

Haplotag: software for haplotype-based genotyping-by-sequencing analysis

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Primary data analysed in this report are available from the NCBI short read archive

(<http://www.ncbi.nlm.nih.gov/sra/>) under project accession number SRP037730. The software described in this report is available from <http://haplotag.aowc.ca>.

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Abstract

Genotyping-by-sequencing (GBS) and related methods are based on high-throughput short-read sequencing of genomic complexity reductions followed by discovery of SNPs within sequence tags. This provides a powerful and economical approach to whole-genome genotyping, facilitating applications in genomics, diversity analysis, and molecular breeding. However, due to the complexity of analysing large data sets, applications of GBS may require substantial time, expertise and computational resources. Haplotag, the novel GBS software described here, is freely available and operates with minimal user-investment on widely-available computer platforms. Haplotag is unique in fulfilling the following set of criteria: (1) operates without a reference genome; (2) can be used in a polyploid species; (3) provides a discovery mode and a production mode; (4) discovers polymorphisms based on a model of local haplotypes within sequenced tags; (5) reports SNPs as well as haplotype-based genotypes; (6) provides an intuitive visual “passport” for each inferred locus.

Summary (100 words): This report describes and makes freely available a novel software application designed to analyze and report results of genotyping-by-sequencing. The software takes a novel approach to discovery and validation of loci based on local haplotypes within sequenced tags. Output from these analysis are formatted as intuitive passports for each cluster of orthologous loci.

Genotyping-by-sequencing (GBS: Elshire, et al., 2011) and similar methods (e.g. RAD: Miller, et al., 2007) have become important strategies for whole genome genetic diversity analysis and related studies in many plant and animal species. These methods are based on high-throughput short-read sequencing of genomic complexity reductions followed by discovery of SNPs within sequence tags. While GBS is powerful and economical, it is also complex: requiring the barcoding and multiplexing of samples, the deconvolution of large data files, the alignment or assembly of short reads (tags), and the discovery and filtering of SNPs.

There are several available bioinformatics pipelines for GBS analysis, including Stacks (Catchen, et al., 2011), TASSEL (Glaubitz, et al., 2014), UNEAK (Lu, et al., 2013) and other custom-designed pipelines (e.g. Poland, et al., 2012; Sonah, et al., 2013). Most pipelines require a reference genome, while UNEAK is designed to operate independently from a reference genome and Stacks has the ability to run with or without a reference genome. Most pipelines require the installation of third party programs (e.g. to assemble or align sequences) while UNEAK requires only the installation of a JAVA run-time environment. Because of this, UNEAK can be run on any computer platform with adequate resources, and it has been popular among researchers studying species where no reference genome is available. However, the UNEAK pipeline excludes all SNPs that belong to multi-locus series, SNPs from tags containing multiple SNPs, or SNPs with more than 2 alleles. In our experience with GBS in hexaploid oat (Huang, et al., 2014) UNEAK excluded at least 30% of potentially useful SNPs that were discovered by an alternate

customized pipeline. Furthermore, the developers of UNEAK (personal communication) have indicated that no further development of UNEAK will be performed.

With high-density genotyping comes the possibility to analyse data based on haplotypes and the ability to impute missing data (Swarts, et al., 2014) which may be of particular importance in GBS analyses where incomplete data are prevalent. Genome wide association studies (GWAS) based on haplotypes could also allow the discovery of cryptic QTL associations that have eluded analysis based on single SNPs (Lorenz, et al., 2010). Because GBS data are acquired from sequenced fragments that often contain multiple SNPs, direct information about localized haplotypes is available within a GBS pipeline. However, to our knowledge, no GBS pipeline provides a simple method to access these haplotypes directly in an output file. Since accurate haplotype inference normally requires a reference genome, the availability to extract haplotypes directly from within GBS fragments could be of particular interest in a species where no reference is available.

Our objective was to develop user-friendly GBS software that operates with minimal user-investment on widely-available computer platforms. Additionally, we intended this software to meet the following requirements: (1) to operate without the requirement for a reference genome; (2) to operate in a polyploid or duplicated genome, distinguishing paralogous loci when an appropriate population filter is available; (3) to provide a discovery mode as well as an efficient production mode for scoring previously-discovered loci; (4) to discover polymorphisms based on models of segregating local haplotypes within GBS sequenced tags; (5) to report

results in a variety of formats, including SNP- and haplotype-based genotypes, and (6) to provide an intuitive “passport” for each inferred locus, enabling visual inspection and validation of discovered GBS loci.

