

PRS harmonization and calculation pipeline to apply weights to genetic data

ESCALATOR container v2.0.0

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General introduction of the pipeline

This is a containerized PRS harmonization pipeline taken from the original pipeline scripts used on Eureka cloud platform at CU Anschutz (<https://github.com/menglin44/escalator>). Generalized scripts adapting non-Eureka environment have been under development and test, for those scripts and if you would like to build the ESCALATOR container image on your own, please visit <https://github.com/MatthewFisher126/ESCALATOR>.

The pipeline takes in weight files in the format supported by PGS catalog, performs harmonization/QC against a target genetic dataset provided by users (in PLINK v2. binary pfile format), computes PRS for each sample in the dataset, and generates short summary files to report the QC process. See the following sections for accepted formats and examples of how to run the container.

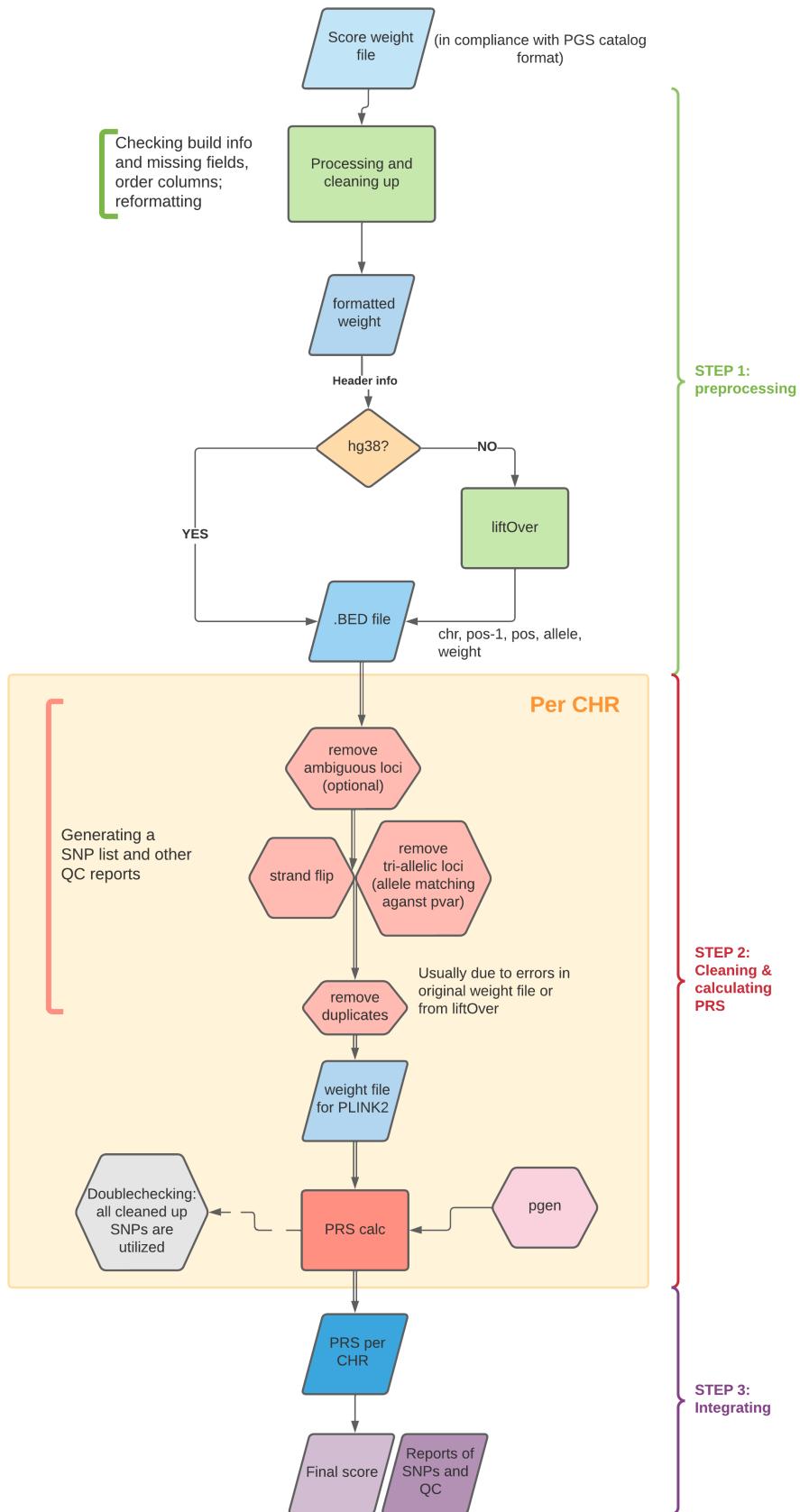
- Some important general pre-assumptions of the current version of the pipeline include -
 - Singularity is already installed in your computational environment.
 - Your test dataset (i.e. genetic data of the samples you wish scores to be calculated on) is on build GRCh38. Weight files can be on any other build, then the weights not on GRCh38 will be lifted by ESCALATOR to this build before being harmonized against the test dataset loci.
 - Your test dataset is parsed by chromosomes, from 1-22 (i.e. autosomes). The parsed files are named with “chr” followed by chromosome number and then the general infix of the file, e.g. chr1_myfile.pvar, chr1_myfile.psam, chr1_myfile.pgen.
 - The containerized pipeline does not include the original function of fetching chromosome and bp position info from dbSNP variant names. Thus the weight file will be expected to contain correct chromosomal and position info itself.

