Sensitive detection of DNA contamination in tumor samples via microhaplotypes

Brett Whitty and John F. Thompson[#1*]

Research and Development, PGDx, 2809 Boston Street, Baltimore, MD 21224

[#1]Current address: Homology Medicines Inc., 1 Patriots Park, Bedford, MA 01730

[*]Corresponding Author: Thompson.john.f@gmail.com

Abstract

Low levels of sample contamination with other human DNAs can have disastrous effects on the accurate identification of somatic variation in tumor samples. Detection of sample contamination in DNA is often based on low frequency variants that indicate if more than a single source of DNA is present. This strategy works with standard DNA samples but can be problematic in solid tumor FFPE samples because there are often huge variations in allele frequency (AF) due to copy number changes arising from gains and losses across the genome. The variable AFs make detection of contamination challenging. To avoid this, we counted microhaplotypes to assess sample contamination. Microhaplotypes are sets of variants on the same sequencing read that can be unambiguously phased. Instead of measuring AF, the number of microhaplotypes is determined. Contamination detection becomes based on fundamental genomic properties, linkage disequilibrium (LD) and the diploid nature of human DNA, rather than variant frequencies. We optimized microhaplotype panel content and selected 164 SNV sets located in regions already being sequenced within a cancer panel. Thus, contamination detection uses existing sequence data. LD data from the 1000 Genomes Project is used to make the panel ancestry agnostic, providing the same sensitivity for contamination detection with samples from individuals of African, East Asian, and European ancestry. Detection of 1% contamination with no matching normal sample is possible. The methods described here can also be extended to other DNA mixtures such as forensic and non-invasive prenatal testing samples where DNA mixes can be similarly detected. The microhaplotype method allows sensitive detection of DNA contamination in FFPE tumor and other samples when deep coverage with Illumina or other high accuracy NGS is used.

31

32 # Introduction

33 In many applications, DNA contamination is not a significant issue because small amounts of
34 extraneous DNA do not affect the experimental outcome. When examining DNA from a single
35 species, it is easy to filter out sequences from other species but within-species contamination is
36 more challenging. When analyzing germ-line variants, a substantial fraction of human DNA from
37 other sources is required to affect variant calls because non-reference calls will be
38 approximately 0%, 50% or 100%. For example, concordance between variant calls made by SNP
39 chips versus pure NGS for high depth samples with 5% contamination fell only from 99.7% to
40 99.2% (1). However, even with low-level DNA contamination, there can be a significant impact
41 on some applications like detecting low frequency somatic variants in tumor DNA samples or
42 deconvoluting complex mixtures in forensic samples. Clinical diagnostic samples are presumed
43 to be pure when they are tested, but there is always a concern that unexpected contamination
44 could affect results and escape standard detection methods. Knowledge of whether a sample is
45 contaminated with another and, if so, the extent of that contamination, is critical for assessing
46 whether somatic variant calls can be trusted.

47 Contamination detection is often carried out by measuring variable lengths of short tandem
48 repeats (STRs) or by genotyping/sequencing single-nucleotide variants (SNVs). When unusual
49 frequencies are observed, the degree of contamination is estimated by quantitating the
50 frequency of the extra variants (2-5). However, accurate estimation becomes difficult at low
51 variant frequency because the true signal can be obscured by the competing noise arising from

52 technical issues like STR stutter or genotyping/sequencing errors. This is especially problematic
53 with analog measurement systems that are often used with STR, genotyping, and classical
54 sequencing methods. In contrast, next-generation sequencing (NGS) methods produce a digital
55 signal, allowing greater sensitivity and accuracy at low contamination levels.

56 In addition to the method described here, there are informatic methods that make use of
57 different types of NGS read data to model whether contamination is likely. These methods are
58 often tied to particular data and/or sample types so may not extrapolate well to other systems
59 like clinical test samples. For example, ContEst (2) is highly sensitive but uses both whole
60 genome and microarray data or matched normal samples. This has recently been replaced by
61 CalculateContamination that relaxes copy number and matched sample requirements.
62 Verifybamid (6) can be used with just NGS data, but its accuracy is dependent on large numbers
63 of SNVs and knowledge of population SNV frequencies and cannot be used with DNA with large
64 copy number changes. It is also computationally intensive; but, VerifyBamID2 was recently
65 developed to address these issues (7). Conpair (8) is much less computationally intensive but
66 still requires large numbers of SNVs as well as tumor-normal pairs. It can be used with exomes
67 or large panels but not smaller panels. Sensitive detection of circulating transplant DNA has
68 been achieved with genome-wide SNVs (9) but this method is hampered when there is copy
69 number variation as is often found in tumor samples. Sehn et al. (10) have taken advantage of
70 closely spaced SNVs that are not in perfect LD, known as microhaplotypes (MH), to assess DNA
71 contamination to a level of 5%. Microhaplotypes can be defined as multiple variants on the
72 same sequence read such that the phasing of the variants is known and the haplotype can be
73 unambiguously determined.

74 Testing clinical tumor samples for somatic variation is complicated by the presence of large
75 regions of the tumor genome that may be highly amplified or deleted. When diagnostic
76 samples have large fractions of the genome with altered copy number, the allelic frequencies
77 (AFs) of SNVs can vary substantially and change from the typical 50-50 AF to much more
78 extreme values. These altered allele ratios can overlap the range of AFs for what is typically
79 seen with contamination and thus a sample that is 100% pure can mistakenly appear to be
80 significantly contaminated if only AF is examined. To avoid these problems, we have optimized
81 an MH counting method that is independent of genotype AF for determining contamination
82 status with tumor DNA and can be carried out with no matching normal sample.

83 For individuals with a normal set of chromosomes, each autosomal region of any size has two
84 sets of alleles. Those alleles may be the same (homozygous) or different (heterozygous); but,
85 under normal circumstances with a few specific exceptions, there should never be more than
86 two haplotypes in a genomic region in any individual. When DNA from two individuals is mixed,
87 accidentally or intentionally, there is a possibility for 1-4 MHs in each region. MHs have been
88 used for forensic purposes to detect contamination and identify individuals (11-17), to detect
89 transplant DNA in a host background and contamination in tumor samples (18), and to
90 determine ancestry (19-23). Use of MHs for routine detection and estimation of contamination
91 in clinical FFPE tumor samples is a powerful application of these methods.

92

93 # Materials and Methods

Sequencing Samples

849 de-identified lung tumor samples and DNA from lung tumors were obtained from several commercial and academic providers. These included BioIVT, Conversant Bio, Cureline, Duke, Folio Bio, Fundacio Institute, Indivumed, iSpecimen, NCI, and Proteogenex. Samples were received either as FFPE material or extracted DNA. If necessary, extraction of DNA from FFPE specimens was carried out using standard kits. DNA was prepared for sequencing using hybridization capture with panels directed primarily at exons in >500 genes implicated in cancer (see https://www.personalgenome.com/assets/resources/elio-tissue-complete-brochure.pdf for a listing of genes). For some genes, intronic and promoter DNA was also targeted. DNA was sequenced using an Illumina NextSeq500. The PGDx elio™ tissue complete assay was used for sample preparation and analysis (24, 25). 15 samples were sequenced on standard runs. For a run to pass, at total of >90 GB sequence with >75% of reads at Q30 or above was required. For an individual sample to pass, >90% of targeted regions were required to have >100x median coverage. Individual reads were analyzed as described (26) with a base quality filter applied to only include bases with a reported Phred quality score >30. Because erroneous reads could artificially inflate the number of $3^{rd}/4^{th}$ MHs, MH calculations included 3rd MHs only if they surpassed a minimum fraction of total calls. We usually set this threshold at >0.2% based on the typical error rate for Illumina NGS (~0.1%). Higher values can be used to reduce noise but that is accompanied by a loss in sensitivity.

Each accepted read covering the positions of all SNVs for each MH set was binned into the appropriate haplotype and counted using Samtools mpileup. After read alignment, mpileup was

115 applied to the first region, the second region, and, if present, the third region. mpileup reads
116 are then combined and separated by basecalls. Each resultant set of base calls (MHs) is then
117 counted to determine MH frequency.

118 *In silico* read mixing

119 Artificially contaminated samples were made by *in silico* mixing of reads at defined
120 contamination levels. Contamination levels of x% were generated by combining randomly
121 selected 100-x% reads from the sample with x% random reads from the contaminant and
122 running the resultant reads through the standard analysis pipeline. It was found that the same
123 results were achieved if only the reads mapping to the MH regions being examined were
124 included in the mixing. These analyses involved much less data so ran on the pipeline much
125 faster. Thus, later experiments used only the truncated data set to speed analysis and allow
126 more conditions to be tested.

127

128 Results

129 DNA samples and MAF-based contamination detection

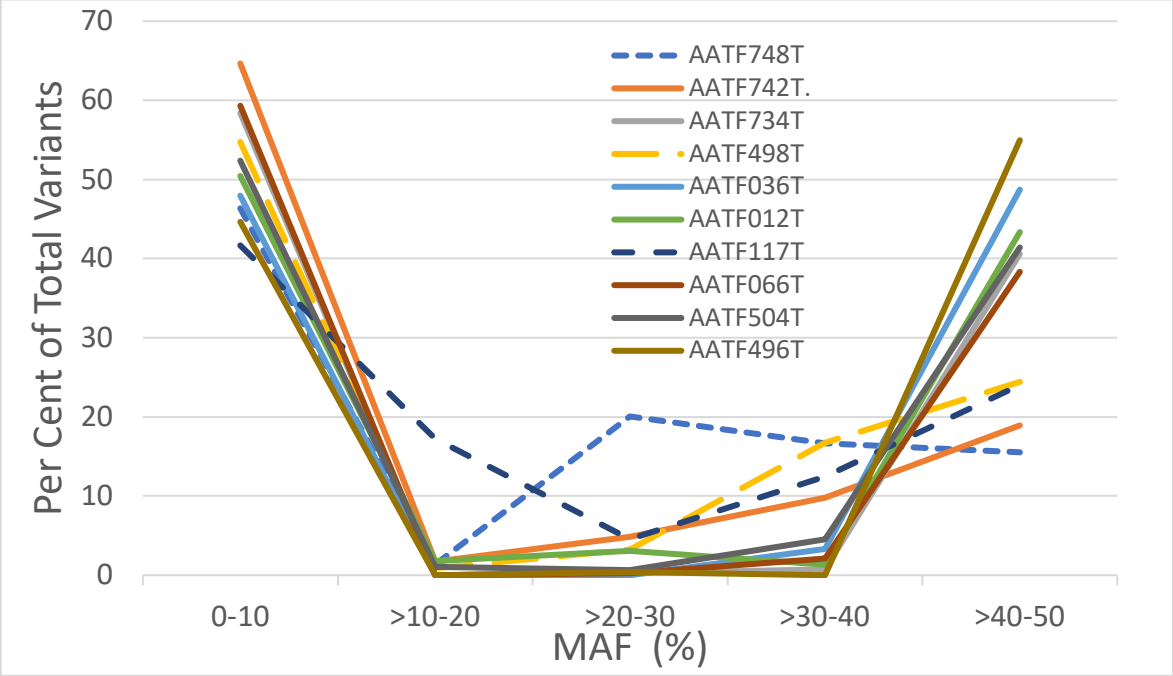
130 849 FFPE tumor DNA samples were examined, but most studies focused on the 51 samples
131 listed in Table 1. All samples that passed QC were amenable to contamination testing, but the
132 number of samples analyzed for mixing had to be limited due to the high compute
133 requirements for analyzing the many *in silico* mixtures. The mixing samples were chosen based
134 on being the most apparently pure and equally representing three of the five geographic

135 ancestral groups described by the 1000 genomes project (African, East Asian, European). DNA
136 quality was highly variable which primarily impacted mixing studies when two samples of much
137 different quality were mixed.

