

# CompoundHetVIP Usage Example

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## Introduction

This example takes users through the steps needed to take a VCF or gVCF file and identify Compound Heterozygous (*CH*) variants. This pipeline is generalized and depending on the data state, some pipeline steps may not be necessary. The data used in this example are from [Genome in a Bottle](#), for an [Ashkenazi Trio](#) (A trio is data from the affected child and both parents). The files are in VCF format and were aligned to GRCh38. However, this pipeline can also process gVCF or VCF files aligned to GRCh37.

The source code for CompoundHetVIP is available at <https://github.com/dmiller903/CompoundHetVIP> and the Docker image is available at <https://hub.docker.com/repository/docker/dmiller903/compound-het-vip>.

The steps of this pipeline assume that you have already installed Docker on the system where you will be processing your data. If you have not installed Docker, see the [Docker Engine overview](#) for installation instructions. In addition, it is assumed that the VCF files used as input have 1-based positions rather than 0-based.

## Set-up

**Pull Docker Image** All steps of the pipeline will use the same Docker image. The Docker image has all the necessary tools for any given step of the pipeline. To obtain the image, use the “docker pull” command.

```
docker pull dmiller903/compound-het-vip:1.0
```

**Download VCF File for Each Member of the Trio** As mentioned in the Introduction, the VCF files that need to be downloaded for this example are for an [Ashkenazi Trio](#). Follow the steps below to obtain the data. **NOTE: ftp links may change over time, if the links below do not work, get the files from: <ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/AshkenazimTrio/>**

```
# Create a directory for the Genome in a Bottle data
mkdir /example/giab

# Download vcf for father
wget -nv ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/AshkenazimTrio/\
HG003_NA24149_father/latest/GRCh38/HG003_GRCh38_GIAB_highconf_CG-Illfb-\
IllsentieonHC-Ion-10XsentieonHC_CHROM1-22_v.3.3.2_highconf.vcf.gz \
-O /example/giab/giab_father.vcf.gz

# Download vcf for mother
wget -nv ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/AshkenazimTrio/\
HG004_NA24143_mother/latest/GRCh38/HG004_GRCh38_GIAB_highconf_CG-Illfb-\
```

```

IllsentieonHC-Ion-10XsentieonHC_CHROM1-22_v.3.3.2_highconf.vcf.gz \
-O /example/giab/giab_mother.vcf.gz

# Download vcf for child
wget -nv ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/release/AshkenazimTrio/\
HG002_NA24385_son/latest/GRCh38/supplementaryFiles/HG002_GRCh38_CHROM1-22_v4.1_highconf.vcf.gz \
-O /example/giab/giab_child.vcf.gz

```

## Step 1: Keep variant-only sites of VCF or gVCF files

This example uses a VCF, but a gVCF may also be used. If input files are VCF and no parent files are available, then this script does not need to be used. However, if files are VCF files and parent files are available, the script will filter each parent VCF file for sites that are in common between the parent and child.

gVCF files are different from VCF files in that they contain information for every position, including non-variant positions. Therefore, these files are large and would take a long time to process if the non-variant positions were included throughout the whole compound heterozygous pipeline. Therefore, if gVCF files are used, this step takes a patient gVCF file and removes all non-variant sites. It outputs the file to a path specified by the user. If parent files for the patient are included, the script will filter each parent gVCF file for sites that are in common between the parent and child.

**Execute Script** The “keep\_variant\_sites.py” script is needed for this step and takes 2 required arguments: 1) the input sample gVCF or VCF file 2) the path where the output should be saved. The optional arguments are: 1) `-parent_1_file`, which is the maternal or paternal gVCF or VCF of the sample 2) `-parent_2_file`, which is the other maternal or paternal gVCF or VCF of the sample. 3) `-output_suffix` which is the suffix for each output file. The default is set to “\_parsed.vcf.gz” 4) `-is_gvcf` which indicates whether or not a gVCF file is used. The default is set to “y”. When argument is set to “y”, all non-variant sites will be filtered out of the sample file and a new VCF will be created.

