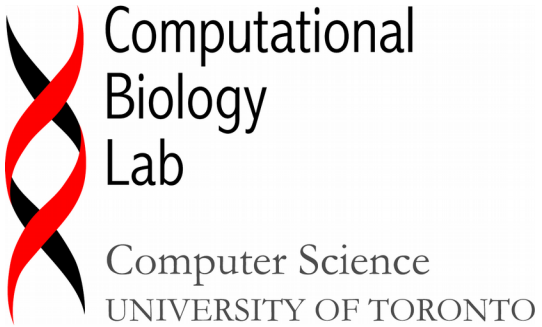
Detecting Copy Number Variation in a Fetal Genome using Maternal Plasma Sequencing



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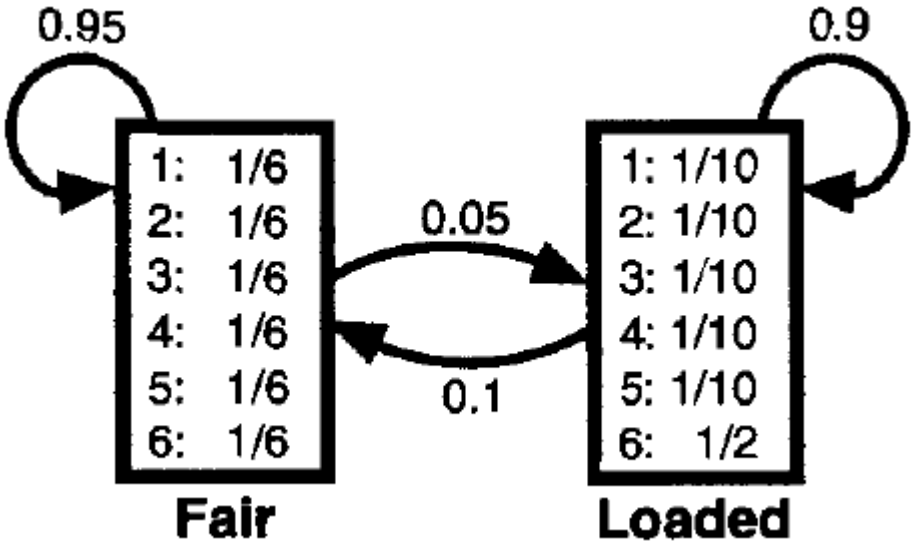
Intro to Hidden Markov Models

- In short an HMM is a probabilistic finite state automaton with “token” emissions in each state
- In an HMM, the state *is not directly visible*, but output that depends on the state *is visible*
- Each state has a probability distribution over the possible output tokens
- Therefore the sequence of tokens generated by an HMM gives some information about the sequence of states

An occasionally dishonest casino dealer

- Suppose a dealer in a casino rolls a die. The dealer use a fair die most of the time, but occasionally he switches to a loaded die. The loaded die has probability 0.5 of a six and probability 0.1 for the numbers 1 to 5.



Intro to Hidden Markov Models (2)

- A path in a HMM is a sequence of states modeled by a Markov chain
- That is (i+1)-th state only depends on the i-th state:

$$P(\ (i+1)\text{-}th\ state = k \mid i\text{-}th\ state = l) =$$
$$= P\ of\ transition\ from\ l\ to\ k$$

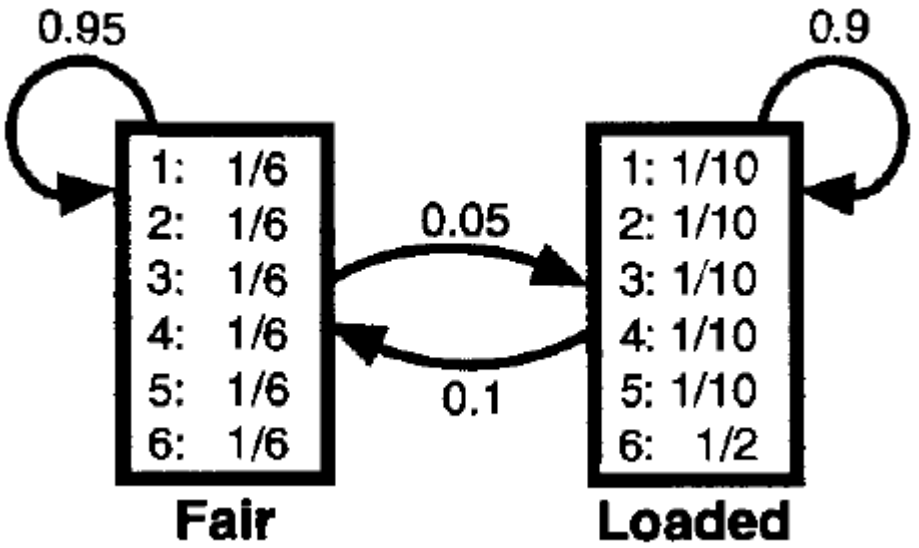
- Emission only depends on the current state:

