

PBScan: Selection outlier scan with population branch statistic

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27th of July 2017

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0. Quick start

```
$ git clone https://github.com/thamala/PBScan.git
$ cd PBScan
$ mv pbscan.* example_data
$ cd example_data
$ gcc pbscan.c -lm -o pbscan
$ ./pbscan -likes example.likes -pop1 list1.txt -pop2 list2.txt -pop3 list3.txt
-out output
$ head output.pbs
```

Chromo	Position	PBS1	PBS2	PBS3
1	220623	0.319226	-0.120292	0.211402
1	445587	0.053947	-0.065290	0.070898
1	734592	-0.028352	0.001156	-0.043844
1	825157	-0.173893	0.251893	0.326334
1	956672	-0.029573	-0.020313	0.212964
1	1069389	0.238198	-0.154341	0.261004
1	1154260	0.192379	-0.125199	0.281174
1	1383999	0.037803	-0.062909	0.448616
1	1587647	0.323904	-0.285808	1.113377

1. Background

PBScan is a program used for detecting selection outliers with population branch statistic (PBS). This measure, introduced by Yi *et al.* (2010), is based on comparing divergence estimates between three populations, two focal ones and an outgroup. PBScan works with diploid NGS data in either genotype call or genotype likelihood formats.

PBScan is a command line tool written in C and is designed to run in UNIX or UNIX-like operating systems, such as macOS and Linux. The program can also run in Windows after compiling the code with e.g MinGW, but it has not been tested in that environment.

1.1 Divergence estimates

Two F_{ST} statistics have been implemented into PBScan: one by Hudson *et al.* (1992) and one by Weir & Cockerham (1984). As default, the pairwise divergence estimates are calculated with Hudson's F_{ST} , using the formula from Bhatia *et al.* (2013):

$$F_{ST} = \frac{(p_1 - p_2)^2 - \frac{p_1(1 - p_1)}{n_1 - 1} - \frac{p_2(1 - p_2)}{n_2 - 1}}{p_1(1 - p_2) + p_2(1 - p_1)}$$

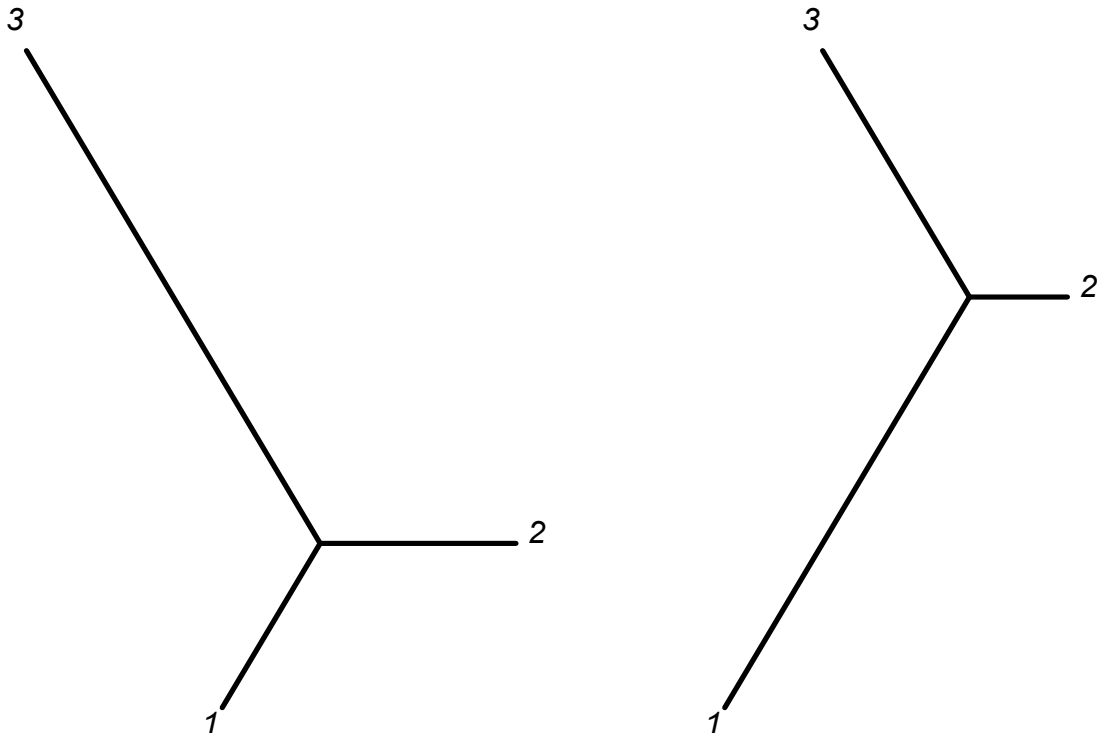
where n_i is the sample size and p_i is the minor allele frequency in the two populations to be compared. Sliding window estimates are based on the weighting method by Reynolds *et al.* (1983). With Hudson's measure, the weighted F_{ST} is: $1 - (\sum_{i=1}^n H_{iW} / \sum_{i=1}^n H_{iB})$, where n is the window size. However, being a relative measure, F_{ST} can be inflated by reduced within population nucleotide diversities, so selection scans can also be conducted using an absolute measure, d_{XY} (Nei 1987). To make this estimator compatible with genotype likelihoods, d_{XY} is calculated from allele frequencies using the following formula (here shown for window of size n):

$$d_{XY} = \frac{1}{n} \sum_{i=1}^n p_{i1}(1 - p_{i2}) + p_{i2}(1 - p_{i1})$$

These estimates are then transformed into relative divergence times: $T = -\ln(1 - X)$, where X is either F_{ST} or d_{XY} , and the PBS for lineage 1 is calculated as:

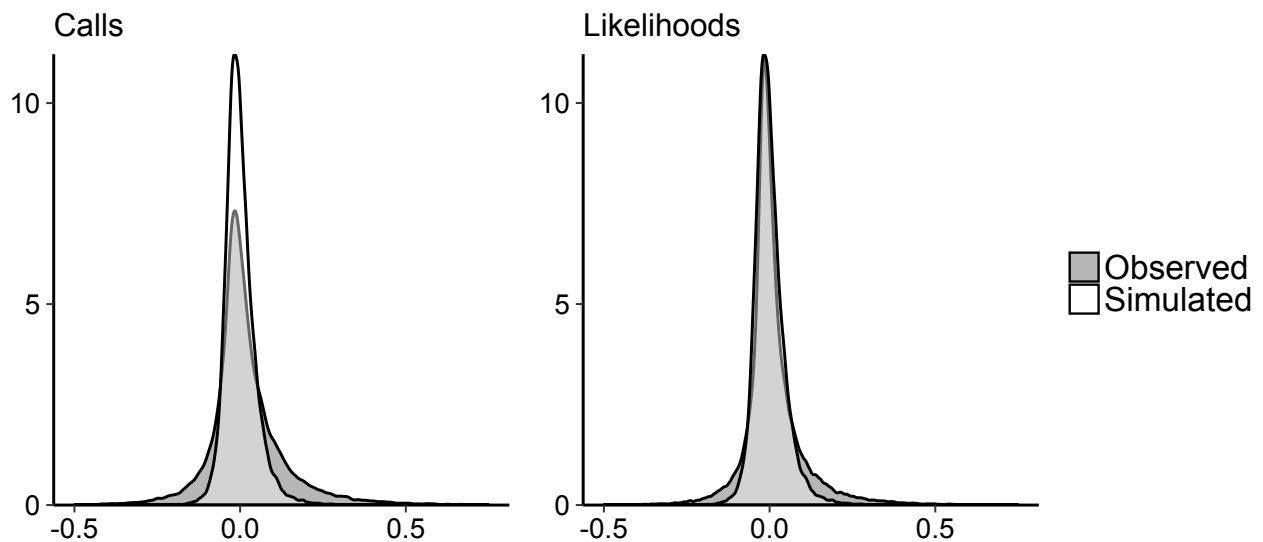
$$PBS = \frac{T_{12} + T_{13} - T_{23}}{2}$$

The obtained value quantifies the magnitude of allele frequency change in population 1 since its divergence from the closely related population 2 and the outgroup 3. The figure below depicts relative branch lengths in a neutral (left) and selected (right) scenarios.



1.2 Genotype likelihoods

A common issue with NGS data is low and often highly variable sequencing coverage. This fact combined with the strict filtering associated with variant calling (most protocols require the called genotype to be ten times more likely than the other ones) can lead to a scenario where heterozygote calls are clearly underrepresented in areas of low coverage, biasing the sampling distributions and thus effecting outlier detection. To prevent this, PBScan utilizes a maximum likelihood model by Kim *et al.* (2011) to estimate allele frequencies directly from genotype likelihoods. This approach bypasses the need for genotype calling, leading to unbiased allele frequency estimates even at very low coverage ($\sim 2\times$). Below is a real data example showing PBS distributions estimated from SNP calls and genotype likelihoods compared against simulated neutral samples. The data has a median coverage of $12\times$ and it was filtered using the following settings: mapping quality 30, site quality 20, genotype quality 20 (SNP calls only), minimum coverage $4\times$.



1.3 Outlier detection

For outlier detection, PBScan utilises a simulation and Monte Carlo based tests. In the latter method, permutations are repeated n number of times, each time randomizing the alleles among individuals. The highest PBS values at each site or window is retained, providing an approximation of maximum genome-wide estimates under neutrality. The p -values are then defined by comparing the observed PBS estimates against quantiles of the simulated distribution. This randomization based approach is robust, applicable to all data, and in most cases more accurate than drawing thresholds directly from the sampling distribution. However, if user has knowledge about demography and recombination parameters, PBScan provides an option to use simulated neutral data in ms format (Hudson 2002) as null distribution.

2. Usage

2.1 Downloading and compiling

PBScan can be cloned from Github:

```
$ git clone https://github.com/thamala/PBScan.git
```

Or downloaded as a ZIP package.

