GENOME ANNOTATION WORKSHOP

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DATA SCIENCE LAB - OCIO

WORKSHOP INFO

INTRODUCTIONS, INFO, CODE OF CONDUCT

WORKSHOP INFO

- GitHub: SmithsonianWorkshops
  https://github.com/SmithsonianWorkshops/smsc_2019_Conservation_Genomics
- Etherpad: We will use to take notes
  https://pad.carpentries.org/2019-Oct-smsc_2019

WORKSHOP INFO

- Code of Conduct: https://docs.carpentries.org/topic_folders/policies/code-of-conduct.html
  - Use welcoming and inclusive language
  - Be respectful of different viewpoints and experiences
  - Gracefully accept constructive criticism
  - Focus on what is best for the community. Show respect and courtesy towards other community members

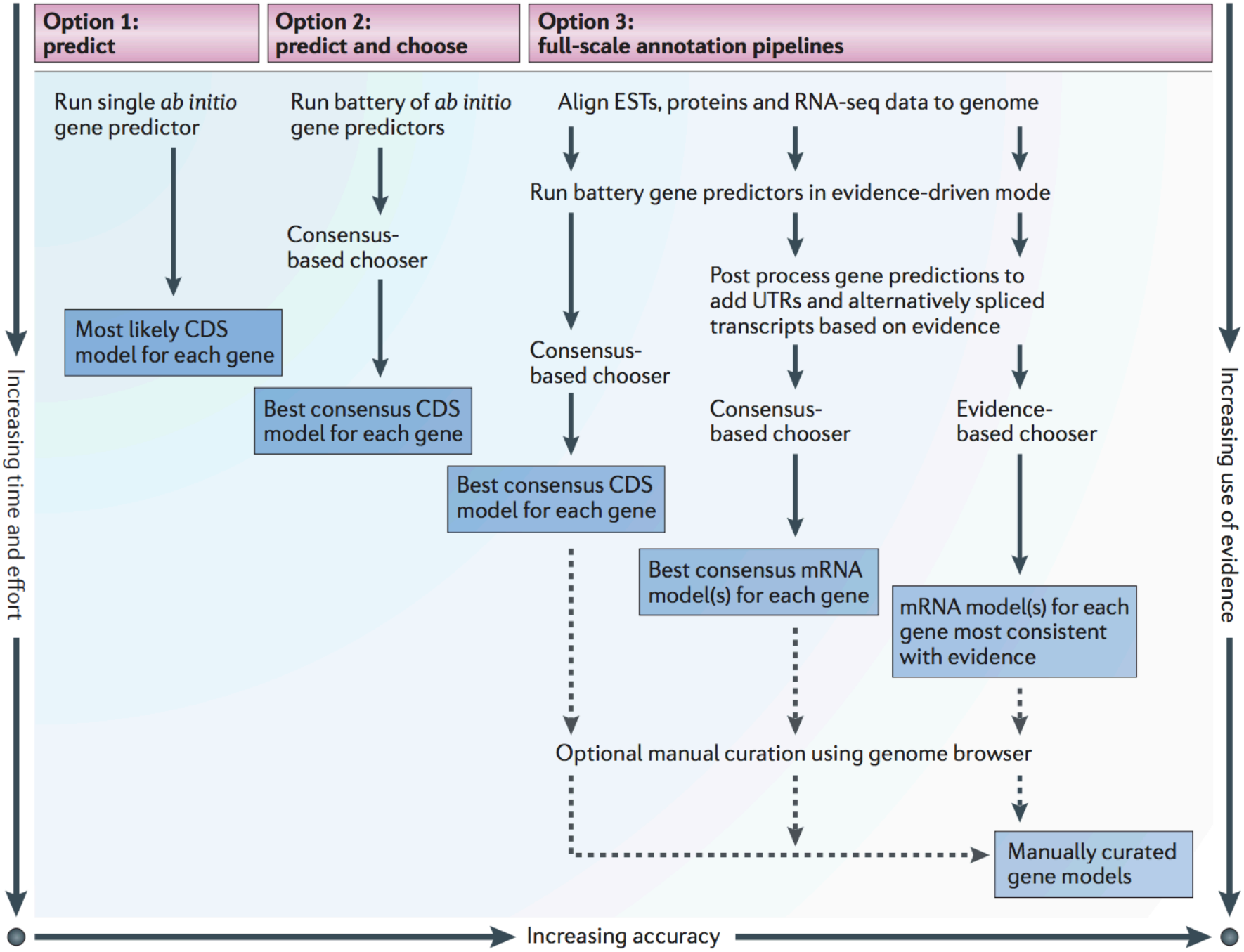
WHAT IS GENOME ANNOTATION?

- Genome annotation is the process of identifying different elements in a genome assembly:
  - Structural
    - Repetitive elements
    - Genes (with introns and exons)
  - Functional
    - What does each gene do?

GENE PREDICTION

ab initio	evidence-driven
• use only the query sequence	• use external evidence

Combining both approaches is the best option


Option 1: predict
Option 2: predict and choose
Option 3: full-scale annotation pipelines
Run single ab initio gene predictor
Most likely CDS model for each gene
Run battery of ab initio gene predictors
Consensus-based chooser
Best consensus CDS model for each gene
Align ESTs, proteins and RNA-seq data to genome
Run battery gene predictors in evidence-driven mode
Consensus-based chooser
Best consensus CDS model for each gene
Post process gene predictions to add UTRs and alternatively spliced transcripts based on evidence
Consensus-based chooser
Best consensus mRNA model(s) for each gene
Evidence-based chooser
mRNA model(s) for each gene most consistent with evidence
Optional manual curation using genome browser
Manually curated gene models
Increasing time and effort
Increasing use of evidence
Increasing accuracy

Chromosome 3R: 23,049,569-23,054,170

ATGTTTGCAAAATATGACAACTCTTTAATAAATTCACTTTTGGCTTTTTTGTACAGAGTATGTTTGTTGTGGTAGACAAAAGTTACGTAGTCACA
TAGAACTGCAGTTAACAACAGTATACTATCAAAAATATAAAAATGTTTTCTTTTCGGCCATCGAACATGCCAAAAGGGGAATATCACCGTTAAA
CGCCTTACGAACAAGGACTAATACAAACTTAAGATACTAATAGCTATGTAGTATACCCCACAGATGCAACACATTTCAAAGGTATCTGATACC
TGGGAGATTGTCGACCAGATCAGAAGTCATGGCAGCTCGTAGGCACGTTCTGAAAGGAAAGTTCGGTAAGTAAAGCTTAATCTCTTTGTCCT
CCGATTCACTGCTTACTCTTAAGGCTCAGCAATTTGTTGGCATGGCGACGTAGATTCTCTTGGTTGGGATTGCGCAGGACTTCAGCCGCCGC
CTCCTTAATCATGCCCTCGATGCTCTGCAGCAGCCATTCCTTATTGGGGCACGTAATAGCCAGACGGTAATCGCCATCCGGTGTCCGCGACA
CGTTCCACTCTGCGGCGGAGTCGTCGCAGTCTCGGCTATCATTGCTCTCCGTGTCCTCCGTCTTTATAGCAATGCACCGACGCACCTTGGAC
GGCTCATCTTCTTCGGCGGCTTCCGGCACGGACTTGCGCTTCTTGCTATTGAGCTGGCGTTCGTCTTGGATGCGATCCCGCTTGGGGCACGT
ACATATTTTAACATGTATAACATGCTGTCCCACGATATCGCCGCTGCAAGAAAGAAAACACGTTAAGCTTTGTTCTAGCCATCTAGAGTTTTGC
TGTCACCTTACCATGCTTTCTCCAGGCAGAAGACTAAGGAAGTTTCTTTTCGCCCGATACACGAGTTTTGGCAGACGAACTTGAAGGCCAGG
GTCTGGCGCGTGAGCCCACTGCGGGTTACAGACCGGCTCATGTTCAGGGGGACTACAACGGAAAAACGCTCGGAAATTCCCTTGCCCTGAG
CATTTCCACAATATACACTGTTGGGATTCTCGCTGCGCAGCAAGCTCTCGCGCATTTTTGCGTTATTGGCCGTCACTAAAGGTTTGTACAAAT
GATAGTTAAATAATCCTGTTCGATCTGTATTGTTATCTTCACTTACAAGGCTCAACGCTAAGGTGATTTTGACAGCGGACCACGGGAGCACTC
ACATCATTGGAGAAGCAAAGGAACACACGCAAATTAAGTGGTTGGATGGGCATTTTAGACTTGAACTGAACGTCCACGTTGAAGGCCTTGTT
CATCCGGATGTAGAGCTTGTTCAGCGGAATCGAGTACATCCAAAGAGACTTGGGCGGCTCATCCAGAACCATGCTGAAGCAATAACCACCGA
TGTTGTGATTCTCTAGCTTGGGCAGCGTGTTCGCCTGGATCTGAATGTCCTGCAGCATCATTTCGCGCAGCACGGATTGCTGTAAGCAAAAA
CAAGATATTATTTGAATGTCAACTTAACTCGCAATCATATAAGGGTTCACACTTTGCGCTCATAGAGCTGATAGAGCTTCATGTTCCAGCTTGC
TCGAGTTACAAATGGACTGGCGATTTGTTTTGTATTCTATTCTATTTTTCACAAGTGCTTGAAATTCTCGCTAAACGATGACGACGGGAAGGAG
TGCACCGTGCTAATCGGCTAGATAAGAGTGTTTTGTTCTGTCCTCCAACTGAAAGTGAACTTCGAAGCGACTTTGACGTCATGTCGCTCTTAT
GAAATTGCAGGCAGCGGCTGAGTCACGCAAGGAATGCCGCTTCCCTCACGCTTCTTATGCTCTTTTGTTTGTGGCTAGTAGTAGCACAACGC
ATGCTCAGTTGGTTTCTGGACGGCCGGTGAAATACATGCGTTTCGCGATGTGTCCCGATAGTGTCCGGCTACTTGACAGGTCCTCTAGATGG
CATCAGTTGGGTAGTTGGAAAACAATTAAGCAGATATTAATGCTGTGGAAAGTGCGCTCACAGTTTGTATTAATTGTTCGAGAGAAATGGACT
TCACCAGCGACTACGCGCATCGGCGTATGGTGAAATTTCTGACGATCATACTGATAGGCTTTATGACCGTCTTCGGACTCCTGGCCAATCGA
TATCGGGCGGGCCGTCGTGAAAGATTCCGCTTCTCAAAGGCAAATCTGGCCTTTGCTTCGCTGTGGGCAATTGCATTCAGCTTGGTTTACGG
GCGGCAAATCTACAAGGAGTACCAGGAGGGTCAGATCAACCTGAAGGACGCCACCACTCTGTACAGCTATATGAACATTACGGTGGCTGTTA
TTAACTATGTGTCGCAAATGATAATCAGTGACCATGTGGCCAAGGTGTTGAGCAAAGTGCCCTTCTTTGATACCCTAAAAGAATTCCGTCTGG
ACAGCAGGTCGCTGTACATATCCATCGTTTTGGCTCTGGTCAAGACCGTGGCTTTTCCTCTAACAATTGAAGTGGCTTTCATACTGCAACAGA
GGCGGCAGCATCCCGAGATGAGCTTGATCTGGACCTTGTACCGGCTGTTTCCCTTAATTATTTCGAATTTTCTCAATAACTGCTACTTTGGCG
CAATGGTGGTGGTGAAGGAGATTCTGTACGCTCTGAACAGACGGCTGGAAGCGCAGCTGCAGGAGGTGAATCTGCTGCAGAGGAAGGACCA
GCTAAAGTTGTACACTAAATACTACCGCATGCAGCGATTTTGCGCCTTGGCGGATGAACTCGACCAGCTGGCGTATCGCTATAGGTTGATATA
TGTGCATTCGGGAAAGTATCTGACCCCAATGTCCTTGTCCATGATTCTGTCGCTCATATGCCACCTGCTCGGAATAACGGTGGGTTTCTACAG
TCTGTACTATGCCATAGCGGACACCTTAATCATGGGCAAGCCGTACGATGGTCTTGGATCGCTGATCAATCTGGTTTTCCTCTCCATCTCGCT
GGCGGAGATCACATTGCTCACGCATTTGTGCAACCACCTATTGGTGGCCACCCGAAGATCGGCAGTCATTCTCCAGGAGATGAATCTCCAGC
ATGCGGACAGCCGCTACCGTCAGGCAGTCCACGGCTTTACTCTGCTGGTCACGGTGACCAAGTACCAAATTAAACCCTTGGGCTTGTACGAG
CTGGACATGCGACTGATCAGCAATGTCTTCTCGGCGGTGGCCAGCTTCCTGCTGATCCTCGTGCAGGCCGATCTGTCCCAGCGCTTCAAGAT
GCAATAGCTAATCGATGTTACCCACCTGGCTGAACTGCATCAGATTCCCGGACTGCGGGGAATAATATTAAATAGTTAGTAAGCTATAGCTTT

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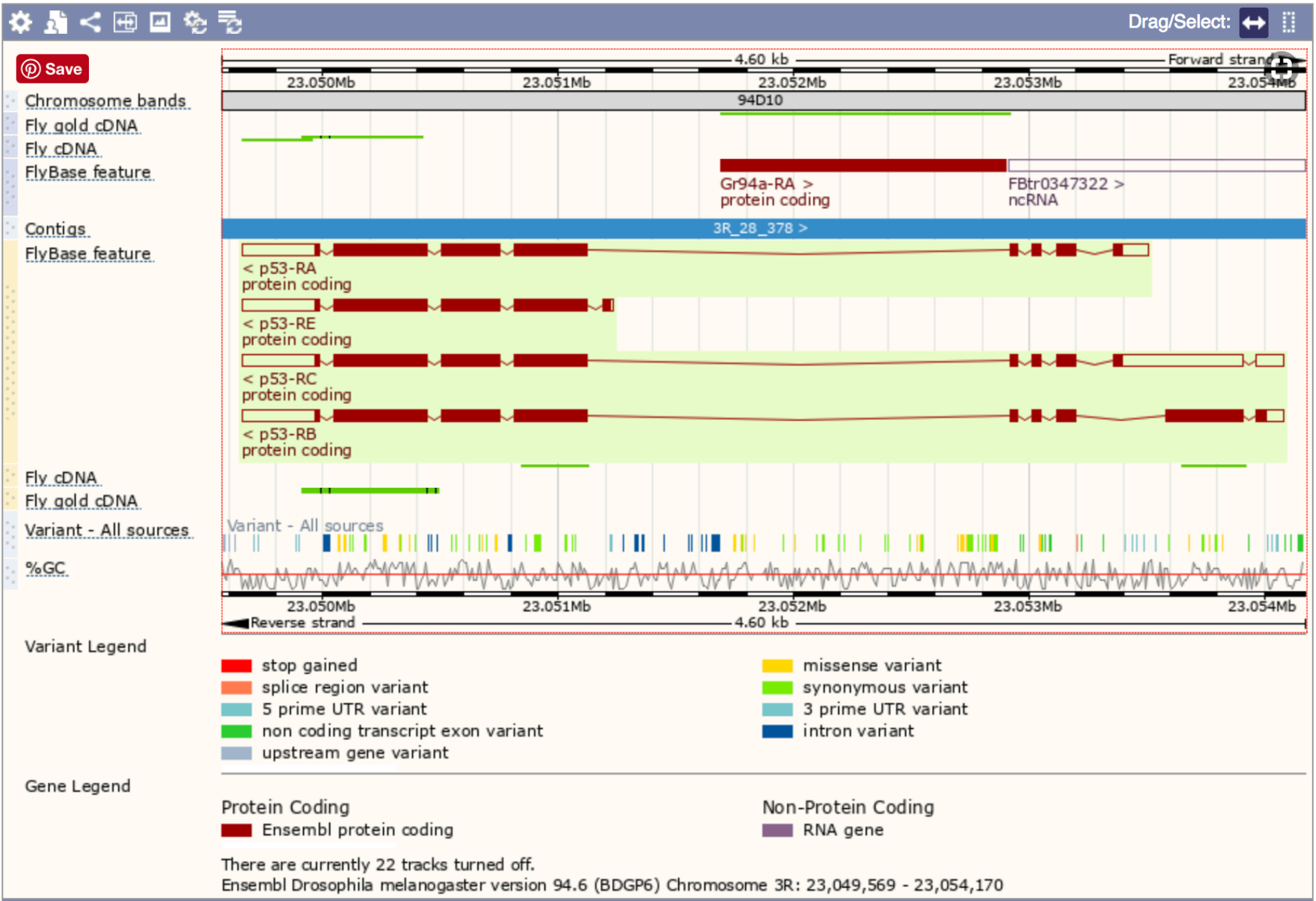