The “docker run” command used for this and subsequent steps use various options. The “-d” option allows the container to run in the background and will save a log number where you direct it to with “>” as seen below. The “-v” option allows you to mount a volume from your local or remote machine to the container. For example, “-v /example:/proj” allows the container to access the “example” directory and all sub directories and is known as “/proj” in the container. “-w” sets the working directory inside the container. At the end of the docker run command, “parse” is the name of the image to execute as a container. Everything after this line, up until “>”, will be executed in the container.

```

docker run -d -v /example:/proj -w /proj -t dmill903/compound-het-vip:1.0 \
python3 /keep_variant_sites.py \
giab/giab_child.vcf.gz \
giab/ \
--parent_1_file giab/giab_father.vcf.gz \
--parent_2_file giab/giab_mother.vcf.gz \
--is_gvcf n \
> giab/keep_variant_sites.out

#This is not necessary but allows you to see any output log information in real-time
CONTAINERID="cat giab/keep_variant_sites.out"
docker logs -f '$CONTAINERID'

```

## Step 2: Combine each trio into a single file

This step is necessary if you have trio data (data from child and both parents). During this step, the VCF of the parents and child will be combined into a single VCF file. This is done so that later, when phasing, a single trio file can be phased.

**Set a new empty directory where reference files are to be downloaded** The script used below executes *GATK4* to combine gVCF files of trios, or *bcftools* to combine VCF files of trios. *GATK4* requires reference files and *bcftools* does not. If using gVCF files, the script will install necessary reference files in the path you indicate with “-v”. This example does not use gVCF files, but if it did, you would create a new empty sub directory within the directory you are mounting to /proj. For example:

```
mkdir /example/giab/references
```

**Execute Script** The “combine\_trio.py” script is needed for this step and takes 4 arguments: 1) sample VCF or gVCF file 2) maternal or paternal VCF or gVCF file 3) other maternal or paternal VCF or gVCF file 4) the name of the combined output file with .gz included at the end. An optional argument `-is_gvcf`, indicates if the files were originally gVCF or not. The default is set to yes, “y”. For this example, this argument is set to “n”.

```
docker run -d -v /example:/proj -w /proj -t dmill903/compound-het-vip:1.0 \  
python3 /combine_trio.py \  
giab/giab_child.vcf.gz \  
giab/giab_father_parsed.vcf.gz \  
giab/giab_mother_parsed.vcf.gz \  
giab/giab_trio.vcf.gz \  
--is_gvcf n \  
> giab/combine_trio.out
```

```
CONTAINERID="cat giab/combine_trio.out"  
docker logs -f '$CONTAINERID'
```

If gVCF files are being use, another “-v” needs to be set to where you want the reference files saved. Above I created the sub directory “references” in my “/example” path. In this case, another “-v” needs to be added, “-v /example/giab/references:/references”. The portion before the colon (:) is where the files will be saved and the portion after the colon is where that path is located in the container and this portion NEEDS to be “/references” for the script to work properly. Notice in the below example, another -v has been added, and `-is_gvcf` has been changed to “y”. **Note: The below code is an example and should not be executed with the example dataset since the example dataset is not gVCF.**

```
docker run -d -v /example:/proj -v /example/giab/references:/references \  
-w /proj -t dmill903/compound-het-vip:1.0 \  
python3 /combine_trio.py \  
giab/giab_child.gvcf.gz \  
giab/giab_father_parsed.gvcf.gz \  
giab/giab_mother_parsed.gvcf.gz \  
giab/giab_trio.vcf.gz \  
--is_gvcf y \  
> giab/combine_trio.out
```

```
CONTAINERID="cat giab/combine_trio.out"  
docker logs -f '$CONTAINERID'
```

### Step 3: Liftover trio files and individual files from GRCh38 to GRCh37

Some phasing programs and other programs such as *GEMINI*, require that VCF or gVCF files be aligned to GRCh37. Therefore, this step takes an input VCF that is aligned to GRCh38 converts it to GRCh37 positions. **Note: If you skipped steps 1-2, and are using this as your first step, you may encounter a “Java, array error” when executing liftover. If you do, ensure that only chromosomes 1-22, and X/Y are in the file. If there are any other chromosomes, liftover may not complete.**

**Use the previously defined reference file directory or set a new empty directory where reference files are to be downloaded** The script used below executes *Picard*’s Liftover tool to convert the file from GRCh38 to GRCh37. *Picard* requires reference files to accomplish liftover. If not already done above, create a new empty sub directory within the directory you are mounting to /proj. For example:

```
mkdir /example/giab/references
```

If you already did this above, you can use the same directory you already created.

