trioPhaser - Supplementary

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Install Docker and download the triophaser Docker image

trioPhaser is executed within a Docker container that contains all necessary software involved in the phasing process. The container can be executed on any operating system as long as the Docker engine is installed on the users system. Instructions for installing Docker are found at https://docs.docker.com/desktop/. Once installed, the Docker image can be downloaded using the following command:

```
docker pull dmill903/triophaser:1.0
```

Phase data from the Genome in a Bottle Consoritum

Download .bam files from the Genome in a Bottle Consortium

We wanted to compare how well trioPhaser phased as compared to the 10X method which has been shown to be one of the most accurate phasing methods. The Genome in a Bottle (GIAB) Consortium contains various file types at different processing stages, therefore, we sought to use the aligned (GRCh38)/index BAM files to generate genotype data that could be input into trioPhaser. We selected the Ashkenazim trio to use in our comparison analysis and downloaded the BAM and BAI files for this trio as seen below:

```
# Create a new directory to store the GIAB files
docker run -v /Data:/proj -w /proj -t \
triophaser mkdir giab_bam

# Download .bam and .bai files for all members of the Ashkenazim trio
docker run -v /Data:/proj -w /proj -t \
triophaser wget --no-check-certificate ftp://ftp-trace.ncbi.nlm.nih.gov/\
ReferenceSamples/giab/data/AshkenazimTrio/analysis/\
10XGenomics_ChromiumGenome_LongRanger2.0_06202016/HG002_NA24385_son/\
NA24385_GRCh38.bam -O giab_bam/son_GRCh38.bam

docker run -v /Data:/proj -w /proj -t \
triophaser wget --no-check-certificate ftp://ftp-trace.ncbi.nlm.nih.gov/\
ReferenceSamples/giab/data/AshkenazimTrio/analysis/\
10XGenomics_ChromiumGenome_LongRanger2.0_06202016/HG002_NA24385_son/\
NA24385_GRCh38.bam.bai -O giab_bam/son_GRCh38.bam.bai

docker run -v /Data:/proj -w /proj -t \
triophaser wget --no-check-certificate ftp://ftp-trace.ncbi.nlm.nih.gov/\
ReferenceSamples/giab/data/AshkenazimTrio/analysis/\
10XGenomics_ChromiumGenome_LongRanger2.0_06202016/\
```

```
HG003_NA24149_father/NA24149_GRCh38.bam -O giab_bam/father_GRCh38.bam

docker run -v /Data:/proj -w /proj -t \
triophaser wget --no-check-certificate ftp://ftp-trace.ncbi.nlm.nih.gov/\
ReferenceSamples/giab/data/AshkenazimTrio/analysis/\
10XGenomics_ChromiumGenome_LongRanger2.0_06202016/\
HG003_NA24149_father/NA24149_GRCh38.bam.bai -O giab_bam/father_GRCh38.bam.bai

docker run -v /Data:/proj -w /proj -t \
triophaser wget --no-check-certificate ftp://ftp-trace.ncbi.nlm.nih.gov/\
ReferenceSamples/giab/data/AshkenazimTrio/analysis/\
10XGenomics_ChromiumGenome_LongRanger2.0_06202016/\
HG004_NA24143_mother/NA24143_GRCh38.bam -O giab_bam/mother_GRCh38.bam

docker run -v /Data:/proj -w /proj -t \
triophaser wget --no-check-certificate ftp://ftp-trace.ncbi.nlm.nih.gov/\
ReferenceSamples/giab/data/AshkenazimTrio/analysis/\
10XGenomics_ChromiumGenome_LongRanger2.0_06202016/\
HG004_NA24143_mother/NA24143_GRCh38.bam.bai -O giab_bam/mother_GRCh38.bam.bai
```

