WGD_Tracker

Summary

Introduction

WGD_Tracker is a tool designed to facilitate genome comparisons and detect and date whole genome duplication events. This tool is fully customizable, allowing users to select stringent or flexible parameters depending on their specific analytical objectives.

This tool comprises four pipelines:

1) **RBBH Pipeline** identifies homologous gene pairs via reciprocal BLAST best hit (RBBH) analysis on a BLAST output file. However, polyploid genomes can contain multiple copies of the same genes, so WGD_Tracker can also search for reciprocal blast best hits (RBH) and not just the best hits in order to highlight all duplicated gene copies.
2) **Ks Pipeline** allows the calculation of the synonymous substitution rate between gene pairs.
3) **Synteny Pipeline** accurately identifies synteny blocks even when they are fragmented.
4) **Dotplot Pipeline** generates graphical plots.
5) **Karyotype Pipeline** generates graphical representation of the syntenic blocks.

Installation

WGD_Tracker does not require any installation, a simple git clone is enough:

```
$ git clone https://github.com/MorganeMilin/WGD_Tracker.git
```

However, WGD_Tracker does **require dependencies**: Python3, Java, PAML, MACSE, R, Singularity and Parallel. However, if you only want to use some of the tool's pipelines, not all dependencies are necessary for them to work properly. The following table shows the dependencies required for each pipeline:

	RBBH	Ks	Synteny	Dotplot	Karyotype
Python 3	X	X	X	X	X
Java		X			
PAML		X			
MACSE		X			
R		X			
Singularity		X			
Parallel	X	X			

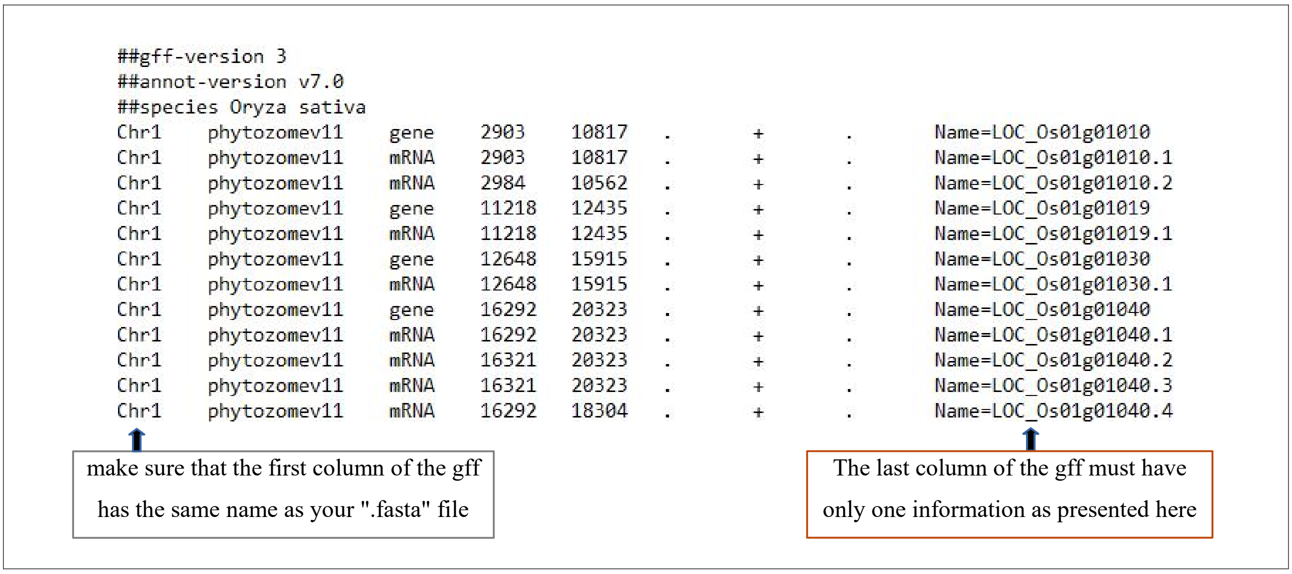
Please find the recommended dependencies version, which was used when developing the tool: Python v3.9, Parallel 20190122, Java v1.8.0, PAML v4.9, MACSE v2.05, R v4.1.0, Singularity v3.8.0