**Execute Script** The “liftover.py” script is needed for this step and takes 2 arguments: 1) an input VCF file 2) the name of the output VCF file with .gz included at the end of the name. Another “-v” needs to be set to where you want the reference files saved. Above I created the sub directory “references” in my “/example” path. In this case, another “-v” needs to be added, “-v /example/giab/references:/references”. The portion before the colon (:) is where the files will be saved and the portion after the colon is where that path is located in the container and this portion NEEDS to be “/references” for the script to work properly.

```
docker run -d -v /example:/proj -v /example/giab/references:/references -w /proj \
-t dm11903/compound-het-vip:1.0 \
python3 /liftover.py \
giab/giab_trio.vcf.gz \
giab/giab_trio_GRCh37.vcf.gz \
> giab/liftover.out
```

```
CONTAINERID="cat giab/liftover.out"
docker logs -f '$CONTAINERID'
```

### Step 4: Remove unplaced, and multiallelic sites and duplicate sites from lifted files

During liftover, some randomly placed sites are included in the VCF file. These randomly placed sites are those that are in GRCh38 but the exact position in GRCh37 is not known. Therefore, for subsequent analysis, these sites are removed. Only sites with known positions, on a known chromosome are kept. In addition, positions that are multiallelic or are duplicates are removed because programs such as PLINK (used next) and SHAPEIT2 (used later) can not handle these types of sites. Sites that contain more than one sample with missing genotype information (i.e. “./.”) are removed to improve phasing accuracy.

**Execute Script** The “remove\_unplaced\_multiallelic.py” script is needed for this step and takes 2 arguments: 1) the input VCF file and the 2) the output VCF file.

```
docker run -d -v /example:/proj -w /proj -t dm11903/compound-het-vip:1.0 \
python3 /remove_unplaced_multiallelic.py \
giab/giab_trio_GRCh37.vcf.gz \
```

```

giab/giab_trio_GRCh37_no_unplaced_or_duplicates.vcf.gz \
> giab/remove_unplaced_multiallelic.out

CONTAINERID="cat giab/remove_unplaced_multiallelic.out"
docker logs -f '$CONTAINERID'

```

## Step 5: Separate VCF file into chromosome files, then generate plink files for each chromosome file

Phasing programs require that chromosomes be phased separately. Some phasing programs, such as *SHAPEIT2*, require PLINK files in order to phase. Therefore, this script separates a VCF into chromosome VCF files. This step also generates the necessary PLINK files needed for phasing (bed, bim, fam).

**Execute Script** The “separate\_chr\_generate\_plink.py” script is needed for this step and takes 2 required arguments: 1) input VCF file 2) the prefix name of output files (no suffix, e.g. “giab/file1”). If using a trio, the optional argument, `-fam_file`, needs to be used to create appropriate PLINK files. If no fam file is included, *PLINK* will output a generic fam file.

```

# Example of how to create fam file
touch giab/giab_trio.fam
echo "giab_trio HG003 0 0 1 1
giab_trio HG004 0 0 2 1
giab_trio INTEGRATION HG003 HG004 1 2" > giab/giab_trio.fam

# Execute container
docker run -d -v /example:/proj -w /proj -t dmill903/compound-het-vip:1.0 \
python3 /separate_chr_generate_plink.py \
giab/giab_trio_GRCh37_no_unplaced_or_duplicates.vcf.gz \
giab/giab_GRCh37 \
--fam_file giab/giab_trio.fam \
> giab/separate_chr_generate_plink.out

CONTAINERID="cat giab/separate_chr_generate_plink.out"
docker logs -f '$CONTAINERID'

```

## Step 6: Phase each of the trios with a haplotype reference panel using SHAPEIT2, Beagle, or Eagle

This pipeline can phase with *SHAPEIT2*, *Eagle2*, and *Beagle*. All programs will integrate 1000 Genomes Project phase 3 haplotype reference panel. The files used for phasing can be found at [https://mathgen.stats.ox.ac.uk/impute/1000GP\\_Phase3.html](https://mathgen.stats.ox.ac.uk/impute/1000GP_Phase3.html) and <ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/>. Files from these sites will be downloaded to your local/remote machine, to the path you designate, the first time one of the phasing scripts is ran.

