



# **CITOGENETICA**

**GENETICA MEDICA 6 ANNO  
UNIVERSITA DI FERRARA**



# Genetic Disorders

## **Cytogenetic Disorders** **chromosomal abnormalities**

### Single Gene Disorders

Classical (Mendelian) inheritance

Non-classical inheritance

Mitochondrial genes

Trinucleotide repeats

Genetic imprinting

# Mutations

A mutation may be defined as a permanent change in the DNA and frequently has a deleterious effect:

- Mutations that affect the **germ cells** are transmitted to the progeny and may give rise to inherited diseases.
- Mutations that occur in **somatic cells** are important in the genesis of cancers and some congenital malformations.

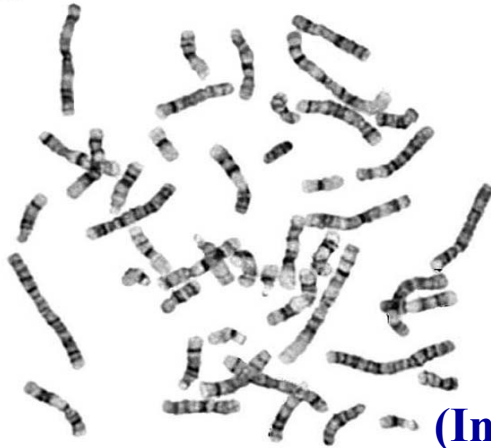


# Citogenetica

- Analisi genomica su base **morfologica**, con la finalità di studiare l'assetto cromosomico delle cellule e definirne il “cariotipo”.
- Il cariotipo è l'insieme dei cromosomi, caratteristico per numero e morfologia, della specie alla quale il genoma appartiene.



Human male  
G-bands



Human male  
G-bands

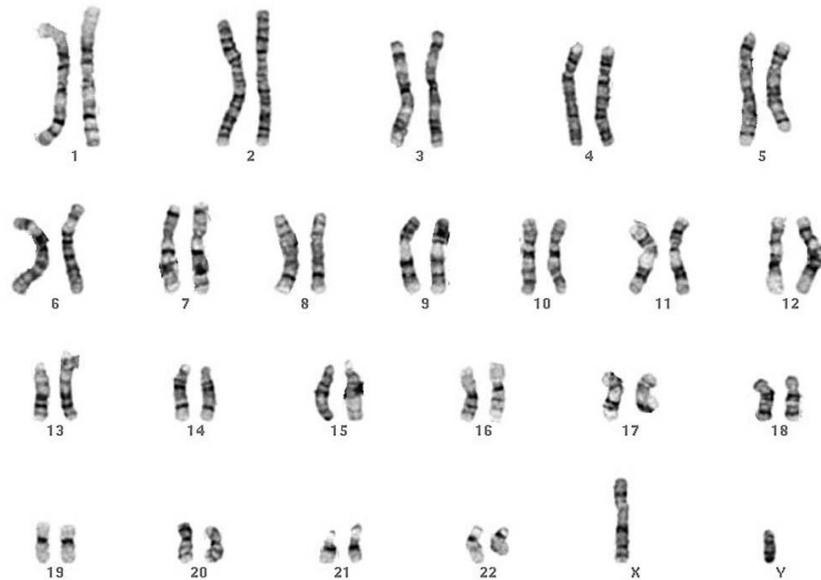


**Bandeggio GTG**

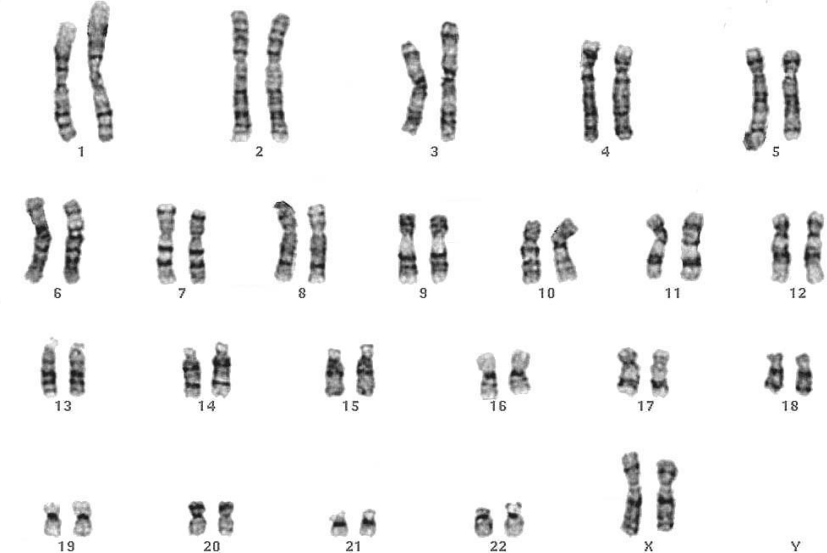
**ISCN**

**(International System for human  
Cytogenomic Nomenclature)**

Human male  
G-bands



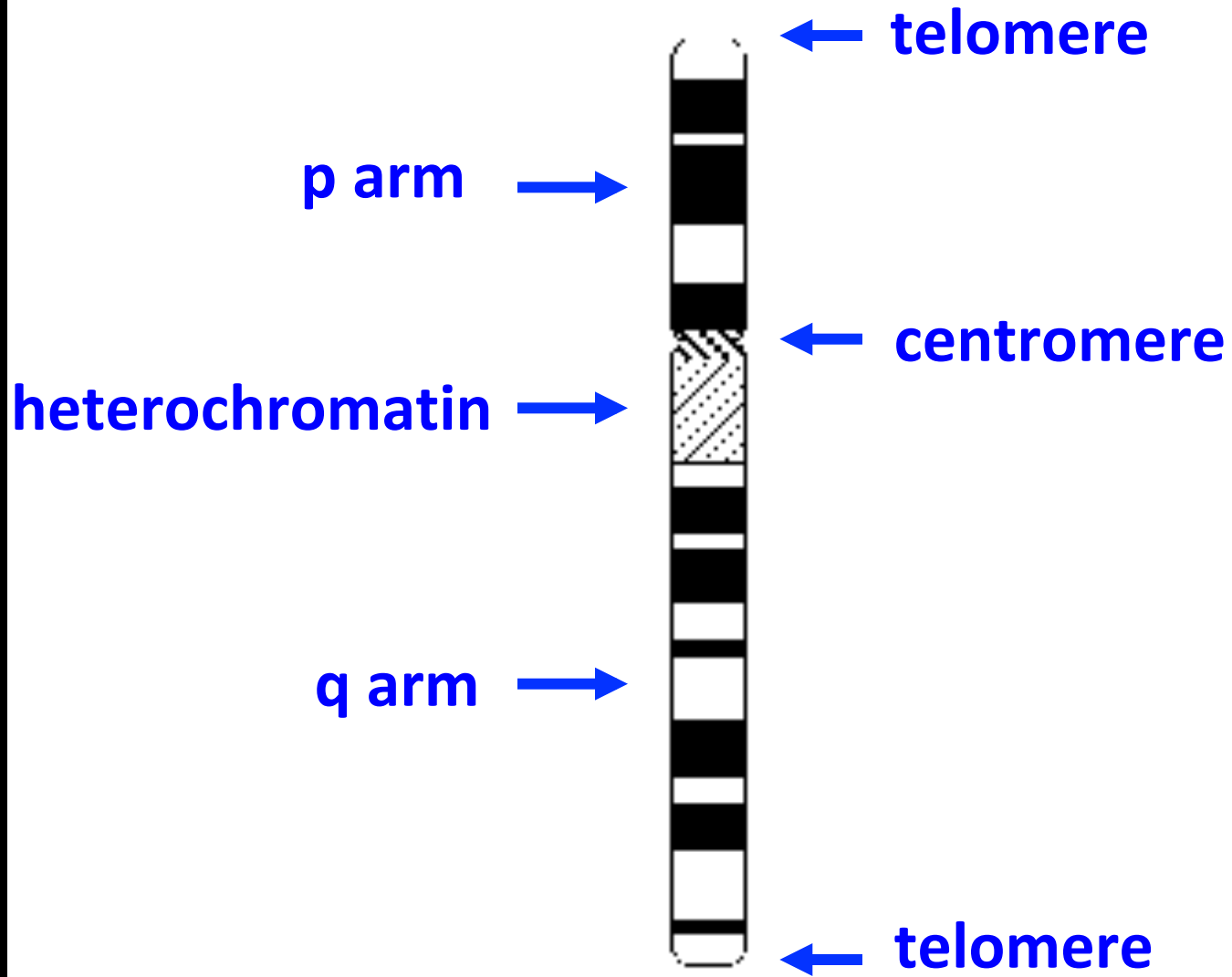
Human female  
G-bands



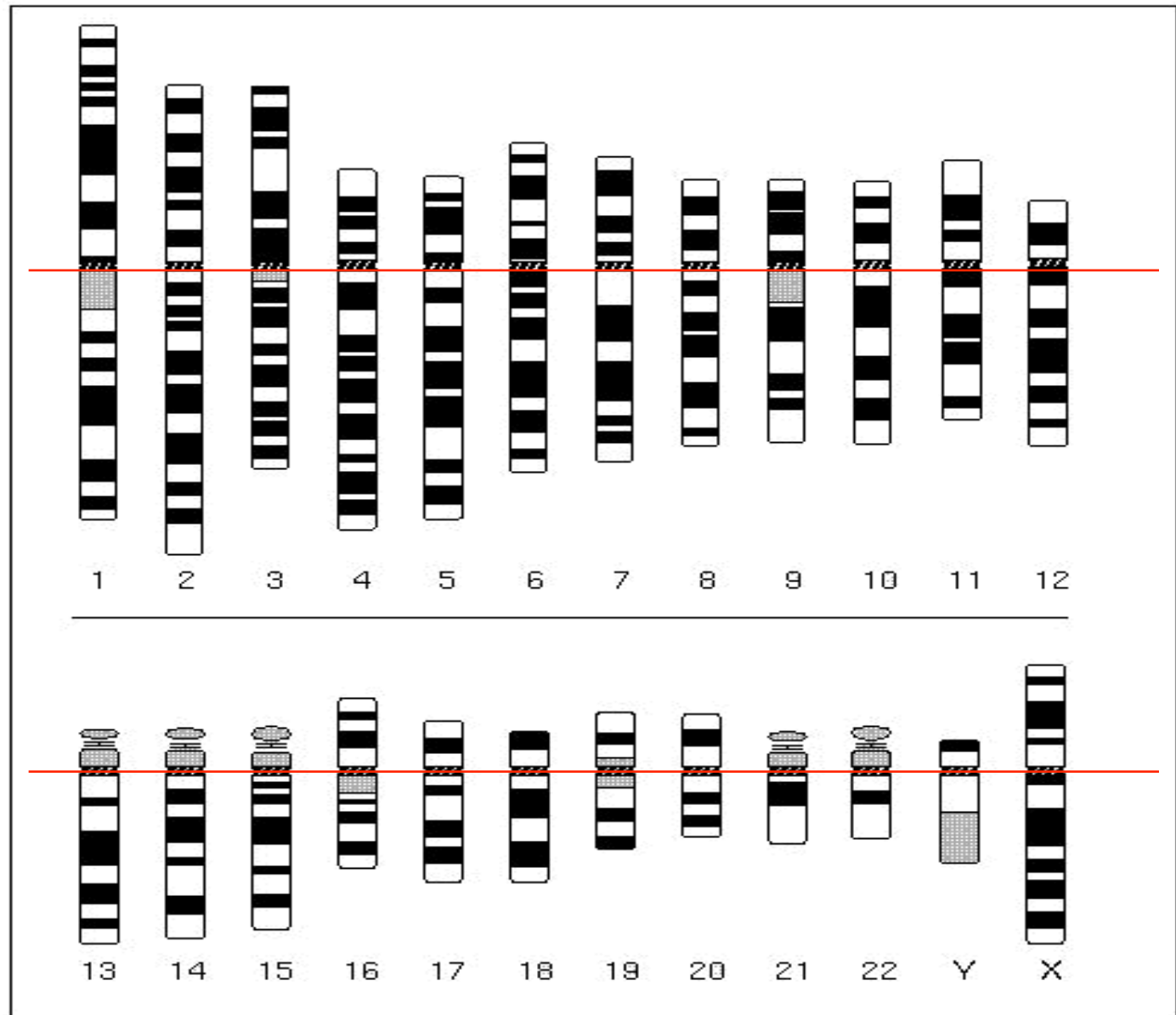
**La standardizzazione internazionale della nomenclatura dei cromosomi umani inizia nel 1960 con la conferenza di Denver, ultimo aggiornamento nel 2016 (ISCN 2016), identifica ciascuna banda e sottobanda cromosomica in 400, 550 ed 850 bande.**

