Annotating Genomes the Ensembl Way

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Biodiversity Genomics Academy 2023

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12:00 - 16:00

Annotating genomes the Ensembl way: General Concepts and Background

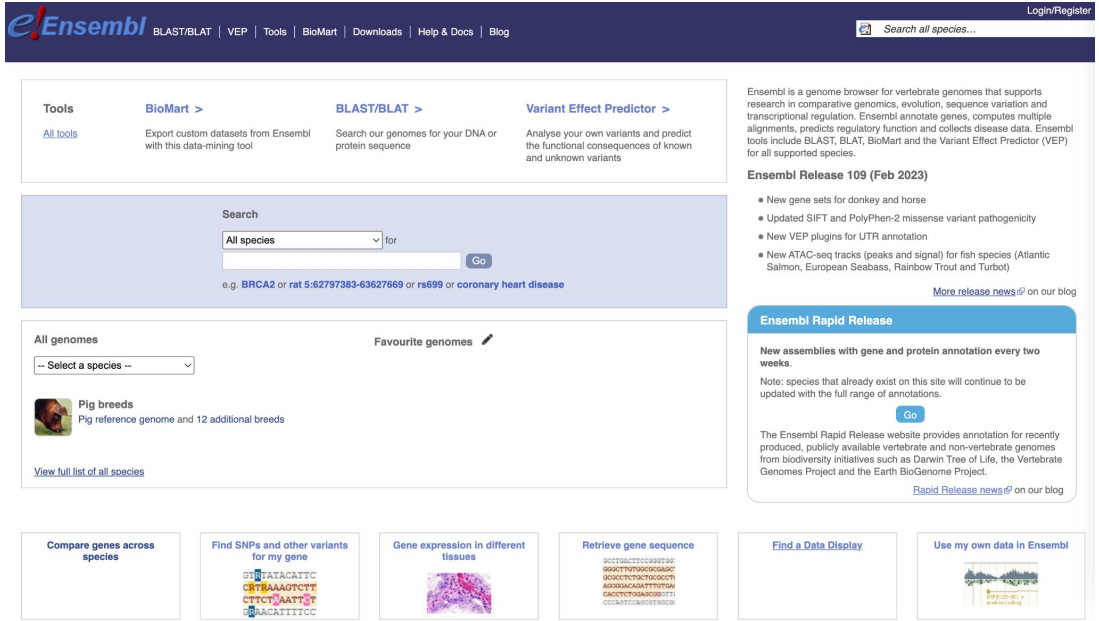
A Case study: Annotating Fungal Genomes - The Challenges!

14:00-16:00

Annotating genomes the Ensembl way: Hands-on - From RNAseq reads to gene models

What is Ensembl?

- A genomics platform for enabling and accelerating downstream science
- Most widely known for our genome browser
- Gene annotation, comparative genomics, variation and regulatory data
- Also provide data via FTP, REST/Perl API, MySQL dumps, BioMart



