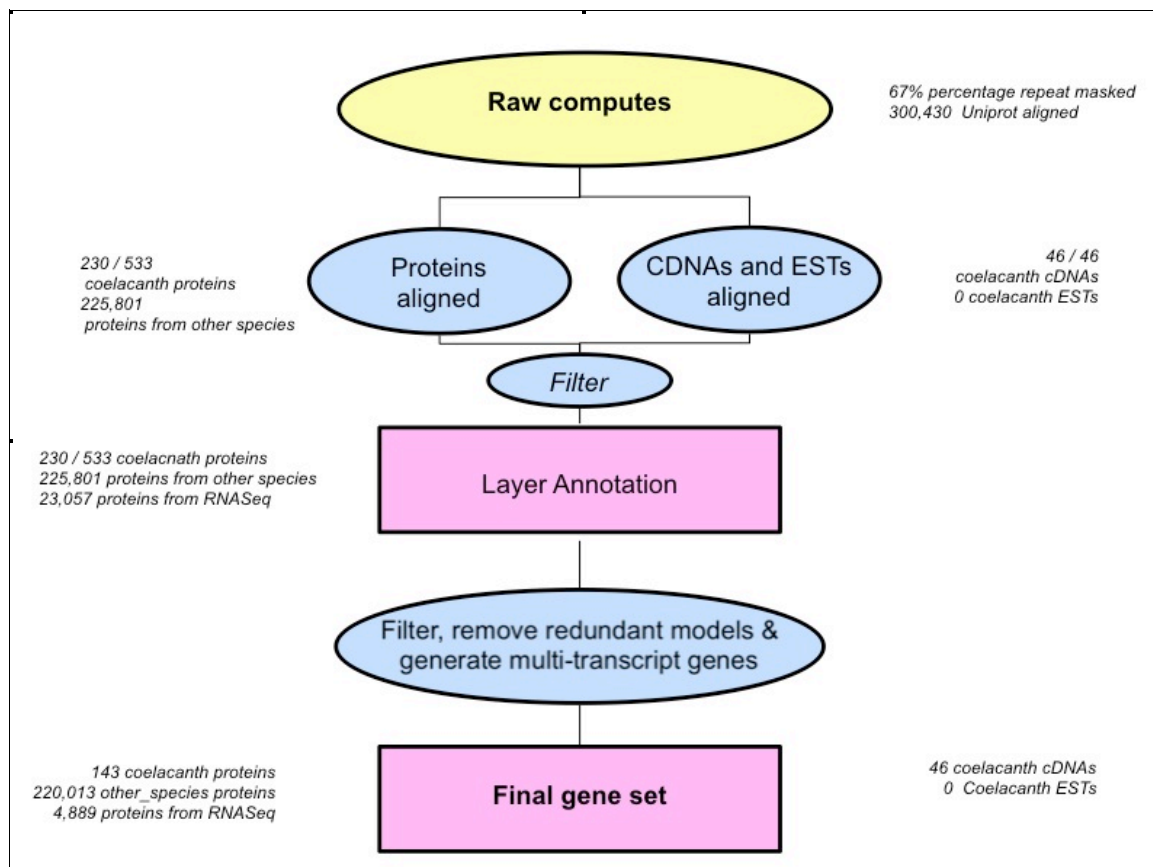


# Ensembl gene annotation project

## *Latimeria chalumnae* (Coelacanth)

### ***Raw Computes Stage: Searching for sequence patterns, aligning proteins and cDNAs to the genome.***

The annotation process of the high-coverage Coelacanth assembly began with the “raw compute” stage [Figure 1] whereby the genomic sequence was screened for sequence patterns including repeats using RepeatMasker [1] (version 3.2.8) with a custom Coelacanth repeat library, Dust [2] and TRF [3]. RepeatMasker masked 41% of the genome. Adding low complexity masking (including gaps) with Dust brings the total masked to 67%.



