Haplotype-based variant detection from short-read sequencing

Erik Garrison and Gabor Marth

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Abstract

The direct detection of haplotypes from short-read DNA sequencing data requires changes to existing small-variant detection methods. Here, we develop a Bayesian statistical framework which is capable of modeling multiallelic loci in sets of individuals with non-uniform copy number. We then describe our implementation of this framework in a haplotype-based variant detector, FreeBayes.

1 Motivation

While statistical phasing approaches are necessary for the determination of large-scale haplotype structure [Browning and Browning, 2007, Delaneau et al., 2012, Howie et al., 2011, Li et al., 2010], sequencing traces provide short-range phasing information that may be employed directly in primary variant detection to establish phase between proximal alleles. Present read lengths and error rates limit this physical phasing approach to variants clustered within tens of bases, but as the cost of obtaining long sequencing traces decreases [Branton et al., 2008, Clarke et al., 2009], physical phasing methods will enable the determination of larger haplotype structure directly using only sequence information from a single sample.

Haplotype-based variant detection methods, in which short haplotypes are read directly from sequencing traces, offer a number of benefits over methods which operate on a single position at a time. Haplotype-based methods ensure semantic consistency among described variants by simultaneously evaluating all classes of alleles in the same context. The use of locally-phased genotype data can lower the computational burden of genotype imputation by reducing the possible space of haplotypes which must be considered. Locally phased genotypes can be used to improve genotyping accuracy in the context of rare variations that can be difficult to impute due to sparse linkage information. Similarly, they can assist in the design of genotyping assays, which can fail in the context of undescribed variation at the assayed locus. Provided sequencing errors are independent, the use of longer haplotypes in variant detection can improve detection by increasing the signal to noise ratio of the genotype likelihood space that is used in analysis. This follows from the fact that the space of possible erroneous haplotypes expands dramatically with haplotype length, while the space of true variation remains constant, with the number of true alleles less than or equal to the ploidy of the sample at a given locus.

The direct detection of haplotypes from alignment data presents several challenges to existing variant detection methods. As the length of a haplotype increases, so does the number of possible alleles within the haplotype, and thus methods designed to detect genetic
variation over haplotypes in a unified context must be able to model multiallelism. However, most variant detection methods establish estimates of the likelihood of polymorphism at a given loci using statistical models which assume biallelism [Li, 2011, Marth et al., 1999] and uniform, typically diploid, copy number [DePristo et al., 2011]. Moreover, improper modeling of copy number impedes the accurate detection of small variants on sex chromosomes, in polyploid organisms, or in locations with known copy-number variations, where called alleles, genotypes, and likelihoods should reflect local copy number and global ploidy.

To enable the application of population-level inference methods to the detection of haplotypes, we generalize the Bayesian statistical method described by Marth et al. [1999] to allow multiallelic loci and non-uniform copy number across the samples under consideration. We have implemented this model in FreeBayes [Garrison, 2012].

2 Generalizing variant detection to multiallelic loci and non-uniform copy number

2.1 Definitions

At a given genetic locus we have \( n \) samples drawn from a population, each of which has a copy number or multiplicity of \( m \) within the locus. We denote the number of copies of the locus present within our set of samples as \( M = \sum_{i=1}^{n} m_i \). Among these \( M \) copies we have \( K \) distinct alleles, \( b_1, \ldots, b_K \) with allele frequencies \( f_1, \ldots, f_K \). Each individual has an unphased genotype \( G_i \) comprised of \( k_i \) distinct alleles \( b_{i1}, \ldots, b_{ik_i} \) and corresponding genotype allele frequencies \( f_{i1}, \ldots, f_{ik_i} \), which may be equivalently expressed as a multiset of alleles \( B_i : |B_i| = m_i \). For the purposes of our analysis, we assume that we cannot accurately discern phasing information outside of the haplotype detection window, so our \( G_i \) are unordered and all \( G_i \) containing equivalent alleles and frequencies are regarded as equivalent. Assume a set of \( s_i \) sequencing observations \( r_{i1}, \ldots, r_{is_i} = R_i \) over each sample in our set of \( n \) samples such that there are \( \sum_{i=1}^{n} s_i \) reads at the genetic locus under analysis. \( Q_i \) denotes the mapping quality, or probability that the read \( r_i \) is mis-mapped against the reference.

