MICHIGAN IMPUTATIONSERVER

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ASHG ANNUAL MEETING 2025
BOSTON, MA • OCTOBER 14-18

Disclosure Slide

Financial Disclosure for:

Christian Fuchsberger

Sebastian Schönherr

Lukas Forer

Cassandra Spracklen

Eleftheria Zeggini

Albert Smith

We have nothing to disclose

Setup

- 6 Sessions:
  (1) Genotype Imputation
  (2) Use the server and the Imputation Bot,
  (3) GWAS,chrX, HLA (4) GWAS pipeline and PGS Server,
  (5) Helmholtz Munich Imputation Server, (6) TOPMed

  - Lectures
  - Demos
  - Interaction
    - PollEv.com/ashg

- Question & Answer session at the end

Section 1

Imputation and the Server

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MICHIGAN IMPUTATIONSERVER

Learning objectives

Participants will

1. Understand the principles of genotype imputation and the Michigan Imputation Server

Genotype imputation

Key method used in GWAS to

- Increase the number of tested variants
- Fine-mapping becomes more complete
- Meta-analysis using different arrays

0. Imputation setting

GWAS Haplotypes

```
. . . . A . . . . . . . A . . . . A . . .
. . . . G . . . . . . . C . . . . A . . .
```

Reference Haplotypes (e.g. TOPMed)

```
C G A G A T C T C C T T C T T C T G T G C
C G A G A T C T C C C G A C C T C A T G G
C C A A G C T C T T T T C T T C T G T G C
C G A A G C T C T T T T C T T C T G T G C
C G A G A C T C T C C G A C C T T A T G C
T G G G A T C T C C C G A C C T C A T G G
C G A G A T C T C C C G A C C T T G T G C
C G A G A C T C T T T T C T T T T G T A C
C G A G A C T C T C C G A C C T C G T G C
C G A A G C T C T T T T C T T C T G T G C
```

1. Identify match among reference

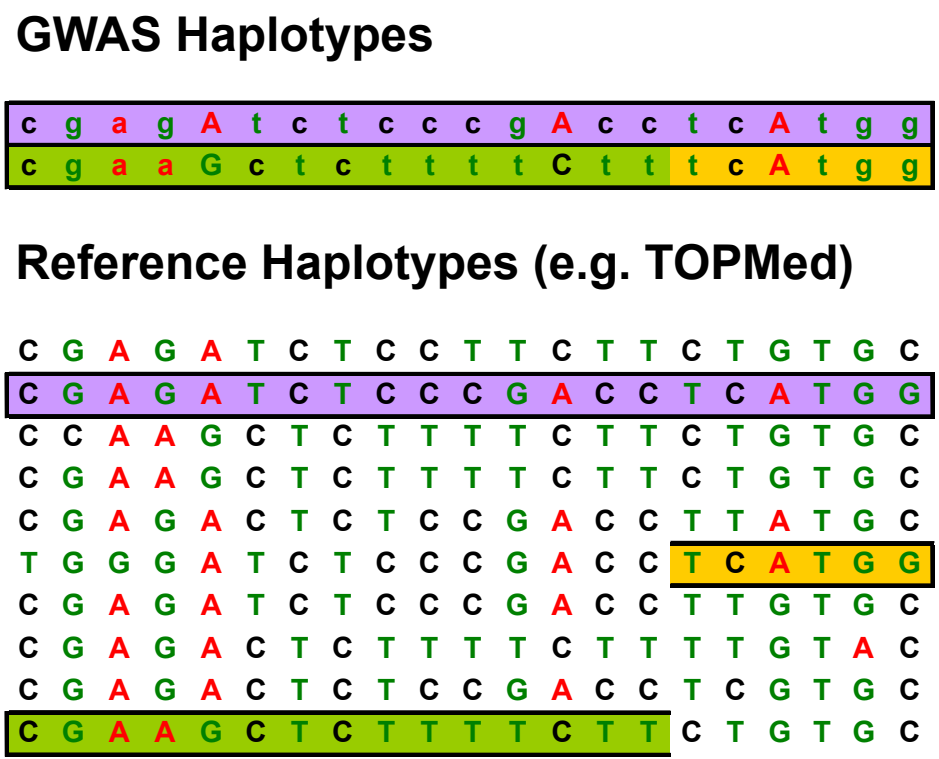
GWAS Haplotypes

```
. . . . A . . . . . . . A . . . . A . . .
. . . . G . . . . . . . C . . . . A . . .
```

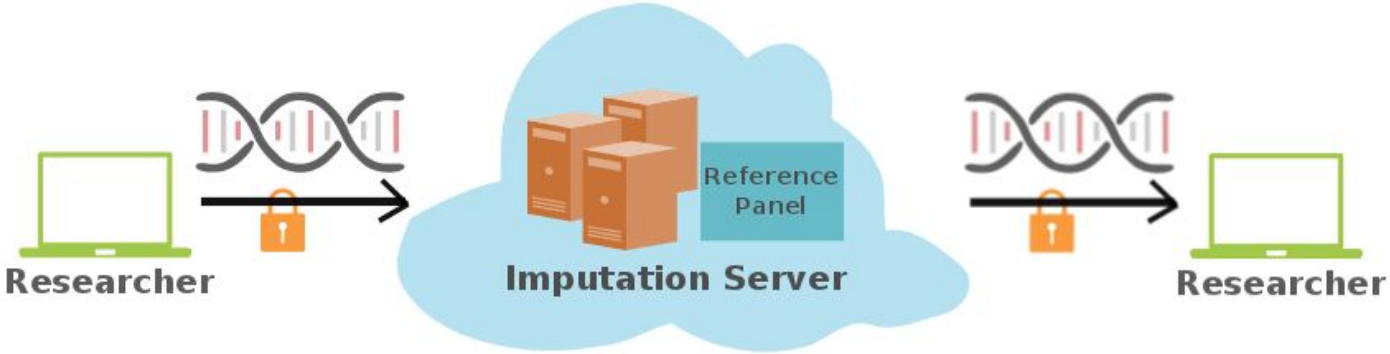
Reference Haplotypes (e.g. TOPMed)

```
C G A G A T C T C C T T C T T C T G T G C
C G A G A T C T C C C G A C C T C A T G G
C C A A G C T C T T T T C T T C T G T G C
C G A A G C T C T T T T C T T C T G T G C
C G A G A C T C T C C G A C C T T A T G C
T G G G A T C T C C C G A C C T C A T G G
C G A G A T C T C C C G A C C T T G T G C
C G A G A C T C T T T T C T T T T G T A C
C G A G A C T C T C C G A C C T C G T G C
C G A A G C T C T T T T C T T C T G T G C
```

2. Impute


GWAS Haplotypes
c g a g A t c t c c c g A c c t c A t g g
c g a a G c t c t t t t C t t t c A t g g
Reference Haplotypes (e.g. TOPMed)
C G A G A T C T C C T T C T T C T G T G C
C G A G A T C T C C C G A C C T C A T G G
C C A A G C T C T T T T C T T C T G T G C
C G A A G C T C T T T T C T T C T G T G C
C G A G A C T C T C C G A C C T T A T G C
T G G G A T C T C C C G A C C T C A T G G
C G A G A T C T C C C G A C C T T G T G C
C G A G A C T C T T T T C T T T T G T A C
C G A G A C T C T C C G A C C T C G T G C
C G A A G C T C T T T T C T T C T G T G C

ASHG 2014: imputation web service



1.

Upload GWAS data

2.

Server performs

- Quality checks
- Pre-phasing
- Imputation
- Encryption

3.

Download results

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Interactive polls

https://pollev.com/ashg

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Have you ever used the Michigan Imputation Server

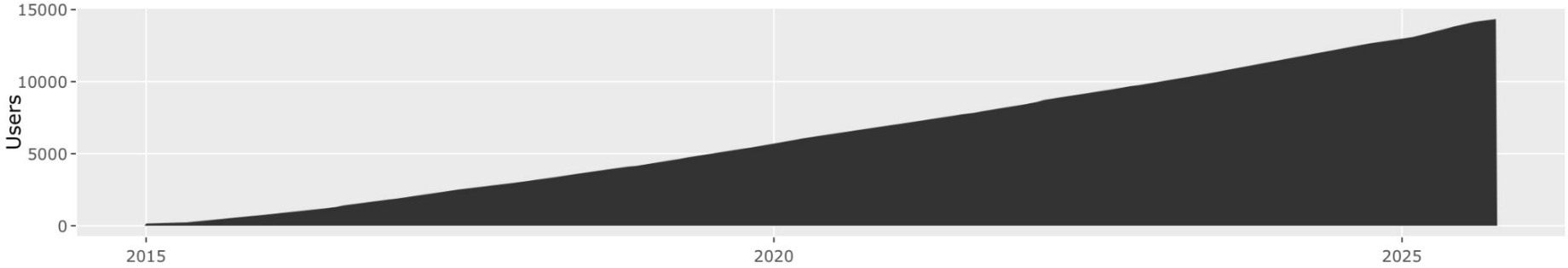
Yes

0%

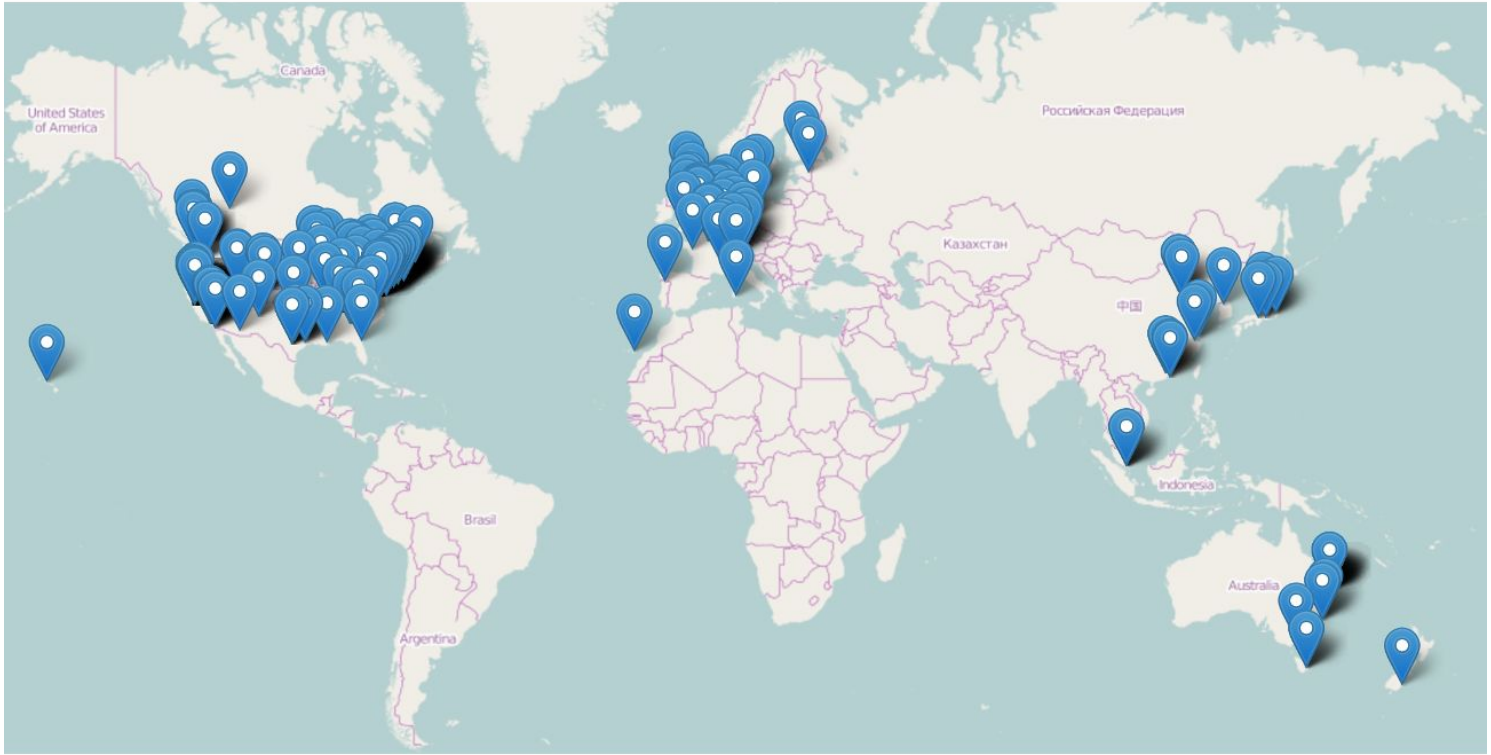
No

0%

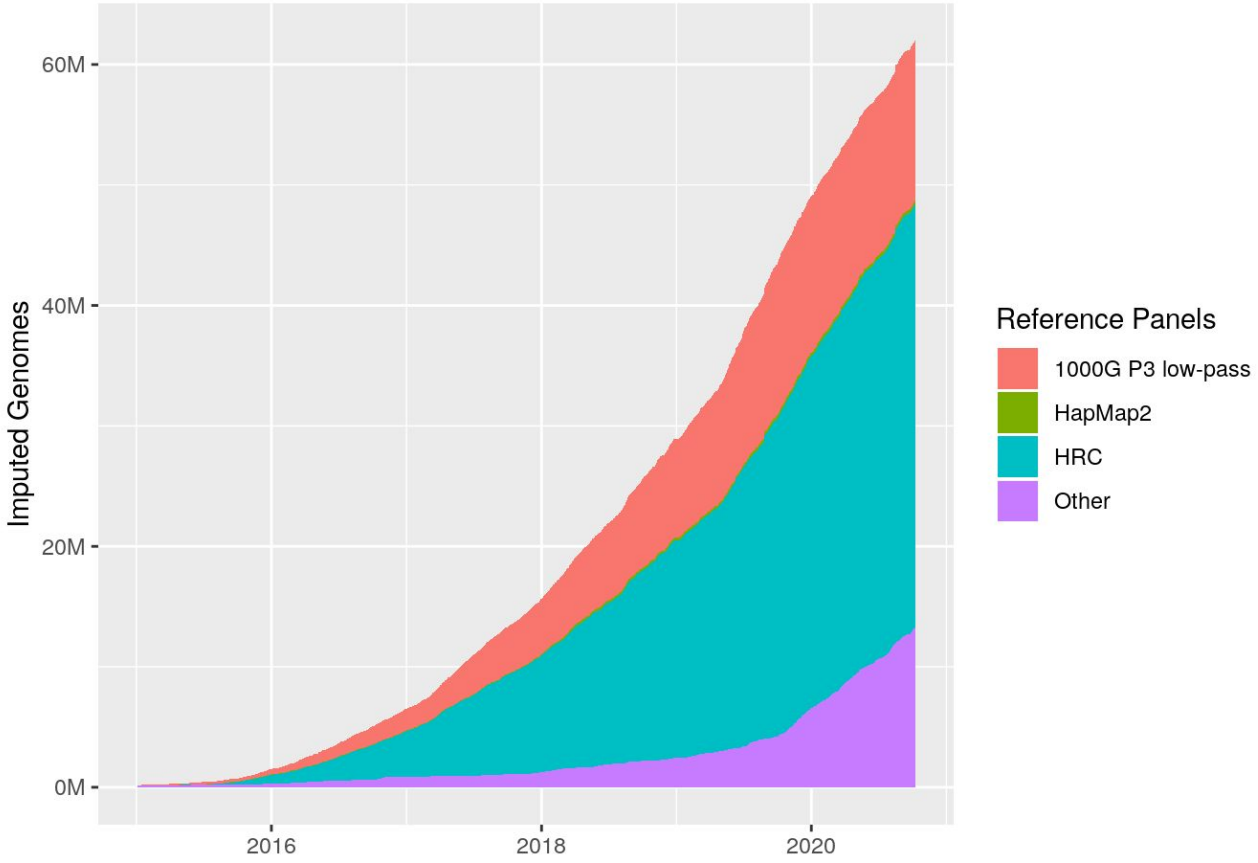
>14,000 users



Used by researcher world-wide



>120M imputed genomes



Summary

Genotype imputation key method in GWAS

Michigan imputation server is easy to use and ensures high quality imputation

Cloud-services will accelerate genetic research so we can devote our time to more interesting tasks

More info and FAQ can be found here:
https://genepi.github.io/michigan-imputationserver/

Section 2

Run a job, Data Preparation and Server Interaction

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Learning objectives

Participants will learn

1. How to submit a genotype imputation job
2. How to prepare your input data
3. Different ways to interact with imputation servers

To run an imputation / PGS jobs, several imputation servers are available (1)

- Michigan Imputation Server (2015)
  https://imputationserver.sph.umich.edu
- TOPMed Imputation Server (2020)
  https://imputation.biodatacatalyst.nhlbi.nih.gov
- Helmholtz Munich Imputation Server (2024)
  https://imputationserver.helmholtz-munich.de
- Mexico City Prospective Study Imputation Server (2025)
  https://imputationserver-reg.sph.umich.edu/

To run an imputation / PGS jobs, several imputation servers are available (2)

- All servers are built on the same software stack
  - version 1: Hadoop based
  - version 2: Nextflow based (since 2024)
- Each server offers different reference panels and applications

- For this workshop, we provide a hands-on tutorial using the Michigan Imputation Server (MIS) instance

Which genotype imputation service have you utilized to date?

Michigan Imputation Server

0%

TOPMed Imputation Server

0%

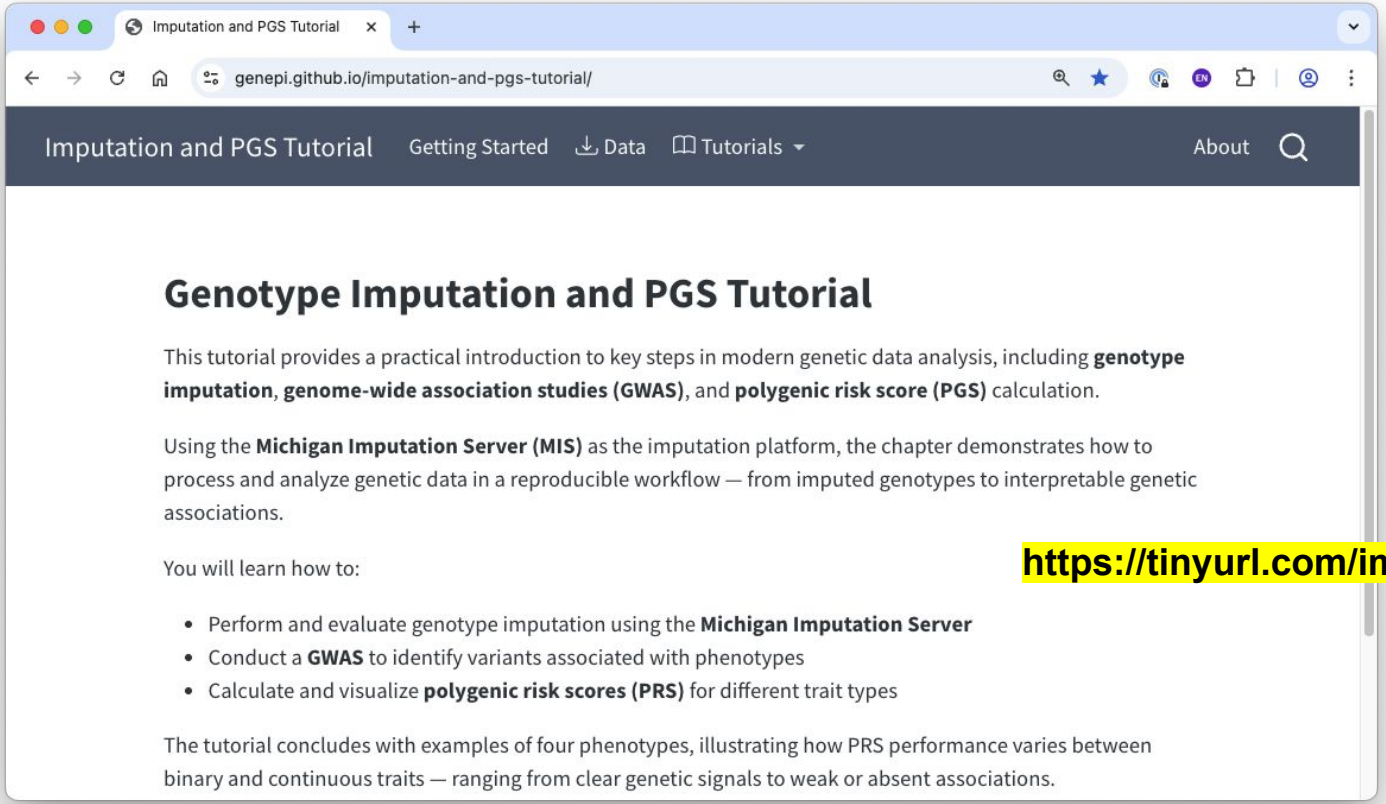
Munich Imputation Server

0%

Other Imputation Service

0%

Hands-On Tutorial



https://tinyurl.com/imputationserver-2025

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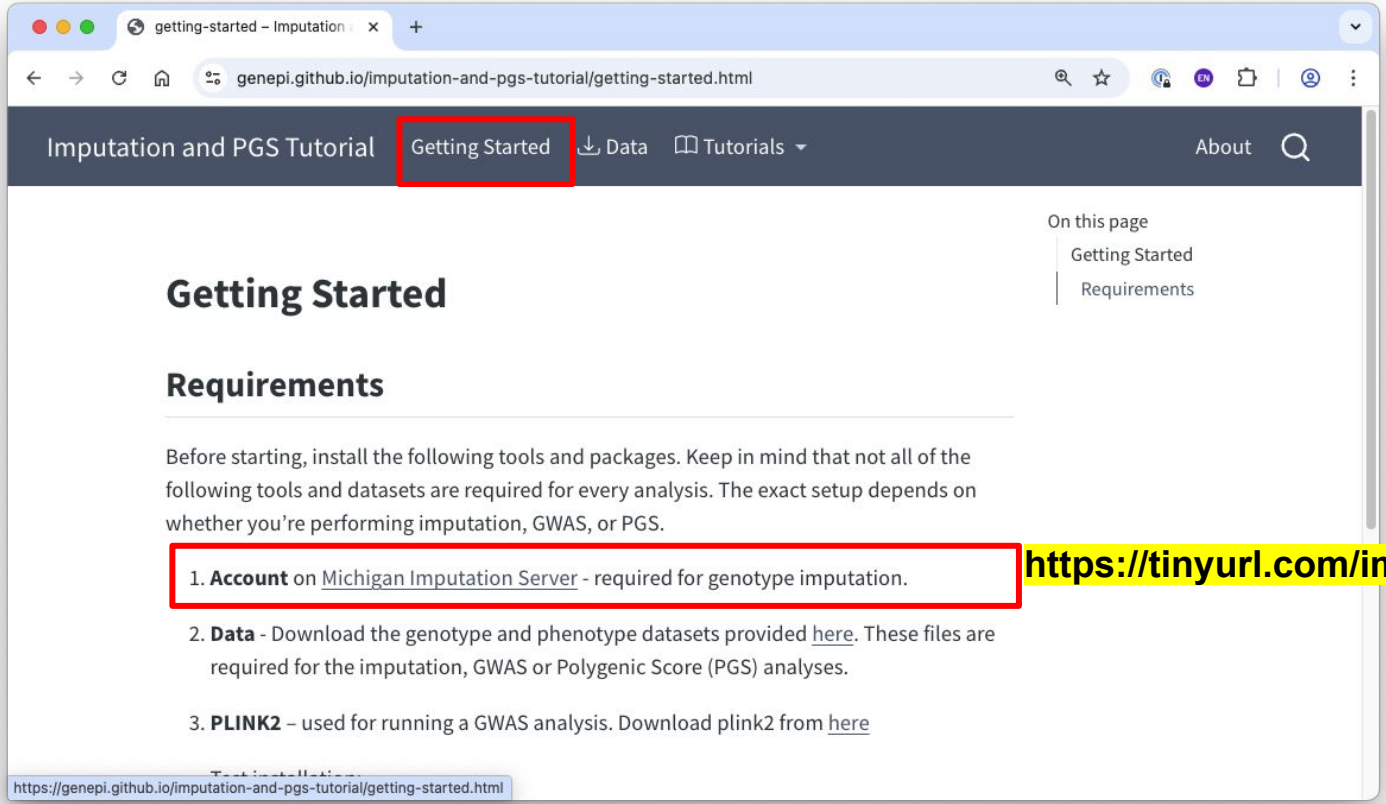
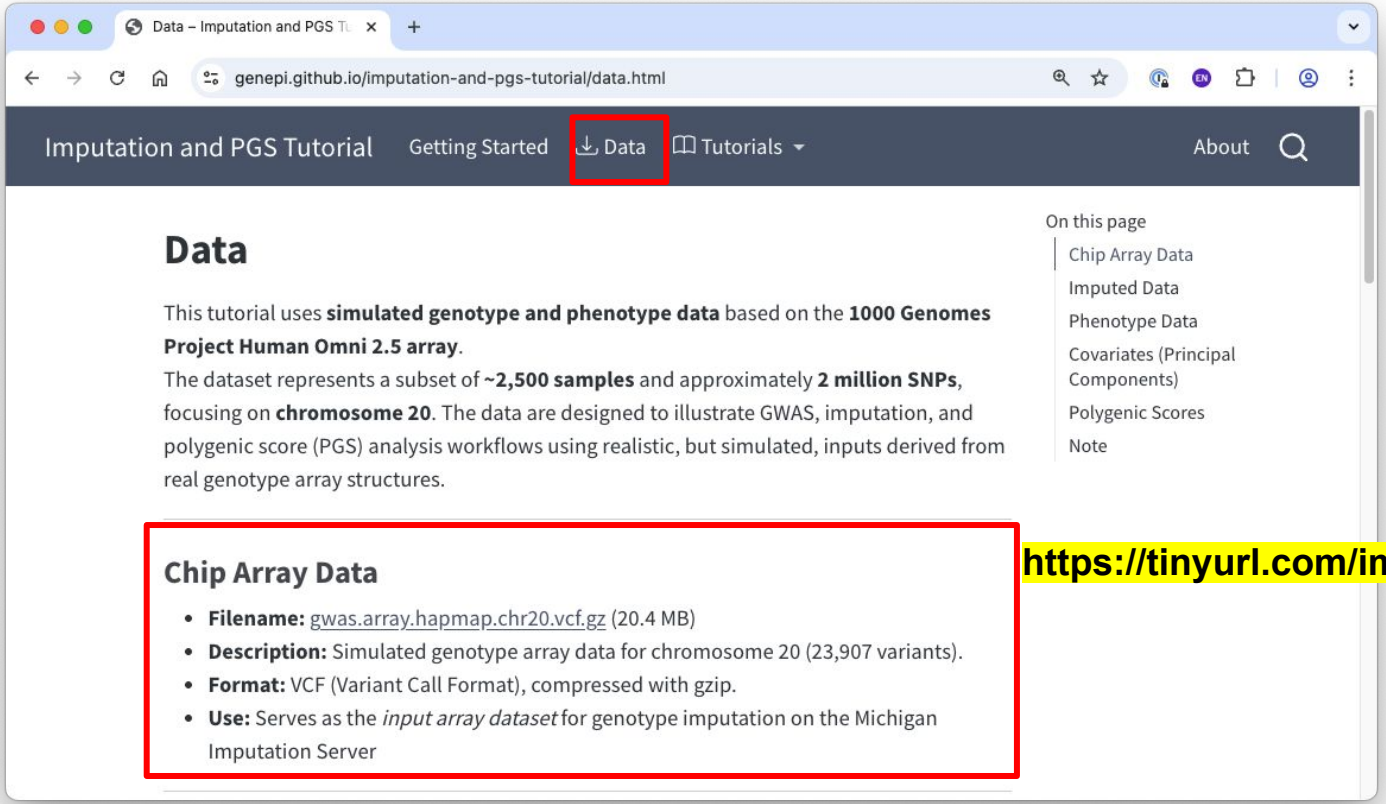
Running Imputation: Register an Account



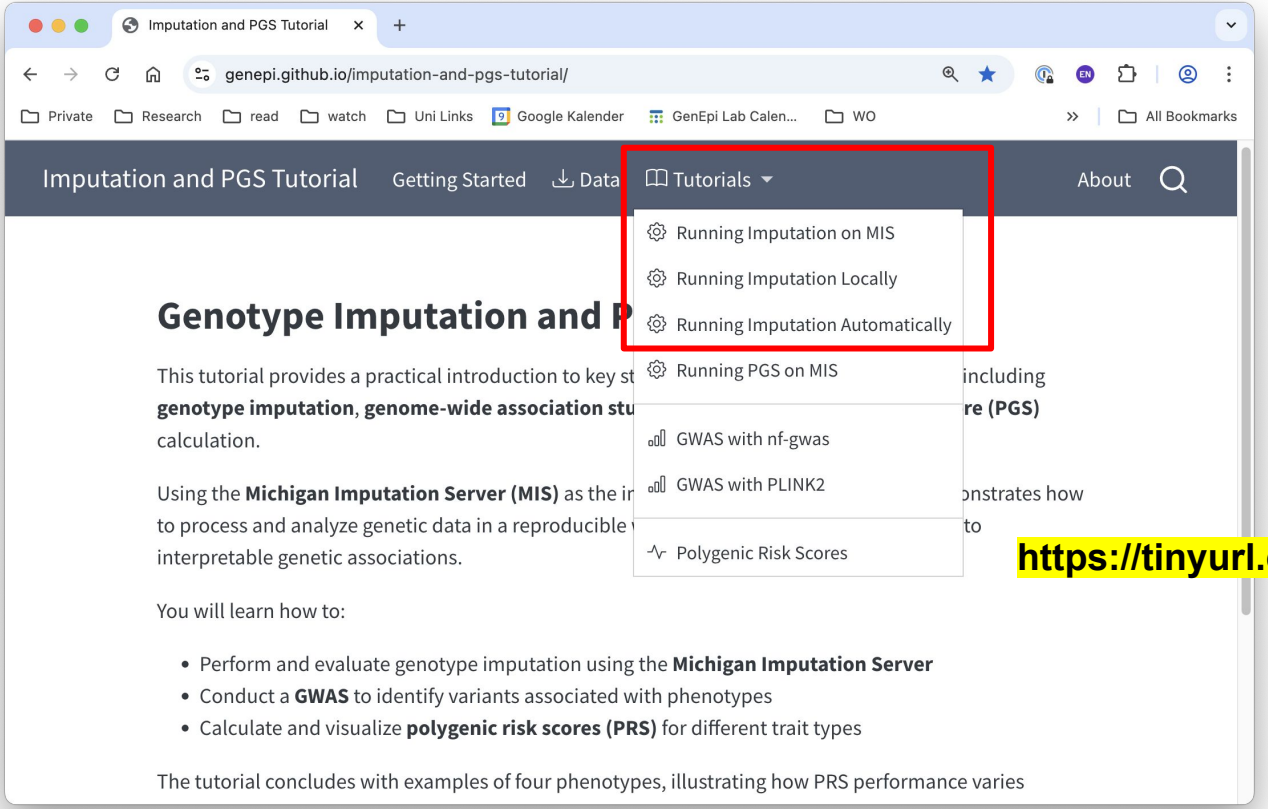
https://tinyurl.com/imputationserver-2025

