MaNaMa Clinical Genetics

Genome diagnosis

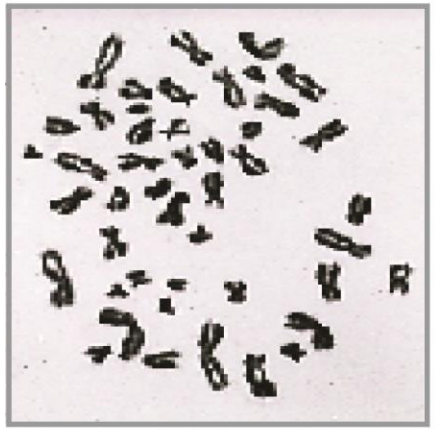
Annelies Dheedene
Sofie Symoens

12 October 2022

Cytogenomic technologies for acquired and constitutional disorders

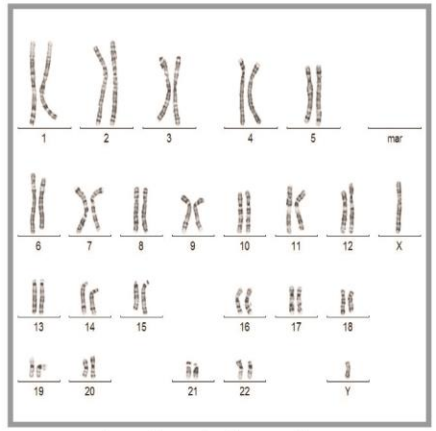
- Karyotyping
- FISH
- Microarray and shallow whole genome sequencing
- QF-PCR
- NIPT

prebanding cytogenetics



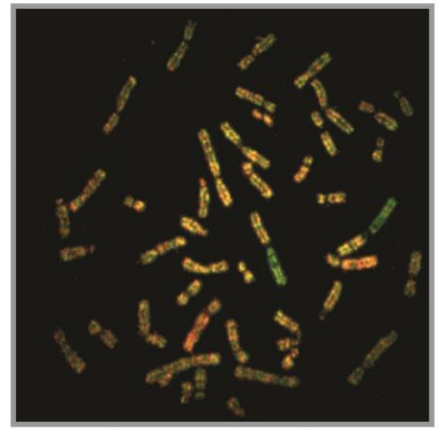
numerical aberrations

high-resolution banding



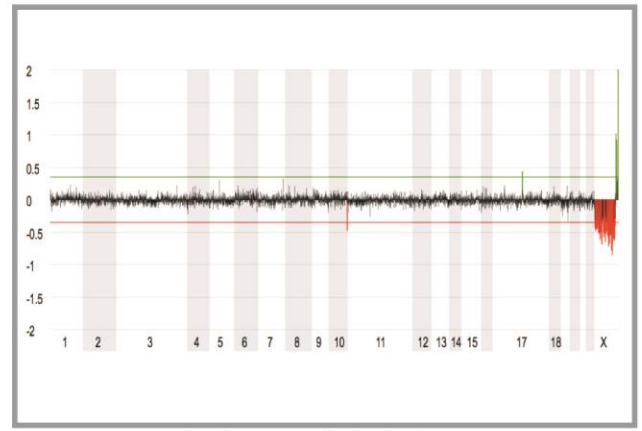
structural aberrations
(3 to 5 Mb)

CGH



structural aberrations
(2 to 10 Mb)

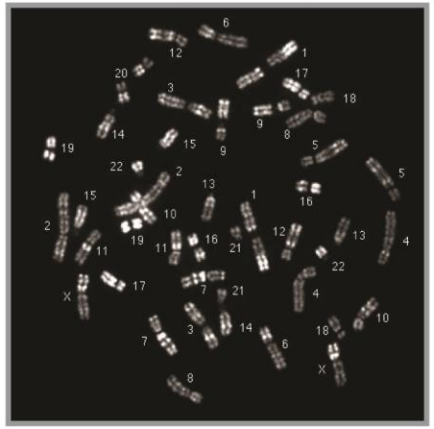
array CGH



submicroscopic imbalances
(50 to 500 kb)

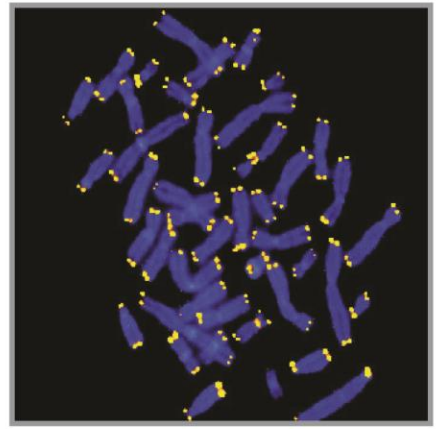
1956 1968 1976 1982 1992 1996 1997 2005

banding techniques



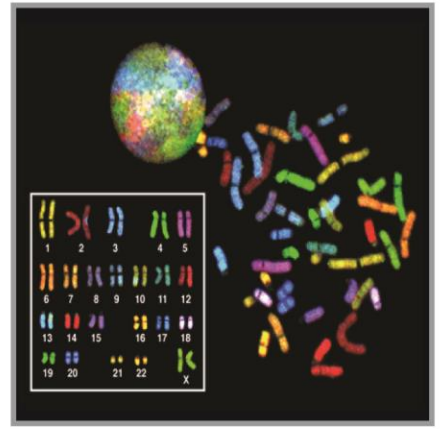
structural aberrations
(5 to 10 Mb)

FISH



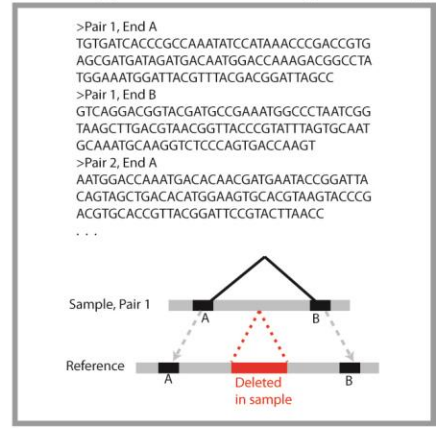
interstitial and (sub)telomeric
imbalances

M-FISH and SKY



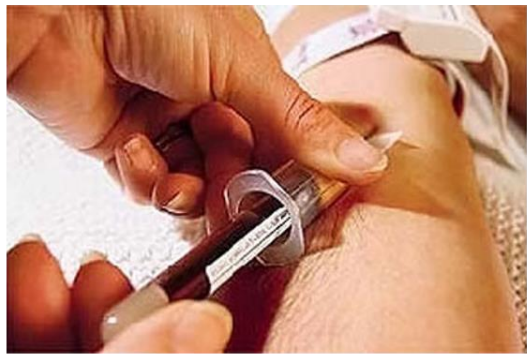
complex rearrangements and
supernumary marker chromosmes

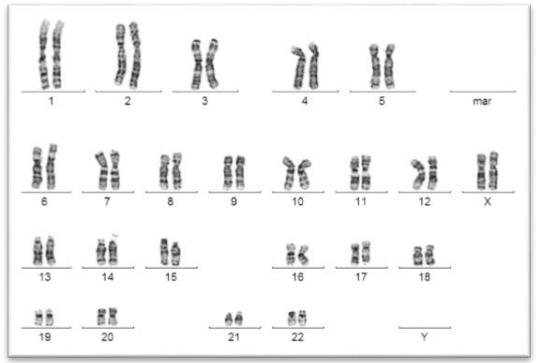
next-generation sequencing



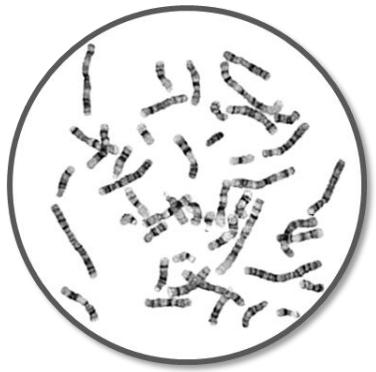
structural variation (>1 kb) and
nucleotide changes

Karyotyping: lymphocytes



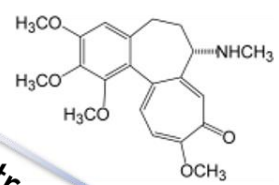


karyotyping



PHA
fytohemagglutinine

culture medium



methotrexate +
thymidine
colchicine + hypotonia
fixative

Giemsa staining

Karyotyping

Constitutional

- Blood
- Fibroblasts
- Amniocytes
- CVS
- EBV celline
- (miscarriage)

