Computational Genomics

Population Genetics:
SNPS Haplotype Inference

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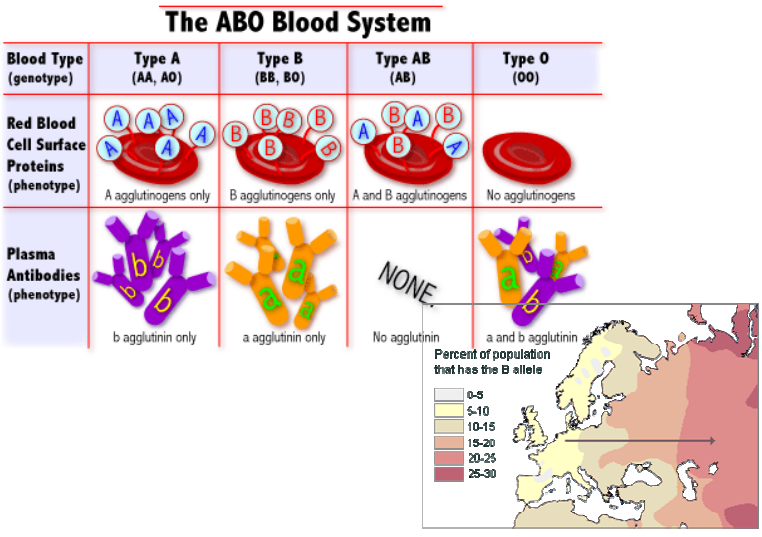
Reading: hand-outs

Genetic Polymorphisms

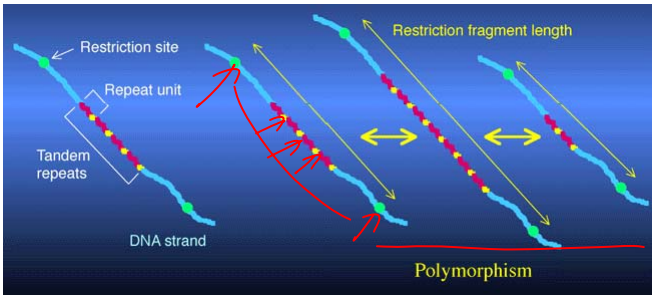


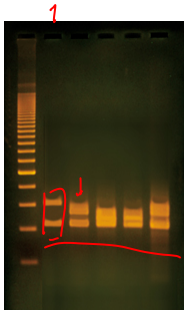
Type of polymorphisms

- Insertion/deletion of a section of DNA
  - Minisatellites: repeated base patterns (several hundred base pairs)
  - Microsatellites: 2-4 nucleotides repeated
  - Presence or absence of Alu segments
- Single base mutation (SNP)
  - Restriction fragment length (RFLP)
  - Creating restriction sites via PCR primer
  - Direct sequencing

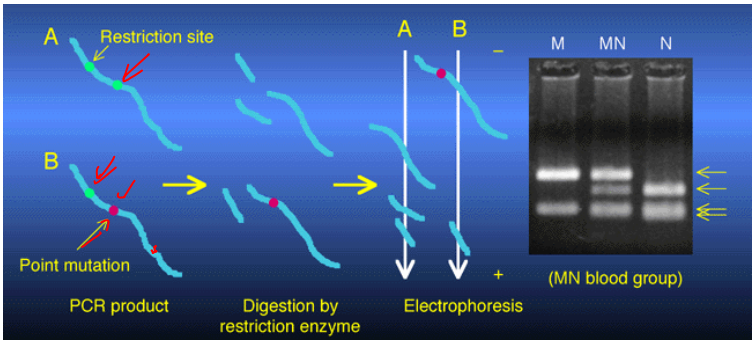
Frequency of SNPs greater than that of any other type of polymorphism

