DNA fetale in plasma, metodi di analisi e applicazioni

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Non-invasive prenatal diagnosis

- It has been known for a while that there is an exchange of cellular material between mother and fetus during pregnancy

- The discovery of the presence of circulating cell-free fetal DNA in maternal plasma/serum opened up no perspectives in this research area (Lo et al.1997)

- Fetal DNA derived from maternal peripheral circulation as source of fetal genetic material for non-invasive prenatal diagnosis

Origin of cffDNA in maternal plasma

- 1997: Y-chromosomal sequences detected in plasma and serum of most pregnant women carrying male fetuses (Lo et al 1997)
- 1998: Quantitation of fetal DNA by real time PCR
  - Higher amounts in Plasma
    - Early pregnancy: 0.4 – 11.9% (mean 3.4%)
    - Late pregnancy: 2.3 – 11.4% (mean 6.2%)
- Placenta specific RNA (ZFY) in maternal plasma (Poon et al. 2000)
- cfDNA from throphoblast (Alberry et al. 2007)

Clearance of cffDNA

- Fetal DNA is cleared very rapidly
- Mean half-life of fetal DNA 16,3 min
- 7/8 women had undetectable levels of circulating fetal DNA by 2 hours postpartum (Lo 1999) all by 2 days.
- Kidney responsible for DNA clearance?: DNA excreted in urine (Botezatu 2000)
- Estimated that fetal DNA is released at a rate of 2.24 $x10^4$ copies/min during pregnancy

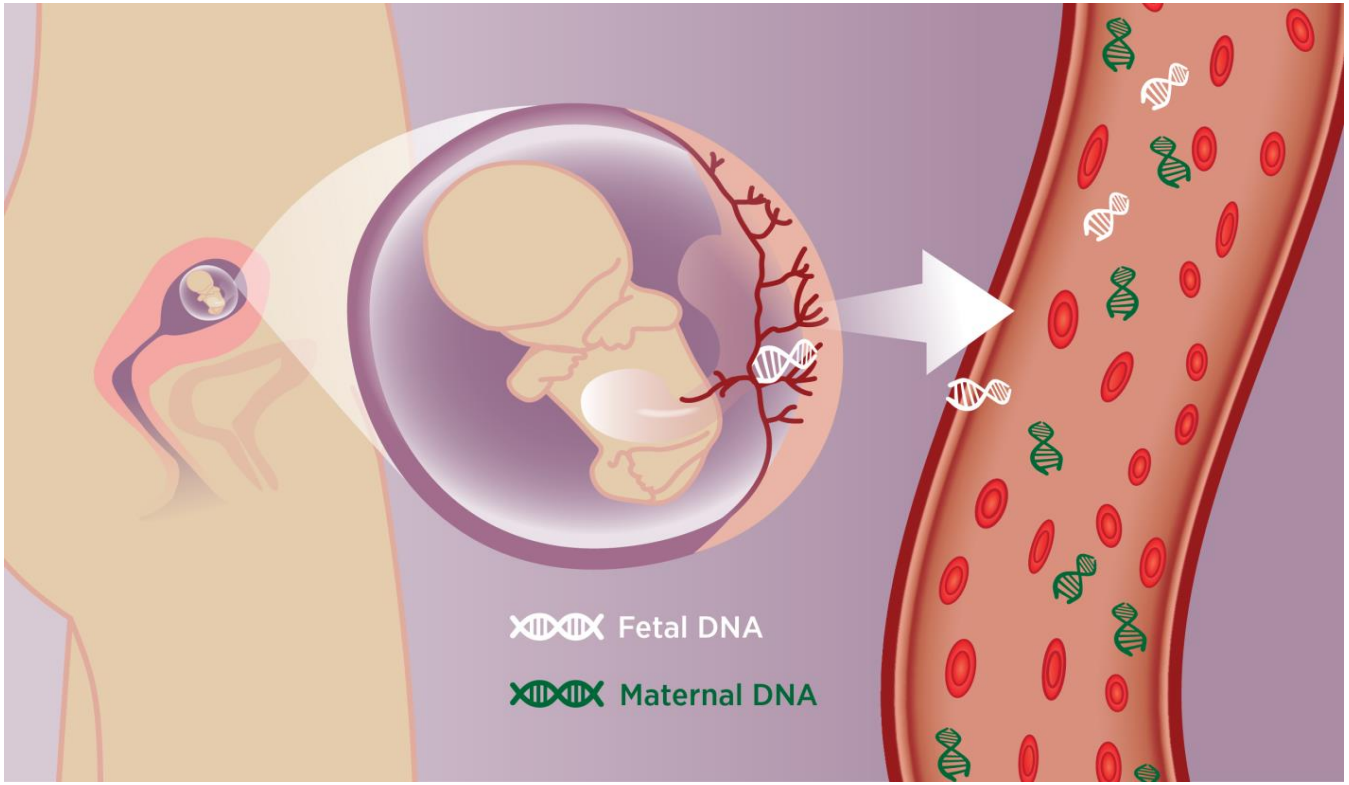
Quantitative abnormalities of fetal DNA in maternal plasma

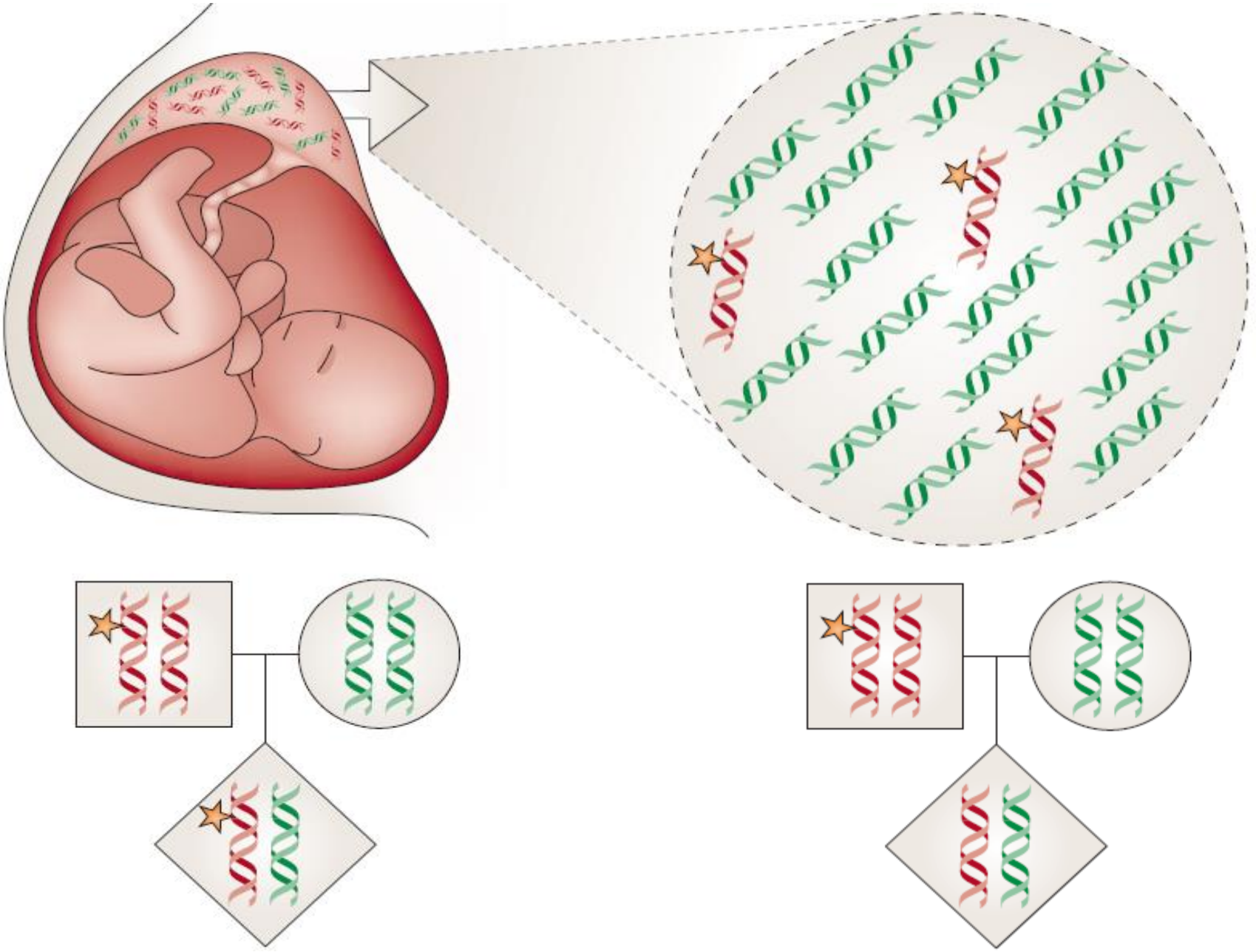
- **The amount of fetal DNA increases during gestation, and also:**

- **Preeclampsia**
- **Preterm labour**
- **Chromosomal aberrations (trisomy 21, 13)**
- **Polyhydramnios**

Cell-free DNA in Maternal Blood

- Cell-free DNA (cfDNA) are short DNA fragments
- Amount of fetal cfDNA present is a small fraction of the maternal cfDNA
- Quickly disappears after delivery





Clinical Applications

- **X-linked Disorders**

The Y chromosome can be detected from 8-10 weeks of gestation in 100% of cases

- **RhD Genotyping**

Lo et al, 1998: Fetal RhD genotyping is possible in all cases during the second trimester of pregnancy.