**Use the previously defined reference file directory or set a new empty directory where reference files are to be downloaded** If not already done above, create a new empty sub directory within the directory you are mounting to /proj. For example:

```
mkdir /example/giab/references
```

If you already did this above, you can use the same directory you created.

### Phase with SHAPEIT2 (Recommended to use as default)

The default parameters for phasing are set so that *SHAPEIT2* uses family relationship genotype information and also uses a haplotype reference panel. *SHAPEIT2* needs to phase each chromosome separately. So this part can be done one chromosome at a time, or scripted so that the user does not need to input each individual chromosome.

The “phase\_with\_shapeit.py” script is needed for this step and takes 3 arguments: 1) the path and name of the files (Either plink files (bed, bim, fam) or a VCF file. Plink files should be used if a trio and no suffix should be included in file name (e.g. giab/giab\_GRCh35\_chr21). For non-trios, VCF can be used as input, and any file suffixes should be included (e.g. giab/giab\_GRCh37\_chr21.vcf.gz)) 2) The output path and name without suffix. 3) the chromosome number to be phased. If the input file used is not a trio, then the optional parameter “-is\_trio” can be set to “n”. If “-is\_trio” is set to “n”, then the input file needs to be a VCF file (include suffix). In the docker command, another “-v” needs to be set to where you want the reference files saved. Above I created the sub directory “references” in my “/example” path. In this case, another “-v” needs to be added, “-v /example/giab/references:/references”. The portion before the colon (:) is where the files will be saved and the portion after the colon is where that path is located in the container and this portion NEEDS to be “/references” for the script to work properly.

```
docker run -d -v /example:/proj -v /example/giab/references:/references -w /proj \
-t dmill903/compound-het-vip:1.0 \
python3 /phase_with_shapeit.py \
giab/giab_GRCh37_chr21 \
giab/giab_GRCh37_chr21_shapeit \
21 \
> giab/giab_GRCh37_chr21_shapeit.out
```

```
CONTAINERID=$(cat giab/giab_GRCh37_chr21_shapeit.out)
docker logs -f "$CONTAINERID"
```

If you want to script this out, you could do something like this with *Python*:

```
import os
for i in range(1, 23):
    os.system("docker run -d -v /example:/proj \
-v /example/giab/references:/references \
-w /proj -t dmill903/compound-het-vip:1.0 \
python3 /phase_with_shapeit.py \
giab/giab_GRCh37_chr{i} \
giab/giab_GRCh37_chr{i}_shapeit \
{i} \
> giab/phase_with_shapeit_chr{i}.out")
```

**Phase with Beagle (optional)** The parameters for phasing are set so that *Beagle* uses a haplotype reference panel. No family relationships are considered. The “phase\_with\_beagle.py” script is needed for this step and takes 3 arguments: 1) the path and name of the vcf file 2) The output path and name without suffix 3) the chromosome number to be phased. This script has not optional parameters. In the

docker command, another “-v” needs to be set to where you want the reference files saved. See “Phase with SHAPEIT2” subheading for an explanation of “-v” usage.

```
docker run -d -v /example:/proj -v /example/giab/references:/references -w /proj \
-t dmill903/compound-het-vip:1.0 \
python3 /phase_with_beagle.py \
giab/giab_GRCh37_chr21.vcf.gz \
giab/giab_GRCh37_chr21_beagle_phased \
21 \
> giab/phase_with_beagle_chr21.out

CONTAINERID="cat giab/phase_with_beagle_chr21.out"
docker logs -f '$CONTAINERID'
```

See “Phase with SHAPEIT2” for an example of how this could be scripted out to do multiple chromosomes with a single command.

**Phase with Eagle2 (optional)** The parameters for phasing are set so that *Eagle2* uses a haplotype reference panel. No family relationships are considered. The “phase\_with\_eagle.py” script is needed for this step and takes 3 arguments: 1) the path and name of the vcf file 2) The output path and name without suffix 3) the chromosome number to be phased. This script has not optional parameters. In the docker command, another “-v” needs to be set to where you want the reference files saved. See “Phase with SHAPEIT2” subheading for an explanation of “-v” usage.

```
docker run -d -v /example:/proj -v /example/giab/references:/references -w /proj \
-t dmill903/compound-het-vip:1.0 \
python3 /phase_with_eagle.py \
giab/giab_GRCh37_chr21.vcf.gz \
giab/giab_GRCh37_chr21_eagle_phased \
21 \
> giab/phase_with_eagle_chr21.out

CONTAINERID="cat giab/phase_with_eagle_chr21.out"
docker logs -f '$CONTAINERID'
```

See “Phase with SHAPEIT2” for an example of how this could be scripted out to do multiple chromosomes with a single command.