Generate gVCF files for the Ashkenazim trio

GATK's haplotypeCaller tool was used to generate gVCF files for each member of the trio using BAM and BAI files as input. Prior to executing GATK, the reference files (contained within the container) were decompressed so GATK could use them:

```
docker run -d -v /Data:/proj -w /proj -t dmill903/triophaser:1.0 /bin/bash -c \
"unzip /fasta_references.zip -d /fasta_references \
&& gzip -d /fasta_references/*.gz \
&& gatk --java-options -Xmx8g HaplotypeCaller \
-R /fasta_references/Homo_sapiens_assembly38.fasta \
-I giab_bam/son_GRCh38.bam \
-O giab_bam/son_GRCh38_test.g.vcf.gz -ERC GVCF" > genotype_son.out

docker run -d -v /Data:/proj -w /proj -t dmill903/triophaser:1.0 /bin/bash -c \
"unzip /fasta_references.zip -d /fasta_references \
&& gzip -d /fasta_references/*.gz \
&& gatk --java-options -Xmx8g HaplotypeCaller \
-R /fasta_references/Homo_sapiens_assembly38.fasta \
-I giab_bam/father_GRCh38.bam \
-O giab_bam/father_GRCh38_test.g.vcf.gz -ERC GVCF" > genotype_father.out

docker run -d -v /Data:/proj -w /proj -t dmill903/triophaser:1.0 /bin/bash -c \
"unzip /fasta_references.zip -d /fasta_references \
&& gzip -d /fasta_references/*.gz \
&& gatk --java-options -Xmx8g HaplotypeCaller \
-R /fasta_references/Homo_sapiens_assembly38.fasta \
-I giab_bam/mother_GRCh38.bam \
-O giab_bam/mother_GRCh38_test.g.vcf.gz -ERC GVCF" > genotype_mother.out
```

Run gVCF files through trioPhaser

Once the gVCF files were created using GATK, the Ashkenazim trio was phased using trioPhaser. "docker run -d -v /Data:/proj -w /proj -t dmill903/triophaser:1.0" is the Docker code needed to execute the container. Everything after this bit of code is being executed within and by the container. The "-d" option allows the container to run in "detached" mode; executing the container in the background. When the "-d" option is used, the container ID is output upon execution. The container ID is output to the terminal, or so you don't have to keep track of the ID, ">" can be used to store the container ID to a file (as done in the example below). On our machine, the GIAB data was stored within the /Data/giab_bam directory. Therefore, we attached the /Data directory to the container using the "-v" option. This allows the container to access all directories and files within the attached directory. We called this directory "/proj" within the container using ":". We then set the "/proj" directory as our working directory within the container using "-w". On our local machine, our working directory is "/Data". "-t" was used to allow the container to execute commands. "dmill903/triophaser:1.0" is the name of the container to execute. The "trio_phaser.py" script is located at the root directory within the container. The "trio_phaser.py" script has 6 required parameters: 1) The gVCF of the child, 2) the gVCF of the father, 3) the gVCF of the mother, 4) the name of the output file, 5) where the reference files are to be stored, and 6) the number of cores available for use. When "trio_phaser.py" is first executed, all necessary reference files are stored to the directory you specify with parameter 5. Therefore, the first time "trio_phaser.py" is executed, the run-time will take longer as reference files are downloaded. However, as long as you point future trioPhaser runs to this reference directory, the previously downloaded files will be used. The default parameter for #6 (the number of cores to use) is 2, however, the more cores you use (up to 22), the faster the run-time. When trioPhaser uses *SHAPEIT4* to phase, if 22 cores are available, all 22 autosomal chromosomes will be phased at once. Because trioPhaser is executed in the background, the log information that "trio_phaser.py" outputs will not be directly output to the terminal on your machine. Instead, all the log information is output within the container. To view the log information information output by "trio_phaser.py", the container ID can be used. In the code below, we save the container ID as a variable, then use the docker command "docker logs {container ID}" to view the log information output by "trio_phaser.py". "trio_phaser.py" outputs helpful information about how many initial variants there were, how many of the initial variants were phased, how many variants were phased by *SHAPEIT4*, how many variants were erroneously phased by *SHAPEIT4* and then correctly phased using Mendelian inheritance, how many variants were phased using Mendelian inheritance that *SHAPEIT4* was unable to phase, and how long trioPhaser took to execute.

```
docker run -d -v /Data:/proj -w /proj -t dmill903/triophaser:1.0 \
  python3 /trio_phaser.py giab_bam/son_GRCh38.g.vcf.gz \
  giab_bam/father_GRCh38.g.vcf.gz \
  giab_bam/mother_GRCh38.g.vcf.gz \
  giab_bam/giab_phased.vcf.gz \
  haplotype_references \
  22 > giab_bam/trio_phaser_giab.out

# Use the container ID to get the log of the "trio_phaser.py" output and store
# the log information to the same file where the container ID was stored.
CONTAINERID="cat giab_bam/trio_phaser_giab.out"
docker logs `$CONTAINERID` >> giab_bam/trio_phaser_giab.out
```

