

# Software for GBS-based relationship calculations

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## Background

R code is available for the analysis of genotyping-by-sequencing (GBS) data, primarily to construct a genomic relationship matrix ('G matrix') for the genotyped individuals. The code can be used on its own, or incorporated into other R programs. There are QC tools (primarily graphical output), relationship estimation tools, pedigree verification tools and pedigree 'mix and match' tools. The latter two operations require additional input information about the samples genotyped.

In this document, 'Individual' or 'sample' generally refers to the genotyping unit (possibly combined, if the same individual or sample is genotyped multiple times). Familial relationships are given the labels 'Father', 'Mother' and 'Offspring' (as appropriate).

The methods used are as described in Dodds *et al.* (2015). Unless specified, relatedness estimates in this documentation refer to those using the 'G5' method of that paper.

## Program structure

There are two separate analysis program files, the first (GBS-Chip-Gmatrix.R) for genotype QC and relationship matrix construction and the second (GBSPedAssign.R) for pedigree verification and/or assignment, based on the related estimates. These programs can be invoked from another program file (using the *source* command), or users can insert all or parts of these programs into their own code. For the purposes of this documentation, it is assumed the first method is used, with calling program named GBSRun.R. If either of these programs are being called multiple times in an R session, care should be taken to make sure any default values still apply for the subsequent call(s), otherwise they should be removed (rm) or set specifically.

## Calling program (GBSRun.R)

Variable command	Type <sup>1</sup>	Description
<b>genofile</b>	V	Name (including path) of the genotype file. Default value is "HapMap.hmc.txt".
<b>gform</b>	V	Type of genotype file. Default is "uneak"; other options are "Tassel" or "Chip".
<b>sampdepth.thresh</b>	V	Minimum mean sample depth for retaining sample results. Default is 0.01.
<b>snpdepth.thresh</b>	V	Minimum mean SNP depth for retaining SNPs. Default is 0.01.
<b>hirel.thresh</b>	V	Lower threshold for reporting highly related individuals, and upper threshold for displaying positive control pairs which don't seem sufficiently related. Default is 0.9.
<b>cex.pointsize</b>	V	Relative value of pointsize used in output graphics. This has a default value of 1.
<b>functions.only</b>	V	Set to TRUE to source GBS-Chip-Gmatrix.R for setting up functions (not reading data etc). Default is FALSE.
<b>outlevel</b>	V	Integer (1-9) determining the level of output created – higher

		numbers give more output. At present only two levels are active; 5 to 9 give the full output while 1 to 4 gives less output. A value less than 8 will suppress the sampled alleles setup and analysis (reducing time). Default is 9 (all available output)
<b>source</b>	C	Invoke GBS-Chip-Gmatrix.R code, to run QC procedures and define functions, e.g. the genomic relationship matrix function ( <i>calcG</i> )
<b>calcG</b>	C	Calculate genomic relationship matrices. May be invoked several times with different options.
<b>pedfile</b>	V	Name of file containing pedigree and/or parent group information
<b>groupsfile</b>	V	Name of file containing which individuals are in which parent groups
<b>GCheck</b>	V	The name (as a string) of the G matrix to use for parent verification or assignment This must be set before calling GBSPedAssign.R.
<b>indsubset</b>	V	The subset of individuals used to calculate the matrix specified in <i>GCheck</i> .
<b>rel.thresh</b>	V	The relatedness threshold to use for parent verification or assignment, if the corresponding parent sex-specific threshold ( <i>rel.threshF</i> or <i>rel.threshM</i> ) has not been set. This has a default value of 0.4.
<b>rel.threshF</b>	V	The relatedness threshold to use for father verification or assignment. This has a default value of <i>rel.thresh</i> .
<b>rel.threshM</b>	V	The relatedness threshold to use for mother verification or assignment. This has a default value of <i>rel.thresh</i> .
<b>mindepth.mm</b>	V	Minimum depth to be used for calculating mismatch proportions in parent matching. Default is 1 (use all results).
<b>snpsubset</b>	V	The subset of SNPs to be used for calculating mismatch rates or for bootstrapping (usually the same set as used for calculating the matrix specified in <i>GCheck</i> ). Default is all SNPs.
<b>emm.thresh</b>	V	The excess mismatch rate threshold to use for parent assignment. This has a default value of 0.01.
<b>emm.thresh2</b>	V	The excess mismatch rate threshold to use for parent-pair assignment. This has a default value 2 x <i>emm.thresh</i> .
<b>emmdiff.thresh2</b>	V	The excess mismatch rate difference (from that for the most related father and most related mother) threshold to use for suggesting an alternate parent-pair assignment. This has a default value of 0.
<b>inb.thresh</b>	V	The lower threshold for the difference between parent relatedness and estimated inbreeding to exclude a parent-pair match with the inbreeding check. This has a default value of 0.2.
<b>boot.thresh</b>	V	If the relatedness with the 2 <sup>nd</sup> best parent is within <i>boot.thresh</i> of that for the best parent, a bootstrapping procedure will be invoked to further compare these possible matches. Default value of 0.05.
<b>depth.min</b>	V	Minimum mean depth of SNPs to be used for bootstrapping. Default value is 0.
<b>depth.max</b>	V	Maximum mean depth of SNPs to be used for bootstrapping. Default value is 0.
<b>puse</b>	V	Allele frequencies to be used bootstrapping. Default is to use <i>p</i> .
<b>nboot</b>	V	Number of bootstrap replicates. Default value is 1000.
<b>boota.thresh</b>	V	The upper threshold on bootstrap reliability for excluding a parent match with the bootstrapping check. This has a default value of 99.
<b>source</b>	C	Invoke GBS-PedAssign.R code to verify parents (if given) or assign parents (if parent groups are given)

<sup>1</sup> Type is V for a variable to be set, or C for a command to be invoked or function to be run.

## Relatedness estimation program (GBS-Chip-Gmatrix.R)

This program performs some QC diagnostics, rudimentary data cleaning and defining a function (*calcG*) for relatedness estimation and reporting. A number of other functions are defined, such as those for checking and report on positive controls (negative control checks, based on a specified sample naming system, are yet to be included). Any procedures or output relating to depth are not implemented for chip data. The use of depth information to construct the GRM can be modified (see *depth2K* section).