## Step 7: Revert REF/ALT to be congruent with reference panel

The phased results from *SHAPEIT2* can have the REF and ALT alleles switched as compared to the reference genome. We are unsure exactly why this occurs. For files with trios, it may be that since each file only has 3 samples, the ALT allele is more common in the trio and becomes the REF. This step ensures that the REF/ALT alleles of the phased VCF files are congruent with the REF/ALT of the reference genome. In addition, sites with Mendel errors are removed.

**Execute Script** The “alt\_ref\_revert.py” script is needed for this step and takes 3 arguments: 1) the input phased VCF file 2) the output VCF file name 3) the chromosome number which is needed so the script can determine which reference file to use. This step assumes you have phased with *SHAPEIT2* and that reference files have already been downloaded. In the docker command, another “-v” needs to be set to where you want the reference files saved. Above I created the sub directory “references” in my “/example” path.

In this case, another “-v” needs to be added, “-v /example/giab/references:/references”. The portion before the colon (:) is where the files will be saved and the portion after the colon is where that path is located in the container and this portion NEEDS to be “/references” for the script to work properly.

```
docker run -d -v /example:/proj -v /example/giab/references:/references -w /proj \
  -t dmiller903/compound-het-vip:1.0 \
  python3 /alt_ref_revert.py \
  giab/giab_GRCh37_chr21_shapeit.vcf.gz \
  giab/giab_GRCh37_chr21_shapeit_reverted.vcf.gz \
  21 \
  > giab/alt_ref_revert_shapeit_chr21.out

CONTAINERID="cat giab/alt_ref_revert_shapeit_chr21.out"
docker logs -f '$CONTAINERID'
```

See “Phase with SHAPEIT2” for an example of how this could be scripted out to do multiple chromosomes with a single command.

## Step 8: Concat and merge phased trio chromosome files into one VCF file

To make subsequent analysis of the phased files easier, this step concatenates all phased chromosomes into a single file using *bcftools*. If you have multiple sample files (e.g. trio1, trio2, trio3, etc.), this script can merge these files into one after concatenating chromosomes together using *bcftools*.

**Execute Script** The “concat\_merge\_phased\_vcf.py” script is needed for this step and takes 2 arguments: 1) A path where all the phased files are stored. Only phased files from a single phasing method should be in this folder. For this example, the files are phased with *SHAPEIT2*. 2) The name of the output file. If you are merging multiple VCF files into one after concatenating chromosomes, then use the optional argument “-merge\_files” and set it to “y”. Default is “n”. If you want a combined .fam file output (written in the order the samples appear in the VCF), then provide a name for this file with the “-output\_fam\_file” argument. Also, if “-merge\_files” and “-output\_fam\_file” are set to “y”, provide .fam file(s) in the same folder the phased files are in.

```
# Create folder where phased files will be moved to
mkdir /example/giab/phased_files
mv /example/giab/*_reverted.vcf.gz /example/giab/phased_files

# Execute docker container to concat files
docker run -d -v /example:/proj -w /proj -t dmiller903/compound-het-vip:1.0 \
  python3 /concat_merge_phased_vcf.py \
  giab/phased_files/ \
  giab/giab_combined.vcf.gz \
  > giab/concat_merge_phased_vcf.out

CONTAINERID="cat giab/concat_merge_phased_vcf.out"
docker logs -f '$CONTAINERID'
```

## Step 9: Trim and normalize VCF file

Prior to annotation and loading a database into *GEMINI* (used later for genotype queries), *GEMINI* recommends left trimming and normalizing VCF files. Files that are not left trimmed and normalized can be



annotated incorrectly and may be incorrectly handled by *GEMINI*. This step uses *vt* tools (using a python script) to trim and normalize the phased VCF file. *vt* tools uses reference files and these sites will be downloaded to your local/remote machine, to the path you designate, the first time one of the phasing scripts is ran.

