AGORA How To

Written by Alexandra LOUIS (alouis {at} biologie {dot} ens {dot} fr), Matthieu MUFFATO (muffato {at} ebi {dot} ac {dot} uk), and Hugues ROEST CROLLIUS (hrc {at} ens {dot} fr).
DYOGEN Laboratory, Institut de Biologie de l'École Normale Supérieure (IBENS)
46 rue d'Ulm, 75005 Paris

Table of contents:

Introduction

History

AGORA stands for "Algorithm for Gene Order Reconstruction in Ancestors" and was developed by Matthieu Muffato during his PhD (2007-2010) in the DYOGEN Laboratory at the École normale supérieure in Paris. Since then it has been constantly used in the group, especially to generate ancestral genomes for the Genomicus online server for comparative genomics. Many algorithms used in AGORA are described in details in Matthieu's thesis, available only in French (Muffato 2010) and the core algorithm used to build and linearise the adjacency graph is described in a separate study (Berthelot et al 2015).

What AGORA does and does not do

AGORA takes as input a set of extant gene lists, a species tree linking the genomes, and phylogenetic gene trees reconciled with the species tree. It can produce linear ancestral gene orders (with transcriptional orientation) at any node of the species tree. This may result in very long successive ancestral adjacencies or CARs (Contiguous Ancestral Regions) if the data allows it (e.g. closely related extant genomes with contiguous sequence assemblies) or very short ones if the data does not allow it (e.g. extant genes distributed in short scaffolds, or very rearranged extant genomes).

AGORA does not reconstruct:

- ancestral nucleotide and protein sequences,

- circular chromosomes, unless they are canonically represented in a linear fashion like the mitochondrial genome.

AGORA can be run in two modes. The first and simplest uses all possible adjacencies found in extant genomes to reconstruct ancestral adjacencies, eventually leading to contiguous ancestral regions. In principle this should work fine if the genomes are perfectly sequenced and annotated, but they rarely are. Also, gene duplications are difficult to resolve accurately in gene phylogenies, and AGORA is sensitive to errors in gene trees. A second, more complex version first identifies "robust" gene familes, on the basis of a user-defined criterion. Typically this can be a requirement that there are as many genes on a tree as there are species, thus limiting the chances that duplications have occurred. AGORA first builds a temporary ancestral genome with these genes (ignoring all other families) as a robust backbone. Then, it use remaining gene families to fill in the space between robust genes, but without breaking a chain of robust genes.

In this HowTo, all the paths are relative to the root of the repository. The individual script commands usually complete within seconds on the example dataset, using less than 300 MB of memory, while the complete reconstructions themselves take between 30 seconds and 1 minute.

Input file formats

To reconstruct ancestral gene orders, AGORA needs 3 kinds of files (see example/data/):

- A species tree, e.g. example/data/Species.nwk
- A set of extant gene trees reconciled with the species tree, e.g. example/data/GeneTreeForest.nhx.bz2.
- The list and positions of the genes of each extant genomes, e.g. example/data/genes/genes.M1.list.bz2. Extant genes that are not in a tree are **not** used for gene order reconstruction.

Species tree

The species tree is expected in Newick format. See the example species tree:

- example/data/Species.nwk – Newick format
- example/data/Species.pdf – Graphical representation

Only the node names matter, as they are used to match extant genomes and name the ancestral genomes.

Warning: Internal labels (e.g. "Amniota" or "Anc659123") have to be unique as they are used to refer to ancestors and name files !

Gene trees

AGORA expects the gene trees to be in NHX format with the following keys:

1. S gives the taxon name, which must exist in the species tree

2. `D` indicates the type of the node:
   - `D=N` for speciation nodes
   - `D=Y` for duplication nodes, which can be marked as "dubious" with an extra `DD=Y`

See an example family:

- example/data/Family1.nhx – NHX format
- example/data/Family1.pdf – Graphical representation

The forest file is merely the concatenation of all the families. See the example forest:

- example/data/GeneTreeForest.nhx.bz2 – NHX format

Gene lists

The *genes* files used by AGORA contain the list of genes on each extant genome. The format is tab-separated values, in 5 mandatory columns (the 6^th is optional). One file must be provided per extant genome.

The fields are:

1. Name of the chromosome (text).
2. Start position of the gene (integer).
3. End position of the gene (integer).
4. Gene orientation (1 or -1)
5. Gene identifier (text)
6. Transcript identifier (text, optional)

AGORA only cares about the order of the genes on each chromosome, so the coordinates can be 0-based or 1-based, inclusive or not, etc, as long as the same convention is used throughout each file.

Warning: The gene identifiers have to be consistent with the ones used in the gene trees.

