TreeClone: Reconstruction of Tumor Subclone Phylogeny Based on Mutation Pairs using Next Generation Sequencing Data

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Abstract

We present TreeClone, a latent feature allocation model to reconstruct tumor subclones subject to phylogenetic evolution that mimics tumor evolution. Similar to most current methods, we consider data from next-generation sequencing of tumor DNA. Unlike most methods that use information in short reads mapped to single nucleotide variants (SNVs), we consider subclone phylogeny reconstruction using pairs of two proximal SNVs that can be mapped by the same short reads. As part of the Bayesian inference model, we construct a phylogenetic tree prior. The use of the tree structure in the prior greatly strengthens inference. Only subclones that can be explained by a phylogenetic tree are assigned non-negligible probabilities. The proposed Bayesian framework implies posterior distributions on the number of subclones, their genotypes, cellular proportions, and the phylogenetic tree spanned by the inferred subclones. The proposed method is validated against different sets of simulated and real-world data using single and multiple tumor samples. An open source software package is available at http://www.compgenome.org/treeclone.

Keywords: Latent feature model; Mutation pair; NGS data; Phylogenetic tree; Subclone; Tumor heterogeneity

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1 Introduction

Initiated by somatic mutations in a single cell, cancer arises through Darwinian-like natural selection. The accumulation of subsequent genetic aberrations and the effects of selection over time result in the sequential clonal expansions of cells, finally leading to the development of a genetically aberrant tumor [Nowell (1976)]. This process, known as tumorigenesis, leads to genetically divergent subpopulations of tumor cells, also known as subclones [Bonavia et al. (2011); Marusyk et al. (2012)].

Deep next generation sequencing (NGS) of bulk tumor DNA samples makes it possible to examine the evolutionary history of individual tumors, based on the set of somatic mutations they have accumulated [Nik-Zainal et al. (2012)]. This is implemented by deconvoluting observed genomic data from a tumor into constituent signals corresponding to various subclones and by then reconstructing the relationship of these subclones in a phylogeny [Deshwar et al. (2015); Marass et al. (2017)]. Apart from phylogenetic relationship, tumor purity, subclones’ genotypes and cellular proportions are also coupled quantities to infer. Uncovering subclonal heterogeneity and their relationship is clinically important for better prognosis [Aparicio and Caldas (2013); Schwarz et al. (2015)]. Therefore there is a pressing need to develop robust methods for a reliable interpretation.

Numerous methods have been proposed for the subclonal reconstruction problem, including SciClone [Miller et al. (2014)], CloneHD [Fischer et al. (2014)], PyClone [Roth et al. (2014)], PyloWGS [Jiao et al. (2014); Deshwar et al. (2015)], Clomial [Zare et al. (2014)], BayClone [Sengupta et al. (2015)], Cloe [Marass et al. (2017)], and PairClone [Zhou et al. (2017)]. The reconstruction is typically based on short reads that are mapped to single nucleotide variants (SNVs) (few methods, for example, CloneHD, also consider somatic copy number aberrations, SCNA). Many methods based on SNV data utilize variant allele fractions (VAFs), that is, the fractions of alleles (or short reads) at each locus that carry mutations. Since humans are diploid, the expected VAFs of short reads for a homogeneous cell population should be 0, 0.5 or 1.0 for any locus in copy number neutral (copy number = 2) regions and after adjusting for tumor purity. Observing VAFs very different from 0, 0.5 or 1.0 is therefore evidence for heterogeneity. Most methods use only marginal SNVs. Recently, Zhou et al. (2017) have proposed to use pairs of proximal SNVs mapped by the same short reads, which carry more information than marginal SNVs, for more accurate subclone reconstruction. In terms of methodology, existing subclone reconstruction methods can be mainly divided into two categories: clustering-based and feature-allocation-based. The two categories are also referred to as indirect and direct reconstructions in Marass et al. (2017), depending on whether the subclonal genotypes are indirectly or directly inferred. Clustering-based methods, in-
cluding SciClone, PyClone and PhyloWGS, first infer SNV clusters based on observed VAFs and then reconstruct subclonal genotypes based on the clusters. The phylogenetic relationship among the subclones can be inferred by imposing hierarchy among the SNV clusters. On the other hand, feature-allocation-based methods (e.g., Clomial, BayClone, Cloe or PairClone) treat subclones as latent features and directly infer subclonal genotypes. Most of the feature-allocation-based methods assume that the features (subclones) are conditionally independent and thus are not able to infer the phylogenetic relationship among the subclones. Recently, Marass et al. (2017) have developed a model allowing for dependency among the features to infer the tumor phylogenetic tree.

In the upcoming discussion we assume that the available data are from $T \geq 1$ samples from a single patient and the main inference goal is intra-tumor heterogeneity. We present a novel approach to reconstruct tumor subclones and their corresponding phylogenetic tree based on mutation pairs. Here a mutation pair refers to a pair of proximal SNVs on the genomes that can be simultaneously mapped by the same paired-end short reads, with one SNV on each end. In other words, mutation pairs can be phased by short reads. See Fig. 1 for an illustration. Short reads mapped to only one of the SNV loci are treated as partially missing paired-end reads and are not excluded from our approach. This idea of working with phased mutation pairs was introduced in Zhou et al. (2017). We build on this work and develop a novel and entirely different inference approach by explicitly modeling the underlying phylogenetic relationship. That is, we model tumor heterogeneity based on a representation of a phylogenetic tree of tumor cell subpopulations. A prior probability model on such phylogenetic trees induces a dependent prior on the mutation profiles of latent tumor cell subpopulations. Part of this construction is that the phylogenetic tree of tumor cell subpopulations is included as a random quantity in the Bayesian model. Like most existing methods, we only consider mutation pairs in copy number neutral region i.e. copy number two. The proposed inference aims to reconstruct (i) subclones defined by the haplotypes across all the mutation pairs, (ii) cellular proportion of each subclone, and (iii) a phylogenetic tree spanned by the subclones.

Next we introduce some notation to formally represent the described data and model structure. Consider an NGS data set with $K$ mutation pairs shared across all $T \geq 1$ samples. We assume that the samples are composed of $C$ homogeneous subclones. The number of subclones $C$ is unknown and becomes one of the model parameters. We use a $K \times C$ matrix $Z$ to represent the subclones, in which each column of $Z$ represents a subclone and each row represents a mutation pair. That is, the $(kc)$ element $z_{kc}$ of the matrix corresponds to subclone $c$ and mutation pair $k$. Each $z_{kc}$ is itself again a matrix. It is a $2 \times 2$ matrix that represents the genotypes of the two alleles of the mutation pair. See
Figure 1: Short reads data from mutation pairs using NGS. Here $s_{tki}$ denotes the $i$-th read for the $k$-th mutation pair in sample $t$. Each $s_{tki}$ is a 2-dimensional vector which corresponds to the two proximal SNVs in the mutation pair, and each component of the vector takes values 0, 1 or –, representing wild type, variant or missing genotype, respectively.

![Mutation Pair 1 and Mutation Pair 2](image)

Fig. 2(b). An important step in the model construction is that the columns (subclones) of $Z$ form a phylogenetic tree $T$. The tree encodes the parent-child relationship across the subclones. A detailed construction of the tree and a prior probability model of $T$ and $Z$ are introduced later. Lastly, we denote with $w_t = (w_{t1}, \ldots, w_{tC})$ the cellular proportions of the subclones in sample $t$ where $0 < w_{tc} < 1$ for all $c$ and $\sum_{c=1}^{C} w_{tc} = 1$. Using NGS data we infer $T$, $C$, $Z$ and $w$ based on a simple idea that variant reads can only arise from subclones with variant alleles consistent with an underlying phylogeny. We develop a tree-based latent feature allocation model (LFAM) to implement this reconstruction. Mutation pairs are the objects of the LFAM, and subclones are the latent features chosen by the mutation pairs.