Organizing folders and files

The working directory must contain the configuration file (**file.config**) and a folder (*e.g.* SP1_vs_SP2) containing all data files. The folder name must be specified in the configuration file (*e.g.* **data_dir**="Sp1_vs_Sp2"). Unlike the configuration file and the folder, which can be named as you wish, the data files must be named in a very specific way. In the comparison file, you need to specify the names of your compared genomes (*e.g.* **SP1**="Osativa_cds"; **SP2**="Sbicolor_cds"; first the short species name (*e.g.* "Osativa" and not "Oryza_sativa") and second the data type: cds, genomic or transcript). You'll need to use the same names for your data files. Thus, for each species, your different data files will be distinguished only by the extension.

The extensions to use are:

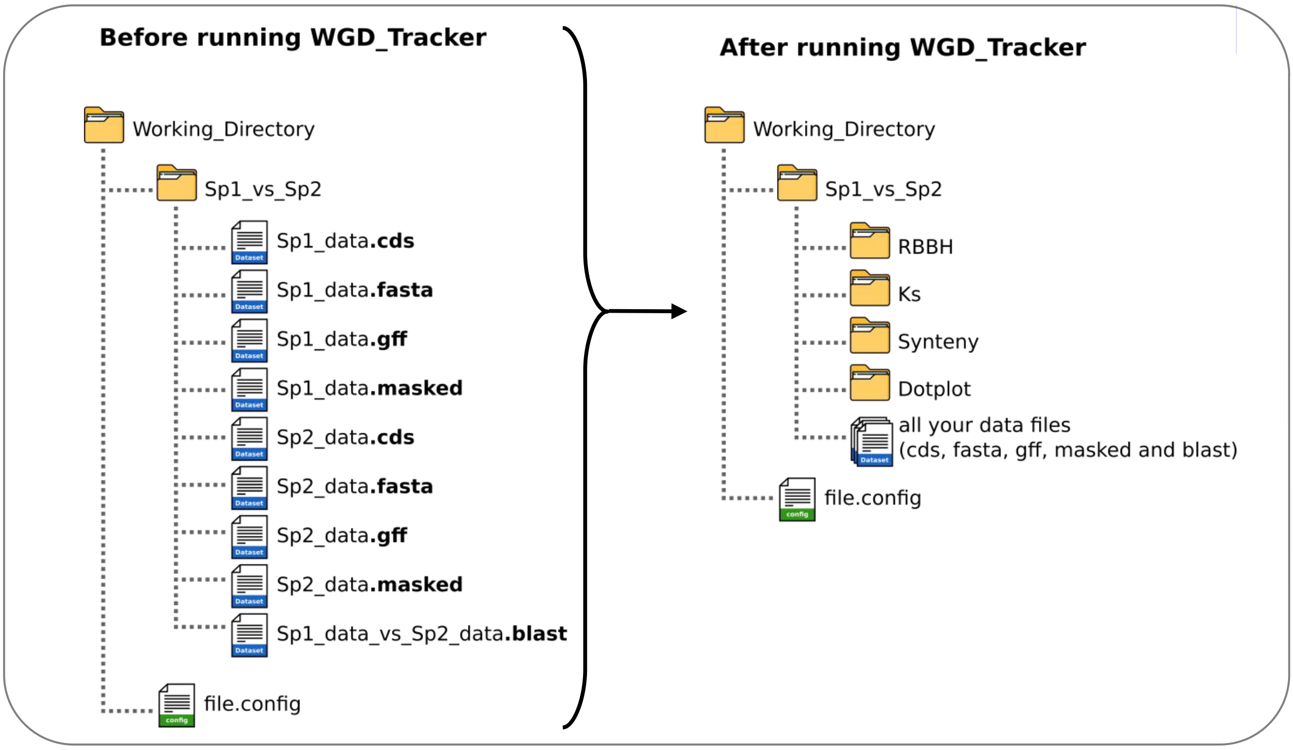
- **.fasta** for your genome assembly in fasta format
- **.cds** for your fasta file containing all your cds sequences (check for transcript)
- **.masked** for your masked genome assembly
- **.gff** for your gff3 files (note that some formatting is required, see below)
- **.blast** for the output file from the BLAST analysis



```
##gff-version 3
##annot-version v7.0
##species Oryza sativa
Chr1    phytozomev11    gene    2903    10817   .       +       .       Name=LOC_Os01g01010
Chr1    phytozomev11    mRNA    2903    10817   .       +       .       Name=LOC_Os01g01010.1
Chr1    phytozomev11    mRNA    2984    10562   .       +       .       Name=LOC_Os01g01010.2
Chr1    phytozomev11    gene    11218   12435   .       +       .       Name=LOC_Os01g01019
Chr1    phytozomev11    mRNA    11218   12435   .       +       .       Name=LOC_Os01g01019.1
Chr1    phytozomev11    gene    12648   15915   .       +       .       Name=LOC_Os01g01030
Chr1    phytozomev11    mRNA    12648   15915   .       +       .       Name=LOC_Os01g01030.1
Chr1    phytozomev11    gene    16292   20323   .       +       .       Name=LOC_Os01g01040
Chr1    phytozomev11    mRNA    16292   20323   .       +       .       Name=LOC_Os01g01040.1
Chr1    phytozomev11    mRNA    16321   20323   .       +       .       Name=LOC_Os01g01040.2
Chr1    phytozomev11    mRNA    16321   20323   .       +       .       Name=LOC_Os01g01040.3
Chr1    phytozomev11    mRNA    16292   18304   .       +       .       Name=LOC_Os01g01040.4
```

