Enigma Protocols for Imputation at each site – v1.1 (last edit 08/12/10)

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##

Before we start, you need to download and install some required programs (which you may already have). The required programs are: Plink, R, Mach, ssh client, mach2qtl. Links to the download sites are available below. Please address any questions to: enigma@lists.loni.ucla.edu.

##

Plink can be downloaded here: http://pngu.mgh.harvard.edu/~purcell/plink/download.shtml.

R can be downloaded here: http://cran.stat.ucla.edu/

Mach can be downloaded here: http://www.sph.umich.edu/csg/abecasis/MACH/download/

An ssh client can be downloaded here (though there are many to choose from): http://www.chiark.greenend.org.uk/~sgtatham/putty/download.html.

Download mach2qtl here: http://www.sph.umich.edu/csg/abecasis/MACH/download/
(run tar -zxvf mach2qtl.tar.gz to decompress the files and then type "make all" in the same directory to build. You will then have an executable called mach2qtl that you should add to your path.)

##

Population substructure checking – paste the blue lines below into a terminal window or shell script

##

Download the HapMap3 data from the following webpages to your working directory which we will call /enigma/genetics/:

http://users.loni.ucla.edu/~jstein/HM3.bed.gz
http://users.loni.ucla.edu/~jstein/HM3.bim.gz
http://users.loni.ucla.edu/~jstein/HM3.fam.gz

##

Filter SNPs out from your dataset which do not meet Quality Control criteria (Minor Allele Frequency < 0.01; Genotype Call Rate < 95%; Hardy-Weinberg Equilibrium < $1x10^{-6}$). Directions assume your data are in binary plink format (bed/bim/fam), if this is not the case try to convert to plink format and contact enigma@lists.loni.ucla.edu with questions.

##

```
export datafileraw=yourrawdata  # replace yourrawdata with the name of the local plink file name
```

```
plink --bfile $datafileraw --hwe 1e-6 --geno 0.05 --maf 0.01 --make-bed --out ${datafileraw}_filtered
```

###

Unzip the HM3 genotypes. Prepare the HM3 and the raw genotype data by extracting only snps that are in common between the two genotype data sets - this avoids exhausting the system memory. We are also removing the strand ambiguous snps from the genotyped data set to avoid strand mismatch among these snps. Your genotype files should be filtered to remove markers which do not satisfy the quality control criteria above.

###

```
bash  # these commands assume you are running bash shell – if you are already running bash then you don’t need to type this (but it won’t hurt)
```

```
cd /enigma/genetics #change directory to a folder with you plink dataset and downloaded HM3 files
```

```
gunzip *.gz
```

```
export datafile=datafile_filtered  # replace datafile_filtered with the name of the local plink file name that has been filtered of SNPs not meeting QC criteria (see above)
```

```
awk '{print $2}' HM3.bim > HM3.snplist.txt
```

```
plink --bfile $datafile --extract HM3.snplist.txt --make-bed --out local
```

```
awk '{ if (($5=="T" && $6=="A")||($5=="A" && $6=="T")||($5=="C" && $6=="G")||($5=="G" && $6=="C")) print $2, "ambig" ; else print $2 ;}' $datafile.bim | grep -v ambig > local.snplist.txt
```

```
plink --bfile HM3 --extract local.snplist.txt --make-bed --out external
```

###
Merge the two sets of plink files – In merging the two files plink will check for strand differences. If any strand differences are found plink will crash with the following error (ERROR: Stopping due to mis-matching SNPs -- check +/- strand?)

Ignore warnings regarding different physical positions

###

```
plink --bfile local --bmerge external.bed  external.bim  external.fam --make-bed --out MDSfile # this step will take a while (less than 1 hour)
```

###
If plink crashed with a strand error (ERROR: Stopping due to mis-matching SNPs -- check +/- strand?) run the following two lines of alternate code

```
plink --bfile local --flip MDSfile.missnp --make-bed --out flipped
```

```
plink --bfile flipped --bmerge external.bed  external.bim  external.fam --make-bed --out MDSfile # this step will take a while (less than 1 hour)
```

```
#####################################################################################
```

Run the MDS analysis

```
#####################################################################################
```

```
plink --bfile MDSfile --cluster --mind .05 --mds-plot 4 --extract local.snplist.txt --out HM3mds # this step will take a while (approx. 1 day)
```