Variable Number of Tandem Repeats (VNTR) Polymorphism

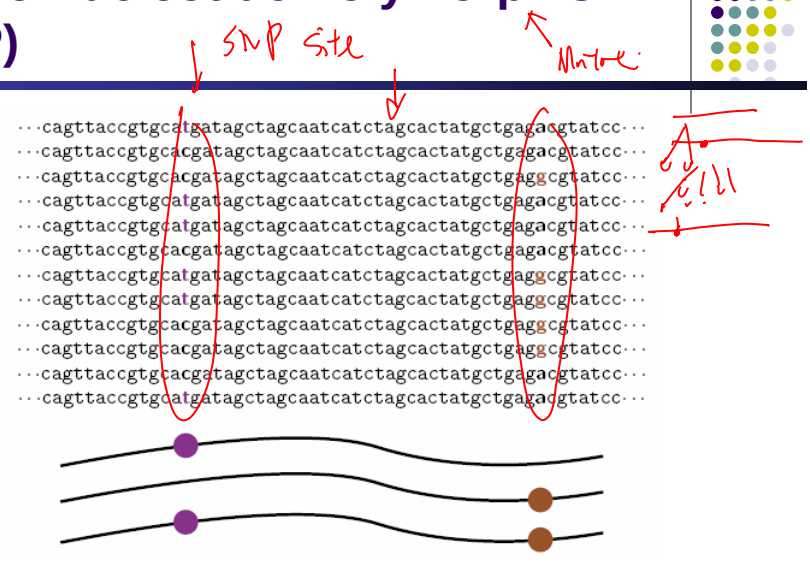




Restriction Fragment Length Polymorphism (RFLP)



Single Nucleotide Polymorphism (SNP)

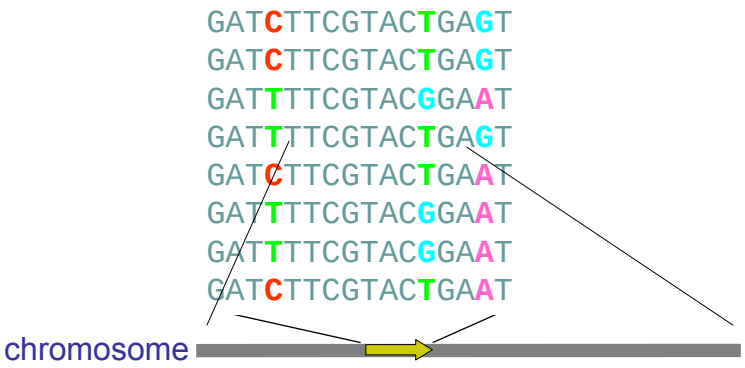


Polymorphism rate: number of letter changes between two different members of a species
Humans: ~1/1,000

Exploiting Genetic Variations

- **Population Evolution**: the majority of human sequence variation is due to substitutions that have occurred **once** in the history of mankind at individual base pairs
  - There can be big differences between populations!
- **Markers for pinpointing a disease**: certain polymorphisms are in "Linkage Disequilibrium" with disease phenotypes
  - Association study: check for differences in SNP patterns between cases and controls
- **Forensic analysis**: the polymorphisms provide individual and familiar signatures

Single Nucleotide Polymorphism (SNP)



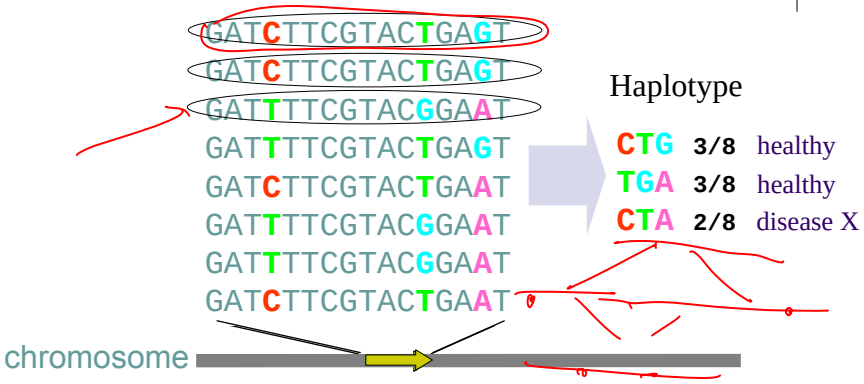
- "Binary" nt-substitutions at a single locus on a chromosome
  - each variant is called an "allele"

Some Facts About SNPs

- More than 5 million common SNPs each with frequency 10-50% account for the bulk of human DNA sequence difference
- About 1 in every 600 base pairs
- It is estimated that ~60,000 SNPs occur within exons; 85% of exons within 5 kb of nearest SNP

What is a haplotype?

