

BRAKER2 User Guide

Katharina J. Hoff

February 23, 2018

Contents

1	Introduction	3
1.1	What is BRAKER2?	3
1.2	Keys to successful gene prediction	3
1.3	Overview of modes for running BRAKER2	4
2	Installation	6
2.1	Supported software versions	6
2.2	BRAKER2	6
2.2.1	Perl pipeline dependencies	6
2.2.2	BRAKER2 components	7
2.3	Bioinformatics software dependencies	8
2.3.1	Mandatory tools	8
2.3.1.1	GeneMark-EX	8
2.3.1.2	AUGUSTUS	8
2.3.1.3	Bamtools	9
2.3.1.4	NCBI BLAST+	10
2.3.2	Optional tools	10
2.3.2.1	Samtools	10
2.3.2.2	GenomeThreader	11
2.3.2.3	Spaln	11
2.3.2.4	Exonerate	11
3	Running BRAKER2	12
3.1	Different BRAKER2 pipeline modes	12
3.1.1	BRAKER2 with RNA-Seq data (only)	12
3.1.2	BRAKER2 with proteins of longer evolutionary distance	12
3.1.3	BRAKER2 with proteins of shorter evolutionary distance	13
3.1.4	BRAKER2 with RNA-Seq and protein data	14
3.1.4.1	Adding protein data of short evolutionary distance to gene prediction step	14
3.1.4.2	Extending training gene set with proteins of short evolutionary distance	15

3.2	Description of selected BRAKER2 command line options	15
3.2.1	--ab_initio	15
3.2.2	--augustus-args='--some_arg=bla'	15
3.2.3	--cores	15
3.2.4	--fungus	15
3.2.5	--softmasking	15
3.2.6	--useexisting	16
3.2.7	--crf	16
4	Output of BRAKER2	17
5	Example data	18
6	Bug reporting	19

Chapter 1

Introduction

1.1 What is BRAKER2?

The rapidly growing number of sequenced genomes requires fully automated methods for accurate gene structure annotation. With this goal in mind, we have developed BRAKER1 [Hoff et al., 2015], a combination of GeneMark-ET [Lomsadze et al., 2014] and AUGUSTUS [Stanke et al., 2008], that uses genomic and RNA-Seq data to automatically generate full gene structure annotations in novel genomes.

However, the quality of RNA-Seq data that is available for annotating a novel genome is variable, and in some cases, RNA-Seq data is not available, at all.

BRAKER2 is an extension of BRAKER1 which allows for **fully automated training** of the gene prediction tools GeneMark-EX and AUGUSTUS from RNA-Seq and/or protein homology information, and that integrates the extrinsic evidence from RNA-Seq and protein homology information into the **prediction**.

In contrast to other available methods that rely on protein homology information, BRAKER2 reaches high gene prediction accuracy even in the absence of the annotation of very closely related species and in the absence of RNA-Seq data.

BRAKER2 can also combine RNA-Seq and protein homology information. This is in an early stage of implementation, though, and will improve with future releases.

1.2 Keys to successful gene prediction

- Use a high quality genome assembly. If you have a huge number of very short scaffolds in your genome assembly, those short scaffolds will likely increase runtime dramatically but will not increase prediction accuracy.
- Use simple scaffold names in the genome file (e.g. `>contig1` will work better than `>contig1|my custom species name|some putative function|/more/information/ | and lots of special characters %&!*(){}).` Make the scaffold names in all your fasta files simple before running any alignment program.
- In order to predict genes accurately in a novel genome, the genome should be masked for repeats. This will avoid the prediction of false positive gene structures in repetitive and low complexity regions. Repeat masking is also essential for mapping RNA-Seq data to a genome. In case of GeneMark-EX and AUGUSTUS, softmasking (i.e. putting repeat regions into lower case letters and all other regions into upper case letters) leads to better results than hardmasking (i.e. replacing letters in repetitive regions by the letter N for unknown nucleotide).

- Many genomes have gene structures that will be predicted accurately with standard parameters of GeneMark-ET and AUGUSTUS within BRAKER2. However, some genomes have clade-specific features, i.e. special branch point model in fungi, or non-standard splice-site patterns. Please read the options section 3.2 in order to determine whether any of the custom options may improve gene prediction accuracy in the genome of your target species.
- Always check gene prediction results before further usage! You can e.g. use a genome browser for visual inspection of gene models in context with extrinsic evidence data.