Materials and Methods

Software named ‘Haplotag’ was written in the Pascal programming language, implemented as Free Pascal (freepascal.org) within the Lazarus programming environment (lazarus-ide.org). Both of these programming packages are open source, available on multiple platforms, and actively supported by developer communities. Most algorithms within Haplotag were written to operate in parallel when executed on a computer with multiple processors. The code was compiled for the Windows 64-bit environment (Microsoft, Redmond WA) and tested with Windows XP, 7, 8, and 10 and server 2008. Haplotag was tested on many different computers, but evaluations reported below were executed on a computer running Windows server 2008 with two Intel (Santa Clara, CA) Xeon X5670 processors running at 2.93 GHz, each having six cores divided into 12 threads (total 24 threads). The test machine contained 96 GB RAM, but all reported analyses were confirmed to run within 24 GB RAM. All input and output data resided on a locally-attached 4TB disk, since prior experience indicated poor performance when reading and writing to a network drive.

Haplotag was evaluated using a set of small simulated demonstration files as well as on the full set of primary GBS reads from oat described by Huang, et al. (2014). The later data contained

894 taxa consisting of 360 diverse oat lines and 534 mapping progeny from six bi-parental populations. Both Haplotag and the UNEAK pipeline were run with a minimum merged tag count of 50, which is higher than the threshold used in the earlier work due to subsequent optimization. Output from both pipelines was filtered across the full population to maintain markers for which genotype calls were $\geq 50\%$ or $\geq 80\%$ complete, heterozygosity was $\leq 10\%$, and minor allele frequency was $\geq 5\%$. The error detection threshold in UNEAK was set to 0.02. Additional filters for Haplotag included a maximum base difference of 3 for aligning orthologs, a maximum of 9 tags per ortholog, a maximum heterozygote frequency on a haplotype basis of 0.25, and a maximum tolerance for tri-zygotes and multi-zygotes of 1% and 0%, respectively.

Data and software availability:

Data analysed in this report were deposited in the NCBI short read archive (<http://www.ncbi.nlm.nih.gov/sra/>) under project accession number SRP037730, and the GBS key for analysis was available in Table S4 of Huang, et al. (2014). Supplemental files (described include: the Haplotag manual (S1), and sample output (S2 and S3). Haplotag is available as an executable distribution for recent versions of Windows 64-bit environments (XP, and versions 7 through 10). The distribution can be obtained from the site <http://haplotag.aowc.ca/> which provides a download links for a compressed file that contains the Windows executable, a user manual (also in S1) and demonstration files. Future updates will be maintained at this site, and a voluntary registration is provided to monitor interest in this software and to enable announcements regarding major revisions. The Pascal source code was made available to

reviewers of this work, and will be provided by request on an as-is basis for any non-commercial use based on an open source license. The source code is expected to be compatible with any operating system where a Free Pascal compiler is available, although minor modifications to the code may be required to adapt it for the file systems of other operating environments.

Results and Discussion

Software execution:

The operation and function of Haplotag is described in the accompanying manual (S1) which references a set of small simulated input files for demonstration purposes. The input files are archived within the software distribution archive. When extracted, the demonstration files fall within three separate subdirectories, each containing a complete self-contained set of demonstration files for one of three primary modes in which Haplotag can operate. Within each subdirectory is a master input file with the default name “*HTinput.txt*” which contains all relevant parameter specifications as well as a set of pipeline commands that Haplotag will follow in the order listed. Based on these commands, Haplotag can read and process data from three starting points (figure 1) representing the three modes of operation.

There is currently a requirement to run part of the UNEAK GBS pipeline prior to running Haplotag in order to de-convolute the raw barcoded sequence data, produce a tag count file for

each sample, and write a merged tag count file for the entire project. The UNEAK pipeline executes these steps very efficiently, thus the replacement of this functionality was not a priority. The current Haplotag distribution provides a small helper utility to assist users in writing the UNEAK script and converting binary output to the text files required by Haplotag. A standalone replacement for UNEAK is being developed which may allow the analysis of tags longer than 64bp, but this tag length is a current limitation of both UNEAK and the current version of Haplotag. Sequencing data with short reads of 100bp is ideal for this type of analysis, since the barcode may occupy up to the first 10 bases, and this allows truncation of lower quality bases at the 3' end of the read. Reads of longer than 100bp can be analysed, but the tags will be truncated at 64 bases.

