Ensembl gene annotation project (*e74*)
Lepisosteus oculatus (spotted gar)

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This document describes the annotation process of the high-coverage spotted gar assembly (LepOcu1), described in Figure 1. The first stage is Assembly Loading where databases are prepared and the assembly loaded into the database.

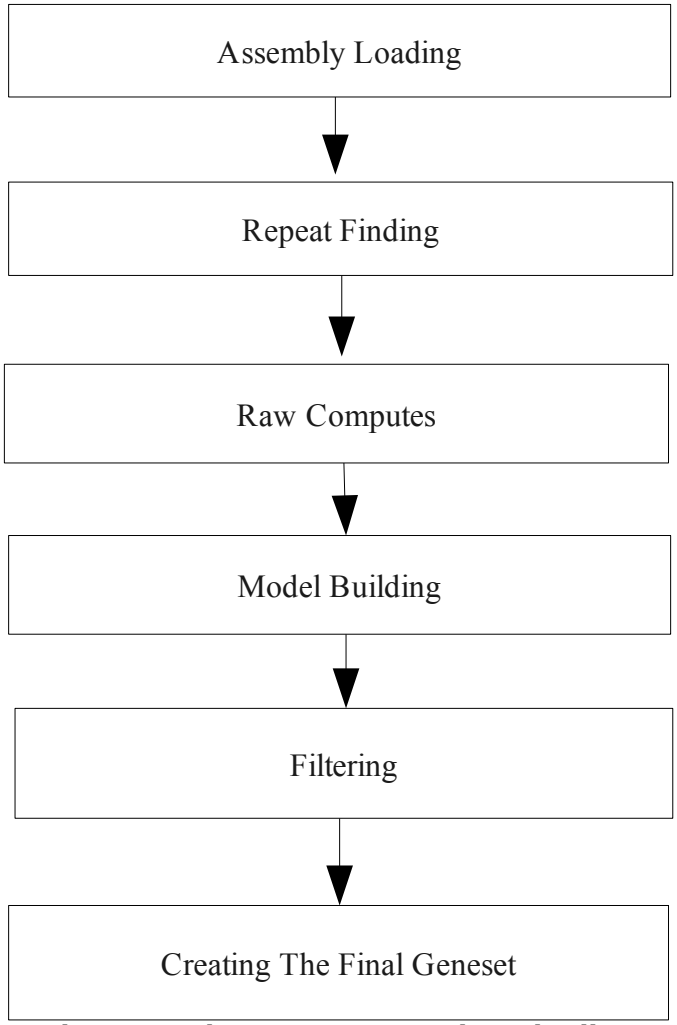


Figure 1: The Gene Annotation Pipeline

Repeat Finding

After loading into a database the genomic sequence was screened for sequence patterns including repeats using RepeatMasker [1] (version 3.2.8 with parameters '`-nolow -species "Danio rerio" -s`') RepeatModeler [2] (version open-1.0.5, to obtain a repeats library, then filtered for an additional RepeatMasker run), Dust [3] and TRF [4]. Both executions of RepeatMasker and Dust combined masked 26% of the species genome.

Raw Computes

Transcription start sites were predicted using Eponine–scan [5] and FirstEF [6]. CpG islands [Micklem, G.] longer than 400 bases and tRNAs [7] were also predicted. The results of Eponine-scan, FirstEF, CpG, and tRNAscan are for display purposes only; they are not used in the gene annotation process.

Genscan [8] was run across repeat masked sequence and the results were used as input for UniProt [9], UniGene [10] and Vertebrate RNA [11] alignments by WU-BLAST [12]. Passing only Genscan results to BLAST is an effective way of reducing the search space and therefore the computational resources required. This resulted in 406,830 UniProt, 318,309 UniGene and 301,972 Vertebrate RNA sequences aligning to the genome.

cDNA and EST Alignments

Spotted gar cDNAs were downloaded from ENA/Genbank/DDBJ, clipped to remove polyA tails, and aligned to the genome using Exonerate. There were only 10 cDNAs available and no ESTs for this species.

