7/12/2023

PhenoDB on Cavatica
User Guide

Overview

PhenoDB is a clinical research tool to analyze VCF files from individuals or families with suspected Mendelian disease. It is now available on Cavatica so data can be analyzed while it remains within the platform's security and computation resources.

Additional information about PhenoDB and its creators is available at available at https://phenodb.org/. Not all PhenoDB analysis functionality in the web version is implemented yet in the Cavatica app.

PhenoDB Apps on Cavatica

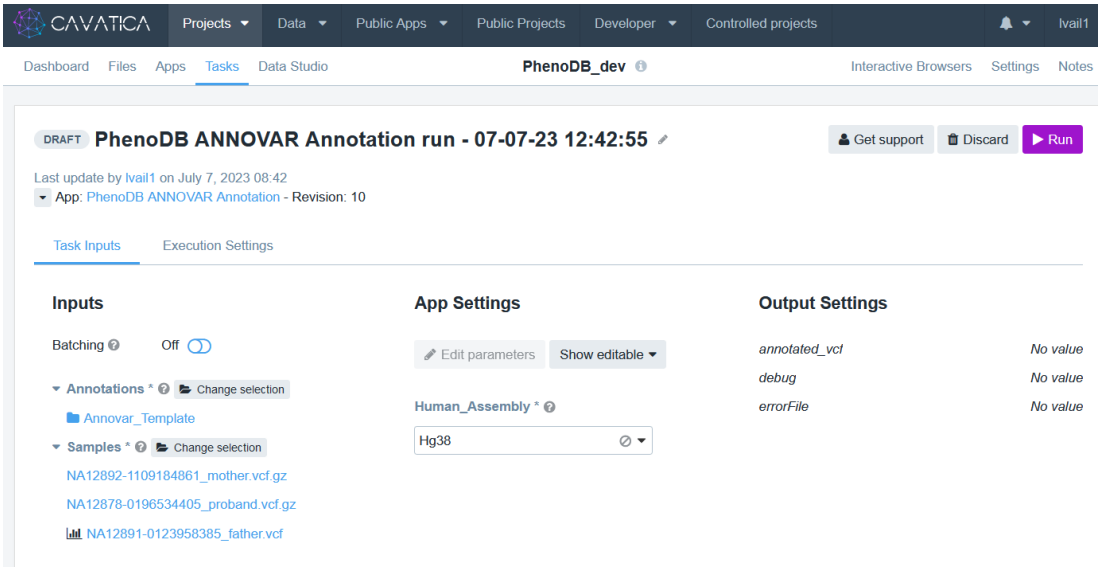
PhenoDB on Cavatica is run in two steps, each in a separate Cavatica app available in this Project

1. PhenoDB ANNOVAR Annotation: annotates VCF files
2. PhenoDB Analysis: finds variants consistent with user-selected inheritance patterns and other parameters.

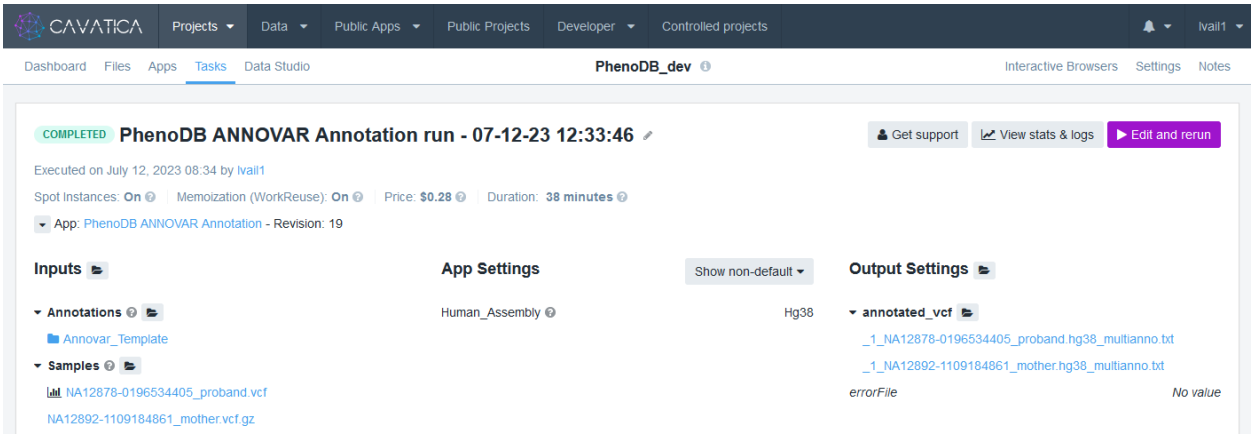
The example inputs used below are in the project's Files in folder 'PhenoDB_Sample_Files.' For assistance, email phenodb@jhmi.edu and Laura Vail (lvail1@jhmi.edu).

