Test genetici: metodologie a confronto

Non-Invasive Prenatal Testing Panorama™

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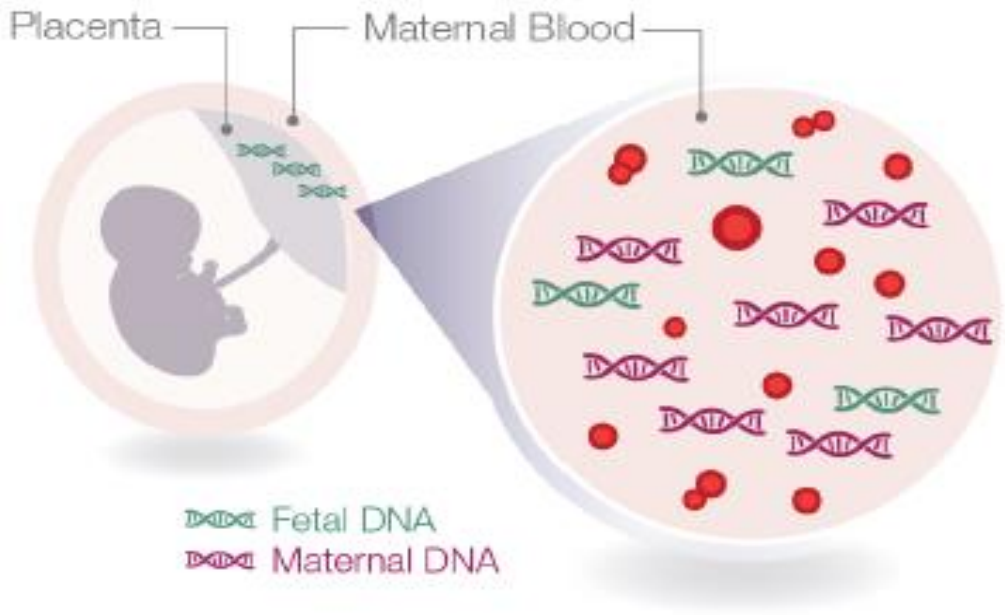
Personal Genomics

Cell-free DNA (cfDNA)



cfDNA comes from apoptotic cells derived from:

- Maternal Circulation
  - Adipocytes
  - White Blood Cells
- Placenta
  - Trophoblasts in the maternal circulation

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Storia del cff DNA

- **1997**. descritto per la prima volta la presenza del cromosoma Y nel plasma di alcune donne con feto di sesso maschile, utilizzando l'analisi del DNA libero presente nel circolo materno (cfDNA)
- Il cffDNA puo essere isolato precocemente a partire dalla **9° settimana**.
- La sua percentuale di **FF** puo variare tra **< 4%** (una quantità non utile per la diagnosi) al 40%, con una media del **10%** alla 12 ° settimana
- La disponibilità **bassa di FF** porterebbe ad un rischio **aumentato di Falsi Negativi**
- Una NON perfetta discriminazione tra **FF vs FM** porterebbe ad un aumento di **Falsi Positivi** e **Falsi Negativi**
- **NON tutti i protocolli di NIPT** al momento disponibili **utilizzano la Frazione Fetale** (FF) come parametro di valutazione. Alcuni test NIPT inseriscono la percentuale della FF nell'algoritmo per la formulazione della probabilità di presenza della trisomia indagata, mentre altriutilizzano fattori di normalizzazione predeterminati. (Dan et al, 2012; Zhang et al, 2015)

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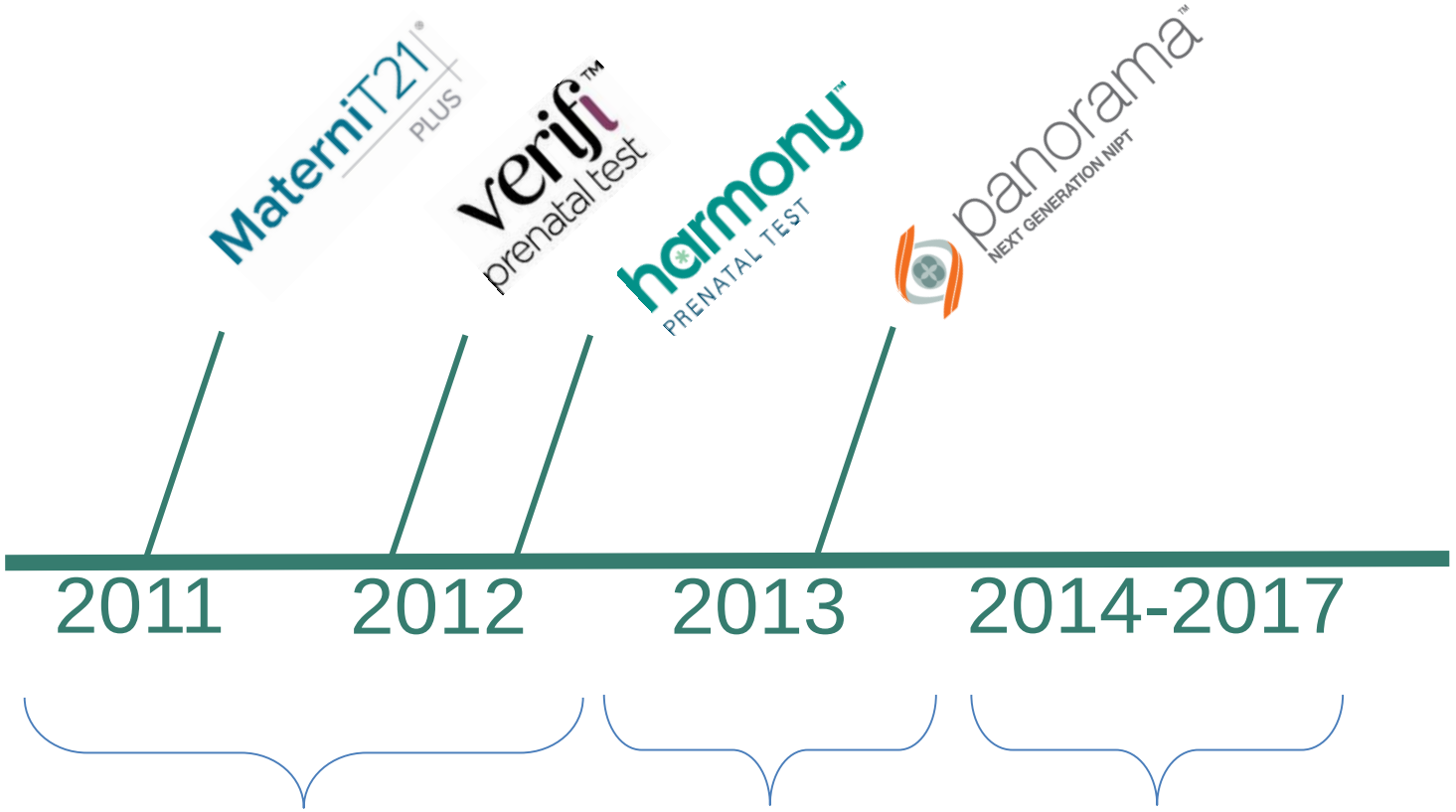
L'evoluzione del NIPT



1st generation: Quantitative or **"Counting"**

2nd generation: Qualitative or **"SNP-based"**

Other labs enter domestic NIPT space using 1st generation counting technologies

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NIPT Methodologies

Counting	SNP
harmony™ PRENATAL TEST MaterniT21® PLUS PrenatalSafe® GENOMA® verifi™ prenatal test CE IVD Vera® PRENATAL TEST NIFTY® Non-Invasive Fetal TrisomY test QNatal™ Advanced NIPS	panorama™ NEXT GENERATION NIPT