Warning: The genes files must be named consistently with the names of the species in the species tree, using the format `prefix.species_name.suffix`.

For example, if the species in the species tree are: `HUMAN`, `MOUSE`, `DOG`, genes files have to be named:

- `prefix.HUMAN.suffix`, e.g. `genes.HUMAN.list`
- `prefix.MOUSE.suffix`
- `prefix.DOG.suffix`

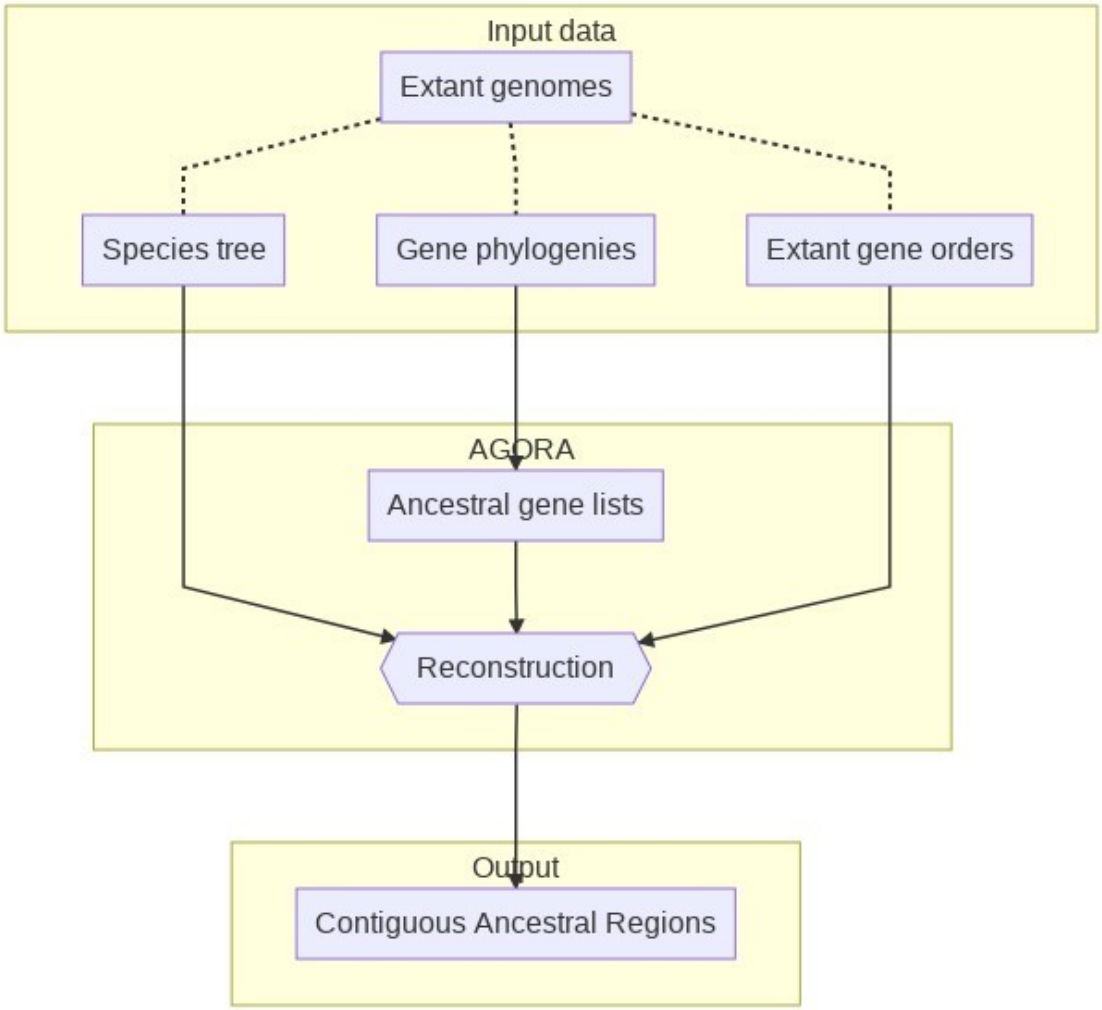
If the species are `Homo.sapiens`, `Mus.musculus` and `Canis.familiaris`, genes files have to be named:

- `prefix.Homo.sapiens.suffix`, e.g. `genes.Homo.sapiens.list`
- `prefix.Mus.musculus.suffix`
- `prefix.Canis.familiaris.suffix`

In example/data, the five species named in the species-tree are `M1`, `M2`, `M3`, `M4`, and `M5`, and the genes files are named genes.M1.list.bz2, etc.

