Annotation of the loblolly pine megagenome

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Annotation of the Genome

Overview

- Alignment of existing resources
- Alignment of *de novo* transcriptome assembly
- Gene space prediction
- Gene family analysis
- Repeat Sequence
  - Repeat Library
  - Interspersed
  - Tandem
- Database Resources
- Community Annotation

Assembly v1.0 (March 2013)

- Approximately 65X coverage
- Total Sequence: 20.1 Gbp
- Total Contig: 2.3 Gbp
- N50 Contig: 8.2 Kbp (11.6 m)
- Total Scaffold: 17.8 Gbp
- N50 Scaffold: 30.7 Kbp (4.8 m)

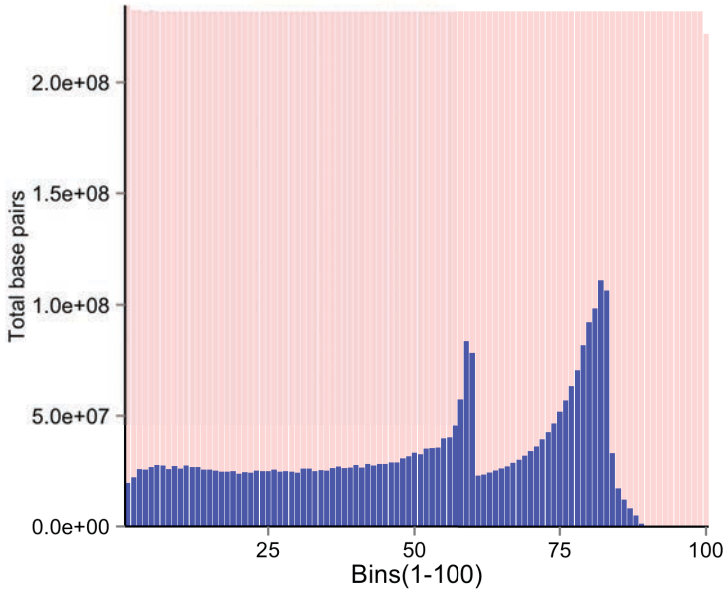
Assembly v1.01

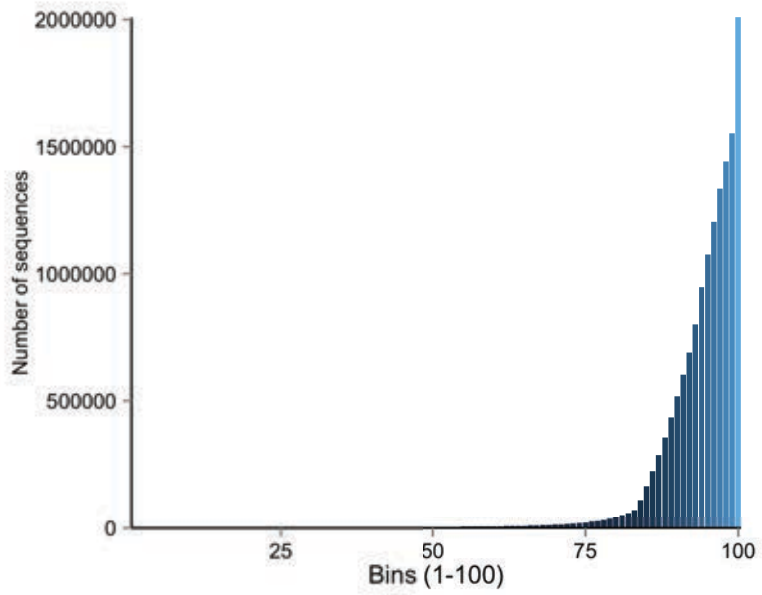
- Total Sequence: 20.1 Gbp
- N50 Contig 8.2 Kbp
- N50 Scaffold: 66.9 Kbp

Annotation of the Genome

Mapping Existing Datasets to the Genome

- Mapping resources initially on unmasked genome (1.0 and 1.01)
  - Divide the sequence into 100 bins of equal size
    - Descending order of scaffold length
      - Parallelize
      - Examine the effects of masking, fragmentation, repetitive sequence





Annotation of the Genome

Mapping Existing Datasets to the Genome

Project	Total sequence	Identity	Coverage	Unique hits	Non-unique hits	Total percent mapped
Pinus taeda (reclustered ESTs)	45,085	98	98	26,700	712	60.8
Pinus taeda (reclustered ESTs)	45,085	98	95	29,676	1,845	69.91
Pinus taeda (reclustered ESTs)	45,085	95	95	31,324	2,074	74.01
Pinus palustris (454)	16,832	95	95	11,242	719	71.06
Pinus palustris (454)	16,832	95	50	11,181	1,949	78.06
Pinus lambertiana (454 + RNASeq)	40,619	95	95	13,134	317	33.11
Pinus lambertiana (454 + RNASeq)	40,619	95	50	23,376	3,792	66.88
Pinus banksiana (Treegenes clusters)	13,040	95	95	9,703	513	78.34
Pinus banksiana (Treegenes clusters)	13,040	95	50	9,470	1,473	83.92
Pinus contorta (Treegenes clusters)	13,570	95	95	9,575	396	73.48
Pinus contorta (Treegenes clusters)	13,570	95	50	9,534	1,083	78.24
Pinus pinaster (Treegenes clusters)	15,648	95	95	9,738	943	68.26
Pinus pinaster (Treegenes clusters)	15,648	95	50	10,221	2,491	81.24

Progressive Transcript Profiling

First comprehensive transcriptome generated (27 unique RNA libraries)

Early Development
seeds
young seedlings

Vegetative Organs
vegetative buds
candles
stems
needles
roots

Reproductive Development
megastrobili
microstrobili

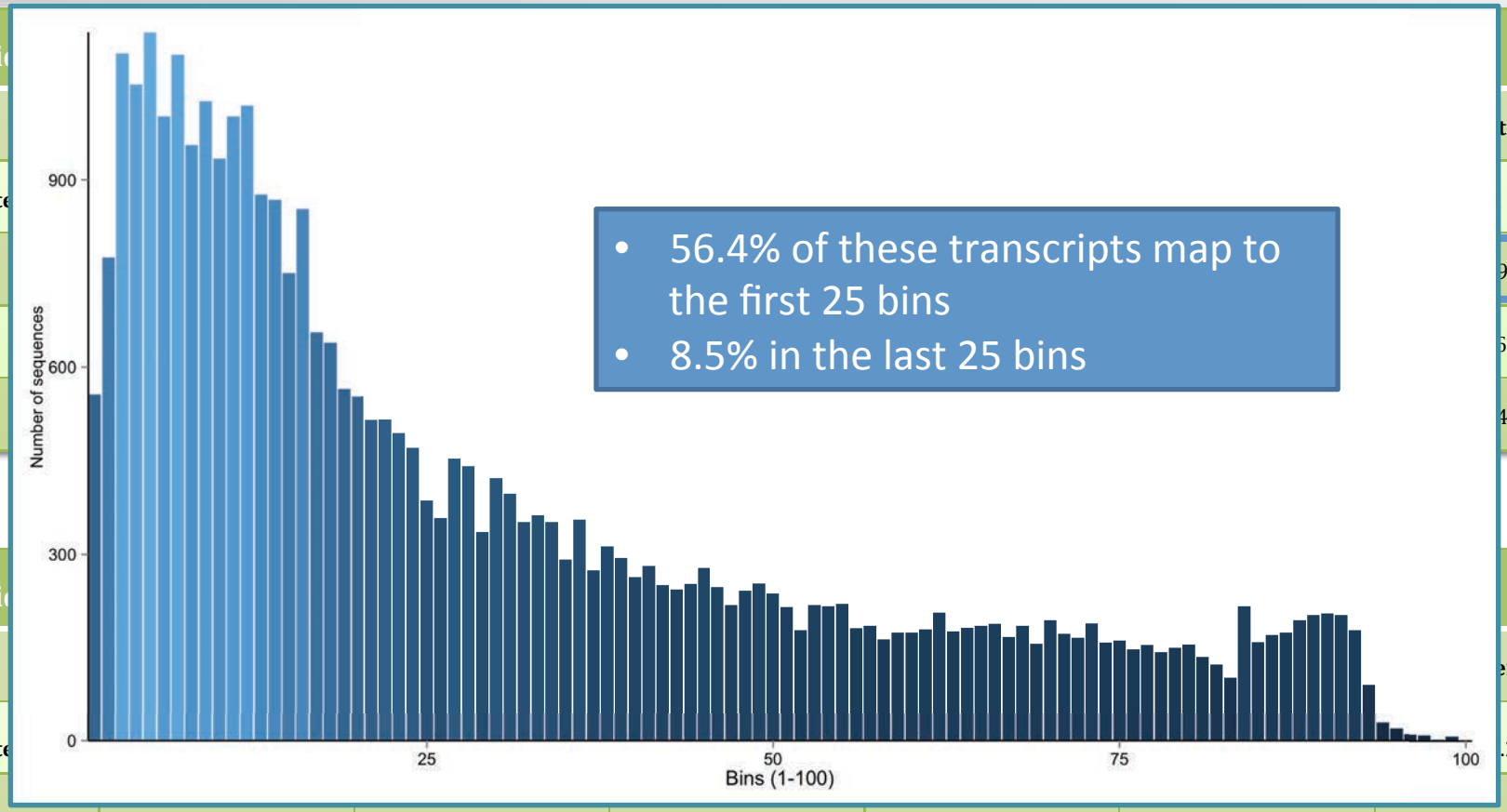
Early Stress Signaling Responses
cold
heat
elevated UV
compression

Transcriptome Assembly

- Considerable variation in *de novo* transcriptome assemblies
  - Influenced by heterozygosity, sampling methods, normalization strategies
  - Used a compare and compete methodology to select the final transcripts
    - Independent, parallel assemblies from 2 Trinity versions and 6 Velvet/Oasis (different k-mer sizes)
  - EvidentialSuite – Full length, unique, protein coding (87,241)
  - 83,285 transcripts aligned at least partially to the genome (v1.01)

Aligning the loblolly pine transcriptome

Results against 1.0 and 1.01



- 56.4% of these transcripts map to the first 25 bins
- 8.5% in the last 25 bins

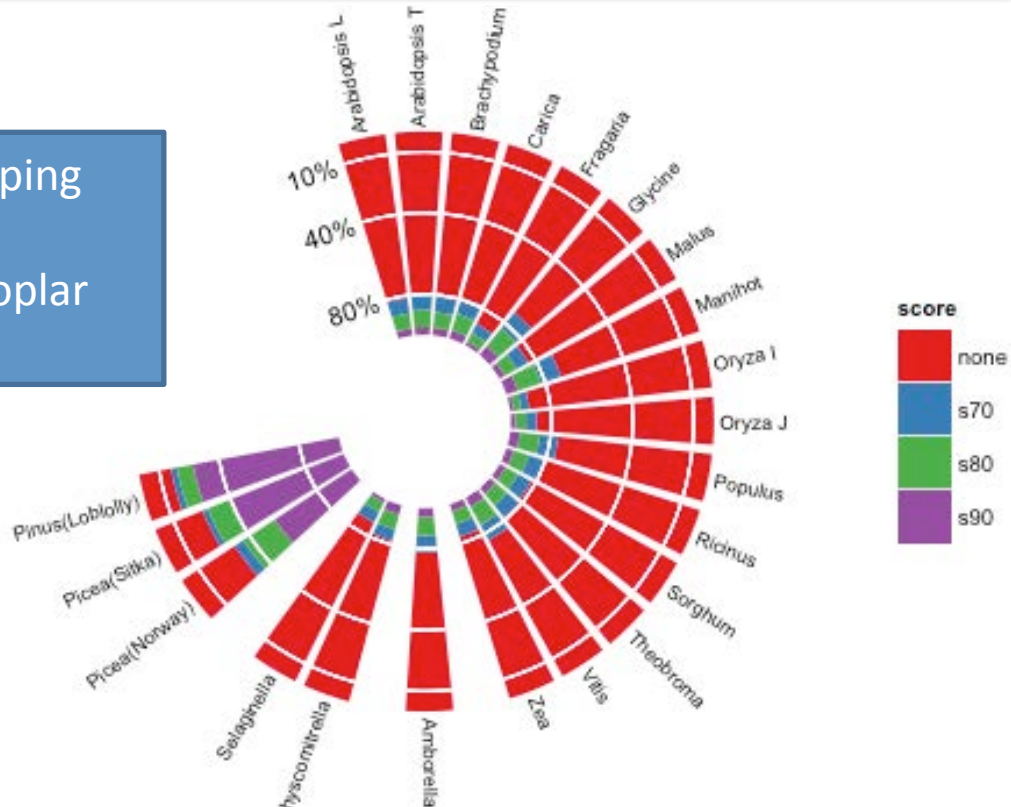
83,285 uni						
Method						t mapped
Blat/Exonerate						
						9
GMAP						6
NUCmer						4
83,285 uni						
Method						ent mapped
Blat/Exonerate						.21
	83,285	98	95	42,822	5,130	57.58
	83,285	95	95	43,972	5,409	59.29
	83,285	95	50	44,469	28,116	87.15

Annotation of the Genome

Mapping Existing Datasets to the Genome

Full-length proteins	Total sequence	Unique hits	Non-unique hits	Total percent mapped	Data source
Picea abies	22,070	11,580	3,638	68.95	Nystedt et al. 2013
Picea sitchensis	10,793	6,516	1,574	74.95	Genbank
Pinus taeda	83,285	45,656	24,427	84.15	Current assembly
PLAZA (24 species)	653,613	90,149	19,492	16.77	Van Bel et al. 2012

Marginally better mapping rates for soybean and cassava followed by poplar and grape.



