

GenSAS

Genome Sequence Annotation Server

*Computational annotation and curation
of genome sequences*

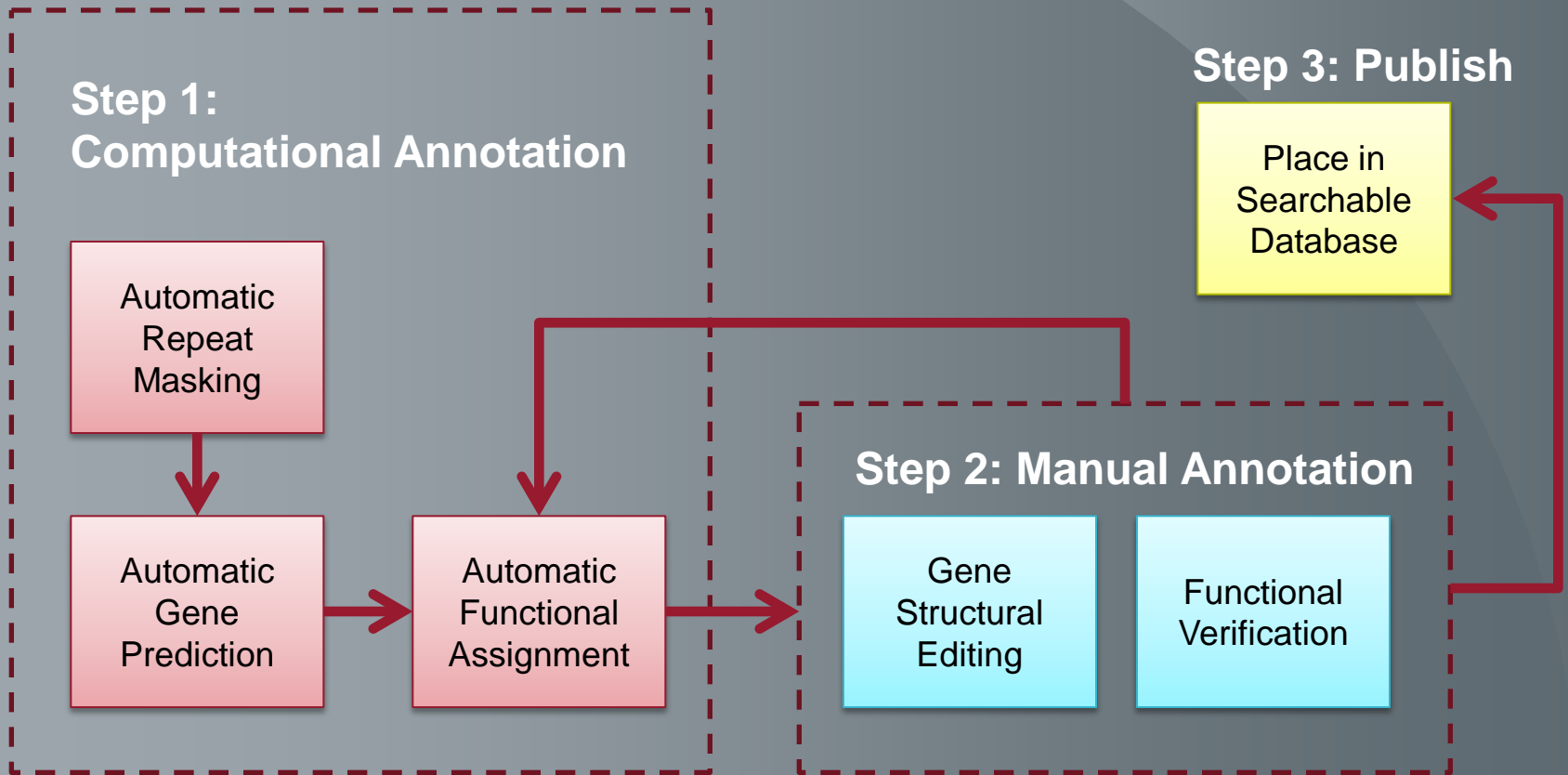
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** Presenting author*

Pine Genome Workshop

PAG XXIII, San Diego CA. Jan 10, 2015.

