Whole Genome Sequencing
Low Pass Sequencing

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Previous Lecture

- Introduction to Whole Genome Sequencing
  - What will we learn from whole genome sequencing?
- Challenges with Read Mapping
- Interpreting Mismatches: Variant or Error
  - Single individual analyses require deep sequencing
  - Multi-individual analyses can use shallower data
- Information contained in paired reads

Questions that Might Be Answered With Complete Sequence Data…

- What is the contribution of each identified locus to a trait?
  - Likely that multiple variants, common and rare, will contribute
- What is the mechanism? What happens when we knockout a gene?
  - Most often, the causal variant will not have been examined directly
  - Rare coding variants will provide important insights into mechanisms
- What is the contribution of structural variation to disease?
  - These are hard to interrogate using current genotyping arrays.
- Are there additional susceptibility loci to be found?
  - Only subset of functional elements include common variants …
  - Rare variants are more numerous and thus will point to additional loci

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

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

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'-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: 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

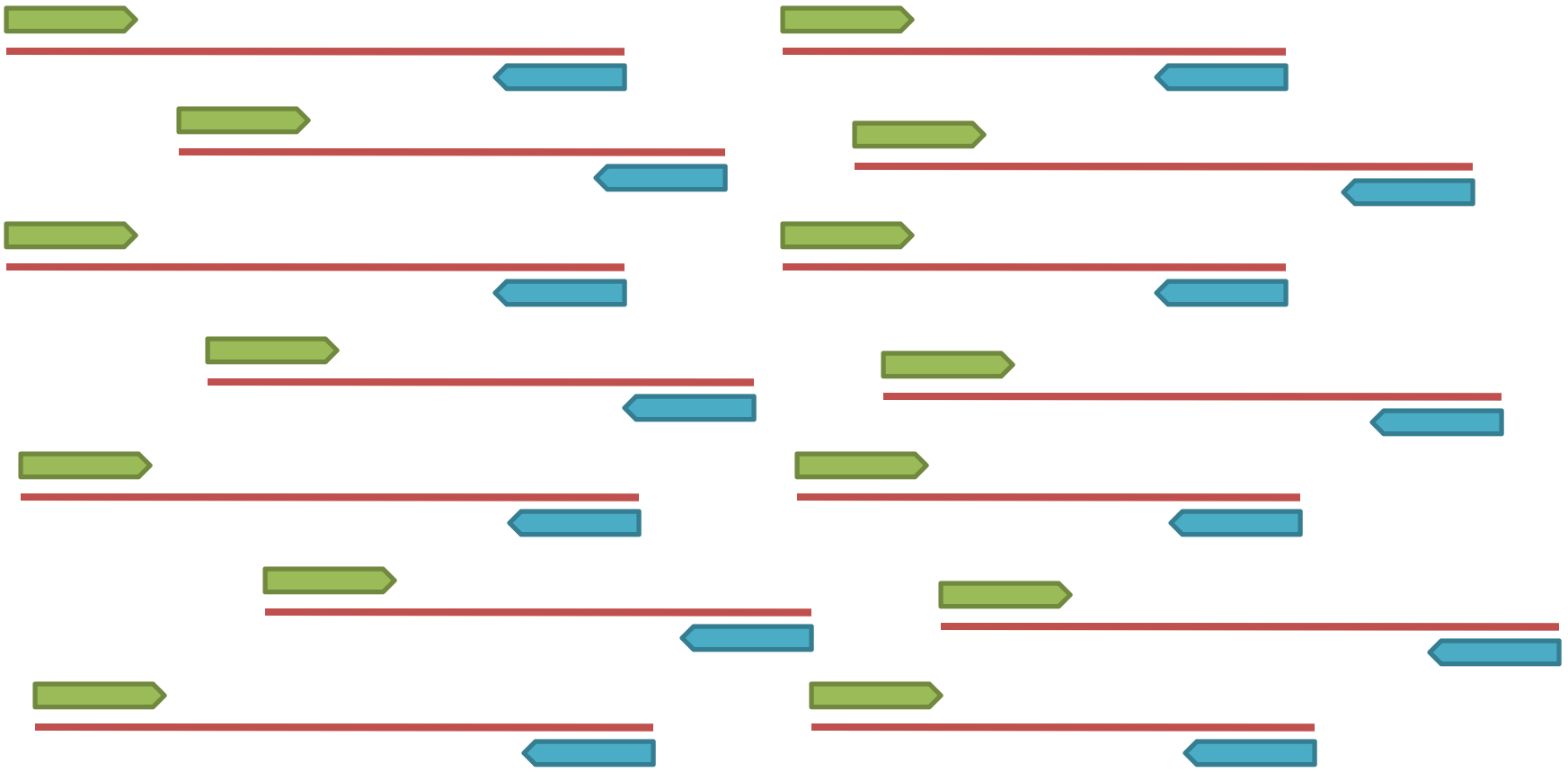
Sequence Based Genotype Calls

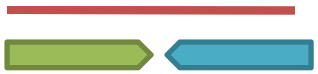
- **Individual Based Prior**
