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## Forensic ancestry analysis with two capillary electrophoresis ancestry informative marker (AIM) panels: Results of a collaborative EDNAP exercise

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- Nineteen laboratories completed a collaborative EDNAP exercise to evaluate two forensic ancestry informative marker (AIM) assays and accompanying statistical tools to infer ancestry from the genotype data.
- Laboratories were sent primers, reference data and five test DNAs of undisclosed origin plus an unmarked DNA mixture (but reported to be one of the samples).
- Fourteen laboratories successfully genotyped the DNAs with a 34-plex SNP assay using SNaPshot, achieving 96.1% profile completeness and 93.5% genotype concordance.
- All laboratories successfully genotyped the DNAs with a 46-plex Indel assay using dye-labelled PCR primers, achieving 99.8% profile completeness and genotype concordance.
- All laboratories identified the mixed DNA sample, indicated by disrupted peak height ratios in the Indel profile and three-allele patterns in SNP rs5030240. 18/19 laboratories assigned the correct ancestry to each of the test DNAs of unknown origin, obtaining likelihood ratios from 80 markers in the range:  $1.25E+07$  to  $1.78E+41$ .

1           **Forensic ancestry analysis with two capillary electrophoresis ancestry informative**  
2           **marker (AIM) panels: Results of a collaborative EDNAP exercise**

3  
4           **Abstract**

5           There is increasing interest in forensic ancestry tests, which are part of a growing number of DNA analyses  
6           that can enhance routine profiling by obtaining additional genetic information about unidentified DNA  
7           donors. Nearly all ancestry tests use single nucleotide polymorphisms (SNPs), but these currently rely on  
8           SNaPshot single base extension chemistry that can fail to detect mixed DNA. Insertion-deletion  
9           polymorphism (Indel) tests have been developed using dye-labeled primers that allow direct capillary  
10          electrophoresis detection of PCR products (PCR-to-CE). PCR-to-CE maintains the direct relationship  
11          between input DNA and signal strength as each marker is detected with a single dye, so mixed DNA is  
12          more reliably detected. We report the results of a collaborative inter-laboratory exercise of 19  
13          participants (15 from the EDNAP European DNA Profiling group) that assessed a 34-plex SNP test using  
14          SNaPshot and a 46-plex Indel test using PCR-to-CE. Laboratories were asked to type five samples with  
15          different ancestries and detect an additional mixed DNA sample. Statistical inference of ancestry was  
16          made by participants using the *Snipper* online Bayes analysis portal plus an optional PCA module that  
17          analyzes the genotype data alongside calculation of Bayes likelihood ratios. Exercise results indicated  
18          consistent genotyping performance from both tests, reaching a particularly high level of reliability for the  
19          Indel test. SNP genotyping gave 93.5% concordance (compared to the organizing laboratory's data) that  
20          rose to 97.3% excluding one laboratory with a large number of miscalled genotypes. Indel genotyping  
21          gave a higher concordance rate of 99.8% and a reduced no-call rate compared to SNP analysis. All  
22          participants detected the mixture from their Indel peak height data and successfully assigned the correct  
23          ancestry to the other samples using *Snipper*, with the exception of one laboratory with SNP miscalls that  
24          incorrectly assigned ancestry of two samples and did not obtain informative likelihood ratios for a third.  
25          Therefore, successful ancestry assignments were achieved by participants in 92 of 95 *Snipper* analyses.  
26          This exercise demonstrates that ancestry inference tests based on binary marker sets can be readily  
27          adopted by laboratories that already have well-established CE regimes in place. The Indel test proved to  
28          be easy to use and allowed all exercise participants to detect the DNA mixture as well as achieving  
29          complete and concordant profiles in nearly all cases. Lastly, two participants successfully ran parallel next-  
30          generation sequencing analyses (each using different systems) and achieved high levels of genotyping  
31          concordance using the exercise PCR primer mixes unmodified.

32  
33          Keywords: Ancestry; SNPs; Indels; AIMs; Bayes analysis; Principal Component Analysis (PCA)

34 **1. Introduction**

35

36 DNA-based forensic ancestry tests have the capacity to provide key information about unidentified DNA  
37 donors, which can be particularly useful when police investigators do not have reliable eyewitness  
38 descriptions or if the STR profiling data fails to give a DNA database match [1]. Therefore, tests for the  
39 inference of ancestry can be grouped alongside forensic DNA phenotyping (FDP) tests such as *HirisPlex* [2]  
40 in a growing array of new technologies that have the potential to take forensic DNA analysis well beyond  
41 simple identification [3,4]. For such tests to be effective in routine forensic use they must be sensitive;  
42 easy to run using validated DNA detection instruments; and, being mainly composed of binary loci, they  
43 should have a reasonably robust way to detect mixed DNA so that apparent heterozygotes are not  
44 mistyped. In addition, the genetic data obtained must be easy to interpret. Ideally, it should be  
45 straightforward to use the genotypes to calculate a set of Bayes likelihoods for particular ancestries (or  
46 phenotypes) in comparison to reference populations whose patterns of genetic variation are already well  
47 defined. Although STRs can provide a degree of ancestry information [5,6] and Y-chromosome/mtDNA  
48 variation is highly differentiated geographically, there are widely discussed reasons why stand-alone  
49 autosomal SNP tests provide more reliable indications of a person's ancestry [7-9].

50 For the last ten years, forensic SNP genotyping has relied on the SNaPshot single base extension system to  
51 create relatively large-scale PCR and extension multiplexes followed by capillary electrophoresis (CE) of  
52 the dye-labeled products using standard run conditions. In this way, FDP and ancestry analysis tests [2,8-  
53 12] have been developed using single-tube amplification reactions that are highly sensitive and use  
54 validated CE regimes [10,13]. One drawback of SNP genotyping with SNaPshot is the inability to  
55 distinguish the highly skewed heterozygote peaks often seen in normal DNA with this technique, from the  
56 imbalanced peaks common to mixtures. This is mainly due to the SNaPshot terminator chemistry using  
57 dyes with much stronger blue/green fluorescence (G/A) compared to yellow/red (C/T) [14]. Therefore,  
58 despite their widespread use and evident sensitivity, forensic SNaPshot tests can be inefficient in  
59 detecting mixtures. Indel tests have been developed in recent years for identification [15-17] and ancestry  
60 analysis [18-20] detecting dye-labeled PCR products sent directly to CE from the amplification stage (PCR-  
61 to-CE). The benefits of short amplicon lengths and high levels of multiplexing that SNPs provide, are kept  
62 with Indel genotyping in this way. However, peak height ratios in heterozygotes are more balanced within  
63 any one locus than those of SNaPshot so mixed DNA is more easily detected from the resulting  
64 imbalanced patterns [17]. Two CE-based forensic ancestry tests have been established that offer  
65 complimentary characteristics: a SNaPshot assay of 34 ancestry informative marker (AIM) SNPs containing  
66 some of the most population-differentiated loci (herein 34-plex, [11]) plus a PCR-to-CE assay of 46 AIM-  
67 Indels [19] that offers comparable population differentiation to AIM-SNPs, but much greater sensitivity to  
68 mixed DNA. This report describes the use of these two assays in an inter-laboratory exercise of 15  
69 participants from the European DNA Profiling (EDNAP) group, and 4 overseas participants, organized by  
70 the University of Santiago de Compostela (USC). As a preamble to the EDNAP exercise, the EUROFORGEN-  
71 NoE Consortium ran a similar small-scale inter-laboratory exercise to establish the test framework and  
72 gauge the transportability of the assay primer sets. As part of the Consortium's networking remit, the  
73 primer mixes used for the EDNAP exercise were purchased, optimized and packaged by USC along with  
74 test DNAs with known ancestries (undisclosed to participants). These test components are freely available  
75 in trial quantities for the forensic community to assess for themselves (available from USC upon request).

76 The exercise had three main goals: i) for laboratories to assess the relative ease-of-use and reliability of  
77 the two assays by genotyping test DNAs, whenever possible, using each participant's own CE regimes; ii)

78 for laboratories to use the statistical ancestry inference tools developed at USC and part of the *Snipper*  
79 data analysis portal [11]; iii) to assess the ability of each assay to detect mixtures by including an  
80 unmarked mixed-donor sample amongst the test DNAs. This third goal was analyzed further by assessing  
81 the Indel heterozygote peak height balance in normal DNA across the range of participant's laboratory  
82 setups, in comparison to peaks in the mixed sample. As well as the 15 European laboratories including  
83 USC, two participants were from Australia, one from New Zealand and one from the USA. All but three  
84 laboratories had participated in the preceding EDNAP *IrisPlex* exercise that applied SNaPshot analysis to  
85 the genotyping of six FDP SNPs [21]. Five EDNAP laboratories, were part of the EUROFORGEN-NoE pilot  
86 ancestry exercise.

## 87 **2. Materials and methods**

### 88 *2.1. Primer sets, test DNA samples and assay protocols*

89 Six quantified DNA samples (10  $\mu$ l volumes at 0.5 ng/ $\mu$ l) plus primer mixes sufficient for 20 reactions were  
90 sent to participants who used their own PCR and SNaPshot reaction components. For the Indel assay,  
91 PCRs only required the combination of 2x Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany)  
92 with the primer mix and DNA. The SNaPshot PCR and extension primer sets plus the Indel PCR primer mix  
93 were prepared as previously described [11,19] and were dispatched with the DNA samples at ambient  
94 temperature. Some package transit times outside Europe exceeded one week, but the stability of both  
95 SNP and Indel primer sets had been previously assessed for the EUROFORGEN-NoE pilot exercise by  
96 carefully testing the profile quality obtained from batches of primers originally sent to the US participant  
97 and one in Australia, who were also part of the subsequent exercise.

98 The test DNAs were given anonymized codes and comprised five volunteer donors, each with a different  
99 continental origin of: East Asian, European, Oceanian, Native American or African ancestries. With the  
100 geographic distribution of these samples, examples of all alleles in 80 markers were observed when  
101 genotyped by USC, except SNP: rs1573020 (all A homozygotes) and Indels: rs35451359 and rs33974167  
102 (all short-allele 'A' homozygotes) plus rs2307998 (all long-allele 'C' homozygotes). In this way, more than  
103 97.6% of component marker alleles could be identifiable in the profiles of the test DNAs. A rare third allele  
104 in Indel: rs25584 was found in one test DNA. The sixth test sample was an artificial mixture combining a  
105 1:3 ratio of additional European and East Asian volunteer donors (herein M1 and M3 respectively). Note  
106 that 34-plex has two tri-allelic SNPs and one: rs5030240 showed three allele patterns in the mixed DNA  
107 sample (other examples in [11]).

108 Participants were told that one sample was mixed and were asked to identify it, then assign ancestries to  
109 the others using Bayes analysis and Principal Component Analysis (PCA) in *Snipper*, as detailed in section  
110 2.2. The above primer volumes were sufficient to allow participants to begin their analyses with the  
111 9947A positive control DNA used in many STR kits.

112 Protocols for PCR, SNaPshot extension reactions and CE were sent in the form of an Excel laboratory  
113 calculator (Supplementary File S1) plus fragment mobility panels-and-bins files (Supplementary Files S2)  
114 that formed templates for participants to adapt to their own CE regimes when necessary. The 9947A DNA  
115 acted as a universal point of reference for the peak patterns typical of both assays and example  
116 electropherograms were provided to participants, as shown in Fig. 1. Although Indel amplified fragments  
117 separate well using all POP polymer types, participants were recommended to use POP-4 for 34-plex  
118 genotyping as peak positions are less well separated at the low size range using POP-7. Supplementary

119 Table S1 lists the CE regimes chosen by participants, indicating that most applied a 3130 or 3500 detector  
120 with POP-4 (13 and 3 respectively), although two used a 3130 with POP-7 and one successfully typed SNPs  
121 with a 3100 and POP-6. Lastly, participants were advised that Indel PCR products could require dilution  
122 prior to CE to obtain optimum peak patterns free from excessive signal pull-up.

## 123 2.2. Preliminary ancestry checks of test DNAs and use of the *Snipper* data analysis portal

124 Although this section reports ancestry analysis results, these analyses were made by USC to evaluate the  
125 ancestry of the exercise test samples prior to their dispatch. This process also checked the reference  
126 population data supplied and ensured test samples were suitably representative of each of the ancestries  
127 the participants were asked to identify.

128 The *Snipper* portal provides a Bayes classifier accessing population reference data in place in the website,  
129 including fixed training sets for three, four or five main continental HGDP-CEPH population groups, for 34  
130 SNPs and/or 46 Indels (these training set genotypes are provided in Supplementary File S3.2). The fixed  
131 data options assess one uploaded profile at a time, which is compared to a training set selected by the  
132 user. Partial profiles can be uploaded with NN genotypes (or partial genotypes, e.g. 'CN'). Indel data has  
133 an identical framework but with 'AC coded' genotypes, where A=short alleles, C=long and is reserved for  
134 novel third alleles. Participants were asked to use the fixed training set option in *Snipper* to make ancestry  
135 inferences. However, no guidance was given on choice of training set, which influences calculation of the  
136 likelihood ratios (herein LRs). For example, selecting a five-group training set for 34-plex SNP data will lead  
137 to lower LRs for East Asian assignments as this marker set lacks AIMs sufficiently differentiated to  
138 distinguish Oceanians and Native Americans from East Asians. As a rule-of-thumb, 34-plex profiles are  
139 optimally analyzed with three-group data (Africa-Europe-East Asia), Indel profiles provide high ancestry  
140 assignment LRs for these groups plus Americans, as this differentiation was targeted in their original  
141 selection [19]. When combined 80-marker data is used, the differentiation of the fifth Oceanian  
142 population group can be accomplished, although Indel data alone can also distinguish Oceanians with  
143 minimal error [19].

144 To check the *Snipper* fixed training sets and test samples used, three ancestry analyses were applied to  
145 the genotype data prior to the exercise and results are summarized in Fig. 2. First, the 80-marker  
146 reference data was cross-validated with *Snipper* (each training set profile removed and classified by  
147 remaining data). The Fig. 2 upper plot shows the distribution of probabilities in ranked order of  $\log_{10}$  LR  
148 values, i.e. the lowest LR from five population comparisons (data in Supplementary File S3.3). The grey  
149 line of LR=1 represents balanced odds, so points below this line show misclassifications. East Asian  
150 training set profiles gave five misclassifications, all assigned as American (5/226=2.2% error). However,  
151 none of their LRs exceeded 750, so applying a threshold value of 1000 led to error-free East Asian  
152 assignments, but a non-classification rate of 3.54% (8/226). Fig. 2 indicates the LRs for test samples,  
153 mixture donors and 9947A tend to fall in the middle to upper range of training set LRs in nearly all cases.

154 In addition to obtaining LRs, it can be helpful to compare patterns of variation in reference population  
155 data to samples of unknown geographic origin by applying *STRUCTURE* and PCA. Both provide an intuitive  
156 way to make such comparisons [3,22,23] and can be useful to alert the analyst that a forensic sample of  
157 unknown origin may be from an admixed individual with co-ancestry. Following review of the  
158 EUROFORGEN-NoE ancestry exercise results, a two-dimensional PCA module (plotting the first two  
159 principal components or PCs) was developed for *Snipper* that allows analysis of multiple profiles plotted  
160 directly onto reference data. The *Snipper* output lists the Bayes analysis data for each profile and their



161 positions are labeled on a PC1-PC2 PCA plot (no PC3 estimates are currently made). Participants were  
162 provided with the input file of training set genotypes and a link to the *Snipper* PCA module to enable  
163 graphical analysis of test DNAs and 9947A.

164 The middle graphics of Fig. 2 show *STRUCTURE* cluster plots (an optimum K=5 genetic clusters inferred  
165 from data) matched to the order of training set LRs charted above. The enlarged cluster plots for samples  
166 A-E on the right indicate an absence of co-ancestry, i.e. their cluster plots have almost no membership to  
167 multiple genetic clusters. Likewise, cluster plots on the left for mixture components M1 and M3 show no  
168 multiple cluster membership, whereas sample F has approximately equal joint membership to the  
169 relevant clusters. The lower graphics show three PCAs made with *Snipper*, with reference cluster colours  
170 matched to the *STRUCTURE* data. PCA plot A is a 3-group analysis of sample F and M1-M3 components.  
171 The position of F highlights the fact that population admixture and mixed DNA can give indistinguishable  
172 PCA patterns, emphasizing the need to efficiently detect mixed DNA in forensic ancestry analysis. PCA plot  
173 B is a 5-group analysis showing samples A-E plus 9947A are distributed into their expected clusters,  
174 although in these 2-PC plots the Oceanian and American clusters show some overlap with East Asians. To  
175 better differentiate these three groups, a PCA can be made of just three possible groups to obtain a more  
176 distinct separation, as shown in PCA plot C analyzing the three test samples from less differentiated  
177 population groups.

178

179 **3. Results**

180

181 *3.1. Genotyping performance of the SNP assay*

182

183 Supplementary Table S1 summarizes the CE regimes used by participants and indicates five did not pursue  
184 SNaPshot genotyping of SNPs but elected to just analyze and report Indel genotypes. Given the  
185 complexities of reading electropherograms consisting of 32 peak pairs plus two triple-peak positions, this  
186 was considered to be a reasonable decision and Indel data alone was collected from these laboratories.  
187 The number of SNaPshot no-calls and miscalls recorded for the five test samples A-E, from 14 participants  
188 reporting SNP data, are summarized in Fig. 3A. SNPs are listed in order of decreasing genotyping  
189 performance for participants, by ranking loci in increasing miscall rate followed by increasing no-calls.  
190 Therefore, rs2065160, rs3785181 and rs8986788 are the most robustly genotyped SNPs in 34-plex, with  
191 all 14 laboratories identifying peaks in five samples, although laboratory #17 had one genotype miscall in  
192 each SNP. At the other extreme, rs239031 was both the most difficult SNP to genotype and the least  
193 reliably genotyped, with laboratories #8 and #21 not assigning genotypes to all or most samples, bringing  
194 the overall call rate down to 80%, well below those of the other 33 SNPs. Genotyping concordance for  
195 rs239031 was also the lowest, with 81.4% of genotypes correctly called. High no-call rates for certain  
196 other SNPs tended to cluster with participants: rs1573020 was not genotyped in laboratory #21; rs881929  
197 in #20; rs1886510 and rs2304925 in #19, despite other laboratories genotyping these SNPs without  
198 problems. Only 1 of 5 genotypes was called by laboratory #13 for rs182549. Average SNP call and  
199 genotype concordance rates shown at the bottom right of Fig. 3A reached 96.3% and 93.5% respectively.  
200 The genotype completeness of ~96% equates to approximately one missing SNP call per 34-plex profile.  
201 Laboratory #17 had evident problems recognizing and accurately calling their SNaPshot  
202 electropherograms with less than half the successful genotype calls made by the other participants  
203 reporting SNPs. Therefore, when considering concordance amongst 13/14 participants, the value rose to  
204 97.3%. Although one other laboratory #6 had slightly below-average genotyping concordance, no obvious  
205 connection could be made between the CE regimes used by participants and miscalls seen in certain SNPs.  
206 Nevertheless, there are known issues previously recognized at USC in some 34-plex SNPs and several of  
207 these were observed in the electropherograms from participants. Certain mobility or non-specific peak  
208 patterns can explain a proportion of the genotype miscalls and these are outlined next.

209

210 Examples of three different challenges for SNP genotyping with 34-plex are shown in Supplementary Fig.  
211 S1. First, SNPs rs10843344-rs239031 run to positions very close together, with the C peak of rs239031  
212 often having a mobility shift that places it very close to the much higher C peak of rs10843344  
213 (Supplementary Fig. S1.1). The same signal imbalance can be seen in the T peaks but the electrophoretic  
214 separation of these peaks remains more distinct. Examination of participant's SNaPshot profiles indicated  
215 some laboratories had missed the lower, shifted rs239031-C peak. Second, rs182549, rs881929 and  
216 rs3827760 have particularly low signal strengths (Supplementary Fig. S1.2) and the three SNPs show  
217 higher than average no-call rates. In the case of rs3827760, there is a very marked disparity in peak  
218 heights between the higher East Asian-informative G allele and the A allele (> 10:1 peak height ratio in the  
219 example shown), so this SNP requires particular care. Third, rs2304925 shows an artifactual G signal in the  
220 negative control very close to the G peak of rs5030240 (Supplementary Fig. S1.3). This peak is much  
221 higher than the T peak of rs2304925 when it is a true allelic extension product but much lower when  
222 artifactual. All participants ran a negative control and most recognized the extra G signal running close to  
223 the G peak of rs5030240, although as this is a tri-allelic SNP, when a homozygous A or C allele is present

224 the genotypes can be mistyped as an AG or CG in the absence of the stronger G peak with which to  
225 compare the artifact signal.

226

### 227 3.2. Genotyping performance of the Indel assay

228 All 19 participants successfully completed the genotyping of the samples with Indels. Supplementary  
229 Table S1 shows that almost half of the laboratories chose to dilute the PCR products 1:5-1:20 prior to CE  
230 detection to control signal pull up. Supplementary Figs. S1.4-5 show two examples of minor challenges  
231 with genotyping of Indels, consisting of the occurrence of dye blobs (broad non-specific peaks around  
232 allele peak positions), identifiable in the negative control, plus signal pull-ups that can occur when the  
233 Indel PCR products are not sufficiently diluted. However, there was no evidence that these two profile  
234 phenomena interfered with the genotyping performance of the Indel tests in any of the 19 laboratories. In  
235 fact, the genotyping completeness and concordance were very high when considering that most  
236 participants were running the test for the first time and required reading 46 different peak sets in each  
237 electropherogram.

238 Fig. 3B summarizes the Indel genotyping performance and shows participants achieved a very high overall  
239 genotyping completeness and concordance rate of 99.8%. Fourteen participants did not have miscalls or  
240 no-calls in any test sample profiles. A slight degree of clustering of genotyping miscalls and no-calls is  
241 discernible in Fig 3B; for example, laboratory #20 chose not to call 3/5 rs2307922 genotypes, and  
242 laboratories #1 and #7 mistyped more than one Indel. It is notable that all 19 participants successfully  
243 identified the rare third allele of rs25584 present in test sample C.

### 244 3.3. Inference of ancestry

245 All participants identified F as the mixed DNA sample and made Bayes analysis to infer the ancestry of  
246 samples A-E using *Snipper*. The majority, but not all, also made comparisons of the genotypes from A-E  
247 with the *Snipper* PCA module using the supplied reference population data. This section summarizes  
248 results for all laboratories using both statistical approaches to illustrate that the SNP and Indel data has a  
249 degree of ancestry-informativeness redundancy, i.e. the Bayes LRs or PCA positions of samples A-E are  
250 very similar despite some genotype miscalls or missing data. Therefore, the ancestry inferences made by  
251 participants were correct in all cases apart from those of laboratory #17 that made incorrect ancestry  
252 inferences for two samples and had PCA positions markedly displaced from the others in most cases.

253 Fig. 4A summarizes SNP profile quality (bar-charts, left-hand scale); Bayes LRs (points superimposed on  
254 bars, right-hand scale); and PCA positions for the SNaPshot assay data of 14 participants, analyzing  
255 samples A-E. Bayes LRs and PCAs from SNP data alone compare African, European and East Asian  
256 ancestries; consequently C and D give lower LRs and edge-of-cluster PCA positions that suggest East Asian  
257 ancestry despite these being Oceanian and American in origin. For 13/14 laboratories, samples A, B and E  
258 give mid-cluster PCA positions and high LRs that varied by four orders of magnitude between  $1E+14$  to  
259  $1E+18$  correctly assigning A as East Asian and B as European, and  $1E+22$  to  $1E+26$  correctly assigning E as  
260 African. The LR values obtained by coordinating laboratory USC for SNP and Indel data are outlined in  
261 Table 1 (the 80-marker LRs for all samples are given separately in Fig. 2). Table 1 indicates sample C gave a  
262 high LR for Oceanian ancestry with just Indel data used in a 5-group comparison.

263 Fig. 4B summarizes Indel profile quality, Bayes LRs and PCA positions for a four group comparison using  
264 the Indel data of all participants. A sixth PCA plot, bottom right, shows the combined 80-marker analysis

265 for Oceanian sample C. Apart from African sample E, Indel data gives lower LR than SNPs and the LR for  
266 samples A and E are from different population likelihoods (bold values in Table 1). The improved  
267 genotyping consistency of Indels amongst participants is reflected in more uniform sets of Bayes LR and  
268 PCA positions that mainly overlay each other (i.e. seen as single points on plots). For the two laboratories  
269 with three Indel miscalls, an effect is seen in the Bayes LR for American sample D and African sample E,  
270 with some PCA displacement, indicating that even with just two markers miscalled it can sometimes affect  
271 the statistical inference made from other correctly called genotypes (~97% of the data). The Oceanian  
272 sample C was correctly identified by 18 participants, with many using both Indel and combined data to  
273 make the inference.

274 Therefore, 18 of 19 laboratories were able to successfully assign ancestry to five samples of undisclosed  
275 geographic origin, obtaining unequivocal Bayes LR and, in most, cases participants constructed PCA plots  
276 providing supplementary analyses with good matches to the Bayes results.