The previous brief outline of data and model structure already highlights two key features of the proposed approach: the use of phylogenetic tree structure and data on mutation pairs. The latter has important advantages. Mutation pairs contain phasing information that improves the accuracy of subclone reconstruction. If two nucleotides reside on the same short read, we know that they must appear in the same DNA strand in a subclone. For example, consider a scenario with one mutation pair and two subclones. Suppose the reference genome allele is (A, G) for that mutation pair, with the notion that A and G are phased by the same DNA strand. Suppose the two subclones have diploid genomes at the two loci and the genotypes for both DNA strands are ((C, G), (A, T)) for subclone $c = 1$, and ((C, T), (A, G)) for $c = 2$. Since in NGS short reads are generated from a single DNA strand, short reads could be any of the four haplotypes (C, G), (A, T), (C, T) or (A, G) for this mutation pair. If indeed relative large counts of short reads with each haplotype are observed, one can reliably infer that there are heterogeneous cell subpopulations in the tumor sample and the mutation pairs are subclonal. In contrast, if we ignore the phasing information and only consider the (marginal) VAFs for each SNV, then the observed VAFs for both SNVs are 0.5, which could be explained by heterogeneous clonal mutations, i.e., the SNVs are present in all tumor cells. In this
paper, we leverage the power of using mutation pairs over single SNVs to incorporate partial phasing information in our model. We assume that mutation pairs and their mapped short reads counts have been obtained using a bioinformatics pipeline, such as LocHap [Sengupta et al. (2016)]. Our aim is to use short reads mapping data on mutation pairs to reconstruct tumor subclones and their phylogeny.

Besides the use of mutation pairs, the other key feature of the proposed approach is that the model is built around phylogenetic tree structure. Imposing the phylogenetic tree structure in the prior of $\mathbf{Z}$ greatly strengthens inference. First, the tree structure improves biological interpretability of the inferred subclones as the evolutionary relationship among the subclones is explicitly modeled. Second, the tree structure improves the identifiability of the problem. In a subclone reconstruction problem, the input signals (observed VAFs) are usually relatively weak, especially when only $T = 1$ sample is available. Different subclone architectures can yield very similar observed data. By explicitly modeling the tree we can put higher prior probability on a subclone structure that follows a more likely phylogenetic tree. Third, the tree structure improves the mixing performance of the Markov chain Monte Carlo (MCMC) simulation used to infer the unknown quantities. As noted in Marass et al. (2017), the likelihood surface of the genotype matrix $\mathbf{Z}$ is highly multi-modal with sharp peaks. Imposing the tree structure, in the MCMC simulation we only need to search from the space of $\mathbf{Z}$ where the tree structure is satisfied, which greatly reduces the dimension of the parameter space of $\mathbf{Z}$ thus improves mixing of the Markov chain.

Finally, for clarification we briefly comment on the proposed model structure versus a traditional use of phylogenetic trees. Phylogenetic trees are usually used to approximate perfect phylogeny for a fixed number of haplotypes [Bafna et al. (2003)]. Most methods lack assessment of tree uncertainties and report a single tree estimate. Also, methods based on SNVs put the observed mutation profile of SNV at the leaf nodes. This is natural if the splits in the tree create subpopulations that acquire or do not acquire a new mutation (or set of mutations). In contrast, we define a tree with all descendant nodes differing from the parent node by some mutations. That is, all nodes, including interior nodes, correspond to tumor cell subpopulations. Finally, we note that the prior structure in our model is different from the phylogenetic Indian Buffet Process (pIBP) [Miller et al. (2008)], which models phylogeny of the objects rather than the features.

The rest of the paper is organized as follows: Section 2 and Section 3 describe the latent feature allocation model and posterior inference, respectively. Section 4 presents two simulation studies. Section 5 reports analysis results for an actual experiment. We conclude with a discussion in Section 6.
2 Statistical Model

2.1 Representation of Subclones

Fig. 2 presents a stylized example of temporal evolution of a tumor, starting from time $T_0$ and evolving until time $T_4$ with the normal clone (subclone $c = 1$) and three tumor subclones ($c = 2, 3, 4$). Each tumor subclone is marked by two mutation pairs with distinct somatic mutation profiles. In Fig. 2 the true phylogenetic tree is plotted connecting the stylized subclones. The true population frequencies of the subclones are marked in parentheses. In panel (b) subclone genomes, their population frequencies and the phylogenetic relationship are represented by $Z$, $w$, and $T$. Next we explain in detail the definition of these parameters.

The entries of $T$ report for each subclone the index of the parent subclone (with $T_1 = 0$ for the root clone $c = 1$). Suppose $K$ mutation pairs with $C$ subclones are present. The subclone phylogeny can be visualized with a rooted tree with $C$ nodes. We use a $C$-dimensional parent vector $T$ to encode the parent-child relationship of a tree, where $T_c = T[e] = j$ means that subclone $j$ is the parent of subclone $c$. The parent vector uniquely defines the topology of a rooted tree. We assume that the tumor evolutionary process always starts from the normal clone, indexed by $c = 1$. The normal clone does not have a parent, and we denote it by $T_1 = 0$. For example, the parent vector representation of the subclone phylogeny in Fig. 2 is $T = (0, 1, 1, 2)$.

We use the $K \times C$ matrix $Z$ to represent the subclone genotypes. Each column of $Z$ defines a subclone, and each row of $Z$ corresponds to a mutation pair. The entry $z_{kc}$ records the genotypes for mutation pair $k$ in subclone $c$. Since each subclone has two alleles $j = 1, 2$, and each mutation pair has two loci $r = 1, 2$, the entry $z_{kc}$ is itself a $2 \times 2$ matrix,

$$z_{kc} = (z_{kcl1}, z_{kcl2}) = \left[ \begin{array}{c} z_{kcl1} \\ z_{kcl2} \end{array} \right]$$

where $(z_{kcl1}, z_{kcl2})$ represent mutation pairs of allele 1 and allele 2, respectively. That is, in $z_{kcjr}$, $j$ and $r$ index the two alleles and the two loci, respectively. Theoretically, each $z_{kcjr}$ can be any one of the four nucleotide sequences, A, C, G, T. However, at a single locus, the probability of having more than two sequences is negligible since it would require the same locus to be mutated twice throughout the life span of the tumor, which is extremely unlikely. Therefore, we assume $z_{kcjr}$ can only take two possible values, with $z_{kcjr} = 1$ (or 0) indicating that the corresponding locus has a mutation (or does not have a mutation) compared to the reference genome, respectively. For example, in Fig. 2, we have $K = 2$ mutation pairs and $C = 4$ subclones. For mutation
Figure 2: Schematic of subclonal evolution and subclone structure. Panel (a) shows the evolution of subclones over time. Panel (b) shows the subclonal structure at $T_4$ with genotypes $Z$, cellular proportions $w$ and parent vector $T$. For each mutation pair $k$ and subclone $c$, the entry $z_{kc}$ of $Z$ is a $2 \times 2$ matrix corresponding to the arrangement in the figure in panel (a), that is, with alleles in the two columns, and SNVs in the rows.

pair $k = 2$ in subclone $c = 4$, the allele $j = 1$ harbors no mutation, while the allele $j = 2$ has a mutation at the first locus $r = 1$, which translates to $z_{24} = (00, 10)$ (writing 00 as a shorthand for (0,0)$_T$, etc.). Altogether, $z_{kc}$ can take $2^4 = 16$ possible values $z_{kc} \in \{(00,00), (00,01), \ldots, (11,11)\}$. Since we do not have phasing information across mutation pairs, the $z_{kc}$ values having mirrored columns lead to exactly the same data likelihood and thus are indistinguishable. This reduces the list of possible values of $z_{kc}$ to the $Q = 10$ values, $z^{(1)} = (00,00), z^{(2)} = (00,01), z^{(3)} = (00,10), z^{(4)} = (00,11), z^{(5)} = (01,01), z^{(6)} = (01,10), z^{(7)} = (10,10), z^{(8)} = (01,11), z^{(9)} = (10,11)$ and $z^{(10)} = (11,11)$.

We assume that the normal subclone has no mutation, $z_{k1} = z^{(1)}$ for all $k$, indicating all mutations are somatic.

Finally, we introduce notation for mixing proportions $w$. Suppose $T$ tissue samples are dissected from the same patient. We assume that the samples are admixtures of $C$ subclones, each sample with a different set of mixing proportions (population frequencies). We use a $T \times C$ matrix $w$ to record the proportions, where $w_{tc}$ represents the population frequencies of subclone $c$ in sample $t$, $0 < w_{tc} < 1$ and $\sum_{c=1}^{C} w_{tc} = 1$. The proportions $w_{t1}$ denotes the proportion of normal cells contamination in sample $t$ (and later we will
still add a weight \( w_{t0} \) for a background clone \( c = 0 \).