  - Assumes all sites have an equal probability of showing polymorphism
  - Specifically, assumption is that about 1/1000 bases differ from reference
  - If reads where error free and sampling Poisson …
  - … 14x coverage would allow for 99.8% genotype accuracy
  - … 30x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

- **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

Paired End Sequencing





Population of DNA fragments of known size (mean + stdev)
Paired end sequences

Paired End Sequencing

Paired Reads

Initial alignment to the reference genome

Paired end resolution

Detecting Structural Variation

- Read depth
  - Regions where depth is different from expected
    - Expectation defined by comparing to rest of genome …
    - … or, even better, by comparing to other individuals
- Split reads
  - If reads are longer, it may be possible to find reads that span the structural variation
- Discrepant pairs
  - If we find pairs of reads that appear to map significantly closer or further apart than expected, could indicate an insertion or deletion
  - For this approach, “physical coverage” which is the sum of read length and insert size is key
- De Novo Assembly

The Challenge

- Whole genome sequence data will greatly increase our understanding of complex traits
- Although a handful of genomes have been sequenced, this remains a relatively expensive enterprise
- 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

Current Genome Scale Approaches

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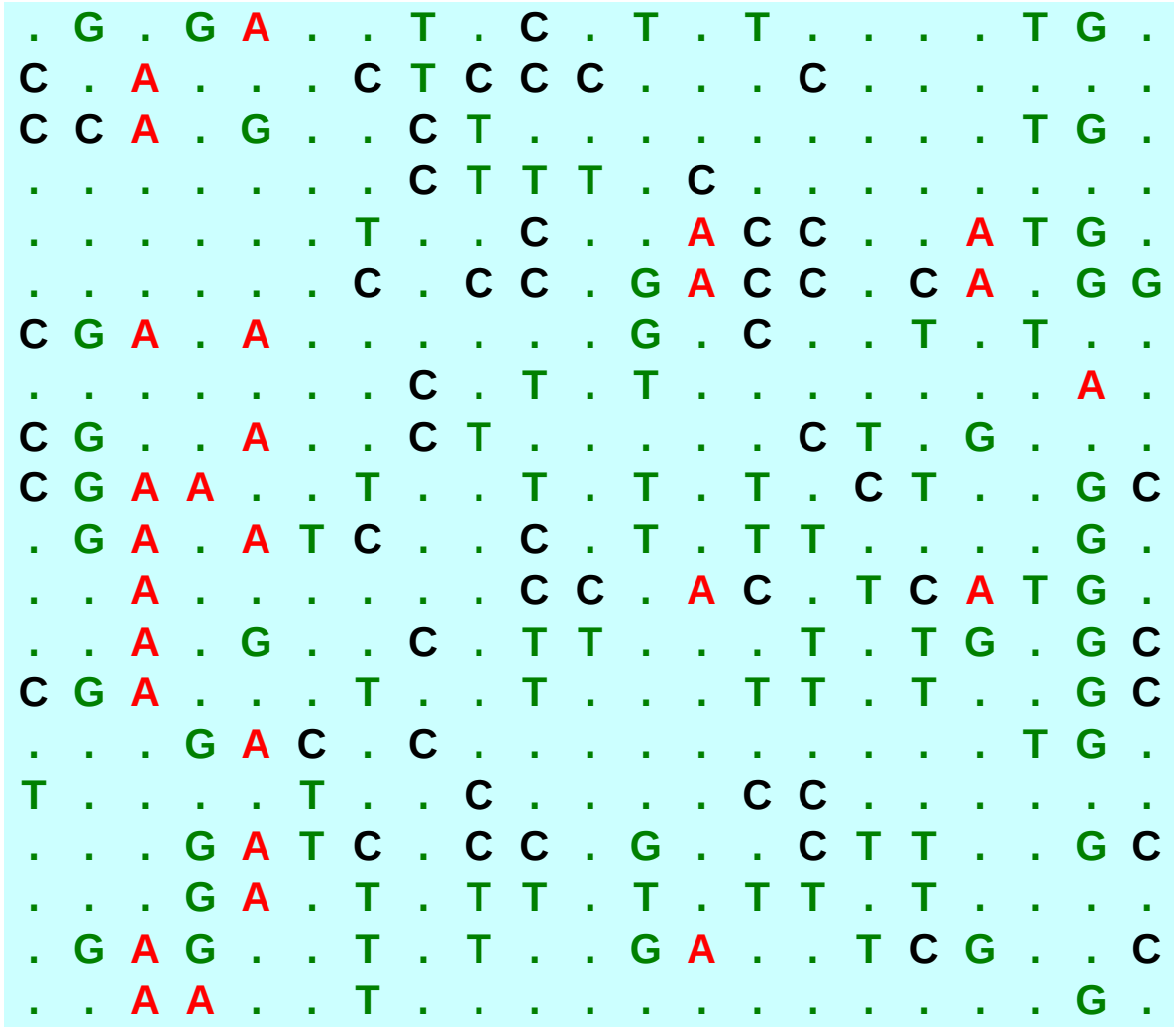
Our Focus For Today

