



NORMENT

Norwegian Centre for
Mental Disorders Research



Pre-imputation QC and imputation

Principles and implementation

Outline

- QC pipeline
- Phasing concept
- Imputation concept
- Phasing+Imputation pipeline

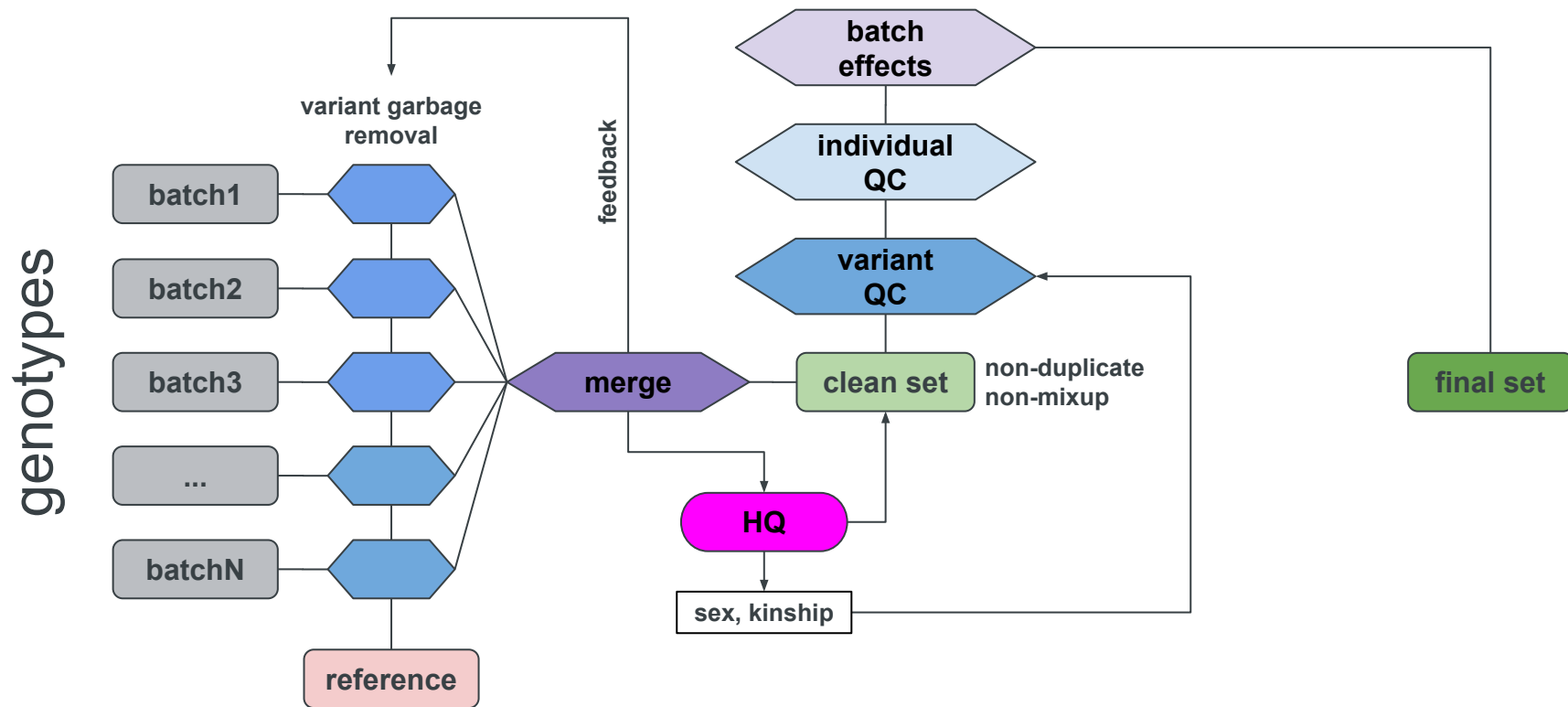


Quality Control (QC)

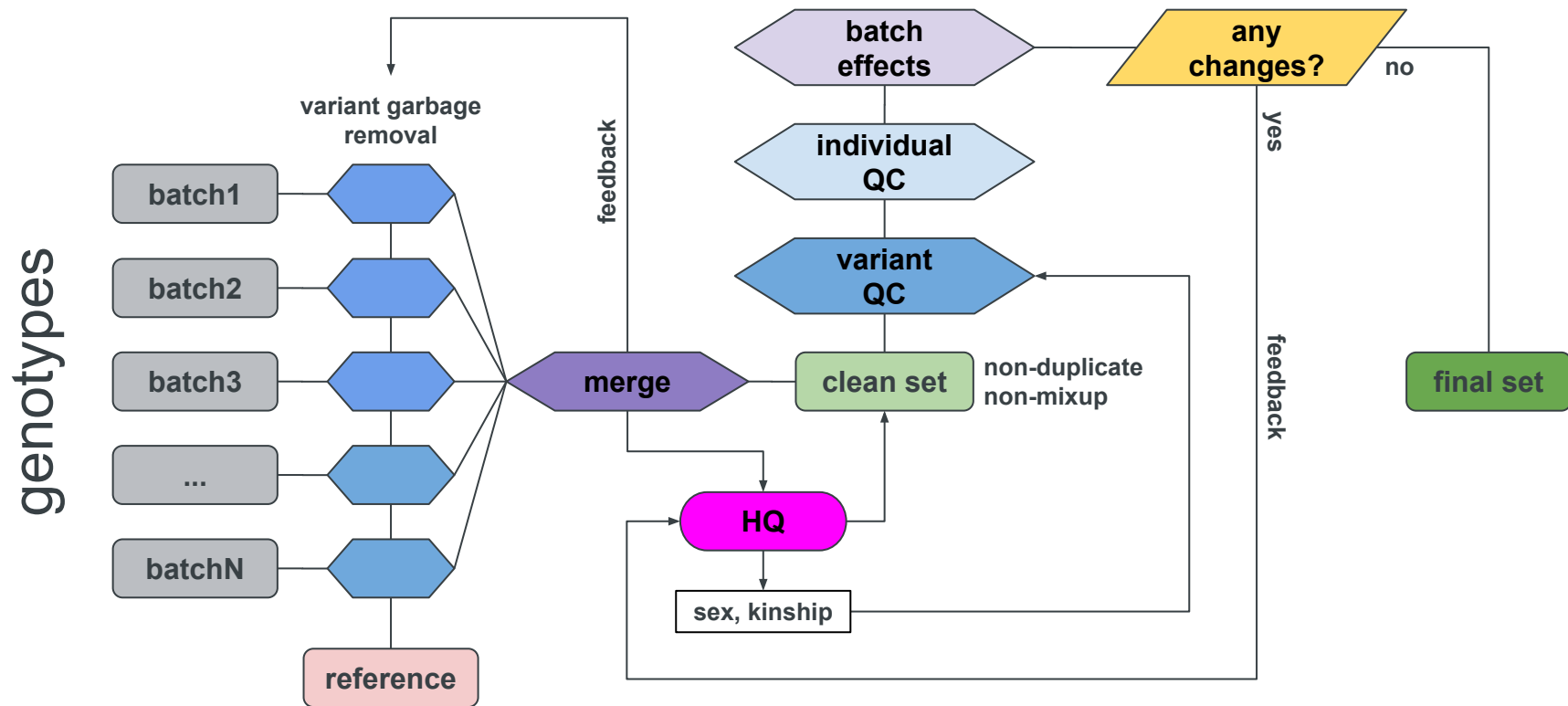
In the beginning..