Samples with very low depth are dropped from the analyses. The threshold is a mean depth of *sampdepth.thresh* (default of 0.01, but can be set in the calling program) or with a maximum depth of one (including those with no genotype calls). Samples that are dropped are reported in the program output, as is the remaining number of samples.

SNPs with no data or with a MAF (minor allele frequency) of zero are dropped. The remaining number of SNPs is reported.

Some basic statistics are reported: Proportion of missing genotypes is the number of SNP x individual combinations with no allele calls; Mean sample depth is the average depth (number of reads of either allele) for a sample.

The default action when sourcing GBS-Chip-Gmatrix.R is to read the data file and run some QC procedures, as well as define various functions. If *functions.only* is set to TRUE, then only the function definition occurs. The default action can then be mimicked using the pair of commands:

```
readGBS()  
GBSSummary()
```

These functions are not yet described in the documentation. Additional processing can be inserted between these statements, for example to manually remove samples or SNPs. The following objects need to be maintained correctly, before *GBSSummary* is run: *nsnps*, *SNP\_Names*, *seqID*, *nind*, *alleles*. If *GBSSummary* has been run once, it could be re-run, e.g. after merging results from the same individual. In that case (detected by the presence of *depth*), processing that uses *alleles* (which is not recalculated in *mergeSamples*) is omitted. This is mainly to obtain *genon* and *depth* which will be assumed to be present and correct, but it should be noted that *p* is not recalculated. *p* should remain unchanged when samples are merged, but could change, for example if the *sampdepth.thresh* is changed between calls to *GBSSummary*.

## Output - files

*SampleStats.csv* contains call rates for each sample, along with mean sample depths (for GBS data).

*AlleleFreq.png* is a plot of allele frequencies calculated using different methods (and as given, if the *uneak* format is used).

*CallRate.png* shows a histogram of sample call rates (proportion of SNPs with a result for a sample).

*SampDepth.png* plots mean sample depth against median sample depth.

*SampDepth-scored.png* plots mean sample depth, over SNPs that are scored for the individual, against mean sample depth over all SNPs for the individual.

*SampDepthHist.png* is a histogram of mean sample depths

*SampDepthCR.png* plots mean sample depth against call rate.

*SNPDepthHist.png* is a histogram of SNP depths (number of reads of either allele averaged over samples)

*SNPCallRate.png* is a histogram of SNP call rates (proportion of samples with a result for a SNP)

*SNPDepth.png* plots SNP depth against mean SNP depth (on a log scale). This may reveal SNPs that are called infrequently, but when they are called have good depth (these SNPs may be near the boundary of a size selection step in the laboratory).

*finplot.png* plots Hardy-Weinberg disequilibrium (HWD) against MAF, shaded by the SNP depth. HWD is the proportion of (reference allele) homozygotes minus the expected proportion (under Hardy-Weinberg equilibrium). HWD is the same whichever allele is used in the calculation. The 'fin plot' may reveal sets of SNPs that do not follow Mendelian inheritance, for example apparent SNPs in duplicated regions.

*HWdisMAFsig.png* is similar to the fin pot, but with shading by the likelihood ratio test statistic for HWD.

*LRT-QQ.png* is a QQ plot for the likelihood ratio test statistic for HWD.

*LRT-hist.png* is a histogram of the likelihood ratio test statistic for HWD.

*MAF.png* is a histogram of the MAFs for each SNP (based on observed genotypes).

### Variables defined

These include:

Variable	Description
<b><i>nind</i></b>	Number of samples analysed (after initial QC)
<b><i>nsnps</i></b>	Number of SNPs analysed (after initial QC)
<b><i>seqID</i></b>	Identifiers for each sample
<b><i>SNP_Name</i></b>	Identifiers for each SNP
<b><i>chrom</i></b>	chromosome label (character), if <i>gform</i> is Tassel
<b><i>pos</i></b>	chromosome position (numeric), if <i>gform</i> is Tassel
<b><i>alleles</i></b>	matrix ( <i>nind</i> x 2* <i>nsnps</i> ) of read counts. The results for each SNP are in consecutive columns.
<b><i>genon</i></b>	matrix ( <i>nind</i> x <i>nsnps</i> ) of numeric genotype calls 0 (homozygous alternate allele), 1 (heterozygous), 2 (homozygous reference allele), NA for missing
<b><i>depth.orig</i></b>	matrix ( <i>nind</i> x <i>nsnps</i> ) of counts for each sample and SNP
<b><i>sampdepth</i></b>	mean depth for each sample
<b><i>snpdepth</i></b>	mean depth for each SNP
<b><i>p</i></b>	allele frequencies on the basis of allele counts
<b><i>pg</i></b>	allele frequencies on the basis of genotype calls

### Function to read TagDigger format files (*readTD*)

This function is for reading TagDigger files. It can be used by the main program (if `functions.only` is FALSE, the default), but can be used to read additional files (e.g. to compare results in two different files). The variables *nsnps*, *seqID*, *nind*, and *alleles* are defined. The ability to generate the other variables required for further analysis is planned. See the section on the TagDigger format for more information.

#### Arguments:

`genofilefn` the name of the file to read. Defaults to *genofile*.

Value: NULL

### Function to remove samples from objects (*samp.remove*)

This function removes samples from the relevant objects (*alleles*, *depth*, *sampdepth*, *seqID* *nind*). It would normally be used between calls to *readGBS* and *GBSsummary*.

Arguments:

<i>samppos</i>	the positions of the samples to remove. Defaults to NULL.
<i>keep</i>	If TRUE, the samples with positions <i>samppos</i> will be kept and other samples removed. Default value is FALSE

### Function to remove SNPs from objects (*snp.remove*)

This function removes samples from the relevant objects (*p*, *nsnps*, *SNP\_Names*, *alleles*, *depth*, and some others). It would normally be used between calls to *readGBS* and *GBSsummary*.

