

How to use functional annotations at the i5k Workspace@NAL

Monica Poelchau 12/13/2022

Agenda

- Background
 - What is functional annotation?
 - What is the AgBase functional annotation pipeline?
 - Why do we use it?
- How to find and use the functional annotations at the i5k Workspace
 - 1. I want to know what functional annotations my protein.
 - 2. I want to find my protein in the functional annotation files.
 - 3. I want to get all the protein accession numbers for a GO category.
 - 4. I want to find all the Schistocerca americana proteins annotated to the ubiquitination pathway.



How do we move from sequence to biology?

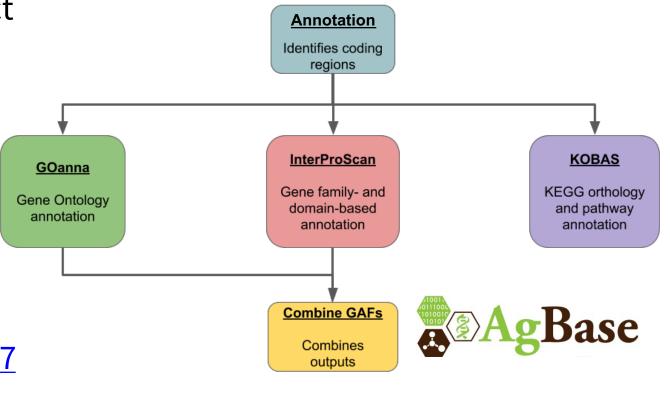
 ARS-University of Arizona joint project to develop common workflows and practices for functionally annotating invertebrate genomes.

 Credits: Fiona McCarthy, Surya Saha, Amanda Cooksey, Anna Childers

 Workflows for Rapid Functional Annotation of Diverse Arthropod Genomes. Saha et al., Insects 2021, 12(8), 748;

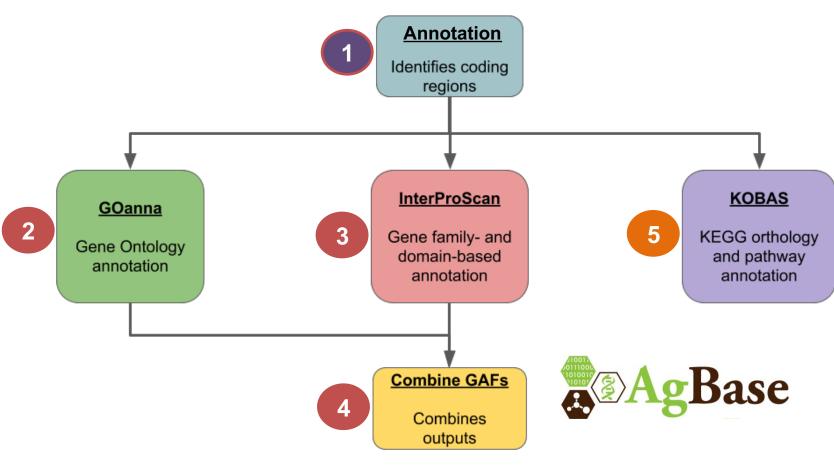
https://doi.org/10.3390/insects120807

<u>48</u>





Functional annotation tools



- 1. Identify proteins
- Transfer function based upon sequence homology
- 3. Assign function based upon functional motifs/domains
- 4. Combine GO, QC, formatting for use
- 5. Pathway information

Slide credit: Surya Saha



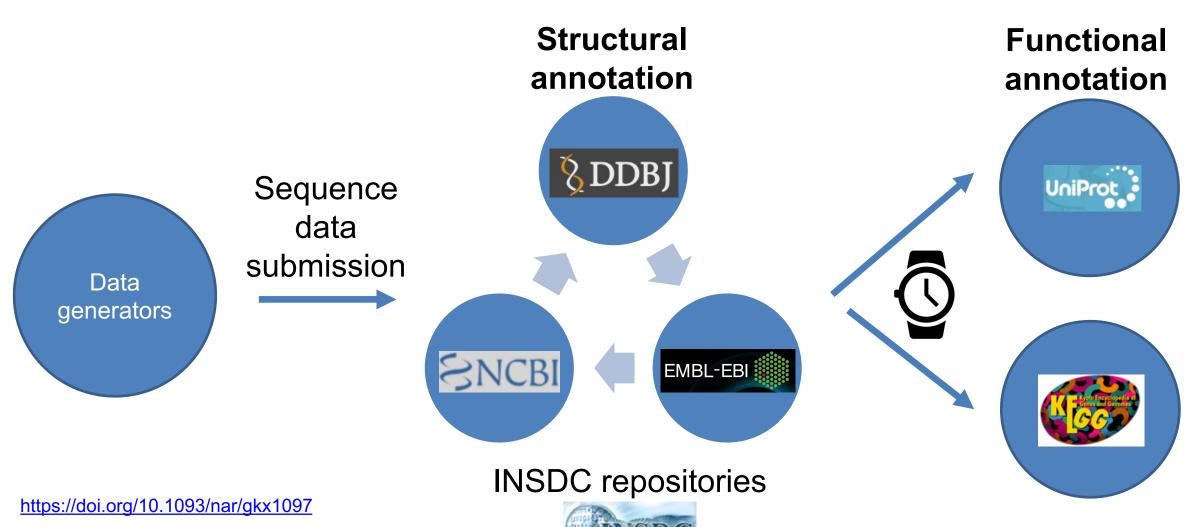
So What Does this Process Get Us?

