GENOMA®
Molecular Genetics Laboratories Group

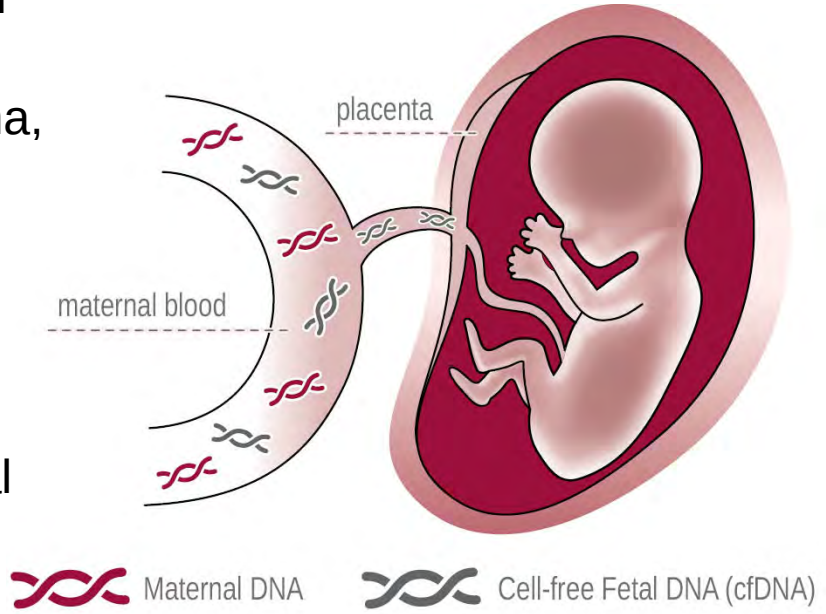
PrenatalSafe®
KARYO

Noninvasive prenatal test (NIPT)
for genome-wide fetal chromosomal abnormalities

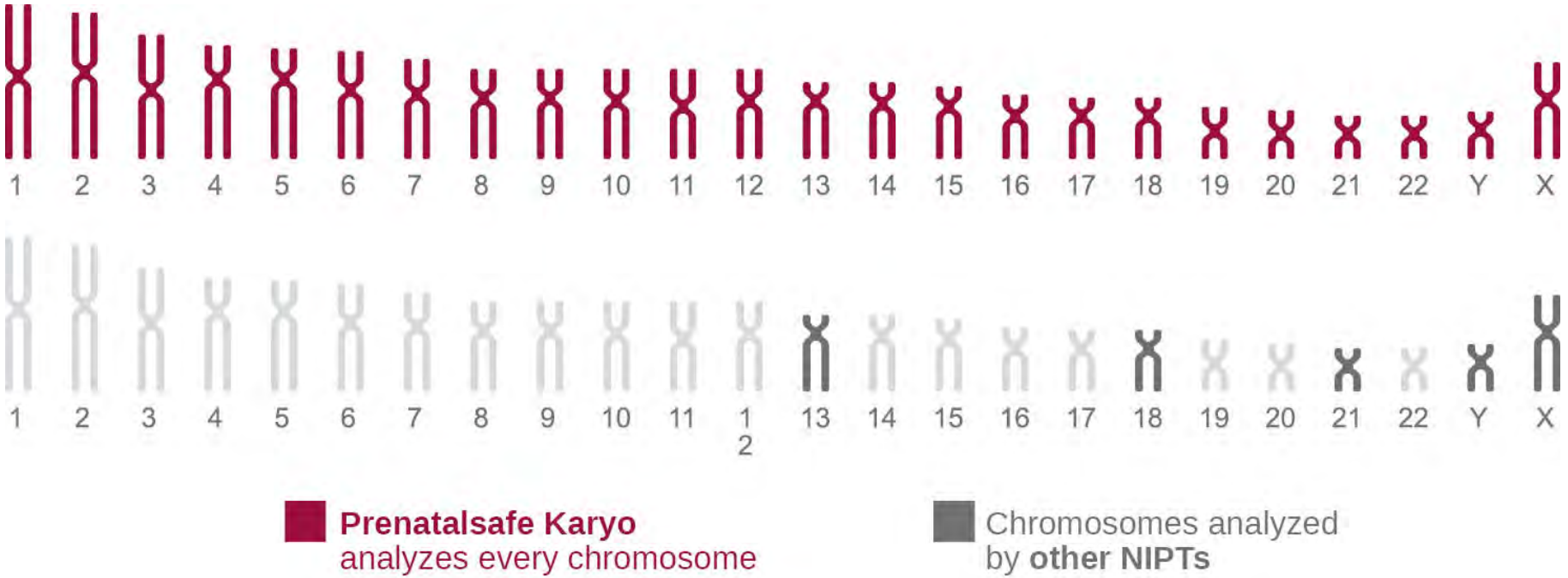
Cell-free fetal DNA analysis from maternal plasma
providing karyotype-level insight

What is PrenatalSafe® KARYO

- PrenatalSAFE® Karyo test is the most technologically advanced **genome-wide NIPT**
- Through cfDNA analysis from maternal plasma, PrenatalSAFE®Karyo detects:
  - **Aneuploidies**
  - **structural chromosomal aberrations** (deletions or duplications) across the fetal genome
- providing **karyotype-level** insight.



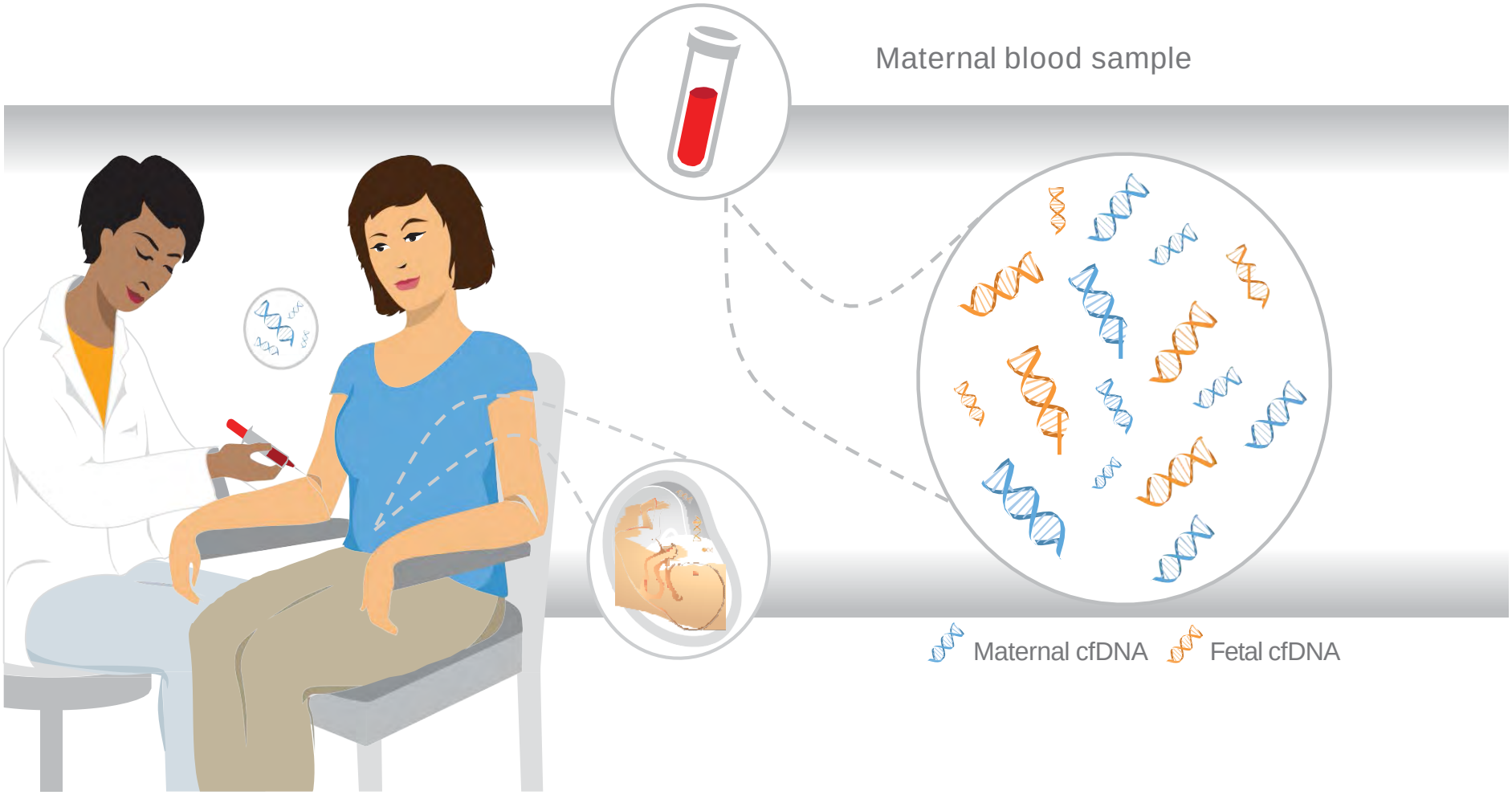
PrenatalSafe® KARYO the next level in noninvasive prenatal testing



- **PrenatalSafe® Karyo** analyzes every chromosome in the genome.
- Unlike any other noninvasive prenatal test available to date, it offers a level of information previously only available from a **fetal karyotype** analysis, performed with invasive prenatal diagnosis procedures (amniocentesis and CVS)

