Ensembl
Eukaryotic Genome Annotation

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Eukaryotic Annotation Team

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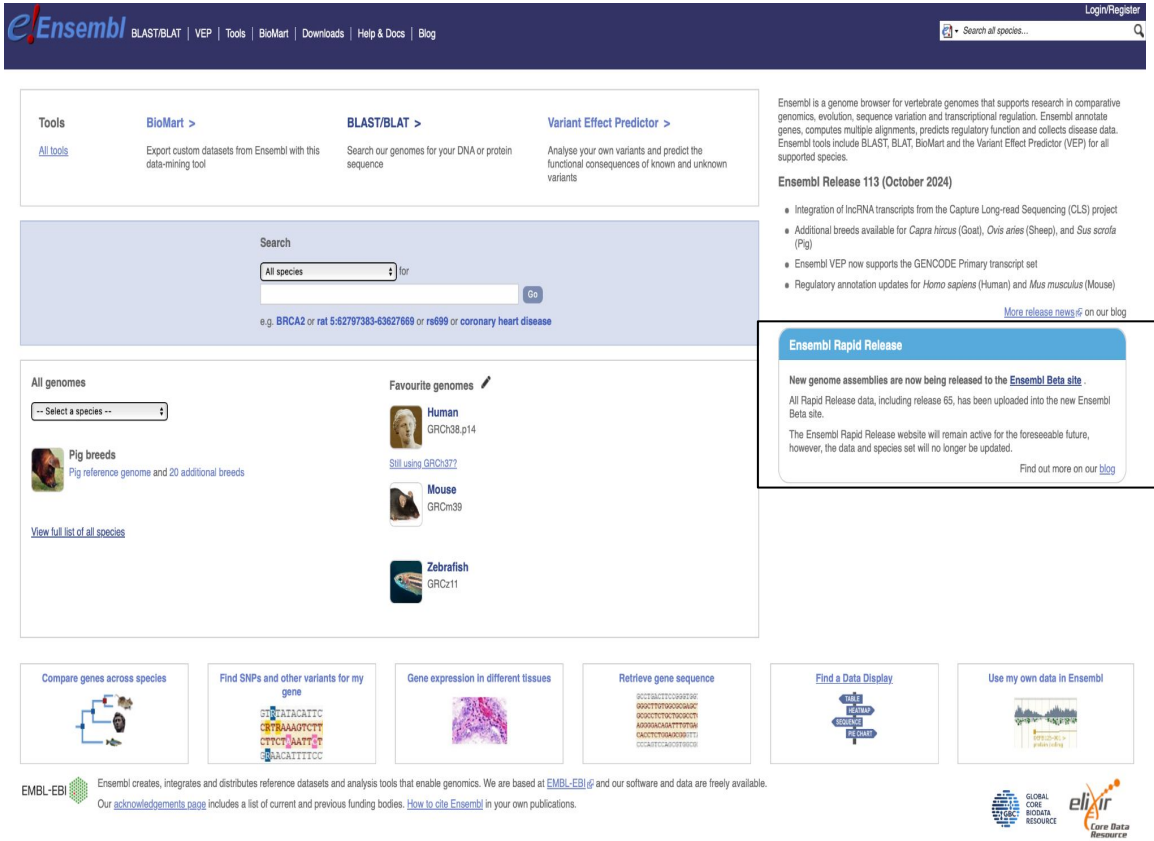
What is Ensembl?

The Ensembl project has following major goals:

1. Provide a comprehensive source of stable genome annotations
2. Enable genomic interpretation
3. Support researcher driven analysis by provide data via FTP, REST/Perl API, MySQL dumps, BioMart



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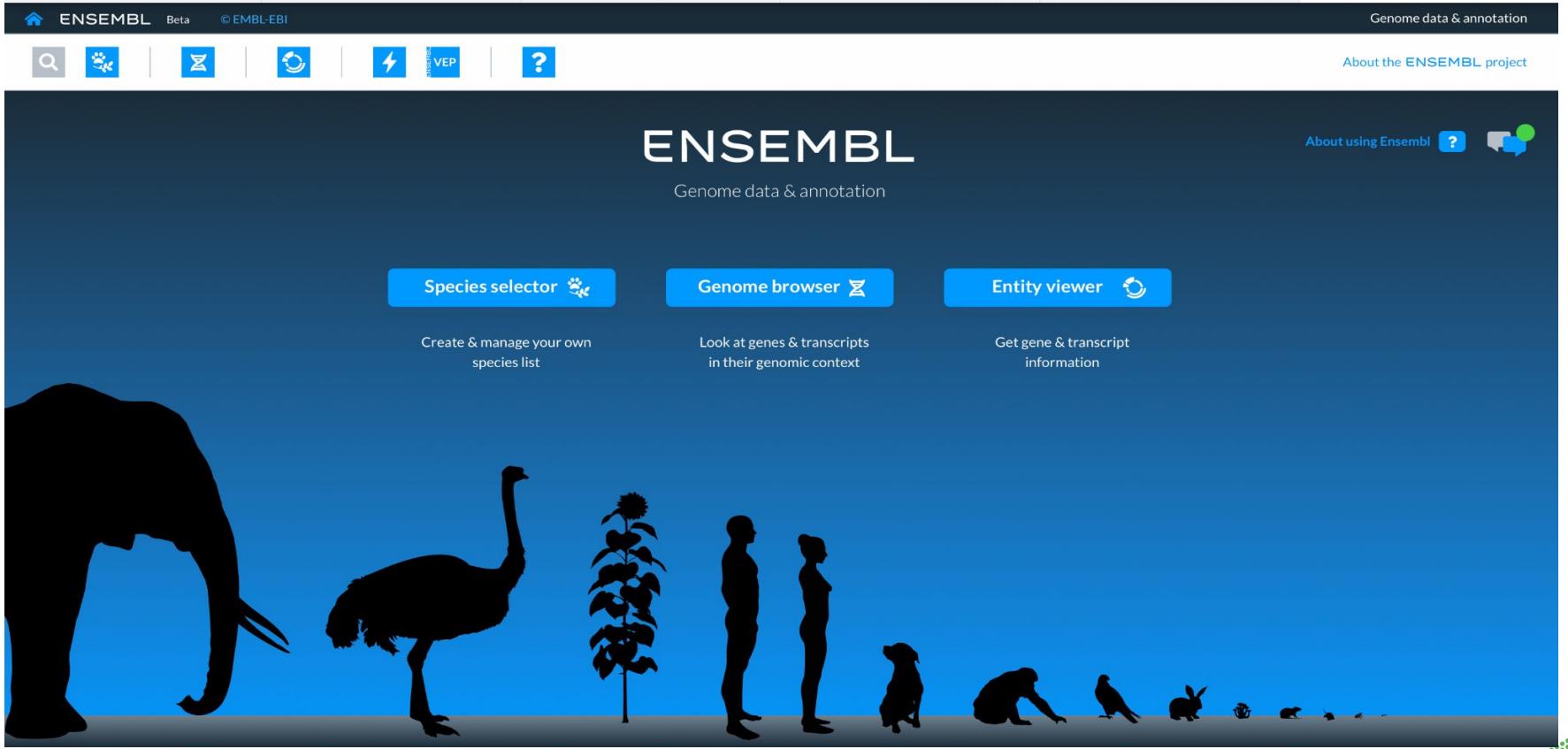
What is Ensembl?

