

CERPO

Centro de Referencia Perinatal Oriente
Facultad de Medicina, Universidad de Chile



EXÁMENES GENÉTICOS I: DNA libre fetal, técnica y rendimiento prenatal

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Dr. Juan Guillermo Rodríguez**



HOJA DE RUTA

- Orígenes e introducción
- Fracción fetal
- Metodología y técnica
- Limitaciones
- Rendimiento
 - Aneuploidías
 - Aneuploidías raras y CNV
 - Sd. Monogénicos
- Screening y Rendimiento
- Caso Clínico
- Conclusiones

Early report

Presence of fetal DNA in maternal plasma and serum

Y M Dennis Lo, Noemi Corbetta, Paul F Chamberlain, Vik Rai, Ian L Sargent, Christopher W G Redman,
James S Wainscoat

ORÍGENES



DNA Libre

Paso sangre fetal

DNA libre fetal

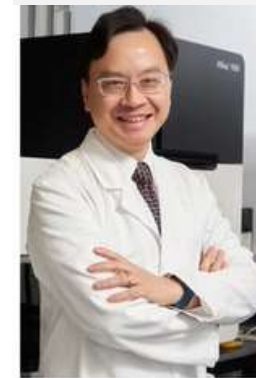
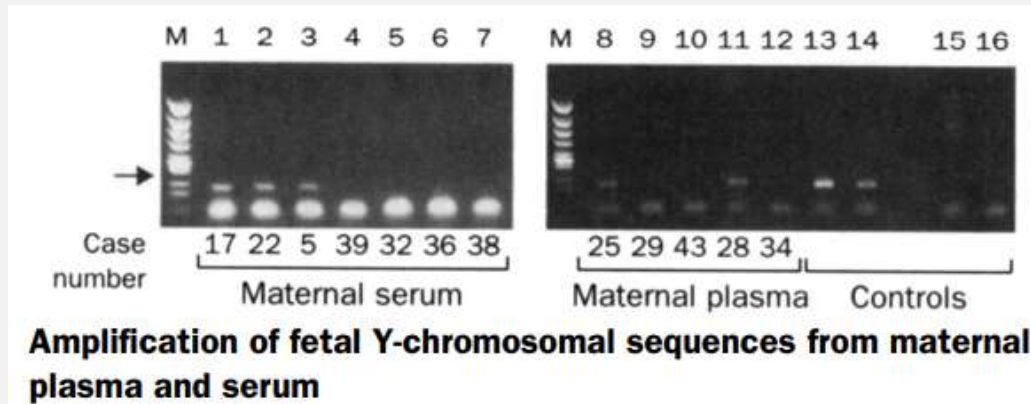
DNA Libre fetal

- 1940
- Pacientes con cáncer

- 1969 paso sangre fetal, determinación sexo fetal

- 1996
- Evaluación SRY

- 2011 se comercializa Amplificación del DNA libre fetal

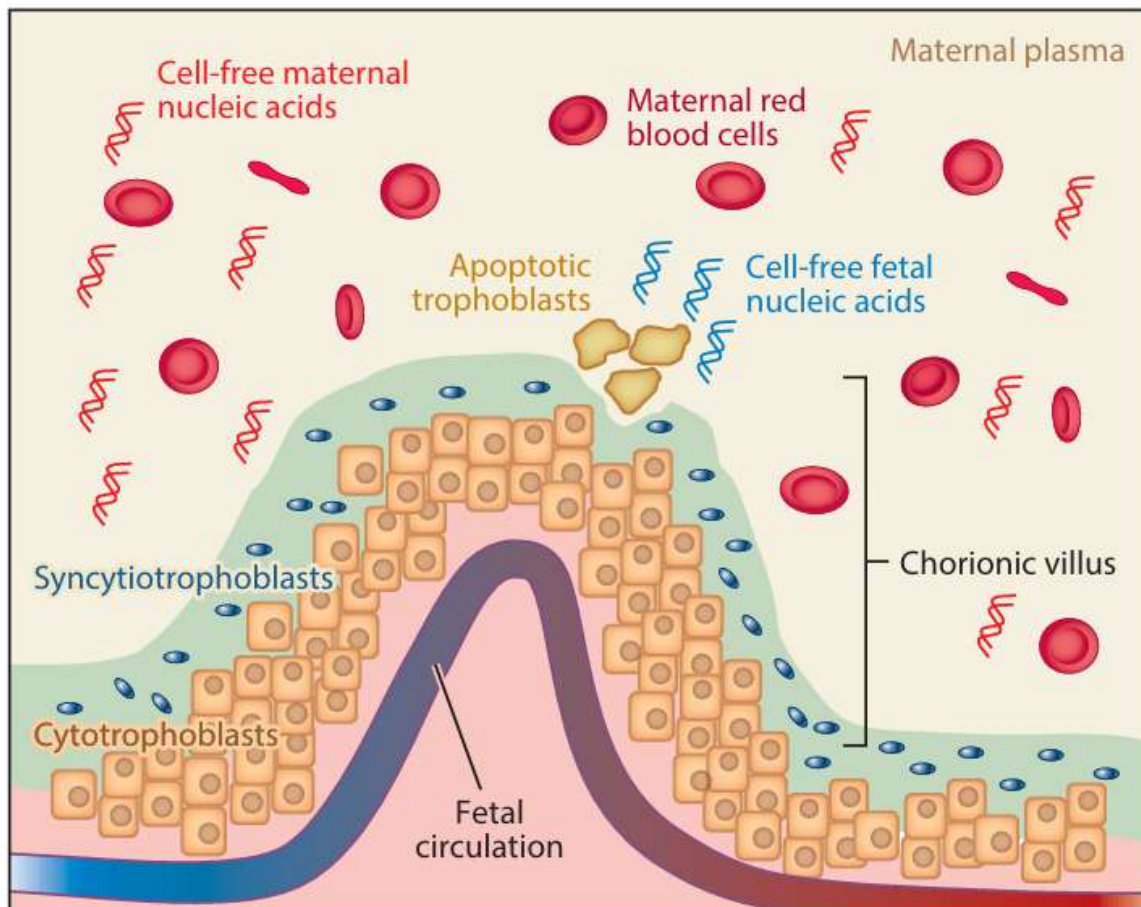


Dennis Lo, MD., PhD.

Lo, Y. D., Corbetta, N., Chamberlain, P. F., Rai, V., Sargent, I. L., Redman, C. W., & Wainscoat, J. S. (1997). Presence of fetal DNA in maternal plasma and serum. *The lancet*, 350(9076), 485-487.

Lo, Y. D., Tein, M. S., Lau, T. K., Haines, C. J., Leung, T. N., Poon, P. M., ... & Hjelm, N. M. (1998). Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *The American Journal of Human Genetics*, 62(4), 768-775.

INTRODUCCIÓN



cffDNA se origina desde la placenta por células apoptóticas del trofoblasto

Fragmentos entre 150-200 pb

Velocidad de liberación a la circulación materna: $2,24 \times 10^4$ copias por minuto.
Desaparece 30 min-2 hrs post parto

Fragmentos más cortos que el ADN materno libre de células: 166 pb v/s 143 pb.

Alberry, M., Maddocks, D., Jones, M., Abdel Hadi, M., Abdel-Fattah, S., Avent, N., & Soothill, P. W. (2007). Free fetal DNA in maternal plasma in anembryonic pregnancies: confirmation that the origin is the trophoblast. *Prenatal Diagnosis: Published in Affiliation With the International Society for Prenatal Diagnosis*, 27(5), 415-418.

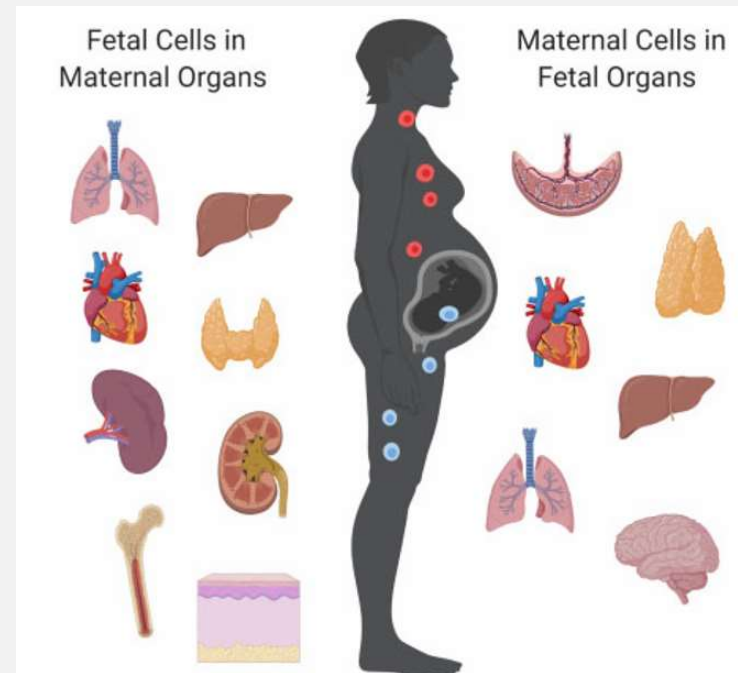
Chan, K. A., Zhang, J., Hui, A. B., Wong, N., Lau, T. K., Leung, T. N., ... & Lo, Y. D. (2004). Size distributions of maternal and fetal DNA in maternal plasma. *Clinical chemistry*, 50(1), 88-92.

