

Proteomics Exercise Part 3

1. Open this GeneName identifier file
"ProteomicsDemoList2.txt." This file has 45 identifiers.

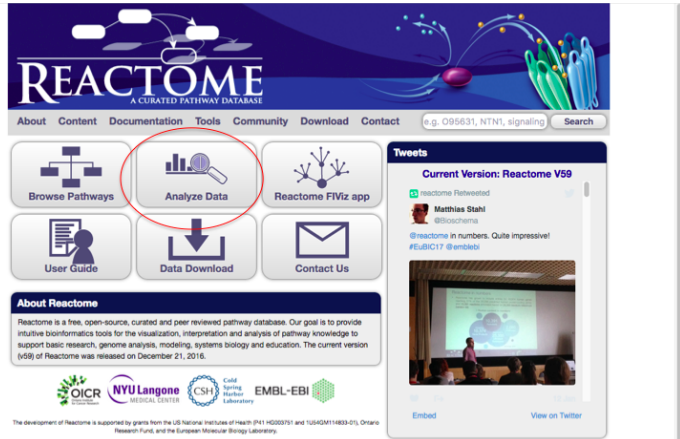
Go to the UniProt website and repeat the steps outlined in Part 1 of this exercise to map them to UniProt Identifiers. Then return for the next steps.

[no image]

2. You should be able to generate an annotated file of 45 UniProt proteins.

Entry	Gene names	Entry name	Status	Protein names	Gene names	Organism	Length
P01730	CD4	CD4_HUMAN	reviewed	T-cell surface glycoprotein CD4 (T-cell surface a	CD4	Homo sapi	458
P01137	TGFB1	TGFB1_HUMAN	reviewed	Transforming growth factor beta-1 (TGF-beta-1) TGF	TGFB1	Homo sapi	390
P01579	IFNG	IFNG_HUMAN	reviewed	Interferon gamma (IFN-gamma) (immune inter) IFNG	IFNG	Homo sapi	166
P12318	FCGR2A	FCGR2A_HUMAN	reviewed	Low affinity immunoglobulin gamma Fc region I FCG	FCGR2A	Homo sapi	317
P08637	FCGR3A	FCGR3A_HUMAN	reviewed	Low affinity immunoglobulin gamma Fc region I FCG	FCGR3A	Homo sapi	254
P15410	CTSL4	CTSL4_HUMAN	reviewed	Cytotoxic T-lymphocyte protein 4 (Cytotoxic T-lym	CTSL4	Homo sapi	223
P29965	CD40LG	CD40LG_HUMAN	reviewed	CD40 ligand (CD40-L) (T-cell antigen Gp39) TNF	CD40LG	Homo sapi	261
P05112	IL4	IL4_HUMAN	reviewed	Interleukin-4 (IL-4) (B-cell stimulatory factor 1) IL	IL4	Homo sapi	153
P01374	IL6	IL6_HUMAN	reviewed	Lymphotxin alpha (LT-alpha) (TNF-beta) (Tum	IL6	Homo sapi	205
P05231	IL6	IL6_HUMAN	reviewed	Interleukin-6 (IL-6) (B-cell stimulatory factor 2) IL	IL6	Homo sapi	212
Q9Y275	TNFSF13B	TNFSF13B_HUMAN	reviewed	Tumor necrosis factor ligand superfamily mem	TNFSF13B	Homo sapi	285
P13194	FCGR2B	FCGR2B_HUMAN	reviewed	Low affinity immunoglobulin gamma Fc region I FCG	FCGR2B	Homo sapi	310
P15510	IL18	IL18_HUMAN	reviewed	Interleukin-1 receptor antagonist protein (IL-18) IL	IL18	Homo sapi	177
Q14116	IL18	IL18_HUMAN	reviewed	Interleukin-18 (IL-18) (Interleukin-18) IL18	IL18	Homo sapi	193
P33681	CD80	CD80_HUMAN	reviewed	T-lymphocyte activation antigen CD80 (Activati	CD80	Homo sapi	288
P11886	MSAA1	MSAA1_HUMAN	reviewed	B-lymphocyte antigen CD20 (B-lymphocyte surf	MSAA1	Homo sapi	297
P42061	CD86	CD86_HUMAN	reviewed	T-lymphocyte activation antigen CD86 (Activati	CD86	Homo sapi	329
P02741	CRP	CRP_HUMAN	reviewed	C-reactive protein (C-reactive pro) CRP	CRP	Homo sapi	224
P43405	SYK	SYK_HUMAN	reviewed	Tyrosine-protein kinase SYK (EC 2.7.10.2) (Splee	SYK	Homo sapi	635
Q07011	TNFRSF9	TNFRSF9_HUMAN	reviewed	Tumor necrosis factor receptor superfamily me	TNFRSF9	Homo sapi	255
P20373	FCGR1G	FCGR1G_HUMAN	reviewed	High affinity immunoglobulin epsilon receptor F	FCGR1G	Homo sapi	86
P25063	CD24	CD24_HUMAN	reviewed	Signal transducer CD24 (Small cell lung carcino	CD24	Homo sapi	80
Q8IXI7	TH1L	TH1L_HUMAN	reviewed	Negative elongation factor C/D (NELF-C/D) (TH1	TH1L	Homo sapi	590
P15163	IPNA2	IPNA2_HUMAN	reviewed	Interferon alpha-2 (IFN-alpha-2) (Interferon alp	IPNA2	Homo sapi	188
Q05998	IL18BP	IL18BP_HUMAN	reviewed	Interleukin-18-binding protein (IL-18BP) (Tat	IL18BP	Homo sapi	194
P05109	S100A8	S100A8_HUMAN	reviewed	Protein S100-A8 (Calgranulin-A) (Calprotectin L	S100A8	Homo sapi	93
P20701	ITGAL	ITGAL_HUMAN	reviewed	Integrin alpha-L (CD11 antigen-like family mem	ITGAL	Homo sapi	1170
P41159	LEP	LEP_HUMAN	reviewed	Leptin (Obese protein) (Obesity factor)	LEP	Homo sapi	167
P02747	PLG	PLG_HUMAN	reviewed	Plasminogen (EC 3.4.21.7) (Circulat	PLG	Homo sapi	810
P08603	CFH	CFH_HUMAN	reviewed	Complement factor H (H factor 1)	CFH	Homo sapi	1231
P02787	TF	TF_HUMAN	reviewed	Serotransferrin (Transferrin) (Beta-1 metal-binc	TF	Homo sapi	698
P40225	THPO	THPO_HUMAN	reviewed	Thrombopoietin (C-mol leucod) (ML) (Mleukarv	THPO	Homo sapi	353