Genome Annotation Workflow



Existing Annotation Tools

- Computational Annotation (Automatic)
 - Maker
 - Ergatis
 - JAMg (Just Annotate My Genome)
 - iPlant DNA Subway
- Manual Annotation (Curation)
 - Apollo
 - WebApollo
 - Manatee
- These tools provide excellent annotation support but an integrated online solution to bridge all three workflow steps is lacking.

GenSAS Goals

- Streamline genomic annotation
 - Integrate all three stages of the annotation workflow
 - An easy-to-use online application
- Simplify pipeline setup for Bioinformaticist
- Support integration of final results into an online genome database for publication.
- Support analysis of very large genomes
 - Such as 22Gb *Pinus taeda* (Loblolly pine)
 - Using high-performance computing clusters
- Educate students and researchers
 - Inline instructions and best practices
 - Online videos & tutorials

GenSAS

The logo for GenSAS features the word "Gen" in black, "S" in green, "A" in red, and "S" in orange. Below the letters are three horizontal bars: a green bar under "Gen", a red bar under "S", and an orange bar under "A".

GenSAS Implementation

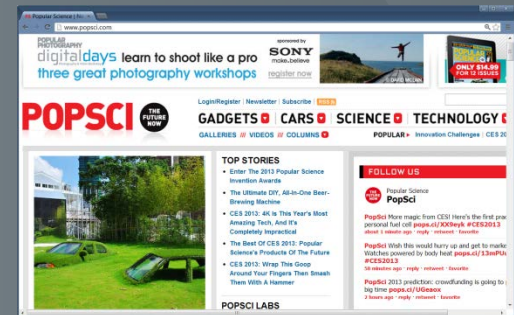
- A Drupal module
 - Drupal is an open-source, free Content Management System (CMS)
 - Used by millions of websites world-wide
 - High profile sites include:



US White House
<http://www.whitehouse.gov>



British Medical Journal
<http://www.bmj.com/>



Popular Science
<http://www.popsci.com/>

- A Drupal site can support social and content needs for a community in addition to functionality provided by GenSAS.
- Integrates easily with Tripal



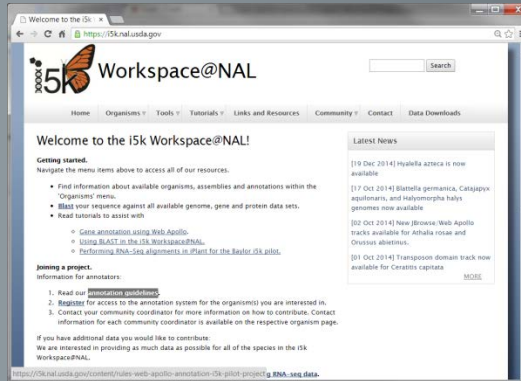
- Construction of online genomic websites
 - A suite of Drupal modules
 - Uses the GMOD Chado schema for data storage
 - Decreases cost and man-power required to create online genomics, genetics and breeding databases
 - Provides an Application Programming Interface (API)
 - Tripal development is currently funded by:
 - NSF DIBBs Award #1443040 (2015-2018)
 - USDA NRSP10 Award (2014-2019)
 - USDA SCRI Award # 2014-51181-22376 (2014-2019)
 - Pending support from several NSF PGRP proposals (include funds to convert Dendrome/TreeGenes to Tripal)