MICROQUIMERISMO FETAL



Presencia de células fetales y maternas como dos poblaciones originadas en individuos genéticamente distintos

- Cicatrización
- Procesos neoplásicos (CaCu, melanoma CA mama)
- Inmunomodulación: pueden alterar el sistema inmune materno o exacerbar patologías



Bianchi, D. W., Khosrotehrani, K., Way, S. S., MacKenzie, T. C., Bajema, I., & O'Donoghue, K. (2021). Forever connected: the lifelong biological consequences of fetomaternal and maternofetal microchimerism. *Clinical chemistry*, 67(2), 351-362.

FETAL FRACTION (FF)



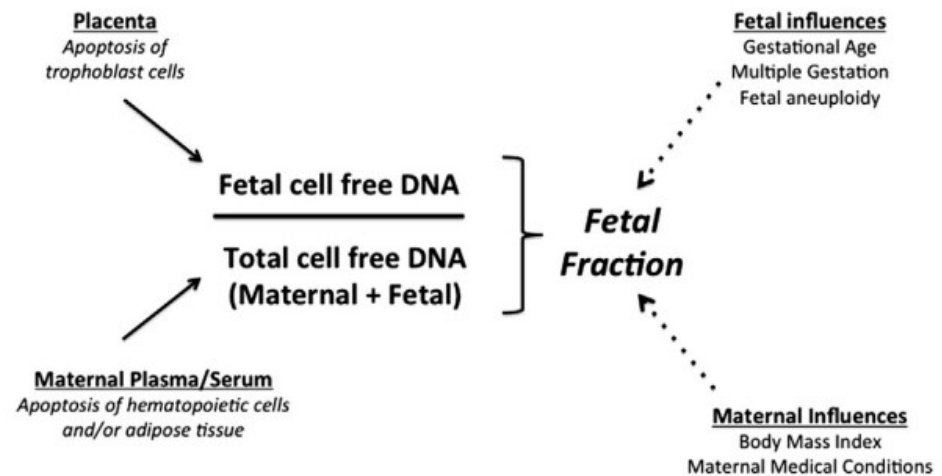
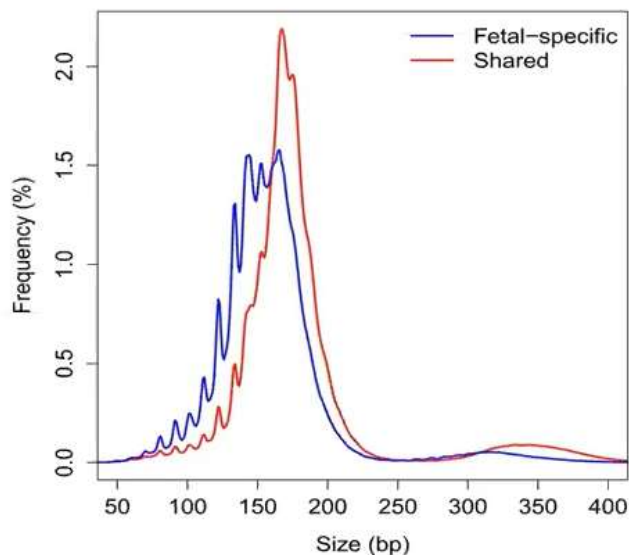
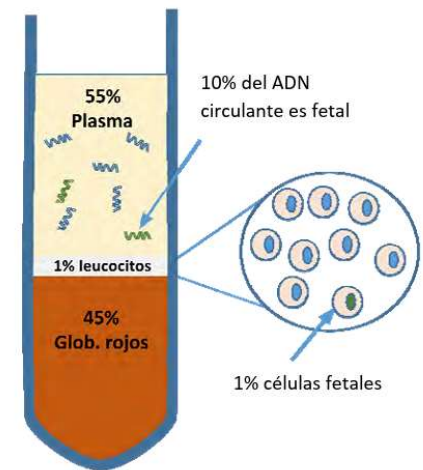
Corresponde al porcentaje de todas las células libres fetales en sangre materna

Este se puede detectar generalmente desde las 9 semanas

Aumenta con la edad gestacional y desaparece rápidamente posterior al nacimiento

•Vida media corta: 16,3 minutos (R: 4-30)

Se sugiere > 4% para estudio válido



Taglauer, E. S., Wilkins-Haug, L., & Bianchi, D. W. (2014). cell-free fetal DNA in the maternal circulation as an indication of placental health and disease. *Placenta*, 35, S64-S68.

FACTORES QUE INFLUYEN EN FF



Edad gestacional precoz

- Menor de 9 semanas

Muestra subóptima

- Tubo morado (EDTA)
- Centrifugado en menos de 6 horas
- Refrigeración -80°C

Obesidad materna

- FF es inversamente proporcional al peso materno

Cariotipo fetal

- En trisomías la FF es menor, T.18, T.13, Sd. Turner y particularmente Triploidías

Table 2 Proportion of women with $\geq 4\%$ fetal cfDNA on repeat blood draw

Maternal weight bin (kg)	# $\geq 4\%$ fetal on 2nd draw	# of total patients	% with $\geq 4\%$ on 2nd draw
<90	30	42	71.4%
$\geq 90 < 100$	14	23	60.9%
$\geq 100 < 110$	13	22	59.1%
$\geq 110 < 120$	10	17	58.8%
$\geq 120 < 130$	2	7	28.6%
$\geq 130 < 140$	5	13	38.5%
≥ 140	2	11	18.2%

Se realizó cffDNA en 22384 embarazos únicos entre las 10 y 21 semanas, calculando FF. Se cuantificó y determinó la curva inversamente proporcional entre la FF y el peso materno

Wang, E., Batey, A., Struble, C., Musci, T., Song, K., & Oliphant, A. (2013). Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. *Prenatal diagnosis*, 33(7), 662-666.

Extracción de sangre (10 ml)

A partir de 10 semanas

Cell-Free DNA BCT

Centrifugado en menos de 6 horas

Refrigeración -80°C

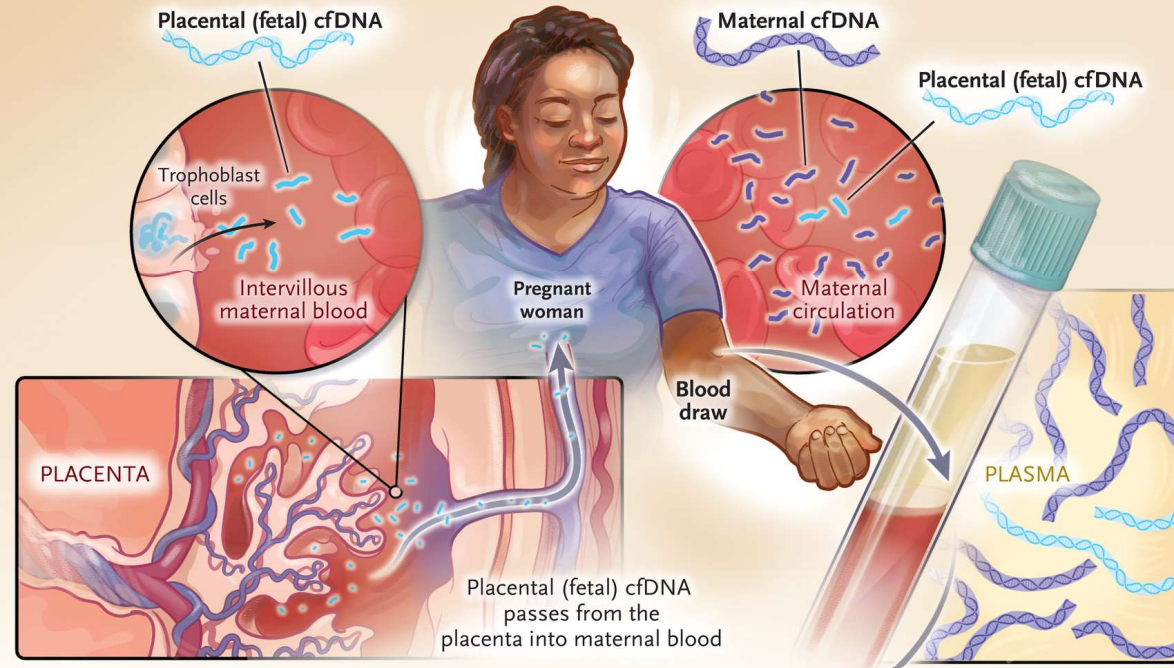
Fracción fetal calculada promedio 10 % (Mínimo 4%)