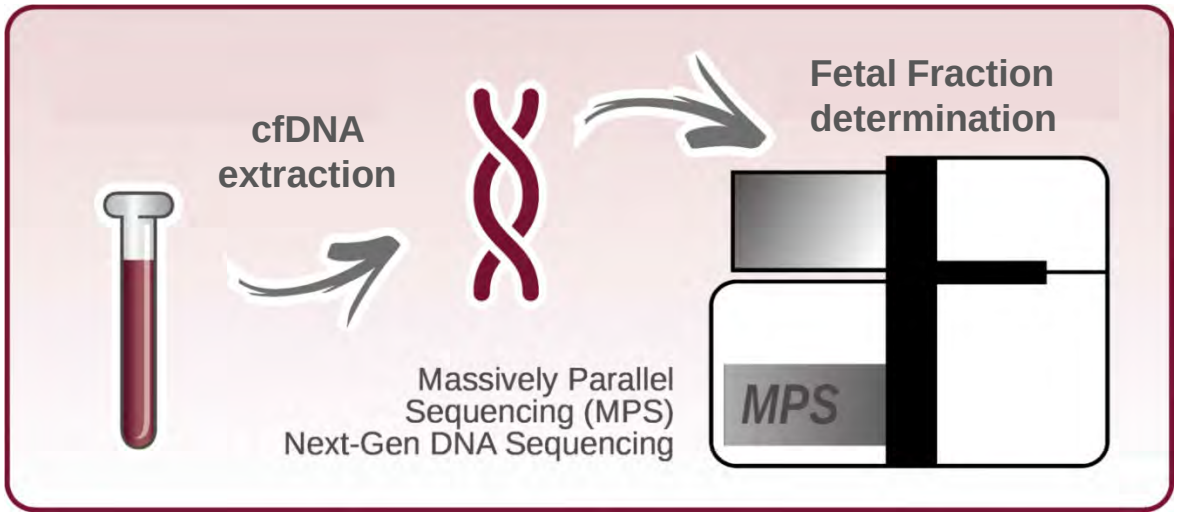
PrenatalSafe® KARYO

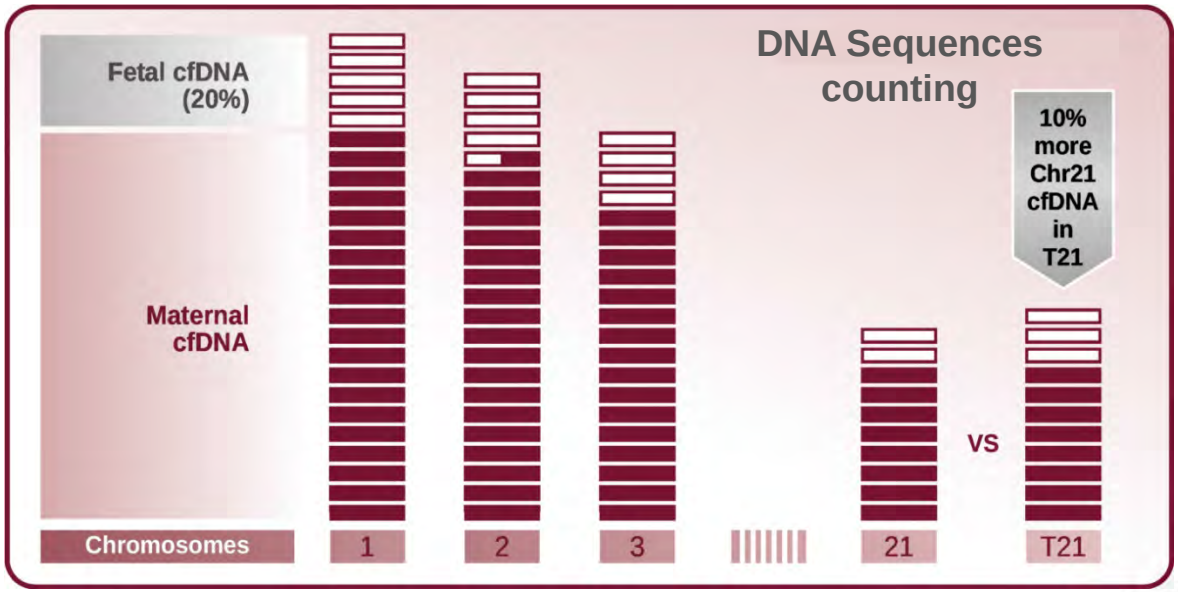
NIPT uses cell-free DNA (cfDNA)



PrenatalSafe® KARYO

Whole-genome sequencing technology





GENOMA® Molecular Genetics Laboratories Group

PrenatalSafe® KARYO — 2 levels of genome-wide screening

PrenatalSafe® KARYO

Common fetal chromosomal aneuploidies

Trisomy 21	Down Syndrome
Trisomy 18	Edwards Syndrome
Trisomy 13	Patau Syndrome
Monosomy X	Turner Syndrome
XXX	Trisomy X
XXY	Klinefelter Syndrome
XYY	Jacobs Syndrome

Other fetal chromosomal aneuploidies

Trisomy 1	Trisomy 9*
Trisomy 4	Trisomy 12
Trisomy 5	Trisomy 16
Trisomy 7	Trisomy 22*

PrenatalSafe® KARYO detects aneuploidies in every chromosome

Chromosomal gains and losses across the fetal genome

PrenatalSafe® KARYO Plus

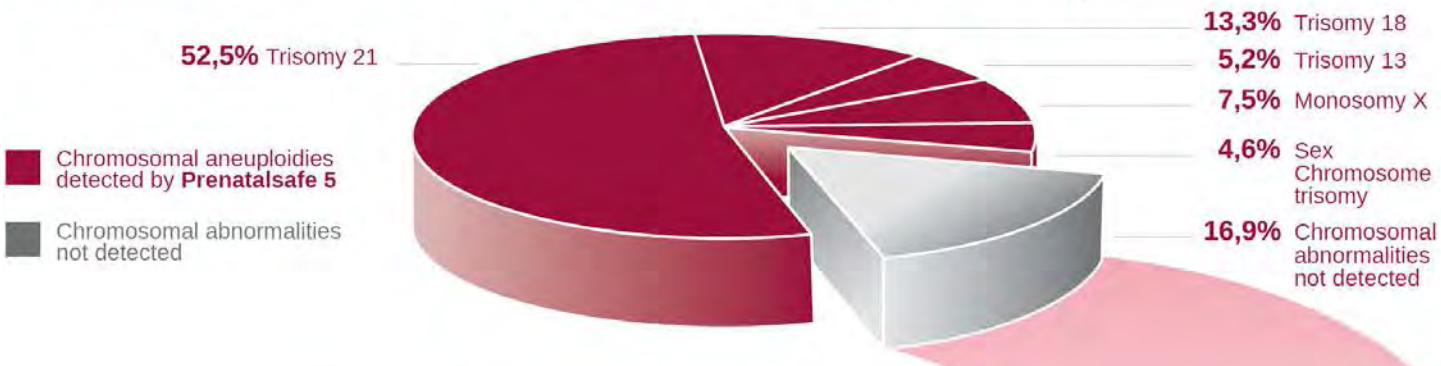
It can also test for **9** common **microdeletion syndromes**

Microdeletion Syndrome	Prevalence
DiGeorge syndrome (22q11.2)	1 in 4,000
1p36 deletion syndrome	1 in 4,000 to 1 in 10,000
Angelman syndrome (15q11.2)	1 in 12,000
Prader-Willi syndrome (15q11.2)	1 in 10,000 to 1 in 25,000
Cri du Chat syndrome (5p15.3)	1 in 20,000 to 1 in 50,000
Wolf-Hirschhorn syndrome (4p16.3)	1 in 50,000
Langer-Giedion syndrome (8q24)	1/200.000
Jacobsen syndrome (11q23)	1/100.000
Smith-Magenis syndrome (17p11.2)	1/15.000 - 1/25.000

