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SVAMP: sequence variation analysis, maps and phylogeny

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1 INTRODUCTION

Associating sequence variants [single nucleotide polymorphisms (SNPs) and indels] with sample metadata such as geographical location and drug susceptibility have played a key role in studying the population structure. SVAMP is a stand-alone Qt/C++ application capable of analysing variants in the context of geography and aiding in making inferences on the population structure. SVAMP is built on the open-source software VarB (Preston et al., 2012).

2 METHODS

Input to SVAMP software is a bundle of multisample VCF file, reference FASTA, annotation general feature format (GFF) and a precalculated SQLite database file. The bundle preparation script included as a part of SVAMP software captures the geographical coordinates, date of isolation and the genome coverage of samples. The files when loaded into SVAMP will aid the user in performing key population genomics analysis in real time and visualize the results. Two popular methods of analysing sample relatedness, principal coordinate analysis [PCoA; Torgerson–Gower scaling (Gower, 1966)] and geo-phylogenetic tree, are integrated into SVAMP. The pairwise dissimilarity matrix D is first computed based on the Hamming distance (Hamming, 1950) (d) between pairs of samples (i, j) using equation

\[ d(i,j) = 1/L \sum_{k=1}^{L} |S_{i,k} \neq S_{j,k}| \]

where \( k \) is the index of the genomic position out of \( L \) considered positions. \( S_{i,k} \) is the genotype called by sample \( i \) at position \( k \) in the genome. Positions that have missing genotype information are ignored in the computation; therefore, the multisample VCF file should ideally consist of samples and variants with reasonably complete data. The matrix D forms the basis for subsequent PCoA and phylogenetid tree reconstruction and consists of N (number of samples) rows and K (number of variant positions) columns.

PCoA, equivalently multidimensional scaling, is computed as per the R function cmdscale, and the phylogenetic tree is constructed using Fitch–Margoliash algorithm (Fitch and Margoliash, 1967). The user is provided with an option to group colours based on a known phenotype (e.g. drug susceptibility) or a custom classification. The ability to perform tree computation using external phylogeny package is also supported by saving alignments in a compatible format and visualizing the tree in SVAMP. The PCoA, phylogenetic tree and exporting alignments can be performed on multiple regions of interest within a subset of samples. Integrating popular bam viewers such as LookSeq (Manske and Kwiatkowski, 2009) to view read alignment evidence for variants is an added feature of SVAMP.

3 RESULTS

We have evaluated the application and scalability of SVAMP using two published datasets: (i) a bacterial population study (Harris et al., 2010) on methicillin-resistant Staphylococcus aureus (commonly known as MRSA) and (ii) a worldwide...
population structure study (Manske et al., 2012) on Plasmodium falciparum malaria parasite. Both these example datasets are available for download at http://cbrc.kaust.edu.sa/svamp as a packaged SVAMP bundle.

3.1 MRSA outbreak analysis using SVAMP
The MRSA dataset visualised in SVAMP as shown in Figure 1 contains 4310 SNP sites determined from 63 isolates obtained from various hospitals across 15 countries, spanning a period of 25 years. The linear phylogenetic tree constructed using SVAMP is shown in Supplementary Figure S1, and the circular tree in Supplementary Figure S2 is consistent with that described in the paper by Harris et al. (2010). Supplementary Figure S3 shows the Portuguese samples on the tree overlaid on the geographical map displaying the year of isolation and location. Supplementary Figure S4 shows the two European isolates DEN907 and TW20 clearly joining the Asian clade. From Supplementary Figure S1, it can also be observed that five isolates from Thailand S21, S24, S39, S42 and S81 obtained from the same hospital cluster together to form a single subclade. Colour coding the isolates based on the country of origin allows the visualization of the geographical map and the tree simultaneously, assisting with making genomic epidemiological inference.

3.2 Exploring the population structure of Malaria isolates using SVAMP
The raw sequencing data obtained from P. falciparum diversity study (Manske et al., 2012) were mapped using samtools. Resulting variants were merged using vcftools. Only coding region variants that do not fall in var, rifin and stevor gene (the hypervariable gene families in malaria) sites were included. After filtering for quality and missing data, 26918 SNPs were retained. This dataset consists of 245 samples from six countries: three from Africa (AFR), two from Southeast Asia (SEA) and Papua New Guinea (PNG). The PCoA analysis using SVAMP in Supplementary Figure S5 clearly shows three different clusters as three different groups AFR, SEA and PNG, as seen in the paper by Manske et al. (2012). As expected, individual continental PCoA analyses demonstrate separation between East and West African samples (Supplementary Fig. S6) and between Thailand and Cambodia samples. The commands and parameters used to obtain the final dataset used in SVAMP are explained in the Supplementary Materials.

3.3 Memory and computational speed of SVAMP on MRSA and malaria datasets
Memory usage and computational speed of SVAMP was evaluated on a laptop computer with 2 cores (4 GB RAM) and on a workstation with 12 CPU cores (96 GB RAM). The results were averaged for both MRSA and malaria datasets and are shown in Table 1.

CONCLUSIONS
By using the sequence variant and associated geographical information, we believe the software SVAMP will aid greatly in analysing isolates from an outbreak, as well as predicting the population structure in epidemiological studies.

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