Los fragmentos se secuencian y alinean con un set de cromosomas de referencia

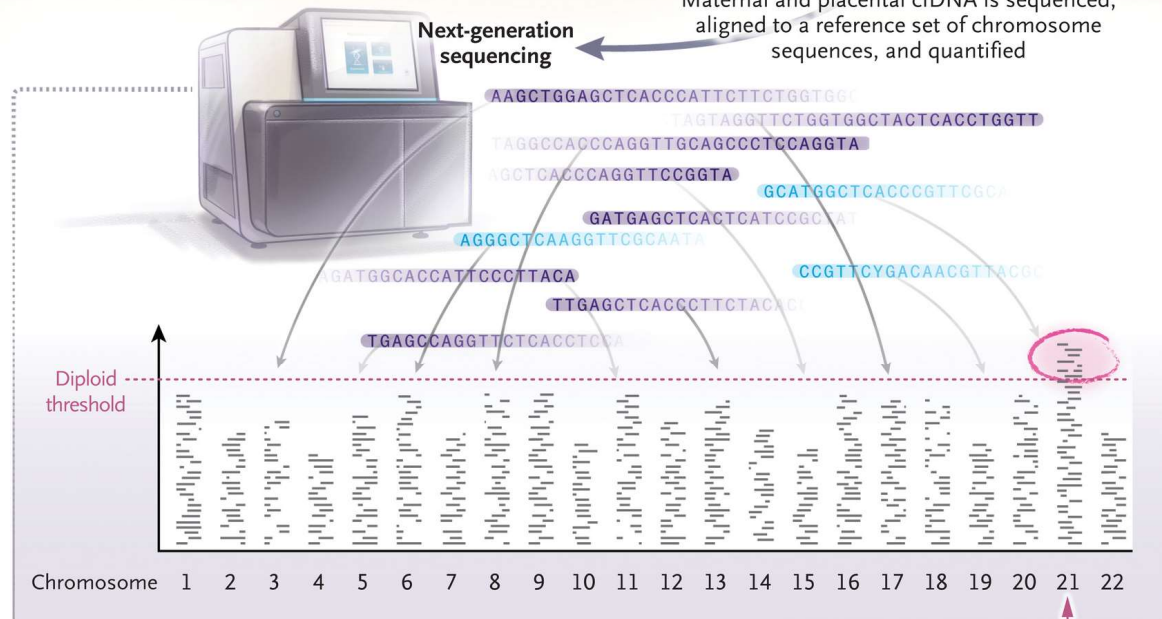
Se cuenta la dosis relativa de cada cromosoma

Norton, M. E. (2022). Circulating cell-free DNA and screening for trisomies. *New England Journal of Medicine*, 387(14), 1322-1324.

Routine turnover of cells releases cfDNA (fragments of genomic DNA) into circulation



Maternal and placental cfDNA is sequenced, aligned to a reference set of chromosome sequences, and quantified



DOSIS RELATIVA DE CROMOSOMAS

Figure 1 Sequence and align

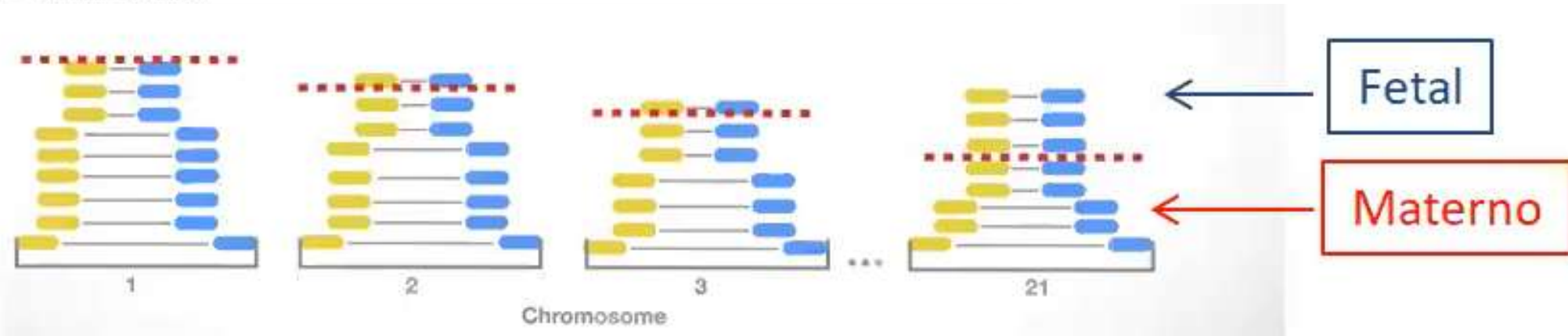
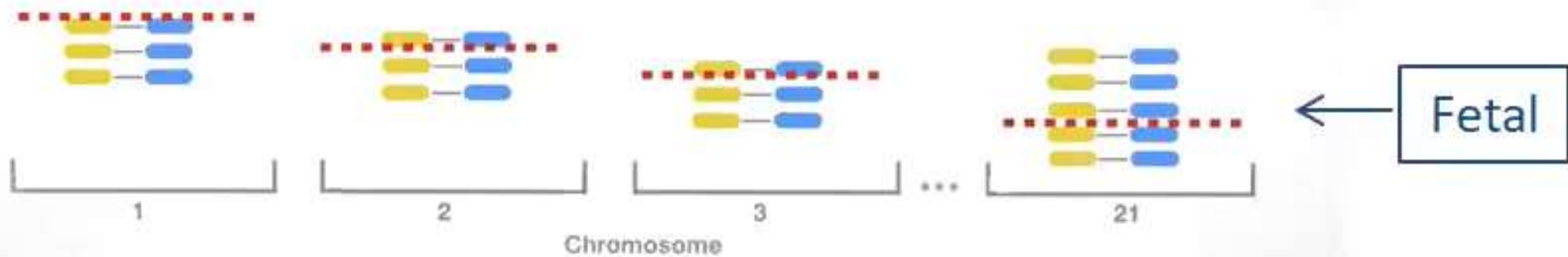
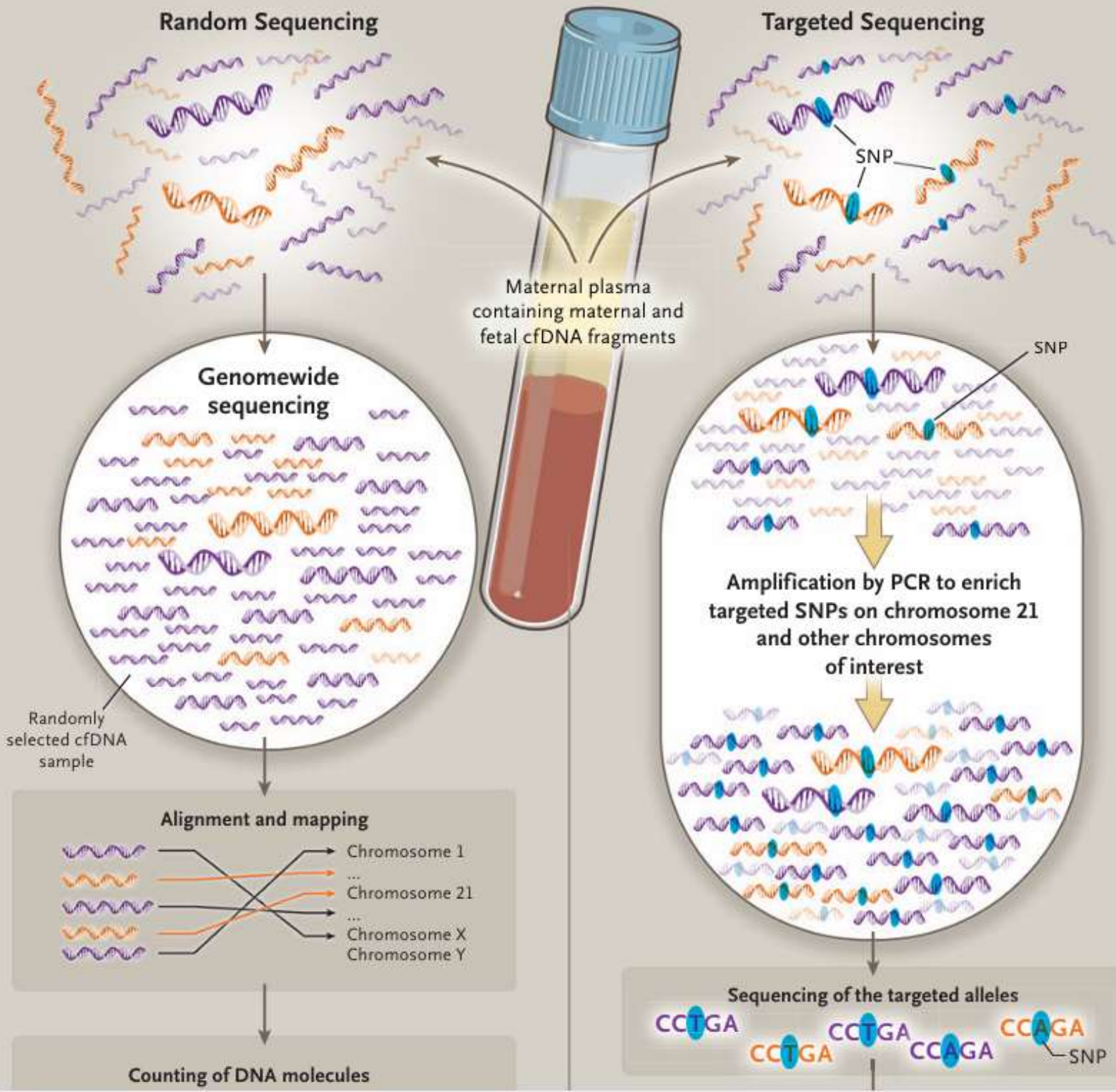
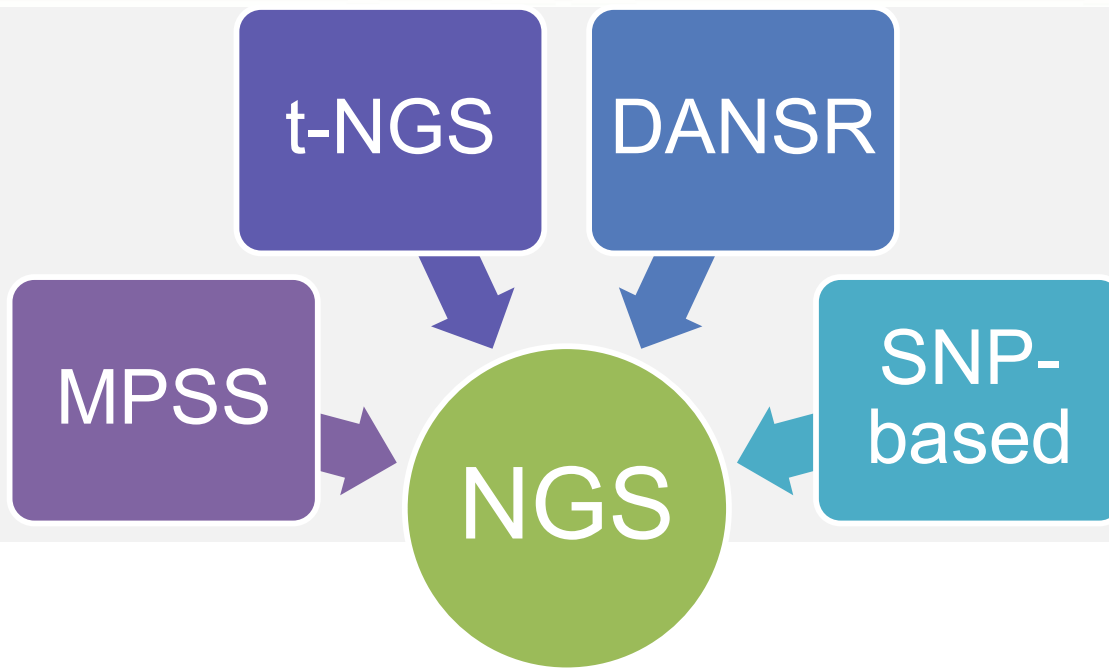
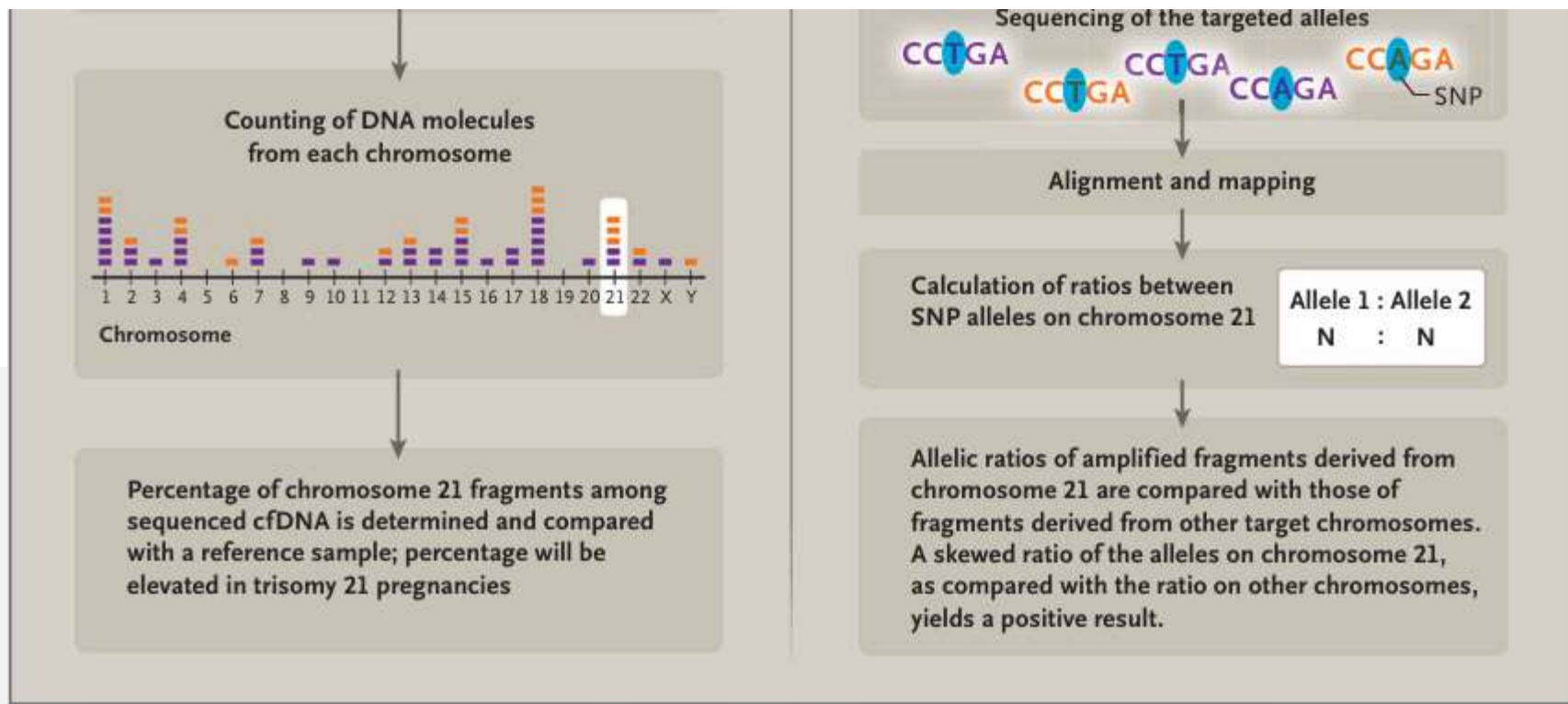


