Appendix 1. Institutional Review Board (IRB) Approvals

Samples collected in accordance with all local privacy and patient protection laws (Gennet [Czech Republic], Fetal Life Science Center [Japan], International Kent Hospital [Turkey]), or under the following IRB approvals: Columbia University Institutional Review Board (Columbia University [New York, NY]), Mount Sinai School of Medicine Institutional Review Board (Mount Sinai School of Medicine [New York, NY]), Ethical and Independent Review Services (Carnegie Hill Imaging for Women [New York, NY], Madonna Perinatal [Mineola, NY], New Beginnings Perinatal Center [Brooklyn, NY], Medical Group Watching Over Mothers and Babies [Tucson, AZ], South Florida Perinatal [Miami, FL], Houston Perinatal [Houston, TX], Daniel R. Bourgue Obstetrics and Gynecology [Lafayette, LA], DBA Soapstone Center for Clinical Research [Decatur, GA], San Francisco Perinatal Associates [San Francisco, CA], St. Vincent Hospital Center for Prenatal Diagnosis [Indianapolis, IN], St. Bernardine Medical Center [San Bernardino, CA], Bellevue Health and Emergency Center [New York, NY], Desert West Obstetrics and Gynecology [Glendale, AZ], Goodman and Partridge Obstetrics and Gynecology [Chandler, AZ], Pacific Fertility Center of San Francisco [San Francisco, CA], Perinatal Fertility Center of Iowa [Waukee, IA], Mayfair Women's Center [Aurora, CO], Maternal-Fetal Medicine Group [San Gabriel, CA]), Baylor College of Medicine Institutional Review Board (Baylor University [Waco, TX]), Stanford Institutional Review Board (Stanford University [Palo Alto, CA]), Einstein Institutional Review Board (Einstein Montefiore Medical Center [New York, NY]), Tufts Health Sciences Campus Institutional Review Board (Tufts University [Medford, MA]), Yale University Human Investigation

Committee (Yale University [New Haven, CT]), New York Methodist Hospital Institutional Review Board (New York Methodist Hospital [New York, NY]), Research Ethics Committee at the Rotunda Hospital in Dublin (Royal College of Surgeons in Ireland, Rotunda Hospital [Dublin, Ireland]), Comite Etico Investigacion Clinical de USP Institute Universitari Dexeus (Institut Universitari Dexeus [Barcelona, Spain]), BioMed Institutional Review Board (StemExpress [Placerville, CA]), Western Internal Review Board (Women's Healthcare Group of Pennysylvania [Wynnewood, PA], Suburban Maternal Fetal Medicine LLC [Hoffman Estates, IL]), Polish Mother's Memorial Hospital Institutional Review Board (Polish Mother's Memorial Hospital-Research Institute [Lodz, Poland]), Oklahoma University Health Science Center Institutional Review Board (University of Oklahoma Health Science Center [Oklahoma City, OK]).

Appendix 2. Patient Demographics

	Overall (n=1064)	Euploid (n=926)	Aneuploid (n=138)
Maternal Age (years)			
Mean ± SD	30.3 ± 7.4	29.6 ± 7.2	35.1 ± 6.5
Median	30.0	29.0	37.0
Range	18-47	18-47	18-46
Gestational Age (weeks)			
Mean ± SD	17.0 ± 4.1	17.2 ± 8.9	15.8 ± 4.6
Median	14.3	14.1	14.6
Range	7.6-40.6	7.6-40.6	8.0-38.9
Karyotype Source			
Amniocentesis/CVS	469 (44.1%)	344	125
Products of conception	455 (42.8%)	443	12
Cord blood/buccal/saliva	140 (13.2%)	139	1
High-risk[1]	543 (51.0%)	414	129
Low-risk[2]	521 (49.0%)	512	9

[1.] High-risk defined after positive serum screen, ultrasound abnormality, and/or maternal age of ≥35 years.

[2.] Low-risk defined as maternal age of <35 years and lacking any reported high-risk indications.

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Appendix 3: Samples Considered Outside the Specifications for Testing at the Time of Analysis and How They Would be Called Using the Current Clinical Protocol

Of the 1,064 cases included in the current study, 13 were considered outside the specifications for testing (Figure 1). These included 6 confirmed triploid samples, 4 samples with a confirmed sex chromosome abnormality other than monosomy X (1 XXX, 2 XXY, 1 XYY), and 3 samples with confirmed fetal mosaicism (1 trisomy 13, 2 monosomy X). Note that the current protocol now also includes detection of XXX, XXY, XYY, and triploidy (1, 2). As such, 10 of 13 samples would generate a call using the latest protocol (Appendix 3): three of the 4 sex chromosome abnormalities, 2 of the 3 mosaic cases, and all 6 confirmed triploidy cases identified as “extremely low fetal fraction” (see Results). However, as is the case with all NIPT approaches, there are some aneuploid pregnancies that would be missed.