PhenoDB ANNOVAR Annotation: Steps

1. Begin with individual VCF files from one or more people
2. Enter your inputs, descriptions below



3. The task may take up to one hour or longer, depending on the size and number of the input files. When completed, there should be one new file for each VCF you entered, with “hg_38_multianno.txt” added to the end of the file's original name



4. These output files can be analyzed with the PhenoDB Analysis app

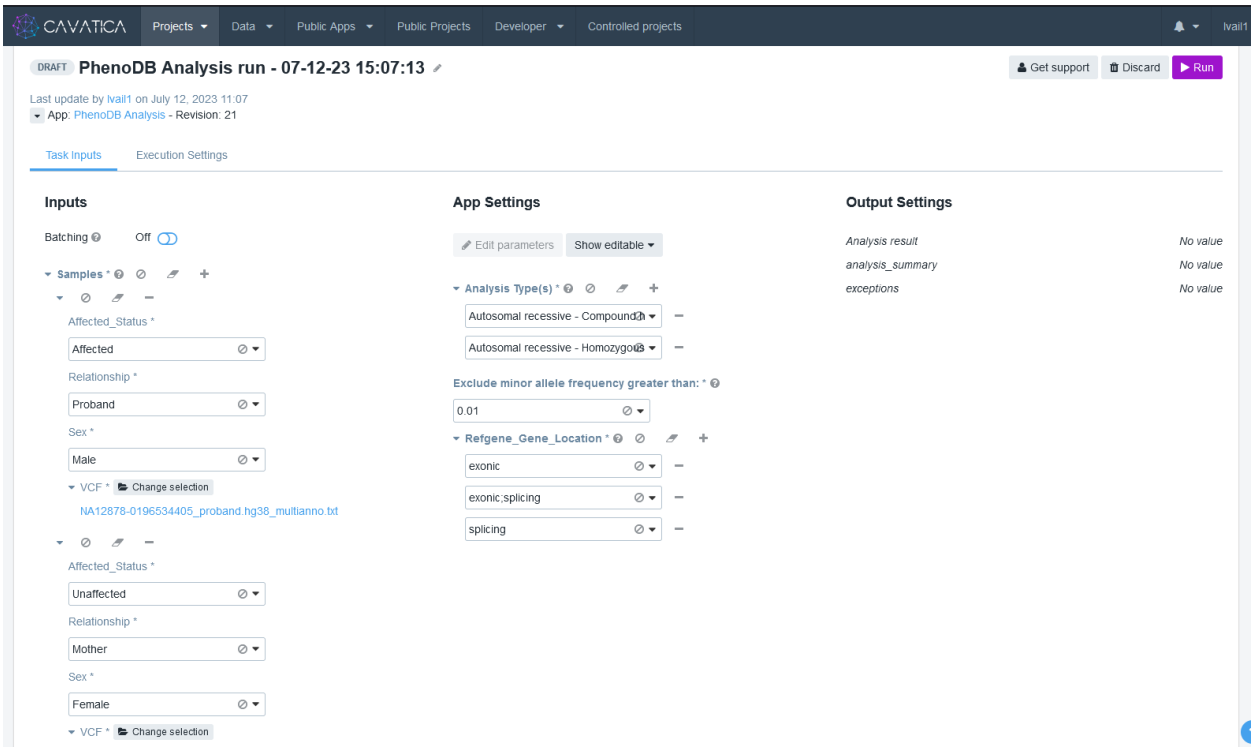
PhenoDB ANNOVAR Annotation: Inputs

- VCF File(s): Individual VCF files for all people included in the analysis
  - This step can be done for multiple families at once
  - The files can be either .gz compressed, or uncompressed
  - Set “Batching” to On for the VCFs, and select “Batch by: File.” This helps the process run faster
- Human_Assembly: Select either Hg19 or Hg38
- Annotations: ANNOVAR reference data to use for the annotation. Select the folder ‘Annovar_Template’ in the project’s Files

