PRIMUS: Pedigree Reconstruction and Identification of a Maximum Unrelated Set

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Abstract

PRIMUS: Pedigree Reconstruction and Identification of a Maximum Unrelated Set

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This dissertation describes the methods and algorithms developed to improve human genetic analysis for both genome-wide association studies (GWAS) and pedigree-based analyses. The new algorithms are included in the software package PRIMUS. There are four main parts of PRIMUS:

1) To identify a maximum unrelated set of individuals within a genetic dataset to improve statistical analyses.
2) To reconstruct pedigrees using genome-wide identity by descent estimates.
3) To improve pedigree reconstruction using mitochondrial and non-recombining Y haplotypes.
4) To extend pedigree reconstruction by PRIMUS beyond third degree relationships using distant pairwise relationship predictions.

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I am grateful for my fellow Nickersonians and everyone in Genomes Sciences and beyond. In particular, Adam Gordon for our countless discussions on research, plots, and science; Colleen Davis for improving everyone of my papers, posters, and talks; Chad Huff for his patience and willingness to help with PADRE in a short time frame; Bruce Weir, Phil Green, Bill Noble, Brian Browning, Larry Ruzzo, Ellen Wijsman, Elizabeth Thompson, Peggy Robertson, Qian Yi, Guillaume Jumenez, Tom Kolar, and Christa Poel for so many helpful discussions and assistance; and the Genome Training Grant and the National Science Foundation for funding my work.

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Chapter 1 Introduction and background

The relationship of genotype to phenotype is central to genetic analysis. Differences in DNA can alter phenotypic expression for simple traits (e.g., those influence by a single gene such as the ability to digest lactose) to more complex traits (e.g., that are modified by variation in thousand of loci such as height)[1]. A small fraction of the changes in DNA result in the expression of abnormal phenotypes and diseases[1]. If identified, these disease-causing DNA variants can provide key insights into human biology and can point to treatments or cures for a disease[2]. To this end, geneticists have tracked the inheritance of disease-causing variants through families and pedigrees[3]. Geneticists use pedigrees and powerful tools like linkage analysis to identify the region(s) in the genome that contains the variant with functional impact. If the pedigree is large with many informative meioses, then it is possible to identify the gene that contains the disease-causing variant with the analysis of the single family and appropriate biological studies.

Today, pedigrees continue to be utilized to determine the heritability and genetic models for traits and disorders[4-6]. Knowing the exact pedigree structure allows investigators to correctly identify the mode of inheritance for a disorder. These approaches utilize powerful statistical tools that require, or benefit from, the correct pedigree structure such as: linkage[7], family-based association[8], pedigree-aware imputation, pedigree-aware phasing, Mendelian error-checking, and, heritability. Furthermore, in many instances, knowing that the pedigree is consistent with the genetic data is crucial to identifying the association variants and genes linked to a disease or disorder[4-6; 9].

The development of large-scale genotyping has brought genome-wide association studies (GWAS) to the forefront. This type of study compares the genotypes of a group of individuals with a disease (cases) to a group of similar people without the disease (controls) or looks at a phenotypic trait. From 2005-2013, the GWAS catalog at http://www.genome.gov/gwastudies/ reported thousands of GWAS have identified more than 14,000 single-nucleotide polymorphisms associated with phenotypic traits[10]. GWAS analysis requires care to not violate the assumption of independence of samples. For instance, interrelatedness can inflate the false positive rate in a GWAS[11; 12], resulting in spurious associations. Interrelatedness can also introduces biases in many statistical analyses, including an inflated false positive rate in burden tests, over-estimation

of relatedness in genome-wide estimates of identity by descent (IBD)[13], and skewed principle component analyses[14]. Interrelatedness must be removed before performing these analyses.

I have developed several new computational tools to improve both the removal of interrelated samples and to identify pedigrees in genetic datasets that can also be used for analyses. First, I address the issue of removing interrelatedness within datasets. Unless modeled in the statistical analysis[15; 16], related samples must be removed prior to genetic analyses to limit potential biases. Given the expense of sample ascertainment, phenotyping as well as the genotyping and/or sequencing, maximizing the number of unrelated samples in any dataset should be a priority. However, available methods and suggestions for removal of related samples do not guarantee the retention of the maximum number of unrelated samples in a dataset. To improve on existing approaches, individuals in a dataset are visualized as nodes in a graph and relationships between individuals as edges between the nodes. Graph theory algorithm developed by Bron and Kerbosch[17] is then applied to identify the maximum unrelated set of individuals from any genetic dataset. I have implement this adapted algorithm in a software package known as Pedigree Reconstruction and Identification of the Maximally Unrelated Set or PRIMUS[18].

Second, I set out to improve approaches to reconstruct pedigrees. Pedigree analysis, through linkage studies, has become a mainstay in human genetics, and the underpinnings of more than 3,500 Mendelian diseases and disorders have been identified (references including OMIM). Significant effort is spent collecting and maintaining accurate sample records for pedigrees. However, despite the best efforts of investigators, pedigree and sample errors are still quite common and require careful examination to avoid a reduction in power to detect linkage[19]. The rate of non-paternities in studies has been reported between 0.8% and 30% (median 3.7%; n=17)[20], with other reports showing more conservative estimates of 1% to 1.5%[21; 22]. Even at the conservative rate of 1%, a pedigree with six children has a 6% chance of being incorrect due to a non-paternity, and the pedigree error rate will be much higher after accounting for other common errors such as sample swaps, duplicate samples, contamination, and other relationship discrepancies.

The standard practice for checking and correcting pedigrees and relationships within genetic datasets is to use pairwise prediction programs[23-27] like RELPAIR[28] and PREST[29] to verify that

the level of relatedness between every pair of individuals falls close to the expected level of relatedness based on the reported pedigree[30-37]. While using pairwise estimates to check relationships in pedigrees is sometimes sufficient, there are four major drawbacks. First, pairwise checking will not catch pedigree errors if there are multiple pedigree structures that fit the genetic data. Second, pairwise relationship checking does not provide, or even suggest, the correct pedigree in the case of inconsistency between the data and the reported pedigree. Third, pairwise inconsistencies between genotyped samples are often resolved by removing the inconsistent sample(s), which can result in the unnecessary loss of samples or in accepting an incorrect pedigree as true. Finally, manually reconstructing an unknown pedigree manually using pairwise relationship comparisons is arduous and error-prone.

A solution to these drawbacks is to use the genetic data to reconstruct the corresponding pedigree structure. Pedigree reconstruction will identify inconsistencies between the reported pedigree, and the genetic data will also provide the correct pedigree. Unfortunately, existing pedigree reconstruction programs have a variety of limitations that prevent them from being broadly applied to reconstructing and verifying human pedigrees. I have developed a pedigree reconstruction method without many of the limitations of previous pedigree reconstruction programs and have incorporated it into PRIMUS. This approach utilizes the power of single nucleotide polymorphism (SNP) arrays or next-generation sequence data to evaluate genome-wide estimates of IBD that are generated by programs such as PLINK[23] or KING[25]. In order to reduce the number of possible pedigrees and increase the chances of identifying the true pedigree structure, I also extended the algorithm to use mitochondrial (mtDNA) and non-recombining Y chromosome (NRY) haplotypes to reduce the number of pedigrees generated by PRIMUS and to improve the chances of identifying the correct pedigree. This decreases runtime and improve the overall reconstruction results.

Finally, I merged the power of PRIMUS with a program that is capable of reconstructing more distant relationships. PRIMUS provides an accurate prediction of relationships up to third-degree (e.g., first-cousins) and can provide complete pedigree structures for individuals in a genetic dataset. However, there are often only clusters of close relationships within genetic datasets, resulting in sparse pedigrees with many relationships more distant than third-degree relationships. These sparse datasets are well suited for pairwise-prediction algorithms that can

accurately predict relationships up to ninth-degree relatives (third-cousins once removed). However, these pairwise prediction programs are not capable of reconstructing close relationships into complete pedigrees, and they are limited to accurate predictions of ninth-degree relatives[38]. I developed and implemented an algorithm that leverages the pedigree reconstruction of first- through third-degree relatives by PRIMUS with the accurate distant relationship predictions by Estimation of Recent Shared Ancestry (ERSA)[38]. This algorithm is known as PADRE, (Pedigree Aware Distant Relationship Estimation) and I have implemented it in PRIMUS. The PADRE algorithm uses ERSA relationship likelihoods to calculate the most likely connection between family networks reconstructed by PRIMUS. By combing the power of pedigree reconstruction with PADRE, we can now connect distantly related pedigrees that can be used for a more powerful linkage analysis.

Chapter 2. Identification of Maximum Unrelated Set

Introduction

Interrelatedness can be a confounding factor in many statistical analyses, including burden tests in sequence data, association studies[11; 12], genome-wide estimates of IBD[13], and principle component analyses[14]. Unless modeled in the statistical analysis[15; 16], interrelatedness must be removed from the data before proceeding with genetic analyses. Given the expense of DNA ascertainment, clinical phenotyping, sequencing and/or genotyping, and data analysis, maximizing the number of unrelated samples utilized in such analyses should be a priority.

Estimates of pairwise IBD, a quantitative measure of relatedness, can reliably detect relatives as distant as first cousins[39]. Over the years, multiple strategies to detect IBD have been developed[23; 25; 39-43], and new methods are emerging that use IBD estimates to confidently detect more distant relatives (up to third-cousins)[39; 40]. With good IBD estimates, relatedness structures that violate the assumption of sample independence can be identified and removed from the dataset through sample pruning.

Methods

Current approaches

We have identified three publicly available methods to produce a set of unrelated individuals given a threshold of tolerated pairwise IBD. The documentation for PLINK[23] (see Web Resources) suggests a method to remove pairwise relatedness by iteratively removing one member of each pair until no pairs remain (Figure 1A). Pemberton et al.[44] suggest generating networks of relatedness in which samples are nodes and pairwise relationships are edges. Relatedness networks are then broken by iteratively removing the most highly connected node, until no edges remain in the dataset (Figure 1B). Finally, the authors of KING[25] describes how they generate a set of unrelated individuals in a recent paper[45]. They first add the person who is related to the fewest other people in the dataset and then proceed to add the individual who is related to the next fewest people in the dataset, as long as the individual to be added is not related to anyone already in the set of unrelated individuals (Figure 1C). However, none of these

approaches maximize the number of retained unrelated samples or selectively retain the most informative samples, as I demonstrate in Figure 1. Solving this problem is proven to be hard (algorithms to solve this problem run in exponential time[46]) and much graph theory has been dedicated to solving it[47].

I have chosen to utilize existing graph theory to solve this problem by formulating the family networks as a graph, where individuals are represented as nodes and relationships between individuals are edges. In graph theory, the maximum unrelated set is referred to as the maximum independent set; the maximum independent set of a graph is the same as the maximum clique of the complement graph. To obtain the complement of a graph, all missing edges are added, and all existing edges of the graph are removed. Here, this is equivalent to forming edges when relationships fall below the user-defined relatedness threshold rather than above it. We then search this complement graph for a maximum clique. A clique is defined as a portion of the graph (subgraph) where each node is connected to every other node in the subgraph. A maximal clique is a clique that is not a subgraph of a larger clique. Finally, a maximum clique is the largest maximal clique. The maximum clique of the complement graph of our relationships networks is thus the maximum unrelated set.

In order to test PRIMUS and compare it to other methods, we programmed each of the three methods as described (Figure 1). This was required because neither of the methods described in PLINK or Pemberton are available in a software package, and the KING program does not allow for the input of user-defined IBD estimates. Rather, KING calculates its own IBD estimates from input genotype data.

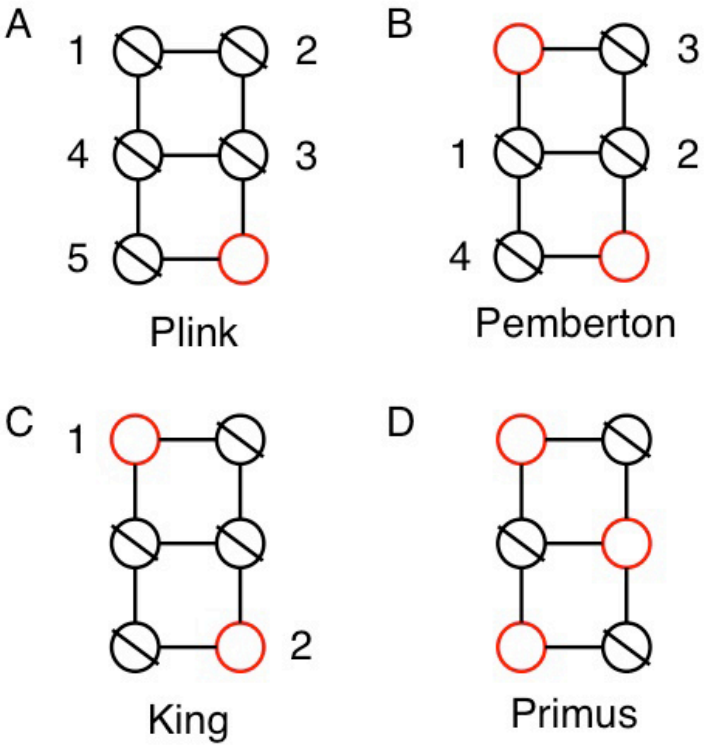


Figure 1. Stepwise selection process of an unrelated set for the three alternative methods and PRIMUS.
In each network, each node represents an individual and an edge represents a familial relationship between two individuals. The red nodes represent the selected set of unrelated individuals. (A and B) The numbers represent one possible ordering that the Pemberton and PLINK methods might use to eliminate individuals from the unrelated set. (C) The numbers indicate one possible ordering for how the KING method selects individuals for inclusion in the unrelated set. (D) PRIMUS will always select a maximum set (n=3) of the graph and will generate the maximum unrelated set of individuals.

New Method

We present a method adapted from graph theory that always identifies the maximum set of unrelated individuals in any dataset, and allows weighting parameters to be utilized in unrelated sample selection (Figure 1D). We implemented this method in a new software package called Pedigree Reconstruction and Identification of a Maximum Unrelated Set (PRIMUS), and it is available online (see Web Resources). We also implemented the other three methods described in this chapter and selected the optimum result of the four algorithms.

PRIMUS reads in user-generated IBD estimates and outputs the maximum possible set of unrelated individuals, given a user-defined threshold of relatedness. PRIMUS converts the IBD relationship file to an undirected graph in which nodes represent individuals and edges represent pairwise relationships; each connected component represents a "family network" or pedigree. PRIMUS writes out each family network to a .dot file to be viewed in graph visualization software like GraphViz (see Web Resources) to generate images of the family networks (see Figure 2).

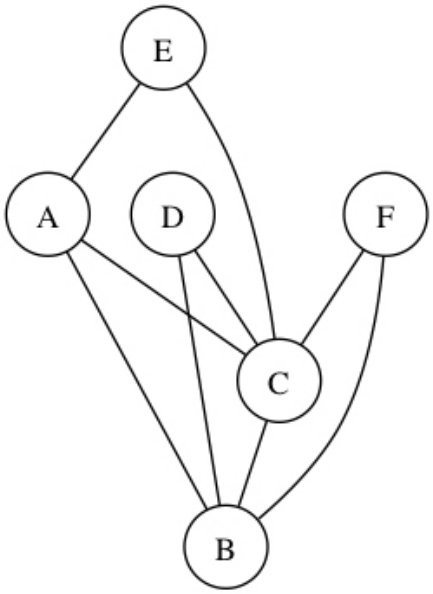


Figure 2. Example of a family network graph.
Each node represents an individual in the family network and each edge shows a relationship between two individuals. A graph like this will be generated for each family network with more than two people.

All individuals within each family network of the data are unrelated to any individual in a different family network (at the user-specified threshold). Thus, the problem of identifying the maximally sized unrelated set is reduced to finding the maximum unrelated set within each family network and then combining the unrelated sets of each family network to get the maximum unrelated set of the entire graph/dataset.

PRIMUS uses the Bron-Kerbosch algorithm[17] with improved pivot selection[48] to enumerate all maximal cliques of each complement family network. The Bron-Kerbosch algorithm using a recursive backtracking algorithm to enumerate all maximal cliques and then selects the largest of the maximal cliques. For each family network, PRIMUS picks the maximum clique or the weighted maximum clique to add to the maximum unrelated set of individuals. Finally, it generates a file containing the maximum set of unrelated individuals.

Weighted Maximum Set Selection

A unique strength of our program is its ability to weight the maximum clique selection using additional criteria. The maximum clique is the clique containing the most samples; however, there are often two or more maximum cliques. Any one of these will produce a maximum unrelated set, and PRIMUS allows for preferential selection of the maximum clique based on additional weighting criteria. In case/control studies this function is particularly useful, because it allows for the retention of the maximum clique with the most affected individuals. Alternatively, the user may wish to select the maximum clique with the lowest missingness rate within the data, or perhaps to first select for affected status and then for lowest missingness. PRIMUS allows specification of as many of these weighting criteria as desired as well as ordering how they are applied in the selection. No other available method for selecting unrelated samples offers weighting functionality.

PRIMUS can also retain the maximum number of unrelated individuals with a desired binary characteristic (e.g. affected status), even if this unrelated set is smaller than the maximum set of unrelated individuals. For example, a study may contain a trio with an affected child and two unaffected parents. The maximum unrelated set would require removing the child and retaining both parents, since the parents are unrelated to each other. It is likely one would wish to retain the single affected child for further analysis instead of both unaffected parents. As a result, the overall unrelated set size will be smaller, but the set will contain more of the affected samples.

Since none of the PLINK, Pemberton, and KING methods has a weighting algorithm, we implemented one for each. These implementations are available upon request. For the PLINK method, we implemented weighting by selecting the individual with the desired trait. For example, to preferentially select affected individuals, the algorithm will keep the affected

individual in a case-control related pair. For the Pemberton method, we implemented a weighting scheme by choosing to remove the node with the less optimal criteria whenever two nodes are equally connected. For the KING method, we implemented weighting by retaining the more desirable individual whenever two individuals are related to the same number of other individuals.

The Approximation Function

The Bron-Kerbosch algorithm is impractical to run on large, sparse family networks due to the algorithm's exponentially increasing computational cost (Figure 3). To remedy this, we implemented an approximation function for networks above a set cutoff size (Table 1). PRIMUS' approximation function takes a similar approach to the Pemberton method[44] by repeatedly removing the highest degree node from the family network until the network is smaller than the approximation function size cutoff or until it breaks into sub-networks smaller than the cutoff. Once the size of the network or sub-networks is below the approximation function size cutoff, PRIMUS uses the Bron-Kerbosch algorithm to obtain an independent set that is approximately the largest. We do acknowledge that we do not know how good this approximation is and would benefit from additional testing.

Family Network Simulations

To compare the performance of PRIMUS to these methods on all types of family networks, we randomly generated 7,500 simulated family networks of varying sizes and network connectivity, which is a measure of how interconnected the network is. Connectivity is the number pairwise relationships that exist in a dataset divided by the total possible number of pairwise relationships. Connectivity can vary widely in family data (Figure 4); some family networks are highly connected (e.g. a father, mother, and 10 offspring), while other family networks may be sparsely connected (e.g. a 'string' of cousins in which each is related through a unique parent). For each network size (5 to 130 by increments of five) we randomly generated 30 simulated networks with the network connectivity proportion ranging from 0.1 (10% of all possible pairwise relationships exist in the network) to 1 (every individual is related to every other individual), and our simulation data is available upon request. For each simulation, we obtained an unrelated set from PRIMUS and the three other methods.

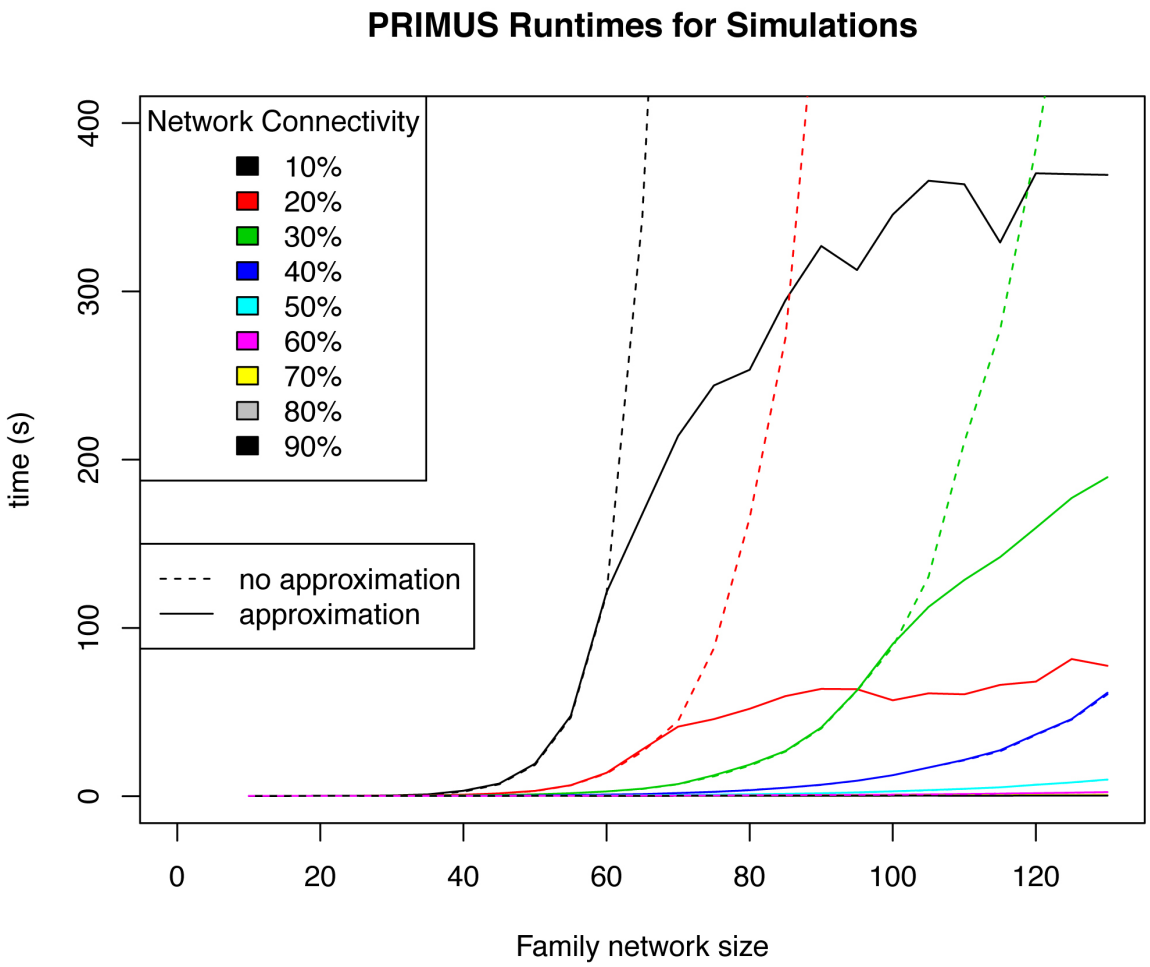


Figure 3. PRIMUS run-times on the simulations.
The dashed lines show the exponential run-time and computational infeasibility of the Bron-Kerbosch algorithm for large network sizes. The solid colored lines show the run-times of PRIMUS. The dashed and solid lines separate when as PRIMUS' approximation function is used to avoid the exponential run-times

Table 1. Minimum network size for which the approximation function of PRIMUS is applied.

Connectivity (<=)	15%	20%	25%	35%	45%	55%	65%	75%	100%
Approximation size cutoff (number of nodes)	60	70	90	100	130	170	230	330	500

Table showing the approximation network size for all ranges of network connectivity that PRIMUS will compute without using the approximation function. For example, a network with 10% connectivity will require the approximation function if the network size is > 60.

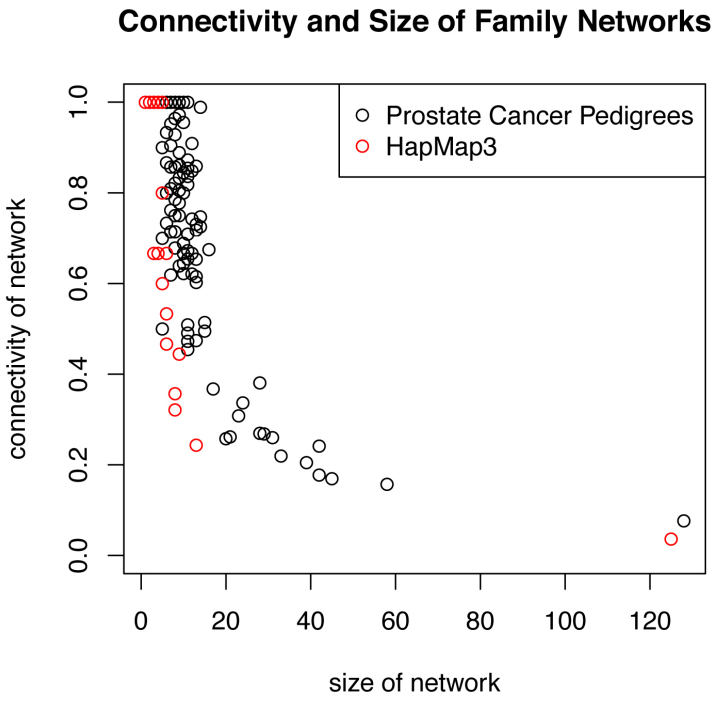


Figure 4. The diversity of sizes and connectivity levels for family networks in real data. The size and connectivity of family networks varies within datasets. We are using connectivity as a measure of how related the individuals are within a family network. Connectivity is the number pairwise relationships that exist in a dataset divided by the total possible number of pairwise relationships. Each circle on the plot represents one family network or pedigree from either the Prostate Cancer Pedigree dataset [49] or the HapMap3 dataset [50].

Results

Simulation Results

In all 6,540 simulations that did not require the use of the approximation function, PRIMUS produced an unrelated set of size equal to or greater than all other approaches. In our simulations, PRIMUS increased the unrelated set size by more than 50% relative to the PLINK method (Figure 5) and by similar amounts relative to the other selection methods (Figure 6). Although PRIMUS provides the greatest improvement as the network size and connectivity increase, even for sparse, small networks PRIMUS typically provides 5-20% improvement compared to the other methods (Figure 6).

Only when the PRIMUS' approximation function was used (960 simulations) do the other methods have the potential to outperform PRIMUS. The Pemberton method never outperforms PRIMUS because PRIMUS' approximation function is very similar to the Pemberton method while the size of the network is above the approximation size thresholds shown in Table 1. Table 2 shows that PRIMUS' approximation function outperforms the other three methods in more than 98.75% of the simulations. To address the 1.25% of cases, we have incorporated each of the other methods into PRIMUS, such that when it recognizes the need to run the approximation function, it will also run each of the other methods and return the largest unrelated set derived from any of the four methods.

We also compared the performance of each method on weighting for a binary and a quantitative trait. Similar to the maximum unrelated set identification, PRIMUS always identifies the largest set of unrelated affected individuals when the approximation function is not needed. PRIMUS retained up to 75% more affected individuals in the weighted comparisons between PRIMUS and each of the other methods (Figure 7).

HapMap3 Results

Finally, we compared the performance of PRIMUS and the other three methods on data from Phase 3 of the Haplotype Mapping Project[50] and the 1000 Genomes Project[51]. For each dataset, PRIMUS obtained the largest set of unrelated individuals (see Table 3). Given our IBD estimates

for these reference datasets, the maximum sample set in which no pair of individuals have a coefficient of relatedness ($\hat{\pi}$) > 0.1 are listed in Table 3 and a link to a list of the sample IDs can be found at the PRIMUS website (see Web Resources).

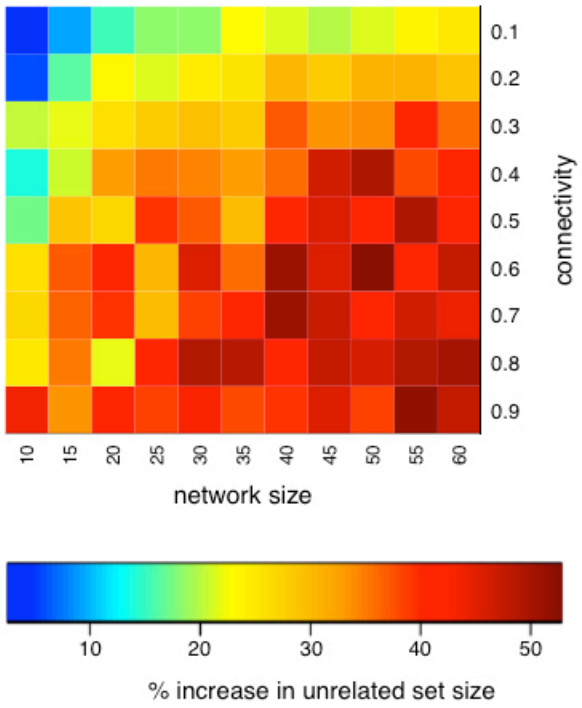
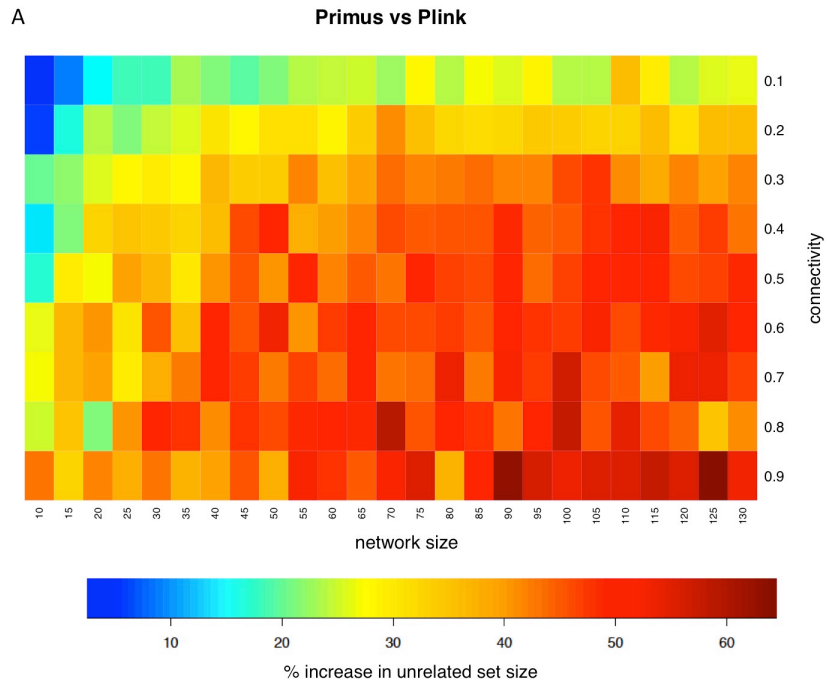
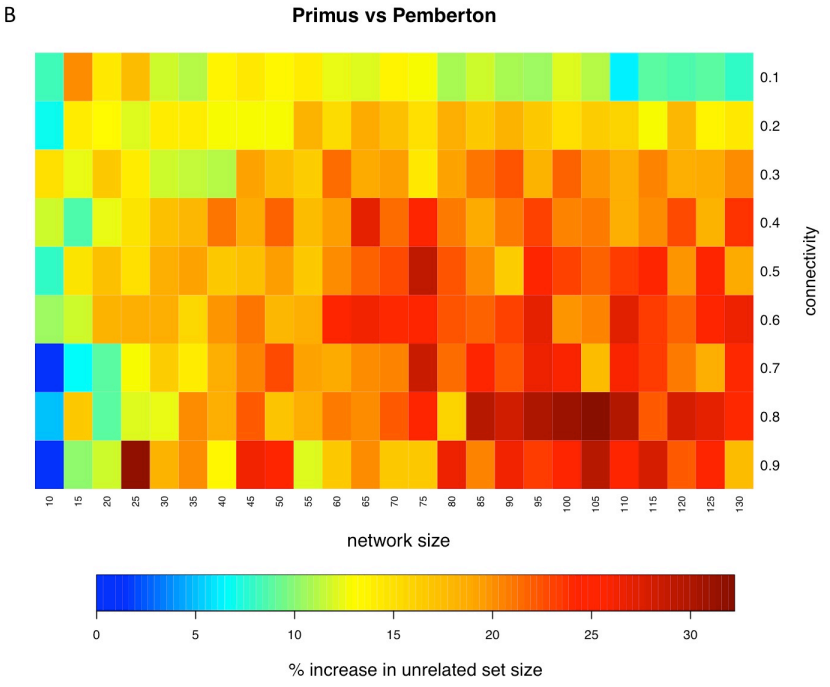


Figure 5. A heatmap showing the percent increase in unrelated sample size by PRIMUS compared to PLINK's suggested method.
The vertical axis is the number of edges in the network divided by the total number of possible edges. The horizontal axis is the size of the simulated network. The color in each square corresponds to the percent increase in the size of the unrelated sample set generated by PRIMUS relative to the set generated by PLINK averaged across 30 randomly generated networks.

A


Primus vs Plink
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
connectivity
10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130
network size
10 20 30 40 50 60
% increase in unrelated set size

B


Primus vs Pemberton
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
connectivity
10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130
network size
0 5 10 15 20 25 30
% increase in unrelated set size

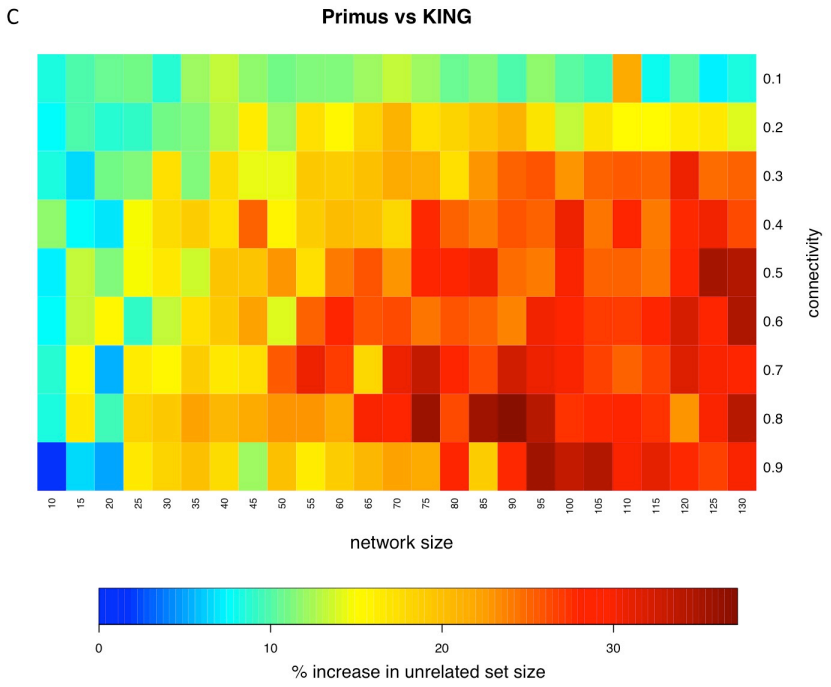


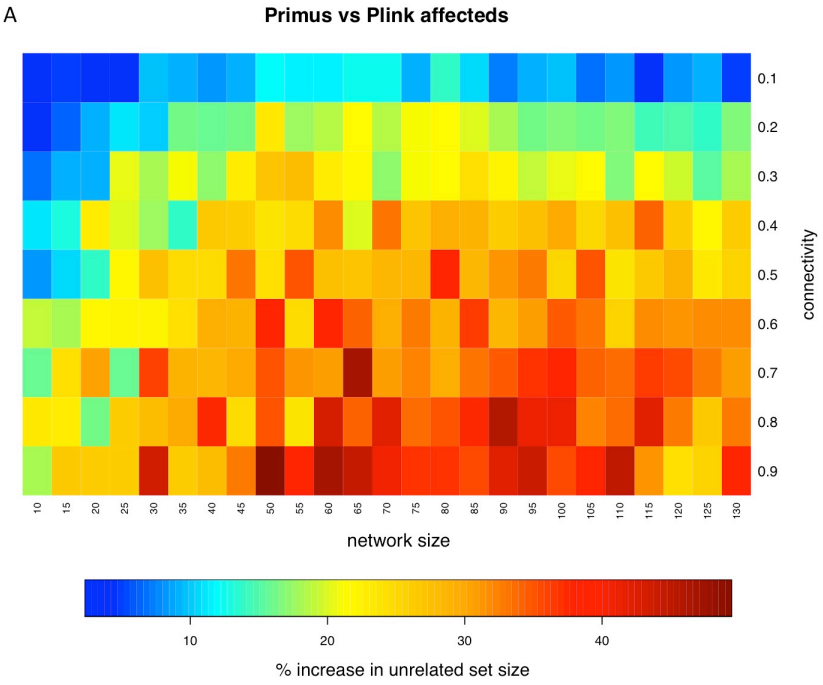
Figure 6. Heatmaps comparing PRIMUS and three other methods on simulated data.
For N retained unrelated samples, the color in each square reflects the value of $\frac{1}{30}\sum_{1}^{30}\frac{(N_{PRIMUS}\text{-}N_{alt})}{N_{alt}}$. (A) A comparison of PRIMUS to the PLINK method. (B) A comparison of PRIMUS and Pemberton. (C) A comparison of PRIMUS and KING.

