Non Invasive
Chromosome Examination

What is NICE® prenatal test?

NICE® is a non-invasive prenatal test (NIPT) that detects fetal DNA in maternal plasma during pregnancy through Next Generation Sequencing (NGS). It can be tested from the 10th week of pregnancy and evaluates fetal chromosomal abnormalities. NICE® screens for common trisomy (such as 21, 18, 13), sex chromosome aneuploidies and analyzes eight clinically important microdeletion regions.

Test Option

	NICE® LITE	NICE® BASIC	NICE® PREMIUM
T13, T18, T21	✓	✓	✓
T9, T16, T22		✓	✓
All Chromosome			✓
*8 Microdeletions			
*116 Microdeletions			
*Sex Chromosome Disorder			

* Any or all can be added to LITE, BASIC, or PREMIUM service

NICE vs traditional prenatal screening

NICE® is a non-invasive prenatal test (NIPT) that detects fetal DNA in maternal plasma during pregnancy through Next Generation Sequencing (NGS). It can be tested from the 10th week of pregnancy and evaluates fetal chromosomal abnormalities. NICE® screens for common trisomy (such as 21, 18, 13) , sex chromosome aneuploidies and analyzes eight clinically important microdeletion regions.

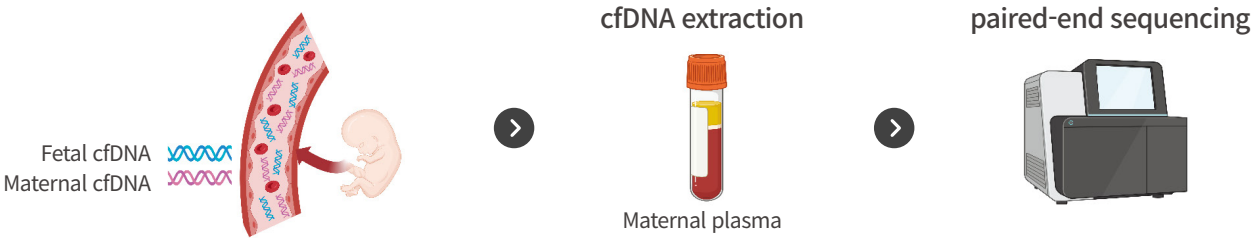
	Screening	How to	Since when	How long	Detection Rate%
NIPT	NICE	Non-invasive	From 10 weeks	7~10 days	>99%
Conventional Blood Test	Triple Screen	Non-invasive	From 11-13 weeks	2days	67~71%
	Quadruple Screen		From 11-13 weeks		79~81%
Integrated Screening Test	Integrated Screen	Non-invasive	From 11-13 week From 11-13 weeks	4~5 weeks	94~96%
Cell Culture Test	Chorionic Screen	Invasive	From 11-13 weeks	4~5 weeks	>99%
	Amniocentesis		From 11-13 weeks		

How is the NICE® performed

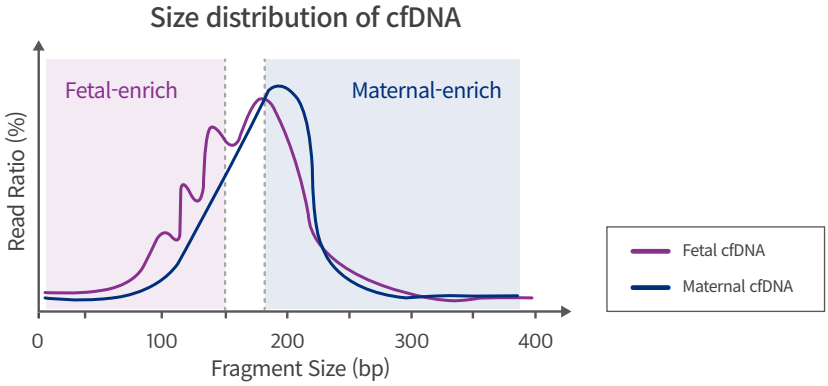
NICE® looks at isolated fetal cfDNA present in maternal blood, collected after 10 weeks of pregnancy from massively parallel WGS. Sequencing data is analyzed by applying the bioinformatics pipeline. After removing the GC bias, normalized reads are used for further analysis. The size selection method is used to separate and analyze fetal-derived cfDNA and maternal-derived cfDNA according to the size distribution of cfDNA. To detect chromosomal aneuploidies, we use a multi-Z method with 21 z-scores for each autosomal chromosome. First, fetal sex is determined using Y-derived reads, and the fetal fraction is analyzed using sex-specific bioinformatics pipelines.