## 2.2 Sampling Model

Let \( \mathbf{N} \) be a \( T \times K \) matrix with \( N_{tk} \) representing read depth for mutation pair \( k \) in sample \( t \). It records the number of times any locus of the mutation pair is covered by sequencing reads (see Fig. 1). Let \( \mathbf{s}_{tki} = (s_{tkir}, r = 1, 2) \) be a specific short read where \( r = 1, 2 \) index the two loci in a mutation pair, \( i = 1, 2, \ldots, N_{tk} \). We use \( s_{tkir} = 1 \) (or 0) to denote a variant (reference) sequence at the read, compared to the reference genome. An important feature of the data is that read \( i \) may overlap in only one locus. We use \( s_{tkir} = -1 \) to represent the missing other sequence on the read. Reads that do not overlap with either of the two loci are not included in the model as they do not contribute any information about the mutation pair. In summary, \( \mathbf{s}_{tki} \) can take \( G = 8 \) possible values, \( \mathbf{s}_{tki} \in \{s^{(1)}, \ldots, s^{(8)}\} = \{00, 01, 10, 11, -0, -1, 0-, 1-\} \).

Among all \( N_{tk} \) reads, let \( n_{tkg} = \sum_i I(\mathbf{s}_{tki} = s^{(g)}) \) be the number of short reads having genotype \( s^{(g)} \). E.g., in Fig. 1 out of a total of \( N_{t1} = 4 \) reads, we have \( n_{t12} = 1, n_{t13} = 1, n_{t16} = 1 \) and \( n_{t18} = 1 \).

We assume a multinomial sampling model for the observed read counts

\[
(n_{tk1}, \ldots, n_{tk8}) \mid N_{tk} \sim \text{Mn}(N_{tk}; p_{tk1}, \ldots, p_{tk8}),
\]

where \( p_{tkg} \) is the probability of observing a short read \( \mathbf{s}_{tki} \) with genotype \( s^{(g)} \). Next we link \( p_{tkg} \) with the underlying subclone structures.

For a short read \( \mathbf{s}_{tki} \), depending on whether it covers both loci or only one locus, we consider three cases: (i) a read covers both loci, taking values \( \mathbf{s}_{tki} \in \{s^{(1)}, \ldots, s^{(4)}\} \) (complete read); (ii) a read covers the second locus, taking values \( \mathbf{s}_{tki} \in \{s^{(5)}, s^{(6)}\} \) (left missing read); and (iii) a read covers the first locus, taking values \( \mathbf{s}_{tki} \in \{s^{(7)}, s^{(8)}\} \) (right missing read). Let \( v_{tk1}, v_{tk2}, v_{tk3} \) denote the probabilities of observing a short read of type (i), (ii) and (iii), respectively. Conditional on cases (i), (ii) or (iii), let \( \tilde{p}_{tkg} \) be the conditional probability of observing \( \mathbf{s}_{tki} = s^{(g)} \). We have \( p_{tkg} = v_{tk1} \tilde{p}_{tkg}, g = 1, \ldots, 4 \), \( p_{tkg} = v_{tk2} \tilde{p}_{tkg}, g = 5, 6 \), and \( p_{tkg} = v_{tk3} \tilde{p}_{tkg}, g = 7, 8 \). We assume non-informative missingness and do not make inference on \( v \)’s. So they remain constants in the likelihood.

We express \( \tilde{p}_{tkg} \) in terms of \( \mathbf{Z} \) and \( \mathbf{w} \) based on the following generative model in three steps. Consider a sample \( t \). To generate a short read, we first select a subclone \( c \) with probability \( w_{tc} \) (step 1). Next we select with probability 0.5 one of the two alleles \( j = 1, 2 \) (step 2). Finally, we record the read \( s^{(g)}, g = 1, 2, 3 \) or 4, corresponding to the chosen allele \( z_{kci} = (z_{kci1}, z_{kci2}) \) (step 3). In the case of left (or right) missing locus we observe
\( s^{(g)} \), \( g = 5 \) or 6 (or \( g = 7 \) or 8), corresponding to the observed locus of the chosen allele. Reflecting steps 2 and 3, we denote the probability of observing a short read \( s^{(g)} \) from subclone characterized by \( z_{kc} \) by
\[
A(s^{(g)}, z_{kc}) = \sum_{j=1}^{2} 0.5 \times I(s_{1}^{(g)} = z_{kcj1}) I(s_{2}^{(g)} = z_{kcj2}),
\]
with the understanding that \( I(- = z_{kcjr}) = 1 \) for missing reads. Implicit in (2) is the restriction \( A(s^{(g)}, z_{kc}) \in \{0, 0.5, 1\} \), depending on the arguments. Finally, proceeding as in step 1 we use the conditional probabilities \( A(\cdot) \) to obtain the marginal probability of observing a short read \( s^{(g)} \) from the tumor sample \( t \) with \( C \) subclones with cellular proportions \( \{w_{tc}\} \) as
\[
\tilde{p}_{tkg} = \sum_{c=1}^{C} w_{tc} A(s^{(g)}, z_{kc}) + w_{t0} \rho_{g}.
\]
The first term in Eq. 3 states that the probability of observing a short read with genotype \( s^{(g)} \) is a weighted sum of the \( A \)'s across all the subclones.

The last term introduces the notion of a background subclone, indexed as \( c = 0 \) and without biological meaning, to account for noise and artifacts in the NGS data (sequencing errors, mapping errors, etc.), and also for tiny subclones that are not detectable given the sequencing depth. In (3), \( w_{t0} \rho_{g} \) stands for the probability of observing \( s^{(g)} \) due to this random noise. We assume the random noise does not differ across different mutation pairs, thus \( \rho_{g} \) does not have an index \( k \). Note that \( \rho_{1} + \ldots + \rho_{4} = \rho_{5} + \rho_{6} = \rho_{7} + \rho_{8} = 1 \).

Finally, we note that if desired, it is straightforward to incorporate data for marginal SNV reads in the sampling model by treating such reads as, for example, right missing reads, i.e. \( s_{tk2} = - \). In this case, \( n_{tk1} = \ldots = n_{tk6} = 0 \), and the multinomial sampling model reduces to a binomial model. The addition of marginal SNV counts does not typically improve inference. See more details in Zhou et al. (2017).

### 2.3 Prior Model

We construct a hierarchical prior model, starting with \( p(C) \), then a prior on the tree for a given number of nodes, \( p(T \mid C) \), and finally a prior on the subclonal genotypes given the phylogenetic tree \( T \). We assume a geometric prior for the number of subclones, \( p(C) = (1 - \alpha)^{C-1} \alpha, C \in \{1, 2, 3, \ldots\} \). Conditional on \( C \), the prior on the tree, \( p(T \mid C) \) is as in Chipman et al. (1998). For a tree with \( C \) nodes, we let
\[
p(T \mid C) \propto \prod_{c=1}^{C} (1 + \eta_{c})^{-\beta},
\]
where $\eta_c$ is the depth of node $c$, or the number of generations between node $c$ and the normal subclone 1. The prior penalizes deeper trees and thus favors parsimonious representation of subclonal structure.

Conditional on $T$ we define a prior for $Z$. The subclone genotype matrix $Z$ can be thought of as a feature allocation for categorical matrices. The mutation pairs are the objects, and the subclones are the latent features chosen by the objects. Each feature has 10 categories corresponding to the $Q = 10$ different genotypes. Given $T$ the construction of the subclone genotype matrix needs to introduce dependence across features to respect the assumed phylogeny. We construct a prior for $Z$ based on the following generative model. We start from a normal subclone denoted by $z_1 = 0$. Now consider a subclone $c > 1$ and defined by $z_c$. The subclone preserves all mutations from its parent $z_{T_c}$, but also gains a Poisson number of new mutations. We assume the new mutations happen randomly at the unmutated loci of the parent subclone. A formal description of prior of $Z$ follows.

