

Oncotator: Cancer Variant Annotation Tool

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ABSTRACT: Oncotator is a tool for annotating genomic point mutations and short nucleotide insertions/deletions (indels) with variant- and gene-centric information relevant to cancer researchers. This information is drawn from 14 different publicly available resources that have been pooled and indexed, and we provide an extensible framework to add additional data sources. Annotations linked to variants range from basic information, such as gene names and functional classification (e.g. missense), to cancer-specific data from resources such as the Catalogue of Somatic Mutations in Cancer (COSMIC), the Cancer Gene Census, and The Cancer Genome Atlas (TCGA). For local use, Oncotator is freely available as a python module hosted on Github (<https://github.com/broadinstitute/oncotator>). Furthermore, Oncotator is also available as a web service and web application at <http://www.broadinstitute.org/oncotator/>. ©2015 Wiley-Liss, Inc.

KEY WORDS: oncotator, annotation, cancer, database, TCGA

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INTRODUCTION

Variant annotation, the aggregation and reporting of data relevant to a given genomic alteration, is a key step in a sequencing data analysis pipeline and is crucial for subsequent interpretation of detected variants. Genome sequencing of cancer samples typically reveal thousands to tens of thousands of somatic mutations per tumor that are often unique to the individual tumor (equivalent to ‘singletons’ in a germline analysis) (Lawrence et al., 2013). Therefore, researchers rely on annotations to filter variants to a subset of alterations that are most important to a given study or application. At the most basic level, variant annotations help researchers identify the genes, transcripts, and genomic regions pertaining to a given variant, as well as predict the impact an alteration has on the translated protein product of a gene. With the emergence of large databases of germline (Sherry et al., 2001; Landrum et al., 2013; NHLBI GO Exome Sequencing Project, 2014) and somatic (Forbes et al., 2010) variation, synthesis of all available clinical and biological information for single variants greatly empowers researchers to distinguish driver mutations from passengers (Imielinski et al., 2014), link specific variants to patient phenotypes (Van Allen et al., 2013; Biesecker and Green, 2014), and uncover unexpected oncogenic mechanisms shared across diseases (Lawrence et al., 2013). For example, variant annotations such as the frequency of a mutation in published cancer genomic studies, or whether a significant functional effect is predicted by algorithms such as Polyphen-2 or SIFT (Kumar et al., 2009; Adzhubei et al., 2010), can be utilized to interpret and prioritize variants. For clinicians, variant annotations can be of immense aid for clinical interpretation of variants as annotations can identify genetic events associated with cancer prognosis/diagnosis or drug sensitivity/resistance (Van Allen et al., 2013; Biesecker and Green et al., 2014).

Numerous tools, such as ANNOVAR, SnpEff, and Variant Effect Predictor, exist for annotating sequencing variants; however, many were developed for general non-cancer applications (Le Pera et al., 2010; McLaren et al., 2010; Wang et al., 2010; Cingolani et al., 2011; Sana et al., 2011). Although cancer sequencing studies use many of these tools, variants may lack cancer-specific annotations that can aid in downstream interpretation.

Here we report Oncotator, a cancer variant annotation pipeline implemented as a command line tool, as well as a web application, which provides both an interactive user interface and a programmatic web service. As currently deployed, Oncotator allows users to annotate variants with a pre-packaged bundle of cancer-relevant information in a single step. Oncotator has been used internally in the Broad Institute’s Cancer Genome Analysis pipeline since 2011, resulting in its use in over 20 published cancer studies, including several large scale (>100 tumors) efforts conducted by TCGA, NHGRI, TARGET, and the Slim Initiative for Genomic Medicine collaboration (Bass et al., 2011; Berger et al., 2011; Cancer Genome Atlas Research Network, 2011, 2013, 2014; Chapman et al., 2011; Hammerman et al., 2011; Stransky et al., 2011; Wang et al., 2011; Banerji et al., 2012; Barbieri et al., 2012; Barretina et al., 2012; Berger et al., 2012; Hodis et al., 2012; Imielinski et al., 2012; Lee et al., 2012; Lohr et al., 2012; Pugh et al., 2012, 2013, 2014; Ciriello et al., 2013; Francis et al., 2013; Lawrence et al., 2013; Ojesina et al., 2013). The goal of this article is to make the scientific community aware of the first public release of Oncotator (version 1.5) that is free to non-profit users. In the past two years, large parts of Oncotator were refactored to support: (i) highly optimized annotations; (ii) customizable data sources; and (iii) deployment outside of the Broad Institute environment.