were plink files

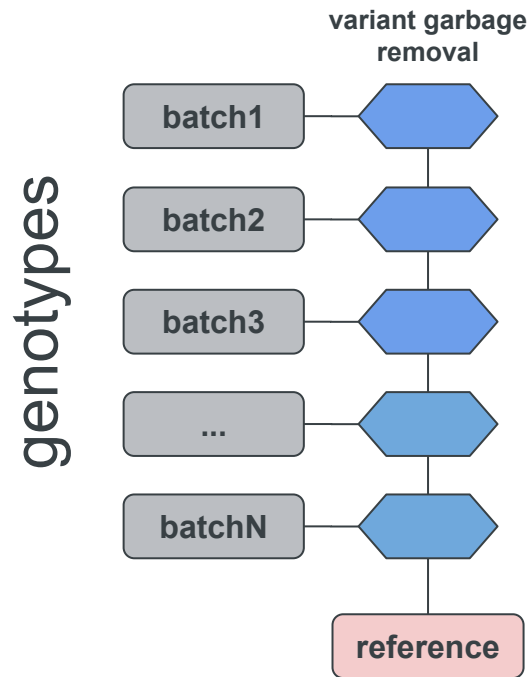
The QC pipeline (current)



The QC pipeline (future)

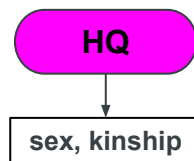


The QC pipeline

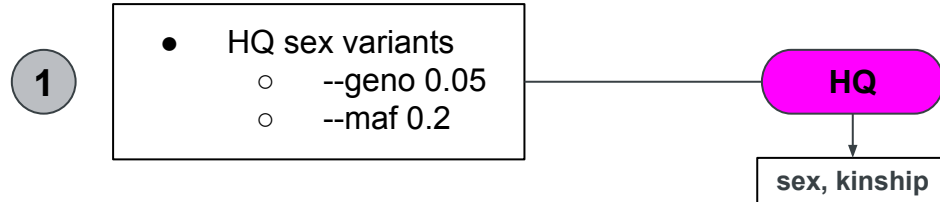


- alignment to reference (strand flips)
- removal of variants with:
 - ambiguous strand
 - different alleles *cfr.* reference
 - incoherent genotypes
 - incoherent info across batches

