KinLinks: Software Toolkit for Kinship Analysis and Pedigree Generation from NGS Datasets

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Abstract

The ability to predict familial relationships from source DNA in multiple samples has a number of forensic and medical applications. Kinship testing of suspect DNA profiles against relatives in a law enforcement database can provide valuable investigative leads, determination of familial relationships can inform immigration decisions, and remains identification can provide closure to families of missing individuals. The proliferation of Next-Generation Sequencing technologies allows for enhanced capabilities to accurately predict familial relationships to the third degree and beyond. KinLinks, developed by MIT Lincoln Laboratory, is an open source software tool that predicts pairwise relationships and reconstructs kinship pedigrees for multiple input samples using single-nucleotide polymorphism (SNP) profiles. The software has been trained and evaluated on a set of 175 subjects (30,450 pairwise relationships), consisting of three multi-generational families and 52 geographically diverse subjects. Though a panel of 5396 SNPs was selected for kinship prediction, KinLinks is highly modular, allowing for the substitution of expanded SNP panels and additional training models as sequencing capabilities continue to progress. KinLinks builds on the SNP-calling capabilities of Sherlock's Toolkit, and is fully integrated with the Sherlock's Toolkit pipeline[1]. KinLinks is also available to download as a standalone application on SourceForge at https://sourceforge.net/projects/kinlinks.

Keywords

Kinship, pedigree, SNP (single nucleotide polymorphism), algorithm, machine learning

Introduction

Kinship testing may be used to identify relatives for immigration cases, or for intelligence in establishing relationships between individuals. For example, terrorist networks are often family centered affairs[2]. Identifying relationships, both immediate and distant, may prove useful in better understanding networks at many levels. Kinship analysis may also enable genotype imputation to increase sample size in pedigreed populations for a number of secondary applications[3]. Kinship testing is needed for older remains identification with matching to second or third generation descendants. Yet another use involves deterring immigration fraud by verifying claimed familial relationships. For these purposes and others, the law enforcement community typically relies on sample identification by sizing of short tandem repeats (STRs) with capillary electrophoresis, usually coupled with searches against the CODIS database or a relative's DNA. However, the ability of STRs to identify relatives is limited. Increasing the number of STR loci improves the statistical power of the analysis, but to a surprisingly modest degree[4]. Large panels of SNP loci may be useful in further resolving extended kinship relationships: chip-based assays using 192,000 loci can identify third-degree relationships [5]. One of the goals of this project was to achieve similar results with a smaller panel of loci detected by NGS sequencing.

A number of tools currently exist to identify first degree kinship relationships – parents [6, 7] or siblings[8], but few tackle the more challenging problem of multi-generation pedigree reconstruction in the presence of missing or incomplete data. For example, MERLIN [9]with the FEST front-end[10] uses sparse trees to represent gene flows in pedigrees. Haplo2PED considers haplotype fragments as genomic markers, and perform whole genome linkage analysis with these haplotype markers dynamically in disease gene mapping[11]. GenoSeq computes kinship from whole genome sequencing data [12], and LDAK provides heritability estimates from genome-wide SNPs by computing LD-adjusted kinships[13]. The MPKin program allows users to select among multiple familial searching strategies to match a sample to a target database of potential relatives: minimum number of shared alleles, moderate stringency matches at all loci, Kinship Coefficient calculation, and other approaches [14]. However, though it has been used extensively by law enforcement agencies for pairwise relationship prediction, it stops short of full pedigree reconstruction.

Other tools go a step beyond pairwise kinship prediction to tackle the problem of automated pedigree reconstruction. IPED[15] and its successor IPEDX[16] use haplotype and identify by descent (IBD) information to reconstruct pedigrees generation-by-generation backwards in time. For each generation, the pairwise relationships are predicted between individuals within the current generation, and parents are created according to the predicted relationships. Though this approach is able to rapidly reconstruct pedigrees in the presence of perfect data and consanguineous marriages, it does not handle more complex relationships such as half-siblings or missing samples, both common phenomena for forensics analysis. The tool, Familias, takes a different approach to solve the problem of disaster victim identification by combining information (if any) prior to DNA with STR profiles in a Bayesian manner to deliver the posterior probabilities for sets of familial relationships[17]. Although Familias addresses both population substructure and mutation, it utilizes STR profiles and cannot be easily extended to work with SNP panels. Furthermore, the 'equal probability model' and 'proportional model' used in Familias are not necessarily the best for STR loci, and the algorithm does not handle relationships of degree three or higher. FRANZ incorporates prior information in addition to genotypes to find parentage combinations that define the maximum likelihood pedigree[18]. The algorithm uses Markov Chain Monte Carlo sampling to estimate statistical confidence for each possible pedigree. FRANZ is designed to operate on wild type populations, where samples may be missing, so is quite promising for forensic applications. However, the algorithm relies on external information, such as age data and a priori estimates of population minor allele frequencies, both of which are often unknown of vary by populations.

The KinLinks software combines some of the standard approaches to kinship prediction used by the above- mentioned tools, such as the Kinship Coefficient, probability of zero identity by state, and likelihood ratio calculation to identify the most likely pedigree from multiple candidates to predict pairwise kinship relationships and automatically reconstruct pedigrees for multiple SNP genotyped samples. KinLinks assumes no a prior knowledge about the relative ages of subjects, pedigree completeness (missing samples are expected and tolerated), or population allele frequencies. The algorithm training module uses a supervised machine learning classifier to build training models for pairwise kinship degree predictions. These pairwise predictions are then combined into multi-generation pedigrees via a set of heuristics to resolve relationship type (half siblings vs avuncular vs grandparent relationships for second degree relationships, for example) and direction (the parent in a parent/child relationship for example). Experimental validation on several hundred samples has yielded perfect prediction of first and second degree relationships as well as the ability to distinguish unrelated vs related individuals. Third degree relationships can be resolved with over 90% accuracy.

Methods/Algorithm Overview

SNP panel design

A panel of 5396 SNP loci was generated utilizing Ampliseq multiplex 150 bp amplicons (**Supplementary Table 1**). This amplicon size was compatible with the current capabilities of the Ion Torrent Proton instrument, which produces ~70 million reads per run. These loci were chosen based on

- low minor allele frequency, based on the hypothesis that fractions of shared minor alleles are indicative of degree of relationship[19],
- low correlation with biogeographic ancestry, and
- maximal spacing along the genome (at least 50 kbp between loci)

Training and test data samples

Source DNA from four groups was purchased from the Coriel Medical Institute to serve as training and test samples for kinship analysis. These include the Family 95 pedigree of 43 individuals across three generations (**Supplementary Figure 1a**), the ALS NINDS0760 family of 30 individuals across 4 generations (**Figure 4a**), the Retinitis pigmentosa families 2110 and 2111 of 52 individuals across 6 generations (**Figure 5a**), and 54 geographically diverse samples (**Supplementary Table 2**). SNP DNA from all individuals was analyzed using the custom-designed 5396-locus Ampliseq panel described above, with sequencing performed on the Ion Torrent Proton instrument.

In phase 1 of the project, the pedigree generation algorithm was trained on 1225 pairwise relationships from Family 95 as well as 2862 pairwise relationships from the geographically diverse samples. The algorithm was tested on 870 pairwise relationships from the ALS family. In phase 2, the ALS samples were added to the training dataset, and algorithm performance was evaluated on the 2652 pairwise relationships within the Retinitis pigmentosa family.

Machine Learning Classifier for pairwise kinship prediction

Ten features were identified for training a one-vs-one multi-class support vector machine classifier. These include:

- probability of identity by state equaling zero P(IBS=0) [20, 21]. This metric measures the number of dissimilar alleles shared between a pair of individuals, where dissimilar is quantified as two individuals presenting with homozygous, opposite allele calls (one individual is homozygous minor, while the other is homozygous major). Unlike the Kinship Coefficient, this metric is able to differentiate parent-child relationships from siblings.
- Kinship Coefficient as calculated by the KING algorithm [22]. The Kinship Coefficient in this model is defined as the number of minor alleles shared between two individuals, divided by the

average number of minor alleles between the two individuals. The Kinship Coefficient was selected because it is not reliant on population-estimated minor allele frequencies. In heterogeneous populations this type of estimation has been shown more accurate and robust to large groups of individuals[23, 24].

- biogeographic ancestry[23] Biogeographic ancestry predictions to the regional level (Americas, Europe, Middle East, East Asia, South Asia, East Africa, West Africa, Oceania) were determined via a genetic algorithm using a separate panel of 96 SNPs, as detailed in Ricke et al[1].
- number of shared loci.
- number of loci where both individuals were homologous for the minor allele.
- number of loci where both individuals were homozygous for the major allele.
- number of loci where both individuals where heterozygous for the minor allele.
- number of loci where one individual was homozygous for the minor allele while the other one was heterozygous.
- number of loci where one individual was homozygous for the major allele while the other was heterozygous.
- number of loci where one individual was homozygous for the major allele while the other was homozygous for the minor allele.

