Tools and statistical approaches for integrating DNA sequencing into clinical care

Doctorate of Philosophy Dissertation Defense

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Renaissance Computing Institute

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Talk Outline

One-minute primer on human genetics
  Genetic material organized into chromosomes
  Central dogma of molecular biology

Copy number variation in exome sequencing
  Background and motivation
  Better capturing the exome
  Novel copy number variant algorithm
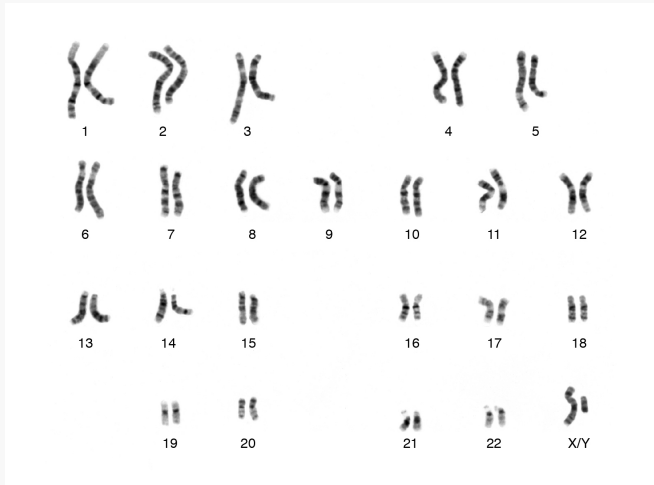
Detecting fetal variation from cell-free DNA
  Background
  Novel maternal-fetal genotyping algorithm
  Shortcomings of noninvasive exome sequencing
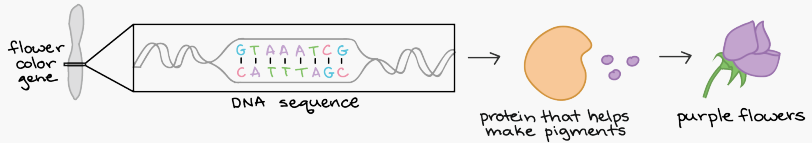
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One-minute primer on human genetics
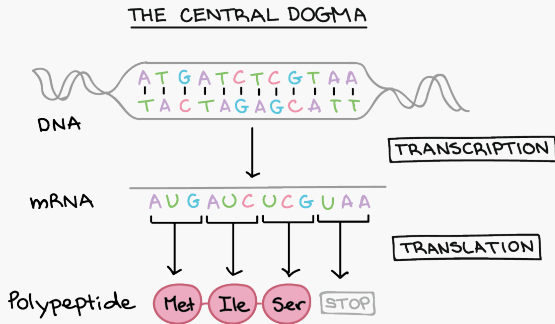
Genetic material organized into chromosomes

(Image taken from NHGRI)

Central dogma of molecular biology

flower color gene

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

DNA sequence

protein that helps make pigments
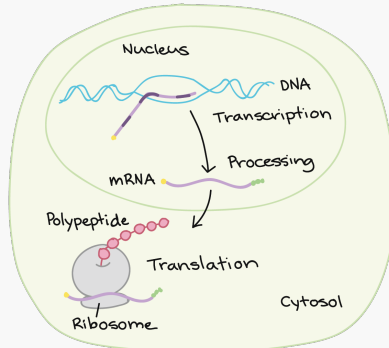
purple flowers

(Image taken from Khan Academy)

Central dogma of molecular biology

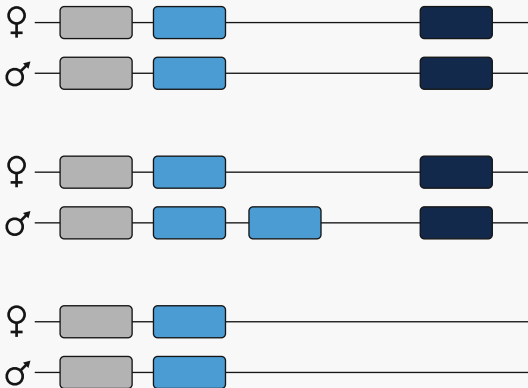(Image taken from Khan Academy)

Central dogma of molecular biology

(Image taken from Khan Academy)

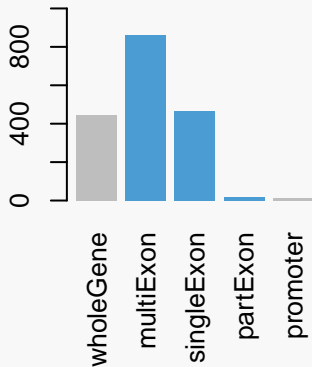# Copy number variation in exome sequencing

What is a copy number variant?

Prevalence and properties of intragenic copy-number variation in Mendelian disease genes

Rebecca Truty, PhD[1], Joshua Paul, PhD[1], Michael Kennemer, MS[1], Stephen E. Lincoln, BS[1], Eric Olivares, PhD[1], Robert L. Nussbaum, MD, FACMG[1,2] and Swaroop Aradhya, PhD, FACMG[1]

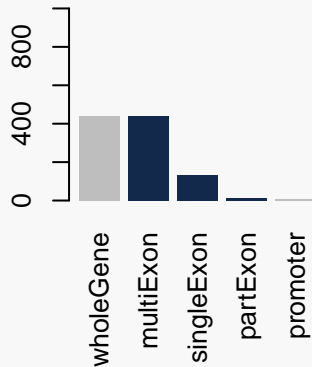*Our analysis identified 2844 intragenic CNVs in 384 clinically tested genes. CNVs were observed in 1.9% of the entire cohort but in a disproportionately high fraction (9.8%) of individuals with a clinically significant result.*
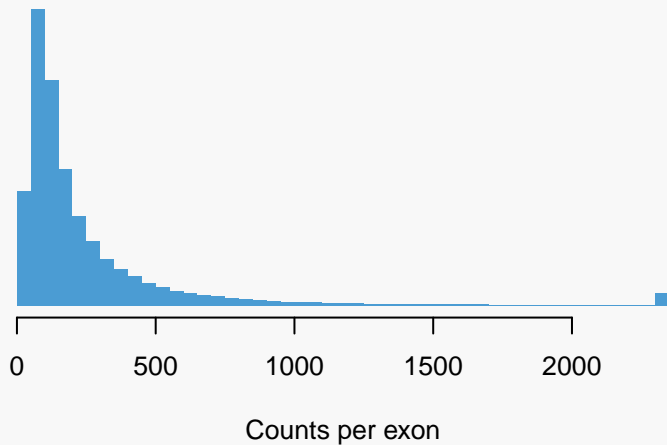
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Deletions / Duplications

Why exome sequencing?