Table 2. The number of simulations where other methods outperform the approximation function in PRIMUS.

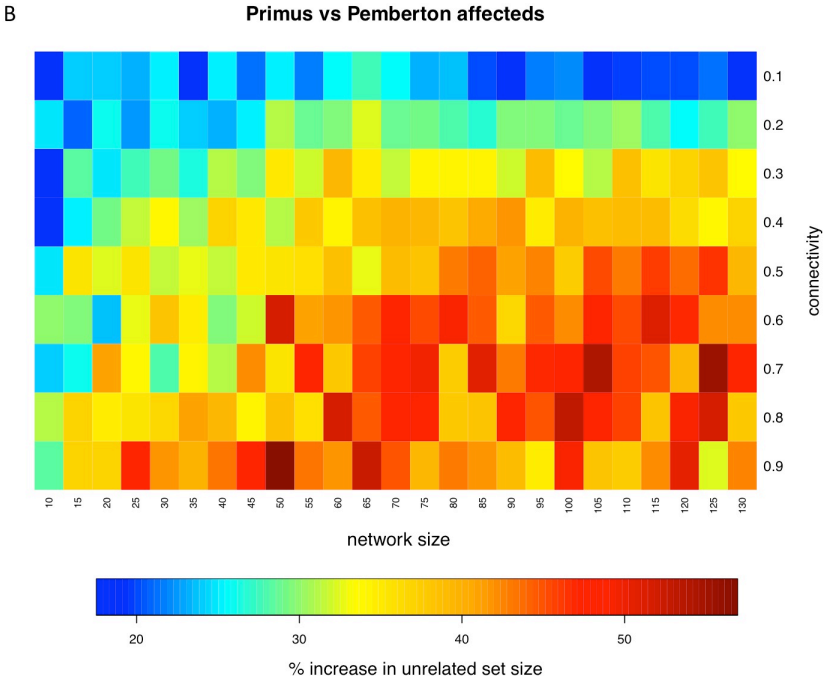
Weighting Criteria	**PLINK**	**Pemberton**	**KING**	**% of total**
No weighting	1/960	0/960	6/960	0.24%
Affected status	51/960	0/960	0/960	1.77%
Low quantitative trait	2/960	0/960	19/960	0.73%

There were 960 simulations that required PRIMUS to use its approximation function. The table shows out of those 960 simulations how many simulations did the other methods outperform PRIMUS.

A


Primus vs Plink affecteds
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
connectivity
10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130
network size
10
20
30
40
% increase in unrelated set size

B


Primus vs Pemberton affecteds
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
connectivity
10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130
network size
20
30
40
50
% increase in unrelated set size

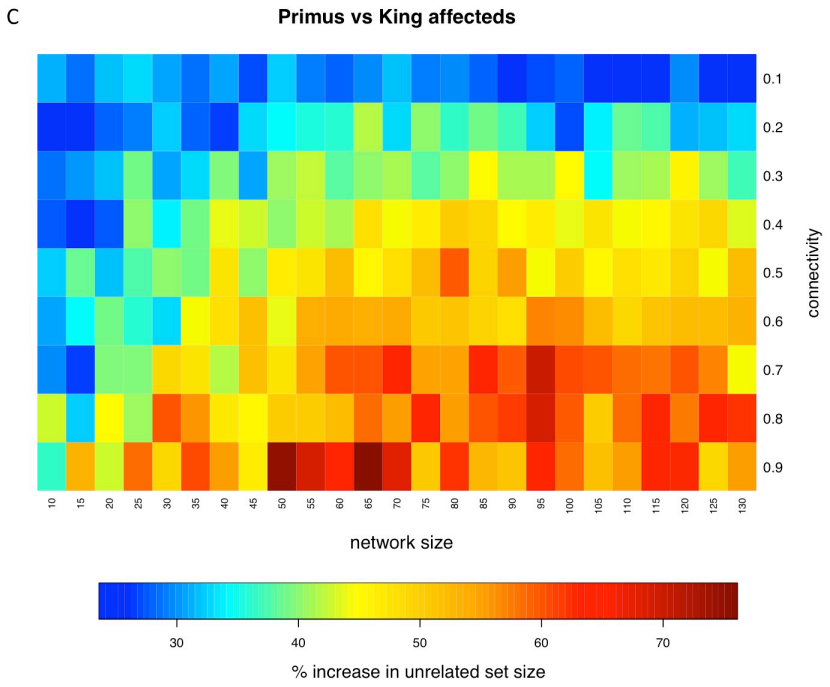


Figure 7. Heatmaps comparing PRIMUS and three other approaches to identify unrelated sets on simulation data when using the binary weighting functions.

The color in each square reflects the value of $\frac{1}{30}\sum_{1}^{30}\frac{(N^{a}_{PRIMUS}-N^{a}_{alt})}{N^{a}_{alt}}$ where N^{a} is the number of affected individuals in the unrelated set. (A) A comparison of PRIMUS to the PLINK method. (B) A comparison of PRIMUS and Pemberton. (C) A comparison of PRIMUS and KING.

Table 3. Comparison of PRIMUS and other methods on publicly available datasets.

Cohort	# of samples	PRIMUS	PLINK	Pemberton	KING
1K Genomes	1094	519	494	518	516
HapMap 3	1184	899	843	893	899
ASW	83	46	44	46	46
CEU	165	112	91	112	112
CHB	84	84	84	84	84
CHD	85	83	83	83	83
GIH	88	84	84	84	84
JPT	86	86	86	86	86
LWK	90	78	78	78	78
MEX	77	47	39	46	47
MKK	171	81	74	77	81
TSI	88	88	88	88	88
YRI	167	110	92	109	110

Table contains the total number of samples (including related individuals) for each cohort and the sizes of the unrelated sets produced by each method. The HapMap 3 cohort is also separated by population. IBD estimates were generated in PLINK, and a pair of individuals was determined to be related if the coefficient of relatedness ($\hat{\pi}$) >= 0.1. In datasets with limited relatedness like the HapMap, all the approaches work well

Discussion

Although PRIMUS will identify the largest unrelated set of samples, as is shown in Figure 5, the performance advantage of PRIMUS depends strongly on the amount of connectivity within the families, the size of the families, and clearly, the presence of family data among the samples (all methods do equally well when the samples are unrelated). PRIMUS provides the greatest benefit on large family networks with moderate to high interrelatedness; however, PRIMUS is useful on all varieties of genetic datasets.

We recommend using PRIMUS to obtain unrelated reference sample sets. For example, many researchers use HapMap3 and 1000 Genomes datasets to impute genotypes, estimate population allele/haplotype frequencies, and run principle component analyses. However, both datasets contain related samples[44], and if the interrelatedness is not removed, then these imputations and estimates will be inaccurate.

Methods exist to account for pedigree structure or interrelatedness when doing association studies[15; 16]. However, in the context of large cohort studies the power gain would be modest since most samples are not related within the last few generations, and the minor power gain may not be worth the computational burden of accounting for the interrelatedness. In such a case, PRIMUS is the best option for removing relatedness.

We also recommend using PRIMUS' weighted maximum set selection to optimize the selection of an unrelated set with a desired characteristic. Specific scenarios include selecting for affected status in a case/control study, selecting for the lowest missingness, or selecting samples in the tails of a distribution in a quantitative trait study.

PRIMUS allows users to specify the level of relatedness in their dataset. Since PRIMUS can take any quantitative measure of relatedness, the selected cutoff should be based on the sensitivity of the tool used to estimate the pairwise relatedness. For example, PLINK is relatively accurate at estimating relationships up to first cousins but less accurate for more distant relationships[39]. Therefore a coefficient of relatedness ($\hat{\pi}$) cutoff of 0.1 is appropriate. We have found that KING has similar sensitivity as PLINK when estimating pairwise relationships; however, KING uses the kinship coefficient, $\hat{\pi}/2$; therefore, the recommended cutoff for KING IBD estimates is

0.05. Other programs[39; 40] are more powerful at accurately detecting more distant relationships, and the user specified cutoff should be adjusted accordingly.

When statistics assume independence among samples, stripping datasets of relatedness observed in the genetic data is a necessary step in quality control and data cleaning. We have developed an efficient and optimal approach that uses user-generated IBD estimates to quickly provide a maximum set of unrelated samples to retain in further analyses. Despite the importance of retaining the largest sample size possible in genetic analyses, we have only found a single published analysis that utilizes this concept[52] to obtain a maximum unrelated set of samples. In addition, our approach provides the option to retain the most informative samples in the resulting dataset (i.e. based on phenotype or data missingness). Furthermore, as a by-product, PRIMUS reports all connected family networks in the data; knowledge of these networks can then be leveraged to improve the power in some analyses by utilizing this familial information[8], or to select the most distantly related affected individuals within each family for exome or whole genome sequencing. Finally, PRIMUS is fast, capable of processing thousands of individuals distributed across hundreds of family networks in minutes or less, making it a practical tool for even the largest and most complex datasets.

Chapter 3. PRIMUS: Rapid Reconstruction of Pedigrees from Genome-wide Estimates of Identity by Descent

Understanding and correctly utilizing relatedness among samples is essential for genetic analysis; however, managing sample records and pedigrees can often be error prone and incomplete. Data sets ascertained by random sampling often harbor cryptic relatedness that can be leveraged in genetic analyses for maximizing power. We have developed a method that uses genome-wide estimates of pairwise identity by descent to identify families and quickly reconstruct and score all possible pedigrees that fit the genetic data by using up to third-degree relatives, and we have included it in the software package PRIMUS (Pedigree Reconstruction and Identification of the Maximally Unrelated Set). Here, we validate its performance on simulated, clinical, and HapMap3 pedigrees. Among these samples, we demonstrate that PRIMUS can verify reported pedigree structures and identify cryptic relationships. Finally, we show that PRIMUS reconstructed pedigrees, all of which were previously unknown, for 203 families from a cohort collected in Starr County, TX (1,890 samples).

Introduction

Following the transmission of variants through a genealogy is at the foundation of modern genetics. Today, pedigrees continue to be utilized to determine the heritability and genetic models for traits and disorders, and knowing the exact pedigree structure allows investigators to correctly identify the genetic mode of disease inheritance as well as utilize powerful genetic analysis tools that require, or benefit from, the true pedigree structure such as linkage[7], family-based association[8], pedigree aware imputation, pedigree aware phasing, Mendelian error checking, and heritability. In many instances, knowing the pedigree that is consistent with the generated genetic data is crucial to solving the disease[4-6; 9]. Additionally, the collection of samples from a limited geographical region for a genetic analysis may introduce biases toward unintentionally obtaining samples of unknown relatedness for which a previously unknown pedigree could be reconstructed and used. As a result, large case/control consortia can harbor cryptic relatedness[11], which will bias the analysis unless the cryptic relatedness is removed or investigators use a method that models a kinship matrix[53]. However, a substantial increase in power may be obtained if the true pedigree structures were known[53].

Given the benefits of family-based studies in genetic research, an enormous amount of effort is spent collecting and maintaining accurate sample records and corresponding pedigrees. However, despite the best efforts of investigators, pedigree and sample errors are still quite common and require careful examination so as to avoid a reduction in power to detect linkage[19]. The rate of non-paternities in studies has been reported between 0.8% and 30% (median 3.7%; n=17)[20], with other reports showing more conservative estimates around 1% to 1.5%[21; 22]. Even at the conservative rate of 1%, a pedigree with six children has a 6% chance of being incorrect due to a non-paternity, and the pedigree error rate will be much higher after accounting for other common errors such as sample swaps, duplicate samples, contamination, and other relationship discrepancies. The standard practice for checking and correcting pedigrees and relationships within genetic datasets is to use pairwise prediction programs[23-27] like RELPAIR[28] and PREST[29] to verify that the level of relatedness between every pair of individuals falls close to the expected level of relatedness based on the reported pedigree[30-37].

While using pairwise estimates to check relationships in pedigrees is sometimes sufficient, there are four major drawbacks that we illustrate in this manuscript. First, pairwise checking will not catch pedigree errors if there are multiple pedigree structures that fit the genetic data, and the reported pedigree structure is among the incorrect possibilities. Second, pairwise relationship checking does not provide, or even suggest, the correct pedigree in the case of inconsistency between the data and the reported pedigree. Instead, these methods flag inconsistent relationships for the investigator to review by hand. Third, pairwise inconsistencies between genotyped samples are often resolved by removing the inconsistent sample(s), which can result in the unnecessary loss of samples or in accepting an incorrect pedigree as true. Finally, manually reconstructing an unknown pedigree using pairwise relationship comparisons requires arduous, error-prone labor. Previous attempts have been made to address this issue. For example, Pemberton *et al.*[44] manually reconstructed cryptic HapMap3 pedigrees, but the authors encountered inconsistencies they could not resolve by hand.

A possible solution to the drawbacks of checking pedigrees by using pairwise comparisons is to use the genetic data to reconstruct the corresponding pedigree structure. Ideally, pedigree reconstruction would not only identify any inconsistencies in a pedigree, but also automatically provide the correct pedigree. Pedigree reconstruction methods exist; however, the reason they are

not the standard for checking pedigrees in genetics studies is that existing methods have limited uses. Current approaches are limited in the number of genetic variants that can be used[54-56], are heavily biased in the presence of linkage disequilibrium between markers[57], cannot reconstruct half-sibling relationships[58; 59], or cannot reconstruct a pedigree if it is connected by individuals for whom no genotype data are available[54-57]. Even the most recent methods (COP/CIP[59], IPED[58]/IPED2, and PREPARE[60]) assume that all genotyped individuals are in the same generation, requiring *a priori* knowledge of the relative generations of the samples or the pedigree structure. Using the age of individuals is not adequate; for example, it is not uncommon to have an uncle/aunt younger than a niece/nephew. The most recent methods are good at reconstructing a small niche of pedigrees structures, but few pedigree structures typical of human genetic studies fall into this niche. Indeed, these are not capable of reconstructing many basic and common pedigree structures (e.g., trios).

We have developed a pedigree reconstruction method without many of the limitations of previous pedigree reconstruction programs and have incorporated it into a software package known as Pedigree Reconstruction and Identification of the Maximally Unrelated Set (PRIMUS)[18]. Our method utilizes the power of single nucleotide polymorphism (SNP) arrays or next-generation sequence data to evaluate genome-wide estimates of identity by descent (IBD) that are generated by programs such as PLINK[23] or KING[25]. Our method assigns relationships using the expected mean and variance for each relationship class and leverages all pairwise relationships within a family (as well as genetically-determined sex) to reconstruct the possible pedigree structures consistent with the observed pairwise sharing. We designed PRIMUS to improve on previous methods in several ways—PRIMUS automatically reconstructs multigenerational pedigrees with genotyped samples in any generation; reconstructs using all individuals connected to a pedigree at a level of 3rd degree relatives or closer; requires no prior knowledge of the pedigree structure; allows for missing (i.e., non-genotyped) individuals in the pedigree; appropriately incorporates half-siblings; allows for, but does not require, additional information such as sex and age of samples to improve reconstruction; and inputs and outputs common file formats to improve usability.

In this report, we validate the performance of PRIMUS on thousands of simulated pedigrees. We also demonstrate its ability to reconstruct clinical pedigrees, HapMap3 pedigrees, and to find

previously unknown relationships in a large population-based study from Starr County, Texas, illustrating that PRIMUS can 1) reconstruct, validate, and correct reported pedigrees, 2) incorporate cryptic relatedness into known pedigrees, and 3) find and reconstruct previously unknown pedigrees that can exist within large genetic datasets.

Methods

Simulated pedigrees

We generated simulated pedigrees for the training and initial testing of PRIMUS using a broad range of known pedigrees that contained different structures, sizes, genotypes, and combinations of missing data among the individuals. In all, thousands of pedigrees were generated for three classes of pedigree structures:

1. Size12 pedigree: a 12-person pedigree containing all relationships from Table 4 (Figure 8).
2. Uniform pedigree: a variable-sized pedigree with no half-sibling relationships in which each pair of parents is expected to have three children. However, to obtain the desired pedigree sizes, there may be a single pair of parents with as few as one child or as many as four children (Figure 9).
3. Half-sibling pedigree: identical to Uniform except there is a 30% chance that one person from each pair of parents has two children with another individual (Figure 9).

For both the Uniform and Halfsib pedigrees, we simulated complete pedigrees of sizes ranging from five to 400 individuals. For each pedigree we created different genotypes for 100 versions of the pedigree structures using the method applied by Morrison[61] (see Web Resources): we randomly selected founder haplotypes with ~1M SNPs from among the unrelated HapMap3 CEU samples, and we simulated recombination as a homogeneous Poisson process disregarding the centromere and using the approximation 1 Mb = 1 cM. We compared the true IBD proportions to those calculated by PLINK for IBD estimates generated from 6K and 1M SNPs (Figure 10). The correlation between the estimates and the true values were $r^2 = 0.999$ with pedigrees of size ten and $r^2 = 0.974$ with pedigrees of size 400. IBD estimates generated from as few as 6K SNPs are still remarkably accurate (Supplementary Table 1), and they improve as the number of SNPs increases. We also tested the accuracy of IBD estimates calculated using the overlap of the approximately one million HapMap3 SNP set and commonly used SNP panels and

found high accuracy levels (Supplementary Table 1). Unless otherwise stated, the complete ~1M SNP sets were used for the simulations.

We also simulated data missingness in each of the Uniform and Halfsib pedigrees. To accomplish this, we created ten additional versions of each pedigree by iteratively removing genetic data for a single sample until we had removed up to ten missing individuals. Data were eligible for removal if the individual had children and if his or her removal did not create a gap in the pedigree larger than a 3rd degree relationship. Eligible samples were removed uniformly at random, creating unique combinations of missing sample data for each pedigree.

Table 4. Expected mean IBD proportions for the outbred familial relationship categories

Familial Relationship	Symbol	IBD0	IBD1	IBD2
Parental	PO	0	1	0
Full-sibling	FS	0.25	0.5	0.25
Half-sibling/avuncular/grandparental	2^{nd}	0.5	0.5	0
1^{st} cousin/great-grandparental/ great-avuncular/half-avuncular	3^{rd}	0.75	0.25	0
Distantly related	DIS	Varies	Varies	0
Unrelated (includes > 3° relationships)	UN	1	0	0

IBD0, IBD1, and IBD2 are the proportions of the genome shared on 0, 1, and 2 chromosomes, respectively, between two individuals. Many relationships share the same expected mean IBD proportions; however, for FS, 2^{nd} degree, and 3^{rd} degree relationships there is a variance around the expected mean that is due to the random nature of recombination events. Genotyping and other technical errors can contribute to this variance.

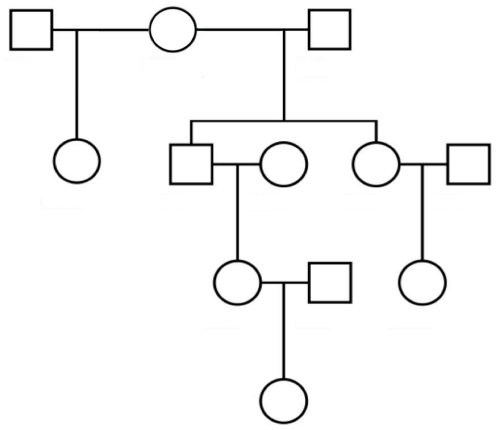


Figure 8. Schematic of a simulated 12-person pedigree.
This pedigree contains all types of familial relationships shown in Table 4. We randomly assigned HapMap3 CEU haplotypes to each of the founders and then simulated recombination events to propagate these genotypes to the children.

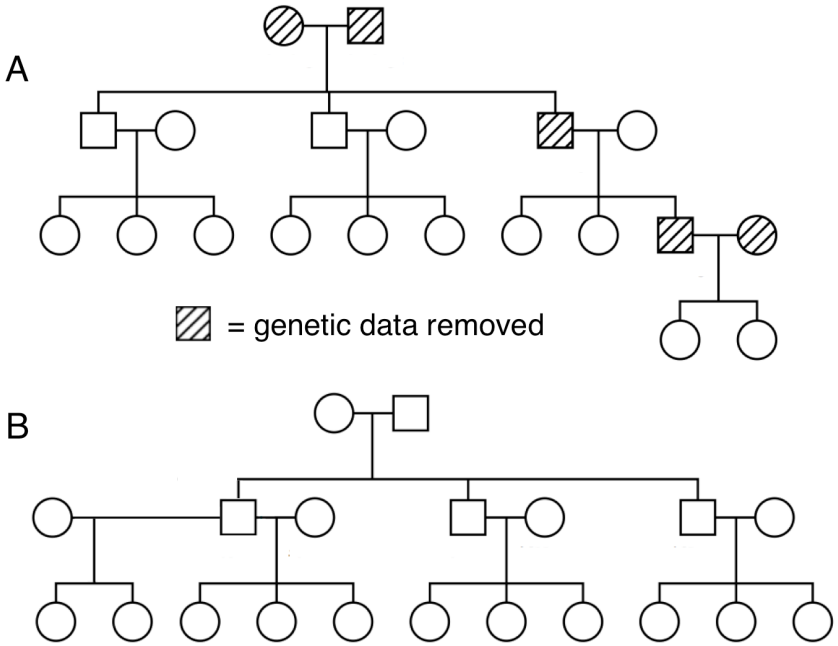


Figure 9. Examples of simulated pedigrees of size 20.
A) Uniform size-20 pedigree with five samples for whom the genetic data was removed. The missing individuals simulated the real world case where you cannot get good genotypes from an individual either due to lack of consent, poor DNA quality, contamination, or absence of the individual. All of the remaining individuals are genotyped and are included in the pedigree and the reconstruction. B) Halfsib size-20 pedigree without any missing individuals.

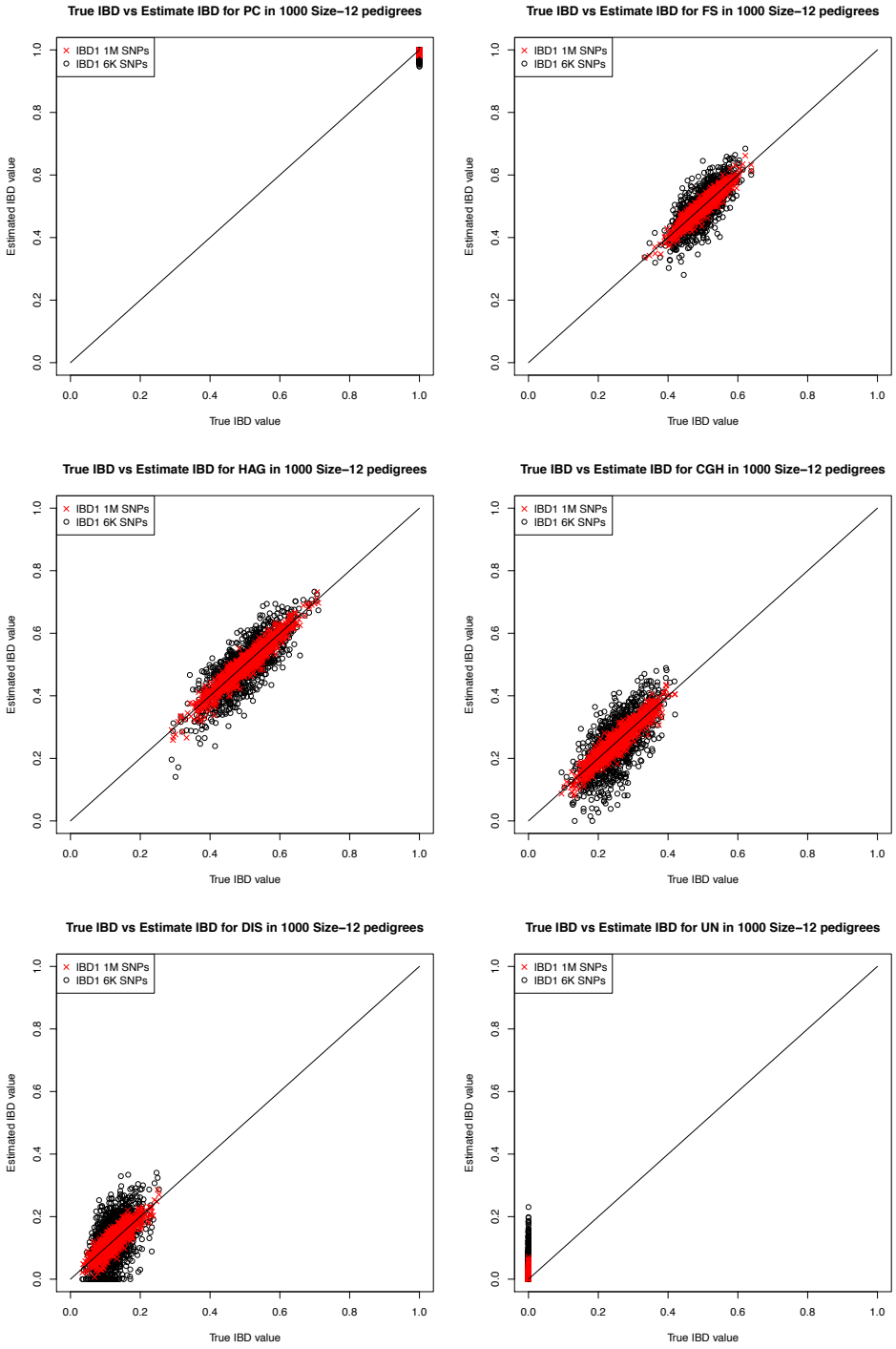


Figure 10. Comparison of the true IBD1 value to the PLINK IBD1 estimates for relationship sampled from 1000 size-12 pedigrees.
Each graph shows the comparison of 6K SNPs and 1 million SNPs to the true IBD value. Each plot shows a different relationship category. IBD estimates generated from 6K SNPs have a much wider variance than the one IBD estimates generated from 1M SNPs. However, the distance that they depart from the expected value appears to remain fairly constant at each degree of relatedness.

IBD estimates

PRIMUS takes input from any program that provides estimates of the proportions of the genome shared IBD on 0, 1, and 2 chromosomes (IBD0/1/2). We note that calculating accurate relationships and estimating pairwise IBD is a non-trivial problem, and one that has been tackled by a number of methodologies[23; 25; 42; 62; 63]. IBD proportions presented here were calculated using the method of moments estimation implemented in PLINK[23]. Although not required for the simulated pedigrees, some pedigrees may require careful analysis of admixture in the samples. In these cases, we applied the approaches recommended by Morrison[61] to remove ancestry informative SNPs that could otherwise bias IBD estimates. The code used to calculate IBD estimates is available for download with the PRIMUS package (Web Resources).

Family network identification

PRIMUS first groups the samples into family networks (or groups) based on the estimated pairwise coefficient of relatedness (two times the kinship coefficient)[18]. An individual is only added to a family network if the sample is related to at least one other person in the network given a user-defined minimum coefficient of relatedness. For example, 0.1875, the midpoint between the mean expected IBD proportion for 2nd and 3rd degree relatives, is a threshold that will capture connections between most 2nd degree relatives or closer. The pedigree reconstruction is then performed independently on each family network within the dataset.

Familial relationship prediction using a kernel density estimation (KDE) function

PRIMUS uses six relationship categories to reconstruct pedigrees based on the expected mean IBD0/1/2 shown in Table 4; however, distantly related and unrelated samples are handled as the same class during reconstruction. Both biological (i.e., recombination events, population substructure, historic inbreeding) and technical (i.e., density and distribution of the genotyped markers) factors contribute to variation around these means.

Given the IBD0, IBD1, and IBD2 estimates for a pair of individuals, PRIMUS predicts the corresponding relationship category using a trained kernel density estimate (KDE; see Web Resources) for each of six familial relationship categories. We chose to use the KDEs to account for the overlapping IBD distributions and the variability of the IBD estimates. We used the

scipy.stats.gaussian_kde function (Web Resources) with two training features: genome-wide estimates of IBD0 and IBD1. The training IBD0 and IBD1 estimates were selected from the IBD estimates generated with 6K SNPs for the 1,000 Size12 simulated pedigrees. We chose to use the lower number of SNPs so that the KDE could better handle the technical noise that comes with estimating IBD. A parent/child, full-sibling, 2nd degree, 3rd degree, distantly related, and unrelated relationship were selected from each of the 1,000 simulated pedigrees and were used to train the respective KDEs. We used these simulated IBD proportions to train a KDE function for each of the six familial relationship categories.

Since the coefficient factor influences the trained KDE, we tested each KDE with different values for the coefficient factor used in calculating the kernel covariance matrices and the results are in Figure 11. Evaluated the performance of each KDE by calculating the false positive (FP) and false negative (FN) relationship predictions when trained with different coefficient factors. We used these predictions to optimize the ability of PRIMUS to accurately identify the relationship between two individuals (true positive = 1 - FN) while minimizing the number of incorrect relationships that it predicts (FP). Since the optimal bandwidth would need to perform well across different likelihood cutoffs, we tested the performance of PRIMUS with likelihood cutoffs ranging from 0.01 to 0.5. We used the scipy.stats.gaussian_kde function (Web Resources) with two training features: genome-wide estimates of IBD0 and IBD1. We tested a range of bandwidths by specifying scalar values 1 through 17 as the "bw_method" option and these values are used as the coefficient that multiplies the data covariance matrix to obtain the kernel covariance matrix. With KDEs trained at each bandwidth coefficient value from 1 to 17, we predicted the relationship category of each relationship in the 100 Uniform size-400 pedigrees at likelihood cutoffs varying from 0.01 to 0.5. We evaluated the relationship prediction of the KDEs trained with different bandwidths by testing their FN (results A-E) and FP (results F-J) rates.

The results can bee seen in Figure 11. The color in each cell indicates the number of relationships from the 100 size-400 Uniform pedigrees that were either FN or FP. The color scale is $\log_{10}$. An FN occurs if the true relationship did not have a likelihood higher than the cutoff. An FP occurs if a relationship other than the true relationship has a likelihood higher than the likelihood cutoff. Parent-offspring relationships did not have any FP or FN predictions, so the

corresponding heat maps are not shown. We selected the covariance factor for each relationship category that minimized the FP and FN predictions, and these are set as the default in PRIMUS: PO = 17; FS = 2; 2nd degree = 6; 3rd degree=5; DIS = 2; UN = 1.

With an initial likelihood threshold higher than 0.3, we found a higher rate of false negative relationship predictions for 2nd degree, 3rd degree, and distantly related relationships in the Uniform size-400 pedigrees (Figure 10). However, lowering this threshold results in more relationships with likelihood scores that exceed the threshold. If there is more than one relationship category that exceeds the likelihood threshold, then PRIMUS will attempt to reconstruct a different version of the pedigree for each possible relationship, resulting in additional computational time. Therefore, we desired a default threshold that was lenient enough to reduce the chance of a false negative prediction, but also stringent enough to minimize the number of false positive relationships that are tested in the reconstruction.

We chose 0.01 as the lower likelihood threshold bound because all relationship categories had 0% false negative rate at this threshold for their selected bandwidth. The strategy for the automatically lowering threshold is designed to capture the true pedigree while minimizing the runtime and the number of possible false positive pedigrees. This strategy assumes that PRIMUS will not output a pedigree structure until all true relationships have a likelihood higher than the likelihood threshold, and, thus, it will be able to reconstruct the true pedigree structure. There are rare scenarios (~0.5% of the simulations, Supplementary Table 2) where PRIMUS did not output a correct pedigree structure before the threshold was low enough to correctly predict all familial relationships. Therefore, in this rare scenario, the true pedigree structure was not among the PRIMUS results. In these instances, PRIMUS can generate the true pedigree structure if the likelihood threshold is initially set low enough (e.g., 0.01). We chose 0.3 as the default because it provides the greatest savings in runtime and reduced number of possible pedigrees for the common uses of PRIMUS, but users can select a different value to fit their custom needs.

These empirical tests allowed us to select the coefficient for the KDE of each relationship category that best optimized reconstruction performance. For the overlapping KDE distributions, we selected the smallest bandwidth that had no false negative predictions of our test dataset at a likelihood cutoff of 0.01 or lower bandwidth. We selected the largest bandwidths possible for

parent/offspring and full-sibling without overlap of the density distributions with other relationship categories. This minimizes the false positive calls for these predictions. Figure 12 shows a density plot for the KDE of each relationship category, which is consistent with previous reports of genome-wide IBD proportions[64].

PRIMUS uses the trained kernels to predict the familial relationship category for each pairwise relationship. For a set of IBD0/1/2 proportions, PRIMUS queries each kernel for the density at the IBD0 and IBD1 values and stores the density for each familial category in a vector. Then PRIMUS normalizes the vector by dividing each density by the sum of all densities, producing a vector of the likelihoods corresponding to each familial category. This relationship likelihood vector is used during both reconstruction and ranking of possible pedigrees.

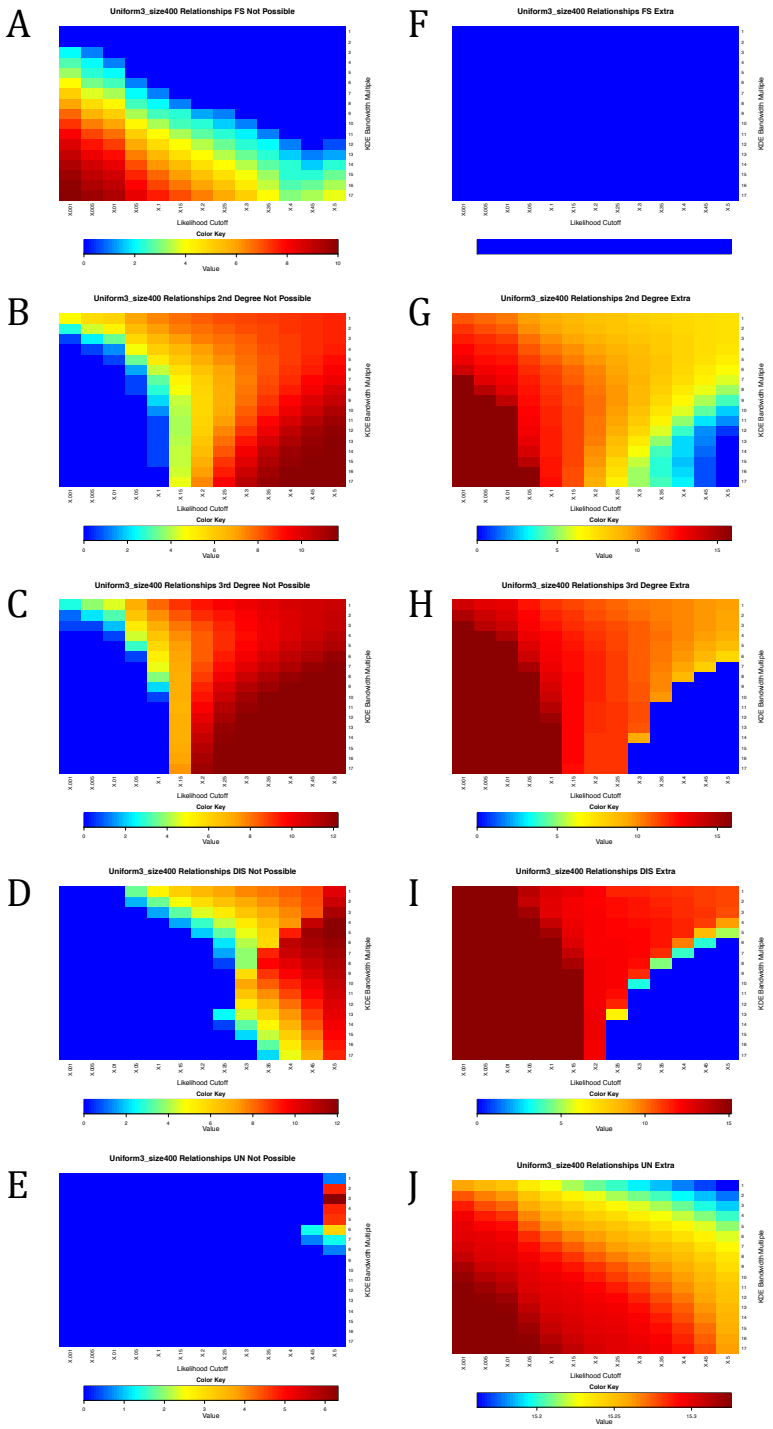


Figure 11. False positive (FP) and false negative (FN) relationship predictions with different KDE bandwidths and likelihood cutoffs for full-sibling (FS), 2nd degree, 3rd degree, distant (DIS) and unrelated (UN) relationships.
We evaluated the relationship prediction of the KDEs trained with different bandwidths by testing their FN (results A-E) and FP (results F-J) rates. The color in each cell indicates the number of relationships from the 100 size-400 Uniform pedigrees that were either FN or FP. The color scale is log10.

