Apollo:
Collaborative Genome Annotation Editing

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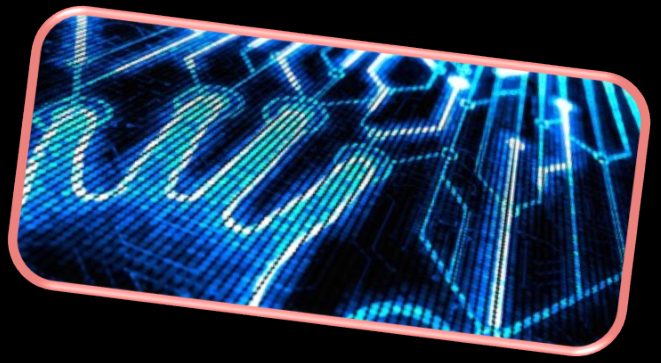
Berkeley Bioinformatics Open-Source Projects
Environmental Genomics and Systems Biology Division
Lawrence Berkeley National Laboratory

A workshop for AGS X. University of Notre Dame, South Bend, IN. 08 June, 2017

Today...

We will learn effective ways to extract valuable information about a genome through curation efforts.



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After this workshop, you will:

- Better understand curation in the context of genome annotation: **assembled genome → automated annotation → manual annotation**
- Become familiar with Apollo's environment and functionality.
- Learn to identify homologs of known genes of interest in your newly sequenced genome.
- Learn how to corroborate and modify automatically annotated gene models using all available evidence in Apollo.

Schedule

1. Genome Curation: 7 minutes
2. Predicting & annotating genes: 7 min.
3. Apollo – intro & examples: 20 min.
4. Hands-on practice 40 min.
5. Break 6 min.
6. Hands-on practice (ctd.) 40 min.


GENOME TRAIN


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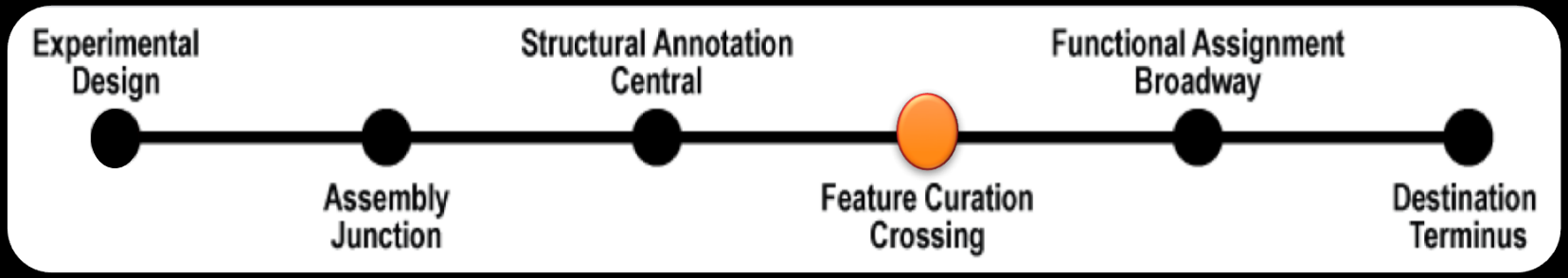
Experimental Design
Assembly Junction
Structural Annotation Central
Feature Curation Crossing
Functional Assignment Broadway
Destination Terminus


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Knowledge

Extracting knowledge from data

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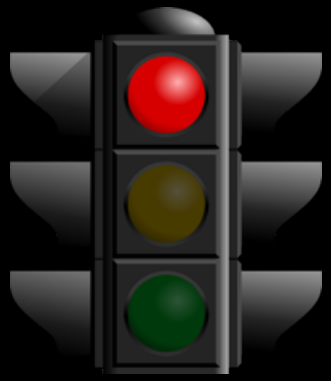
Extracting knowledge from data



South-facing traffic light at *St Mary's Rd*. has turned red

Information

Data

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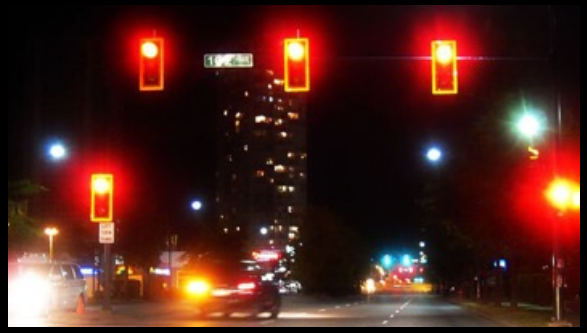
Extracting knowledge from data



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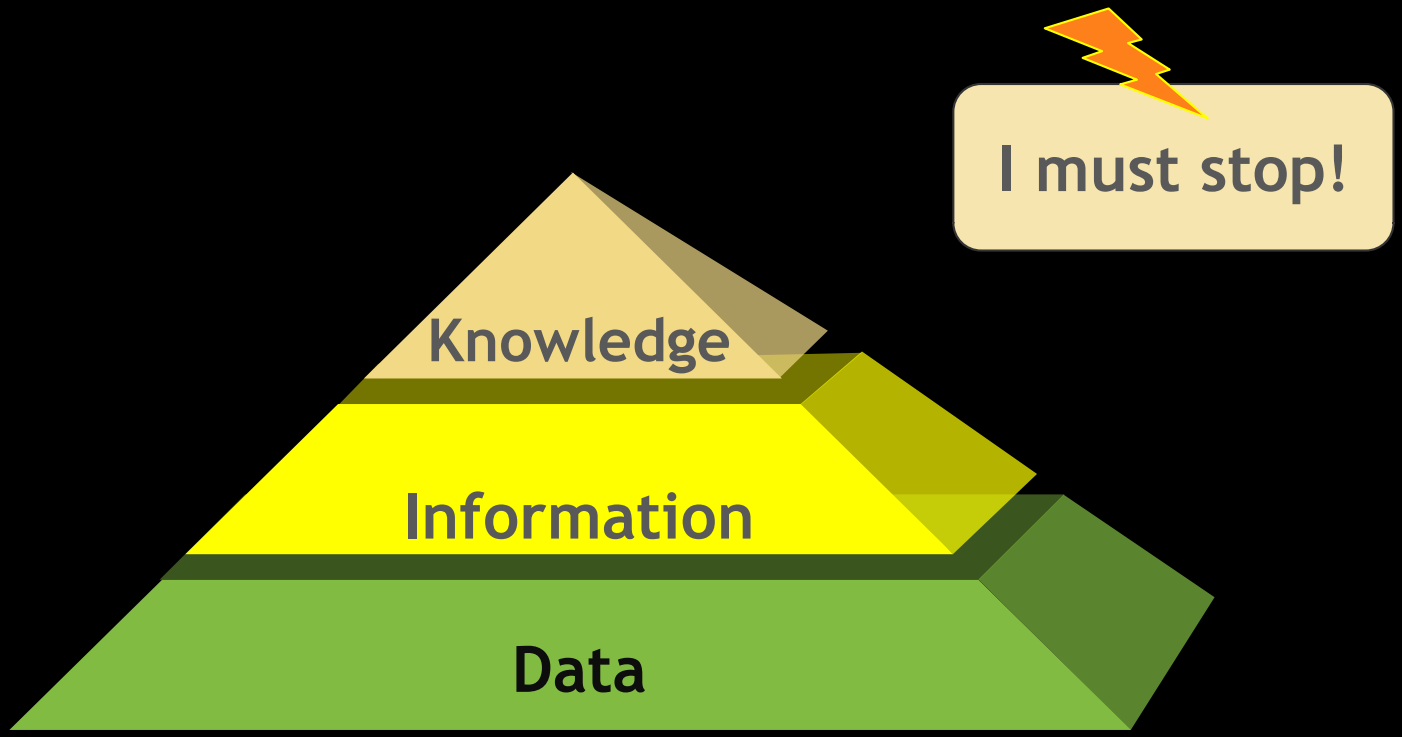
Extracting knowledge from data



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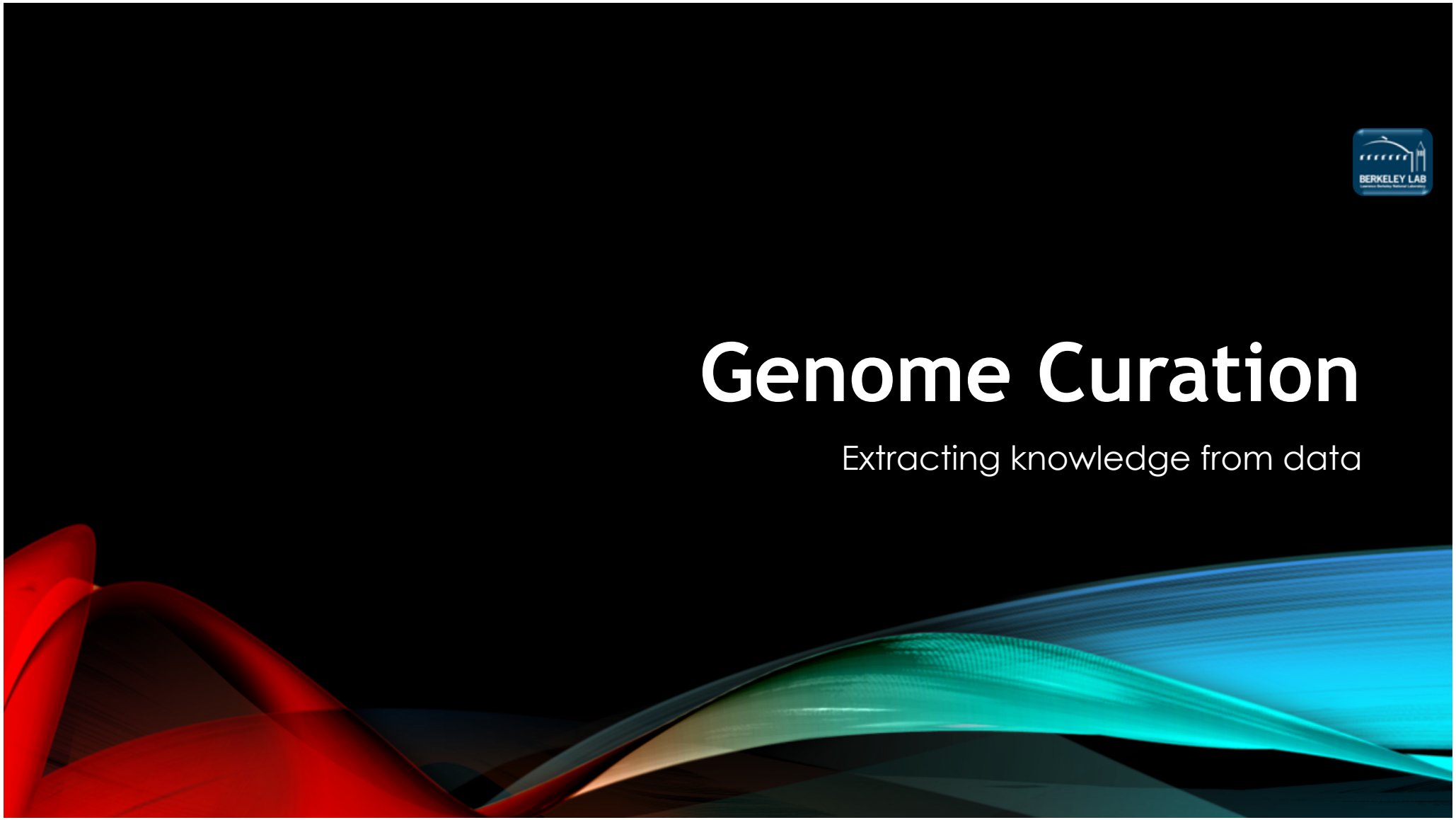
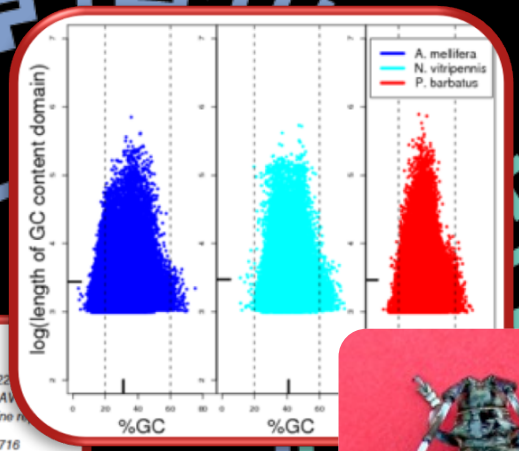
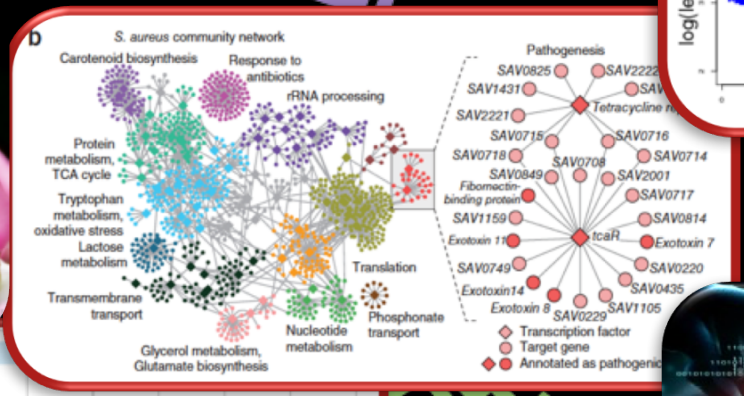


