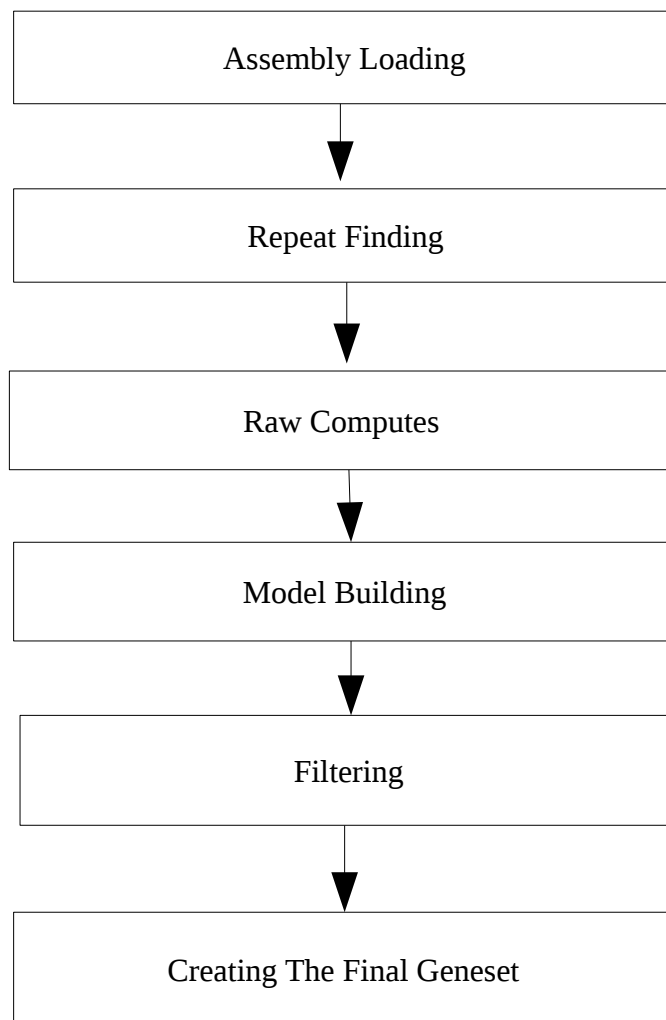


# Ensembl gene annotation project (e73)

## *Ficedula albicollis*

### (Flycatcher)

This document describes the annotation process of the high-coverage flycatcher assembly, 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 "aves" -s'), a custom RepeatModeler [2] library we generated (version open-1.0.5), Dust [3] and TRF [4]. The RepeatMasker aves library and custom RepeatModeler library combined to mask 6.6% of the flycatcher 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 RepeatMasked 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 14,397,460 UniProt, 11,736,241 UniGene and 11,453,693 Vertebrate RNA sequences aligning to the genome.

## Model Generation

Our gene annotation system is evidence-based; all protein coding and non-coding RNA gene models are supported by biological sequences from public databases. Input data for the protein coding gene models came from UniProt, ENA, Uppsala University and the Ensembl release 71 and 68 databases for chicken and zebra finch respectively. Data from each source were aligned to the genome and filtered in order to generate gene models. The number of preliminary gene models (before filtering) generated from each data source/pipeline are outlined in Table 1.

<b>Pipeline</b>	<b>Source</b>	<b>Number of Models</b>
Similarity	6329148 Uniprot PE level 1,2 proteins	194425
RNAseq	Uppsala University	174504
Ensembl Longest Translations	17404 Ensembl Release 68 proteins for finch, 14656 Ensembl Release 71 proteins for chicken	32060

**Table 1: Gene Model Generation Overview**

### ***Similarity Pipeline: Generating coding models using proteins from related species***

Coding models were generated using data from related species. The UniProt alignments from the Raw Computes step were filtered and only those sequences belonging to UniProt's Protein Existence (PE) classification level 1 and 2 were kept. WU-BLAST was rerun for these sequences and the results were passed to Genewise [14] to build coding models.

### ***RNAseq Pipeline***

RNAseq data provided by Uppsala University [15] were used in the annotation. These comprised of paired end data from samples including: brain, embryo, kidney, liver, lung, muscle, ovary, skin, testis and a pooled of 9 tissues from a number of individuals. 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 RNAseq pipeline produced 174504 transcript models in total. The predicted open reading frames were compared to Uniprot Protein Existence (PE) classification level 1 and 2 proteins using WU-BLAST. Models with poorly scoring or no BLAST alignments were split into a separate class and not used in the final gene set.

### ***Ensembl Longest Translations***

The longest translation for each protein coding gene in Ensembl proteins

release e68 and e71 for zebra finch and chicken respectively were downloaded. These proteins were aligned against the flycatcher genome using Exonerate [13] to produce a set of 32060 models.