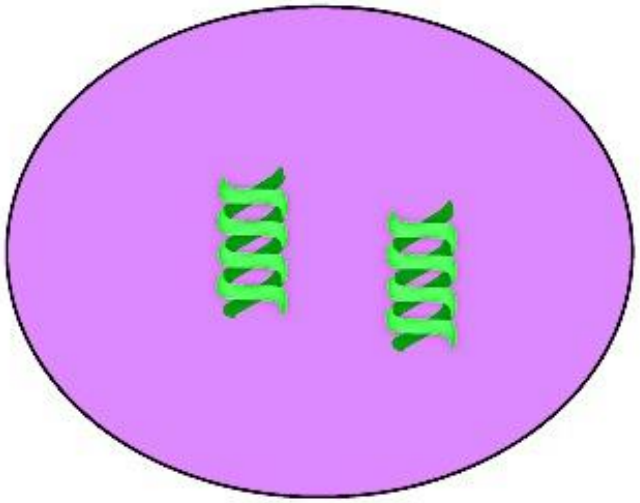
Repeatedly confirmed over the years, first routine application of NIPD (British National Blood Service 2001)

Detection of Paternal Mutations

- Myotonic Distrophy
- Acondroplasia
- Cystic Fibrosis
- ß Thalasemia
- Congenital Adrenal Hiperplasia

- Anomalías Cromosómicas

Normal



