Whole Genome Sequencing Studies

Goncalo Abecasis

University of Michigan School of Public Health

Shotgun Sequence Data

TAGCTGATAGCTAG**A**TAGCTGATGAGCCCGAT

ATAGCTAG**A**TAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCC

AGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

A/C

Predicted Genotype

Shotgun Sequence Data

Sequence Reads

★

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A, read mapped)= 1.0

P(reads|A/C, read mapped)= 1.0

P(reads|C/C, read mapped)= 1.0

Possible Genotypes

Shotgun Sequence Data

★

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A, read mapped)= P(C observed|A/A, read mapped)

P(reads|A/C, read mapped)= P(C observed|A/C, read mapped)

P(reads|C/C, read mapped)= P(C observed|C/C, read mapped)

Possible Genotypes

Shotgun Sequence Data

★

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A, read mapped)= 0.01

P(reads|A/C, read mapped)= 0.50

P(reads|C/C, read mapped)= 0.99

Possible Genotypes

Shotgun Sequence Data

★

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A, read mapped)= 0.0001

P(reads|A/C , read mapped)= 0.25

P(reads|C/C , read mapped)= 0.98

Possible Genotypes

Shotgun Sequence Data

★

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A , read mapped)= 0.000001

P(reads|A/C , read mapped)= 0.125

P(reads|C/C , read mapped)= 0.97

Possible Genotypes

Shotgun Sequence Data

★

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A , read mapped)= 0.00000099

P(reads|A/C , read mapped)= 0.0625

P(reads|C/C , read mapped)= 0.0097

Possible Genotypes

Shotgun Sequence Data

★

TAGCTGATAGCTAG**A**TAGCTGATGAGCCCGAT

ATAGCTAG**A**TAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCC

AGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A , read mapped)= 0.00000098

P(reads|A/C , read mapped)= 0.03125

P(reads|C/C , read mapped)= 0.000097

Possible Genotypes

Shotgun Sequence Data

★

TAGCTGATAGCTAGATAGCTGATGAGCCCGAT

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A, read mapped)= 0.00000098

P(reads|A/C, read mapped)= 0.03125

P(reads|C/C, read mapped)= 0.000097

Combine these likelihoods with a prior incorporating information from other individuals and flanking sites to assign a genotype.

Shotgun Sequence Data

★

TAGCTGATAGCTAG**A**TAGCTGATGAGCCCGAT

ATAGCTAG**A**TAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCC

AGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

$$P(Genotype|reads) = \frac{P(reads|Genotype)Prior(Genotype)}{\sum_G P(reads|G)Prior(G)}$$

Combine these likelihoods with a prior incorporating information from other individuals and flanking sites to assign a genotype.

Ingredients That Go Into Prior

- Most sites don’t vary
  - P(non-reference base) ~ 0.001
- When a site does vary, it is usually heterozygous
  - P(non-reference heterozygote) ~ 0.001 * 2/3
  - P(non-reference homozygote) ~ 0.001 * 1/3
- Mutation model
  - Transitions account for most variants (C↔T or A↔G)
  - Transversions account for minority of variants

From Sequence to Genotype: Individual Based Prior

TAGCTGATAGCTAGATAGCTGATGAGCCCGAT

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A)** = 0.00034	**Posterior(A/A)** = <.001
P(reads|A/C)= 0.03125	**Prior(A/C)** = 0.00066	**Posterior(A/C)** = 0.175
P(reads|C/C)= 0.000097	**Prior(C/C)** = 0.99900	**Posterior(C/C)** = 0.825

Individual Based Prior: Every site has 1/1000 probability of varying.

From Sequence to Genotype: Individual Based Prior

TAGCTGATAGCTAG**A**TAGCTGATGAGCCCGAT

ATAGCTAG**A**TAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCC

AGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A) =** 0.00034	**Posterior(A/A) =** <.001
P(reads|A/C)= 0.03125	**Prior(A/C) =** 0.00066	**Posterior(A/C) =** 0.175
P(reads|C/C)= 0.000097	**Prior(C/C) =** 0.99900	**Posterior(C/C) =** 0.825

Individual Based Prior: Every site has 1/1000 probability of varying.

Sequence Based Genotype Calls

- **Individual Based Prior**
  - Assumes all sites have an equal probability of showing polymorphism
  - Specifically, assumption is that about 1/1000 bases differ from reference
  - If reads where error free and sampling Poisson …
  - … 14x coverage would allow for 99.8% genotype accuracy
  - … 30x coverage of the genome needed to allow for errors and clustering

From Sequence to Genotype: Population Based Prior

TAGCTGATAGCTAG**A**TAGCTGATGAGCCCGAT

ATAGCTAG**A**TAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCC

AGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A)** = 0.04	**Posterior(A/A)** = <.001
P(reads|A/C)= 0.03125	**Prior(A/C)** = 0.32	**Posterior(A/C)** = 0.999
P(reads|C/C)= 0.000097	**Prior(C/C)** = 0.64	**Posterior(C/C)** = <.001

Population Based Prior: Use frequency information from examining others at the same site.

In the example above, we estimated P(A) = 0.20

From Sequence To Genotype: Population Based Prior

TAGCTGATAGCTAGATAGCTGATGAGCCCGAT

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A)** = 0.04	**Posterior(A/A)** = <.001
P(reads|A/C)= 0.03125	**Prior(A/C)** = 0.32	**Posterior(A/C)** = 0.999
P(reads|C/C)= 0.000097	**Prior(C/C)** = 0.64	**Posterior(C/C)** = <.001

Population Based Prior: Use frequency information from examining others at the same site.

In the example above, we estimated P(A) = 0.20

Sequence Based Genotype Calls

- **Individual Based Prior**
  - Assumes all sites have an equal probability of showing polymorphism
  - Specifically, assumption is that about 1/1000 bases differ from reference
  - If reads where error free and sampling Poisson …
  - … 14x coverage would allow for 99.8% genotype accuracy
  - … 30x coverage of the genome needed to allow for errors and clustering

- **Population Based Prior**
  - Uses frequency information obtained from examining other individuals
  - Calling very rare polymorphisms still requires 20-30x coverage of the genome
  - Calling common polymorphisms requires much less data

Shotgun Sequence Data

Haplotype Based Prior

★

TAGCTGATAGCTAG**A**TAGCTGATGAGCCCGAT

ATAGCTAG**A**TAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCC

AGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A)** = 0.81	**Posterior(A/A)** = <.001
P(reads|A/C)= 0.03125	**Prior(A/C)** = 0.18	**Posterior(A/C)** = 0.999
P(reads|C/C)= 0.000097	**Prior(C/C)** = 0.01	**Posterior(C/C)** = <.001

Haplotype Based Prior: Examine other chromosomes that are similar at locus of interest.
In the example above, we estimated that 90% of similar chromosomes carry allele A.