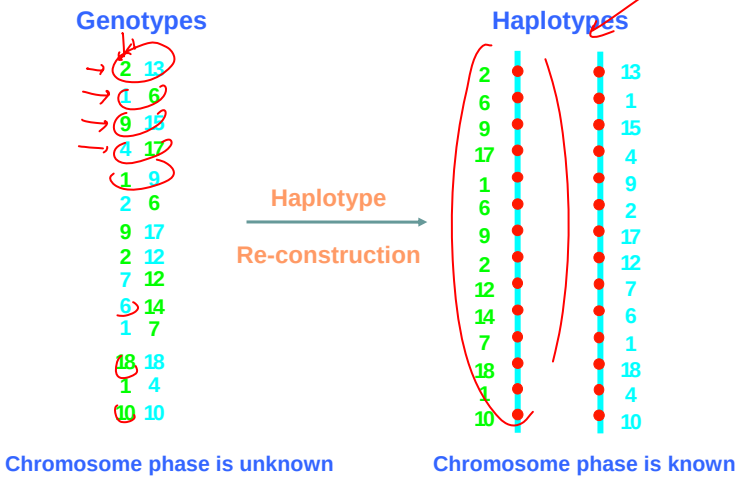
-- a more discriminative state of a chromosomal region



- Consider J binary markers in a genomic region
- There are 2^J possible haplotypes
  - but in fact, far fewer are seen in human population
- Good genetic marker for population, evolution and hereditary diseases …

Haplotype and Genotype

- A collection of alleles derived from the same chromosome

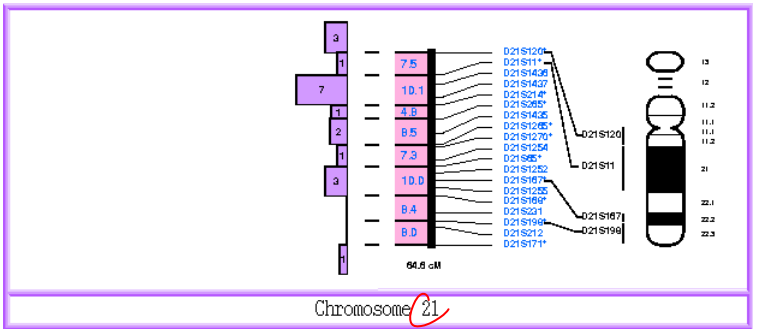


Linkage Disequilibrium

- LD reflects the relationship between alleles at different loci.
  - Alleles at locus A: frequencies $p_1,\ldots, p_m$
  - Alleles at locus B: frequencies $q_1,\ldots,q_n$
  - Haplotype frequency for A_iB_j:
    - equilibrium value: $p_i q_j$
    - Observed value: h_{ij}
    - Linkage disequilibrium: $h_{ij} - p_i q_j$
  - Linkage disequilibrium is an allelic association measure (difference between the actual haplotype frequency and the equilibrium value)
  - More precisely: **gametic association**
- Association studies.
  - If inheriting a certain allele at the disease locus increases the chance of getting the disease, and the disease and marker loci are *in LD*, then the frequencies of the marker alleles will **differ** between diseased and non-diseased individuals.

Use of Polymorphism in Gene Mapping

- 1980s – RFLP marker maps
- 1990s – microsatellite marker maps



Advantages of SNPs in genetic analysis of complex traits

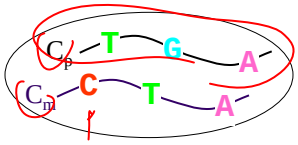
- Abundance: high frequency on the genome
- Position: throughout the genome (level of influence of type of SNP, e.g. coding region, promoter site, on phenotypic expression?)
- Ease of genotyping
- Less mutable than other forms or polymorphisms
- Allele frequency drift (different populations)
- Haplotypic patterns

Haplotype analyses

- Haplotype analyses
  - Linkage disequilibrium assessment
  - Disease-gene discovery
  - Genetic demography
  - Chromosomal evolution studies

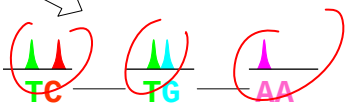
- Why Haplotypes
  - Haplotypes are more powerful discriminators between cases and controls in disease association studies
  - Use of haplotypes in disease association studies reduces the number of tests to be carried out.
  - With haplotypes we can conduct evolutionary studies

Phase ambiguity
-- haplotype reconstruction for individuals



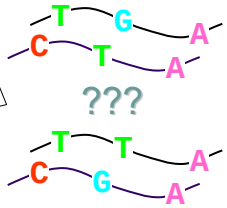
Heterozygous diploid individual

sequencing



Genotype g

pairs of alleles with association of alleles to chromosomes unknown



haplotype $h \equiv (h_1, h_2)$

possible associations of alleles to chromosome

Inferring Haplotypes

- Genotype: AT//AA//CG
  - Maternal genotype: TA//AA//CC
  - Paternal genotype: TT//AA//CG
  - Then the haplotype is AAC/TAG.