$$P(\ i\text{-}th\ emission \mid i\text{-}th\ state)$$

Decoding problem in HMM

- Input:
  - HMM given by transition and emission probabilities
  - Sequence of L observations X
- Question:
  - Find the most probable path Z* for X
    i.e. Z* maximizes the joint probability P(X, Z) among all possible paths
- Solution:
  - Viterbi algorithm (simple dynamic programming)

An occasionally dishonest casino dealer




```
Rolls   651166453132651245636664631636663162326455236266666625151631
Die     LLLLLLFFFFFFFFFFFFLLLLLLLLLLLLLLLLLFFFLLLLLLLLLLLLLLFFFFFFFFF
Viterbi LLLLLLFFFFFFFFFFFFLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLFFFFFFF
```

Outline

- **Introduction to non-invasive prenatal testing**
- Methods published so far
- Our novel probabilistic method
- Experiments and results
- Summary and future directions

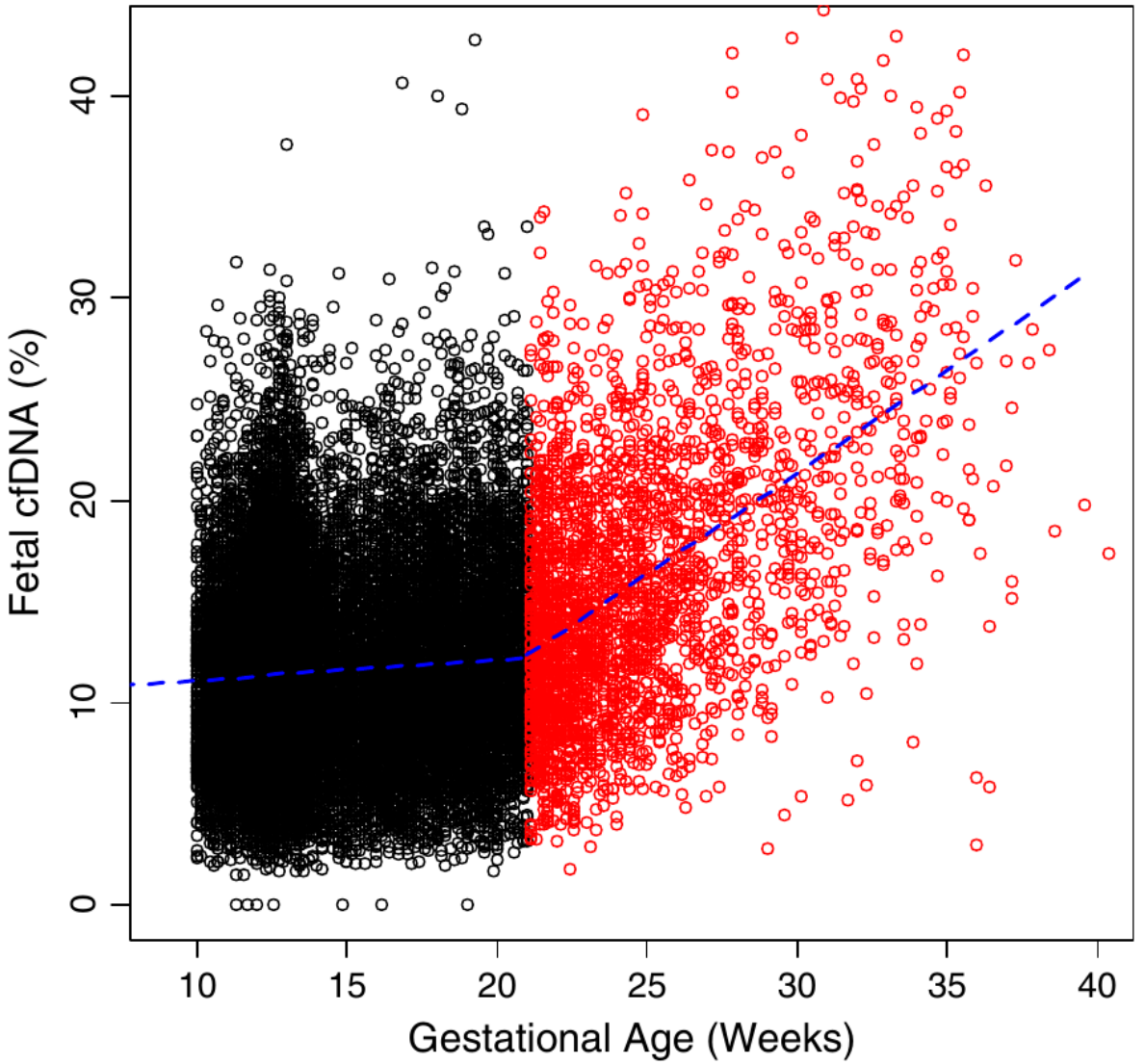
Invasive prenatal diagnosting

- Invasive methods obtain:
  - fetal cells from placenta – *Chorionic villus sampling* (usually at 10-12 weeks' gestation, higher risk)
  - amniotic fluid – *Amniocentesis* (16-22 weeks, lower risk than CVS but still ~0.06%)
- Provide diagnostic quality results (at cost of $1-3k)
- Carry nontrivial risk of miscarriage or other complications
- Amniocentesis is performed relatively late for safe abortion (the majority of abortions are before 12th week)

Noninvasive prenatal testing

- What's needed:
  - Maternal blood plasma during pregnancy
  - Parental genomes (depends on the method)
- Fetal **cell free** cf-DNA is present in maternal plasma
- Admixture ratio is significantly influenced by gestational age and maternal body weight
  + varies largely

Fetal admixture and pregnancy age



Wang, E., Batey, A., Struble, C., Musci, T., Song, K., Oliphant, A.: Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. Prenatal diagnosis (2013)

Fetal admixture vs. maternal weight

Maternal weight bin (kg)	*n*	Pregnancies with ≥4% fetal cell-free DNA (%)