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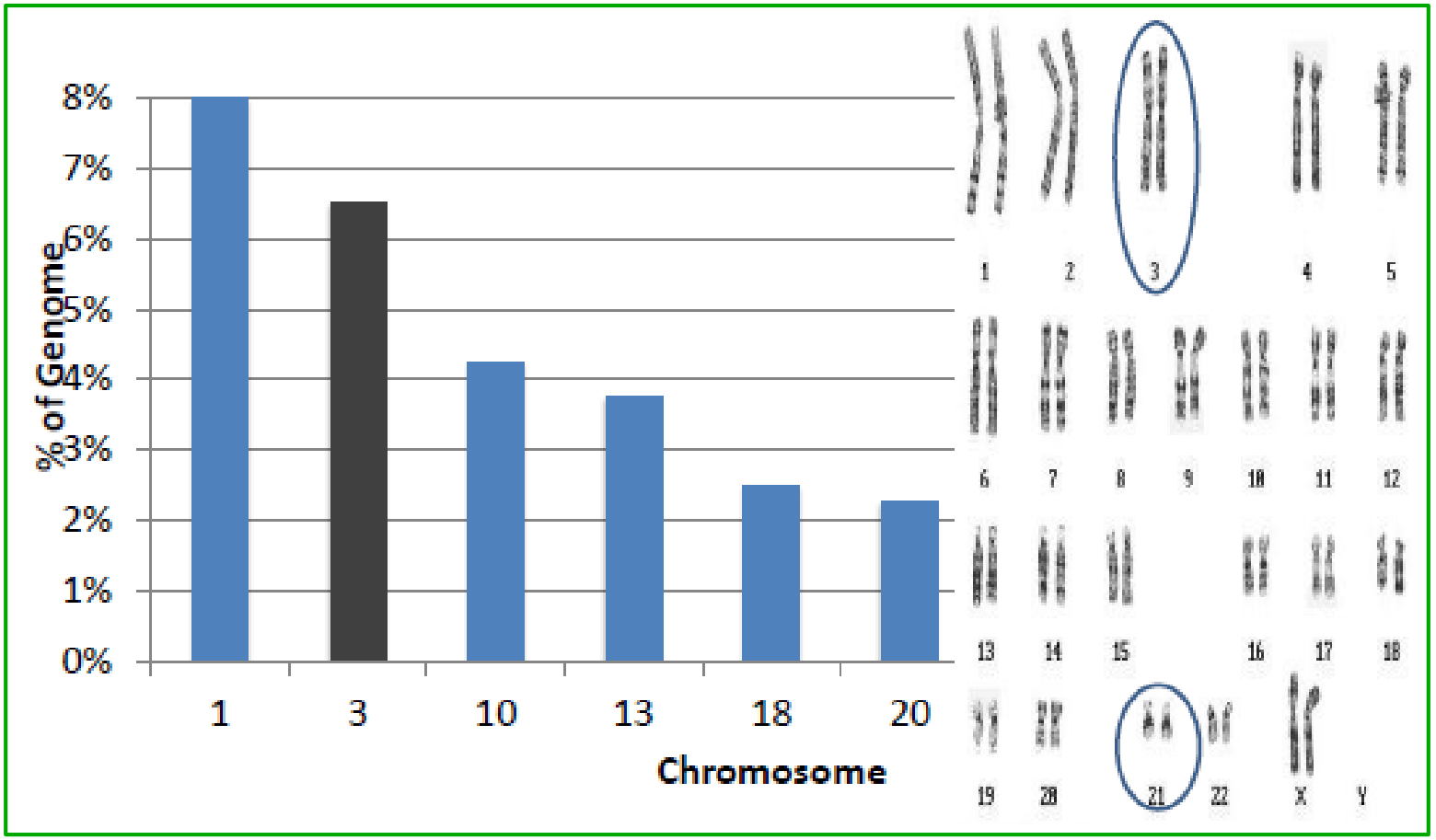
Conteggio vs SNP's method



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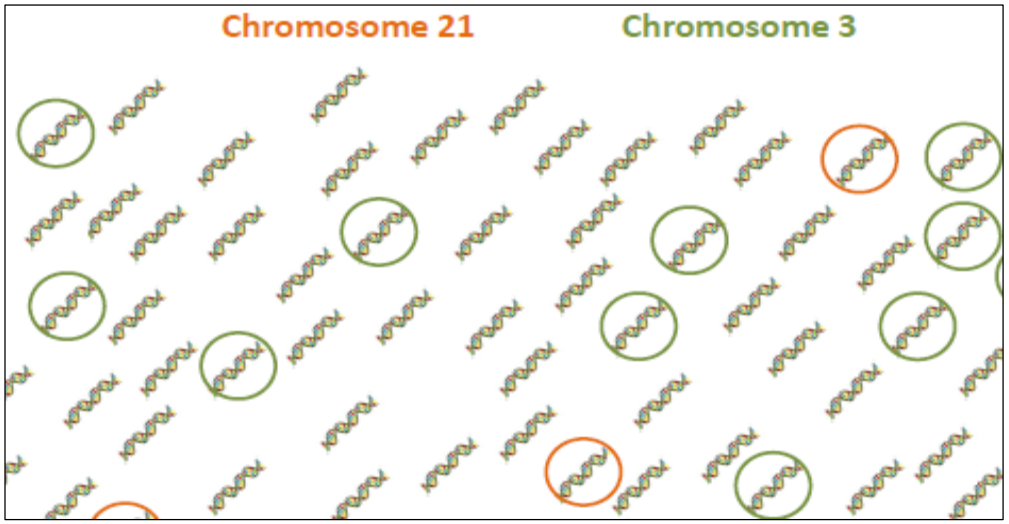
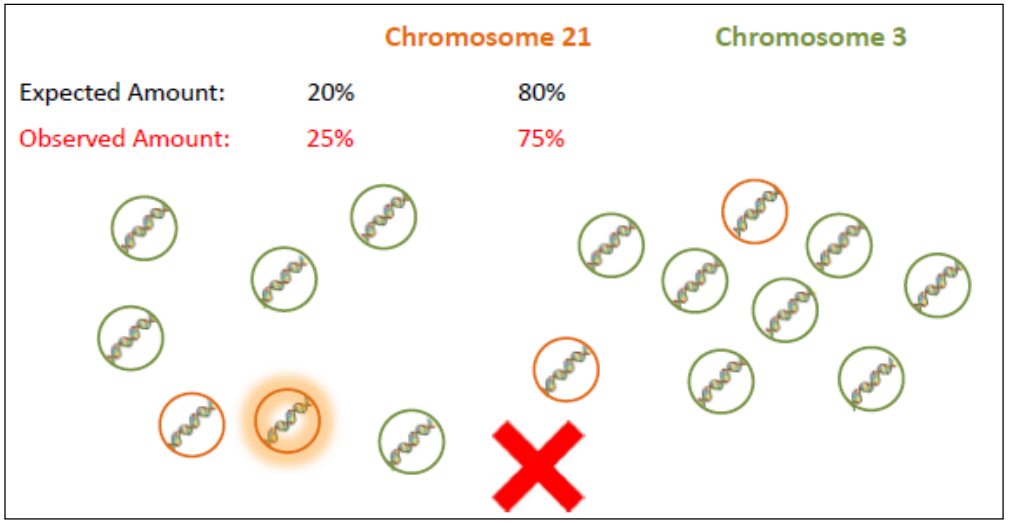
Metodo del Conteggio





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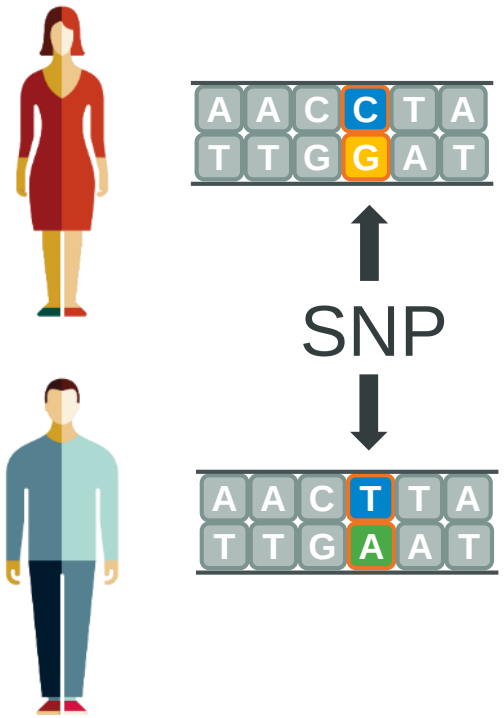
SNP = Single Nucleotide Polymorphism



- A DNA sequence variation occurring when a single base pair is changed
- Normal genetic changes that occur in every person
- Panorama™ analyzes more than 13,000 SNPs

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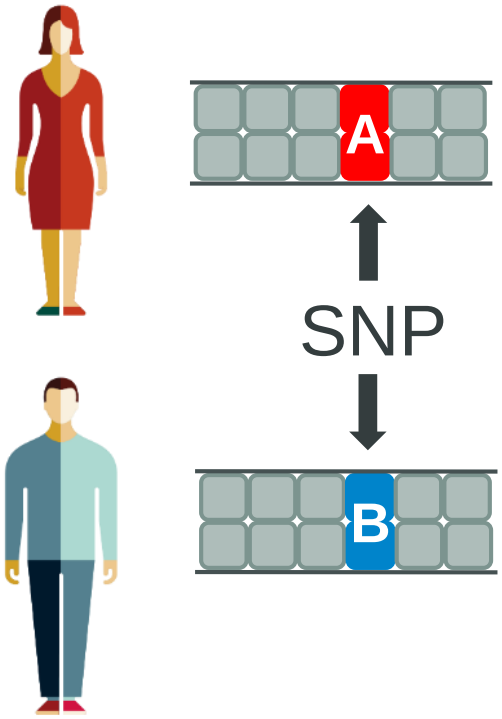
SNP = Single Nucleotide Polymorphism



- All analyzed SNPs are assumed to be biallelic
- For simplicity, we designate this as **A** and **B**