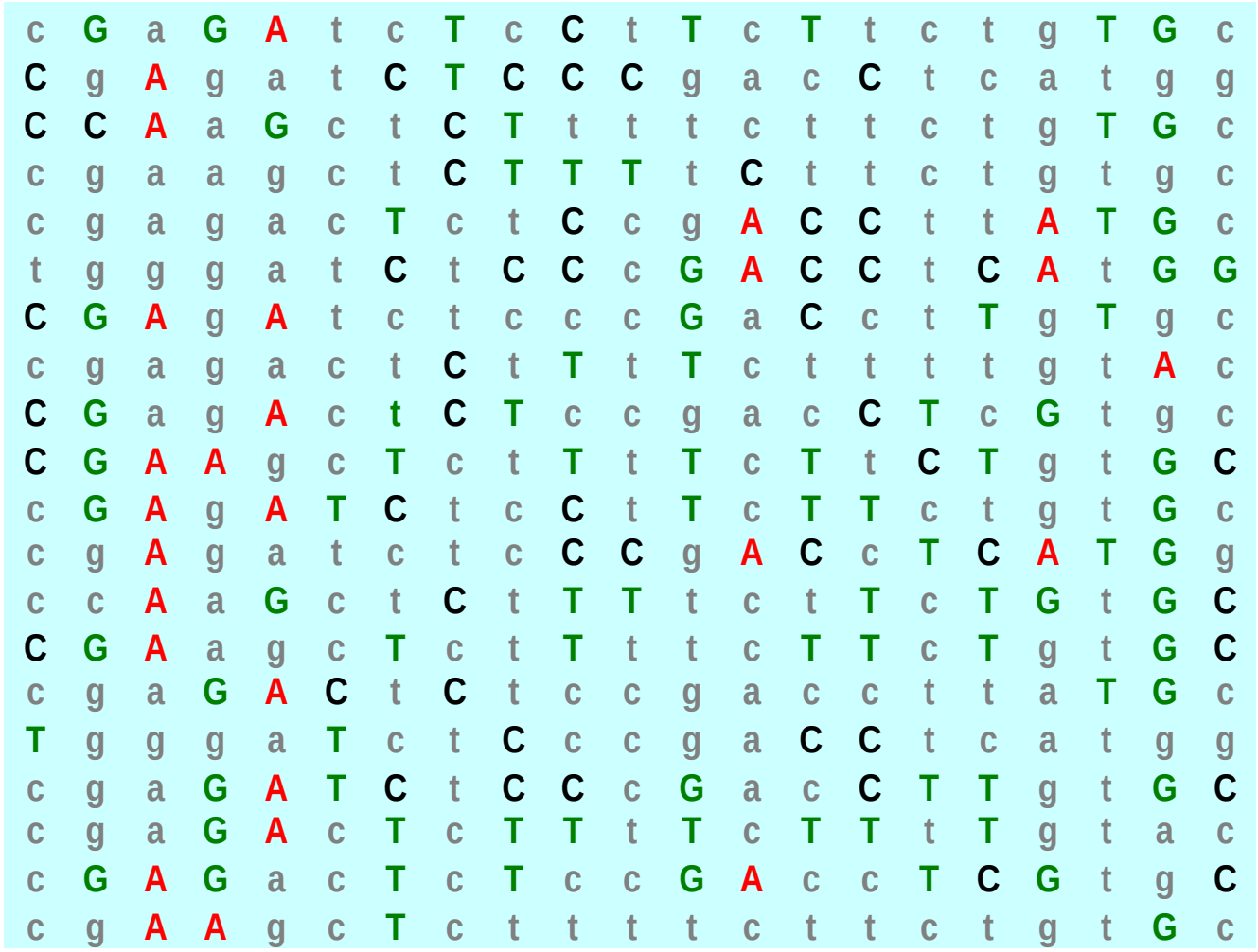
Recipe For Imputation With Shotgun Sequence Data