<50	809	99.8
≥50 < 60	4825	99.6
≥60 < 70	6224	99.2
≥70 < 80	4313	98.8
≥80 < 90	2574	98.2
≥90 < 100	1608	96.3
≥100 < 110	921	93.9
≥110 < 120	508	89.8
≥120 < 130	298	87.9
≥130 < 140	172	81.4
≥140	132	71.2

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Noninvasive prenatal testing (2)

- Specialized methods for particular CNVs:
  - Require low depth WGS of plasma or targeted deep sequencing
  - Aneuploidy tests are getting wide-spread, though still “only” testing and not diagnostic quality

2012: Whole fetal genome reconstruction from maternal plasma sample

- Kitzman et al. (Science 2012) – first proof-of-concept for complete fetal genotype reconstruction
  - Used paternal saliva and a tube of maternal blood sampled at 18.5 weeks gestation (78x DOC)
  - Achieved over 99% accuracy after phasing of maternal genotype
- Fan et al. (Nature 2012) – similar results by using “haplotype counting method”, tested on exome sequenced to ~200x and ~600x DOC (!)

2013: Genome-wide detection of fetal CNVs from maternal plasma

- Srinivasan et al. (2013):
  - deep sequencing of 11 plasma samples
  - no need for parental genomes
  - simple binning approach leveraging the number of samples available
  - claim resolution down to 300kb CNVs
- Chen et al. (2013):
  - 1311 low coverage samples as currently used for aneuploidy detection
  - reliable for detection of >10Mb events

CNVs and depth of coverage

- DOC is a standard measure for CNV calling, but there are challenges in its application in this scenario:
  - A CNV signal from single plasma sample is weak due to relatively low fetal fraction in plasma
  - Additionally, distribution of DOC per a region of fixed size is much wider for cf-DNA sequencing than for standard WGS

CNVs and depth of coverage (2)

- Solution: Use multiple plasma samples to estimate statistically significant baselines for CNV calling
- That is what Srinivasan et al. and Chen et al. methods do in their respective way