- Genotype: AT//AA//CG
  - Maternal genotype: AT//AA//CG
  - Paternal genotype: AT//AA//CG
  - Cannot determine unique haplotype

- **Problem**: determine Haplotypes without parental genotypes

Identifiability

Genotype representations

0/0 → 0
1/1 → 1
0/1 → 2

Genotypes of 14 individual

```
21 2 222 02
02 1 111 22
11 0 000 01
02 1 111 22
21 2 222 02
02 1 111 22
11 0 000 01
02 1 111 22
21 2 222 02
22 2 222 21
21 1 222 02
02 1 111 22
22 2 222 21
21 2 222 02
|| | ||| ||
```

Identifiability

```
01 1 111 00
11 0 000 01

01 1 111 00
00 1 111 11

11 0 000 01
11 0 000 01

01 1 111 00
00 1 111 11

01 1 111 00
11 0 000 01

00 1 111 11
01 1 111 00

11 0 000 01
11 0 000 01

01 1 111 00
00 1 111 11

01 1 111 00
11 0 000 01

00 1 111 11
11 0 000 01

11 1 000 01
01 1 111 00

00 1 111 11
01 1 111 00

11 0 000 01
00 1 111 11

01 1 111 00
11 0 000 01
```

```
11 0 000 01
|| | ||| ||   11

01 1 000 00
|| | ||| ||   10

00 1 111 11
|| | ||| ||   7
```

?

Parsimonious solution

```
01 1 101 00
11 0 010 01

01 1 111 00
00 1 111 11

11 0 000 01
11 0 000 01

01 1 111 00
00 1 111 11

01 0 111 00
11 1 000 01

00 1 111 11
01 1 111 00

11 0 000 01
11 0 000 01

01 1 111 00
00 1 111 11

01 1 111 00
11 0 000 01

00 1 111 11
11 0 000 01

11 1 000 01
01 1 111 00

00 1 111 11
01 1 111 00

11 0 000 11
00 1 111 01

01 1 111 00
11 0 000 01
```

```
11 0 000 01
|| | ||| ||   8

11 0 010 01
||.|.|||.||   1

11 1 000 01
||.|.|||.||   1

11 0 000 11
||.|.|||.||   1

01 1 111 00
|| | ||| ||   8

01 1 101 00
||.|.|||.||   1

01 0 111 00
||.|.|||.||   1

00 1 111 11
|| | ||| ||   6

00 1 111 01
||.|.|||.||   1
```

Three Problems

- Frequency estimation of all possible haplotypes
- Haplotype reconstruction for individuals
- How many out of all possible haplotypes are plausible in a population

Given a random sample of multilocus genotypes at a set of SNPs

Haplotype reconstruction: Clark (1990)

- Choose individuals that are homozygous at every locus (e.g. TT//AA//CC)
  - Haplotype: TAC
- Choose individuals that are heterozygous at just one locus (e.g. TT//AA//CG)
  - Haplotypes: TAC or TAG
- Tally the resulting known haplotypes.
- For each known haplotype, look at all remaining unresolved cases: is there a combination to make this haplotype?
  - Known haplotype: TAC
    - Unresolved pattern: AT//AA//CG
    - Inferred haplotype: TAC/AAG. Add to list.
  - Known haplotype: TAC and TAG
    - Unresolved pattern: AT//AA//CG
    - Inferred haplotypes: TAC and TAG. Add both to list.
- Continue until all haplotypes have been recovered or no new haplotypes can be found this way.

Problems: Clark (1990)

- No homozygotes or single SNP heterozygotes in the sample
- Many unresolved haplotypes at the end
- Error in haplotype inference if a crossover of two actual haplotypes is identical to another true haplotype
- Frequency of these problems depend on avg. heterozygosity of the SNPs, number of loci, recombination rate, sample size.
- Clark (1990): algorithm "performs well" even with small sample sizes.

