small sizes'
- Manter as outras como estão
- Clicar em 'Apply'



# Vai aparecer a seguinte tela

File Edit View Select Layout Apps Tools Help

Control Panel

Network Style Select Annotation

Enter search terms for NDEx...

1 of 1 Network selected

Plan1

Plan1 8 50

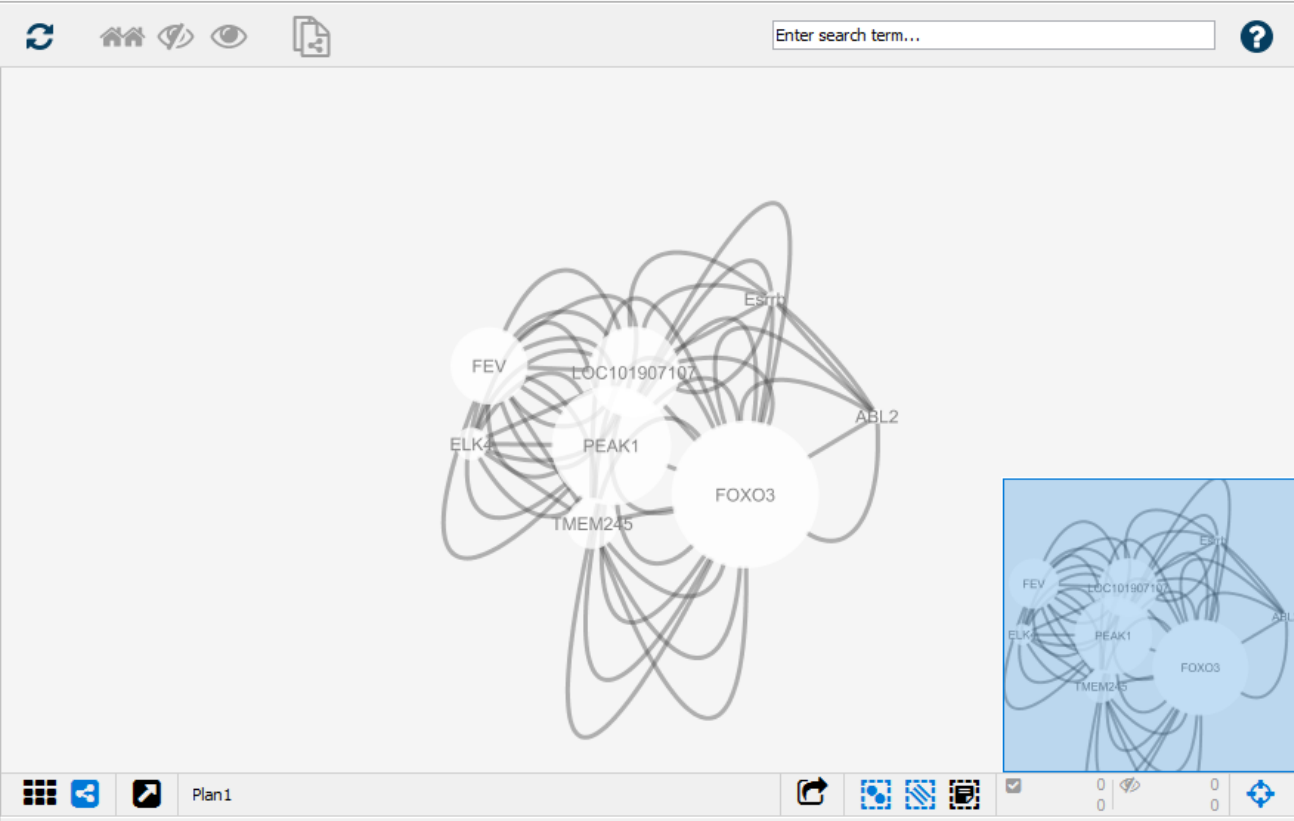


Table Panel

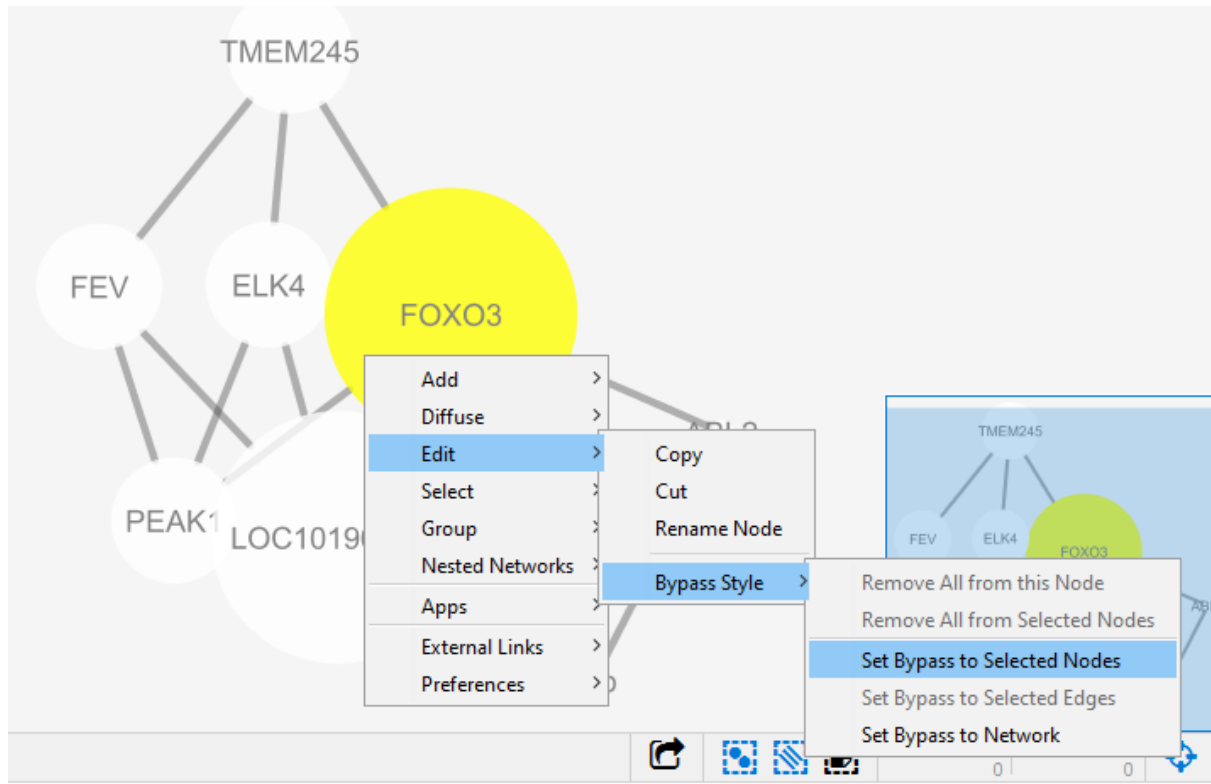
shared name	name	AverageShc	ClusteringC <sub>i</sub>	ClosenessC <sub>i</sub>	IsSingleNod	PartnerOfM	Selfloops	Eccentricity	Stress	Degre
ABL2	ABL2	2.0	0.0	0.5	☐	2	0	3	6	...

Node Table Edge Table Network Table

Memory

# Se quiser editar as cores para identificar os genes e fatores de transcrição

- Ao clicar nas bolinhas com cada gene ou fator de transcrição, elas ficam com cor
- Para alterar a cor dos genes e fatores de transcrição:
- Clicar com o botão direito em cima da bolinha → Edit → Bypass Style → Set Bypass to Selected Nodes



Control Panel

Network Style Select Annotation

NetworkAnalyzer Style: Merged Network

Properties

Def.	Map.	By.	
			Border Paint
0.0			Border Width
			Fill Color

Column	name
Mapping Type	Discrete Mapping
APM2	
APBA3	
ARHGEF12	
ATCAY	
ATP6V0A2	
CA11	
CACNG3	
CACTIN	
CBFA2T3	
CDH15	

Merged Network

Table Panel

STAT3

NO11 SPACA4 CREB3L3 SULT2B1 PPIE FZR1 CACNG3 DDHD1 TMED2 ATCAY FUF1