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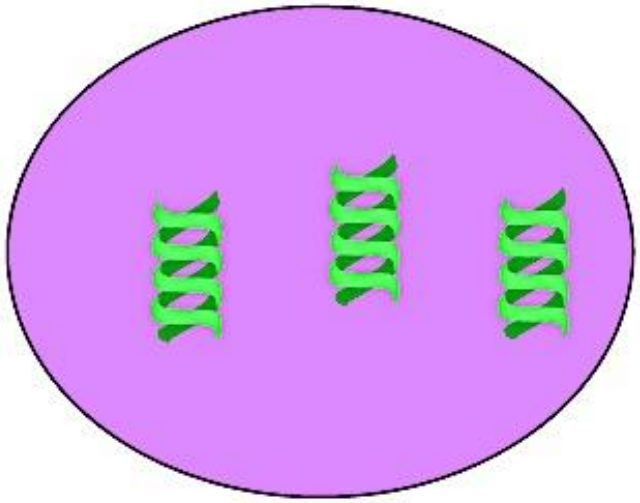
Trisomy 21



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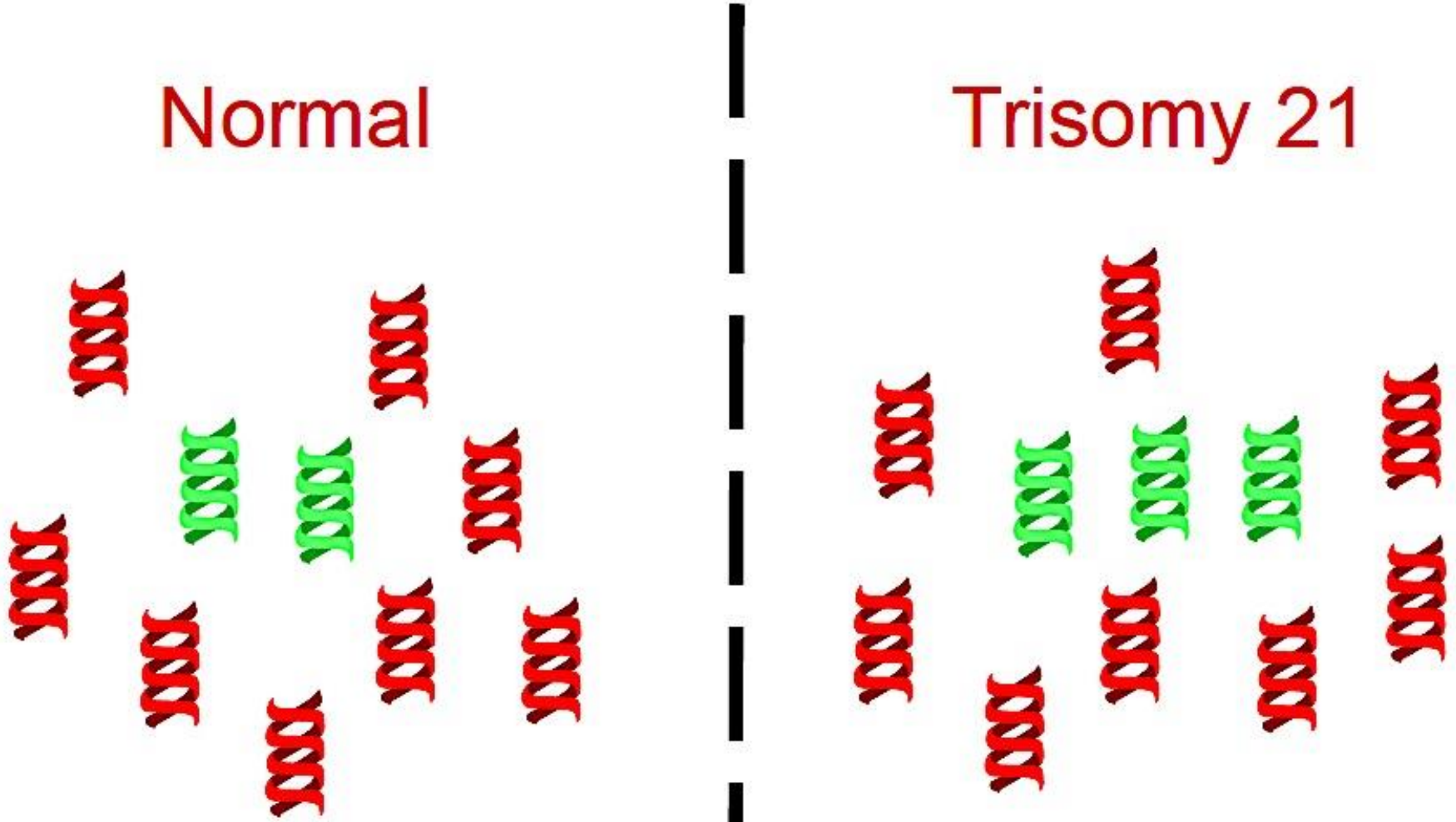
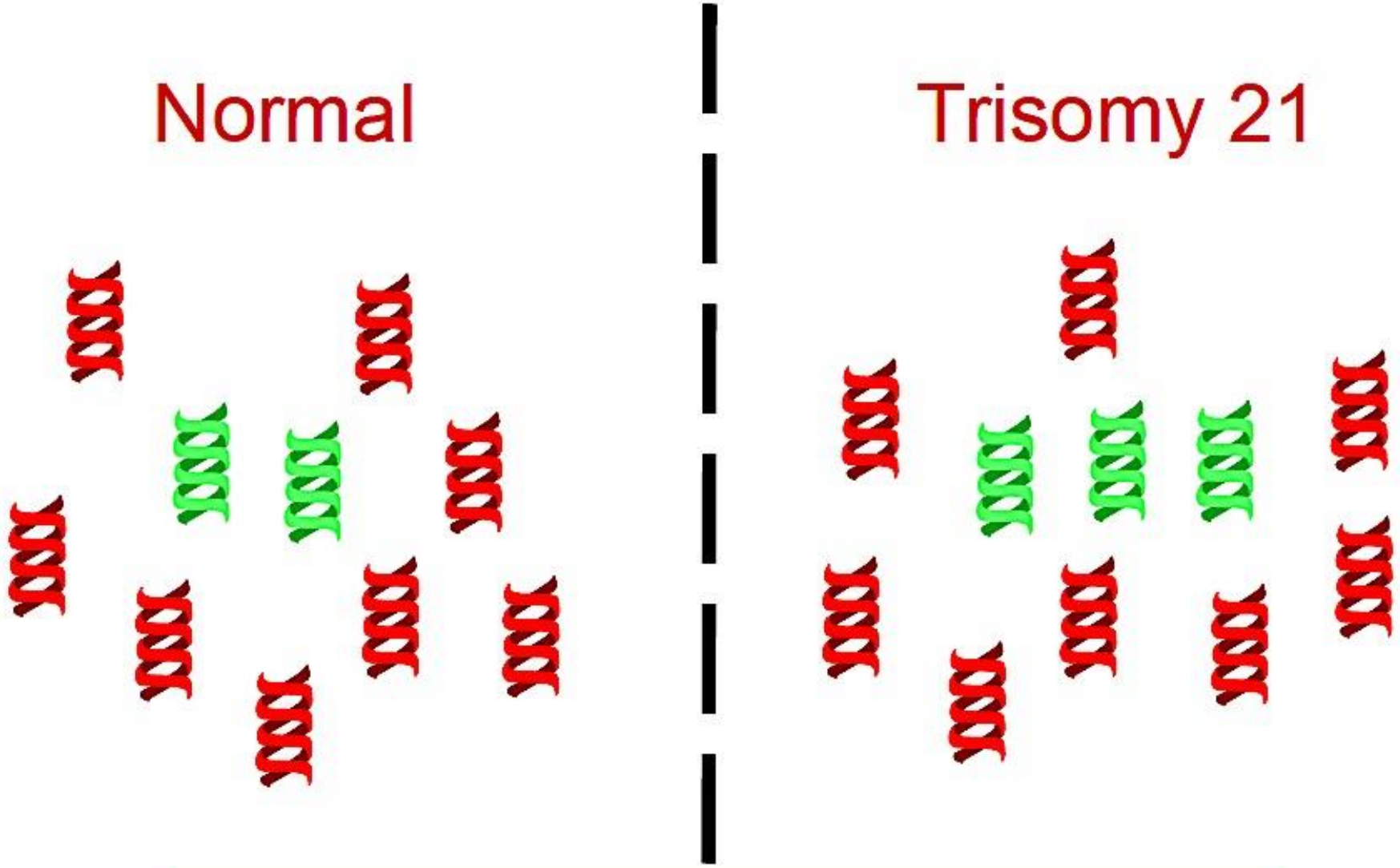
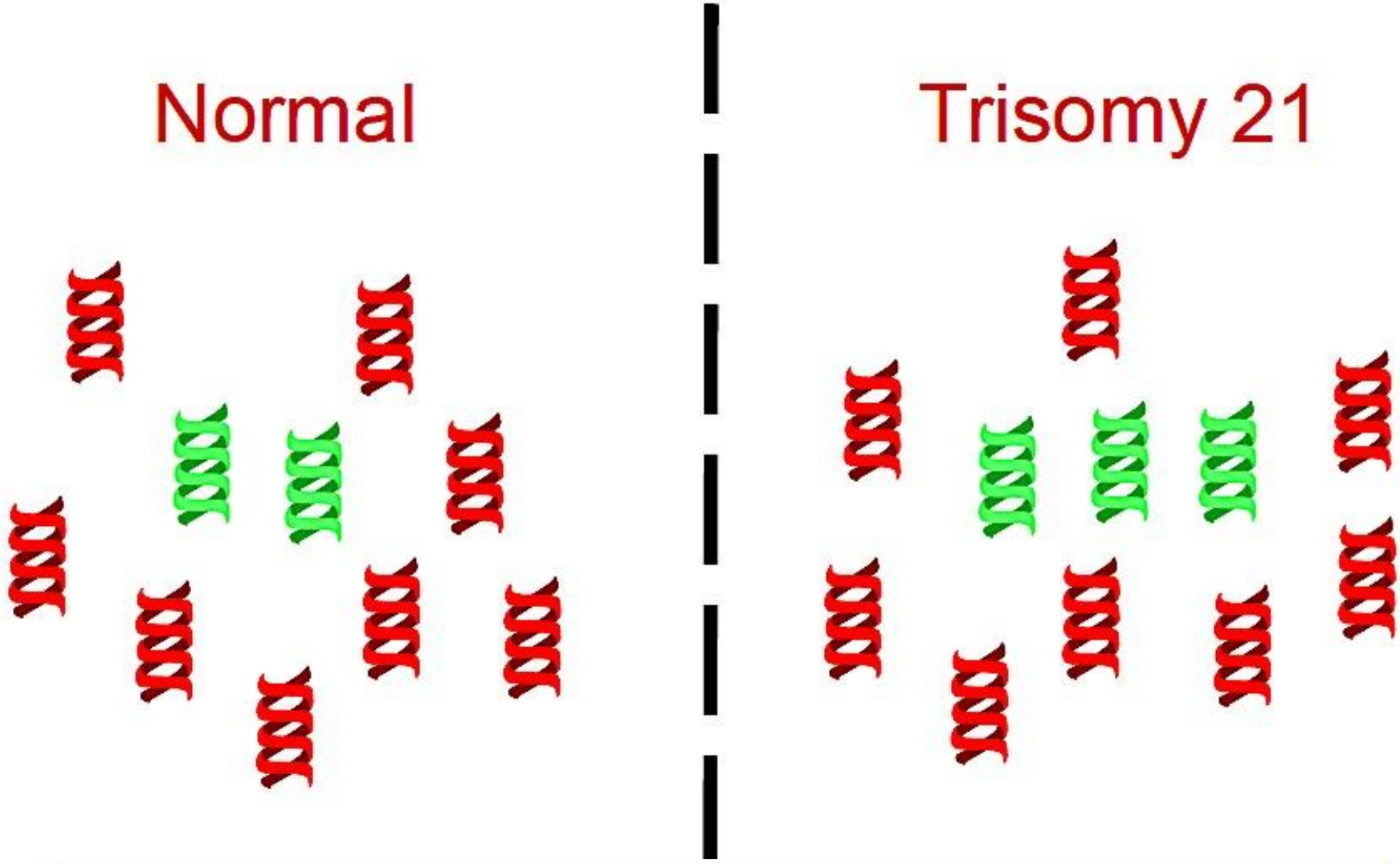
Pure fetal DNA sample (e.g. CVS):
50% ↑ in chr21 DNA