138 Prior to using MHs, we had assessed DNA contamination based on finding low level germline
139 SNVs that were assumed to come from contaminating DNA. However, FFPE tumor samples can
140 have high levels of copy number variation that can have a serious impact on germline SNV AFs
141 as shown in Fig 1. For 10 FFPE tumor samples, all variants in the VCF with a dbSNP number were
142 examined. Having an assigned dbSNP number was used as a surrogate for variation that is most
143 likely real since it has been observed previously. Variants were sorted by MAF into 10% bins.
144 Most samples had the expected distribution where >80% of the variants had 0-10% MAF with
145 this low-level variation arising from sequencing errors, FFPE artifacts or somatic variants. In
146 addition, there are also many with 40-50% MAF arising from heterozygous germline variants.
147 However, three samples had much more widely distributed MAFs including one sample
148 (AATF748T) where >20% of the variants had MAFs between 20 and 30%. These are germline
149 variants as confirmed by matching normal DNA samples that were also sequenced for these
150 samples. When we used a method for detecting DNA contamination based on low frequency
151 germline variants, these samples were discarded based on supposed high contamination levels
152 when, in fact, they are close to 100% pure based on MH analysis. The “contamination” signal is
153 actually copy number variation.

154 **Figure 1 MAF for 10 FFPE tumor samples**

155 All variants with dbSNP identifiers were binned by AF for the less common allele. Bins were 10%
156 ranges. These samples had 348-561 such variants in the VCF with the vast majority being
157 germline as confirmed by matching normal samples. The percentage of variants in each bin is
158 shown. Additional information about these samples is in Table 1.



159

160 **Choice of variant sets for microhaplotype analysis**

161 There are many MHs that have been characterized in the literature, and their usefulness for
162 distinguishing individuals and ancestries is well documented (13, 20, 23). However, most of the
163 well-characterized MHs are not in regions chosen for commercial cancer panel sequencing. In
164 addition, the criteria for selecting MHs for discriminating individuals and ancestry are not the
165 same as for detecting contamination where the goal is not distinguishing individuals but
166 maximizing the number of MHs with potentially >2 versions. Since high depth sequencing is
167 required for sensitive contamination detection using this approach, regions already targeted for

168 analysis were examined so that additional, non-productive sequencing in MH regions outside of
169 the pre-existing panel could be avoided. For gene panel sequencing, this means that literature
170 MH data is of limited value. Each novel MH set we chose was tested for behavior across
171 samples and ancestries. For these experiments, we have used a >500 gene, 2.23 Mb cancer
172 panel directed against FFPE tumor DNA
173 (https://www.personalgenome.com/assets/resources/elio-tissue-complete-brochure.pdf), but
174 any pre-existing panel can be examined in a similar manner to identify useful SNV sets. This
175 cancer panel included regions outside of exons and these regions were also inspected for
176 potentially informative SNV sets.

177 Candidate MH sets were chosen manually, but these steps can be automated if many or larger
178 panels are desired. The criteria for inclusion in the initial set of variants to be tested included
179 maximizing the frequency of 3rd MHs while minimizing the likelihood of sequencing errors in
180 those variants. This was achieved by avoiding regions with higher inherent sequencing error
181 rates such as insertions, deletions, and variants within homopolymeric regions and other
182 repetitive elements. The regions targeted for cancer panel sequencing were examined in the
183 gnomAD database (gnomad.broadinstitute.org/; 27) for multiple SNVs within 200 bp of the
184 targeted regions and of each other and with 3-97% AF. The desired final AF (5-95%) was higher,
185 but we wanted to ensure that MHs from populations underrepresented in gnomAD could still
186 be identified. The gnomAD database, at the time initially queried, had a higher proportion of
187 individuals of European ancestry (~60%) than individuals with other geographic ancestries.
188 Because of this, variation in European ancestry individuals had a larger impact on the initial
189 choice of SNVs than variation in other ancestries. Thus, there is a possibility that samples from

190 different ancestral backgrounds could respond differently when not filtered based on ancestry.
191 Once the candidate MH sets were identified, they were balanced for ancestry so that detection
192 of contamination in all groups would behave similarly.

193 Variants not in a segmental duplication or a low confidence region as defined by gnomAD were
194 examined for LD in the 1000 Genomes Project samples (ldlink.nci.nih.gov/?tab=ldhap; 28). This
195 site provides data on the frequency of each haplotype in each ancestry. Pairs and triplets of
196 SNVs with at least three haplotypes and the 3rd and greater haplotypes having a total frequency
197 of >5% in any individual ancestral grouping in the 1000 genomes project were advanced for
198 evaluation in real samples sequenced as described. SNV sets with median coverage >250x for all
199 relevant variants on individual reads were examined further. SNV sets with high frequency
200 3rd/4th MHs in many purportedly pure samples were eliminated because they might produce
201 high noise relative to signal. With these samples, SNV sets with more than 5 samples with >2
202 MHs were eliminated (26 of 264 evaluated SNV sets). SNV sets in close physical proximity to
203 each other in high LD would provide duplicate information. LD was considered too high if the
204 haplotype frequency was unchanged when the SNVs were combined and examined together.
205 SNV sets with shorter homopolymers, higher coverage, and fewer 3rd/4th MHs in pure samples
206 were included in the panel when high LD alternatives were considered. SNV sets that passed
207 these initial evaluations are shown in Supplementary Table 1. In addition to the genomic
208 coordinates and dbSNP identifiers, the frequency of MHs is shown for the five major ancestry
209 groupings defined by 1000 Genomes as well as the median coverage for the relevant SNVs
210 across 849 samples, the number of times the SNV set generated more than 2 MHs in “pure”
211 samples, and the DNA length separating the SNVs.

212 Once these criteria were met, SNV sets were balanced for ancestral frequency. While it would
213 have been desirable to use all population groupings from 1000 Genomes in this analysis, there
214 were not enough SNV sets to allow balancing of all five major ancestry groupings so only data
215 from African, East Asian, and European ancestry were assessed to achieve balanced variation.
216 SNV sets that adversely affected ancestry balance were eliminated. The balanced panel of 164
217 SNV sets is shown in Supplementary Table 1. The average frequency of $3^{rd}/4^{th}$ MHs was 13.9%
218 for each of the three balanced ancestries.

219

220 Assessing contamination and estimating levels

221 In order to determine how best to detect contamination, *in silico* mixing of reads from
222 individual pure samples was carried out so that the precise level of contamination would be
223 known and the sensitivity and accuracy of the MH assay could be determined. The initial
224 samples used for establishing contamination methods need to be as close to 100% pure as
225 possible for maximizing sensitivity. 51 samples were chosen for *in silico* mixing experiments to
226 examine this in more detail. Samples with self-declared ancestry were chosen to achieve
227 ancestral diversity and the fewest number of >2 MH sets. The performance of 51 reportedly
228 pure samples from individuals of known, self-declared ancestry is shown in Table 1.

229 The self-declared ancestry of samples was confirmed using Principle Component Analysis (PCA)
230 of the SNV sets based on 1000 Genome MH frequencies. The self-declared and PCA-determined
231 ancestries of all samples agreed. However, with some samples, the self-declared ancestry was
232 Asian and the PCA-determined ancestry was South Asian rather than East Asian. For analysis of

233 individuals who listed Asian ancestry, only samples with PCA-confirmed East Asian ancestry
234 were used in analyses. In addition to diverse ancestral histories, samples were selected for
235 mixing experiments based on having the fewest SNV sets with >2 MHs.

236 For these samples, the mean number of 3rd/4th MHs is over 20. Because these samples are
237 supposed to be pure, the 3rd/4th MHs are likely often due to artifactual false calls that arise due
238 to the high coverage depth for many SNV sets or FFPE-induced errors. This is supported by the
239 mean 3rd MH frequency which is only 0.003 for the panel. To minimize the number of artifactual
240 3rd MHs, we set a lower limit of 0.2% for counting 3rd/4th MHs based on the typical Illumina
241 error rate of ~0.1%. Using a threshold of >0.2% eliminates over 1/3 of 3rd MHs in these pure
242 samples, most of which are likely to be artifactual. When this threshold is employed, the
243 median 3rd MH frequency increases but remains less than 0.6% for all samples with >20 3rd
244 MHs. The need for a minimum threshold for the number of 3rd/4th MHs is highlighted by the
245 three examples from these 51 samples where the median 3rd MH frequency is >0.01. All three
246 have five or fewer 3rd MHs, suggesting that outlier artifactual MHs are driving the median
247 frequency when few real 3rd MHs are present. Based on this concern, we instituted a minimum
248 threshold of 25 SNV sets with >2 MHs with frequency >0.2%. 25 SNV sets with >2 MHs is not
249 intended to distinguish contaminated from pure samples but rather ensure that the 3rd MH
250 frequency is more reliable. 5 of the 51 pure DNAs in Table 1 have > 25 3rd/4th MHs (>0.2%
251 minimum threshold for inclusion as a 3rd MH). This threshold should never eliminate a truly
252 contaminated DNA but is intended only to ensure there are enough SNV sets to establish a
253 reliable 3rd MH median frequency. The low rate of 3rd/4th MHs across pure samples support the
254 concept that, at least at the sites analyzed, NGS and FFPE errors occur at a very low rate. All *in*

255 *silico* mixtures tested at the 0.5% contamination level using the samples in Table 1 had more
256 than 25 MHs. As seen in Fig 2, the average value for the number of 3rd MHs among all
257 ancestries with 1% contamination is >45 with this panel (range 29-69). Thus, this threshold
258 should not eliminate any samples contaminated at >0.5% and allows the 3rd MH frequency
259 value to be more reliable.