An one-vs-one support vector machine multi-class classifier with a linear kernel was implemented using the sklearn Python toolkit[25]. The classifier was trained via leave-one-out cross-validation on the set of pairwise relationships in Family 95 and the set of geographically diverse samples, as described above. Support vector machine parameters gamma and C were determined via a parameter sweep in two-dimensional space.

Two classifiers were developed – the first classifier predicts relationship degree (i.e. pairs of 1st, 2nd, 3rd degree relatives or unrelated individuals) and the second classifier predicts the exact relationship among a pair of samples (i.e. parent/child, sibling, grandparent, avuncular, cousin, unrelated). Both classifiers use the above-mentioned feature values for a set of samples as inputs. The first classifier predicts the degree of relation between each pair of samples and the second classifier predicts the relationship between each pair of samples.

Heuristics for pedigree generation

An algorithm was implemented to automatically generate pedigrees from the pairwise kinship predictions. As a first step, a set of heuristics was implemented to determine relationship direction for first and second degree relatives (i.e. for a first-degree relationship, who are the parents, who are children, and who are siblings). These include:

- Spouse married into family: a sample has a first degree relationship to one or more other samples (children), but no relationship of any degree to other samples in the dataset.
- Siblings: siblings are expected to share 2 alleles at 25% of the loci, 1 allele at 50% of the loci, and 0 alleles at 25% of the loci.
- Direct descent: A child will share 50% alleles with a parent, 25% of alleles with a grandparent, 12.5% alleles with a great grandparent, 6.25 % allele with a great grandparent.

- Trio pattern: If two unrelated samples both have a first degree relationship with a third sample, and each unrelated sample shares ~50% of its alleles with the third sample, the first two samples are the parents of the third sample.

The python graph_tool library (v. 2.2.36) [27] was used to generate a semi-directed acyclic graph to represent the pairwise relationship predictions for a set of samples (**Figure 2**). Nodes represent samples used for the analysis. Blue nodes indicate males, while pink nodes indicate females. Sex was inferred by analyzing sample genotypes at 30 loci present on the X chromosome (**Supplementary Table 1, rows 2-38**). If a heterozygous genotype was observed at one or more of these loci, the sample was designated a female. Otherwise, it was designated as male. Edges between the nodes represent predicted relationships. In cases where the above-mentioned heuristics allow the determination fo relationship direction, the edge is directed accordingly. Edges are color-coded to indicate degree of relationship.

Additional heuristics were generated to infer connections between nuclear families, allowing the generation of fully-connected pedigrees for Coriel Family 95 (**Supplementary Figure 1b**), ALS family (**Figure 4b**), and the Retinitis pigmentosa family (**Figure 5b**). The auto-generated pedigrees were graphed as well as in family-tree format using the PyGraphViz toolkit (v 1.3rc2)[26].

Cousin relationships were resolved by identifying a unique pattern of allele sharing. All cousin relationships across the 72 individuals analyzed for Family 95 and ALS met the following criteria:

- Relationship identified as second or third degree by machine learning algorithm in the first step of kinship analysis
- $\frac{m_{00}}{N_{shared}} < 0.70$
- $\frac{m_{01}}{N_{shared}} > 0.21$
- $\frac{m_{11}}{N_{shared}} < 0.05$

Nshared indicates the total number of loci in the kinship panel where both subjects had an allele call. With m_{00} representing the total number of loci where both subjects had 0 minor alleles. And, m_{01} representing the number of loci where one subject had 0 minor alleles, while the other subject had 1 minor allele. And, m_{11} representing the number of loci where both subjects had 1 major allele and 1 minor allele. If this pattern was observed between two nodes in the pedigree, the minimum number of nodes needed to create a cousin relationship were added to the pedigree. In cases where multiple assignments of nodes were possible, nodes were added in a way as to avoid any conflicts with existing high-confidence (nuclear family) relationships as well as to minimize conflicts with the degree designations of the machine learning algorithm in step 1. Some contradiction with the machine learning calls was tolerated, as the machine learning algorithm miscalls a fraction of relationships of degree 3 or higher.

Once cousins had been added to the graph, power calculations were performed to resolver relationships of the same degree. The purpose of this step was to determine whether each second degree relationship called by the machine learning algorithm was avuncular, grandparent-grandchild, or half-sibling. No difference in allele sharing patterns was observed between these three types of relationships, so the distinction was made by relying on graph connectivity and a modified form of the gradient ascent algorithm. The disjoint pedigree was treated as a set of connected components. The weighted degree of each node was computed, considering only nodes present in the other components (i.e. the nuclear families of which the node was not a member). Edges were weighted by the inverse of degree of relationship (i.e. a 2nd degree relationship was assigned w=1/2). This led to the observation that the weighted degree was higher for older individuals than for younger individuals in the same nuclear family, providing a means to infer age within nuclear families (**Supplementary Figure 2**). Furthermore, only individuals who had married into the family had a weighted degree of 0. The weightings also provide a set of constraints for resolution of second degree relationships. For example, grandparents will have degree weightings three to four times higher than their grandchildren. Half siblings will have similar degree weightings if the shared parent is part of the pedigree. If the shared parent is not part of the pedigree, one of the siblings will have a degree weight of 0, while the other will have a non-zero degree weight. This gives rise to the following set of constraints:

- If two nodes have similar degree weightings, they do not share a grandparent/grandchild relationship.
- If one node has a degree weight of 0, while the other has a non-zero degree weight, they are half-siblings related through a parent that married into the family.
- If two nodes have degree weightings of ratio > 2, but neither weighting is 0, they are not half-siblings.
- If two nodes share a grandparent/grandchild relationship, the grandparent node will have a higher degree weight.

Given this set of constraints, a set of second degree relationships were selected to avoid contradictions with established nuclear family relationships and minimize contradictions with the machine learning algorithm degree calls from step one. For example, if node A and B have similar degree weightings, they may be either half-siblings or have an avuncular relationship. The minimum possible number of nodes is added to the pedigree to support each relationship and any contradictions are computed. The relationship that minimizes contradictions is assigned. In case of ties, a relationship is not assigned for the two nodes. All second degree relationships are examined, and the algorithm is repeated until no additional second degree relationships can be resolved.

Results

Figure 1 illustrates KING algorithm plots for Kinship Coefficient versus probability of zero identify by descent for pairwise relationships within Family 95 and the ALS family. This analysis, using 5400 SNPs, produces results similar to chips using 192,000 SNPs[5]. It does have the particular advantage of not relying directly on minor allele frequency values, which are expected to vary to a certain degree between populations. All parent/child relationships fall within the expected range, KC ~=0.25, P(IBS=0)~=0. Approximately half of sibling relationships fall within the expected range, KC=0.25, P(IBS=0)~=0.25. The predictions for relationships of degree 3 and higher have below 50 percent accuracy, reflecting the challenges faced by many current kinship analysis algorithm of accurately predicting relationships beyond the second degree.

Supplementing the traditional Kinship Coefficient and P(IBS=0) metrics with the other 8 features used for the one-vs-one machine learning classifier yields improved results, as presented in **Tables 1,2** and

Supplementary Table 3. **Table 1b** indicates that all 44 parent-child relationships and 25 sibling relationships were correctly identified by the algorithm for the ALS family, when trained on Family 95 and unrelated geographically diverse samples. For second degree relationship predictions, 100% accuracy was also achieved, though 4 of the second degree relationships (9%) were classified as a different relationship within the same degree (2 grandparent relationships were classified as avuncular and vice versa). Out of 84 third degree relationships, 71 (85%) were classified correctly. An additional set of 7 relationships (9%) were classified as second degree, while the final 6 (8%) were classified as unrelated. Accuracy falls to below 50% in classifying relationships of degree four and higher. However, no pair of unrelated individuals is classified as related, all 120 pairs of unrelated individuals within the ALS family are classified as such. Consequently, for the ALS test dataset, the KinLinks algorithm has a false positive rate of 0% and an overall false negative rate of 25%, where the false negative rate is the defined as the failure to identify a relationship among two individuals with relationship of degree 6 or lower.

Similar results can be observed when KinLinks is trained on both the Family 95 and ALS datasets, along with 54 geographically diverse unrelated samples, and tested on the Retinitis pigmentosa family. All parent/child and sibling relationships are predicted correctly, over 94% of unrelated individuals are identified as such, and 77% of second degree relationships are classified correctly, while the remaining 23% are classified to within 1 degree of relation (either 1st or 3rd degree relatives).