make sure that the first column of the gff has the same name as your ".fasta" file

The last column of the gff must have only one information as presented here



Once the analyses are complete, you will find the results in the same folder that contains your data files. Each pipeline creates a specific folder containing all output files.

Usage

To run the RBBH Pipeline:

```
$ sbatch --cpus-per-task=2 ./WGD_Tracker/RBBH_Pipeline.txt ./file.config
```

To run the Ks Pipeline:

```
$ sbatch --cpus-per-task=20 ./WGD_Tracker/Ks_Pipeline.txt ./file.config
```

To run the Synteny Pipeline:

```
$ sbatch ./WGD_Tracker/Synteny_Pipeline.txt ./file.config
```

To run the Dotplot Pipeline:

```
$ sbatch ./WGD_Tracker/Dotplot_Pipeline.txt ./file.config
```

To run the Karyotype Pipeline:

```
$ sbatch ./WGD_Tracker/Karyotype_Pipeline.txt ./file.config
```

RBBH Pipeline

Inputs

Several input files must be provided for the RBBH pipeline to run properly:

1. The ".fasta" and/or ".cds" file(s) used to generate the BLAST analysis
2. The BLAST output file (tabular output format 6 without header, **"-outfmt 6"**)
3. Optional - The masked fasta file, if you want to remove hits located in repeated regions
4. The gff file(s), properly formatted as described on page 2
5. The configuration file

Configuration file

It is important to fill in the configuration file according to your dataset and goals. Only a few parameters need to be specified in the configuration file (***i.e.*** **data_dir, tool_dir, Nb_CPU, intragenomic, SP1 and SP2**), the other parameters do not need to be specified unless you want to use a different setting than the one defined by default.