SOFTWARE/WRAPPERS

Augustus

SNAP

Glimmer HMM

Genemark -ES

FGenesh

Gnomon

OTHER ALTERNATIVES

NCBI

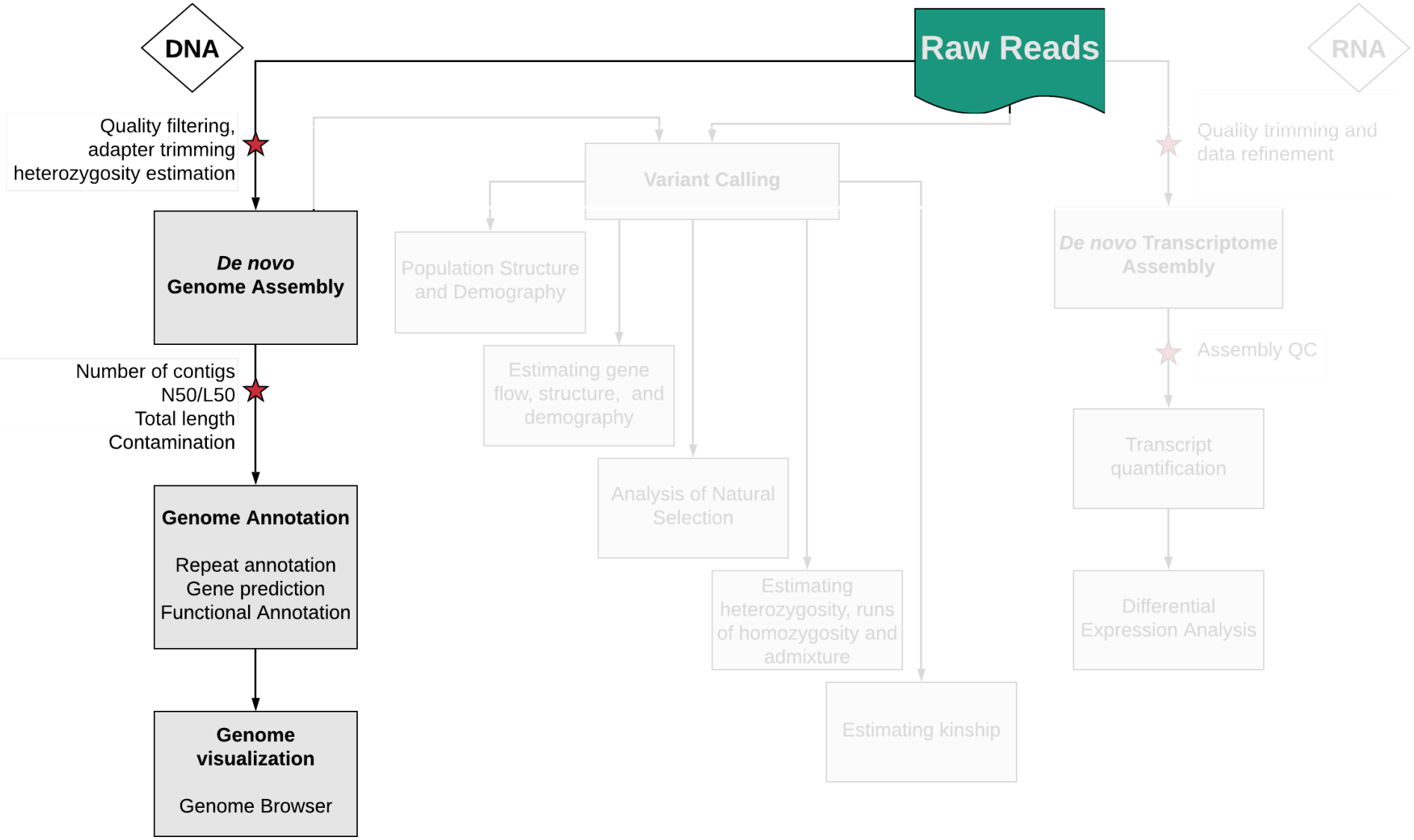
e!Ensembl

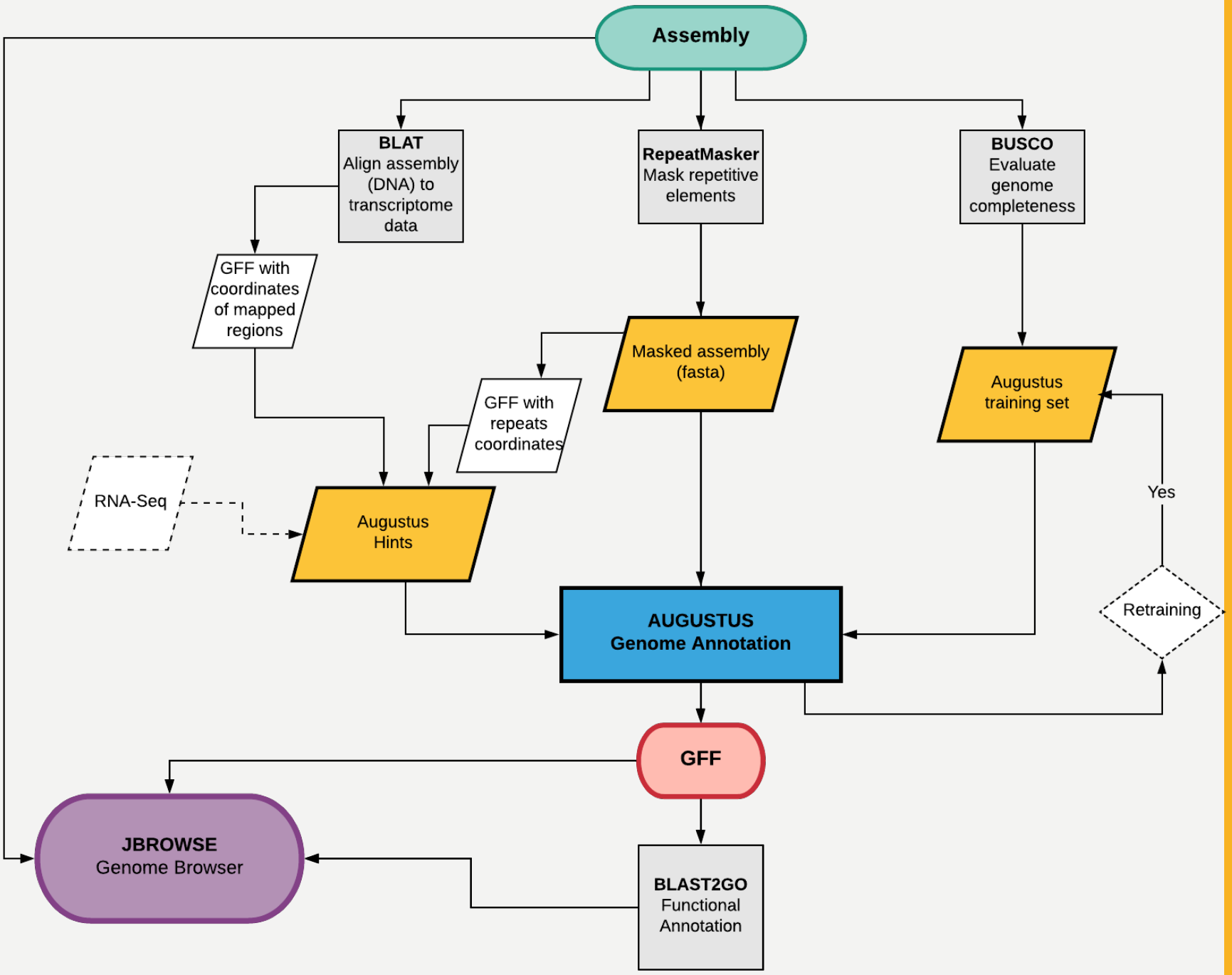
Pros

- Standardized
- Free

Limitations

- Quality requirements
- Taxonomic priorities


DNA
Raw Reads
RNA
Quality filtering,
adapter trimming
heterozygosity estimation
Quality trimming and
data refinement
Variant Calling
De novo
Genome Assembly
Population Structure
and Demography
De novo Transcriptome
Assembly
Number of contigs
N50/L50
Total length
Contamination
Estimating gene
flow, structure, and
demography
Assembly QC
Analysis of Natural
Selection
Transcript
quantification
Genome Annotation
Repeat annotation
Gene prediction
Functional Annotation
Estimating
heterozygosity, runs
of homozygosity and
admixture
Differential
Expression Analysis
Estimating kinship
Genome
visualization
Genome Browser


Assembly
BLAT
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RepeatMasker
Mask repetitive elements
BUSCO
Evaluate genome completeness
GFF with coordinates of mapped regions
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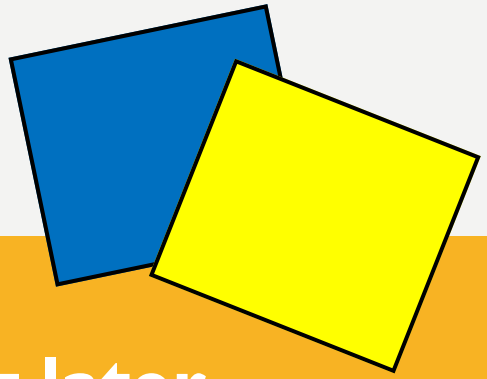
Augustus training set
GFF with repeats coordinates
RNA-Seq
Augustus Hints
Yes
Retraining
AUGUSTUS
Genome Annotation
GFF
JBROWSE
Genome Browser
BLAST2GO
Functional Annotation

LET’S START!

FOLDER STRUCTURE

1. Create the folder **genome_annot** inside the smsc_2019 folder:
2. Then, create the following folders inside genome_annot:

- assembly
- busco
- repmasker
- blat
- blast
- b2go
- augustus
- jbrowse
- logs
- jobs

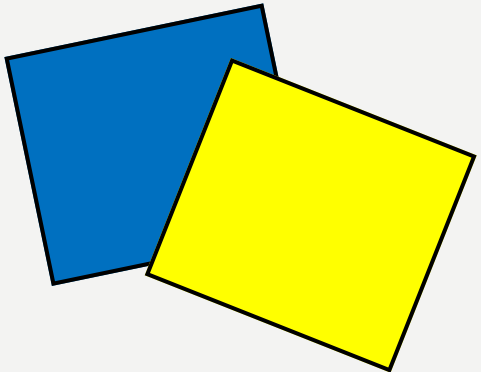


Why?
It is easier to find everything later.

ASSEMBLY STATS

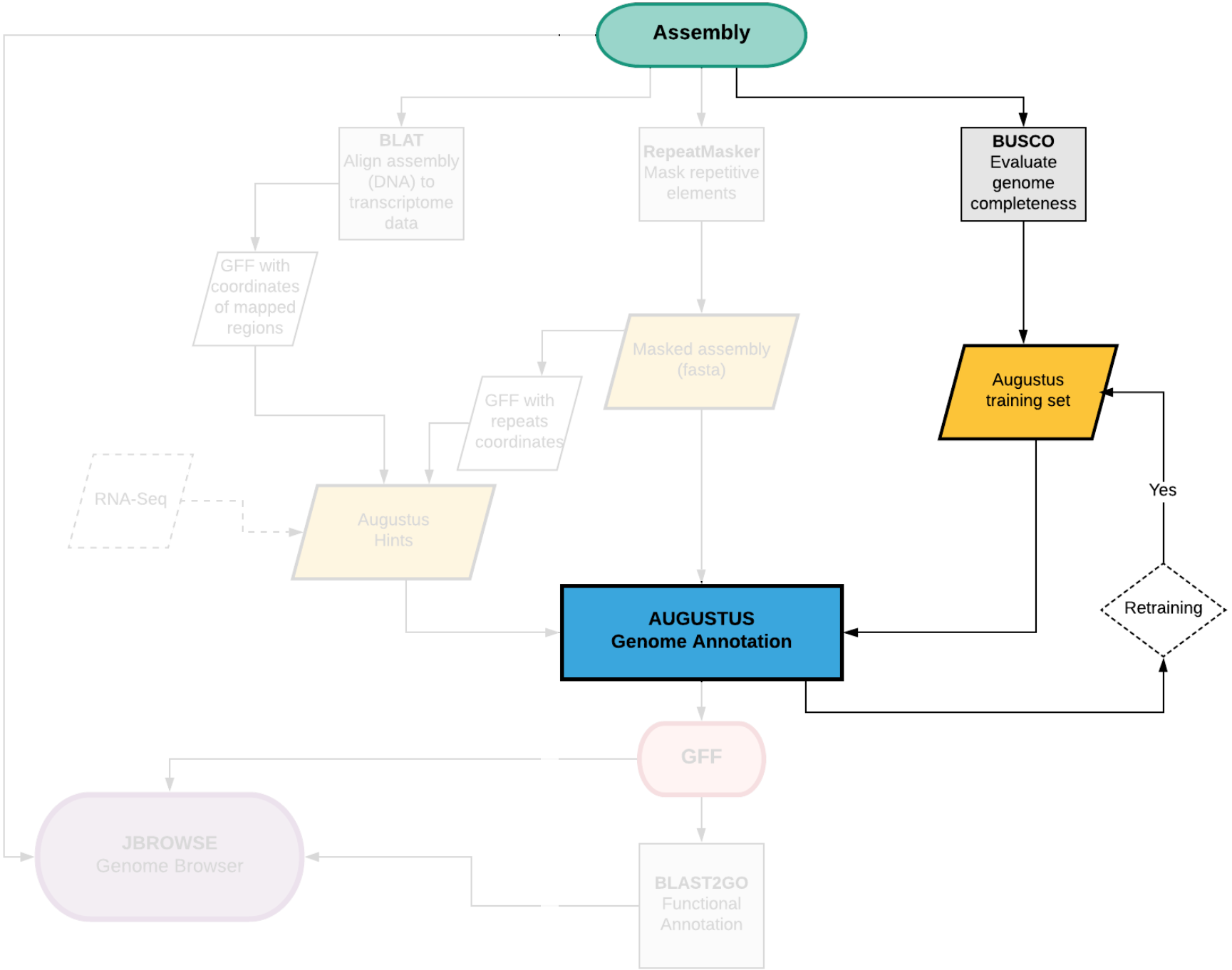
```
genome_annot
     |__ assembly
     |__ augustus
     |__ blast
     |__ b2go
     |__ blat
     |__ busco
     |__ jbrowse
     |__ jobs
     |__ logs
     |__ repmasker
```

- ASSEMBLY
  - Species: *Spinus cucullatusi* (siskin_10largest.fasta)
  - Copy the file to your folder assembly. File location: /data/genomics/workshops/SMSC_2019/siskin_10largest.fasta
- We will use a python script (available in the module assembly_stats) to get some basic info about this genome.
- We will run this part from the interactive queue. Login using qrsh



BUSCO

BENCHMARKING UNIVERSAL SINGLE-COPY ORTHOLOGS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BUSCO

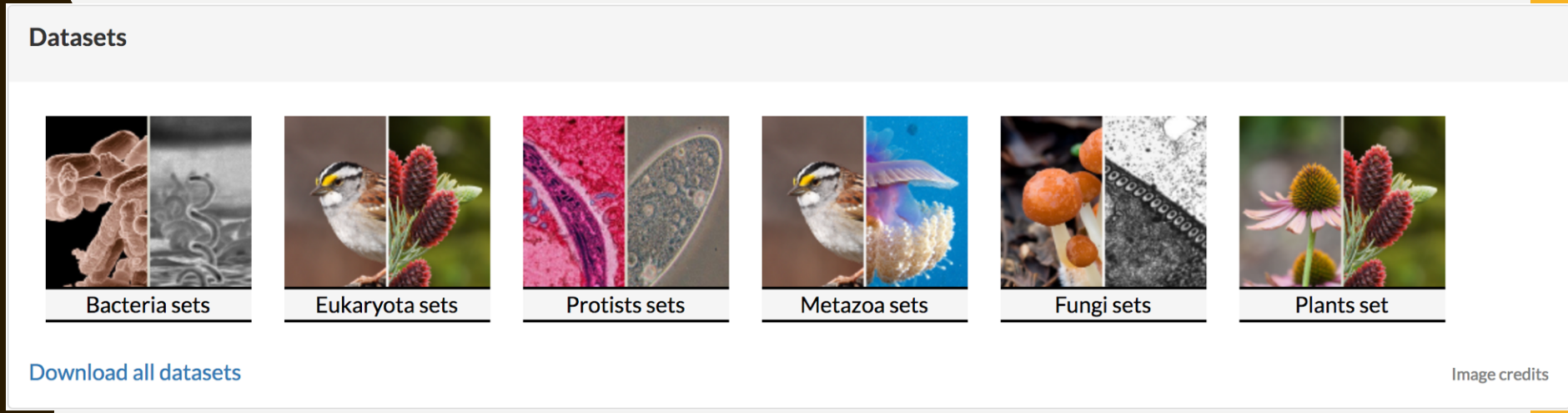
- Benchmarking Universal Single-Copy Orthologs

What is a ortholog?

Orthologs are genes in different species that evolved from a common ancestral gene by speciation.

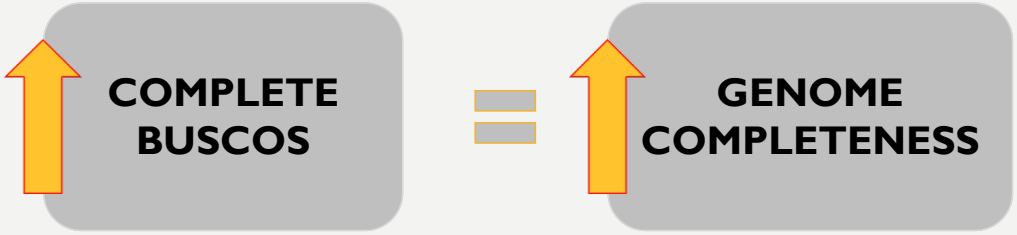
BUSCO:
HOW COMPLETE IS THE ASSEMBLY?

- Database: taxon-specific single copy orthologs



BUSCO:
HOW COMPLETE IS THE ASSEMBLY?

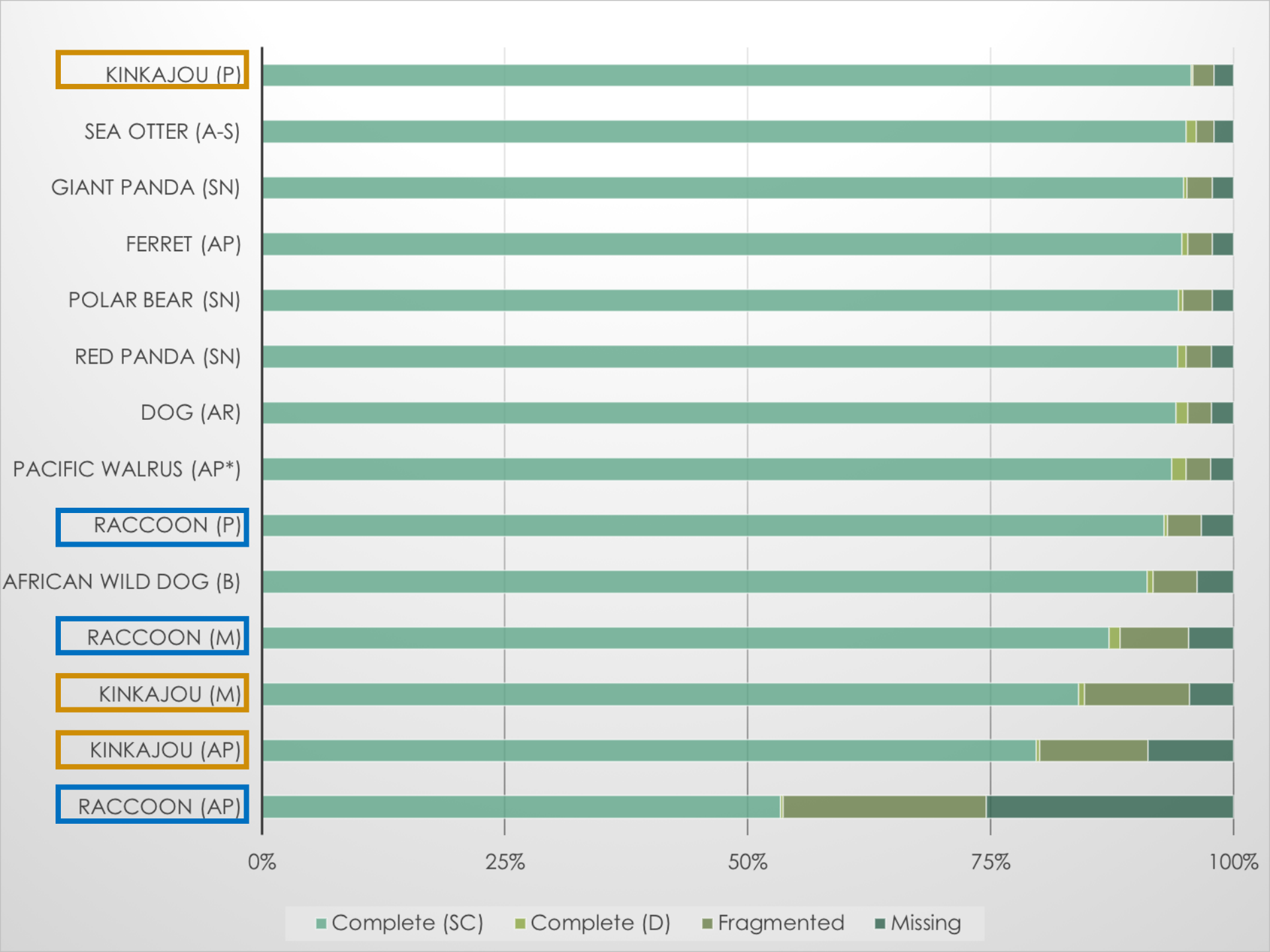
- Assessment:
  - Complete (single copy or duplicate)
  - Fragmented
  - Missing