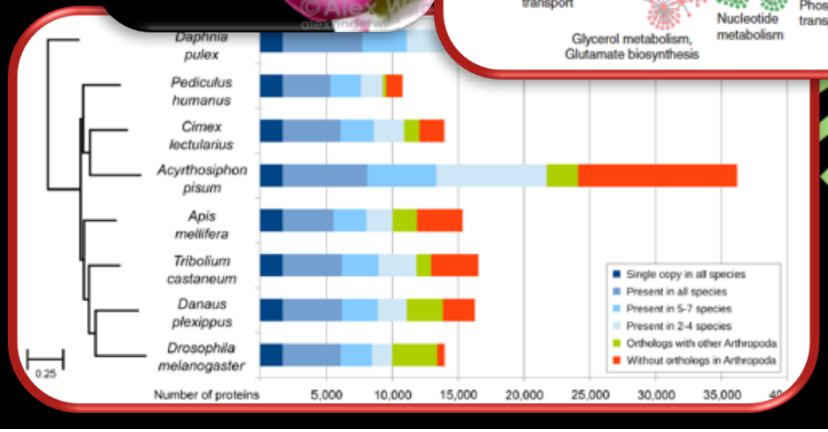
Genome Curation

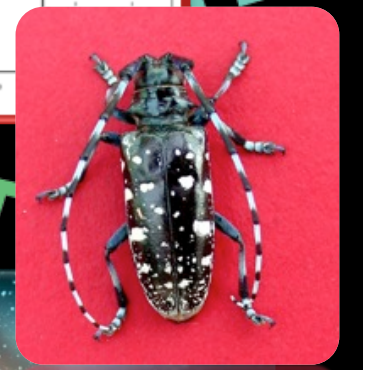
Extracting knowledge from data

Unlocking genomes









Marbach et al. 2011. *Nature Methods* | Shutterstock.com | Alexander Wild

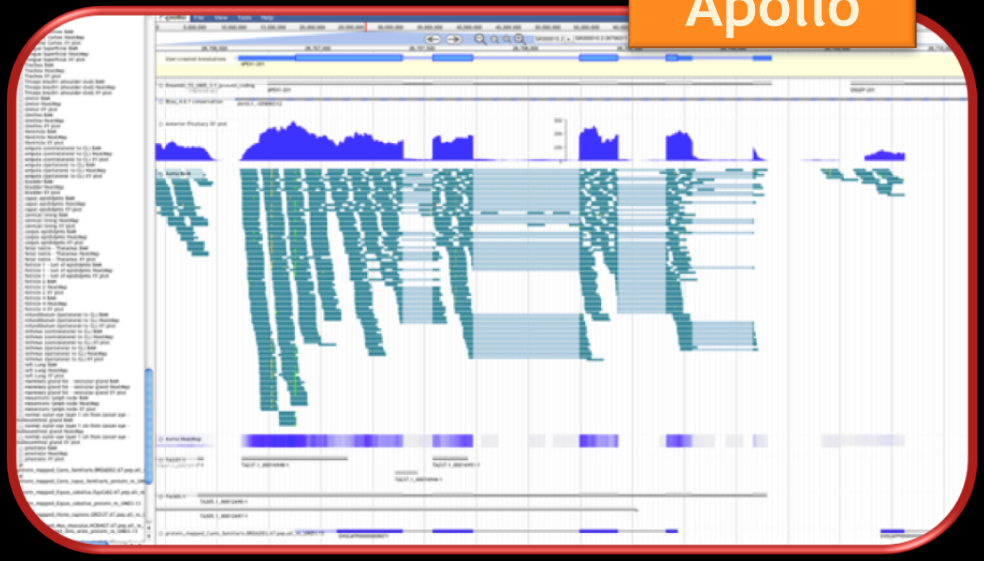
Good genes are required!

1. **Generate gene models**
   - A few rounds of gene prediction.
2. **Annotate gene models**
   - Function, expression patterns, metabolic network memberships.
3. **Manually review them**
   - Structure & Function.

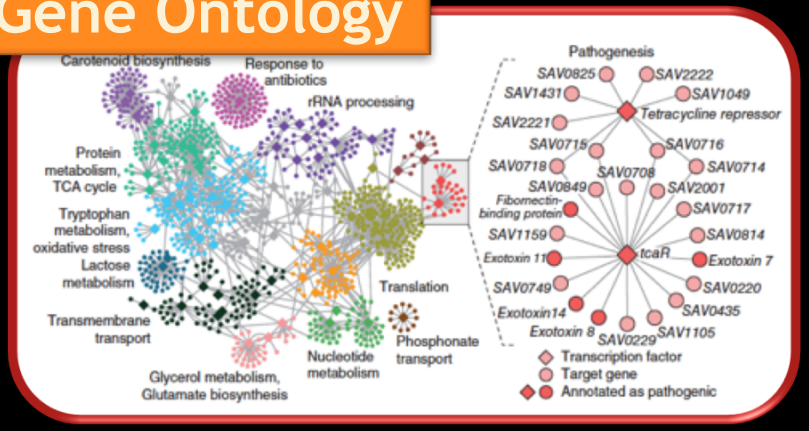
Curation improves quality

Best representation of biology & removal of elements reflecting errors in automated analyses.

Apollo



Gene Ontology



Functional assignments through comparative analysis using literature, databases, and experimental data.

Curation is valuable:

- To make accurate orthology assessments
- To accurately annotate expanded / contracted gene families
- To identify novel genes, species-specific isoforms
- To efficiently take advantage of transcriptomic analyses

Curation is inherently collaborative

- It is impossible for a single individual to curate an entire genome with precise biological fidelity.

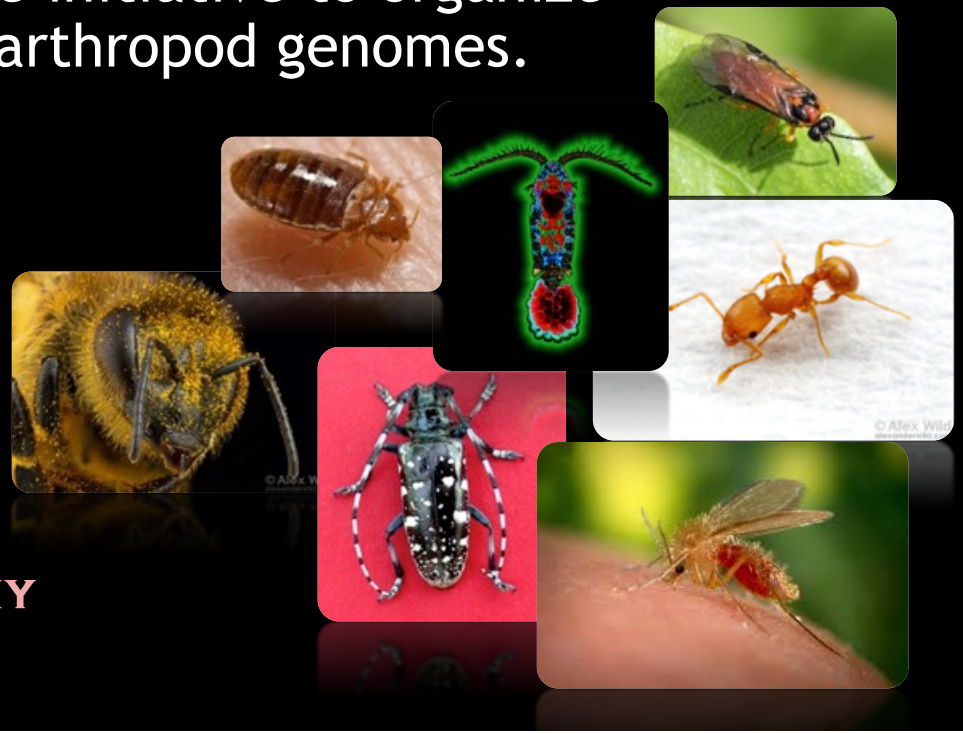
- Curators need second opinions and insights from colleagues with domain and gene family expertise.

i5k - five thousand arthropod genomes

http://i5k.github.io

- Transformative, broad, & inclusive initiative to organize sequencing and analysis of 5,000 arthropod genomes.