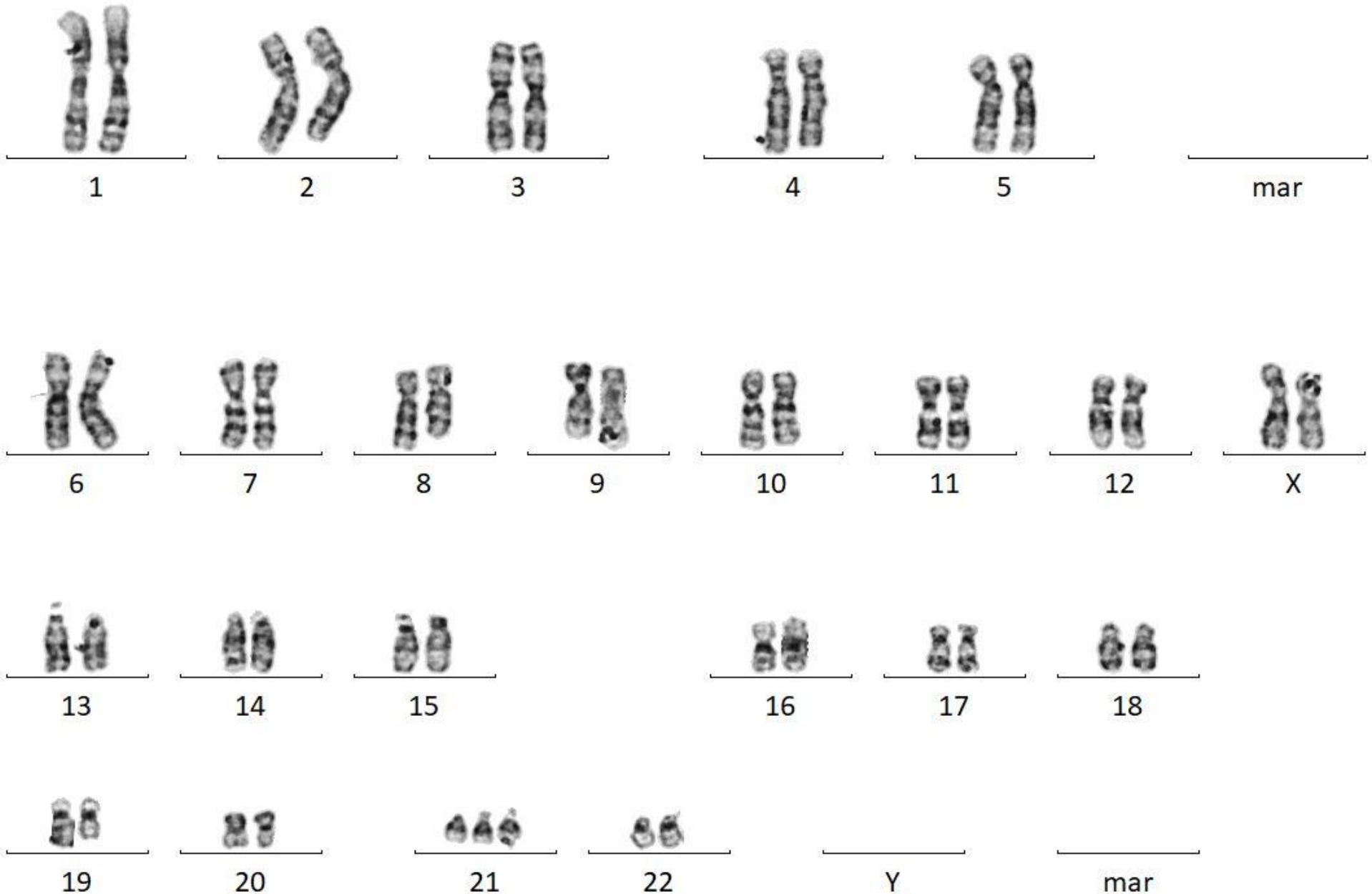
Acquired

- Bone marrow
- (maligne) blood

prophase → pro-metaphase → metaphase

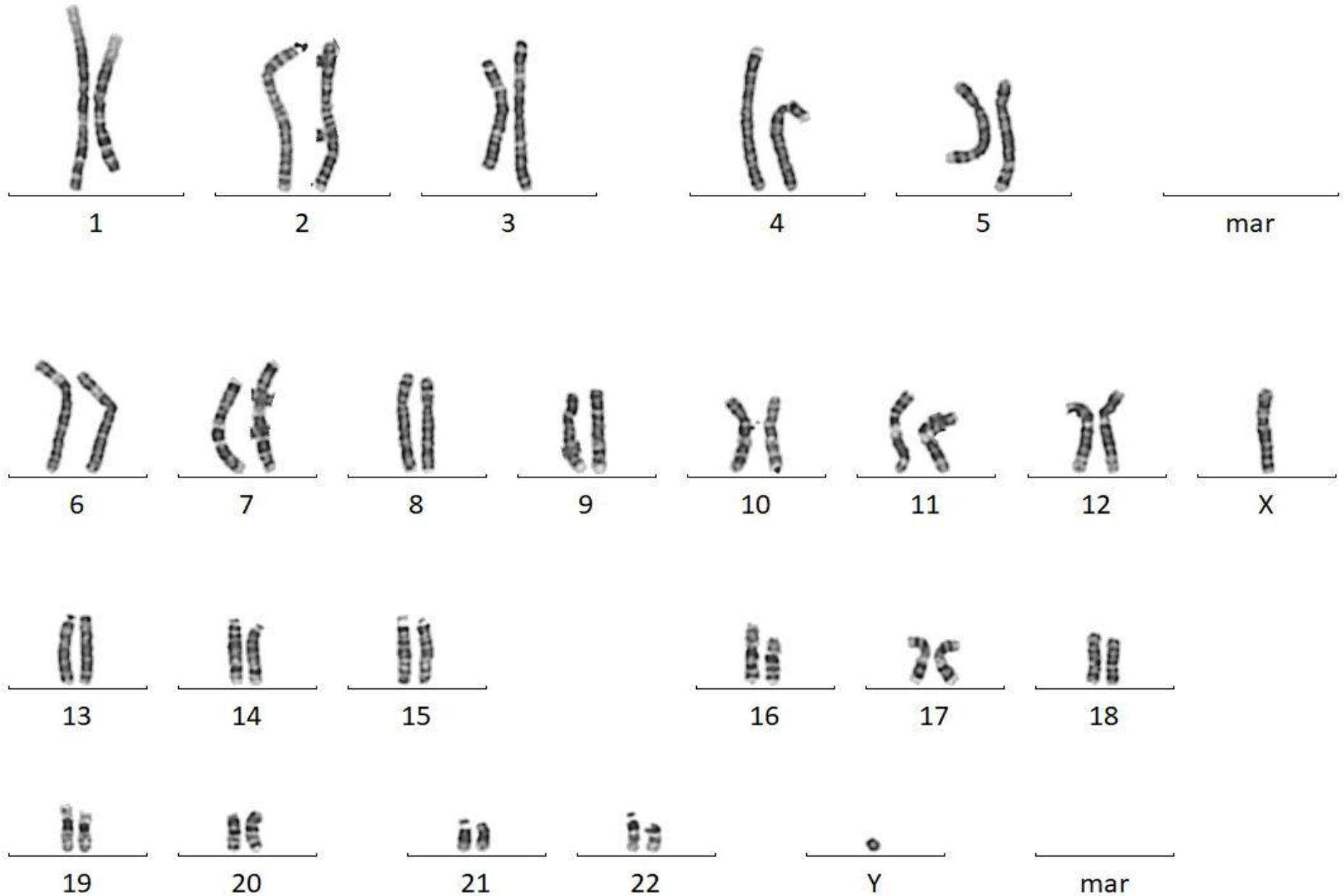
800 bands → 400 bands

G-bands per haploid karyotype

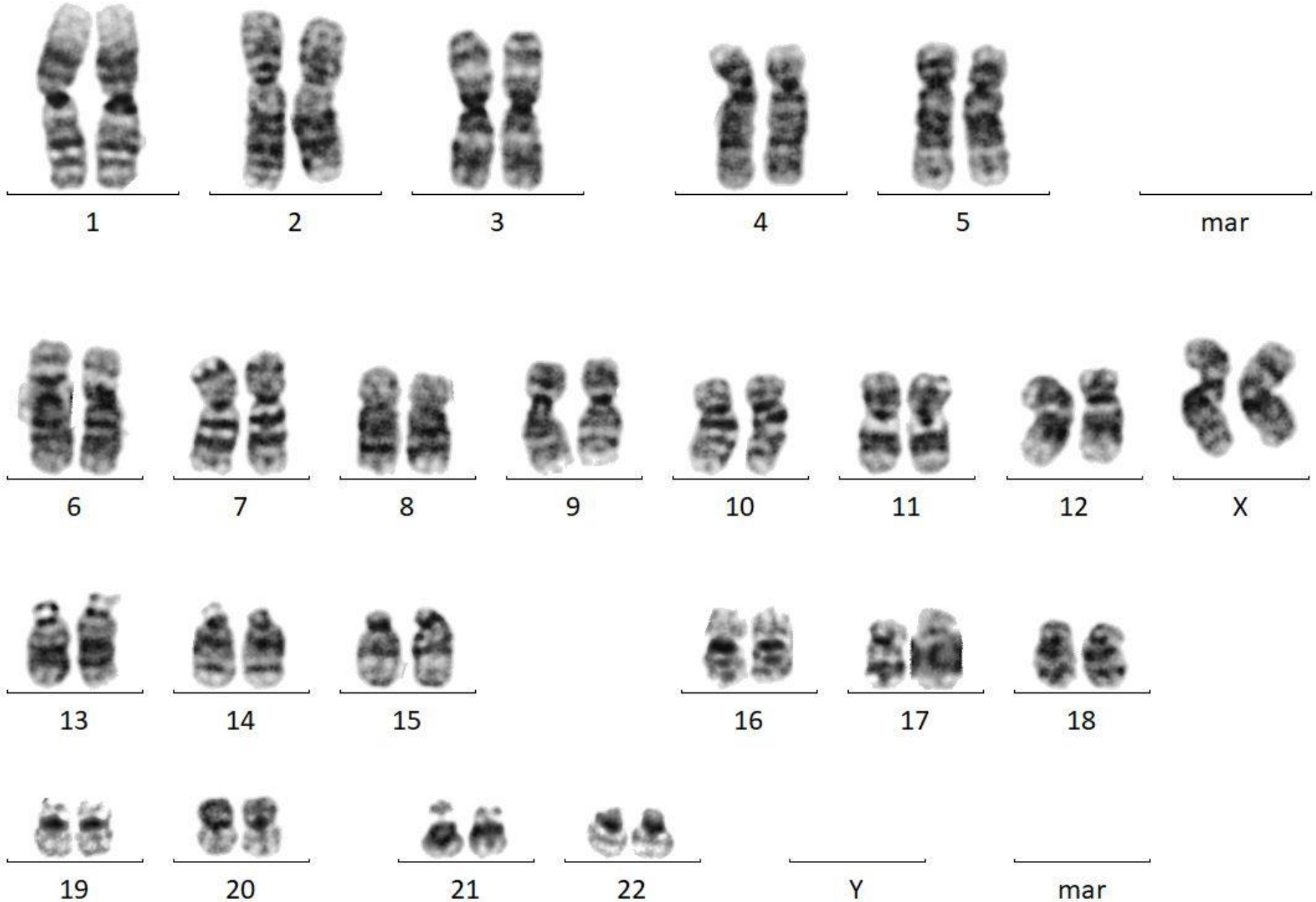


Constitutional blood – short term culture

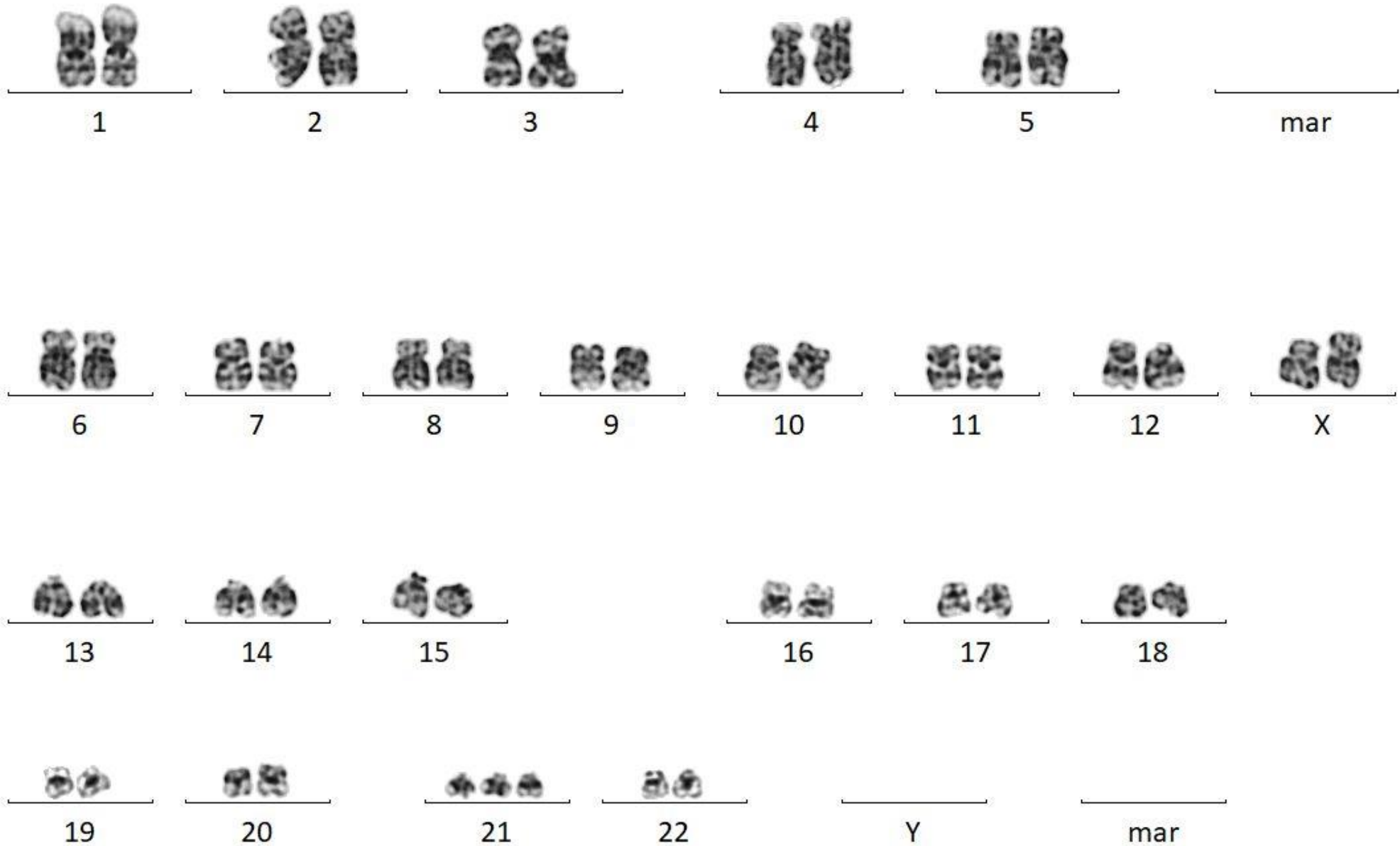
46,X,i(Y)(p10)



Constitutional blood



Bone marrow



Prenatal amniocytes

Karyotyping

Resolution is dependent on tissue type and culture conditions

Interpretation by skilled personnel

Many manual laboratory steps

General Guidelines and Quality Assurance for Cytogenetics

A common European framework for quality assessment for constitutional, acquired and molecular cytogenetic investigations.

E.C.A. Permanent Working Group for Cytogenetics and Society

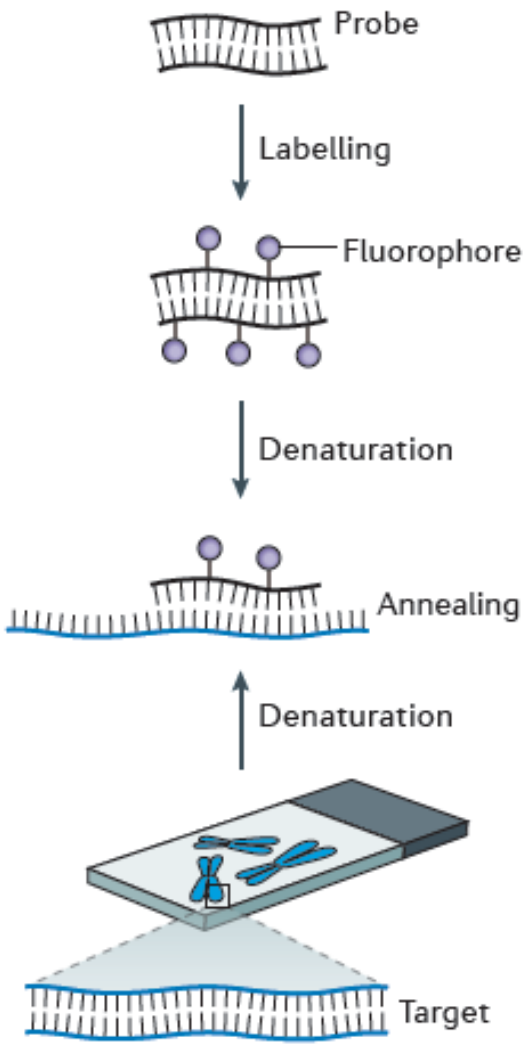
Table 5

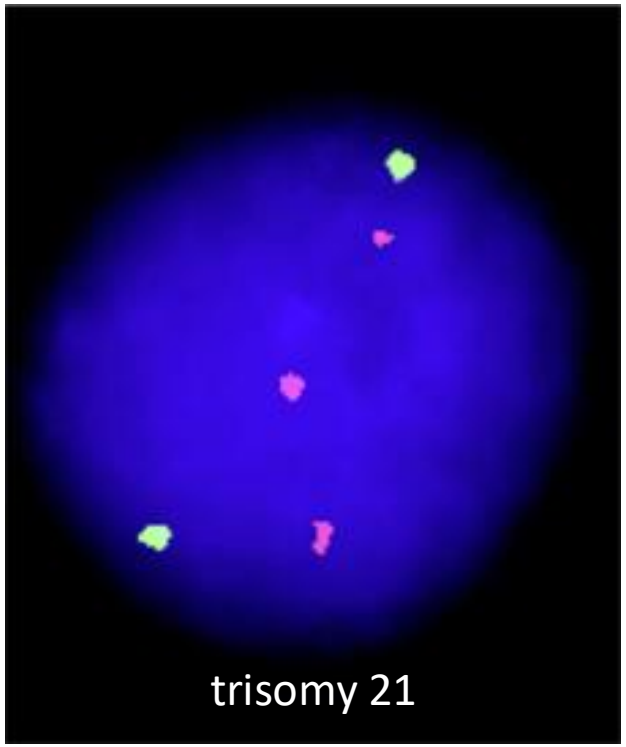
Amniotic fluid and long term CVS cultures	17 days
Lymphocytes cultures	28 days
Bone marrows and solid tumour cultures	21 days
Solid tissue culture	28 days
Short term CVS cultures (directs)	7 days
Urgent* lymphocyte, cord blood cultures	7 days
Urgent* bone marrows cultures (diagnostic samples)	7 days
Prenatal aneuploidy FISH screening/QF-PCR	4 days

ISCN: examples of abnormal karyotypes

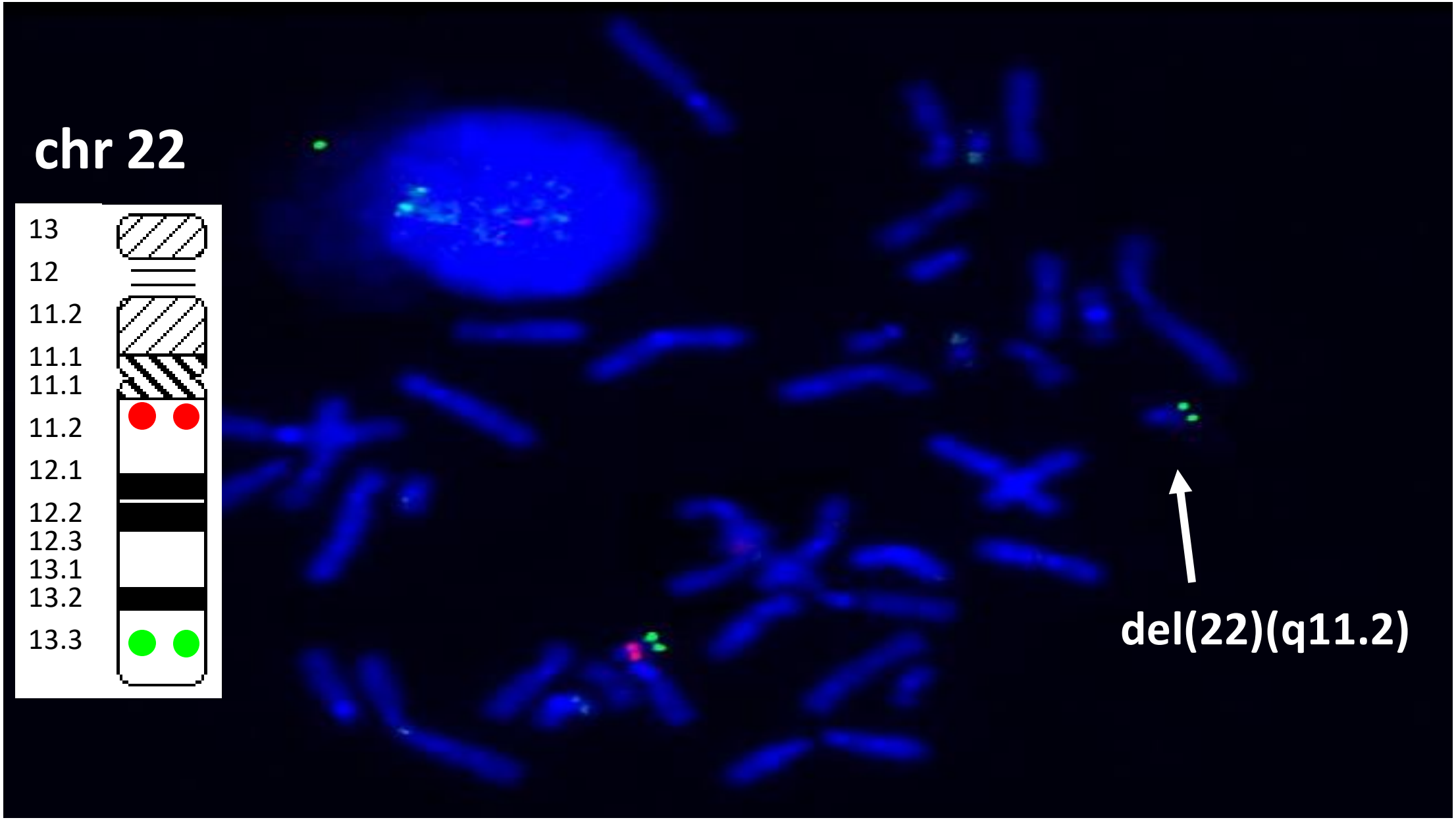
- 47,XY,+21
- 45,XX,der(13;21)(q10;q10)
- 46,XY,der(13;21)(q10;q10),+21
- 45,X
- 46,XY,t(6;9)(q24;p23)
- 46,XY,der(6)t(6;9)(q24;p23)dmat
- 46,XY,del(6)(q24q31)
- 46,XY,inv(7)(p14q25)
- 46,XY,inv(17)(q12q22)
- 47,XXY,del(22)(q11.2)
- 46,XX,del(9)(ptelp23)

Fluorescent in-situ hybridization (FISH)