#### 277 *3.4. Mixture detection and analysis of participant's Indel peak height data*

278 Although the exercise was not a fully blinded test (i.e. where the presence of a mixed sample is not  
279 disclosed), all participants were able to identify sample F as the mixture from the observation of  
280 imbalanced signals in the heterozygote peak pairs of the Indel profile. Therefore, despite a lack of  
281 familiarity with Indel peak patterns in most laboratories, there was sufficient contrast between the mixed  
282 sample F and the unmixed A-E DNAs for the mixture to be discernible by all participants. In addition, 7 of  
283 14 laboratories reported an ACG triple-peak pattern in the tri-allelic SNP rs5030240, one reported an AC  
284 with possible G, one a GG result and the other five gave no-calls. A typical sample F peak pattern for  
285 rs5030240 is shown in Supplementary Fig. S1.6.

286 The detection of peak height imbalances that can indicate mixed DNA has been stated to be an advantage  
287 of direct PCR-to-CE Indel genotyping compared to SNaPshot tests [15,17,19], however such patterns have  
288 not been properly assessed across a range of CE detectors. For this reason, we decided to ask participants  
289 to provide their heterozygote peak height data and then compiled the variation in peak height ratios  
290 (PHRs, highest/lowest peaks) recorded in the five unmixed and single mixed DNAs from the range of CE  
291 regimes used. Furthermore, when analyzing binary markers the number of heterozygotes observed in  
292 mixtures is invariably higher than normal unmixed samples. Although PHR values were distinct between  
293 A-E and F, three factors complicated the straightforward statistical comparison of patterns of  
294 heterozygosity observed amongst the test samples. First, there was variation in the number of  
295 heterozygotes recorded in sample F. Specifically, laboratory #1 identified 18 heterozygotes; #15: 17; #18:  
296 21; and #20: 17, compared to an average number of heterozygotes identified by the other fifteen  
297 laboratories of 27. Second, the lower number of identified heterozygotes for F in some participant's data  
298 affects the minimum-maximum and average PHR values, particularly when the PHR is extreme and a very  
299 low peak is discounted when reading the profile. Four example peak pairs that were recorded as single  
300 allele genotypes by one participant but as heterozygotes by the others, are shown in Fig. 5A. Third, due to  
301 the contrasting frequencies of most of the 46 Indels between population groups, sample A showed lower  
302 numbers of heterozygotes and sample B higher numbers than those seen in C-E.

303 The numbers of heterozygotes and PHR values are plotted in Fig. 5B. This chart shows data from 15/19  
304 laboratories (excluding #1, #15, #18 and #20). The same chart with all 19 participant's data is shown in  
305 Supplementary File S4.A. The dark grey bars mark the data from 3500 detectors and indicates that no  
306 difference in peak height ratios are discernible in comparison with 31x CE data.

307 Statistical assessment of the number of heterozygotes in A-E vs. F was made with a unilateral 2-sample  
308 test for equality-of-proportions (with continuity correction). The resulting grid of  $p$ -values for pairwise  
309 comparisons across all 19 laboratories is shown in Supplementary File S4.B, along with the Fig 5B chart re-  
310 plotted for full data from all laboratories (Supplementary File S4.A). It can be seen from the  
311 Supplementary File S4.A chart that the numbers of sample F heterozygotes recorded by laboratories #1,  
312 #15 and #20 is lower than the average number in unmixed sample B. Inclusion of this data has a direct  
313 effect on the distribution of significant  $p$ -values obtained from pairwise comparisons. Laboratories #1, #15  
314 and #20 sample F heterozygote numbers are significantly different to those of most of the other  
315 laboratories, but not different to heterozygote numbers in unmixed samples B-E, while #18 data for  
316 sample F is not significantly different to samples B and C. The high number of heterozygotes in sample B is  
317 reflected in significant differences only found for comparisons to those of laboratories #8, #13, #14, and  
318 #5, who recorded 29 or more heterozygotes in their sample F profiles. Therefore, we opted to remove #1,  
319 #15, #18 and #20 data from the statistical assessment of PHR differences between A-E and F.

320 The average PHRs shown in Fig. 5B indicate a quite distinct contrast between samples A-E and F, with  
321 values of 1.15 compared to 3.14 respectively, which suggests a ratio of 1:2.73 that approximates the  
322 actual 1:3 contributor ratio well. Although the PHR values give a clearly discernible difference between  
323 mixed and unmixed samples, we completed a formal statistical test of this difference. An ANOVA test is a  
324 standard approach for assessing continuous values such as PHR measurements, but a Shapiro-Wilks test  
325 indicated some of the data was not normally distributed (data not shown). Therefore, a Kruskal-Wallis  
326 rank sum test was applied and the grid of pairwise  $p$ -values comparing the average PHRs of A-E with  
327 individual PHRs of F is shown in Supplementary File S4.C. The results are completely consistent: the  
328 pairwise comparisons of mixed vs. unmixed PHRs give significant  $p$ -values in every case and none were  
329 detected for comparisons within each sample set.

330 In summary, despite the need to adjust statistical comparisons by removing 4 of 19 participant's data due  
331 to under-reported heterozygote peak pairs, the other laboratories provided a ratio of average peak  
332 heights close to 1:3. This ratio is consistent with the mixture that was constructed for the exercise and is  
333 statistically significant for all signal strength comparisons made.

### 334 *3.5. Additional Next Generation Sequencing experiments applied to test DNAs by two laboratories*

335 Two laboratories decided to use their remaining PCR primers to genotype one or both marker sets with  
336 different Next Generation Sequencing (NGS) systems, as outlined in Supplementary File S5. One assessed  
337 34-plex SNP typing using an unmodified PCR followed by library preparation and massively parallel  
338 sequencing with the Illumina MiSeq system. The other assessed 34-plex SNPs and Indel genotyping in the  
339 same way (unmodified PCR in each case) with the Thermo Fisher Scientific-Life Technologies (TFS-LT) Ion  
340 PGM™ system.

341 The 34-plex SNP sequence analyses were successful to a very large degree, as all genotypes were  
342 identified and almost fully concordant with each laboratory's SNaPshot data. Sample F was observed to  
343 be distinct in a major proportion of its allele-pair sequence ratios (defined as the second allele exceeding  
344 10% of sequence reads), compared to A-E. Supplementary File S5 indicates there were only 5/14  
345 sequence ratios of 1.5 or less (i.e. in the range: 0.4:0.6-0.5:0.5) in the Ion PGM™ data and 3/17 in the  
346 MiSeq data. This equates to 64% and 82% of sequence ratios exceeded those of most normal DNA  
347 heterozygotes seen in Ion PGM™ and MiSeq respectively, giving unequivocal signals of a mixture in F.  
348 Both systems also detected displaced sequence ratios in each of the two tri-allelic SNPs.

349 The Indel analysis with NGS gave three discordant genotypes in samples B and C, plus an average 8.7% no-  
350 calls (coverage too low) and 2.9% missing data (undetected sequence), although not all samples gave the  
351 same non-detection rates. Overall, 84% of the NGS genotypes matched the CE calls. However, the  
352 alignment of sequences that contain short insertions and deletions is particularly challenging in NGS  
353 sequence analysis and it was not possible to be sure how many miscalls or no-calls were due to  
354 misalignment issues. Supplementary File S5 shows assessments of Indel sequence ratios for sample F  
355 compared to A-E. Given that sequence coverage was low in some loci and this is the first NGS experiment  
356 with this type of forensic marker, results need cautious interpretation. However, patterns suggest a  
357 degree of displacement in F away from the perfect sequence balance midline (0.5:0.5) compared to many  
358 of the heterozygote sequence ratios detected in A-E.

359 **4. Discussion**

360 As forensic NGS analysis gains greater traction, it is the right moment for the forensic community to use  
361 inter-laboratory exercises to assess the binary marker sets that will start to add complementary genetic  
362 data to conventional STR polymorphisms. Ancestry inference is seen as a key part of the enhanced  
363 characterization of forensic DNA that NGS will allow. Therefore, it is important to evaluate the robustness  
364 of existing CE-based ancestry-informative SNP and Indel multiplexes in terms of how easily they can be  
365 adopted in laboratories not previously experienced with binary marker genotyping. The statistical analysis  
366 of the genotype data obtained from AIMs also needs to be easy to use and interpret by forensic  
367 laboratories. The most straightforward approach for inferring ancestry uses Bayesian LR comparisons  
368 between the two geographic origin hypotheses with the highest likelihoods. Lastly, binary variation has a  
369 reduced capacity to detect mixtures since homozygotes in combination can look like heterozygotes and  
370 only a few non-binary SNPs or Indels currently offer the chance to observe more than two alleles.  
371 Therefore, the exercise findings for genotyping reliability, ease-of-use of the recommended ancestry  
372 inference tools and ability to detect mixed DNA are all relevant to the progress towards adoption of AIMs  
373 in forensic analysis.

374 The principal finding of this exercise was that each of the participants readily established the AIM-Indel  
375 46-plex test in their laboratory. All participants achieved good quality profiles that reached the high level  
376 of genotyping concordance of 99.8% and then efficiently detected mixed sample F. In contrast, SNaPshot  
377 typing was both more challenging and for many participants less reliable, despite most laboratories  
378 having successfully genotyped six SNPs for the preceding *IrisPlex* EDNAP exercise [21]. Miscalled  
379 genotypes with SNaPshot produced an overall genotyping concordance rate of 97.3% when a single  
380 participant's results were excluded (13/14 laboratories).

381 We have no explanation for the very high number of SNP miscalls from this one laboratory but it resulted  
382 in their statistical analyses producing the only incorrect ancestry inferences for two test DNAs and one  
383 uninformative LR of 1.2. All other participants produced correct ancestry predictions from the Bayes LRs  
384 calculated in *Snipper* and, for those that created PCA plots, obtained cluster patterns and profile positions  
385 that corresponded to these LRs. Therefore, from the review of exercise reports returned from 19  
386 laboratories, we can recommend the use of both of these statistical approaches to ancestry inference, as  
387 these proved easy to use and allowed correct ancestry assignments of samples with undisclosed  
388 geographic origin in 92 of 95 cases.

389 Mixture detection achieved from Indel peak patterns was particularly successful, with sample F giving a  
390 clear signal of mixed DNA for all participants. Our analysis of peak height ratios made after the exercise  
391 finished, gave a good approximation of the actual mixture component ratio, averaging 1.15 and 3.14 for  
392 PHRs in unmixed samples and the mixture respectively. The much higher number of heterozygotes in F  
393 could mainly be due to the different ancestries of the mixture contributors. Nevertheless, recording a  
394 higher number of heterozygotes than in normal DNA samples and observing PHRs markedly above ~1.2  
395 gives a simple and easily adopted system to detect mixtures with Indels. SNaPshot does not offer the  
396 same direct relationship between peak heights and input DNA so there is a risk that simple two-person  
397 mixtures mimic the patterns seen in individuals with co-ancestry due to population admixture, as revealed  
398 by the PCA plot of sample F in Fig. 2 (plot A). Obviously, single sample experiments are not fully indicative  
399 of how well Indels will perform with a range of forensic samples, mixture ratios or component ancestry  
400 combinations, but the fact that most participants were running Indels for the first time and all detected

401 the mixture indicates sensitivity to mixed DNA with this assay.

402 Although the NGS findings from two participants are a set of parallel genotyping experiments using  
403 exercise materials that were not part of the study plan, results are included in this report to highlight the  
404 enhanced sensitivity to mixtures obtained for SNP analysis with NGS. It is also interesting to note that  
405 existing optimized forensic multiplexes work very well in NGS without the need for any modification,  
406 confirming the results of a recent study that found the 34-plex PCR primers, amongst four other forensic  
407 SNP multiplexes, provide good quality output with the Ion PGM™ system [24]. In addition, the relative  
408 success of the initial Indel genotyping experiments with NGS indicate dye-labeled PCR primers do not  
409 interfere with library preparation and subsequent sequencing chemistry of the Ion PGM™. This suggests  
410 existing forensic CE multiplexes for a range of markers, including STR kits, could be used to prepare target  
411 DNA for experimental NGS sequencing runs.

412 Until NGS systems that incorporate AIMs are widely adopted for forensic use, the results from this EDNAP  
413 inter-laboratory exercise indicate the PCR-to-CE Indel test is by far the best current option for forensic  
414 ancestry analysis. The Indel multiplex provides a simple, reliable and informative test from a  
415 comparatively large marker set that is analyzed using validated CE regimes. Detection of simple two-  
416 component mixed DNA from scrutiny of Indel peak patterns was a task accomplished by all exercise  
417 participants and gives Indel genotyping a key additional advantage over SNP-based ancestry tests.

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419

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**Table 1.** Lowest LR values produced from *Snipper* Bayes analysis of the full SNP and Indel profiles of samples A-E and 9947A with their ancestry inferences. Participant LR values for the same samples are plotted in Figs. 4A/B. Bold values for A and E highlight different population ratios giving the lowest LRs when SNP, 3-group or Indel, 4-group comparisons are made. With Indel, 4-group comparisons the second lowest LRs for samples A and E are based on the same population ratios as the lowest LRs for SNP, 3-group comparisons. Sample C is correctly inferred to be Oceanian with Indel data alone but most participants reported the LR from 80 marker data.

Inference:	34-plex SNPs, 3-group	
European	9947A is 2,118,840,589,047,061,020,672 times more likely EUROPEAN than E ASIAN	
East Asian	<b>A is 361,148,635,069,545,024 times more likely E ASIAN than EUROPEAN</b>	
European	B is 64,191,487,284,485,608 times more likely EUROPEAN than E ASIAN	
East Asian	C is 13,115,706 times more likely E ASIAN than AFRICAN	
East Asian	D is 248,539,593,557 times more likely E ASIAN than EUROPEAN	
African	<b>E is 556,454,701,312,037,054,117,314,560 times more likely AFRICAN than E ASIAN</b>	
	46-plex Indels, 4-group	46-plex, 4-group (second lowest LR)
European	9947A is 1,937,432,967,198 times more likely EUROPEAN than E ASIAN	
East Asian	<b>A is 6,993,957 times more likely E ASIAN than AMERICAN</b>	A is 37,290,377,821,078,192,128 times more likely E ASIAN than EUROPEAN
European	B is 143,659,679,122 times more likely EUROPEAN than E ASIAN	
LR too low	C is 131 times more likely E ASIAN than EUROPEAN	
American	D is 944,698,134 times more likely AMERICAN than E ASIAN	
African	<b>E is 3,229,841,442,838,053,650,432 times more likely AFRICAN than EUROPEAN</b>	E is 5,715,694,248,335,998,122,459,136 times more likely AFRICAN than E ASIAN
	46-plex, 5-group	80 Markers, 5-group
Oceanian	C is 24,880,402 times more likely OCEANIAN than E ASIAN	C is 153,747,536,542,653 times more likely OCEANIAN than E ASIAN

508

509 **Figure legends**

510

511 **Fig. 1.** Electropherograms from the Indel test (upper panel) and the 34-plex SNP test for the 9947A control  
512 DNA. Peak positions are labeled with the internal codes used for each marker (internal code-rs-number  
513 lists are provided in Fig. 3A; Supplementary Files S3; *Snipper* and in [11]).

514

515 **Fig. 2.** Ancestry analysis of exercise test samples. 80-marker genotypes were analyzed and HGDP-CEPH  
516 training set data was as supplied to participants (Supplementary File S3.2). Top plot shows ranked  
517 *Snipper* Bayes analysis LRs from training set cross validation or test profile analysis (black points). Grey  
518 points in East Asians/Oceanians indicate LRs below a threshold value of 1000 (the grey shaded log LR  
519 range around balanced odds line of LR=1). Red points indicate East Asian training set LRs that misclassified  
520 as Americans. Middle plots show *STRUCTURE* analysis aligned directly to the LR distributions above  
521 with separate plots for mixture components, left and test samples, right. Lower plots show 2D PCA  
522 analyses of test samples in 3-group or 5-group comparisons. Plot A shows a 3-group comparison of sample  
523 F, positioned mid-cluster between contributors M1 and M3. Plot B shows the full 5-group PCA of samples  
524 A-E plus 9947A. Plot C shows a restricted comparison of just East Asian, Oceanian and American data to  
525 obtain better differentiation of reference population clusters and A, C, D; all more closely distributed in  
526 plot B.

527

528 **Fig. 3.** (A) Genotyping performance of the 34-plex test arranged by SNP (rows) and by 14 participants  
529 (columns). Cells record miscalls on the left, and no-calls right. The bar plots on the right summarize total  
530 genotype completeness and concordance for each SNP and at the bottom, for each participant. SNPs are  
531 ordered by diminishing performance (i.e. decreasing concordance then completeness). Overall genotype  
532 concordance is given for 14 and 13 laboratories separately, excluding participant #17 with a very high  
533 number of SNP miscalls. (B) Genotyping performance grid for Indel test data from all 19 laboratories.  
534 Miscalls are shown as dark grey cells, no-calls light grey.

535

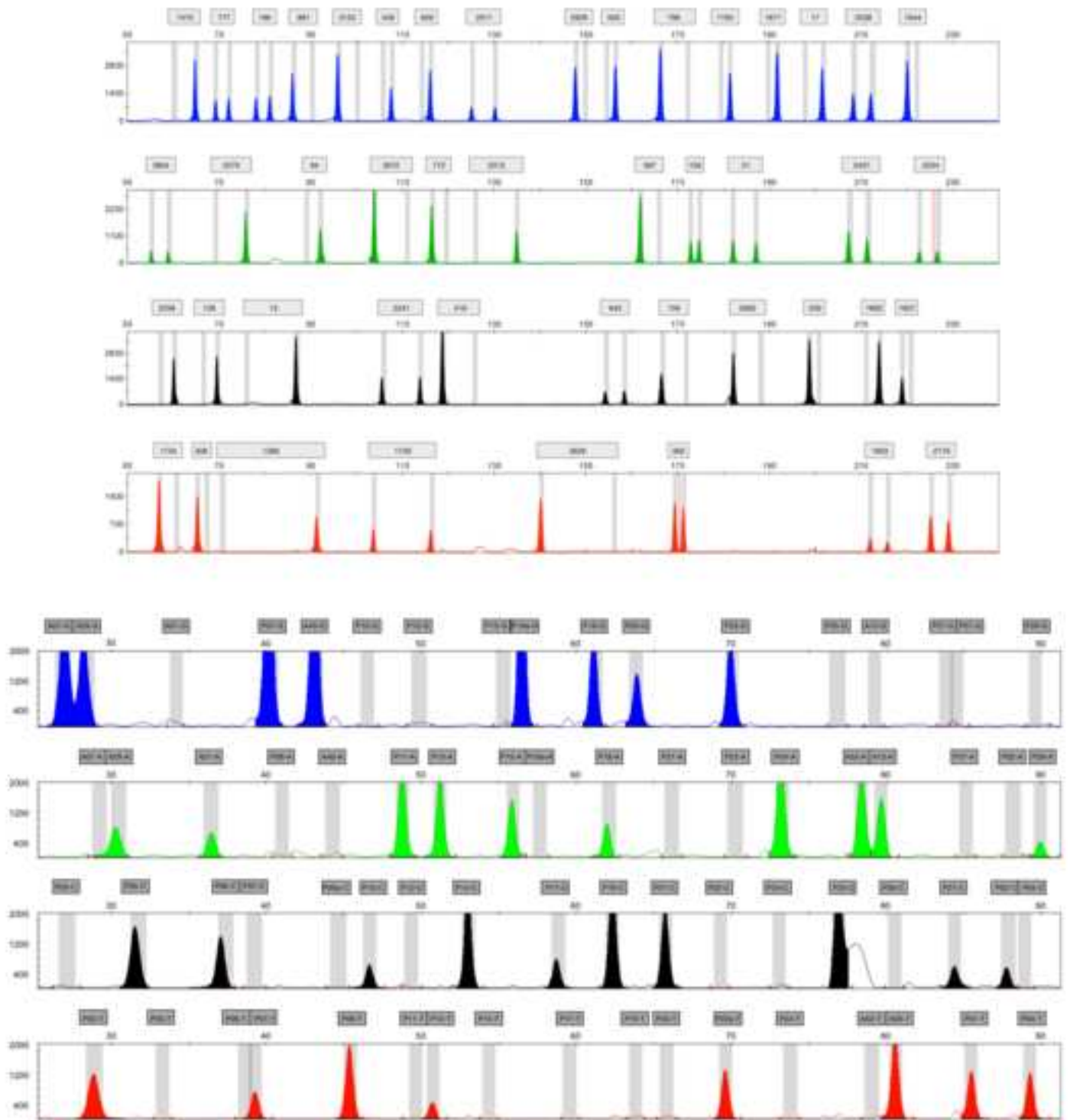
536 **Fig. 4.** (A) Participant's SNP-based Bayes LRs and PCA positions for three-group comparisons (AFR-EUR-E  
537 ASN) analyzing samples A-E. Genotype completeness and concordance rates are shown as bar charts (left-  
538 hand scales) and ancestry assignment LRs (i.e. lowest values) as overlaid points (right-hand scales).  
539 Laboratories with some displacement of a sample position from the main PCA cluster are individually  
540 labeled and incorrect positions/assignments from miscalled genotypes are shown in red. (B) Participant's  
541 Indel-based Bayes LRs and PCAs for 4-group comparisons (including Americans) analyzing A-E. The sixth  
542 plot, lower right, shows a 5-group PCA of sample C (adding Oceanian reference data) using 80-marker  
543 genotypes. Laboratories only reporting Indel data have Bayes LRs shown in green and one uninformative  
544 LR shown in blue.

545

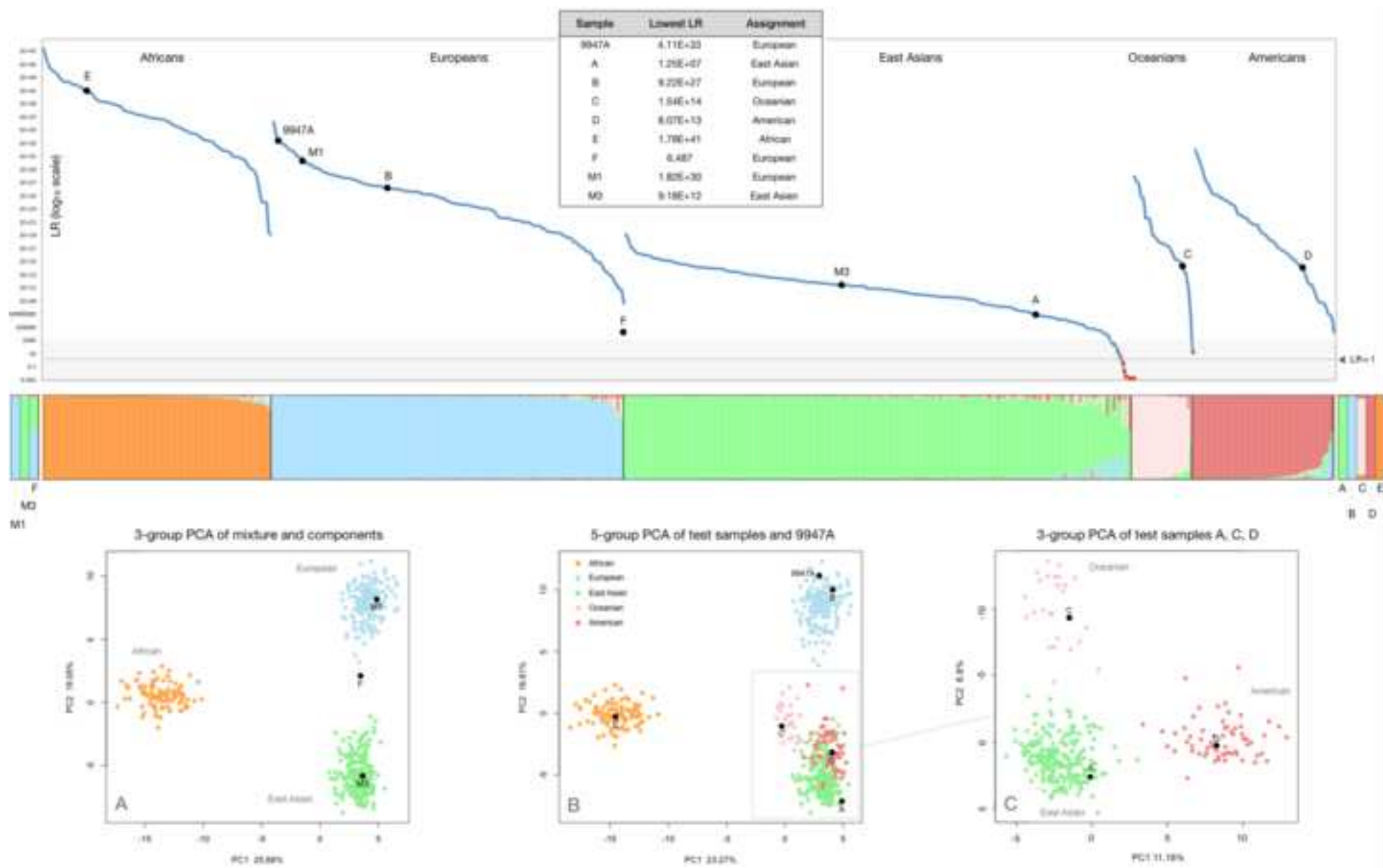
546 **Fig. 5.** (A) Example Indel peak pairs for sample F discounted as heterozygotes by one participant. (B)  
547 Numbers of Indel heterozygotes (bars) and their peak height ratios (PHR: points) recorded by 15  
548 participants. Unmixed samples A-E are average values from all data and sample F values are shown  
549 individually as different numbers of peak pairs were recognized as heterozygotes amongst laboratories.