**Figure 1: Summary of Coelacanth gene annotation project.**

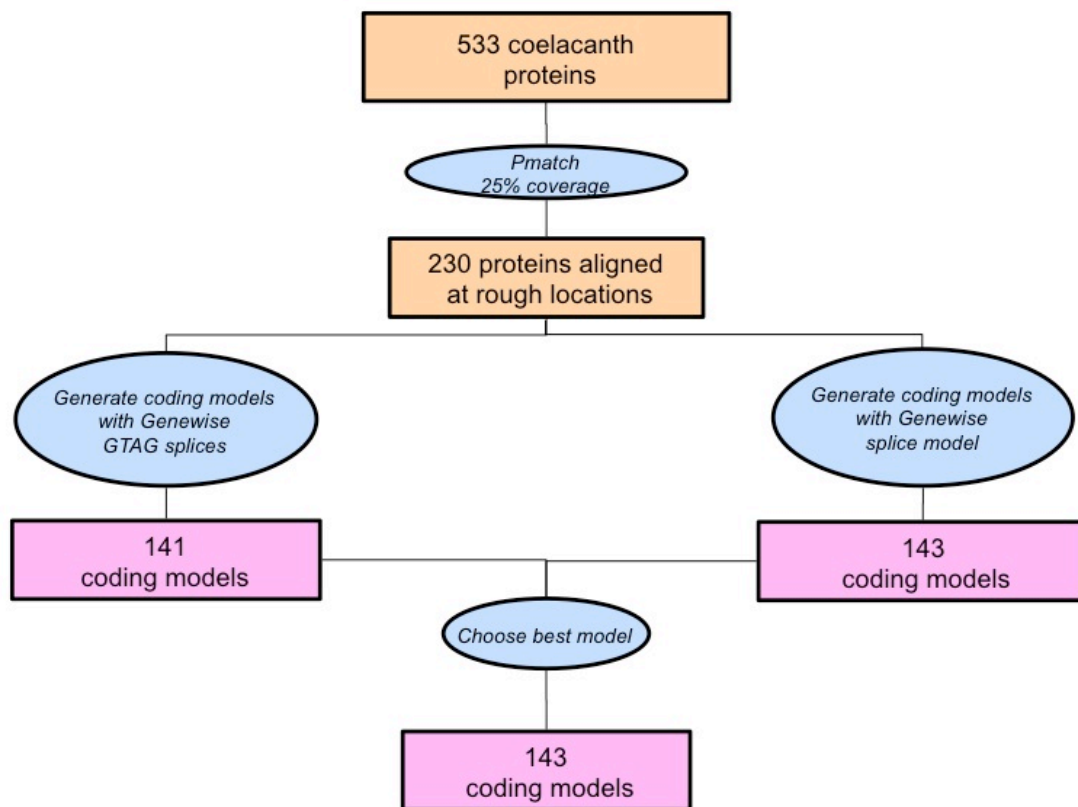
Transcription start sites were predicted using Eponine-scan [4], and FirstEF [5] CpG islands and tRNAs [6] were also predicted.

Genscan [7] was run across RepeatMasked sequence and the results were used as input for UniProt [8], UniGene [9], and Vertebrate RNA [10] alignments by WU-BLAST [11] (Passing only Genscan results to BLAST is an effective way of reducing the search space and therefore the computational resources required). This resulted in 300,430 UniProt vertebrate and 98,486 Uniprot non-vertebrate sequences, 378,875 UniGene and 342,925 Vertebrate RNA sequences aligning to the genome.

### ***Targeted Stage: Generating coding models from Coelacanth evidence***

Coelacanth protein sequences were downloaded from public databases (UniProt SwissProt/TrEMBL [8] and Genbank) and filtered to remove sequences based on predictions. The Coelacanth sequences were mapped to the genome using Pmatch as indicated in Figure 2.

Models of the coding sequence (CDS) were produced from the proteins using Genewise [13]. 2 sets of models were produced, one with only consensus splice sites and one where non-consensus splices were allowed; where a single protein sequence had generated two different coding models at the same locus, the BestTargetted module was used to select the coding model that most closely matched the source protein to take through to the next stage of the gene annotation process. The generation of transcript models using Coelacanth-specific data is referred to as the "Targeted stage". This stage resulted in 143 of 533 Coelacanth proteins used to build 153 coding models.



**Figure 2: Targeted stage using Coelacanth specific proteins**

### ***cDNA and EST Alignment***

Coelacanth cDNAs were downloaded from Genbank, clipped to remove polyA tails, and aligned to the genome using Exonerate. Of these, 46 of 46 Coelacanth cDNAs aligned with a cut-off of 90% coverage and 97% identity. No ESTs were found.

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

Due to the small number of Coelacanth specific protein and cDNA evidence the majority of the gene models were based on proteins from other species. UniProt alignments from the Raw Compute step were filtered to favor proteins classed by UniProt's Protein Existence (PE) classification level 1 and 2. Proteins from other PE levels were used where no other evidence was available; similarly,