For a subclone $c$, let $\ell_{kc} = \sum_{j,r} z_{kcjr}$ denote the number of mutations in mutation pair $k$, and let $L_c = \{k : \ell_{kc} < 4\}$ denote the mutation pairs in subclone $c$ that have less than four mutations. Let $m_{kc} = \ell_{kc} - \ell_{kT_c}$ denote the number of new mutations that mutation pair $k$ gains compared to its parent, and let $m_c = \sum_k m_{kc}$. We assume (i) The child subclone should acquire at least one additional mutation compared with its parent (otherwise subclone $c$ would be identical to its parent $T_c$). (ii) If the parent has already acquired all four mutations for a given $k$, then the child can not gain any more new mutation. That is, if $\ell_{kT_c} = 4$, then $m_{kc} = 0$. (iii) Each mutation pair can gain at most one additional mutation in each generation, $m_{kc} \in \{0, 1\}$. Based on these assumptions, given a parent subclone $z_{T_c}$, we construct a child subclone $z_c$ as follows. Let $M_c = \{k : m_{kc} = 1\}$ be the set of mutation pairs in subclone $c$ where new mutations are gained. Let $\text{Choose}(L, m)$ denote a uniformly chosen subset of $L$ of size $m$, and let $X \sim \text{Trunc-Pois}(\lambda; [a, b])$ represent a Poisson distribution with mean $\lambda$, truncated to $a \leq X \leq b$. We assume

$$m_c | z_{T_c}, T, C \sim \text{Trunc-Pois}(\lambda; [1, |L_{T_c}|]),$$

$$M_c | m_c, z_{T_c}, T, C \sim \text{Choose}(L_{T_c}, m_c).$$

The lower bound and upper bound of the truncated Poisson reflect assumptions (i) and (ii) respectively. Also, Eq. 4 implicitly captures assumption (iii).

Next, for a mutation pair that gains one new mutation, we assume the new mutation randomly arises in any of the unmutated loci in the parent subclone. Let $Z_{kc} = \{(j, r) : z_{kcjr} = 0\}$, and let $\text{Unif}(A)$ denote a uniform distribution over the set $A$. We first choose

$$(j^*, r^*) | z_{T_c}, T, C \sim \text{Unif}(Z_{kT_c}),$$
and then set $z_{kcj} r^* = 1$. So we have

$$p(Z \mid T, C) \propto \prod_{c=2}^{C} \text{Trunc-Pois}(m_c; |1, |L_{T_c}|) \cdot \prod_{k \in M_c} \frac{1}{|Z_{kT_c}|} \cdot \frac{1}{m_c}.$$

The prior construction is completed with a prior for $w$ and $\rho$. We design the prior $p(w)$ of $w$ in such a manner that we could put an informative prior for $w_{t1}$ if a reliable estimate for tumor purity is available based on some prior bioinformatics pipeline (e.g. Van Loo et al. (2010); Carter et al. (2012)). Recall that $c = 1$ is the normal subclone, i.e., $w_{t1}$ is the normal subclone proportion (or $1$ minus tumor purity), and that $\sum_{c=0, c \neq 1}^C w_{tc} + w_{t1} = 1$. We assume a Beta-Dirichlet prior on $w$ such that,

$$w_{t1} \sim \text{Be}(a_p, b_p); \quad \text{and} \quad \frac{w_{tc}}{(1 - w_{t1})} \sim \text{Dir}(d_0, d, \ldots, d),$$

where $c = 0, 2, 3, \ldots, C$. We set $d_0 << d$ as $w_{t0}$ is only a correction term to account for background noise and model mis-specification term.

Finally, to specify a prior for $\rho = \{\rho_g\}$ we consider complete reads, left missing reads and right missing reads separately and assume $(\rho_1, \ldots, \rho_4) \sim \text{Dir}(d_1, \ldots, d_1), (\rho_5, \rho_6) \sim \text{Dir}(2d_1, 2d_1)$, and $(\rho_7, \rho_8) \sim \text{Dir}(2d_1, 2d_1)$.

### 3 Posterior Inference

Let $x = (Z, w, \rho)$ denote the unknown parameters except for the number of subclones $C$ and the tree $T$. Markov chain Monte Carlo (MCMC) simulation from the posterior $p(x \mid n, T, C)$ is used to implement posterior inference. Gibbs sampling transition probabilities are used to update $Z$, and Metropolis-Hastings transition probabilities are used to update $w$ and $\rho$. For example, we update $Z$ by row with

$$p(z_k \mid z_{k-}, \ldots) \propto \prod_{t=1}^T \prod_{g=1}^G \left[ \sum_{c=1}^C w_{tc} A(h_g, z_{kc}) + w_{t0} \rho_g \right]^{n_{k,g}} \cdot p(z_k \mid z_{k-}, T, C),$$

where $z_k$ is a row of $Z$ satisfying the phylogeny $T$.

Since the posterior distribution $p(x \mid n, T, C)$ is expected to be highly multi-modal, we utilize parallel tempering [Geyer (1991)] to improve the mixing of the chain. Specifically, we use OpenMP parallel computing API [Dagum and Menon (1998)] in C++, to implement a scalable parallel tempering algorithm.

Updating $C$ and $T$ is more difficult. In general, posterior MCMC on tree structures can be very challenging to implement [Chipman et al. (1998); Denison et al. (1998)].
However, the problem here is manageable since plausible numbers for \( C \) constrain \( T \) to moderately small trees. We assume that the number of nodes is \textit{a priori} restricted to \( C_{\text{min}} \leq C \leq C_{\text{max}} \). Conditional on the number of subclones \( C \), the number of possible tree topologies is finite. Let \( \mathcal{T} \) denote the (discrete) sample space of \((T, C)\). Updating the values of \((T, C)\) involves trans-dimensional MCMC. At each iteration, we propose new values for \((T, C)\) from a uniform proposal, \( q(\tilde{T}, \tilde{C} \mid T, C) \sim \text{Unif}(\mathcal{T}) \).

In order to search the space \( \mathcal{T} \) for the number of subclones and trees that best explain the observed data, we follow a similar approach as in Lee et al. (2015); Zhou et al. (2017) (motivated by fractional Bayes’ factor in O’Hagan (1995)) that splits the data into a training set and a test set. Recall that \( n \) represents the read counts data. We split \( n \) into a training set \( n' \) with \( n'_{tkg} = bn_{tkg} \), and a test set \( n'' \) with \( n''_{tkg} = (1 - b)n_{tkg} \). Let \( p_b(\mathbf{x} \mid T, C) = p(\mathbf{x} \mid n', T, C) \) be the posterior evaluated on the training set only. We use \( p_b \) in two instances. First, \( p_b \) is used as an informative prior instead of the original prior \( p(\mathbf{x} \mid T, C) \), and second, \( p_b \) is used as a proposal distribution for \( \tilde{x} \), \( q(\tilde{x} \mid \tilde{T}, \tilde{C}) = p_b(\tilde{x} \mid \tilde{T}, \tilde{C}) \). Finally, the acceptance probability of proposal \( (\tilde{T}, \tilde{C}, \tilde{x}) \) is evaluated on the test set. Importantly, in the acceptance probability the (intractable) normalization constant of \( p_b \) cancels out, making this approach computationally feasible.

\[
p_{\text{acc}}(T, C, x, \tilde{T}, \tilde{C}, \tilde{x}) = 1 \wedge \frac{p(n'' \mid x, \tilde{T}, \tilde{C}) \cdot p(\tilde{T}, \tilde{C}) p_b(x \mid \tilde{T}, \tilde{C})}{p(n'' \mid x, T, C) \cdot p(T, C) p_b(x \mid T, C)} \cdot \frac{q(T, C \mid \tilde{T}, \tilde{C}) q(x \mid \tilde{T}, \tilde{C})}{q(T, \tilde{C} \mid T, C) q(x \mid T, \tilde{C})}.
\]

Here we use \( p_b \) as an informative proposal distribution for \( \tilde{x} \) to achieve a better mixing Markov chain Monte Carlo simulation with reasonable acceptance probabilities. Without the use of an informative proposal, the proposed new tree is almost always rejected because the multinomial likelihood with the large sample size is very peaked. Under the modified prior \( p_b(\cdot) \), the resulting conditional posterior on \( x \) remains entirely unchanged, \( p_b(x \mid T, C, n) = p(x \mid T, C, n) \) [Zhou et al. (2017)].

The described uniform tree proposal is in contrast to usual search algorithms for trees that generate proposals from neighboring trees. Such algorithms have the important drawback that they quickly gravitate towards a local mode and then get stuck. A possible approach to addressing this problem is to repeatedly restart the algorithm from different starting trees. See Chipman et al. (1998) for more details. Our uniform tree proposal combined with the data splitting scheme is another way to mitigate this challenge, efficiently searching the tree space while keeping a reasonable acceptance probability.

