Ensembl gene annotation project

Otolemur garnettii (Bushbaby)

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

Approximate time: 3 weeks

The annotation process of the high-coverage bushbaby 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 "mammal" -s`'), Dust [2.] and TRF [3.]. RepeatMasker and Dust combined masked 42.5% of the species genome.
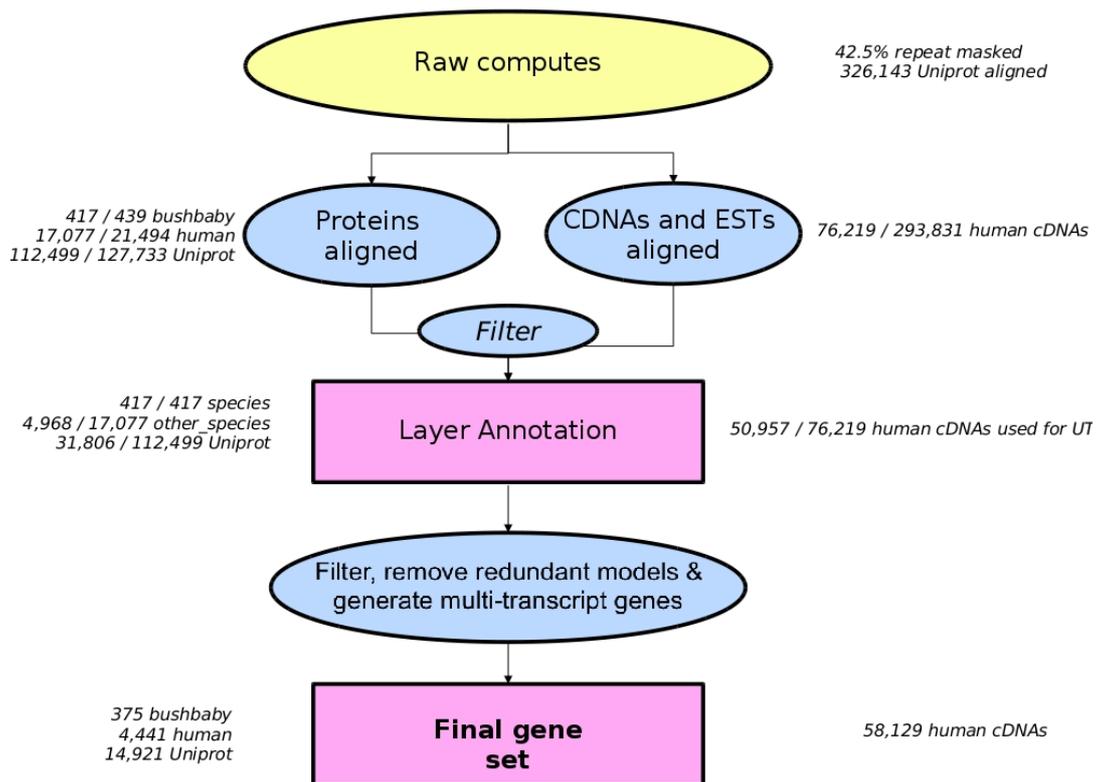
Figure 1: Summary of bushbaby gene annotation project.

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 326,143 UniProt, 330,766 UniGene and 331,022 Vertebrate RNA sequences aligning to the genome.

Targetted Stage: Generating coding models from bushbaby and human evidence

Approximate time: 3 weeks

Next, bushbaby protein sequences were downloaded from public databases (UniProt SwissProt/TrEMBL [8.] and RefSeq [9.]). Also, human translations were downloaded from Ensembl (e!63) The bushbaby and human protein sequences were mapped to the genome using Pmatch as indicated in [Figure 2] and [Figure 3].
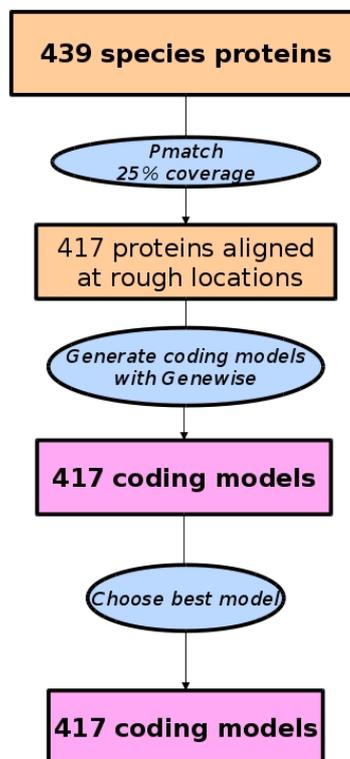
Figure 2: Targetted stage using bushbaby protein sequences.

