USDA National Agricultural Library
U.S. DEPARTMENT OF AGRICULTURE

BPRI Annotation Training

3/30/2023

BPRI annotation jamboree goals and outcomes

- Goals:
  - Educational goal: everyone is educated on how to look at a genome with a question in mind, make a tree, generate an phylogenetic insight and perhaps design some primers, RNAi or crispr reagent
  - Integration goal: integrate the genomes in a phylogenomic manner with all other BPRI research.
- Outcomes:
  - We hope that each group will have a list of 5-20 genes, for a total of > 100.
  - The aim will be a phylogenetic tree for each gene family, and a paragraph of text summarizing why you looked at those genes, what you expected based on other species, what was found.

Agenda

1. The basics: Apollo registration; Schistocerca datasets available; NCBI annotation pages
2. Using NCBI and i5k resources to find genes in your species in Apollo
    1. Example 1: Orco
    2. Example 2: ebony
3. Naming
4. Annotation outcomes
5. Q&A

Other Apollo training resources

- Our previous Apollo tutorials go in-depth on how to use various Apollo functions.
- Using the Apollo2 manual annotation tool:
  - Slides: https://i5k.nal.usda.gov/sites/default/files/presentations/apollo_training_november_2021.pdf
  - Recording: https://www.zoomgov.com/rec/share/8ZxP3FhFE9ahBpVTUFDP8HwKNUS1prZlhAflINmrQaZMKeVoyo4F97RtmwJYdwQK.936mXuEkaOcjfCDW
    Passcode: hm3tu1A@
- In-depth annotation techniques:
  - Slides: https://i5k.nal.usda.gov/sites/default/files/presentations/Apollo_webinar_9-20-2022.pdf
  - Recording: https://www.zoomgov.com/rec/share/HckZOnI1po3QZL1Xh6OZCukps6IzKPPRBHfnTCM-UbefiwSi6x4eLcvIlqY1OPw.WfMjRxdDGA2qhd9v
    Passcode: 1%yC!#w#

The Basics

- Ask new annotators to register here: https://i5k.nal.usda.gov/web-apollo-registration
  - Approval process: ideally, send me a pre-approved list of emails
- Datasets
  - Schistocerca americana example: https://i5k.nal.usda.gov/bio_data/1394317
  - RNA-Seq alignments in Jbrowse: https://apollo.nal.usda.gov/apollo/4615055/jbrowse/index.html
- The RefSeq gene predictions
  - Schistocerca nitens AR example: https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Schistocerca_nitens/100/

Finding your gene in Apollo - Orco

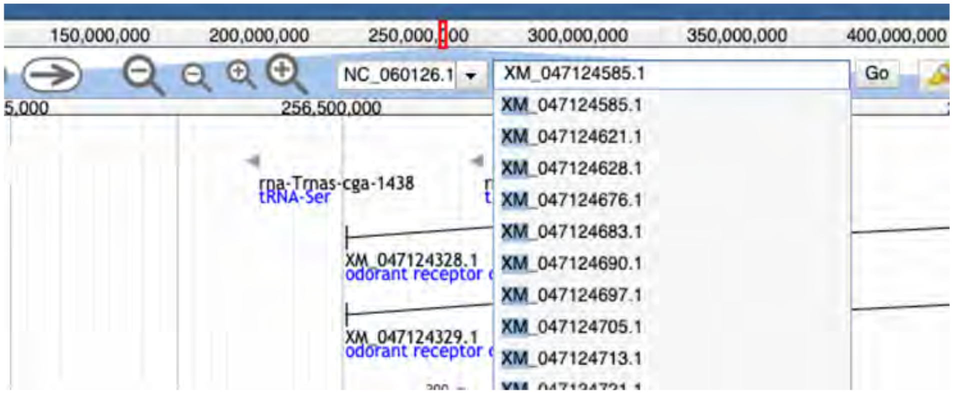
- Using NCBI's gene name search (I don't recommend it)
  - Can try using the advanced search in both protein and gene databases
  - I have contacted NCBI about some inconsistencies in their search results
  - That said, nomenclature inconsistencies between different sources (RefSeq vs. self-submitted) still make a comprehensive name-based search impractical