- Only 1-2% of our DNA codes for protein (exome)
- Sequencing only the exome is cost-efficient and easier to interpret

Counts per exon

A robust model for read count data in exome sequencing experiments and implications for copy number variant calling

Vincent Plagnol[1,*], James Curtis[2], Michael Epstein[1,3], Kin Y. Mok[4], Emma Stebbings[2], Sofia Grigoriadou[5], Nicholas W. Wood[4], Sophie Hambleton[6], Siobhan O. Burns[7], Adrian J. Thrasher[7], Dinakantha Kumararatne[8], Rainer Doffinger[8] and Sergey Nejentsev[2]

- ExomeDepth builds a comparative model based on the beta-binomial distribution
- Begins by finding highly-correlated samples to build a control vector
- Defaults to an expected CNV size of 50kb – not expected to perform well for small (exon level) variation

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Can we better capture exomes?

Independent Captures

Multiplexed Capture

Barcoding   Capture   Sequencing

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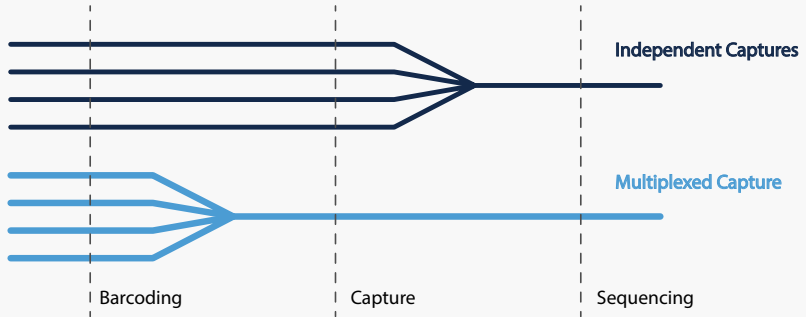
Can we better capture exomes?

pool	capture	N	medExon	medTotal	rsdTotal
IDT-IC[†]	IDT	16	143	55,149,058	22.4
IDT-MC	IDT	16	93	29,772,684	64.2
IDT-RR	IDT	16	272	79,079,629	22.9
NCGENES[†]	Agilent	112	93	24,451,245	27.6
Pool1	Agilent	16	56	13,265,614	18.5
Pool2	Agilent	16	86	21,076,056	27.6
SMA1	Agilent	8	56	12,256,002	6.2
SMA2	Agilent	8	25	5,622,040	10.4
WGS	Agilent	16	196	46,406,224	16.4

Can we better capture exomes?
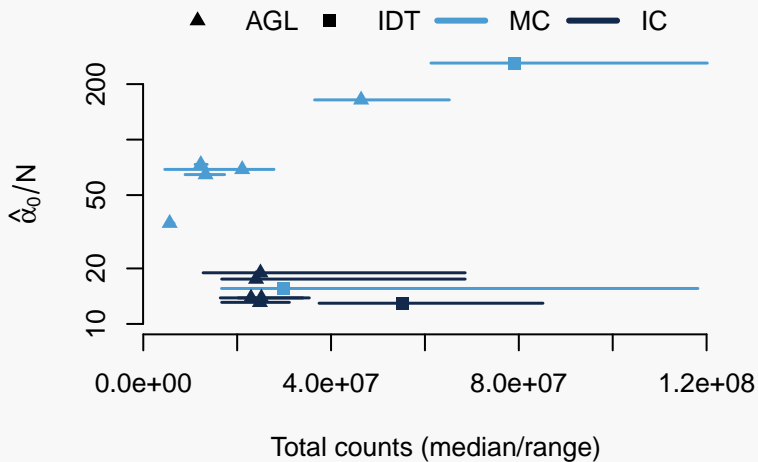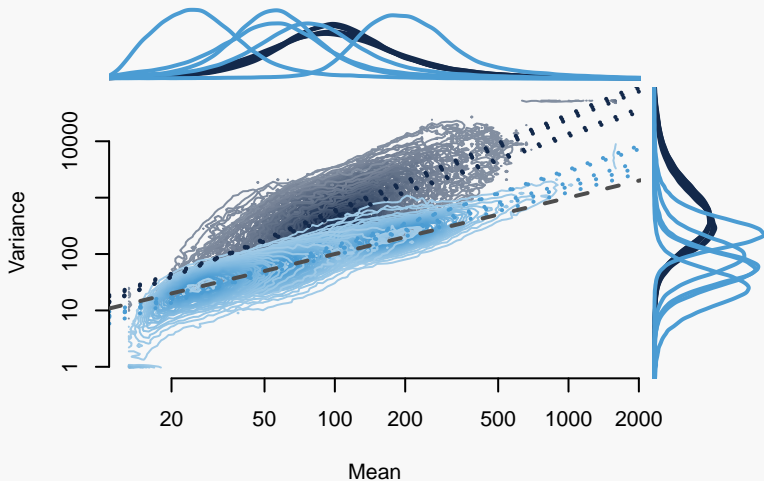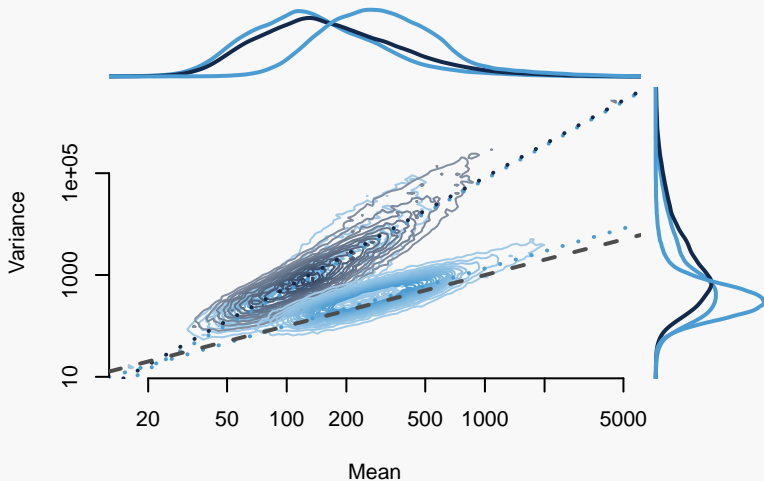
Can we better capture exomes?
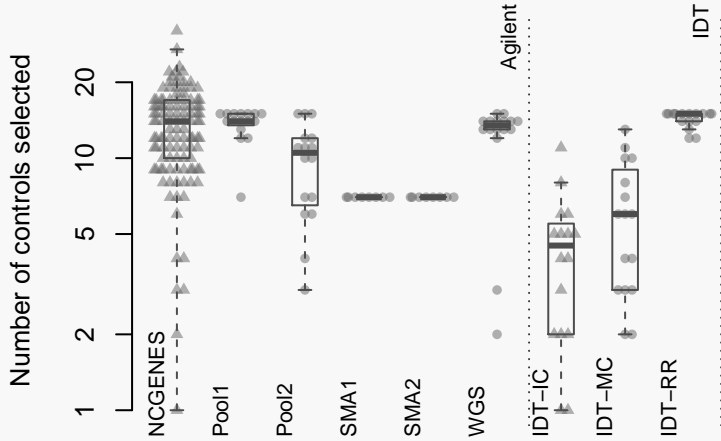
Can we better capture exomes?
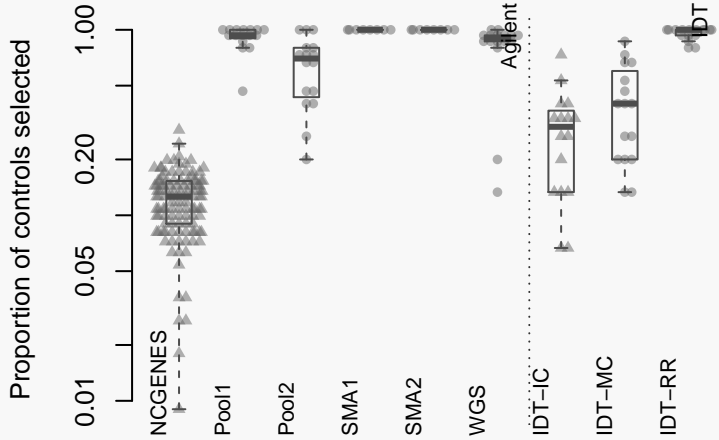
Can we better capture exomes?
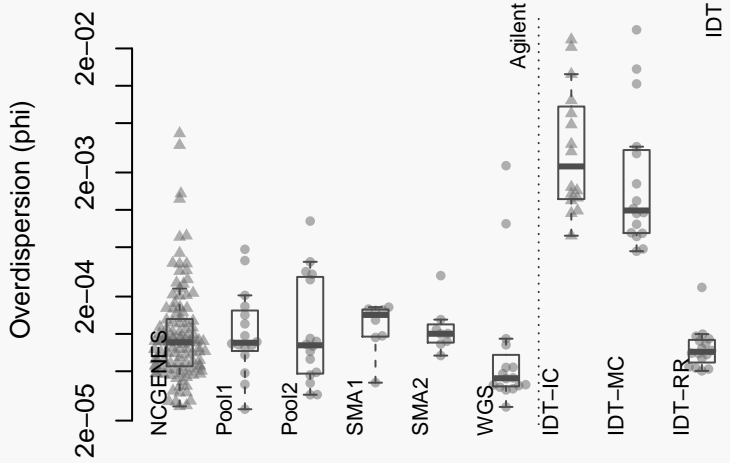
Can we better capture exomes?