Running AGORA

General AGORA workflow



The AGORA method is wrapped up in a script named `agora.py`, which runs all the steps of the reconstructions according to a configuration file.

The reconstruction itself can be perfomed with different approaches, explained below, and the output is a set of CARs. AGORA comes with three different configuration files of increasing complexity:

- AGORA with no selection of robust families

- AGORA with selection of robust families
- AGORA with multiple selection of robust families

Notes

All AGORA scripts automatically creates the necessary output directories given to them as command line arguments. This excludes standard output / error shell redirections, which must still be valid paths.

AGORA supports several compression formats for input and output files:

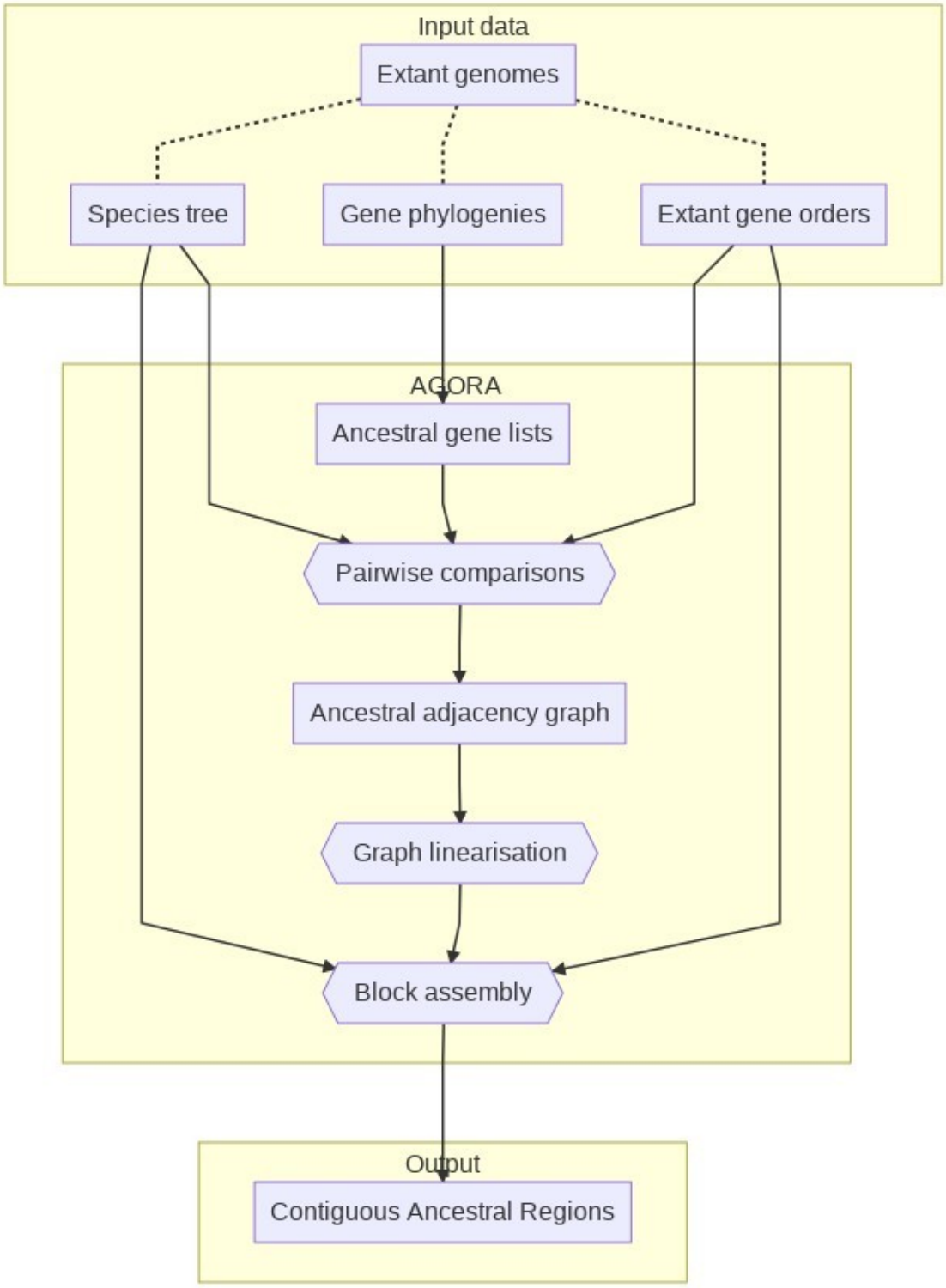
- gzip – `.gz` extension
- bzip2 – `.bz2` extension
- LZMA – `.lzma` and `.xz` extensions

Compression / decompression costs extra CPU time, but decreases file transfer times and storage footprint (typically 6x with bzip2). Compression is also supported on the standard output by adding `+gz`, `+bz2`, `+lzma`, or `+xz` on the command-line.

