SOFTWARE

PopGenPlayground: a population genomics analysis pipeline

Walter W. Wolfsberger[1]‡, Khrystyna Shchubelka[1,2]‡ Olga T. Oleksyk[3], Yaroslava Hasynets [2], Silvia Patskun[2], Mykhailo Vakerych[2], Roman Kish[2], Violeta Mirutenko[2], Vladislav Mirutenko[2], Coralia Adina Cotoraci[4], Calin Pop[4], Olimpia Neagu[4], Cornel Baltă[4], Hildegard Herman[4], Paula Mare[4], Simona Dumitra4, Horatiu Papiu[4], Anca Hermenean[4] and Taras K. Oleksyk [1,2] †

[1] Department of Biological Sciences, Oakland University, Rochester, MI 48309, USA.
[2] Department of Biological Sciences, Uzhhorod National University, Uzhhorod 88000, Ukraine.
[3] A. Novak Transcarpathian Regional Clinical Hospital, Uzhhorod 88000, Ukraine.
[4] ”Vasile Goldiş” Western University of Arad, 94-96, Revoluției Bld., Arad, 310025, Romania

‡ Authors contributed equally to this work

† Correspondence should be addressed to oleksyk@oakland.edu

Abstract

Background: Population genomic projects are essential in the current drive to map the genome diversity of human populations across the globe. Various barriers persist hindering these efforts, and the lack of bioinformatic expertise and reproducible standardized population-scale analysis is one of the major challenges limiting their discovery potential. Scalable, automated, user-friendly pipelines can help researchers with minimum programming skills to tackle these issues without extensive training.

Results: *PopGenPlayground* (PGP), is a streamlined, single-command computation pipeline designed for human population genomics analysis based on *Snakemake* workflow management system. Developed to automate secondary analysis of a previously published national genome project, it leverages the publicly available genomic databases for comparative analysis and annotation of variant calls.

Conclusions: PGP presents a multi-platform robust population analysis pipeline, that reduces the time and the expertise levels to perform the main core of population analysis for a national genome project. PGP provides a comprehensive secondary analysis tool and can be used to perform analysis on a personal computer or using a remote high-performance computing platform.

Background

The recent rise of population genomics can largely be attributed to major developments in sequencing technology and data analysis, and the exponential increase in the general volume of the data generated in the field (McGuire et al., 2020). In its nature (i.e. analysis of large datasets from multitude of samples), it is closely tied to the evolution of bioinformatics approaches and proliferation of modern computation techniques (Bartlett et al., 2017). Using new bioinformatics software and algorithms, researchers can efficiently analyze torrents of raw sequence data, identify variants, predict their functional effects, and examine patterns of genetic variation within and across populations of interest. Human population genomics projects are the necessary step towards providing information for and developing approaches in personalized medicine and advanced medical care (Polimanti et al., 2014).

Bioinformatics approaches facilitated the integration of different types of data in population genomics studies and opened the door to answering multi-domain research questions. For example, it is now possible to integrate genomic data with environmental, phenotypic, and geographic (Van Assche et al., 2015) data to study how various factors influence genetic variation within and among populations. This integrative approach has greatly enriched our understanding of population genomics. However, the growing complexity of modern research combined with the relative novelty and lack of standardized approaches for analyses establishes complex barriers to research groups starting their population genomics projects. Bioinformatics has made it possible but not necessarily made it accessible.

A typical bioinformatic analysis for any research study involves a multitude of tools and intermediary data manipulation steps, introducing an ever-growing bar in terms of expertise, forcing bioinformatics expert so specialize to narrow niches(Bartlett et al., 2017). One of the solutions to this problem aims to combine multiple steps and repetitive procedures in such analysis into a workflow. Streamlining, standardizing, and scaling up bioinformatics workflows, along with widespread knowledge sharing, can greatly improve the field's accessibility. These initiatives have the potential to democratize genomics research, allowing a broader spectrum of researchers to participate in genome wide analysis of and reap the benefits of the big data approach.

Purpose of PopGenPlayground

PopGenPlayground (PGP) is designed to facilitate comprehensive population genomics analysis using Variant Call Files (VCF). PGP suite is hosted on GitHub (Wolfsberger, 2023) and integrates a wide array of population genomics approaches (Table 1) into a single, user-friendly workflow. This pipeline automates key processes in population genomics analysis, including data wrangling, visualization, format conversion, and phasing, which reduces manual work and increases efficiency. *PGP* uses the *Snakemake* workflow management system (Köster et al., 2021) to ensure consistent computational environments throughout each analysis stage, limiting the impact of external variables. The workflow is scalable, capable of supporting high-performance computing environments, making it adaptable for large datasets. *PGP* features a simple configuration and does not require the user to have an advanced bioinformatics expertise to run.