Figure 2 Sequence counting



Grati, F. R., Bajaj, K., Simoni, G., Maggi, F., Gross, S. J., & Ferreira, J. C. P. B. (2019). Noninvasive prenatal testing by cell-free DNA: technology, biology, clinical utility, and limitations. In *Human Reproductive and Prenatal Genetics* (pp. 627-652). Academic Press.



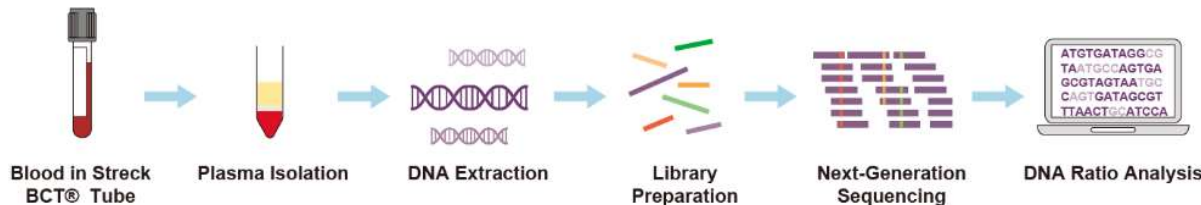
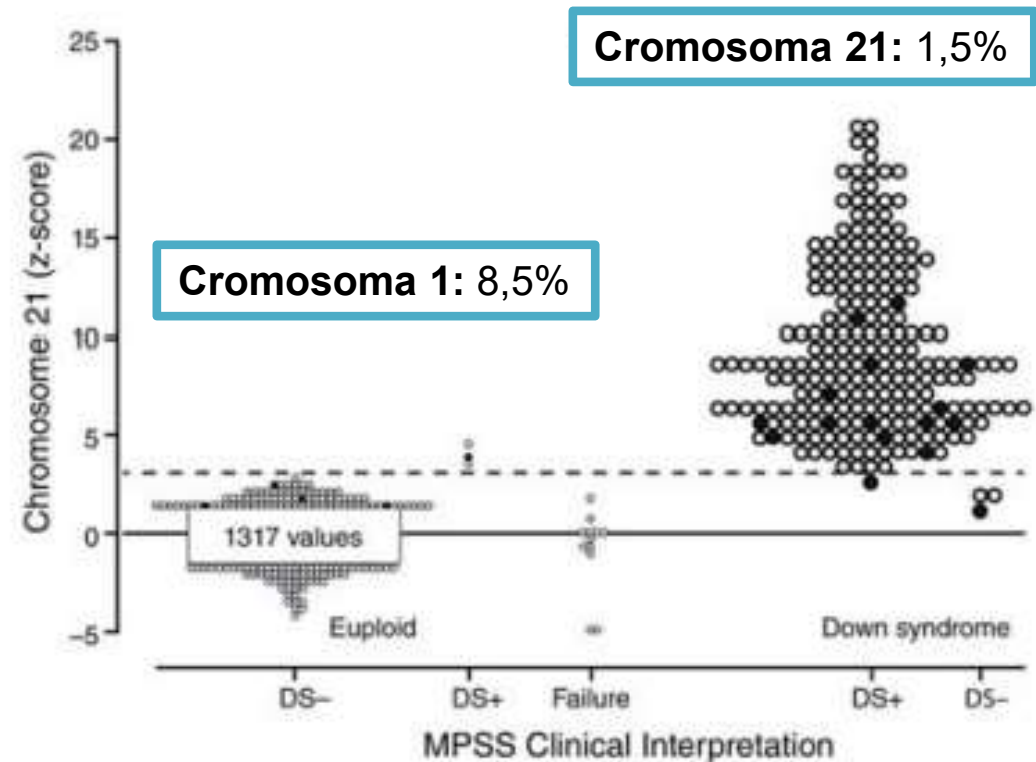


MASSIVELY PARALLEL SHOTGUN SEQUENCING (MPSS)