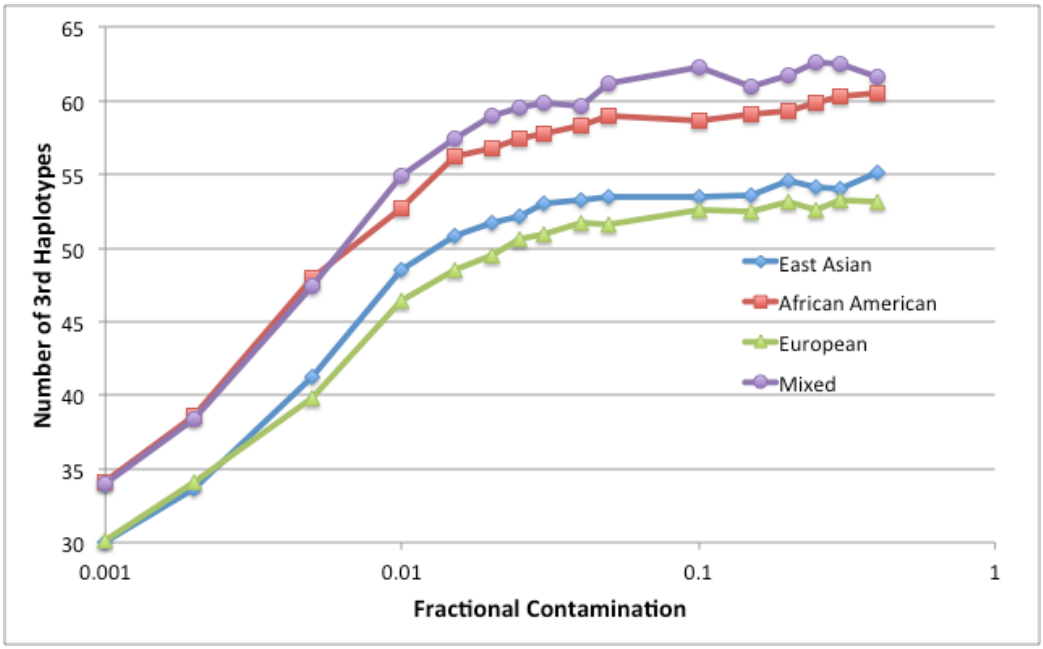
260 Precise levels of read mixing can be generated *in silico* but the functional level of contamination
261 was found to often differ significantly from the known mixing proportions. This issue was
262 particularly obvious when samples with divergent DNA qualities were mixed. The longer, higher
263 quality DNA appeared to have a higher concentration than what had been added to the mix.
264 This arises because longer reads map better and are more likely to contain all the variants being
265 examined and thus this DNA appears to be relatively more frequent than the shorter, more
266 degraded DNA. This is consistent with previous results that showed DNA with reduced
267 extraction yields tend to be lower quality and more susceptible to contamination (10). To
268 minimize the impact of this effect, all DNA mixes were examined in a pairwise fashion where
269 one sample was the primary sample and the second was the contaminant. These roles were
270 then switched and the results averaged. This allowed an over-contamination with one sample
271 set to be partially mitigated by the paired under-contamination of the opposite sample set.

272 15 DNA sets from each ancestry were mixed as sample/contaminant pairs with averaged results
273 shown in Fig 2. As expected, the East Asian fraction of >2 MHs was nearly identical to the
274 European results. The African American samples that we examined had a higher frequency of
275 >2 MHs than the other groupings, but nearly identical to the samples that were mixed across
276 ancestries. This is predicted by 1000 Genomes data. Their subgroupings of African ancestry

277 included seven different sub-populations, only one of which was US-based. When the African
278 American subgrouping is compared to the other African populations, it has a higher 3rd/4th MH
279 frequency (0.155 vs 0.139, Supplementary Table 1). The higher frequency in our population
280 reflects the fact our African ancestry samples are primarily from African American individuals.

281 **Figure 2 Number of SNV sets with >2 MHs for individual samples**

282 The number of 3rd MHs for pairs of individuals of East Asian, African American, and European
283 ancestry is shown for mixes of 0.1 – 40%. Mixing results from pairs of individuals with different
284 ancestry is included as "Mixed", all on a log scale.



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286 Even at the lowest tested contamination level, 0.1%, the mean number of 3rd/4th MHs is over 30
287 compared to 13 (range 2-39) for the pure samples without mixing (Table 1). At 1%
288 contamination, the mean number of 3rd/4th MHs is over 45 for all groupings with a range for
289 individual mixes of 29-69. Because a small number of 3rd/4th MHs can cause a median values to

290 be impacted greatly by outliers, we have set a minimum threshold for the number of 3rd/4th
291 MHs before a contamination level is calculated. Based on these data, 25 is set as the minimum
292 number before a sample is considered potentially contaminated because it is less than all
293 samples mixed at 1% and high enough to minimize the impact of individual outliers.

294 While the number of 3rd MHs is relevant for assessing whether a sample is potentially
295 contaminated, it is less useful for estimating the level of contamination as it reaches a
296 maximum around 2% contamination with these coverage levels. In contrast, median 3rd MH
297 frequency changes as a function of contamination level so is more useful in this regard.

298 The *in silico* mixing studies provide an empirical calibration, but it is also useful to understand
299 the theoretical basis for those findings. As shown in Fig 3, the expected frequency of the
300 contaminating 3rd MH depends on the nature of the starting and incoming genotypes. Some
301 combinations will generate no 3rd MH, others will generate a 3rd MH with a frequency half the
302 level of incoming contaminant, and others will generate a genotype that is the same frequency
303 as the contaminant. Since most variant combinations will be at the 50% level, examination of
304 the median value for the 3rd MH should yield a value that is half of the true contamination level.

305 **Figure 3 Expected frequencies for 3rd MHs based on genotype**

306 A primary sample can be either homozygous for one MH or heterozygous for two versions. For
307 an incoming contaminating sample with at least one third MH, there are four different
308 possibilities for each starting primary sample as shown. Most of these generate a
309 contamination signal at half the frequency of the starting DNA. When the four alleles are all
310 different, the 3rd MH comes in at half the frequency while the combined 3rd/4th frequency is 1x.

311 If there is a 4th MH, it should have the same frequency as the 3rd MH as long as there are no

312 technical artifacts.

Primary Sample		Contaminant (X)		Rate of 3rd MH Accumulation
MH1/MH1	+	MH1/MH3	→	no 3rd MH
		MH2/MH3	→	0.5 X
		MH3/MH3	→	no 3rd MH
		MH4/MH3	→	0.5 X
MH1/MH2	+	MH1/MH3	→	0.5 X
		MH2/MH3	→	0.5 X
		MH3/MH3	→	1X
		MH4/MH3	→	0.5X

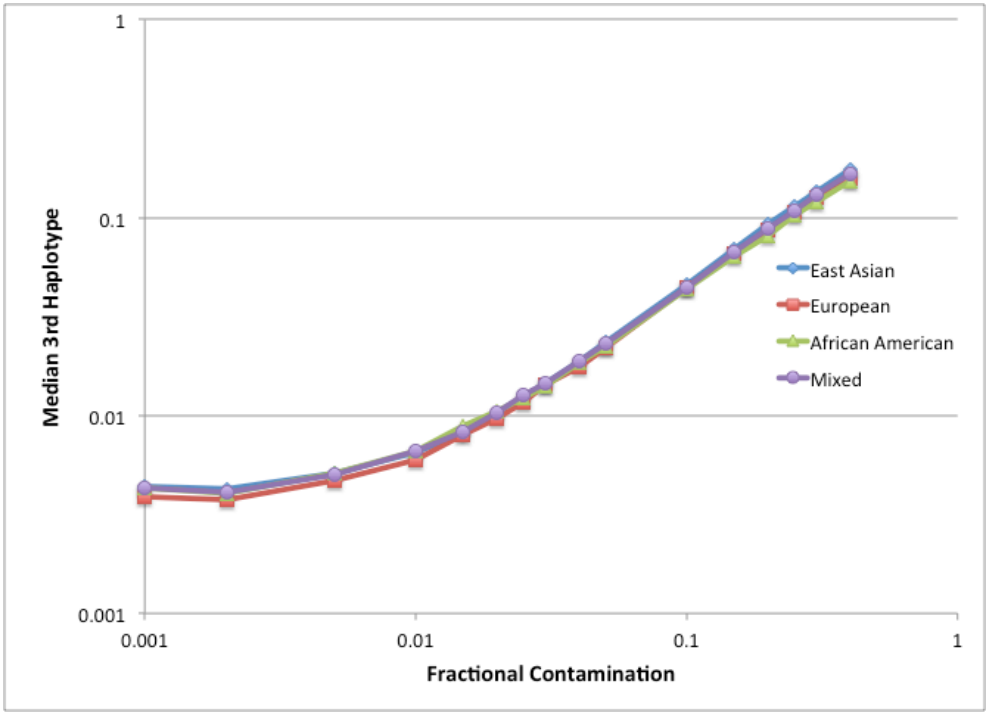
313

314 **Figure 4_ Contamination Detection as a function of ancestry**

315 The median frequency of 3rd MHs for pairs of individuals of East Asian, African American, and

316 European ancestry is shown. Results from pairs of individuals with different ancestry is included

317 as "Mixed", all on a log scale for mixing levels of 0.1 – 40%.



The primary limitations on the sensitivity of contamination detection are the practical sequencing depths for the SNV sets in question and the inherent error rates for sequencing. The higher the sensitivity desired, the greater sequencing depth required. The usefulness of added depth is limited by sequencing error rates that are typically on the order of 0.1% for Illumina. It is possible to employ various error correction methods to improve sensitivity, but the need for that is dependent on the sensitivity required for the application. In addition, there is no value in sequencing a sample more deeply than the starting number of input molecules, which can be a limitation in some situations. Based on these considerations, we have aimed for detection of contamination at least as low as 1% in these samples.

As shown in Fig 4, the median 3rd MH level is independent of ancestry with this panel. All three ancestries and mixed samples behave nearly identically. These data are combined in Supplementary Table 2. 15 sample/contaminant pairs were tested for each ancestry with the

331 same number of each mixed ancestry pairs. When all samples are aggregated, 40% of samples
332 with 0.5% contamination were detected. If the threshold is set at 1%, all contaminated samples
333 were detected. The detection threshold can be set wherever required based on the detection
334 needs and the likely impact of contamination on final results. However, the core limitations of
335 sequencing accuracy, coverage depth, and input DNA molecules combine to ultimately limit
336 sensitivity.