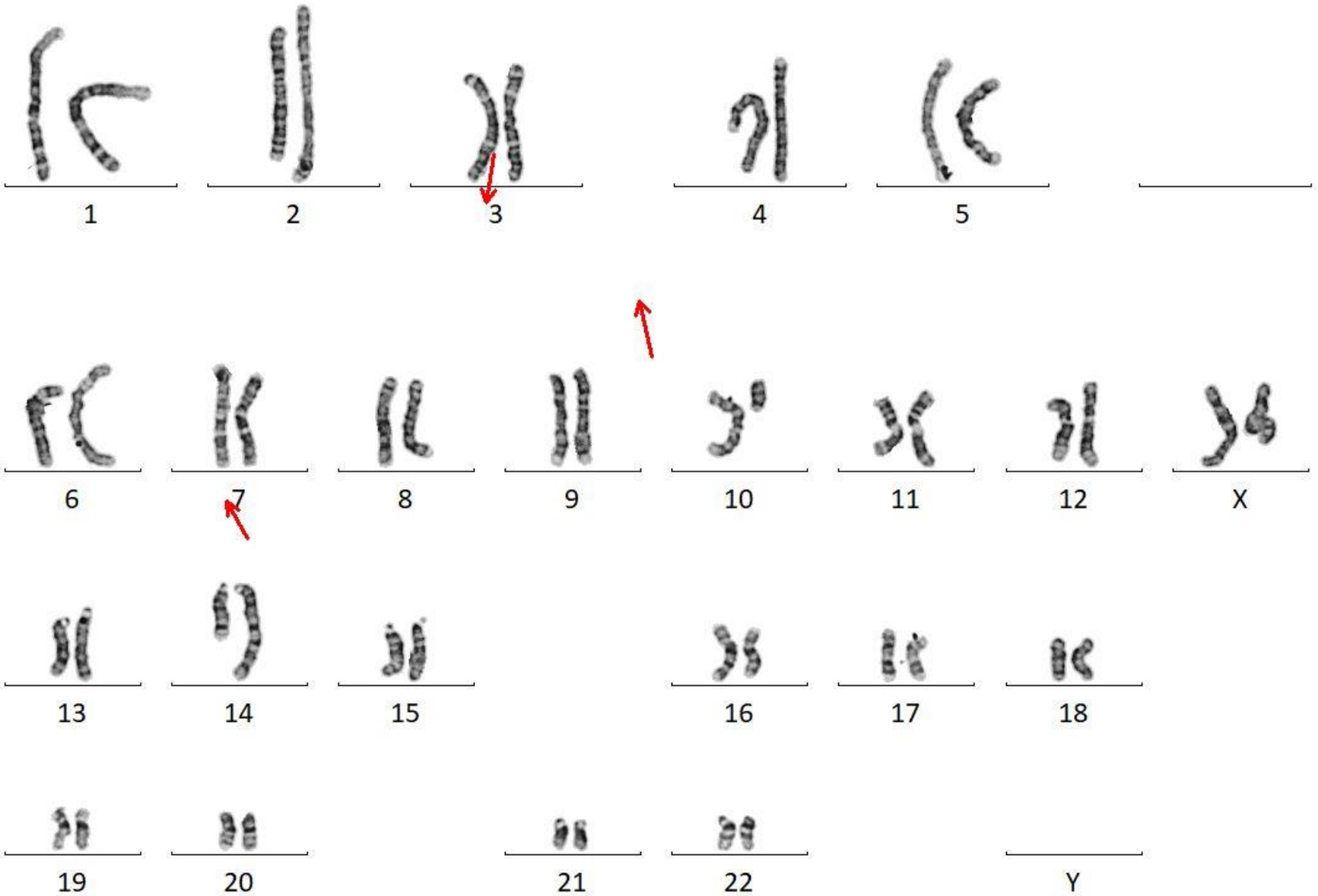




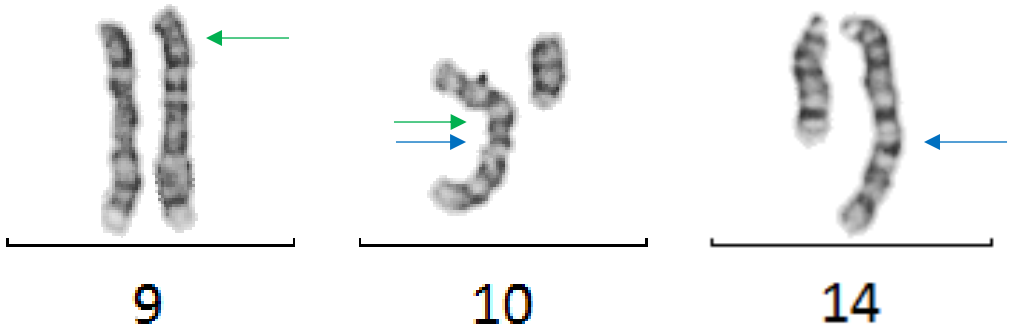
Vermeesch, Nat Gen Rev, 2016


chr 22
13
12
11.2
11.1
11.1
11.2
12.1
12.2
12.3
13.1
13.2
13.3
del(22)(q11.2)

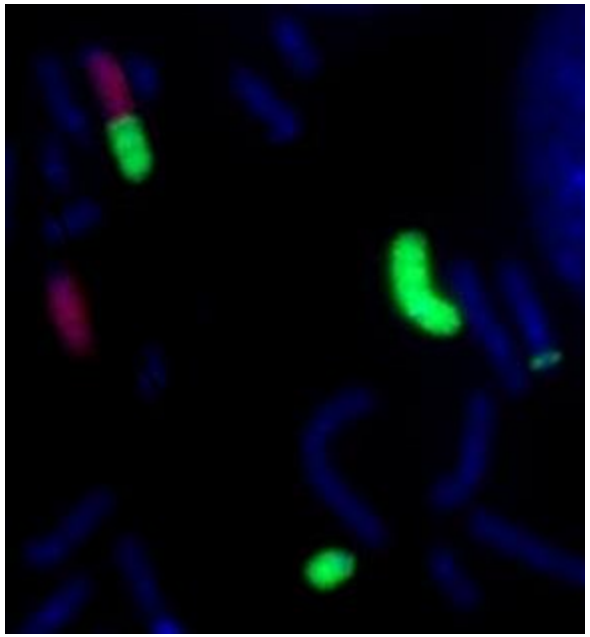
Indication: implantation failure



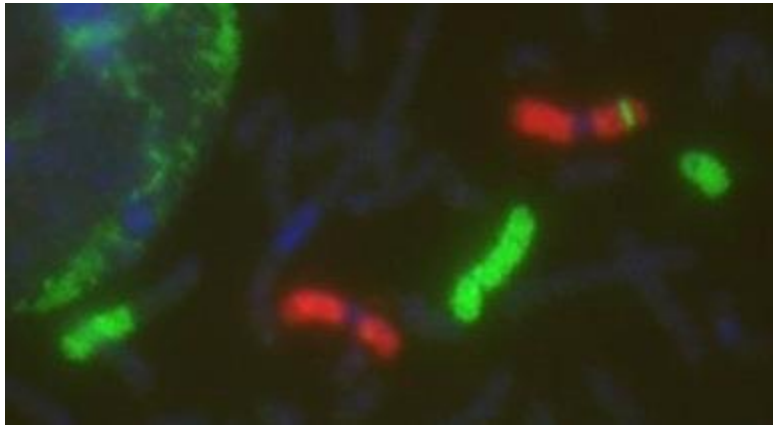
L1801919 (V)



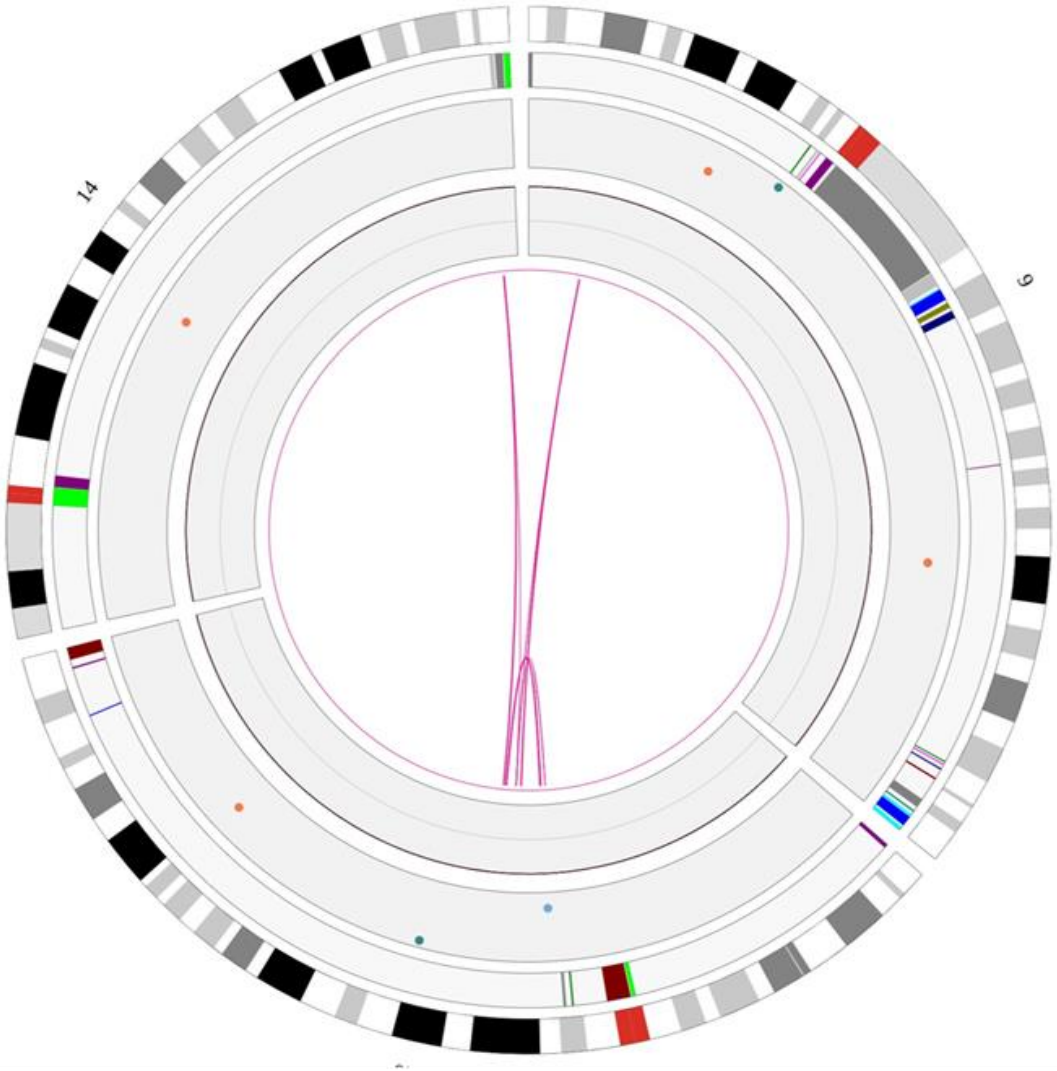
chr10 = green; chr 14 = red



FISH: chr10 = green; chr9 = red



46,XX,ins(9;10)(p22;q11.2q21.2),t(10;14)(q?;q32.3)



Filter Criteria

BED SV OverlapPrecision(Kbp):	12
BED CNV OverlapPrecision(Kbp):	500
SV MaskingFilter:	nonmasked
CopyNumberType:	all
CopyNumberConfidence:	0.99
CopyNumberMin Size (bp):	500000
Self MoleculeCount:	0
% in Control:	0
% in ControlforEnzyme:	0
SV ChimericScore:	all
Foundin Self Molecules:	all
OverlapGenes:	all

SV Filter	Confidence	Min Size (bp)
Insertion	-1	
Deletion	-1	
Inversion	-1	
Duplication	-1	
Intra-Translocation	-1	
Inter-Translocation	-1	

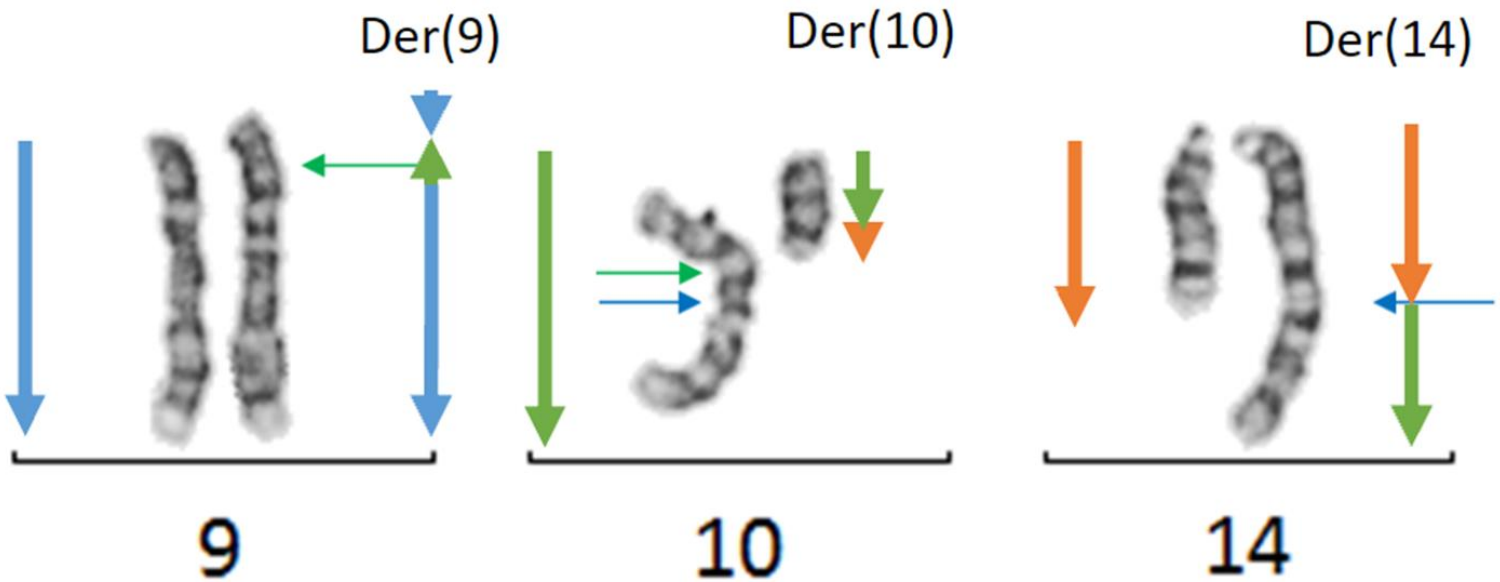
BED File	SV	CNV	Action
hg38cnvmasks			

AddBED

Samples

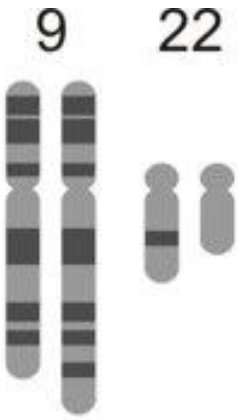
Reference: hg38_DLE1_0kb_0labe

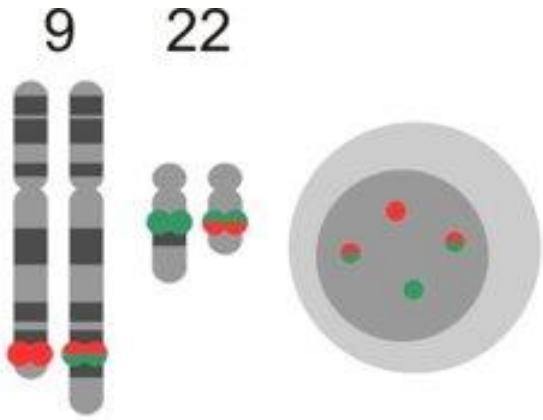
18.22565_denovo_assembly_sv	Count
Insertion	2
Deletion	6
Inversion	1
Duplication	0
Intra-Translocation	5
Inter-Translocation	6
CNV Gain Segment	0
CNV Loss Segment	0