1.3 Overview of modes for running BRAKER2

BRAKER2 mainly features semi-supervised, extrinsic evidence data (RNA-Seq **or** protein spliced alignment information¹) supported training of GeneMark-EX and subsequent training of AUGUSTUS with integration of extrinsic evidence in the final gene prediction step. However, there are now a number of additional pipelines included in BRAKER2. In the following, we give an overview:

- genome and RNA-Seq file from the same species (see figure 1.1A); this approach is suitable for RNA-Seq libraries with a good coverage of the transcriptome,
- genome file and database of proteins that may be of longer evolutionary distance to the target species (see figure 1.1B; this approach is suitable if no RNA-Seq data is available, and if no protein data from a very closely related species is available,
- genome file and file with proteins of short evolutionary distance (see figure 1.2D); this approach is suitable if RNA-Seq data is not available and if the reference species is very closely related,
- genome and RNA-Seq file and proteins of short evolutionary distance (see figures 1.2C and 1.2E). In both cases, GeneMark-ET is trained supported by RNA-Seq data, and the resulting gene predictions are used for training AUGUSTUS. In approach C), protein alignment information is used in the gene prediction step with AUGUSTUS, only. In approach E), protein spliced alignment data is used to complement the training set for AUGUSTUS. The latter approach is in particular suitable if RNA-Seq data does not produce a sufficiently high number of training gene structures for AUGUSTUS, and if a very closely related and already annotated species is available. **Carefully inspect results of BRAKER2 runs with RNA-Seq and protein data! Compare to runs with only one of those data sources. The optimal result is not always the one from integrating both sources of evidence. (This will change in future versions of BRAKER2.)**

¹using both sources of evidence for GeneMark-EX is not supported by this release of BRAKER2.

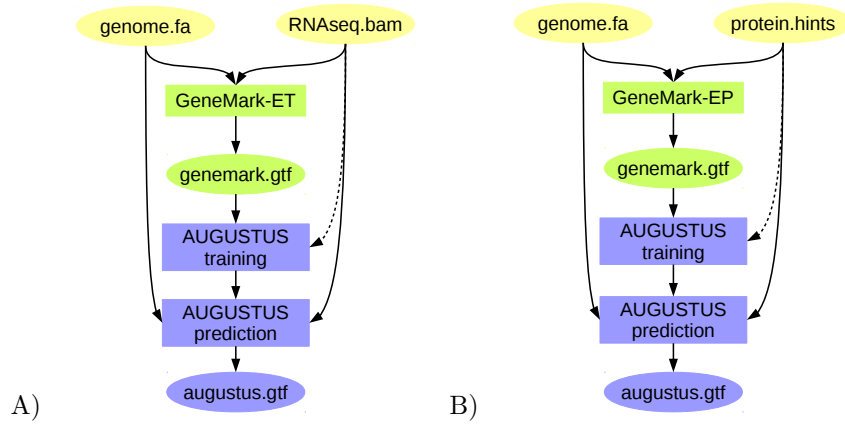


Figure 1.1: BRAKER2 pipeline A) training GeneMark-ET supported by RNA-Seq spliced alignment information, prediction with AUGUSTUS with that same spliced alignment information, B) training GeneMark-EP on protein spliced alignment information, prediction with AUGUSTUS with that same spliced alignment information. Proteins used for B) can be of longer evolutionary distance.

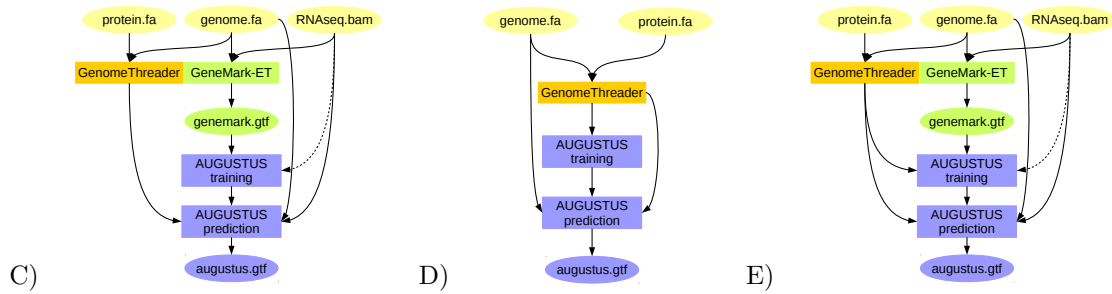


Figure 1.2: BRAKER2 pipeline C) training GeneMark-ET supported by RNA-Seq spliced alignment information, prediction with AUGUSTUS with spliced alignment information from RNA-Seq data and with gene features determined by alignments from proteins of a very closely related species against the target genome, D) training AUGUSTUS on the basis of spliced alignment information from proteins of a very closely related species against the target genome, E) training GeneMark-ET on the basis of RNA-Seq spliced alignment information, training AUGUSTUS on a set of training gene structures compiled from RNA-Seq supported gene structures predicted by GeneMark-ET and spliced alignment of proteins of a very closely related species.