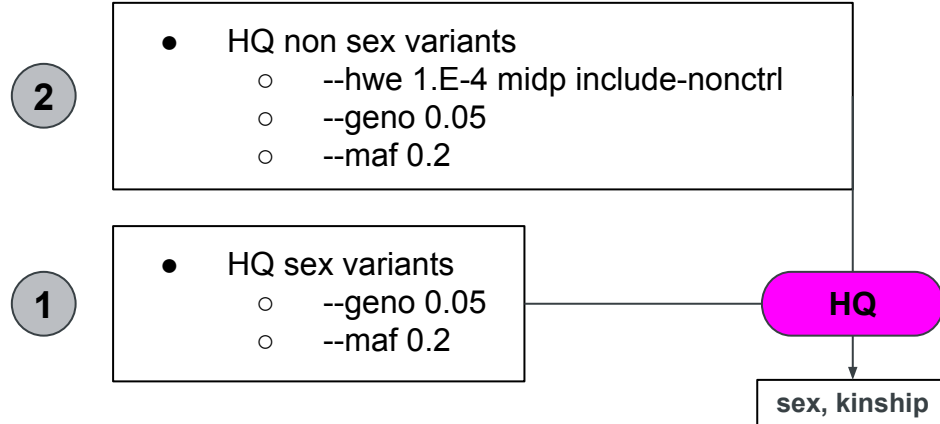
The QC pipeline



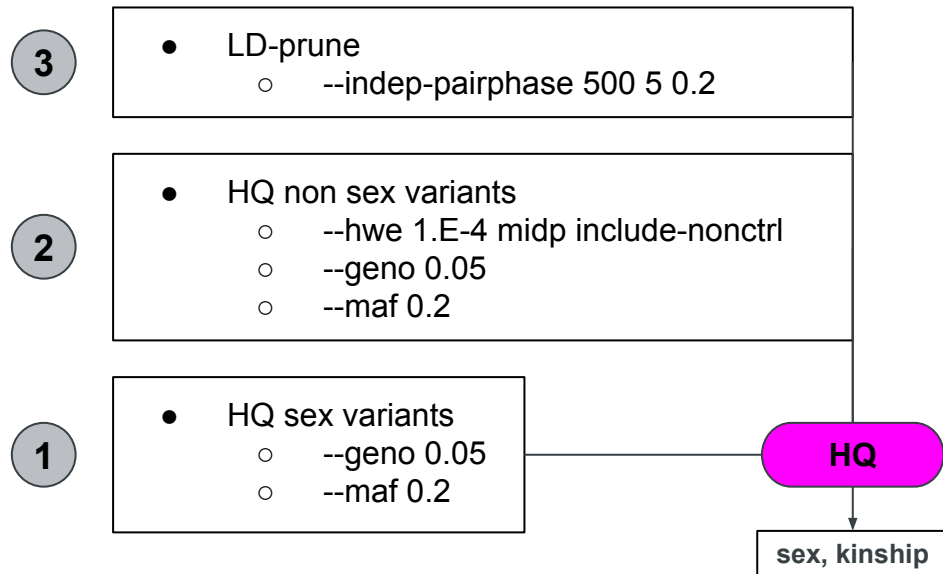
The QC pipeline



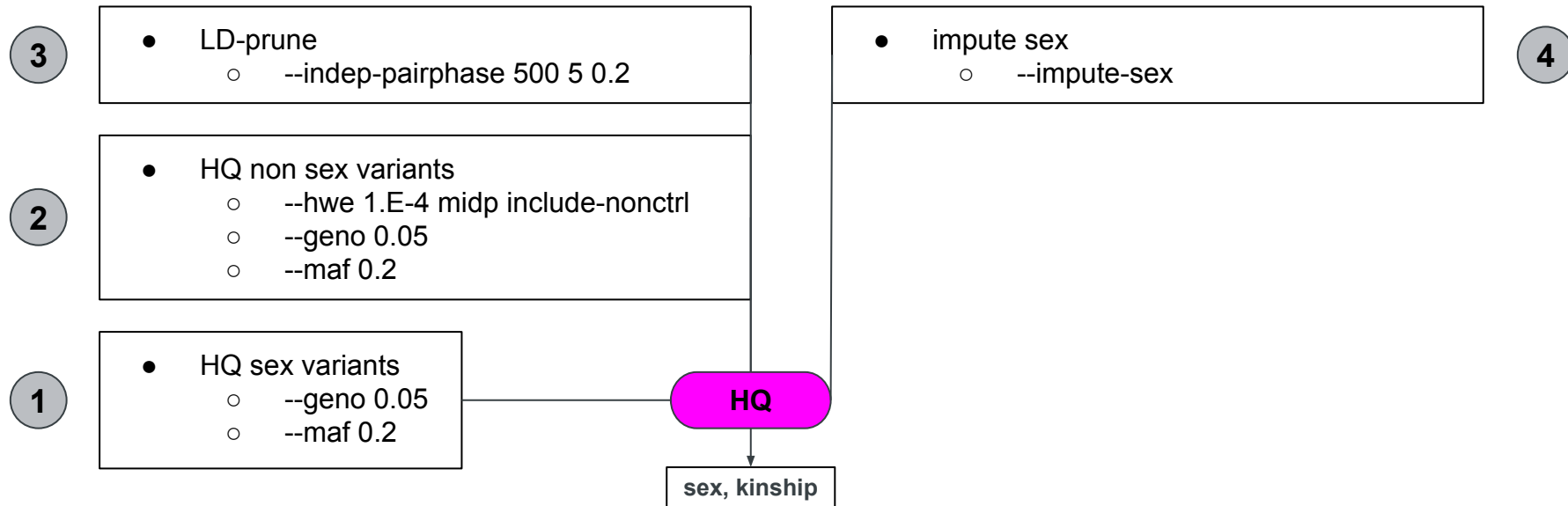
The QC pipeline



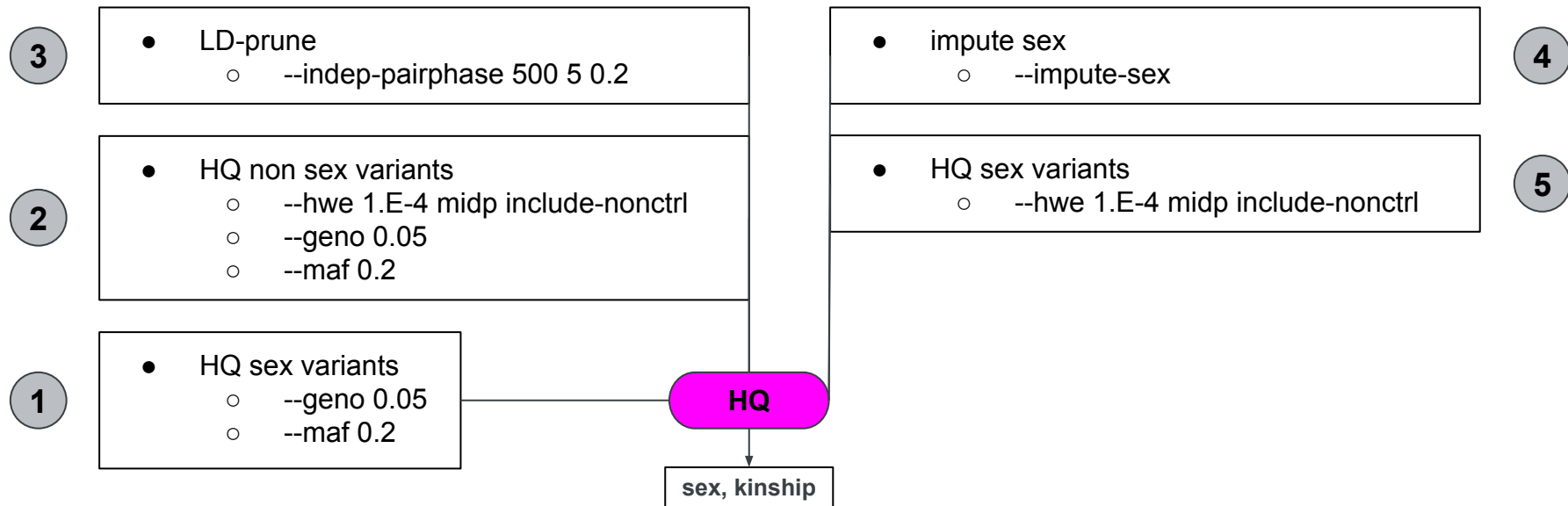
The QC pipeline



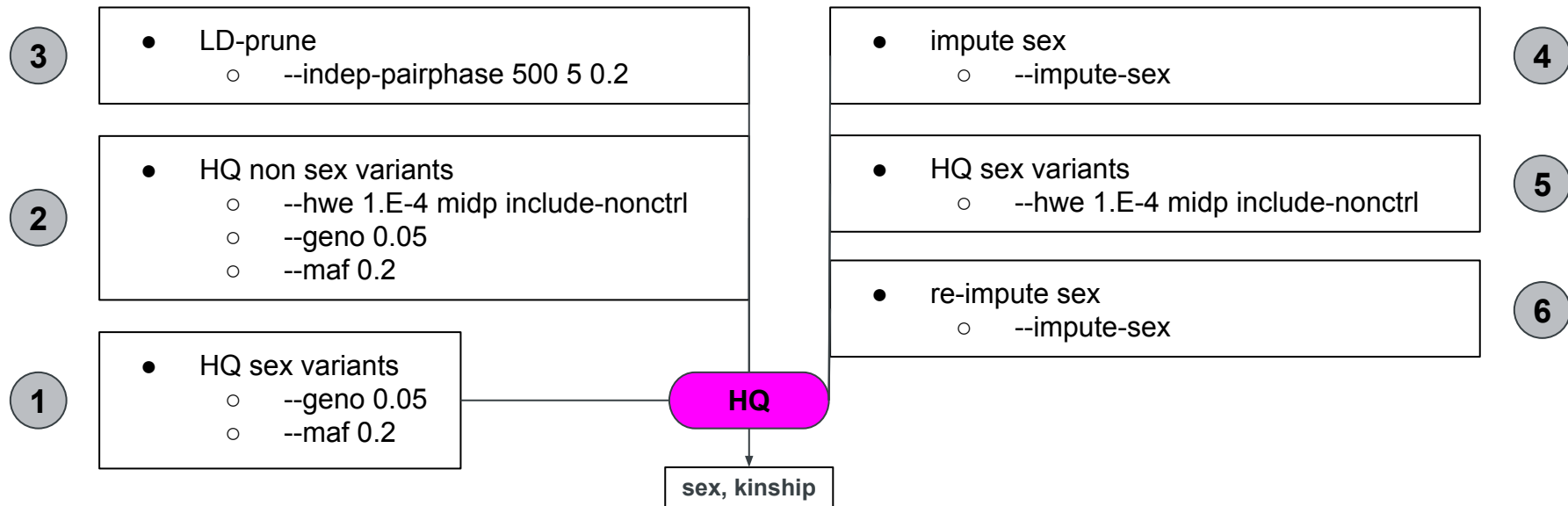
The QC pipeline



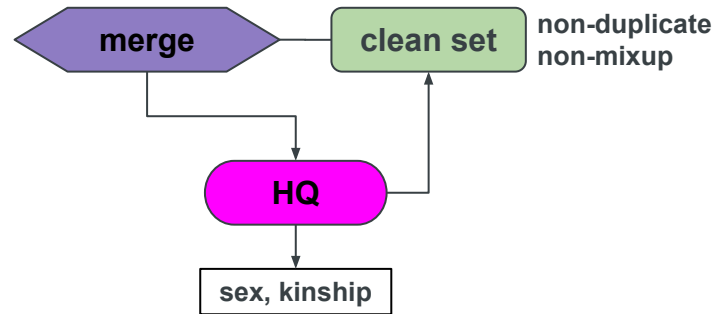
The QC pipeline



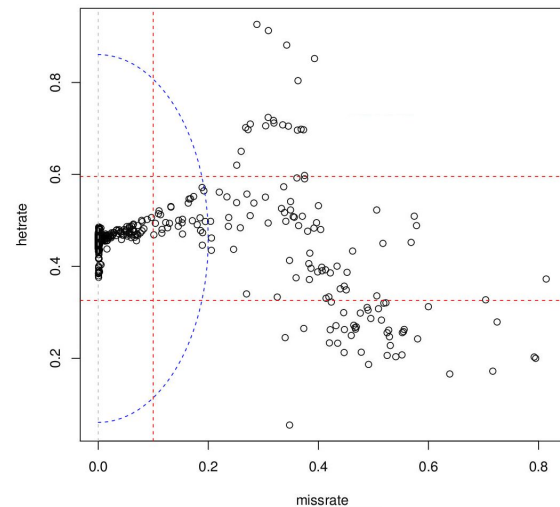
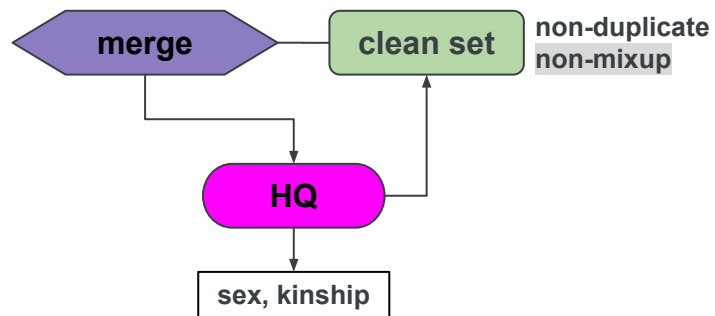
The QC pipeline