- Support for comparative genomics
 - Motif/domain information for comparative & evolutionary studies -> Evolution of gene families
- Support for functional genomics
 - GO information for GO enrichment
 - Pathway information for pathways enrichment
- Targets for genome annotation

Slide credit: Surya Saha

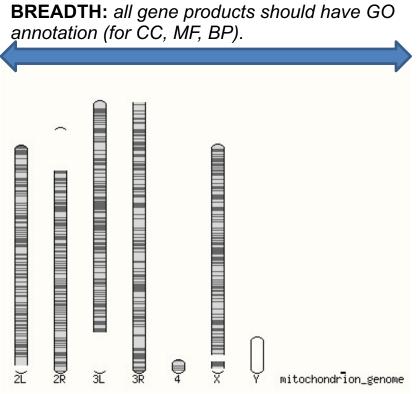


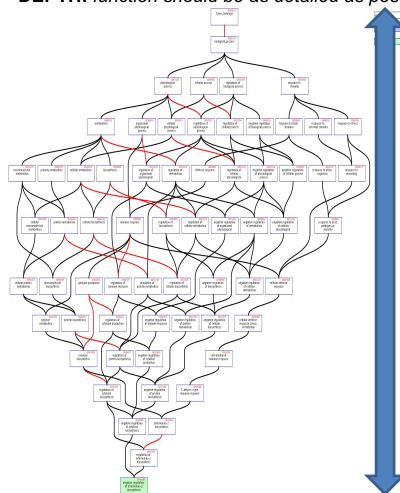
Typical flow of sequence data



How do we Measure GO Quality?

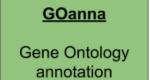
DEPTH: function should be as detailed as possible.





EVIDENCE: Published experiments provide direct evidence of function in that species.

Code	Code definition	Evidence code rank
IDA	Inferred from Direct Assay	5
IGI	Inferred from Genetic Interaction	5
IMP	Inferred from Mutant Phenotype	5
IPI	Inferred from Physical Interaction	5
IC	Inferred by Curator	4
TAS	Traceable Author Statement	4
IEP	Inferred from Expression Pattern	3
RCA	Inferred from Reviewed Computational Analysis	3
IGC	Inferred from Genomic Context	3
ISS	Inferred from Sequence or Structural Similarity	2
IEA	Inferred from Electronic Annotation	2
NAS	Non-traceable Author Statement	2
NR	Not Recorded	1
ND	No Biological data available	0



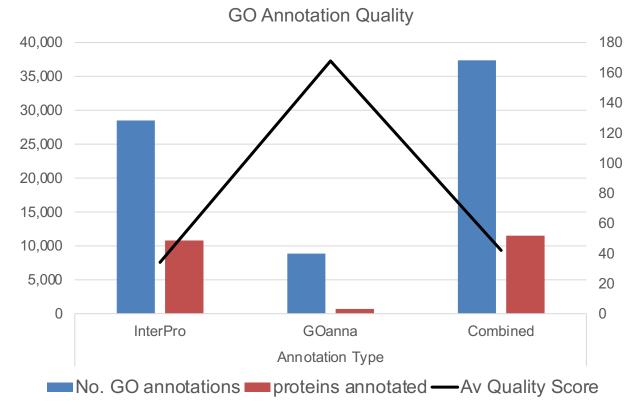
rank

Slide credit: Surya Saha Buza et al 2008. Gene Ontology annotation quality analysis in model eukaryotes. Nucleic acids research, 36(2), e12-e12.



Adding Details to InterProScan GO: GOanna

Interpro & GOanna are **complementary** approaches.
InterProScan provides "breadth" (some GO annotation for most proteins)
GOanna provides "depth" (more detailed GO terms)



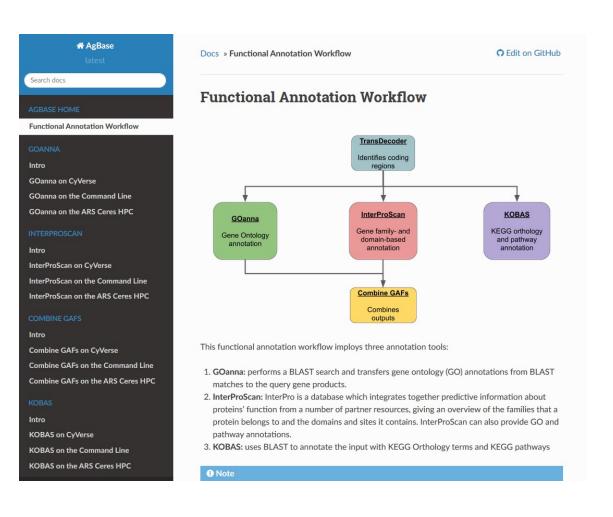
CO Quality Analysis	Annotation Type		
GO Quality Analysis	InterPro	GOanna	Combined
No. GO annotations	28,494	8,866	37,360
proteins annotated	10,810	691	11,500
Av Quality Score	34.064	167.751	42.085



Slide credit: Surya Saha



Accessing functional annotation tools





agbase-docs.readthedocs.io



de.cyverse.org



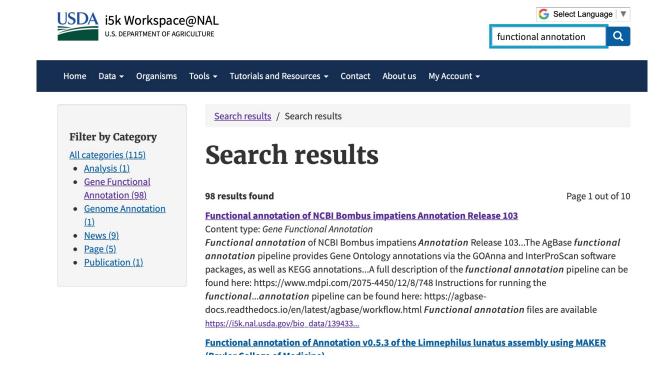
hub.docker.com/u/agbase

How to find and use the functional annotations at the i5k Workspace



15k Workspace functional annotations

- Functional annotations are available for 98 datasets;
- All new i5k Workspace organisms and assemblies will be functionally annotated.