Chapter 2

Installation

2.1 Supported software versions

At the time of release, this BRAKER2 version was tested with:

- AUGUSTUS xxx
- GeneMark-EX xxx
- BAMTOOLS 2.4.1
- SAMTOOLS 0.1.19-96b5f2294a
- GenomeThreader 1.1.6
- Spaln 2.3.1
- Exonerate 2.2.0
- NCBI BLAST+ xxx

2.2 BRAKER2

2.2.1 Perl pipeline dependencies

BRAKER2 is implemented in Perl. Running BRAKER2 requires a Linux-system with `bash` and Perl. Furthermore, BRAKER2 requires the following CPAN-Perl modules to be installed:

- `File::Spec::Functions`
- `Hash::Merge`
- `List::Util`
- `Logger::Simple`
- `Module::Load::Conditional`
- `Parallel::ForkManager`
- `POSIX`
- `Scalar::Util::Numeric`

- **YAML**

On Ubuntu, for example, install the modules with CPANminus¹: `sudo cpanm [Module::Name]`, e.g. `sudo cpanm Hash::Merge`.

BRAKER2 also uses a Perl module `helpMod.pm` that is not available on CPAN. This module is part of the BRAKER2 release and does not require separate installation.

2.2.2 BRAKER2 components

BRAKER2 is a collection of Perl scripts and a Perl module. The main script that will be called in order to run BRAKER2 is `braker.pl`. Additional Perl components are:

- `align2hints.pl`
- `filterGenemark.pl`
- `filterIntronsFindStrand.pl`
- `startAlign.pl`
- `helpMod.pm`

All Perl scripts (files ending with `*.pl`) that are part of BRAKER2 must be executable in order to run BRAKER2. This should already be the case if you download BRAKER2 from our website. Executability may be overwritten if you e.g. transfer BRAKER2 on a USB-stick to another computer. In order to check whether required files are executable, run the following command in the directory that contains BRAKER2 Perl scripts:

```
ls -l *.pl
```

The output should be similar to this:

```
-rwxr-xr-x 1 braker braker 13802 Jan 12 14:23 align2hints.pl
-rwxr-xr-x 1 braker braker 159944 Jan 16 16:42 braker.pl
-rwxr-xr-x 1 braker braker 21217 Jul 3 2017 filterGenemark.pl
-rwxr-xr-x 1 braker braker 5716 Jul 12 2017 filterIntronsFindStrand.pl
-rwxr-xr-x 1 braker braker 32123 Jan 12 14:23 startAlign.pl
```

It is important that the `x` in `-rwxr-xr-x` is present for each script. If that is not the case, run

```
chmod a+x *.pl
```

in order to change file attributes.

You may find it helpful to add the directory in which BRAKER2 perl scripts reside to your `$PATH` environment variable. For a single bash session, enter:

```
PATH=/your_path_to_braker/:$PATH
export PATH
```

To make this `PATH` modification available to all bash sessions, add the above lines to a startup script (e.g. `sim/.bashrc`).

¹install with `sudo apt-get install cpanminus`

2.3 Bioinformatics software dependencies

BRAKER2 calls upon various bioinformatics software tools that are not part of BRAKER2. Some tools are obligatory, i.e. BRAKER2 will not run at all if these tools are not present on your system. Other tools are optional. Please install all tools that are required for running BRAKER2 in the mode of your choice.

2.3.1 Mandatory tools

2.3.1.1 GeneMark-EX

Download GeneMark-EX from http://exon.gatech.edu/GeneMark/license_download.cgi. Unpack and install GeneMark-EX as described in GeneMark-EX's README file.

If already contained in your `$PATH` variable, BRAKER2 will guess the location of `gmes_petap.pl`, automatically. Otherwise, BRAKER2 can find GeneMark-EX executables either by locating them in an environment variable `GENEMARK_PATH`, or by taking a command line argument (`--GENEMARK_PATH=/your_path_to_GeneMark-EX/gmes_petap/`). In order to set the environment variable for your current Bash session, type:

```
export GENEMARK_PATH=/your_path_to_GeneMark-ET/gmes_petap/
```

Add the above lines to a startup script (e.g. `~/.bashrc`) in order to make it available to all bash sessions.²

2.3.1.2 AUGUSTUS

Download AUGUSTUS from <http://bioinf.uni-greifswald.de/augustus/downloads/index.php>. Unpack AUGUSTUS and install AUGUSTUS according to AUGUSTUS README.TXT.

AUGUSTUS comes with pre-compiled binaries (located in the `augustus-x.x.x/bin` folder). However, you should compile AUGUSTUS on your own system in order to avoid problems with versions of libraries used by AUGUSTUS. Compilation instructions are provided in the AUGUSTUS README.TXT file (`augustus-x.x.x/README.txt`).