Finding your gene in Apollo - Orco

- BLAST-base sequence retrieval
  - D. melanogaster record in RefSeq: NP_524235.2
  - NCBI BLAST: https://blast.ncbi.nlm.nih.gov/Blast.cgi
    - Gives you a more comprehensive search, allows you to find proteins beyond the RefSeq predictions
  - I5k BLAST: https://i5k.nal.usda.gov/webapp/blast
    - Allows you to limit your BLAST search only to the models in Apollo, find the identifiers you need

Finding your gene in Apollo - Orco

- To find your gene in Apollo, you need the name and/or the NCBI accession number
  - – starts with XM - nucleotide - or XP – protein
- Search for either of these in the search box



NC_060126.1 XM_047124585.1 Go

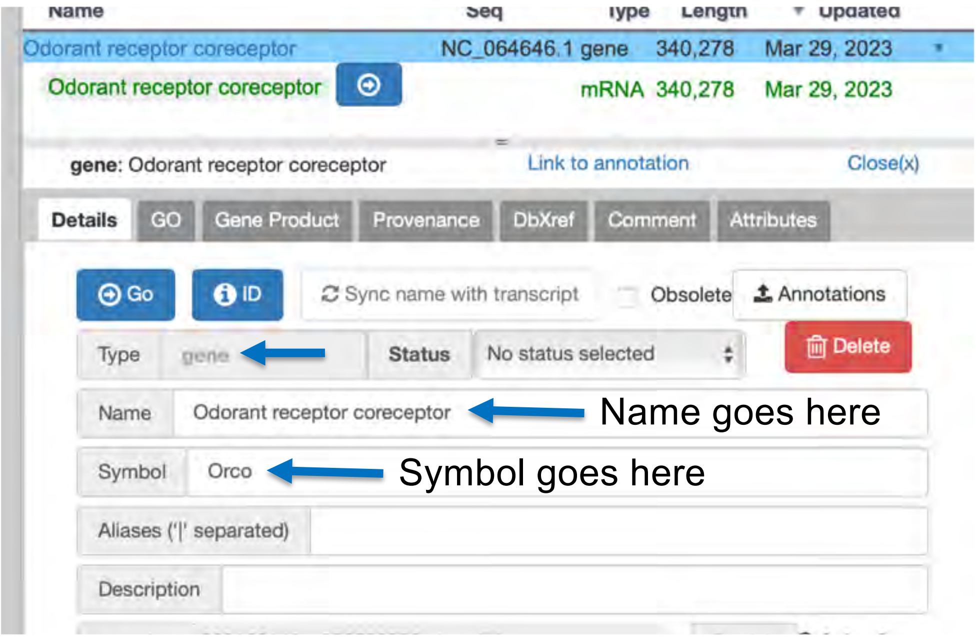
Finding your gene in Apollo - ebony

- No search results for ‘Schistocerca americana’ and ‘ebony’ in NCBI
- BLAST approach with Fly ebony protein sequence (NP_524431.2)
- Best S. americana hit: XP_046995316.1/mycosubtilin synthase subunit C isoform X2 [Schistocerca americana]
- Search for XP_046995316.1 in Apollo

Naming

Names vs. symbols vs. accession numbers

- **Name**: Describes the function of a gene or protein, e.g. “odorant receptor coreceptor”.
- **Symbol**: A short form of the Name, e.g. ‘Orco’. Only applies to the gene (not protein).
- **Accession number**: The permanent, unique identifier for a feature, e.g. XM_050086732.1. These are provided and maintained by NCBI.