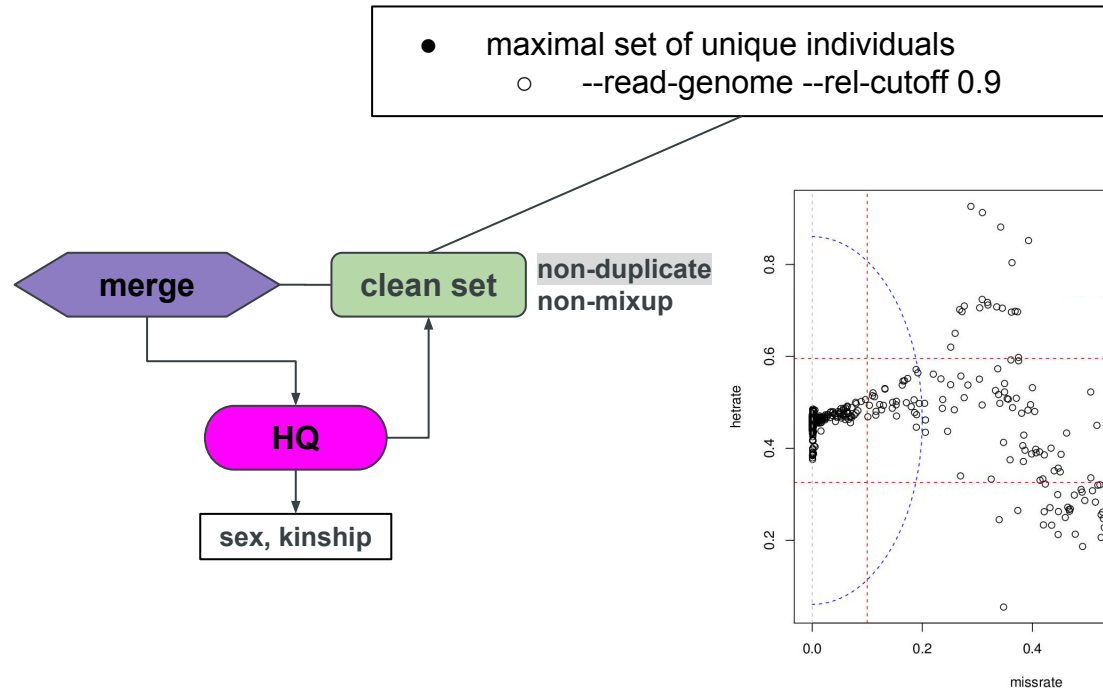
The QC pipeline



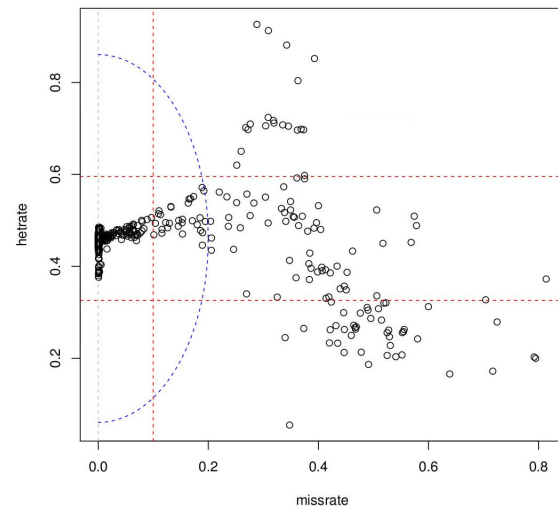
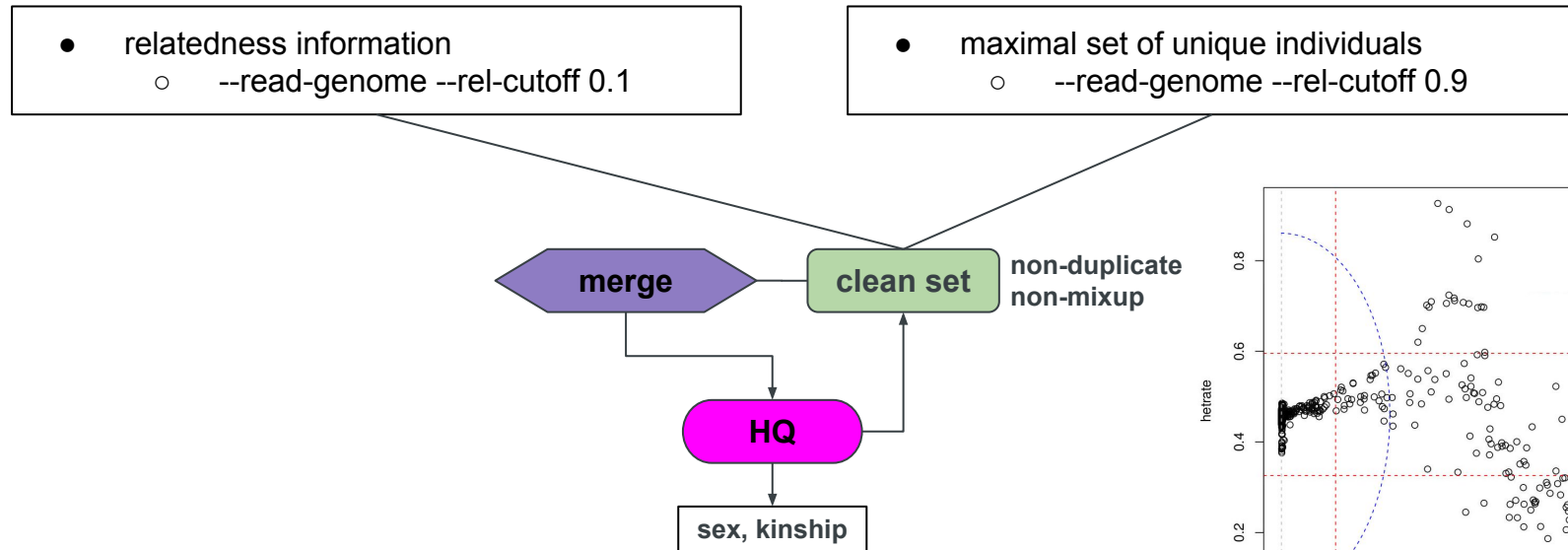
The QC pipeline