The Retinitis pigmentosa family presents two additional challenges for classification. The first is the presence of consanguineous marriages[27], annotated by red arrows in **Figure 5a.** Consequently, a number of relationships do not fit the training model generated by Family 95 relationships as well as the ALS family. For the ALS family, no cases were observed where the predicted degree of a relationship was more than 1 degree closer than the truth degree. However, for the Retinitis pigmentosa family, 1.8% of predictions exhibit this error. In the most extreme cases, 4 pairs of unrelated individuals were predicted as second degree relatives, and 9 pairs were predicted as third degree relatives.

A second challenge can be attributed to the higher proportion of non-sequenced samples (non-highlighted nodes in **Figure 5a**) in the Retinitis pigmentosa family. Consequently, while KinLinks is able to automatically regenerate the connected pedigree for the ALS family (**Figure 4b**), four sub-pedigrees are generated for the Retinitis pigmentosa family (**Figure 5b**). As illustrated in **Figure 4b,** KinLinks is able to interpolate missing nodes, denoted by “Unknown” labels, that connect second degree relatives and cousins. Such missing nodes are also interpolated in **Figure 5b** for the Retinitis pigmentosa family, but not for relationships of degree four or higher.

Relationship predictions for Family 95 and the Retinitis pigmentosa family illustrate the ability of the KinLinks software to identify pedigree errors and to serve as a quality checking tool for truth data. Two sample mix-ups were correctly identified by KinLinks. For Family 95, the truth pedigree initially reported individuals NA10725 and NA10724 are full siblings, but the algorithm correctly identified them as half-siblings. For the Retinitis pigmentosa family, an off-by-one sample labeling error led individual NA9776 to be placed incorrectly in the truth pedigree (**Figure 6**). KinLinks classified NA9776 as unrelated to the supposed mother and sister. However, the sample was identified as having a parent-child relationship with node NA8990, a second-degree relationship with nodes NA9003 and NA9007, and a third degree relationship with nodes NA8983 and NA8936. It turns out that NA9976 was swapped with NA9765, initially not sequenced and shown in gray in **Figure 6**. Subsequent sequencing of NA9765 showed a parent-child relationship with NA9783 and a sibling relationship with NA9760, confirming the KinLinks prediction.

The influence of biogeographic ancestry on kinship prediction was examined (**Figure 3**). In the first KinLinks iteration, biogeographic ancestry predictions were not included as a feature for the one-vs-one SVM classifier. When the algorithm was trained on Family 95, of European descent, as well as 54 geographically diverse individuals **(Supplementary Table 2)**, a number of errors were present in the relationship predictions **(Figure 3a)**. Unrelated individuals from the same world region frequently showed up as having a second, third, or fourth degree relationship. A cluster of 6 South American subjects (purple nodes) were predicted as related to each other, as was a cluster of five European subjects. Subsequently, a genetic algorithm was implemented to predicted sample biogeographic ancestry to major world region [1]. A test of the algorithm on the ALS family in conjunction with the 54 geographically diverse samples removed all relationships between unrelated pairs of individuals, with the exception of two (**Figure 3b**). One remaining second degree relationship was between a pair of Quechua individuals, and the second remaining third degree relationship was between a pair of Druze samples. Since relationship annotations used to generate the truth data are self-reported, it could not be established whether these are indeed false positives or unreported true positive relationships.

Resource Requirements

KinLinks is implemented in Python (version 2.7), and was evaluated on a machine running Fedora 7. The training phase of the algorithm was executed in 43 minutes, when training on a set of 124 samples (15252 pairwise relationships) using a single core and 8 GB of RAM. A training model can be generated once and stored for repeated use with test samples. This allows the test phase of the algorithm to be executed in under 1 minute for an equal number of relationships.

Discussion

As illustrated in **Figure 7**, the current panel size of 5396 SNPs is sufficient to resolve relationships of the first and second degree with 100% percent accuracy (for the ALS family test dataset). Unrelated individuals can also be identified with 100% accuracy, and third degree relatives can be called with over 95% accuracy. However, the algorithm is unable to resolve relationships of degree 4 or higher with accuracy over 50%[28]. Algorithm performance was evaluated for varying SNP panel sizes, ranging from a 674 SNP subset of the panel (1/8 of the SNPs) to the full 5396 SNPs. By fitting three-dimensional splines to the performance curves, performance for larger SNP panels can be extrapolated. This method suggests that doubling the panel size to 10792 SNPs will enable resolution of 4th degree relationships to within 1 degree of relatedness. Tripling the panel size to 15000 SNPs is likely to enable perfect resolution of 4th degree relationships. However, the extrapolation suggests that a panel size of 690,000 SNPs would be necessary to resolve 5th degree relationships to within 1 degree.

Improving SNP panel design provides an alternative to increasing panel size for resolution of higher degree relationships. Analysis of linked SNPs[29] or shared chromosome segments [30] has the potential to increase power of resolution. Advances in microhaplotype analysis techniques enabled by NGS sequencing may be useful for resolving higher degree relationships as well[31]. Furthermore, the current panel includes 30 SNPs from the X chromosome, and no SNPs from the Y chromosome. By including a sub-panel of X and Y SNPs in the analysis, patterns of X/Y inheritance can be traced through a pedigree and used to generate additional features for training the one-vs-one SVM classifier. Work has been initiated on a second iteration of KinLinks, with a second panel of X and Y SNPs that will be used to resolve relationships within a predicted degree (i.e. grandparent vs avuncular). Thirdly, as the cost of exome and whole genome sequencing continues to drop, KinLinks can be modified to work with whole genome sequence data[32].

One of the greatest challenges encountered during the development of KinLinks was the dearth of publically available multi-generation pedigree DNA samples. Though repositories such as the 1000 Genomes Project, CEPH, and HapMap provide genotypes for multiple sets of trios and nuclear families, an extensive search did not yield any set of genotypes for a family with over four generations for download, and few are available from commercial vendors. As KinLinks relies on supervised machine learning algorithms, the eventual availability of additional training data will increase the power of relationship predictions. All three families used for training and evaluation were of European descent, and future efforts will focus on evaluating the algorithm on families with diverse biogeographic ancestries.

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Figures 6a and S2 list cell lines/DNA samples that were obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research.

