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ntRoot: Computational Inference of Human Ancestry at Scale from Genomic Data

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Abstract

Ancestry information is essential to large cohort studies, yet it is often unavailable or inconsistently measured. For studies with a genome sequencing component, current ancestry prediction approaches are hindered by high computational demands and complex input requirements. We present ntRoot, a computationally-lightweight method for inferring human super-population-level ancestry from whole genome assemblies or raw short or long sequencing data. Utilizing an alignment-free variant detection framework, ntRoot employs a succinct Bloom filter data structure to efficiently query diverse genomic data inputs. Demonstrated on over 600 human genome sequencing datasets—including complete genomes, draft assemblies, and over 280 independently-generated datasets—ntRoot accurately predicts geographic labels, a descriptor of human populations, and shows high concordance with traditional methods such as ADMIXTURE ($R^2 = 0.9567$) when predicting ancestry fractions. It achieves these predictions within 30 minutes for complete and draft genomes and within 1 hour and 15 minutes for 30X sequencing data, using a maximum of 13GB and 68GB of RAM, respectively. ntRoot offers both global and local ancestry inference, delivering high-resolution predictions across genomic loci. This paradigm fills a critical gap in cohort studies by enabling rapid, resource-efficient, and accurate ancestry inference at scale, advancing the characterization of continental-level ancestry in the genomic era.

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Introduction

Our ancestry information, encoded within our DNA, is crucial for genomic studies. Individuals

from different ancestries may have a different genetic makeup and associated disease risk

factors, yet association studies do not always take ancestry into consideration or simply leave it

as a confounding variable, either because that information is costly to compute, is based on

incomplete genotype data, or is not reliably measured. On a population scale, the HapMap

Project (1) was the first to profile common patterns of variation between individuals.

Subsequently, the 1000 Genomes Project (1kGP) (2) and the Simons Genome Diversity Project

(SGDP) (3) catalogued human genomic variation across the whole genomes of healthy

individuals from various populations, to generate a baseline for the study of population genetics.

Ancestry admixture inference can be divided into Local Ancestry Inference (LAI) and

Global Ancestry Inference (GAI). LAI is the estimation of ancestry along a DNA sequence. This

paradigm is based on the fact that during chromosomal crossover, proximal regions of DNA are

carried together. Hence, even if an individual's ancestors were admixed generations ago, DNA

fragments may be sourced from single ancestry. While GAI offers a genome-wide summary,

LAI provides higher resolution ancestry inferences across genomic loci (4).

Most existing computational approaches developed for ancestry inference are based on

single nucleotide variants (SNVs), leveraging the availability of population-scale genotype data

from HapMap (1) and 1kGP projects (5). Tools for GAI analysis include the widely-applied

model-based STRUCTURE program (6), principal component analysis-based utilities Rye (7)

and EIGENSTRAT (8), and ADMIXTURE for likelihood model-based estimation of ancestry in

unrelated individuals (9). HAPAA (10) and HAPMIX (11), both designed for LAI, use Hidden

Markov Models (HMMs) and explicitly incorporate linkage disequilibrium (LD), the degree of

correlation between allelic SNVs. RFMix (12) is an HMM-based admixture model with

conditional random forest that leverages phase and LD information (13) for both GAI and LAI,

and SNVstory (14) uses machine learning-based models to compute GAI from sequencing data.

Additional LAI methods include G-Nomix, FLARE, and SALAI-Net (15–17). While these

utilities have their strengths, large-scale studies and routine applications may benefit from more

flexible genomic input and smoother execution to better align with current data throughputs and

computational demands in the genomic era.

Here, we present ntRoot, a scalable and flexible method for super-population-level

(continental, as defined by 1kGP) LAI and GAI analysis from whole genome assemblies or raw

genome sequencing datasets. It uses a sequence alignment-free genome variant detection

framework (18) that employs a succinct Bloom filter (19) data structure, which contains a

reduced sequence representation. This filter is first used to identify SNVs and then leverages

integrated variant call sets (IVC) from 1kGP to estimate continental ancestry based on

established 1kGP labels (Figs. 1a, and S1). Bloom filters are probabilistic data structures

commonly used in bioin-formatics software because they offer a low-memory alternative, ena-

bling scalable applications in the era of large sequencing datasets (20,21). The ntRoot paradigm

offers both global and local ancestry inference, providing high-resolution predictions across

genomic loci. In our manuscript, the term "ancestry" does not refer to genetic ancestry. Instead,

it refers to group labels of geographical locations used to describe a population, as established by

the 1kGP and in accordance to The National Academies of Sciences, Engineering, and Medicine

guidelines (22).

