Title: Inferring biogeographic ancestry with compound markers of slow and fast evolving polymorphisms.

Authors: Moriot A, Santos C, Freire-Aradas A, Phillips C, Hall D

Journal: European journal of human genetics : EJHG

Year: 2018 Nov

Issue: 26

Volume: 11

Pages: 1697-1707

DOI: 10.1038/s41431-018-0215-2

Inferring biogeographic ancestry with compound markers of slow and fast evolving polymorphisms

Amandine Moriot[1¶], Carla Santos[2¶], Ana Freire-Aradas[2], Christopher Phillips[2], Diana Hall[1*]

[1] Unité de Génétique Forensique, Centre Universitaire Romand de Médecine Légale, Centre Hospitalier Universitaire Vaudois et Université de Lausanne, Lausanne, Switzerland

[2] Forensic Genetics Unit, Institute of Forensic Science, University of Santiago de Compostela, Santiago de Compostela, Spain

[¶]These authors contributed equally to this work.

[*]Corresponding author

Diana Hall

Centre Universitaire Romand de Médecine Légale, Centre Hospitalier Universitaire Vaudois et Université de Lausanne
Ch. de la Vulliette 4
1000, Lausanne
Switzerland

Telephone: +41 021 314 7081, FAX: +41 021 314 7090

Email: Diana.Hall@chuv.ch

Conflict of interest The authors declare that they have no conflict of interest.

Abstract

Bio-geographic ancestry is an area of considerable interest in the medical genetics, anthropology and forensics. Although genome-wide panels are ideal as they provide dense genotyping data, small sets of ancestry informative marker provide a cost-effective way to investigate genetic ancestry and population structure. Here, we investigate the performance of a reduced marker set that combine different types of autosomal markers through haplotype analysis. In particular, recently described DIP-STR markers should offer the advantage of comprising both, low mutation rate Indels (DIPs), to study human history over longer time scale; and high mutation rate STRs, to trace relatively recent demographic events.

In this study, we assessed the ability of an initial set of 23 DIP-STRs to distinguish major population groups using the HGDP-CEPH reference samples. The results obtained applying the STRUCTURE algorithm show that the discrimination capacity of the DIP-STRs is comparable to currently used small-scale ancestry informative markers by approaching seven major demographic groups. Yet, the DIP-STRs show an improved success rate in assigning individuals to populations of Europe and Middle East.

These data show a remarkable ability of a preliminary set of 23 DIP-STR markers to infer major biogeographic origins. A novel set of DIP-STRs preselected to contain ancestry information should lead to further improvements.

Key Words

DIP-STR, ancestry inference, HGDP-CEPH, population structure, Indels

Introduction

Bio-geographic ancestry inference has largely contributed to controlling for population structure in disease or trait association studies and to infer human evolutionary history [1-4]. Sets of small-scale ancestry informative markers (AIM) are valuable to provide most of the information in a cost-effective manner when sample sets have not been typed with genome-wide arrays of SNPs or when DNA amount is limited as in forensic science. Targeted association studies – such as candidate gene studies or replication studies following up genome-wide scans typically analyse a much smaller number of markers than genome-wide scans, making it difficult to infer ancestry in order to correct for stratification. AIMs are also used to select samples for follow-up studies before genome-wide scans are performed. In criminal investigations or missing persons identifications, in the absence of any other investigative leads (no database or suspect match), AIM genotypes obtained from evidential material could indicate the likely ancestry of the donor, and therefore help direct the course of investigations [5,6].

Numerous AIM panels composed of dozens of autosomal SNPs, Indels or STRs have been published [7-15]. Briefly, these and other studies have shown that populations clustering patterns are robust, provided that at least about 60-150 markers are used [16-18], or about 30 or fewer if markers are preselected to have a high information content about ancestry [8,12,15,19]. Most of small AIM sets are able to structure global populations into five major geographic regions: America, Sub-Saharan Africa, East Asia, Oceania and a cluster composed of Europe, Central-South Asia and Middle East populations within Eurasia [7].

Nevertheless, different types of markers offer different population structure resolution. In the field of molecular evolution, it has long been recognized that markers

78 with relatively low mutation rates (SNP, Alu, Indel) serve as best loci for the analysis of
79 human history over longer time scales (therefore to provide a biogeographical resolution
80 at the level of continents), whereas rapidly evolving markers (STR and mtDNA)
81 provide the greatest resolution over shorter time scales (regional resolution) [20].
82 Haplotypes composed of both slow- and fast-evolving loci should combine the benefits
83 of two types of markers. For example, in the case of linked SNP-STR, the instability of
84 the STR should generate many alleles, in proportion to the populations' divergence
85 time, while the more stable flanking SNP should allow greater certainty in tracing the
86 lineage of each haplotype. In addition, the number of possible haplotypes formed by the
87 combination of these markers is much greater than the number of alleles at each
88 individual locus. Such high variability increases the likelihood of rare haplotypes, that
89 are easily lost during population founding and bottleneck events.

90 The first example of compound genetic markers are the SNP-STRs residing in the
91 nonrecombining region of the human Y chromosome [20]. However, the uniparental
92 transmission of the Y chromosome, like the mitochondrial genome, has the limit of
93 reflecting only a fraction of individual's ancestry with the risk of misinterpreting the
94 overall ancestry in admixed individuals [6,21]. To overcome such limitation Mountain et
95 al. [22] proposed autosomal SNP-STR markers. In a pilot study they were able to show
96 that two such compound markers were sufficient to provide support for the "out of
97 Africa" human evolutionary hypothesis. Unfortunately, the application of SNP-STR for
98 ancestry inference found limited success because of the difficulties initially encountered
99 in genotyping SNPs linked STRs.

100 The current high throughput DNA sequencing technology finally enables genetic
101 practitioners to consider multiple polymorphisms grouped into haplotypes. Many

benchtop DNA sequencing platforms provide today continuous runs of a hundred base pairs or more on a single DNA molecule that allows to directly inferring the phase of the multiple markers within a small DNA segment. Minihaplotypes and microhaplotypes encompassing two to four SNPs spanning less than 10 kb and 200 bp, respectively, were recently described [23,24]. Haplotype systems based on multiple SNPs have proven a forensically useful DNA marker for family or lineage inference [24,25] and in anthropology for population relationships [19,24,26,27]. A recent study showed that assignment of individuals to candidate populations significantly improves through combining linked SNPs. For example, incorrectly assigned individuals from empirical data of French and German populations can be decreased by 73% using haplotypes [28].

In this study, we evaluated the ability of an additional compound genetic marker recently described named DIP-STR, to serve as useful marker for ancestry inference. Originally, DIP-STRs were proposed as typing method for the characterization of DNA mixtures from two individuals present in very different proportions [29-33]. These mixtures are commonly encountered in forensic investigations during mixed trace analyses; in cases of peripheral blood DNA microchimerism during pregnancy or induced by solid organ transplant. Because of PCR amplification bias, the genetic identification of a DNA that contributes trace amounts to a mixed sample (minor DNA) represents a tremendous challenge. The DIP-STRs have the innovative feature of combining the analysis of a DIP (Deletion/Insertion Polymorphism) and a closely linked STR polymorphism. To target the analysis of the minor DNA contributor of the mixture, PCR primers overlap the deleted/inserted sequence (S or L) to produce allele-specific amplifications of one or two minor DIP-STR haplotypes comprising the DIP allele that is not shared with the major DNA. Allele-specific amplifications of DIP-STR

haplotypes enable the characterization a minor DNA in the presence of more than 1000-fold excess of a major DNA contributor.

Here we present the results of 23 DIP-STR markers typed in the global HGDP-CEPH reference samples. A comparative HGDP-CEPH analysis is shown using existing AIM SNP and AIM Indel marker sets.

Materials and Methods

DIP-STR marker selection

DIP-STRs were selected from three groups: 14 from the first two sets of DIP-STRs developed for the analysis of unbalanced DNA mixtures (Table 1). Three markers from the first set were not included because of the large amplicon size (over 600 bp) and one appeared not interesting based on balanced allele frequencies of the DIP across the major geographic regions as reported by data from the 1000 Genomes project. Five additional DIP-STRs were rescued from the preliminary marker selection of the study [33]. In this previous study, several markers were initially eliminated because of the negative results of the first amplification assay; here they produced positive results with newly designed PCR primers. Among these candidates, we selected those markers with larger DIP allele frequencies differences between continents using the 1000 Genomes dataset. Finally, four DIP-STRs were selected considering previously described sets of AIM Indels [12-14,34-39]. DIPs of interest were located near a repeated sequence, showed similar genotyping conditions and skewed global allele frequencies among continents.

Population samples

The CEPH Human Genome Diversity panel (CEPH-HGDP) contains 1,064 individuals from African, European, North African/Middle Eastern, Central-South

Asian, East Asian, Native American and Oceanian populations [40]. For all data analyses purposes we considered only 952 individuals (H952 subset) after exclusion of duplicates, first- and second-degree relatives [41]. Populations were combined into continental-based groups which have been previously established [16] with the following composite populations, sample sizes and labels: 6 African (105 AFR), 8 European (158 EUR), 4 North African/Middle Eastern (162 ME), 9 Central-South Asian (202 CSA), 17 East Asian (230 EAS), 2 Oceanian (28 OCE) and 5 Native American (64 NAM). For all subjects, blood cell samples were obtained according to protocols and informed-consent procedures approved by institutional review boards, and were labelled with an anonymous code number linked only to demographic information and sex.

Marker typing

DNA samples were genotyped for the 23 DIP-STRs and also for the 23 DIPs alone in order to further control for allele-specificity of the S- and L-DIP-STR amplifications. PCR reactions were performed in 20 μL final volume. This contained 1× PCR Buffer containing 1.5 mM $MgCl_2$ (Thermo Fisher Écublens, Switzerland), 250 μM dNTP (Thermo Fisher Écublens, Switzerland), 1.2 U AmpliTaq Gold DNA Polymerase (Thermo Fisher Écublens, Switzerland) and 0.5 ng DNA. Primers' sequences, quantities and multiplexes are indicated in Supplementary Table 1 and Supplementary Table 2. PCR thermal cycling conditions were: 5 min at 95°C, 1 min at 94°C, 1 min at annealing temperature specific to the markers set to be genotyped, 1 min at 72°C for a number of PCR cycles that also varied across multiplex and a final extension of 30 min at 72°C. Annealing temperatures and number of cycles are indicated in Supplementary Table 2.