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Figures

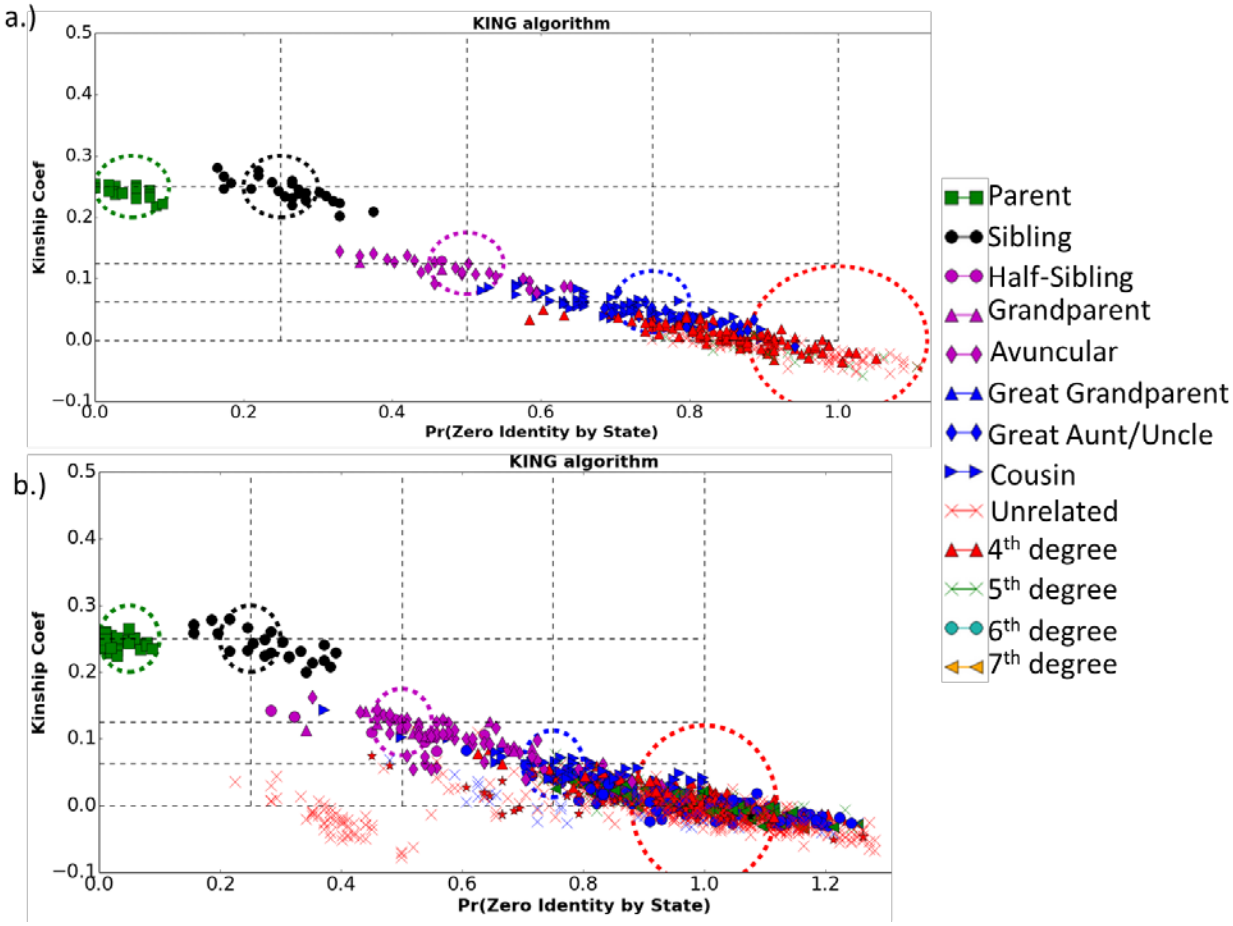


Figure 1: KING algorithm plots for two multi-generation families. Probability of zero identity by descent is plotted along the x-axis. Kinship Coefficient is plotted along the y-axis. Dashed circles indicate expected location of parents (green), siblings (black), second degree relatives (purple), third degree relatives (blue), higher degree/unrelated individuals (red). a) KING algorithm plot for 29 members of ALS Family 95. b) KING plot for 52 members of the Retinitis pigmentosa Family.

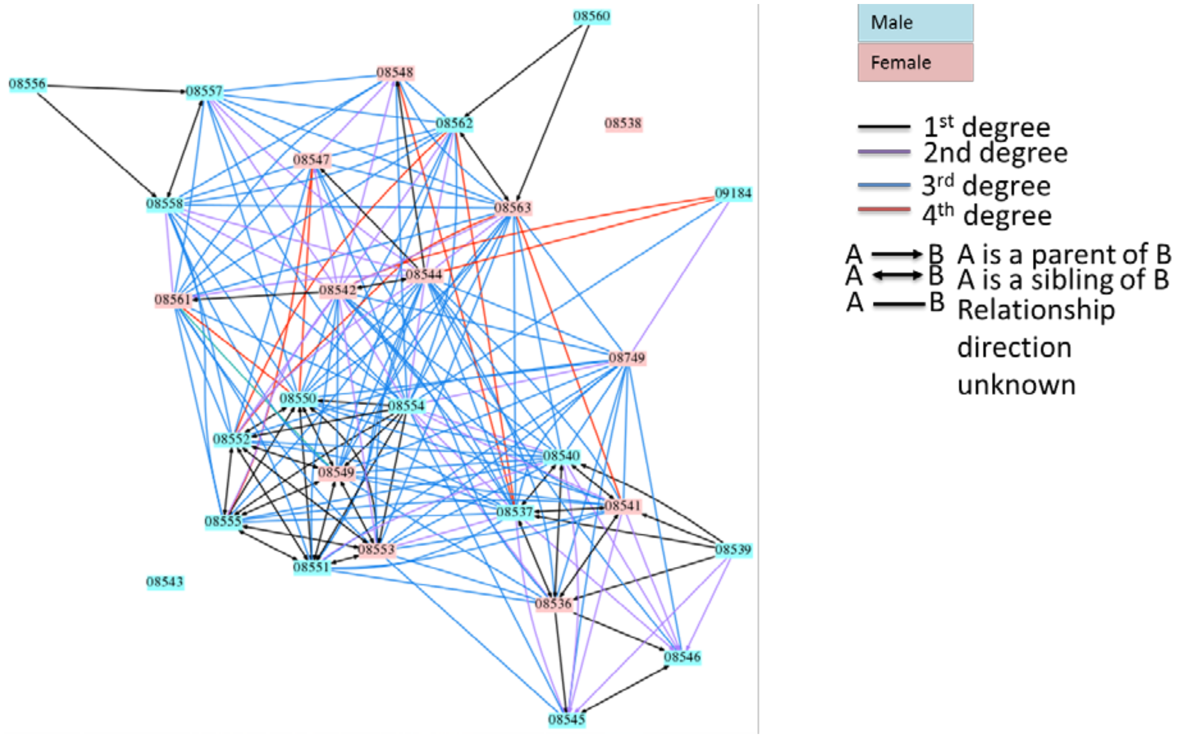


Figure 2: Graph representation of predicted relationships in the ALS family (29 individuals evaluated). Arrow color represents predicted degree of relationship between two samples (black –1st degree, purple – 2nd degree, blue—3rd degree, red—4th degree). Arrow direction (if shown) represents the direction of the relationship. An arrow without direction signifies that the direction of the relationship cannot be determined. Pink vertices represent female subjects, blue vertices represent male subjects.

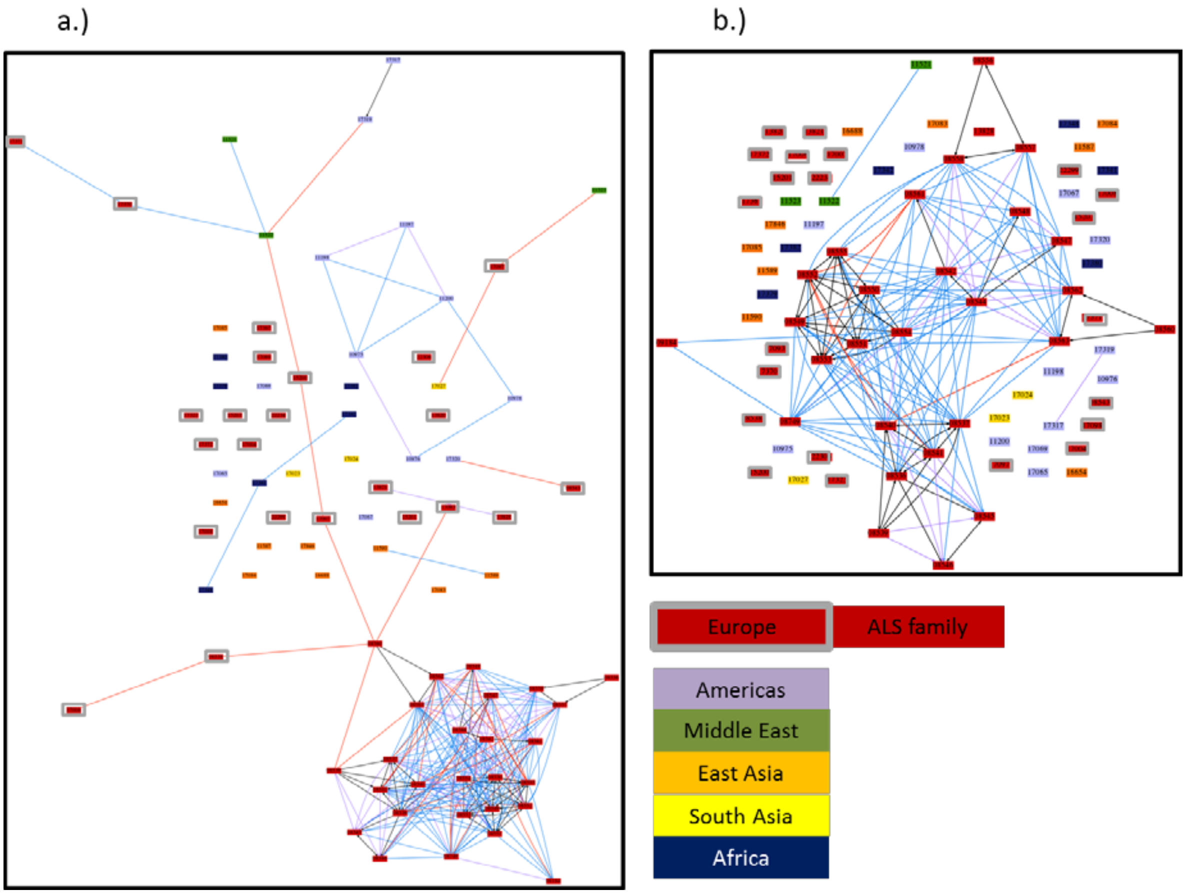


Figure 3: Effect of biogeographic ancestry on classifier performance. Predictions were made for 29 samples from the ALS family (red nodes), 17 unrelated European samples (red nodes with gray outline), 12 Central/South American samples (purple nodes), three Middle Eastern samples (green nodes), 9 East Asian samples (orange nodes), three South Asian samples (yellow nodes), 6 African samples (dark blue nodes). a) Relationship predictions when biogeographic ancestry was not utilized. b) Relationship predictions after biogeographic ancestry was added to the classification algorithm.