337 In addition to detecting contamination, it is also possible to use this method to identify sources
338 of contamination. A partial set of genotypes for the contaminating DNA can be generated from
339 the MH data and then matched with samples that have been tested previously or
340 contemporaneously to determine if they are the source of contamination. In a contaminated
341 sample, MHs with 1 or 4 different haplotypes can be assigned their correct genotypes directly.
342 MHs with 2 or 3 different haplotypes can generally be assigned a partial or complete genotype
343 based on the frequency of the individual MHs. The frequency of the $2^{nd}/3^{rd}$ haplotypes will
344 provide at least one and sometimes two genotypes corresponding to the contaminant.
345 Comparison of the known and partial genotypes with other samples allows them to be ruled in
346 or out as potential sources of contamination. This procedure has been applied to multiple
347 contaminated samples to successfully determine the source in real clinical laboratory
348 situations. Often, the contamination source can be shown not to arise from any samples known
349 to have been handled in the laboratory so the contamination must have originated prior to
350 sample receipt.

351

352 # Discussion

353 Cross-contamination of DNA samples is a well-known issue among clinical testing laboratories
354 and hence standard clinical testing guidelines generally suggest that precautions should be
355 taken to be prevent it. Two recent guidelines for somatic variant testing (29, 30) recommend
356 checking for handling-induced contamination and, suggest some possible methods for
357 detecting contamination (6, 10, 31). However, none of the methods mentioned is sensitive
358 enough to ensure that somatic variants (32) can be distinguished from sample contamination.
359 Thus, simple, sensitive methods for reliable detection of contamination are needed.

360 The degree of DNA contamination that causes problems varies depending on the application. In
361 some cases, the impact of contamination on results is obvious. With other applications, signals
362 arising from contamination can be easily confused with real signals. With diagnosis of somatic
363 variants in cancer, this is especially a problem because variant identification is attempted at and
364 below the very limits of the NGS technology. Being able to separate the tumor signal from any
365 contamination noise is critical for the proper diagnosis of tumors. Because tumor samples often
366 have highly unusual copy number patterns, use of simple genotype frequencies can cause
367 errors in contaminant detection. In our experience, many FFPE samples that had been
368 determined to be contaminated using raw genotype frequencies were found to be pure by MH
369 analysis (Fig 1). In contrast to FFPE tumor samples, circulating-free DNA (cfDNA) purified from
370 the plasma of cancer patients is not as prone to the same issues. Most cfDNA purified from
371 plasma is from normal cells so typical Mendelian ratios are observed with variants. MH analysis
372 in such samples is still valuable for sensitive contamination detection, but such samples are not

373 as likely to generate false positives as DNA from FFPE tumor samples where copy number
374 variation is much more significant because of the high tumor DNA content.

375 For most purposes, the approximation that the percent contamination is twice the median
376 frequency for the 3rd MH is sufficient. This relationship arises from the likelihood that a 3rd MH
377 is most likely heterozygous so is present on only one of the two incoming alleles. The
378 uncertainty around this probability introduces the potential for multiple sources of error and
379 those should be considered if a more exact measurement is necessary. At low coverage, two
380 confounding factors arise. First, low coverage leads to high AF variability due to the stochastic
381 nature of read accumulation in poorly covered SNV sets. Only SNV sets with 3rd MHs are
382 included in the median calculation rather than all SNV sets so, at low coverage, AF inflation
383 occurs among the variants that are counted. The observed AF is higher than reality because
384 there are not enough reads to generate the proper AF value. By requiring a large number of 3rd
385 MHs prior to calling a sample contaminated, this effect is mitigated. Superimposed on this
386 effect is the counting of homozygous 3rd MHs before heterozygous 3rd MHs as coverage
387 increases. If coverage is deep, this is not an issue but, at low coverage, homozygous variants will
388 appear first because they are being double counted. Further complicating the relationship is the
389 presence of low frequency technical artifacts and the copy number issues that can occur with
390 cancer samples. The copy number variations will make SNV sets appear at different fractional
391 rates depending on whether the 3rd MH is under- or over-represented. The more SNV sets used
392 in the calculations, the more likely it is that these effects can be averaged out.

393 The MH approach is perfectly suited for use when somatic variants are sought because such
394 studies use very high coverage in order to detect rare variants. The sensitivity of contamination

395 detection is limited by the coverage of the regions used in the analysis. Other panel-based and
396 exome NGS approaches can be made more reliable by using similar contamination detection
397 methods. A typical whole genome approach with less than 50x coverage would not be
398 amenable to sensitive detection using individual MH sets because the low coverage would
399 prevent the observation of infrequent 3rd MHs. To overcome this, combinations of MH sets
400 could be used. The simplest approach would be to combine SNV sets that are in high LD. Other
401 methods for pooling SNV sets could also be used.

402 The theoretical limitation on the sensitivity of the MH assay is the number of DNA molecules
403 available for study. The assay can only be as sensitive as the amount of input DNA allows. In
404 addition to that limitation (which is significant for some samples), there are also technology
405 limitations. When looking at a pair of SNVs, either one could be called incorrectly resulting in
406 false 3rd MHs. If panels with 3 or more SNVs in close proximity were available, this would
407 minimize such errors. However, there are other potential errors introduced in NGS processing.
408 It is not unusual for barcodes used for multiplexing samples in the same run to be
409 contaminated at 1% or more, resulting in misassignment of reads to samples. Index hopping
410 and chimeric PCR reads can also affect sensitivity (33, 34).

411 The same techniques used for these cancer samples can also be used with forensic samples to
412 detect contributors to a sample. Unlike tumor samples, copy number is not an issue. Like tumor
413 samples, the major and minor contributors to a sample may have different DNA qualities that
414 can affect the accuracy of MH frequencies, but this is less important in forensic situations.
415 Panels designed with a larger number of >4 potential MHs would be appropriate when forensic
416 samples are being examined to clarify the number of contributors.

417 Another useful application of the MH methods is for detection of CNV anomalies in fetal DNA in
418 Non-invasive prenatal testing **(**NIPT). MH methods are already in use for paternity testing (35-
419 38). For NIPT, panels can be designed to focus on genomic regions with the most likely copy
420 number variation (e. g. chr 5, 13, 18, or 21) and the frequency of 3rd MHs in targeted regions
421 compared to the rest of the genome. In genomic regions where the mother is heterozygous and
422 the father's contribution is a different allele, there will be a low frequency 3rd MH.

423 The methods described here highlight the factors that are important for examining DNA
424 mixtures in a variety of contexts. The use of MHs in these applications requires different
425 properties than other uses (39). Most early studies with MHs focus on distinguishing individuals
426 and assigning ancestry. The MH properties required in those situations are different with a
427 need for MHs specific for particular populations. With MHs used for contamination detection, it
428 is preferable to have MHs that are common to all populations so that detection is uniform
429 across individuals. The methods described herein allow the appropriate choice of MHs leading
430 to the ability to detect low levels of secondary DNA, which can be valuable in many
431 applications.

432

433 *Abbreviations*

434 **AdAm:** Admixed American

435 **AF:** Allele Frequency

436 **AfAm**: African American

437 **Afri:** African

438 **cfDNA:** circulating free DNA

439 **EaAs:** East Asian

440 **Euro:** European

441 **FFPE:** Formalin-fixed Paraffin-embedded

442 **FP:** False Positive

443 **LD:** Linkage Disequilibrium

444 **MH:** Microhaplotype

445 **NGS:** Next-generation Sequencing

446 **NIPT:** Non-invasive Prenatal Testing

447 **PCA:** Principle Component Analysis

448 **SNV:** Single Nucleotide Variant

449 **SoAs:** South Asian

450 **STR:** Short Tandem Repeat

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552 **Table 1 Microhaplotypes and Coverage for Pure Samples**

553 Performance of the 164 SNV set panel is shown with 51 pure samples with known ancestries. Comparisons include the median and

554 average coverage, the number of SNV sets with 1-4 MHs, and the median frequency of 3rd MHs among individuals of African (Afri),

555 East Asian (EaAs), and European (Euro) ancestry as defined by the 1000 Genomes project. The number and median 3rd MH

556 frequency for MHs with frequency >0.2% is shown. ND=Not Done; NA=Not Applicable

557

			Number of MHs									
Sample	M/F	Ancestry	1	2	3	4	Med 3rd MH	# 3rd MHs >0.002	Med 3rd >0.002	#	Med Cov	Ave Cov
AATF748T		Afri	52	87	21	3	0.0033	18	0.0081	163	313	620
AATF094T	F	Afri	36	90	36	0	0.0027	29	0.0031	162	421	533
AATF115T	F	Afri	67	82	12	0	0.0027	7	0.0059	161	202	286
AATF066T	F	Afri	48	92	21	0	0.0041	17	0.0044	161	305	337
AATF117T	F	Afri	37	##	22	1	0.0053	18	0.0065	163	439	605
AATF498T	F	Afri	68	76	19	0	0.0018	8	0.0037	163	674	959
AATF308T	M	Afri	42	96	24	0	0.0022	14	0.0033	162	531	683
AATF562T		Afri	46	##	14	1	0.0021	8	0.0029	163	574	706
AATF486T	F	Afri	39	88	36	0	0.0023	21	0.0034	163	709	924
PGRD0454T	M	Afri	58	90	14	0	0.0018	6	0.0027	162	797	930
AATF218T	F	Afri	42	88	33	1	0.0029	24	0.0034	164	405	555
AATF778T		Afri	60	91	11	1	0.0023	7	0.0055	163	462	520

AATF219T	M	Afri	33	95	34	1	0.0022	19	0.0039	163	553	668
AATF217T	M	Afri	57	90	15	0	0.0018	5	0.0029	162	609	996
AATF073T	F	EaAs	74	78	12	0	0.0021	7	0.0028	164	415	726
AATF503T	F	EaAs	62	76	24	1	0.0031	16	0.0037	163	426	545
AATF504T	M	EaAs	70	84	8	1	0.0042	9	0.0042	163	517	758
AATF027T	M	EaAs	58	92	13	1	0.0024	8	0.0052	164	331	443
AATF012T	F	EaAs	66	91	7	0	0.0029	5	0.0050	164	542	826
AATF029T	M	EaAs	42	74	46	1	0.0033	37	0.0035	163	379	590
AATF024T	F	EaAs	31	82	48	2	0.0037	39	0.0051	163	452	724
AATF023T	M	EaAs	33	91	36	3	0.0025	26	0.0028	163	500	765
AATF022T	M	EaAs	42	92	30	0	0.0023	19	0.0046	164	510	768
AATF021T	M	EaAs	56	78	29	0	0.0027	19	0.0041	163	528	792
AATF733T	F	EaAs	53	93	17	0	0.0047	12	0.0086	163	170	257
AATF595T	F	EaAs	53	84	27	0	0.0038	19	0.0098	164	201	390
AATF742T	F	EaAs	51	##	10	0	0.0020	5	0.0128	163	210	352
AATF734T	M	EaAs	60	89	13	1	0.0029	11	0.0032	163	344	431
AATF594T		EaAs	64	92	7	0	0.0028	5	0.0030	163	390	533
AATF735T	M	EaAs	68	88	6	1	0.0025	5	0.0027	163	399	614
AATF597T	F	EaAs	54	94	14	1	0.0021	8	0.0062	163	399	502
AATF606T	F	EaAs	62	85	15	0	0.0023	10	0.0029	162	443	659
AATF601T	F	EaAs	58	85	20	0	0.0022	11	0.0030	163	457	570
AATF599T	F	EaAs	58	84	21	1	0.0025	15	0.0042	164	487	648
AATF600T	F	EaAs	52	94	16	1	0.0024	10	0.0028	163	605	752
AATF417T	M	Euro	67	90	5	1	0.0142	5	0.0176	163	187	239
AATF391T	M	Euro	47	95	20	1	0.0019	10	0.0023	163	476	754