Pre-processing (Sequencing)

Figure 1. outlines the bioinformatics process from maternal blood collection to reporting of test results.



Size selection method

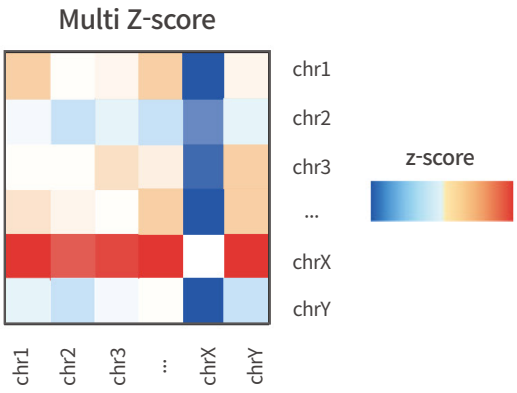


Bioinformatics anslysis

Figure 2. The workflow of NICE® pipeline based on size selection method using cfDNA fragment size.

	chr1	chr2	chr3	...	chrX	chrY
chr1	chr1/ chr1	chr1/ chr2	chr1/ chr3	...	chr1/ chrX	chr1/ chrY
chr2	chr2/ chr1	chr2/ chr2	chr2/ chr3	...	chr2/ chrX	chr2/ chrY
...	...	...	...	...	...	...
chrX	chrX/ chr1	chrX/ chr2	chrX/ chr3	...	chrX/ chrX	chrX/ chrY
chrY	chrY/ chr1	chrY/ chr2	chrY/ chr3	...	chrY/ chrX	chrY/ chrY

Matrix of mapping read ratio



Why NICE® is different – Strengths

If there is an abnormality in the chromosomes derived from the placenta or the mother, false negatives and false positives may appear as a result of NIPT. Although the proportion of false- negative and false-positive results in NIPT are very small, the reporting of false results cannot be ignored in clinical setting.

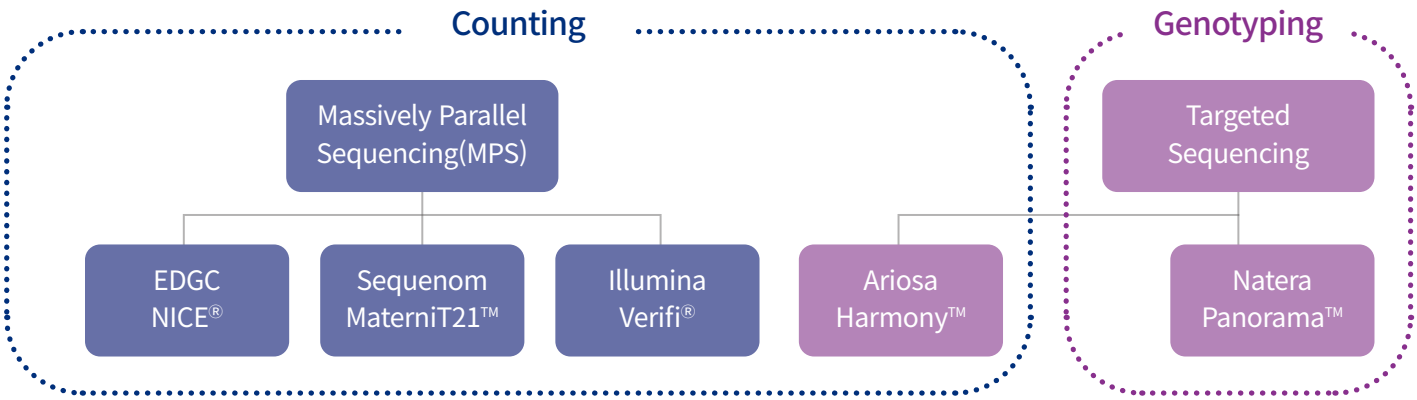
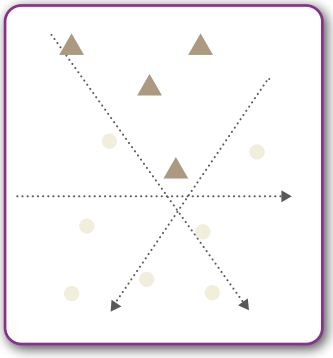


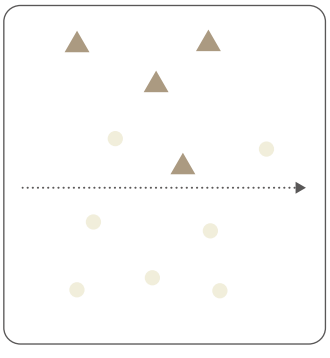
Table1. Differences between NICE® and targeted sequencing methods