Shotgun Sequence Data

Haplotype Based Prior

TAGCTGATAGCTAG**A**TAGCTGATGAGCCCGAT

ATAGCTAG**A**TAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAG**C**TAGCTGATGAGCC

AGCTGATAGCTAG**C**TAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAG**C**TAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

P(reads|A/A)= 0.00000098	**Prior(A/A) =** 0.81	**Posterior(A/A) =** <.001
P(reads|A/C)= 0.03125	**Prior(A/C) =** 0.18	**Posterior(A/C) =** 0.999
P(reads|C/C)= 0.000097	**Prior(C/C) =** 0.01	**Posterior(C/C) =** <.001

Haplotype Based Prior: Examine other chromosomes that are similar at locus of interest.

In the example above, we estimated that 90% of similar chromosomes carry allele A.

Sequence Based Genotype Calls

- **Individual Based Prior**
  - Assumes all sites have an equal probability of showing polymorphism
  - Specifically, assumption is that about 1/1000 bases differ from reference
  - If reads where error free and sampling Poisson …
  - … 14x coverage would allow for 99.8% genotype accuracy
  - … 30x coverage of the genome needed to allow for errors and clustering

- **Population Based Prior**
  - Uses frequency information obtained from examining other individuals
  - Calling very rare polymorphisms still requires 20-30x coverage of the genome
  - Calling common polymorphisms requires much less data

- **Haplotype Based Prior or Imputation Based Analysis**
  - Compares individuals with similar flanking haplotypes
  - Calling very rare polymorphisms still requires 20-30x coverage of the genome
  - Can make accurate genotype calls with 2-4x coverage of the genome
  - Accuracy improves as more individuals are sequenced

The Challenge

- Whole genome sequence data will greatly increase our understanding of complex traits
- Variants of large effect are typically extremely rare
- Common variants typically have extremely small effects
- Dissecting complex traits will require whole genome sequencing of 1,000s of individuals
- **How to sequence 1,000s of individuals cost-effectively?**

Current Genome Scale Approaches

- Deep whole genome sequencing
    - Can only be applied to limited numbers of samples
    - Most complete ascertainment of variation
- Exome capture and targeted sequencing
    - Can be applied to moderate numbers of samples
    - SNPs and indels in the most interesting 1% of the genome
- Low coverage whole genome sequencing
    - Can be applied to moderate numbers of samples
    - Very complete ascertainment of shared variation
    - Less complete ascertainment of rare variants

Simulation Results: Common Sites

- Detection and genotyping of Sites with MAF >5% (2116 simulated sites/Mb)

  - **Detected Polymorphic Sites: 2x coverage**
  - 100 people 2102 sites/Mb detected
  - 200 people 2115 sites/Mb detected
  - 400 people 2116 sites/Mb detected

  - **Error Rates at Detected Sites: 2x coverage**
  - 100 people 98.5% accurate, 90.6% at hets
  - 200 people 99.6% accurate, 99.4% at hets
  - 400 people 99.8% accurate, 99.7% at hets

Yun Li

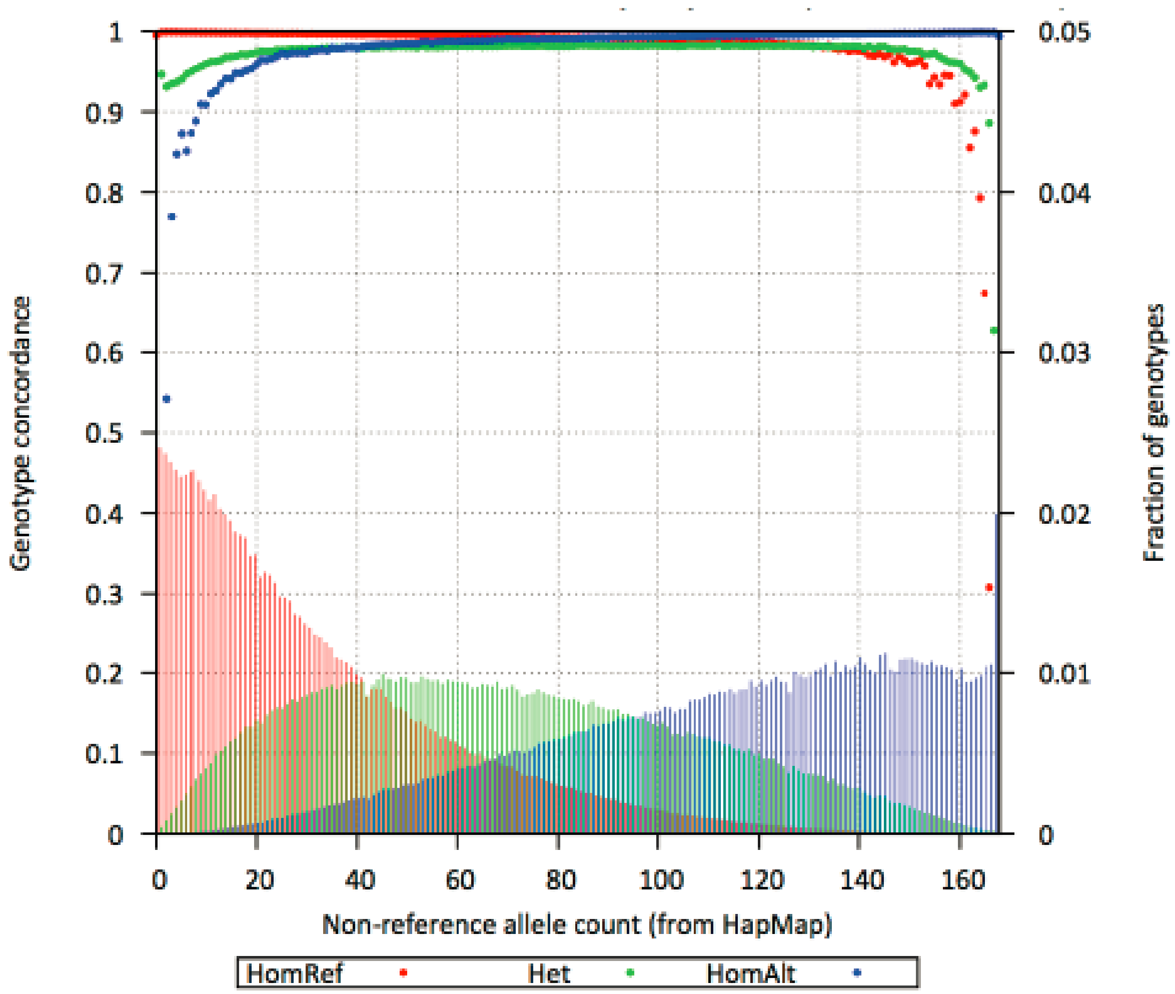
That’s The Theory … Show Me The Data!