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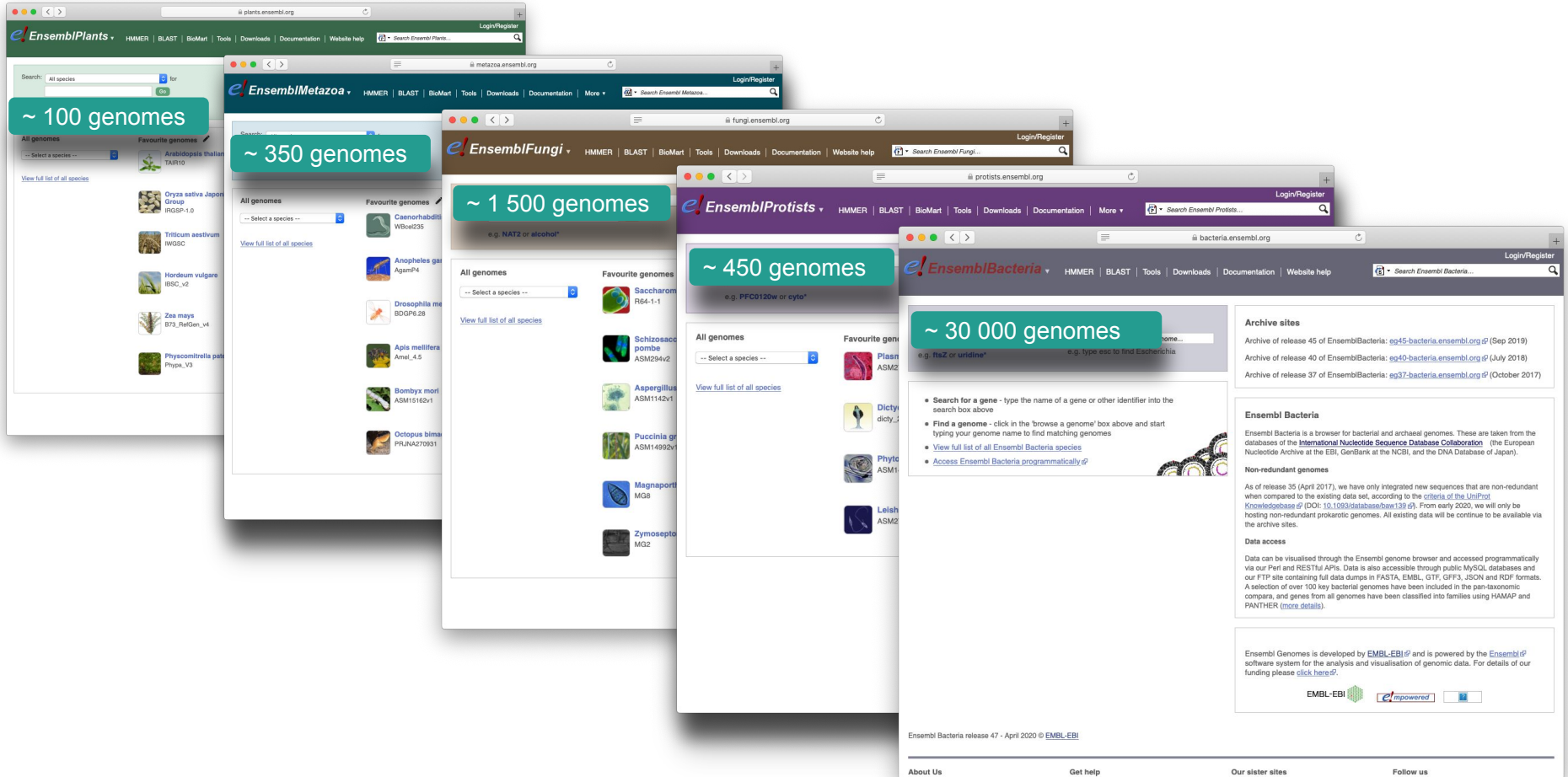
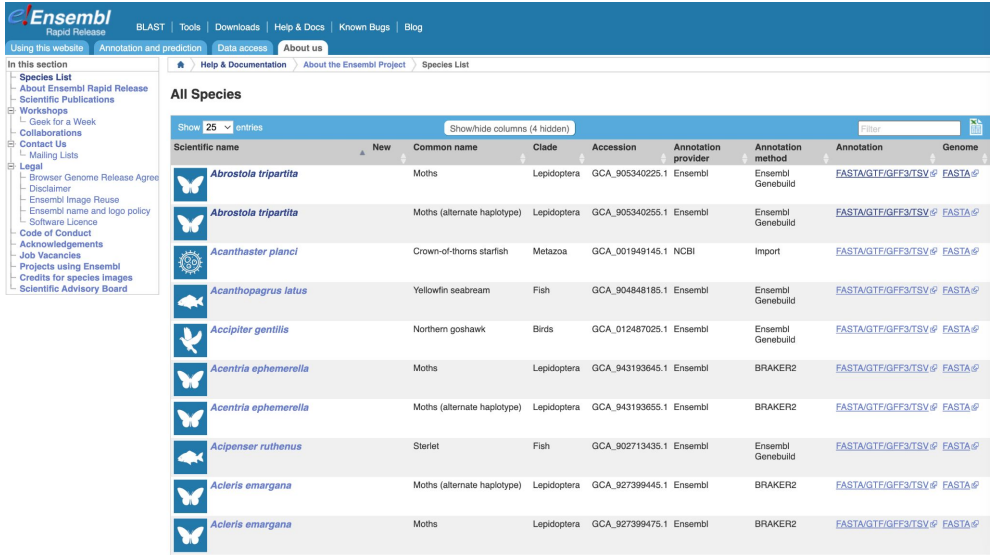
Ensembl Division Sites



Rapid Release (rapid.ensembl.org)

- Runs on a two-week release cycle
- For deploying both verts/non-verts
- >1,700 genomes for >1000 different species since June 2020

- Current functionality
  - Gene annotation
  - Homologues
  - Repeat tracks
  - Protein feature annotation
  - BLAST
  - HAL multiple alignments for certain clades
  - Variation data for human, fruit fly and pigeon pea



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Genome Annotation

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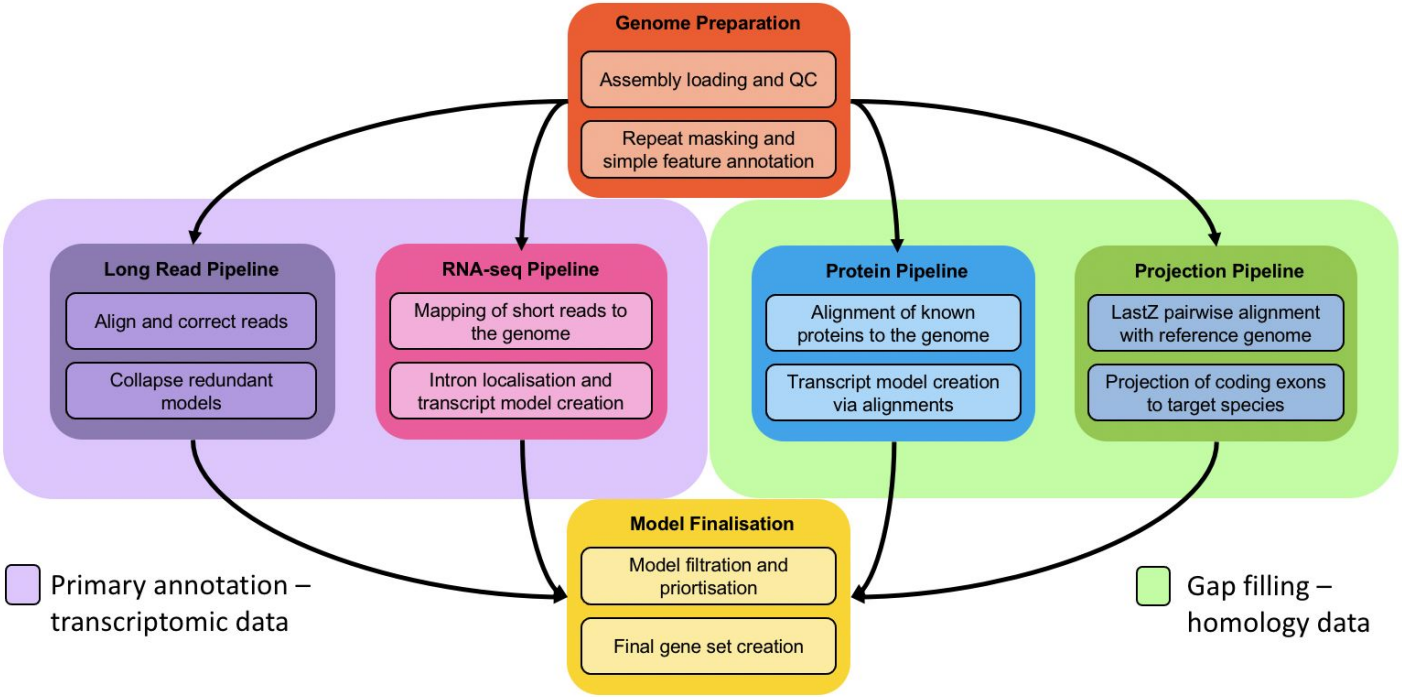
Genome Annotation