Arguments:

<i>snppos</i>	the positions of the SNPs to remove. Defaults to NULL.
<i>keep</i>	If TRUE, the SNPs with positions <i>snppos</i> will be kept and other SNPs removed. Default value is FALSE

### Depth functions (*depth2K*, *depth2Kbb*, *depth2Kmodp*, *depth2Kchoose*)

The GBS-Chip-Gmatrix.R program defines a default function for calculating “*K* values”, as well as alternate functions (using alternate allele sampling models) and a function to reset the default to one of the alternatives. These functions are relevant for used both self-relatedness estimation and pedigree assignment diagnostics. If a different depth model is required for calculating the self-relatedness, this *depth2K* function should be re-defined before using the *calcG* function (defined below). *K* is the probability of observing an AA genotype, given that the true genotype is AB and the read depth is *k*. These models will be discussed in more detail elsewhere. The function is used within *calcG* for calculating the self-relatedness for G5, and in the pedigree assignment program, for calculating expected mismatch rates.

A function *depth2K* is defined. This function takes a vector of read depths and returns the corresponding set of *K* values. Initially the function is defined using a binomial sampling model (the number of A alleles is binomial with probability parameter 0.5 and sample size the read depth).

*depth2Kbb* is an alternate depth function which uses a beta-binomial model. This model has two parameters,  $\alpha$  and  $\beta$ , but here these are set to be equal, so that  $P(AA|AB, k=1) = 0.5$ .

Usage: *depth2Kbb* (depthvals, alph=Inf)

Arguments:

<i>depthvals</i>	a vector of read depths
<i>alph</i>	the value of $\alpha$ (and also $\beta$ ) – the default is to use Inf, in which case the binomial model is used.

*depth2Kmodp* is an alternate depth function which uses a modified *p* value for 2<sup>nd</sup> and subsequent reads. The modified *p* can be thought of as the probability of seeing the same allele as in the previous read (for that SNP) for a true AB genotype, although because we are only interesting in the probability of all reads being the same allele, it is also the probability of seeing the same allele as *all* previous reads (for a true AB genotype).

Usage: *depth2Kbb* (depthvals, alph=Inf)

Arguments:

<i>depthvals</i>	a vector of read depths
<i>modp</i>	the modified probability – the default is 0.5, which gives the binomial model. Normally a value $\geq 0.5$ would be used to reflect an increased chance of seeing the same allele as in the previous read.

*depth2Kchoose* is function to re-define *depth2K* to one of the alternative models.

Usage: *depth2K* <- *depth2Kchoose* (dmodel="bb", param)

Arguments:



dmodel	the model to use, either “modp” (to use <i>depth2Kmodp</i> ), or “bb” to use <i>depth2Kbb</i> – the default is “bb” (also used if any other string is used)
param	the parameter to use for the alternative function, used for alph for the bb model, and modp for the modp model.

### Function for reporting on positive controls (*posCreport*)

A function, *posCreport*, for reporting on samples which are supposedly from the same individual. These will normally be one or more positive controls, but may also be repeat runs.

Usage *posCreport*(*mergeIDs*, *Guse*, *indsubset*)

Arguments:

<i>mergeIDs</i>	a vector of identifiers, ordered as in <i>Guse</i> , where samples from the same individuals are given the same identifier
<i>Guse</i>	the G matrix for comparing samples
<i>sfx</i>	text to be included in output file names to allow output from multiple calls or runs to be identified
<i>indsubset</i>	a vector of integers (between 1 and <i>nind</i> , inclusive) of the individuals in <i>Guse</i> . Only this subset of <i>mergeIDs</i> will be compared. The default is to use all individuals.

Value: a data frame containing columns *mergeID* (the ID given in *mergeIDs*), *nresults* (the number of runs with this ID), *selfrel* (the average self-relatedness), *meanrel* (the mean relatedness between all pairs with the given value of *mergeID*), *minrel* (the minimum relatedness between all pairs with the given value of *mergeID*). Only values of *mergeID* with *nresults* >1 are included.

Details: The function displays pairs of results where the estimated relatedness is  $\leq$  *hirel.thresh*, and outputs the files:

*posCchecks<sfx>.txt* a copy of the results displayed on the default output (i.e. low relatedness pairs)

*posCreport<sfx>.csv* contains the data frame that waws returned by the function

*SelfRel<sfx>.png* a plot of *meanrel* against *selfrel*. The line of identity is shown in red.

### Function for merging results for the same individual (*mergeSamples*)

A function, *mergeSamples*, for merging samples from the same individual.

Usage *mergeSamples* (*mergeIDs*)

Arguments:

<i>mergeIDs</i>	a vector of identifiers, ordered as in <i>Guse</i> , such that samples that have the same identifier are to be merged
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Value: a list of the following objects:

<i>mergeIDs</i>	a vector of identifiers, as per the input, but ordered as in the other output objects (and with unique values)
<i>nind</i>	the length of <i>mergeIDs</i>
<i>seqID</i>	normally one of the <i>seqIDs</i> that correspond to the <i>mergeIDs</i> . If the <i>seqIDs</i> can be broken into five parts, using an underscore ( <code>_</code> ) as a separator, then the second part will be replaced by “merged”, the third part by the number of results merged and the fourth part by “0”
<i>genon</i>	genotype (0/1/2) matrix after merging
<i>depth.orig</i>	depth matrix after merging
<i>sampdepth</i>	sample mean read depths after merging
<i>snpdepth</i>	SNP mean read depths after merging
<i>pg</i>	allele frequencies based on genotype calls, after merging
<i>nmerged</i>	number of results merged (1, if not merged) for each individual.

Normally these objects would be used to replace their corresponding values before the merge, but this is not done automatically (it is up to the user). Note that some objects are not merged

(e.g. the allele depth matrix, *alleles*) and that the diagnostics produced when sourcing GBS-Chip-Gmatrix.R are not re-done by this function.

### Allele frequency function (*calcp*)

A function, *calcp*, for calculating allele frequencies (for all SNPs), is defined.

Usage: *calcp*(indsubset, pmethod="A")

Arguments:

indsubset	a vector of integers (between 1 and <i>nind</i> , inclusive) of the individuals for which are to be used for allele frequency estimation. The default is to use all individuals.
pmethod	a method for calculating the frequencies, being one of "A" (calculate on the basis of allele counts – the default method) or "G" (calculate on the basis of genotype calls)

Value: a vector of allele frequencies

Warning when using this after *mergeSamples*: pmethod A uses the object *alleles*, which is not recreated during the merge, so indsubset refers to sample positions prior to the merge. pmethod G uses *genon* whose positions are those following the merge.

