Data QC and Exploratory Data Analysis

(Afternoon session)

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Outline

- Haplotype estimation
- Genotype imputation
- Refining genotype calls
- Imputation performance
- Reference panels for imputation
- Information on the practical

Haplotype estimation

We observe genotypes for each individual

G 2 1 1 2 2 1 1 1 0 2 1 2 2 1 1 1 2 1 1 0

Haplotype estimation

We observe genotypes for each individual

G 2 1 1 2 2 1 1 1 0 2 1 2 2 1 1 1 2 1 1 0

1 0 0 1 1 1 0 1 0 1 0 1 1 0 1 1 1 0 1 0

1 1 1 1 1 0 1 0 0 1 1 1 1 1 0 0 1 1 0 0

We want to recover the underlying haplotypes

<u>Complex problem:</u>

N heterozygotes -> 2^N haplotypes

N heterozygotes -> 2^{N-1} pairs of haplotypes

Haplotypes in disease genetics

Estimation of haplotypes from array-based and sequencing studies is an important problem for multiple reasons:

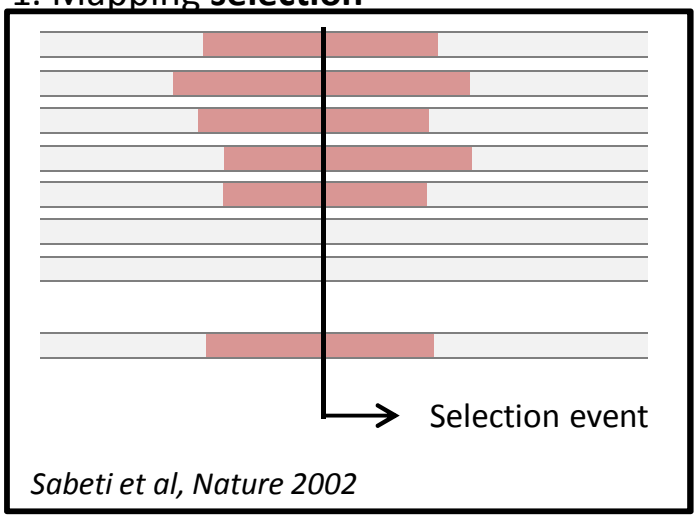
1. Construction of reference panels of haplotypes (HapMap, 1000 Genomes, UK10K, GoNL, etc…).

2. Imputation of Genome Wide Association Studies (i.e. GWAS).

3. Detection of novel associations in GWAS or fine-mapping of known effects e.g. compound heterozygotes.

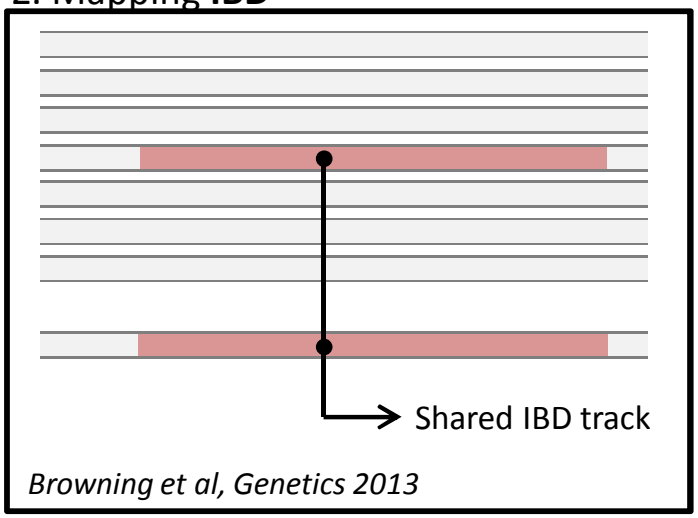
Haplotypes in population genetics

1. Mapping **selection**



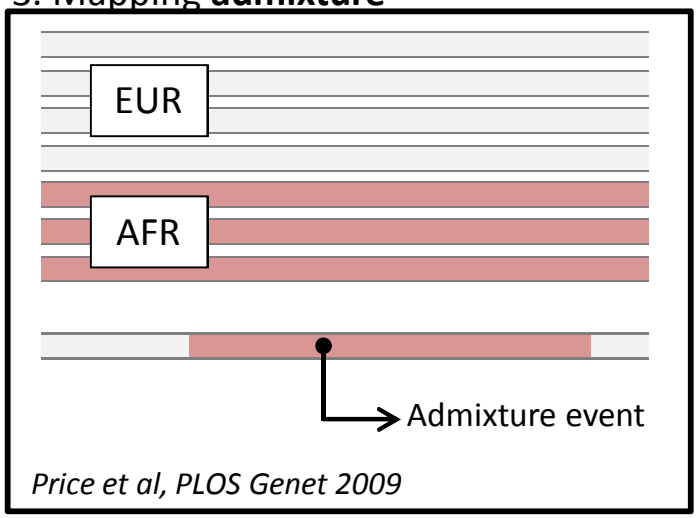
Sabeti et al, Nature 2002

2. Mapping **IBD**



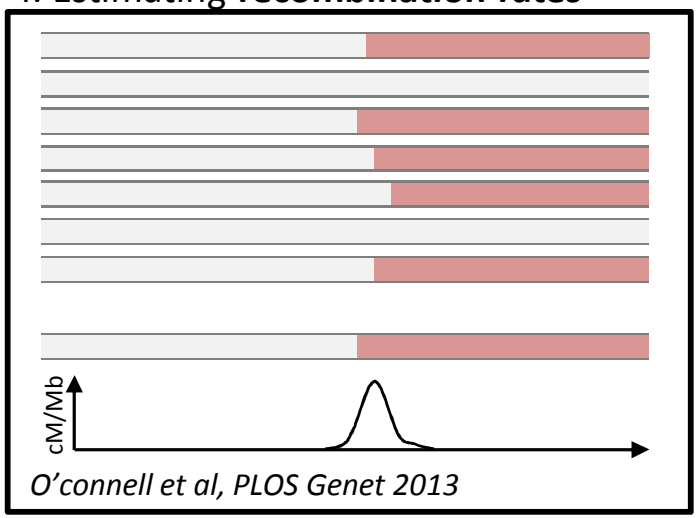
Browning et al, Genetics 2013

3. Mapping **admixture**



Price et al, PLOS Genet 2009

4. Estimating **recombination rates**



O'connell et al, PLOS Genet 2013

Basic idea of phasing

H

0	1	0	1	1	1	0	1	0	1	1	1	1	0	0	0	1	0	1	0
0	1	0	1	1	1	0	1	0	1	1	1	1	0	0	0	1	0	1	0
1	0	1	1	1	0	1	0	0	1	0	1	1	1	1	1	1	1	0	0
1	0	1	0	0	0	1	0	1	0	0	0	0	1	1	1	0	1	0	1
0	1	0	1	1	1	0	0	0	1	0	1	1	1	1	1	1	1	0	0
1	0	1	1	1	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0
0	1	0	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	0	0
1	0	1	1	1	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0

G

2	1	1	2	2	1	1	1	0	2	1	2	2	1	1	1	2	1	1	0

Haplotype estimation works iteratively.

Basic idea of phasing

H

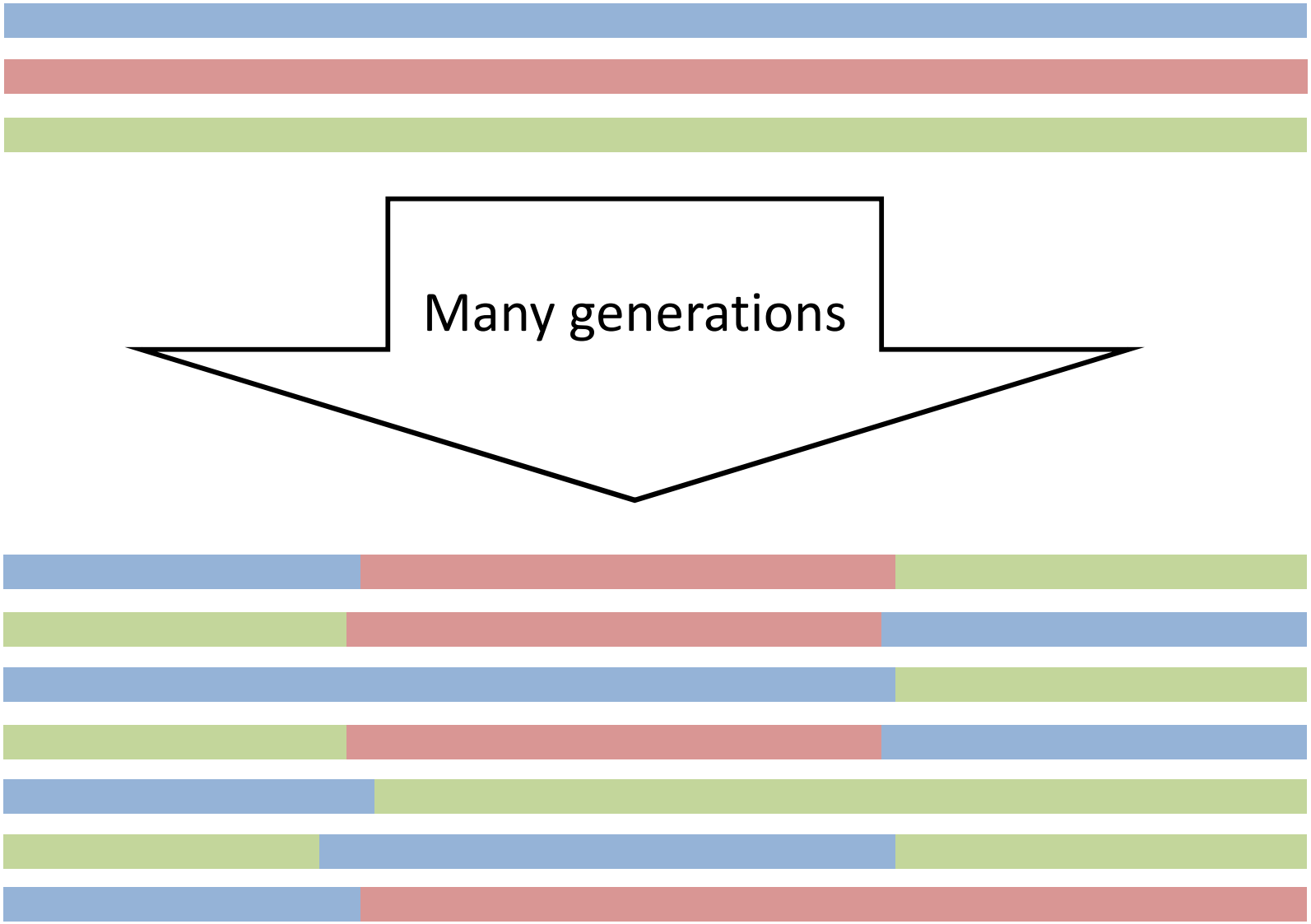
0 1 0 1 1 1 0 1 0 1 1 1 1 0 0 0 1 0 1 0
0 1 0 1 1 1 0 1 0 1 1 1 1 0 0 0 1 0 1 0
1 0 1 1 1 0 1 0 0 1 0 1 1 1 1 1 1 1 0 0
1 0 1 0 0 0 1 0 1 0 0 0 0 1 1 1 0 1 0 1
0 1 0 1 1 1 0 0 0 1 0 1 1 1 1 1 1 1 0 0
1 0 1 1 1 0 1 0 1 0 0 0 0 1 0 0 1 0 1 0
0 1 0 1 1 1 0 1 0 1 1 1 1 0 1 1 1 1 0 0
1 0 1 1 1 0 1 0 1 0 0 0 0 1 0 0 1 0 1 0

G

2 1 1 2 2 1 1 1 0 2 1 2 2 1 1 1 2 1 1 0

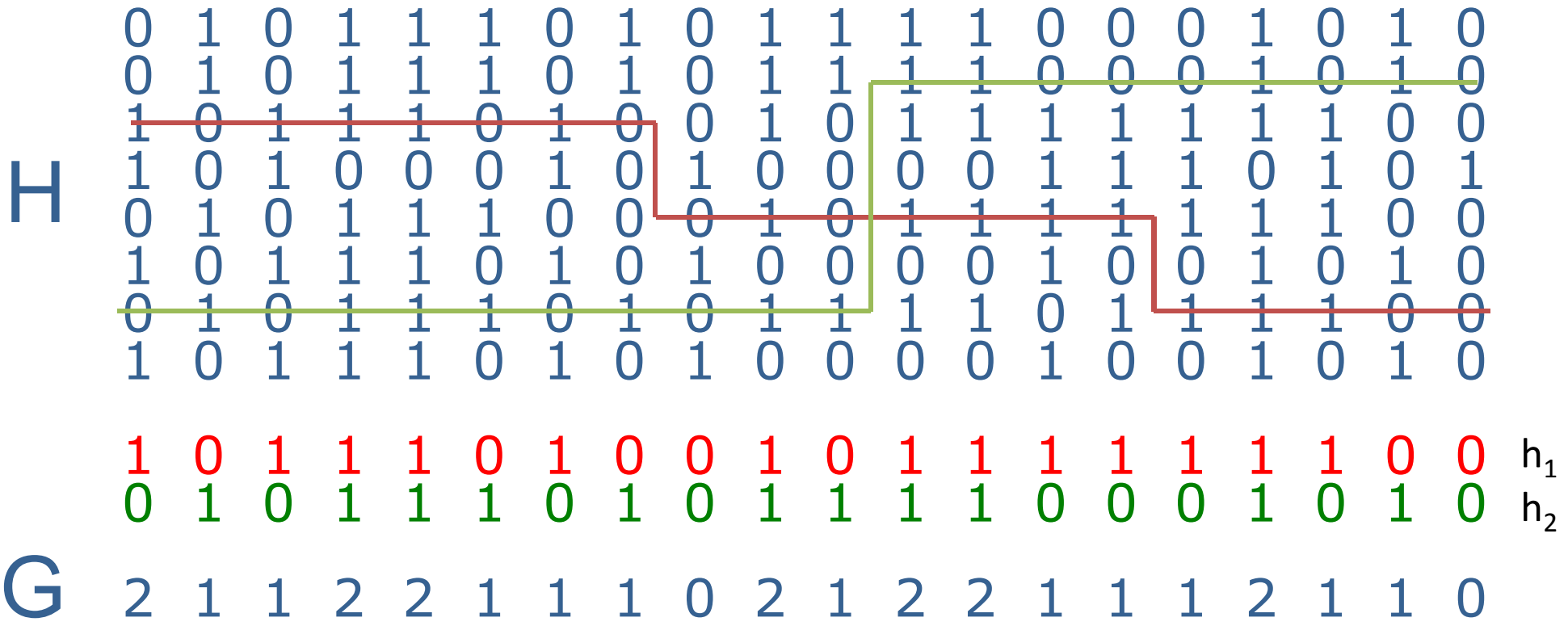
It takes each individuals genotype vector (G) in turn and estimates the underlying haplotypes using the current haplotype estimates of other individuals (H).

Haplotype structure



Individuals share stretches of haplotypes inherited from common ancestors

Basic idea of phasing



It uses Hidden Markov Models (*Li and Stephens, 2005*) that models the underlying haplotypes as mosaics of haplotypes of other individuals.