PCR fragments were separated by capillary electrophoresis after adding 1 μL PCR amplicon to 8.5 μL deionized formamide HI-DI (Thermo Fisher Écublens, Switzerland)

and to 0.5 µL 600 LIZ size standard (Thermo Fisher Écublens, Switzerland). Capillary electrophoresis was performed using an ABI PRISM 3130*xl* Genetic Analyzer (Thermo Fisher Écublens, Switzerland) according to the manufacturer's instruction and analyzed using the GeneMapper® ID v3.2.1 software (Thermo Fisher Écublens, Switzerland), with a minimum peak height threshold of 50 RFU. The commercial DNA CEPH 1347-02 (Thermo Fisher Écublens, Switzerland) was added to two empty positions in each PCR plate as positive control of amplification and internal standard for allele calls, at least one empty well per plate was used a negative control of amplification. Markers information and genotypes are available at the HGDP-CEPH database (http://www.cephb.fr/en/hgdp_panel.php#basedonnees).

Data analysis

In order to assess the predictive value of the 23 DIP-STR marker set, cluster analysis was performed using the STRUCTURE program version 2.3.4 [42,43]. Runs consisted of 50 000 Markov Chain steps after a burn-in of length 50 000 with ten replicates for K=5 and K=7, using the *admixture* ancestry model and *correlated allele frequencies*. The DIP-STR allele names indicated the DIP variant, either S (deletion) or L (insertion) and the STR allele expressed in DNA fragment size, this last corresponds to the STR allele size that one would obtain using primers located around the repeated sequence (allele names can be found in Figure 1 and Supplementary Figure 1). For the STRUCTURE analysis DIP-STR alleles were recoded with serial numbers from 1 to *n* starting from the shortest L-DIP-STR to the longest S-DIP-STR. The bar plot was prepared using CLUMPAK (http://clumpak.tau.ac.il/).

To estimate the success rate in assigning the population of origin of each individual considering the genetic information of 23 DIP-STRs the likelihood-based approach

implemented by Phillips et al. [44] in the SNIPPER App Suite (http://mathgene.usc.es/snipper/) was used. With this tool, a single, unknown genetic profile can be compared to a set of references populations, the "training set". The software calculates individual maximum likelihoods estimates for the inclusion of the unknown sample into each reference population. A cross-validation has been performed using the frequency-based classifier in *Snipper* app suite. Each sample was tested in turn as unknown sample against the training set containing all the remaining samples. No marker deviated from the Hardy–Weinberg equilibrium and linkage disequilibrium. For comparative analysis, both STRUCTURE and *Snipper* based analyses were repeated for two previously published AIM markers sets, these include a 34 AIM SNP set [44,45] and a 46 AIM Indel set [37].

Results

Patterns of DIP-STR variability

The number of DIP-STR markers studied here is 23 (Table 1). Of the 368 alleles present more than once in the dataset, 27.5% appeared in all major regions represented, these are Africa, Europe, Middle East, Central-South Asia, East Asia, Oceania and Native America. Those exclusive to one region were 8.7%; region-specific alleles showed a median relative frequency of 1.4% in their region of occurrence.

As previously observed, Africa was the most variable region [46,47]. Number of alleles, and mean number of private alleles followed a common trend: they were highest in the African samples, were somewhat lower in Europeans and East Asians, and were lowest in Amerindians and Oceanians. This was especially true for 13 markers (Supplementary Figure 1). Note that Oceanians population size is about half the Native

Americans and between one fourth and one eight of the other major geographic regions. In Figure 1a is showed the allele frequency distribution of marker MID1013-D5S490 representative of the pattern described above. Conversely, seven markers showed a higher number of alleles outside Africa. For these markers the DIP variant is not polymorphic in Africa therefore all the DIP-STR haplotypes containing the DIP allele that probably appeared outside Africa, are missing in this group. Here as well, the number of observed alleles decreases with increasing distance from Eurasia. An example is reported in Figure 1b, marker rs112604544-STR.

Population clustering analysis

We assessed the ability of the DIP-STR genotypes to cluster the global HGDP-CEPH reference population samples applying the widely used STRUCTURE Bayesian grouping algorithm. Previous studies including several types of ancestry informative markers suggest that when limited numbers of markers are analyzed it is appropriate to aim to assign individuals to five major population groups in the first instance, these are Africans, Europeans, East Asians, Oceanians and Native Americans. To obtain the clearest pattern of group membership from STRUCTURE, the study population complexity was reduced by excluding geographically close populations such as Middle East and Central-South Asia. These are known to show higher misclassification rates since they occupy regions in the middle of a continuum of variability. The STRUCTURE results with the highest likelihood at K= 5 shows a clear pattern of five clusters corresponding to major geographical regions (Figure 2a). These results are comparable to currently used AIM markers sets comprising 34 AIM SNPs (Figure 2b) or 46 AIM Indels (Figure 2c). The results at K=7 including the complete HGDP-CEPH dataset show that although a “private” ancestry component is present in Middle East and

Central South Asia, clustering patterns are less distinct and Europe loses clear definition incorporating the additional inferred cluster as partial degrees of ancestry (Figure 3a). These data are comparable to the results obtained using the 34 AIM SNP set (Figure 3b), yet they show an improved clustering capacity of the European and Middle East groups when compared to the results obtained with the 46 AIM Indels (Figure 3c).

Individual ancestry assignment analysis

Snipper cross-validation classification success values for five and seven HGDP-CEPH reference samples obtained using the 23 DIP-STRs considered in this study are in agreement with the clustering obtained using the STRUCTURE classification algorithm. For the five group analysis the classification success rates are higher than 99% for all populations. These results are similar to those obtained with 34 AIM SNPs and 46 AIM Indels, with a somewhat better classification success rate for the East Asian group (Table 2). For the seven group analysis, samples with Eurasians origin were more difficult to classify, yet about 96% of Europeans 86% of Middle Easterns are correctly classified (Table 3). These values are lower when using 34 AIM SNPs (70% and 62%) and 46 AIM Indels (40% and 46%).

Separate STRUCTURE analysis of the 23 DIP set and the 23 STR set

The independent contribution to the clustering of the HGDP-CEPH populations of the 23 DIPs and the 23 STRs composing the haplotypes analyzed before, was investigated (Figure 4). This is possible because haplotypes are named based on the respective DIP and STR comprising alleles. The data at K=5 after excluding geographically close populations of Middle East and Central South Asia, show similar results for the DIPs and the STRs. The populations of Africa are distinguished from the other main geographical regions while part of Native Americans are clustered with the

Oceanians. The populations of Eurasia form a unique cluster with a high degree of noise and some indication of admixture with the Native Americans, especially when using the DIPs.

Discussion

The aim of this study was to explore the contribution to small-scale marker sets based biogeographic inference of DIP-STR haplotype markers. We hypothesized that the compound nature of DIP-STRs may represent an attractive feature not only for DNA mixture resolutions as we originally proposed, but also for studies of population structure. Briefly, each DIP-STR haplotype is provided with a slow and fast mutating variant that should confer higher biogeographic information compared to the use of each single type of polymorphism. Here, the survey of the HGDP-CEPH global reference samples with an initial set of 23 DIP-STRs allowed us to determine the relative value of our markers with respect to other validated AIM marker sets.

Overall, the clustering patterns observed with the STRUCTURE algorithm and the population assignments obtained with the Snipper program were in good correlation with each other as well as with worldwide population structure [8,12,15,19,37]. The prediction of bio-geographic ancestry was achieved for five major populations using a smaller set of markers (23 DIP-STRs *versus* 34 to 46 markers of validated AIM SNP and AIM Indel marker sets). Moreover, the seven group analysis shows that with these markers the global pattern is approaching seven clusters and some additional selected DIP-STRs may produce stable and reproducible distinction of the populations of Middle East and Central-South Asia within Eurasia. The discrimination of these groups still represents a challenge for most available AIM panels [7], as also indicated by our comparative

294 analysis using 34 AIM SNPs and 46 AIM Indels. However, according to Snipper cross-
295 validation success values the DIP-STRs show an improved success rate in assigning
296 individuals to populations of Europe and Middle East. It should be noted that the results
297 showed here, were obtained with DIP-STR markers not selected for distinguishing
298 continental or intra-continental structures. Yet, these results are comparable to those
299 obtained with larger sets of SNPs and Indels produced after refined selections using
300 allele frequency data from worldwide population surveys. Finally, the fact that the
301 obtained resolution is not due to single composing markers (either DIPs or STRs) with
302 no advantage of considering the combined information, is also showed by the
303 corresponding cluster analysis using the DIP marker set and the STR marker set,
304 separately.

305 It is generally accepted that identifying SNPs that can distinguish among four or
306 five continental groups of populations is not difficult [48]. Many different non-
307 overlapping sets of SNPs capable of inferring continental ancestry were described (see
308 references in the Introduction). However, previous studies showed the difficulty of
309 developing a single panel comprising a limited number of SNPs or Indels that is capable
310 of differentiating populations both globally and within regions. Note that, the HGDP
311 collection has the drawback of not sampling densely within each geographic region;
312 however, the use of a large number of markers showed the possibility of a sub-
313 continental estimation of bio-geographic ancestry.

314 To reach such a result with DIP-STR markers; first of all, they need to be selected
315 for ancestry purposes. Large whole genome sequencing data will provide the basis for
316 annotating novel DIP-STRs and estimating allele frequencies across populations from
317 long read data; alternatively, additional marker selecting criteria can be formulated

318 based on annotated lists of unphased DIP and STR markers. For example: a tentative
319 approach could be a marker selection based on DIP skewed allele frequencies among
320 large geographical regions (this would include a mixture of old and young DIP
321 mutations) linked to highly polymorphic STRs.

322 Finally, the value of a small ancestry panel is also measured based on the capacity
323 of analyzing trace amount of DNA with a cost-effective method easy to implement in
324 routine laboratories. Most of the DIP-STRs genotyped here were analyzed in short
325 fragments by standard PCR and capillary electrophoresis analysis and were validated
326 for forensic contact traces [32]. Yet, several multiplex were used to produce the data. This
327 is because: on one side, markers were progressively added to the study; on the other
328 side, the typing of 23 DIP-STRs conserving S- and the L-specific amplifications require
329 some developmental efforts. We plan to work on a compact multiplex method once
330 final DIP-STRs and minimum number of marker are identified.