Since the gVCF files for the Ashkenazim trio are too large to upload to GitHub (>19 GB/per file), we have provided gVCF files for chromosome 22 that can be used to test trioPhaser. These files can be found at https://github.com/dmiller903/trioPhaser/tree/main/validate and within the Docker container at "/trioPhaser/validate/". These files do not include all chromosome 22 positions. Positions were removed so each file was less than 100 MB in size (max file size supported by GitHub). These example files can be executed using the commands below. Change "/Data" to the path you want the container to be able to access on your local machine (i.e. the path where the "haplotype_references" directory is located (or whatever you named this directory)). In this example, we set "/proj" as the working directory within the

container. This directory is equivalent to "/Data" on our local machine. We use "/Data" as the working directory on our local machine. Within the "/Data" directory, the "haplotype_references" directory exists. The output file "giab_phased_chr22.vcf.gz" will be found at "/Data/giab_phased_chr22.vcf.gz" on the local machine when trioPhaser is done executing. The log file, "trio_phaser_giab_chr22.out" will be at "/Data/trio_phaser_chr22.out".

```
docker run -d -v /Data:/proj -w /proj -t dmill903/triophaser:1.0 \
  python3 /trio_phaser.py /trioPhaser/validate/son_GRCh38_chr22.g.vcf.gz \
  /trioPhaser/validate/father_GRCh38_chr22.g.vcf.gz \
  /trioPhaser/validate/mother_GRCh38_chr22.g.vcf.gz \
  giab_phased_chr22.vcf.gz \
  haplotype_references \
  22 > trio_phaser_giab_chr22.out
```

Download the Ashkenazim child's 10X phased VCF file

The 10X-phased VCF file of the Ashkenazim child was needed in order to compared it to the trioPhaser-phased VCF file. The file was downloaded with the following code:

```
docker run -v /Data:/proj -w /proj -t dmill903/triophaser:1.0 \
  wget --no-check-certificate ftp://ftp-trace.ncbi.nlm.nih.gov/ReferenceSamples/\
  giab/data/AshkenazimTrio/analysis/\
  10XGenomics_ChromiumGenome_LongRanger2.0_06202016/\
  HG002_NA24385_son/NA24385_GRCh38.vcf.gz -O giab_bam/son_GRCh38_longRanger.vcf.gz
```

Compare trioPhaser phase results to 10X

The giab_phased.vcf.gz (output by trioPhaser) file was compared to the son_GRCh38_longRanger.vcf.gz (10X phased file) file using the "compare_trioPhaser_to_10X.py" script.

```
docker run -v /Data/KidsFirst/trioPhaser:/proj -w /proj -t \
  dmill903/triophaser:1.0 \
  python3 /trioPhaser/validate/compare_trioPhaser_to_10X.py \
  giab_bam/giab_phased.vcf.gz \
  giab_bam/son_GRCh38_longRanger.vcf.gz \
  giab_bam/
```

Phase data from the Gabriella Miller Kids First Data Resource Center

Download gVCF files from the Gabriella Miller Kids First Data Resource Center

Controlled-access gVCF files of 50 trios were downloaded from the Gabriella Miller Kids Frist (GMKF) Data Resource Center. These files were generated from WGS data of normal tissue. The child in each trio had neuroblastoma. Trios were stored in a directory named with the family ID (e.g. FM_AC8MB9RH), and gVCF file names were changed to indicate which family the individual belonged to (e.g. FM_AC8MB9RH) and family relationship (e.g. father). Original file names and updated file names are seen in the table below:

original_file_name	download_name
057a1584-5a10-4f8a-af6c-a2e60872adcb.g.vcf.gz	FM_AC8MB9RH/FM_AC8MB9RH_father.g.vcf.gz
0781ea81-95b6-4ed5-a2f9-3466b81c8784.g.vcf.gz	FM_2JR5YVMZ/FM_2JR5YVMZ_father.g.vcf.gz