Popular phasing methods

There are several popular methods for haplotype estimation from genotype data:

MACH - http://www.sph.umich.edu/csg/abecasis/MACH/

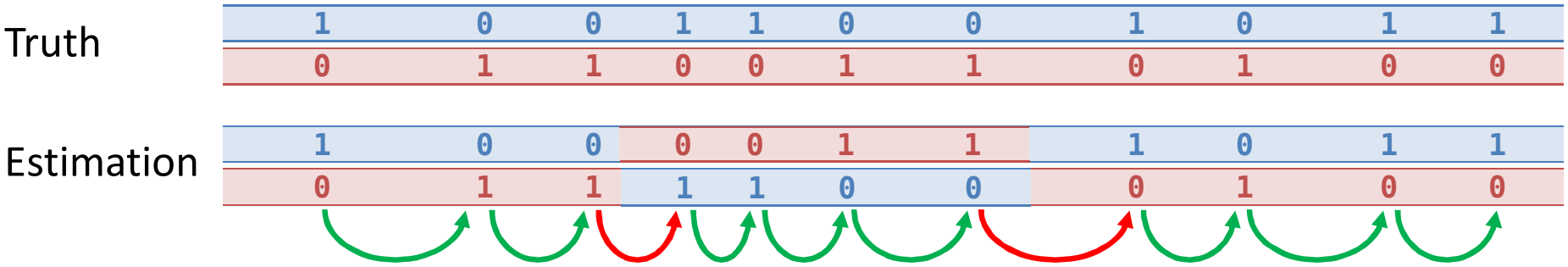
BEAGLE - http://faculty.washington.edu/browning/beagle/beagle.html

SHAPEIT2 - http://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html

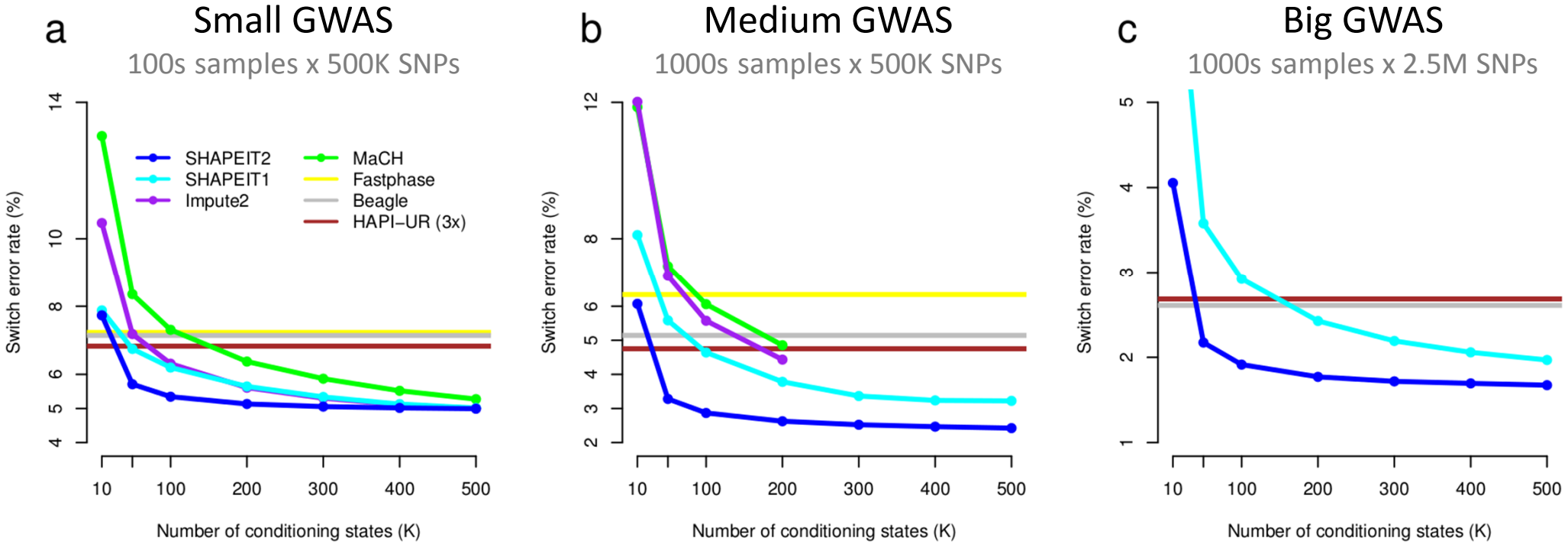
EAGLE - https://data.broadinstitute.org/alkesgroup/Eagle/

SHAPEIT3 - http://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html

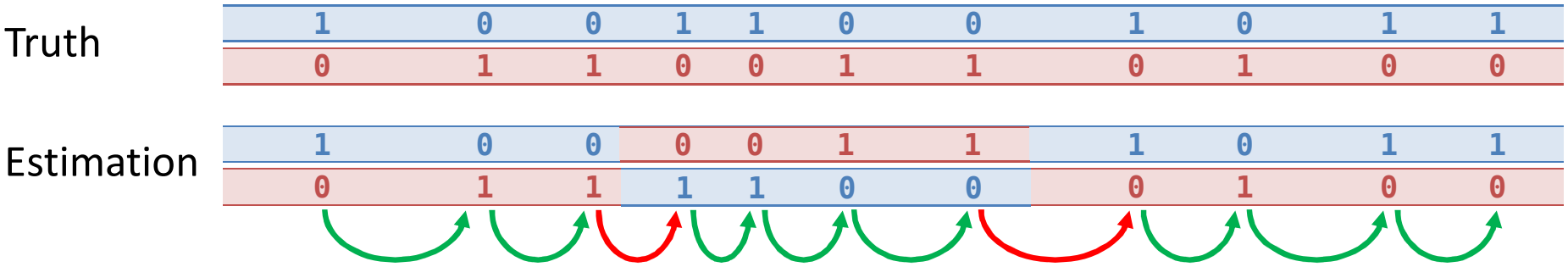
Measuring performance



Switch error rate = 2/10 = 20%



Measuring performance



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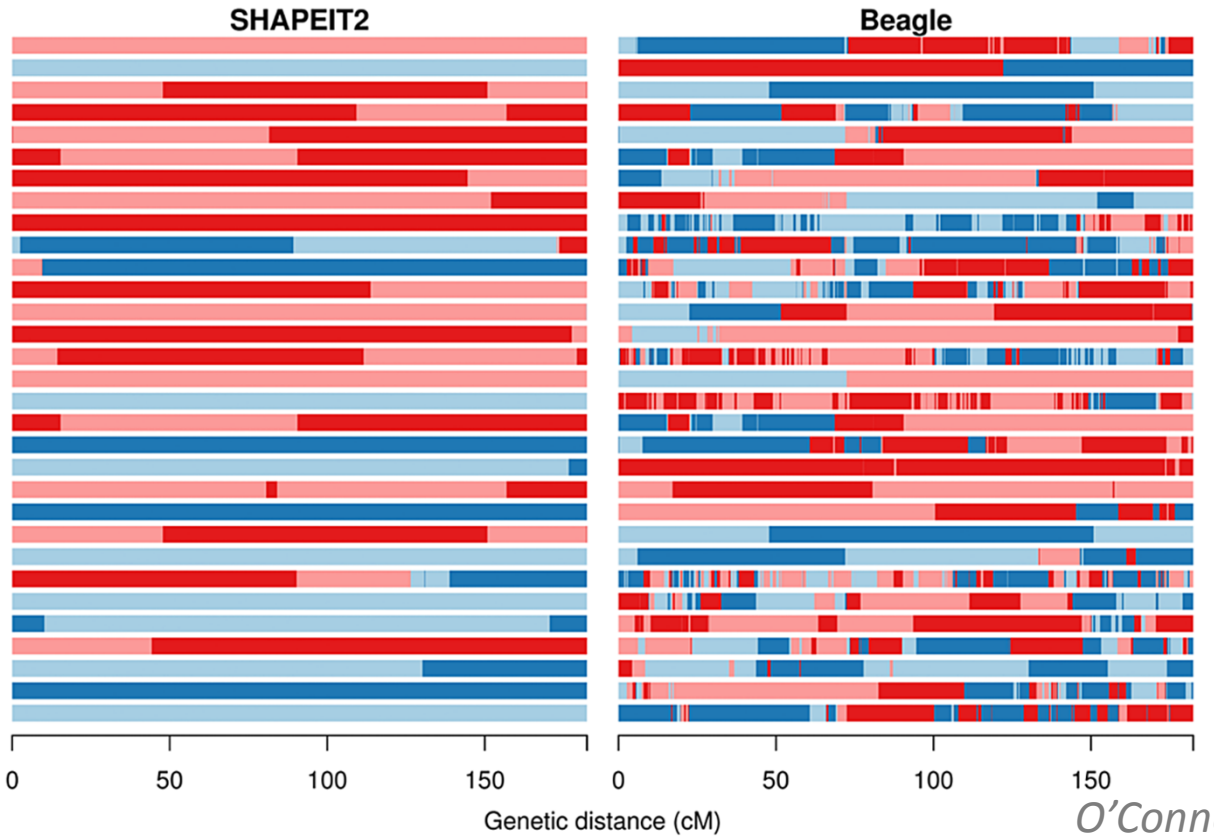
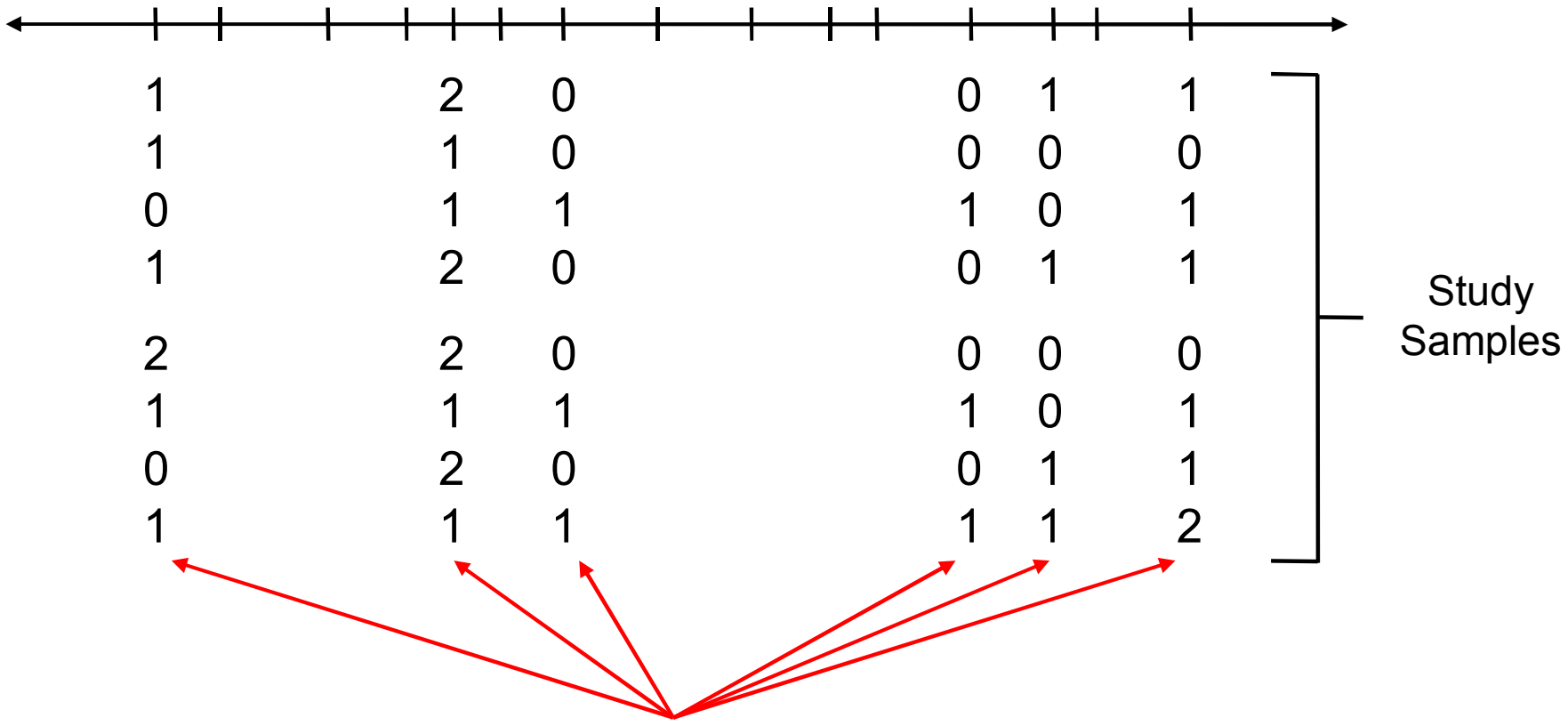