Methods

We adapted the base-editing utility ntEdit (18) for SNV prediction and ancestry inference (Figs.

1a and S1). This mode leverages k-mer (sequence words of length k)-based analysis to identify

alternate base possibilities across genomic datasets, bypassing the error-checking step otherwise

used for genome polishing in ntEdit. As with the polishing mode, the SNV calling feature of

ntEdit requires a primary Bloom filter (BF) built from k-mers of a user-specified length k,

derived from a sequencing data input. After BF construction, each and every k-mer of the

provided reference sequence is queried, 5' to 3', and the last, 3'-most base is permutated to

identify potential alternate bases at that position (Fig. S1). Alternate bases are reported in a

variant call format (VCF) file when their observed frequency surpasses a user-defined threshold.

The method does not explicitly rely on paired-end read information or alignment-based

positional context, focusing on k-mer presence/absence from the BF instead (more details

below).

After cross-referencing the putative SNVs identified with the 1kGP IVC (23,24), we

average the SNV allele frequency (AF) within each of the five continental super-populations

labels, globally for the genome under scrutiny, and locally for genomic windows (details below).

We also keep an independent tally of non-zero AF SNVs at both local (per genomic window of a

certain size, referred to as *tile* hereafter) and global (whole genome) levels. We rank the super-

populations separately for each tile and the genome using those combined metrics, as an

indicator of the most likely ancestry. We note that ntRoot can also predict ancestry from a user

input VCF file generated from other sources (i.e., not just ntEdit; Fig. 1a), but this feature is not

presented in the current study.

Single nucleotide variant detection with ntEdit v2

The functionality of ntEdit was extended to include single nucleotide variant detection. In this

mode, each genomic base of the human reference genome (GRCh38), represented by individual

k-mers, is interrogated in turn using the k-mer representation from the individual of interest,

stored in a BF. Reference k-mers and their constituent overlapping k-mers (e.g., a 25-mer

contains 25 overlapping k-mers) query the BF for presence/absence. Putative SNVs are recorded

when alternate 3'-end A, T, C, or G base positions on the original k-mer show sufficient support.

Specifically, at any given position in the genome, each k-mer variant and its associated

overlapping k-mers are queried, while shifting to the next k-mer by j bases. Once k/j k-mers have

been queried, a possible SNV is recorded after assessing the proportion of successful Bloom

filter k-mer hits (k_{ct}/j) and confirming that it meets or exceeds the user-defined $-Y$ parameter (set

to 0.55 or 55% by default). Putative SNVs are then cross-referenced with integrated variant

callsets from the 1kGP, marking the concordant positions in an output VCF file (Fig. S1).

Fig. 1. Ancestry inference from genomic data. **a**, Schematic of the ntRoot ancestry inference workflow using whole genome sequencing (WGS) or assembly (WGA) data. Using ntEdit in SNV mode (ntEdit-SNV), each genomic base of the human genome reference GRCh38 queries a Bloom filter built from an

individual WGS or WGA data with either ntHits or btllib, respectively; Identified SNVs are cross-

referenced with the 1kGP IVC *(23,24)*. Optionally, ntRoot can take a third-party VCF input (dashed lines)

to cross-reference the 1kGP integrated variant call set for ancestry predictions (not presented in this

study). Human ancestry composition estimates derived from LAI on **b**, the 266 WGS 1kGP validation set

grouped by 5 super-populations (1kGP continental ancestry labels AMR:American; AFR:African;

EUR:European; SAS:South Asian; EAS:East Asian) and 26 populations (1kGP labels CLM:Colombian;

MXL:Mexican-American; PEL:Peruvian; PUR:Puerto Rican; ACB:African-Caribbean; ASW:African-

American South West; ESN:Esan; GWD:Gambian; LWK:Luhya; MSL:Mende; YRI:Yoruba; CEU:CEPH

(Centre d'Étude du Polymorphisme Humain – Utah); FIN:Finnish; GBR:British; IBS:Spanish;

TSI:Tuscan; BEB:Bengali; GIH:Gujarati; ITU:Indian; PJL:Punjabi; STU:Sri Lankan; CDX:Dai Chinese;

CHB:Han Chinese; CHS:Southern Han Chinese; JPT:Japanese; KHV:Kinh Vietnamese), and **c**, 279

SGDP WGS discovery set from 129 distinct populations **d**, shown in the global context (using 1kGP

continental ancestry labels), with inset regions zoomed-in.