2.2 A Bayesian approach

To genotype the samples at a specific locus, we could simply apply a Bayesian statistic relating \( P(G_i|R_i) \) to the likelihood of sequencing errors in our reads and the prior likelihood of specific genotypes. However, this maximum-likelihood approach limits our ability to incorporate information from other individuals in the population under analysis, which can improve detection power.

Given a set of genotypes \( G_1, \ldots, G_n \) and observations observations \( R_1, \ldots, R_n \) for all individuals at the current genetic locus, we can use Bayes’ theorem to related the probability of a specific combination of genotypes to both the quality of sequencing observations and \( a \) priori expectations about the distribution of alleles within a set of individuals sampled from the same population:
\[
P(G_1, \ldots, G_n|R_1, \ldots, R_n) = \frac{P(G_1, \ldots, G_n)P(R_1, \ldots, R_n|G_1, \ldots, G_n)}{P(R_1, \ldots, R_n)} \tag{1}
\]

\[
P(G_1, \ldots, G_n|R_1, \ldots, R_n) = \frac{P(G_1, \ldots, G_n)\prod_{i=1}^{n} P(R_i|G_i)}{\sum_{G_1, \ldots, G_n} P(G_1, \ldots, G_n)\prod_{i=1}^{n} P(R_i|G_i)} \tag{2}
\]

Under this decomposition, \(P(R_1, \ldots, R_n|G_1, \ldots, G_n) = \prod_{i=1}^{n} P(R_i|G_i)\) represents the likelihood that our observations match a given genotype combination (our data likelihood), and \(P(G_1, \ldots, G_n)\) represents the prior likelihood of observing a specific genotype combination. We estimate the data likelihood as the joint probability that the observations for a specific individual support a given genotype. We use a neutral model of allele diffusion conditioned on an estimated population mutation rate to estimate the prior probability of sampling a given collection of genotypes.

Except for situations with small numbers of samples and alleles, we avoid the explicit evaluation of the posterior distribution as implied by (2), instead using a number of optimizations to make the algorithm tractable to apply to very large datasets (see section 3.3).

2.3 Estimating the probability of a sample genotype given sequencing observations, \(P(R_i|G_i)\)

Given a set of reads \(R_i = r_{i1}, \ldots, r_{ik_i}\) of a sample at a given locus, we can extract a set of \(k_i\) observed alleles \(B'_i = b'_1, \ldots, b'_k\) which encapsulate the potential set of represented variants at the locus in the given sample, including erroneous observations. Each of these observed alleles \(b'_j\) has a frequency \(o'_j\) within the observations of the individual sample : \(\sum_{j=1}^{k_i} o'_j = s_i\) and corresponds to a true allele \(b_i\).

If we had perfect observations of a locus, \(P(R_i|G_i)\) for any individual would approximate the probability of sampling observations \(R_i\) out of \(G_i\) with replacement. This probability is given by the multinomial distribution in \(s_i\) over the probability \(P(b_i)\) of obtaining each allele from the given genotype, which is \(\frac{f_{i_j}}{m_i}\) for each allele frequency in the frequencies which define \(G_i, f_{i_1}, \ldots, f_{i_{k_i}}\)

\[
P(R_i|G_i) \approx P(B'_i|G_i) = \frac{s_i!}{\prod_{j=1}^{k_i} o'_j!} \prod_{j=1}^{k_i} \left(\frac{f_{i_j}}{m_i}\right)^{o'_j} \tag{3}
\]

Our observations are not perfect, and thus we must account for the probability of errors in the reads. We can use the per-base quality scores provided by sequencing systems to develop the probability that an observed allele is drawn from an underlying true allele, \(P(B'_i|R_i)\). We assume a mapping between sequencing quality scores and allele qualities such that each observed allele \(b'_j\) has a corresponding quality \(q_i\) which approximates the probability that the observed allele is incorrect.

Furthermore, we must sum \(P(R_i|G_i)\) for all possible \(R_i\) combinations \(\forall(R_i \in G_i : |R_i| = k_i)\) drawn from our genotype to obtain the joint probability of \(R_i\) given \(G_i\), as each \(\prod_{i=1}^{s_i} P(b'_i|b_i)\) only accounts for the marginal probability of the a specific \(R_i\) given \(B'_i\).
This extends \( P(R_i|G_i) \) as follows:

\[
P(R_i|G_i) = \sum_{\forall (R_i \in G_i)} \left( s_i! \prod_{j=1}^{k_i} \left( \frac{f_{ij}}{m_{ij}} \right)^{s_{ij}} \prod_{l=1}^{\pi_j} P(b_l'|b_l) \right) \tag{4}
\]

In summary, the probability of obtaining a set of reads given an underlying genotype is proportional to the probability of sampling the set of observations from the underlying genotype, scaled by the probability that our reads are correct. As \( q_l \) approximates the probability that a specific \( b_l \) is incorrect, \( P(b_l'|b_l) = 1 - q_l \) when \( b_l \in G_i \) and \( P(b_l'|b_l) = q_l \) when \( b_l \notin G_i \).