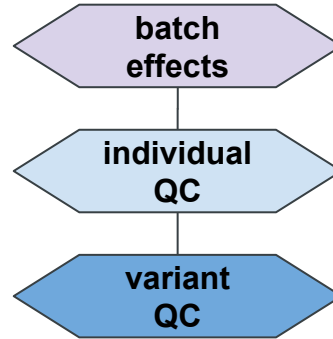
The QC pipeline



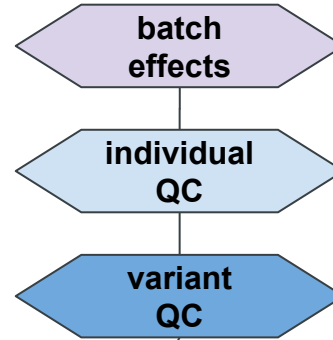
The QC pipeline



The QC pipeline

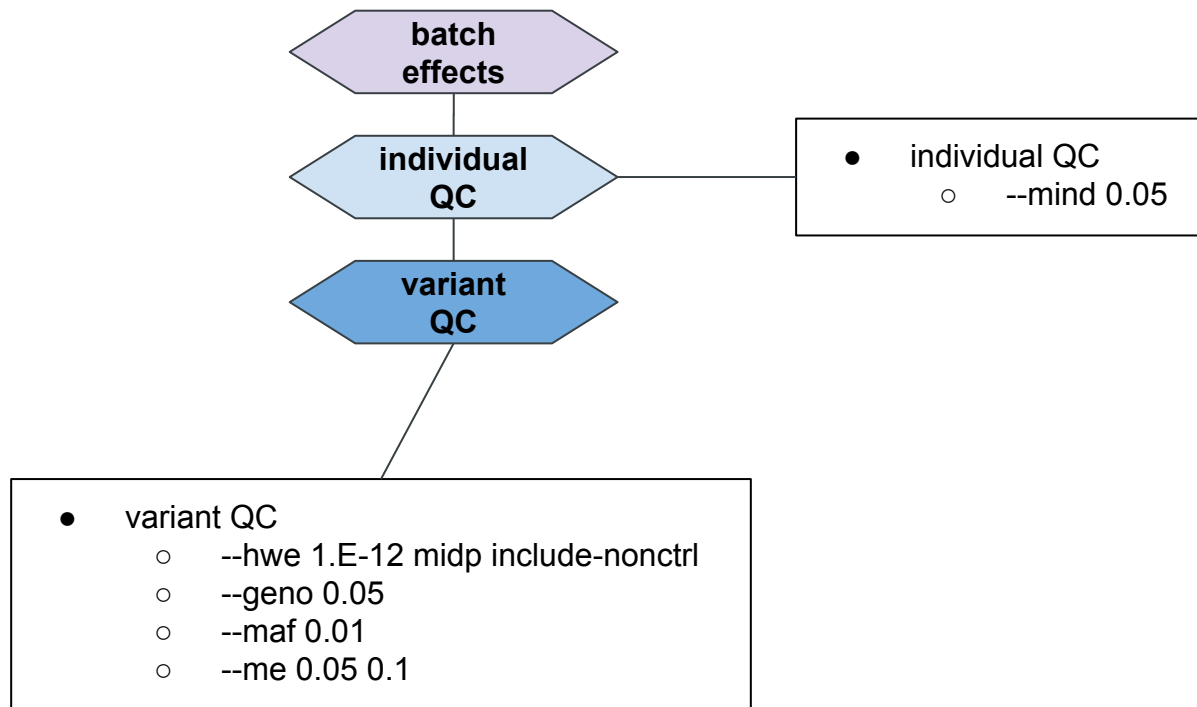


The QC pipeline

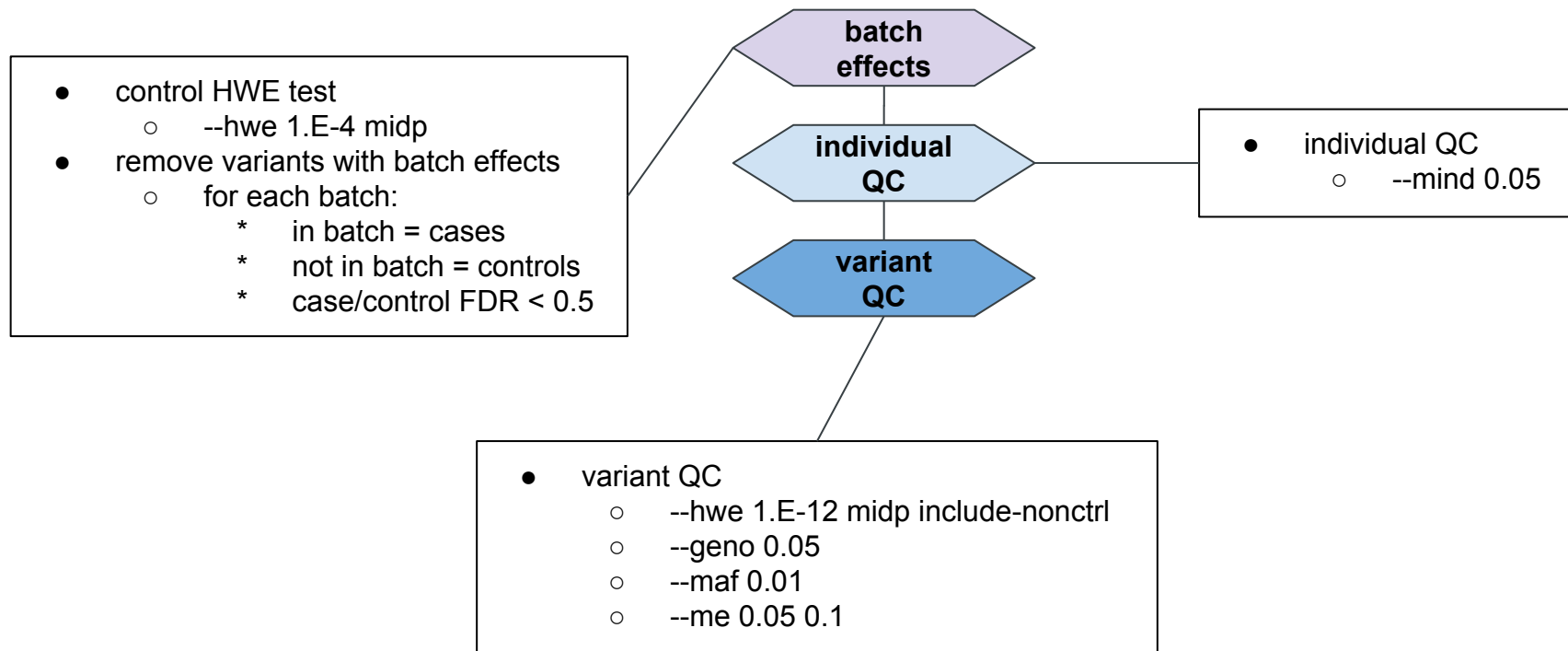


- variant QC
 - `--hwe 1.E-12 midp include-nonctrl`
 - `--geno 0.05`
 - `--maf 0.01`
 - `--me 0.05 0.1`

The QC pipeline



The QC pipeline



The QC pipeline: notes

no prior removal of individuals with:

- sex issues
- relatedness issues
- ethnic issues

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- ← well handled (software, reference)

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- ← do no harm
- ← well handled (software, reference)

these are *annotated* for use in downstream analyses

The QC pipeline: notes

000UID	FID	IID	PEDSEX	SNPSEX	STATUS	F	MISMIX	RELSHIP	DUPORG	COV	PC1	PC2	PC3	PC4
AMD00001	AMD00001	AMD00001	0	2	PROBLEM	0.07613	OK	NA	ORG	YES	-0.00147879	0.00164313	0.0128753	-0.00402758
AMD00002	AMD00002	AMD00002	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00163578	-0.00290372	-0.00193555	0.00373585
AMD00003	AMD00003	AMD00003	0	2	PROBLEM	0.01625	OK	AMD00007_AMD00007(0.937)	ORG	YES	-0.00238496	-0.00157558	-0.00417843	0.000328896
AMD00004	AMD00004	AMD00004	0	2	PROBLEM	0.02266	OK	NA	ORG	YES	-0.00256515	-0.00171294	-0.0039789	0.00138859
AMD00005	AMD00005	AMD00005	0	1	PROBLEM	1	OK	PACGRXH_DG_NUR6007(0.1046)	ORG	YES	-0.00172506	-0.00241938	-0.00381866	0.00275083
AMD00006	AMD00006	AMD00006	0	2	PROBLEM	-0.06074	OK	NA	ORG	YES	-0.00189367	-0.00187384	-0.00316229	0.000993657
AMD00007	AMD00007	AMD00007	0	1	PROBLEM	1	OK	AMD00003_AMD00003(0.937)	DUP	0	NA	NA	NA	NA
AMD00008	AMD00008	AMD00008	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00191639	-0.00166818	-0.00180217	0.00269283
AMD00009	AMD00009	AMD00009	0	2	PROBLEM	-0.01797	OK	AMD02266_AMD02266(0.2555),AMD02435_AMD02435(0.2472)	ORG	YES	-0.00174973	-0.00212934	-0.00167149	0.000634928