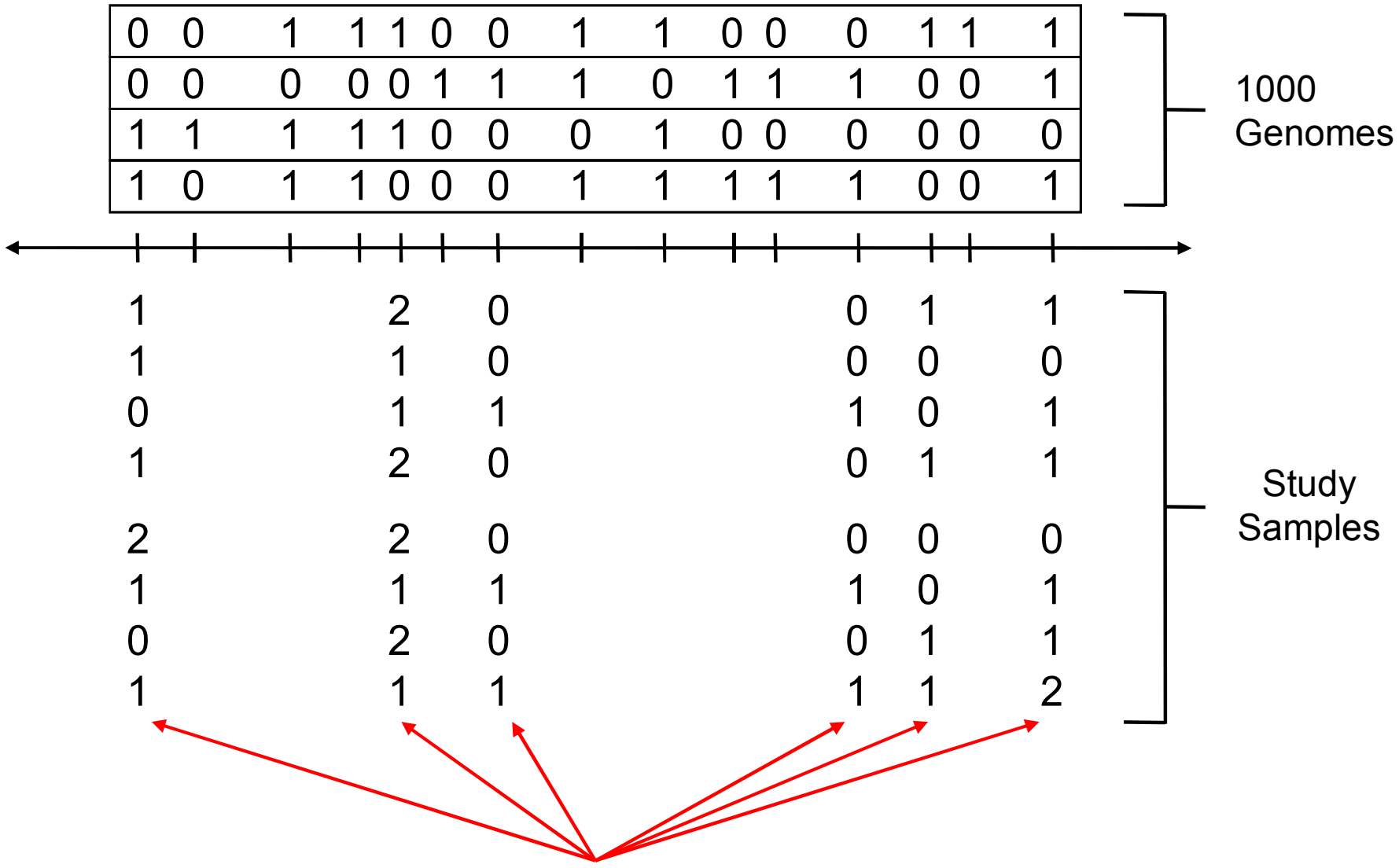
O'Connell et al, Plos Gen. 2014

Limitation of genotyping data



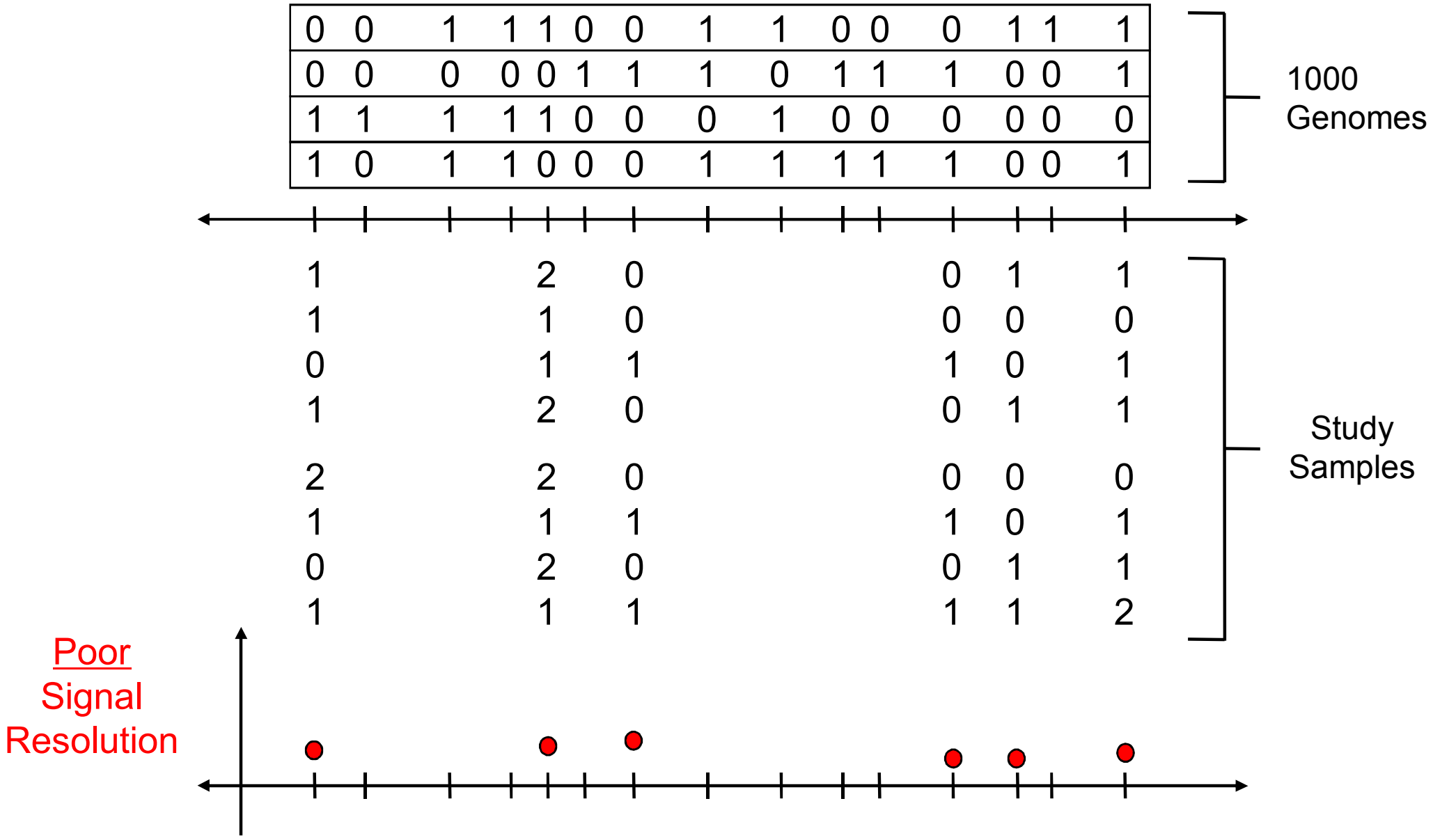
SNPs genotyped in a genomic study: 1 to 5M variants, 100s to 1000s of samples

Limitation of genotyping data

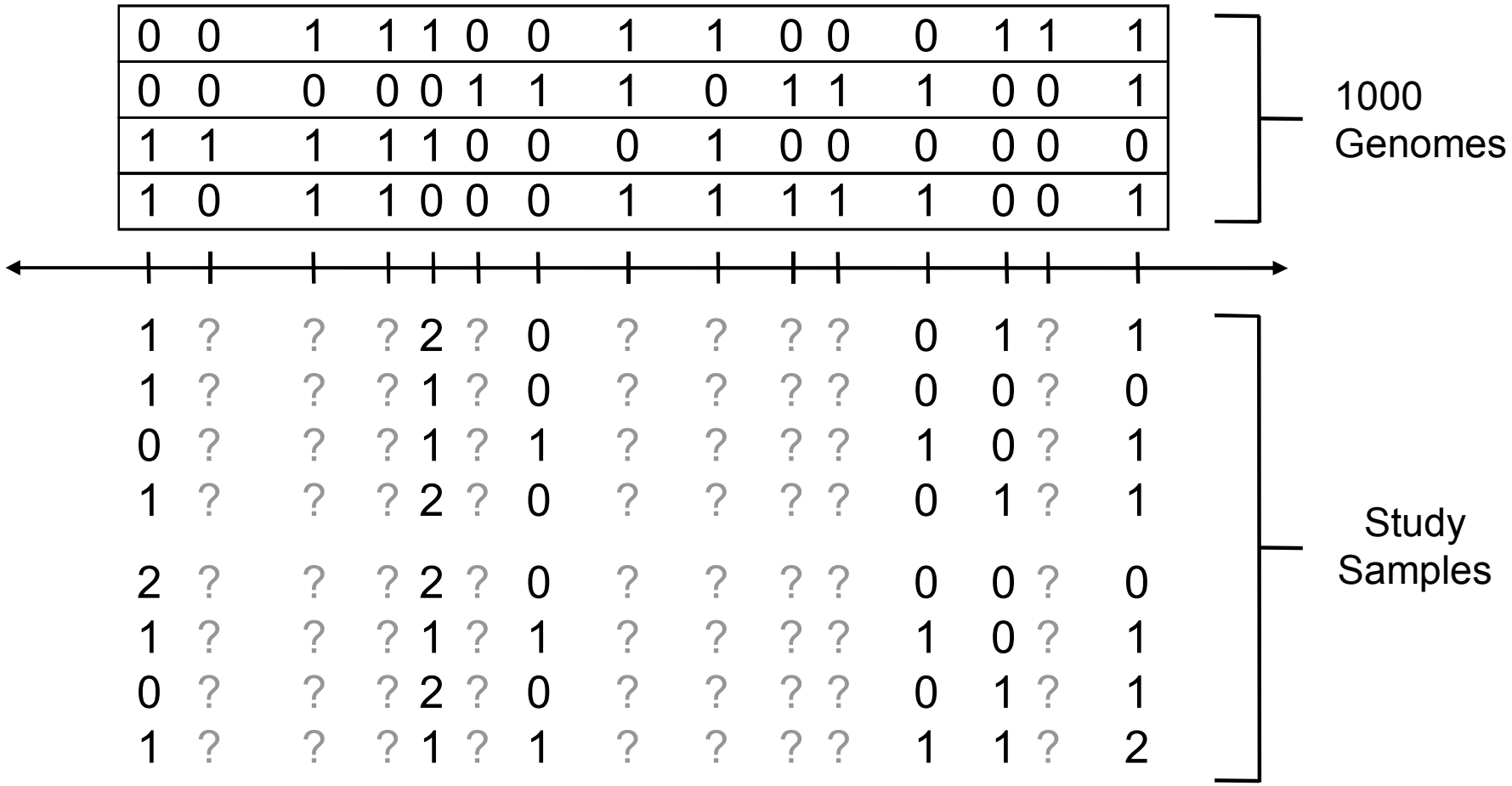


This is far less that what we've got in sequencing based studies

Limitation of genotyping data

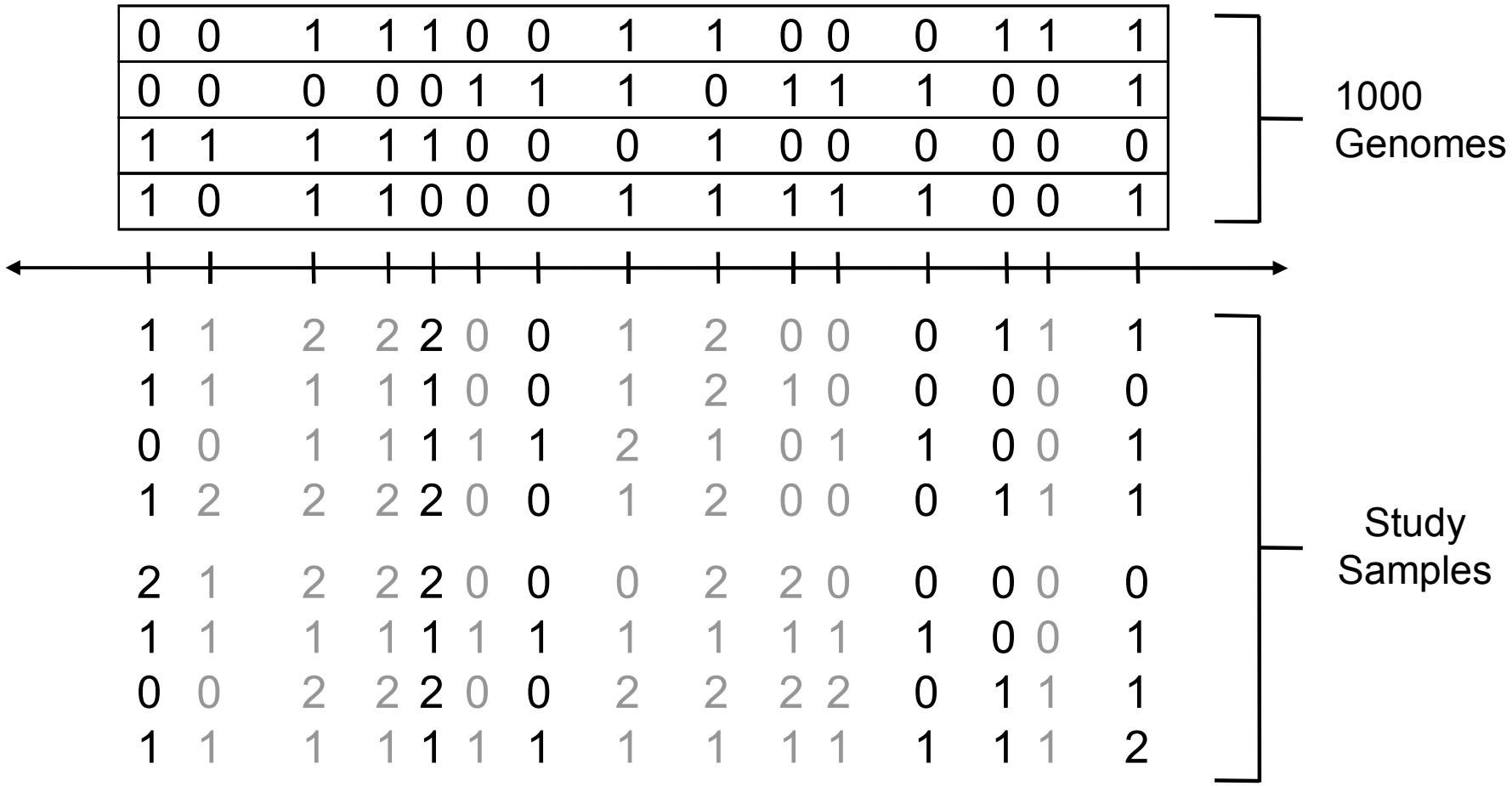


Solution: Imputation



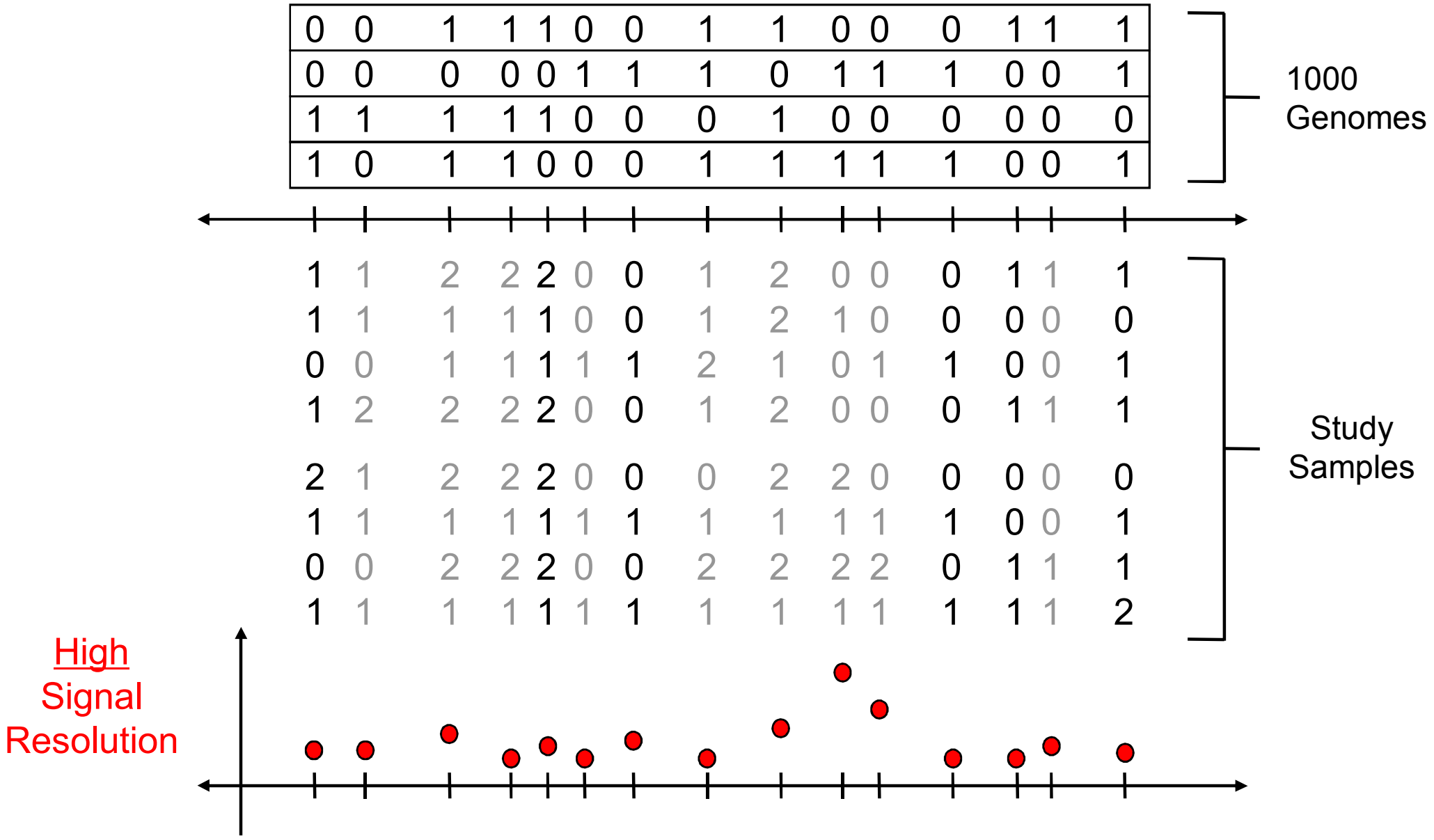
Un-typed variants are treated as missing data.