**Use the previously defined reference file directory or set a new empty directory where reference files are to be downloaded** If not already done above, create a new empty sub directory within the directory you are mounting to /proj. For example:

```
mkdir /example/giab/references
```

If you already did this above, you can use the same directory you created.

**Execute Script** The “vt\_split\_trim\_left\_align.py” script is needed for this step and requires 2 arguments: 1) input VCF name 2) output VCF name. In the docker command, another “-v” needs to be set to where you want the reference files saved. Above I created the sub directory “references” in my “/example” path. In this case, another “-v” needs to be added, “-v /example/giab/references:/references”. The portion before the colon (:) is where the files will be saved and the portion after the colon is where that path is located in the container and this portion NEEDS to be “/references” for the script to work properly.

```
docker run -d -v /example:/proj -v /example/giab/references:/references -w /proj \
-t dmill903/compound-het-vip:1.0 \
python3 /vt_split_trim_left_align.py \
giab/giab_combined.vcf.gz \
giab/giab_combined_vt.vcf.gz \
> giab/vt_split_trim_left_align.out

CONTAINERID="cat giab/vt_split_trim_left_align.out"
docker logs -f '$CONTAINERID'
```

## Step 10: Annotate with snpEff

*snpEff* is used to annotate. Annotation provides information on the effects of variants on known genes. *GEMINI* allows users to explore genetic variation based on the annotations of the genome.

**Create a new, empty, sub directory within the references path created earlier** Using the same path set up earlier for references, add a sub directory to this path, such as “snpEff\_data”. This will help keep files organized but isn’t necessary.

```
mkdir /example/giab/references/snpEff_data
```

**Execute Script** The “annotate.py” script is needed for this step and requires 2 arguments: 1) name of input VCF 2) name of annotated VCF file. *snpEff* requires annotation reference files in order to annotate. These will be downloaded when the “annotate.py” script is executed for the first time. In the docker command, another “-v” needs to be set to where you want the reference files saved. Above I created the sub directory “snpEff\_data” in my “/example/giab/references” path. In this case, another “-v” needs to be added, “-v /example/giab/references/snpEff\_data:/snpEff/./data/GRCh37.75”. The portion before the colon (:) is where the files will be saved and the portion after the colon is where that path is located in the container and this portion NEEDS to be “/snpEff/./data/GRCh37.75” for the script to work properly.

```

docker run -d -v /example:/proj \
  -v /example/giab/references/snpEff_data:/snpEff/./data/GRCh37.75 -w /proj \
  -t dmill903/compound-het-vip:1.0 \
  python3 /annotate.py \
  giab/giab_combined_vt.vcf.gz \
  giab/giab_annotated.vcf \
  > giab/annotate.out

CONTAINERID="cat giab/annotate.out"
docker logs -f '$CONTAINERID'

```

## Step 11: Load VCF as GEMINI database

*GEMINI* is used to load the VCF into a GEMINI database. The created GEMINI database includes minor allele frequency, CADD score, and other information for each variant position. This information is used in Step 12 to query for *CH* variants.

**Create a new, empty, sub directory within the references path created earlier** Using the same path set up earlier for references, add a sub directory to this path, such as “gemini\_data”. This will help keep files organized but isn’t necessary.

```
mkdir /example/giab/references/gemini_data
```

**Execute Script** The “gemini\_load.py” script is needed for this step and requires 2 arguments: 1) name of annotated VCF file 2) Name of output database (name needs to end in .db). There are 3 optional arguments: 1) “-fam\_file” should be used if your VCF file contains family samples. GEMINI will use this file to keep track of family relationships and this allows for *CH* and *de Novo* variant identification. 2) GEMINI databases can take a while to be created, but can be significantly sped up with the “-num\_cores” argument. The default is 2. 3) snpEff is how the files are annotated in this pipeline, but if a user chooses to annotate outside of the pipeline with VEP, the “-anno\_program” can be used to indicate that VEP was used instead of snpEff. In order to load a GEMINI database, annotation files are necessary. In addition, CADD scores are required by us, not GEMINI, because many of our queries involve CADD scores. These files will be downloaded the first time the “gemini\_load.py” script is executed first time. In the docker command, another “-v” needs to be set to where you want the reference files saved. Above I created the sub directory “gemini\_data” in my “/example/giab/references” path. In this case, another “-v” needs to be added, “-v /example/giab/references/gemini\_data:/usr/local/share/gemini/gemini\_data”. The portion before the colon (:) is where the files will be saved and the portion after the colon is where that path is located in the container and this portion NEEDS to be “/usr/local/share/gemini/gemini\_data” for the script to work properly.

```

docker run -d -v /example:/proj \
  -v /example/giab/references/gemini_data:/usr/local/share/gemini/gemini_data \
  -w /proj -t dmill903/compound-het-vip:1.0 \
  python3 /gemini_load.py \
  giab/giab_annotated.vcf \
  giab/giab.db \
  --fam_file giab/giab_trio.fam \
  --num_cores 43 \
  > giab/gemini_load.out