- Our problem: Only two plasma samples available
- This limits power of our DOC-based predictor, however it is still an important complimentary signal to the allelic distributions

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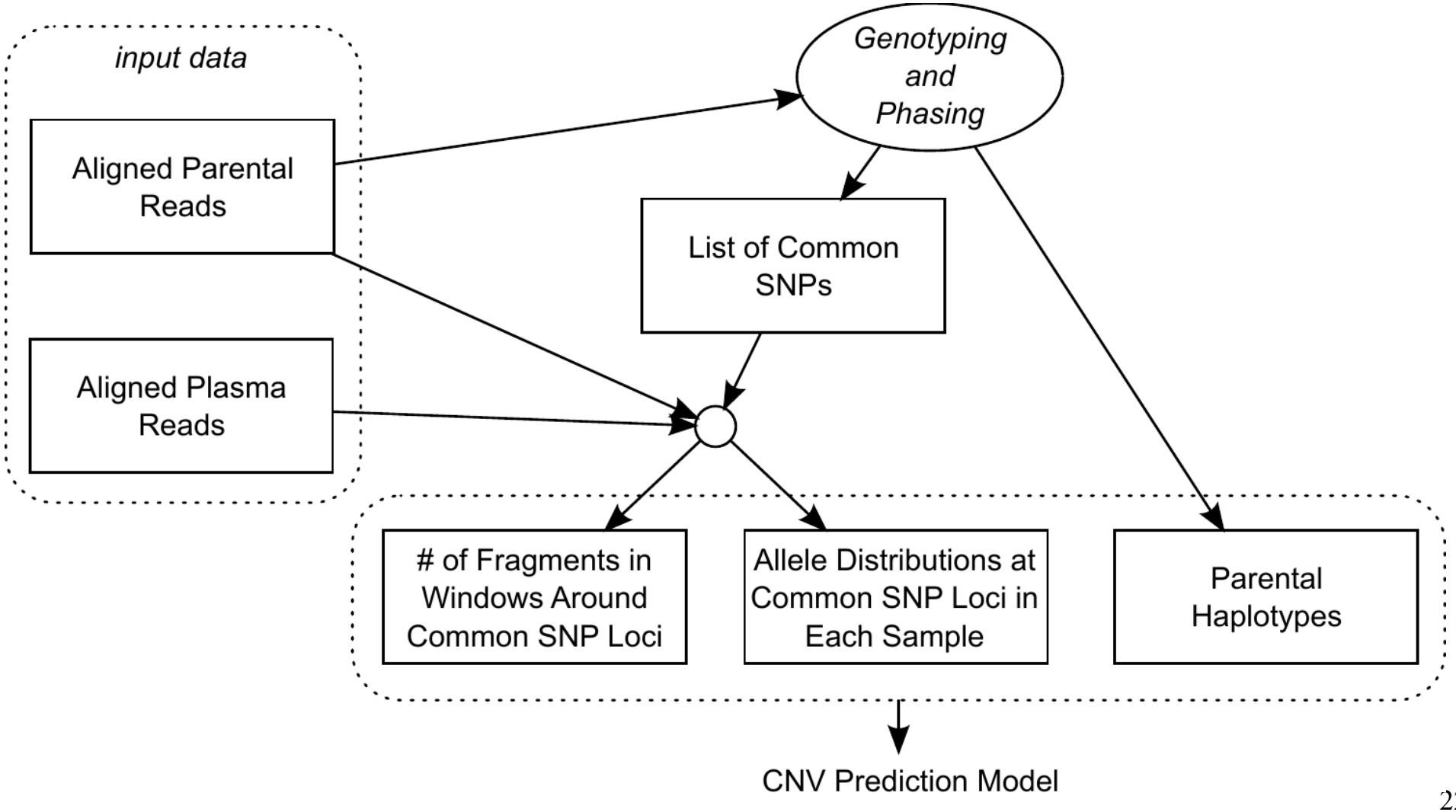
What we address

- Provide confident calls of CNV with mid and large size (several hundreds kilobase and more in size)
- While not relying on availability of multiple datasets (we are using only one reference plasma sample)
- Model additional source of information – **SNP allelic ratios**

Overview of data preprocessing



Overview of our method

- We model 2 types of signal from the data:
  i. change of the allele distributions at SNP loci
  ii. change in number of fragments sequenced from a larger genomic region
- These are combined into a single hidden Markov model where:
  - (i) is interpreted as emission probability
  - (ii) is used as a prior of each HMM state