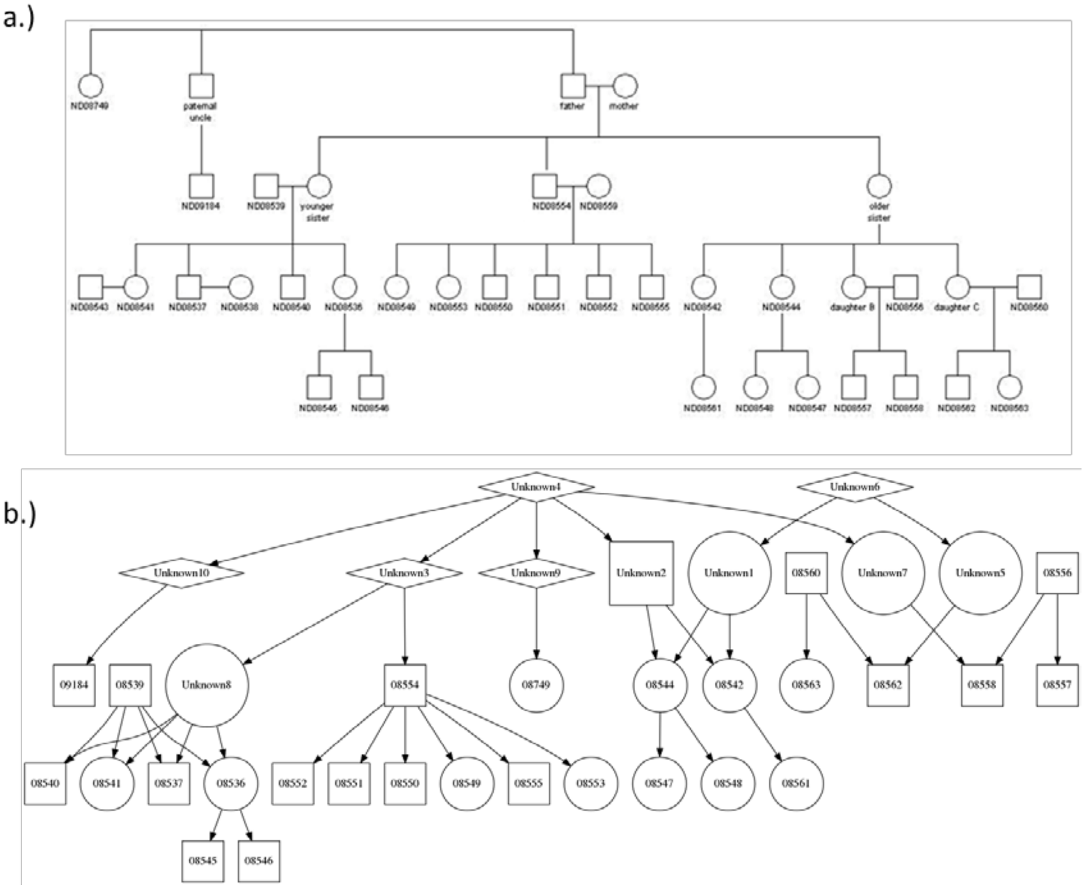


Figure 4: Automated pedigree generation for 29 individuals from the ALS family. a) Truth pedigree for the ALS family. Individuals identified by the "ND" prefix are those for which sequencing data was obtained. b) Automatically generated pedigree for the sequenced ALS individuals. Circles represent predicted females, squares represent predicted males, diamonds represent undetermined sex. Arrows point from parents to children.

]

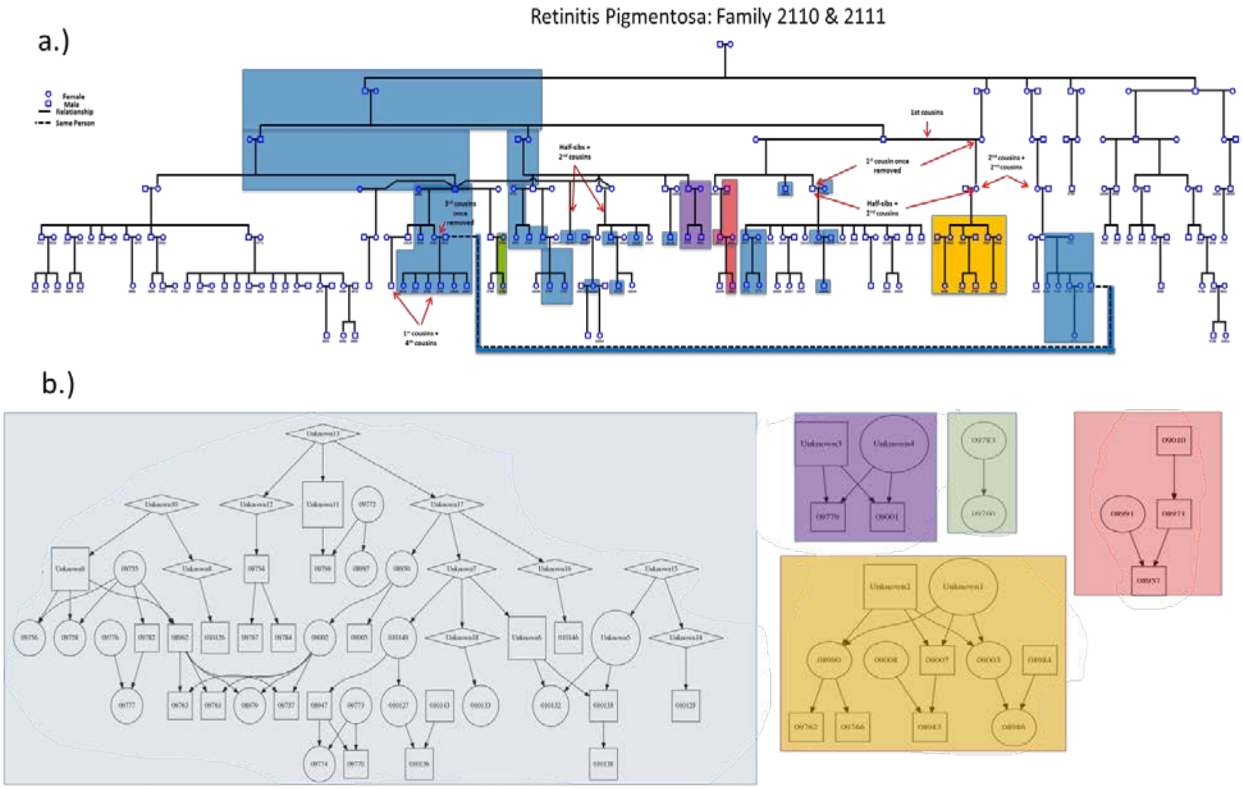


Figure 5. Automated pedigree generation for 52 members from the Retinitis pigmentosa family. a) Truth pedigree for the Retinitis pigmentosa family. b) Pedigrees for groups of sequenced profiles that were automatically generated by the kinship algorithm. Pedigrees are color-coded to correspond with truth data.

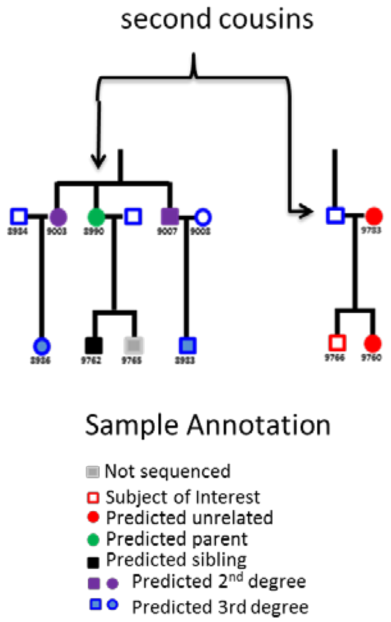


Figure 6: Predictions involving individual “9760”. Strong evidence suggests that the location of this individual in the reference pedigree is not correct (red circles on the mother and sister nodes indicate that these individuals were predicted as unrelated to 9760). More likely, 9760 is the child of 8990 (green circle), sibling of 9762 (black square).

