Ancient Migrations - The first complete genome assembly, annotation and variants of the Zoroastrian-Parsi community of India

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Abstract

With the advent of Next Generation Sequencing, many population specific whole genome sequences published thus far, predominantly represent individuals of European ancestry. While sequencing efforts of underrepresented communities in genomes datasets, like the Yoruba West-African, Han Chinese, Tibetan, South Korean, Egyptian and Japanese have recently added to the public genomic repositories, a comprehensive understanding of human genomic diversity and discovery of trait-associated variants necessitates the need for additional population specific analysis. In this context, the genomics of the population from the Indian sub-continent, given its genetic heterogeneity needs further elucidation.

In this context, the endogamous Zoroastrian-Parsi community of India, offer an exceptional insight into a homogenous population that has culturally, socially, and genetically remained intact, for 13 centuries amidst the genomic, social and cultural Indian landscape, consequent to their migration from the ancient Persian plateau.

Notwithstanding longevity as a trait, this endangered community is highly susceptible to cancers, rare genetic disorders, and display a documented high incidence of neurodegenerative and autoimmune conditions. The community as a matter of cultural practice abstains from smoking.

Here, we describe the assembly and annotation of the genome of an adult female, Zoroastrian-Parsi individual sequenced at a high depth of 173X using a combination of short Illumina reads (160X) and long nanopore reads (13X). Using a combination of hybrid assemblers, we created a new, population-specific human reference genome, The Zoroastrian-Parsi Genome Reference Female, AGENOME-ZPGRF, contains 2,778,216,114 nucleotides as compared to 3,096,649,726 in GRCh38 constituting 93.235% of the total genomic fraction. Annotation identified 20833 genomic features, of which 14996 are almost identical to their counterparts on GRCh38 while 5837 genomic features were covered in partial. AGENOME-ZPGRF contained 5,426,310 variants of which the majority were SNP's (4,291,601) and 960,867 SNPs were AGENOME-ZPGRF specific personal variants not listed in dbSNP.

We present, AGENOME-ZPGRF as a whole reference for any genetic studies involving Zoroastrian-Parsi individuals extending their application to identify disease relevant prognostic biomarkers and variants in global population genomics studies.

Introduction

Recent technological advances in high-throughput genome sequencing have brought a steep decline in the cost of genetic information[1], while increasing the predictive power and path to clinical translation of risk estimates for common variants found in genome wide association studies[2]. Most massively parallel sequencing approaches use simple alignment of short reads to a reference genome to study genomic variation. While the approach has been successful[3], an exhaustive study of structural variants and SNPs at a high depth, coverage, and confidence is essential for translation to precision medicine. The increase in long read sequencing technologies, as part of the 3rd generation genomic approaches have facilitated the assembly of large eukaryotic genomes in the last decade[4,5]. These advanced genomic platforms have given us powerful methods to generate long reads that compliment short accurate reads like those from Illumina sequencing chemistry to complete gaps and get better contiguity for the overall human genome.

Medical genetics has taken a leap forward in personalized medicine with the information of whole genome sequence for inheritable conditions, birth defects and chromosomal disorders[6]. Personalized genome assembly has shed light on the effects that non-genetic, disease-linked etiologies like methylation of CpG base pair islands have on gene availability for transcription[7,8]. With the advances in genomic sequencing, the importance of understanding genetic variability across extant human genomic diversity has become crucial[9], especially since the current reference genome assembly GRCh38[10] and variants cover only a sub-section of global population sub-types due to its mosaic nature of Caucasian and African genomic admixtures. Therefore, approaches focusing on understanding the minor, ethnic population groups, hitherto unrepresented in major genome variation studies, such as HapMap[11], 1000 Genome Initiative[12], Human Genome Diversity Project[13] and disease specific variation studies like TCGA[14] has become a prerogative in population genomics. This approach has been extended to sequence whole genome population references from Chinese[15], Ashkenazi[16], Korean[17], Japanese[18], Turkish[19], Egyptian[20], South Indian Asian-Indian[21,22] and many more draft genomes in the recent years. The availability of reference genomes from multiple human populations greatly aids attempts to find genetic causes of traits that are over- or under-represented in those populations, including susceptibility to disease.

The Indian subcontinent is a hotspot of social, ethnic and genetic diversity with waves of migration to Southeast Asia through India[23]. The genetic landscape of this region is mainly constituted from Austro-Asiatic (AA), Indo-European (IE), Tibeto-Burman (TB), and Dravidian (DR) families with cultural and social frameworks that discourage and at times prohibit intermarriages between ethnic groups[24,25]. The extensive genetic diversity of India with genetically isolated subpopulations, makes it an ideal for population genomic studies to explore the disease–variants relationships.

The Zoroastrian-Parsi of India represent one such endogamous, genetically homogenous community. While the community members have a longer median life span, their present numbers in India are dwindling making a genomic study of the community critical for population genomics. This community in India trace their origins to migrations from the Persian plateau (~847 AD), from Pars and Khorasan through the island of Hormuz to India where they settled as Parsis and practiced their faith, Zoroastrianism. The venerate Fire as the medium of worship and practice ostracism against smokers, therefore representing an important genomic biobank in understanding diseases associated with nicotine dependence. The community has a high prevalence of cardiovascular disorders, Autoimmune disorders like Rheumatoid Arthritis, Neurological/Neurodegenerative conditions like Parkinson's Disease, Alzheimes Disease and different types of cancers.

Here, we describe the assembly and annotation of the genome of an adult female, Zoroastrian-Parsi individual sequenced at a high depth of 173X using a combination of short Illumina reads (160X) and long nanopore reads (13X). Using a combination of hybrid assemblers, we created a new, population-specific human reference genome. The Zoroastrian-Parsi reference genome, AGENOME-ZPGRF, contains 2,778,216,114 nucleotides as compared to 3,096,649,726 in GRCh38. Annotation identified 20674 genomic features, of which 15235 are > 99% identical to their counterparts on GRCh38, while the remaining genes were found to covered in partial. AGENOME-ZPGRF contained 5,426,310 variants of which the majority were SNPs (4,291,601) and 960,867 SNPs were AGENOME-ZPGRF specific not listed in dbSNP.

Materials and Methods

Sample collection and ethics statement

The donor is a healthy, non-smoking Parsi female volunteer (age: 65 y.o), invited to attend blood collection camps at the Zoroastrian center in the city of Bangalore, India under the auspices of The Avestagenome Project®. The adult female (>18 years) underwent height and weight measurements and answered an extensive questionnaire designed to capture her medical, dietary, and life history. The subject provided written informed consent for the collection of samples and subsequent analysis. All health-related data collected from the cohort questionnaire were secured in The Avestagenome Project® database to ensure data privacy.