3. Go to www.reactome.org. Select the "Analyze Data" option.



4. Copy/Paste your Accession list into the box. You can add a header line at the top following a # symbol.

Select "Continue."

On the next page, select "Analyze" (all IDs are for Human).

5. The display should look similar to this image. You can change color profile settings as shown if you want to see overview better. I use barium salts. The darker the color, the more significant the pathway.

Note: some IDs were not found. You can ignore these—not all gene/proteins exist in a defined pathway yet.

The central image is an overview display of all their pathways so you can quickly see what pathways your genes fall into. On the left you have all the pathways in their hierarchy. Note that Reactomes pathways are organized in a loose hierarchy (not all pathways resources do this).

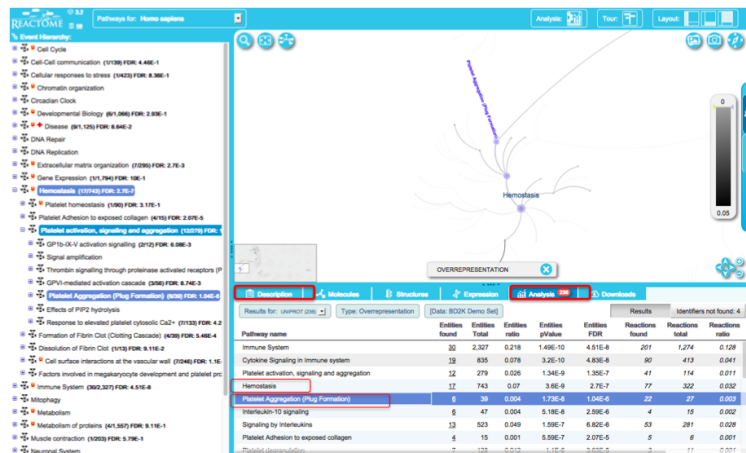
On the bottom you have the analysis results pane, which is similar to the GO analysis we did and shows an overrepresentation of genes in a pathway compared to random results with the number of entities (proteins/genes) found, expected, and p-value, etc.

