ProCARs Documentation

Release 0.1

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July 17, 2014

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CHAPTER

ONE

WHAT IS PROCARS ?

ProCars is a program used to reconstruct Ancestral gene orders as *CARs* (Contiguous Ancestral Regions) with a progressive homology-based method. The method runs from a phylogeny tree (without branch lengths needed) with a marked ancestor and a block file.

This homology-based method is based on *iteratively* detecting and assembling ancestral adjacencies, while allowing some micro-rearrangements of synteny blocks at the extremities of the progressively assembled CARs.

The method starts with a set of blocks as the initial set of CARs, and detects iteratively the potential ancestral adjacencies between extremities of CARs, while building up the CARs *progressively* by adding, at each step, new non-conflicting adjacencies that induce the less homoplasy phenomenon. The species tree is used, in some additional internal steps, to compute a score for the remaining conflicting adjacencies, and to detect other reliable adjacencies, in order to reach completely assembled ancestral genomes.

CHAPTER

TWO

USING PROCARS

ProCARs is a Python package, which has been developed using Python 2.7.5.

2.1 Installing ProCARs

To run ProCARs on your dataset(s), you have two possibilities, depending on your use: installing it or running it from the sources.

For a classic use, you can install the package `procars`. For that, you also have two possibilities: installing it on your system as every Python package, or installing it in development mode. You can see here-below the difference between these two types of installations, and choose the one you need, according to your use.

Note: Note that you can always *uninstall ProCARs* if you want to install a new version instead.

2.1.1 Development mode

If you want to install the package `procars` while still working on modifying the scripts, or being able to download and run latest versions, just do:

```
sudo pip install -e .
```

or:

```
sudo python setup.py develop
```

Your changes will then be taken into account. As you installed the package, you will be able to run ProCARs from any directory in your computer.

2.1.2 Final installation

To install the package `procars`, and all its dependances, just do:

```
sudo pip install .
```

or:

```
sudo python setup.py install
```

You will then be able to use ProCARs from any directory in your computer, just as any other software.

Warning: This must be done only if you downloaded a stable version of the package, and won't do any more changes on the scripts and modules. Indeed, by installing the package, the changes done after won't be taken into account while running the scripts. If you plan to work on the scripts, choose the deployment installation (see above).

Note: You can always *uninstall ProCARs* if you want to install a new version, or work on the scripts.

2.2 Uninstalling ProCARs

Whatever the way you installed ProCARs, you can uninstall it by running:

```
sudo pip uninstall procars
```

2.3 Running ProCARs

When you have installed ProCARs (in final or development mode, see here-above for more information) you can run it from anywhere in your computer, by calling the following command:

```
procars_main -t tree_file -b block_file -r result_directory
```

where *tree_file* and *block_file* are the paths to your input files, for the phylogeny and the orthology blocks respectively. *result_directory* is the directory in which you want to save all ProCARs results.

If you want to work on the ProCARs code, and do not want to install the package, you must run ProCARs from its root directory (same directory as `procars`, `bin`, `setup.py` etc.), and use the following command:

```
PYTHONPATH+=. python bin/procars_main -t tree_file -b block_file -r result_directory
```

2.4 ProCARs file formats

The input files are :

- a phylogeny tree, with Newick format, and with a @ at the required ancestor node
- a block file, containing all blocks position and orientation for all extant genomes of the tree

You also have to specify a directory where you want to store all results from ProCARs run. If the specified directory does not already exist, it will be created.

Note: If you run ProCARs twice on the same result directory, it will erase the last results and replace them by the new ones.

The output is a Q-tree, corresponding to the ancestor's reconstructed genome, composed of CARs.

2.4.1 Input files

You can find examples of those input files into the `Examples` directory of ProCARs.

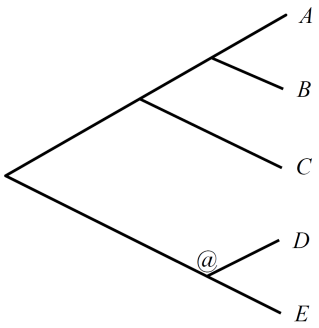
Phylogenic tree

The phylogenic tree is at the *Newick tree format*. You only need to give the phylogeny, with all extant species names, but no branch lengths are needed. You also have to tag an ancestor node with a @ to specify which ancestor's genome you want ProCARs to reconstruct. The ancestor must be a node splitting the tree into three subtrees: two ingroups and one outgroup.

