Genome Annotation

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Objectives:

1. Understand steps involved in genome annotation
2. Demonstrate the use of data and tools that can be used in genome annotation
3. Learn how to QC genome assemblies and annotations
4. Understand how to derive functional predictions for genes

Goals of genome annotation

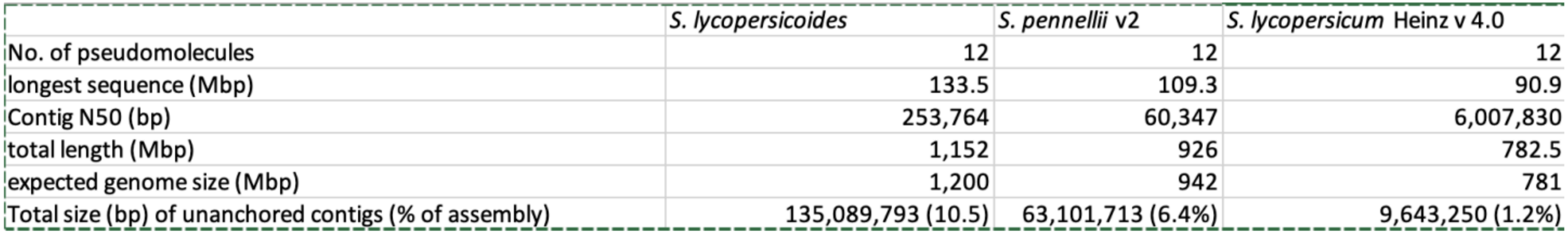
1. Predict, categorize, and mask repetitive elements
2. Determine gene structures as accurately as possible
3. Predict putative functions of predicted genes
4. Associate gene ontology terms, domains, etc for downstream analyses

Annotation Workflow Overview

1. Assembly QC - is it good enough to annotate?
2. Structural annotation - tools, input, outputs
3. Annotation QC - are we capturing most of the gene models accurately?
4. Functional annotation - tools, input, outputs

1. Assembly QC

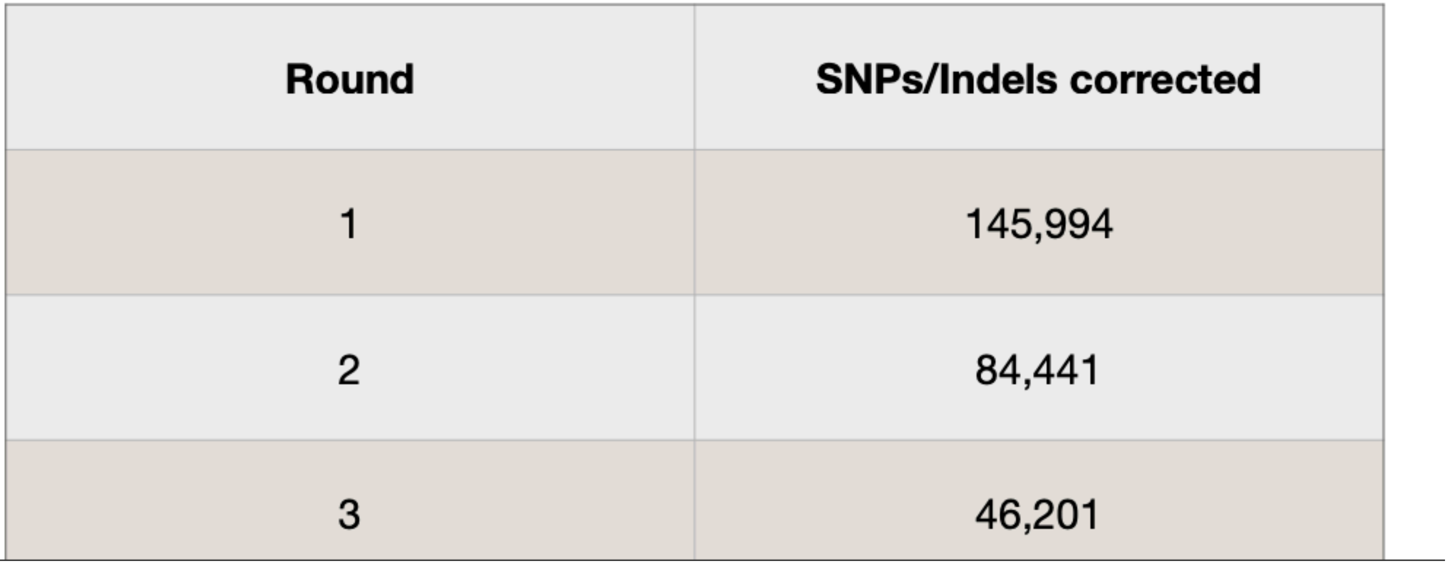
- Assembly quality (total length, N50, etc)



	S. lycopersicoides	*S. pennellii* v2	*S. lycopersicum* Heinz v 4.0
No. of pseudomolecules	12	12	12
longest sequence (Mbp)	133.5	109.3	90.9
Contig N50 (bp)	253,764	60,347	6,007,830
total length (Mbp)	1,152	926	782.5
expected genome size (Mbp)	1,200	942	781
Total size (bp) of unanchored contigs (% of assembly)	135,089,793 (10.5)	63,101,713 (6.4%)	9,643,250 (1.2%)

1. Assembly QC

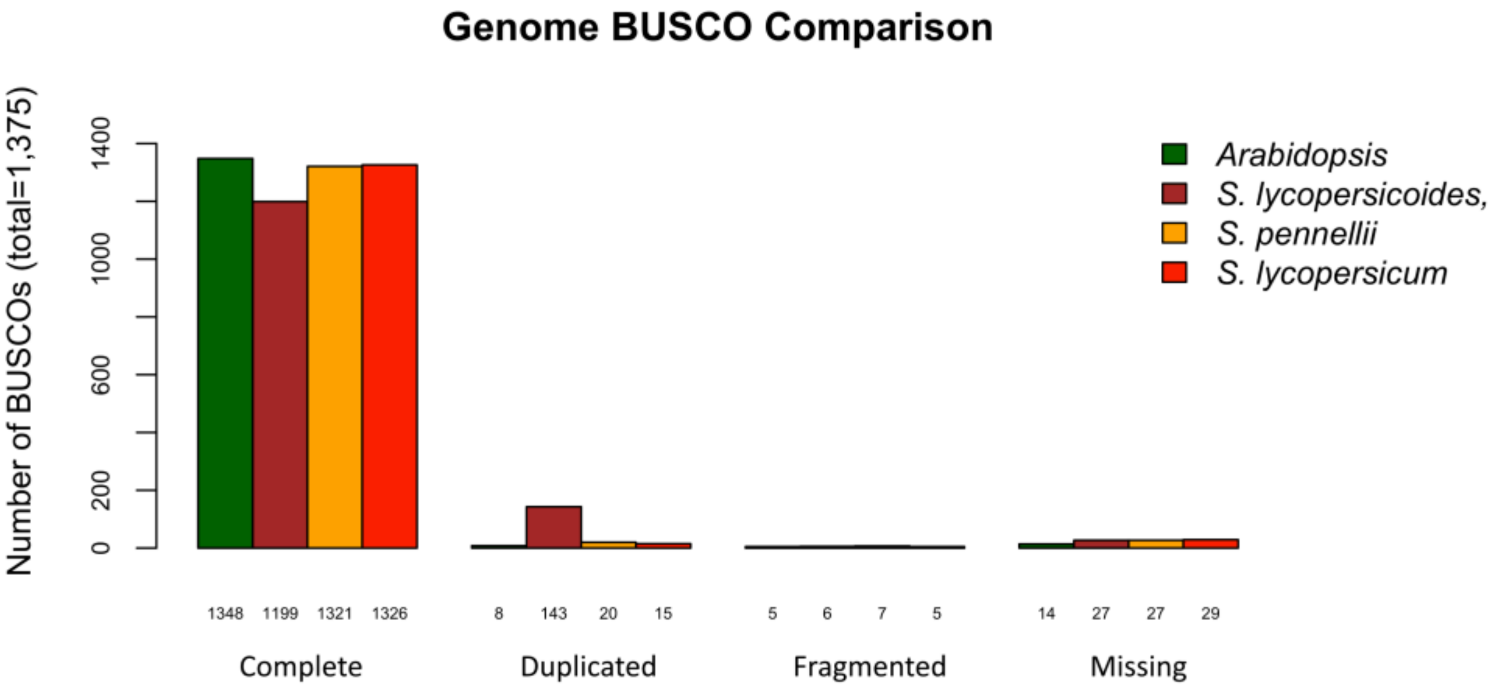
- Assembly Errors - correction
  - Tools: Pilon - Illumina https://github.com/broadinstitute/pilon/wiki
    - Polca - part of MaSurCA package
    - Arrow - PacBio https://github.com/PacificBiosciences/GenomicConsensus
    - Nanopolish - nanopore https://github.com/jts/nanopolish
- Example: tomato Nanopore/Illumina hybrid assembly polished with Illumina reads:

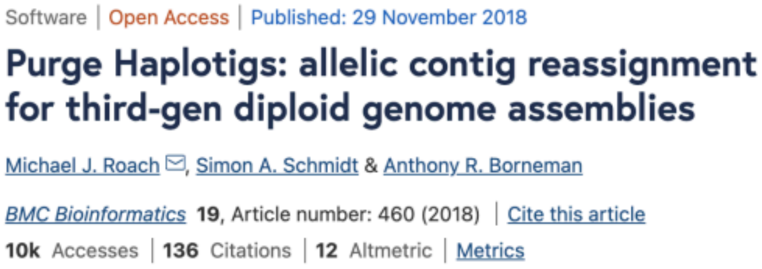


Round	SNPs/Indels corrected
1	145,994
2	84,441
3	46,201

1. Assembly QC

- Assembly BUSCO metrics https://gitlab.com/ezlab/busco_biocontainer




Software | Open Access | Published: 29 November 2018

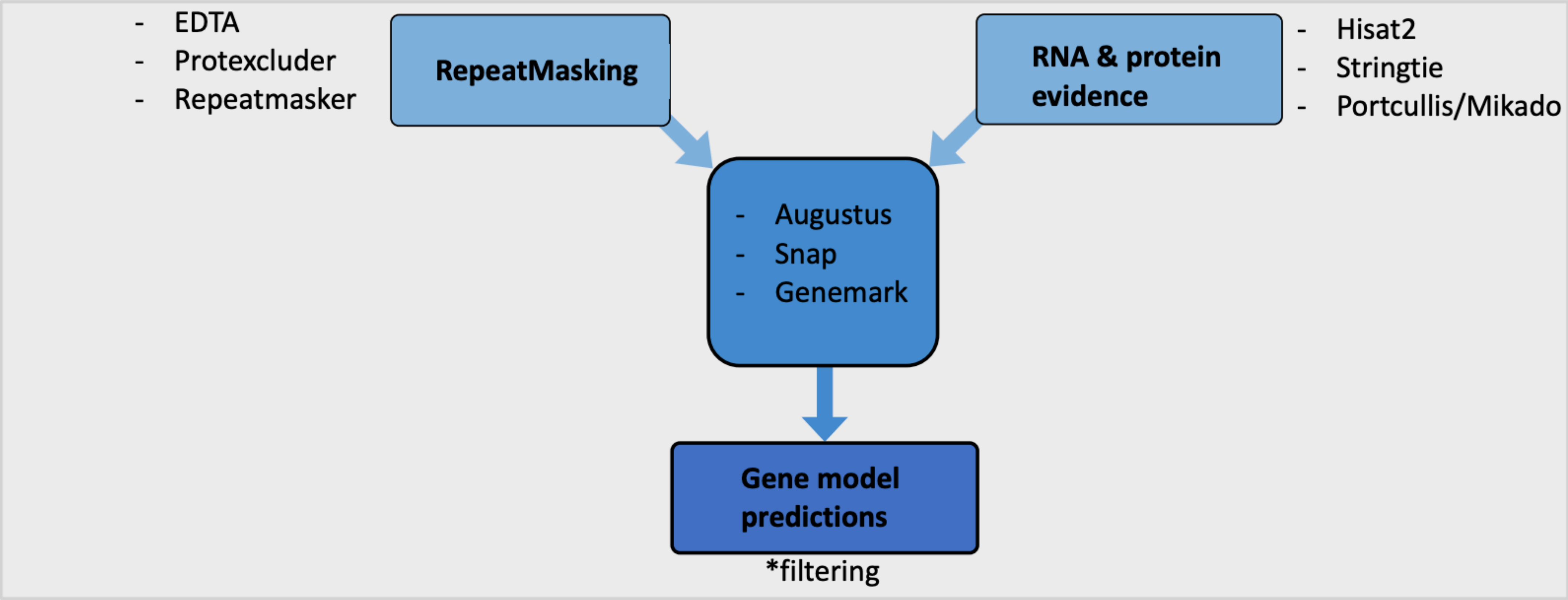
Purge Haplotigs: allelic contig reassignment for third-gen diploid genome assemblies

Michael J. Roach, Simon A. Schmidt & Anthony R. Borneman

BMC Bioinformatics **19**, Article number: 460 (2018) | Cite this article

10k Accesses | **136** Citations | **12** Altmetric | Metrics

2. Structural Annotation



- EDTA
- Protexcluder
- Repeatmasker

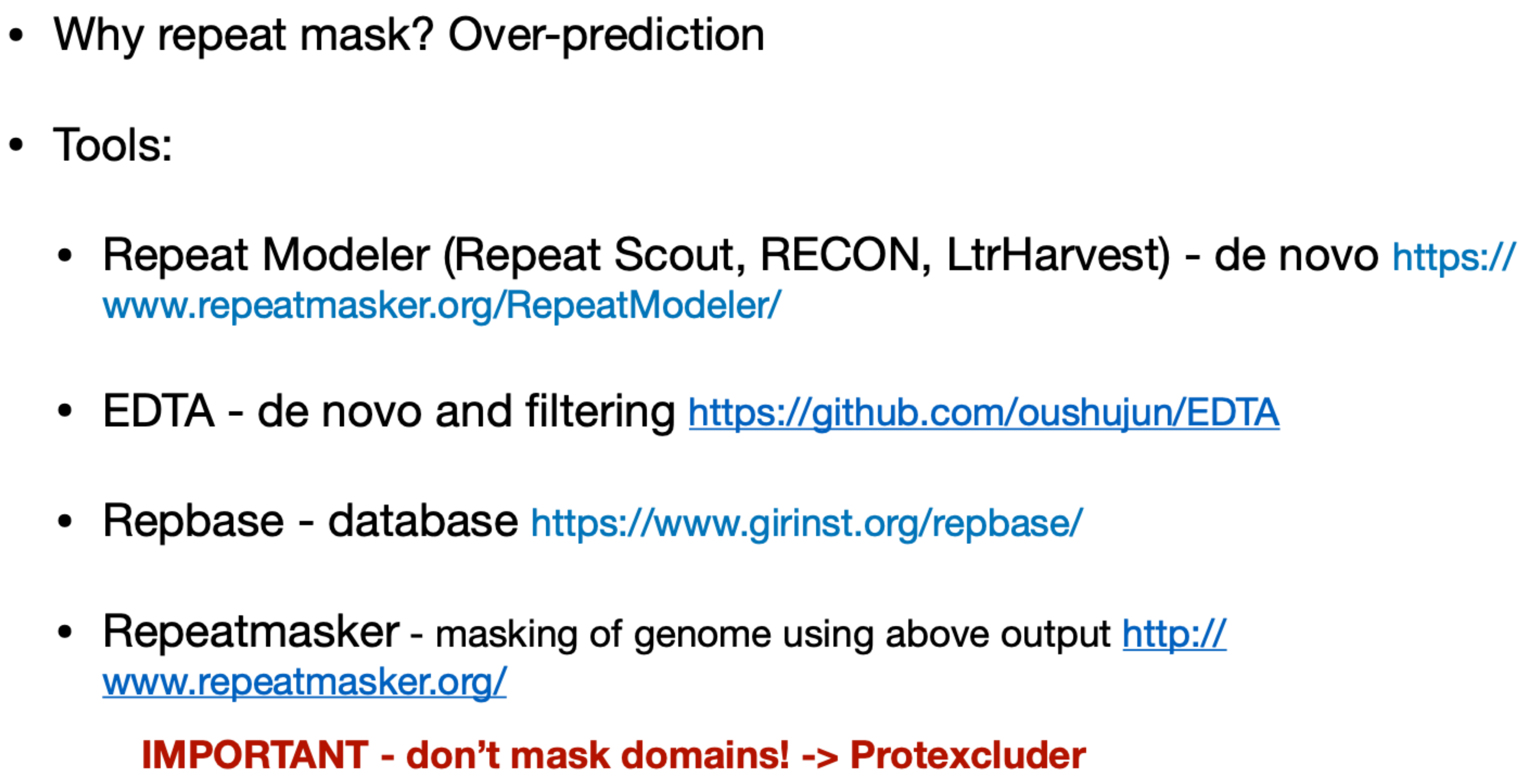
- Hisat2
- Stringtie
- Portcullis/Mikado

- Augustus
- Snap
- Genemark

*filtering

Annotation pipelines:
Maker
Braker

2. Structural Annotation: Repeat Masking

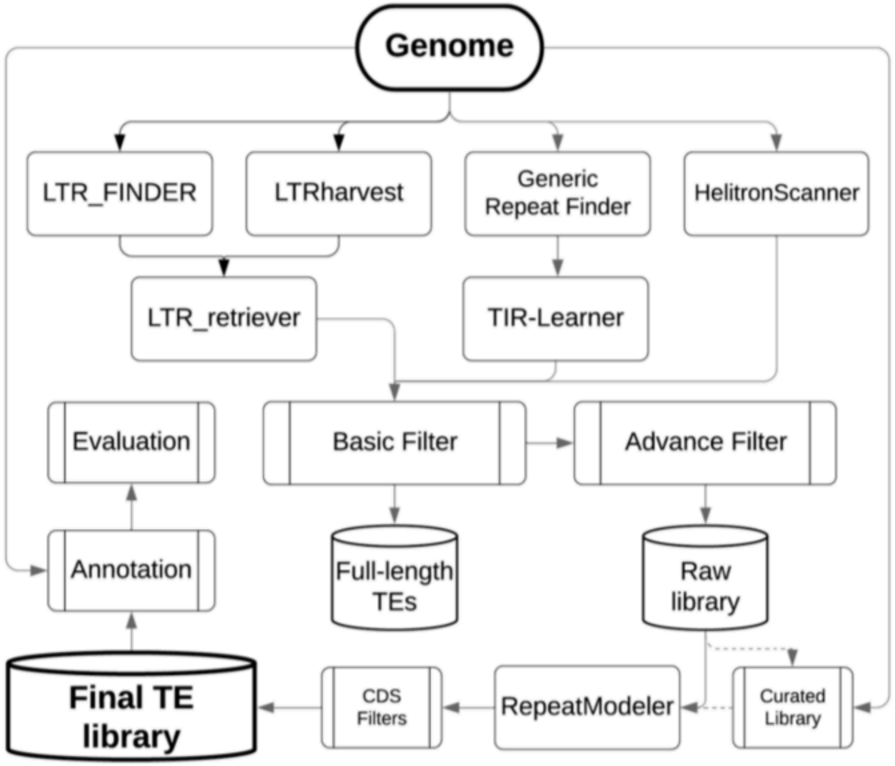


- Why repeat mask? Over-prediction
- Tools:
  - Repeat Modeler (Repeat Scout, RECON, LtrHarvest) - de novo https://www.repeatmasker.org/RepeatModeler/
  - EDTA - de novo and filtering https://github.com/oushujun/EDTA
  - Repbase - database https://www.girinst.org/repbase/
  - Repeatmasker - masking of genome using above output http://www.repeatmasker.org/

IMPORTANT - don’t mask domains! -> Protexcluder

2. Structural Annotation: Repeat Masking

The Extensive *de novo* TE Annotator (EDTA)



https://github.com/oushujun/EDTA

2. Structural Annotation: *Ab initio* Prediction

- Why *ab initio*? Similarity-based methods may not be applicable, propagation of errors, use statistical models to predict gene models
  - Training: “*ab initio* gene predictors use organism-specific genomic traits, such as codon frequencies and distributions of intron– exon lengths, to distinguish genes from intergenic regions and to determine intron–exon structures.” -Yandell and Ence 2012
- Tools:
  - Snap - easy to train https://github.com/KorfLab/SNAP
  - Augustus - difficult to train https://github.com/Gaius-Augustus/Augustus
  - Genemark http://exon.gatech.edu/GeneMark/