Ancestry inference workflow

On whole genome sequencing (WGS) read datasets, the ntRoot workflow (driven by the python

script, ntroot --reads) launches ntEdit variant calling, followed by the ancestry inference step.

The ntEdit pipeline first executes ntHits (v1.0.2, user-defined parameter k=55,

https://github.com/bcgsc/ntHits), which outputs a BF (19) (BF) of robust (non-error) read k-

mers. ntEdit (v2.0.0, https://github.com/bcgsc/ntEdit) loads the generated BF and, using the

human genome (hg) reference GRCh38, interrogates every k-mer it comprises in turn, querying

the BF for presence/absence as per above to generate the variants in VCF format. ntRoot loads a

succinct version of the 1kGP integrated variant call sets where reported SNVs have an allele

frequency (AF) of 1% or more in at least one human super-population label (EAS, AFR, EUR,

SAS or AMR) and the SNVs reported by ntEdit are cross-referenced against the 1kGP call set

input VCF. On genome sequence assemblies (WGA) and other 1X representation of an

individual's genome, ntRoot builds a BF using the btllib common code library (25) (ntroot --

genome). The remaining steps in WGA follow the same process as WGS (Fig. 1a). Once the

ntEdit execution has completed, the ntEdit output VCF file is parsed, summing and averaging the

variant AF for each of the five super-populations while tracking the number and rate of non-zero

(termed nz in [1] and [2]) AF SNVs over all cross-referenced SNVs. Separate tallies are kept for

the whole genome and for each 5 Mbp tile (user-defined parameter --tile, default 5 Mbp). Global

(GAI) and local (LAI) ancestry inference scores are calculated as:

163
$$Scores = Average\ AF\ x\ nz\ SNV\ rate \qquad [1]$$

nz: non-zero

SNV: single nucleotide variant

AF: SNV allele frequency

Where Average AF=(Sum of AF for each SNV) / (Total number of SNVs) for a given label

for the whole genome and locally, for each tile. For each super-population label, the non-zero

SNV rate is defined as:

171
$$nz\ SNV\ rate_{label} = \frac{nz\ AF\ SNV\ count_{label}}{total\ nz\ AF\ SNV\ count} \qquad [2]$$

173   The GAI and LAI scores are bounded within the range [0, 1], with 0 and 1 representing lowest

174   and highest confidence, respectively. The highest-scoring ancestry is reported for the former

175   (GAI), globally, and the associated super-population label assigned to each tile for the latter

176   (LAI), locally. When each tile has been assigned, the LAI super-population fraction for each

177   label is reported as:

178
$$LAI\_fraction_{label} = \frac{\sum_{i=1}^{num\_tiles} length(tile_i)\ if\ tile_{i_{label}} == label}{\sum_{i=1}^{num\_tiles} length(tile_i)} \qquad [3]$$

The GAI and LAI scores are output in a tab-separated variable (TSV) output file (_ancestry-

predictions_tile<tile size>.tsv). When the --lai option is specified, ntRoot will additionally output

the ancestry predictions and scores per tile (_ancestry-predictions-tile-resolution_tile<tile

size>.tsv).

Data

In our study we tested ntRoot on over 600 data sets, which we broadly separated into whole

genome sequence (WGS) and whole genome assembly (WGA). The WGS set included 3

different cohorts: **validation**, **discovery** and **benchmark**. The WGS validation and benchmark

sets consisted of 266 and 100 1kGP sequence datasets, respectively and were used to test the

accuracy and compute performance of the method, respectively. The WGS discovery dataset

(n=279) is independent from 1kGP and was sequenced as part of the Simons Genome Diversity

Project (SGDP). The WGA sets comprised complete and assembled genomes in draft stages

from both independent (e.g., HuRef, KOREF, CN1) and 1kGP sources (e.g., HG02055 and

HG002). Details on the datasets, software commands and run parameters are included in Table

S1 and Supplementary Methods.