AGORA with no selection of robust families

This is the simplest and quickest reconstruction. AGORA compares all extant genomes pairwise to extract conserved adjacencies, generates the ancestral adjacency graphs and linearises them to produce CARs.

AGORA workflow with no selection of robust families



In this mode, the reconstruction is composed of three steps akin to a genome assembly:

- The pairwise comparisons provide “reads” of the ancestral genomes
- The reads are assembled into contigs (“Graph linearisation” step)
- The contigs are assembled into scaffolds (“Block assembly” step)

All in one: agora.ini

The agora.ini configuration file sets AGORA to run these three steps sequentially. The only parameters that have to be changed are the paths to the input files (species tree and gene trees). Then run AGORA with the name of output directory (which will be automatically created):

```
src/agora.py conf/agora.ini -workingDir=output_dir
```

To regenerate the reference output of the example dataset, simply run:

```
src/agora.py conf/agora.ini -workingDir=example/results -nbThreads=1
```

By default, AGORA uses all the cores available on the machine. Use the `-nbThreads=XX` option to change this.

Step by step

The scripts can also be run step by step. In the following command lines, `A0` represents the name of the target ancestor for the reconstructions (here the root of the example species tree). This ancestor and all its descendants will be reconstructed.

Extraction of ancestral gene content

The first step in AGORA is to identify all ancestral genes for all ancestral genomes, and print them in one file per target ancestral genome. The `ALL.extractGeneFamilies.py` script takes as input the species tree, the forest of gene trees and a template to name the output files.

```
mkdir -p example/results/ancGenes
src/ALL.extractGeneFamilies.py \
  example/data/Species.nwk \
  example/data/GeneTreeForest.nhx.bz2 \
  -OUT.ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2
\
  +bz2 \
  > example/results/GeneTreeForests.withAncGenes.nhx.bz2 \
  2> example/results/ancGenes/ancGenes.log
```

Be careful to provide the correct path to write the *ancGenes* files (`ancGenes/all/ancGenes.%s.list.bz2`), it will be important if you use AGORA on *robust* family in a second step (see article). The `%s` is automatically replaced by the extant and ancestral species name, as indicated in the species tree.

ancGenes files are tab-separated files, with the following two fields:

1. Ancestral gene names (generated by AGORA)
2. A space separated list of extant copies of this ancestral gene, in the genome of extant species.

On the standard output, the script produces the forest of gene trees, rewritten with the ancestral gene names at each node, in NHX format. Note that the rest of the scripts will use these ancGenes files rather than the forest of gene trees.

Pairwise comparisons

This step compares extant genomes in all possible pairwise combinations to identify conserved adjacencies.

```
mkdir -p example/results/pairwise/pairs-all/
src/buildSynteny.pairwise-conservedPairs.py \
  example/data/Species.nwk \
  A0 \
  -OUT.pairwise=example/results/pairwise/pairs-all/%s.list.bz2 \
  -genesFiles=example/data/genes/genes.%s.list.bz2 \
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/pairwise/pairs-all/log
```

Graph linearisation

This step integrates all the pairwise comparisons identified above for each ancestor and combine them into adjacency graphs, from which a first set of CARs are derived.

```
mkdir -p example/results/integrDiags/denovo-all/
src/buildSynteny.integr-denovo.py \
  example/data/Species.nwk \
  A0 \
  example/results/pairwise/pairs-all/%s.list.bz2 \
  +searchLoops \
  -OUT.ancDiags=example/results/integrDiags/denovo-all/diags.
%s.list.bz2 \
  -LOG.ancGraph=example/results/integrDiags/denovo-all/graph.
%s.log.bz2 \
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/integrDiags/denovo-all/log
```

Block assembly

In this step, we basically reiterate the same process (pairwise comparisons and integration into an adjacency graph) but on the previous CARs, which allows finding higher-level adjacencies. The result is a set of CARs made of CARs, that are much longer than in the previous steps.