<http://tripal.info>

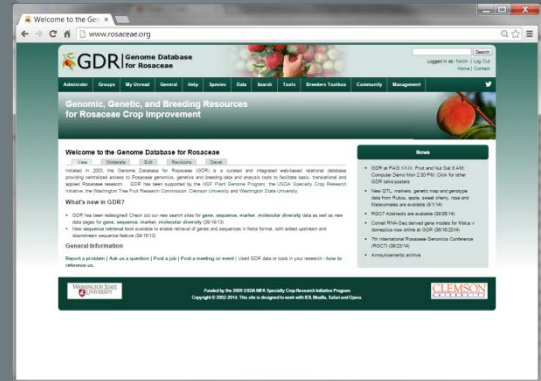
Example Sites Using Tripal



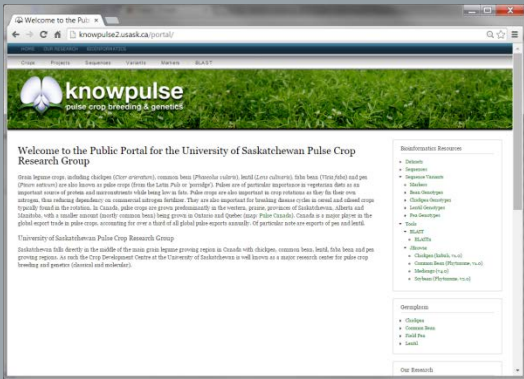
Banana Genome Hub
<http://banana-genome.cirad.fr/>



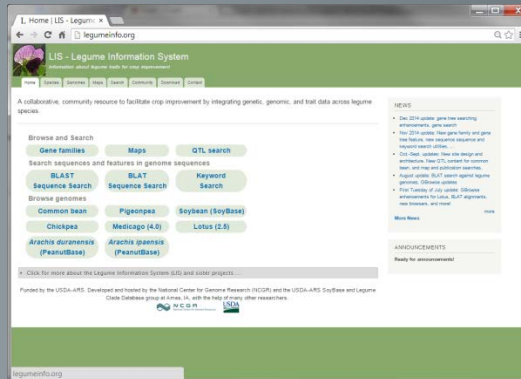
i5K Workspace@NAL
<http://i5k.nal.usda.gov/>



Genome Database for Rosaceae
<http://www.rosaceae.org>



Knowpulse: pulse crop genomics & breeding
<http://knowpulse2.usask.ca/portal>



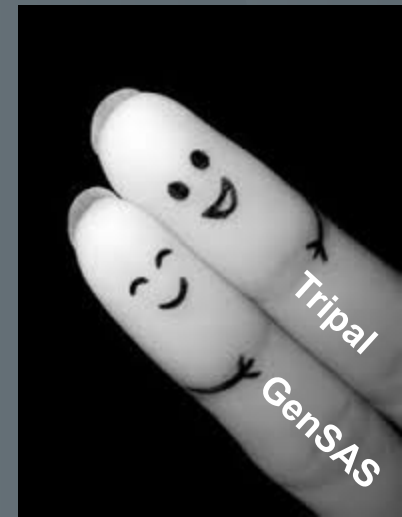
Legume Information System
<http://legumeinfo.org/>



CottonGen
<http://www.cottongen.org>

GenSAS + Tripal

- Completes the “publish” stage
 - GenSAS fully integrates into the same Drupal site that uses Tripal
 - Results generated by GenSAS are 100% compatible
 - Versioning of results in GenSAS ensures no conflict with future annotation updates.
 - Tripal provides searching and pages (e.g. gene pages)
- Three options:
 - GenSAS expanded later with Tripal for full publication of the genomic annotations
 - GenSAS added to an existing Tripal site
 - GenSAS can be installed as a separate tool for non Tripal sites.



GenSAS Availability

- Public server:
 - <http://gensas.bioinfo.wsu.edu>
 - Resources are currently limited, thus accounts are given on a case-by-case basis
 - Anyone can request an account
 - Currently houses *Pinus taeda* Maker annotations



Welcome to GenSAS

The Genome Sequence Annotation Server (GenSAS) is an online tool that provides a pipeline for whole genome structural annotation. Users can upload genome sequences and select from a variety of tools for repeat masking, prediction of gene models and other structural features. GenSAS integrates with JBrowse and WebApollo to provide visualization and editing. Full support for functional annotation is coming soon!