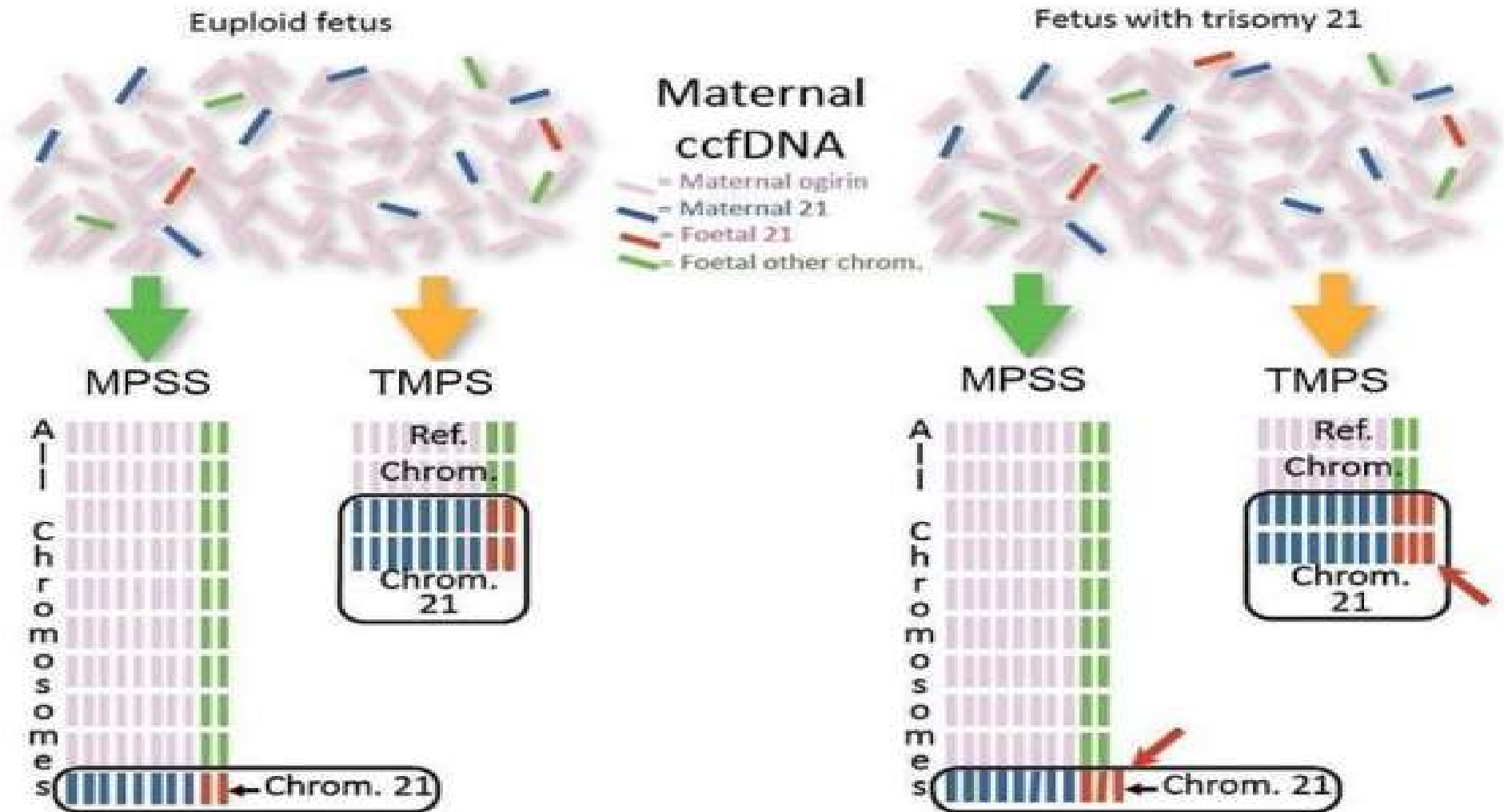
Secuencia genoma completo comparándolo con genoma de referencia

Se otorga puntuación Z-Score



Bianchi, D. W., & Chiu, R. W. (2018). Sequencing of circulating cell-free DNA during pregnancy. *New England Journal of Medicine*, 379(5), 464-473.

Targeted NGS o MPSS (t-NGS o t-MPSS)



Fetal Trisomy Risk Determination			Fetal Fraction Determination			Technical Aspects			Examples of Tests		
Technology	Principle	Statistics	Triploids	Principle	DNA Contamination Detection—FF Estimation of Each Fetus in Dizygotic Twin Pregnancies	Sample Analysis	Counting Depth	Maximum Sample Throughput		Possibility to Add Further Clinically Validated Targets	
MPSS	Counting ratio	z-score/NCV	Not detectable	Multiple approaches other than SNP-genotyping	No as SNPs are not tested	Multiplex by NGS	Variable ^a	48 samples/run	Possible by unlocking bioinformatic analysis of the fragment counts belonging from other chromosomes	HiSeq/NextSeq (Illumina) or Ion Proton™ (Thermo Fisher Scientific) MPSS platform-based technologies which are offered under multiple local brands (e.g., VeriSeq, Verifi, Neobona, MaterniT, G-test, Tranquility, IONA, InformaSeq, Prelude, SafeTest, etc.)	
Targeted-NGS	Counting ratio	z-score/NCV	Not detectable	SNP-genotyping or fragment size	Yes if SNPs are tested	Multiplex by NGS	Variable ^a	192 sample/run		Possible by adding new complementary molecular probes in the assay	Clarigo (Multiplicom, Agilent), Veracity (NIPD Genetics)
DANSR	Counting ratio	FORTE (incorporating FF%)—Odds ratio	Not detectable	SNP-genotyping	Yes	Singleplex by microarray	Standardized	384 samples/run		Possible by adding new complementary DANSR probes in the assay	Harmony Prenatal Test (Roche)
SNP-counting	SNP genotyping	NATUS (incorporating FF%)—Bayesian statistics with Maximum Likelihood Estimation	Detectable	SNP-genotyping	Yes	Multiplex by NGS	Variable ^a	48 samples/run		Possible if enough informative SNPs are present in the target chromosome region	Panorama (Natera)

LIMITACIONES

Detección de anomalías no clásicas

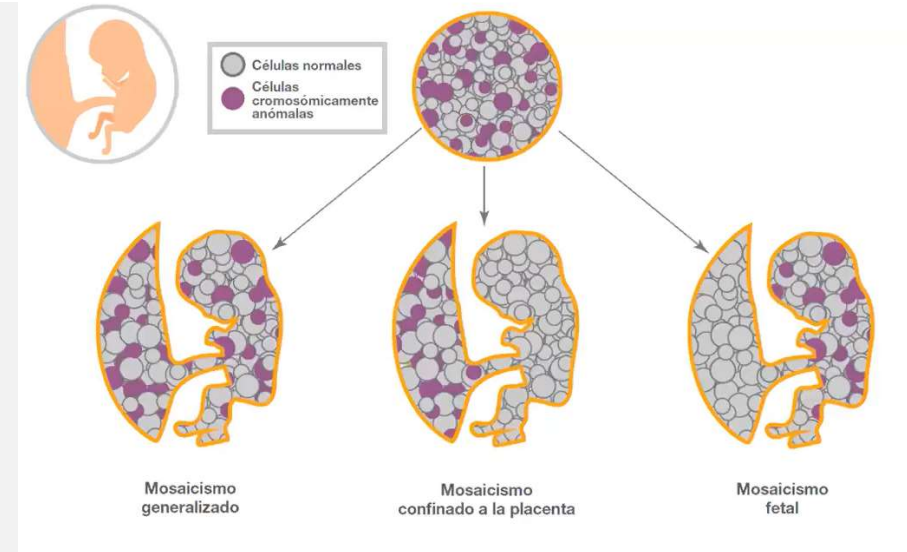
- Microdeleciones/microduplicaciones (CNV)
- Translocaciones
- Triploidías digénicas

Falsos Positivos/Negativos

- Mosaicos confinados a placenta
- Gemelares discordantes
- Fetos evanescentes
- Tumores maternos

FF baja

- Obesidad
- Aneuploidias
- Muestra subóptima
- Edad gestacional



Embarazo Múltiple:

- Gestaciones dicigóticas: cada gemelo puede tener su propia FF
- Fetos evanescentes
- Muerte de 1 gemelo
- No se puede saber cuál de los dos es XX-XY el Gemelo 1 o el 2

RENDIMIENTO

Detección de aneuploidías fetales

- T.13, T.18, T.21, X, Y

Detección de autosómicas “raras” (no 21, 18, 13)

Detección de síndromes microdelecionales (CNV > 7 Mb)

Detección de síndromes monogénicos

¡Aplicación dependerá de la tecnología utilizada!

1. Massively parallel shotgun sequencing (MPSS)
2. Targeted NGS (t-NGS)
3. Digital analysis of selected regions (DANSR)
4. Single nucleotide polymorphisms (SNP)-based.