Genomic DNA extraction

Genomic DNA from the buffy coat of peripheral blood was extracted using the Qiagen Whole Blood and Tissue Genomic DNA Extraction kit (cat. #69504). Extracted DNA samples were assessed for quality using the Agilent Tape Station and quantified using the Qubit™ dsDNA BR Assay kit (cat. #Q32850) with the Qubit 2.0® fluorometer (Life Technologies™). Purified DNA was subjected to both long-read (Nanopore GridION-X5 sequencer, Oxford Nanopore Technologies, Oxford, UK) and short-read (Illumina Technologies)

Library preparation and sequencing on the Nanopore platform

Libraries of long reads from genomic DNA were generated using standard protocols from Oxford Nanopore Technology (ONT) using the SQK-LSK109 ligation sequencing kit. Briefly, 1.5 µg of high-molecular-weight genomic DNA was subjected to end repair using the NEBNext Ultra II End Repair kit (NEB, cat. #E7445) and purified using 1x AmPure beads (Beckman Coulter Life Sciences, cat. #A63880). Sequencing adaptors were ligated using NEB Quick T4 DNA ligase (cat. #M0202S) and purified using 0.6x AmPure beads. The final libraries were eluted in 15 µl of elution buffer. Sequencing was performed on a GridION X5 sequencer (Oxford Nanopore Technologies, Oxford, UK) using a SpotON R9.4 flow cell (FLO-MIN106) in a 48-hr sequencing protocol.

Nanopore raw reads (fast5 format) were base called (fastq5 format) using Guppy v2.3.4 software. Samples were run on two flow cells and generated a dataset of ~14 GB.

Library preparation and sequencing on the Illumina platform

Genomic DNA samples were quantified using the Qubit fluorometer. For each sample, 100 ng of DNA was fragmented to an average size of 350 bp by ultrasonication (Covaris ME220 ultrasonicator). DNA sequencing libraries were prepared using dual-index adapters with the TruSeq Nano DNA Library Prep kit (Illumina) as per the manufacturer's protocol. The amplified libraries were checked on a Tape Station (Agilent Technologies) and quantified by real-time PCR using the KAPA Library Quantification kit (Roche) with the QuantStudio-7flex Real-Time PCR system (Thermo). Equimolar pools of sequencing libraries were sequenced using S4 flow cells in a Novaseq 6000 sequencer (Illumina) to generate 2 x 150-bp sequencing reads for 30x genome coverage per sample.

Raw fastq files Illumina and nanopore reads

The sample genome was of an adult female from the endogamous Parsi community which was used for the construction of 173X Zoroastrian Parsi Whole Genome Assembly. Illumina HiSeq with a read length of 2 X 150 bp is used for obtaining short reads for the genome. We obtained a total of 2.2 Billion sequences from the Illumina HiSeq platform (160X) and a total of 6.8 Million reads from the Nanopore platform. For the long reads, the library preparation was according to the standard protocol and the sequencing of the genome was performed using the Oxford Nanopore Minion platform

Quality trimming and Quality control of the reads

Quality trimming and adapter removal of the short Illumina platform reads was performed using AdapterRemoval (version 2.2.2)[26] with minlength 30, trimwindow size 30 and reads lesser than quality score of Q30 were discarded. For adapter removal of long Oxford nanopore reads, Porechop tool (V0.2.4)[27] with default options was used. The long error prone reads from Oxford

Nanopore cannot cross the quality score of Q20 hence the cutoff was kept to 8 in this case. All the quality scores are checked using FastQC (version 0.11.5)[28] and FastP (V0.20.1)[29] tool (**Supplementary Figure 1, 2**).

Whole Genome assembly

The quality trimmed and adapter removed short and long reads were processed for Hybrid assembly. The choice of hybrid assembly was made using relevant literature study where short read alone, long read alone and short-long read hybrid assemblies were compared for different cases[30] and hybrid assemblies outperformed and gave better QC statistics reflecting better quality of the assembled genomes. The raw data was sub sampled to 60X coverage with length cutoff of 60 bp using fastP according to the instruction on the Wengan GitHub repository. The processed reads were assembled with Wengan[31] using D mode (uses DiscovarDenovo short-read assembler) with options -l ontraw, -g 3000 (3Gbp). An alternative assembly was generated by using HASLR, Wtdbg2[32], WenganA and WenganM assemblers.

Removing mis-assemblies at segmental duplications and centromere regions

Centromere regions were downloaded from UCSC web browser for GRCh38 version of human reference genome. Segmental duplications in a BED format flat file was downloaded from the GitHub repository (segDupPlots/ucsc.collapsed.sorted.segdups). A python script20 was used to remove miss-assemblies from Segmental duplications and centromere regions. Identification and annotations of repetitive elements was obtained using REPEATMASKER (V4.1.1)33 by aligning the genome sequences against known library of repeats in humans.

Read mapping and variant calling for Illumina sequencing reads

The variant detection for the AGENOME-ZPGRF female sample was carried out using GATK pipeline (V4.1.5.0)[35], Picard (2.21.9) and Samtools (1.3.1)[36]. The GATK pipeline included read mapping and variant processing. Single-nucleotide variants (SNVs) and indels were called by local reassembly of haplotypes using HaplotypeCaller of GATK V4.1.5.0.

The following workflow was used for variant calling, the raw reads were pre-processed, converted to unaligned BAM and readgroup information were assigned using FastqToSam. The adapters were tagged using Markilluminadapter function and the bam file were converted to interleaved fastq sequences to map to the reference genome. The reference genome (GRCh38) is indexed using BWA index and samtools, further the sequence dictionary for reference was obtained using picard CreateSequenceDictionary function. Mapping the FASTQ reads to reference genome was performed by BWA-MEM (version 0.7.17-r1188). Information from unaligned BAM and the aligned BAM were merged using MergeBaMAlignment to retain the raw read information. The duplicate reads through experimental artefacts are tagged using MarkDuplicates (picard) module. The base quality score recalibration (BQSR) was applied to overcome the errors associated with base quality score due to sequencing errors. The BAM file were further indexed to identify variants by HaplotypeCaller. The variants obtained from HaplotypeCaller were annotated using SnpEff (4.3t), a genetic variant annotation and effect prediction toolbox.

Structural variants

SVs were called using DELLY2[33] with default parameters on duplicate marked bam file for germline SV calling (https://github.com/dellytools/delly).

Pharmacogenomics relevance

To assess the pharmacogenomics relevance, we obtained common variants in AGENOME-ZPGRF and dbSNP-138 database. These variants were annotated based on PharmGKB (www.pharmgkb.org) database[34] to obtain pharmacogenomics association. The variants that were classified as conflicting-interpretation, uncertain significance and benign were removed. The variants that had Pharmacokinetic (PK) and Phamacodynamic (PD) associations were considered to obtain actionable SNPs.