- Worldwide Agriculture
- Food Safety
- Medicine
- Energy Production
- Models in Biology
- Most Ecosystems
- Every Branch of the Phylogeny



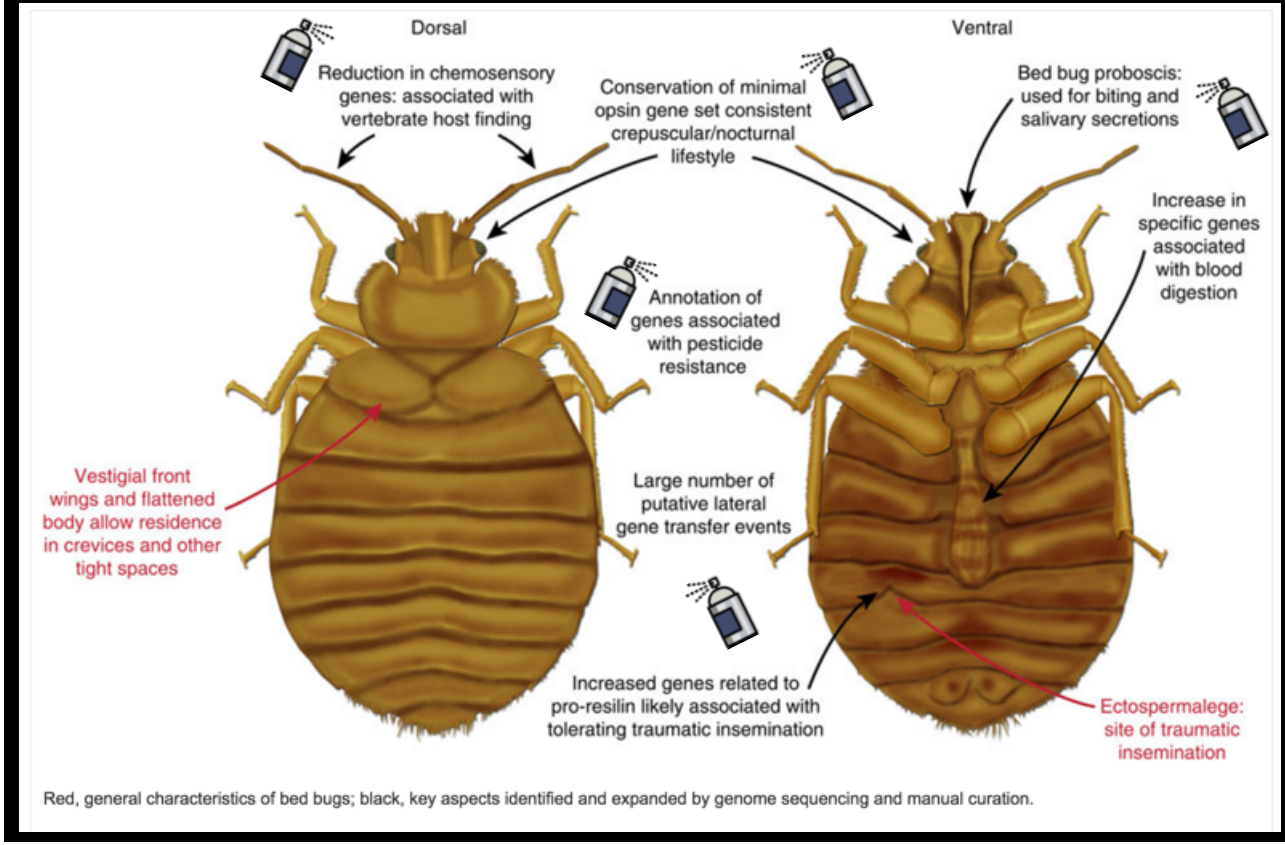
The bed bugs, they're back!

~80 Curators!

- International Travel and Commerce
- Increased Insecticide Resistance

http://i5k.github.io

Benoit et al. (2015) *Nature Communications*. *doi:10.1038/ncomms10165*



Red, general characteristics of bed bugs; black, key aspects identified and expanded by genome sequencing and manual curation.

- Timely resource for biology of human ectoparasites.
- Discovery of new targets for control.
- Common lab strain collected before introduction of pyrethroid insecticides.
  - What triggered the current bed bug resurgence?
    Did bed bugs originate from one or multiple sources?
  - Studies on mechanisms that hinder vertebrate pathogen survival & proliferation and transmission.

Predicting & annotating gene structures

Gene Prediction & Gene Annotation

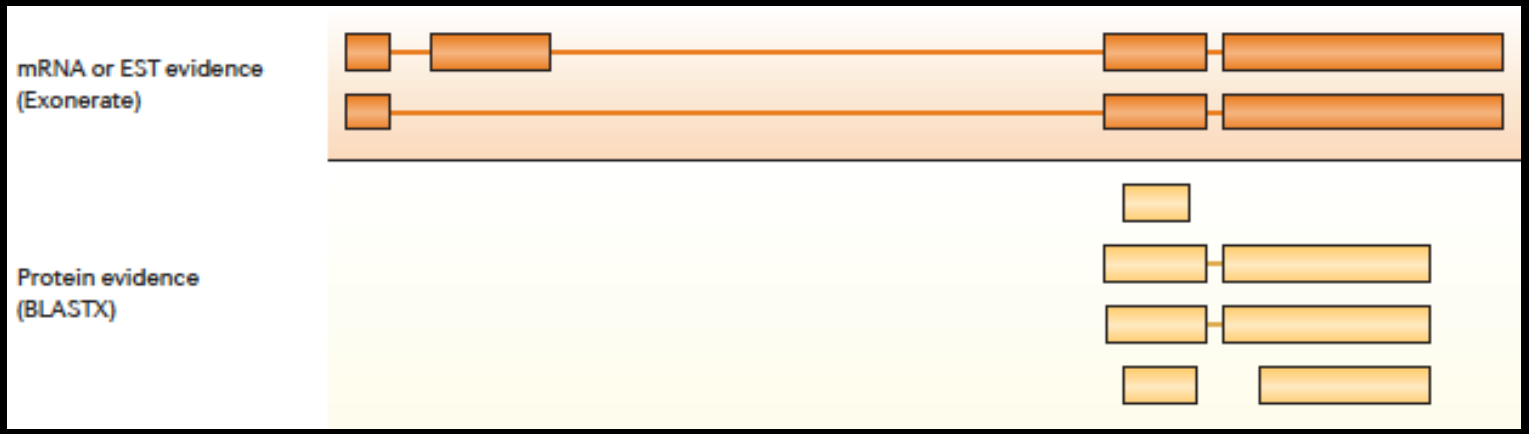
Identification and annotation of genomic elements:

- Primarily focuses on protein-coding genes.
- Also identifies RNAs (tRNA, rRNA, long and small non-coding RNAs (ncRNA)), regulatory motifs, repetitive elements, etc.
- Happens in 2 steps:
  - Computation phase
  - Annotation phase

Computation Phase

1) Experimental data are aligned to the genome:

RNA-sequencing reads, proteins, etc.



Yandell & Ence. *Nature Rev* 2012 doi:10.1038/nrg3174

Computation Phase

2) Gene predictions are generated:

2a) *Ab initio*: based on nucleotide sequence and composition
e.g. Augustus, fgenesh, etc.

2b) Using experimental evidence: identifying domains and motifs
e.g. SGP2, JAMg, fgenesh++, etc.

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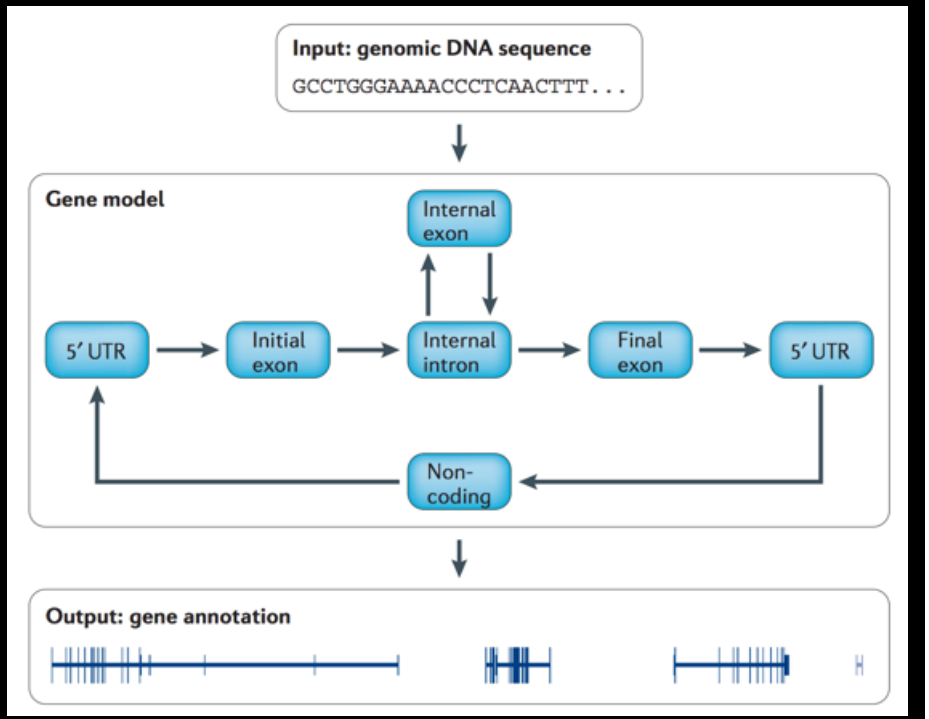
Yandell & Ence. *Nature Rev* 2012 doi:10.1038/nrg3174

Gene Prediction - methods for discovery

2a) *Ab initio*:

- Based on DNA composition
- Deals strictly with genomic sequences
- Makes use of statistical approaches (e.g. HMM) to search for coding regions and typical gene signals

  - E.g. Augustus, fgenesh, etc.



Gene Prediction - methods for discovery

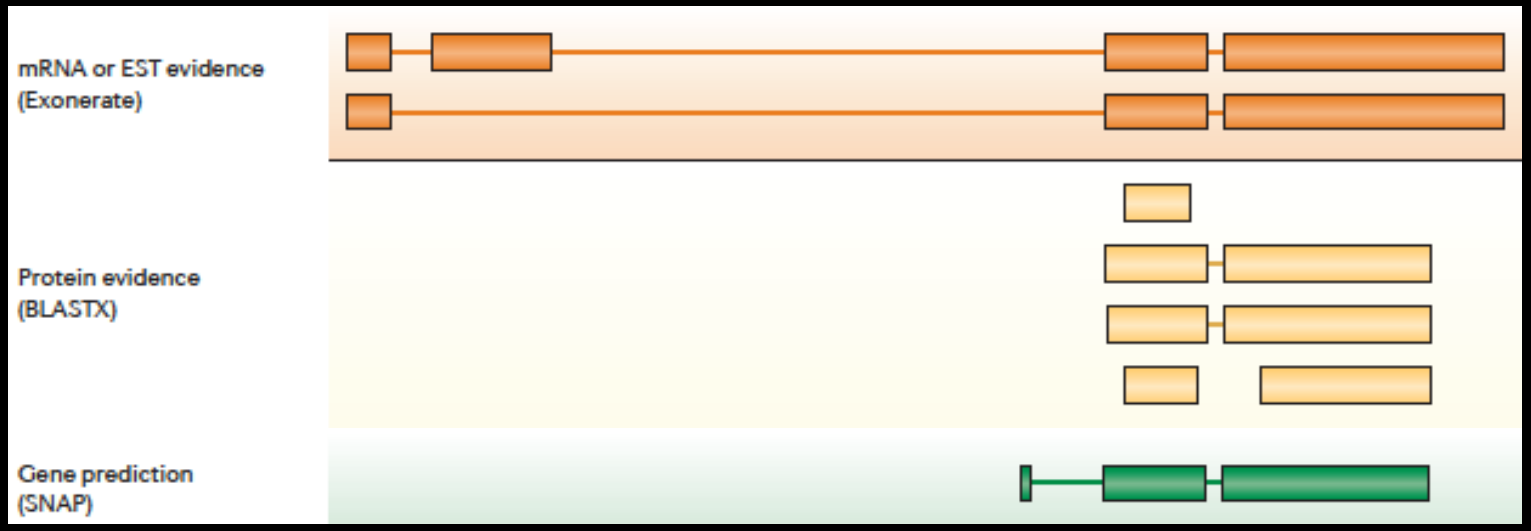
2b) Evidence-based:

Finds genes using either similarity searches against public databases or other experimental data sets e.g. RNAseq.

E.g: SGP2, fgenesh++, JAMg, etc.