Yes!

We recommend

1. Multiplexing roughly 16 samples prior to exome capture

2. Checking for library balance prior to exome capture (from our limited data, we suggest RSD 30%)

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mcCNV algorithm

1. Assumes multiplexed capture
2. Models observed counts using the negative binomial model
3. Uses a shrinkage estimator for the dispersion

Can we better analyze exomes?

Can we better analyze exomes?
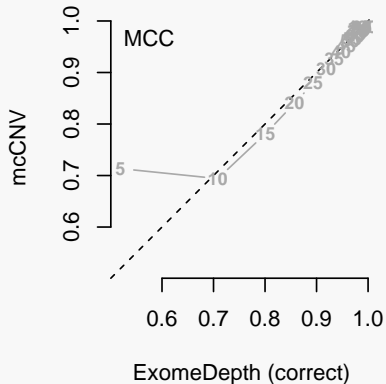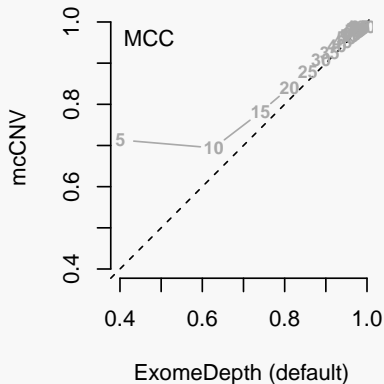
Can we better analyze exomes?
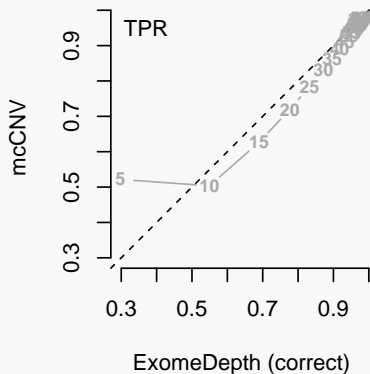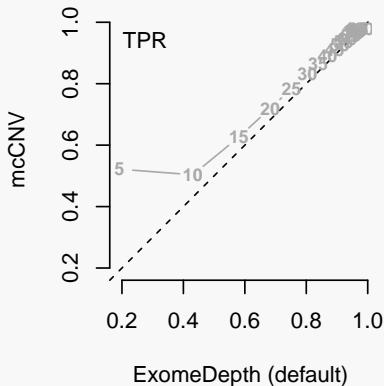
Can we better analyze exomes?
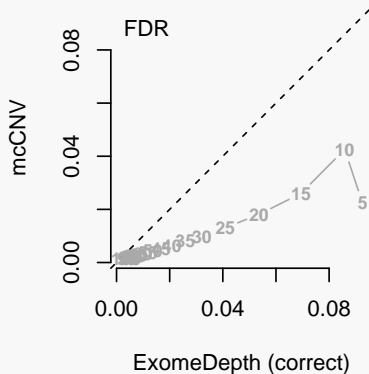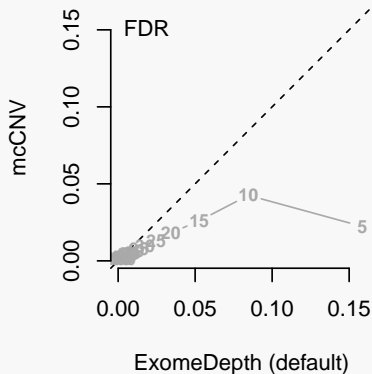
ARTICLE