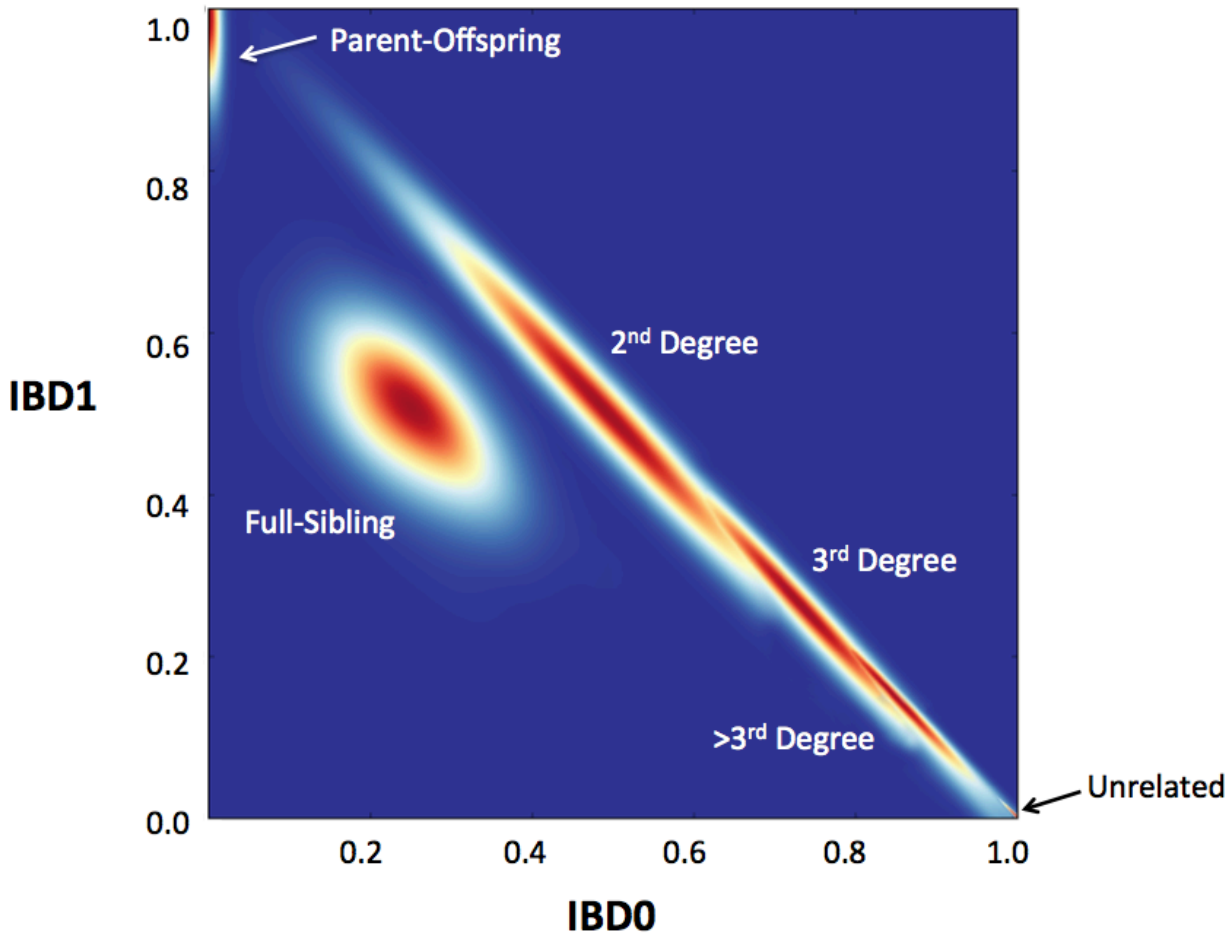


Figure 12. Kernel density distributions of the trained kernel density estimates for each familial relationship category.
Parent-offspring and full-sibling are viably separated from the other density clusters. 2nd Degree and 3rd Degree are labeling the distribution of IBD estimates for 2nd and 3rd degree relationships, respectively. >3rd degree and "Unrelated" label the distributions of IBD estimates for relatives more distant that 3rd degree or unrelated, respectively.

Pedigree reconstruction algorithm

For each family network, PRIMUS uses the relationship likelihood vectors of all pairwise relationships to reconstruct all possible pedigrees, subject to the restrictions that a) only relatives up to 3rd degrees are considered and b) the likelihood of each relationship class considered must exceed a minimum likelihood threshold (initial default of 0.3). We chose 0.3 as a good initial likelihood threshold based on the relationship predictions of the Uniform size-400 pedigrees (see Figure 11 for details).

The reconstruction is an iterative process of identifying a pairwise relationship within the family network that has not yet been incorporated into the pedigree, fitting that relationship into the pedigree, and testing that all of the relationships generated by adding the individual are compatible with the relationship likelihood vectors and sex data for all of the samples. If the addition of a relationship is incompatible with the relationship likelihood vectors or if two individuals of the same sex have offspring, the pedigree is rejected and removed from the set of possible pedigrees. The reconstruction continues until all pairwise relationships from the family network are represented in each possible pedigree or until there are no possible pedigrees left to continue reconstructing.

PRIMUS reconstructs in three phases. Phase 1 uses parent-offspring (PO) and full-sibling (FS) relationships. These two types of relationships are the most accurately predicted because POs have no biological variance around the expected proportion of sharing, and FS are the only non-consanguineous relationship with IBD2 greater than 0. Phase 1 creates a backbone on which to build the more distant relationships. A PO relationship between individuals A and B is added to the pedigree by creating a version of the pedigree in which A is the parent of B and another version in which B is the parent of A. Missing individuals are added as necessary so that each individual in the family network has zero or two parents. In phase 2, PRIMUS reconstructs 2nd degree relationships: half-sibling, avuncular, and grand-parental. The algorithm tests all possible re-arrangements for each 2nd degree relationship within the pedigree and adds missing individuals to connect portions of the pedigree as necessary. Phase 3 is identical to phase 2, except that it considers 3rd degree relationships: first cousins, half-avuncular, great-avuncular, and great-grandparental. Since PRIMUS always checks every possible way that a sample can be

added to the pedigree and eliminates pedigrees that do not fit, it is effectively exploring the entire search space of possible pedigrees. At present, PRIMUS does not reconstruct complex relationships (e.g., half-sibling plus first cousin or double 1st cousins), consanguineous relationships, or relationships more distant than 3rd degree relatives. If one of these relationships is present in the dataset, PRIMUS will match it to one of the relationship categories in Table 4 and fit the relationship into the pedigree accordingly.

Automatically adjusting likelihood threshold

If PRIMUS reaches the end of reconstruction and has zero possible pedigrees remaining, then it will automatically lower the likelihood threshold from the default of 0.3 to 0.2 and will rerun, allowing PRIMUS to consider additional possible pairwise relationships with likelihoods between 0.2 and 0.3. PRIMUS will continue to gradually drop the likelihood threshold until it produces a possible pedigree or it reaches a threshold below 0.01. If no possible pedigrees result from reconstruction after lowering the threshold below 0.01, then PRIMUS stops reconstruction. For further details, see Figure 11.

Pedigree scoring

For many families, there is only one possible pedigree that fits the data and the true pedigree. However, due to the unknown directionality of some relationships and missing data for individuals, PRIMUS can reconstruct more than one possible pedigree that fits the genetic data with the true pedigree among them. We attempt to increase the chances that the true pedigree is near the top of the list by ranking the possible pedigrees using the relationship likelihood vectors to obtain a pedigree score.

PRIMUS will rank the pedigrees according to a pedigree score calculated by summing the log of the likelihood value of each relationship in the pedigree. This score is used to provide a ranking of the pedigrees. For example, if a pedigree has only two individuals, and they have a 0.6 likelihood of being 2nd degree relatives and a 0.4 likelihood of being 3rd degree relatives, then all pedigrees where they are 2nd degree relatives will be ranked higher than pedigrees in which they are 3rd degree relatives. Additionally, if the ages of individuals are provided, then PRIMUS will flag and rank all pedigrees where the ages are inconsistent (e.g., a child is older than the parent).

PRIMUS results and output

PRIMUS uses Cranefoot[65] (Web Resources) to provide an image of each pedigree and provides the corresponding PLINK-formatted FAM file. Summary results are provided for each family network and the entire dataset, as well as a list of the possible relationships for each pair of related individuals similar to Supplementary Table 3. See the PRIMUS documentation for a complete list and description of output files and formats (Web Resources).

Pedigree checking program

PRIMUS also has the ability to check that a reported pedigree is among the produced reconstructed pedigrees. The user provides the reported pedigree in the form of a PLINK FAM or PED file, and PRIMUS compares it to each of the reconstructed pedigrees to see if there is a match. In the case that the reconstruction included additional samples that were not part of the reported pedigree, PRIMUS will find the match and report that there are additional genotyped samples included in the pedigree.

Reconstructing authentic pedigrees

We tested the ability of PRIMUS to reconstruct several different pedigrees using real genetic data. IBD estimates were obtained from genotypes generated with a HumanCytoSNP-12 BeadChip for all available pedigrees obtained by the University of Washington Center for Mendelian Genomics (UW CMG) with the exception of 49 pedigrees for which only exome sequence data were generated (see Boston EOCOPD samples). UW CMG studies were approved by the institutional review boards of the University of Washington, and informed consent was obtained from participants or their parents. The Boston Early-onset COPD Study participants provided written informed consent, and the Partners HealthCare Human Research Committee approved the study.

IBD estimates for HapMap3 were generated using pHapMap3 release 2 data (Web Resources). We used PLINK to calculate all IBD estimates using SNPs with a minor allele frequency >1% and a call rate >90%. We used PRIMUS to identify the maximum unrelated set for each HapMap3 population, and the allele frequencies from the unrelated samples were used for the IBD analysis of their own respective populations.

The Starr County Health Studies' Genetics of Diabetes Study is composed of 1,890 cases and representative control samples obtained from a systematic survey in Starr County, Texas, conducted from 2002 to 2006[66]. However, the types of relationships and potential families in the study were unknown. IBD estimates for the Starr County samples were generated from genotypes called from the Affymetrix Genome-Wide SNP Array 6.0[66]. We used PLINK to calculate all IBD estimates using SNPs with a minor allele frequency >1% and a call rate >90%. We used PRIMUS[18] to identify the maximum unrelated set for the Starr County data, and the allele frequencies from the unrelated samples were used for the IBD estimations. The Starr County Health Studies' participants provided written informed consent, and the institutional review boards of the University of Texas Health Science Center at Houston approved the study.

Exome sequence data and corresponding pedigrees

The Boston Early-Onset Chronic Obstructive Pulmonary Disease (EOCOPD) Study[67] (see Web Resources) is an extended pedigree study of genetic susceptibility to EOCOPD. All available first-degree relatives (siblings, parents, and children), older second-degree relatives (half-siblings, aunts, uncles, and grandparents), and other relatives diagnosed with EOCOPD were invited to participate in the study. For this project, 351 subjects from 49 pedigrees were sequenced at the UW CMG.

Exome sequencing was performed (NimbleGen v2 in-solution hybrid capture and Illumina HiSeq 2000 sequencing)[68], sequences were aligned to the human reference genome (hg19)[69], and single-nucleotide and insertion-deletion variants were called with GATK[70]. VCFtools[71] was used to select only PASS SNPs with a minimum and maximum depth of eight and 300, respectively, and converted to a PLINK[23] formatted .ped and .map file. IBD estimates were then calculated in PLINK using the 56,516 SNPs with a minor allele frequency >1% and a call rate >90%. SNP allele frequencies for the IBD analysis were calculated from 81 of the 351 exome sequenced samples that made up the maximum unrelated set as calculated by PRIMUS[18] using a coefficient of relatedness cutoff of 0.1.

Results

Reconstructing simulated pedigrees

To test and evaluate the performance of PRIMUS on a broad range of known pedigrees, we simulated two types of pedigree structures (Uniform and Halfsib) of varying sizes, different numbers of markers, and varying combinations of missing data for individuals in the pedigrees (see Methods for details). Figure 13 shows the simulation results for reconstruction of size-20 and size-40 Uniform pedigrees with $\leq$ 20% missing samples. PRIMUS reconstructed the true pedigree as the only pedigree or the highest scoring pedigree in 89% of the simulations. For another 5.6% of these simulations, the true pedigree was tied as the highest scoring pedigree with one other pedigree. Only 2.5% of these simulations failed to run to completion either due to too many possible pedigrees (>100,000), too long of a runtime (>36 hours), or using up too much memory (e.g., exceeding 12Gb of memory). These incomplete reconstructions were then rerun using a relatedness cutoff of 0.375 to generate partial reconstructions for each. A partially reconstructed pedigree typically consists of two to six pieces of the larger pedigree where the individuals are connected by 1st degree relationships. It would require connecting these pieces with 2nd and 3rd degree relationships to achieve a complete reconstruction of the true pedigree.

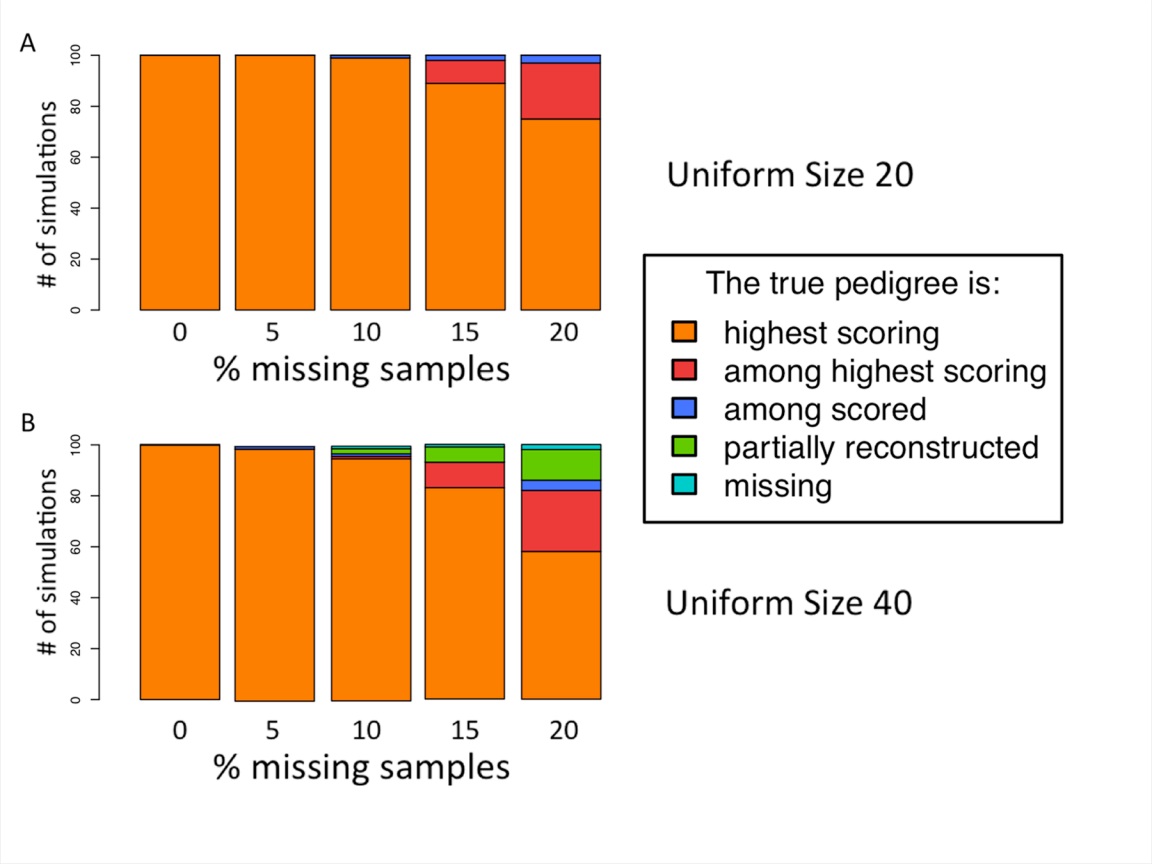


Figure 13 (Figure 1). A summary of the reconstructions for a thousand simulated pedigrees by PRIMUS.

All simulated pedigrees for the Uniform size-20 (A) and Uniform size-40 (B) with up to 20% missing samples were reconstructed with PRIMUS. We ran 100 simulations for each size and % of missing samples. For each simulation, we determined where the true pedigree fell among the ranked reconstruction results. Each bar displays the proportion of the 100 simulations that corresponds to the five reconstruction outcomes defined as follows:

"highest scoring" – The true pedigree is the highest scoring pedigree.

"among highest scoring" – PRIMUS output contained more than one possible pedigree and the true pedigree is tied as the highest scoring pedigree with one or more other pedigrees.

"among scored" – The true pedigree is not the highest scoring pedigree, but is among the pedigrees generated by PRIMUS.

"partial reconstruction" – The complete reconstruction either resulted in too many possible pedigrees, ran out of memory, or took longer than 36 hours to run, and as a result only a partial reconstruction using 1st degree relationships was generated.

"missing" – PRIMUS reconstructed one or more possible pedigrees, but the true pedigree was not among them.

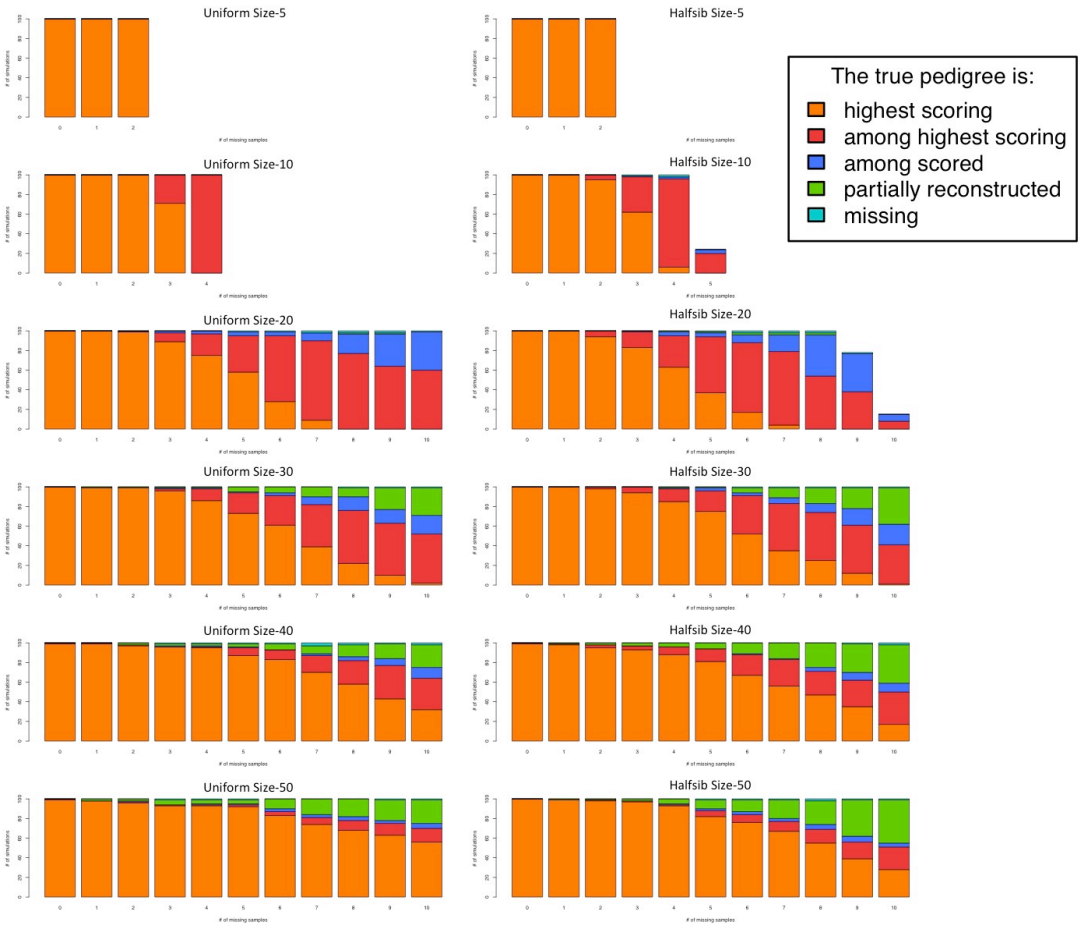


Figure 14. Results from the reconstruction of simulated pedigrees.
We simulated 100 pedigrees for each size from five to 50 and for both Uniform and Halfsib pedigree structures. We removed up to ten samples from each pedigree and reconstructed each in PRIMUS. For each simulation we determined where the true pedigree fell among the ranked reconstruction results. Each bar displays the proportion of the 100 simulations that corresponded to the five reconstruction outcomes. Some of the Halfsib pedigree structures allowed for more samples to be removed than others due to the random nature of how they were simulated. As a result, Halfsib size-10 with five missing samples and size-20 with nine and ten missing samples do not have 100 unique simulations. The different outcomes are defined as in Figure 13.

Across all of the Uniform and Halfsib simulated pedigrees of size five to 50 (~10,000 pedigrees), PRIMUS reconstructed the true pedigree as the highest scoring or tied for highest scoring pedigree in 88.7% of the simulations (Supplementary Table 2 and Figure 14). Only 6.3% of all simulations led to partial reconstructions, and PRIMUS completed, but did not reconstruct the true pedigree in only 0.5% of the simulations. We found that if PRIMUS outputs a single possible pedigree, then that pedigree is the true pedigree in 99.83% of the simulations.

Two trends were seen within the simulation results with respect to the size of pedigree being reconstructed and the proportion of individuals without genetic data. First, PRIMUS identified the true pedigree as the most likely pedigree in 94.9% of the simulations of pedigrees up to size-20 and up to 20% missing sample data, and identified the highest or tied for highest scoring pedigree in 99.4% of the simulations. As the proportion of individuals without genetic data increases to 50%, the true pedigree is more often tied for the highest scoring pedigree rather than being the highest scoring pedigree, as you would expect. Frequently, additional information such as ages will help rule out many of the tied pedigrees to identify the true pedigree structure.

Second, even with size-50 pedigrees and 20% missing samples, more often than not, PRIMUS identifies the correct pedigree as the single most likely pedigree. These results can be further improved with greater computational capabilities; PRIMUS tends to produce partial reconstructions as the size of the pedigree increases. For example, size-50 pedigrees with 20% missing samples require more run time (> 36 hrs) and memory (>12 Gb) to traverse the entire space of possible pedigrees as compared to size-20 with 50% missing samples.

There are very few simulations that completed reconstruction yet failed to find the true pedigree among the possible pedigrees (~0.5%), and the occurrence of these was not linked to pedigree size or the number of missing samples. This occurs when the initial likelihood threshold is set higher than the likelihood calculated by the KDE for one or more of the relationships in the true pedigree. Running PRIMUS with an initial likelihood threshold of 0.01 would result in the true pedigree being among the reconstructed pedigrees. As expected, we find that PRIMUS runtime tends to increase exponentially with pedigree size and the amount of missing sample data (Figure 15). Pedigrees up to size 20 and 20% missing samples reconstruct in a matter of seconds.

Confirming and correcting clinically ascertained pedigrees

To demonstrate the ability of PRIMUS to verify the genetic information for clinical pedigrees, we have reconstructed and confirmed or corrected more than a hundred pedigrees submitted to the UW CMG. The genetic information used by PRIMUS can be derived from either chip-based (Figure 16) or sequence-based (Figure 17 and Figure 18) technologies. Genome-wide IBD estimates for the samples in the pedigree in Figure 16 were generated using genotypes from the HumanCytoSNP BeadChip for each non-missing sample. PRIMUS used these IBD estimates for all pairs of samples to reconstruct the possible pedigree. Only one pedigree fit the data, and it matched the clinically provided pedigree, supporting our hypothesis that it is the correct pedigree. This reconstruction took nine seconds on a 2.3 GHz Intel Core i7 processor. Importantly, PRIMUS also introduced the five missing individuals necessary to connect the final pedigree and correctly identified a cycle in the pedigree caused by individual III-3 having children with the two cousins III-2 and III-4 (Figure 16).

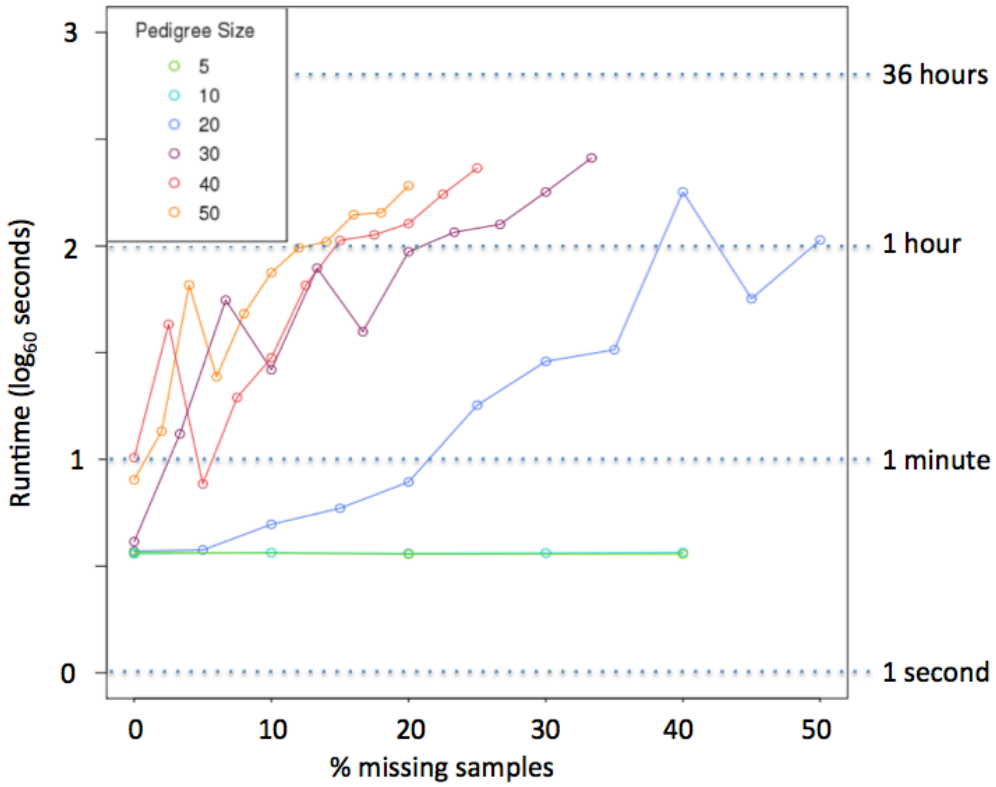


Figure 15. Simulation runtime results.
These simulations were run on a single Intel Xeon CPU X5690 @ 3.47GHz with up to 35GB of RAM.

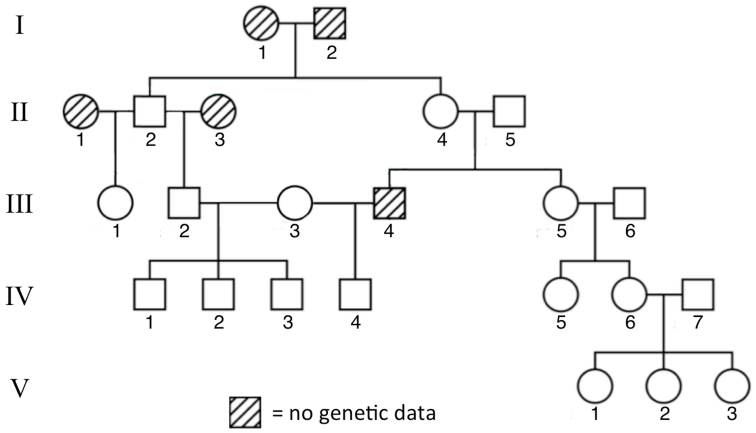


Figure 16. A pedigree correctly reconstructed by PRIMUS in nine seconds.
PRIMUS used chip-based genotype data to verify this clinically ascertained pedigree, which included the presence of five individuals for whom no genetic data were available (individuals marked with diagonal lines) as well as a cycle in the pedigree generated by individual III-3 having children with both III-2 and III-4.

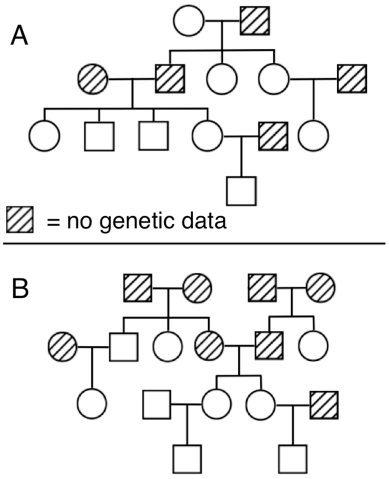


Figure 17. Two EOCOPD pedigrees verified by PRIMUS
(A) This pedigree was the only pedigree generated from PRIMUS. (B) This pedigree was tied as the highest scoring pedigree with five other pedigrees.

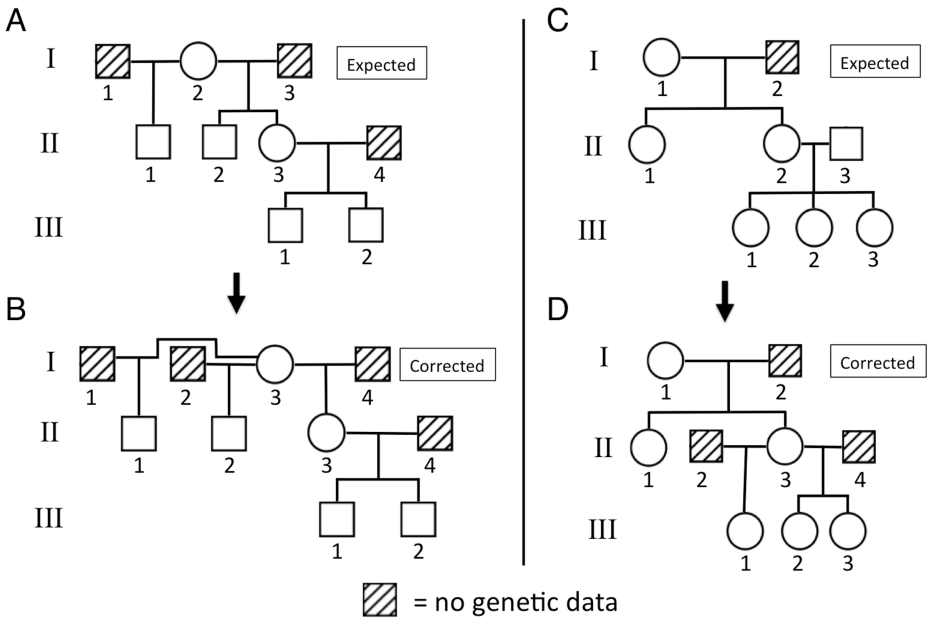


Figure 18**.** Two of the six EOCOPD pedigrees corrected by PRIMUS.
The reported pedigree is depicted above with the corrected pedigree below. Expected pedigree A has a non-paternity error so samples II-2 and II-3 are actually half-siblings rather than full-siblings in the correct pedigree (B). B was the top ranked pedigree in the PRIMUS output. Expected pedigree C contains not only a non-paternity that resulted in sample III-1 being a half-sibling to III-2 and III-3, but also a sample swap that resulted in sample II-3's DNA being swapped for DNA of a sample in an entirely different pedigree. Corrected pedigree D was the only pedigree generated by PRIMUS. The investigators have independently confirmed the corrected pedigrees.

Using variant data obtained from exome sequencing generated by the UW CMG, PRIMUS validated 49 pedigrees consisting of 351 individuals ascertained through a proband with severe EOCOPD. The pedigrees range from size four with 50% missing samples to size 23 with 35% missing samples. PRIMUS confirmed that 43 of the pedigrees matched the reported pedigrees collected in the study. Among the remaining six pedigrees, PRIMUS found and corrected five non-paternity errors, one sample swap, and one duplicate sample. These findings were consistent with the corrections independently made by the Boston EOCOPD Study investigators who compared estimates of IBDs obtained using PLINK with theoretical IBDs obtained using the KINSHIP2 package (Web Resources). A summary of the EOCOPD reconstruction including size, number of possible pedigrees, and where the true pedigree ranked in the possible pedigrees is provided in Supplementary Table 4.

Figure 17 shows two reported pedigrees from the EOCOPD dataset that were verified by PRIMUS. The pedigree depicted in Figure 17A was the only pedigree generated by PRIMUS and the pedigree in Figure 17B was among the highest scoring pedigrees. Figure 18 shows two of the reported pedigrees (A and C) that were corrected with PRIMUS (B and D). Figure 18A had a non-paternity error so individuals A and B are actually half-siblings rather than full-siblings (Figure 18B). For the reported pedigree in Figure 18C, PRIMUS not only corrected a non-paternity revealing that individual B was a half-sibling to C and D, but also identified a sample swap that resulted from A's DNA being replaced with DNA from another individual in the dataset. This corrected pedigree was the only pedigree generated by PRIMUS for these samples.

Reconstructing and incorporating cryptic relatedness

To evaluate whether PRIMUS could incorporate cryptic relationships into known pedigrees, we reconstructed pedigrees using the HapMap3 data[72]. Although the HapMap samples were collected to contain trios, duos, and unrelated individuals, cryptic relatedness among these samples is well established[6, 19, 44]. For example, the 10-person pedigree from individuals of Mexican Ancestry in Los Angeles (MXL, Figure 19) has been manually reconstructed using pairwise relationship predictions by several groups[24; 44; 62].

We used PRIMUS to automatically reconstruct all pedigrees within each HapMap3 population, and PRIMUS reconstructed cryptic pedigrees in nine of the eleven populations (Supplementary Table 5). PRIMUS confirmed the relationships reported by the HapMap Consortium as well as the cryptic 1st through 3rd degree relationships reported by Pemberton *et al.*[44] and Kyriazopoulou-Panagiotopoulou *et al.*[24] (Supplementary Table 5). However, because PRIMUS uses all pairwise relationships up to 3rd degree relatives to reconstruct the entire pedigree, it can consider each relationship in the context of all others. This enabled our approach to correct one misspecified 1st degree and two 2nd degree relationships reported by Pemberton *et al.* In addition to these corrections, PRIMUS was able to increase the specificity of 13 2nd and 3rd degree relationship predictions. For example, Pemberton et. al reported that MKK individuals NA21312 and NA21370 had an unknown relationships status, but PRIMUS identified them as half-siblings. PRIMUS eliminated all other 2nd degree relationships using the context of the other pairwise relationships in the pedigree.

PRIMUS also identified 85 previously unreported[24; 44] potential 3rd degree relationships among the HapMap3 samples (Supplementary Table 5). Although we cannot be certain these relationships are precise, our results provide strong evidence that relationships do exist and are an improvement over the common assumption that these samples are unrelated. We have made all reconstructed HapMap3 pedigrees available for download on the PRIMUS website (see Web Resources).

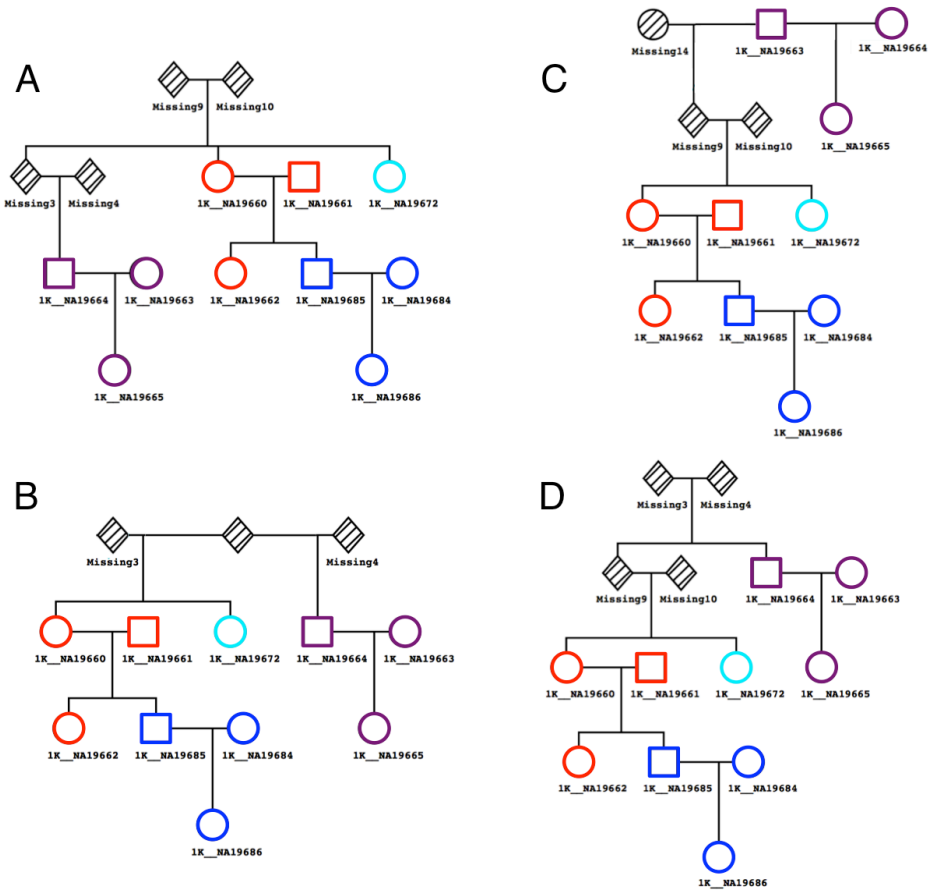


Figure 19. A ten-person MapMap3 MXL pedigree obtained from the HapMap3 and 1000 Genomes samples.
The pedigree includes three reported trios (each colored differently) from HapMap3 and an additional individual from the 1000 Genomes Project. The pedigree shown in A is one of four possible pedigrees that fit the estimated IBD proportions and is the pedigree previously reported[24]. Alternative possible pedigrees that fit the genetic data are shown in B, C, and D. For these MXL samples, PREPARE reported a single sibling relationship (NA19662, NA19685), the two first-cousin relationships (NA19662-NA19664 and NA19664-NA19685). PREPARE is presented to have automatically reconstructed the nine-person HapMap3 MXL pedigree reported in the CARROT paper[24], but they only show that NA19686 (incorrectly labeled as NA19685 in Figure 14 of their paper[60]) and NA19665 are 2nd cousins. Unlike the PRIMUS automatic reconstruction (Figure 19), they do not show how the other eight individuals fit into the pedigree, nor do they acknowledge that there are four different pedigree structures that fit the genetic data.