AATF375T	M	Euro	34	97	32	1	0.0052	30	0.0056	164	305	463
AATF088T	F	Euro	41	99	21	1	0.0029	16	0.0038	162	288	406
AATF389T	F	Euro	39	94	30	1	0.0021	16	0.0030	164	446	741
AATF567T	M	Euro	68	84	10	0	0.0035	8	0.0053	162	312	704
AATF107T	M	Euro	51	98	13	1	0.0034	9	0.0066	163	445	518
AATF079T	F	Euro	58	94	10	0	0.0016	4	0.0041	162	494	641
AATF496T	M	Euro	62	94	8	0	0.0018	2	0.0322	164	601	674
AATF036T	M	Euro	44	97	20	2	0.0029	15	0.0030	163	538	603
AATF042T	M	Euro	39	93	31	1	0.0020	16	0.0027	164	613	747
AATF039T	M	Euro	49	##	11	0	0.0020	5	0.0035	162	693	842
AATF038T	F	Euro	50	94	16	2	0.0015	7	0.0031	162	673	789
AATF731T	F	Euro	59	87	17	1	0.0024	12	0.0027	164	352	495
AATF378T	F	Mix	52	94	17	1	0.0028	12	0.0042	164	418	707
AATF097T	F	Mix	35	98	30	0	0.0035	22	0.0042	163	416	486
Median			52	91	17	1	0.0025	11	0.0038	163	445	641
Mean			52	90	20	1	0.0030	13	0.0052	163	450	623

560 **Supplementary Table 1 Detailed characterization of MH sets**

561 The genomic locations of all SNV sets tested are shown in column 1. Median coverage in 849 samples and the number of instances
562 of more than 2 MHs are shown in the next columns. The distance between SNVs and the dbSNP identifiers are listed in the next
563 columns. The final columns show the sum of frequencies for all 3rd and 4th MHs from the 1000 genome database and the reason for
564 why SNVs were removed from the final panel, if applicable.

565 **Supplementary Table 2 Detection of in silico mixtures versus 3rd MH threshold**

566 Pure samples were mixed in 15 different combinations from each ancestry group (Afri, EaAs, Euro) as well as 15 different mixed
567 ancestry combinations. For each threshold 3rd MH frequency, the percent of all samples that scored as contaminated at that level of
568 contamination are listed at each level of in silico mixing.

569

570 Supplementary Table 1

Location	Final Panel	Med Cov	MH >2	SNV Len	SNV1	SNV2	SNV3	Afri 3+4	EaAs 3+4	Euro 3+4	AdAm 3+4	SoAs 3+4	AfAm 3+4	Reason for Omitting
chr1:23885498-23885599		692	25	102	rs11574	rs2067053		0.011	0.028	0.242	0.261	0.081	0.016	MH>2
chr1:46641827-46641837		296	1	11	rs12030928	rs12752892		0.017	0.000	0.125	0.071	0.080	0.057	Balance
chr1:120057158-120057246		689	3	89	rs6203	rs45609334		0.033	0.082	0.235	0.205	0.157	0.090	Balance
chr1:156846120-156846233	Yes	1526	2	114	rs1800880	rs6334		0.105	0.139	0.065	0.117	0.244	0.098	
chr1:226573364-226573402	Yes	2011	1	39	rs1805414	rs1805408		0.143	0.205	0.159	0.147	0.183	0.164	
chr1:226589833-226589958	Yes	361	2	126	rs1805407	rs1805404		0.115	0.251	0.154	0.147	0.100	0.164	
chr2:16042003-16042051	Yes	392	1	49	rs2693006	rs67056216		0.113	0.177	0.177	0.159	0.264	0.123	
chr2:16073257-16073263	Yes	1546	2	7	rs12986946	rs12986949		0.052	0.000	0.101	0.058	0.115	0.098	
chr2:16112814-16112828	Yes	835	1	15	rs16863159	rs6716344		0.022	0.276	0.088	0.244	0.131	0.148	
chr2:16113594-16113723	Yes	368	4	130	rs34339850	rs6741005		0.052	0.284	0.217	0.183	0.245	0.066	
chr2:29416366-29416481		677	2	116	rs1881421	rs1881420		0.240	0.000	0.150	0.127	0.027	0.312	Balance
chr2:29416481-29416615		750	15	135	rs1881420	rs56132472		0.078	0.000	0.123	0.065	0.024	0.090	MH>2
chr2:29446184-29446202	Yes	2130	0	19	rs2276550	rs4622670		0.259	0.054	0.236	0.222	0.203	0.033	
chr2:29446701-29446721	Yes	686	1	21	rs12619049	rs4665447		0.412	0.081	0.026	0.062	0.015	0.320	
chr2:29447108-29447253	Yes	448	1	146	rs4387740	rs6723311		0.390	0.141	0.254	0.232	0.173	0.426	
chr2:47800577-47800603	Yes	1072	0	27	rs56239373	rs3814360		0.077	0.154	0.042	0.065	0.086	0.057	
chr2:47852559-47852643	Yes	293	5	85	rs6722699	rs10165802		0.110	0.076	0.093	0.104	0.061	0.139	
chr2:47856243-47856328		572	8	86	rs11892597	rs11887626		0.209	0.078	0.273	0.203	0.261	0.205	MH>2
chr2:48010488-48010558		1461	2	71	rs1042821	rs1042820		0.020	0.000	0.175	0.114	0.065	0.033	Balance
chr2:112754828-112754880		366	1	53	rs3811632	rs3811633		0.103	0.106	0.287	0.189	0.230	0.156	Balance
chr2:112754943-112755001		747	3	59	rs3811634	rs2230515		0.104	0.106	0.287	0.189	0.230	0.156	Balance
chr2:113980645-113980736		550	0	92	rs12472361	rs56865224		0.017	0.073	0.110	0.117	0.129	0.057	
chr2:113982584-113982608		540	0	25	rs4849177	rs4849178		0.017	0.077	0.164	0.138	0.183	0.057	
chr2:113983937-113984033	Yes	776	1	97	rs3748915	rs3748916		0.203	0.086	0.163	0.135	0.229	0.213	
chr2:113984503-113984594	Yes	1400	0	92	rs2241975	rs67776659		0.142	0.013	0.110	0.087	0.038	0.074	

chr2:113985170-113985186		1536	2	17	rs4849179	rs4849180		0.020	0.077	0.163	0.138	0.183	0.066	LD, homopolymer
chr2:113989236-113989267	Yes	1009	2	32	rs2863242	rs2863243		0.017	0.074	0.163	0.138	0.183	0.057	
chr2:113990242-113990261		2923	0	20	rs2166421	rs7421852		0.011	0.074	0.162	0.135	0.183	0.041	LD, homopolymer
chr2:202122956-202122995	Yes	1337	0	40	rs3769824	rs3769823		0.000	0.000	0.047	0.114	0.043	0.016	
chr3:12641425-12641518		346	1	94	rs5746223	rs2246390		0.040	0.030	0.111	0.125	0.065	0.090	Balance
chr3:12642945-12643013		277	3	69	rs3773345	rs904464	rs904452	0.145	0.031	0.192	0.114	0.052	0.148	Balance
chr3:12649857-12649937	Yes	567	2	81	rs2055311	rs963959		0.225	0.028	0.164	0.310	0.125	0.221	
chr3:36986932-36986992	Yes	2760	4	61	rs2276809	rs2276808		0.073	0.077	0.115	0.160	0.216	0.090	
chr3:36986992-36987083		2802	6	92	rs2276808	rs2276807		0.210	0.000	0.048	0.078	0.000	0.303	MH>2
chr3:37022803-37022864		345	0	62	rs4678921	rs4678922		0.082	0.000	0.000	0.026	0.000	0.213	Balance
chr3:71247257-71247304	Yes	1098	0	48	rs939845	rs2037474		0.163	0.104	0.064	0.202	0.044	0.139	
chr3:138327951-138328016	Yes	634	1	66	rs61699523	rs111398337		0.167	0.020	0.028	0.071	0.110	0.295	
chr3:142277536-142277575	Yes	642	0	40	rs2227929	rs2227930		0.147	0.118	0.200	0.154	0.158	0.188	
chr3:178968634-178968660		1223	0	27	rs7645550	rs1170672		0.041	0.000	0.095	0.038	0.170	0.057	Balance
chr3:178984575-178984679	Yes	2320	2	105	rs7612684	rs7646600		0.302	0.011	0.177	0.131	0.132	0.213	
chr3:178986121-178986203	Yes	623	5	83	rs73188921	rs9830427	rs9830432	0.158	0.119	0.054	0.076	0.190	0.131	
chr3:178990402-178990462	Yes	1179	1	61	rs2864411	rs6443633		0.017	0.142	0.000	0.050	0.045	0.098	
chr3:183211906-183212026		536	2	121	rs1520101	rs2256061		0.128	0.000	0.182	0.123	0.162	0.238	Balance
chr4:1745492-1745500	Yes	4202	2	9	rs4865466	rs4865467		0.126	0.144	0.217	0.306	0.229	0.139	
chr4:1747971-1748069		1482	1	99	rs4572936	rs4558926		0.049	0.000	0.156	0.095	0.082	0.098	Balance
chr4:1748200-1748266		2245	8	67	rs4507432	rs4597899	rs62285133	0.328	0.159	0.392	0.311	0.273	0.336	MH>2
chr4:1750487-1750584	Yes	1702	3	98	rs7680647	rs73202803		0.042	0.161	0.235	0.180	0.121	0.057	
chr4:1785738-1785791		2874	2	54	rs1867926	rs61407096		0.063	0.000	0.158	0.157	0.087	0.074	Balance
chr4:1788994-1789044	Yes	678	4	51	rs11248077	rs11248078		0.249	0.233	0.383	0.346	0.377	0.254	
chr4:1796629-1796636	Yes	319	1	8	rs3135841	rs3135842		0.254	0.051	0.094	0.141	0.061	0.262	

