

The Large Scale Blast Score Ratio (LS-BSR) pipeline

Citation:

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What does it do?

The LS-BSR pipeline was designed as a way to quickly compare the genetic content between a large number of bacterial genomes. LS-BSR can calculate several pan-genome statistics in a population and the output can be easily visualized with a variety of third-party tools. Additionally, LS-BSR can be used to query a set of genes against a large set of genomes to identify gene distribution and conservation. LS-BSR was developed to be easy to run and interpret.

Installation

-The code is kept here:

<https://github.com/jasonsahl/LS-BSR.git>

-You can clone the repository to your own system with git:

\$git clone <https://github.com/jasonsahl/LS-BSR.git>

-Enter the directory, then:

\$python setup.py install

-if your install directory is /Users/jsahl/LS-BSR, run:

\$export PYTHONPATH=/Users/jsahl/LS-BSR:\$PYTHONPATH

-You can add this to your .bashrc or .profile

-You can test your installation by running the tests:

\$python /Users/jsahl/LS-BSR/tests/test_all_functions.py

-If your installation is correct, all tests should pass

Dependencies

1. Python >2.7 and <3.0
2. USEARCH (tested version is 6.0.307), path is passed as command-line option - **only required if a set of gene sequences is not supplied**. 32-bit version should be sufficient for most applications, including the analysis of 1000 *E. coli* genomes. Tested successfully with versions v7.0.959 and 7.0.1090. Can be freely obtained for academics/non-profits from:
<http://www.drive5.com/usearch/>
3. BioPython, must be in \$PYTHONPATH environmental variable. Can be freely obtained from:
http://biopython.org/wiki/Main_Page
4. blastall (tested version is 2.2.25), must be in path as 'blastall' –**only required if you are using BLASTN or TBLASTN, and not BLAT**. Errors have been observed with v2.2.26. v2.2.5 can be obtained from: <ftp://ftp.ncbi.nlm.nih.gov/blast/executables/release/2.2.25/>
5. BLAT (tested version is v. 35x1), must be in path as 'blat' – **only required if you use choose blat for your alignment method**. Can be obtained from:
<http://hgdownload.cse.ucsc.edu/admin/exe/>
6. Prodigal (tested version is 2.60), must be in path as 'prodigal' - **only required if a set of gene sequences is not supplied**. Can be obtained from: <https://code.google.com/p/prodigal/>
7. Numpy, must be in PythonPath. **Numpy is only required for the post-process compare matrices tool**. If you don't want to install numpy, you should be fine, but won't be able to run the compare script. Can be obtained from: www.numpy.org

Command Line options

-d DIRECTORY, --directory=DIRECTORY : the directory to your fasta files, all must end in ".fasta". Can either be complete genomes or draft assemblies. Scaffolds are discouraged.

-i ID, de-replication clustering value for USEARCH, defaults to 0.9 (range from 0.0-1.0)

-f FILTER, whether to use BLAST filtering, default is "F" or filter, turn off with "T". Turning this to "T" should speed up the analysis, but may throw out highly repetitive sequences.

-p PROCESSORS, number of processors to use, defaults to 2.

-g GENES, if you have a list of genes to screen, supply a nucleotide fasta file. Each gene sequence must be in frame, or questionable results will be obtained (only true for TBLASTN). If this flag is not invoked, then the *de novo* gene prediction method is invoked

-b BLAST, which blast method to use. Default is 'tblastn', can be changed to 'blastn'. Can be used with either a list of supplied genes, or with the *de novo* method.

-q PENALTY, blast mismatch penalty, default is -4, only works with blastn. Optimized to return longer matches. Only certain q/r ratios are allowed. See BLAST documentation for more details.

-r REWARD, blast reward value, default is 5, only works with blastn. Optimized to return longer matches. Only certain q/r ratios are allowed. See BLAST documentation for more details.