Some other features that do not affect the validity of the use of a score, but are worth clarifying for this version -

- Please note that the output score values generated by **PLINK2 score function used in ESCALATOR is halved (see here)**, as PLINK2 divides scores $a^T G$ by the number of allele observations. In the majority of PRS-related analyses, this does not impact the final results, as scores need to be standardized or scaled before use. Yet if other methods of using scores is involved, such as summing two scores that require them to be on the same scale to reflect the raw weight per variant, users should be aware of how PLINK handles the averaged score and consider multiplying the score values by two.

- If there are missing genotypes in variants of interest among individuals, they will NOT be auto-imputed based on population allele frequency. Auto-imputation is a default in PLINK2 for missing genotypes (see <https://www.cog-genomics.org/plink/2.0/score>), but is shut off in this pipeline. If you wish to enable this function, you can build your own container by going to the generalized scripts branch on our github, and deleting the `no-mean-imputation` flag in the plink step in the master shell script before containerization.

A general overview of the pipeline is visualized as below -



1. Explanations of the QC steps

- Build liftover

Genome coordinates of chromosome and positions of variants in weights files will be lifted over to GRCh38/hg38, if not on this build already. Rather than matching the content of weight files against each build reference to detect the genome build of the input, the pipeline currently relies on an indication of build info in the header, such as `# Original Genome Build` or `# genome_build` (see the section of *Example of running the pipeline*)

The pipeline supports input files with builds on [GRCh38/hg38](#), [GRCh37/hg19](#), [NCBI36/GRCh36/hg18](#), [NCBI35/hg17](#).

- Removing ambiguous loci

Loci with risk & reference alleles that are A/T, C/G in the weight files will be removed.

- Removing duplicated loci that have the same risk allele, but each was assigned a different weight value

This has been a very rare observation, but it can happen from an error in the original weight files or liftOver process - duplicated loci with the same coordinates / names having the same allele codes, but the weight of the risk allele appears different.

- Strand matching with the test dataset

Strand flip will be performed on variants from the input file to match the same locus in the test dataset.

- Removing loci with mismatching allele code with the test dataset

If either strand flipping the input variant or not does not match with the allele code of the same locus in the test dataset (e.g. a tri-allelic situation where input locus has allele A / G, the test dataset has allele A / C), the variant will be removed. This is because all weight value of a risk allele is only relative to that of the correct reference allele, as estimated by initial association studies.

- Removing the loci not found in the test dataset

2. Score calculation after harmonization

ESCALATOR leverages external software of PLINK2, which is included in the container.

3. Format of an input file

The input file can be either in plain text or gzipped text file (ending with .gz), providing weight and variant information in the format concordant with that in PGS catalog, as described here. The format supported by PGS catalog prior to early 2022 is slightly different, referred as **version 1** here, compared to the format at the later stage(**version 2**). If using the catalog's preharmonized version (example), this would be version **version 3**. Providing a version number of 1, 2, or 3 is a required argument for ESCALATOR input (alternatively, "F" is also accepted if the input weight file is already formatted by ESCALATOR).