### Genomic relatedness function (*calcG*)

A function, *calcG*, for calculating the genomic relatedness, is defined.

Usage: *calcG*(snpsubset, sfx="", puse, indsubset, depth.min=0, depth.max=Inf, npc=0, calclevel=9, cocall.thresh=0, mdsplot=FALSE)

Arguments:

snpsubset	a vector of integers (between 1 and <i>nsnps</i> , inclusive) of the SNPs to use in the calculation. The default is to use all SNPs.
sfx	A suffix to use in output file names to identify which function call has produced that output.
puse	a vector of (reference) allele frequencies to use in the calculations. The default is to use allele frequencies calculated on the basis of allele counts. The values (for the snps in <i>indsubset</i> ) should be greater than 0 and less than 1. This is for the full set of snps (it is subsetted using <i>indsubset</i> ).
indsubset	a vector of integers (between 1 and <i>nind</i> , inclusive) of the individuals for which relatedness matrices will be calculated. The default is to calculate for all individuals.
depth.min	The minimum depth for a SNP result for an individual to be used.
depth.max	The maximum depth for a SNP result for an individual to be used.
npc	The number of principal components of the 'G5' relatedness matrix to display. If <i>npc</i> ≤ 0, then the heatmap plot is omitted, but otherwise <i> npc </i> is used for <i>npc</i> . If <i>npc</i> = 0 (the default) the principal component analysis is omitted.
calclevel	specifies the amount of calculation and output produced: 1 gives G5 (see below) and intermediate results only, 2 gives G5 and reports using G5, 3 gives all types of G available and 9 gives these and all reporting available.
cocall.thresh	Samples may be removed so that co-call rates (the proportion of SNPs with a call in both of a pair of samples) for heatmap and PCA analyses are above this value. Firstly, if <i>cocall.thresh</i> ≥ 0, samples with a maximum SNP depth of 1 are removed. The further samples are removed successively, with the sample appearing the most often in pairs not meeting the criterion removed at each step, until all pairs meet the criterion. The removal of these samples under the default threshold allows the heatmap and PCA analyse to be performed (no NAs in the relationship matrix used).
mdsplot	if TRUE and the conditions for plotting the principal components is met, a two dimensional multidimensional scaling plot is also plotted. The default is FALSE.

Value: a list of relatedness structures: G1, G4d (diagonal elements of G4), G5, samp.removed (positions of samples removed to ensure the cocall.thresh criterion) and PC, the output of the



principal components analysis (if  $|npc| > 0$ ). The  $G_n$  relatedness matrices are described in Dodds *et al.* (2015), except that a range of allele sampling models can be incorporated for the diagonal of  $G_5$  – see the depth2K section below.

Details: The function also produces a set of output files, as follows.

*Co-call-<sfx>.png* is a histogram of co-call rates (the proportion of SNPs with a call in both of a pair of samples) for all sample pairs.

*MAF<sfx>.png* is a histogram of the MAFs for the subset of SNPs used (if not all SNPs).

*HighRelatedness<sfx>.csv* contains pairs of samples and their  $G_5$  relatedness, where this relatedness is  $> hirel.thresh$  (default value of *hirel.thresh* is 0.9).

*Heatmap-G5<sfx>.png* is a heatmap plot using  $G_5$  relatedness. This is not produced if  $npc \leq 0$ . If *fcolo* for the relevant individuals has more than one colour, colour bars are added to the plot.

*HeatmapOrder<sfx>.csv* contains a list of the samples in the order they are plotted on the heatmap. *rowInd* is the index values (written on the heatmap plot), *seqIDInd* is the position of the individual in *seqID.csv*; *seqID* is also included. For “standard” cases, where all *seqID* samples are included, the values of *rowInd* and *seqIDInd* will be the same.

*Gcompare<sfx>.png* is a plot comparing relatedness estimates for  $G_1$ ,  $G_3$  and  $G_5$ .

*G<sfx>-diag.png* is a plot of diagonal elements (self-relatedness estimates) of  $G_4$  against those of  $G_5$  (illustrating the effect of correcting for depth).

*G<sfx>diagdepth.png* is a plot of diagonal elements of  $G_5$  against the logged sample depth. We do not expect there to be a relationship between these variables (unless planned) so this serves as a diagnostic for e.g. non-Mendelian SNPs and/or the assumption of random sampling of alleles during sequencing.

*PC1v2G5<sfx>.png* (if  $npc > 0$ ) is a plot of 2<sup>nd</sup> versus the 1<sup>st</sup> principal components. If only one component was requested, a histogram of the 1<sup>st</sup> component is produced.

*PCG5<sfx>.pdf* (if  $npc > 2$ ) is a scatterplot matrix of the first *npc* principal components.

*MDS1v2G5<sfx>.png* (if  $npc > 0$ ) is a plot of 2<sup>nd</sup> versus the 1<sup>st</sup> principal components. If only one component was requested, a histogram of the 1<sup>st</sup> component is produced.

There is a vector *fcolo* (length *nind*) of colours to be used for the individuals in these plots. It defaults to all black, but can be reset after sourcing the program and before calling *calcG*.

### Output genomic relationship matrix (*writeG*)

A function, *writeG*, for saving genomic relationship matrices, is defined.

Usage: *writeG* (*Guse*, *outname*, *outtype*=0, *indsubset*, *IDuse*)

Arguments:

<i>Guse</i>	the $G$ matrix of relationships to output, should be a square matrix
<i>outname</i>	text used in the naming of the output file(s)
<i>outtype</i>	constant or vector containing the type(s) of output required. If <i>outtype</i> contains any of the following values, the corresponding output is produced:
1	an R datasets file containing the $G$ matrix and corresponding <i>seqID</i>
2	a .csv file containing the $G$ matrix with row and column headings
3	a .csv file containing the $G$ matrix in “long” format, i.e. one row for every (unique) relationship pair including selfs; columns are IDs of first

	and second individual, followed by the relatedness value
	4 a .csv file containing inbreeding for each individual; first column contains IDs, second column contains inbreeding estimates
indsubset	a vector of integers (between 1 and <i>nind</i> , inclusive) of the individuals in the G matrix. The default assumes all individuals.
IDuse	a vector of IDs to use in the output, corresponding to the order in <i>Guse</i> , the default is to use values of <i>seqID</i> as the identifiers (in which case <i>seqID</i> must exist)

Details: One or more files are written to the default directory, according to *outtype*:

*<outname>.RData* an R data file containing the G matrix and corresponding *IDuse* values, produced when *outtype* contains a 1. The G matrix is named based on the object specified in *Guse*, removing text up to \$ and from [, if either of these are present. As an example using *writeG(Gfull\$G5[1:100,1:100],outtype=1)* will result in the G matrix being named G5.