```

```
CONTAINERID="cat giab/gemini_load.out"
docker logs -f '$CONTAINERID'
```

## Step 12: Identify *CH* variants

To identify *CH* variants, use “identify\_CH\_variants.py”. This script has 2 arguments: 1) name of GEMINI database to query 2) the output name prefix for query results. There are also 4 optional arguments: 1) “-cadd” which allows users to set the CADD cut-off. 15 is the default. 2) “-maf” which allows users to set the minor allele frequency cut-off. 0.01 is the default. 3) “-fam\_file” which is necessary when trios are being used. When a .fam file is used, the parental haplotypes are taken into consideration when identifying *CH* variants. If either parent is compound heterozygous or homozygous alternate in a gene where the child has a *CH* variant, the gene is not included in the final output. 4) “-upper\_bound” which allows for scenarios where one variant in a *CH* pair can be rare and the other common. This argument allows you to choose the highest maf that a variant can be. One of the variants in a *CH* pair can be as high as the value you set, and the other has to be below your “-maf” argument. 0.01 is the default for “-upper\_bound”.

```
docker run -d -v /example:/proj -w /proj -t dmill903/compound-het-vip:1.0 \
python3 /identify_CH_variants.py \
giab/giab.db \
giab/giab_CH_maf01_cadd15.tsv \
--fam_file giab/giab_trio.fam \
> giab/identify_CH_variants.out
```

```
CONTAINERID="cat giab/identify_CH_variants.out"
docker logs -f '$CONTAINERID'
```

We have also included scripts to identify *de Novo* (“identify\_deNovo\_variants.py”) and homozygous alternate (“identify\_homAlt\_variants.py”) variants. “identify\_homAlt\_variants.py” has the same required and optional arguments as the “identify\_CH\_variants.py” script. For “identify\_deNovo\_variants.py”, a .fam file is required, but all other required and optional arguments are the same as “identify\_CH\_variants.py” and “identify\_homAlt\_variants.py”. An example of its use is below:

```
docker run -d -v /example:/proj -w /proj -t dmill903/compound-het-vip:1.0 \
python3 /identify_deNovo_variants.py \
giab/giab.db \
giab/giab_deNovo_maf01_cadd15.tsv \
giab/giab_trio.fam \
> giab/identify_deNovo_variants.out
```

```
CONTAINERID="cat giab/identify_CH_variants.out"
docker logs -f '$CONTAINERID'
```

When using the “identify\_homAlt\_variants.py” and have trio data, if either parent has homozygous alternate variants in the same gene that the child has homozygous alternate variant(s) in, that gene is excluded in the final output.

## Step 13: Add Gene Damage Index Scores and Gene lengths to files

The Gene Damage Index (GDI) is a way to rank genes based on their likelihood of harboring disease-causing variants. The “add\_GDI\_and\_gene\_lengths.py” script adds GDI scores to a GEMINI queried file and also adds CDS and total gene length information. The “add\_GDI\_and\_gene\_lengths.py” script requires 2

arguments: 1) the input file, 2) the output file. The optional argument, “--anonymize” will anonymize some identifying information such as the actual patient ID, family ID, etc. The default is set to “n” (no).

```
docker run -d -v /example:/proj -w /proj -t dmill903/compound-het-vip:1.0 \  
python3 /add_GDI_and_gene_lengths.py \  
giab/giab_CH_maf01_cadd15.tsv \  
giab/giab_CH_maf01_cadd15_GDI.tsv \  
> giab/add_GDI_and_gene_lengths.out  
  
CONTAINERID="cat giab/add_GDI_and_gene_lengths.out"  
docker logs -f '$CONTAINERID'
```