Running Imputation: Download Array Data



https://tinyurl.com/imputationserver-2025

Running Imputation on MIS



https://tinyurl.com/imputationserver-2025

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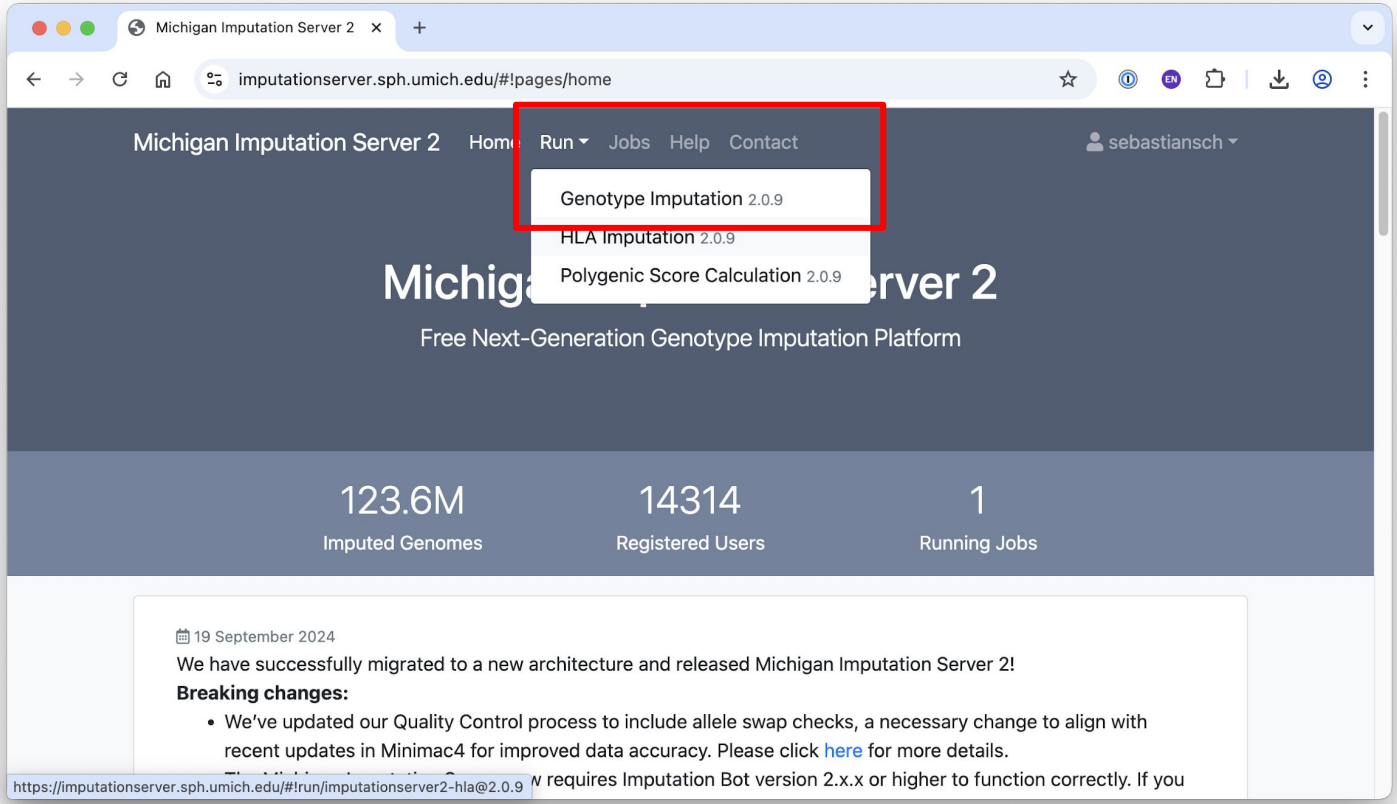
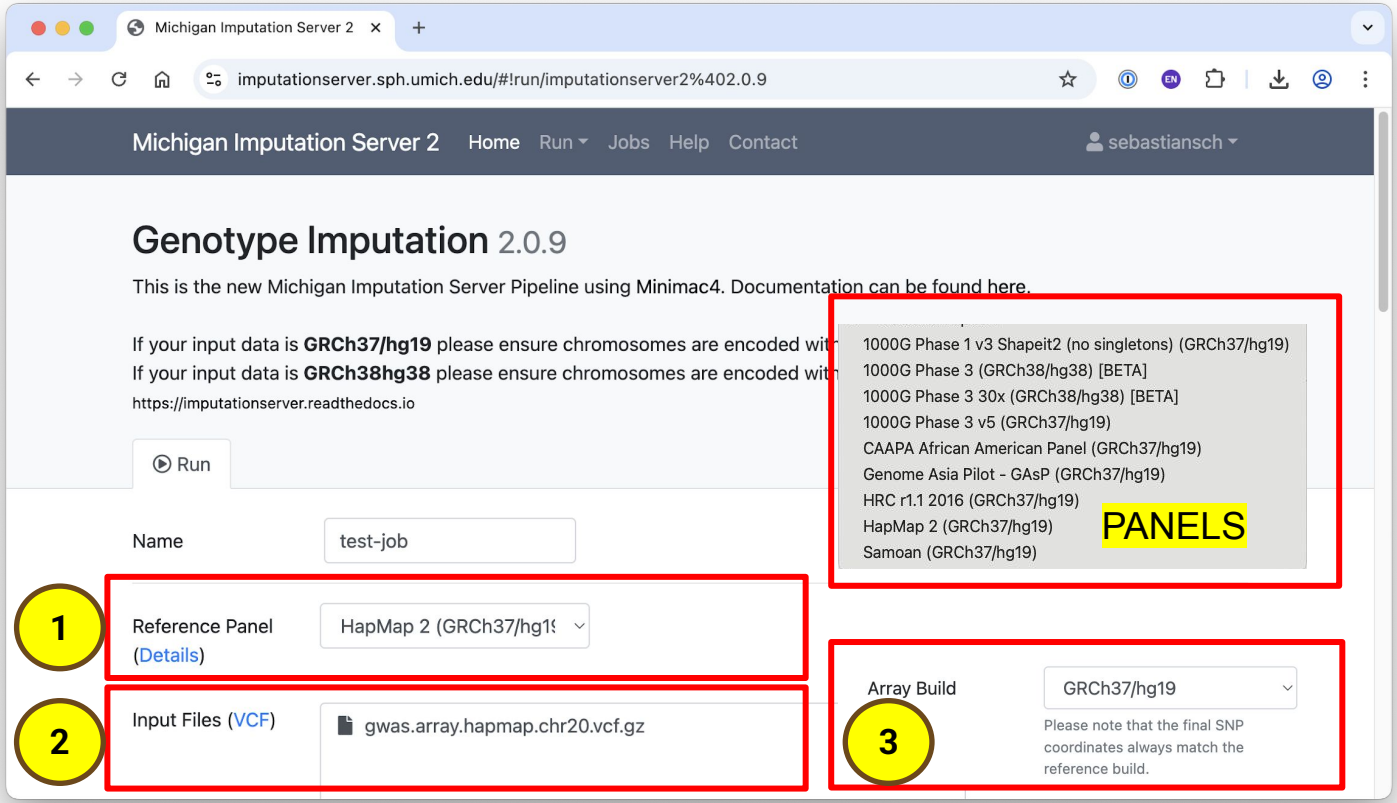
Running Imputation on MIS

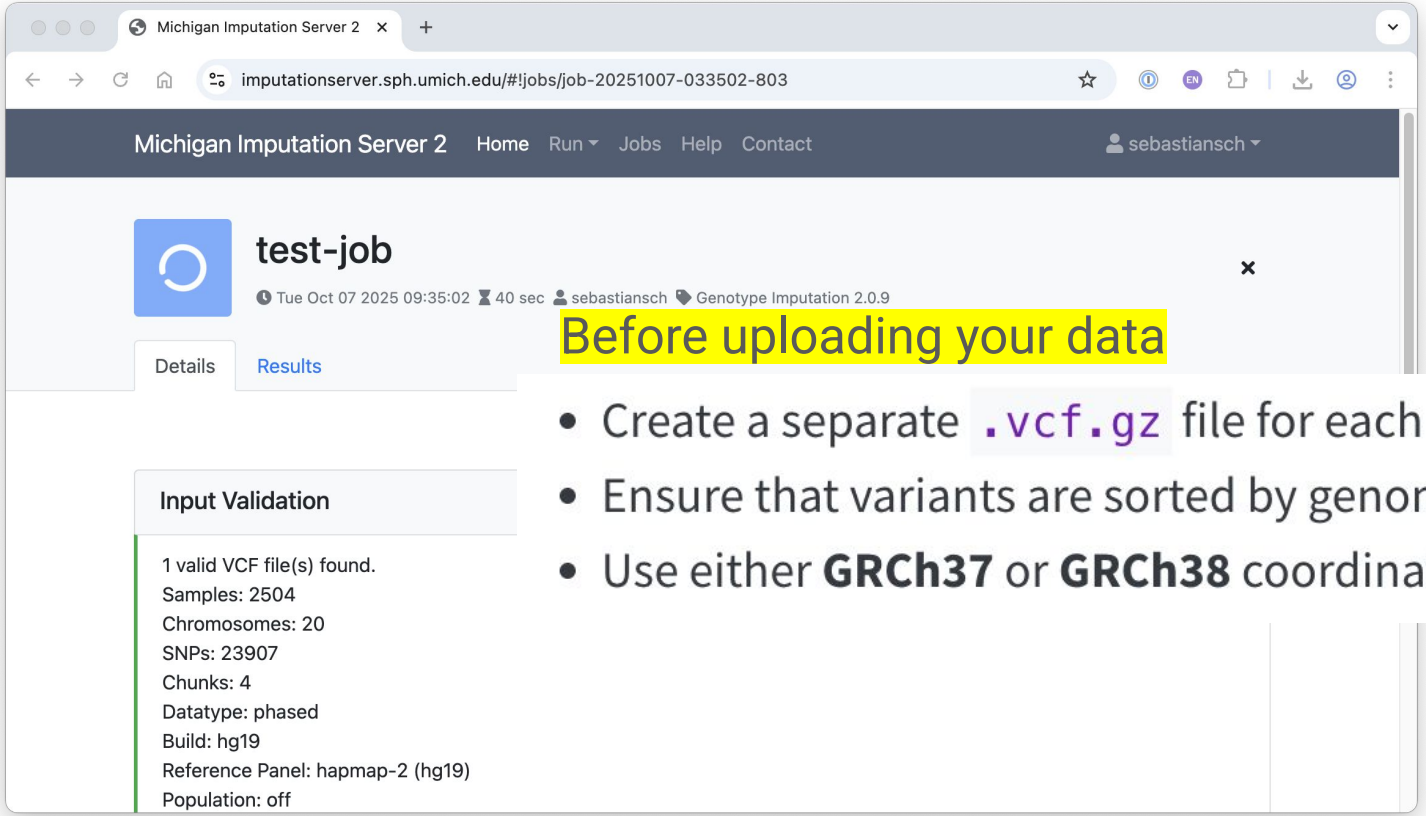
Running Imputation: Submit a first job



Upload Data and Select Reference Panel



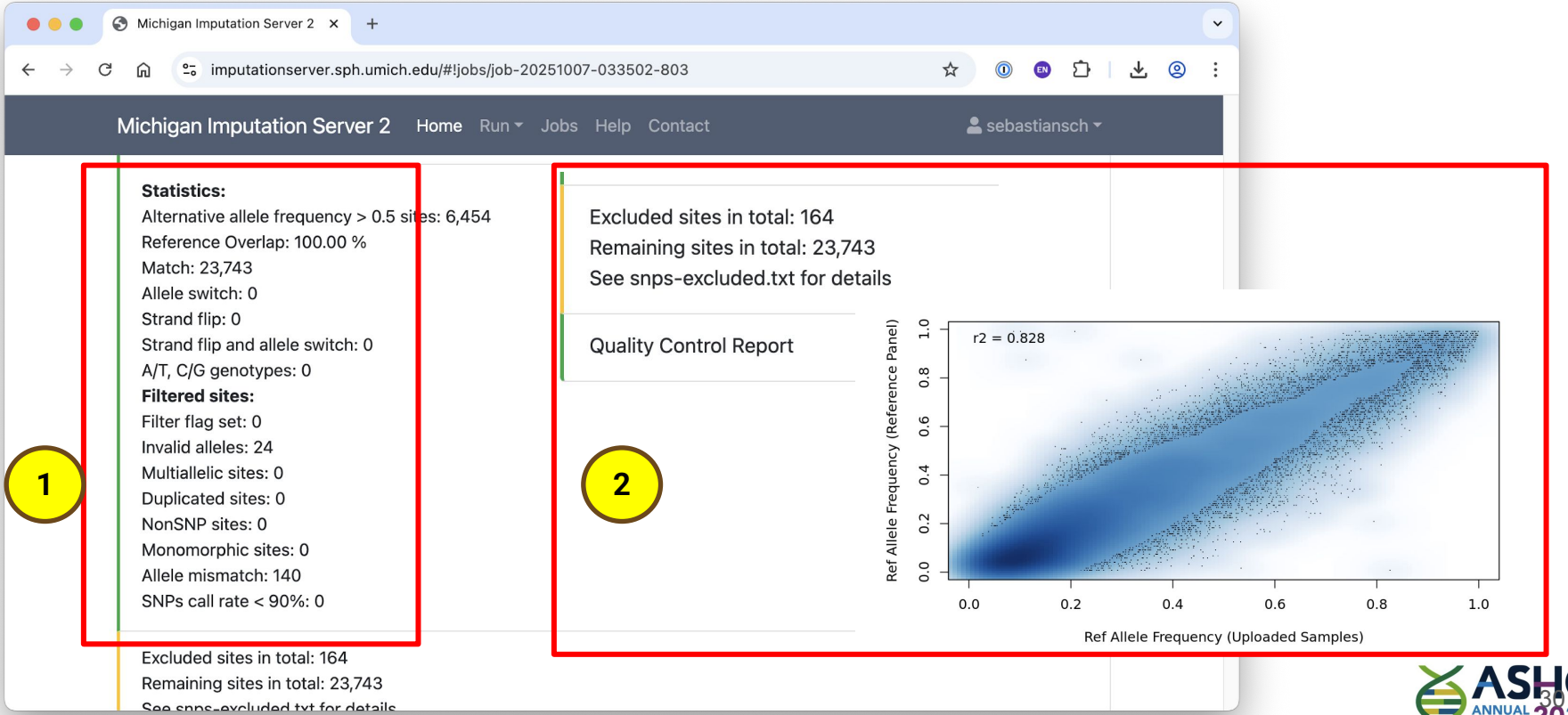
Step 1: Input Validation



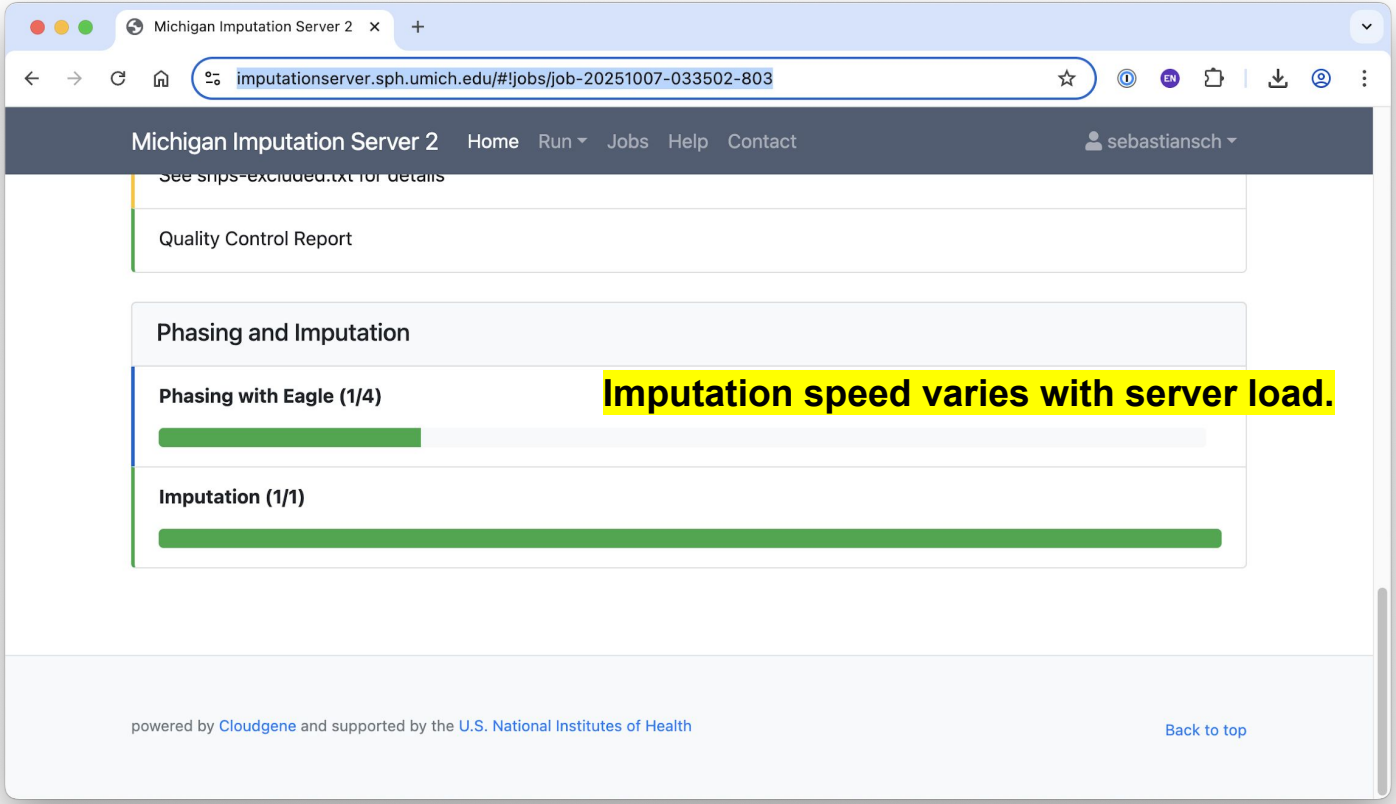
Before uploading your data

- Create a separate `.vcf.gz` file for each chromosome.
- Ensure that variants are sorted by genomic position.
- Use either **GRCh37** or **GRCh38** coordinates.

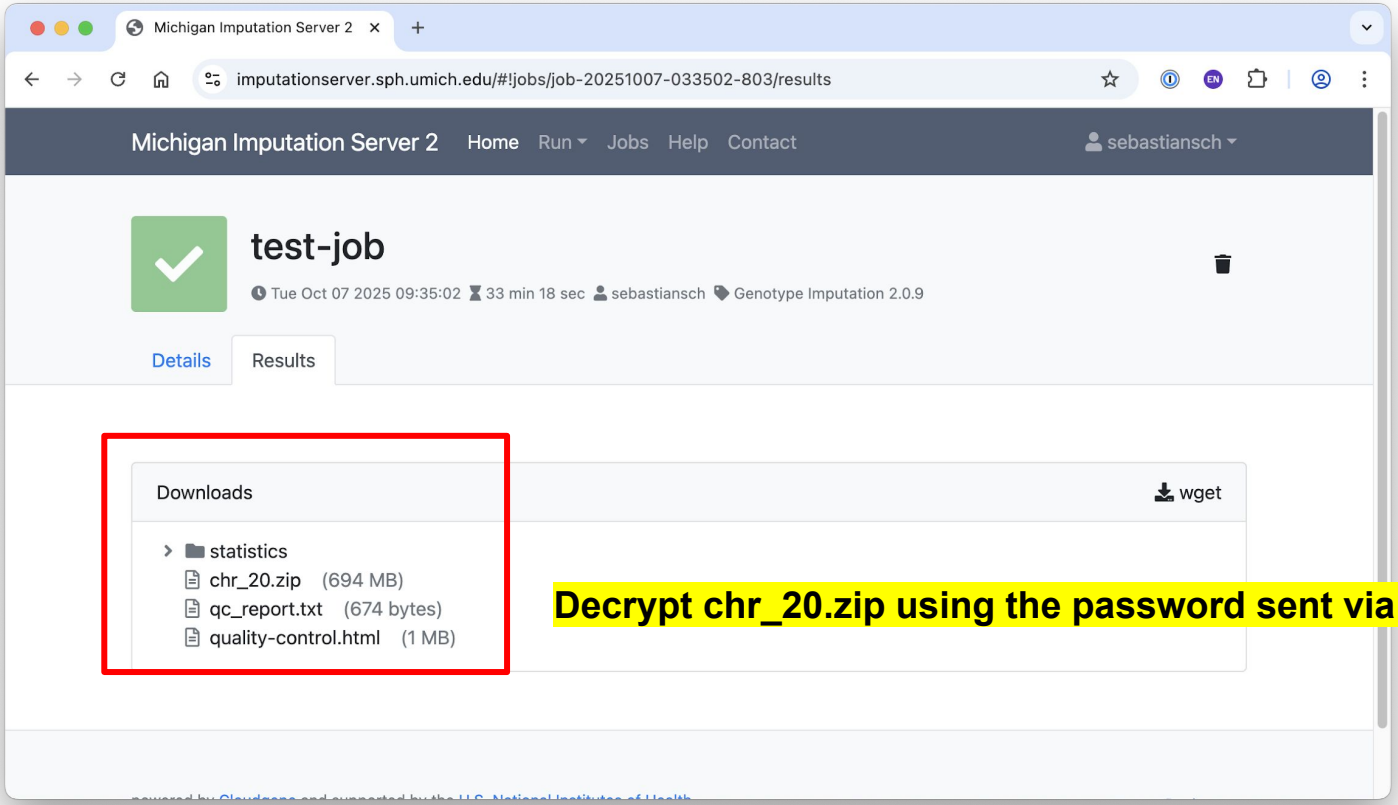
Step 2: Quality Control



Step 3 + 4: Phasing, Imputation, Encryption



Download Results

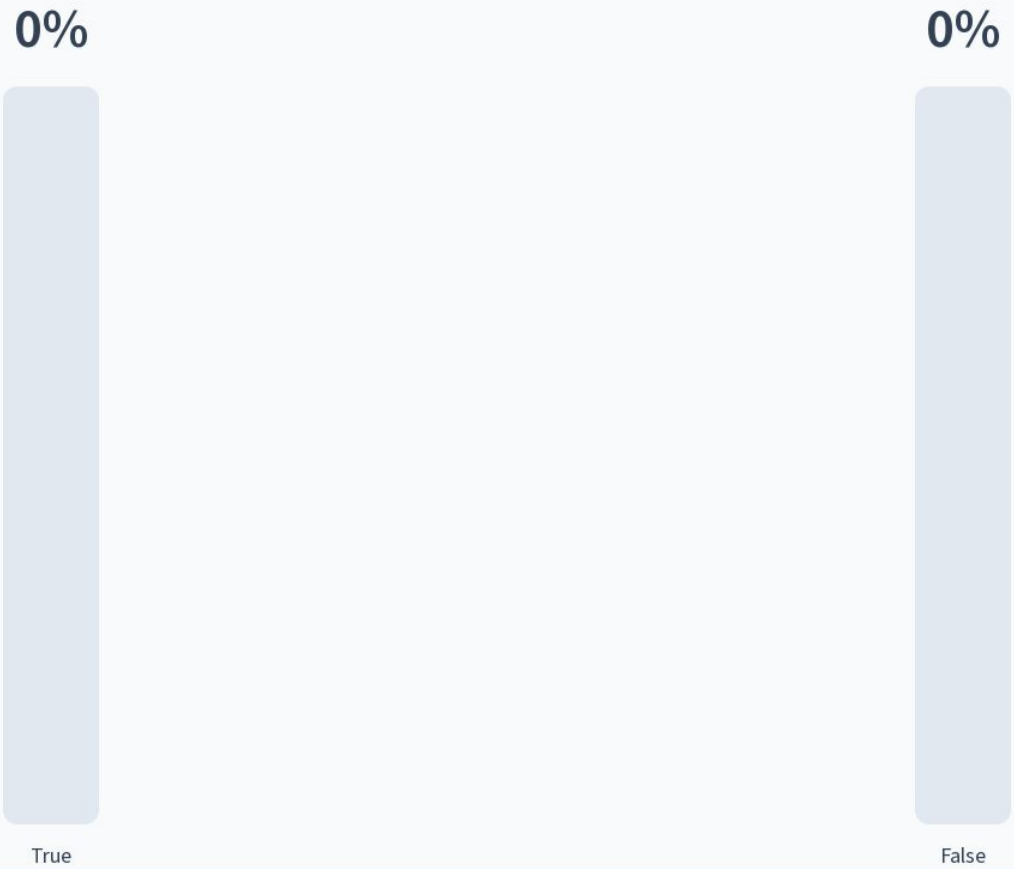


Decrypt chr_20.zip using the password sent via email.

How many jobs are failing?

- 40% in 2015; 20% in 2019; 7% 2020-2024; >10% 2025
- Reason for job failures: Something wrong with your input data ***or*** phasing/imputation issue on our side

For users: Have you ever encountered a failed imputation job?



Input Validation

Input Validation

The provided VCF file is malformed. Error during index creation: [tabix] was bgzip used to compress this file? (see Help).

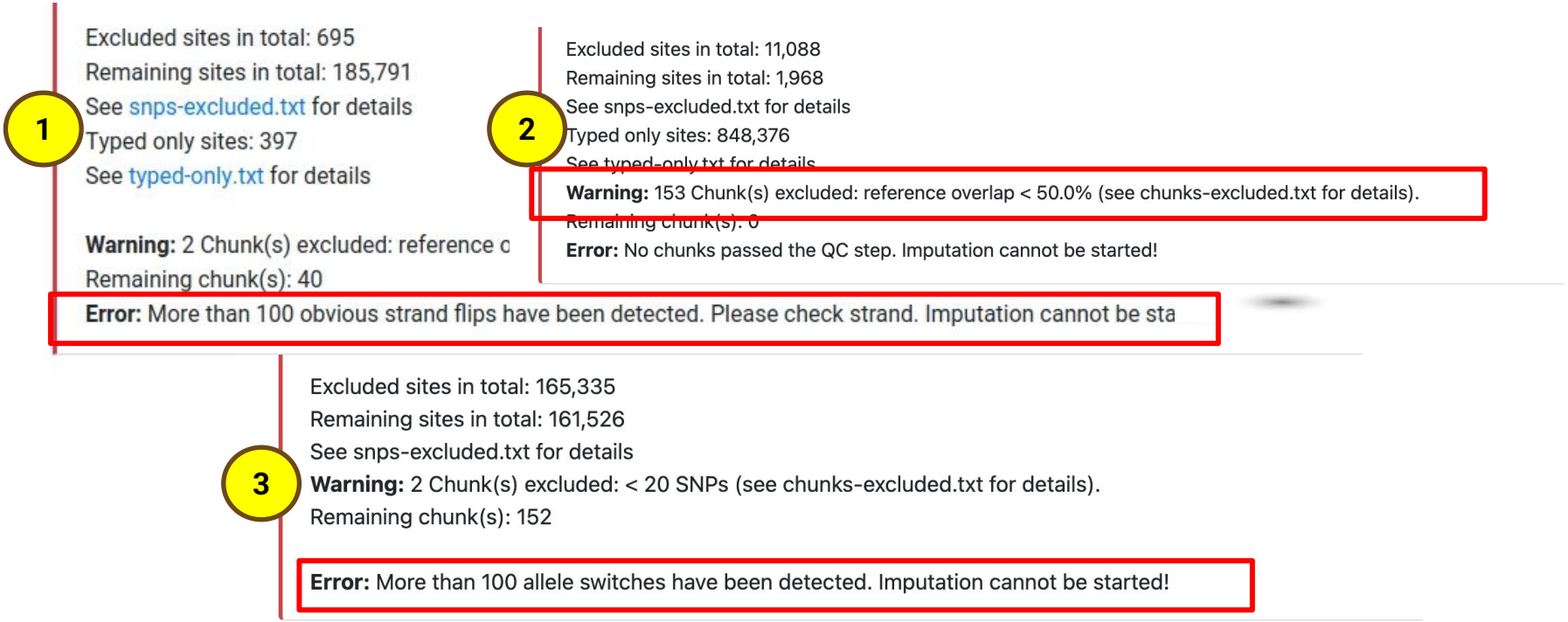
Input Validation

The provided VCF file contains more than one chromosome. Please split your input VCF file by chromosome (see Help).

Input Validation

Unable to parse header with error: Your input file has a malformed header: We never saw the required CHROM header line (starting with one #) for the input VCF file (see Help).

QC Step



How to fix input files?