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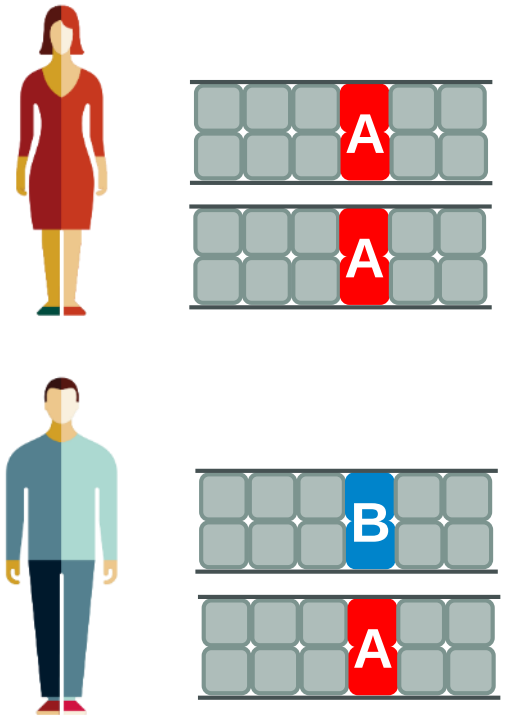
SNP = Single Nucleotide Polymorphism



- Since chromosomes come in pairs, we would inherit two SNPs at the same location, and could be **A**/**A**, **A**/**B**, or **B**/**B** for each SNP

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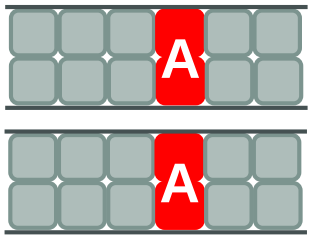
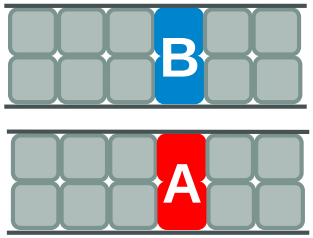
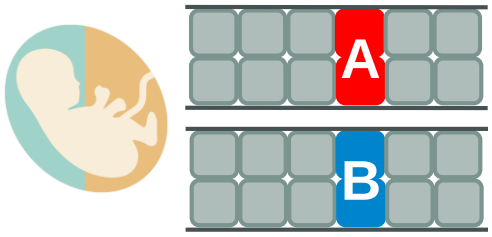
SNP = Single Nucleotide Polymorphism





- Baby inherits one SNP from mom and one from dad. In this example baby inherits **A** from mom and **B** from dad. Baby is **A/B** for this SNP.



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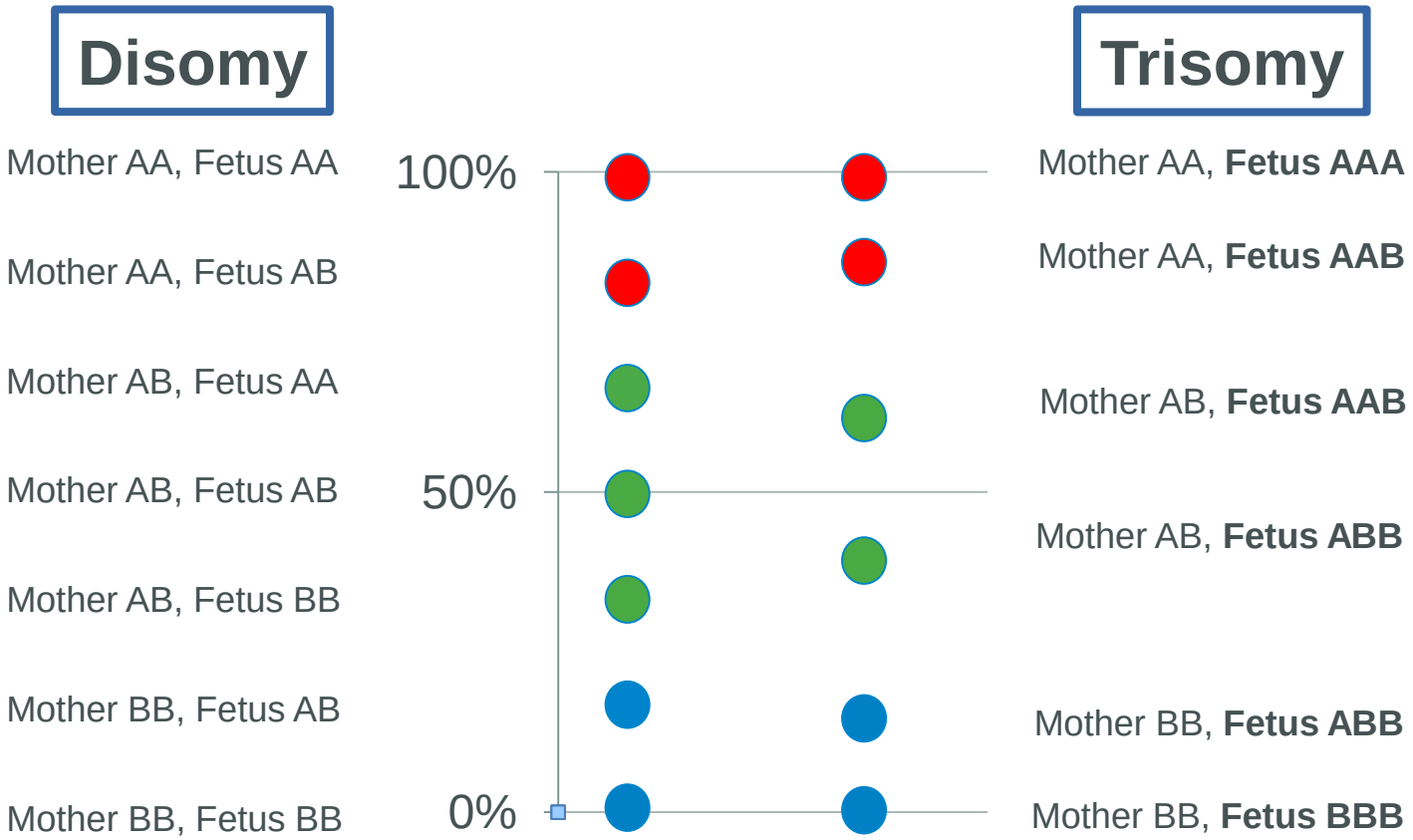
Relative amount of each of two alleles at a SNP



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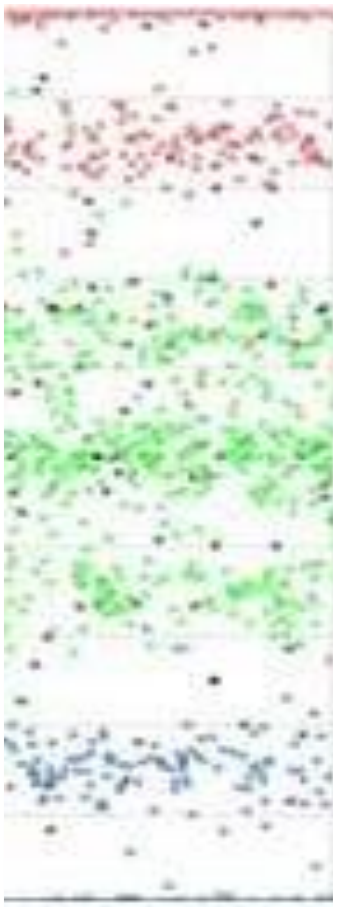
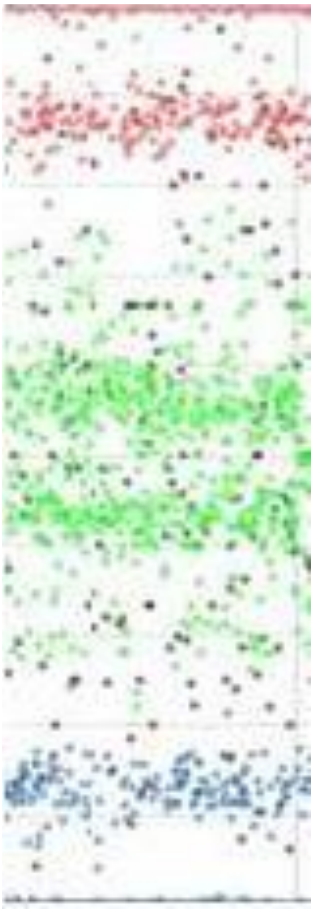
SNP profile

	Disomy	Trisomy	
Mother AA, Fetus AA			Mother AA, Fetus AAA
Mother AA, Fetus AB			Mother AA, Fetus AAB
Mother AB, Fetus AA			
Mother AB, Fetus AB			Mother AB, Fetus AAB
Mother AB, Fetus BB			Mother AB, Fetus ABB
Mother BB, Fetus AB			Mother BB, Fetus ABB
Mother BB, Fetus BB			Mother BB, Fetus BBB