ASSEMBLERS

		ALLPATHS-LG	Platanus	MaSuRCA
Raccoon (34X)	Number	42,696	50,007	277,099
	N50 (Mb)	0.11	1.45	0.38
	Longest (Mb)	1.83	10.59	3.43
	Total Length (Gb)	1.79	2.25	2.78
Kinkajou (48X)	Number	23,505	15,879	67,074
	N50 (Mb)	0.29	3.55	0.12
	Longest (Mb)	3.91	15.44	1.01
	Total Length (Gb)	2.05	2.21	2.3

3 paired end libraries (350 bp) + 2 mate pair libraries (3 kb and 8 kb)

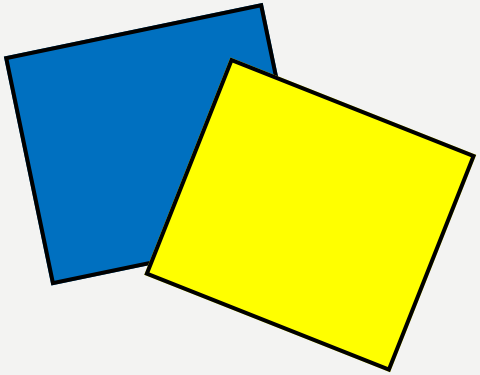

KINKAJOU (P)
SEA OTTER (A-S)
GIANT PANDA (SN)
FERRET (AP)
POLAR BEAR (SN)
RED PANDA (SN)
DOG (AR)
PACIFIC WALRUS (AP*)
RACCOON (P)
AFRICAN WILD DOG (B)
RACCOON (M)
KINKAJOU (M)
KINKAJOU (AP)
RACCOON (AP)
0%
25%
50%
75%
100%
Complete (SC)
Complete (D)
Fragmented
Missing

BUSCO: TASKS

```
genome_annot
     |__ assembly
     |__ augustus
     |__ blast
     |__ b2go
     |__ blat
     |__ busco
     |__ jbrowse
     |__ jobs
     |__ logs
     |__ repmasker
```

1. Copy the augustus config folder to YOUR augustus folder

```
cp -r /share/apps/bioinformatics/augustus/conda/3.3.2/config/ .
```



BUSCO: TASKS

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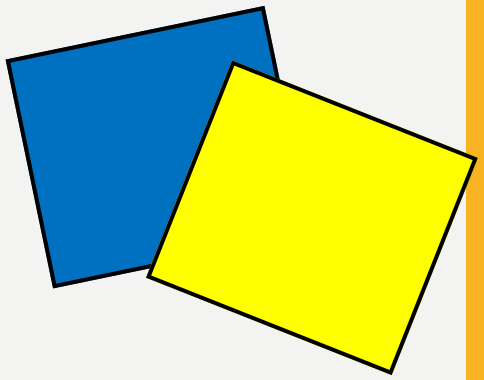
    |__ augustus
          |__ config
    |__ blast
    |__ b2go
    |__ blat
    |__ busco
    |__ jbrowse
    |__ jobs
    |__ logs
    |__ repmasker
```

1. Download the most appropriate database to your busco folder (in this case, we will use Aves)
2. Create and submit the the BUSCO job:
   a) One for the contig

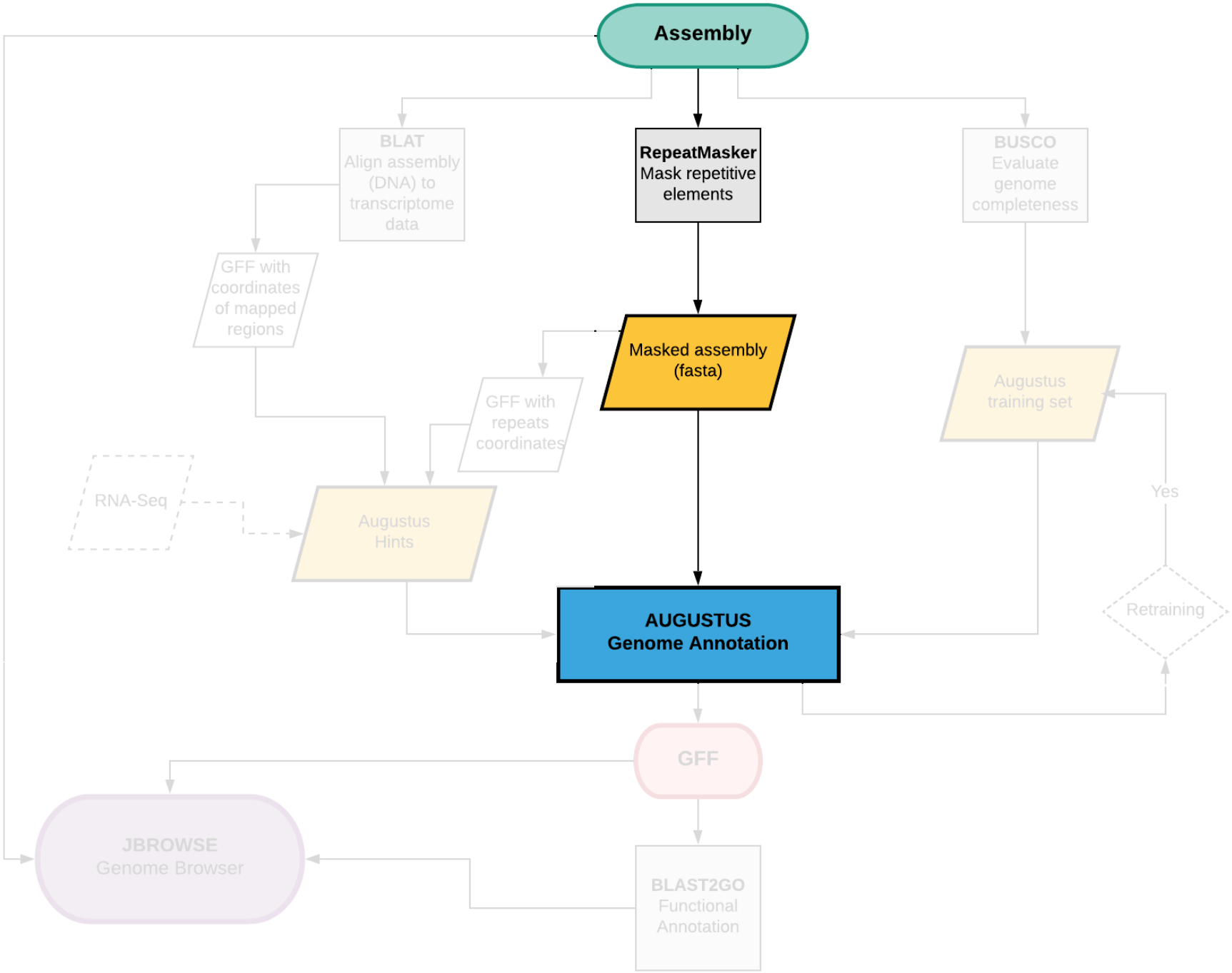


BUSCO

- Tomorrow we will look at the BUSCO results. It will take about ~12 hours for it to run.

MASKING AND ANNOTATION OF REPETITIVE ELEMENTS

REPEATMASKER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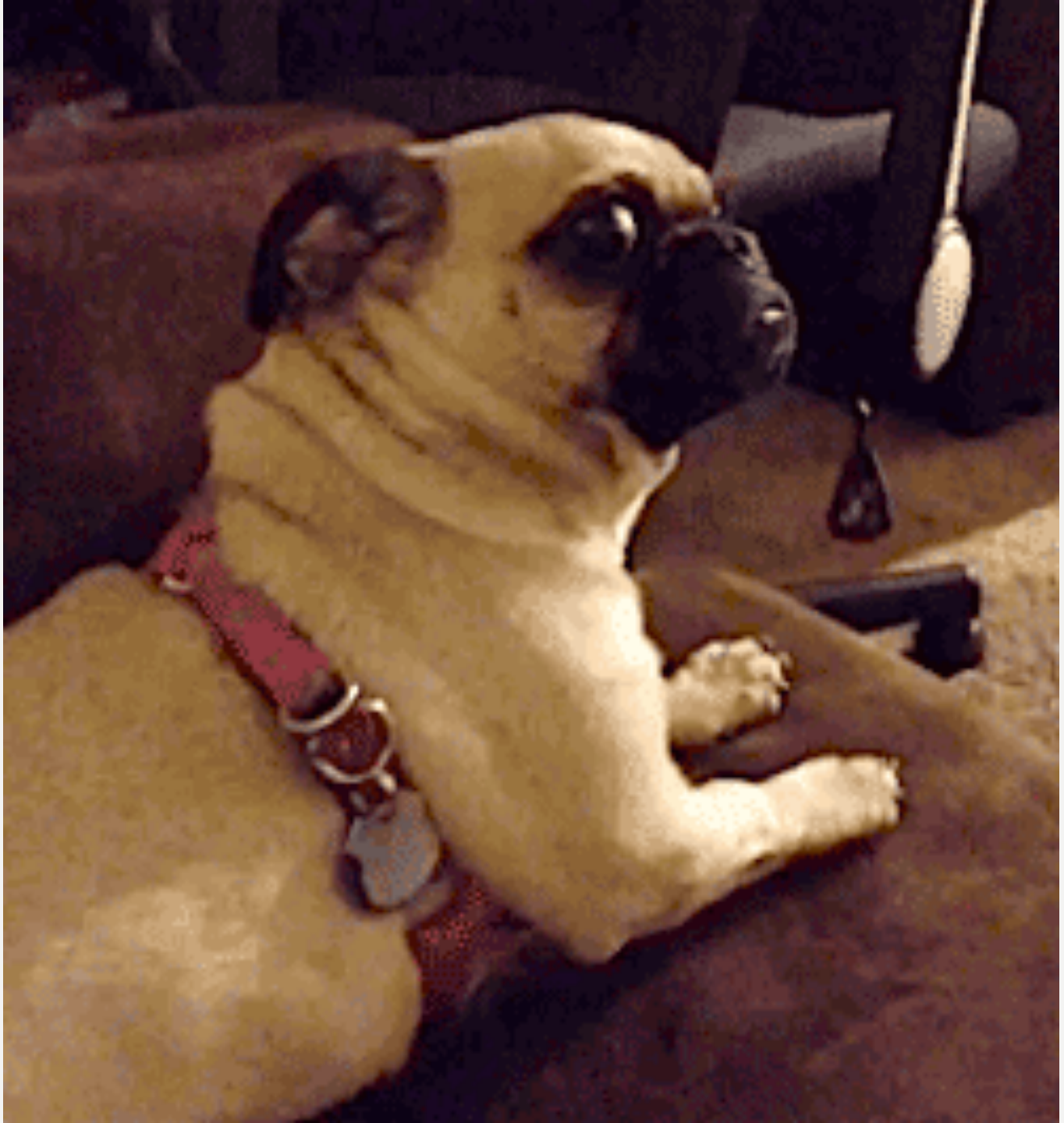
REPEATMASKER

- RepeatMasker is a program that screens DNA sequences for interspersed repeats and low complexity DNA sequences.

(from the RepeatMasker website)



>Contig3141_pilon
GACGGTCTGTGCTAGTTCAGCACTCATCCACTGTTCCAGCTGGCAGCCCG
CTCGAAGGTGAAAAAATACTATAGATAGATATGTAGATGTAAAAAGCAGA
TGAGAACCGGTCGCATATATAGACAAGCTCGCGTAACTCTGGAGGAGGAG
AGTGAGCCCTGCGCTCCAGAGATGTCCTTAGAGACTCCGCCGCTCTGATA
CTGTGTCTAGCCATATATAGACAACCAACGAGTCAACCCACTACCGAAAA
GTTGCGTGGTACTGAACGCTCATTCGCGCTTGCCACGCTCTCTCTCGAGA
ACAGCTCTAGCACCAAATATAAGGACCATCCGCACATCGAGCACCTATGG
AGGCTATCTCACCCGCGTGGTGGCGAACACTCAGCGCTCACACACTATGC
GTAAGCCTCTGATTCTACTAGCCAATTGTAACATCAGCCCAAAACAGCAT
TGTTTGAGAACTTGCCCTCTACTTCTACAGATTATCGCTCAAATCTAACA
TATGCTGAGGTTCAATCTGCCCTCCGCTACTTTAGTGGAGACACATGTAA
ATACAGTCGAGGTGCATCACATTAGTAACTCTCGAGAGACTGAGTCTCTA
CAGctctctctctctccctctatctATGCCTATATGCGAGTGCTGAGA
GGACTAGCTAGTGACACGTGCTGGTATCTCTATGCTCTCTCTCATTGCTA
GATGGACACATCGTGGGTGGCAGCGCACGCGAGAGACGCATACTTAGCTG
AACGTCTCCATTATTCACTCCCTTACATCAGATAGCATAACATCTAGAAG
TTACTGTGGTAGCACCCAtttctatttatttttttttttttatttttttt
ttttttCCTACAGAAGTGCTATCTTCAGCACTGAGCGGCGAAAACAGCTA
TTTTGCAGATATTCCATCGAAAATAGACTCCAAAAACATCATCATGAAGA
TCGACCACTTCCCCCTGATATAGGATTTCACAAGTTAAGATGGAAGATGG
CTATTTTCAGGTAGCAGTGGAAGCTGCTTCCTCTTCTCTCGCCTTGGTAG
CAGCCGACGGCTGGATTTGGGCCTCCACATTGCACCCCCAGGTCTTGGCT
GCAAGGACAAGAGAGAGATACTTGGCAAAAAAATCCACGGTTGGTGCTCT
CTGTCCCCCTCTCGGCCTTTGACCTTGTTATGACTTCCTGAGGTATATAT

```
file name: siskin_10largest.fasta
sequences:            10
total length:   41463291 bp  (41459480 bp excl N/X-runs)
GC level:         42.07 %
bases masked:    1027439 bp ( 2.48 %)
==================================================
```

	number of elements*	length occupied	percentage of sequence
DNA transposons	256	40450 bp	0.10 %
hobo-Activator	41	7588 bp	0.02 %
Tc1-IS630-Pogo	13	2092 bp	0.01 %
En-Spm	0	0 bp	0.00 %
MuDR-IS905	0	0 bp	0.00 %
PiggyBac	0	0 bp	0.00 %
Tourist/Harbinger	31	5322 bp	0.01 %
Other (Mirage, P-element, Transib)	0	0 bp	0.00 %
Rolling-circles	1	108 bp	0.00 %
Unclassified:	100	15272 bp	0.04 %
Total interspersed repeats:		538158 bp	1.30 %
Small RNA:	110	13149 bp	0.03 %
Satellites:	0	0 bp	0.00 %
Simple repeats:	8441	395878 bp	0.95 %
Low complexity:	1650	88173 bp	0.21 %

```
==================================================

* most repeats fragmented by insertions or deletions
  have been counted as one element


The query species was assumed to be gallus gallus
RepeatMasker Combined Database: Dfam_3.0

run with rmblastn version 2.9.0+
```

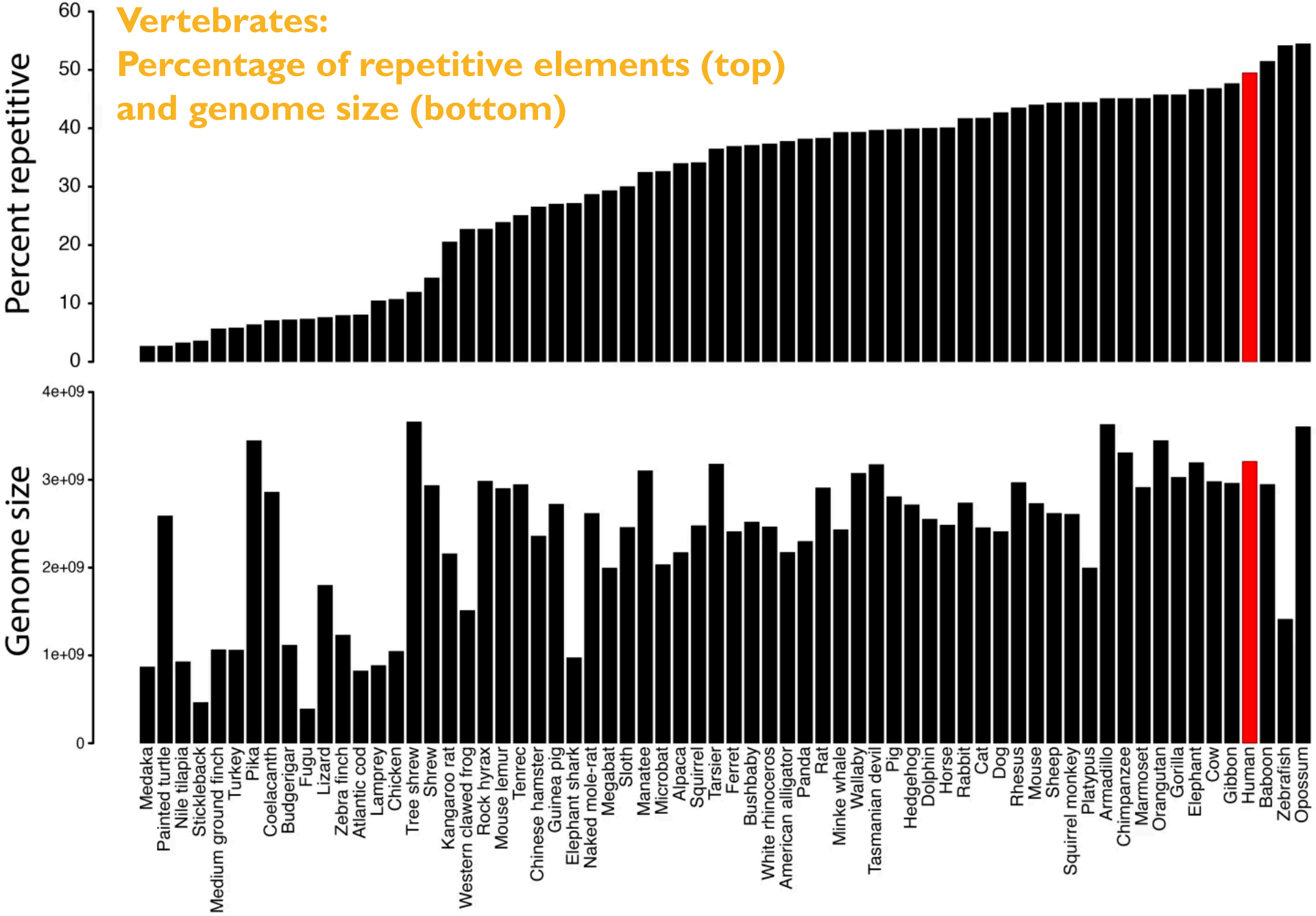
Run information: input file, total length, % bases masked

Types of repetitive elements, quantity, length and percentage of sequence

Repeat database and program version

```
[tsuchiyam@hydra-login01 repmasker]$ cat siskin_10largest.fasta.out
   SW   perc perc perc  query             position in query           matching            repeat              position in repeat
score   div. del. ins.  sequence          begin   end        (left)   repeat              class/family      begin  end    (left)    ID