Solution: Imputation

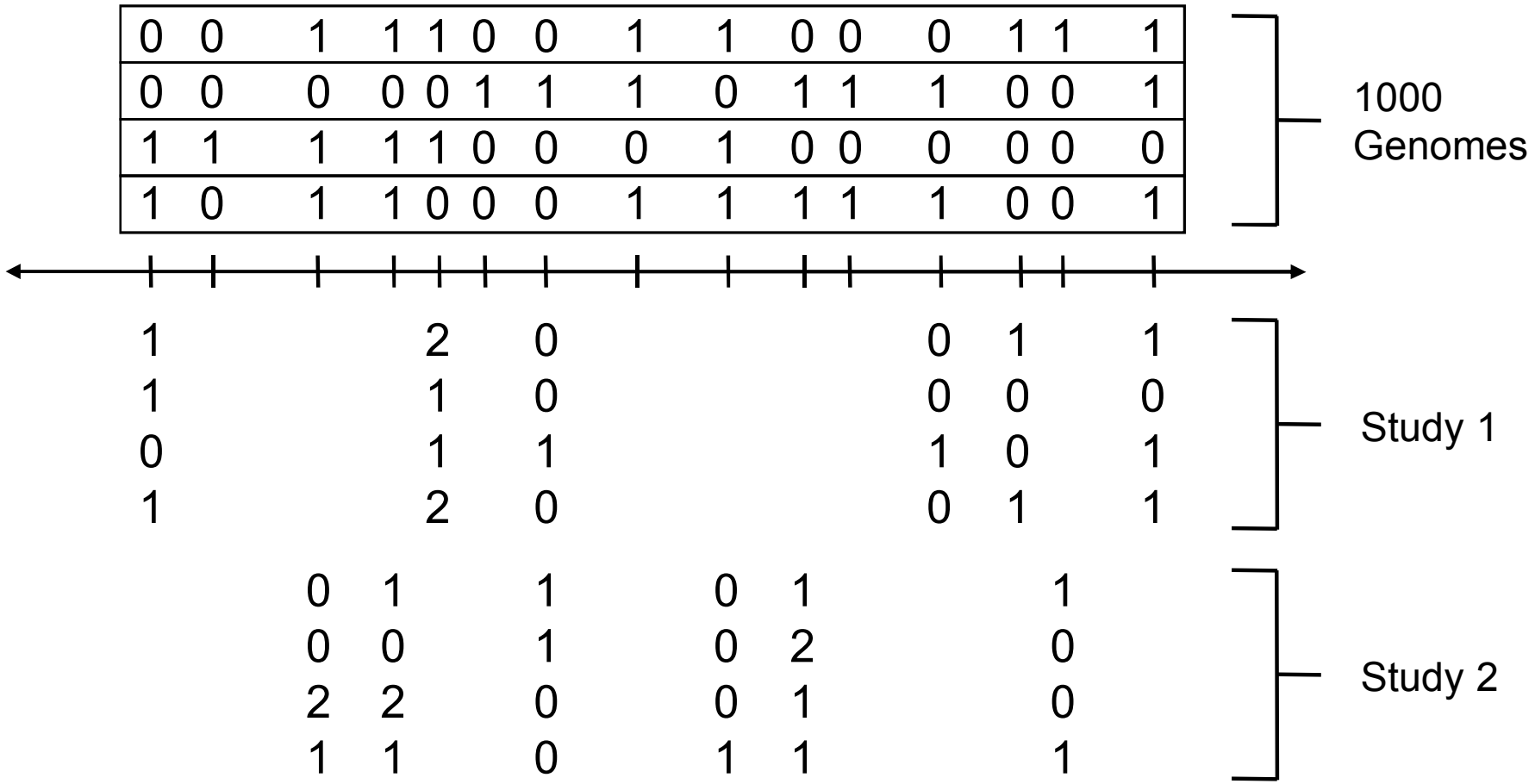


The goal of imputation is to estimate the missing genotypes at these variants

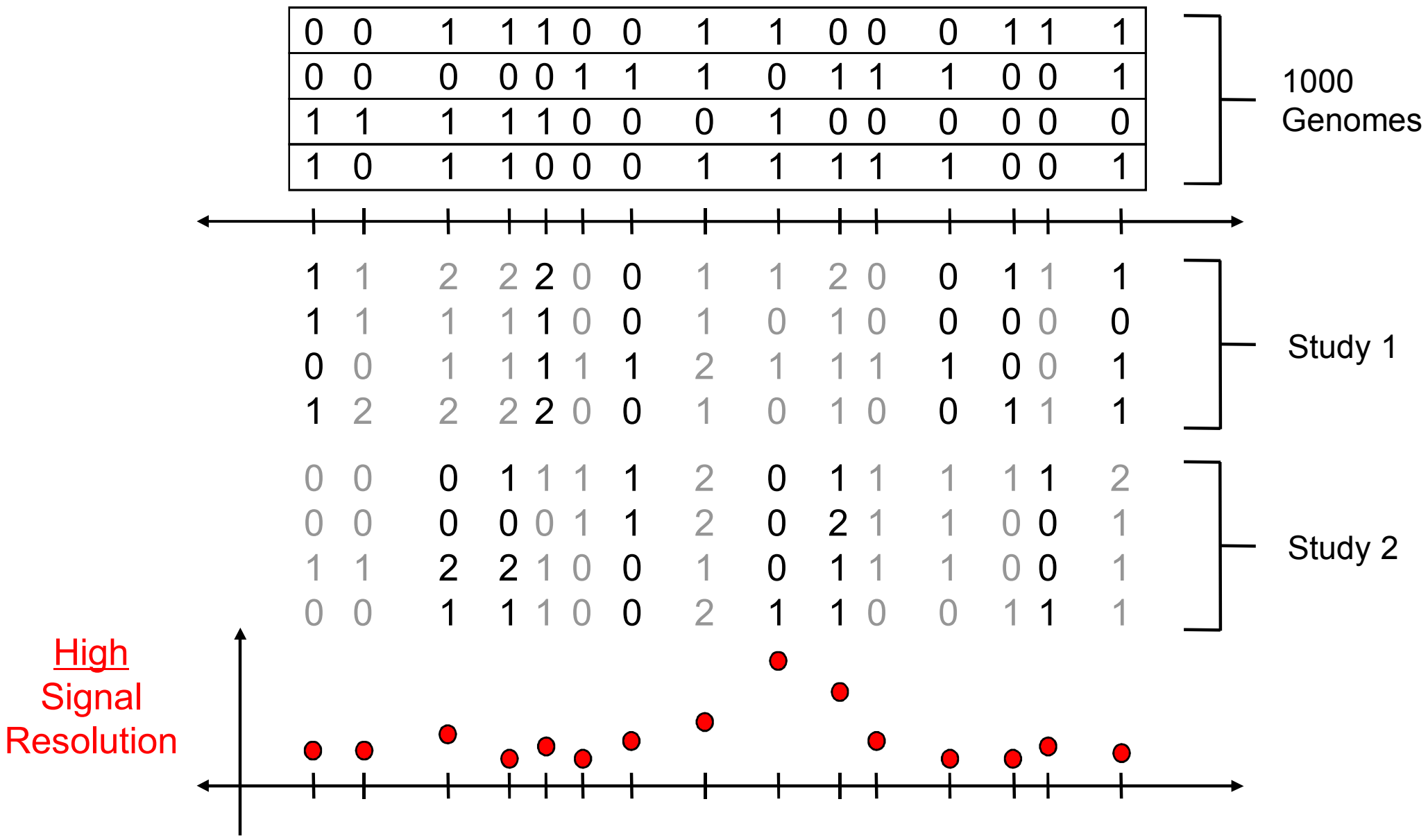
Solution: Imputation



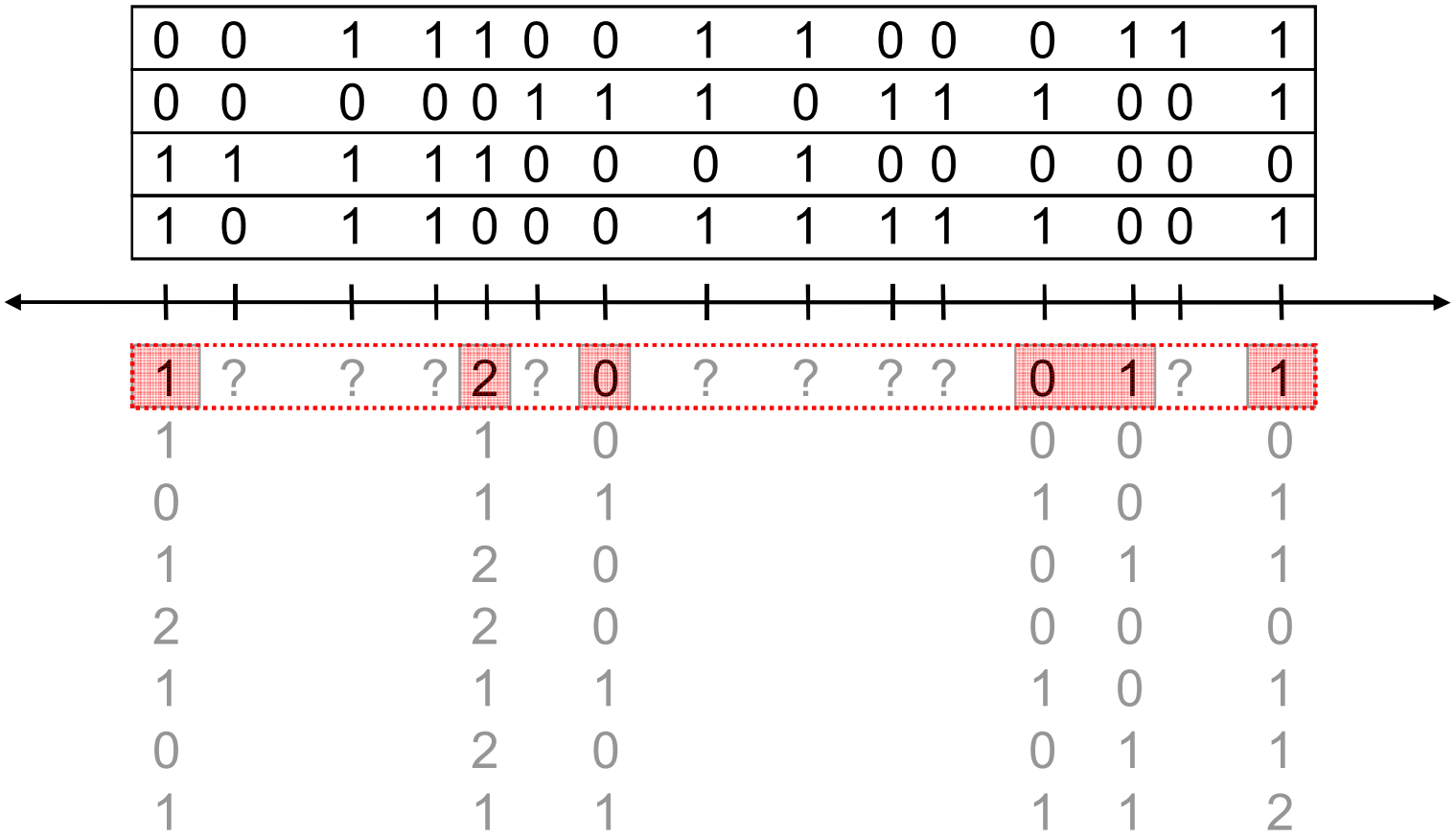
Combining genotype data sets



Combining genotype data sets



How does it work?



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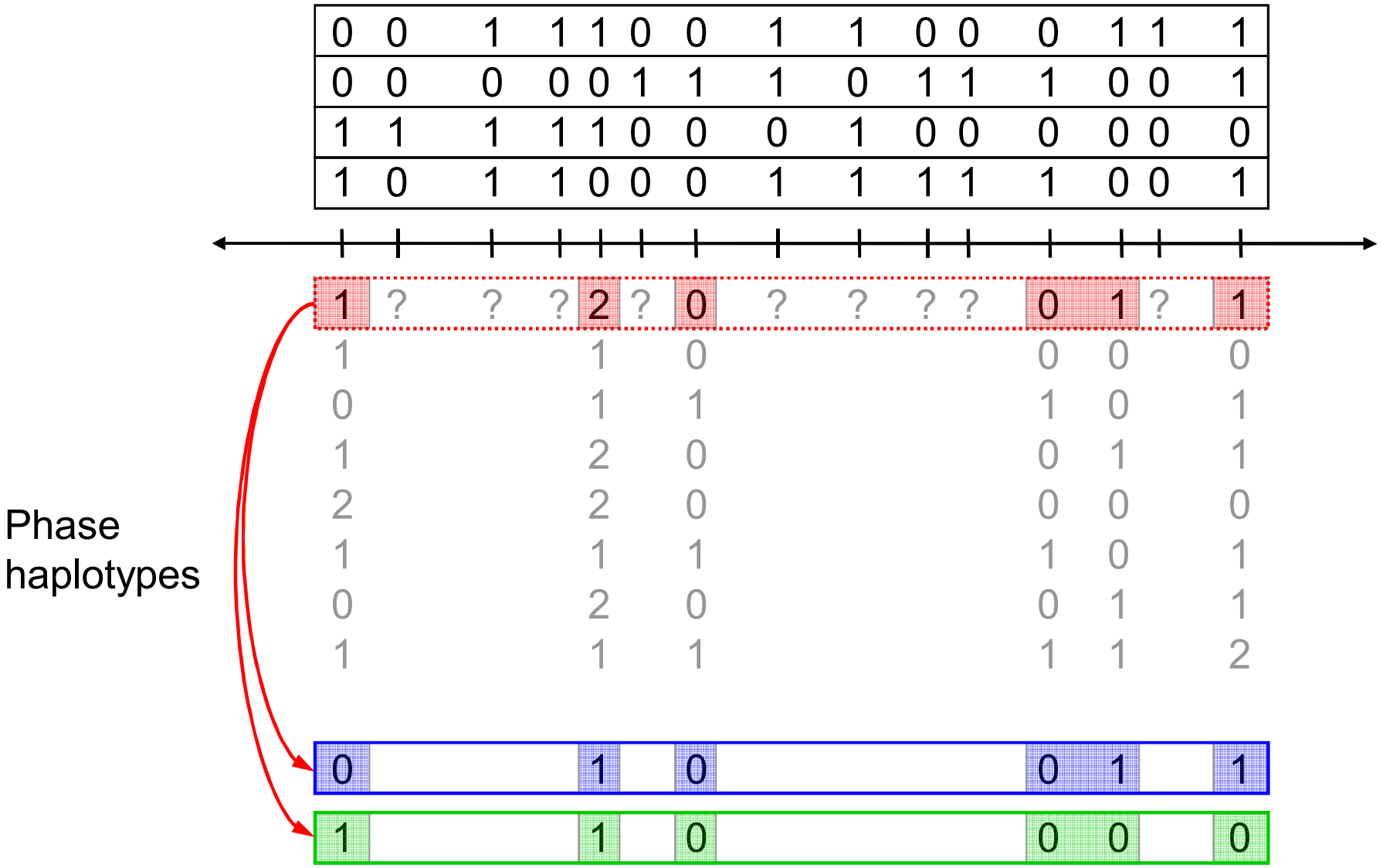