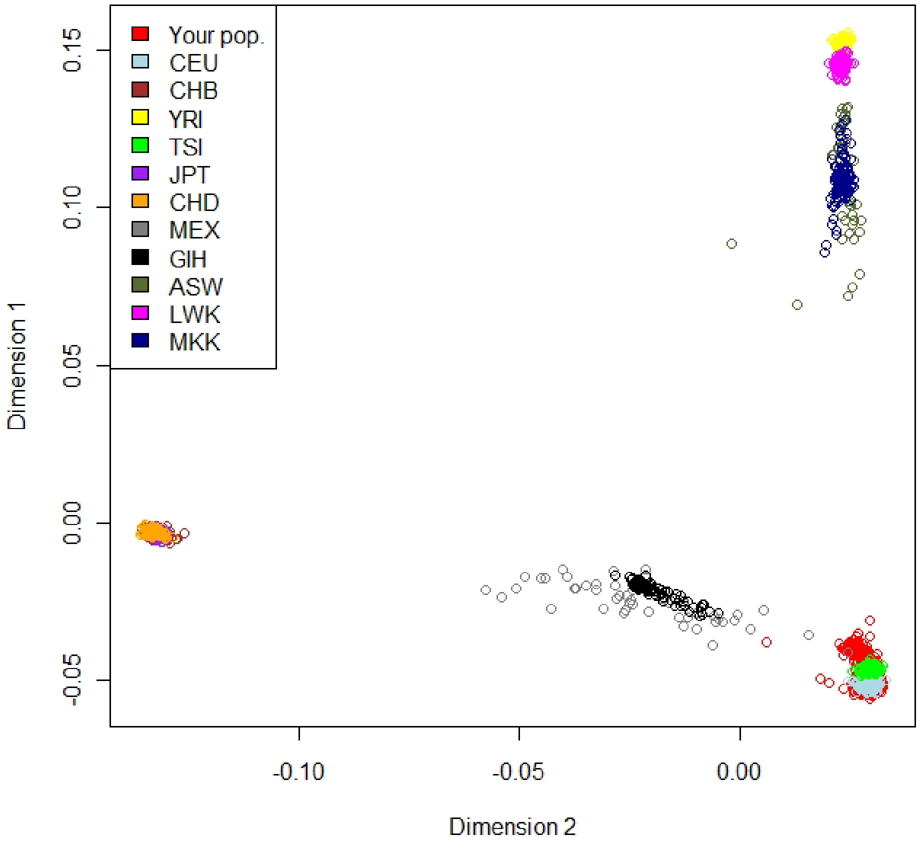
```
#####################################################################################
```

Plot the MDS results using R into a file called mdsplot.eps and mdsplot.pdf (Note: type R to start R in unix and q() followed by n to close the R session after the plot has been made)

```
awk '{print $1, $2, $3, $4, $5, $6, $7}' >> HM3mds2R.mds HM3mds.mds #This formats the plink output into an R compatible format.
```

```
R

mds.cluster = read.table("HM3mds2R.mds", header=T);

ur.num = length(mds.cluster$C1) - 985;

colors = c(rep("red", ur.num), rep("lightblue", 112), rep("brown", 84), rep("yellow", 112), rep("green", 88), rep("purple", 86), rep("orange", 84), rep("grey50", 50), rep("black", 88), rep("darkolivegreen", 49), rep("magenta", 89), rep("darkblue", 143));

postscript(file="mdsplot.eps", paper="special", width=10, height=10, horizontal=FALSE) #Use this file to upload to the ENIGMA website since it is better for publication

plot(mds.cluster$C2, mds.cluster$C1, col=colors, ylab="Dimension 1", xlab="Dimension 2")

legend("topleft", c("Your pop.", "CEU", "CHB", "YRI", "TSI", "JPT", "CHD", "MEX", "GIH", "ASW", "LWK", "MKK"), fill=c("red", "lightblue", "brown", "yellow", "green", "purple", "orange", "grey50", "black", "darkolivegreen", "magenta", "darkblue"))

dev.off()

pdf(file="mdsplot.pdf") #Use this file to look at your MDS plot if you do not have an EPS viewer (for example if you are working on a PC and do not have ghostview)

plot(mds.cluster$C2, mds.cluster$C1, col=colors, ylab="Dimension 1", xlab="Dimension 2")

legend("topleft", c("Your pop.", "CEU", "CHB", "YRI", "TSI", "JPT", "CHD", "MEX", "GIH", "ASW", "LWK", "MKK"), fill=c("red", "lightblue", "brown", "yellow", "green", "purple", "orange", "grey50", "black", "darkolivegreen", "magenta", "darkblue"))

dev.off()
```