550 **Supplementary Files**  
551  
552 **Supplementary Figs. S1.** Examples of genotyping challenges in 34-plex or Indel profiles.  
553  
554 **Supplementary Table S1.** Capillary electrophoresis (CE) details for participating laboratories. Grey bars for  
555 34-plex denote five participants not completing SNP genotyping with SNaPshot.  
556  
557 **Supplementary File S1.** Laboratory protocol guide in the form of an Excel calculator for reaction setups  
558 provided to exercise participants.  
559  
560 **Supplementary File S2.** CE fragment mobility panels-and-bins files provided to exercise participants.  
561  
562 **Supplementary Files S3.** SNP and Indel genotypes used in the exercise as reference population data plus  
563 test DNA data established by USC. Worksheets are:  
564 **File S3.1** PCA input  
565 **File S3.2** The 5-group training set data for Bayes analysis  
566 **File S3.3** Cross-validation data plotted in Fig. 2.  
567 Note that to use the files for *Snipper* analysis each must be moved to 'worksheet position 1'.  
568 PCA: <http://mathgene.usc.es/snipper/analysismultipleprofiles.html>,  
569 Bayes custom or fixed training set data: [http://mathgene.usc.es/snipper/analysispopfile\\_new.html](http://mathgene.usc.es/snipper/analysispopfile_new.html)  
570 <http://mathgene.usc.es/snipper/popchoosing5groups.html>).  
571  
572 **Supplementary File S4.** Statistical analysis of participant's Indel heterozygote peak height ratio data.  
573  
574 **Supplementary File S5.** Details and results of NGS analyses of 34-plex and Indel markers made by two  
575 laboratories.

Figure 1  
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**Figure 2**  
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**Figure 3A**  
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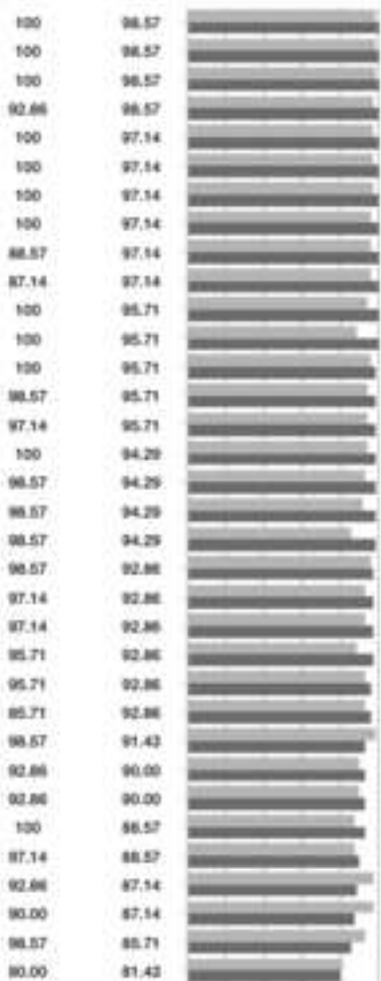
34 SNPs miscalls | no-calls

14 labs

34-plex SNPs ordered by decreasing genotyping performance for participants

		1	2	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19	20	21
P10	rs2065100	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				1/0	0/0	0/0	0/0	0/0
P10	rs3788181	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				1/0	0/0	0/0	0/0	0/0
P14	rs896788	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				1/0	0/0	0/0	0/0	0/0
P10	rs1573020	1/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				0/0	0/0	0/0	0/0	0/0
P24	rs1428654	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				2/0	0/0	0/0	0/0	0/0
P16a	rs2572267	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				2/0	0/0	0/0	0/0	0/0
P04	rs2614778	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				2/0	0/0	0/0	0/0	0/0
P26	rs730670	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				2/0	0/0	0/0	0/0	0/0
A11	rs1886510	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				2/0	0/0	0/0	0/0	0/0
P08	rs2827700	0/0	0/1	0/0		0/0	0/0	0/1	0/1	0/0		0/0				0/0	0/0	0/0	0/1	0/0
A40	rs2040411	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				0/0	0/0	0/0	0/0	1/0
P14	rs2065962	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				0/0	0/0	0/0	0/0	0/0
P08	rs7667596	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				0/0	0/0	0/0	0/0	0/0
P09a	rs1978606	0/0	0/0	0/0		0/0	0/0	0/0	0/0	1/0		0/0				0/0	0/0	0/0	0/1	0/0
P23	rs2026721	0/0	0/0	0/0		0/0	0/0	0/1	0/0	0/0		0/0				0/0	0/0	0/0	0/1	1/0
P24	rs18891982	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				4/0	0/0	0/0	0/0	0/0
P08	rs12813632	1/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				0/0	0/0	0/0	0/1	0/0
P03	rs1321333	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				4/0	0/0	0/0	0/1	0/0
P10	rs772608	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				4/0	0/0	0/1	0/0	0/0
P17	rs2302798	0/0	0/0	0/0		0/0	0/0	1/1	0/0	0/0		0/0				4/0	0/0	0/0	0/0	0/0
A02	rs1335873	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				4/0	0/0	0/0	0/0	1/0
P21	rs1486444	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/0	1/0					2/0	0/0	0/0	0/2	0/0
P24	rs4540055	1/0	0/0	0/0		0/0	0/0	1/2	0/0	0/0		0/0				0/0	0/0	0/1	0/0	0/0
P02	rs5967008	0/0	1/1	0/0		0/0	1/0	0/1	0/0	0/0		0/0				0/0	0/0	0/0	0/1	1/0
P20	rs81928	0/0	0/1	0/0		0/0	0/0	1/1	0/0	0/0		0/0				4/0	0/0	0/1	0/0	0/0
A28	rs1024116	0/0	0/0	0/0		0/0	0/0	0/0	0/0	0/1		0/0				0/0	0/0	0/0	0/0	1/0
P09a	rs10843944	1/0	0/0	0/0		1/0	0/0	1/0	0/0	0/0		0/0				4/0	0/0	0/1	0/1	0/0
P12	rs182548	0/0	0/0	0/0		4/0	0/0	0/0	0/1	0/0		0/0				0/0	0/0	0/0	0/0	0/0
P11	rs10141763	0/0	0/0	0/0		0/0	0/0	1/0	0/0	0/0		0/0				4/0	0/0	0/0	0/0	0/0
A21	rs722088	1/1	0/0	0/0		0/0	0/0	0/0	0/0	0/0		0/0				0/0	0/0	0/0	0/1	0/0
P07	rs600040	0/0	0/0	0/0		1/0	0/0	0/0	0/0	0/1	1/0					0/0	0/1	0/0	0/1	0/0
P01	rs2304820	0/0	1/0	1/0		0/0	0/0	0/0	0/0	0/1	1/0					0/0	0/0	0/0	1/1	1/0
A07	rs817116	1/0	0/0	0/0		0/0	0/0	1/0	0/0	0/0		0/0				0/0	0/0	0/0	0/1	0/0
P02	rs239021	0/1	0/0	0/0		0/0	1/0	0/1	0/1	0/0		0/0				0/0	0/0	1/1	0/0	0/0

Genotype completeness  
 Genotype concordance



Profile completeness	96.82	96.24	100		100	96.82	90.59		96.24	96.47						98.24	99.41	91.58	86.47	82.94	<b>96.3</b>	
Profile concordance	95.29	97.06	99.41		90.59	96.82	94.12		99.41	96.24						44.71	98.82	99.41	96.24	95.29		<b>93.5</b> (14 labs)





Figure 3B  
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46 Indels miscalls | no-calls

19 labs

		1	2	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19	20	21	
MID-1470	rs2307966	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-777	rs1810663	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-196	rs18635	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-681	rs1810965	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1430	rs20451388	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-648	rs140637	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-628	rs1160893	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2011	rs2308203	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2009	rs23074167	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-593	rs1160832	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-736	rs1810884	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1103	rs2067280	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1071	rs2308067	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-17	rs4182	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2038	rs2054057	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1444	rs2307840	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2654	rs00612434	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2275	rs3033053	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-44	rs14384	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2072	rs24611675	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-772	rs1810899	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2213	rs20452715	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-387	rs230621	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1636	rs2307932	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-61	rs16343	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2421	rs3031979	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2384	rs24122927	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2288	rs123062	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-138	rs6490	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-15	rs4181	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2241	rs3030026	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-419	rs140708	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-940	rs1811628	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-139	rs18438	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2005	rs2306181	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-200	rs18687	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1882	rs2307966	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1887	rs2307963	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1734	rs2307900	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-406	rs230630	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1388	rs2307582	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1728	rs2307922	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2629	rs11257928	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-380	rs230884	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-1982	rs2307799	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
MID-2719	rs24541393	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

Genotype completeness  
 Genotype concordance

Profile completeness	99.1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Profile concordance	98.7	100	100	100	100	98.7	100	100	100	100	99.8	100	100	100	100	100	100	100	100	100	100

99.8

99.8

**Figure 4A**  
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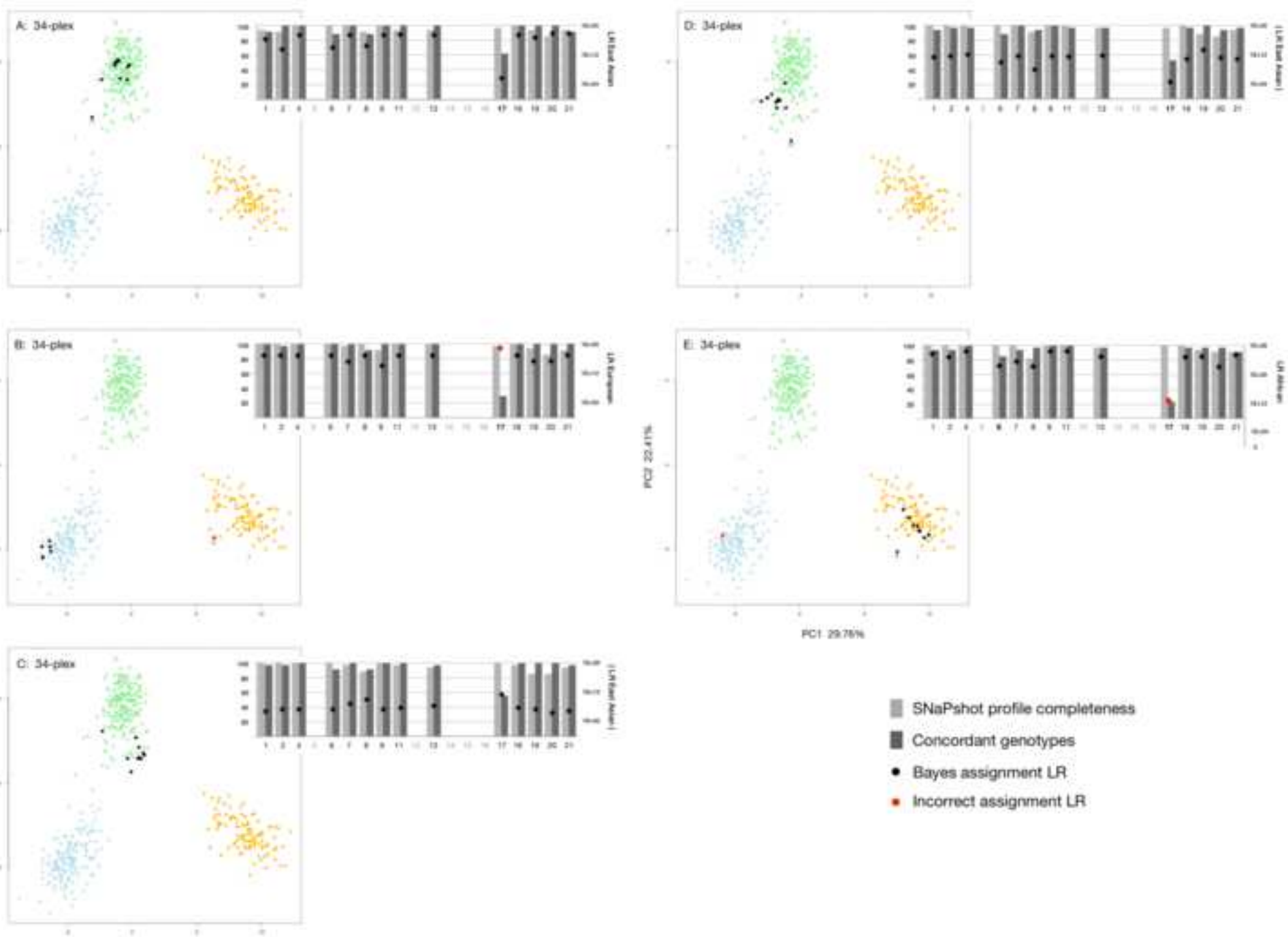
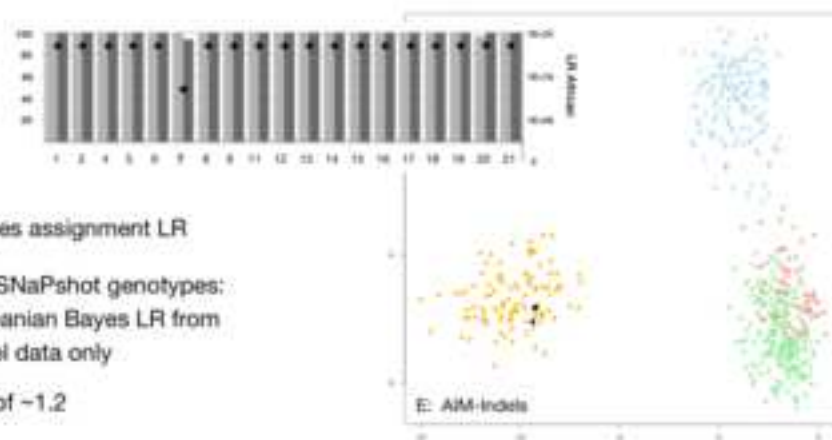
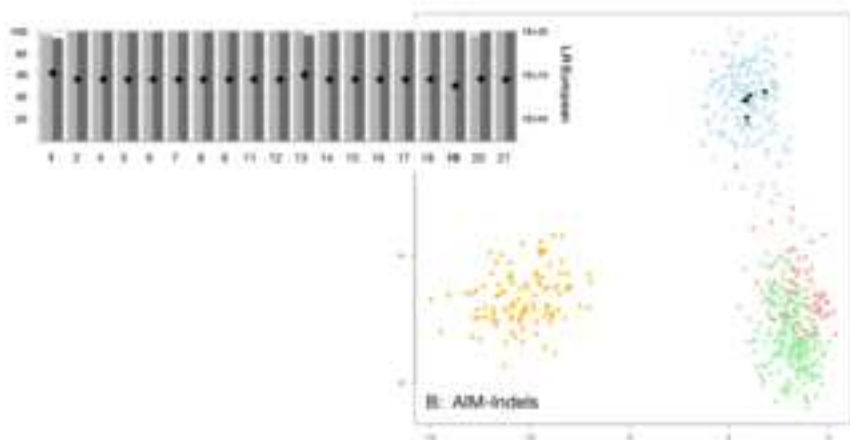
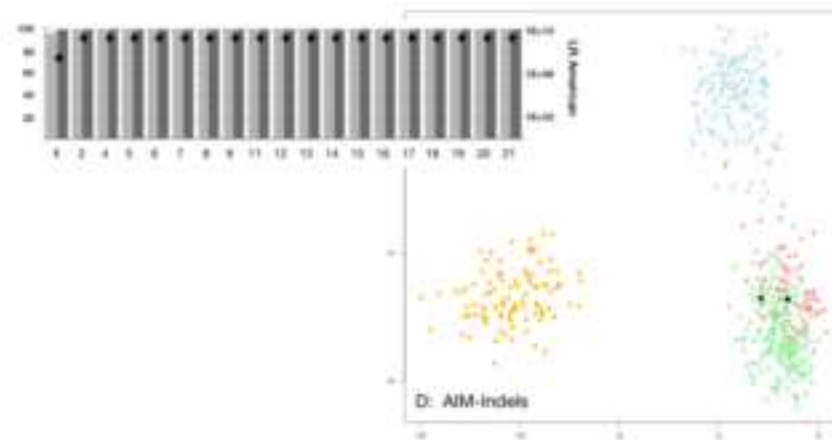
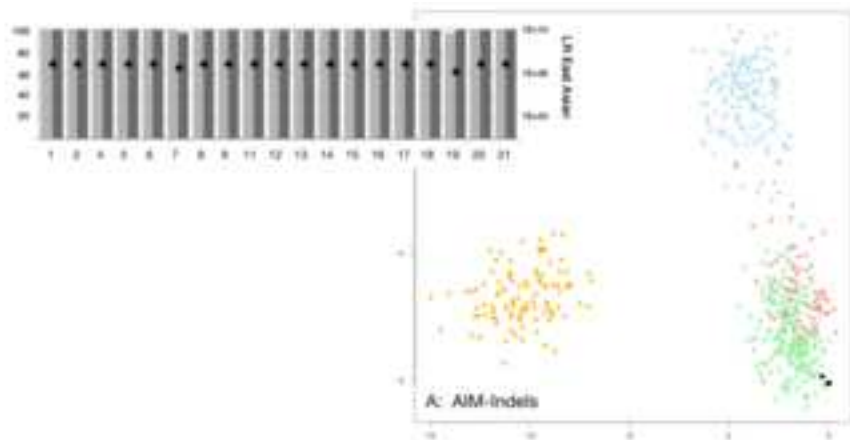
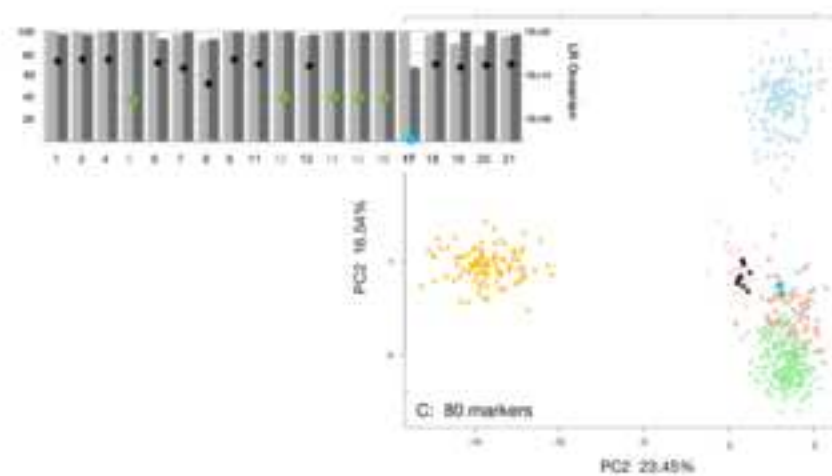
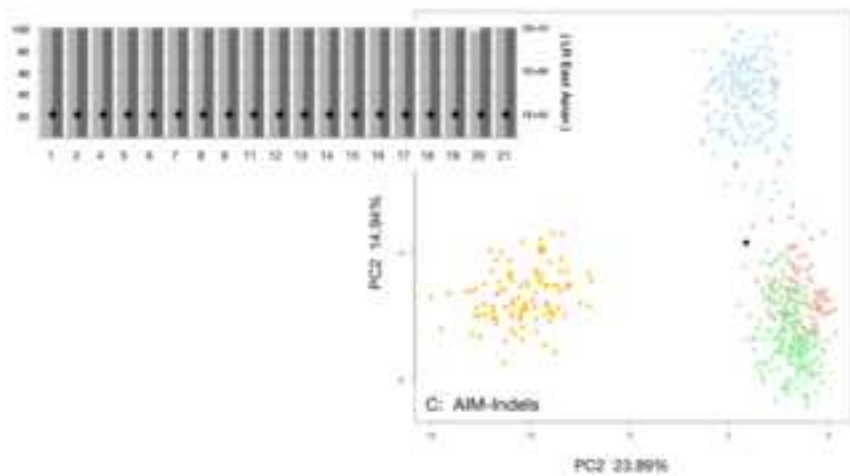


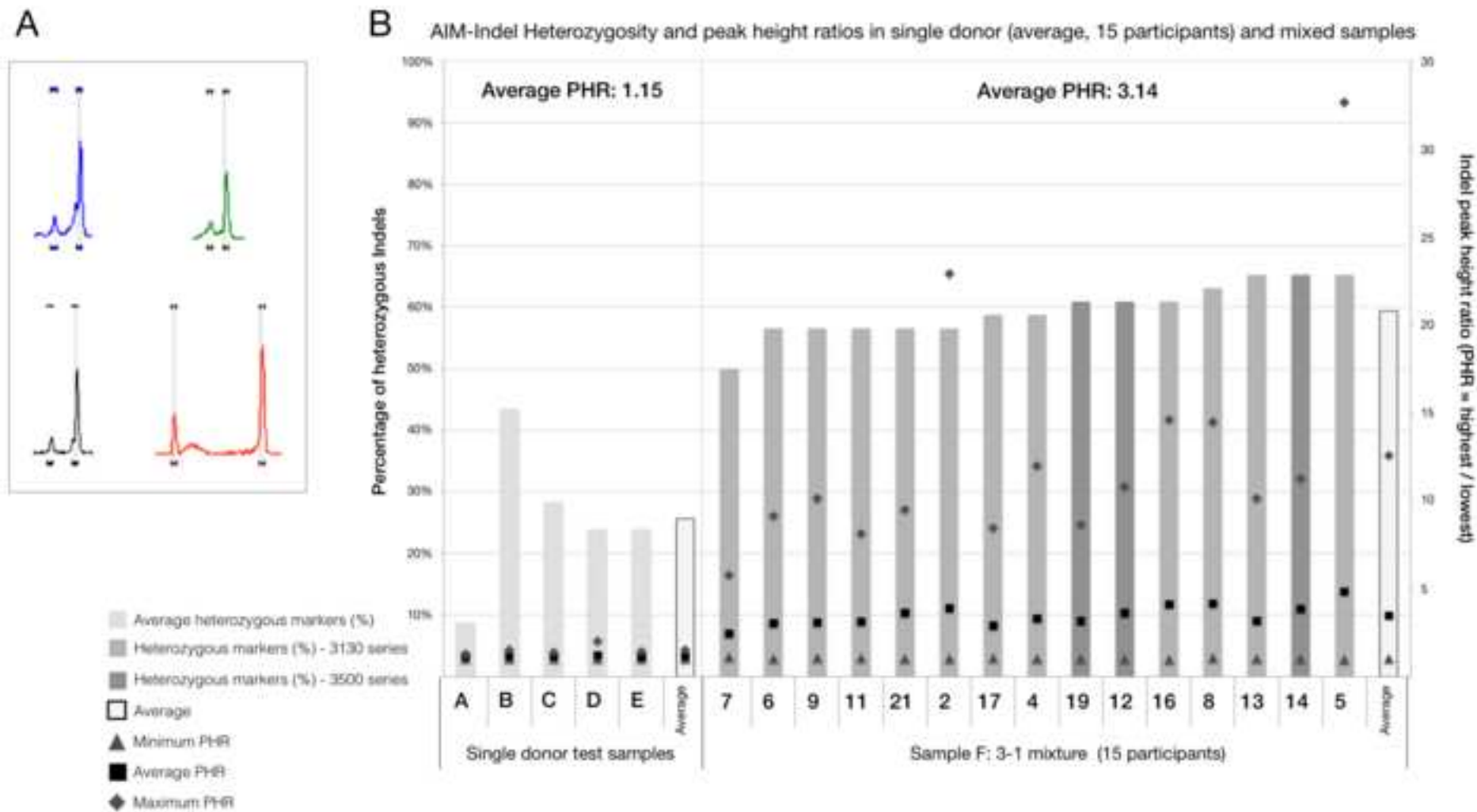
Figure 4B  
[Click here to download high resolution image](#)



- Bayes assignment LR
- No SNaPshot genotypes:  
Oceanian Bayes LR from  
Indel data only
- LR of ~1.2