chr4:1797741-1797852	Yes	995	4	112	rs3135848	rs743682		0.227	0.056	0.092	0.144	0.062	0.271	
chr4:54269096-54269173	Yes	557	1	78	rs10001201	rs62325166		0.050	0.133	0.140	0.105	0.046	0.082	
chr4:54526892-54526902		647	2	11	rs12649494	rs2668565		0.023	0.093	0.153	0.079	0.117	0.082	Balance
chr4:54589337-54589371		445	21	35	rs2590827	rs75219949		0.054	0.223	0.140	0.208	0.207	0.115	MH>2
chr4:54657737-54657790	Yes	288	5	54	rs28489910	rs4864823		0.233	0.111	0.209	0.226	0.148	0.254	
chr4:55049698-55049751		310	3	54	rs13131212	rs7659596		0.028	0.061	0.215	0.159	0.242	0.041	Balance
chr4:55208737-55208788	Yes	284	3	52	rs2412560	rs10018115	rs73234206	0.202	0.247	0.200	0.270	0.317	0.361	
chr4:55477000-55477073		991	4	74	rs17827038	rs2703460		0.020	0.072	0.134	0.098	0.195	0.090	Balance
chr4:55501109-55501195	Yes	357	5	87	rs6554196	rs6554197		0.110	0.110	0.200	0.163	0.223	0.230	
chr4:55582037-55582068	Yes	714	3	32	rs76272262	rs3134889		0.040	0.172	0.036	0.051	0.081	0.025	
chr4:55619846-55619859	Yes	892	3	14	rs11732442	rs4353958		0.125	0.109	0.109	0.069	0.212	0.139	
chr4:55916001-55916063		317	0	63	rs7664996	rs7665169		0.164	0.000	0.201	0.177	0.149	0.172	Balance
chr4:55977759-55977800		278	3	42	rs3943404	rs2034965		0.210	0.215	0.258	0.264	0.227	0.213	Balance
chr4:55982752-55982784	Yes	651	1	33	rs11133360	rs34945396		0.044	0.204	0.194	0.144	0.190	0.041	
chr4:56026865-56026914	Yes	565	1	50	rs4864958	rs75371420	rs34743464	0.216	0.200	0.284	0.180	0.453	0.238	
chr4:106196829-106196951	Yes	534	0	123	rs34402524	rs2454206		0.066	0.047	0.140	0.089	0.090	0.090	
chr4:143043340-143043404		351	0	65	rs2270658	rs13133767		0.016	0.075	0.082	0.105	0.089	0.057	Balance
chr4:187534362-187534375	Yes	2353	0	14	rs2249916	rs2249917		0.195	0.281	0.110	0.189	0.084	0.197	
chr4:187629497-187629538	Yes	1727	0	42	rs458021	rs3733413		0.128	0.085	0.070	0.091	0.031	0.098	
chr5:231111-231143	Yes	2366	1	33	rs1126417	rs2288459		0.164	0.058	0.111	0.241	0.079	0.139	
chr5:256472-256509		1282	9	38	rs6961	rs6962		0.269	0.059	0.105	0.108	0.075	0.197	MH>2
chr5:35861068-35861159	Yes	351	3	92	rs1494558	rs11567705	rs969128	0.328	0.191	0.413	0.349	0.239	0.443	
chr5:35871190-35871273	Yes	255	1	84	rs1494555	rs2228141		0.069	0.153	0.144	0.166	0.062	0.164	
chr5:56178111-56178217	Yes	473	0	107	rs3822625	rs832583		0.119	0.108	0.075	0.078	0.055	0.049	
chr5:57754808-57754851		359	2	44	rs697133	rs702722		0.230	0.105	0.104	0.069	0.098	0.205	Balance

chr5:67477132-67477234	Yes	371	0	103	rs34721946	rs34166422	rs73126524	0.017	0.247	0.035	0.105	0.072	0.033	
chr5:67492589-67492652	Yes	677	2	64	rs13188623	rs58409263		0.105	0.293	0.121	0.180	0.118	0.130	
chr5:67517563-67517646	Yes	275	1	84	rs6449959	rs831227		0.243	0.018	0.187	0.161	0.100	0.303	
chr5:67522722-67522851	Yes	262	1	130	rs706713	rs706714		0.130	0.051	0.012	0.029	0.060	0.098	
chr5:67534039-67534057	Yes	887	0	19	rs7709243	rs10940158	rs12652661	0.216	0.154	0.212	0.272	0.097	0.156	
chr5:67553771-67553827	Yes	584	1	57	rs6893676	rs34303		0.090	0.168	0.173	0.143	0.106	0.074	
chr5:79950497-79950512		1392	8	16	rs2250063	rs1105524		0.036	0.047	0.248	0.206	0.231	0.098	MH>2
chr5:149456772-149456811	Yes	1109	3	40	rs60844779	rs3829987		0.223	0.068	0.031	0.215	0.051	0.221	
chr5:176517326-176517461		652	3	136	rs422421	rs446382		0.169	0.000	0.078	0.040	0.033	0.148	Balance
chr5:176523562-176523597	Yes	1990	0	36	rs31777	rs31776		0.137	0.000	0.076	0.038	0.033	0.115	
chr5:176531772-176531857	Yes	284	3	86	rs7708357	rs165943		0.168	0.046	0.242	0.248	0.183	0.197	
chr5:176539212-176539286		922	0	75	rs244730	rs351864		0.031	0.027	0.121	0.140	0.207	0.066	Balance
chr5:176721198-176721272		1806	1	75	rs28580074	rs11740250		0.011	0.000	0.119	0.146	0.181	0.041	Balance
chr5:180046209-180046344		765	12	136	rs446003	rs448012		0.100	0.057	0.083	0.075	0.135	0.098	MH>2
chr5:180051003-180051118		2483	2	116	rs307826	rs728986		0.015	0.000	0.037	0.055	0.044	0.016	Balance
chr5:180057231-180057293		1518	0	63	rs3736061	rs34221241		0.000	0.000	0.081	0.033	0.041	0.016	Balance
chr6:26056549-26056708	Yes	524	2	160	rs10425	rs2230653	rs12204800	0.048	0.309	0.227	0.344	0.256	0.082	
chr6:30865115-30865204	Yes	461	5	90	rs2239517	rs2267641		0.120	0.244	0.038	0.063	0.094	0.098	
chr6:32188603-32188642	Yes	1185	1	40	rs520803	rs520692	rs520688	0.000	0.047	0.000	0.000	0.011	0.000	
chr6:32190390-32190484	Yes	2363	5	95	rs915894	rs8192569		0.330	0.232	0.102	0.141	0.205	0.328	
chr6:41924853-41924931	Yes	922	2	79	rs4623235	rs16895130		0.095	0.110	0.210	0.156	0.138	0.107	
chr6:41941980-41941993		1079	1	14	rs4415146	rs72853828		0.000	0.089	0.221	0.125	0.132	0.049	Balance
chr6:42013020-42013049	Yes	530	0	30	rs9381126	rs6919122	rs6942118	0.351	0.421	0.381	0.504	0.390	0.254	
chr6:42039487-42039542	Yes	651	3	56	rs9349215	rs66472208		0.023	0.245	0.020	0.048	0.127	0.057	
chr6:42039551-42039666	Yes	292	1	116	rs66489927	rs7763360	rs2492927	0.192	0.148	0.300	0.248	0.322	0.221	

chr6:42044204-42044273		717	1	70	rs11968793	rs9471750		0.184	0.057	0.046	0.045	0.014	0.221	Balance
chr6:42052577-42052667	Yes	305	0	91	rs9357387	rs2493841	rs9381136	0.050	0.163	0.176	0.161	0.139	0.123	
chr6:117725448-117725578		277	4	131	rs1998206	rs2243378		0.076	0.181	0.150	0.143	0.197	0.197	Balance
chr6:152382311-152382325		279	2	15	rs2273206	rs2273207		0.137	0.039	0.026	0.039	0.055	0.066	Balance
chr7:6026775-6026942		720	19	168	rs2228006	rs1805323		0.000	0.122	0.046	0.017	0.106	0.016	MH>2
chr7:6026942-6026988	Yes	3560	3	47	rs1805323	rs1805321		0.000	0.303	0.046	0.017	0.153	0.016	
chr7:55220177-55220202	Yes	1118	0	26	rs11506105	rs845561		0.115	0.265	0.254	0.304	0.413	0.148	
chr7:55236774-55236862		458	5	89	rs845550	rs1815156		0.096	0.000	0.114	0.062	0.042	0.123	Balance
chr7:55251541-55251648	Yes	672	4	108	rs2877261	rs13222385	rs11771471	0.200	0.076	0.233	0.183	0.090	0.189	
chr7:55306599-55306625		577	6	47	rs2037700	rs2037699	rs2037698	0.368	0.083	0.104	0.124	0.045	0.271	MH>2
chr7:100410597-100410657		1469	8	61	rs2230585	rs770657085		0.164	0.056	0.000	0.043	0.156	0.197	MH>2
chr7:100416139-100416250	Yes	1438	3	112	rs3857809	rs144173		0.185	0.059	0.000	0.301	0.173	0.172	
chr7:116336880-116336947	Yes	666	1	68	rs2237708	rs39749		0.036	0.209	0.257	0.228	0.242	0.115	
chr7:116471122-116471227	Yes	297	4	106	rs41773	rs62470772		0.129	0.093	0.206	0.115	0.148	0.131	
chr7:116480433-116480524		319	7	92	rs41777	rs35099490	rs41778	0.267	0.217	0.279	0.268	0.283	0.288	MH>2
chr8:30999122-30999123	Yes	554	3	2	rs3024239	rs2737335		0.149	0.024	0.060	0.032	0.085	0.246	
chr8:31024638-31024654	Yes	432	0	17	rs1801196	rs1346044		0.147	0.104	0.266	0.173	0.283	0.221	
chr8:38299624-38299715	Yes	1668	5	92	rs60527016	rs6987534		0.028	0.286	0.236	0.219	0.076	0.074	
chr8:38310910-38311001	Yes	1289	0	92	rs10958700	rs4733930		0.029	0.323	0.260	0.249	0.074	0.074	
chr8:38317398-38317476		1504	4	79	rs7012413	rs7388222	rs3758101	0.213	0.088	0.076	0.225	0.115	0.205	Balance
chr8:38350292-38350315	Yes	580	2	24	rs35305468	rs7830964		0.039	0.249	0.180	0.118	0.138	0.107	
chr8:38359468-38359529		1045	3	62	rs7845393	rs35804490		0.137	0.172	0.125	0.114	0.282	0.180	LD
chr8:38361379-38361430	Yes	1456	2	52	rs328294	rs328293		0.309	0.172	0.126	0.115	0.283	0.213	
chr8:128700175-128700233	Yes	496	2	59	rs13282849	rs7005394		0.208	0.179	0.063	0.084	0.201	0.287	
chr8:128713221-128713364	Yes	796	5	144	rs28548827	rs7820045		0.254	0.057	0.028	0.101	0.111	0.148	