The new [Ensembl Beta site]



Useful links to Ensembl Outreach training

- The training site (https://training.ensembl.org/)
- The training material can be found here (https://training.ensembl.org/events/upcoming/)
- Ensembl workshops and hosting details (https://training.ensembl.org/hosting).
- Keep up to date on virtual open workshops using
    - Bluesky (https://bsky.app/profile/ensembl.bsky.social)
    - LinkedIn (https://www.linkedin.com/company/ensemblgenomebrowser/)

Genome Annotation

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Genome Annotation

Definition: Identifying and labeling genomic features to understand structure and function.

🧬 Coordinate-Based Annotation:

- Defines **physical locations** of genomic elements.
- Includes **repeats, genes, transcripts, exons, variants, regulatory regions**.

📖 Knowledge-Based Annotation:

- Assigns **biological meaning** to genomic features.
- Includes **gene function, variant effect, repeat classification**.

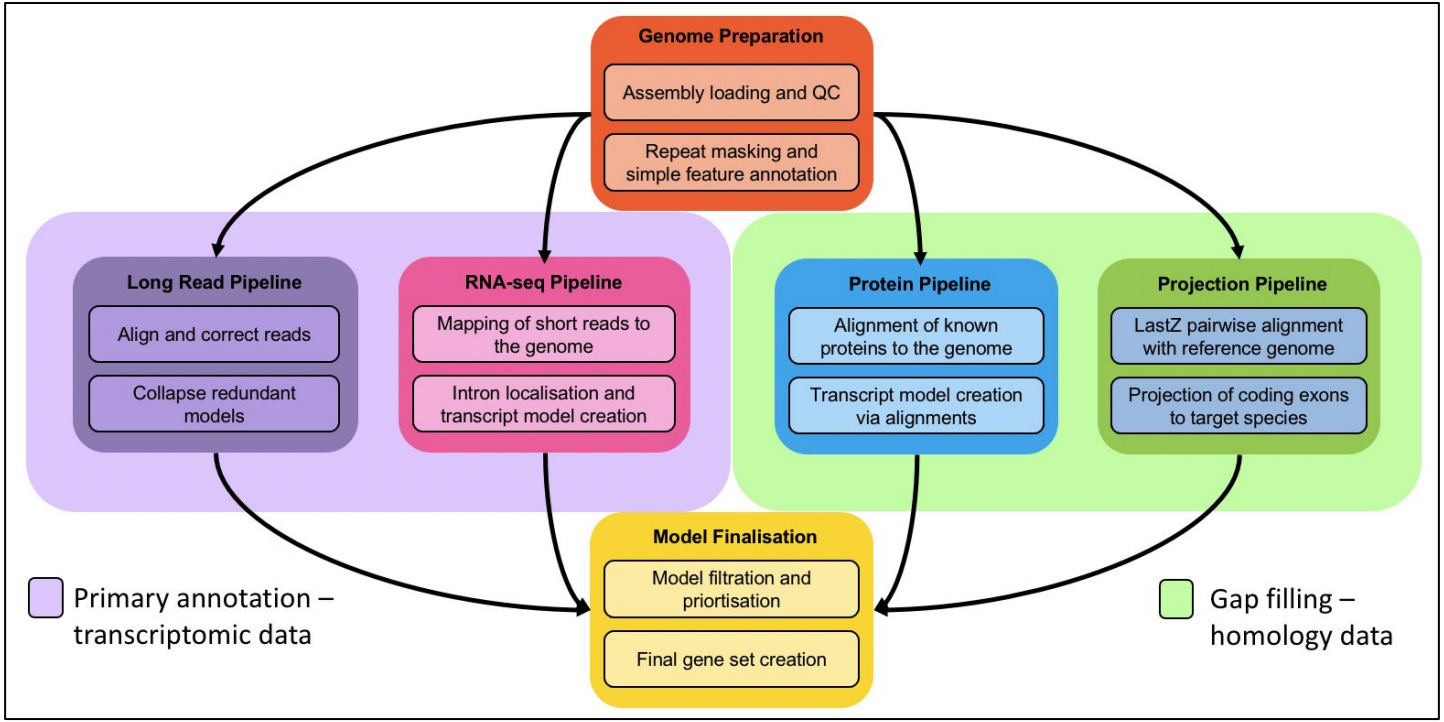
🌍 Context-Based Annotation:

- Uses **comparative genomics** to analyze evolutionary relationships.
- Includes **orthology/paralogy, synteny**.