data_dir

Mandatory
String expected
The absolute path to the Working Directory containing all data files (".fasta", ".gff", ".blast", etc.)

tool_dir

Mandatory
String expected
The absolute path to the WGD_Tracker tool folder

Nb_CPU

Mandatory
Integer expected (default: 2)
The number of cores to use for the analysis. Note that the value specified in the configuration file must be the same as the value specified in the sbatch line of code that executes the pipeline

intragenomic

Mandatory
Boolean expected: "True" or "False"
Specify whether this is an intragenomic (*e.g.* "True") or intergenomic (*e.g.* "False") analysis

SP1 and **SP2**

Mandatory
String expected
The name(s) of your compared genome(s) must be specified here (*e.g.* **SP1**="Osativa_cds"; **SP2**="Sbicolor_cds"; first the short species name (*e.g.* "Osativa" and not "Oryza_sativa") and second the data type: cds, genomic or

transcript). You'll need to use the same names for your data files. Thus, for each species, your different data files will be distinguished only by the extension

identity

Optional

Integer expected (default: 70)

Minimum identity (in %) value that will be kept in the dataset

len_align

Optional

Integer expected (default: 60)

Minimum alignment length (in nucleotide) that will be kept in the dataset

len_ratio

Optional

List of two values expected (default: not used)

- 1st value corresponds to the minimum coverage percentage requested (integer expected: within 0 and 100)
- 2nd value corresponds to the number of CDS and/or transcript files used (string expected: “single” or “double”)

Example:

Genomic Comparisons	Settings that must be used
Species1 CDS *versus* Species2 Genomic	len_ratio="[%coverage, 'simple']"
Species1 CDS *versus* Species2 Transcript	len_ratio="[%coverage, 'double']"
Species1 CDS *versus* Species2 CDS	len_ratio="[%coverage, 'double']"
Species1 CDS *versus* Species1 CDS (*i.e.* intragenomic)	len_ratio="[%coverage, 'double']"

Filter the dataset based on blast alignment coverage (only if at least one dataset used in BLAST are CDS and/or Transcripts)

corr_intra

Optional

List of two values expected (default: not used)

- 1st value corresponds to a pattern to target (*e.g.* “.” or “-“)
- 2nd value corresponds to the number of elements to be deleted

Example:

Sequence name	Settings	Results
sequence1.1 sequence1.2 sequence1.3	corr_intra="['.', -1]"	sequence1 sequence1 sequence1
sequence1.1.2 sequence1.1.25 sequence1.1.256	corr_intra="['.', -2]"	sequence1 sequence1 sequence1
sequence1-1 sequence1-2 sequence1-1.2 sequence1-1-2	corr_intra="['-', -1]"	sequence1 sequence1 sequence1 sequence1-1

Useful for intragenomic analysis only. Multiple conformations can be found for a given sequence (*e.g.* LOC_Os01g01010.1, LOC_Os01g01010.2, etc). This parameter ensures that alignments against themselves are removed, including alignments of identical sequences with different conformations

coding

Optional

Boolean expected: “True” or “False” (default: False)

coding=“True” allows to keep only alignments located in coding region (only if dataset used in BLAST are genome assembly)

coding_type

Optional

String expected: “simple” or “double” (default: not used)

Specify how many genomes you wish to filter

SP1_coding_infos and SP2_coding_infos

Optional

List of three values expected (default: not used)

1st value corresponds to the Species name (*e.g.* Osativa)

2nd value corresponds to the targeted motif of the third column in the gff file (*e.g.* “gene”, “mRNA”)

3rd value corresponds to the minimum length (in nucleotides) necessary to confirm that the alignment is in a coding region

Example:

Genomic Comparisons	Example of the settings that must be used

Species1 Genomic *versus* Species2 CDS	coding="True"
	coding_type="simple"
	SP1_coding_infos="['Species1','mRNA',60]"