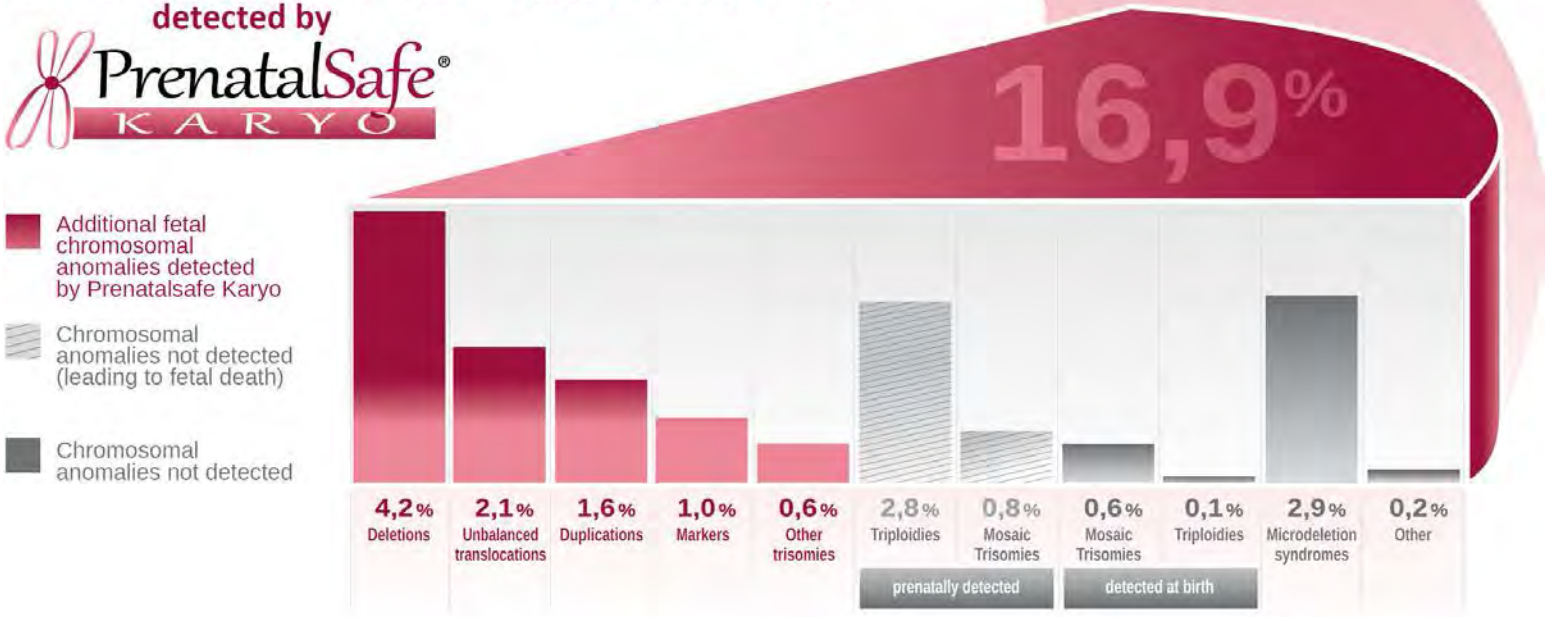
PrenatalSafe® KARYO

Detection rate

Prevalence of fetal chromosomal aneuploidies detected by PrenatalSafe® 5

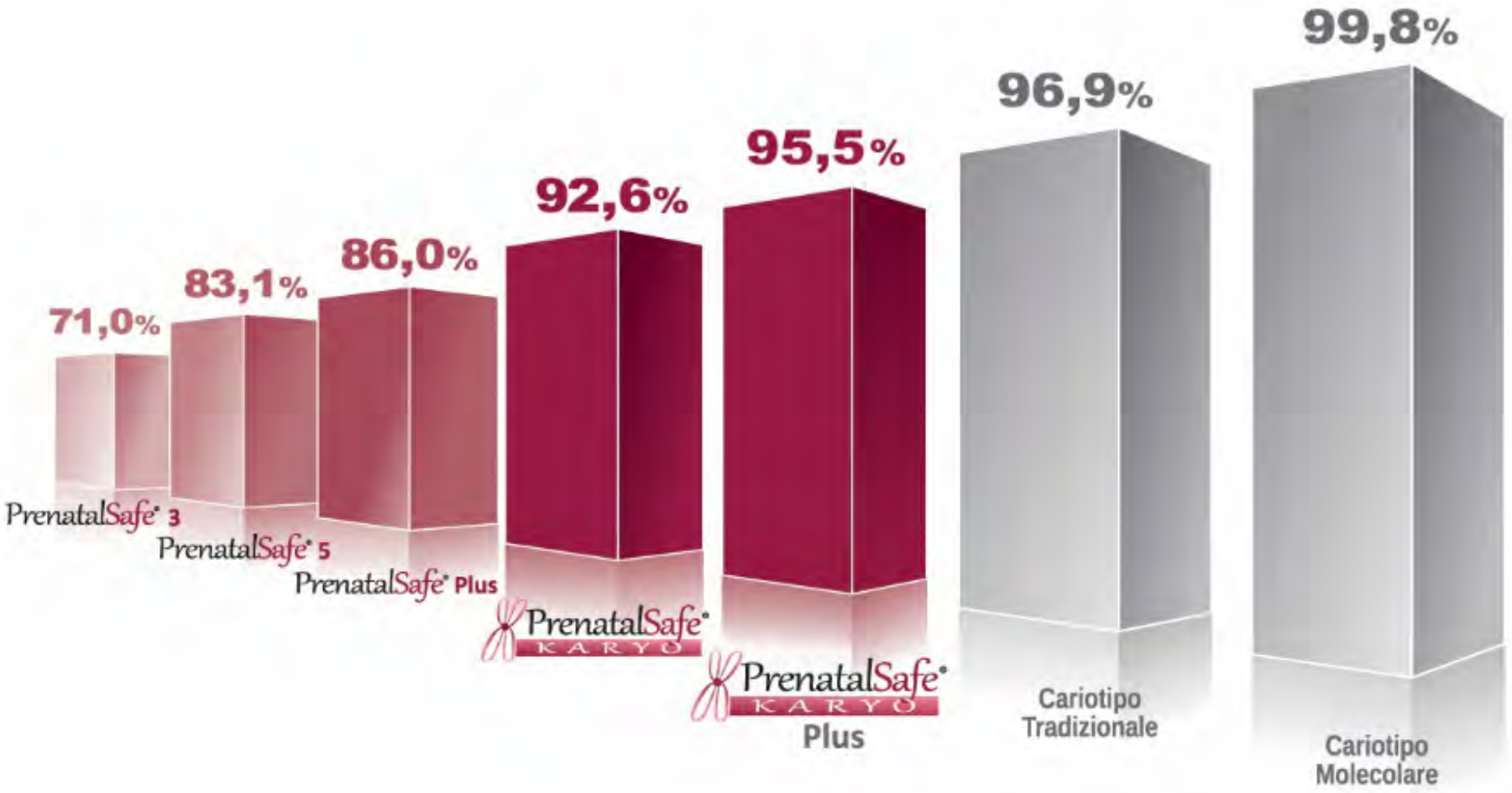


Prevalence of additional fetal chromosomal anomalies detected by PrenatalSafe® KARYO



GENOMA® Molecular Genetics Laboratories Group

Detection rate

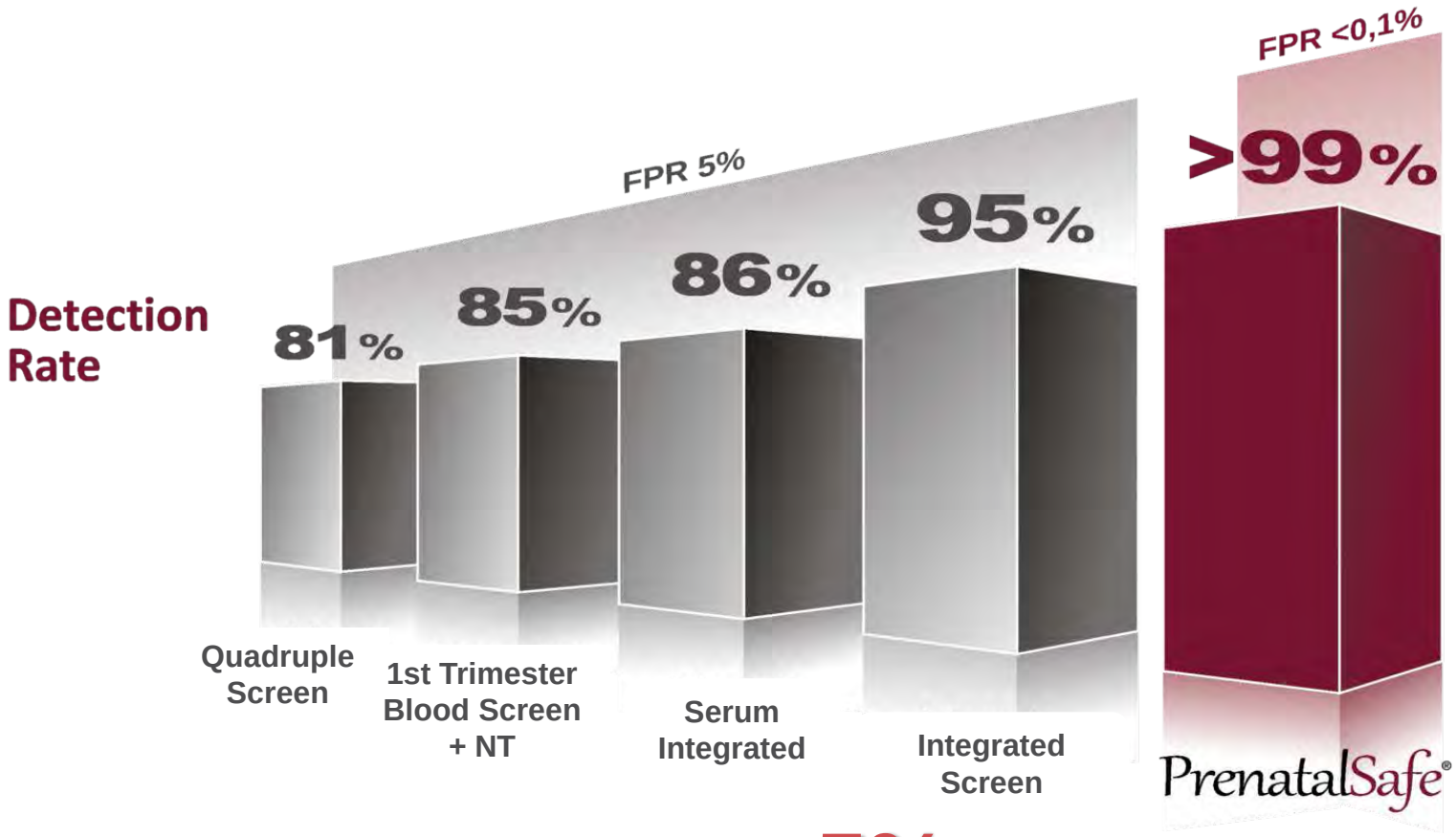


PrenatalSAFE® Karyo Plus test identifies **95.5%** of chromosomal anomalies **prenatally detected** and **99.1%** of those anomalies observed **at birth**, achieving a level of detection rate very closed to the **traditional fetal karyotyping** (**96.9%**), obtained with invasive, prenatal diagnostic techniques.