Results

Benchmarking of hybrid assemblers for Whole Genome Assembly using Chr22

De novo assembly was performed on a female Parsi whole-genome sequencing data. The data was in the form of Illumina-paired end 160X coverage and Oxford nanopore data of 13X. To standardize the pipeline, Chromosome 22 data (genome size of 52 Mbp) was extracted from the whole genome data and tested with iterative combination of different assembler strategy. The Illumina paired-end short reads with a read length of 150 bp were assembled using the Abyss[35] assembler resulting in a total length of 44,331,422 bp and a contig N50 of 15,753 bp.

We proceeded to use Hybrid assemblers known to perform scaffolding using long reads and polishing using short reads. Wengan assembler outperformed all other hybrid assemblers by producing a total length of 32,346,746 bp with 194 contigs greater than 50,000 bp. Quickmerge[36] meta-assembler was applied on Wengan assembly which gave the lowest number of contigs versus length and Abyss (160X) gave the longest length with the higher number of contigs improving the contiguity of the assembly. The results for this exercise produced a total length of 50,216,737 bp which is close to 90% of the total length of Chr22 and 2,675 contigs (**Appendix 1**).

De novo assembly of the First complete Zoroastrian Parsi Whole Genome Reference Female (AGENOME-ZPGRF)

Following the assembly of Chr22, we extended our meta-assembly strategy to assemble the whole genome reference (**Figure 1**). Our Zoroastrian-Parsi genome (AGENOME-ZPGRF) is based on high-quality *de novo* assembly from one female Parsi individual. The assembly was generated from a combination of short and long read data sets: 2x150 bp Illumina paired-end reads (160X), Oxford Nanopore (13X) reads averaging over 5,784 bp in length (**Table 1**).

We initially created five hybrid assemblies using Illumina short reads and Oxford Nanopore Technology long reads. Three assemblies were based on Synthetic Scaffolding Graph approach using Wengan hybrid genome assembler, one assembly based on synthetic paired end reads using HASLR and one assembly based on fuzzy De-Brujin graph method using wtdbg2. WenganD gave the best results with respect to total length of 2.7 Giga bases (Gbp), N50 of 2 Mega bases (Mbp)

and genomic fraction of 93.2%. The annotation based on QUAST-LG identified 14,996 complete and 5,837 partial number of genomic features (**Table 2**). The Parsi reference genome, AGENOME-ZPGRF, contains 2,778,216,114 nucleotides as compared to 3,096,649,726 in GRCh38. This assembly, designated AGENOME-ZPGRF, was the basis for all subsequent refinements and analysis.

Completeness of the genome

Gene completeness was measured with BUSCO54 v.4.1.4[37] using the Primates ODB-10 gene set. BUSCO provides intuitive metrics to describe genome, gene set or transcriptome completeness[38]. We observed 88.3% of genome completeness, 12,165 complete BUSCOs, 12,113 complete and single copy BUSCOs, 52 complete and duplicated BUSCOs, 461 fragmented BUSCOs, 1,154 missing BUSCOs. The total BUSCO group searched was 13,780.

Repeated elements in AGENOME-ZPGRF

When annotating repeats with REPEATMASKER, about 48.34% of the genome (**Table 3**) was identified as repetitive, with its results similar to those from EGYPTRef, AK1 and YORUBA genome assemblies. Most of the repetitive elements comprised of 21.80% Long Interspersed Nuclear Elements (LINEs), 13.45% of Short Interspersed Nuclear Elements (SINEs) and the rest in ALU elements, Mammalian-wide Interspersed Repeats (MIRs), Long Terminal Repeats (LTR) elements, DNA elements, small RNAs, satellites and simple repeats. Further, we found that about two-thirds of the SNPs identified in the repeat regions were found in long interspersed elements (LINE; 21.80%; majority occurring in LINE1 elements) or short interspersed elements.

Variant identification

We used the GATK variant calling pipeline, performed according to GATK best-practice recommendations and the HaplotypeCaller tool was employed for identifying putative variants, followed by Snpeff (build 2017-11-24) to annotate and make functional predictions. Our analysis revealed 5,426,310 variants of which 79% are SNPs (4,291,601) and 21% are indels, multiple-

nucleotide polymorphism (MNPs) and mixed variants (**Figure 2**). The transitions (297,330), transversions (251,540), Ts/Tv, ratio was 1.18, resembling expected figures in similar studies. Among the identified SNPs, 41.40% were intronic and 40.78% were intergenic while the rest were SNPs in the upstream (8.84%) and downstream region (7.19%). SNPs in the exonic region constituted only 0.5% of the total SNP count and the SNPs in the untranslated 3′ or 5′ region made up the rest (**Figure 2, Supplementary Figure 3**). Of these SNPs, 14,572 were missense (non-synonymous), 13,321 were silent (synonymous) substitutions and 189 nonsense SNPs. This is consistent with a non-syn:syn (dN/dS) ratio of ~1 expected of a normal genome[39].

Based on the SNPEff[40] annotation, we sought to identify the functional impact of the SNP's in terms of "high", "low", "moderate" impact based on their occurrence on the genome. High impact SNPs occur when (i) the variant hits a splice acceptor/donor site, (ii) a start codon is changed into a nonstart codon, or (iii) a stop codon is gained or lost due to the variant. We identified 1652 SNPs with a high-impact effect (**Appendix 2**), 16128 with low impact and 14856 with moderate impact. The majority of the high impact variants occurred on the gene *DPP6* (n=2920, **Appendix 4, 6**), variants of which have been reported to be associated to familial idiopathic ventricular fibrillation[41].

Out of the ~4.2 million SNPs identified, there were 960,867 potentially novel SNPs that did not exist in dbSNP[42] (**Appendix 3, 7**). Further analysis of the AGENOME-ZPGRF specific novel SNPs showed, 50.9% and 31.2% were in intergenic and intronic regions, respectively. We found 9.2% were upstream, 7.17% downstream of a gene, and 4202 (or 0.49%) of the SNPs were found to be in coding regions (**Table 5**). Among the 4202 SNPs in coding regions, we could further classify 31 nonsense SNPs and a total of 415 SNPs with a high impact (**Appendix 5**). Most of the variants (both genomewide and AGENOME-ZPGRF specific variants) occurred on Chr1, while the highest frequency of distribution occurred on Chr22 (**Table 4**). We identified 1,133,653 indels, which consisted of 546,985 insertions and 586,668 deletions. Of these indels, 503,214 (or 44%) were found to be novel. Majority of the unique variants occurred on LOC105379427 (n=1552, **Appendix 4**) that code for zinc finger protein 717-like proteins (putative) and *DUX4L18/DUX4L19/CNTNAP3B* genes which have been implicated in cognitive disorders.