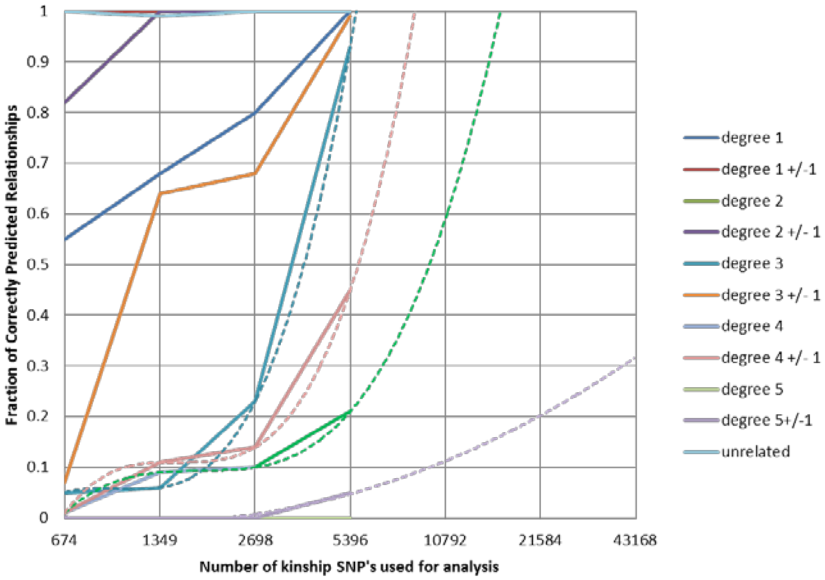
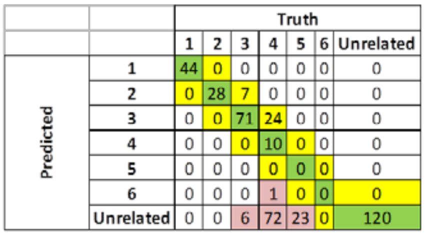


Figure 7: KinLinks performance for varying SNP panel sizes. Panel size was varied from 674 SNPs (1/8th of the original panel size) to 5396 SNPs, and the fraction of correct kinship predictions among 30 members of the ALS family was computed for each degree of relationship. The fraction of predictions correct to within 1 degree of truth is also shown (i.e. 1st or 3rd degree predictions for 2nd degree relatives). Three-dimensional splines, dotted lines, were fit to the performance curves to extrapolate algorithm performance for larger SNP panels.

Tables

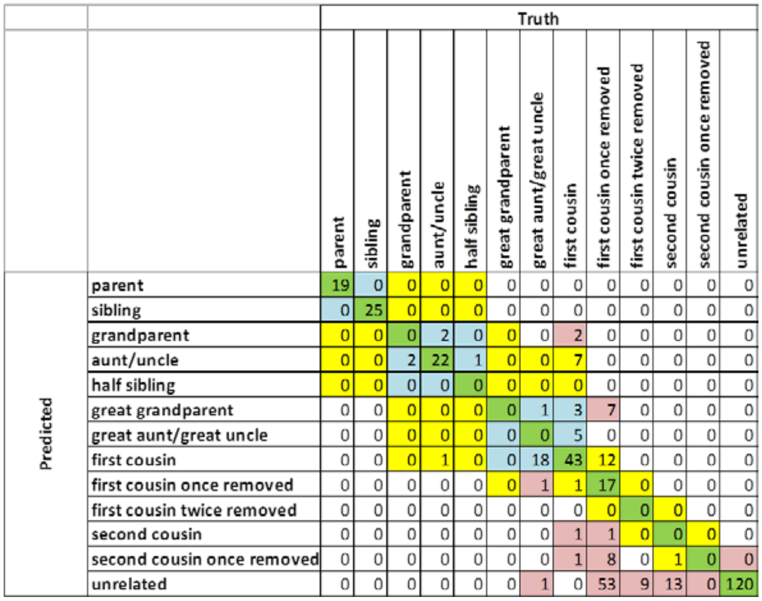
Table 1: Confusion matrices for relationship prediction for 29 members of the ALS family. Correctly predicted relationship pairs are highlighted in green. Predictions that differ by one degree of relationship from the truth are highlighted in yellow. Predictions that differ by more than 1 degree from the truth are highlighted in red. Predicted relationships that differ from the truth but share the same degree of relation are highlighted in blue. a) Confusion matrix for predicted degree of relationship. b) Confusion matrix for predicted named relationships.

a.)



		Truth						
		1	2	3	4	5	6	Unrelated
Predicted	1	44	0	0	0	0	0	0
	2	0	28	7	0	0	0	0
	3	0	0	71	24	0	0	0
	4	0	0	0	10	0	0	0
	5	0	0	0	0	0	0	0
	6	0	0	0	1	0	0	0
	Unrelated	0	0	6	72	23	0	120

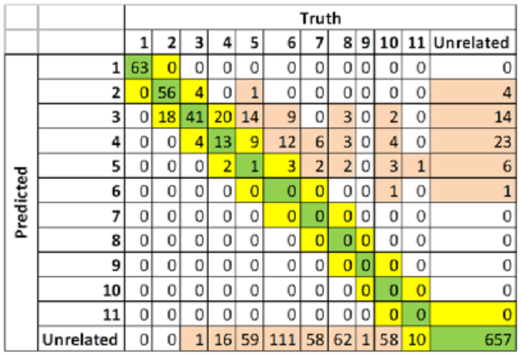
b.)



		Truth												
		parent	sibling	grandparent	aunt/uncle	half sibling	great grandparent	great aunt/great uncle	first cousin	first cousin once removed	first cousin twice removed	second cousin	second cousin once removed	unrelated
Predicted	parent	19	0	0	0	0	0	0	0	0	0	0	0	0
	sibling	0	25	0	0	0	0	0	0	0	0	0	0	0
	grandparent	0	0	0	2	0	0	0	2	0	0	0	0	0
	aunt/uncle	0	0	2	22	1	0	0	7	0	0	0	0	0
	half sibling	0	0	0	0	0	0	0	0	0	0	0	0	0
	great grandparent	0	0	0	0	0	0	1	3	7	0	0	0	0
	great aunt/great uncle	0	0	0	0	0	0	0	5	0	0	0	0	0
	first cousin	0	0	0	1	0	0	18	43	12	0	0	0	0
	first cousin once removed	0	0	0	0	0	0	1	1	17	0	0	0	0
	first cousin twice removed	0	0	0	0	0	0	0	0	0	0	0	0	0
	second cousin	0	0	0	0	0	0	0	1	1	0	0	0	0
	second cousin once removed	0	0	0	0	0	0	0	1	8	0	1	0	0
	unrelated	0	0	0	0	0	0	1	0	53	9	13	0	120

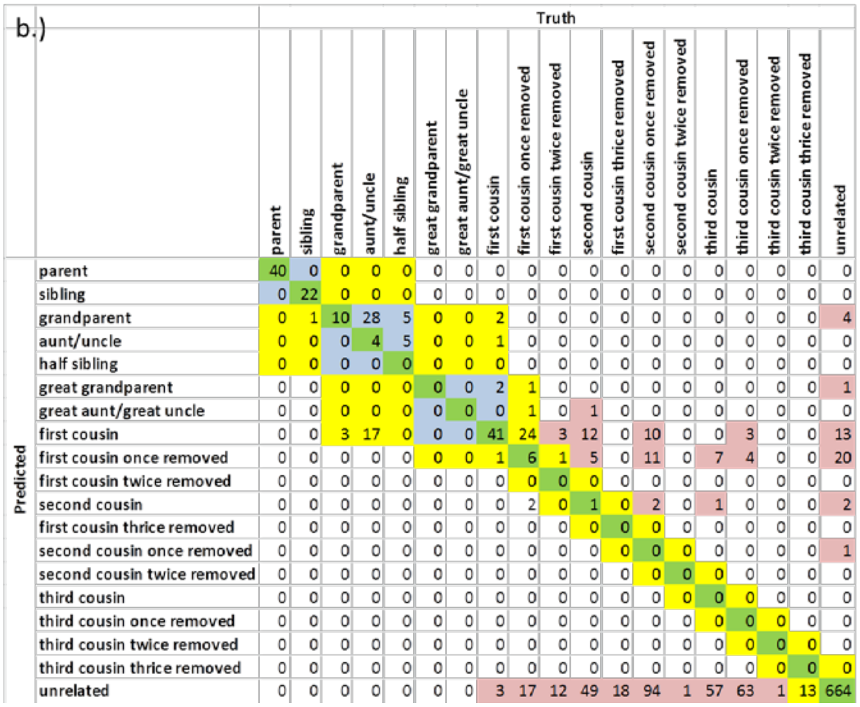
Table 2: Confusion matrix for 52 samples from the Retinitis pigmentosa family. The predictions were made with a classifier trained on Family 95, ALS, and 54 unrelated samples of diverse biogeographic backgrounds. Relationships that were predicted correctly are highlighted in green, predictions that differed by 1 degree from the truth are highlighted in yellow, predictions that differed from the truth by 2 or more degrees are highlighted in red, predictions that are the same degree as the truth relationship are highlighted in blue. a) Predictions of degree of relation. b) Exact relationship predictions.

a.)