Name	Seq	Type	Length	Updated
Odorant receptor coreceptor	NC_064646.1	gene	340,278	Mar 29, 2023
Odorant receptor coreceptor		mRNA	340,278	Mar 29, 2023

gene: Odorant receptor coreceptor

Type: gene

Status: No status selected

Name: Odorant receptor coreceptor

Symbol: Orco

Aliases ('|' separated):

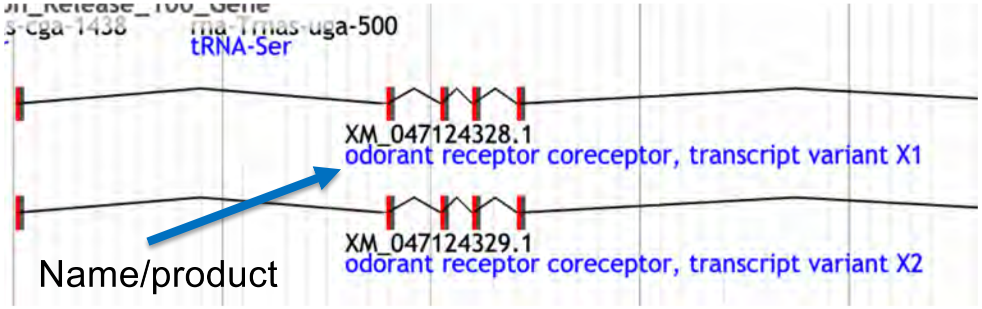
Description:

Name goes here

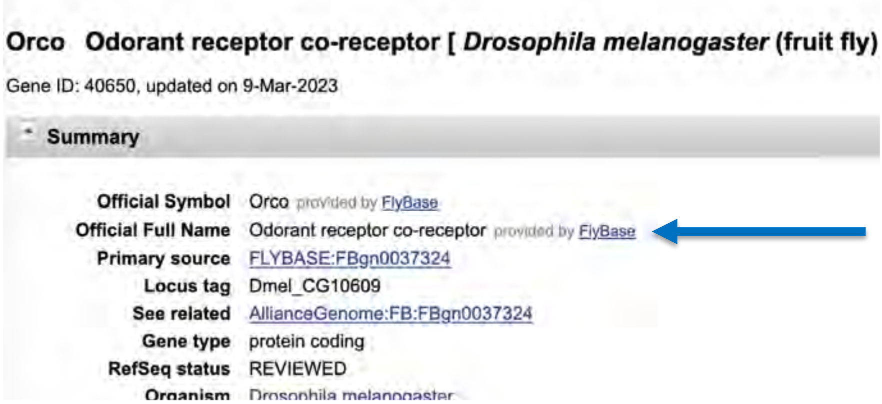
Symbol goes here

Names vs. symbols vs. accession numbers

- The protein name (aka product in NCBI terms) is in blue.
- The accession number is in black.
- Change the NCBI name in Apollo if it is incorrect.


n_Release_100_Gene
s-cga-1438
rna-Trnas-uga-500
tRNA-Ser
XM_047124328.1
odorant receptor coreceptor, transcript variant X1
XM_047124329.1
odorant receptor coreceptor, transcript variant X2

Name/product



Orco Odorant receptor co-receptor [*Drosophila melanogaster* (fruit fly)