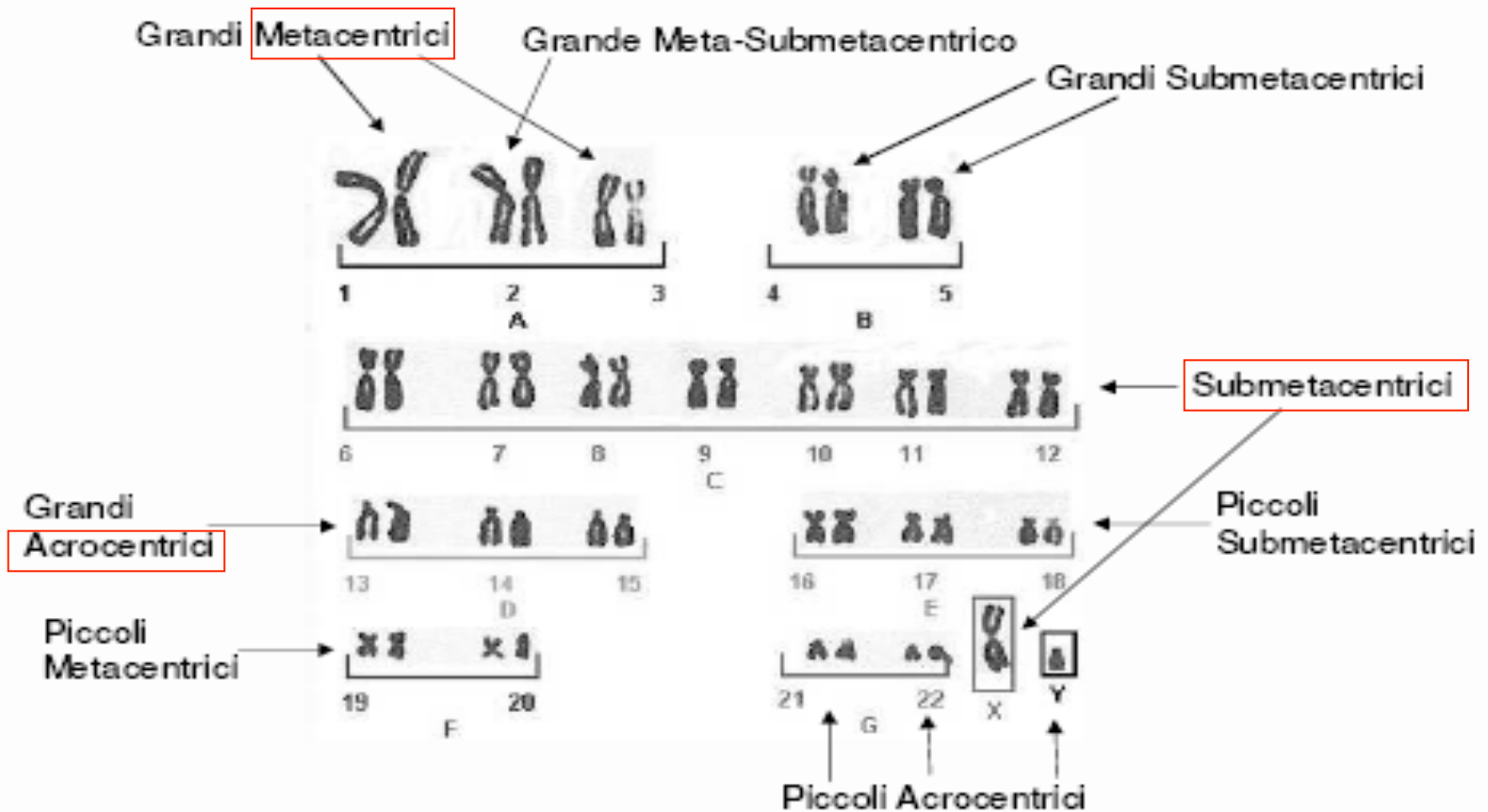


## Naming of parts



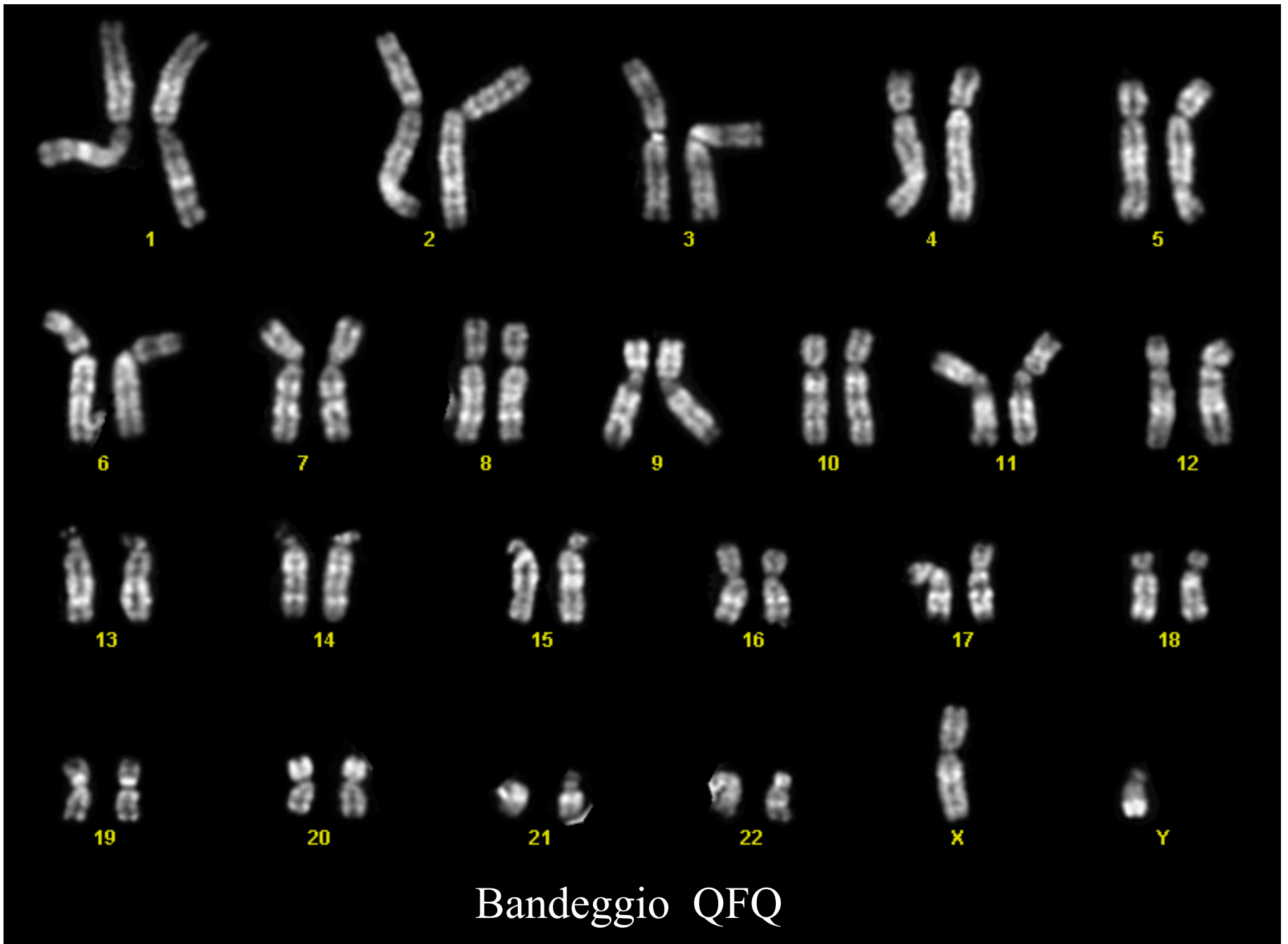
# Ideogramma del cariotipo umano



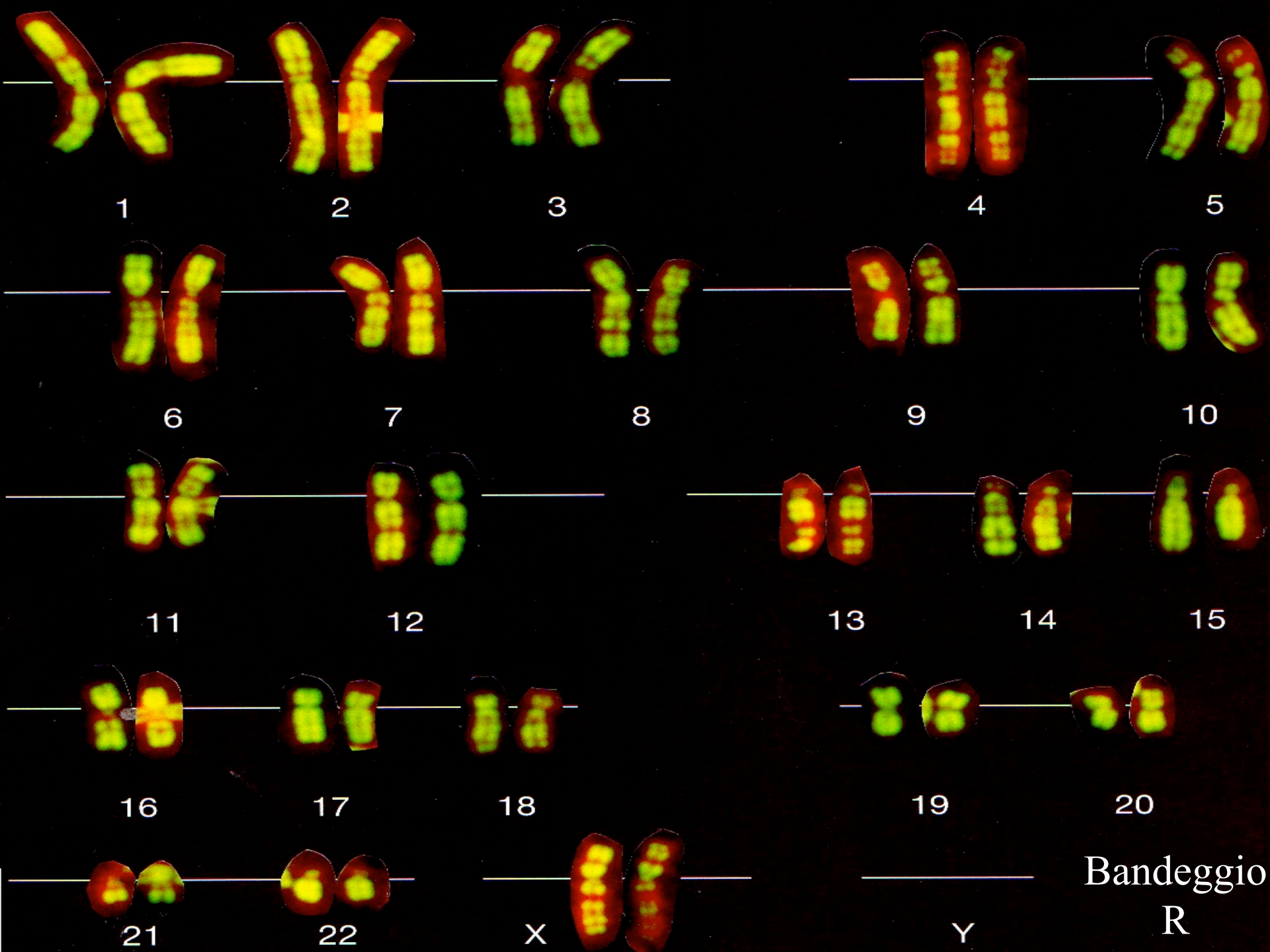


GRUPPO	CROMOSOMI	CARATTERISTICHE
A	1, 2, 3	Cromosomi grandi con centromeri in posizione mediana.
B	4, 5	Cromosomi grandi con centromeri sub-mediani.
C	6, 7, 8, 9, 10, 11, 12	Cromosomi di media grandezza con centromeri sub-mediani
D	13, 14, 15	Cromosomi di media grandezza con centromeri terminali.
E	16, 17, 18	Cromosomi piccoli con centromeri mediani e sub-mediani.
F	19, 20	Cromosomi piccoli con centromeri mediani.
G	21, 22	Cromosomi molto piccoli e acrocentrici.





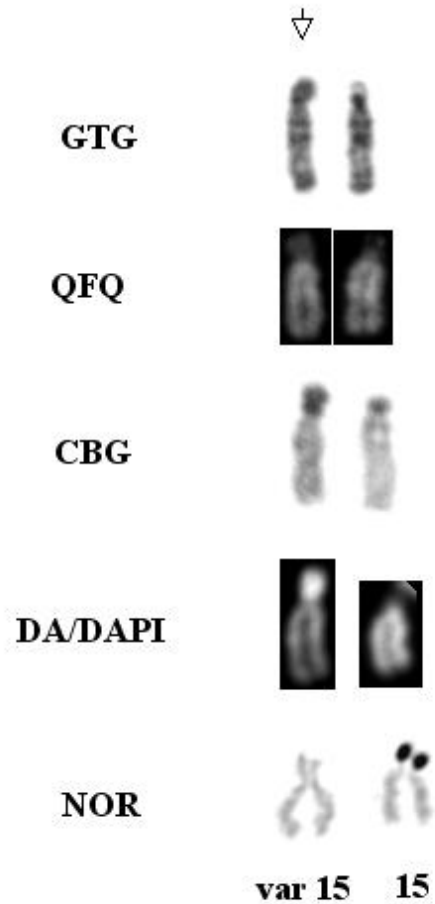
Bandeggio QFQ



# Varianti cromosomiche

Variante cromosomica  
riscontrata in LA

15ph+



15



# THE WAY TO MOLECULAR KARYOTYPE

classical cytogenetics

molecular cytogenetics

1960

1980

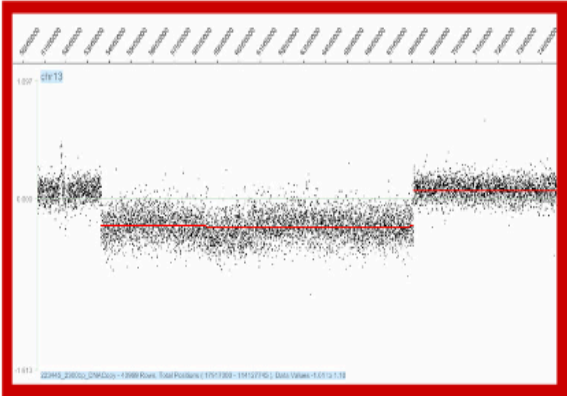
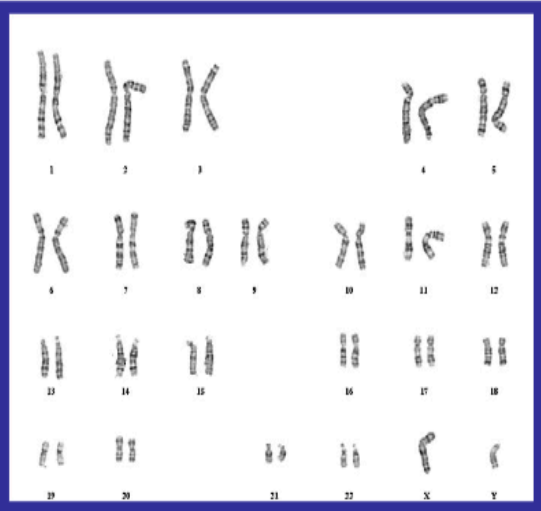
1990

2000

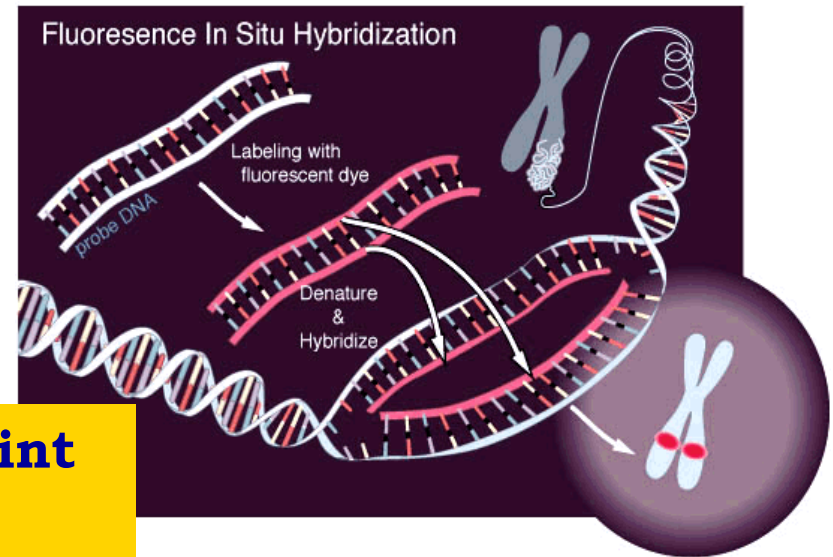
Chromosome banding

FISH

Array- CGH



# Fluorescence In Situ Hybridization (FISH)



- **Fluorescent molecules paint chromosomes to detect abnormalities**