2. Structural Annotation: Evidence Aligners

- Why/What? Tools to align RNA and protein evidence to genome, usually output to gff3 or bam

- Tools:

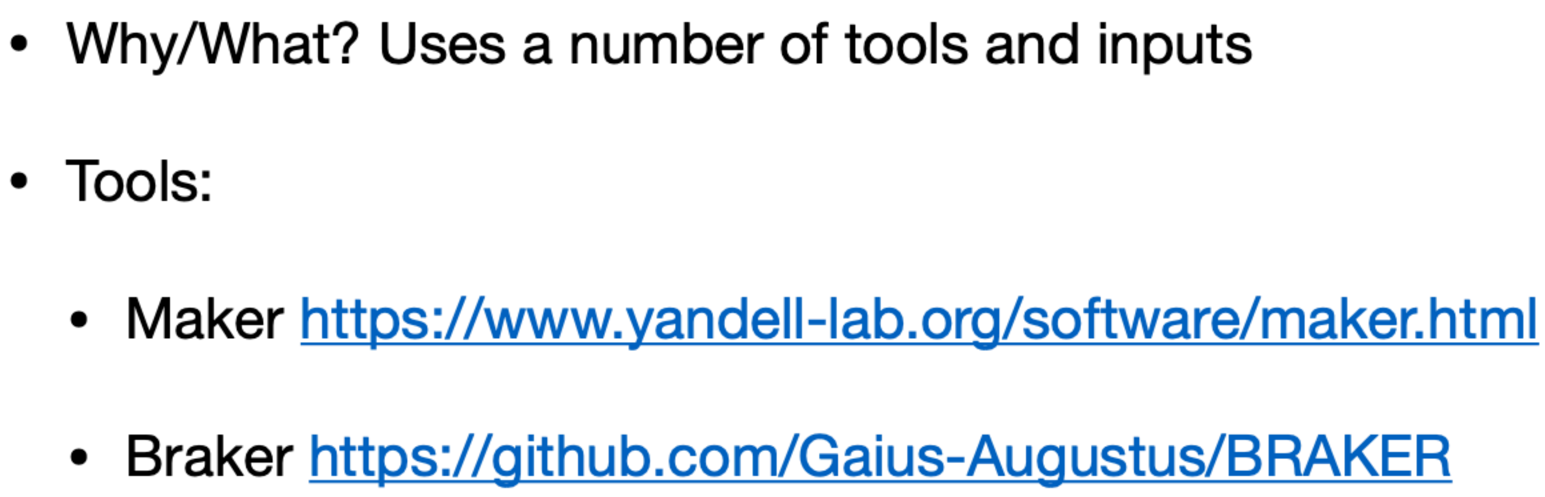
  - Hisat2 - align RNA-seq http://daehwankimlab.github.io/hisat2/

  - Gmap - align mRNA https://academic.oup.com/bioinformatics/article/21/9/1859/409207

  - Mikado/Portcullis - RNA-seq clean-up https://mikado.readthedocs.io/en/stable/

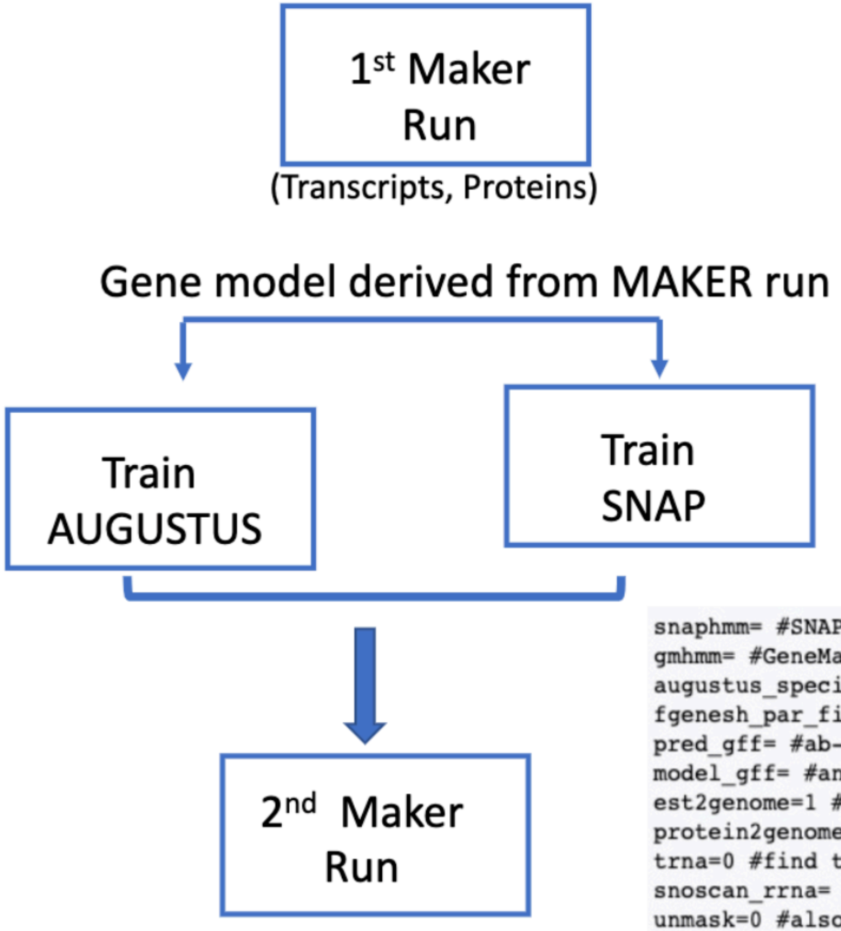
  - Pasa https://github.com/PASApipeline/PASApipeline/blob/master/docs/index.asciidoc

2. Structural Annotation: Pipelines

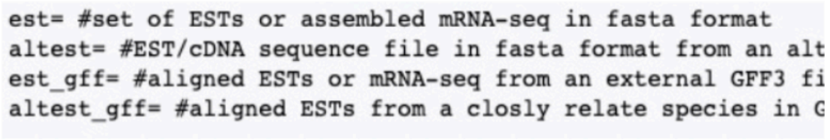


- Why/What? Uses a number of tools and inputs
- Tools:
  - Maker https://www.yandell-lab.org/software/maker.html
  - Braker https://github.com/Gaius-Augustus/BRAKER

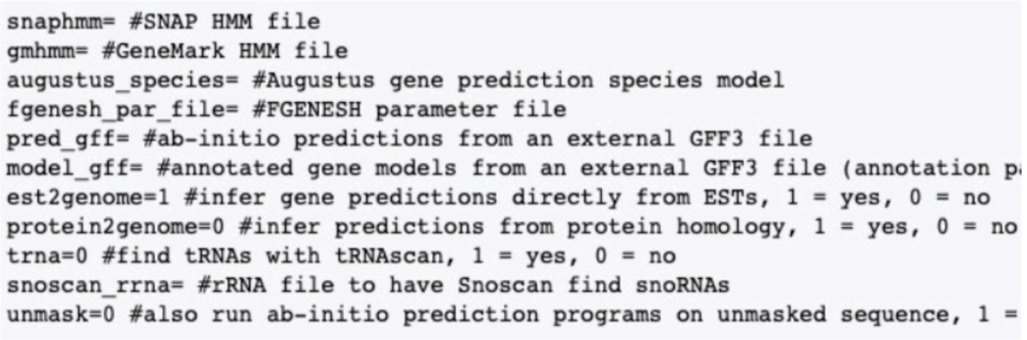
MAKER pipeline



maker_opt.ctl:



```
est= #set of ESTs or assembled mRNA-seq in fasta format
altest= #EST/cDNA sequence file in fasta format from an alt
est_gff= #aligned ESTs or mRNA-seq from an external GFF3 fi
altest_gff= #aligned ESTs from a closly relate species in G
```



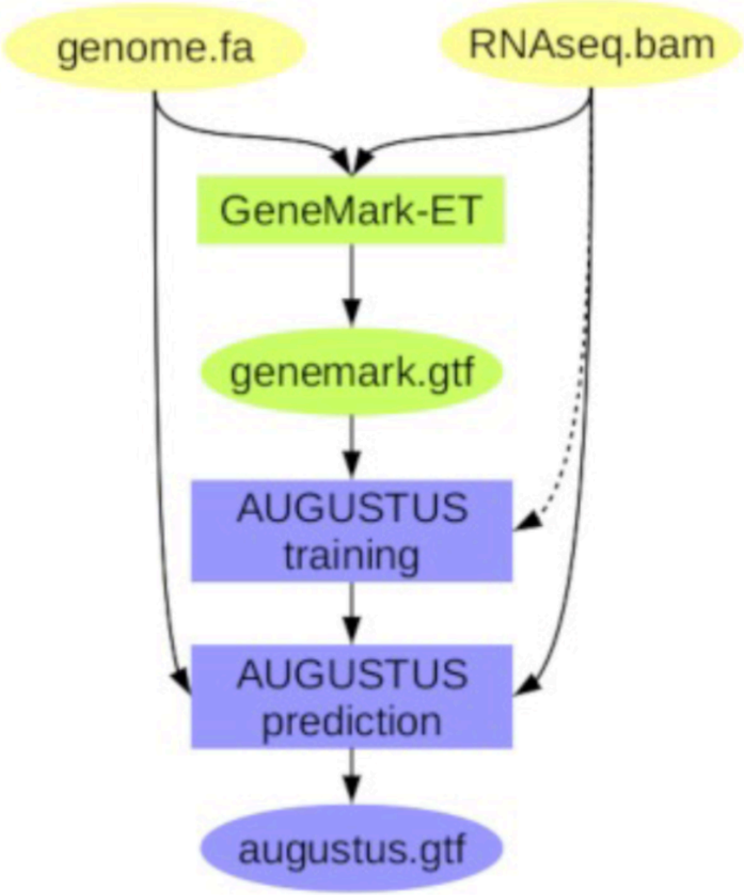
```
snaphmm= #SNAP HMM file
gmhmm= #GeneMark HMM file
augustus_species= #Augustus gene prediction species model
fgenesh_par_file= #FGENESH parameter file
pred_gff= #ab-initio predictions from an external GFF3 file
model_gff= #annotated gene models from an external GFF3 file (annotation p
est2genome=1 #infer gene predictions directly from ESTs, 1 = yes, 0 = no
protein2genome=0 #infer predictions from protein homology, 1 = yes, 0 = no
trna=0 #find tRNAs with tRNAscan, 1 = yes, 0 = no
snoscan_rrna= #rRNA file to have Snoscan find snoRNAs
unmask=0 #also run ab-initio prediction programs on unmasked sequence, 1 =
```