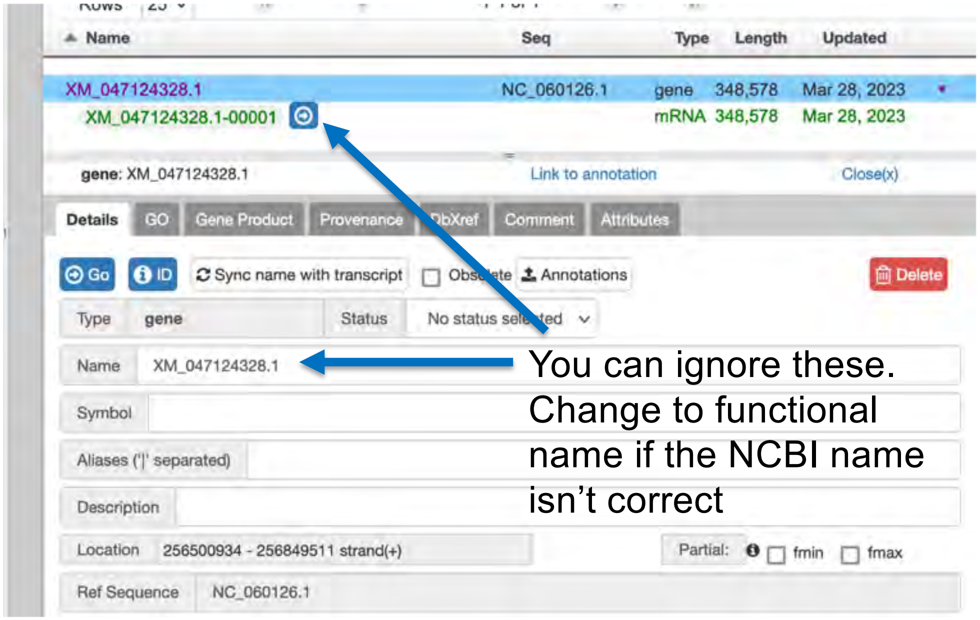
Gene ID: 40650, updated on 9-Mar-2023

Summary

Official Symbol	Orco provided by FlyBase
Official Full Name	Odorant receptor co-receptor provided by FlyBase
Primary source	FLYBASE:FBgn0037324
Locus tag	Dmel_CG10609
See related	AllianceGenome:FB:FBgn0037324
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Drosophila melanogaster

A word on Apollo default ‘names’

- Apollo adds placeholder names when you create a model
  - the NCBI accession for gene names
  - the NCBI accession plus a number for the mRNA.
- This is just Apollo’s default behavior – this is NOT the new name of the model.
- I will remove placeholder names before submitting to NCBI.
- Please change these if you need to make a change to the actual name (e.g. odorant receptor co-receptor)



Name	Seq	Type	Length	Updated
XM_047124328.1	NC_060126.1	gene	348,578	Mar 28, 2023
XM_047124328.1-00001		mRNA	348,578	Mar 28, 2023

gene: XM_047124328.1 Link to annotation Close(x)

Type: gene
Status: No status selected
Name: XM_047124328.1
Symbol:
Aliases ('|' separated):
Description:
Location: 256500934 - 256849511 strand(+)
Partial: □ fmin □ fmax
Ref Sequence: NC_060126.1

You can ignore these. Change to functional name if the NCBI name isn’t correct

I5k Workspace Guidelines - Names

Are you adopting a name from a homolog?

- You can re-use existing, established names (e.g. from *Drosophila melanogaster*)
- Don’t add a species prefix (although okay to use in your manuscript for clarity)
- If you want to imply uncertainty, you can append ‘-like’ to the name
- Good: “Ultraspiracle” 🙂
- Okay: “Ultraspiracle-like”
- Bad: “Clec-ultraspiracle” or “similar to ultraspiracle”🚫

https://i5k.nal.usda.gov/i5k-workspace-gene-and-protein-naming-guidelines

I5k Workspace Guidelines - Names

- Are you naming an isoform?
  - use the suffix “isoform A”, “isoform B”, etc.
- Are you naming a fragmented gene?
  - include a *comment* 'Part X of Y', where Y is the total number of fragments, and X is the ordinal number for that gene.
  - Don’t add ‘partial’ or ‘part of’ to the name.