MAKER-P

Identification of the Gene Space

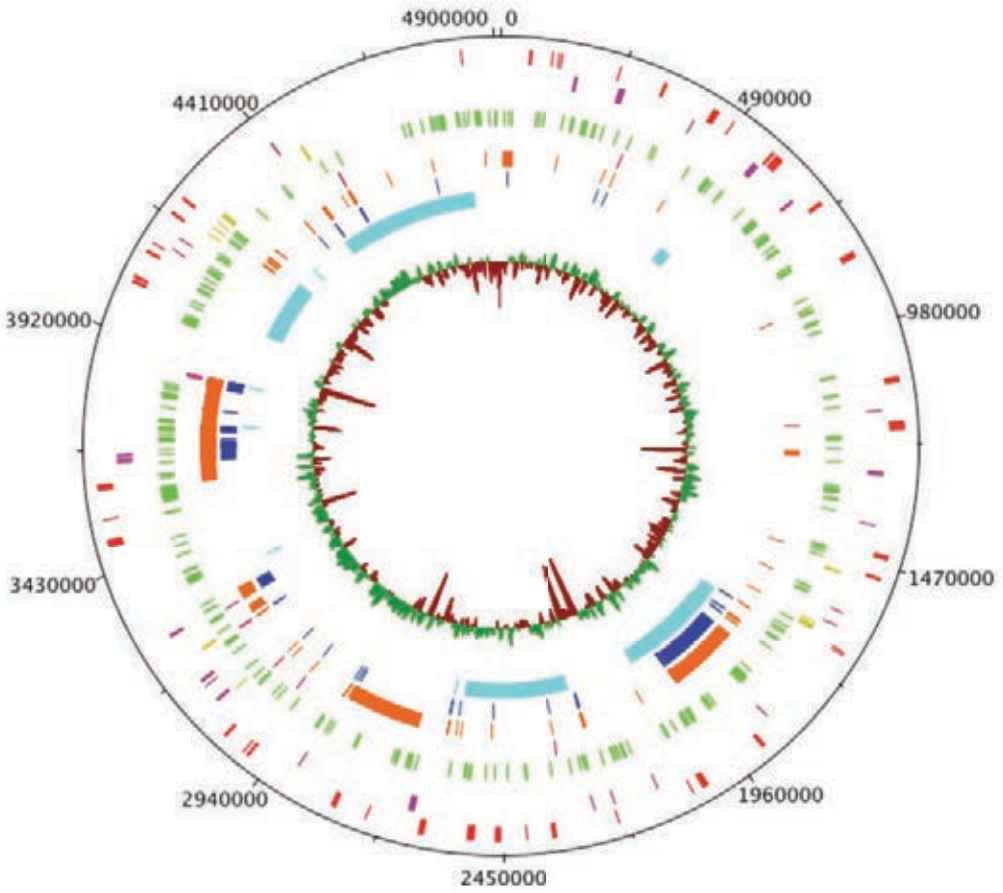
MAKER
Annotate this!

Provided *ab initio* and reference guided annotations

Texas Advanced Computing Center (TACC) 8,640 CPUs (216 CPUs for each of 40 jobs) 17 hours total run time

Produced over 90,000 gene models that were further filtered to reduce the false positives associated with Pseudogenes

- Multi-exonic
- Recognizable protein domain
- One of more forms of supporting Evidence

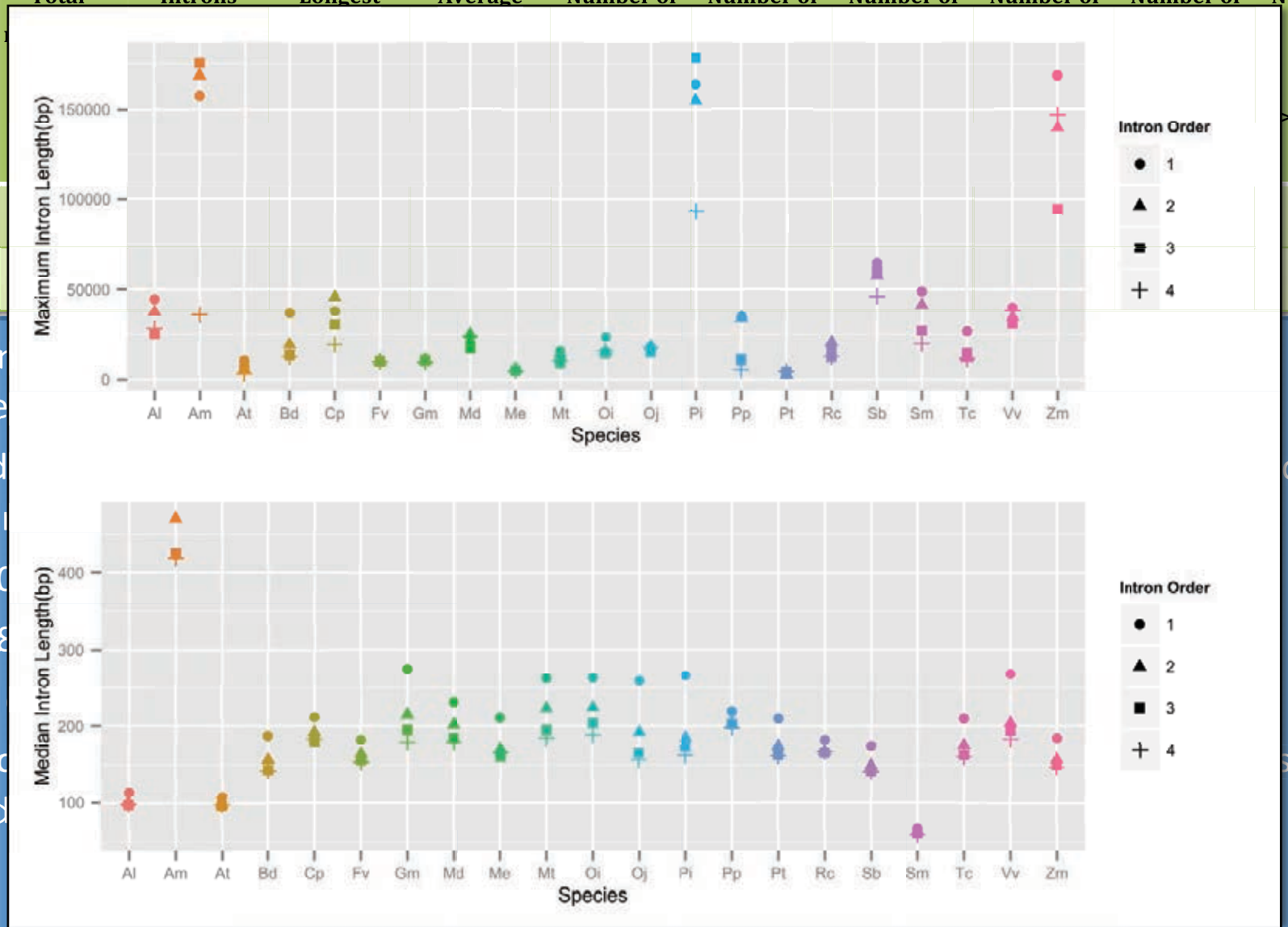


- 50,172 gene models mapped to 31,284 scaffolds at least 10 Kbp in length
- 3,835 scaffolds contained three or more genes

MAKER-P

Final Gene Models

Total Sequences	Total	Introns	Longest	Average	Number of	Number of	Number of	Number of	Number of	Number of FIRST introns >100Kbp
50,172										63
15,653										13



- Intron
  expre
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  - 40
  - 38
  - 8
- Transc
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Comparing Plant Genomes

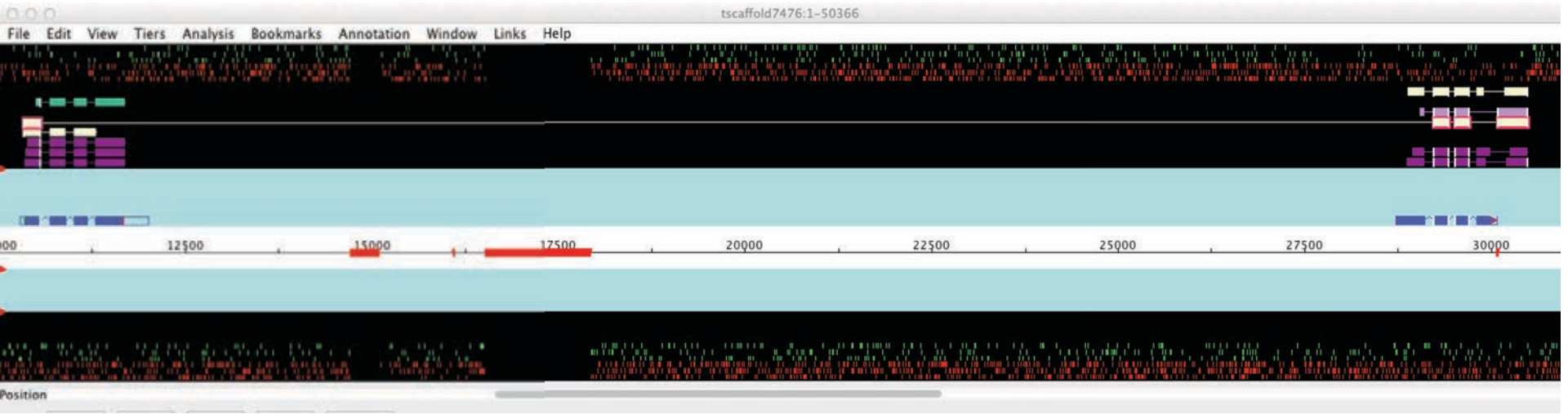
Gene Space

	Pinus taeda	*Picea abies*	*Arabidopsis thaliana*	*Populus trichocarpa*	*Vitis vinifera*	*Amborella trichopoda*
Genome size (assembled)(Mbp)	20,148	12,019	135	423	487	706
Chromosomes	12	12	5	19	19	13
G+C content (%)	38.2	37.9	35.0	33.3	36.2	35.5
TE content (%)	79	70	15.3	42	41.4	N/A
Number of genes	50,172	58,587	27,160	36,393	25,663	25,347
Average CDS length (bps)	965	723	1102	1143	1095	969
Average intron length (bps)	2,741	1,020	182	366	933	1,538
Maximum intron length (bps)	318,524	68,269	10,234	4,698	38,166	175,748

- **How many genes are in the pine genome?**
  - Fragmentation, Pseudogenes, Repeats still present issues in constructing complete models
  - Bias for known protein domains and intron-less genes (up to 20%)
  - Previous estimates in literature range from 30K to over 90K