Overview of our method (2)

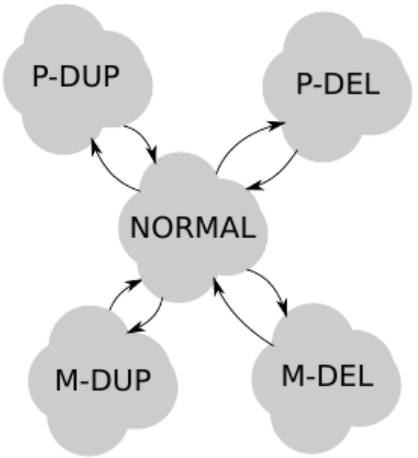
- We model 4 possible inheritances other than normal; **deletion / duplication** of **maternal / paternal** origin
- Thus we require phased parental genomes
- This yields a total of 20 **phased patterns = HMM states**
  - e.g. the following 6 states for maternal duplication:

$$M_A M_A P_A,\ M_A M_B P_A,\ M_B M_B P_A,$$
$$M_A M_A P_B,\ M_A M_B P_B,\ M_B M_B P_B$$

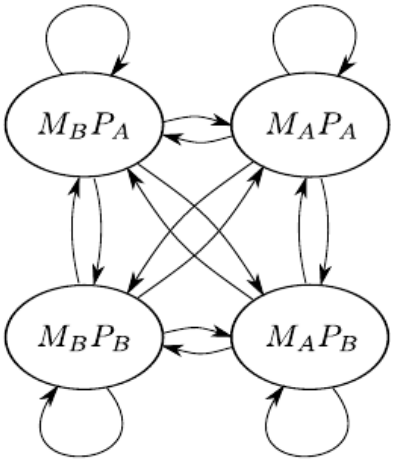
Hidden Markov model for fetal CNV inference

- **20 states** = 20 phased inheritance patterns
- **Emissions** = # of reads supporting individual SNP variants
- **Emission distributions**: expected distributions at SNP loci w.r.t. a particular phased pattern
- **Transition probabilities**: default probabilities set by hand, then for each SNP position we multiply the transition probabilities into the state by the copy number priors obtained by DOC-based predictor
- Viterbi decoding for CNV inference

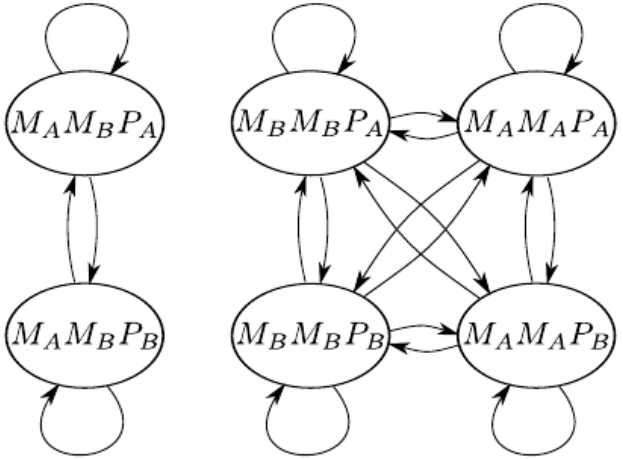
Hidden Markov model for fetal CNV inference (2)