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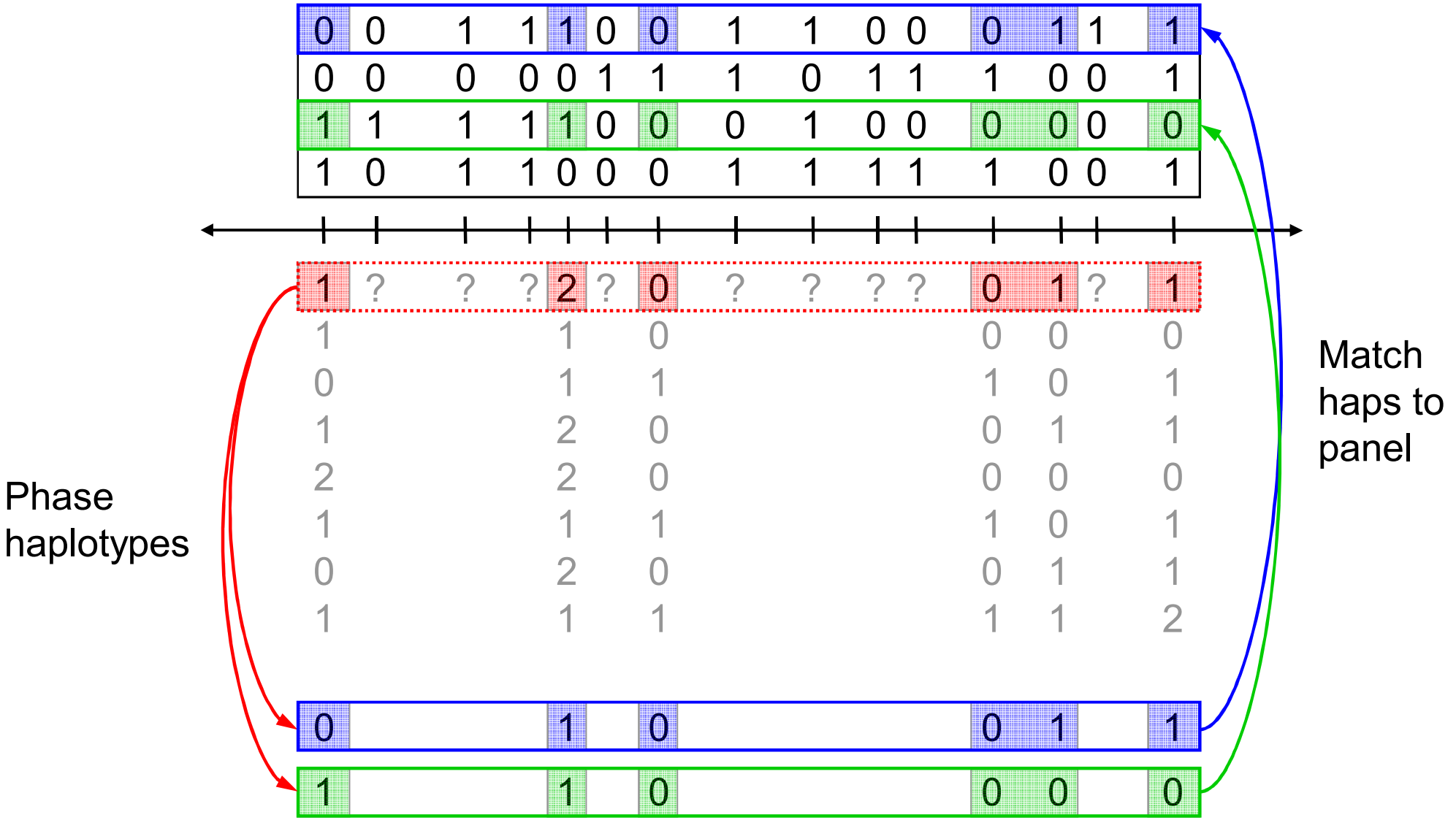
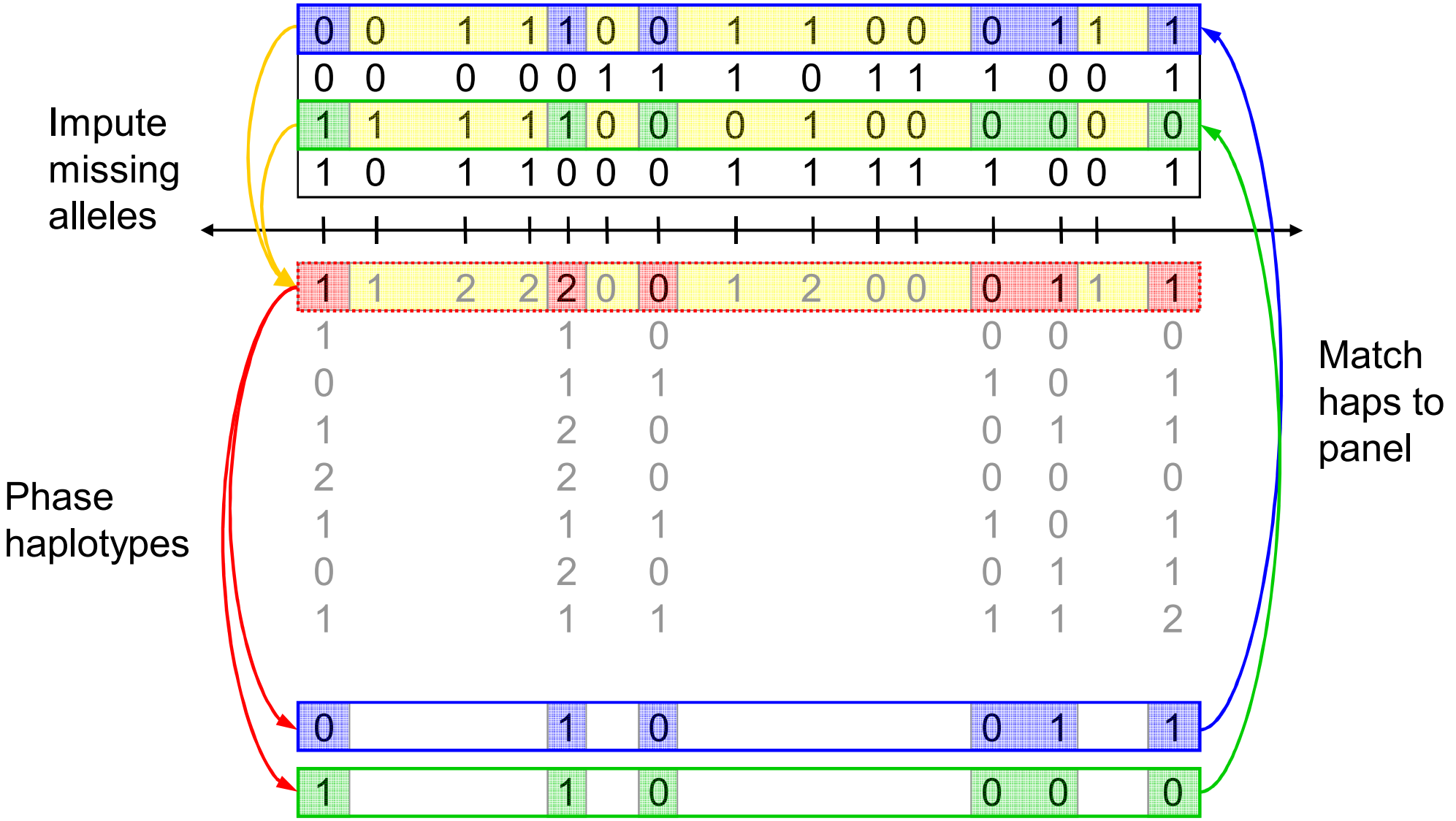


How does it work?



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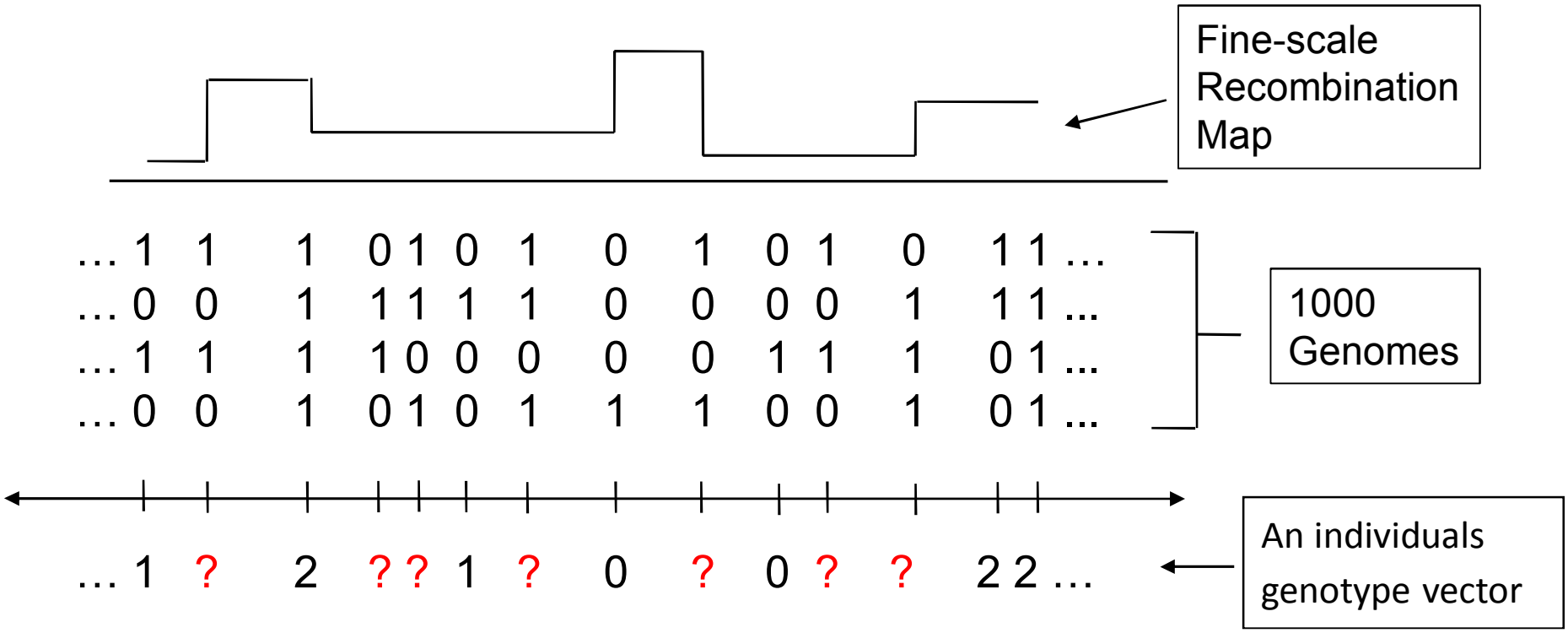

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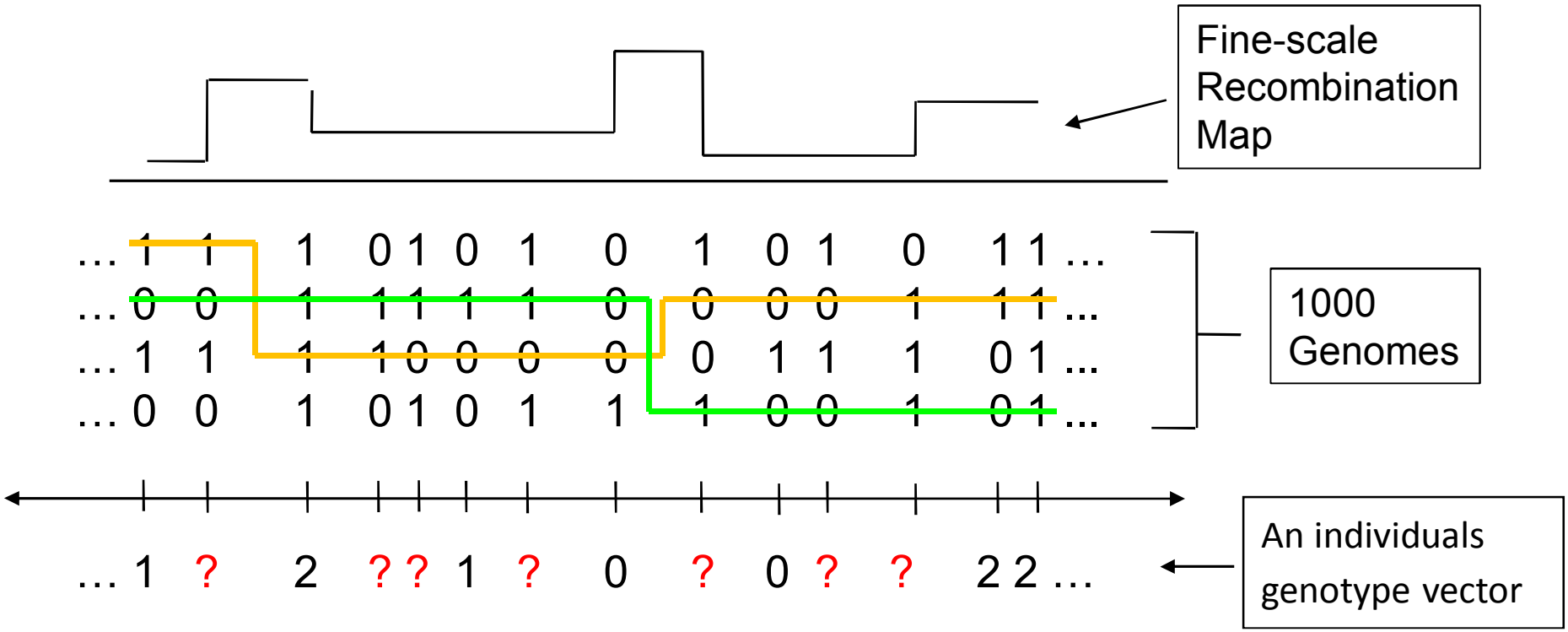
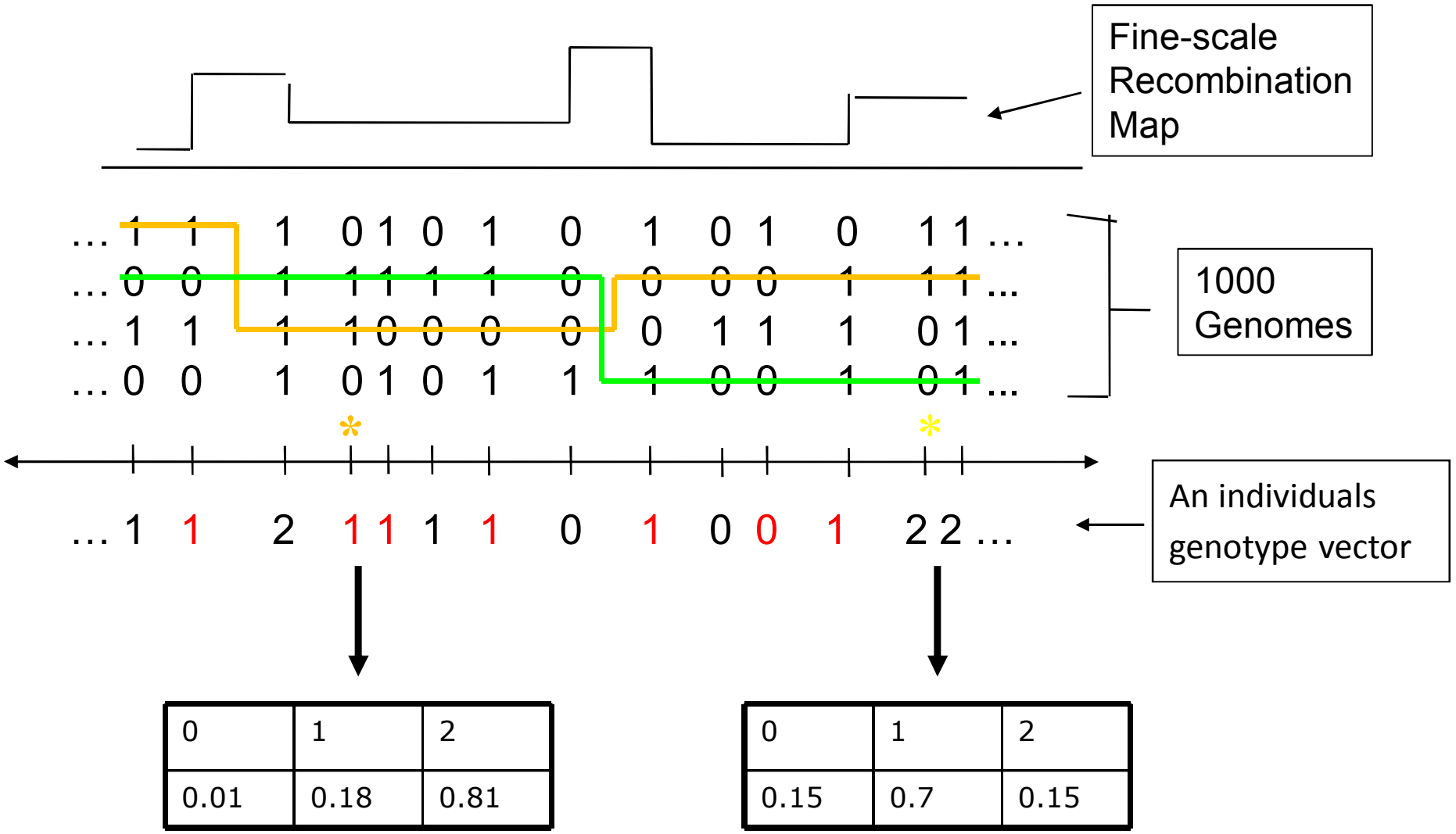


The model says that an individuals genotype is constructed by copying alleles along two paths through the space of haplotypes. The switch rates of the paths are controlled by the recombination map. Mutation events are also allowed.

How does it work?