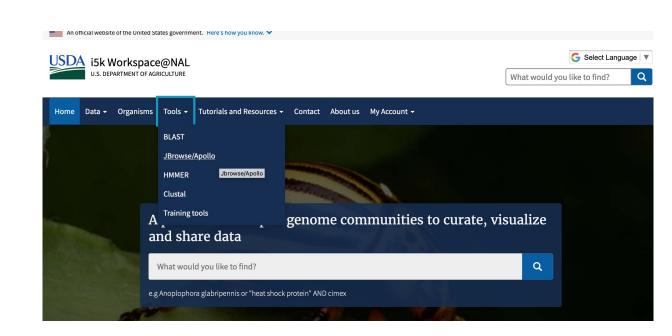


I5k Workspace functional annotation long-term storage and citation

- The functional annotation datasets are intended to be ephemeral, as the underlying GO databases update regularly. Therefore, we do not provide longterm storage of these datasets.
- If you plan on using a particular functional annotation dataset in a publication, we can archive this dataset for you at the Ag Data Commons.
- If you use the functional annotations in a publication, please cite the following:
 - The i5k Workspace@NAL—enabling genomic data access, visualization and curation of arthropod genomes. Poelchau et al., Nucleic Acids Research, Volume 43, Issue D1, 28 January 2015, Pages D714–D719, https://doi.org/10.1093/nar/gku983
 - Workflows for Rapid Functional Annotation of Diverse Arthropod Genomes. Saha et al.,
 Insects 2021, 12(8), 748; https://doi.org/10.3390/insects12080748

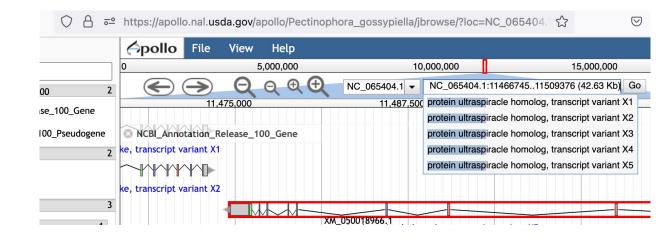
1. I want to know what functional annotations my protein has

- Example protein: ultraspiracle in the pink bollworm, *Pectinophora gossypiella*
- Go to https://i5k.nal.usda.gov/ ->
 Tools -> Jbrowse/Apollo -> Find a
 genome browser: Available
 genome browsers
- Find organism (*Pectinophora gossypiella*) in list

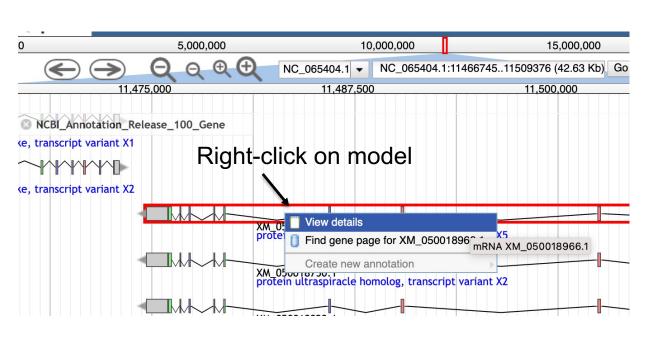


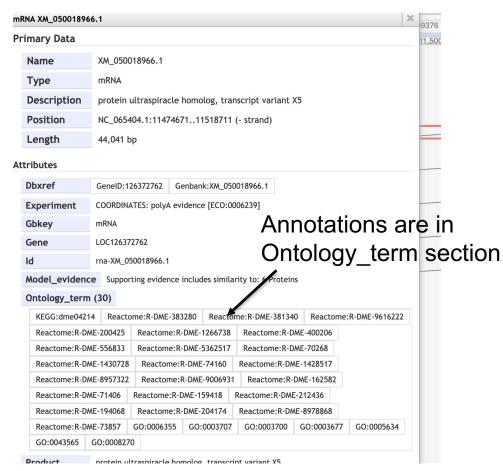
1. I want to know what functional annotations my protein has

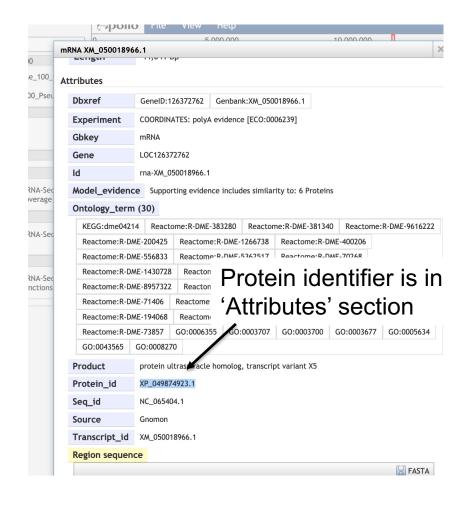
- search for 'ultraspiracle' in the Jbrowse search bar (tip – if you can't find it, add 'protein' before the name)
- Protein or mRNA accession numbers are also typically searchable