NICE® - Massively Parallel Sequencing	Targeted Sequencing
All chromosomal abnormalities can be detected	Only major chromosomal abnormalities can be detected
Unlike other MPS-based NIPT tests, it reports using 21 z-score thresholds	Reported as a risk score similar to serum screening
There is also no effect due to differences between ethnicities	Depending on the SNP, it may be affected by differences between ethnicities
Amplification of fetal-derived cfDNA/maternalderived cfDNA by size selection method using paired-end sequencing	Inability to isolate fetal-derived cfDNA and maternal-derived cfDNA

Multi-Z method

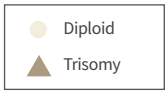


NICE® prenatal test <Multi-Z Threshold>



Other NIPT tests <Single Threshold>

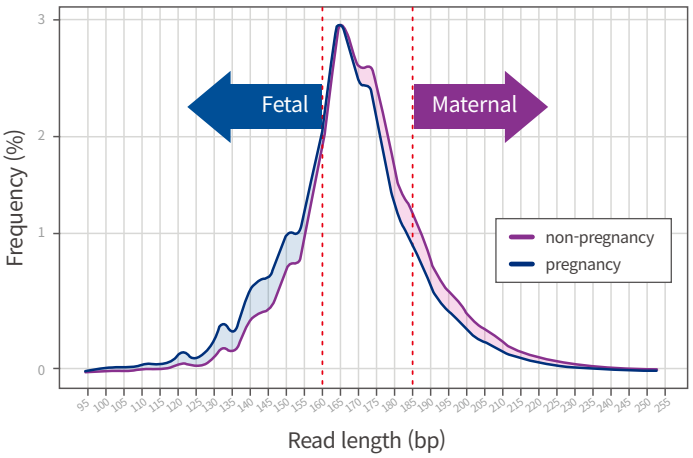
The multi-Z method has the advantage of being more precise by using a 21 z-scores compared to the existing methods using one or two z-score thresholds by other labs.

