

Ensembl gene annotation project

Ailuropoda melanoleuca (Giant panda)

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

Approximate time: 1 week

The annotation process of the high-coverage panda 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 parameters '-nolow -species "Ailuropoda melanoleuca" -s'), Dust [2.] and TRF [3.]. RepeatMasker and Dust combined masked 41% of the panda genome.

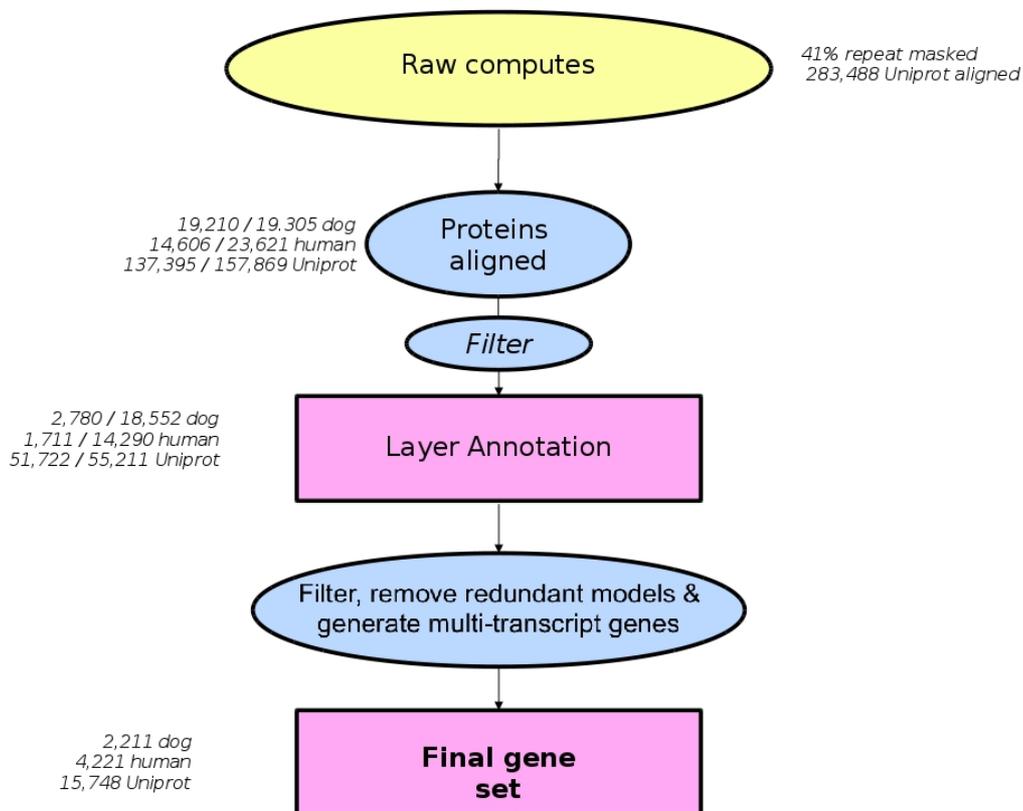


Figure 1: Summary of panda gene annotation project.

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 283,488 UniProt, 336,453 UniGene and 332,810 Vertebrate RNA sequences aligning to the genome.

Exonerate Stage: Generating coding models from dog and human evidence

Approximate time: 1 week

Next, dog and human protein sequences were downloaded from Ensembl (e! 56). The dog and human protein sequences were aligned to the genome using Exonerate [12.] as indicated in [Figure 2] and [Figure 3].