Approaches for Genome Annotation

Annotation Method	Pros	Cons
Ab-initio Prediction	✅ No prior knowledge needed ✅ Works for novel genomes	❌ High false positives ❌ Struggles with complex genes & splicing
Protein-to-Genome Alignment	✅ High accuracy for conserved genes ✅ Uses existing protein data	❌ Misses novel genes ❌ Poor performance in divergent species
Projection & Liftover	✅ Fast for well-annotated genomes ✅ Good for closely related species	❌ Limited to known annotations ❌ Cannot detect novel genes
Transcriptomic Annotation	✅ Identifies expressed genes accurately ✅ Captures UTRs & isoforms	❌ Misses non-expressed/low-expression genes ❌ Requires high-quality RNA-seq

Ensembl Annotation Pipeline



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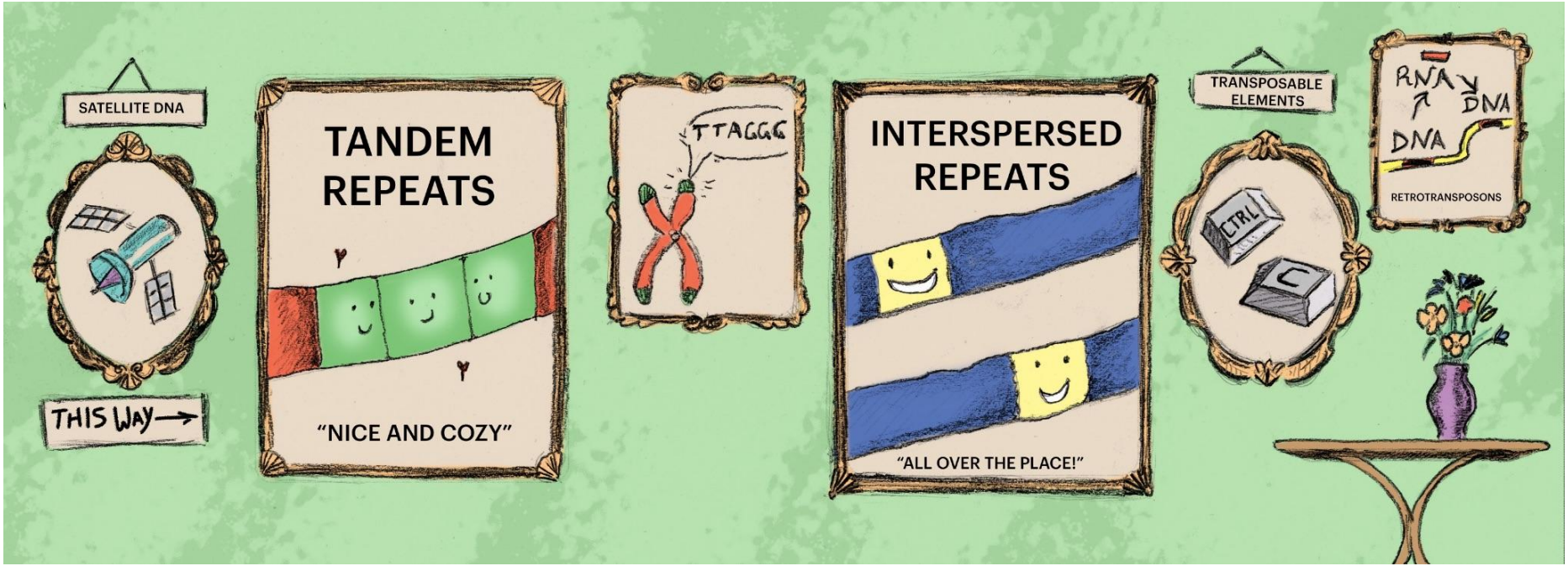

Repeat Annotation

Source of Image

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Types of Repeats & Importance of Repeat Annotation

Types of Repeats

- **Low Complexity Regions** – Poly-purine/pyrimidine stretches, extreme AT/GC content
- **Transposable Elements**
  - **Class I** (Retrotransposons: LINEs, SINEs, LTRs)
  - **Class II** (DNA Transposons: TIRs, MITEs, Helitrons)
- **Satellite DNA** – Short & long tandem repeats