Reconstruction of previously unknown pedigrees from Starr County

We used the Starr County Health Study to demonstrate the ability of PRIMUS to reconstruct previously unknown pedigrees from a large genetic dataset. We calculated IBD estimates among all 1,890 samples using genotypes obtained for the individuals (Affymetrix Genome-Wide SNP Array 6.0[66]). PRIMUS used these estimates to group 458 samples into 203 family networks of two or more samples. Using only these genetic data, PRIMUS reconstructed a single possible pedigree for 120 of these families in less than four minutes, and, based on our simulation results, we expect that ~99.83% of these are the true pedigrees. When ages are provided to PRIMUS, it flags pedigrees that are impossible given the ages of the samples (e.g., a parent being younger than a child). Using the age information collected for the Starr County Heart Study dataset, PRIMUS ruled out these incorrect pedigrees and identified a single possible pedigree for an additional 73 families for a total of 193 novel pedigrees that range in size from two to five individuals.

Comparing PRIMUS to competing methods

We compared the results of PRIMUS to those generated by RELPAIR, a program commonly used to check relationships in genetic data. Using the method employed by Pemberton *et al.*[44], we compared the accuracy of the pairwise predictions of RELPAIR to the accuracy of the pairwise relationships in the top ranked reconstructed pedigree produced by PRIMUS (Figure 20 and Supplementary Table 3). Both methods had 100% accuracy when distinguishing between 1st degree relationships; however, PRIMUS outperformed RELPAIR when 2nd degree relationships were considered. While RELPAIR makes the distinction between the 1st and 2nd degree relationships, it labels all third degree relationships as cousins. PRIMUS distinguishes between the four 3rd degree relationships and also gives directionality to the relationship (e.g., individual II-2 is the grandfather of individual IV-2 in Figure 16). Therefore, to make a fair comparison between the ability of PRIMUS and RELPAIR to predict 3rd degree relationships, we compared only the degree of the relationship predicted by PRIMUS to the "cousin" prediction of RELPAIR. PRIMUS outperformed RELPAIR when classifying relationships as 3rd degree, and as >3rd degree/unrelated (Figure 20; Supplementary Table 3).

We also compared PRIMUS to the latest pedigree reconstruction programs, PREPARE and IPED2 (see Web Resources). Of the 9,717 simulated pedigrees of size ten to 50, only 43 pedigrees had all genotyped samples in a single generation, and all of these pedigrees had at least one half-sibling relationship. Therefore, PREPARE and IPED2 could only attempt to correctly reconstruct <0.5% of the simulated pedigrees; PRIMUS correctly reconstructed 9,008 of the 9,717 (92.7%) simulated pedigrees. Figure 21 shows PRIMUS reconstructions for additional simple, common pedigree structures that PREPARE and IPED2 cannot completely reconstruct.

Additionally, neither PREPARE nor IPED2 can completely reconstruct any of the real data presented in this manuscript because all of these pedigrees have genotyped samples from multiple generations. PREPARE and IPED2 can provide a partial reconstruction by dropping samples from higher generations and using only extant individuals, as the PREPARE authors did with the MXL pedigree (Figure 14 of the PREPARE paper[60]; Figure 19). In order to reconstruct relationships, PREPARE requires *a priori* information about which individuals are in the same generation prior to reconstruction and cannot connect these pairwise relationships into a single, multigenerational pedigree. PRIMUS completely reconstructs these pedigrees (*e.g.* Figure 19). PREPARE and IPED2 provide limited utility to check reported pedigree structures and to reconstruct previously unknown pedigrees *de novo*.

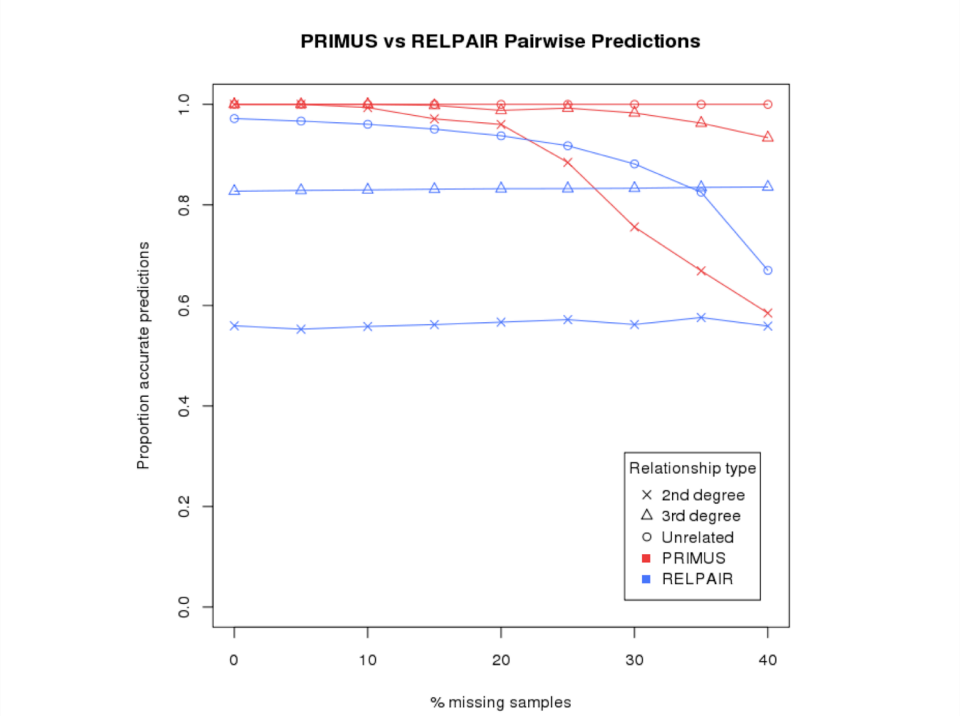


Figure 20**.** Comparison of relationship prediction accuracies for simulated pedigrees using RELPAIR and PRIMUS.
For this comparison, Halfsib size-20 pedigrees with 0% to 40% missing samples were used to test pairwise relationship prediction accuracy. For PRIMUS, we tested whether the relationships in the highest ranked pedigree matched the true simulated relationships. For RELPAIR, we used the method employed by Pemberton et al.[44] to obtain the prediction and compared that to the true simulated relationship. A 2nd degree relationship prediction is correct if the relationship type matches the true relationship type. A 3rd degree relationship prediction is correct if the predicted relationship degree matches the true relationship degree. A Distantly/Unrelated prediction is correct if the true relationship is more than a 3rd degree relationship.

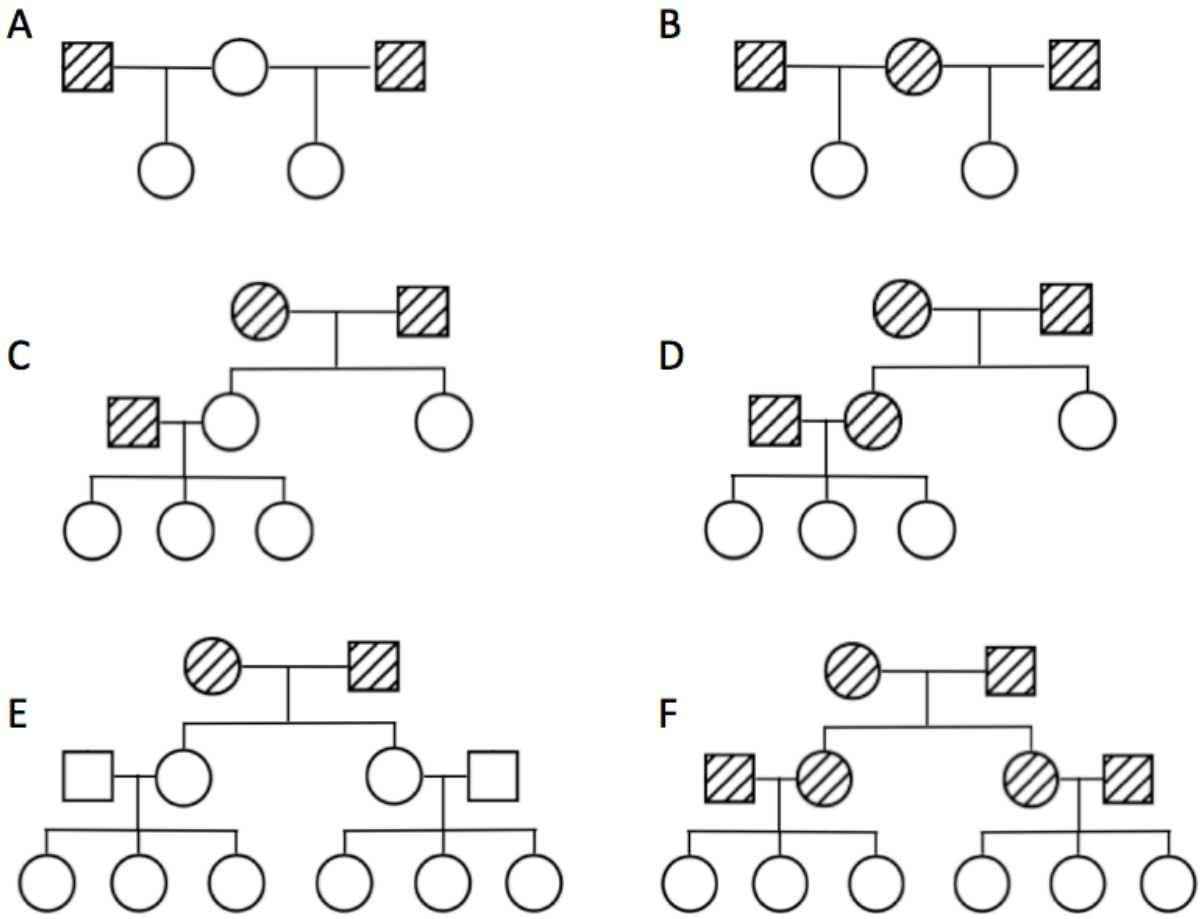


Figure 21. Examples of simple and common pedigrees structures.
Diagonal lines through an individual's symbol indicate that DNA data are unavailable for that individual. PRIMUS can easily reconstruct all six pedigree structures. PREPARE[60] and IPED2 (He et al., in press) can reconstruct pedigrees B and F because they require that all genotyped samples be in the same generation. If we had prior knowledge of each of these pedigrees, ages of the samples, or knowledge of who was in which generation, then PREPARE and IPED2 could do partial reconstructions of the lowest generation of each pedigree and the middle generation of C by discarding the other genotyped individuals. IPED[58] and COP/CIP[59] can only reconstruct pedigree F, because they are unable to handle half-sibling relationships, but could do the same partial reconstruction of the same pedigrees as PREPARE and IPED, except for A.

Discussion

PRIMUS is designed to reconstruct non-consanguineous pedigrees of arbitrary size and structure from pairwise estimates of IBD for samples up to 3rd degree relatives. It can also reconstruct some consanguineous pedigrees with children whose parents are 3rd degree relatives (Figure 22). PRIMUS provides major advances in reconstructing, testing, and correcting pedigrees. While pairwise predictions provided by commonly applied programs such as RELPAIR and PREST can test whether two individuals are related at the expected degree of relatedness, they are much weaker at distinguishing between relationship types within the same degree of relatedness (e.g., avuncular vs. grandparental) and cannot provide information of the directionality of a relationship (i.e., individual A is the grandparent of B). As a result, they are not able to detect all pedigree inconsistencies or to suggest corrections to pedigrees. Additionally, using pairwise relationships to check pedigrees can result in the unnecessary loss of data (Figure 23) or in accepting an incorrect pedigree as true (Figure 24).

PRIMUS improves on the pairwise predictions by using all the pairwise relationships to reconstruct the pedigree. The context of all the pairwise relationships in the family improves the prediction accuracy of each relationship pair. We show that the reconstructed pedigrees obtained by PRIMUS were more accurate than those obtained with RELPAIR (Figure 20; Supplementary Table 3). In the case of HapMap3, PRIMUS corrected and improved several of the pairwise relationship predictions made by RELPAIR and CARROT (Supplementary Table 5).

PRIMUS is also a major step forward when compared to existing pedigree reconstruction programs since the existing methods require a small number of markers, completely genotyped pedigrees, no half-siblings, and/or they require that all genotyped samples are in the same generation. For these reasons, no other pedigree reconstruction program we tested is capable of reconstructing the variety of pedigrees we illustrate in this paper that represent some of the most common pedigrees found in human genetic studies.

Importantly, pedigree reconstruction by PRIMUS depends on the quality of the IBD estimates, which are influenced by several factors including the number of genetic markers, population substructure[25], admixture[62], and reference minor allele frequencies[73]. For best results, users should obtain high quality IBD estimates before reconstructing pedigrees with PRIMUS. IBD

estimates can be obtained by PRIMUS or by another program (PLINK[23], KING[25], or REAP[62]) that uses the appropriate allele frequencies for the ancestry of the samples and accounts for potential admixture and population substructure among the data.

We designed PRIMUS to reconstruct up to 3rd degree relationships for several reasons. First, the distance between the expected mean genome-wide IBD proportions for more distant relationships (e.g., 4th degree and 5th degree) is small while the variation around these means is large. Therefore, the overlap between the distributions of these distant relationships precludes highly accurate relationship assignments of any relationship beyond 3rd degree. Second, as the relationship distance increases beyond 3rd degree, the number of possible relationships increases rapidly (Supplementary Table 6), and pedigree reconstruction quickly becomes computationally challenging. For more distant relationships, it is possible to apply programs such as BEAGLE[42] and ERSA[27] to connect the sub-pedigrees obtained by PRIMUS that are distantly related to one another, and we have incorporated this feature in a follow-up release of PRIMUS described in Chapter 5. Additionally, programs like RELPAIR[28] could improve the pairwise relationship prediction because they model recombination events to distinguish between 2nd degree relationships. The improved relationship predictions could then be used to improve the scoring of possible pedigrees.

We have identified two limitations of PRIMUS and their corresponding remedies. First, due to computational restraints, PRIMUS was unable to complete the reconstruction of 6.3% of simulations using 3rd degree relatives or closer. The vast majority of these pedigrees had ≥30 individuals with >20% missing sample data. Investigators can still greatly benefit from partial reconstructions of these pedigrees. Users can obtain a partial reconstruction, as we did, by using a higher relatedness threshold to reconstruct using just 1st or 2nd degree relationships. Second, for a very small proportion of the simulations (~0.5%), PRIMUS did not output the true pedigree among the results because the initial likelihood threshold was set too high. Yet by lowering the initial likelihood threshold used to predict familial relationships, PRIMUS was able to reconstruct each of these pedigree structures. Therefore, for a very small percentage of pedigrees run on PRIMUS, it may be necessary to depart from the default initial likelihood threshold to obtain a reported pedigree.

PRIMUS provides an immediate benefit to the genetics community in two ways: pedigree verification and pedigree discovery. Because PRIMUS computationally verifies reported pedigrees using genotype data and identifies and corrects inconsistencies, PRIMUS saves a significant amount of time and effort that would otherwise be spent on manual verification of pedigrees. This is especially beneficial when large, complex pedigrees are being studied similar to the Boston EOCOPD Study pedigrees. For example, PRIMUS has identified and corrected non-paternities, under-related samples, samples swaps, duplicate samples, and unexpected consanguinity in clinical pedigrees (Figure 19 and Figure 20). In many cases, such corrections can result in a correction of the genetic model and assumptions used for downstream analysis, improving the chances of finding the genetic cause of the disease.

Moreover, PRIMUS can reconstruct previously unknown pedigrees using only genetic data, as demonstrated in the HapMap3 and Starr County datasets. Although, PRIMUS cannot guarantee that these pedigrees are the true pedigrees, the pedigrees can be treated as a hypothesis to be confirmed with supporting independent evidence. This application of PRIMUS is particularly useful in large-scale genetic studies where substantial cryptic relatedness may exist. In the case of the Starr County data, we can now use powerful family-based analyses that leverage the information contained in nearly 200 previously unknown pedigrees.

Incomplete understanding of relatedness structures (i.e., pedigrees) within genetic data can result in a vast array of analytic problems, from dramatically biased effects of rare variants to power loss in pedigree-based methods. With the introduction of PRIMUS, we hope to address many of the limitations of prior pedigree reconstruction frameworks and pairwise comparison algorithms in a fast, tractable, and easy to use algorithm, enabling investigators to better assess the information present within their data.

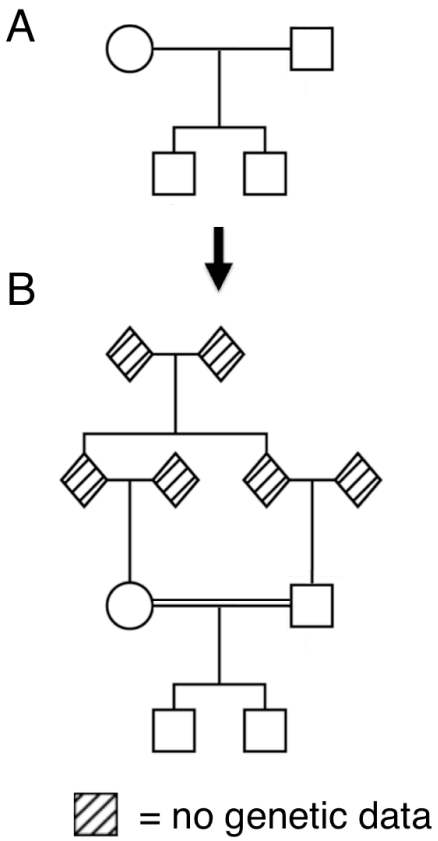


Figure 22. A pedigree from the University of Washington Center for Mendelian Genomics. The parents were reported as unrelated individuals by the clinician as depicted in pedigree A, but PRIMUS reconstructed them as first cousins, as depicted in pedigree B.

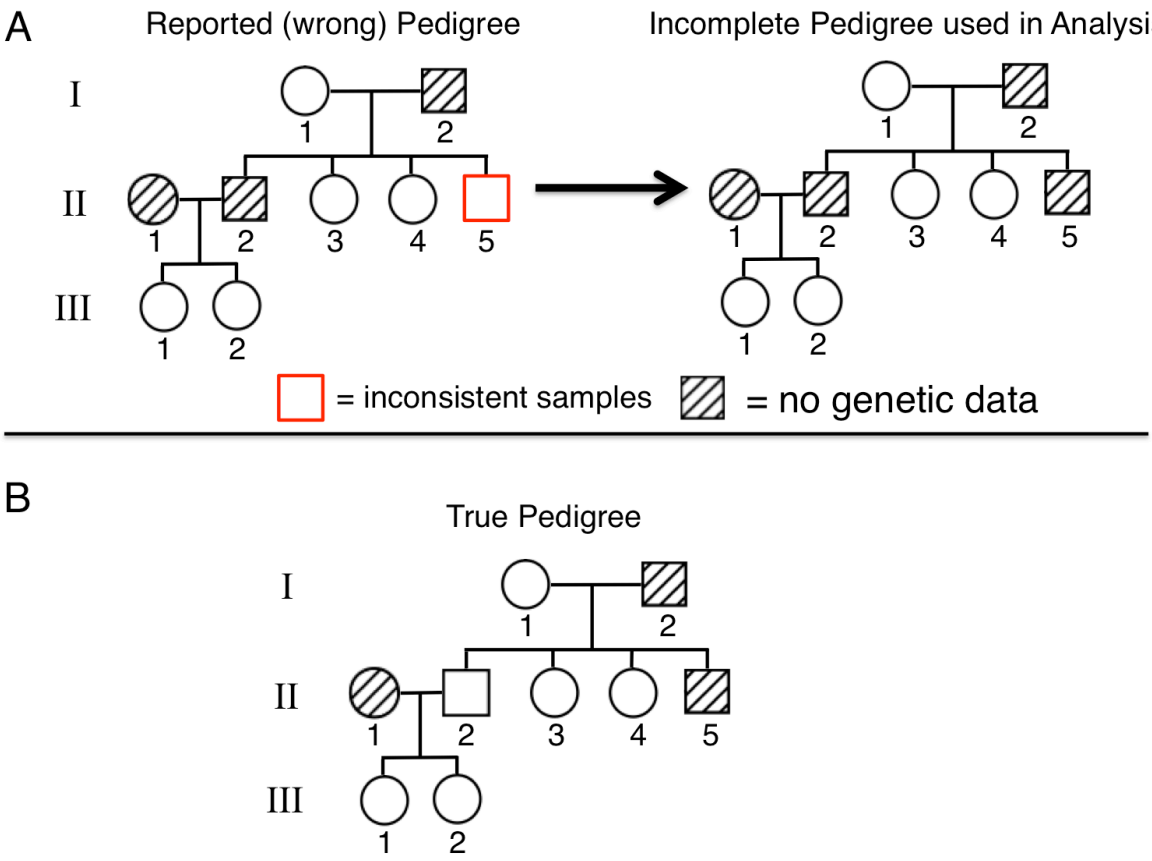


Figure 23. An example where pairwise relationship checking and removal of an inconsistent sample results in an analysis loss.
Panel A shows the reported pedigree as provided by the investigator. Pairwise relationship checking reveals that all relationships are correct except between sample II-5 and the siblings III-1 and III-2. Standard practice is to remove inconsistent samples, in this case sample II-5, resulting in the 5-person pedigree on the right. Panel B shows the true pedigree where sample II-5 was actually the father of the siblings III-1 and III-2 instead of individual II-2, who was not successfully genotyped, being the father. The mix-up could realistically be explained by a sample swap or by misspecified paternity for the two children, and these types of errors are common. Pedigree reconstruction would have revealed the inconsistency and would have easily reconstructed the true pedigree. Therefore, rather than discarding 17% of the data, the investigator could have retained all samples.

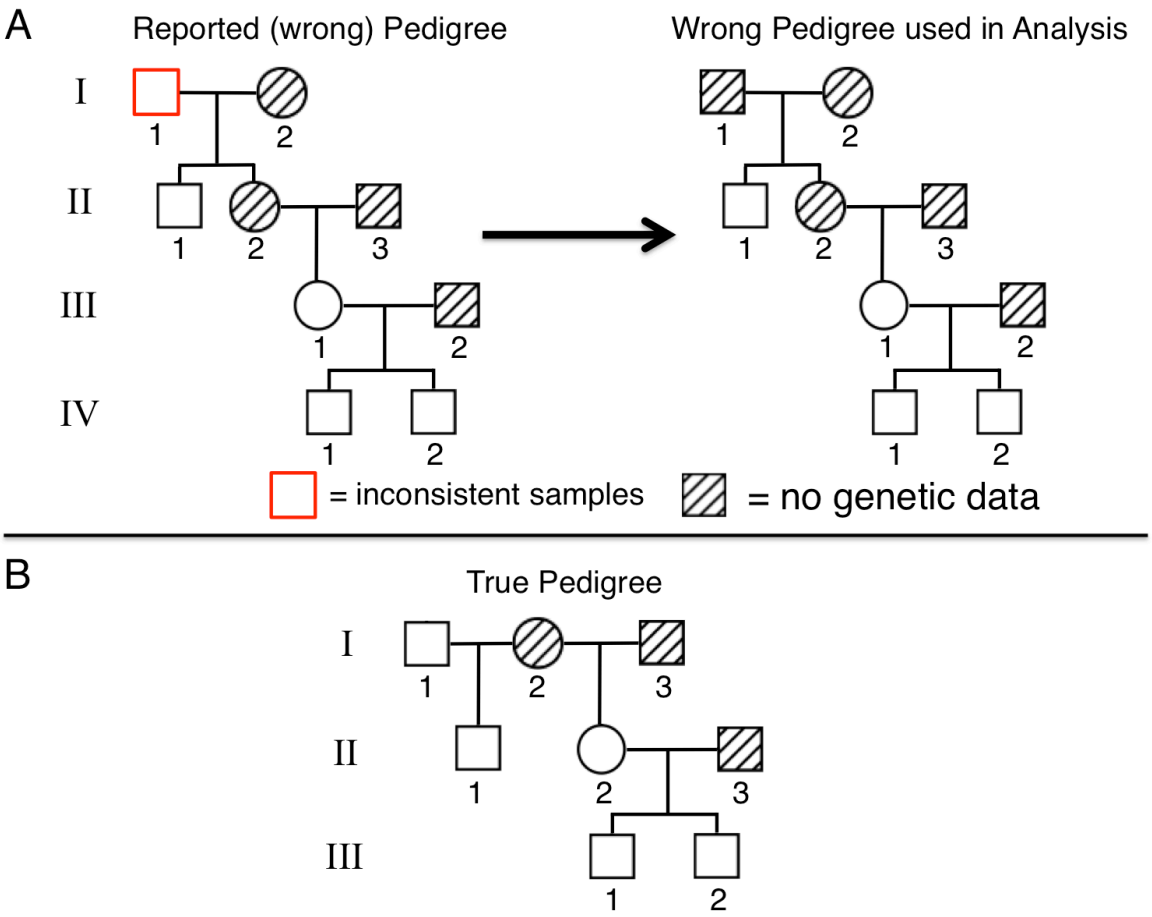


Figure 24. An example where pairwise relationship checking and removal of an inconsistent samples results in an unnecessary data loss and the use of an incorrect pedigree.
Panel A shows the pedigree as reported by the investigator. Pairwise relationship checking reveals that all relationships are correct except between sample I-1 and samples III-1, IV-1, and IV-2. Standard practice is to remove inconsistent samples, in this case sample I-1, resulting in the 4-person pedigree on the right. Panel B shows the true pedigree. The error in the original pedigree was that sample II-1 was incorrectly assigned as the uncle to sample III-1, when, in fact, they were half-siblings. This mix-up could realistically be explained if the family incorrectly reported their family history or by a clerical error, and these types of errors are common. Pedigree reconstruction would easily have revealed the inconsistency and would have reconstructed the true pedigree. Therefore, rather than discarding 20% of the data and assuming an incorrect pedigree, the investigator would have retained all samples and used the true pedigree in further analyses.

Chapter 4. Incorporating mtDNA and NRY Haplotypes into pedigree reconstruction

PRIMUS is a pedigree reconstruction algorithm that uses estimates of genome-wide identity by descent to reconstruct pedigrees consistent with the observed genetic data. However, when the genetic data for a set of individuals within a pedigree are missing, multiple pedigrees can be reconstructed. We report a major expansion of PRIMUS that uses mitochondrial (mtDNA) and non-recombining Y chromosome (NRY) haplotypes to eliminate many of the pedigree structures that are inconsistent with the genetic data. We demonstrate that discordances in mtDNA and NRY haplotypes substantially reduce the number of potential pedigrees, and can often lead to the identification of the correct pedigree.

Introduction

Correctly determining pedigree structures is key to identifying the causes of genetic disorders[2-6; 9]. In some cases, reported pedigree structures are inconsistent with the samples[20-22], resulting in a loss of power that could result in failure to find a disease causing variant[19]. Unexpected relationships among samples in large genetic datasets are also common. Cryptic relatedness within datasets or between pedigrees, as well as sample swaps, is frequently observed. Pedigree reconstruction can find cryptic relationships and correctly fit them into a pedigree structure[74]. Early methods for checking pedigrees applied pairwise comparison methods[28; 29]; however, pedigree reconstruction can more accurately predict relationships, find the correct pedigree, and identify cryptic pedigrees within the data[74]. PRIMUS uses estimates of genome-wide identity by descent (IBD) to identify families and reconstruct all possible pedigrees that fit the IBD estimates up to 3rd degree relationships.

Missing genetic data for a set of people in a family can often lead to the identification of multiple pedigrees that fit the genetic data. We show that using inconsistencies in the inheritance patterns of mitochondrial DNA (mtDNA) and non-recombining Y chromosome (NRY) haplotypes, which are captured by genotyping arrays or by high-throughput sequencing [75], improves the accuracy of pedigree reconstruction. We have developed a new module for the pedigree

reconstruction in PRIMUS (v1.8.0) that utilizes mtDNA and NRY data to reduce the number of possible pedigrees and improve the chance of identifying the correct pedigree structure.

Methods

Identifying concordant and discordant mtDNA and NRY haplotypes

PRIMUS supports standard, easy-to-use formats that now include data with mtDNA and NRY haplotypes. NRY and mtDNA can be encoded as chromosome 24 and 26, respectively, in a PLINK-formatted data file or in any other format accepted by PLINK. For a pair of individuals, PRIMUS calculates the concordance of mtDNA and NRY haplotypes. The percent concordance of a haplotype is calculated as the percentage of matching mtDNA and NRY nucleotide positions across the total number of variable mtDNA and NRY positions and excludes positions with missing calls. A "discordant" prediction between the NRY or mtDNA haplotype of two individuals occurs when concordance is below a user definable cutoff (default is 99%); otherwise, the NRY or mtDNA haplotype is predicted to be "concordant." The discordant status indicates that a pair of individuals has not inherited the mtDNA or NRY haplotypes from a recent common ancestor. Therefore, PRIMUS can eliminate any pedigree structure where a pair of individuals has discordant mtDNA or NRY haplotypes.

By default, PRIMUS only eliminates pedigree structures that are inconsistent with the discordant predictions of mtDNA and NRY, which we demonstrate are very informative and reliable. For example, in both sequencing and genotyping datasets, we observed a nearly 100% haplotype concordance between individuals who have inherited mtDNA or NRY from a recent common ancestor (i.e., fewer than four generations of separation; Table 5); therefore, individuals with discordant predictions are very unlikely to be related to a recent common ancestor of the sex that corresponds to the discordant mtDNA or NRY prediction.

Although discordant mtDNA and NRY haplotypes are very useful in rejecting genetically inconsistent pedigrees, concordant haplotypes are less so. Because recombination does not influence mtDNA and NRY haplotypes, a single haplotype can be passed unchanged through a family for generations. Therefore, distant relatives can have concordant mtDNA and NRY haplotypes while they share little or no autosomal-DNA with detectable IBD (Figure 25). If

PRIMUS requires all concordant mtDNA and NRY predictions to be represented by a recent common ancestor, then distant, concordant ancestral mtDNA and NRY haplotypes can cause PRIMUS to reject the correct pedigree structure. Therefore, by default concordant mtDNA and NRY haplotypes are not used to rule out pedigree structures.

mtDNA and NRY checking

PRIMUS uses mtDNA and NRY discordance to improve pedigree reconstructions by checking whether the discordance is consistent with the expected mtDNA and NRY inheritance patterns within the pedigree. For example, if half-siblings are genotyped and have discordant mtDNA prediction, then their parent in common must be the father. To check whether this discordant prediction is consistent with a pedigree structure, PRIMUS finds the shortest first-degree-relative inheritance path connecting two individuals, A and B. Discordant predictions require an interruption in the transmission of the haplotype in the pedigree. For example, if A and B have a discordant NRY prediction, then there must be a female somewhere in the NRY inheritance path. The logic that applies to NRY inheritance paths through males also applies to mtDNA inheritance paths through females, except that the sex of A and B does not matter unless one is the direct ancestor of the other; in which case, the direct ancestor must be female. We illustrate valid and invalid inheritance paths within a pedigree in Figure 26.

Results

We modified the pedigree simulations described in Staples *et al.* [74], to explore the affects of using mtDNA and NRY during reconstruction of pedigrees with different structures, genotypes and combinations of missing samples. We selected pedigrees of size 20 and masked the genotypes for 0-50% of individuals in the pedigree. We modified the simulations by permuting the sex of the individuals, while maintaining the biological integrity of the pedigree. For each simulation, we assigned a unique NRY haplotype to each male founder and a unique mtDNA haplotype to all founders and propagated these genotypes through the pedigree. We ascertained mtDNA and NRY haplotypes from the phase 1 release of unrelated CEU and TSI haplotypes [18] with individual-level call rates >90% from the 1000 Genomes Project [51]. The haplotypes consisted of 2,832 mtDNA and 8,665 NRY variants with quality score ≥30 and call rates >95%, and with a minor allele frequency >1% for inclusion.

We considered pedigree reconstruction performance on the autosomal data alone as the baseline and compared this to the performance when we added additional information such as sex status, mtDNA and NRY. We see a moderate reduction in the number of possible pedigrees with mtDNA and NRY individually, but the synergistic effects of mtDNA and NRY with sex status exceeded the combined individual improvements (Figure 27). Our results show the largest improvement in pedigrees with more missing samples (35-40%) where a 37% reduction in the mean number of genetically consistent pedigrees is seen (Figure 27). The improvement declines beyond 40% missing samples because the pedigrees become too sparse for the discordant mtDNA and NRY haplotypes to rule out possible pedigrees. We also see a substantial improvement in ranking the correct pedigree structure (Figure 28). In fact, when mtDNA and NRY are combined with individual sex status, we see a 4.5-fold increase over sex status alone in the number of simulations that reconstructed to only the true pedigree (Figure 28).

The improvements in pedigree specificity using mtDNA and NRY genotypes are remarkable. As shown in Figure 29, a dataset that reconstructed to 58 possible pedigrees using autosomal DNA and sex status resolved to the single correct pedigree with the addition of mtDNA data. The new implementation of PRIMUS identified this pedigree by eliminating the 57 pedigrees that were inconsistent with the mtDNA data. NRY and mtDNA provide a substantial improvement in automated pedigree reconstruction with PRIMUS.