AUGUSTUS consists of `augustus`, the gene prediction tool, additional C++ tools located in `augustus/auxprogs` and Perl scripts located in `augustus/scripts`. Perl scripts must be executable (see instructions in section 2.2.2 on page 7).

The C++ tool `bam2hints` is an essential component of BRAKER2. Sources are located in `augustus-x.x.x/auxprogs/bam2hints`. Make sure that you compile `bam2hints` on your system (it should be automatically compiled when AUGUSTUS is compiled, but in case of problems with `bam2hints`, please read troubleshooting instructions in `augustus-x.x.x/auxprogs/bam2hints/README`).

Since BRAKER2 is a pipeline that trains AUGUSTUS, i.e. writes species specific parameter files, BRAKER2 needs writing access to the configuration directory of AUGUSTUS that contains such files (`augustus-x.x.x/config/`). If you install AUGUSTUS globally on your system, the `config` folder will typically not be writable by all users. Either make the directory where `config` resides recursively writable to users of AUGUSTUS, or copy the `config/` folder (recursively) to a location where users have writing permission.

AUGUSTUS will locate the `config` folder by looking for an environment variable `$AUGUSTUS_CONFIG_PATH`. If the `$AUGUSTUS_CONFIG_PATH` environment variable is not set, then BRAKER2 will look in the path `../config` relative to the directory in which it finds an AUGUSTUS executable. Alternatively, you can

²GeneMark-EX is not a mandatory tool if AUGUSTUS is to be trained from GenomeThreader alignments with the option `--trainFromGth`.

supply the variable as a command line argument to BRAKER2 (`--AUGUSTUS_CONFIG_PATH=/your_path_to_AUGUSTUS/augustus/config/`). We recommend that you export the variable e.g. for your current bash session:

```
export AUGUSTUS_CONFIG_PATH=/your_path_to_AUGUSTUS/augustus/config/
```

In order to make the variable available to all Bash sessions, add the above line to a startup script, e.g. `~/ .bashrc`.

Important: BRAKER2 expects the entire `config` directory of AUGUSTUS at `$AUGUSTUS_CONFIG_PATH`, i.e. the subfolders `species` with its contents (at least `generic`) and `extrinsic`! Providing an writable but empty folder at `$AUGUSTUS_CONFIG_PATH` will not work for BRAKER. If you need to separate augustus binary and `$AUGUSTUS_CONFIG_PATH`, we recommend that you recursively copy the un-writable config contents to a writable location.

Example:

You have a system-wide installation of AUGUSTUS at `/usr/bin/augustus`, an unwritable copy of `config` sits at `/usr/bin/augustus_config/`. The folder `/home/yours/` is writable to you. Copy with the following command (and additionally set the then required variables):

```
cp -r \texttt{/usr/bin/augustus_config/ /home/yours/
export AUGUSTUS_CONFIG_PATH=/home/yours/augustus_config
export AUGUSTUS_BIN_PATH=/usr/bin
export AUGUSTUS_SCRIPTS_PATH=/usr/bin/augustus_scripts
```

Modification of \$PATH. Adding adding directories of AUGUSTUS binaries and scripts to your `$PATH` variable enables your system to locate these tools, automatically. It is not a requirement for running BRAKER2 to do this, because BRAKER2 will try to guess them from the location of another environment variable (`$AUGUSTUS_CONFIG_PATH`), or both directories can be supplied as command line arguments to `braker.pl`, but we recommend to add them to your `$PATH` variable. For your current bash session, type:

```
PATh=:/your_path_to_augustus/bin:/your_path_to_augustus/scripts/:$PATH
export PATH
```

For all your BASH sessions, add the above lines to a startup script (e.g. `sim/ .bashrc`).

2.3.1.3 Bamtools

Download BAMTOOLS (e.g. `git clone https://github.com/pezmaster31/bamtools.git`). Install BAMTOOLS by typing the following in your shell:

```
cd your-bamtools-directory
mkdir build
cd build
cmake ..
make
```

If already in your `$PATH` variable, BRAKER2 will find bamtools, automatically. Otherwise, BRAKER2 can locate the bamtools binary either by using an environment variable `$BAMTOOLS_PATH`, or by taking a command line argument (`--BAMTOOLS_PATH=/your_path_to_bamtools/bin/3`). In order to set the environment variable e.g. for your current bash session, type:

```
export BAMTOOLS_PATH=/your_path_to_bamtools/bin/
```

Add the above line to a startup script (e.g. `~/ .bashrc`) in order to set the environment variable for all bash sessions.

2.3.1.4 NCBI BLAST+

On Ubuntu, install with `sudo apt-get install ncbi-blast+`.