ANEUPLODÍAS

Table 1 Performance of NIPS in a general-risk population for trisomy 21, trisomy 18, and trisomy 13 calculated in random-effects meta-analyses

Test Statistic	No. of Studies	Result (%) (95% CI)	I ² (%)
Trisomy 21			
Sensitivity	17	98.80 (97.81-99.34)	0.0
Specificity	14	99.96 (99.92-99.98)	75.9
PPV	28	91.78 (88.43-94.23)	68.3
NPV	14	100 (99.99-100)	0.0
FPR	14	0.04 (0.02-0.08)	75.9
Accuracy	14	99.94 (99.91-99.96)	80.2
DOR ^a	14	110,000 (44,000-260,000); P < .0001	55.7
Trisomy 18			
Sensitivity	6	98.83 (95.45-99.71)	0.0
Specificity	7	99.93 (99.83-99.97)	94.9
PPV	17	65.77 (45.29-81.68)	88.5
NPV	7	100 (100-100)	0.0
FPR	7	0.07 (0.03-0.17)	75.9
Accuracy	6	99.91 (99.73-99.97)	95.7
DOR ^a	6	29,000 (4800-180,000); P < .0001	94.9
Trisomy 13			
Sensitivity	7	100 (0-100)	0.0
Specificity	8	99.96 (99.92-99.98)	81.5
PPV	18	37.23 (26.08-49.93)	71.9
NPV	8	100 (100-100)	0.0
FPR	8	0.04 (0.02-0.08)	81.5
Accuracy	8	99.95 (99.90-99.97)	82.2
DOR ^a	7	29,000 (8900-94,000); P < .0001	0

Tabla 1. Resultado del ADNfl en gestantes con embarazo único

Cribado combinado	Muestras estudiadas	No valorables	Alto riesgo DNA fetal	No aceptan invasivo	Aceptan invasivo	PV ¹	
RA	1019	29 (2,8%)	68 (6,7%)	8/68 (11,8%)	60/68 (88,2%)	56/60	→ Reducción test invasivos
RI	4602	104 (2,3%)	38 (0,8%)	1/38 (2,6%)	37/38 (97,4%)	27/37	→ 7% falsos positivos
Total	5621	133 (2,4%)	106 (1,9%)	9/106 (8,5%)	97/106 (91,5%)	83 (1,5%)	→ 27% falsos positivos

PV: positivo verdadero; - se excluyen las gestantes sin test invasivo

Tabla 2. Resultado del ADNfl en gestantes con embarazo gemelar

Cribado combinado	Muestras recibidas	Alto riesgo DNA fetal	Aceptan invasivo	No aceptan invasivo
RA	23	1 (4,3%)	1 (100%)	0
RI	108	3 (2,8%)	2 (66,7%)	1 (33,3%)
Total	131	4 (3%)	3 (75%)	1 (25%)

Sensibilidad:

- Trisomía 21: 99%
- Trisomía 18: 97-99%
- Trisomía 13: 95-97%
- Monosomía X: 95%
- CNV: 74%

Especificidad:

- Trisomía 21: 99.9%
- Trisomía 18: 99.9%
- Trisomía 13: 99.9%
- Monosomía X: 90%
- CNV: 99%

Jacobsson, B., MacPherson, C., Egbert, M., Malone, F., Wapner, R. J., Roman, A. S., ... & Norton, M. E. (2022). Cell-free DNA screening for trisomies 21, 18, and 13 in pregnancies at low and high risk for aneuploidy with genetic confirmation. *American journal of obstetrics and gynecology*, 227(2), 259-e1.



A

TRID
 Non-
 Scree

Rare autosomal trisomies: comparison of detection through cell-free DNA analysis and direct chromosome preparation of chorionic villus samples

me-Wide

P. BENN¹*, F. MALVESTITI², B. GRIMI², F. MACCI², G. SIMONI² and F. R. GRATI²

¹Department of Genetics and Genome Sciences, University of Connecticut Health Center, Farmington, CT, USA; ²Unit of Research and Development, Cytopgenetics and Medical Genetics, TOMA, Advanced Biomedical Assays S.p.A., Busto Arsizio, Varese, Italy

Estudio	Gestaciones cribradas	Pérdidas fetales	Anomalia confirmada	Fenotipo anormalo	DUP significativa	CIR o bajo peso al nacimiento	RN vivo aparentemente normal	Resultado desconocido	Anomalia cromosómica materna	Total RATs
Fiorentino (2017)	12.114	7	3	0	1	0	6	0	0	17
Pertile (2017)	16.885	26	5	1	1	2	14	8	1	58
van Opstal (2018)	2.527	0	3	5	0	8	9	1	0	26
Scott (2018)	23.388	6	2 ^v	5	0	6	9	0	0	28
Wan (2018)	15.362	2	1	0	0	-	18	32	0	53
Chatron (2019)	1.617	0	0	0	1	3	6	0	0	10
Total	71.893	41	14	11	3	19	62	41	1	192
% de casos con resultado conocido (n=151)		27%	9.3%	7.3%	2.0%	15%	41%	-	0.7%	100%
Casos cribrados por casos patológico (1/n)		1.753	5.135	6.536	23.964	2.975 ^o	1.160		71.893	

Benn, P., Malvestiti, F., Grimi, B., Maggi, F., Simoni, G., & Grati, F. R. (2019). Rare autosomal trisomies: comparison of detection through cell-free DNA analysis and direct chromosome preparation of chorionic villus samples. *Ultrasound in Obstetrics & Gynecology*, 54(4), 458-467.

MICRODELECCIONES Y MICRODUPLICACIONES (CNVs)



The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis

- Asociación con discapacidades y retrasos del neurodesarrollo. Se distribuyen por todo el genoma y no están necesariamente asociadas con la edad materna
- Limitaciones para la detección
- Muchos laboratorios no pueden identificar los desequilibrios cromosómicos que tienen un tamaño de < 7 Mb
- S 66-86%, E 98,0-99,8%, VPP 31-45%

SÍNDROMES DE MICRODELECCIÓN

Sd. De DiGeorge

Sd. De Cri-Du-Chat

Sd. Prader Willi

Sd. Angelman

Sd. Deleción 1p36

Sd. De Wolf-Hirschhorn

Raymond, Y. C., Acreman, M. L., Bussolaro, S., Mol, B. W., Fernando, S., Menezes, M., ... & Rolnik, D. L. (2023). The accuracy of cell-free DNA screening for fetal segmental copy number variants: A systematic review and meta-analysis. *BJOG: an international journal of obstetrics and gynaecology*.

SINGLE-GENE DESORDERS



- Se han podido hacer ciertas detecciones
- Gen CFTR
- Gen FGFR3, COL2a1
- Gen DMD
- Muy pocos estudios

Síndromes Monogénicos

Espectro Noonan

Desórdenes esqueléticos

Alagille

Esclerosis tuberosa

CHARGE Syndrome

Sotos y Rett Syndrome

Cornelia de Lange Syndrome

Scotchman, E., Shaw, J., Paternoster, B., Chandler, N., & Chitty, L. S. (2020). Non-invasive prenatal diagnosis and screening for monogenic disorders. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 253, 320-327.

Mohan, P., Lemoine, J., Trotter, C., Rakova, I., Billings, P., Peacock, S., ... & Benn, P. (2022). Clinical experience with non-invasive prenatal screening for single-gene disorders. *Ultrasound in Obstetrics & Gynecology*, 59(1), 33-39.

PRUEBA DE SCREENING



Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics

- Tasa de falsos positivos
- Limitaciones en otras alteraciones genómicas
- Limitaciones con Mosaicismo placentario (1-2%)
- Un gemelo fallecido, cuyo cffDNA se puede detectar entre 8-13 sem post muerte
- Mosaicismo materno
- Cáncer materno

Gregg, A. R., Skotko, B. G., Benkendorf, J. L., Monaghan, K. G., Bajaj, K., Best, R. G., ... & ACMG Noninvasive Prenatal Screening Work Group. (2016). Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. *Genetics in medicine*, 18(10), 1056-1065.

Rose, N. C., Barrie, E. S., Malinowski, J., Jenkins, G. P., McClain, M. R., LaGrave, D., ... & Guidelines Committee. (2022). Systematic evidence-based review: The application of noninvasive prenatal screening using cell-free DNA in general-risk pregnancies. *Genetics in Medicine*, 24(7), 1379-1391.

¿SE DEBERÍA OFRECER A TODAS LAS PACIENTES?



Low risk patients

- PPV T.21 → 50-81% (N=55244)

High risk patients

- PPV T.21 → 94% (N = 72382)

Screening convencional vs NIPT

- PPV 80.9% vs 90% (N = 15841)

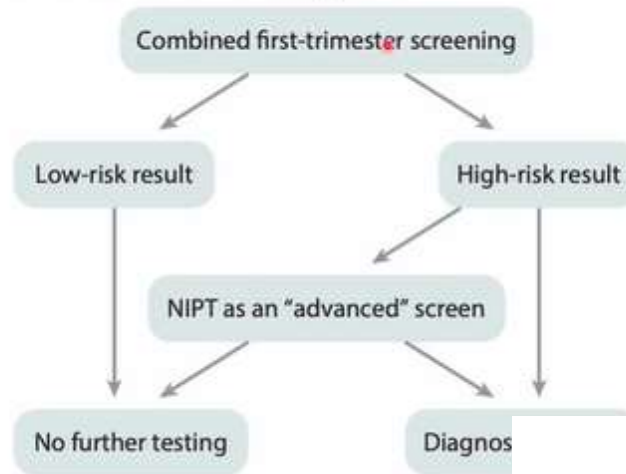
- Recomendaciones ACMG:
 - Informar a las pacientes sobre NIPT como un método de Screening
 - Derivar en caso de hallazgos en NIPT a genetista
 - Ofrecer test diagnóstico frente a hallazgo
 - No recomendar laboratorios que no ofrezca: DR, SPEC, PPV y NPV

Zhang, H., Gao, Y., Jiang, F., Fu, M., Yuan, Y., Guo, Y., ... & Wang, W. (2015). Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146 958 pregnancies. *Ultrasound in Obstetrics & Gynecology*, 45(5), 530-538.