   35    5.1  0.0  0.0  Contig1977_pilon      392     432 (3768949) + (GCCGCT)n             Simple_repeat         1     41    (0)      1
   24   14.2  3.5  3.5  Contig1977_pilon     6593    6650 (3762731) + (CCG)n                Simple_repeat         1     58    (0)      2
   18   13.3  7.0  2.2  Contig1977_pilon     7122    7174 (3762207) + (AT)n                 Simple_repeat         1     55    (0)      3
   15    5.6  0.0  0.0  Contig1977_pilon     9974    9992 (3759389) + (T)n                  Simple_repeat         1     19    (0)      4
   15   19.7  0.0  0.0  Contig1977_pilon    20766   20794 (3748587) + (T)n                  Simple_repeat         1     29    (0)      5
   17    3.8  3.7  0.0  Contig1977_pilon    27637   27663 (3741718) + (TTTTG)n              Simple_repeat         1     28    (0)      6
   12   12.5  0.0  3.7  Contig1977_pilon    31589   31616 (3737765) + (TTTGG)n              Simple_repeat         1     27    (0)      7
  230   29.3  5.8  6.9  Contig1977_pilon    32387   32591 (3736790) + CR1-3_Croc            LINE/CR1            950   1152 (2453)     8
  236   30.6 11.1  0.0  Contig1977_pilon    32767   32874 (3736507) + CR1-L3A_Croc          LINE/CR1           3284   3403  (885)     9
   12   14.7  3.3  0.0  Contig1977_pilon    33646   33675 (3735706) + (CTGCTG)n             Simple_repeat         1     31    (0)     10
   13   17.0  0.0  5.7  Contig1977_pilon    40521   40557 (3728824) + (CCGCC)n              Simple_repeat         1     35    (0)     11
   14    4.0  7.7  0.0  Contig1977_pilon    42391   42416 (3726965) + (CACA)n               Simple_repeat         1     28    (0)     12
   13   18.9  0.0  0.0  Contig1977_pilon    43596   43625 (3725756) + (TTA)n                Simple_repeat         1     30    (0)     13
   13   26.2  4.9  1.6  Contig1977_pilon    46810   46870 (3722511) + (GCCCG)n              Simple_repeat         1     63    (0)     14
   14   16.3  4.7  2.3  Contig1977_pilon    46897   46939 (3722442) + (GGCGG)n              Simple_repeat         1     44    (0)     15
   13   15.9  2.7  2.7  Contig1977_pilon    53355   53391 (3715990) + (TTTCTT)n             Simple_repeat         1     37    (0)     16
  264   16.3  1.4 17.2  Contig1977_pilon    58730   58803 (3710578) C CR1-3_Croc            LINE/CR1             (1)   3604   3541     17
   24   16.4  0.0  0.0  Contig1977_pilon    83019   83059 (3686322) + (T)n                  Simple_repeat         1     41    (0)     18
   22   21.9  4.6  1.5  Contig1977_pilon    83423   83487 (3685894) + (AT)n                 Simple_repeat         1     67    (0)     19
   30    0.0  0.0  0.0  Contig1977_pilon    84441   84468 (3684913) + (T)n                  Simple_repeat         1     28    (0)     20
   88    0.0  0.0  0.0  Contig1977_pilon    85203   85279 (3684102) + (AAT)n                Simple_repeat         1     77    (0)     21
   12   10.3  6.1  2.9  Contig1977_pilon    87513   87545 (3681836) + (ACTC)n               Simple_repeat         1     34    (0)     22
   32   23.3  1.5  7.1  Contig1977_pilon    91116   91248 (3678133) + (GCCCCG)n             Simple_repeat         1    126    (0)     23
  125    0.0  3.1  7.6  Contig1977_pilon    94671   94863 (3674518) + (TCCTT)n              Simple_repeat         1    185    (0)     24
   11   10.6  6.2  8.5  Contig1977_pilon    98745   98792 (3670589) + (TCTGATAA)n           Simple_repeat         1     47    (0)     25
   78   21.1  2.8  2.3  Contig1977_pilon   105934  106149 (3663232) + (GCAGG)n              Simple_repeat         1    217    (0)     26
   24   13.6  9.2  0.0  Contig1977_pilon   106238  106302 (3663079) + (CCCGCGC)n            Simple_repeat         1     71    (0)     27
   21   20.5  0.0  0.0  Contig1977_pilon   110256  110295 (3659086) + A-rich               Low_complexity        1     40    (0)     28
  267   22.9  4.3  0.0  Contig1977_pilon   111594  111663 (3657718) + CR1-3_Croc           LINE/CR1           2683   2755  (850)     29
   92    0.0  0.0  0.0  Contig1977_pilon   116028  116108 (3653273) + (AAAAT)n             Simple_repeat         1     81    (0)     30
   12   11.1  0.0  9.4  Contig1977_pilon   119685  119719 (3649662) + (GCTGA)n             Simple_repeat         1     32    (0)     31
   13   28.4  0.0  0.0  Contig1977_pilon   122110  122152 (3647229) + A-rich               Low_complexity        1     43    (0)     32
   11    9.7  8.6  2.7  Contig1977_pilon   126315  126349 (3643032) + (GGAGGCT)n           Simple_repeat         1     37    (0)     33
   43    2.5  0.0  0.0  Contig1977_pilon   136676  136716 (3632665) + (TA)n                Simple_repeat         1     41    (0)     34
   13   10.8  3.1  3.1  Contig1977_pilon   154695  154726 (3614655) + (TTGGT)n             Simple_repeat         1     32    (0)     35
   13    0.0  7.7  3.7  Contig1977_pilon   157644  157669 (3611712) + (CTAAT)n             Simple_repeat         1     27    (0)     36
   15    5.5  0.0  0.0  Contig1977_pilon   157828  157846 (3611535) + (AC)n                Simple_repeat         1     19    (0)     37
   12    5.5  0.0  0.0  Contig1977_pilon   176097  176115 (3593266) + (CCA)n               Simple_repeat         1     19    (0)     38
   15   24.4  5.8  1.4  Contig1977_pilon   176667  176735 (3592646) + A-rich               Low_complexity        1     72    (0)     39
   13    7.8  7.4  0.0  Contig1977_pilon   177664  177690 (3591691) + (CTC)n               Simple_repeat         1     29    (0)     40
  225   11.3  6.5  4.3  Contig1977_pilon   179927  179972 (3589409) + CR1-3_Croc           LINE/CR1           1299   1345 (2260)     41
   15   24.1  0.0  2.2  Contig1977_pilon   182585  182630 (3586751) + G-rich               Low_complexity        1     45    (0)     42
```



https://davetang.org/muse/2014/05/22/repetitive-elements-in-vertebrate-genomes/

REPEATMASKER

- RepeatMasker has several repetitive elements databases (Eukaryotic species only)

- Commonly used species include: mammal, carnivore, rodentia, rat, cow, pig, cat, dog, chicken, fugu, danio, "ciona intestinalis" drosophila, anopheles, elegans, diatoaea, artiodactyl, arabidopsis, rice, wheat, and maize

HOW TO KNOW WHICH SPECIES TO USE?

REPEATMASKER

- To query the RepeatMasker database by taxonomy, you can use the following command:

```
queryTaxonomyDatabase.pl -species Spinus
```

- You can also see the repeat library available for your species

```
queryRepeatDatabase.pl -species Spinus
```

REPEATMASKER

- Better way to query:

```
Repbase_RepeatQuery_Taxonomy_MTNT.sh spinus
```

(Copy the script from /data/genomics/workshops/SMSC_2019/)

PARAMETERS

RepeatMasker

-species chicken: RepBase species

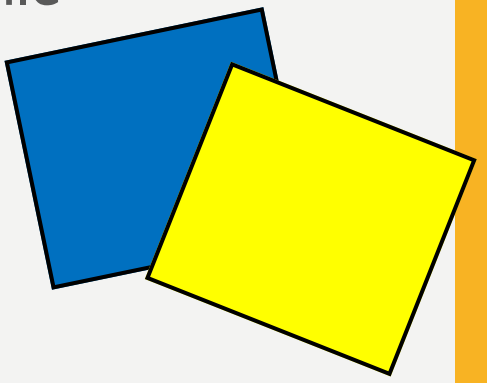
-xsmall : soft-masking (repetitive elements are masked in low caps instead of replaced by N)

-gff: additional output in gff2 format

-pa $NSLOTS: number of cpus

-dir /path/: output the results to the specified folder

../assembly/Contig3141_pilon.fasta: input file



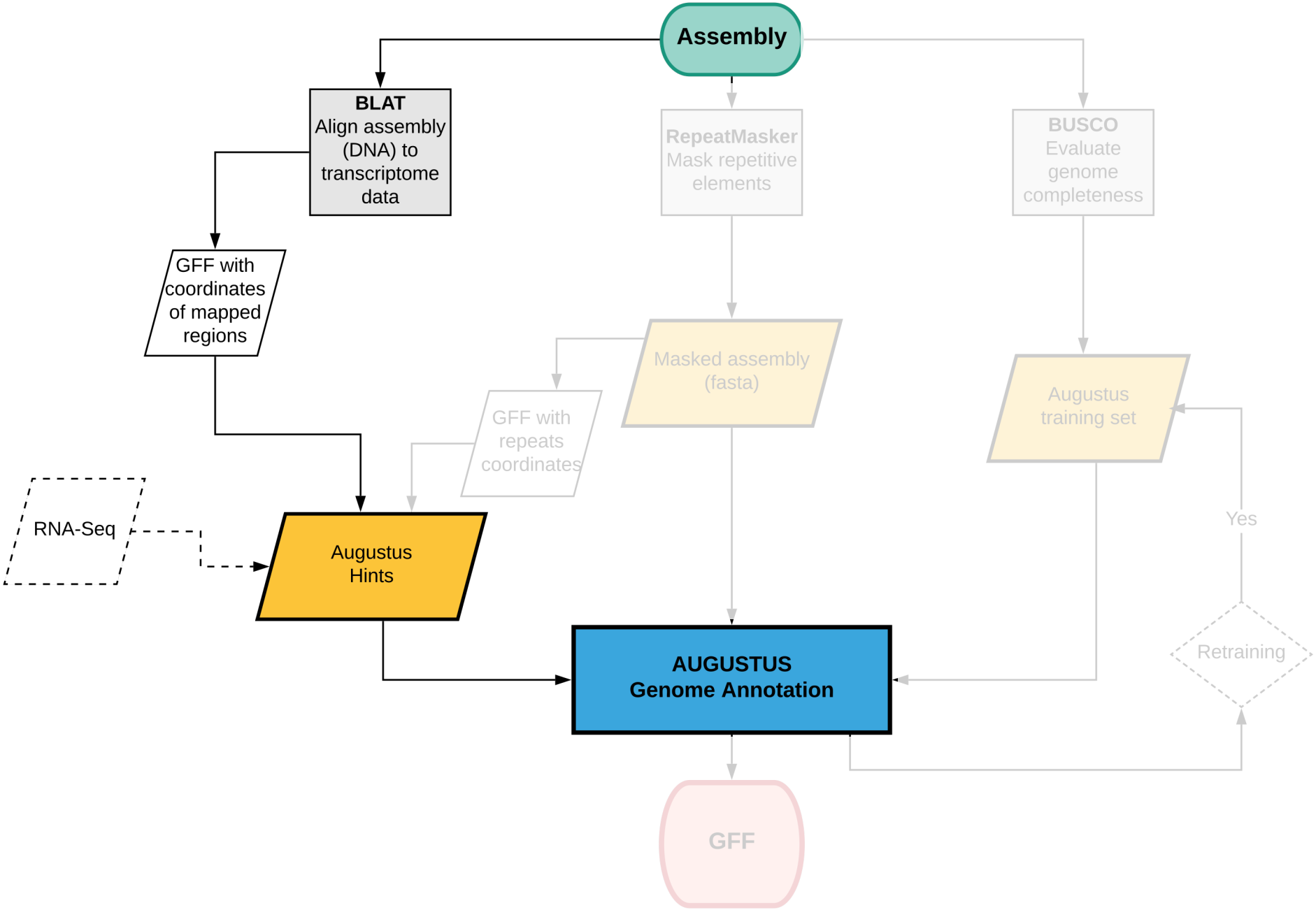
BLAT

BLAST-LIKE ALIGNMENT TOOL

OTHER SOURCES OF EVIDENCE

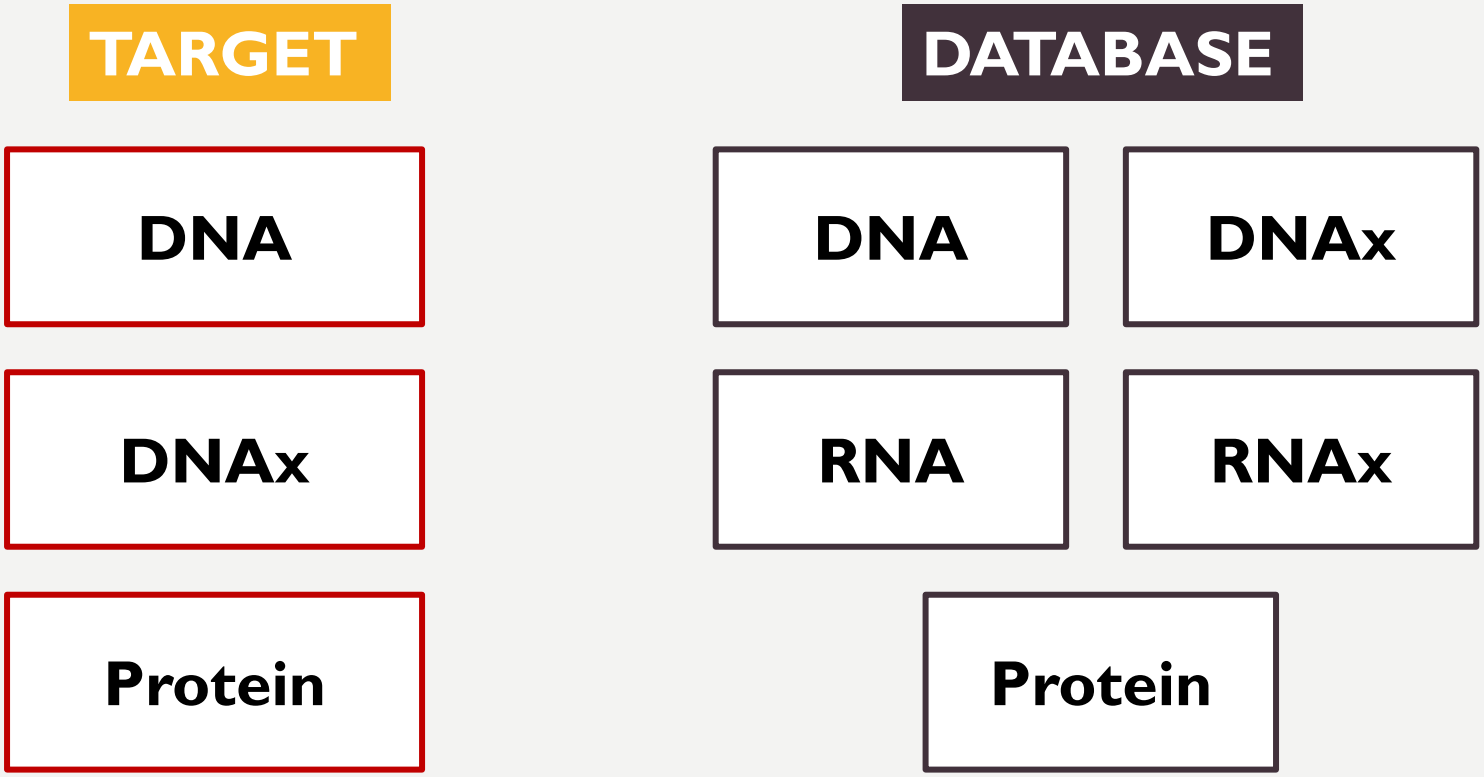
- RNA-Seq
- Transcriptomes

Today we will use the transcriptome of a different species to generate another source of information for the annotation


Assembly
BLAT
Align assembly (DNA) to transcriptome data
RepeatMasker
Mask repetitive elements
BUSCO
Evaluate genome completeness
GFF with coordinates of mapped regions
Masked assembly (fasta)
Augustus training set
GFF with repeats coordinates
RNA-Seq
Augustus Hints
Yes
Retraining
AUGUSTUS
Genome Annotation
GFF

BLAT

- BLAST-like Alignment Tool



DNAx and RNAx correspond to 6-frame translated sequences

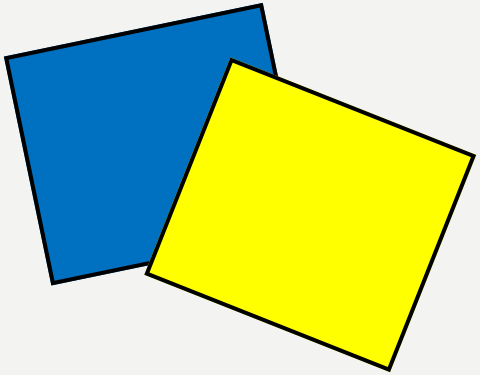
BLAT: WHAT DOES IT TELL US?

- It provides information regarding exons and introns, based on the alignment of the transcriptome sequence to our assembly.

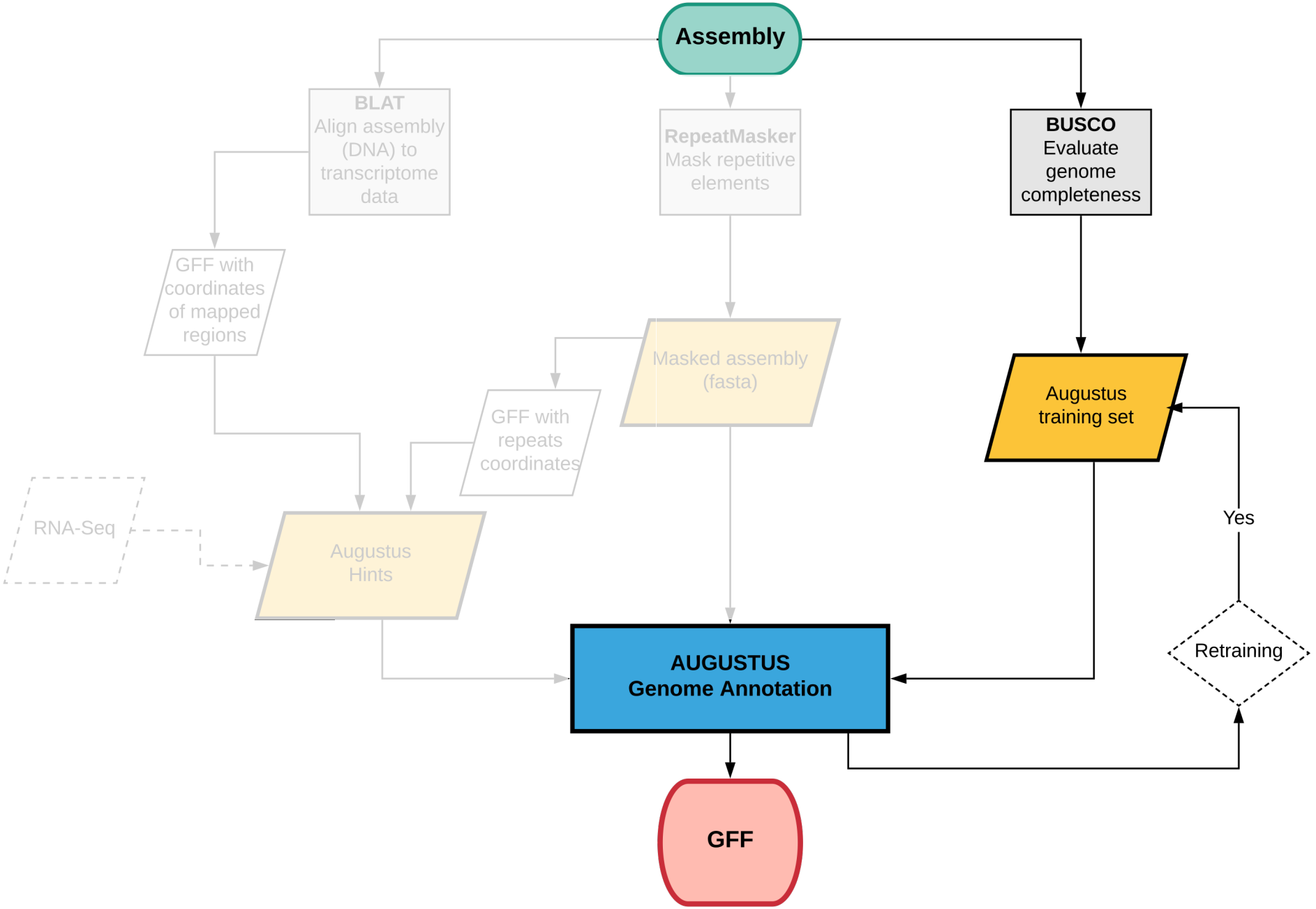
BLAT: TASKS