Species1 Genomic *versus* Species2 Transcript	

Species1 Genomic *versus* Species2 Genomic	coding="True"
	coding_type="double"
	SP1_coding_infos="['Species1','mRNA',60]"
	SP2_coding_infos="['Species2','mRNA',60]"
	
	If you only want to check if Species2 alignment are in coding region:
	coding="True"
	coding_type="simple"
	SP1_coding_infos="['Species2','mRNA',60]"
	
	If Intragenomic:
	intragenomic="True"
	coding="True"
	coding_type="double"
	SP1_coding_infos="['Species1','mRNA',60]"

TErm

Optional

Boolean expected: “True” or “False” (default: False)

TErm=“True” allows to remove alignments present in regions containing repeated elements

TErm_type

Optional

String expected: “simple” or “double” (default: not used)

Specify how many genomes you wish to filter

SP1_TErm and SP2_TErm

Optional

List of three values expected (default: not used)

1st value corresponds to the Species name (*e.g.* Osativa)

2nd value corresponds to the targeted motif of the third column in the gff file (*e.g.* “gene”, “mRNA”)
3rd value corresponds to the percentage of repeat authorized in the alignment

Example:

Genomic Comparisons	Example of the settings that must be used

if you want to check both Species1 and Species2 alignment	TErm="True"
	TErm_type="double"
	SP1_TErm="['Species1','mRNA',25]"
	SP2_TErm="['Species2','mRNA',25]"

if you only want to check Species1 alignment	TErm="True"
	TErm_type="simple"
	SP1_TErm="['Species1','mRNA',25]"

if you only want to check Species2 alignment	TErm="True"
	TErm_type="simple"
	SP1_TErm="['Species2','mRNA',25]"

if intragenomic analysis	intragenomic="True"
	TErm="True"
	TErm_type="double"
	SP1_TErm="['Species1','mRNA',25]"

If the dataset is a genome assembly, the percentage of repeats on the alignment is checked, whereas if the dataset is a CDS sequences, the percentage of repeats on the entire CDS sequence is verified and not just the part of the sequence aligned by blast

BH_limit

Optional
Integer expected (default: 1)
Number of best hit to keep for (1) each CDS or Transcript (if CDS and/or Transcript used in BLAST) or (2) for each non-overlapping windows (if genome assembly in BLAST)

interval

Optional
Integer expected (default: 50000)
Non-overlapping windows size (in nucleotides) to be used to analyzed each chromosome. Required only if the analysis was done on a genomic assembly

Outputs

The output files of this pipeline will be located in the RBBH folder:

- Several output files from filtration steps
    - pFlt_*.txt is the filter output file for identity and alignment length values
    - cds_*.txt and non_coding_*.txt is the output file separating hits found in coding and non-coding regions
    - TErm_*.txt corresponds to the output file after deletion of hits found in repeated regions
- **BH_*.txt** corresponds to the output file retrieving the best hit(s) for each sequence
- **RBBH_*.txt** is the final output file providing the Reciprocal Blast Hits

Output file(s) format is the same as BLAST output, plus a few columns in BH_*.txt and RBBH_*.txt file(s):

1. query or source (gene) sequence id
2. subject or target (reference genome) sequence id
3. percentage of identical positions
4. alignment length (sequence overlap)
5. number of mismatches
6. number of gap openings
7. start of alignment in query
8. end of alignment in query
9. start alignment in subject
10. end alignment in subject
11. expect value
12. bit score
13. best hit number

If the dataset used are genome assemblies:

14. length of the reciprocal alignment in the query sequence
15. start of the reciprocal alignment in the query sequence
16. end of the reciprocal alignment in the query sequence
17. length of the reciprocal alignment in the subject sequence
18. start of the reciprocal alignment in the subject sequence
19. end of the reciprocal alignment in the subject sequence

Ks Pipeline

Inputs

Several input files must be provided for the RBBH pipeline to run properly:

1. The “.cds” file(s)
2. The RBBH output file (the format is the same as a BLAST output file)
3. The configuration file

Configuration file

It's important to mention that the Ks computation step generates many small files, which can saturate the server and cause the analysis to fail. However, most of these files are not essential, so we divide all fasta files into different folders. For example, we'll set the maximum number of folders to analyze to 10,000 and the maximum number of fasta files per folder to 2,000. The folders are then analyzed one by one, and once a folder is analyzed, the Ka, Ks and ratio values are retrieved and the folder is zipped to limit the risk of server saturation. However, since this Ks calculation step can be parallelized, it's advisable to create several analysis folders with a smaller number of fasta files, so that you can analyze a larger number of folders in parallel, reducing the analysis time without running the risk of saturating your server.

data_dir

Mandatory
String expected
The absolute path to the Working Directory containing all data files (“.fasta”, “.gff”, “.blast”, etc.)

tool_dir

Mandatory
String expected
The absolute path to the WGD_Tracker tool folder

Nb_CPU

Mandatory
Integer expected (default: 2)
The number of cores to use for the analysis. Note that the value specified in the configuration file must be the same as the value specified in the sbatch line of code that executes the pipeline.
It is recommended that you take advantage of the parallelization of this pipeline to reduce the analysis time. To do so, simply increase this parameter according to the size of your dataset and the performance of your computer

intragenomic

Mandatory

Boolean expected: “True” or “False”

Specify whether this is an intragenomic (*e.g.* “True”) or intergenomic (*e.g.* “False”) analysis

SP1 and SP2

Mandatory

String expected

The name(s) of your compared genome(s) must be specified here (*e.g.* **SP1**="Osativa_cds"; **SP2**="Sbicolor_cds"; first the short species name (*e.g.* "Osativa" and not "Oryza_sativa") and second the data type: cds, genomic or transcript). You'll need to use the same names for your data files. Thus, for each species, your different data files will be distinguished only by the extension

Ks_begin

Optional

String expected (default: "fasta_Extract")

Specify the analysis step to start with. Either step 1 **"fasta_Extract",** which generates all fasta files to be analyzed; or step 2 **"Ks_calculation"**, which calculates the Ka and Ks for each pair of genes, or the last step **"Ks_distribution"**, which generates a Ks distribution and estimate the mode of the peak(s).

Ks_folder_limit

Optional

Integer expected (default: 10000)

Maximum number of folders

Ks_file_limit

Optional

Integer expected (default: 2000)

Number of gene pair to analyze per folder

mxt_ksmin

Optional

Float or integer expected (default: 0.01)

Minimum Ks value kept for the analysis. Parameter needed to generate a graphical representation of the data and estimate the mode of the peak(s)

mxt_ksmax

Optional

Float or integer expected (default: 3)

Maximum Ks value kept for the analysis. Parameter needed to generate a graphical representation of the data and estimate the mode of the peak(s)

mxt_kmin

Optional

Integer expected (default: 2)

Minimum number of peaks expected. Parameter needed to generate a graphical representation of the data and estimate the mode of the peak(s)

mxt_kmax

Optional

Integer expected (default: 4)

Maximum number of peaks expected (Warning: analysis time increases with k). Parameter needed to generate a graphical representation of the data and estimate the mode of the peak(s)

mxt_boots

Optional

Integer expected (default: 1000)

Bootstrapping effort during search for optimal number of peaks. (Warning: this is time consuming. Recommended value is 1000). Parameter needed to generate a graphical representation of the data and estimate the mode of the peak(s)

mxt_epsilon

Optional

Integer expected (default: 1e-3)

Convergence criterion; heuristics are stopped when loglik is improved by less than epsilon. Parameter needed to generate a graphical representation of the data and estimate the mode of the peak(s)

mxt_breaks

Optional

Integer expected (default: 300)

Number of breaks on the histogram. Parameter needed to generate a graphical representation of the data and estimate the mode of the peak(s).