Para preencher as cores:

Se quiser todos da mesma cor é só selecionar a cor no primeiro quadrinho (onde está verde na imagem). Para preencher um por um:

Clicar na setinha ao lado de 'Fill Color' → Ao lado de 'Column' selecionar 'name' → Ao lado de 'Mapping Type' selecionar 'Discrete Mapping'

Clicar na segunda coluna (ao lado do nome de cada gene) → Clicar nos 3 pontinhos → Selecionar a cor (o mesmo vale para outras alterações, ex.: forma = Shape)

- Para mudar a cor de fundo:
- Clicar com o botão direito na imagem
- Edit → Bypass Style → Set Bypass to Network
- No menu à esquerda:
- Em 'Background Paint' selecionar a cor desejada

The screenshot displays the NetworkAnalyzer application interface. The main window shows a network graph with two prominent yellow nodes, STAT3 and EGR1, and several green nodes including SULT2B1, PPIE, FZR1, CACNG3, and DDHD1. The Control Panel on the left is set to 'NetworkAnalyzer Style: Merged Network' and has the 'Background Paint' property selected. The Table Panel at the bottom shows a table with columns for 'shared name', 'name', 'Matching.Attribute', 'AverageShortestPathLength', 'ClusteringCoefficient', 'ClosenessCentrality', and 'IsSingle'. The table contains one row of data for 'SHD'.