- Start with some plausible configuration for each individual
- Use Markov model to update one individual conditional on all others
- Repeat previous step many times
- Generate a consensus set of genotypes and haplotypes for each individual

Silly Cartoon View of Shot Gun Data



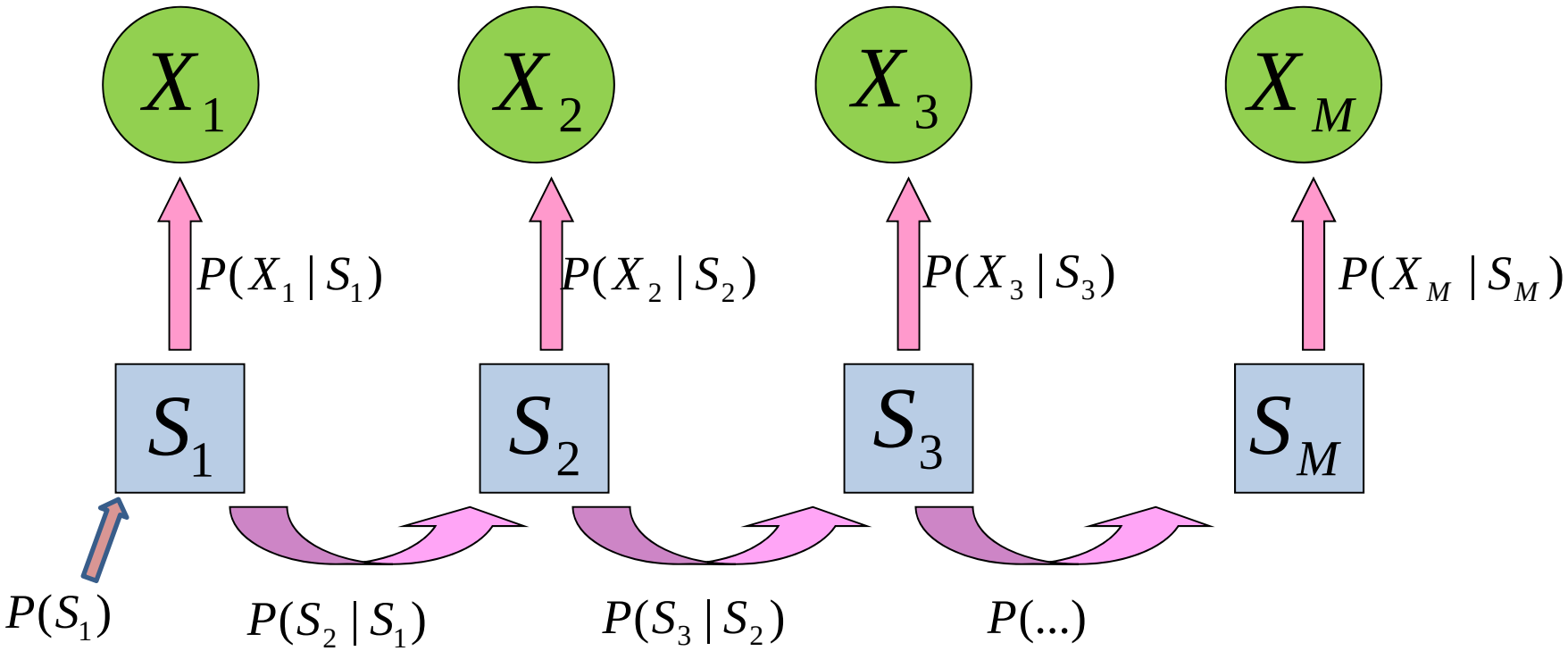
Cartoon View of Shot Gun Data



How Do We Update One Pair Of Haplotypes?

- Markov model is very similar to that used for analysis of genotype imputation analysis
- To carry out an update, select one individual
  - Let X_i be observed bases overlapping position *i* for individual
- Assume (temporarily) that current haplotype estimates for all other individuals are correct
- Model haplotypes for individual being updated as mosaic of the other available haplotypes
  - $S_i = (S_{i1}, S_{i2})$ denotes the pair of haplotypes being copied

Markov Model



The final ingredient connects template states along the chromosome …

Likelihood

$$L = \sum_{S_1} \sum_{S_2} \cdots \sum_{S_M} P(S_1) \prod_{i=2}^{M} P(S_i \mid S_{i-1}) \prod_{i=1}^{M} P(X_i \mid S_i)$$

- $P(S_1) = 1 / H^2$ where H is the number of template haplotypes
- $P(S_i \mid S_{i-1})$ depends on estimated population recombination rate
- $P(X_i \mid S_i)$ are the genotype likelihoods

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

Simulation Results: Rarer Sites

- Detection and genotyping of Sites with MAF 1-2% (425 simulated sites/Mb)
  - **Detected Polymorphic Sites: 2x coverage**
  - 100 people 139 sites/Mb detected
  - 200 people 213 sites/Mb detected
  - 400 people 343 sites/Mb detected
  - **Error Rates at Detected Sites: 2x coverage**
  - 100 people 98.6% accurate, 92.9% at hets
  - 200 people 99.4% accurate, 95.0% at hets
  - 400 people 99.6% accurate, 95.9% at hets

