**Supplementary Table 1**. Databases and prediction tools in use for effector gene mining.

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| --- | --- | --- | --- |
| **Name** | **Description** | **URL** | **References** |
| **Fungal gene prediction and annotation** | | | |
| Blast2GO | A platform for functional annotation of genomic datasets. | www.blast2go.com/ | Conesa et al., 2005 |
| Evidence  Modeller | EVM integrates different prediction results into a consensus gene set. | evidencemodeler.github.io | Haas et al., 2008 |
| Galaxy | A platform designed with a user-friendly interface for researchers, could be used for effector mining. | usegalaxy.org/ | Blankenberg et al., 2010 |
| TransDecoder | Predict open reading frame from transcript sequences. | github.com/TransDecoder/TransDecoder/wiki | Haas et al., 2013 |
| SnowyOwl | An analysis pipeline customized to fungal gene prediction. | snowyowl.sourceforge.io | Reid et al., 2014 |
| CodingQuarry | Gene predictor in fungal genomes to capture most effector genes. | codingquarry.sourceforge.io | Testa et al., 2015 |
| BlastKOALA | A KEGG’s tool for annotating genome and metagenome sequences. | www.kegg.jp/blastkoala | Kanehisa et al., 2016 |
| AmiGO 2 | Gene ontology and annotation database. | amigo.geneontology.org/amigo | Carbon and Mungall, 2018 |
| BRAKER2 | An automatic protein coding gene prediction for fungal genome. | github.com/Gaius-Augustus/BRAKER | Brůna et al., 2021 |
| DESeq | Differential gene expression analysis based on the negative binomial distribution. Effectors are characterized by high expression in infection/stage specific/haustoria expressed. | bioconductor.org/packages/DESeq2 | 10.18129/B9.bioc.DESeq2 |
| **Analysis of homologs or orthologs** | | | |
| PSI-BLAST | Provide a means of detecting distant relationships between proteins. | ncbi.nlm.nih.gov/BLAST | Altschul et al., 1997 |
| HHblits | Detect protein sequence homologues via HMM-HMM alignment. | github.com/soedinglab/hh-suite | Remmert et al., 2012 |
| NCBI-BLAST | General sequence identification and similarity searches. | blast.ncbi.nlm.nih.gov/Blast.cgi | Boratyn et al., 2013 |
| HMMER | Search sequence databases for homologs and make alignments. | www.ebi.ac.uk/Tools/hmmer | Potter et al., 2018 |
| Orthovenn2 | Identify and compare orthologous genes clusters from multiple species. | orthovenn2.bioinfotoolkits.net | Xu et al., 2019 |
| EffectorDB | Database of predicted rare orthologous groups or lateral gene transfer groups in fungal effectors. | effectordb.com |  |
| **Analysis of conserved motif, domain, and protein family** | | | |
| TribeMCL | A method for clustering proteins into protein families. | metacpan.org/pod/Bio::Tools::Run::TribeMCL | Enright et al., 2003 |
| MEME Suite | Motif discovery, enrichment, and analysis tools. | meme-suite.org | Bailey et al., 2015 |
| NCBI-CDD | ‘Conserved Domain Database’ and domain analysis tool. | ncbi.nlm.nih.gov/cdd | Lu et al., 2020 |
| InterPro | Classify proteins into families and predict domains. | www.ebi.ac.uk/interpro | Blum et al., 2021 |
| Pfam 35.0 | Database for finding conserved and annotated domains in proteins. | pfam.xfam.org | Mistry et al., 2021 |
| SMART | A tool for detecting protein domains and domain architectures. | smart.embl.de | Letunic et al., 2021 |
| **Genomic analysis** | | | |
| MUMmer | General purpose pairwise genome alignment.  Useful for finding presence absence variations. | mummer.sourceforge.net | Kurtz et al., 2004 |
| PAML 4 | Maximum likelihood methods for detection of diversifying selection via CODEML. | abacus.gene.ucl.ac.uk/software/paml.html | Yang, 2007 |
| RIPCAL | Software for calculating repeat-induced point mutation in fungal genes. | sourceforge.net/projects/ripcal | Hane and Oliver, 2008 |
| SnpEff | General SNP annotation and methods for predicting SNP effects. | pcingola.github.io/SnpEff | Cingolani et al., 2012 |
| Progressive  Cactus | Multiple genome alignment appropriate for eukaryotic genomes.  Useful for finding presence absence variations. | github.com/glennhickey/progressiveCactus | Nguyen et al., 2014 |
| Occultercut | Detection of AT-rich regions in genomes, which may contain effectors. | sourceforge.net/projects/occultercut | Testa et al., 2016 |
| **Prediction of effector-like protein properties** | | | |
| LED | A database about lipases and lipase associated proteins. | www.led.uni-stuttgart.de | Fischer and Pleiss, 2003 |
| PredGPi | A Glycosylphosphatidylinositol (GPI) anchor predictor for fungal proteins. | gpcr.biocomp.unibo.it/predgpi/pred.htm | Pierleoni et al., 2008 |
| DFVF | Database of virulence factors in fungal pathogens. | sysbio.unl.edu/DFVF | Lu et al., 2012 |
| FungiDB | An integrated bioinformatics resource for fungi. | FungiDB.org | Stajich et al., 2012 |
