

# Introduction

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## What is batch\_brb?

batch\_brb is a command-line application for automated best reciprocal BLAST and phylogenetic analysis using FastTree. Common ortholog identification methods can have organism availability limitations and difficulty identifying divergent sequences. These issues can be overcome with manual searching but this is a time consuming and tedious process making it unsuitable for moderate to larger scale analyses. batch\_brb provides tools to automate the data collection process while maintaining flexibility in hit selection criteria, enabling the analysis of greater numbers of sequences.

batch\_brb performs ortholog identification using best reciprocal BLAST. Sequences of interest are searched against a user created database. The top x hits per query per organism are extracted and filtered by y coverage of the query where x and y are specified by the user. Identical hits do not contribute to the hit count. These hits are searched against the organism of the original query sequences. The top x hits per query are filtered by y query coverage as above. Where the hits from the first and reverse BLAST match, the sequences are considered orthologs. batch\_brb is designed to enable maximum coverage and requires user analysis of hits for the exclusion of mishits and paralogs.

## Typical workflow

- Create BLAST database - batch\_makeblastdb
- Create alias database - aliasdb\_pipeline
- Retrieve accessions - accession\_retrieve
- Ortholog identification - orthology\_pipeline
- Genome walk - orthology\_pipeline, merge\_results
- Build phylogenetic trees - fasttree\_pipeline

## User support

To report issues please use the [GitHub issue tracker](#), for general enquiries please contact [ebutterfield@dundee.ac.uk](mailto:ebutterfield@dundee.ac.uk)

## Citing batch\_brb

A protocol publication describing batch\_brb is currently in preparation. In the meantime please cite the [GitHub repository](#). Please also cite the dependency publications.

## Acknowledgements

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## License

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## How to use batch\_brb

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### Installation

batch\_brb is available as a Bioconda package and requires [Conda](#) and [Bioconda](#). On Linux/Unix-like operating system:

```
conda create -n batch_brb batch_brb
conda activate batch_brb
```

Change into the directory where batch\_brb should be located and run:

```
batch_brb_setup
```

### Dependencies

batch\_brb has several dependencies, listed below. These are automatically installed during the batch\_brb installation. If you use batch\_brb please reference the dependencies.

- bash >= 5.0.018
- biopython >= 1.78
- blast >= 2.10.1
- fasttree >= 2.1.10
- muscle >= 3.8.1551
- numpy >= 1.19.2
- pandas >= 1.1.3
- python >= 3.8.6
- sed >= 4.8
- seqkit >= 0.13.2
- sqlite >= 3.33.0

- perl-fast

## Command reference

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### batch\_brb

This command is for the setup of batch\_brb. This will create a batch\_brb directory which will contain four directories: jobs, databases, templates and documentaion. jobs is the directory where jobs results will be located. databases is where organism fasta files (files provided by the user which are retrieved from repositories or sourced in-house) and the resulting databases the user generates will be located. templates contains the template files for the pipelines in both CSV and Excel formats. documentation contains the license and manual for user reference.

### show

This command will show the GPL3 license.

### batch\_makeblastdb

This script will create a BLAST database from a fasta file and add the accessions to a SQLite3 database.

USAGE: batch\_makeblastdb [options]

options:

-h, --help                show brief help  
-csv                      csv file of parameters, required  
                         csv must be in format: INFILE, DB  
                         INFILE: Required, input fasta file  
                         DB: Optional, default = accession\_db.db

This is to be used in conjunction with the 01\_batch\_makeblastdb\_template file located in the templates folder. Templates are available as both Excel files and CSVs.

infile    Name of fasta file to convert to BLAST database  
db        Name of SQLite3 database, OPTIONAL, default = accession\_db.db

File must be saved as a csv, ensure no excess columns included

## aliasdb\_pipeline

This script will generate a BLAST alias database and input the details into a SQLite3 database.

USAGE: aliasdb\_pipeline [options]

options:

-h, --help      show brief help  
-csv            csv file of parameters, required  
                csv must be in format: DBLIST\_FILE, DBTYPE, TITLE, OUTPUT, SQLITE3\_DATABASE  
                The following fields are optional, default values are shown, all other fields are required:  
                SQLITE3\_DB: default = accession\_db.db

This is to be used in conjunction with the 02\_make\_aliasdb\_template file located in the templates folder. Templates are available as both Excel files and CSVs.

dblist_file	Name of file containing list of databases to include in alias database (include file extension), include _database in the database names within the text file (dblist_file)
dbtype	prot or nucl depending on protein or nucleotide respectively
title	Descriptive title for the database – this is not the new database name
output	Name for the alias database
SQLite3_db	Name of SQLite3 database, OPTIONAL, default = accession_db.db

File must be saved as a csv, ensure no excess columns included

## accession\_retrieve

This script will retrieve matching accessions from the SQLite3 database, if they are not found BLAST is performed to enable accession identification by user.

USAGE: accession\_retrieve [options]

options:

-h, --help      show brief help  
-csv            csv file of parameters, required  
                csv must be in format: FASTA\_FILE, JOB\_NAME, BLAST\_DATABASE\_NAME, SQLITE3\_DATABASE, EVALUATE, MAX, NUM\_THREADS

The following fields are optional, default values are shown, all other fields are required:  
 SQLITE3\_DB: SQLite3 database, default = accession\_db.db  
 EVALUE: Expect value for BLAST, default = 0.1  
 MAX: int, maximum number of sequences to retrieve in BLAST, default = 5  
 NUM\_THREADS: int, number of threads to use, default = 4

This is to be used in conjunction with the O3\_accession\_retrieve\_template file located in the templates folder. Templates are available as both Excel files and CSVs.