Of the three confirmed fetal mosaic cases, the algorithm generated high-confidence high-risk results for both monosomy X mosaic cases, but did not return a result due to low fetal fraction for the trisomy 13 case. The only mosaic case with a confirmed non-mosaic fetus (and thus included in analyses) was the trisomy 18 case, where the algorithm generated a high-confidence low-risk result for trisomy 18 at a fetal fraction of 18%. This case was diagnosed as mosaic after direct sampling of the products of conception identified approximately 60% trisomy 18 and 40% euploid cells in the placenta, whereas the fetus was found to be non-mosaic trisomy 18 (data not

shown). This supports previous reports that fetal cell-free DNA originates from a subset of placental cells (3).

Appendix 4: Samples Considered Outside the Specifications for Testing at the Time of Analysis and How They Would be Called Using the Current Clinical Protocol

Sample	Call Using Current Protocol
Confirmed triploid	Extremely low fetal fraction
Confirmed triploid	Extremely low fetal fraction
Confirmed triploid	Extremely low fetal fraction
Confirmed triploid	Extremely low fetal fraction
Confirmed triploid	Extremely low fetal fraction
Confirmed triploid	Extremely low fetal fraction
Confirmed XXX	XXX
Confirmed XXY	XXY
Confirmed XXY	No-call due to low fetal fraction
Confirmed XYY	XYY
Confirmed fetal mosaic trisomy 13	No-call due to low fetal fraction
Confirmed fetal mosaic monosomy X	Monosomy X
Confirmed fetal mosaic monosomy X	Monosomy X

Appendix 5: No-Calls

		No-Calls				
Total Samples* (N=85)	**No-Calls, Total**	**Trisomy 21 (n=8 [1])**	**Trisomy 18 (n=7)**	**Trisomy 13 (n=2)**	**Monosomy X (n=2)**	**Euploid (n=66)**
Low fetal fraction	64	6	6	2	1	49
Low input DNA	12	1	0	0	0	11
Contamination	6	1	0	0	0	5
LOH	2	0 [1]*	1	0	1	0
Poor model fit	1	0	0	0	0	1

*Excludes no-calls only on single chromosomes, including one trisomy 21 sample that was a no-call only on chromosome 21 due to loss of heterozygosity.

LOH, loss of heterozygosity.

Appendix 6. Performance of the Externally Blinded Cohort

Of the 487 externally blinded samples (441 euploid and 46 aneuploid: 29 trisomy 21, 7 trisomy 18, 5 trisomy 13, 5 monosomy X), only 3.5% (17/487) did not return a result based upon a single sampling. This included 9 euploid and 8 aneuploid (3 trisomy 21, 3 trisomy 18, 1 trisomy 13, 1 monosomy X) samples.

Of the 470 externally blinded samples that passed quality control, 39 were aneuploid (26 trisomy 21, 4 trisomy 18, 4 trisomy 13, and 5 monosomy X). The Next-generation Aneuploidy Test Using SNPs algorithm accurately identified fetal copy number for all autosomes, but made one false negative call on monosomy X. No false positives calls were made. When considering only the autosomal chromosomes, the overall sensitivity was 100% (34/34, CI: 89.7-100%); when including monosomy X, the sensitivity was 97.4% (38/39, CI: 86.2-99.9%). The specificity for all syndromes was 100% (431/431 CI: 99.15-100%) on a per-sample basis. This was improved performance when compared to the overall cohort.