- The process of identifying and labelling information on a genome
- Coordinate-based: repeats, genes, transcripts, exons, variants, regulatory regions
- Knowledge-based: gene function, variant effect, repeat type
- Context-based: orthology/paralogy, synteny

Annotation Approaches

Approach	Main strengths	Main weaknesses
Ab initio	• Fast • Only genome required as input	• Large numbers of false positives • Large amounts of missing/spurious exons
Protein-genome alignments	• Fast • Uses existing data • Can give excellent coverage	• No UTR/lncRNAs • Accuracy decreases significantly as evolutionary distance increases • Highly dependent on the proteins available • Doesn't capture novelty
Projection/liftover	• Can be highly accurate even at moderate distances • Can leverage high quality reference annotations	• Expensive if using a full WGA • Doesn't capture novelty • Genes not covered in the WGA not projected
Transcriptomic	• Fast and accurate for finding structures • Captures novel genes and transcripts • Annotation of non-coding elements • Allows for tissue/timepoint-specific tracks • Can be used to help validate structures identified through other approaches	• Difficult to get high coverage of full gene set • Sampling/Cost • Fragmentation • Short read data requires inference of structures

Ensembl Annotation Pipelines



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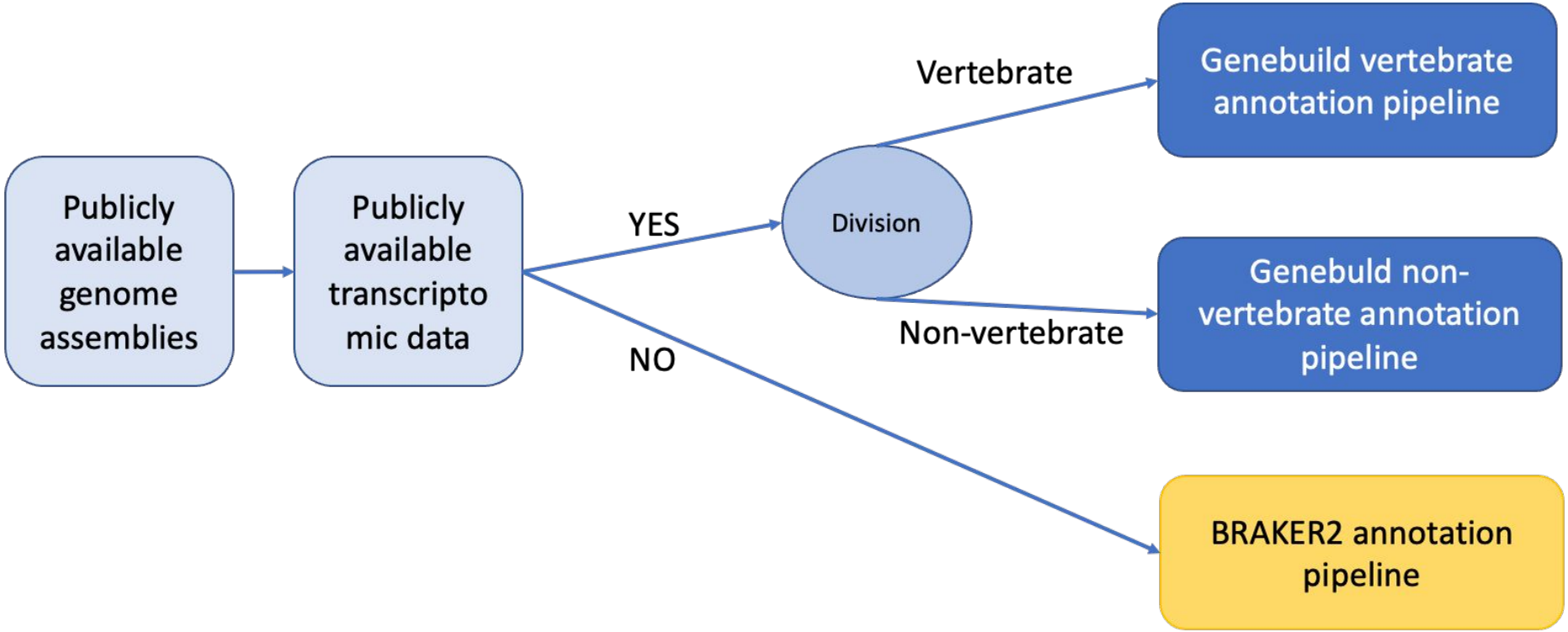
Ensembl Annotation Pipelines