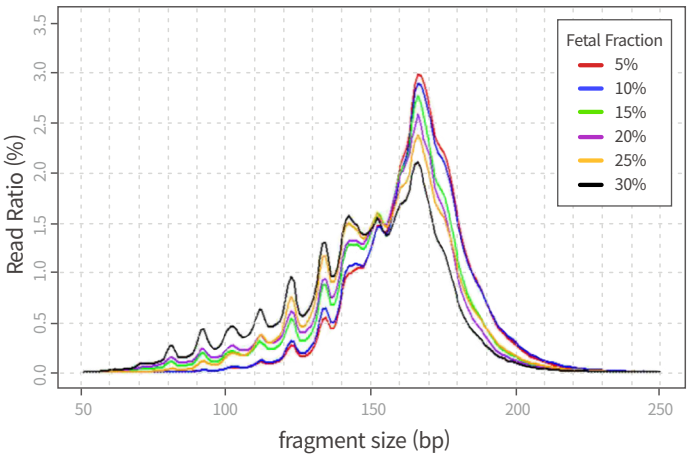


Why NICE® is different – Strengths

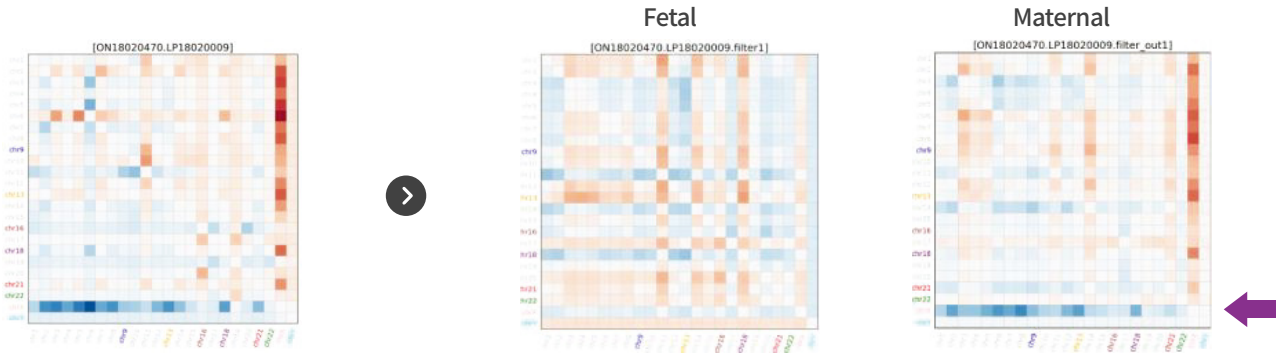
Size selection method

The bioinformatics pipeline of the NICE test aimed to improve the accuracy of NIPT by dividing and analyzing fetal or maternal cfDNA based on cfDNA size differences. Through the size selection method, fetal-derived cfDNA analysis had the effect of increasing the fetal DNA fraction, and maternal-derived cfDNA analysis had the effect of removing factors affecting maternal chromosomal abnormalities and increased NIPT accuracy. In addition, when gDNA is contaminated, the distribution of cfDNA fragments is different, so the quality of sequencing data is controlled through the distribution of cfDNA.

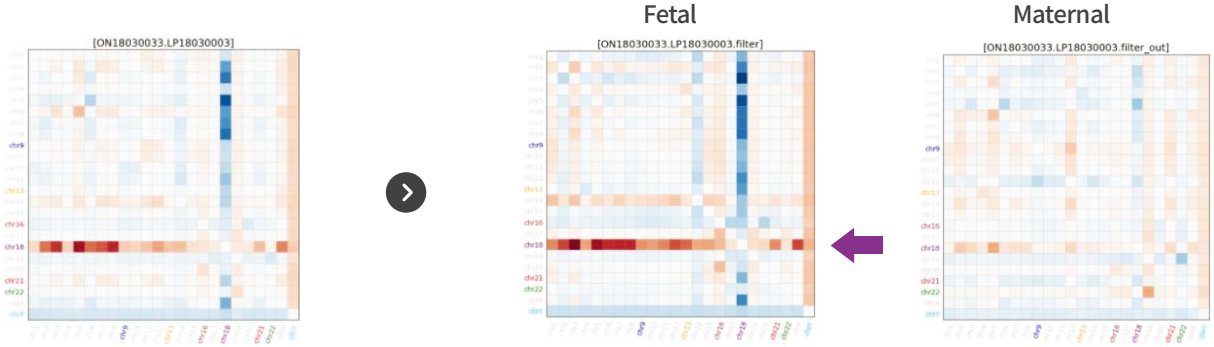




XO Case : Not detected (Maternal Mosaicism)



Trisomy 18 : 2-step confirmation



Factors Affecting False Results

If NIPT is performed too early in the pregnancy (less than 10 weeks)[1,2] women with high BMI[1,3,4] and long-term storage of blood samples[5,6] may result in low fetal fraction and increase the false negative rate. In addition, this method has limitations in that maternal malignancies, maternal mosaicism, and placental mosaicism my cause an inconsistency between fetal karyotype and NIPT results, resulting in some false positive or negative results.[7,8]

Maternal

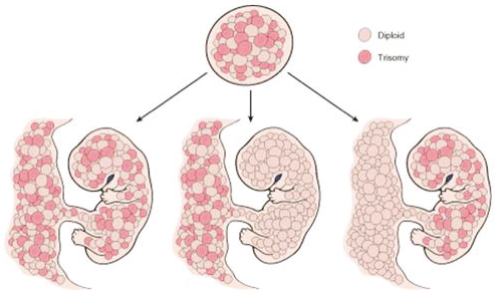
- Maternal trisomy/SCA mosaicism
- Maternal genetic variation (CNV, dup/del)
- Maternal malignancy (Cancer)
- Maternal sex chromosome aneuploidy

Placental / Fetal

- Confined placental mosaicism (CPM)
- Fetoplacental mosaicism
- Fetal mosaicism
- Low fetal fraction
- Vanishing twin / co-twin demise