chr8:128718068-128718102		457	2	35	rs9642880	rs78635199		0.000	0.035	0.129	0.062	0.171	0.041	Balance
chr8:128889285-128889371	Yes	1835	1	87	rs6470587	rs6470588		0.081	0.165	0.210	0.202	0.230	0.156	
chr8:145737636-145737816	Yes	485	0	181	rs4925828	rs4251691		0.000	0.203	0.000	0.072	0.000	0.000	
chr8:145741702-145741765		2058	6	64	rs4244612	rs4244613		0.081	0.027	0.108	0.094	0.129	0.148	MH>2
chr9:5408242-5408358	Yes	344	3	117	rs10758685	rs10975098	rs10975099	0.084	0.349	0.257	0.320	0.409	0.205	
chr9:5415025-5415111	Yes	372	3	87	rs78298180	rs10758687		0.104	0.161	0.054	0.052	0.199	0.066	
chr9:5420254-5420266	Yes	1180	1	13	rs10121219	rs11790878		0.064	0.227	0.222	0.248	0.218	0.172	
chr9:5456523-5456587		277	1	65	rs17742278	rs7023227	rs12002985	0.165	0.083	0.337	0.320	0.305	0.262	Balance
chr9:5458035-5458095	Yes	323	3	61	rs7042084	rs10481593		0.268	0.132	0.220	0.249	0.131	0.205	
chr9:5484100-5484203	Yes	395	4	104	rs11793113	rs11790610	rs10122509	0.139	0.151	0.094	0.084	0.167	0.156	
chr9:5505508-5505583		262	5	76	rs7025653	rs59338043	rs6476984	0.228	0.188	0.234	0.298	0.215	0.205	Balance
chr9:5514839-5514919		561	7	81	rs2381282	rs56381807		0.275	0.204	0.128	0.203	0.190	0.303	MH>2
chr9:87478135-87478172	Yes	1016	4	38	rs7048015	rs10780690		0.023	0.251	0.184	0.258	0.216	0.139	
chr9:93641175-93641199		693	2	25	rs2306041	rs2306040		0.062	0.000	0.064	0.058	0.020	0.066	Balance
chr9:98238358-98238379	Yes	3840	0	22	rs2066836	rs1805155		0.011	0.083	0.109	0.076	0.060	0.074	
chr9:139401504-139401577	Yes	1346	1	74	rs3124596	rs7870145	rs3829116	0.310	0.000	0.163	0.117	0.264	0.238	
chr9:139403268-139403280		500	1	13	rs3125000	rs11145765		0.046	0.000	0.095	0.059	0.238	0.025	Balance
chr9:139405093-139405261	Yes	626	3	169	rs36119806	rs3125001		0.150	0.012	0.102	0.065	0.184	0.156	
chr9:139410424-139410589	Yes	327	2	166	rs3125006	rs4880099		0.088	0.052	0.115	0.068	0.215	0.049	
chr9:139411714-139411880		428	5	167	rs11145767	rs9411254		0.209	0.000	0.000	0.025	0.000	0.213	Balance
chr10:43611708-43611865	Yes	629	2	158	rs741968	rs2256550		0.060	0.218	0.161	0.212	0.284	0.139	
chr10:43615505-43615633	Yes	463	5	129	rs2472737	rs1800863		0.105	0.121	0.193	0.187	0.160	0.115	
chr10:70332580-70332672	Yes	549	1	93	rs10823229	rs12773594		0.023	0.173	0.185	0.151	0.271	0.090	
chr10:104386934-104387019	Yes	250	0	86	rs17114803	rs12414407		0.224	0.250	0.093	0.238	0.240	0.197	
chr10:123194558-123194609	Yes	384	0	52	rs7911440	rs6585731		0.051	0.211	0.242	0.082	0.243	0.107	

chr10:123199092-123199095	Yes	1151	2	4	rs4752560	rs2114689		0.283	0.023	0.075	0.156	0.160	0.131	
chr10:123242724-123242780		415	2	57	rs3135811	rs3135810		0.154	0.000	0.064	0.030	0.000	0.148	Balance
chr10:123275662-123275666	Yes	320	1	5	rs2912761	rs2981453		0.211	0.000	0.000	0.050	0.000	0.197	
chr10:123335839-123335866	Yes	1055	1	28	rs45631611	rs10886946		0.017	0.113	0.071	0.055	0.114	0.041	
chr10:123346116-123346190	Yes	420	0	75	rs2981575	rs1219648		0.195	0.048	0.000	0.022	0.013	0.090	
chr10:123396636-123396715		259	5	80	rs10788193	rs2454809		0.059	0.176	0.195	0.192	0.246	0.139	Coverage
chr10:123396728-123396806	Yes	331	2	79	rs1909670	rs1614303		0.029	0.176	0.100	0.131	0.073	0.057	
chr10:123406645-123406663	Yes	699	4	19	rs10788194	rs7923788		0.084	0.227	0.151	0.192	0.125	0.139	
chr11:534197-534242	Yes	2026	1	46	rs41258054	rs12628		0.000	0.153	0.056	0.137	0.076	0.025	
chr11:8246326-8246343		287	6	18	rs34544683	rs3816490		0.022	0.098	0.125	0.219	0.133	0.123	MH>2
chr11:69412090-69412124	Yes	2968	1	35	rs79274134	rs7112989		0.254	0.232	0.000	0.127	0.031	0.254	
chr11:69446719-69446766		1480	2	48	rs609581	rs11826558		0.070	0.015	0.058	0.079	0.187	0.139	Balance
chr11:69448373-69448445		563	7	73	rs654240	rs654648		0.188	0.029	0.136	0.112	0.210	0.221	MH>2
chr11:69508082-69508159		574	3	78	rs7930020	rs10908198		0.000	0.041	0.125	0.267	0.045	0.066	Balance
chr12:4333159-4333203		1194	4	45	rs7955734	rs7970219	rs7954847	0.268	0.092	0.026	0.085	0.100	0.229	Balance
chr12:4346169-4346177	Yes	646	0	9	rs11063052	rs11832328		0.318	0.079	0.038	0.072	0.080	0.213	
chr12:4351884-4352027	Yes	468	5	144	rs7955545	rs4766223		0.051	0.113	0.033	0.076	0.092	0.139	
chr12:4376089-4376091	Yes	306	2	3	rs4238013	rs12818766		0.119	0.033	0.181	0.161	0.147	0.139	
chr12:4399036-4399054	Yes	1619	2	52	rs3217859	rs3217860	rs3217861	0.325	0.391	0.414	0.491	0.479	0.303	
chr12:4399917-4399970	Yes	892	2	54	rs3217867	rs3217868	rs3217869	0.173	0.041	0.220	0.133	0.188	0.213	
chr12:4411639-4411683	Yes	1376	1	45	rs3217925	rs3217926		0.127	0.068	0.253	0.172	0.227	0.188	
chr12:4417127-4417232	Yes	1224	1	106	rs7133323	rs9668504		0.449	0.324	0.237	0.282	0.142	0.418	
chr12:4463020-4463036		850	31	17	rs7297048	rs7296130	rs12823973	0.115	0.089	0.193	0.303	0.117	0.131	MH>2
chr12:12009741-12009874	Yes	379	2	134	rs2238126	rs743614		0.181	0.240	0.190	0.249	0.079	0.180	
chr12:12013572-12013612	Yes	647	3	41	rs2855708	rs6488463		0.232	0.196	0.211	0.347	0.146	0.262	

chr12:12016008-12016089	Yes	1488	3	82	rs2238130	rs2416944	rs2238131	0.125	0.248	0.144	0.216	0.104	0.197	
chr12:12020114-12020170	Yes	637	1	57	rs2723805	rs7973930		0.241	0.111	0.075	0.066	0.054	0.238	
chr12:12033536-12033570		2184	4	35	rs11832110	rs4763730		0.232	0.000	0.042	0.074	0.000	0.189	Balance
chr12:12035649-12035664	Yes	2052	1	16	rs2710310	rs2739085		0.126	0.271	0.194	0.251	0.159	0.180	
chr12:18656174-18656225		381	1	52	rs11044141	rs11044142		0.099	0.000	0.000	0.000	0.000	0.041	Balance
chr12:56494991-56494998		3387	6	8	rs2271189	rs773123		0.073	0.000	0.110	0.066	0.070	0.066	MH>2
chr12:69169222-69169316	Yes	404	3	95	rs6581833	rs73334654		0.256	0.016	0.059	0.078	0.000	0.328	
chr12:69265196-69265278	Yes	768	0	83	rs3817605	rs2293637		0.310	0.192	0.022	0.111	0.106	0.271	
chr12:69277127-69277165	Yes	773	1	39	rs10878875	rs1663588		0.126	0.162	0.124	0.133	0.215	0.180	
chr12:121416622-121416650	Yes	3076	2	29	rs1169289	rs1169288		0.082	0.049	0.132	0.112	0.151	0.131	
chr12:121431272-121431300	Yes	1774	0	29	rs2071190	rs1169301		0.118	0.255	0.236	0.272	0.182	0.189	
chr12:121435427-121435475		3503	1	49	rs2464196	rs2464195		0.014	0.000	0.062	0.046	0.092	0.049	Balance
chr12:121437114-121437221		1919	0	108	rs55834942	rs1169304		0.012	0.000	0.166	0.082	0.023	0.025	Balance
chr12:133208886-133208979	Yes	739	2	94	rs5745023	rs5745022		0.173	0.105	0.135	0.219	0.049	0.180	
chr12:133226159-133226196	Yes	587	2	38	rs4883613	rs4883537		0.105	0.107	0.135	0.222	0.050	0.180	
chr12:133253995-133254083	Yes	448	1	89	rs5744751	rs5744750		0.000	0.105	0.100	0.045	0.042	0.025	
chr13:21562832-21562948		1715	3	117	rs2770928	rs558614		0.175	0.000	0.080	0.087	0.153	0.156	Balance
chr13:32854998-32855018		333	1	21	rs17692070	rs11617975		0.129	0.000	0.098	0.128	0.122	0.123	Balance
chr13:32986219-32986340	Yes	313	0	122	rs206319	rs206320	rs615762	0.107	0.204	0.175	0.244	0.262	0.107	
chr14:35872792-35872926		643	1	135	rs2233415	rs1050851		0.020	0.019	0.213	0.130	0.146	0.033	Balance
chr14:102568296-102568367		969	0	72	rs10873531	rs8005905		0.278	0.049	0.017	0.068	0.123	0.303	Balance
chr14:104165753-104165927		765	4	175	rs861539	rs1799796		0.114	0.073	0.295	0.229	0.189	0.115	Balance
chr14:105239146-105239192	Yes	521	5	47	rs3803304	rs2494732		0.169	0.097	0.171	0.290	0.302	0.230	
chr14:105258892-105258893	Yes	737	1	2	rs2494748	rs2494749		0.120	0.122	0.092	0.231	0.245	0.123	
chr15:41857216-41857303		1528	2	88	rs11639399	rs2277536		0.096	0.012	0.308	0.172	0.236	0.139	Balance