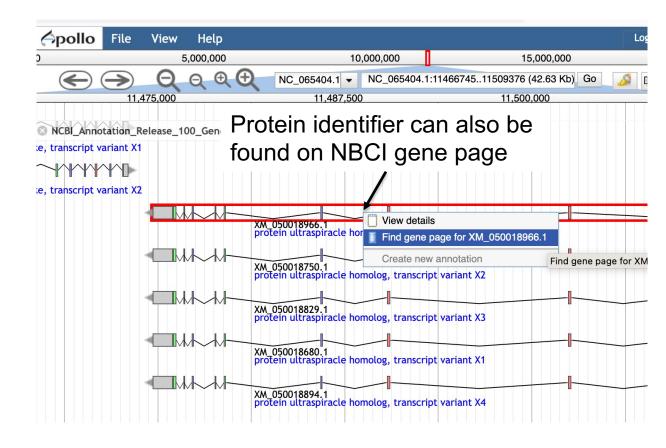


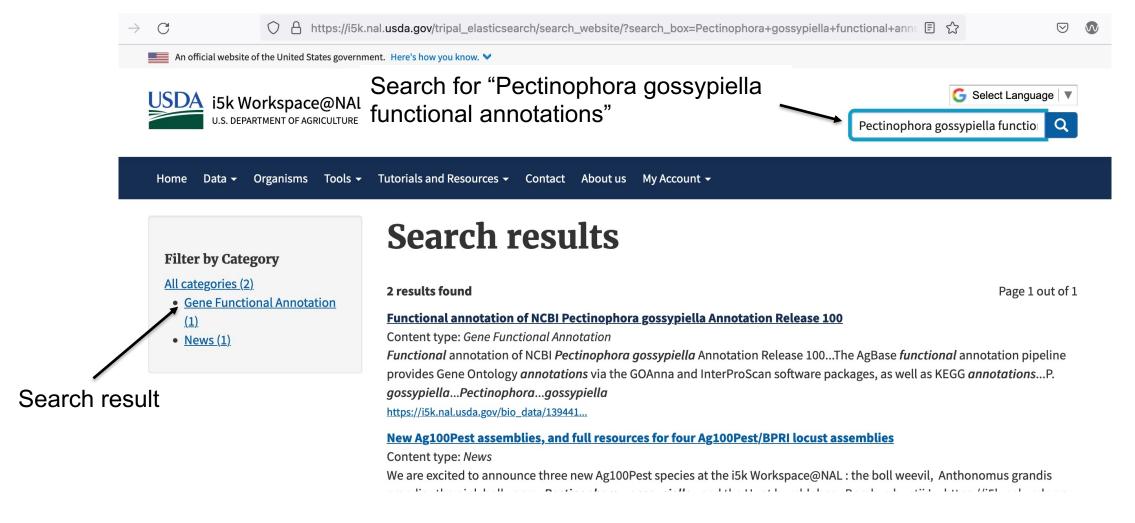
1. I want to know what functional annotations my protein has

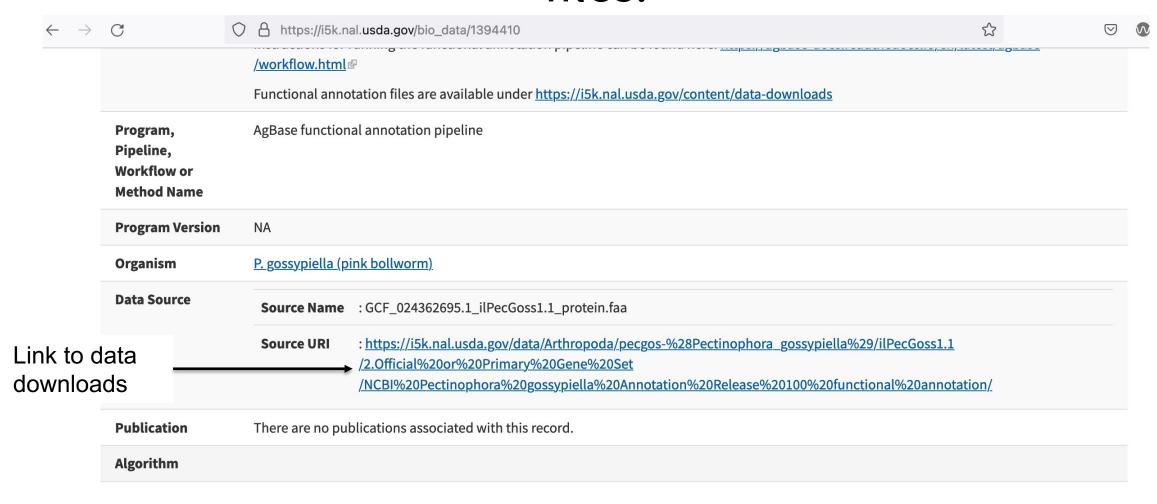










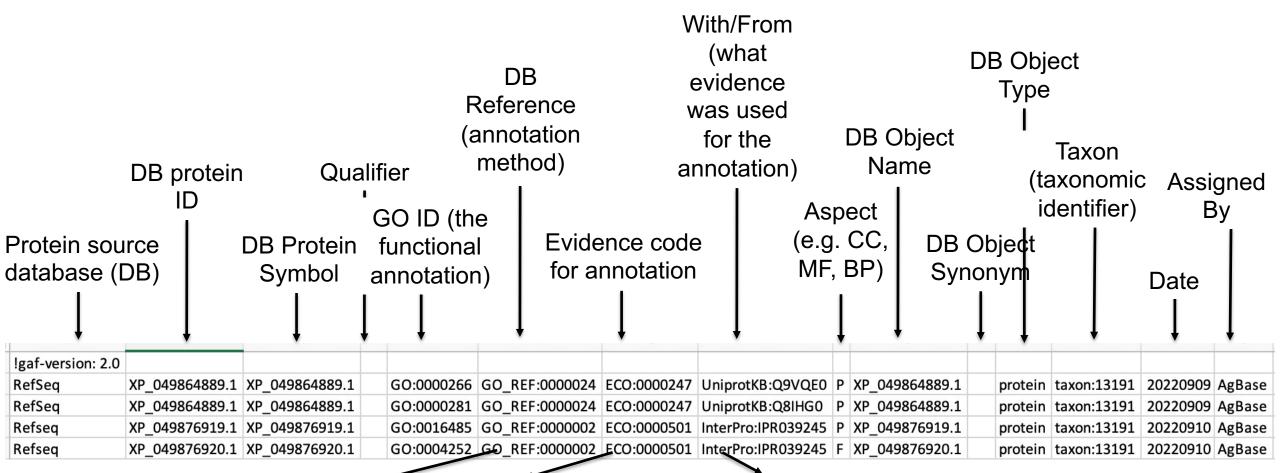


- We'll download the 'complete GAF' file, and search for protein accession XP_049874923.1
- GAF file format specification: <u>http://geneontology.org/docs/go-annotation-file-gaf-format-2.2/</u>

