

A MAKER pipeline for prediction of protein-coding genes in parasitic worm genomes

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Method Article

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Abstract

Once a genome has been sequenced, a challenging task is to predict protein-coding genes in the genome assembly. If several genomes of related species have been sequenced, it is desirable that the pipeline is scalable to multiple species, and will give consistent results across species. Here we describe a consistent, scalable and automated computational pipeline, based on the MAKER software, for prediction of protein-coding genes in parasitic worm (nematode and platyhelminth) genome assemblies. This protocol can be used to generate a relatively accurate and complete gene set, for a broad range of nematode and platyhelminth species. Furthermore, it does not require RNAseq data and does not require training using a curated set of genes of known structure.

Introduction

Once a genome has been sequenced, a challenging task is to predict protein-coding genes in the genome assembly. If several genomes of related species have been sequenced, it is desirable that the pipeline is scalable to multiple species, and will give consistent results across species. Here we describe a consistent, scalable and automated computational pipeline, based on the MAKER software, for prediction of protein-coding genes in parasitic worm (nematode and platyhelminth) genome assemblies. In our pipeline, gene predictions are generated using a pipeline that uses MAKER version 2.2.28 (Holt et al 2011). Our MAKER annotation pipeline consists of four steps, taking into account evidence from multiple sources (Figure 1). First, repetitive elements in the genome are identified and masked using RepeatMasker (www.repeatmasker.org) by scanning scaffolds for matches to repeats from a repeat library generated using RepeatModeler (www.repeatmasker.org/RepeatModeler.html). Second, *ab initio* gene models to be used as evidence within MAKER are generated using Augustus 2.5.5 (Stanke et al 2006), GeneMark-ES 2.3a (self-trained) (Ter-Hovhannisyanyan et al 2008), and SNAP 2013-02-16 (Korf 2004). Further gene models to use as MAKER input are generated using comparative algorithms genBlastG (She et al 2011) (using comparisons to *C. elegans* gene models from WormBase; Yook et al 2012) and RATT (Otto et al 2011; transferring gene models from the taxonomically nearest published 'reference' genome from the list: *Haemonchus contortus* for clade V parasites; *Ascaris suum* for Ascaridomorpha; *Brugia malayi* (and *Onchocerca volvulus*) for Spiruromorpha; *Trichuris muris* for clade I; *Strongyloides ratti* for clade IV; *Hymenolepis microstoma* for cestodes except *Echinococcus multilocularis* for *Taenia* species; *Schistosoma mansoni* for trematodes). Third, species-specific ESTs and cDNAs from INSDC (Cochrane et al 2016), and proteins from related species (see below), are aligned against the genome using BLASTN and BLASTX (Altschul et al 1997), respectively, and these alignments are further refined with respect to splice sites using exonerate (Slater & Birney 2005). Last, the EST and protein homology alignments, comparative gene models, and *ab initio* gene predictions are integrated and filtered by MAKER to produce a gene set for the species, with just one transcript for each gene.  The four-step MAKER pipeline is run three consecutive times (Figure 1). The first run is performed using the *est2genome* option with species-specific ESTs and cDNAs and the *protein2genome* for nematode protein sequences from UniProt's UniRef 90 clusters for nematodes \

(UniProt Consortium, 2015). For this first MAKER run, Augustus and SNAP are trained using CEGMA \ (Parra _et al_ 2007) gene models for KOGs, as well as 'nematode orthologous groups' \ (NOGs) \ (Martin & Mitreva 2018), 'trematode orthologous groups' \ (TROGs), or 'cestode orthologous groups' \ (CEOGs) as appropriate. Gene models obtained from the first MAKER run are used to train SNAP, and MAKER is run a second time, using the same nematode proteins as in the first run. Gene models from the second run are then used to train Augustus. Using the trained versions of SNAP and Augustus, MAKER is run a third time, using a taxonomically broader protein set that includes proteins from metazoans with complete proteomes from UniProt and proteins from helminths from GeneDB \ (Logan-Klumpler _et al_ 2012). The resulting MAKER gene set is then filtered to remove less reliable gene models. This protocol can be used to generate a relatively accurate and complete gene set, for a broad range of nematode and platyhelminth species. Unlike gene-finding approaches that require extensive use of RNAseq data \ (for example, use of Augustus in the _Strongyloides_ project; Hunt _et al_ 2016), this protocol does not require RNAseq data, so is suitable when RNAseq data is not available. Furthermore, unlike many gene-finding approaches, it does not require training using a curated set of genes of known structure \ (e.g. confirmed using cDNAs or RNAseq), as it uses conserved genes from KOGs/NOGs/TROGs/CEOGs for an initial round of training, and then its own gene MAKER models for later rounds of training.