Apollo

Manual Annotation Needed

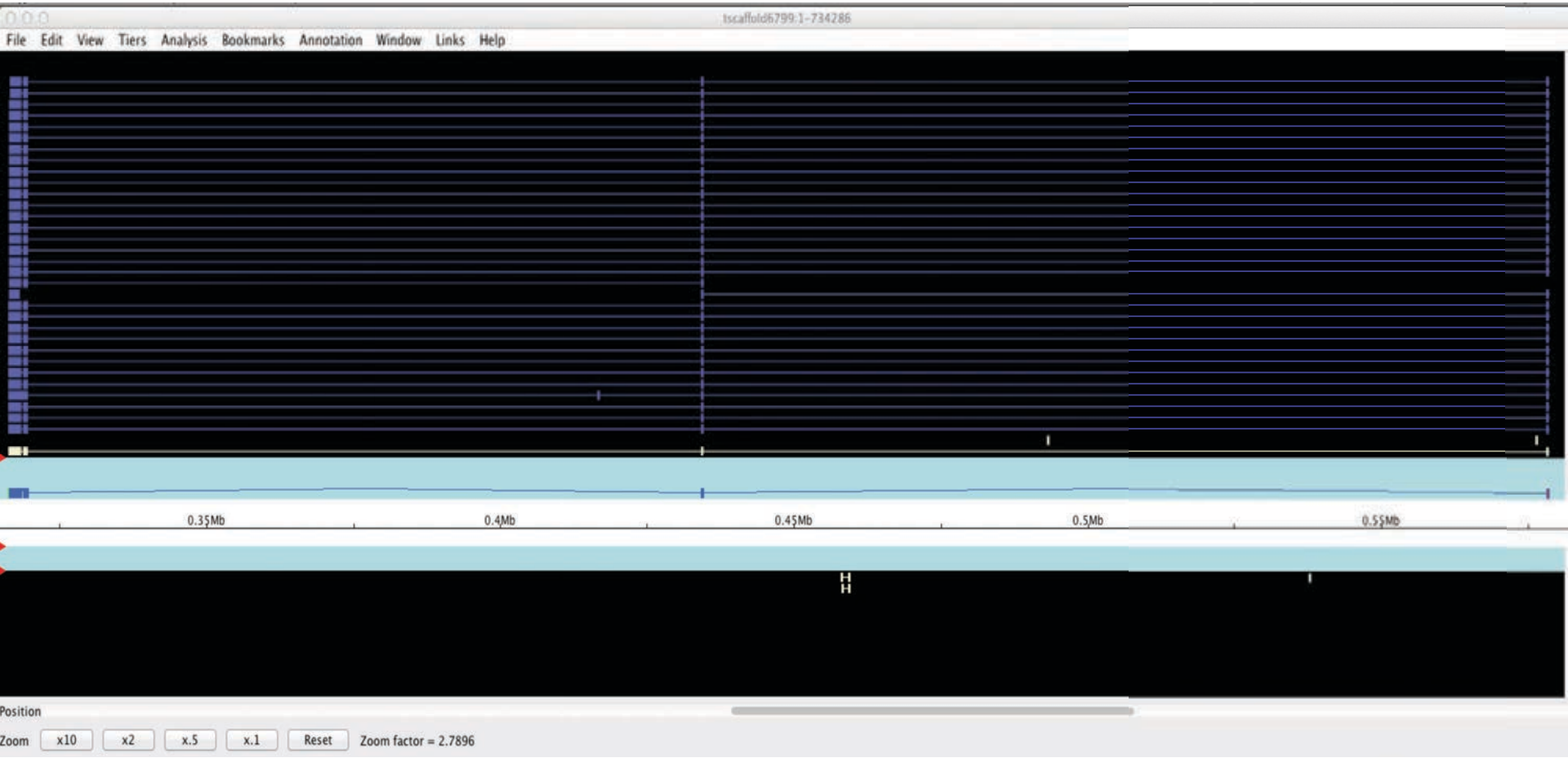


Yellow = Loblolly pine transcriptome
Green = High quality Norway spruce protein
Purple = Low Quality PLAZA proteins (70/70)
Blue-Violet = MAKER annotation

Long introns, over 100k

De novo transcript evidence and orthologous proteins

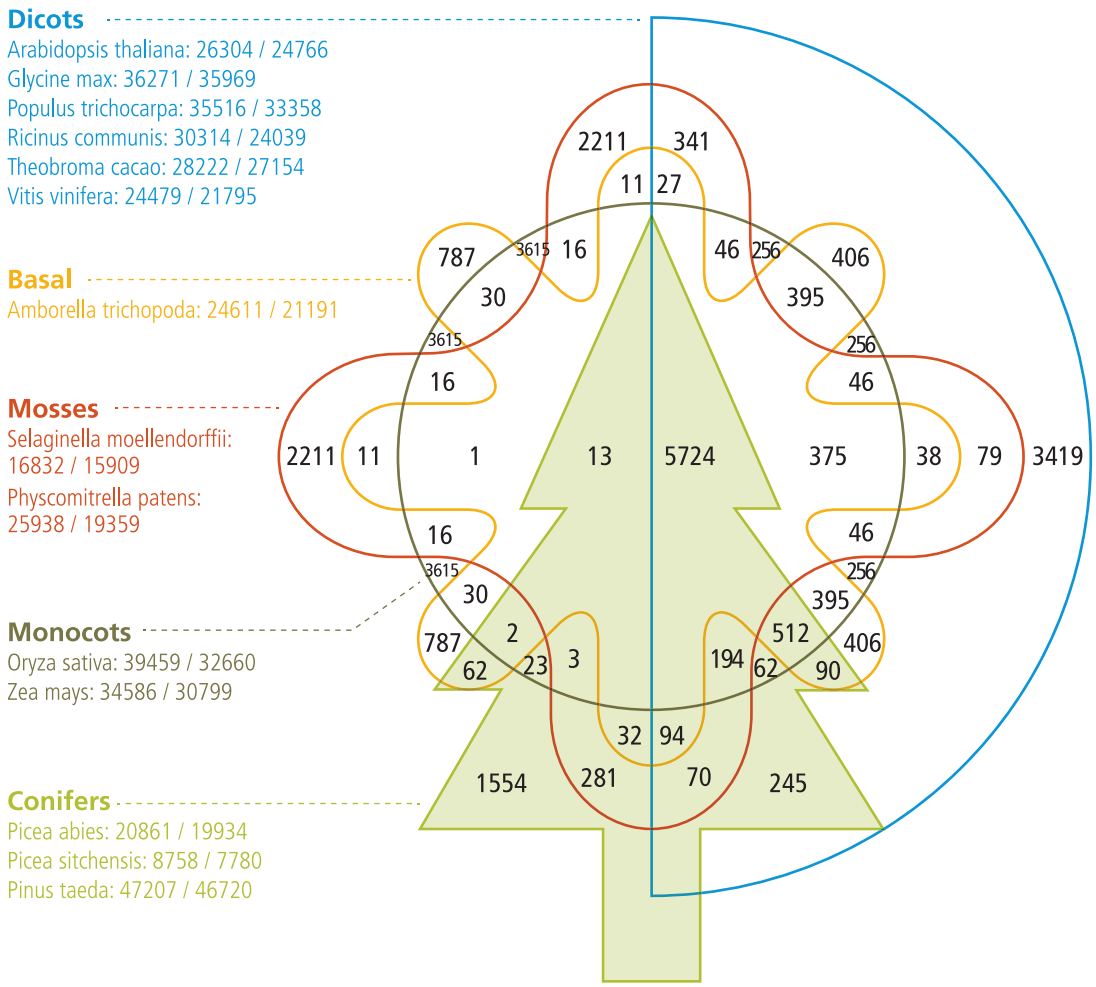
Pinus sylvestris phytochrome (NCBI BLASTP)



Orthologous Proteins

Examining Gene Families

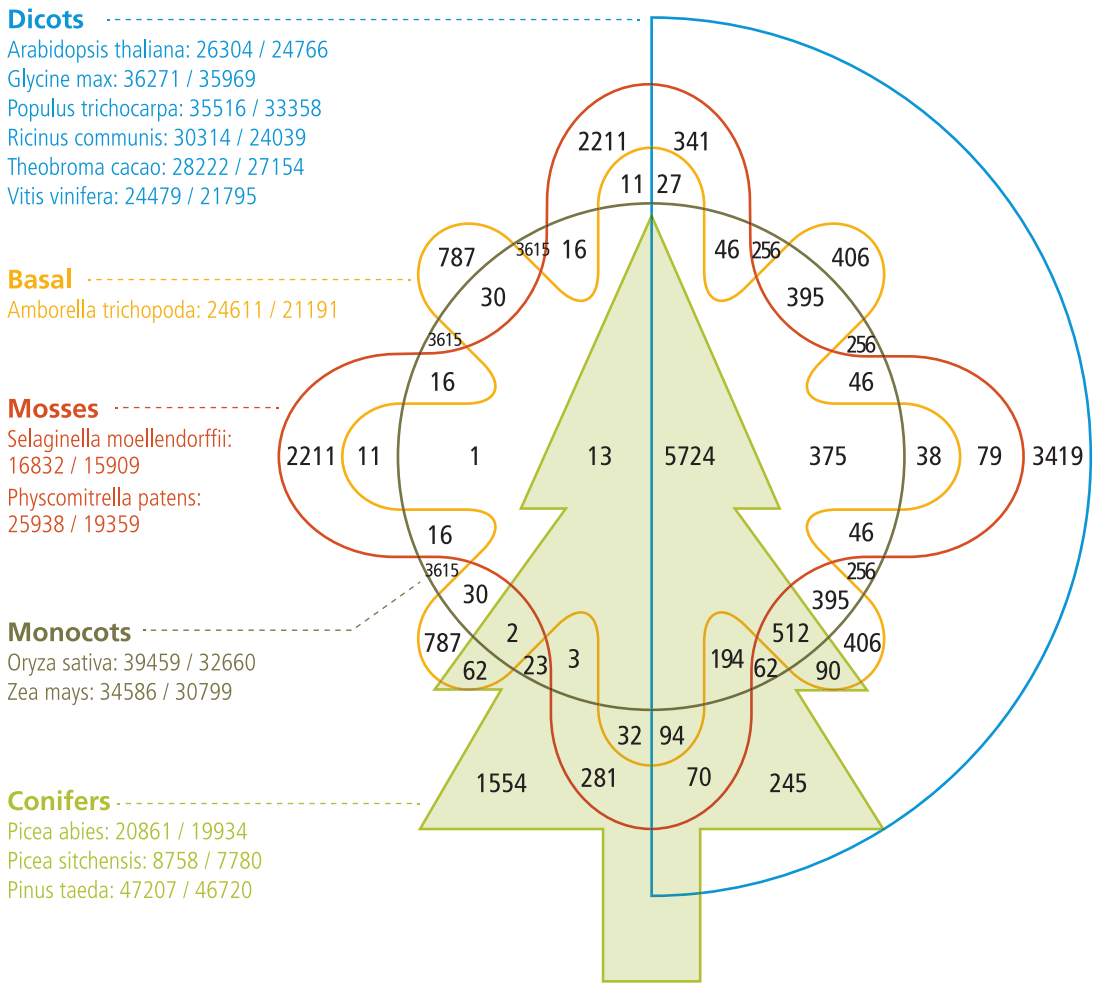
- 50K gene models to the 352,151 proteins curated from 13 plant species resulted in **20,646 unique gene families**
- 11 PLAZA species + Amborella + Norway spruce + Sitka spruce
- Grouped into dicots, basal, mosses, monocots, and conifers



Orthologous Proteins

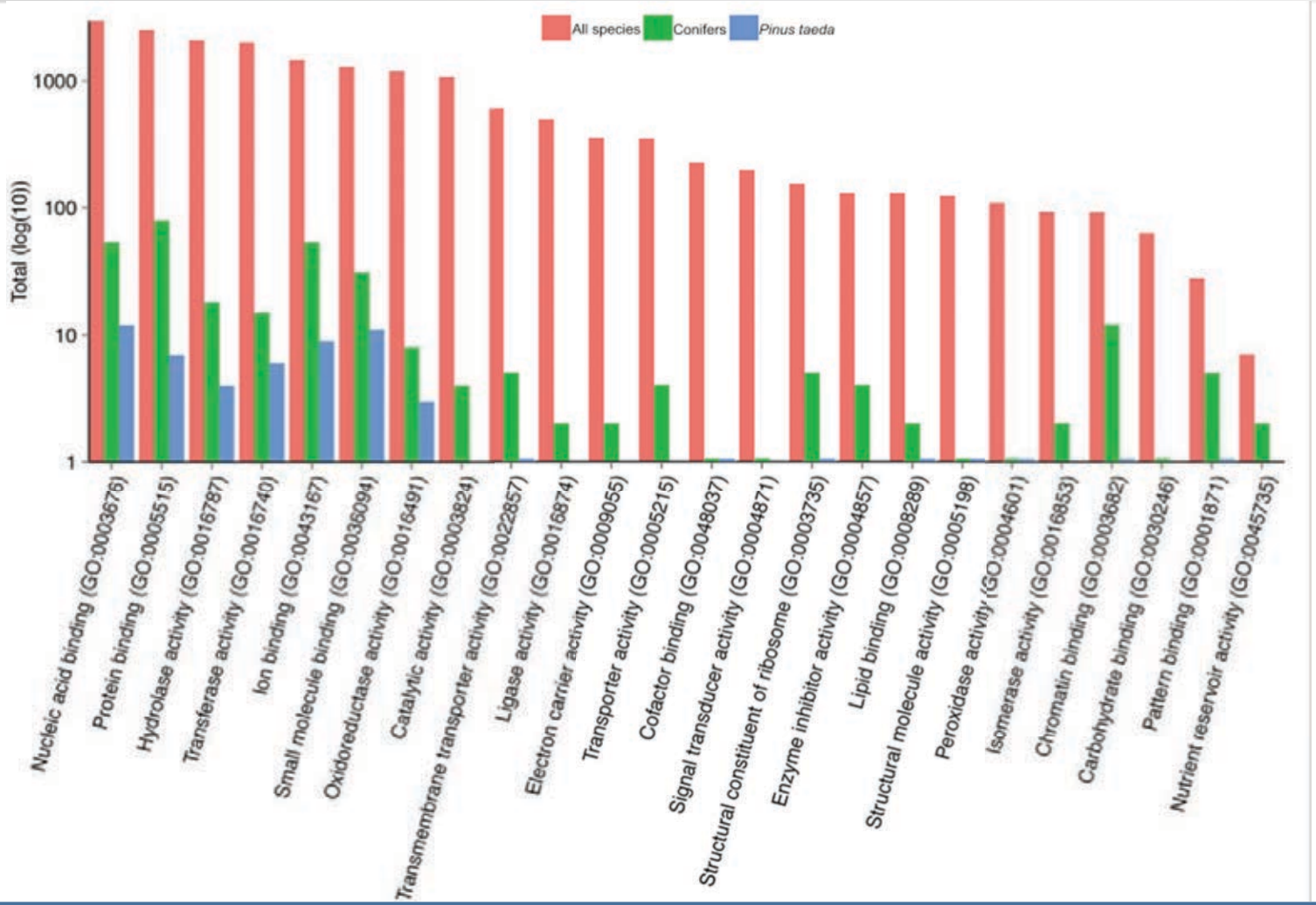
Examining Gene Families

- **20,646 unique gene families contained 90% gene set** (361,433 proteins) with an average of 17 genes/ family
- 1,554 conifer specific families
- Of these, 152 were unique to loblolly pine (32 with 5 more members)