original_file_name	download_name
0a8ccf6b-a510-4afb-ae64-a6fe101971bb.g.vcf.gz	FM_XBG76ESE/FM_XBG76ESE_mother.g.vcf.gz
0c465840-8133-497d-b7ae-9a49510a86a5.g.vcf.gz	FM_RF8RS3YT/FM_RF8RS3YT_child.g.vcf.gz
15312212-ec1c-4c53-b0df-c66177d0da78.g.vcf.gz	FM_0FKGXBZB/FM_0FKGXBZB_mother.g.vcf.gz
16e7875f-6c31-45b1-a6f5-2c316b894987.g.vcf.gz	FM_1DG7K3TV/FM_1DG7K3TV_father.g.vcf.gz
17e821f2-8b3e-4e0c-b654-e97a116d3133.g.vcf.gz	FM_EWX4PAC4/FM_EWX4PAC4_father.g.vcf.gz
18cc83be-7ba2-475a-a695-b5fe971ac647.g.vcf.gz	FM_XBG76ESE/FM_XBG76ESE_child.g.vcf.gz
190f149e-82d9-4291-9af6-f7bbd6d6bf72.g.vcf.gz	FM_068BMXVN/FM_068BMXVN_child.g.vcf.gz
19f89a26-adb3-4214-b17a-e720c39f6783.g.vcf.gz	FM_VM04HHXA/FM_VM04HHXA_child.g.vcf.gz
1d3d816c-b8aa-46b7-884c-c996b7fc5a07.g.vcf.gz	FM_6G8GWTMG/FM_6G8GWTMG_father.g.vcf.gz
1e2e05ad-1fb0-4538-85d8-7f39bdf8467f.g.vcf.gz	FM_WR20QYM1/FM_WR20QYM1_child.g.vcf.gz
1fc33481-ebd3-419a-9ed8-640a37455e94.g.vcf.gz	FM_QNFTR3R4/FM_QNFTR3R4_mother.g.vcf.gz
21406e96-2f13-459d-b6d0-4118ee015767.g.vcf.gz	FM_9H9QAMW8/FM_9H9QAMW8_child.g.vcf.gz
2152ed58-d971-47d1-812f-071ddf43c89f.g.vcf.gz	FM_2VKNQ3DF/FM_2VKNQ3DF_father.g.vcf.gz
26c127a0-d576-48d1-98dd-af0b362f9619.g.vcf.gz	FM_T36W3RK9/FM_T36W3RK9_father.g.vcf.gz
2ba32ce1-9a7f-489e-ac41-53efac14721f.g.vcf.gz	FM_BW30548P/FM_BW30548P_child.g.vcf.gz
2c9650b7-eee0-4fc8-bf24-096592560307.g.vcf.gz	FM_8NEWFAZ7/FM_8NEWFAZ7_child.g.vcf.gz
2c9910fe-897d-430a-a00f-96e6f40a2f92.g.vcf.gz	FM_MA1QF65E/FM_MA1QF65E_mother.g.vcf.gz
2cd5c46b-bac6-4b40-baac-e59cb74e6665.g.vcf.gz	FM_JS4PRGFS/FM_JS4PRGFS_mother.g.vcf.gz
2d1a8e6d-7615-49a8-97b1-6f145957bf91.g.vcf.gz	FM_JHCE7KM4/FM_JHCE7KM4_mother.g.vcf.gz
2eec8c1b-7eef-4bf0-9c3a-289be86f03ba.g.vcf.gz	FM_F20MF8G6/FM_F20MF8G6_child.g.vcf.gz
2fe52015-4ab9-4281-87da-f09cb52a7afc.g.vcf.gz	FM_9XR6YJ29/FM_9XR6YJ29_mother.g.vcf.gz
2ffef012-86e3-4155-9327-738d432b88e3.g.vcf.gz	FM_9XR6YJ29/FM_9XR6YJ29_child.g.vcf.gz
303b961c-9585-4547-b8cb-a8f98519561f.g.vcf.gz	FM_8NEWFAZ7/FM_8NEWFAZ7_father.g.vcf.gz
33134715-aea3-439a-973a-2067056b3166.g.vcf.gz	FM_1TQ4YE5Q/FM_1TQ4YE5Q_child.g.vcf.gz
34419302-d8c1-44e3-97d2-c9b93c64b386.g.vcf.gz	FM_2VKNQ3DF/FM_2VKNQ3DF_mother.g.vcf.gz
34e4407e-bfd9-4418-ae36-57a3f0eb0692.g.vcf.gz	FM_BHTM6B9K/FM_BHTM6B9K_child.g.vcf.gz
