

# Legacy Data Confounds Modern Genomics Studies

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**Abstract** In investigating a population genetics signal, we noticed some discrepancies between the Japanese samples from the 1000 Genomes Project (1kGP) and a more recent high quality dataset. We found that the variants causing the signal were only present in individuals with low average sequencing quality. We identified 625  $p < 10^{-8}$  and 1048  $p < 10^{-6}$  single nucleotide polymorphisms (SNPs) that were associated to quality in the 1kGP dataset Japanese individuals. These variants are present in nearly all of the populations from the 1kGP. We then turned our attention to the rest of the 1000 Genomes populations and saw that there were similar batch effects in many of these populations. Some of these variants are being imputed onto genotype data and reach genome wide significance in recent publications. [\\*update values for all populations\\*](#)

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## Introduction

### Reference Cohorts

The last 5 years has seen an increase in the number of individuals genotyped through private companies or medical research cohorts. Larger sample sizes allow researchers to identify finer resolution statistically significant differences between groups of individuals. Mutations associated to a disease can be identified by comparing the genomes of healthy individuals to those afflicted by a disease. The mutations commonly found in patients and rarely in controls might be associated to the disease in question. However, demonstrating that these mutations are biologically relevant can be difficult. Especially with the increasing size of cohorts, spurious associations are increasingly becoming an issue. For this reason, careful consideration must be taken when including individuals from different ancestral origins in these association studies. Benign mutations at high frequencies in one populations might be exceedingly rare in another population. Therefore, population wide differences in mutations must be included as covariates to avoid spurious associations.

### Imputation

Genome wide associations using large cohorts have lead to developments in the identification of rare genetic diseases as well as the risk prediction to certain types of cancer and diseases. Despite drastic reductions in cost of whole genome sequencing, it remains an expensive test for large sample sizes. For this reason, genotype data is often imputed to increase power for association studies in a cost effective way.

[Luke: cut paragraph to discussion](#)

Combining data can be an issue when the quality of the data produced can vary between sequencing technologies and even sequencing centres. The errors in one dataset don't disappear when they are combined with another higher quality one. Many of the legacy datasets produced

41 using dated sequencing technologies have been known to contain a higher rate of false positives  
42 than their more recent counterparts. Is it time to retire legacy data?

### 43 **Mutation Spectrum**

44 A genome wide mutational signature can be measured by taking the sum of all the different  
45 types of mutations in an individual. The signatures from individuals in the same population will  
46 have a tendency to be more similar due to shared ancestry. In 2015, Harris et al reported an  
47 overrepresentation of the TCC to TTC mutation in European populations. Harris and Pritchard  
48 published a follow up study comparing how various ethnic groups differ from one another with  
49 respect to their mutational spectrum. In this paper, they proposed that not only is there a distinct  
50 mutational spectrum difference in Europeans, but that these changes are present in most human  
51 populations. One result was of particular interest to our research group : the heterogeneously  
52 distributed signal found in a subset of Japanese individuals. A mutational signature that is present  
53 unevenly across a population is unexpected because it suggested strong population structure;  
54 unlikely for a dense population living on an archipelago.

### 55 **Motivations**

56 This project was motivated by this unusual population genetics observation in a recent publication  
57 by Harris et. al, that the 1000 Genomes Project (1kGP) Japanese population seem to be partitioned  
58 in two clusters of diff mutation rates. This heterogeneously distributed mutational signal was  
59 unexpected as a signal of this nature could either be due to population structure, a mutagen,  
60 or a technical bias. In investigating this mutational signature, we were unable to reproduce the  
61 results using a larger and higher quality dataset and concluded that this signal can be attributed to  
62 sequencing error.

63 This study is the result of an investigation in the quality of the 1kGP dataset. To begin, we will  
64 discuss the discrepancy between the Japanese samples from the 1kGP and a more recent high  
65 quality dataset. We then consider methods to discriminate sequencing errors resulting from dated  
66 technologies. Next, we explore more broadly how low quality variants remain embedded in other  
67 populations of the 1kGP. Finally, we will discuss the impact these variants have on modern analyses.