Control files:

- maker_exe.ctl: path for the underlying executables
- maker_bopt.ctl: stat for BLAST and Exonerate
- maker_opt.ctl: path for input genome files + training parameters

https://weatherby.genetics.utah.edu/MAKER/wiki/index.php/Main_Page

BRAKER with RNA-Seq reads

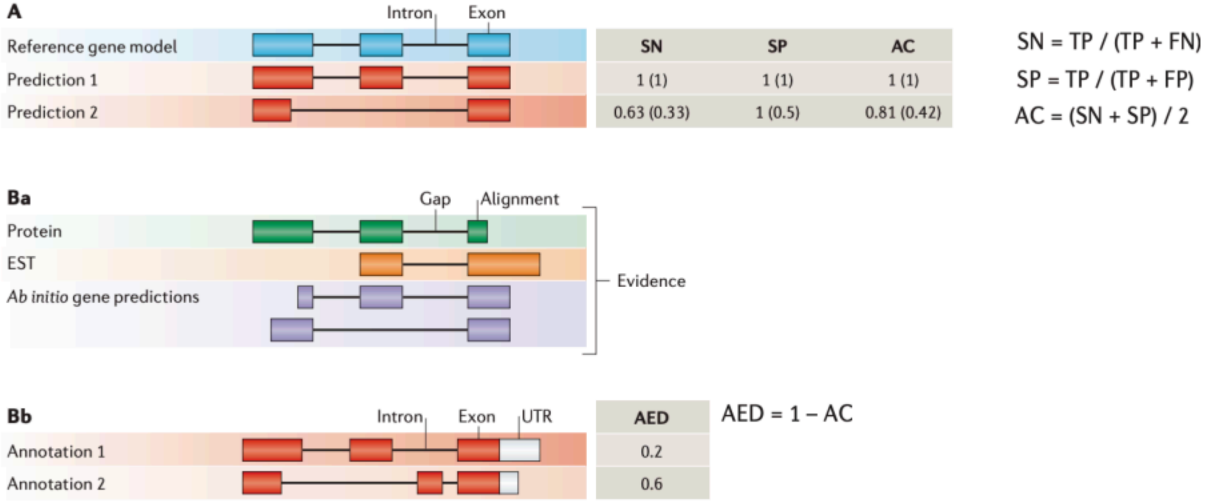


https://github.com/Gaius-Augustus/BRAKER

Slide courtesy of Bikash Shrestha

3. Annotation WC: Postprocessing, Cleanup, and QC

- Remove
  - Transposons
  - incomplete gene models
  - Genes with no match to nr (<e-20) an FPKM <0.1 and no InterProScan domain
- Sensitivity, specificity, accuracy, AED value



	SN	SP	AC
Reference gene model			
Prediction 1	1 (1)	1 (1)	1 (1)
Prediction 2	0.63 (0.33)	1 (0.5)	0.81 (0.42)

SN = TP / (TP + FN)

SP = TP / (TP + FP)