		Truth											
		1	2	3	4	5	6	7	8	9	10	11	Unrelated
Predicted	1	63	0	0	0	0	0	0	0	0	0	0	0
	2	0	56	4	0	1	0	0	0	0	0	0	4
	3	0	18	41	20	14	9	0	3	0	2	0	14
	4	0	0	4	13	9	12	6	3	0	4	0	23
	5	0	0	0	2	1	3	2	2	0	3	1	6
	6	0	0	0	0	0	0	0	0	0	1	0	1
	7	0	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	0	0	0
	Unrelated	0	0	1	16	59	111	58	62	1	58	10	657

b.)

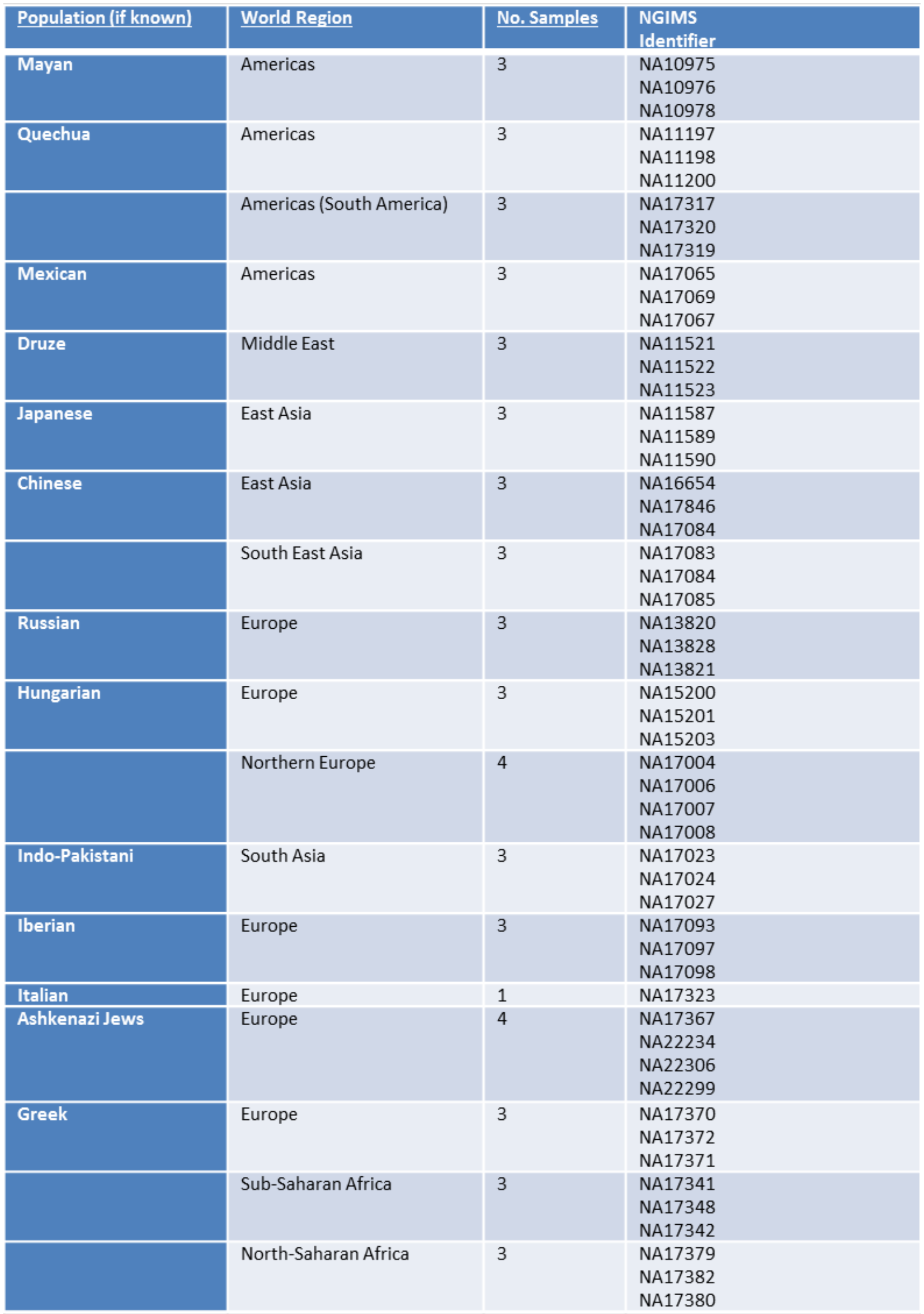


		Truth																		
		parent	sibling	grandparent	aunt/uncle	half sibling	great grandparent	great aunt/great uncle	first cousin	first cousin once removed	first cousin twice removed	second cousin	first cousin thrice removed	second cousin once removed	second cousin twice removed	third cousin	third cousin once removed	third cousin twice removed	third cousin thrice removed	unrelated
Predicted	parent	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	sibling	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	grandparent	0	1	10	28	5	0	0	2	0	0	0	0	0	0	0	0	0	0	4
	aunt/uncle	0	0	0	4	5	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	half sibling	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	great grandparent	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	1
	great aunt/great uncle	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
	first cousin	0	0	3	17	0	0	0	41	24	3	12	0	10	0	0	3	0	0	13
	first cousin once removed	0	0	0	0	0	0	0	1	6	1	5	0	11	0	7	4	0	0	20
	first cousin twice removed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	second cousin	0	0	0	0	0	0	0	0	2	0	1	0	2	0	1	0	0	0	2
	first cousin thrice removed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	second cousin once removed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	second cousin twice removed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	third cousin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	third cousin once removed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	third cousin twice removed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	third cousin thrice removed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unrelated	0	0	0	0	0	0	0	3	17	12	49	18	94	1	57	63	1	13	664

Supplementary Materials

Table S1: Included as a separate file.

Table S2: 54 geographically diverse samples were obtained from the Coriell Medical Insitute. Samples are grouped by population (left column) and major geographic region (middle column).



Population (if known)	World Region	No. Samples	NGIMS Identifier
Mayan	Americas	3	NA10975 NA10976 NA10978
Quechua	Americas	3	NA11197 NA11198 NA11200
	Americas (South America)	3	NA17317 NA17320 NA17319
Mexican	Americas	3	NA17065 NA17069 NA17067
Druze	Middle East	3	NA11521 NA11522 NA11523
Japanese	East Asia	3	NA11587 NA11589 NA11590
Chinese	East Asia	3	NA16654 NA17846 NA17084
	South East Asia	3	NA17083 NA17084 NA17085
Russian	Europe	3	NA13820 NA13828 NA13821
Hungarian	Europe	3	NA15200 NA15201 NA15203
	Northern Europe	4	NA17004 NA17006 NA17007 NA17008
Indo-Pakistani	South Asia	3	NA17023 NA17024 NA17027
Iberian	Europe	3	NA17093 NA17097 NA17098
Italian	Europe	1	NA17323
Ashkenazi Jews	Europe	4	NA17367 NA22234 NA22306 NA22299
Greek	Europe	3	NA17370 NA17372 NA17371
	Sub-Saharan Africa	3	NA17341 NA17348 NA17342
	North-Saharan Africa	3	NA17379 NA17382 NA17380