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Repeat Annotation

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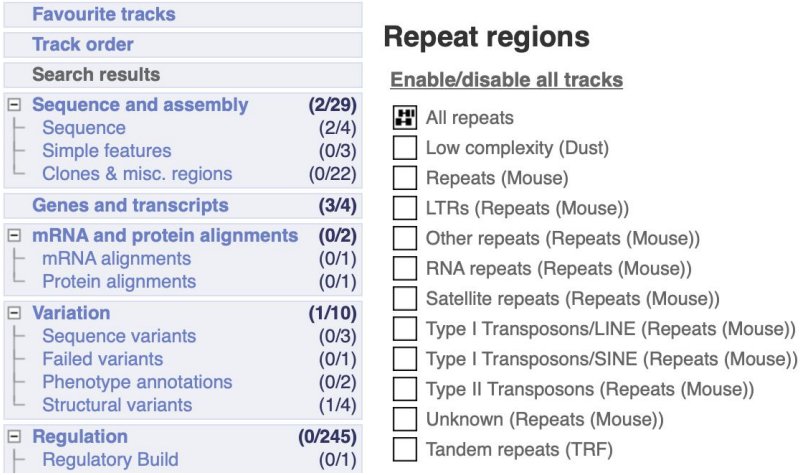
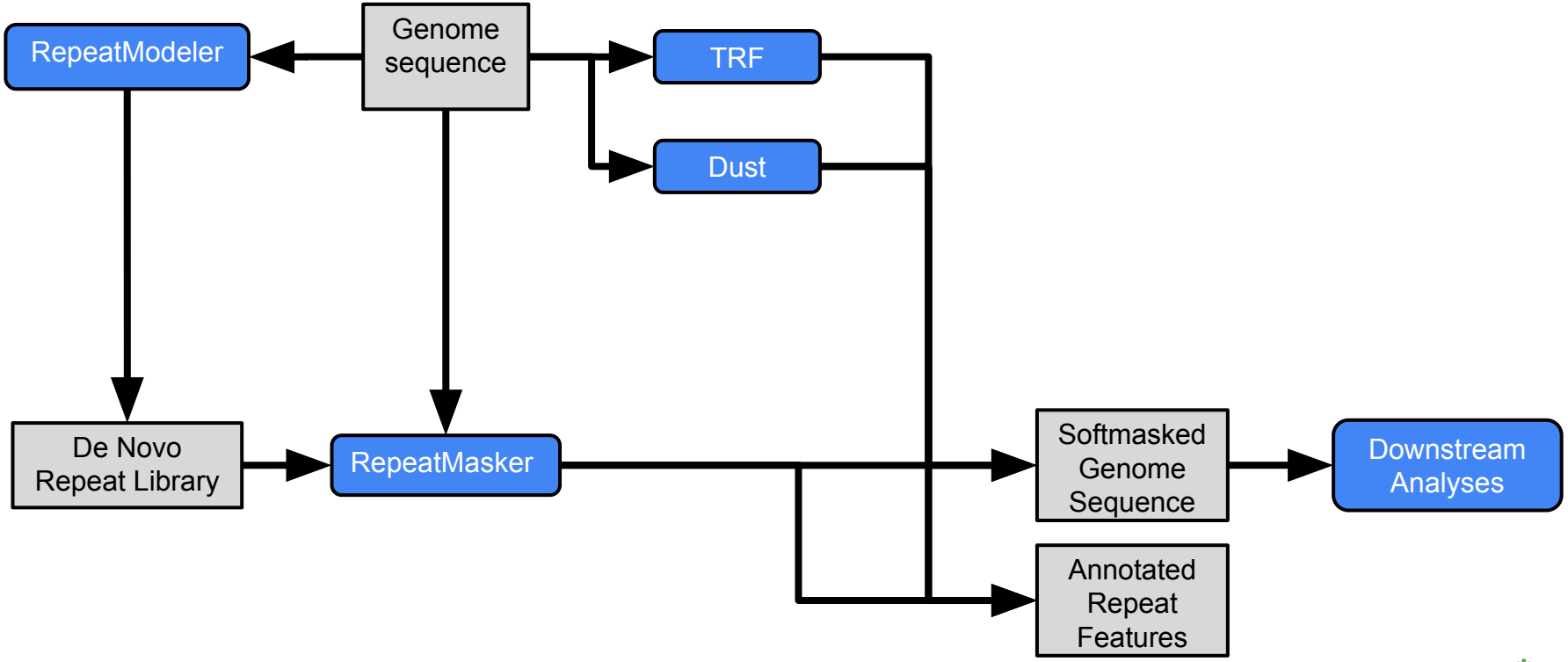
Repeat Annotation

Types of Repeats

- Low complexity regions
  - Poly-purine or poly-pyrimidine stretches
  - Regions of extremely high AT or GC content
- Transposable elements
  - Class I retrotransposons (“copy-and-paste”)
  - Class II DNA transposons (“cut-and-paste”)
- Satellite DNA
  - Short and long tandem repeats



Repeat Annotation



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Repeat Annotation

- Repeat masking an important initial step in genome annotation
- Repeat annotation presents a lot of challenges
  - It is computationally very costly
  - Repeat libraries can sometimes contain gene families
  - Huge volume of software, but only a few long lasting/well supported ones
- Red (REpeatDetector) is extremely efficient tool if masking alone is the goal