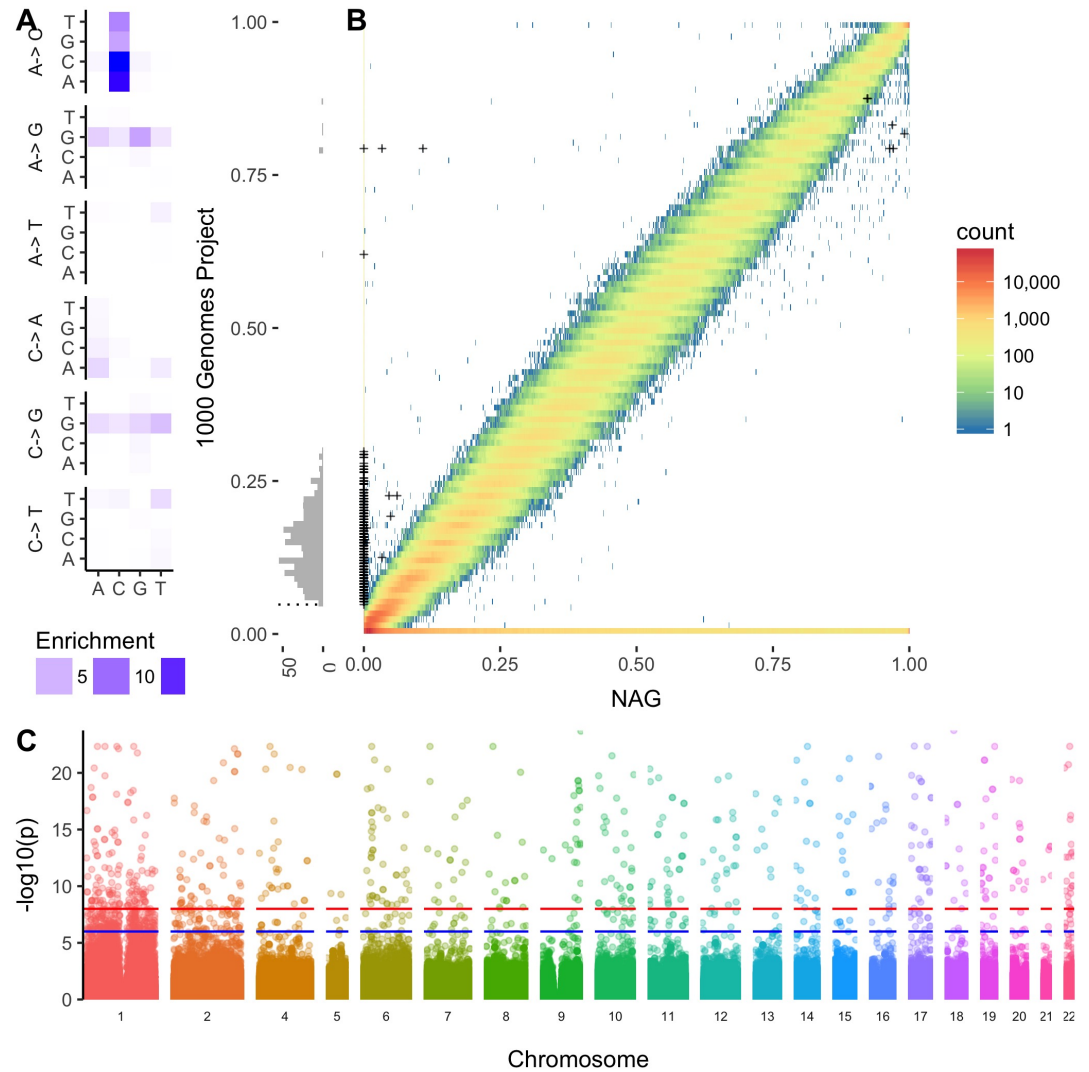
## 68 **Results**

### 69 **A peculiar mutational signature in Japan**

70 If two groups of individuals are sampled from the same population, we expect there to be little  
71 deviation in allele frequencies between the two groups. However, when comparing the allele  
72 frequencies between the Japanese individuals from the 1kGP and a more recent dataset, we  
73 observed an unusually large number of private single nucleotide polymorphisms (SNPs), only found  
74 in one of the two groups. Surprisingly, the variants responsible for the signal observed by Harris  
75 et. al (2017) were missing in the more recent dataset 1. We also noticed that the individuals  
76 carrying these mutations all had lower average quality scores of mapped bases. This suggested  
77 that individuals with lower quality were driving this signal.