Orthologous Proteins

Examining Gene Families

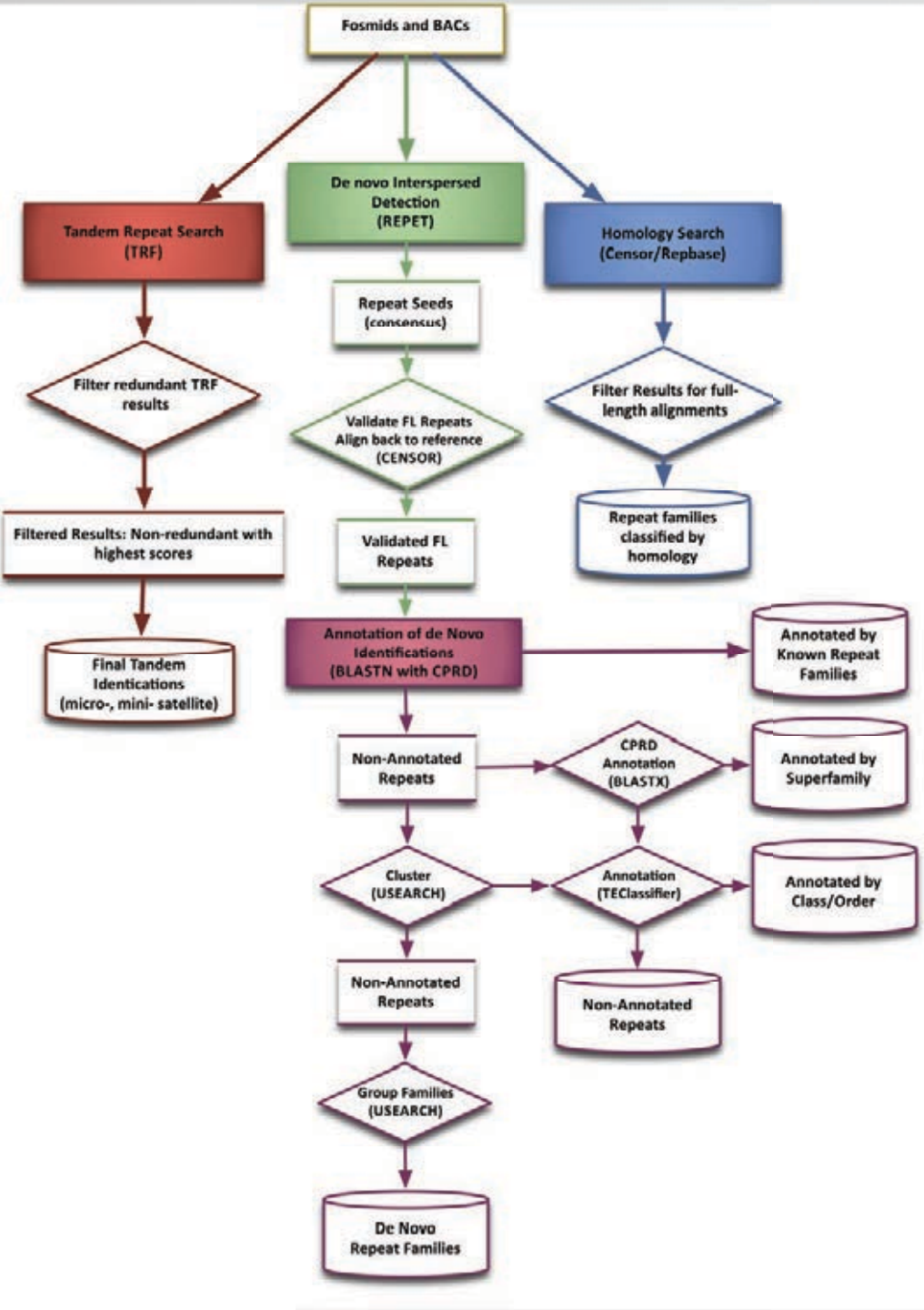


The *COPI C* family (58 members) was the largest exclusively identified in loblolly pine. Vesicle coat protein complexes containing *COPI* family members mediate transport between the ER and golgi, and interact with Ras-related transmembrane proteins, p23 and p24

Prior Studies in Gymnosperms

Sequence Type	Species	Paper	Size of Resource	Repetitive Content Estimate	Elements Identified
BACs	Picea glauca	Hamberger et al (2009)	150 Kb	40%	
BACs	Pinus taeda	Morse et al (2009)	1,612 Kb		Gymny
BACs	Pinus taeda	Kovach et al (2010)	887 Kb	24-80%	PtIFG7
BACs	Taxodium disitchum	Liu et al (2011)	580 Kb	90%	
Fosmids	Taxus mairei	Hao et al (2010)	1,923 Kb	20.8%	
Southern Hybridization	Pinus pinaster	Rocheta et al (2006)			PpRT1
Southern Hybridization	Pinus elliottii	Kamm et al (1996)			TPE1

Similarity and De Novo Repeat Identification



Tandem Repeat Finder (TRF)

Homology (Censor against RepBase)

Summary of Repbase v17.07

- Number of entries: 28,155
- Number of species represented: 715
- Number of repeat families: 280
  - Angiosperm entries: 131
  - **Gymnosperm entries (conifer):13**

De Novo (REPET/TEannot)

- Self-alignment (all vs all) with BLAST to find HSPs is followed by clustering with Grouper, Recon, and Piler
- 3 sets of clusters are aligned with a MSA (MAP) to derive a consensus sequence
- Structural search runs simultaneously (LTR Harvest) to detect highly diverged LTRs
- Final Blastclust to cluster potential sequences

Genomic Sequence

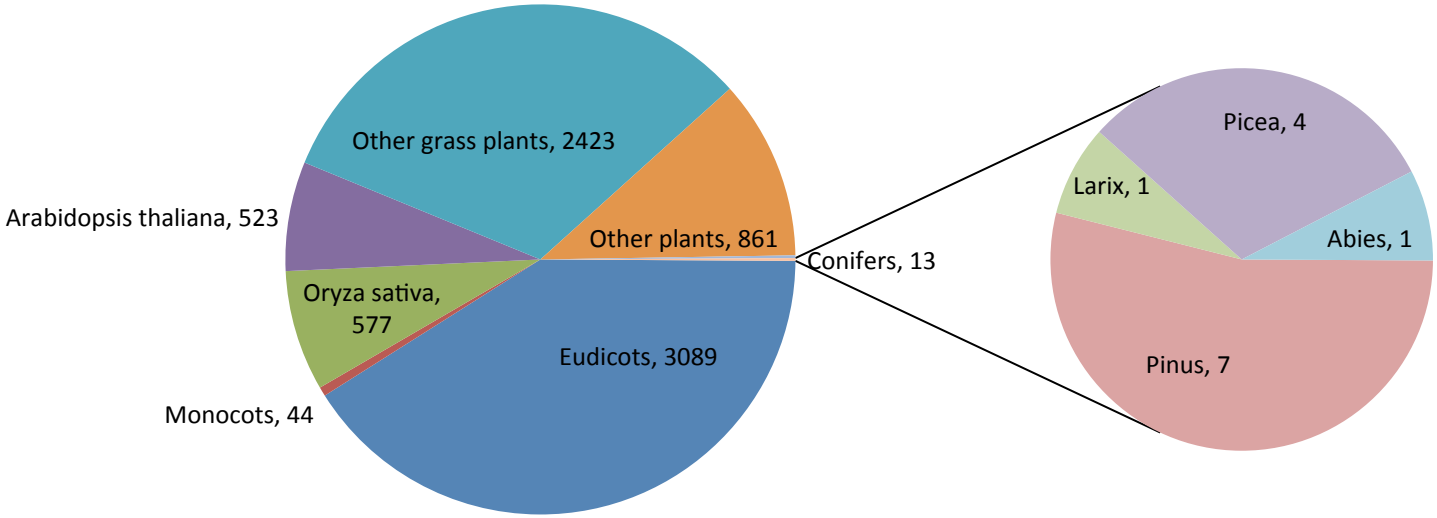
Pinus taeda BACs and Fosmids

	Pinus taeda BACs	*Pinus taeda* Fosmids
Total number of sequences	103	90,973
Average sequence length	115,130	2,918
Median sequence length	118,782	475
N50 sequence length (bp)	127,167	16,204
Shortest sequence length	1,392	201
Longest sequence length	235,088	75,791
Total length (bp)	11,858,447	265,511,345
GC %	37.98%	38.09%
A : C : T : G%	31.27 : 18.79 : 31.32 : 18.62	30.94:19.07:30.97:19.03

Combined sequence resource represents roughly 1% of the estimated genome

Similarity Search Analysis

RepBase

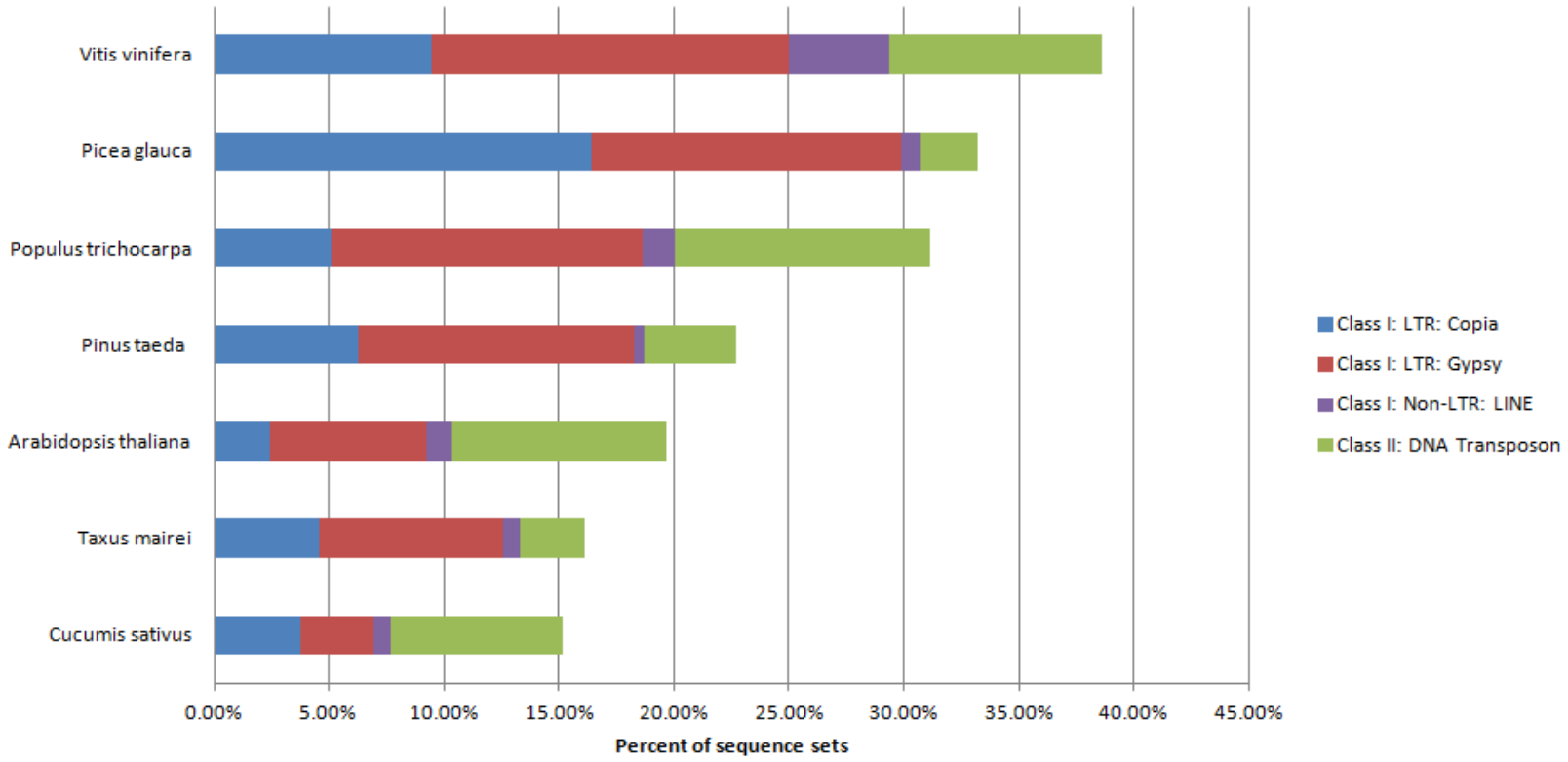


Pinus elliotti	Pinus thunbergii	Pinus radiata	Picea abies	Picea glauca	Abies veitchii	Larix
TY1_PE (Copia-LTR)	PLN1_PT (LINE) PLN2_PT (LINE) PLN3_PT (LINE) RT_PT (Copia-LTR)	IFG7_I (Gypsy-LTR) IFG7_LTR (Gypsy-LTR)	AlISEI_LTR(Gypsy-LTR) ALISEI_I(Gypsy-LTR)	PGGYPSYX1 (Gypsy-LTR) PCOPIAX1 (Copia-LTR)	ROMANIAV1 (Gypsy-LTR)	SAT1_LM (Satellite)