The high impact SNPs across the AGENOME-ZPGRF genome are distributed among 1,014 protein-coding genes in the genome and 311 non-coding regions. We next classified the high

impact variants to understand the significance of the coding (sSNPs) and non-coding SNPs (nsSNPs), in terms of their distribution in protein class, pathways, biochemical activity with KEGG[43] pathways using DAVID[44]. The distribution of both high impact sSNPs and nsSNPs was significantly enriched in G protein coupled receptor pathway genes, olfactory transduction. Our finding is consistent with studies that demonstrate higher levels of polymorphism observed in human olfactory gene family[45]. In addition, we found enrichment for pathways associated with neuroactive ligand-receptor and osteoclast differentiation. The majority of the high impact coding SNPs belonged to transmembrane helices, transmembrane and receptor protein classes. Reactome based pathway analysis[46] showed that pathways for antigen presentation: Folding, assembly and peptide loading constituted the major pathway implicated for genes harboring the coding and non-coding SNPs **(Figure 4**).

Genetic structural variation in AGENOME-ZPGRF

Using short-read sequencing data of AGENOME-ZPGRF, we called 69,148 SVs using DELLY2 structural variant prediction tool[33] (**Figure 3**). We observed that while most of the SVs were deletions (n=40070), we found other SVs categorized as inversions (n=6004), duplications (n=5808), insertions (n=2129) and translocations (n=15137). No mis-assemblies were observed outside centromeric regions and segmental duplication regions.

Pharmacogenomics and drug risk assessment using AGENOME-ZPGRF SNPs

One of the aims of personalized genomics is to assess the individuals SNPs for disease and drug reaction assessment aiding drug dosage regimens. Using the variant-drug risk correlation annotation in the PharmGKB database[34] and KEGG database, we sought to understand SNPs of pharmacogenomic relevance in the AGENOME-ZPGRF. We identified 20 unique SNPs (**Appendix 8**) associated with 12 genes distributed across 9 chromosomes (chr1,2,4,7,8,11,14,15 and 16) with pharmacogenomic relevance based on PharmGKB (**Appendix 7**). We identified 10 actionable SNPs from literature as it pertains to treatment with various drugs, some of which are also represented in the PharmGKB (Table 3).

Discussion

We present the first, high depth whole Zoroastrian-Parsi Genome Reference Female sequence, AGENOME-ZPGRF for the Zoroastrian-Parsi population of India. The AGENOME-ZPGRF is a high depth whole genome sequence at 173X, combining genomic reads from short read (160X; Illumina) and long read (8X; 5X; Oxford Nanopore Technologies) sequencing technologies. AGENOME-ZPGRF represents the first high depth whole genome sequence from the Indian subcontinent, where there have been previous genome assemblies of male[47,22] and female[21] at <40X. AGENOME-ZPGRF, contains 2,778,216,114 nucleotides as compared to 3,096,649,726 in GRCh38. AGENOME-ZPGRF is unique as it is derived almost entirely from a single individual unlike GRCh38, which represents a mosaic of multiple individuals, thereby, adding further insight into personal genome and variant-disease association approaches. We have extended our study to sequence the first Zoroastrian-Parsi male genome, presently 2,589,561,354 bp in length with 87.46% genome fraction mapping to GRCh38 (**Appendix 9**).

The genome completeness is 93.25% which is on par compared to other high resolution whole genomes from the Ashkenazi[16], Egypt ref[48] and Yoruba genome[49] assembly projects. Our assembly quality is further enhanced by BUSCO completeness score of 88.3% validating our benchmarking process for genome completeness. Annotation identified 20,833 genomic features, of which 14,996 are > 99% identical to their counterparts on GRCh38. Most of the remaining genes were partial. This assembly, designated AGENOME-ZPGRF, was the basis for all subsequent refinements. Our analysis revealed 5,426,310 variants of which 79% are SNPs (4,291,601) and 21% are indels, MNPs and mixed variants. AGENOME-ZPGRF had 960,867 novel/personal SNPs not listed on dbSNP. The AGENOME-ZPGRF reference standard of this endogamous, socially, genetically divergent community adds valuable insights into human population genetic diversity as compared to other global populations. Furthermore, our study adds information to the catalogue of genomic variation derived from the 1000 human genome project consortium, which also includes samples of Indian origin (1000 Genomes Project Consortium).

Besides the nuclear genome, we had previously studied AGENOME-ZPGRF mitochondrial genome variants[50] as the first *de novo* Zoroastrian-Parsi mitochondrial genome, AGENOME-ZPMS-HV2a-1 (Genbank accession, MT506314). Our analysis showed that the AGENOME-ZPGRF belongs to haplogroup HV2a and that showed 28 unique variants compared with the

revised Cambridge Reference Standard (rCRS). HV2a is an extremely rare haplogroup, and prevalent among the Zoroastrians-Parsis in our study cohort. The haplogroup HV2a is closely associated with Caucasian descent, with its documented prevalence dating back to ancient Scythians who were geographically distinct group of nomads joined by common cultural expressions[57]. They date back to about the 9th century BCE until the 4th century CE[56] tracing their origins to the Caspian Pontic Steppes and the Altai mountains[51], indicative of the unique genomic landscape of the contemporary Zoroastrian-Parsi among Indian and European communities.

Variants in personal genomes can be used to assess disease risk, carrier status and drug response/interaction contributing to a pharmacogenomic insights in clinical genetics. We have assessed the AGENOME-ZPGRF genome using OMIM, PharmaGKB and KEGG databases for SNPs with health and disease consequences. We identified high risk for Multiple sclerosis, among other diseases that include cancers and neurodegenerative diseases. We found two *CHRNA3*, *CHRNA5* alleles: rs16969968, rs1051730 gene variants that have been associated with cognition, possibly mediating in part risk for developing Nicotine Dependence[52,53]. We also found a C>A variant in *C11orf65* located near ATM gene regulating metformin response in Type 2 diabetics[54]. Additionally, we found intronic variant (rs762551) in *CYP1A2* associated with leflunomide induced toxicity in treatment for Rheumatoid Arthritis[55]. In the context of preventive pharmacogenomics association, we found the AGENOME-ZPGRF, harbored a SNP (C>T) in *DPYD;DPYD-AS1* implicated in fatal consequences to 5-Fluorouracil (5-FU)-based treatments (4%-5%, early onset-severe to 0.3%, fatal) in patients with dihydropyrimidine dehydrogenase (DPD) deficiency.