Gene Annotation

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Gene Annotation

- Can be broken into a number of approaches:
  - Transcriptomic – using long/short reads
  - Homology – Mapping/lifting data from other species
  - Ab initio – Using HMMs or other predictive methods
  - Hybrid – Using a combination of the above

Gene Annotation
Transcriptomic

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Gene Annotation - *Transcriptomic*

- Two major types of data – **short reads** (usually Illumina) and **long reads** (PacBio Iso-Seq, ONT)

- Short reads generally through two initial phases:
  - Align to genome to find exon positions
  - Reconstruction into potential transcript isoforms

- Long reads have one initial phase and one optional:
  - Align to the genome to find transcript structures
  - Error correcting the identified structures

- After the initial phases both data types have putative transcript models, then usually:
  - Collapse redundant/fragmented models
  - Identify longest ORF
  - Classify ORF as coding/non-coding

Gene Annotation - *Transcriptomic*

Approach	Main strengths	Main weaknesses
Short reads	• Better coverage • Cheap • High accuracy • Can be easier to validate structures from other approaches	• Inferring transcripts, not observing them • UTRs difficult to call
Long reads	• Full length transcript structures • More accurate UTRs • Can be more accurate for spliced lncRNAs	• Lots of single exon transcriptional noise • High error rate for ONT data

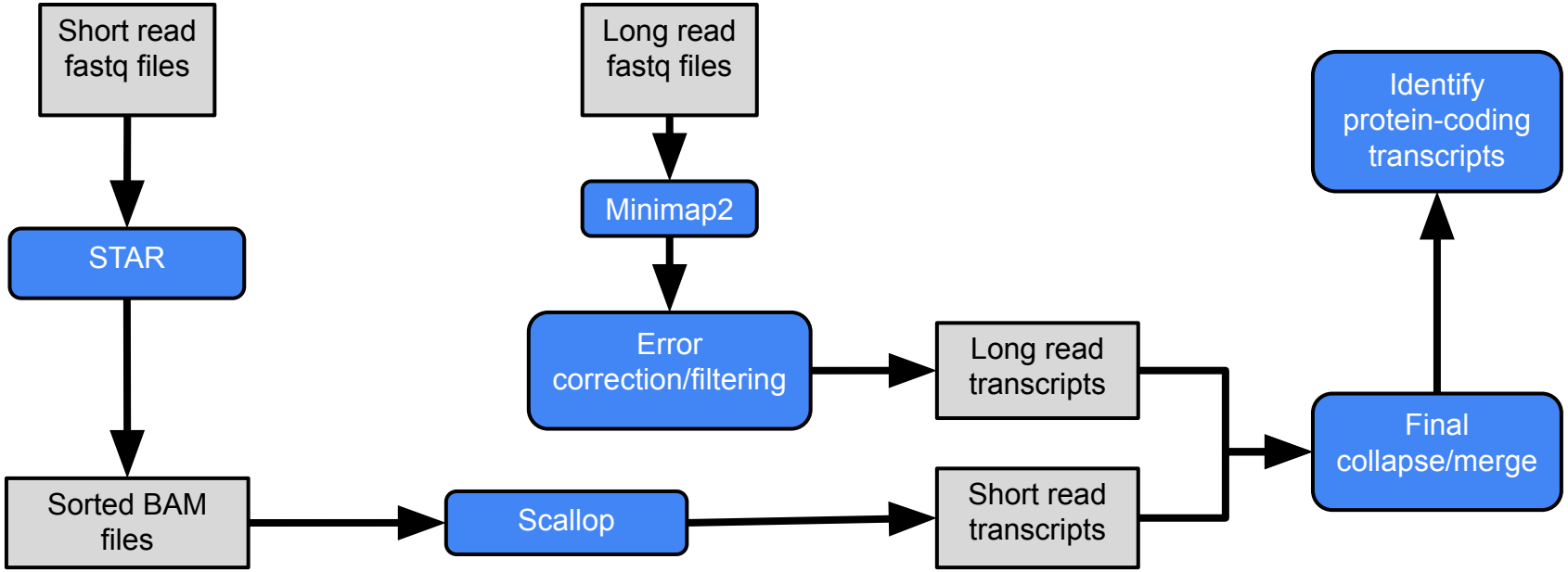
Gene Annotation - *Transcriptomic*

Disclaimer: This applies to vertebrates!!!

- **Minimal**
  - Short read data only
  - Highest value: brain, gonads, lung/gill, embryo
  - Lowest value: liver, muscle, blood
  - 100-150bp
  - 100 million reads+ per tissue

- **Ideal**
  - Short and long read data
  - 5+ tissues
  - Dev stages if possible
  - The more reads the better
  - Preference for consensus/cleaned over raw reads