- **Labeled probes hybridize to complementary DNA**
- **Three types of probes: locus specific, centromeric repeat, whole chromosome**



## **Metaphase FISH**

**Detect microdeletions beyond resolution of routine cytogenetics**

**Determine a simple deletion or a subtle or complex rearrangement**

**Detect specific rearrangements in certain cancers**

**Number of microdeletion syndromes diagnosed by FISH is expanding rapidly**

**Probe may be specific for the gene as in Williams Syndrome a deletion has been shown in the elastin gene in 96% of individuals with a firm diagnosis.**



## Interphase FISH

**Determine the chromosomes number**

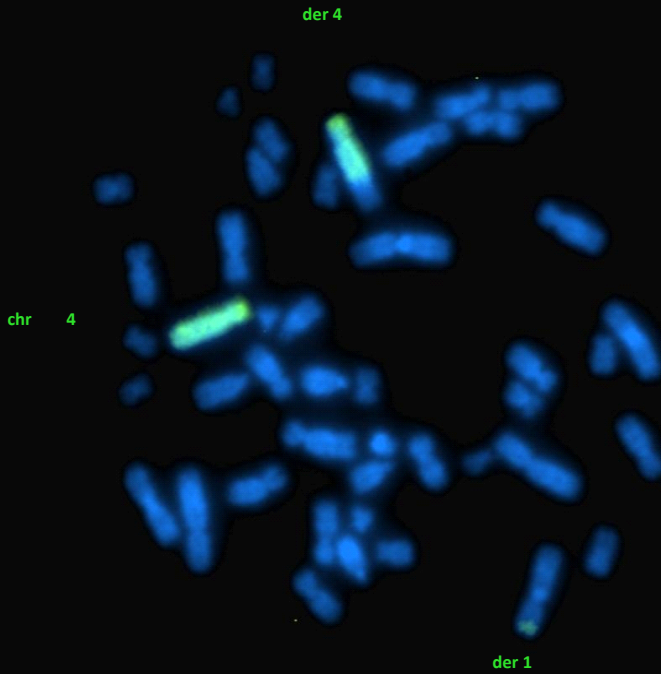
**Detects specific rearrangements in certain cancers**

**Advantage is that it is rapid**

**Aneuploid Screen on amniotic fluid cells**

*Nuclei denatured and hybridized with probes for chromosomes 13, 18, 21, X, and Y and results usually obtained within 24 hours*

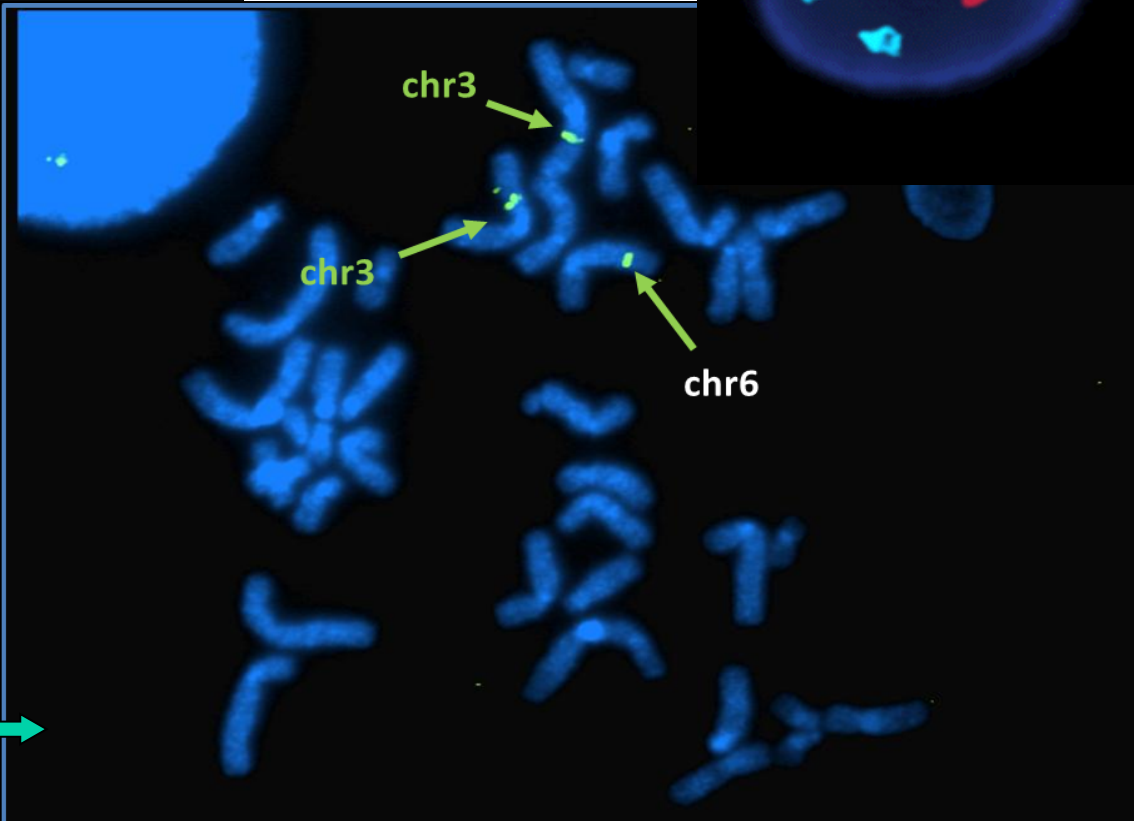
**Extensive work-up in PND to determine the level of a mosaic trisomy**