Finite mixture model

- The probability of a genotype *g*:

$$p(g) = \sum_{h_1,h_2 \in \mathcal{H}} p(h_1,h_2)\, p(g \mid h_1,h_2)$$

Population haplotype pool | Haplotype model | Genotyping model

- Standard settings:
  - $p(g|h_1,h_2) = \mathbf{1}(h_1 \oplus h_1 = g)$ noiseless genotyping
  - $p(h_1,h_2) = p(h_1)p(h_2) = f_1 f_2$ Hardy-Weinberg equilibrium, multinomial
  - $|\mathcal{H}| = K$ fixed-sized population haplotype pool

$$p(g) = \sum_{\substack{h_1,h_2 \in \mathcal{H} \\ h_1 \oplus h_2 = g}} f_1 f_2$$

EM algorithm:

Excoffier and Slatkin (1995)

- Numerical method of finding maximum likelihood estimates for parameters given incomplete data.

1. Initial parameter values: Haplotype frequencies: $f_1,\ldots,f_h$
2. Expectation step: compute expected values of missing data based on initial data
3. Maximization step: compute MLE for parameters from the complete data
4. Repeat with new set of parameters until changes in the parameter estimates are negligible.

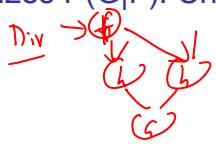
- Beware: local maxima.

EM algorithm efficiency

- Heavy computational burden with large number of loci? (2^L possible haplotypes for L SNPs)
- Accuracy and departures from HWE?
- Error between EM-based frequency estimates and their true frequencies
- Sampling error vs. error from EM estimation process

Bayesian Haplotype reconstruction

- Bayesian model to approximate the posterior distribution of haplotype configurations for each phase-unknown genotype.
- $G = (G_1, \ldots, G_n)$ observed multilocus genotype frequencies
- $H = (H_1, \ldots, H_n)$ corresponding unknown haplotype pairs
- $F = (F_1, \ldots, F_M)$ M unkown population haplotype frequencies
- EM algorithm: Find F that maximizes P(G|F). Choose H that maximizes $P(H|F^{EM}, G)$.



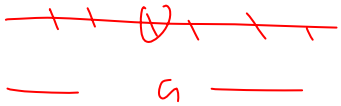
Gibbs sampler

- Initial haplotype reconstruction $H^{(0)}$.
- Choose and individual i, uniformly and at random from all ambiguous individuals.
- Sample $H_i^{(t+1)}$ from $P(H_i|G,H_{-i}^{(t)})$, where H_{-i} is the set of haplotypes excluding individual i.
- Set $H_j^{(t+1)} = H_j^{(t)}$ for j=1,…,i-1,i+1,…,n.

HAPLOTYPER:

Bayesian Haplotype Inference (Niu et al.2002)

- Bayesian model to approximate the posterior distribution of haplotype configurations for each phase-unknown genotype.
- Dirichlet priors $\beta=(\beta_1,\ldots,\beta_M)$ for the haplotype frequencies $F=(f_1,\ldots,f_M)$.
- Multinomial model (as in EM algorithm) for individual haplotypes:
- product over n individuals,
- and multilocus genotype probabilities are sums of products of pairs of haplotype probabilities.



Gibbs sampler

- Haplotypes H are "missing:"

$$P(G,H\mid F)\sim\prod_{i=1,\ldots,n} f_{h_{i1}} f_{h_{i2}} \prod_{j=1,\ldots,n} f_j^{\beta_j-1}$$

- Sample h_{i1} and h_{i2} for individual *i*:

$$P(h_{i1}=g,h_{i2}=h\mid F,G_i)=\frac{f_g f_h}{\sum_{g'\oplus h'=G_i} f_{g'} f_{h'}}$$

- Sample H given $H^{updated}$ Improving efficiency (Niu et al.)

Gibbs sampler

- **Predictive updating (Gibbs sampling)**:
  - (N(H)=vector of haplotype counts)

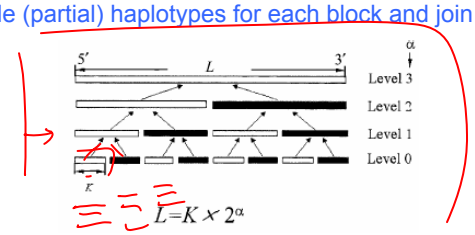
    $P(G,H) \sim \Gamma(|\beta+N(H)|)/\ \Gamma(\beta+N(H))$
  - Pick an individual i, update haplotype h_i:

    $P(h_i=(g,h)|H_{-i},G) \sim (n_g+\beta_g)(n_h+\beta_h)$

    (n_g =count of g in H_{-i})
    - **Prior Annealing**:
  - use high pseudo counts at the beginning of the iteration and progressively reduce them at a fixed rate as the sampler continues.