https://i5k.nal.usda.gov/i5k-workspace-gene-and-protein-naming-guidelines

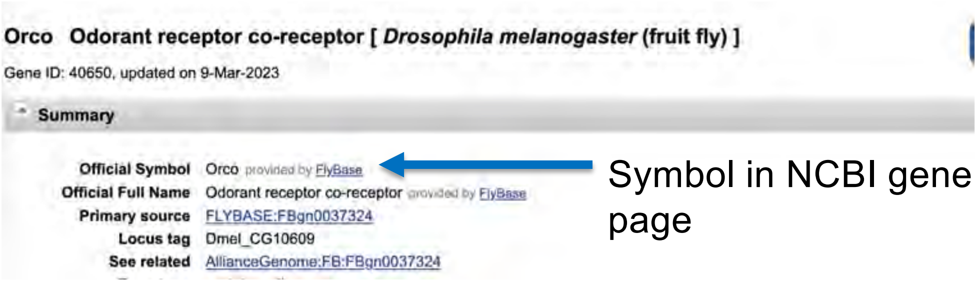
I5k Workspace Guidelines - Names

- Are you naming a ‘new’ gene?
  - Choose a name that could be propagated to all orthologous proteins; try not to make it species- or tissue-specific
    - **Good: “magnesium transporter”** 🙂
    - Bad: “diapause-associated protein” 🚫
- Are you naming a gene from a gene family?
  - Check if a naming system already exists: http://www.uniprot.org/docs/nomlist.txt
  - Use Arabic numbers to specify the different members encoded by a multigene family.

https://i5k.nal.usda.gov/i5k-workspace-gene-and-protein-naming-guidelines

Symbols

- Symbol:
  - a short form of the name, e.g. Orco
  - Is assigned to the gene (not protein)
  - We don't recommend coining new symbols – okay to adopt existing ones, though
- You can add a symbol at the gene level in Apollo if there is an existing one in an ortholog. This may help with searching in NCBI in the long run.



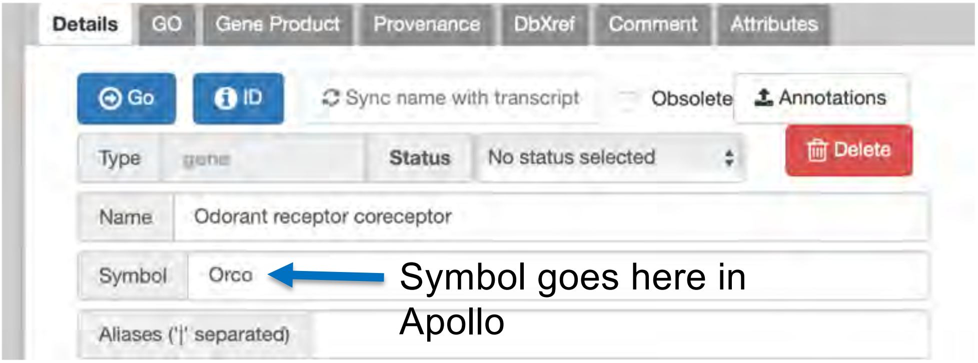
Orco Odorant receptor co-receptor [*Drosophila melanogaster* (fruit fly)]

Gene ID: 40650, updated on 9-Mar-2023

Summary

Official Symbol Orco provided by FlyBase
Official Full Name Odorant receptor co-receptor provided by FlyBase
Primary source FLYBASE:FBgn0037324
Locus tag Dmel_CG10609
See related AllianceGenome:FB:FBgn0037324

Symbol in NCBI gene page



Type gene
Status No status selected
Name Odorant receptor coreceptor
Symbol Orco
Aliases ('|' separated)

Symbol goes here in Apollo

I5k Workspace Guidelines - Symbols

- We do not recommend coining new symbols for newly named genes.
- However, if a name from an orthologous gene was adopted, you may use this gene's symbol, as well.
- Don't use species prefixes in Apollo (e.g. Clec-Pepck). Okay to use in publications to distinguish between species, though.
- Examples: Orco, Pepck, Ser12