**Chromosome specific  
Probe (painting FISH)**

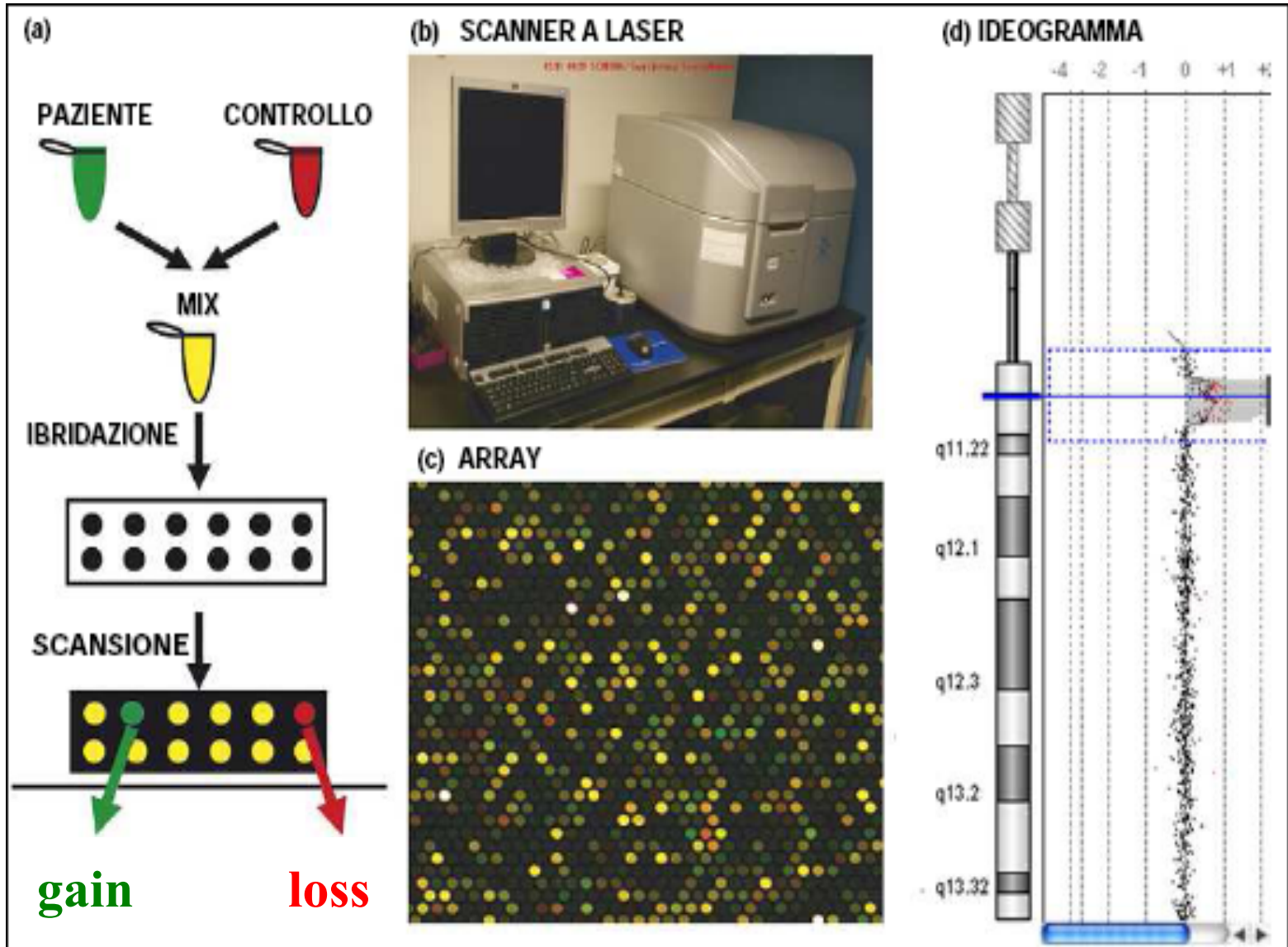
**Locus specific Probe**

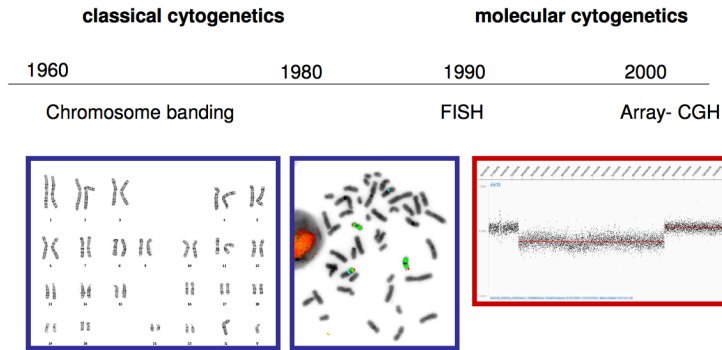
**Centromeric  
Probe**





# Array CGH method





## COMPARATIVE GENOMIC HYBRIDISATION (molecular karyotype)

Current cytogenetic genome screen - karyotyping  
- even the best has low resolution (5MB)

FISH – better resolution (3MB), but only targeted  
regions

Chromosomal Micro-Array (CMA) - struggle with  
larger sized fragments and whole genome  
analysis

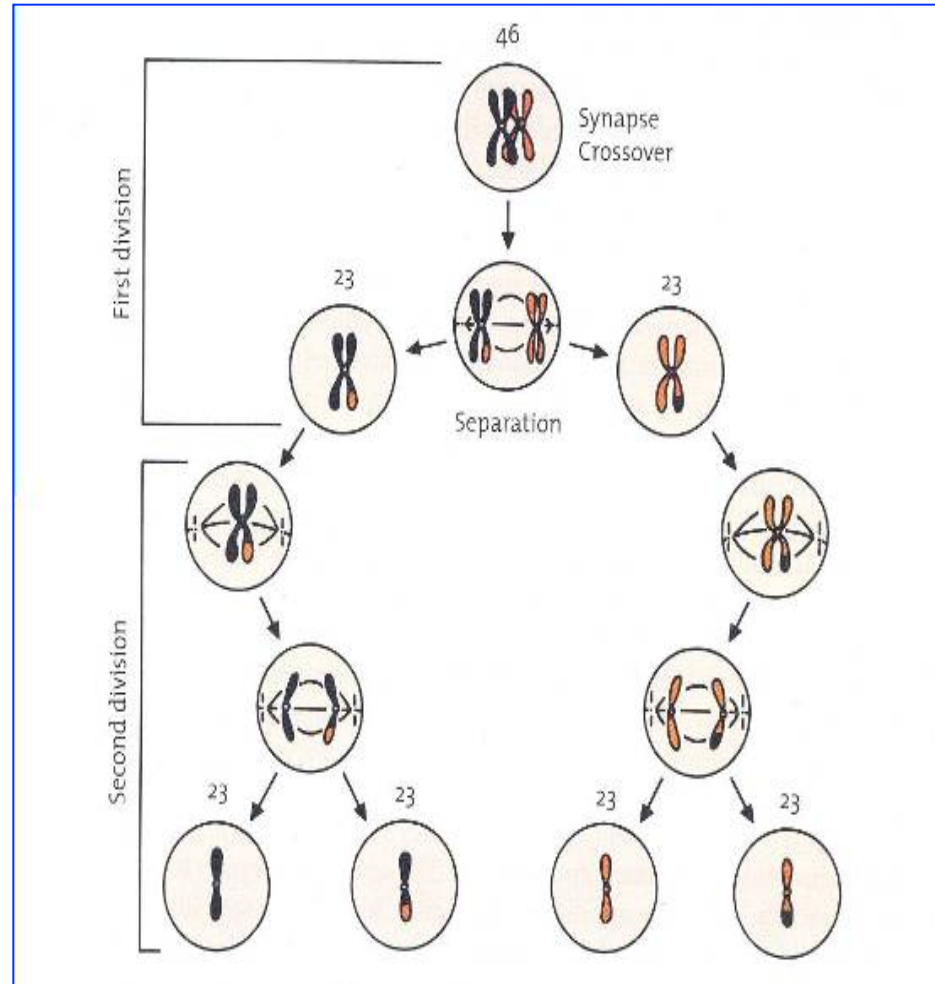
# Meiosis

- Meiosis begins by replicating the chromosomes: the number of chromosomes doubles.

- During the first meiotic division the homologous chromosomes pair and crossing-over can occur

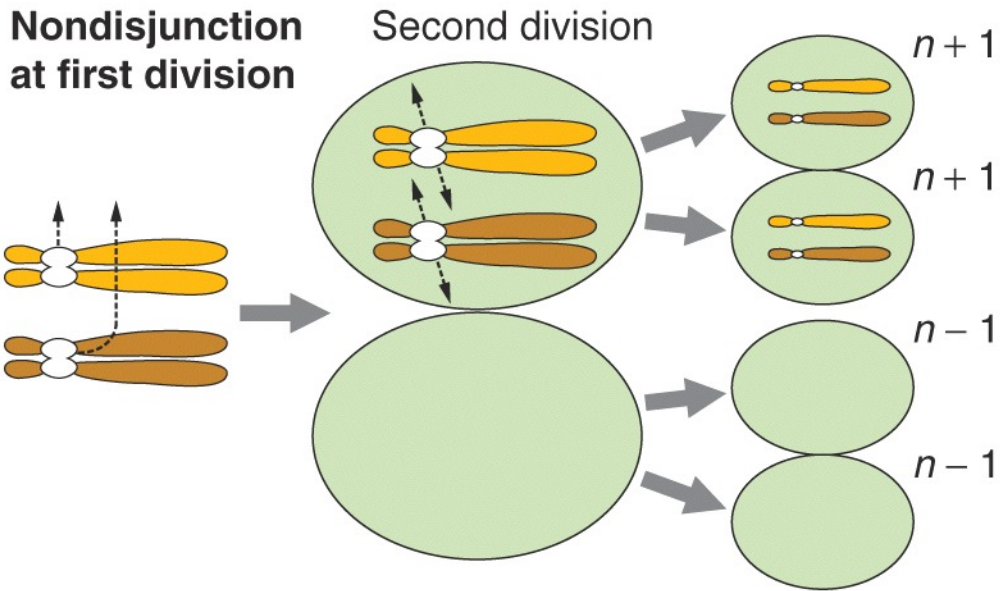
- At the end of the first meiotic division the homologous chromosomes segregate

- At the end of the second meiotic division, the sister chromatids separate forming the gametes with 23 chromosomes