All posterior inference is contained in the posterior distribution for \( x, C \) and \( T \). For example, the marginal posterior distribution of \( C \) and \( T \) gives updates posterior probabilities for all possible values of \( C \) and \( T \). But it is still useful to report point estimates.
We use the posterior modes (\( \hat{C}, \hat{T} \)) as point estimates for (\( C, T \)), and conditional on \( \hat{C} \) and \( \hat{T} \), we use the maximum a posteriori (MAP) estimator as an estimation for the other parameters. The MAP is approximated as the MCMC sample with highest posterior probability. Let \( \{x^{(l)}, l = 1, \ldots, L\} \) be a set of MCMC samples of \( x \), and

\[
\hat{l} = \arg \max_{l \in \{1, \ldots, L\}} p(n \mid x^{(l)}, \hat{T}, \hat{C}) p(x^{(l)} \mid \hat{T}, \hat{C}).
\]

We report point estimates as \( \hat{Z} = Z^{(\hat{l})} \), \( \hat{w} = w^{(\hat{l})} \) and \( \hat{\rho} = \rho^{(\hat{l})} \).

4 Simulation Studies

We carried out three simulation studies to assess the performance of TreeClone. We simulate multiple datasets with different number of subclones (\( C \)), number of samples (\( T \)), average read depths (\( \bar{N}_{tk} \)) and left and right missing rates (\( v_{tk2}, v_{tk3} \)) to test the performance of the proposed model in different scenarios. In all simulation studies, we generate hypothetical read count data for dozens of mutation pairs (\( K = 100 \) for simulations 1 and 2, and \( K = 69 \) for simulation 3), which is a typical number of mutation pairs in a real tumor sample.

4.1 Simulation 1, Convergence Diagnostic and Sensitivity Analysis

Simulation 1(a). We first validate TreeClone on 9 simulated datasets, one for each combination of \( C \in \{3, 4, 5\} \) and average read depth \( \bar{N}_{tk} \in \{50, 200, 1000\} \). For all 9 datasets, we consider \( T = 5 \) samples and \( K = 100 \) mutation pairs. We randomly generate the phylogenetic tree \( T \) and the genotype matrix \( Z \) from the prior model. The subclone proportions are simulated from \( w_t \sim \text{Dir}(0.01, \sigma(15, 10, 5)), \text{Dir}(0.01, \sigma(15, 10, 8, 5)), \text{Dir}(0.01, \sigma(15, 10, 8, 5, 3)) \) for \( C = 3, 4 \) and 5, respectively. Here \( \sigma(x_1, \ldots, x_n) \) stands for a random permutation of \( x_1, \ldots, x_n \). The noise fractions \( \rho \) are generated from the prior with \( d_1 = 1 \). We mimic a typical rate of left (or right) missing reads by setting \( v_{tk2} = v_{tk3} = 0.3 \), for all \( t \) and \( k \). The read depth \( N_{tk} \) is generated from a negative-binomial distribution, \( N_{tk} \sim \text{NB}(r_N, p_N) \), to reflect the over-dispersion of read depth in sequencing data. We fix \( r_N \) and \( p_N \) such that \( E(N_{tk}) = \bar{N}_{tk} \) and \( \text{sd}(N_{tk}) = \bar{N}_{tk}/5 \) for \( \bar{N}_{tk} = 50, 200 \) and 1000. Finally, the read counts \( \{n_{tkg}\} \) are simulated from the multinomial sampling model (1).

We fit the model with the following hyperparameters: \( \alpha = 0.5, \beta = 0.5, d = 0.5, d_0 = 0.03, d_1 = 1 \), where the values of \( \alpha \) and \( \beta \) imply mild penalty for deep and bushy trees [Chipman et al. (1998)], and other hyperparameters are default non-informative.
choices. We set $a_p = d, b_p = d_0 + (C - 1)d$ for given $C$ as a non-informative prior choice and set $\lambda = 2K/C$ to express our prior belief that about half of the mutations occur uniformly at each generation. We use $C_{\text{min}} = 2$ and $C_{\text{max}} = 5$ as the range of $C$ for computational efficiency. In principle $C_{\text{max}}$ can be any finite number. However, the size of the tree space grows exponentially with $C_{\text{max}}$, so that the time needed for achieving a good mixing Markov chain grows exponentially. We set the training set fraction (in the trans-dimensional MCMC implementation) to $b = 0.95$, which we found to performs well in all simulation studies. We run a total of 8000 MCMC iterations. Discarding the first 3000 draws as initial burn-in, we have a Monte Carlo sample with 5000 posterior draws.

We evaluate the performance of TreeClone in estimating the number of subclones $C$, phylogeny $T$, genotype $Z$ and subcone proportions $w$. To this end, we define the reconstruction error rates $C_{\text{err}} = I(\hat{C} \neq C), T_{\text{err}} = I(\hat{T} \neq T)$,

$$Z_{\text{err}} = \frac{1}{K(C - 1)} \min_{\sigma} \left( \sum_{k,c} I(\hat{z}_{k\sigma(c)} \neq z_{kc}) \right)$$

and $w_{\text{err}} = \sum_{t,c} |\hat{w}_{t\sigma(c)} - w_{tc}|/(TC)$, similar to Marass et al. (2017). Here $\sigma$ is a permutation of columns of $Z$ to account for label-switching of subclones, and the same permutation is imposed on columns of $w$. The simulation results are summarized in Table 1. For all 9 simulated datasets TreeClone nicely recovers the truth and attains reasonably low reconstruction errors.

| $\bar{N}_{tk}$ | $C$ | 3 | 4 | 5 |
|---|---|---|---|---|
| 50 | 0, 0, 0.00, 0.09 | 0, 0, 0.03, 0.07 | 0, 0, 0.07, 0.06 |
| 200 | 0, 0, 0.00, 0.15 | 0, 0, 0.00, 0.09 | 0, 0, 0.00, 0.05 |
| 1000 | 0, 0, 0.00, 0.14 | 0, 0, 0.00, 0.10 | 0, 0, 0.00, 0.08 |

Table 1: Simulation 1(a). Summary of simulation results for 9 combinations of $C$ and $\bar{N}_{tk}$. Each cell of the table reports $(C_{\text{err}}, T_{\text{err}}, Z_{\text{err}}, w_{\text{err}})$ as an average over three runs of TreeClone with different random number seeds.

To assess the convergence of the MCMC algorithm, for each simulated dataset we run the algorithm three times with different random seeds. We use the log-posterior values to calculate a potential scale reduction factor (PSRF) [Gelman and Rubin (1992)] for the three Markov chains. A PSRF close to 1 indicates convergence of the Markov chain to the target distribution. The results are reported in Table 2. Next, to assess the identifiability of the parameters in the TreeClone model, we calculate frequentist coverage probabilities
of 95% posterior credible intervals for $p_{tkg}$. The results are shown as the second entry in each cell of Table 2.

| $\bar{N}_{tk}$ | 3      | 4      | 5      |
|--------------|--------|--------|--------|
| 50           | 1.06, 100% | 1.08, 85% | 1.33, 96% |
| 200          | 1.13, 92%  | 1.04, 86%  | 1.38, 88%  |
| 1000         | 1.05, 100% | 1.11, 83%  | 3.72, 94%  |

Table 2: Simulation 1(a). Convergence diagnostic and frequentist coverage probabilities for 9 simulated datasets. For each simulated dataset, we run TreeClone three times with different random seeds. Each cell of the table reports the PSRF (for log-posterior values) and average coverage rate (of 95% credible intervals for $p_{tkg}$), averaged over the three chains.