Implementation

The pipeline is developed with *Snakemake* workflow management system (Köster et al., 2021) using the Python-based rule definition language. The pipeline integrates various bioinformatics instruments, described in detail in **Table 1** to perform the analysis, and handles all the data wrangling and transformation steps internally using Unix shell commands, and Python programming language with *Pandas*, and associated data analysis libraries and the Fisher Exact Test (FET) statistics calculation module. The pipeline uses publicly available genomic databases. The International Genome Sample Resource or IGSR (Fairley et al., 2020) project was used for developing and evaluating the comparative analysis steps of the pipeline, databases associated with *Ensembl VEP* (McLaren et al., 2016) were used for genome annotations, while *NCBI ClinVar* data (Landrum et al., 2016) was integrated for annotation of clinical medical variation within the data.

Table 1. Analyses incorporated in the *PopGenPlayground* pipeline.

Software\Tool	**Class**	**Purpose**	**Reference**
BCFtools	Data wrangling, VCF analysis	Provides an ability to merge and intersect Variant File Call, outputs statistics for comparative studies	(Danecek et al., 2021)
Whole Genome Sequencing Variant Call Files IGSR database	Comparative analysis	Provides populations to run the comparative analysis with other instruments	(Fairley et al., 2020)
PLINK2	Pedigree, Identity by descent/state, data wrangling, PCA test	Estimating inbreeding and relatedness, estimating structure, LD pruning, data conversion	(Chang et al., 2015)
ADMIXTURE	Clustering and characterizing admixture	Grouping individuals in clusters maximizing HW equilibrium and LD between loci	(Alexander et al., 2009)
detectRUNS (R package)	Runs of Homozygosity, Inbreeding based on runs of homozygosity	Estimating inbreeding and relatedness, detection of signals of selection	(Chang et al., 2015; Marras et al., 2015)
Shapeit4	Variant call phasing,	haplotype detection, missing data imputation	(Delaneau et al., 2008)
Ensembl VEP	Variant annotation	Combines multiple annotation approaches to assess the impact of variation on phenotype	(McLaren et al., 2016)
Python programming language and *Pandas* data analysis library	Data analysis tools	Report generation, data visualization, data wrangling	-

Steps of the PGP analysis are defined as rules and direct the system on how to produce the desired output files. Dependencies of the rules are determined automatically, based on the input and output files defined. The integration of *Conda* package management system ("Anaconda Software Distribution," 2020) handles the software dependencies of each workflow step. The pipeline adopts the "lazy" approach to fulfilling the rules, resolving them backwards from the final desired output file, and scanning the data folder for results of previous completed steps. This means an interruption of analysis can be resumed without the repetition of previously successful steps. Self-contained reports provide full transparency from results down to used steps, parameters, code, and software.

The pipeline uses minimalistic requirements in its configuration. After the initial installation of *Snakemake* and it's requirements (Köster et al., 2021), a simple markup language format file is filled by the user. The input includes the VCF file of the population in study, and the three letter codes of populations from IGSR (Fairley et al., 2020) project. If there is no need to perfom all of the steps of the latter analysis, the configuration file can be given a simple binarry variable to enable-disable specific analytical steps. The pipeline follows the order of commands, provided in **Figure 1**.