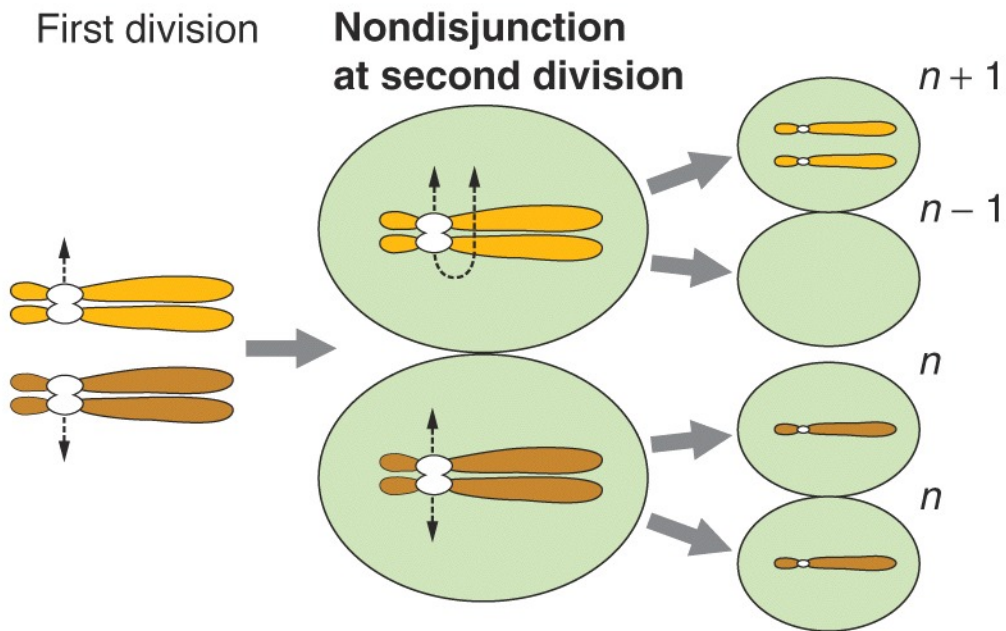




- **Meiosis** results in genetic variation by **shuffling** of maternal and paternal chromosomes by a random assortment and eventually by **crossing over**.
- Crossing-over and random assortment lead to **new combinations of DNA**.
- No daughter cells formed during meiosis are genetically identical to either mother or father.
- During sexual reproduction, fusion of gametes containing a unique haploid assortment of chromosomes produces truly unique offspring.



**100% of  
aneuploid  
gametes**



**50% of  
aneuploid  
gametes**

**50% of  
normal  
gametes**



## Constitutional karyotype analysis

- post natal  
BLOOD SAMPLE
  
- pre natal  
AMNIOCYTES  
CORIONIC VILLUS  
FOETAL BLOOD



# **ANOMALIE CROMOSOMICHE**

## **- Di numero (aneuploidie)**

**Triploidie**

**Tetraploidie**

**Trisomie**

**Monosomie**

**Mosaicismi**

## **- Di struttura**

**Traslocazioni**


**Inversioni**

**Delezioni**

**Duplicazioni**

**Ring (cromosomi ad anello)**

# Numerical abnormalities

- 
- ⌘ The category of chromosome changes which involve the addition or loss of complete sets of chromosomes.
  - **triploidy**
    - ⌘ The possession of one complete extra set of chromosomes.
    - ⌘ Usually caused by polyspermy, the fertilisation of an egg by more than one sperm.
    - ⌘ Such embryos will usually spontaneously abort.
  - **tetraploidy**
    - ⌘ Usually the result of a failure of the first zygotic division. It is also lethal to the embryo.
    - ⌘ Any other cell division may also fail to complete properly and in consequence a very small proportion of tetraploid cells can sometimes be found in normal individuals.



# Numerical abnormalities

## Triploidia

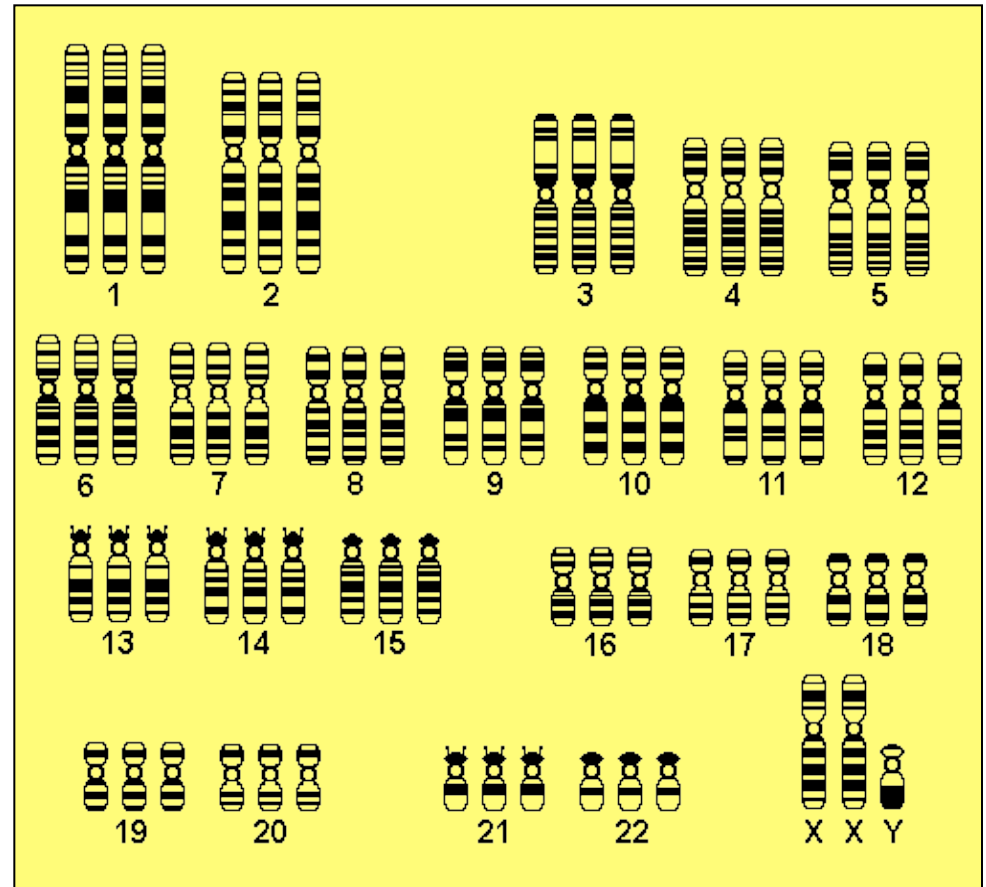
*Frequenza alla nascita = 1/10.000*

*Frequenza negli aborti = 1/14*

Cariotipo 69,XXY 57%

Cariotipo 69,XXX 40%

Cariotipo 69,XYY 3%



# Numerical abnormalities

## ANEUPLOIDY

The category of chromosome changes which do not involve whole sets. It is usually the consequence of a failure of a single chromosome (or bivalent) to complete division.

- **MONOSOMIES**

- ⌘ **All autosomal monosomies are lethal in very early embryogenesis. They do not even feature in the table of frequencies which follows because they abort too early even to be recognised as a conception.**

- **TRISOMIES**



# Numerical abnormalities

## MOSAIC

**An individual with more than one cytogenetically-distinct population of cells**

**The fraction each genotype is variable**

**Large proportion of abnormal cells will manifest disease**

**Small number of normal cells may prevent or reduce disease**



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## Nondisjunction syndrome frequencies

Syndrome	Description	Chromosomes	Incidences (newborns)
Down	Mental retardation; wide, flat face with upper eyelid fold, short stature; abnormal palm creases	Trisomy 21	1/800
Patau	Malformed internal organs, face, and head; extra digits; mental retardation	Trisomy 13	1/15,000
Edward	Malformed internal organs, face, and head; extreme muscle tone	Trisomy 18	1/6,000
Turner	Short stature; webbed neck; broad chest; no sexual maturity	XO	1/6,000
Klinefelter	Breast development possible; testes underdeveloped; no facial hair	XXY (or XXXY)	1/1,500
Triplo-X	Tall and thin with menstrual irregularities	XXX (or XXXX)	1/1,500
Jacob	Taller than average; persistent acne; speech and learning problems possible	XYY	1/1,000

b.

From Robert F. Weaver and Phillip W. Hedrick, *Genetics*, 2nd ed. Copyright 1992 WCB.

# Numerical abnormalities

## Down syndrome, trisomy 21

- ⌘ Most cases arise from non disjunction in the first meiotic division, the father contributing the extra chromosome in 15% of cases.
- ⌘ A small proportion of cases are mosaic and these probably arise from a non disjunction event in an early zygotic division.
- ⌘ About 4% of cases arise by inheritance of a translocation chromosome from a parent who is a balanced carrier.
- ⌘ The symptoms include characteristic facial dysmorphism, and an IQ of less than 50. Down syndrome is responsible for about 1/3 of all cases of moderate to severe mental handicap.



# Numerical abnormalities

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## Incidence of Down's syndrome with increasing maternal age

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maternal age	at CVS	at birth
20	~1 in 750	1 in 1500
30	~1 in 450	1 in 900
35	1 in 240	1 in 400
40	1 in 110	1 in 100
45	1 in 13	1 in 30

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## Numerical abnormalities

### Trisomy 13, Patau syndrome

- ⌘ The incidence is about 1 in 5000 live births.
- ⌘ 50% of these babies die within the first month and very few survive beyond the first year. There are multiple dysmorphic features.
- ⌘ Most cases, as in Down's syndrome, involve maternal non-disjunction.
- ⌘ Again, a significant fraction have a parent who is a translocation carrier.

### Trisomy 18, Edwards Syndrome

- ⌘ Incidence ~1 in 3000
- ⌘ Most babies die in the first year and many within the first month.



# Numerical abnormalities

## Sex chromosome aneuploidies

### Turner syndrome 45,X

- ⌘ The incidence is about 1 in 2000 female births but this is only the tip of the iceberg because 95% of Turner syndrome embryos are spontaneously aborted.
- ⌘ Individuals are very short, they are usually infertile. Characteristic body shape changes include a broad chest with widely spaced nipples and may include a webbed neck.
- ⌘ IQ and lifespan are unaffected.



# Numerical abnormalities

## Klinefelter's syndrome 47,XXY

- ⌘ The incidence at birth is about 1 in 1000 males.
- ⌘ Testes are small and fail to produce normal levels of testosterone which leads to breast growth (gynaecomastia) in about 40% of cases and to poorly developed secondary sexual characteristics.  
There is no spermatogenesis.
- ⌘ These males are taller and thinner than average and may have a slight reduction in IQ. Klinefelter males lead a normal life.
- ⌘ Very rarely more extreme forms of Klinefelter's syndrome occur where the patient has 48, XXXY or even 49, XXXXY karyotype. These individuals are generally severely retarded.