Fetal DNA is the minority in maternal plasma



Maternal plasma:
50% ↑ in **fetal** derived chr21 DNA



↑ in **total** chr21 DNA in maternal plasma:

½ of fetal DNA fraction

Increase of Cr.21 DNA in maternal plasma

Sample	Fetal DNA content	↑ chr21 (%)	↑ chr21 (fold)
CVS	100%	50%	1.5
Maternal plasma	~ 10%	5%	1.05

Fetal Trisomy Detection by MPSS

Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood

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Communicated by Leonard A. Herzenberg, Stanford University School of Medicine, Stanford, CA, August 22, 2008 (received for review July 13, 2008)

Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma

Rossa W. K. Chiu[a,b], K. C. Allen Chan[a,b], Yuan Gao[c,d], Virginia Y. M. Lau[a,b], Wenli Zheng[a,b], Tak Y. Leung[e], Chris H. F. Foo[f], Bin Xie[c], Nancy B. Y. Tsui[a,b], Fiona M. F. Lun[a,b], Benny C. Y. Zee[f], Tze K. Lau[e], Charles R. Cantor[g,1], and Y. M. Dennis Lo[a,b,1]

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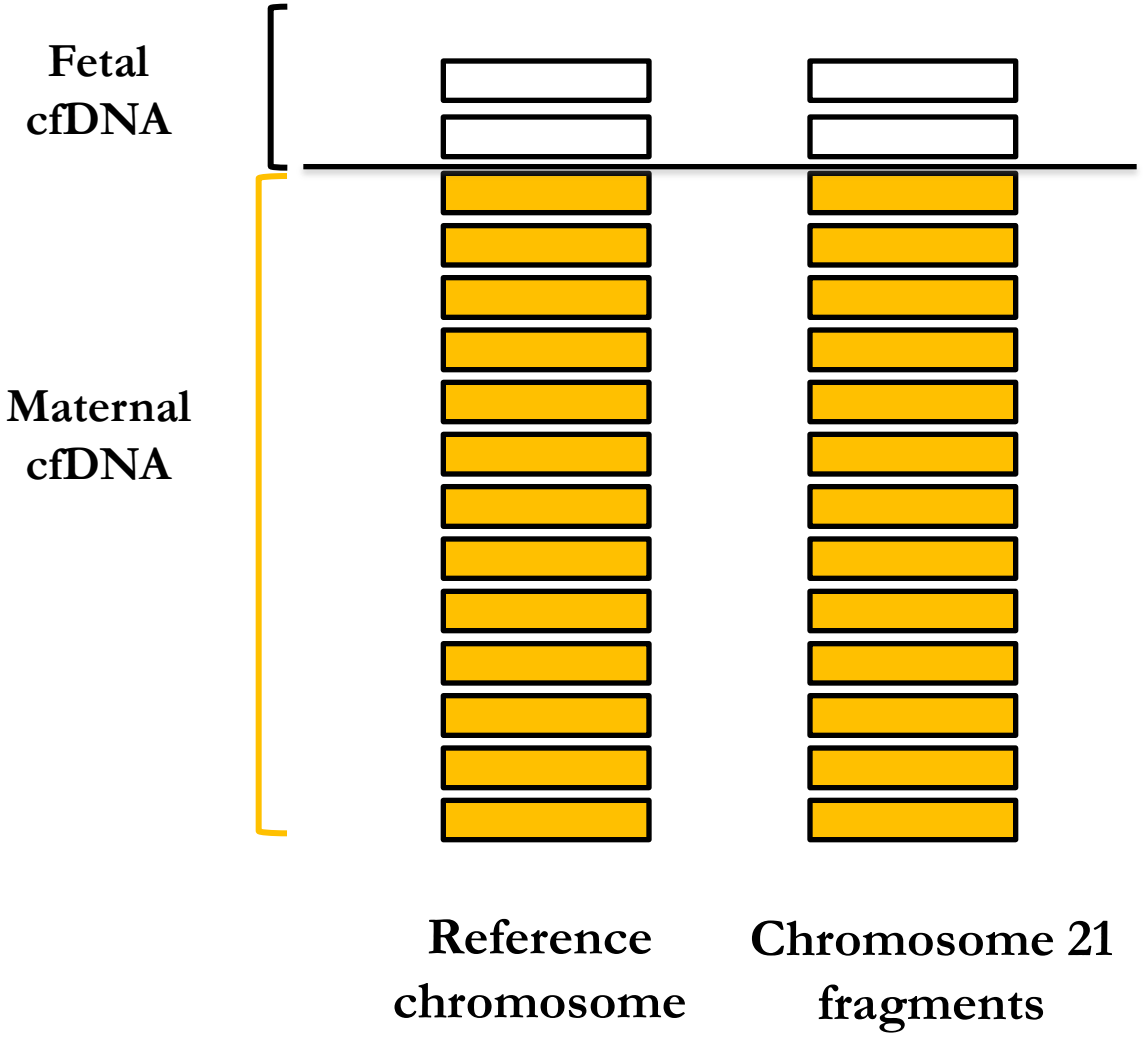
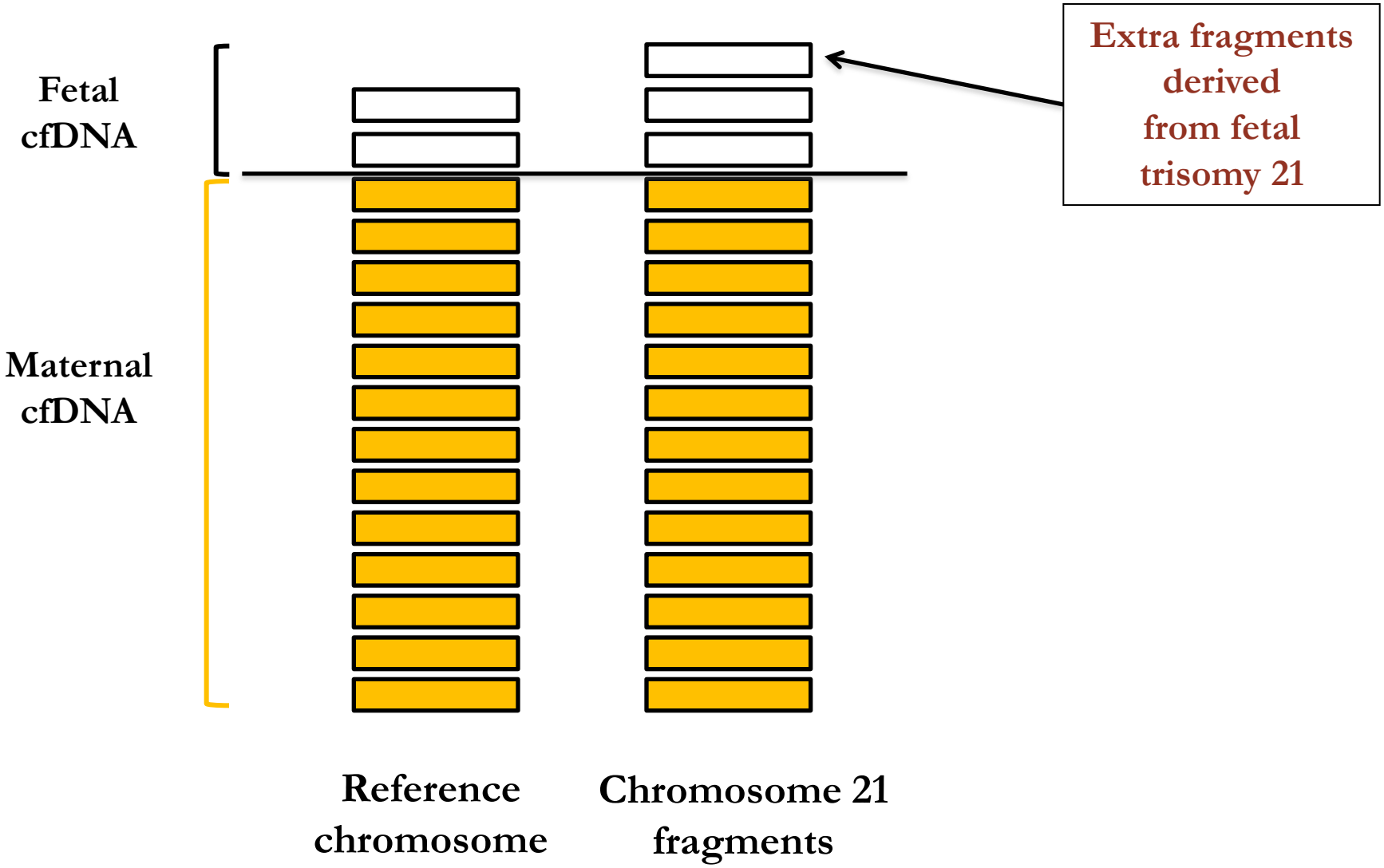
Contributed by Charles R. Cantor, October 22, 2008 (sent for review September 29, 2008)