Gene Annotation - *Transcriptomic*



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Gene Annotation
Homology

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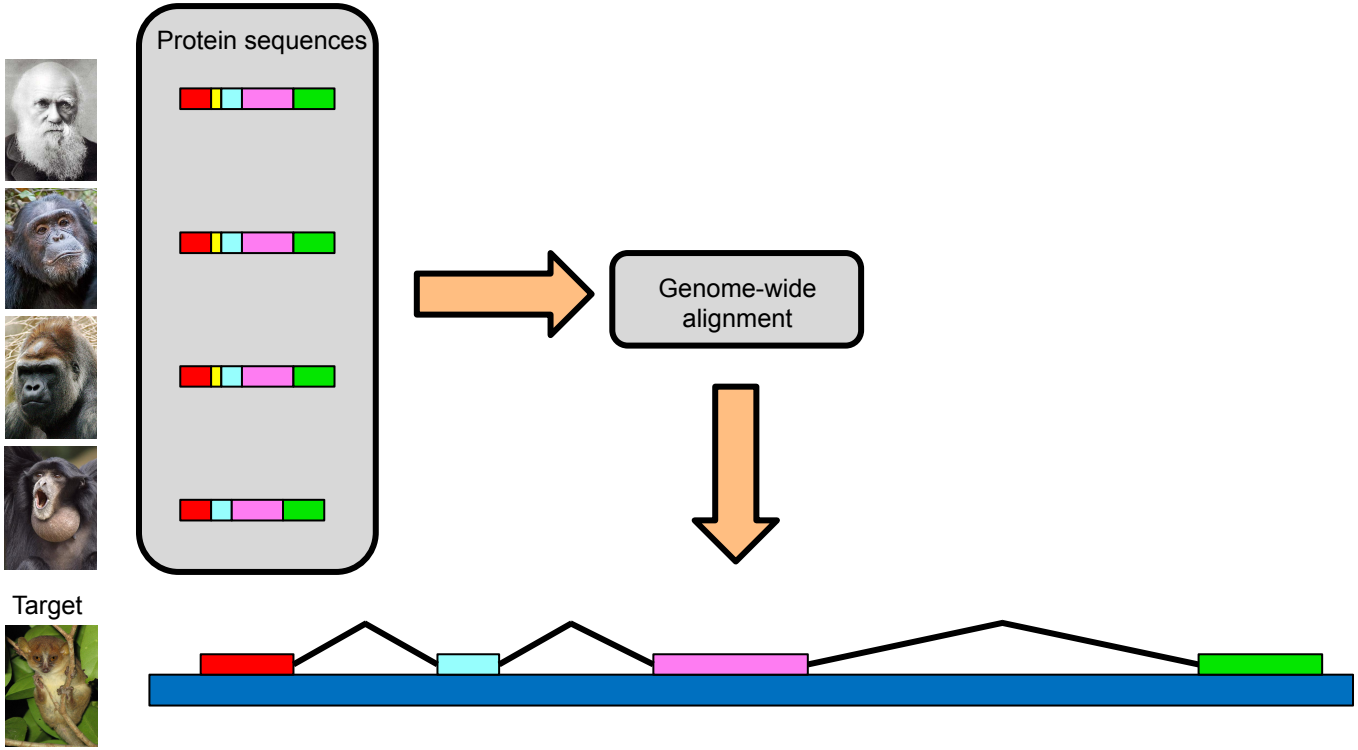
Gene Annotation - *Homology*

- Cross species protein alignments
  - Protein sequences are more conserved than nucleotide sequences
  - There are vast numbers of available protein sequences, with varying levels of evidence

Cross Species Protein Alignments



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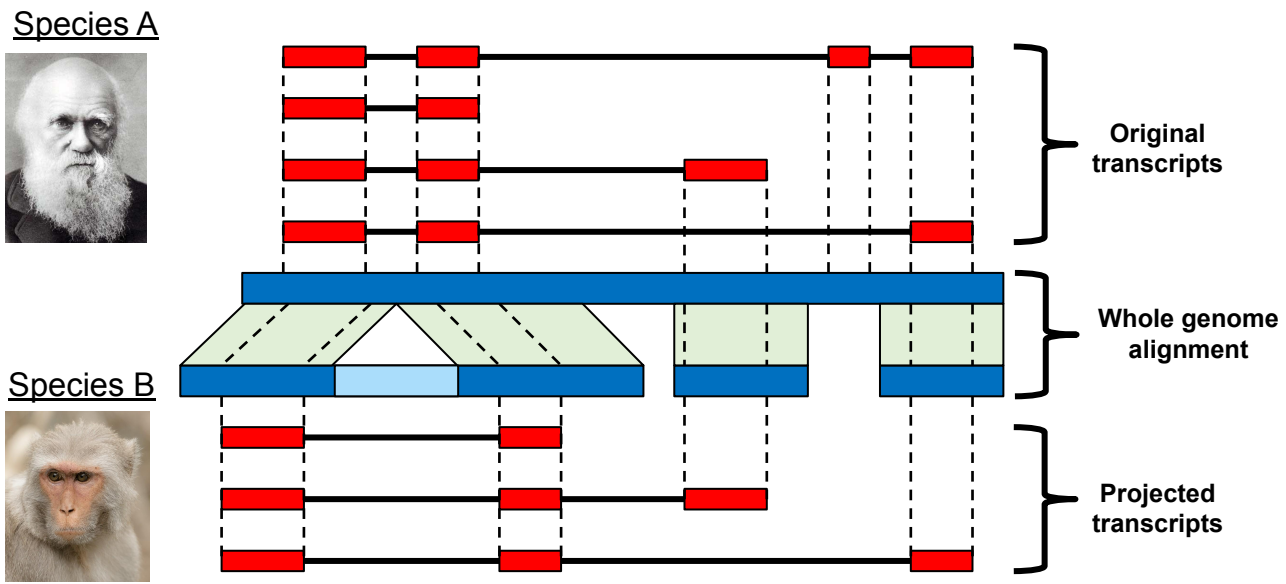
Gene Annotation - *Homology*

- Projection from a reference
  - Projection/liftover methods generally are based off a whole genome alignment
  - Generally involve high quality reference assembly and annotation being projected to a target assembly
  - Some methods focus on breeds/strains/haplotypes, and allow projection of coding and non-coding features
  - Others focus on only the coding exons to allow projection across greater evolutionary distance