## Numerical abnormalities

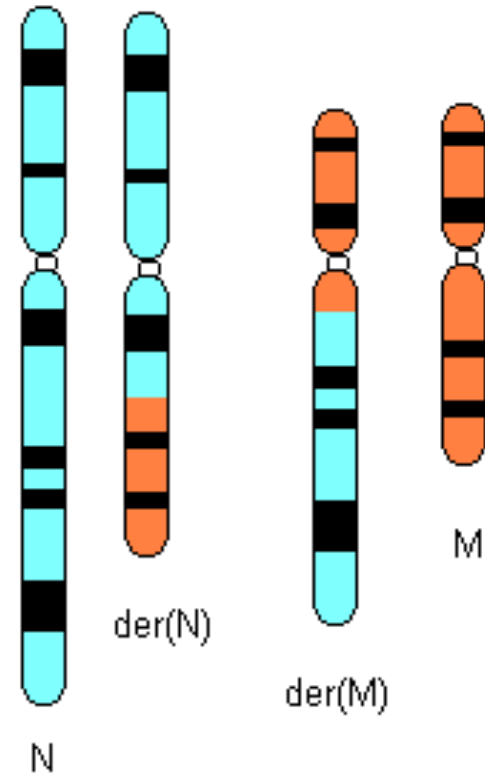
### **XXX females**

- ⌘ About one woman in 1000 has an extra X chromosome. It seems to do little harm, individuals are fertile and do not transmit the extra chromosome.
- ⌘ They do have a reduction in IQ comparable to that of Klinefelter's males.

## Structural aberrations

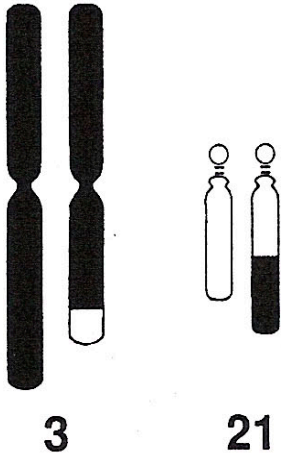
- Translocations
  - ⌘ In a balanced translocation there is no net gain or loss of chromosomal material, two chromosomes have been broken and rejoined in the wrong combination

reciprocal translocation  
between chromosomes N and M

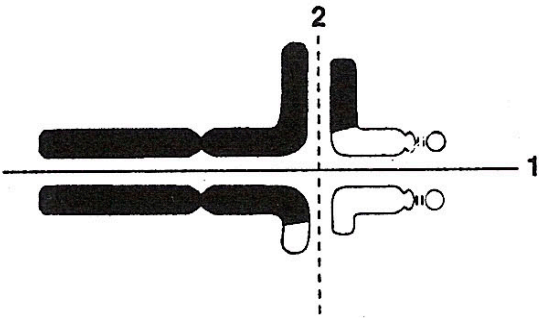


# Structural aberrations

TRASLOCAZIONE RECIPROCA

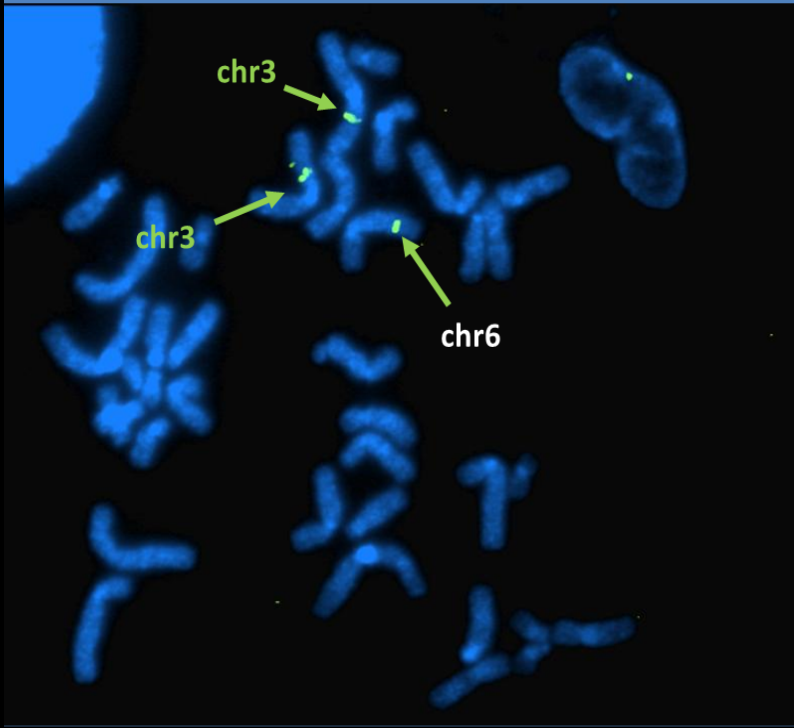


APPAIAMENTO ALLA MEIOSI



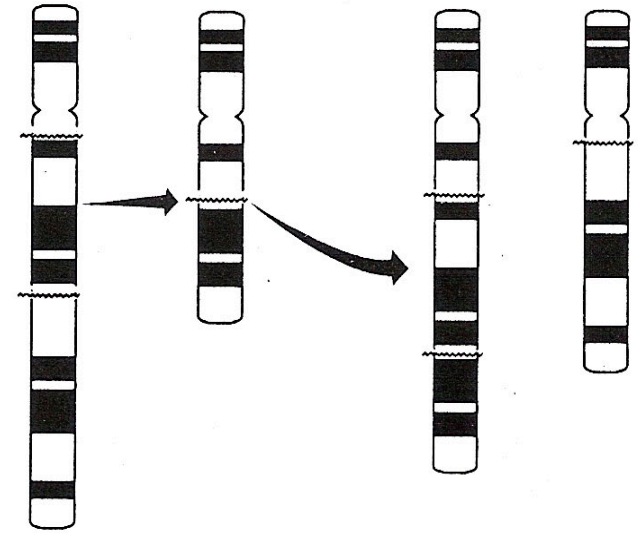
SEGREGAZIONE	ALTERNATA		ADIACENTE 1		ADIACENTE 2	
	NORMALE	BILANCIATA	SBILANCIATA		SBILANCIATA	
GAMETI						
FIGLI	NORMALE	TRASLOCAZIONE BILANCIATA	TRISOMIA 3q MONOSOMIA 21q	MONOSOMIA 3q TRISOMIA 21q	TRISOMIA 3 MONOSOMIA 21	MONOSOMIA 3 TRISOMIA 21

- maggiori sono le dimensioni cromosomiche, minore è la possibilità di una gravidanza a termine
- minori sono le dimensioni, maggiore è il rischio di un feto malformato
- Sesso del genitore donna > uomo (gli spermatozoi hanno il 7.5% di difetti contro l'1% degli oociti, ma sono selezionati)
- Il rischio aumenta se il difetto è stato accertato a partire da un figlio precedente con cariotipo sbilanciato

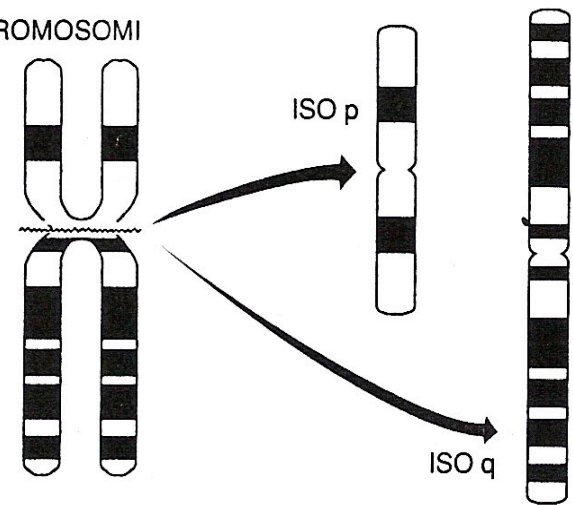


**Traslocazioni  
inserzionali  
e  
Isocromosomi**

INSERZIONE

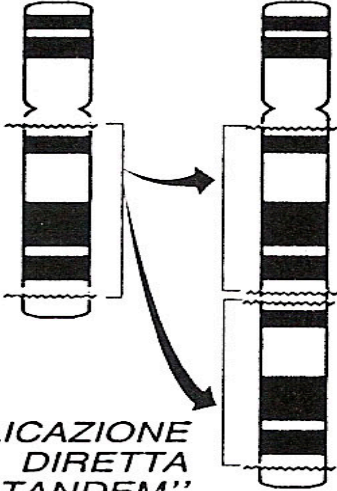


ISOCROMOSOMI

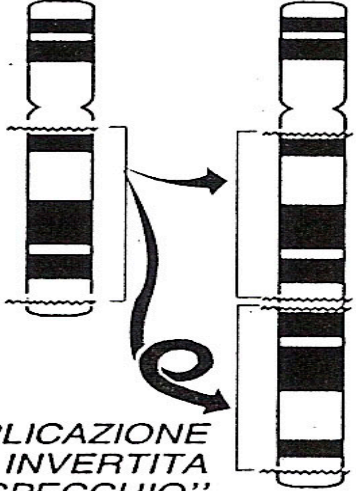


# Structural aberrations

## DUPLICAZIONE

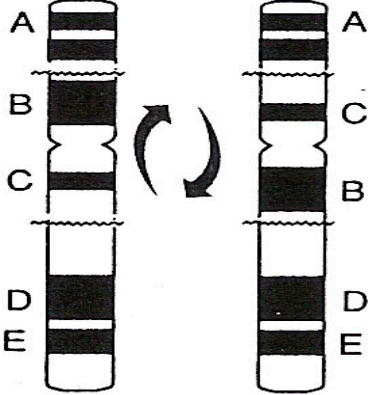


*DUPLICAZIONE DIRETTA "TANDEM"*

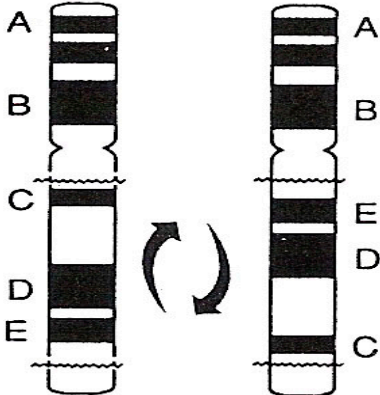


*DUPLICAZIONE INVERTITA "A SPECCHIO"*

## INVERSIONE



*PERICENTRICA*



*PARACENTRICA*

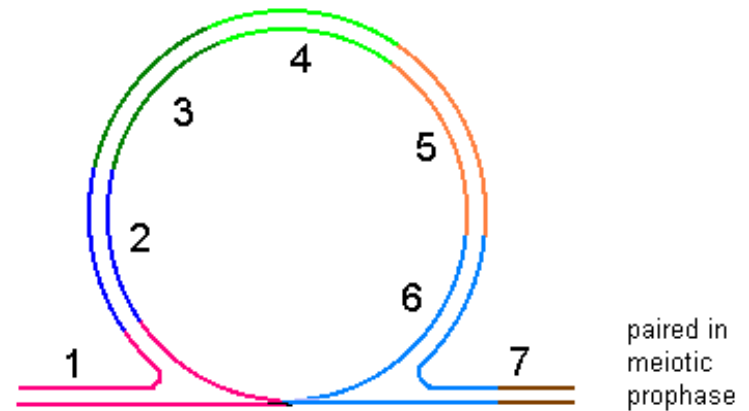
# Structural aberrations

## Inversions

⌘ In an inversion, a piece of chromoson has been lifted out, turned around and reinserted.

⌘ If this includes the centromere then th inversion is termed **pericentric**.