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

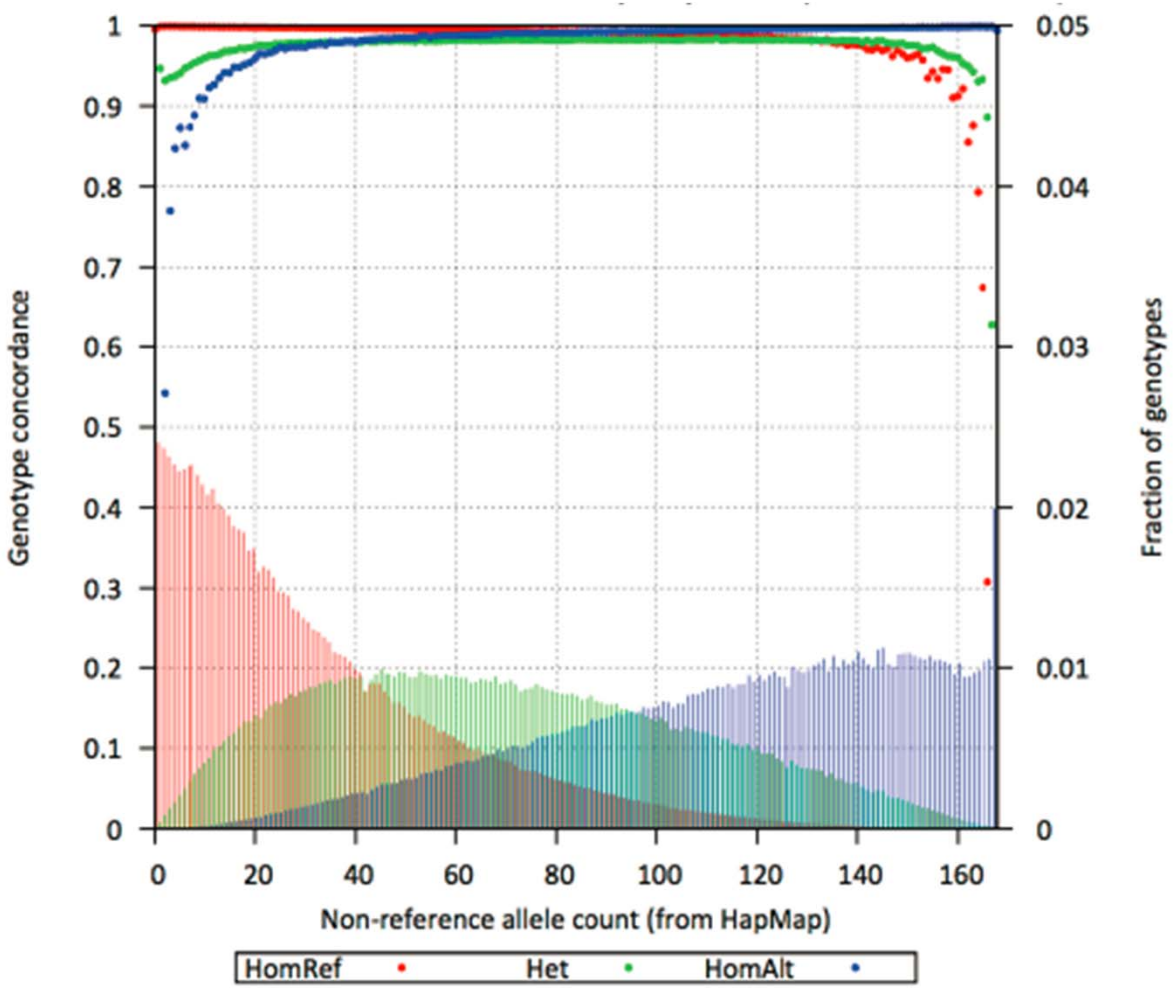
- Define haplotype structure in the genome

1000 Genomes Pilot Completed

- 2 deeply sequenced trios
- 179 whole genomes sequenced at low coverage
- 8,820 exons deeply sequenced in 697 individuals