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The QC pipeline: notes

000UID	FID	IID	PEDSEX	SNPSEX	STATUS	F	MISMIX	RELSHIP	DUPORG	COV	PC1	PC2	PC3	PC4
AMD00001_AMD00001	AMD00001	AMD00001	0	2	PROBLEM	0.07613	OK	NA	ORG	YES	-0.00147879	0.00164313	0.0128753	-0.00402758
AMD00002_AMD00002	AMD00002	AMD00002	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00163578	-0.00290372	-0.00193555	0.00373585
AMD00003_AMD00003	AMD00003	AMD00003	0	2	PROBLEM	0.01625	OK	AMD00007_AMD00007(0.937)	ORG	YES	-0.00238496	-0.00157558	-0.00417843	0.000328896
AMD00004_AMD00004	AMD00004	AMD00004	0	2	PROBLEM	0.02266	OK	NA	ORG	YES	-0.00256515	-0.00171294	-0.0039789	0.00138859
AMD00005_AMD00005	AMD00005	AMD00005	0	1	PROBLEM	1	OK	PACGRXH_DG_NUR6007(0.1046)	ORG	YES	-0.00172506	-0.00241938	-0.00381866	0.00275083
AMD00006_AMD00006	AMD00006	AMD00006	0	2	PROBLEM	-0.06074	OK	NA	ORG	YES	-0.00189367	-0.00187384	-0.00316229	0.000993657
AMD00007_AMD00007	AMD00007	AMD00007	0	1	PROBLEM	1	OK	AMD00003_AMD00003(0.937)	DUP	0	NA	NA	NA	NA
AMD00008_AMD00008	AMD00008	AMD00008	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00191639	-0.00166818	-0.00180217	0.00269283
AMD00009_AMD00009	AMD00009	AMD00009	0	2	PROBLEM	-0.01797	OK	AMD02266_AMD02266(0.2555),AMD02435_AMD02435(0.2472)	ORG	YES	-0.00174973	-0.00212934	-0.00167149	0.000634928

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The QC pipeline: notes

because no sex was
annotated to begin with

000UID	FID	IID	PEDSEX	SNPSEX	STATUS	F	MISMIX	RELSHIP	DUPORG	COV	PC1	PC2	PC3	PC4
AMD00001	AMD00001	AMD00001	0	2	PROBLEM	0.07613	OK	NA	ORG	YES	-0.00147879	0.00164313	0.0128753	-0.00402758
AMD00002	AMD00002	AMD00002	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00163578	-0.00290372	-0.00193555	0.00373585
AMD00003	AMD00003	AMD00003	0	2	PROBLEM	0.01625	OK	AMD00007_AMD00007(0.937)	ORG	YES	-0.00238496	-0.00157558	-0.00417843	0.000328896
AMD00004	AMD00004	AMD00004	0	2	PROBLEM	0.02266	OK	NA	ORG	YES	-0.00256515	-0.00171294	-0.0039789	0.00138859
AMD00005	AMD00005	AMD00005	0	1	PROBLEM	1	OK	PACGRXH_DG_NUR6007(0.1046)	ORG	YES	-0.00172506	-0.00241938	-0.00381866	0.00275083
AMD00006	AMD00006	AMD00006	0	2	PROBLEM	-0.06074	OK	NA	ORG	YES	-0.00189367	-0.00187384	-0.00316229	0.000993657
AMD00007	AMD00007	AMD00007	0	1	PROBLEM	1	OK	AMD00003_AMD00003(0.937)	DUP	0	NA	NA	NA	NA
AMD00008	AMD00008	AMD00008	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00191639	-0.00166818	-0.00180217	0.00269283
AMD00009	AMD00009	AMD00009	0	2	PROBLEM	-0.01797	OK	AMD02266_AMD02266(0.2555),AMD02435_AMD02435(0.2472)	ORG	YES	-0.00174973	-0.00212934	-0.00167149	0.000634928

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The QC pipeline: notes

000UID	FID	IID	PEDSEX	SNPSEX	STATUS	F	MISMIX	RELSHIP	DUPORG	COV	PC1	PC2	PC3	PC4
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AMD00002	AMD00002	AMD00002	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00163578	-0.00290372	-0.00193555	0.00373585
AMD00003	AMD00003	AMD00003	0	2	PROBLEM	0.01625	OK	AMD00007_AMD00007(0.937)	ORG	YES	-0.00238496	-0.00157558	-0.00417843	0.000328896
AMD00004	AMD00004	AMD00004	0	2	PROBLEM	0.02266	OK	NA	ORG	YES	-0.00256515	-0.00171294	-0.0039789	0.00138859
AMD00005	AMD00005	AMD00005	0	1	PROBLEM	1	OK	PACGRXH_DG_NUR6007(0.1046)	ORG	YES	-0.00172506	-0.00241938	-0.00381866	0.00275083
AMD00006	AMD00006	AMD00006	0	2	PROBLEM	-0.06074	OK	NA	ORG	YES	-0.00189367	-0.00187384	-0.00316229	0.000993657
AMD00007	AMD00007	AMD00007	0	1	PROBLEM	1	OK	AMD00003_AMD00003(0.937)	DUP	0	NA	NA	NA	NA
AMD00008	AMD00008	AMD00008	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00191639	-0.00166818	-0.00180217	0.00269283
AMD00009	AMD00009	AMD00009	0	2	PROBLEM	-0.01797	OK	AMD02266_AMD02266(0.2555),AMD02435_AMD02435(0.2472)	ORG	YES	-0.00174973	-0.00212934	-0.00167149	0.000634928

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The QC pipeline: notes

duplicates are removed

000UID	FID	IID	PEDSEX	SNPSEX	STATUS	F	MISMIX	RELSHIP	DUPORG	COV	PC1	PC2	PC3	PC4
AMD00001	AMD00001	AMD00001	0	2	PROBLEM	0.07613	OK	NA	ORG	YES	-0.00147879	0.00164313	0.0128753	-0.00402758
AMD00002	AMD00002	AMD00002	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00163578	-0.00290372	-0.00193555	0.00373585
AMD00003	AMD00003	AMD00003	0	2	PROBLEM	0.01625	OK	AMD00007_AMD00007(0.937)	ORG	YES	-0.00238496	-0.00157558	-0.00417843	0.000328896
AMD00004	AMD00004	AMD00004	0	2	PROBLEM	0.02266	OK	NA	ORG	YES	-0.00256515	-0.00171294	-0.0039789	0.00138859
AMD00005	AMD00005	AMD00005	0	1	PROBLEM	1	OK	PACGRXH_DG_NUR6007(0.1046)	ORG	YES	-0.00172506	-0.00241938	-0.00381866	0.00275083
AMD00006	AMD00006	AMD00006	0	2	PROBLEM	-0.06074	OK	NA	ORG	YES	-0.00189367	-0.00187384	-0.00316229	0.000993657
AMD00007	AMD00007	AMD00007	0	1	PROBLEM	1	OK	AMD00003_AMD00003(0.937)	DUP	0	NA	NA	NA	NA
AMD00008	AMD00008	AMD00008	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00191639	-0.00166818	-0.00180217	0.00269283
AMD00009	AMD00009	AMD00009	0	2	PROBLEM	-0.01797	OK	AMD02266_AMD02266(0.2555),AMD02435_AMD02435(0.2472)	ORG	YES	-0.00174973	-0.00212934	-0.00167149	0.000634928