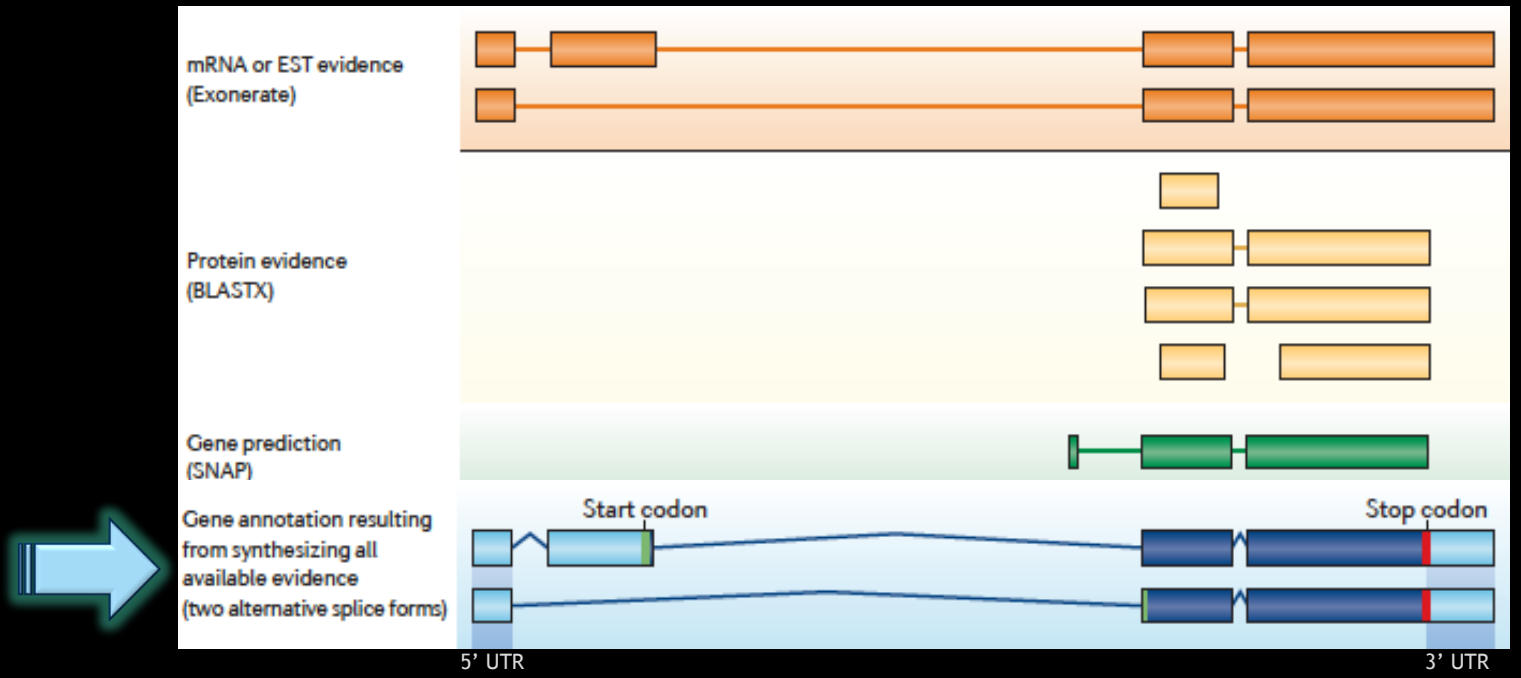
Computation Phase: result

- The single most likely coding sequence, no UTRs, no isoforms.



Annotation Phase

- Data from experimental evidence and prediction tools are synthesized into a reliable set of **structural** gene annotations.



Result: gene models that generally include UTRs, isoforms, evidence trails.

Consensus Gene Sets

Gene models may be organized into sets using:

- Combiners for automatic integration of predicted sets

  e.g: GLEAN, EvidenceModeler, etc.

- Tools packaged into pipelines

  e.g: MAKER, PASA, Gnomon, Ensembl, etc.

Challenges

Ab initio

+ can capture species-specific or highly-divergent genes
- false positive predictions (incomplete predictions, readthrough predictions)
- not enough on its own to establish orthology

Reference-guided

+ uses reliable gene orthologs from better-annotated species
- can miss species-specific genes and other sequences
- not enough on its own to establish orthology

Some suggestions

- **Hybrid** reference-guided & *ab initio* gene prediction

- **Generate transcriptomic data** to confirm predictions, extend & improve models, identify new expressed loci.
  - the more tissues, the better!

- **Review synteny** to verify orthologous assignments
  - largely manual for now.

Annotating gene functions

Functional Annotation

Attaching metadata to structural annotations for the purpose of assigning a particular function.

- Assignments do not necessarily have to be supported by your own experimental data.
- Sequence similarity approaches must be informed and validated by evolutionary theory, not just a score value.

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Gene Ontology

GeneOntology.org

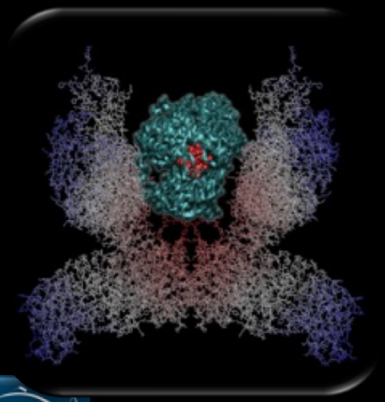
Terms (classes) arranged in a graph: molecular functions, biological processes, cellular locations, and the relationships connecting them all, in a species-independent manner.

~150 Contributors!

1. Molecular Function

An elemental activity or task or job

- protein kinase activity
- insulin receptor activity

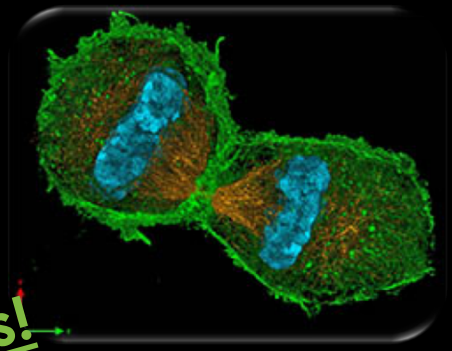


Insulin Receptor
Petrus et al, 2009, *ChemMedChem*

2. Biological Process

A commonly recognized series of events

- cell division

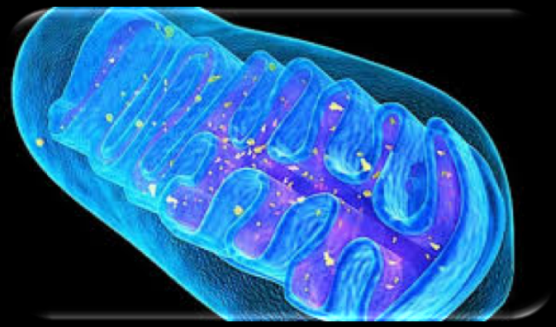


End of Telophase.
Lothar Schermelleh

3. Cellular Component

Where a gene product is located

- mitochondria
- mitochondrial matrix
- mitochondrial inner membrane



Mitochondrion.
PaisekaScience Photo Library

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Collaboratively curating gene structures

General process of curation

1. Select or find a **region of interest** (e.g. scaffold).
2. Select appropriate **evidence** tracks to review the genome element to annotate (e.g. gene model).
3. Determine whether a feature in an existing evidence track will provide a reasonable **gene model to start** working.
4. If necessary, **adjust** the gene model.
5. Check your edited gene model for **integrity and accuracy** by comparing it with available homologs.
6. **Comment** and finish.

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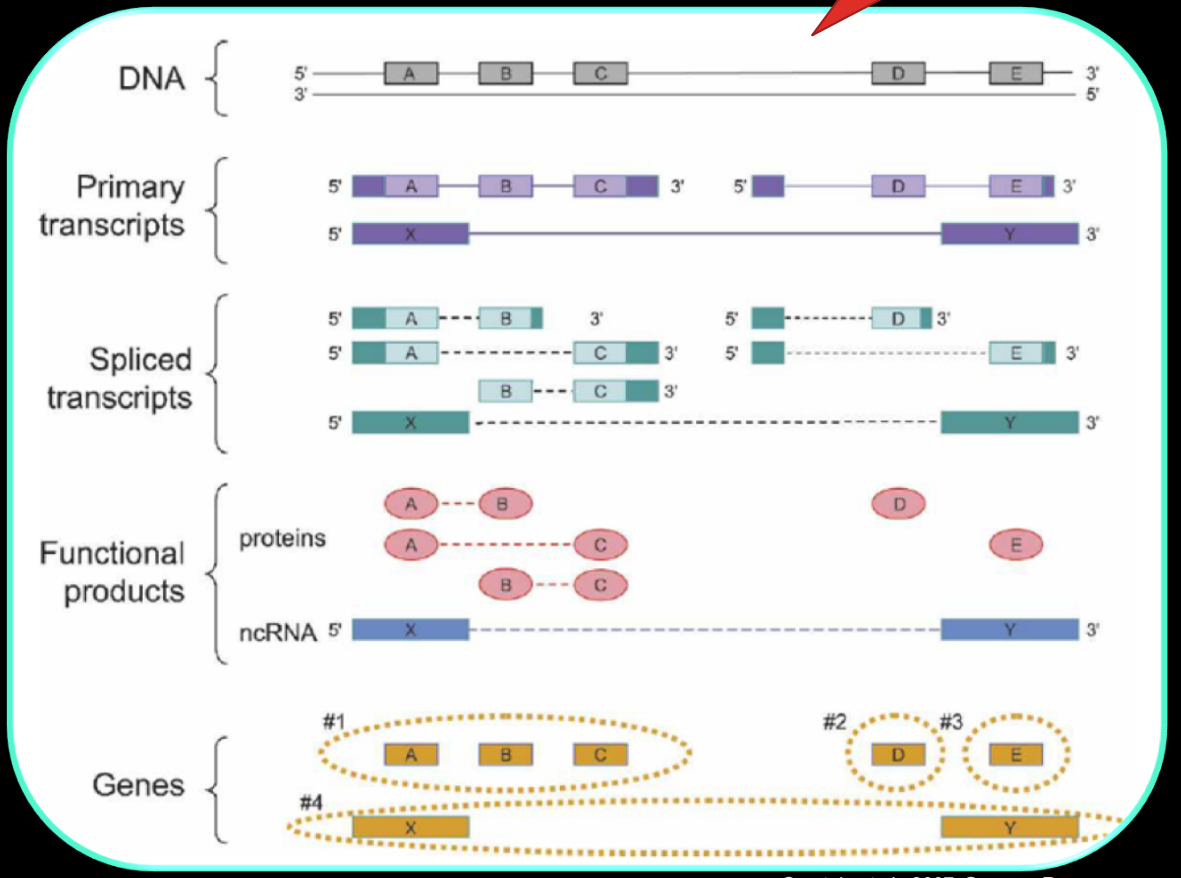
Biorefresher

A brief refresher

The gene: *a moving target*

Biorefresher

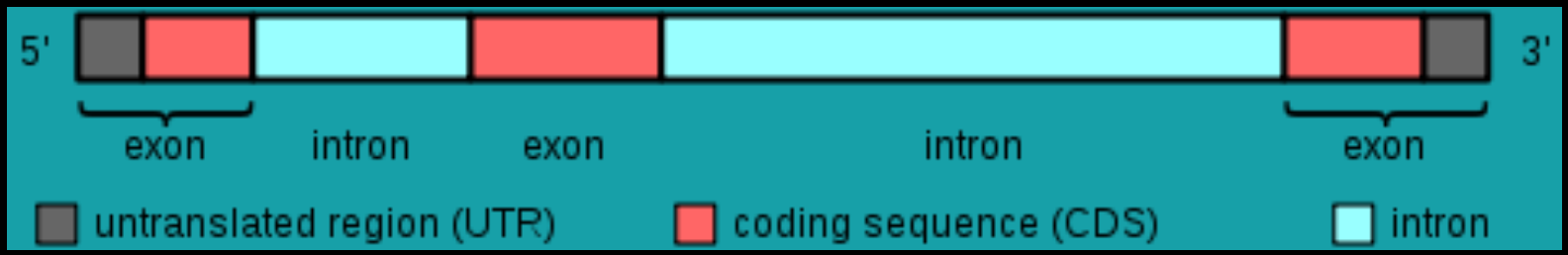
"The gene is a union of genomic sequences encoding a coherent set of potentially overlapping functional products."