The cluster discovery mode (Figure 1A) is designed for applications where complete *de-novo* SNP discovery is required. This de-novo clustering step is multi-threaded, but it may still run slowly on very large data sets. The haplotype discovery mode (Figure 1B) reduces the scale of analysis by seeding the clusters with a set of pre-determined tags. This feature is useful for maintaining the legacy nomenclature of reference sequences from prior GBS analyses. It could also be used to seed the assembly of orthologs using predicted fragments from a sequenced genome. Alternatively, this step could incorporate consensus sequences from an alternate or more efficient clustering algorithm. The production mode (Figure 1C) is designed for applications where SNPs and Haplotypes have already been discovered by Haplotag using a large and diverse set of populations, and where the objective is to genotype new samples while maintaining exactly the same nomenclature of loci, haplotypes, and SNPs. No new haplotypes

will be discovered in production mode, so it is not recommended for an application where the diversity of new taxa falls outside of the diversity where the model was built.

What distinguishes Haplotag from other GBS pipelines is the treatment of the tags as haplotypes, and the development of locus models using a population filter to validate the diploid segregation these haplotypes. Prior to the model discovery, tags are deliberately over-assembled into clusters that potentially represent multiple orthologous loci. Then Haplotag tests every possible combination of haplotypes within each cluster to identify mutually exclusive groups of haplotypes that behave as single loci. This model testing is based on a population filter, which specifies threshold parameters for heterozygosity, allele frequency, and genotype completeness. The result can be a single locus within a single orthologous cluster, or multiple loci within the same orthologous cluster. The latter is common in polyploid or recently duplicated genomes. Results of locus prediction and genotype scoring are summarized within a single passport file for each ortholog cluster (see below). Although the model selection within ortholog clusters does not incorporate sequence divergence, the population filter invariably identifies loci having haplotypes that diverge less within loci and more among loci.

Software function, as illustrated by passport files:

Another important and unique feature of Haplotag is the automated production of a ‘passport’ file for each orthologous cluster of loci. This is illustrated by one passport from the analysis of the included demonstration data (Figure 2). Passport files are formatted in plain HTML, such

that they can be viewed in any web browser. They are indexed in a master HTML file which can also be opened and searched in any browser. While these files can be opened directly from a local disk, they could also be uploaded to a website in order to provide external access to the results of an analysis. Individual passport files can be inspected to determine if program parameters are appropriate, or to explore the metadata and genotypes of specific loci. In our experience, these files also serve as intuitive graphical presentations that can assist in explaining the GBS concept and the program function to a lay audience.

For example, in Figure 2, we would first explain that the six sequences at the top (TagID 1 to 6) constitute all of the unique 64-base tags from the experiment that assembled into a single cluster. Potential SNPs in this cluster are highlighted, and counts of each tag are shown at the left. We would then explain that the species from which these tags are generated is polyploid, such that we suspect these tags may come from more than one locus. We might then click on the “details of model” link (which would open table S2) to illustrate how Haplotag has inspected all 57 possible combinations (“models”) of two or more tags from the available six tags. This step is referred to as a “population filter”, since it allows the exclusion of inappropriate models based on whether the tags in a model segregate in a diploid manner within the tested population. Parameters for population filtering (S1) include completeness, allele frequency, and heterozygosity. Here (S2) each model was evaluated based on whether it would pass this filter (yes or no). Next, the acceptable model having complete data for the greatest number of taxa (Model 42 in S2) was assigned as Locus-1. All models that overlapped with Model 42 were then removed, and remaining acceptable models were inspected. Of these,

the next best model was assigned to a locus (in this case, Model 48 is assigned as Locus-2). The above process is iterated indefinitely until no acceptable models remain. We would then point out that Locus-1 contains two SNPs, which could theoretically form four haplotypes, of which three haplotypes were observed. Locus-2 contains only one SNP, and thus, two haplotypes.