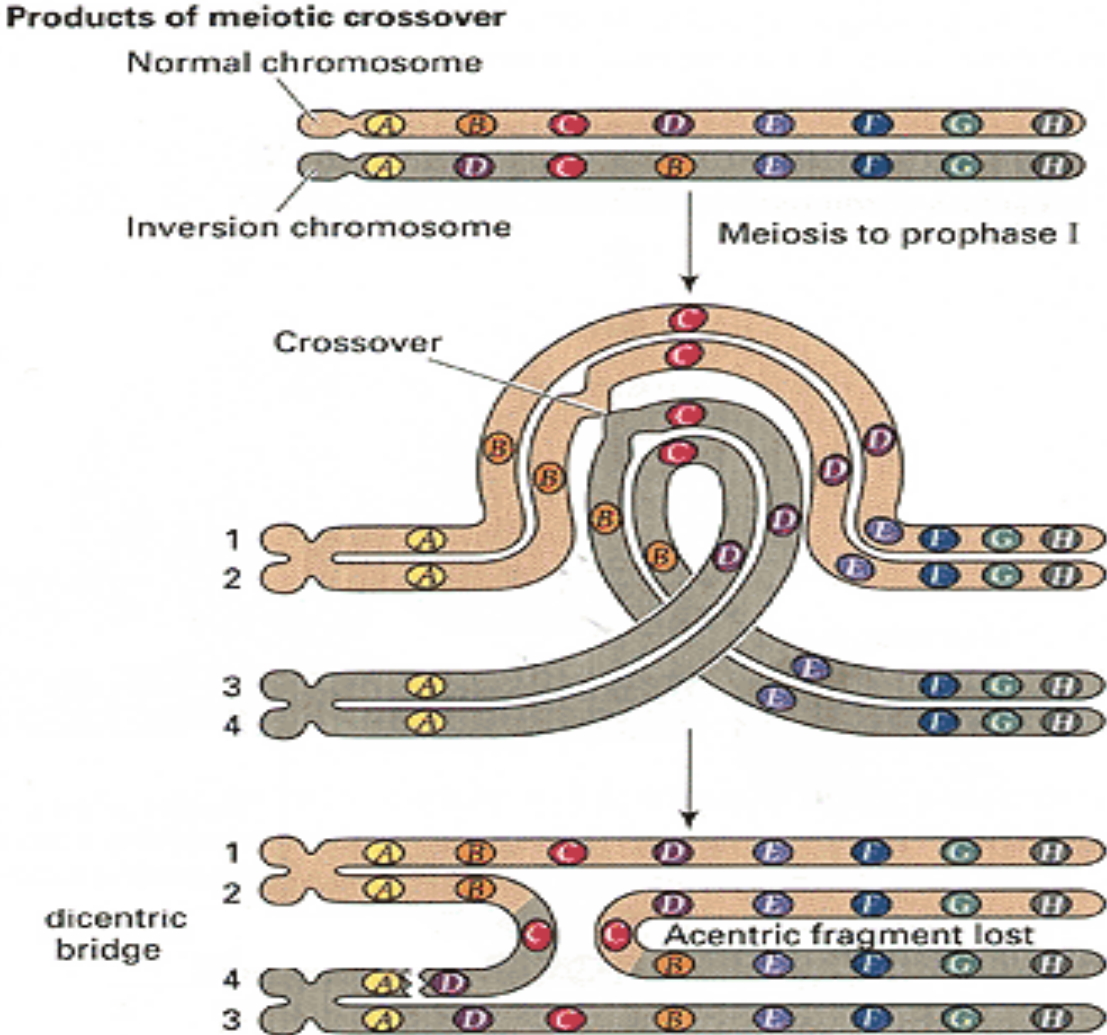
⌘ If it excludes the centromere then it is a **paracentric** inversion.





# Structural aberrations

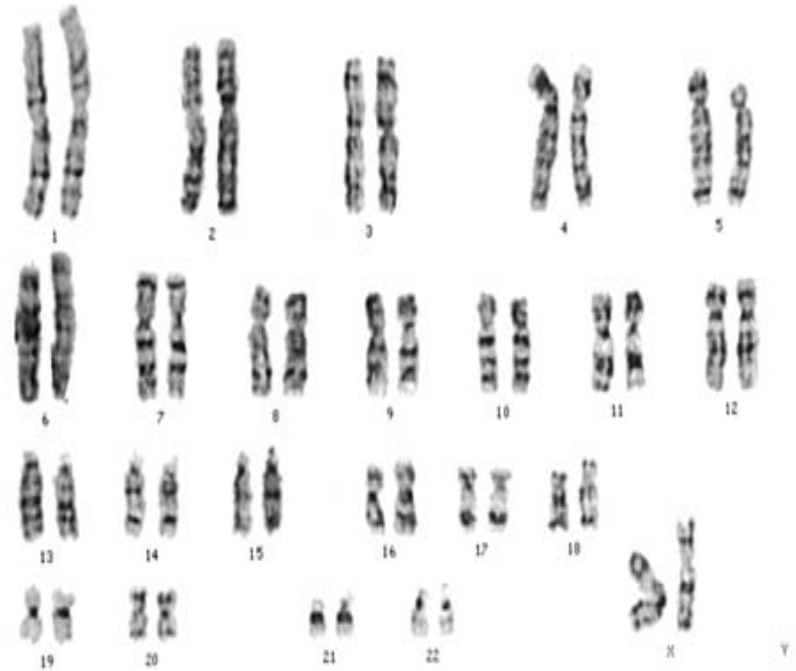
## Paracentric inversions

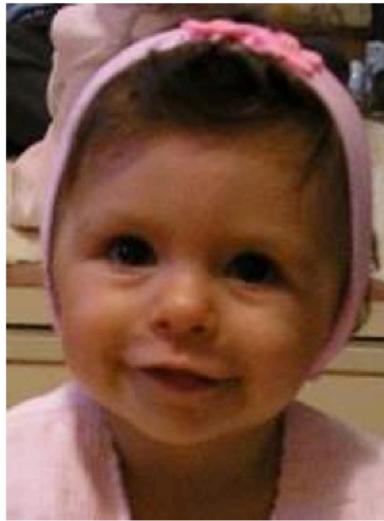


# Unbalanced rearrangements

## Deletions

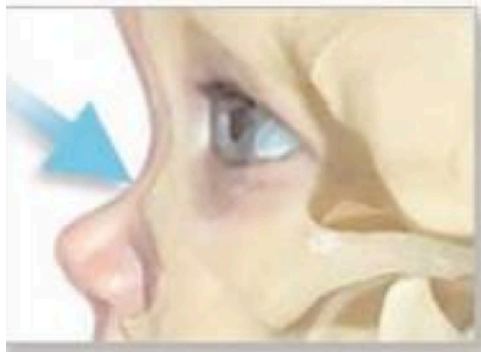
- ⌘ Deletions may either be either interstitial or terminal.
- ⌘ *If big enough to be visible a deletion must be removing many genes and will probably give rise to a severe phenotype*
- ⌘ An example of an **interstitial deletion** is Williams syndrome
- ⌘ A **terminal deletion** involves the loss of material at the end of the short arm of chromosome 4. It causes “Wolf Hirschhorn” syndrome.





## Williams-Beuren

- prevalenza alla nascita 1/7500-1/20.000, ma può non essere diagnosticata





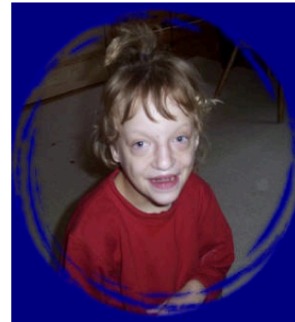
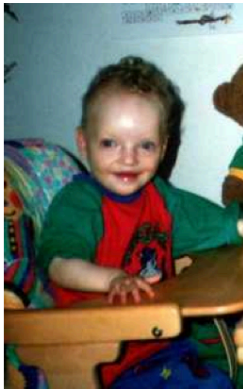
## Williams aspetto e segni

- Faccia da elfo
  - Occhi blu (77%) con pattern stellato dell'iride (74%) ma questo vale per i nordeuropei, strabismo (40%)
  - Naso con la punta bulbosa
  - bocca larga e guance piene
  - microdontia e micrognazia
  - Statura 10 cm in meno del normale
  - ipercalcemia
  - stenosi periferica delle arterie polmonari
  - stenosi aortica sopravvalvolare
- 
- delezione "de novo"
  - trasmissione autosomica dominante
  - delezione di 1.6MB da 21 geni contigui in eterozigosi a 7q11.23
    - gene dell'elastina
    - LIM kinase 1 (LIMK1)
    - CLIP-115 che lega i microtubuli
    - Fattori di trascrizione GTF2I e GTF2IRD1
    - effetto posizionale su altri geni circostanti la delezione

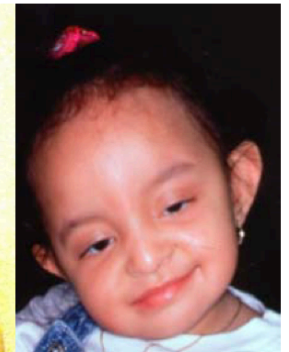



## Wolf-Hirschhorn

- Scarso accrescimento
- Ritardo mentale, ipotonia
- Labbro leporino
- Conformazione ad elmo di guerriero greco



**Wolf-Hirschhorn  
delezione a 4p16.3**





**Microdeletion Syndromes  
Currently  
Diagnosable with FISH**

Cri-du-Chat

Miller-Dieker Syndrome

Smith-Magenis Syndrome

Steroid Sulfatase Deficiency

DiGeorge/Velo-Cardio-  
Facial/CATCH-22/  
Shprintzen Syndrome

Kallman Syndrome

Williams Syndrome

Wolf-Hirschhorn

Prader-Willi/Angelman  
Syndrome

## traslocazioni robertsoniane (rob)

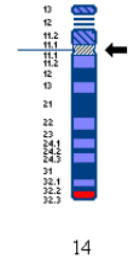
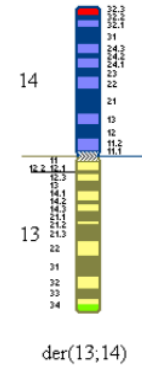
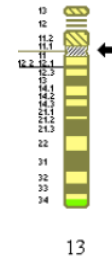
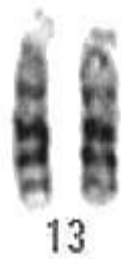
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- coinvolgono i cromosomi acrocentrici 13, 14, 15, 21 e 22
- nessuna regione cromosomica è assente, perché questi contengono un braccio corto privo di geni che può risultare perduto con la fusione dei bracci q di due cromosomi acrocentrici
- La più frequente traslocazione Robertsoniana è la rob(13q14q) che rappresenta il 75% di tutte le rob
- segue poi la rob(14q21q) e la rob(21q21q)
- si formano in genere durante la meiosi femminile e comportano infertilità maschile o abortività ripetuta.