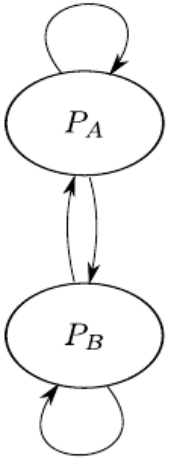
(a) Overall HMM architecture



(b) States for normal inheritance



(c) States for maternal duplication

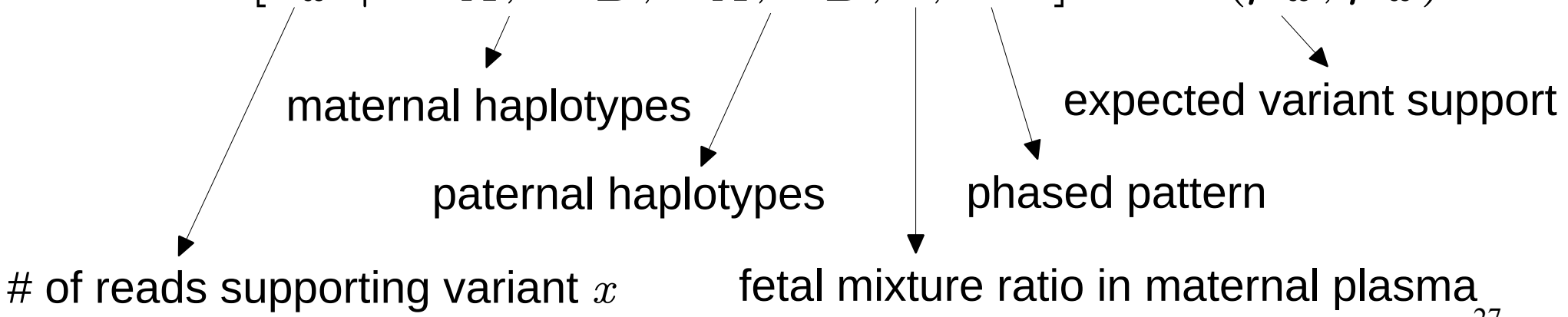


(d) States for maternal deletion

SNP allele distribution w.r.t. a phased pattern

- For every SNP locus we observe a distribution of nucleotides in maternal plasma reads
- Model the probability of the observation given a particular phased pattern *PP* as a Poisson distribution (and approximated by a Gaussian):

$$Pr[k_x \mid M_A, M_B; P_A, P_B; r; PP] \sim \mathcal{N}(\mu_x, \mu_x)$$



Expected variant support μ_x

- Variant support at a SNP position with a duplication in the fetal genome:

$$M_A M_A M_A M_A M_A M_A M_A M_A M_A M_A M_A ... M_A \,|\, M_B M_B M_B M_B M_B M_B M_B M_B M_B M_B M_B ... M_B \,|\, F_1 F_1 F_1 \,|\, F_2 F_2 F_2 \,|\, F_3 F_3 F_3$$

from reads of maternal origin

derived from fetus,

F_1, F_2, F_3 are specified by the phased pattern

- Given parental haplotypes, fetal mixture ratio, overall number of mapped reads, and phased inheritance pattern we can compute the expected μ_x

DOC-based predictor

- For a window of size 1kb centered to a SNP position we compute the probability of the observed number of mapped fragments conditional on number of fetal haplotypes given by the inheritance pattern (HMM state)
- We use *window ratio value* statistic that is corrected for GC content bias and independent of average sample sequencing depth:

fragments aligned to the window W_i

$$WRV_i = \frac{N_{W_i}}{\sum_{W \in neigh_{\mathtt{GC}}^{200}(W_i)} N_W}$$

200 windows with GC content closest to that of W_i

- This measure is analogous to that used by Srinivasan et al.

DOC-based predictor (2)

- Window ratio values obtained for a particular window from two different samples are approximately equal assuming the same underlying copy count in the two samples
- We can adjusted the reference *WRV* w.r.t. fetal copy count given by a particular phased pattern and then compute the conditional probability of the observed *WRV*

DOC-based predictor (3)

- *Problem*: Biased when the copy count in the reference sample is not two (in either maternal of fetal genome)
- Especially when using small window size of 1kb
- *Possible solution*: call CNVs in the reference trio and interrogated trio's maternal sample, then adjust the window ratio values accordingly
- Currently we are not doing any corrections here
- We take this noisy DOC-based predictor as a weak prior to help break ties between phased patterns for which the SNP allele distributions are similar