We would then draw attention to the inferred genotypes and segregation of these five haplotypes at two putative loci within the population of taxa, which are shown in the table at the bottom of the passport (Figure 2). In this idealized example, the genotypes of all 10 taxa are complete at both accepted loci. The numbers in each cell show the total counts of tags observed for each taxon under each haplotype within a selected locus. Those with non-zero counts for two (or more) haplotypes (e.g. Taxa TJ, under Locus 1) are scored as heterozygotes. These inferred genotypes are written to a simple text-based file called "HTgenos.txt". Since many programs for genetic analysis cannot read haplotypes, an alternate genotype file is written where genotypes are defined by SNP calls from within the haplotypes. In the example in Figure 2, three SNP calls would be written, with Locus-1 being converted to two sub-loci, identified by their SNP positions. Nomenclature output files are also written, such that all dependencies are represented in a hierarchical naming system. These files are designed with shared fields such that they could easily be loaded into a relational database designed for this purpose.

Parameter selection:

It is well known that results of SNP identification, especially in a polyploid without a reference genome, are highly dependent on methods and parameters (Huang, et al., 2014; Tinker, et al., 2014). As with other methods for SNP identification, there is no formal way to optimize the selection of model parameters within Haplotag. However, parameters need to be selected carefully, possibly using iterative testing, in order to obtain good results and avoid artefacts. In our experience, the best results from Haplotag are obtained when it is run across a large composite base population consisting of a mixture of bi-parental populations and diverse taxa representative of target germplasm. The bi-parental populations will allow validation of Mendelian segregation and mapping of the polymorphisms, while the diversity samples will ensure discovery of alternate haplotypes. The parameters used for the oat data presented below were based on recursive optimization for this type of experiment. If bi-parental populations are analyzed, then the minimum allele frequency filter can be raised appropriately. If the analysis is restricted to a single bi-parental population, then the filter could be set to achieve a specific chi-square cut-off. Setting the maximum heterozygote frequency to a low value is very useful to exclude non-Mendelian models, but this can only be applied effectively within inbred lines where the expected heterozygote frequency is significantly lower than 50%.

Evaluation of Haplotag using data from hexaploid oat:

Data from 894 taxa reported by Huang, et al. (2014) were reanalyzed to compare performance and output of Haplotag to that of the UNEAK pipeline. The first two steps of the UNEAK pipeline (production of tag counts and merged tag counts) were run to produce a common starting

point for both pipelines, requiring approximately 6h hours to run on the test environment from the raw sequence files. The UNEAK pipeline is not multi-threaded so the presence of 24 processors on this machine was not relevant. The remaining steps in the UNEAK pipeline took only 5min. The total count of SNP loci from the UNEAK pipeline passing the population filter at a completeness of 50% was 12,780. At a threshold of 80% presence, the count of filtered loci was 4260.

Running on the same machine, but utilizing 23 processors, the full Haplotag pipeline in cluster discovery mode took 6.9h. The cluster discovery step (assembly of tag clusters) took most of this execution time. After applying the same population filter, the number of haplotype-based locus calls from Haplotag was 29,421 with a completeness of 50% or 11950 with a completeness of 80%. When translated to SNP loci, the number of calls was 43,378 at 50% completeness or 17,117 at 80% completeness. The larger number of SNPs relative to haplotypes is due to the presence of multiple SNPs within the same haplotype.

In comparing the filtered SNPs called by UNEAK to the SNP calls from Haplotag, 4204 (94%) of the 4260 UNEAK SNPs filtered at 80% completeness were identical to those called by Haplotag at the same filtering level. In contrast, UNEAK identified only 24% of the 17,117 Haplotag SNPs filtered at 80% completeness. In general, Haplotag calls most of the SNPs called by UNEAK because these represent the orthologous clusters in Haplotag with exactly two haplotypes having only a single SNP difference. The small number of UNEAK SNPs that are missed by Haplotag are a result of the cluster building parameters in Haplotag, which may assemble

additional rare haplotypes into an orthologous cluster. In some cases this will result in a complex orthologous cluster that is excluded because it exceeds the tolerance of Haplotag. The UNEAK pipeline has a different network-based strategy that is intended to exclude rare haplotypes, because it is designed to seek models with two haplotypes and a single SNP. While it is possible to adjust Haplotag parameters to increase the coverage of UNEAK SNPs, this would be at the expense of a greater number of multi-haplotype models that are called by UNEAK.