-l LENGTH, minimum BSR value to be called a duplicate, defaults to 0.7. The BSR of the "duplicate" divided by the reference bit score must be greater than this value to be called a duplicate

-m MAX_PLOG, maximum value to be called a paralog, defaults to 0.85. If the BSR value is greater than this value, then it is considered to be an ortholog

-n MIN_HLOG, minimum BLAST ID to be called a homolog, defaults to 75. If the BLAST ID is below this value, it is considered a remote homolog

-t F_PLOG, filter ORFs with a paralog from BSR matrix? Default is F (do not filter), values can be T (filter paralogs) or F

-k KEEP, keep or remove temp files, choose from T or F, defaults to False (F)

Test data – give LS-BSR a whirl on small datasets

-Test data is present in the test_data directory. This data consists of:

- Genomes (4 *E.coli* genomes from 4 different pathogenic variants). Genomes are:

- H10407 - enterotoxigenic *E. coli* (ETEC)
- E2348/69 - enteropathogenic *E. coli* (EPEC)
- O157:H7 sakai - shiga toxin *E. coli* (STEC)
- SSON046 - *Shigella sonnei*

-Genes (5 different markers that delineate between the variants). These include:

- IpaH3 - *Shigella* invasion antigen. Mostly present in *Shigella* spp.
- LT - heat-labile toxin. Only present in ETEC (not all)
- ST2 - heat-stable toxin. Only present in ETEC (not all)
- bfpB - bundle forming pilus. Only present on plasmid in EPEC
- stx2a - shiga toxin. Present in STEC

-You can test out the LS-BSR functionality in 4 different ways:

1. Test the gene screen method with tblastn:

-enter test_data directory, run LS-BSR

```
$python /Users/jsahl/LS-BSR/lb_bsr.py -d genomes -g genes/ecoli_markers.fasta
```

-the output should show how each gene is only present in the correct pathovar

2. Test the gene screen method with blastn:

-enter test_data directory, run LS-BSR

```
$python /Users/jsahl/LS-BSR/lb_bsr.py -d genomes -g genes/ecoli_markers.fasta -b blastn
```

3. Test the de novo gene prediction method:

-enter test_data directory, run LS-BSR

```
$python /Users/jsahl/LS-BSR/lb_bsr.py -d genomes -u /usr/local/bin/usage6
```

-To inspect the output, you can look up the following entries in the BSR matrix. They should correspond with the results obtained with the gene screen methods (TBLASTN only):

IpaH3 -> centroid_1724 LT -> centroid_11953 ST2 -> centroid_19265 bfpB -> centroid_1922
stx2a -> centroid_7471

-Sample output for each method is shown in the test_data directory

Visualization of output:

1. The output of LS-BSR can be visualized in many different ways. One popular method to visualize the output as a heatmap is through integration with R. Many users find R a difficult interface, and this link by Kat Holt provides some excellent workflows on how to build heatmaps, and correlate the output with phylogenies:

<http://bacpathgenomics.wordpress.com/2012/05/25/displaying-data-associated-with-phylogenetic-trees/>

2. The Interactive Tree of Life (ITOL) project has an interface that is very user-friendly and can directly take LS-BSR output and a phylogeny and create publication ready figures. ITOL can be found here:

<http://itol.embl.de/>

3. Finally, MeV is designed as a way to visualize expression data, but can just as easily create heatmaps from LS-BSR output. MeV is platform independent.

<http://www.tm4.org/>

Post-matrix scripts

1. compare_bsr.py

-what does it do? Looks for CDS differences between two user-defined populations. Differences can be set by user-defined thresholds for presence and absence. The “names.txt” file contains the names as they should be listed in your separate groups file

-what do you need for the script to run? Requirements include:

- BSR matrix
- Two new-line delimited group files, taken from “names.txt”
- FASTA file of all CDS sequences

-what does output look like? Perhaps the most interesting output includes two separate files with any FASTA entries unique to each defined group

```
$python compare_BSR.py -1 group1.txt -2 group2.txt -f consensus.fasta -b  
bsr_matrix_values.txt
```

2. filter_BSR_variome.py

-what does it do? Filters out the conserved regions of the pan-genome, if you are only interested in looking at the “variome” or accessory genome

-what do you need for the script to run?