```
genome_annot
    |__ assembly
    |__ augustus
    |__ blast
    |__ b2go
    |__ blat
    |__ busco
    |__ jbrowse
    |__ jobs
    |__ logs
    |__ repmasker
```

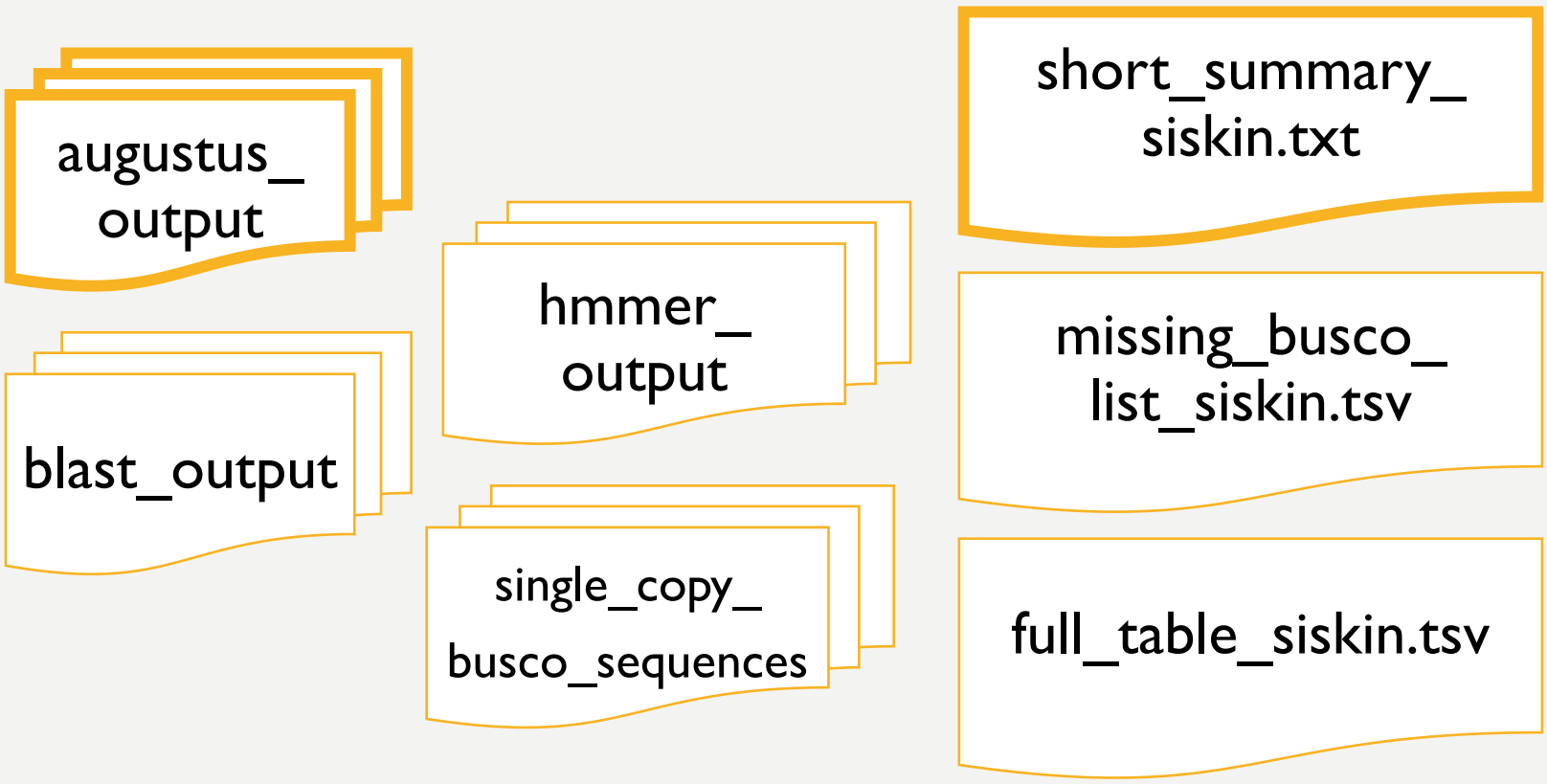
1. Download the transcriptome of *Taeniopygia guttata* from Genbank. Extract the file.
2. Create the BLAT job
3. Submit the job



BUSCO - RESULTS


Assembly
BLAT
Align assembly (DNA) to transcriptome data
RepeatMasker
Mask repetitive elements
BUSCO
Evaluate genome completeness
GFF with coordinates of mapped regions
Masked assembly (fasta)
Augustus training set
GFF with repeats coordinates
RNA-Seq
Augustus Hints
Yes
Retraining
AUGUSTUS
Genome Annotation
GFF

BUSCO OUTPUT: RUN_SISKIN



BUSCO - RESULTS

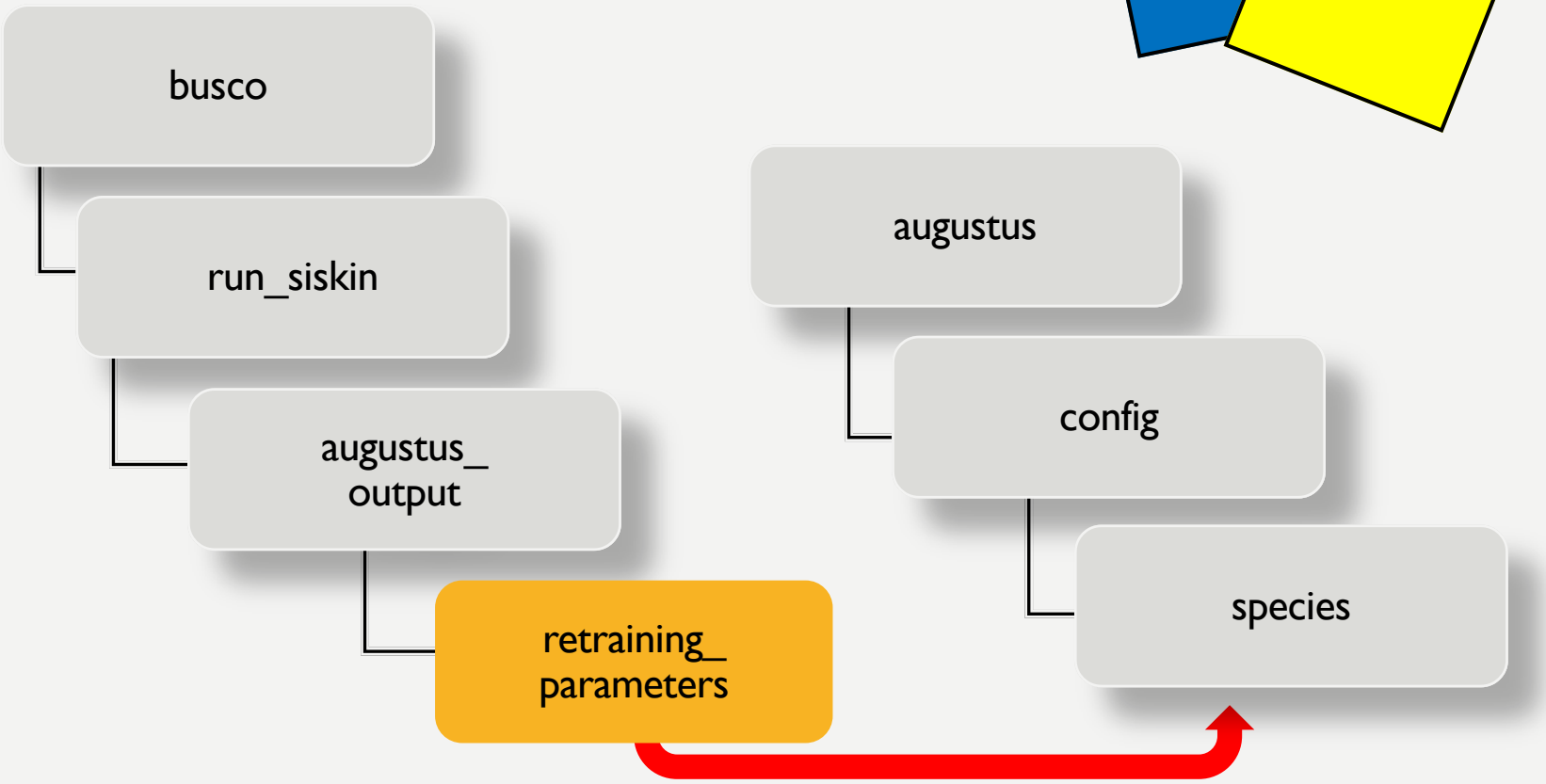
- Check the results of your BUSCO run in the short_summary_siskin.txt
  - How many Complete (C), Duplicated (D), Fragmented (F) and Missing (M)?
  - Do you think this is a good or a bad assembly?

BUSCO - RESULTS

- Compare it to the results from the entire assembly:

FROM BUSCO TO AUGUSTUS

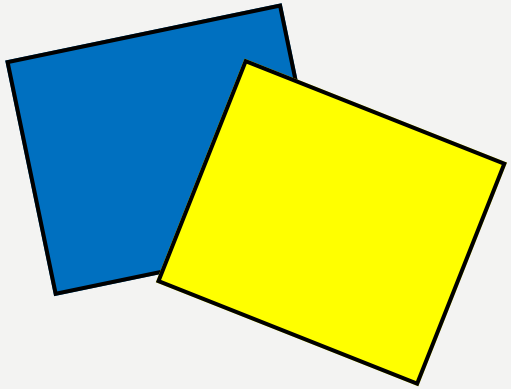
1. Copy the folder retraining_parameters to augustus/config/species



FROM BUSCO TO AUGUSTUS

2. Rename the folder retraining_parameters using the prefix that appears in all files.

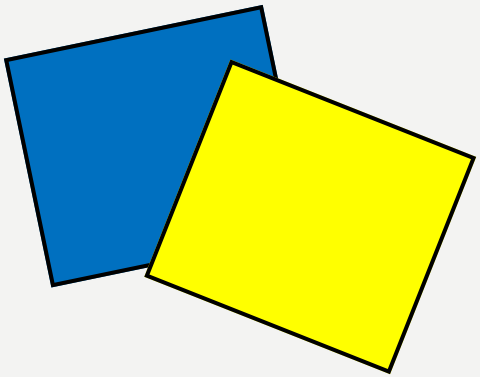
You can find this info by looking at the file prefix inside the folder. In this case, we will rename the folder BUSCO_siskin_2598564919



CREATING HINTS FOR AUGUSTUS

AUGUSTUS: FOLDER STRUCTURE

- Create the following folders in your augustus directory:
  - hints
  - output
  - scaffolds



AUGUSTUS: FOLDER STRUCTURE

- The command ls should return the following result:

  config

  hints

  output

  scaffolds

IMPORTANT QUESTIONS

- What sources of extrinsic evidence do we have?
- How do those files look like?

We need to convert the hints into a format that augustus can read

GFF

- General Feature Format. Fields:
  - **seqname**
  - **source**
  - **feature**
  - **start**
  - **end**
  - **score**
  - **strand**
  - **frame**
  - **attribute**

GFF3 - REPEATMASKER

```
[tsuchiyam@compute-8-31 repmasker]$ cat test.gff3
##gff-version 3
##sequence-region Contig3141_pilon 1 5638391
Contig3141_pilon        RepeatMasker    dispersed_repeat        604     627     17      +       .       Target=(CT)n 1 24
Contig3141_pilon        RepeatMasker    dispersed_repeat        819     856     26      +       .       Target=(T)n 1 38
Contig3141_pilon        RepeatMasker    dispersed_repeat        3372    3459    30      +       .       Target=(TGTT)n 1 89
Contig3141_pilon        RepeatMasker    dispersed_repeat        3852    3879    12      +       .       Target=(GCCT)n 1 28
Contig3141_pilon        RepeatMasker    dispersed_repeat        5845    6049    780     +       .       Target=CR1-X2 3923 4133
Contig3141_pilon        RepeatMasker    dispersed_repeat        6253    6290    18      +       .       Target=(ATT)n 1 38
Contig3141_pilon        RepeatMasker    dispersed_repeat        17123   17168   14      +       .       Target=G-rich 1 44
Contig3141_pilon        RepeatMasker    dispersed_repeat        23515   23627   459     +       .       Target=CR1-X1 4023 4133
Contig3141_pilon        RepeatMasker    dispersed_repeat        28192   28600   1488    -       .       Target=CR1-F2 4088 4497
Contig3141_pilon        RepeatMasker    dispersed_repeat        28926   28983   254     -       .       Target=UCON24 206 263
Contig3141_pilon        RepeatMasker    dispersed_repeat        30324   30384   20      +       .       Target=A-rich 1 59
Contig3141_pilon        RepeatMasker    dispersed_repeat        40281   40303   21      +       .       Target=(T)n 1 23
Contig3141_pilon        RepeatMasker    dispersed_repeat        42725   43050   969     -       .       Target=CR1-X2 3783 4098
Contig3141_pilon        RepeatMasker    dispersed_repeat        44280   44308   15      +       .       Target=(TCTTC)n 1 28
Contig3141_pilon        RepeatMasker    dispersed_repeat        44314   44343   32      +       .       Target=(T)n 1 30
Contig3141_pilon        RepeatMasker    dispersed_repeat        44531   44573   13      +       .       Target=(CTGCTG)n 1 46
Contig3141_pilon        RepeatMasker    dispersed_repeat        47527   47560   13      +       .       Target=(CCTCCC)n 1 33
Contig3141_pilon        RepeatMasker    dispersed_repeat        51189   51380   631     +       .       Target=CR1-H 4602 4798
Contig3141_pilon        RepeatMasker    dispersed_repeat        52831   52858   19      +       .       Target=(AACA)n 1 27
Contig3141_pilon        RepeatMasker    dispersed_repeat        57134   57152   15      +       .       Target=(T)n 1 19
Contig3141_pilon        RepeatMasker    dispersed_repeat        60288   60487   1008    +       .       Target=CR1-C4 4289 4508
Contig3141_pilon        RepeatMasker    dispersed_repeat        64723   64739   16      +       .       Target=(T)n 1 17
Contig3141_pilon        RepeatMasker    dispersed_repeat        64868   64896   15      +       .       Target=A-rich 1 29
Contig3141_pilon        RepeatMasker    dispersed_repeat        65872   65906   12      +       .       Target=(CTTTA)n 1 34
Contig3141_pilon        RepeatMasker    dispersed_repeat        72051   72093   47      +       .       Target=(T)n 1 43
Contig3141_pilon        RepeatMasker    dispersed_repeat        72913   73192   47      +       .       Target=(GT)n 1 276
Contig3141_pilon        RepeatMasker    dispersed_repeat        76071   76299   429     -       .       Target=GGLTR8B 2 234
Contig3141_pilon        RepeatMasker    dispersed_repeat        78999   79042   38      +       .       Target=(A)n 1 44
Contig3141_pilon        RepeatMasker    dispersed_repeat        80244   80288   28      +       .       Target=(A)n 1 45
Contig3141_pilon        RepeatMasker    dispersed_repeat        80590   80640   35      +       .       Target=(A)n 1 51
Contig3141_pilon        RepeatMasker    dispersed_repeat        84211   84687   1021    +       .       Target=CR1-C4 3956 4516
Contig3141_pilon        RepeatMasker    dispersed_repeat        90620   90684   235     +       .       Target=Chompy-2_Croc 6 72
```

GFF3 - BLAT

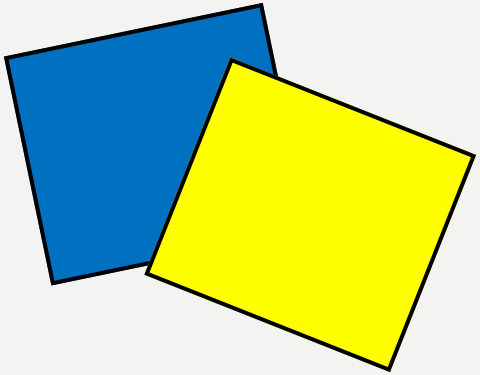
mtsuchiya — tsuchiyam@login-30-1:/scratch/genomics/tsuchiyam/RepeatMasker/RM_hints — ssh tsuchiyam@hydra-login01.si.edu — 144×44