We produce estimates of genotype uncertainty at both untyped and typed genotypes

Accounting for genotype uncertainty

There are several ways the imputed genotype probabilities can be used.

1. Threshold the probability distribution to give genotype calls
2. Use the expected allele counts

AA	AB	BB
0.01	0.18	0.81

0 x 0.01 + 1 x 0.18 + 2 x 0.81 = **1.8**

3. Average over uncertainty
   - Can be done in both the Frequentist and Bayesian frameworks
   - Very few implementations (SNPtest)

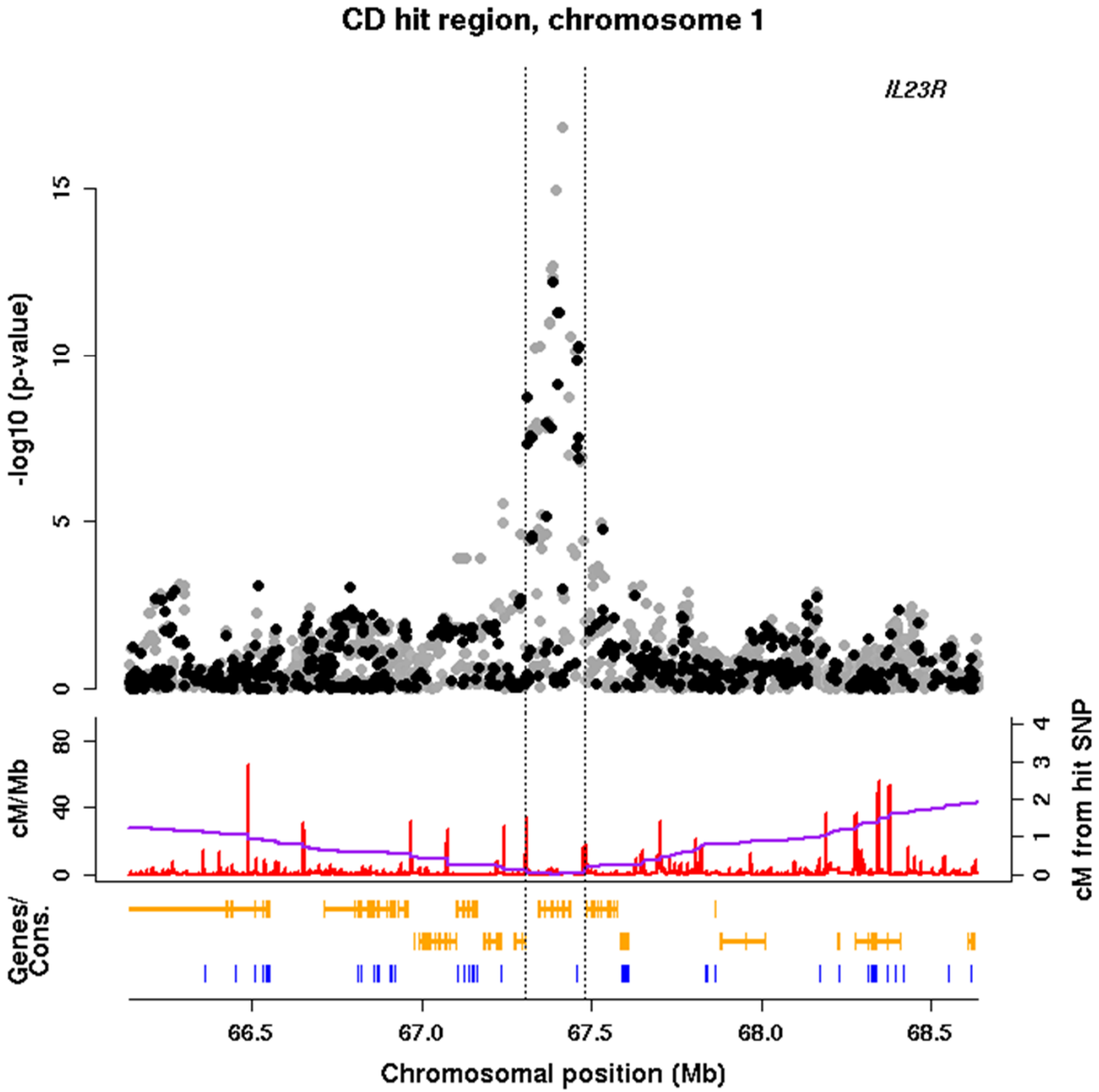


Image from The Wellcome Trust Case Control Consortium (2007) Genomewide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447;661-78. DOI: 10.1038/nature05911.

Popular imputation methods

There are several popular methods for genotype imputation:

IMPUTE2 - https://mathgen.stats.ox.ac.uk/impute/impute_v2.html

MINIMACH - http://genome.sph.umich.edu/wiki/Minimac

BEAGLE - http://faculty.washington.edu/browning/beagle/beagle.html

Note1: Genome build

The Human Reference Sequence is updated periodically, each version is referred to a ‘genome build’.

Positions of SNPs can change between builds.

Almost all imputation programs align SNPs between the reference panels and the GWAS datasets using the position of SNPs.

So it is very important that the genotypes of your GWAS are mapped to the same genome build as the reference panel you are using.

Currently, all the most commonly used reference panels use build 37.

Note2: Strand issue

Genotypes from SNP chips are called relative to either the + or – strand of the human reference genome.

Maternal chromosome	ACGTAGCTCTCTGATCGAT	+ strand
	TGCATCGAGAGACTAGCTA	- strand
Paternal chromosome	ACATAGCTCTCTGAACGAT	+ strand
	TGTATCGAGAGACTTGCTA	- strand

+ strand GA
- strand CT

+ strand TA
- strand AT

Haplotype reference panels have alleles aligned to the + strand.
Genotype chips can have a mixture of genotypes from + and - strand
This needs to be fixed prior to imputation.
The strand info about all chips can be found here

http://www.well.ox.ac.uk/~wrayner/strand/

Note3: Information metric

Once imputation has been carried out, it is a good idea to try and measure how well imputed the genotypes are at each SNP. IMPUTE produces an information measure for each SNP in the range [0,1]:

1 means there is no uncertainty at all in any of the imputed genotypes.

0 means there is complete uncertainty for all of the genotypes.

Approximately, an information measure of α means the imputed genotypes contain as much information as αN genotypes that are completely certain (where N is the total number of genotypes).

In recent published studies (especially those that have used imputation for meta-analysis), variants with info<0.4 have been excluded from the study.

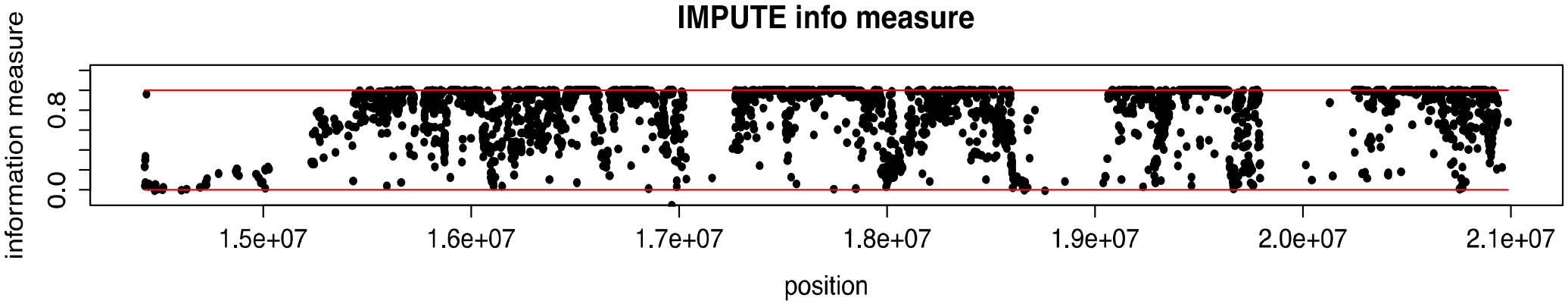


Image from J. Marchini and B. Howie (2010) Genotype imputation for genome-wide association studies. Nature Reviews Genetics doi:10.1038/nrg2796

1000 Genomes project

nature International weekly journal of science 2015

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Archive > Volume 526 > Issue 7571 > Articles > Article

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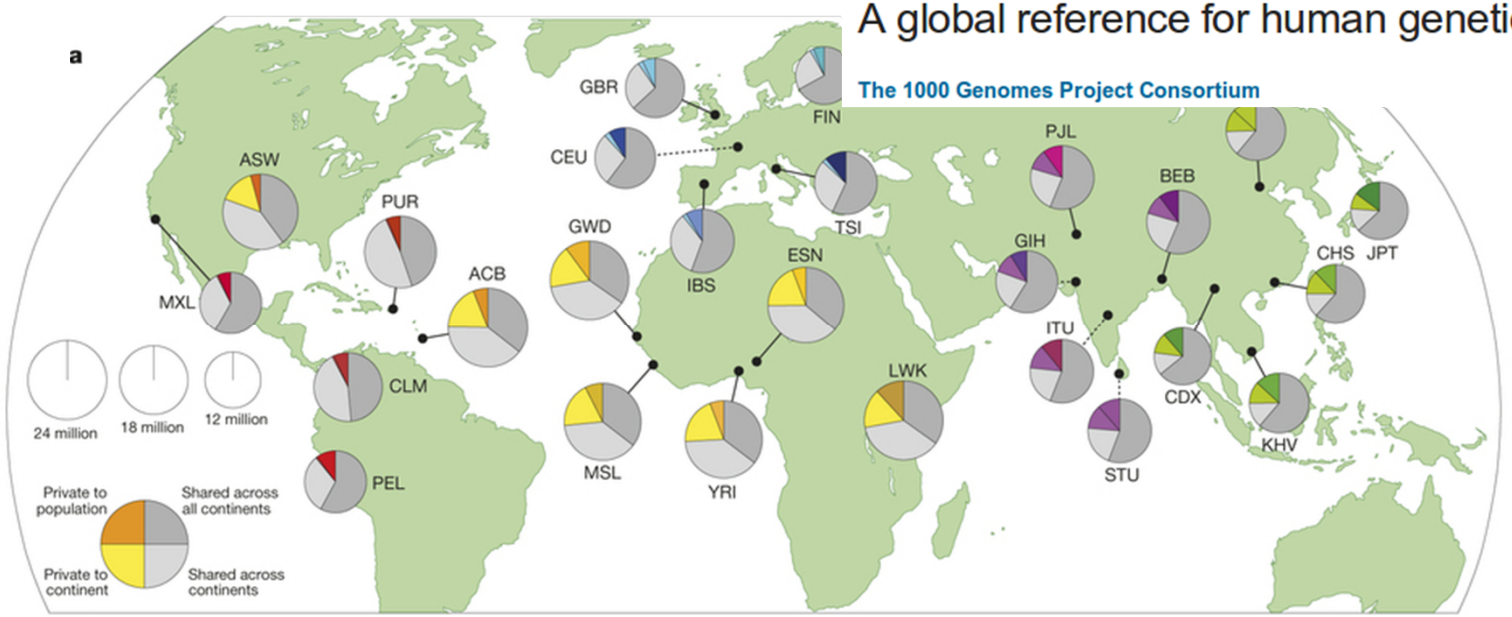
日本語要約

A global reference for human genetic variation

The 1000 Genomes Project Consortium



Population	Number of samples
African	661
Americas	347
Est Asian	504
European	503
South Asian	489
Total	**2,504**

Type of variants	Number of variants
Bi-allelic SNPs	81,102,777
Bi-allelic Indels	3,196,364
Multi-allelic SNPs	274,425
Multi-allelic Indels	169,601
Structural variants	58,713
Total	**84,801,880**

Why did we phase 1000 Genomes?

1. To get fully resolved haplotypes, obviously,

ATGC**A**TCGAGCT**G**ACTGAGG**A**CTGGACTAG**C**GATCAG
ATGC**T**TCGAGCT**T**ACTGAGG**C**CTGGACTAG**G**GATCAG

ATGC**A**TCGAGCT**G**ACTGAGG**A**CTGGACTAG**C**GATCAG
ATGC**A**TCGAGCT**T**ACTGAGG**A**CTGGACTAG**G**GATCAG

Why did we phase 1000 Genomes?

1. To get fully resolved haplotypes, obviously,
2. But also to refine the genotype calls.

How does it work?

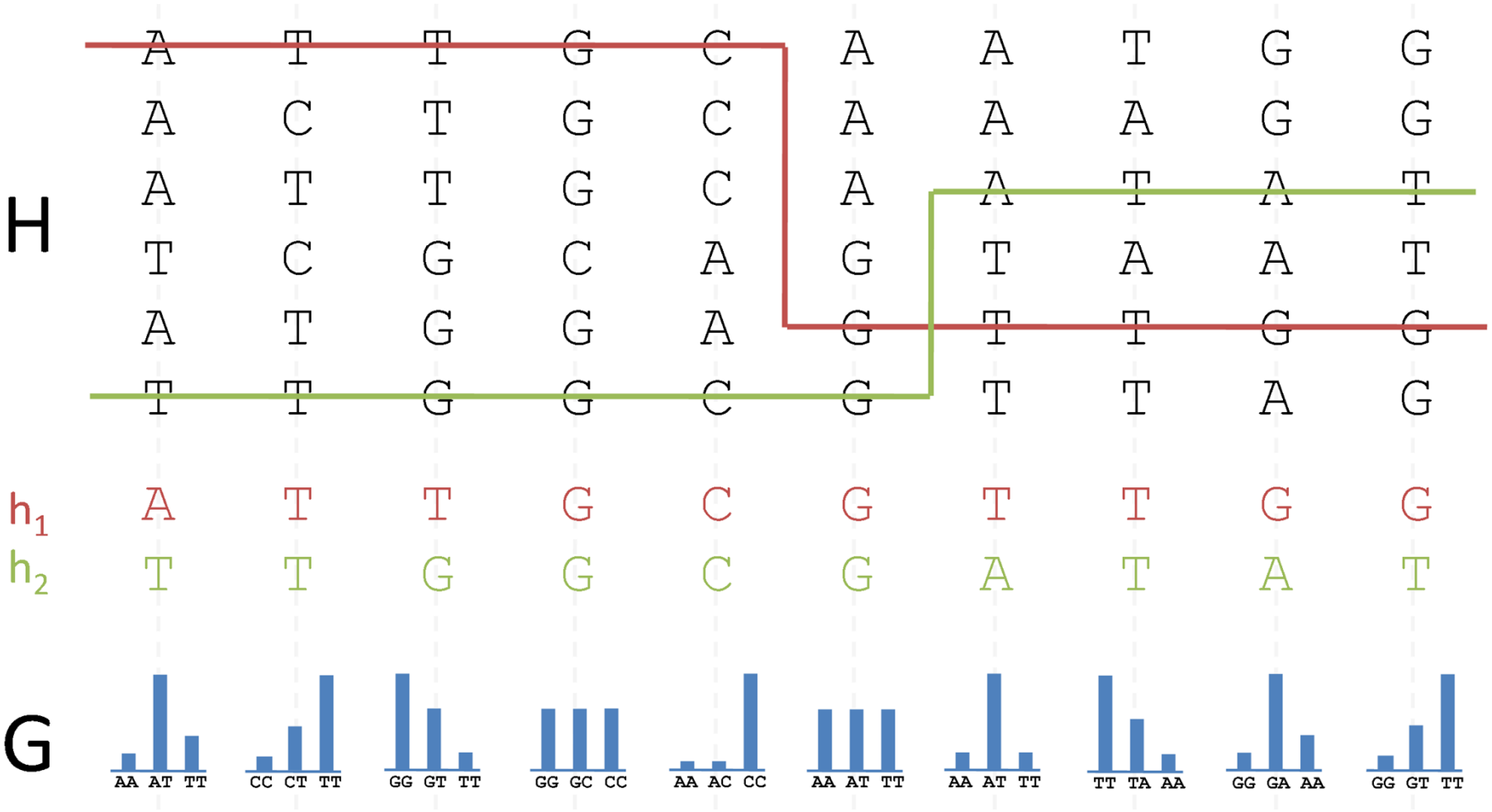
H

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

G

Uncertainty in genotypes is modeled with genotype likelihoods (GLs)

How does it work?