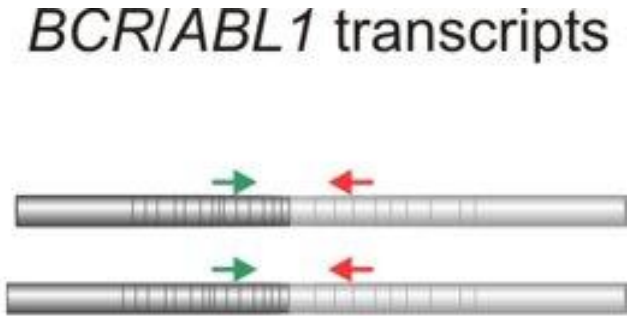


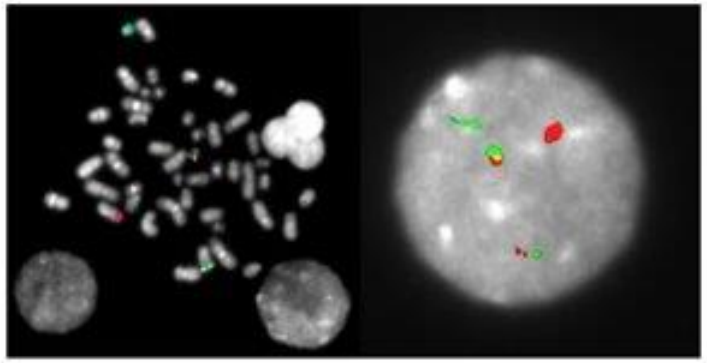
FISH Philadelphia chromosome

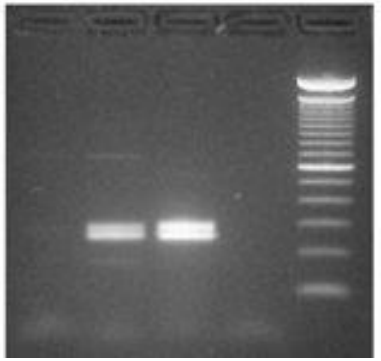
Dual Fusion Translocation Probe



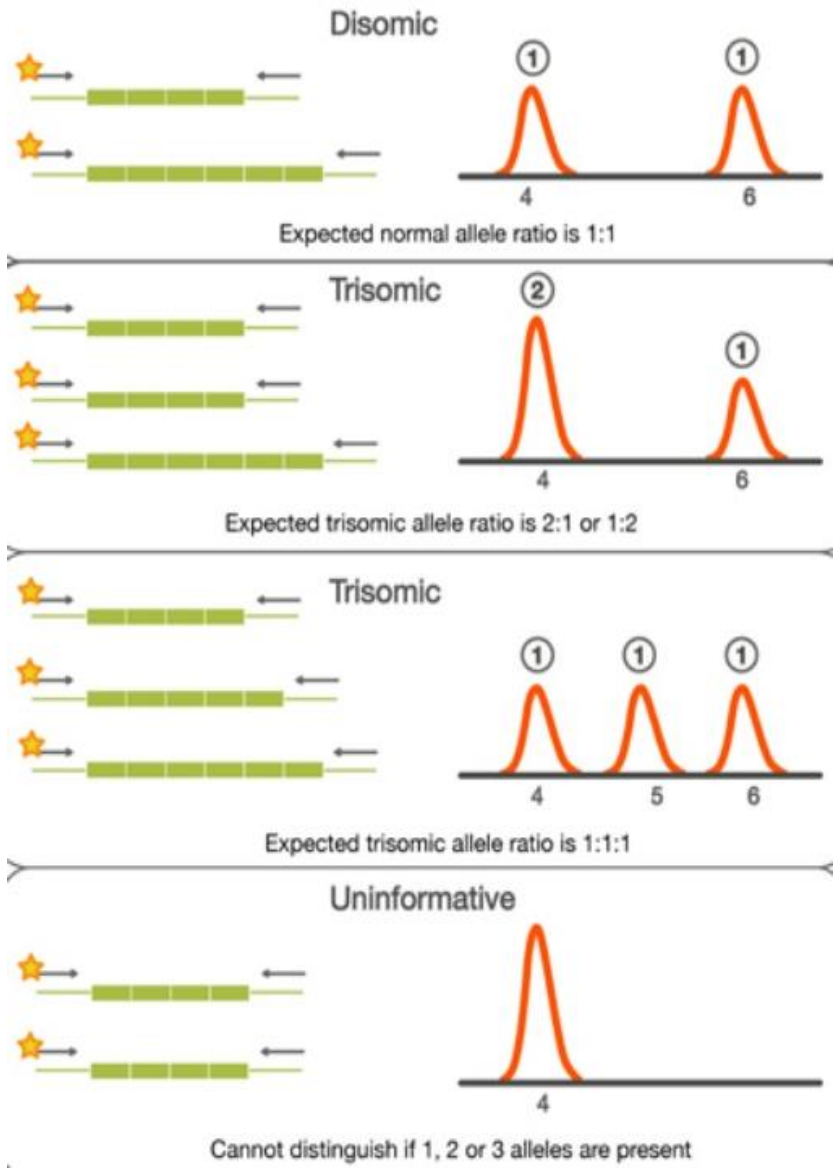








QF-PCR

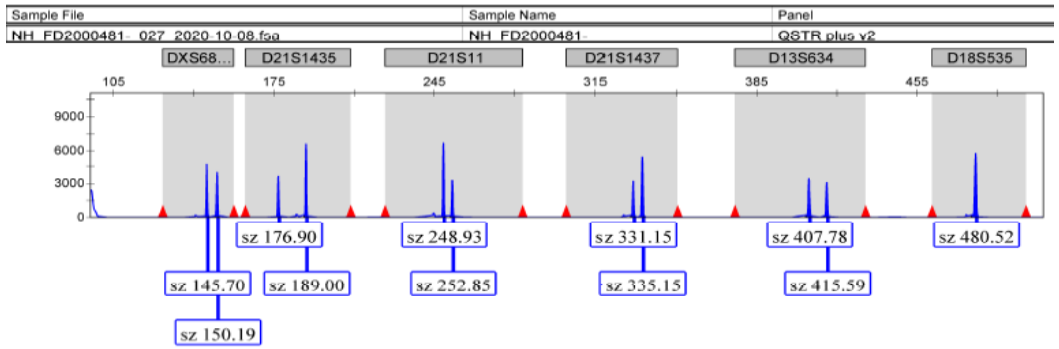


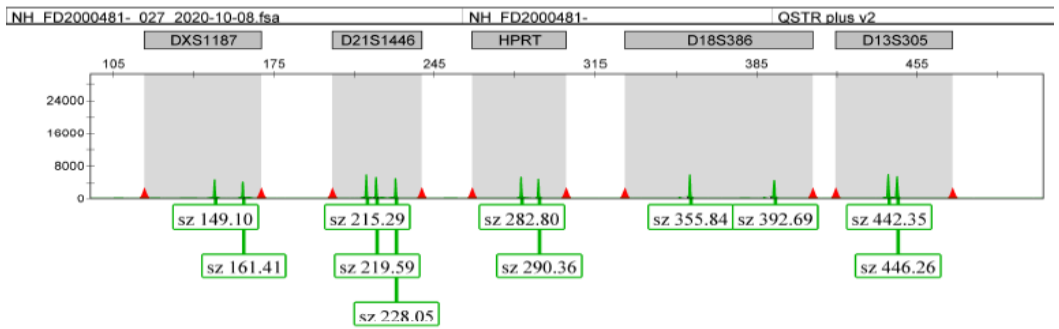
- Fast, multiplex PCR
- Aneuploidy detection for chromosome 13, 18, 21, X and Y
- Maternal contamination
- (Low) mosaicism not detectable
- Structural rearrangements not detectable
- Excercise: diagnosis?
  - Tip: marker D13Sxxx = marker from chr 13, D18Sxxx = marker from chr 18 etc.

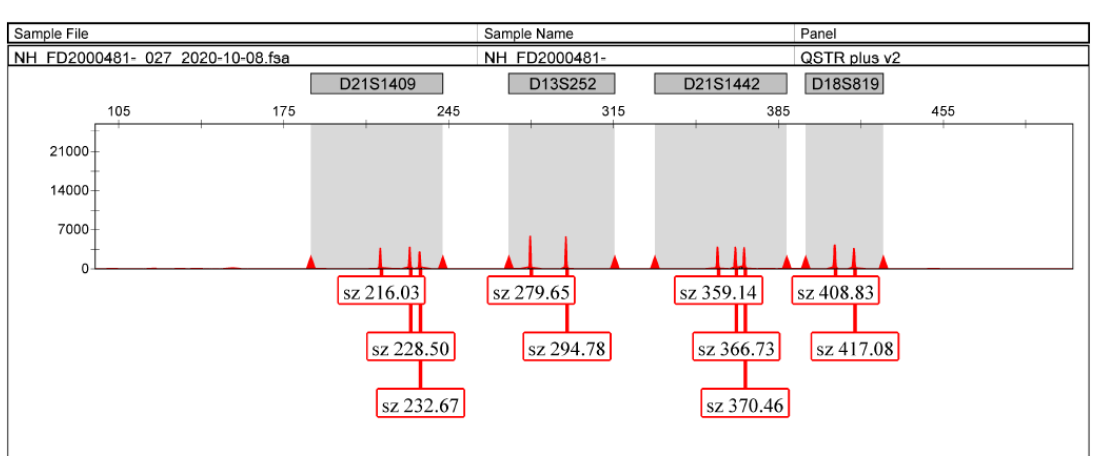
Applied Biosystems

neMapper Software 6

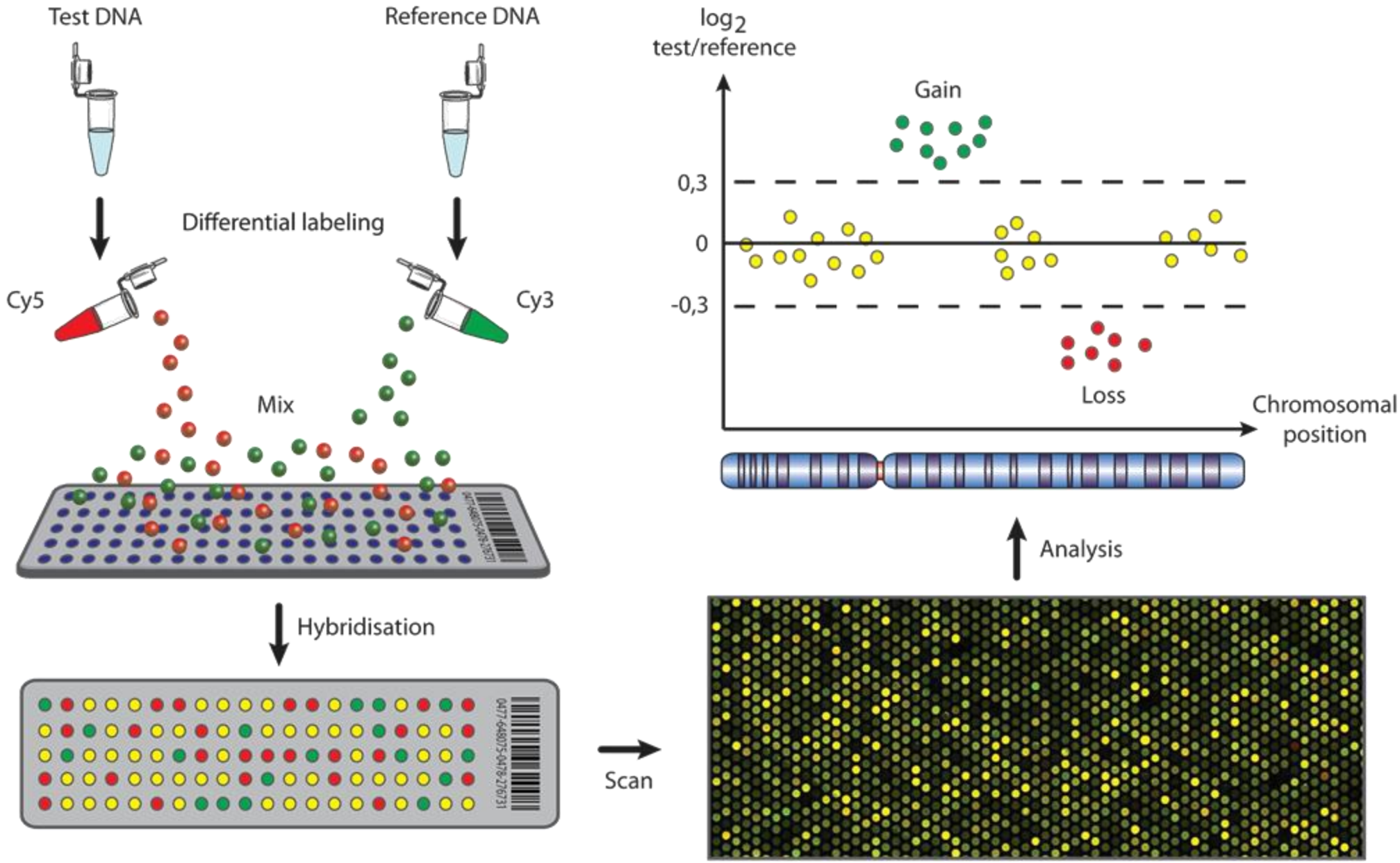
frag_prenatal_20201008



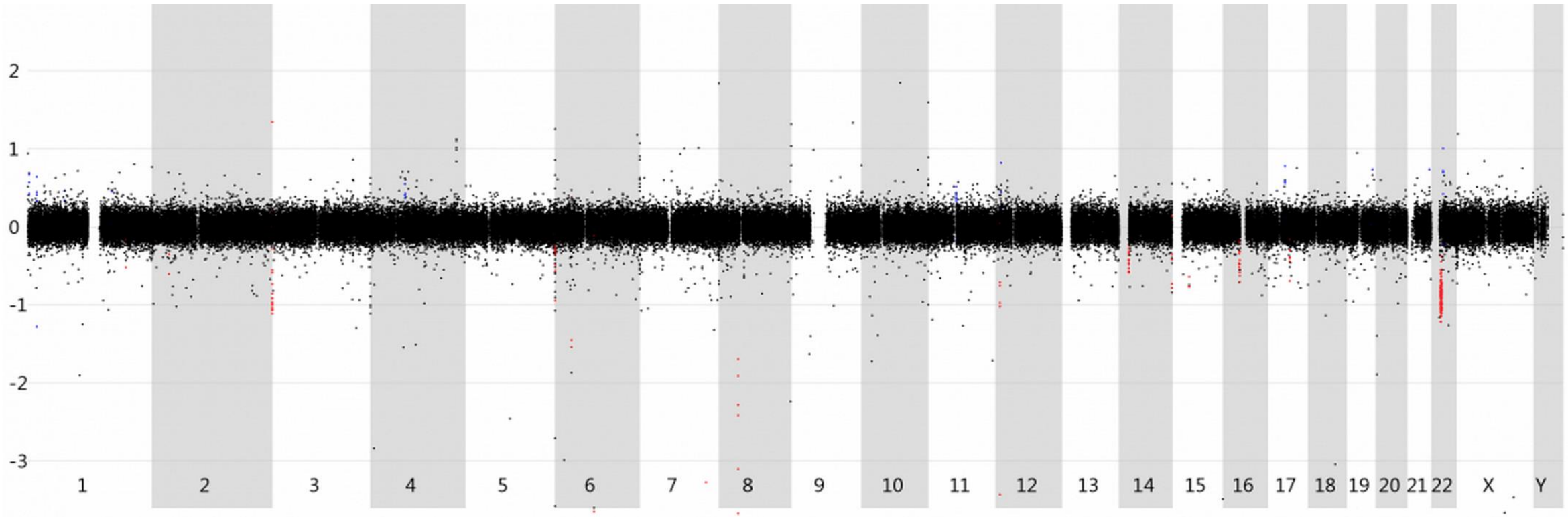




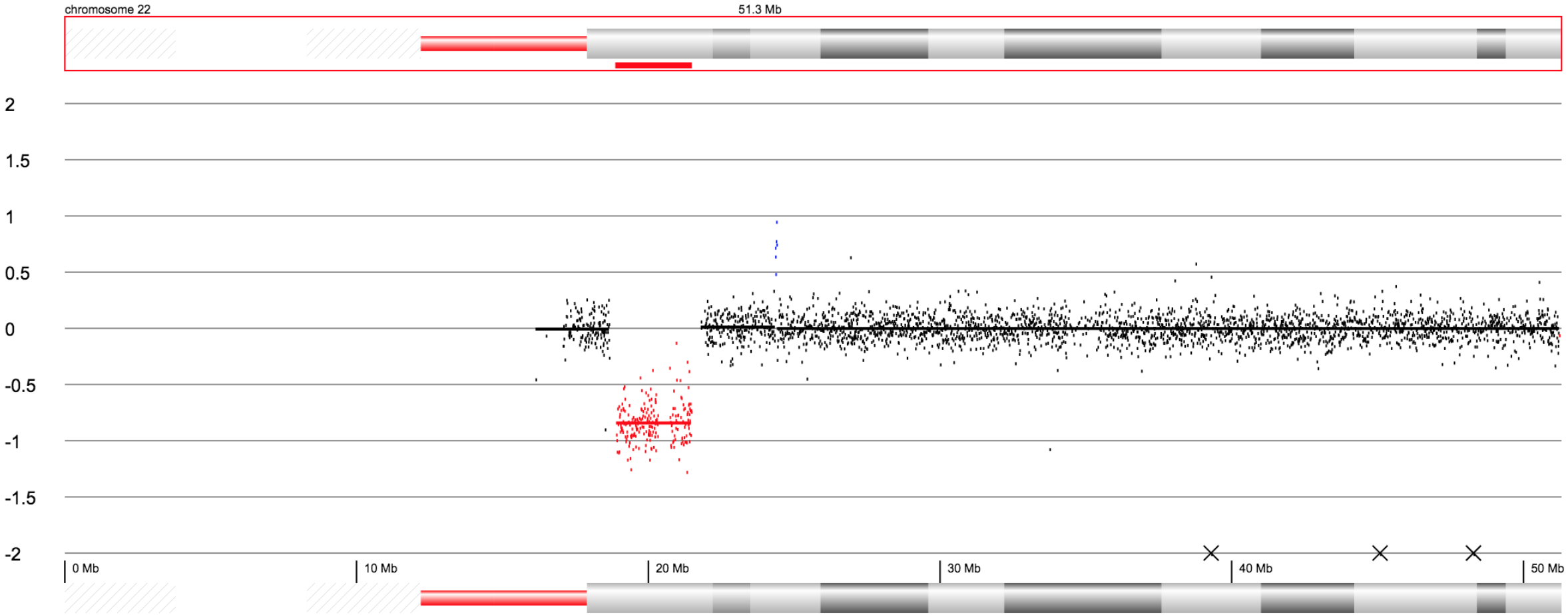
Array Comparative Genomic Hybridization (aCGH)



arr[GRCh37] 22q11.21(18915001_21465000)x1



chr 22


chromosome 22
51.3 Mb
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
0 Mb
10 Mb
20 Mb
30 Mb
40 Mb
50 Mb

Prenatal genomic microarray

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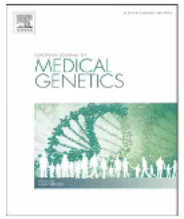
journal homepage: http://www.elsevier.com/locate/ejmg



Review

Implementation of genomic arrays in prenatal diagnosis: The Belgian approach to meet the challenges

Olivier Vanakker[a], Catheline Vilain[d], Katrien Janssens[b], Nathalie Van der Aa[b], Guillaume Smits[d], Claude Bandelier[h], Bettina Blaumeiser[b], Saskia Bulk[g], Jean-Hubert Caberg[g], Anne De Leener[d], Marjan De Rademaeker[c], Thomy de Ravel[f], Julie Desir[e], Anne Destree[e], Annelies Dheedene[a], Stéphane Gaillez[g], Bernard Grisart[e], Ann-Cécile Hellin[g], Sandra Janssens[a], Kathelijn Keymolen[c], Björn Menten[a], Bruno Pichon[d], Marie Ravoet[h], Nicole Revencu[h], Sonia Rombout[e], Catherine Staessens[c], Ann Van Den Bogaert[c], Kris Van Den Bogaert[f], Joris R. Vermeesch[f], Frank Kooy[b], Yves Sznajer[h], Koen Devriendt[f,*]

[a] Center for Medical Genetics, Universiteit Gent, Belgium
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[e] Center for Medical Genetics, IPG, Charleroi Gosselies, Belgium
[f] Center for Medical Genetics, Katholieke Universiteit Leuven, Belgium
[g] Center for Medical Genetics, Université de Liège, Belgium
[h] Center for Medical Genetics, Université Catholique de Louvain, Belgium