Homology Search Results

Censor (BLAST-style) comparisons against Repbase

Partial and Full-length Interspersed Alignments (compared across species)

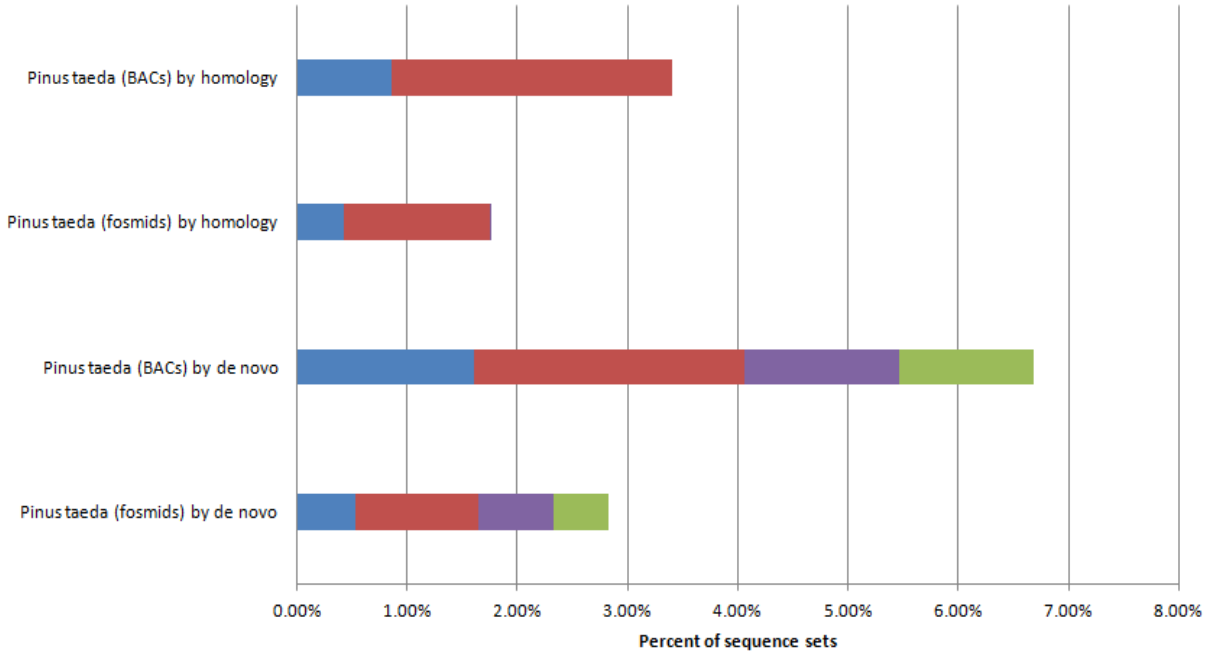


Summary of Combined Homology and De Novo Approach

	P + F (Homology)	Filtered (Homology)	P + F (de novo)	Filtered (de novo)
Class I	20.41%	1.39%	73.39%	21.82%
Class II	4.03%	0%	1.52%	0.53%
Other (Tandem)	3.06%	2.6%	12.97%	6.22%
Total	**27.50%**	**3.99%**	**87.89%**	**28.58%**

Full Length Sequences
80-80-80 Rule (Wicker et al. 2007)

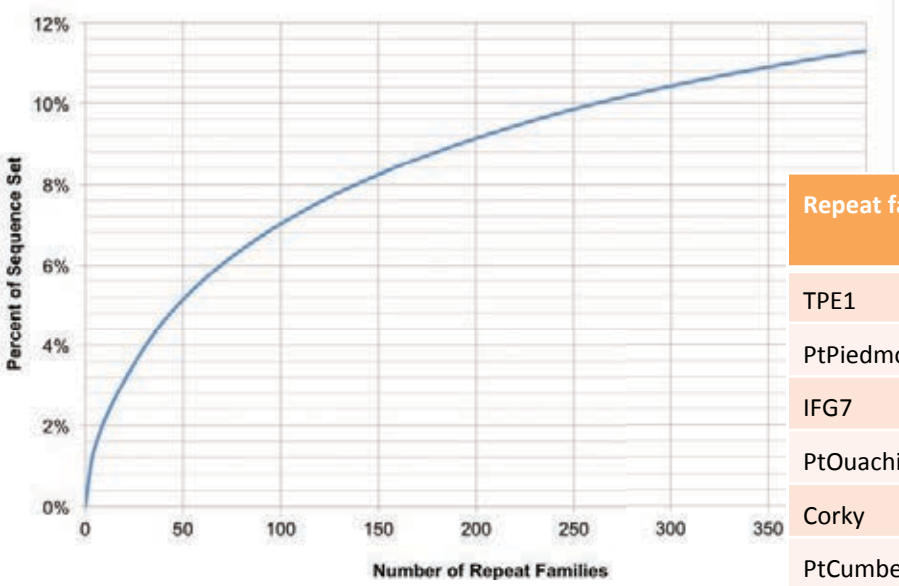
- 80 bp in length
- 80% identity
- 80% coverage



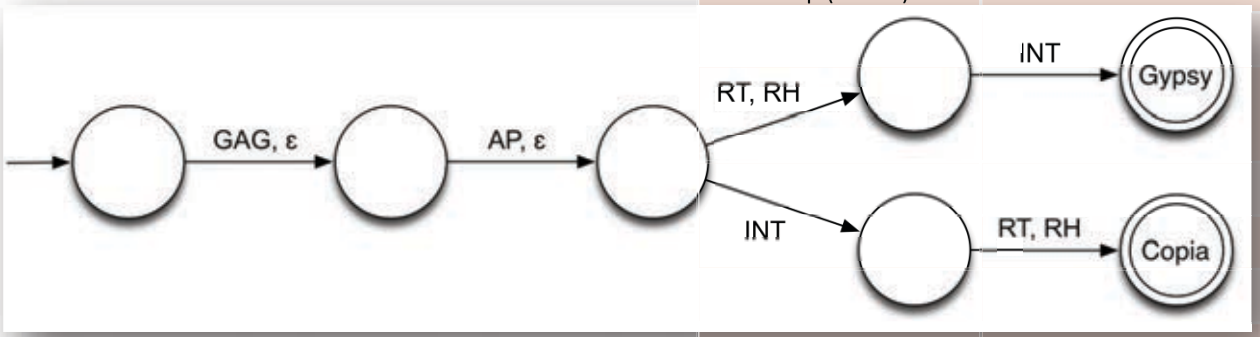
Novel Repeat Elements

Diverged LTRs are annotated as 6,270 novel families

Top 400 elements only cover 12% of the combined sequence sets

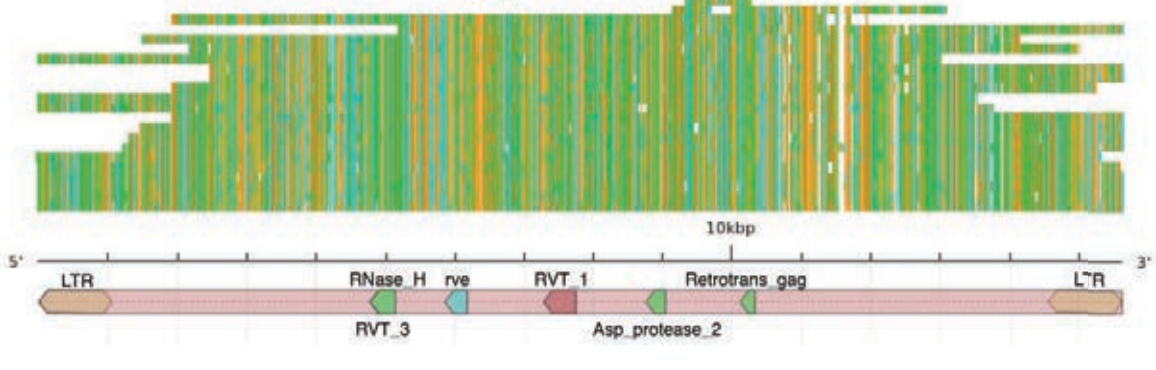


Repeat family	Full-Length Copies	Length (bp)	Percent of Sequence Set
TPE1	159	1,077,598	0.39%
PtPiedmont (93122)	133	969,109	0.35%
IFG7	162	956,018	0.34%
PtOuachita (B4244)	47	576,871	0.21%
Corky	78	469,286	0.17%
PtCumberland (B4704)	67	431,492	0.16%
PtBastrop (82005)	38	378,631	0.14%
		378,020	0.14%
		367,653	0.13%
		322,632	0.12%
		309,248	0.11%
		291,479	0.11%
PtConagree (B3341)	50	285,850	0.10%
PtTalladega (215311)	33	274,826	0.10%
Total	982	7,088,713	2.56%

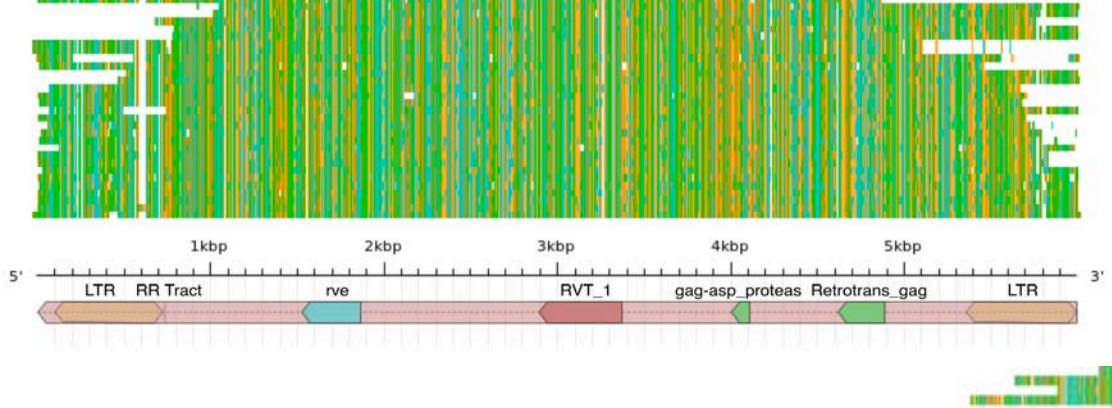


Novel Repeat Elements

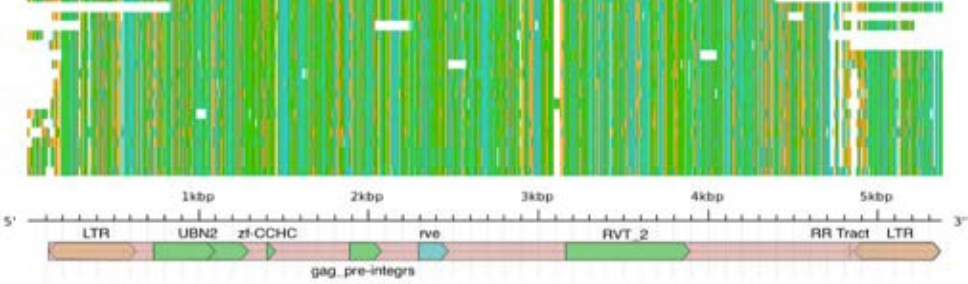
Multiple sequence alignment with annotations of the novel Gypsy LTR - PtOuachita



Multiple sequence alignment with annotations of the novel Gypsy LTR - PtAppalachian



Multiple sequence alignment with annotations of the novel Copia LTR - PtPineywoods