If already in your `$PATH` variable, BRAKER2 will find blastp, automatically. Otherwise, BRAKER2 can locate the blastp binary either by using an environment variable `$BLAST_PATH`, or by taking a command line argument (`--BLAST_PATH=/your_path_to_blast/`). In order to set the environment variable e.g. for your current bash session, type:

```
export BLAST_PATH=/your_path_to_blast/
```

Add the above line to a startup script (e.g. `~/ .bashrc`) in order to set the environment variable for all bash sessions.

2.3.2 Optional tools

2.3.2.1 Samtools

Samtools is not required for running BRAKER2 if all your files are formatted, correctly (i.e. all sequences should have short and unique fasta names). If you are not sure whether all your files are formatted correctly, it might be helpful to have Samtools installed because BRAKER2 can automatically fix certain format issues by using Samtools.

As a prerequisite for Samtools, download and install `htslib` (e.g. `git clone https://github.com/samtools/htslib.git`, follow the `htslib` documentation for installation).

Download and install Samtools (e.g. `git clone git://github.com/samtools/samtools.git`), subsequently follow Samtools documentation for installation).

If already in your `$PATH` variable, BRAKER2 will find samtools, automatically. Otherwise, BRAKER2 can find Samtools either by taking a command line argument (`--SAMTOOLS_PATH=/your_path_to_samtools/`), or by using an environment variable `$SAMTOOLS_PATH`. For exporting the variable, e.g. for your current bash session, type:

```
export SAMTOOLS_PATH=/your_path_to_samtools/
```

Add the above line to a startup script (e.g. `~/ .bashrc`) in order to set the environment variable for all bash sessions.

³The binary may e.g. reside in `bamtools/build/src/toolkit`

2.3.2.2 GenomeThreader

This tool is required, only, if you would like to run protein to genome alignments with BRAKER2 using GenomeThreader. This is a suitable approach if an annotated species of short evolutionary distance to your target genome is available. Download GenomeThreader from <http://genomethreader.org/>. Unpack and install according to `gth/README`.

BRAKER2 will try to locate the GenomeThreader executable by using an environment variable `$ALIGNMENT_TOOL_PATH`. Alternatively, this can be supplied as command line argument (`--ALIGNMENT_TOOL_PATH=/your/path/to/gth`).

2.3.2.3 Spaln

This tool is required, only, if you would like to run protein to genome alignments with BRAKER2 using Spaln. This is a suitable approach if an annotated species of short evolutionary distance to your target genome is available. (We recommend the usage of GenomeThreader instead of Spaln.) Download Spaln from http://www.genome.ist.i.kyoto-u.ac.jp/~aln_user. Unpack and install according to `spaln/doc/SpalnReadMe22.pdf`.

BRAKER2 will try to locate the Spaln executable by using an environment variable `$ALIGNMENT_TOOL_PATH`. Alternatively, this can be supplied as command line argument (`--ALIGNMENT_TOOL_PATH=/your/path/to/spaln`).

2.3.2.4 Exonerate

This tool is required, only, if you would like to run protein to genome alignments with BRAKER2 using Exonerate. This is a suitable approach if an annotated species of short evolutionary distance to your target genome is available. (We recommend the usage of GenomeThreader instead of Exonerate because Exonerate is comparably slower and has lower specificity than GenomeThreader.) Download Exonerate from <https://github.com/nathanweeks/exonerate>. Unpack and install according to `exonerate/README`. (On Ubuntu, download and install by typing `sudo apt-get install exonerate`.)

BRAKER2 will try to locate the Exonerate executable by using an environment variable `$ALIGNMENT_TOOL_PATH`. Alternatively, this can be supplied as command line argument (`--ALIGNMENT_TOOL_PATH=/your/path/to/exonerate`).

Chapter 3

Running BRAKER2

3.1 Different BRAKER2 pipeline modes

In the following, we describe “typical” BRAKER2 calls for different input data types.

3.1.1 BRAKER2 with RNA-Seq data (only)

.

This approach is suitable for genomes of species for which RNA-Seq libraries with a good coverage of the transcriptome are available. The pipeline is illustrated in figure 1.1A) on page 5.

BRAKER2 can either extract RNA-Seq spliced alignment information from **bam** files, or it can use such extracted information, directly.

In order to run BRAKER2 with RNA-Seq data supplied as **bam** file(s) (in case of multiple files, separate them by comma), run:

```
braker.pl --species=yourSpecies --genome=genome.fasta --bam=file1.bam,file2.bam
```

In order to run BRAKER2 with RNA-Seq spliced alignment information that has already been extracted, run:

```
braker.pl --species=yourSpecies --genome=genome.fasta \
--hints=hints1.gff,hints2.gff
```

The format of such a hints file must be as follows (tabulator separated file):

```
chrName b2h intron 6591 8003 1 + . pri=4;src=E
chrName b2h intron 6136 9084 11 + . mult=11;pri=4;src=E
...
```

The source **b2h** in the second column and the source tag **src=E** in the last column are essential for BRAKER2 to determine whether a hint has been generated from RNA-Seq data.