In sum, our present study has delivered the first, complete, *de novo*, high depth genome assembly AGENOME-ZPGRF for Zoroastrian Parsi community of India. Analysis of the variants in AGENOME-ZPGRF indicated 960,867 novel/personal SNPs, some of which were found associated with adverse drug interactions in separate studies. We have also completed the whole genome assembly of a Zoroastrian-Parsi male, whose variant annotation is underway. Further analysis of personal variant-disease-drug response annotations that are gender specific made using clinically validated variants will complement current healthcare practices with personalized pharmacogenomics. This will lead to safe, accurate, drug dosage and treatment regimen by physicians and clinical trials.

Declarations:

Ethics approval and consent to participate.

We would like to state that this ethics review board is not affiliated with a commercial entity, and we confirm that the Ethics Review was sought from an independent ethics review board not affiliated with the funder or the commercial entity, in line with Declaration of Helsinski that the "committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified".

The study was approved by the Institutional BioEthics Committee constituted by the Department of Biotechnology, Government of India (BIAG-CSP-033). The committee constituted is compliant with the scientific, medical, ethical, legal and social requirements of the research proposal and in line with the 1964 Helsinki declaration and its later amendments. All subjects have provided written informed consent for the collection of samples and subsequent analysis.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

Figure 1

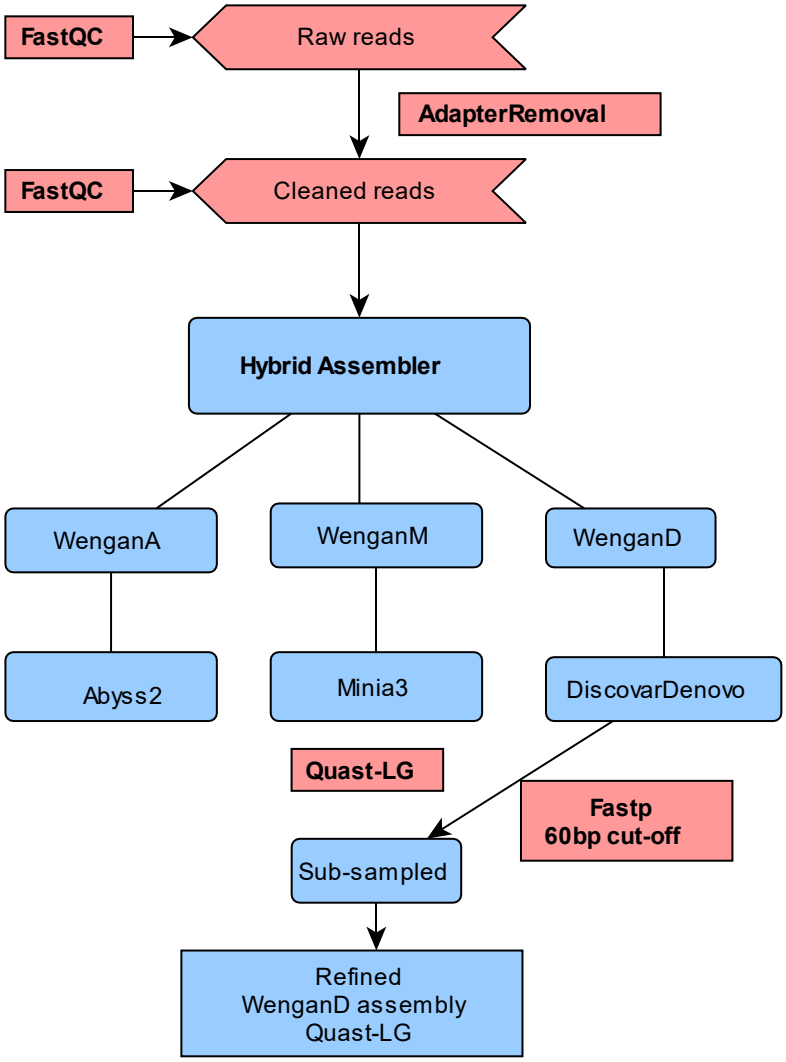

Figure 1: Workflow detailing meta-assembly protocol using iterative combinations of hybrid assemblers to generate the first Zoroastrian-Parsi Genome, AGENOME-ZPGRF

579 **Figure 2**

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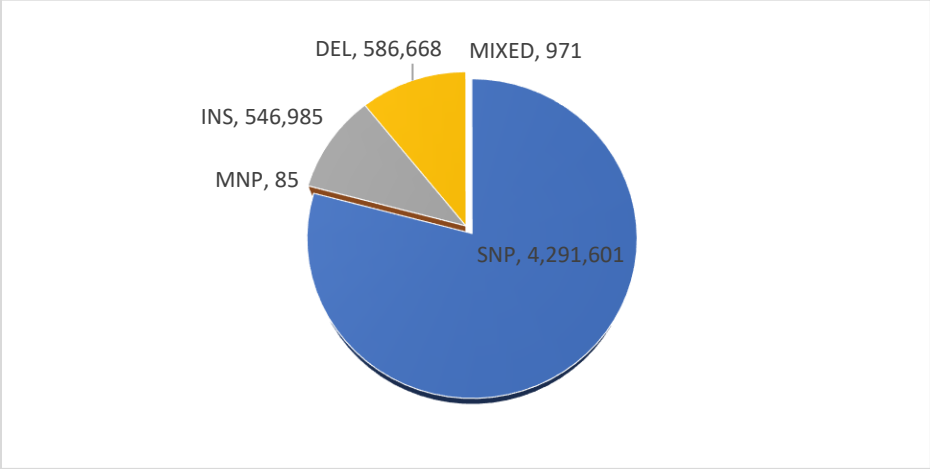
581 **A)**



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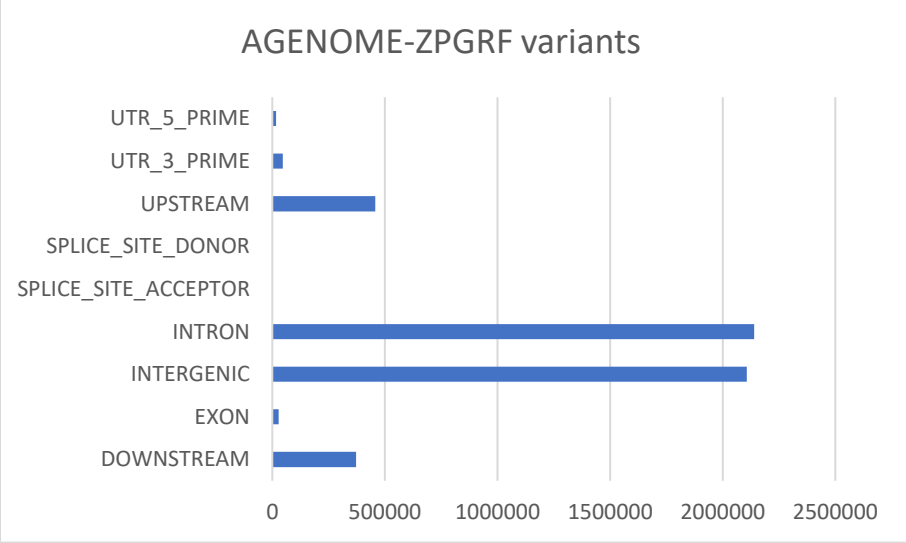
583 **B)**