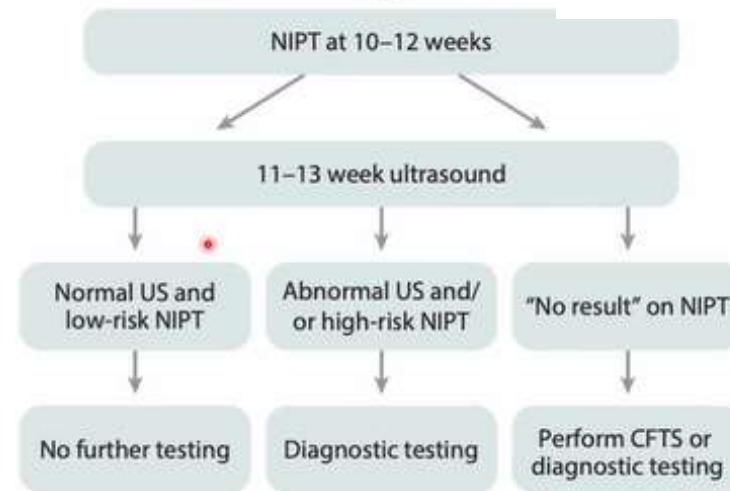


ALTERNATIVAS CLÍNICAS

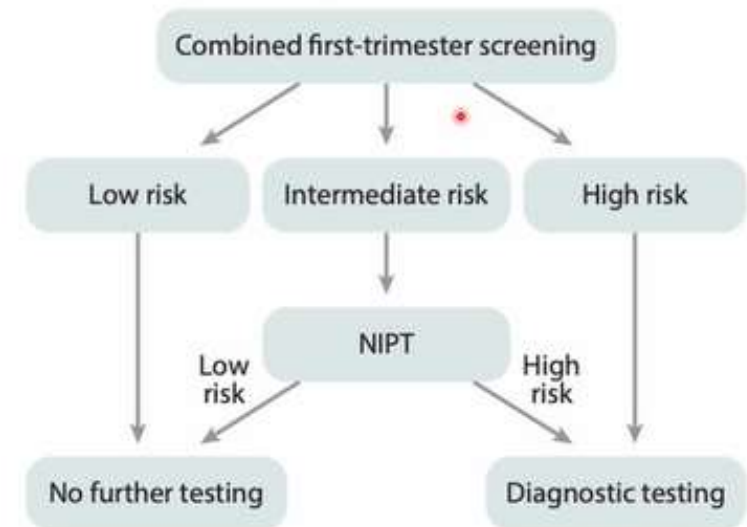
a Advanced screening test



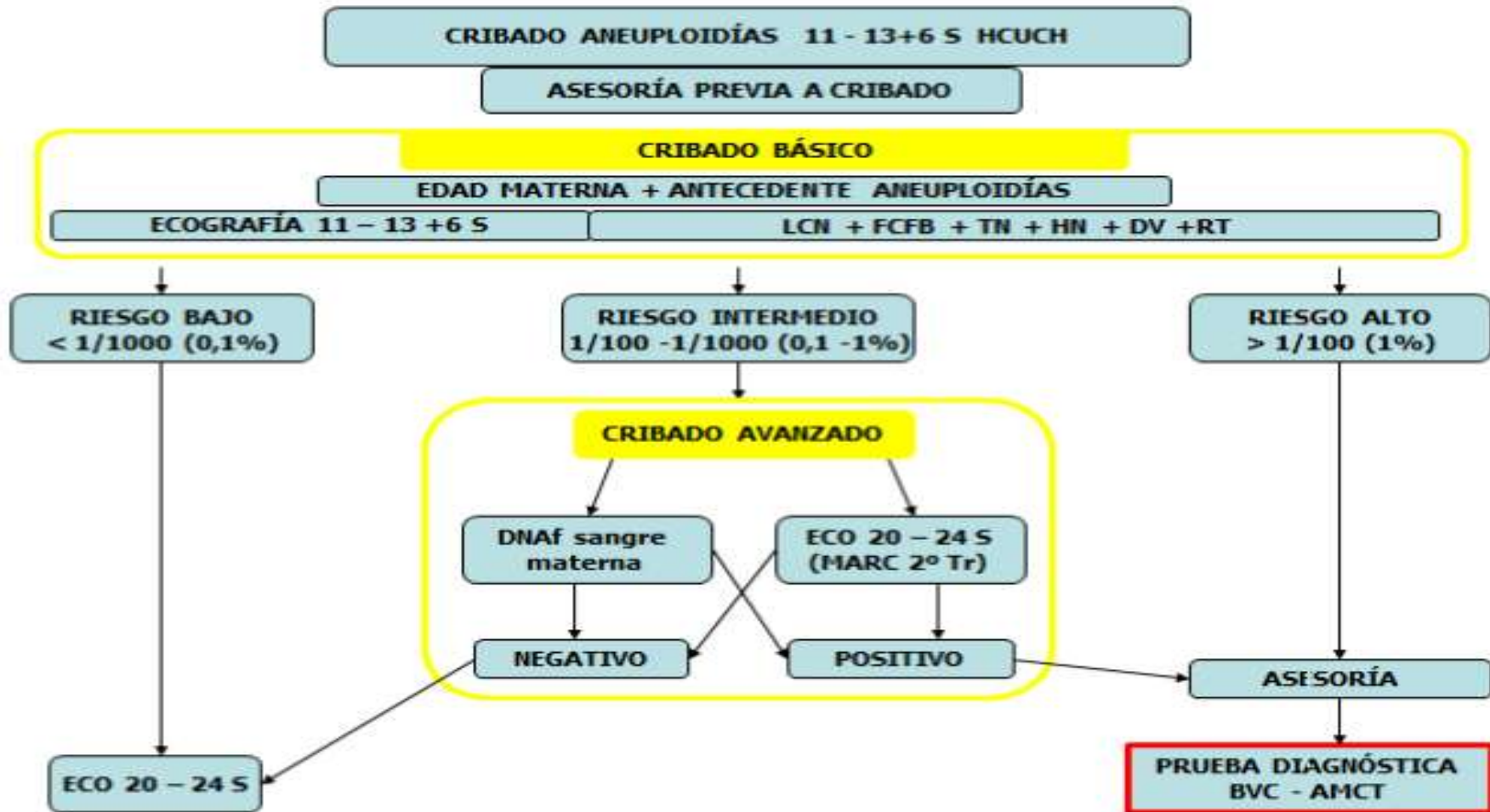
b Universal screening test



c Contingent model



¿Y EN CHILE?



CASO CLÍNICO

- Primigesta
- Emb 13+1 sem
- Ecografía 11-14: normal, SHE
- Riesgo combinado T.21: 1/1053, T.18: 1/8577
- DNA Fetal: T.13, T.18, T.21 → riesgo bajo

- Eco 22-24: **Pie equinovaro bilateral**
- Resto de exploración normal



¿QUÉ RECOMENDAMOS?

1. Realiza test invasivo por la aparición de anomalías ecográficas
2. No recomendar Test Invasivo porque DNA fetal libre ya ha descartado trisomías clásicas
3. Seguimiento ecográfico

- Eco 26+2 sem: Pie equinovaro bilateral
- Eco Cardio normal
- Desarrollo cortical normal

- Eco 29+2 sem: Piel equinovaro bilateral
- Desarrollo cortical
- Cuerpo calloso morfología normal
- Percentil 16

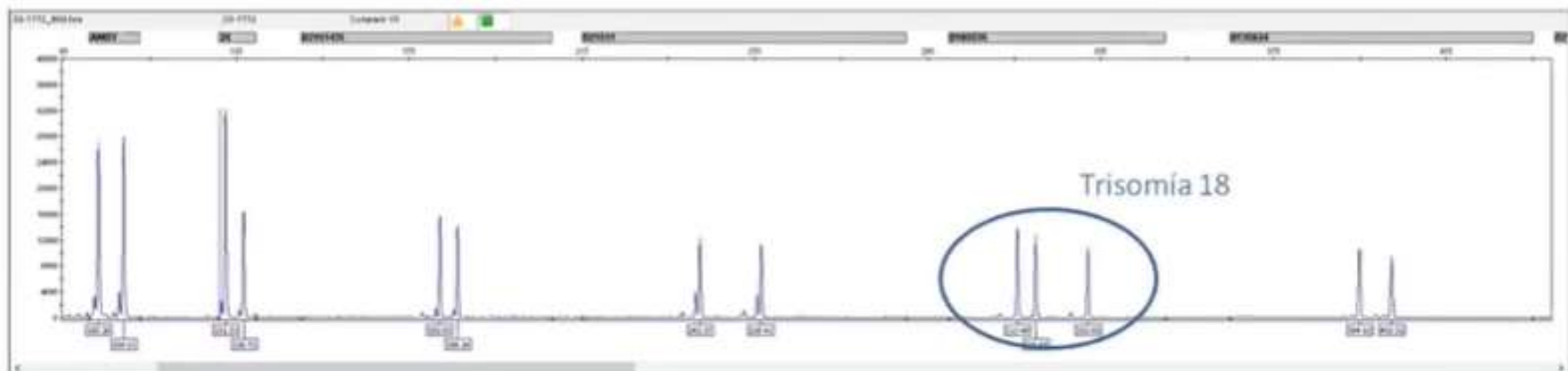
- Eco 32+6: Pie equinovaro bilateral
- Percentil 11

**PACIENTE
RECHAZA** ☹️

- Eco35-36 sem → AMCT
- RNM: Cerebelo y vermis hipoplásico, impresiona microftalmia bilateral y pie equinovaro