3.1.2 BRAKER2 with proteins of longer evolutionary distance

This approach is suitable for genomes of species for which no RNA-Seq libraries are available and for which no closely related and well annotated genome is available. A database of proteins with longer

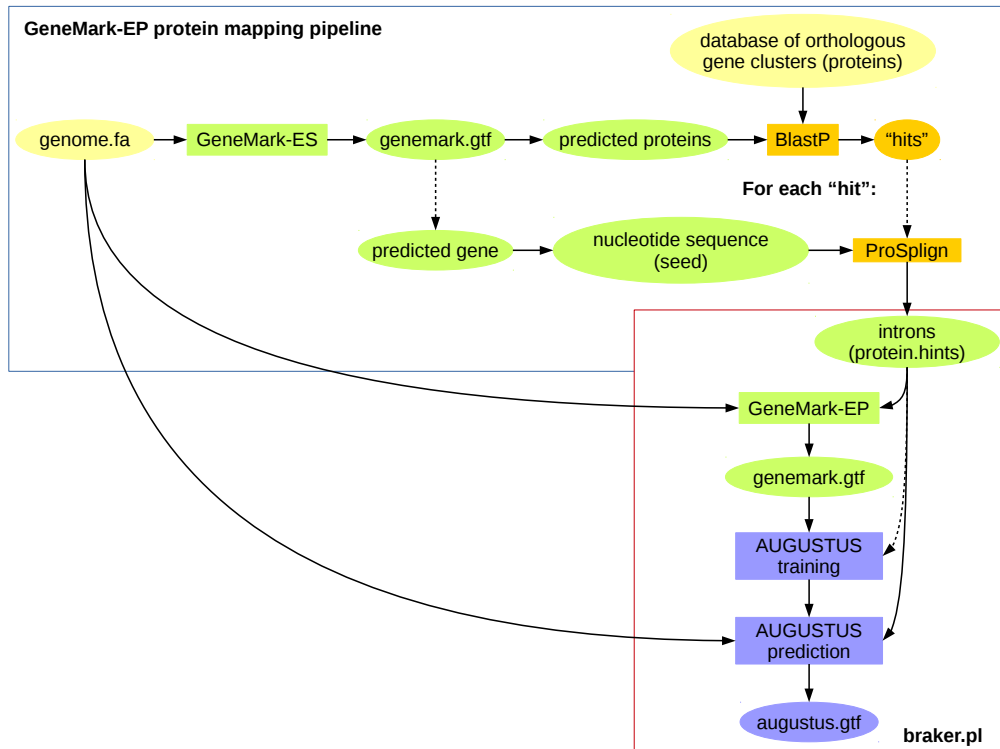


Figure 3.1: BRAKER2 and GeneMark-EP protein mapping pipeline.

evolutionary distance to the target species may be used in this case. The pipeline is illustrated in figure 3.1 on page 13.

Running BRAKER2 with proteins of longer evolutionary distance requires the preparation of “protein hints” before running BRAKER2, itself. Preparing protein hints is in this case not part of BRAKER2 because in contrast to BRAKER2, which can run on a work station with one or multiple cores, the GeneMark-EP specific protein mapping pipeline requires a cluster for execution.

For running BRAKER2 in this mode, type:

```
braker.pl --species=yourSpecies --genome=genome.fasta \
--hints=hints.gff --epmode
```

The format of such a hints file must be as follows (tabulator separated file):

```
chrName ProSplign intron 6591 8003 5 + . mult=5;pri=4;src=P
chrName ProSplign intron 6136 9084 11 + . mult=11;pri=4;src=P
...
```

The source `ProSplign` in the second column and the source tag `src=P` in the last column are essential for BRAKER2 to determine whether a hint has been generated from remote homology protein data.

3.1.3 BRAKER2 with proteins of shorter evolutionary distance

This approach is suitable if RNA-Seq data for the species of the target genome is not available and if a well annotated and very closely related reference species is available. The pipeline is illustrated in figure 1.2D) on page 5.