Warning: The underscore _ is a required parameter. It tells AGORA to consider all extant species under A0 for this step.

```
mkdir -p example/results/integrDiags/denovo-all.groups/
src/buildSynteny.integr-groups.py \
  example/data/Species.nwk \
  A0 _ \
  -IN.ancDiags=example/results/integrDiags/denovo-all/diags.
%s.list.bz2 \
  -OUT.ancDiags=example/results/integrDiags/denovo-all.groups/diags.
%s.list.bz2 \
  -LOG.ancGraph=example/results/integrDiags/denovo-all.groups/graph.
%s.log.bz2 \
  -genesFiles=example/data/genes/genes.%s.list.bz2 \
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/integrDiags/denovo-all.groups/log
```

Conversion to ancestral genomes

The previous script outputs the ancestral reconstructions as *diags* files There is a last script to convert these files to a format very similar to the input *genes* files, named *ancGenomes*:

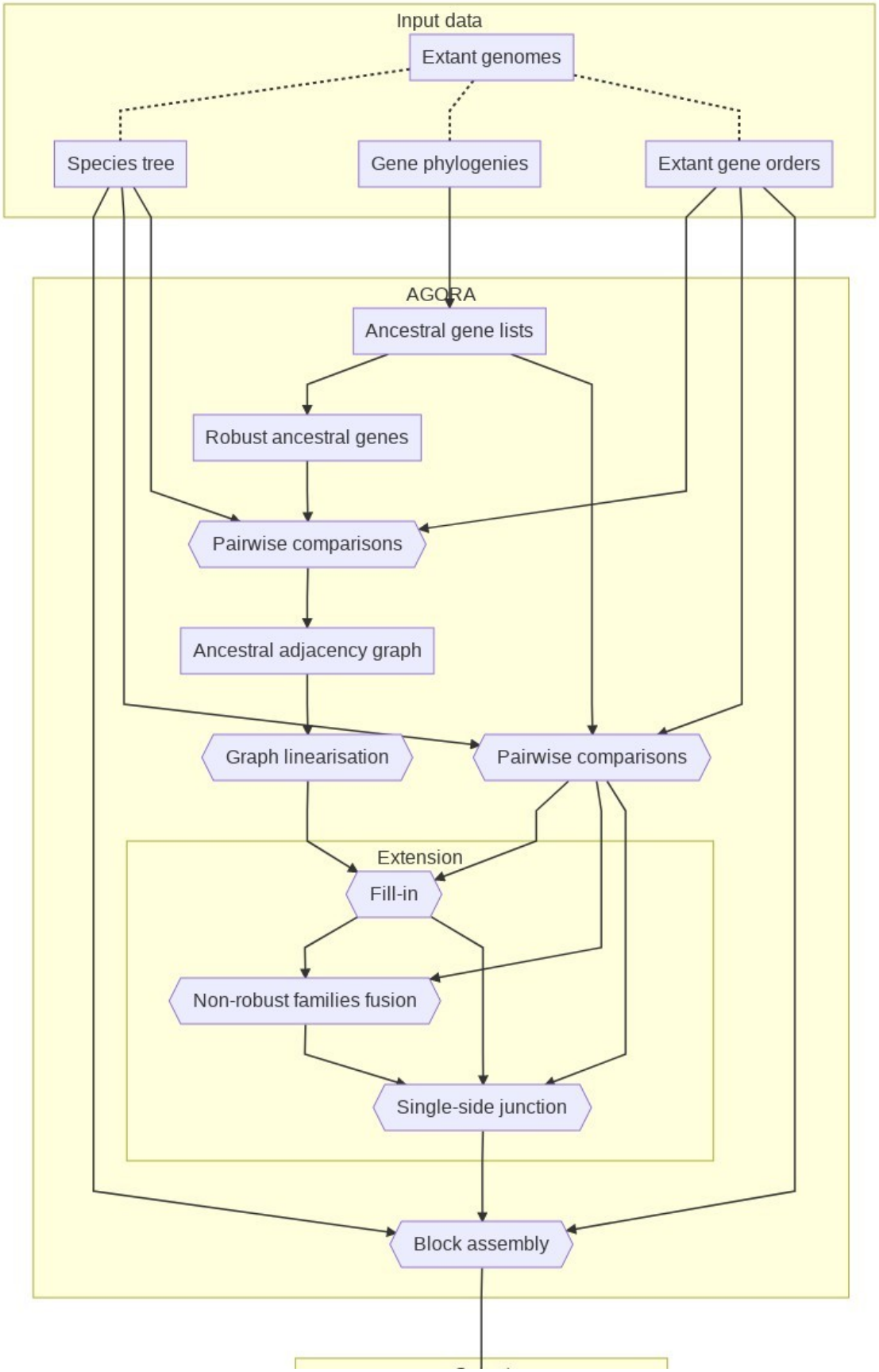
```
mkdir -p example/results/ancGenomes/standard
src/convert.ancGenomes.diags-genes.py \
  example/data/Species.nwk \
  A0 \
  -IN.ancDiags=example/results/integrDiags/denovo-all.groups/diags.
%s.list.bz2 \
  -OUT.ancGenomes=example/results/ancGenomes/standard/ancGenome.
%s.list.bz2 \
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/ancGenomes/standard/log
```

More information about these files in Output file formats below.