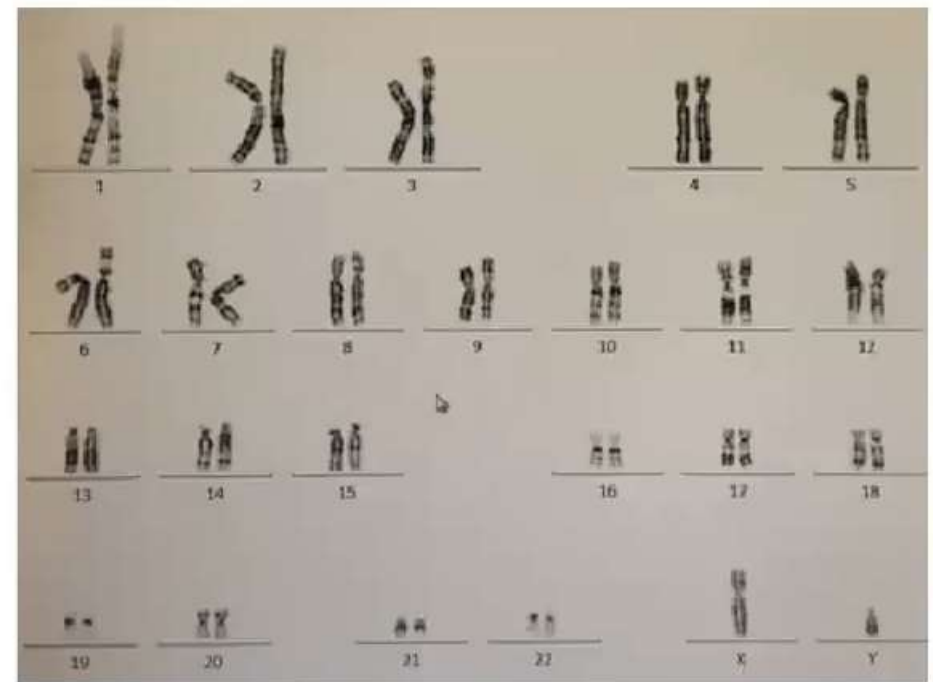
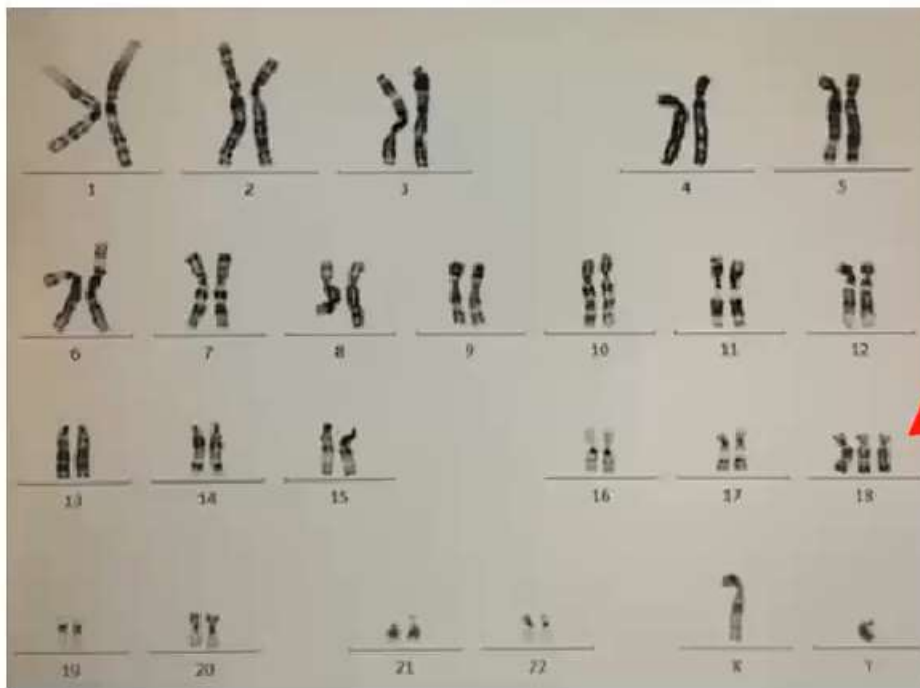
**PACIENTE
ACEPTA** 😊

QF-PCR en líquido amniótico



¿QUÉ RECOMENDAMOS?

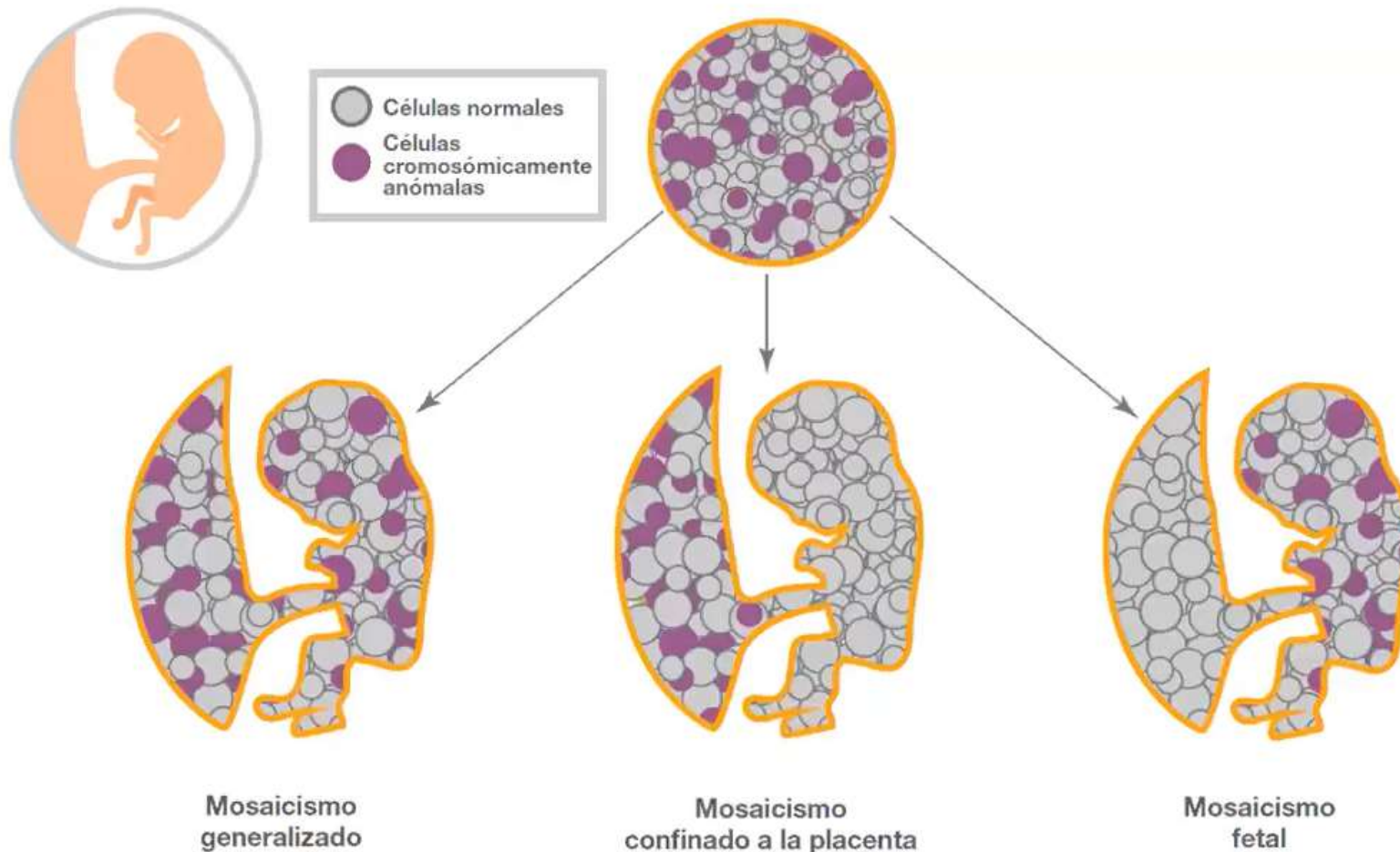
1. Cariotipo convencional en muestra de líquido amniótico
2. Repetir DNA fetal con nueva muestra
3. Segunda AMCT
4. arrayCGH



47,XY,+18[96]/46,XY[4]

¿POR QUÉ HUBO DISCORDANCIA ENTRE DNA FETAL Y CARIOTIPO?

1. Mosaico generalizado
2. Mosaico confinado a placenta
3. Mosaico fetal
4. 1 y 3 son correctas



CONCLUSIONES



- NIPT es un estudio de screening no invasivo válido para pesquisa de anomalías congénitas
- Su uso se está haciendo más frecuente y el conocimiento e interpretación es y será fundamental para el manejo y orientación de pacientes
- Su uso podría disminuir la cantidad de procedimientos invasivos
- Su tasa de detección, S, E, VVP, VPN en otras anomalías congénitas podría ir en aumento conforme vayan aumentando los estudios de éstos

CERPO

Centro de Referencia Perinatal Oriente

Facultad de Medicina, Universidad de Chile



EXÁMENES GENÉTICOS I: DNA libre fetal, técnica y rendimiento prenatal

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Results

V4.2n.1a.4a

Location	Disease	Result	Location	Disease	Result
1p36	1p36 deletion syndrome	High Risk	11q23	Jacobsen syndrome	Low Risk
2q33.1	2q33.1 deletion syndrome	Low Risk	15q11.2-q13	Prader-willi / Angelman syndrome	Low Risk
4p16.3	Wolf-Hirschhorn syndrome	Low Risk	22q11.2	DiGeorge syndrome	Low Risk
5p-	Cri Du Chat syndrome	Low Risk	Others	Other syndrome sites	Low Risk
7q11.23	Williams-Beuren syndrome	Low Risk			



Interpretation

A deletion of approximately 6 Mb in size was detected on chromosome 1 ([GRCh37] 1p36 (1,510,001-7,510,001)). To a definitive diagnosis, amniocentesis CMA (chromosomal microarray analysis) should be necessary.