For running BRAKER2 in this mode, type:

```
braker.pl --species=yourSpecies --genome=genome.fasta \
  --prot_seq=proteins.fa --prg=gth --ALIGNMENT_TOOL_PATH=/path/to/gth/binary \
  --trainFromGth
```

It is possible to generate protein alignments externally, prior running BRAKER2, itself. The compatible command for running GenomeThreader prior running BRAKER2, is:

```
gth -genomic genome.fa -protein protein.fa -gff3out -skipalignmentout -o gth.aln
```

In order to use such externally created alignment files, run:

```
braker.pl --species=yourSpecies --genome=genome.fasta \
  --prot_aln=proteins.aln --prg=gth --trainFromGth
```

It is also possible to run BRAKER2 in this mode using an already prepared hints file. In this case, run:

```
braker.pl --species=yourSpecies --genome=genome.fasta \
  --hints=hints.gff --prg=gth --trainFromGth
```

Format of the hints file should look like this:

```
chrName  gth2h  CDSpart 105984 106633 . - . src=P;grp=FBpp0285205;pri=4
chrName  gth2h  start  106646 106648 . - . src=P;grp=FBpp0285205;pri=4
```

Supported features are intron, CDSpart, start, stop.

3.1.4 BRAKER2 with RNA-Seq and protein data

BRAKER2 with RNA-Seq and protein data is currently still under development. BRAKER2 currently does not train GeneMark-EX from protein and RNA-Seq data, yet. However, if RNA-Seq data of the target species and protein data of a very closely related reference species are available, BRAKER2 already supports the following to modes.

3.1.4.1 Adding protein data of short evolutionary distance to gene prediction step

This pipeline is illustrated in figure 1.2C) on page 5.

In general, add the options

```
--prot_seq=proteins.fa --prg=(gth|exonerate|spaln)
```

to the BRAKER2 call that is described in section 3.1.1. Select one protein alignment tool from GenomeThreader (**gth**, recommended), Spaln (**spaln**) or Exonerate (**exonerate**). Of course, you may also specify the protein information as protein alignment files or hints files as described in section 3.1.3 on page 13). This may result in a call similar to:

```
braker.pl --species=yourSpecies --genome=genome.fasta --bam=file1.bam,file2.bam \
  --prot_seq=proteins.fa --prg=(gth|exonerate|spaln)
```

3.1.4.2 Extending training gene set with proteins of short evolutionary distance

If the number of training gene structures identified by RNA-Seq data, only, seems to be too small, you may add training gene structures generated by protein alignments with GenomeThreader to the training gene set. This pipeline is illustrated in 1.2E) on page 5.

In general, add the options

```
--prot_seq=proteins.fa --prg=gth --gth2traingen
```

to the BRAKER2 call that is described in section 3.1.1. This may result in a call similar to:

```
braker.pl --species=yourSpecies --genome=genome.fasta --bam=file1.bam,file2.bam \
--prot_seq=proteins.fa --prg=gth --gth2traingen
```

3.2 Description of selected BRAKER2 command line options

Please run `braker.pl --help` to obtain a full list of options.

3.2.1 --ab_initio

Compute AUGUSTUS *ab initio* predictions in addition to AUGUSTUS predictions with hints (additional output files: `augustus.ab_initio.*`). This may be useful for estimating the quality of training gene parameters when inspecting predictions in a Browser.

3.2.2 --augustus-args='--some_arg=bla'

One or several command line arguments to be passed to AUGUSTUS, if several arguments are given, separated by whitespace, i.e. `'--first_arg=sth --second_arg=sth'`. This may be useful if you know that gene prediction in your particular species benefits from a particular AUGUSTUS argument during the prediction step.

3.2.3 --cores

Specifies the maximum number of cores that can be used during computation. BRAKER2 has to run some steps on a single core, others can take advantage of multiple cores. The optimal core number of all steps is 8. If you use more than 8 cores, this will not speed up all parallelized steps, in particular, the time consuming `optimize_augustus.pl` will not use more than 8 cores. However, if you don't mind some cores being idle, using more than 8 cores will speed up other steps.

3.2.4 --fungus

GeneMark-EX option: run algorithm with branch point model. Use this option if your genome is a fungus.

3.2.5 --softmasking

Softmasking option for soft masked genome files. Set to 'on' or '1'. Recommended to use this option!

3.2.6 --useexisting

Use the present config and parameter files if they exist for 'species'. This step will skip training AUGUSTUS and instead use pre-trained parameters.

3.2.7 --crf

Execute CRF training for AUGUSTUS; resulting parameters are only kept for final predictions if they show higher accuracy than HMM parameters. This increases runtime!