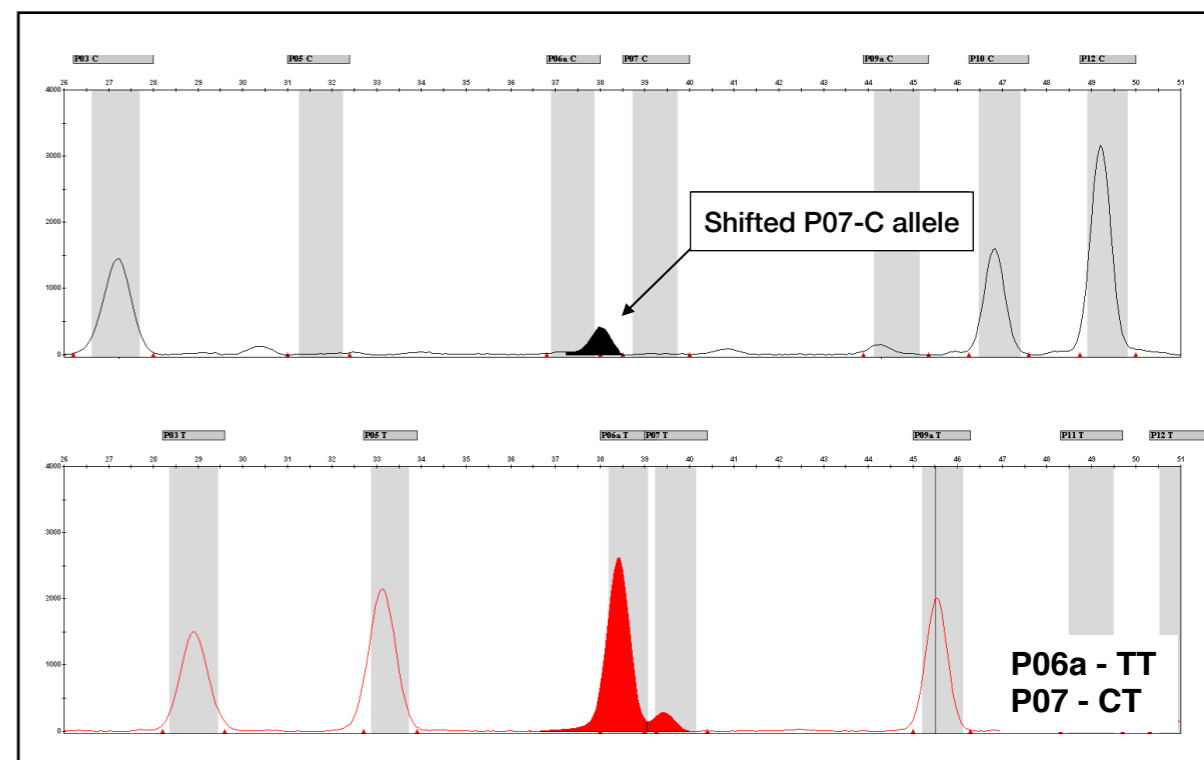
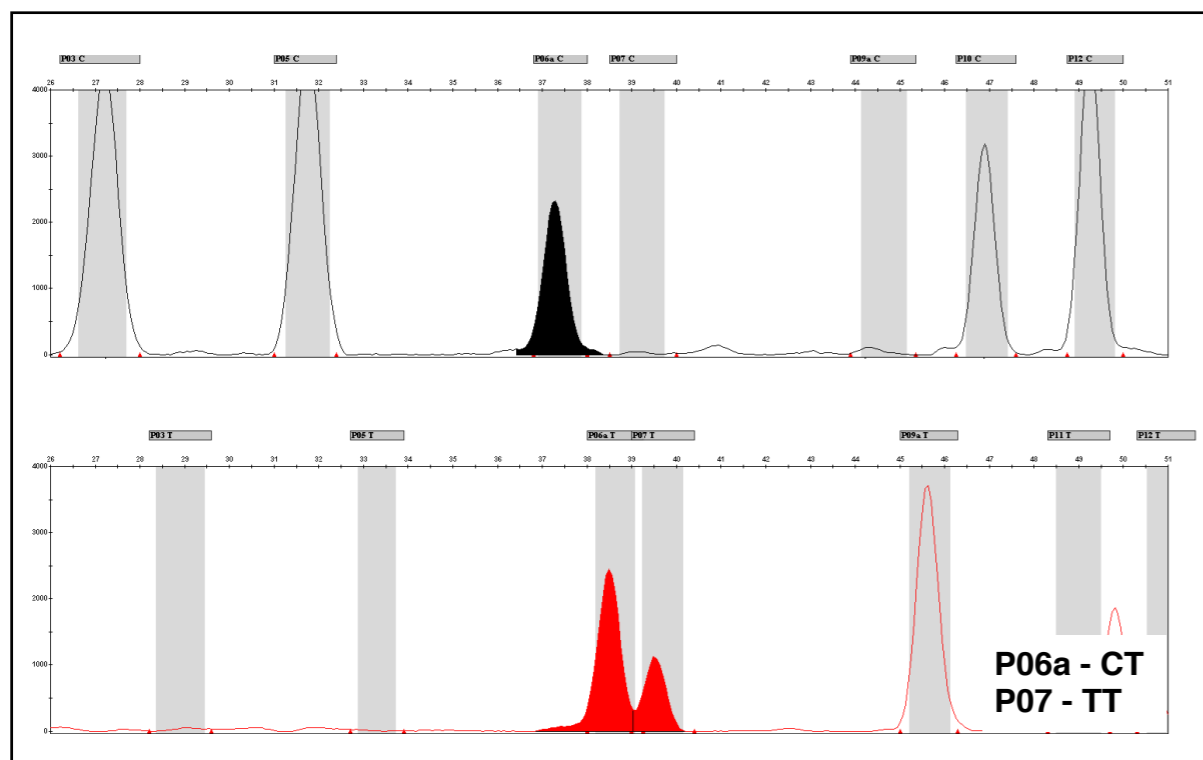
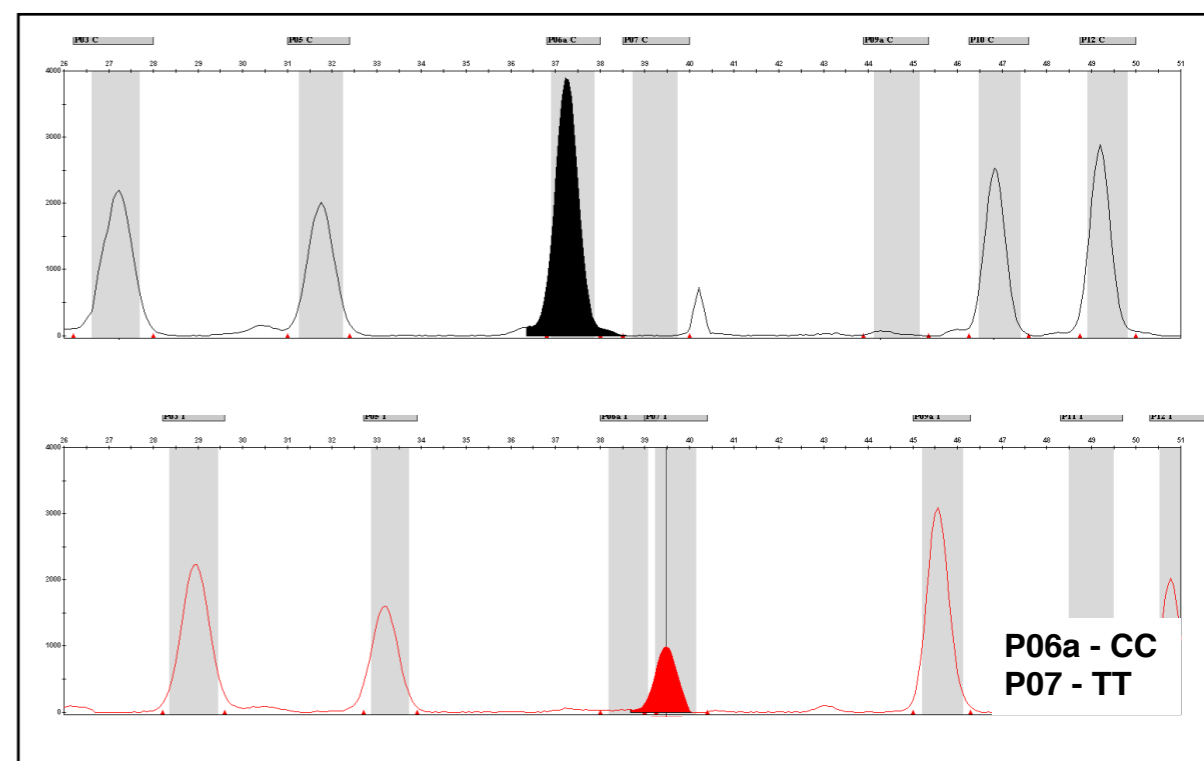
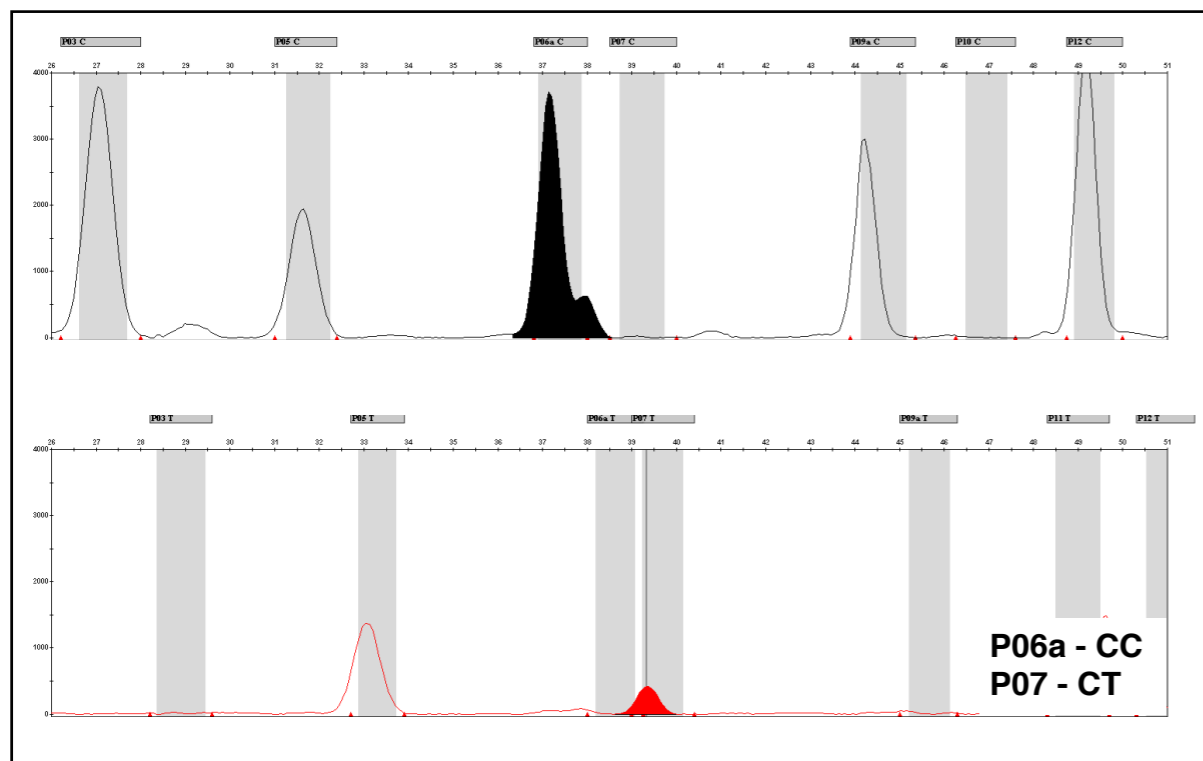


**Figure 5**  
[Click here to download high resolution image](#)

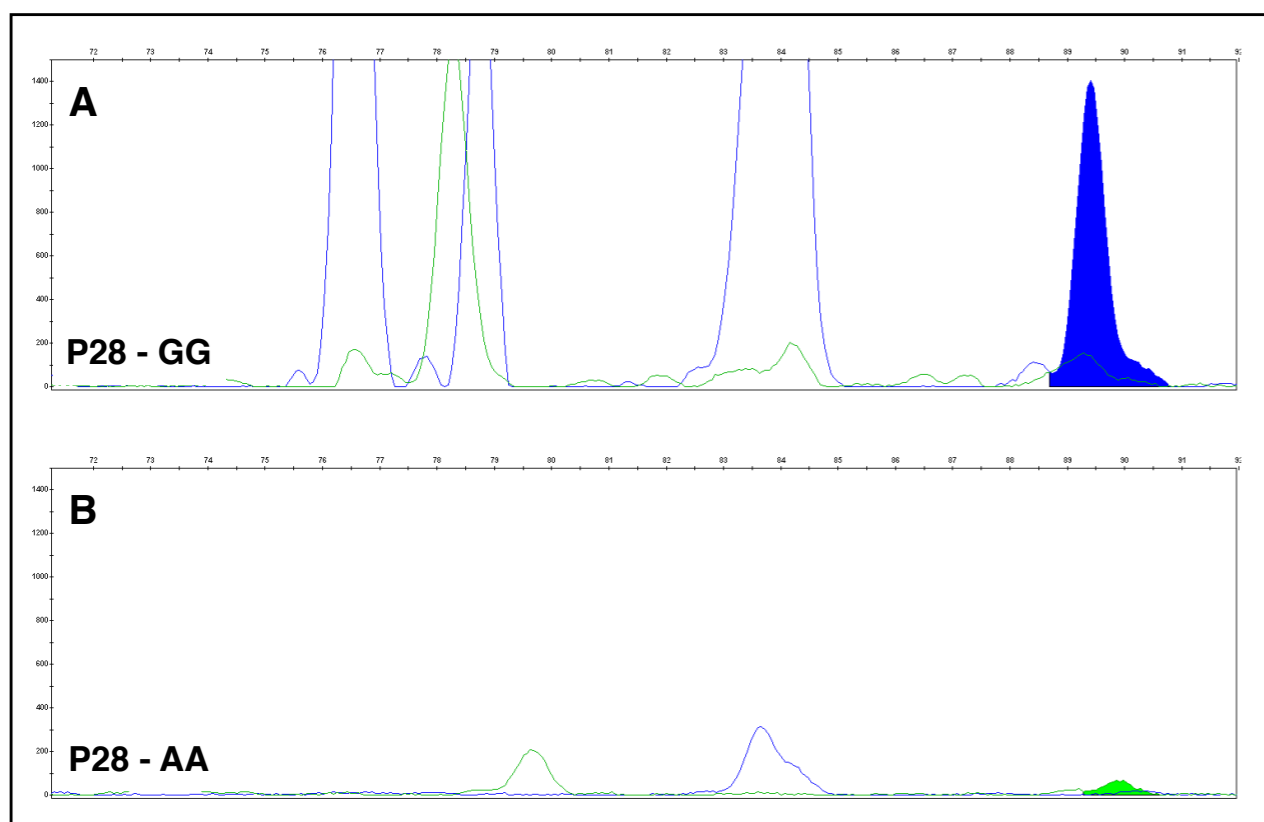
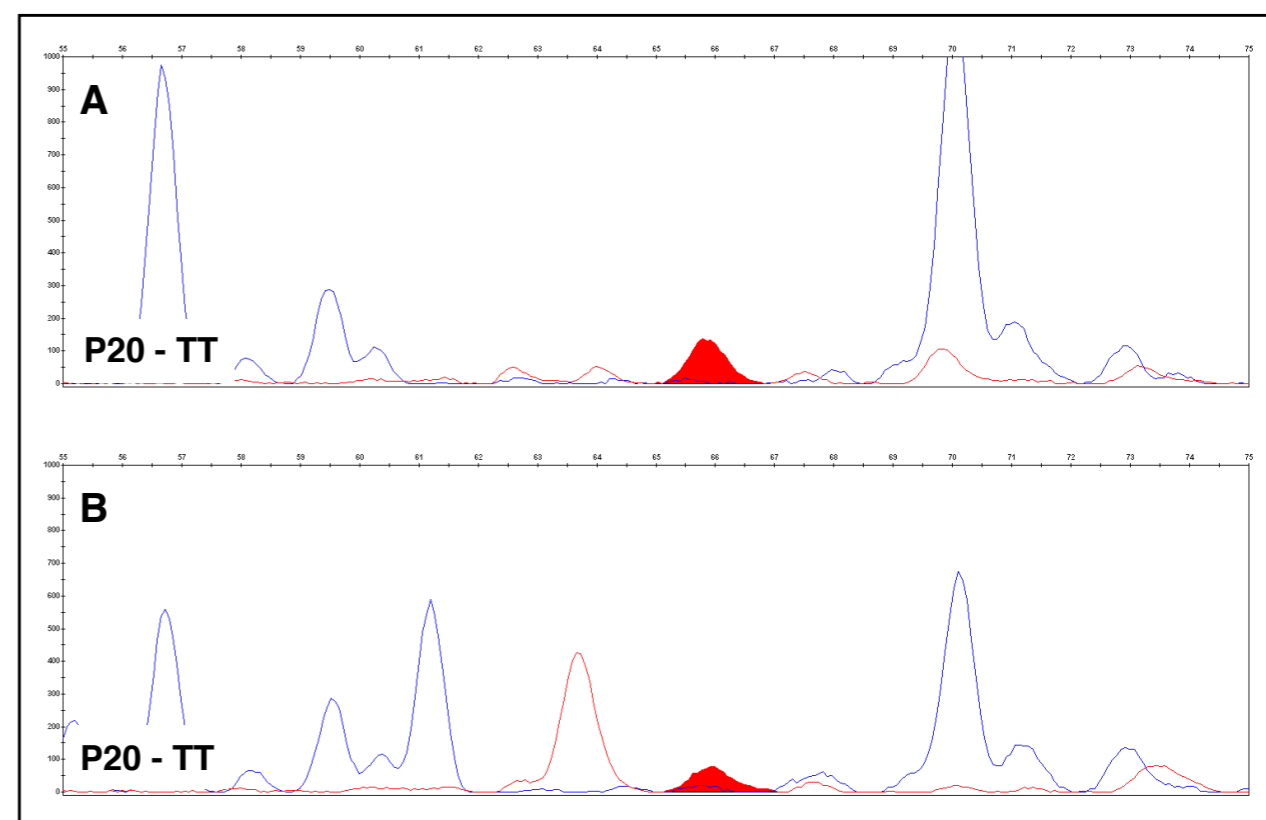
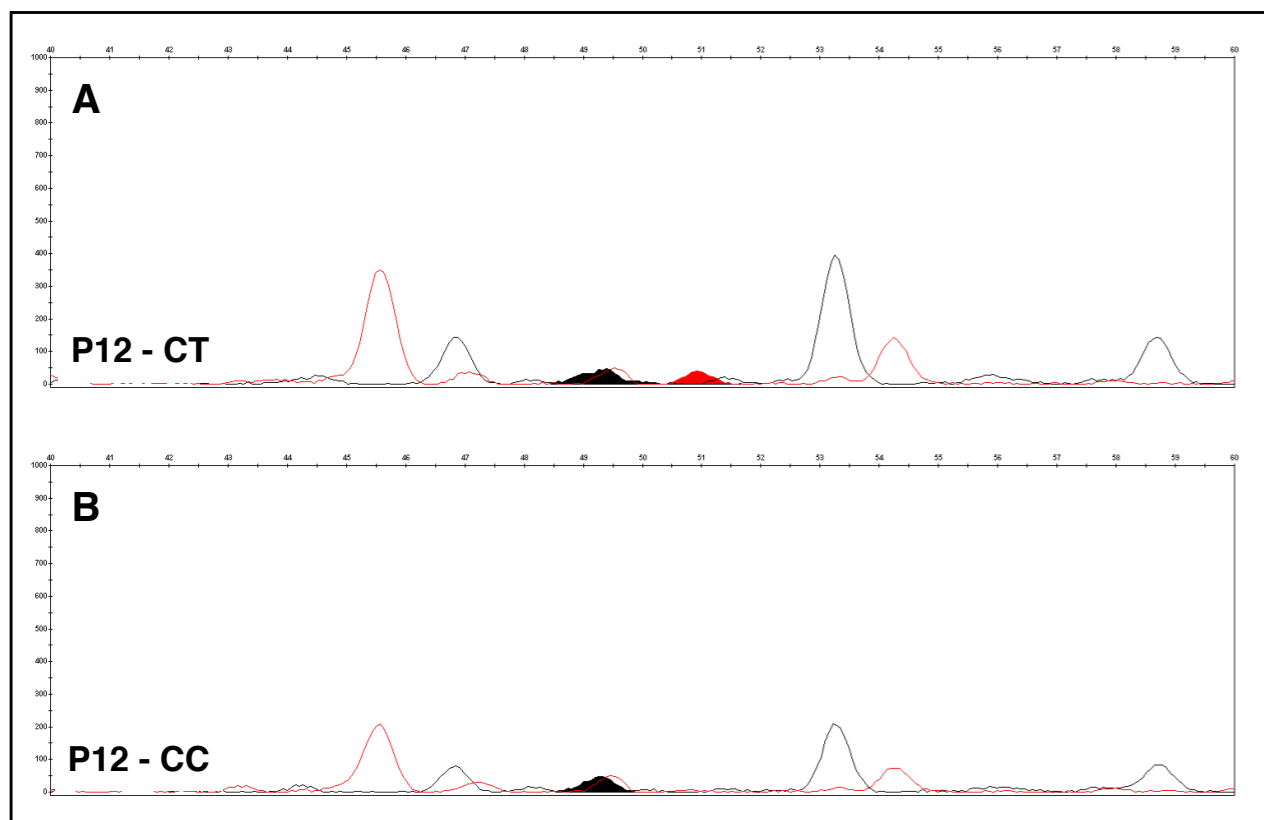