Summary of Combined Homology and De Novo Approach

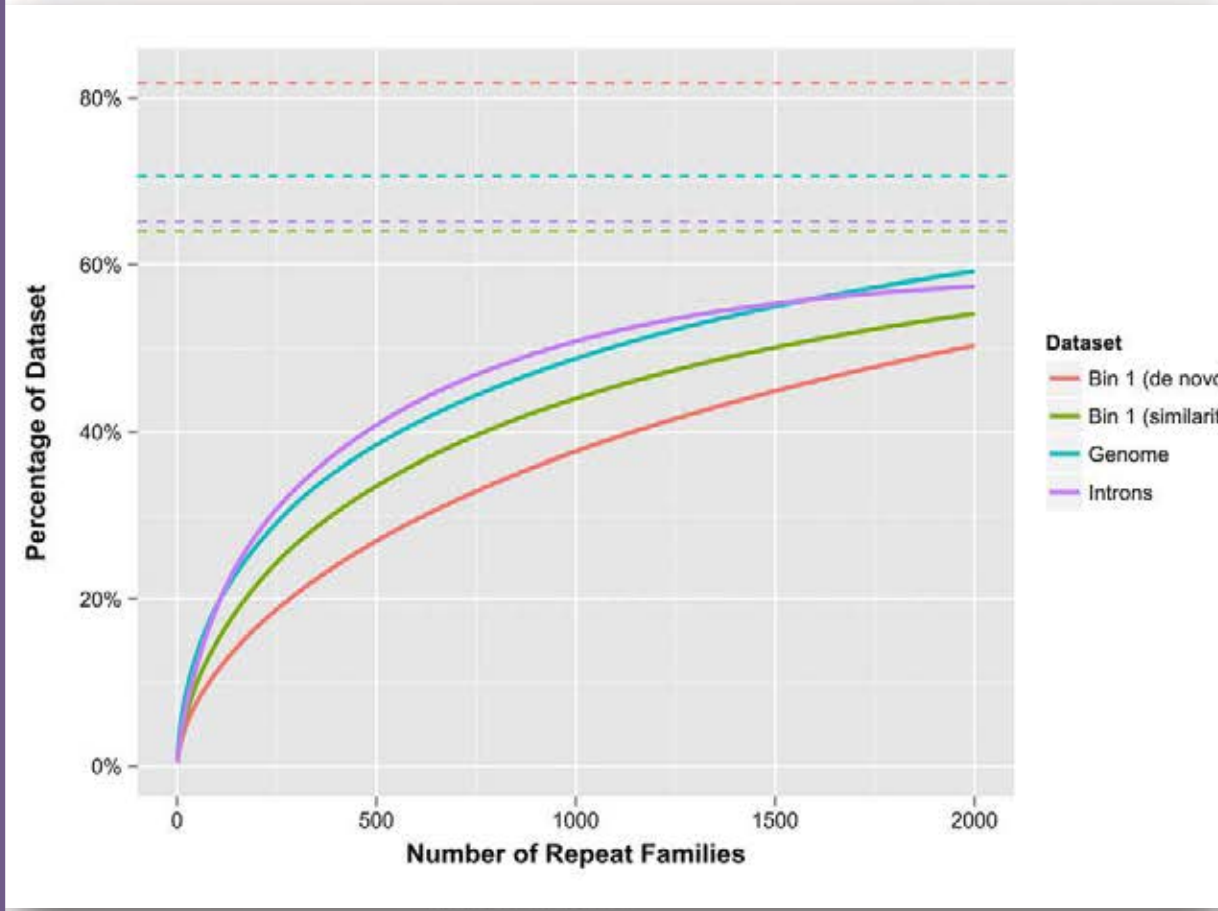
- 88% repetitive (partial and full-length)
- 29% repetitive (full-length only defined by 80-80-80)
  - 87% of the full-length content is characterized as LTR retrotransposon (Gypsy and Copia)
- Repeats are highly diverged
  - Only 23% identified by homology for full and partial elements
  - Repbase contains just 15 (+5) gymnosperm elements
  - 6,270 novel families discovered with no homology
    - 5,155 are single copy
- Nested repeats common in LTR retrotransposons

PIER library

Pine Interspersed Element Repeat Library (PIER 2.0)

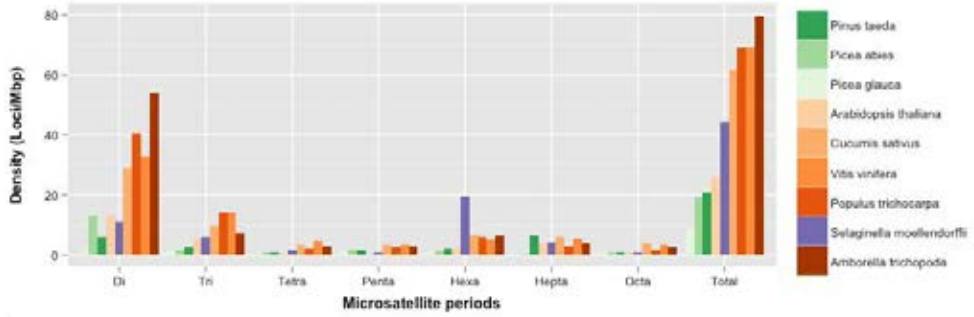
5,280 elements belonging to six families (plnrep, grasrep, mcotrep, dcotrep, oryrep, and athrep) from Repbase (17.07), 5 additional elements previously characterized, and 9,415 elements characterized in loblolly pine BAC/fosmids. Total = 14,700

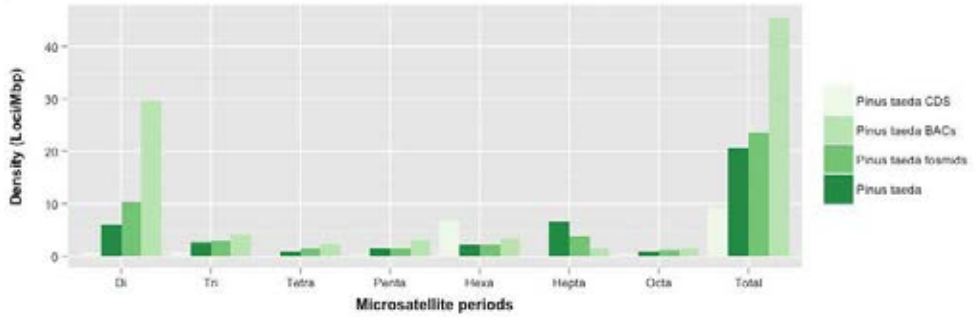
- Similarity analysis alone revealed just over 60% of the genome to be repetitive
  - Much less full length content identified than in BACs/ fosmids
- De novo analysis applied to bin1 estimates 82% repetitive
- Repeat element families are very diverged
- High copy and highest coverage elements different than those identified in BACs/fosmids originally
  - First 100 elements account for less than 10% of the genome
  - TPE1 and PtConagree



Tandem Repeats

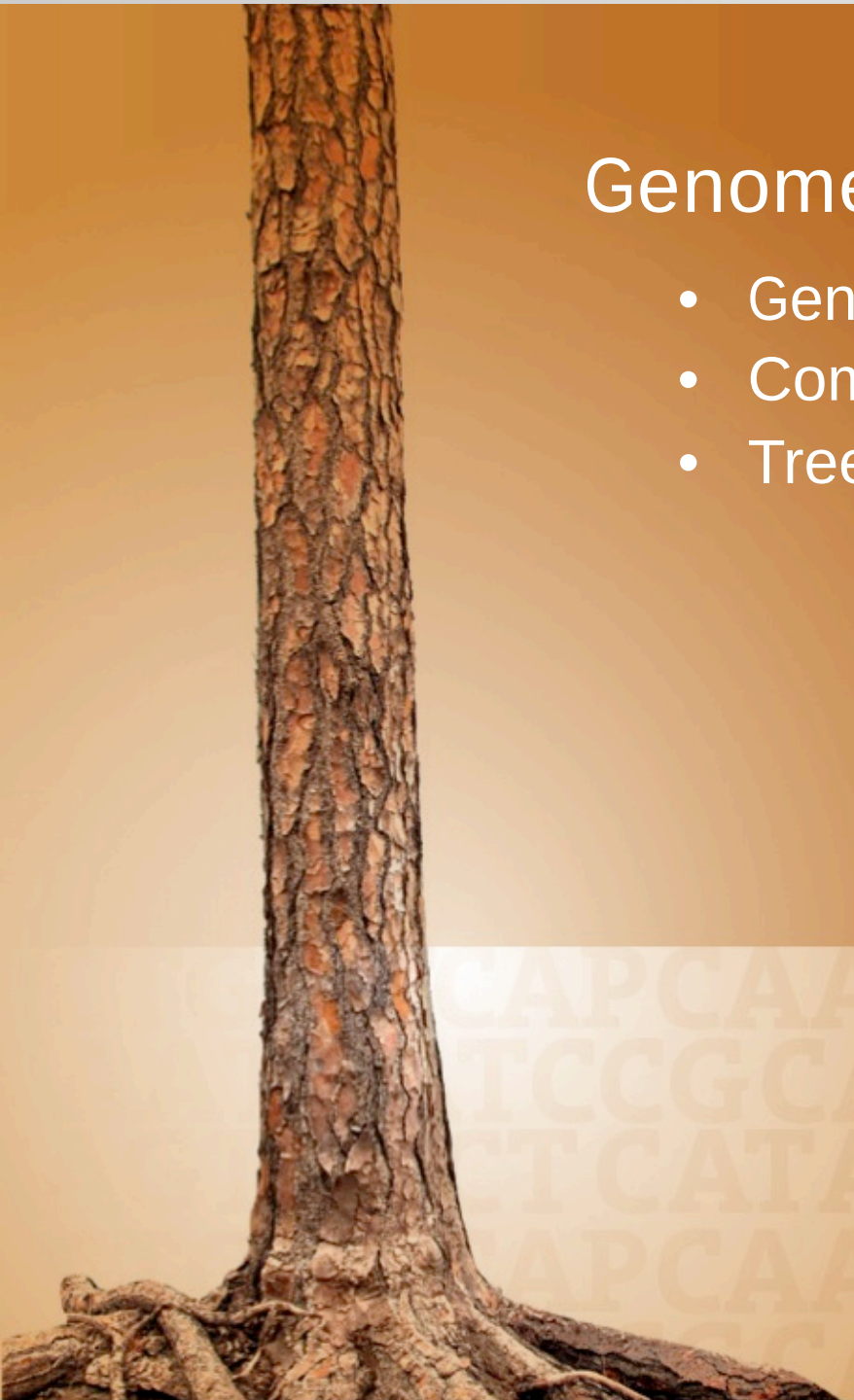
Comparison across sequenced angiosperms and other gymnosperms





Total tandem content for loblolly pine:
3.31% of BACs
2.59% of fosmids
2.86% of genome

	Pinus taeda v1.01			*Picea glauca v1.0*			*Picea abies v1.0*		
	Micro	**Mini**	**Sat**	**Micro**	**Mini**	**Sat**	**Micro**	**Mini**	**Sat**
Most frequent period size	7	21	123	2	21	102	2	50	101
Cumulative length (Mbp)	8.93	20.09	8.66	2.26	8.70	4.89	10.38	29.00	5.25
Num. of loci	145,992	361,356	27,422	62,592	179,716	23,256	255,380	285,648	24,895
Total cumulative length (Mbp)	27.33	396.42	221.01	7.48	357.90	198.83	18.63	299.25	151.81
Total %	0.12%	1.76%	0.98%	0.04%	1.72%	0.96%	0.10%	1.53%	0.77%
Total overall content	**2.86%**			**2.71%**			**2.40%**		