Chapter 4

Output of BRAKER2

BRAKER2 produces three important output files in the working directory:

hintsfile.gff	- The extrinsic evidence data extraced from RNAseq.bam and/or protein data. The introns are used for training GeneMark-EX, all features are used for predicting genes with AUGUSTUS. The file is in gff format.
GeneMark-ET/genemark.gtf	- Genes predicted by GeneMark-EX in gtf format (RNA-Seq trained)
GeneMark-EP/genemark.gtf	- Genes predicted by GeneMark-EX in gtf format (Protein trained)
augustus.hints.gff	- Genes predicted by AUGUSTUS with hints from given extrinsic evidence in gtf format

For details about gtf format, see <http://www.sanger.ac.uk/Software/formats/GFF/>. A gtf format file contains one line per predicted exon. Example:

HS04636	AUGUSTUS	initial	966	1017	.	+	0	transcript_id "g1.1"; gene_id "g1";
HS04636	AUGUSTUS	internal	1818	1934	.	+	2	transcript_id "g1.1"; gene_id "g1";

The columns (fields) contain:

seqname	source	feature	start	end	score	strand	frame	transcript ID and gene ID
---------	--------	---------	-------	-----	-------	--------	-------	---------------------------

Chapter 5

Example data

Due to file size, example data for testing BRAKER2 is separately available for download at <http://bioinf.uni-greifswald.de/augustus/downloads/index.php> and <http://exon.gatech.edu/> as archive `BRAKER2examples.tar.gz`.

After extraction, test BRAKER2 with the following commands:

1) For usage with RNA-Seq, only (BRAKER1 functionality):

```
perl braker.pl --genome=/path/to/examples/genome.fa --bam=/path/to/examples/RNAseq.bam
```

2) For usage with RNA-Seq and proteins (BRAKER2 functionality, adapt command to aligner of your choice):

```
perl braker.pl --genome=/path/to/examples/genome.fa --bam=/path/to/examples/RNAseq.bam \
  --prot_seq=/path/to/examples/proteins.fa --prg=gth --ALIGNMENT_TOOL_PATH=/path/to/aligner
```

The runtime of this example should be around 46 hours on a 2.27 GHz single CPU.

Chapter 6

Bug reporting

If you found a bug, please contact katharina.hoff@uni-greifswald.de.

Information worth mentioning in your bug report:

Check in `braker/yourSpecies/braker.log` at which step `braker.pl` crashed.

There are a number of other files that might be of interest, depending on where in the pipeline the problem occurred. Some of the following files will not be present if they did not contain any errors.

- `braker/yourSpecies/errors/bam2hints.*.stderr` - will give details on a bam2hints crash (step for converting bam file to intron gff file)
- `braker/yourSpecies/hintsfile.gff` - is this file empty? If yes, something went wrong during hints generation - does this file contain hints from source “b2h” and of type “intron”? If not: GeneMark-ET will not be able to execute properly.
- `braker/yourSpecies/startAlign.stderr` - if you provided a protein fasta file and this file is not empty, something went wrong during protein alignment
- `braker/yourSpecies/startAlign.stdout` - may give clues on at which point protein alignment went wrong
- `braker/yourSpecies/(align_gth|align_exonerate|align_spaln)/*err` - errors reported by the alignment tools gth/exonerate/spaln
- `braker/yourSpecies/errors/GeneMark-ET.stderr` - errors reported by GeneMark-ET
- `braker/yourSpecies/errors/GeneMark-ET.stdout` - may give clues about the point at which errors in GeneMark-ET occurred
- `braker/yourSpecies/GeneMark-ET/genemark.gtf` - is this file empty? If yes, something went wrong during executing GeneMark-ET
- `braker/yourSpecies/GeneMark-ET/genemark.f.good.gtf` - is this file empty? If yes, something went wrong during filtering GeneMark-ET genes for training AUGUSTUS
- `braker/yourSpecies/genbank.good.gb` - try a “`grep -c LOCUS genbank.good.gb`” to determine the number of training genes for training AUGUSTUS, should not be low
- `braker/yourSpecies/errors/firstetraining.stderr` - contains errors from first iteration of training AUGUSTUS
- `braker/yourSpecies/errors/secondetraining.stderr` - contains errors from second iteration of training AUGUSTUS

- `braker/yourSpecies/errors/optimize_augustus.stderr` - contains errors `optimize_augustus.pl` (additional training set for AUGUSTUS)
- `braker/yourSpecies/errors/augustus*.stderr` - contain AUGUSTUS execution errors

Bibliography

- [Hoff et al., 2015] Hoff, K. J., Lange, S., Lomsadze, A., Borodovsky, M., and Stanke, M. (2015). Braker1: unsupervised rna-seq-based genome annotation with genemark-et and augustus. *Bioinformatics*, 32(5):767–769.
- [Lomsadze et al., 2014] Lomsadze, A., Burns, P. D., and Borodovsky, M. (2014). Integration of mapped rna-seq reads into automatic training of eukaryotic gene finding algorithm. *Nucleic acids research*, 42(15):e119–e119.
- [Stanke et al., 2008] Stanke, M., Diekhans, M., Baertsch, R., and Haussler, D. (2008). Using native and syntenically mapped cdna alignments to improve de novo gene finding. *Bioinformatics*, 24(5):637–644.