# Supplementary Fig. S1 Examples of genotyping challenges in 34-plex or Indel profiles

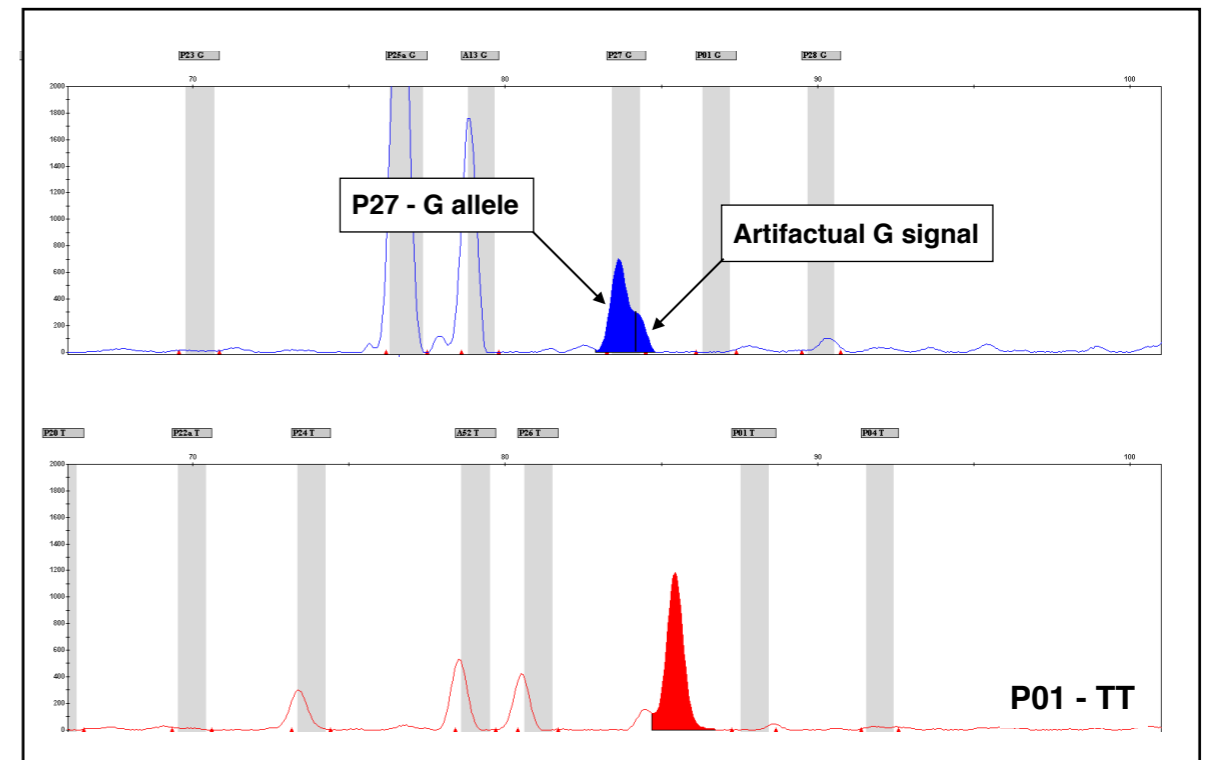
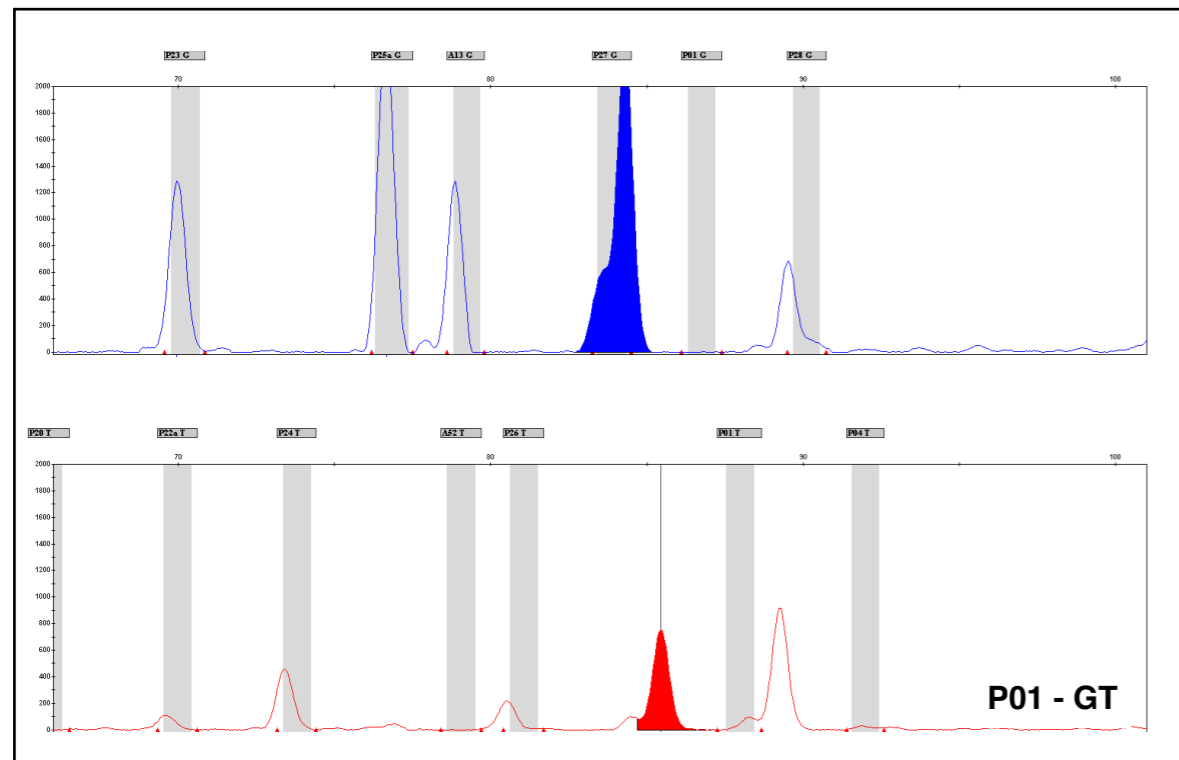
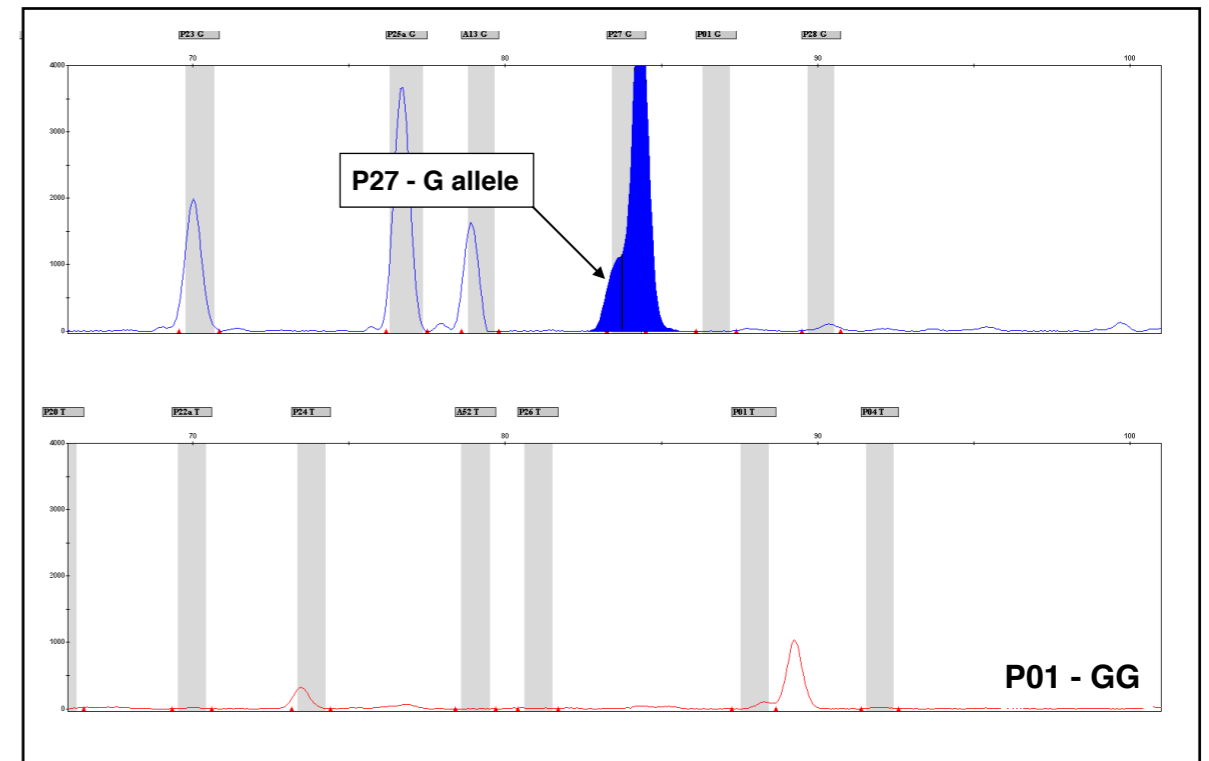
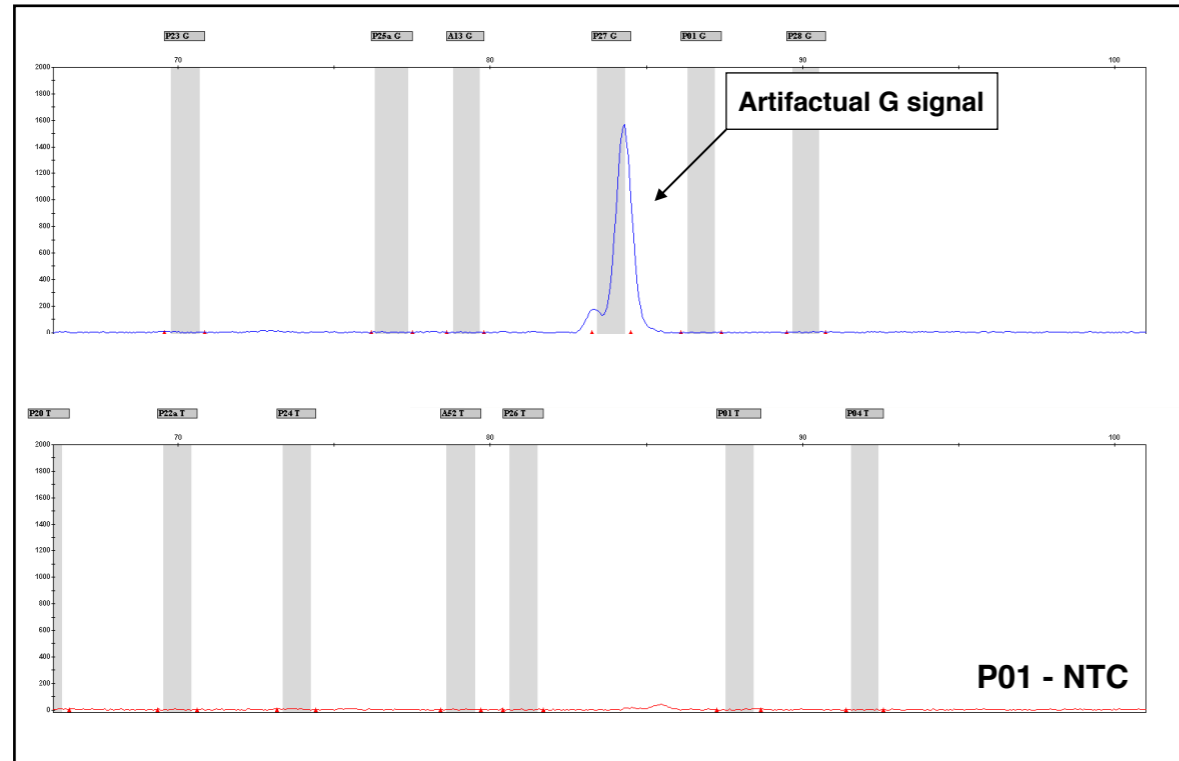
## S1.1 SNPs P06a-P07 (rs10843344-rs239031) peak pairs run very close together



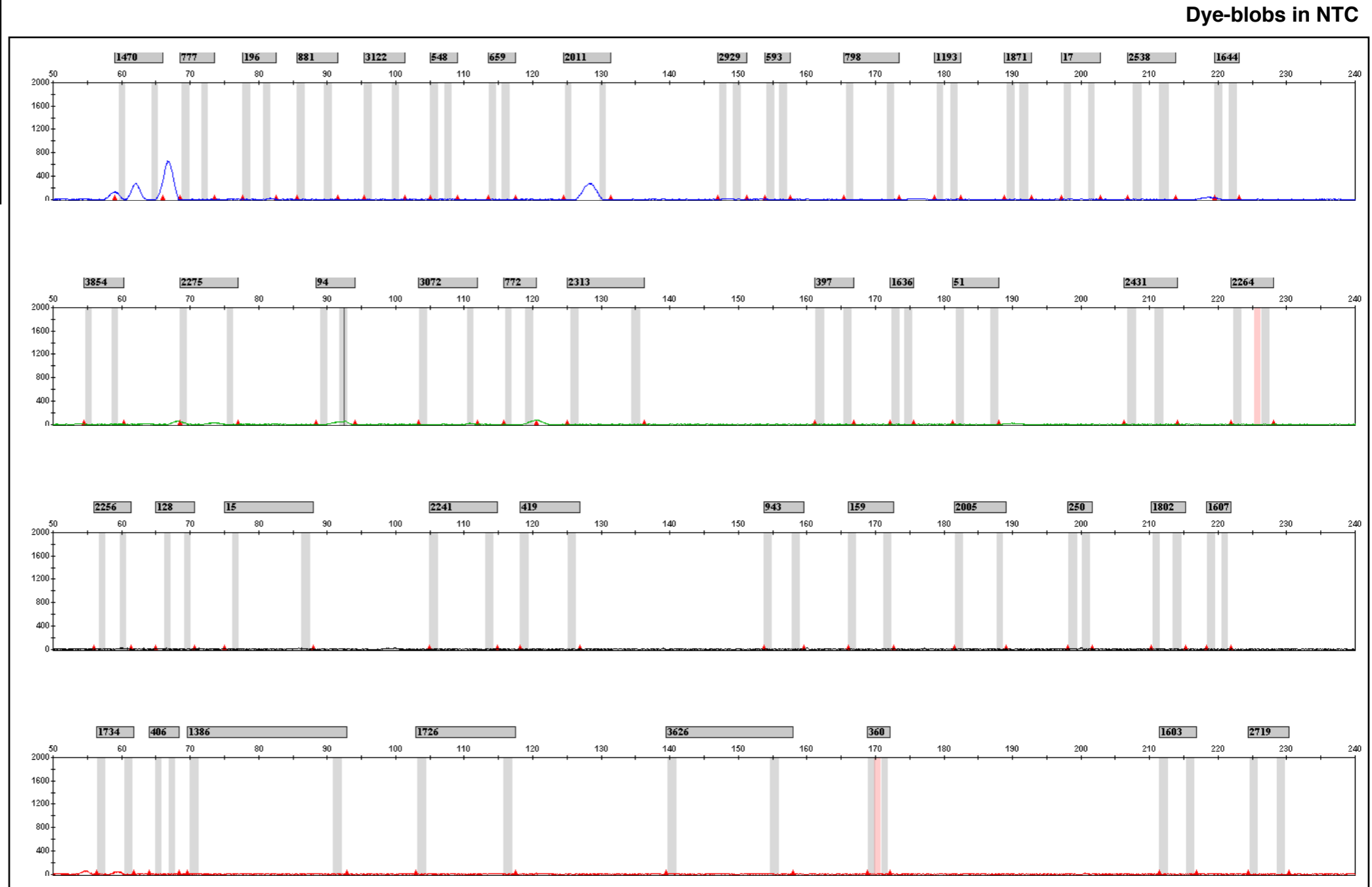
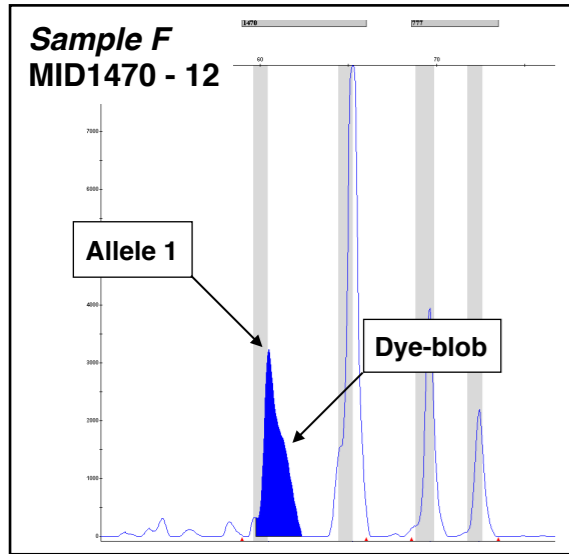
**S1.2** SNPs P12/P20/P28 (rs182549/rs1881929/rs3827760) show very low peaks for one or both alleles, particularly allele A in SNP P28.



**S1.3** P01 (rs2304925) shows an artifactual G signal in the negative control very close to the G peak of P27 (rs5030240-rs2304925). This peak is much higher than the T peak when a true allelic extension product but much lower when artifactual.

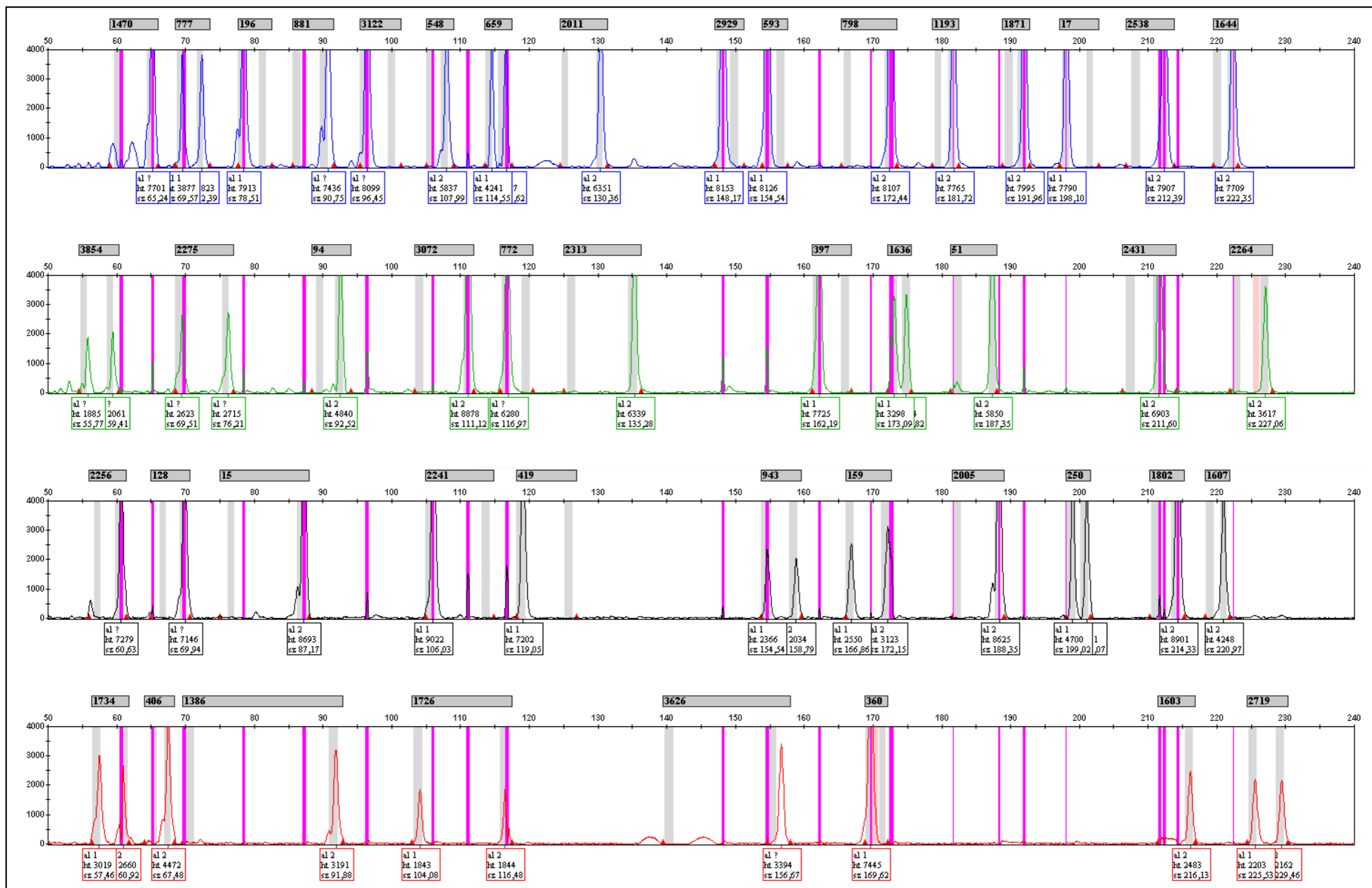


**S1.4** Indel dye-blobs present in non-allelic positions in a typical negative template control (NTC) profile. One example of a dye-blob is shown in the inset top-left. This is very close to an allele of the short-amplicon Indel MID1470 (rs2307666), influencing its estimated mid-peak position slightly.

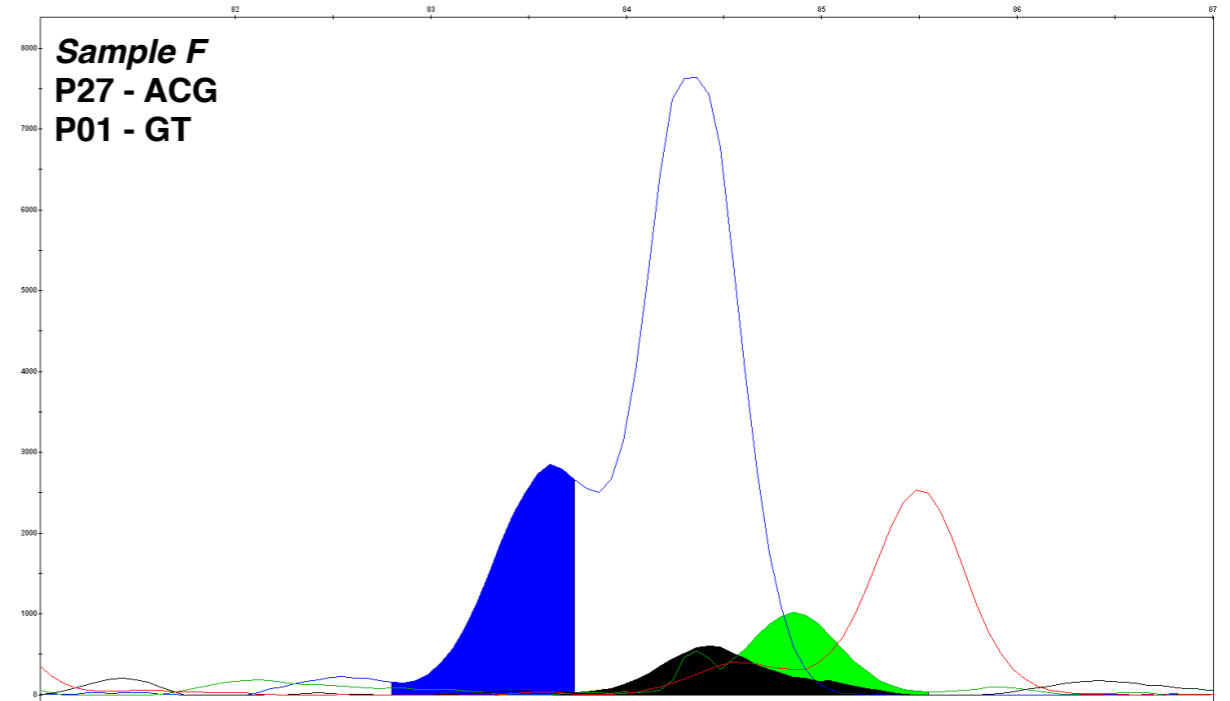
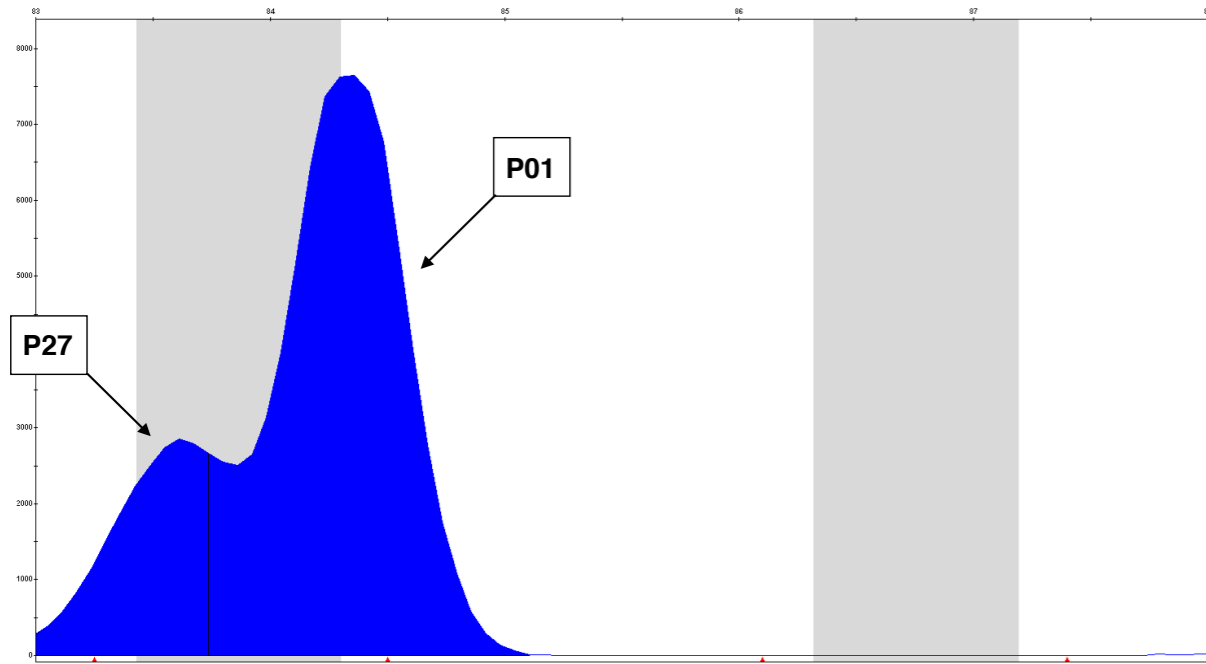




# S1.5 Example of strong signal pull-up in an Indel profile due to an overloaded sample



**S1.6** SNP P27 (rs5030240) is a tri-allelic marker that showed three alleles in mixed sample F but has low relative peak height for the C allele compared to those of A and G alleles. Peak patterns shown left give another example of the close peak positions shown in S1.3.



**Supplementary Table S1**[Click here to download e-component: Supplementary Table S1.docx](#)

**Supplementary Table S1.** Capillary electrophoresis (CE) details for participating laboratories. Grey bars for 34-plex denote five participants not completing SNP genotyping with SNaPshot.

Lab.	CE Detector	Polymer	Dilution factor	
			34-plex*	AIM-Indels
1	3130xl	POP-4	None	None
2	3130xl	POP-4	None	1:10 (A-E) 1:5 (F & NTC)
4	3100	POP-6	None	None
5	3130xl	POP-4		None
6	3130xl	POP-7	None	1:10
7	3130	POP-4	None	None
8	3130xl	POP-4	None	1:20
9	3130xl	POP-7	None	None
11	3130xl	POP-4	None	1:10 (E & F)
12	3500	POP-4		None
13	3130	POP-4	1:10	1:10
14	3500xl	POP-4		1:5
15	3130xl	POP-4		(Not reported)
16	3130xl	POP-4		None
17	3130xl	POP-4	None	1:20
18	3130	POP-4	None	None
19	3500xl	POP-4	None	1:20
20	3130xl	POP-4	None	1:10
21	3130	POP-4	None	None

\* 5/19 laboratories marked did not run the 34-plex SNP assay, but this does not show a relationship to the choice of polymer or CE detector used.