| fPoxDB | ‘Fungal peroxidase database’ fabricated via a prediction on fungal genomes. | peroxidase.riceblast.snu.ac.kr | Choi et al., 2014 |
| dbCAN2 | A web of ‘Carbohydrate-active enzyme’ (CAZymes) for automated annotation. | bcb.unl.edu/dbCAN2 | Zhang et al., 2018 |
| MEROPS | Database for peptidases, inhibitors, and substrates of peptidases. | www.ebi.ac.uk/merops | Rawlings et al., 2018 |
| NetSurfP 2.0 | Predict the surface accessibility and secondary structure of protein sequence. | www.cbs.dtu.dk/services/NetSurfP | Klausen et al., 2019 |
| PSIPRED | Secondary structure prediction and various analysis. | bioinf.cs.ucl.ac.uk/psipred | Buchan and Jones, 2019 |
| PHI-base | ‘Pathogen Host Interaction database’ provides information about experimentally tested pathogenicity, virulence and effector genes of pathogens infecting various host organisms. | phi-base.org | Urban et al., 2020 |
| EffectorP 3.0 | Fungal effector prediction via machine learning method. | effectorp.csiro.au | Sperschneider et al., 2021 |
| NetGPI 1.1 | NetGPI is a prediction tool of GPI-anchoring or glypiation. | github.com/mhgislason/netgpi-1.1 | Gíslason et al., 2021 |
| CAZy | CAZymes database of enzymes that degrade, modify, or create glycosidic bonds. | www.cazy.org | Drula et al., 2022 |
| **Secretome analysis** | | | |
| SPScan | SPScan scans protein sequences for the presence of secretory signal peptides. | rothlab.ucdavis.edu/genhelp/spscan.html | Nielsen, et al., 1997 |
| PrediSi | An online software for the prediction of signal peptides. | predisi.de | Hiller et al., 2004 |
| SecretomeP | Ab initio prediction of non-classical protein secretion. | www.cbs.dtu.dk/services/SecretomeP | Bendtsen et al., 2005 |
| Signal-CF | A method for predicting protein signal peptide and its cleavage site. | www.csbio.sjtu.edu.cn/bioinf/Signal-CF | Chou and Shen, 2007 |
| FSD | A platform for studying fungal secretome using the data of stored fungal species. | fsd.snu.ac.kr | Choi et al., 2010 |
| FunSecKB | A database contains predicted fungal secreted proteins from NCBI RefSeq. | proteomics.ysu.edu/secretomes | Lum and Min, 2011 |
| Secretool | Prediction and characterization of fungal secreted proteins. | genomics.cicbiogune.es/SECRETOOL | Cortázar et al., 2014 |
| TOPCONS | Prediction of membrane protein topology and signal peptides. | octopus.cbr.su.se | Tsirigos et al., 2015 |
| SignalP 6.0 | N-terminus secretion signal prediction based on artificial neural networks and protein language models. A common first step in effector prediction. | www.cbs.dtu.dk/services/SignalP | Teufel et al., 2022 |
| **Prediction for removing secreted proteins with transmembrane domains** | | | |
| PRED-TMR2 | A system for the classification of transmembrane proteins. | athina.biol.uoa.gr/PRED-TMR2/input.html | Pasquier and Hamodrakas, 1999 |
| TMHMM 2.0 | Prediction of transmembrane helices of proteins. | www.cbs.dtu.dk/services/TMHMM | Krogh et al., 2001 |
| MINNOU | Sequence-based prediction of relative lipid accessibility. | minnou.cchmc.org | Cao et al., 2006 |
| Phobius | A combined transmembrane topology and signal peptide predictor. | phobius.sbc.su.se | Käll et al., 2007 |
| SOMRuler | A novel interpretable transmembrane helices predictor. | www.csbio.sjtu.edu.cn/bioinf/SOMRuler | Yu et al., 2011 |
| MemBrain | A web server developed for transmembrane protein structure prediction. | www.csbio.sjtu.edu.cn/bioinf/MemBrain | Feng et al., 2020 |
| **Subcellular localization prediction** | | | |
| ProtComp 9.0 | Predict the subcellular localization for fungi and plant proteins. | linux1.softberry.com |  |
| Cello | Prediction of subcellular localization of secretory proteins. | cello.life.nctu.edu.tw | Yu et al., 2006 |
| WoLF PSORT | Prediction of subcellular localization of proteins. | wolfpsort.hgc.jp | Horton et al., 2007 |
| cNLS Mapper | Prediction of nuclear localization signals in proteins. | nls-mapper.iab.keio.ac.jp | Kosugi et al., 2009 |
| YLoc | A prediction system for protein subcellular localization prediction. | github.com/KohlbacherLab/YLoc | Briesemeister et al., 2010 |
| Cello2GO | Prediction of subcellular localization of proteins with annotation. | cello.life.nctu.edu.tw/cello2go | Yu et al., 2014 |
| LOCALIZER | Subcellular localization prediction of plant and effector proteins. | localizer.csiro.au | Sperschneider et al., 2017 |
| ApoplastP | Prediction of proteins localized into apoplast region of plant. | apoplastp.csiro.au | Sperschneider et al., 2018 |
| TargetP 2.0 | Prediction of subcellular or extracellular localization of proteins. | www.cbs.dtu.dk/services/TargetP | Almagro Armenteros et al., 2019 |

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