User login

Username *

Password *

- [Create new account](#)
- [Request new password](#)

- Source Code
 - <http://drupal.org/project/gensas>
 - Available soon...
 - download and install on local computational resources.

Functionality Overview

- GenSAS v3.0 provides:
 - Repeat Finding & Masking: repeat libraries and *de novo*
 - *RepeatMasker, RepeatModeler*
 - Gene Prediction
 - Intrinsic
 - using tools the use heuristics and the genomic sequence
 - *Augustus, FgeneSH (parsing only), Genscan, Glimmer3, GlimmerM, SNAP, tRNAScan, getorf*
 - Extrinsic
 - using tools that use transcript or protein libraries to assist with gene prediction.
 - *BLAT, BLAST*
 - Gene Consensus Prediction
 - *EvidenceModeler*
 - Gene Visualization and Curation
 - *WebApollo, Jbrowse*
 - Publish
 - Generates GFF3 & FASTA files with properly versioned naming.

Using GenSAS

Project Sequences Files Repeats Masking Genes Consensus Refine Functional Annotate Publish

Hello GenSASuser Account Page | Site Home | Log out

Current Project : N/A

Welcome! x Project



Tabs will appear here to provide the content needed for each stage. Some tabs can be closed and re-opened later but others will always remain. The tabs will provide instructions for each workflow stage.



Click the buttons above to move through the annotation workflow. As you complete a stage the next button becomes available. Click the **Project** button to begin.



Welcome to Genome Sequence Annotation Server !

Click the **Job Queue**



to view the analysis jobs for the project. Click the **Browser** tab to view the predicted features aligned to the genomic sequence. Click the **Sharing** tab to share the project with other GenSAS users. Click the **Manage Sequences** tab to manage the uploaded sequences.

Job Queue

Please start a project

Browser

Sharing

Manage Sequences

- View the GenSAS Computer Demo C03 at 2:10pm today in California Room for further detail, plus Poster # P1153

Step 1: Start a project

Welcome! x Project

▼ Begin a New Project

Project Name

Please provide a name for this project

Project Description

Please provide a description for this project.

Project Type

Please select the project type that best describes the organism and type of sequences that will be provided.

▼ Organism Details

Genus *

The genus name of the organism for this project.

Species *

The species name of the organism for this project.

Common Name

The common name for this species.

Step 2: Upload Sequences

Project Sequences Files Repeats Masking Genes Consensus Refine Functional Annotate Publish

Welcome! x Sequences

Upload Sequences

FASTA Sequence File *
 No file chosen

Upload a FASTA file either as plain text or with gzip compression.

FASTA file names : File names must include only alpha numeric characters, dashes or periods in the file name. File extension must be one of the followings : fasta, fna, fa, fas and gz
Sequence names (the first non-space characters in the definition line) : contain only alpha numeric characters, periods, underscores and dashes. Please ensure no colons are present in a sequence name.

Sequence Type *

Please select the type of sequences you are providing. Below is a list describing each type:

- pseudomolecule: A partial DNA sequence assembly of a chromosome or full genome, which contains gaps that are filled with N's. Sequence Ontology ID: [SO:0001876](#)
- scaffold: One or more contigs that have been ordered and oriented using end-read information. Contains gaps that are filled with N's. Sequence Ontology ID: [SO:0000148](#)
- contig: A contiguous sequence derived from sequence assembly. Has no gaps, but may contain N's from unavailable bases. Sequence Ontology ID: [SO:0000149](#)

Sequence Versioning Instructions

Assembly Version Major Number *

Please provide the major version number for the sequence set.

Assembly Version Minor Number *

Please provide the minor version number for the sequence set.

Other Version Identifier *

If your sequences do not have a version number with a major and minor version and if it is not possible to use such a version system. Please provide a unique identifier to indicate the version of the sequence set.