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586 **Figure 2:** Distribution of variant types (A) and location in genomic regions (B) identified in the

587 AGENOME-ZPGRF

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590 **Figure 3**

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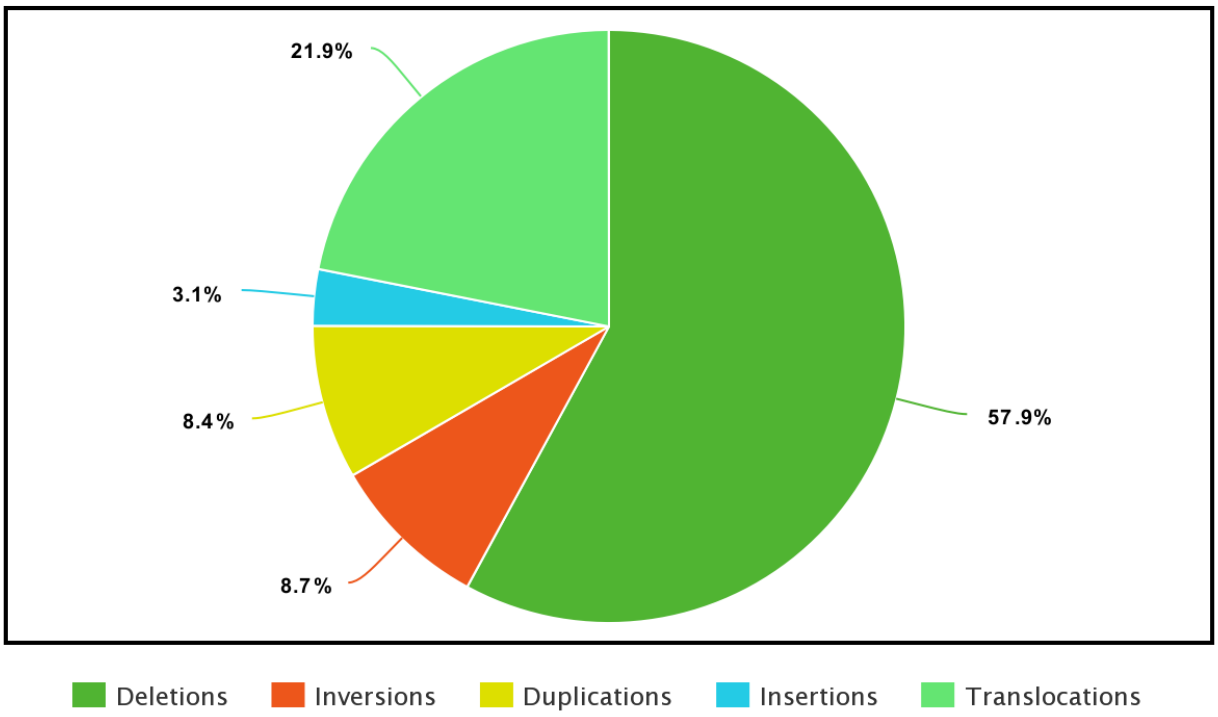

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595 **Figure 3**: Distribution of Structural Variant (SV) calls in the assembled first Zoroastrian-Parsi
596 Genome, AGENOME-ZPGRF; The breakdown of the SV's is as follows: deletions (n=40070),
597 inversions (n=6004), duplications (n=5808), Insertion (n=2129) and translocations (n=15137).

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Figure 4:

A)

Term	Count	P-Value	Fold Enrichment
GOTERM_BP_DIRECT			
detection of chemical stimulus involved in sensory perception of smell	8	2.50E-07	1.40E+01
G-protein coupled receptor signaling pathway	9	2.90E-06	7.50E+00
adaptive immune response	3	1.20E-02	1.10E+01
sensory perception of smell	3	1.40E-02	1.10E+01
cell adhesion	3	9.40E-02	3.70E+00
INTERPRO			
G protein-coupled receptor, rhodopsin-like	10	5.90E-08	1.00E+01
GPCR, rhodopsin-like, 7TM	10	7.20E-08	9.90E+00
Olfactory receptor	8	4.30E-07	1.30E+01
Immunoglobulin-like domain	8	2.10E-05	7.30E+00
Immunoglobulin subtype	6	2.40E-04	8.10E+00
KEGG_PATHWAY			
Olfactory transduction	8	6.80E-08	1.20E+01
Osteoclast differentiation	1	1.00E+00	0.00E+00
Neuroactive ligand-receptor interaction	1	1.00E+00	0.00E+00
UP_KEYWORDS			
Glycoprotein	22	2.60E-12	4.00E+00
Disulfide bond	19	1.40E-10	4.50E+00
Transmembrane helix	22	2.10E-10	3.20E+00
Transmembrane	22	2.20E-10	3.20E+00
Membrane	23	3.30E-09	2.50E+00
Receptor	13	3.90E-08	6.20E+00
G-protein coupled receptor	10	1.50E-07	9.20E+00
Transducer	10	2.60E-07	8.60E+00
Olfaction	8	3.70E-07	1.40E+01
Cell membrane	15	8.60E-07	3.80E+00
Sensory transduction	8	3.10E-06	9.90E+00

B)

Pathway name	Entities mapped	Total Entities	Entities pValue	Entities FDR
Antigen Presentation: Folding, assembly and peptide loading of class I MHC	35	102	1.11E-16	5.55E-15
Endosomal/Vacuolar pathway	35	82	1.11E-16	5.55E-15
ER-Phagosome pathway	35	165	1.11E-16	5.55E-15
Antigen processing-Cross presentation	35	187	1.11E-16	5.55E-15
Class I MHC mediated antigen processing & presentation	36	465	5.44E-15	2.39E-13
Adaptive Immune System	45	1003	2.34E-10	9.37E-09
Cytokine Signaling in Immune system	45	1108	5.12E-09	1.84E-07
Vpr-mediated induction of apoptosis by mitochondrial outer membrane p	2	4	0.001913956	0.063161
Immune System	59	2713	0.005544705	0.166341

Figure 4: Enrichment of AGENOME-ZPGRF novel high impact variant across different databases like (A) DAVID and (B) Reactome

Table 1

Sequence technology	Total reads (bp)	Mean read length(bp)	Coverage
Illumina-library-1	1422887753	151	100X
Illumina-library-2	322537951	150	30X
Illumina-library-3	479650511	151	30X
ONT-library-1	3318994	4566	5X
ONT-library-2	3515835	7002	8X