## **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.

As no species-specific data were available in terms of proteins, cDNAs or ESTs, RNAseq and similarity data provided the supporting evidence for the models. As such both RNAseq and similarity models were ordered high in the layering process. A basic overview of the final layering is as follows:

- Strong pooled RNAseq and bird-specific similarity models
- Mammal and other vertebrate similarity models
- Strong tissue-specific RNAseq models
- Zebra finch and chicken Ensembl models
- Weaker RNAseq models
- Non-vertebrate similarity models

In the above ordering strong RNAseq models were ones where we had a matching BLAST alignment to a Uniprot PE1,2 protein that had both a hit coverage and percent identity of greater than or equal to 80 percent. For the weak RNAseq models the hit coverage and percent identity of the PE1,2 BLAST alignments were between 50 to 80 percent.

### ***Addition of UTR to coding models***

The set of coding models was extended into the untranslated regions (UTRs) using RNAseq sequences. At the UTR addition stage 55,582 gene models out of total of 70,825 non-RNAseq pipeline generated gene models had UTR added.

### ***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 15,400 genes with 16,095 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 66 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 [19].

### ***Cross-referencing***

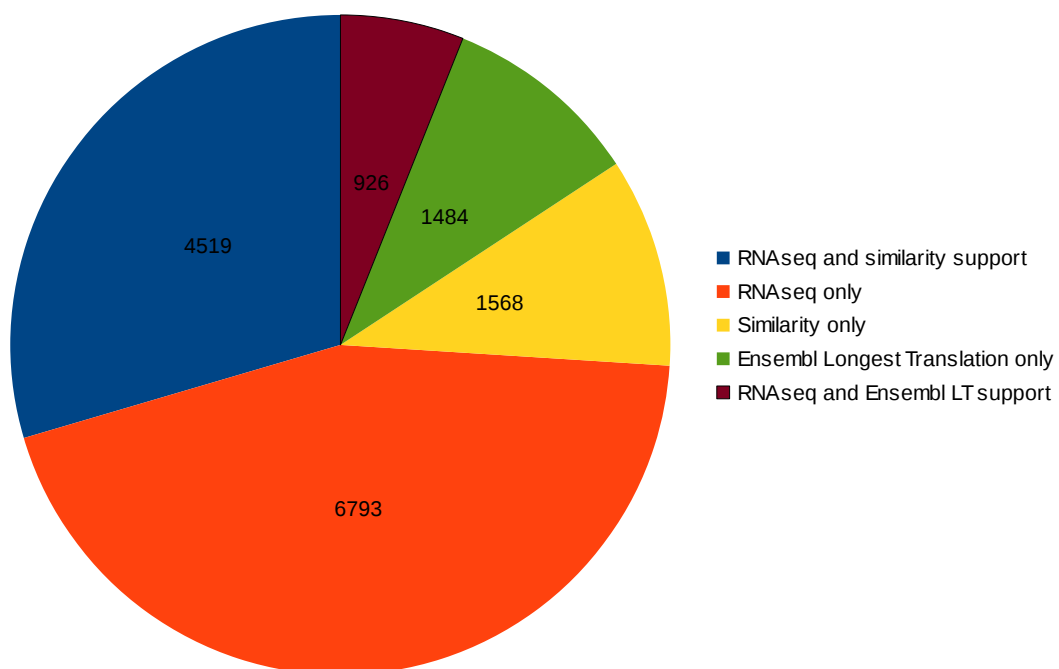
Before public release the transcripts and translations were given external references (cross-references to external databases). Translations were searched for signatures of interest and labelled where appropriate. Databases searched include: Seg, SignalP, Ncoils, Tmhmm, Prints, Pfscan, Pfam, Tigrfam, Superfamily, Smart and Pirsf.

### ***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 15290 protein-coding genomic and 13 protein-coding mitochondrial models. These represent 15983 transcripts. A total of 24 non-coding mitochondrial genes were imported. Pseudogene analysis identified 66 pseudogenes. A total of 873 ncRNAs were added by the ncRNA pipeline.



**Figure 2: Supporting evidence for genomic protein-coding models.**



## ***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 be 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.

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
  - o A higher coverage usually indicates a more complete assembly.
  - o Using Sanger sequencing only, a coverage of at least 2x is preferred.
2. N50 of contigs and scaffolds
  - o A longer N50 usually indicates a more complete genome assembly.
  - o 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
  - o A lower number of top-level sequences usually indicates a more complete genome assembly.
4. Alignment of cDNAs and ESTs to the genome
  - o 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, Eyraas 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\\_gene\\_build\\_process.txt?root=ensembl&view=co](http://cvs.sanger.ac.uk/cgi-bin/viewvc.cgi/-doc/pipeline_docs/the_gene_build_process.txt?root=ensembl&view=co)

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