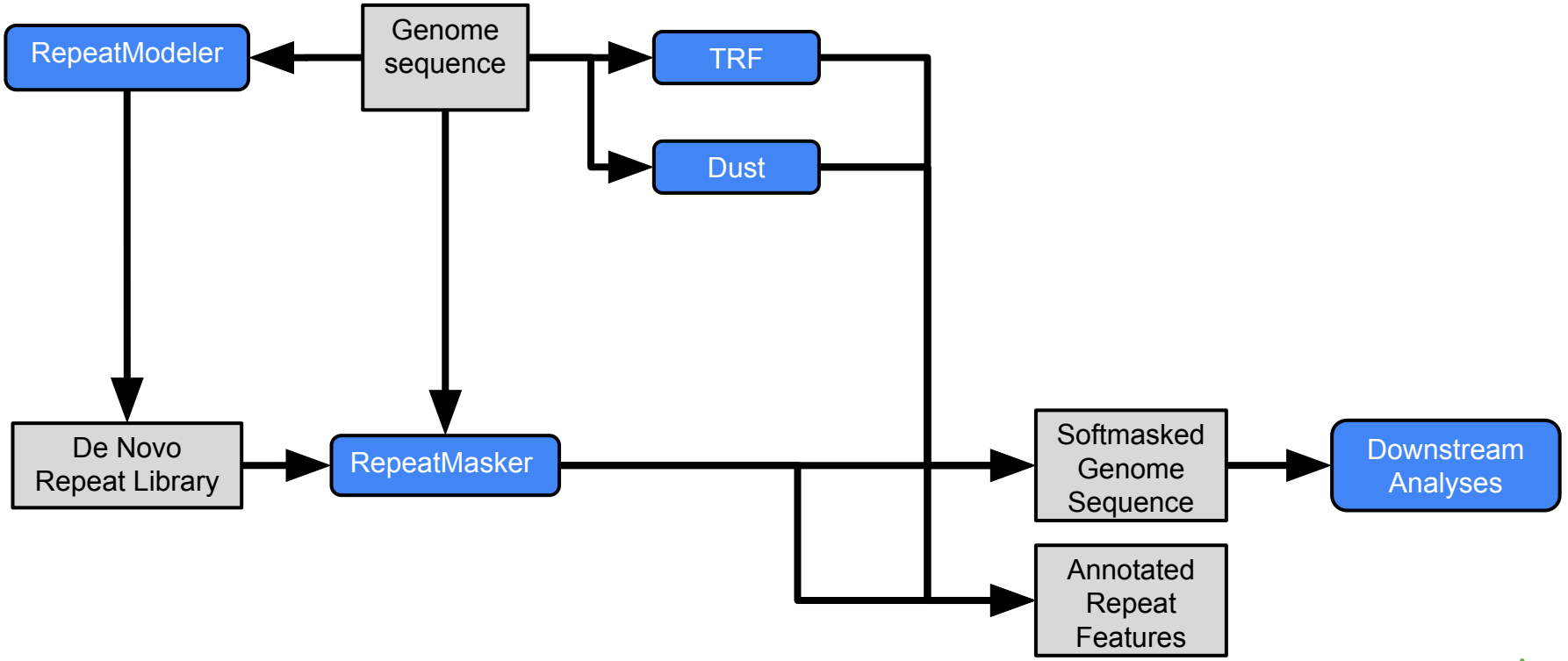
Why Annotate Repeats?

★ **Prevents Spurious Alignments** – Reduces false gene annotations

★ **Optimizes Computational Resources** – Improves efficiency of downstream analysis

★ **Reveals Evolutionary Insights** – Helps study genome plasticity & regulatory evolution

Repeat Annotation



Red (REpeatDetector) – Extremely efficient for repeat masking tasks

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Repeat Annotation

Importance of Repeat Masking

- **Critical Initial Step** – Prevents spurious alignments and false gene annotations
- **Improves Annotation Accuracy** – Essential for downstream genomic analyses

Challenges in Repeat Annotation

- **Computationally Expensive** – High resource demand for large genomes
- **Complex Libraries** – Some repeat libraries may include gene families, complicating annotation
- **Vast Software Landscape** – Numerous tools, but only a few are well-supported and long-lasting

Gene Annotation

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Gene Annotation

Ab Initio Annotation

- **Predicts Genes** based on genomic sequence
    - Uses **Hidden Markov Models (HMMs)** or other predictive algorithms

Homology-Based Annotation

- **Maps or Lifts Data** from well-annotated genomes of related species
    - Relies on **sequence similarity** for functional predictions

Transcriptomic Annotation

- **Utilizes RNA-Seq Data**
    - Uses data from **long-read** or **short-read** sequencing technologies

Hybrid Annotation

- **Combines Methods** – Merges **transcriptomic, homology, and ab initio** approaches for enhanced accuracy

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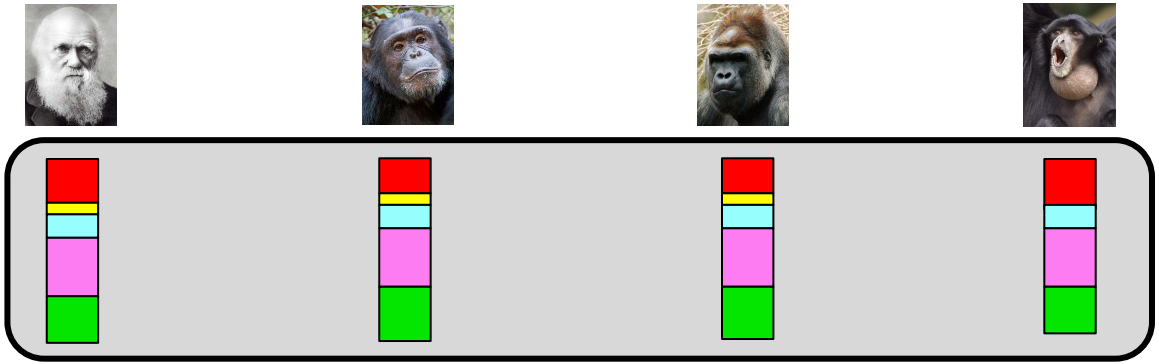
Cross Species Protein Alignments