- 15M SNPs, 1M indels, 20,000 structural variants

Accuracy of Low Pass Genotypes



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

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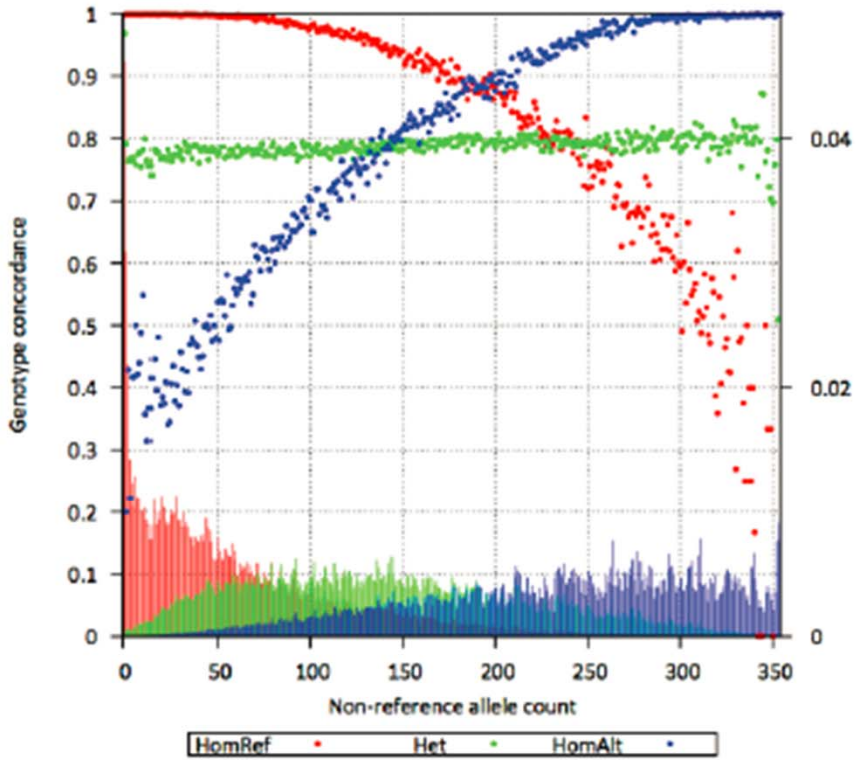
Does Haplotype Information Really Help?