*<outname>.csv* a csv file containing the G matrix, produced when *outtype* contains a 2. The first column is labelled with the name of the object passed to *IDuse* and contains the values of *IDuse*. The other columns are labelled with the values of *IDuse*.

*<outname>-long.csv* a csv file containing the unique relatedness values, one row for every pair of individuals (including selfs), produced when *outtype* contains a 3. The columns are labelled ID1, ID2 and rel. *IDuse* is used for the ID values.

*<outname>-Inbreeding.csv* a csv file containing inbreeding values (self-relatedness minus 1), produced when *outtype* contains a 4. The first column is labelled with the name of the object passed to *IDuse* and the second column as Inbreeding. *IDuse* is used for the ID values.

### Relatedness comparison function (*GCompare*)

This is a function to help make comparisons between different estimates of relatedness on the same set (or overlapping subsets) of individuals. These different estimates may come from different genotyping technologies (e.g. SNP chip vs GBS), different protocols (e.g. GBS with different restriction enzymes, different levels of multiplexing samples, different SNP callers) or using different SNP filters.

The program inputs a set of (genomic) relationship matrices (GRMs) and a corresponding set of individual IDs. The output is a set of scatterplots (possibly as scatterplot matrices) and corresponding regression output. The relatedness estimates between each pair of (different) individuals for each pair of GRMs are compared, as are those for the self-relatedness estimates for each individual. For any pair of GRM, all individuals common to the GRM are used. There needs to be at least two such individuals for the code to work.

Additionally, if the *MethComp* (Carstensen, 2015) R package is installed, there can be corresponding sets of plots using this package, with scatterplots of relatedness estimates below the diagonal, and 'Bland-Altman' (BA) plots (Altman and Bland, 1983) above the diagonal. The Bland-Altman plots have the differences on the vertical and the means on the horizontal axis, for the two relatedness estimates. These plots take a lot more CPU time than the regression plots.

Usage: *GCompare* (*Glist*,*IDlist*,*Gnames* = *paste0*("G.",1:length(*Glist*)), *plotname* = "", *whichplot*="both", *doBA*=FALSE, ...)

### Arguments:

<i>Glist</i>	a list of G matrices
<i>IDlist</i>	a list of ID variables, paired to the G matrices and in the same order as the data in the corresponding G matrix
<i>Gnames</i>	a set of labels to use for the G matrices (defaults to G1, G2, ...)
<i>plotname</i>	text to use in the naming of output files

whichplot	variable to choose which plot types are produced, can be one of: “diag”: compare diagonals (self-relatedness) “off”: compare off-diagonals (relatedness between individuals) “both”: compare both diagonals and off-diagonals. This is the default.
doBA	Additionally produce Bland-Altman plots. The default is FALSE.
...	Arguments to be passed to the plotting functions (e.g. col= for coloring). These need to be relevant to the plot types being produced (e.g. if a vector of colours, then it should not be used with <i>whichplot</i> =“both”).

Details: One or more plots are produced, depending on the options used. A set of ignorable warnings is issued.

*Gcompare- <plotname>-diag.png* a plot of the diagonal comparison(s). If more than 2 G matrices, this will be a scatterplot matrix with regression results in the upper matrix panels.

*Gcompare- <plotname>-offdiag.png* a plot of the diagonal comparison(s). If more than 2 G matrices, this will be a scatterplot matrix with regression results in the upper matrix panels.

*GcompareBA- <plotname>-diag.png* a scatterplot matrix BA plot of the diagonal comparison(s). The regression plots are in the lower diagonal and the BA plots in the upper diagonal. A grey line indicates equality ( $y=x$  for lower plots,  $y=0$  for upper plots). The BA plots have 3 additional horizontal lines being the mean & mean  $\pm 1.96sd$  ('95% limits of agreement).

*GcompareBA- <plotname>-offdiag.png* a scatterplot matrix BA plot of the off-diagonal comparison(s). See description of the BA plot for the diagonals for more details.

## Pedigree program (GBSPedAssign.R)

This program uses a relatedness matrix to verify given pedigrees and/or to find the best matching parents from groups of potential parents. Both these tasks require a pedigree file (with name given in *pedfile*). For parent matching a groups file (with name given in *groupsfile*) is also required. See below for the formats for these files. Father (Mother) verification is undertaken if the pedigree file contains a FatherID (MotherID) variable. Father (Mother) matching is undertaken if a groups file is given and the pedigree file contains a FatherGroup (MotherGroup) variable. The Group fields are read as text fields; if they are not present this causes a warning which can be ignored.

For parent matching, mismatch statistics are calculate for reporting and using, in addition to relatedness values, for assigning parentage. The 'raw' mismatch rate is the proportion of apparent (i.e. using observed genotypes) mismatches (i.e., genotypes inconsistent with parentage). 'Excess' rates are the differences between raw rates and rates that are expected given the genotype uncertainty due to the GBS process (manuscript in prep). A number of variables (see below) control how the mismatch rates are calculated and used. Mismatch rates are calculated for offspring-parent pairs and for offspring-parent trios (if matching to both parents). If both parents are being matched, the apparent parent-pair mismatch rates (offspring and parent genotypes incompatible) are given for each combination of the best two matching parents.