Gerstein et al., 2007. Genome Res

mRNA

Biorefresher



"Gene structure" by Daycd- Wikimedia Commons

Biorefresher

Reading frames

In eukaryotes, only one reading frame per section of DNA is biologically relevant at a time: can be transcribed into RNA and translated into protein.

OPEN READING FRAME (ORF)

ORF = Start signal + coding sequence (divisible by 3) + Stop signal

Biorefresher

Splice sites

Splicing “signals” (from the point of view of an intron):

- 5’ end splice “signal” (site): usually GT (less common: GC)
- 3’ end splice site: usually AG

…]5’ - GT / AG - 3’[…

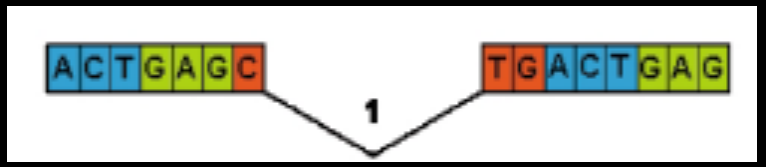
Alternatively bringing exons together produces more than one protein from the same genic region: isoforms.

Exons and Introns

Biorefresher

- Introns can interrupt the reading frame of a gene by inserting a sequence between two consecutive codons

- Between the first and second nucleotide of a codon



- Or between the second and third nucleotide of a codon

Biorefresher

Obstacles to transcription and translation

- Premature *Stop* codons in the message: A process called **non-sense mediated decay** checks and corrects them to avoid incomplete splicing, DNA mutations, transcription errors, and leaky scanning of ribosome – which can cause changes in the reading frame (frame shifts).

- Insertions and deletions (**indels**) can cause frame shifts when the indel is not divisible by three. As a result, the peptide can be abnormally long, or abnormally short – depending on when the first in-frame *Stop* signal is located.

Apollo

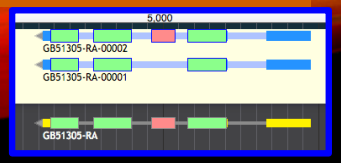
Functionality overview

Apollo Genome Annotation Editor

Collaborative, instantaneous, web-based, built on top of JBrowse.

GenomeArchitect.org

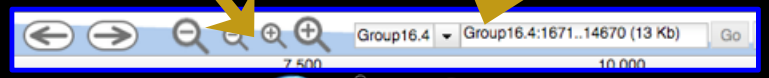
Color by CDS frame, toggle strands, set color scheme and highlights.



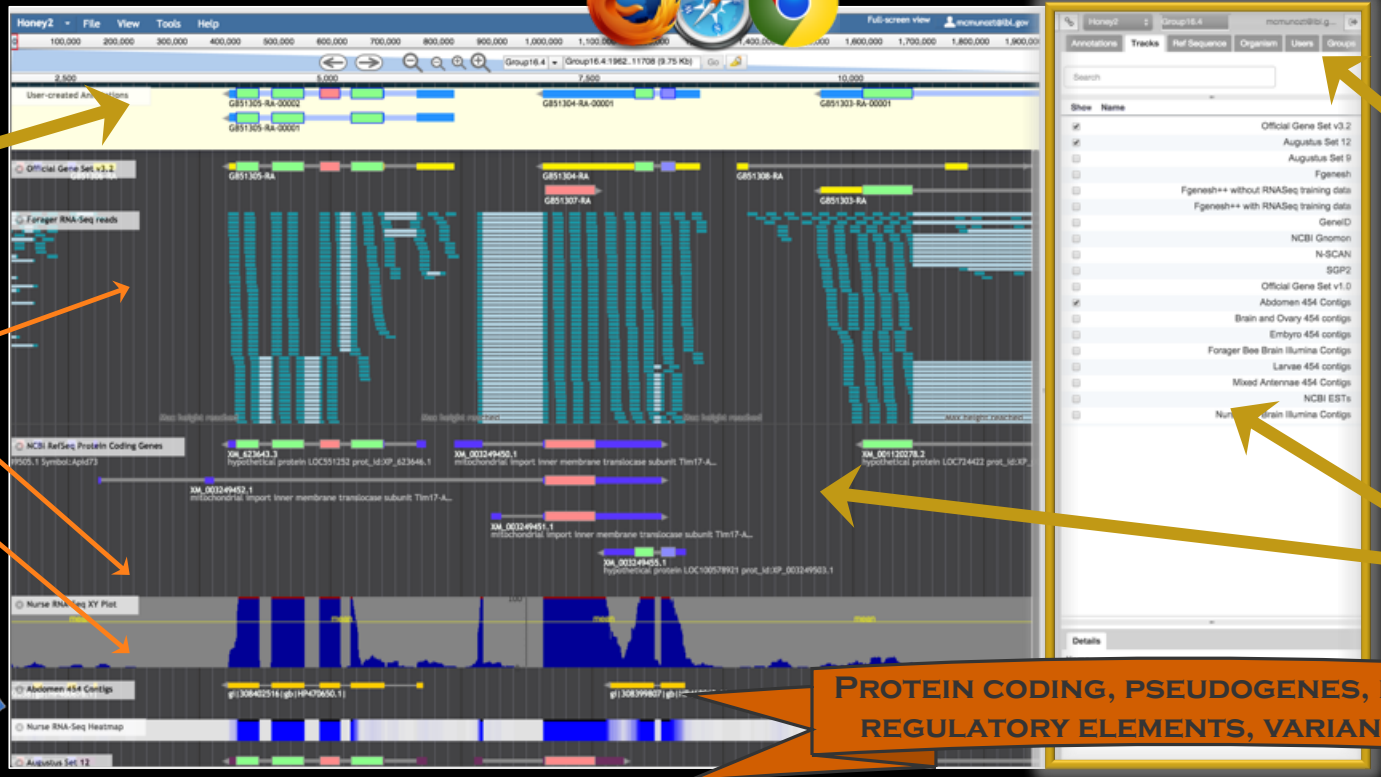
Query the genome using **BLAT.**

Navigate and zoom.

Search for a gene model or a scaffold.



Upload evidence files (GFF3, BAM, BigWig), add **combination** and **sequence search** tracks.



User-created annotations.

Stage and cell-type specific transcription data.

PROTEIN CODING, PSEUDOGENES, NCRNAS, REGULATORY ELEMENTS, VARIANTS, ETC.

Apollo

JBrowse

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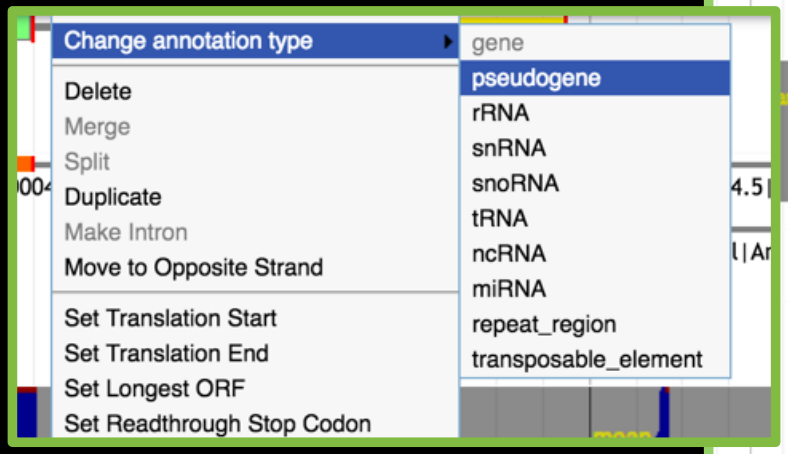
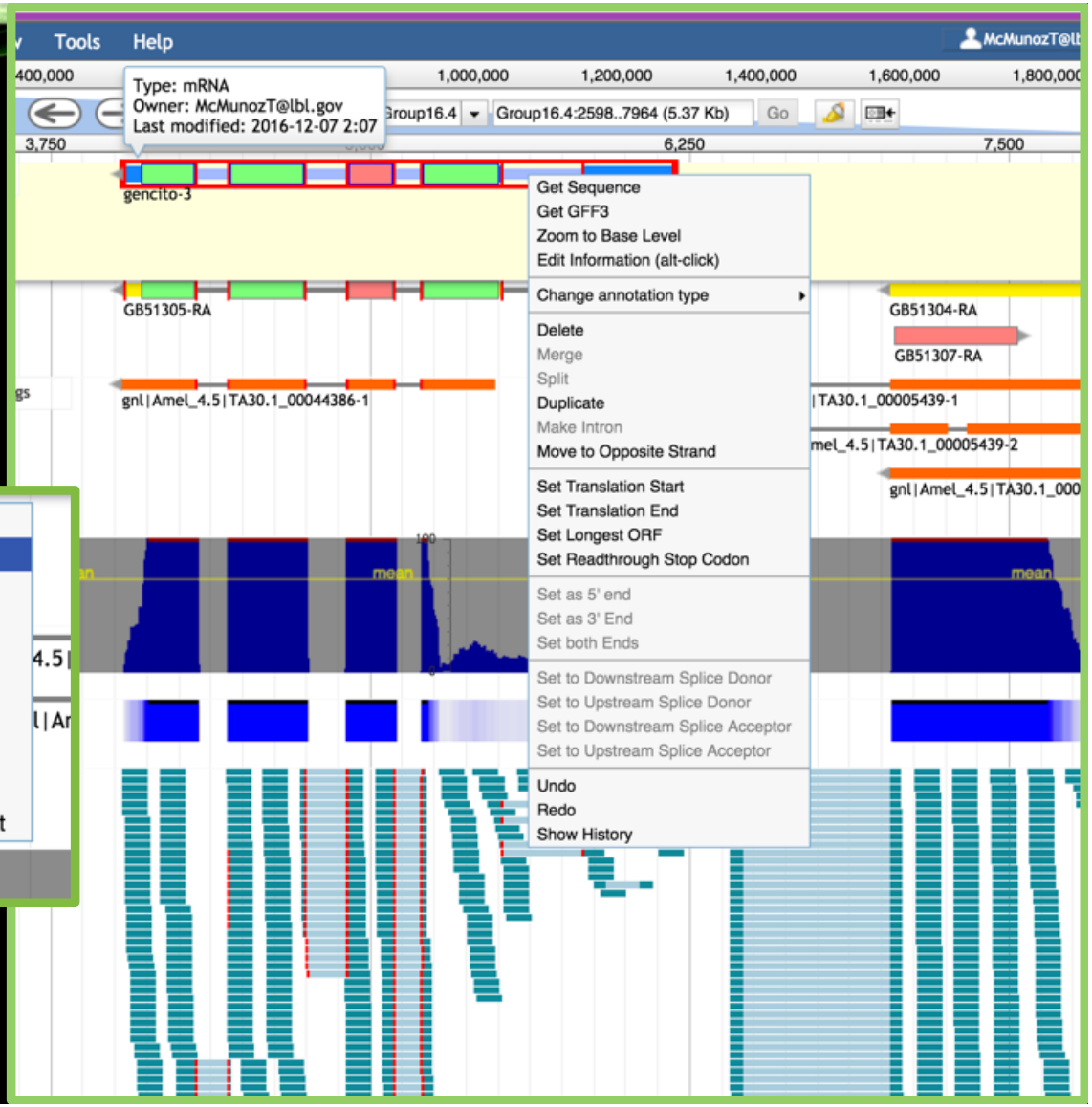
Right-click functionality