331 As stated before, significant progress has been made in the research of ancestry
332 marker sets; therefore the effort that the development of AIM-DIP-STRs requires is
333 justified only by an advantage over existing methods. Besides the hope of providing
334 better (more refined and robust) resolution of bio-geographic ancestry that can be
335 assessed with simple and cost-effective methods, these markers have the attractive
336 feature of combining ancestry estimates and unbalanced DNA mixture resolution,
337 especially useful in forensics. Although, ancestry and identity DIP-STRs are may not to
338 be the same marker sets, one can apply DIP-STR markers to first, detect the minor DNA
339 of the trace sample; for example, the DNA of a man who sexually assaulted a woman,
340 retrieved from a gynecological sample. Second, if the DIP-STR profile does not find a
341 match in the list of suspects, additional ancestry DIP-STRs can be used as intelligence

tool to further guide the investigation. A caveat to this is that the ancestry inference in the context of mixture resolution would produce results for a reduced number of markers (those that contain DIP alleles unique to the minor DNA) and therefore the validation of redundant DIP-STRs may be required to have sufficient markers for ancestry resolution.

Our study provides another example of forensic markers capable of providing substantial biogeographic information. The fact that individual identifiability and population identifiability may be correlated has been observed before for forensic microsatellite markers [49] and it has been extensively studied by Algee-Hewitt and colleagues [50]. These authors identified in the high level of polymorphism the potential for high population identifiability. In the case of DIP-STRs the number of possible haplotypes formed by the combination of two markers is indeed much greater than the number of alleles at each individual locus, but the combination of slow- and fast-evolving loci is also expected to play a role.

In conclusion, our study demonstrates that DIP-STRs can provide a useful adjunct to AIM panels aiming at providing a better resolution and, in forensic science, at combining the function of mixture deconvolution to bio-geographic ancestry estimates. We showed here that continental regions can be readily distinguished, while more markers are necessary to improve the classification of closely related populations such as Eurasians.

This represents an exceptional result for the first evaluation of this set of marker that was not selected to contain information about ancestry. The research described here should be considered a pilot study to encourage more efforts on DIP-STRs marker

discovery and multiplex development. Finally, these data provide references of global DIP-STR allele frequencies including the set proposed for forensic casework analysis.

Acknowledgments This work was financially supported by funding from the University Center of Legal Medicine of the University Hospital of Lausanne and the Faculty of Biology and Medicine of the University of Lausanne (Pro-Femmes fellowship to Diana Hall).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Hellenthal G, Busby GB, Band G *et al*: A genetic atlas of human admixture history. *Science* 2014; **343:** 747-751.

2. Li JZ, Absher DM, Tang H *et al*: Worldwide human relationships inferred from genome-wide patterns of variation. *Science* 2008; **319:** 1100-1104.

3. Tishkoff SA, Kidd KK: Implications of biogeography of human populations for 'race' and medicine. *Nat Genet* 2004; **36:** S21-27.

4. Elhaik E, Tatarinova T, Chebotarev D *et al*: Geographic population structure analysis of worldwide human populations infers their biogeographical origins. *Nat Commun* 2014; **5:** 3513.

5. Kayser M, de Knijff P: Improving human forensics through advances in genetics, genomics and molecular biology. *Nature reviews Genetics* 2011; **12:** 179-192.

6. Phillips C, Prieto L, Fondevila M *et al*: Ancestry analysis in the 11-M Madrid bomb attack investigation. *PLoS One* 2009; **4:** e6583.

7. Phillips C: Forensic genetic analysis of bio-geographical ancestry. *Forensic science international Genetics* 2015; **18:** 49-65.

8. de la Puente M, Santos C, Fondevila M *et al*: The Global AIMs Nano set: A 31-plex SNaPshot assay of ancestry-informative SNPs. *Forensic science international Genetics* 2016; **22:** 81-88.

9. Pakstis AJ, Kang L, Liu L *et al*: Increasing the reference populations for the 55 AISNP panel: the need and benefits. *Int J Legal Med* 2017.

10. Phillips C, Parson W, Lundsberg B *et al*: Building a forensic ancestry panel from the ground up: The EUROFORGEN Global AIM-SNP set. *Forensic science international Genetics* 2014; **11:** 13-25.

11. Lao O, van Duijn K, Kersbergen P, de Knijff P, Kayser M: Proportioning whole-genome single-nucleotide-polymorphism diversity for the identification of geographic population structure and genetic ancestry. *Am J Hum Genet* 2006; **78:** 680-690.

12. Santos C, Phillips C, Oldoni F *et al*: Completion of a worldwide reference panel of samples for an ancestry informative Indel assay. *Forensic science international Genetics* 2015; **17:** 75-80.

13. Zaumsegel D, Rothschild MA, Schneider PM: A 21 marker insertion deletion polymorphism panel to study biogeographic ancestry. *Forensic science international Genetics* 2013; **7:** 305-312.

14. Yang N, Li HZ, Criswell LA *et al*: Examination of ancestry and ethnic affiliation using highly informative diallelic DNA markers: application to diverse and admixed populations and implications for clinical epidemiology and forensic medicine. *Human Genetics* 2005; **118:** 382-392.

15. Londin ER, Keller MA, Maista C *et al*: CoAIMs: a cost-effective panel of ancestry informative markers for determining continental origins. *PLoS One* 2010; **5:** e13443.

16. Rosenberg NA, Pritchard JK, Weber JL *et al*: Genetic structure of human populations. *Science* 2002; **298:** 2381-2385.

17. Halder I, Shriver M, Thomas M, Fernandez JR, Frudakis T: A panel of ancestry informative markers for estimating individual biogeographical ancestry and admixture from four continents: Utility and applications. *Human Mutation* 2008; **29:** 648-658.

18. Kosoy R, Nassir R, Tian C *et al*: Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America. *Hum Mutat* 2009; **30:** 69-78.

19. Kidd KK, Pakstis AJ, Speed WC *et al*: Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics. *Forensic science international Genetics* 2014; **12:** 215-224.

20. de Knijff P: Messages through bottlenecks: On the combined use of slow and fast evolving polymorphic markers on the human Y chromosome. *American Journal of Human Genetics* 2000; **67:** 1055-1061.

21. King TE, Parkin EJ, Swinfield G *et al*: Africans in Yorkshire? The deepest-rooting clade of the Y phylogeny within an English genealogy. *Eur J Hum Genet* 2007; **15:** 288-293.

22. Mountain JL, Knight A, Jobin M *et al*: SNPSTRs: Empirically derived, rapidly typed, autosomal Haplotypes for inference of population history and mutational processes. *Genome Research* 2002; **12:** 1766-1772.

23. Kidd KK, Speed WC, Pakstis AJ *et al*: Evaluating 130 microhaplotypes across a global set of 83 populations. *Forensic science international Genetics* 2017; **29:** 29-37.

24. Pakstis AJ, Fang R, Furtado MR, Kidd JR, Kidd KK: Mini-haplotypes as lineage informative SNPs and ancestry inference SNPs. *Eur J Hum Genet* 2012; **20:** 1148-1154.

25. Kidd KK, Pakstis AJ, Speed WC *et al*: Microhaplotype loci are a powerful new type of forensic marker. *Forensic Science International: Genetics Supplement Series* 2013; **4:** e123-e124.

26. Kidd JR, Friedlaender F, Pakstis AJ *et al*: Single nucleotide polymorphisms and haplotypes in Native American populations. *Am J Phys Anthropol* 2011; **146:** 495-502.

27. Schlebusch CM, Soodyall H: Extensive population structure in San, Khoe, and mixed ancestry populations from southern Africa revealed by 44 short 5-SNP haplotypes. *Hum Biol* 2012; **84:** 695-724.

28. Gattepaille LM, Jakobsson M: Combining markers into haplotypes can improve population structure inference. *Genetics* 2012; **190:** 159-174.

29. Castella V, Gervaix J, Hall D: DIP-STR: highly sensitive markers for the analysis of unbalanced genomic mixtures. *Hum Mutat* 2013; **34:** 644-654.

30. Cereda G, Biedermann A, Hall D, Taroni F: An investigation of the potential of DIP-STR markers for DNA mixture analyses. *Forensic science international Genetics* 2014; **11:** 229-240.

31. Cereda G, Biedermann A, Hall D, Taroni F: Object-oriented Bayesian networks for evaluating DIP-STR profiling results from unbalanced DNA mixtures. *Forensic science international Genetics* 2014; **8:** 159-169.

32. Oldoni F, Castella V, Grosjean F, Hall D: Sensitive DIP-STR markers for the analysis of unbalanced mixtures from "touch" DNA samples. *Forensic science international Genetics* 2017; **28:** 111-117.

33. Oldoni F, Castella V, Hall D: A novel set of DIP-STR markers for improved analysis of challenging DNA mixtures. *Forensic science international Genetics* 2015; **19:** 156-164.

34. Bastos-Rodrigues L, Pimenta JR, Pena SDJ: The genetic structure of human populations studied through short insertion-deletion polymorphisms. *Annals of Human Genetics* 2006; **70:** 658-665.

35. Francez PA, Ribeiro-Rodrigues EM, dos Santos SE: Allelic frequencies and statistical data obtained from 48 AIM INDEL loci in an admixed population from the Brazilian Amazon. *Forensic science international Genetics* 2012; **6:** 132-135.

36. Pereira R, Phillips C, Alves C, Amorim A, Carracedo A, Gusmao L: A new multiplex for human identification using insertion/deletion polymorphisms. *Electrophoresis* 2009; **30:** 3682-3690.

37. Pereira R, Phillips C, Pinto N *et al*: Straightforward inference of ancestry and admixture proportions through ancestry-informative insertion deletion multiplexing. *PLoS One* 2012; **7:** e29684.

38. Rosenberg NA, Mahajan S, Ramachandran S, Zhao CF, Pritchard JK, Feldman MW: Clines, clusters, and the effect of study design on the inference of human population structure. *Plos Genetics* 2005; **1:** 660-671.

39. Santos NPC, Ribeiro-Rodrigues EM, Ribeiro-dos-Santos AKC *et al*: Assessing Individual Interethnic Admixture and Population Substructure Using a 48-Insertion-Deletion (INSEL) Ancestry-Informative Marker (AIM) Panel. *Human Mutation* 2010; **31:** 184-190.