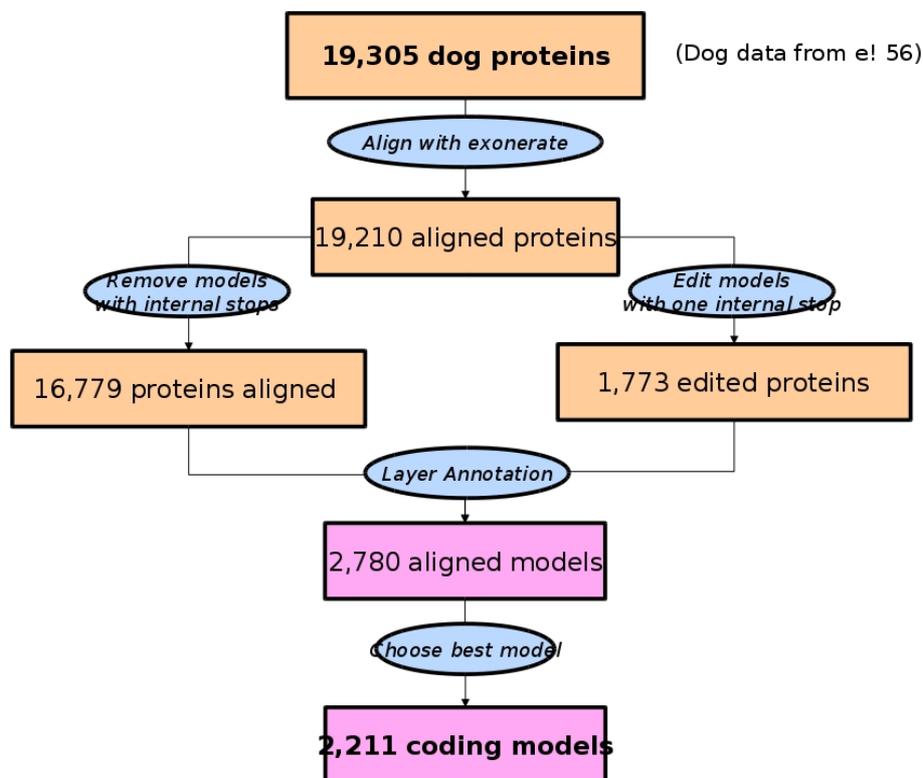


Figure 2: Alignment and filtering of dog proteins.

This stage resulted in 2,780 (of 18,552) dog proteins and 4,349 (of 14,290) human proteins used to build coding models to be taken through to the genebuild stage.

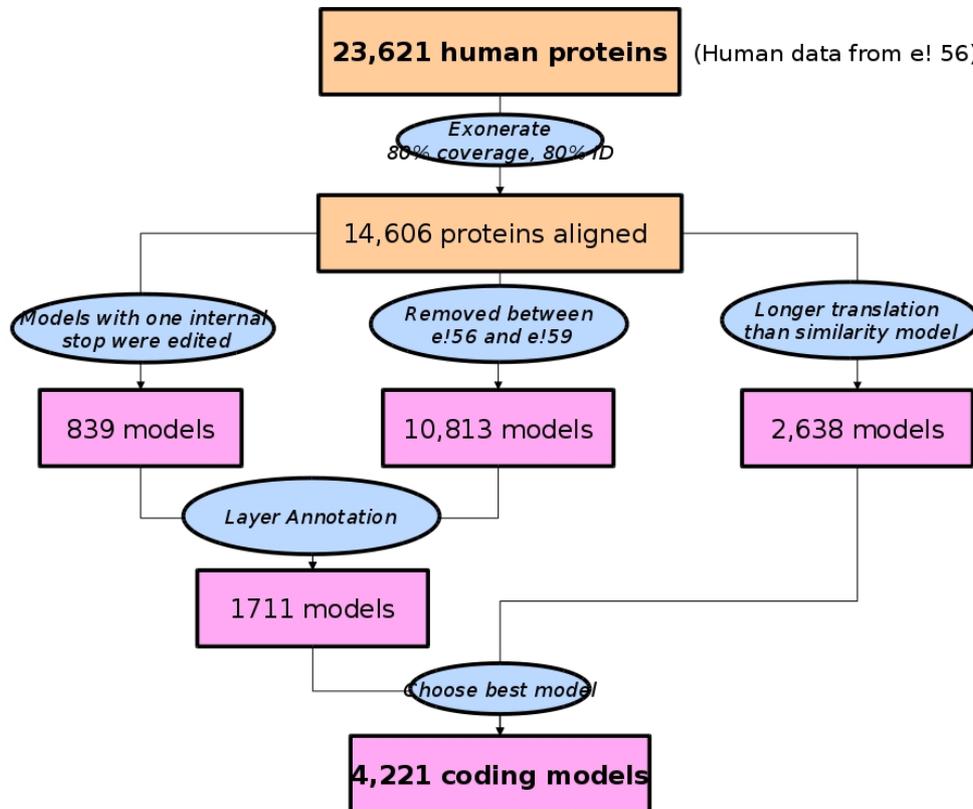


Figure 3: Alignment and filtering of human proteins.

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

Approximate time: 1 week

Following the human and dog Targetted alignments, additional coding models were generated as follows. 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 [13.] 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 103,160 mammalian and 34,289 non-mammalian coding models.

Filtering Coding Models

Approximate time: 1 week

Coding models from the Similarity stage were filtered using modules such as TranscriptConsensus and LayerAnnotation. The Apollo software [14.] was used to visualise the results of filtering.

Generating multi-transcript genes

Approximate time: 2-3 weeks

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 19,521 genes included 2,062 genes with at least one transcript supported by dog proteins, a further 3,687 genes without dog evidence but with at least one transcript supported by human evidence. The remaining 13,772 genes had transcripts supported by proteins from other sources [Figure 4].