List susceptibility loci

https://www.college-genetics.be/

To report

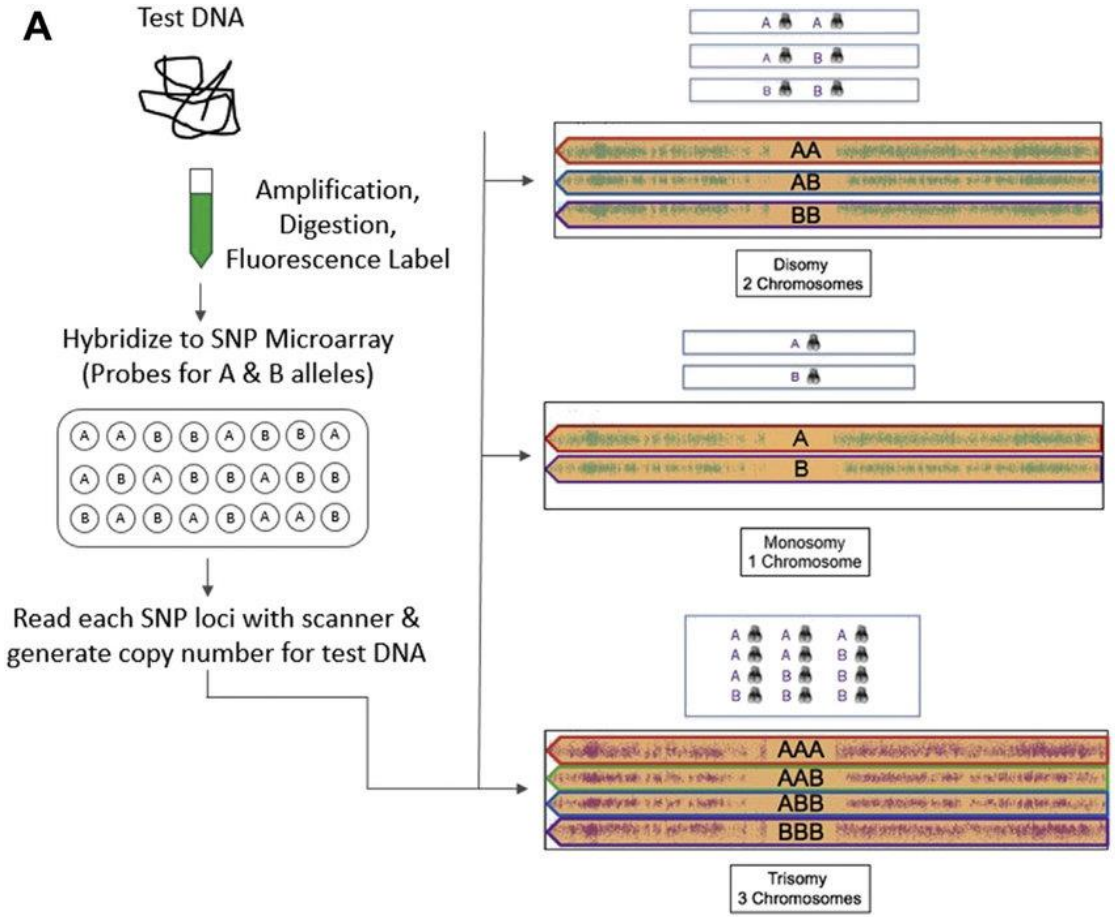
Not to report

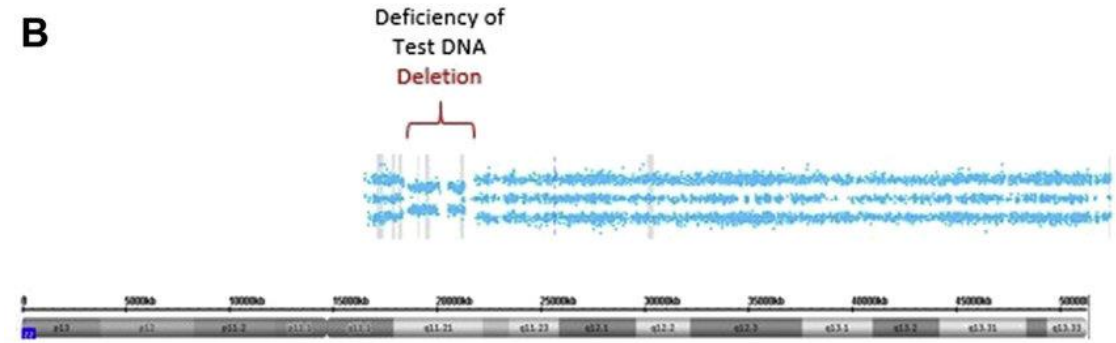
chr	start in Mb (hg19)	stop in Mb (hg 19)	size in kb	CNV	gene	phenotype	morph. anomaly	return?	OMIM	update May 2017
1	146,57	147,39	820	distal 1q21.1 dup	*GJA5 (CX40)*	ID, DD, ASD, schizophrenia	macrocephaly, CHD	YES	612475	YES
1	146,57	147,39	820	distal 1q21.1 del	*GJA5 (CX40)*	ID, DD, ASD, SZ, facial dysmorphism	microcephaly, CHD, renal and urinary tract anomalies	YES	612474	YES
1	171,81	172,38(?)	57	1q24.3 del	*DNM3*	ID	IUGR, microcephaly, brachydactyly	YES		
15	31,13	32,48	1350	15q13.3 del	*CHRNA7*	DD, ID, ASD, epilepsy, SZ	microcephaly, CHD	YES	612001	YES
15	99,36	102,52	3160	15q26 del	*IGF1R*	MR	IUGR	YES		YES
16	28,74	28,96	220	16p11.2 distal del	*SH2B1*	obesity, DD, ID, SZ	none	YES	613444	YES
16	29,59	30,19	600	16p11.2 proximal dup	*TBX6*	ASD, ID, DD, SZ, anorexia	microcephaly	~~NO~~ YES	614671	moved to YES since actionable; penetrance del and dup comparable
16	29,59	30,19	600	16p11.2 proximal del	*TBX6*	ID, DD, ASD, obesity, SZ, speech delay	macrocephaly, vertebra	YES	611913	YES
17	34,82	36,21	1390	17q12 deletion syndrome RCAD (renal cysts & diabetes)	*TCF2*	facial dysmorphy, genital abnormalities, ID, DD, ASD, MODY	renal anomalies	YES	614527	YES
22	19,02	20,29	1270	22q11.2 dup	*TBX1*	ASD, ID, DD, dysmorphic features	microcephaly, CHD	YES	608363	YES
1	144,97	146,61	1640	1q21.1 dup	*HFE2*	DD, ASD	CHD	NO		NO
2	50	51,11	1110	2p16.3 del	*NRXN1*	ID, ASD, SZ, DD, dysmorphic features	none	NO	614332	NO
2	110,87	110,98	110	2q13 dup	*NPHP1*	ASD, ID	none	NO		NO
3	197,2	198,84	1600	3q29 dup		MR, DD	none	NO		NO
13	20,81	21,01	1200	13q12 dup	*CRYL1*	?	?	NO		NO
15	22,8	23,09	290	15q11.2 dup	*NIPA1*	DD, motor delay, speech delay, ASD	none	NO		NO (likely benign)
15	22,8	23,09	290	15q11.2 del	*NIPA1*	ID, DD, epilepsy	CHD	NO	615656	NO (likely benign)
15	31,13	32,48	1350	15q13.3 dup	*CHRNA7*	ADHD, ID, DD, ASD	none	NO		NO (likely benign)
16	14,98	16,48	1500	16p13.11 dup	*MYH11*	ID, ASD, SZ, ADHD	aorta dilatation	NO		NO
16	14,98	16,48	1500	16p13.11 del	*MYH11*	ID, DD, ASD, epilepsy	microcephaly	NO		NO
16	21,94	22,46	520	16p12.2 dup	*EEF2K, CDR2*	?	?	NO		NO (likely benign)
16	21,94	22,46	520	16p12.2 del	*EEF2K, CDR2*	DD, speech dealy	cranofacial and skeletal abnormalities, CHD	NO	136570	NO

Single Nucleotide Polymorphism array (SNParray)

SNP genotyping:

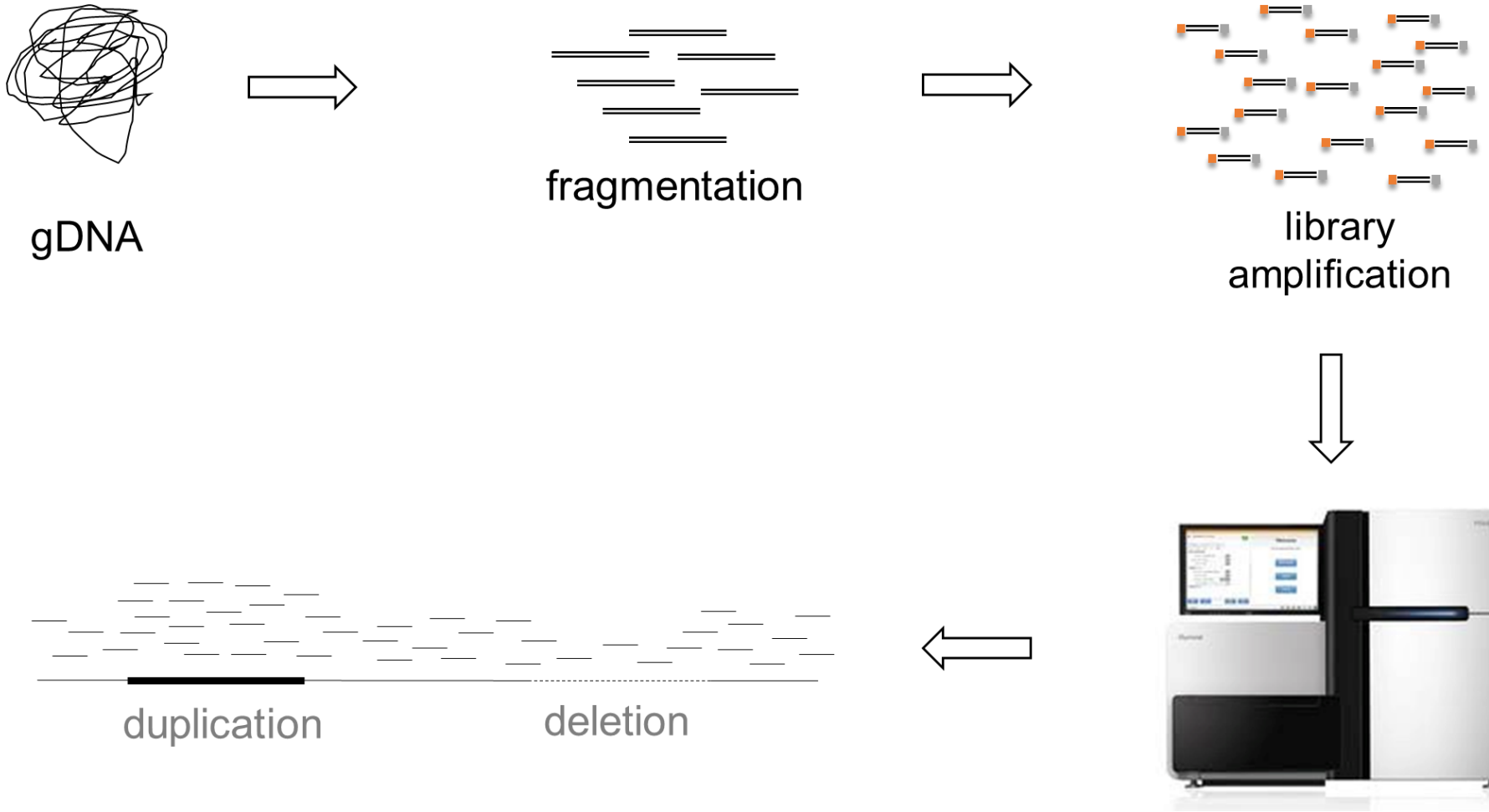
- UPD analysis
- homozygosity mapping

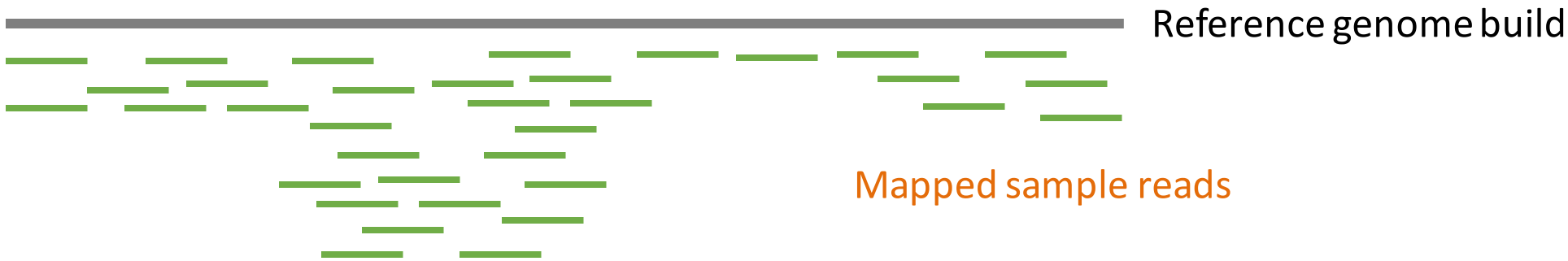


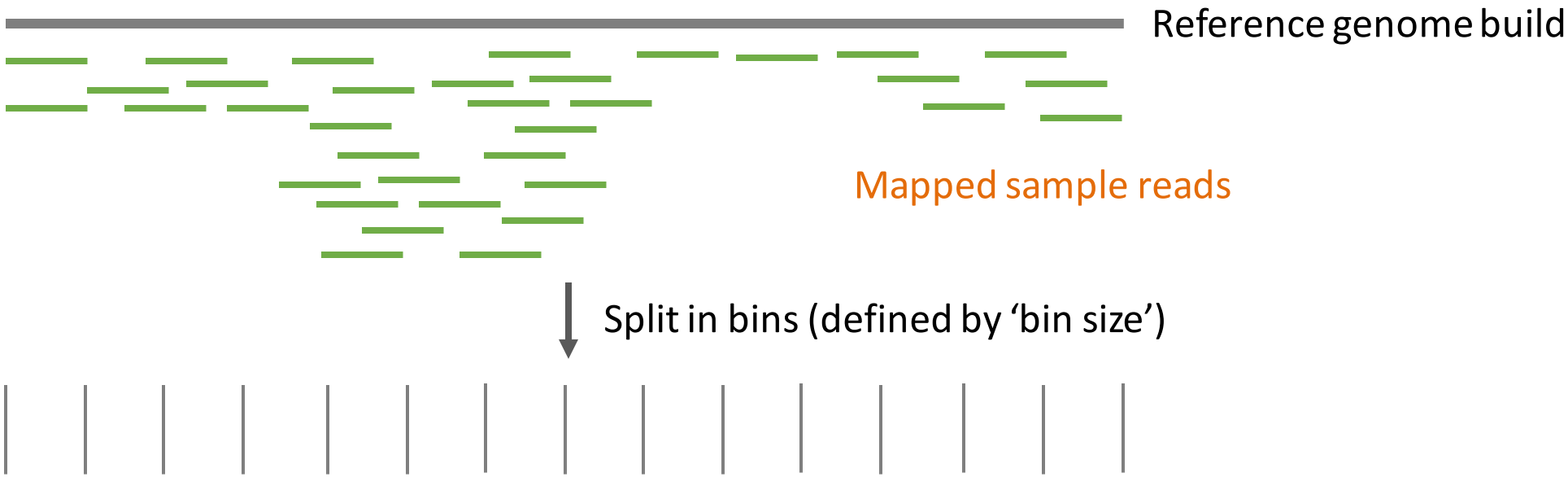


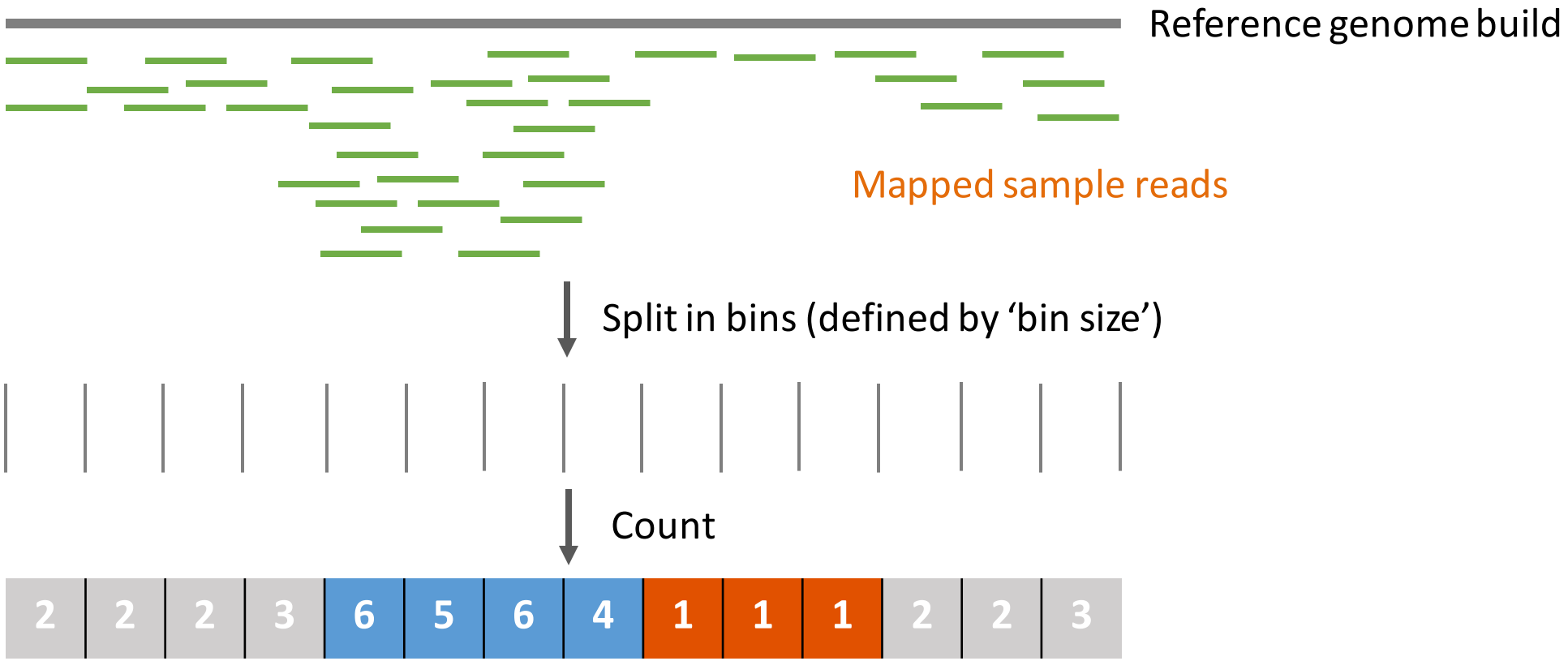
Stosic et al 2017

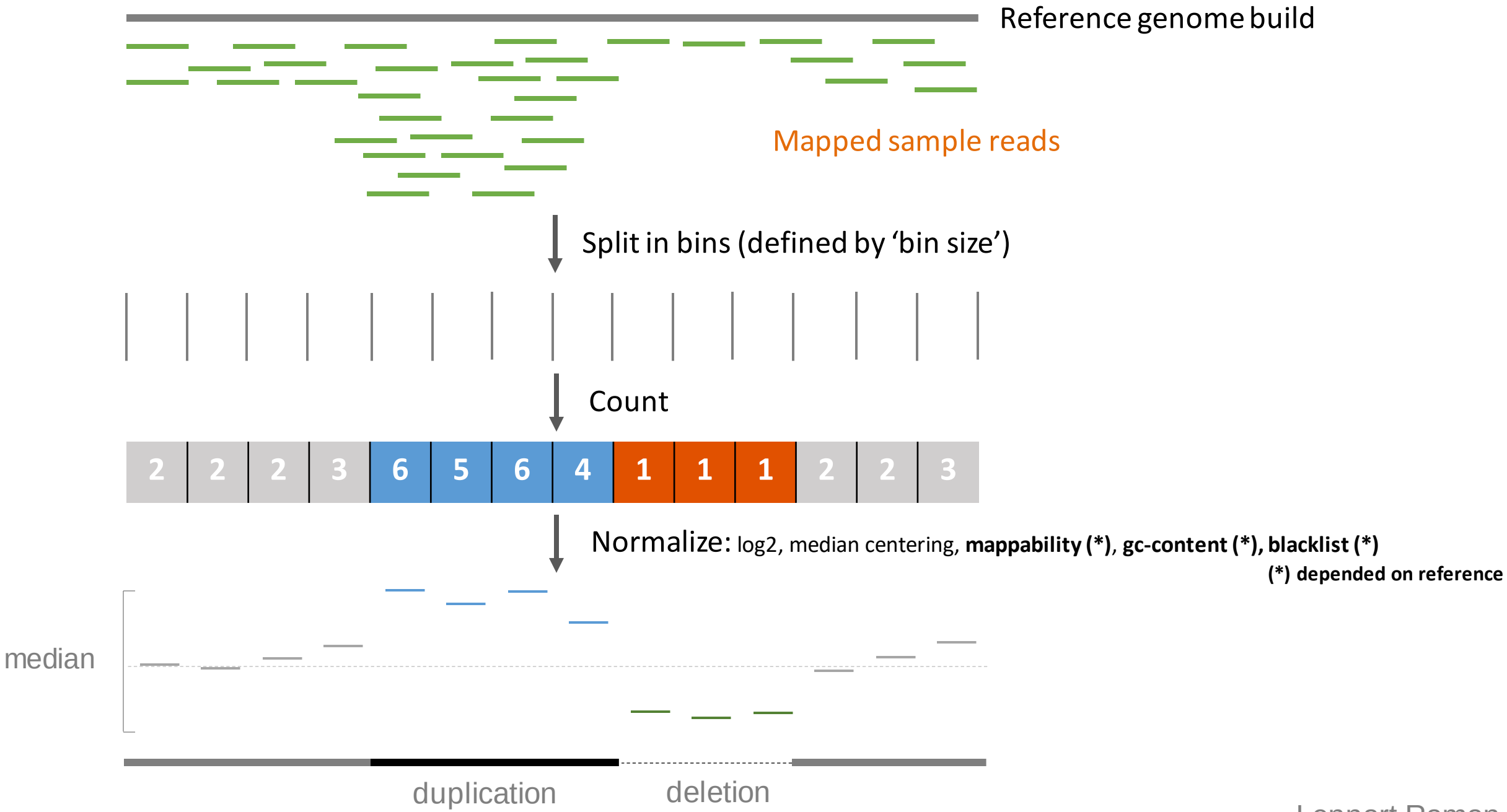
SHALLOW WHOLE GENOME SEQUENCING (0,1X - 10X COVERAGE)




Reference genome build
Mapped sample reads

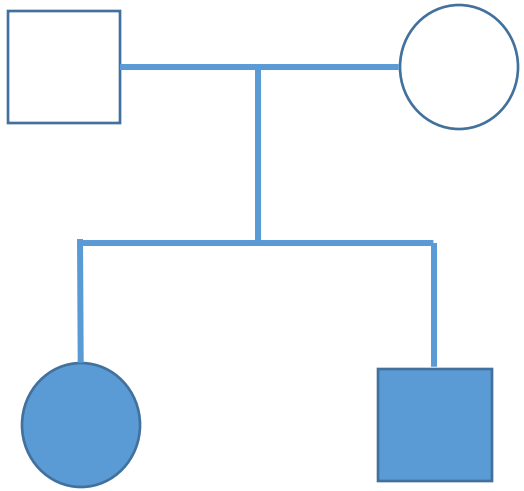

Reference genome build
Mapped sample reads
Split in bins (defined by ‘bin size’)


Reference genome build
Mapped sample reads
Split in bins (defined by ‘bin size’)
Count
2 2 2 3 6 5 6 4 1 1 1 2 2 3



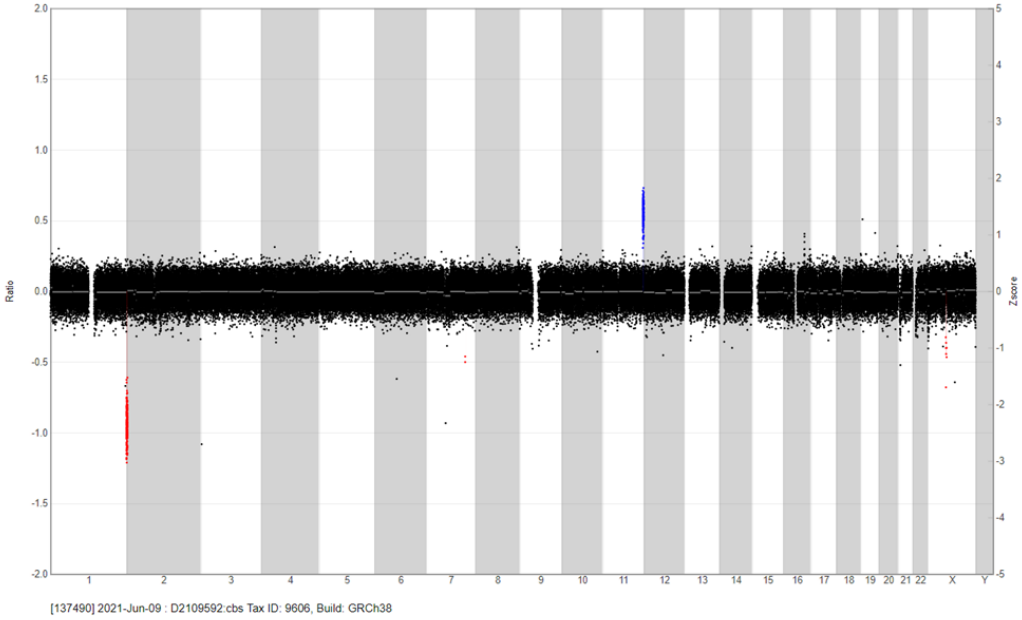
Lennart Raman

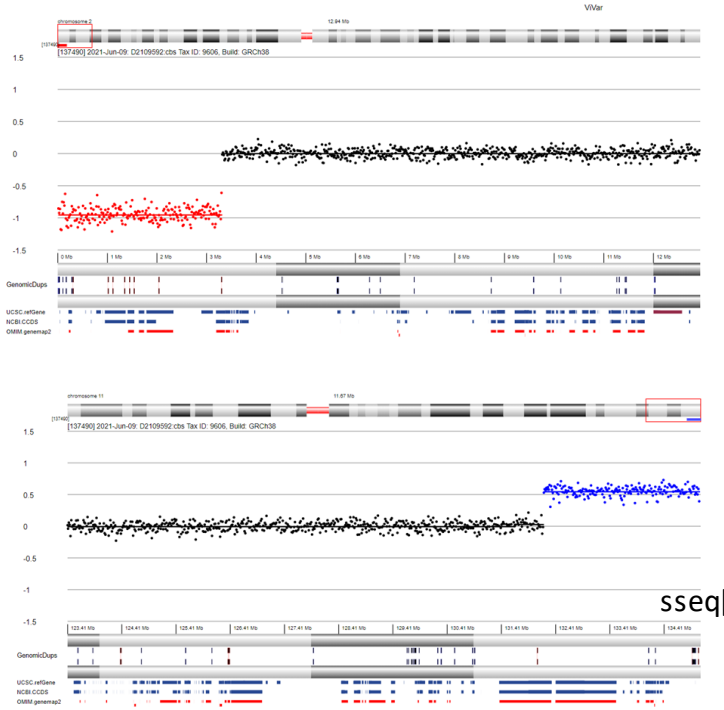
The cytogenomics toolbox: an example



° 2018
Developmental delay
Language delay

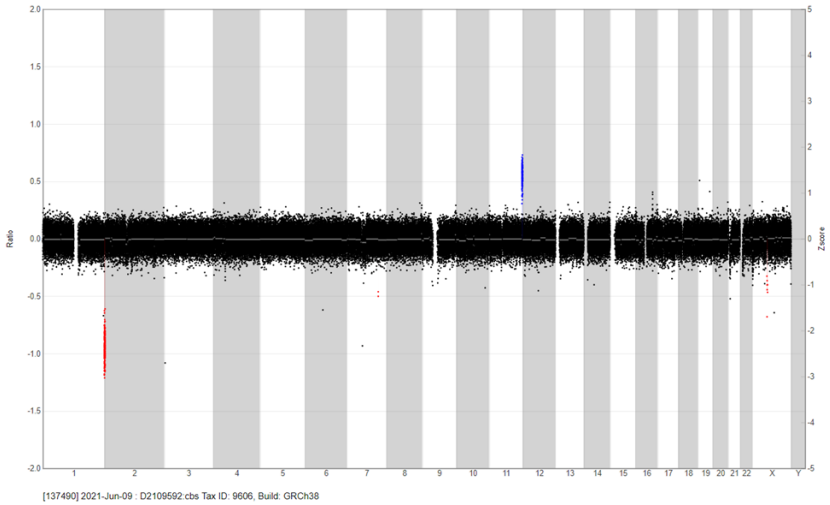
° 2020
Developmental delay
Overgrowth

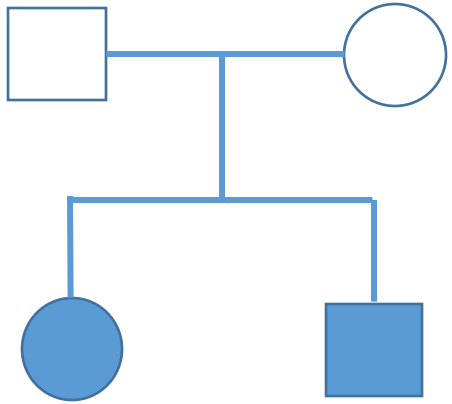


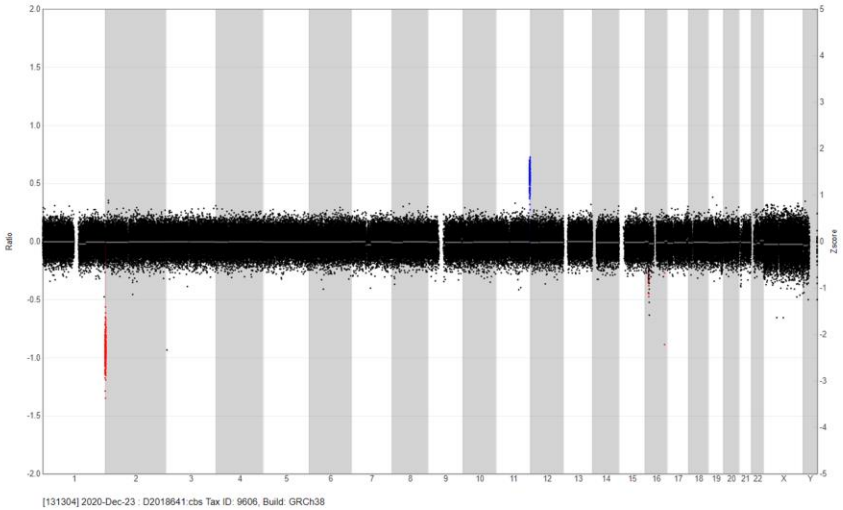


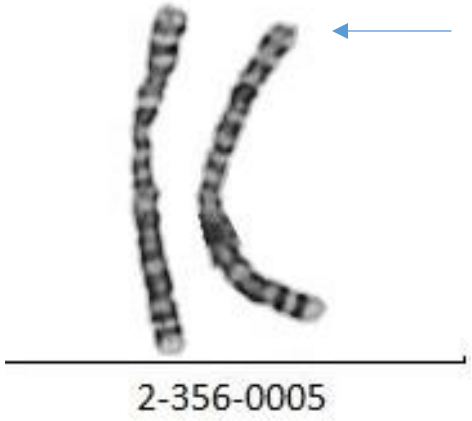
sseq[GRCh38] 2p25.3(15001_3315000)x1

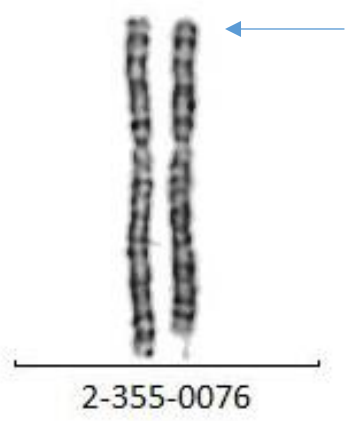
sseq[GRCh38] 11q25(132195001_135075000)x3


[137490] 2021-Jun-09 : D2109592.cbs Tax ID: 9606, Build: GRCh38



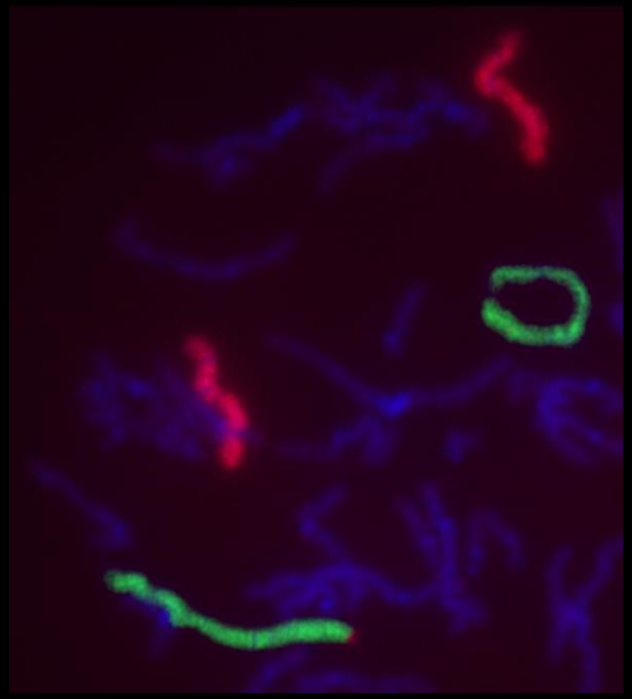

[131304] 2020-Dec-23 : D2018641.cbs Tax ID: 9606, Build: GRCh38


?
2-356-0005

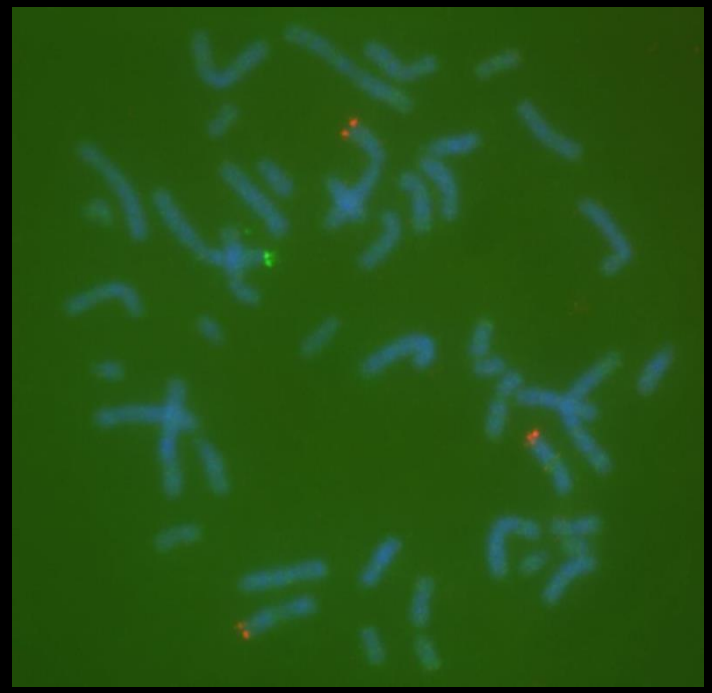

2-355-0076

- FISH:

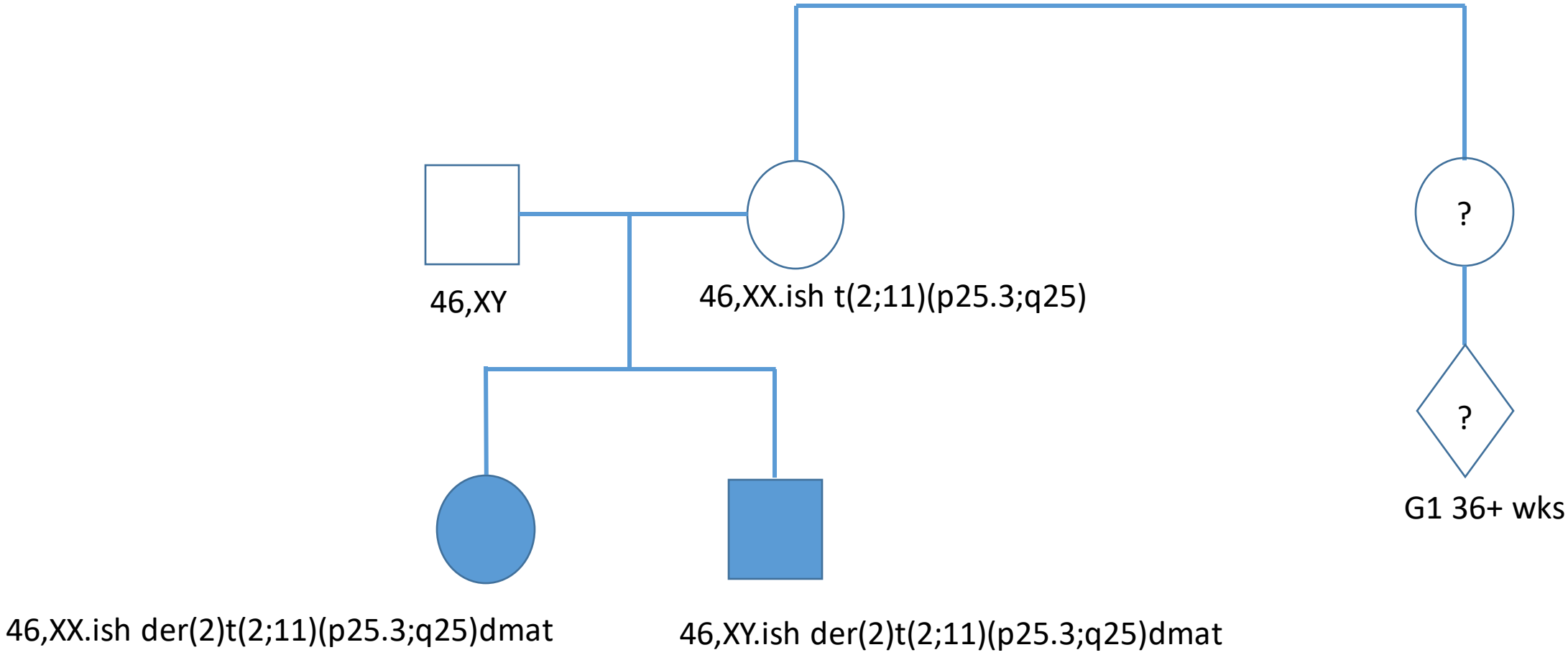
#2 + #11



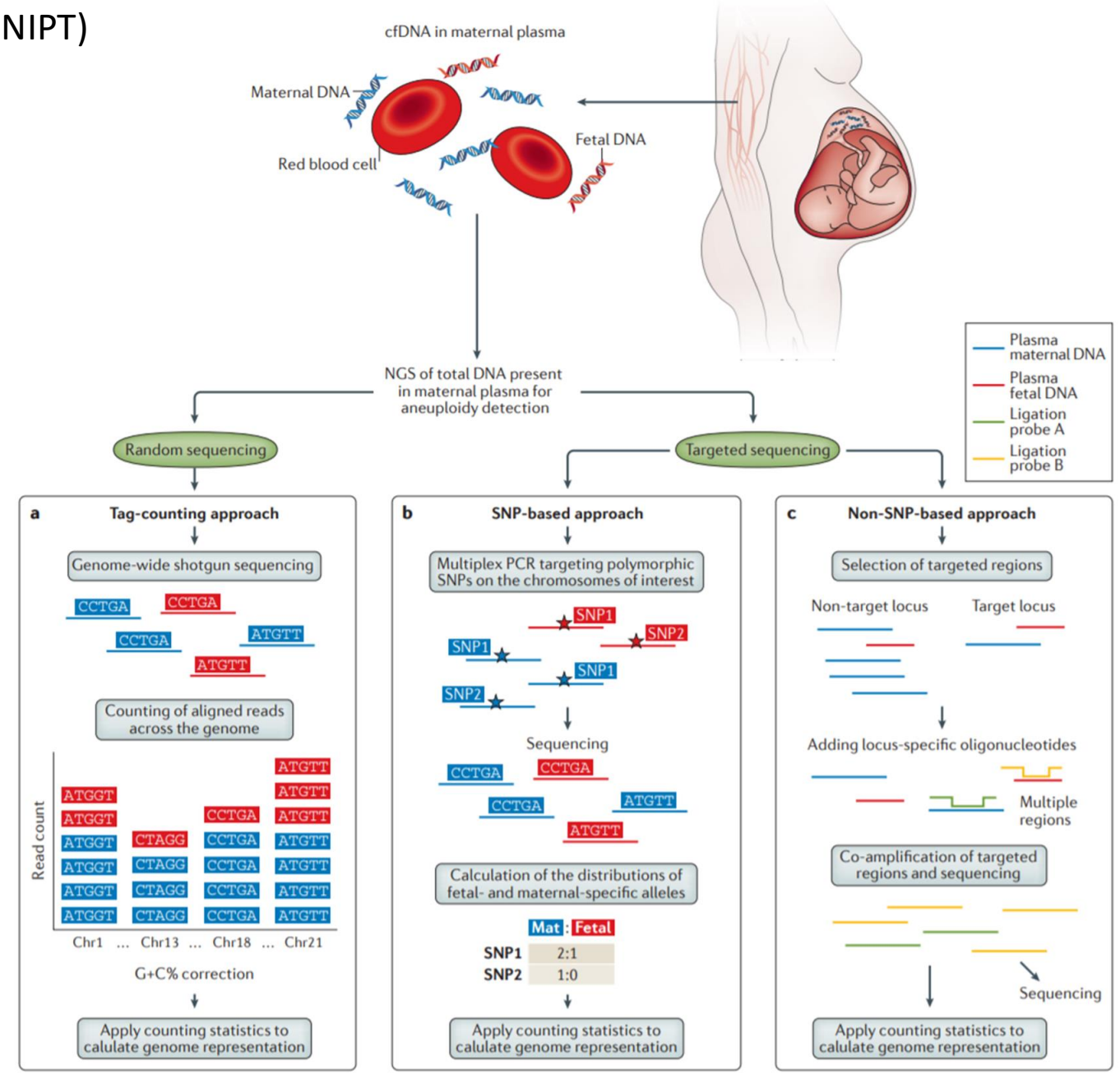
2ptel + 11qtel



→ 46,XY/XX.ish der(2)t(2;11)(p25.3;q25)


?
?
46,XY
46,XX.ish t(2;11)(p25.3;q25)
G1 36+ wks
46,XX.ish der(2)t(2;11)(p25.3;q25)dmat
46,XY.ish der(2)t(2;11)(p25.3;q25)dmat

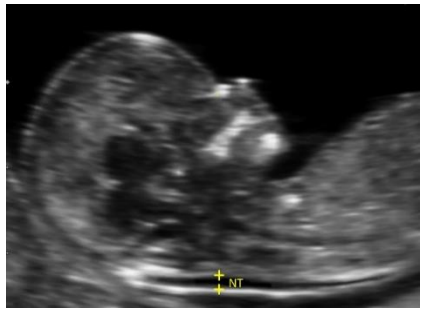
Non-invasive prenatal testing (NIPT)



Vermeesch, Nat Gen Rev, 2016

1st trimester screening

NIPT



+

maternal age

75 – 85% sensitivity

5% false positives

Table 1. Meta-analysis of diagnostic accuracy of cell-free fetal DNA–based non-invasive prenatal test demonstrated by sensitivity and specificity ratio of common tests[37].

Test	Sensitivity	Specificity
Fetal sex	0.989 (95% CI 0.980–0.994)	0.996 (95% CI 0.989–0.998)
Rhesus D	0.993 (95% CI 0.982–0.997)	0.984 (95% CI 0.964–0.993)
Trisomy 21	0.994 (95% CI 0.983–0.998)	0.999 (95% CI 0.999–1.000)
Trisomy 18	0.977 (95% CI 0.952–0.989)	0.999 (95% CI 0.998–1.000)
Trisomy 13	0.906 (95% CI 0.823–0.958)	1.00 (95% CI 0.999–0.100)
Monosomy X	0.929 (95% CI 0.741–0.984)	0.999 (95% CI 0.995–0.999)

CI, confidence interval.

Pös, F1000 res, 2019

NIPT in Belgium

Genetics inMedicine

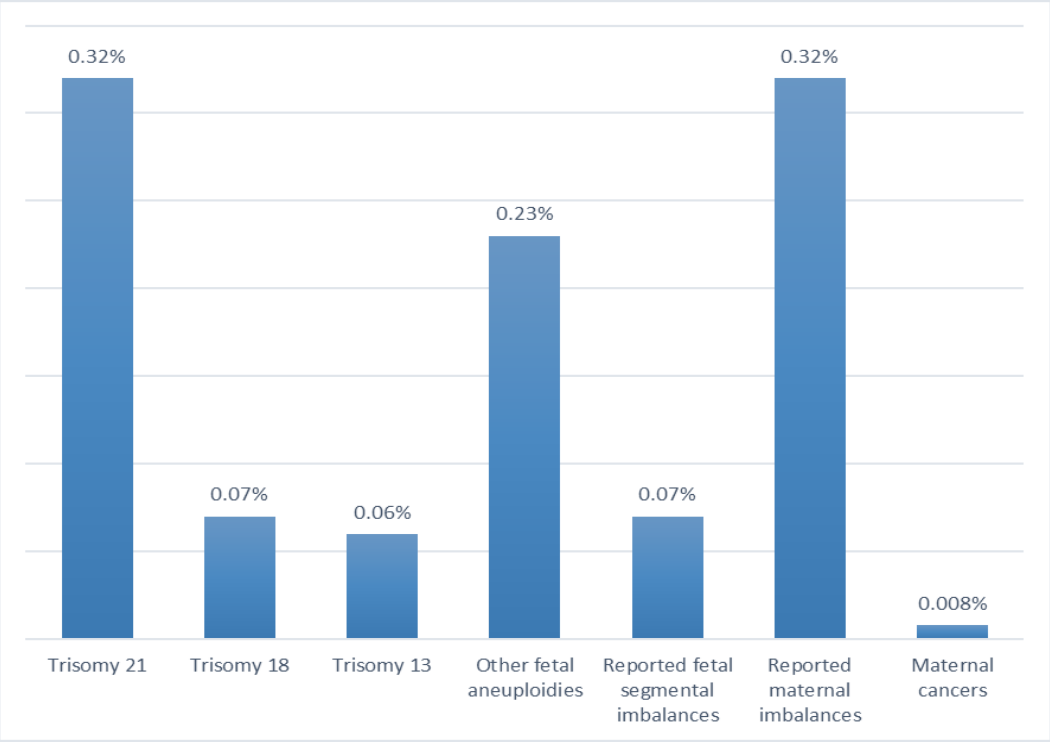
www.nature.com/gim

Check for updates

ARTICLE

Outcome of publicly funded nationwide first-tier noninvasive prenatal screening

Kris Van Den Bogaert[1], Lore Lannoo[2], Nathalie Brison[1], Vincent Gatinois[1], Machteld Baetens[3], Bettina Blaumeiser[4,5], François Boemer[6], Laura Bourlard[7], Vincent Bours[6], Anne De Leener[8], Marjan De Rademaeker[5], Julie Désir[7,9], Annelies Dheedene[3], Armelle Duquenne[8], Nathalie Fieremans[10], Annelies Fieuw[10], Jean-Stéphane Gatot[6], Bernard Grisart[9], Katrien Janssens[4], Sandra Janssens[3], Damien Lederer[9], Axel Marichal[9], Björn Menten[3], Colombine Meunier[9], Leonor Palmeira[6], Bruno Pichon[7], Eva Sammels[10], Guillaume Smits[7], Yves Sznajer[8], Elise Vantroys[10], Koenraad Devriendt[1] and Joris Robert Vermeesch[1]✉

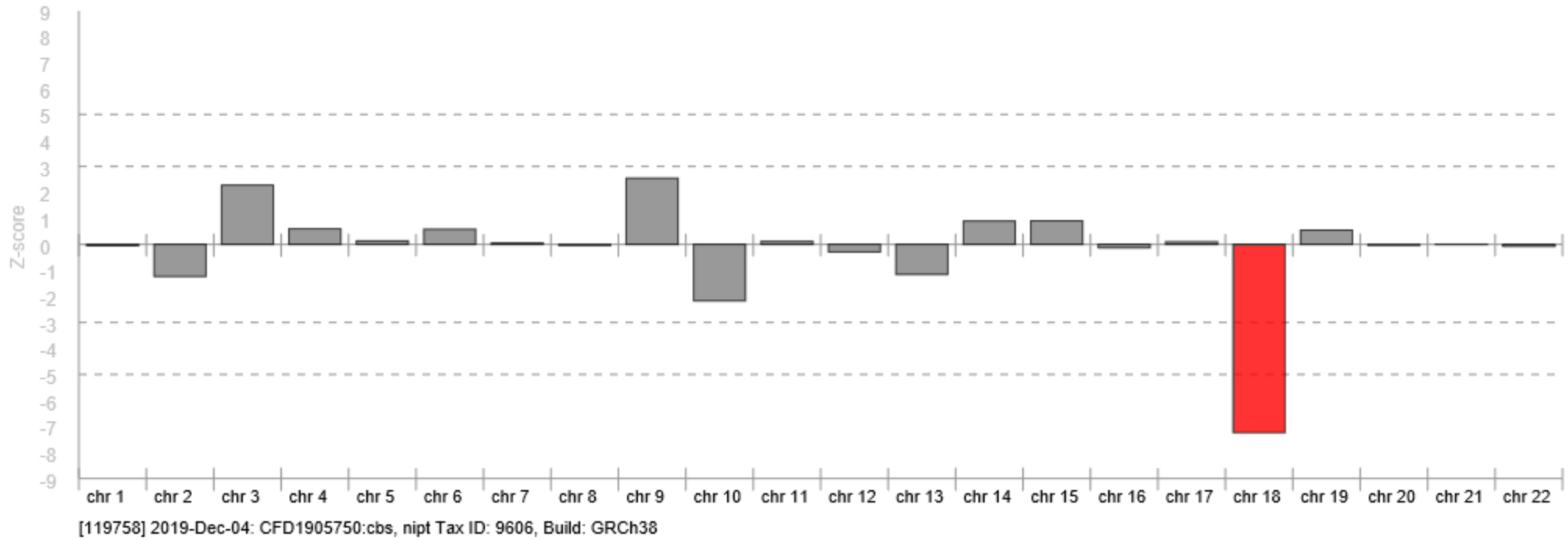


Percentage of pregnancies with a fetal or maternal imbalance from first-tier genome-wide NIPT.