Your output will look something like this when viewed either as an EPS file or a PDF file:



If you have any questions about your MDS plot (what to do with outliers, which population to choose for imputation, color-blindness limits your interpretative capabilities, etc.) please email the authors of this protocol (Sarah Medland, Jason Stein, Alejandro Arias Vasquez, and Derrek Hibar) , and the ENIGMA listserv enigma@lists.loni.ucla.edu for help and friendly advice.

##

Running the first stage of the imputation

##

The genotype data need to be written out in merlin format for imputation

– at this stage you want to drop any snps that are not in the Hapmap reference set

- you also want to drop any strand ambiguous snps

In the first stage of the imputation process, the genotyped data are compared to the reference data and error rate and recombination files are produced summarising the differences between the two data sets

Randomly select 300 people to be used in the first stage of the imputation process. Create a file (referenceIDs.list) containing the famID and ID of these individuals (this needs to be a space or tab delimited file) . If your sample is less than 500 people you may want to use all you participants at this stage rather than a subset.

##

#To generate the referenceIDs.list file with randomly selected subjects (unrelated only, if possible) or your whole sample if < 500 subjects, we first need to generate a plink file for manipulation.

```
plink --bfile local --recodeA --chr 22 --out 4selectsubset
```

#Download and run the randomlysplit.R code from the enigma website (add to your enigma directory):

http://users.loni.ucla.edu/~jstein/randomlysplit.R

```
R --no-save --slave < randomlysplit.R
```

#Split up files by chromosome and put in Merlin compatible format using the subject list outputted by randomlysplit.R

```
for x in {1..22}

do

plink --bfile local --extract local.snplist.txt --recode --chr ${x}  --make-founders  --keep referenceIDs.list  --out reference${x}

echo "S1 dummy_phenotype" > reference${x}.dat

awk '{print "M", $2}' reference${x}.map >> reference${x}.dat

done
```

##

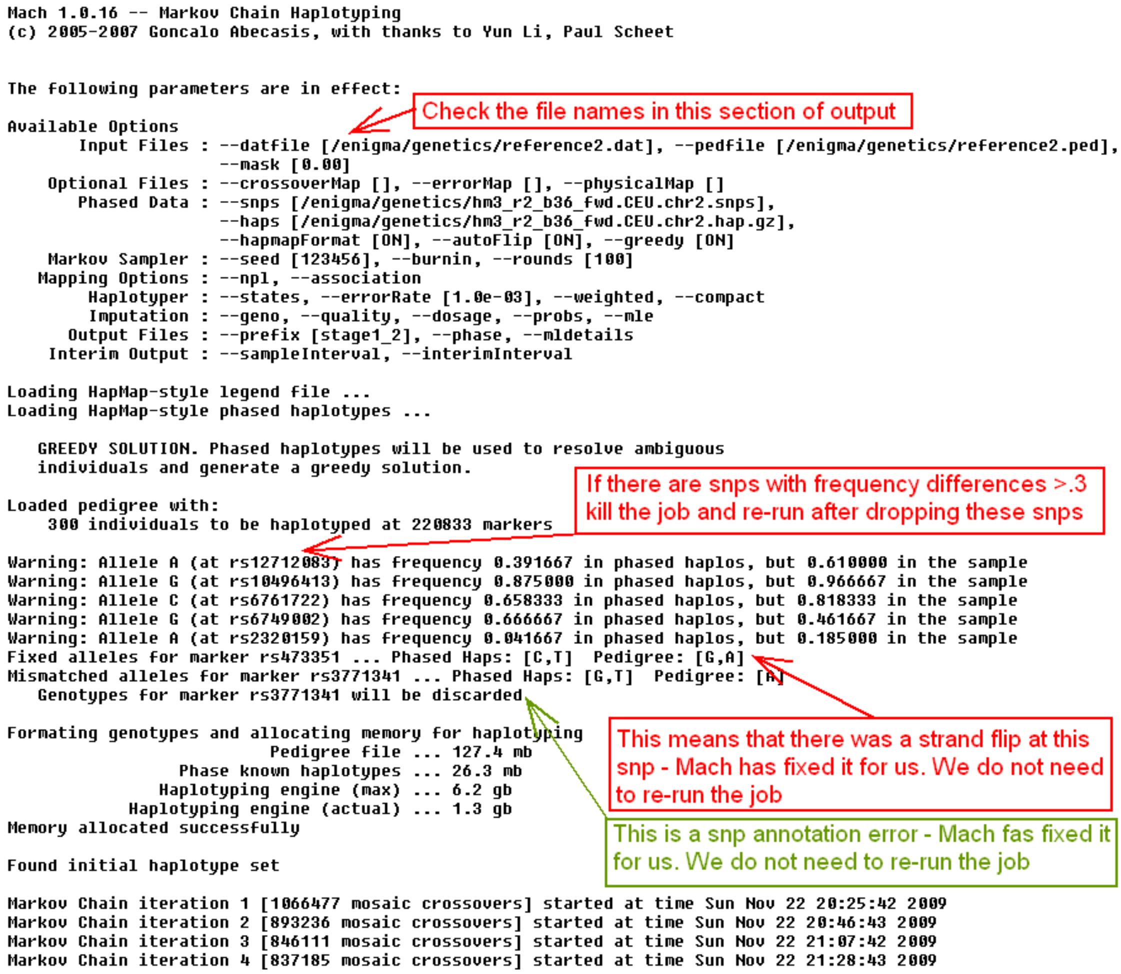
Download and decompress (tar -zxvf *.tar.gz) the relevant Hapmap3 reference files for your sample from the http://enigma.loni.ucla.edu/protocols/genetics-protocols/imputation-protocol/hapmap-downloads/ website and set stage 1 running