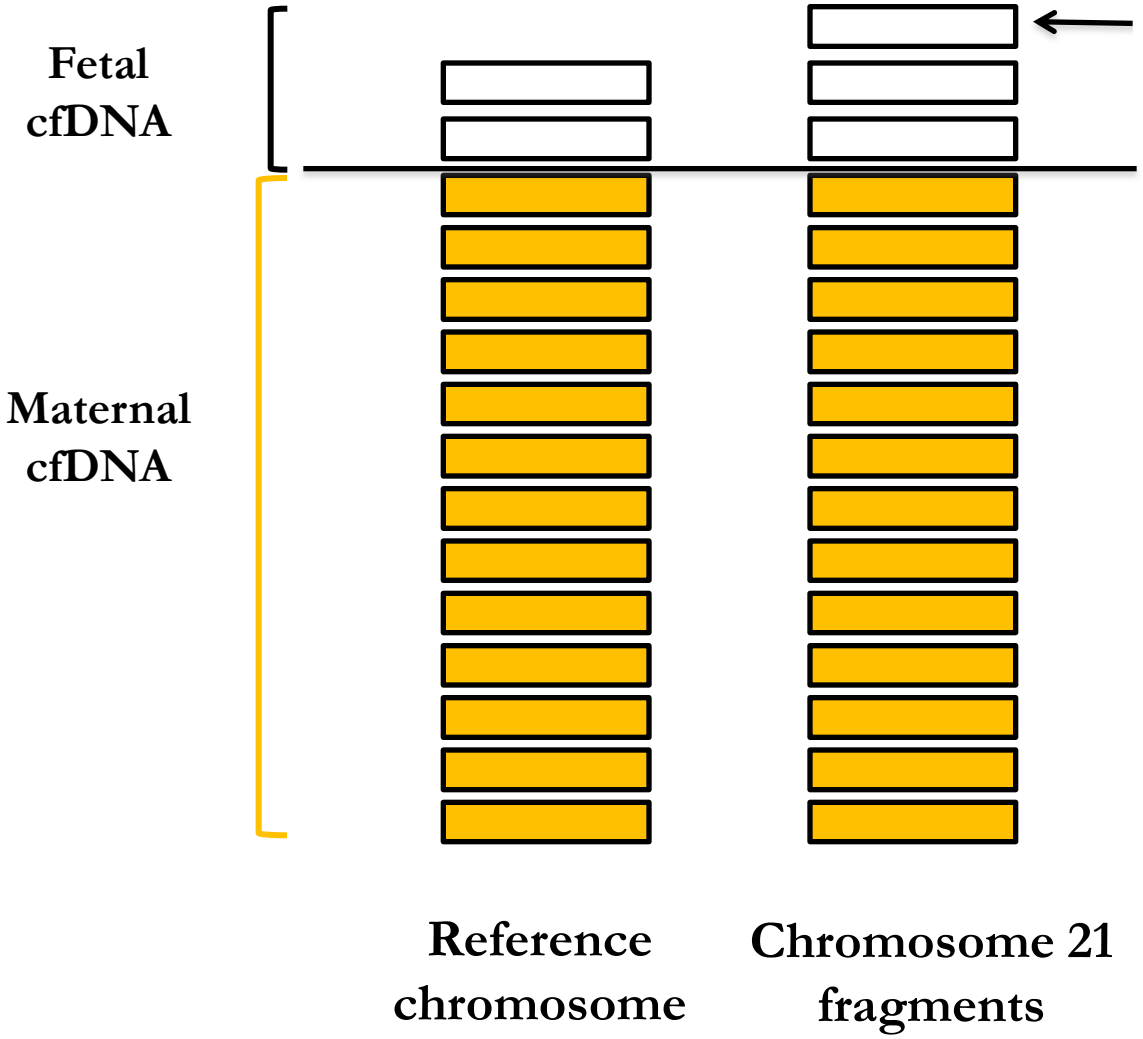
Fetal Trisomy Detection with cfDNA



Fetal Trisomy Detection with cfDNA



Fetal Trisomy Detection with cfDNA



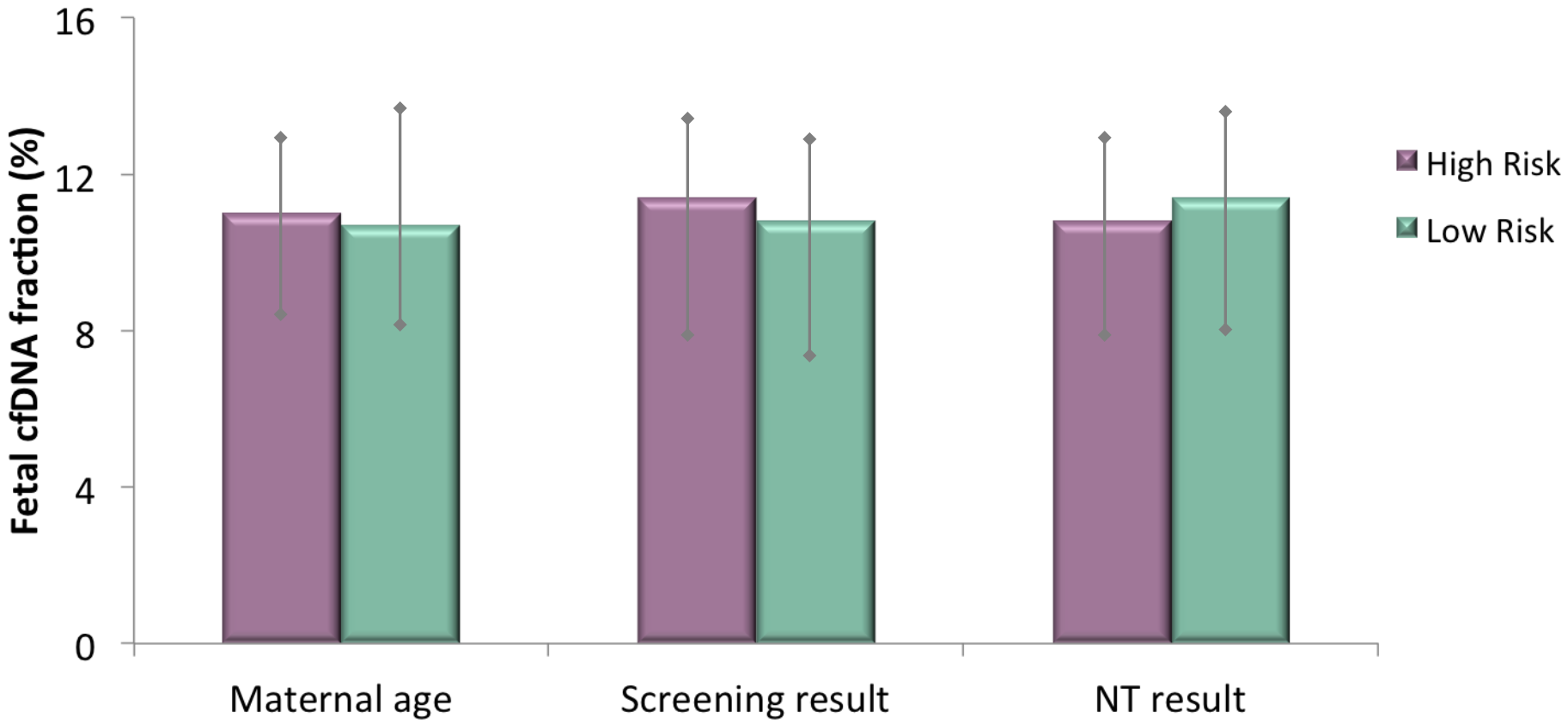
Fetal Fraction	Expected ratio for Trisomy
4%	1.02
10%	1.05
20%	1.10
40%	1.20

Importance of Fetal Fraction

A minimum amount of fetal cfDNA (>4%) is needed for reliable testing with any NIPT

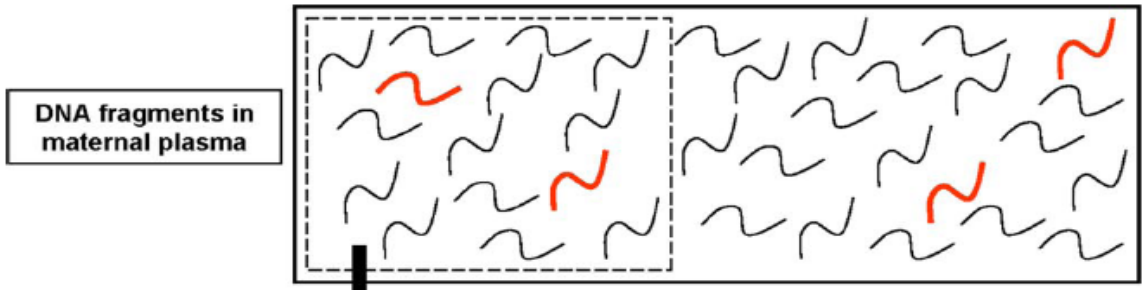
- **Fetal cfDNA measurement is a required basic laboratory quality metric, but not measured by all labs**
- **Factors leading to low fetal fraction of cfDNA (<4%)**
  - **Maternal weight**
  - **Gestational age**
  - **Suboptimal blood collection and shipping**
  - **Other biological and environmental factors**

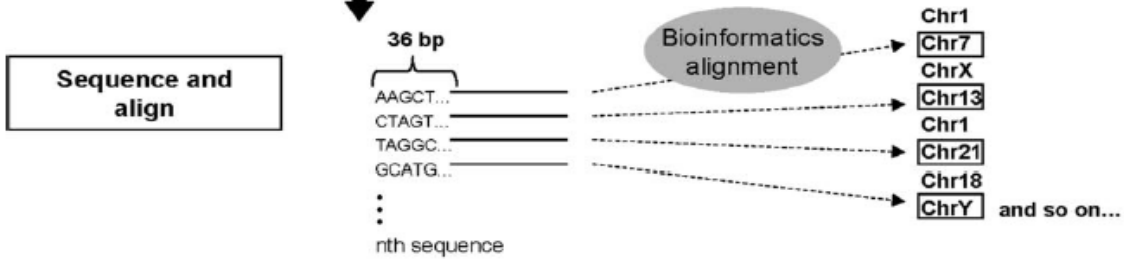
Fetal Fraction Constant Across Risk Groups

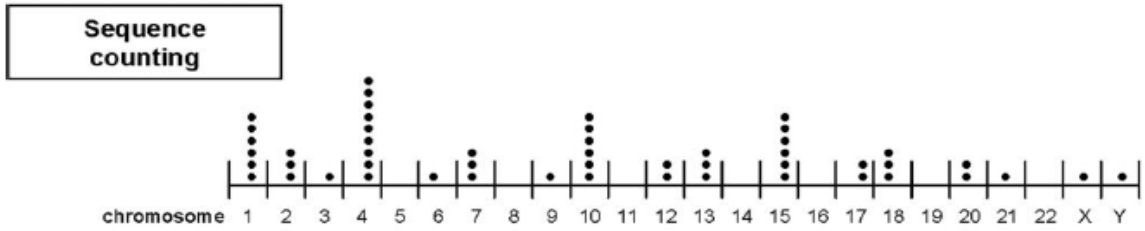


Provides support that NIPT performance is consistent between "High" and "Low" risk pregnancies

Brar H et al., J Matern Fetal Neonatal Med. 2013 Jan;26(2):143-5.





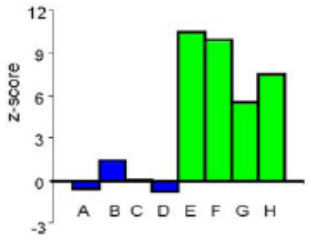


% representation of unique sequences mapped to a chromosome

$$\%\ \text{chrN} = \frac{\text{Unique count for chrN}}{\text{Total unique count}}$$