Conventional Prenatal Screening vs

Detection Rates for Trisomy 21

PrenatalSafe® KARYO



False Positive Rate (FPR): **5%**

GENOMA® Molecular Genetics Laboratories Group

Fetal Karyotyping vs PrenatalSafe® KARYO

	Traditional Karyotyping	PrenatalSafe® KARYO	PrenatalSafe® KARYO Plus
Analyzes every chromosome	✓	✓	✓
Detects chromosomal gains or losses >7 Mb	✗	✗	✓
Detects chromosome gains or losses >10 Mb	✓	✓	✓
Requires an invasive procedure	✗	✓	✓
Detects unbalanced translocations	✓	✓	✓
Detects segmental deletions/duplications	✓	✓	✓
Detects mosaic trisomies	✓	✗	✗
Detects marker chromosomes	✓	✓	✓
Detects microdeletion syndromes	✗	✗	✓
Detects triploidy	✓	✗	✗
Considered diagnostic	✓	✗	✗

PrenatalSafe® KARYO

Pre-clinical Validation performance

	Trisomy 21 (n=1419)	Trisomy 18 (n=1419)	Trisomy 13 (n=1419)	SCA (n=1419)	CNV (n=1419)
True Positive	100	31	14	36	37
False Positive	0	0	0	0	0
True Negative	1319	1388	1405	1383	1382
False Negative	0	0	0	0	0
Sensitivity (95% CI)	100,00% (96.38% - 100.00%)	100,00% (88.78% - 100.00%)	100,00% (76.84% - 100.00%)	100,00% (90.26% to 100.00%)	100,00% (90.51% to 100.00%)
Specificity (95% CI)	100,00% (99.72% - 100.00%)	100,00% (99.73% - 100.00%)	100,00% (99.74% - 100.00%)	100,00% (99.73% to 100.00%)	100,00% (99.73% to 100.00%)
PPV (95% CI)	100,00% (96.38% - 100.00%)	100,00% (88.78% - 100.00%)	100,00% (76.84% - 100.00%)	100,00% (90.26% to 100.00%)	100,00% (90.51% to 100.00%)
NPV (95% CI)	100,00% (99.72% - 100.00%)	100,00% (99.73% - 100.00%)	100,00% (99.74% - 100.00%)	100,00% (99.73% to 100.00%)	100,00% (99.73% to 100.00%)

PPV: Positive Predictive Value; NPV: Negative Predictive Value; SCA: Sex Chromosomes Aneuploidy. CNV: Copy Number Variation

PrenatalSafe® Performance Standard protocol

Follow-up March 2016

	Trisomy 21 (n=31.800)	Trisomy 18 (n=31.800)	Trisomy 13 (n=31.800)	Monosomy X (n=31.800)	SCA (n=31.800)
True positive	257	47	32	77	160
False positive	6	6	7	48	58
True negative	31536	31746	31746	31675	31582
False negative	1	1	0	0	0
Sensitivity (95% CI)	99,61% (97.86% - 99.99%)	97,92% (88.93% - 99.95%)	100,00% (89.11% - 100.00%)	100,00% (95.32% - 100.00%)	100,00% (99.72% - 100.00%)
% False Negative	0,39%	2,08%	0,00%	0,00%	0,00%
Specificity (95% CI)	99,98% (99.96% - 99.99%)	99,98% (99.96% - 99.99%)	99,98% (99.95% - 99.99%)	99,85% (99.80% - 99.89%)	99,82% (99.76% - 99.86%)
% False Positive	0,02%	0,02%	0,02%	0,15%	0,18%
PPV (95% CI)	97,72% (95.10% - 99.16%)	88,68% (76.97% - 95.73%)	82,05% (66.47% - 92.46%)	61,60% (52.48% - 70.16%)	73,39% (67.01% - 79.13%)
NPV (95% CI)	100,00% (99.98% - 100.00%)	100,00% (99.98% - 100.00%)	100,00% (99.99% - 100.00%)	100,00% (99.99% - 100.00%)	100,00% (99.99% - 100.00%)

PPV: Positive Predictive Value; NPV: Negative Predictive Value; SCA: Sex Chromosomes Aneuploidy

PrenatalSafe® Performance FAST protocol

Follow-up March 2016

	Trisomy 21 (n=15.258)	Trisomy 18 (n=15.258)	Trisomy 13 (n=15.258)	Monosomy X (n=15.258)	SCA (n=15.258)
True positive	156	30	20	57	91
False positive	2	1	1	18	22
True negative	15100	15227	15279	15183	15145
False negative	0	0	0	0	0
Sensitivity (95% CI)	100,00% (88.43% - 100.00%)	100,00% (88.43% - 100.00%)	100,00% (83.16% - 100.00%)	100,00% (93.73% - 100.00%)	100,00% (96.03% - 100.00%)
% False Negative	0,0%	0,0%	0,0%	0,0%	0,0%
Specificity (95% CI)	99,99% (99.96% - 100.00%)	99,99% (99.96% - 100.00%)	99,99% (99.96% - 100.00%)	99,88% (99.81% - 99.93%)	99,85% (99.78% - 99.91%)
% False Positive	0,01%	0,01%	0,01%	0,12%	0,15%
PPV (95% CI)	98,73% (83.30% - 99.92%)	96,77% (83.30% - 99.92%)	95,24% (76.18% - 99.88%)	76,00% (64.75% - 85.11%)	80,53% (72.02% - 87.38%)
NPV (95% CI)	100,00% (99.98% - 100.00%)	100,00% (99.98% - 100.00%)	100,00% (99.98% - 100.00%)	100,00% (99.98% - 100.00%)	100,00% (99.98% - 100.00%)

PPV: Positive Predictive Value; NPV: Negative Predictive Value; SCA: Sex Chromosomes Aneuploidy

PrenatalSafe® KARYO

Published validation studies

Original Research ajog.org

OBSTETRICS

Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants

Roy B. Lefkowitz, PhD; John A. Tynan, PhD; Tong Liu, PhD; Yijin Wu, PhD; Amin R. Mazloom, PhD; Eyad Almasri, MS; Grant Hogg, MS; Vach Angkachatchai, PhD; Chen Zhao, PhD; Daniel S. Grosu, MD; Graham McLennan, MS; Mathias Ehrich, MD

European Journal of Human Genetics (2015), 1–8

www.nature.com/ejhg

ARTICLE

Noninvasive prenatal testing using a novel analysis pipeline to screen for all autosomal fetal aneuploidies improves pregnancy management

Baran Bayindir[1,2,4], Luc Dehaspe[1,4], Nathalie Brison[1,4], Paul Brady[1,4], Simon Ardui[1], Molka Kammoun[1], Lars Van der Veken[3], Klaske Lichtenbelt[3], Kris Van den Bogaert[1], Jeroen Van Houdt[1], Hilde Peeters[1], Hilde Van Esch[1], Thomy de Ravel[1], Eric Legius[1], Koen Devriendt[1] and Joris R Vermeesch*[,1]

Clinical Chemistry 61:4
608–616 (2015)

Molecular Diagnostics and Genetics

Detection of Fetal Subchromosomal Abnormalities by Sequencing Circulating Cell-Free DNA from Maternal Plasma

Chen Zhao,[1] John Tynan,[1] Mathias Ehrich,[2] Gregory Hannum,[1] Ron McCullough,[1] Juan-Sebastian Saldivar,[1] Paul Oeth,[1] Dirk van den Boom,[2*] and Cosmin Deciu[1*]

DOI: 10.1002/pd.4110

PRENATAL DIAGNOSIS

ORIGINAL ARTICLE

A method for noninvasive detection of fetal large deletions/duplications by low coverage massively parallel sequencing

Shengpei Chen[1,7†], Tze Kin Lau[2†], Chunlei Zhang[1†], Chenming Xu[3], Zhengfeng Xu[4], Ping Hu[4], Jian Xu[5], Hefeng Huang[4], Ling Pan[5], Fuman Jiang[1], Fang Chen[1,8], Xiaoyu Pan[1,6], Weiwei Xie[1], Ping Liu[1], Xuchao Li[1], Lei Zhang[1], Songgang Li[1], Yingrui Li[1], Xun Xu[1], Wei Wang[1], Jun Wang[1,8,9,10], Hui Jiang[1,8*] and Xiuqing Zhang[1*]

[1]BGI-Shenzhen, Shenzhen, China
[2]Fetal Medicine Centre, Paramount Clinic, Hong Kong
[3]Key Laboratory of Reproductive Genetics, Zhejiang University, Ministry of Education, Hangzhou, China
[4]State Key Laboratory of Reproductive Medicine, Center of Prenatal Diagnosis, Nanjing Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University, Nanjing, China
[5]Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China
[6]School of Bioscience and Bioengineering, South China University of Technology, Guangzhou, China
[7]State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing, China
[8]Department of Biology, University of Copenhagen, Copenhagen, Denmark
[9]King Abdulaziz University, Jeddah, Saudi Arabia
[10]The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark
*Correspondence to: Hui Jiang. E-mail: jianghui@genomics.org.cn or Xiuqing Zhang. E-mail: zhangxq@genomics.org.cn
†These authors contributed equally to this work.