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GenomeArchitect.org

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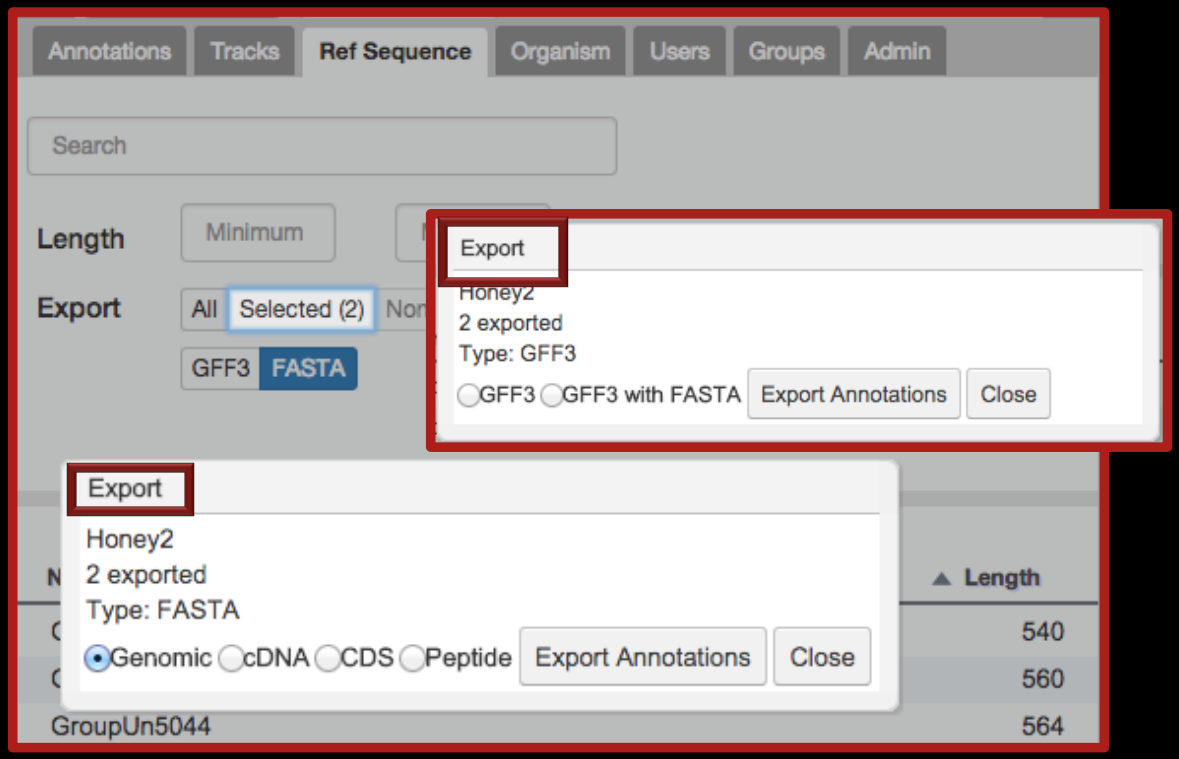
Export



GenomeArchitect.org

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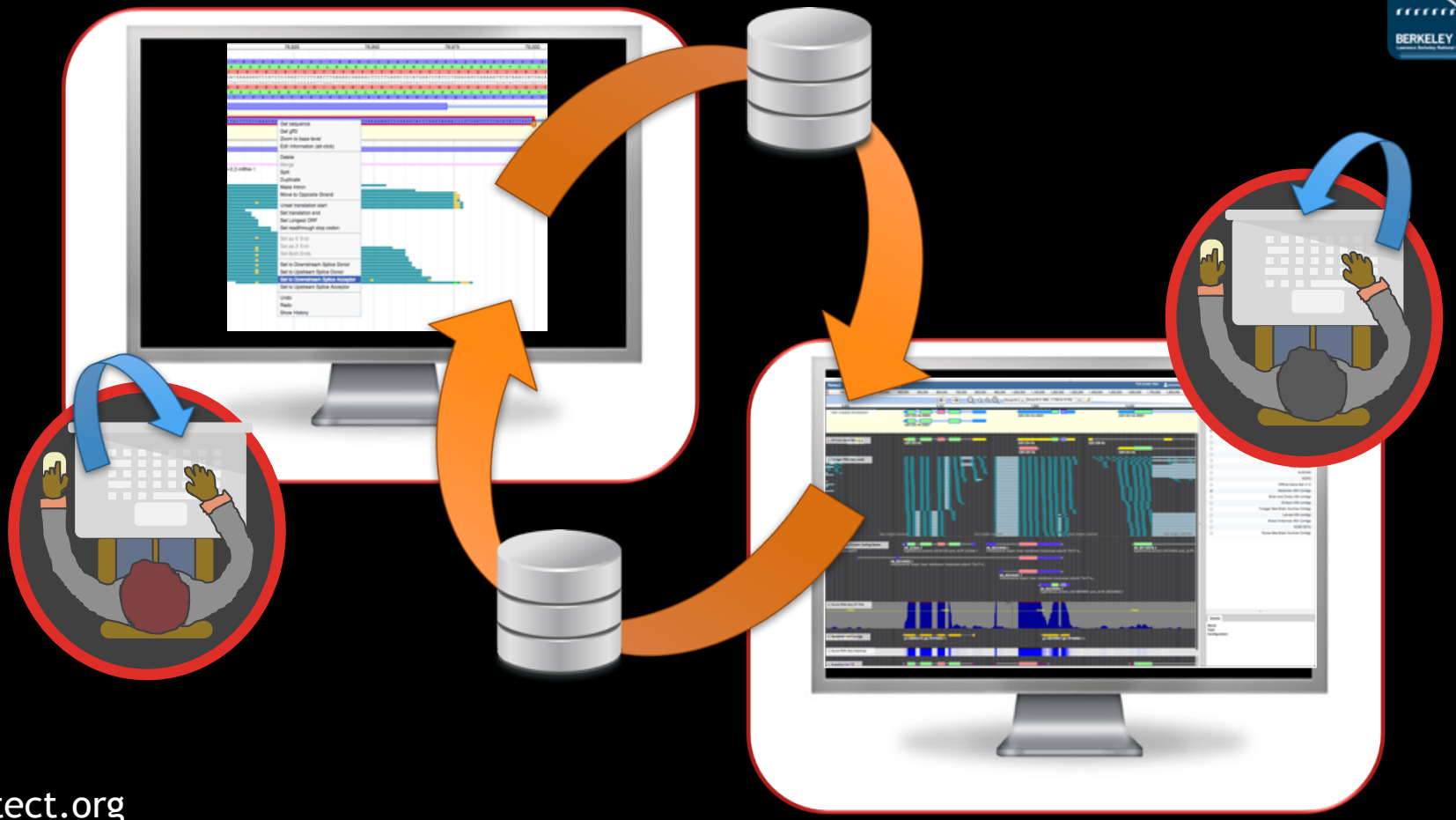
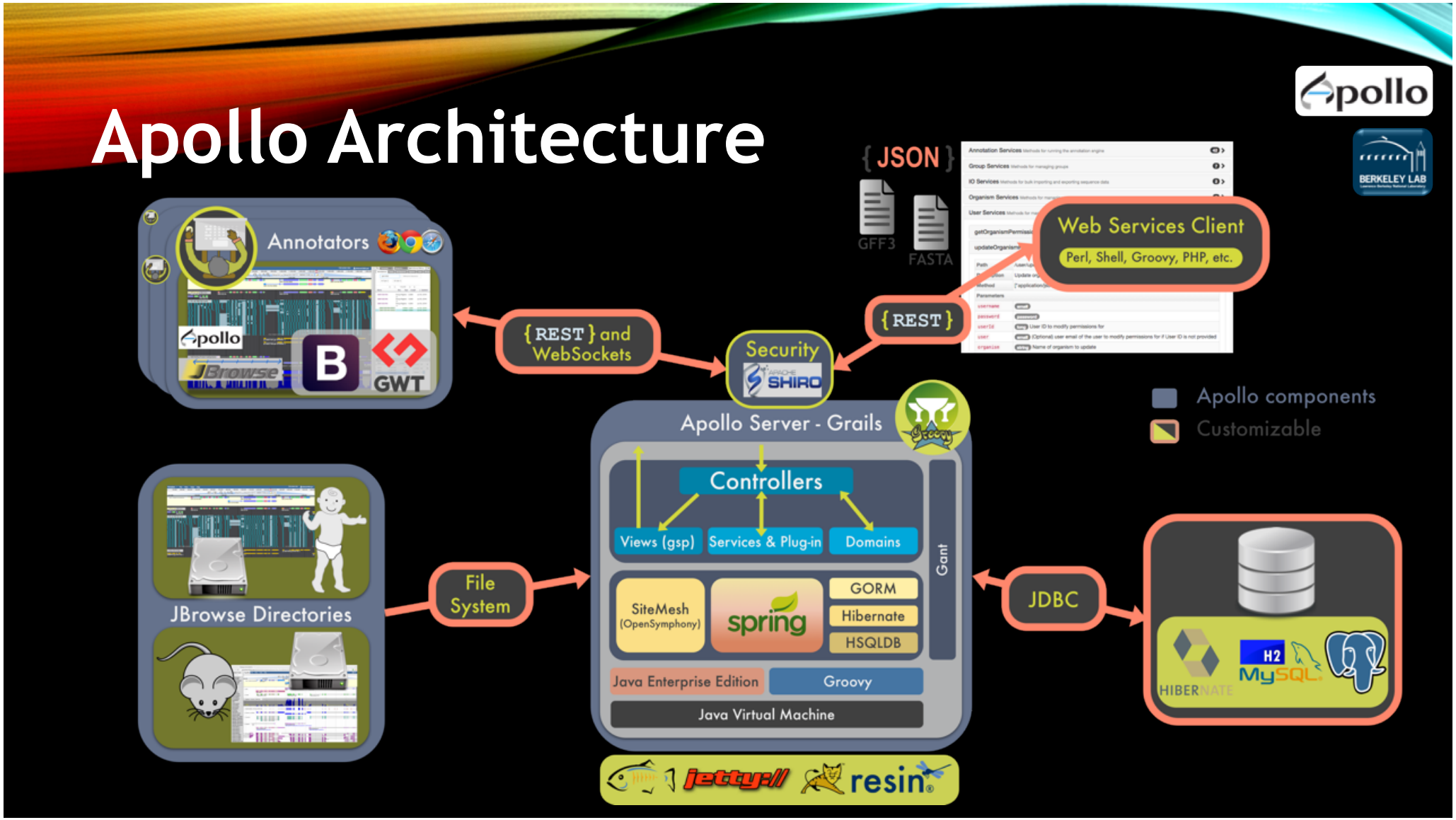
Collaboration in real time