Step 3: Upload Supporting Files

▼ Evidence Files

One of the best ways to identify genes is to provide transcript (e.g. ESTs, full length cDNA, RNA-seq) and protein files from the species or from closely related species. These files should be provided in FASTA or FASTQ format, and when aligned to the genomic sequence serve as direct evidence of gene expression. Transcripts and proteins of closely related species can be provided when species-specific files are not available or are insufficient. Supplying a species-specific (or closely-related species) file of known repeats, in FASTA format, can help with identification of repetitive elements more accurate for the species.

Uploaded Evidence Files

File	Type	Size	Action
There are currently no files uploaded.			

▶ Repeat Library

▶ EST Evidence

▶ Protein Evidence

▶ RNA-seq Evidence (currently unavailable)

Step 3: Upload Supporting Files

▼ Gene Predictions, Repeats, or Alignments

If you have already identified repetitive elements, protein or transcript alignments or gene predictions for your sequences outside of GenSAS and you have results in a GFF3 file you may upload them here. Upload as many GFF3 files as needed. A job for parsing of the GFF3 file will be added to the job queue for each GFF3 file you upload. Please be sure that the landmark sequences (first column in the GFF3 file) matches the sequence names used with this project. GenSAS uses the set of characters up to the first space as the sequence name.

▶ Upload Repeats in GFF3 Format

▼ Upload Alignments, Genes or Other Predictions in GFF3 Format

Job Name

Please provide a name for this GFF3 import.

GFF3 Type

Gene Prediction ▼

What type of annotations are contained in this GFF3 file?

GFF3 Prediction File

Choose File No file chosen

Upload

Please provide the GFF file.

Import GFF3 File

Current *Pinus taeda* Maker predictions loaded in this way

Step 4: Repeat Finding

▼ RepeatMasker

RepeatMasker is a program that screens DNA sequences for interspersed repeats and low complexity DNA sequences. The output of the program is a detailed annotation of the repeats that are present in the query sequence as well as a modified version of the query sequence in which all the annotated repeats have been masked (default: replaced by Ns). On average, almost 50% of a human genomic DNA sequence currently will be masked by the program. Sequence comparisons in RepeatMasker are performed by one of several popular search engines including, cross_match, ABBlast/WUBlast, RMBlast and Decypher.

Job Name

Please provide a name for this RepeatMasker job. It is used to distinguish between two or more RepeatMasker jobs.

Search Engine

The search engine to use (e.g. wublast, cross_match)

Speed / Sensitivity

The speed vs. sensitivity parameter

DNA Source

Specify the species or clade of the input sequence. The species name must be a valid NCBI Taxonomy Database species name and be contained in the RepeatMasker repeat database.

Repeat Options

Select the types of repeats you would like to mask.

Step 5: Masking

Repeat Masking

▶ Instructions

Final Masking Selection

Select the repeat masking jobs that you want to include in masking of the genomic sequence prior to gene discovery.

Masking Job	Status
<input type="checkbox"/> RepeatMasker-slow	Completed
<input type="checkbox"/> RepeatMasker	Completed

Mask Sequences

Skip Repeat Masking Step

Step 6: Gene Prediction

The screenshot displays the GenSASu web interface. At the top, a navigation bar contains the following steps: Project, Sequences, Files, Repeats, Masking, Genes (highlighted in green), Consensus, Refine, Functional, Annotate, and Publish. The user's name, "Hello GenSASu", is visible in the top right corner. Below the navigation bar, there is a tabbed interface with tabs for "Welcome!", "Feature Tools", "WebApollo", and "JBrowse". The main content area is titled "Instructions" and features a sidebar on the left with the heading "Gene Prediction". The sidebar contains three sub-sections: "Transcript Alignments", "Protein Alignments", and "Other Features". The main content area lists six gene prediction methods, each with a right-pointing arrow: Augustus, FGENESH, Genscan, Glimmer3, GlimmerM, and SNAP. At the bottom left of the main content area, there is a button labeled "Move on to Gene Consensus".