Index of /data/Arthropoda/pecgos-(Pectinophora_gossy Primary Gene Set/NCBI Pectinophora gossypiella Ann annotation/



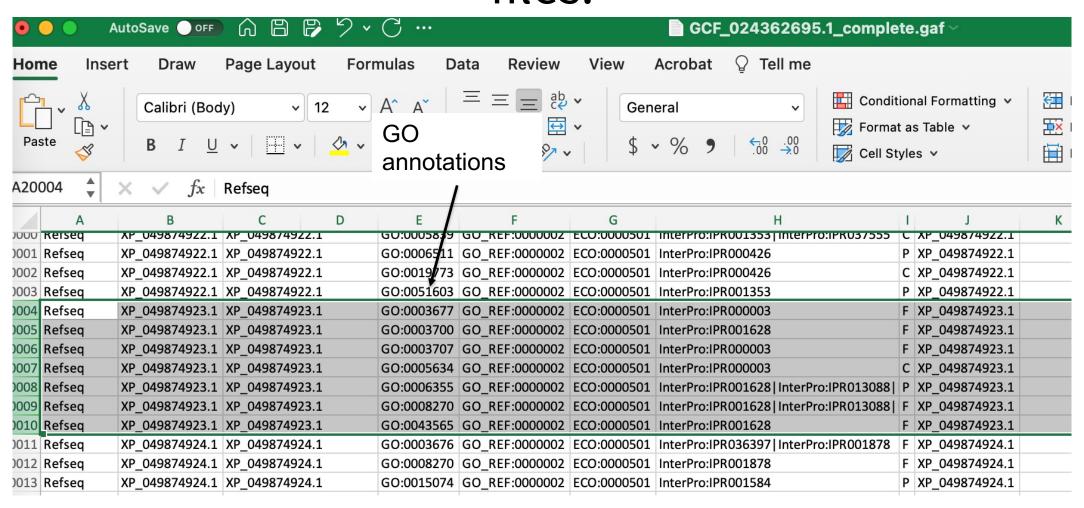
The GAF format.



GO_REF:0000024 – GOAnna GO_REF:0000002 - InterProScan

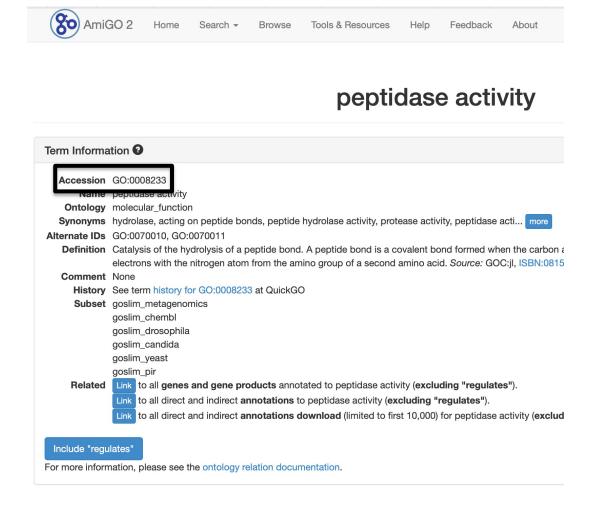
ECO:0000247 - GOAnna ECO:0000501 - InterProScan

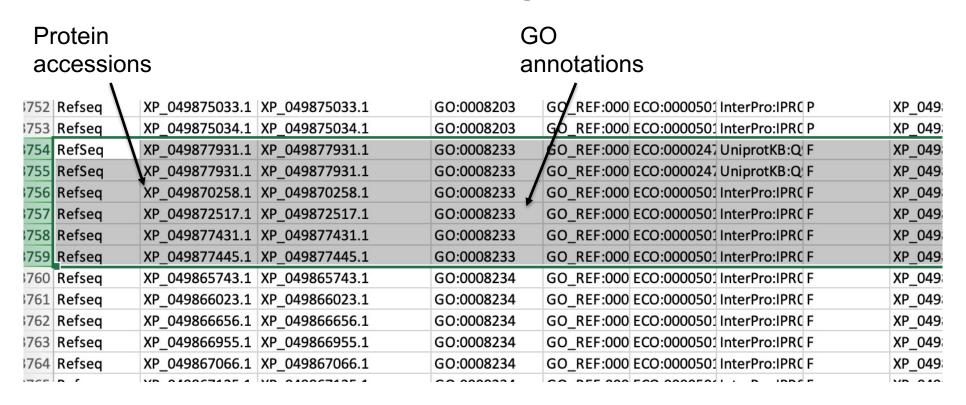
GOAnna: accession $\overline{\#}$ of the exp. characterized sequences(s) that match the query InterProScan: individual seqs, seq objects, methods, keyword mapping files, etc. that underlie the annotation.