A Comprehensive Workflow for Read Depth-Based Identification of Copy-Number Variation from Whole-Genome Sequence Data

Brett Trost,[1] Susan Walker,[1] Zhuozhi Wang,[1] Bhooma Thiruvahindrapuram,[1] Jeffrey R. MacDonald,[1] Wilson W.L. Sung,[1] Sergio L. Pereira,[1] Joe Whitney,[1] Ada J.S. Chan,[1,2] Giovanna Pellecchia,[1] Miriam S. Reuter,[1] Si Lok,[1] Ryan K.C. Yuen,[1] Christian R. Marshall,[1,3] Daniele Merico,[1,4,6] and Stephen W. Scherer[1,2,5,6,*]

- Combines calls from two algorithms: ERDS and CNVnator
- Excludes repetitive and low-complexity regions (RCLRs)
- Followed their recommendations, and removed all exons overlapping their defined RLCRs

Can we better analyze exomes?

subject	Total			Duplications			Deletions		
	MC	ED	WG	MC	ED	WG	MC	ED	WG
NCG_00012	90	106	143	61	73	121	29	33	22
NCG_00237	82	101	165	50	64	129	32	37	36
NCG_00525	68	74	151	30	33	110	38	41	41
NCG_00593	45	58	142	22	28	81	23	30	61
NCG_00676	66	78	112	38	46	92	28	32	20
NCG_00790	5,156	2,204	121	19	37	92	5,137	2,167	29
NCG_00819	68	76	134	30	41	100	38	35	34
NCG_00840	78	92	157	44	52	115	34	40	42
NCG_00851	1,151	859	141	28	51	102	1,123	808	39
NCG_00857	59	75	119	10	15	81	49	60	38
NCG_00976	46	58	114	25	37	93	21	21	21
NCG_01023	59	95	143	32	60	113	27	35	30
NCG_01043	73	94	128	40	64	105	33	30	23
NCG_01076	36	57	105	7	22	78	29	35	27
NCG_01077	135	157	230	103	121	184	32	36	46
NCG_01117	95	101	154	72	78	129	23	23	25

Can we better analyze exomes?

			MCC	TPR	FDR	PPV
ALL	Total	MC	0.185	0.335	0.897	0.1030
		ED	0.263	0.363	0.809	0.1910
	Sub	MC	0.487	0.345	0.311	0.6890
		ED	0.482	0.378	0.383	0.6170
DUP	Total	MC	0.396	0.236	0.334	0.6660
		ED	0.347	0.240	0.496	0.5040
	Sub	MC	0.404	0.246	0.333	0.6670
		ED	0.384	0.266	0.446	0.5540
DEL	Total	MC	0.180	0.639	0.949	0.0509
		ED	0.219	0.558	0.914	0.0861
	Sub	MC	0.683	0.661	0.294	0.7060
		ED	0.541	0.554	0.471	0.5290

Can we better analyze exomes?

DUPLICATIONS

DELETIONS

MC ED WG
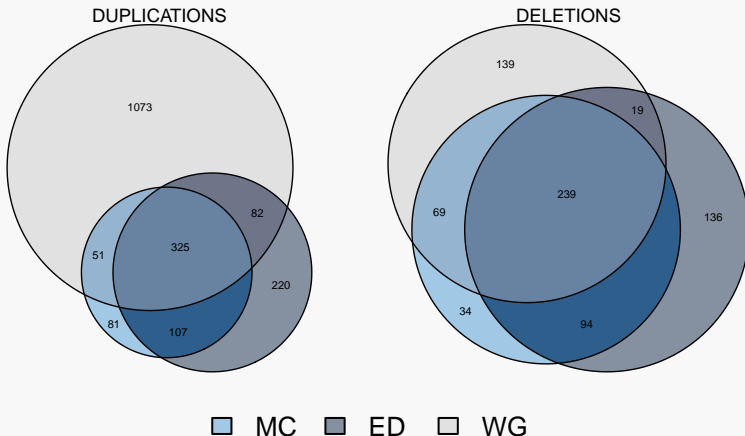
Possibly...

- Found comparable performance overall to ExomeDepth
- However! We do not require prior information

Detecting fetal variation from cell-free DNA

Early report

Presence of fetal DNA in maternal plasma and serum

Y M Dennis Lo, Noemi Corbetta, Paul F Chamberlain, Vik Rai, Ian L Sargent, Christopher W G Redman, James S Wainscoat

Noninvasive testing can now detect:

- aneuploidy and large chromosomal deletions ($>$5Mb)

- autosomal dominant single-gene disorders

- autosomal recessive single-gene disorders using relative haplotype dosing (RHDO)

No one has demonstrated accurate fetal genotyping from cell-free DNA without additional parental sequencing

Fetal genotyping using only cell-free DNA would
1. enable population-level screening for Mendelian disorders, allowing immediate neonatal management;
2. provide more targets for developing *in utero* therapies.
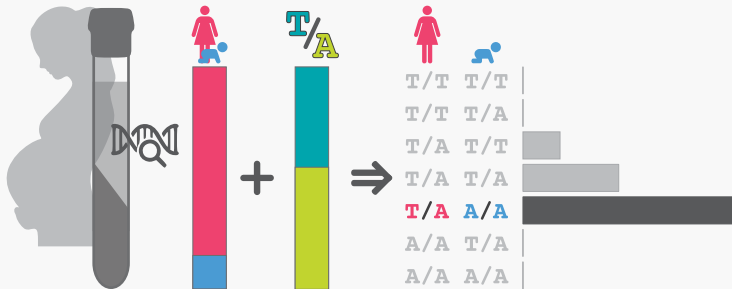
Estimating fetal fraction

Represent maternal and fetal genotype pairs with capital and lowercase letters, where 'A' and 'B' represent the major and minor alleles (e.g. 'AAab' represents the fetus uniquely heterozygous for the minor allele). Define the fetal fraction and PMAR as the random variables F and M.

$$\mathsf{E}[M|G = \mathsf{AAab}, F = f] = \frac{f}{2} \tag{1}$$

$$\mathsf{E}[M|G = \mathsf{ABaa}, F = f] = \frac{1-f}{2} \tag{2}$$

$$\mathsf{E}[M|G = \mathsf{ABab}, F = f] = \frac{1}{2} \tag{3}$$

$$\mathsf{E}[M|G = \mathsf{ABbb}, F = f] = \frac{1+f}{2} \tag{4}$$

$$\mathsf{E}[M|G = \mathsf{BBab}, F = f] = 1 - \frac{f}{2} \tag{5}$$

Estimating fetal fraction

Novel maternal-fetal genotyping algorithm

- Perform empirical Bayes EM routine to identify unique fetal heterozygosity
- Estimate fetal fraction as the median across all sites with fetal heterozygosity
- Estimate maternal-fetal genotypes as the maximal-likelihood given the fetal fraction estimate