Models of the coding sequence (CDS) were produced from the proteins using Genewise [13.] and Exonerate [12.]. Where one protein sequence had generated more than one coding model at a 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 species-specific (in this case bushbaby and human) data is referred to as the "Targetted stage". This stage resulted in 417 (of 439) bushbaby proteins and 17,077 (of 21,494) human proteins used to build coding models to be taken through to the UTR addition stage.
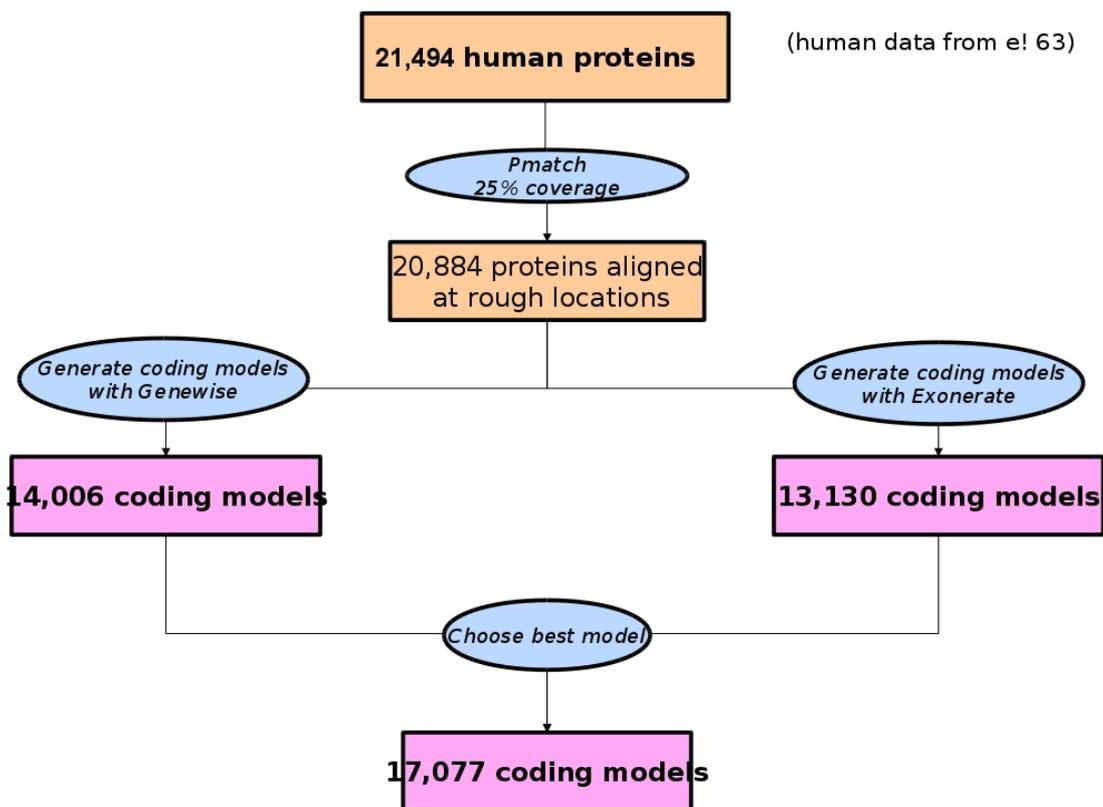
Figure 3: Alignment and filtering of human proteins.

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

Approximate time: 2 weeks

Following the bushbaby and human 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 39,872 primates, 50,831 mammals and 21,796 vertebrates coding models.

cDNA Alignment

Approximate time: 1 week

Human cDNAs were downloaded from ENA/Genbank/DDBJ, clipped to remove polyA tails, and aligned to the genome using Exonerate [Figure 4].

Human cDNAs

293,831

PolyA clipping

291,127

Align with Exonerate 90% coverage, 90% ID
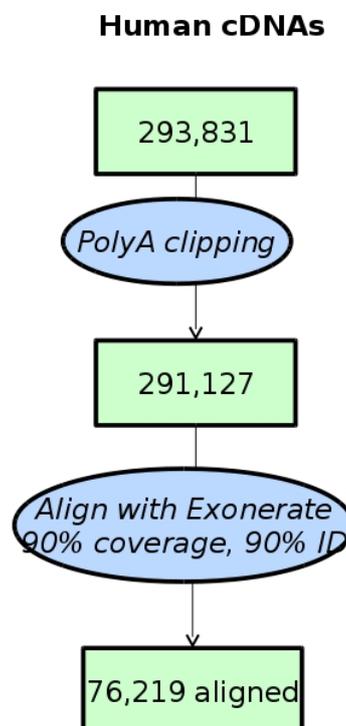
76,219 aligned

Figure 4: Alignment of human cDNAs to the bushbaby genome.

Of these, 76,219 (of 293,831) human cDNAs aligned. Alignments were at a cut-off of 90% coverage and 90% identity.

Filtering Coding Models

Approximate time: 5 weeks

Coding models from the Similarity stage were filtered using modules such as TranscriptConsensus and LayerAnnotation. The Apollo software [15.] was used to visualise the results of filtering.

Addition of UTR to coding models

Approximate time: 1 week

The set of coding models was extended into the untranslated regions (UTRs) using human cDNA. This resulted in 143 (of 417) bushbaby coding models with UTR, 6,371 (of 17,077) human coding models with UTR, and 42,552 (of 112,499) UniProt coding models with UTR.