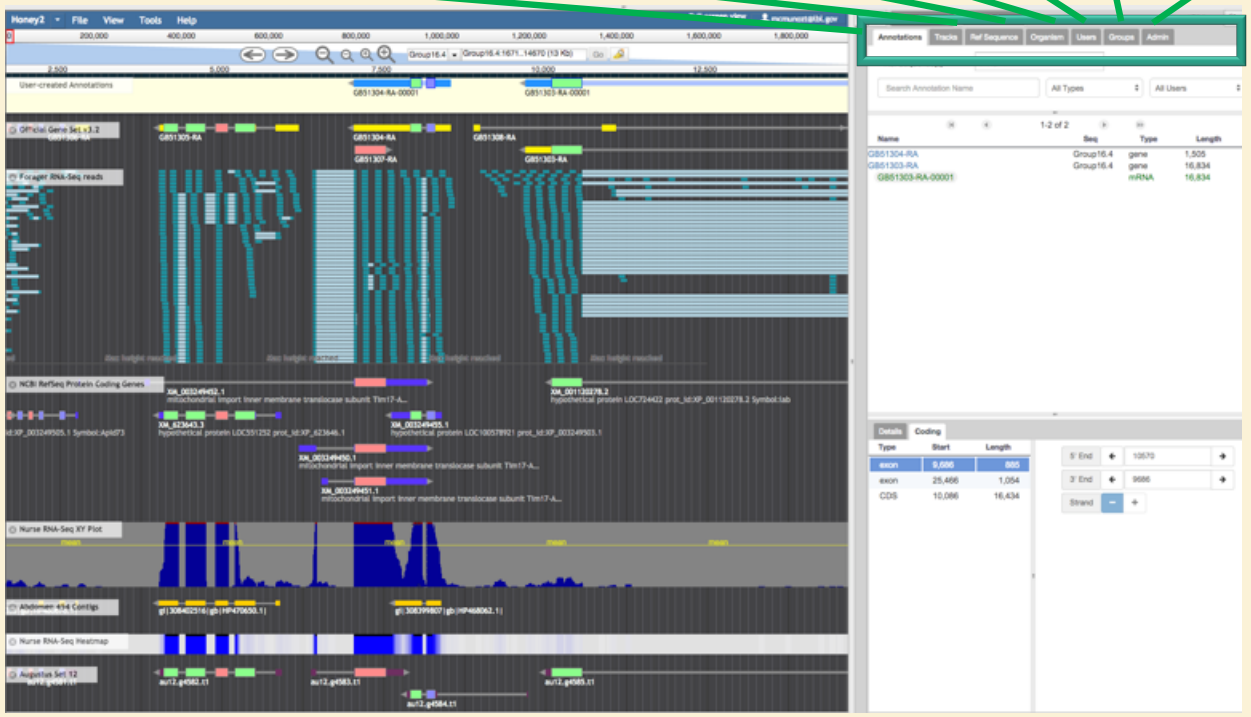
GenomeArchitect.org

Apollo Architecture



Removable *Annotator Panel*

Annotations | Tracks | Reference Sequence | Organism | Users | Groups | Admin



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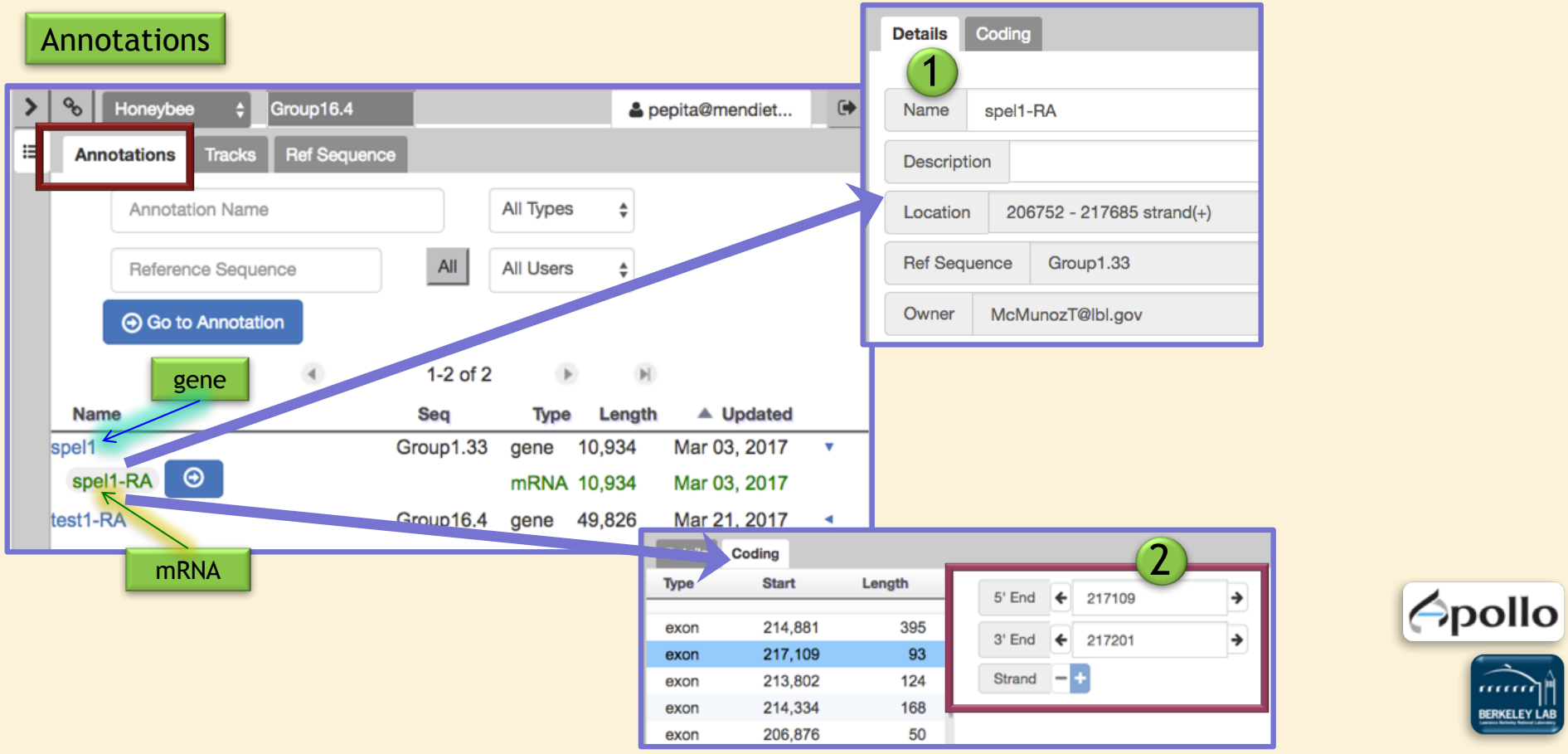
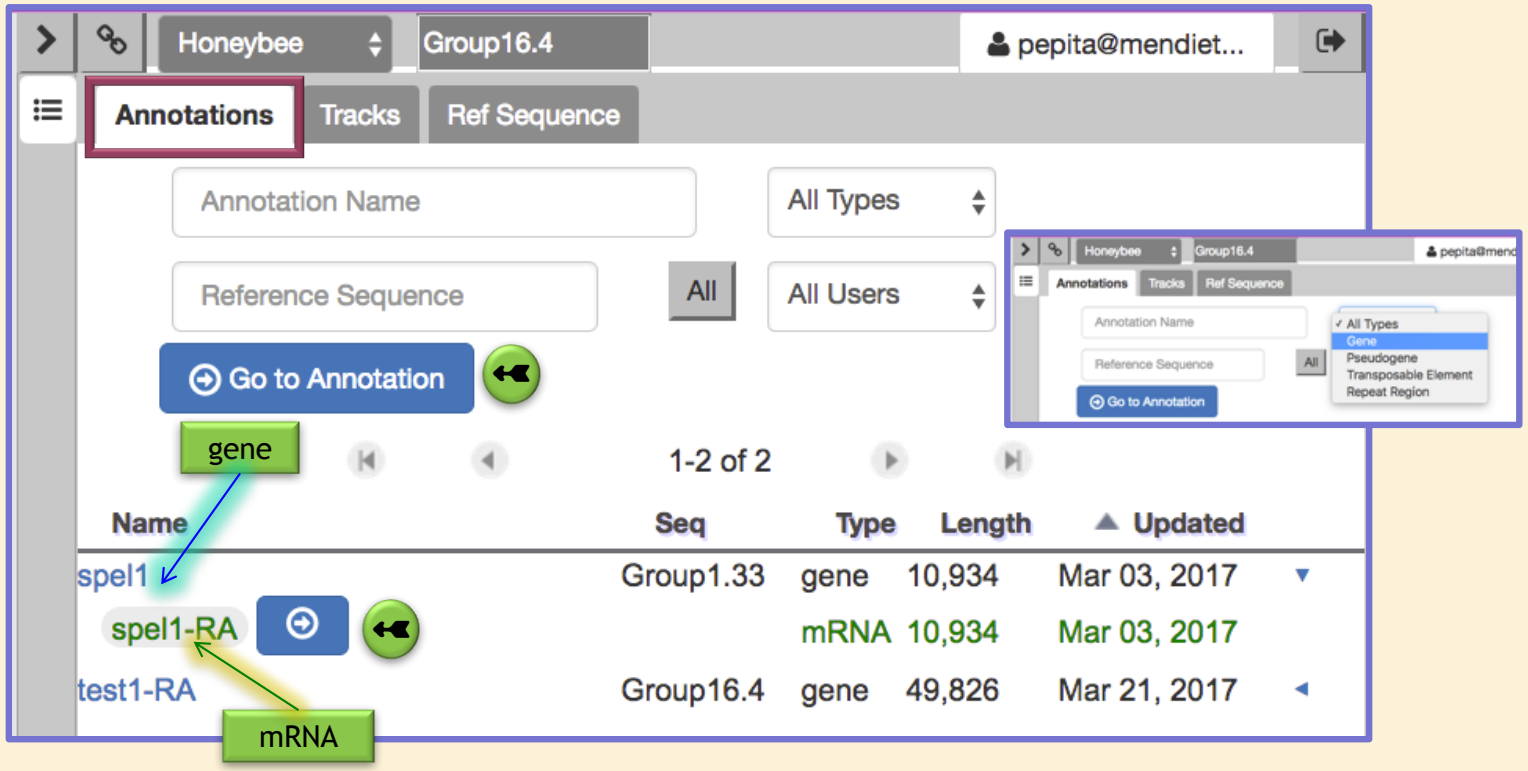
Annotation details & exon boundaries