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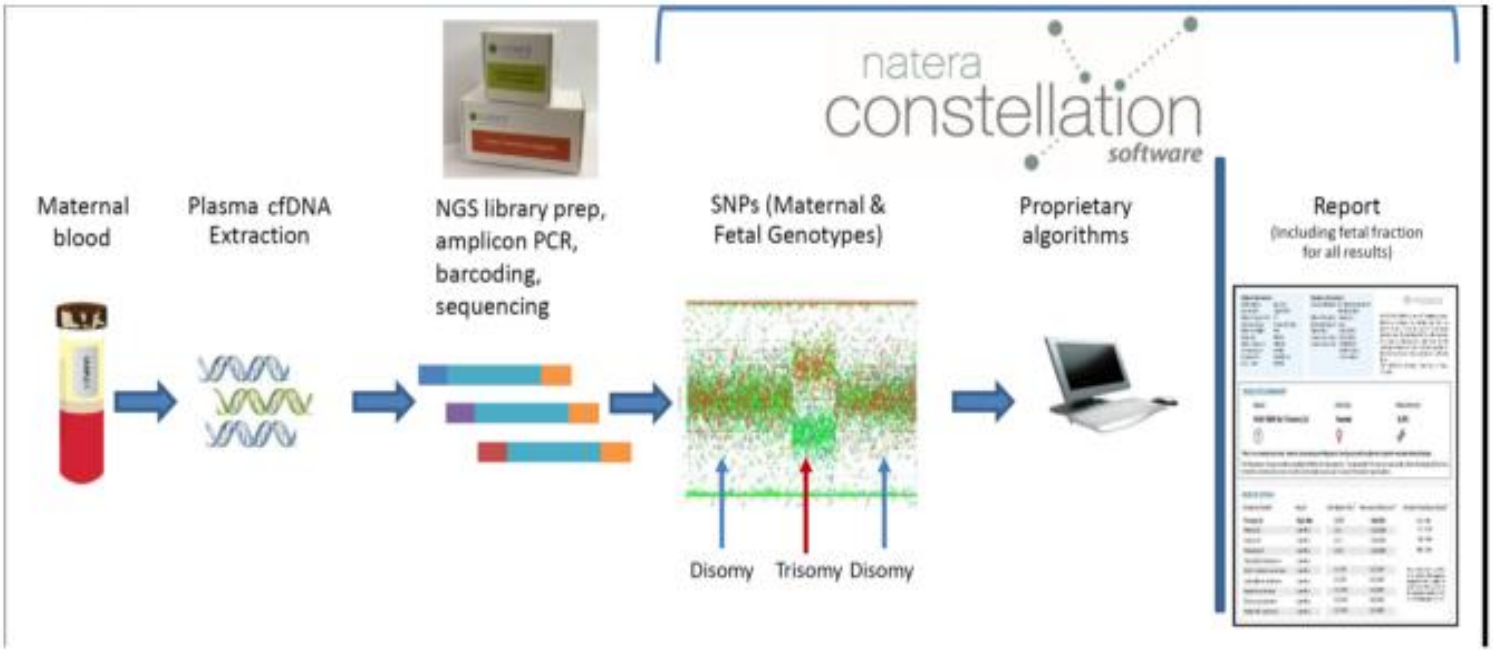
Panorama Test Workflow



Proprietary SNP analysis distinguishes between maternal & fetal DNA

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Vantaggi Clinici di SNP method

Panorama® uniquely differentiates between maternal and fetal DNA

Fetal fraction

Maternal contribution

Vanishing twins

Fetal sex accuracy

Triploidy/complete mole

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Vantaggi Clinici degli SNP

Panorama™ uniquely differentiates between maternal and fetal DNA

- Fetal fraction
- Maternal contribution
- Vanishing twins
- Fetal sex accuracy
- Triploidy
- Twins with zygosity

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L'Importanza della Frazione Fetale (FF)

"…the measurement of fetal cfDNA is a basic quality metric required to ensure reliable interpretation of test results."[1]

$$\frac{\text{Fetal DNA}}{\text{Fetal DNA} + \text{Maternal DNA}}$$

- Average fetal fraction is 10–12%[2]
- Varies by gestational age, maternal weight, placental and pregnancy factors[3]
- Panorama™ cutoff: 2.8%

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NIPT on Non-Pregnant Women

NIPT	Patient 1		Patient 2	
	Test result available	Details	Test result available	Details
Panorama™	No	Unable to report due to low fetal fraction	No	Unable to report due to low fetal fraction
Array-NIPT	No	Insufficient fetal cfDNA for accurate NIPT evaluation	No	Insufficient fetal cfDNA for accurate NIPT evaluation
WGS1-NIPT	Yes	Negative, consistent with female fetus (fetal fraction 4.3% reported on request)	Yes	Negative, consistent with female fetus (fetal fraction 3.9% reported on request)
WGS2-NIPT	Yes	No aneuploidy detected, two sex chromosomes (XX)	Yes	No aneuploidy detected, two sex chromosomes (XX)

Takoudes et al., Ultrasound Obstet Gynecol 2015; 45: 112–116

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Vantaggi Clinici degli SNP

Panorama™ uniquely differentiates between maternal and fetal DNA

- Fetal fraction
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Vanishing twins

- 36% of first-trimester twin pregnancies result in one deceased (“vanishing”) twin[1]
- Quantitative, or counting approaches cannot distinguish source of additional DNA sequences
- Incorrect fetal sex
- Increased possibility of false positive or false negative results

1. Multifetal gestations: twin, triplet, and higher-order multifetal pregnancies. Practice Bulletin No. 144. American College of Obstetricians and Gynecologists. Obstet Gynecol. 2014; 123:1118-1132.

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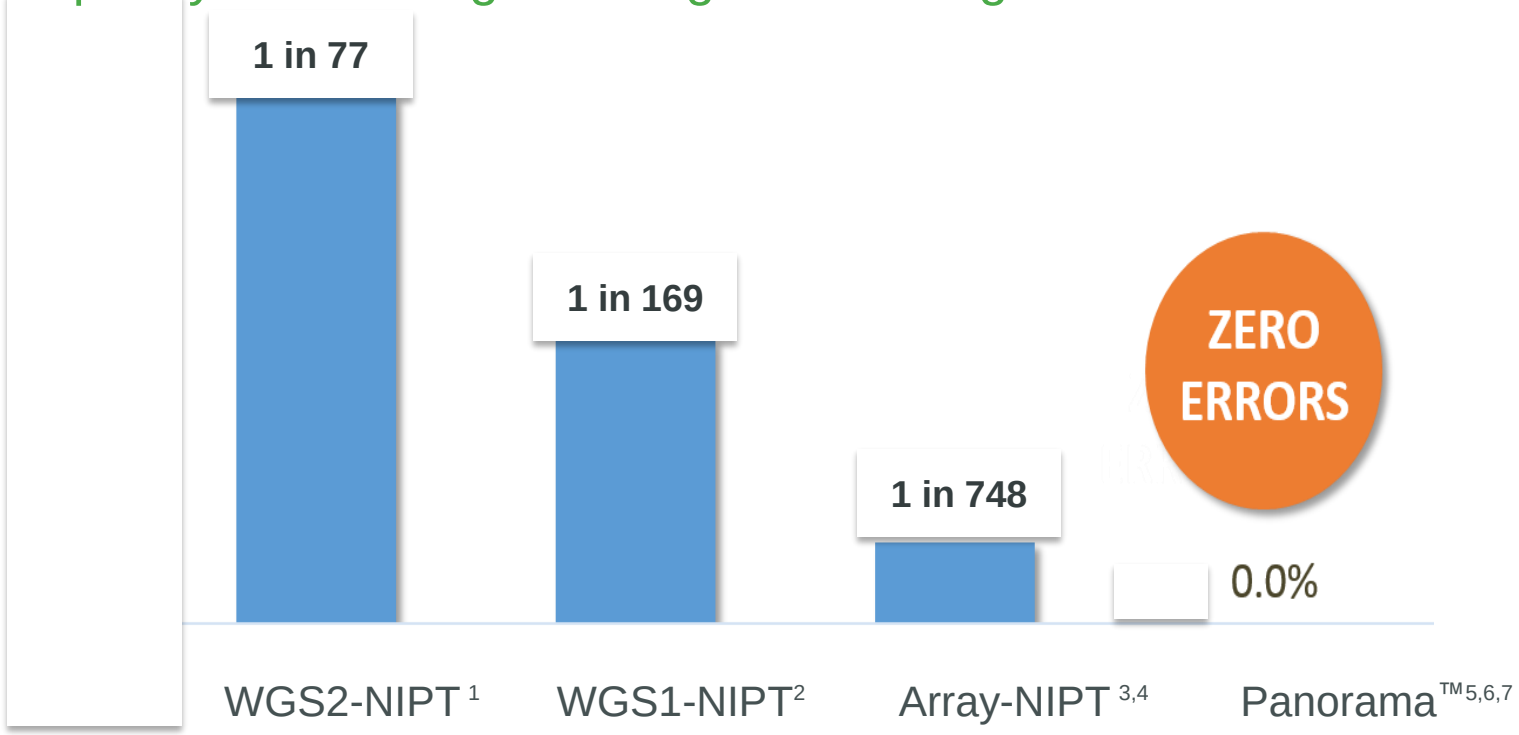
Error Rate nella determinazione del Sesso

In validation studies, as many as 1 in 77 cases can have gender discrepancy when using counting methodologies.