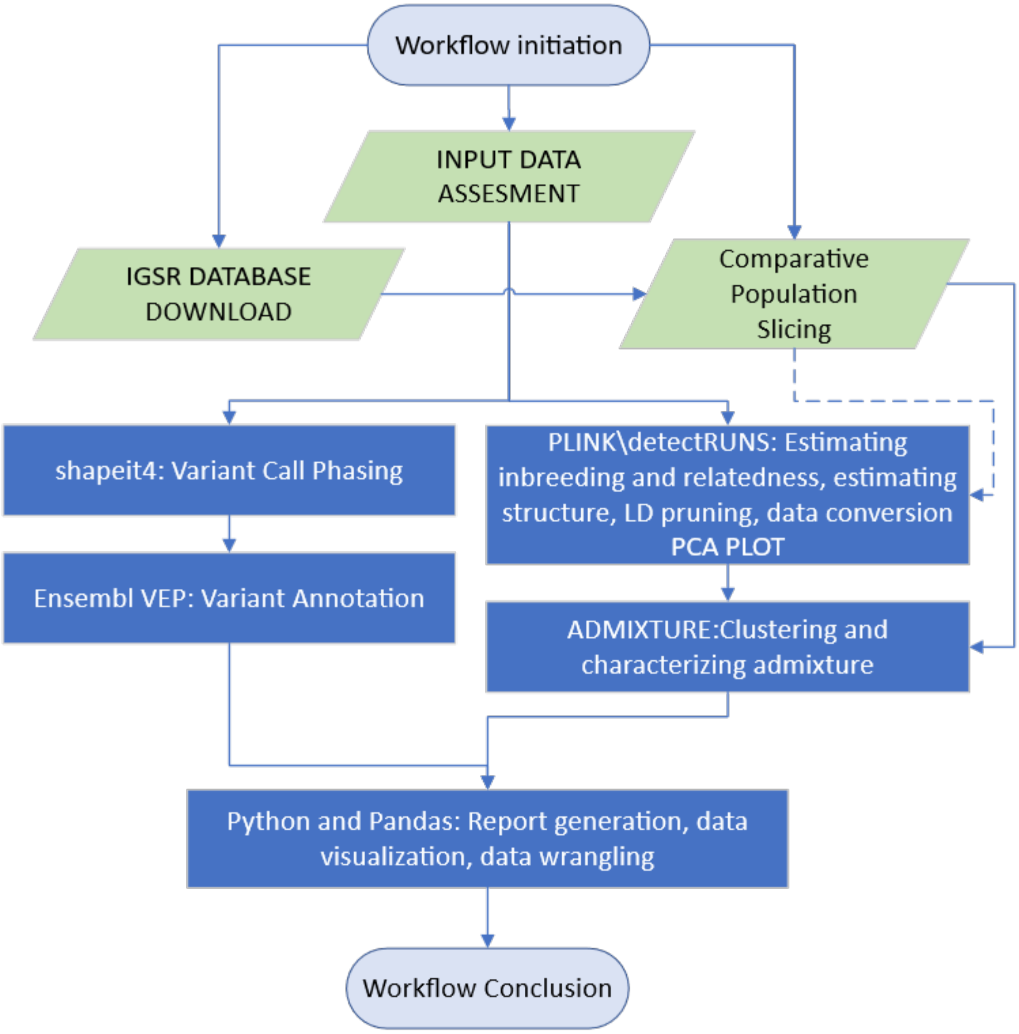

Workflow initiation
INPUT DATA ASSESMENT
IGSR DATABASE DOWNLOAD
Comparative Population Slicing
shapeit4: Variant Call Phasing
PLINK\detectRUNS: Estimating inbreeding and relatedness, estimating structure, LD pruning, data conversion PCA PLOT
Ensembl VEP: Variant Annotation
ADMIXTURE:Clustering and characterizing admixture
Python and Pandas: Report generation, data visualization, data wrangling
Workflow Conclusion