```
Contig3141_pilon        b2h     ep      617     623     0       .       .       grp=XR_003076074.1;pri=4;src=E
Contig3141_pilon        b2h     ep      617     623     0       .       .       grp=XR_003076075.1;pri=4;src=E
Contig3141_pilon        b2h     ep      617     623     0       .       .       grp=XR_003076077.1;pri=4;src=E
Contig3141_pilon        b2h     ep      617     623     0       .       .       grp=XR_003076078.1;pri=4;src=E
Contig3141_pilon        b2h     ep      3406    3423    0       .       .       grp=XM_003641786.3;pri=4;src=E
Contig3141_pilon        b2h     ep      3409    3435    0       .       .       grp=XM_015291998.2;pri=4;src=E
Contig3141_pilon        b2h     ep      3409    3435    0       .       .       grp=XM_025153839.1;pri=4;src=E
Contig3141_pilon        b2h     ep      3409    3435    0       .       .       grp=XM_025153840.1;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=NM_001278026.1;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=NM_001278028.1;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_015296983.2;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_015296984.2;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_015296985.2;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_015296986.2;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_015296988.2;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_015296989.2;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_025142718.1;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_025142719.1;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_025142720.1;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_025142721.1;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_025142722.1;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_025142723.1;pri=4;src=E
Contig3141_pilon        b2h     ep      5916    5932    0       .       .       grp=XM_417523.6;pri=4;src=E
Contig3141_pilon        b2h     intron  28014   28314   0       .       .       mult=5;pri=4;src=E
Contig3141_pilon        b2h     ep      28316   28334   0       .       .       grp=XR_001469930.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28316   28334   0       .       .       grp=XR_003071673.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28325   28336   0       .       .       grp=XR_001464165.2;pri=4;src=E
Contig3141_pilon        b2h     ep      28325   28340   0       .       .       grp=XR_003071393.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28325   28340   0       .       .       grp=XR_003071394.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28325   28341   0       .       .       grp=XR_003074136.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28325   28345   0       .       .       grp=XR_003072657.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28325   28348   0       .       .       grp=XR_003074120.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28335   28348   0       .       .       grp=XR_003073844.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28328   28349   0       .       .       grp=XR_003073482.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28328   28349   0       .       .       grp=XR_003073483.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28328   28349   0       .       .       grp=XR_003073484.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28315   28350   0       .       .       grp=XR_003074104.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28315   28350   0       .       .       grp=XR_003074105.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28315   28350   0       .       .       grp=XR_003074106.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28315   28350   0       .       .       grp=XR_003074107.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28315   28350   0       .       .       grp=XR_003074108.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28325   28350   0       .       .       grp=XM_015286437.2;pri=4;src=E
Contig3141_pilon        b2h     ep      28325   28350   0       .       .       grp=XM_025148848.1;pri=4;src=E
Contig3141_pilon        b2h     ep      28325   28354   0       .       .       grp=XM_025149296.1;pri=4;src=E
```

AUGUSTUS HINTS: BLAT

1. Log to the interactive queue
2. Sort the .psl file
3. Load the augustus/3.3 module
4. Run the script blat2hints.pl

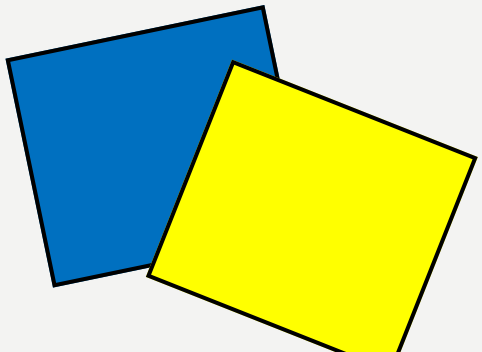
```
genome_annot
    |__ assembly
    |__ augustus
          |__ config
          |__ hints
          |__ output
          |__ scaffolds
    |__ blast
    |__ b2go
    |__ blat
    |__ busco
    |__ jbrowse
    |__ jobs
    |__ logs
    |__ repmasker
```



AUGUSTUS HINTS: REPEATMASKER

```
genome_annot
    |__ assembly
    |__ augustus
          |__ config
          |__ hints
          |__ output
          |__ scaffolds
    |__ blast
    |__ b2go
    |__ blat
    |__ busco
    |__ jbrowse
    |__ jobs
    |__ logs
    |__ repmasker
```

1. Load the module repeatmasker
2. Use the script rmOutToGFF3.pl to convert your .out file into GFF3
3. Use the script gff2hints to make the final conversion

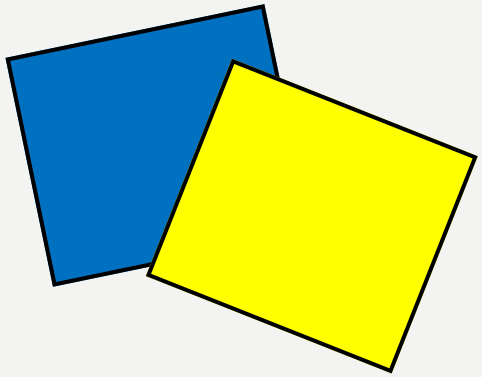


AUGUSTUS: COMBINING HINTS

```
genome_annot
    |__ assembly
    |__ augustus
          |__ config
          |__ hints
          |__ output
          |__ scaffolds
    |__ blast
    |__ b2go
    |__ blat
    |__ busco
    |__ jbrowse
    |__ jobs
    |__ logs
    |__ repmasker
```

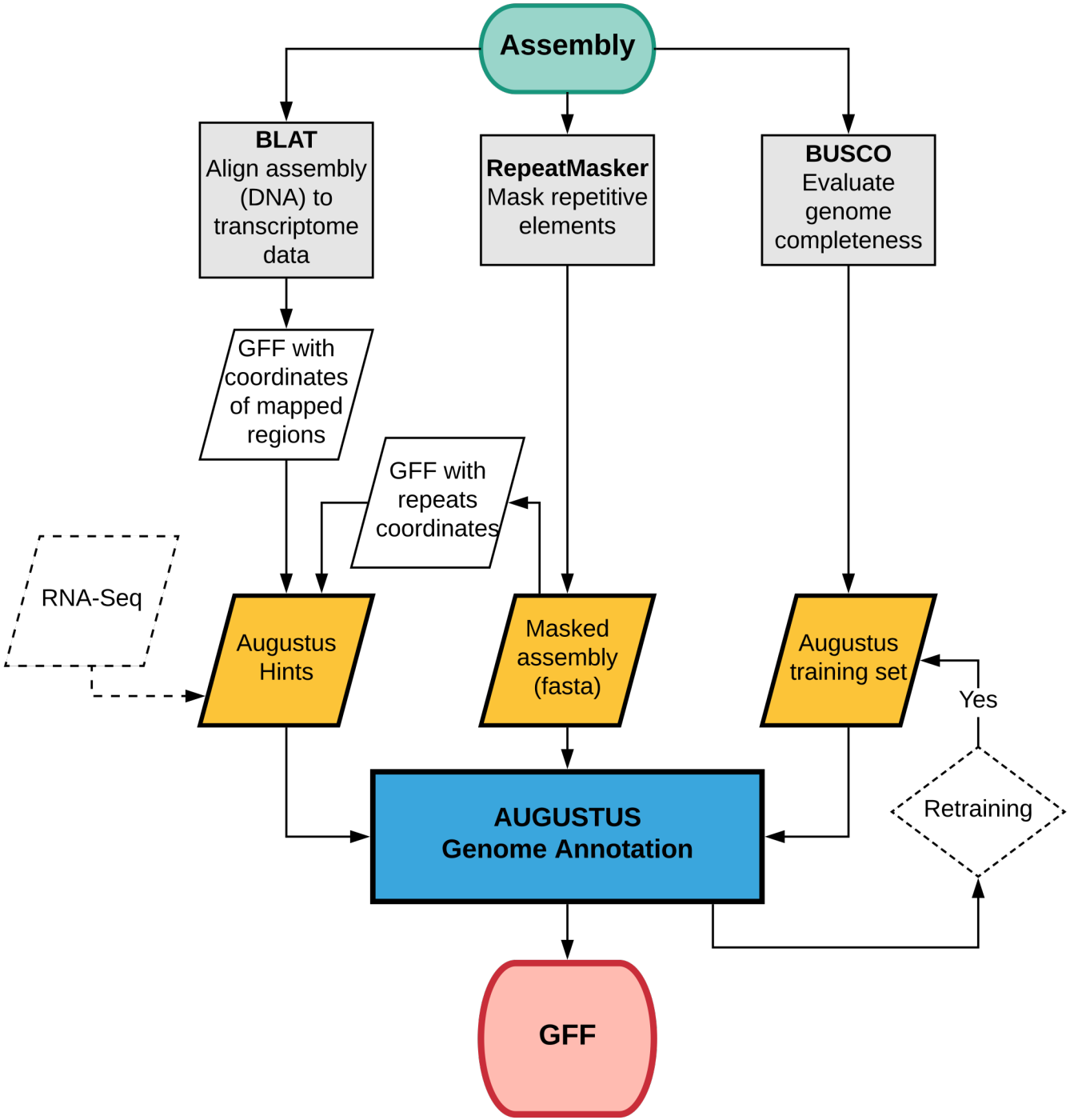
- Merge both files:

cat Dhydei_RM_hints.out Dhydei_blat_hints.out | sort -k1,1 -k4,4n > Dhydei_hints_RM_E.gff3



PHEW…

- What do we have now?
  - Masked fasta from RepeatMasker
  - Hints file
  - Training set from BUSCO
- What else do we need?


Assembly
BLAT
Align assembly (DNA) to transcriptome data
RepeatMasker
Mask repetitive elements
BUSCO
Evaluate genome completeness
GFF with coordinates of mapped regions
GFF with repeats coordinates
RNA-Seq
Augustus Hints
Masked assembly (fasta)
Augustus training set
Yes
Retraining
AUGUSTUS
Genome Annotation
GFF

AUGUSTUS

- ab initio (internal) + evidence-driven(external)

AUGUSTUS is based on a generalized hidden Markov model (GHMM), which defines probability distributions for the various sections of genomic sequences. Introns, exons, intergenic regions, etc. correspond to states in the model and each state is thought to create DNA sequences with certain pre-defined emission probabilities.

AUGUSTUS

- Augustus needs to be trained:
  - Training consists on the generation of a training set that will more accurately predict genes.

(BUSCO solved the training issue for us)

AUGUSTUS

- Inputs:
  - Masked fasta (repeatmasker output)
  - Hints file
  - Training set

AUGUSTUS EXTRINSIC FILE

- Defines how the information from the hints will be weighted:

- It assigns "bonus" and "malus" (penalty) values to each hint used
  - M: manual annotation
  - W: RNA-Seq coverage information
  - E: EST/cDNA database hit
  - R: retroposed genes
  - RM: repeat masking

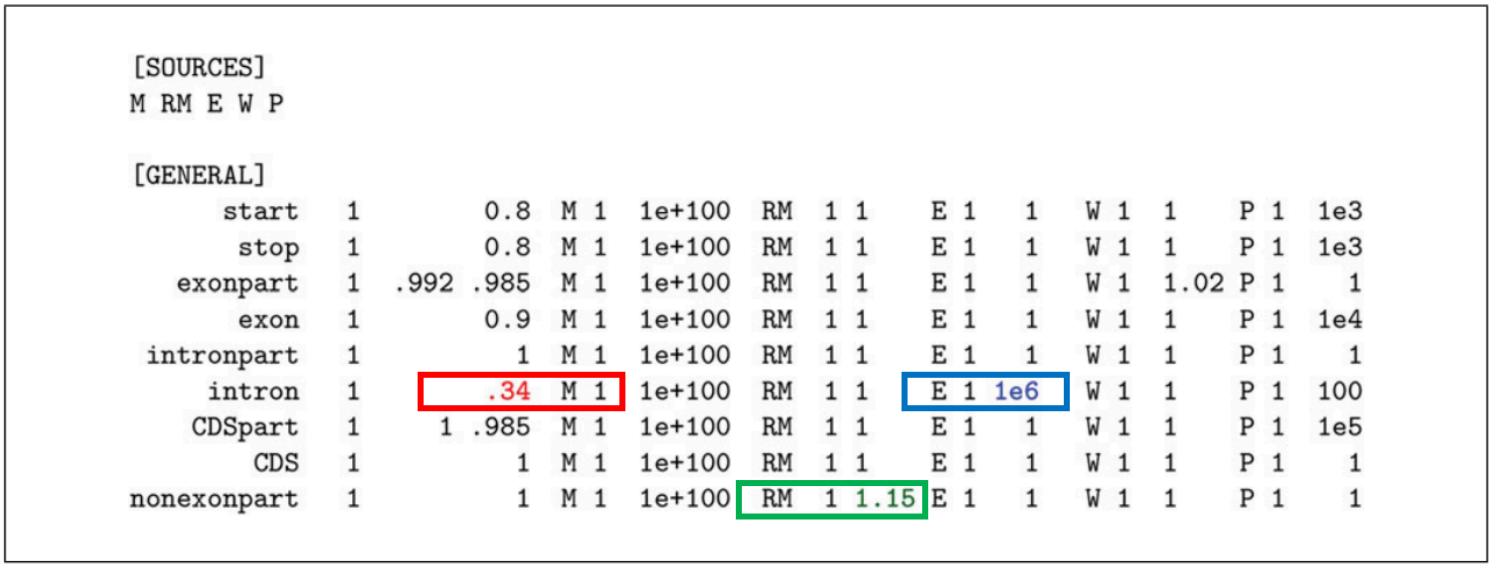
```
[SOURCES]
M RM E

#
# individual_liability: Only unsatisfiable hints are disregarded. By default this flag is not set
# and the whole hint group is disregarded when one hint in it is unsatisfiable.
# 1group1gene: Try to predict a single gene that covers all hints of a given group. This is relevant for
# hint groups with gaps, e.g. when two ESTs, say 5' and 3', from the same clone align nearby.
#
[SOURCE-PARAMETERS]


#   feature        bonus         malus   gradelevelcolumns
#               r+/r-
#
# the gradelevel colums have the following format for each source
# sourcecharacter numscoreclasses boundary    ...  boundary    gradequot  ...  gradequot
#

[GENERAL]
      start      1          1  M    1  1e+100  RM  1     1    E 1    1
       stop      1          1  M    1  1e+100  RM  1     1    E 1    1
        tss      1          1  M    1  1e+100  RM  1     1    E 1    1
        tts      1          1  M    1  1e+100  RM  1     1    E 1    1
        ass      1      1 0.1  M    1  1e+100  RM  1     1    E 1    1
        dss      1      1 0.1  M    1  1e+100  RM  1     1    E 1    1
   exonpart      1  .992 .985  M    1  1e+100  RM  1     1    E 1  1e2
       exon      1          1  M    1  1e+100  RM  1     1    E 1  1e4
 intronpart      1          1  M    1  1e+100  RM  1     1    E 1    1
     intron      1        .34  M    1  1e+100  RM  1     1    E 1  1e6
    CDSpart      1     1 .985  M    1  1e+100  RM  1     1    E 1    1
        CDS      1          1  M    1  1e+100  RM  1     1    E 1    1
    UTRpart      1     1 .985  M    1  1e+100  RM  1     1    E 1    1
        UTR      1          1  M    1  1e+100  RM  1     1    E 1    1
     irpart      1          1  M    1  1e+100  RM  1     1    E 1    1
nonexonpart      1          1  M    1  1e+100  RM  1     1.15 E 1    1
  genicpart      1          1  M    1  1e+100  RM  1     1    E 1    1