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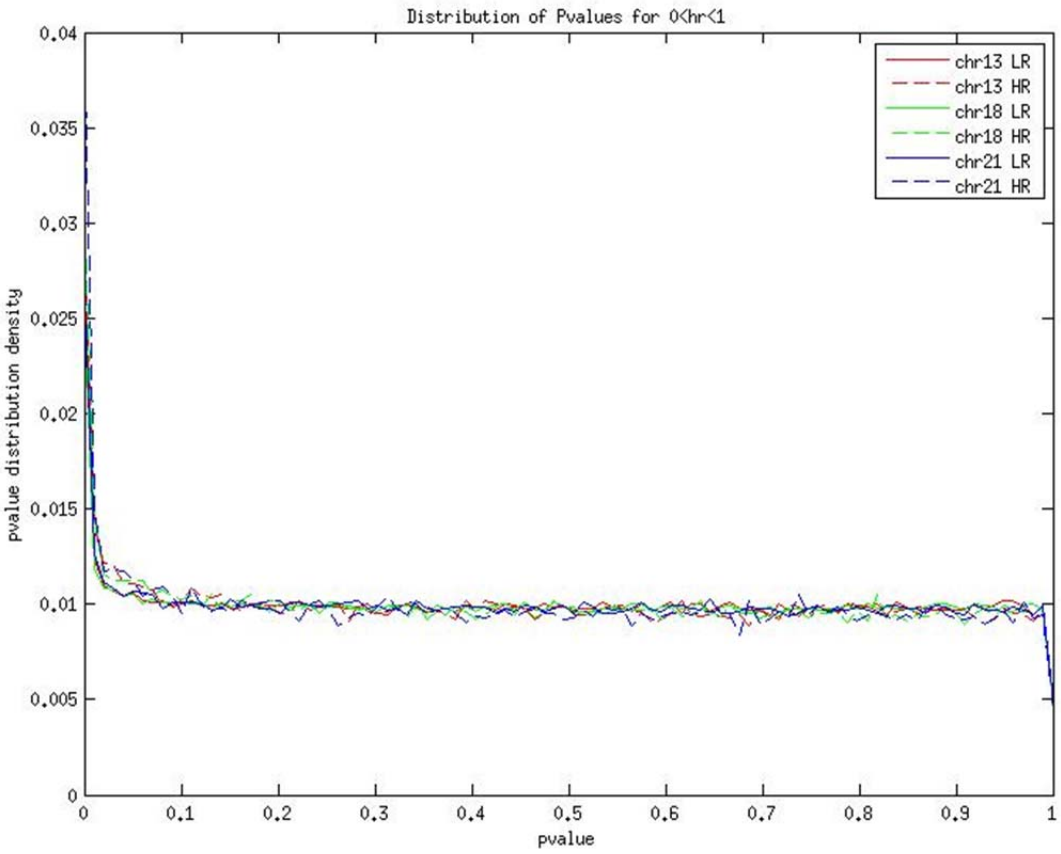
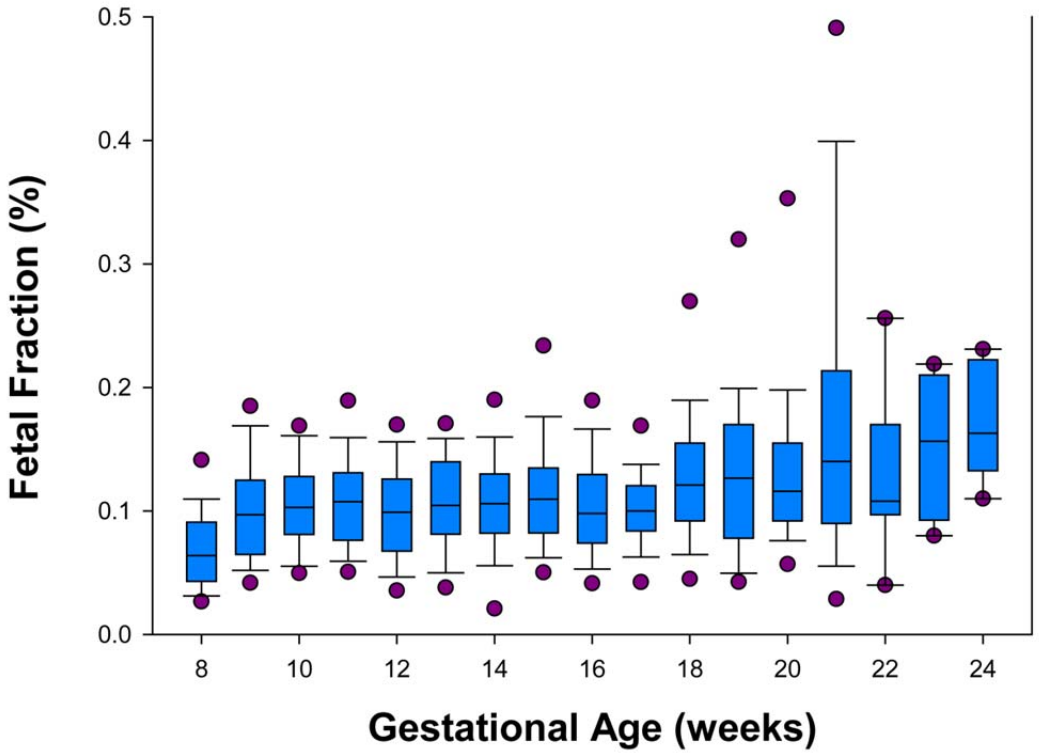
Appendix 7. Model Fit P-value Distributions in High-Risk and Low-Risk Women Were Equivalent