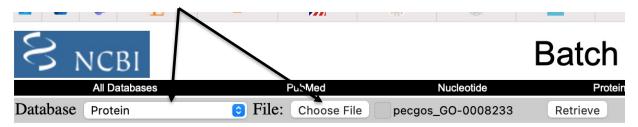
- The Amigo site can be used to search for relevant GO categories (http://amigo.geneontology.org/)
- Here, I searched for "peptidase activity" (GO:0008233)





Copy all protein accessions to a text file

Select protein database and upload file with protein accessions

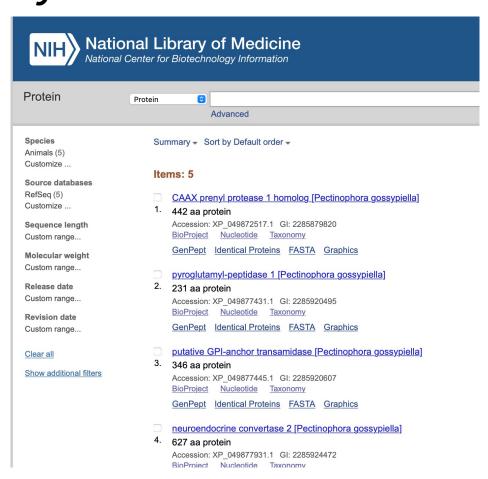


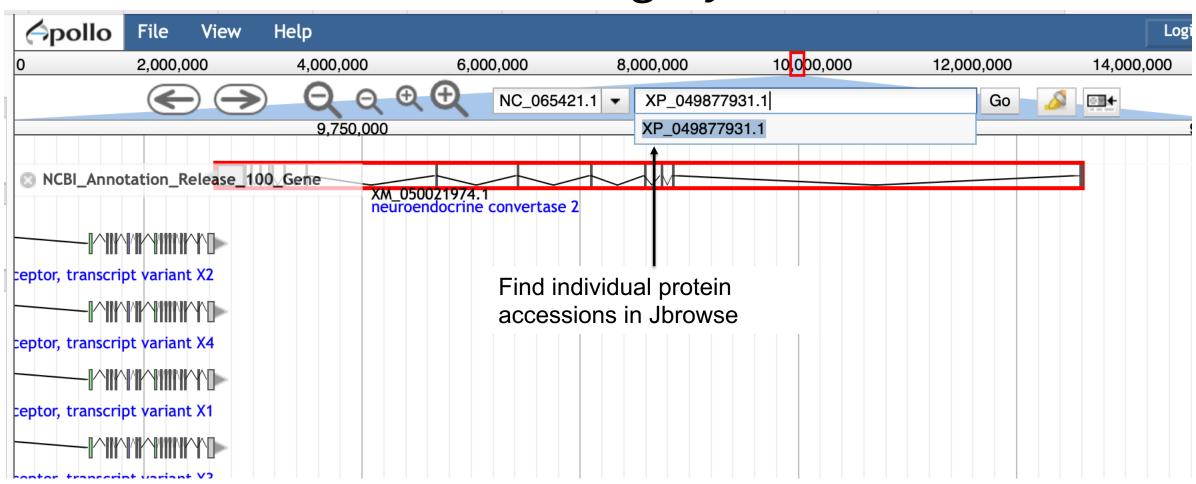
Batch Entrez

Given a file of Entrez accession numbers or other identifiers, Batch Entrez downloads the corresponding records.

Instructions

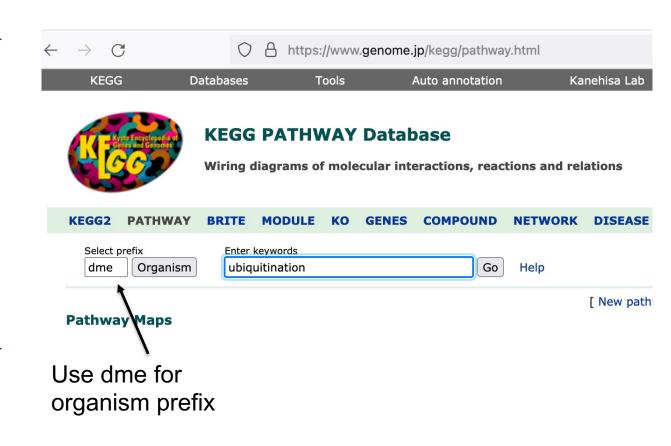
https://www.ncbi.nlm.nih.gov/sites/batchentrez

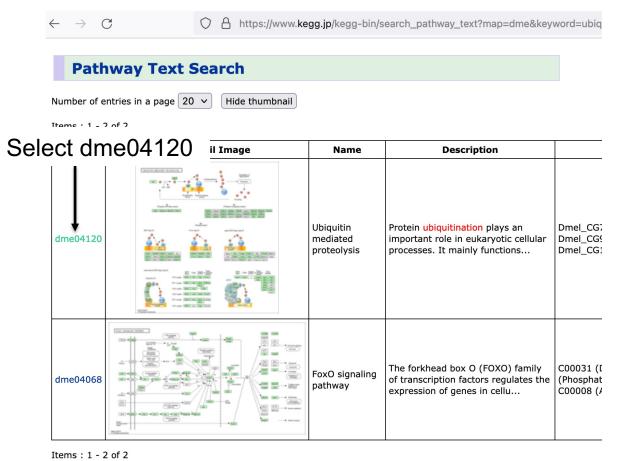




- Start with KEGG2: <u>https://www.genome.jp/kegg/pathway.html</u>
- Search for ubiquitination
- Choose 'dme' for prefix –
 Drosophila melanogaster
- Comparison paper:

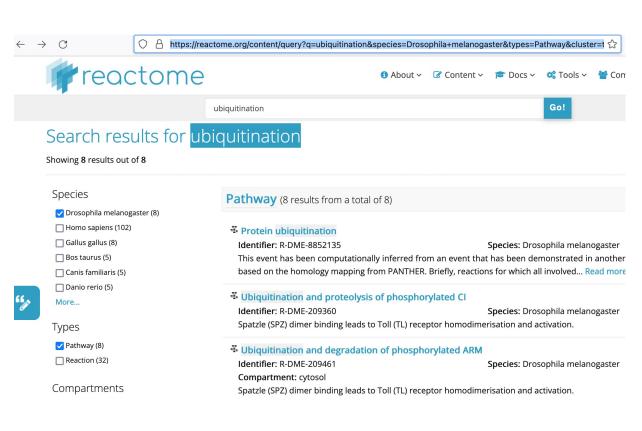
 https://www.biorxiv.org/content/1
 0.1101/2021.10.11.464014v1.abstra
 ct

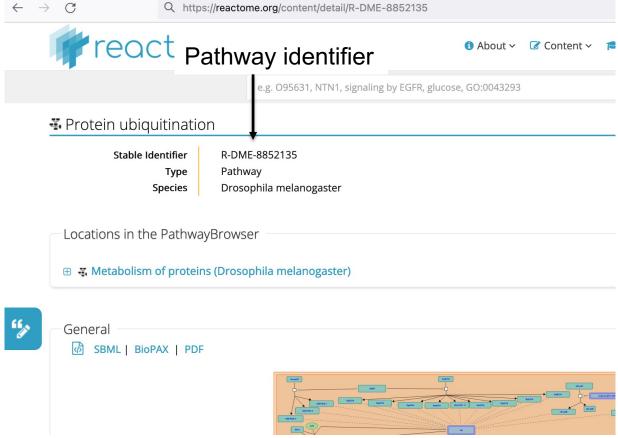




PATHWAY: dme04120 Entry dme04120 Pathway Ubiquitin mediated proteolysis - Drosophila melanogaster (fruit Name fly) Description Protein ubiquitination plays an important role in eukaryotic cellular processes. It mainly functions as a signal for 26S proteasome dependent protein degradation. The addition of ubiquitin to proteins being degraded is performed by a reaction cascade consisting of three enzymes, named E1 (ubiquitin activating enzyme), E2 (ubiquitin conjugating enzyme), and E3 (ubiquitin ligase). Each E3 has specificity to its substrate, or proteins to be targeted by ubiquitination. Many E3s are discovered in eukaryotes and they are classified into four types: HECT type, U-box type, single RING-finger type, and multi-subunit RING-finger type. Multi-subunit RING-finger E3s are exemplified by cullin-Rbx E3s and APC/C. They consist of a RING-finger-containing subunit (RBX1 or RBX2) that functions to bind E2s, a scaffold-like cullin molecule, adaptor proteins, and a target recognizing subunit that binds substrates. Class Genetic Information Processing; Folding, sorting and degradation BRITE hierarchy Pathway map dme04120 Ubiquitin mediated proteolysis

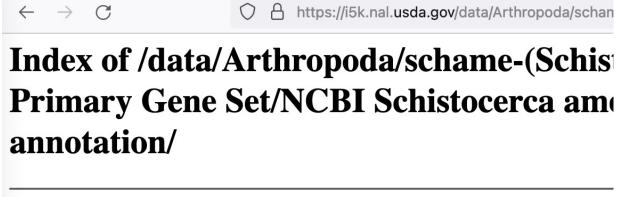
https://www.kegg.jp/entry/dme04120





- KEGG annotations (e.g. Drosophila melanogaster annotations computed by KEGG) are primarily based on sequence similarity to ortholog groups
- *D. melanogaster* Reactome annotations are based primarily on sequence similarity to human proteins
- FlyBase has additional pathway annotations.
- Delineations of the pathways themselves, and what proteins are assigned to a pathway, differ between all three resources.





```
### GOanna/

GOanna/

Interproscan/

KOBAS/

GCF_021461395.2_complete.gaf.tsv

README.txt

README.txt

README.txt

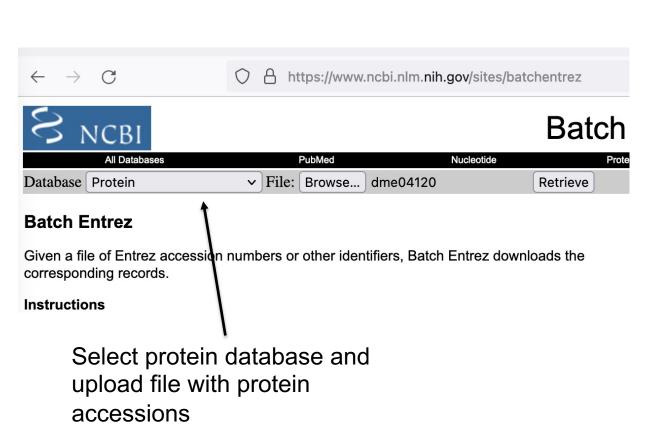
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23-Sep-2022 23:20
27-Apr-2022 16:12
27-Apr-2022 16:12
```