363e424c-266c-43a9-ac3b-bd3959648dbb.g.vcf.gz	FM_H67TXYYJ/FM_H67TXYYJ_child.g.vcf.gz
37d2510f-faa1-4003-8357-6e6fb96e0b14.g.vcf.gz	FM_02AXWKRM/FM_02AXWKRM_child.g.vcf.gz
394421cb-58d2-47fe-a311-8a674b52dd0b.g.vcf.gz	FM_FR0MMM3E/FM_FR0MMM3E_child.g.vcf.gz
39bdf5ab-bc45-4e88-afab-82c4a84825a2.g.vcf.gz	FM_MA1QF65E/FM_MA1QF65E_child.g.vcf.gz
3e5ec4f2-527b-4a21-8667-dfb5ebf93c8b.g.vcf.gz	FM_NZABV4W7/FM_NZABV4W7_father.g.vcf.gz
3f5a0fa7-180c-4c21-b1d9-e0faef7fdf84.g.vcf.gz	FM_5A7HFR0N/FM_5A7HFR0N_father.g.vcf.gz
40be2fd3-6b20-43ff-9e34-320f04ccc594.g.vcf.gz	FM_9XR6YJ29/FM_9XR6YJ29_father.g.vcf.gz
433b9cec-fe89-468f-b6e8-2207abc79651.g.vcf.gz	FM_H67TXYYJ/FM_H67TXYYJ_father.g.vcf.gz
445a06dc-5460-43dd-a763-101e9d45b6ca.g.vcf.gz	FM_068BMXVN/FM_068BMXVN_mother.g.vcf.gz
4517c80d-63bb-43d6-b404-358e7bf99250.g.vcf.gz	FM_RHPD85WC/FM_RHPD85WC_child.g.vcf.gz
45a3d2da-4dc9-4521-b8f5-235c562d5936.g.vcf.gz	FM_TEGCS8FR/FM_TEGCS8FR_mother.g.vcf.gz
46d4751c-39c8-4701-bc5e-6ea9575f1eb3.g.vcf.gz	FM_XBG76ESE/FM_XBG76ESE_father.g.vcf.gz
482a1d4d-fcb1-4621-bd36-b77be700ba5a.g.vcf.gz	FM_RF8RS3YT/FM_RF8RS3YT_father.g.vcf.gz
4927c134-114a-46d9-8f53-a0ae70eb14a8.g.vcf.gz	FM_BHTM6B9K/FM_BHTM6B9K_mother.g.vcf.gz
4b0c0f8a-861c-4149-a575-f1e896af9f69.g.vcf.gz	FM_C0QC9C8B/FM_C0QC9C8B_father.g.vcf.gz
4c0e37e1-56f5-43a3-bae6-86b8f434ac6a.g.vcf.gz	FM_02AXWKRM/FM_02AXWKRM_mother.g.vcf.gz
4e7f01f9-28f4-4051-b5c5-1dd83a8a9a67.g.vcf.gz	FM_1DG7K3TV/FM_1DG7K3TV_child.g.vcf.gz
501f7bb5-13a0-4506-b923-e4b06201020f.g.vcf.gz	FM_8F2EF55Z/FM_8F2EF55Z_father.g.vcf.gz
5177fff7-2d6d-4c88-9167-71a677666571.g.vcf.gz	FM_WR20QYM1/FM_WR20QYM1_mother.g.vcf.gz
53a7c633-07aa-4880-81b0-b914f5a15021.g.vcf.gz	FM_TEGCS8FR/FM_TEGCS8FR_child.g.vcf.gz
54f42659-9e05-4a3a-9b7e-24368fe6598c.g.vcf.gz	FM_0FKGXBZB/FM_0FKGXBZB_child.g.vcf.gz
56f33db9-2a29-4b4a-9eec-e6632c84acd0.g.vcf.gz	FM_6VKD1S0D/FM_6VKD1S0D_mother.g.vcf.gz
57eb3cb3-65c0-4c71-80a8-37a9d76fcd0a.g.vcf.gz	FM_GWXRTT53/FM_GWXRTT53_father.g.vcf.gz
5874586d-c23f-419b-badc-8653c1462343.g.vcf.gz	FM_QNFTR3R4/FM_QNFTR3R4_child.g.vcf.gz
5894f13f-faaa-4ea0-a0c0-1aff9ffd866d.g.vcf.gz	FM_P5JX9P4J/FM_P5JX9P4J_mother.g.vcf.gz
59c22c44-59b0-4781-ae12-8cf01b913431.g.vcf.gz	FM_6VKD1S0D/FM_6VKD1S0D_child.g.vcf.gz