Prenatal Diagnosis 2013, 33, 584–590

OPEN ACCESS Freely available online

PLOS ONE

Noninvasive Prenatal Molecular Karyotyping from Maternal Plasma

Stephanie C. Y. Yu[1,2], Peiyong Jiang[1,2], Kwong W. Choy[3], Kwan Chee Allen Chan[1,2], Hye-Sung Won[4], Wing C. Leung[5], Elizabeth T. Lau[6], Mary H. Y. Tang[6], Tak Y. Leung[3], Yuk Ming Dennis Lo[1,2], Rossa W. K. Chiu[1,2]*

1 Centre for Research into Circulating Fetal Nucleic Acids, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China, 2 Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China, 3 Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China, 4 Department of Obstetrics and Gynecology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea, 5 Kwong Wah Hospital, Kowloon, Hong Kong SAR, China, 6 Tsan Yuk Hospital, Department of Obstetrics and Gynaecology, University of Hong Kong, Hong Kong SAR, Hong Kong

GENOMA® Molecular Genetics Laboratories Group

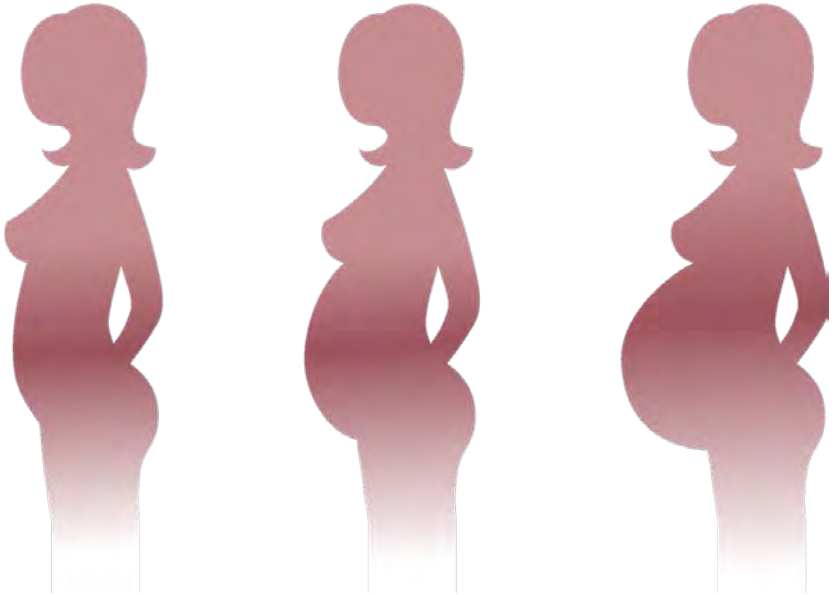
Which Patients Should Be Offered?

This test is intended for patients at **10 weeks** or greater gestation who meet any of the following criteria:

- Maternal age-related risks (e35 years)
- Positive results on maternal serum screening
- Abnormal ultrasound finding(s)
- Prior pregnancy with aneuploidy
- Parental translocation
- Low risk pregnancies
  - Patients wanting early, accurate testing and are at average risk of aneuploidy

pregnancy characteristics

- **The test** is suitable for both **single** and **twin** pregnancies.
- It can be performed in patients whose pregnancies have been achieved by **IVF** techniques, including pregnancies with **egg donation**.



PrenatalSafe® KARYO

Free services supplied

- **RhSafe test**
  for pregnant women Rh(D) positive and partners Rh(D) negative
- **Shipper kits with Streck™ BCT Tubes**
- **Genetic counseling pre- and post-test**
- **Follow-up**
  follow-up of anbormal results, performed with both traditional and molecular karyotyping

GENOMA® Molecular Genetics Laboratories Group

Case study

Case Study 1

31 y.o. patient

↓ **12^ weeks gestation**

PrenatalSafe® **5**

↓

Negative Result

↓ **20^ week gestation**

Fetal abnormal ultrasound findings
(cerebellar ipoplasia; Ventriculomegaly)

↙ ↘

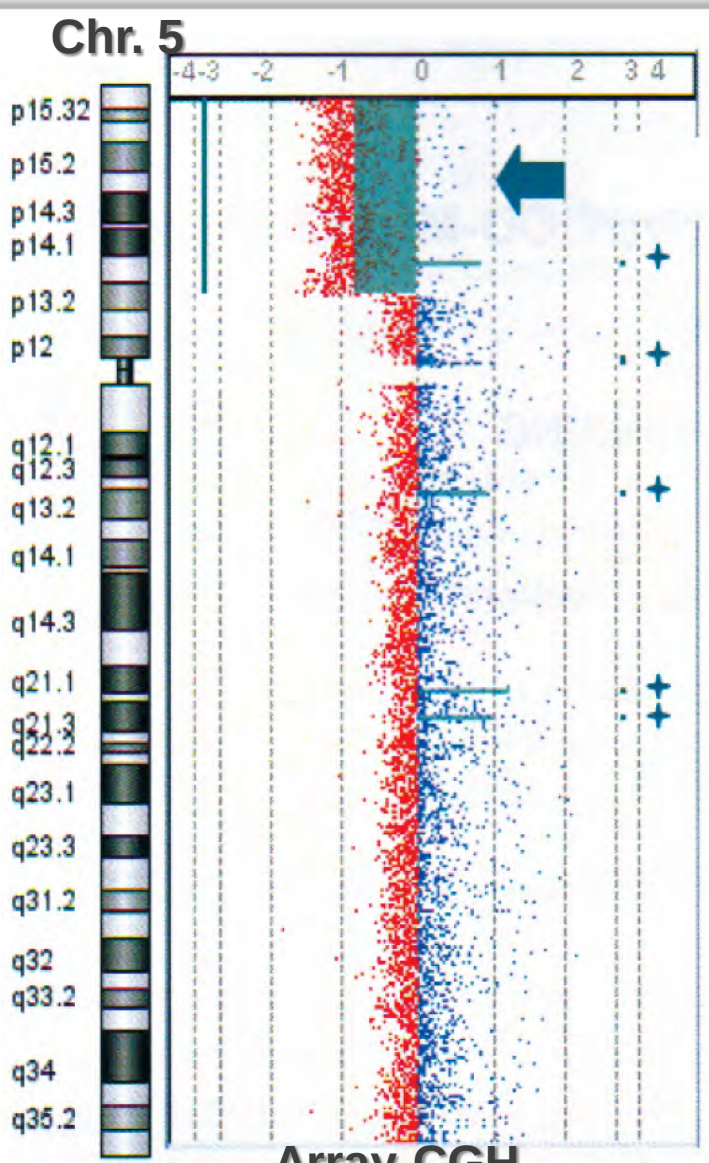
Amniocentesis

Reassessment of the NIPT data with

PrenatalSafe® KARYO

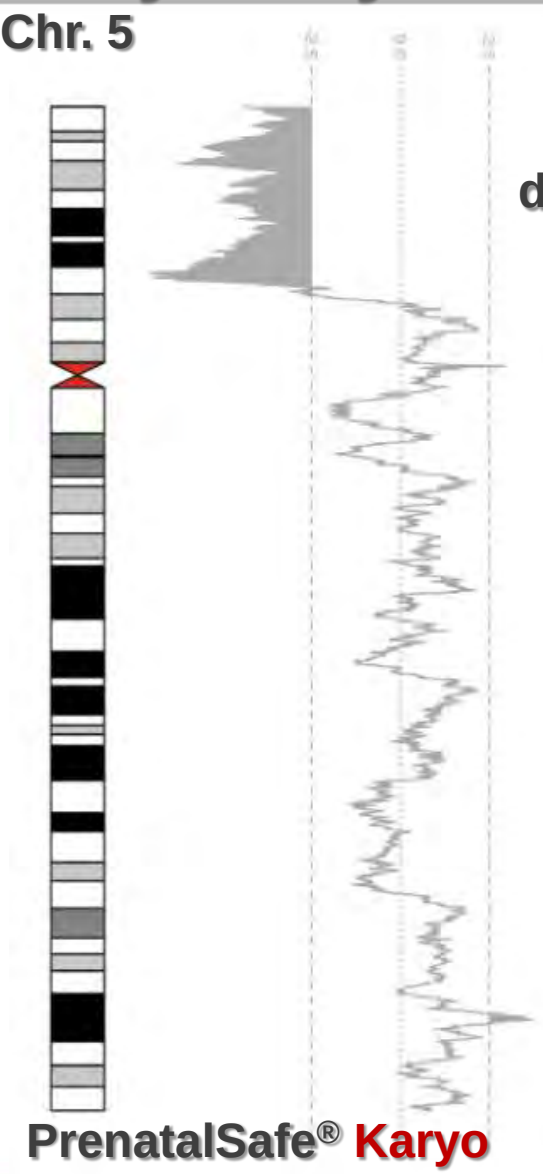
GENOMA® Molecular Genetics Laboratories Group