40. Cann HM, de Toma C, Cazes L *et al*: A human genome diversity cell line panel. *Science* 2002; **296:** 261-262.

41. Rosenberg NA: Standardized subsets of the HGDP-CEPH Human Genome Diversity Cell Line Panel, accounting for atypical and duplicated samples and pairs of close relatives. *Ann Hum Genet* 2006; **70:** 841-847.

42. Falush D, Stephens M, Pritchard JK: Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 2003; **164:** 1567-1587.

43. Pritchard JK, Stephens M, Donnelly P: Inference of population structure using multilocus genotype data. *Genetics* 2000; **155:** 945-959.

44. Phillips C, Salas A, Sanchez JJ *et al*: Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs. *Forensic Science International-Genetics* 2007; **1:** 273-280.

45. Fondevila M, Phillips C, Santos C *et al*: Revision of the SNPforID 34-plex forensic ancestry test: Assay enhancements, standard reference sample genotypes and extended population studies. *Forensic science international Genetics* 2013; **7:** 63-74.

46. Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL: High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 1994; **368:** 455-457.

47. Calafell F, Shuster A, Speed WC, Kidd JR, Kidd KK: Short tandem repeat polymorphism evolution in humans. *Eur J Hum Genet* 1998; **6:** 38-49.

48. Soundararajan U, Yun L, Shi M, Kidd KK: Minimal SNP overlap among multiple panels of ancestry informative markers argues for more international collaboration. *Forensic science international Genetics* 2016; **23:** 25-32.

49. Phillips C, Fernandez-Formoso L, Garcia-Magarinos M *et al*: Analysis of global variability in 15 established and 5 new European Standard Set (ESS) STRs using the CEPH human genome diversity panel. *Forensic science international Genetics* 2011; **5:** 155-169.

50. Algee-Hewitt BF, Edge MD, Kim J, Li JZ, Rosenberg NA: Individual Identifiability Predicts Population Identifiability in Forensic Microsatellite Markers. *Curr Biol* 2016; **26:** 935-942.

Titles and legends to the figures

Figure 1 Allele frequency distributions of two representative DIP-STR markers estimated for the seven groups of Africa (AFR), Europe (EUR), Middle East (ME), Central-South Asia (CSA), East Asians (EAS), Oceania (OCE) and Native America (NAM). (a) MID1013-D4S490. (b) rs112604544-STR

Figure 2 STRUCTURE analyses of the HGDP-CEPH reference populations at K = 5, after exclusion of Middle Eastern and Central-South Asian groups. Analyses were computed using the *admixture* ancestry model and *correlated allele frequencies*. (a) Results obtained using 23 DIP-STRs. (b) 34 AIM SNPs. (c) 46 AIM Indels.

Figure 3 STRUCTURE analyses of the HGDP-CEPH reference populations at K = 7. Analyses were computed using the *admixture* ancestry model and *correlated allele frequencies*. (a) Results obtained using 23 DIP-STRs. (b) 34 AIM SNPs. (c) 46 AIM Indels.

Figure 4 STRUCTURE analyses of the HGDP-CEPH reference populations at K = 5, after exclusion of Middle Eastern and Central-South Asian groups using 23 markers. (a) Results obtained using only the 23 DIP genotypes of the DIP-STRs. (b) Results obtained using only the 23 STRs genotypes of the DIP-STRs.

Supporting information

Supplementary Table 1 DIP-STR primers

Supplementary Table 2 Marker information, PCR primer concentration, number of

cycles and multiplex groups

Supplementary Table 3 STRUCTURE individual ancestry proportions of HGDP-CEPH individuals analyzed for three marker sets: 23 DIP-STRs, 34 AIM SNPs and 46 AIM Indels at K=5 and K=7

Supplementary Figure 1 DIP-STRs haplotype frequencies for seven HGDP-CEPH major population groups

Table 1 DIP-STR marker list

DIP-STR	Chr. Loc.	DIP S/L sequence	STR repeat	DIP-STR size (bases)	Reference
MID1013[a]-D5S490	5q23.2	-/CCAG	GT	299-345	Castella et al. 2013
rs11277790-D10S530	10q25.1	-/TCCAACT	GT	340-371	Castella et al. 2013
rs60194384-D15S1514	15q26.2	-/TCTTAA	TATC	283-325	Castella et al. 2013
rs66679498-D2S342	2q32.3	-/CCAACTTTCTCCTAC	CA	331-359	Castella et al. 2013
rs35032587-STR	15q26.1	-/TATT	AGAT	239-271	Oldoni et al. 2015
rs142543564-STR	2q34	-/TACT	ATAA	210-238	Oldoni et al. 2015
rs34212659-STR	7p14.1	-/AGG	TGAA	182-199	Oldoni et al. 2015
rs112604544-STR	1q25.3	-/TTTAA	TTCC	134-204	Oldoni et al. 2015
rs111478323-STR	2p25.3	-/GAGA	TTTA	229-265	Oldoni et al. 2015
rs146332920-STR	9q31.3	-/AGG	TAAA	179-207	Oldoni et al. 2015
rs71070706-STR	1p12	-/TGT	AAAG	212-264	Oldoni et al. 2015
rs72406828-STR	4q21.3	-/ATTG	AATTT	178-250	Oldoni et al. 2015
rs145423446-STR	16p13.2	-/AGTC	GATA	230-256	Oldoni et al. 2015
rs2308142-STR	20p13	-/ATT	TTA	205-238	Oldoni et al. 2015
rs71725104-STR	13q31.3	-/ATAG	AAAT	211-235	
rs72534187-STR	5p13.1	-/ACAGGCC	ATAG	208-236	
rs139592446-STR	2q24.2	-/ACTTAGTC	CATC	154-174	
rs36194161-STR	2q32.1	-/CTC	TTTA	138-178	
rs138331044-STR	1p12	-/CATATGC	AGAT	266-302	
MID473[a]-STR	6q16.1	-/TTACATTT	AGGA	179-227	Francez et al. 2012 [b] Rosemberg et al. 2005 [b] Santos et al. 2010 [b]
MID2538[a]-STR	15q25.3	-/TGTT	AC	299-311	Santos et al. 2015 [b]
MID2824[a]-STR	11p13	-/AGGACT	AAAC	197-222	Zaumsegel et al. 2013 [b]
MID73[a]-STR	22q12.3	-/GAA	CCACT	362-442	Rosemberg et al. 2005 [b]

[a] Marker name is from the Marshfield database and corresponds to rs1611095, rs140762, rs3054057, rs11278940, rs16365, respectively

[b] References for the DIP marker only

Table 2 *Snipper* cross-validation classification success values for five HGDP-CEPH major population groups using 23 DIP-STRs, 34 AIM SNPs and 46 AIM Indels.

Population of origin		Population assignment				
		AFR	EUR	EAS	OCE	NAM
23 DIP-STRs						
	AFR	**100.00%**	-	-	-	-
	EUR	-	**99.37%**	0.63%	-	-
	EAS	-	0.43%	**99.57%**	-	-
	OCE	-	-	-	**100.00%**	-
	NAM	-	-	-	-	**100.00%**
34 AIM SNPs						
	AFR	**100.00%**	-	-	-	-
	EUR	-	**99.37%**	**-**	-	0.63%
	EAS	-	0.43%	**94.71%**	0.44%	4.85%
	OCE	-	-	-	**100.00%**	-
	NAM	-	-	-	-	**100.00%**
46 AIM Indels						
	AFR	**100.00%**	-	-	-	-
	EUR	-	**100%**	**-**	-	-
	EAS	-	0.44%	**96.07%**	2.62%	0.87%
	OCE	-	-	-	**100.00%**	-
	NAM	-	-	-	-	**100.00%**

The most likely major component of ancestry was considered independently of the admixture proportions

Populations are AFR-Africa, EUR-Europe, EAS-East Asia, OCE-Oceania and NAM- Native America

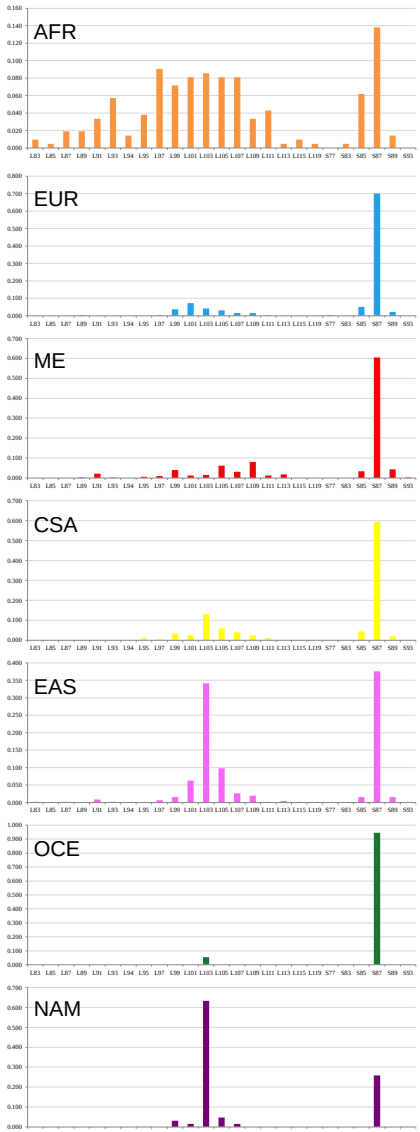
Table 3 *Snipper* cross-validation classification success values for seven HGDP-CEPH major population groups using 23 DIP-STRs, 34 AIM SNPs and 46 AIM Indels.