AGORA with selection of robust families

This approach builds ancestral adjacencies considering a subset of the genes. The idea here is to build “robust” ancestral adjacency scaffolds, and to insert within these adjacencies the remaining ancestral genes.

AGORA workflow with selection of robust families


Input data
Extant genomes
Species tree
Gene phylogenies
Extant gene orders
AGORA
Ancestral gene lists
Robust ancestral genes
Pairwise comparisons
Ancestral adjacency graph
Graph linearisation
Pairwise comparisons
Extension
Fill-in
Non-robust families fusion
Single-side junction
Block assembly

From the complete list of ancestral genes, AGORA identifies a subset of robust genes according to a user-defined criterion. It compares all extant genomes pairwise (considering all genes and robust genes separately), build the adjacency graphs on the comparisons of robust genes and linearise them to obtain robust contigs. It then *fills these in* with non-robust genes, builds contigs of non-robust genes (*non-robust families fusion*) and inserts these in the filled-in robust contigs (*single side junction*). Finally it assembles the resulting contigs (block assembly) into Contiguous Ancestral Regions (CARs).

All in one: `agora-robust.py`

The whole workflow can be run automatically with `agora.py` and agora-robust.ini. First edit the paths to the input files (species tree and gene trees), then run:

```
src/agora.py conf/agora-robust.ini -workingDir=output_dir
```

This configuration file is set to select the genes families that have exactly the same number of extant genes as extant species (i.e. *minSize* and *maxSize* parameters equal to 1). Having undergone fewer losses and duplications, the synteny signal of those families is less ambiguous and their adjacencies easier to compare and more conserved. These two parameters can be changed according to the dynamics of the species considered.

To regenerate the reference output of the example dataset, simply run:

```
src/agora.py conf/agora-robust.ini -workingDir=example/results -
nbThreads=1
```

Step by step

Selection of robust genes

This script filters the complete set of ancestral genes and selects the ones that match the required number of extant genes (relative to the number of extant species.

Warning: this assumes you have already extracted the ancestral genes from the gene trees (see running AGORA with no selection of robust families).

```
src/ALL.filterGeneFamilies-size.py \
  example/data/Species.nwk \
  A0 \
  example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  example/results/ancGenes/size-%s-%s/ancGenes.%s.list.bz2 \
  1.0 \
  1.0 \
  2> example/results/ancGenes/size.log
```

Pairwise comparison

This step is run once for all ancestral genes, and once for the set of robust families.

For all ancestral genes:

```
mkdir -p example/results/pairwise/pairs-all
src/buildSynteny.pairwise-conservedPairs.py \
  example/data/Species.nwk \
  A0 \
  -OUT.pairwise=example/results/pairwise/pairs-all/%s.list.bz2 \
  -genesFiles=example/data/genes/genes.%s.list.bz2 \
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/pairwise/pairs-all/log
```

For the robust gene families:

```
mkdir -p example/results/pairwise/pairs-size-1.0-1.0
src/buildSynteny.pairwise-conservedPairs.py \
  example/data/Species.nwk \
  A0 \
  -OUT.pairwise=example/results/pairwise/pairs-size-1.0-
1.0/%s.list.bz2 \
  -genesFiles=example/data/genes/genes.%s.list.bz2 \
  -ancGenesFiles=example/results/ancGenes/size-1.0-1.0/ancGenes.
%s.list.bz2 \
  2> example/results/pairwise/pairs-size-1.0-1.0/log
```

Graph linearisation

This step integrates all the pairwise comparisons of robust genes identified above for each ancestor and combines them into adjacency graphs, from which a first set of CARs are derived.

```
mkdir -p example/results/integrDiags/denovo-size-1.0-1.0
src/buildSynteny.integr-denovo.py \
  example/data/Species.nwk \
  A0 \
  example/results/pairwise/pairs-size-1.0-1.0/%s.list.bz2 \
  -OUT.ancDiags=example/results/integrDiags/denovo-size-1.0-1.0/diags.
%s.list.bz2 \
  -LOG.ancGraph=example/results/integrDiags/denovo-size-1.0-1.0/graph.
%s.log.bz2 \
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/integrDiags/denovo-size-1.0-1.0/log
```