In short, the weight file should contain **tab-separated** columns of chromosome and bp positions, effect allele, risk allele, and weight of the risk allele. A header line with build info is also required. Irrelevant fields or headers will be ignored. Examples are provided as below (the order of columns does not matter):

- Provide chromosome, bp position, risk and other allele codes, and weight of risk allele

- **version 1**

```
# Original Genome Build = hg38
chr_name chr_position effect_allele reference_allele effect_weight
11 69516650 T C 0.1622
...

```

- **version 2**

```
# genome_build = GRCh38
chr_name chr_position effect_allele other_allele effect_weight
11 69516650 T C 0.1622
...

```

- **version 3**

```
#HmPOS_build=GRCh38
hm_chr hm_pos effect_allele other_allele effect_weight
11 69516650 T C 0.1622
...

```

4. Output files

4.1 A list of PRS score of all samples in the test dataset

Depending on the input plink2 files containing one column of ID (“IID”) or two (“FID” and “IID”), [prefix]_prs.sscore consists of additional three columns of number of alleles across scored variants (“ALLELE_CT”), sum of named allele dosages (“NAMED_ALLELE_DOSAGE_SUM”), and PRS score equivalent as the dot product of weight and genotype matrix (“SCORE”).

4.2 A record of variants being used in PRS calculation

[prefix]_cleaned_forRecord.list contains a complete record of final variants being used for PRS calculation, after QC. Columns include:

CHR(hg38): Chromosome position of the variant, based on hg38

BP(hg38): Base pair position of the variant, based on hg38

OriginalSNPID: SNP ID, or variant name, from the input file, if not present, a chr:bp:ref:alt matching the test dataset will be assigned.

UpdatedSNPID: SNP ID, or variant name, used in the test dataset

UpdatedRiskAllele: Risk allele code with strand matching the test dataset

UpdatedRefAllele: Reference/Other allele code with strand matching the test dataset

Weight: Weight value of the updated risk allele

4.3 Variants discarded / changed during QC

These files will be stored in a **sub-directory recordfiles/** nested under the user-specified bucket directory. **Note: if a folder named “recordfiles/” already exists within the destination directory, please make sure it has the written permission granted to you as the pipeline will try to output files in it.**

[prefix]_flipped.list records variants that are used but with strand flipped to match the test dataset.

[prefix]_mismatch.list records variants with allele codes not matching the test dataset, and thus are not included in final PRS calculation.

[prefix]_missing_in_pvar.list records variants that are not present in the test dataset, and thus are not included in final PRS calculation.

[prefix]_at_cg.list records variants that are removed because they have ambiguous allele codes, such as A and T as the risk and other alleles, respectively. This file will be empty (with only one header line), if the user has chosen not to remove ambiguous variants.

4.4 Log file

[prefix]_prs.log contains a summary of counts of variants during QC, and change of build (i.e. liftover). An example of log file:

Total number of input weight file: 52.

Lifting over from hg19 to hg38.

Discarded unmatched variants from liftOver: 0

Number of non-autosomal variants being discarded: 0

Number of ambiguous A/T, C/G loci that are removed: 6

Number of weight SNPs that are flipped to the other strand: 2

Number of SNPs with mismatched allele codes against pgen that are removed: 0

Number of SNPs in weight file not found in pgen that are removed: 1

Number of duplicated entries with same position and alleles (and are removed): 0

Number of final variants used for PRS: 45

4.5 In situations of unsuccessful runs

A PRS might not be calculated successfully in the following situations, and would have a message generated in the last line of the corresponding log file.

- Genome build is not listed / unclear

Didn't detect the genome build of the input file

- Some of the essential column information is missing in the original weight file (see section 2)

Reformatting of the input file failed

- No variants are left in the weight file after QC / none of the variants are found the in target pfile

No variants are left for PRS score

5. Example of running the pipeline

```
singularity exec escalaTor-v2.sif masterPRS_v4.sh .sh 2 \
/home/user/score_weights \
PGS002155.txt.gz \
/home/user/score_output \
pigmentation \
/home/user/genetic_data_plink \
freeze3_imputed_dosages \
T \
NA
```