Fasta_file	Name of the fasta file (include extension)
job_name	Name for the job
BLAST_database_name	Name of the organism database to retrieve sequences from, do not include _database in the name, it will be added automatically
SQLite3_db	Name of SQLite3 database, OPTIONAL, default = accession_db.db
Evalue	Expect value, OPTIONAL, default = 0.1
max	Maximum number of sequences to retrieve from BLAST, OPTIONAL, default = 5
num_threads	Number of cores for BLAST, OPTIONAL, default = 4

File must be saved as a csv, ensure no excess columns included

## orthology\_pipeline

This script will calculate putative orthologs using best reciprocal BLAST with the option to generate phylogenetic trees using FastTree.

USAGE: orthology\_pipeline -csv [options]

options:

-h, --help            show brief help  
 -csv                csv file of parameters, required  
 csv must be in format: JOB\_NAME, ACCESSION\_LIST, FB\_DATABASE, RB\_DATABASE, EVALUE, HITS, COVERAGE, SQLITE3\_DB, NUM\_THREADS, MAX, TREE, FREQUENCY, MODEL  
 The following fields are optional, default values are shown, all other fields are required:  
 SQLITE3\_DB: SQLite3 database, default = accession\_db.db  
 EVALUE: expect value, default = 0.1  
 NUM\_THREADS: int, number of threads to use, default = 4  
 MAX: int, maximum number of sequences to retrieve in BLAST, default = 150  
 TREE: boolean (y/n), perform phylogenetic analysis, default = n

```

FREQUENCY: float, frequency of gaps allowed per residue,
if TREE selected default = 0.25
MODEL: model to use for phylogenetic analysis,
OPTIONS lg or wag for protein or
gtr for nucleotide, if TREE selected default = JTT
for protein and JC for nucleotide

```

This is to be used in conjunction with the 04\_orthology\_pipeline\_form\_template file located in the templates folder. Templates are available as both Excel files and CSVs.

Job_name	Name for the job
Accession_list	Name of the accession list file (include extension). Accessions must be those retrieved with the accession_retrieve pipeline
FB_database	Name of the first BLAST database, include _database in the name if not an alias database, do not include extension for either database type
RB_database	Name of the reverse BLAST database, MUST be a single organism database, do not include _database in the name
Evalue	Expect value, OPTIONAL, default = 0.1
Hits	Number of hits for orthology calculation
Coverage	Percentage coverage of query for orthology calculation
SQLite3_db	Name of SQLite3 database, OPTIONAL, default = accession_db.db
Num_threads	Number of threads, OPTIONAL, default = 4
Max	Maximum number of hits to retrieve for BLAST, OPTIONAL, default = 150
Tree (y/n)	y or n, include fasttree_pipeline, OPTIONAL, default = n
Frequency	float, frequency of gaps allowed per residue, if TREE selected default = 0.25
Model	model to use for phylogenetic analysis, OPTIONS lg or wag for protein or gtr for nucleotide, if TREE selected default = JTT for protein and JC for nucleotide

File must be saved as a csv, ensure no excess columns included

## merge\_results

```

usage: merge_results [-h] in1 in2 outfile

Combine csv results produced by the BLAST pipeline

positional arguments:
  in1          First csv file
  in2          Second csv file

```

```
outfile      Name of output
```

```
optional arguments:
```

```
-h, --help  show this help message and exit
```

## fasttree\_pipeline

This script will generate FastTree phylogenetic trees from input fasta or accession lists specified in text files or orthology results in a CSV.

```
USAGE: fasttree_pipeline [options]
```

```
options:
```

```
-h, --help          show brief help
-db, --database      BLAST database to retrieve sequences from,
                     required for text or csv files
-f, --frequency      OPTIONAL, frequency of gaps allowed per residue,
                     default is 0.25
-csv                OPTIONAL, CSV of ortholog results, the first column
                     must be query accessions with the heading Accession,
                     remaining columns must be the
                     results with one column per organism,
                     the first row should be organism names
-m                  OPTIONAL, model for phylogenetic analysis
                     (choice of lg or wag for protein or gtr
                     for nucleotide), default if not supplied is
                     JTT for protein and JC for nucleotide
```

## delete\_db

This script will delete a BLAST database and remove the corresponding information from the SQLite3 database.

```
USAGE: delete_db [options]
```

```
options:
```

```
-h, --help          show brief help
-csv                csv file of parameters, required
                     csv must be in format: BLAST_DB, SQLITE3_DB
                     BLAST_DB: Required, name of BLAST database to delete
                     SQLITE3_DB: Optional, SQLite3 database where data is
                     stored, default = accession_db.db
```

This is to be used in conjunction with the delete\_database\_template file located in the templates folder. Templates are available as both Excel files and CSVs.

```
BLAST_db      REQUIRED, Name of BLAST database to delete,  
              do not include _database in the name, do not include extension  
SQLite3_DB    Name of SQLite3 database, OPTIONAL, default = accession_db.db
```

File must be saved as a csv, ensure no excess columns included