Population of origin		Population assignment						
		AFR	EUR	ME	CSA	EAS	OCE	NAM
23 DIP-STRs								
	AFR	**100.00%**	-	-	-	-	-	-
	EUR	-	**95.57%**	1.90%	1.90%	0.63%	-	-
	ME	1.23%	5.56%	**86.42%**	6.17%	0.62%	-	-
	CSA	-	7.92%	7.43%	**82.18%**	2.48%	-	-
	EAS	-	0.43%	-	0.87%	**98.70%**	-	-
	OCE	-	-	-	-	-	**100.00%**	-
	NAM	-	-	-	-	-	-	**100.00%**
34 AIM SNPs								
	AFR	**100.00%**	-	-	-	-	-	-
	EUR	-	**69.62%**	25.95%	4.43%	-	-	-
	ME	0.62%	1.85%	**61.73%**	35.19%	0.62%	-	-
	CSA	-	4.95%	3.47%	**84.16%**	3.96%	1.49%	1.98%
	EAS	-	-	0.43%	3.04%	**86.96%**	1.74%	7.83%
	OCE	-	-	-	-	-	**96.43%**	3.57%
	NAM	-	-	-	-	-	-	**100.00%**
46 AIM Indels								
	AFR	**100.00%**	-	-	-	-	-	-
	EUR	-	**40.51%**	39.87%	19.62%	-	-	-
	ME	1.23%	0.61%	**46.01%**	52.15%	-	-	-
	CSA	-	-	0.50%	**90.10%**	7.43%	1.49%	0.50%
	EAS	-	-	-	-	**96.94%**	0.87%	2.18%
	OCE	-	-	-	-	-	**100.00%**	-
	NAM	-	-	-	1.56%	-	-	**98.44%**

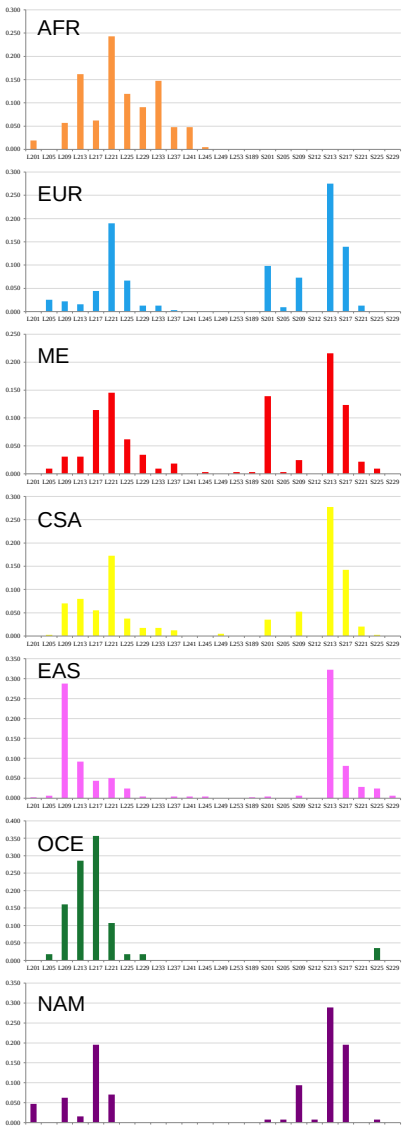
The most likely major component of ancestry was considered independently of the admixture proportions

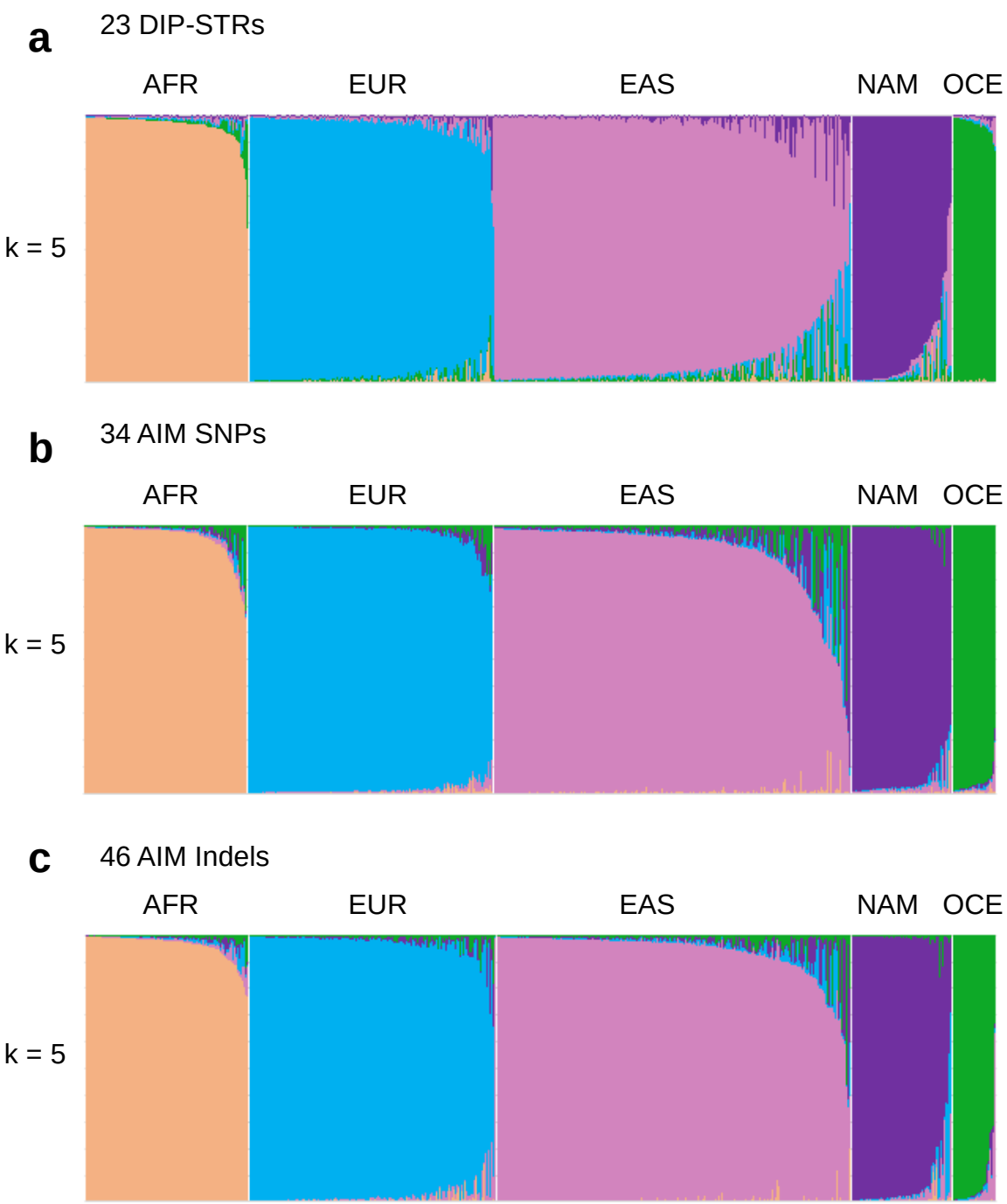
Populations are AFR-Africa, EUR-Europe, ME-Middle East, CSA-Central-South Asia, EAS-East Asia, OCE-Oceania and NAM- Native America

a

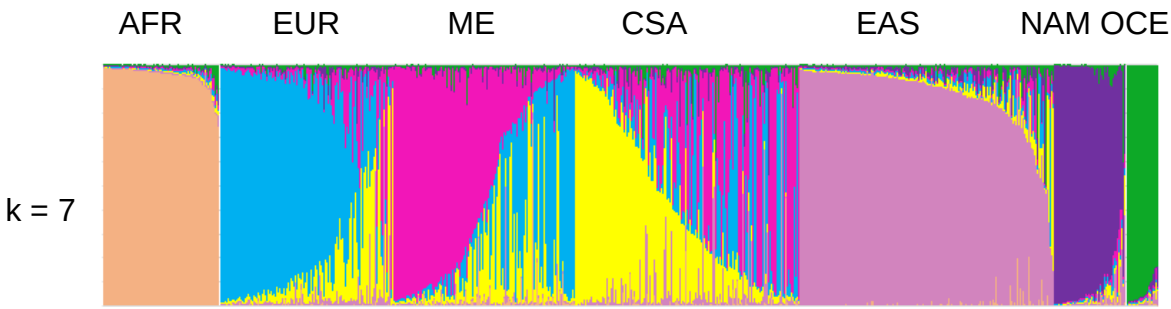


b

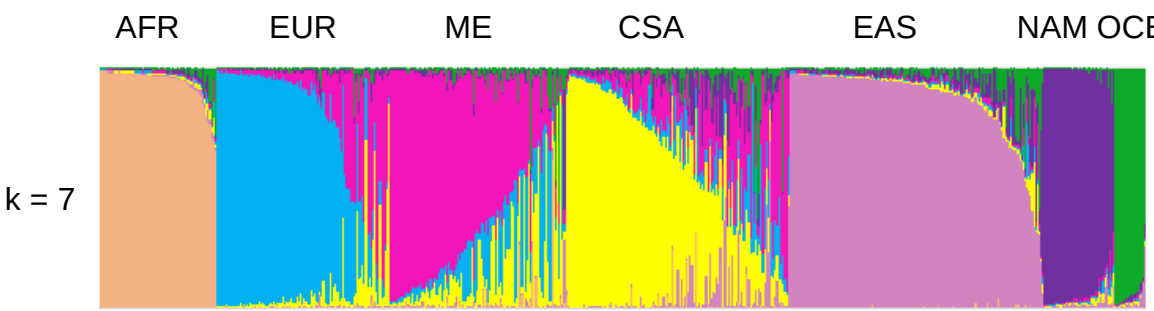



a
23 DIP-STRs
AFR
EUR
EAS
NAM
OCE
k = 5
b
34 AIM SNPs
AFR
EUR
EAS
NAM
OCE
k = 5
c
46 AIM Indels
AFR
EUR
EAS
NAM
OCE
k = 5