Species	cDNA/EST	Sequences Downloaded	Sequences Aligned
spotted gar	cDNA	10	10
spotted gar	EST	0	0

Table 1: cDNA/EST alignments

Model Generation

Various sources of transcript and protein data were investigated and used to develop gene models using a variety of techniques. The data and techniques employed to generate models are outlined here. The numbers of gene models generated is described in Table 2.

Pipeline	Source	Number of Models
Similarity	34,535,400 UniProt proteins	389,015
RNASeq	Broad Institute	194,131
Ensembl Longest Translations	26,210 Ensembl Release 67 proteins for zebrafish	16,058

Table 2: Gene Model Generation Overview

Similarity Pipeline: Generating coding models using proteins from related species

Coding models were generated using data from related species. WU-BLAST was re-run for these sequences and the results were passed to Genewise [14] to build coding models.

RNASeq Pipeline

RNASeq data provided by the Broad Institute was used in the annotation. This comprised a mixture of single and paired end data from samples including: a pool of 10 tissues, eye, liver, larvae, muscle, brain, skin, heart, embryo, testis and kidney. The available reads were aligned to the genome using BWA. The Ensembl RNASeq pipeline was used to process the BWA alignments and create further split read alignments using Exonerate.

The split reads and the processed BWA alignments were combined to produce 194,131 transcript models in total. The predicted open reading frames were compared to UniProt using WU-BLAST. Models with poorly scoring or no BLAST alignments were split into a seperate class.

Ensembl Longest Translations

The longest translation for each protein coding gene in Ensembl proteins release 67 for zebrafish were downloaded. These proteins were aligned against the spotted gar genome using Exonerate [13] to produce a set of coding models.

Addition of UTR to coding models

The set of coding models was extended into the untranslated regions (UTRs) using RNASeq and cDNA sequences. At the UTR addition stage 25,028 gene models out of total of 51,018 non-RNASeq pipeline generated gene models had UTR added.

Filtering the Models

The filtering phase decided the subset of protein-coding transcript models, generated from the model-building pipelines, that comprise the final protein-coding gene set.

Models were filtered using the TranscriptConsensus, LayerAnnotation and GeneBuilder modules.

Apollo software [16] was used to visualise the results of filtering.

LayerAnnotation

The LayerAnnotation module was used to define a hierarchy of input data sets, from most preferred to least preferred. The output of this pipeline included all transcript models from the highest ranked input set. Models from lower ranked input sets are included only if their exons do not overlap a model from an input set higher in the hierarchy.

The top layer contained the top rated RNASeq models and good similarity models (PE 1 & 2 and Fish PE3, 4 & 5). The remaining model sets were used in the following order:

- Lower rated RNASeq models
- Ensembl Longest Translation models from zebrafish
- Other similarity models made from UniProt PE 3,4 and 5

Generating multi-transcript genes

The above steps generated a large set of potential transcript models, many of which overlapped one another. Redundant transcript models were collapsed and the remaining unique set of transcript models were clustered into multi-transcript genes where each transcript in a gene has at least one coding exon that overlaps a coding exon from another transcript within the same gene.

At this stage the gene set comprised of 18,383 genes with 22,531 transcripts.

Pseudogenes

The Pseudogene module was run to identify processed pseudogenes from within the set of gene models – these were labelled as pseudogenes. A total of 37 genes were labelled as pseudogenes.

Creating The Final Gene Set

ncRNAs

Small structured non-coding genes were added using annotations taken from RFAM [17] and miRBase [18]. WU-BLAST was run for these sequences and models built using the Infernal software suite [20].