You can adjust pane sizes as needed. See [Reactome tutorials](#) for full details on the interface.



6. Note: the 3 panes are linked. If I click on one pathway, the other panels change also.

Hover over a term in the analysis results pane, and you will see it highlighted on the overview pane. Click on it, and the overview and hierarchy pane change to zoom in on more details.



7. In the analysis results pane, select "Hemostasis."

In the hierarchy pane we see the pathway we selected highlighted. Hemostasis is a high-level pathway in the hierarchy under which more specific pathways are grouped.

8. Select "Description," and you get a description of the pathway with references about what this top level pathway is about.

[no image]

Hemostasis is a physiological response that culminates in the arrest of bleeding from an injured vessel.

9. Select "Analysis" to go back to Analysis results.

[no image]

Select "Platelet Aggregation (Plug Formation)" and you see in the hierarchy pane that it is one of the more specific pathways in the "Hemostasis" hierarchy under "Platelet activation, signaling and aggregation," which is also highlighted and on our list of significant hits.

Note: Results here are slightly different in order of significance than in the demo video as the Reactome database and pathways has been updated since the demo video was recorded. Slight changes like this are normal if the resources are improving themselves. It is important to note the database version and date you are using for any analysis you want to publish.

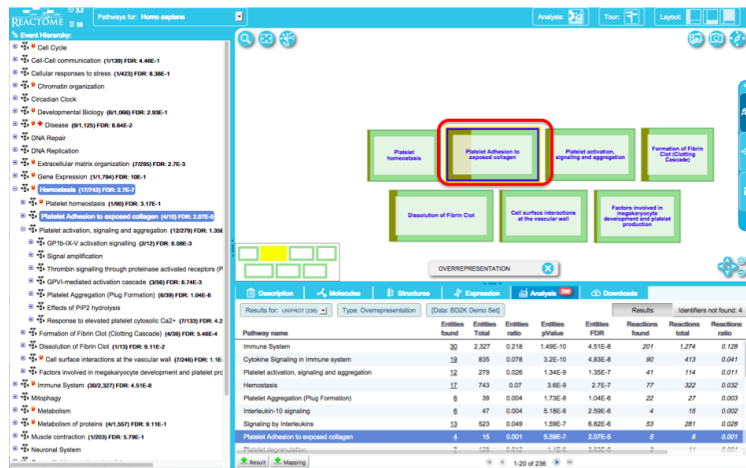
[no image]

10. Double-click on the hemostasis pathway in the "Analysis" pane. Now you see 7 boxes in graphics pane illustrating that hemostasis is really a high-level collection of more detailed pathways related to hemostasis.

The brown shading illustrates the proportion of genes/protein in our list that is in a pathway.

"Platelet Adhesion to exposed collagen" seems to have the most shading. Double click on it to see details.

Now we see some details of the pathway. Again, the brown shading shows genes that are in our list. The orange/pink color illustrates where the reaction occurs, cytosol, nucleus, membrane, extracellular, etc. Not all are shown in all pathways.