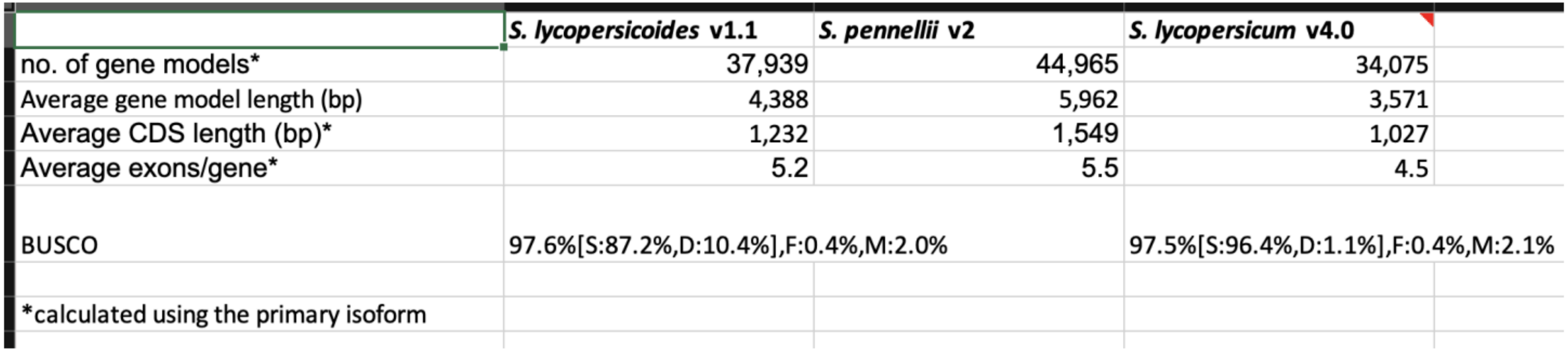
AC = (SN + SP) / 2

	AED
Annotation 1	0.2
Annotation 2	0.6

AED = 1 – AC

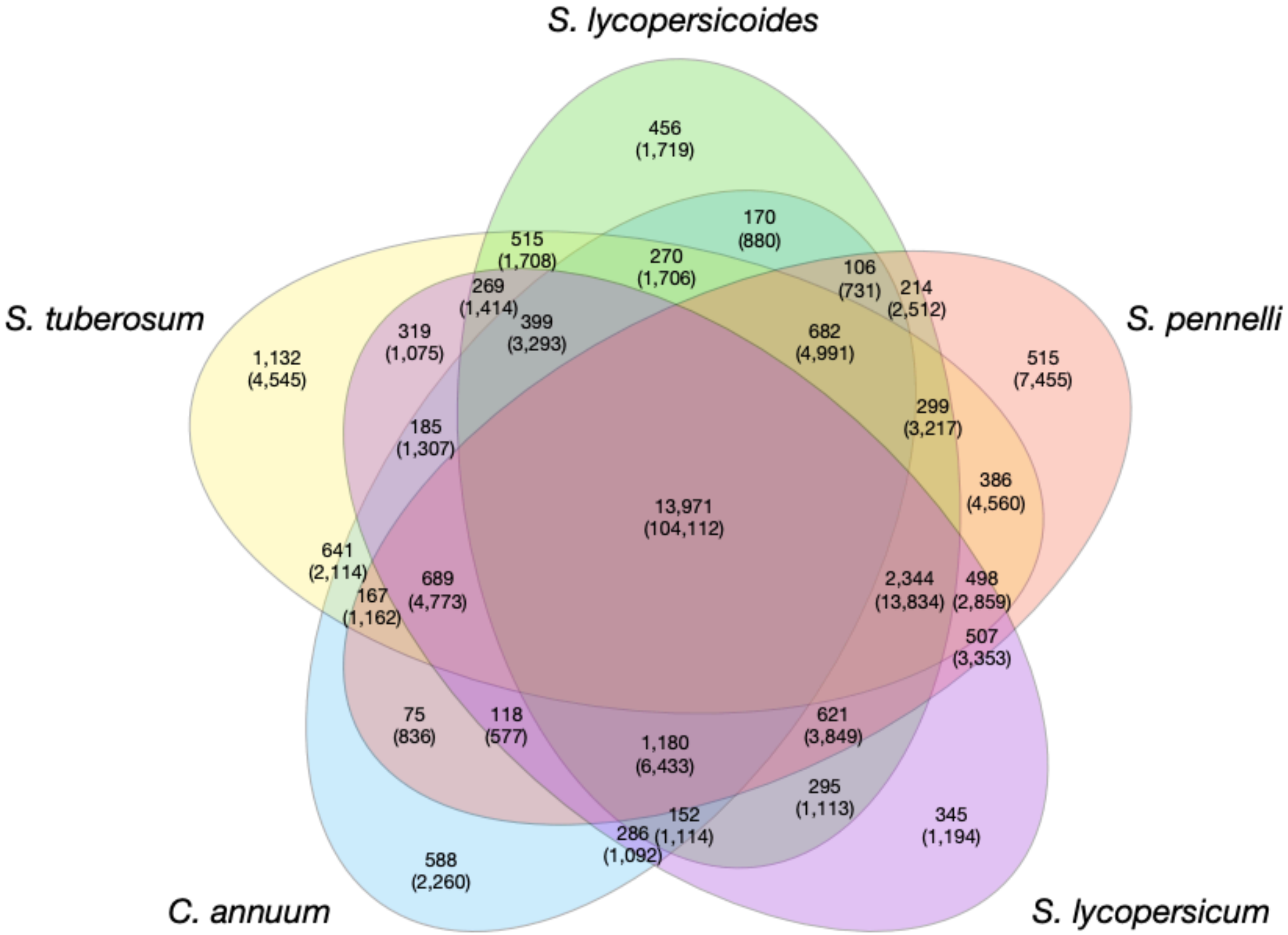
Yandell and Ence 2012

3. Annotation QC: Comparison to a related species

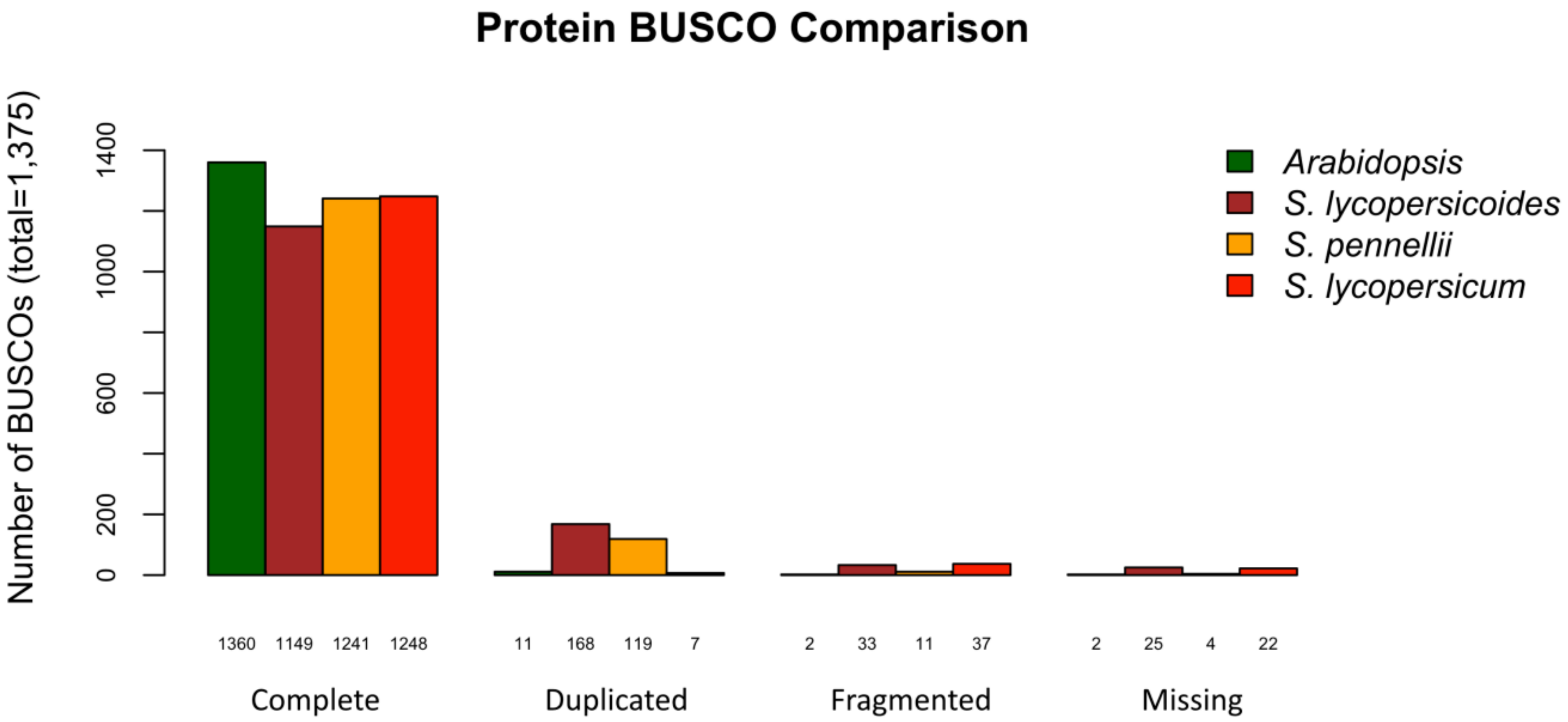


	S. lycopersicoides v1.1	*S. pennellii* v2	*S. lycopersicum* v4.0
no. of gene models*	37,939	44,965	34,075
Average gene model length (bp)	4,388	5,962	3,571
Average CDS length (bp)*	1,232	1,549	1,027
Average exons/gene*	5.2	5.5	4.5
BUSCO	97.6%[S:87.2%,D:10.4%],F:0.4%,M:2.0%		97.5%[S:96.4%,D:1.1%],F:0.4%,M:2.1%
*calculated using the primary isoform			

3. Annotation QC: Gene Families



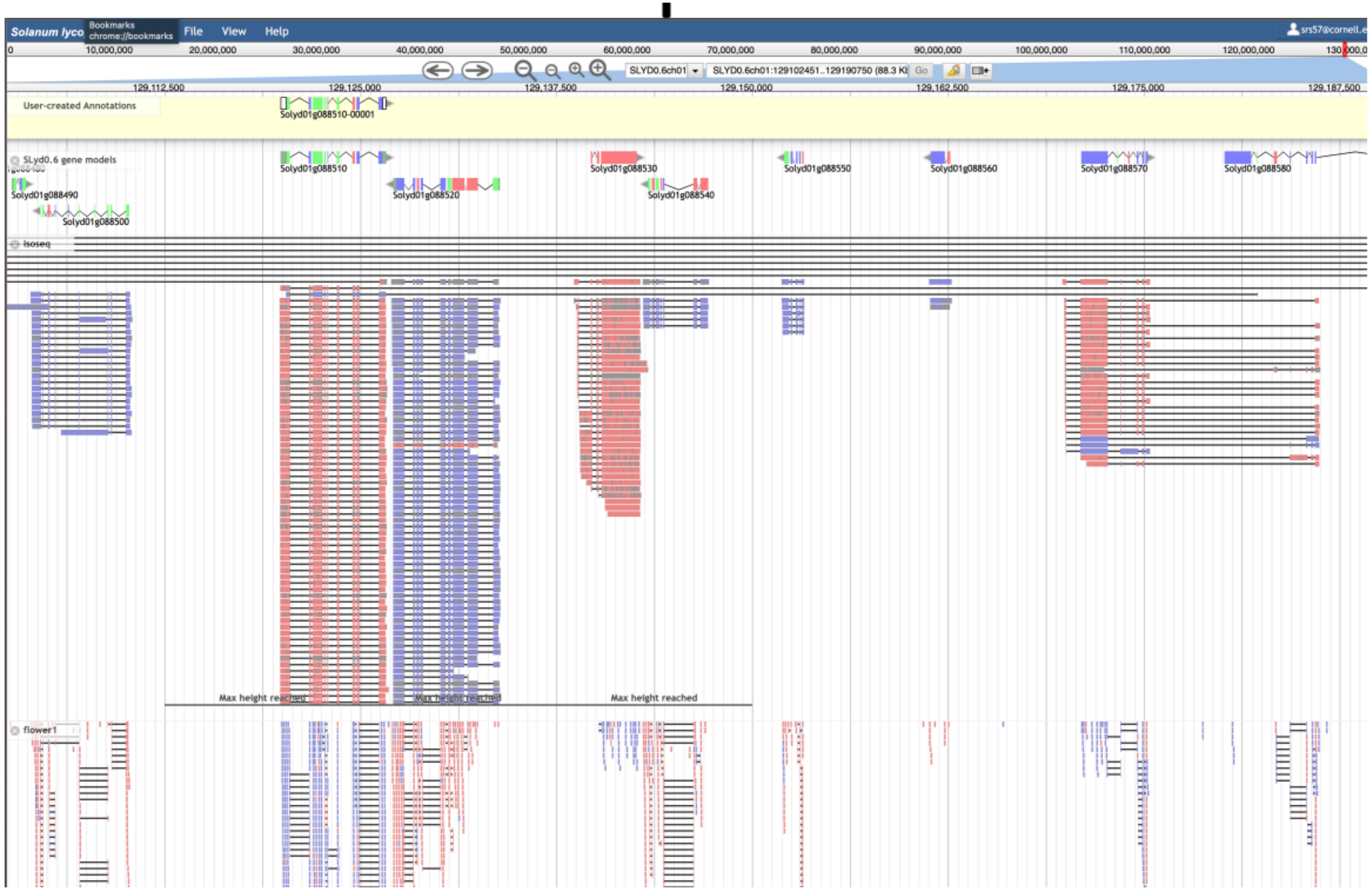
3. Annotation QC: BUSCO



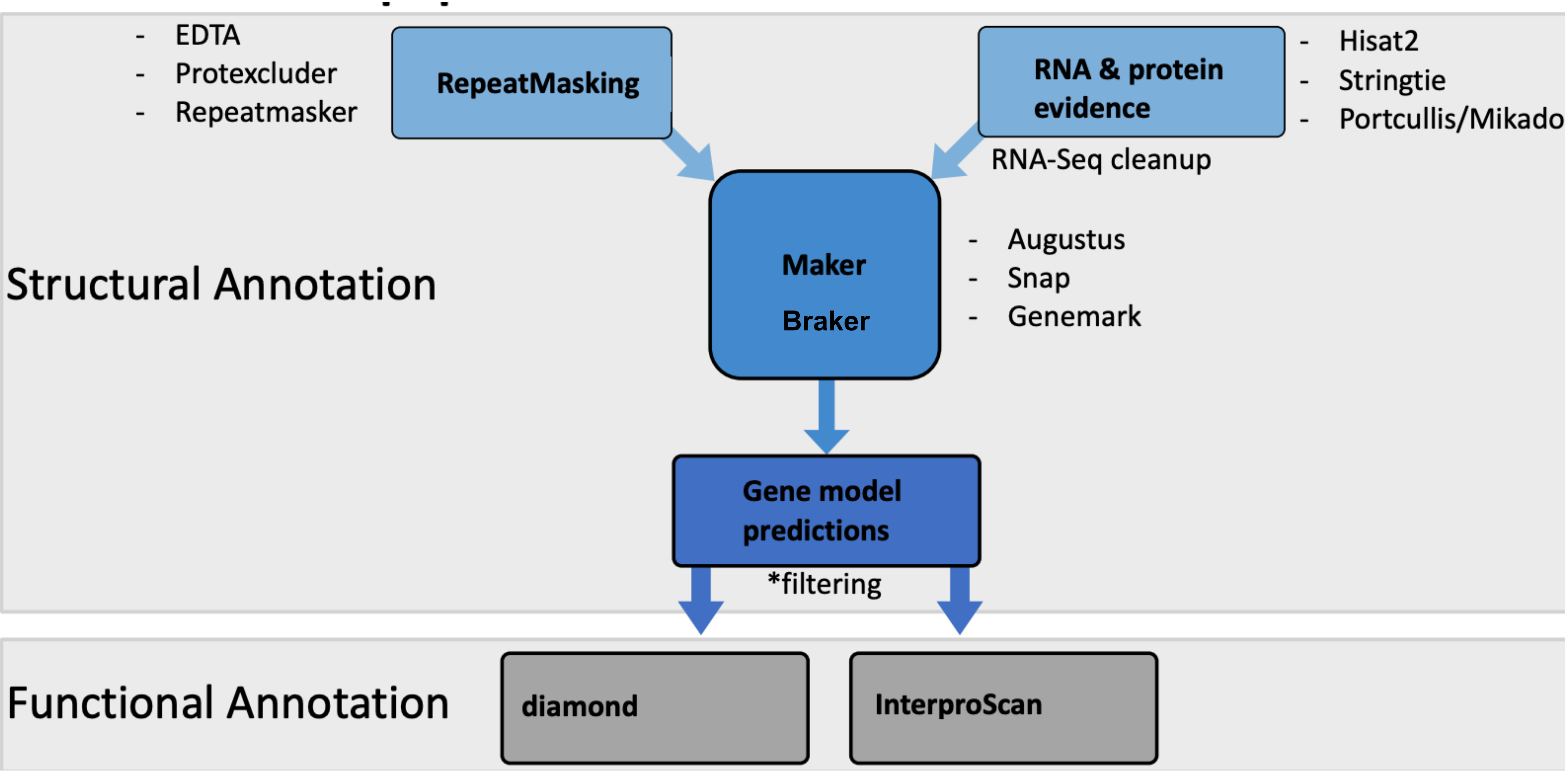
3. Annotation QC: Post-processing, Clean-up, and QC