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Applying genotyping algorithm to cell-free exome sequencing

	GA	Clinical findings	Genetic diagnosis	FF	Dep	%Dup	%Filt
1	32w2d	5 prior pregnancies affected with X-linked recessive Menke's syndrome	Menke's syndrome; del. ATP7A exon 1	0.117	241	42.80	21.96
2	24w5d	Fetal sonogram at 21w5d showed femoral bowing with shortened length ($<3\%$ for GA) bilaterally	Osteogenesis imperfecta type VIII; P3H1 c.1120G$>$T (rs140468248)	0.122	152	33.32	22.09
3	34w0d	Fetal sonogram at 19w0d showed bilateral club foot with bilateral upper limb arthrogryposis	None, to date, despite exome and genome sequencing of newborn	0.169	330	53.67	32.65

Applying genotyping algorithm to cell-free exome sequencing
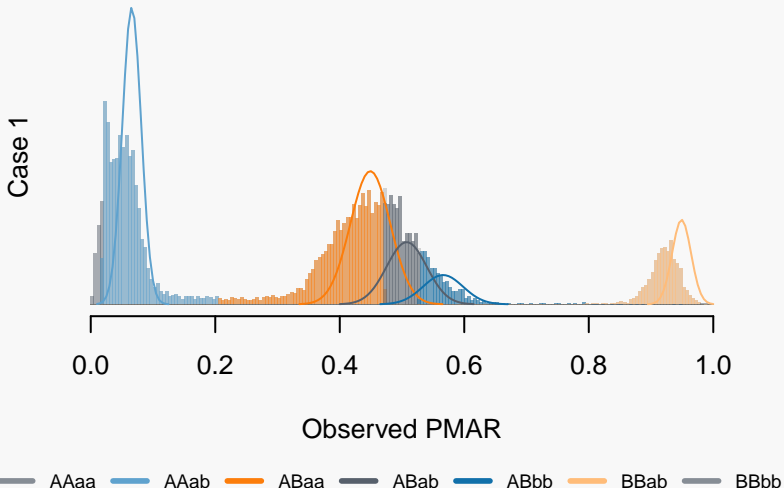
Case 1

Observed PMAR

— AAaa — AAab — ABaa — ABab — ABbb — BBab — BBbb

Applying genotyping algorithm to cell-free exome sequencing
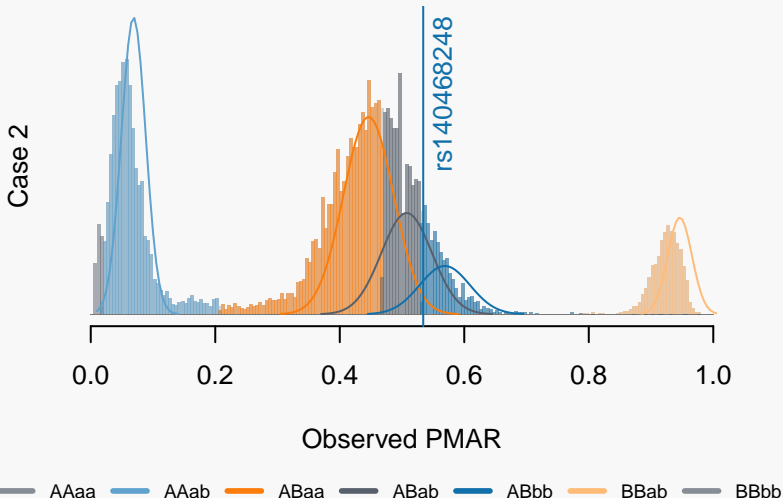
Applying genotyping algorithm to cell-free exome sequencing
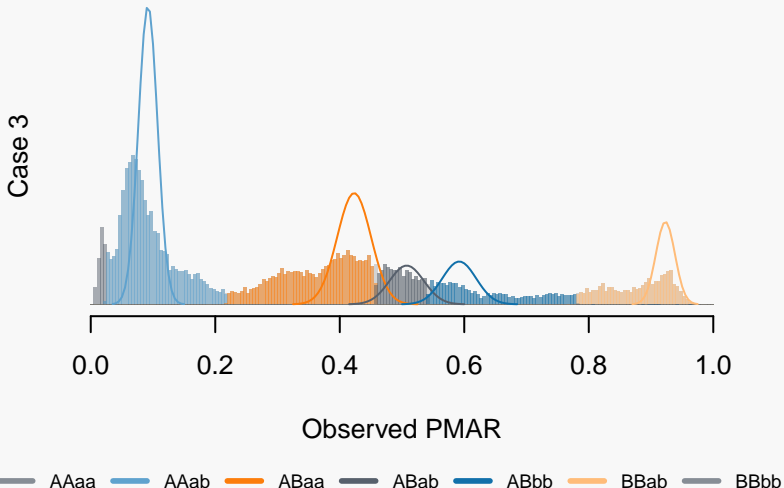
Applying genotyping algorithm to cell-free exome sequencing

- In Case 3, we also have individual exome sequencing for the mother, father, and fetus
- Overall, we found a 50.91% genotyping accuracy

		Cell-free		
		0/0	0/1	1/1
	0/0	1,063	1,857	9
Fetal	0/1	3,598	7,079	1,454
	1/1	76	2,197	1,391