METHODS

As a starting point for annotation, Oncotator requires the genomic position, reference allele, and variant allele as input in TSV, VCF (Danecek et al., 2011), or muTect call_stats (Cibulskis et al., 2013) formats. Variants are currently annotated with data from 14 different resources (Table 1), described briefly below. Annotated variants can be output in TCGA MAF or VCF formats, regardless of which input format is used (with the unintended consequence that researchers often use the tool for format conversion). Names and versions of the datasources are included in the header of output files.

Oncotator uses a local indexed database of reference transcripts derived from GENCODE to map variants to specific genes (Harrow et al., 2012). Each variant is assigned a “variant classification” (e.g. “Splice_Site” or “Nonsense_Mutation”) based on the mutation’s position relative to an overlapping gene and the expected consequence, if any, that the mutation has on a translated protein product. The model of reference transcript selection used is user-defined by a command line argument or can be left to automatically use the model with the greatest deleterious effect. Variant classification terms defined by the TCGA are used and nomenclature adheres to specifications defined by the Human Genome Variation Society (<http://www.hgvs.org/mutnomen>) (Dunnen et al., 2000).

In addition to basic transcript annotations described above, Oncotator will annotate variants with annotations derived from sources that can be beneficial to researchers looking to prioritize variants. To identify common Single Nucleotide Polymorphism (SNP) variants (which are less likely to contribute to tumorigenesis), Oncotator utilizes data from dbSNP, 1000 Genomes Project, and National Heart, Lung, and Blood Institute’s Exome Sequence Project (Sherry et al., 2001; 1000 Genomes Project Consortium. 2010; NHLBI GO Exome Sequencing Project, 2014). Oncotator can also annotate variants with the local GC content (within 100 base-pairs, by default) and surrounding nucleotide context (within 10 base-pairs, by default). Such annotations can be helpful for identifying biological mutational processes with sequence-specific mutation (Lawrence et al., 2013; Alexandrov et

al., 2013) or artifactual mutation biases such as oxidation of guanine bases during sequencing library construction (OxoG) (Costello et al., 2013).

Table 1. Oncotator Datasources

Annotation Category	Resource	URL	Comments
Genomic	GENCODE	http://www.encodegenes.org/	GENCODE/ENSEMBL transcripts and annotations for hg19
	ref_context		Can be used for artifact inference
	gc_content		Can be used for artifact inference
	Human DNA Repair Genes	http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html	Alteration in such genes can help explain higher overall mutation rates in specific samples
Protein	UniProt	http://www.uniprot.org/	Includes Drugbank & GO annotations
	dbNSFP	https://sites.google.com/site/jpopgen/dbNSFP	Contains pre-computed conservation scores, prediction classifications, and other information
Cancer Variant	COSMIC	http://www.sanger.ac.uk/genetics/CGP/cosmic/	
	Cancer Gene Census	http://www.sanger.ac.uk/genetics/CGP/Census/	
	CCLE	http://www.broadinstitute.org/ccle/home	Cancer cell line annotations. Can be used to identify cell line models containing variants of interest
	Familial Cancer Database	http://www.familialcancerdatabase.nl/	
	ClinVar	http://www.ncbi.nlm.nih.gov/clinvar/	
Non-Cancer Variant	dbSNP	http://www.ncbi.nlm.nih.gov/projects/SNP/	b142 release for human (9606)
	1000 Genomes	http://www.1000genomes.org/data	Phase 3 variant set
	NHLBI GO Exome Sequencing Project (ESP)	https://esp.gs.washington.edu/drupal/	

Predicting the functional impact of somatic mutations in cancer can be aided by mapping coding DNA sequence variants in genes onto amino acid sequences and proteins they encode. For example, knowledge of the specific protein regions that variants affect can be used to identify particular protein domains or active sites that are enriched for mutations across multiple samples or even across genes containing similar domains. To this end, Oncotator can annotate genomic variants with protein-specific annotations derived from UniProt human protein sequence records (UniProt Consortium, 2011). Oncotator maps genomic variants to protein position-based annotations derived from the feature table section of a UniProt record. Protein annotations added include “region” (e.g. protein kinase domain), “site” (e.g. ATP binding site), “natural variation” (e.g. Y → F in Pfeiffer syndrome), and “experimental” (e.g. Y → F: 50% decrease in interaction with PIK3C2B) data, if available. Furthermore, UniProt records are utilized to derive Gene Ontology (GO) annotations, describing the biological process, cellular component, and molecular function of a gene; and DrugBank annotations, pertaining to small molecules known to target the protein of interest (Knox et al., 2011). Through the dbNSFP (Liu et al., 2011) Oncotator datasource, variants can also be annotated with pre-computed results derived from many functional prediction and conservation score algorithms (PolyPhen-2, SIFT, MutationTaster, Mutation Assessor, FATHMM, LRT, SiPhy, GERP++ and PhyloP), which can be used to classify variants most likely to have an impact on a protein’s function (Siepel et al., 2006; Chun and Fay, 2009; Garber et al., 2009; Kumar et al., 2009; Adzhubei et al., 2010; Davydov et al., 2010; Schwarz et al., 2010; Reva et al., 2011; Shihab et al., 2013).