78 To further investigate the extent to which low quality individuals were associated to spurious  
79 mutations, we performed a genome-wide association (GWA) study 1. GWA studies are commonly  
80 used to find genomic regions associated to biologically relevant traits. In this case, we are using  
81 this analysis in a more unconventional way to identify regions of the genome that are associated to  
82 biologically irrelevant traits like SNPs associated to individuals with low quality.

83 Using the linear GWA study function offered by PLINK, we were able to identify 625  $p < 10^{-8}$  and  
84 1048  $p < 10^{-6}$  SNPs that were associated to the average quality of SNPs mapped for an individual 1.  
85 This GWA study included 104 individuals, \*000\* of them were sequenced in phase 1 while the rest  
86 were sequenced in phase 3 of the 1000 Genomes Project. The variants that are associated to the  
87 quality of mapped bases have an enrichment in \*AC→\*CC mutations.



**Figure 1. A** Joint frequency spectrum plot of the Japanese from the 1000 Genomes Project and a more recent dataset. Crosses ( + ) are variants that reached genome wide significance in a GWA of sequencing quality. The histogram on the left of the plot is the distribution of significant variants. The lowest frequency variant able to reach genome wide significance is 5%. **B** Mutation spectrum of the 1048 variants that reached a genome wide significance with a p value less than in a in a GWA of sequencing quality. There is a *\*significant\** enrichment in *\*AC→\*CC* mutations. **C** Genome wide association of the average quality of mapped bases for the 104 Japanese individuals included in the 1000 Genomes Project. This GWA study identified 625  $p < 10^{-8}$  and 1048  $p < 10^{-6}$  SNPs that were associated to the average quality of SNPs mapped for an individual. The same analysis was performed independently for each of the populations in the 1000 Genomes Project.

88 Despite removing the variants identified as being low quality, the signal persists. When we  
89 remove the variants significantly associated to low quality, the signal identified by Harris et. al  
90 (2017) the enrichment in \*AC→\*CC mutations. When we remove individuals with average quality of  
91 mapped SNPs below 30, the signal goes away. We suspect that this has to do with the lack of power  
92 we have for more rare alleles. The lowest frequency variants that are significantly associated to  
93 quality are at 5%. When we remove all the variants above 1% in 1kGP but missing from the NAG  
94 data, the signal disappears. This suggests that while the GWA approach can identify some of the  
95 low hanging bad apples, there are likely more of these false positives nested inside these legacy  
96 cohorts.

97 One way to assess the validity of these unusual variants is to see if they are present in a higher  
98 quality dataset. Upon comparing the Japanese 1000 Genomes cohort to a higher quality and  
99 larger cohort, we identified \*000\* more variants that are beyond the expected frequency spectrum  
100 deviation for individuals from the same population.

## 101 Sequencing quality over time

102 We turned our attention to the other populations in the 1kGP, we found that the sequencing done  
103 in phase 1 was more variable and overall tended to include lower quality sequencing data 2. The  
104 sequencing quality of individuals increases over time 2. \*What sequencers were used in each  
105 phase?\* By 2011 the sequencing quality seems to level off.

## 106 Overlap of significant SNPs

107 Comparing the results of each independent GWA study, we were able to identify over \*0000\* vari-  
108 ants that were independently associated to low quality in multiple populations. This confirmation  
109 using more than one GWAS is a strongly suggests that these variants might not be genuine 3.

## 110 Imputation

111 30% of the SNPs we identified as being associated with low quality were found to be imputed using  
112 the Michigan Imputation Server. These should be removed from reference database.