The “SNP-fit” of samples from the high-risk and low-risk cohorts were compared by comparing the observed allele distribution to that predicted by the algorithm. Specifically, the two-sample Kolmogorov-Smirnov distribution test was used to compare SNP-fit for high- and low-risk cohorts over chromosomes 13, 18 and 21. “SNP-fit” was defined as the p-value of observed allele counts on a particular SNP, belonging to the distribution predicted by the method, given a specific fetal fraction (predicted by the algorithm), noise parameters (predicted by the algorithm), observed depth-of-read, and observed mother and child genotypes. Given no differences in performance, the SNP-fit distributions for each sample should be close to uniform. The figure above shows the SNP-fit distribution, i.e. SNP p-values for high- and low-risk cohorts, for chromosomes 13, 18, and 21. The x-axis depicts the p-value range. The y-axis depicts the p-value distribution density (percent of SNPs with a given p-value). For chromosomes 13, 18, 21, the null hypotheses of no difference between SNP-fit distributions for low- versus high-risk cohorts yielded p-values of 93%, 91%, and 76% respectively; this supported

that there was no difference in performance between low- and high-risk cohorts on each of the chromosomes. The null hypothesis of no differences between SNP-fit distributions for chromosome 13 versus 18 and 18 versus 21 yielded p-values of 87% and 20% respectively; this supported that there was no difference in performance between high- and low-risk cohorts between chromosomes.

Appendix 8: Fetal Fraction by Gestational Age



Fetal fraction showed high variance at all gestational ages, and was positively correlated with gestational age. Regression analysis (see Materials and Methods) revealed a significant positive correlation between gestational age and fetal fraction: average fetal fraction increased by 2.6% per week from 8 to 10 weeks of gestation (r^2=0.195, p=<0.001), 0.2% per week from 10 to 20 weeks of gestation (r^2=0.015, p=<0.005), and 0.7% per week beyond 20 weeks of gestation (r^2=0.216, p=<0.001).

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Appendix 9. Breakdown of Samples per Study

	Trisomy 21				Trisomy 18				Trisomy 13			
	TP	**FP**	**TN**	**FN**	**TP**	**FP**	**TN**	**FN**	**TP**	**FP**	**TN**	**FN**
Chiu *et al.*	86	3	143	0	-	-	-	-	-	-	-	-
Ehrich *et al.*	39	1	409	0	-	-	-	-	-	-	-	-
Palomaki *et al.*	209	3	1468	3	-	-	-	-	-	-	-	-
Palomaki *et al.*	-	-	-	-	59	5	1683	0	11	16	1672	1
Bianchi *et al.*	89	0	404	0	35	0	460	1	11	0	485	3
Sparks *et al.*	36	0	123	0	8	0	123	0	-	-	-	-
Ashoor *et al.*	50	0	297	0	49	0	297	1	-	-	-	-
Norton *et al.*	81	1	2887	0	37	2	2886	1	-	-	-	-
Ashoor *et al.*	-	-	-	-	-	-	-	-	8	1	1938	2
Nicolaides	25	0	197	0	3	0	226	0	1	0	228	0

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et al.												
Present study	58	0	907	0	24	1	940	1	12	0	953	0

TP, true positive; FP, false positive; TN, true negative; FN, false negative.

Appendix 10. Reported False-Positive and Unaffected Samples for the Quantitative Methods[4–12] and This Single-Nucleotide Polymorphism-Based Method Discussed in the Present Study[13]

	False Positive	False Positive+True Negative
Quantitative methods		
Trisomy 21	8	5739
Trisomy 18	7	5456
Trisomy 13	17	4112
SNP		
Trisomy 21	0	1104
Trisomy 18	1	1167
Trisomy 13	0	1181

Appendix 11. Projected Aggregate Specificities for the Quantitative Methods[4–12] and This Single-Nucleotide Polymorphism-Based Method Discussed in the Present Study[13]

Quantitative methods (n=4112)	
Trisomy 21	5.73
Trisomy 18	5.28
Trisomy 13	17
Sum	28.01
Specificity[1]	99.32% (1 - 28.01/4112)
95% CI	99.02-99.55%
Single nucleotide polymorphism (n=1105)	
Trisomy 21	0
Trisomy 18	0.95
Trisomy 13	0
Sum	0.95
Specificity	99.91% (1 - 0.95/1104)

95% CI	99.50-100.00%

CI, confidence interval. [1.]False positives conservatively adjusted for cohort size.

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