Genome-wide alignment

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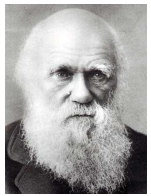
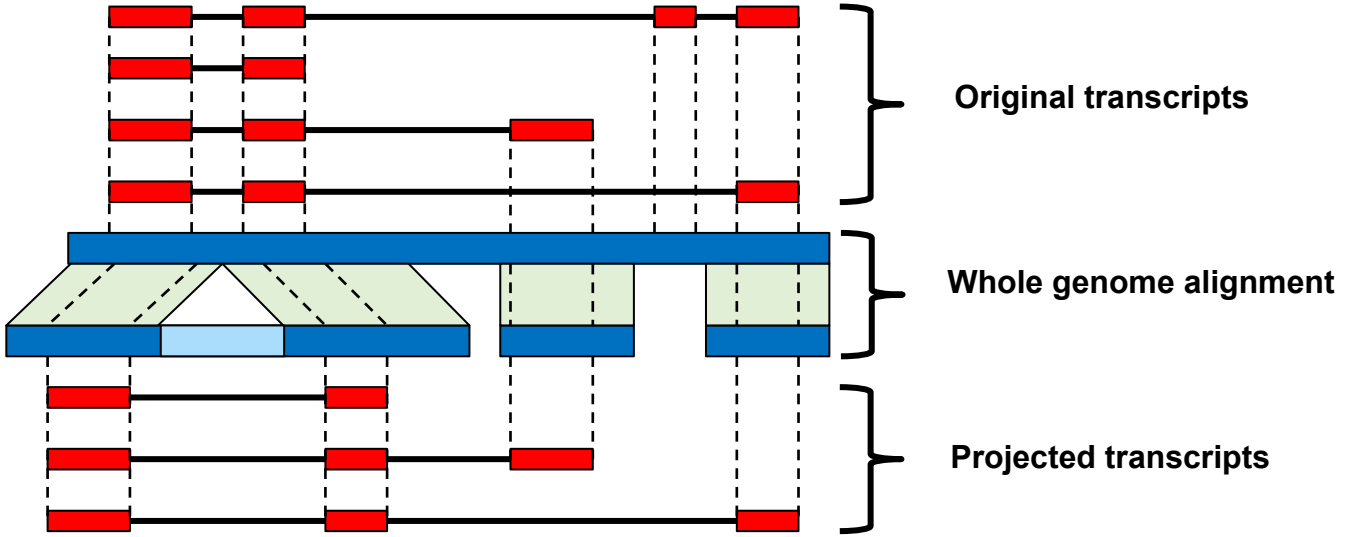
Projection From a Reference

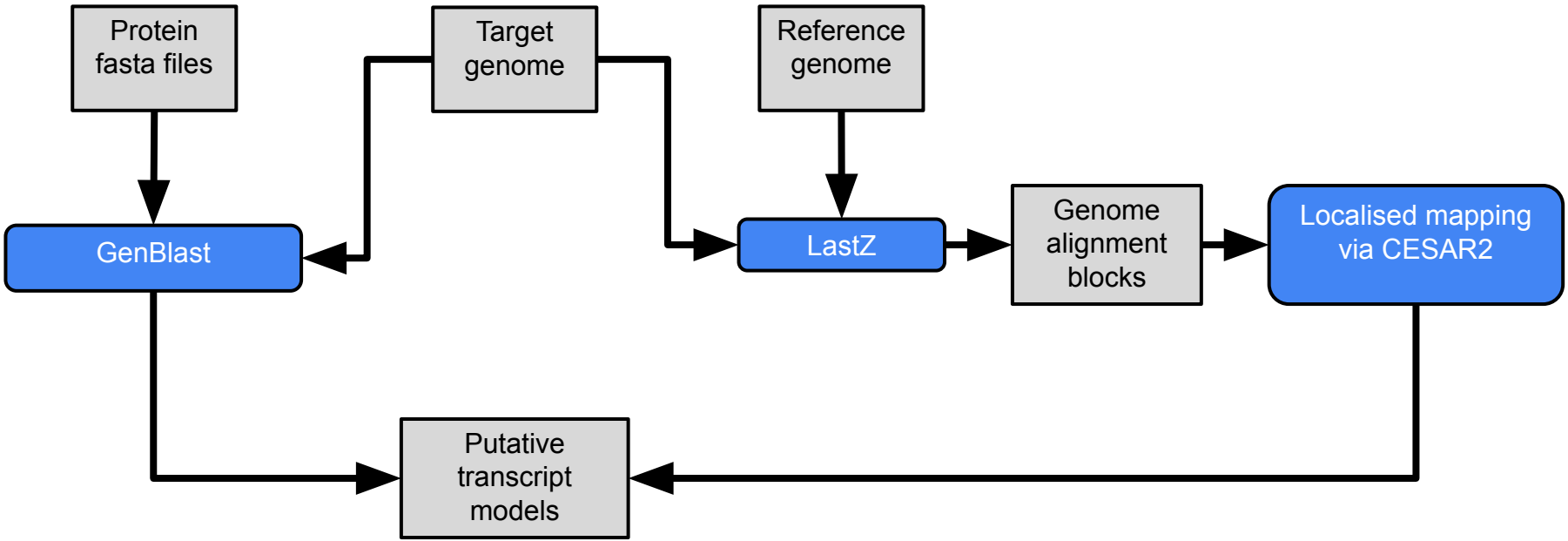


Species A

Species B



Homology based Gene Annotation



Another fast and efficient protein to genome alignment method is **Miniprot**.