Results from 1000 Genomes Project

Project Goals

- >95% of accessible genetic variants
  with a frequency of >1%
  in each of multiple continental regions
- Extend discovery effort to lower frequency variants in coding regions of the genome
- Define haplotype structure in the genome

Accuracy of Low Pass Genotypes



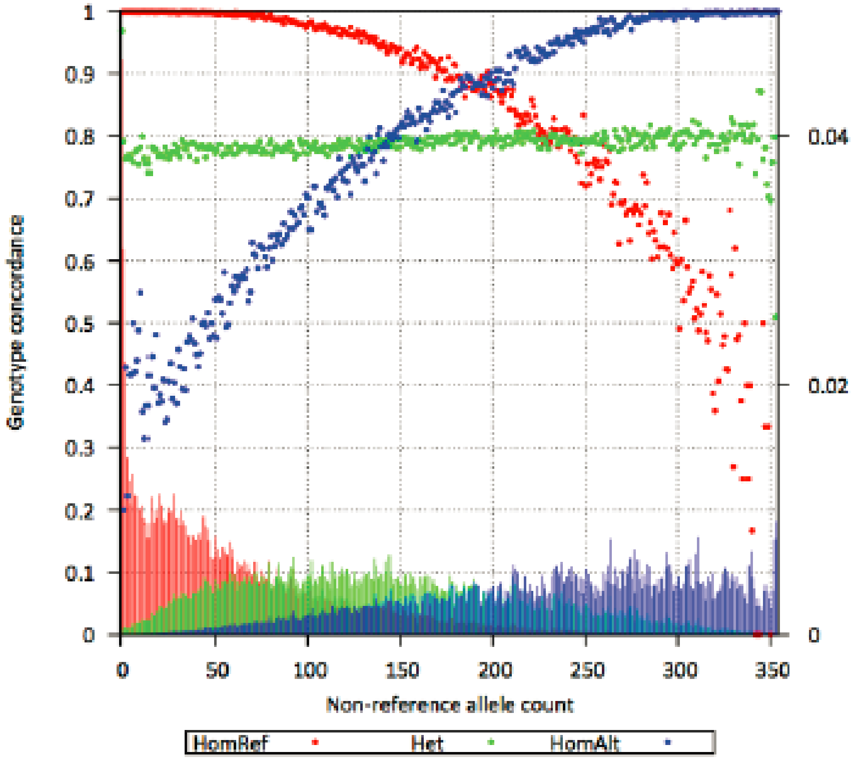
Genotype accuracy for rare genotypes is lowest, but definition of rare changes as more samples are sequenced.

Hyun Min Kang

Does Haplotype Information Really Help?

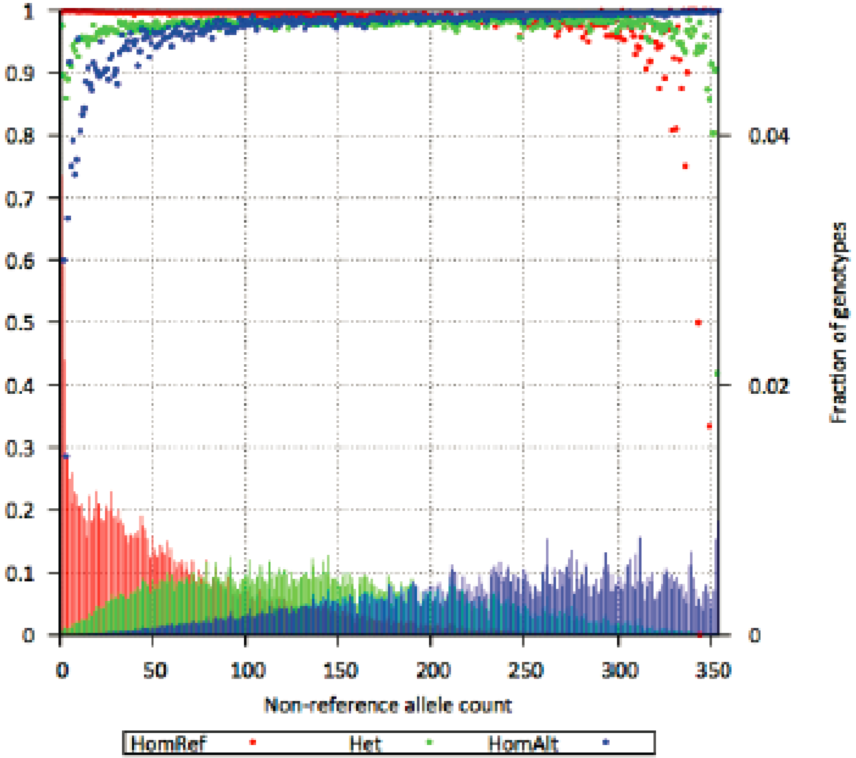
Single Site Analysis

– 21.4% HET errors



Haplotype Aware Analysis

– 2.0% HET errors



As More Samples Are Sequenced, Low Pass Genotypes Improve

Analysis	#SNPs	dbSNP%	Missing HapMap %	Ts/Tv	Accuracy at Hets*
March 2010 Michigan/EUR 60	9,158,226	63.5	7.0	1.91	96.74
August 2010 Michigan/EUR 186	10,537,718	52.5	5.6	2.04	97.56
October 2010 Michigan/EUR 280	13,276,643	50.1	1.8	2.20	97.91**

Accuracy of Low Pass Genotypes Generated by 1000 Genomes Project,
When Analyzed Here At the University of Michigan

What Was Optimal Model for Analyzing Pilot Data?