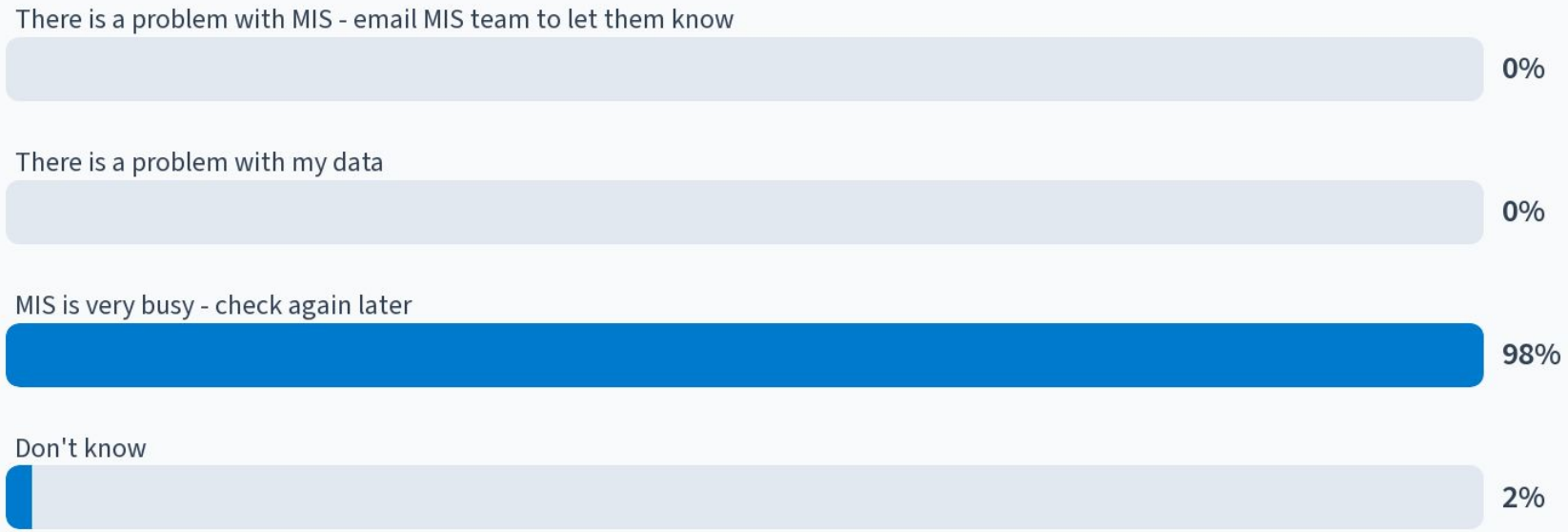
Imputation Preparation Tool

TUTORIAL AVAILABLE ON MIS

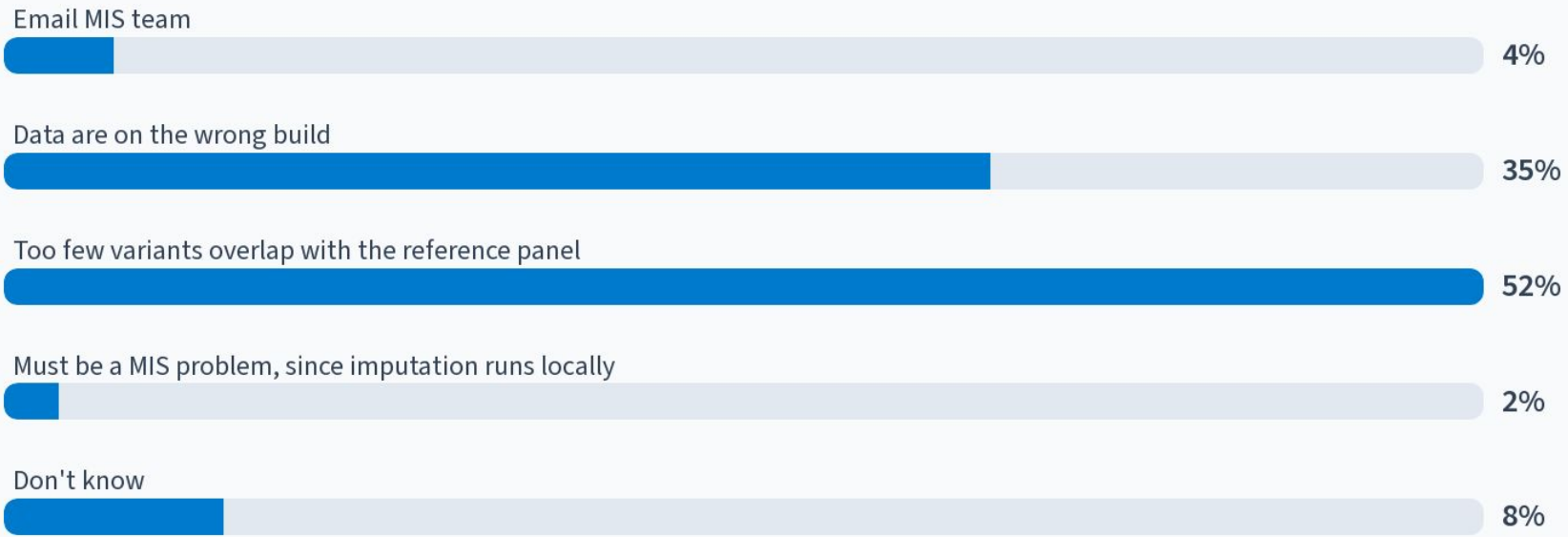
- Developed by W. Rayner
- Works for all major reference panels (HRC, TOPMed, Asia, CAAPA, 1000G)
- Checks for consistency between input data and a reference panel
- Updates/removes SNPs, Updates strand, position and ref/alt assignment
- Input Data in PLINK Binary Format (bim, bed, fam)

https://www.well.ox.ac.uk/~wrayner/tools/HRC-1000G-check-bim-v4.3.0.zip

Uploaded my data 5 mins ago and my job is still waiting...

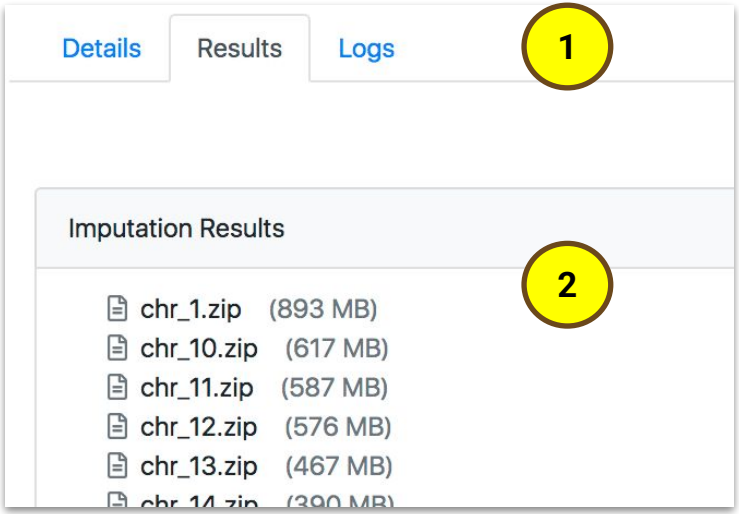


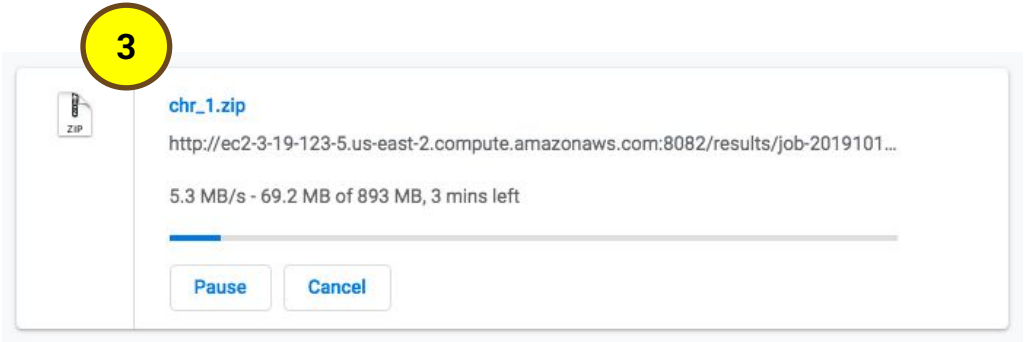
Error: No chunks passed the QC step. Imputation cannot be started!



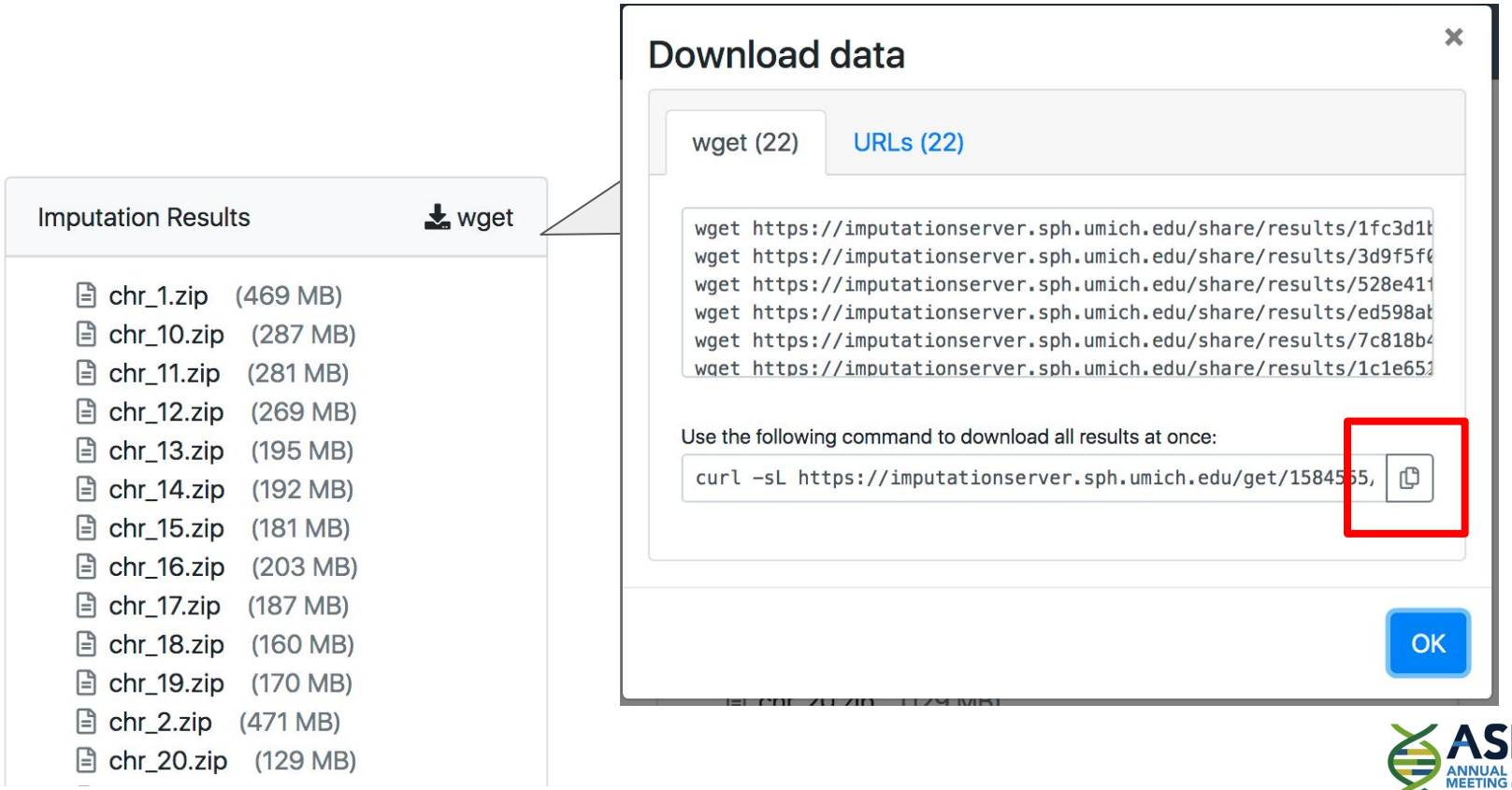
How to download the imputed genotypes?

Option 1: Web-Interface

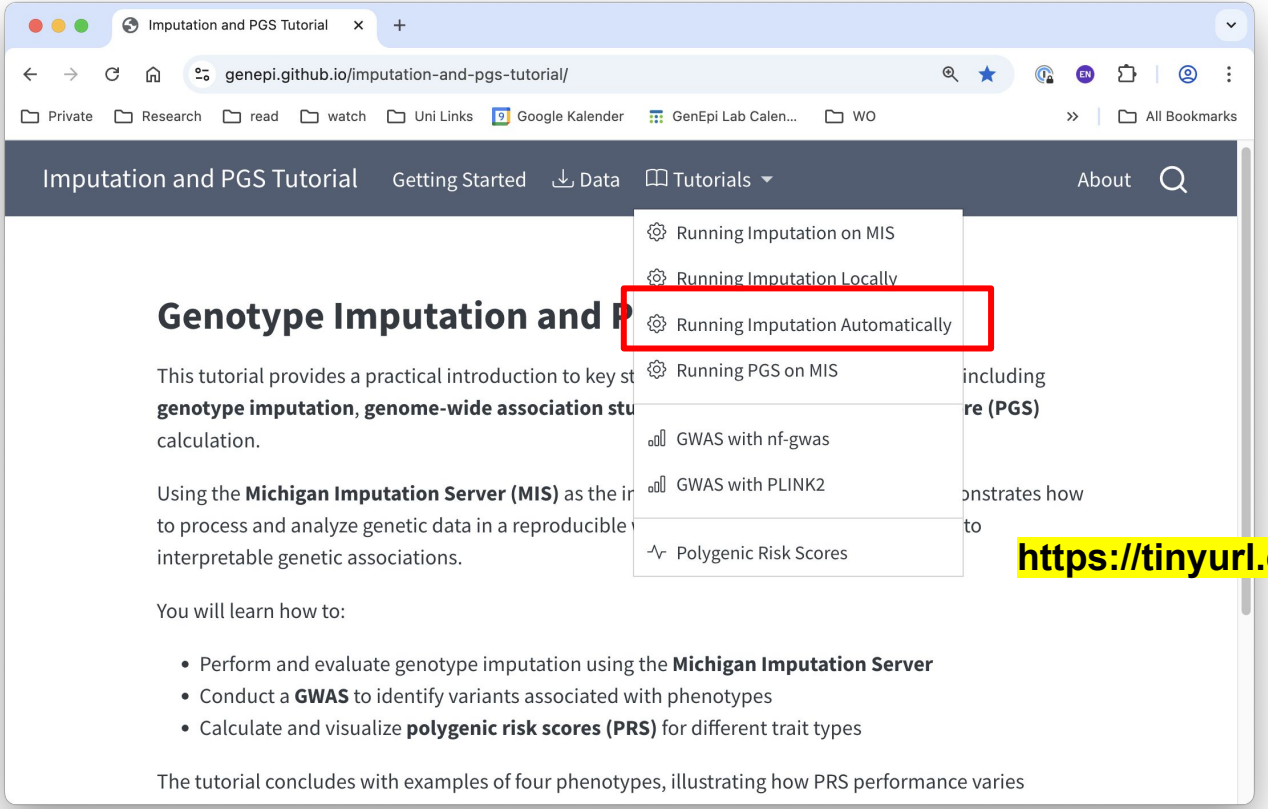




Option 2: Batch Download



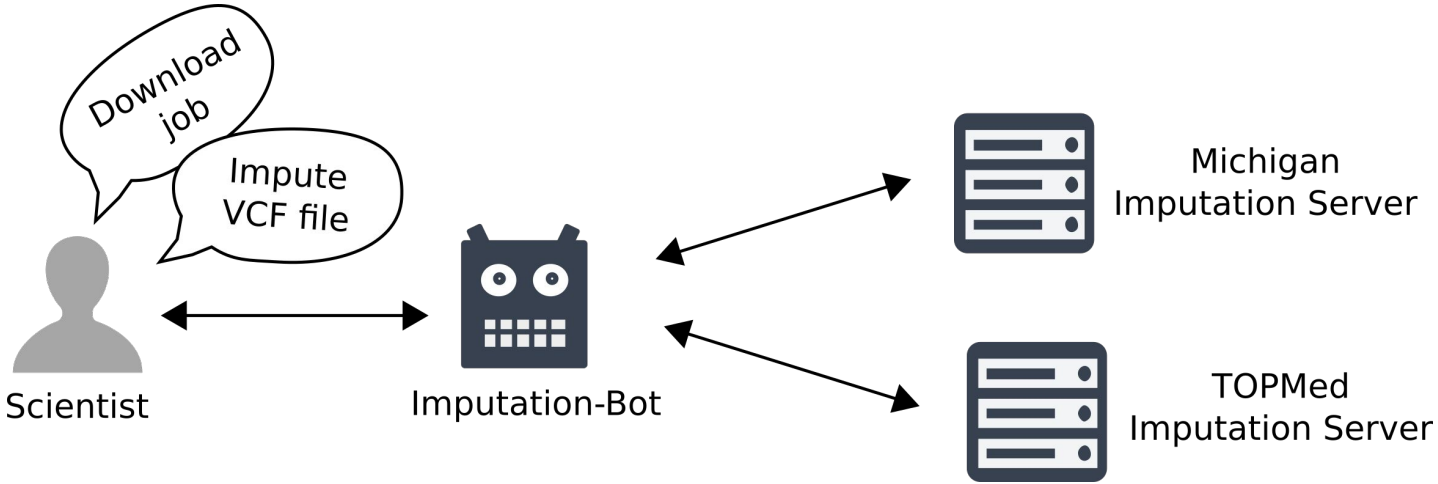
Option 3: Use Imputationbot

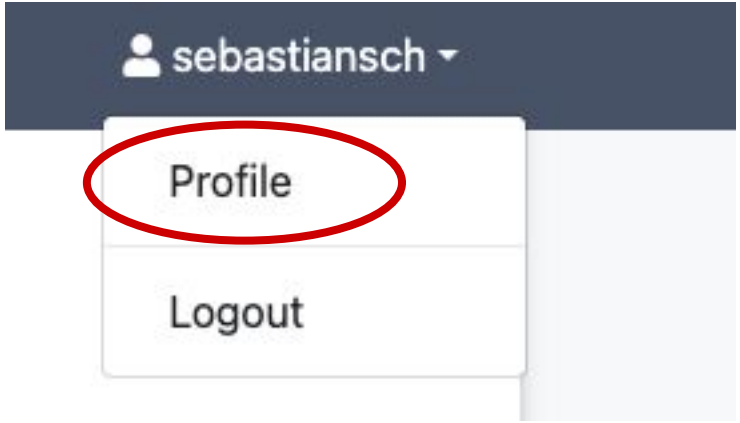


https://tinyurl.com/imputationserver-2025

Imputation Bot

- Automate remote imputation
- Submit and monitor jobs from the command line
- Different commands can easily be combined



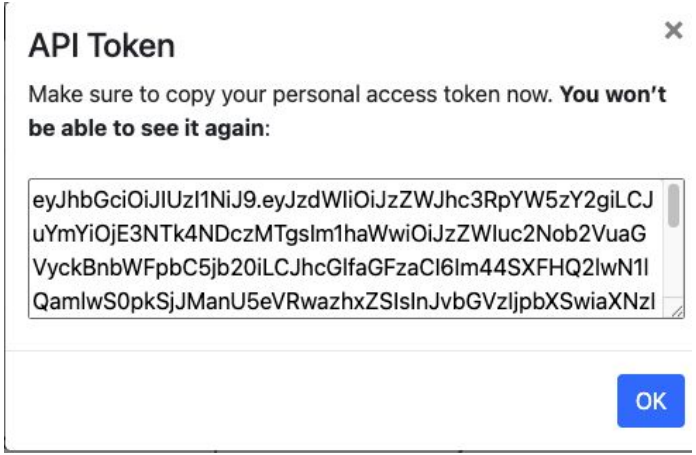


API Access

This service provides a rich RestAPI to submit, monitor and download jobs.

You need a access token to use the API. Learn more.

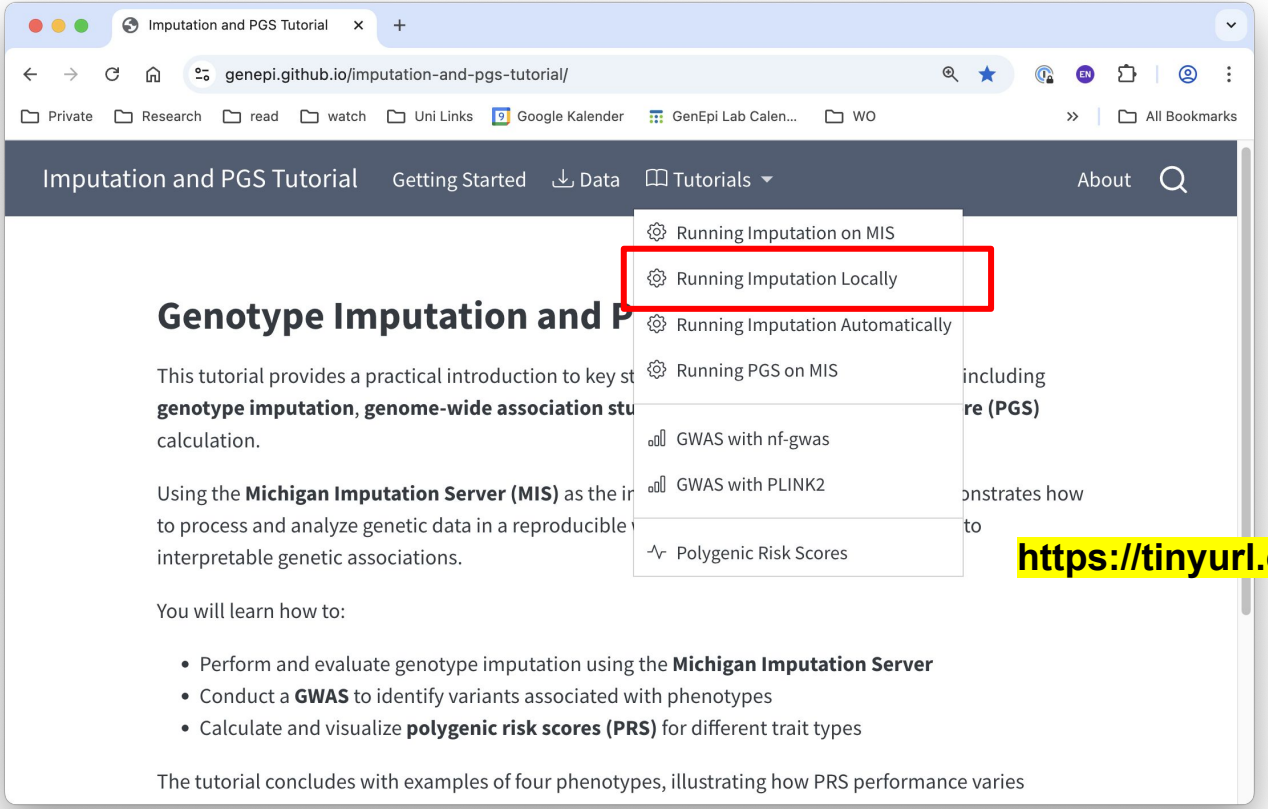
Create API Token Expires in 30 days



Workflow

- curl -sL imputationbot.now.sh/v2 | bash
- ./imputationbot add-instance
- ./imputationbot impute --files gwas.array.hapmap.chr20.vcf.gz --refpanel hapmap-2 --population eur
- ./imputationbot download job-20251007-104433-914 --password ABCD

Running Imputation Locally



https://tinyurl.com/imputationserver-2025

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Running Imputation Locally

- Since 2024 MIS Workflow is based on Nextflow
- Nextflow is a workflow management system for scalable and reproducible bioinformatics pipelines
- Requirements
  - Install Nextflow
  - Use your own/publicly available reference panel
  - Run Imputation on the commandline

Running Imputation Locally


```
# Create temp directory
mkdir imputationserver
cd imputationserver
# Download Input Data
wget https://genepi.i-med.ac.at/downloads/imputation/gwas.array.hapmap.chr20.vcf.gz
# Download and unzip Reference Panel
wget https://imputationserver.sph.umich.edu/resources/ref-panels/imputationserver2-h
unzip imputationserver2-hapmap2.zip -d hapmap2
```

1

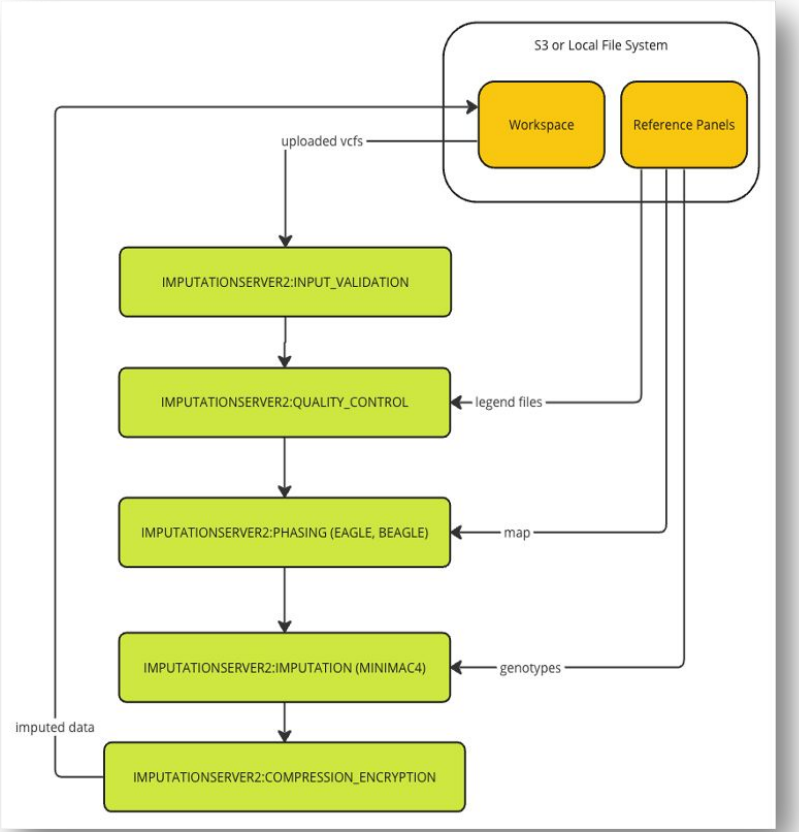
```
params {
    project                    = "my-test-project"
    build                      = "hg19"
    files                      = "gwas.array.hapmap.chr20.vcf.gz"
    allele_frequency_population = "eur"
    mode                       = "imputation"
    refpanel_yaml              = "hapmap2/imputation-hapmap2.yaml"
    output                     = "results"
    encryption.enabled         = false
}
```

2

3

```
nextflow run genepi/imputationserver2 -r v2.0.9  -c imputation.config -profile docker
```

Running Imputation Locally




```
N E X T F L O W  ~  version 24.04.2

Launching `main.nf` [fabulous_mendel] DSL2 - revision: 16c60661b1

Loading reference panel from file /Users/seb/repositories/nf-imputationserver/tests/data
Welcome to Imputation Server 2 (v2.0.6)
executor >  local (5)
[0a/d99e6b] INPUT_VALIDATION:INPUT_VALIDATION_VCF                [100%] 1 of 1 ✔
[91/a9fe51] QUALITY_CONTROL:QUALITY_CONTROL_VCF                  [100%] 1 of 1 ✔
[cf/093d23] QUALITY_CONTROL:QUALITY_CONTROL_REPORT               [100%] 1 of 1 ✔
[6c/fd767c] PHASING:EAGLE (chunk_20_0040000001_0060000000.vcf.gz) [ 25%] 1 of 4
[-        ] IMPUTATION:MINIMAC4                                  [  0%] 0 of 1
[-        ] ENCRYPTION:COMPRESSION_ENCRYPTION_VCF                -
```

Summary

- MIS Web Interface provides a fast and reliable way to impute data
- We provide tutorials to run imputation on MIS, locally and via the imputationbot
- MIS applies a strict Quality Control with the goal to return high quality imputation data

Section 3

Performing GWAS using imputed data

Cassandra Spracklen
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Learning objectives

Participants will learn to:

- Identify and understand the use of variant imputation quality information following imputation in the MIS
- Distinguish between some of the available options for GWAS
- Troubleshoot common GWAS errors

Have you ever performed a GWAS?

Yes

0%

No

0%

I'm trying

0%

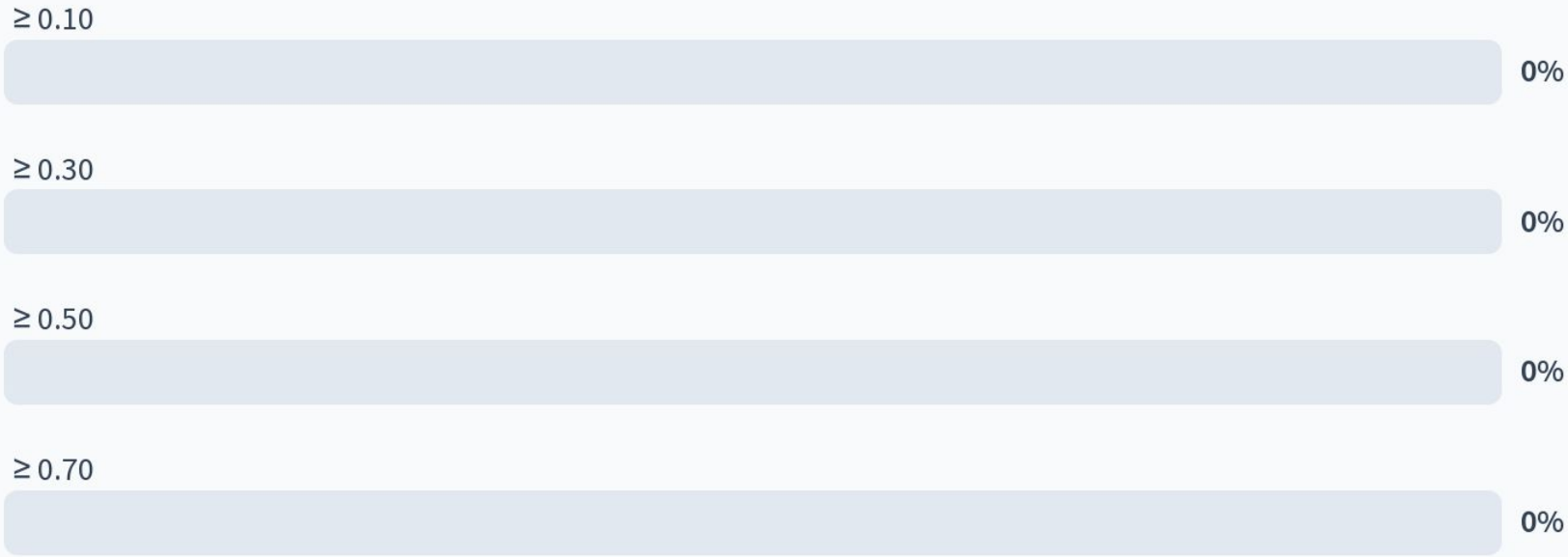
Imputation Quality

- For each variant, how confident can we be that the imputation dosages are sufficiently “accurate” for association analyses?
- Measure of confidence in imputed dosages: “Rsq” column [range 0-1]

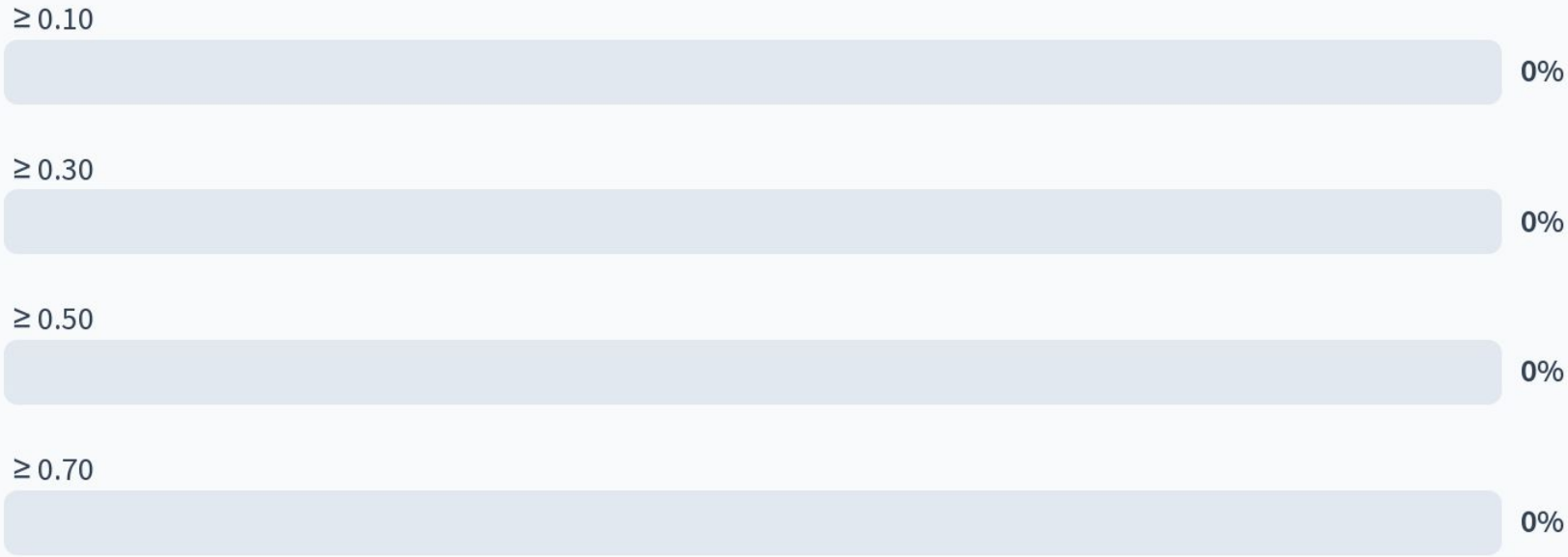
SNP	REF(0)	ALT(1)	ALT_Frq	MAF	AvgCall	Rsq	Genotyped	...
20:61795:G:T	G	T	0.26318	0.26318	0.88455	0.54658	Imputed	...
20:63231:T:G	T	G	0.03843	0.03843	0.98342	0.67736	Imputed	...
20:63244:A:C	A	C	0.16132	0.16132	0.91761	0.49907	Imputed	...