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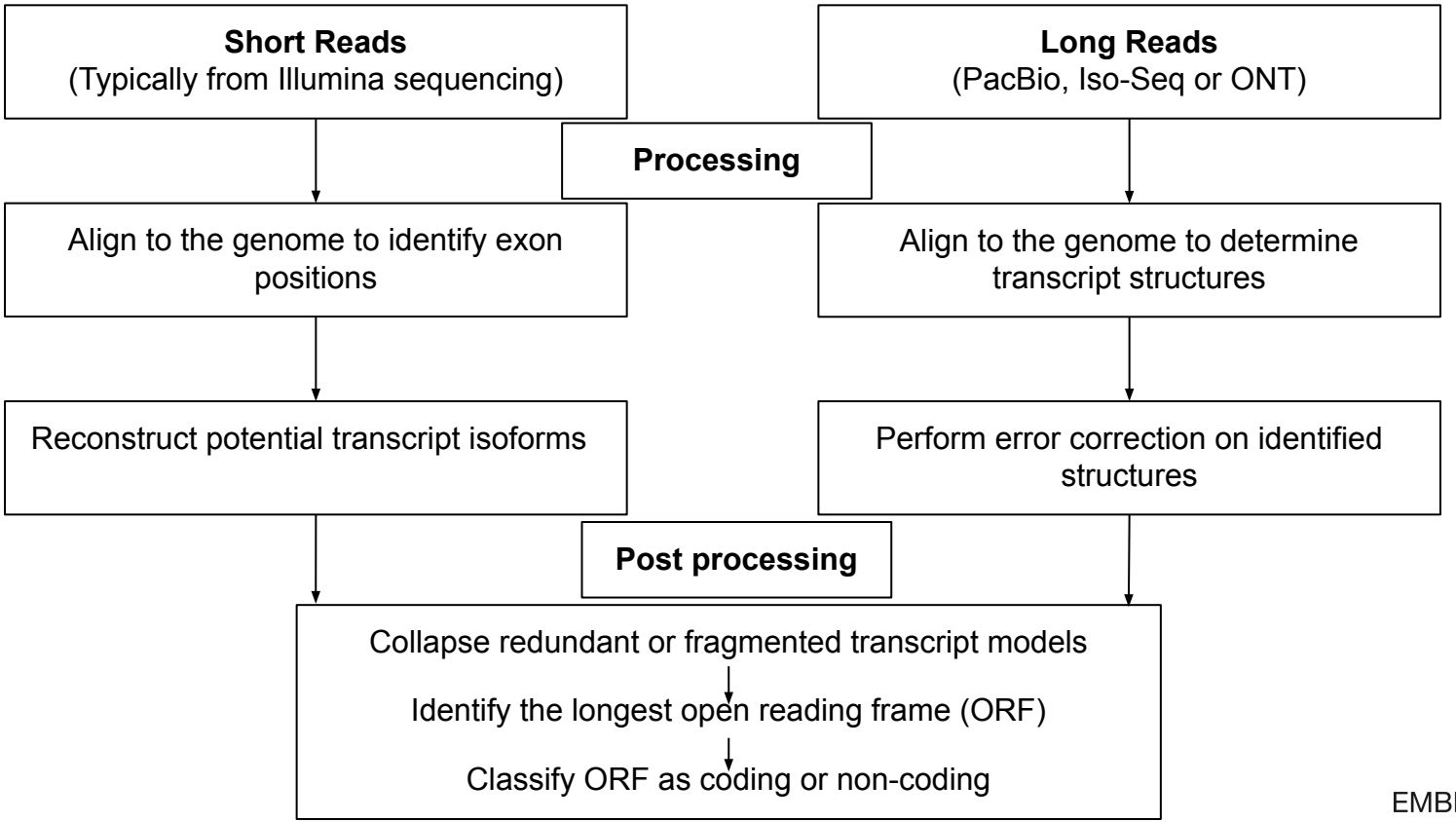
Transcriptomic based Gene Annotation



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Transcriptomic based Gene Annotation

Approach	Strengths	Weaknesses
Short Reads	• High accuracy with low error rates • Cost-effective for large-scale sequencing • Generates high-depth coverage	• Short length makes isoform reconstruction difficult • Struggles with repetitive and GC-rich regions • Difficult to call UTRs • More reliant on transcript assembly algorithms
Long Reads	• Captures full-length transcripts and complex isoforms • Resolves repetitive and structural variants • Less reliance on transcript assembly algorithms	• Higher error rates, requiring error correction • More expensive and lower throughput compared to short reads • Requires more DNA input for high-quality results