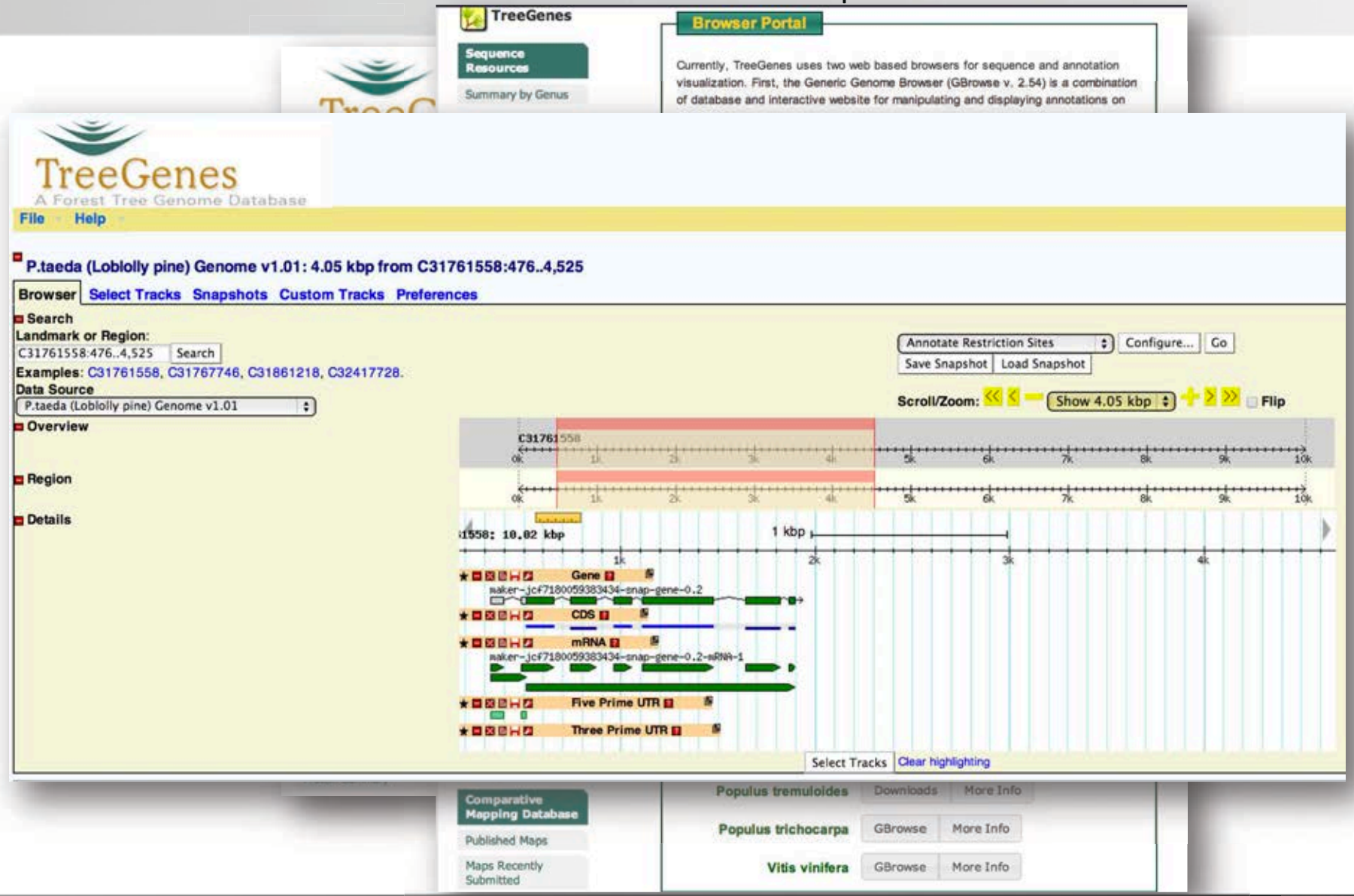
PineRefSeq

Genome Database (TreeGenes)

- Genome/Transcriptome Delivery
- Community-level annotation
- TreeGenes resources