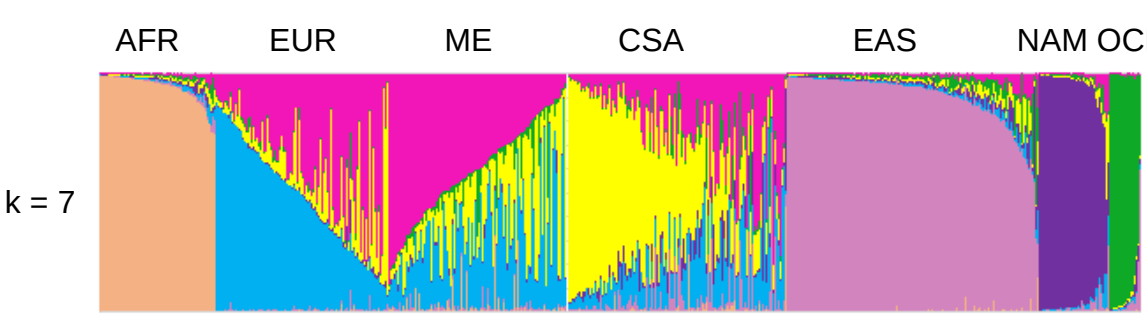
a 23 DIP-STRs

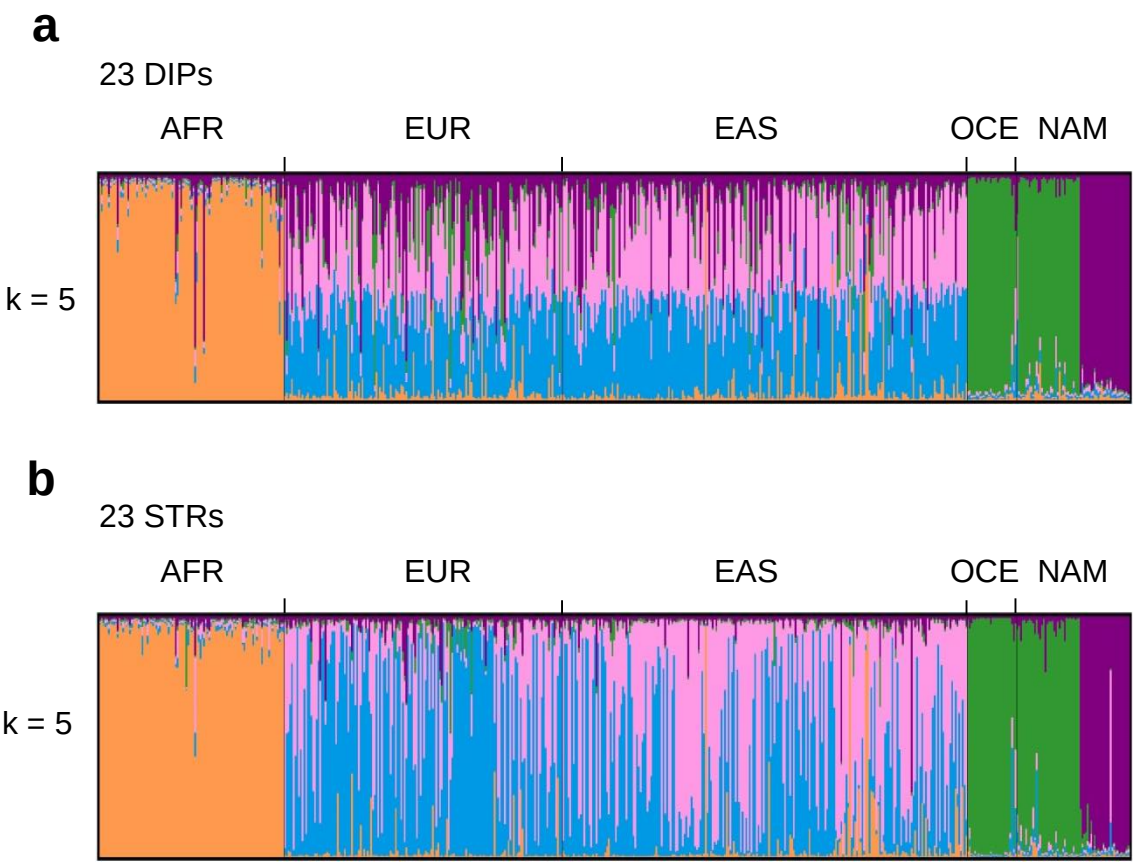


b 34 AIM SNPs



c 46 AIM Indels




a
23 DIPs
AFR
EUR
EAS
OCE
NAM
k = 5
b
23 STRs
AFR
EUR
EAS
OCE
NAM
k = 5

Supplementary Table 1 DIP-STR primers

Marker	DIP primers	STR primers	S-DIP allele-specific primer L-DIP allele-specific primer DIP-STR reverse primer
rs71725104-STR	*TTTTTGCCACAAAACTAAATT GCAGCTCCTGCAAAAATTTC	*GTGTGGTGGTAGCTGGGACT TTTTGTGGCAAAAACTTTGG	CCTTTCCTTCTATTCTTGCTTTAT.TTT CCTTTCCTTCTATTCTTGCTTTATCTA *GTGTGGTGGTAGCTGGGACT
rs72534187-STR	*GCTCATGCAATTGATCAAACC GCTTTGGGCTTGATACAGAAA	*GGTATGCAATCTATCCTGATGTGA TGCATGAGCCAATTTATCTGA	TCTCTGGTTCTCTAGCTTGTAGAT.TAC CTCTAGCTTGTAGATGGCCTGTTA *AGGCCAAAATTGACATTATAGTTTA
rs139592446-STR	*ACTGGGAAAACTATGAGAAACAAA CCTTCCTTTTATCTTCTATCACACA	*GATTTAGGAGGGGATGTGGT TTTCCCAGTGTCTGCTCAA	CCATTTTGCCCCACTAGT.TC TGCCCCACTAGTGACTAAGTTC *TAGCCTTCTGCCCAAACATC
rs36194161-STR	*CCAAGATTGTGCCACTGC TCACCAGGTCTTGGGTATGTT	*AAAACATACCCAAGACCTGGTG TTTAAACCTCTTTCCTGCTTGC	AAATATTACTAGTTGTATTAGTCTGTT.ACG TATTACTAGTTGTATTAGTCTGTTCTCAC *TGCAGTGAGCAGGGTGAC
rs138331044-STR	*ACAATCGCTGCTCACTGAAG GCCGAAGCAGGTGTATTTCT	*AGCACATAGCAGGCACTAGC GCGATTGTGCCACTACACAG	ATTAGCTGGGCTTAGTG.CCTGT TAGCTGGGCTTAGTGGCATATG *GCACTAGCTGTTAGTTCCTTTTCTG
MID473[a]-STR	*AAATGTTAAGCCTCCCCTGTC CCACTGACAGCAACAACCAA	*CCTTGTCTTGGTTGTTGCTG TGCAGGCAGATTTTAAAGGAA	TGGGCTTTCTA.TTACATTTTTAGC TGGGCTTTCTATTACATTTTTACAT *TGCAGGCAGATTTTAAAGGAA
MID2538[a] -STR	*AACAATCTTGGCACCCATTT GCTCGCAAAGTAGGCAAGTT	*TCATTACCTTCTCTGCATTGGA GTGCCAAGATTGTTGGTGTG	GTTCAAAATCACAATCACTCA.TTT TCAAAATCACAATCACTCAAACA *TGGAATCACTCATTACCTTCTCTG
MID2824[a] -STR	*TGTTCCACTTCTGCCATGTG TCTAGTGGGGGTTTGCAGAG	*ACTTGGGAGGCTGAGACAGA CACATGGCAGAAGTGGAACA	TCCAAGATGAGCACTG.GGC CCTCCAAGATGAGCACTGAGTC *CCAGCCTGGCAACAGAGTA
MID73[a] -STR	*TGTGTTTCTAAGGAGCGCTGT CACAGTGAGGAGAAGGAAGGA	*CCATTCTCTCCTTCCTTCTCC CCTGGTGCCAGAGCACAT	CATACTCAGAACTGCCTT.GAAAAG CATACTCAGAACTGCCTTGAAGAA *GCACATGGCTCTTTAATACACTG

[a] Marker name is from the Marshfield database and corresponds to rs140762, rs3054057, rs11278940, rs16365, respectively

* Fluorescent labeled primers. In the last column dots indicate the insertion/deletion point and underlined is the inserted sequence. Primers for markers previously published were not changed

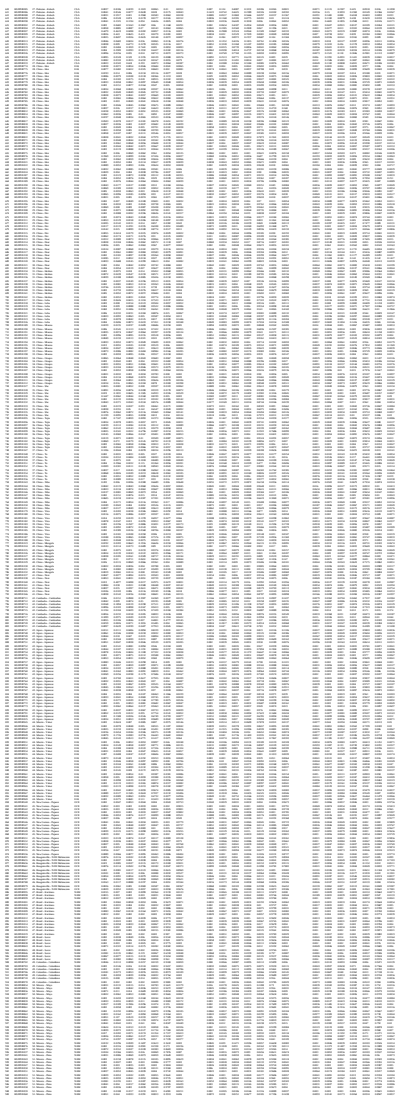
Supplementary Table 2 Marker information, PCR primer concentration, number of cycles and multiplex groups