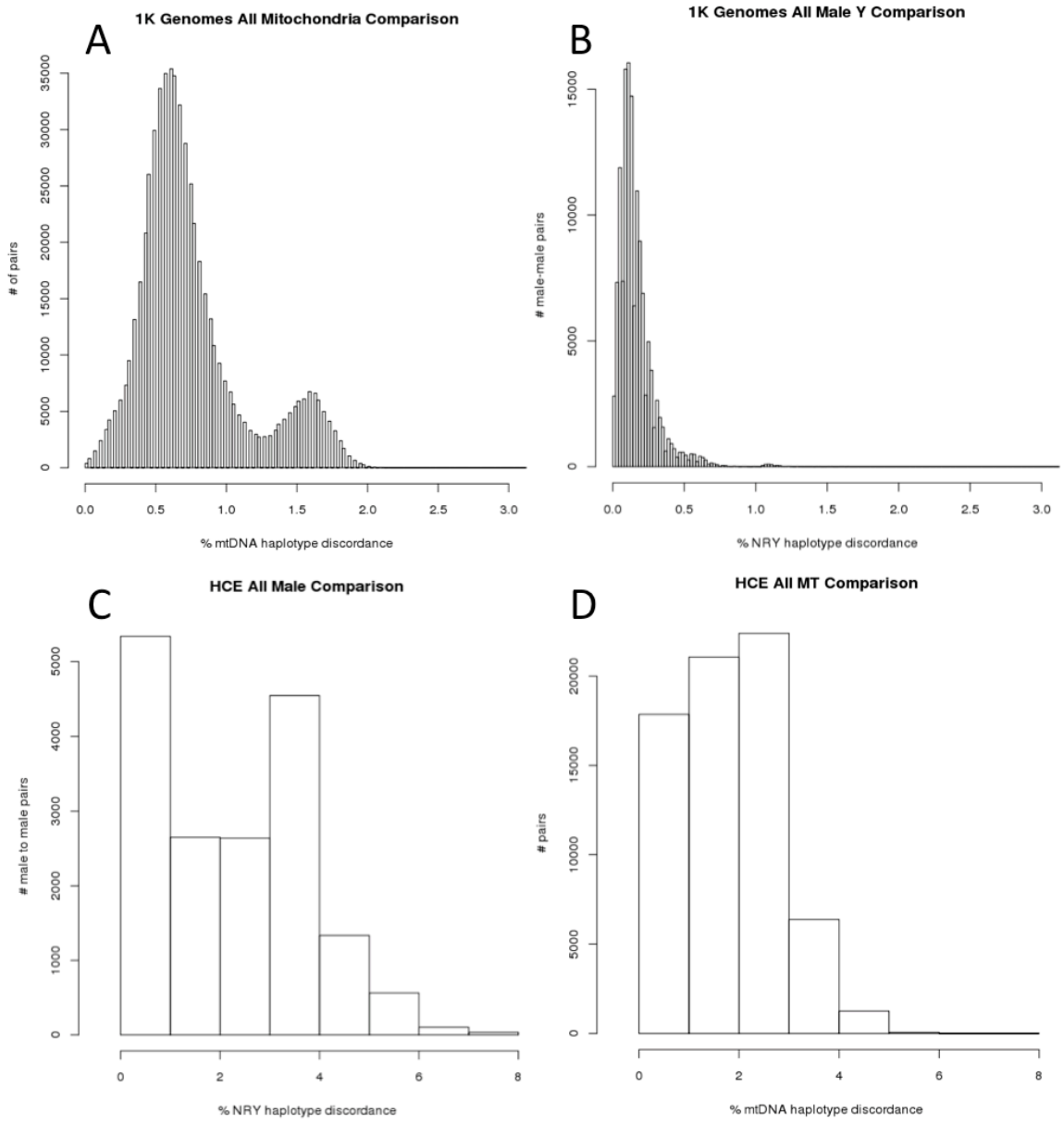


Figure 25. Summary of the haplotype discordance between pairs of individuals.
Panel A and B show the haplotype discordance for the low-pass 1000 Genomes Project [51] phase 1 release using 2,832 high quality mtDNA sites and 8,665 high quality NRY sites from 548 females and 526 males. Panel C and D show haplotype discordance for 372 individuals genotyped with the Illumina Infinium HumanCoreExome-24 BeadChips (HCE) array for 205 high quality mtDNA sites and 126 high quality NRY sites genotyped in 185 female and 187 male samples. (A) Of the ~576,000 mtDNA pairwise comparisons, 99.5% are more than 0.1% discordant; however, 2,684 pairs of individuals have haplotypes that are >99.9% identical. The majority of these pairs are in individuals that are more distantly related than 3rd degree relatives. (B) All male/male NRY chromosome comparisons in 1000 Genomes. (C) ~69,000 pairwise comparisons between 372 individuals from HCE dataset. (D) All male/male NRY chromosome comparisons in HCE dataset.

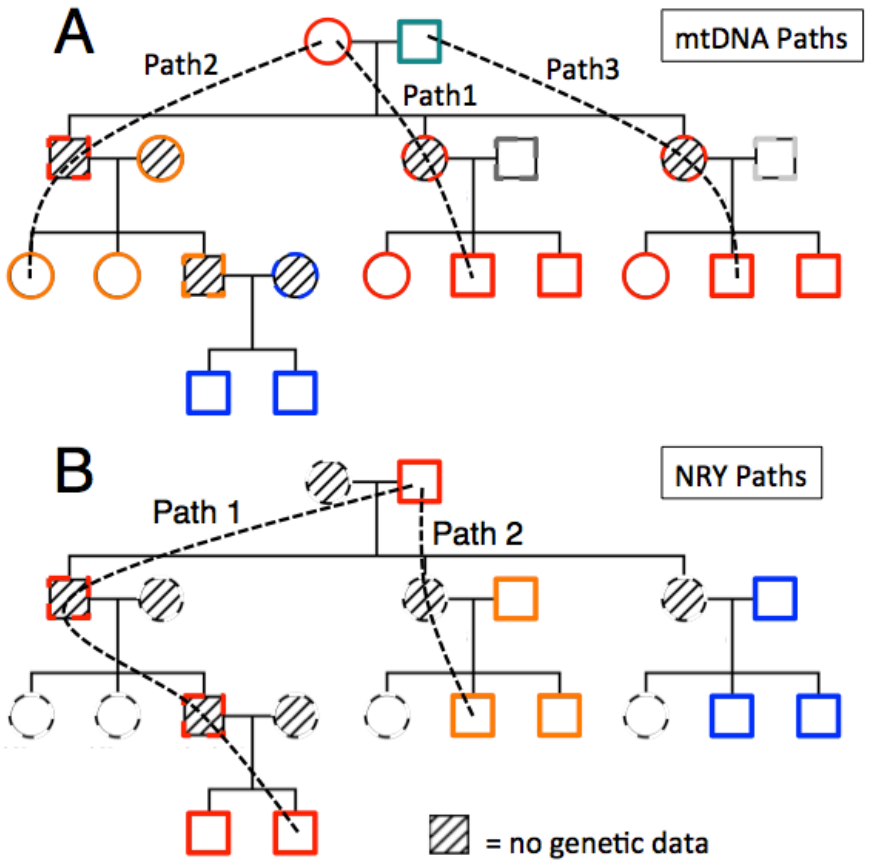


Figure 26. Examples of (A) mtDNA and (B) NRY inheritance paths in a pedigree. Individuals who share identical mtDNA or NRY haplotypes have the same symbol color. Diagonal lines through the symbol indicate that there was no genetic data available for the individual; therefore, sex of those individuals cannot be determined prior to reconstruction with mtDNA and NRY data. The dotted lines passing through multiple individuals represent examples of possible inheritance paths for mtDNA and NRY. (A) mtDNA is passed from mother to child. Path 1 shows a valid mtDNA inheritance path that passes from a grandmother to her grandson through the grandson's mother. If the grandmother's and grandson's mtDNA were discordant, then PRIMUS would reject this pedigree because it is inconsistent with a mtDNA inheritance path. Path 2 shows an invalid mtDNA inheritance that passes through a male where the grandmother and granddaughter have discordant mtDNA haplotypes. Path 3 shows an invalid mtDNA inheritance path because the most recent ancestral person in the path is male. (B) Path 1 shows a valid NRY inheritance path where the males at the end of the path have concordant predictions; if not, then this pedigree would be inconsistent with the NRY haplotypes, and it would be rejected. Path 2 shows an invalid NRY inheritance path for the two males because the grandson could not inherit the grandfather's NRY through the mother.

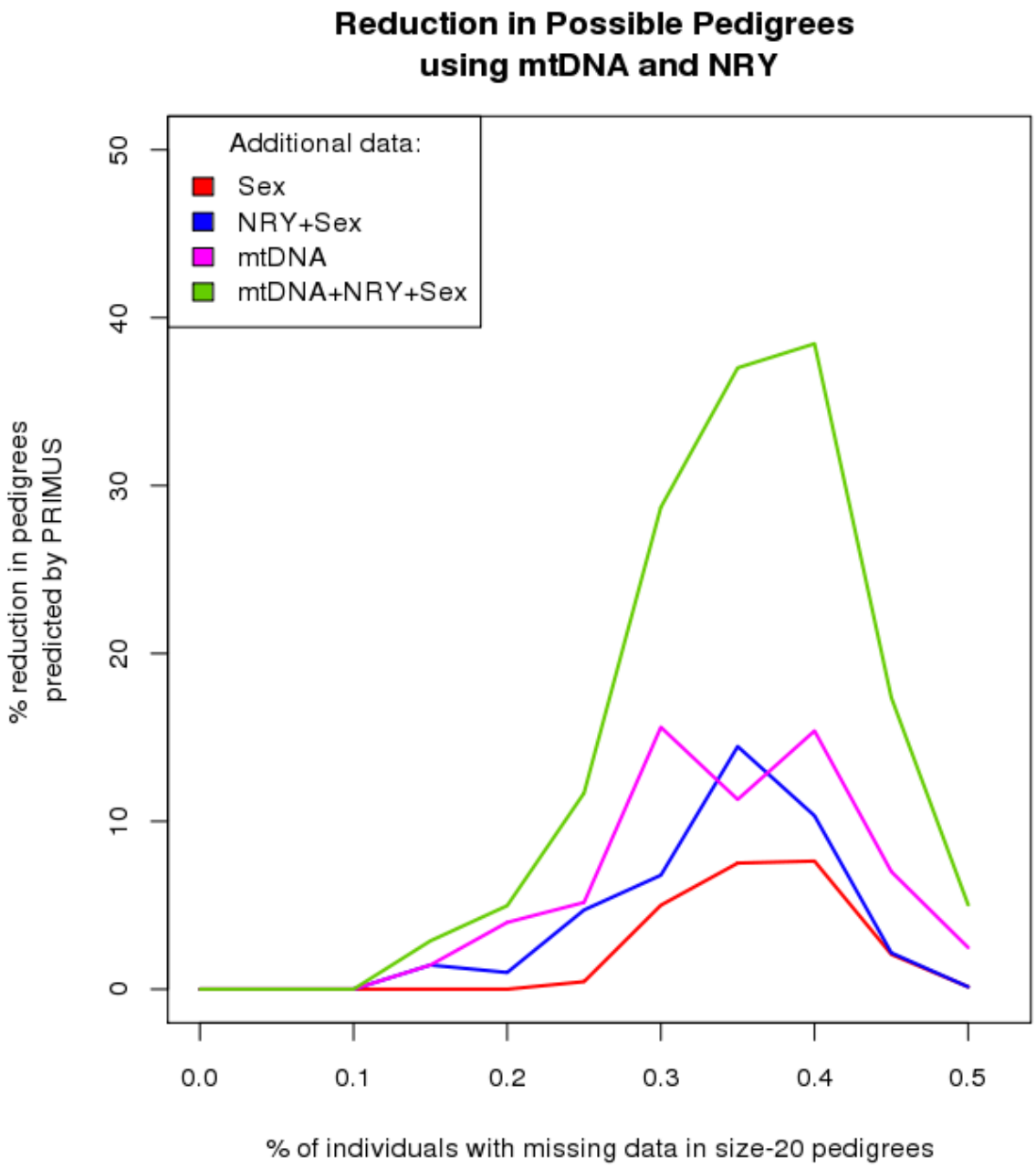


Figure 27. A summary of the percent reduction in the average number of possible pedigrees when data from mtDNA haplotypes, NRY halpotypes, sex or all of these are applied in pedigree analysis.
The addition of either mtDNA or NRY data outperforms the addition of only sex status. The greatest reduction in the number of possible pedigrees is obtained when mtDNA, NRY and sex status are combined, eliminating nearly 40% of the incorrect pedigrees.

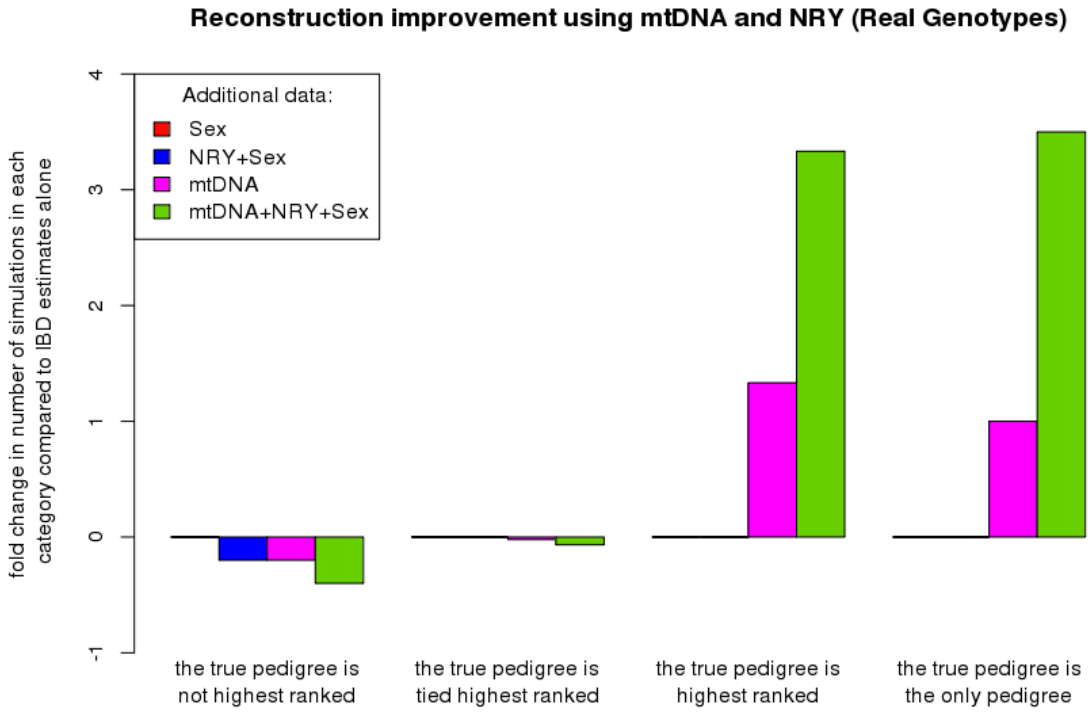


Figure 28. Relative improvement of reconstruction of simulated pedigrees with 35% masked samples with additional data (i.e., sex status, NRY and mtDNA haplotypes).
The ideal would be to reconstruct as few pedigrees as possible while retaining the correct pedigree, and to rank the correct pedigree as the highest when multiple pedigrees are consistent with the genetic data. When the correct pedigree is not the highest rank, or tied to the highest rank pedigree, the addition of mtDNA and mtDNA + NRY + Sex provides a substantial improvement in the number of pedigrees that become the highest ranked or only pedigree by eliminating pedigrees that are not compatible with the observed mtDNA, or mtDNA and NRY haplotypes and Sex.

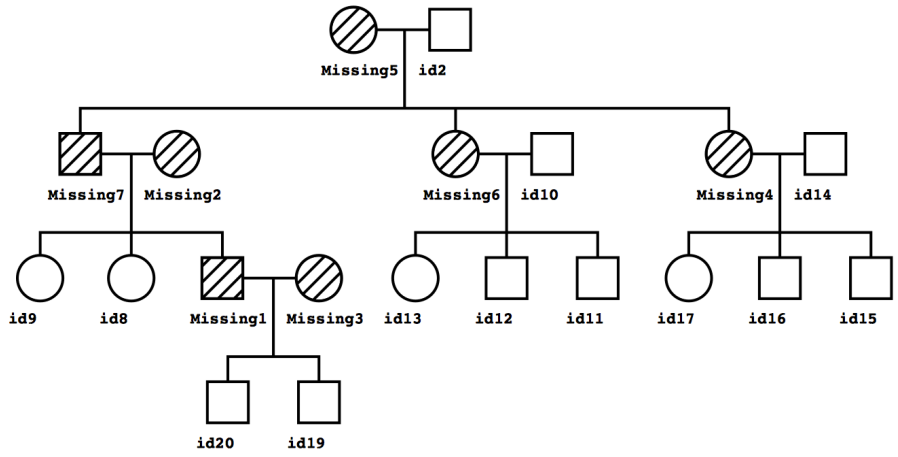


Figure 29. A reconstructed simulated pedigree uniquely identified with mtDNA data. This pedigree reconstruction using autosomal data and sex status generated 58 possible pedigrees. By including discordant mtDNA pairings, PRIMUS identified the correct pedigree and rejected all the others. In the simulations, ~24% had greater than a 10% reduction in the number of pedigrees reconstructed by PRIMUS when using mtDNA, NRY and sex status.

Table 5. A summary of the concordance between pairs of individuals known to have concordant mtDNA or NRY haplotypes.

	# of pairs	% of pairs 100% concordant	% of pairs >99.8% concordance
A. 1KG mtDNA mother/child and full-siblings	21	62%	100%
B. 1KG NRY father/son and male full-siblings	5	40%	100%
C. HCE mtDNA mother/child and full-siblings	67	100%	100%
D. HCE NRY father/son and make full-siblings	23	100%	100%

Summary of the sequence identity between pairs of individuals from the low-pass 1000 Genomes Project [51] phase 1 release (A and B) and from 372 individuals with Illumina Infinium HumanCoreExome-24 BeadChips (HCE) chip data (C and D). We used 2,832 high quality mtDNA sites and 8,665 high quality NRY sites from the 1000 Genomes Project. The HCE data contain 205 high quality mtDNA sites and 126 high quality NRY sites from 185 female and 187 male samples. Every pairwise relationship included in the figures is expected to be nearly identical since they were either directly passed from an individual to the other or both inherited mtDNA or NRY from mother and dad, respectively. (A) 1000 Genomes mother/child and siblings have the same mtDNA DNA and should, therefore, have very few, if any, differences in the mtDNA sequence. These results show that there is less than 0.2% sequence difference between these pairs of individuals. (B) 1000 Genomes Father/son comparison; there are less than 0.2% sequence difference among these pairs. (C). HCE Mother/child or full-sibling pairs have 100% mtDNA genotype identity. (D) HCE Father/child and male full-sibling pairs have 100% NRY genotype identity.

Chapter 5. PADRE: Pedigree Aware Distant Relationship Prediction

Accurate estimation of shared ancestry is important in genetics studies. Current relationship prediction programs have the ability to accurately estimate pairwise relationships as distant as ninth-degree relatives. We demonstrate that combining distant relationship estimates with pedigree reconstruction can provide improved accuracy and power to estimate more distant relationships (i.e., more than ninth-degree relatives) depending on the depth of the pedigrees. We have developed a Pedigree Aware Distant Relationship Estimation (PADRE) algorithm that uses relationship likelihoods generated by Estimation of Recent Shared Ancestry (ERSA) to identify the highest likelihood connection between family networks reconstructed by PRIMUS (Pedigree Reconstruction and Identification of a Maximum Unrelated Set). We used PADRE to estimate relationships from SNP genotypes for both simulated pedigrees and 169 individuals from three previously described extended pedigrees. PADRE provides a substantial improvement in both the accuracy and power to detect distant relationships compared to ERSA alone, by correctly predicting 20% more of the fourth- through ninth-degree simulated relationships within one degree. PADRE also correctly predicts 59% of the tenth- through thirteenth-degree simulated relationships within one degree of relatedness, compared to 4% with ERSA alone. The improvement seen in the real pedigrees is broadly consistent with the improvement seen in simulations. We also used PADRE to estimate the distant relationships among the HapMap3 CEU samples, illustrating how the different trios and individuals are interconnected. PADRE greatly expands the range of relationships that can be estimated from genetic data that contain pedigrees and, it is implemented in the PRIMUS software package.

Introduction

Accurate prediction and verification of relationships among individuals is essential in a variety of genetic studies. Errors in pedigrees are common[20-22] and have adverse consequences, including a drop in the power to detect linkage[19]. Therefore, ensuring that the genetic relationships among the DNA samples match the reported pedigree structure is critical and improve linkage analysis and other forms of genetic analysis[74]. Identifying cryptic relationships can be useful as well. Genetic relationships identified in population studies can be also leveraged

for improved phasing and imputation. The identification of relatives also plays an important role in forensics in criminal investigations[76] and with victims of mass disaster[77].

With close relationships (first- through third-degree), pedigree reconstruction can provide the structure of the individuals in a genetic dataset[74]. However, genetic datasets often contain relationships that are more distant than third-degree, resulting in sparsely connected pedigrees that are unsuitable for reconstruction. Segmental sharing algorithms, such as ERSA[27; 38], can accurately predict pairwise relationships up to ninth-degree relatives (third-cousins once removed), but do not reconstruct pedigrees nor can they utilize information from known or observed pedigree structures in the data. We report the development of a new algorithm that leverages the pedigree reconstruction of first- to third-degree relatives by PRIMUS[74] with the accurate distant relationship predictions by ERSA[38], known as PADRE (Pedigree Aware Distant Relationship Estimation), which we have implemented in PRIMUS. PADRE uses ERSA-generated relationship likelihoods to identify the highest likelihood connection between family networks reconstructed by PRIMUS, which significantly expands the relationships that can be predicted.

Methods

Pedigree Aware Distant Relationship Estimation (PADRE) algorithm

PADRE combines reconstructed pedigree information with distant pairwise relationship predictions to determine the most likely way that two pedigrees may be distantly related. The algorithm is implemented within PRIMUS (v1.8.0) and requires results from ERSA (v2.1) as input. When PRIMUS reconstructs a dataset into two family networks, Net1 and Net2, both networks can have one or more possible pedigrees that fit the genetic data, annotated here with subscripts (i.e., Net1_1, Net1_2,…, and Net1_n).

The ERSA pairwise relationship model has two additional degree of freedom (relationship distance and number of shared segments) compared to the model that specifies two individuals are unrelated. Therefore, PADRE uses the Akaike Information Criterion (AIC) to penalize the related ERSA model for having two additional degrees of freedom. For a statistical model, the AIC value is $AIC = 2\text{k} - 2lnl(L)$, where k is the number of parameter in the model and L is the

maximum likelihood value of the function. For a pair of founders, *x* and *y,* related as *N*th-degree relatives, PADRE calculates the AIC with Eq 1.

$$AIC_{combined}(x, y|N) = \sum_{\substack{\forall a \in Net1_i \\ \forall b \in Net2_j}} AIC(a, b|D), \quad (1)$$

where a and b are individuals in the pedigrees $Net1_i$ and $Net2_j$, respectively, *D* is the degree of relatedness between a and b given the two networks and that founders *x* and *y* are *N*th-degree relatives, and $AIC(a, b|D)$ is calculated as:

$$AIC(a, b|D, D < 10) = 4 - 2lnl(a, b|D), \quad (2)$$

where the $lnl(a, b|D)$ is the maximum log-likelihood from ERSA of a and b being related as D^{th}-degree relatives. Thus, the AIC in Eq 1 is calculated from the composite likelihood of all cross-network pairwise relationship likelihoods in ERSA conditioning on an *Nth*-degree relationship between *x* and *y*. The AIC for two unrelated individuals is equal to $lnl(a, b|unrelated)$.

To identify the best fitting model, PADRE calculates the AIC for each possible fourth- through ninth-degree relationship between the founders of the two pedigrees using Eq 1. However, each network may have more than one possible pedigree, so we must evaluate all pairs of possible pedigrees identified by PRIMUS for each network and identify the pair of pedigrees that minimizes the AIC of the two networks. We also incorporated the PRIMUS calculated composite log-likelihoods of both reconstructed pedigree by summing them with the $AIC_{combined}$. Thus, the equation for this is:

$$AIC_{min}(Net1, Net2) = \min_{\substack{4 \le D \le 9 \\ 1 \le i \le Net1_n \\ 1 \le j \le Net2_n}} \left\{AIC_{combined}(x, y|N) + lnl(Net1_i) + lnl\left(Net2_j\right)\right\}, \quad (3)$$

for each founder *x* from Net1$_i$ and founder *y* from Net2$_j$, where *lnl(Net1$_i$)* and *lnl(Net2$_j$)* are the composite log-likelihoods of the pedigrees obtained from PRIMUS.

Because most tenth-degree relatives share no segments of their autosomal DNA IBD (in humans)[27], models involving relationships more distant than ninth-degree require special consideration. Although such models also include two additional parameters, the maximum likelihood estimate for the number of shared genetic segments is typically 0 resulting in a compressed free parameter space. Minimizing the AIC of such models without accounting for the reduced free parameter space over-penalizes such distant relationships. We address this problem with the following approximation. Given that two individuals *a* and *b* are genetic ninth-degree relatives, the unconditional likelihood of the tenth-degree relationship for individual *a* and the offspring of individual *b* is as follows: with probability one half, the shared segment is inherited by the offspring of individual *b,* and the likelihood is equal to the ninth-degree relationship likelihood. Otherwise, the likelihood is equal to the unrelated likelihood. This leads to the following formula to approximate the AIC of tenth-degree and more distant relationships:

$$AIC(a,b|D,D>9) = ln\left[e^{\left((0.5)^{D-9}AIC(a,b|9)\right)} + e^{\left(\left(1-(0.5)^{D-9}\right)\left(AIC(a,b|unrelated)\right)\right)}\right] \quad (4)$$

PADRE will output the pair of founders, their degree of relatedness, and the two possible pedigrees that resulted in the AIC_{min} for each pair of networks. In a separate output file, PADRE provides the degree of relatedness between each pair of samples that resulted in the AIC_{min}.

Controlling for Type 1 error

PADRE requires that the evidence for a relationship between two networks meet a specified significance threshold. If founders *x* and *y* are predicted to be *N*th-degree relatives based on the max composite likelihood, then PADRE will perform a likelihood ratio test to compare the null model that the founders are unrelated to the alternative model that they are *N*th degree relatives using the ERSA likelihoods: $-2\left(lnl(x,y|unrelated) - lnl(x,y|N)\right)$. PADRE evaluates the significance of this statistic using a chi-square approximation with two degrees of freedom, as previously described[27]. If the p-value is below the specified threshold (by default 0.05), then

PADRE includes the relationship between the pair of networks in the results. For the CEU pedigrees, we applied a Bonferroni correction of $p = 5.5x10^{-6}$ (0.05 / 9074 founder-to-founder relationships) to account for the number of founder-to-founder relationships tested in the CEU pedigree reconstruction results.

Simulations

We simulated pedigrees to evaluate the accuracy and relative benefit of using PADRE to detect distant relationships compared to ERSA alone. We used two identical 13-person, 3-generation pedigree structures and connected a founder in each pedigree by varying the number of generations to their recent common ancestor. Figure 30 illustrates a simulated pedigree where founders A2 and B2 are ninth-degree relatives. To test the full range of ERSA predictions beyond third-degree, we generated versions of the pedigree where individuals A2 and B2 are fourth- through ninth-degree relatives. For each of these versions of simulated pedigree structures, we created 100 different sets of genotypes using the method applied by Morrison[61] (see Web Resources). We randomly selected haplotypes with ~1M SNPs from among the unrelated HapMap3[72] CEU samples and assigned them to the two founders and anyone who married into the simulated pedigrees. The unrelated set of CEU samples was determined by running ERSA on all the HapMap3 CEU samples and then running the IMUS algorithm within PRIMUS[18] to identify the maximum unrelated set. We then propagated the genotypes from the founders through the pedigree by simulating recombination events as a homogeneous Poisson process using the genetic map provided with the HapMap3 data, but disregarding the centromere. Genotypes were removed for all individuals not included in either of the 13-person pedigrees. IBD estimates were calculated using PLINKv1.9, and all simulated pedigrees were reconstructed with PRIMUS. We obtained ERSA results as described above for each simulation.

To test improvements in relationship predictions by PADRE as the size and density of genotyped individuals increased, we first used PRIMUS, ERSA, and PADRE to analyze individuals A6 and B6 in each simulated pedigree (Figure 30). We then included genotypes of a randomly selected first- or second-degree relative of A6 and B6 and reran the analysis and comparison. We repeated this process until all first- and second-degree relatives of A6 and B6 were included, which results in two 10-person pedigrees connected by a single fourth- through ninth degree

relationship between A2 and B2. We then performed a final analysis and comparison using all 13 individuals in each pedigree.

Extended Pedigree Samples

We analyzed Affymetrix 6.0 SNP microarray data on 169 individuals from three previously described extended pedigrees with predominantly northern European ancestry (Huff, Witherspoon, et al., 2010). The three pedigrees were composed of 24, 30, and 115 genotyped individuals and included a total of 7,266 pairs of related individuals.

HapMap3 CEU samples

Using 165 CEU individuals from HapMap3 release 2[72] obtained from http://hapmap.ncbi.nlm.nih.gov, we reconstructed pedigree structures within this dataset using PRIMUS as described below using the default settings. We also ran ERSA on the dataset as described above, using the CEU dataset as its own control file to identify the regions for masking[38].

Pedigree reconstruction with PRIMUS

PRIMUS reconstructs multiple generation pedigrees with genotyped individuals in any generation[74]. PRIMUS uses genome-wide identity by descent (IBD) estimates to identify families and reconstruct all possible pedigrees that fit the genetic data using relationships as distant as third-degree relatives. We used the prePRIMUS IBD pipeline[74] to generate IBD estimates between all samples in each pedigree and PRIMUS to reconstruct pedigrees. Due to the sparse number of individuals genotyped in the three European ancestry pedigrees and in many of the simulations, we applied a relatedness cutoff in PRIMUS of second-degree to both datasets. We used the default relatedness cutoff of third-degree relatives for the HapMap3 CEU dataset[72].

Distant relationships prediction with ERSA

We applied the IBD detection pipeline described Glusman et al.[38] by first phasing all genetic data with Beagle (v3.3.2)[78] and then analyzing the phased data in GERMLINE (v1.4.0)[79] with the following parameters: err_het = 1, err_hom = 2, and min_m = 2.5 Mb. We then analyzed the GERMLINE output files with ERSA (v2.1) to calculate the likelihood of each possible pairwise

relationship (from first- through thirty-ninth-degree) among all samples in the dataset. We controlled for potential false-positive IBD segments by masking genomic regions from the 1000 Genomes Project[51] CEU samples with excess pairwise IBD (mask_region_threshold = 4) as previously described[38].

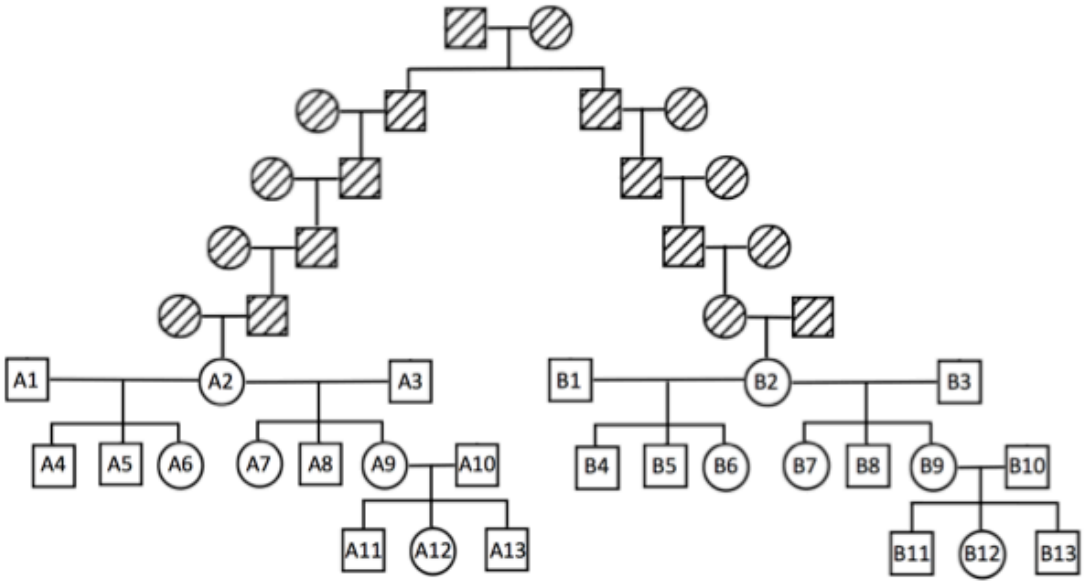


Figure 30. This pedigree structure was used to simulate ninth-degree pedigrees.
One hundred ninth-degree pedigrees, each with different genotypes, were generated with A2 and B2 related as ninth-degree relatives. The same pedigree structures for samples A1-A13 and B1-B13 were also used to generate 100 pedigrees, each with different genotypes, where A2 and B2 were fourth-, fifth-, sixth-, seventh-, eighth-, and ninth-degree relatives. The number of ancestral relatives was adjusted to account for the different degree of relatedness.

Results

Simulations

To evaluate the improvements in relationship prediction, we ran PADRE on all 600 simulated pedigrees each with ten different patterns of genotyped individuals, and we evaluated the accuracy of the pairwise relationship predictions. Figure 31A shows that PADRE and ERSA alone have the same accuracy when the individuals do not have any other first- or second-degree relatives in the pedigree. However, as additional genotyped individuals are included in the pedigrees, PADRE achieves much higher relationship prediction accuracy as well as the ability

to predict relationships that are too distant for ERSA alone to predict. Figure 31B shows that PADRE provides a substantial increase in power to detect a sixth- through thirteenth-degree relationships between two individuals when one or more first- or second-degree relatives are included in the analysis regardless of accuracy of degree prediction. The ERSA results fluctuate because of we are adding in additional pairs of individuals as described with the generation of the simulations.

Figure 31 shows the results up to 20 individuals in the pedigree, and Figure 32 summarizes the ERSA and PADRE results for the simulated pedigrees when all 26 individuals are included in the analysis. PADRE predicted the exact degree of relationship for 20% additional fourth- through ninth-degree relationships relative to ERSA alone. For tenth- through thirteenth-degree relationships, ERSA accurately predicted to within one degree only 4% of tenth- though thirteenth-degree relationships. In comparison, PADRE accurately predicted to within one degree 59% of the tenth- through thirteenth-degree relationships. Thus, with deeply genotyped pedigrees, PADRE frequently predicts very distant genealogical relationships that are effectively undetectable in ERSA alone.

Extended pedigree results

We ran PADRE on the real data from the extended pedigrees described in Methods. ERSA and PADRE attained the same accuracy for pairs of individuals who did not have first- or second-degree relatives genotyped in the pedigrees. However, when we looked at pairs of individuals who had two first- or second-degree relatives, we see a substantial improvement in accuracy with PADRE (Figure 33), while ERSA's accuracy remained the same. The accuracy improvement with the real data broadly matches the improvement we see with the simulations (Figure 33). PADRE correctly predicted 39% of the 10^{th} degree relationships within one degree of relatedness when the individuals had two first- or second-degree relatives in the pedigree, which was nearly three times as many relationships as ERSA alone. PADRE was also able to correctly predict 9% of the eleventh-degree relationships, while ERSA alone could not detect any.

Results for HapMap3 CEU samples

We previously reconstructed 51 separate pedigrees within the HapMap3 CEU dataset[72]. These pedigrees contain between two and six individuals. PADRE is able to connect 40 pairs of pedigrees with fourth- through ninth-degree relationships (Figure 34), consisting of 594 pairs of individuals with relationships beyond third-degree. Figure 35 illustrates how PADRE predicts relationships among four CEU pedigrees.

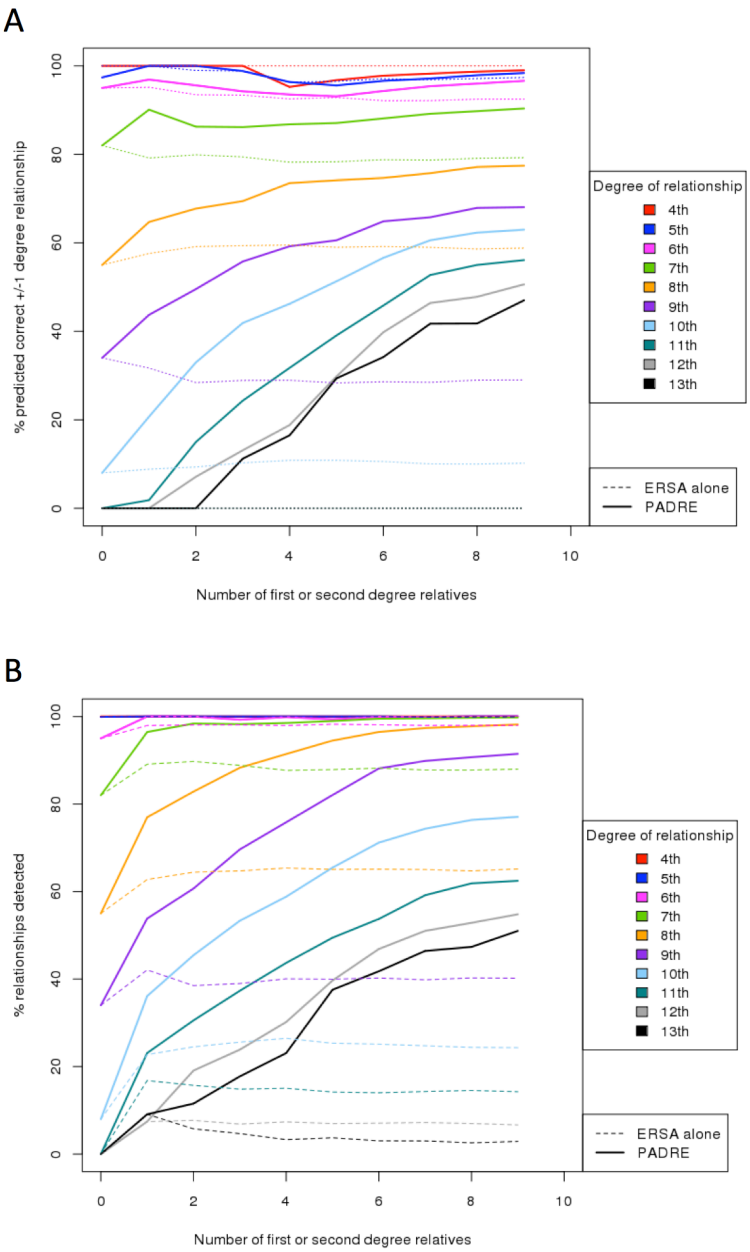


Figure 31. ERSA and PADRE performance on simulations.
A) ERSA alone and PADRE predictions by degree of relationships within the simulated pedigrees. The observed accuracy in the pedigree predictions increases as additional first- and second-degree relatives are added. B) Power of PADRE and ERSA alone to detect simulated relationships as additional first- and second-degree relatives were added to the pedigree. The ERSA results fluctuate because of we are adding in additional pairs of individuals as described with the generation of the simulations.