From a chr20.info.gz file

Minimally accepted Rsq value for common (MAF≥5%) variants?



Minimally accepted Rsq value for low frequency (MAF<5%) and rare variants?



Imputation Quality

- Minimal Rsq value for common variants
  - ≥ 0.30
- Minimal Rsq value for low frequency/rare variants
  - ≥0.50
- Before performing GWAS, remove variants that do not meet these thresholds
  - Suggested program: VCFtools
  - Saves computational time when performing GWAS

Which GWAS program(s) have you used?

Performing the GWAS

- Each program has its own input, output formats, and options
- Typical input files
  - Genotype file (.vcf; .bgen; .bed/.bim/.fam)
  - Phenotype/covariate file (.txt; .ped)
  - Some programs use separate phenotype and covariate files
  - Kinship/relationship matrix (EPACTS, SAIGE)

Available GWAS Programs

No File Reformatting (VCF from MIS)

- EPACTS
- Rvtests
- SNPTEST
- SAIGE

File Formatting Required

- BOLT-LMM
- BGENIE
- regenie
- PLINK

**May not be all-inclusive*

Each GWAS Program Has Strengths, Limitations

EPACTS/Rvtests

+ Many model options - single variant, gene-based
+ Chr X analyses
+ Phenotypic transformation (e.g inverse normal; Rvtests only)
+ Linear mixed model for sample relatedness (quantitative traits only)
+ Generate covariance matrices for downstream analyses (e.g conditional analyses; Rvtests only)

- Memory intensive
- Sample size ~≤20,000 (better ≤10,000)

EPACTS: https://genome.sph.umich.edu/wiki/EPACTS
Rvtests: https://genome.sph.umich.edu/wiki/Rvtests

Each GWAS Program Has Strengths, Limitations

SAIGE

+ Similar to Rvtests, but for very large sample sizes (e.g. biobanks)
+ Able to account for sample relatedness for binary traits
+ Designed to handle unbalanced number of cases and controls
+ Chr X analyses

- Should not be used to examine heritability (biased variance estimates)
- Computational time can vary widely between phenotypes and sample sizes
- Can be conservative for extremely unbalanced case and control ratio
- Odds ratios estimated to conserve computational time

SAIGE: https://github.com/weizhouUMICH/SAIGE

Each GWAS Program Has Strengths, Limitations

SNPTEST

+ Frequentist and bayesian methods supported
+ Chr X analyses

- Limited to unrelated individuals
- Computationally intensive

SNPTEST: https://www.well.ox.ac.uk/~gav/snptest/#introduction

Each GWAS Program Has Strengths, Limitations

BOLT-LMM/BGENIE/Regenie

+ Great for very large sample sizes (e.g. biobanks)
+ Chr X analyses
+ Computationally efficient (Regenie)

- Requires files to be in BGEN or PLINK format
- Nextflow pipeline for regenie using VCF: https://github.com/genepi/nf-gwas
- Not optimal for extremely unbalanced case control ratio (especially with rare variants)

BOLT-LMM: https://data.broadinstitute.org/alkesgroup/BOLT-LMM/#x1-5600011
BGENIE: https://jmarchini.org/bgenie/
Regenie: https://github.com/rgcgithub/regenie

Each GWAS Program Has Strengths, Limitations

PLINK

+ Quick
+ Multiple versions; often as intermediary tool to the other programs
+ Can run on the command line (unix not required)
+ Chr X analyses

- Requires files to be in PLINK format (.bed/.bim/.fam)
- Limited model options

PLINK: https://www.cog-genomics.org/plink/2.0/

Summary of common GWAS analysis tools

	EPACTS	Rvtests	SNPTEST	SAIGE	BOLT-LMM	Bgenie	Regenie
Input VCF	Y	Y	Y				
Sample relatedness (Quantitative outcome)	Y	Y		Y	Y	Y	Y
Sample relatedness (Binary outcome)				Y		Y	Y
Case control imbalance				Y			Y
Large sample size (>20,000)				Y	Y	Y	Y

Which program(s) would be best?

A researcher new to genetic analyses and unfamiliar to the UNIX environment wants to perform a GWAS on total cholesterol using a cohort of 5,500 unrelated individuals.

EPACTS/Rvtests 0%

SAIGE 0%

BOLT-LMM 0%

PLINK 0%

Which program(s) would be best?
Researchers want to perform a GWAS using a cohort of 10,000 individuals with household based recruitment (i.e. includes related individuals).

EPACTS/Rvtests 0%

SAIGE 0%

BOLT-LMM 0%

PLINK 0%

Which program(s) would be best?
Researchers want to perform a GWAS using data from BioBank Japan (>200,000 individuals)

(A) EPACTS/Rvtests 0%

(B) SAIGE 0%

(C) BOLT-LMM 0%

(D) PLINK 0%

(E) Regenie/Bgenie 0%

Common Errors When Running a GWAS

- Wording of error messages vary by program, but the same issues will cause errors throughout all of the program
- [Unix] Errors independent of GWAS program
  - File permissions
    - Correct by changing file permissions
  - Directory/file not found
    - Correct by making sure all of the file locations and names are accurate
  - Not enough memory/time
    - Correct by restarting job with adequate memory/time allocation

Common Errors When Running a GWAS

- Common errors
  - IDs don’t match
    - Correct by ensuring that the ID in the phenotype, genotype, covariance, kinship matrix are consistent format in all files

Common Errors When Running a GWAS

- Common errors
  - IDs don’t match
    - Correct by ensuring that the ID in the phenotype, genotype, covariance, kinship matrix are consistent format in all files
  - File format(s) incorrect
    - Correct by making sure the format of all files are as the program is expecting (e.g. columns, delimiters, headers, file extension

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  - Improperly specified options/command
    - Correct by checking all needed options are specified, correct order, no typos

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  - Peripheral programs not available (e.g. R with EPACTS, SAIGE)
    - Correct by installing other peripheral programs

Common Errors When Running a GWAS

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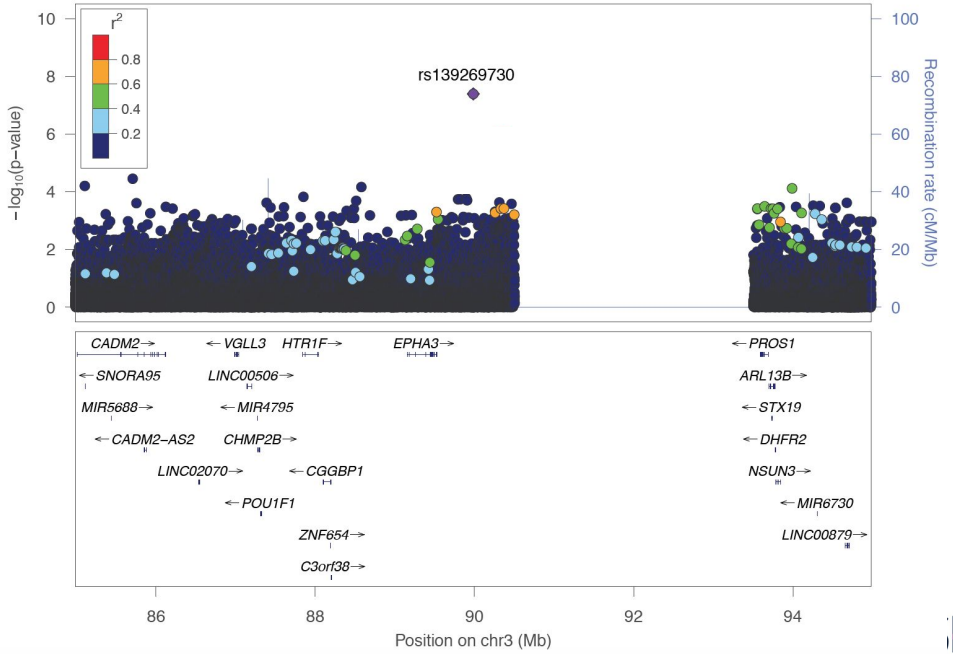
  - Improperly specified options/command
    - Correct by checking all needed options are specified, correct order, no typos
  - Peripheral programs not available (e.g. R with EPACTS, SAIGE)
    - Correct by installing other peripheral programs
  - Invalid estimate (e.g. heritability in BOLT-LMM)
    - Sample too related and/or sample size too small
    - Correct by using a different program

Interpreting GWAS Results

- GWAS results must be carefully reviewed for:
  - Imputation quality!
  - Genomic inflation
  - False positives

- Replication datasets
- PheWas



Sex chromosomes

- Humans have two sex chromosomes, X and Y, that in combination determine the sex of an individual.
  - To ensure balanced expression, one of the female's X-chromosome is randomly selected to undergo inactivation(either paternal or maternal determined at early embryonic development).
  - Pseudoautosomal regions (PAR1 and PAR2) are short regions on the X- and Y-chromosomes that are inherited in an autosomal rather than a sex-linked manner

X-chromosome - genotype calls and QC

- Genotype calls on X-chromosomes
  - One copy in males, two copies in females
- Clarity of genotype calls on X-Chromosome
  - 0/1/2 allele coding in females; 0/1 or 0/2 allele coding in males
  - 0/0.5/1 allele coding in females; 0/0.5 allele coding in males
  - Comparing standard error with autosomal data
- Analysis plan to be specific on allelic coding
- Develop QC checks before association analyses, e.g., similar allele frequencies between males and females (differential calls bias)

X-chromosome - imputation

Chromosome X Pipeline

Additionally to the standard QC, the following per-sample checks are executed for chrX:

- Ploidy Check: Verifies if all variants in the nonPAR region are either haploid or diploid.
- Mixed Genotypes Check: Verifies if the amount of mixed genotypes (e.g. 1/.) is < 10 %.

For phasing and imputation, chrX is split into three independent chunks (PAR1, nonPAR, PAR2). These splits are then automatically merged by Michigan Imputation Server and are returned as one complete chromosome X file. Only Eagle is supported.

https://imputationserver.readthedocs.io/en/latest/pipeline/#chromosome-x-pipeline

Interpretation of association

- Biological considerations:
  - Assumption of total X-inactivation but there is evidence of escape and compensation mechanisms
  - Different inheritance/activation in different tissues?
  - Sex-biased gene expression? Unclear if it is real differences in gene expression or influence of hormones.
- Methodology development
  - Statistical methods to estimate the inactivation when estimating the effects; adjusting for sex

Summary

- Variants must be filtered post-imputation to remove those with poor imputation quality
- There are many GWAS programs available, each with their own strengths and limitations - so be sure to pick one that fits your analyses needs
- As these GWAS programs are widely used or adopted by consortia, there are tutorials and help-pages available
- X-chr imputation and analyses are also possible!

More info and FAQ can be found here:
https://imputationserver.readthedocs.io

HLA Imputation

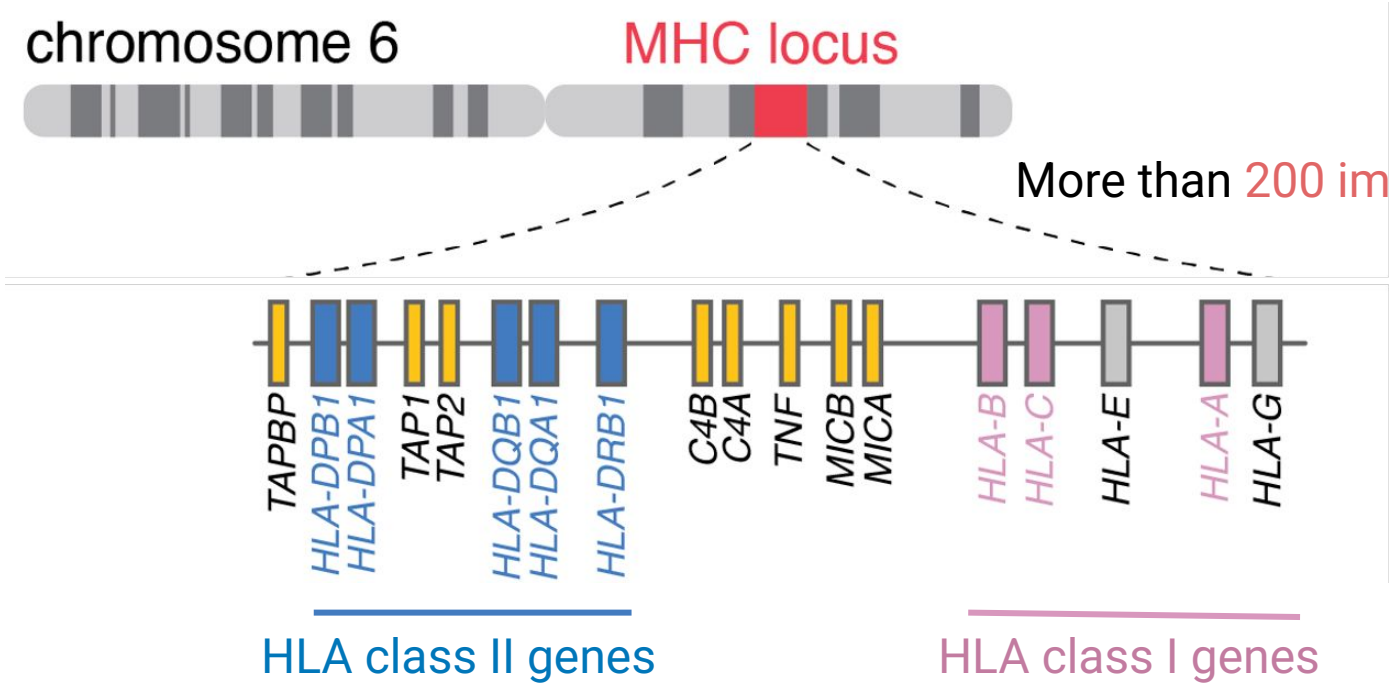
Slides Created By:

Saori Sakaue
Broad Institute
ssakaue@broadinstitute.org

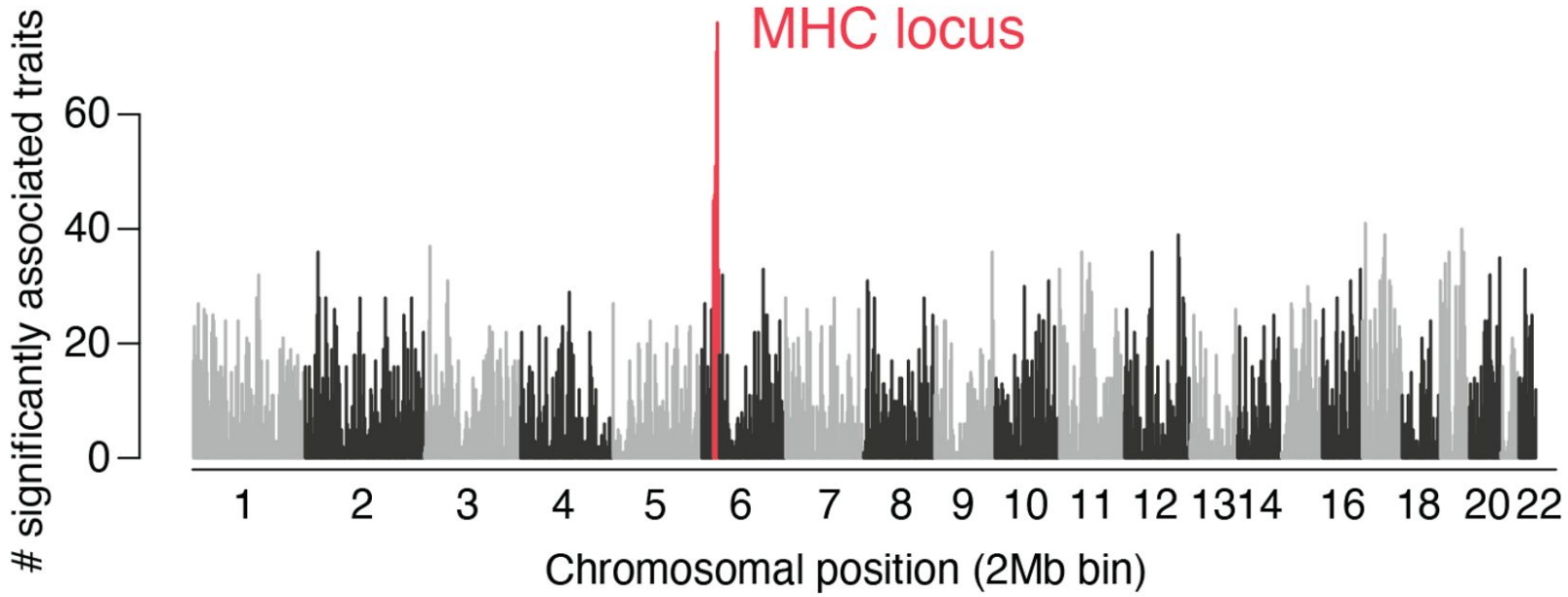
MICHIGAN IMPUTATIONSERVER

ASHG ANNUAL MEETING 2025
BOSTON, MA • OCTOBER 14-18

HLA genes are within MHC (major histocompatibility complex) locus on chromosome 6



MHC locus confers the largest number of associations of any locus genome-wide



In UK Biobank, Sakaue et al. Nat Genet 2021, Sakaue et al. Nature Protocols 2023

Genotype Imputation of HLA Region is great, but not perfect

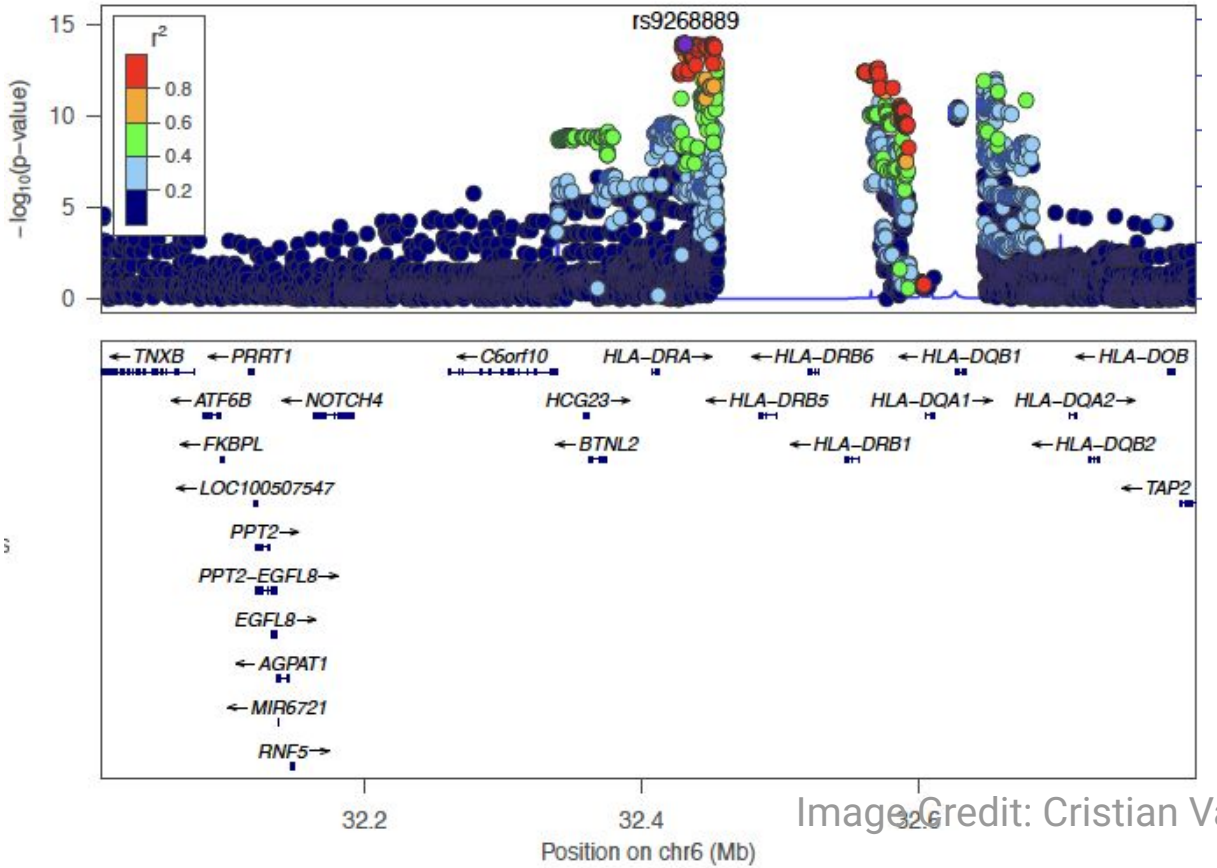


Image Credit: Cristian Valencia

Are you planning to impute the HLA region?

No

0%

Yes

0%

Already imputed!

0%

Genotype imputation in a nutshell

	Genotype imputation
Known	Genotyped SNPs
Given	Reference haplotype with whole-genome SNPs
Unknown (Impute!)	Untyped SNPs

HLA imputation in a nutshell

	Genotype imputation	HLA imputation
Known	Genotyped SNPs	Genotyped SNPs
Given	Reference haplotype with whole-genome SNPs	Reference haplotype with HLA alleles and amino acid sequences
Unknown (Impute!)	Untyped SNPs	Untyped HLA alleles and amino acid sequences

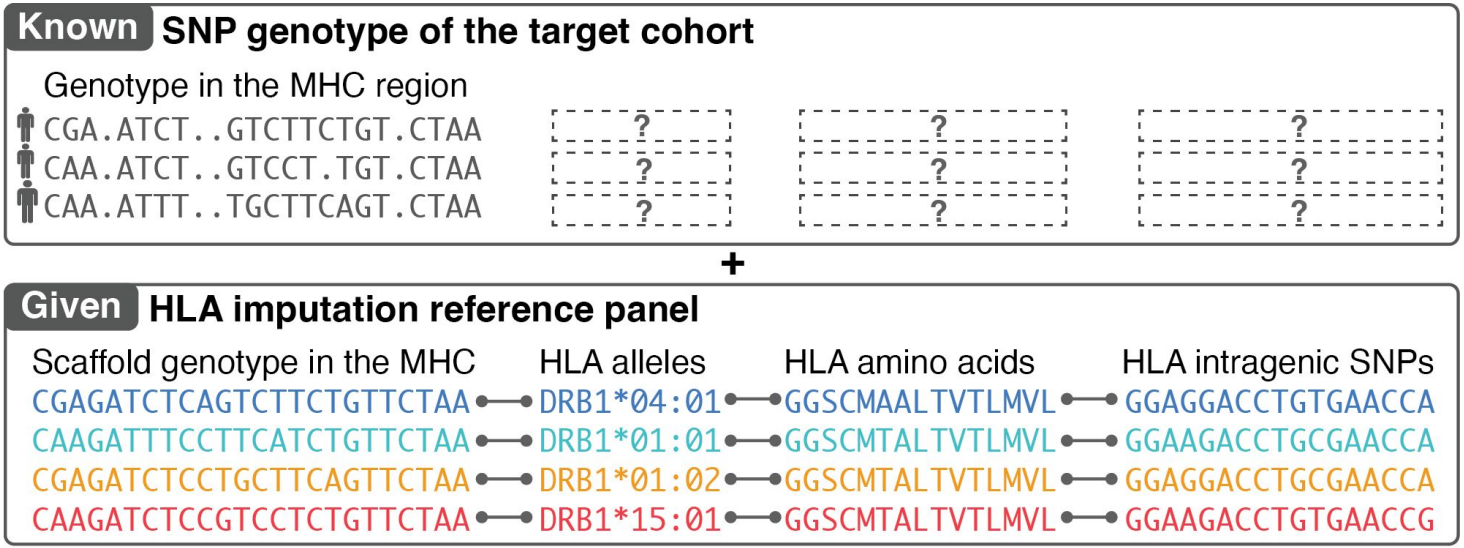
HLA imputation in a nutshell

Known **SNP genotype of the target cohort**

Genotype in the MHC region	HLA alleles	HLA amino acids	HLA intragenic SNPs
CGA.ATCT..GTCTTCTGT.CTAA	?	?	?
CAA.ATCT..GTCCT.TGT.CTAA	?	?	?
CAA.ATTT..TGCTTCAGT.CTAA	?	?	?

QCed Plink *.{bed,bim,fam}

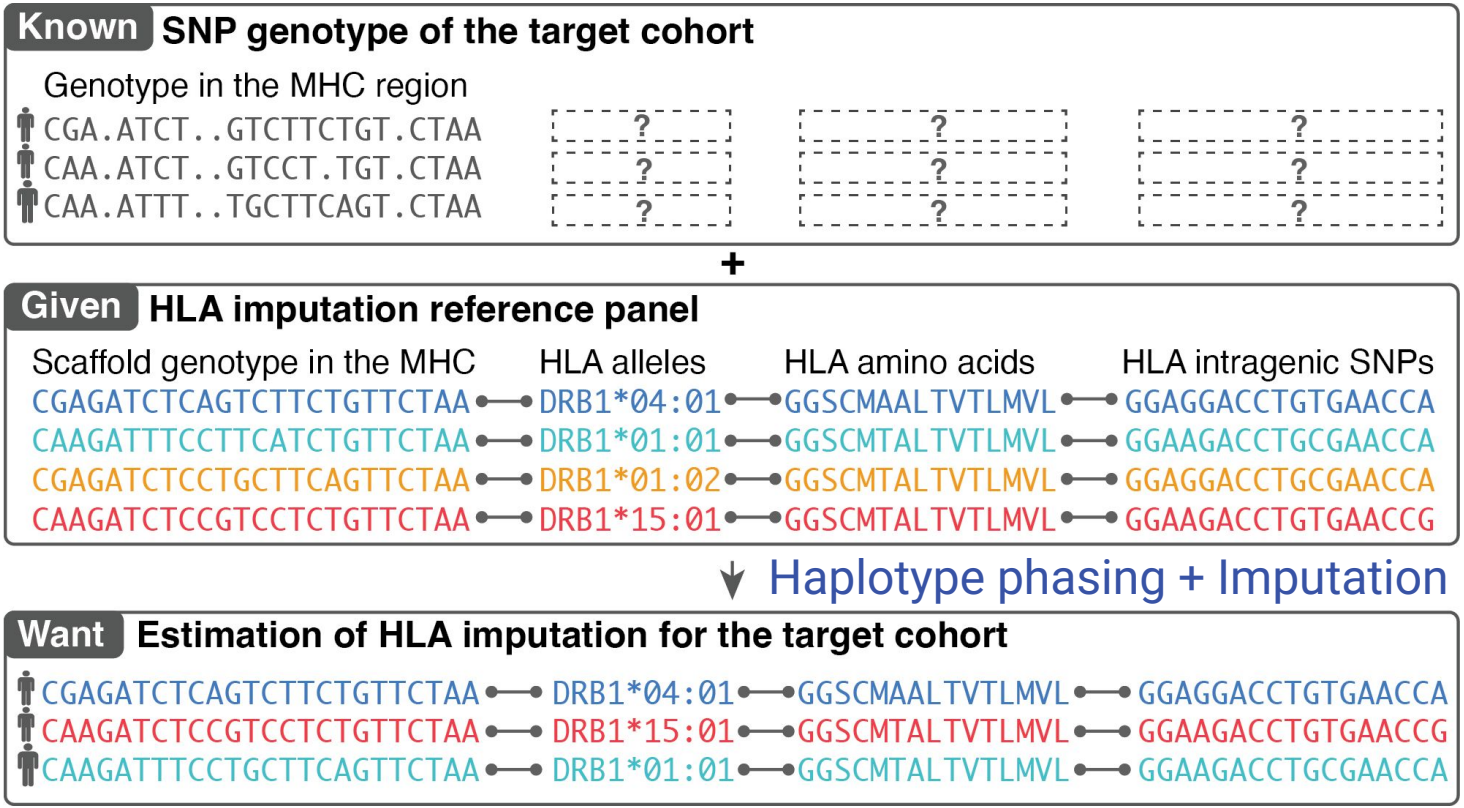
HLA imputation in a nutshell



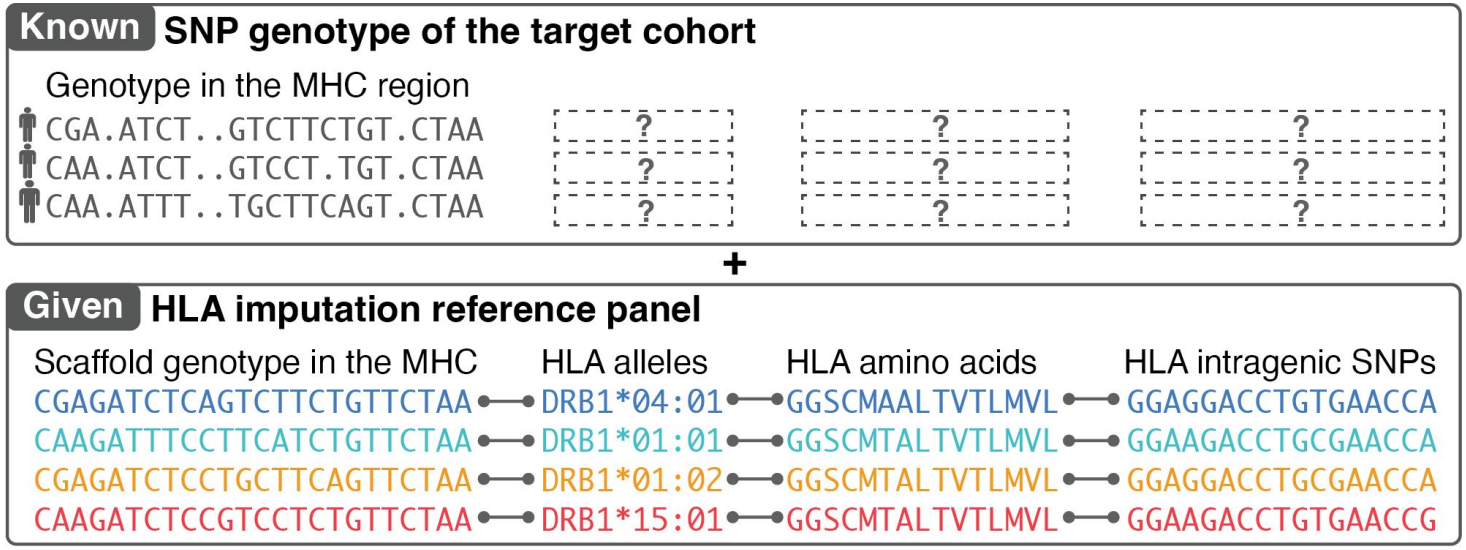
Multi-ancestry panel
(EUR, EAS, SAS, AFR, LAT)

Luo et al. Nat Genet 2021, Sakaue et al. Nat Protocols 2023

HLA imputation in a nutshell



HLA imputation in a nutshell



- Beagle
- SHAPEIT
- Eagle

- Beagle
- Minimac

Browning & Browning. AJHG 2007, Dalaneau et al. Nat Methods 2013, Loh et al. Nat Genet 2016, Das et al. Nat Genet 2016

HLA imputation in a nutshell

Known **SNP genotype of the target cohort**

Genotype in the MHC region

CGA.ATCT..GTCTTCTGT.CTAA	?	?	?
CAA.ATCT..GTCCT.TGT.CTAA	?	?	?
CAA.ATTT..TGCTTCAGT.CTAA	?	?	?