## 34-plex AIM-SNPs

**PCR mix:** 1 <  $\mu$ l decimals (1 or 2)  
5 < add preferred % pipetting top-up here  
x1 sample 16 < add sample multiple here

<b>Buffer 10x</b>	0.69 $\mu$ l	11.6 $\mu$ l	11.59	11.60	
			1.5	1.50	
<b>BSA (1.6 <math>\mu</math>g/<math>\mu</math>l)</b>	0.69 $\mu$ l	11.6 $\mu$ l			
<b>MgCl<sub>2</sub> (25 mM)</b>	1.63 $\mu$ l	27.4 $\mu$ l	27.38	27.40	
<b>dNTPs (10 mM)</b>	0.43 $\mu$ l	7.2 $\mu$ l	7.22	7.20	
				1.50	
<b>PCR primer mix</b>	1 $\mu$ l	16.8 $\mu$ l	16.80	16.80	
				1.50	
<b>AmpliTaq Gold</b>	0.1 $\mu$ l	1.7 $\mu$ l	1.68	1.70	
			0.86	0.90	14.45
<b>H<sub>2</sub>O</b>	0.9 $\mu$ l	14.4 $\mu$ l	5.40	5.40	

□

5.4  $\mu$ l Mix  
Total  
Volume

+

<b>Optimum DNA input is 0.75 ng</b>	1.5 $\mu$ l
---	-------------

□

6.9  $\mu$ l  
Total  
Volume

0.5 < add DNA concentration

### Exo-SAP purification:

*low-cost*

	x1 sample	x1 sample (non evidential DNA)
<b>ExoSAPit</b>	1.3 $\mu$ l	0.65 $\mu$ l
<b>PCR product</b>	2.5 $\mu$ l	1.25 $\mu$ l

### EXT mix:

x1 sample 16 x

**Supplementary Files S2.1**[Click here to download e-component: AIM-indelplex\\_POP4\\_bins.txt](#)

Version GM v 3.0

Chemistry Kit AIM-indelplex

BinSet Name AIM-indelplex

Panel Name AIM-indelplex

Marker Name 1470

1 60.17 0.5 0.76

2 65.1 0.5 0.5

Marker Name 777

1 69.33 0.5 0.5

2 72.33 0.5 0.5

Marker Name 196

1 78.37 0.5 0.5

2 81.37 0.5 0.5

Marker Name 881

1 86.37 0.5 0.5

2 90.37 0.5 0.5

Marker Name 3122

1 96.18 0.5 0.5

2 100.18 0.5 0.5

Marker Name 548

1 105.8 0.5 0.5

2 107.8 0.5 0.5

Marker Name 659

1 114.3 0.5 0.5

2 116.3 0.5 0.5

Marker Name 2011

1 125.29 0.5 0.5

2 130.29 0.5 0.5

Marker Name 2929

1 147.91 0.5 0.5

2 149.91 0.5 0.5

Marker Name 593

1 154.56 0.5 0.5

2 156.56 0.5 0.5

Marker Name 798

1 166.38 0.5 0.5

2 172.28 0.5 0.5

Marker Name 1193

1 179.47 0.5 0.5

2 181.47 0.5 0.5

Marker Name 1871

1 189.75 0.5 0.5

2 191.75 0.5 0.66

Marker Name 17

1 197.81 0.5 0.5

2 201.81 0.5 0.5

Marker Name 2538

1 208.38 0.5 0.75

2 212.38 0.5 0.75

Marker Name 1644

1 220.2 0.5 0.5

2 222.2 0.5 0.5

Marker Name	3854			
1	55.4	0.5	0.5	
2	59.21	0.59	0.5	
Marker Name	2275			
1	69.3	0.5	0.5	
2	76.09	0.5	0.5	
Marker Name	3072			
1	104.07	0.5	0.5	
2	111.07	0.5	0.5	
Marker Name	772			
1	116.6	0.5	0.5	
2	119.6	0.5	0.5	
Marker Name	2313			
1	126.01	0.5	0.5	
2	135.01	0.5	0.5	
Marker Name	397			
1	161.99	0.5	0.5	
2	165.99	0.5	0.5	
Marker Name	1636			
1	172.86	0.5	0.5	
2	174.86	0.5	0.5	
Marker Name	51			
1	182.11	0.5	0.5	
2	187.11	0.5	0.5	
Marker Name	2431			
1	207.47	0.5	0.75	
2	211.47	0.5	0.75	
Marker Name	2264			
1	222.8	0.5	0.5	
3	225.8	0.4	0.4	mutant
2	226.8	0.4	0.66	
Marker Name	2256			
1	57.38	0.5	0.5	
2	60.38	0.5	0.5	
Marker Name	128			
1	66.67	0.5	0.5	
2	69.67	0.5	0.5	
Marker Name	15			
1	76.1	0.5	0.5	
2	87.0	0.5	0.64	
Marker Name	2241			
1	106.0	0.64	0.5	
2	114.01	0.5	0.5	
Marker Name	419			
1	118.84	0.5	0.65	
2	125.84	0.5	0.5	
Marker Name	943			
1	154.51	0.56	0.5	
2	158.51	0.5	0.5	
Marker Name	159			
1	166.85	0.61	0.5	
2	171.9	0.5	0.5	

Marker Name	2005			
1	182.28	0.5	0.55	
2	188.28	0.64	0.5	
Marker Name	250			
1	198.85	0.5	0.5	
2	200.85	0.5	0.5	
Marker Name	1802			
1	211.13	0.5	0.5	
2	214.13	0.5	0.5	
Marker Name	1607			
1	219.02	0.5	0.5	
2	220.95	0.62	0.5	
Marker Name	406			
1	65.39	0.5	0.5	
2	67.39	0.5	0.5	
Marker Name	1386			
1	70.71	0.5	0.71	
2	91.57	0.5	0.5	
Marker Name	1726			
1	103.82	0.5	0.5	
2	116.5	0.5	0.5	
Marker Name	3626			
1	140.36	0.5	0.5	
2	156.25	0.5	0.5	
Marker Name	360			
1	169.42	0.4	0.4	
3	170.42	0.4	0.4	mutant
2	171.42	0.4	0.4	
Marker Name	1603			
1	212.05	0.5	0.56	
2	215.97	0.5	0.5	
Marker Name	2719			
1	225.38	0.5	0.5	
2	229.38	0.5	0.5	
Marker Name	1734			
1	57.11	0.5	0.5	
2	60.79	0.5	0.5	
Marker Name	94			
1	89.14	0.5	0.5	
2	92.14	0.5	0.56	



Version GM v 3.0

Kit type: MICROSATELLITE

Chemistry Kit AIM-indelplex none

Panel AIM-indelplex none

1470	blue	59.498930343000005	65.81825256900001	-	9	0.0	none
777	blue	68.55843072	73.031835852	-	9	0.0	none
196	blue	77.588872041	82.088429436	-	9	0.0	none
881	blue	85.617309136	91.15076970400001	-	9	0.0	none
3122	blue	95.320690594	100.900499185-	9	0.0	none	
548	blue	105.057912973108.532867596-	9	0.0	none		
659	blue	113.556264981117.080076701-	9	0.0	none		
2011	blue	124.498164706131.041303657-	9	0.0	none		
2929	blue	146.955519673150.824584052-	9	0.0	none		
593	blue	153.83022589700002	157.14313298399998	-	9	0.0	none
798	blue	165.32897286899998	173.501119818-	9	0.0	none	
1193	blue	178.658800433182.45524446299999	-	9	0.0	none	
1871	blue	188.823452509192.72466145	-	9	0.0	none	
17	blue	197.084416798202.680731973-	9	0.0	none		
2538	blue	206.773228342213.827474758-	9	0.0	none		
1644	blue	219.483987195223.06873612500002	-	9	0.0	none	
3854	Green	54.61523001	59.995735289	-	9	0.0	none
2275	Green	68.507204408	76.863106493	-	9	0.0	none
3072	Green	103.268780394111.960791133-	9	0.0	none		
772	Green	115.766532729120.46989852499999	-	9	0.0	none	
2313	Green	125.024081548136.274250832-	9	0.0	none		
397	Green	161.189615282166.818202663-	9	0.0	none		
1636	Green	172.08629923200002	175.600073663-	9	0.0	none	
51	Green	181.211398852188.025739155-	9	0.0	none		
2431	Green	206.293870962214.07491917299998	-	9	0.0	none	
2264	Green	221.926816896228.05683674	-	9	0.0	none	
2256	Yellow	56.680080642	61.154696174	-	9	0.0	none
128	Yellow	65.93894781700001	70.400997293	-	9	0.0	none
15	Yellow	75.5	87.975809403	-	9	0.0	none
2241	Yellow	104.973806014114.835216709-	9	0.0	none		
419	Yellow	118.07762833899999	126.850206737-	9	0.0	none	
943	Yellow	153.696434559159.341659618-	9	0.0	none		
159	Yellow	166.02409311300002	172.70691387	-	9	0.0	none
2005	Yellow	181.501371715189.06208221400001	-	9	0.0	none	
250	Yellow	198.065914853201.572590476-	9	0.0	none		
1802	Yellow	210.217316797215.228136625-	9	0.0	none		
1607	Yellow	218.273123059221.87207210900002	-	9	0.0	none	
406	Red	64.678876207	68.176704628	-	9	0.0	none
1386	Red	69.56966599	92.812677351	-	9	0.0	none
1726	Red	102.977336125117.29484620400001	-	9	0.0	none	
3626	Red	139.43282453700002	156.750843119-	9	0.0	none	
360	Red	168.792202855172.14353350399998	-	9	0.0	none	
1603	Red	211.384383599216.862882022-	9	0.0	none		
2719	Red	224.388680709230.34325627	-	9	0.0	none	
1734	Red	56.285110841	61.814691114000006	-	9	0.0	none
94	Green	88.363699841	92.99304501099999	-	9	0.0	none

**Supplementary Files S2.3**[Click here to download e-component: 34plex\\_POP4\\_\\_bins.txt](#)

Version GM v 3.0

Chemistry Kit	34plex_POP4		
BinSet Name	34plex_POP4		
Panel Name	34plex_POP4		
Marker Name	P01 T		
T	85.75	0.4	0.4
Marker Name	A07 G		
G	26.92	0.53	0.48
Marker Name	A07 A		
A	29.26	0.46	0.42
Marker Name	P03 C		
C	27.22	0.6	0.47
Marker Name	P03 T		
T	28.98	0.62	0.46
Marker Name	P04 C		
C	88.76	0.4	0.4
Marker Name	P04 T		
T	89.56	0.4	0.4
Marker Name	A29 G		
G	28.44	0.46	0.45
Marker Name	A29 A		
A	30.49	0.49	0.47
Marker Name	P05 C		
C	31.79	0.53	0.45
Marker Name	P05 T		
T	33.3	0.42	0.41
Marker Name	A21 G		
G	34.18	0.4	0.41
Marker Name	A21 A		
A	36.46	0.51	0.47
Marker Name	P06a C		
C	37.39	0.48	0.47
Marker Name	P06a T		
T	38.65	0.45	0.42
Marker Name	P08 G		
G	40.37	0.45	0.41
Marker Name	P08 A		
A	41.01	0.41	0.42
Marker Name	P07 C		
C	39.23	0.5	0.5
Marker Name	P07 T		
T	39.67	0.43	0.47
Marker Name	A40 G		
G	43.22	0.42	0.42
Marker Name	A40 A		
A	44.34	0.51	0.41
Marker Name	P09a C		
C	44.64	0.5	0.5
Marker Name	P09a T		
T	45.67	0.46	0.44
Marker Name	P10 G		
G	46.52	0.42	0.42

Marker Name	P10 C		
C	46.94	0.46	0.46
Marker Name	P11 A		
A	49.0	0.5	0.47
Marker Name	P11 T		
T	49.63	0.4	0.4
Marker Name	P12 C		
C	49.34	0.43	0.46
Marker Name	P12 T		
T	50.97	0.44	0.41
Marker Name	P13 G		
G	49.85	0.5	0.5
Marker Name	P02 A		
A	88.17	0.5	0.5
Marker Name	P02 C		
C	87.87	0.46	0.45
Marker Name	P01 G		
G	84.55	0.4	0.4
Marker Name	P13 A		
A	51.5	0.46	0.45
Marker Name	P14 C		
C	53.37	0.43	0.46
Marker Name	P14 T		
T	54.34	0.4	0.41
Marker Name	P15 G		
G	55.29	0.47	0.45
Marker Name	P15 A		
A	56.1	0.47	0.43
Marker Name	P16a G		
G	56.79	0.42	0.45
Marker Name	P16a A		
A	57.64	0.4	0.4
Marker Name	P17 C		
C	58.84	0.44	0.42
Marker Name	P17 T		
T	59.56	0.44	0.43
Marker Name	P18 G		
G	61.26	0.41	0.42
Marker Name	P18 A		
A	62.14	0.47	0.41
Marker Name	P19 C		
C	62.57	0.46	0.41
Marker Name	P19 T		
T	63.82	0.4	0.4
Marker Name	P20 G		
G	63.87	0.45	0.42
Marker Name	P20 T		
T	65.82	0.43	0.45
Marker Name	P21 A		
A	66.17	0.47	0.43
Marker Name	P21 C		
C	66.01	0.46	0.43

Marker Name	P22a C		
C	69.27	0.4	0.4
Marker Name	P22a T		
T	69.98	0.44	0.43
Marker Name	P23 G		
G	70.23	0.45	0.45
Marker Name	P23 A		
A	70.24	0.5	0.5
Marker Name	P24 A		
A	73.53	0.47	0.4
Marker Name	P24 C		
C	73.08	0.4	0.4
Marker Name	P24 T		
T	73.83	0.46	0.4
Marker Name	A52 A		
A	78.77	0.43	0.48
Marker Name	A52 T		
T	79.01	0.4	0.49
Marker Name	P25a G		
G	76.83	0.51	0.52
Marker Name	P25a C		
C	77.18	0.48	0.46
Marker Name	P26 C		
C	80.5	0.4	0.47
Marker Name	P26 T		
T	81.07	0.44	0.43
Marker Name	A13 G		
G	79.24	0.43	0.4
Marker Name	A13 A		
A	80.07	0.4	0.47
Marker Name	P27 G		
G	83.88	0.45	0.42
Marker Name	P27 A		
A	85.12	0.42	0.42
Marker Name	P27 C		
C	84.71	0.42	0.43
Marker Name	P28 G		
G	89.59	0.4	0.4
Marker Name	P28 A		
A	90.5	0.43	0.45

Version GM v 3.0

Kit type: MICROSATELLITE

Chemistry Kit 34plex\_POP4 none

Panel 34plex\_POP4 none

P01 T	Red	85.0	86.5	-	2	0.0	rs2304925	-
A07 G	Blue	25.95	27.5	-	2	0.0	rs917118	-
A07 A	Green	28.5	29.88	-	2	0.0	rs917118	-
P03 C	Yellow	26.2	28.0	-	2	0.0	rs1321333	-
P03 T	Red	28.2	29.6	-	2	0.0	rs1321333	-
P04 C	Yellow	88.1	89.5	-	2	0.0	rs2814778	-
P04 T	Red	88.7	90.5	-	2	0.0	rs2814778	-
A29 G	Blue	27.8	29.17	-	2	0.0	rs1024116	-
A29 A	Green	29.8	31.1	-	2	0.0	rs1024116	-
P05 C	Yellow	31.0	32.4	-	2	0.0	rs7897550	-
P05 T	Red	32.7	33.9	-	2	0.0	rs7897550	-
A21 G	Blue	33.6	34.71	-	2	0.0	rs722098	-
A21 A	Green	35.75	37.1	-	2	0.0	rs722098	-
P06a C	Yellow	36.8	38.0	-	2	0.0	rs10843344	-
P06a T	Red	38.0	39.25	-	2	0.0	rs10843344	-
P08 G	Blue	39.8	41.01	-	2	0.0	rs12913832	-
P08 A	Green	40.4	41.65	-	2	0.0	rs12913832	-
P07 C	Yellow	38.5	40.0	-	2	0.0	rs239031	-
P07 T	Red	39.0	40.4	-	2	0.0	rs239031	-
A40 G	Blue	42.6	43.85	-	2	0.0	rs2040411	-
A40 A	Green	43.6	44.93	-	2	0.0	rs2040411	-
P09a C	Yellow	43.9	45.35	-	2	0.0	rs1978806	-
P09a T	Red	45.0	46.3	-	2	0.0	rs1978806	-
P10 G	Blue	45.9	47.2	-	2	0.0	rs773658	-
P10 C	Yellow	46.25	47.6	-	2	0.0	rs773658	-
P11 A	Green	48.3	49.7	-	2	0.0	rs10141763	-
P11 T	Red	48.8	50.5	-	2	0.0	rs10141763	-
P12 C	Yellow	48.75	50.0	-	2	0.0	rs182549	-
P12 T	Red	50.3	51.6	-	2	0.0	rs182549	-
P13 G	Blue	49.1	50.7	-	2	0.0	rs1573020	-
P02 A	Green	87.5	88.85	-	2	0.0	rs5997008	-
P02 C	Yellow	87.19	88.5	-	2	0.0	rs5997008	-
P01 G	Blue	84.1	85.3	-	2	0.0	rs2304925	-
P13 A	Green	50.85	52.2	-	2	0.0	rs1573020	-
P14 C	Yellow	52.75	54.0	-	2	0.0	rs896788	-
P14 T	Red	53.75	54.95	-	2	0.0	rs896788	-
P15 G	Blue	54.65	55.9	-	2	0.0	rs2065160	-
P15 A	Green	55.4	56.75	-	2	0.0	rs2065160	-
P16a G	Blue	56.2	57.45	-	2	0.0	rs2572307	-
P16a A	Green	56.7	58.2	-	2	0.0	rs2572307	-
P17 C	Yellow	58.2	59.4	-	2	0.0	rs2303798	-
P17 T	Red	59.0	60.2	-	2	0.0	rs2303798	-
P18 G	Blue	60.65	61.9	-	2	0.0	rs2065982	-
P18 A	Green	61.5	62.75	-	2	0.0	rs2065982	-
P19 C	Yellow	62.0	63.2	-	2	0.0	rs3785181	-
P19 T	Red	63.22	64.4	-	2	0.0	rs3785181	-
P20 G	Blue	63.22	64.55	-	2	0.0	rs881929	-
P20 T	Red	65.2	66.5	-	2	0.0	rs881929	-

P21 A	Green	65.5	66.8	-	2	0.0	rs1498444	-
P21 C	Yellow	65.45	66.6	-	2	0.0	rs1498444	-
P22a C	Yellow	68.65	69.85	-	2	0.0	rs1426654	-
P22a T	Red	69.35	70.6	-	2	0.0	rs1426654	-
P23 G	Blue	69.55	70.85	-	2	0.0	rs2026721	-
P23 A	Green	69.55	70.95	-	2	0.0	rs2026721	-
P24 A	Green	72.85	74.15	-	2	0.0	rs4540055	-
P24 C	Yellow	72.5	73.65	-	2	0.0	rs4540055	-
P24 T	Red	73.17	74.4	-	2	0.0	rs4540055	-
A52 A	Green	78.15	79.4	-	2	0.0	rs1335873	-
A52 T	Red	78.4	79.7	-	2	0.0	rs1335873	-
P25a G	Blue	76.2	77.5	-	2	0.0	rs16891982	-
P25a C	Yellow	76.5	77.8	-	2	0.0	rs16891982	-
P26 C	Yellow	79.9	81.15	-	2	0.0	rs730570	-
P26 T	Red	80.4	81.7	-	2	0.0	rs730570	-
A13 G	Blue	78.6	79.8	-	2	0.0	rs1886510	-
A13 A	Green	79.5	80.75	-	2	0.0	rs1886510	-
P27 G	Blue	83.25	84.5	-	2	0.0	rs5030240	-
P27 A	Green	84.5	85.75	-	2	0.0	rs5030240	-
P27 C	Yellow	84.1	85.35	-	2	0.0	rs5030240	-
P28 G	Blue	89.0	90.5	-	2	0.0	rs3827760	-
P28 A	Green	89.9	91.15	-	2	0.0	rs3827760	-

**Supplementary Files S2.7**[Click here to download e-component: 34-PLEX\\_POP7\\_bins.txt](#)

Version GM v 3.0

Chemistry Kit	34-PLEX			
BinSet Name	34-PLEX			
Panel Name	34-Plex Electrophoretic Shift			
Marker Name	01rs1321333			
ASR	34.77	37.61		
C	34.77	35.9	Yellow	
T	36.39	37.61000000000001	Red	
Marker Name	02rs917118			
ASR	32.6	35.67		
G	32.6	33.809999999999995	Blue	
A	34.870000000000005	35.67	Green	
Marker Name	03rs1024116			
ASR	33.35	36.39		
G	33.35	34.54	Blue	
A	35.17	36.39	Green	
Marker Name	04rs7897550			
ASR	37.53	40.05		
C	37.53	38.36	Yellow	
T	38.96	40.050000000000004	Red	
Marker Name	05rs722098			
ASR	37.7	40.78		
G	37.7	38.5	Blue	
A	39.980000000000004	40.78	Green	
Marker Name	06rs10843344			
ASR	41.68	44.0		
C	41.68	42.48	Yellow	
T	43.2	44.0	Red	
Marker Name	07rs239031			
ASR	42.35	45.17		
C	42.35	43.15	Yellow	
T	44.36	45.17	Red	
Marker Name	08rs12913832			
ASR	44.16	45.64		
G	44.160000000000004	44.96	Blue	
A	44.84	45.64	Green	
Marker Name	09rs2040411			
ASR	46.48	48.49		
G	46.480000000000004	47.78	Blue	
A	47.690000000000005	48.49	Green	
Marker Name	10rs1978806			
ASR	48.63	50.43		
C	48.629999999999995	49.75	Yellow	
T	49.63	50.43	Red	
Marker Name	11rs773658			
ASR	49.49	51.02		
G	49.49	50.29	Blue	
C	50.22	51.019999999999996	Yellow	
Marker Name	12rs10141763			
ASR	52.01	54.21		
A	52.010000000000005	53.08	Green	
T	52.669999999999995	54.21	Red	

Marker Name	13rs182549		
ASR	51.8	53.81	
C	51.8	52.92	Yellow
T	53.01	53.809999999999995	Red
Marker Name	14rs1573020		
ASR	53.09	54.94	
G	53.09	54.59	Blue
A	53.69	54.94	Green
Marker Name	15rs896788		
ASR	55.66	57.75	
C	55.66	57.03	Yellow
T	56.900000000000006	57.75	Red
Marker Name	16rs2065160		
ASR	56.85	58.04	
G	56.849999999999994	57.75	Blue
A	57.24	58.04	Green
Marker Name	17rs2572307		
ASR	58.03	59.66	
G	58.03	58.83	Blue
A	58.86	59.66	Green
Marker Name	18rs2303798		
ASR	59.78	61.12	
C	59.78	60.58	Yellow
T	60.32	61.12	Red
Marker Name	19rs2065982		
ASR	62.31	63.86	
G	62.31	63.11	Blue
A	63.06	63.86	Green
Marker Name	20rs3785181		
ASR	63.89	66.12	
C	63.89	64.79	Yellow
T	65.32	66.12	Red
Marker Name	21rs881929		
ASR	64.29	67.39	
G	64.29	65.26	Blue
T	66.58999999999999	67.39	Red
Marker Name	22rs1498444		
ASR	67.19	68.17	
A	67.19	67.990000000000001	Green
C	67.36999999999999	68.17	Yellow
Marker Name	23rs1426654		
ASR	70.48	72.13	
C	70.47999999999999	71.28	Yellow
T	71.33	72.130000000000001	Red
Marker Name	24rs2026721		
ASR	71.28	72.65	
G	71.28	72.080000000000001	Blue
A	71.85	72.65	Green
Marker Name	25rs4540055		
ASR	74.18	76.08	
A	74.5	75.300000000000001	Green
C	74.17999999999999	74.98	Yellow



T	75.28	76.08000000000001	Red
Marker Name	26rs16891982		
ASR	77.66	79.03	
G	77.66	78.46000000000001	Blue
C	78.22999999999999	79.03	Yellow
Marker Name	27rs1335873		
ASR	79.89	81.1	
A	79.89	80.69000000000001	Green
T	80.3	81.10000000000001	Red
Marker Name	28rs1886510		
ASR	80.52	82.01	
G	80.52000000000001	81.31	Blue
A	81.17999999999999	82.01	Green
Marker Name	29rs730570		
ASR	82.12	83.68	
C	82.12	83.06	Yellow
T	82.67	83.67999999999999	Red
Marker Name	30rs5030240		
ASR	85.05	86.71	
G	85.05	85.67	Blue
A	86.05	86.71	Green
C	85.95	86.58	Yellow
Marker Name	31rs2304925		
ASR	87.25	89.32	
G	87.25	87.78999999999999	Blue
T	88.55	89.32000000000001	Red
Marker Name	32rs5997008		
ASR	88.14	88.85	
A	88.14	88.83	Green
C	88.23	88.85000000000001	Yellow
Marker Name	33rs3827760		
ASR	90.46	91.43	
G	90.46	91.02	Blue
A	90.73	91.42999999999999	Green
Marker Name	34rs2814778		
ASR	91.78	93.27	
C	91.78	92.77000000000001	Yellow
T	92.39	93.27	Red
Panel Name	34-PLEX		
Marker Name	01rs1321333		
ASR	32.62	35.85	
C	32.620000000000005	33.75	Yellow
T	34.62999999999995	35.85	Red
Marker Name	02rs917118		
ASR	31.54	34.26	
G	31.540000000000003	32.75	Blue
A	33.46	34.26	Green
Marker Name	03rs1024116		
ASR	31.87	34.99	
G	31.870000000000005	33.06	Blue
A	33.77	34.99	Green
Marker Name	04rs7897550		

ASR 36.44 39.3  
 C 36.440000000000005 37.27 Yellow  
 T 38.21 39.300000000000004 Red  
 Marker Name 05rs722098  
 ASR 36.94 39.87  
 G 36.940000000000005 37.74 Blue  
 A 39.07 39.87 Green  
 Marker Name 06rs10843344  
 ASR 40.55 43.09  
 C 40.550000000000004 41.35 Yellow  
 T 42.29 43.089999999999996 Red  
 Marker Name 07rs239031  
 ASR 41.21 44.01  
 C 41.21 42.01 Yellow  
 T 43.199999999999996 44.01 Red  
 Marker Name 08rs12913832  
 ASR 43.28 44.56  
 G 43.28 44.08 Blue  
 A 43.76 44.559999999999995 Green  
 Marker Name 09rs2040411  
 ASR 45.69 47.51  
 G 45.690000000000005 46.99 Blue  
 A 46.71 47.51 Green  
 Marker Name 10rs1978806  
 ASR 47.22 49.46  
 C 47.22 48.34 Yellow  
 T 48.660000000000004 49.46 Red  
 Marker Name 11rs773658  
 ASR 48.36 49.88  
 G 48.36 49.16 Blue  
 C 49.08 49.879999999999995 Yellow  
 Marker Name 12rs10141763  
 ASR 50.6 53.45  
 A 50.6 51.669999999999995 Green  
 T 51.91 53.45 Red  
 Marker Name 13rs182549  
 ASR 51.13 53.81  
 C 51.129999999999995 52.25 Yellow  
 T 53.01 53.809999999999995 Red  
 Marker Name 14rs1573020  
 ASR 52.36 54.19  
 G 52.36 53.86 Blue  
 A 52.94 54.19 Green  
 Marker Name 15rs896788  
 ASR 55.02 57.07  
 C 55.019999999999996 56.39 Yellow  
 T 56.220000000000006 57.07 Red  
 Marker Name 16rs2065160  
 ASR 56.85 58.04  
 G 56.849999999999994 57.75 Blue  
 A 57.24 58.04 Green  
 Marker Name 17rs2572307

