COMMUNICATIONS

WGSA: an annotation pipeline for human genome sequencing studies

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DNA sequencing technologies continue to make progress in both increased throughput and quality, and decreased cost. As we transition from whole exome capture sequencing to whole genome sequencing (WGS), our ability to convert machine-generated variant calls, including single nucleotide variant (SNV) and insertion-deletion variants (indels), into human-interpretable knowledge has lagged far behind the ability to obtain enormous amounts of variants. To help narrow this gap, here we present WGSA (WGS Annotator), an functional annotation pipeline for human genome sequencing studies, which is runnable out-of-box on the Amazon Compute Cloud and freely downloadable at https://sites.google.com/site/jpopgen/wgsa/.

Functional annotation is a key step in a WGS analysis. In one way, annotation helps the analyst filter to a subset of elements of particular interest (e.g., cell type specific enhancers), in another way annotation helps the investigators to increase the power of identifying phenotype-associated loci (e.g., association test using functional prediction score as a weight) and interpret potentially interesting findings. Currently, there are several popular gene model based annotation tools, including ANNOVAR [1], SnpEff [2] and the Ensembl Variant Effect Predictor (VEP) [3]. These can annotate a variety of gene models both protein coding and non-coding from a range of species. It is well known among practitioners that different databases (e.g., RefSeq [4] and Ensembl [5]) use different models for the same gene. Even when the same gene structure is implemented, predicted consequences of a given variant from different annotation tools may not be in agreement [6]. Therefore, it has been suggested to obtain annotation from tools across multiple databases for a more complete interpretation of the variants discovered in WGS [6]. Annotations of both coding and non-coding variants include scores pertaining to functionality, conservation, population allele frequencies and disease-related annotations, i.e., known disease-causing variants and disease-associated variants identified in genome-wide association analyses (GWAS). Recent large-scale epigenomics projects provide rich datasets of cell-specific regulatory elements. Unfortunately, there are currently few tools available to integrate all those functional annotation resources and provide a convenient and efficient pipeline for annotating millions of variants discovered in a WGS study.

To facilitate the functional annotation step of WGS, we developed WGSA. Currently WGSA supports the annotation of SNVs and indels locally without remote database requests, allowing it to scale up for large WGS studies. The overview of the WGSA pipeline is presented in **figure 1**. The complete list of the resources (and their references) contained in WGSA can be found in **online supplementary table S1**. For gene-model based annotation, WGSA integrates the outputs from three annotation tools (ANNOVAR, SnpEff and VEP) versus two databases (RefSeq and Ensembl), and provides a summary of variant consequences from the six annotation results. To further speed up the process for large-scale WGS studies, we have pre-computed annotations for all potential human SNVs (a total of 8,584,031,106) based on human reference hg19 non-N bases and use it as a local database. For SNV-centric resources, WGSA integrates five functional prediction scores, eight conservation scores, allele frequencies from four large-scale sequencing studies, variants in four disease-related databases, among others (**figure 1** and **online supplementary table S1**). For regulatory region-centric resources, WGSA includes cell type specific transcription factor binding sites, DNAse I hypersensitivity regions and chromosome activity predictions from three epigenomics projects (**figure 1** and **online supplementary table S1**). WGSA also contains rich functional annotations for non-synonymous SNVs and genes from our dbNSFP database [7, 8].

Annotating the consequences of indels raises special challenges. In addition to the allele frequencies and consequences predicted by the three annotation tools (ANNOVAR, SnpEff and VEP), we take an approach to first “translate” an indel to local SNVs by incorporating their effect (insertion, deletion, replacement) on nearby flanking sequences, annotating those SNVs with other available resources, and then summarizing these results for the indel (**figure 1**, **online supplementary figure S1** and details in **online supplementary notes**). Although this approach is a simplification of potentially complicate impact of an indel, it will provide additional information on an indel regarding the focal region it resides, such as whether it is a STR (short tandem repeat) mutation, whether it breaks a TFBS (transcription factor binding site), local functional prediction scores, local conservation scores, etc.