Before calling the program, the variable *GCheck* must be set to the name (as a string) of the G matrix to use. If this is for a subset of individuals, *indsubset* must be set to the indices of those individuals (as used in *calcG*). In addition, *rel.thresh* (and/or *rel.threshF* and/or *rel.threshM* for fathers and mothers, respectively) may be set to override the default relatedness value of 0.4 for declaring a parentage verification (or to allow parent assignment). A number of other variables control calculated results and reporting for parent matching. *mindepth.mm* may be set to override the default minimum depth (1) for a SNP for the individuals being compared when calculating (excess) mismatch rates for parentage matching. The default value is recommended for calculating excess rates, but raw rates are likely to be more useful when using a higher

threshold. *snpsubset* may be set to indices of SNPs to be considered for use in calculating mismatch rates and for bootstrapping (see below, this will usually be the same subset as used for calculating the G matrix being used). The excess mismatch rate thresholds for declaring parentage are set by *emm.thresh* (parent-offspring pair; default value of 0.01) and *emm.thresh2* (parent-offspring trio; default value of twice *emm.thresh*). An alternative parentage is suggested when a possible pair (mother and father) have an excess mismatch rate that is lower than that for the best (i.e., most highly related) father and best mother by more than *emmdiff.thresh2* (default value of 0).

For parent pair matching, the estimated relatedness between the parent pairs (all four combinations of best and 2<sup>nd</sup> best matching fathers and mothers) are calculated. The relatedness for the best matching pair of parents is compared with the estimated inbreeding for the individual. High values of parent relatedness (compared with the inbreeding of the individual) may indicate that one of the parents has been incorrectly assigned to a relative of the other parent. A parent-pair match will be excluded as a match if the parent relatedness exceeds offspring inbreeding by at least *inb.thresh* (default value 0.2).

A bootstrapping procedure is available to provide a metric on the closeness of parent-offspring match compared to that with the 2<sup>nd</sup> best parent. The procedure resamples SNPs (with replacement), recalculates the relatedness values (for the offspring and each of the two best parents) and reports the percentage of times that the best parent is still the better of the two among the bootstrap replicates. As bootstrapping is quite time-consuming, it is invoked only when there are 2 possible parents with similar (within *boot.thresh*) parent-offspring relatedness values. The number of bootstrap replicates is set by *nboot* (default value 1000). Three other variables (*depth.min*, *depth.max*, *puse*) mirror those used in calcG to allow the bootstrapping to calculate relatedness in the same way as was used for the G matrix being used in parentage assignment. These variables should be set to the same values as those used for calculating the G matrix. An assignment is flagged (see below) if the best parent is the better one in the bootstrap samples in less than *boota.thresh* percent (default value 99) of the replicates.

The output files contain variables to indicate whether the parentage should be accepted. These variables are called *FatherAssign* and *MotherAssign* for single parent matching of fathers and mothers, respectively. The codes used as values for these variables are:

Assign code	Description
<b>N</b>	Relatedness estimate for best matching parent is below <i>rel.threshF</i> or <i>rel.threshM</i> (for fathers and mothers, respectively).
<b>E</b>	Excess mismatch rate for best matching parent exceeds <i>emm.thresh</i> .
<b>A</b>	Alternate assignment: the 2 <sup>nd</sup> best parent appears acceptable. This parent has relatedness exceeding <i>rel.threshF</i> or <i>rel.threshM</i> (for fathers and mothers, respectively) and excess mismatch rate that is lower than <i>emm.thresh</i> when the best parent had excess mismatch rate exceeding this threshold.
<b>B</b>	Best matching parent is the better one in less than <i>boota.thresh</i> % of the bootstrap replicates.
<b>Y</b>	Best matching parent passes all assignment criteria

The variable for indicating whether a parent-pair match should be accepted is *BothAssign* and takes values as shown:

Assign code	Description
<b>N</b>	Relatedness estimate for best matching parent is below <i>rel.threshF</i> or <i>rel.threshM</i> (for fathers and mothers, respectively).
<b>M</b>	Mother assigned, father not assigned.
<b>F</b>	Father assigned, mother not assigned.
<b>E</b>	Excess mismatch rate for best matching parent-pair exceeds <i>emm.thresh2</i> ,

	except when one parent assigned and the other has an E code, then the parent assignment is made.
<b>A</b>	An alternate parent-pair appears acceptable. This pair has excess mismatch rate less than <i>emm.thresh2</i> and lower than that for the best parent-pair by more than <i>emmdiff.thresh2</i> . If the alternate pair also passes the other checks, the pair is indicated by the value of <i>Alternate</i> , e.g. a value of F1M2 indicates that the alternate pair is the best father and 2 <sup>nd</sup> best mother.
<b>B</b>	At least one of the parents has a B code and the other is not assigned.
<b>I</b>	The best parent-pair relatedness exceeds the offspring inbreeding by at least <i>inb.thresh</i> .
<b>Y</b>	Best matching parent passes all assignment criteria

Where than one of the assign codes is possible, the one that ranks the highest (in the order given in the above tables) is used.

This program outputs summary statistics and a number of files. The %s of verified fathers and mothers are given, as well as the mean relatedness estimates for matching and non-matching fathers and mothers. The files, where relevant, are as follows:

*PedVerify.csv* returns the pedigree file with additional columns, as shown below:

Variable name	Description
<b>FatherRel</b>	Relatedness estimate between individual and it's specified father
<b>FatherMatch</b>	TRUE if <i>FatherRel</i> > <i>rel.threshF</i>
<b>MotherRel</b>	Relatedness estimate between individual and it's specified mother
<b>MotherMatch</b>	TRUE if <i>MotherRel</i> > <i>rel.threshM</i>

*FatherVerify.png* is a scatterplot matrix showing *FatherRel* (see above), the position of the individual in the pedigree file and the position of the recorded father in the pedigree file. This is useful for seeing the distribution of relatedness values, and possibly for detecting sample tracking issues (if the order in the pedigree file relates to the order samples are processed at a particular stage).

*MotherVerify.png* is a scatterplot matrix like *FatherVerify.png* but for mother verification.