Experiments and Analysis

- gDNA contamination
- Sample mix up/human error
- Limitation of NIPT



A Fetoplacental mosaicism

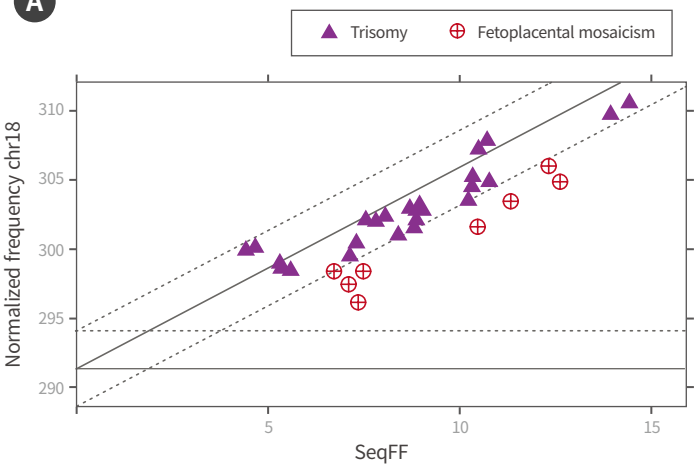
B Confined placental mosaicism

C Fetal mosaicism

Mosaicism prediction method

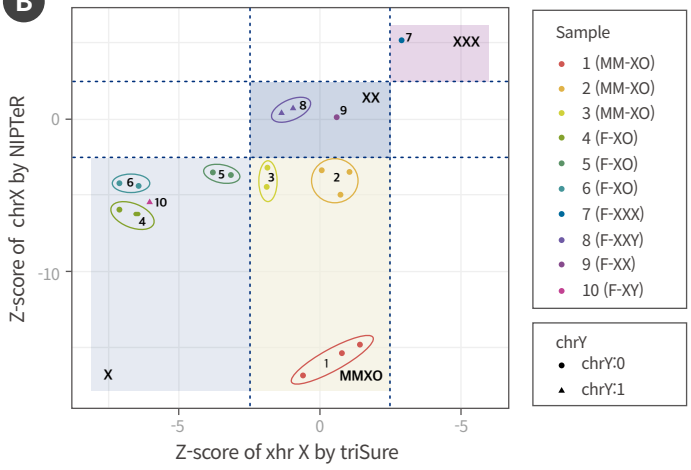
NICE®uses two methods to predict mosaicism. Figure A is a graph showing the correlation between Fetal Fraction and z-score. If it deviates from the straight line distributed by trisomy, mosaicism can be predicted. Figure B shows a method to distinguish between fetal monosomy X and maternal mosaic monosomy X by calculating true values.

A



Prenat Diagn. 2020 Jan;40(2):155-163

B



Prenat Diagn. 2019 Mar;39(4):324-327

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Why choose NICE®?

NICE® improved the accuracy of results and test success rate by using a multi-Z score through the size-selection method, developed through research and development by EDGC. By selectively enriching short cfDNA fragments, the fetal DNA fraction was increased by relatively enriching fetal-derived cfDNA. In addition, by selectively enriching long cfDNA fragments and enriching maternal-derived cfDNA, factors causing abnormal chromosomal abnormalities in the mother were eliminated. Therefore, this method reduced false- positive results by more than half, had higher sensitivity, specificity, and accuracy over than other NIPTs, and could improve overall performance.

Available after 10 weeks pregnant

More than 99% test success rate

Safe and easy test with maternal blood

Bioinformatics pipeline accuracy

Table2. NICE® vs Other NIPT services

Sensitivity (False-positive rates)	EDGC NICE®	Sequenom MaterniT21™	Illumina Verifi®	Ariosa Harmony™	Natera Panorama™
Trisomy 21 Down syndrome	>99% (0%)	99.1% (0.2%)	>99.9% (0.3%)	>99% (<0.1%)	99% (0%)
Trisomy 18 Edwards syndrome	96.5% (0%)	96.9% (0.1%)	97.3% (0.4%)	96.7% (<0.1%)	94.1% (<0.1%)
Trisomy13 Patau syndrome	92.31% (0%)	89.3% (0.3%)	87.5% (0.1%)	80% (0.05%)	>99% (0%)
Monosomy X Turner syndrome	>99.99% (0%)	94.7% (0.5%)	95% (1.0%)	96.7% (unreported)	94.7% (<0.1%)
Sex chromosome Trisomies	>99.99% (0%)	>99.9%	67-100%	67-100%	73.1%
Female	>99% (0%)	97.9% (0.5%)	97.6% (0.8%)	>99% (unreported)	>99.9% (0%)
Male	>99% (0%)	99.4% (2.1%)	99.1% (1.1%)	>99% (unreported)	>99.9% (0%)

NICE® prenatal test performance

NICE® has verified the accuracy of results in multiple clinical samples and provides reliable results.

* The total number of samples in singletons is 75,067.
* The total number of samples for twin pregnancy is 1,711.
* PPV performance was calculated based on amniocentesis and QF-PCR results.
* Due to insufficient number of amniocentesis samples, Sensitivity was calculated by estimating True Positive (TP) based on PPV.
* Samples presumed to be mosaicism were excluded from the calculation.