1000 Genomes Call Set (CEU)	Homozygous Reference Error	Heterozygote Error	Homozygous Non-Reference Error
Broad	0.66	4.29	3.80
Michigan	0.68	3.26	3.06
Sanger	1.27	3.43	2.60
Majority Consensus	0.45	2.05	2.21

- Pilot analyzed with different haplotype sharing models
  - Sanger (QCALL), Michigan (MaCH/Thunder), Broad (BEAGLE)
  - Consensus of the three callers clearly bested single callers

Implications for
Whole Genome Sequencing Studies

- Suppose we could afford 2,000x data (6,000 GB)
- We could sequence 67 individuals at 30x

	Sequencing of 67 individuals at 30x depth			
Minor Allele Frequency	**0.5 – 1.0%**	**1.0 – 2.0%**	**2.0 – 5.0%**	**>5%**
Proportion of Detected Sites	59.3%	90.1%	96.9%	100.0%
Genotyping Accuracy	100.0%	100.0%	100.0%	100.0%
…. Heterozygous Sites Only	100.0%	100.0%	100.0%	100.0%
Correlation with Truth (r^2)	99.8%	99.9%	99.9%	100.0%
Effective Sample Size ($n \cdot r^2$)	67	67	67	67

Implications for Whole Genome Sequencing Studies

- Suppose we could afford 2,000x data (6,000 GB)
- We could sequence 1000 individuals at 2x

	Sequencing of 1000 individuals at 2x depth			
Minor Allele Frequency	**0.5 – 1.0%**	**1.0 – 2.0%**	**2.0 – 5.0%**	**>5%**
Proportion of Detected Sites	79.6%	98.8%	100.0%	100.0%
Genotyping Accuracy	99.6%	99.5%	99.5%	99.8%
…. Heterozygous Sites Only	78.8%	89.5%	95.9%	99.8%
Correlation with Truth (r^2)	56.7%	76.1%	88.2%	97.8%
Effective Sample Size ($n \cdot r^2$)	567	761	882	978

Given Fixed Capacity, Should We Sequence Deep or Shallow?

	.5 – 1%	**1 – 2%**	**2-5%**
400 Deep Genomes (30x)			
Discovery Rate	100%	100%	100%
Het. Accuracy	100%	100%	100%
Effective N	400	400	400
3000 Shallow Genomes (4x)			
Discovery Rate	100%	100%	100%
Het. Accuracy	90.4%	97.3%	98.8%
Effective N	2406	2758	2873

Li et al, *Genome Research,* 2011

Design A Whole Genome Sequencing Study in Sardinia

Gonçalo Abecasis

David Schlessinger

Francesco Cucca

SardiNIA Whole Genome Sequencing

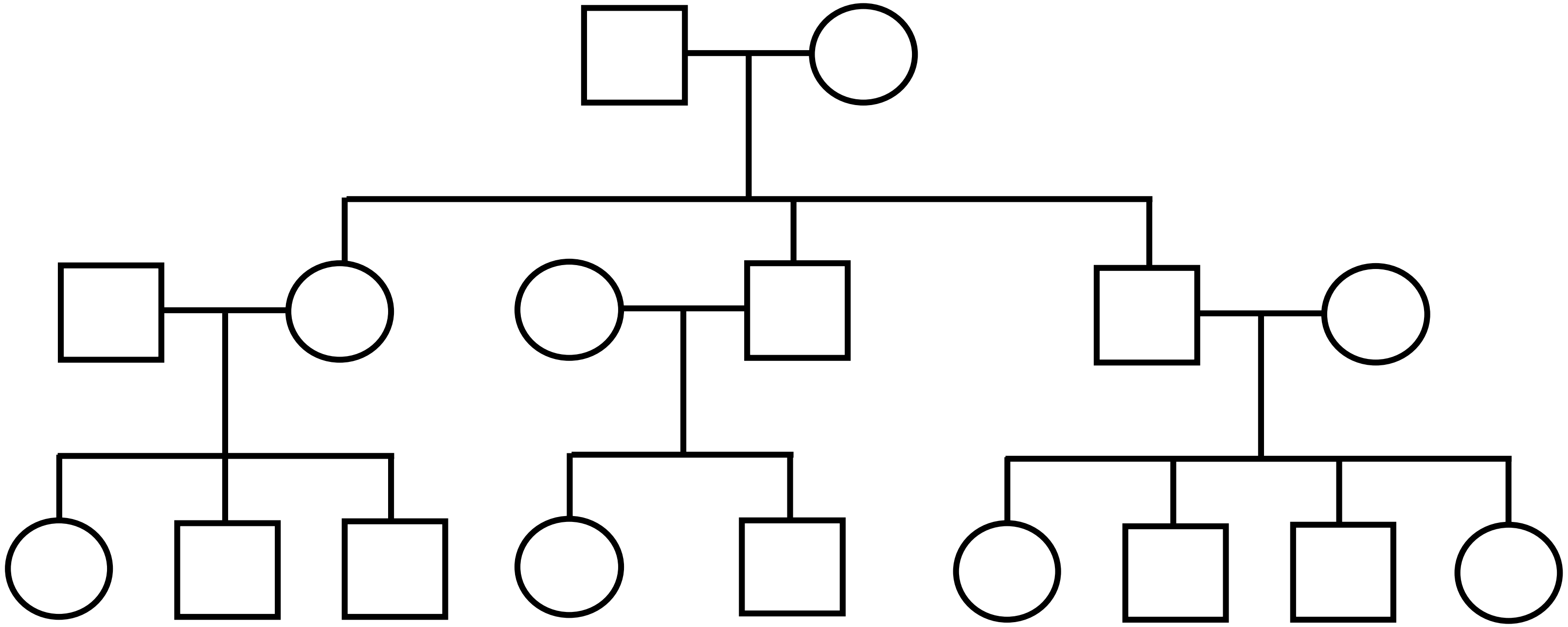
- 6,148 Sardinians from 4 towns in the Lanusei Valley, Sardinia
  - Recruited among population of ~9,841 individuals
  - Sample includes >34,000 relative pairs

- Measured ~100 aging related quantitative traits

- Original plan:
  - Sequence >1,000 individuals at 2x to obtain draft sequences
  - Genotype all individuals, impute sequences into relatives

Who To Sequence?