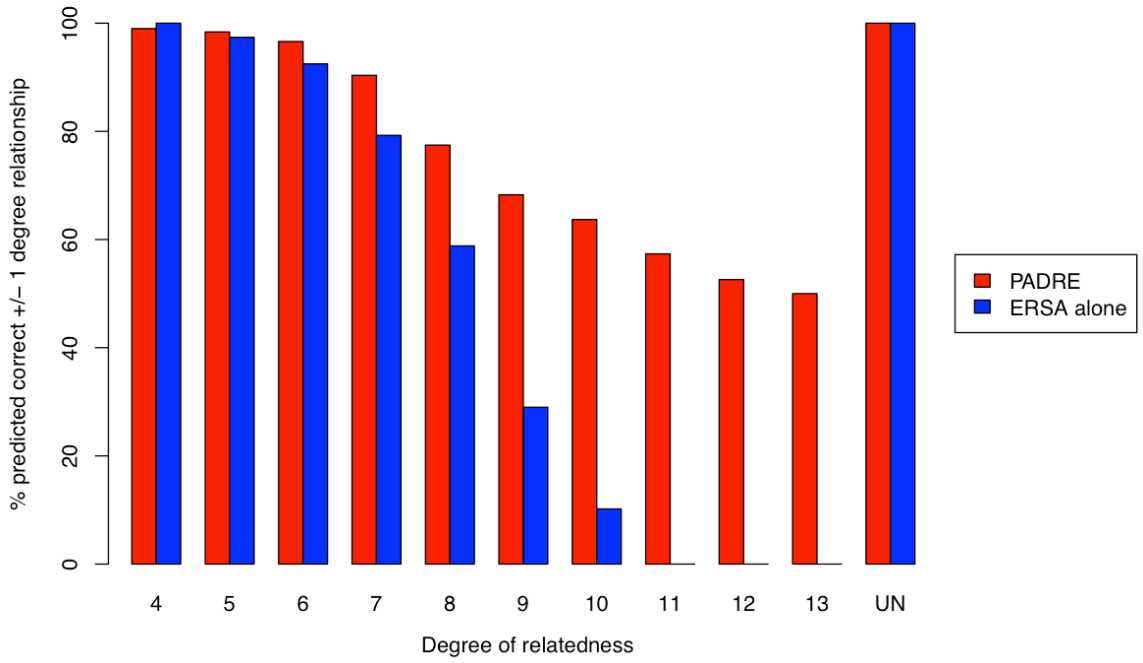


Figure 32. PADRE and ERSA results on complete simulated pedigrees.
PADRE more accurately predicts fifth- through tenth-degree relationships relative to ERSA and frequently identifies eleventh- through thirteenth-degree relatives that were undetectable in ERSA.

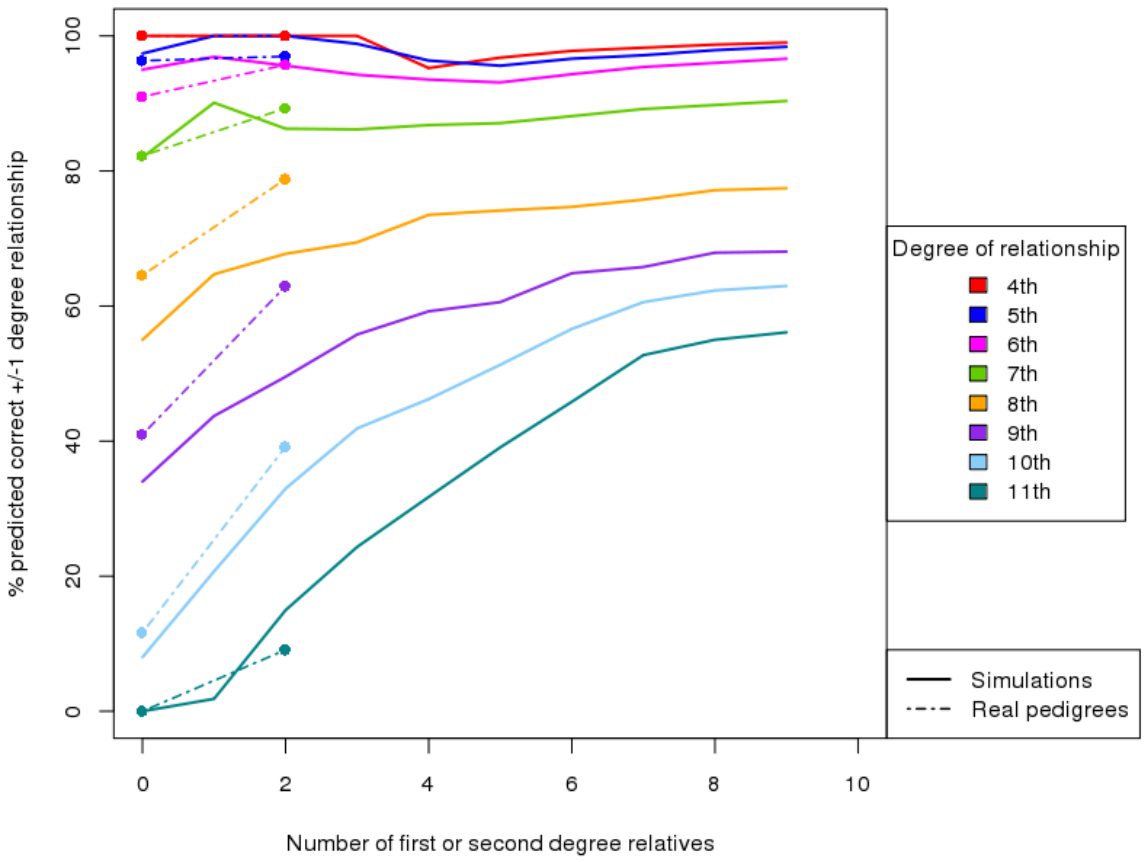


Figure 33. Percentage of correct relationship estimations by PADRE on extended European ancestry pedigrees compared to the simulated pedigree results.
The accuracy observed in the extended pedigrees as additional first- and second-degree relatives were added was broadly consistent with the simulated pedigree accuracy.

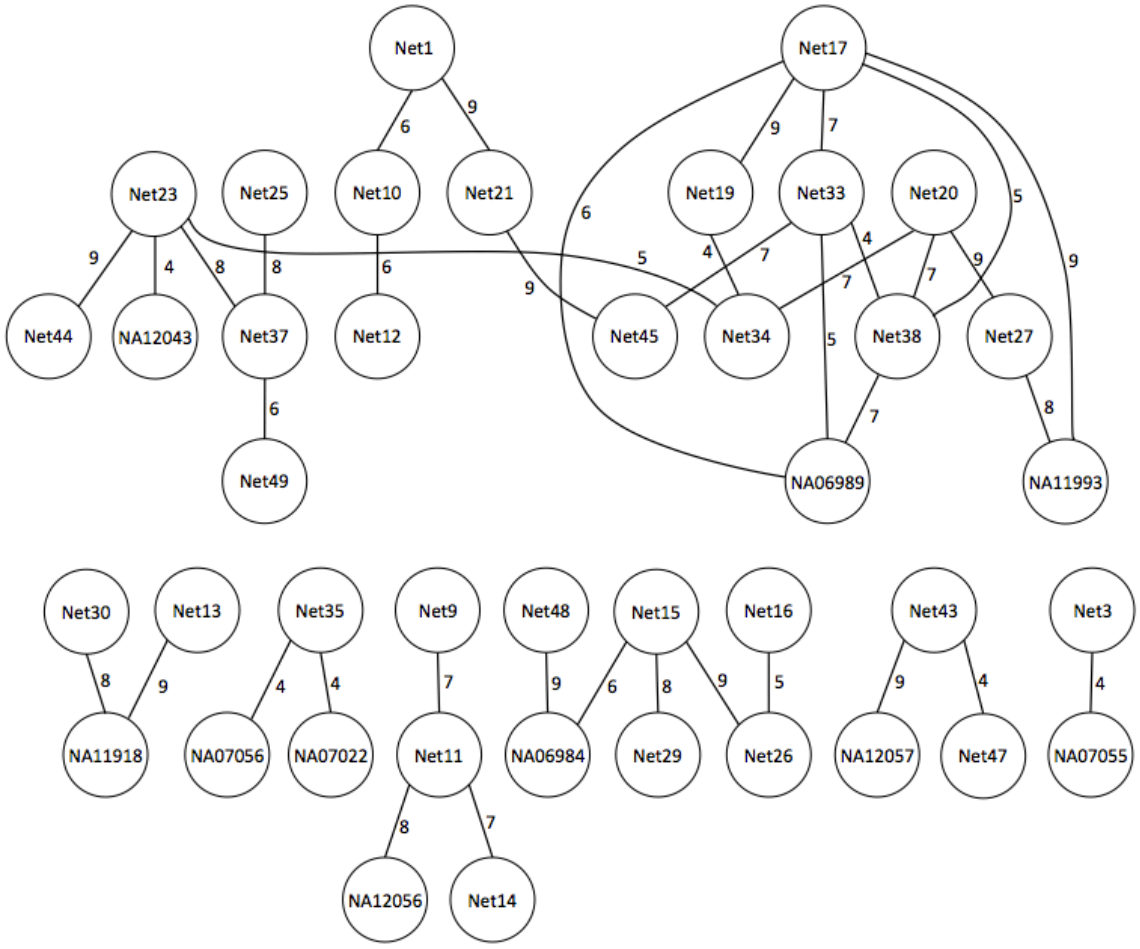


Figure 34. A graph of PADRE estimated relationships among the CEU samples using a Bonferroni adjusted threshold of $\alpha = 0.05/9090 = 5.5 \times 10^{-6}$.
Each node corresponds to a PRIMUS reconstructed network number, and an edge between nodes indicates a significant relationship predicted by PADRE using pairwise relationship likelihoods obtained by ERSA. The number next to each edge indicates the degree of relationship connecting a founder in the reconstructed pedigree of each network. This type of network graph is the standard output of PADRE.

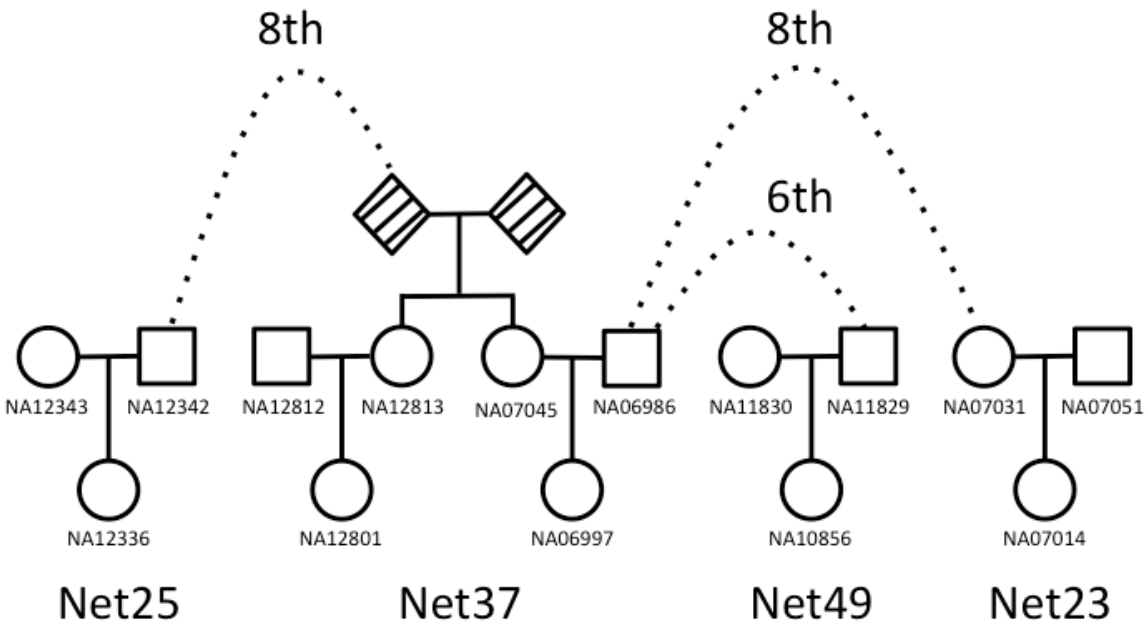


Figure 35. An example of four distantly related HapMap3 CEU pedigrees with relationships predicted by PADRE.
Although the trios and the full-sibling relationship between NA12813 and NA07045 have been previously reported, PADRE is able to identify statistically significant relationships connecting these distantly related pedigrees. The related pairs of founders are marked with the dotted lines and the degree of relationship is labeled next to the line.

Discussion

We have demonstrated through simulations and actual pedigrees that PADRE can leverage pedigree reconstruction results from PRIMUS and distant pairwise relationship predictions from ERSA to improve both the power to detect distant relationships and the accuracy of relationship estimation. The power to detect relationships more distant than ninth-degree relatives is dependent on the number of generations in the pedigrees that PADRE connects. For instance, PADRE detected up to thirteenth-degree relationships in the simulated pedigrees because each pedigree had three generations and the founders of the pedigrees (A2 and B2, Figure 30) were ninth-degree relatives. It stands to reason that as the depth of the pedigrees being connected by PADRE increases, so will the distance of relationships that PADRE will be able to predict. Therefore, we expect that the maximum degree of relationship PADRE can detect is ninth degree plus the sum of generations in the two pedigrees PADRE connects. PADRE relationship estimation accuracy improves as the number of genotyped individuals within each pedigree increases (Figure 31 and Figure 33). PADRE is most accurate when all individuals within a pedigree are genotyped (Figure 32).

We note that PADRE does not look for distant relationships within the same reconstructed pedigree structures, as it assumes no consanguinity. However, these types of relationships can be detected in other ways, for example using ISCA[80] and ERSA (v2) to evaluate regions of the genome that are shared IBD2 between individuals.

Existing pairwise prediction programs use the number and size of shared IBD segments between two individuals to estimate their degree of relatedness. However, as the degree of relatedness increases, the number of shared segments drops to zero. Most tenth-degree relatives share no segments of their autosomal DNA IBD (in humans); therefore, their degree of relatedness cannot be estimated by existing pairwise comparison programs. PADRE uses reconstructed pedigrees to estimate relationships between individuals who share no portion of their genomes with detectable IBD as long as their ancestors can be accurately estimated as ninth-degree relatives or closer.

PADRE provides an immediate benefit to human genetic analysis. This is particularly true in large case-control studies. Supposedly unrelated individuals can in fact have relationships at the level of fourth- through thirteenth-degree relatives, and these cryptic relationships can be

detected even in small datasets, as shown in our analysis of the CEU data (Figure 34 and Figure 35). By identifying these relationships, studies can avoid artifactual findings due to cryptic relatedness[11]. In addition, techniques that explicitly model relationships may provide more power[81], particularly for relatively high penetrance alleles segregating in pedigrees.

Chapter 6. Summary and future directions

Significant Contributions

PRIMUS features an implementation of a graph theory algorithm adapted to find the maximum unrelated set of individuals within a genetic dataset. PRIMUS retains up to 50% more samples when used over existing and suggested methods.

PRIMUS also features pedigree reconstruction capabilities. It can read in genome-wide estimates of IBD, and infer the familial relationships from these estimates, and piece these relationships together to generate all the possible pedigree structures that fit the genetic data. This method overcomes several of the limitations of other pedigree reconstruction programs and provides a tool capable of using real world family structures. For instance, of the 10,000 simulated pedigrees generated to validate PRIMUS, the leading pedigree reconstruction programs could only reconstruct 43 (<0.5%) while PRIMUS fully reconstructed over 92% of them. PRIMUS can reconstruct non-consanguineous, multi-generational pedigrees using up to third-degree relationships, regardless of the pattern of missing samples. PRIMUS even outperformed the standard programs used for verifying pedigrees by demonstrating that it can more accurately predict relationships within a pedigree structure when more than 50% of the samples are genotyped. PRIMUS also allows the user to specify the sex and age of the individuals to improve the chances of identifying the correct pedigree structure.

PRIMUS is the first pedigree reconstruction program to incorporate genome-wide, high-density data with mtDNA and non-recombining Y chromosome (NRY) haplotypes. The addition of these data greatly improves reconstruction by eliminating pedigrees that fit the autosomal inheritance pattern, but are inconsistent with the inheritance pattern of mtDNA and NRY haplotypes. For example, one simulated pedigree had 58 pedigree structures that fit the autosomal data, but with the addition of just mtDNA, PRIMUS was able to identify the true pedigree structure by ruling out the 57 incompatible structures.

Finally, PRIMUS provides an excellent tool for reconstructing pedigrees using up to third-degree relationships, but with addition of a new algorithm known as PADRE (Pedigree Aware Distant Relationship Estimation), I sought a way to connect the reconstructed pedigrees using a distant

relationships prediction method: Estimation of Recent Shared Ancestry (ERSA). Importantly, PADRE extends the relationship predictions beyond the ninth-degree relationship limit of ERSA alone. With PADRE, predictions to thirteenth-degree were possible within the simulations. PADRE also improves the accuracy of predictions for fifth- through ninth-degree relationships by 25% for simulated data compared to ERSA alone.

Future Directions

As sequencing technology continues to improve and drives down the cost of medical sequencing, I expect that within the next two decades most, if not all, people in developed countries will have their genomes sequenced. Having access to this level of data would provide unprecedented power to detect, solve, and treat human genetic diseases. However, this volume of data presents enormous bioinformatics challenges. The challenge most relevant to my research would be obtaining and maintaining accurate records of how genetic data match reported family histories and pedigrees. Pedigree reconstruction with PRIMUS provides an initial solution, but advances in the field of pedigree reconstruction are necessary in order to handle the volume, distance, and variety of family relationships found across the entire human population.

PRIMUS was designed to reconstruct pedigrees with first- through third-degree relationships from outbred populations. However, I believe that pedigree reconstruction can, and must, be expanded to incorporate three additional classes of relationships: complex, consanguineous, and distant relationships up to sixth-degree relatives.

Complex relationships

First, complex relationships include double first cousins (i.e., the children of two sisters married to two brothers) and half-sibling-plus-first-cousin (i.e., children of an individual who had children with a pair of siblings). Although these relationships are not extremely common in the United States, they often occur in pedigrees of individuals in other cultures. Both of these relationships have expected mean IBD proportions that are distinct from the non-complex first- through third-degree relationships, and they can be incorporated into pedigree reconstruction using the same framework laid out with PRIMUS.

Consanguineous relationships

There are two types of consanguinity that will need to be handled differently. First, there are recent consanguineous relationships. These relationships will have mean expected IBD proportions and, therefore, could be accurately predicted using the framework already laid out by PRIMUS; however, I expect that the IBD estimate distributions for the various consanguineous relationships will significantly overlap with one another. This problem can be remedied as DNA from more individuals in the pedigree are collected and genotyped/sequenced. The second type of consanguinity is an in-bred population background often caused by a bottlenecked and isolated population, for example the Hutterites[82]. This consanguineous background results in individuals looking more closely related to one another than they should be given their recent family history. One potential solution is to mask regions of the genome common among all individuals of the population. Another solution may be to identify regions of each individual's genome that happen to be detected IBD with many other individuals in the population. The impact that these regions of the individual's genome have on IBD estimates can be down weighted in order to counter act the inbred background affect on IBD estimates. Both of these potential solutions to the inbred background problem require a substantial amount of sequence or genotype data for each population of interest in order to know which regions of the genome to mask or down weight. However, with the continued advancement in sequencing technology and the amount of data being generated worldwide, this does not seem unrealistic for most populations within the next decade.

Distant relationships

Distant relationships pose a significant challenge in pedigree reconstruction for two reasons: they are more challenging to accurately estimate (Figure 31), and there are more relationship types as the degree of relatedness increases (Supplementary Table 6). However, with improved phasing and IBD detection, I expect that detection up to sixth-degree relationship can be nearly 100% accurate since we have power to detect all sixth-degree relationships with the PADRE algorithm (Figure 2B from PADRE paper). That said, piecing together many sixth-degree relationships would generate far too many pedigrees. Instead of reconstructing them, fourth- through sixth-degree relationships could be used to reduce the number of possible pedigrees generated in

pedigree reconstruction by eliminating the pedigrees that are inconsistent with the relationship estimations. Addition of mtDNA and Y haplotypes could also be helpful in piece back together distant relationships and reducing the number of pedigree structures that fit the genetic data.

Conclusion

The field of human genetics is expanding rapidly and is in need of powerful computational tools to manage, verify, and leverage the massive datasets being generated. I have developed a powerful tool called PRIMUS that has proven effective at using several types of genetic data to reconstruct and verify pedigrees as well as connect distantly related pedigrees with PADRE. PRIMUS is a big step forward towards tackling the need to reconstruct and verify pedigrees within large existing datasets like the Jackson Heart Study and the Geisinger Health System. However, in order to handle the size and wide variety of pedigree structures that exist within the world's populations, additional advancements in IBD detection and complex/inbred relationship reconstruction will be necessary.

Web Resources

The URLs for data presented herein are as follows:

Boston Early-Onset COPD Study, http://bostoncopd.org

CraneFoot, http://www.finndiane.fi/software/cranefoot/

GraphViz, www.graphviz.org

International HapMap Project, http://hapmap.ncbi.nlm.nih.gov

IPED2, http://www.cs.ucla.edu/~danhe/Software/IPED2.html

Kinship2, http://cran.r-project.org/package.kinship2

PLINK 1.07, http://pngu.mgh.harvard.edu/purcell/plink/

PRIMUS, http://primus.gs.washington.edu

PRIMUS maximum unrelated set of individuals for the publically available datasets, http://sourceforge.net/projects/primus-beta/datasets/

PRIMUS simulations, the link to the code used for generating simulations, and the reconstructed HapMap3 pedigrees, http://sourceforge.net/projects/primus-beta/files/

SciPy, http://www.scipy.org

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Supplementary Tables

Supplementary Table 1. True IBD vs. Estimated IBD for different SNP sets

# of SNPs	IBD0 r^2	IBD1 r^2	IB2 r^2
6K	0.987	0.981	0.961
10K	0.992	0.99	0.984
20K	0.995	0.993	0.99
50K	0.997	0.996	0.997
100K	0.997	0.997	0.997
1000K	0.998	0.998	0.997
Linkage Panel IV	0.979	0.974	0.97
Affy 6.0	0.998	0.997	0.993
CytoSNP	0.998	0.997	0.994
HumanCore	0.998	0.997	0.996
Omni Express	0.998	0.998	0.995
Omni 2.5	0.998	0.998	0.995

SNP sets 6K-1000K were generated using PLINK to trim the HapMap3 dataset down to the desired number of SNPs. The remaining SNP sets were generated by taking the intersection of SNPs in those panels and HapMap3. IBD estimates were generated with PLINK using SNPs with a minor allele frequency >1% and a call rate >90%. In statistical package R, we plotted the true IBD proportion to the estimated IBD proportion for each relationship in the 100 halfsib size-20 pedigrees. We then calculated r^2 based on the deviation from Y=X.

Supplementary Table 2. Combined simulation reconstruction results

Size	structure	0 Missing samples	1 Missing samples	2 Missing samples	3 Missing samples	4 Missing samples	5 Missing samples	6 Missing samples	7 Missing samples	8 Missing samples	9 Missing samples	10 Missing samples
5	highest scoring	200	200	200	NA	NA	NA	NA	NA	NA	NA	NA
5	among highest scoring	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
5	among scored	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
5	partially reconstructed	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
5	missing	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
10	highest scoring	200	200	195	133	6	0	NA	NA	NA	NA	NA
10	among highest scoring	0	0	5	65	190	20	NA	NA	NA	NA	NA
10	among scored	0	0	0	1	2	4	NA	NA	NA	NA	NA
10	partially reconstructed	0	0	0	0	0	0	NA	NA	NA	NA	NA
10	missing	0	0	0	1	2	0	NA	NA	NA	NA	NA
20	highest scoring	200	200	193	172	138	95	45	13	0	0	0
20	among highest scoring	0	0	6	25	54	94	138	156	131	102	68
20	among scored	0	0	1	3	7	8	12	25	62	72	46
20	partially reconstructed	0	0	0	0	1	1	2	2	3	1	0
20	missing	0	0	0	0	0	2	3	4	4	3	1
30	highest scoring	200	199	197	190	171	148	113	74	47	22	3
30	among highest scoring	0	0	2	8	25	42	69	91	103	102	90
30	among scored	0	0	0	1	2	4	6	14	23	31	40
30	partially reconstructed	0	1	1	1	2	6	11	20	25	43	65
30	missing	0	0	0	0	0	0	1	1	2	2	2
40	highest scoring	198	197	192	189	183	168	150	126	105	78	49
40	among highest scoring	2	2	4	4	9	21	31	44	48	61	65
40	among scored	0	0	0	1	1	1	1	3	8	15	20
40	partially reconstructed	0	1	4	5	6	9	17	24	37	44	62
40	missing	0	0	0	1	1	1	1	3	2	2	4
50	highest scoring	199	197	194	190	186	174	159	141	123	102	84
50	among highest scoring	1	0	2	2	2	8	12	17	24	29	37
50	among scored	0	0	1	0	2	3	6	6	9	9	9
50	partially reconstructed	0	3	3	7	9	13	22	35	42	58	68
50	missing	0	0	0	1	1	2	1	1	2	2	2

We combined the reconstruction results for both the Uniform and Halfsib pedigrees. Some of the Halfsib pedigree structures allowed for more samples to be removed than others due to the random nature of how they were simulated. As a result, Halfsib size-10 with five missing samples and size-20 with nine and ten missing samples do not add up to 200 simulations. We ran 100 simulations for each size and % of missing samples. For each simulation we determined where the true pedigree fell among the ranked reconstruction results. Each bar displays the proportion of the 100 simulations that corresponded to the five reconstruction outcomes defined as follows:

"highest scoring" – The true pedigree is the highest scoring pedigree
"among highest scoring" – PRIMUS output contained more than one possible pedigree and the true pedigree is tied as the highest scoring pedigree with one or more other pedigrees
"among scored" – the true pedigree is not the highest scoring pedigree, but is among the pedigrees generated by PRIMUS
"partial reconstruction" – the complete reconstruction either resulted in too many possible pedigrees, ran out of memory, or took longer than 36 hours to run and as a result only a partial reconstruction using 1st degree relationships was generated
"missing" – PRIMUS reconstructed one or more possible pedigrees, but the true pedigree was not among them

Supplementary Table 3. The accuracy of PRIMUS and RELPAIR relationship predictions with Halfsib size-20 pedigrees

PRIMUS									
Percent Missing samples	0%	5%	10%	15%	20%	25%	30%	35%	40%
1st degree category	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
2nd degree category	100.00%	100.00%	100.00%	100.00%	99.79%	99.63%	99.55%	99.35%	99.55%
3rd degree category	100.00%	100.00%	100.00%	99.79%	98.81%	99.26%	98.30%	96.27%	93.37%
Distantly/unrelated	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
1st degree relationship type	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
2nd degree relationship type	100.00%	100.00%	99.37%	97.12%	96.00%	88.45%	75.61%	66.88%	58.48%
3rd degree relationship type	100.00%	100.00%	98.13%	95.42%	92.46%	85.68%	63.67%	46.73%	26.54%
1st degree relationship type + direction	100.00%	100.00%	100.00%	99.86%	99.84%	99.57%	99.54%	99.52%	99.92%
2nd degree relationship type + direction	100.00%	100.00%	99.41%	97.80%	96.89%	91.18%	80.92%	73.76%	61.95%
3rd degree relationship type + direction	100.00%	100.00%	96.87%	93.37%	91.90%	86.43%	68.29%	45.41%	14.15%
RELPAIR									

Percent Missing samples	0%	5%	10%	15%	20%	25%	30%	35%	40%
1st degree category	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
2nd degree category	99.98%	99.98%	99.97%	99.97%	99.97%	99.96%	100.00%	100.00%	100.00%
3rd degree category	82.72%	82.89%	82.98%	83.13%	83.22%	83.25%	83.32%	83.48%	83.57%
Distantly/unrelated	97.17%	96.67%	96.04%	95.07%	93.75%	91.77%	88.16%	82.52%	66.98%
1st relationship type	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
2nd relationship type	55.95%	55.27%	55.81%	56.18%	56.68%	57.17%	56.20%	57.60%	55.89%
3rd relationship type	NA	NA	NA	NA	NA	NA	NA	NA	NA
1st degree relationship type + direction	NA	NA	NA	NA	NA	NA	NA	NA	NA
2nd degree relationship type + direction	NA	NA	NA	NA	NA	NA	NA	NA	NA
3rd degree relationship type + direction	NA	NA	NA	NA	NA	NA	NA	NA	NA

Halfsib size-20 pedigrees with 0% to 40% missing samples were used to test the pairwise relationship prediction accuracy of both PRIMUS and RELPAIR[28]. We compared the pairwise relationship of the highest ranked pedigree in PRIMUS to the true simulated relationship. We used the method employed by Pemberton et al.[44] to obtain the RELPAIR prediction and then compared that to the true simulated relationship. The table shows accuracy of each method at correctly predicting each relationship in the pedigree by the degree of relatedness (e.g., A and B are first degree relatives), the type of relationship (e.g., A and B have a parental relationship), and the type and directionality of the relationship (e.g., A is the parent of B). The results have been grouped by the degree of the relationships. RELPAIR does not make a distinction between the four 3rd degree relationships nor is it able to predict the directionality of pairwise relationships; therefore, NA is used for those results. The highlighted results are the ones plotted in Figure 2.

Supplementary Table 4. EOCOPD pedigree reconstruction summary

Fam FID	Reconstructed Correctly	Number of Genotyped Samples	Reconstructed FIDs	Expected Pedigree Rank	Number of Possible Pedigrees	Explanation
Fam1	1	11	1	1	2	
Fam2	1	8	2	1	1	
Fam3	1	13	3	2	2	
Fam4	1	3	4	1	1	
Fam5	1	15	5	1	1	
Fam6	0	9	6	NA	20	NON PATERNITY CAUGHT: half sib is actually full sib
Fam7	1	10	7	20	28	
Fam8	1	9	8	1	1	
Fam9	1	8	9	1	1	
Fam10	1	9	10	1	1	
Fam11	1	4	11	3	7	
Fam12	1	2	12	1	1	
Fam13	1	3	13	1	1	
Fam14	1	5	14	1	1	
Fam15	1	2	15	2	2	
Fam16	1	2	16	1	1	
Fam17	1	8	17	1	1	
Fam18	1	6	18	1	2	
Fam19	1	5	19	32	33	
Fam20	1	15	20	4	11	
Fam21	1	5	21	1	1	
Fam22	1	13	22	1	2	
Fam23	1	8	23	2	3	
Fam24	0	10	24	NA	5	NON PATERNITY

						CAUGHT: half avuncular instead of avuncular
Fam25	1	5	25	1	1	
Fam26	1	6	26	1	2	
Fam27	1	6	27	1	1	
Fam28	1	14	28	1	1	
Fam29	1	3	29	1	1	
Fam30	1	11	30	1	2	
Fam31	1	5	31	1	1	
Fam32	0	6	32	NA	4	NON PATERNITY CAUGHT
Fam33	1	3	33	1	1	
Fam34	1	4	34	1	4	
Fam35	0	6	35,39	NA	1	Sample missing because of duplicate; Also non-paternity;
Fam36	1	3	36	1	1	
Fam37	1	2	37	2	5	
Fam38	1	3	38	1	1	
Fam39	1	15	39	3	3	Contains duplicate sample
Fam40	1	5	40	1	1	
Fam41	1	6	41	1	3	
Fam42	1	5	42	1	1	
Fam43	1	9	43	4	6	
Fam44	1	14	44	1	2	
Fam45	0	7	45	NA	1	NON PATERNITY CAUGHT

Fam46	1	3	46	1	1	
Fam47	1	6	47	4	4	
Fam48	1	4	48	1	1	
Fam49	1	10	49	1	1	

Supplementary Table 5. Comparison of HapMap3 pairwise relationships

Population	Network	IID1	Sex	Hapmap Reported	PRIMUS Predicted	Pemberton Predicted	CARROT Predicted	IID2	Sex	Hapmap Reported	PRIMUS Predicted	Pemberton Predicted	CARROT Predicted	Notes
ASW	1	NA19916	M	P	P	O,P	-	NA19918	M	O	O	O,P	-	
ASW	1	NA19917	F	P	P	O,P	-	NA19918	M	O	O	O,P	-	
ASW	2	NA19834	M	P	P	O,P	-	NA19836	F	O	O	O,P	-	
ASW	2	NA19835	F	P	P	O,P	-	NA19836	F	O	O	O,P	-	
ASW	3	NA20279	M	-	O	O,P	-	NA20282	F	-	P	O,P	-	
ASW	3	NA20279	M	-	H	H	-	NA20284	M	-	H	H	-	R*
ASW	3	NA20279	M	-	N	N	-	NA20301	F	-	A	A	-	R
ASW	3	NA20279	M	-	1C	-	1C	NA20302	M	-	1C	-	1C	
ASW	3	NA20282	F	P	P	O,P	-	NA20284	M	O	O	O,P	-	
ASW	3	NA20282	F	-	F	F	-	NA20301	F	-	F	F	-	
ASW	3	NA20282	F	-	A	A	-	NA20302	M	-	N	N	-	R*
ASW	3	NA20284	M	-	N	N	-	NA20301	F	-	A	A	-	R
ASW	3	NA20284	M	-	1C	-	1C	NA20302	M	-	1C	-	1C	
ASW	3	NA20301	F	P	P	O,P	-	NA20302	M	O	O	O,P	-	
ASW	4	NA19703	M	P	P	O,P	-	NA19705	M	O	O	O,P	-	
ASW	4	NA19704	F	P	P	O,P	-	NA19705	M	O	O	O,P	-	
ASW	6	NA19900	M	P	P	O,P	-	NA19902	F	O	O	O,P	-	
ASW	6	NA19901	F	P	P	O,P	-	NA19902	F	O	O	O,P	-	
ASW	9	NA20287	F	P	O,P	O,P	-	NA20288	M	O	O,P	O,P	-	
ASW	14	NA19713	F	-	A	A	-	NA19714	F	-	N	N	-	R
ASW	14	NA19713	F	P	P	O,P	-	NA19983	F	O	O	O,P	-	
ASW	14	NA19713	F	-	F	F	-	NA19985	F	-	F	F	-	R
ASW	14	NA19714	F	-	1C	-	1C	NA19983	F	-	1C	-	1C	
ASW	14	NA19714	F	O	O	O,P	-	NA19985	F	P	P	O,P	-	
ASW	14	NA19982	M	P	P	O,P	-	NA19983	F	O	O	O,P	-	
ASW	14	NA19983	F	-	N	N	-	NA19985	F	-	A	A	-	R*
ASW	15	NA20340	M	-	1C,GA,GC,GG,GN,H A,HN,UN	-	U	NA20344	F	-	1C,GA,GC,GG,GN,H A,HN,UN	-	U	
ASW	15	NA20340	M	-	1C,GA,GC,GG,GN,H A,HN,UN	-	-	NA20349	M	-	1C,GA,GC,GG,GN,H A,HN,UN	-	-	N
ASW	15	NA20344	F	P	P	O,P	-	NA20345	M	O	O	O,P	-	
ASW	15	NA20344	F	-	F	F	-	NA20349	M	-	F	F	-	
ASW	15	NA20344	F	-	A	A	-	NA20350	M	-	N	N	-	R
ASW	15	NA20345	M	-	N	N	-	NA20349	M	-	A	A	-	R*
ASW	15	NA20345	M	-	1C	-	1C	NA20350	M	-	1C	-	1C	
ASW	15	NA20349	M	P	P	O,P	-	NA20350	M	O	O	O,P	-	

					1C,GA,GC ,GG,GN,H						1C,GA,GC ,GG,GN,H			
ASW	16	NA20281	M	-	A,HN,UN	-	-	NA20297	M	-	A,HN,UN	-	-	N
ASW	17	NA19908	M	P	P	O,P	-	NA19919	M	O	O	O,P	-	
ASW	17	NA19909	F	P	P	O,P	-	NA19919	M	O	O	O,P	-	
ASW	20	NA19818	M	P	P	O,P	-	NA19828	M	O	O	O,P	-	
ASW	20	NA19819	F	P	P	O,P	-	NA19828	M	O	O	O,P	-	
ASW	29	NA20294	F	P	O,P	O,P	-	NA20295	M	O	O,P	O,P	-	
ASW	30	NA20334	F	P	P	O,P	-	NA20335	M	O	O	O,P	-	
ASW	30	NA20334	F	-	F	F	-	NA20336	F	-	F	F	-	R
ASW	30	NA20334	F	-	A	A	-	NA20337	F	-	N	N	-	R
ASW	30	NA20335	M	-	N	N	-	NA20336	F	-	A	A	-	R
ASW	30	NA20335	M	-	1C	-	1C	NA20337	F	-	1C	-	1C	
ASW	30	NA20336	F	P	P	O,P	-	NA20337	F	O	O	O,P	-	
ASW	44	NA19700	M	P	P	O,P	-	NA19702	M	O	O	O,P	-	
ASW	44	NA19701	F	P	P	O,P	-	NA19702	M	O	O	O,P	-	
ASW	46	NA20289	F	P	P	O,P	-	NA20290	F	O	O	O,P	-	
ASW	46	NA20289	F	-	F	F	-	NA20341	F	-	F	F	-	R
ASW	46	NA20290	F	-	1C,GC,GG ,GN,HN,U N	-	U	NA20333	F	-	1C,GA,GC ,GG,HA,U N	-	U	P
ASW	46	NA20290	F	-	N	N	-	NA20341	F	-	A	A	-	R
ASW	46	NA20332	F	P	O,P	O,P	-	NA20333	F	O	O,P	O,P	-	
ASW	46	NA20332	F	-	A,G,H	U	-	NA20343	M	-	C,H,N	U	-	R,P
ASW	46	NA20332	F	-	1C,UN	-	-	NA20346	M	-	1C,UN	-	-	N
ASW	46	NA20333	F	-	1C,GG,HA ,HN	-	U	NA20343	M	-	1C,GC,HA ,HN	-	U	P
ASW	46	NA20342	M	P	P	O,P	-	NA20343	M	O	O	O,P	-	
ASW	46	NA20343	M	-	1C,GC,GG ,GN,HN,U N	-	U	NA20346	M	-	1C,GA,GC ,GG,HA,U N	-	U	P
ASW	46	NA20346	M	P	P	O,P	-	NA20347	M	O	O	O,P	-	
ASW	46	NA20347	M	-	1C,GC,GN ,HA,HN,U N	-	-	NA20359	F	-	1C,GA,GG ,HA,HN,U N	-	-	N
ASW	46	NA20347	M	-	1C,GC,GG ,GN,HA,H N,UN	-	-	NA20360	M	-	1C,GA,GC ,GG,HA,H N,UN	-	-	N
ASW	46	NA20347	M	-	C,H,N	H	-	NA20363	F	-	A,G,H	H	-	R,?
ASW	46	NA20347	M	-	1C,GC,HA ,HN	-	HA	NA20364	F	-	1C,GG,HA ,HN	-	HN	?
ASW	46	NA20359	F	P	O,P	O,P	-	NA20360	M	O	O,P	O,P	-	
ASW	46	NA20359	F	-	A,C,G,H,N	A	-	NA20363	F	-	A,C,G,H,N	N	-	R,?
ASW	46	NA20359	F	-	1C,GA,GC ,GG,HA,H N,UN	-	GA	NA20364	F	-	1C,GC,GG ,GN,HA,H N,UN	-	GN	?
ASW	46	NA20360	M	-	1C,GC,GG ,GN,HA,H N,UN	-	1C	NA20363	F	-	1C,GA,GC ,GG,HA,H N,UN	-	1C	?