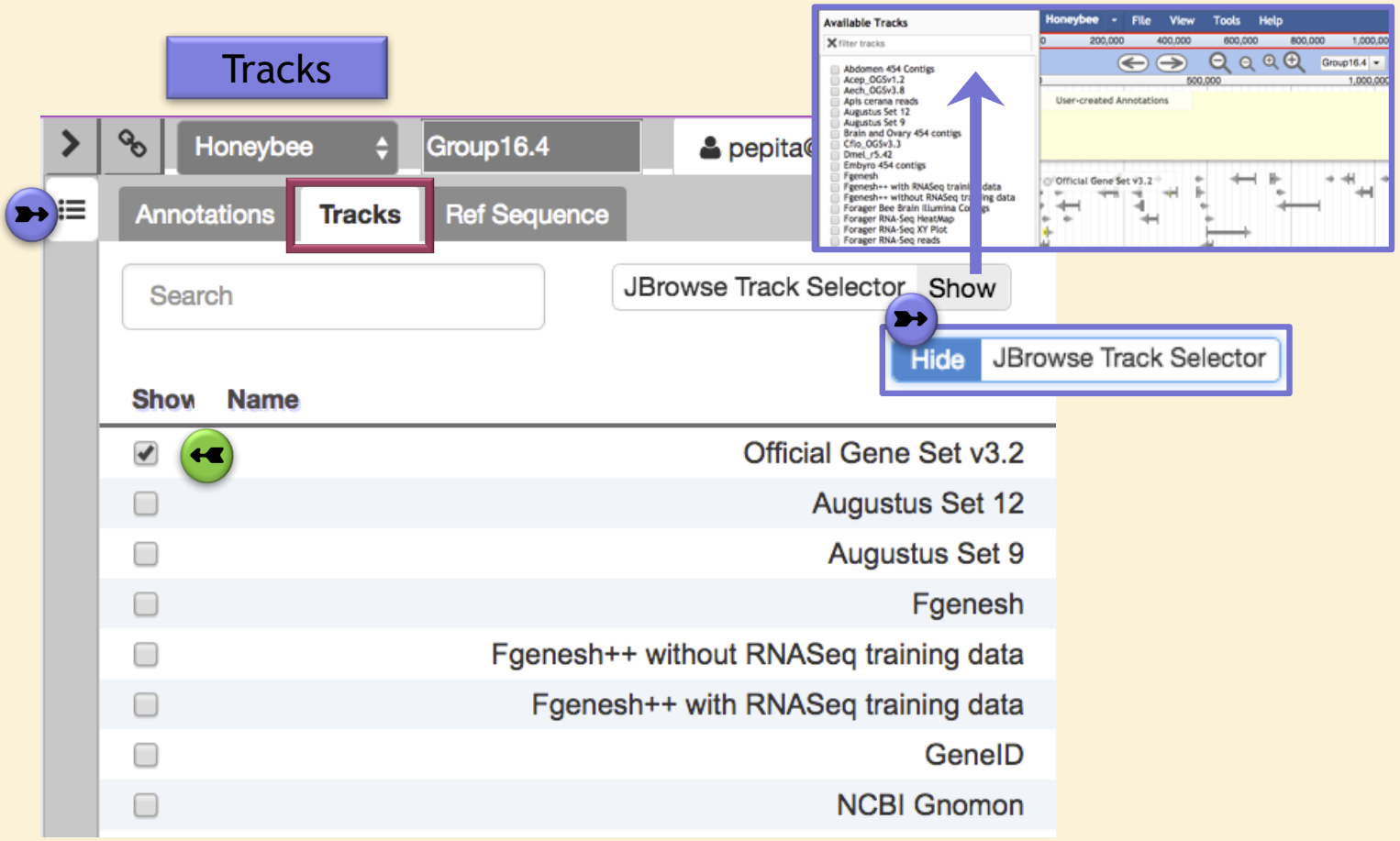


Navigating to an annotation

Annotations

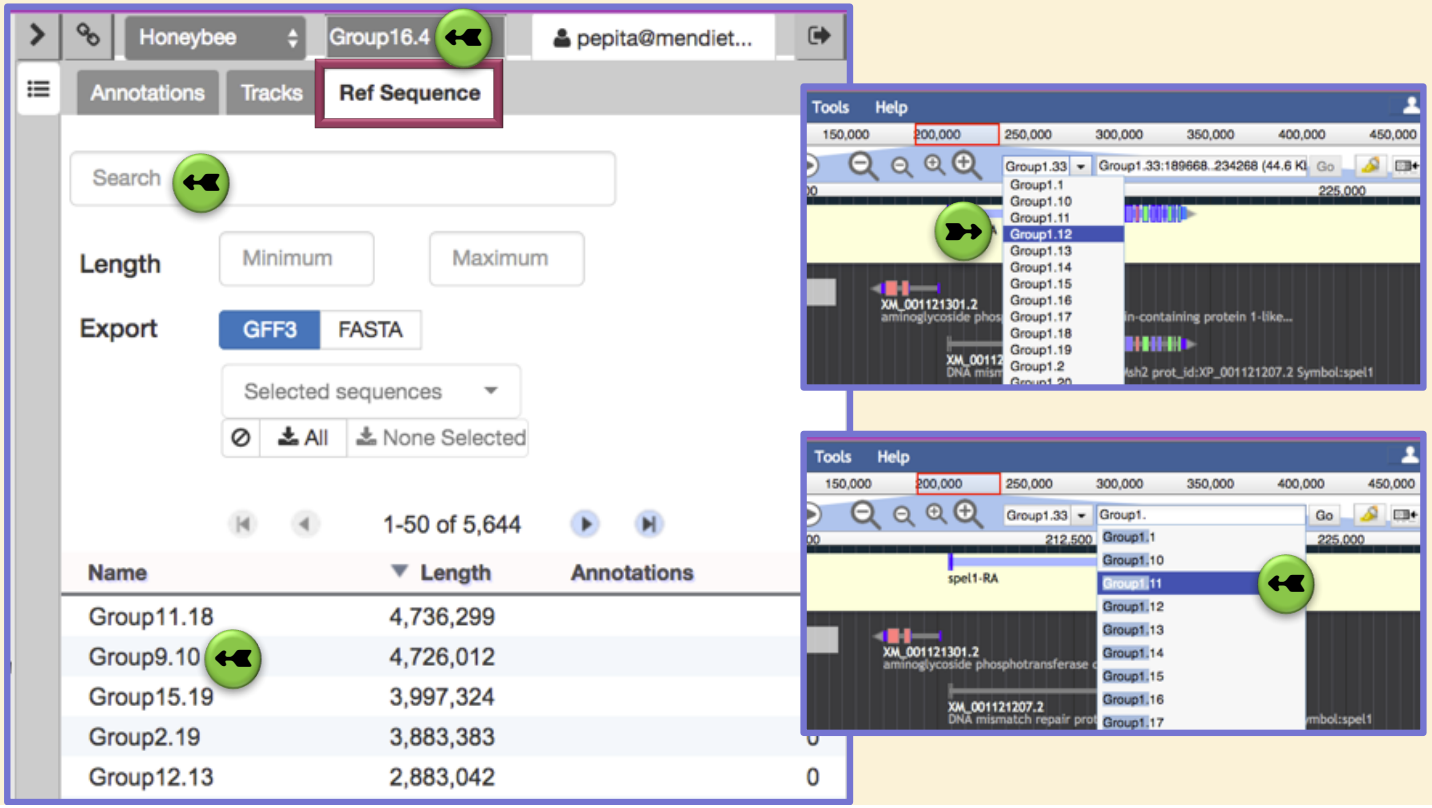


Displaying tracks with supporting data

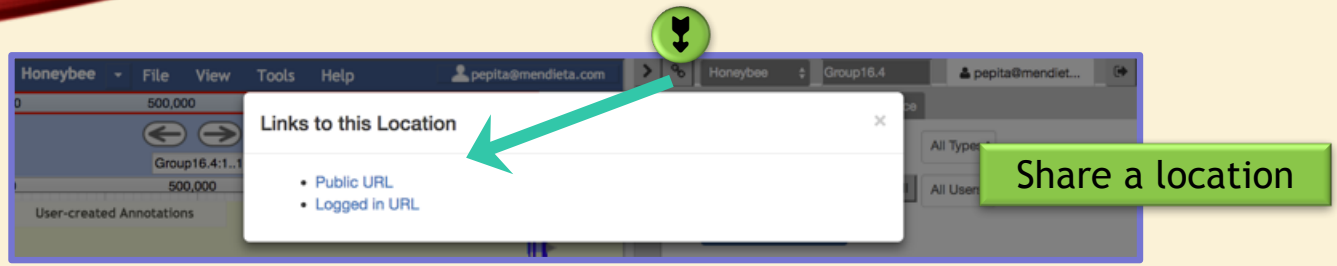


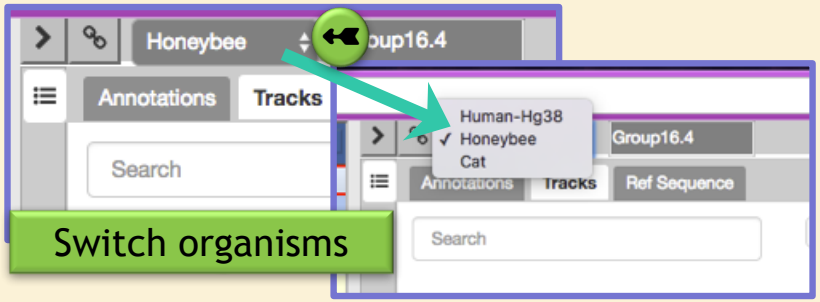
Navigating to ‘Reference Sequence’
(i.e. assembly fragments: scaffolds, chromosomes, etc.)

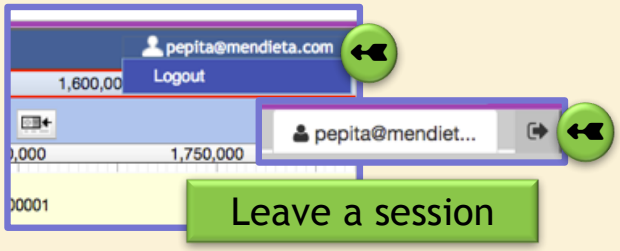
Ref Sequence

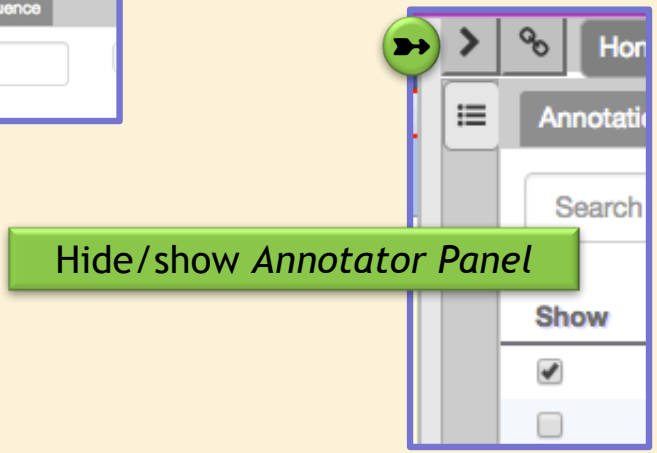


Additional functionality









Follow along

Access Apollo

Your number	Email	Password	Server	Organism	Begin at
1	user.one@example.com	userone	1	Honey0	1
2	user.two@example.com	usertwo	2	Honey0	1
3	user.three@example.com	userthree	3	Honey0	1
4	user.four@example.com	userfour	4	Honey0	1
5	user.five@example.com	userfive	5	Honey0	1
6	user.six@example.com	usersix	1	Honey1	7
7	user.seven@example.com	userseven	2	Honey1	7
8	user.eight@example.com	usereight	3	Honey1	7
9	user.nine@example.com	usernine	4	Honey1	7
10	user.ten@example.com	userten	5	Honey1	7
11	user.eleven@example.com	usereleven	1	Honey2	1
12	user.twelve@example.com	usertwelve	2	Honey2	1
13	user.thirteen@example.com	userthirteen	3	Honey2	1
14	user.fourteen@example.com	userfourteen	4	Honey2	1
15	user.fifteen@example.com	userfifteen	5	Honey2	1
16	user.sixteen@example.com	usersixteen	1	Honey3	7
17	user.seventeen@example.com	userseventeen	2	Honey3	7
25	user.twentyfive@example.com	usertwentyfive	5	Honey4	1
26	user.twentysix@example.com	usertwentysix	1	Honey5	7
27	user.twentyseven@example.com	usertwentyseven	2	Honey5	7
28	user.twentyeight@example.com	usertwentyeight	3	Honey5	7
29	user.twentynine@example.com	usertwentynine	4	Honey5	7
30	user.twentynine@example.com	usertwentynine	5	Honey5	7

Server	URL
1	http://tinyurl.com/apollo-ags1
2	http://tinyurl.com/apollo-ags2
3	http://tinyurl.com/apollo-ags3
4	http://tinyurl.com/apollo-ags4
5	http://tinyurl.com/apollo-ags5

Your number	Email	Password	Server	Organism	Begin at
1	user.one@example.com	userone	1	Honey0	1
2	user.two@example.com	usertwo	2	Honey0	1

Apollo

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Thank You.

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- Monica Poelchau, USDA/NAL (Apollo)
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- i5k Community

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BBOP Projects

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- **GeneOntology.org (GO)**
  - Assigning function to genes in all organisms (including Noctua)
- **GenomeArchitect.org (Apollo)**
  - Collaborative curation of genomes and gene models
- **MonarchInitiative.org**
  - Using comparative phenomics to illuminate human diseases
- **INCA**
  - Intelligent Concept Assistant for application of metadata
- **Planteome.org**
  - (Prime: OSU) Common reference ontologies & annotations
- **AllianceGenome.org (AGR)**
  - Unified Model Organism Databases
- **NCATS Translator**
  - Automating the translation of mechanistic biological knowledge to clinical applications

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