Here is an example of a species tree input:

```
((C,(A,B)),(D,E)@);
```

corresponding to this tree:



Note: If your extant genomes have long names with several words, separate the words with _ instead of a space.

Orthology blocks

The orthology blocks have the following format:

```
>*block_number*
*species1_name*.*chromosome_name*:*start*-*end* *orientation*
*species2_name*.*chromosome_name*:*start*-*end* *orientation*
*species3_name*.*chromosome_name*:*start*-*end* *orientation*
*species4_name*.*chromosome_name*:*start*-*end* *orientation*

>*block_number*
*species1_name*.*chromosome_name*:*start*-*end* *orientation*
*species2_name*.*chromosome_name*:*start*-*end* *orientation*
*species3_name*.*chromosome_name*:*start*-*end* *orientation*
*species4_name*.*chromosome_name*:*start*-*end* *orientation*
```

where *start* and *end* are the nucleotide positions of the current block on the chromosome containing it, and *orientation* is either + or − according to the orientation of the block on the species chromosome. For example, the following could be the beginning of an orthology block input file for the tree in the previous example:

```
>1
A.1:1-99 +
B.1:1-99 +
C.1:1-99 +
D.1:1-99 +
E.1:1-99 +

>2
A.1:101-199 +
B.1:101-199 +
C.1:101-199 +
D.1:101-199 -
E.1:101-199 +
```

2.4.2 Output files

The output is a Q-tree, corresponding to the ancestor's reconstructed genome, composed of CARs. Each CAR is an ordered list of signed blocks. The format of a Q-tree is the following:

```
#CAR1
_Q block1 block2 block3 block4 ... Q_
#CAR2
_Q block1 block2 block3 block4 ... Q_
#CAR3
_Q block1 block2 block3 block4 ... Q_
```

where each block is a signed integer, indicating its orientation. For example:

```
#CAR1
_Q 1 2 3 -5 Q_
#CAR2
_Q -7 -6 4 Q_
#CAR3
_Q 8 Q_
```

In your result directory, you will also find a subfolder called `procars_steps`. In this directory, you can find all intermediate ancestor trees (*S_PQtree_ancestor_* + step number of ProCARs in which the Q-tree was computed), with the same format as the output file. You will also find files describing at which step each adjacency was added, and its type (*S_Adjacencies_ancestor...* + step number of ProCARs at which the adjacency file was created). The adjacency files have the following format:

```
block1_adj1 block2_adj1 car1_adj1 car2_adj1 type_adj1 step_adj1 labels_adj1
block1_adj2 block2_adj2 car1_adj2 car2_adj2 type_adj2 step_adj2 labels_adj2
block1_adj3 block2_adj3 car1_adj3 car2_adj3 type_adj3 step_adj3 labels_adj3
```

where *block1 block2* is the block adjacency (the two blocks involved in the adjacency added to the Q-tree) and *car1 car2* is the car adjacency corresponding to the block adjacency. *type_adj* is the type of the given adjacency. It is either `1` for a fully conserved adjacency, `0` for a partly conserved adjacency, or `2` for a DCJ-reliable adjacency. Finally, *labels_adj* are indicating if the given adjacency is present (`1`) or absent (`0`) in each of the extant genomes. Hence, there are as many labels as extant genomes. Here is an example of an adjacency file, for the input tree example (5 extant genomes):

```
6 7 6 7 0 1 1 1 1 1 1
1 2 1 2 1 1 1 1 1 0 1
2 3 2 3 1 1 1 1 1 0 1
3 -5 3 -5 1 1 0 0 0 1 1
-4 6 -4 6 1 1 0 0 0 1 1
```

You will finally find other adjacency files, with a suffix `_discarded.txt`, or `_discarded_used.txt`. The first one corresponds to the conflicting adjacencies saved by a step a) of ProCARs. If, in the same step, the set of non-conflicting adjacencies is empty, ProCARs will go to the step b) (resolve conflicts) and hence use this file. At the end of this step b), the file suffixed by `_discarded.txt` is empty (conflicts are resolved), but its content is saved to the file suffixed by `_discarded_used.txt`, if you need to check which adjacencies were involved in a resolved conflict. All these adjacency files have the same format, described here-above.