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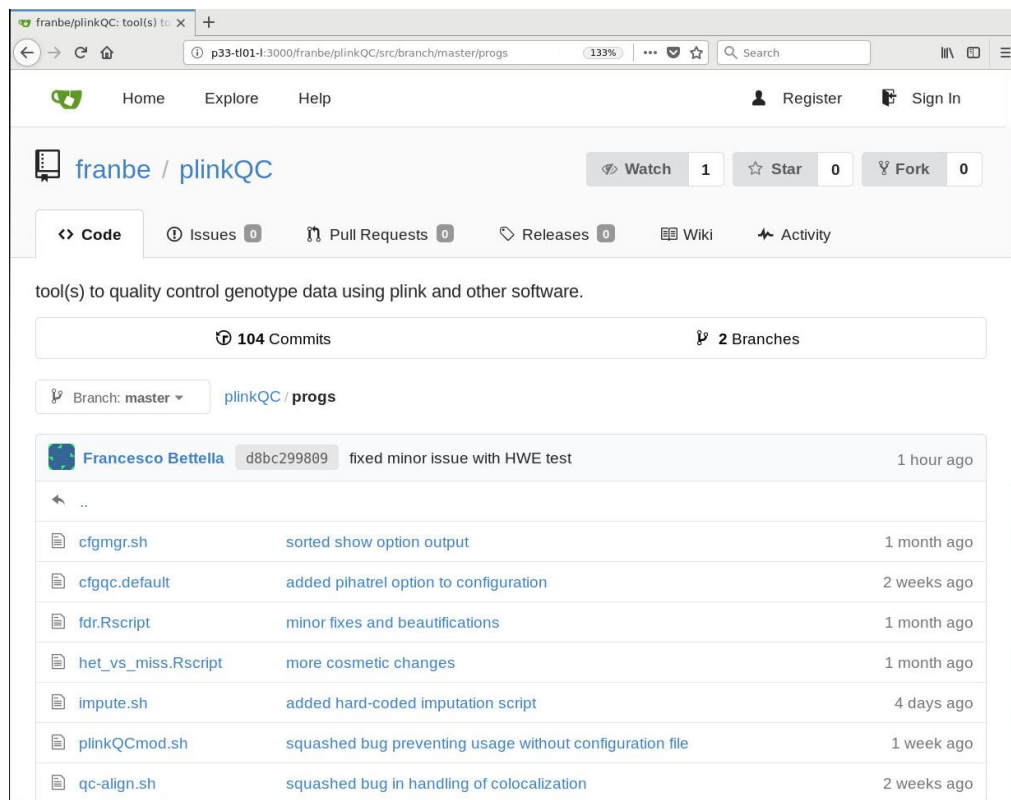
The QC pipeline: notes

some participant in the DemGene project
has a third cousin in the HRC

000UID	FID	IID	PEDSEX	SNPSEX	STATUS	F	MISMIX	RELSHIP	DUPORG	COV	PC1	PC2	PC3	PC4
AMD00001	AMD00001	AMD00001	0	2	PROBLEM	0.07613	OK	NA	ORG	YES	-0.00147879	0.00164313	0.0128753	-0.00402758
AMD00002	AMD00002	AMD00002	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00163578	-0.00290372	-0.00193555	0.00373585
AMD00003	AMD00003	AMD00003	0	2	PROBLEM	0.01625	OK	AMD00007_AMD00007(0.937)	ORG	YES	-0.00238496	-0.00157558	-0.00417843	0.000328896
AMD00004	AMD00004	AMD00004	0	2	PROBLEM	0.02266	OK	NA	ORG	YES	-0.00256515	-0.00171294	-0.0039789	0.00138859
AMD00005	AMD00005	AMD00005	0	1	PROBLEM	1	OK	PACGRXH_DG_NUR6007(0.1046)	ORG	YES	-0.00172506	-0.00241938	-0.00381866	0.00275083
AMD00006	AMD00006	AMD00006	0	2	PROBLEM	0.06074	OK	NA	ORG	YES	-0.00189367	-0.00187384	-0.00316229	0.000993657
AMD00007	AMD00007	AMD00007	0	1	PROBLEM	1	OK	AMD00003_AMD00003(0.937)	DUP	0	NA	NA	NA	NA
AMD00008	AMD00008	AMD00008	0	1	PROBLEM	1	OK	NA	ORG	YES	-0.00191639	-0.00166818	-0.00180217	0.00269283
AMD00009	AMD00009	AMD00009	0	2	PROBLEM	-0.01797	OK	AMD02266_AMD02266(0.2555),AMD02435_AMD02435(0.2472)	ORG	YES	-0.00174973	-0.00212934	-0.00167149	0.000634928

these are *annotated* for use in downstream analyses

The QC pipeline



The screenshot shows the GitHub repository page for 'franbe/plinkQC'. The repository is described as 'tool(s) to quality control genotype data using plink and other software.' It has 104 commits and 2 branches. The current branch is 'master'. The repository is owned by 'franbe' and has 1 watch, 0 stars, and 0 forks. The repository is categorized as 'Code'.

The commit history is as follows:

Commit Hash	Author	Message	Time Ago
d8bc299809	Francesco Bettella	fixed minor issue with HWE test	1 hour ago
..
cfgmgr.sh	..	sorted show option output	1 month ago
cfgqc.default	..	added pihatrel option to configuration	2 weeks ago
fdr.Rscript	..	minor fixes and beautifications	1 month ago
het_vs_miss.Rscript	..	more cosmetic changes	1 month ago
impute.sh	..	added hard-coded imputation script	4 days ago
plinkQCmod.sh	..	squashed bug preventing usage without configuration file	1 week ago
qc-align.sh	..	squashed bug in handling of colocalization	2 weeks ago

The QC pipeline

```
[p33-franbe@p33-tl02-l]#317 18:37:39 (plinkQC): progs/plinkQCmod.sh
you may have neglected to provide any input genotype files.
USAGE: plinkQCmod.sh [OPTIONS] <bed|bcf|vcf file(s)>
    where <bed|bcf|vcf file(s)> are the genotype files to be merged and qc'd.

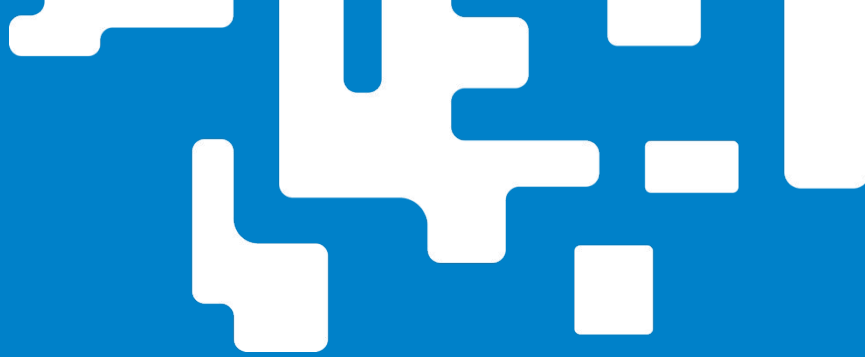
OPTIONS:
  -c <config file>    optional configuration file
  -m                  reduce variant set to the minimal one common to all
  -w <sample file>    optional white list of individuals to restrict qc to
  -o <output prefix>  optional output prefix [default: 'plinkqc']
  -h                  show help message

CONFIGURATION:
  the <config file> may contain custom definitions for a number of variables.
  [see default configuration file 'cfgqc.default' for more information]

[p33-franbe@p33-tl02-l]#318 18:39:10 (plinkQC):
```

The screenshot shows the GitHub repository page for 'franbe/plinkQC'. The repository has 104 commits and 2 branches. The 'master' branch is selected. A list of recent commits is shown, with the commit 'd8bc299809' by Francesco Bettella, titled 'fixed minor issue with HWE test', highlighted. Below the commit list, a table of files is displayed, including 'cfgmgr.sh', 'cfgqc.default', 'fdr.Rscript', 'het_vs_miss.Rscript', 'impute.sh', 'plinkQCmod.sh', and 'qc-align.sh'. The file 'plinkQCmod.sh' is circled in orange, and an arrow points from it to the terminal window on the left.