Transcriptomic based Gene Annotation

Minimal vs. Ideal Scenarios for input transcriptomic data

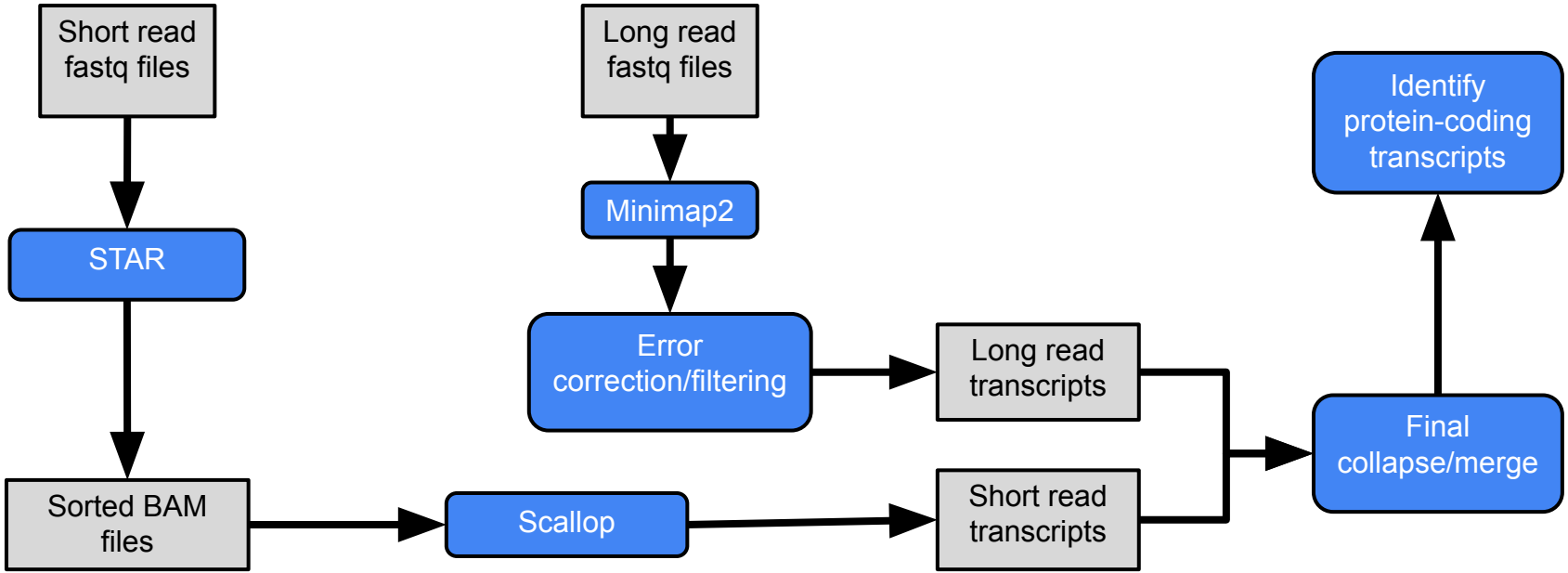
Minimal Scenario (Short Reads Only)

- **Tissues with Highest Value:** Brain, gonads, lung/gill, embryo
- **Tissues with Lowest Value:** Liver, muscle, blood
- **Read Length:** 100–150 bp
- **Coverage:** 100+ million reads per tissue

Ideal Scenario (Short + Long Reads)

- **Diverse Tissues:** At least 5+ tissues
- **Developmental Stages:** If available
- **Read Length:** 100–150 bp (short reads), 10–30 kb+ (long reads)
- **Coverage:** Deep sequencing preferred (e.g., 200M+ short reads, high-depth long reads)
- **Data Quality:** Preference for consensus/cleaned reads over raw data

Transcriptomic based Gene Annotation



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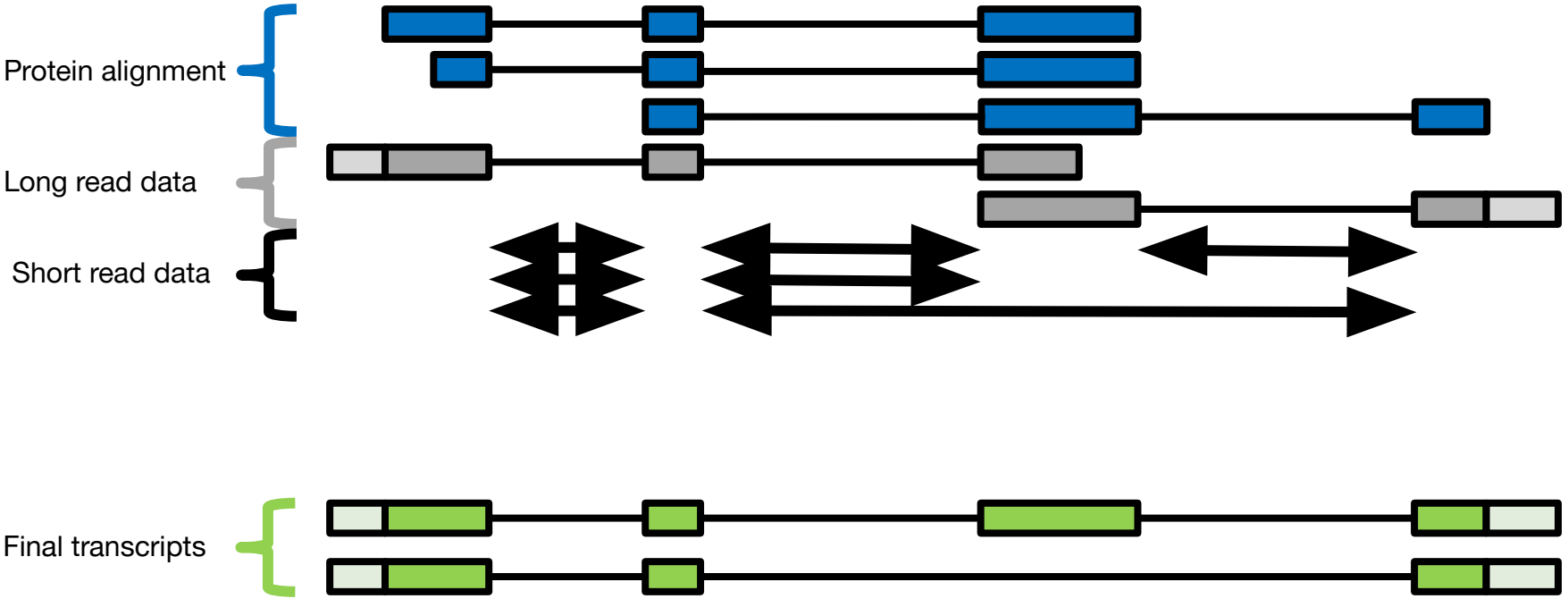

Hybrid Gene Annotation

- **Enhanced Accuracy** – Reduces false positives and improves gene model predictions.
- **Comprehensive Gene Discovery** – Identifies both conserved (homology) and novel (transcriptomics) genes.
- **Better Isoform & UTR Prediction** – Transcriptomics helps define **alternative splicing** and **5'/3' UTR regions**, improving gene structure resolution.
- **Improved Functional Annotation** – Homology provides gene function insights, while transcriptomics validates expression.
- **Robust Annotation in Low-Quality Genomes** – Compensates for incomplete references using expression and conservation data.