Circular genome representations

For all aforementioned experiments and for each human super-population, we tracked tile

assignments and SNV density in TSV files for all non-zero AF SNVs reported by ntRoot and

cross-referenced with the 1kGP integrated variant call set. That information was then plotted in

R using circlize (26). All scripts developed to support our study are available on zenodo

(10.5281/zenodo.10869033).

204 *Benchmarking*

205 ntRoot was mainly (WGA and 1kGP validation set) benchmarked on a server with 144 Intel(R)

206 Xeon(R) Gold 6150 2.70GHz CPUs with 2.1TB RAM, using 48 threads. Additionally, we used a

207 server-class system with 144 Intel(R) Xeon(R) Gold 6254 3.1 GhZ CPUs with 2.9 TB RAM for

208 processing the large SGDP WGS data volumes and benchmarking SNVstory and ADMIXTURE.

209 We extracted the wall clock run time and peak memory from the output of the UNIX time

210 command preceding all ntRoot, SNVstory and ADMIXTURE processes.

212 **Results and Discussion**

213 To benchmark the performance of ntRoot, we derived super-population-level (label-based

214 continental/geographic) ancestry for a random subset of the 1kGP whole genome sequencing

215 (WGS) dataset (27) organized into the 26 human populations as defined by the 1kGP (WGS

216 validation set, Fig. S2, Table S2, n=266, 10-12 per population) and observed congruent ancestry

217 inferences for all 266 individuals compared to 1kGP assigned labels when derived globally (Fig.

218 S3, Tables S2-S4). The ancestry composition was inferred locally (i.e., LAI) using 5Mbp or

219 2Mbp tiles (Table S5), and the estimated super-population contributions were reported for the

220 entire genomes of the 266 and 279 WGS 1kGP and SGDP datasets, respectively (Fig. 1b-d, Fig.

221 S4, Table S6). We note that, based on LAI, the ancestry compositions of Americans (Fig. 1b,

222 AMR), Europeans (EUR) and South Asians (SAS) are more diverse than are those of Africans

223 (AFR) and East Asians (EAS), consistent with historical accounts of population movement (28).

224 Inferring super-population ancestries locally (i.e., LAI) is information-rich (Fig. 2) but we also

225 caution about deriving a GAI label from LAI (i.e., largest pie chart slice) because, out of the 266

226 1kGP WGS validation datasets, one (CLM:Columbian, 0.4%) and three labels (1 CLM, 2

227 PUR:Puerto Rican, 1.1%) are inconsistent with the highest super-population fraction, when using

Fig. 2. Circular genome representations of ancestry inference using WGA for **a,** European HuRef (Levy *et al.*, 2007) and HuRefPrime haplotypes, **b,** Han Chinese CN1 (Yang *et al.*, 2023) (mat:maternal, pat:paternal) haplotypes and **c,** simulated HuRef/CN1 diploid rearrangement. The outer track shows ntRoot local ancestry assignments (LAI) along each chromosome using 5Mbp tiles (AMR:American; AFR:African; EUR:European; SAS:South Asian; EAS:East Asian), with each subsequent inner track showing the corresponding SNV density for each super-population. Inner pie charts report the predicted global ancestry composition (GAI).

5Mbp vs. 2Mbp tiles, respectively (Table S5). Interestingly, all four incongruent samples represent individuals from the Americas, where population admixture has occurred (28) and hence, genetic ancestry is likely less homogeneous. This is the case for HG01243, a Puerto Rican individual with a recently documented (29) mixed, but primarily African (AFR) ancestry, originally assigned an American (AMR) label by the 1kGP consortium.

ADMIXTURE and ntRoot GAI and ancestry fractions for this individual are consistent and align with the revised and recently published AFR ancestry assignment (29) (Fig. S5, Tables S7-S8).

ntRoot LAI using the chromosome-scale diploid HuRef (30) (Fig. 2a), CN1 (31) (Fig. 2b)

and haploid KOREF (32) (Fig. S6) genome assemblies of three individuals revealed prevalent

ancestry based on their highest composition (EUR:61%, EAS:95%, EAS:95%, respectively),

consistent with both the highest GAI scores, and the published European (30), Chinese (31) and

Korean (32) ancestry for each (Tables S7-S8). Furthermore, ntRoot correctly inferred EAS and

EUR as the two most prevalent super-population ancestries from synthetic diploid genome

admixtures of HuRef and CN1 (Fig. 2c, Table S8).