Fill-in

This step inserts non-robust genes in each interval of the ancestral contigs, following paths in the complete ancestral adjacency graph.

```
mkdir -p example/results/integrDiags/denovo-size-1.0-1.0.refine-all
src/buildSynteny.integr-refine.py \
  example/data/Species.nwk \
  A0 \
  example/results/pairwise/pairs-all/%s.list.bz2 \
  -IN.ancDiags=example/results/integrDiags/denovo-size-1.0-1.0/diags.
%s.list.bz2 \
  -OUT.ancDiags=example/results/integrDiags/denovo-size-1.0-
1.0.refine-all/diags.%s.list.bz2 \
  -LOG.ancGraph=example/results/integrDiags/denovo-size-1.0-
1.0.refine-all/graph.%s.log.bz2 \
  2> example/results/integrDiags/denovo-size-1.0-1.0.refine-all/log
```

Non-robust families fusion

This step takes all the remaining singletons, which are mostly non-robust genes, and tries to assemble them into contigs.

```
mkdir -p example/results/integrDiags/denovo-size-1.0-1.0.refine-
all.extend-all
src/buildSynteny.integr-extend.py \
  example/data/Species.nwk \
  A0 \
  example/results/pairwise/pairs-all/%s.list.bz2 \
  -IN.ancDiags=example/results/integrDiags/denovo-size-1.0-1.0.refine-
all/diags.%s.list.bz2 \
  -OUT.ancDiags=example/results/integrDiags/denovo-size-1.0-
1.0.refine-all.extend-all/diags.%s.list.bz2 \
  -LOG.ancGraph=example/results/integrDiags/denovo-size-1.0-
1.0.refine-all.extend-all/graph.%s.log.bz2 \
  2> example/results/integrDiags/denovo-size-1.0-1.0.refine-
all.extend-all/log
```

Single-side junction

This step inserts the contigs of non-robust families created above and inserts them in the CARs.

```
mkdir -p example/results/integrDiags/denovo-size-1.0-1.0.refine-
all.extend-all.halfinsert-all
src/buildSynteny.integr-halfinsert.py \
  example/data/Species.nwk \
  A0 \
  example/results/pairwise/pairs-all/%s.list.bz2 \
  -IN.ancDiags=example/results/integrDiags/denovo-size-1.0-1.0.refine-
```

```
all.extend-all/diags.%s.list.bz2 \
  -REF.ancDiags=example/results/integrDiags/denovo-size-1.0-
1.0.refine-all/diags.%s.list.bz2 \
  -OUT.ancDiags=example/results/integrDiags/denovo-size-1.0-
1.0.refine-all.extend-all.halfinsert-all/diags.%s.list.bz2 \
  -LOG.ancGraph=example/results/integrDiags/denovo-size-1.0-
1.0.refine-all.extend-all.halfinsert-all/graph.%s.log.bz2 \
  2> example/results/integrDiags/denovo-size-1.0-1.0.refine-
all.extend-all.halfinsert-all/log
```

Block assembly

Like in non-robust mode, this step does pairwise comparisons and a graph linearisation of the CARs themselves, which allows finding higher-level adjacencies.

Warning: Here as well the underscore _ must be given. It tells AGORA to consider all extant species

```
mkdir -p example/results/integrDiags/denovo-size-1.0-1.0.refine-
all.extend-all.halfinsert-all.groups
src/buildSynteny.integr-groups.py \
  example/data/Species.nwk \
  A0 _ \
  -IN.ancDiags=example/results/integrDiags/denovo-size-1.0-1.0.refine-
all.extend-all.halfinsert-all/diags.%s.list.bz2 \
  -OUT.ancDiags=example/results/integrDiags/denovo-size-1.0-
1.0.refine-all.extend-all.halfinsert-all.groups/diags.%s.list.bz2 \
  -LOG.ancGraph=example/results/integrDiags/denovo-size-1.0-
1.0.refine-all.extend-all.halfinsert-all.groups/graph.%s.log.bz2 \
  -genesFiles=example/data/genes/genes.%s.list.bz2 \
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/integrDiags/denovo-size-1.0-1.0.refine-
all.extend-all.halfinsert-all.groups/log
```

Conversion to ancestral genomes

This step converts the *diags* files to *ancGenomes*:

```
mkdir -p example/results/ancGenomes/robust
src/convert.ancGenomes.diags-genes.py \
  example/data/Species.nwk \
  A0 \
  -IN.ancDiags=example/results/integrDiags/denovo-size-1.0-1.0.refine-
all.extend-all.halfinsert-all.groups/diags.%s.list.bz2 \
  -OUT.ancGenomes=example/results/ancGenomes/robust/ancGenome.
%s.list.bz2 \
```

```
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/ancGenomes/robust/log
```

More information about these files in Output file formats below.

AGORA with multiple selection of robust families

The process can be further tuned to use multiple sets of robust genes for specific ancestors. Along the *1.0-1.0* robust families used above, we can define other, more relaxed, sets, like *0.9-1.1*, which tolerates a 10% deviation between the number of extant genes and extant species, and so forth.