1. Verinata white paper. Analytical validation of the Verifi prenatal tes: enhanced test performance for detecting trisomies 21, 18 and 13 and the option for classification of sex chromosome status. 2012
2. Mazloom et. al. 2013. Prenat Diagn.
3. Nicolaides et. al. 2013. Fetal Diagn Ther.
4. Hooks et al. 2014. Prenat. Diagn.
5. Pergament et al. 2014. Obstetrics & Gyn.
6. Nicolaides et. al. 2013. Prenat Diagn
7. Ryan et al. Fetal Diagn Ther. 2016;40(3):219-223.

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Vantaggi Clinici degli SNP

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- Fetal fraction
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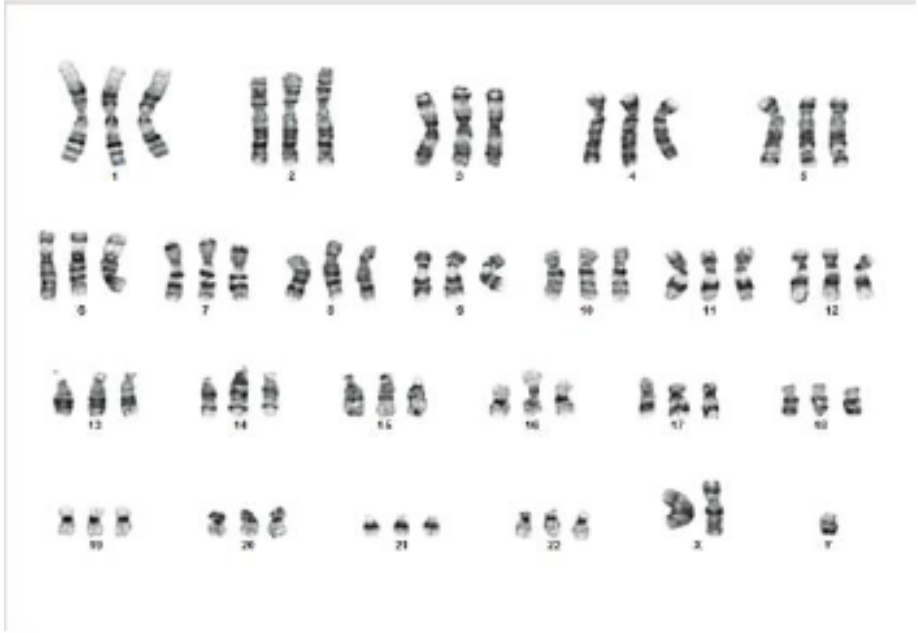
Triploidia

- Three copies of each chromosome present instead of two.



- Associated with severe birth defects, preeclampsia, postpartum hemorrhage, and other complications.
- Most miscarry, but incidence at 10 weeks is 1 in 1,000.[1]
- Clinical symptoms can overlap with routine miscarriage.
- Paternal triploidy
  - Up to 5% risk for persistent gestational trophoblastic disease.[2,3]
  - Risk for malignant tumors.
- Maternal triploidy: risk for recurrence in future pregnancies.[4]

[1]Snijders, et al. Fetal Diagn Ther 1995; 10:357-9.
[2]Berkowitz, RS and Goldstein, DP, Cancer 1995; 76: 2079–2085.
[3]Soper, J. Obstet Gynecol 2006; 108:176–87
[4]Chromosome Abnormalities and Genetic Counseling, Gardner and Sutherland, 2004.

Vantaggi Clinici degli SNP

Panorama™ uniquely differentiates between maternal and fetal DNA

- Fetal fraction
- Maternal contribution
- Vanishing twins
- Fetal sex accuracy
- Triploidy
- Twins with zygosity

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Vantaggi Clinici degli SNP

- Panorama identifies unique DNA fingerprints.
- Does not rely on reference chromosomes.

- **Fewer false negatives**
  - Fetal fraction measurement
- **Identifies conditions in fetus that are associated with maternal complications:**
  - Triploidy
  - Complete molar pregnancy

Fewer false positives:

- Maternal contribution (e.g. maternal mosaicism)
- Vanishing twin

Unparalleled fetal sex accuracy

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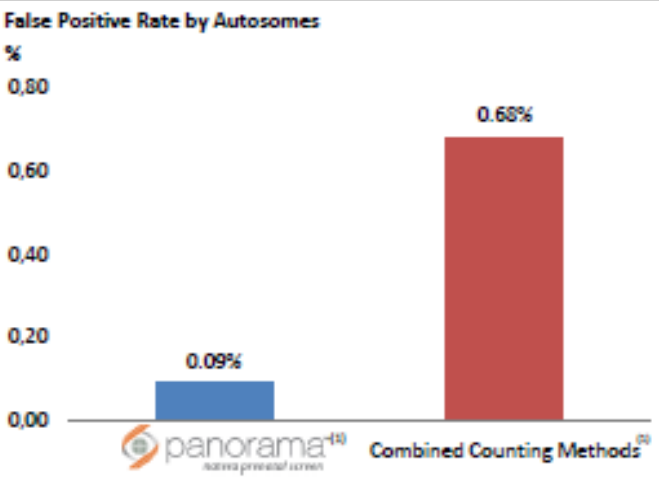
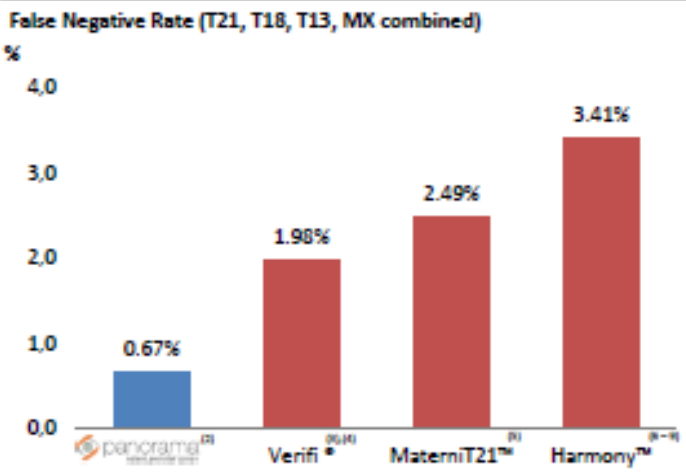
Superiorita' Clinica

Fewer False Positives



Fewer False Negatives



"... Combined specificity for the three autosomal trisomies was 99.91% (1,103/1,104 total negative samples, CI: 99.5-100%); the overall specificity of the combined quantitative methods was 99.32% (4,084/4,112, CI: 99.02-99.55%). This is a statistically significant difference (p=0.0085)." [1]

PPV	T21	T18	T13	MX
	90.9%	93.1%	38.1%	50.0%

[4]

1. Pergament et al. Obstet Gynecol 2014
2. Dar et al. Am J Obstet Gynecol 2014
3. Futch et al. Prenat Diagn 2013
4. Bianchi et al. NEJM 2014
5. Porreco et al. Am J Obstet Gynecol 2014
6. Verweij et al. Prenat Diagn 2013
7. Gil et al. Ultrasound Obstet Gynecol 2013
8. Jackson et al. Prenat Diagn 2013
9. Norton et al. NEJM 2015