Table3. Sensitivity, specificity, accuracy, and PPV(positive predictive value)

Aneuploidy	Sensitivity	95% CI	Specificity	95% CI	Accuracy	95% CI	PPV	95% CI
Singletons								
T21	99.72% (705/707)	98.98-99.97	>99.99 (73637/73640)	99.99-100	99.99%	99.98-100	98.72% (231/234)	96.13-99.58
T18	99.04% (206/208)	96.57-99.88	>99.99% (74637/74639)	99.99-100	99.99%	99.99-100	96.49% (55/57)	87.30-99.10
T13	98.84% (85/86)	93.69-99.97	>99.99% (74868/74870)	99.99-100	>99.99%	99.99-100	85.71% (12/14)	59.80-96.03
T9	>99.99% (15/15)	78.20-100	>99.99% (75037/75037)	99.99-100	>99.99%	99.99-100	>99.99% (2/2)	15.81-100
T16	>99.99% (3/3)	29.24-100	>99.99% (75055/75056)	99.99-100	>99.99%	99.99-100	50% (1/2)	12.35-87.65
T22	>99.99% (19/19)	82.35-100	>99.99% (75029/75029)	99.99-100	>99.99%	99.99-100	>99.99% (2/2)	15.81-100
XO	>99.99% (79/79)	95.44-100	>99.99% (74859/74865)	99.98-100	99.99%	99.98-100	76% (19/25)	58.72-87.58
XXX	>99.99% (61/61)	94.13-100	>99.99% (74945/74945)	99.99-100	>99.99%	99.99-100	>99.99% (18/18)	81.47-100
XXY	>99.99% (114/114)	96.82-100	>99.99% (74839/74839)	99.99-100	>99.99%	99.99-100	>99.99% (29/29)	88.06-100
XYY	>99.99% (32/32)	89.11-100	>99.99% (75003/75003)	99.99-100	>99.99%	99.99-100	>99.99% (7/7)	59.04-100
Twin estations								
T21	>99.99% (18/18)	81.47-100	>99.99% (1675/1675)	99.78-100	>99.99%	99.78-100	>99.99% (4/4)	39.76-100
T18	>99.99% (2/2)	15.81-100	>99.99% (1707/1707)	99.78-100	>99.99%	99.78-100	>99.99% (1/1)	2.50-100
T13	Nan (0/0)	Nan	>99.99% (1707/1707)	99.78-100	Nan	Nan	Nan (0/0)	Nan

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Microdeletion syndromes

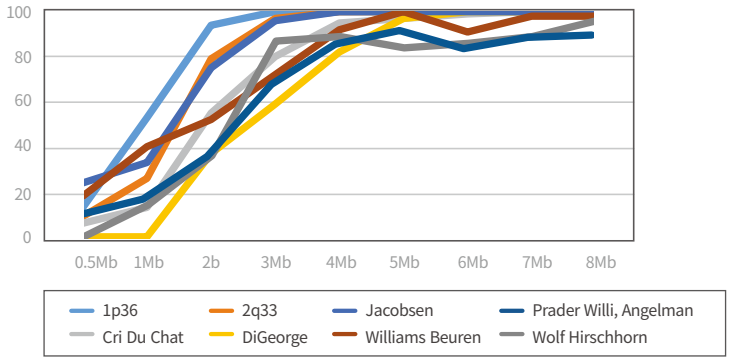
Due to the lack of clinical samples, we generated 28,800 artificial data referencing known areas from DECIPHER and OMIM. Shallow-depth NGS sequencing methods have chromosomal regions that are difficult to map to reference sequences depending on chromosomal characteristics. Sensitivity gradually increases with increasing CNV size and fetal fraction.

* Artificial data were created with combinations of 0.5, 1, 2, 3, 4, 5, 6, 7, 8 Mb deletion units and 3, 5, 7, and 10% fetal fractions.
* Low fetal fraction or short CNV size is a technical limitation.