Using orthogonal datasets distinct from the 1kGP data, WGS-based SGDP results (WGS

discovery set, Table S6) and WGA-based GAI/LAI results (Tables S7 and S8, respectively)

independently validate our approach. The SGDP discovery data set uses slightly different

geographic names compared to 1kGP, but despite this and the fact that we are using 1kGP

integrated variant call VCFs to analyze the continental-level composition of SGDP, the location

and ancestry fraction is congruent (Fig. 1d). Further, the full SGDP set comprises multiple

individuals from the same populations, and the ntRoot predictions are consistent between these

biological replicates (Table S6).

Unlike existing tools, ntRoot can infer ancestries directly from a genome assembly, even

when it is in a draft stage (Fig. S7). Processing haploid WGA with ntRoot averaged 29m and

consumed at most 13 GB of RAM, while it ran in under 1h36m requiring at most 68 GB of

RAM, on average, on 279 SGDP WGS datasets (22–87-fold genome coverage, average 44-fold).

Despite varying WGS sequencing coverage, ntRoot produced robust predictions, using as little as

22X coverage (Table S6). A more controlled sequencing coverage titration experiment using

Illumina WGS data from the HG002 (also known as GM24385 and huAA53E0) cell line derived

from an Ashkenazi Jewish individual of primarily European ancestry reveals that ntRoot's

predictions remain robust even at 12.5-fold coverage. The LAI EUR fraction, which represents

the GAI and the largest ancestry fraction, shows a delta of 2.62% between full coverage

(44.83%) and 12.5-fold coverage (42.21%), demonstrating the stability of ntRoot's ancestry

predictions at reduced sequencing depths (Table S10). Further, the predictions appear to be

sequencing data type-agnostic, as long-read WGS data from Illumina, PacBio (HiFi), and Oxford

Nanopore Technologies (ONT, V14 kit) instruments yield consistent ntRoot predictions of

ancestry fractions for HG002. The largest percentage variation observed is 0.7%, seen in both the

LAI AFR fraction (Illumina vs. ONT) and the LAI EUR fraction (Illumina vs. ONT). This

consistency is also observed in KOREF, where both Illumina and PacBio WGS data yield nearly

identical ancestry fraction predictions. For example, the LAI EAS fraction, which represents the

GAI and the largest ancestry fraction for KOREF, is 97.72% for Illumina and 97.90% for

PacBio, with the LAI AFR, AMR, and EUR fractions showing 100% consistency across both

platforms (Tables S1, S7-S8). ntRoot ancestry predictions are influenced by the parameter k,

with the GAI being accurately inferred at higher k values (e.g., k45-k70). However, as k

decreases, the fraction compositions can fluctuate. This is expected, as smaller k values generally

increase the recall of ancestry-discriminant SNVs, but at the cost of precision. Using the Genome

in a Bottle SNV benchmarks for HG002 (33), we found k=55 to be a Pareto-optimal choice,

representing a good balance between sensitivity (0.8051) and precision (0.8262) for identifying

ancestry-discriminant SNVs (Figs. S8 and Table S11). Based on these findings, we recommend

running ntRoot with the default or higher k values on all datasets.

Compared to SNVstory (14), one of the more recent GAI-based utility that was shown to

have more consistent performance on continental and subcontinental ancestry inference tasks

compared to RFMix and ADMIXTURE, ntRoot has the same or similar accuracy as SNVstory

and ADMIXTURE (100% and 99% concordance with 1kGP labels, respectively) while running

~4X and ~250X faster (1h09m vs. 4h25m vs. 273h42m, for ntRoot, SNVstory and

ADMIXTURE) and with 1.3-6.7X less compute memory when inferring the ancestry of 100

1kGP individuals from WGS data (WGS benchmark set, Tables S9, S12-S13). We note that

while SNVstory outputs probabilities for GAI predictions—often 0.99 or 1 on the WGS

benchmark set—these values do not provide insights into ancestry composition. In contrast,

ntRoot and ADMIXTURE offer more detailed ancestry fractions, which are useful for

characterizing populations and individuals with significant ancestry admixtures (Fig. S9, Table