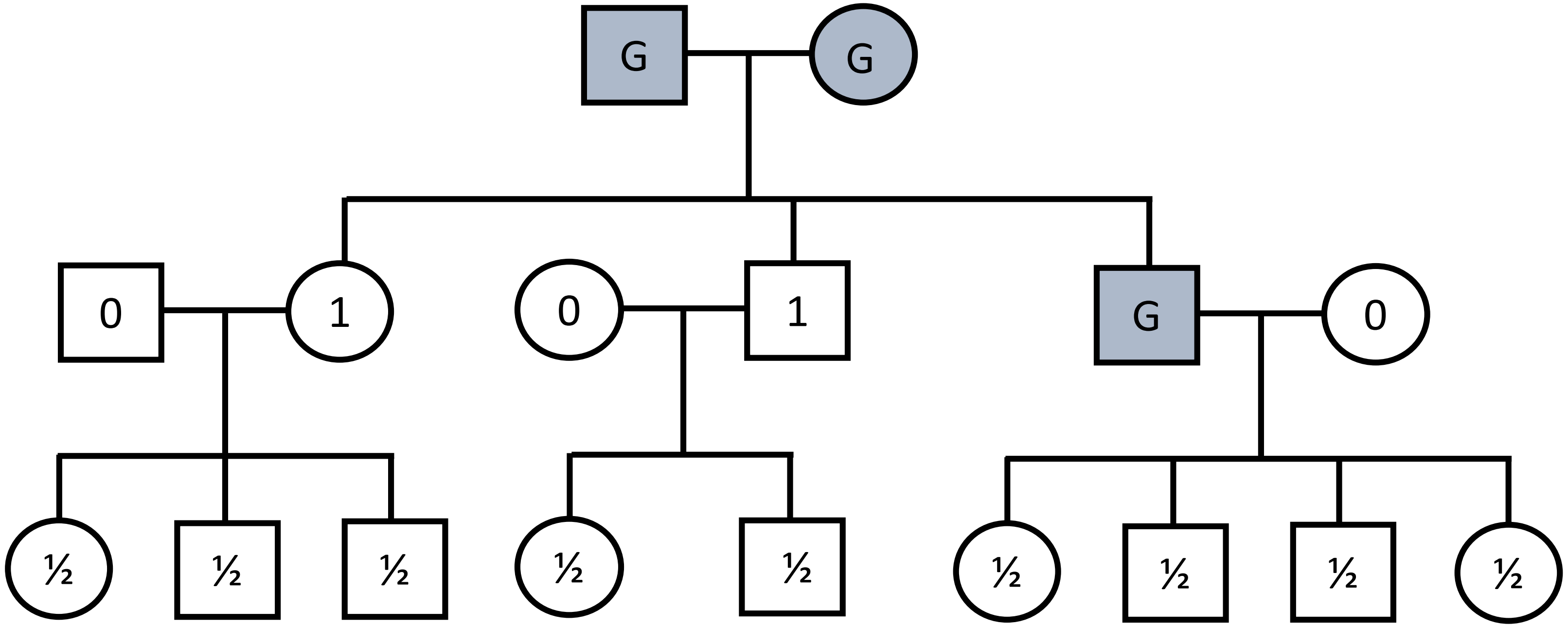
Assuming All Individuals Have Been Genotyped



0 Genomes Sequenced, 0 Genomes Analyzed

Who To Sequence?

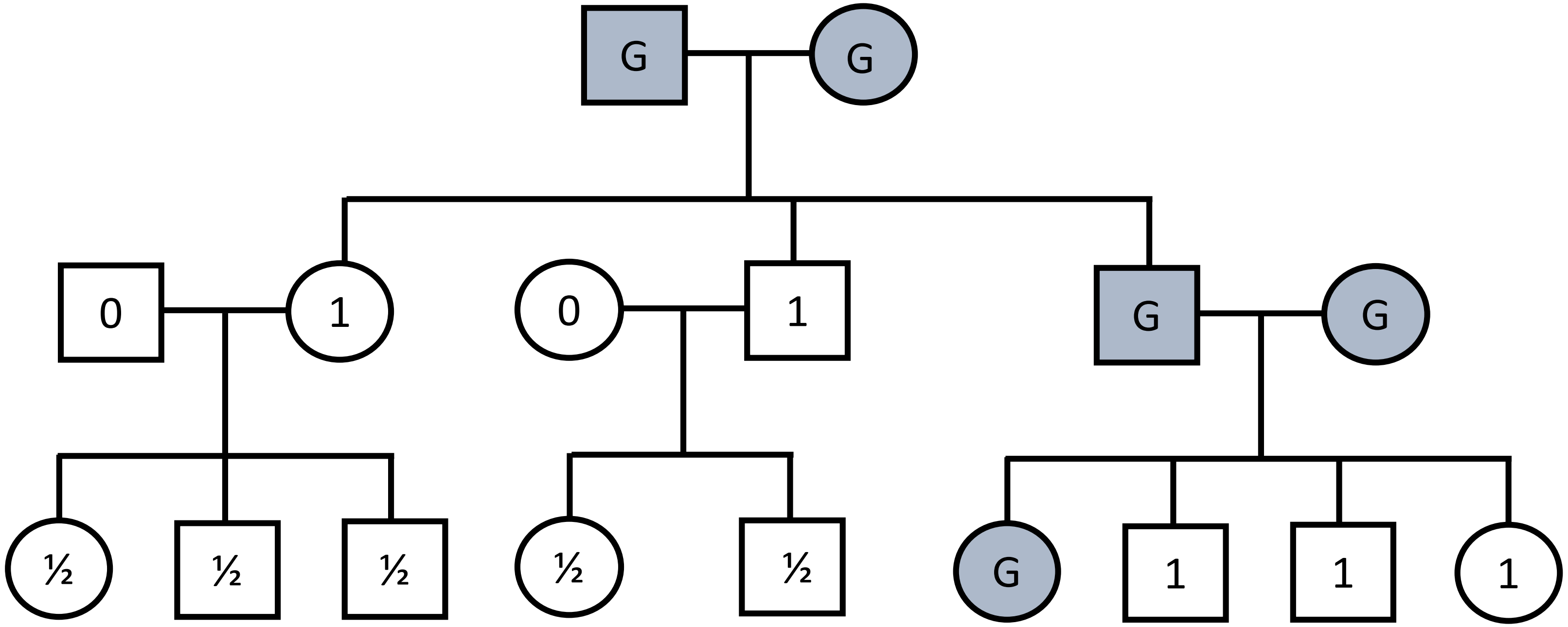
Assuming All Individuals Have Been Genotyped



3 Genomes Sequenced, 9.5 Genomes Analyzed

Who To Sequence?