ASR	58.03	59.66		
G	58.03	58.83	Blue	
A	58.86	59.66	Green	
Marker Name	18rs2303798			
ASR	59.78	61.12		
C	59.78	60.58	Yellow	
T	60.32	61.12	Red	
Marker Name	19rs2065982			
ASR	62.31	63.86		
G	62.31	63.11	Blue	
A	63.06	63.86	Green	
Marker Name	20rs3785181			
ASR	63.89	66.12		
C	63.89	64.79	Yellow	
T	65.32	66.12	Red	
Marker Name	21rs881929			
ASR	64.29	67.39		
G	64.29	65.26	Blue	
T	66.58999999999999	67.39	Red	
Marker Name	22rs1498444			
ASR	67.19	68.17		
A	67.19	67.99000000000001	Green	
C	67.36999999999999	68.17	Yellow	
Marker Name	23rs1426654			
ASR	70.48	72.13		
C	70.47999999999999	71.28	Yellow	
T	71.33	72.13000000000001	Red	
Marker Name	24rs2026721			
ASR	71.28	72.65		
G	71.28	72.08000000000001	Blue	
A	71.85	72.65	Green	
Marker Name	25rs4540055			
ASR	74.18	76.08		
A	74.5	75.30000000000001	Green	
C	74.17999999999999	74.98	Yellow	
T	75.28	76.08000000000001	Red	
Marker Name	26rs16891982			
ASR	77.66	79.03		
G	77.66	78.46000000000001	Blue	
C	78.22999999999999	79.03	Yellow	
Marker Name	27rs1335873			
ASR	79.89	81.1		
A	79.89	80.69000000000001	Green	
T	80.3	81.10000000000001	Red	
Marker Name	28rs1886510			
ASR	80.52	82.01		
G	80.52000000000001	81.31	Blue	
A	81.17999999999999	82.01	Green	
Marker Name	29rs730570			
ASR	82.12	83.68		
C	82.12	83.06	Yellow	
T	82.67	83.67999999999999	Red	



ASR	69.9	71.5		
G	69.89999999999999	70.55	Blue	
A	70.80000000000001	71.5	Green	
Marker Name	03rs1024116			
ASR	32.4	34.99		
G	32.400000000000006	33.24	Blue	
A	33.77	34.99	Green	
Marker Name	05rs722098			
ASR	36.67	39.4		
G	36.67	37.47	Blue	
A	38.6	39.4	Green	
Marker Name	06rs10843344			
ASR	39.25	41.42		
C	39.25	40.05	Yellow	
T	40.620000000000005	41.42	Red	
Marker Name	07rs239031			
ASR	43.27	45.87		
C	43.27	44.07	Yellow	
T	45.06	45.870000000000005	Red	
Marker Name	13rs182549			
ASR	51.13	53.81		
C	51.129999999999995	52.25	Yellow	
T	53.01	53.809999999999995	Red	
Marker Name	10rs1978806			
ASR	47.0	49.9		
C	47.0	48.0	Yellow	
T	48.9	49.9	Red	
Panel Name	34-Plex Electrophoretic Shift 2			
Marker Name	01rs1321333			
ASR	32.86	36.83		
C	32.86	33.989999999999995	Yellow	
T	35.61	36.830000000000005	Red	
Marker Name	02rs917118			
ASR	32.6	35.67		
G	32.6	33.809999999999995	Blue	
A	34.870000000000005	35.67	Green	
Marker Name	03rs1024116			
ASR	33.35	36.39		
G	33.35	34.54	Blue	
A	35.17	36.39	Green	
Marker Name	04rs7897550			
ASR	36.47	39.46		
C	36.47	37.3	Yellow	
T	38.37	39.46	Red	
Marker Name	05rs722098			
ASR	36.72	39.59		
G	36.72	37.519999999999996	Blue	
A	38.79	39.589999999999996	Green	
Marker Name	06rs10843344			
ASR	41.68	44.0		
C	41.68	42.48	Yellow	
T	43.2	44.0	Red	

Marker Name 07rs239031  
 ASR 42.35 45.17  
 C 42.35 43.15 Yellow  
 T 44.36 45.17 Red  
 Marker Name 08rs12913832  
 ASR 44.16 45.64  
 G 44.160000000000004 44.96 Blue  
 A 44.84 45.64 Green  
 Marker Name 09rs2040411  
 ASR 46.48 48.49  
 G 46.480000000000004 47.78 Blue  
 A 47.690000000000005 48.49 Green  
 Marker Name 10rs1978806  
 ASR 47.82 49.26  
 C 47.82 48.940000000000005 Yellow  
 T 48.46 49.26 Red  
 Marker Name 11rs773658  
 ASR 48.55 50.04  
 G 48.550000000000004 49.35 Blue  
 C 49.24 50.04 Yellow  
 Marker Name 12rs10141763  
 ASR 50.32 52.76  
 A 50.32 51.389999999999999 Green  
 T 51.58 52.76 Red  
 Marker Name 13rs182549  
 ASR 51.0 53.29  
 C 51.0 52.120000000000005 Yellow  
 T 52.49 53.29 Red  
 Marker Name 14rs1573020  
 ASR 51.23 53.38  
 G 51.23 52.73 Blue  
 A 52.13 53.38 Green  
 Marker Name 15rs896788  
 ASR 54.01 56.51  
 C 54.01 55.38 Yellow  
 T 55.660000000000004 56.51 Red  
 Marker Name 16rs2065160  
 ASR 56.36 57.68  
 G 56.36 57.260000000000005 Blue  
 A 56.88 57.68 Green  
 Marker Name 17rs2572307  
 ASR 58.03 59.66  
 G 58.03 58.83 Blue  
 A 58.86 59.66 Green  
 Marker Name 18rs2303798  
 ASR 59.78 61.12  
 C 59.78 60.58 Yellow  
 T 60.32 61.12 Red  
 Marker Name 19rs2065982  
 ASR 62.31 63.86  
 G 62.31 63.11 Blue  
 A 63.06 63.86 Green

Marker Name 20rs3785181  
 ASR 63.89 66.12  
 C 63.89 64.79 Yellow  
 T 65.32 66.12 Red  
 Marker Name 21rs881929  
 ASR 64.29 67.39  
 G 64.29 65.26 Blue  
 T 66.58999999999999 67.39 Red  
 Marker Name 22rs1498444  
 ASR 67.19 68.17  
 A 67.19 67.99000000000001 Green  
 C 67.36999999999999 68.17 Yellow  
 Marker Name 23rs1426654  
 ASR 70.48 72.13  
 C 70.47999999999999 71.28 Yellow  
 T 71.33 72.13000000000001 Red  
 Marker Name 24rs2026721  
 ASR 71.28 72.65  
 G 71.28 72.08000000000001 Blue  
 A 71.85 72.65 Green  
 Marker Name 25rs4540055  
 ASR 74.18 76.08  
 A 74.5 75.30000000000001 Green  
 C 74.17999999999999 74.98 Yellow  
 T 75.28 76.08000000000001 Red  
 Marker Name 26rs16891982  
 ASR 77.66 79.03  
 G 77.66 78.46000000000001 Blue  
 C 78.22999999999999 79.03 Yellow  
 Marker Name 27rs1335873  
 ASR 79.89 81.1  
 A 79.89 80.69000000000001 Green  
 T 80.3 81.10000000000001 Red  
 Marker Name 28rs1886510  
 ASR 80.52 82.01  
 G 80.52000000000001 81.31 Blue  
 A 81.17999999999999 82.01 Green  
 Marker Name 29rs730570  
 ASR 82.12 83.68  
 C 82.12 83.06 Yellow  
 T 82.67 83.67999999999999 Red  
 Marker Name 30rs5030240  
 ASR 85.05 86.71  
 G 85.05 85.67 Blue  
 A 86.05 86.71 Green  
 C 85.95 86.58 Yellow  
 Marker Name 31rs2304925  
 ASR 86.42 88.52  
 G 86.42 86.96 Blue  
 T 87.75 88.52000000000001 Red  
 Marker Name 32rs5997008  
 ASR 88.14 88.85

A 88.14 88.83 Green  
C 88.23 88.85000000000001 Yellow  
Marker Name 33rs3827760  
ASR 90.46 91.43  
G 90.46 91.02 Blue  
A 90.73 91.42999999999999 Green  
Marker Name 34rs2814778  
ASR 90.16 92.14  
C 90.16 91.15 Yellow  
T 91.26 92.14 Red  
Panel Name 34-plex Elec Feb 2014  
Marker Name 01rs1321333  
ASR 32.62 35.85  
C 32.620000000000005 33.75 Yellow  
T 34.629999999999995 35.85 Red  
Marker Name 02rs917118  
ASR 31.54 34.26  
G 31.540000000000003 32.75 Blue  
A 33.46 34.26 Green  
Marker Name 03rs1024116  
ASR 31.87 34.99  
G 31.870000000000005 33.06 Blue  
A 33.77 34.99 Green  
Marker Name 04rs7897550  
ASR 36.44 39.3  
C 36.440000000000005 37.27 Yellow  
T 38.21 39.300000000000004 Red  
Marker Name 05rs722098  
ASR 36.94 39.87  
G 36.940000000000005 37.74 Blue  
A 39.07 39.87 Green  
Marker Name 06rs10843344  
ASR 40.55 43.09  
C 40.550000000000004 41.35 Yellow  
T 42.29 43.089999999999996 Red  
Marker Name 07rs239031  
ASR 41.21 44.01  
C 41.21 42.01 Yellow  
T 43.199999999999996 44.01 Red  
Marker Name 08rs12913832  
ASR 43.28 44.56  
G 43.28 44.08 Blue  
A 43.76 44.559999999999995 Green  
Marker Name 09rs2040411  
ASR 45.69 47.51  
G 45.690000000000005 46.99 Blue  
A 46.71 47.51 Green  
Marker Name 10rs1978806  
ASR 47.22 49.46  
C 47.22 48.34 Yellow  
T 48.660000000000004 49.46 Red  
Marker Name 11rs773658



ASR 48.36 49.88  
 G 48.36 49.16 Blue  
 C 49.08 49.87999999999995 Yellow  
 Marker Name 12rs10141763  
 ASR 50.6 53.45  
 A 50.6 51.66999999999995 Green  
 T 51.91 53.45 Red  
 Marker Name 13rs182549  
 ASR 51.13 53.81  
 C 51.12999999999995 52.25 Yellow  
 T 53.01 53.80999999999995 Red  
 Marker Name 14rs1573020  
 ASR 52.36 54.19  
 G 52.36 53.86 Blue  
 A 52.94 54.19 Green  
 Marker Name 15rs896788  
 ASR 55.02 57.07  
 C 55.019999999999996 56.39 Yellow  
 T 56.220000000000006 57.07 Red  
 Marker Name 16rs2065160  
 ASR 56.85 58.04  
 G 56.849999999999994 57.75 Blue  
 A 57.24 58.04 Green  
 Marker Name 17rs2572307  
 ASR 58.03 59.66  
 G 58.03 58.83 Blue  
 A 58.86 59.66 Green  
 Marker Name 18rs2303798  
 ASR 59.78 61.12  
 C 59.78 60.58 Yellow  
 T 60.32 61.12 Red  
 Marker Name 19rs2065982  
 ASR 62.31 63.86  
 G 62.31 63.11 Blue  
 A 63.06 63.86 Green  
 Marker Name 20rs3785181  
 ASR 63.89 66.12  
 C 63.89 64.79 Yellow  
 T 65.32 66.12 Red  
 Marker Name 21rs881929  
 ASR 64.29 67.39  
 G 64.29 65.26 Blue  
 T 66.58999999999999 67.39 Red  
 Marker Name 22rs1498444  
 ASR 67.19 68.17  
 A 67.19 67.990000000000001 Green  
 C 67.369999999999999 68.17 Yellow  
 Marker Name 23rs1426654  
 ASR 70.48 72.13  
 C 70.479999999999999 71.28 Yellow  
 T 71.33 72.130000000000001 Red  
 Marker Name 24rs2026721

ASR	71.28	72.65		
G	71.28	72.08000000000001	Blue	
A	71.85	72.65	Green	
Marker Name	25rs4540055			
ASR	74.18	76.08		
A	74.5	75.30000000000001	Green	
C	74.17999999999999	74.98	Yellow	
T	75.28	76.08000000000001	Red	
Marker Name	26rs16891982			
ASR	77.66	79.03		
G	77.66	78.46000000000001	Blue	
C	78.22999999999999	79.03	Yellow	
Marker Name	27rs1335873			
ASR	79.89	81.1		
A	79.89	80.69000000000001	Green	
T	80.3	81.10000000000001	Red	
Marker Name	28rs1886510			
ASR	80.52	82.01		
G	80.52000000000001	81.31	Blue	
A	81.17999999999999	82.01	Green	
Marker Name	29rs730570			
ASR	82.12	83.68		
C	82.12	83.06	Yellow	
T	82.67	83.67999999999999	Red	
Marker Name	30rs5030240			
ASR	85.05	86.71		
G	85.05	85.67	Blue	
A	86.05	86.71	Green	
C	85.95	86.58	Yellow	
Marker Name	31rs2304925			
ASR	86.12	87.95		
G	86.12	86.66	Blue	
T	87.17999999999999	87.95	Red	
Marker Name	32rs5997008			
ASR	87.74	88.78		
A	87.74	88.42999999999999	Green	
C	88.16	88.78	Yellow	
Marker Name	33rs3827760			
ASR	90.46	91.29		
G	90.46	91.02	Blue	
A	90.59	91.28999999999999	Green	
Marker Name	34rs2814778			
ASR	89.64	91.85		
C	89.64	90.63000000000001	Yellow	
T	90.97	91.85	Red	

**Supplementary Files S2.8**[Click here to download e-component: 34-PLEX\\_POP7\\_Panels.txt](#)

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Kit type: SNP

Chemistry Kit 34-PLEX none

Panel 34-Plex Electrophoretic Shift none

01rs1321333 - none

02rs917118 - none

03rs1024116 - none

04rs7897550 - none

05rs722098 - none

06rs10843344 - none

07rs239031 - none

08rs12913832 - none

09rs2040411 - none

10rs1978806 - none

11rs773658 - none

12rs10141763 - none

13rs182549 - none

14rs1573020 - none

15rs896788 - none

16rs2065160 - none

17rs2572307 - none

18rs2303798 - none

19rs2065982 - none

20rs3785181 - none

21rs881929 - none

22rs1498444 - none

23rs1426654 - none

24rs2026721 - none

25rs4540055 - none

26rs16891982 - none

27rs1335873 - none

28rs1886510 - none

29rs730570 - none

30rs5030240 - none

31rs2304925 - none

32rs5997008 - none

33rs3827760 - none

34rs2814778 - none

Panel 34-PLEX none

01rs1321333 - none

02rs917118 - none

03rs1024116 - none

04rs7897550 - none

05rs722098 - none

06rs10843344 - none

07rs239031 - none

08rs12913832 - none

09rs2040411 - none

10rs1978806 - none

11rs773658 - none

12rs10141763 - none

13rs182549 - none

14rs1573020	-	none	
15rs896788	-	none	
16rs2065160	-	none	
17rs2572307	-	none	
18rs2303798	-	none	
19rs2065982	-	none	
20rs3785181	-	none	
21rs881929	-	none	
22rs1498444	-	none	
23rs1426654	-	none	
24rs2026721	-	none	
25rs4540055	-	none	
26rs16891982	-	none	
27rs1335873	-	none	
28rs1886510	-	none	
29rs730570	-	none	
30rs5030240	-	none	
31rs2304925	-	none	
32rs5997008	-	none	
33rs3827760	-	none	
34rs2814778	-	none	
Panel 34-PLEX Extra		none	
02rs917118	-	none	
03rs1024116	-	none	
05rs722098	-	none	
06rs10843344	-	none	
07rs239031	-	none	
13rs182549	-	none	
28rs1886510	-	none	
Panel 34-PLEX Extra Mod		none	
02rs917118	-	none	
03rs1024116	-	none	
05rs722098	-	none	
06rs10843344	-	none	
07rs239031	-	none	
13rs182549	-	none	
10rs1978806	-	none	
Panel 34-Plex Electrophoretic Shift 2		none	
01rs1321333	-	none	
02rs917118	-	none	
03rs1024116	-	none	
04rs7897550	-	none	
05rs722098	-	none	
06rs10843344	-	none	
07rs239031	-	none	
08rs12913832	-	none	
09rs2040411	-	none	
10rs1978806	-	none	
11rs773658	-	none	
12rs10141763	-	none	
13rs182549	-	none	
14rs1573020	-	none	

15rs896788	-	none	
16rs2065160	-	none	
17rs2572307	-	none	
18rs2303798	-	none	
19rs2065982	-	none	
20rs3785181	-	none	
21rs881929	-	none	
22rs1498444	-	none	
23rs1426654	-	none	
24rs2026721	-	none	
25rs4540055	-	none	
26rs16891982	-	none	
27rs1335873	-	none	
28rs1886510	-	none	
29rs730570	-	none	
30rs5030240	-	none	
31rs2304925	-	none	
32rs5997008	-	none	
33rs3827760	-	none	
34rs2814778	-	none	
Panel 34-plex Elec Feb 2014		none	
01rs1321333	-	none	
02rs917118	-	none	
03rs1024116	-	none	
04rs7897550	-	none	
05rs722098	-	none	
06rs10843344	-	none	
07rs239031	-	none	
08rs12913832	-	none	
09rs2040411	-	none	
10rs1978806	-	none	
11rs773658	-	none	
12rs10141763	-	none	
13rs182549	-	none	
14rs1573020	-	none	
15rs896788	-	none	
16rs2065160	-	none	
17rs2572307	-	none	
18rs2303798	-	none	
19rs2065982	-	none	
20rs3785181	-	none	
21rs881929	-	none	
22rs1498444	-	none	
23rs1426654	-	none	
24rs2026721	-	none	
25rs4540055	-	none	
26rs16891982	-	none	
27rs1335873	-	none	
28rs1886510	-	none	
29rs730570	-	none	
30rs5030240	-	none	
31rs2304925	-	none	

32rs5997008	-	none
33rs3827760	-	none
34rs2814778	-	none

**Supplementary Files S2.5**[Click here to download e-component: AIM-indelplex\\_POP7\\_bins.txt](#)

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Chemistry Kit AIM-indelplex

BinSet Name AIM-indelplex

Panel Name AIM-indelplex

Marker Name 1470

1 61.28 0.5 0.76

2 66.54 0.5 0.5

Marker Name 777

1 70.9 0.5 0.5

2 73.85 0.5 0.5

Marker Name 196

1 81.19 0.5 0.5

2 84.3 0.5 0.5

Marker Name 881

1 89.41 0.5 0.5

2 93.46 0.5 0.5

Marker Name 3122

1 98.52 0.5 0.5

2 103.05 0.5 0.5

Marker Name 548

1 107.97 0.5 0.5

2 109.91 0.5 0.5

3 104.91 0.5 0.5 mutant

Marker Name 659

1 116.64 0.5 0.63

2 118.92 0.5 0.5

Marker Name 2011

1 127.45 0.5 0.5

2 132.61 0.5 0.5

Marker Name 2929

1 150.73 0.5 0.5

2 152.95 0.5 0.5

Marker Name 593

1 156.67 0.5 0.5

2 158.85 0.5 0.5

Marker Name 798

1 168.67 0.5 0.5

2 174.57 0.5 0.5

Marker Name 1193

1 181.46 0.5 0.5

2 183.5 0.5 0.5

Marker Name 1871

1 191.3 0.5 0.5

2 193.25 0.5 0.66

Marker Name 17

1 200.28 0.5 0.5

2 204.2 0.5 0.5

Marker Name 2538

1 210.23 0.5 0.75

2 213.79 0.5 0.75

Marker Name 1644

1 222.95 0.5 0.5

2	224.81	0.5	0.5	
Marker Name	3854			
1	56.54	0.97	0.5	
2	60.12	0.59	0.5	
Marker Name	2275			
1	70.9	0.5	0.5	
2	77.92	0.5	0.5	
Marker Name	3072			
1	106.57	0.5	0.5	
2	113.5	0.5	0.5	
Marker Name	772			
1	118.4	0.5	0.5	
2	121.6	0.5	0.5	
Marker Name	2313			
1	128.4	0.5	0.5	
2	137.67	0.5	0.5	
Marker Name	397			
1	164.01	0.5	0.5	
2	168.03	0.5	0.5	
Marker Name	1636			
1	174.2	0.5	0.5	
2	175.88	0.5	0.5	
Marker Name	51			
1	184.47	0.5	0.5	
2	189.56	0.5	0.5	
Marker Name	2431			
1	209.32	0.5	0.75	
2	213.6949191730.5	0.5	0.75	
Marker Name	2264			
1	225.25	0.5	0.5	
3	228.0	0.4	0.4	mutant
2	229.12	0.4	0.66	
Marker Name	2256			
1	58.21	0.5	0.5	
2	61.374696174	0.5	0.5	0.5
Marker Name	128			
1	68.29	0.5	0.5	
2	71.41	0.5	0.5	
Marker Name	15			
1	78.8	0.5	0.5	
2	89.72	0.5	0.64	
Marker Name	2241			
1	107.93	0.64	0.5	
2	115.84	0.5	0.5	
Marker Name	419			
1	120.77	0.5	0.65	
2	127.68	0.5	0.5	
Marker Name	943			
1	156.89	0.56	0.5	
2	160.77	0.5	0.5	
Marker Name	159			
1	168.75	0.61	0.5	



2	173.8	0.5	0.5	
Marker Name 2005				
1	184.78	0.5	0.55	
2	190.57	0.64	0.5	
Marker Name 250				
1	201.03	0.5	0.5	
2	203.1	0.5	0.5	
Marker Name 1802				
1	213.67	0.5	0.5	
2	216.64	0.5	0.5	
Marker Name 1607				
1	221.42	0.5	0.5	
2	223.03	0.62	0.5	
Marker Name 406				
1	66.96	0.5	0.5	
2	68.77	0.5	0.5	
Marker Name 1386				
1	72.66	0.5	0.71	
2	94.14	0.5	0.5	
Marker Name 1726				
1	105.6	0.5	0.5	
2	118.28	0.5	0.5	
Marker Name 3626				
1	142.49	0.5	0.5	
2	158.53	0.5	0.5	
Marker Name 360				
1	170.34	0.4	0.4	
3	171.2	0.4	0.4	mutant
2	172.15	0.4	0.4	
Marker Name 1603				
1	214.33	0.5	0.56	
2	218.0	0.5	0.5	
Marker Name 2719				
1	227.71	0.5	0.5	
2	231.5	0.5	0.5	
Marker Name 1734				
1	57.94	0.5	0.5	
2	61.77	0.5	0.5	
Marker Name 94				
1	91.31	0.5	0.5	
2	94.25	0.5	0.56	

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Kit type: MICROSATELLITE

Chemistry Kit AIM-indelplex none

Panel AIM-indelplex none

1470	blue	59.498930343000005	68.0	-	9	0.0	none	
777	blue	68.55843072	76.0	-	9	0.0	none	
196	blue	77.588872041	85.0	-	9	0.0	none	
881	blue	85.617309136	94.0	-	9	0.0	none	
3122	blue	95.320690594	103.8	-	9	0.0	none	
548	blue	104.2	110.8	-	9	0.0	none	
659	blue	113.556264981	120.0	-	9	0.0	none	
2011	blue	124.498164706	135.0	-	9	0.0	none	
2929	blue	146.955519673	153.8	-	9	0.0	none	
593	blue	154.7	160.0	-	9	0.0	none	
798	blue	165.32897286899998	176.0	-	9	0.0	none	
1193	blue	178.658800433	186.0	-	9	0.0	none	
1871	blue	188.823452509	195.5	-	9	0.0	none	
17	blue	197.084416798	205.0	-	9	0.0	none	
2538	blue	206.773228342	216.0	-	9	0.0	none	
1644	blue	219.483987195	226.0	-	9	0.0	none	
3854	Green	54.61523001	64.0	-	9	0.0	none	
2275	Green	68.507204408	80.0	-	9	0.0	none	
3072	Green	103.268780394	114.0	-	9	0.0	none	
772	Green	115.766532729	123.0	-	9	0.0	none	
2313	Green	125.024081548	139.5	-	9	0.0	none	
397	Green	161.189615282	170.0	-	9	0.0	none	
1636	Green	172.086299232	200.002	177.5	-	9	0.0	none
51	Green	181.211398852	191.0	-	9	0.0	none	
2431	Green	206.293870962	216.0	-	9	0.0	none	
2264	Green	221.926816896	231.0	-	9	0.0	none	
2256	Yellow	56.680080642	64.0	-	9	0.0	none	
128	Yellow	66.0	72.0	-	9	0.0	none	
15	Yellow	76.5	91.0	-	9	0.0	none	
2241	Yellow	104.973806014	116.5	-	9	0.0	none	
419	Yellow	118.07762833899999	129.0	-	9	0.0	none	
943	Yellow	153.696434559	162.0	-	9	0.0	none	
159	Yellow	166.024093113	200.002	175.0	-	9	0.0	none
2005	Yellow	181.501371715	192.0	-	9	0.0	none	
250	Yellow	198.065914853	204.0	-	9	0.0	none	
1802	Yellow	210.217316797	218.0	-	9	0.0	none	
1607	Yellow	218.9	224.0	-	9	0.0	none	
406	Red	64.678876207	69.5	-	9	0.0	none	
1386	Red	70.5	96.0	-	9	0.0	none	
1726	Red	104.0	121.0	-	9	0.0	none	
3626	Red	141.0	160.0	-	9	0.0	none	
360	Red	168.792202855	174.0	-	9	0.0	none	
1603	Red	213.0	219.0	-	9	0.0	none	
2719	Red	226.0	232.5	-	9	0.0	none	
1734	Red	56.285110841	63.5	-	9	0.0	none	
94	Green	88.363699841	96.0	-	9	0.0	none	