- Change gene model names once structural annotation is completed.
  - Ex: maker-Contig3008-exonerate_est2genome-gene-0.0-mRNA-1 VS Solyd03g00650
- Versioning of genome and annotation (and keeping them in sync) – very important
- Apollo https://genomearchitect.readthedocs.io/en/latest/

3. Annotation QC: Manual curation with Apollo



4. Functional Annotation



Structural Annotation

- EDTA
- Protexcluder
- Repeatmasker

RepeatMasking

RNA & protein evidence

- Hisat2
- Stringtie
- Portcullis/Mikado

RNA-Seq cleanup

Maker

Braker

- Augustus
- Snap
- Genemark

Gene model predictions

*filtering

Functional Annotation

diamond

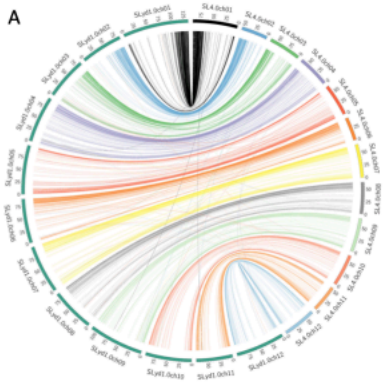
InterproScan

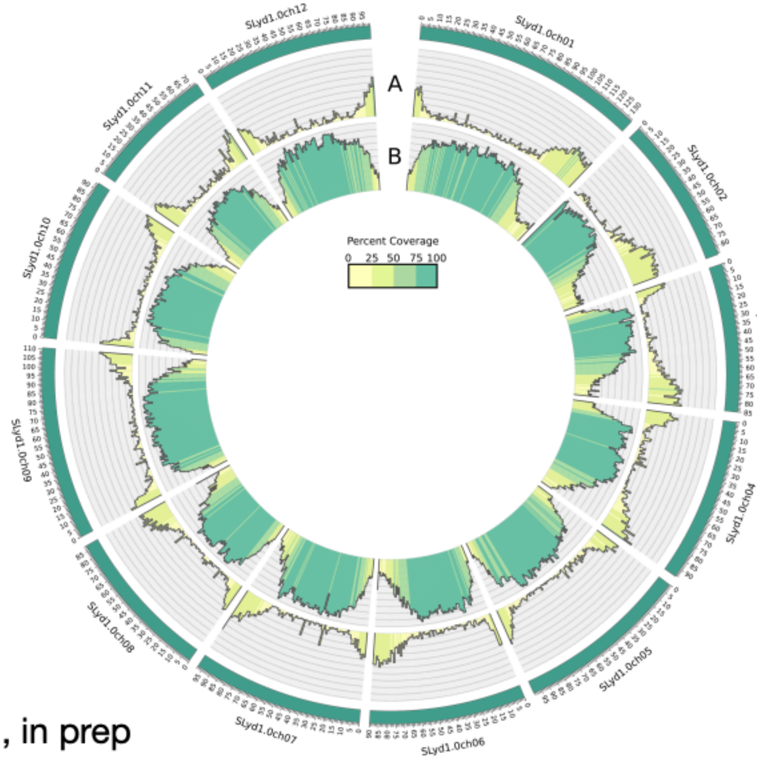
4. Functional Annotation: Tools

- Sequence searches
  - Diamond/BLAST
  - Databases: Swiss-prot, Trembl, nr, InterPro
- Domain searches
  - InterProScan
  - domains, GO terms, pathways
- Gene families
  - Orthofinder

Publishing your plant genome

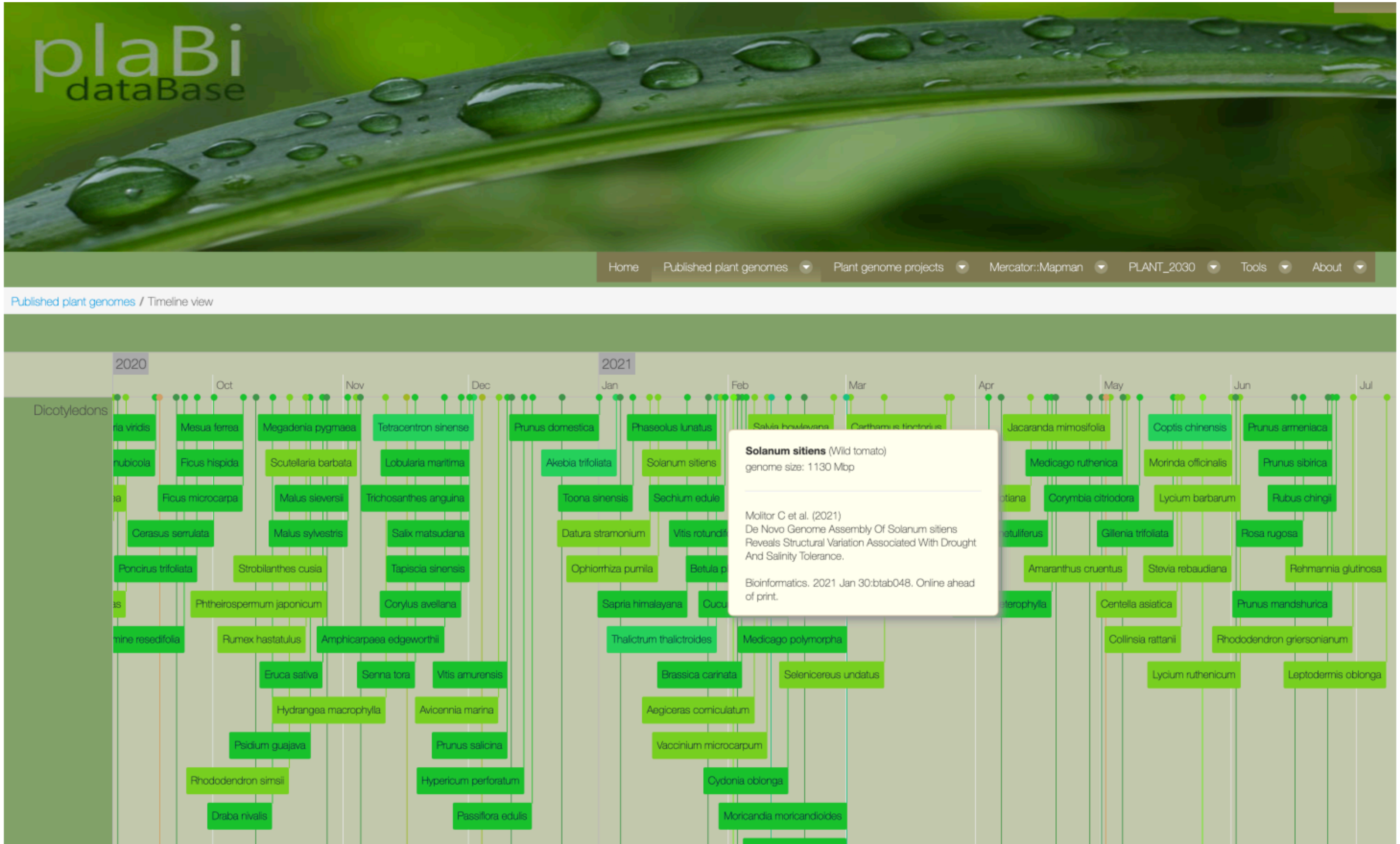
- Typical tables/figures (N50, gaps, etc, repeat content, gene families (expansion/ contraction), BUSCO, comparisons to reference)
- Circos plots
- Nice to have a biology hook
- Where to publish? https://plabipd.de/portal/sequenced-plant-genomes
- Submitting to Genbank: Project ID for publication
  - All supporting raw reads, annotation files, fasta files
- Organism-specific database
  - JBrowse
  - Apollo
  - Blast
- CyVerse/CoGe





Powell et al, in prep

Publishing your plant genome



Solanum sitiens (Wild tomato)
genome size: 1130 Mbp

Molitor C et al. (2021)
De Novo Genome Assembly Of Solanum sitiens Reveals Structural Variation Associated With Drought And Salinity Tolerance.

Bioinformatics. 2021 Jan 30:btab048. Online ahead of print.

Let’s annotate our *U. gibba* FLYE assembly!

- Genome file: Ugibba_FLYE_assembly.fasta.PolcaCorrected.fa.cat.all.gz
- RNA-seq from shoots and traps: https://www.ncbi.nlm.nih.gov/sra/SRX2368915[accn]
- All this stuff plus some output files in /home/user/work/data/iplant/home/shared/Botany2020NMGWorkshop/