Reagents

WormBase database \ (www.wormbase.org) INSDC database \ (www.insdc.org) UniProt database \ (www.uniprot.org) GeneDB database \ (www.genedb.org)

Equipment

Computer cluster.

Procedure

Gene predictions are generated using MAKER version 2.2.28 \ (Holt _et al_ 2011). The MAKER annotation pipeline consists of four steps, taking into account evidence from multiple sources. The four-step MAKER pipeline is run three consecutive times \ ('round 1', 'round 2', 'round 3' in Figure 1). **Step 1** 1. Generate a repeat library for the genome of interest using RepeatModeler \ (www.repeatmasker.org/RepeatModeler.html). 2. Identify and mask repetitive elements in the genome using RepeatMasker \ (www.repeatmasker.org) by scanning scaffolds for matches to the repeats in the repeat library. **Step 2** 1. For the first MAKER run \ ('round 1' in Figure 1), train SNAP 2013-02-16 \ (Korf 2004) and Augustus 2.5.5 \ (Stanke _et al_ 2006) using CEGMA \ (Parra _et al_ 2007) gene models for KOGs, as well as 'nematode orthologous groups' \ (NOGs) \ (Martin & Mitreva 2018), 'trematode orthologous groups' \ (TROGs), or 'cestode orthologous groups' \ (CEOGs) as appropriate. For the second MAKER run \ ('round 2'), first train SNAP using the MAKER gene models obtained from the first MAKER run \ ('round 1'). For the third MAKER run \ ('round 3'), first train Augustus using the MAKER gene models obtained from the second MAKER run \ ('round 2'). 2. Generate _ab initio_ gene models to be used as

evidence within MAKER, using Augustus, GeneMark-ES 2.3a (self-trained) (Ter-Hovhannisyan et al 2008), and SNAP. 3. Generate further gene models to use as MAKER input, using comparative algorithms genBlastG (She et al 2011) (using comparisons to *C. elegans* gene models from WormBase; Yook et al 2012) and RATT (Otto et al 2011; transferring gene models from the taxonomically nearest published 'reference' genome from the list: *Haemonchus contortus* for clade V parasites; *Ascaris suum* for Ascaridomorpha; *Brugia malayi* (and *Onchocerca volvulus*) for Spiruromorpha; *Trichuris muris* for clade I; *Strongyloides ratti* for clade IV; *Hymenolepis microstoma* for cestodes except *Echinococcus multilocularis* for *Taenia* species; *Schistosoma mansoni* for trematodes). **Step 3** 1. Align species-specific ESTs and cDNAs from INSDC (Cochrane et al 2016), and proteins from related species (see below), against the genomes using BLASTN and BLASTX (Altschul et al 1997), respectively. The first and second MAKER runs ('round 1' and 'round 2' in Figure 1) are performed using the est2genome option with species-specific ESTs and cDNAs, and the protein2genome for nematode protein sequences from UniProt's UniRef 90 clusters for nematodes (UniProt Consortium, 2015). The third MAKER run ('round 3') is performed using species-specific ESTs and cDNAs, and a taxonomically broader protein set that includes proteins from metazoans with complete proteomes from UniProt and proteins from helminths from GeneDB (Logan-Klumpler et al 2012). 2. Refine these alignments further with respect to splice sites using exonerate (Slater & Birney 2005). **Step 4** 1. The EST and protein homology alignments, comparative gene models, and *ab initio* gene predictions are integrated and filtered by MAKER to produce a gene set for the species, with just one transcript for each gene. **Step 5** After running the MAKER pipeline above (Steps 1-4) three times (rounds 1-3), filter the resulting MAKER gene set to remove less reliable gene models, as follows: 1. Discard any MAKER gene models that were based on exonerate or BLASTX alignments, and do not overlap any Augustus, genBlastG or RATT gene model (as they were probably due to spurious alignments). 2. Discard MAKER gene models that encode proteins of shorter than 30 amino acids. 3. If two different MAKER gene models overlap in their coding sequence, discard the gene model with the worst MAKER score (i.e. AED score).