Detection of a deletion 5p confirmed by array-CGH



Amniotic Fluid

Array-CGH



del5p15.33p13.2

34 Mb

cfDNA

PrenatalSafe® Karyo

Case Study 2

32 y.o. patient

↓

13^ weeks gestation

PrenatalSafe® 5

↓

Negative Result

↓

21^ weeks gestation

Multiple fetal abnormal ultrasound findings

↓

Reassessment of the NIPT data with

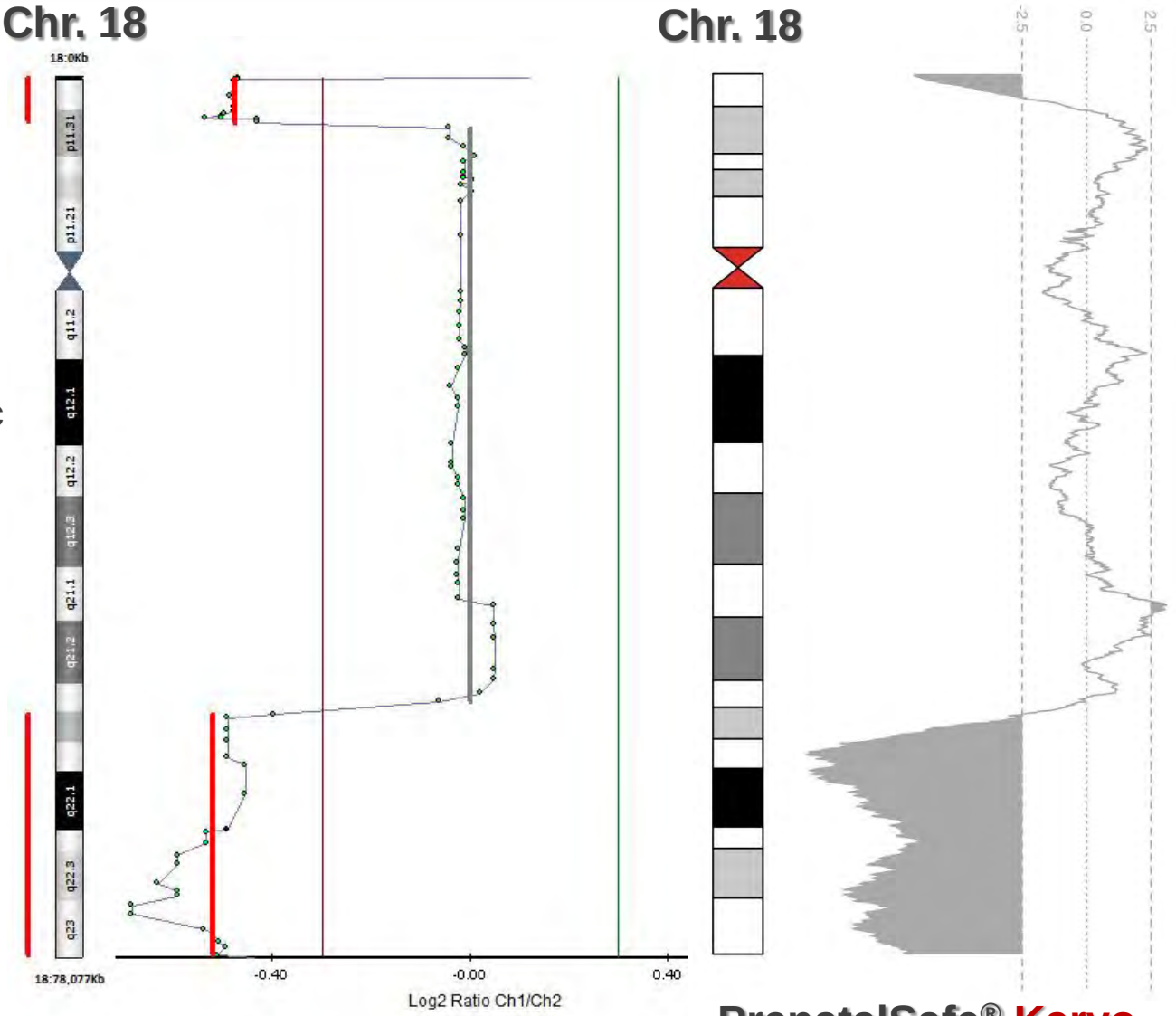
PrenatalSafe® KARYO

↓

Amniocentesis

Identification of 2 deletions (18p / 18q) confirmed by array-CGH

Amniotic Fluid



cfDNA

Del18p11.32-p11.31
3.8 Mb

Del18q21.32-q23
21.3 Mb

GENOMA® Molecular Genetics Laboratories Group

Case Study 3

32 y.o patient

Carrier of a reciprocal translocation 46,XX,t(7;9)(p15;q22)

11^ weeks gestation

PrenatalSafe® KARYO

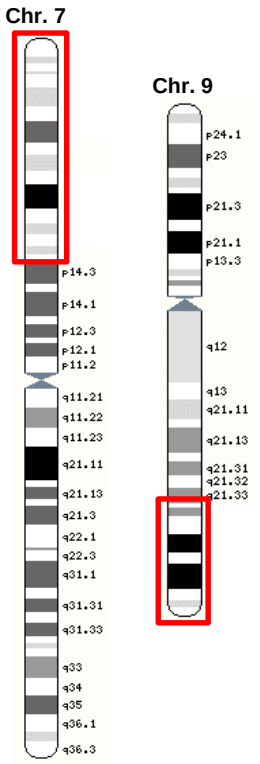
Abnormal Result

detected fetal karyotype with an unbalanced translocation

Villocentesis

PrenatalSafe® KARYO

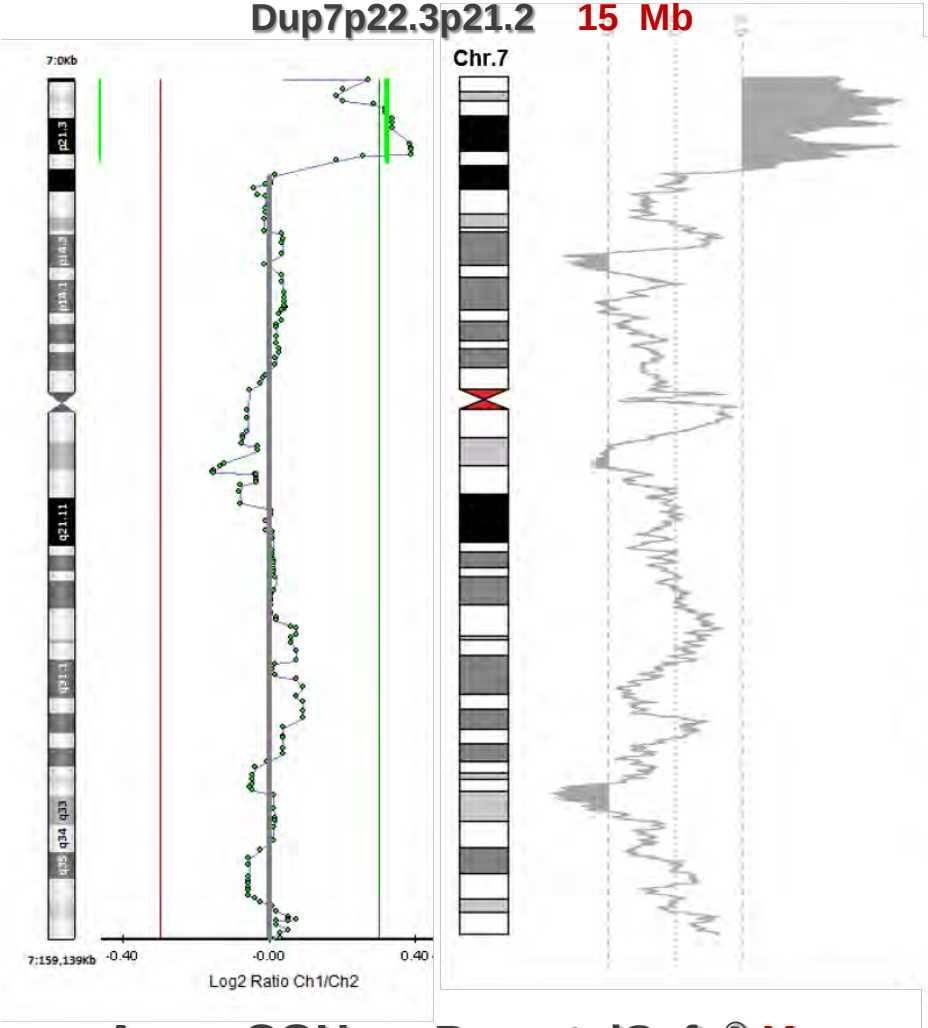
Results confirmed



GENOMA® Molecular Genetics Laboratories Group

Detected a fetal karyotype with an unbalanced translocation confirmed by array-CGH