Projection From a Reference



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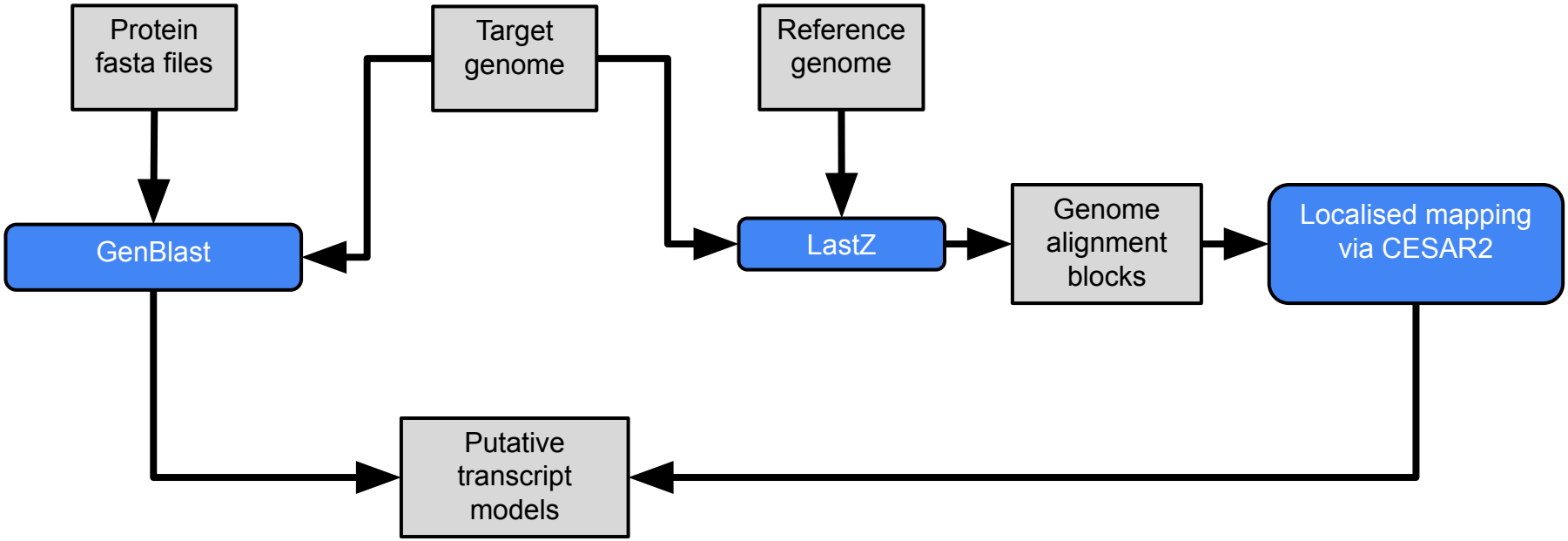
Gene Annotation - *Homology*



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Gene Annotation
Combined Evidence

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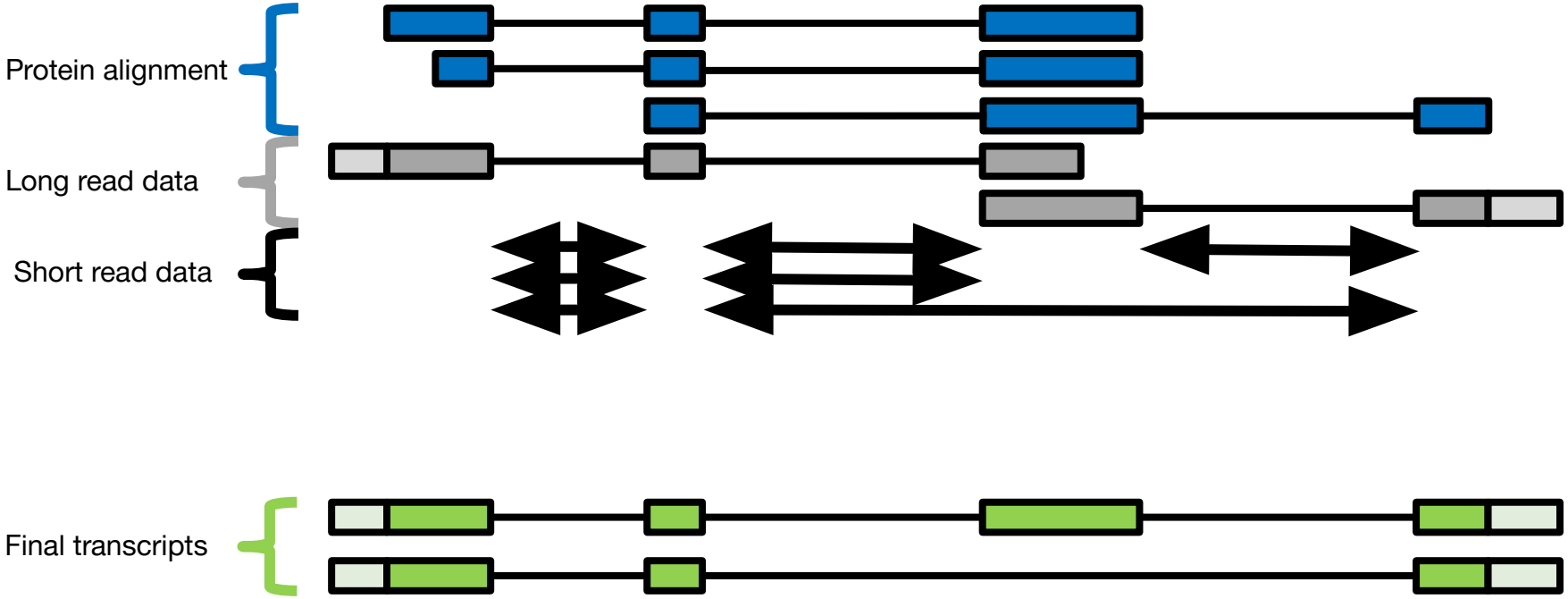
Gene Annotation - *Combined Evidence*