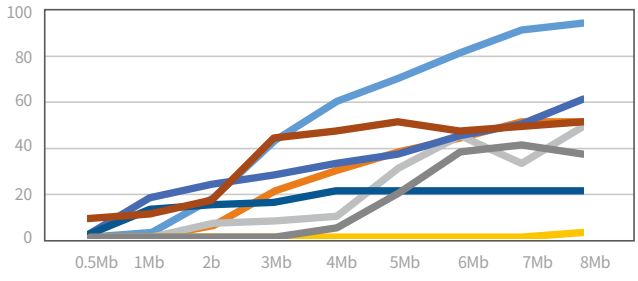
Disease	deletion location and length (DECIPHER)	LOD length (Mb)	LOD Fetal Fraction	sensitivity	specificity
1p36 deletion syndrome	1:10001-12840259 (12.83Mb)	≥3	≥3%	42~100%	100%
2q33.1 deletion syndrome	2:196925121-205206939 (8.23Mb)	≥3	≥3%	20~97%	100%
Wolf-Hirschhorn syndrome (4p16.3 deletion)	4:1569197-2110236 (0.54Mb)	≥3	≥5%	19~87%	100%
Cri du chat syndrome (5p15.3 deletion)	5:10001-12533304 (12.52Mb)	≥4	≥5%	44~95%	100%
Williams beuren syndrome (7q11.23 deletion)	7:72744455-74142672 (1.39Mb)	≥4	≥3%	46~92%	100%
Jacobsen syndrome (11q23 deletion)	11:110470724-121170709 (10.69Mb)	≥3	≥3%	27~96%	100%
Prader-Willi / Angelman syndrome (15q11.2 deletion)	15:22749354-28438266 (5.68Mb)	≥4	≥3%	20~87%	100%
DiGeorge syndrome (22q11.2 deletion)	22:19009792-21452445 (2.44Mb)	≥5	≥5%	34~97%	100%

Among them, 22q.11 deletion (DiGeorge syndrome) has a sensitivity of 34% when 5 Mb units are detected in 5% of the fetal fraction, and has a sensitivity of 97% when more than 8 Mb units are detected in 10% of the fetal fraction.

Stair-matrix [10% fetal fraction]



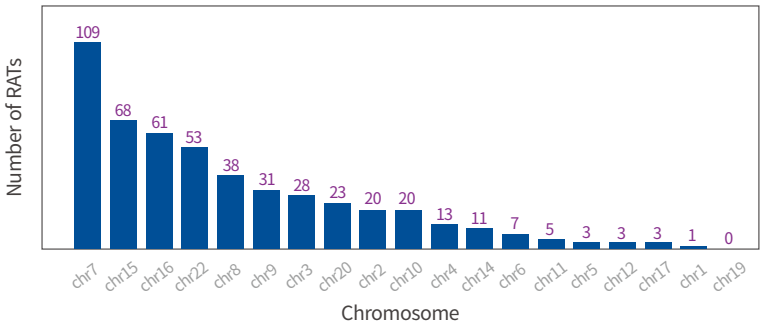
Stair-matrix [3% fetal fraction]