+

Given **HLA imputation reference panel**

Scaffold genotype in the MHC	HLA alleles	HLA amino acids	HLA intragenic SNPs
CGAGATCTCAGTCTTCTGTTCTAA	DRB1*04:01	GGSCMAALTVTLMVL	GGAGGACCTGTGAACCA
CAAGATTTCCTTCATCTGTTCTAA	DRB1*01:01	GGSCMTALTVTLMVL	GGAAGACCTGCGAACCA
CGAGATCTCCTGCTTCAGTTCTAA	DRB1*01:02	GGSCMTALTVTLMVL	GGAGGACCTGCGAACCA
CAAGATCTCCGTCCTCTGTTCTAA	DRB1*15:01	GGSCMTALTVTLMVL	GGAAGACCTGTGAACCG

↓ Haplotype phasing + Imputation

Want Estimation of HLA imputation for the target cohort

- Beagle
- SHAPEIT
- Eagle

- Beagle
- Minimac

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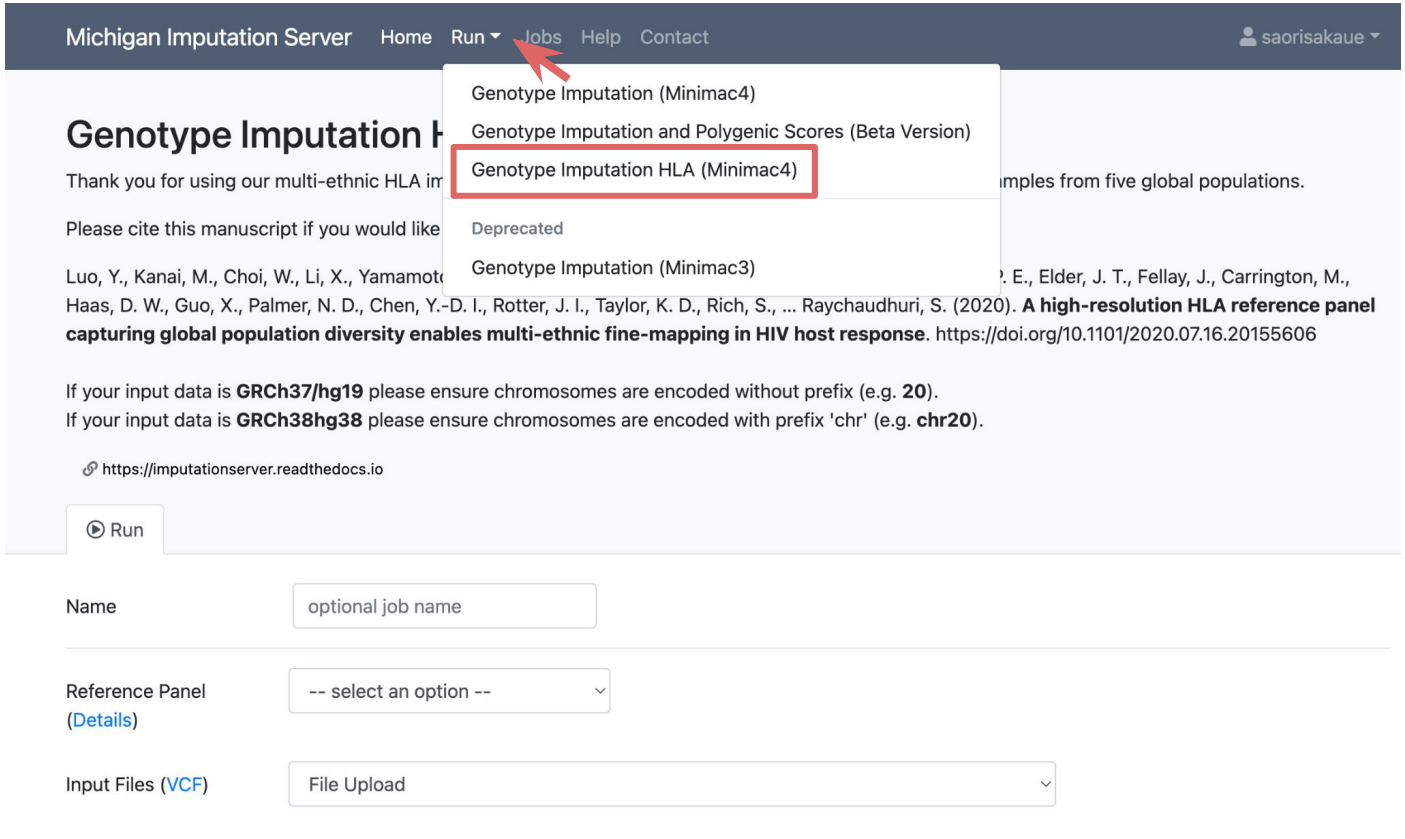
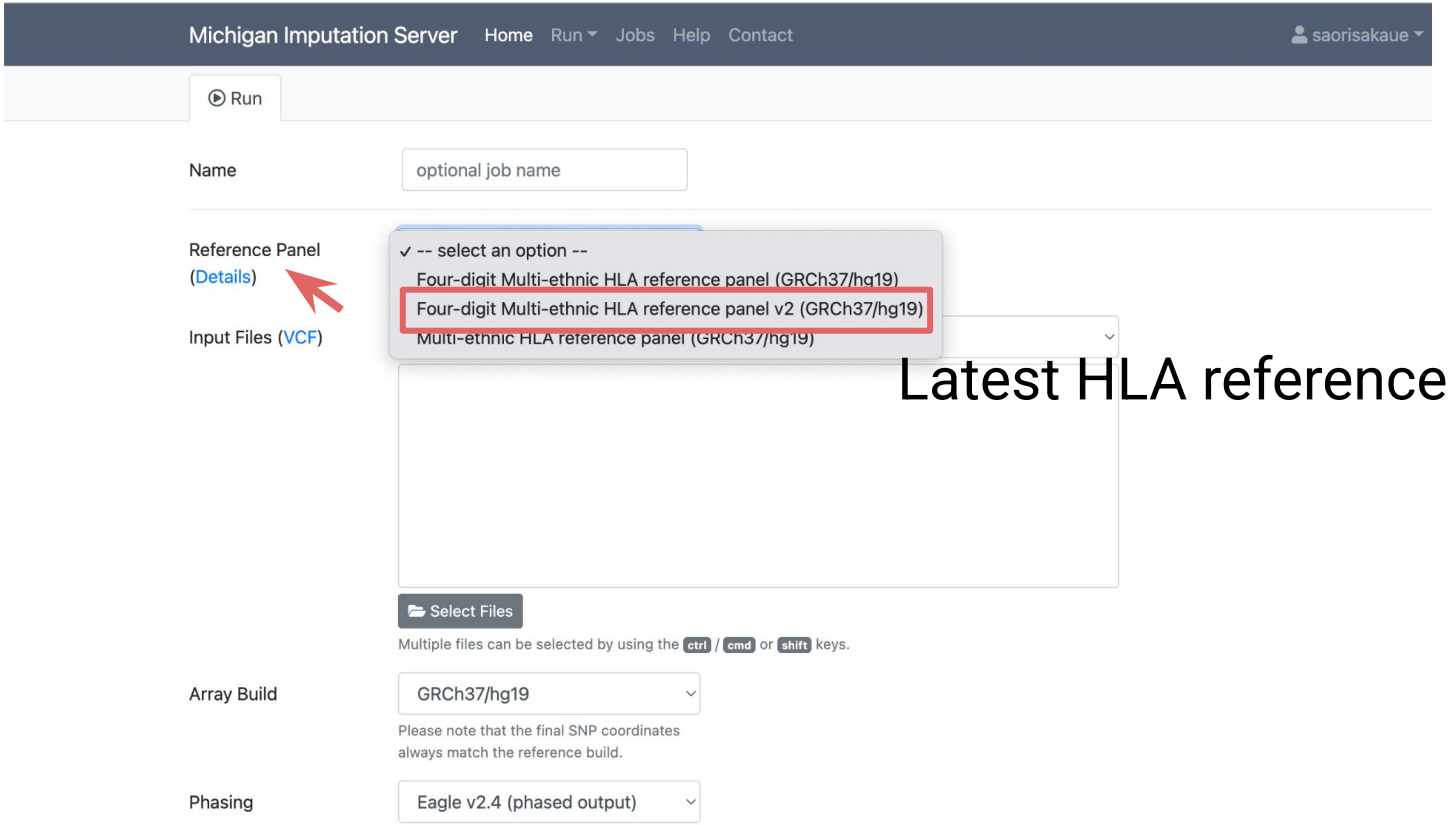
Sakaue et al. Nat Protocols 2023

HLA imputation in MIS

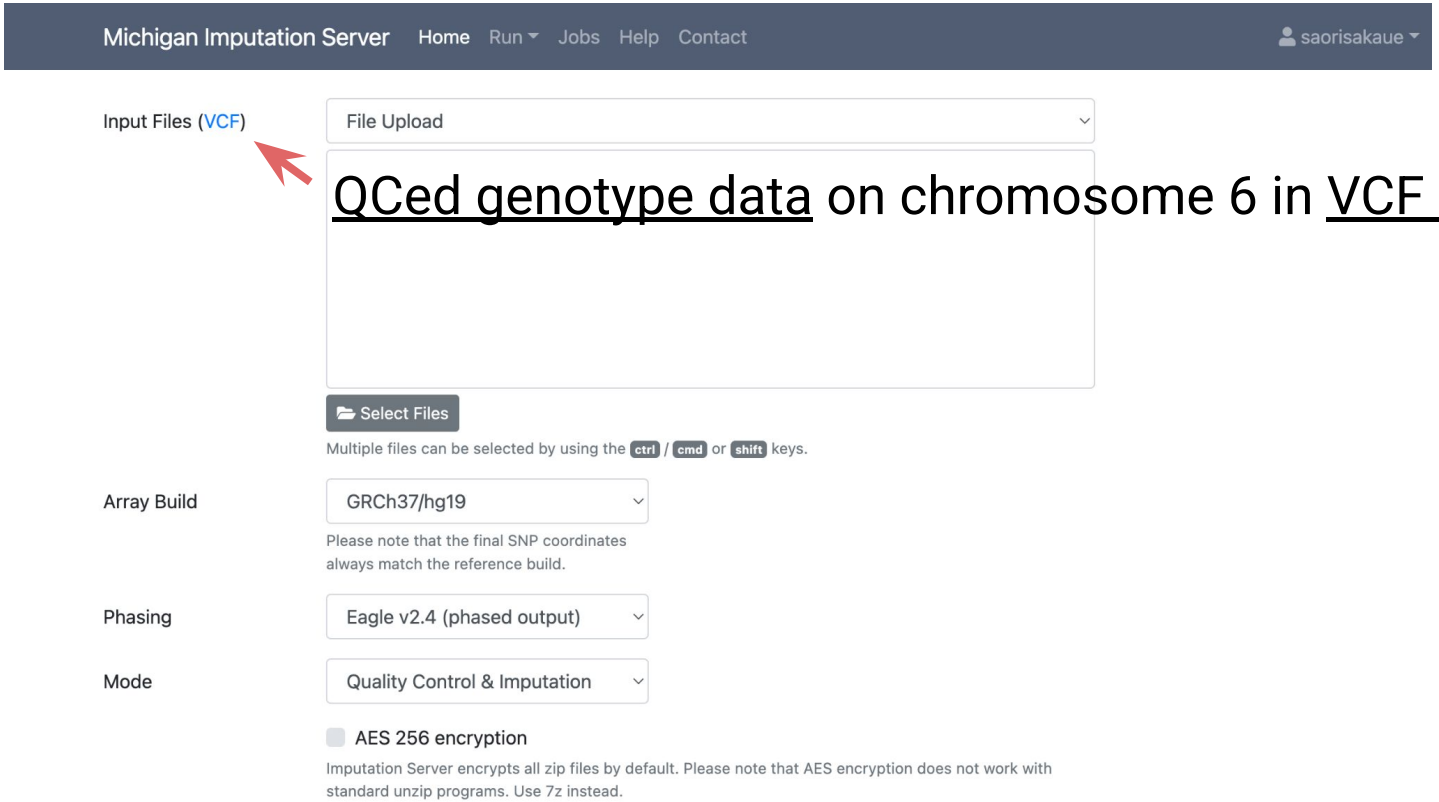


HLA imputation in MIS

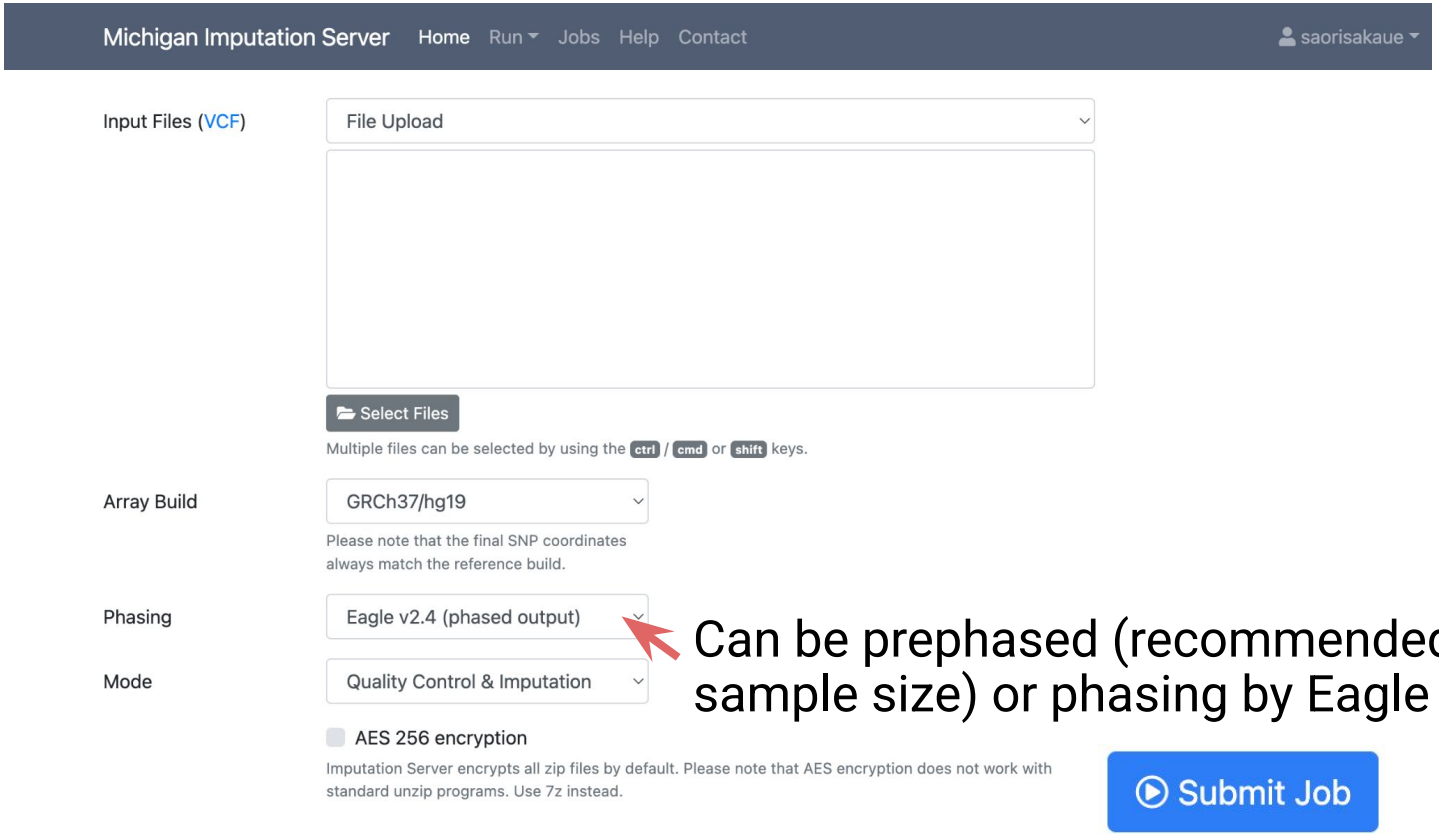


Latest HLA reference panel

HLA imputation in MIS

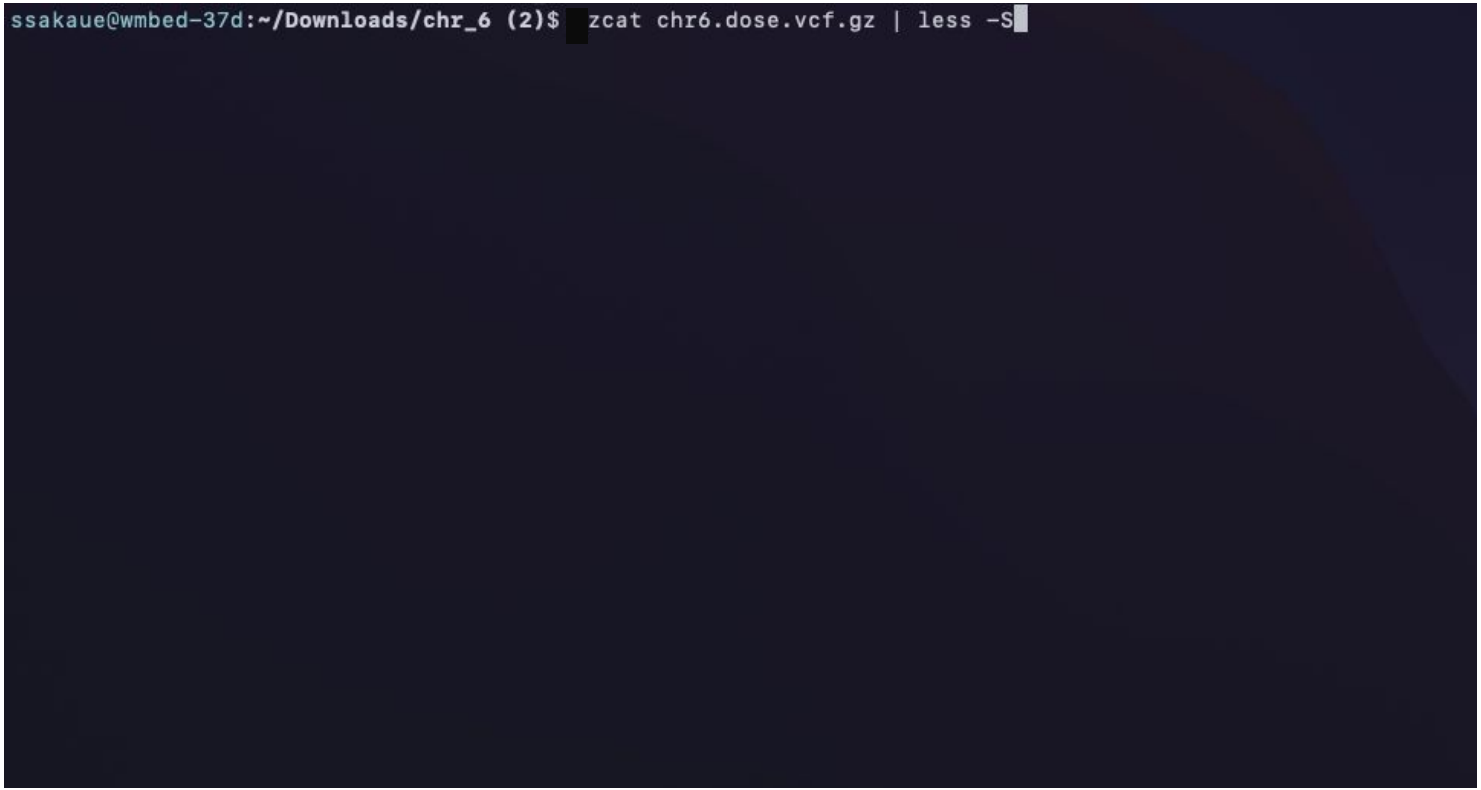


HLA imputation in MIS



Can be prephased (recommended in small sample size) or phasing by Eagle at MIS

Let’s look at the output from MIS!



Let's look at the output from MIS!

```
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##filedate=2022.11.14
##contig=<ID=6>
##INFO=<ID=AF,Number=1,Type=Float,Description="Estimated Alternate Allele Frequency">
##INFO=<ID=MAF,Number=1,Type=Float,Description="Estimated Minor Allele Frequency">
##INFO=<ID=R2,Number=1,Type=Float,Description="Estimated Imputation Accuracy (R-square)">
##INFO=<ID=ER2,Number=1,Type=Float,Description="Empirical (Leave-One-Out) R-square (available only for genotyped variants)">
##INFO=<ID=IMPUTED,Number=0,Type=Flag,Description="Marker was imputed but NOT genotyped">
##INFO=<ID=TYPED,Number=0,Type=Flag,Description="Marker was genotyped AND imputed">
##INFO=<ID=TYPED_ONLY,Number=0,Type=Flag,Description="Marker was genotyped but NOT imputed">
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##FORMAT=<ID=DS,Number=1,Type=Float,Description="Estimated Alternate Allele Dosage : [P(0/1)+2*P(1/1)]">
##FORMAT=<ID=HDS,Number=2,Type=Float,Description="Estimated Haploid Alternate Allele Dosage ">
##FORMAT=<ID=GP,Number=3,Type=Float,Description="Estimated Posterior Probabilities for Genotypes 0/0, 0/1 and 1/1 ">
##pipeline=michigan-imputationserver-1.5.8
##imputation=minimac4-1.0.2
##phasing=eagle-2.4
##panel=apps@multiethnic-hla-panel-4digit-v2@1.0.0
##r2Filter=0.0
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  010061321010_R01C01_10007854    010061321010_R02C01_10020793    010061321010_
6       27970031        rs149946        G       T       .       PASS    AF=0.22479;MAF=0.22479;R2=0.99214;IMPUTED       GT:DS:HDS:GP    0|0:0:0,0:1,0
6       27976200        rs9380032       G       T       .       PASS    AF=0.02975;MAF=0.02975;R2=0.97885;IMPUTED       GT:DS:HDS:GP    0|0:0:0,0:1,0
6       27979188        rs4141691       A       G       .       PASS    AF=0.11754;MAF=0.11754;R2=0.94642;IMPUTED       GT:DS:HDS:GP    0|0:0.003:0.0
6       27979625        rs10484402      A       G       .       PASS    AF=0.04041;MAF=0.04041;R2=0.92706;IMPUTED       GT:DS:HDS:GP    0|0:0.003:0.0
6       27981673        rs9368540       G       A       .       PASS    AF=0.03706;MAF=0.03706;R2=0.98634;IMPUTED       GT:DS:HDS:GP    0|0:0:0,0:1,0
6       27984726        rs74505854      A       C       .       PASS    AF=0.00719;MAF=0.00719;R2=0.94391;IMPUTED       GT:DS:HDS:GP    0|0:0:0,0:1,0
6       27984907        rs17765055      T       C       .       PASS    AF=0.04632;MAF=0.04632;R2=0.99897;ER2=0.97293;TYPED     GT:DS:HDS:GP    0|0:0
6       27986199        rs72848791      C       T       .       PASS    AF=0.04361;MAF=0.04361;R2=0.99732;ER2=0.97465;TYPED     GT:DS:HDS:GP    0|0:0
6       27986529        rs9368544       A       C       .       PASS    AF=0.04631;MAF=0.04631;R2=0.99885;IMPUTED       GT:DS:HDS:GP    0|0:0:0,0:1,0
6       27998258        rs149990        G       A       .       PASS    AF=0.11782;MAF=0.11782;R2=0.99974;ER2=0.99765;TYPED     GT:DS:HDS:GP    0|0:0
6       27999044        rs9368545       A       T       .       PASS    AF=0.04627;MAF=0.04627;R2=0.99825;IMPUTED       GT:DS:HDS:GP    0|0:0:0,0:1,0
6       27999421        rs16893573      C       T       .       PASS    AF=0.02852;MAF=0.02852;R2=0.98601;IMPUTED       GT:DS:HDS:GP    0|0:0:0,0:1,0
6       28001003        rs17708949      A       C       .       PASS    AF=0.03124;MAF=0.03124;R2=0.98465;IMPUTED       GT:DS:HDS:GP    0|0:0:0,0:1,0
6       28001610        rs149942        T       C       .       PASS    AF=0.26963;MAF=0.26963;R2=0.99868;ER2=0.98731;TYPED     GT:DS:HDS:GP    0|0:0
6       28002388        rs149943        G       A       .       PASS    AF=0.11781;MAF=0.11781;R2=0.99984;ER2=0.99889;TYPED     GT:DS:HDS:GP    0|0:0
6       28003271        rs183926        T       A       .       PASS    AF=0.00996;MAF=0.00996;R2=0.99551;ER2=0.95540;TYPED     GT:DS:HDS:GP    0|0:0
```
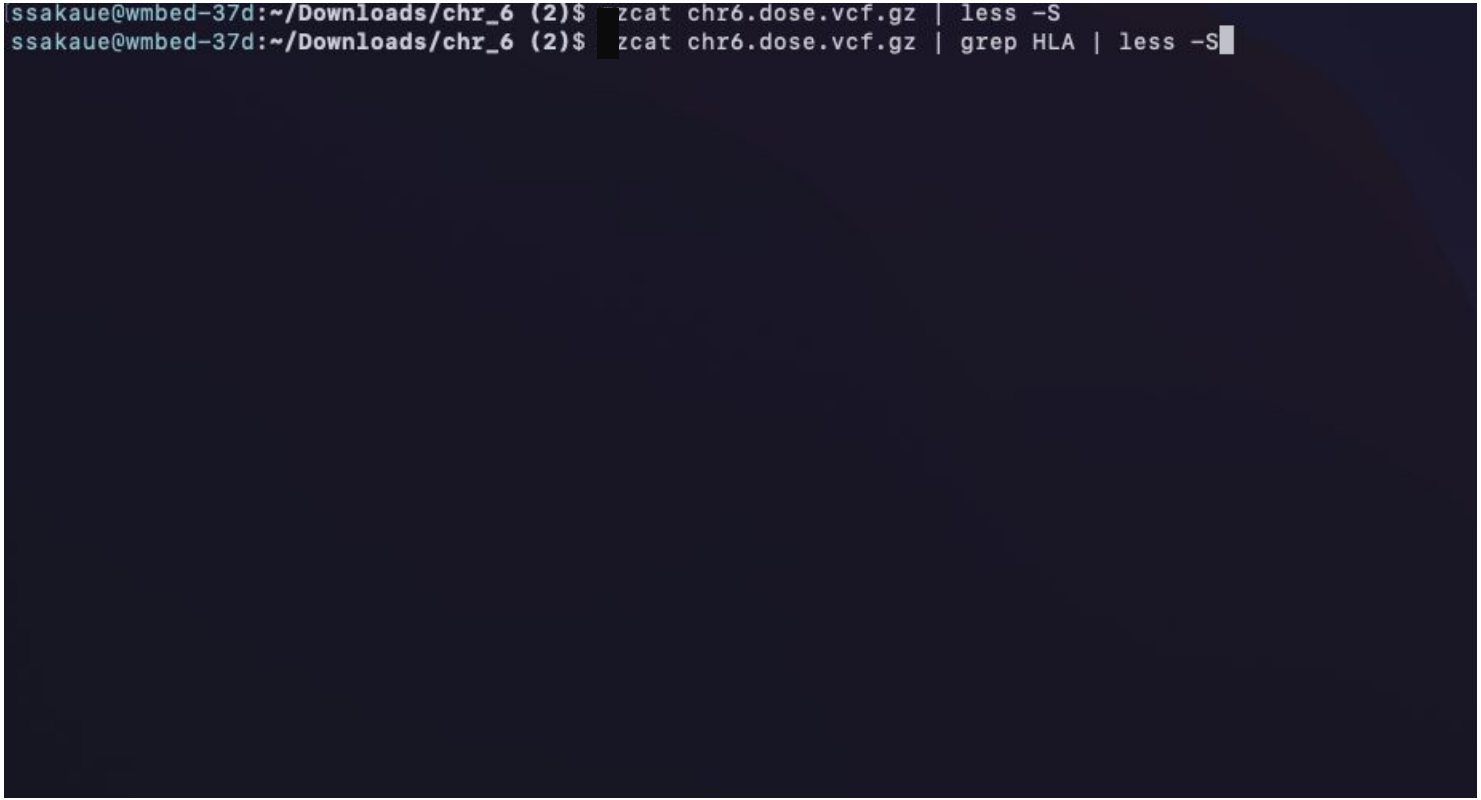

Imputed SNPs within MHC

Let’s look at the output from MIS!