2.4 Priors for unphased genotype combinations, \( P(G_1, \ldots, G_n) \)

2.4.1 Decomposition of prior probability of genotype combination

Let \( G_1, \ldots, G_n \) denote the set of genotypes at the locus and \( f_1, \ldots, f_k \) denote the set of allele frequencies which corresponds to these genotypes. We estimate the prior likelihood of observing a specific combination of genotypes within a given locus by decomposition into resolvable terms:

\[
P(G_1, \ldots, G_n) = P(G_1, \ldots, G_n \cap f_1, \ldots, f_k) \tag{5}
\]

The probability of a given genotype combination is equivalent to the intersection of that probability and the probability of the corresponding set of allele frequencies. This identity follows from the fact that the allele frequencies are derived from the set of genotypes and we always will have the same \( f_1, \ldots, f_k \) for any equivalent \( G_1, \ldots, G_n \).

Following Bayes’ Rule, this identity further decomposes to:

\[
P(G_1, \ldots, G_n \cap f_1, \ldots, f_k) = P(G_1, \ldots, G_n|f_1, \ldots, f_k)P(f_1, \ldots, f_k) \tag{6}
\]

We now can estimate the prior probability of \( G_1, \ldots, G_n \) in terms of the genotype combination sampling probability, \( P(G_1, \ldots, G_n|f_1, \ldots, f_k) \), and the probability of observing a given allele frequency in our population, \( P(f_1, \ldots, f_k) \).

2.4.2 Genotype combination sampling probability \( P(G_1, \ldots, G_n|f_1, \ldots, f_k) \)

The multinomial coefficient \( \binom{M}{f_1, \ldots, f_k} \) gives the number of ways which a set of alleles with frequencies \( f_1, \ldots, f_k \) may be distributed among \( M \) copies of a locus. For phased genotypes \( \hat{G}_i \) the probability of sampling a specific \( \hat{G}_1, \ldots, \hat{G}_n \) given allele frequencies \( f_1, \ldots, f_k \) is thus provided by the inverse of this term:

\[
P(\hat{G}_1, \ldots, \hat{G}_n|f_1, \ldots, f_k) = \binom{M}{f_1, \ldots, f_k}^{-1} \tag{7}
\]

However, our model is limited to unphased genotypes because our primary data only allows phasing within a limited context. Consequently, we must adjust (7) to reflect the number of phased genotypes which correspond to the unphased genotyping \( G_1, \ldots, G_n \). Each
unphased genotype corresponds to as many phased genotypes as there are permutations of
the alleles in \( G_i \). Thus, for a given unphased genotyping \( G_1, \ldots, G_n \), there are \( \prod_{i=1}^{n} m_{i_1} \cdots m_{i_k} \) phased genotypings.

In conjunction, these two terms provide the probability of sampling a particular unphased
 genotype combination given a set of allele frequencies:

\[
P(G_1, \ldots, G_n | f_1, \ldots, f_k) = \left( \frac{M}{f_1, \ldots, f_k} \right)^{-1} \prod_{i=1}^{n} \left( \frac{m_i}{f_{i_1}, \ldots, f_{i_k}} \right) = \frac{1}{M!} \prod_{l=1}^{k} f_l! \prod_{i=1}^{n} \frac{m_{i_l}!}{\prod_{j=1}^{k_i} f_{i_j}}
\]

(8)

In the case of a fully diploid population, the product of all possible multiset permutations
of all genotypes reduces to \( 2^h \), where \( h \) is the number of heterozygous genotypes, simplifying
(8) to:

\[
P(G_1, \ldots, G_n | f_1, \ldots, f_k) = 2^h \left( \frac{M}{f_1, \ldots, f_k} \right)^{-1}
\]

(9)

2.4.3 Derivation of \( P(f_1, \ldots, f_k) \) by Ewens’ sampling formula

Provided our sample size \( n \) is small relative to the population which it samples, and the
population is in equilibrium under mutation and genetic drift, the probability of observing a given
set of allele frequencies at a locus is given by Ewens’ sampling formula [Ewens, 1972]. Ewens’
sampling formula is based on an infinite alleles coalescent model, and relates the probability
of observing a given set of allele frequencies to the number of sampled chromosomes at the
locus (\( M \)) and the population mutation rate \( \theta \).