Marker	dbSNP ID of the DIP marker	Genome hg38 position of the DIP marker	Genbank ID of the STR marker	Genome hg38 position of the STR marker
MID1013-D5S490	rs1611095:insCCAG	chr5:g.127507347_127507348insCCAG	Z23637 [GT]	chr5:127507078-127507107
rs2308142-STR	rs2308142:insATT	chr20:g.3484768_3484769insATT	G08041 [TTA]	chr20:3484910-3484948
rs11277790-D10S530	rs11277790:delTCCAACT	chr10:g.105760904_105760910delTCCAACT	Z23432 [GT]	chr10:105761139-105761184
rs112604544-STR	rs112604544:insTTTAA	chr1:g.185716220_185716221insTTTAA	Simple Tandem Repeat [TTCC]	chr1:185716115-185716167
rs34212659-STR	rs34212659:delAGG	chr7:g.38563258_38563260delAGG	Simple Tandem Repeat [TGAA]	chr7:38563129-38563173
rs145423446-STR	rs145423446:delAGTC	chr16:g.8094810_8094813delAGTC	Simple Tandem Repeat [GATA]	chr16:8094861-8094992
rs111478323-STR	rs111478323:insGAGA	chr2:g.2619471_2619472insGAGA	Simple Tandem Repeat [TTTA]	chr2:2619638-2619680
rs146332920-STR	rs146332920:delAGG	chr9:g.111781629_111781631delAGG	Simple Tandem Repeat [TAAA]	chr9:111781725-111781753
rs35032587-STR	rs35032587:insTATT	chr15:g.93295596_93295597insTATT	Simple Tandem Repeat [AGAT]	chr15:93295413-93295457
rs142543564-STR	rs142543564:delTACT	chr2:g.211170402_211170405delTACT	Simple Tandem Repeat [ATAA]	chr2:211170264-211170317
rs72406828-STR	rs72406828:delATTG	chr4:g.86985935_86985938delATTG	Simple Tandem Repeat [AATTT]	chr4:86986026-86986089
rs71070706-STR	rs71070706:delTGT	chr1:g.117804313_117804315delTGT	Simple Tandem Repeat [AAAG]	chr1:117804149-117804266
MID473-STR	rs140762:delTTACATTT	chr6:g.94529411_94529418delTTACATTT	Simple Tandem Repeat [AGGA]	chr6:94529500-94529560
MID2538-STR	rs3054057:delTGTT	chr15:g.85467307_85467310delTGTT	Simple Tandem Repeat [AC]	chr15:85467076-85467101
MID2824-STR	rs11278940:delAGGACT	chr11:g.36029406_36029411delAGGACT	Simple Tandem Repeat [AAAC]	chr11:36029245-36029271
rs60194384-D15S1514	rs60194384:delTCTTAA	chr15:g.95010636_95010641delTCTTAA	G10630 [TATC]	chr15:95010830-95010878
rs66679498-D2S342	rs66679498:delCCAACTTTCTCCTAC	chr2:g.195019736_195019750delCCAACTTTCTCCTA	Z23993 [CA]	chr2:195019569-195019613
rs139592446-STR	rs139592446:delACTTAGTC	chr2:g.162477942_162477949delACTTAGTC	Simple Tandem Repeat [CATC]	chr2:162477813-162477859
rs71725104-STR	rs71725104:delATAG	chr13:g.93633710_93633713delATAG	Simple Tandem Repeat [AAAT]	chr13:93633636-93633677
rs36194161-STR	rs36194161:delCTC	chr2:g.187252184_187252186delCTC	Simple Tandem Repeat [TTTA]	chr2:187252243-187252287
rs72534187-STR	rs72534187:delACAGGCC	chr5:g.42202012_42202018delACAGGCC	Simple Tandem Repeat [ATAG]	chr5:42201874-42201943
rs138331044-STR	rs138331044:delCATATGC	chr1:g.119135915_119135921delCATATGC	G07791 [AGAT]	chr1:119135695-119135762
MID73-STR	rs16365:delGAA	chr22:g.34308579_34308581delGAA	Simple Tandem Repeat [CCATC]	chr22:34308746-34308839

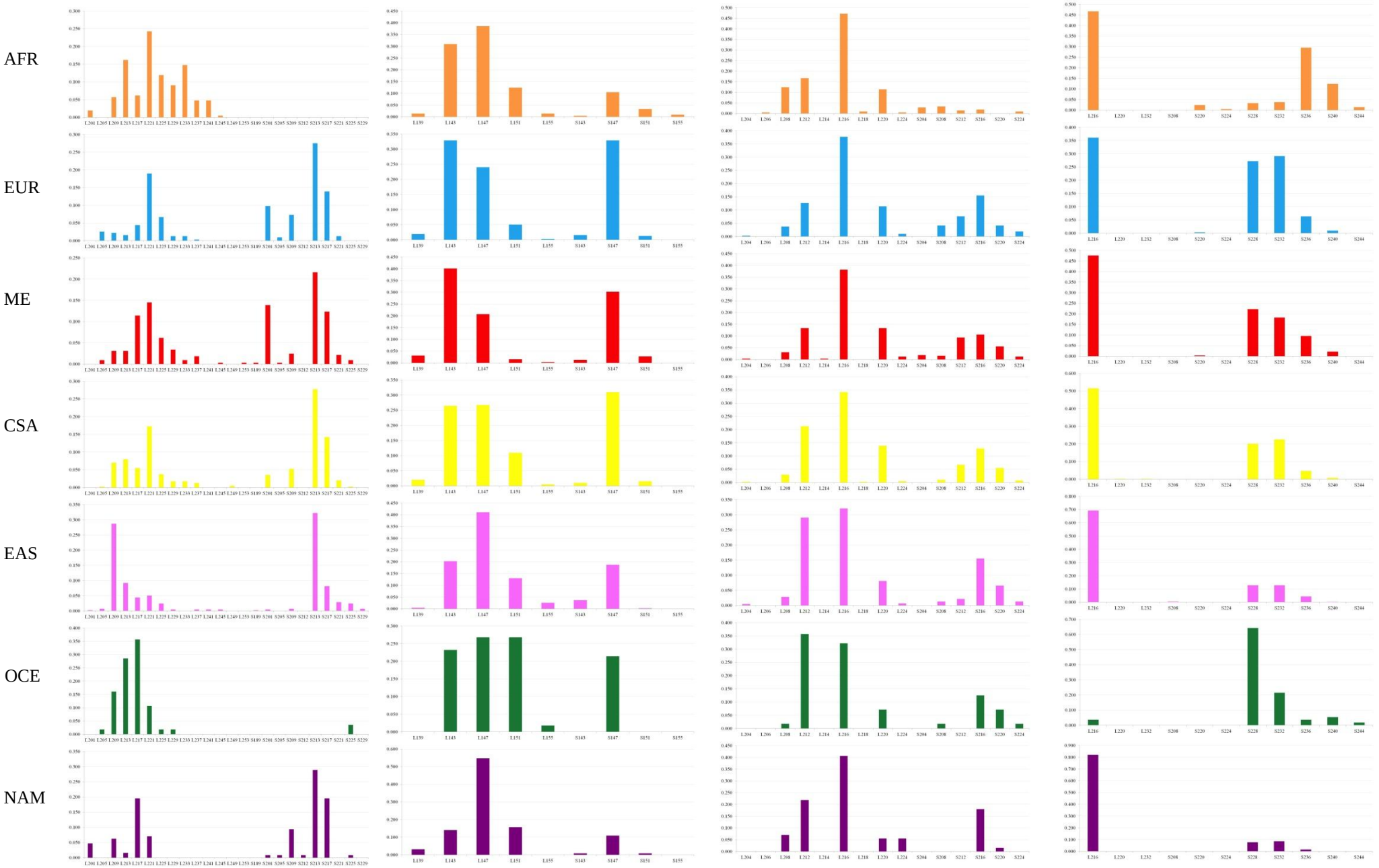
[a]Annealing temperatures and number of cycles are the same for the S- and L-specific DIP-STR amplifications

Supplementary Table 2 (continued)

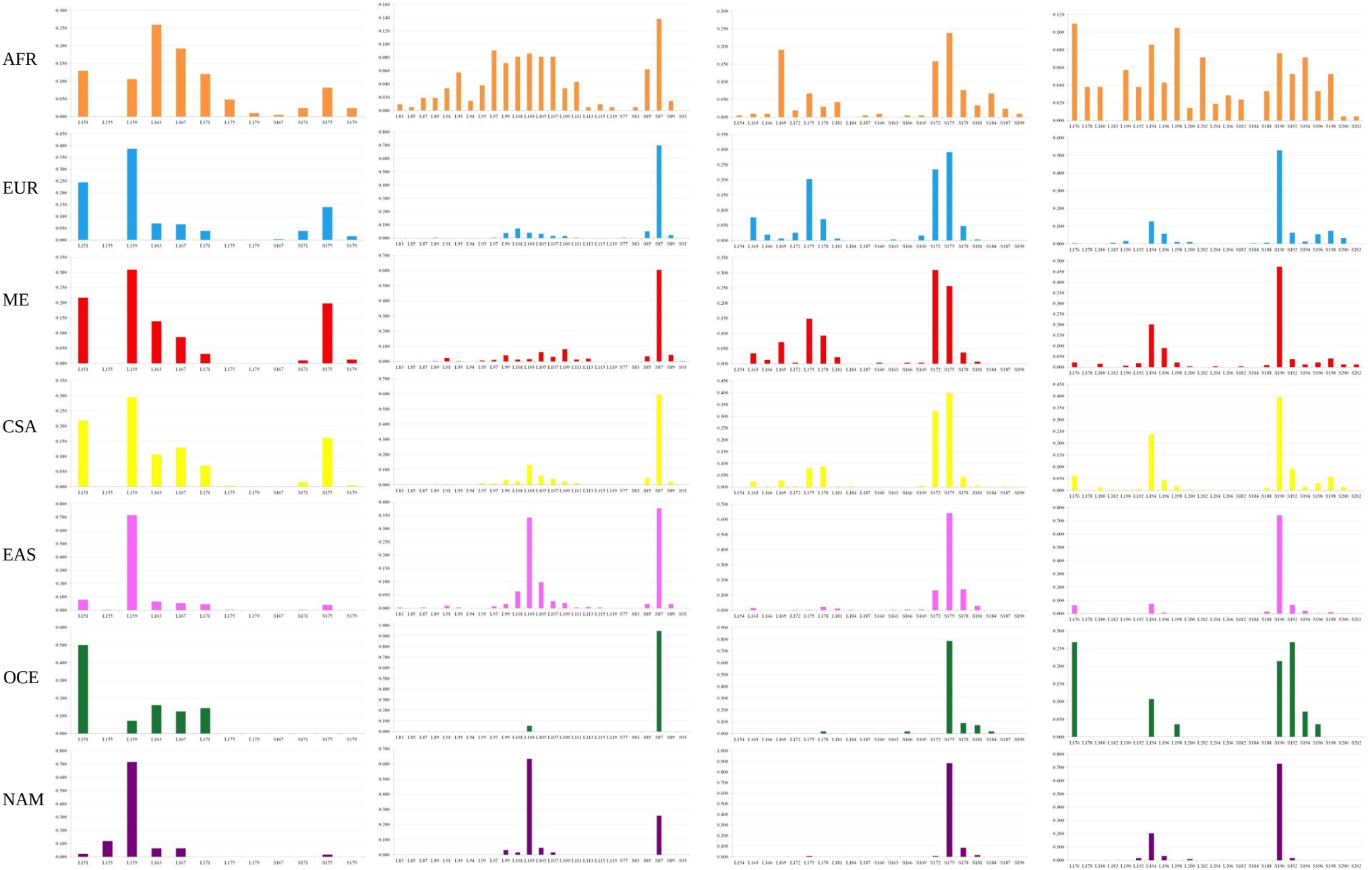
		Primers DIP	Annealing			Primers S-DIP-STR		Primers L-DIP-STR	Annealing[a]	
Marker	multiplex group	concentration (nM)	temperature (°C)	N cycles	multiplex group	concentration (nM)	multiplex group	concentration (nM)	temperature (°C)	N cycles[a]
MID1013-D5S490	1	100	52	28	6	100	5	100	52	34
rs2308142-STR	1	200	52	28	5	100	6	100	52	34
rs11277790-D10S530	1	100	52	28	5	100	6	100	52	34
rs112604544-STR	2	100	55	34	7	100	8	150	55	34
rs34212659-STR	2	100	55	34	7	100	8	100	55	34
rs145423446-STR	2	100	55	34	7	100	8	100	55	34
rs111478323-STR	2	50	55	34	7	100	8	100	55	34
rs146332920-STR	2	50	55	34	7	100	8	100	55	34
rs35032587-STR	2	200	55	34	9	100	10	100	55	34
rs142543564-STR	2	100	55	34	9	100	10	100	55	34
rs72406828-STR	2	150	55	34	9	100	10	100	55	34
rs71070706-STR	2	50	55	34	9	200	10	200	55	34
MID473-STR	3	100	55	30	11	50	12	50	55	34
MID2538-STR	3	100	55	30	11	200	12	200	55	34
MID2824-STR	3	400	55	30	11	50	12	50	55	34
rs60194384-D15S1514	1	200	52	28	11	200	12	200	55	34
rs66679498-D2S342	1	200	52	28	11	100	12	500	55	34
rs139592446-STR	4	150	55	34	13	50	14	37	58	34
rs71725104-STR	4	150	55	34	13	75	14	50	58	34
rs36194161-STR	4	150	55	34	13	100	14	150	58	34
rs72534187-STR	4	75	55	34	13	100	14	100	58	34
rs138331044-STR	4	120	55	34	13	100	14	500	58	34
MID73-STR	3	400	55	30	15	200	16	100	59	34