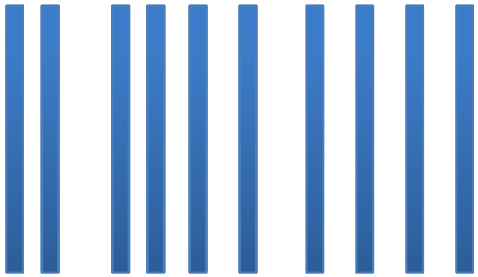
Estimating haplotypes produces genotype calls.

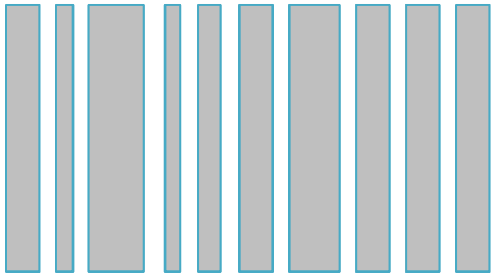
Measuring imputation performance

Complete Genomics Genotypes

Measuring imputation performance

Genotypes on Illumina 1M



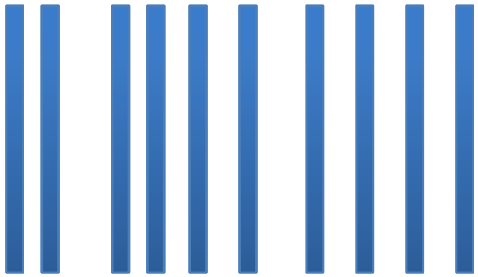


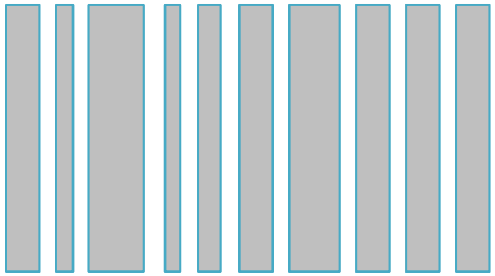
Genotypes NOT on Illumina 1M

Measuring imputation performance

1000 Genomes haplotypes

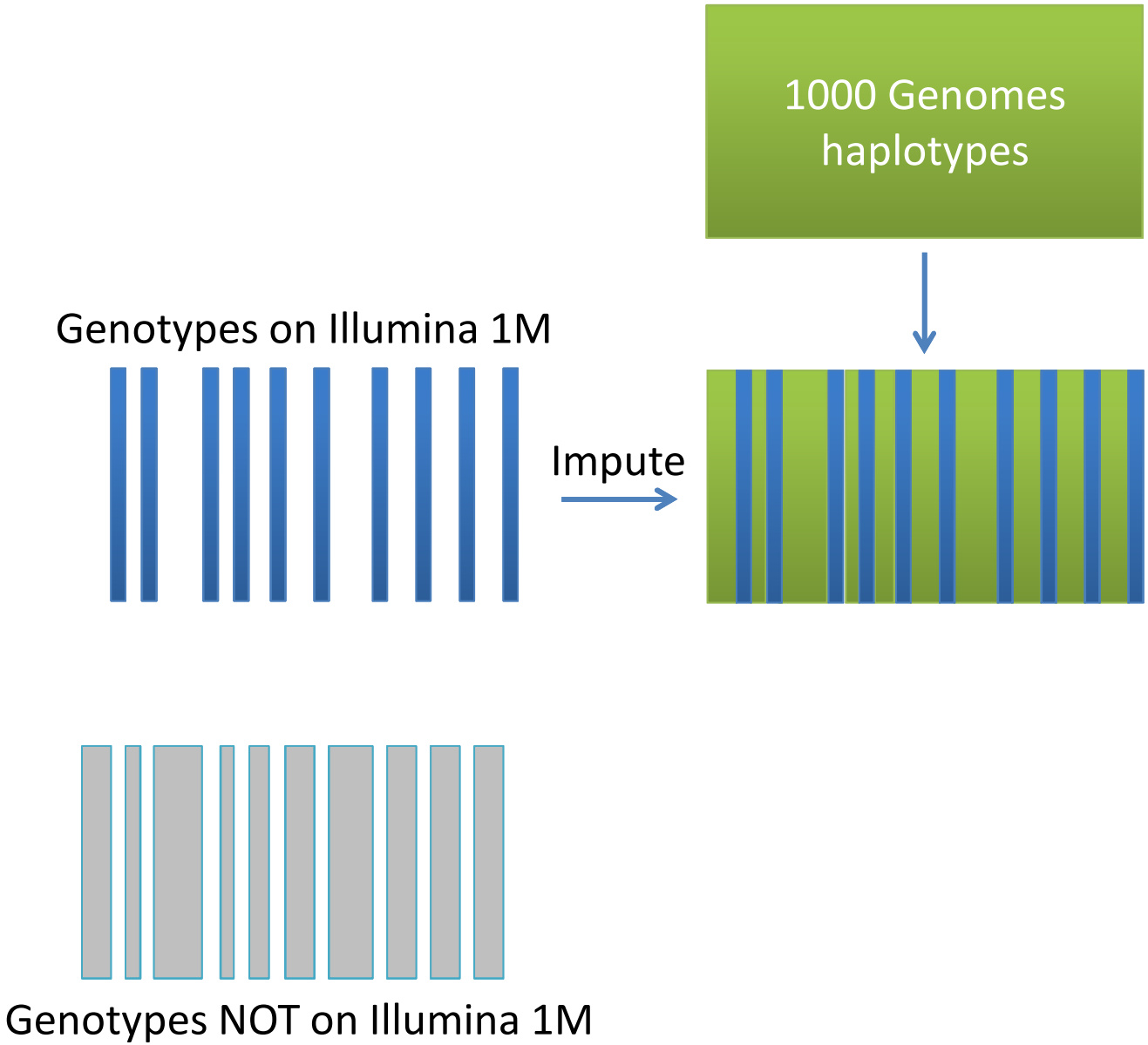
Genotypes on Illumina 1M



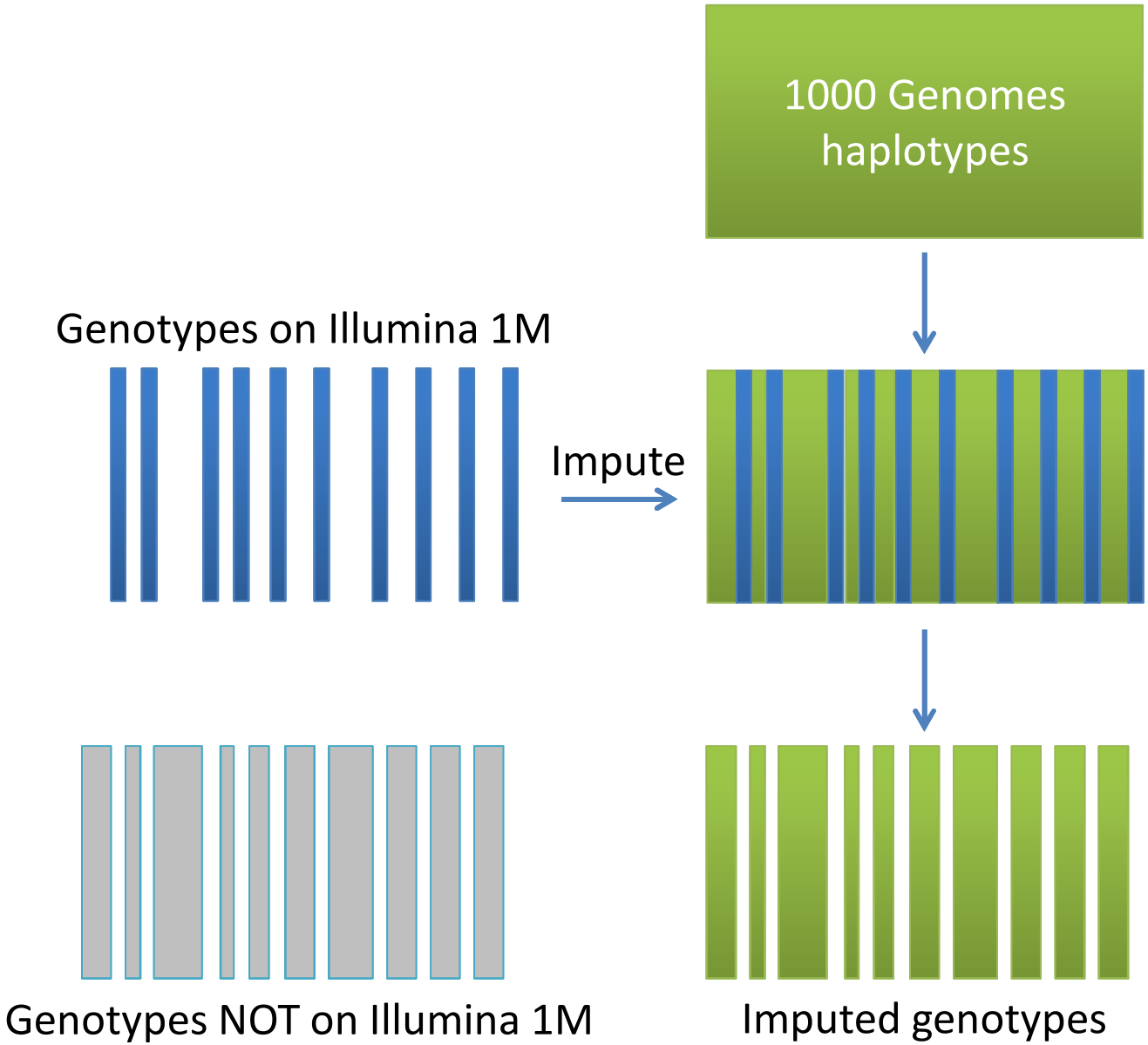


Genotypes NOT on Illumina 1M

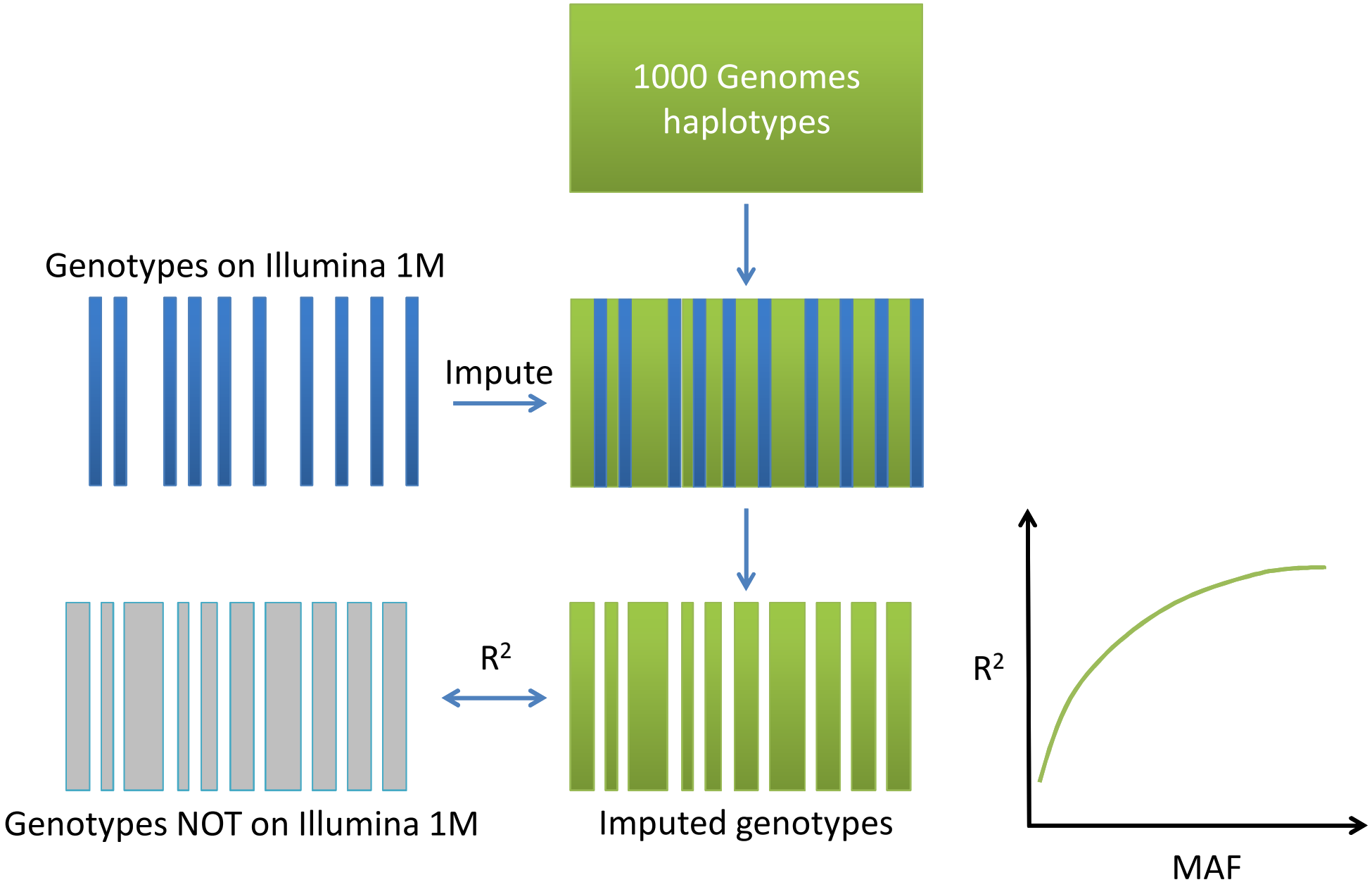
Measuring imputation performance

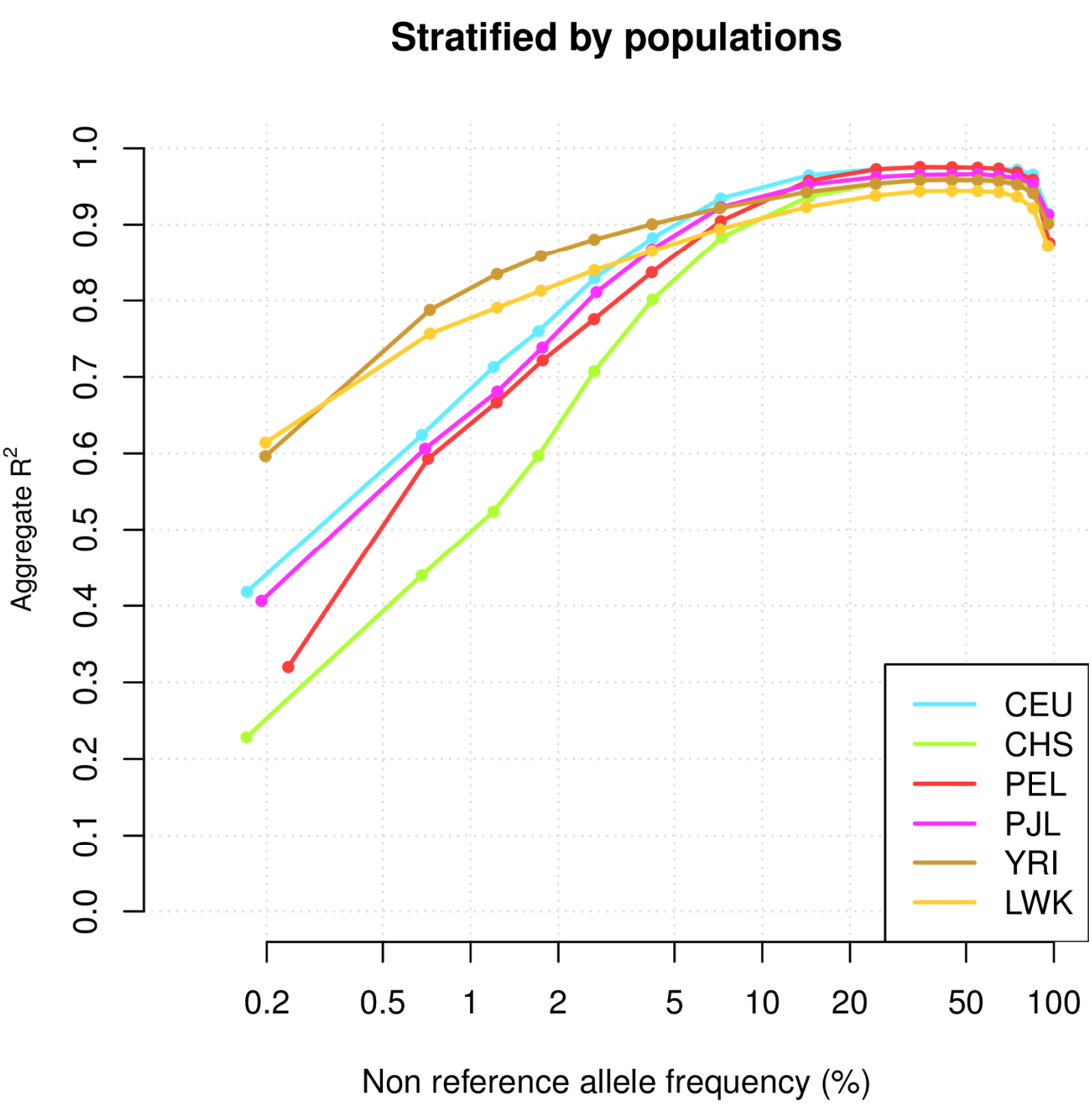


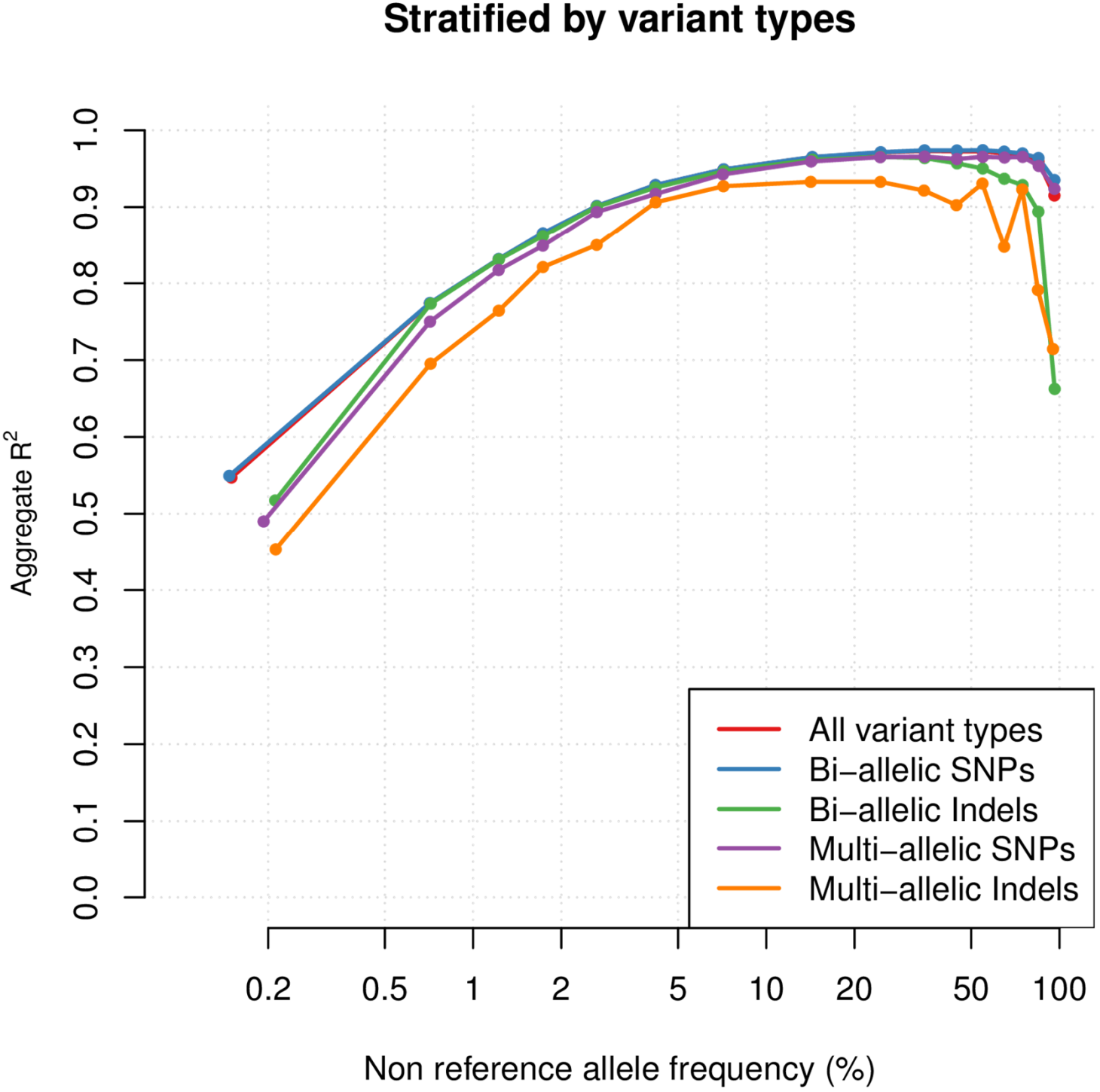
Measuring imputation performance

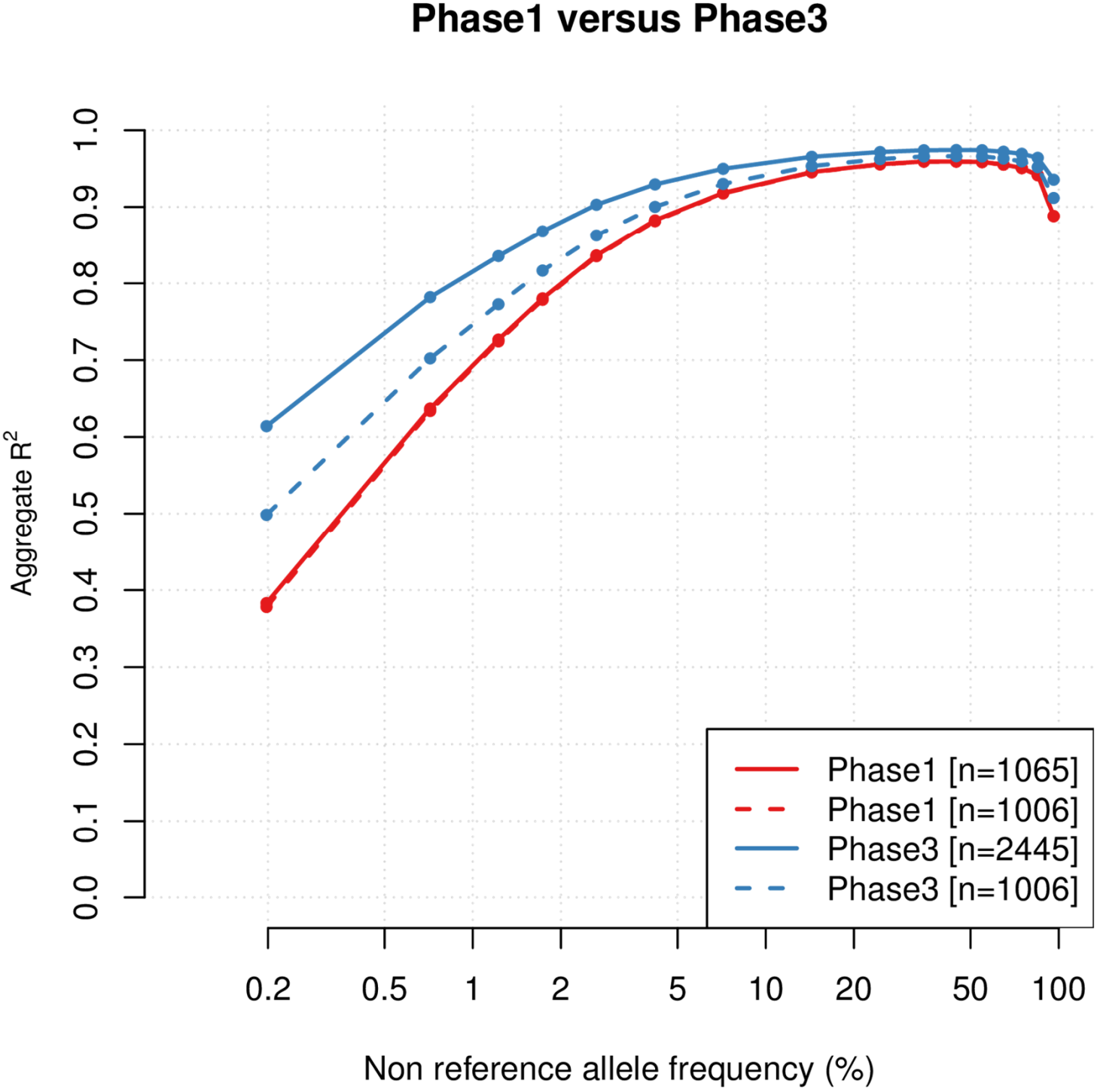


Measuring imputation performance




Stratified by populations
Aggregate R^2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.2
0.5
1
2
5
10
20
50
100
Non reference allele frequency (%)
CEU
CHS
PEL
PJL
YRI
LWK


Stratified by variant types
Aggregate R^2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.2
0.5
1
2
5
10
20
50
100
Non reference allele frequency (%)
All variant types
Bi−allelic SNPs
Bi−allelic Indels
Multi−allelic SNPs
Multi−allelic Indels


Phase1 versus Phase3
Aggregate R2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.2
0.5
1
2
5
10
20
50
100
Non reference allele frequency (%)
Phase1 [n=1065]
Phase1 [n=1006]
Phase3 [n=2445]
Phase3 [n=1006]

Haplotype reference panels

Reference	Year	# haplotypes	# populations	# variants
HAPMAP 2	2006	420	3	2,139,483
HAPMAP 3	2009	2,368	11	1,440,616
1000GP Pilot	2010	358	3	14,894,361
1000GP 2010	2010	1,258	14	22,242,654
1000GP Interim	2011	2,186	14	38,558,931
1000GP Phase 1	2012	2,186	14	38,219,282

Reference	Year	# haplotypes	populations	# variants
1000GP Final	2014	~5,000	25	~80,000,000