The application of Ewens’ formula to our context is straightforward. Let \( a_f \) be the
number of alleles among \( b_1, \ldots, b_k \) whose allele frequency within our set of samples is \( f \).
We can thus transform our set of frequencies \( f_1, \ldots, f_k \) into a set of non-negative frequency
 counts \( a_1, \ldots, a_M : \sum_{f=1}^{M} f a_f = M \). As many \( f_1, \ldots, f_k \) can map to the same \( a_1, \ldots, a_M \),
this transformation is not invertible, but it is unique from \( a_1, \ldots, a_M \) to \( f_1, \ldots, f_k \).

Having transformed a set of frequencies over alleles to a set of frequency counts over
frequencies, we can now use Ewens’ sampling formula to approximate \( P(f_1, \ldots, f_k) \) given \( \theta \):

\[
P(f_1, \ldots, f_k) = P(a_1, \ldots, a_M) = \frac{M!}{\theta \prod_{z=1}^{M-1} (\theta + z)} \prod_{j=1}^{M} \frac{\theta^{a_j}}{j^{a_j} a_j!}
\]

(10)

In the bi-allelic case in which our set of samples has two alleles with frequencies \( f_1 \) and
\( f_2 \) such that \( f_1 + f_2 = M \):

\[
P(a_{f_1} = 1, a_{f_2} = 1) = \frac{M!}{\prod_{z=1}^{M-1} (\theta + z)} \frac{\theta}{f_1 f_2}
\]

(11)

While in the monomorphic case, where only a single allele is represented at this locus in
our population, this term reduces to:
\[ P(a_M = 1) = \frac{(M-1)!}{\prod_{z=1}^{M-1} (\theta + z)} \]  

(12)

In this case, \( P(f_1, \ldots, f_k) = 1 - \theta \) when \( M = 2 \). This is sensible as \( \theta \) represents the population mutation rate, which can be estimated from the pairwise heterozygosity rate of any two chromosomes in the population \[\text{Tajima, 1983, Watterson, 1975}\].

3 Direct detection of phase from short-read sequencing

By modeling multiallelic loci, this Bayesian statistical framework provides the foundation for the direct detection of longer, multi-base alleles from sequence alignments. In this section we describe our implementation of a haplotype-based variant detection method based on this model.

Our method assembles haplotype observations over minimal, dynamically-determined, reference-relative windows which contain multiple segregating alleles. To be used in the analysis, haplotype observations must be derived from aligned reads which are anchored by reference-matching sequence at both ends of the detection window. These haplotype observations have derived quality estimations which allow their incorporation into the general statistical model described in section 2. We then employ a gradient ascent method to determine the maximum \textit{a posteriori} estimate of a mutual genotyping over all samples under analysis and establish an estimate of the probability that the loci is polymorphic.

3.1 Parsing haplotype observations from sequencing data

In order to establish a range of sequence in which multiple polymorphisms segregate in the population under analysis, it is necessary to first determine potentially polymorphic windows in order to bound the analysis. This determination is complicated by the fact that a strict windowing can inappropriately break clusters of alleles into multiple variant calls. We employ a dynamic windowing approach that is driven by the observation of multiple proximal reference-relative variations (SNPs and indels) in input alignments.

Where reference-relative variations are separated by less than a configurable number of non-polymorphic bases in an aligned sequence trace, our method combines them into a single haplotype allele observation, \( H_i \). The observational quality of these haplotype alleles is given as \( \min(q_i \forall b'_i \in H_i, Q_i) \), or the minimum of the supporting read’s mapping quality and the minimum base quality of the haplotype’s component variant allele observations.