Imputed HLA alleles

6	29910247	HLA_A*01	A	T	.	PASS	AF=0.15456;MAF=0.15456;R2=0.99719;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910248	HLA_A*01:01	A	T	.	PASS	AF=0.15234;MAF=0.15234;R2=0.99517;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910249	HLA_A*01:02	A	T	.	PASS	AF=0.00110;MAF=0.00110;R2=0.85198;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910250	HLA_A*01:136	A	T	.	PASS	AF=0.00002;MAF=0.00002;R2=0.04644;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910251	HLA_A*02	A	T	.	PASS	AF=0.26026;MAF=0.26026;R2=0.99741;IMPUTED	GT:DS:HDS:GP 0|1:0.998:0.0
6	29910252	HLA_A*02:01	A	T	.	PASS	AF=0.22914;MAF=0.22914;R2=0.98881;IMPUTED	GT:DS:HDS:GP 0|1:0.996:0.0
6	29910253	HLA_A*02:02	A	T	.	PASS	AF=0.00471;MAF=0.00471;R2=0.99686;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910254	HLA_A*02:03	A	T	.	PASS	AF=0.00094;MAF=0.00094;R2=0.88473;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910255	HLA_A*02:04	A	T	.	PASS	AF=0.00017;MAF=0.00017;R2=0.89691;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910256	HLA_A*02:05	A	T	.	PASS	AF=0.01556;MAF=0.01556;R2=0.99839;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910257	HLA_A*02:06	A	T	.	PASS	AF=0.00364;MAF=0.00364;R2=0.98705;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910258	HLA_A*02:07	A	T	.	PASS	AF=0.00133;MAF=0.00133;R2=0.94265;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910259	HLA_A*02:10	A	T	.	PASS	AF=0.00001;MAF=0.00001;R2=0.06774;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910260	HLA_A*02:11	A	T	.	PASS	AF=0.00072;MAF=0.00072;R2=0.79302;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910261	HLA_A*02:135	A	T	.	PASS	AF=0.00005;MAF=0.00005;R2=0.22275;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910262	HLA_A*02:17	A	T	.	PASS	AF=0.00108;MAF=0.00108;R2=0.91078;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910263	HLA_A*02:195	A	T	.	PASS	AF=0.00003;MAF=0.00003;R2=0.00997;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910264	HLA_A*02:20	A	T	.	PASS	AF=0.00019;MAF=0.00019;R2=0.41177;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910265	HLA_A*02:22	A	T	.	PASS	AF=0.00026;MAF=0.00026;R2=0.84742;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910266	HLA_A*02:279	A	T	.	PASS	AF=0.00008;MAF=0.00008;R2=0.24552;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910267	HLA_A*02:55	A	T	.	PASS	AF=0.00001;MAF=0.00001;R2=0.02045;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910268	HLA_A*02:56	A	T	.	PASS	AF=0.00007;MAF=0.00007;R2=0.08360;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910269	HLA_A*02:60	A	T	.	PASS	AF=0.00004;MAF=0.00004;R2=0.49123;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910270	HLA_A*02:76	A	T	.	PASS	AF=0.00030;MAF=0.00030;R2=0.40308;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910271	HLA_A*02:87	A	T	.	PASS	AF=0.00001;MAF=0.00001;R2=0.00984;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910272	HLA_A*03	A	T	.	PASS	AF=0.12657;MAF=0.12657;R2=0.99727;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910273	HLA_A*03:01	A	T	.	PASS	AF=0.12061;MAF=0.12061;R2=0.99073;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910274	HLA_A*03:02	A	T	.	PASS	AF=0.00441;MAF=0.00441;R2=0.97833;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910275	HLA_A*03:36N	A	T	.	PASS	AF=0.00063;MAF=0.00063;R2=0.40664;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910276	HLA_A*03:89	A	T	.	PASS	AF=0.00006;MAF=0.00006;R2=0.05589;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910277	HLA_A*11	A	T	.	PASS	AF=0.06332;MAF=0.06332;R2=0.99759;IMPUTED	GT:DS:HDS:GP 1|0:1.000:1.0
6	29910278	HLA_A*11:01	A	T	.	PASS	AF=0.05966;MAF=0.05966;R2=0.96821;IMPUTED	GT:DS:HDS:GP 1|0:0.958:0.9
6	29910279	HLA_A*11:02	A	T	.	PASS	AF=0.00059;MAF=0.00059;R2=0.84655;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910280	HLA_A*11:32	A	T	.	PASS	AF=0.00021;MAF=0.00021;R2=0.04209;IMPUTED	GT:DS:HDS:GP 0|0:0.024:0.0
6	29910281	HLA_A*11:50Q	A	T	.	PASS	AF=0.00250;MAF=0.00250;R2=0.41268;IMPUTED	GT:DS:HDS:GP 0|0:0.018:0.0
6	29910282	HLA_A*23	A	T	.	PASS	AF=0.02822;MAF=0.02822;R2=0.99569;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910283	HLA_A*23:01	A	T	.	PASS	AF=0.02797;MAF=0.02797;R2=0.99150;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0
6	29910284	HLA_A*23:15	A	T	.	PASS	AF=0.00000;MAF=0.00000;R2=0.00005;IMPUTED	GT:DS:HDS:GP 0|0:0:0,0:1,0

REF ALT

"T": Presence of the allele

"A": Absence of the allele

Imputed HLA alleles

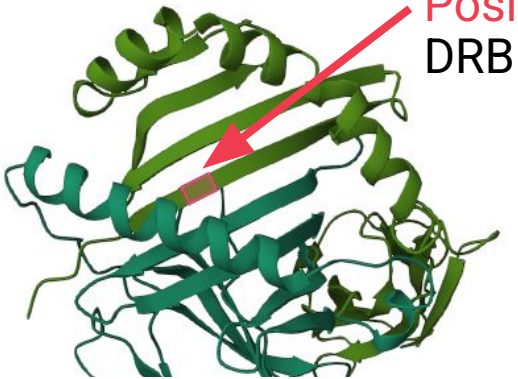
6	29910247	HLA_A*01	A	T	.	PASS	AF=0.15456;MAF=0.15456;R2=0.99719;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910248	HLA_A*01:01	A	T	.	PASS	AF=0.15234;MAF=0.15234;R2=0.99517;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910249	HLA_A*01:02	A	T	.	PASS	AF=0.00110;MAF=0.00110;R2=0.85198;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910250	HLA_A*01:136	A	T	.	PASS	AF=0.00002;MAF=0.00002;R2=0.04644;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910251	HLA_A*02	A	T	.	PASS	AF=0.26026;MAF=0.26026;R2=0.99741;IMPUTED	GT:DS:HDS:GP	0|1:0.998:0.0
6	29910252	HLA_A*02:01	A	T	.	PASS	AF=0.22914;MAF=0.22914;R2=0.98881;IMPUTED	GT:DS:HDS:GP	0|1:0.996:0.0
6	29910253	HLA_A*02:02	A	T	.	PASS	AF=0.00471;MAF=0.00471;R2=0.99686;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910254	HLA_A*02:03	A	T	.	PASS	AF=0.00094;MAF=0.00094;R2=0.88473;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910255	HLA_A*02:04	A	T	.	PASS	AF=0.00017;MAF=0.00017;R2=0.89691;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910256	HLA_A*02:05	A	T	.	PASS	AF=0.01556;MAF=0.01556;R2=0.99839;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910257	HLA_A*02:06	A	T	.	PASS	AF=0.00364;MAF=0.00364;R2=0.98705;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910258	HLA_A*02:07	A	T	.	PASS	AF=0.00133;MAF=0.00133;R2=0.94265;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910259	HLA_A*02:10	A	T	.	PASS	AF=0.00001;MAF=0.00001;R2=0.06774;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910260	HLA_A*02:11	A	T	.	PASS	AF=0.00072;MAF=0.00072;R2=0.79302;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910261	HLA_A*02:135	A	T	.	PASS	AF=0.00005;MAF=0.00005;R2=0.22275;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910262	HLA_A*02:17	A	T	.	PASS	AF=0.00108;MAF=0.00108;R2=0.91078;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910263	HLA_A*02:195	A	T	.	PASS	AF=0.00003;MAF=0.00003;R2=0.00997;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910264	HLA_A*02:20	A	T	.	PASS	AF=0.00019;MAF=0.00019;R2=0.41177;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910265	HLA_A*02:22	A	T	.	PASS	AF=0.00026;MAF=0.00026;R2=0.84742;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910266	HLA_A*02:279	A	T	.	PASS	AF=0.00008;MAF=0.00008;R2=0.24552;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910267	HLA_A*02:55	A	T	.	PASS	AF=0.00001;MAF=0.00001;R2=0.02045;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910268	HLA_A*02:56	A	T	.	PASS	AF=0.00007;MAF=0.00007;R2=0.08360;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910269	HLA_A*02:60	A	T	.	PASS	AF=0.00004;MAF=0.00004;R2=0.49123;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910270	HLA_A*02:76	A	T	.	PASS	AF=0.00030;MAF=0.00030;R2=0.40308;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910271	HLA_A*02:87	A	T	.	PASS	AF=0.00001;MAF=0.00001;R2=0.00984;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910272	HLA_A*03	A	T	.	PASS	AF=0.12657;MAF=0.12657;R2=0.99727;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910273	HLA_A*03:01	A	T	.	PASS	AF=0.12061;MAF=0.12061;R2=0.99073;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910274	HLA_A*03:02	A	T	.	PASS	AF=0.00441;MAF=0.00441;R2=0.97833;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910275	HLA_A*03:36N	A	T	.	PASS	AF=0.00063;MAF=0.00063;R2=0.40664;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910276	HLA_A*03:89	A	T	.	PASS	AF=0.00006;MAF=0.00006;R2=0.05589;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910277	HLA_A*11	A	T	.	PASS	AF=0.06332;MAF=0.06332;R2=0.99759;IMPUTED	GT:DS:HDS:GP	1|0:1.000:1.0
6	29910278	HLA_A*11:01	A	T	.	PASS	AF=0.05966;MAF=0.05966;R2=0.96821;IMPUTED	GT:DS:HDS:GP	1|0:0.958:0.9
6	29910279	HLA_A*11:02	A	T	.	PASS	AF=0.00059;MAF=0.00059;R2=0.84655;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910280	HLA_A*11:32	A	T	.	PASS	AF=0.00021;MAF=0.00021;R2=0.04209;IMPUTED	GT:DS:HDS:GP	0|0:0.024:0.0
6	29910281	HLA_A*11:50Q	A	T	.	PASS	AF=0.00250;MAF=0.00250;R2=0.41268;IMPUTED	GT:DS:HDS:GP	0|0:0.018:0.0
6	29910282	HLA_A*23	A	T	.	PASS	AF=0.02822;MAF=0.02822;R2=0.99569;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910283	HLA_A*23:01	A	T	.	PASS	AF=0.02797;MAF=0.02797;R2=0.99150;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0
6	29910284	HLA_A*23:15	A	T	.	PASS	AF=0.00000;MAF=0.00000;R2=0.00005;IMPUTED	GT:DS:HDS:GP	0|0:0:0,0:1,0

REF ALT

"T": Presence of the allele

"A": Absence of the allele

How are imputed HLA variants associated with disease?



Disease ↔ ?

1 HLA alleles — **2 HLA amino acid positions (Omnibus test)**

```
HLA-DRB1*01:01 ⟷ GGSCMAALTVTLMVLSSP
HLA-DRB1*04:01 ⟷ GGSCMTALTVTLMVLSSP
HLA-DRB1*15:01 ⟷ GGSCMTALTVTLMVLSSP
```

3 HLA haplotypes (Conditional haplotype test)

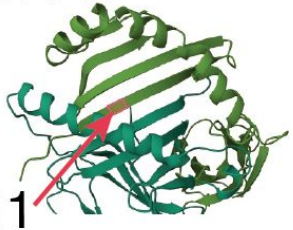
Omnibus test to ask which HLA amino acid position affects the disease

Full model: $log(odds_i) = \beta_0 + \sum_k \beta_k covariate_{k,i} + \sum_{m=1}^{M-1} \beta_m AM_{m,i}$

Reduced model: $log(odds_i) = \beta_0 + \sum_k \beta_k covariate_{k,i}$

ANO

HLA-DRB1



pos 11

RFLWQLKFECH
RFLEYSTSECH
RFLEQVKHECH
RFLWQGKYKCH
RFLKQDKFECH
RFLWQPKRECH

M = 6 possible amino acid residues

M - 1

	L	S	V	G	D	(P)
	0.1	0.0	0.9	0.0	0.0	1.0
	0.9	0.0	0.0	0.0	1.0	0.1
	0.0	1.0	0.0	0.9	0.0	0.1
	0.0	0.0	1.9	0.0	0.1	0.0
	2.0	0.0	0.0	0.0	0.0	0.0

Omnibus test to ask which HLA amino acid position affects the disease

Full model: $log(odds_i) = \beta_0 + \sum_k \beta_k covariate_{k,i} + \sum_{m=1}^{M-1} \beta_m AM_{m,i}$

Reduced model: $log(odds_i) = \beta_0 + \sum_k \beta_k covariate_{k,i}$

ANOVA(Full model, Reduced model)

How much does this amino acid position's polymorphism increase the explained variance for the trait?

→ Determine the single most significant amino acid position

Summary

1. HLA amino acid sequences and alleles characterize antigen presentation and disease risk within HLA.
2. HLA amino acid sequences and alleles can be accurately imputed from genotyped SNPs by MIS.
3. Imputed HLA alleles can be used to fine-map causal disease mechanisms.

Summary

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3. Imputed HLA alleles can be used to fine-map causal disease mechanisms.

Sakaue et al. *Nature Protocols* 2023

Section 4

nf-gwas and PGS Server

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MICHIGAN IMPUTATIONSERVER

ASHG ANNUAL MEETING 2025 BOSTON, MA • OCTOBER 14-18

Learning objectives

Participants will

1. Learn how to run a GWAS pipeline
2. Learn how to use the PGS extension
3. Learn how to analyze PGS calculated by the server

Summary of common GWAS analysis tools

	EPACTS	Rvtests	SNPTEST	SAIGE	BLOT-LMM	Bgenie	regenie
Input VCF	Y	Y	Y				
Sample relatedness (Quantitative outcome)	Y	Y		Y	Y	Y	Y
Sample relatedness (Binary outcome)				Y		Y	Y
Case control imbalance				Y			Y
Large sample size (>20,000)				Y	Y	Y	Y

nf-gwas

- A GWAS pipeline based on REGENIE and works with VCF input
- Includes several pre- and post-processing steps
- Based on Nextflow
  - Allows to build a portable, reproducible, scalable pipeline
  - Runs on clusters (e.g. SLURM) or in the cloud (e.g. AWS Batch)

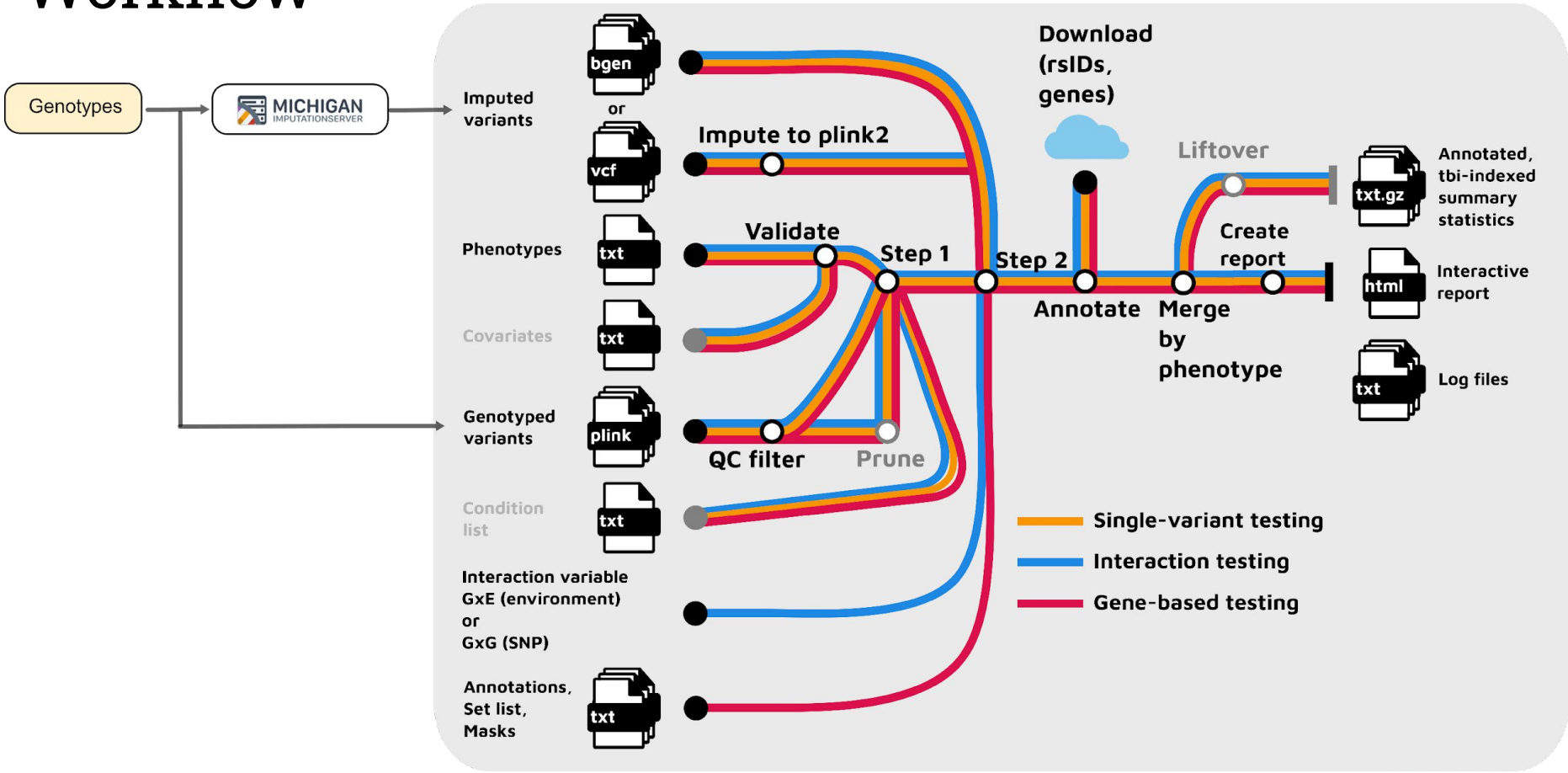
JOURNAL ARTICLE

Performing highly parallelized and reproducible GWAS analysis on biobank-scale data

Sebastian Schönherr, Johanna F Schachtl-Riess, Silvia Di Maio, Michele Filosi, Marvin Mark, Claudia Lamina, Christian Fuchsberger, Florian Kronenberg, Lukas Forer
Author Notes

NAR Genomics and Bioinformatics, Volume 6, Issue 1, March 2024, lqae015,
https://doi.org/10.1093/nargab/lqae015

Workflow


Genotypes
MICHIGAN IMPUTATIONSERVER
Imputed variants
bgen
or
vcf
Phenotypes
txt
Covariates
txt
Genotyped variants
plink
Condition list
txt
Interaction variable GxE (environment) or GxG (SNP)
Annotations, Set list, Masks
txt
Impute to plink2
Validate
Step 1
QC filter
Prune
Step 2
Download (rsIDs, genes)
Annotate
Liftover
Merge by phenotype
Create report
txt.gz
Annotated, tbi-indexed summary statistics
html
Interactive report
txt
Log files
Single-variant testing
Interaction testing
Gene-based testing

Download Phenotypes

Phenotype Data

- **Filename:** phenotypes.txt (131.6 KB)
- **Description:** Simulated phenotype file containing four traits.
- **Format:** Plain text (tab-delimited).
- **Columns:**
  - `sample_id` — individual sample identifiers
  - `pheno_1`, `pheno_2`, `pheno_3`, `pheno_4` — four simulated phenotypes
- **Use:** Provides phenotype data for GWAS and PGS evaluation.

Covariates (Principal Components)

- **Filename:** covariates.txt (292.3 KB)
- **Description:** Covariate file containing the first 10 genetic principal components (PCs)
- **Format:** Tab-delimited, compressed with gzip.
- **Columns:**
  - `sample` — individual identifiers matching the genotype data
  - `PC1` – `PC10` — first ten principal components representing population structure
- **Use:** Used as covariates in association analyses to correct for population stratification

You can also download the pre-imputed data

Simulated Phenotypes

Phenotype	Type	Highly Genetic?
pheno_1	Binary (e.g., case/control)	✅ Yes
pheno_2	Continuous (e.g., height)	✅ Yes
pheno_3	Binary or categorical	❌ No
pheno_4	Continuous	❌ No

https://tinyurl.com/imputationserver-2025

Easy to configure

my-gwas.config

```
params {
  project                       = 'test-gwas-with-pcs'
  genotypes_association         = 'gwas.imputed.chr20.dose.vcf.gz'
  genotypes_association_format  = 'vcf'
  association_build             = 'hg19'
  phenotypes_filename           = 'phenotypes.txt'
  phenotypes_columns            = 'pheno_2,pheno_4'
  covariates_filename           = 'covariates.txt'
  covariates_columns            = 'PC1,PC2,PC3,PC4,PC5,PC6,PC7,PC8,PC9,PC10'
  regenie_test                  = 'additive'
  regenie_min_imputation_score  = 0.3
  regenie_skip_predictions      = true
  rsids_filename                = "rsids-v154-hg19-chr20.index.gz"
  binning_size                  = 50000
}
```

Imputed genotypes from Imputation Server

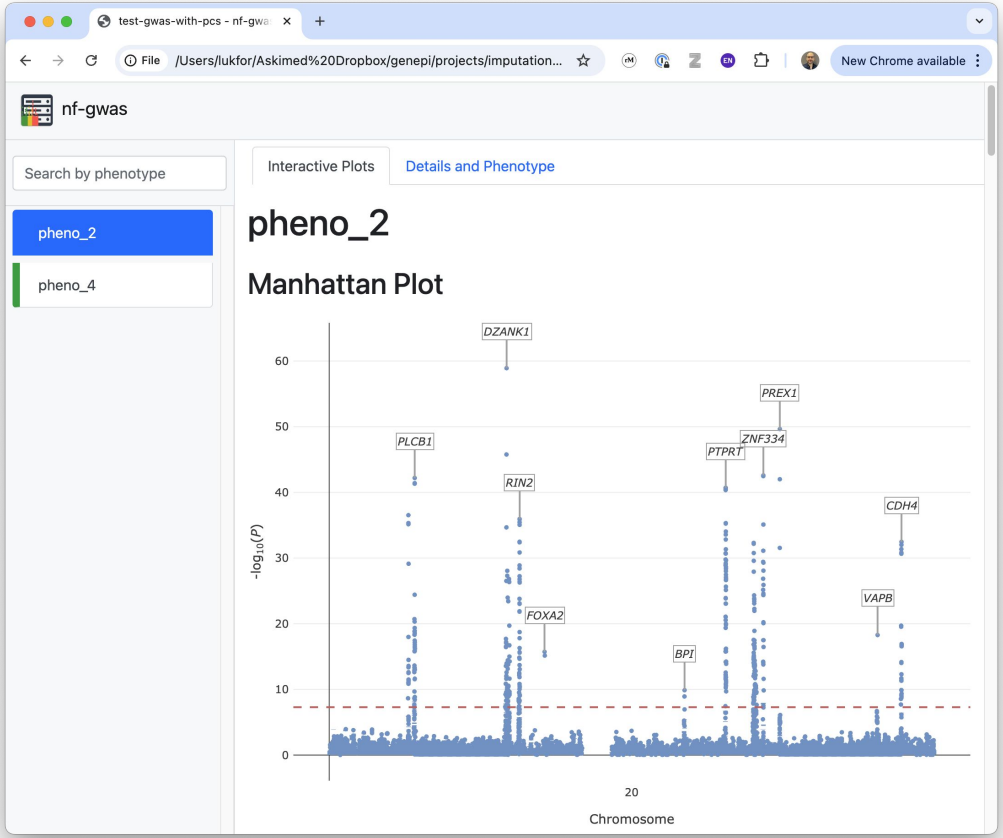
phenotypes

covariates

Annotation and visualization

```
nextflow run genepi/nf-gwas -r v1.0.11 -profile docker -c my-gwas.config
```

Results Phenotype 2

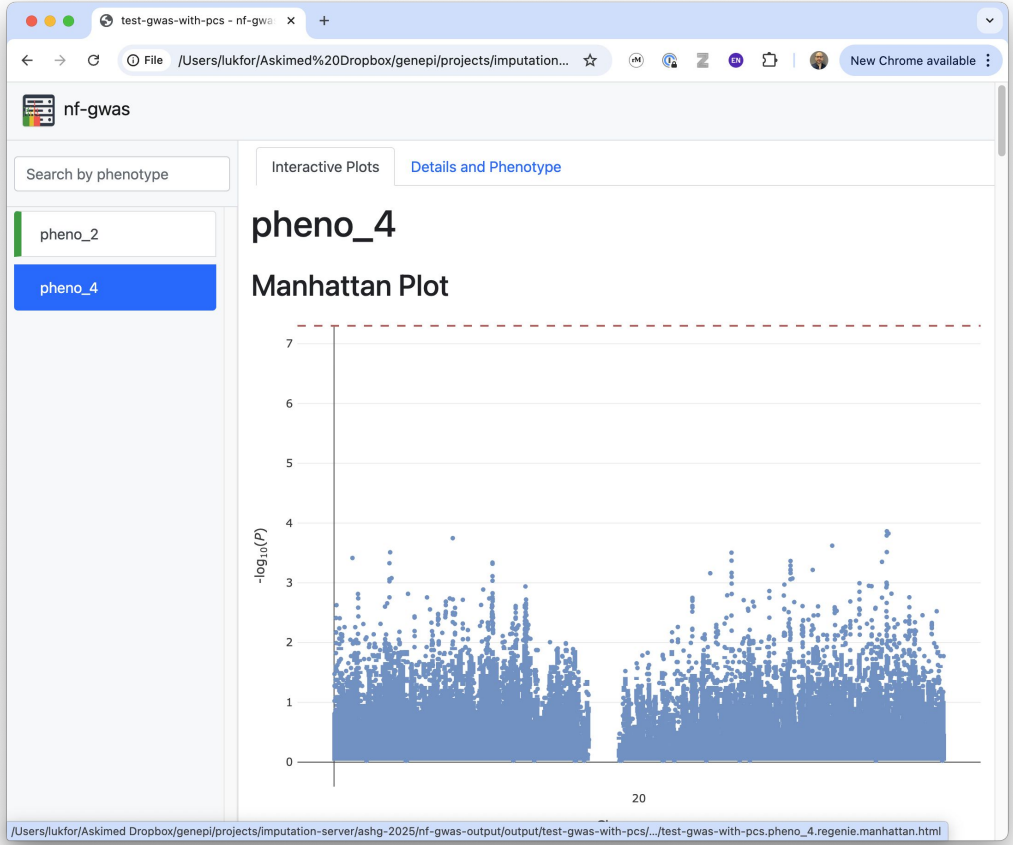


Top Loci

Variant	ID	Ref Allele	Effect Allele	Nearest Gene	-log10(P)	Beta
20:18445266	rs6111973	C	T	DZANK1	58.9235	-0.522676
20:46858654	rs7269193	A	G	PREX1	49.648	0.626932
20:45142619	rs847055	A	T	ZNF334	42.5918	0.493058
20:8887891	rs6056246	T	C	PLCB1	42.2005	0.437334
20:41227725	rs6102924	C	T	PTPRT	40.7084	-0.627067
20:19800969	rs1884767	A	G	RIN2	35.9524	-0.385179
20:59507557	rs6071441	C	T	CDH4	32.4673	0.480876
20:57020044	rs6070466	C	G	VAPB	18.2868	0.925181
20:22392054	rs13038903	A	G	FOXA2	15.7283	-3.3011
20:36950990	rs6024863	A	T	BPI	9.8814	0.225461
20:1728212	rs605739	T	G	SIRPG	3.95055	0.124383
20:4453463	rs297660	C	T	PRNP	3.89483	-0.14028
20:2513877	rs6083567	A	G	TMC2	3.79769	-0.217792
20:21387531	rs13040764	A	G	NKX2-4	3.7689	-0.763363
20:31440180	rs6088022	T	G	MAPRE1	3.69196	-0.442159
20:25957535	rs2252934	G	C	ZNF337	3.57123	-0.184881
20:29859763	rs6089068	C	T	DEFB115	3.51671	0.189548
20:53303956	rs6023507	C	T	DOK5	3.47376	0.357591
20:25221547	rs6050477	T	C	PYGB	3.47251	-0.120575

Loci are defined ±200 kb around lead SNPs

Results Phenotype 4



No significant loci.