https://i5k.nal.usda.gov/i5k-workspace-gene-and-protein-naming-guidelines

Annotation outcomes

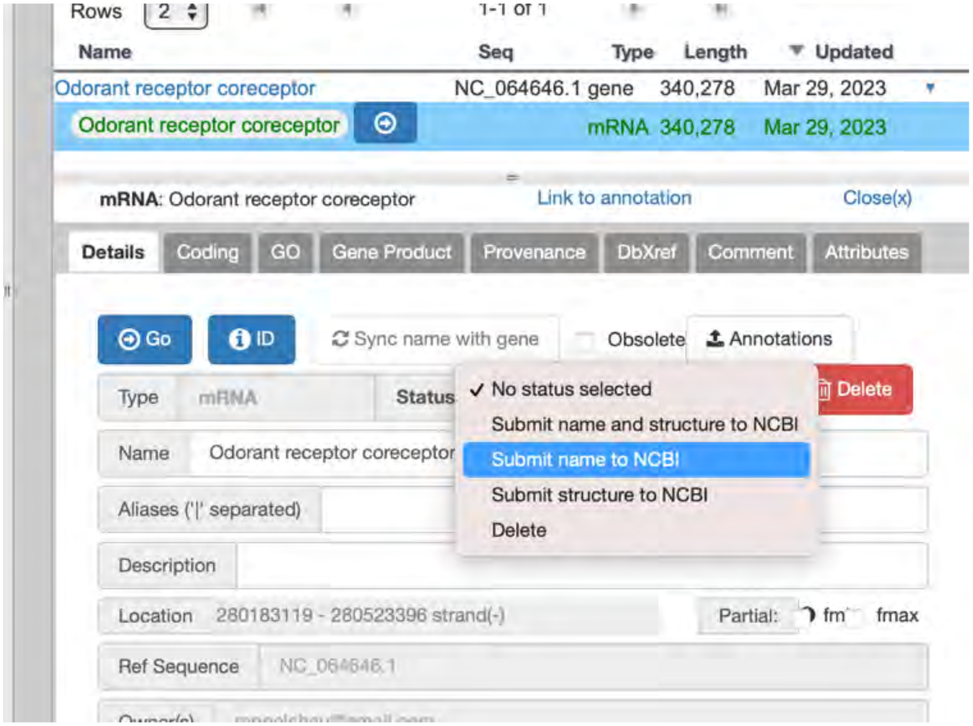
How to handle annotations with 1) different types of changes and 2) different publication outcomes

Annotation outcomes

- Will you refer to a changed gene model in a published paper, or should others use your changes?
- If yes, then the i5k Workspace should submit any changes to the gene model back to NCBI.
- If we don’t submit your changes to NCBI, they can be submitted to a generic repository such as Ag Data Commons or Dryad – but it’s much harder to re-use sequence data from these locations.

The ‘Status’ field

- We will use the ‘Status’ field in Apollo to indicate what should be submitted to NCBI.
  - Submit name and structure to NCBI
  - Submit name to NCBI
  - Submit structure to NCBI



Rows 2 1-1 of 1

Name	Seq	Type	Length	Updated
Odorant receptor coreceptor	NC_064646.1	gene	340,278	Mar 29, 2023
Odorant receptor coreceptor		mRNA	340,278	Mar 29, 2023

mRNA: Odorant receptor coreceptor

Type mRNA

Status

Name Odorant receptor coreceptor

Aliases ('|' separated)