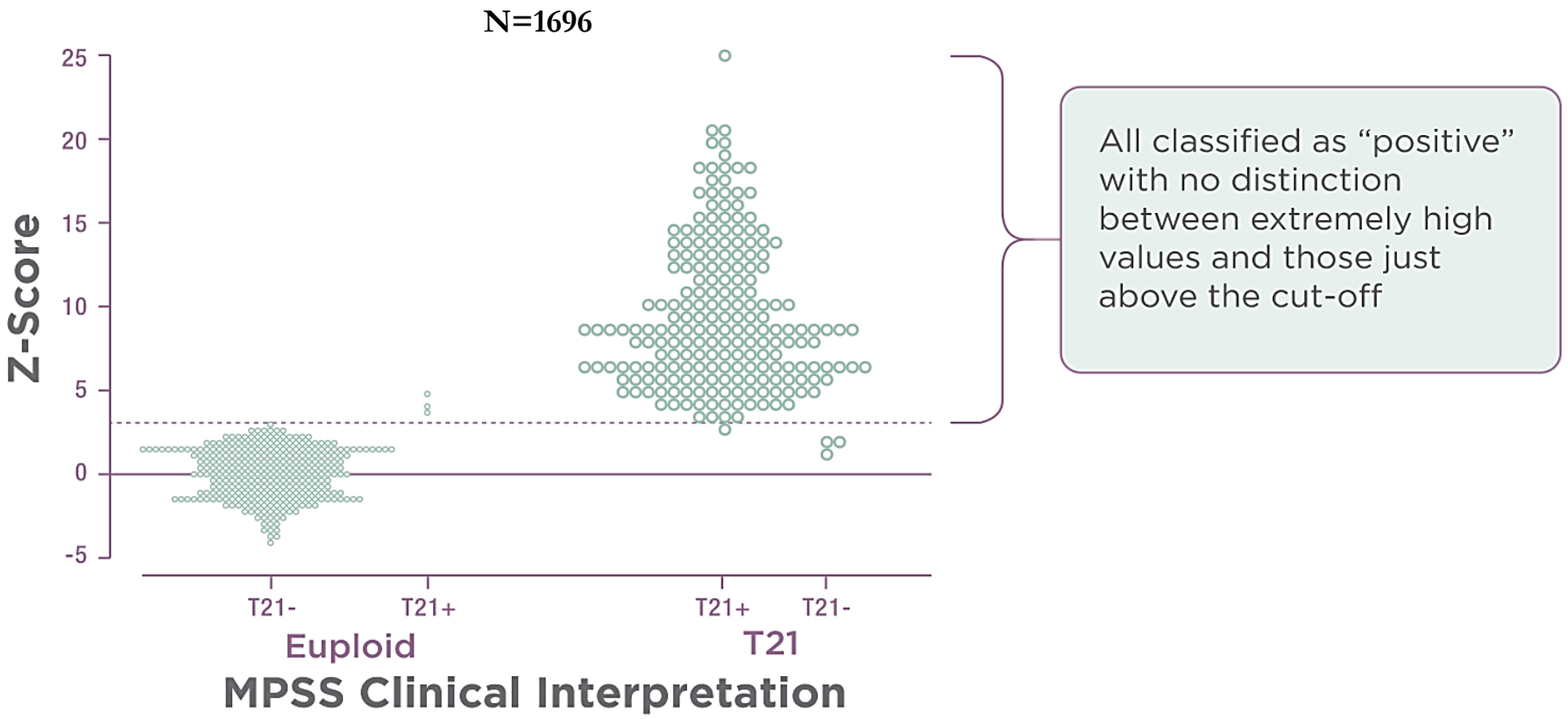
Disease status determination

$$\text{chrN z-score for test sample} = \frac{\%\ \text{chrN}_{\text{sample}} - \text{mean}\ \%\ \text{chrN}_{\text{reference}}}{\text{S.D.}\ \%\ \text{chrN}_{\text{reference}}}$$



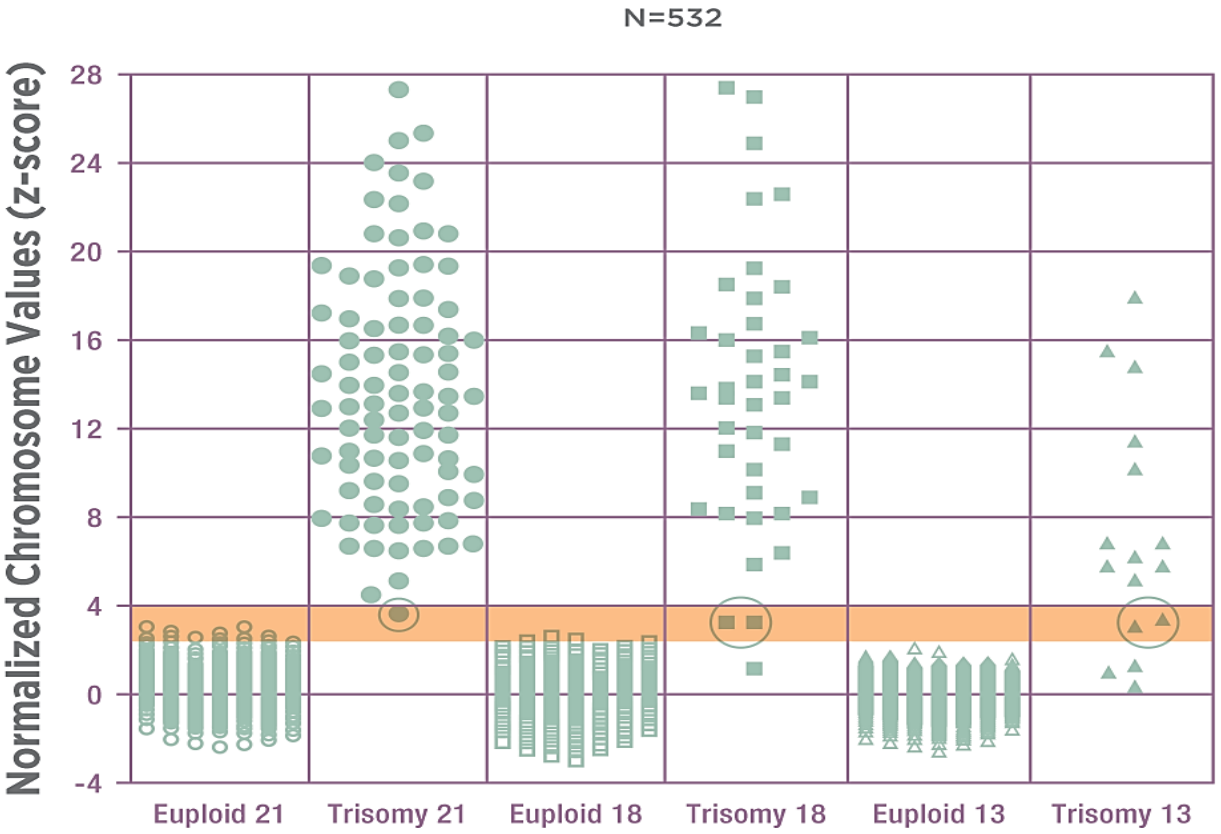
Massively Parallel Shotgun Sequencing (MPSS)

- **MPSS is a random sampling of cfDNA fragments**
- **An arbitrary z-score value is used as a cut-off for trisomy**



Palomaki et al.. Genet Med. 2011 Nov;13(11):913-20.

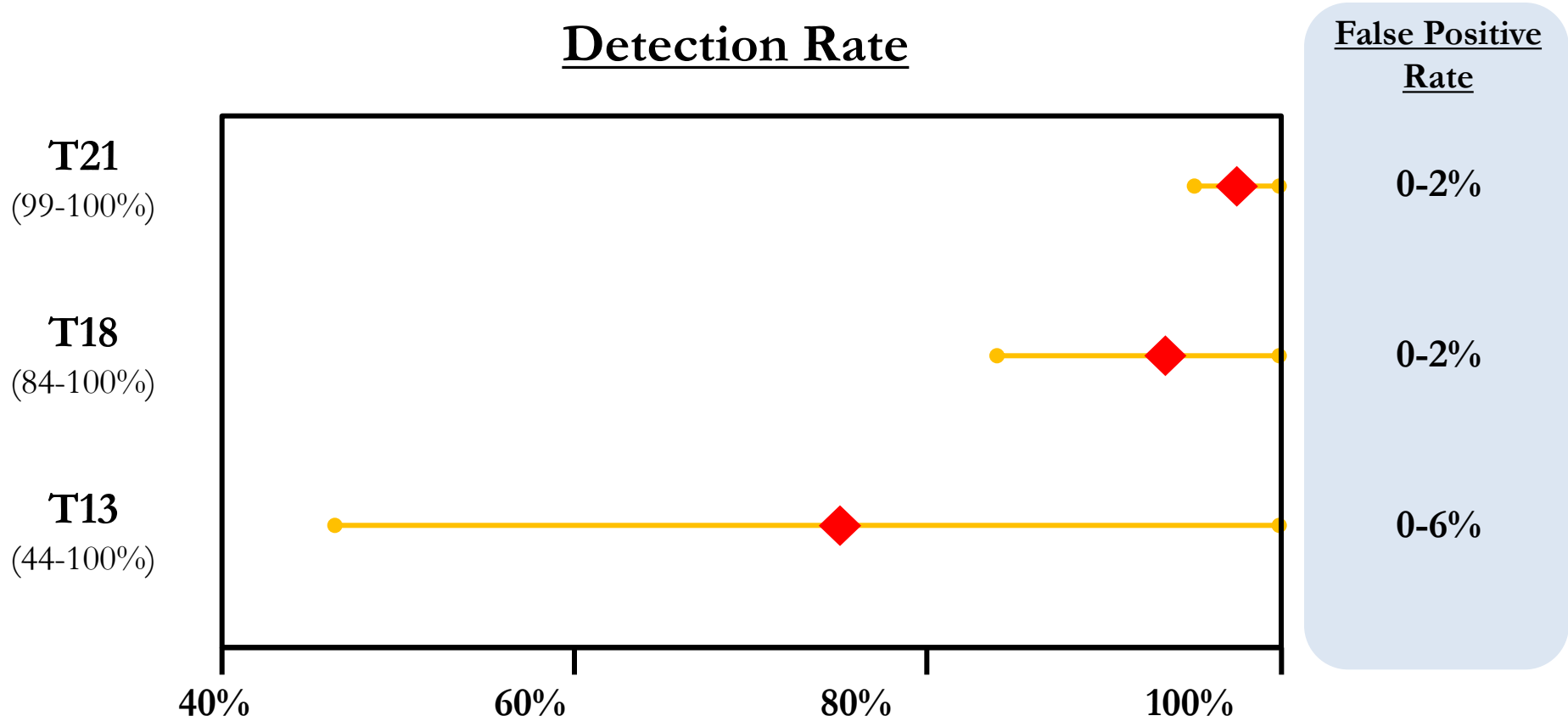
MPSS Unclassified Values



- "Unclassified" zone for values between 2.5-4
- Disproportionate number of positives in this zone

Bianchi, DW, et al.. Obstet Gynecol. 2012 May;119(5):890-901.