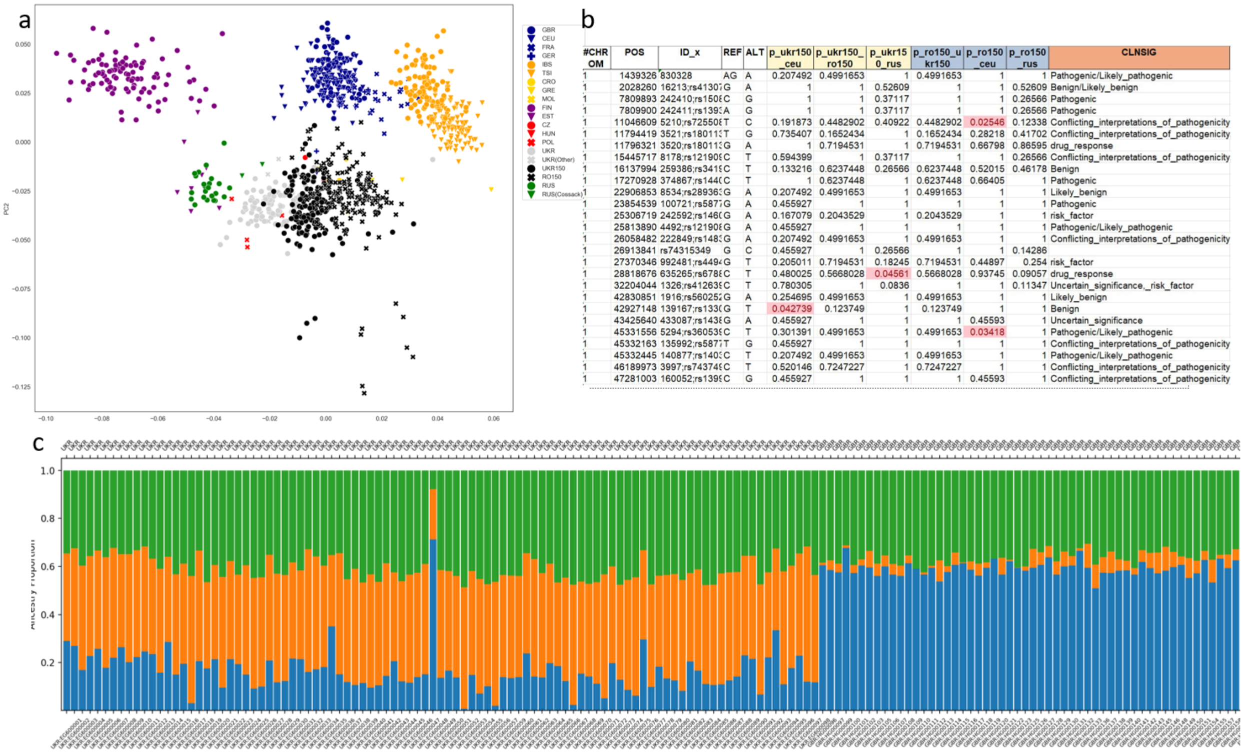
Figure 1. The schematic outline of the PopGenPlayground (PGP) pipeline

Results

Originally intended to replicate the results of the existing national genomics project analysis, the PGP pipeline can replicate the complete of the population genomics analysis performed in that study (Oleksyk et al., 2021). Using the pipeline replicates requires a minimal expertise, only the basic understanding of Unix shell-like command line. PGP leverages existing datasets and produces output providing valuable discoveries for the projects.

PGP provides a summary of variation population whole-genome sequences, offering insights into the total sequence reads, mean coverage, and variations such as SNPs, bi-allelic, multi-allelic, small indels, deletions, insertions, and structural variants. Additional table describes the summary annotation of different genomic elements, detailing the number of alleles and their distribution across various genomic locations like exons, introns, and intergenic regions. Lastly, it provides a dataset of comparative pairwise analysis of the population study, and all populations extracted from the IGSR (Fairley et al., 2020), highlighting the variants statistical different in their frequencies according to a Fisher Exact test.

Additionally, PGP produces various figures and associated datasets, using Python programming language, and various data analysis libraries. Clustering analysis results are summarized in PCA (**Figure 2.A**) and ADMIXTURE plots (**Figure 2.C**), illustrating the relationship between the samples within the population, and their comparative analysis with populations extracted from the IGSR. Variant calls with annotation and pairwise Fisher Exact Test (FET) information for allele frequencies are generated in a text table format. An example of a pre-publication figure output produced by PGP is shown in **Figure 2.**