## 113 Found to be included in other GWA studies

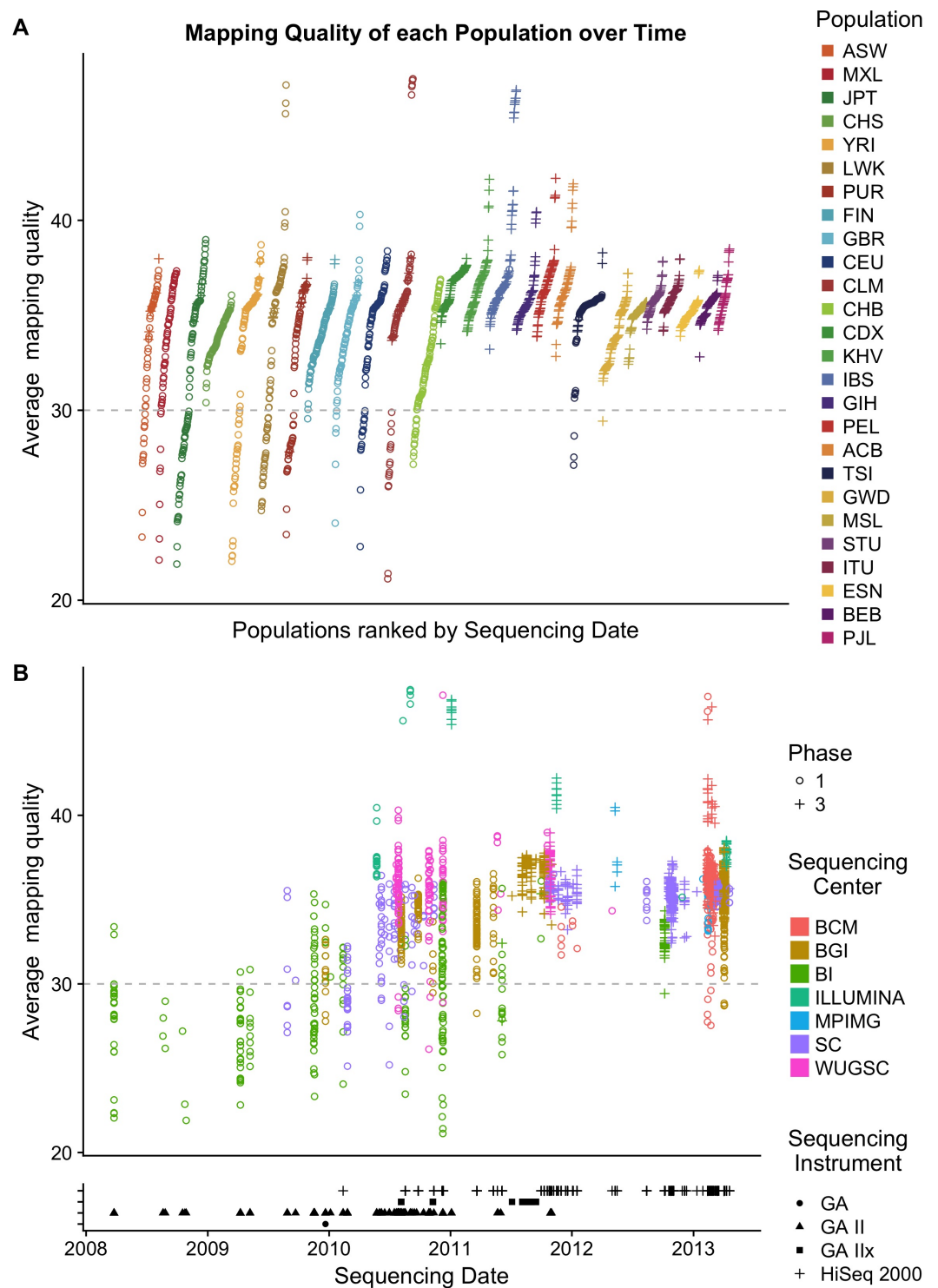
114 Once we identified SNPs that were clearly associated with low quality, we searched the literature for  
115 any GWA studies that might have called these erroneous variants as being significantly correlated  
116 with some biological trait. Using the NHGRI-EBI Catalog of published genome-wide association  
117 studies we queried the rsIDs of the SNPs we identified as being low quality and found 6 recent  
118 publications that had found at least one of the variants to have reached genome wide significance  
119 in their study.

120 Five of these studies used the 1000 Genomes Project as the reference database for imputation  
121 and one used the 1000 Genomes Project cell cultures and sequence data. They used strict quality  
122 thresholds, including population genetic statistical tests such as the Hardy-Weinberg equilibrium  
123 test, allele frequency differences using reference populations. They also removed rare alleles and  
124 alleles with high degrees of missingness. Despite using the state of the art quality controls, these  
125 erroneous variants managed not only to be imputed onto real genotype data, but they also reached  
126 genome wide significance for biological traits.