ASW	46	NA20363	F	P	O,P	O,P	-	NA20364	F	O	O,P	O,P	-
ASW	48	NA20356	M	P	P	O,P	-	NA20358	M	O	O	O,P	-
ASW	48	NA20357	F	P	P	O,P	-	NA20358	M	O	O	O,P	-
ASW	49	NA20291	M	P	O,P	O,P	-	NA20292	F	O	O,P	O,P	-
ASW	62	NA19921	F	P	O,P	O,P	-	NA20129	F	O	O,P	O,P	-
ASW	65	NA20317	F	P	O,P	O,P	-	NA20319	F	O	O,P	O,P	-
ASW	71	NA20126	M	P	P	O,P	-	NA20128	F	O	O	O,P	-
ASW	71	NA20127	F	P	P	O,P	-	NA20128	F	O	O	O,P	-
ASW	74	NA19914	F	P	O,P	O,P	-	NA19915	M	O	O,P	O,P	-
ASW	80	NA20276	F	P	O,P	O,P	-	NA20277	F	O	O,P	O,P	-
CEU	1	NA10865	M	O	O	O,P	-	NA11891	M	P	P	O,P	-
CEU	1	NA10865	M	O	O	O,P	-	NA11892	F	P	P	O,P	-
CEU	8	NA10836	F	O	O,P	O,P	-	NA12275	F	P	O,P	O,P	-
CEU	12	NA10852	F	O	O,P	O,P	-	NA12045	M	P	O,P	O,P	-
CEU	22	NA10837	M	O	O	O,P	-	NA12272	M	P	P	O,P	-
CEU	22	NA10837	M	O	O	O,P	-	NA12273	F	P	P	O,P	-
CEU	26	NA12766	M	O	O	O,P	-	NA12775	M	P	P	O,P	-
CEU	26	NA12766	M	O	O	O,P	-	NA12776	F	P	P	O,P	-
CEU	27	NA12344	M	O	O	O,P	-	NA12347	M	P	P	O,P	-
CEU	27	NA12344	M	O	O	O,P	-	NA12348	F	P	P	O,P	-
CEU	28	NA12817	M	O	O	O,P	-	NA12827	M	P	P	O,P	-
CEU	28	NA12817	M	O	O	O,P	-	NA12828	F	P	P	O,P	-
CEU	29	NA10840	F	O	O	O,P	-	NA12286	M	P	P	O,P	-
CEU	29	NA10840	F	O	O	O,P	-	NA12287	F	P	P	O,P	-
CEU	32	NA12708	F	O	O,P	O,P	-	NA12718	F	P	O,P	O,P	-
CEU	37	NA06995	M	O	O	O,P	-	NA07037	F	P	P	O,P	-
CEU	37	NA06995	M	O	O	O,P	-	NA07435	M	P	P	O,P	-
CEU	44	NA12375	M	O	O,P	O,P	-	NA12383	F	P	O,P	O,P	-
CEU	46	NA12335	M	O	O	O,P	-	NA12340	M	P	P	O,P	-
CEU	46	NA12335	M	O	O	O,P	-	NA12341	F	P	P	O,P	-
CEU	47	NA12767	F	O	O	O,P	-	NA12777	M	P	P	O,P	-
CEU	47	NA12767	F	O	O	O,P	-	NA12778	F	P	P	O,P	-
CEU	50	NA12877	M	O	O	O,P	-	NA12889	M	P	P	O,P	-
CEU	50	NA12877	M	O	O	O,P	-	NA12890	F	P	P	O,P	-
CEU	52	NA07346	F	P	P	O,P	-	NA07349	M	O	O	O,P	-
CEU	52	NA07347	M	P	P	O,P	-	NA07349	M	O	O	O,P	-
CEU	54	NA12739	M	O	O	O,P	-	NA12748	M	P	P	O,P	-
CEU	54	NA12739	M	O	O	O,P	-	NA12749	F	P	P	O,P	-
CEU	55	NA10864	F	O	O	O,P	-	NA11893	M	P	P	O,P	-
CEU	55	NA10864	F	O	O	O,P	-	NA11894	F	P	P	O,P	-
CEU	59	NA10853	M	O	O,P	O,P	-	NA11843	M	P	O,P	O,P	-
CEU	61	NA12818	F	O	O	O,P	-	NA12829	M	P	P	O,P	-
CEU	61	NA12818	F	O	O	O,P	-	NA12830	F	P	P	O,P	-

CEU	65	NA10843	F	O	O	O,P	-	NA11919	M	P	P	O,P	-	
CEU	65	NA10843	F	O	O	O,P	-	NA11920	F	P	P	O,P	-	
CEU	66	NA12376	F	O	O	O,P	-	NA12489	F	P	P	O,P	-	
CEU	66	NA12376	F	O	O	O,P	-	NA12546	M	P	P	O,P	-	
CEU	70	NA12832	F	O	O	O,P	-	NA12842	M	P	P	O,P	-	
CEU	70	NA12832	F	O	O	O,P	-	NA12843	F	P	P	O,P	-	
CEU	74	NA07014	F	O	O	O,P	-	NA07031	F	P	P	O,P	-	
CEU	74	NA07014	F	O	O	O,P	-	NA07051	M	P	P	O,P	-	
CEU	77	NA12386	F	O	O	O,P	-	NA12399	M	P	P	O,P	-	
CEU	77	NA12386	F	O	O	O,P	-	NA12400	F	P	P	O,P	-	
CEU	80	NA12336	F	O	O	O,P	-	NA12342	M	P	P	O,P	-	
CEU	80	NA12336	F	O	O	O,P	-	NA12343	F	P	P	O,P	-	
CEU	82	NA10845	M	O	O	O,P	-	NA11930	M	P	P	O,P	-	
CEU	82	NA10845	M	O	O	O,P	-	NA11931	F	P	P	O,P	-	
CEU	86	NA10847	F	O	O	O,P	-	NA12146	M	P	P	O,P	-	
CEU	86	NA10847	F	O	O	O,P	-	NA12239	F	P	P	O,P	-	
CEU	87	NA10859	F	O	O	O,P	-	NA11881	M	P	P	O,P	-	
CEU	87	NA10859	F	O	O	O,P	-	NA11882	F	P	P	O,P	-	
CEU	89	NA12707	M	O	O,P	O,P	-	NA12716	M	P	O,P	O,P	-	
CEU	90	NA10830	M	O	O,P	O,P	-	NA12154	M	P	O,P	O,P	-	
CEU	91	NA12753	F	O	O	O,P	-	NA12762	M	P	P	O,P	-	
CEU	91	NA12753	F	O	O	O,P	-	NA12763	F	P	P	O,P	-	
CEU	94	NA12865	F	O	O	O,P	-	NA12874	M	P	P	O,P	-	
CEU	94	NA12865	F	O	O	O,P	-	NA12875	F	P	P	O,P	-	
CEU	96	NA10831	F	O	O	O,P	-	NA12155	M	P	P	O,P	-	
CEU	96	NA10831	F	O	O	O,P	-	NA12156	F	P	P	O,P	-	
CEU	106	NA12752	M	O	O	O,P	-	NA12760	M	P	P	O,P	-	
CEU	106	NA12752	M	O	O	O,P	-	NA12761	F	P	P	O,P	-	
CEU	107	NA06985	F	P	P	O,P	-	NA06991	F	O	O	O,P	-	
CEU	107	NA06991	F	O	O	O,P	-	NA06993	M	P	P	O,P	-	
CEU	108	NA10838	M	O	O,P	O,P	-	NA12003	M	P	O,P	O,P	-	
CEU	111	NA06986	M	P	P	O,P	-	NA06997	F	O	O	O,P	-	
CEU	111	NA06997	F	O	O	O,P	-	NA07045	F	P	P	O,P	-	
CEU	111	NA06997	F	-	1C	-	1C	NA12801	M	-	1C	-	1C	
CEU	111	NA06997	F	-	N	N	-	NA12813	F	-	A	A	-	R
CEU	111	NA07045	F	-	A	A	-	NA12801	M	-	N	N	-	R
CEU	111	NA07045	F	-	F	F	-	NA12813	F	-	F	F	-	
CEU	111	NA12801	M	O	O	O,P	-	NA12812	M	P	P	O,P	-	
CEU	111	NA12801	M	O	O	O,P	-	NA12813	F	P	P	O,P	-	
CEU	115	NA10863	F	O	O	O,P	-	NA12234	F	P	P	O,P	-	
CEU	115	NA10863	F	O	O	O,P	-	NA12264	M	P	P	O,P	-	
CEU	117	NA12802	F	O	O	O,P	-	NA12814	M	P	P	O,P	-	
CEU	117	NA12802	F	O	O	O,P	-	NA12815	F	P	P	O,P	-	

CEU	122	NA10846	M	O	O	O,P	-	NA12144	M	P	P	O,P	-	
CEU	122	NA10846	M	O	O	O,P	-	NA12145	F	P	P	O,P	-	
CEU	127	NA10854	F	O	O	O,P	-	NA11839	M	P	P	O,P	-	
CEU	127	NA10854	F	O	O	O,P	-	NA11840	F	P	P	O,P	-	
CEU	131	NA10855	F	O	O	O,P	-	NA11831	M	P	P	O,P	-	
CEU	131	NA10855	F	O	O	O,P	-	NA11832	F	P	P	O,P	-	
CEU	132	NA06994	M	P	P	O,P	-	NA07029	M	O	O	O,P	-	
CEU	132	NA07000	F	P	P	O,P	-	NA07029	M	O	O	O,P	-	
CEU	137	NA12740	F	O	O	O,P	-	NA12750	M	P	P	O,P	-	
CEU	137	NA12740	F	O	O	O,P	-	NA12751	F	P	P	O,P	-	
CEU	139	NA10839	F	O	O	O,P	-	NA12005	M	P	P	O,P	-	
CEU	139	NA10839	F	O	O	O,P	-	NA12006	F	P	P	O,P	-	
CEU	141	NA07345	F	P	P	O,P	-	NA07348	F	O	O	O,P	-	
CEU	141	NA07348	F	O	O	O,P	-	NA07357	M	P	P	O,P	-	
CEU	143	NA12878	F	O	O	O,P	-	NA12891	M	P	P	O,P	-	
CEU	143	NA12878	F	O	O	O,P	-	NA12892	F	P	P	O,P	-	
CEU	147	NA12864	M	O	O	O,P	-	NA12872	M	P	P	O,P	-	
CEU	147	NA12864	M	O	O	O,P	-	NA12873	F	P	P	O,P	-	
CEU	150	NA10856	M	O	O	O,P	-	NA11829	M	P	P	O,P	-	
CEU	150	NA10856	M	O	O	O,P	-	NA11830	F	P	P	O,P	-	
CEU	152	NA10835	M	O	O	O,P	-	NA12248	M	P	P	O,P	-	
CEU	152	NA10835	M	O	O	O,P	-	NA12249	F	P	P	O,P	-	
CEU	156	NA10861	F	O	O	O,P	-	NA11994	M	P	P	O,P	-	
CEU	156	NA10861	F	O	O	O,P	-	NA11995	F	P	P	O,P	-	
CHD	2	NA17981	F	-	F	F	-	NA17986	M	-	F	F	-	R
CHD	16	NA17980	M	-	A,C,G,H,N	U	-	NA18150	F	-	A,C,G,H,N	U	-	R
GIH	54	NA20909	M	-	O,P	U	-	NA20910	F	-	O,P	U	-	R
GIH	61	NA20882	F	-	P	P	-	NA20900	F	-	O	O	-	R
GIH	61	NA20891	M	-	P	P	-	NA20900	F	-	O	O	-	R
GIH	61	NA20891	M	-	A,C,G,H,N	A	-	NA20907	F	-	A,C,G,H,N	N	-	R,?
GIH	61	NA20900	F	-	1C,GC,GN ,HA,HN	-	-	NA20907	F	-	1C,GA,GG ,HA,HN	-	-	N
GIH	71	NA20874	F	-	F	F	-	NA20879	F	-	F	F	-	R
LWK	3	NA19027	M	-	A,C,G,H,N	U	-	NA19311	M	-	A,C,G,H,N	U	-	R
LWK	13	NA19396	F	-	F	F	-	NA19397	M	-	F	F	-	R
LWK	22	NA19380	M	-	1C,C,GA, GC,GG,H A,HN,N	-	-	NA19381	F	-	1C,A,G,G C,GG,GN, HA,HN	-	-	N
LWK	22	NA19380	M	-	A,C,G,H,N	H	-	NA19382	M	-	A,C,G,H,N	H	-	R,?
LWK	22	NA19381	F	-	O,P	U	-	NA19382	M	-	O,P	U	-	R
LWK	38	NA19347	M	-	F	F	-	NA19352	M	-	F	F	-	R
LWK	45	NA19313	F	-	A,C,G,H,N	U	-	NA19334	M	-	A,C,G,H,N	U	-	R
LWK	60	NA19443	M	-	A	A	-	NA19469	F	-	N	N	-	R
LWK	60	NA19443	M	-	F	F	-	NA19470	F	-	F	F	-	R

LWK	60	NA19469	F	-	O	O	-	NA19470	F	-	P	P	-	R
LWK	69	NA19434	F	-	F	F	-	NA19444	M	-	F	F	-	R
					1C,GA,GC ,GG,GN,H						1C,GA,GC ,GG,GN,H			
LWK	71	NA19451	M	-	A,HN,UN	-	-	NA19452	M	-	A,HN,UN	-	-	N
LWK	80	NA19373	M	-	F	F	-	NA19374	M	-	F	F	-	R
					1C,GA,GC ,GG,GN,H						1C,GA,GC ,GG,GN,H			
LWK	82	NA19309	M	-	A,HN,UN	-	-	NA19359	M	-	A,HN,UN	-	-	N
MEX	0	NA19660	F	P	P	O,P	-	NA19662	F	O	O	O,P	-	
MEX	0	NA19660	F	-	A,C,G,H,N	A	-	NA19664	M	-	A,C,G,H,N	N	-	R,?
					1C,GA,GG						1C,GC,GN			
MEX	0	NA19660	F	-	,HA,HN	-	GA	NA19665	F	-	,HA,HN	-	GN	?
MEX	0	NA19660	F	-	P	P	-	NA19685	M	-	O	O	-	R
MEX	0	NA19660	F	-	G	G	-	NA19686	F	-	C	C	-	R
MEX	0	NA19661	M	P	P	O,P	-	NA19662	F	O	O	O,P	-	
MEX	0	NA19661	M	-	P	P	-	NA19685	M	-	O	O	-	R
MEX	0	NA19661	M	-	G	G	-	NA19686	F	-	C	C	-	R
					1C,GC,GN						1C,GA,GG			
MEX	0	NA19662	F	-	,HA,HN	-	1C	NA19664	M	-	,HA,HN	-	1C	?
MEX	0	NA19662	F	-	F	F	-	NA19685	M	-	F	F	-	R
MEX	0	NA19662	F	-	A	A	-	NA19686	F	-	N	N	-	R
MEX	0	NA19663	F	P	P	O,P	-	NA19665	F	O	O	O,P	-	
MEX	0	NA19664	M	P	P	O,P	-	NA19665	F	O	O	O,P	-	
					1C,GA,GG						1C,GC,GN			
MEX	0	NA19664	M	-	,HA,HN	-	1C	NA19685	M	-	,HA,HN	-	1C	?
MEX	0	NA19684	F	P	P	O,P	-	NA19686	F	O	O	O,P	-	
MEX	0	NA19685	M	P	P	O,P	-	NA19686	F	O	O	O,P	-	
MEX	4	NA19722	F	P	P	O,P	-	NA19724	M	O	O	O,P	-	
MEX	4	NA19723	M	P	P	O,P	-	NA19724	M	O	O	O,P	-	
MEX	5	NA19649	M	P	O,P	O,P	-	NA19650	M	O	O,P	O,P	-	
MEX	6	NA19669	F	P	P	O,P	-	NA19671	F	O	O	O,P	-	
MEX	6	NA19670	M	P	P	O,P	-	NA19671	F	O	O	O,P	-	
MEX	9	NA19657	F	P	P	O,P	-	NA19659	F	O	O	O,P	-	
MEX	9	NA19658	M	P	P	O,P	-	NA19659	F	O	O	O,P	-	
MEX	11	NA19719	F	P	P	O,P	-	NA19721	F	O	O	O,P	-	
MEX	11	NA19720	M	P	P	O,P	-	NA19721	F	O	O	O,P	-	
MEX	16	NA19759	M	P	O,P	O,P	-	NA19760	F	O	O,P	O,P	-	
MEX	20	NA19675	F	P	P	O,P	-	NA19677	F	O	O	O,P	-	
MEX	20	NA19675	F	-	O	O	-	NA19678	F	-	P	P	-	R
MEX	20	NA19675	F	-	O	O	-	NA19679	M	-	P	P	-	R
MEX	20	NA19675	F	-	F	F	-	NA19680	F	-	F	F	-	R
MEX	20	NA19676	M	P	P	O,P	-	NA19677	F	O	O	O,P	-	
MEX	20	NA19677	F	-	C	C	-	NA19678	F	-	G	G	-	R*
MEX	20	NA19677	F	-	C	C	-	NA19679	M	-	G	G	-	R*
MEX	20	NA19677	F	-	N	N	-	NA19680	F	-	A	A	-	R

MEX	20	NA19678	F	P	P	O,P	-	NA19680	F	O	O	O,P	-	
MEX	20	NA19679	M	P	P	O,P	-	NA19680	F	O	O	O,P	-	
MEX	23	NA19651	F	P	P	O,P	-	NA19653	F	O	O	O,P	-	
MEX	23	NA19652	M	P	P	O,P	-	NA19653	F	O	O	O,P	-	
MEX	26	NA19725	F	P	P	O,P	-	NA19727	M	O	O	O,P	-	
MEX	26	NA19726	M	P	P	O,P	-	NA19727	M	O	O	O,P	-	
MEX	28	NA19755	F	P	P	O,P	-	NA19757	M	O	O	O,P	-	
MEX	28	NA19756	M	P	P	O,P	-	NA19757	M	O	O	O,P	-	
MEX	32	NA19773	F	P	P	O,P	-	NA19775	F	O	O	O,P	-	
MEX	32	NA19774	M	P	P	O,P	-	NA19775	F	O	O	O,P	-	
MEX	35	NA19776	F	P	P	O,P	-	NA19778	M	O	O	O,P	-	
MEX	35	NA19777	M	P	P	O,P	-	NA19778	M	O	O	O,P	-	
MEX	38	NA19782	F	P	P	O,P	-	NA19784	M	O	O	O,P	-	
MEX	38	NA19783	M	P	P	O,P	-	NA19784	M	O	O	O,P	-	
MEX	45	NA19779	F	P	P	O,P	-	NA19781	F	O	O	O,P	-	
MEX	45	NA19780	M	P	P	O,P	-	NA19781	F	O	O	O,P	-	
MEX	54	NA19681	F	P	P	O,P	-	NA19683	F	O	O	O,P	-	
MEX	54	NA19682	M	P	P	O,P	-	NA19683	F	O	O	O,P	-	
MEX	55	NA19746	F	P	P	O,P	-	NA19748	F	O	O	O,P	-	
MEX	55	NA19747	M	P	P	O,P	-	NA19748	F	O	O	O,P	-	
MEX	59	NA19716	F	P	O,P	O,P	-	NA19718	F	O	O,P	O,P	-	
MEX	61	NA19794	F	P	P	O,P	-	NA19796	M	O	O	O,P	-	
MEX	61	NA19795	M	P	P	O,P	-	NA19796	M	O	O	O,P	-	
MEX	63	NA19654	F	P	O,P	O,P	-	NA19656	F	O	O,P	O,P	-	
MEX	64	NA19749	F	P	P	O,P	-	NA19751	M	O	O	O,P	-	
MEX	64	NA19750	M	P	P	O,P	-	NA19751	M	O	O	O,P	-	
MEX	67	NA19761	F	P	P	O,P	-	NA19763	F	O	O	O,P	-	
MEX	67	NA19762	M	P	P	O,P	-	NA19763	F	O	O	O,P	-	
MEX	69	NA19770	F	P	P	O,P	-	NA19772	M	O	O	O,P	-	
MEX	69	NA19771	M	P	P	O,P	-	NA19772	M	O	O	O,P	-	
MEX	73	NA19788	F	P	P	O,P	-	NA19790	F	O	O	O,P	-	
MEX	73	NA19789	M	P	P	O,P	-	NA19790	F	O	O	O,P	-	
MKK	8	NA21399	M	P	P	O,P	-	NA21401	M	O	O	O,P	-	
MKK	8	NA21399	M	-	F	F	-	NA21402	M	-	F	F	-	R
MKK	8	NA21399	M	-	A	A	-	NA21404	F	-	N	N	-	R*
MKK	8	NA21399	M	-	F	F	-	NA21405	M	-	F	F	-	R
MKK	8	NA21400	F	P	P	O,P	-	NA21401	M	O	O	O,P	-	
MKK	8	NA21401	M	-	N	N	-	NA21402	M	-	A	A	-	R*
MKK	8	NA21401	M	-	1C	-	-	NA21404	F	-	1C	-	-	N
MKK	8	NA21401	M	-	N	N	-	NA21405	M	-	A	A	-	R
MKK	8	NA21402	M	P	P	O,P	-	NA21404	F	O	O	O,P	-	
MKK	8	NA21402	M	-	F	F	-	NA21405	M	-	F	F	-	R
MKK	8	NA21403	F	P	P	O,P	-	NA21404	F	O	O	O,P	-	

MKK	8	NA21404	F	-	N	N	-	NA21405	M	-	A	A	-	R*
MKK	16	NA21716	M	P	P	O,P	-	NA21718	M	O	O	O,P	-	
MKK	16	NA21716	M	-	A,C,G,H,N	A	-	NA21741	M	-	A,C,G,H,N	N	-	R,?
MKK	16	NA21717	F	P	P	O,P	-	NA21718	M	O	O	O,P	-	
MKK	16	NA21718	M	-	1C,GC,GN ,HA,HN	-	-	NA21741	M	-	1C,GA,GG ,HA,HN	-	-	N
MKK	25	NA21723	F	-	A,C,G,H,N	H	-	NA21733	F	-	A,C,G,H,N	H	-	R,?
MKK	26	NA21307	M	P	P	O,P	-	NA21309	F	O	O	O,P	-	
MKK	26	NA21307	M	-	A,C,G,H,N	A	-	NA21616	M	-	A,C,G,H,N	N	-	R,?
MKK	26	NA21308	F	P	P	O,P	-	NA21309	F	O	O	O,P	-	
MKK	26	NA21308	F	-	A,C,G,H,N	A	-	NA21379	F	-	A,C,G,H,N	N	-	R,?
MKK	26	NA21308	F	-	A,C,G,H,N	H	-	NA21517	F	-	A,C,G,H,N	H	-	R,?
MKK	26	NA21309	F	-	1C,GC,GN ,HA,HN	-	-	NA21379	F	-	1C,GA,GG ,HA,HN	-	-	N
MKK	26	NA21309	F	-	1C,GC,GN ,HA,HN	-	-	NA21517	F	-	1C,GA,GG ,HA,HN	-	-	N
MKK	26	NA21309	F	-	1C,GC,GN ,HA,HN	-	-	NA21616	M	-	1C,GA,GG ,HA,HN	-	-	N
MKK	26	NA21379	F	-	1C,UN	-	-	NA21517	F	-	1C,UN	-	-	N
MKK	31	NA21357	F	-	F	F	-	NA21509	M	-	F	F	-	R
MKK	40	NA21381	M	P	P	O,P	-	NA21383	M	O	O	O,P	-	
MKK	40	NA21382	F	P	P	O,P	-	NA21383	M	O	O	O,P	-	
MKK	40	NA21382	F	-	1C,GA,GG ,HA,HN	-	-	NA21384	M	-	1C,GC,GN ,HA,HN	-	-	N
MKK	40	NA21382	F	-	A,C,G,H,N	N	-	NA21387	M	-	A,C,G,H,N	A	-	R,?
MKK	40	NA21382	F	-	1C,GA,GG ,HA,HN	-	-	NA21389	M	-	1C,GC,GN ,HA,HN	-	-	N
MKK	40	NA21383	M	-	1C,GC,GN ,HA,HN	-	-	NA21387	M	-	1C,GA,GG ,HA,HN	-	-	N
MKK	40	NA21384	M	P	P	O,P	-	NA21386	F	O	O	O,P	-	
MKK	40	NA21384	M	-	O	O	-	NA21387	M	-	P	P	-	R
MKK	40	NA21384	M	-	O	O	-	NA21388	F	-	P	P	-	R
MKK	40	NA21384	M	-	F	F	-	NA21389	M	-	F	F	-	R
MKK	40	NA21385	F	P	P	O,P	-	NA21386	F	O	O	O,P	-	
MKK	40	NA21386	F	-	C	C	-	NA21387	M	-	G	G	-	R
MKK	40	NA21386	F	-	C	C	-	NA21388	F	-	G	G	-	R
MKK	40	NA21386	F	-	N	N	-	NA21389	M	-	A	A	-	R*
MKK	40	NA21387	M	P	P	O,P	-	NA21389	M	O	O	O,P	-	
MKK	40	NA21388	F	P	P	O,P	-	NA21389	M	O	O	O,P	-	
MKK	43	NA21521	M	-	A,C,G,H,N	U	-	NA21599	M	-	A,C,G,H,N	U	-	R
MKK	43	NA21521	M	-	1C,GA,GG ,HA,HN	-	-	NA21601	F	-	1C,GC,GN ,HA,HN	-	-	N
MKK	43	NA21599	M	P	P	O,P	-	NA21601	F	O	O	O,P	-	
MKK	43	NA21600	F	P	P	O,P	-	NA21601	F	O	O	O,P	-	
MKK	57	NA21620	F	-	A,C,G,H,N	U	-	NA21719	M	-	A,C,G,H,N	U	-	R
MKK	68	NA21574	F	-	O,P	U	-	NA21575	M	-	O,P	U	-	R
MKK	101	NA21457	F	-	F	F	-	NA21683	F	-	F	F	-	R

MKK	105	NA21363	F	-	O,P	U	-	NA21415	F	-	O,P	U	-	R
MKK	114	NA21440	M	P	P	O,P	-	NA21442	M	O	O	O,P	-	
MKK	114	NA21441	F	P	P	O,P	-	NA21442	M	O	O	O,P	-	
MKK	115	NA21359	M	P	P	O,P	-	NA21361	F	O	O	O,P	-	
MKK	115	NA21360	F	P	P	O,P	-	NA21361	F	O	O	O,P	-	
MKK	119	NA21391	F	-	A,C,G,H,N	H	-	NA21421	F	-	A,C,G,H,N	H	-	R,?
MKK	119	NA21391	F	-	1C,UN	-	-	NA21478	M	-	1C,UN	-	-	N
MKK	119	NA21391	F	-	1C,GA,GC ,GG,GN,H A,HN	-	-	NA21485	M	-	1C,GA,GC ,GG,GN,H A,HN	-	-	N
MKK	119	NA21391	F	-	1C,GA,GC ,GG,GN,H A,HN,UN	-	-	NA21488	M	-	1C,GA,GC ,GG,GN,H A,HN,UN	-	-	N
MKK	119	NA21421	F	-	1C,UN	-	-	NA21478	M	-	1C,UN	-	-	N
MKK	119	NA21421	F	-	A,C,G,H,N	U	-	NA21485	M	-	A,C,G,H,N	U	-	R
MKK	119	NA21421	F	-	1C,GA,GG ,HA,HN	-	-	NA21487	F	-	1C,GC,GN ,HA,HN	-	-	N
MKK	119	NA21421	F	-	1C,UN	-	-	NA21488	M	-	1C,UN	-	-	N
MKK	119	NA21475	M	P	P	O,P	-	NA21477	M	O	O	O,P	-	
MKK	119	NA21475	M	-	1C,GC,GN ,HA,HN	-	-	NA21478	M	-	1C,GA,GG ,HA,HN	-	-	N
MKK	119	NA21475	M	-	1C,GC,GN ,HA,HN	-	-	NA21485	M	-	1C,GA,GG ,HA,HN	-	-	N
MKK	119	NA21475	M	-	O	O	-	NA21488	M	-	P	P	-	R
MKK	119	NA21475	M	-	O	O	-	NA21489	F	-	P	P	-	R
MKK	119	NA21475	M	-	F	F	-	NA21490	M	-	F	F	-	R
MKK	119	NA21476	F	P	P	O,P	-	NA21477	M	O	O	O,P	-	
MKK	119	NA21477	M	-	C	C	-	NA21488	M	-	G	G	-	R
MKK	119	NA21477	M	-	C	C	-	NA21489	F	-	G	G	-	R
MKK	119	NA21477	M	-	N	N	-	NA21490	M	-	A	A	-	R
MKK	119	NA21478	M	P	P	O,P	-	NA21480	F	O	O	O,P	-	
MKK	119	NA21478	M	-	1C,GA,GC ,GG,GN,H A,HN,UN	-	-	NA21485	M	-	1C,GA,GC ,GG,GN,H A,HN,UN	-	-	N
MKK	119	NA21478	M	-	GC,UN	-	-	NA21487	F	-	GG,UN	-	-	N
MKK	119	NA21478	M	-	A,C,G,H,N	U	-	NA21488	M	-	A,C,G,H,N	U	-	R
MKK	119	NA21478	M	-	1C,GA,GG ,HA,HN	-	-	NA21490	M	-	1C,GC,GN ,HA,HN	-	-	N
MKK	119	NA21479	F	P	P	O,P	-	NA21480	F	O	O	O,P	-	
MKK	119	NA21479	F	-	A,C,G,H,N	H	-	NA21685	M	-	A,C,G,H,N	H	-	R,?
MKK	119	NA21480	F	-	1C,GC,GN ,HA,HN	-	-	NA21488	M	-	1C,GA,GG ,HA,HN	-	-	N
MKK	119	NA21480	F	-	1C,GC,GN ,HA,HN	-	-	NA21685	M	-	1C,GA,GG ,HA,HN	-	-	N
MKK	119	NA21485	M	P	P	O,P	-	NA21487	F	O	O	O,P	-	
MKK	119	NA21485	M	-	A,C,G,H,N	U	-	NA21488	M	-	A,C,G,H,N	U	-	R
MKK	119	NA21485	M	-	1C,GA,GG ,HA,HN	-	-	NA21490	M	-	1C,GC,GN ,HA,HN	-	-	N
MKK	119	NA21486	F	P	P	O,P	-	NA21487	F	O	O	O,P	-	

					1C,GC,GN						1C,GA,GG				
MKK	119	NA21487	F	-	,HA,HN	-	-	NA21488	M	-	,HA,HN	-	-	N	
MKK	119	NA21488	M	P	P	O,P	-	NA21490	M	O	O	O,P	-		
MKK	119	NA21489	F	P	P	O,P	-	NA21490	M	O	O	O,P	-		
MKK	125	NA21352	M	-	A,C,G,H,N	U	-	NA21414	M	-	A,C,G,H,N	U	-	R	
					1C,GA,GG						1C,GC,GN				
MKK	125	NA21352	M	-	,HA,HN	-	-	NA21527	M	-	,HA,HN	-	-	N	
MKK	125	NA21352	M	-	A,C,G,H,N	U	-	NA21583	M	-	A,C,G,H,N	U	-	R	
MKK	125	NA21414	M	-	1C,UN	-	-	NA21583	M	-	1C,UN	-	-	N	
MKK	125	NA21526	F	P	P	O,P	-	NA21527	M	O	O	O,P	-		
MKK	125	NA21527	M	O	O	O,P	-	NA21583	M	P	P	O,P	-		
					1C,GA,GG						1C,GC,GN				
					,HA,HN,U						,HA,HN,U				
MKK	131	NA21300	F	-	N	-	-	NA21312	M	-	N	-	-	N	
					1C,GA,GG						1C,GC,GN				
					,HA,HN,U						,HA,HN,U				
MKK	131	NA21300	F	-	N	-	-	NA21370	M	-	N	-	-	N	
MKK	131	NA21300	F	-	1C,UN	-	-	NA21435	M	-	1C,UN	-	-	N	
MKK	131	NA21300	F	-	A,G,H,N	G	-	NA21520	M	-	A,C,H,N	C	-	R*,?	
MKK	131	NA21300	F	-	A,G,H,N	G	-	NA21613	F	-	A,C,H,N	C	-	R,?	
MKK	131	NA21300	F	-	A,C,G,H,N	H	-	NA21617	F	-	A,C,G,H,N	H	-	R,?	
MKK	131	NA21300	F	-	1C,UN	-	-	NA21647	M	-	1C,UN	-	-	N	
MKK	131	NA21300	F	-	1C,UN	-	-	NA21686	F	-	1C,UN	-	-	N	
MKK	131	NA21300	F	-	1C,UN	-	-	NA21825	F	-	1C,UN	-	-	N	
MKK	131	NA21301	M	P	P	O,P	-	NA21302	F	O	O	O,P	-		
MKK	131	NA21301	M	-	F	F	-	NA21344	M	-	F	F	-	R	
MKK	131	NA21301	M	-	A	A	-	NA21366	M	-	N	N	-	R*	
MKK	131	NA21302	F	O	O	O,P	-	NA21303	F	P	P	O,P	-		
MKK	131	NA21302	F	-	N	N	-	NA21344	M	-	A	A	-	R*	
MKK	131	NA21302	F	-	1C	-	-	NA21366	M	-	1C	-	-	N	
MKK	131	NA21311	M	-	G,H	H	-	NA21312	M	-	C,H	H	-	R*,?	
MKK	131	NA21311	M	-	GG,HA	-	-	NA21313	M	-	GC,HN	-	-	N	
MKK	131	NA21311	M	-	O,P	O	-	NA21314	M	-	O,P	P	-	R,?	
MKK	131	NA21311	M	-	G,H	C	-	NA21320	F	-	C,H	G	-	R,?	
					1C,GC,GG						1C,GA,GC				
					,GN,HA,H						,GG,HA,H				
MKK	131	NA21311	M	-	N,UN	-	-	NA21367	M	-	N,UN	-	-	N	
MKK	131	NA21311	M	-	1C,UN	-	-	NA21424	F	-	1C,UN	-	-	N	
MKK	131	NA21311	M	-	1C,UN	-	-	NA21596	M	-	1C,UN	-	-	N	
MKK	131	NA21312	M	P	P	O,P	-	NA21313	M	O	O	O,P	-		
MKK	131	NA21312	M	-	O	O	-	NA21314	M	-	P	P	-	R	
MKK	131	NA21312	M	-	G,H	C	-	NA21320	F	-	C,H	G	-	R*,?	
					1C,GC,GN						1C,GA,GG				
MKK	131	NA21312	M	-	,HA,HN	-	-	NA21367	M	-	,HA,HN	-	-	N	
MKK	131	NA21312	M	-	H	U	-	NA21370	M	-	H	U	-	R,P	
					1C,GA,GC						1C,GA,GC				
					,GG,GN,H						,GG,GN,H				
MKK	131	NA21312	M	-	A,HN,UN	-	-	NA21423	M	-	A,HN,UN	-	-	N	