Cross-referencing

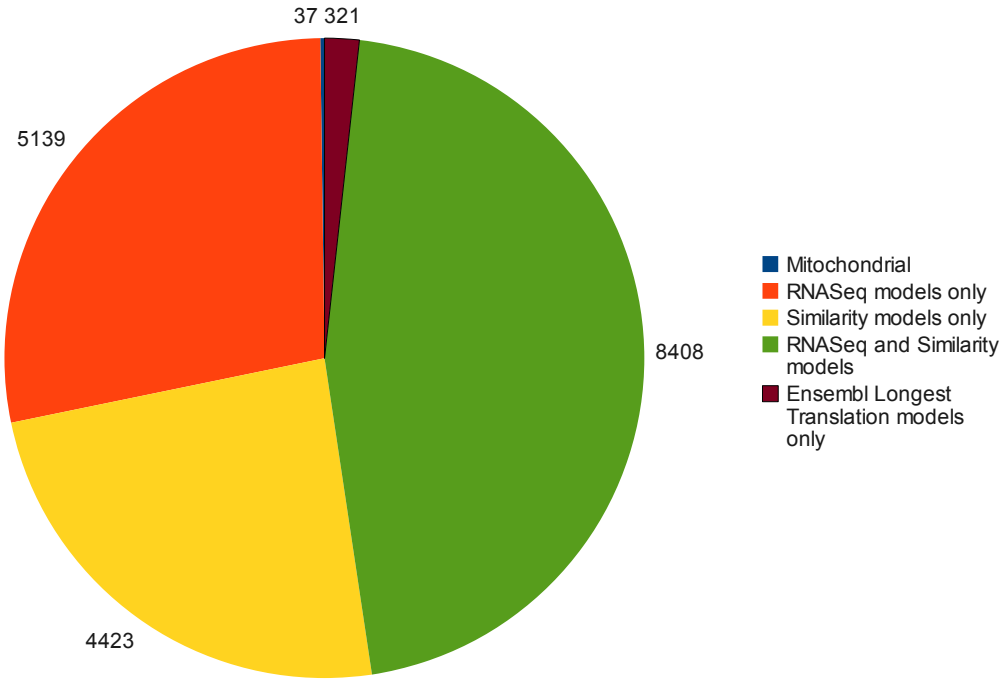
Before public release the transcripts and translations were given external references (cross-references to external databases). Translations were searched for signatures of interest and labelled where appropriate.

Stable Identifiers

Stable identifiers were assigned to each gene, transcript, exon and translation. When annotating a species for the first time, these identifiers are auto-generated. In all subsequent annotations for a species, the stable identifiers are propagated based on comparison of the new gene set to the previous gene set.

Final Gene Set Summary

The final gene set consists of 18,328 protein coding genes, including 37 mitochondrial genes. These contain 22,470 transcripts. A total of 37 pseudogenes were identified. 2,595 ncRNAs were added by the ncRNA pipeline.



Further information

The Ensembl gene set is generated automatically, meaning that gene models are annotated using the Ensembl gene annotation pipeline. The main focus of this pipeline is to generate a conservative set of protein-coding gene models, although non-coding genes and pseudogenes may also annotated.

Every gene model produced by the Ensembl gene annotation pipeline is supported by biological sequence evidence (see the “Supporting evidence” link on the left-hand menu of a Gene page or Transcript page); *ab initio* models are not included in our gene set. *Ab initio* predictions and the full set of cDNA alignments to the genome are available on our website.

The quality of a gene set is dependent on the quality of the genome assembly. Genome assembly can be assessed in a number of ways, including:

1. Coverage estimate
    - A higher coverage usually indicates a more complete assembly.
    - Using Sanger sequencing only, a coverage of at least 2x is preferred.
2. N50 of contigs and scaffolds
    - A longer N50 usually indicates a more complete genome assembly.
    - Bearing in mind that an average human gene may be 10-15 kb in length, contigs shorter than this length will be unlikely to hold full-length gene models.
3. Number of contigs and scaffolds
    - A lower number toplevel sequences usually indicates a more complete genome assembly.
4. Alignment of cDNAs and ESTs to the genome

- A higher number of alignments, using stringent thresholds, usually indicates a more complete genome assembly.

More information on the Ensembl automatic gene annotation process can be found at:

- Curwen V, Eyras E, Andrews TD, Clarke L, Mongin E, Searle SM, Clamp M: **The Ensembl automatic gene annotation system.** *Genome Res.* 2004, **14(5):**942-50. [PMID: 15123590]
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- http://cvs.sanger.ac.uk/cgi-bin/viewvc.cgi/-doc/pipeline_docs/the_genebuild_process.txt?root=ensembl&view=co

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