Explaining the poor performance
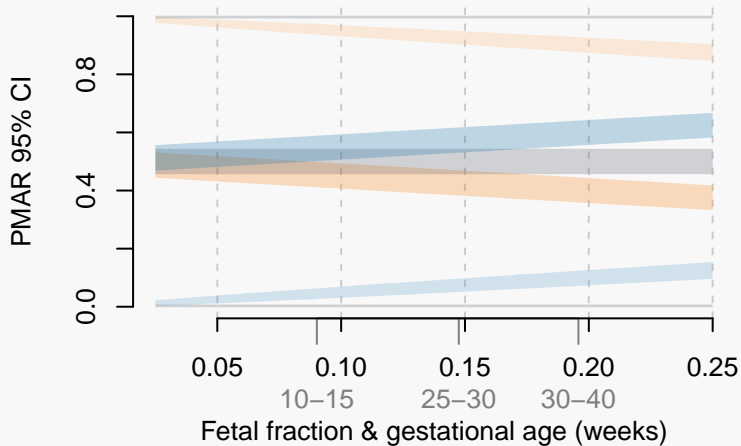
Explaining the poor performance
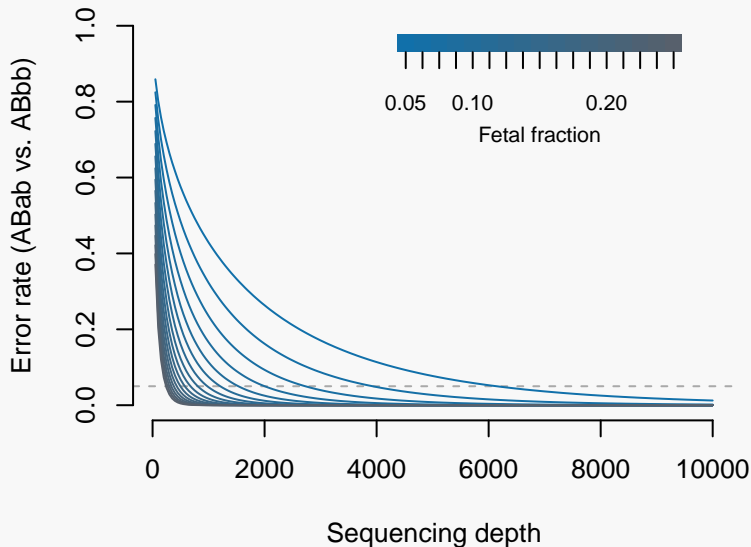
Size Distributions of Maternal and Fetal DNA in Maternal Plasma

K.C. Allen Chan,[1†] Jun Zhang,[1†] Angela B.Y. Hui,[2] Nathalie Wong,[3] Tze K. Lau,[4]
Tse N. Leung,[4] Kwok-Wai Lo,[2] Dolly W.S. Huang,[3] and Y.M. Dennis Lo[1*]

Method

Bayesian-based noninvasive prenatal diagnosis of single-gene disorders

Tom Rabinowitz,[1] Avital Polsky,[1] David Golan,[2] Artem Danilevsky,[1] Guy Shapira,[1]
Chen Raff,[1] Lina Basel-Salmon,[1,3] Reut Tomashov Matar,[3] and Noam Shomron[1]

Does fragment length matter?
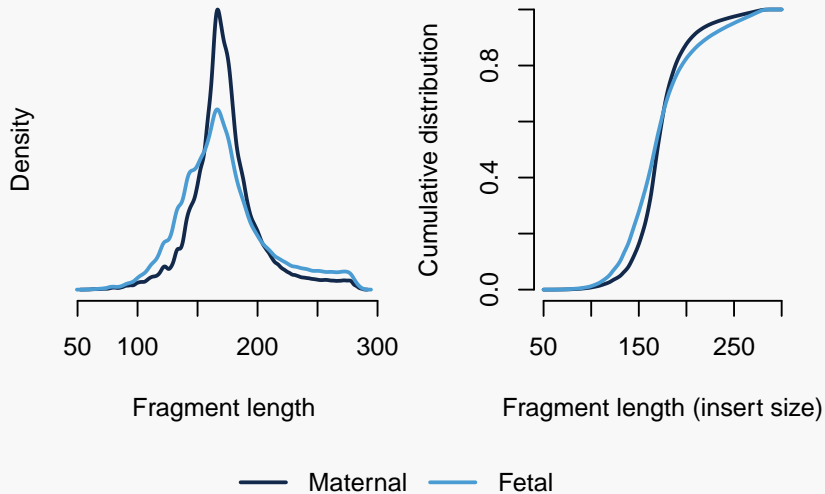
Does fragment length matter?
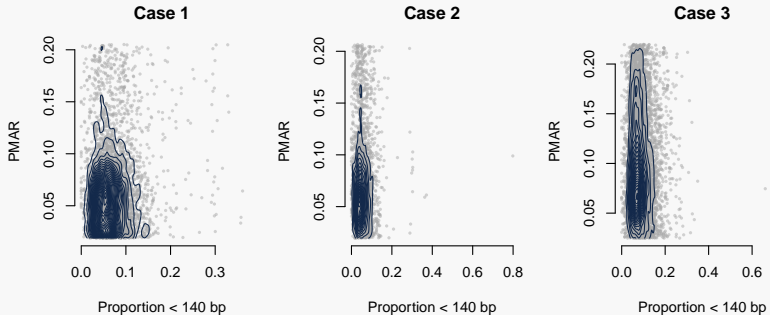
- Rabinowitz et al. found the difference in accuracy varied from -0.25% to 1.89% when using versus not using fragment length in their exome analyses.
- Correcting for fragment length will not overcome the bounds of the binomial distribution

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Summary

- Noninvasive exome sequencing would require cost-prohibitive sequencing depths
- Despite suggestions by others, simply correcting for fragment length will not facilitate noninvasive genome/exome sequencing in the clinic
- We recommend a more targeted approach

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Acknowledgments

- Kirk Wilhelmsen
  - Fenshen Kuo
  - Chris Bizon
  - Jeff Tilson
  - Darius Bost
  - Kimberly Robasky
  - Phil Owen
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  - Bradford Powell (Chair)
  - Stan Ahalt
  - Yun Li
  - Neeta Vora
- Jonathan Berg
  - Christian Tilley
  - Alicia Brandt

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  - Jonathan Cornett
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  - Fernando Pardo-Manuel de Villena
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  - Toni Darville
  - Mohanish Deshmuk
  - Alison Reagan
  - Carol Herrion
  - Amber Brosius
- **Family & Friends**

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Appendix

Appendix

Median count per exon

Negative binomial distribution

Produce simulated exomes

mcCNV algorithm

Venn diagrams with all samples

Aglient capture versus IDT capture

Maternal-fetal genotyping algorithm

All case 3 calls
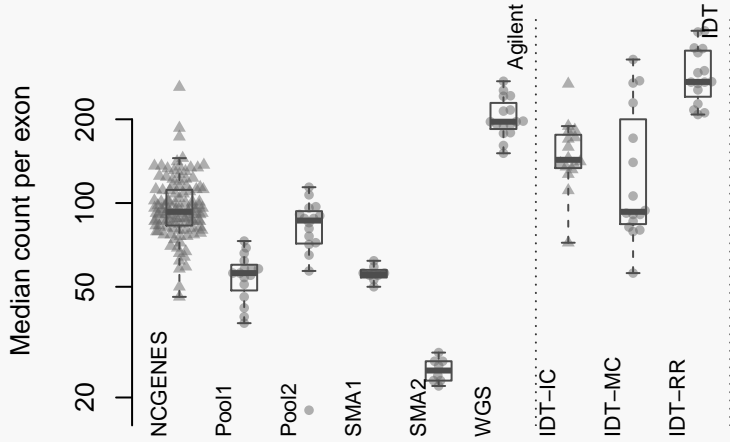
Negavtive binomial distribution