original_file_name	download_name
5b9337d8-3a98-495e-951a-c75d55b34507.g.vcf.gz	FM_T36W3RK9/FM_T36W3RK9_mother.g.vcf.gz
5cacc6e6-cf59-475b-b0df-278e75019180.g.vcf.gz	FM_P5JX9P4J/FM_P5JX9P4J_father.g.vcf.gz
5e1646ca-9007-4f5d-bb91-a70656df457e.g.vcf.gz	FM_6VKD1S0D/FM_6VKD1S0D_father.g.vcf.gz
5e82d3d0-c1f4-43a8-bae3-f18e6cb21246.g.vcf.gz	FM_1TQ4YE5Q/FM_1TQ4YE5Q_father.g.vcf.gz
600706ea-e048-4ebc-b028-1e7dbf739230.g.vcf.gz	FM_6G8GWTMG/FM_6G8GWTMG_child.g.vcf.gz
611d8b78-cf31-4e28-9f47-12b5b1ad46c4.g.vcf.gz	FM_VM04HHXA/FM_VM04HHXA_father.g.vcf.gz
6469fec8-43f7-4a05-a753-124f13ef1575.g.vcf.gz	FM_69MH3P0P/FM_69MH3P0P_father.g.vcf.gz
64dcb088-bb01-498c-9b98-d708727d5e60.g.vcf.gz	FM_7MF9WPNH/FM_7MF9WPNH_mother.g.vcf.gz
662d595f-5b74-4696-8b21-471ab70c48fc.g.vcf.gz	FM_0FKGXBZB/FM_0FKGXBZB_father.g.vcf.gz
673f2920-c3fb-489f-98c1-90afbc669677.g.vcf.gz	FM_58WE88C4/FM_58WE88C4_father.g.vcf.gz
68631b06-312f-4b33-8898-062653d5fe4b.g.vcf.gz	FM_QNFTR3R4/FM_QNFTR3R4_father.g.vcf.gz
6c19a5e7-b53b-47d9-93b0-86e4beddafef.g.vcf.gz	FM_RNMD1436/FM_RNMD1436_mother.g.vcf.gz
6c82ce2e-ff31-41b3-920b-3c01f5c9ac1c.g.vcf.gz	FM_C0QC9C8B/FM_C0QC9C8B_child.g.vcf.gz
6eb36ab5-60ac-4200-9bf1-35320285da66.g.vcf.gz	FM_Z53CBVAP/FM_Z53CBVAP_mother.g.vcf.gz
70ca874b-1e51-4c66-88a3-93fec1211e0b.g.vcf.gz	FM_H4GA6GWQ/FM_H4GA6GWQ_mother.g.vcf.gz
71737c84-fd85-459d-a4bf-0bec216643ee.g.vcf.gz	FM_RHPD85WC/FM_RHPD85WC_father.g.vcf.gz
721c423b-d95c-4d30-9a4d-30f82730fc1f.g.vcf.gz	FM_41RNEZ6B/FM_41RNEZ6B_mother.g.vcf.gz
73aeeb93-ffb5-4234-977d-666b2dc99731.g.vcf.gz	FM_AC8MB9RH/FM_AC8MB9RH_child.g.vcf.gz
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9a80cac2-3416-4e93-ba88-b60bdd50a678.g.vcf.gz	FM_41RNEZ6B/FM_41RNEZ6B_father.g.vcf.gz
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a9d22b4c-55c3-4c11-b7ec-161c617e57a4.g.vcf.gz	FM_5A7HFR0N/FM_5A7HFR0N_mother.g.vcf.gz
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ad4e29a2-9878-4aae-88ce-cc7ae4b946da.g.vcf.gz	FM_2JR5YVMZ/FM_2JR5YVMZ_child.g.vcf.gz
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ba7829ae-1545-4972-a4f0-388bcf1f53d7.g.vcf.gz	FM_VM04HHXA/FM_VM04HHXA_mother.g.vcf.gz
baeef16f-2d1e-4fb1-bd57-91d92ba4a93c.g.vcf.gz	FM_3055H3PW/FM_3055H3PW_mother.g.vcf.gz
bfab7f0f-d1ad-47b1-be29-c4f3ccb6f87a.g.vcf.gz	FM_02AXWKRM/FM_02AXWKRM_father.g.vcf.gz