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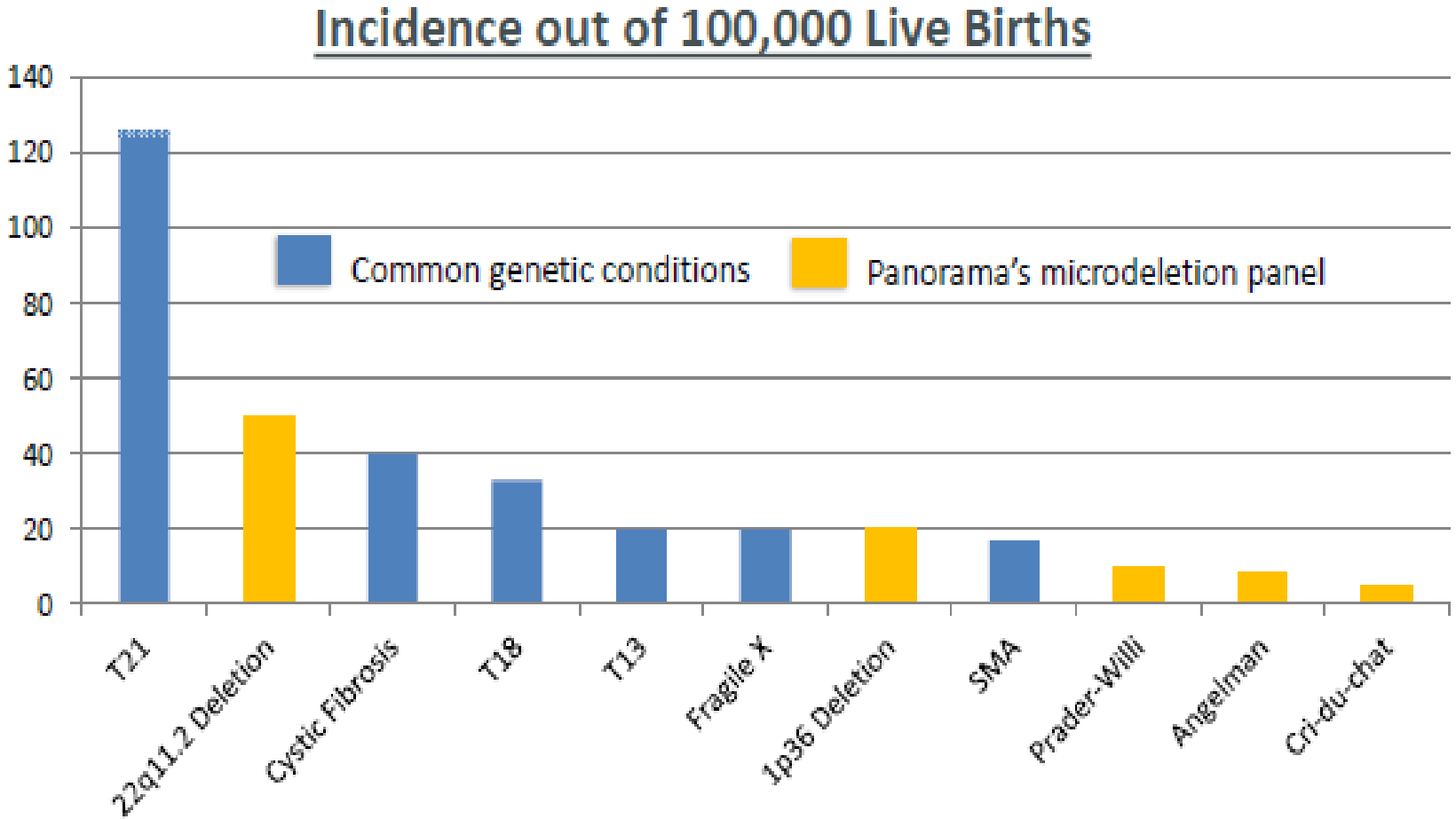
Incidence out of 100,000 Live Births
140
120
100
80
60
40
20
0
Common genetic conditions
Panorama's microdeletion panel
T21
22q11.2 Deletion
Cystic Fibrosis
T18
T13
Fragile X
1p36 Deletion
SMA
Prader-Willi
Angelman
Cri-du-chat

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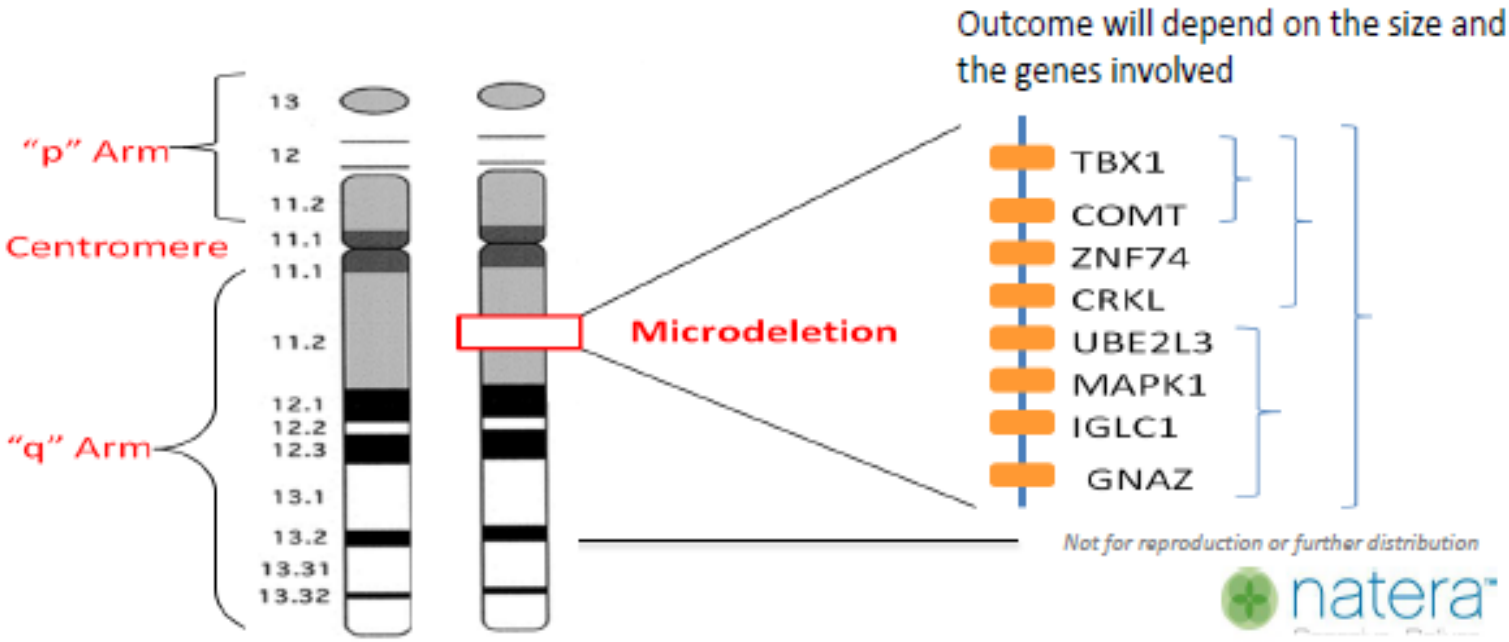
Microdelezione 22q11.2

- 1MB (megabase) = 1 million base pairs
- Microdeletions are 100kb to several MB
- Karyotype can usually only detect ≥7-10 MB changes



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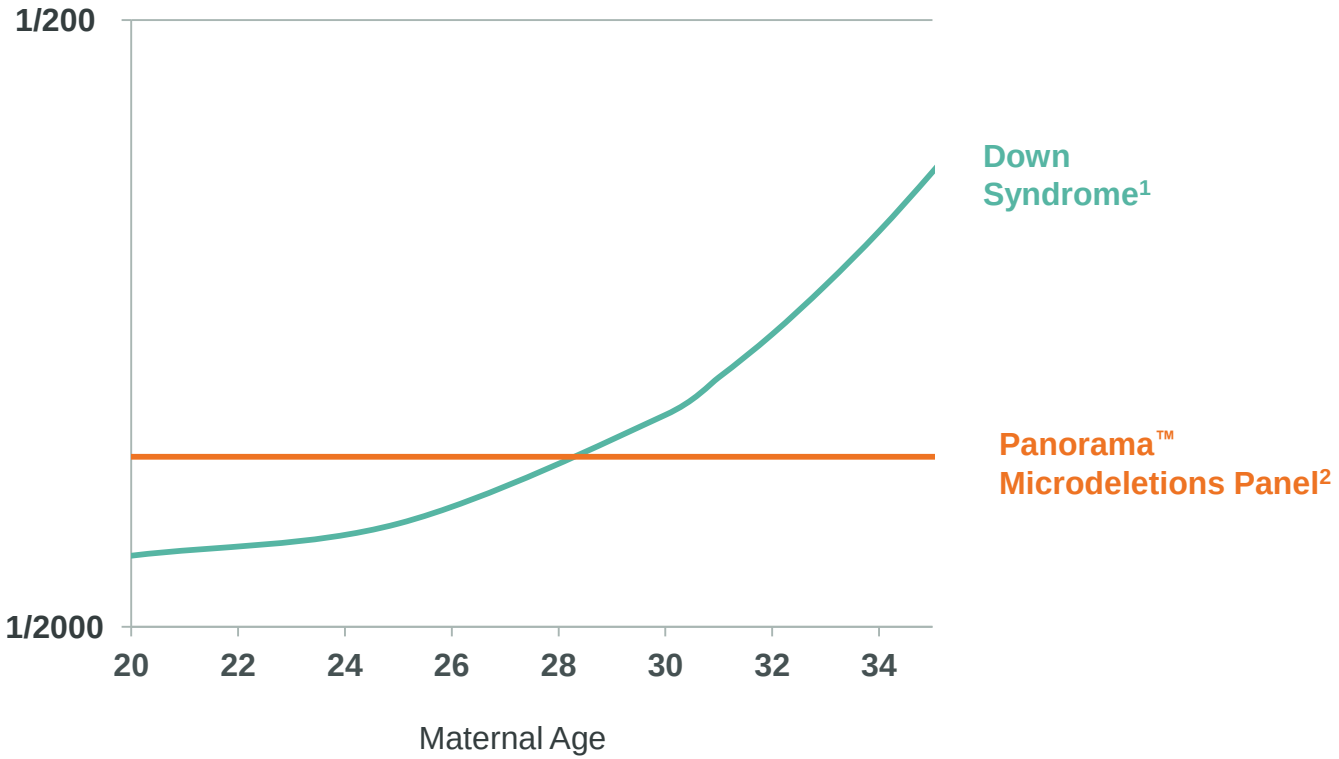
Rethink screening

Microdeletions are more common than Down syndrome in younger women



Snijders, et al. Ultrasound Obstet Gynecol 1999;13:167–170.
Gross et al. Prenatal Diagnosis 2011; 39, 259-266*
https://ghr.nlm.nih.gov/condition/1p36-deletion-syndrome#statistics*
*Combined prevalence using higher end of published ranges; Total prevalence may range from 1/1071 - 1/2206