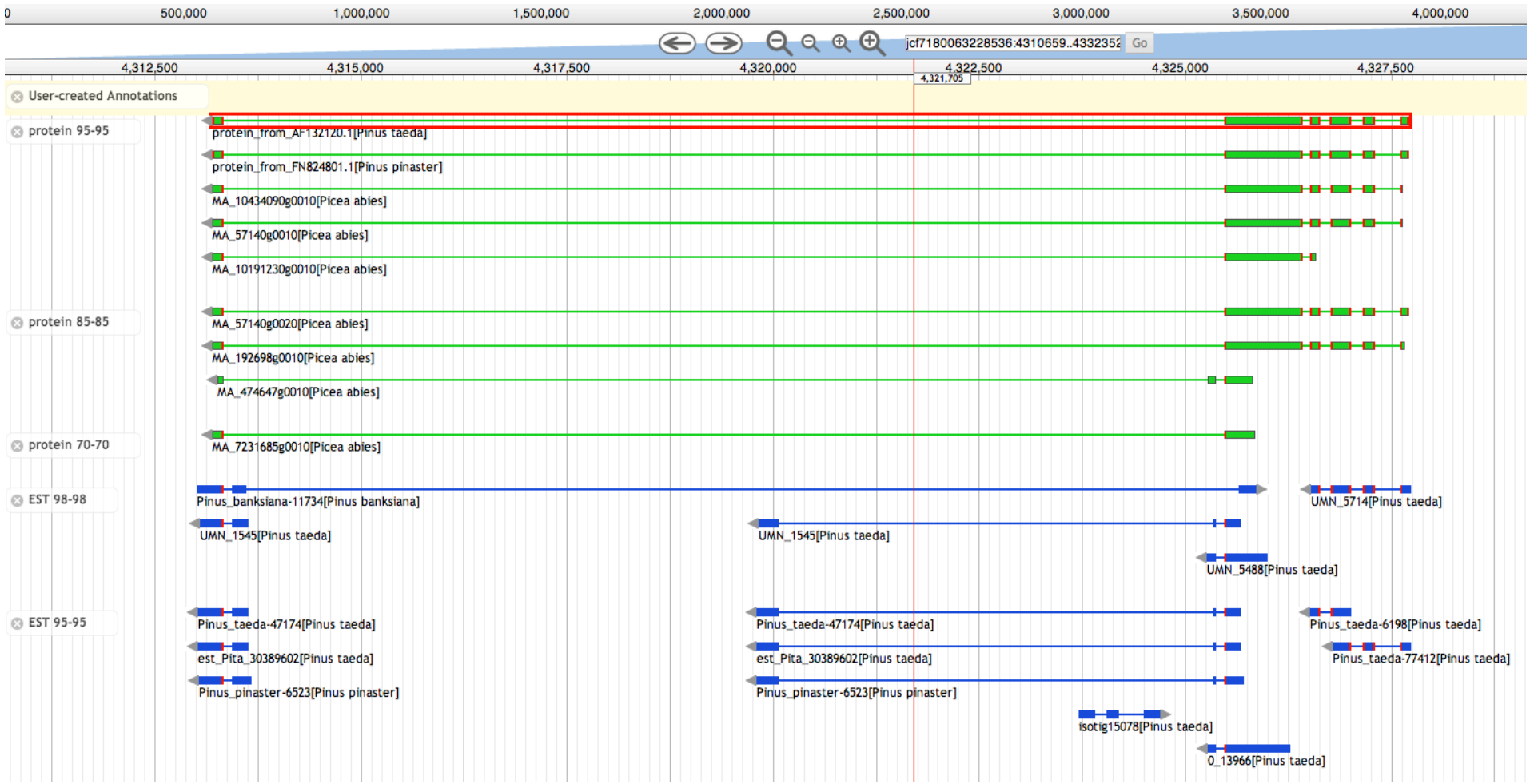
Dendrome Project

TreeGenes Database to Distribute Transcriptome and Genome



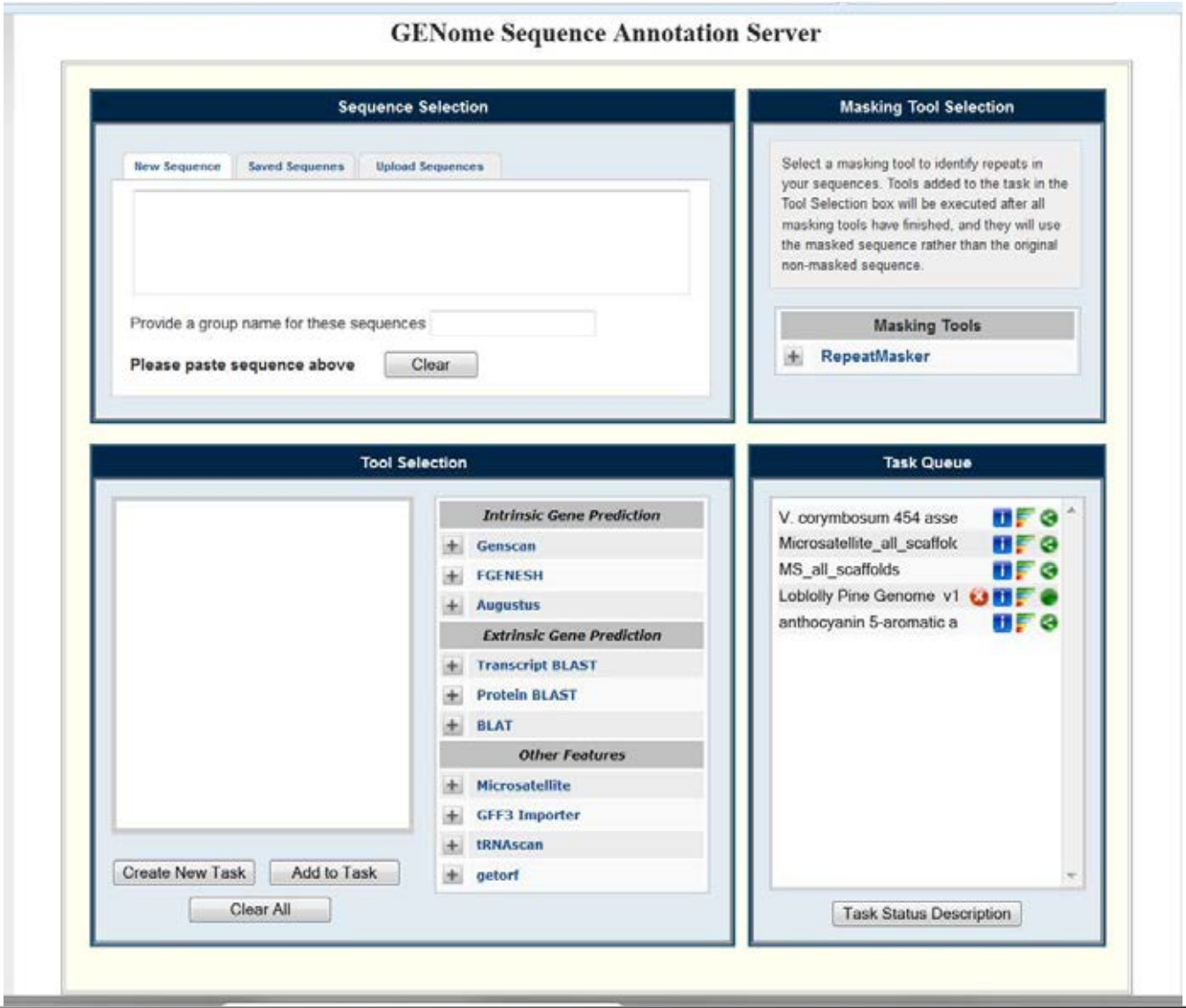
WebApollo on TreeGenes

Conifer specific proteins



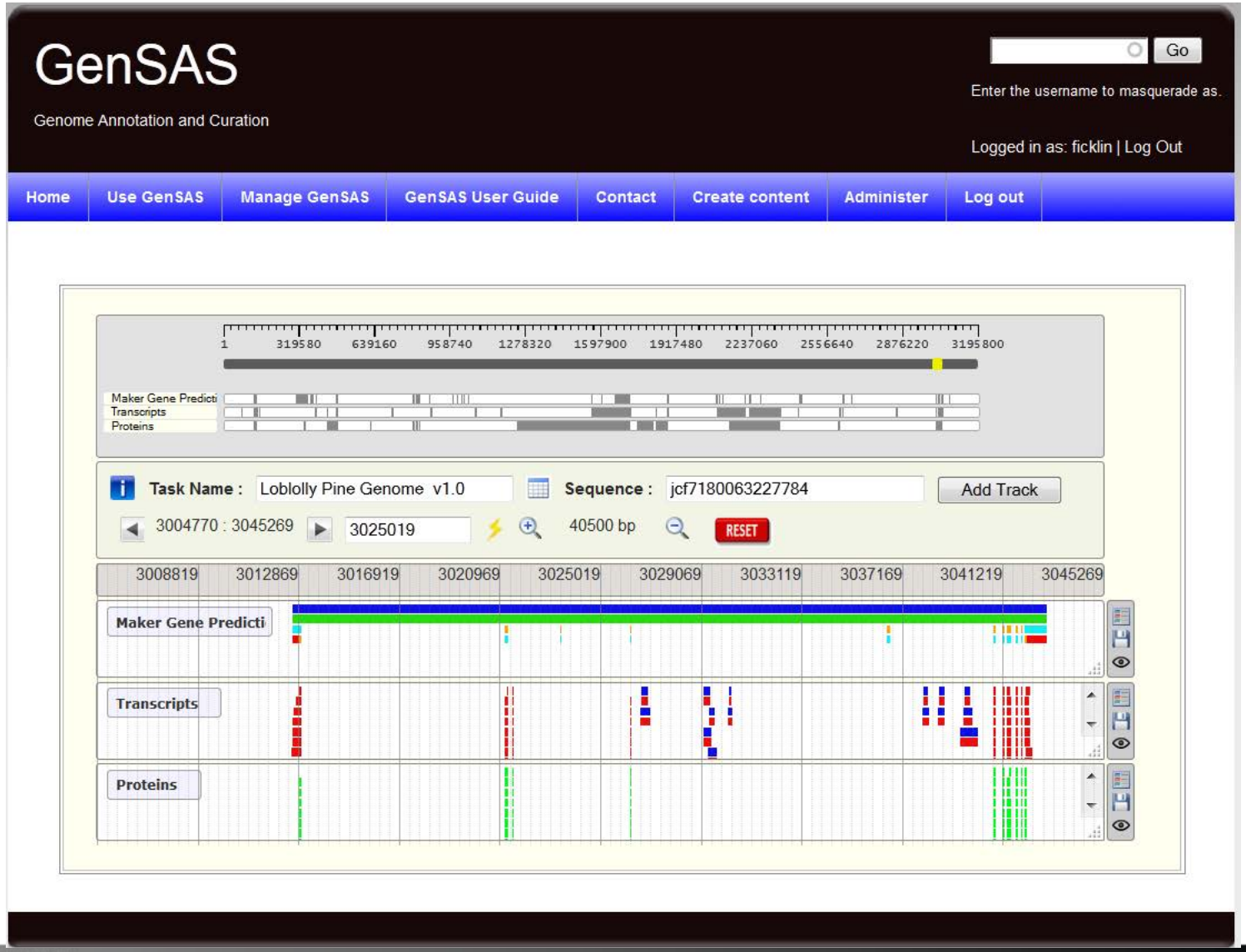
GenSAS

GENome Sequence Annotation Server



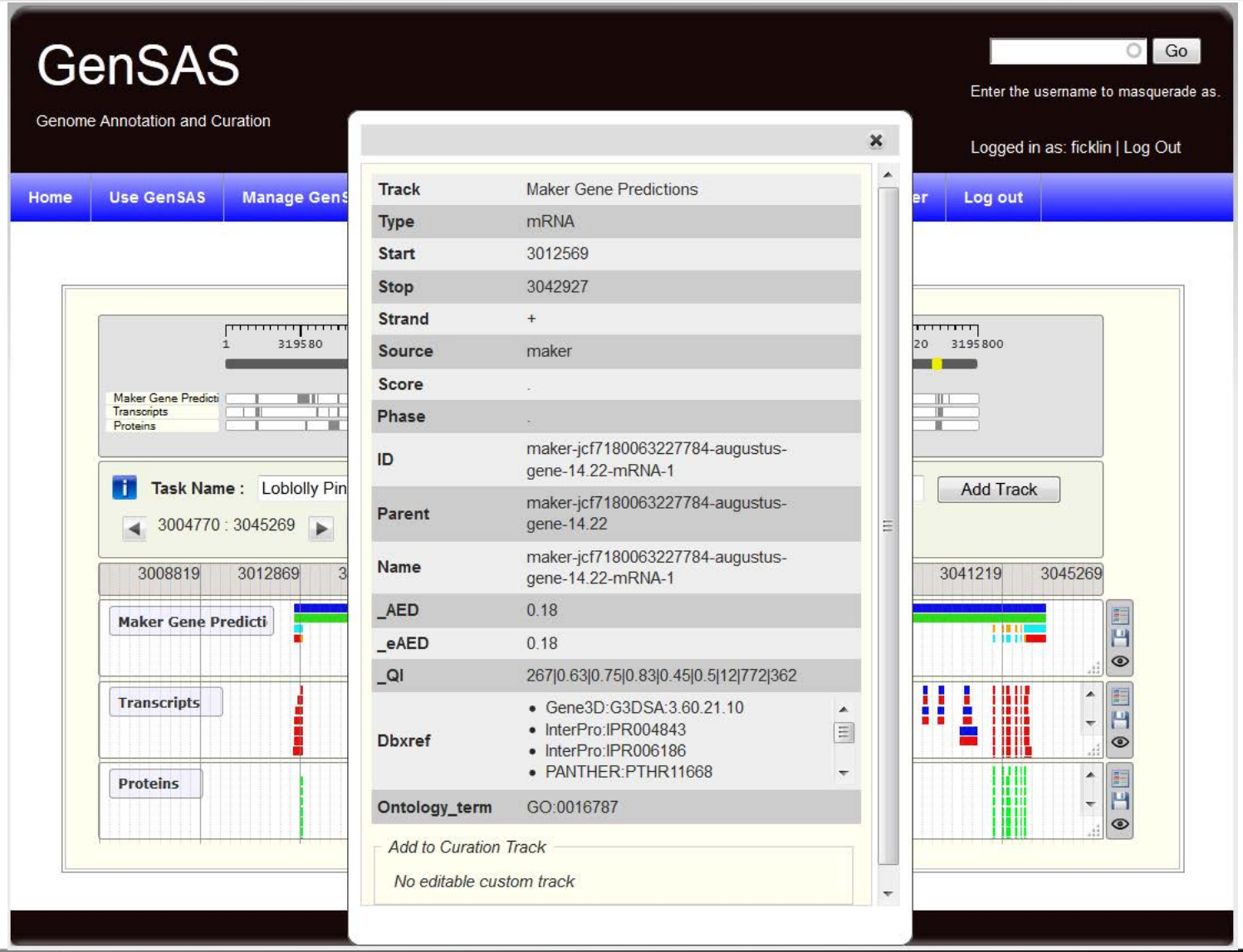
GenSAS

GENome Sequence Annotation Server



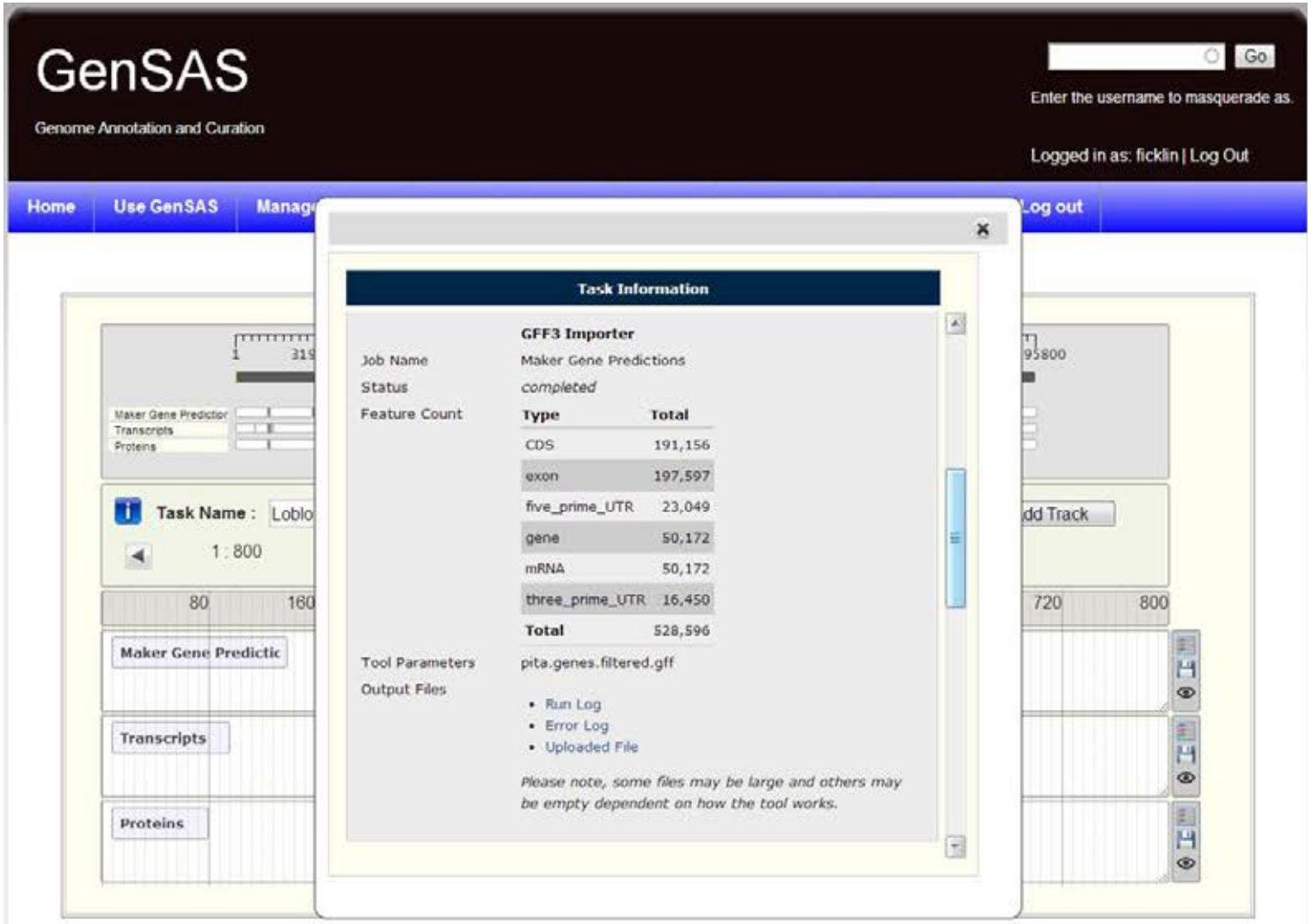
GenSAS

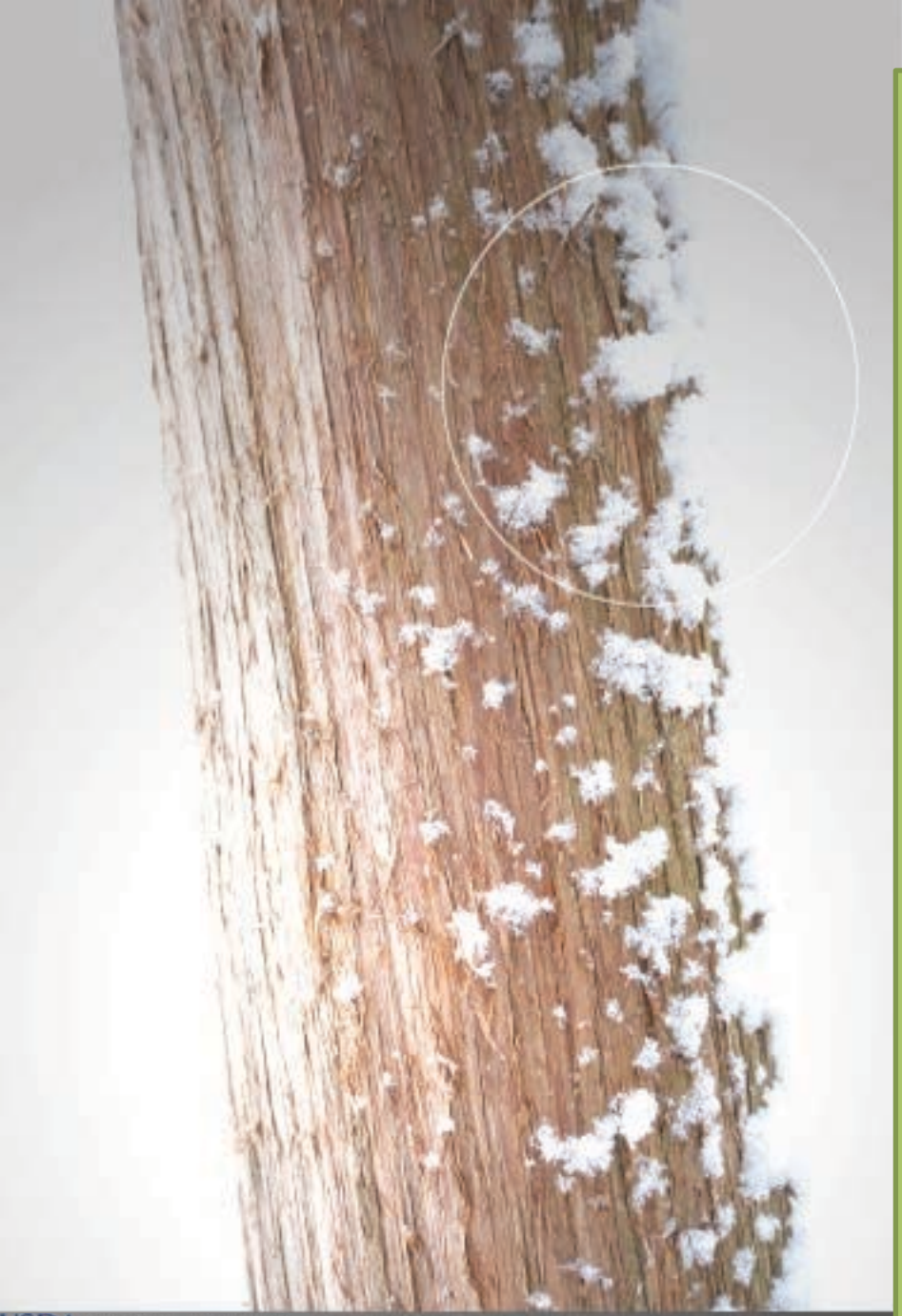
GENome Sequence Annotation Server



GenSAS

GENome Sequence Annotation Server





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PineRefSeq

Thank you!

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