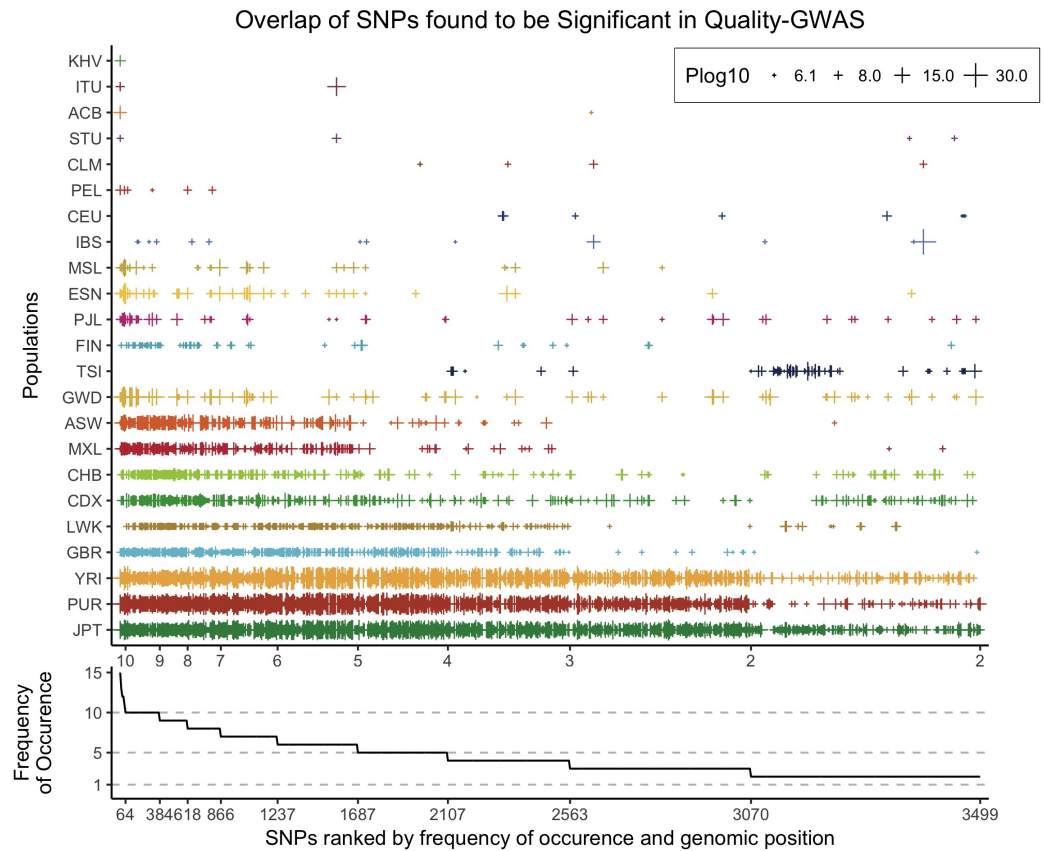
## 127 Discussion

### 128 Why do we care?

129 These SNPs matter because they reached genome wide significance for medically relevant traits.  
130 Could lead to false diagnosis at worst, or spurious correlations at least. Polygenic risk scores  
131 take into account all snps reaching significance, without much manual curation. It's likely that the  
132 variants we have identified are being included in these multi-locus risk scores.



**Figure 2. A** The average mapping quality of each individual per population included in the 1000 Genomes sequencing project. The x-axis is ranked by populations with the least to the most variance, followed by average mapping quality per individual. **B** Same data as in **A** except the x-axis is sorted by sequencing date. The colors indicate the sequencing centers that produced the data for each individual.



**Figure 3.** Overlap of SNPs identified independently to be associated with quality. The populations that have the most low-quality individuals also have the most low-quality variants. What is interesting here, is that the same variants identified as being low quality independently in each population are found in other populations.

133 Despite these variants reaching genome wide significance, the majority of researchers did not  
134 pursue these variants for further analysis. This is likely because these variants have no peak in LD  
135 that is characteristic of a biologically significant variant.

## 136 Recommendations

137 The most conservative approach would be to remove all individuals that don't meet the quality  
138 threshold as well as all the variants associated to low quality. In this case, we used a cut off of an  
139 average quality of mapped bases over 30. This threshold has been *\*previously used by studies\**. It  
140 is the minimum requirements for *\*GATK variant calling\** for them to have a minimum quality of 30.

## 141 Imputation

142 Imputation of genotype data is a probabilistic method that infers the bases of a given genome  
143 based on its similarity to a set of reference genomes. While on average, two humans differ in about  
144 1/10,000 bases, this number is *\*lower\** in closely related individuals, and *\*higher\** in individuals  
145 from different continental origins. Modern chip sequencing will provide the genotype information  
146 for over 1 million bases of the genome. The unique combination of genotyped bases can be enough  
147 to identify haplotype blocks that are identical by descent in individuals from the reference database.

