**Supplemental Methods**

*Enet*

Enet is a regularized regression that is a combination of LASSO (least absolute shrinkage and selection operator) and ridge regression. Using the Enet procedure([1](#_ENREF_16)), a model was built to predict fetal DNA fraction as estimated by male euploid chromosome Y representation([2](#_ENREF_15)). Specifically, we utilized the R package ‘glmnet’ (version 1.9-5) with an alpha parameter of 0.01. We then employed ten-fold cross-validation to estimate the appropriate lambda parameter based on the “one-standard-error” rule([1](#_ENREF_16)). The final model may be written as

where β is determined by Enet for *ith* 50kb genomic region amongst *n* autosomal bins, *X* is the normalized counts for bin *i*, *Y* is the estimated chromosome Y representation(2), and δ is a correction parameter defined by Enet. These model parameters are learned from the laboratory developed test dataset.

*WRSC*

The WRSC method(3,4) is a two-step prediction method which uses a multivariate response model. Instead of predicting the fetal DNA fraction directly, the individual bin values for chromosome Y are predicted using Reduced-Rank Regression with the rank determined by the criterion, WRSC, using the close-form formula for the tuning parameter. This model takes into account not only the correlation between autosomal bins as predictors but also the correlation between chromosome Y bins.

This model may be written as

where is a matrix of samples and chromosome Y bins(2) and is a matrix of normalized sequence read counts for samples and autosomal bins(5). The error matrix, *E*, is assumed to have terms with mean zero and covariance . The unknown matrices and are full-rank matrices of sizes and with unknown and are determined from the training data set. The combined matrix is a reduced-rank matrix of rank . The unknown matrix of coefficients may be solved by the WRSC which is

where is a matrix of weights corresponding to the covariance of , is the tuning parameter, and is the rank of the matrix . The weight matrix estimated by the sample covariance of the response variables provides good predictive results. These model parameters are derived from the laboratory developed test dataset. The predicted chromosome Y bins were then used to estimate the fetal fraction as described in Mazloom *et al.*([2](#_ENREF_15)).

**SupplementalFigure1.** SeqFF comparisons between Enet and WRSC.

**SupplementalFigure2.** (Left) Comparison of SeqFF with Jensen *et al.* or Alkan *et al.* data normalization method. (Right) Comparison of SeqFF with Alkan et al. against chromosome Y-based fetal DNA fraction based on Mazloom *et al.*

**SupplementalFigure3.** Distribution of Enet model coefficients partitioned into 25% quantiles of fragment ratios per 50kb bins.

**SupplementalFigure4.** Distribution of WRSC model coefficients partitioned into 25% quantiles of fragment ratios per 50kb bins.

**SupplementalFigure5.** Similarities of WRSC and Enet model coefficients. Bins with zero weights in Enet are removed.

**SupplementalTable1.** SNP information and primer sequence for the SNP-based fetal DNA fraction assay.

**SupplementalTable2.** Genomic coordinates, fragment ratios, GC content, exon density for each 50kb genomic region.

SeqFF.R – R script to calculate SeqFF from a sam file or tabulated counts of aligned reads for each 50kb genomic region.

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2) Mazloom AR, Dzakula Z, Oeth P, Wang H, Jensen T, Tynan J, et al. Noninvasive prenatal detection of sex chromosomal aneuploidies by sequencing circulating cell-free DNA from maternal plasma. Prenatal diagnosis. 2013 Jun;33(6):591-7. PubMed PMID: 23592550. Epub 2013/04/18. eng.

3) Geis J. ProQuest Dissertation Accession Order No AAT 3519397.

4) Izenman AJ. Reduced-rank regression for the multivariate linear model. Journal of Multivariate Analysis. 1975 6//;5(2):248-64.

5) Jensen TJ, Zwiefelhofer T, Tim RC, Dzakula Z, Kim SK, Mazloom AR, et al. High-throughput massively parallel sequencing for fetal aneuploidy detection from maternal plasma. PloS one. 2013;8(3):e57381. PubMed PMID: 23483908. Pubmed Central PMCID: PMC3590217. Epub 2013/03/14. eng.