S12). Moreover, ntRoot demonstrates high concordance with ADMIXTURE overall (Fig. S10a,

$R^2 = 0.9567$) and across each ancestry (Fig. S10b), with R^2 values ranging from 0.9237 (AMR) to

0.9974 (AFR). Additionally, a mean squared error (MSE) analysis further supports this

concordance, with an overall MSE of 0.78%, and individual MSE values for each ancestry as

follows: EAS – 0.44%, SAS – 0.70%, AMR – 1.19%, AFR – 0.06%, and EUR – 1.48%. We

note, however, that ntRoot's LAI-based fraction is different from the fine-scale genetic structure

analysis previously reported (34) and, in its current implementation, does not have the resolution

to characterize genetic ancestry at that level. Because ntRoot cross-references the SNVs

identified with an integrated variant call set collated from 2,709 1kGP individuals (23,24), exact

ancestry compositions are not expected. This is especially true given that the continental (and

population) labels have been assigned by 1kGP based on the geographical location where the

original 1kGP samples were taken/where the individuals lived at the time of collection, and that

the information that ntRoot uses to cross-reference its SNVs is built on a relatively limited

number (n=2,709) of those individuals. Given a human population size of eight billion in mid-

November 2022 (35) and that large human migrations have taken place in the past 10,000 years,

2,709 individuals are not expected to represent the full breadth of nucleotide variations in

humans. We also stress that ntRoot is designed to predict the likely continental (super-

population)-level ancestry based on pre-assigned labels, which may or may not be representative

of true genetic ancestry. To mitigate these limitations, potential strategies include providing

ntRoot with an IVC built from other or additional sources (e.g., the Genome Aggregation

Database - gnomAD or The International Genome Sample Resource - IGSR), ensuring it is

compiled from more comprehensive and diverse reference panels (36,37).

The ntRoot framework automates each step via a driver script, markedly simplifying the

execution of ancestry prediction pipelines compared to the often convoluted workflows of other

modern predictors (Table S13). It builds on top of the sequence alignment-free ntEdit paradigm

(18), for identifying and cross-referencing sequence variants in whole genome sequencing and

whole genome assembly datasets with an efficient use of computational resources. It

accomplishes the feat by using Bloom filters generated from either ntHits (38) (in WGS mode)

or btllib (25) (in WGA mode). Bloom filters, widely used in bioinformatics applications and the

genomics realm (21,39), are popular, succinct probabilistic data structures that accommodate

large datasets with a light compute memory footprint, as demonstrated herein. In previous work

(18), we showed how Bloom filter false positive rates do not affect downstream ntEdit analyses.

This is because ntEdit requires k-mer redundancy at each base for its nucleotide base

identification. Further, ntRoot cross-references the variants it identifies (or inputs) with

integrated variant call sets, which only looks at a subset of human variations characterized and

curated by third parties (23,24). As observed with ntRoot predictions from WGA, even a 1X

genome representation is sufficient to accurately predict continental-level ancestry. We also find

that, in a typical ntRoot run that uses either WGA or WGS as input, roughly 2-3M SNVs are

cross-referenced with the 1kGP integrated variant call sets and used to derive adequate ancestry

inferences (Table S7; SNV count column).

Conclusion

With its streamlined, memory-efficient, fast, and easy-to-execute workflow, ntRoot generates

GAI and LAI-based admixture profiles that provide detailed insights into population genetics.

We anticipate that ntRoot will broadly facilitate geographic-level ancestry inference, offering

reliable and objective demographic information from the sequencing data of modern

technologies. By enabling rapid and accurate pedigree inference at scale, ntRoot addresses a

critical gap in cohort studies and holds promise for advancing super-population-level ancestry

predictions for association studies in the genomic era.

Data and Code Availability

The data used in this study is summarized in Supplementary Material, Table S1.

ntRoot is freely available on GitHub (https://github.com/bcgsc/ntroot).

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Supporting information captions

Supplementary Methods

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results on representative 1kGP whole-genome sequencing datasets (n=266, WGS validation

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Supplementary Table S3. **Continental, super-population level summary of ntRoot GAI**

results on representative 1kGP whole-genome sequencing datasets (n=266, WGS validation

dataset), compared to their Assigned Label Counts

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