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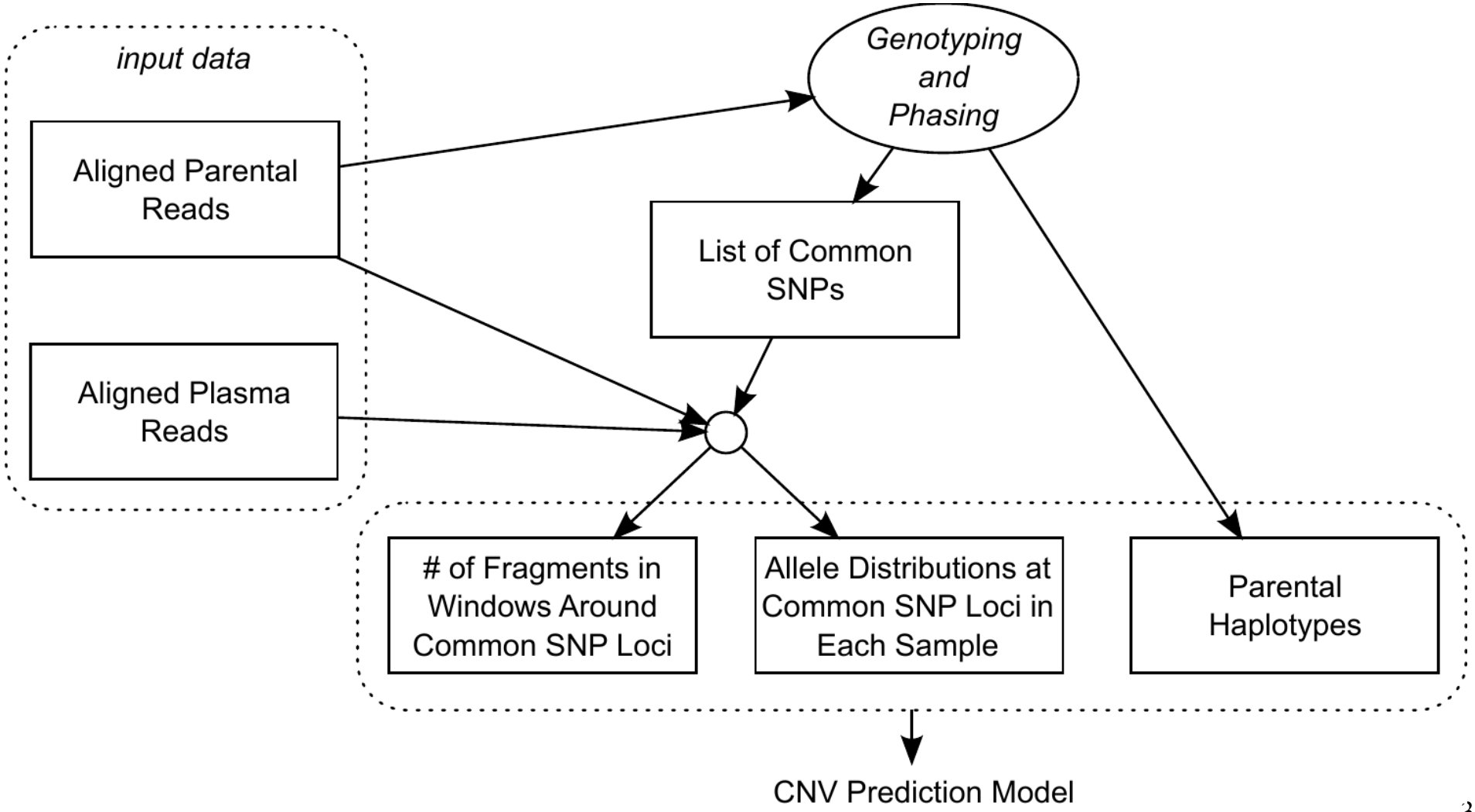
Experiments:
Datasets and Processing

- We have used whole genome sequencing data of two mother-father-child trios I1 and G1, published by Kitzman et al. (2012)
- Interrogated I1 trio:

Individual	*Sample*	*DOC*
Mother	Plasma (18.5 weeks, 13% admixture)	78
	Whole blood	32
Father	Saliva	39
Child	Cord blood at delivery	40

Experiments:
Datasets and Processing (2)



Experiments: Datasets and Processing (3)

- We simulated 360 CNVs in the chr1 of I1 maternal plasma sample:
  - 6 size categories:
    100kb, 300kb, 500kb, 1Mb, 5Mb, 10Mb
  - 6 CNV types:
    duplication of M/P haplotype A/B, deletion of fetal haplotype A/B
  - 10 cases for each category combination, randomly placed in chr1 outside telomers and centromere positions
- We generated an analogous data set after down-rating the fetal admixture in the plasma from 13% to 10%