#CHROM	POS	ID_x	REF	ALT	p_ukr150_ceu	p_ukr150_ro150	p_ukr150_rus	p_ro150_ukr150	p_ro150_ceu	p_ro150_rus	CLNSIG
1	1439326	830328	AG	A	0.207492	0.4991653	1	0.4991653	1	1	Pathogenic/Likely_pathogenic
1	2028260	16213;rs41307	G	A	1	1	0.52609	1	1	0.52609	Benign/Likely_benign
1	7809893	242410;rs1508	C	G	1	1	0.37117	1	1	0.26566	Pathogenic
1	7809900	242411;rs1393	A	G	1	1	0.37117	1	1	0.26566	Pathogenic
1	11046609	5210;rs72550	T	C	0.191873	0.4482902	0.40922	0.4482902	0.02546	0.12338	Conflicting_interpretations_of_pathogenicity
1	11794419	3521;rs180113	T	G	0.735407	0.1652434	1	0.1652434	0.28218	0.41702	Conflicting_interpretations_of_pathogenicity
1	11796321	3520;rs180113	G	A	1	0.7194531	1	0.7194531	0.66798	0.86595	drug_response
1	15445717	8178;rs121909	C	T	0.594399	1	0.37117	1	1	0.26566	Conflicting_interpretations_of_pathogenicity
1	16137994	259386;rs3419	C	T	0.133216	0.6237448	0.26566	0.6237448	0.52015	0.46178	Benign
1	17270928	374867;rs1440	C	T	1	0.6237448	1	0.6237448	0.66405	1	Pathogenic
1	22906853	8534;rs289363	G	A	0.207492	0.4991653	1	0.4991653	1	1	Likely_benign
1	23854539	100721;rs5877	G	A	0.455927	1	1	1	1	1	Pathogenic
1	25306719	242592;rs1460	G	A	0.167079	0.2043529	1	0.2043529	1	1	risk_factor
1	25813890	4492;rs121908	G	A	0.455927	1	1	1	1	1	Pathogenic/Likely_pathogenic
1	26058482	222849;rs1483	G	A	0.207492	0.4991653	1	0.4991653	1	1	Conflicting_interpretations_of_pathogenicity
1	26913841	rs74315349	G	C	0.455927	1	0.26566	1	1	0.14286	
1	27370346	992481;rs4494	G	T	0.205011	0.7194531	0.18245	0.7194531	0.44897	0.254	risk_factor
1	28818676	635265;rs6788	C	T	0.480025	0.5668028	0.04561	0.5668028	0.93745	0.09057	drug_response
1	32204044	1326;rs412639	C	T	0.780305	1	0.0836	1	1	0.11347	Uncertain_significance,_risk_factor
1	42830851	1916;rs560252	G	A	0.254695	0.4991653	1	0.4991653	1	1	Likely_benign
1	42927148	139167;rs1330	G	T	0.042739	0.123749	1	0.123749	1	1	Benign
1	43425640	433087;rs1438	G	A	0.455927	1	1	1	0.45593	1	Uncertain_significance
1	45331556	5294;rs360539	C	T	0.301391	0.4991653	1	0.4991653	0.03418	1	Pathogenic/Likely_pathogenic
1	45332163	135992;rs5877	T	G	0.455927	1	1	1	1	1	Conflicting_interpretations_of_pathogenicity
1	45332445	140877;rs1403	C	T	0.207492	0.4991653	1	0.4991653	1	1	Pathogenic/Likely_pathogenic
1	46189973	3997;rs743749	C	T	0.520146	0.7247227	1	0.7247227	1	1	Conflicting_interpretations_of_pathogenicity
1	47281003	160052;rs1399	C	G	0.455927	1	1	1	0.45593	1	Conflicting_interpretations_of_pathogenicity

Figure 2. An example of the output produced by the *PopGenPlayground (PGP)*. a) PCA plot of populations in study combined with populations from the IGSR database (Fairley et al., 2020); b) Variant calls with annotation and pairwise Fisher Exact Test information for allele frequencies; c) A fragment of the Admixture plot of the population in study and IGSR-extracted population.

Conclusions and future directions

To answer the rising demand in expertise and low accessibility of modern population genomics analysis approaches, we developed a bioinformatic pipeline *PopGenPlayground* (PGP). *PGP* is a robust bioinformatics tool providing user-friendly and efficient access to population genomics analyses. While utilizing the *Snakemake* workflow management system, PGP effectively combines diverse data types and integrates multiple bioinformatics tools, streamlining the analysis process. The design of the pipeline is based on previous expertise of analysis in a published national genomics project (Oleksyk et al., 2021) and integrates various public genomic databases for comparative analysis and variant annotation. Hosted on GitHub (Wolfsberger, 2023), PGP promotes collaboration and future growth of the pipeline. The field of population genomics is constantly changing, with new tools and important datasets being incorporated in them. Availability of new tools that can be incorporated into the analysis ensures the continuous development for the future growth of PGP pipeline, integrating more recent datasets, and expanding its analysis profile to include new instruments and data analyses.

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Declarations

Authors Contributions

W.W.W. developed the pipeline and wrote the first draft. W.W.W. and K.S. prepared and analyzed the data. All other authors contributed ideas to the development of the pipeline and reviewed the ppeline and the manuscript. T.K.O. contributed to the original ideas, writing, and final editing of the manuscript.

Software Availability

The complete pipeline and instructions for PopGenPlayground (PGP) pipeline use are available from the GitHub (*https://github.com/wwolfsberger/OU_popgen_playground*)(Wolfsberger, 2023)

List of Abbreviations

PGP: PopGenPlayground; VCF: Variant Call Format.

Competing Interests

The authors declare that they have no competing interests.

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