**Simulation 1(b).** Next, we vary average read depth $\bar{N}_{tk}$ and $T$. Again we simulate 9 datasets, one for each combination of $T$ and $\bar{N}_{tk}$ with $T \in \{1, 3, 5\}$ and $\bar{N}_{tk} \in \{50, 200, 1000\}$. We assume the same genotype matrix $Z$ for all the 9 datasets with $C = 4$ subclones and $K = 100$ mutation pairs. The parameters are generated from the prior model, and $N_{tk}$ is generated from NB($r_N, p_N$), as in simulation 1(a).

| $\bar{N}_{tk}$ | 1      | 3      | 5      |
|--------------|--------|--------|--------|
| 50           | 0, 0, 0.29, 0.05 | 0, 0, 0.11, 0.09 | 0, 0, 0.03, 0.08 |
| 200          | 0, 0, 0.16, 0.04 | 0, 0, 0.01, 0.07 | 0, 0, 0.00, 0.08 |
| 1000         | 0, 0, 0.13, 0.02 | 0, 0, 0.00, 0.12 | 0, 0, 0.00, 0.10 |

Table 3: Simulation 1(b). Summary of posterior inference for 9 combinations of $T$ and $\bar{N}_{tk}$. Each cell of the table reports $(C_{err}, T_{err}, Z_{err}, w_{err})$.

The simulation results are summarized in Table 3. Even with only one sample and low read depth, TreeClone can reliably estimate $C$ and $T$ (although it does not perfectly recover $Z$ due to the limited amount of data).

**Simulation 1(c).** Finally, we assess sensitivity with respect to the rates of left and right missing mutation pairs $v_{tk2}$ and $v_{tk3}$. Missingness is simply due to the length of a short read is not long enough to cover both loci in a mutation pair. Thus, missingness is
non-informative, and we assume \( v_{tk2} = v_{tk3} \) in the simulation for simplicity. We simulate 5 datasets with missing rates \( v_{tk2} = v_{tk3} = 0, 0.1, 0.25, 0.4 \) and 0.5. The extreme case \( v_{tk2} = v_{tk3} = 0.5 \) implies that no complete mutation pair reads are recorded. For all the 5 datasets, we consider \( T = 5 \) samples and average read depth \( \bar{N}_{tk} = 200 \), and we assume the same genotype \( Z \) with \( C = 4 \) subclones and \( K = 100 \) mutation pairs. The parameters are generated from the prior model as in simulation 1(a) and (b). The simulation results are summarized in Table 4. TreeClone maintains high reconstruction accuracy across all scenarios.

\[
\begin{array}{|c|c|c|c|c|}
\hline
v_{tk2} = v_{tk3} & 0 & 0.1 & 0.25 & 0.4 & 0.5 \\
0, 0, 0.00, 0.08 & 0, 0, 0.00, 0.09 & 0, 0, 0.00, 0.08 & 0, 0, 0.00, 0.07 & 0, 0, 0.12, 0.07 \\
\hline
\end{array}
\]

Table 4: Simulation 1(c). Summary of posterior inference in simulated datasets under a range of values for \( v_{tk2} = v_{tk3} \). Each entry reports \((C_{err}, T_{err}, Z_{err}, w_{err})\).

**Using only marginal SNVs.** We consider another simulation assuming that we observe only marginal SNVs that are not phased. We treat the marginal SNVs as right missing reads, i.e. \( v_{tk2} = 0 \) and \( v_{tk3} = 1 \). We simulate one dataset under this scenario. The reconstruction errors are \((C_{err}, T_{err}, Z_{err}, w_{err}) = (0, 0, 0.46, 0.1)\). The number of subclones and phylogenetic tree are correctly identified. The genotype reconstruction error \( Z_{err} \) is high because the data do not provide any information for inferring the unobserved locus \( z_{kcj2} \) for all \( k, c \) and \( j \). For a fair comparison we re-define \( Z_{err}^{SNV} = \min_{\sigma} \left( \sum_{k,c} I(\hat{z}_{kcj1} \neq z_{kcj1} \text{ for } j = 1 \text{ or } 2) \right) / (K(C - 1)) \). The re-defined reconstruction error is \( Z_{err}^{SNV} = 0.00 \).

### 4.2 Simulation 2 and comparison with alternatives

In the second simulation study, we compare the performance of TreeClone with existing methods. We consider \( T = 1 \) sample, which is the case for most real-world tumor cases (due to the challenge in obtaining multiple samples from a patient). In practice we notice that methods using only marginal SNV data find it hard to identify branching in a phylogenetic tree. We use this simulation to illustrate the information gain by using mutation pairs data.

We consider \( K = 100 \) mutation pairs and assume a simulation truth with \( C = 4 \) latent subclones with a true phylogenetic tree as
Fig. 3(a) shows the true underlying subclonal genotypes. We use a heatmap to show the subclone matrix $Z$, where colors from light gray to red to black are used to represent genotypes $z^{(1)}$ to $z^{(10)}$. The subclone weights are simulated from $\text{Dir}(0.01, \sigma(15, 10, 8, 5))$. For the single sample in this simulation we get $\mathbf{w} = (0.000, 0.135, 0.169, 0.470, 0.226)$. We generate $\mathbf{p}$ from the prior model with $d_1 = 1$, and we set $\rho_{tk} = \rho_{tk} = 0.3$, for all $t$ and $k$. We generate the read depth $N_{tk} \sim \text{NB}(r_N, p_N)$ with $E(N_{tk}) = 500$ and $\text{sd}(N_{tk}) = 100$.

The hyperparameters are set as in Simulation 1. To explore a larger tree space, we set $C_{\text{max}} = 6$, run a total of 13000 MCMC iterations and discard the first 3000 draws as initial burn-in.

Posterior inference is summarized in Fig. 3(b, c). Fig. 3(c) shows the top three tree topologies and corresponding posterior probabilities. The posterior mode recovers the true phylogeny. Fig. 3(b) shows the estimated genotypes conditional on the posterior modes $(\hat{C}, \hat{T})$. Some mismatches are due to the single sample and limited read depth. The estimated subclone proportions are $\hat{\mathbf{w}} = (0.000, 0.103, 0.162, 0.498, 0.237)$, which agrees with the truth.

Figure 3: Simulation 2. Simulation truth $Z$ (a) and posterior inference under TreeClone (b, c).

Comparison with alternatives. There is no other subclone calling method based on paired-end read data that also infers phylogeny. We therefore compare with other similar model-based approaches. In particular, we use Cloe [Marass et al. (2017)], PhyloWGS [Jiao et al. (2014); Deshwar et al. (2015)] and PairClone [Zhou et al. (2017)] for inference
with the same simulated data. Cloe and PhyloWGS infer phylogeny but take marginal SNV data as input. For these methods we therefore discard the phasing information in mutation pairs and only record marginal mutation counts for SNVs. The simulation truth for Cloe and PhyloWGS is shown in Fig. 4(a). The orange color means a heterozygous mutation at the corresponding SNV locus. PairClone takes the same mutant read counts and read depths for mutation pairs as input but uses a different probability model that does not allow to infer the phylogenetic tree.

Cloe infers clonal genotypes and phylogeny based on a similar feature allocation model. We run Cloe with the default hyperparameters for the same number of 13000 iterations with the first 3000 draws as initial burn-in. Based on the MAP estimate (over $2 \leq C \leq 6$), Cloe reports $C = 3$ subclones with phylogeny $1 \rightarrow 2 \rightarrow 3$, and the subclone genotypes are shown in Fig. 4(b) with subclone proportions $w_{\text{Cloe}} = (0.555, 0.223, 0.222)$.

PhyloWGS, on the other hand, infers clusters of mutations and phylogeny. One can then make phylogenetic analysis to conjecture subclones and genotypes. Let $\tilde{\phi}_i$ denote the fraction of cells with a variant allele at locus $i$. The $\tilde{\phi}_i$’s are latent quantities related to the observed VAF for each SNV. PhyloWGS infers the phylogeny by clustering SNVs with matching $\tilde{\phi}_i$’s under a tree-structured prior for the unique values $\phi_j$. In particular, they use the tree-structured stick breaking process (TSSB) [Adams et al. (2010)]. The TSSB implicitly defines a prior on the formation of subclones, including a prior on $C$ and the number of novel loci that arise in each subclone (in contrast, TreeClone explicitly defines these model features, allowing easier prior control on $C$ and $M_c$). We run PhyloWGS with the default hyperparameters and 2500 iterations with a burn-in of 1000 samples. We only consider loci with VAF > 0 as for PhyloWGS the other loci do not provide information on clustering. We then report cluster sizes and phylogeny based on MAP estimate. PhyloWGS reports 3 subclones with phylogeny $0 \rightarrow 1(79, 0.438) \rightarrow 2(53, 0.220)$, where 0 refers to the normal subclone, and the numbers in the brackets refer to the cluster sizes and cellular prevalences. The conjectured subclone genotypes are shown in Fig. 4(c), with subclone proportions $w_{\text{PWGS}} = (0.562, 0.218, 0.220)$.