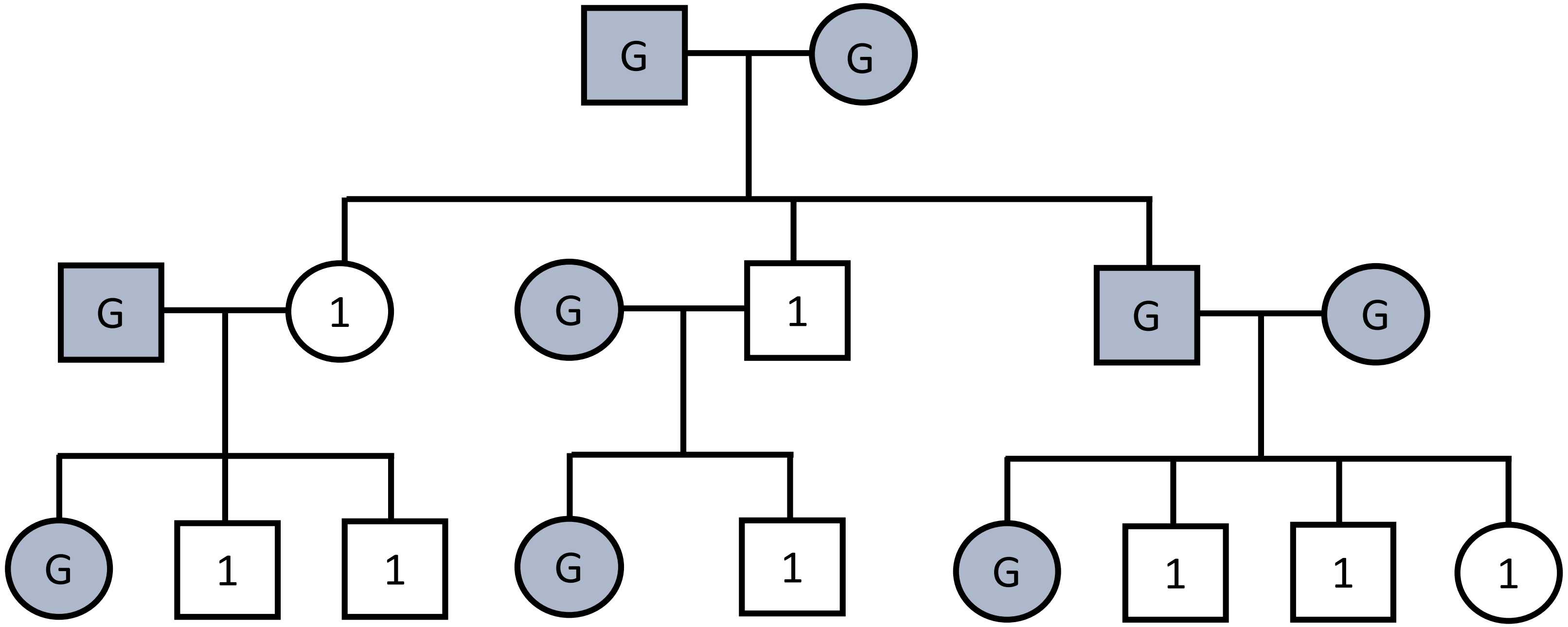
Assuming All Individuals Have Been Genotyped



5 Genomes Sequenced, 12.5 Genomes Analyzed

Who To Sequence?

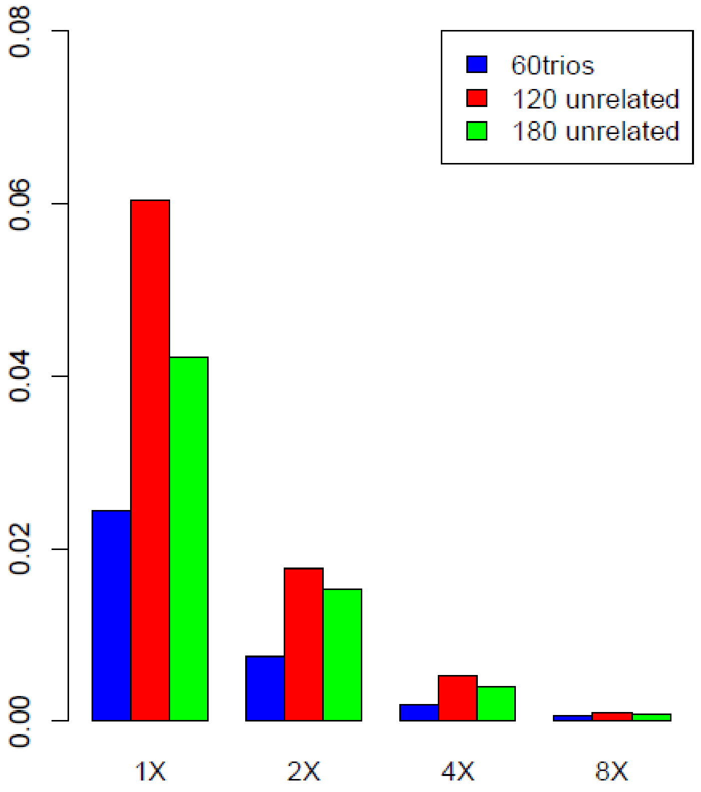
Assuming All Individuals Have Been Genotyped



9 Genomes Sequenced, 17 Genomes Analyzed

Anything to Gain from Sequencing Trios?

Improved Accuracy at Heterozygous Sites



- Sequencing trios improves genotype call accuracy
  - At low coverage …
  - Smaller gain w/deep coverage
- Leads to similar numbers of detected variants
  - At low coverage …
  - No gain w/deep coverage
- Improved haplotype accuracy

Wei Chen and Bingshan Li

How Did Sequencing Progress?

- NHGRI estimates of sequencing capacity and cost …
  - Since 2006, for fixed cost …
  - … ~4x increase in sequencing output per year

- In our own hands…
  - Mapped high quality bases
  - March 2010: ~5.0 Gb/lane
  - May 2010: ~7.5 Gb/lane
  - September 2010: ~8.6 Gb/lane
  - January 2011: ~16 Gb/lane
  - Summer 2011: ~45 Gb/lane

- Other small improvements
  - No PCR libraries increase genome coverage, reduce duplicate rates