Table 1: Sequence data for assembly of *De novo* Zoroastrian-Parsi Genome Reference Female genome (AGENOME-ZPGRF)

638 **Table 2**

Genome statistics	AGENOME-ZPGRF	EGYPT	AK1	YORUBA
# contigs	**4806**	**3235**	**2832**	**1647**
# contigs (>= 0 bp)	4806	3724	2832	1741
# contigs (>= 1000 bp)	4806	3560	2832	1726
# contigs (>= 5000 bp)	4775	2776	2832	1562
# contigs (>= 10000 bp)	4017	1917	2832	1347
# contigs (>= 25000 bp)	3335	1069	1570	799
# contigs (>= 50000 bp)	2966	734	747	288
Largest contig	15222969	88566048	113921103	248986603
Total length	**2778216114**	**2836714529**	**2904207228**	**3088335497**
Total length (>= 0 bp)	2778216114	2837486204	2904207228	3088495238
Total length (>= 1000 bp)	2778216114	2837367164	2904207228	3088485407
Total length (>= 5000 bp)	2778073401	2834831880	2904207228	3087990629
Total length (>= 10000 bp)	2772661904	2828723737	2904207228	3086359078
Total length (>= 25000 bp)	2761939683	2815431970	2882817238	3076876085
Total length (>= 50000 bp)	2748699427	2803817652	2855011855	3059626724
N50	**2012108**	**25502944**	**44846623**	**155338310**
N75	937275	8329420	19924750	114367800
L50	369	29	21	8
L75	875	77	46	14
GC (%)	40.85	40.81	40.88	40.88
Misassemblies				
# misassemblies	3322	1276	1952	1756
# misassembled contigs	777	484	782	374
Misassembled contigs leng	1398700724	2137050584	2657569650	3053643982
# local misassemblies	10110	10797	5004	5679
# scaffold gap ext. mis.	0	0	15	3
# scaffold gap loc. mis.	0	0	108	435
# possible TEs	250	330	256	296
# unaligned mis. contigs	33	333	455	228
Unaligned				
# fully unaligned contigs	128 + 2501 part	1207	402	412
Fully unaligned length	27948040	12541896	9234968	10329457
Genome fraction (%)	**93.235**	94.174	95.177	95.391
Duplication ratio	1.001	1.01	1.023	1.088
# genomic features	**14996 + 5837 part**	**17682 + 3226 part**	**19651 + 1396 part**	**19356 + 1721 part**
Largest alignment	9191143	75492126	58219133	65512502
Total aligned length	2749835393	2800100449	2829006639	2832740986
NG50	1734895	20857787	39609866	145208384
NG75	610541	5007910	14897232	114367800
NA50	1339017	12942852	15098581	19529238
NA75	659297	5094247	5183319	9114594
NGA50	1175915	11187777	13028687	19529238
NGA75	435352	3192669	3932304	8890200
LG50	455	35	24	9
LG75	1193	109	54	14
LA50	584	60	59	43
LA75	1316	146	140	96
LGA50	712	71	66	43

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640 **Table 2:** AGENOME-ZPGRF assembly statistics using Quast

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642 **Table 3**

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Repetitive elements	Number of elements	Length occupied	Percentage of sequence
SINEs:	1592285	367249056 bp	13.45 %
ALUs	1078242	289089690 bp	10.58 %
MIRs	506412	77200621 bp	2.83 %
LINEs:	924026	595272136 bp	21.80 %
LINE1	517835	482717596 bp	17.67 %
LINE2	345948	98455152 bp	3.60 %
L3/CR1	45096	10230478 bp	0.37 %
LTR elements:	485651	250839692 bp	9.18 %
ERVL	107081	55227511 bp	2.02 %
ERVL-MaLRs	241322	104424519 bp	3.82 %
ERV_class I	104268	76806564 bp	2.81 %
ERV_class II	6733	7378587 bp	0.27 %
DNA elements:	419798	102266826 bp	3.74 %
hAT-Charlie	214680	44096448 bp	1.61 %
TcMar-Tigger	96328	35713313 bp	1.31 %
Unclassified:	9436	4642192 bp	0.17 %
Total interspersed repeats:		1320269902 bp	48.34 %
Small RNA:	11816	1234995 bp	0.05 %
Satellites:	4807	10740343 bp	0.39 %
Simple repeats:	621517	35759325 bp	1.31 %
Low complexity:	94569	5807986 bp	0.21 %

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646 **Table 3:** Repetitive elements in AGENOME-ZPGRF identified using REPEATMASKER

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650 **Table 4**

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AGENOME-ZPGRF Total Variants			AGENOME- ZPGRFPersonal Variants	
Chromosome	Length (bp)	Variant Count	Chromosome	Variant Count
Chr1	248956422	406844	Chr1	80130
Chr2	242193529	405925	Chr2	55166
Chr3	198295559	330471	Chr3	44518
Chr4	190214555	356369	Chr4	39089
Chr5	181538259	300512	Chr5	40208
Chr6	170805979	310307	Chr6	37739
Chr7	159345973	288693	Chr7	45470
Chr8	145138636	248099	Chr8	28697
Chr9	138394717	229576	Chr9	42833
Chr10	133797422	260117	Chr10	41812
Chr11	135086622	244294	Chr11	32123
Chr12	133275309	245744	Chr12	34417
Chr13	114364328	203749	Chr13	38176
Chr14	107043718	161817	Chr14	25147
Chr15	101991189	148767	Chr15	22335
Chr16	90338345	153943	Chr16	23462
Chr17	83257441	148518	Chr17	33152
Chr18	80373285	146714	Chr18	21699
Chr19	58617616	111838	Chr19	19212
Chr20	64444167	136692	Chr20	45802
Chr21	46709983	93091	Chr21	21527
Chr22	50818468	93011	Chr22	34511
ChrX	156040895	172036	ChrX	42009

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654 **Table 4:** Chromosome-wise distribution of variants in AGENOME-ZPGRF and novel variants
655 in AGENOME-ZPGRF

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Table 5

Type (alphabetical order)	Count	Percent (%)
DOWNSTREAM	61074	7.176438102
EXON	4202	0.493751726
INTERGENIC	433381	50.92399255
INTRON	266354	31.29765521
SPLICE_SITE_ACCEPTOR	70	0.008225279
SPLICE_SITE_DONOR	51	0.005992703
UPSTREAM	78750	9.25343846
UTR_3_PRIME	5392	0.633581463
UTR_5_PRIME	1761	0.20692451
TOTAL	851035	

Number of effects by impact		
Type (alphabetical order)	Count	Percent
HIGH	415	0.043318195
LOW	1702	0.177656788
MODERATE	2150	0.224419562
MODIFIER	953760	99.55460545
TOTAL	958027	