Haplotag was also tested in production mode. Complete execution of Haplotag in production mode took only 11 minutes. Genotypes for 24,412 or 7,343 haplotype-based loci (at 50% or 80% completeness, respectively) were generated, which translated to 31,685 and 8,872 SNP loci, respectively. The reduced number of loci compared to the Haplotype ortholog discovery mode is due to the fact that the starting files for this analysis (HTLoci and HTAlleles) were produced from a previous analysis where Haplotag was run in haplotype discovery mode. In that analysis, orthologs were built from the full set of SNP reference sequences resulting from the merging of two GBS pipelines reported by Huang, et al. (2014), as well as from reference sequences discovered in subsequent work from a total of 3327 taxa. This strategy was used in order to preserve SNP nomenclature with prior published and submitted work. The disadvantage of this strategy, which we have now demonstrated, is that the current production files have not incorporated a large number of high quality “new” SNPs that are discoverable only by Haplotag. This new result will be considered in future GBS work in oat, and will require careful addition of new orthologs, loci, and haplotypes to the existing production files, while still preserving the legacy nomenclature.

Each Haplotag run produces a complete index of passport files for each called locus. While this index is written in HTML format, it can easily be manipulated into a table, which we have demonstrated in Supplement S3. This table provides links to the passport files for the 7,343 haplotype-based loci called in production mode and filtered at 80% completeness. We have chosen this output because it contains legacy SNPs and nomenclature (from Huang, et al., 2014) to which we have added known map positions. By loading all passport files to a web server, they do not need to be downloaded and duplicated by users of this resource. This strategy will be used in future to provide passports and metadata for public GBS data sets loaded into the T3/Oat database (<https://triticeaetoolbox.org/oat/>).

Figure Captions

Figure 1. Flow chart showing input files (green), output files (blue) and dependencies (connecting lines) associated with 'Haplotag' GBS discovery software. Default file names are shown in yellow, and are normally appended by ".txt" in the Windows file system. Three alternative pipelines (A, B, and C) are available, with required input labeled for each. The cluster discovery pipeline (A) and the haplotype discovery pipeline (B) start by clustering a complete inventory of tags (A) or a reduced inventory of tags from prior work (B) to produce clusters of orthologs. In (B), the complete inventory is then assembled against this template to increase the sampling of new haplotypes. A complete tag-by-taxa matrix of tag counts (HTBT) is then

formed for all tags belonging to clusters of two or more tags. Other output files are then created based on haplotype model fitting. In the production pipeline, only the files labelled by (C) are required, since genotyping is based on counting copies of haplotype-tags in the output files from previous discovery work.

Figure 2. Passport file produced by Haplotag from simulated demonstration files. Here, six tags (potential haplotypes) are identified at the top. After model fitting by population-based filtering, two locus-models are selected. When Haplotag is run in ‘verbose’ mode, the details of model selection are written in a separate file (see S2). Locus-1 contains three haplotypes and Locus-2 contains two. SNP positions are identified by color. The table at the bottom of the passport shows the tag counts at the presumed haplotypes within each locus. Counts greater than one are shaded, indicating that they are scored as “present”.

Supplementary Material

File S1. Complete user manual for Haplotag. Future updates may be available at <http://haplotag.aowc.ca> where the latest version of Haplotag software can also be downloaded.

File S2: Details of model selection from the passport file presented in Figure 2. A total of 57 models were evaluated, which represent all possible combinations with 2 or more members of the 6 potential haplotypes. Of these, 5 models met the filtering criteria. Model 42 was

selected as the first valid locus with the greatest number of complete genotypes. Other models containing overlapping haplotypes from model 47 were then eliminated, and the process was iterated to select model 48 as a second valid locus.

Table S3. Index of haplotype based locus calls from the software Haplotag. Calls were made from primary sequence data originating from 894 taxa, described by Huang, et al. (2014). Data were analysed in the Haplotag production mode, such that SNP nomenclature from the previous work was preserved.

References

Catchen, J.M., *et al.* Stacks: building and genotyping loci de novo from short-read sequences.

G3: Genes, Genomes, Genetics 2011;1(3):171-182.

Elshire, R.J., *et al.* A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS one* 2011;6(5):e19379.

Glaubitz, J.C., *et al.* TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline.

PLoS one 2014;9(2):e90346.

Huang, Y.F., *et al.* Using genotyping-by-sequencing (GBS) for genomic discovery in cultivated oat. *PLoS ONE* 2014;9(7):e102448.