Tutorial: Setup, Parameters, and Example Analyses

- On the workshop website, you'll find a step-by-step tutorial on how to:
  - Setup and install the pipeline
  - Explore all available parameters
  - Run association tests for binary traits (e.g. pheno_1 and pheno_2)
  - Generate and interpret QQ plots

- Also available for PLINK2

https://tinyurl.com/imputationserver-2025

JOURNAL ARTICLE

Imputation Server PGS: an automated approach to calculate polygenic risk scores on imputation servers

Lukas Forer, Daniel Taliun, Jonathon LeFaive, Albert V Smith, Andrew P Boughton, Stefan Coassin, Claudia Lamina, Florian Kronenberg, Christian Fuchsberger, Sebastian Schönherr ✉

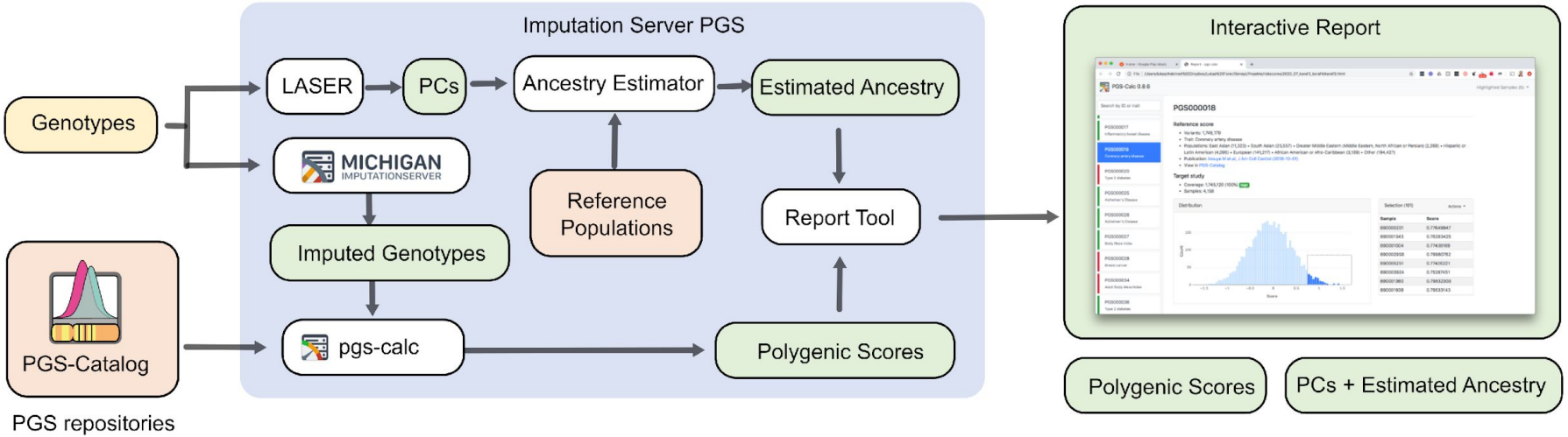
Nucleic Acids Research, Volume 52, Issue W1, 5 July 2024, Pages W70–W77,
https://doi.org/10.1093/nar/gkae331

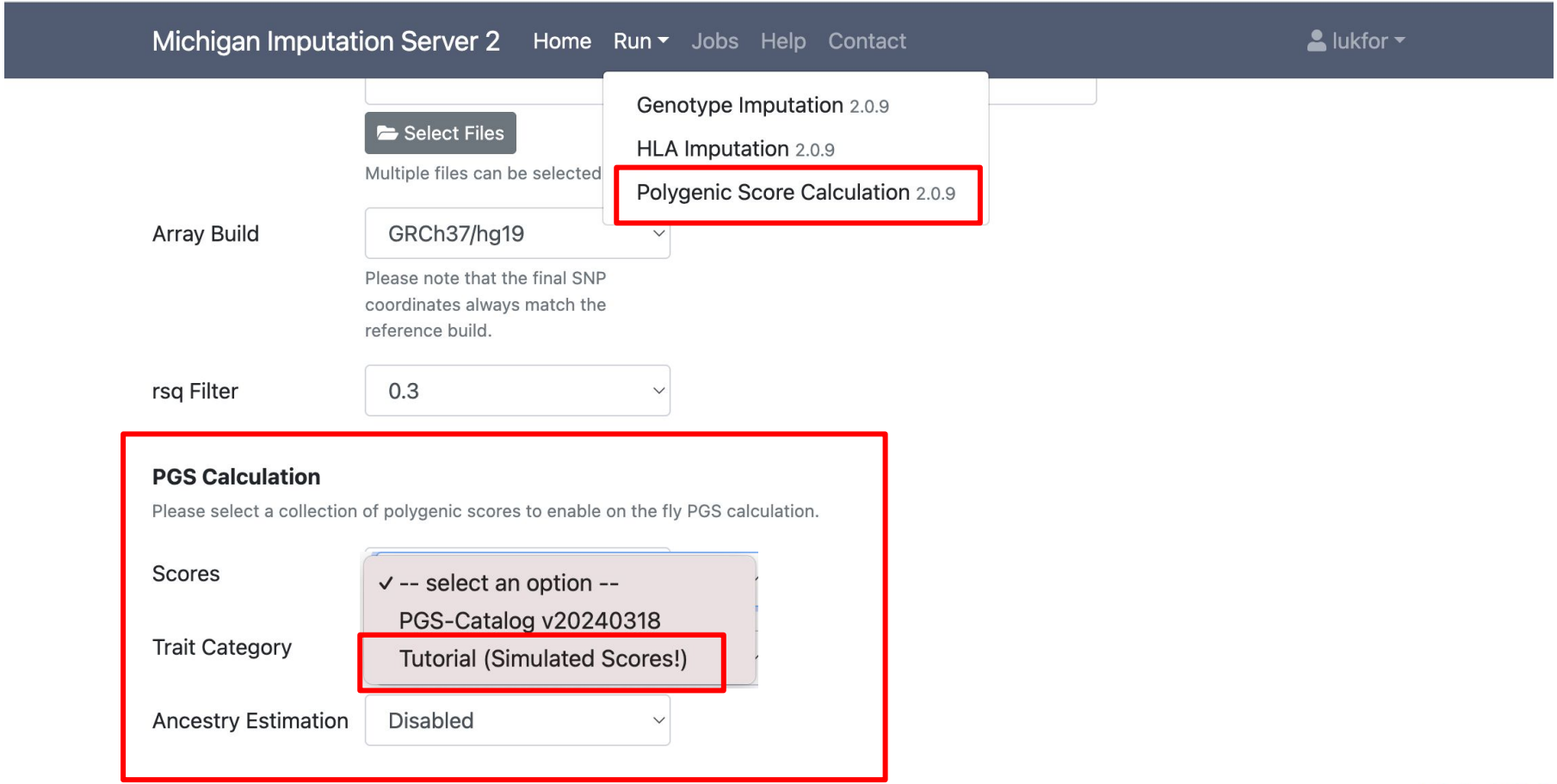
Imputation Server PGS

- Makes it easier to use polygenic scores (PGS) with imputed genotypes by calculating them directly on the server
- PGS: aggregates the effects of many genetic variants into a single number which predicts genetic predisposition for a phenotype
- Available since April 2022
- More than 5,000 submitted jobs
- Supports more than 3,000 scores

Have you previously used the PGS tool on the Michigan Imputation Server?

Workflow


Imputation Server PGS
Genotypes
LASER
PCs
Ancestry Estimator
Estimated Ancestry
MICHIGAN IMPUTATIONSERVER
Reference Populations
Report Tool
Imputed Genotypes
PGS-Catalog
pgs-calc
Polygenic Scores
PGS repositories
Interactive Report
Polygenic Scores
PCs + Estimated Ancestry


Michigan Imputation Server 2
Home
Run
Jobs
Help
Contact
lukfor
Genotype Imputation 2.0.9
HLA Imputation 2.0.9
Polygenic Score Calculation 2.0.9
Select Files
Multiple files can be selected
Array Build
GRCh37/hg19
Please note that the final SNP coordinates always match the reference build.
rsq Filter
0.3
PGS Calculation
Please select a collection of polygenic scores to enable on the fly PGS calculation.
Scores
-- select an option --
PGS-Catalog v20240318
Tutorial (Simulated Scores!)
Trait Category
Ancestry Estimation
Disabled

Results

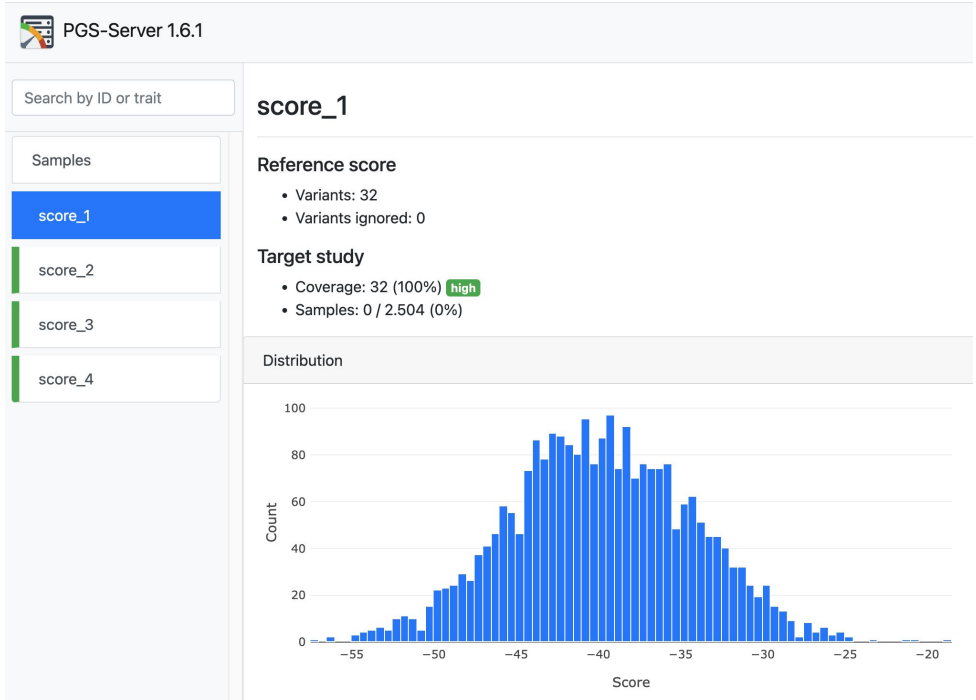
Details | Results | Logs

Downloads

- statistics
- qc_report.txt (674 bytes)
- quality-control.html (1 MB)
- scores.coverage (222 bytes)
- scores.html (32 KB)
- scores.info (1 KB)
- scores.report.zip (175 KB)
- scores.txt (228 KB)

Logs

- step1-nextflow.log (59 KB)
- step1-report.html (2 MB)
- step1-timeline.html (251 KB)
- step1-trace.csv (3 KB)



What can we do with these scores now?

Example: compare how well each score explains the simulated phenotypes

- Merge scores with phenotypes

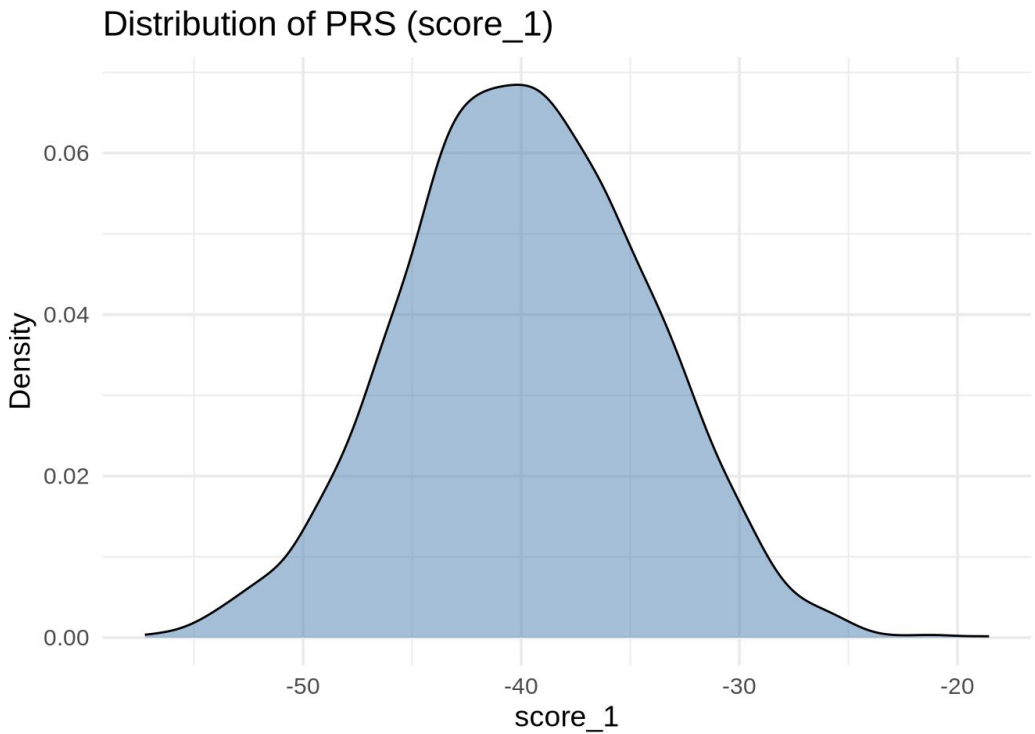
```
# Read PRS scores and phenotypes
scores <- read_csv("data/scores.txt")
phenos <- read_table("data/phenotypes.txt")

# Merge datasets by sample ID
merged <- inner_join(scores, phenos, by = c("sample" = "IID"))
```

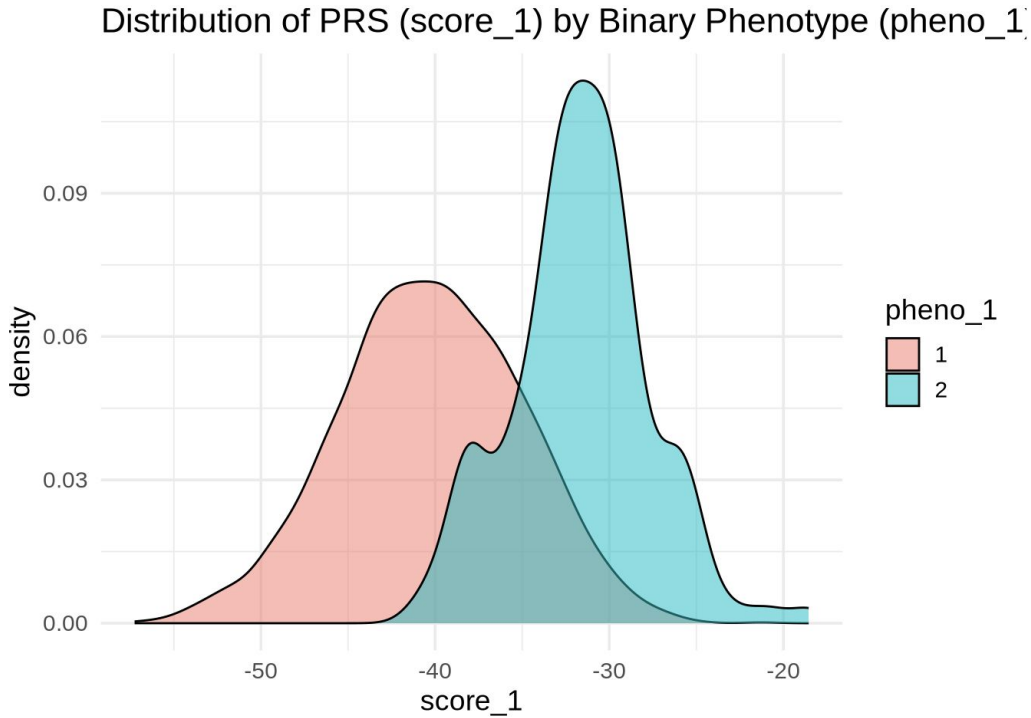
- You can also download the calculated polygenic scores from the website

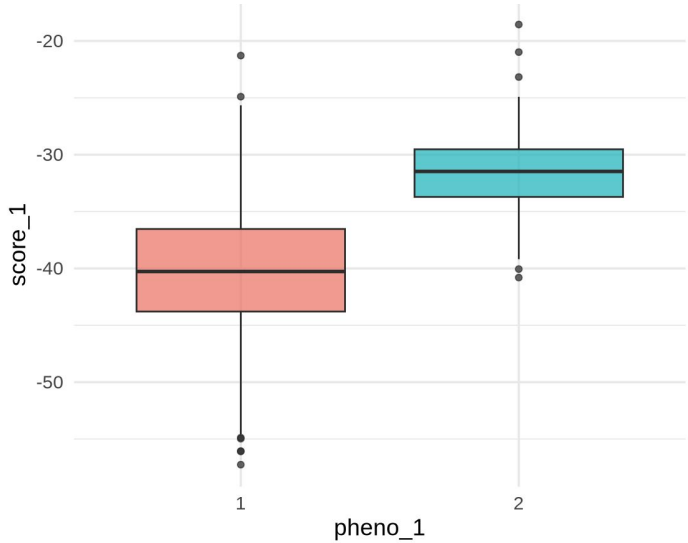
https://tinyurl.com/imputationserver-2025

```r
ggplot(merged, aes(x = score_1)) +
  geom_density(alpha = 0.5, fill = "steelblue") +
  labs(
    title = "Distribution of PRS (score_1)",
    x = "score_1",
    y = "Density"
  )
```

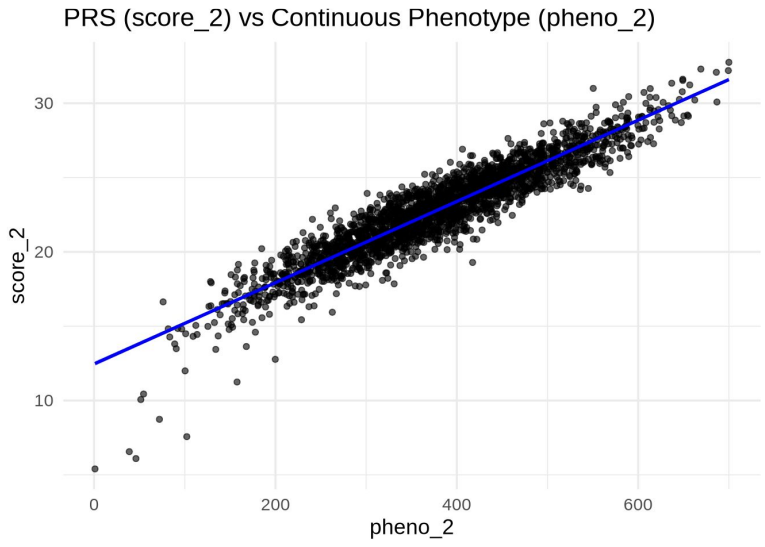


```r
ggplot(merged, aes(x = score_1, fill = as.factor(pheno_1))) +
  geom_density(alpha = 0.5) +
  labs(
    title = "Distribution of PRS (score_1) by Binary Phenotype (pheno_1)",
    x = "score_1",
    fill = "pheno_1"
  )
```

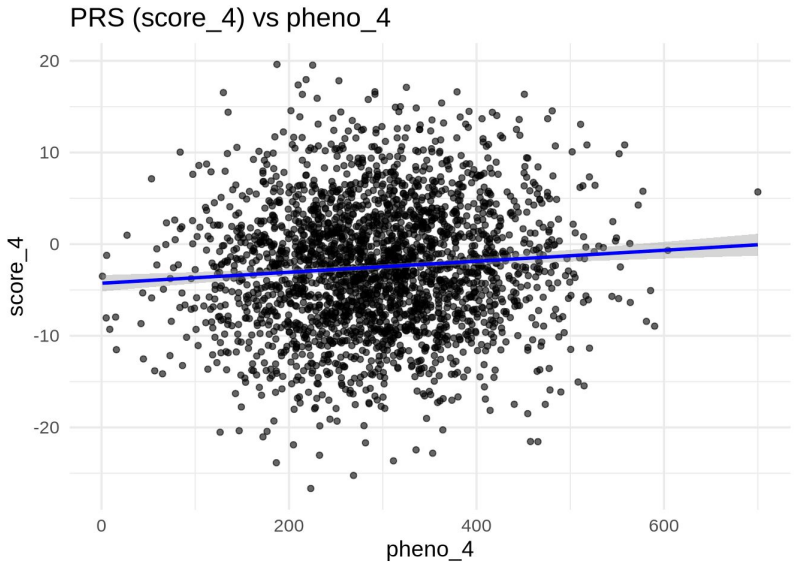




```r
ggplot(merged, aes(x = pheno_2, y = score_2)) +
  geom_point(alpha = 0.6) +
  geom_smooth(method = "lm", se = TRUE, color = "blue") +
  labs(
    title = "PRS (score_2) vs Continuous Phenotype (pheno_2)",
    x = "pheno_2",
    y = "score_2"
  )
```




```r
ggplot(merged, aes(x = pheno_4, y = score_4)) +
  geom_point(alpha = 0.6) +
  geom_smooth(method = "lm", se = TRUE, color = "blue") +
  labs(
    title = "PRS (score_4) vs pheno_4",
    x = "pheno_4",
    y = "score_4"
  )
```



https://tinyurl.com/imputationserver-2025

ASHG American Society of Human Genetics

Summary

- Imputation Servers are easy to use and ensure high-quality imputation
- GWAS and PGS play a key role in modern genetic research

- Several pipelines and extensions of the Imputation Server

Aim: Taking the burden out of genetic analysis

Section 5

Helmholtz Munich Imputation Server

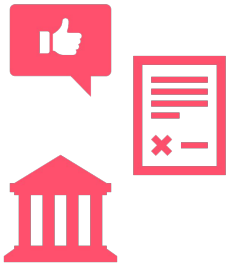
Eleftheria Zeggini

Director, Institute of Translational Genomics

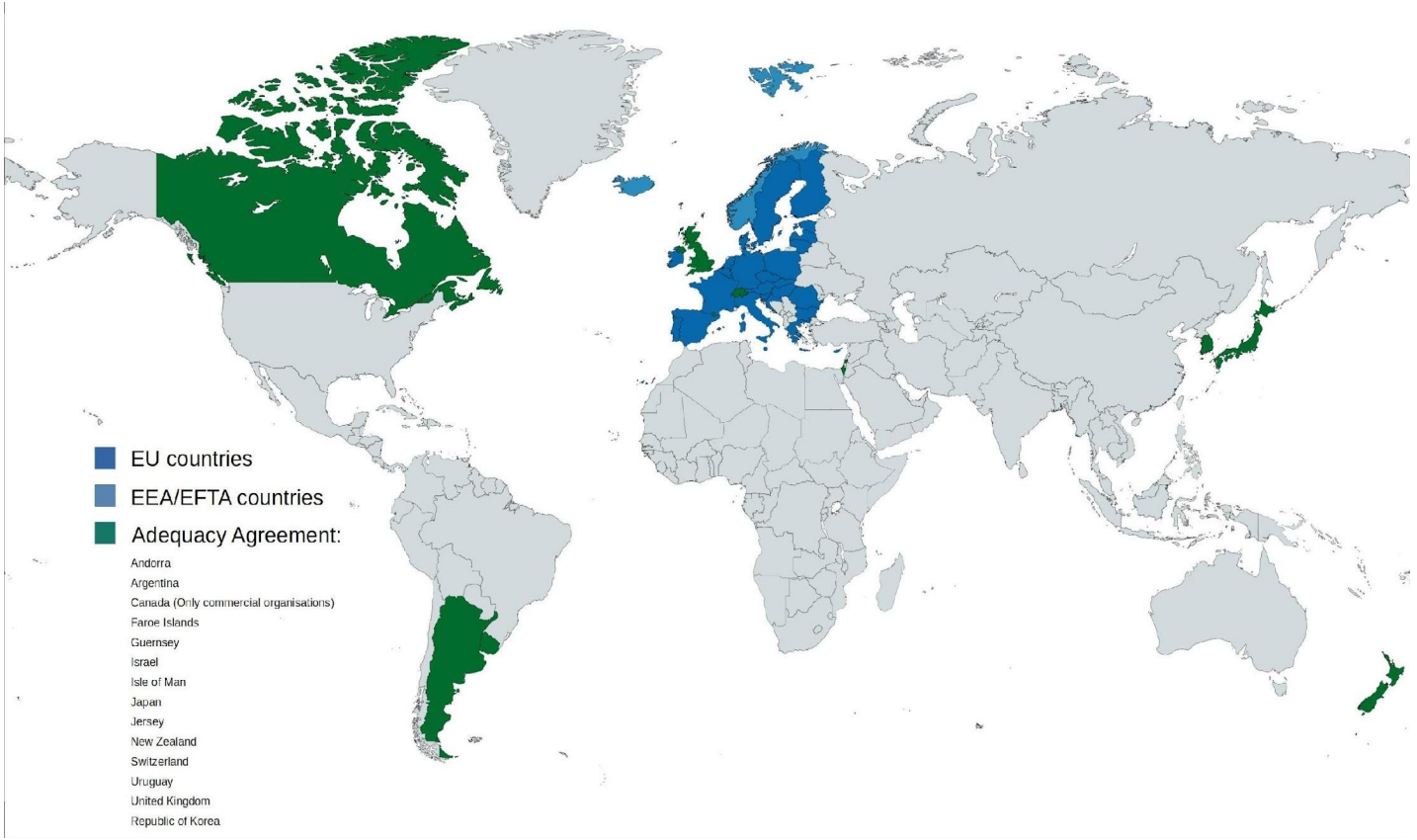
HELMHOLTZ MUNICH

Why do we need another imputation server?

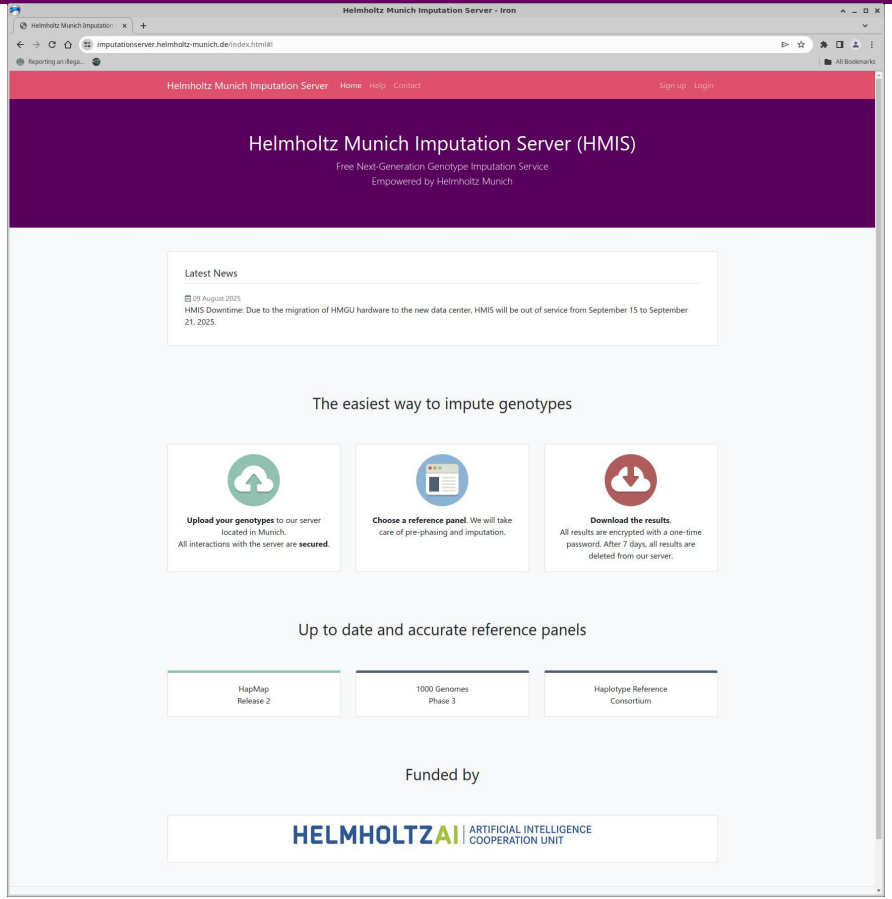
- The transfer of personal data to countries outside the European Economic Area (EEA) is generally prohibited by default
- Therefore, all personal data on EU citizens must reside and be processed on servers physically located within the EU
- Human genetic data are considered a special category of personal data subject to stricter requirements
- There are reasons when personal data can be moved outside the EEA:
  - Consent is given by the individual for the transfer
  - A Standard Contractual Clause (SCC) has been agreed between the two parties
  - There is adequacy agreement with country



EU Adequacy Agreements



Helmholtz Munich Imputation Server



The server is designed to assist users in the EU to comply with the 2016 General Data Protection Regulation (GDPR) law

nature genetics

Correspondence | Published: 15 November 2024

Toward GDPR compliance with the Helmholtz Munich genotype imputation server

N. William Rayner, Young-Chan Park, Christian Fuchsberger, Andrei Barysenka & Eleftheria Zeggini

Nature Genetics **56**, 2580–2581 (2024) | Cite this article

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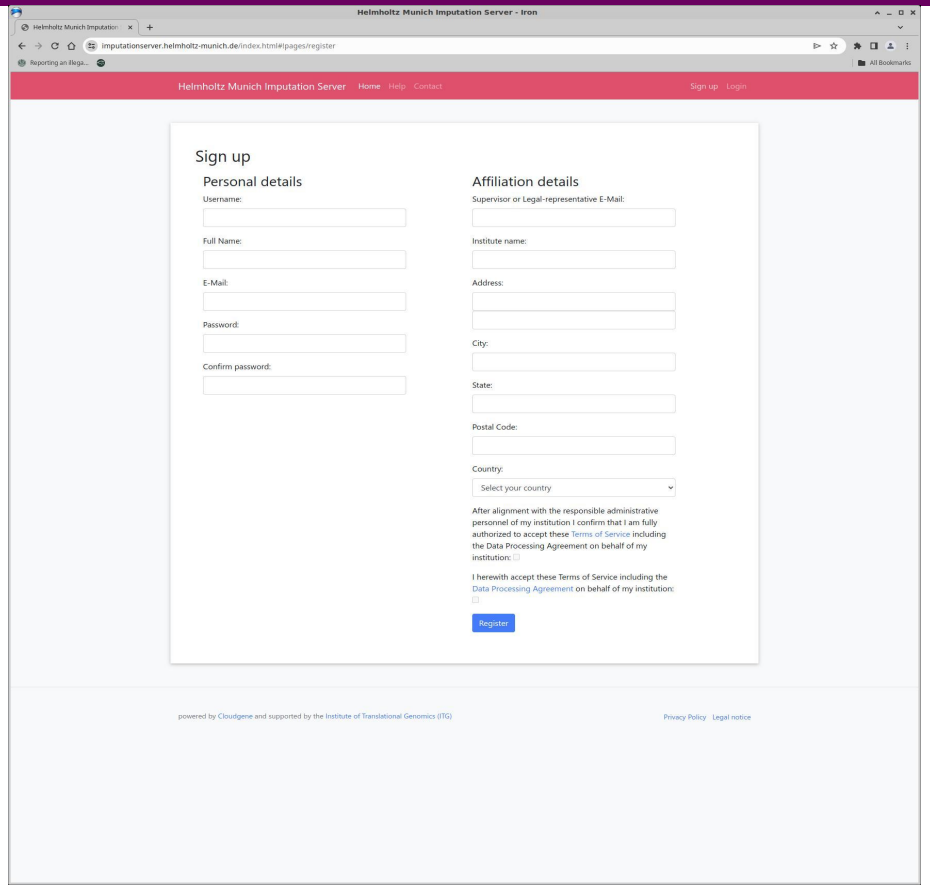
Helmholtz Munich Imputation Server