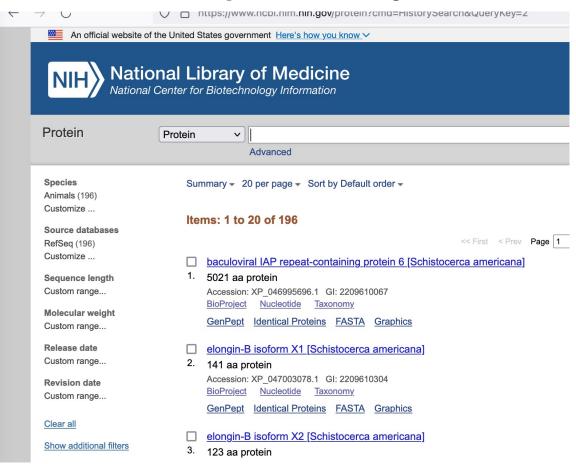


Select file organized by pathway

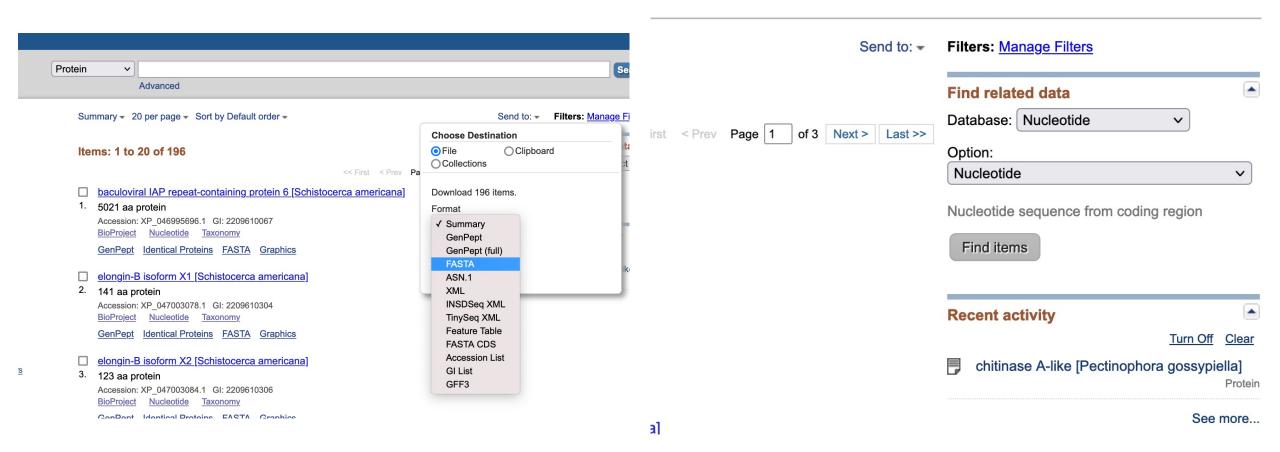
```
Q~ dme04120
0.1, XP_046991197.1, XP_046999343.1, XP_046981228.1, XP_046984995.1, XP_0469
EGG: dme04120
P 046993085.1,XP 046981059.1,XP 046994848.1,XP 046999012.1,XP 047000354
8.1,XP 047003904.1,XP 046990850.1,XP 046995563.1,XP 046999469.1,XP 0469
005094.1,XP 047000014.1,XP 046996878.1,XP 046979735.1,XP 046994184.1,XP
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P_046992967.1,XP_047001201.1,XP_046981442.1,XP_047000355.1,XP_046999825
7.1,XP 046982722.1,XP 046980112.1,XP 046999468.1,XP 046998185.1,XP 0469
981105.1,XP 046996813.1,XP 046991011.1,XP 046996696.1,XP 047003446.1
eactome: R-DME-209228
                        XP 046985860.1,XP 046997807.1,XP 046997808.1,XP
EGG: dme00280
```

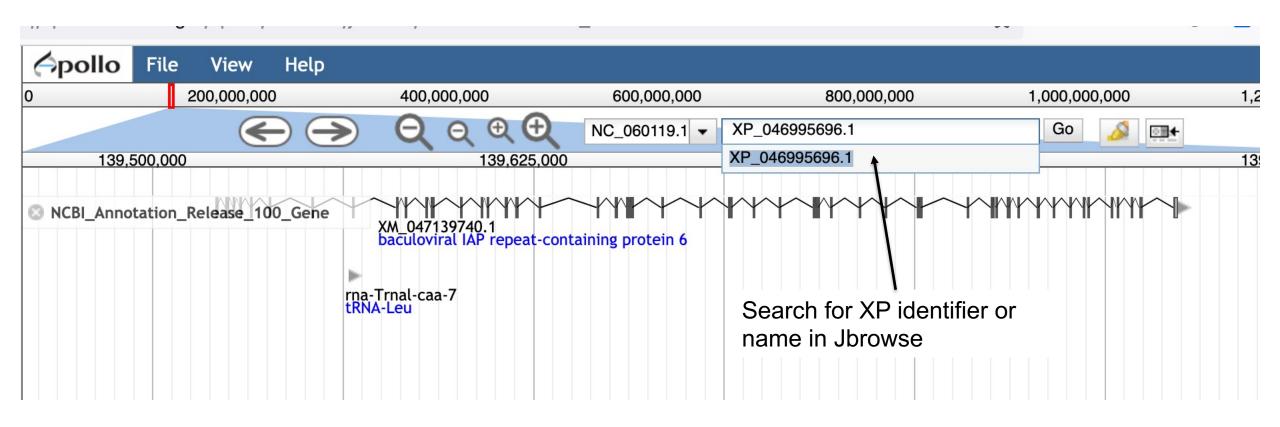
```
Q~ R-DME-8852135
35.1
Reactome: R-DME-2980766
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Reactome: R-DME-69109
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57.1,XP_046999463.1,XP_047001215.1,XP_046991794.1,XP_046979774
                         XP 046988142.1.XP 046990669.1.XP 046
Reactome: R-DME-8939246
Reactome: R-DME-9607240
KP_046993897.1, XP_046992770.1, XP_047004650.1, XP_046997292.1, XF
```













Questions?

How are you planning to use the functional annotations?

Acknowledgements



Anna Childers



Fiona M McCarthy





Amanda Cooksey



Surya Saha

- The i5k Workspace@NAL team
- All of our data contributors!