Number of effects by functional class		
Type (alphabetical order)	Count	Percent
MISSENSE	2009	56.83168317
NONSENSE	31	0.876944837
SILENT	1495	42.29137199
TOTAL	3535	

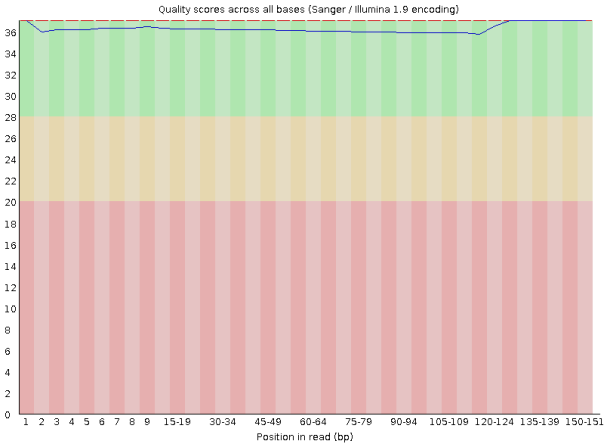
Table 5: Genomic location, functional class and impact of unique variants in AGENOME-ZPGRF

Supplementary Figures

Supplementary Figure 1

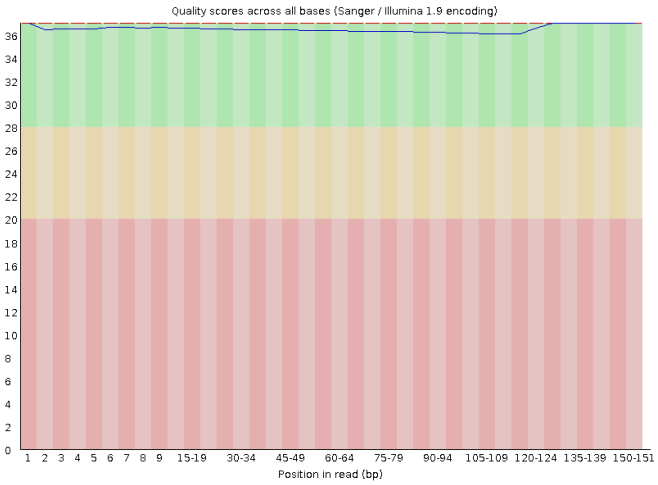
Fastqc for cleaned 100 x female short reads -R1

Total Sequences	1255218629
Average coverage	62.76093145



Fastqc for cleaned 30 x female short reads

Total Sequences	450173738
Average coverage	22.5086869



Supplementary Figure 1: Representative read quality plot from FastQC after cleaning of reads using AdapterRemoval

Supplementary Figure 2:

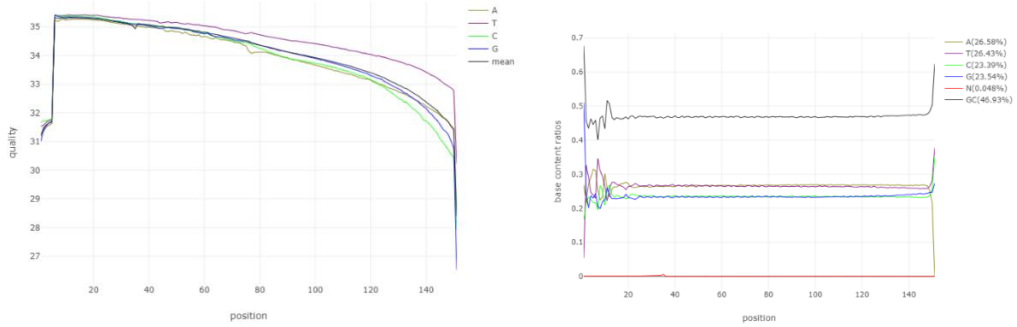
A)

Trimming and Filtering

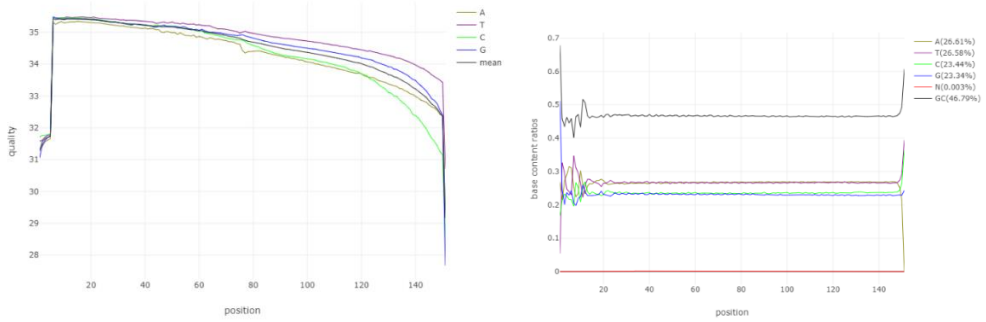
	Raw	Trimmed and Filtered
No. of Reads (Raw)	16.76 M	16.03 M
Total Base Pairs (Raw)	1.81 G	1.72 G
Mean Length (bp)	108bp; 108bp	107bp; 107bp
Q20 bases (%)	94.37	96.36
Q30 bases (%)	91.98	94.18
GC content (%)	47.06	46.79

B)

Before filter



After filter



Supplementary Figure 2: Read sequencing and Analysis statistics. A) Table indicating read processing and QC of sequencing data pre and post filtering B) Fastp output of read quality and base counts

703 **Supplementary Figure 3:**

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Type (alphabetical order)	Count	Percent (%)
DOWNSTREAM	371789	7.196359982
EXON	27640	0.535000739
INTERGENIC	2106964	40.78246374
INTRON	2139107	41.4046247
SPLICE_SITE_ACCEPTOR	281	0.005439045
SPLICE_SITE_DONOR	301	0.005826166
UPSTREAM	456935	8.844448729
UTR_3_PRIME	47060	0.910894891
UTR_5_PRIME	16271	0.314942005
TOTAL	5166348	100

Number of effects by impact		
Type (alphabetical order)	Count	Percent
HIGH	1652	0.031058581
LOW	16128	0.303215973
MODERATE	14856	0.279301618
MODIFIER	5286345	99.38642383
TOTAL	5318981	100

Number of effects by functional class		
Type (alphabetical order)	Count	Percent
MISSENSE	14572	51.89089096
NONSENSE	189	0.673028987
SILENT	13321	47.43608005
TOTAL	28082	100

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706 **Supplementary Figure 3:** AGENOME-ZPGRF **g**enome wide distribution of variants across
707 different genomic regions, by impact and functional classes

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