Poisson process: count the number of discrete events in a specific time frame.

$$K \sim \text{Poisson}(\lambda) = e^{-\lambda}\frac{\lambda^k}{k!}; k \in \{0, 1, 2, ...\}$$

$$\text{mean}(K) = \text{variance}(K) = \lambda$$

Negavtive binomial distribution

Negative binomial process: count the number of expected failures before a set number of successes.

$$\Pr\{X = x | p, r\} = \binom{r + x - 1}{x}(1 - p)^x p^r$$

$$\mathsf{E}[X] = \frac{r(1 - p)}{p}$$

$$\mathsf{Var}(X) = \frac{r(1 - p)}{p^2}$$

Where p is the probability of success for each trial & r is the set number of successful trials.

Negavtive binomial distribution

However, we can redefine the probability and size as follows:

$$p = \frac{1}{\mu\phi + 1}, \quad r = \frac{1}{\phi}$$

then,

$$\Pr\{X = x | \mu, \phi\} = \binom{\frac{1}{\phi} + x - 1}{x} \left(\frac{\mu\phi}{\mu\phi + 1}\right)^x \left(\frac{1}{\mu\phi + 1}\right)^{\frac{1}{\phi}}$$

$$\mathsf{E}[X] = \mu$$
$$\mathsf{Var}(X) = \mu + \mu^2\phi$$

Negative binomial distribution

Poisson($\lambda = 10$)

NegBinom($\mu = 10$, $\phi = 0.15$)
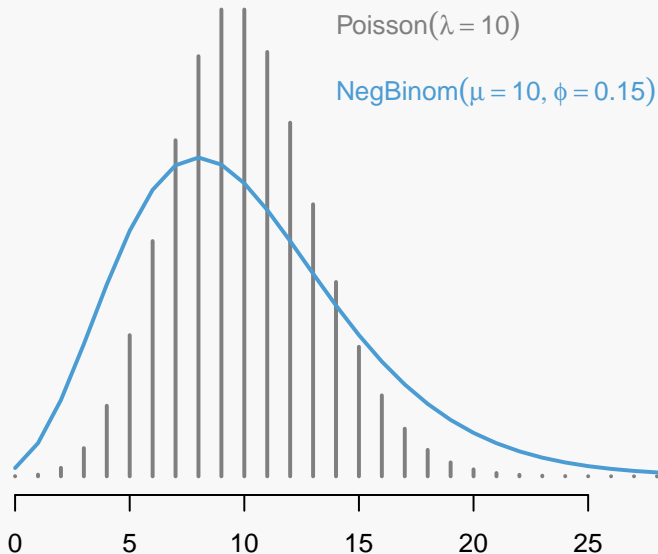
Produce simulated exomes

We use an alternate definition of copy state, such that 1 represents the normal diploid state. Let N represent the total number of molecules (read pairs) and $e_j \in \mathbb{E}$ represent the probability of capturing target j, then for each subject, i:

1. Randomly select $s_{ij} \in \mathbb{S}_i$ from $S = \{0.0, 0.5, 1, 1.5, 2\}$ as the copy number at target j

2. Adjust the subject-specific capture probabilities by the copy number, $\mathbb{E}_i = \frac{\mathbb{E} \odot \mathbb{S}_i}{\sum_j \mathbb{E} \odot \mathbb{S}_i}$

3. Draw N times from Multinomial($\mathbb{E}_i$), giving the molecule counts at each target j for sample i, $c_{ij} \in \mathbb{C}_i$

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mcCNV algorithm

The mcCNV algorithm adjusts the sSEQ probability model by adding a multiplier for the copy state:

$$C_{ij} \sim \mathcal{NB}(f_i s_{ij} \hat{\mu}_j, \tilde{\phi}_j / f_i)$$

where the random variable C_{ij} represents observed molecule counts for subject i at target j, f_i is the size factor for subject i, s_{ij} is the copy state, μ_j is the expected mean under the diploid state at target j, and $\tilde{\phi}_j$ is the shrunken phi at target j. We observe c_{ij} and wish to estimate s_{ij}, $\hat{s}_{ij}$.

mcCNV algorithm

Initialize by setting $\hat{s}_{ij} = 1$ for all i, j. Then,

1. Adjust the observed values for the estimated copy-state,

$$c'_{ij} = \frac{c_{ij}}{\hat{s}_{ij}}.$$

2. Subset c'_{ij} such that $c'_{ij} > 10, \ \hat{s}_{ij} > 0$

3. Calculate the size-factor for each subject

$$f_i = \text{median} \left(\frac{c'_{ij}}{g_j} \right),$$

   where g_j is the geometric mean at each exon.

4. Use method of moments to calculate the expected dispersion

$$\hat{\phi}_j = \max \left(0, \frac{\hat{\sigma}_j^2 - \hat{\mu}_j}{\hat{\mu}_j^2} \right)$$

   where $\hat{\mu}_j$ and $\hat{\sigma}_j^2$ are the sample mean and variance of c'_{ij}/f_i.

mcCNV algorithm

5. Let J represent the number of targets. Shrink the phi values to

$$\tilde{\phi}_j = (1 - \delta)\hat{\phi}_j + \delta\hat{\xi}$$

such that

$$\delta = \frac{\sum\limits_{j} \left(\hat{\phi}_j - \frac{1}{n_j} \sum\limits_{j} \hat{\phi}_j \right)^2 /(J-1)}{\sum\limits_{j} \left(\hat{\phi}_j - \hat{\xi} \right)^2 /(n_j - 2)}$$

and

$$\hat{\xi} = \underset{\xi}{\text{argmin}} \left\{ \frac{d}{d\xi} \frac{1}{\sum\limits_{j} \left(\hat{\phi}_j - \xi \right)^2} \right\}.$$

6. Update $\hat{s}_{ij}$,

$$\underset{s \in S}{\mathrm{argmax}} \left\{ \mathcal{L}(s|c_{ij}, f_i, \hat{\mu}_j, \tilde{\phi}_j) \right\}$$

   where $S = \{0.001, 0.5, 1, 1.5, 2\}$.