original_file_name	download_name
bfd86a7e-380d-4ef2-98f3-3a6d321bbe5f.g.vcf.gz	FM_RF8RS3YT/FM_RF8RS3YT_mother.g.vcf.gz
c29c76de-a22f-4631-91b6-2d48ca262876.g.vcf.gz	FM_BW30548P/FM_BW30548P_mother.g.vcf.gz
c523eef5-b72e-4ae5-8993-ea8956d92835.g.vcf.gz	FM_8F2EF55Z/FM_8F2EF55Z_mother.g.vcf.gz
c6a77235-a9ce-400f-91e1-90d9e797cf47.g.vcf.gz	FM_Z53CBVAP/FM_Z53CBVAP_father.g.vcf.gz
c70e5a82-41fa-4ce7-82f4-c30eecd56666.g.vcf.gz	FM_GWXRTT53/FM_GWXRTT53_mother.g.vcf.gz
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cd75f800-0e75-4f18-a682-61414778a5a2.g.vcf.gz	FM_MA1QF65E/FM_MA1QF65E_father.g.vcf.gz
cd8adb95-737b-47c2-a5ea-bb2c8c389106.g.vcf.gz	FM_GWXRTT53/FM_GWXRTT53_child.g.vcf.gz
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d038d7aa-c439-4b24-bde3-1c6f72e0650b.g.vcf.gz	FM_JHCE7KM4/FM_JHCE7KM4_child.g.vcf.gz
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d967df2c-8999-4db7-a7c3-b2d3bed18083.g.vcf.gz	FM_RNMD1436/FM_RNMD1436_father.g.vcf.gz
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db7ab99c-2bd0-44da-8d95-15dda9320f9c.g.vcf.gz	FM_JS4PRGFS/FM_JS4PRGFS_father.g.vcf.gz
dd1c4fc1-360c-46b9-93b4-b86a5a02225d.g.vcf.gz	FM_HPZPPFHN/FM_HPZPPFHN_child.g.vcf.gz
ddaa38f2-2243-4a73-baa7-1437dfee1381.g.vcf.gz	FM_58WE88C4/FM_58WE88C4_child.g.vcf.gz
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de306be5-1cb3-4662-a18a-27273014cd04.g.vcf.gz	FM_068BMXVN/FM_068BMXVN_father.g.vcf.gz
de5711d7-c1ae-4311-bca3-ce300f9d1881.g.vcf.gz	FM_NZABV4W7/FM_NZABV4W7_mother.g.vcf.gz
deda254c-e91c-4faf-8425-491e75e7ee06.g.vcf.gz	FM_TEGCS8FR/FM_TEGCS8FR_father.g.vcf.gz
e182fe38-a33b-43bb-9e0b-aa2cc0d369ee.g.vcf.gz	FM_RNMD1436/FM_RNMD1436_child.g.vcf.gz
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e48371e4-9832-4704-b95f-e88f5e31156a.g.vcf.gz	FM_1DG7K3TV/FM_1DG7K3TV_mother.g.vcf.gz
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ff2cb823-7195-4fc4-a9d3-66803d7c8d96.g.vcf.gz	FM_HPZPPFHN/FM_HPZPPFHN_father.g.vcf.gz