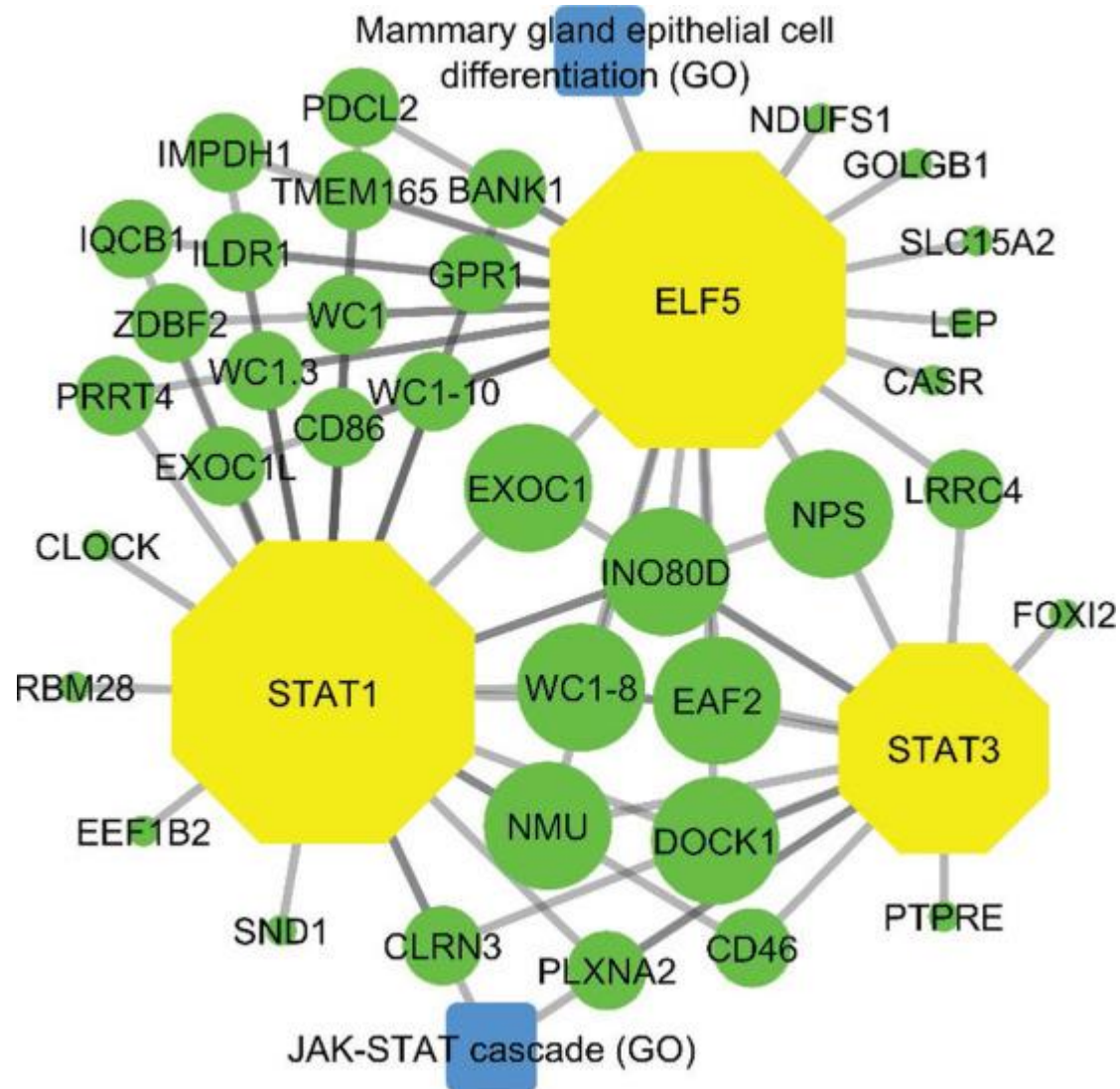
shared name	name	Matching.Attribute	AverageShortestPathLength	ClusteringCoefficient	ClosenessCentrality	IsSingle
SHD	SHD	[SHD]	2.61818182	0.0	0.38194444	



# Exemplo:

Figure 3. Gene-transcription factor (TF) network: Genes located in the top 10 windows for 305-d milk yield (kg; green circle nodes) and their associated TF (yellow octagon nodes). Node size corresponds to network analyses (Cytoscape; [Shannon et al., 2003](#)), in which larger nodes denotes a higher edge density associated with the number of TF binding sites. Blue square nodes show the gene ontology (GO) biological processes related to TF.

Otto et al. (2020)  
<https://doi.org/10.3168/jds.2019-17890>



# Considerações finais

- Sobre os genes identificados, principalmente os que tem processo biológicos relacionados às características estudadas, seria interessante realizar estudos futuros avaliando a expressão gênica