File	Description	Time
cfmgr.sh	sorted show option output	1 month ago
cfgqc.default	added phlatel option to configuration	2 weeks ago
fdr.Rscript	minor fixes and beautifications	1 month ago
het_vs_miss.Rscript	more cosmetic changes	1 month ago
impute.sh	added hard-coded imputation script	4 days ago
plinkQCmod.sh	squashed bug preventing usage without configuration file	1 week ago
qc-align.sh	squashed bug in handling of colocalization	2 weeks ago



Phasing

Chip genotypes

A = 0 → reference allele
a = 1 → alternative allele

diploid (two copies)

101001120222000101212020100010

Chip genotypes

phasing problem

A AA aAaaaAAA A a aAaA AAA A

A AA aAaaaAAA A a aAaA AAA A

↑ ↑↑ ↑↑↑↑↑↑↑↑ ↑ ↑ ↑↑↑↑ ↑↑↑ ↑

101001120222000101212020100010

Chip genotypes

phasing problem

A	AA	aAaaaAAA	A	a	aAaA	AAA	A			
?	?	??		?	?	?		?		?
A	AA	aAaaaAAA	A	a	aAaA	AAA	A			

↑ ↑

101001120222000101212020100010

Chip genotypes

phasing problem

A	AA	aA	aaa	AAA	aA	a	aA	aA	AAA	A
?	?	??			?	?		?		?
A	AA	aA	aaa	AAA	AA	a	aA	aA	AAA	A

↑ ↑

101001120222000101212020100010

Chip genotypes

phasing problem

A	AA	aAaaaAAA	A	A	a	aAaA	AAA	A
?	?	??			?	?		?
A	AA	aAaaaAAA	a	A	a	aAaA	AAA	A

↑ ↑

101001120222000101212020100010

Chip genotypes

phasing problem

A	AA	aAaaaAAA	A	a	aAaA	AAA	A
?	?	??		?	?		?
A	AA	aAaaaAAA	a	a	aAaA	AAA	A



another job for Captain Markov!

Inbox - checco74@gmail.co presentations - Google Driv Workshop_Dec11 - Google Eagle v2.4.1 User Manu X + - □ X

← → ↻ ⌂ https://data.broadinstitute.org/alkesgroup/Eagle/ 📖 ☆ ⚙️ ✍️ ↶ 🔴 ...

Eagle v2.4.1 User Manual

Po-Ru Loh

November 18, 2018

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Chip genotypes

phasing problem

aAA**A**A**aA**aAaAaA**A**A**aA**a**A**aAaA**A**A**A**A**A** **52%**

AA**A**A**aA**aAaAaA**A**A**aA**a**A**aAaA**A**A**A**A**A** **24%**

aAA**A**A**A**aAaAaA**A**A**aA**a**A**aAaA**aA**A**A**A**A** **16%**

aAA**A**A**A**aAaAaA**A**A**A**a**aA**aAaA**aA**A**A**A**A** **8%**

Chip genotypes

phasing problem

aAA**A**A**aA**aAa**aA**A**aA**a**A**aAa**A**A**A**A**A**A**A** **52%**

AA**A**A**aA**aAa**aA**A**A**a**aA**aAa**A**A**A**A**A**A**A** **24%**

aAA**A**A**A**aAa**aA**A**A****A**a**aA**aAa**aA**A**A**A**A**A**A** **16%**

aAA**A**A**A**aAa**aA**A**A****A**a**aA**aAa**aA**A**A**A**A**A**A** **8%**

Chip genotypes

phasing problem

aA**A**A**a**A**a**A**a**a**A**A**a**A**a**A**a**A**A**A**A**A**A**

Chip genotypes

phasing problem

aAAA**aA**aAaaaAA**aA****aA**aAaA**AAAA****AA**

AA**aAA****Aa**aAaaaAA**AA****AA****a**aAaA**aAAA****aA**

Chip genotypes

phasing problem

aA**A**A**a**A**a**A**a**a**A**A**a**A**a**A**a**A**A**A**A**A**A**

AA**a**A**A**A**a**A**a**a**a**A**A**A**A**A**a**A**a**A**a**A**a**A**A**A**a**A

Phased haplotypes



Imputation

Phased haplotypes

imputation problem

aAAAAaAaAaaaAAAAaAaaAaAaAAAAAA

Phased haplotypes

imputation problem

a??AAAA??aAa???AaaaA??A?AaAaaAaAa??AAA??AAA?A

Phased haplotypes

imputation problem

a??AAAA??aAa???AaaaA??A?AaAaaAaAa??AAA??AAA?A

A diagram consisting of a red rounded rectangle containing the haplotype string. Below the rectangle, a central point has several thin grey lines radiating upwards to connect to specific characters in the string: the first 'a', the first 'A' of the first 'AAAA' block, the 'a' of 'aAa', the first 'A' of the second 'AaaaA' block, the 'A' of 'A?', the 'a' of 'Aa', the first 'a' of 'Aaa', the 'A' of 'AaAa', the first 'A' of the second 'AAA' block, and the first 'A' of the third 'AAA' block.

based in linkage in a reference data set,
we want to guess what lies under '?'


Phased haplotypes

imputation problem

a??AAAA??aAa???AaaaA??A?AaAaaAaAa??AAA??AAA?A



another job for Captain Markov!



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Minimac4

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Introduction

Minimac4 is a latest version in the series of genotype imputation software - preceded by [Minimac3](#) (2015), [Minimac2](#) (2014), [minimac](#) (2012) and [MaCH](#) (2010). **Minimac4** is a lower memory and more computationally efficient implementation of the original algorithms with comparable imputation quality.

The Minimac3 mailing list has been renamed as the Minimac4 mailing list. If you were already a member, no need to re-join. If not, please join our [mailing list](#) to get updates about future releases or report possible bugs or email them to [Ketian Yu](#) or [Sayantan Das](#).

Download

Minimac4 (version 1.0.0, released 2.14.2018) is currently available for download. Commonly used reference panels in M3VCF format are available for download in [Reference Panels](#).

The imputation pipeline

phasing

for each chromosome:

eagle

```
--chrom ${chromosome}  
--geneticMapFile ${genmap}  
--vcfRef ${bcfref}  
--vcfTarget ${bcftarget}  
--outPrefix ${out}  
--numThreads 4
```

imputation

for each chromosome:

for each subsample:

minimac

```
--chr ${chromosome}  
--haps ${phasedvcf}  
--refHaps ${vcfref}  
--rounds 5 --states 200  
--prefix ${out}
```

phasing and imputation are handled by 'impute.sh'

The screenshot shows the GitHub repository for 'franke/plinkQC'. The commit history is displayed, with the 'impute.sh' file highlighted by an orange circle. An arrow points from the 'minimac' section of the imputation pipeline to this file. The commit history includes the following entries:

Commit Hash	Author	Message	Time
d8bc299809	Francesco Bettella	fixed minor issue with HWE test	1 hour ago
..
cfgmgr.sh		sorted show option output	1 month ago
cfgqc.default		added phlatrel option to configuration	2 weeks ago
fdr.Rscript		minor fixes and beautifications	1 month ago
het_vs_miss.Rscript		more cosmetic changes	1 month ago
impute.sh		added hard-coded imputation script	4 days ago
plinkQCmod.sh		squashed bug preventing usage without configuration file	1 week ago
qc-align.sh		squashed bug in handling of colocalization	2 weeks ago

Useful links

- <https://genome.sph.umich.edu/wiki/Minimac4>
- <https://data.broadinstitute.org/alkesgroup/Eagle>
- <https://www.cog-genomics.org/plink2>
- <https://samtools.github.io/bcftools/bcftools>
- <https://www.r-project.org>



NORMENT

Norwegian Centre for
Mental Disorders Research

That's all folks!

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