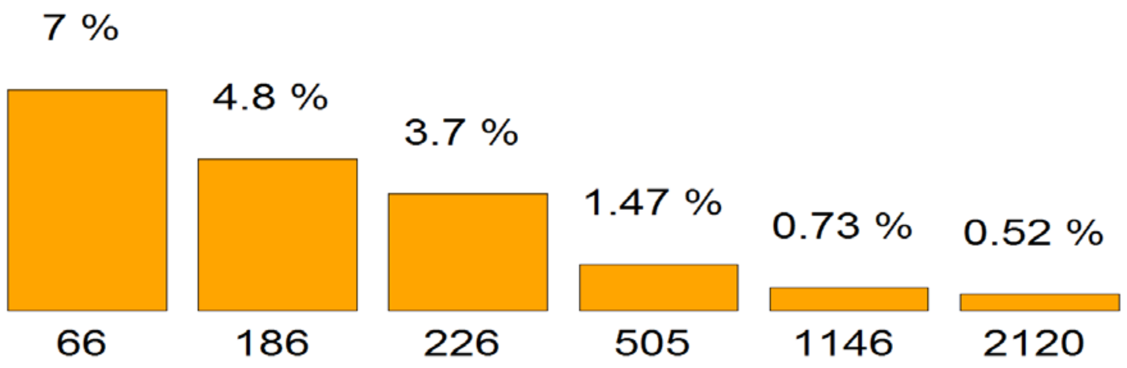
Fabio Busonero, Andrea Maschio

Assembling Sequences In Sardinia

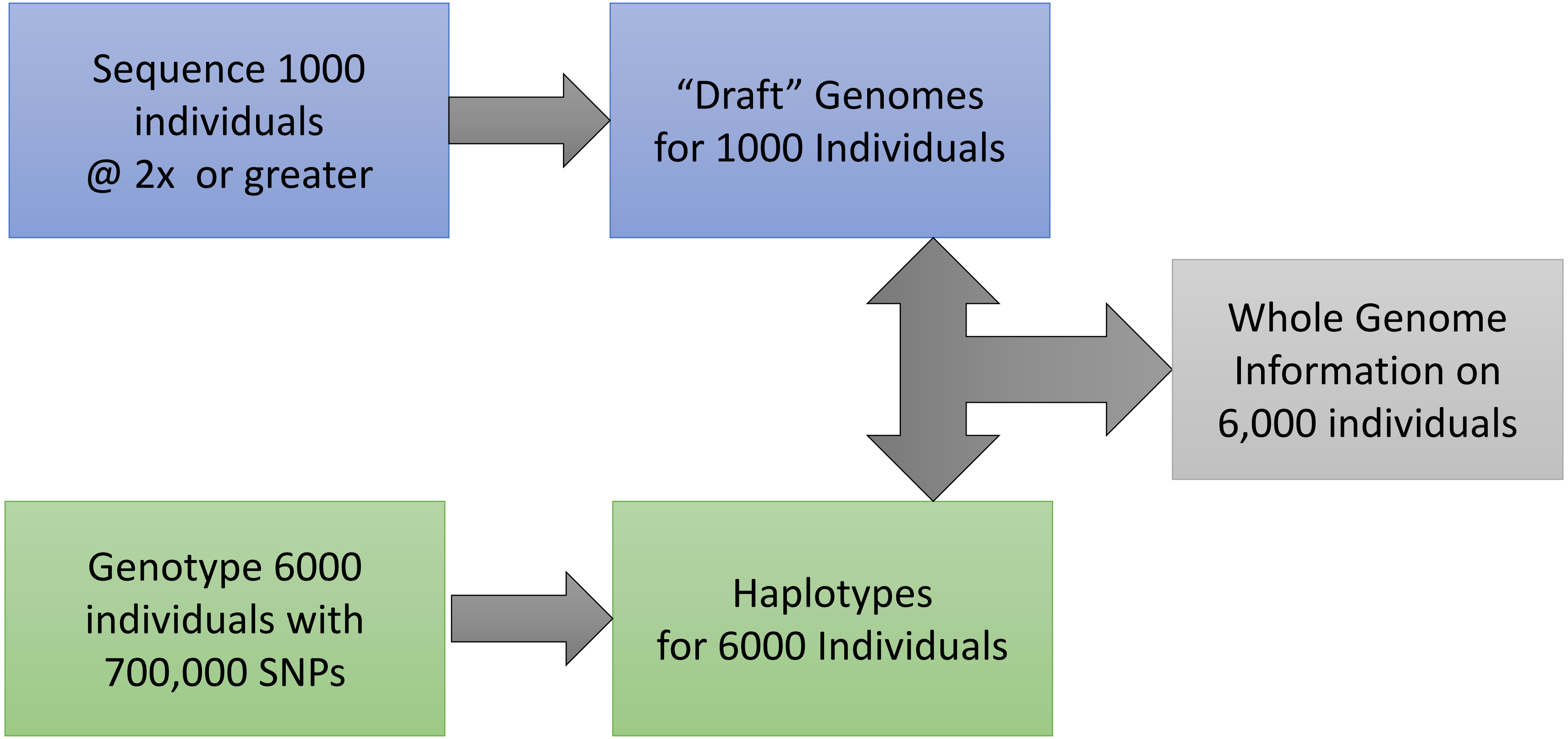
Sardinian team led by Francesco Cucca, Serena Sanna, Chris Jones

As more samples are sequenced, Accuracy increases

Heterozygous Mismatch Rate (in %)