UK10K	2013/14	~8,000	UK	~50,000,000
GoT2D	2013/14	~5,600	European	~27,000,000
GoNL	2013/14	1,000	NL	~20,000,000

Q Which reference panel to use for imputation into GWAS?

The **H**aplotype **R**eference **C**onsortium

http://www.haplotype-reference-consortium.org/

- The HRC data will NOT be publically available, as HapMap and 1000GP haplotypes are, due to consent issues.
- Currently 2 imputation servers exist that allow users to upload genotypes from their GWAS samples, and have imputation carried out remotely and efficiently
  - https://imputation.sanger.ac.uk/
  - https://imputationserver.sph.umich.edu
- A phasing server for phasing high coverage sequenced samples is available at:
  - https://phasingserver.stats.ox.ac.uk/

McCarthy et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat. Gen. 2016

The **H**aplotype **R**eference **C**onsortium

Dataset	Samples	Coverage
IBD	4514	2-4x
UK10K	3781	6.5x
Sardinia	3514	4x
GoT2D	2874	4x + Exome
1000GP Phase 3	2535	4x + Exome
BRIDGES	2489	6-8x
AMD	2099	4x
Finland	1941	4-6x
MCTFR	1339	10x
HUNT	1024	4x
GECCO	954	4-6x
Project MinE	943	45x
GPC	767	30x
GoNL	748	12x
inCHIANTI	680	7x
Orkney	399	4x
Neptune	253	4x
FVG	250	4-10x
MANOLIS	249	4x
Val Borbera	225	6x
	32,488	

Goal : create a European haplotype map of over 50,000+ haplotypes by combining together many low-coverage sequencing studies.

Release 1

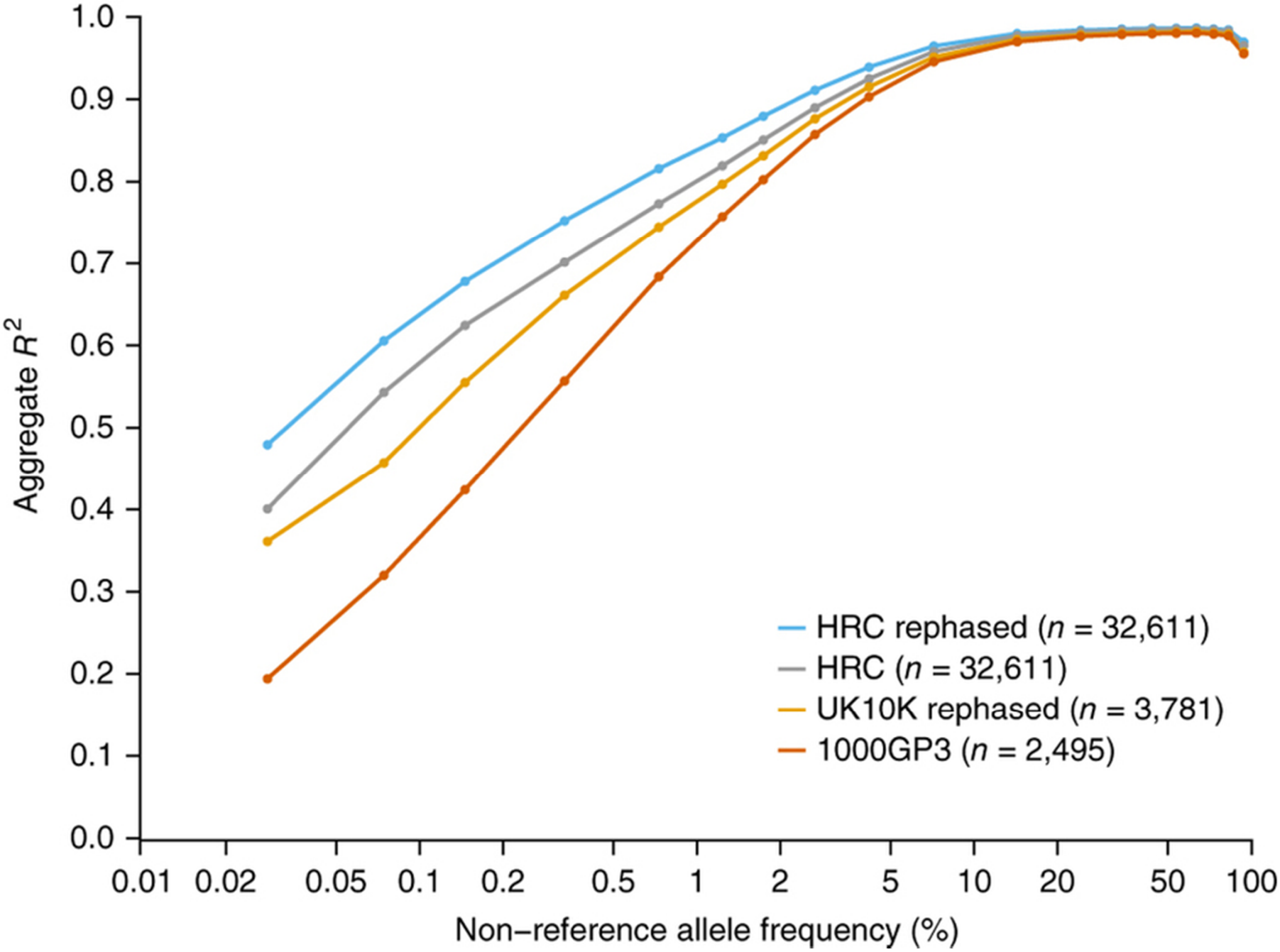
64,976 haplotypes

39,235,157 SNPs

estimated MAC >= 5

McCarthy et al. (2016) A reference panel of 64,976 haplotypes for genotype imputation. Nature Genetics 10.1038/ng.3643

The **H**aplotype **R**eference **C**onsortium



The afternoon practical

- The goal of the practical of this afternoon is to impute the data we QCed this morning
- We will use multiple approaches for genotype imputation
- Look at ***/embo/data/olivier***, everything you need is there.