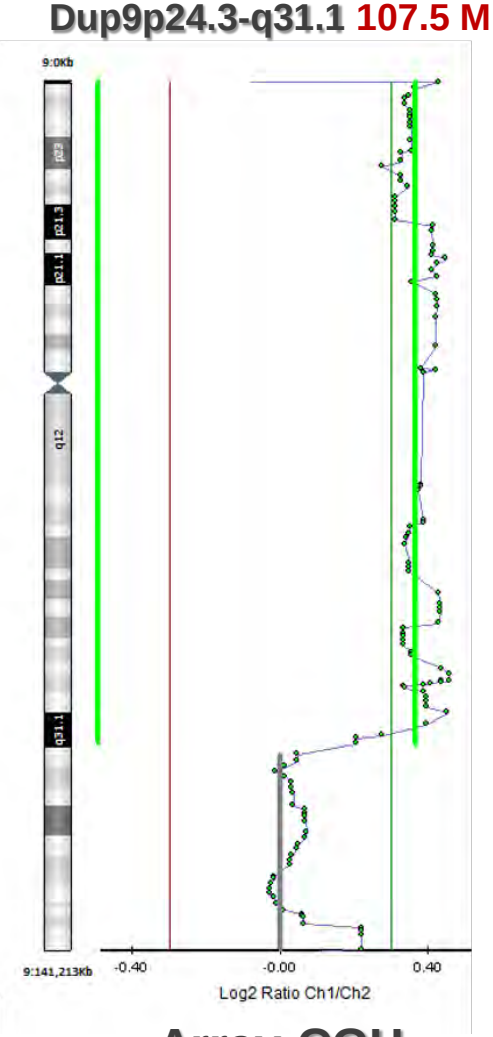
Dup7p22.3p21.2 15 Mb



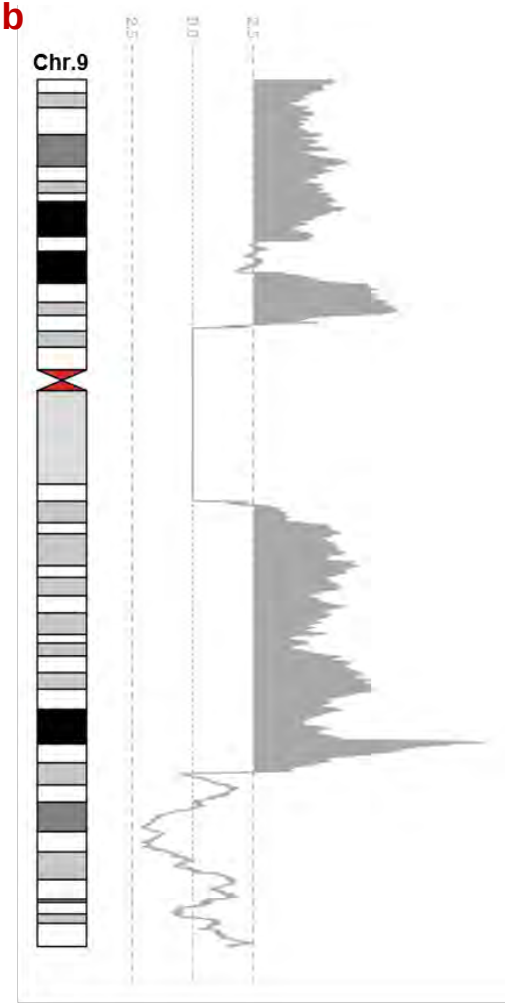
Array-CGH
CVS

PrenatalSafe® Karyo
cfDNA

Dup9p24.3-q31.1 107.5 Mb



Array-CGH
CVS



PrenatalSafe® Karyo
cfDNA

GENOMA®
Molecular Genetics Laboratories Group

Case Study 4

35 y.o patient

↓ **13^ weeks gestation**

Amniocentesis
(traditional karyotyping)

↓

Negative Result
(Fetale karyotype 46, XY)

↓ **20^ weeks gestation**

Fetal abnormal ultrasound findings
(Suspected DiGeorge syndrome)

↓

PrenatalSafe® KARYO

↓

Identified deletion 22q11.2 (compatible with DiGeorge syndrome)

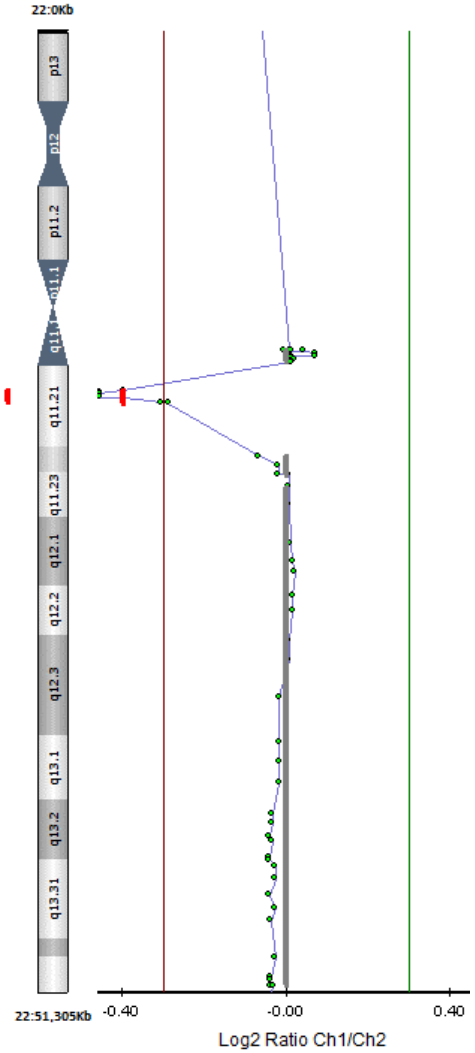
↓

Amniocentesis

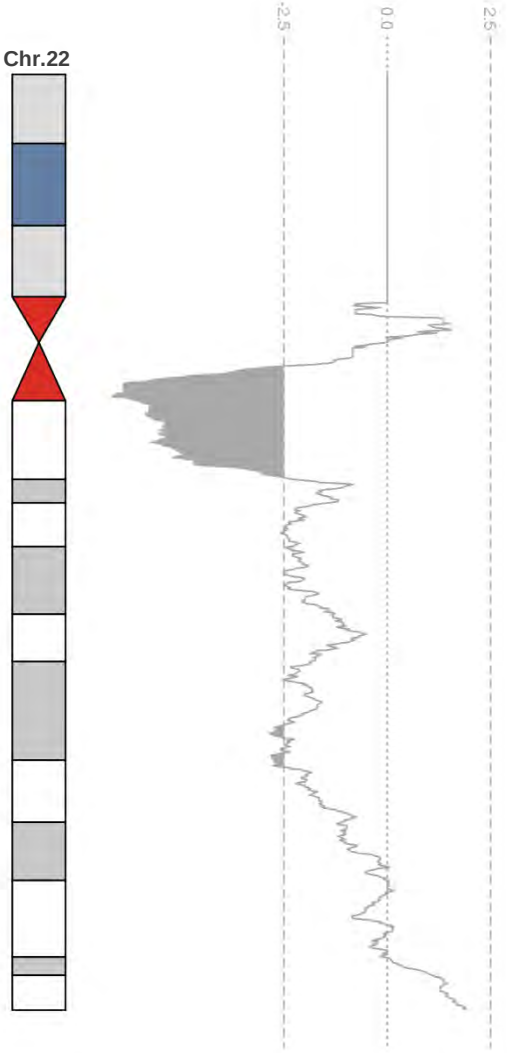
PrenatalSafe® KARYO Plus

Identification of 22q11.2 deletion confirmed by Array-CGH

Amniotic Fluid



Array-CGH



PrenatalSafe® Karyo

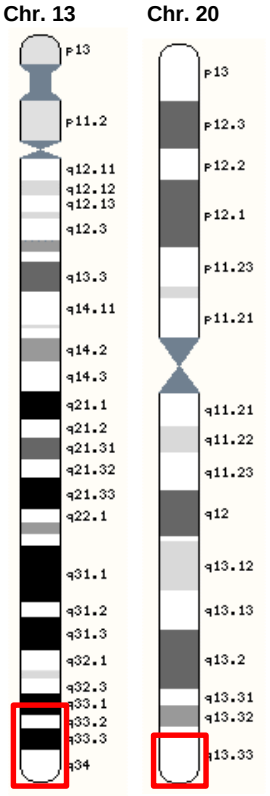
del22q11.21
DiGeorge Syndrome

4 Mb

cfDNA

GENOMA® Molecular Genetics Laboratories Group

Case Study 5



Paziente portatrice di traslocazione reciproca 46,XX,t(13;20)(q33;q13.3)

33 y.o. patient

(She was not aware to be carrier of a chromosomal translocation)