MPSS Performance



Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med* 2011 Nov;13(11):913–20.;
Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP, Genome-Wide Fetal Aneuploidy Detection by Maternal Plasma DNA Sequencing. *Obstet Gynecol.* [Epub ahead of print] 2012 Feb 22.;
Chiu et. Al BMJ 2011;342:c7401 Chen et.al (2011) http://www.plosone.org/article/info:doi/10.1371/journal.pone.0021791

NIPT: New Techniques

Massively Parallel Shotgun Sequencing (MPSS)

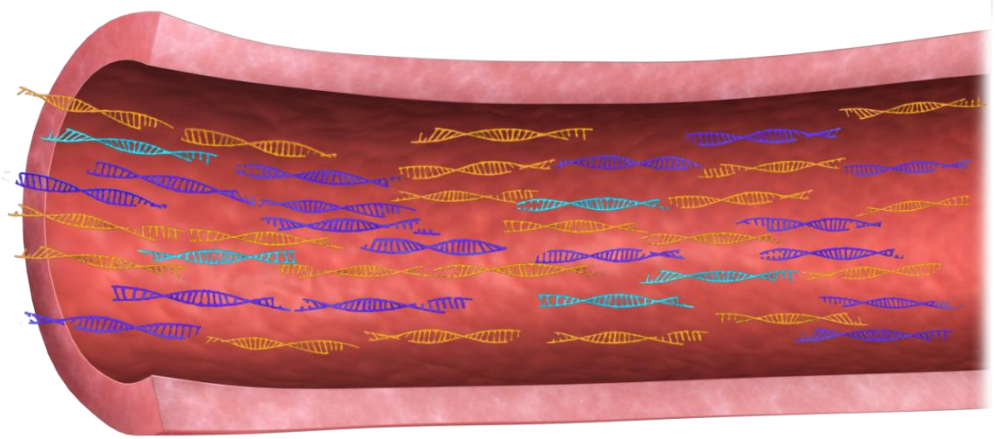
Targeted Approach

Key differences

Binary +/- result based on z-score

Risk classification and risk score

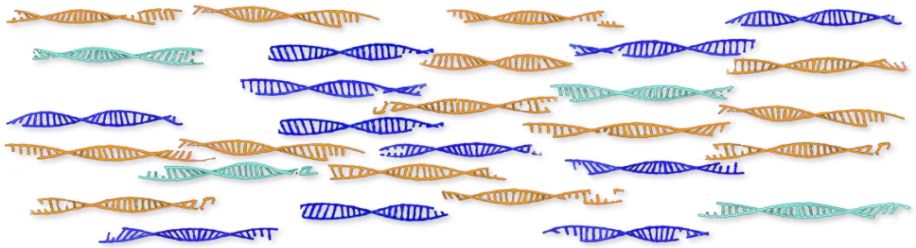
Assay Comparison – Targeted vs MPSS



cfDNA in blood

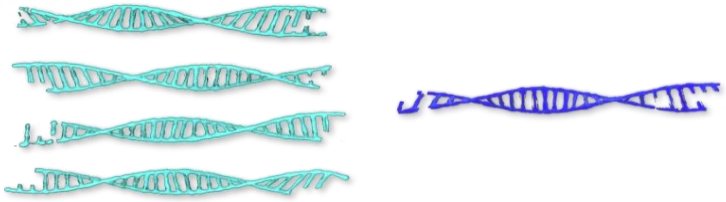
- Chr 21, 18, 13, X & Y cfDNA
- Other Chr cfDNA
- Unmapped cfDNA

MPSS (shotgun)



Random analysis of cfDNA

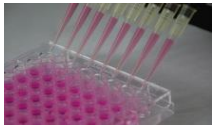
Directed (Harmony)



More efficient

TARGETED NIPT 21, 18, 13, XY

DANSR™



(Digital ANalysis of Selected Regions)

- **Directed assay for cfDNA isolation and analysis.**
- **Targeted method allows for high throughput DNA sequencing**

FORTE™

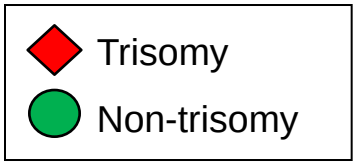
(Fetal-fraction Optimized Risk of Trisomy Evaluation)

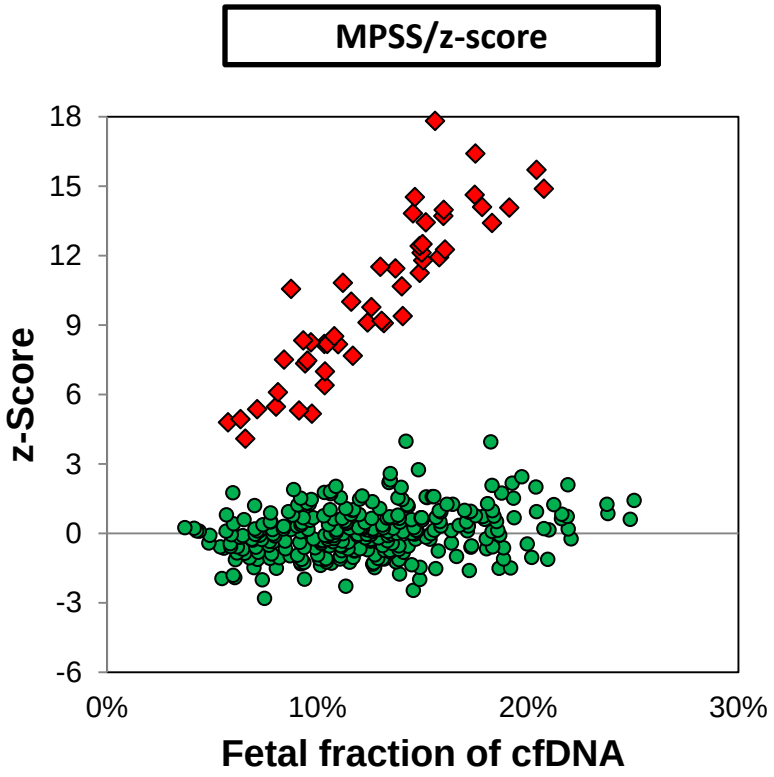
- **New analysis that provides a trisomy risk score**
- **Incorporates DANSR assay results (chromosome counts, fetal fraction), maternal and gestational age**

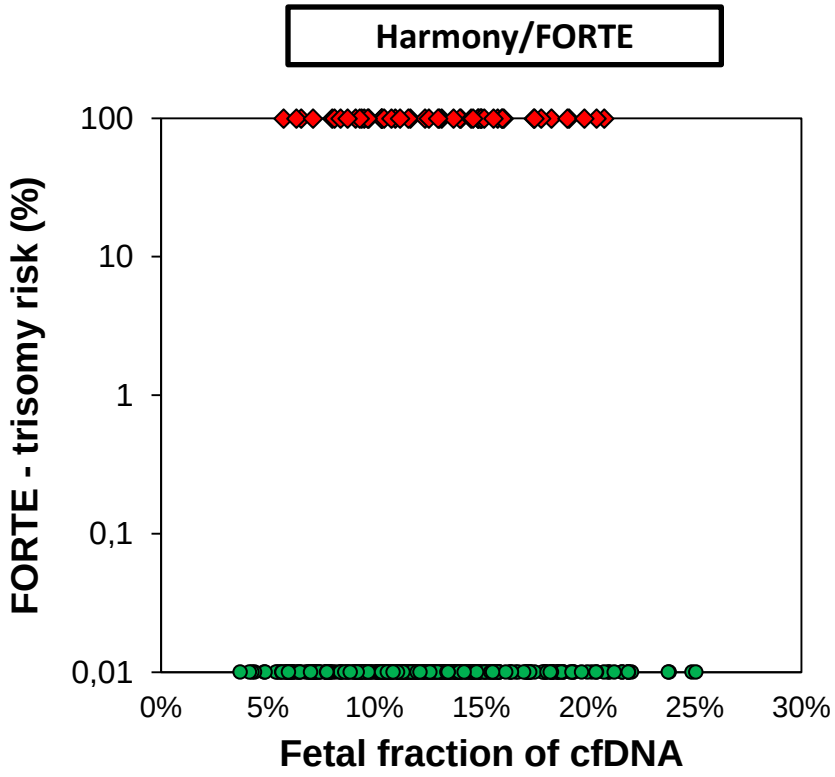
High throughput and scalable test
Clinically interpretable results to patients

Advantages of FORTE

- FORTE incorporates fetal fraction into the results allowing for a more robust analysis







Ashoor G et al., Am J Obstet Gynecol. 2012 Apr;206(4):322.e1-5.