The agora-multirobust.ini configuration file does this. It demonstrates how to use multiple filters on different ancestors, and how to conbine the results.

```
src/agora.py conf/agora-multirobust.ini -workingDir=output_dir
```

Indicative steps

The most efficient way of extracting multiple sets of robust families is to do all at once, for instance:

```
src/ALL.filterGeneFamilies-size.py \
  example/data/Species.nwk \
  A0 \
  example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  example/results/ancGenes/size-%s-%s/ancGenes.%s.list.bz2 \
  1.0,0.9,0.77 \
  1.0,1.1,1.33 \
  2> example/results/ancGenes/multi-size.log
```

Pairwise comparisons would have to be run on each set independently. Then these different sets can be used on different ancestors to generate the first set of ancestral adjacencies, e.g.:

```
mkdir -p example/results/integrDiags/denovo-size-1.0-1.0
src/buildSynteny.integr-denovo.py \
  example/data/Species.nwk \
  =A3 \
  example/results/pairwise/pairs-size-1.0-1.0/%s.list.bz2 \
  -OUT.ancDiags=example/results/integrDiags/denovo-size-1.0-1.0/diags.
%s.list.bz2 \
  -LOG.ancGraph=example/results/integrDiags/denovo-size-1.0-1.0/graph.
%s.log.bz2 \
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/integrDiags/denovo-size-1.0-1.0/log
```

```
mkdir -p example/results/integrDiags/denovo-size-0.9-1.1
src/buildSynteny.integr-denovo.py \
  example/data/Species.nwk \
  =A1,=A2 \
  example/results/pairwise/pairs-size-0.9-1.1/%s.list.bz2 \
  -OUT.ancDiags=example/results/integrDiags/denovo-size-0.9-1.1/diags.
%s.list.bz2 \
  -LOG.ancGraph=example/results/integrDiags/denovo-size-0.9-1.1/graph.
%s.log.bz2 \
  -ancGenesFiles=example/results/ancGenes/all/ancGenes.%s.list.bz2 \
  2> example/results/integrDiags/denovo-size-0.9-1.1/log
```

These sets can be combined by running the copy script multiple times, like this:

```
mkdir -p example/results/integrDiags/denovo-size-custom
src/buildSynteny.integr-copy.py \
  example/data/Species.nwk \
  =A3 \
  -IN.ancDiags=example/results/integrDiags/denovo-size-1.0-1.0/diags.
%s.list.bz2 \
  -OUT.ancDiags=example/results/integrDiags/denovo-size-custom/diags.
%s.list.bz2 \
  2> example/results/integrDiags/denovo-size-custom/log
```

Output file formats

The *diags* files

These files are present under `example/results/integrDiags/*`. Each of these contains a file per ancestral reconstructed genome (e.g. `diags.A0.list.bz2`). The files are tab-separated, and values in each field are further separated by single spaces. The term *diag* historically refers to the diagonal lines that appear in 2 dimensional matrices comparing 2 genomes and reflecting successive conserved adjacencies.

The fields are:

1. Name of the ancestral species.
2. Number of genes in the ancestral block.
3. List of gene IDs. Each ID corresponds to the line number in the corresponding *ancGenes* file (the full one) of this ancestor (starting from 0).
4. Gene transcriptional orientation (strand) within the block.
5. A relative confidence index for each inter-block linkage.
   - The values in parenthesis are the size of the initial blocks.
   - The values without parenthesis represent the number of time the two adjacent blocks are adjacent in extant species.

The sum of the lengths of the initial blocks (numbers in parenthesis) is thus equal to the size of the whole block (field number 2)

For instance, the following line represents a block of 8 genes in A0 made of 2 sub-blocks (of respectively 5 and 3 genes) linked by an adjacency of score 6.

```
A0  8   4559 4179 10099 15638 1304 10998 5675 13765 -1 -1 -1 1 1 -1 -1
1    (5) 6 (3)
```

The *ancGenome* files

The *ancGenomes* files are a simpler way of accessing the content of the ancestral genomes, and can be found under `example/results/ancGenomes/*`.

They are very similar to the input *genes* files. They are tab-separated and contain 5 columns:

1. Name of the ancestral block.
2. Relative start position of the ancestral gene.
3. Relative end position of the ancestral gene.
4. Ancestral gene orientation within the block.
5. Ancestral gene names, separated by a space. The first name corresponds to the ancestral gene, subsequent ones are the list of extant copies of this ancestral gene, in the genome of extant species.

Coordinates follow the same convention as BED files. The start coordinate is 0-based while the end coordinate is 1-based. Thus the first gene in a block has got the coordinates 0 and 1, and the sixth gene 5 and 6.