*FatherMatches.csv* shows the results of the father matching. It returns the first two columns of the pedigree file with additional columns, as shown below:

Variable name	Description
<b>BestFatherMatch</b>	IndivID of the father from the <i>FatherGroup</i> having the highest estimated relatedness to the individual
<b>FatherMatch2nd</b>	IndivID of the father from the <i>FatherGroup</i> having the 2 <sup>nd</sup> highest estimated relatedness to the individual
<b>Fatherrel</b>	The estimated relatedness for <i>BestFatherMatch</i>
<b>Fatherrel2nd</b>	The estimated relatedness for <i>FatherMatch2nd</i>
<b>Father12rel</b>	The estimated relatedness between <i>BestFatherMatch</i> and <i>FatherMatch2nd</i> .
<b>mmrateFather</b>	The (raw) mismatch rate for <i>BestFatherMatch</i>
<b>mmnumFather</b>	The number of snps used to calculate <i>mmrateFather</i>
<b>exp.mmrateFather</b>	The expected mismatch rate for <i>BestFatherMatch</i>
<b>mmrateFather2</b>	The (raw) mismatch rate for <i>FatherMatch2nd</i>
<b>exp.mmrateFather2</b>	The expected mismatch rate for <i>FatherMatch2nd</i>
<b>Fathersd</b>	The bootstrap sd of <i>Fatherrel</i> values (for bootstrapped cases, the variable is present only if there are bootstrapped caess)



<b>FatherReliability</b>	The % of bootstrap results where <i>Fatherrel</i> > <i>Fatherrel2nds</i> (for bootstrapped cases, the variable is present only if there are bootstrapped caess)
<b>FatherAssign</b>	The code for father assignment.

*MotherMatches.csv* shows the results of the mother matching (with columns as for *FatherMatches.csv* but for mothers instead of fathers).

*BothMatches.csv* shows the results of both father and mother matching (for individuals with both *FatherGroup* and *MotherGroup*). It contains the columns of *FatherMatches.csv* and *MotherMatches.csv* with additional columns, as shown below:

Variable name	Description
<b>mmrateF&lt;fatherrank&gt;M&lt;motherrank&gt;</b>	The (raw) mismatch rate for possible parent matches, where <fatherrank> is 1 to indicate <i>BestFatherMatch</i> and 2 to indicate <i>FatherMatch2nd</i> , and similarly for <motherrank>.
<b>mmnumF&lt;fatherrank&gt;M&lt;motherrank&gt;</b>	The number of SNPs used to calculate <i>mmrateF&lt;fatherrank&gt;M&lt;motherrank&gt;</i>
<b>exp.mmrateF&lt;fatherrank&gt;M&lt;motherrank&gt;</b>	The expected mismatch rate corresponding to <i>mmrateF&lt;fatherrank&gt;M&lt;motherrank&gt;</i>
<b>relF&lt;fatherrank&gt;M&lt;motherrank&gt;</b>	The estimated relatedness between the pair of possible parents
<b>Inb</b>	The estimated inbreeding of the offspring
<b>BothAssign</b>	The code for the parent-pair assignment
<b>Alternate</b>	An alternative (to F1M1) parent pair

*GroupsParentCounts.csv* returns the groups file with additional columns, as shown below:

Variable name	Description
<b>FatherFreq</b>	Number of offspring where this father is the <i>BestFatherMatch</i> in this group
<b>MotherFreq</b>	Number of offspring where this mother is the <i>BestMotherMatch</i> in this group

*BestFatherMatches.png* is a plot of the raw mismatch rate for *BestFatherMatch* against the estimated relatedness (*Fatherrel*). Points are coloured using *fcolo* and a grey vertical line indicates the value of *rel.thresh* used.

*BestFatherMatchesE.png* is the same *BestFatherMatches.png* except that the excess mismatch rate is plotted. A grey horizontal line indicates the value of *emm.thresh* used.

*Best2FatherMatches.png* is a plot of the estimated relatedness for *FatherMatch2nd* (*Fatherrel2nd*) against that for *BestFatherMatch* (*Fatherrel*). Points are coloured using a scale based on the excess mismatch rate (*mmrateFather* - *exp.mmrateFather*) for father-offspring and the line of equality is drawn (by definition all points fall below the line). Vertical and horizontal grey lines indicate the value of *rel.threshF* or *rel.threshM* (for fathers and mothers, respectively) used.

*ExpMM-Father.png* is a plot of the raw mismatch rate against the expected mismatch rate for *BestFatherMatch*. A red line shows where these are equal and a grey line shows the boundary for an E assign code. Points are coloured using *fcolo* and the symbols indicate *FatherAssign*.

*BestMotherMatches.png*, *BestMotherMatchesE.png*, *Best2MotherMatches.png* and *ExpMM-Mother.png* are the corresponding plots to *BestFatherMatches.png*, *BestFatherMatchesE.png* and *Best2FatherMatches.png* and *ExpMM-Father.png*, respectively, for mothers.



*ParRel-Inb.png* is a plot of estimated parent-pair relatedness against offspring estimated inbreeding. Points are coloured according to the mean depth in the offspring (as depth is more critical for inbreeding than relatedness estimation), and with a symbol corresponding to *BothAssign* (see *ExpMM-Both.png* for a key).

*MMrateBoth.png* is a scatterplot matrix plot of the four combinations of parent-pair raw mismatch rates that were saved in *BothMatches.csv*. Points are coloured using *fcolor* and the lines of equality are drawn (in red).

*MMrateBothE.png* is a scatterplot matrix plot of the four combinations of parent-pair excess mismatch rates. Points are coloured using *fcolor*, the lines of equality are drawn (in red), a grey line shows the boundary for an E assign code and the symbols for the points denote *BothAssign*. The key for the symbols can be found in *ExpMM-Both.png*.

*ExpMM-BothE.png* is a plot of raw versus expected parent-pair mismatch rates. Points are coloured using *fcolor* and the symbols for the points denote *BothAssign*.

## Input formats

The genotype input format is set with *gform*, one of “uneak” (the default), “Tassel”, “TagDigger” or “Chip”.