chr15:41860411-41860490		860	2	80	rs7171675	rs12148316		0.095	0.011	0.134	0.131	0.110	0.139	Balance
chr15:67457335-67457485	Yes	475	4	151	rs1065080	rs2289261		0.133	0.238	0.139	0.087	0.220	0.156	
chr15:73996066-73996101		3299	3	36	rs11574483	rs10083681		0.147	0.000	0.000	0.017	0.000	0.148	Balance
chr15:88488326-88488428	Yes	1800	1	103	rs8042993	rs1369426		0.088	0.135	0.153	0.097	0.261	0.066	
chr15:88549118-88549151	Yes	1763	0	34	rs11073758	rs12324332		0.266	0.015	0.124	0.133	0.079	0.303	
chr15:88554878-88554886		2501	3	9	rs4989257	rs898707		0.040	0.014	0.124	0.128	0.082	0.164	Balance
chr15:88646922-88647038	Yes	975	1	117	rs16941255	rs76506232		0.110	0.132	0.000	0.010	0.000	0.115	
chr15:88667852-88667948	Yes	1099	0	97	rs3784411	rs3784410		0.192	0.100	0.217	0.225	0.151	0.172	
chr16:2138218-2138269		1617	14	52	rs1800718	rs1748		0.136	0.000	0.090	0.063	0.024	0.074	MH>2
chr16:2138269-2138398		941	4	130	rs1748	rs13332221		0.249	0.000	0.116	0.017	0.123	0.197	Balance
chr16:2138398-2138422	Yes	2026	0	25	rs13332221	rs13332222		0.118	0.000	0.000	0.013	0.000	0.090	
chr16:81819768-81819820	Yes	2558	1	53	rs1143685	rs4294811		0.140	0.141	0.282	0.271	0.126	0.148	
chr16:89806343-89806347	Yes	601	2	5	rs11647746	rs7195906		0.161	0.013	0.074	0.035	0.134	0.156	
chr16:89849480-89849629		275	2	150	rs2239359	rs12448860		0.032	0.013	0.064	0.370	0.352	0.319	Balance
chr16:89858505-89858525		698	3	21	rs6500452	rs1800287		0.177	0.012	0.073	0.043	0.133	0.221	Balance
chr17:1782952-1782957	Yes	1284	1	6	rs5030755	rs2230930		0.000	0.000	0.102	0.020	0.024	0.016	
chr17:37807698-37807707		1082	2	10	rs9972882	rs75397452		0.141	0.000	0.047	0.042	0.000	0.131	Balance
chr17:37832279-37832315	Yes	1408	1	37	rs1495100	rs2934953		0.194	0.000	0.016	0.062	0.053	0.188	
chr17:37833562-37833567		2351	2	6	rs11656146	rs907088		0.000	0.000	0.092	0.030	0.011	0.016	Balance
chr17:37834357-37834367		666	0	11	rs732084	rs732083		0.181	0.000	0.000	0.043	0.011	0.156	Balance
chr17:37834715-37834808	Yes	1558	5	94	rs12150603	rs72832915		0.042	0.153	0.308	0.196	0.235	0.131	
chr17:37853048-37853097		1655	1	50	rs2643194	rs2517951		0.188	0.000	0.017	0.085	0.081	0.303	Balance
chr17:37893458-37893484		499	8	27	rs67597968	rs4795393		0.209	0.000	0.053	0.069	0.098	0.221	MH>2
chr17:41616392-41616456	Yes	1646	1	65	rs76280498	rs7222604		0.000	0.150	0.106	0.110	0.181	0.033	
chr17:78820329-78820374	Yes	3252	0	46	rs3751945	rs2589156		0.082	0.000	0.107	0.078	0.115	0.066	

chr17:78865546-78865630	Yes	631	3	85	rs2289764	rs2289765		0.289	0.044	0.111	0.110	0.115	0.303	
chr17:78896488-78896529	Yes	2726	4	42	rs2271602	rs2271603		0.154	0.196	0.321	0.291	0.307	0.197	
chr17:78897547-78897561	Yes	1725	0	15	rs7217786	rs6565491		0.031	0.199	0.122	0.111	0.249	0.049	
chr17:78921117-78921211	Yes	1576	2	95	rs4969231	rs9912373		0.022	0.079	0.124	0.114	0.060	0.082	
chr19:2226676-2226772	Yes	2349	1	97	rs3815308	rs2302061		0.034	0.182	0.143	0.172	0.203	0.090	
chr19:3110349-3110361		2345	5	13	rs11085000	rs41276846		0.018	0.193	0.112	0.063	0.188	0.016	
chr19:3119184-3119239	Yes	1438	1	56	rs308046	rs4900		0.166	0.233	0.135	0.101	0.275	0.271	
chr19:3119405-3119406		884	33	2	rs370286	rs451778		0.000	0.233	0.135	0.087	0.280	0.025	MH>2
chr19:5210622-5210782		740	2	161	rs2302224	rs1143698		0.166	0.066	0.126	0.134	0.090	0.131	Balance
chr19:5210762-5210782		4185	0	21	rs1143699	rs1143698		0.222	0.000	0.099	0.081	0.056	0.205	Balance
chr19:5212380-5212482		1945	1	103	rs1064300	rs2230611		0.115	0.000	0.124	0.135	0.126	0.098	Balance
chr19:7163154-7163230	Yes	810	2	77	rs2963	rs2245648		0.186	0.025	0.065	0.068	0.141	0.148	
chr19:7166376-7166388	Yes	1028	2	13	rs2059806	rs2229429		0.179	0.065	0.191	0.144	0.262	0.197	
chr19:10267011-10267077	Yes	265	0	67	rs4804490	rs2228611		0.171	0.281	0.068	0.184	0.224	0.172	
chr19:17937758-17937786		1721	0	29	rs3212798	rs3212797		0.074	0.000	0.052	0.033	0.012	0.066	Balance
chr19:17955001-17955021	Yes	1946	1	21	rs3212713	rs3212712	rs3212711	0.197	0.000	0.000	0.022	0.000	0.139	
chr19:30253901-30253998	Yes	768	2	98	rs117342492	rs4805475		0.000	0.221	0.000	0.104	0.073	0.033	
chr19:30255068-30255090	Yes	495	2	23	rs8103966	rs8099838		0.043	0.310	0.250	0.232	0.252	0.082	
chr19:30290349-30290357	Yes	2732	1	9	rs1473201	rs111640872		0.085	0.106	0.247	0.180	0.213	0.066	
chr19:30319892-30319896		284	5	5	rs56285422	rs8106054		0.085	0.106	0.248	0.180	0.213	0.066	LD
chr19:30340381-30340412	Yes	593	3	32	rs929813	rs929814		0.216	0.087	0.121	0.293	0.263	0.271	
chr19:30361995-30362112	Yes	290	2	118	rs255270	rs255271		0.184	0.104	0.037	0.068	0.012	0.271	
chr19:41724820-41724885	Yes	2049	0	66	rs2301236	rs28364580		0.094	0.179	0.224	0.148	0.275	0.172	
chr19:41781493-41781579	Yes	1040	2	87	rs8103839	rs9304592		0.067	0.073	0.000	0.066	0.064	0.066	
chr19:50919797-50919828	Yes	2886	5	32	rs3218776	rs3218760		0.125	0.139	0.075	0.148	0.275	0.156	

chr20:9543622-9543681	Yes	813	5	60	rs2297345	rs2297346		0.122	0.214	0.088	0.174	0.059	0.139	
chr20:30729488-30729523	Yes	3150	2	36	rs6089193	rs6089194		0.206	0.085	0.026	0.137	0.053	0.189	
chr20:40714479-40714540	Yes	1095	1	62	rs2016647	rs1569548		0.114	0.074	0.242	0.167	0.138	0.066	
chr20:40714539-40714540		1134	12	2	rs1569547	rs1569548		0.000	0.073	0.231	0.150	0.120	0.016	MH>2
chr20:57478807-57478939		711	8	133	rs7121	rs3730168		0.186	0.091	0.286	0.120	0.169	0.205	MH>2
chr21:42845374-42845383	Yes	6069	0	10	rs2298659	rs17854725		0.173	0.115	0.230	0.218	0.189	0.220	
chr21:42876400-42876447	Yes	2128	0	48	rs7277080	rs395584		0.287	0.017	0.019	0.235	0.212	0.229	
chr21:45643008-45643052		288	31	45	rs1048710	rs2298564		0.069	0.237	0.045	0.140	0.043	0.066	MH>2
chr22:17640022-17640045	Yes	1258	0	24	rs11550530	rs7287672		0.125	0.035	0.086	0.130	0.058	0.066	
chr22:21337266-21337325	Yes	565	4	60	rs178280	rs13054014		0.116	0.200	0.259	0.223	0.234	0.189	
chr22:21348914-21349037		1246	25	124	rs4822790	rs178292		0.105	0.224	0.135	0.112	0.142	0.139	MH>2
chr22:24158895-24158899	Yes	713	2	5	rs9608192	rs2070457		0.098	0.059	0.115	0.071	0.153	0.090	
chr22:29690246-29690345	Yes	259	0	100	rs73156524	rs131189		0.032	0.281	0.086	0.053	0.034	0.090	
Final Panel	164	768	1.78	59				0.139	0.139	0.139	0.156	0.153	0.155	

Supplementary Table 2

		Threshold Median 3rd MH Frequency				
		0.5	1	1.5	2	2.5
In silico Mixing Levels	0.5	40	0	0	0	0
	1	100	0	0	0	0
	1.5	100	7	0	0	0
	2	100	63	0	0	0
	2.5	100	100	2	0	0
	3	100	100	35	0	0
	4	100	100	95	15	0
	5	100	100	100	88	7
	10	100	100	100	100	100