Project Sequences Files Repeats Masking **Genes** Consensus Refine Functional Annotate Publish Hello GenSASu

Welcome! x Feature Tools WebApollo x JBrowse x

▸ [Instructions](#)

Gene Prediction

- Transcript Alignments
- Protein Alignments
- Other Features

▸ Augustus

▸ FGENESH

▸ Genscan

▸ Glimmer3

▸ GlimmerM

▸ SNAP

Move on to Gene Consensus

View Results

Project Sequences Files Repeats Masking **Genes** Consensus Refine Functional Annotate Publish Hello GenSAS

Welcome! x Project WebApollo x JBrowse x

Reload JBrowse | View JBrowse outside of GenSAS

Available Tracks

- filter by text
- Gene Predictions 5
 - Augustus
 - Augustus-complete genes only
 - Genscan
 - GlimmerM
 - SNAP
- Repeats 2
 - RepeatMasker
 - RepeatMasker-slow
- Transcript Alignments 2
 - BLAST (nucleotide)
 - BLAT

apollo File View Help Login

0 20,000 40,000 60,000 80,000 100,000 120,000 140,000

TAIR10_c1_150kb TAIR10_c1_150kb:60024..90767 (30.75 Kb) Go

62,500 75,000 87,500

Augustus At_1150_000120g.m01 At_1150_000140g.m01 At_1150_000170g.m01 At_1150_000180g.m01 At_1150_000190g.m01 At_1150_000130g.m01 At_1150_000150g.m01 At_1150_000160g.m01

Augustus-complete genes only At_1151_000140g.m01 At_1151_000170g.m01 At_1151_000180g.m01 At_1151_000190g.m01 At_1151_000130g.m01 At_1151_000150g.m01 At_1151_000160g.m01

GlimmerM

SNAP At_1154_000140g.m01 At_1154_000160g.m01 At_1154_000190g.m01 At_1154_000200g.m01 At_1154_000150g.m01 At_1154_000170g.m01 At_1154_000180g.m01 At_1154_000210g.m01 At_1154_000220g.m01

Step 7: Consensus

Identification of Genes and other Features

Instructions

Select the gene finding jobs that you want to include in building the consensus gene predictions and provide a numerical weight indicating the expected accuracy of the predictions. Higher weights indicate higher accuracy.

Gene Finding Job	Status	Weight
Gene Prediction		
Augustus	Completed	<input type="text"/>
Augustus-complete genes only (Augustus)	Completed	<input type="text"/>
Genscan	Completed	<input type="text"/>
GlimmerM	Completed	<input type="text"/>
SNAP	Completed	<input type="text"/>
Protein Alignments		
BLAST (proteins)	Completed	<input type="text"/>
Transcript Alignments		
BLAST (nucleotide)	Completed	<input type="text"/>
BLAT	Completed	<input type="text"/>

Step 8: Manual Curation

Curation Track

The screenshot displays the Apollo genome annotation software interface. The top navigation bar includes tabs for 'Welcome!', 'Annotate', 'WebApollo', and 'JBrowse'. The main window features a menu bar with 'File', 'View', 'Tools', and 'Help', and a user profile for 'GenSASuser'. A scale bar at the top indicates genomic coordinates from 0 to 140,000, with a red box highlighting the current view range from 60,000 to 80,000. Below the scale bar, navigation controls (back, forward, zoom) and a search box containing 'TAIR10_c1_150kb' are visible. The main display area shows several tracks:

- User-created Annotations:** A track showing blue boxes representing exons and lines representing introns for the gene At000150g.m01. A red arrow points to a specific exon at approximately 66,250 coordinates.
- Consensus Gene Set:** A track showing the consensus gene set for At000150g.m01.
- Augustus-complete genes only:** A track showing the Augustus-complete gene set for At_1151_000130g.m01.
- BLAST (proteins):** A track showing protein alignments for Q6ZLP5, Q2QY53, Q2RAX3, Q93VD3, Q84VQ3, and Q10SC8.
- BLAST (nucleotide):** A track showing nucleotide alignments for NM_099996.3 and NM_179240.1.