Lorenz, A.J., Hamblin, M.T. and Jannink, J.-L. Performance of single nucleotide polymorphisms versus haplotypes for genome-wide association analysis in barley. *PLoS one* 2010;5(11):e14079.

Lu, F., *et al.* Switchgrass genomic diversity, ploidy, and evolution: novel insights from a network-based SNP discovery protocol. *PLoS genetics* 2013;9(1):e1003215.

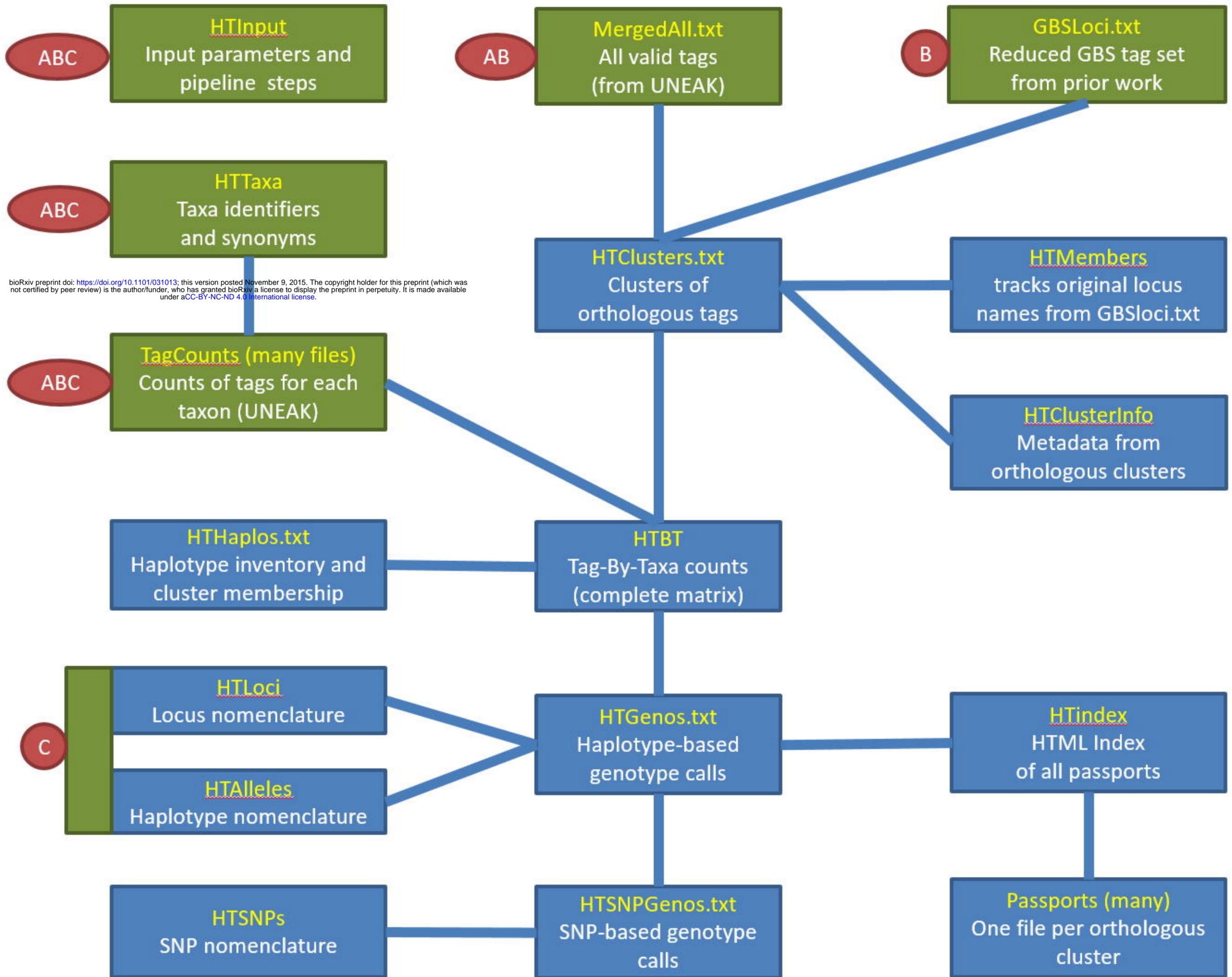
Miller, M.R., *et al.* Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome research* 2007;17(2):240-248.