General | Nipt | Info

#	Date	Name	Fetal Gender	Statistics
[119758]	04-12-2019	CFD1905750	male	**Qscore**: 0.55 **FFY**: 9.11% **FFX**: 8.78% **PREFACE**: 8.16%

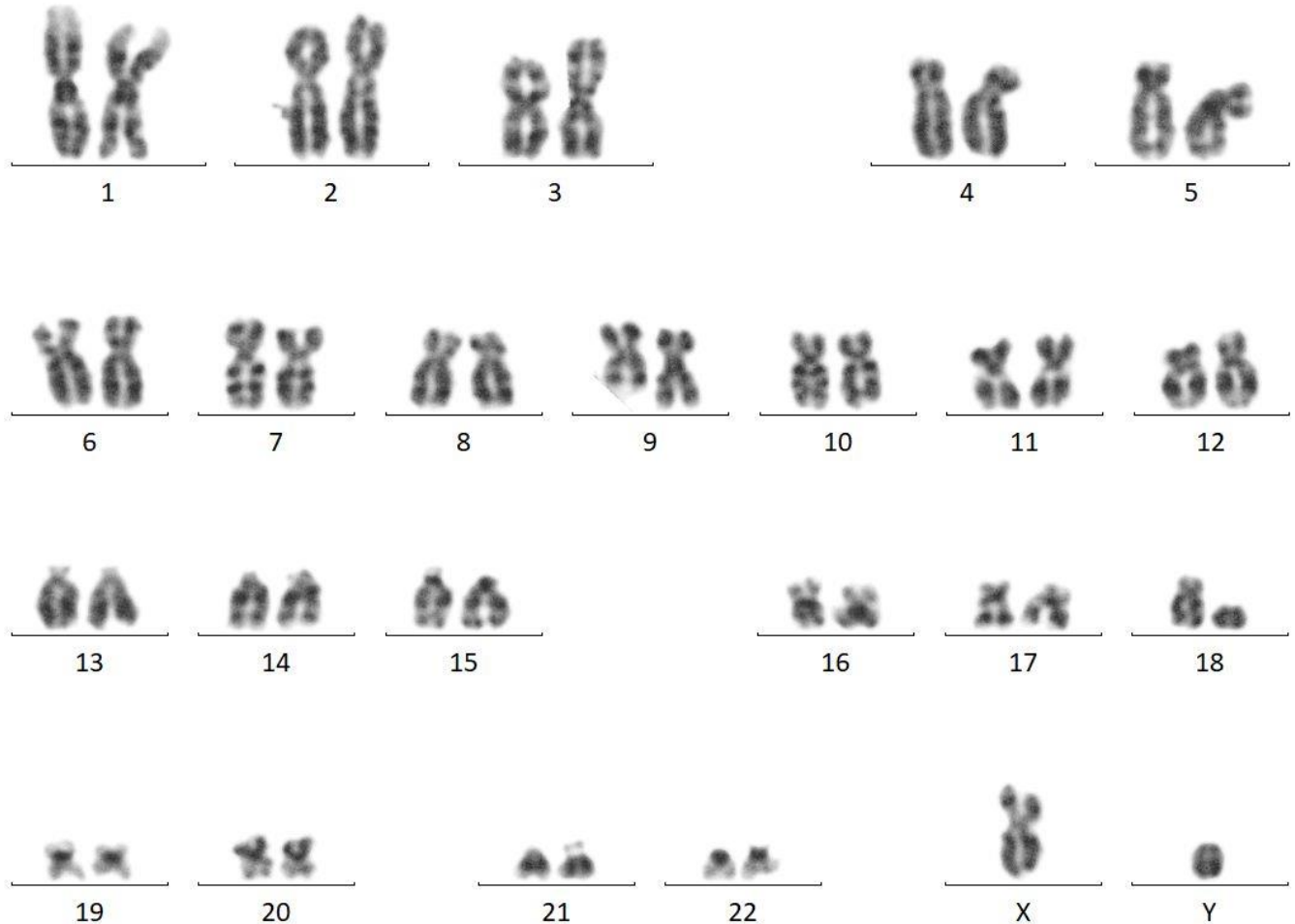
Z-scores



[119758] 2019-Dec-04: CFD1905750:cbs, nipt Tax ID: 9606, Build: GRCh38

NIPT chr 18 in detail

Amniocentesis (15w GA) chr 18 in detail
NT=3,6mm


1
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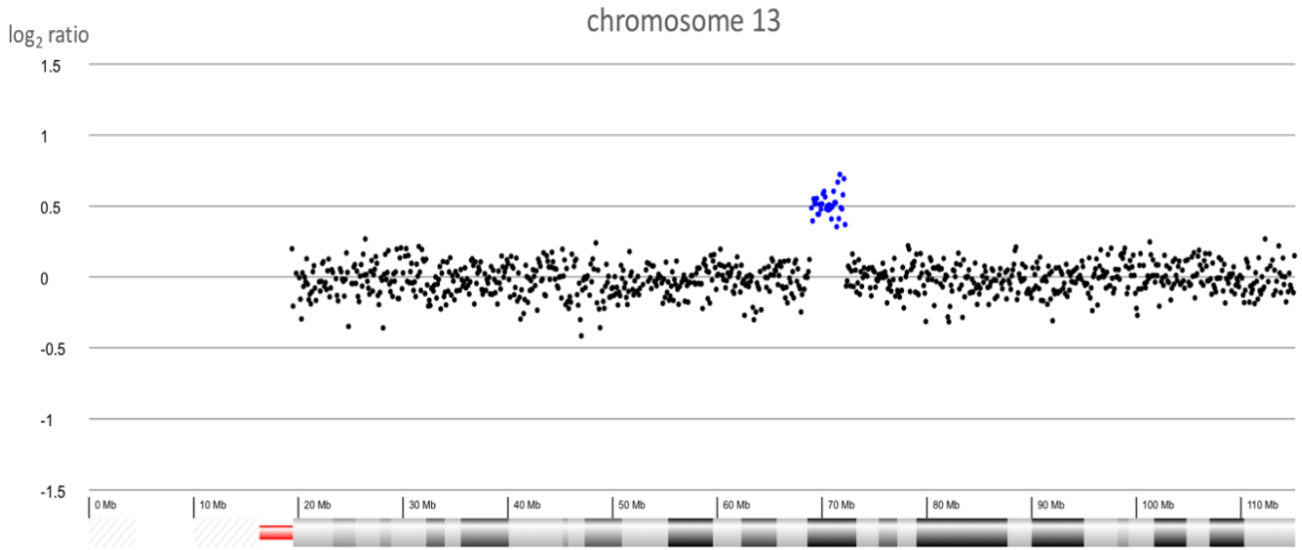
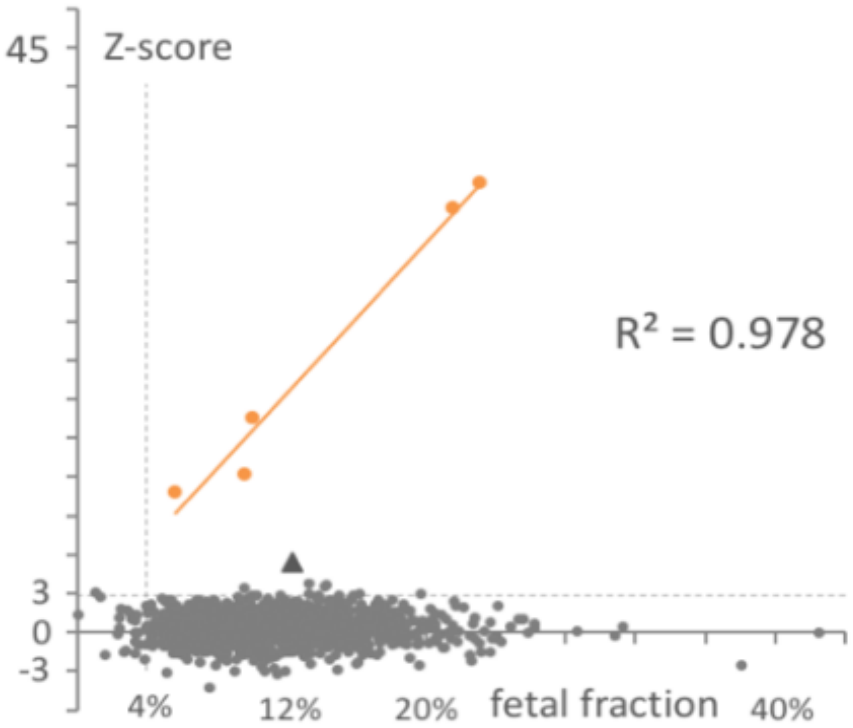
21
22
X
Y

maternal duplication as reason for false positive





Z13 = 5.41 → trisomy 13

FF = 12.3%

Dheedene et al. Pren. Diagn. 2016

Ultrasound Obstet Gynecol 2019; **54**: 458–467
Published online 3 September 2019 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.20383

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

P. BENN[1], F. MALVESTITI[2], B. GRIMI[2], F. MAGGI[2], G. SIMONI[2] and F. R. GRATI[2]

Table 4 Observed rates of uniparental disomy (UPD) identified in amniotic fluid, following detection of trisomy in chorionic villus sample

Imprinted chromosome	*Investigated cases* (n)	*Cases with UPD* (n)	*UPD rate (% (95% CI))*
Trisomy 6	3	0	0 (0–56)
Trisomy 7	93	0	0 (0–4)
Trisomy 11	6	0	0 (0–39)
Trisomy 14	15	5*	33 (15–58)
Trisomy 15	32	3†	9 (3–24)
Trisomy 20	34	0	0 (0–10)
Total	183	8	4.4 (2.2–8.4)

*Three cases with rare autosomal trisomies (RAT) in cytotrophoblasts and two cases with RAT in mesenchyme. †Two cases with RAT in cytotrophoblasts and one case with RAT in mesenchyme.

“incidental” findings in the mother

Brief Report

September 2015

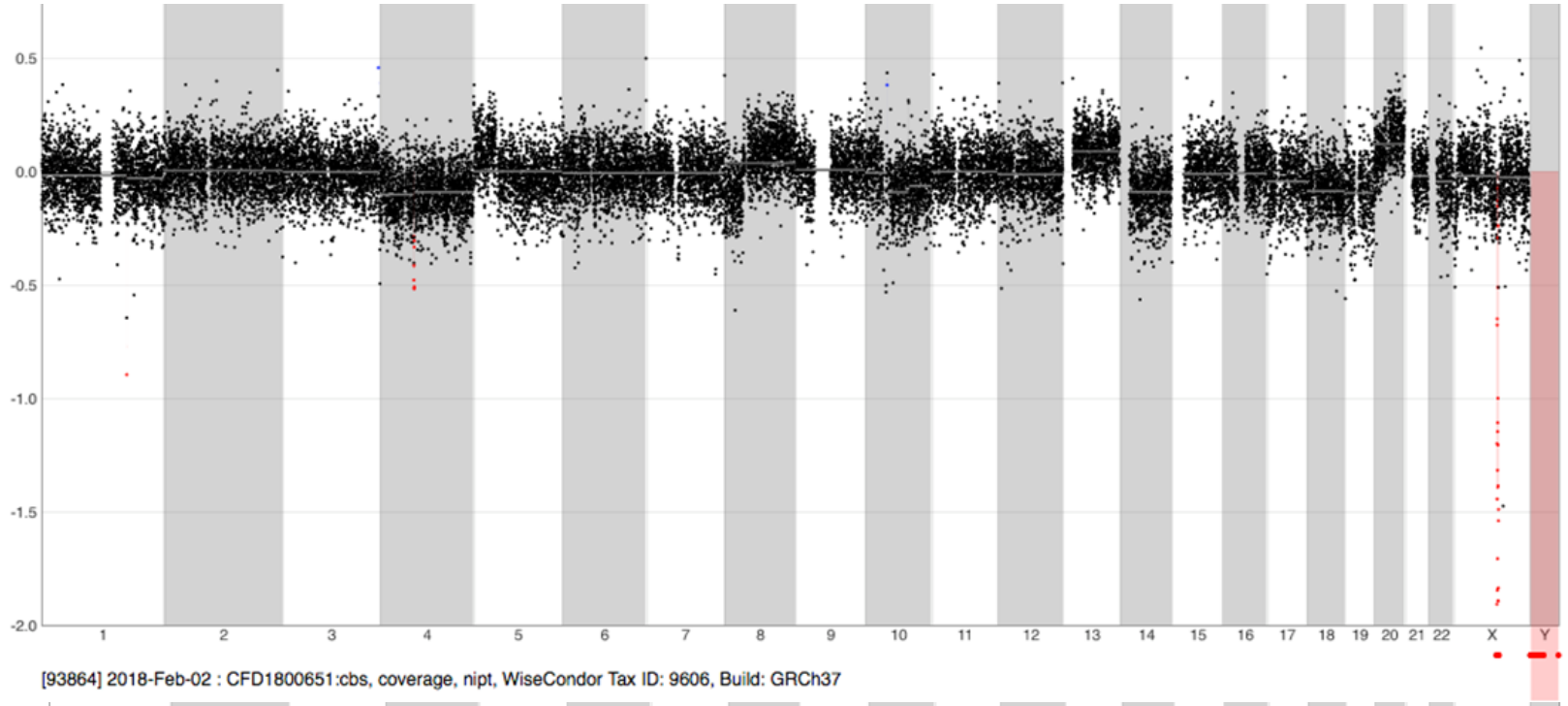
Presymptomatic Identification of Cancers in Pregnant Women During Noninvasive Prenatal Testing

Frédéric Amant, MD, PhD[1]; Magali Verheecke, MD[1]; Iwona Wlodarska, PhD[2]; Luc Dehaspe, PhD[2]; Paul Brady, PhD[2]; Nathalie Brison, PhD[2]; Kris Van Den Bogaert, PhD[2]; Daan Dierickx, MD, PhD[3]; Vincent Vandecaveye, MD, PhD[4]; Thomas Tousseyn, MD, PhD[5]; Philippe Moerman, MD, PhD[5]; Adriaan Vanderstichele, MD[2]; Ignace Vergote, MD, PhD[2]; Patrick Neven, MD, PhD[2]; Patrick Berteloot, MD[6]; Katrien Putseys, MD[7]; Lode Danneels, MD[8]; Peter Vandenberghe, MD, PhD[2,3]; Eric Legius, MD, PhD[2]; Joris Robert Vermeesch, PhD[2]

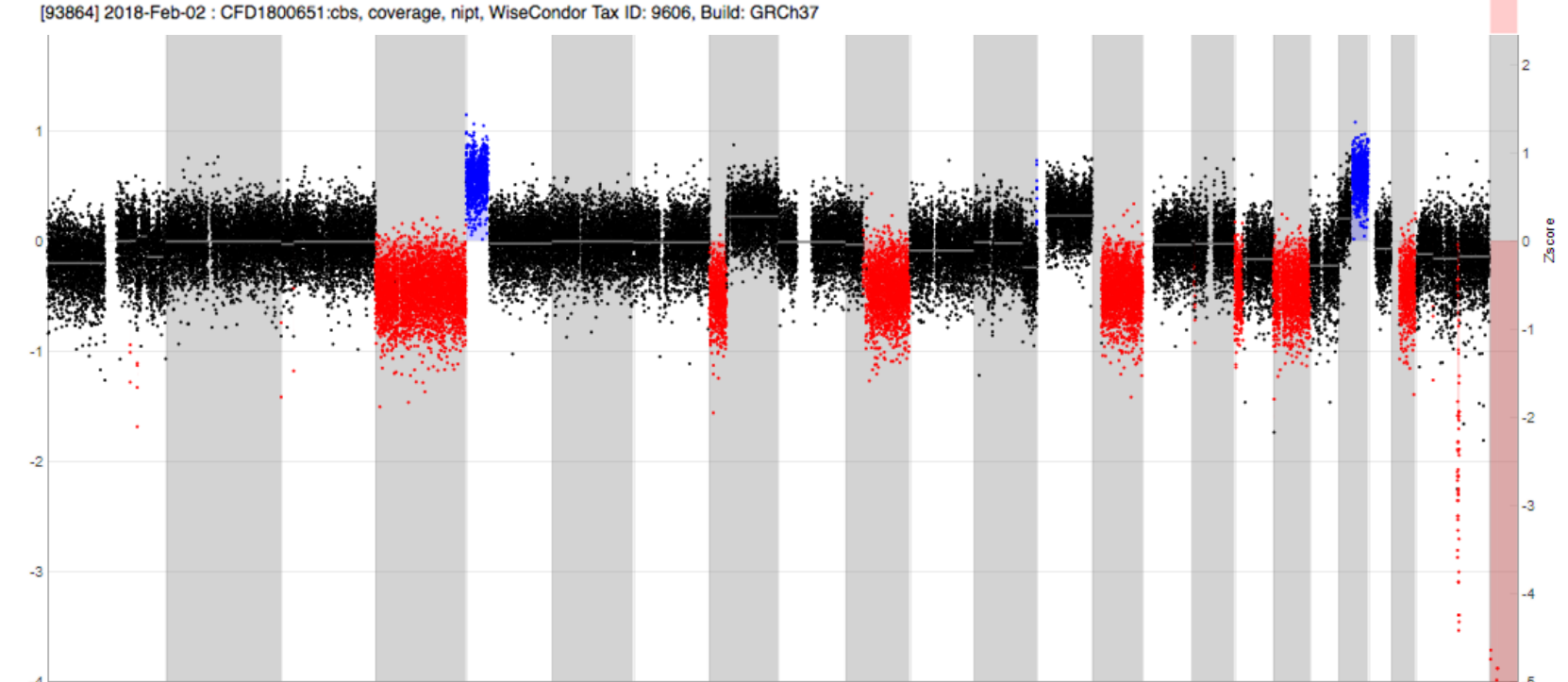
» Author Affiliations

JAMA Oncol. 2015;1(6):814-819. doi:10.1001/jamaoncol.2015.1883

NIPT: suspicion maternal malignancy



Molecular karyotype: Resection polyp



→ Liquid biopsy