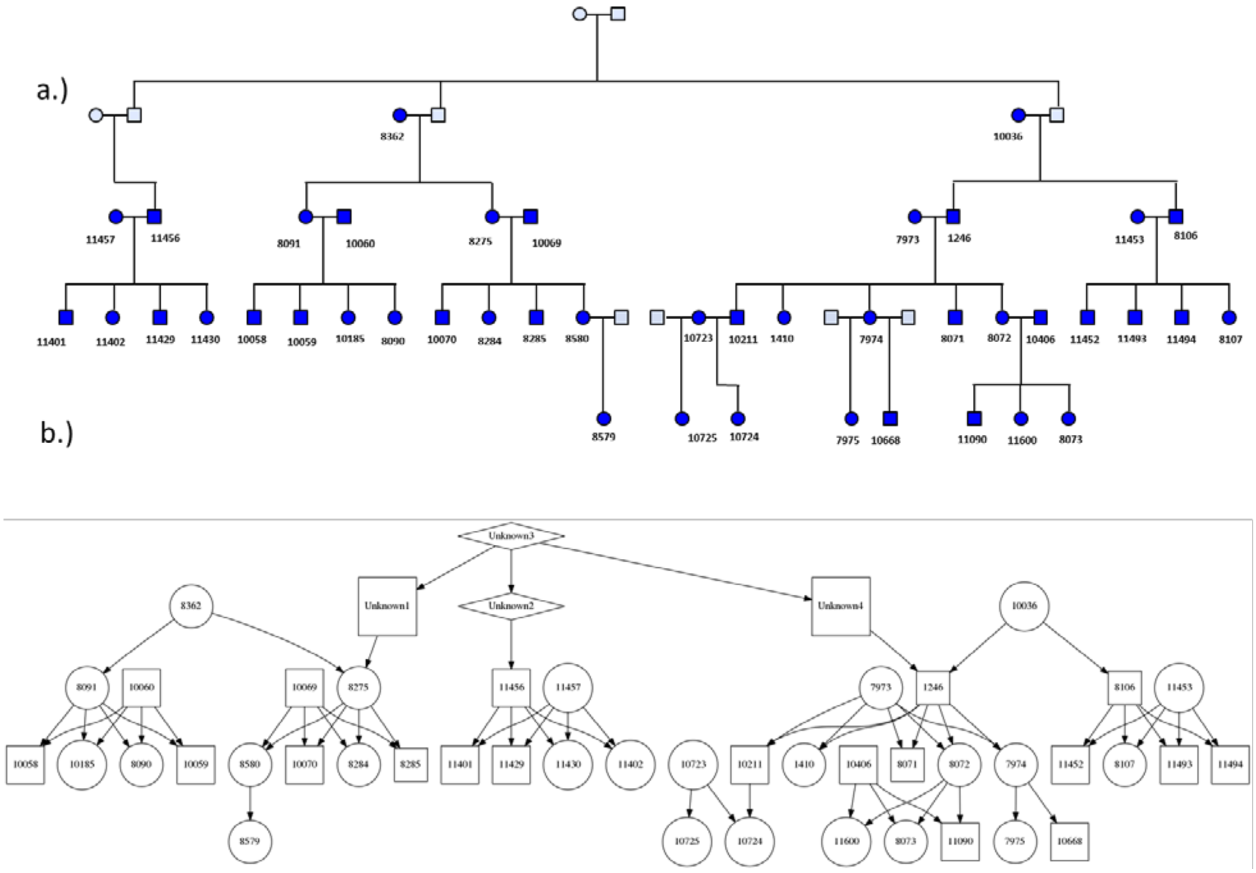
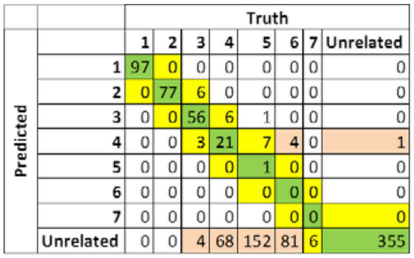


Figure S1: Automatically generated pedigree for 42 individuals for Coriell Family 95. This family, along with a set of 50 geographically diverse individuals, served as training data for the kinship K-means classifier. a.) Reference pedigree. Individuals with available sequence data are highlighted in dark blue. b.) Automatically generated pedigree.

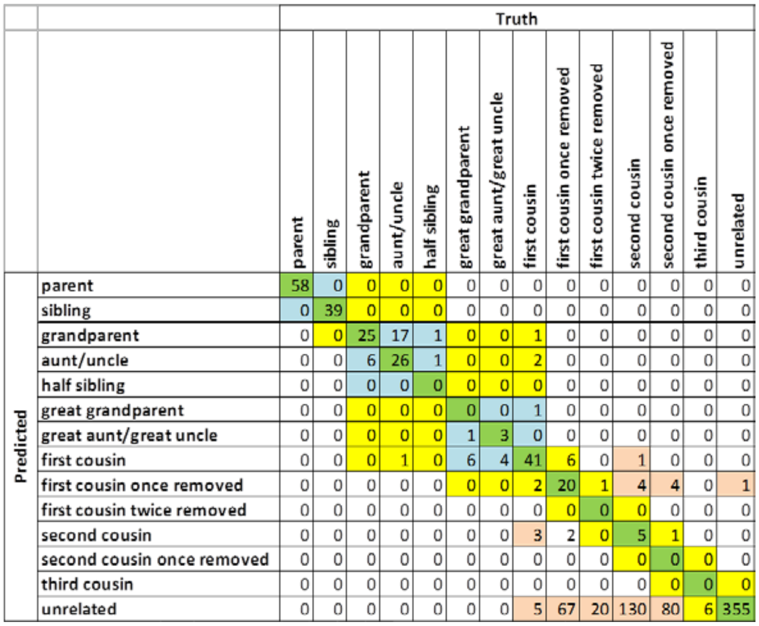
Table S3: Confusion matrices for relationship prediction for 42members of the Coriell Family 95. Correctly predicted relationship pairs are highlighted in green. Predictions that differ by one degree of relationship from the truth are highlighted in yellow. Predictions that differ by more than 1 degree from the truth are highlighted in red. Predicted relationships that differ from the truth but share the same degree of relation are highlighted in blue. a.) Confusion matrix for predicted degree of relationship. b.) Confusion matrix for predicted named relationships.

a.)



		Truth							
		1	2	3	4	5	6	7	Unrelated
Predicted	1	97	0	0	0	0	0	0	0
	2	0	77	6	0	0	0	0	0
	3	0	0	56	6	1	0	0	0
	4	0	0	3	21	7	4	0	1
	5	0	0	0	0	1	0	0	0
	6	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0
	Unrelated	0	0	4	68	152	81	6	355

b.)



		Truth													
		parent	sibling	grandparent	aunt/uncle	half sibling	great grandparent	great aunt/great uncle	first cousin	first cousin once removed	first cousin twice removed	second cousin	second cousin once removed	third cousin	unrelated
Predicted	parent	58	0	0	0	0	0	0	0	0	0	0	0	0	0
	sibling	0	39	0	0	0	0	0	0	0	0	0	0	0	0
	grandparent	0	0	25	17	1	0	0	1	0	0	0	0	0	0
	aunt/uncle	0	0	6	26	1	0	0	2	0	0	0	0	0	0
	half sibling	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	great grandparent	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	great aunt/great uncle	0	0	0	0	0	1	3	0	0	0	0	0	0	0
	first cousin	0	0	0	1	0	6	4	41	6	0	1	0	0	0
	first cousin once removed	0	0	0	0	0	0	0	2	20	1	4	4	0	1
	first cousin twice removed	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	second cousin	0	0	0	0	0	0	0	3	2	0	5	1	0	0
	second cousin once removed	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	third cousin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	unrelated	0	0	0	0	0	0	0	5	67	20	130	80	6	355

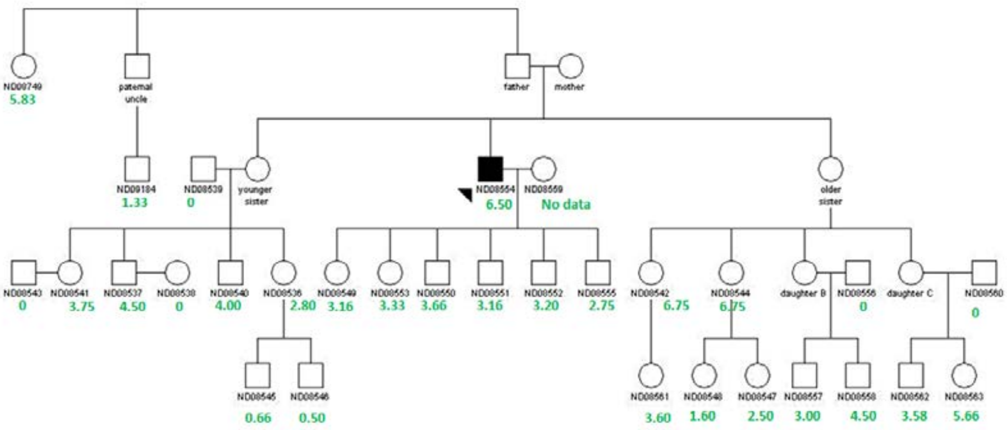


Figure S2: Node degree for the ALS pedigree. Weighted degree computed for each node in the ALS family using the nuclear families derived through the kinship algorithm. Married-in individuals have a degree of 0. Degree is higher for older individuals within a nuclear family.