Poland, J.A., *et al.* Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS one* 2012;7(2):e32253.

Sonah, H., *et al.* An improved genotyping by sequencing (GBS) approach offering increased versatility and efficiency of SNP discovery and genotyping. *PLoS one* 2013;8(1):e54603.

Swarts, K., *et al.* Novel methods to optimize genotypic imputation for low-coverage, next-generation sequence data in crop plants. *The Plant Genome* 2014;7(3).

Tinker, N.A., *et al.* A SNP genotyping array for hexaploid oat. *The Plant Genome* 2014;7(3).



Demo Output: GBS passport file for tag cluster: HC1

Cluster Consensus: TGCAGAAAAAAAAAAWTCAAATTABAATGCAGTCACYTTTGTAAAGTTTCTSGTTAAGATCAAAG

TagID	Count*	1	2	3	4	5	6
1	8	TGCAGAAAAAAAAAA	TCAAATTAT	AATGCAGTCAC	T	TTTGTAAAGTTTCT	CGTTAAGATCAAAG
2	7	TGCAGAAAAAAAAAA	TCAAATTAC	AATGCAGTCAC	T	TTTGTAAAGTTTCT	GGTTAAGATCAATG
3	3	TGCAGAAAAAAAAAA	TCAAATTAC	AATGCAGTCAC	C	TTTGTAAAGTTTCT	GGTTAAGATCAATG
4	2	TGCAGAAAAAAAAAA	TCAAATTAT	AATGCAGTCAC	T	TTTGTAAAGTTTCT	GGTTAAGATCAAAG
5	4	TGCAGAAAAAAAAAA	TCAAATTAT	AATGCAGTCAC	T	TTTGTAAAGTTTCT	GGTTAAGATCAATG
6	5	TGCAGAAAAAAAAAA	TCAAATTAC	AATGCAGTCAC	T	TTTGTAAAGTTTCT	GGTTAAGATCAATG

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*Count = number of taxa that contain this haplotype
(For details of model selection click [HERE](#))

Best model #1 fits 10 genotypes, with 10% heterozygotes.

Consensus: TGCAGAAAAAAAAAAATCAAATTAKAATGCAGTCAC TTTTGTAAAGTTTCTGGTTAAGATCAAAG

Locus 1	TagID	Count	1	2	3	4	5	6
	4	2	TGCAGAAAAAAAAAAATCAAATTAT	AATGCAGTCAC	TTTTGTAAAGTTTCT	GGTTAAGATCAAAG		
	5	4	TGCAGAAAAAAAAAAATCAAATTAT	AATGCAGTCAC	TTTTGTAAAGTTTCT	GGTTAAGATCAATG		
	6	5	TGCAGAAAAAAAAAAATCAAATTAG	AATGCAGTCAC	TTTTGTAAAGTTTCT	GGTTAAGATCAATG		

Best model #2 fits 10 genotypes, with 0% heterozygotes.

Consensus: TGCAGAAAAAAAAAAATTCAAATTACAATGCAGTCACYTTTGTAAAGTTTCTGGTTAAGATCAATG

Locus 2	TagID	Count	1	2	3	4	5	6
	2	7	TGCAGAAAAAAAAAAATTCAAATTACAAT	GCAGTCAC	TTTTGTAAAGTTTCT	GGTTAAGATCAATG		
	3	3	TGCAGAAAAAAAAAAATTCAAATTACAAT	GCAGTCAC	TTTTGTAAAGTTTCT	GGTTAAGATCAATG		

Literal segregation of tag presence in test population

Best selected models are on the left ----- haplotypes excluded from the selected locus model(s) are on the right

TaxaID	Project	TaxaName	Locus Model and haplotype IDs					
			Locus-1			Locus-2		No-model
			4	5	6	2	3	1
TA	Div	A	7	0	0	12	0	0
TB	Div	B	4	0	0	11	0	16
TC	Div	C	0	6	0	4	0	16
TD	Div	D	0	0	6	0	2	16
TE	BP	E	0	0	5	5	0	16
TF	BP	F	0	2	0	3	0	16
TG	BP	G	0	0	7	0	1	16
TH	BP	H	0	9	0	0	3	16
TI	BP	I	0	0	2	7	0	16
TJ	BP	J	0	1	1	3	0	0