2.5 Running tests

If you want to work on ProCARs scripts, you can use the tests provided with the software, used to check each of its functionalities. To run the tests, run, from the root of the project:

```
PYTHONPATH+=. py.test test/
```

Or, if you installed the package (final or development mode):

```
py.test test/
```

CHAPTER

THREE

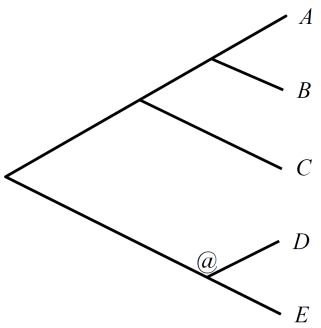
EXAMPLES

The `Examples` directory contains examples of data sets on which ProCARs can be run. You will find two different examples: a fictive data set, and a real data set.

3.1 Fictive_data

3.1.1 Data set description

This data set is composed of 5 genomes (A, B, C, D and E) with 8 synteny blocks. It corresponds to the following phylogeny, with the ancestor to reconstruct marked with an `@`:



You will find in this directory a species tree called `tree.ph` (corresponding to the tree here-above), and a block file called `orthology_blocks.txt`. This data set corresponds to the example given in the paper's method part.

3.1.2 Run data set

To run this data set from the *Examples* directory (if you previously installed ProCARs):

```
procars_main --tf=Fictive_data/tree.ph
--bf=Fictive_data/orthology_blocks.txt
-r Fictive_data/results
```

If you did not install ProCARs, run:

```
PYTHONPATH+=.. python ../bin/procars_main --tf=Fictive_data/tree.ph
--bf=Fictive_data/orthology_blocks.txt
-r Fictive_data/results
```

3.1.3 Results

With this dataset, ProCARs runs in 3 steps:

- **step1** founds:
  - a set of 1 fully conserved and 4 partly conserved non-conflicting adjacencies, which are added to the PQtree: see `Fictive_data/results/procars_steps/S_Adjacencies_ancestor_1.txt` and `Fictive_data/results/procars_steps/S_PQtree_ancestor_1.txt`.
  - a set of 2 discarded adjacencies: see `Fictive_data/results/procars_steps/S_Adjacencies_ancestor_1.txt_discarded.txt`
- **step2** founds:
  - an empty set of non-conflicting adjacencies
  - a set of 2 discarded adjacencies (the sames as in step1): see `Fictive_data/results/procars_steps/S_Adjacencies_ancestor_2.txt_discarded_used.txt`
  - Hence, it **runs step b)** (resolve conflicts), using the set of discarded adjacencies, and retains 1 adjacency, giving the PQtree `Fictive_data/results/procars_steps/S_PQtree_ancestor_2.txt`
- **step3** founds:
  - empty sets of non-conflicting and conflicting adjacencies
  - Hence, it runs step c) (find DCJ-reliable adjacencies), but does not find any adjacency to add.

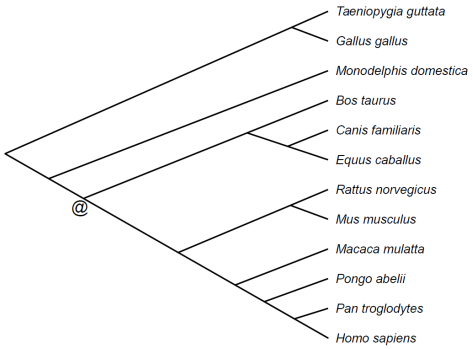
The output file is hence `Fictive_data/results/ProCars_PQtree.txt`, which is the same as `Fictive_data/results/procars_steps/S_PQtree_ancestor_2.txt`. The ancestor genome contains 2 CARs with 4 blocks each.

3.2 Boreoeutherian_ancestor

3.2.1 Data set description

This data set is used to compute a set of CARs for the boreoeutherian ancestral genome using the gene orders of twelve amniote genomes.

We chose those twelve genomes assembled and present in a Pecan multiple alignement of 20 amniote genomes available in the release 73 of the Ensembl Compara database. The phylogenetic tree (*tree_boreoeutherian_ancestor.ph*) was obtained from the National Center for Biotechnology Information taxonomy, and is the following:



We constructed a set of synteny blocks (*orthology_blocks.txt*) using the multiple alignements as seeds. This resulted in a set of 12 genomes made of 689 blocks for species *Homo sapiens*, *Pan troglodytes*, *Pongo abelii*, *Macaca mulatta*,

Mus musculus, *Rattus norvegicus*, *Equus caballus*, *Canis familiaris*, *Bos taurus*, *Monodelphis domestica*, *Gallus gallus* and *Taeniopygia guttata*.