To provide convenient access for a broad community, we have built an Amazon Machine Image (AMI) for running WGSA on the cloud via Amazon Web Services (AWS). To run WGSA, the user only needs to upload a variant list file (or a .vcf file) and a configuration file, containing a list of the resources to be included in the annotation. The annotation pipeline can be run with just two command line calls. We also provide WGSA as a downloadable version at https://sites.google.com/site/jpopgen/wgsa for bioinformaticians who prefer to build WGSA locally. It can be used as a foundation resource for customized annotation pipelines, as is the case for Baylor College of Medicine's Human Genome Sequencing Center annotation software, Cassandra (https://www.hgsc.bcm.edu/software/cassandra). The runtime of two experimental runs with 46.6 million variants is shown in **online supplementary table S2**. WGSA was written in Java so that most of its annotation modules (the whole SNV annotation and most of the indel annotation except VEP) can be easily run across different platforms. Detailed protocols for using WGSA in the cloud and building it locally can be found in **online supplementary notes** and at https://sites.google.com/site/jpopgen/wgsa/. As changes inevitably occur in the source databases as well as new annotation resources emerge, WGSA will be updated in response. Users will be able to receive update notice and detailed instruction on updating steps (for local version).

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Competing interests The authors declare that they have no competing interests.

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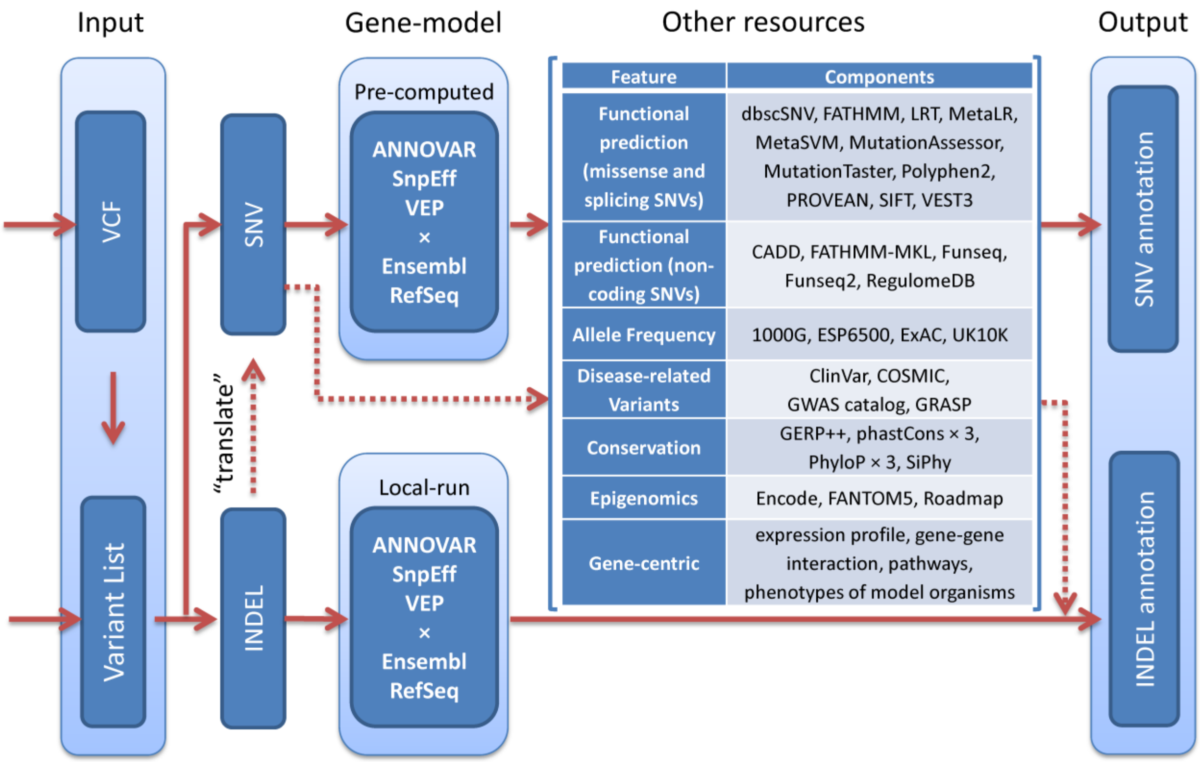

Feature	Components
Functional prediction (missense and splicing SNVs)	dbscSNV, FATHMM, LRT, MetaLR, MetaSVM, MutationAssessor, MutationTaster, Polyphen2, PROVEAN, SIFT, VEST3
Functional prediction (non-coding SNVs)	CADD, FATHMM-MKL, Funseq, Funseq2, RegulomeDB
Allele Frequency	1000G, ESP6500, ExAC, UK10K
Disease-related Variants	ClinVar, COSMIC, GWAS catalog, GRASP
Conservation	GERP++, phastCons × 3, PhyloP × 3, SiPhy
Epigenomics	Encode, FANTOM5, Roadmap
Gene-centric	expression profile, gene-gene interaction, pathways, phenotypes of model organisms

Figure 1: Flowchart of the WGSA annotation pipeline. Dotted lines show the “detour” of the indel annotation via the SNV annotation pipes.