The program is then compiled using the following command (the header file `pbscan.h` needs to be in the same folder):

```
$ gcc pbscan.c -lm -o pbscan
```

2.2 Input data

PBScan supports three input data formats: genotype likelihoods in Beagle format, genotype calls in VCF 4.1 format and genotype calls in native PBScan format.

Example of a Beagle format input file, shown here for one individual (Ind0) and three markers (Chromosome 1, base pairs 24, 47 and 91):

marker	allele1	allele2	Ind0	Ind0	Ind0
1_24	3	0	0.999878	0.000122	0.000000
1_47	2	0	0.999992	0.000008	0.000000
1_91	2	0	1.000000	0.000000	0.000000

Beagle format files can be produced e.g. with ANGSD (Korneliussen *et al.* 2014):
http://www.popgen.dk/angsd/index.php/Beagle_input

VCF 4.1 is the de facto genotype call format and it can be produced e.g. with GATK (McKenna *et al.* 2010) or Freebayes (Garrison & Marth 2012).

The flexibility of the VCF format can, however, sometimes cause issues with algorithmic reading, so there is an option to use native PBScan format as an input (shown here for six individuals and three markers):

CHR	BP	Ind0	Ind1	Ind2	Ind3	Ind4	Ind5
1	69270	11	10	00	00	11	11
1	69761	11	11	11	11	11	10
1	183598	10	00	00	00	00	10

A VCF file can also be transformed into a native format by using `-vcfp` input argument. This way the user has an option to check that the data has been read correctly, and the native format can be used as a faster input option in subsequent runs.

All input data files are assumed to be sorted according to chromosome and position. VCFtools (Danecek *et al.* 2011) or Picard (<http://broadinstitute.github.io/picard/>) can be used to sort VCF files.

2.3 Population lists

Three lists are required to define which individuals belong to which populations. Each list should be a plain text file (.txt) with UNIX line endings (LF) containing individual names corresponding to names found in data input files (one name per line). The focal populations are defined with lists `-pop1` and `-pop2` and the outgroup is defined with a list `-pop3`.

2.4 Simulated neutral data

Simulated neutral samples can be used in the outlier detection. Data are required to be in ms format (Hudson 2002), which may be produced also by other simulation programs, such as MSMS (Ewing & Hermisson 2010) and SLiM 2 (Haller & Messer 2017). Simulated data must have the same number of individuals as the observed data and they must be in the order defined by the populations lists. For example, if `-pop1` list has ten names, first ten individuals in the ms file are assumed to belong to population 1. The neutral PBS distributions can be printed to file by using `-msp` argument.

2.5 Output

PBScan run without `-win` `-ms` or `-mc` options produces the simple output format, with chromosome, position in base pair and PBS estimates for the three populations:

Chromo	Position	PBS1	PBS2	PBS3
1	220623	0.319226	-0.120292	0.211402
1	445587	0.053947	-0.065290	0.070898
1	734592	-0.028352	0.001156	-0.043844

If `-win` (defined in number of SNPs) is used, start, middle and end positions of the sliding window, along with window length in number of base pairs, are also printed to file:

Chromo	Beginning	Middle	End	Length	PBS1	...
1	220623	477608	734592	513969	0.107262	...
1	825157	947273	1069389	244232	0.009635	...
1	1154260	1370954	1587647	433387	0.202560	...

And when `-ms` or `-mc` argument is present, *p*-values are also printed:

Chromo	Beginning	Middle	End	Length	PBS1	P1	...
1	220623	477608	734592	513969	0.107262	0.042000	...
1	825157	947273	1069389	244232	0.009635	0.460000	...
1	1154260	1370954	1587647	433387	0.202560	0.008000	...

2.6 Parameters

Required parameters:

-likes [file]*	Genotype likelihoods in Beagle format
-vcf [file]*	Genotype calls in VCF 4.1 format
-vcfp [file]*	Same as '-vcf', except prints out a native PBScan file (suffix .pbscan)
-in [file]*	Genotype calls in native PBScan format
-pop1 [file]	List of individuals (one per line) from focal population one
-pop2 [file]	List of individuals (one per line) from focal population two
-pop3 [file]	List of individuals (one per line) from the outgroup population
-out [string]	Name for the output file (suffix .pbs)

*one of these is required

Optional parameters:

-win [int]	SNP based window size (def 1)
-step [int]	SNP based step size (def 1)
-ms [file]	Simulated neutral data in ms format
-msp [file]	Same as '-ms', except prints the null-distributions to file (suffix .nulldist)
-perm [int]	Number of permutation cycles for Monte Carlo testing (cannot be used with '-ms')
-div [int]	Divergence measure: [0] Hudson's F_{ST} [1] Weir & Cockerham's F_{ST} [2] d_{xy} (def 0)
-min [int]	Minimum number of individuals required per population (def 1)
-maf [double]	Minimum minor allele frequency required per site (def 0.01)

Usage example:

```
./pbscan -likes example.likes -pop1 list1.txt -pop2 list2.txt -pop3 list3.txt -out output -win 50 -step 51 -ms  
example.ms -div 2 -min 5 -maf 0.05
```


3. References

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