Validacion/Aplicación Clínica

Study	Subjects	Reference
NICE - Cohort validation study	3,228	Norton M et al., AJOG 2012
General screening population, 1st trimester	2,049	Nicolaides et al, AJOG 2012
Trisomy 13	1,949	Ashoor et al., Ultra Obstet Gyn 2013
Kypros Nicolaides clinical implementation	701	Gil et al, Ultra Obstet Gyn (in press)
EU-NITE - European study	520	Verweij et al., (submitted)
High-risk population, 1st trimester	400	Ashoor et al., AJOG 2012
FORTE	338	Sparks et al., AJOG 2012
DANSR	298	Sparks et al., Prenat Diagn 2012
Ob/Gyn real world experience	289	Fairbrother et al., Prenat Diagn 2013
Maternal weight effects - commercial data	22,000	Wang et al., Prenat Diag (in press)
Consistent in high and low-risk women	3,007	Brar et al, J Mat Fet Neonat Med 2013
Maternal weight and fetal factors, study 2	1,949	Ashoor et al. Ultras Obstet Gyn 2013
Maternal weight and fetal factors, study 1	400	Ashoor et al., Fetal Diagn Ther 2012

NICE Study

- 50 participating clinical sites in U.S. and Europe
- Largest cohort study to date – All eligible subjects evaluated
- Study population was women undergoing invasive testing for any indication and thus included low risk women

	Sensitivity	Specificity	False Positive Rate
Trisomy 21	**100%** (81/81)	**99.97%** (2887/2888)	**0.03%** (1/2888)
Trisomy 18	**97%** (37/38)	**99.93%** (2886/2888)	**0.07%** (2/2888)

Norton ME et al. (2012) American Journal of Obstetrics and Gynecology

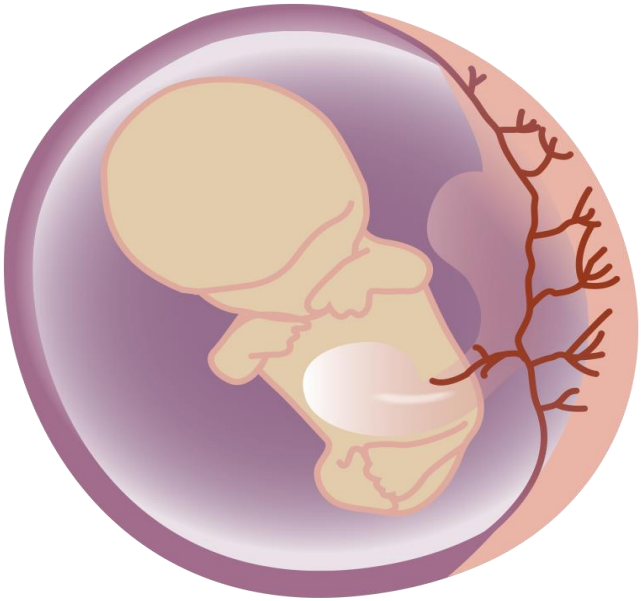
Overall cfDNA Screening Performance

	Detection rate	FPR
Trisomy 21	590 / 594 (99.5%)	0.1%
Trisomy 18	222 / 230 (97%)	0.1%
Trisomy 13	30 / 38 (79%)	0.1%

Chiu et al, 2011; Chen et al, 2011; Ehrich et al, 2011; Palomaki et al, 2011; Bianchi et al, 2012; Sparks et al, 2012; Ashoor et al, 2012; Norton et al, 2012

cfDNA analysis does not always correlate with fetal genotype

T18, T13 – Confined Placental Mosaicism



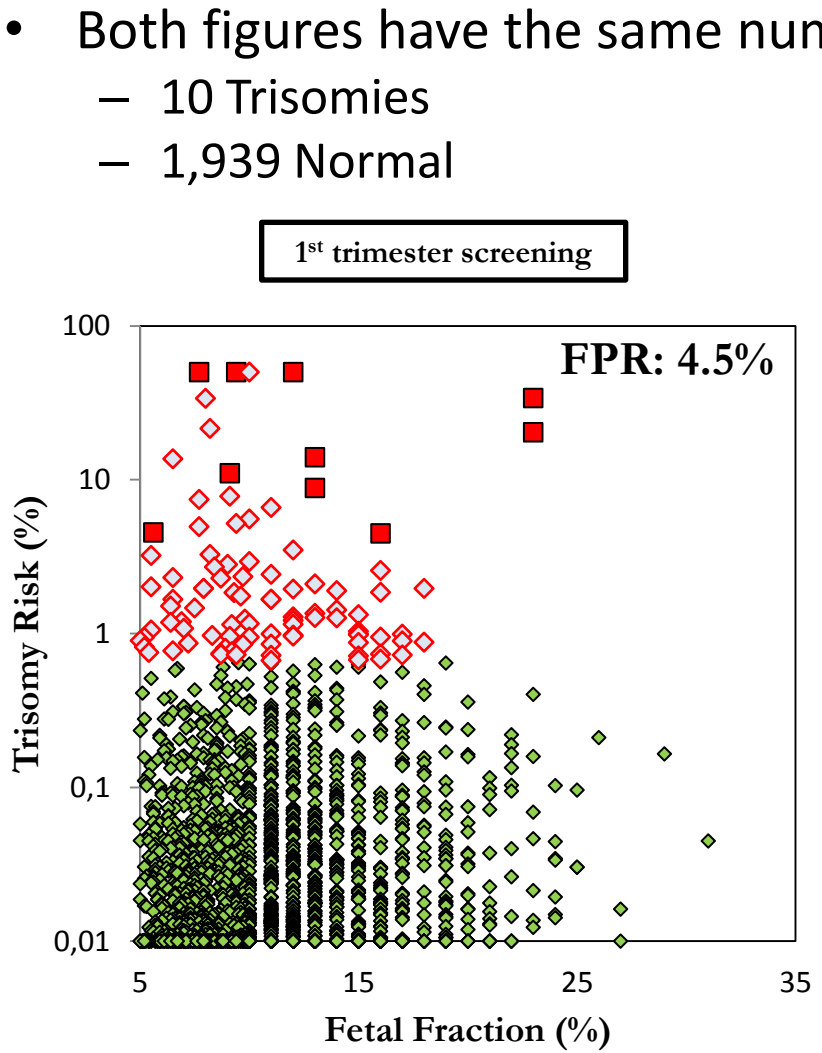
- cfDNA originates from placenta
  - Likely to be from trophoblast
  - Similar to STC of CVS
- Placenta and fetal genotypes can be different
- Occurs more frequently with chromosomes 13 and 18, as compared to chromosome 21

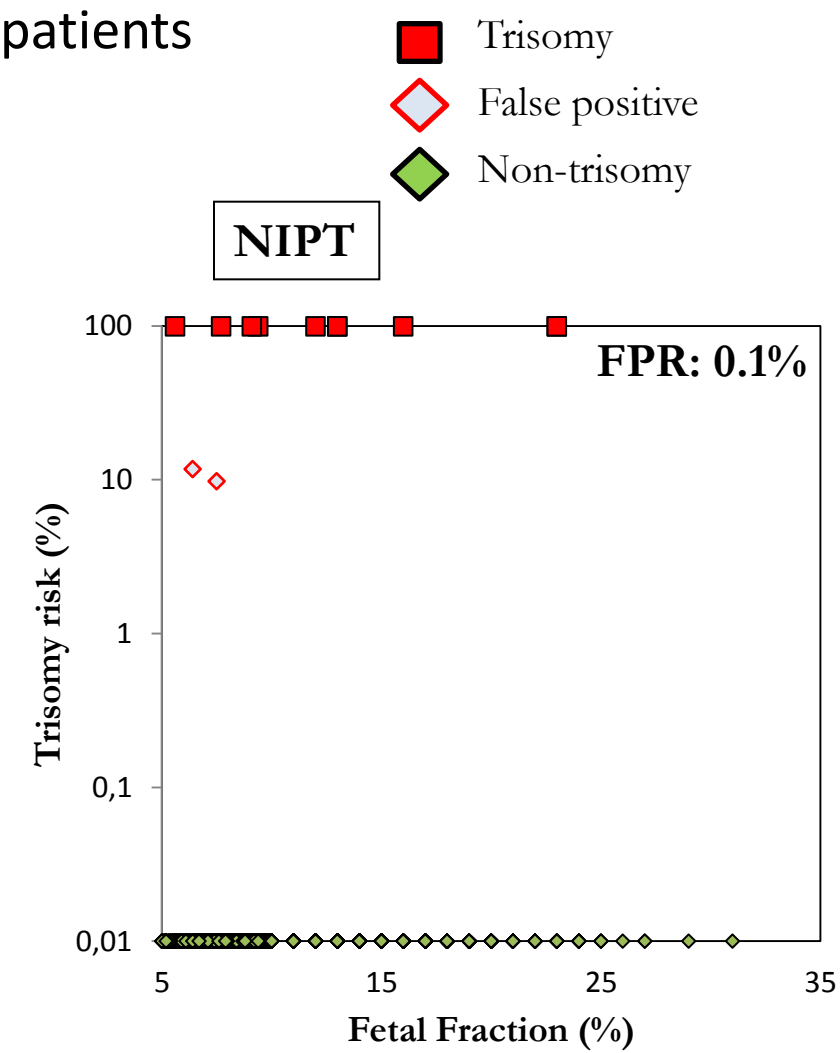
Can cause false positive and negative results

1. Kalousek DK et al., Am J Hum Genet. 1989 Mar;44(3):338-43. 2. Wirtz et al, Prenat Diag. 1991 Aug;11(8):563-7.

Average Risk Study – Risk Score Comparison

- Both figures have the same number of patients
  - 10 Trisomies
  - 1,939 Normal





Nicolaides KH et al. (2012) American Journal of Obstetrics and Gynecology

Screening for Aneuploidies by cfDNA in maternal blood

By Far the best available option for T21 and 18

100.000 Pregnancies

Trisomy 21 N=200 | **99.800 Normal**

METHOD OF SCREENING	DR	Detected	False Positive	
Serum biochemistry at 16 wks	70%	140	5%	4990
Combined test at 12 wks	90%	180	5%	4990
Combined plus at 12 wks	97%	194	3%	2994
Cell-free DNA	>99%	>199	<0.1%	<100

- Can be offered to all women irrespective of risk
- Can provide result in the 1st trimester of pregnancy

K. Nicolaides SMFM SF 2013

ispd Position Statement - NIPT

- The test is Advanced Screening not Diagnostic
- Only detects a fraction of Chromosome Abnormalities detected by AF/CVS in women at high risk
- Suitable for selected pregnancies but only after Genetic Counseling

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