HAPLOTYPER Discussions

- Missing marker data:
  - PCR dropouts->absence of both alleles,
  - one allele is unscored
  - Gibbs sampler adapts nicely
- Ligation
  - Problem: large number of loci.
  - Partition L loci into blocks of 8 and carry out block level haplotype reconstruction.
  - Record the B most probable (partial) haplotypes for each block and join them
    - Progressive ligation.
    - Hierarchical ligation.



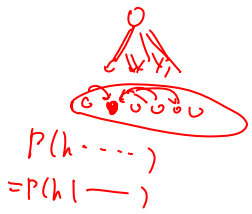
Phase

Coalescence-based Bayesian Haplotype inference: Stephens et al (2001)

- What is $P(H_i | G, H_{-i}^{(t)})$?
- For a haplotype $H_i=(h_{i1},h_{i2})$ consistent with genotypes G_i: $P(H_i|G,H_{-i}) \sim P(H_i|H_{-i}) \sim \pi(h_{i1}|H_{-i})\,\pi(h_{i2}|h_{i1},H_{-i})$
- $\pi(.|H)$=conditional distribution of a future sampled haplotype given previously sampled haplotypes H.
- r=total number of haplotypes, r_α=number of haplotypes of type α, θ=mutation rate, then a choice for

  $$\pi(\alpha|H) = (r_\alpha + \theta\,\mu_\alpha)/(r+\theta),$$

  where μ_α=prob. of type α.



PHASE, details

- This is not working when the number of possible values H_i is too large: 2^{J-1}, J=number of loci at which individual i is heterozygous. Alternatively,

$$\pi(h \mid H) = \sum_{\alpha \in E} \sum_{s=0}^{\infty} \frac{r_\alpha}{r} \left(\frac{\theta}{r+\theta}\right)^s \frac{r}{r+\theta} \left(P^s\right)_{\alpha h}$$

  - where *E*=set of types for a general mutation model, *P*=reversible mutation matrix.

- I.e. future haplotype *h* is obtained by applying a random number of mutations, *s* (sampled from geometric distribution), to a randomly chosen existing haplotype, r_α (coalescent).
- Problems: estimation of *θ*, dimensionality of *P* (dim P = M, the number of possible haplotypes).

PHASE Discussion

- Key: unresolved haplotypes are similar to known haplotypes
- HWE assumption, but robust to "moderate" levels of recombinations
- More accurate than EM,Clark's and Haplotyper algorithms
- Provides estimates of the uncertainty associated with each phase call
- Problem (of both Bayesian model): dimensionality

Summary: Algorithms

- Clark's parsimony algorithm:
  - simple, effective,
  - depends on order of individuals in the data set,
  - need sufficient number of homozygous individuals,
  - Disadvantage: individuals may remain phase indeterminate, biased estimates of haplotype frequencies
- EM algorithm:
  - accurate in the inference of common haplotypes
  - Allows for possible haplotype configurations that could contribute to a phase-unknown genotype.
  - Cannot handle a large number of SNPs.

Summary: Algorithms

Haplotyper:

- Bayesian model to approximate the posterior distribution of haplotype configurations
- Prior annealing helps to escape from local maximum
- Partitions long haplotypes into small segments: block-by-block strategy
- Gibbs sampler to reconstruct haplotypes within each segment. Assembly of segments.
- http://www.people.fas.harvard.edu/~junliu/index1.html#ComputationalBiology

Summary: Algorithms

PHASE:

- Bayesian model to approximate the posterior distribution of haplotype configurations
- based on the coalescence theory to assign prior predictions about the distributions of haplotypes in natural populations,
- may depend on the order of the individuals,
- pseudo posterior probabilities (-> pseudo Gibbs sampler),
- lacks a measure of overall goodness.
- http://www.hgmp.mrc.ac.uk/Registered/Option/phase.html

Reference

- Stephens, M., Smith, N., and Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. American Journal of Human Genetics, 68, 978--989.
- T. Niu, Z.S. Qin, X. Xu, and J. Liu (2002) Bayesian Haplotype Inference for Multiple Linked Single Nucleotide Polymorphisms. Am. J. Hum. Genet
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