Outputs

The output files of this pipeline will be located in the Ks folder:

- **Res_compil_NG_Ks_total.txt** - Output file containing the Ka, Ks and ratio values for each pair of genes compared.
- **Ks_distribution_NG.pdf** - Distribution of the Ks values and estimates the mode of the peak(s) using the R mixtools package.
- **Ks_distribution_NG_log_transformed.pdf** - Distribution of the Ks values (with a dataset logarithmic transformation) and estimates the mode of the peak(s) using the R mixtools package.
- **Ks_distribution_NG_sqrt_transformed.pdf** - Distribution of the Ks values (with a dataset square root transformation) and estimates the mode of the peak(s) using the R mixtools package.

Res_compil_NG_Ks_total.txt format:

1. gene1 - gene name used in the gene pair comparison
2. gene2 - second gene name used in the gene pair comparison
3. Ka - number of non-synonymous substitutions (altering) per non-synonymous site
4. Ks - number of synonymous substitutions per synonymous site
5. Ka/Ks - ratio used to assess selection pressure on coding regions

Synteny Pipeline

Inputs

Several input files must be provided for the RBBH pipeline to run properly:

1. The gff file(s), properly formatted as described on page 2
2. The configuration file

Configuration file

data_dir

Mandatory
String expected
The absolute path to the Working Directory containing all data files (".fasta", ".gff", ".blast", etc.)

tool_dir

Mandatory
String expected
The absolute path to the WGD_Tracker tool folder

intragenomic

Mandatory
Boolean expected: "True" or "False"
Specify whether this is an intragenomic (*e.g.* "True") or intergenomic (*e.g.* "False") analysis

SP1 and **SP2**

Mandatory
String expected
The name(s) of your compared genome(s) must be specified here (*e.g.* **SP1**="Osativa_cds"; **SP2**="Sbicolor_cds"; first the short species name (*e.g.* "Osativa" and not "Oryza_sativa") and second the data type: cds, genomic or transcript). You'll need to use the same names for your data files. Thus, for each species, your different data files will be distinguished only by the extension

sp1_motif and **sp2_motif**

Mandatory
For each species specify the targeted motif of the third column in the gff file (*e.g.* "gene", "mRNA")

corr_SB

Optional
Integer expected (default: 100)
Maximum number of genes with no hits tolerated between two consecutive hits of the same syntenic block

gap

Optional
Integer expected (default: 100)
Maximum number of genes with no hits tolerated between two consecutive hits of the same syntenic block

gene_nb

Optional
Integer expected (default: 5)

Minimum number of hits required to define a syntenic block

Ks_min

Optional
Float or integer expected (default: 0.01)
Minimum Ks value kept for the analysis. Parameter needed to generate a graphical representation of the data and estimate the mode of the peak(s)

Ks_max

Optional
Float or integer expected (default: 3)
Maximum Ks value kept for the analysis. Parameter needed to generate a graphical representation of the data and estimate the mode of the peak(s)

Outputs

The output files of this pipeline will be located in the Synteny folder. There are several output files corresponding to intermediate steps. There are two important files to keep, which correspond to syntenic hit results, presented in two different formats:

Res_compil_NG_Syntenic_blocks_STEP_3.txt format:

1. gene1 - gene name used in the gene pair comparison
2. gene2 - second gene name used in the gene pair comparison
3. Ka - number of non-synonymous substitutions (altering) per non-synonymous site
4. Ks - number of synonymous substitutions per synonymous site
5. Ka/Ks - ratio used to assess selection pressure on coding regions

Syntenic_blocks_STEP_3.txt format:

1. Reference id
2. Species1 Chromosome
3. Species2 Chromosome
4. Syntenic Block number
5. orientation
6. gene number in the block
7. Species1 gene rank list
8. Species1 gene name list
9. Species2 gene rank list
10. Species2 gene name list
11. Ks