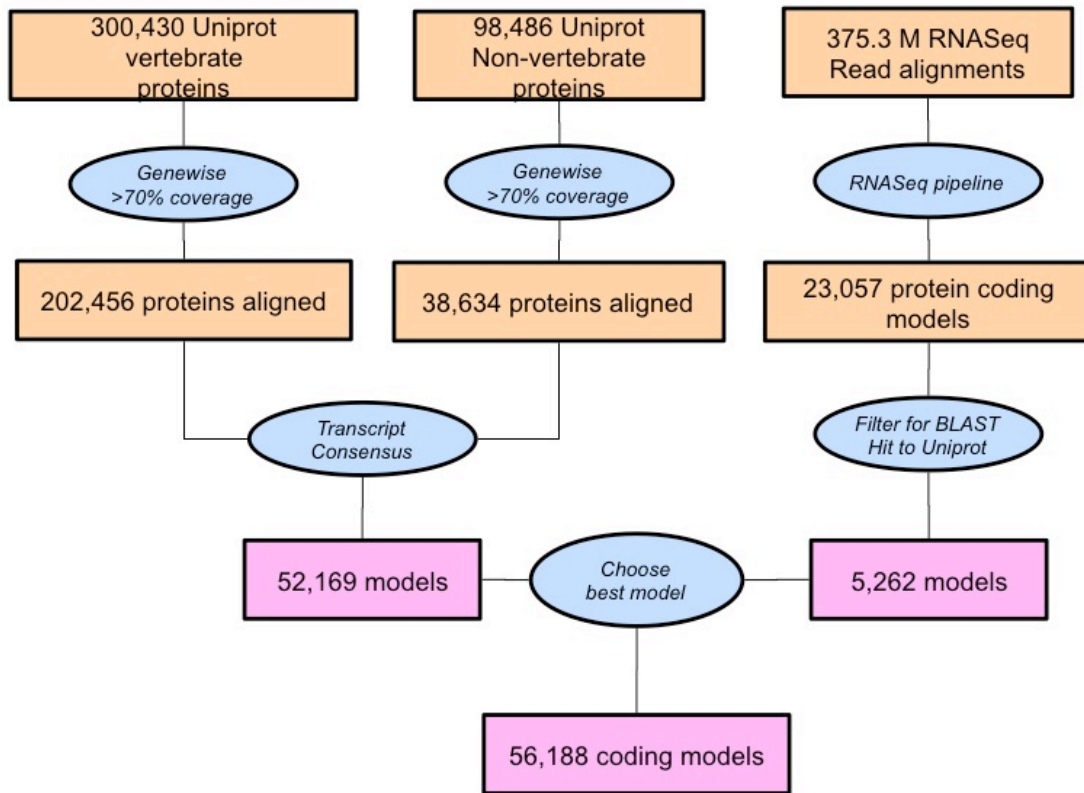
mammalian proteins were favored over non-mamalian. WU-BLAST was rerun for these sequences and the results were passed to Genewise to build coding models. The generation of transcript models using data from related species is referred to as the "Similarity stage". This stage resulted in 297,885 coding models.

### ***Filtering Coding Models***

Coding models from the Similarity stage were filtered using modules such as TranscriptConsensus, RNA-Seq spliced alignments supporting introns were used to help filter the set. 247,112 models were rejected as a result of filtering. The Apollo software [15] was used to visualise the results of filtering. [Figure 3]

### ***Addition of RNA-Seq models***

The largest set of Coelacanth specific evidence was from Illumina paired end RNASeq, this was used where appropriate to help inform our gene annotation. A set of 375 million reads that passed QC were aligned to the genome using BWA resulting in 225 million reads aligning and properly pairing. The Ensembl RNA-Seq pipeline was used to process the BWA alignments and create a further 30 million split read alignments using Exonerate. The split reads and the processed BWA alignments were combined to produce 23,057 transcript models in total; one transcript per loci. The predicted open reading frames were compared to Uniprot Protein Existence (PE) classification level 1 and 2 proteins using WU-BLAST, models with no BLAST alignment or poorly scoring BLAST alignments were discarded. The resulting models were added into the gene set where they produced a novel model or splice variant, in total 4,994 models were added.



**Figure 3: Alignment and filtering of other species proteins and addition of RNASeq models**

### ***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 removed 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. The final gene set of genes included 11 genes built only using Coelacanth proteins, a further 14,279 genes built only using proteins from other species, and 3,291 genes built only from RNA-Seq evidence. 1,671 genes had a mixture of RNA-Seq and other species proteins and 10 genes were a mixture of Coelacanth specific proteins and proteins from other species, 1 gene contained only coelacanth specific and RNASeq transcripts, and 2 genes contained transcripts from all 3 sources.