Mach will produce a series of erate and rec files which will be used in stage 2 of the imputation

- If the program runs out of memory add the –compact command
- The following commands will produce a series of scripts that can be run sequentially or in parallel. Note: this part of the process can be very slow depending on your sample size and may run for more than one day.
- If you have a cluster environment, you will have to use the commands tailored to your system to submit to multiple nodes.
- To run sequentially, after you generate the files:

  ```
  for x in {1..22}; do ./compute_mach_chr${x}.sh; done
  ```

- Regardless of computing environment, it is probably best to test run a single script in case you need to make any of the adjustments or do error checking as mentioned above.
- Mach will automatically check your data file against the reference data before starting the imputation process. After Mach has been running for 20-30 minutes it is a good idea to check the log files which are produced. If errors are present or there are major differences in MAF between the files it is a good idea to fix these and then re-run Mach
  Some example MACH log output is given below



```
Mach 1.0.16 -- Markov Chain Haplotyping
(c) 2005-2007 Goncalo Abecasis, with thanks to Yun Li, Paul Scheet


The following parameters are in effect:

Available Options
       Input Files : --datfile [/enigma/genetics/reference2.dat], --pedfile [/enigma/genetics/reference2.ped],
                     --mask [0.00]
    Optional Files : --crossoverMap [], --errorMap [], --physicalMap []
       Phased Data : --snps [/enigma/genetics/hm3_r2_b36_fwd.CEU.chr2.snps],
                     --haps [/enigma/genetics/hm3_r2_b36_fwd.CEU.chr2.hap.gz],
                     --hapmapFormat [ON], --autoFlip [ON], --greedy [ON]
    Markov Sampler : --seed [123456], --burnin, --rounds [100]
   Mapping Options : --npl, --association
        Haplotyper : --states, --errorRate [1.0e-03], --weighted, --compact
        Imputation : --geno, --quality, --dosage, --probs, --mle
      Output Files : --prefix [stage1_2], --phase, --mldetails
    Interim Output : --sampleInterval, --interimInterval

Loading HapMap-style legend file ...
Loading HapMap-style phased haplotypes ...

   GREEDY SOLUTION. Phased haplotypes will be used to resolve ambiguous
   individuals and generate a greedy solution.

Loaded pedigree with:
    300 individuals to be haplotyped at 220833 markers

Warning: Allele A (at rs12712083) has frequency 0.391667 in phased haplos, but 0.610000 in the sample
Warning: Allele G (at rs10496413) has frequency 0.875000 in phased haplos, but 0.966667 in the sample
Warning: Allele C (at rs6761722) has frequency 0.658333 in phased haplos, but 0.818333 in the sample
Warning: Allele G (at rs6749002) has frequency 0.666667 in phased haplos, but 0.461667 in the sample
Warning: Allele A (at rs2320159) has frequency 0.041667 in phased haplos, but 0.185000 in the sample
Fixed alleles for marker rs473351 ... Phased Haps: [C,T]  Pedigree: [G,A]
Mismatched alleles for marker rs3771341 ... Phased Haps: [G,T]  Pedigree: [A]
   Genotypes for marker rs3771341 will be discarded

Formating genotypes and allocating memory for haplotyping
                          Pedigree file ... 127.4 mb
                 Phase known haplotypes ... 26.3 mb
               Haplotyping engine (max) ... 6.2 gb
            Haplotyping engine (actual) ... 1.3 gb
Memory allocated successfully

Found initial haplotype set

Markov Chain iteration 1 [1066477 mosaic crossovers] started at time Sun Nov 22 20:25:42 2009
Markov Chain iteration 2 [893236 mosaic crossovers] started at time Sun Nov 22 20:46:43 2009
Markov Chain iteration 3 [846111 mosaic crossovers] started at time Sun Nov 22 21:07:42 2009
Markov Chain iteration 4 [837185 mosaic crossovers] started at time Sun Nov 22 21:28:43 2009
```