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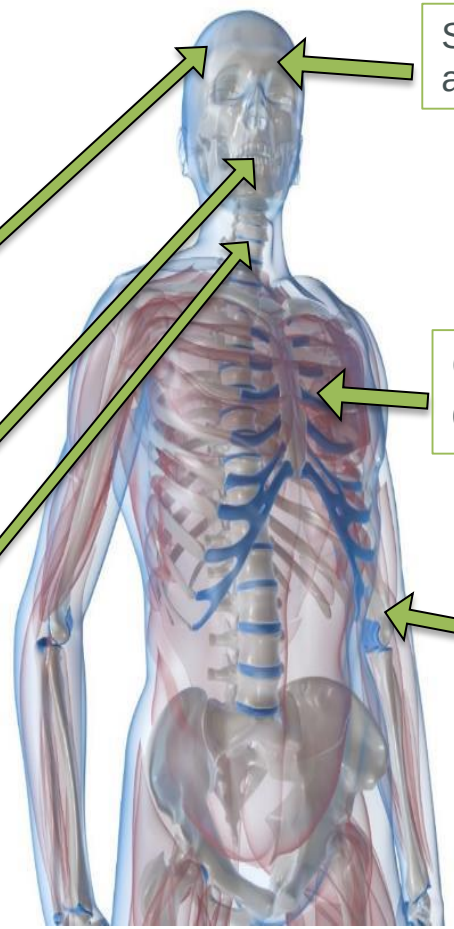
Microdel 22q11.2: DeGeorge Syndrome

"…occurs in approximately in every 2,000 to 4,000 live births, although this is likely a gross underestimate of its prevalence. It is thought to be almost as common as Down syndrome."[1]



Schizophrenia in adulthood (25%)

Congenital heart defects (74%)

Hypocalcemia (50%)

Developmental delay and learning disabilities (70-90%)

Palatal abnormalities (69%)

Immune deficiencies (77%)

1. International 22q11.2 Foundation – Handbook and www.22q.org
2. https://www.ncbi.nlm.nih.gov/books/NBK1523/ (applies to all boxes)

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Prevenzione

- Deliver baby at tertiary care center
- Delay in administering live vaccines
- Monitor calcium levels
- Check palate for clefting and velopharyngeal insufficiency

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SNP-based Aneuploidy Screening for Twins

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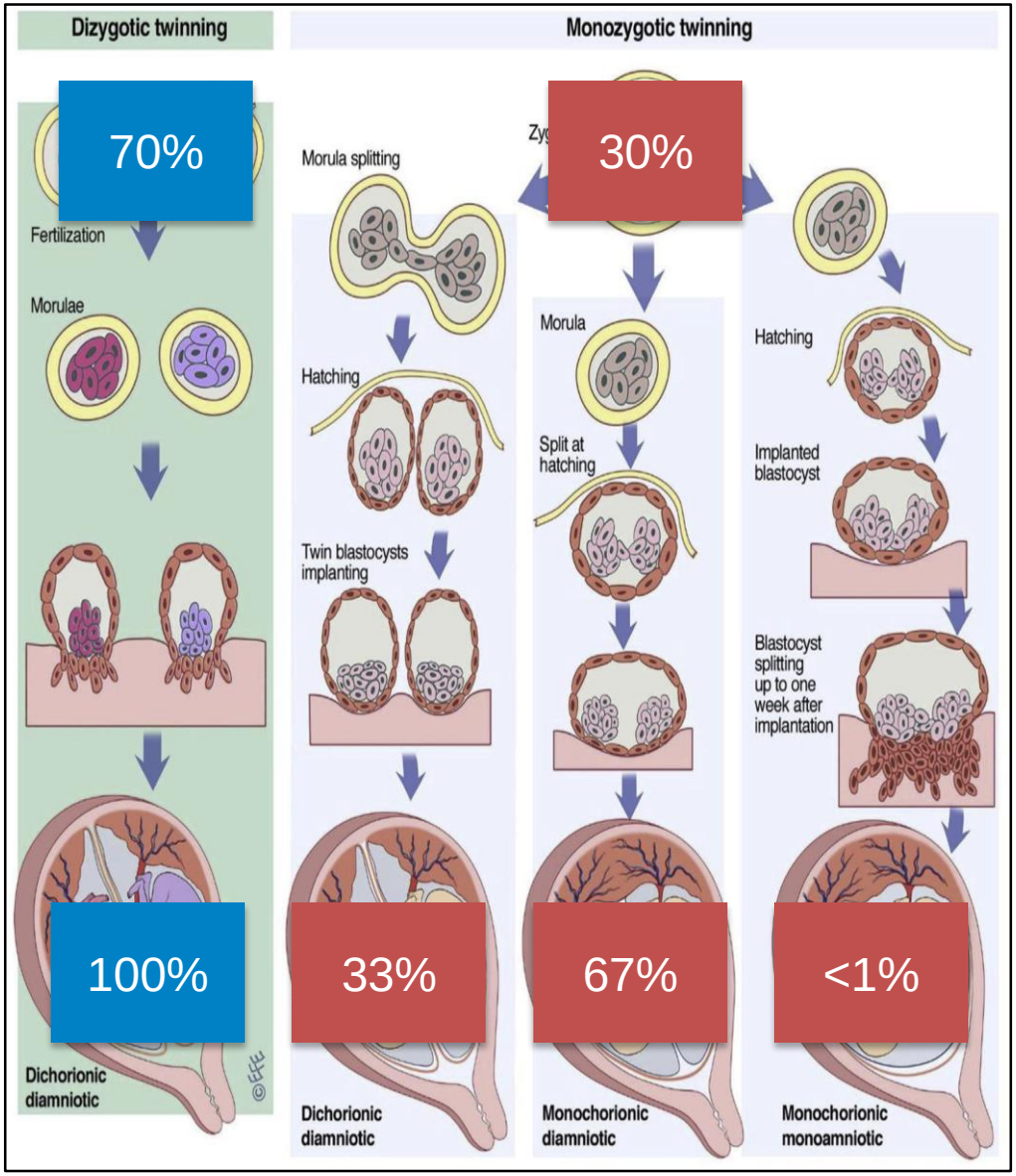
Twin Gestations

- 1/30 live births in the US[1]
- Increase over time associated with assisted reproductive technologies and advancing maternal age[1]
- Perinatal mortality rate for twins is 5–7 times greater than singleton pregnancy[2]

1. Fosler et al, Ultrasound Obstet Gynecol 2017;49:470-477
2. ACOG Practice Bulletin Number 169, Obstet Gynecol VOL. 128, NO. 4, Oct 2016

Prevalence

- 45% of all twins have concordant sex
- DZ prevalence varies among populations
- MZ prevalence is stable worldwide at 3–5/1,000 births



C. Mcnamara, Helen & Kane, Stefan & Craig, Jeffrey & Short, Roger & Umstad, Mark. (2016). Mechanisms of twinning. Society for Maternal-Fetal Medicine, Clinical guideline: Twin-twin transfusion syndrome, Jan 2013.
American College of Obstetricians and Gynecologists and Society for Maternal-Fetal Medicine, Practice Bulletin No. 169, Oct 2016.
Blumenfeld et al. J Ultrasound in Med. 2014 Dec;33(12):2187-92.
Oldenburg et al. Ultrasound Obstet Gynecol 2012; 39: 69–74.
Chasen, Chervenak. Twin pregnancy: Prenatal issues. In: UpToDate, Post, CL, DL (Ed), UpToDate, Aug 2017.
Cunningham et al. *Williams Obstetrics.* 24th edition. New York: McGraw-Hill Education, 2014.

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NEXT GENERATION NIPT
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NIPT test sui GEMELLARI

Panorama fornisce uno screening più completo per le gravidanze gemellari

Solo Panorama determina:

- ☑ Informazioni sullo zigotismo
- ☑ Frazioni fetali individuali per i gemelli eterozigoti
- ☑ Sesso fetale per ciascun gemello
- ☑ Probabilità di monosomia X per gemelli monozigoti

Panorama ha eseguito uno screening per le trisomie 21, 18 e 13 nelle gravidanze gemellari con una sensibilità combinata inferiore al 99% e una specificità inferiore al 99% nell'ambito di uno studio di convalida.[1]