- Based on the freely available Michigan software
- Currently version 2 based on Cloudgene and Hadoop
- Moving to version 3, currently integrating the changes we have made from the standard Michigan software

Notable Differences vs Michigan

- More information is required on sign up to comply with GDPR

HMIS sign up



Institute name and address

+

email of legal representative and country are required so as to meet our obligations under GDPR

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Notable Differences vs Michigan

- More information is required on sign up to comply with GDPR
- Short and long (>25,000 samples) processing queues introduced to ensure fair access to the resource
- Longer more complex passwords are now required, >15 characters

Current Reference Panels

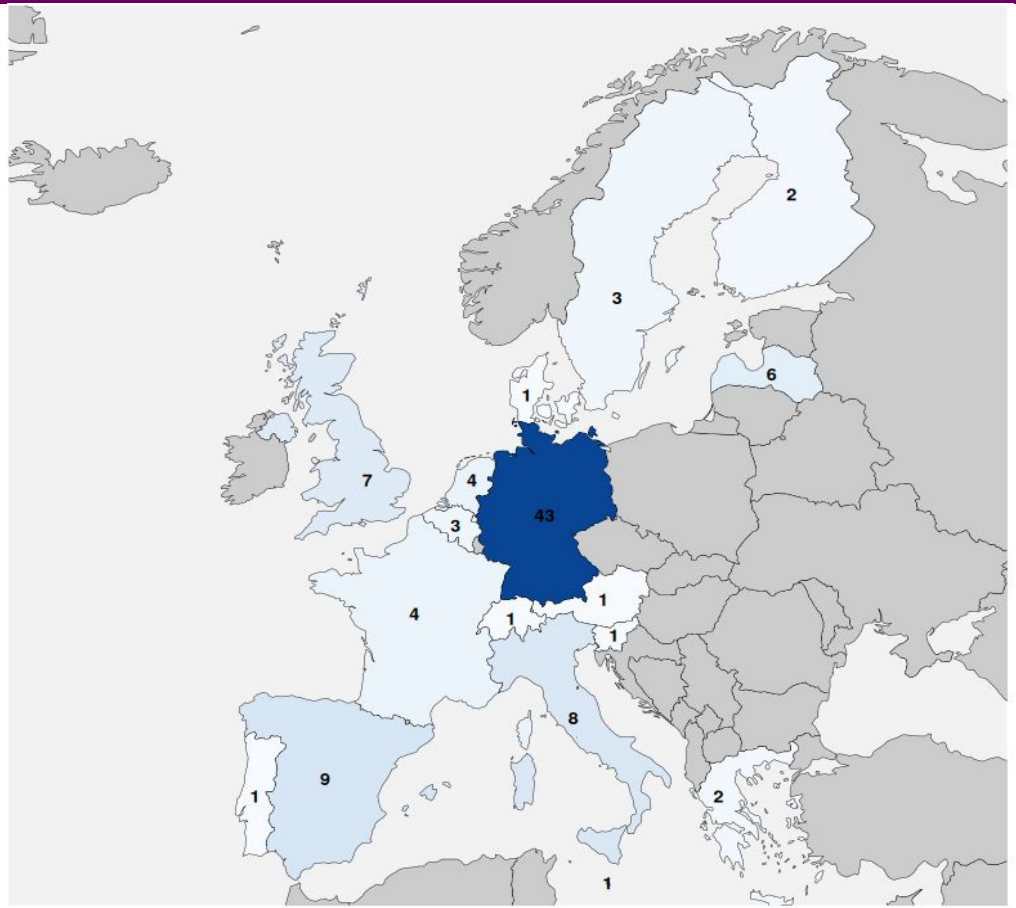
- 1000G GRCh37
- 1000G GRCh38
- HRC
- HapMap2

Upcoming Reference Panels

- German National Cohort (NAKO)
  ~15,500
  Will be expanded to 30,000

- Genome of Europe

Registered Users



We have 101 registered users, largely from across the EU

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Number of genomes

- Over 900,000 genomes imputed to date

Direct future developments

0 surveys completed

0 surveys underway

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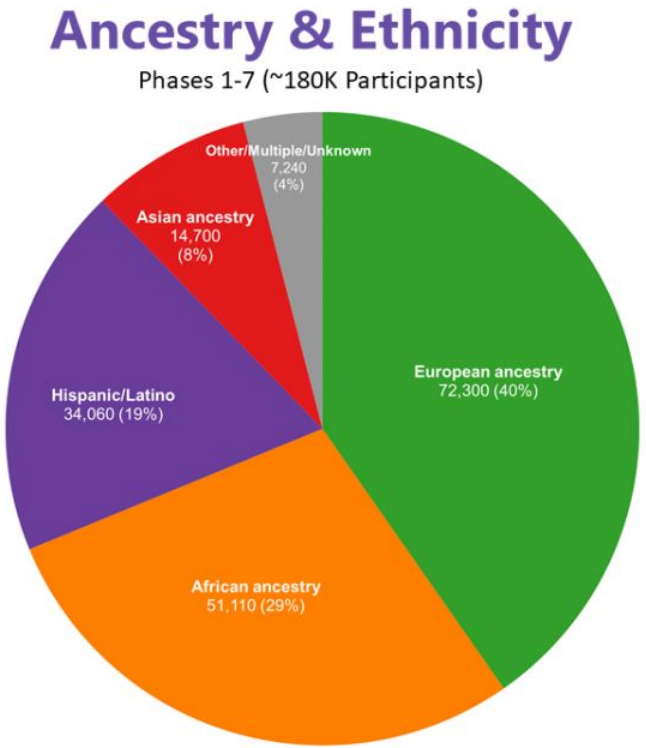
Section 6

The TOPMed Imputation Server

Albert Smith
University of Michigan
albertvs@umich.edu
@avsmith

TOPMed Program

- Trans-Omics for Precision Medicine (TOPMed) Program
- A Precision Medicine Initiative sponsored by National Heart, Lung and Blood Institute
- Integrating whole-genome sequencing and other omics data
- >180k participants from >90 studies



TOPMed Imputation

- Current reference panel based on TOPMed Freeze 8 Calls
- Michigan Imputation Server ported to Amazon Web Services
- R2 panel released April 2020
- Updated R3 panel released December 2023
- https://imputation.biodatacatalyst.nhlbi.nih.gov
- Registration as before, open access to TOPMed panel

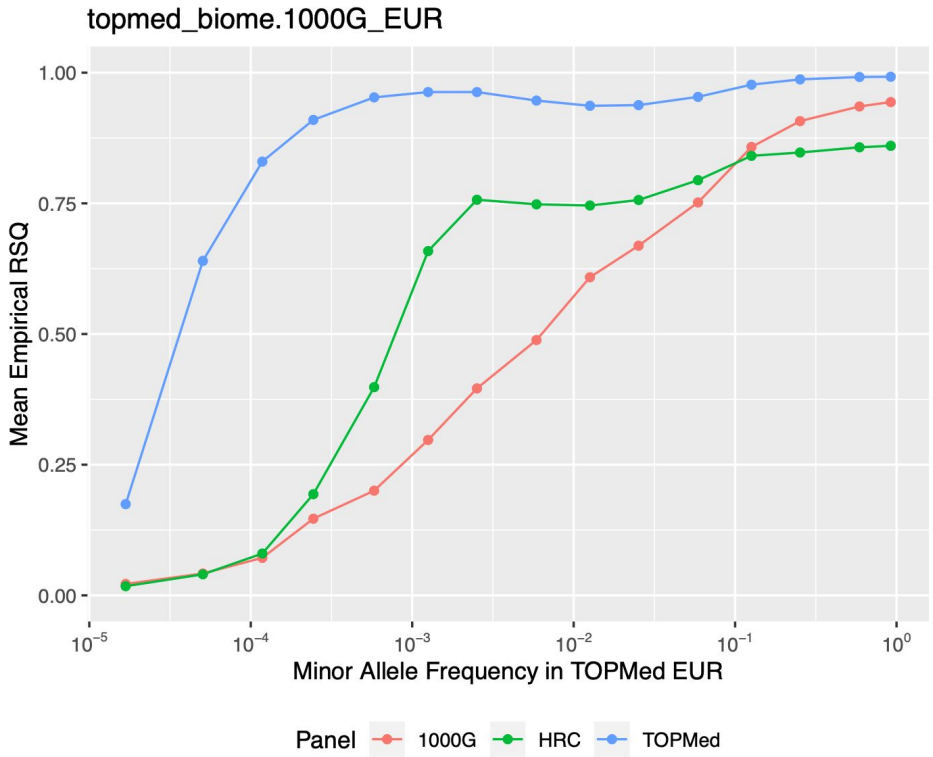
TOPMed Panel Compared

	TOPMed_r2	HRC	1000G Genomes
N samples	97K	39K	2,500
Ancestry	Multiethnic	European	Multiethnic
N variants	308M	39M	88M
Avg. depth	38X	8X	4X
Genome build Position	b38	b37	b37

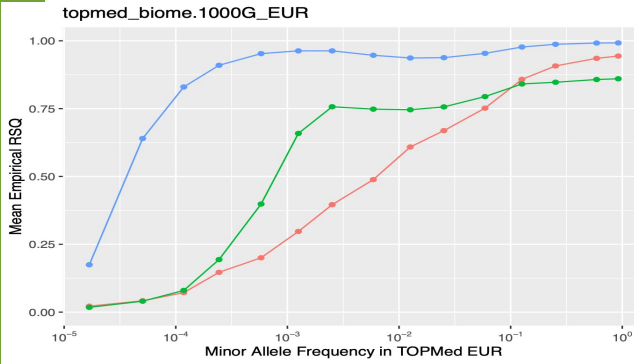
TOPMed Panel Freq Distribution

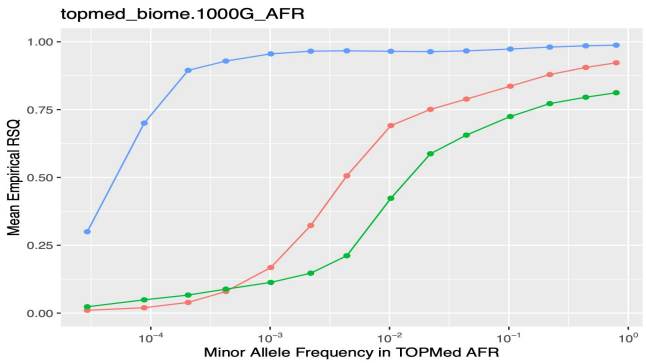
Variation type	Non-reference allele frequency bins				
	(0, 0.005]	(0.005, 0.01]	(0.01, 0.05]	(0.05, 1)	Totals
SNVs	270,352,495	3,365,284	5,330,340	7,020,861	286,068,980
Insertions	5,462,262	74,150	130,506	148,595	5,815,513
Deletions	15,406,052	185,606	297,186	333,748	16,222,592
Totals	**291,220,809**	**3,625,040**	**5,758,032**	**7,503,204**	**308,107,085**

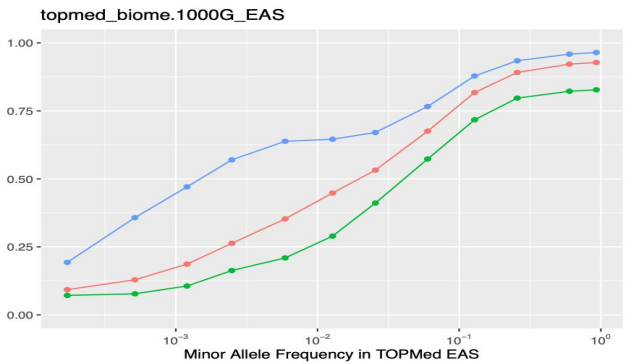
Imputation Panel Quality

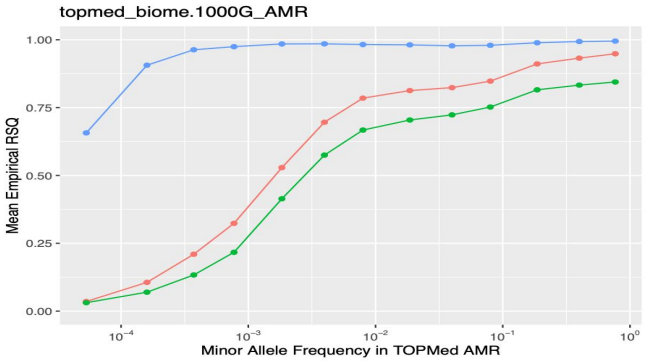


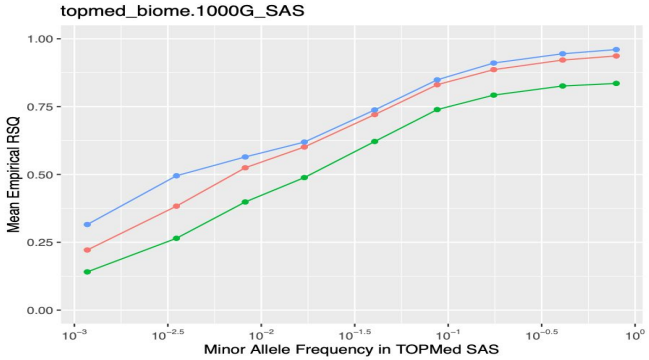
Imputation Panel Quality





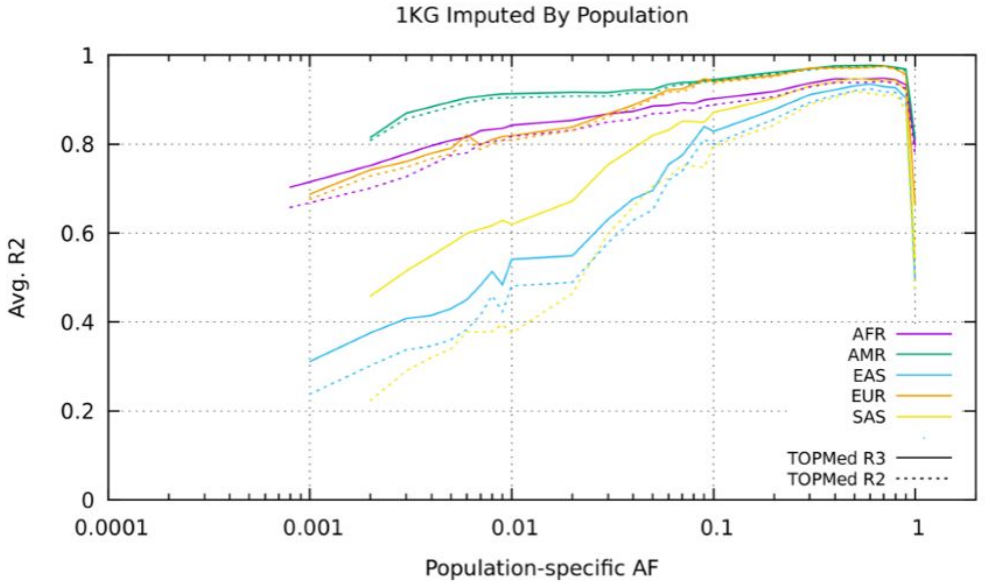






Updated TOPMed R3 Panel

- Existing panel has better coverage for EUR, AFR and AMR samples
- Targeted improvement for SAS and EAS samples
- Expanded panel (97k => 143k)
- Released Dec 2023 (R2 deprecated)



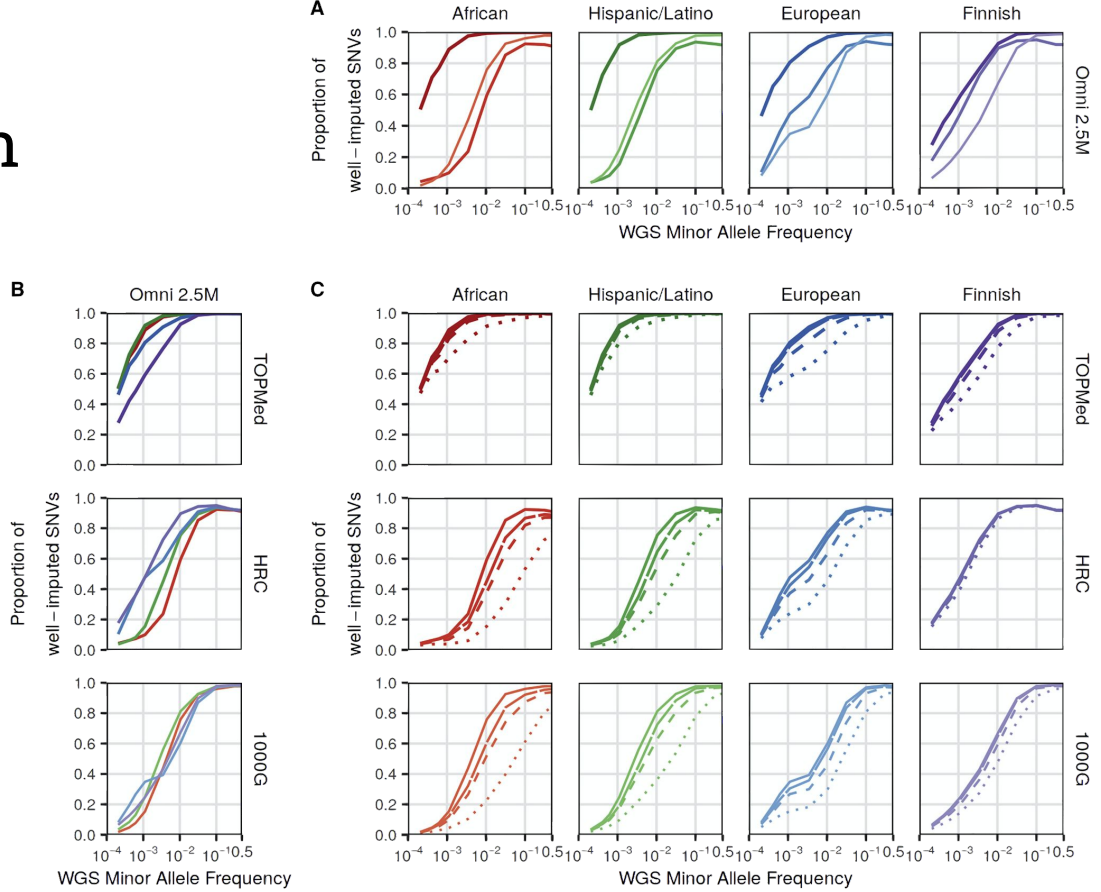
TOPMed Panel Compared

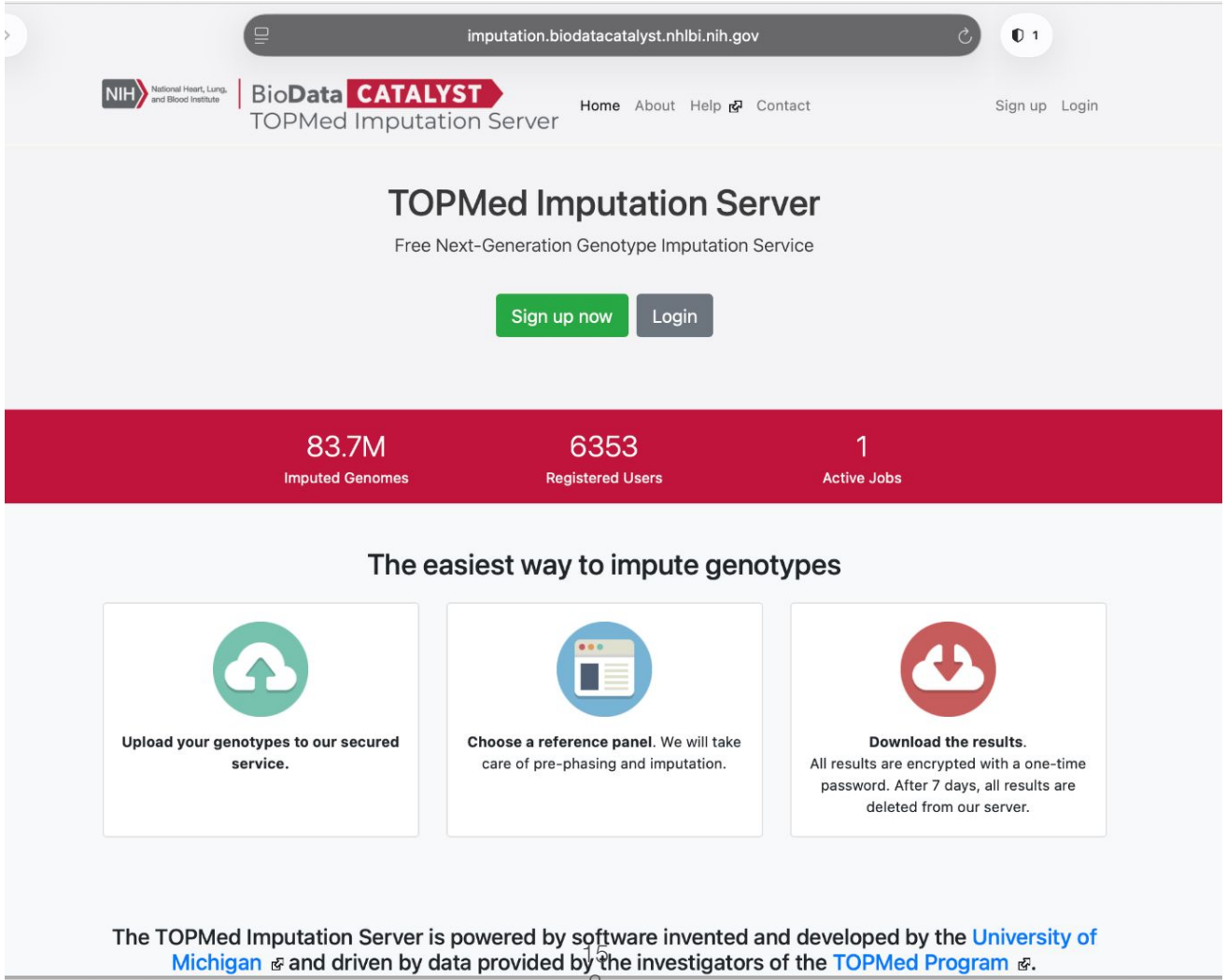
	TOPMed_r3	TOPMed_r2	HRC	1000G Genomes
N samples	143K	97K	39K	2,500
Ancestry	Multiethnic	Multiethnic	European	Multiethnic
N variants	414M	308M	39M	88M
Avg. depth	38X	38X	8X	4X
Genome build Position	b38	b38	b37	b37

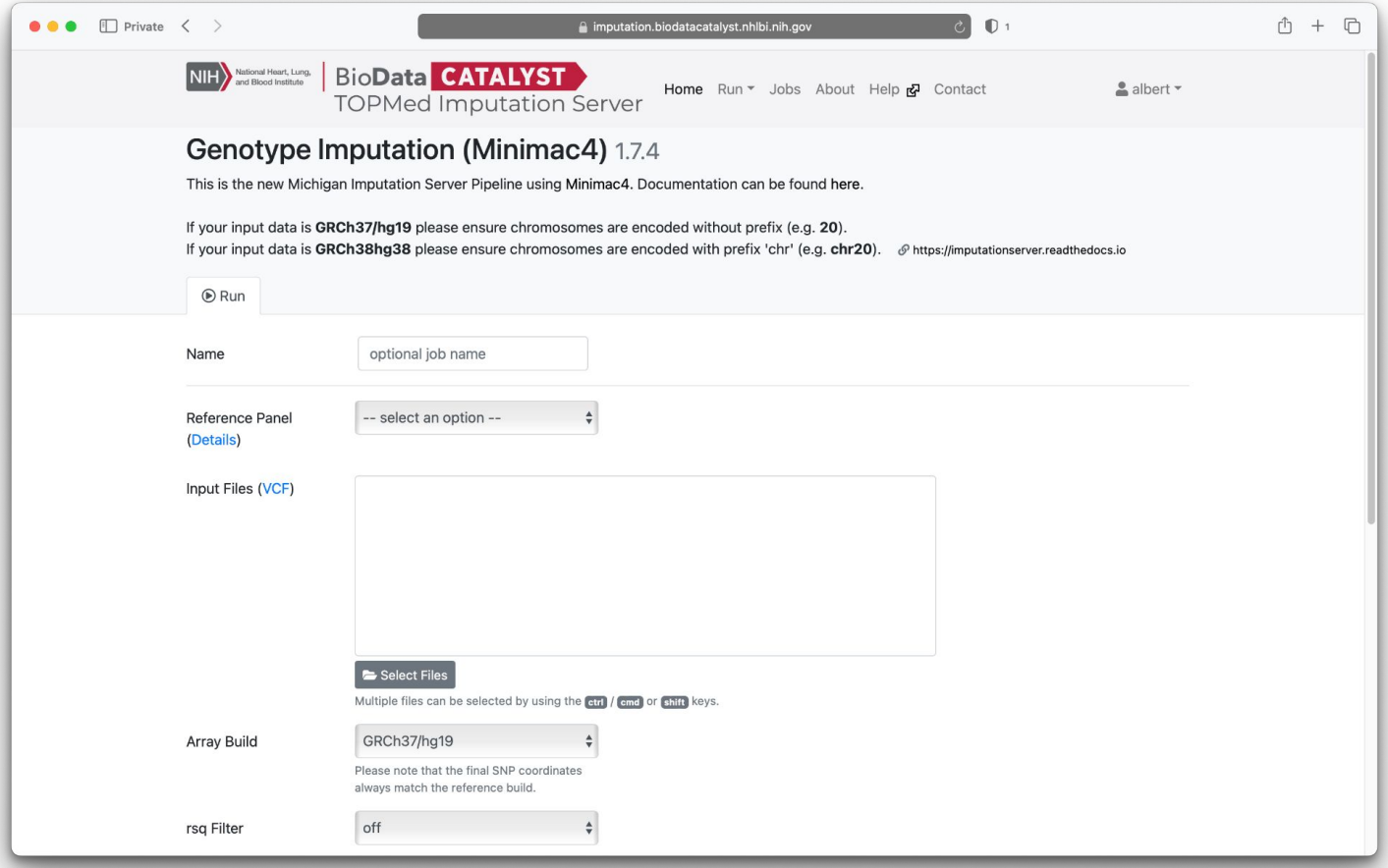
TOPMed_r2: Deprecated Dec 2025
TOPMed_r3: Contains HRC and HGDP
HRC: Haplotype Reference Consortium Panel
1000G: 1000 Genomes Project Reference Panel

TOPMed Imputation Compared to WGS

- Proportion well imputed (r2 > 0.8) down to MAF:
  - 0.14% in African
  - 0.11% in Hispanic/Latino
  - 0.35% in European
  - 0.85% in Finnish
- Similar performance for arrays with >700k variants
- Source: Hanks et al. https://doi.org/10.1016/j.ajhg.2022.07.012

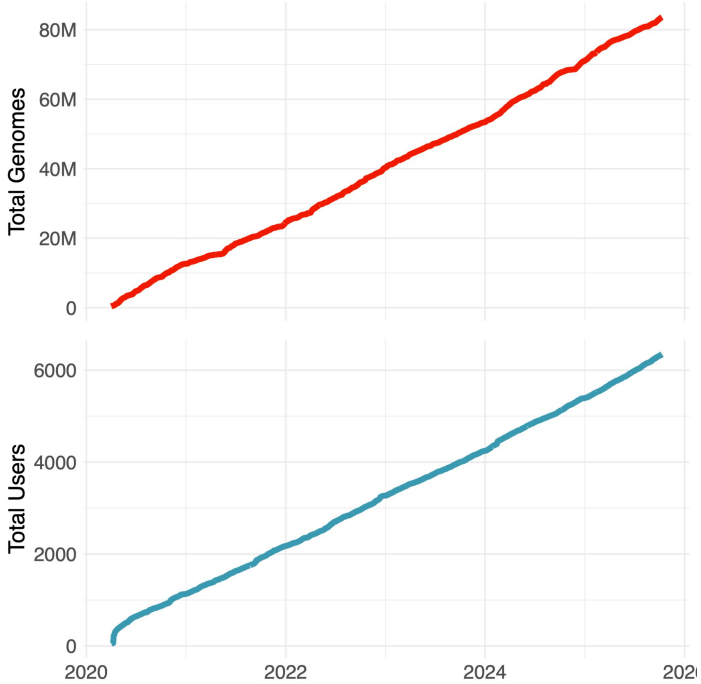



imputation.biodatacatalyst.nhlbi.nih.gov
1
NIH National Heart, Lung, and Blood Institute
BioData CATALYST
TOPMed Imputation Server
Home
About
Help
Contact
Sign up
Login
TOPMed Imputation Server
Free Next-Generation Genotype Imputation Service
Sign up now
Login
83.7M
Imputed Genomes
6353
Registered Users
1
Active Jobs
The easiest way to impute genotypes
Upload your genotypes to our secured service.
Choose a reference panel. We will take care of pre-phasing and imputation.
Download the results.
All results are encrypted with a one-time password. After 7 days, all results are deleted from our server.
The TOPMed Imputation Server is powered by software invented and developed by the University of Michigan and driven by data provided by the investigators of the TOPMed Program.



TOPMed Imputation

- 84M genomes imputed
- Supplanted 1000g & HRC imputation for many studies
- Particularly benefits ethnically diverse cohorts
- Satisfying GDPR-related concerns of European users remains a challenge
  (Better served by Munich Imputation Server)



TOPMed Imputation

- TOPMed Server migrating to Nextflow based architecture in near future
- Beta testers needed
- If you have imputation ready samples, contact me <albertvs@umich.edu> or our support address <imputationserver@umich.edu>
- NOTE: *This will be the same R3 panel. Testers should have new samples.*

Imputation Resources

- Michigan Imputation Server
  https://imputation.sph.umich.edu/
- Helmholtz Munich Imputation Server
  https://imputationserver.helmholtz-munich.de/
- TOPMed Imputation Server
  https://imputation.biodatacatalyst.nhlbi.nih.gov/
- MCPS Imputation Server
  https://imputationserver-reg.sph.umich.edu/

Your questions:

Nobody has responded yet.

Hang tight! Responses are coming in.

Thank you!

Your questions

If we run out of time, please send your questions to

Christian Fuchsberger - cfuchsb@umich.edu