Generating multi-transcript genes

Approximate time: 3 weeks

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. The final gene set of 19506 genes included 383 genes with at least one transcript supported by bushbaby proteins, a further 4373 genes without species evidence but with at least one transcript supported by human evidence. The remaining 14750 genes had transcripts supported by proteins from other sources [Figure 5].

Evidence for genes
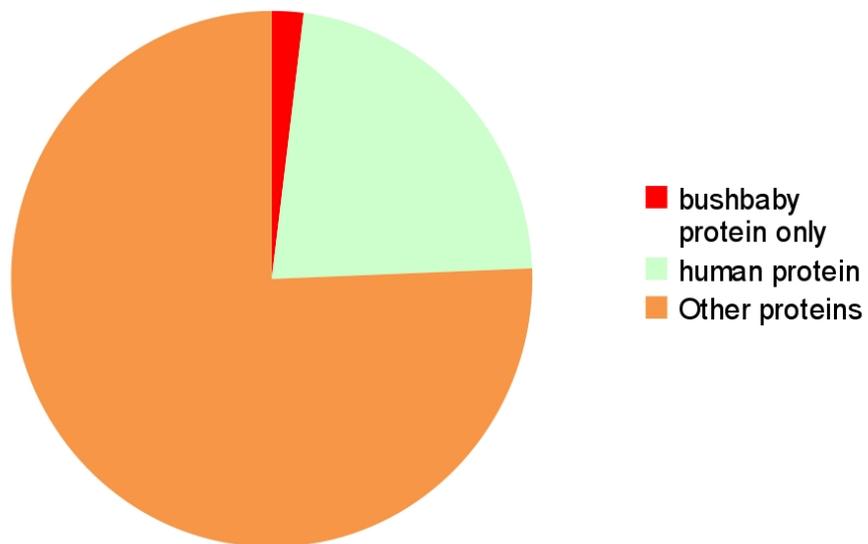
Figure 5: Supporting evidence for bushbaby final gene set.

The final transcript set of 19986 transcripts included 385 transcripts with support from bushbaby proteins, 4446 transcripts with support from human proteins and 15155 transcripts with support from UniProt SwissProt [Figure 6].

Evidence for transcripts
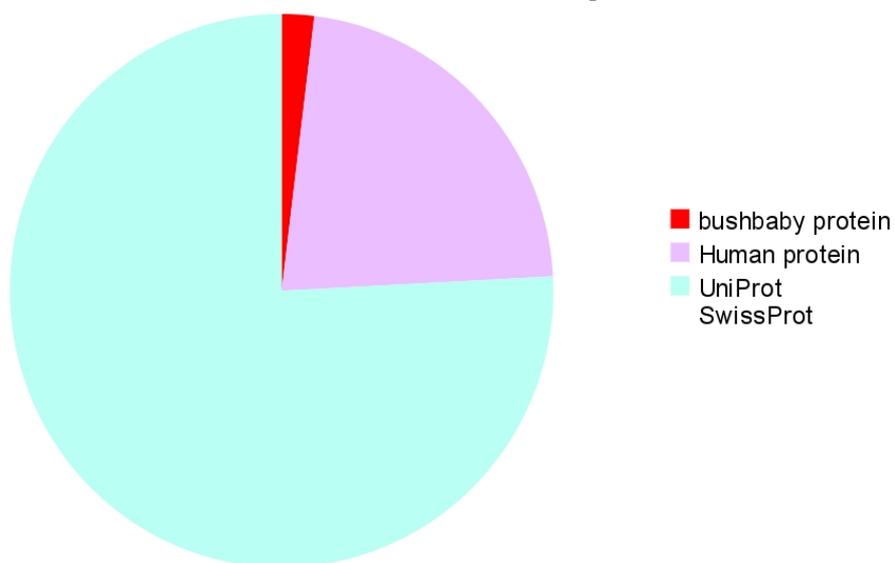
Figure 6: Supporting evidence for bushbaby final transcript set.

Pseudogenes, Protein annotation, Cross-referencing, Stable Identifiers

Approximate time: 1 week

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 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
   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 toplevel 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, 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

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10. http://www.ebi.ac.uk/ena/

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12. Slater GS, Birney E: **Automated generation of heuristics for biological sequence comparison.** *BMC Bioinformatic*s 2005, **6:**31. [PMID: 15713233]

13.   Birney E, Clamp M, Durbin R: **GeneWise and Genomewise.** *Genome Res.* 2004, **14(5):**988-995. [PMID: 15123596]

14.   Eyras E, Caccamo M, Curwen V, Clamp M. **ESTGenes: alternative splicing from ESTs in Ensembl.** *Genome Res.* 2004 **14(5):**976-987. [PMID: 15123595]

15.   Lewis SE, Searle SM, Harris N, Gibson M, Lyer V, Richter J, Wiel C, Bayraktaroglir L, Birney E, Crosby MA, Kaminker JS, Matthews BB, Prochnik SE, Smithy CD, Tupy JL, Rubin GM, Misra S, Mungall CJ, Clamp ME: **Apollo: a sequence annotation editor.** *Genome Biol.* 2002, **3(12):**RESEARCH0082. [PMID: 12537571]