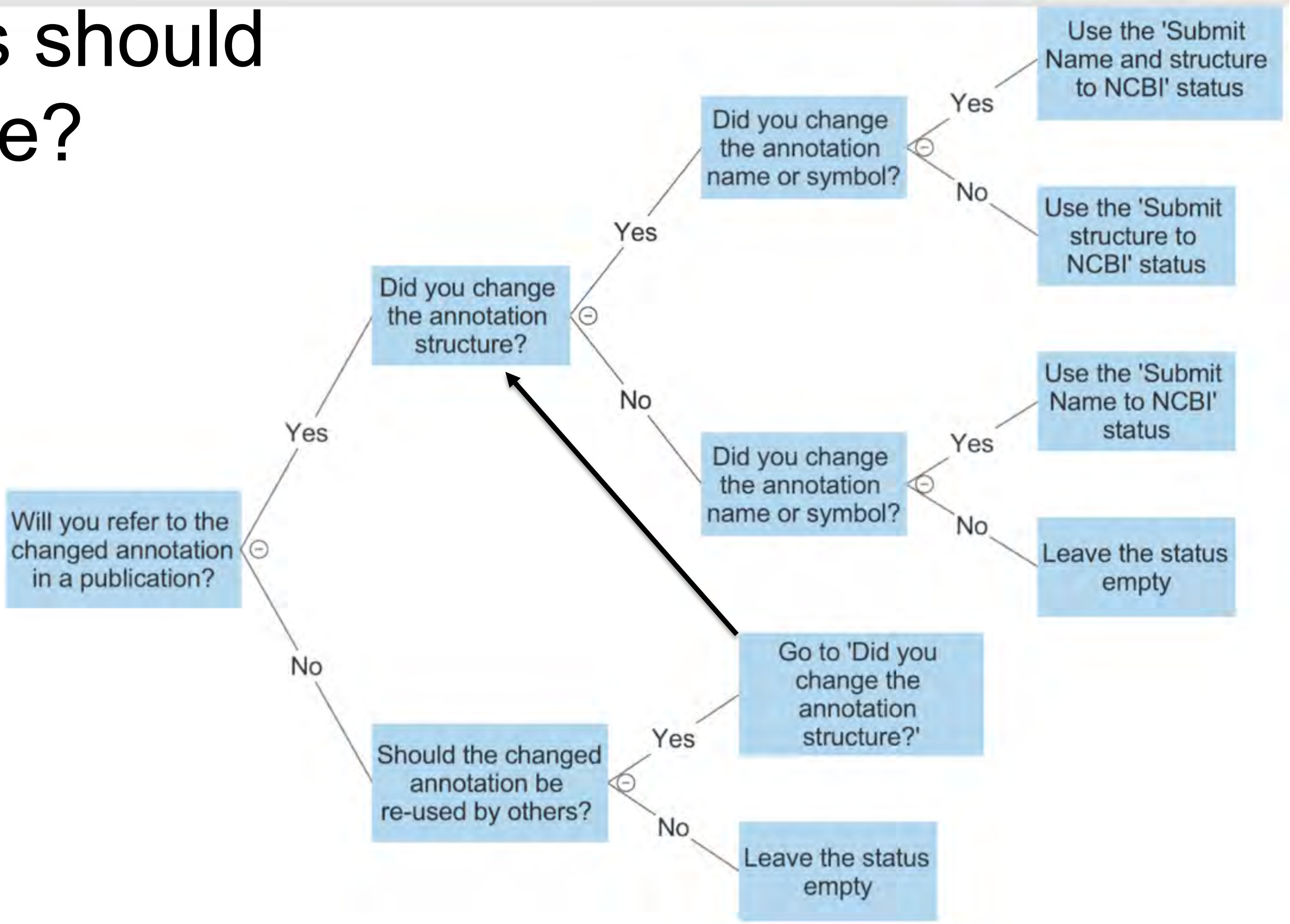
Description

Location 280183119 - 280523396 strand(-)

Partial: fmin fmax

Ref Sequence NC_064646.1

What Status should you use?



Will you refer to the changed annotation in a publication?

Yes

Did you change the annotation structure?

Yes

Did you change the annotation name or symbol?

Yes

Use the 'Submit Name and structure to NCBI' status

No

Use the 'Submit structure to NCBI' status

No

Did you change the annotation name or symbol?

Yes

Use the 'Submit Name to NCBI' status

No

Leave the status empty

No

Should the changed annotation be re-used by others?