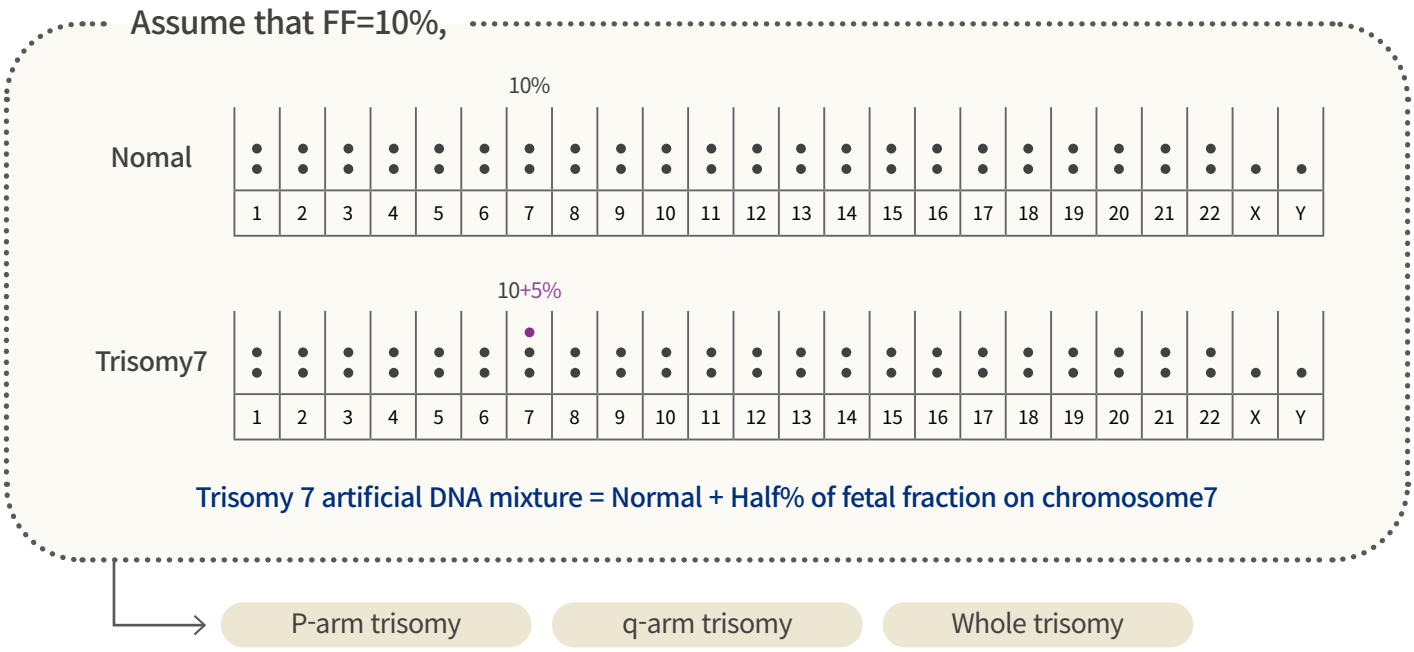
All chromosomes

Summary of RAT(Rare Autosomal Trisomies)

The p-arm, q-arm and whole trisomy regions of RAT were investigated through several literatures and case reports. The incidence of trisomy 7 and trisomy 15 was higher than that of trisomy 9, trisomy 16, and trisomy 22 provided by the NICE test.

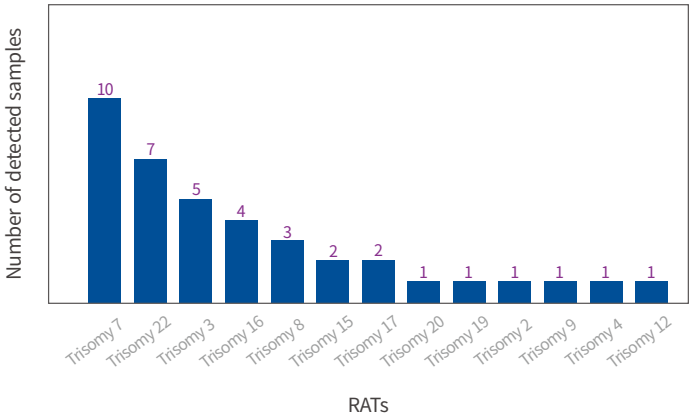


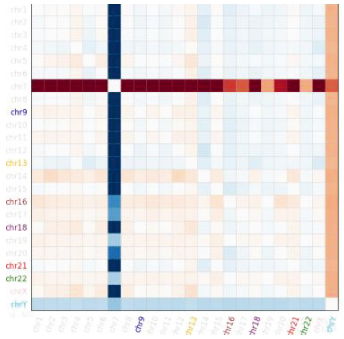
Make artificial samples

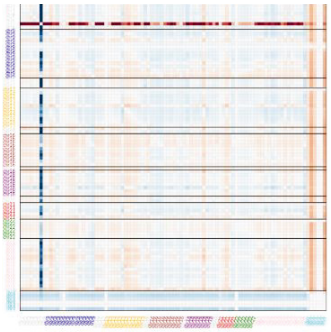


Validation

8700 samples were validated, and Trisomy7 was detected the most with 10.







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References

1. Bianchi, D. W. & Wilkins-Haug, L. Integration of Noninvasive DNA Testing for Aneuploidy into Prenatal Care: What Has Happened Since the Rubber Met the Road? Clinical Chemistry **60**, 78–87 (2014).

2. Canick, J. A., Palomaki, G. E., Kloza, E. M., Lambert-Messerlian, G. M. & Haddow, J. E. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. Prenatal Diagnosis **33**, 667–674 (2013).

3. Ashoor, G., Syngelaki, A., Poon, L. C. Y., Rezende, J. C. & Nicolaides, K. H. Fetal fraction in maternal plasma cell-free DNA at 11–13 weeks' gestation: relation to maternal and fetal characteristics. Ultrasound in Obstetrics & Gynecology **41**, 26–32 (2013).

4. Wang, E. et al. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. Prenatal Diagnosis **33**, 662–666 (2013).

5. Fernando, M. R. et al. A new methodology to preserve the original proportion and integrity of cell-free fetal DNA in maternal plasma during sample processing and storage. Prenatal Diagnosis **30**, 418–424 (2010).

6. Barrett, A. N., Zimmermann, B. G., Wang, D., Holloway, A. & Chitty, L. S. Implementing Prenatal Diagnosis Based on Cell-Free Fetal DNA: Accurate Identification of Factors Affecting Fetal DNA Yield. PLOS ONE **6**, e25202 (2011).

7. Bianchi, D. W. et al. Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies. JAMA **314**, 162–169 (2015).

8. Wang, Y. et al. Maternal mosaicism is a significant contributor to discordant sex chromosomal aneuploidies associated with noninvasive prenatal testing. Clin Chem **60**, 251–259 (2014).