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Gene Annotation - *Combined Evidence*

- Data rich:
  - More transcript isoforms
  - More genes captured per class, including timepoint/tissue specific ones
  - UTR features captured
  - Better exon capture, especially around start/end exons
  - Ability to measure confidence of the gene set

- Data poor:
  - Often focused on protein coding genes
  - Generally one isoform per gene
  - Often missing exons/misalignments of start/end exons
  - No UTRs
  - False positives

Gene Annotation
Assessing Quality

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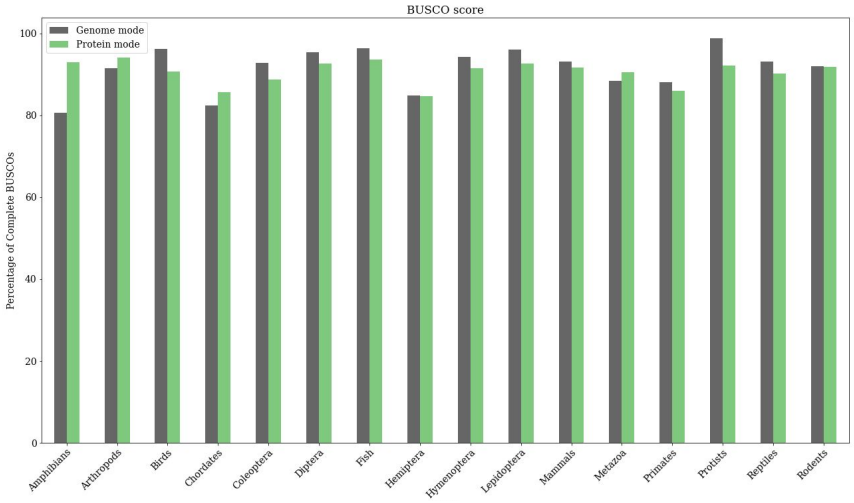
Gene Annotation - *Assessing Quality*

- Assessing the quality of a gene set can be difficult
- The closer to a well annotated reference, the easier the assessment
- Things to look for:
  - One-to-one orthologues (or reciprocal best BLAST hits) with references
  - Long/split/orphan gene counts
  - Average coding exons/genomic span/CDS length
  - BUSCO/ OMArk completeness for most appropriate taxonomic group

Gene Annotation - *Assessing Quality*

BUSCO

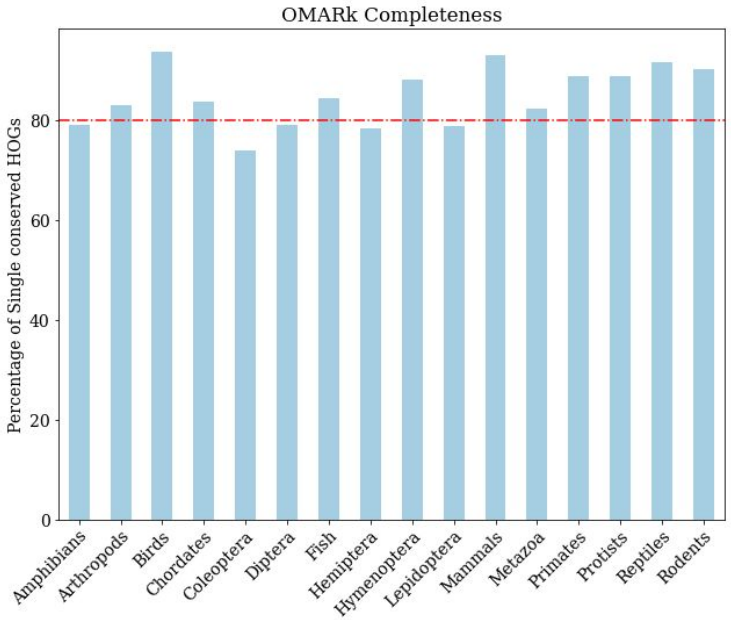
- A measure for quantitative assessment of genome assembly and annotation completeness based on evolutionarily informed expectations of gene content.
- Based on the concept of single-copy orthologs that should be highly conserved among the closely related species



Gene Annotation - *Assessing Quality*

OMArk

- Estimate the proteome completeness by comparison to conserved orthologous groups defined using Hierarchical Orthologous Groups (HOGs).
- Estimate the proportion of accurate and erroneous gene models in the proteome by comparing to the known gene families of the selected ancestral lineage
- Detect possible contamination from other species in the proteome.



Summary

- **Repeat annotation** is usually the first step in genome annotation
- Many sources of repeat libraries and tools, but RepeatModeler and RepeatMasker combo is most popular approach

- Gene annotation can be done with a variety of approaches, with **transcriptomic data** being most valuable

- The **quality of annotation** will always be dependent on the quality of the input data used
- Important to take into account the quality for planning downstream analyses

The Eukaryotic Annotation Team

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