Design



Sequence 1000 individuals @ 2x or greater

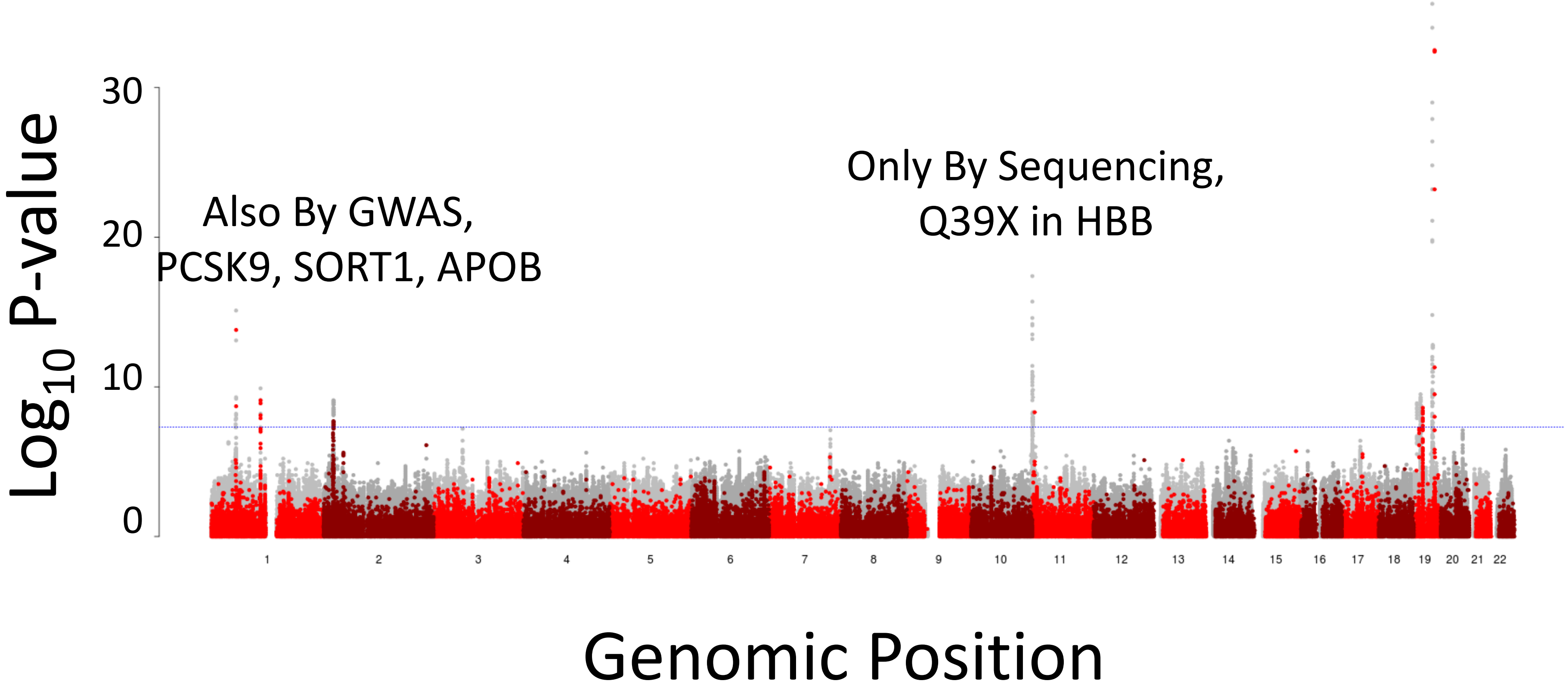
“Draft” Genomes for 1000 Individuals

Whole Genome Information on 6,000 individuals

Genotype 6000 individuals with 700,000 SNPs

Haplotypes for 6000 Individuals

What Do We See Genomewide?
LDL Cholesterol



Also By GWAS,
PCSK9, SORT1, APOB

Only By Sequencing,
Q39X in HBB

Also By GWAS,
LDLR, APOE

LDL Genetics In Lanusei Valley, Sardinia, Current Sequenced Based View

Locus	Variants	MAF	Effect Size (SD)	H^2
HBB	**Q39X**	.04	0.90	8.0%??
APOE	R176C, C130R	.04, .07	0.56, 0.26	3.3%
PCSK9	R46L, rs2479415	.04, .41	0.38, 0.08	1.2%
LDLR	rs73015013, **V578R**	.14, .005	0.16, 0.62	1.2%
SORT1	rs583104	.18	0.15	0.6%
APOB	rs547235	.19	0.19	0.5%

- Most of these variants are important across Europe, extensively studied.
- **Q39X** variant in HBB is especially enriched in Sardinia.
- **V578R** in LDLR is a Sardinia specific variant, particularly common in Lanusei.

Summary

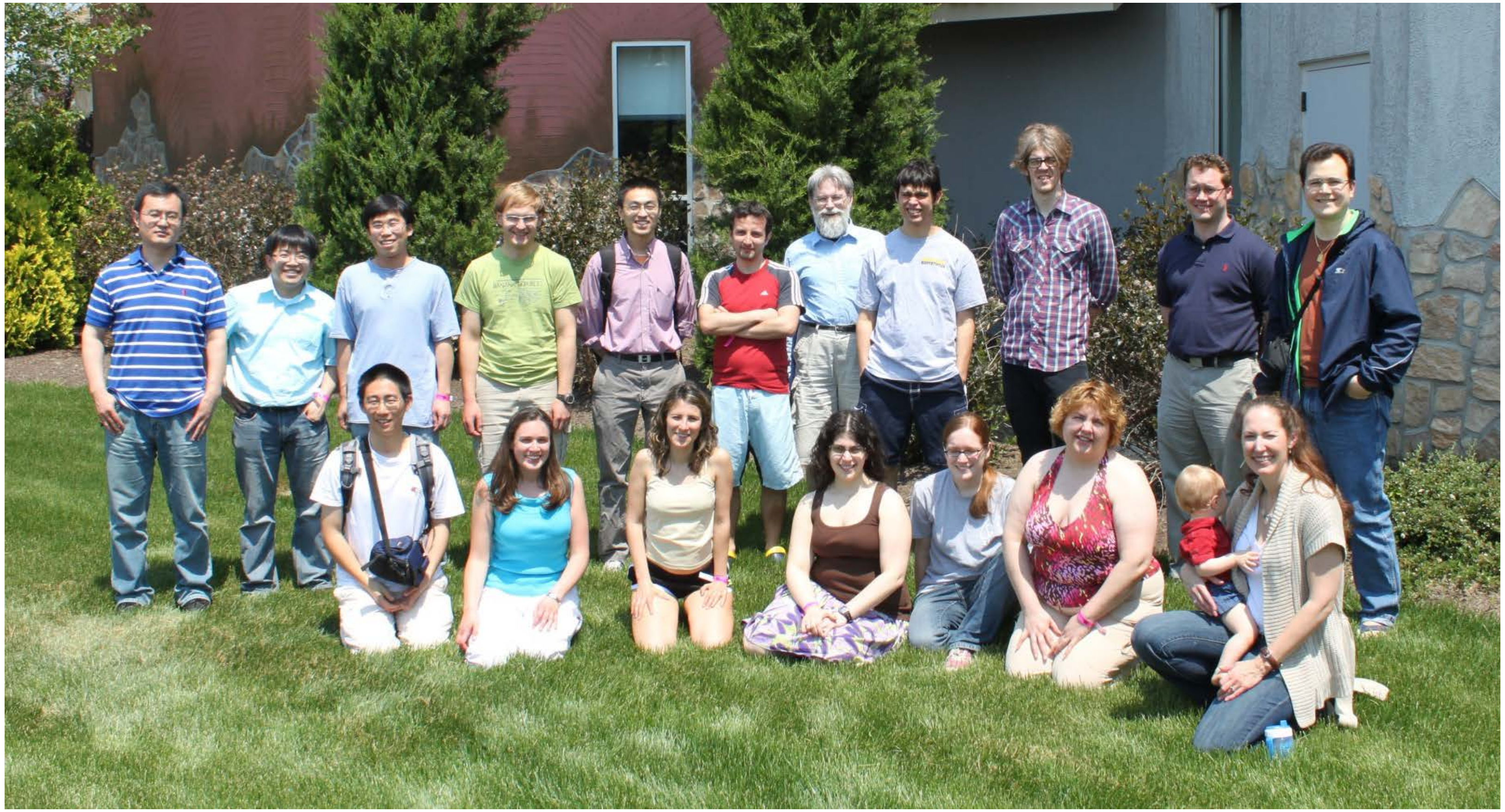
- Challenges and opportunities in genetic association studies.

- Great need for statistical and computational method development.

- In a specific examples, we …
    - Designed method to combine sequence information across samples.
    - Applied the method to sequence an interesting population in Sardinia.

    - Designed method to infer ancestry from small amounts of sequence.
    - Applied the method to identify additional controls for sequencing study.

Acknowledgements



Thank you to the National Institutes of Health (NEI, NHGRI, NHLBI), GlaxoSmithKline and the University of Michigan for funding our work.