11. Now you see the pathway and reactions in more detail.

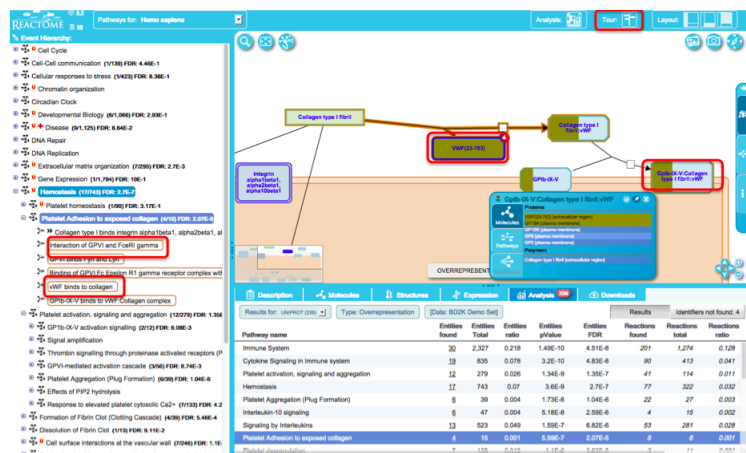
Open the pathway in the hierarchy pane and to see each reaction and click on the reaction "vWF binds to Collagen". This will zoom into the reaction. Adjust panes as needed.

Hover over "VWF" (von Willebrand Factor), one of the proteins in our list in the extra cellular space.

Double-click on the VWF box, and we see the reaction it participates in. VWF binds to Collagen type 1 to make collagen IV vWF complex, which then binds to another complex called GPIb-IX-V complex to form the GPIb-IX-V:vWF:Collagen IV complex on the plasma membrane.

Hover over GPIb-IX-V:vWF:Collagen IV complex or other box and you see an arrow on the right. Click to see the contents of the molecule or complex and which molecules are in our list (highlighted in brown) and which are missing (in blue). You can also see if the complex is involved in another pathway or other interactions.

Note: Red numbers on VWF (or other proteins) indicates that there are 4 proteins known to interact with the molecule in the IntAct database. More information can be found in the demo video.



Please investigate other options on the interface and/or take the tour or other tutorials Reactome offers.

Now lets take a quick look at the "Interaction of GPVI and FcεRI gamma" reactions in this pathway. Click on the reaction "Interaction of GPVI and FcεRI gamma" in the hierarchy pane.

12. Now lets take a quick look at the "Interaction of GPVI and FcεRI gamma" reactions in this pathway.

Click on the reaction "Interaction of GPVI and FcεRI gamma" in the hierarchy pane.

[no image]

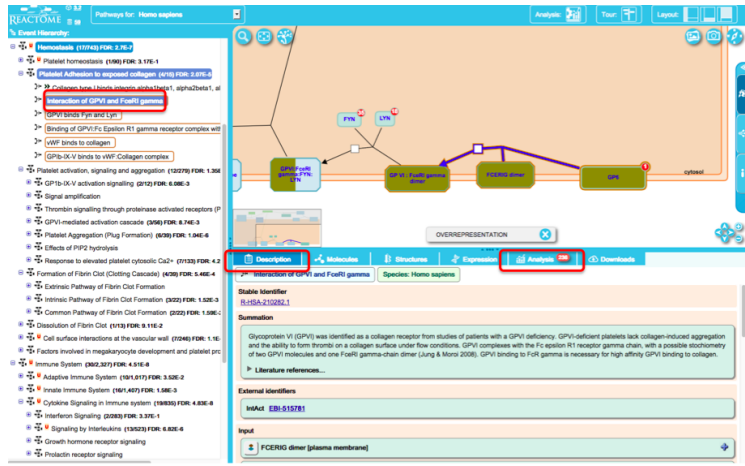
You can go through the individual steps of the reaction on your own.

13. Now let's look at the description for this reaction by clicking on the "Description" button.

Glycoprotein VI (on our list) was identified as a collagen receptor from studies of patients with a GPVI deficiency. GPVI-deficient platelets lack collagen-induced

aggregation and the ability to form thrombi (blood clots) on a collagen surface under flow conditions. GPVI complexes with the Fc epsilon R1 receptor gamma chain, with a possible stoichiometry of two GPVI molecules and one FcεRI gamma-chain dimer (Jung & Moroi 2008). GPVI binding to FcR gamma is necessary for high affinity GPVI binding to collagen.

Note: Publications are provided.



14. Go back to the analysis results.

This gives you a flavor of the level of detail you can get from a Reactome pathway analysis. Obviously many hours maybe needed to investigate this more, but let's back up and look at the big picture again.

Review the other significant hits on the "Analysis" pane and "Hierarchy" panes. What biological processes are related to this gene list? Lots of pathways related to hemostasis, platelets and immune system processes.