### GBS via UNEAK

The default input format (‘uneak’) is a ‘hapmap count’ formatted file as produced by the UNEAK pipeline (Lu *et al.* 2013). This is a tab-separated flat text file with the first column being the SNP identifier, then a column for each genotyped individual (or sample, or other genotyping unit), followed by 5 columns of summary information (HetCount\_allele1, HetCount\_allele2, Count\_allele1, Count\_allele2, Frequency). Only the last of these 5 is used. Each row is for a different SNP. The column for each individual contains the genotype information as the allele depth (number of reads of that allele) for the ‘reference’ and ‘alternate’ alleles, respectively. The designation of reference and alternate is arbitrary for this software. The numbers of reads are separated by a pipe symbol (“|”). There is a header line, which, for the genotype columns, is taken as the identifiers of the individuals.

### GBS via Tassel

An additional format(‘Tassel’) is available that may be easier to use for GBS data that has been manipulated in Tassel. It is similar to the uneak format, but allele depths in a genotype are separated by a comma (“,”), has two columns before genotype data (, and no columns following the genotype data. The first two columns are the chromosome and position (which together, separated by an underscore, serve as the SNP identifier), respectively. As with the “uneak” format, this is a tab- separated flat text file with a header row.

A python helper script *vcf2ra\_ro\_ao.py* is available to convert .vcf files (containing either AD – allelic depth – or AO and RO fields) to the ‘Tassel’ format.

### GBS via TagDigger

TagDigger (<https://github.com/lvclark/tagdigger>, Clarke and Sacks, 2016) is a tool for SNP calling from a given set of tags (sequences). It is likely to be used in a production environment, where the set of SNPs being called is unlikely to change much with additional samples being added. The ‘TagDigger’ format requires a comma delimited file with sample results in rows and SNP results in pairs of columns (count of reference allele, count of alternate allele). The first column contains the sample identifier. The header row, apart from the first value, contains SNP/allele

identifiers. It is assumed that these identifiers have a SNP identifier followed by an underscore, followed by the allele identifier. The text preceding the underscore is taken as the SNP name (the other text is ignored).

TagDigger files will be read with the `fread` function from the `data.table` package, if that package is installed. This is faster than the method used when the package is not available. Files compressed with the `gzip` (.gz) format can be read by both methods on linux platforms, but not for `fread` on other platforms.

## Chip

Fully recorded genotypes can be entered via the “Chip” format. This comma-separated format has results for each individual in the rows and SNP results in a column. There is a header row (SNP identifiers) and the first column contains individual identifiers. Subsequent columns contain the SNP results. Genotype data is given in 0/1/2 format, representing first homozygote, heterozygote and second homozygote, respectively. Designation of which allele is the ‘first’ is arbitrary.

## Pedigree file

An optional pedigree file can be given, and will be used to verify or find parent matches. This is a comma separated file (csv). All individuals to be considered as offspring or parents need to have a row in this file. The columns of this file are specified below. The names must be exactly as specified. Additional columns may be present in the file.

Variable name	Required?	Description
<b><i>IndivID</i></b>	Y	identifies individuals in the pedigree and groups files
<b><i>seqID</i></b>	Y	matches <i>IndivID</i> to the identifier in the genotype file
<b><i>FatherID</i></b>	N	Recorded <i>IndivID</i> of father
<b><i>MotherID</i></b>	N	Recorded <i>IndivID</i> of mother
<b><i>FatherGroup</i></b>	N	Group label for group of potential fathers for the given <i>IndivID</i>
<b><i>MotherGroup</i></b>	N	Group label for group of potential mothers for the given <i>IndivID</i>

Father and mother group labels should be distinct. If required, they are entered for the progeny. The information linking these labels to the set of possible parents is placed in the groups file.

## Groups file

If parent matching is required, then a groups file describing the group labels in the pedigree file is required. This is a comma separated file (csv). The columns (both required) of this file are specified below. The names must be exactly as specified. Additional columns may be present in the file.

Variable name	Description
<b><i>IndivID</i></b>	identifier for potential parent, matching <i>IndivID</i> in the pedigree file
<b><i>ParGroup</i></b>	Group label for the group that <i>IndivID</i> belongs to

There should be one row for each group a potential parent belongs to.

## Example

The example given is based on an earlier version of the code. Files in directory :  
GBSRun.R HapMap.hmc.txt.gz Ped-GBS.csv Ped-Groups.csv

## GBSRun.R

```
genofile <- "HapMap.hmc.txt.gz"

source("<source directory>/GBS-Chip-Gmatrix.R")
Gfull <- calcG()
GHwdgm.05 <- calcG(which(HWdis > -0.05), "HWdgm.05", npc=4) #
recalculate using Hardy-Weinberg disequilibrium cut-off at -0.05

pedfile <- "Ped-GBS.csv"
groupsfile <- "Ped-Groups.csv"

rel.thresh <- 0.2
GCheck <- "GHwdgm.05$G5"
source("<source directory>/GBSPedAssign.R")
```

<source directory> should be replaced with the location of the relevant .R files before running.

linux command:

R CMD BATCH --no-save GBSRun.R &

Files in directory after running code:

AlleleFreq.png	GcompareHwdgm.05.png	MotherVerify.png
Best2FatherMatches.png	Gcompare.png	PC1v2G5Hwdgm.05.png
Best2MotherMatches.png	Gdiagdepth.png	PCG5Hwdgm.05.pdf
BestFatherMatchesE.png	G-diag.png	Ped-GBS.csv
BestFatherMatches.png	GHwdgm.05diagdepth.png	Ped-Groups.csv
BestMotherMatchesE.png	GHwdgm.05-diag.png	PedVerify.csv
BestMotherMatches.png	GroupsParentCounts.csv	SampDepthCR.png
CallRate.png	HapMap.hmc.txt.gz	SampDepthHist.png
Co-call-Hwdgm.05.png	Heatmap-G5Hwdgm.05.png	SampDepth.png
Co-call-.png	Heatmap-G5.png	SampDepth-scored.png
ExpMM-Father.png	HighRelatedness.csv	SampleStats.csv
ExpMM-Mother.png	HWdisMAFsig.png	seqID.csv
FatherMatches.csv	LRT-hist.png	SNPCallRate.png
FatherVerify.png	LRT-QQ.png	SNPDepthHist.png
finplot.png	MAFHwdgm.05.png	SNPDepth.png
GBSRun.R	MAF.png	
GBSRun.Rout	MotherMatches.csv	

A workshop using this example was given at the [2015 MapNet meeting](#). [Instructions](#) and [course notes](#) are available.

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