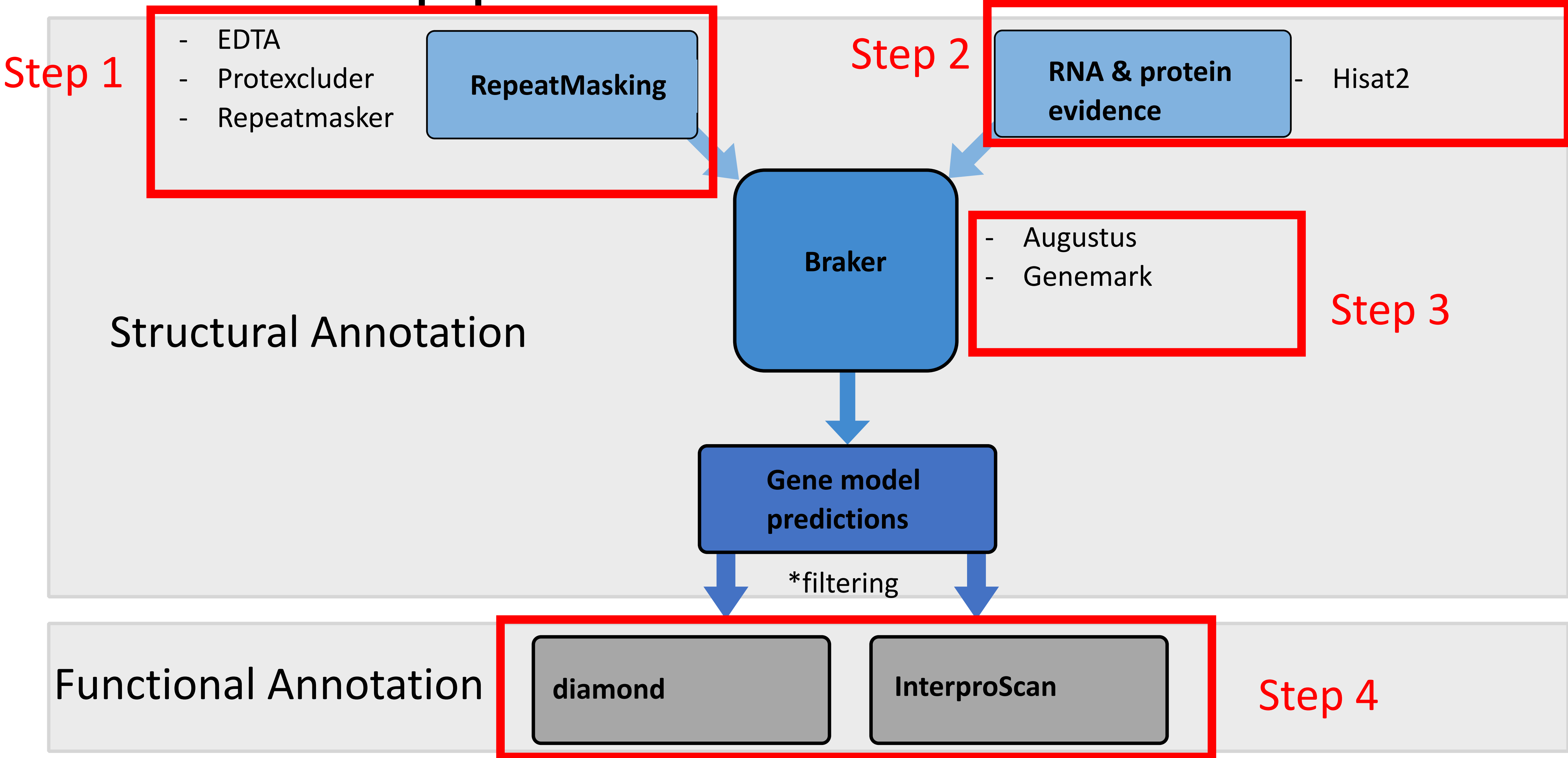
All scripts are on GitHub

```
$ cd ~/
$ git clone https://github.com/bcbc-group/Botany2022NMGWorkshop.git
$ cd Botany2022NMGWorkshop
```

QC of FLYE *U. gibba* assembly

- Size = 85,700,758 bp
- N50 = 4,134,757 bp
- BUSCO = 93.6% complete

Annotation pipeline



Step 1

- EDTA
- Protexcluder
- Repeatmasker

RepeatMasking

Step 2

RNA & protein evidence

- Hisat2

Braker

- Augustus
- Genemark

Step 3

Structural Annotation

Gene model predictions

*filtering

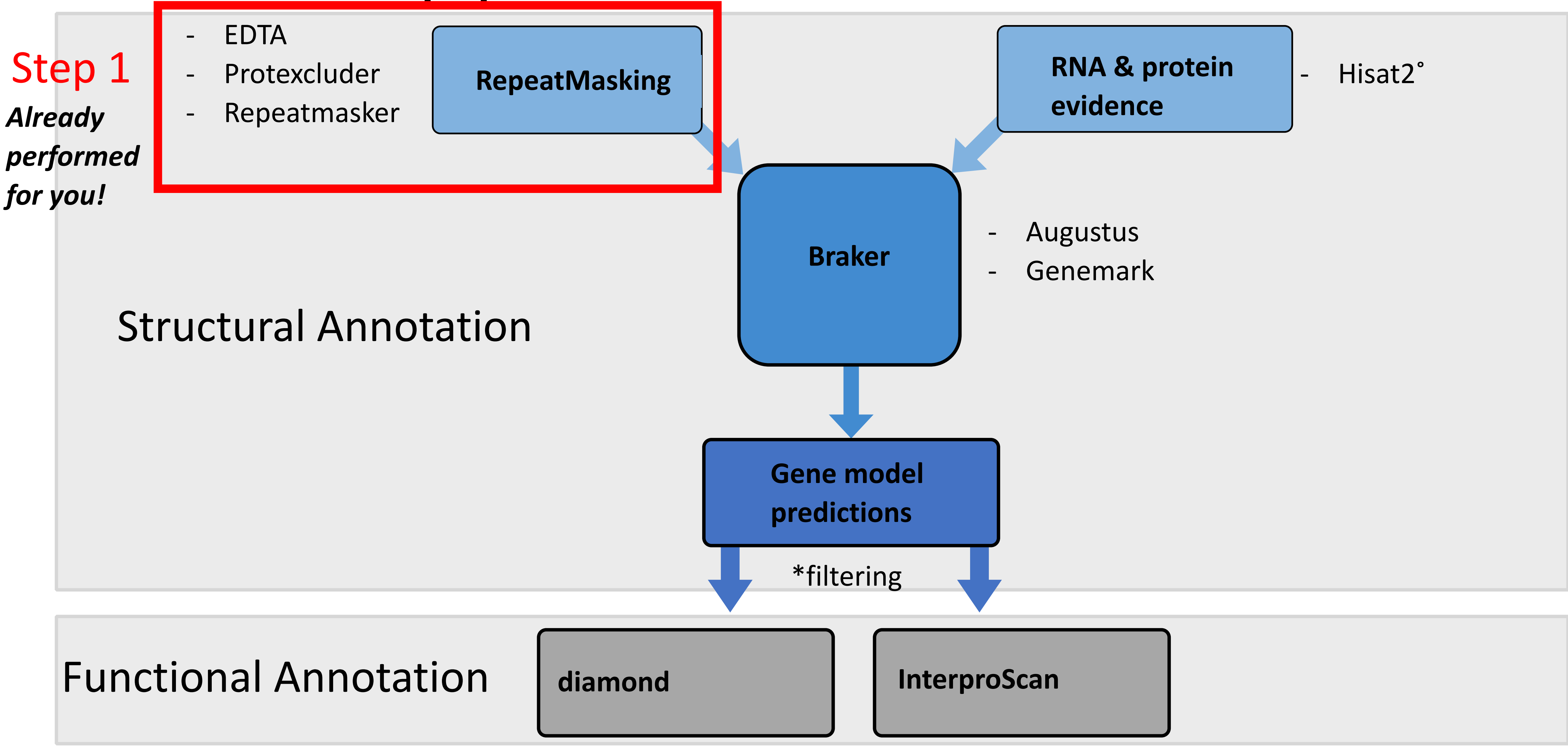
Functional Annotation

diamond

InterproScan

Step 4

Annotation pipeline



Step 1

Already performed for you!

- EDTA
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RepeatMasking

RNA & protein evidence

- Hisat2°

Braker

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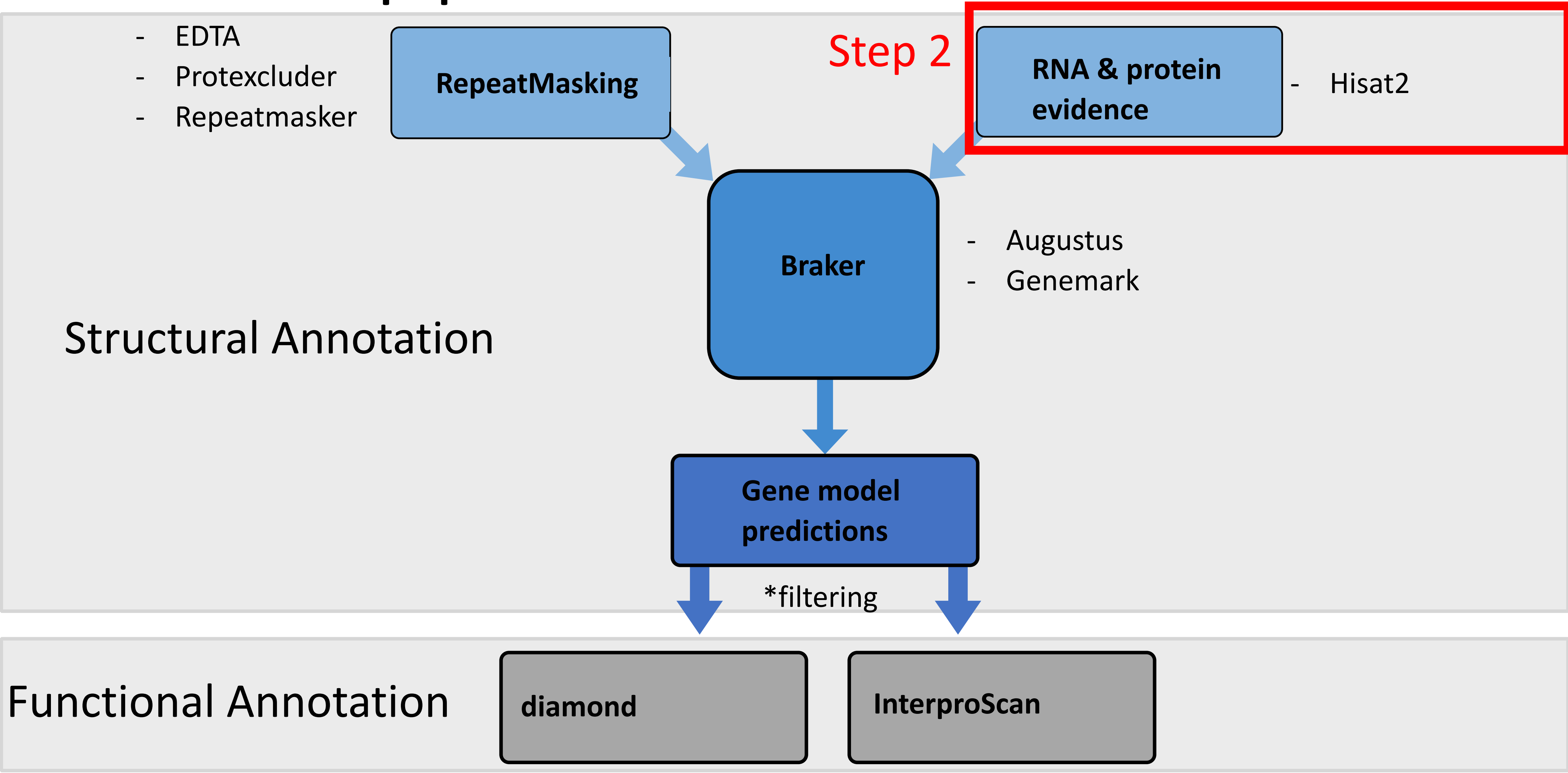
InterproScan