Results: recall

- For each simulated CNV in chr1 we run our algorithm on a window starting 20Mb before and ending 20Mb after the CNV location

mixture ratio	length		Paternal Del (20)		Paternal Dup (40)		Maternal Del (20)		Maternal Dup (20)	
			ratios	combined	ratios	combined	ratios	combined	ratios	combined
13%	50k - 400k	recall	55	50	55	58	10	15	25	25
		precision	73	19	25	79	67	100	2	4
	400k - 3M	recall	100	100	98	98	30	45	73	68
		precision	100	100	100	100	86	100	23	100
	>3M	recall	95	100	93	98	95	95	100	100
		precision	100	100	100	100	100	100	100	100
10%	50k - 400k	recall	50	45	48	55	0	0	15	13
		precision	71	20	23	82	NA	NA	2	2
	400k - 3M	recall	100	100	90	90	5	15	38	33
		precision	100	100	95	100	100	75	10	87
	>3M	recall	95	100	100	100	45	30	93	85
		precision	100	100	100	100	100	100	97	97

Results: precision

- To test precision we run our algorithm on the whole plasma sample
- Then we compared the calls to CNVnator calls on maternal and paternal genome

Combined Model		50-200k	200-400k	400-750K	750k-3M	3M-7.5M	10M+
in silico CNV recall	Maternal origin	3%	40%	50%	70%	97%	100%
	Paternal origin	40%	70%	100%	97%	97%	100%
WG calls and their (M, P) overlap		51 (6, 3)	13 (2, 2)	5 (1, 0)	3 (1, 2)	0 (0, 0)	0 (0, 0)

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Summary

- New method for *de novo* fetal CNV detection from maternal plasma
- Combines 3 types of information:
  - allelic distribution observed at SNP positions
  - phasing to combine multiple SNP positions signals
  - depth of coverage
- Accurate *in silico* CNV simulation
- Results show sensitivity of 97.5% for CNVs >400kb, and 57.5% for 50-400kb

Future directions

- Better parameter estimation / training
- Try Input/Output HMMs and CRFs
- Mainly for combining DOC and SNP alleles ratio signal
- Improve both signal processing:
  - In SNP alleles ratio, the numbers of reads supporting individual variants are not independent
  - For DOC, add a correction for CNVs in the reference plasma

Thank You!

SNP allele distribution w.r.t. a phased pattern (computation)

$$r' = \frac{|PP| \cdot r/2}{|PP| \cdot r/2 \ + \ (1-r)} \tag{1}$$

$$p_x = \sum_{i \in \{A,B\}} [x \text{ equals } M_i] \cdot m_i(1-r')/2 + \sum_{i=1}^{|PP|} [x \in PP] \cdot r'/|PP| \tag{2}$$

$$m_i = \frac{\alpha + \#\text{reads supporting } M_i \text{ in maternal sequencing}}{2\alpha + \sum_{j \in \{A,B\}} \#\text{reads supporting } M_j \text{ in maternal sequencing}} \tag{3}$$

$$\mu_x = p_x \cdot \#\text{mapped reads} \tag{4}$$

DOC-based predictor (details)

- We compute the probability of sample's WRV_i^S being generated from an event with fetal allele copy count $|PP|$ as

$$\mathcal{N}(\, WRV_i^{R,|PP|} - \, WRV_i^S; \mu = 0, \sigma_{\text{noise}})$$

- Where $WRV_i^{R,|PP|}$ is adjusted reference WRV_i^R w.r.t. fetal copy count given by a particular phased pattern

$$WRV_i^{R,|PP|} = \, WRV_i^R \cdot \big(1 + (|PP| - 2) \cdot r/2\big)$$

CNV simulation

- We performed the *in silico* CNV simulation on the reads level
- Complete trio phasing used to identify haplotypes
- **Duplication**:
  - we first determined the region and target maternal / paternal haplotype to be duplicated
  - separated reads from the maternal / paternal sample originating from this haplotype
  - added expected number of these reads to the plasma sample to simulate a duplication in the fetus

CNV simulation (2)

- **Deletion**:
  - we probabilistically identified reads originating from the target fetal haplotype in plasma sample
  - these reads were then removed from the plasma sample
- **Down-rating fetal admixture** in the plasma sample:
  - similar to deletion
  - uniformly down-sampled reads originating from the fetal haplotypes

Experiments:
Datasets and Processing (2)

- Reads were aligned to the hg19 genome using BWA
- Genotyping was done by Samtools and Vcftools (we only considered sites identified as variable within the 1000 Genomes Project)
- We phased the haplotypes using Beagle 4 with reference haplotype panels from 1000 Genomes Project