SI Appendix

Derivations

Calculating fetal DNA fraction in maternal plasma of male pregnancies:

i. With digital PCR TaqMan assays

Digital PCR is the amplification of single DNA molecule. DNA sample is diluted and distributed across multiple compartments such that on average there is less than 1 copy of DNA per compartment. A compartment displaying fluorescence at the end of a PCR represents the presence of at least one DNA molecule.

Assay for Total DNA: EIF2C1 (Chromosome 1)
Assay for Fetal DNA: SRY (Chromosome Y)

The count of positive compartments from the microfluidic digital PCR chip of each assay is converted to the most probable count according to the method described in the supporting information of the following reference:
Warren L, Bryder D, Weissman IL, Quake SR (2006) Transcription factor profiling in individual hematopoietic progenitors by digital RT-PCR. *Proc Nat Acad Sci* 103: 17807-12.

Fetal DNA Fraction ε= (SRY count) / (EIF2C1 count / 2)

ii. With sequence tags

From ChrX:

Let fetal DNA fraction be ε

	Maternal Contribution	Male Fetus Contribution	Female Fetus Contribution
ChrX	$2(1-\varepsilon)$	ε	2ε

Male pregnancies ChrX sequence tag density (fetal and maternal) = $2(1-\varepsilon) + \varepsilon = 2 - \varepsilon$
Female pregnancies ChrX sequence tag density (fetal and maternal) = $2(1- \varepsilon) + 2\ \varepsilon = 2$
Let x be the ratio of ChrX sequence tag density of male to female pregnancies. In this study, the denominator of this ratio is taken to be the median sequence tag density of all female pregnancies.

Thus, fetal DNA fraction $\varepsilon = 2(1-x)$

From ChrY:

Fetal DNA fraction ε =
(sequence tag density of ChrY in maternal plasma/sequence tag density of ChrY in male plasma)

Note that in these derivations, we assume that the total number of sequence tags obtained is the same for all samples. In reality, the total number of sequence tags obtained for different sample is different, and we have taken into account such differences in our estimation of fetal DNA fraction by normalizing the sequence tag density of each chromosome to the median of the autosomal sequence tag densities for each sample (see Methods).

Calculating fetal DNA fraction in maternal plasma of aneuploid (trisomy) pregnancies:

Let fetal DNA fraction be ε

	Maternal Contribution	Trisomic Fetus Contribution	Disomic Fetus Contribution
Trisomic Chromosome	$2(1-\varepsilon)$	3ε	2ε

Trisomic pregnancies trisomic chromosome sequence counts (fetal and maternal)
$= 2(1-\varepsilon) + 3\varepsilon = 2 + \varepsilon$
Disomic pregnancies trisomic chromosome sequence counts (fetal and maternal)
$= 2(1- \varepsilon) + 2\ \varepsilon = 2$
Let x be the ratio of trisomic chromosome sequence counts (or sequence tag density) of trisomic to disomic pregnancies. In this study, the denominator of this ratio is taken to be the median sequence tag density of all disomic pregnancies.

Thus, fetal DNA fraction $\varepsilon = 2(x-1)$