Step 1: Repeat Masking

https://github.com/bcbc-group/Botany2022NMGWorkshop/blob/main/5.Annotation/1_repeatmasking.sh

*this has already been performed to conserve time

Annotation pipeline



- EDTA
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RepeatMasking

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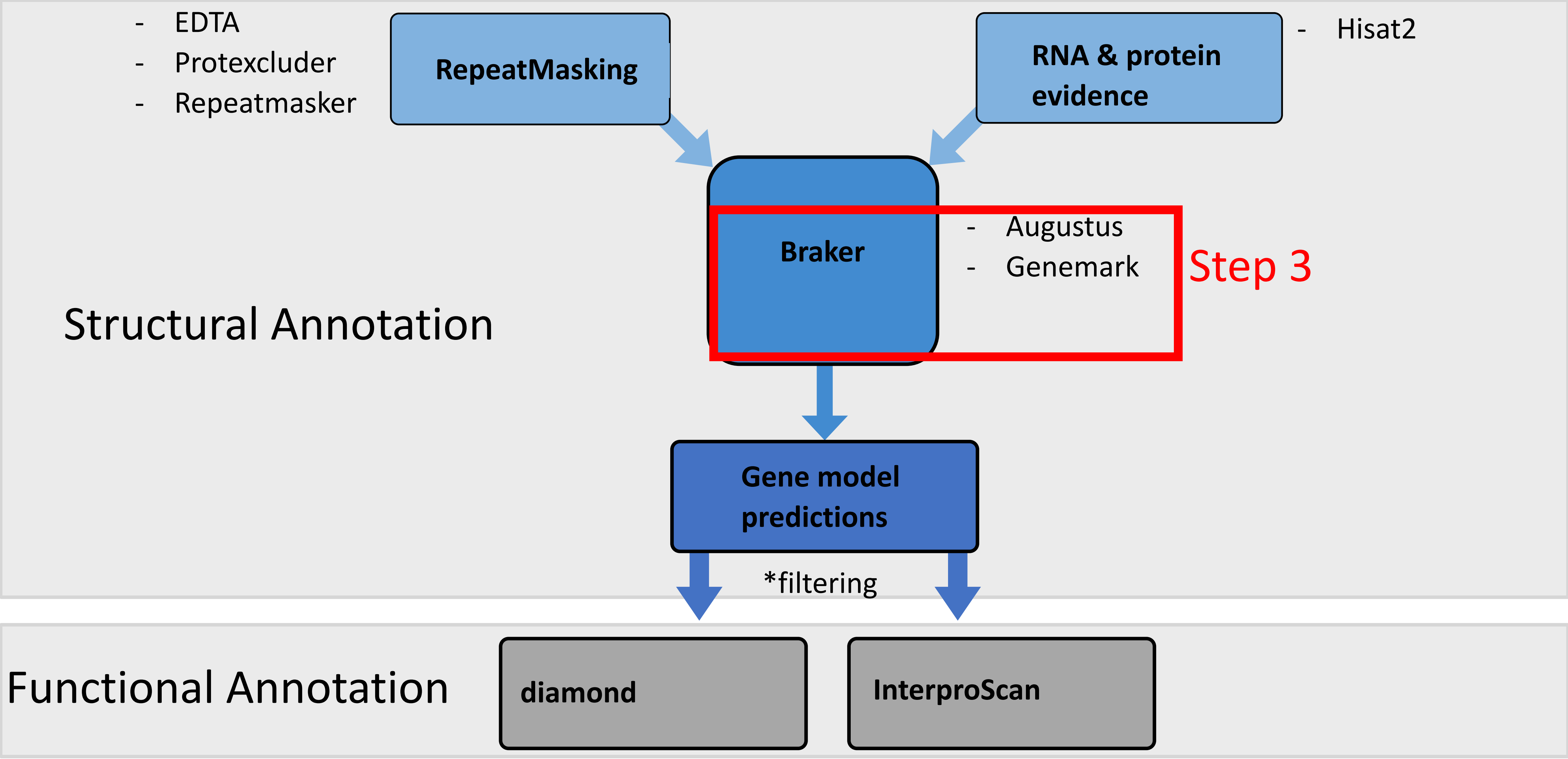
Step 2: RNA-Seq read mapping

https://github.com/bcbc-group/Botany2022NMGWorkshop/blob/main/5.Annotation/2_hisat_pe_annot.sh

To run:

```
$cd ~/
$bash Botany2022NMGWorkshop/5.Annotation/2_hisat_pe_annot.sh
```

Annotation pipeline



Step 3: Running Braker

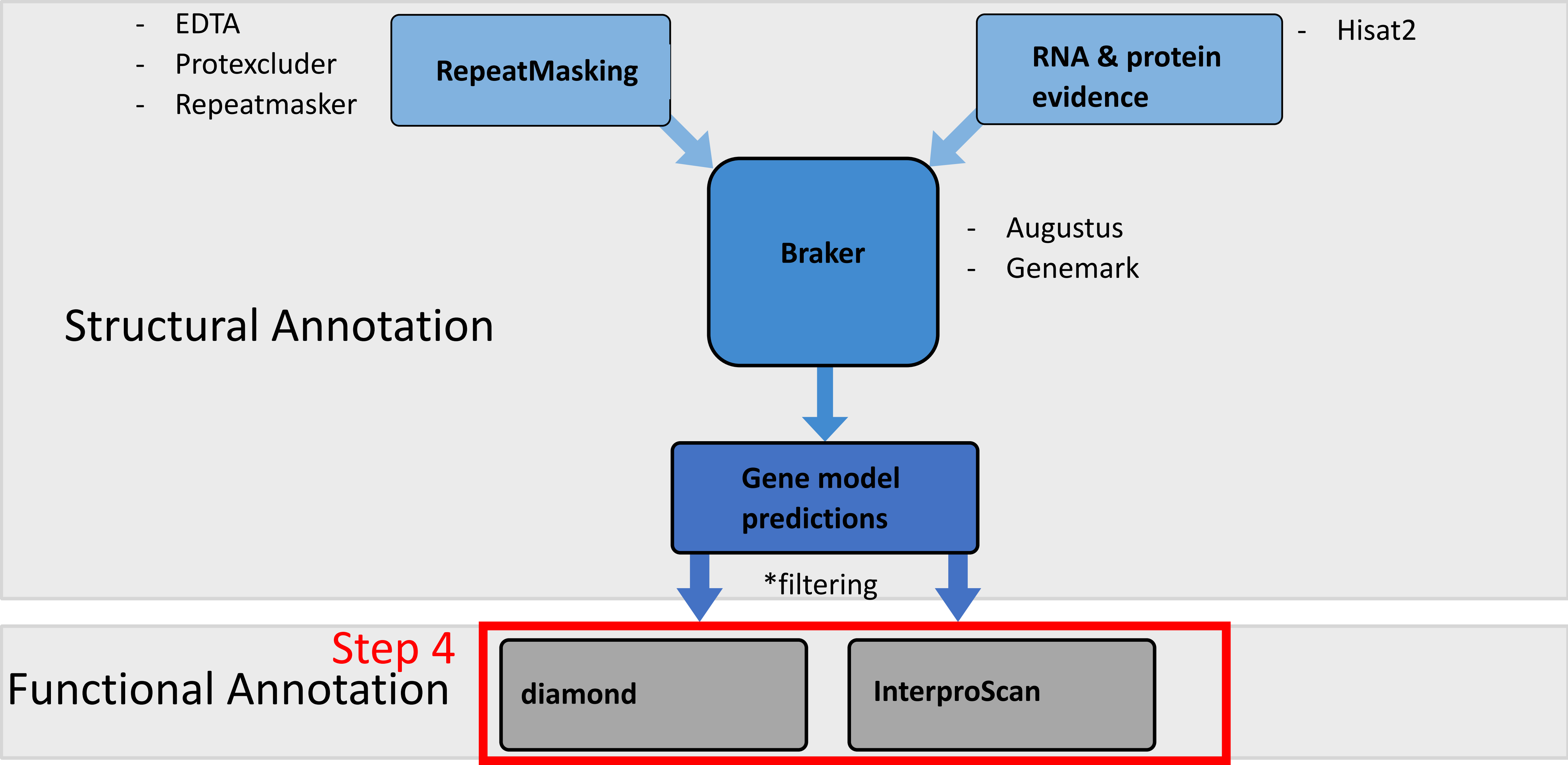
- https://github.com/bcbc-group/Botany2022NMGWorkshop/blob/main/5.Annotation/3_braker.sh

To run:

```
$cd ~/
$bash Botany2022NMGWorkshop/5.Annotation/3_braker.sh
```

- Runs a long time! Let’s look at output from last year’s workshop: https://github.com/bcbc-group/Botany2021NMGWorkshop/tree/main/5.Annotation/Output/braker_ouput

Annotation pipeline



- EDTA
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RepeatMasking

RNA & protein evidence

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Structural Annotation

Gene model predictions

*filtering

Step 4

Functional Annotation

diamond

InterproScan

Step 4: Functional annotation

- https://github.com/bcbc-group/Botany2022NMGWorkshop/blob/main/5.Annotation/4_functional_annot.sh

- Maker also has several scripts for postprocessing files under:
- /opt/maker/bin

Postprocessing, Cleanup, and QC

- Remove Transposons
- complete genes only
- match to nr, e-20
- FPKM > 0.1
- AED value
- InterProScan domain
- Comparison to relative, length and number of genes
- Gene families
- BUSCO
- Change gene model names once structural annotation is completed.
- Versioning –very important
- Apollo

Maker

- If you are interested in running maker check out: https://github.com/bcbc-group/Botany2021NMGWorkshop/tree/main/5.Annotation/maker_scripts