- BSR matrix
- Sometimes if a single genome is missing a value, it is still of interest. You can change the number of missing values that can still be considered as core, and therefore filtered

-what does output look like? A new BSR matrix, with only variable positions included

```
$python filter_BSR_variome.py -b bsr_matrix_values.txt
```

3. filter_column_BSR.py

-what does it do? Can remove a column from a BSR matrix, in the case where a genome doesn't belong, or is of poor quality

-what do you need for the script to run?

- BSR matrix
- Prefix for output file
- New line delimited file of genome(s) to remove

-what does output look like? A new BSR matrix, with genome columns removed

```
$python filter_column_BSR.py -b bsr_matrix_values.txt -p pruned -g to_remove.txt
```

4. isolate_uniques_BSR.py

-what does it do? Isolates CDSs only present in a single genome, using a user defined threshold for the definition of absence

-what do you need for the script to run?

- BSR matrix
- Threshold for absence, defaults to 0.4

-what does the output look like? A new BSR matrix, with only CDSs present in a single genome

```
$python isolate_uniques_BSR.py -b bsr_matrix_values.txt
```

5. pan_genome_stats.py

-what does it do? Calculates several popular pan-genome stats, based on the BSR matrix

-what do you need for the script to run?

- BSR matrix
- Upper and lower threshold for BSR values

-what does output look like? Several stats are printed to screen. The script also creates two files for the IDs of core and unique CDSs. The frequency_data.txt file can be graphed in order to see the distribution of CDSs across the pan-genome

```
$python pan_genome_stats.py -b bsr_matrix_values.txt
```

6. BSR_to_PANGP.py

-what does it do? Converts a BSR matrix to something that can be easily visualized with PanGP (<http://PanGP.big.ac.cn>). CDSs that are above a given threshold are converted to a "1" and below that threshold are converted to a "-".

-What do you need for the script to run?

- BSR matrix
- Threshold for presence/absence

-what does output look like? A new matrix compatible with PanGP

```
$python BSR_to_PANGP.py -b bsr_matrix_values.txt
```

7. BSR_to_gene_accumulation_scatter.py

-what does it do? For a given number of iterations, the script randomly samples genomes at various depths, and reports back the number of core and unique CDSs in the pan-genome. The script also determines the gene accumulation in the pan-genome. The output can be easily graphed in Excel.

-what do you need for script to run?

- BSR matrix
- Upper and lower bounds for presence/absence

-what does output look like? The mean for each sampling depth is printed to screen. The script can be run to output accumulation, core, uniques, or all (default).

```
$python BSR_to_gene_accumulation_scatter.py -b bsr_matrix_values -i 100
```

8. quantify_BSR_uniques.py

-what does it do? Prints out the number of unique CDSs, sorted by a given tree. Nice way of annotating a tree with where unique CDSs are present

-what do you need for script to run?

- BSR matrix
- Newick tree

-what does output look like? The script prints to a file: uniques_sorted_by_tree.txt, which shows the taxa name and the number of unique CDSs

```
$python quantify_BSR_uniques.py -b bsr_matrix_values.txt -r test.tree
```

9. reorder_BSR_matrix_by_tree.py

-what does it do? Transposes a BSR matrix and re-orders the matrix based on the order of the taxa in a newick-formatted tree

-what do you need for script to run?

- BSR matrix
- Newick tree

-what does output look like? The script prints a reordered BSR matrix to file. Note that since the matrix is transposed, the number of columns can be significant. Best for smaller analyses.

```
$python reorder_BSR_matrix_by_tree.py -b bsr_matrix_values.txt -t test.tree
```

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