Inferences under Cloe and PhyloWGS do not entirely recover the truth. Let $M_c$ denote the new mutations that are gained by subclone $c$. The reason for the failure to recover the simulation truth is probably that the common mutations of subclones 2 and 4 ($M_2$ with a cellular prevalence of 0.169 + 0.226) have a similar cellular prevalence as the mutations of subclone 3 ($M_3$ with a cellular prevalence of 0.470). Therefore, Cloe infers that $M_2$ and $M_3$ belong to the same subclone ($M_2^{\text{Cloe}} \approx M_2 \cup M_3$ and $M_3^{\text{Cloe}} \approx M_4$). Similarly, PhyloWGS clusters $M_2$ and $M_3$ together. Using more informative mutation pairs data, TreeClone can infer that $M_2$ and $M_3$ belong to different subclones. The inclusion of phasing information from the paired-end read data increases statistical power.
in recovering the underlying structure.

**PairClone** uses the same mutation pairs data and same sampling model to infer clonal genotypes. However, PairClone uses a finite categorical Indian buffet process prior for \( Z \), which assumes independence among the subclones and does not infer phylogeny. PairClone infers 3 subclones with genotypes shown in Fig. 4(d). The estimated subclone proportions are \( \hat{w}_{\text{PairClone}} = (0.594, 0.229, 0.177) \), similar to Cloe and PhyloWGS’s results. PairClone does not entirely recover the truth, probably because not imposing the tree structure reduces the identifiability of the problem.

**Comparison using additional marginal SNVs.** An NGS dataset contains many more marginal SNVs than phased mutation pairs. These additional marginal SNVs can be utilized by methods such as Cloe and PhyloWGS. For an illustration of the information gain by using more SNVs, we run Cloe and PhyloWGS with a larger set of SNVs that contains 400 SNVs in addition to the 200 SNVs that are part of the 100 mutation pairs, i.e., a total of 600 SNVs. We assume a genotype matrix \( Z_+ = (Z, Z, Z)' \), i.e. repeating the rows of \( Z \) three times, and keep the other parameters unchanged from before.

Using 600 SNVs, Cloe infers two subclones with phylogeny \( 1 \rightarrow 2 \) and proportions \( \hat{w}_{\text{Cloe}} = (0.641, 0.359) \). PhyloWGS infers three (conjectured) subclones with phylogeny \( 1 \rightarrow 2 \rightarrow 3 \) and proportions \( \hat{w}_{\text{PWGS}} = (0.562, 0.212, 0.226) \). The results suggest that the additional 400 SNVs do not contribute much information about the branching in the phylogenetic tree.

### 4.3 Simulation 3

In the third simulation, we evaluate the performance of the proposed approach on multiple samples. We generate the simulated data by mimicking a real-world lung cancer data...
(see Section 5). Following that dataset, we consider \( T = 4 \) tissue samples and \( K = 69 \) mutation pairs. The simulation truth \( Z \) and \( w \) are generated by fitting TreeClone on the lung cancer dataset. We get \( C = 6 \) subclones with phylogeny

\[
\begin{array}{c}
1 \\
\leftarrow 2 \rightarrow 3 \\
5 \\
\rightarrow 4 \\
\rightarrow 6.
\end{array}
\]

Fig. 5(a, b) shows the simulation truth \( Z \) and \( w \) in the form of heatmaps, respectively. We show \( w \) with a light gray to deep blue scale. A darker blue color indicates higher abundance of a subclone in a sample (the proportions \( w_{t0} \) of the background subclone are tiny and not shown). Read depths \( \{N_{tk}\} \) and left and right missing rates \( \{v_{tk2}, v_{tk3}\} \) are taken to be exactly the same as in the real data. The average read depth is \( \hat{N}_{tk} = 156 \).

The hyperparameters are set as in Simulation 1. To explore a larger tree space, we set \( C_{\text{max}} = 7 \), run a total of 30000 MCMC iterations and discard the first 3000 draws as initial burn-in.

Posterior inference is summarized in Fig. 5(c, d, e). Fig. 5(c) shows the top three tree topologies and corresponding posterior probabilities. The posterior mode recovers the true phylogeny. Fig. 5(d, e) shows the estimated genotypes \( \hat{Z} \) and subclone proportions \( \hat{w} \) conditional on \( (\hat{C}, \hat{T}) \). There are some mismatches for subclones 2, 4 and 5, as they only take small proportions in all the samples \( (w_{t2}, w_{t5} < 0.02 \text{ and } w_{t4} < 0.11 \text{ for all } t) \). The reconstruction errors are \( Z_{\text{err}} = 0.21 \) and \( w_{\text{err}} = 0.01 \) (considering only subclones 1, 3 and 6 the reconstruction error becomes \( Z_{\text{err}}^{(1,3,6)} = 0.04 \)).

For comparison we again run Cloe, PhyloWGS and PairClone on the same data. Cloe infers three subclones with phylogeny \( 1 \rightarrow 2 \rightarrow 3, \) where Cloe’s subclones 1, 2 and 3 roughly correspond to the true subclones 1, 6 and 3, respectively. Cloe’s result is reasonable since subclones 2, 4 and 5 have small proportions and there is not much statistical power to estimate them. Similar to the definition of \( Z_{\text{err}} \), we define \( Z_{\text{err, Cloe}} \) for SNVs. Comparing \( \hat{Z}_{\text{Cloe}} \) with subclones 1, 6 and 3 we calculate the reconstruction error \( Z_{\text{err, Cloe}}^{(1,3,6)} = 0.04 \), indicating good model fit of Cloe. By allowing mutation loss, Cloe infers a linear phylogenetic tree, which is still reasonable. On the other hand, PhyloWGS infers the phylogeny as \( 0 \rightarrow 1 \rightarrow 2 \rightarrow 3 \rightarrow 4 \) (details not shown), which approximates but misses some detail in the simulation truth. Finally, PairClone infers three subclones corresponding to the true subclones 1, 3 and 6, shown in Fig. 5(f). PairClone also reasonably recovers the truth but does not infer phylogeny. Comparing \( \hat{Z}_{\text{PairClone}} \) with subclones 1, 3 and 6 we calculate the reconstruction error \( Z_{\text{err, PairClone}}^{(1,3,6)} = 0.14 \), which is higher than \( Z_{\text{err}}^{(1,3,6)} \).
5 Lung Dataset

We use whole-exome sequencing (WES) data generated from four ($T = 4$) surgically dissected tumor samples taken from a single patient diagnosed with lung adenocarcinoma. DNA is extracted from all four samples and the exome library is sequenced on an Illumina HiSeq 2000 platform in paired-end fashion. Each read is 100 base-pairs long. We use BWA [Li and Durbin (2009)] and GATK’s UniformGenotyper [McKenna et al. (2010)] for mapping and variant calling, respectively. In order to find mutation pair locations along with their genotypes and the number of reads supporting them, we use a bioinformatics tool called LocHap [Sengupta et al. (2016)]. LocHap searches for two or three SNVs that are scaffolded by the same reads. When the scaffolded SNVs, known as local haplotypes, exhibit more than two haplotypes, it is known as local haplotype variant (LHV). Using the individual BAM and VCF files LocHap finds a few hundreds LHVs on average in a WES sample. We select LHVs with two SNVs as we are interested in mutation pairs
only. On those LHV s, we run the bioinformatics filters suggested by LocHap to keep the mutation pairs with high calling quality. We focus our analysis in copy number neutral regions. In the end, we get 69 mutation pairs for the sample and record the read count data from the output of LocHap. Figure 6 shows the histograms of read depths, left missing rates and right missing rates. The average read depth, left missing rate and right missing rate are 156, 0.21 and 0.23, respectively. Simulation 1 showed that with $T = 4$ samples TreeClone should provide useful inference with this combination of moderate read depth and left/right missing rates.

![Histograms of Read Depths, Left Missing Rates, and Right Missing Rates](image)

(a) Histogram of $\{N_{tk}\}$  (b) Histogram of $\{v_{tk2}\}$  (c) Histogram of $\{v_{tk3}\}$

Figure 6: Some summary plots of the lung cancer dataset. Histograms of read depths (a), left missing rates (b) and right missing rates (c). The red dashed line in (a) is a negative binomial density, showing that it is not unreasonable to assume the read depths follow a negative binomial distribution.