Genome Annotation: Assessing Quality

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Genome Annotation - Assessing Quality

Challenges

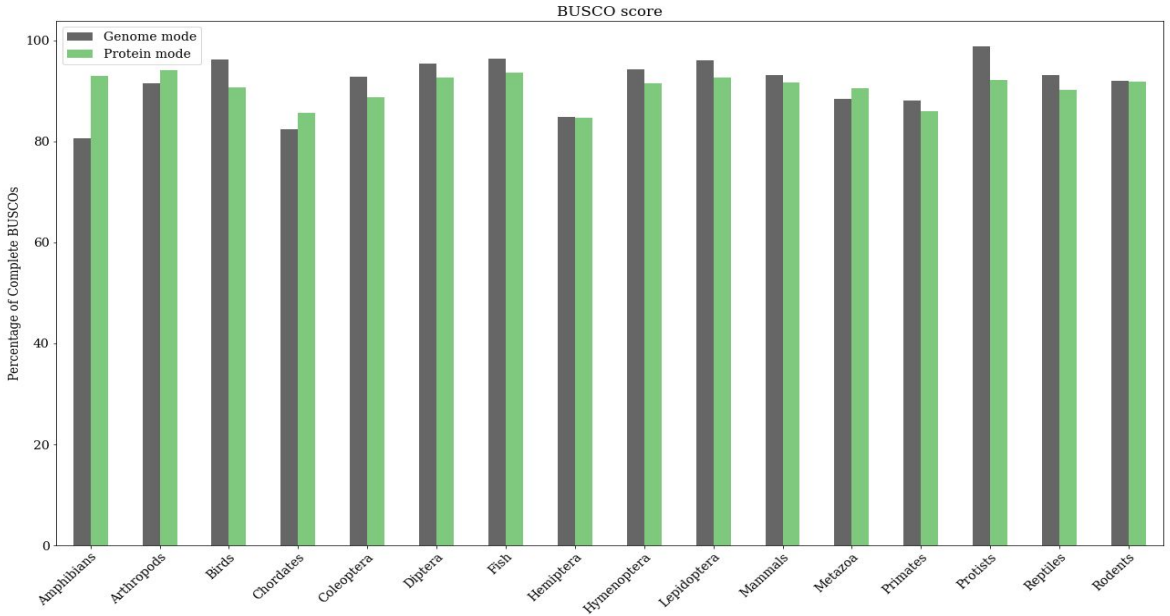
- Evaluating gene set quality is complex.
- Easier when comparing against a well-annotated reference genome.

Key Quality Metrics	
Orthology Analysis	One-to-one orthologs or reciprocal best BLAST hits with reference genomes.
Gene Structure Metrics	Long genes, split genes, orphan gene counts
Exon & CDS Statistics	Average coding exons per gene, genomic span, and CDS length.
Completeness Metrics	**BUSCO/OMArk** scores for assessing annotation quality within the appropriate taxonomic group.
Functional Annotation Coverage	Percentage of genes with GO terms, Pfam domains, or known functional annotations.

Genome 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



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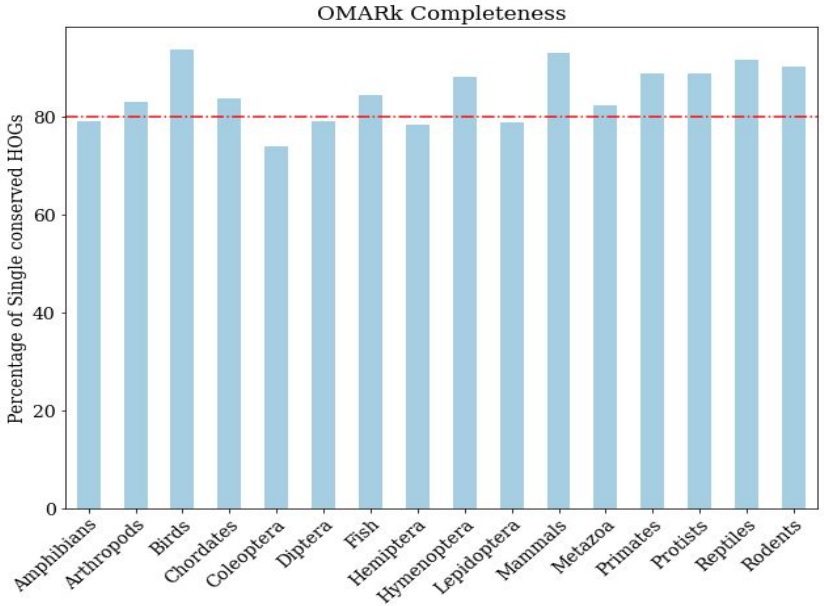
Genome Annotation - Assessing Quality

OMArk

- Estimate the proteome completeness by comparison to conserved orthologous groups
- 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.



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Summary

- **Repeat Annotation Comes First** – Essential to prevent false alignments and improve gene prediction accuracy.
    - **Popular Tools** – RepeatModeler + RepeatMasker is the most widely used approach.
- **Gene Annotation Methods** – Transcriptomic data is the most valuable for accurate gene predictions.
- **Quality Depends on Input Data** – Better sequencing depth and accuracy lead to more reliable annotations.
- **Impacts Downstream Analyses** – High-quality annotation is crucial for functional studies and comparative genomics.

The Eukaryotic Annotation Team

The Automated Annotation Team

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Bioinformatician

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National Human Genome Research Institute

Co-funded by the European Union

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