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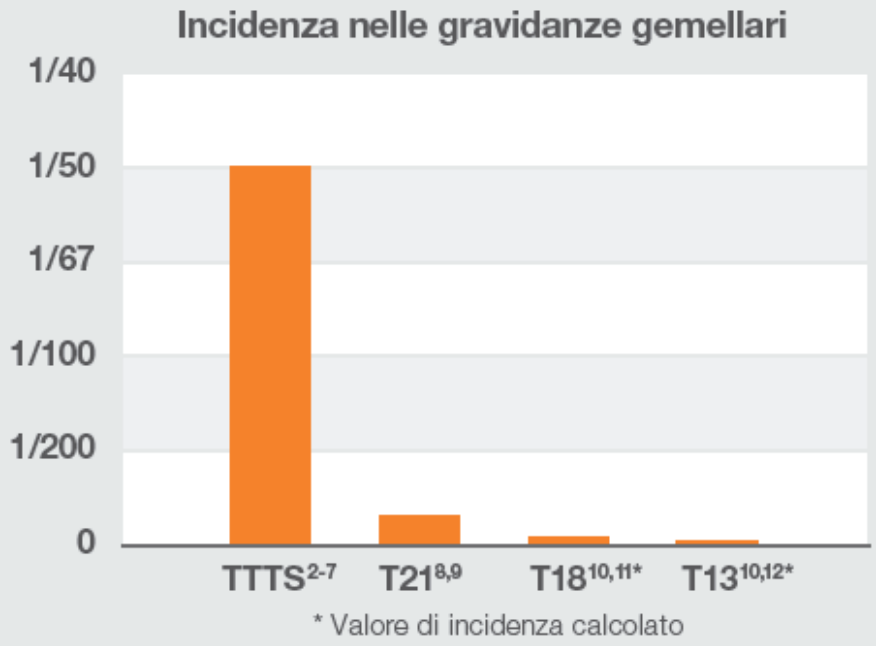
TTTS: Sindrome da Trasfusione Feto Feta

Panorama aiuta i medici a identificare la più ampia probabilità di condizioni che interessano più di

1 su 45

gravidanze gemellari[2-12]



I gemelli monozigoti possono avere un maggior rischio di complicazioni durante la gravidanza, che includono il ritardo di crescita intrauterina, i difetti di nascita e la sindrome da trasfusione feto fetale (TTTS).[2–7]

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NIPT GEMELLARE

STUDIO di VALIDAZIONE:

Panorama ha identificato gemelli MONOZIGOTI con sensibilità e specificità >99%

Condizioni sottoposte a screening per gravidanze gemellari, con ovodonazione e in affitto:	
	· Trisomia 21
	· Trisomia 18
	· Trisomia 13
	· Monosemia X*
	· Trisomia del cromosoma sessuale (segnalato quando è visto)*
	· Sindrome da delezione 22q11.2 (opzionale)*

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Panorama™ Logistics

Twins, Egg Donor and Surrogate Pregnancies

- For egg donor/surrogate pregnancies, Fetal Fraction (FF) cut off is 2.8%
- For dizygotic twin pregnancies both fetal fractions must be above 2.8% for aneuploidy risk assessment
- Determination of only zygosity requires a FF of >1% on each twin, with one having at least 1.8% FF.

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Zero Zygosity Call Errors in Validation Study

	Monozygotic result	Dizygotic result
True Monozygotic	**29**	**0**
True Dizygotic	**0**	**64**

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Aneuploidy Performance in Validation Study

- ✓ *Correctly identified 96 of 96 known euploid samples*
- ✓ *Correctly identified 11 of 11 known aneuploid samples*

	Trisomy 21	Trisomy 18	Trisomy 13
Aneuploid Twin Pregnancies (11)	**5/5**	**5/5**	**1/1**

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Panorama™ for Twins

Zero Fetal Sex Errors in Validation Study

	Result Female-Female	Result Female-Male	Result Male-Male
True Female-Female	**28**	**0**	**0**
True Female-Male	**0**	**34**	**0**
True Male-Male	**0**	**0**	**40**

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Panorama™ for Egg Donor/Surrogate

Aneuploidy	Sample size	Incorrect call	Specificity	95% CI
T21, T18, T13	37	0	100%	[90.5%, 100%]

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Low Probability: Dizygotic Twins

FINAL RESULTS SUMMARY: TWINS

Result	*Zygosity*	*Fetal Sex*	*Fetal Fraction(s)*
LOW RISK	Dizygotic	♂ Male	8.3%, 8.4%
✓	*FRATERNAL TWINS*	♀ Female	

Notes by the clinical reviewer, if any, will be shown here.

RESULT DETAILS: ANEUPLOIDIES

Condition tested[1]	*Result*	*Risk Before Test*[2]	*Risk After Test*[3]
Trisomy 21	Low Risk	1/101	<1/4,000
Trisomy 18	Low Risk	1/236	<1/10,000
Trisomy 13	Low Risk	1/744	<1/10,000

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High Probability Trisomy 21: Dizygotic Twins

FINAL RESULTS SUMMARY: TWINS

Result	*Zygosity*	*Fetal Sex*	*Fetal Fraction(s)*
HIGH RISK for Trisomy 21	**Dizygotic**	♂ **Male**	**8.3%, 8.4%**
⚠	*FRATERNAL TWINS*	♀ **Female**	

This is a screening test only. Genetic counseling and diagnostic testing for both fetuses should be offered to further evaluate these findings.

Panorama analyzes DNA from the placenta. In some cases placental DNA can differ from that of the fetus; therefore, no irreversible decisions should be made based upon results of this screening test alone.

RESULT DETAILS: ANEUPLOIDIES

Condition tested[1]	*Result*	*Risk Before Test*[2]	*Risk After Test*[3]
Trisomy 21	**High Risk**	1/101	**7/10**
Trisomy 18	Low Risk	1/236	<1/10,000
Trisomy 13	Low Risk	1/744	<1/10,000

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Comparison of NIPT for Twins

	Non-SNP methodology NIPT	Panorama™ SNP-based NIPT
Zygosity	✗	✓
Fetal sex reported for each twin	✗	✓
Individual fetal fraction reported	✗	✓
T21, T13, T18	✓	✓
SCAs	✗	✓ (Monozygotic)
22q11.2 deletion syndrome	✓ (lab dependent)	✓ (Monozygotic)

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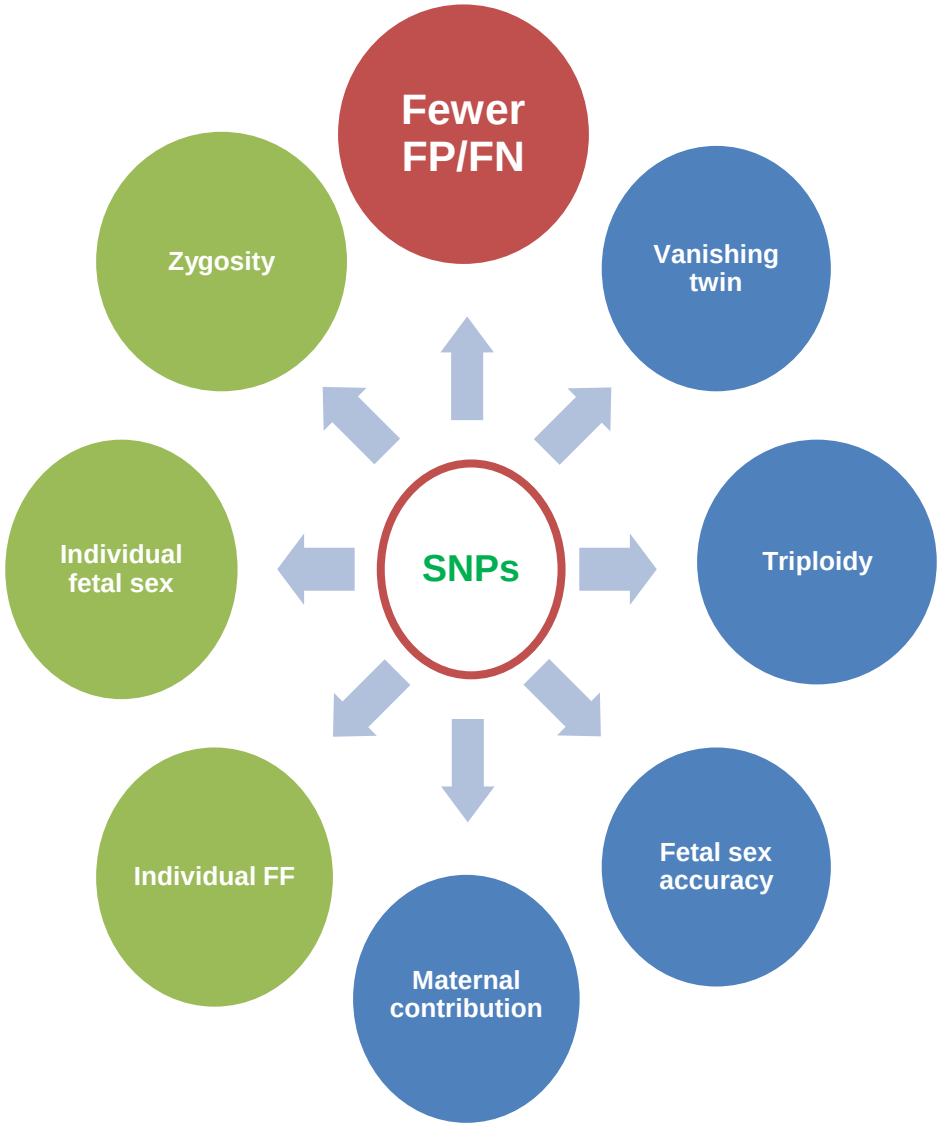
The SNP Story

The same technology that is used in our Panorama singleton screen allows us to provide valuable information for twins



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