3.2.2 Run data set

To run this data set from the *Examples* directory (if you previously installed ProCARs):

```
procars_main --tf=Boreoeutherian_ancestor/tree_boreoeutherian_ancestor.ph
 --bf=Boreoeutherian_ancestor/orthology_blocks.txt
 -r Boreoeutherian_ancestor/results
```

If you did not install ProCARs, run:

```
PYTHONPATH+=.. python ../bin/procars_main
--tf=Boreoeutherian_ancestor/tree_boreoeutherian_ancestor.ph
 --bf=Boreoeutherian_ancestor/orthology_blocks.txt
 -r Boreoeutherian_ancestor/results
```

3.2.3 Results

With this dataset, ProCARs runs in 5 steps:

- **step1**:
  - a set of 498 fully conserved and 149 partly conserved non-conflicting adjacencies which are added to the PQtree: see `Boreoeutherian_ancestor/results/procars_steps/S_Adjacencies_ancestor_1.txt` and `Boreoeutherian_ancestor/results/procars_steps/S_PQtree_ancestor_1.txt`.
  - a set of 11 discarded conflicting adjacencies: see `Boreoeutherian_ancestor/results/procars_steps/S_Adjacencies_ancestor_1.txt_discarded.txt`
- **step2**:
  - a set of 10 partly conserved non-conflicting adjacencies which are added to the PQtree: see `Boreoeutherian_ancestor/results/procars_steps/S_Adjacencies_ancestor_2.txt` and `Boreoeutherian_ancestor/results/procars_steps/S_PQtree_ancestor_2.txt`.
  - a set of 6 discarded conflicting adjacencies: see `Boreoeutherian_ancestor/results/procars_steps/S_Adjacencies_ancestor_2.txt_discarded.txt`
- **step3**:
  - an empty set of non-conflicting adjacencies.
  - a set of 6 discarded conflicting adjacencies: see `Boreoeutherian_ancestor/results/procars_steps/S_Adjacencies_ancestor_3.txt_discarded_used.txt`
  - **runs step b)** (resolve conflicts), and retains 3 adjacencies, added to the PQtree: see `Boreoeutherian_ancestor/results/procars_steps/S_Adjacencies_ancestor_3.txt` and `Boreoeutherian_ancestor/results/procars_steps/S_PQtree_ancestor_3.txt`.
- **step4**:
  - an empty set of non-conflicting adjacencies.
  - an empty set of conflicting adjacencies.

  - **runs step c)** (Find DCJ-reliable adjacencies), and retains 3 DCJ-reliable adjacencies, added to the PQtree: see `Boreoeutherian_ancestor/results/procars_steps/S_Adjacencies_ancestor_4.txt` and `Boreoeutherian_ancestor/results/procars_steps/S_PQtree_ancestor_4.txt`.

- **step5**:
  - a set of 2 partly conserved non-conflicting adjacencies which are added to the PQtree: see `Boreoeutherian_ancestor/results/procars_steps/S_Adjacencies_ancestor_5.txt` and `Boreoeutherian_ancestor/results/procars_steps/S_PQtree_ancestor_5.txt`.
  - an empty set of conflicting adjacencies.

In step6, all sets of adjacencies are empty, and no DCJ-reliable adjacency is found: the algorithm stops. The output is `Boreoeutherian_ancestor/results/ProCars_PQtree.txt`, which is the same as (`Boreoeutherian_ancestor/results/procars_steps/S_Adjacencies_ancestor_5.txt`). ProCARs found 25 CARs for this Boreoeutherian ancestor's genome, with a number of blocks per ACR ranging from 2 to 68.

CHAPTER

FOUR

ABOUT PROCARS

The ProCARs program is available at http://bioinfo.lifl.fr/procars.

If you use ProCARs, please cite:

Amandine Perrin, Jean-Stéphane Varré, Samuel Blanquart and Aïda Ouangraoua, "ProCARs: Progressive Reconstruction of Ancestral Gene Orders", 2014, submitted to BMC genomics (ISCB-LA)

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