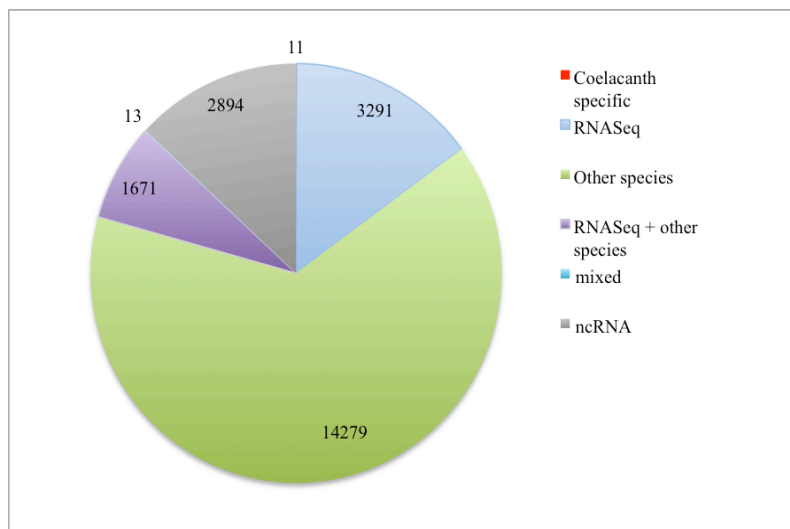
## ***Pseudogenes, Protein annotation, non-coding genes, Cross referencing, Stable Identifiers***

The gene set was screened for potential pseudogenes. Before public release the transcripts and translations were given external references cross references to external databases), while translations were searched for domains/signatures of interest and labeled where appropriate. 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 the stable identifiers are propagated based on comparison of the new gene set to the previous gene set.)

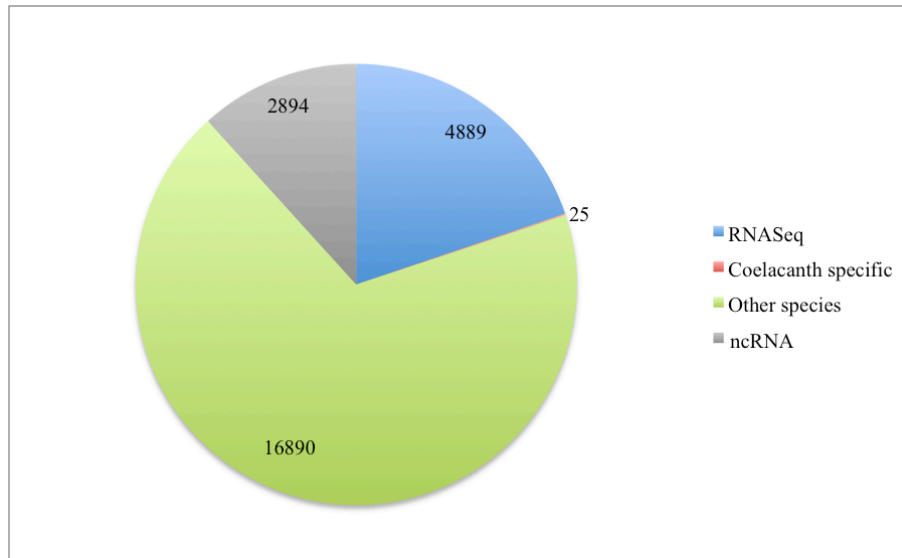
Small structured non-coding genes were added using annotations taken from RFAM [16] and miRBase [17].

The final gene set consists of 19,033 protein coding genes including mitochondrial genes, these contain containing 21,817 transcripts. A total of 141 pseudogenes were identified and 2,894 ncRNAs. Of the protein coding transcripts 4,889 were made from RNASeq, 16,890 came from proteins from other species; some 4,172 of which had UTR added from RNASeq. 25 transcripts were Coelacanth specific, 13 transcripts were mitochondrial.

**Figure 4: Composition of Coelacanth gene set.**



**Figure 5: Composition of Coelacanth transcripts**



***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. Ab initio predictions and the full set of cDNA and EST 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 of top-level 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 indicate 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]
- Potter SC, Clarke L, Curwen V, Keenan S, Mongin E, Searle SM, Stabenau A, Storey R, Clamp M. The Ensembl analysis pipeline. *Genome Res.* 2004, 14(5):934-41. [PMID: 15123589]
- [http://www.ensembl.org/info/docs/genebuild/genome\\_annotation.html](http://www.ensembl.org/info/docs/genebuild/genome_annotation.html)
- \* [http://cvs.sanger.ac.uk/cgi-bin/viewvc.cgi/ensembl/doc/pipeline\\_docs/the\\_genebuild\\_process.txt?root=ensembl&view=co](http://cvs.sanger.ac.uk/cgi-bin/viewvc.cgi/ensembl/doc/pipeline_docs/the_genebuild_process.txt?root=ensembl&view=co)

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