The left sidebar contains the 'Available Tracks' section, which is currently filtered by text. The tracks listed are:

- Gene Predictions (6):
 - Augustus
 - Augustus-complete genes only
 - Consensus Gene Set
 - Genscan
 - GlimmerM
 - SNAP
- Protein Alignments (1):
 - BLAST (proteins)
- Repeats (2):
 - RepeatMasker
 - RepeatMasker-slow
- Transcript Alignments (2):
 - BLAST (nucleotide)
 - BLAT

Step 9: Publish

Available Results for Publishing

Please choose the jobs to be included in the published release for this project.

Consensus Gene Predictions

Consensus Gene Set (EvidenceModeler)

The consensus gene set will be the primary set of genes in your published annotation set, therefore, it is selected by default to be published.

Consensus Masking

Masked Consensus

The repeat masked consensus job created the FASTA sequence on which all other predictions were made. This job should be included in any published release.

Gene Predictions

Augustus (Augustus)

Genscan (Genscan)

GlimmerM (GlimmerM)

SNAP (SNAP)

Because you have a consensus gene prediction set you do not need to publish additional singular gene prediction results. The consensus will be the set used by others and will be considered candidates for future annotations. However, these gene predictions help provide evidence for how the consensus genes were constructed. If you would like to provide this information please include any gene prediction jobs as desired.

Repeats & Masking

RepeatMasker (RepeatMasker)

RepeatModeler (RepeatModeler)

Jobs that were used in construction of the consensus masked sequence are selected by default.

Protein Alignments

BLAST (proteins) (BLAST (proteins))

Protein alignments help provide evidence for the predicted gene models and provide clues for functional assignment. Consider including protein alignments that help provide support. Avoid including any with an overwhelming set of alignments such as alignments against an all-inclusive protein database. It is best to include alignments to species-specific or species-related alignments.

Step 9: Publish

Annotation version number

In order to prevent naming conflicts with past or future annotations for the same genomic sequence assembly version, the annotations should have a separate version. If this is the first time that the genomic sequence will be annotated then the annotation version should be 1. When a new set of predictions are created for the same genomic sequence then the annotation version number should be incremented by 1. GenSAS will include both the sequence version number and the annotation version number in the names of predicted features. For example, if the genomic sequence version is v1.0 and this is the first annotation set, GenSAS will include "v1.0.a1" in the feature names to indicate the genomic sequence and annotation set to which the predicted features belong.

Publish

Ongoing Work

- **Functional Annotation**
 - Does not yet fully support functional annotation.
 - Requires addition of functional tools and result viewers.
 - Currently under development
 - Will be available in a future release of GenSAS
- **Support of High-Performance Computing**
 - Currently executes on stand-alone server
 - Will integrate with two types of HPC schedulers: PBS, GE
 - Currently under development
 - Will be available in a future release of GenSAS
- **Support of RNA-Seq Datasets**
- **Integration with PASA for gene refinements**
- **Full Integration with Tripal**
 - Currently only exported files are compatible with Tripal loaders
 - Integrate so that publish button can automatically import into Tripal.

Funding

- **Current Funding:**
 - The work presented herein was funded by the SDA/NIFA (2011-67009-30030) subaward to Dorrie Main at Washington State University (PI: David Neale at University of California, Davis)
- **Continued Funding**
 - Further funding for development and implementation of GenSAS is provided by an USDA National Research Support Project (NRSP10) to WSU (Dorrie Main PI) through 2019.

GenSAS for Pine Annotation

- Current capabilities

- *Pinus Taeda v1.01* Maker annotations loaded
- Pine community can annotate the predicted genes using integrated WebApollo
- WebApollo manages user access (via GenSAS integration)
- Annotation training may be available via WebApollo outreach for community.