12^ weeks gestation

PrenatalSafe® KARYO

Abnormal Result

detected fetal karyotype with an unbalanced translocation

Villocentesis

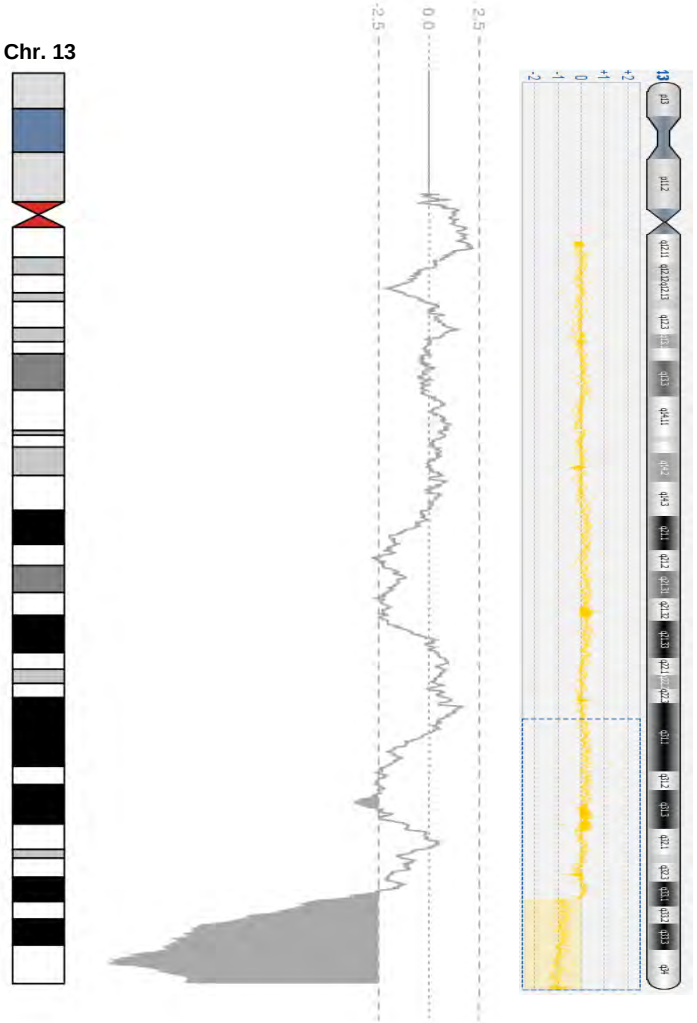
PrenatalSafe® KARYO

Results confirmed

GENOMA® Molecular Genetics Laboratories Group

PrenatalSafe® KARYO — Detected a fetal karyotype with an unbalanced translocation confirmed by array-CGH

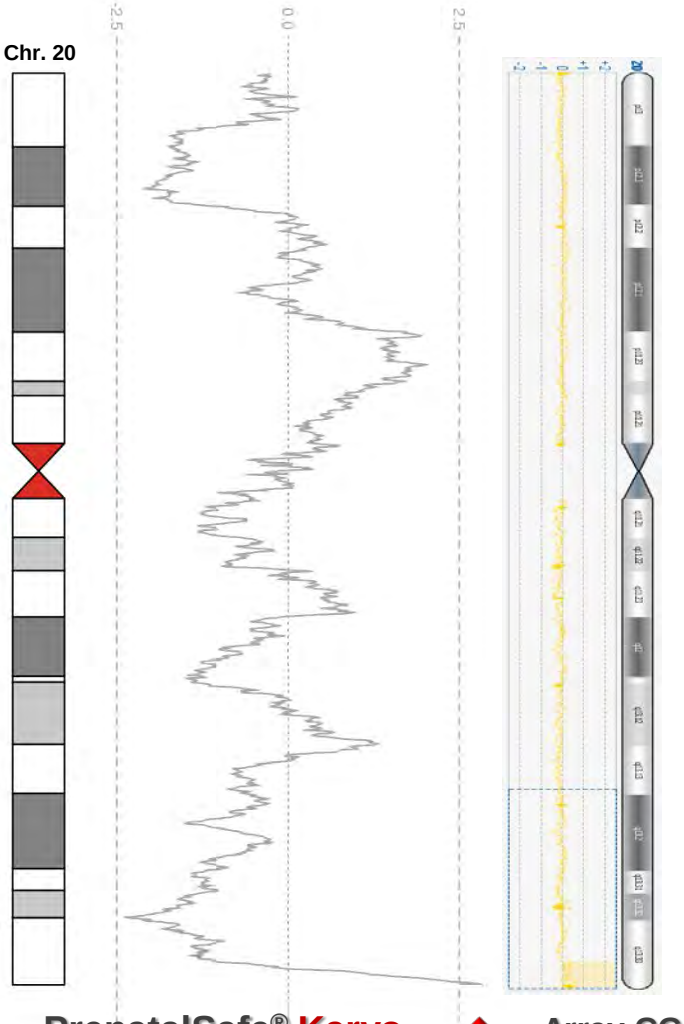
Del13q33.1q34 11.4 Mb



PrenatalSafe® Karyo (cfDNA)

Array-CGH (CVS)

Dup20q13.33 1.9 Mb



PrenatalSafe® Karyo (cfDNA)

Array-CGH (CVS)

GENOMA® Molecular Genetics Laboratories Group

Case Study 6

40 y.o. patient

↓ **13^ weeks gestation**

NIPT
(5 chromosomes screening)

↓ **Test performed by a different lab**

Positive Result
High risk for Trisomy 21

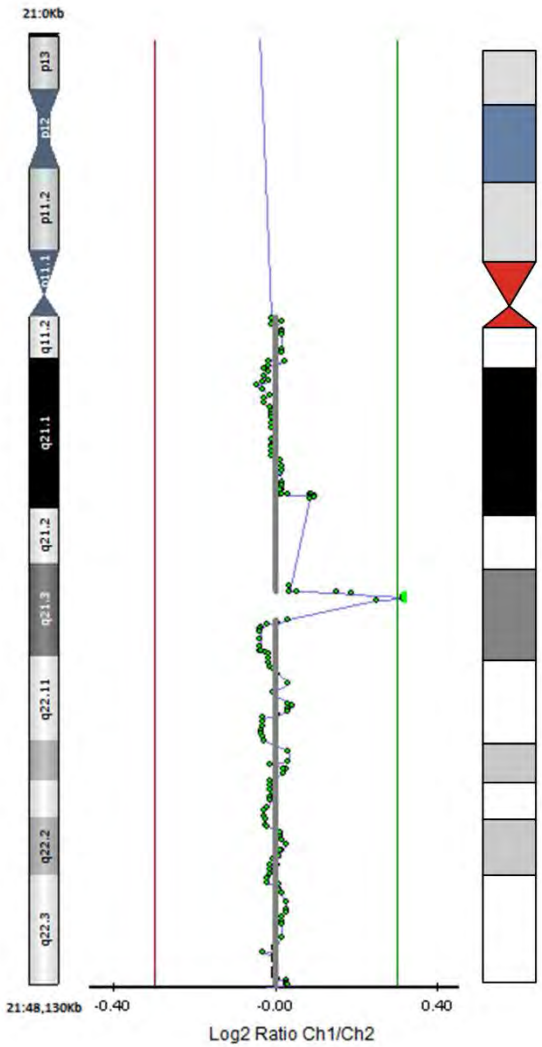
↓

PrenatalSafe® KARYO

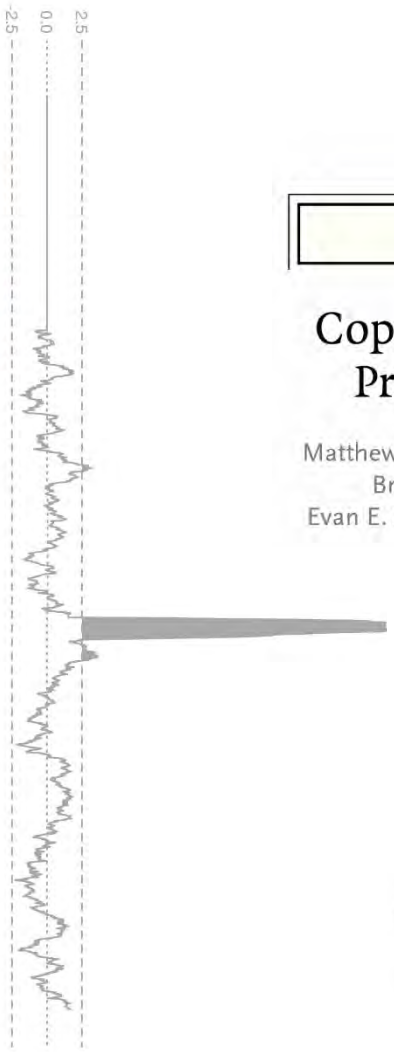
↓

Trisomy 21 not detected
Maternal CNV detected
causing the false positive result

Lower the risk of false positive results determined by maternal CNVs



Array-CGH
Maternal blood



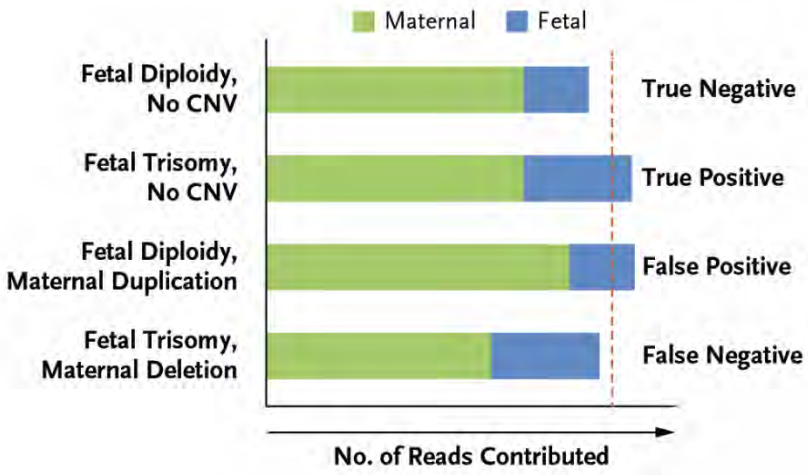
PrenatalSafe® Karyo
cfDNA

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Copy-Number Variation and False Positive Prenatal Aneuploidy Screening Results

Matthew W. Snyder, M.S., LaVone E. Simmons, M.D., Jacob O. Kitzman, Ph.D., Bradley P. Coe, Ph.D., Jessica M. Henson, B.S., Riza M. Daza, B.S., Evan E. Eichler, Ph.D., Jay Shendure, M.D., Ph.D., and Hilary S. Gammill, M.D.



GENOMA® Molecular Genetics Laboratories Group