3.2 Determining a window over which to assemble haplotype observations

At each position in the reference, we collect allele observations derived from alignments as described in 3.1. To improve performance, we apply a set of input filters to exclude alleles from the analysis which are highly unlikely to be true. These filters require a minimum number of alternate observations and a minimum sum of base qualities in a single sample in order to incorporate a putative allele and its observations into the analysis.
We then determine a haplotype length over which to genotype samples by a bounded iterative process. We first determine the allele passing the input filters which is longest relative to the reference. For instance, a longer allele could be a multi-base indel or a composite haplotype observation flanked by SNPs. Then, we parse haplotype observations from all the alignments which fully overlap this window, finding the rightmost end of the longest haplotype allele which begins within the window. This rightmost position is used to update the haplotype window length, and a new set of haplotype observations are assembled from the reads fully overlapping the new window. This process repeats until the rightmost end of the window is not partially overlapped by any haplotype observations which pass the input filters. This method will converge given reads have finite length and the only reads which fully overlap the detection window are used in the analysis.

3.3 Detection and genotyping of local haplotypes

Once a window for analysis has been determined, we parse all fully-overlapping reads into haplotype observations which are anchored at the boundaries of the window. Given these sets of sequencing observations \( r_{i_1}, \ldots, r_{i_s} = R_i \) and data likelihoods \( P(R_i|G_i) \) for each sample and possible genotype derived from the putative alleles, we then determine the probability of polymorphism at the locus given the Bayesian model described in section 2.

To establish a maximum a posteriori estimate of the genotype for each sample, we employ a convergent gradient ascent approach to the posterior probability distribution over the mutual genotyping across all samples under our Bayesian model. This process begins at the genotyping across all samples \( G_1, \ldots, G_n \) where each sample’s genotype is the maximum-likelihood genotype given the data likelihood \( P(R_i|G_i) \):

\[
G_1, \ldots, G_n = \arg\max_{G_i} P(R_i|G_i)
\]  

(13)

The posterior search then attempts to find a genotyping \( G_1, \ldots, G_n \) in the local space of genotypings which has higher posterior probability under the model than this initial genotyping. In practice, this step is done by searching through all genotypings in which a single sample has up to the \( N \)th best genotype when ranked by \( P(R_i|G_i) \), and \( N \) is a small number (e.g. 2). This search starts with some set of genotypes \( G_1, \ldots, G_n = \{G\} \) and attempts to find a genotyping \( \{G\}' \) such that:

\[
P(\{G\}'|R_1, \ldots, R_n) > P(\{G\}|R_1, \ldots, R_n)
\]  

(14)

\( \{G\}' \) is then used as a basis for the next update step. This search iterates until convergence, but in practice must be bounded at a fixed number of steps in order to ensure optimal performance. As the quality of input data increases in coverage and confidence, this search will converge more quickly because the maximum-likelihood estimate will lie closer to the maximum a posteriori estimate under the model.

This method incorporates a basic form of genotype imputation into the detection method, which in practice improves the quality of raw genotypes produced in primary allele detection and genotyping relative to methods which only utilize a maximum-likelihood method to determine genotypes. Furthermore, this method allows for the determination of marginal
genotype likelihoods via the marginalization of assigned genotypes for each sample over the posterior probability distribution.

### 3.4 Probability of polymorphism

Provided a maximum _a posteriori_ estimate of the genotyping of all the individuals in our sample, we might like establish an estimate of the quality of the genotyping. For this, we can use the probability that the locus is polymorphic, which means that the number of distinct alleles at the locus, $K$, is greater than 1. While in practice the space of possible genotypings is too great to integrate over, it is possible to derive the probability that the loci is polymorphic in our samples by summing across the monomorphic cases:

$$P(K > 1|R_1, \ldots, R_n) = 1 - P(K = 1|R_1, \ldots, R_n)$$  (15)

Equation (15) thus provides the probability of polymorphism at the site, which is provided as a quality estimate for each evaluated locus in the output of FreeBayes.

### 3.5 Marginal likelihoods of individual genotypes

Similarly, we can establish a quality estimate for a single genotype by summing over the marginal probability of that specific genotype and sample combination under the model. The marginal probability of a given genotype is thus:

$$P(G_j|R_i, \ldots, R_n) = \sum_{\forall\{G\} : G_j \in \{G\}} P(\{G\}|R_i, \ldots, R_n)$$  (16)

In implementation, the development of this term is more complex, as we must sample enough genotypings from the posterior in order to obtain well-normalized marginal likelihoods. In practice, we marginalize from the local space of genotypings in which each individual genotype is no more than a small number of steps in one sample from the maximum _a posteriori_ estimate of $G_i, \ldots, G_n$. This space is similar to that used during the posterior search described in section 3.3. We apply (16) to it to estimate marginal genotype likelihoods for the most likely individual genotypes, which are provided for each sample at each site in the output of our implementation.

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