- Future capabilities

- Functional Annotation:
 - Execute functional tools inside GenSAS
 - Result viewers for tools such as InterProScan, SignalP, TargetP, Pfam, blastp, etc.
- Tools will execute on high-performance computing to decrease time requirements
- Future pine genomes can be annotated directly in GenSAS

Loblolly Pine in GenSAS

GenSAS x GenSAS x

gensas2.bioinfo.wsu.edu/gensas

Project Sequences Files Repeats Masking Genes Consensus Refine Functional Annotate Publish

Hello fickl Account Page | Site Home | Log out
Current Project : Loblolly v1.01 Assembly & Annotation

Welcome! x Project WebApollo x JBrowse x Maker High Quality Whole Genes results x

Reload JBrowse | View JBrowse outside of GenSAS

Available Tracks

filter by text

Gene Predictions 4

- Maker High Quality Partial Genes
- Maker High Quality Whole Genes
- Maker Low Quality Partial Genes
- Maker Low Quality Whole Genes

Other Features 1

- InterProScan

Protein Alignments 2

- blastx
- protein2genome

Repeats 1

- RepeatRunner

Transcript Alignments 2

- blastn
- est2genome

pollo File View Help Login

0 500,000 1,000,000 1,500,000 2,000,000 2,500,000 3,000,000

tsscaffold1547 tsscaffold1547:2771756..2880569 (108.8) Go

2,775,000 2,800,000 2,825,000 2,850,000 2,875,000

Maker High Quality Whole Genes

Pita_1135_197200g.m01 Pita_1135_197180g.m01

InterProScan

IPR000109 Oligopeptide transporter IPR005135 Endonuclease/exonuclease/phosphatase IPR006384:match_part:3

588 IPR005135 Endonuclease/exonuclease/phosphatase IPR000109:match_part:331223 IPR005135 Endonuclease/exonuclease/phosphatase

exonuclease/phosphatase IPR000109:match_part:442364 IPR000109 Oligopeptide transporter IPR005135 Endonuclease/exonuclease/phosphatase

413 IPR005135:match_part:522379 IPR006384 Pyridoxal phosphate phosphatase-related IPR005135:match_part:120492

414 IPR005135:match_part:522380 IPR006384:match_part:331230 IPR005135:match_part:75551

IPR005135:match_part:522381 IPR006384:match_part:331231 IPR005135:match_part:75552

IPR016196 Major facilitator superfamily, general substrate transporter IPR005135:match_part:120493

IPR016196:match_part:442367 IPR000109:match_part:331222 IPR005135:match_part:199432

IPR018456 PTR2 family proton/oligopeptide symporter, conserved site IPR005135 Endonuclease/exonuclease/phosphatase

IPR018456:match_part:442362 IPR000109:match_part:331224 IPR005135:match_part:120491

IPR016196:match_part:442368 IPR006384 Pyridoxal phosphate phosphatase-related

Job Queue

[View full report](#) | [Update status](#)

Repeats & Masking

Job Name	Status
RepeatMasker	Parsing
RepeatRunner	Completed
Repeats	Loading

Genes & Other Predictions

Job Name	Status
blastn	Completed
blastx	Completed
est2genome	Completed
InterProScan	Completed
Maker High Quality Partial Genes	Completed
Maker High Quality Whole Genes	Completed
Maker Low Quality Partial Genes	Completed
Maker Low Quality Whole Genes	Completed
protein2genome	Completed

Browser

Sharing

Management Sequences

Thank You!

Gen SAS



More GenSAS at PAG

- Computer Demo: C03 at 2:10pm today, Jan 10th in California Room for further details
- Poster: P1153. Meet w/ Jodi Humann from 3.00-4.30 PM Monday, Jan 12th
- Contact Info: dorrie@wsu.edu, stephen.ficklin@wsu.edu, jhumann@wsu.edu