**Supplementary File S3.** SNP and Indel genotypes used in the exercise as reference population

**File S3.1.** PCA input file formatted for use with *Snipper* at: <http://math>

**File S3.2.** Training set file applicable to custom analyses (and used as fixed reference)

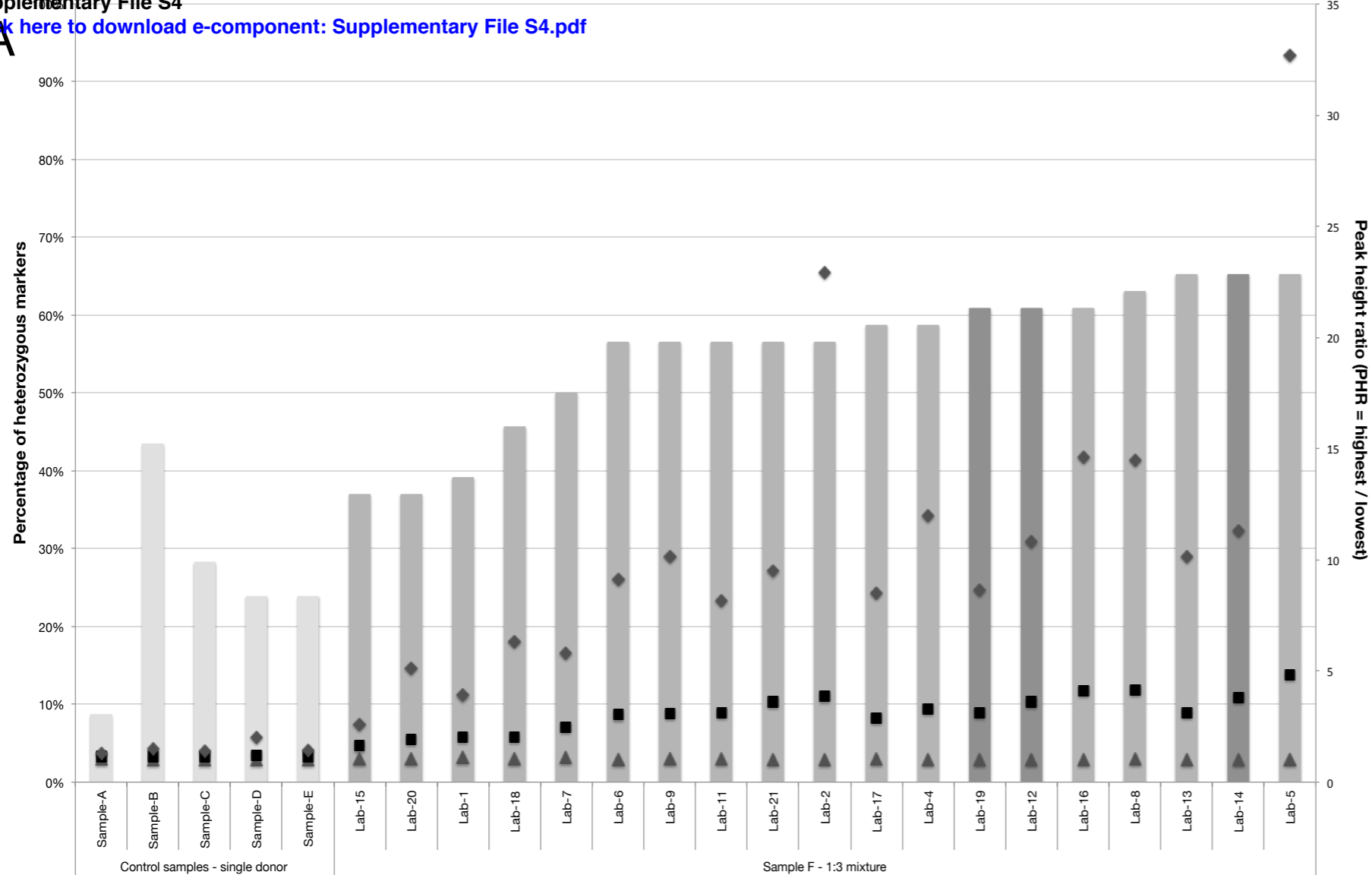
**File S3.3.** Likelihood ratios from cross-validation of PCA input file genotypes (comparison)

**Note:** Worksheets S3.1 and S3.2 need to be placed in 'position 1' to use make the genotype

LRs obtained with *Snipper* from complete profile data in Supplementary File S3.1

<b>Inference</b>	<b>34-plex, 3-group</b>
European	9947A is 2,118,840,589,047,061,020,672 times more likely EUROPE than EAST ASIA
East Asian	<b>A is 361,148,635,069,545,024 times more likely EAST ASIA than EUROPE</b>
European	B is 64,191,487,284,485,608 times more likely EUROPE than EAST ASIA
East Asian	C is 13,115,706 times more likely EAST ASIA than AFRICA
East Asian	D is 248,539,593,557 times more likely EAST ASIA than EUROPE
African	<b>E is 556,454,701,312,037,054,117,314,560 times more likely AFRICA than EUROPE</b>
	<b>46-plex, 4-group</b>
European	9947A is 1,937,432,967,198 times more likely EUROPE than EAST ASIA
East Asian	<b>A is 6,993,957 times more likely EAST ASIA than AMERICA</b>
European	B is 143,659,679,122 times more likely EUROPE than EAST ASIA
East Asian	C is 131 times more likely EAST ASIA than EUROPE
American	D is 944,698,134 times more likely AMERICA than EAST ASIA
African	<b>E is 3,229,841,442,838,053,650,432 times more likely AFRICA than EUROPE</b>
	<b>80 Markers, 5-group</b>
Oceanian	C is 153,747,536,542,653 times more likely OCEANIA than EAST ASIA

### Supplementary File S4



**A.** Numbers of heterozygotes (bars) and PHR values (points) plotted for all participant's AIM-Indel data. Samples A-E are average values, sample F is per participant and includes laboratories #15, #20, #1 and #18 removed from the PHR comparisons made with the Kruskal-Wallis rank sum test summarized in C.

**B.** Grid of  $p$ -values for the pairwise comparison of numbers of heterozygotes in A-E (average number from 19 laboratories) and individual numbers per laboratory for sample F, applying a unilateral 2-sample test for equality-of-proportions (with continuity correction). Grey cells mark significant  $p$ -values.

	Sample-A	Sample-B	Sample-C	Sample-D	Sample-E	Lab-15	Lab-20	Lab-1	Lab-18	Lab-7	Lab-6	Lab-9	Lab-11	Lab-21	Lab-2	Lab-17	Lab-4	Lab-19	Lab-12	Lab-16	Lab-8	Lab-13	Lab-14	
Sample-B	0.00018																							
Sample-C	0.01582	0.90390																						
Sample-D	0.04519	0.96120	0.59380																					
Sample-E	0.04519	0.96120	0.59380	0.50000																				
Lab-15	0.00144	0.66470	0.25230	0.12860	0.12860																			
Lab-20	0.00144	0.66470	0.25230	0.12860	0.12860	0.50000																		
Lab-1	0.00074	0.58380	0.18880	0.08909	0.08909	0.50000	0.50000																	
Lab-18	8.85e-05	0.50000	0.06527	0.02441	0.02441	0.26260	0.26260	0.33650																
Lab-7	1.88e-05	0.33800	0.02726	0.00875	0.00875	0.14650	0.14650	0.20070	0.41730															
Lab-6	1.50e-06	0.14860	0.00568	0.00146	0.00146	0.04729	0.04729	0.07201	0.20210	0.33800														
Lab-9	1.50e-06	0.14860	0.00568	0.00146	0.00146	0.04729	0.04729	0.07201	0.20210	0.33800	0.50000													
Lab-11	1.50e-06	0.14860	0.00568	0.00146	0.00146	0.04729	0.04729	0.07201	0.20210	0.33800	0.50000	0.50000												
Lab-21	1.50e-06	0.14860	0.00568	0.00146	0.00146	0.04729	0.04729	0.07201	0.20210	0.33800	0.50000	0.50000	0.50000											
Lab-2	1.50e-06	0.14860	0.00568	0.00146	0.00146	0.04729	0.04729	0.07201	0.20210	0.33800	0.50000	0.50000	0.50000	0.50000										
Lab-17	6.09e-07	0.10540	0.00313	0.00075	0.00075	0.03016	0.03016	0.04761	0.14830	0.33800	0.50000	0.50000	0.50000	0.50000	0.50000									
Lab-4	6.09e-07	0.10540	0.00313	0.00075	0.00075	0.03016	0.03016	0.04761	0.14830	0.33800	0.50000	0.50000	0.50000	0.50000	0.50000	0.50000								
Lab-19	2.39e-07	0.07201	0.00166	0.00037	0.00037	0.01851	0.01851	0.03028	0.10500	0.20070	0.41620	0.41620	0.41620	0.41620	0.41620	0.50000	0.50000							
Lab-12	2.39e-07	0.07201	0.00166	0.00037	0.00037	0.01851	0.01851	0.03028	0.10500	0.20070	0.41620	0.41620	0.41620	0.41620	0.41620	0.50000	0.50000	0.50000						
Lab-16	2.39e-07	0.07201	0.00166	0.00037	0.00037	0.01851	0.01851	0.03028	0.10500	0.20070	0.41620	0.41620	0.41620	0.41620	0.41620	0.50000	0.50000	0.50000	0.50000					
Lab-8	9.09e-08	0.04729	0.00085	0.00017	0.00017	0.01091	0.01091	0.01851	0.07144	0.14650	0.33530	0.33530	0.33530	0.33530	0.33530	0.41540	0.41540	0.50000	0.50000	0.50000				
Lab-13	3.34e-08	0.02980	0.00041	7.98e-05	7.98e-05	0.00616	0.00616	0.01084	0.04667	0.10280	0.26080	0.26080	0.26080	0.26080	0.26080	0.33380	0.33380	0.41450	0.41450	0.41450	0.50000			
Lab-14	3.34e-08	0.02980	0.00041	7.98e-05	7.98e-05	0.00616	0.00616	0.01084	0.04667	0.10280	0.26080	0.26080	0.26080	0.26080	0.26080	0.33380	0.33380	0.41450	0.41450	0.41450	0.50000	0.50000		
Lab-5	3.335e-08	0.02980	0.00041	7.98e-05	7.98e-05	0.006161	0.006161	0.01084	0.04667	0.10280	0.26080	0.26080	0.26080	0.26080	0.26080	0.33380	0.33380	0.41450	0.41450	0.41450	0.50000	0.50000	0.50000	

C

C. Grid of  $p$ -values for pairwise comparisons of PHR values applying a Kruskal-Wallis rank sum test (grey cells: significant values)

	Sample-A	Sample-B	Sample-C	Sample-D	Sample-E	Lab-7	Lab-6	Lab-9	Lab-11	Lab-21	Lab-2	Lab-17	Lab-4	Lab-19	Lab-12	Lab-16	Lab-8	Lab-13	Lab-14
Sample-B	0.24530																		
Sample-C	0.36500	0.05085																	
Sample-D	0.60150	0.26500	0.40090																
Sample-E	0.36080	0.86880	0.31060	0.41180															
Lab-7	0.00777	1.61e-06	3.58e-05	0.00033	5.56e-05														
Lab-6	0.01237	2.36e-06	9.51e-05	0.00033	8.82e-05	0.33620													
Lab-9	0.00341	1.92e-07	3.36e-06	5.80e-05	1.15e-05	0.37810	0.89810												
Lab-11	0.00727	6.92e-07	2.04e-05	0.00017	3.78e-05	0.17310	0.88360	0.67380											
Lab-21	0.01237	2.93e-06	0.00011	0.00026	8.82e-05	0.05445	0.38970	0.30540	0.36020										
Lab-2	0.00341	1.05e-07	3.36e-06	5.80e-05	1.15e-05	0.13820	0.71430	0.49830	0.78370	0.54590									
Lab-17	0.00672	5.98e-07	1.58e-05	0.00018	3.07e-05	0.29770	0.90090	1.00000	0.73530	0.24030	0.60590								
Lab-4	0.01128	1.29e-06	3.01e-05	0.00023	8.05e-05	0.28880	0.97160	0.88680	0.76230	0.34570	0.70870	0.86950							
Lab-19	0.00524	2.40e-07	1.24e-05	8.41e-05	2.52e-05	0.12990	0.87620	0.65260	0.86260	0.44620	1.00000	0.56700	0.74910						
Lab-12	0.01670	1.23e-06	2.64e-05	0.00020	8.41e-05	0.29780	0.86260	0.71620	0.97240	0.57960	0.88980	0.78760	0.89290	0.98040					
Lab-16	0.01034	8.91e-07	2.06e-05	0.00012	6.48e-05	0.08159	0.43600	0.38670	0.42580	0.98620	0.60350	0.32880	0.41900	0.55520	0.62300				
Lab-8	0.00582	3.67e-07	9.82e-06	9.93e-05	2.08e-05	0.07849	0.43810	0.31990	0.50010	0.94620	0.64900	0.32120	0.45560	0.58730	0.62070	0.97450			
Lab-13	0.00882	6.68e-07	1.14e-05	0.00017	4.31e-05	0.55370	0.81810	0.84370	0.64550	0.30840	0.46980	0.81050	0.71320	0.57540	0.58600	0.37510	0.28170		
Lab-14	0.00276	3.69e-08	1.49e-06	2.58e-05	5.86e-06	0.13170	0.53240	0.41140	0.63380	0.78000	0.88250	0.47200	0.63160	0.68580	0.72040	0.80340	0.87950	0.34400	
Lab-5	0.00544	2.62e-07	7.86e-06	8.05e-05	1.74e-05	0.11850	0.59910	0.45970	0.74250	0.69340	0.96070	0.51230	0.64300	0.75560	0.68580	0.82750	0.91550	0.38310	0.92930

## **Supplementary File S5** Next generation sequencing experiments using exercise PCR multiplexes.

### **1. Post-PCR DNA processing for SNP analysis with the MiSeq**

Libraries were prepared directly from PCR products using the Illumina TruSeq ChIP sample preparation kit. Libraries were then run on the MiSeq with the 300 cycle version 2 reagent kit and sequences aligned to a custom 'genome' containing the reference sequences for all 34 SNPs in a single unified strand using Burrows-Wheeler alignment. SNP genotypes were called using GATK.

### **2. Post-PCR DNA processing for Indel and SNP analysis with the TFS-LT Ion PGM™**

Libraries were prepared directly from PCR products using the TFS-LT Ion Xpress™ Plus gDNA fragment library preparation protocol applying the Ion Xpress™ Plus Fragment Library Kit. This kit processes DNA not amplified using AmpliSeq primers by enabling ligation and nick repair reactions. Thereafter sequencing followed standard protocols using kits: Ion OneTouch™ 200 Template v2 and Ion PGM™ Sequencing 200 v2. Sequences were aligned to custom BED files and genotypes called from human genome build hg19 using TFS-LT Torrent Suite™ 4.0.2.

### **3. Genotyping performance for samples A-E**

Both NGS systems gave comparable high levels of genotyping performance for SNP analysis. Ion PGM™ had just singleton no-calls or missing data (no sequences detected carrying expected SNP sites), while MiSeq gave the only miscalled genotype in rs5030240, where a sequence ratio of A=5810 / G=9866 was recorded as a GG, although it was detected as atypical.

Indel genotyping performance with the Ion PGM™ was slightly lower, but this could be due to alignment issues. The Indel rs60612424 (MID-3854) was not detected in any samples, while C had a disproportionately high number of no-calls that might be the result of population-specific flanking indels blocking secure alignment to the reference sequence.

4. Summary tables of genotyping performance from SNP analysis with both NGS systems and Indel analysis with Ion PGM™. Matches count concordant genotype calls made in both CE and NGS.

Ion PGM™ sequence data for 34 SNPs

	A	B	C	D	E	F
<b>NGS no-calls</b>	1	0	0	1	1	1
<b>SNaPshot no-calls</b>	1	0	0	0	0	0
<b>Ion PGM miscalls</b>	0	0	0	0	0	4
<b>Missing data</b>	0	1	1	1	0	1
<b>NGS-CE genotype matches *</b>	32	33	33	32	33	28
<b>Heterozygote number</b>	10	5	10	9	10	14

MiSeq sequence data for 34 SNPs

	A	B	C	D	E	F
<b>NGS no-calls</b>	0	0	0	0	0	0
<b>SNaPshot no-calls</b>	1	0	0	0	0	0
<b>Ion PGM miscalls</b>	0	0	1	0	0	4
<b>Missing data</b>	0	0	0	0	0	0
<b>NGS-CE genotype matches *</b>	33	34	33	34	34	30
<b>Heterozygote number</b>	10	5	9	10	10	17

Ion PGM™ sequence data for 46 Indels

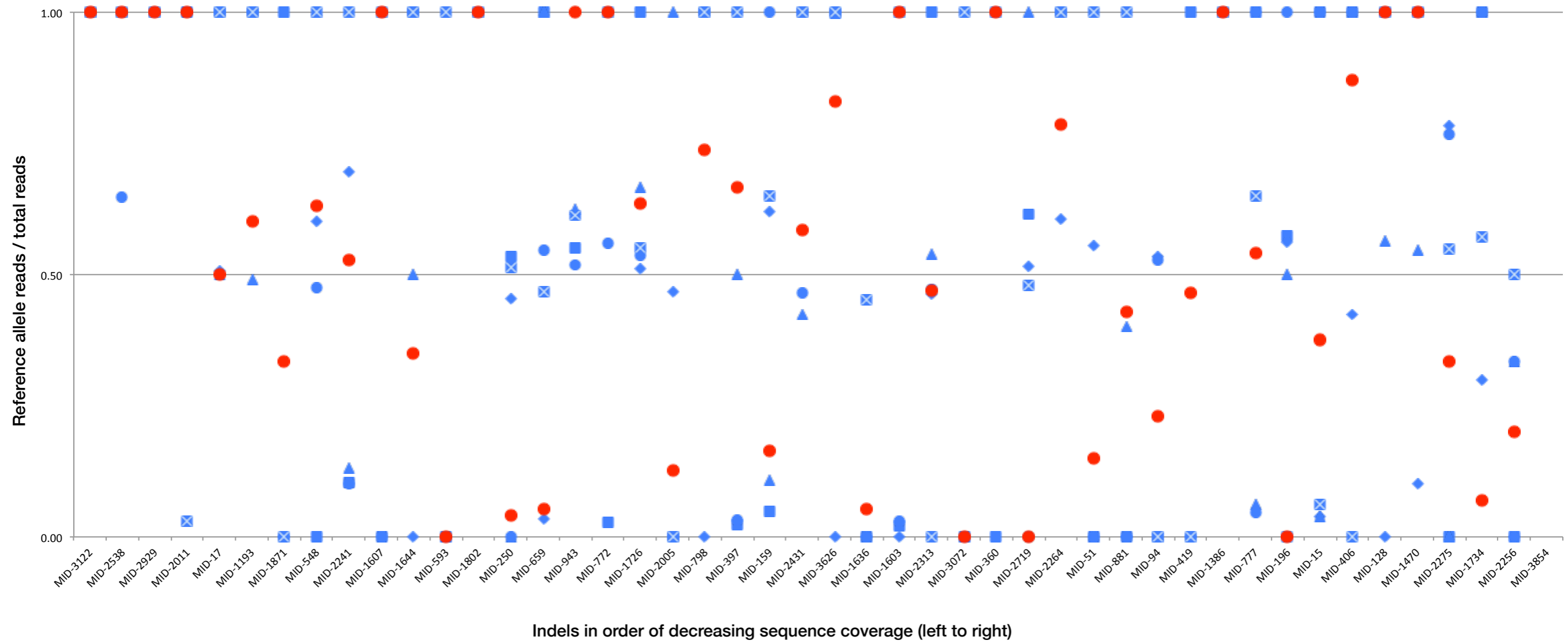
	A	B	C	D	E	F
<b>Ion PGM no-calls</b>	4	1	12	3	1	3
<b>PCR-to-CE no-calls</b>	0	0	0	0	0	0
<b>Ion PGM miscalls</b>	0	3	3	0	0	6
<b>Missing data</b>	1	2	2	1	1	1
<b>NGS-CE genotype matches *</b>	41	40	29	42	44	36
<b>Heterozygote number</b>	4	17	11	10	10	21

\* Matching genotypes denote calls concordant with conventional CE analysis

**5.** NGS analysis of mixed sample F indicated a higher number of displaced sequence ratios positioned outside ranges around an ideal midline ratio of 0.5:0.5, although Indel genotyping showed several irregular ratios for A-E likely due to alignment issues. SNP genotyping with both platforms was very sensitive to imbalanced sequence ratios in F. In samples A-E, Ion PGM™ detected **4/44** peak pairs outside nominal 0.4:0.6-0.5:0.5 sequence ratio ranges, and MiSeq **2/44** (discounting tri-allelics). In sample F by contrast, Ion PGM™ detected **8/14** and MiSeq **14/17** peak pairs outside these ratios, providing clear indications of a mixture that is largely absent from SNaPshot data. Both systems detected displaced ratios in the two tri-allelic SNPs, as well as a remarkably well matched pattern of sample F sequence ratios across the 34 SNPs.

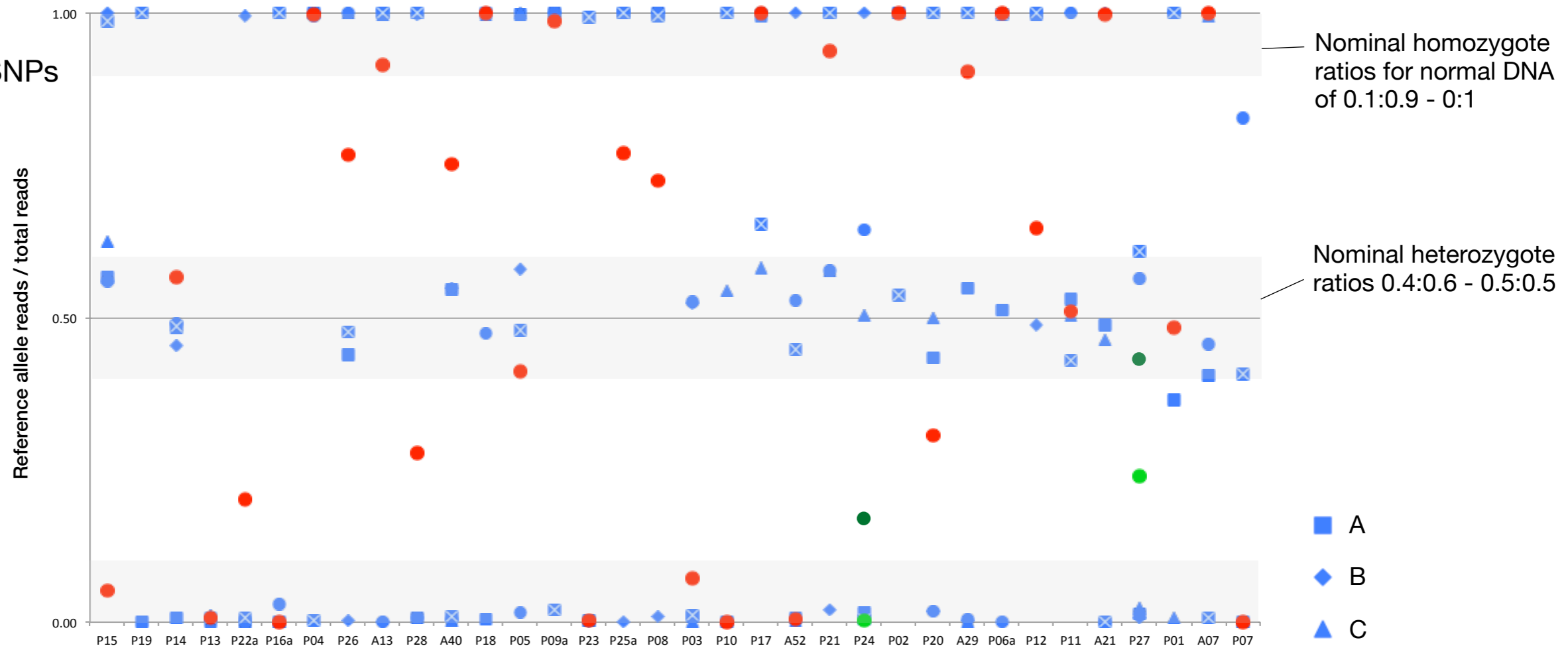
- A
- ◆ B
- ▲ C
- D
- ⊠ E
- F

Ion PGM™ 46 Indels





Ion PGM™ 34 SNPs



MiSeq 34 SNPs