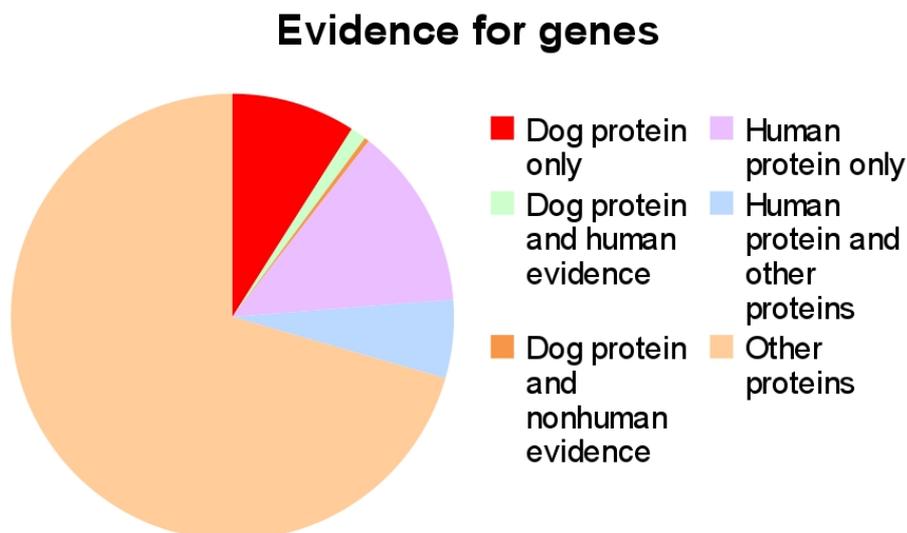


Figure 4: Supporting evidence for panda final gene set.

The final transcript set of 21,344 transcripts included 2,088 transcripts with support from dog proteins, 3,977 transcripts with support from human proteins and 15,279 transcripts with support from UniProt SwissProt [Figure 5].

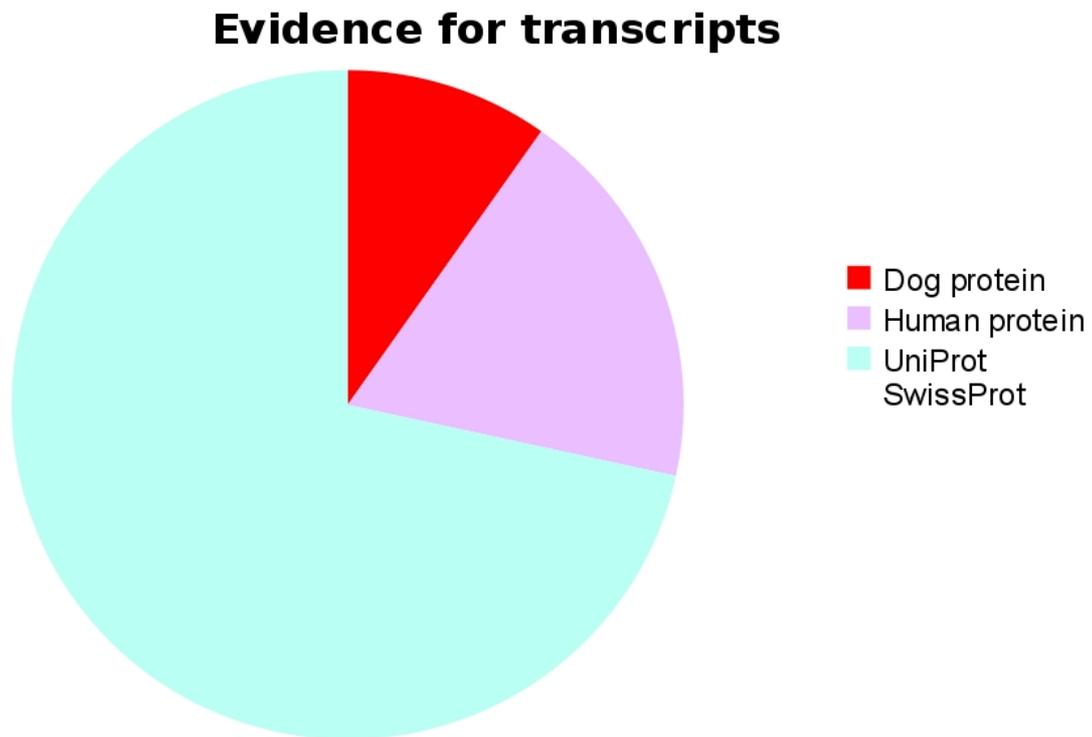


Figure 5: Supporting evidence for panda final transcript set.

Pseudogenes, Protein annotation, Cross-referencing, Stable Identifiers

Approximate time: 2 weeks

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 labelled 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 for a species, the stable identifiers

are propagated based on comparison of the new gene set to the previous gene set.)

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 noncoding 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 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]
- 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://cvs.sanger.ac.uk/cgi-bin/viewvc.cgi/ensembl-doc/pipeline_docs/the_genebuild_process.txt?root=ensembl&view=co

References

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3. Benson G. **Tandem repeats finder: a program to analyze DNA sequences.** *Nucleic Acids Res.* 1999, **27(2)**:573-580. [PMID: 9862982]. <http://tandem.bu.edu/trf/trf.html>
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5. Davuluri RV, Grosse I, Zhang MQ: **Computational identification of promoters and first exons in the human genome.** *Nat Genet.* 2001, **29(4)**:412-417. [PMID: 11726928]

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10. <http://www.ebi.ac.uk/ena/>
11. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: **Basic local alignment search tool.** *J Mol Biol.* 1990, **215(3)**:403-410. [PMID: 2231712.]
12. Slater GS, Birney E: **Automated generation of heuristics for biological sequence comparison.** *BMC Bioinformatics* 2005, **6**:31. [PMID: 15713233]
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