148 The accuracy of imputation depends on the size of the reference database; this varies signifi-  
149 cantly from one population to another. This means that individuals with ancestry that is less well  
150 represented in the reference database will have lower accuracy of imputation. To overcome this  
151 bias, reference databases are often combined to increase the sample size and in turn, the accuracy  
152 of imputation.

## 153 GWA studies from other papers

154 Since these variants are present in more than one population from the 1000 genomes project, they  
155 are more likely to be associated to biological traits as they would appear to be like any other variant  
156 that is shared among multiple populations. The only way to distinguish some of these more covert  
157 false positives is to use statistical tests associating the quality metrics of each position relative to  
158 each individual.

159 *Luke: due to the temporal nature of the batch effects, and because entire populations were*  
160 *sequenced in one centre on one day, the false positives are more likely to cluster with population*  
161 *structure or case/control?*

## 162 Conclusion

163 Our method identifies spurious mutations by correlating mutations with data quality metrics. We  
164 propose including our quality control methods to identify possible false positives in sequencing  
165 data. We have focused on the 1000 Genomes Project dataset as its quality metrics were feely  
166 available, however the issues of quality control are not limited to this one consortium. This study  
167 only used one dataset and one quality metric, but using this same approach can be used to identify  
168 more false positives in many more datasets.

169 As more and more large scale genotyping efforts are being imputed on the same legacy datasets,  
170 we must scrutinize the quality of the reference databases to avoid the amplification of false positives.  
171 These results bring forth many questions regarding the reliability of legacy datasets. Moreover,  
172 since there are so many broad applications of imputation, it frames the question for reference data  
173 turnover.

## 174 Methods

### 175 Metadata

176 The metadata used in this analysis was compiled from each of the index files from the 1000  
177 Genomes file system. Average quality of mapped bases per sample was obtained from the BAS



178 files associated with each alignment file. Each BAS file has metadata regarding each sequencing  
179 event for each sample. If a sample was sequenced more than once, we took the average of the  
180 each quality score from each sequencing instance. The submission dates and sequencing centres  
181 for each sample in the analysis was available in the sequence index files. This file also has multiple  
182 entries per sample, however, we were unable to match the individual sequencing runs between the  
183 bas files and the index file, which lead us to take the average of the quality scores and only kept  
184 the earliest sequencing date per sample. The dates of the sequencing are only used to plot Figure.  
185 [\\*Average Quality of mapped bases: How was it calculated?\\*](#)

## 186 **Data Availability**

187 Index of BAS files [available here](#).  
188 Phase3 analysis sequence index file [available here](#)  
189 [\\*link to my compiled metadata file here\\*](#)

## 190 **Quality Controls**

191 We reproduced the quality control pipelines used by Harris et. al as they applied the current  
192 state of the art quality thresholds to remove questionable sequences especially for the high  
193 standards for detecting population level differences. Several mask files were applied to remove  
194 regions of the genome that might be lower quality, or might have very different mutation rates  
195 or basepair complexity compared to the rest of the genome. The 1000 Genomes [strict mask](#)  
196 was used to remove low quality regions of the genome , highly conserved regions were removed  
197 using the [phastCons100way](#) mask file and highly repetitive regions were also removed using the  
198 [NestedRepeats](#) mask file from RepeatMasker. Furthermore, only diallelic autosomal SNPs were  
199 considered, with missingness below 0.01, MAF less than 0.1, and MAF greater than 0.9.

## 200 **Genome Wide Association**

201 Using PLINK v1.90b4.4 we ran a linear genome wide association study independently for each  
202 population of the 1000 Genomes Project. We used the average quality of mapped bases per  
203 individual as the phenotype for the analysis. We also controlled for population structure by  
204 including the first 4 principle components of a PCA of each population using genotype data. [\\*NOT  
205 DONE YET\\*](#)

## 206 **Mutation Spectrum**

207 We calculated the mutation spectrum for each list of significant SNPs for each population.  
208 We also compared the mutation spectrum ratio between populations using a modified version  
209 of the methods used in Harris et al. 2017.

## 210 **Imputation**

211 Using the Michigan Imputation Server, we imputed the genotype data from 1000 Genomes Project

## 212 **Code Availability**

213 [\\*here is where I will put my git hub\\*](#)

## 214 **Acknowledgments**

215 We would like to thank Kelly Harris for sharing her mutation spectrum pipelines.