Generate phase data for each trio

"docker run -d -v /Data:/proj -w /proj -t dmill903/triophaser:1.0" is the Docker code needed to execute the container. Everything after this bit of code is being executed within and by the container. The "-d" option allows the container to run in "detached" mode; executing the container in the background. When the "-d" option is used, the container ID is output upon execution. The container ID is output to the terminal, or

so you don't have to keep track of the ID, ">" can be used to store the container ID to a file (as done in the example below). On our machine, each trio directory was stored within the /Data directory. Therefore, we attached the /Data directory to the container using the "-v" option. This allows the container to access all directories and files within the attached directory. We called this directory "/proj" within the container using ":". We then set the "/proj" directory as our working directory within the container using "-w". "-t" was used to allow the container to execute commands. "triophaser" is the name of the container to execute. The "trio_phaser.py" script is located at the root directory within the container. The "trio_phaser.py" script has 6 required parameters: 1) The gVCF of the child, 2) the gVCF of the father, 3) the gVCF of the mother, 4) the name of the output file, 5) where the reference files are to be stored, and 6) the number of cores available for use. When "trio_phaser.py" is first executed, all necessary reference files are stored to the directory you specify with parameter 5. Therefore, the first time "trio_phaser.py" is executed, the run-time will take longer as reference files are downloaded. However, as long as you point future trioPhaser runs to this reference directory, the previously downloaded files will be used. The default parameter for #6 (the number of cores to use) is 2, however, the more cores you use (up to 22), the faster the run-time. When trioPhaser uses *SHAPEIT4* to phase, if 22 cores are available, all 22 autosomal chromosomes will be phased at once. Because trioPhaser is executed in the background, the log information that "trio_phaser.py" outputs will not be directly output to the terminal on your machine. Instead, all the log information is output within the container. To view the log information information output by "trio_phaser.py", the container ID can be used. In the code below, we save the container ID as a variable, then use the docker command "docker logs {container ID}" to view the log information output by "trio_phaser.py". "trio_phaser.py" outputs helpful information about how many initial variants there were, how many of the initial variants were phased, how many variants were phased by *SHAPEIT4*, how many variants were erroneously phased by *SHAPEIT4* and then correctly phased using Mendelian inheritance, how many variants were phased using Mendelian inheritance that *SHAPEIT4* was unable to phase, and how long trioPhaser took to execute.

Each trio was ran through trioPhaser. The example below shows the code used to phase one trio:

```
docker run -d -v /Data:/proj -w /proj -t \
  dmill903/triophaser:1.0 python3 /trio_phaser.py \
  FM_RF8RS3YT/FM_RF8RS3YT_child.g.vcf.gz \
  FM_RF8RS3YT/FM_RF8RS3YT_father.g.vcf.gz \
  FM_RF8RS3YT/FM_RF8RS3YT_mother.g.vcf.gz \
  FM_RF8RS3YT/FM_RF8RS3YT_phased.vcf.gz \
  haplotype_references \
  22 \
  > FM_RF8RS3YT/trio_phaser_FM_RF8RS3YT.out # Outputs container ID to a file

# Use the container ID to get the log of the "trio_phaser.py" output and store
# the log information to the same file where the container ID was stored.
CONTAINERID="cat FM_RF8RS3YT/trio_phaser_FM_RF8RS3YT.out"
docker logs `$CONTAINERID` >> FM_RF8RS3YT/trio_phaser_FM_RF8RS3YT.out
```

Average phasing results across all trios

"trio_phaser.py" outputs helpful information about how many initial variants there were, how many of the initial variants were phased, how many variants were phased by *SHAPEIT4*, how many variants were erroneously phased by *SHAPEIT4* and then correctly phased using Mendelian inheritance, how many variants were phased using Mendelian inheritance that *SHAPEIT4* was unable to phase, and how long trioPhaser took to execute. This information was averaged across all affected children using the "summarize_neuroblastoma_log_files.py" script. This script takes 2 arguments, 1) where the family/trio directories are located, and 2) where the summary stats should be saved. The summary stats file includes all the information contained in the log output by "trio_phaser.py", but does so in a tidy format.

```
docker run -v /Data:/proj -w /proj -t \
  dmill903/triophaser:1.0 \
  python3 /trioPhaser/validate/summarize_neuroblastoma_log_files.py \
  /proj/ \
  /proj/summary_stats.tsv
```