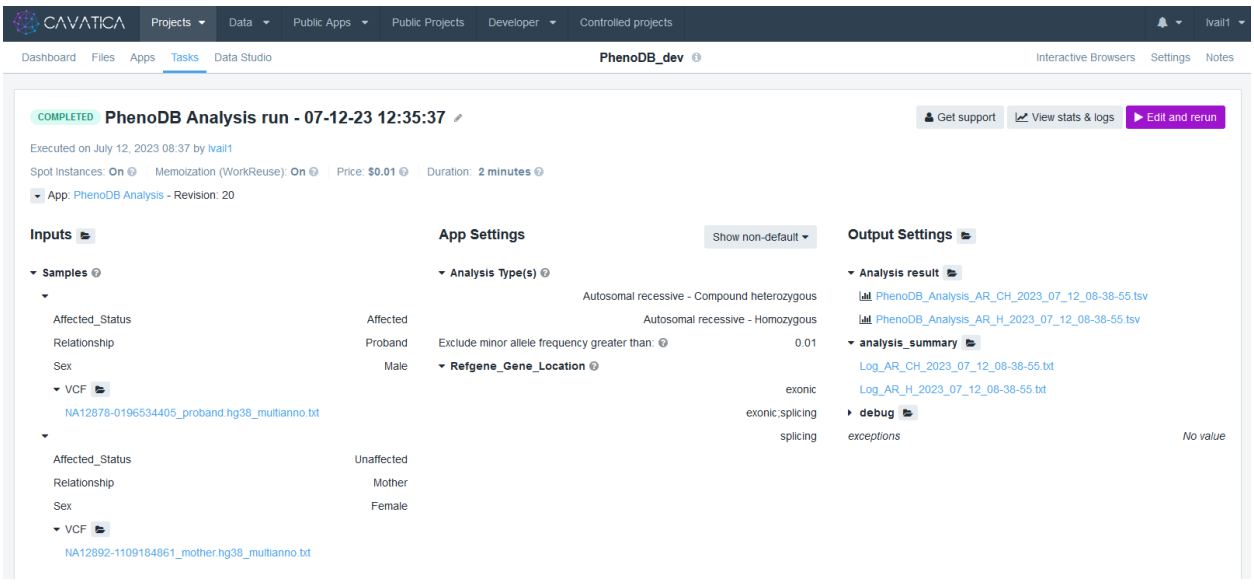
PhenoDB Analysis: Steps

1. Begin with annotated VCF files generated by the 'PhenoDB ANNOVAR Annotation' app

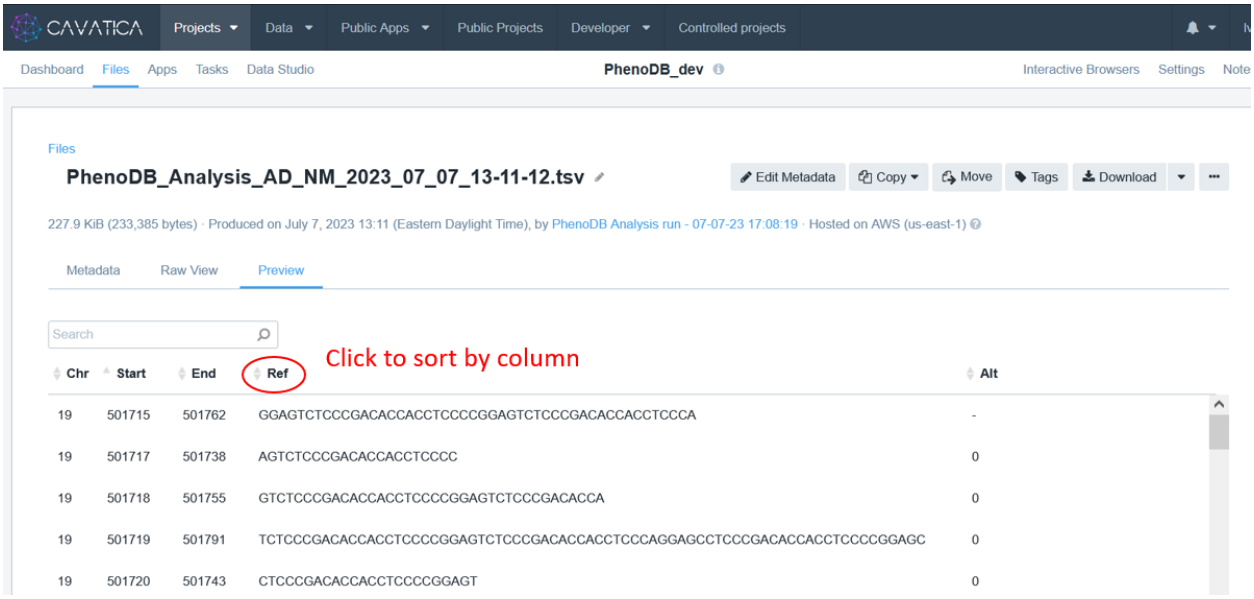
2. Enter your inputs, descriptions below