#
# Explanation: see original extrinsic.cfg file
```



```
[SOURCES]
M RM E W P

[GENERAL]
      start   1         0.8  M 1  1e+100  RM  1 1    E 1    1   W 1  1     P 1  1e3
       stop   1         0.8  M 1  1e+100  RM  1 1    E 1    1   W 1  1     P 1  1e3
   exonpart   1  .992 .985  M 1  1e+100  RM  1 1    E 1    1   W 1  1.02  P 1    1
       exon   1         0.9  M 1  1e+100  RM  1 1    E 1    1   W 1  1     P 1  1e4
 intronpart   1           1  M 1  1e+100  RM  1 1    E 1    1   W 1  1     P 1    1
     intron   1        .34   M 1  1e+100  RM  1 1    E 1 1e6    W 1  1     P 1  100
    CDSpart   1     1 .985   M 1  1e+100  RM  1 1    E 1    1   W 1  1     P 1  1e5
        CDS   1           1  M 1  1e+100  RM  1 1    E 1    1   W 1  1     P 1    1
nonexonpart   1           1  M 1  1e+100  RM  1 1.15 E 1    1   W 1  1     P 1    1
```

Figure 5 An excerpt of an extrinsic configuration file. In this example, each number to the right of the column filled with M's that is different from 1 specifies a *bonus*. A bonus is a relative factor that the un-normalized joint probability of gene structure candidate gets for being compatible with a hint of that type and source. For example, the blue *1e6* in the intron row after the source letter E means that for each intron hint with source tag E (src=E), gene structures that have an intron with both boundaries given as in the hint are rewarded by a factor of 10^6 relatively to gene structures disregarding the intron hint. A high bonus has the effect that many of the respective hints are respected by AUGUSTUS. The green 1.15 in the non-exonpart row after the tag RM (repeat masking) specifies that for each non exonpart hint, every gene structure gets a relative bonus factor of 1.15 *for each base* that is not an exon and not in a repeat. This discourages—but does not exclude—the overlap of exons and repeats. Repeat masking evidence can be given explicitly with hints of source RM, or implicitly with a soft-masked genome and the option *softmasking* turned on. The number(s) immediately to the left of the M column other than 1 specifies a penalty (malus) for gene structures with unsupported features. For example, the red .34 in the intron row means that every intron candidate that has no intron hints supporting it is penalized by multiplying its unnormalized probability with the factor 0.34. If you decrease this number even more (say from .3 to .001) then fewer introns unsupported by hints should be predicted. This would likely decrease the false positive intron rate, but, also, more true unsupported introns would be missed. For more information, see the file *config/extrinsic/extrinsic.cfg*.

EXTRINSIC FILE

- For practical purposes, copy the extrinsic file below to your augustus/config/extrinsic folder:

/data/genomics/workshops/GAworkshop/extrinsic.M.RM.E.cfg

AUGUSTUS

- Masked fasta:
  - Copy the file from the repeatmasker
  - cp ../repeatmasker/assembly.fasta.masked .

LET'S RUN AUGUSTUS

- augustus --strand=both --singlestrand=true \
- --hintsfile= siskin_RM_E.hints \
- --extrinsicCfgFile=extrinsic.M.RM.E.cfg \
- --alternatives-from-evidence=true \
- --gff3=on \
- --uniqueGeneId=true \
- --softmasking=1 \
- --species= BUSCO_siskin_busco_2684346740 \
- assembly.fasta.masked > siskin_Contig3141.gff

WHAT DO WE DO WITH THE ANNOTATION?

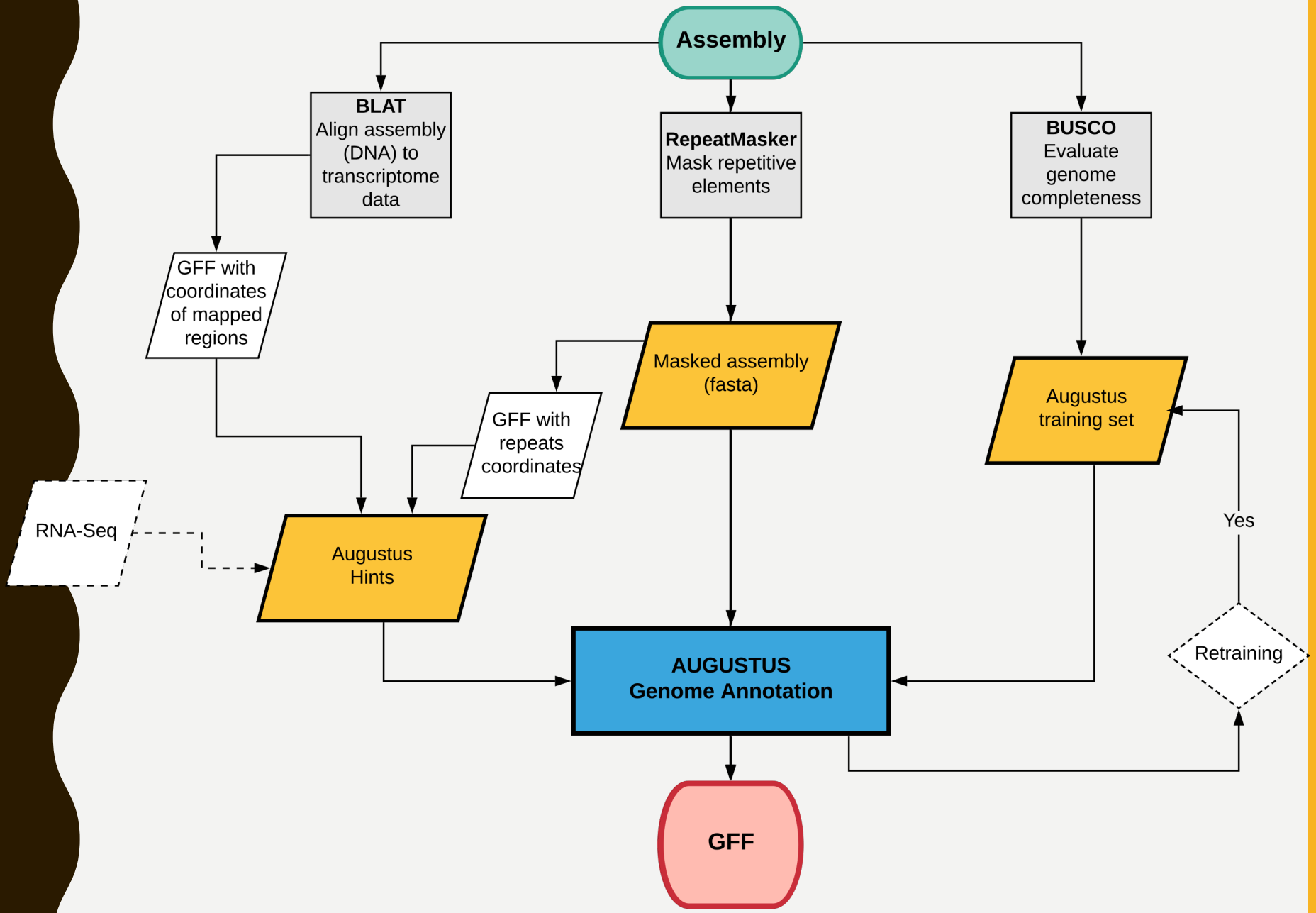
- The next step is to understand the functions of the genes we identified

- For functional annotation, we used both BLAST and BLAST2GO

GENOME ANNOTATION WORKSHOP

MIRIAN T. N. TSUCHIYA
DATA SCIENCE POSTDOCTORAL FELLOW
DATA SCIENCE LAB - OCIO

WHERE ARE WE?


Assembly
BLAT
Align assembly (DNA) to transcriptome data
RepeatMasker
Mask repetitive elements
BUSCO
Evaluate genome completeness
GFF with coordinates of mapped regions
Masked assembly (fasta)
Augustus training set
GFF with repeats coordinates
RNA-Seq
Augustus Hints
Yes
AUGUSTUS
Genome Annotation
Retraining
GFF

WHAT DID WE DO

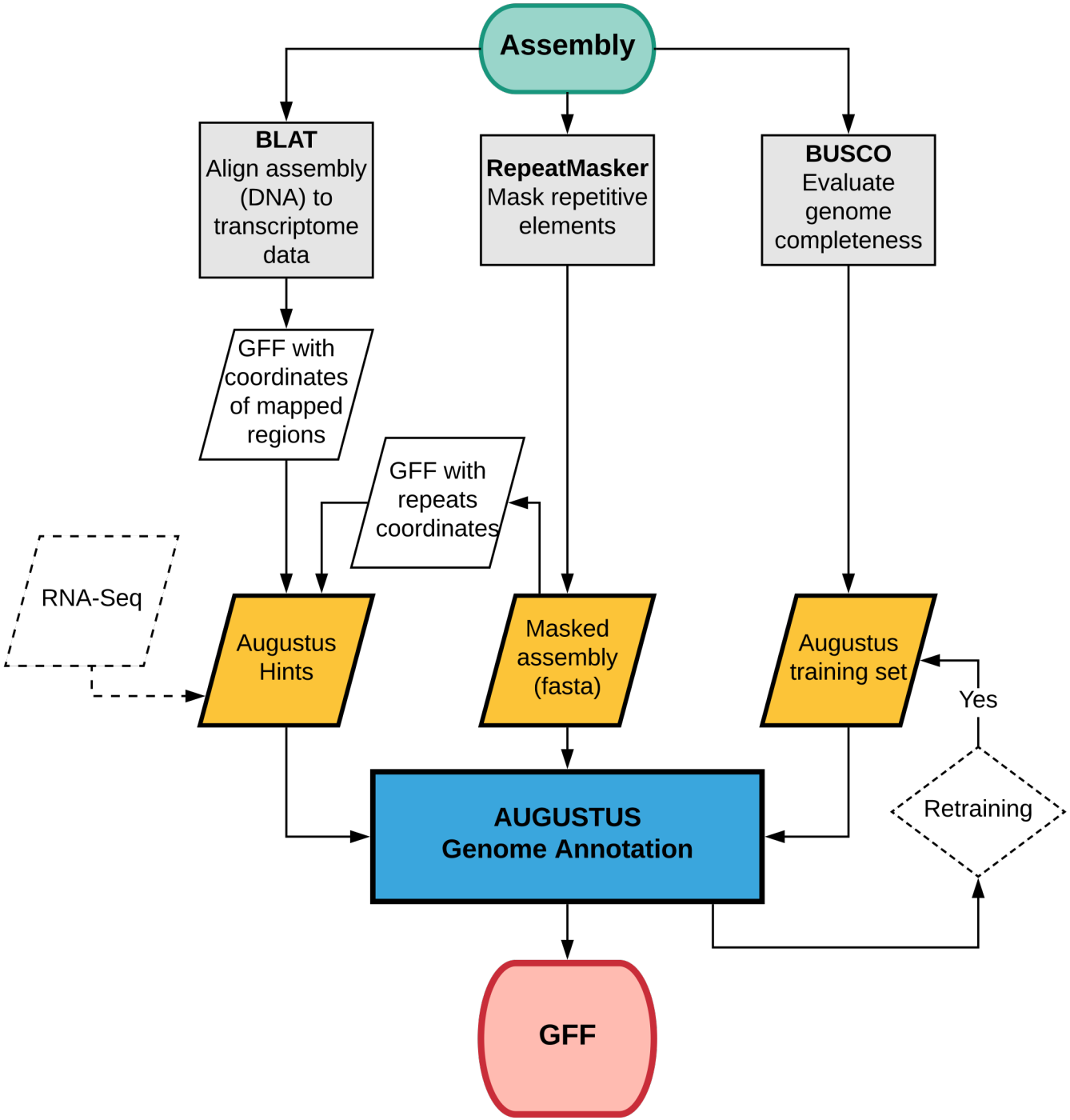
1. BUSCO
    - BUSCO summary ✓
    - Retraining parameters ✓
2. BLAT
    - Hints ✓
3. RepeatMasker
    - Masked assembly 

WHAT ARE WE DOING TODAY?

1. Setting up an embarrassingly parallel AUGUSTUS run
2. Combine the results
3. Visualize it all using JBrowse

AUGUSTUS

FINALLY!


Assembly
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GFF

AUGUSTUS

- ab initio (internal) + evidence-driven(external)

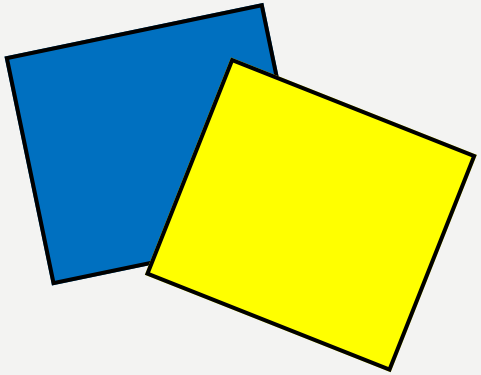
AUGUSTUS is based on a generalized hidden Markov model (GHMM), which defines probability distributions for the various sections of genomic sequences. Introns, exons, intergenic regions, etc. correspond to states in the model and each state is thought to create DNA sequences with certain pre-defined emission probabilities.