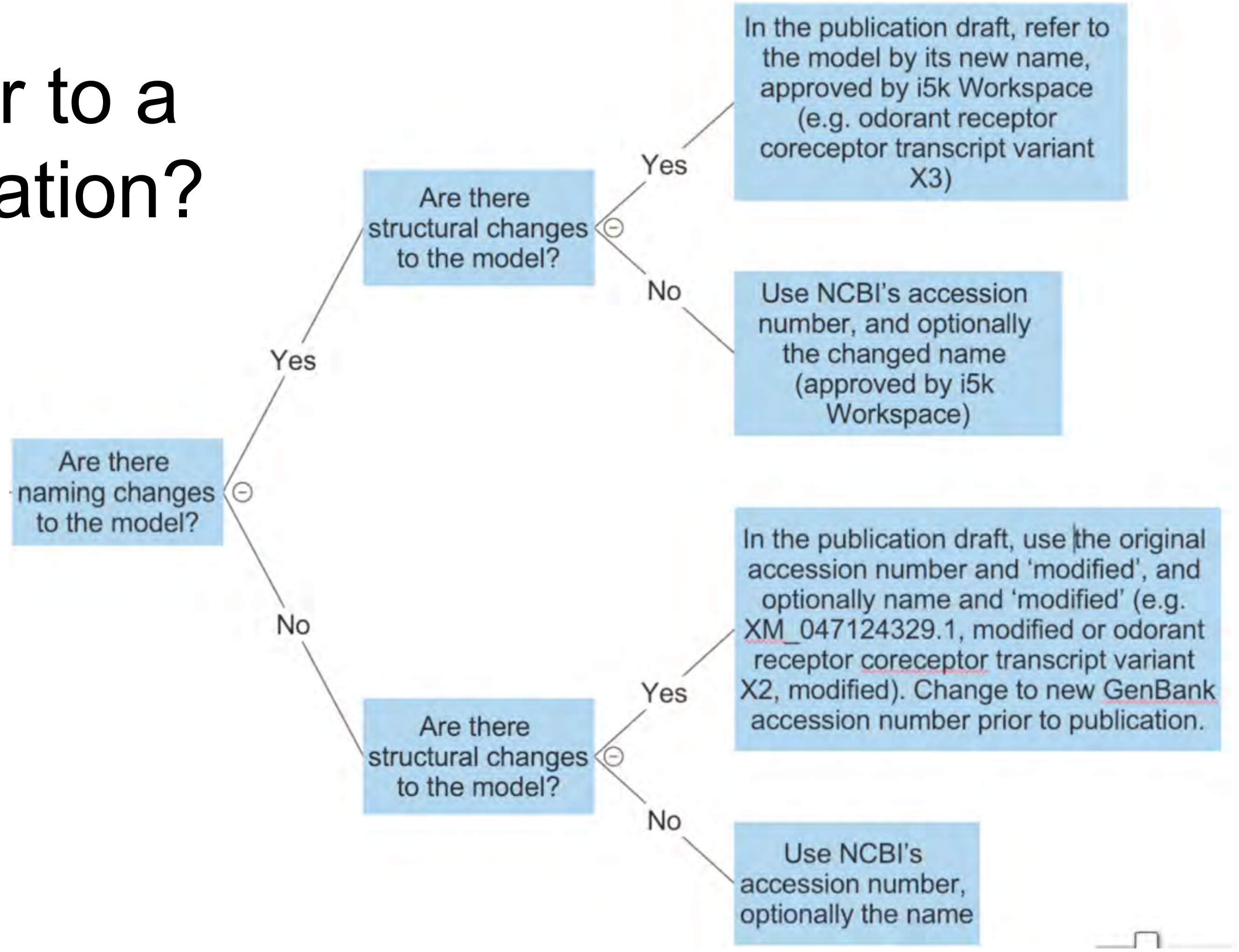
Yes

Go to 'Did you change the annotation structure?'

No

Leave the status empty

How do you refer to a model in a publication?



Are there naming changes to the model?

Are there structural changes to the model?

In the publication draft, refer to the model by its new name, approved by i5k Workspace (e.g. odorant receptor coreceptor transcript variant X3)

Use NCBI's accession number, and optionally the changed name (approved by i5k Workspace)

Are there structural changes to the model?

In the publication draft, use the original accession number and 'modified', and optionally name and 'modified' (e.g. XM_047124329.1, modified or odorant receptor coreceptor transcript variant X2, modified). Change to new GenBank accession number prior to publication.

Use NCBI's accession number, optionally the name

Other procedural notes

- It would help to send me a list of annotator emails
- We're missing S. gregaria RNA-Seq, and protein alignments
- Keep in touch with me if you have any questions
- Let me know when you'd like me to review your annotations that should be submitted to NCBI – this can take some time.
- Keep me up to date on your publication timelines, so I can communicate with NCBI

Thank you!

- Questions?