Supplementary Fig. 1 DIP-STRs haplotype frequencies in seven major population groups

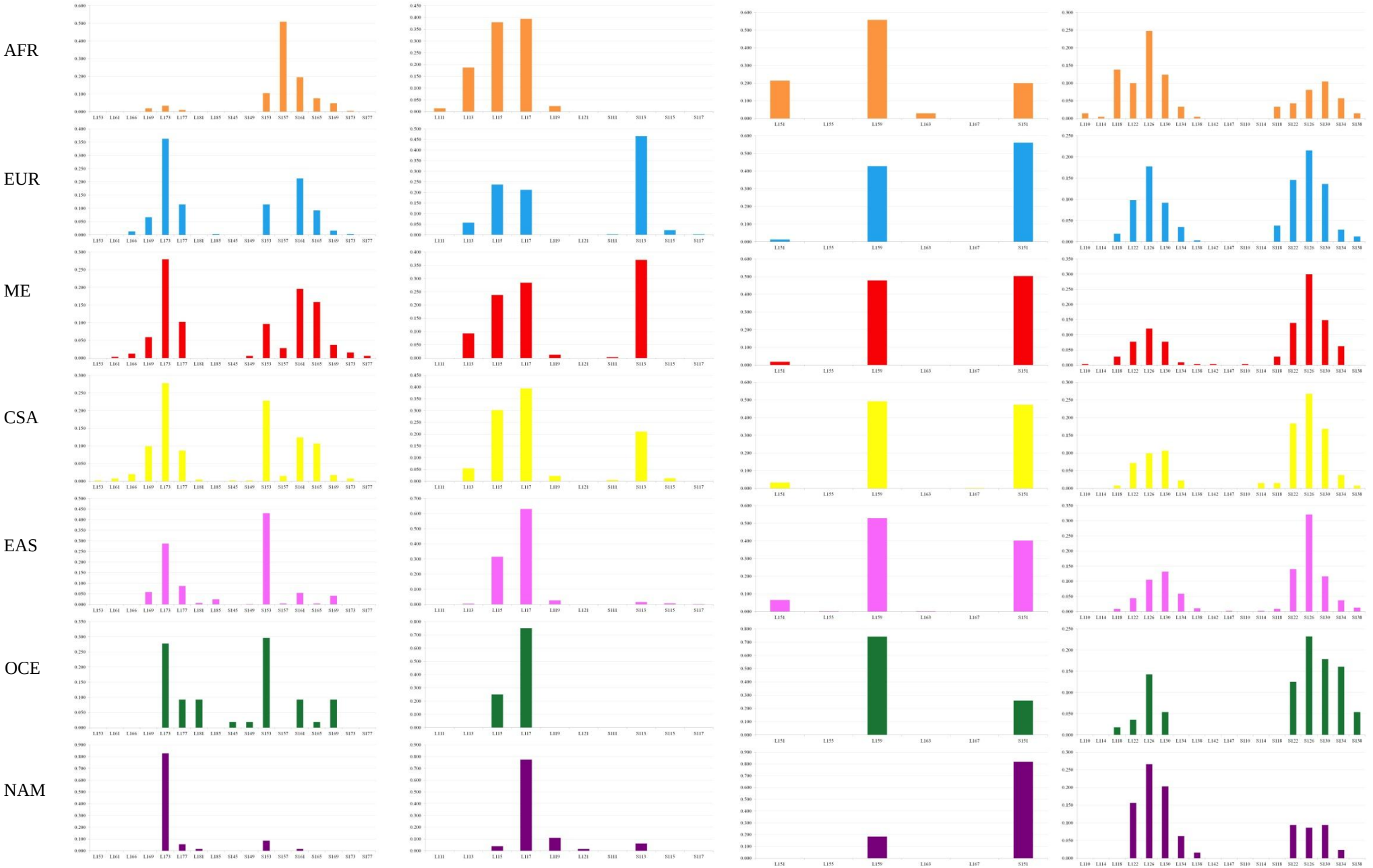


Supplementary Fig. 1 (continued)

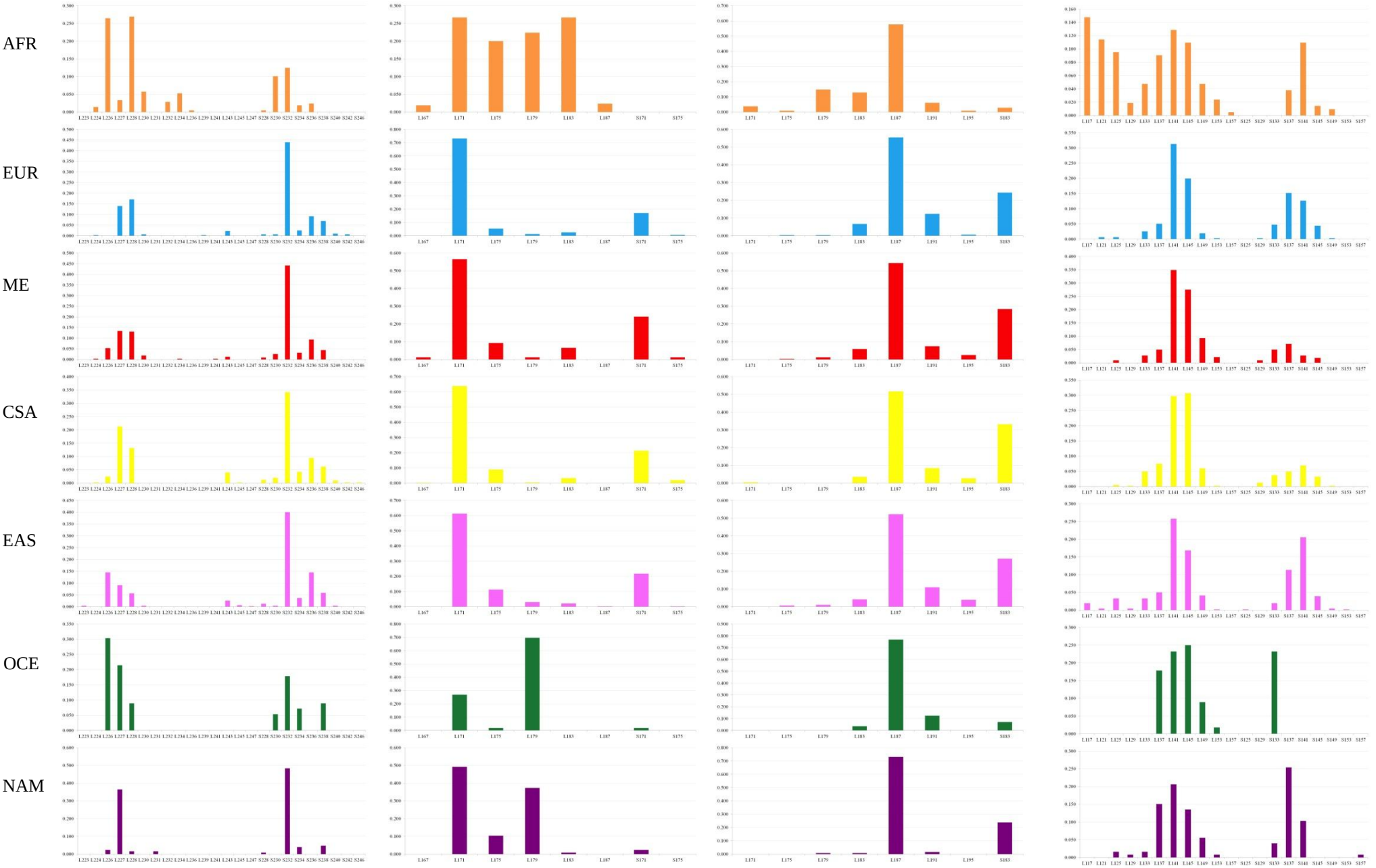


Supplementary Fig. 1 (continued)

Supplementary Fig. 1 (continued)



Supplementary Fig. 1 (continued)



Supplementary Fig. 1 (continued)