Troubleshooting

It is likely that in a highly fragmented genome assembly, many genes will be split into multiple partial gene predictions. To correct for the effect of assembly fragmentation on the gene count, the gene count in your assembly can be normalised, by dividing the total proteome length by the mean protein length for *C. elegans* (409.82 amino acids). This should give a more accurate estimate of the true gene count in your species.

Anticipated Results

The output from the protocol is a set of protein-coding gene predictions for one or more genome assemblies of interest.

References

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Figures

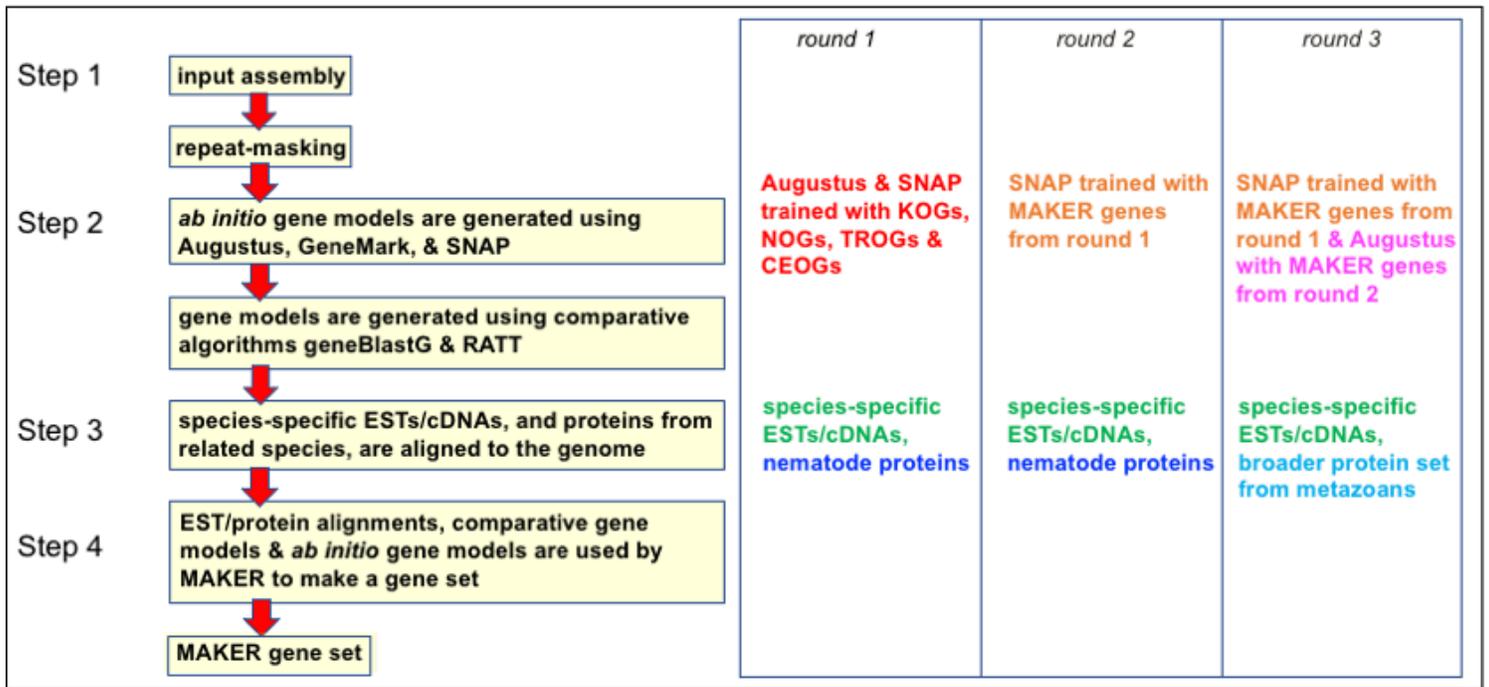


Figure 1

Flowchart of protocol Four-step MAKER pipeline.