The general command with required arguments used above is:

```
singularity exec escalaTor-v2.sif masterPRS_v4.sh [format version of weight] \
[weight file directory] \
[weight file] \
[output directory] \
[output prefix] \
[test dataset directory] \
[test dataset infix] \
[whether to remove variants with ambiguous codes] \
[frequency file to impute missing genotypes, or can be left as 'NA']
```

- Explanations of each argument:

1. *[format version of weight file]* refers to the different version of **v1**, **v2** or **v3** format from PGS catalog as Section 3.
2. *[weight file directory]* and *[weight file]*: The directory and file name of the score weight file.
3. *[output directory]*: The directory under which the calculated score, and the other records files will be written.
4. *[output prefix]*: Prefix of the output file names.
 - For example, it often can be the name of the phenotype (“pigmentation”) or score number from the PGS catalog (e.g. “PGS00001”).
 - In case of this argument being specified as “unknown”, the prefix of the output files will be determined by ESCALATOR scanning for header lines of **# PGS ID =**, or **# pgs_id =** (only when **# format_version=2.0** is also present).
5. *[test dataset directory]*: The directory where the genetic dataset of samples of interest rests.
6. *[test dataset infix]*: The infix of the genetic dataset, parsed by each chromosome from 1-22.
 - E.g. An infix of “myfile” will let the pipeline automatically search for *chr1 myfile.psam*, *chr1 myfile.pvar*, *chr1 myfile.pgen* ... all the way to *chr22 myfile.psam*, *chr22 myfile.pvar*, *chr22 myfile.pgen* in the specified test dataset directory.
7. *[whether to remove variants with ambiguous codes]*: T or F as to whether to remove variants with ambiguous allele codes (A/T, C/G).

8. [frequency file for PLINK to impute missing genotypes]: If your dataset has a sample size < 50, a frequency file acceptable by PLINK –read-freq is required (e.g. .afreq or .freq files generated by PLINK) is required for imputing missing genotypes. Otherwise, you can leave the argument as ‘NA’. If a frequency file is to be submitted, it is expected to be found in the [weight file directory].

Alternatively, to see general help information from the container, type -

```
singularity run-help escalator-v2.sif
```

6. Miscellaneous

If your original files are in VCF format and do not come with variant names to be converted to PLINK2 file (for example, all variant names are “.” in 1000 Genomes low coverage as below),

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	HG00096
22	18516173	.	A	G	.	PASS	AC=121;AN=59%;DP=8283;AF=0.02;EAS_AF=0;EUR_AF=0.02;AFR_AF=0.02;SAS_AF=0;VT=SNP;NS=2548	GT	0 0
22	18522217	.	G	A	.	PASS	AC=89;AN=59%;DP=9885;AF=0.02;EAS_AF=0;EUR_AF=0;AFR_AF=0.07;AMR_AF=0;SAS_AF=0;VT=SNP;NS=2548	GT	0 0
22	18526445	.	A	G	.	PASS	AC=4948;AN=59%;DP=7978;AF=0.97;EAS_AF=0.98;EUR_AF=1;AFR_AF=0.93;AMR_AF=0.97;SAS_AF=1;VT=SNP;NS=2548	GT	1 1
22	18527834	.	G	T	.	PASS	AC=271;AN=59%;DP=5974;AF=0.05;EAS_AF=0.01;EUR_AF=0.03;AFR_AF=0.11;AMR_AF=0.03;SAS_AF=0.07;VT=SNP;NS=2548	GT	0 0
22	18527838	.	C	G	.	PASS	AC=267;AN=59%;DP=5947;AF=0.05;EAS_AF=0.08;EUR_AF=0.03;AFR_AF=0.01;AMR_AF=0.07;SAS_AF=0.08;VT=SNP;NS=2548	GT	0 0

you can use the extra flags `--set-all-var-ids chr@:#:\$r:\$a --new-id-max-allele-len 150`

such in the example of

```
for i in {1..22}
do
./plink2 --vcf ALL.chr${i}.shapeit2_integrated_snvindels_v2a_27022019.GRCh38.phased.vcf.gz \
--set-all-var-ids chr@:#:\$r:\$a --new-id-max-allele-len 150 \
--make-pgen --out chr${i}_tgp_snvindels
done
```

when converting VCF to PLINK2 file.

For other questions, please contact meng.lin@cuanschutz.edu and matthew.j.fisher@cuanschutz.edu