3. When complete, there should be two files for each selected analysis type: one ‘Analysis result’ tsv containing the variants of interest for that inheritance pattern, and one ‘Analysis summary’ file describing the filtering of the variants that led to that result



4. Cavatica has built-in preview for text files, including column sort in the .tsv analysis results. Click on the names of the files, and select “Preview” to view and sort the .tsv files, and “Raw View” for the .txt files. In the .tsv preview, dynamically sort the results by clicking on column headers



PhenoDB Analysis: Inputs

- Samples: One or more individuals to be analyzed together. Each Sample has four attributes to be entered:
    - Affected Status: Affected, Unaffected, or Unknown
    - Relationship: Proband, or relationship to proband (Mother, Father, Other Relative)
    - Sex: Male, Female, Unknown or not XX/XY
    - VCF: Individual's annotated VCF file from the 'PhenoDB ANNOVAR Annotation' app
- Analysis Type(s): Choose one or more inheritance patterns for the analysis, described below. Recommended default: Autosomal recessive compound heterozygous and Autosomal recessive homozygous
- Exclude minor allele frequency greater than: Choose your cutoff point. Data sources are the ExAC, esp6500siv2, 1000g2014oct, and 1000g2015aug databases. Recommended default: 0.01
- RefGene gene location: Select one or more to include. Recommended default, three locations: "exonic", "exonic;splicing", and "splicing"
- Inheritance Types: Choose as appropriate for your analysis from the below descriptions. Recommended default:

Inhertiance Types: Description and Samples Required

AR_CH: Autosomal Recessive Compound Heterozygous

- Requires: At least proband

- Variants in Result: Includes only the heterozygous variants identified in the proband (assumed to be affected); if there is more than one affected family members, the analysis include only the variants that are identified in all affected members; next, the analysis includes only the genes that have more than one variant in the proband but if the same set of variants in a gene is found in one of the parents or in other unaffected family member then this gene (and its variants) is excluded of the analysis (Sobreira et al., 2015)

AR_H: Autosomal Recessive Homozygous

- Requires: At least proband
- Variants in Result: Identifies homozygous variants that are shared by all affected individuals and excludes variants that are homozygous in an unaffected individual (Sobreira et al., 2015)

AD_NM: Autosomal Dominant New Mutation

- Requires: Proband and two parents (trio), and neither parent is affected
- Variants in Result: Includes heterozygous variants that are identified in the proband but not in the parents (Sobreira et al., 2015)

AD_IM: Autosomal Dominant Inherited Mutation

- Requires: Proband plus at least one affected or unaffected relative, not necessarily a parent
- Variants in Result: Retains heterozygous variants that are shared by affected individuals and excludes those found in unaffected individuals (Sobreira et al., 2015)

AD_V: Autosomal Dominant Variant

- Requires: Should be used when only one individual is being analyzed.
- Variants in Result: Retains heterozygous variants with a minor allele frequency (MAF) less than the threshold selected for the ExAC, esp6500siv2, 1000g2014oct, and 1000g2015aug databases