REQUIRED FILES

1. Masked assembly ✓
2. Hints file ✓
3. Retraining parameters ✓
4. Extrinsic file ✓

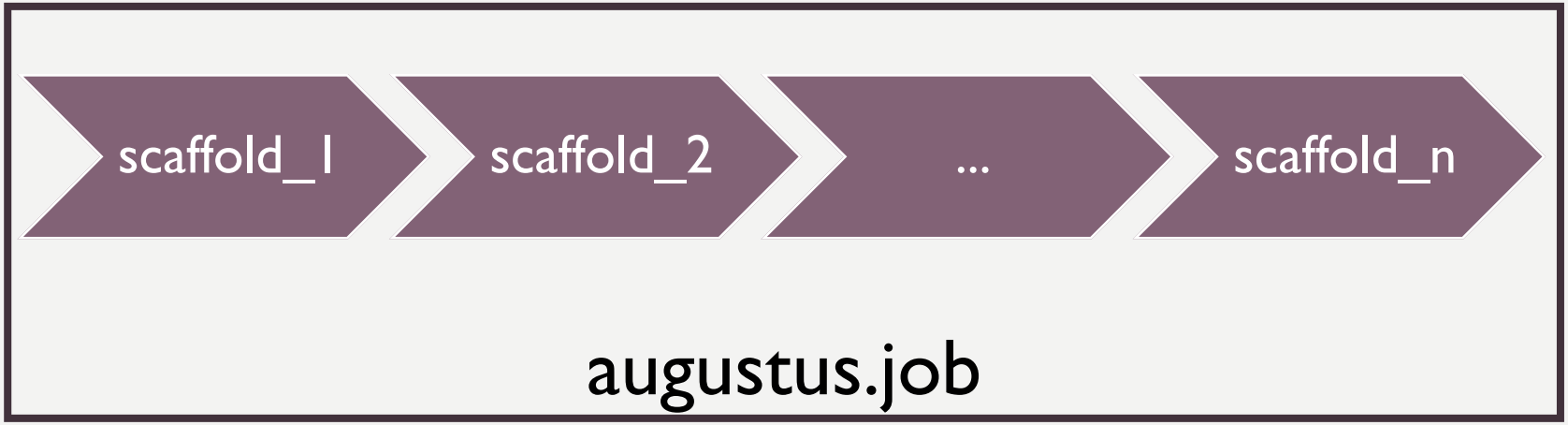
TASKS

1. Copy the masked assembly to your augustus folder
2. Download and extract EVidenceModeler to your augustus folder



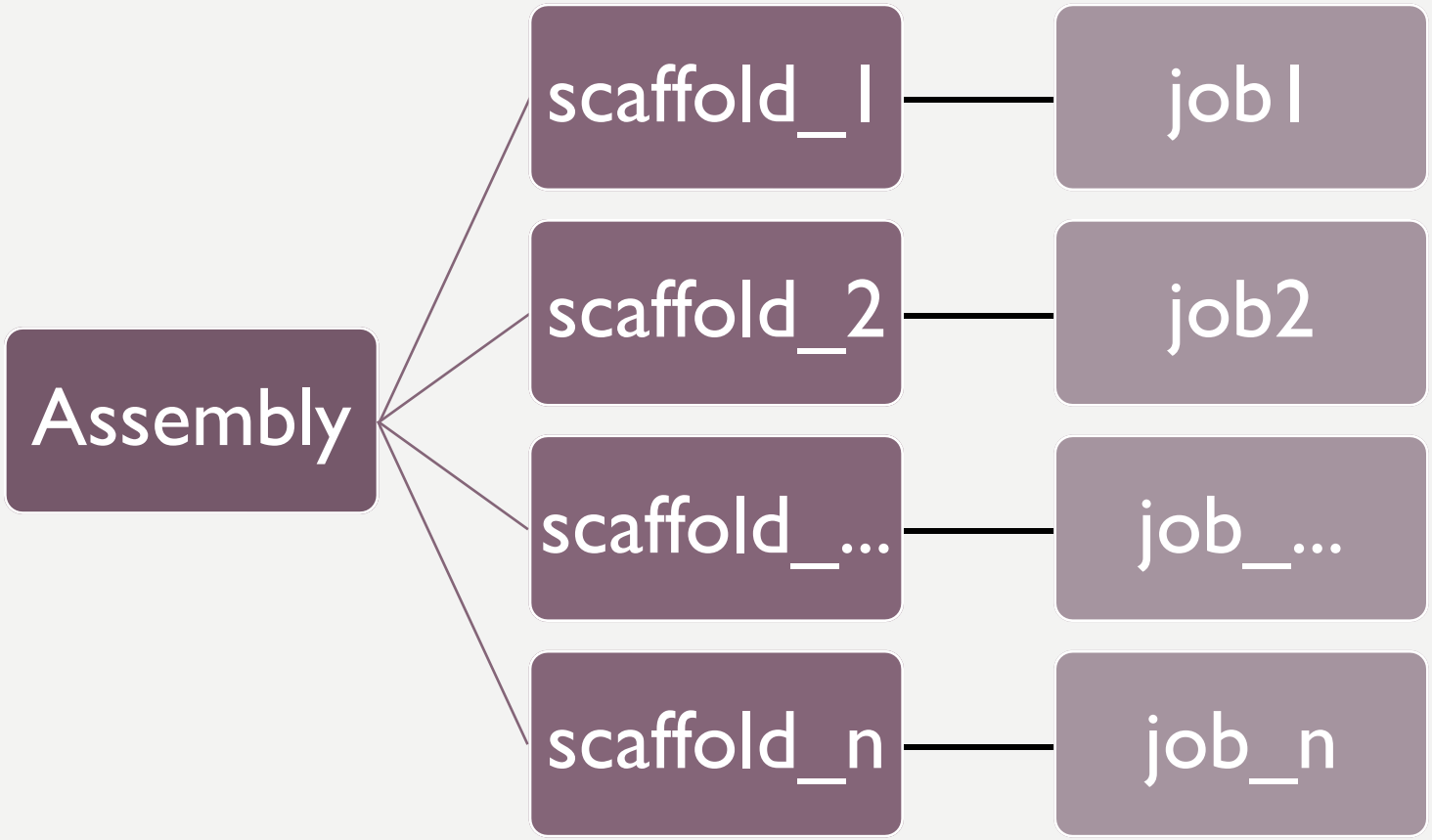
EMBARRASSINGLY PARALLEL

- AUGUSTUS runs serially (aka one scaffold at a time)



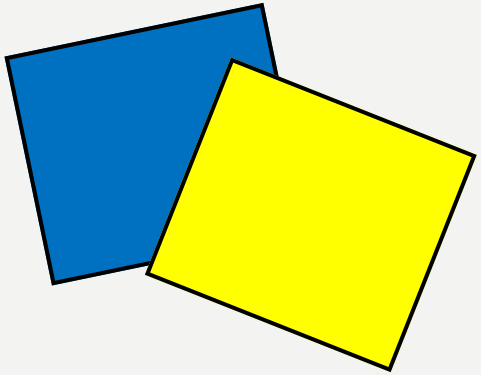
EMBARRASSINGLY PARALLEL

- But we can "force" it to run in parallel



TASKS

- Login to the interactive queue
- Run the EVM script `partition_EVM_inputs.pl` from your scaffolds folder
  - Don't forget that you copied the masked assembly to your augustus folder. Adjust the paths accordingly.



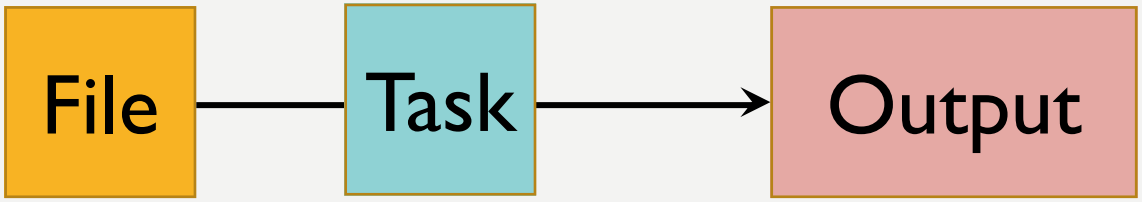
SCAFFOLDS FOLDER

- How many folders do we have?
- Take a look in the folder scaffold_110.
  - What's inside?
  - Use less to look at the files.

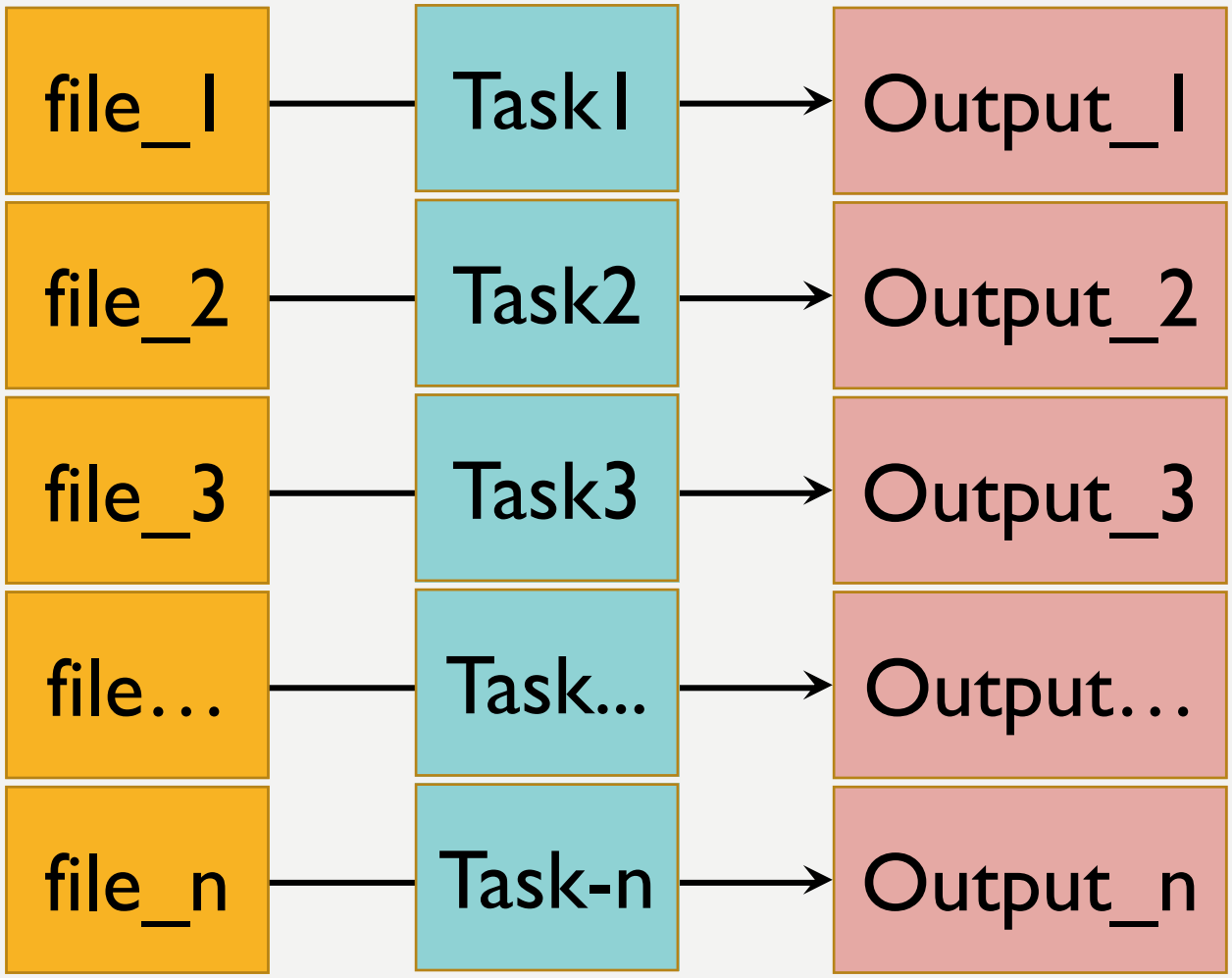
AUGUSTUS JOB FILE

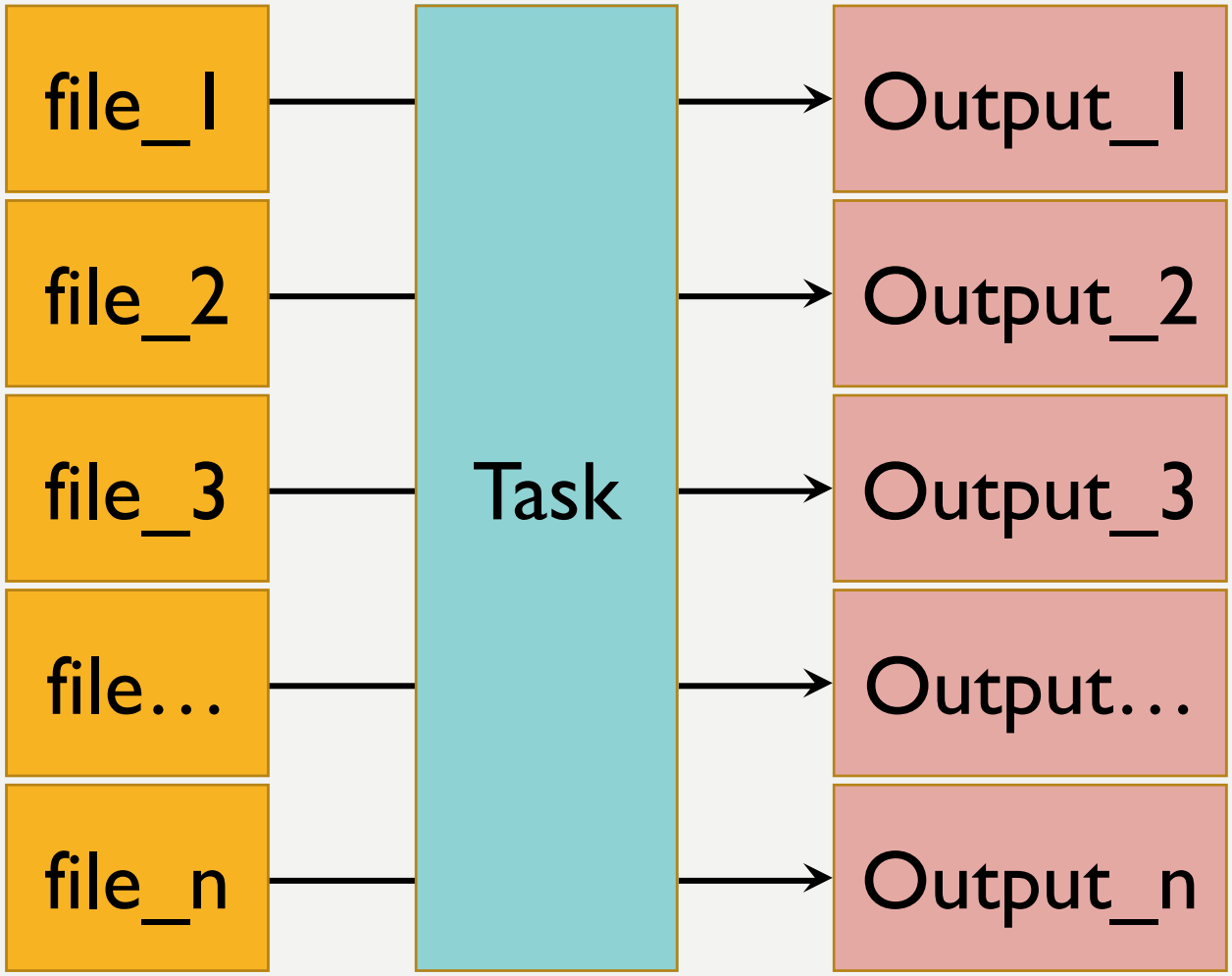
- How are we running augustus on each scaffold?
  - Option 1: Create one job file for each scaffold… manually
  - Option 2: Create one job file and use a loop to submit it.

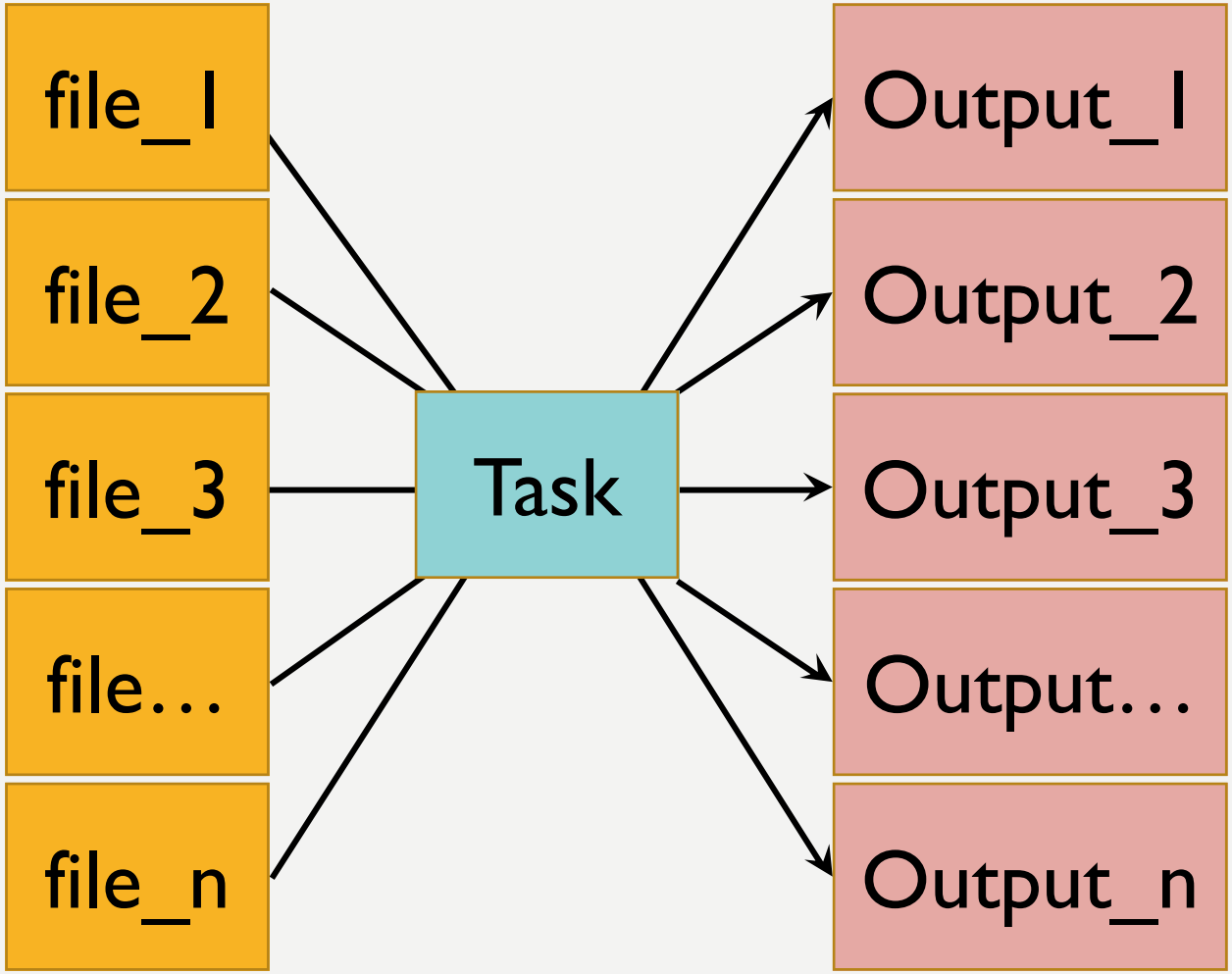
BRIEF INTRO ABOUT LOOPS



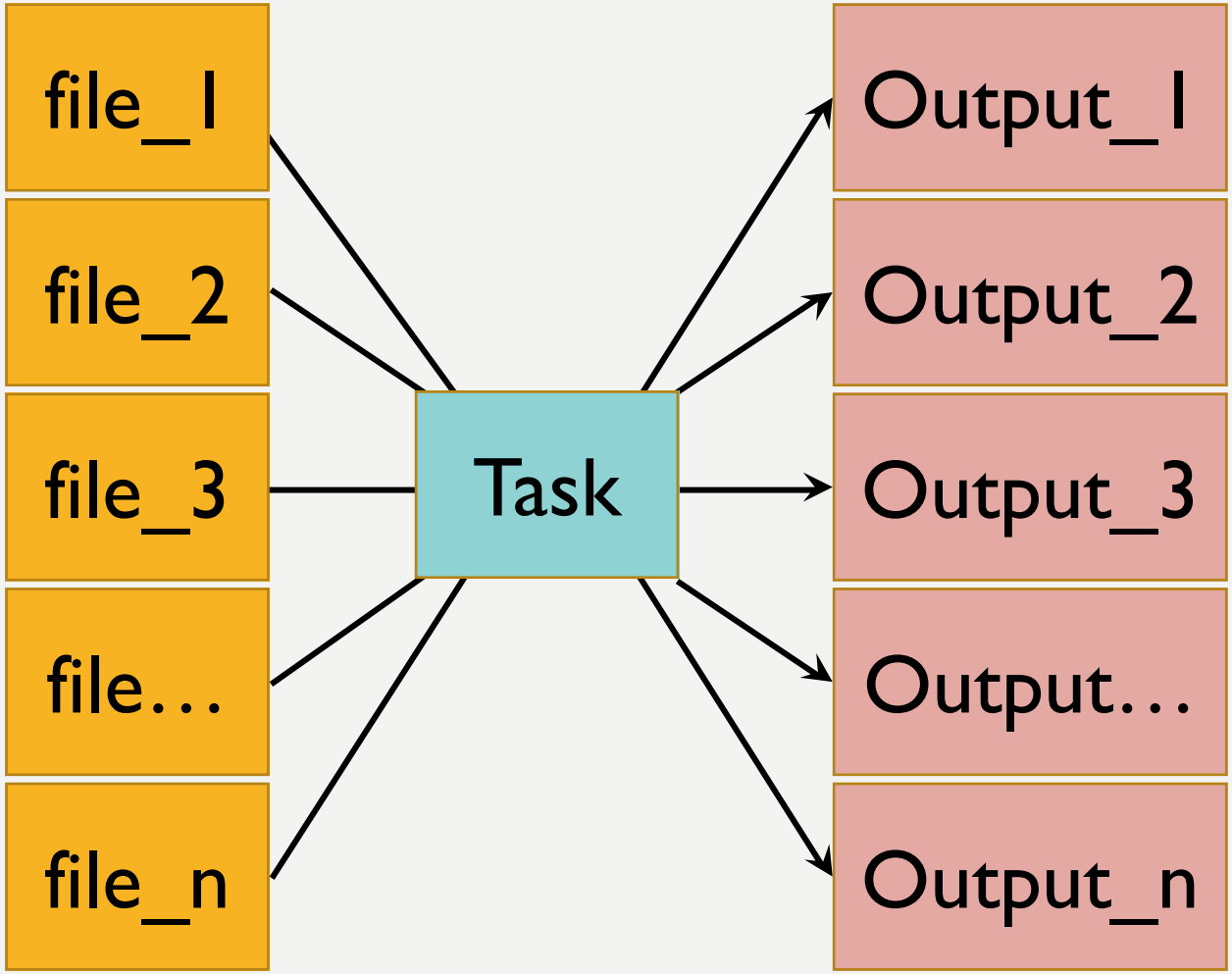
One file = One job


file_1
Task1
Output_1
file_2
Task2
Output_2
file_3
Task3
Output_3
file…
Task...
Output…
file_n
Task-n
Output_n


file_1
file_2
file_3
file…
file_n
Task
Output_1
Output_2
Output_3
Output…
Output_n


file_1
file_2
file_3
file…
file_n
Task
Output_1
Output_2
Output_3
Output…
Output_n

for f in file_*; do task > ${f}.output; done



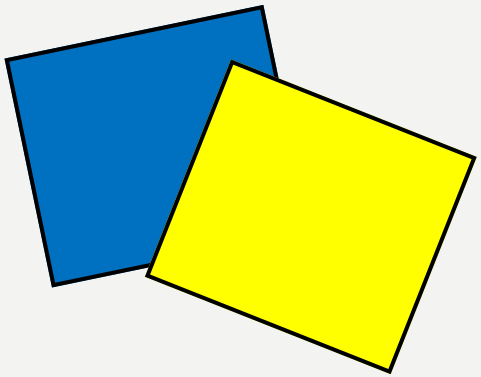
```
FOR DOG IN PUPPY_*; DO
  PLAY ${DOG} > ${DOG}.HAPPY;
DONE
```

BACK TO AUGUSTUS

- We will create one job (augustus.job) and will submit it using a for loop that will iterate over each scaffold - all at the same time.

TASKS

- Create the augustus job and save it in the jobs folder.
- You will submit the job from the folder scaffolds.

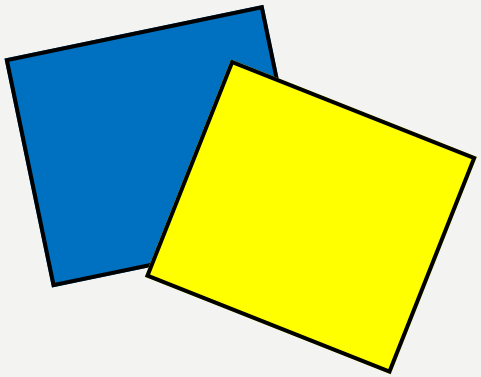


AUGUSTUS RESULTS

- List the files in the folder outputs. You should have one gff file per scaffold.
- Use cat or less to visualize file contents.

COMBINING THE RESULTS

- Now we want to combine all the gffs into a single gff file. Dhydei_augustus_all.gff3
- We will use the script `join_aug_pred.pl` from AUGUSTUS to combine the results.



FROM THE FOLDER AUGUSTUS

cp -r /data/genomics/workshops/Gaworkshop/augustus/outputs outputs2

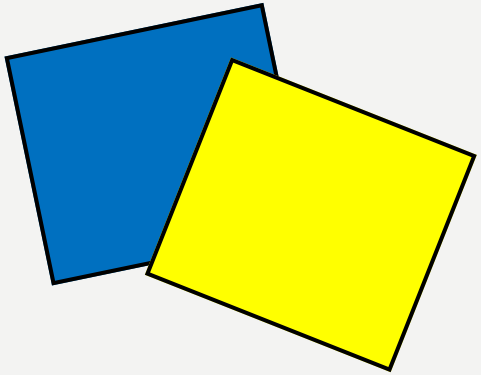
JBROWSE

JBROWSE


Available Tracks
filter tracks
BAM 8
BAM - paired-read test pattern at 28kb
BAM - volvox-longreads.bam
BAM - volvox-sorted SNPs/Coverage
BAM - volvox-sorted.bam
BAM - volvox-sv.bam
CRAM - volvox-longreads.cram
CRAM - volvox-sorted.cram
CRAM - volvox-sv.cram
Miscellaneous 15
BEDTabix - volvox.bed in-memory adaptor
BigBed - volvox genes
ChromHMM
FromConfig - Features in Configuration
GFF3 - sub-subparts
GFF3 - volvox.gff3 in-memory adaptor
GFF3Tabix - volvox.gff3 in-memory adaptor
GTF - volvox.gtf in-memory adaptor
HTMLFeatures - ESTs
HTMLFeatures - Example Features
HTMLFeatures - Example motifs
HTMLFeatures - Features with right-click menus
HTMLFeatures - Fingerprinted BACs
HTMLFeatures - Name test track has a really long track label
HTMLFeatures - SNPs
Quantitative 4
Density 2
BigWig Density - volvox_microarray
BigWig Density - volvox_sine
XY Plot 2
BigWig XY - volvox_microarray
BigWig XY - volvox_sine
Reference sequence 1
Reference sequence
Transcripts 5
Genome
Track
View
Help
Volvox Example
Share
ctgA
ctgA:4411..6492 (2.08 Kb)
Go
Reference sequence
Zoom in to see sequence
CanvasFeatures - transcripts
BigWig Density - volvox_microarray
BigWig XY - volvox_microarray
mean
VCF - volvox-sorted variants
SNV C -> G
BAM - volvox-sorted SNPs/Coverage
BAM - volvox-sorted.bam

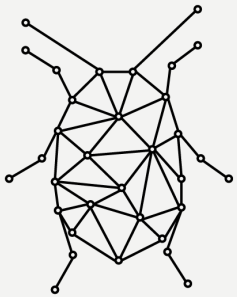
TASKS

- Run the JBrowse scripts on your files. You need:
  - Assembly
  - GFF (final)
- Compress the file and copy it to your Desktop.



JBROWSE

- You can deploy an instance locally (from your computer) or you can use a cloud service (AWS, Azure, etc)



OCIO
DATA
SCIENCE
LAB

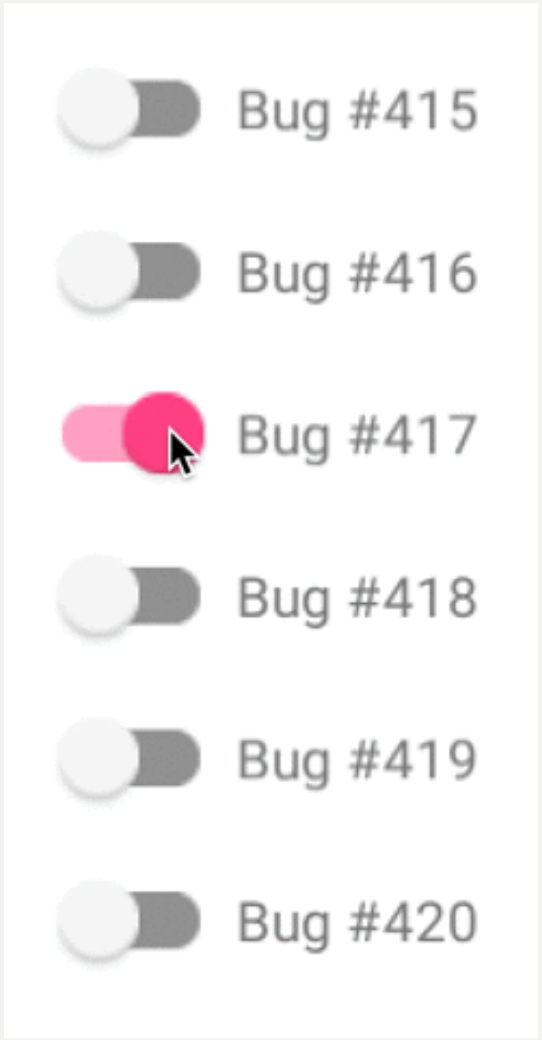
@SIDatascience

datascience.si.edu

tsuchiyam@si.edu

@MirianTsuchiya

Hydra help: SI-HPC@si.edu


Bug #415
Bug #416
Bug #417
Bug #418
Bug #419
Bug #420


99 little bugs in the code.
99 little bugs in the code.
Take one down, patch it around.
127 little bugs in the code...