Check the file names in this section of output

If there are snps with frequency differences >.3 kill the job and re-run after dropping these snps

This means that there was a strand flip at this snp - Mach has fixed it for us. We do not need to re-run the job

This is a snp annotation error - Mach fas fixed it for us. We do not need to re-run the job

##

export hm3pop=hm3_r2_b36_fwd.CEU #change this so that it reflects the root file name of your hm3 population of interest

export fulldir=/enigma/genetics/ #you will have to edit this to reflect the location of your enigma folder with all of the files generated previously

export hapdir=/enigma/genetics/CEU/ #edit this to reflect the location of the folder containing the hap and snp folders from the tar output of your hm3 population

#note: you need to have mach1 in your path for this to work or give the full path to the mach executable in place of “mach1” below.

for ((i=1;i<=22;i++))

```
do

echo "mach1 -d ${fulldir}reference"$i".dat -p ${fulldir}reference"$i".ped -s ${hapdir}snps/${hm3pop}.chr"$i".snps -h ${hapdir}hap/${hm3pop}.chr"$i".hap.gz --greedy --autoFlip -r 100 -o ${fulldir}stage1_"$i" > ${fulldir}mach.stage1.c"$i".log" > ${fulldir}compute_mach_chr"$i".sh

sed -i '1i#!/bin/bash\n\n' compute_mach_chr"$i".sh

chmod 755 compute_mach_chr"$i".sh

done

#Now execute these scripts
```

Running the second stage of the imputation

(Note this step needs to be done for everyone regardless of the size of your reference sample)

###

Write out the genotype data for your full sample in merlin format. The files must contain exactly the same snps as used in your reference sample. We will reuse the dat file that was made in the previous step.

###

```
for ((i=1;i<=22;i++))

do

plink --bfile local --extract local.snplist.txt --recode --chr "$i"  --make-founders    --out population"$i"

done
```

###

Run stage 2

- Note: stage 2 runs much faster than stage1

Mach will produce a series of mldose mlprob mlinfo and mlgeno files – the mlprob and mlgeno files can be zipped and archived. The mldose and mlinfo files will be used for the analysis.

###

```
export hm3pop=hm3_r2_b36_fwd.CEU #change this so that it reflects the root file name of your hm3 population of interest

export hapdir=/enigma/genetics/CEU/ #edit this to reflect the location of the folder containing the hap and snp folders from the tar output of your hm3 population

for ((i=1;i<=22;i++))
```

```
do

 mach1 -d reference"$i".dat  -p population "$i".ped  -s ${hapdir}snps/${hm3pop}.chr"$i".snps
-h ${hapdir}hap/${hm3pop}.chr"$i".hap.gz  --greedy --autoFlip --errorMap stage1_"$i".erate
--crossoverMap stage1_"$i".rec  --mle  --mldetails -o enigmaImp_"$i" > mach.stage2.c"$i".log

done
```

```
##################################################################################################
```

Instructions for performing the tests of association, either in family or population-based studies, can be found in a separate protocol.