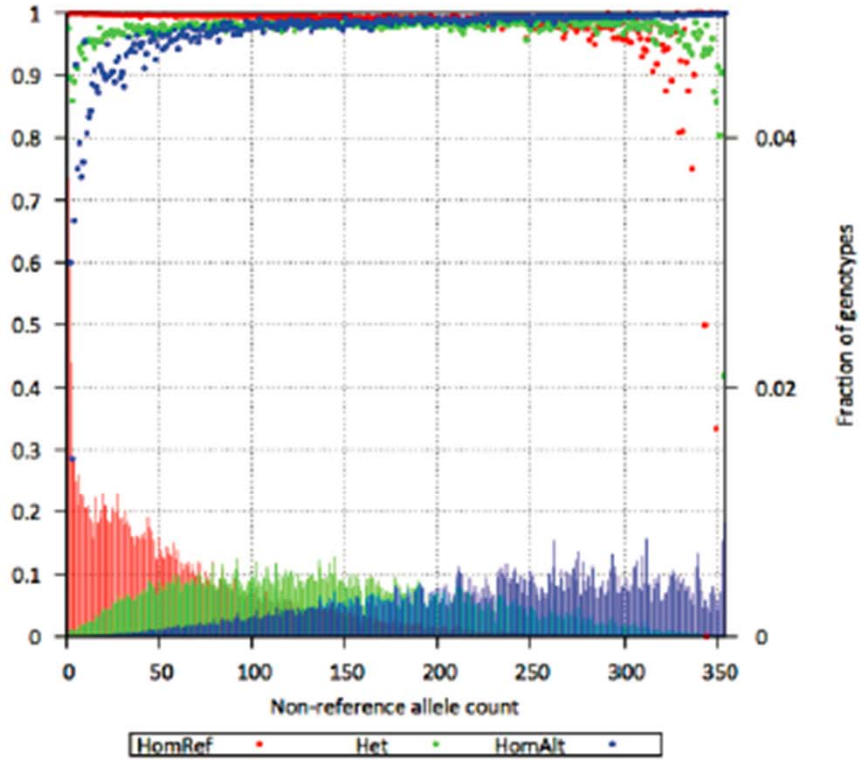
Single Site Analysis

– 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 Analysed At the University of Michigan

Some Important Notes

- The Markov model we described is one of several possible models for analysis of low pass data
- Alternative models, based on E-M algorithms or local clustering of individuals into small groups exist
- Currently, the best possible genotypes produced by running multiple methods and generating a consensus across analysis their results.

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

Summary for Today

- Analysis of Low Pass Sequence Data
  - Single sample analyses produce poor quality variants.
  - Single site analyses produce poor quality genotypes.
  - Multi-sample, multi-sample analyses can work quite well.

- Why low pass analyses are attractive for complex disease association studies.

Recommended Reading

- The 1000 Genomes Project (2010) A map of human genome variation from population-scale sequencing. *Nature* **467**:1061-73

- Li Y, Willer CJ, Ding J, Scheet P and Abecasis GR (2010) MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol* **34**:816-834

- Le SQ and Durbin R (2010) SNP detection and genotyping from low-coverage sequencing data on multiple diploid samples. *Genome Research* (in press)