7. Repeat until the number of changed states falls below a threshold or a maximum number of iterations is reached.

8. After convergence, calculate p-values for the diploid state, $\pi_{ij} = \mathrm{Pr}(s_{ij} = 1)$.

9. Adjust p-values using the Benjamini–Hochberg procedure and filter to a final call-set such that adjusted p-values fall below some threshold, α.

DUPLICATIONS

1232

86

328

79

299

95

109

DELETIONS

77   159

264   34

3903

2452

713
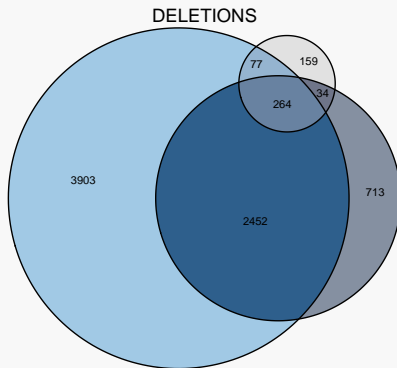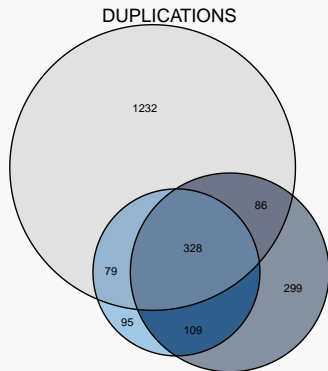
□ MC   ■ ED   □ WG

Aglient capture versus IDT capture

Maternal-fetal genotyping algorithm

Represent maternal and fetal genotype pairs, given by the random variable G, with capital and lowercase letters, where 'A' and 'B' represent the major and minor alleles (e.g. 'AAab' represents the fetus uniquely heterozygous for the minor allele).
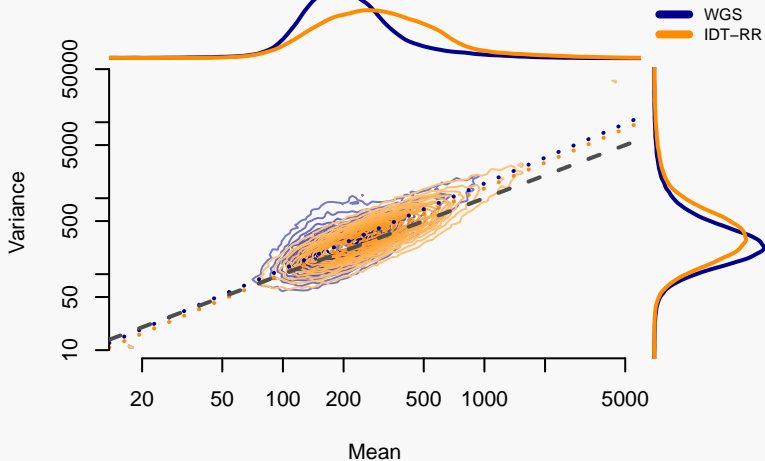
Let X, Y be random variables for major and minor allele read counts. Define the fetal fraction and PMAR as the random variables F and M. We employ an empirical Bayesian expectation-maximization algorithm to identify loci with unique fetal heterozygosity, i.e. $g \in \{\mathsf{AAab}, \mathsf{BBab}\}$. We pick reasonable starting values for the fetal fraction, $F = f$, and the average minor allele frequency, then iteratively update the expected allele distribution and expected PMAR values until some convergence:

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1. Initialize the genotype probabilities, $p_g^* = \Pr\{G = g\}$, and the expected PMAR, $m_g^* = m_g$, based on reasonable estimates for the average minor allele frequency and fetal fraction

2. Update $\hat{\mathbb{G}}$:

$$\hat{g}_i = \underset{g \in G}{\operatorname{argmax}} \left\{ p_g^* \mathcal{L}(g | m_g^*, x_i, y_i) \right\}, Y_i \sim \operatorname{Bin}(x_i + y_i, m_g^*)$$

3. Update the genotype probabilities:

$$p_g^* = \frac{\sum_i \mathsf{I}(\hat{g} = g) + N\Pr\{G = g\} - 1}{\sum_g \left\{ \sum_i \mathsf{I}(\hat{g} = g) + N\Pr\{G = g\} - 2 \right\}}$$

where N is the weight given to the initial estimate of the genotype probability, $\Pr\{G = g\}$.

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4. Update the expected PMAR:

$$m_g^* = \frac{\sum_i y_i \mathsf{I}(\hat{g} = g) + N m_g - 1}{\sum_i (x_i + y_i) \mathsf{I}(\hat{g} = g) + N - 2}$$

where N is the weight given to the initial estimate of the PMAR, m_g.

5. Continue updating $\hat{\mathbb{G}}$, p_g^*, and m_g^* until $\hat{\mathbb{G}}$ converges.

6. For all loci j, such that $\hat{g} \in \{\mathsf{AAab}, \mathsf{BBab}\}$, calculate $\hat{f}_j$:

$$\hat{f}_j = \begin{cases} \dfrac{2y_j}{x_j + y_j}, & \hat{g} = AAab \\[2mm] 2 - \dfrac{2y_j}{x_j + y_j}, & \hat{g} = BBab \end{cases}$$

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7. Let
$$\hat{f} = \text{median}\left(\hat{f}_j\right)$$

8. Calculate the expected PMAR using the fetal fraction estimate,
$$m_g = \mathsf{E}[M|\hat{f}, g]$$

9. Finally, for all loci, i, estimate $\hat{g}_i \in \widehat{\mathbb{G}}$,
$$\hat{g}_i = \underset{g \in G}{\text{argmax}}\left\{\mathcal{L}(g|m_g, x_i, y_i)\right\}, Y_i \sim \text{Bin}(x_i + y_i, m_g)$$

All case 3 calls

Mat	Fet	Cff	N
0/0	0/0	0/0	468
		0/1	1,159
	0/1	0/0	64
		0/1	352
	1/1	0/0	1
		0/1	2
1/1	0/0	0/1	1
		1/1	2
	0/1	0/0	3
		0/1	1,308
		1/1	648
	1/1	0/1	1,601
		1/1	23

Mat	Fet	Cff	N
0/1	0/0	0/0	387
		0/1	107
		1/1	6
	0/1	0/0	3,072
		0/1	1,967
		1/1	713
	1/1	0/0	68
		0/1	458
		1/1	1,291