We use the same hyperparameters as in Simulation 1. To explore a larger tree space, we set $C_{\text{max}} = 7$, run a total of 30000 MCMC iterations and discard the first 3000 draws as initial burn-in. Fig. 7(c) shows the top three tree topologies and corresponding posterior probabilities. The posterior mode is

$$
1 \rightarrow 2 \rightarrow 3 \\
5 \rightarrow 6
$$

with $C = 6$ subclones. Fig. 7(a, b) show the estimated subclone genotypes $\hat{Z}$ and cellular proportions $\hat{\omega}$, respectively ($\hat{\omega}_{t0} < 3 \times 10^{-3}$ are not shown). The rows for $\hat{Z}$ are reordered for better display. The cellular proportions of the subclones show strong similarity across the 4 samples, indicating homogeneity of the samples. This is plausible as the samples are dissected from proximal sites. Subclone 1, which is the normal subclone, takes a
small proportion in all 4 samples, indicating high purity of the tumor samples. Subclones 2 and 5 are also included in only small proportions. They have almost vanished in the samples. However, as parents of subclones 3, 4 and 6, respectively, they are important for the reconstruction of the subclone phylogeny. Subclones 3, 4 and 6 are the three main subclones. They share a large proportion of common mutations, but each one has some private mutations, consistent with the estimated tree.

**Test of fit.** Finally, Fig. 7(d) shows a histogram of residuals, where we calculate empirical values $\hat{p}_{tkg} = n_{tkg}/N_{tk}$ and plot the difference $(\hat{p}_{tkg} - \bar{p}_{tkg})$. The residuals are centered at zero with little variation, indicating a good model fit. For a more formal goodness-of-fit test, we carried out the Bayesian $\chi^2$ test proposed in Johnson (2004). Recall that the observations in our case are short reads $s_{tki}$ taking $G = 8$ discrete values \{00, 01, 10, 11, −0, −1, 0−, 1−\}. We count the number of short reads that fall into each of these categories. Let $M_g$ denote these counts, and let $x^{(l)}$ be a posterior sample of $x = (Z, w, \rho)$. The statistic $R^B$ is defined as

$$R^B(x^{(l)}) = \sum_{g=1}^{G} \left[ \frac{M_g - Nq_g(x^{(l)})}{\sqrt{Nq_g(x^{(l)})}} \right]^2,$$

where $N = \sum_{t,k} N_{tk}$ and $q_g(x^{(l)}) = \sum_{t,k} N_{tk}p_{tkg}(x^{(l)})/N$ is the expected proportion of short reads in category $g$ calculated by $x^{(l)}$. Under the null hypothesis of a good model fit the statistic should follow a $\chi^2$-distribution with $G − 1 = 7$ degrees of freedom. Fig. 7(e) shows a quantile-quantile plot of posterior samples of $R^B$ against expected order statistics from a $\chi^2_7$ distribution. In addition, find the proportion of posterior samples of $R^B$ exceeding the 95% quantile of a $\chi^2_7$ distribution to be 0.054. There is no evidence of a lack of fit.

**Cloe and PhyloWGS.** For comparison, we run Cloe and PhyloWGS on the same data set with default hyperparameters. Cloe infers four subclones with phylogeny 1 → 2 → 3 → 4. Fig. 8(a, b) show the estimated genotypes $\hat{Z}^{\text{Cloe}}$ and cellular proportions $\hat{w}^{\text{Cloe}}$, respectively. The estimated subclones 2, 3 and 4 under Cloe match with subclones 6, 4 and 3, respectively, under TreeClone. Cloe infers a linear phylogenetic tree since it allows mutation loss. PhyloWGS estimates 6 clusters (and a cluster 0 for normal subclone) of the SNVs with phylogeny

$$0 \rightarrow 1 \rightarrow 2 \rightarrow 3 \rightarrow 4 \rightarrow 5 \rightarrow 6.$$

Fig. 8(c) summarizes the cluster sizes and cellular prevalences. In light of the earlier simulation studies we believe that the inference on $T$ under TreeClone is more reliable.
Figure 7: Posterior inference with TreeClone for lung cancer data set.

However, results from Cloe and PhyloWGS confirm that the four samples have similar proportions of all the subclones, indicating little inter-sample heterogeneity. Also, Cloe and PhyloWGS infer very small normal cell proportion, confirming the high tumor purity found by TreeClone. Finally, the same lung cancer dataset was analyzed under PairClone in Zhou et al. (2017). PairClone infers three subclones corresponding to TreeClone’s subclones 1, 3 and 6 and also confirms the 4 tissue samples are highly homogenous. PairClone gives reasonable result but can not infer phylogeny.

6 Discussion

In this work, using a tree-based LFAM we infer subclonal genotypes structure for mutation pairs, their cellular proportions and the phylogenetic relationship among subclones. This is the first attempt to generate a subclonal phylogenetic structure using mutation pair data. We show that more accurate inference can be obtained using mutation pairs data compared to using only marginal counts for single SNVs. The model can be easily
extended to incorporate more than two SNVs. Another way of extending the model is to encode mutation times inside the length of the edges of phylogenetic tree.

The major motivation for accurate estimation of heterogeneity in tumor is personalized medicine. The next step towards this goal is to use varying estimates of subclonal proportions across patients to drive adaptive treatment allocation.

Currently the heterogeneity is measured mostly with SNV and CNA data. However, structural variants (SVs) like deletion, duplication, inversion, translocation and other large genome rearrangement arguably provide more accurate data for VAF estimation [Fan et al. (2014)], which is the key input for characterizing tumor heterogeneity. Therefore incorporation of SVs into the current model could significantly improve the outcome of tumor heterogeneity analysis. Recently, in Brocks et al. (2014) the authors attempted to explain intratumor heterogeneity in DNA methylation and copy-number pattern by a unified evolutionary process. The current genome centric definition of tumor heterogeneity could be extended by incorporation of methylation, DNA mutation, and RNA expression data in an integromics model.

Finally in the era of big data it is important to factor computation into the research effort, and build efficient computational models that could handle millions of SNVs. Linear response variational Bayes [Giordano et al. (2015)] or MAD-Bayes [Broderick et al. (2013); Xu et al. (2015)] methods could be considered as alternative computational strategies to tackle the problem.
Appendix

Glossary of terms

**SNV.** A single nucleotide variant or SNV is a DNA sequence variation where one nucleotide is replaced by another nucleotide. This is very similar to a single nucleotide polymorphism or SNP. In our paper, the term SNV is preferred over SNP because the latter includes an additional interpretation about variants in a population. This means that an SNV could potentially be a SNP but this cannot be determined at the point where the variant is detected in a single sample.

**SCNA.** Copy number aberration (CNA) is gain or loss of large segments of the genome ranging in size from a few kilobases to a whole chromosomes. Somatic CNA (SCNA) that occurs during the lifetime of an individual is a major contributor to cancer development, particularly for solid tumors.

**Short-read.** Short DNA sequences produced by the sequencing machine. The range of the read length of a short-read sequencing instrument is between 100 and 600 bp.

**Phasing.** Phasing helps to identify the copy of the chromosome (paternal or maternal chromosome) to which a particular allele belongs or alternatively, which alleles appear together on the same chromosome. In short-read sequencing, for example, it is difficult to resolve the haplotype of two heterozygous SNPs if they have not been covered by the same read. If you observe A/C and G/T, it is difficult to know whether we have AG and CT or CG and AT.

**VAF.** Variant allele frequency or VAF is the relative frequency of an alternative allele at a particular genomic locus expressed as a fraction or percentage. The quantity is calculated as the fraction of short-reads overlapping a genomic locus that support the non-reference (mutant/alternate) allele.

**Purity.** Tumor purity is an essential component in finding tumor heterogeneity. The tumor micro-environment contains other non-cancerous cell types including fibroblasts, immune cells, endothelial cells and normal epithelial cells along with cancer cells. Tumor purity is the proportion of cancer cells in the sample.

**Phylogenetic tree.** A phylogenetic tree of a tumor sample is a branching diagram or “tree” showing the evolutionary relationships among various subclones that co-exist in
Subclone calling method. Subclone calling method refers to a computation algorithm that is used to infer the subclonal structure in a tumor sample.

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