Oncotator also annotates variants with data from several cancer-specific resources that may aid in interpreting variants. Using data from COSMIC, Oncotator identifies variants reported in published studies and reports their observed frequency across all cancers and within each tissue type (Forbes et al., 2010). Overlapping breakpoint and fusion genes in COSMIC are also provided. Cancer researchers can also benefit from knowledge of relevant cancer cell line models in which to perform follow-up in vitro experiments with. To this end, Oncotator utilizes data from the Cancer Cell Line Encyclopedia to identify if a variant has been previously observed in a cell line (Barretina et al., 2012) (<http://www.broadinstitute.org/ccle/home>). Other cancer-specific resources utilized include the Cancer Gene Census (Futreal et al., 2004), ClinVar (Landrum et al., 2014), the Familial Cancer Database (<http://www.familialcancerdatabase.nl/>), and a curated set of DNA repair genes (Wood et al., 2005).

IMPLEMENTATION

Oncotator is available as a command line tool written in the Python programming language (<https://www.python.org/>). This tool is recommended for advanced users and is ready for inclusion into automated pipelines, since the annotation options, selection of data sources, and file formats are more flexible. The Oncotator software is an annotation framework that is broken into a three-stage workflow: (i) convert the input data into an internal model of mutations; (ii) annotate the mutation objects with a collection of pre-processed datasources (which can be locus-, variant- or gene-specific); and (iii) render the mutations to the specified output format (VCF or MAF). The software architecture encapsulates each step, which allows easy implementation of input and output formats by decoupling file formats from the actual annotation engine. The encapsulation also eased the development of hundreds of automated test modules, some testing hundreds of scenarios, that allow developers to make code changes and be confident that their changes have not unintentionally broken previous functionality (regression tests). Using the default corpus of datasources on a machine with a solid-state drive, Oncotator will take approximately 30 minutes to annotate 100,000 variants (~18 ms per variant). This runtime can be greatly reduced to approximately 11 minutes (~6.6 ms per variant) if a cache is utilized (with the ‘-u’ option).

We recognize that researchers would like to extend variant annotations beyond the current available datasources in Oncotator. Therefore, we included in Oncotator tools for creating new datasources from TSV, VCF, and GTF files. Most of the default Oncotator datasources were created using these tools. New versions of datasources are created from scratch and are not updated in-place, however, this typically only requires the updated data and making minor adjustments to the datasource creation command. Users are encouraged to contribute to the project via a publicly available Github repository (<https://github.com/broadinstitute/oncotator>). Although the tool was initially developed for cancer research, Oncotator can address non-cancer needs and additional non-cancer datasources can be easily introduced. Periodically, we make updated and new datasources available, as part of the versioned default corpus. In the future, we plan to add datasources specific to whole genome sequencing analysis, such as conservation scores outside of the exome, as well as add functionality for annotation of genomic regions.

Oncotator is also available as a web application at <http://www.broadinstitute.org/oncotator/>. Users can input a tab-delimited text file containing genomic coordinates and allele genotypes for each variant. Annotation results are presented as interactive tables. Annotation runtimes using the web application vary according to the input size but users can monitor the time to completion with a continuously updated progress bar on the report page. Users can also download a tab-delimited file containing multiple columns corresponding to the different annotations that are aggregated. The Oncotator web service is implemented using a REST-like architecture to facilitate integration with existing applications and pipelines. Users can also retrieve variant annotations programmatically using HTTP requests in the form: http://www.broadinstitute.org/oncotator/mutation/<chromosome>_<start_position>_<end_position>_<reference_allele>_<observed_allele>. Results are returned as JSON objects (<http://json.org/>) which can be easily parsed by users.

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