MKK	131	NA21312	M	-	1C,UN	-	-	NA21424	F	-	1C,UN	-	-	N
MKK	131	NA21312	M	-	1C,UN	-	-	NA21447	M	-	1C,UN	-	-	N
					1C,GC,GN						1C,GA,GG			
					,HA,HN,U						,HA,HN,U			
MKK	131	NA21312	M	-	N	-	-	NA21520	M	-	N	-	-	N
MKK	131	NA21312	M	-	1C,UN	-	-	NA21596	M	-	1C,UN	-	-	N
					1C,GC,GN						1C,GA,GG			
					,HA,HN,U						,HA,HN,U			
MKK	131	NA21312	M	-	N	-	-	NA21613	F	-	N	-	-	N
MKK	131	NA21312	M	-	C,H,N	U	-	NA21617	F	-	A,G,H	U	-	R,P
MKK	131	NA21313	M	-	C	C	-	NA21314	M	-	G	G	-	R
MKK	131	NA21313	M	-	HA,HN	-	-	NA21320	F	-	HA,HN	-	-	N
MKK	131	NA21313	M	O	O	O,P	-	NA21362	F	P	P	O,P	-	
MKK	131	NA21313	M	-	HN	-	-	NA21370	M	-	HA	-	-	N
					1C,GC,GN						1C,GA,GG			
MKK	131	NA21313	M	-	,HA,HN	-	-	NA21438	F	-	,HA,HN	-	-	N
					GC,GN,H						GA,GG,H			
MKK	131	NA21313	M	-	N	-	-	NA21617	F	-	A	-	-	N
MKK	131	NA21314	M	-	P	O	-	NA21320	F	-	O	P	-	R
MKK	131	NA21314	M	-	A,C,G,H,N	H	-	NA21367	M	-	A,C,G,H,N	H	-	R,?
MKK	131	NA21314	M	-	1C,UN	-	-	NA21378	M	-	1C,UN	-	-	N
					1C,GA,GC						1C,GA,GC			
					,GG,GN,H						,GG,GN,H			
MKK	131	NA21314	M	-	A,HN,UN	-	-	NA21423	M	-	A,HN,UN	-	-	N
MKK	131	NA21314	M	-	1C,UN	-	-	NA21424	F	-	1C,UN	-	-	N
MKK	131	NA21314	M	-	GC,UN	-	-	NA21425	F	-	GG,UN	-	-	N
MKK	131	NA21314	M	-	1C,UN	-	-	NA21447	M	-	1C,UN	-	-	N
MKK	131	NA21314	M	-	1C,UN	-	-	NA21493	F	-	1C,UN	-	-	N
MKK	131	NA21314	M	-	1C,UN	-	-	NA21596	M	-	1C,UN	-	-	N
MKK	131	NA21316	M	P	P	O,P	-	NA21317	M	O	O	O,P	-	
MKK	131	NA21316	M	-	F	F	-	NA21318	M	-	F	F	-	R
MKK	131	NA21316	M	-	A,C,H,N	C	-	NA21519	M	-	A,G,H,N	G	-	R,?
MKK	131	NA21316	M	-	1C,UN	-	-	NA21619	M	-	1C,UN	-	-	N
					1C,GA,GC						1C,GA,GC			
					,GG,GN,H						,GG,GN,H			
MKK	131	NA21316	M	-	A,HN,UN	-	-	NA21635	F	-	A,HN,UN	-	-	N
					1C,GA,GC						1C,GA,GC			
					,GG,GN,H						,GG,GN,H			
MKK	131	NA21316	M	-	A,HN,UN	-	-	NA21678	M	-	A,HN,UN	-	-	N
MKK	131	NA21317	M	-	N	N	-	NA21318	M	-	A	A	-	R
					1C,GC,GN						1C,GA,GG			
MKK	131	NA21317	M	-	,HN	-	-	NA21519	M	-	,HA	-	-	N
MKK	131	NA21317	M	O	O	O,P	-	NA21580	F	P	P	O,P	-	
MKK	131	NA21318	M	-	A,C,H,N	C	-	NA21519	M	-	A,G,H,N	G	-	R,?
MKK	131	NA21318	M	-	1C,UN	-	-	NA21619	M	-	1C,UN	-	-	N
					1C,GA,GC						1C,GA,GC			
					,GG,GN,H						,GG,GN,H			
MKK	131	NA21318	M	-	A,HN,UN	-	-	NA21635	F	-	A,HN,UN	-	-	N

					1C,GA,GC ,GG,GN,H						1C,GA,GC ,GG,GN,H			
MKK	131	NA21318	M	-	A,HN,UN	-	-	NA21678	M	-	A,HN,UN	-	-	N
MKK	131	NA21320	F	-	H,N	H	-	NA21365	F	-	A,H	H	-	R,?
MKK	131	NA21320	F	-	1C,HA	-	-	NA21366	M	-	1C,HN	-	-	N
MKK	131	NA21320	F	-	1C,GC,GN ,HA,HN	-	-	NA21367	M	-	1C,GA,GG ,HA,HN	-	-	N
MKK	131	NA21320	F	-	1C,GA,GC ,GG,GN,H	-	-	NA21423	M	-	1C,GA,GC ,GG,GN,H	-	-	N
MKK	131	NA21320	F	-	1C,UN	-	-	NA21424	F	-	1C,UN	-	-	N
MKK	131	NA21320	F	-	1C,UN	-	-	NA21447	M	-	1C,UN	-	-	N
MKK	131	NA21320	F	-	H,N	H	-	NA21523	M	-	A,H	H	-	R*,?
MKK	131	NA21320	F	-	1C,HA	-	-	NA21525	M	-	1C,HN	-	-	N
MKK	131	NA21320	F	-	1C,UN	-	-	NA21596	M	-	1C,UN	-	-	N
MKK	131	NA21344	M	P	P	O,P	-	NA21366	M	O	O	O,P	-	
MKK	131	NA21362	F	-	A,C,G,H,N	H	-	NA21438	F	-	A,C,G,H,N	H	-	R*,?
MKK	131	NA21362	F	-	1C,GA,GG ,HA,HN	-	-	NA21439	M	-	1C,GC,GN ,HA,HN	-	-	N
MKK	131	NA21362	F	-	1C,GN,HA ,HN,UN	-	-	NA21528	M	-	1C,GA,HA ,HN,UN	-	-	N
MKK	131	NA21362	F	-	1C,GN,HA ,HN,UN	-	-	NA21587	M	-	1C,GA,HA ,HN,UN	-	-	N
MKK	131	NA21365	F	P	P	O,P	-	NA21366	M	O	O	O,P	-	
MKK	131	NA21365	F	-	F	F	-	NA21523	M	-	F	F	-	R
MKK	131	NA21365	F	-	A	A	-	NA21525	M	-	N	N	-	R*
MKK	131	NA21366	M	-	N	N	-	NA21523	M	-	A	A	-	R
MKK	131	NA21366	M	-	1C	-	-	NA21525	M	-	1C	-	-	N
MKK	131	NA21367	M	-	1C,GA,GN ,HA,HN,U N	-	-	NA21378	M	-	1C,GA,GN ,HA,HN,U N	-	-	N
MKK	131	NA21367	M	-	1C,UN	-	-	NA21423	M	-	1C,UN	-	-	N
MKK	131	NA21367	M	-	1C,UN	-	-	NA21424	F	-	1C,UN	-	-	N
MKK	131	NA21367	M	-	1C,UN	-	-	NA21447	M	-	1C,UN	-	-	N
MKK	131	NA21367	M	-	1C,GA,GN ,HA,HN,U N	-	-	NA21493	F	-	1C,GA,GN ,HA,HN,U N	-	-	N
MKK	131	NA21367	M	-	1C,GG,U 1C,GC,UN	-	-	NA21596	M	-	1C,GG,U N	-	-	N
MKK	131	NA21370	M	-	H	H	-	NA21494	F	-	H	H	-	R
MKK	131	NA21370	M	-	1C,GC,GN ,HA,HN,U N	-	-	NA21520	M	-	1C,GA,GG ,HA,HN,U N	-	-	N
MKK	131	NA21370	M	-	O	O	-	NA21522	M	-	P	P	-	R
MKK	131	NA21370	M	-	1C,GN,HA ,HN	-	-	NA21528	M	-	1C,GA,HA ,HN	-	-	N
MKK	131	NA21370	M	-	1C,GN,HA ,HN	-	-	NA21587	M	-	1C,GA,HA ,HN	-	-	N
MKK	131	NA21370	M	-	1C,GC,GN ,HA,HN,U N	-	-	NA21613	F	-	1C,GA,GG ,HA,HN,U N	-	-	N

MKK	131	NA21370	M	-	C,H,N	U	-	NA21617	F	-	A,G,H	U	-	R,P
MKK	131	NA21370	M	-	C,H	H	-	NA21682	M	-	G,H	H	-	R,?
MKK	131	NA21378	M	-	A,C,H,N	C	-	NA21448	M	-	A,G,H,N	G	-	R,?
MKK	131	NA21378	M	-	A,C,H,N	U	-	NA21453	M	-	A,G,H,N	U	-	R§,P
MKK	131	NA21378	M	-	1C,GA,HA ,HN	-	-	NA21455	F	-	1C,GN,HA ,HN	-	-	N
MKK	131	NA21378	M	-	F	F	-	NA21493	F	-	F	F	-	R
MKK	131	NA21378	M	-	A	A	-	NA21494	F	-	N	N	-	R*
MKK	131	NA21423	M	P	P	O,P	-	NA21425	F	O	O	O,P	-	
MKK	131	NA21423	M	-	1C,GA,GG ,HA,HN	-	-	NA21439	M	-	1C,GC,GN ,HA,HN	-	-	N
MKK	131	NA21423	M	-	A,C,G,H,N	A	-	NA21447	M	-	A,C,G,H,N	N	-	R,?
MKK	131	NA21424	F	P	P	O,P	-	NA21425	F	O	O	O,P	-	
MKK	131	NA21424	F	-	F	F	-	NA21596	M	-	F	F	-	R
MKK	131	NA21425	F	-	1C,GC,GN ,HA,HN	-	-	NA21447	M	-	1C,GA,GG ,HA,HN	-	-	N
MKK	131	NA21425	F	-	N	N	-	NA21596	M	-	A	A	-	R
MKK	131	NA21435	M	-	1C,UN	-	-	NA21520	M	-	1C,UN	-	-	N
MKK	131	NA21435	M	-	1C,UN 1C,GA,GC ,GG,GN,H	-	-	NA21613	F	-	1C,UN 1C,GA,GC ,GG,GN,H	-	-	N
MKK	131	NA21435	M	-	A,HN,UN	-	-	NA21617	F	-	A,HN,UN	-	-	N
MKK	131	NA21435	M	-	A,C,G,H,N 1C,GA,GG	U	-	NA21634	M	-	A,C,G,H,N 1C,GC,GN	U	-	R
MKK	131	NA21435	M	-	,HA,HN 1C,A,C,G, GA,GC,G G,GN,H,H	-	-	NA21636	F	-	,HA,HN 1C,A,C,G, GA,GC,G G,GN,H,H	-	-	N
MKK	131	NA21435	M	-	A,HN,N 1C,GA,GG ,HA,HN,U	U	-	NA21647	M	-	A,HN,N 1C,GC,GN ,HA,HN,U	U	-	R
MKK	131	NA21435	M	-	N	-	-	NA21648	M	-	N	-	-	N
MKK	131	NA21435	M	-	A,C,G,H,N	H	-	NA21825	F	-	A,C,G,H,N	H	-	R,?
MKK	131	NA21438	F	P	P	O,P	-	NA21439	M	O	O	O,P	-	
MKK	131	NA21439	M	O	O	O,P	-	NA21447	M	P	P	O,P	-	
MKK	131	NA21448	M	-	A,C,G,H,N 1C,GA,GG	H	-	NA21453	M	-	A,C,G,H,N 1C,GC,GN	H	-	R,?
MKK	131	NA21448	M	-	,HA,HN	-	-	NA21455	F	-	,HA,HN	-	-	N
MKK	131	NA21448	M	-	A,G,H,N 1C,GA,GG	G	-	NA21493	F	-	A,C,H,N 1C,GC,GN	C	-	R,?
MKK	131	NA21448	M	-	,HA	-	-	NA21494	F	-	,HN	-	-	N
MKK	131	NA21453	M	P	P	O,P	-	NA21455	F	O	O	O,P	-	
MKK	131	NA21453	M	-	A,G,H,N 1C,GA,GG	U	-	NA21493	F	-	A,C,H,N 1C,GC,GN	U	-	R§,P
MKK	131	NA21453	M	-	,HA	-	-	NA21494	F	-	,HN	-	-	N
MKK	131	NA21454	F	P	P 1C,GN,HA	O,P	-	NA21455	F	O	O 1C,GA,HA	O,P	-	
MKK	131	NA21455	F	-	,HN	-	-	NA21493	F	-	,HN	-	-	N
MKK	131	NA21493	F	P	P	O,P	-	NA21494	F	O	O	O,P	-	
MKK	131	NA21494	F	O	O	O,P	-	NA21522	M	P	P	O,P	-	

					1C,GN,HA						1C,GA,HA				
MKK	131	NA21494	F	-	,HN	-	-	NA21528	M	-	,HN	-	-	N	
					1C,GN,HA						1C,GA,HA				
MKK	131	NA21494	F	-	,HN	-	-	NA21587	M	-	,HN	-	-	N	
MKK	131	NA21494	F	-	C,H	H	-	NA21682	M	-	G,H	H	-	R,?	
MKK	131	NA21519	M	-	1C,UN	-	-	NA21619	M	-	1C,UN	-	-	N	
					1C,C,GA,						1C,G,GA,				
					GC,GG,G						GC,GG,G				
					N,HA,HN,						N,HA,HN,				
MKK	131	NA21519	M	-	UN	G	-	NA21635	F	-	UN	C	-	R,?	
					GC,HN,U						GG,HA,U				
MKK	131	NA21519	M	-	N	-	-	NA21636	F	-	N	-	-	N	
					1C,GA,GC						1C,GA,GC				
					,GG,GN,H						,GG,GN,H				
MKK	131	NA21519	M	-	A,HN,UN	G	-	NA21678	M	-	A,HN,UN	C	-	R*,?	
MKK	131	NA21520	M	-	F	F	-	NA21613	F	-	F	F	-	R	
MKK	131	NA21520	M	-	A,C,H,N	U	-	NA21617	F	-	A,G,H,N	U	-	R§,P	
					1C,GA,HA						1C,GN,HA				
MKK	131	NA21520	M	-	,HN	-	-	NA21648	M	-	,HN	-	-	N	
MKK	131	NA21520	M	-	A,C,H,N	N	-	NA21686	F	-	A,G,H,N	A	-	R,?	
MKK	131	NA21520	M	-	1C,UN	-	-	NA21825	F	-	1C,UN	-	-	N	
MKK	131	NA21522	M	-	A,G,H,N	H	-	NA21528	M	-	A,C,H,N	H	-	R,?	
MKK	131	NA21522	M	-	A,G,H,N	H	-	NA21587	M	-	A,C,H,N	H	-	R*,?	
MKK	131	NA21522	M	-	O,P	P	-	NA21682	M	-	O,P	O	-	R,?	
MKK	131	NA21523	M	P	P	O,P	-	NA21525	M	O	O	O,P	-		
MKK	131	NA21524	F	P	P	O,P	-	NA21525	M	O	O	O,P	-		
MKK	131	NA21528	M	-	F	F	-	NA21587	M	-	F	F	-	R	
					1C,GA,GC						1C,GG,G				
					,HA,HN,U						N,HA,HN,				
MKK	131	NA21528	M	-	N	-	-	NA21682	M	-	UN	-	-	N	
					1C,GA,GC						1C,GG,G				
					,HA,HN,U						N,HA,HN,				
MKK	131	NA21587	M	-	N	-	-	NA21682	M	-	UN	-	-	N	
MKK	131	NA21613	F	-	A,C,H,N	U	-	NA21617	F	-	A,G,H,N	U	-	R§,P	
					1C,GA,HA						1C,GN,HA				
MKK	131	NA21613	F	-	,HN	-	-	NA21648	M	-	,HN	-	-	N	
MKK	131	NA21613	F	-	A,C,H,N	N	-	NA21686	F	-	A,G,H,N	A	-	R,?	
MKK	131	NA21613	F	-	1C,UN	-	-	NA21825	F	-	1C,UN	-	-	N	
MKK	131	NA21617	F	-	1C,UN	-	-	NA21647	M	-	1C,UN	-	-	N	
MKK	131	NA21617	F	-	1C,UN	-	-	NA21686	F	-	1C,UN	-	-	N	
					1C,GA,GC						1C,GA,GC				
					,GG,GN,H						,GG,GN,H				
MKK	131	NA21617	F	-	A,HN,UN	-	-	NA21825	F	-	A,HN,UN	-	-	N	
MKK	131	NA21619	M	-	A,C,G,H,N	H	-	NA21635	F	-	A,C,G,H,N	H	-	R*,?	
					1C,GA,GG						1C,GC,GN				
MKK	131	NA21619	M	-	,HA,HN	-	-	NA21636	F	-	,HA,HN	-	-	N	
					A,G,GN,H						A,C,GA,H,				
MKK	131	NA21619	M	-	,N	H	-	NA21678	M	-	N	H	-	R,?	
MKK	131	NA21634	M	P	P	O,P	-	NA21636	F	O	O	O,P	-		
MKK	131	NA21634	M	-	A,C,G,H,N	U	-	NA21647	M	-	A,C,G,H,N	U	-	R	

					1C,GA,GG						1C,GC,GN			
MKK	131	NA21634	M	-	,HA,HN	-	-	NA21648	M	-	,HA,HN	-	-	N
MKK	131	NA21634	M	-	1C,UN	-	-	NA21825	F	-	1C,UN	-	-	N
MKK	131	NA21635	F	P	P	O,P	-	NA21636	F	O	O	O,P	-	
MKK	131	NA21635	F	-	F	F	-	NA21678	M	-	F	F	-	R
					1C,GC,GN						1C,GA,GG			
MKK	131	NA21636	F	-	,HA,HN	-	-	NA21647	M	-	,HA,HN	-	-	N
MKK	131	NA21636	F	-	N	N	-	NA21678	M	-	A	A	-	R
MKK	131	NA21647	M	P	P	O,P	-	NA21648	M	O	O	O,P	-	
MKK	131	NA21647	M	-	1C,UN	-	-	NA21825	F	-	1C,UN	-	-	N
MKK	131	NA21648	M	O	O	O,P	-	NA21686	F	P	P	O,P	-	
MKK	169	NA21573	M	-	F	F	-	NA21577	M	-	F	F	-	R
YRI	2	NA19184	M	P	P	O,P	-	NA19186	M	O	O	O,P	-	
YRI	2	NA19185	F	P	P	O,P	-	NA19186	M	O	O	O,P	-	
YRI	3	NA19146	M	P	P	O,P	-	NA19148	F	O	O	O,P	-	
YRI	3	NA19147	F	P	P	O,P	-	NA19148	F	O	O	O,P	-	
YRI	11	NA19178	M	P	P	O,P	-	NA19180	F	O	O	O,P	-	
					1C,GA,GC						1C,GA,GC			
					,GG,GN,H						,GG,GN,H			
YRI	11	NA19178	M	-	A,HN,UN	-	U	NA19200	M	-	A,HN,UN	-	U	
YRI	11	NA19178	M	-	GC,UN	-	U	NA19202	F	-	GG,UN	-	U	P
YRI	11	NA19179	F	P	P	O,P	-	NA19180	F	O	O	O,P	-	
YRI	11	NA19180	F	-	GG,UN	-	U	NA19200	M	-	GC,UN	-	U	P
YRI	11	NA19200	M	P	P	O,P	-	NA19202	F	O	O	O,P	-	
YRI	11	NA19201	F	P	P	O,P	-	NA19202	F	O	O	O,P	-	
YRI	12	NA18484	F	O	O	O,P	-	NA18486	M	P	P	O,P	-	
YRI	12	NA18484	F	O	O	O,P	-	NA18488	F	P	P	O,P	-	
YRI	15	NA19189	M	P	P	O,P	-	NA19191	M	O	O	O,P	-	
YRI	15	NA19190	F	P	P	O,P	-	NA19191	M	O	O	O,P	-	
YRI	20	NA19113	M	P	P	O,P	-	NA19115	F	O	O	O,P	-	
YRI	20	NA19114	F	P	P	O,P	-	NA19115	F	O	O	O,P	-	
YRI	24	NA19095	F	P	P	O,P	-	NA19097	F	O	O	O,P	-	
YRI	24	NA19096	M	P	P	O,P	-	NA19097	F	O	O	O,P	-	
YRI	26	NA18909	F	P	P	O,P	-	NA18911	M	O	O	O,P	-	
YRI	26	NA18910	M	P	P	O,P	-	NA18911	M	O	O	O,P	-	
YRI	29	NA19247	F	P	P	O,P	-	NA19249	M	O	O	O,P	-	
YRI	29	NA19248	M	P	P	O,P	-	NA19249	M	O	O	O,P	-	
YRI	31	NA18485	M	O	O	O,P	-	NA18487	M	P	P	O,P	-	
YRI	31	NA18485	M	O	O	O,P	-	NA18489	F	P	P	O,P	-	
YRI	32	NA19181	M	P	P	O,P	-	NA19183	F	O	O	O,P	-	
YRI	32	NA19182	F	P	P	O,P	-	NA19183	F	O	O	O,P	-	
YRI	33	NA19256	M	P	P	O,P	-	NA19258	M	O	O	O,P	-	
YRI	33	NA19257	F	P	P	O,P	-	NA19258	M	O	O	O,P	-	
YRI	34	NA19117	M	P	P	O,P	-	NA19174	M	O	O	O,P	-	
YRI	34	NA19118	F	P	P	O,P	-	NA19174	M	O	O	O,P	-	

YRI	38	NA18518	F	O	O	O,P	-	NA18519	M	P	P	O,P	-
YRI	38	NA18518	F	O	O	O,P	-	NA18520	F	P	P	O,P	-
YRI	39	NA19213	M	P	P	O,P	-	NA19215	F	O	O	O,P	-
YRI	39	NA19214	F	P	P	O,P	-	NA19215	F	O	O	O,P	-
YRI	41	NA19197	F	P	P	O,P	-	NA19199	F	O	O	O,P	-
YRI	41	NA19198	M	P	P	O,P	-	NA19199	F	O	O	O,P	-
YRI	46	NA19121	M	P	P	O,P	-	NA19123	M	O	O	O,P	-
YRI	46	NA19122	F	P	P	O,P	-	NA19123	M	O	O	O,P	-
YRI	47	NA18916	F	P	P	O,P	-	NA18930	F	O	O	O,P	-
YRI	47	NA18917	M	P	P	O,P	-	NA18930	F	O	O	O,P	-
YRI	48	NA18933	F	P	P	O,P	-	NA18935	M	O	O	O,P	-
YRI	48	NA18934	M	P	P	O,P	-	NA18935	M	O	O	O,P	-
YRI	49	NA18923	M	P	P	O,P	-	NA18925	M	O	O	O,P	-
YRI	49	NA18924	F	P	P	O,P	-	NA18925	M	O	O	O,P	-
YRI	52	NA18497	M	O	O	O,P	-	NA18498	M	P	P	O,P	-
YRI	52	NA18497	M	O	O	O,P	-	NA18499	F	P	P	O,P	-
YRI	62	NA18867	F	P	P	O,P	-	NA18869	M	O	O	O,P	-
YRI	62	NA18868	M	P	P	O,P	-	NA18869	M	O	O	O,P	-
YRI	65	NA19107	M	P	P	O,P	-	NA19109	F	O	O	O,P	-
YRI	65	NA19108	F	P	P	O,P	-	NA19109	F	O	O	O,P	-
YRI	66	NA19235	F	P	P	O,P	-	NA19237	F	O	O	O,P	-
YRI	66	NA19236	M	P	P	O,P	-	NA19237	F	O	O	O,P	-
YRI	71	NA19224	M	O	O	O,P	-	NA19225	F	P	P	O,P	-
YRI	71	NA19224	M	O	O	O,P	-	NA19226	M	P	P	O,P	-
YRI	81	NA18509	M	O	O,P	O,P	-	NA18511	F	P	O,P	O,P	-
YRI	83	NA19149	F	P	P	O,P	-	NA19151	F	O	O	O,P	-
YRI	83	NA19150	M	P	P	O,P	-	NA19151	F	O	O	O,P	-
YRI	85	NA18873	F	P	P	O,P	-	NA18875	F	O	O	O,P	-
YRI	85	NA18874	M	P	P	O,P	-	NA18875	F	O	O	O,P	-
YRI	86	NA18503	M	O	O	O,P	-	NA18504	M	P	P	O,P	-
YRI	86	NA18503	M	O	O	O,P	-	NA18505	F	P	P	O,P	-
YRI	91	NA19137	F	P	P	O,P	-	NA19139	M	O	O	O,P	-
YRI	91	NA19138	M	P	P	O,P	-	NA19139	M	O	O	O,P	-
YRI	92	NA19152	F	P	P	O,P	-	NA19154	M	O	O	O,P	-
YRI	92	NA19153	M	P	P	O,P	-	NA19154	M	O	O	O,P	-
YRI	94	NA19221	F	O	O	O,P	-	NA19222	F	P	P	O,P	-
YRI	94	NA19221	F	O	O	O,P	-	NA19223	M	P	P	O,P	-
YRI	95	NA18500	M	O	O,P	O,P	-	NA18501	M	P	O,P	O,P	-
YRI	105	NA18870	F	P	P	O,P	-	NA18872	M	O	O	O,P	-
YRI	105	NA18871	M	P	P	O,P	-	NA18872	M	O	O	O,P	-
YRI	109	NA18861	F	P	P	O,P	-	NA18863	M	O	O	O,P	-
YRI	109	NA18862	M	P	P	O,P	-	NA18863	M	O	O	O,P	-
YRI	110	NA18855	F	P	O,P	O,P	-	NA18857	M	O	O,P	O,P	-

YRI	115	NA19171	M	P	P	O,P	-	NA19173	M	O	O	O,P	-	
YRI	115	NA19172	F	P	P	O,P	-	NA19173	M	O	O	O,P	-	
YRI	117	NA18515	M	O	O	O,P	-	NA18516	M	P	P	O,P	-	
YRI	117	NA18515	M	O	O	O,P	-	NA18517	F	P	P	O,P	-	
YRI	122	NA18912	F	P	P	O,P	-	NA18914	M	O	O	O,P	-	
YRI	122	NA18913	M	P	P	O,P	-	NA18914	M	O	O	O,P	-	
YRI	122	NA18913	M	-	O,P	O,P	-	NA19238	F	-	O,P	O,P	-	
YRI	122	NA18913	M	-	G,H	H	-	NA19240	F	-	C,H	H	-	R,?
YRI	122	NA18914	M	-	C,H	C	-	NA19238	F	-	G,H	G	-	R,?
YRI	122	NA18914	M	-	HA,HN	-	HN	NA19240	F	-	HA,HN	-	HA	?
YRI	122	NA19238	F	P	P	O,P	-	NA19240	F	O	O	O,P	-	
YRI	122	NA19239	M	P	P	O,P	-	NA19240	F	O	O	O,P	-	
YRI	131	NA19209	F	P	P	O,P	-	NA19211	M	O	O	O,P	-	
YRI	131	NA19210	M	P	P	O,P	-	NA19211	M	O	O	O,P	-	
YRI	133	NA18506	M	O	O	O,P	-	NA18507	M	P	P	O,P	-	
YRI	133	NA18506	M	O	O	O,P	-	NA18508	F	P	P	O,P	-	
YRI	134	NA19159	F	P	P	O,P	-	NA19161	M	O	O	O,P	-	
YRI	134	NA19160	M	P	P	O,P	-	NA19161	M	O	O	O,P	-	
YRI	141	NA18858	F	P	P	O,P	-	NA18860	M	O	O	O,P	-	
YRI	141	NA18859	M	P	P	O,P	-	NA18860	M	O	O	O,P	-	
YRI	149	NA19127	F	P	P	O,P	-	NA19129	F	O	O	O,P	-	
YRI	149	NA19128	M	P	P	O,P	-	NA19129	F	O	O	O,P	-	
YRI	150	NA19130	M	P	P	O,P	-	NA19132	F	O	O	O,P	-	
YRI	150	NA19130	M	-	A,C,G,H,N	AV	-	NA19192	M	-	A,C,G,H,N	AV	-	?
YRI	150	NA19130	M	-	1C,GA,GG ,HA,HN	-	1C	NA19194	M	-	1C,GC,GN ,HA,HN	-	1C	?
YRI	150	NA19131	F	P	P	O,P	-	NA19132	F	O	O	O,P	-	
YRI	150	NA19132	F	-	1C,GC,GN ,HA,HN	-	GN	NA19192	M	-	1C,GA,GG ,HA,HN	-	GA	?
YRI	150	NA19192	M	P	P	O,P	-	NA19194	M	O	O	O,P	-	
YRI	150	NA19193	F	P	P	O,P	-	NA19194	M	O	O	O,P	-	
YRI	152	NA19116	F	P	P	O,P	-	NA19120	M	O	O	O,P	-	
YRI	152	NA19119	M	P	P	O,P	-	NA19120	M	O	O	O,P	-	
YRI	155	NA19093	F	P	O,P	O,P	-	NA19094	F	O	O,P	O,P	-	
YRI	157	NA18852	F	P	P	O,P	-	NA18854	M	O	O	O,P	-	
YRI	157	NA18853	M	P	P	O,P	-	NA18854	M	O	O	O,P	-	
YRI	160	NA19140	F	P	P	O,P	-	NA19142	M	O	O	O,P	-	
YRI	160	NA19141	M	P	P	O,P	-	NA19142	M	O	O	O,P	-	
YRI	164	NA19206	F	P	P	O,P	-	NA19208	M	O	O	O,P	-	
YRI	164	NA19207	M	P	P	O,P	-	NA19208	M	O	O	O,P	-	
YRI	165	NA19101	M	P	P	O,P	-	NA19103	M	O	O	O,P	-	
YRI	165	NA19102	F	P	P	O,P	-	NA19103	M	O	O	O,P	-	

Notes column codes:
P – PRIMUS provides more precise relationship prediction than other methods.
P* - PRIMUS provides corrected relationship results.
? – One of the other methods reported a more precise relationship prediction than PRIMUS; however, we found several instances where these predictions are incomplete (i.e., the authors failed to recognize that there are more than one possible way to fit the pairwise relationships into a pedigree) or inaccurate.
R – Pemberton et al. prediction was based on RELPAIR results.
R* - The Pemberton et al. reported relationship is based on manually reconstructed pedigrees, and it disagrees with the relationship that RELPAIR predicted.
R§ - Pemberton et al. could not reconcile the predicted 2nd degree relationship with their manually reconstructed pedigree structure.
N – A possible 3rd degree relationship that was unreported in Pemberton et al.[44] and Kyriazopoulou-Panagiotopoulou et al.[24]. However, the MKK population is reported as a small, isolated population, which results in a low level of background relatedness among the samples. The background relatedness can make individuals appear more closely related than they actually are.

Code	Relationship
P	Parent
O	Off-spring
F	Full-sibling
G	Grandparent
C	Grandchild
A	Uncle/Aunt
N	Neice/Nephew
H	Half-sibling
GG	Great-Grandparent
GC	Great-Grandchild
GA	Great-Aunt/Uncle
GN	Great-Neice/Nephew
HA	Half-uncle/aunt
HN	half-neice/nephew
1C	First cousin
U	Uncertain
UN	Unrelated (4th degree or more distant relative)

Each pair of individuals that is predicted to be related in at least one possible pedigree is represented in this table. The table lists the reported relationships from HapMap3, Pemberton et al.[44], Kyriazopoulou-Panagiotopoulou et al.[24] (CARROT), and PRIMUS. The relationships in the PRIMUS column are the aggregate of all relationships from the possible pedigrees, and they are listed as what their relationship is to the other person on the same line. For example, the first row shows that NA19916 is the parent (P) of NA19918, and NA19918 is the offspring (O) of NA19916. We used a minimum coefficient of relatedness of 0.09875 (3rd degree relatives or closer) to build the relationship networks for all of HapMap3; however, one family network in the Maasai in Kinyawa, Kenya, (MKK population) contained 126 individuals connected by 3rd degree relationships or closer, and it resulted in a number of possible pedigrees that were computationally infeasible. So, for the MKK population we used a minimum coefficient of relatedness of 0.168 (to include all 2nd degree relatives and closer), resulting in more manageable family network sizes. One MKK family still contained 61 individuals (Network 16 in this table).

To reconstruct this network, we broke it into nine sub-networks each containing four to eight closely related samples. We ran PRIMUS on each pair of sub-networks in order to reconstruct relationships between the sub-networks.

Supplementary Table 6. Possible combinations of pairwise 2nd and 3rd degree family relationships considered during pedigree reconstruction.

2nd degree relationship between A and B	A	B
1. Half-sib through mother	Half-sib	Half-sib
2. Half-sib through father	Half-sib	Half-sib
3. Avuncular through mother	Nephew	Uncle
4. Avuncular through mother	Uncle	Nephew
5. Avuncular through father	Nephew	Uncle
6. Avuncular through father	Uncle	Nephew
7. Grandparent though father	Grandfather	Grandson
8. Grandparent though father	Grandson	Grandfather
9. Grandparent though mother	Grandfather	Grandson
10. Grandparent though mother	Grandson	Grandfather
3rd degree relationship between A and B	A	B
1. Cousins through A mom and B mom	Cousins	Cousins
2. Cousins through A mom and B dad	Cousins	Cousins
3. Cousins through A dad and B mom	Cousins	Cousins
4. Cousins through A dad and B dad	Cousins	Cousins
5. Great-grandparental through mom's mom	Great-grandfather	Great-grandson
6. Great-grandparental through dad's mom	Great-grandfather	Great-grandson
7. Great-grandparental through mom's dad	Great-grandfather	Great-grandson
8. Great-grandparental through dad's dad	Great-grandfather	Great-grandson
9. Great-grandparental through mom's mom	Great-grandson	Great-grandfather
10. Great-grandparental through dad's mom	Great-grandson	Great-grandfather
11. Great-grandparental through mom's dad	Great-grandson	Great-grandfather
12. Great-grandparental through dad's dad	Great-grandson	Great-grandfather
13. Grand-avuncular through mom's mom	Grand-uncle	Grand-nephew
14. Grand-avuncular through dad's mom	Grand-uncle	Grand-nephew
15. Grand-avuncular through mom's dad	Grand-uncle	Grand-nephew
16. Grand-avuncular through dad's dad	Grand-uncle	Grand-nephew

17. Grand-avuncular through mom's mom	Grand-nephew	Grand-uncle
18. Grand-avuncular through dad's mom	Grand-nephew	Grand-uncle
19. Grand-avuncular through mom's dad	Grand-nephew	Grand-uncle
20. Grand-avuncular through dad's dad	Grand-nephew	Grand-uncle
21. Half-avuncular through mom's mom	Half-uncle	Half-nephew
22. Half-avuncular through dad's mom	Half-uncle	Half-nephew
23. Half-avuncular through mom's dad	Half-uncle	Half-nephew
24. Half-avuncular through dad's dad	Half-uncle	Half -nephew
25. Half-avuncular through mom's mom	Half-nephew	Half-uncle
26. Half-avuncular through dad's mom	Half-nephew	Half-uncle
27. Half-avuncular through mom's dad	Half-nephew	Half-uncle
28. Half-avuncular through dad's dad	Half -nephew	Half-uncle

As the degree of relatedness increases from 2nd to 3rd degree, there are far more relationships to test during reconstruction. Continuing to 4th degree relatives would require testing even more relationships.