# Robertsonian translocations

Involve the **acrocentric** chromosomes







## Percentuale alla nascita di figli con cariotipo sbilanciato da genitori con traslocazione robertsoniana

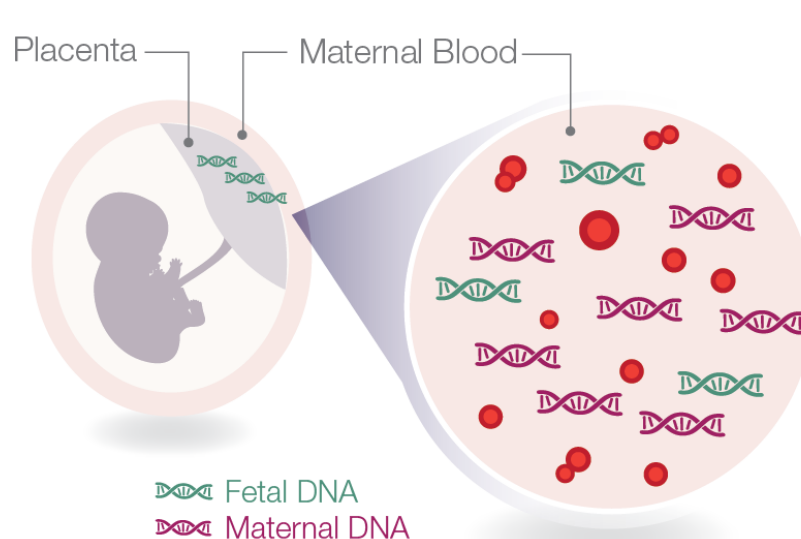
- $t(13;14)$  M=F 1%
- $t(14;21)$  F 15% M 2%
- $t(21;22)$  F 10% M 5%
- $t(21;21)$  M=F 100%

# NON INVASIVE PRENATAL TEST

## NIPT cff DNA

- < 1 % del DNA totale nel circolo materno è fetale
- 5-30 % del cell-free DNA nel circolo materno è fetale
- La NIPT misura il rapporto tra le sequenze che appartengono ai cromosoma 21, 18 e 13 rispetto alle sequenze di controllo

# Fetal and maternal cell-free DNA (cffDNA and cfmDNA) physiologically circulate in the maternal blood



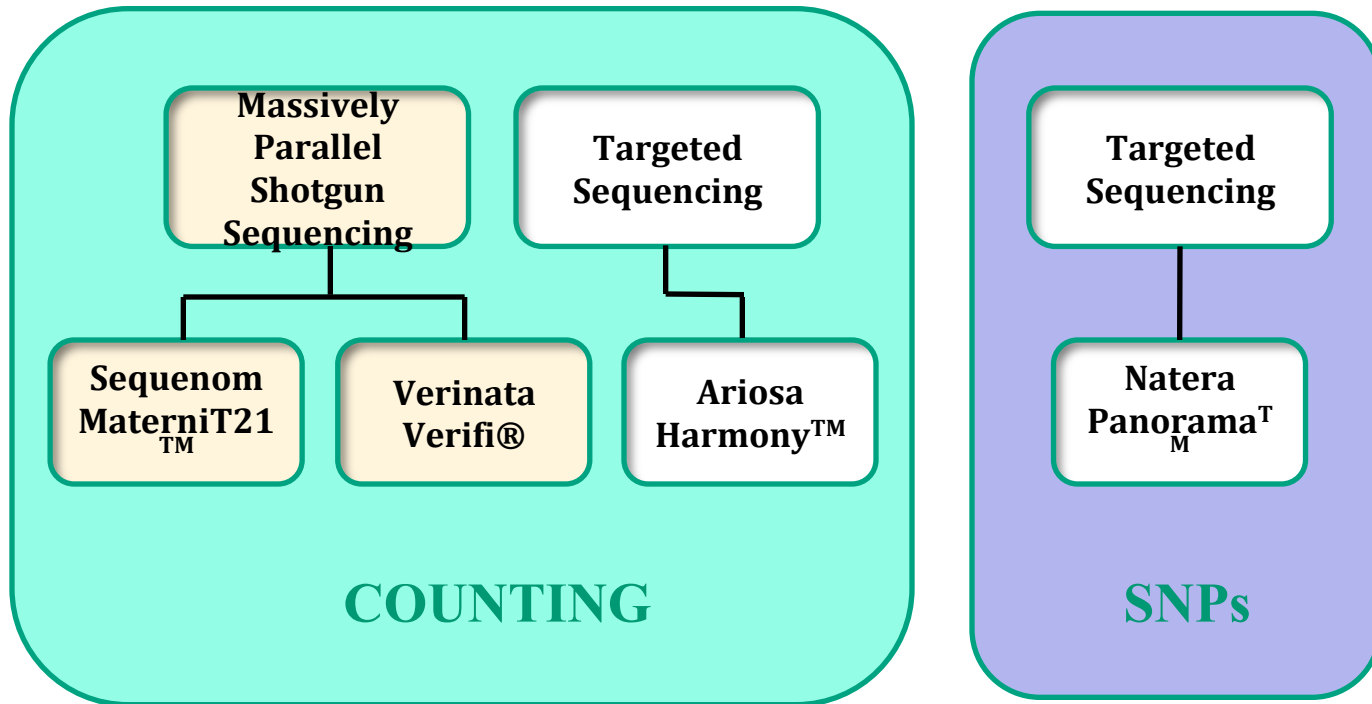
**cfDNA comes from apoptotic cells derived from:**

- Maternal Circulation
  - Adipocytes
  - White Blood Cells
- Fetal
  - Placental cells (trophoblasts) in the maternal circulation

It can be identified and isolated from the 10<sup>th</sup> week of pregnancy by blood test in the mother

# Differentiating NIPT Methodologies

fcfDNA is heavily methylated and thus can be separated from the maternal DNA



[Lancet](#). 1997 Aug 16;350(9076):485-7.

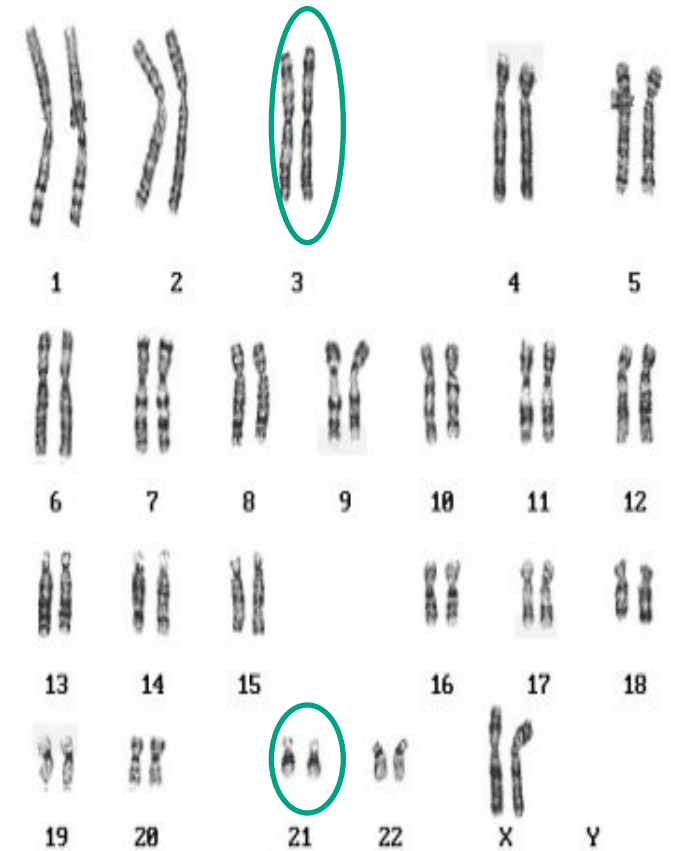
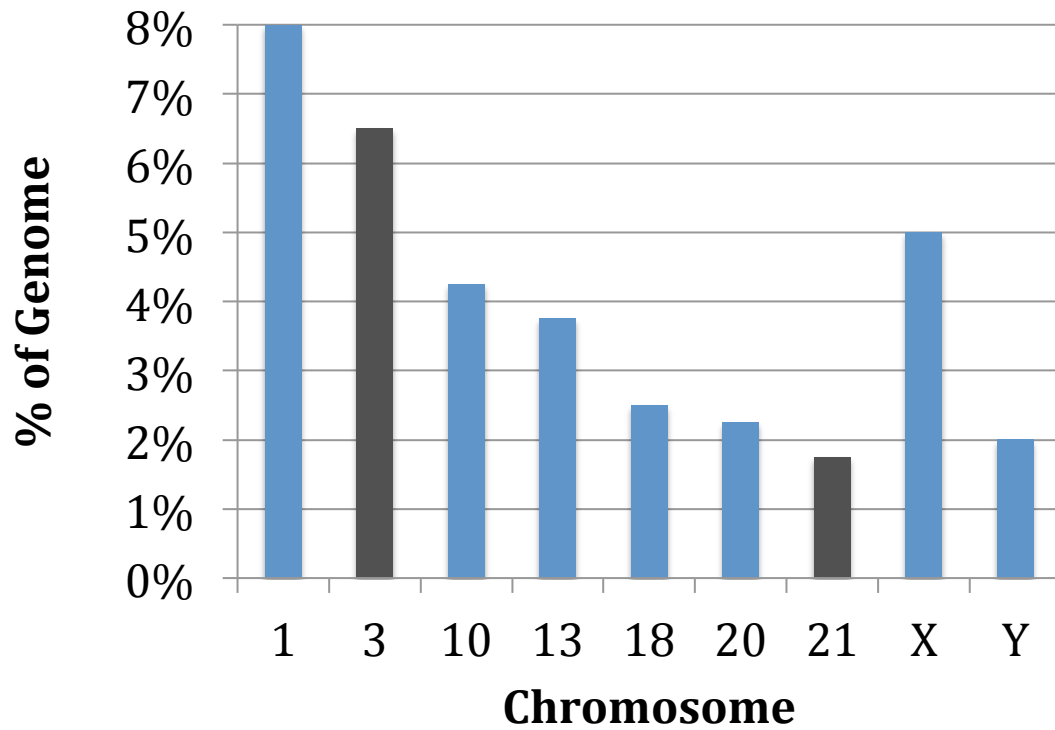
**Presence of fetal DNA in maternal plasma and serum.**

[Lo YM](#), [Corbetta N](#), [Chamberlain PF](#), [Rai V](#), [Sargent IL](#), [Redman CW](#), [Wainscoat JS](#).

Nuffield Department of Clinical Biochemistry, John Radcliffe Hospital, University of Oxford, UK.

# Relative Size of Chromosomes

COUNTING



# Counting

**Chromos  
ome 21**

**Chromo  
some 3**



# Counting chromosome-specific (21,13,18, X) copy numbers

**Chromosome 21** Chromosome 3

Expected Amount:

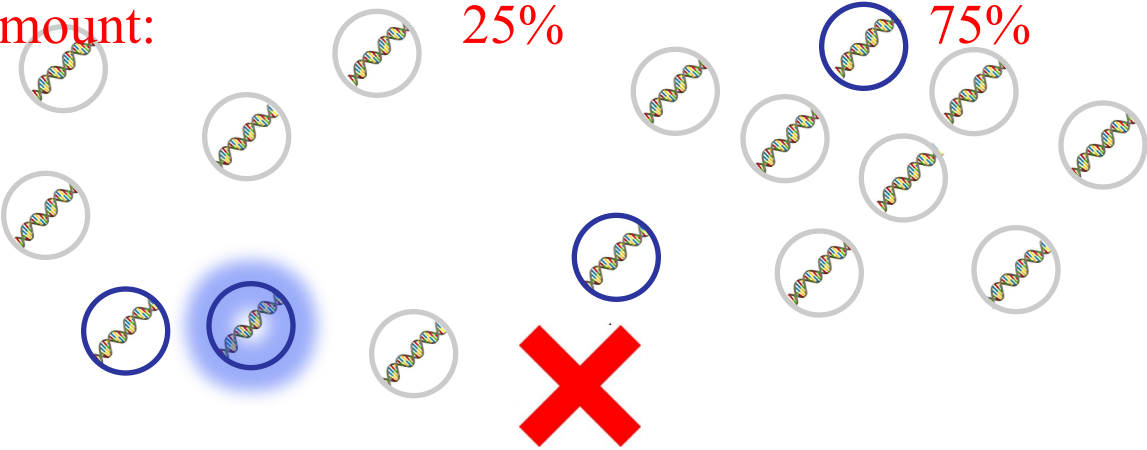
20%

80%

Observed Amount:

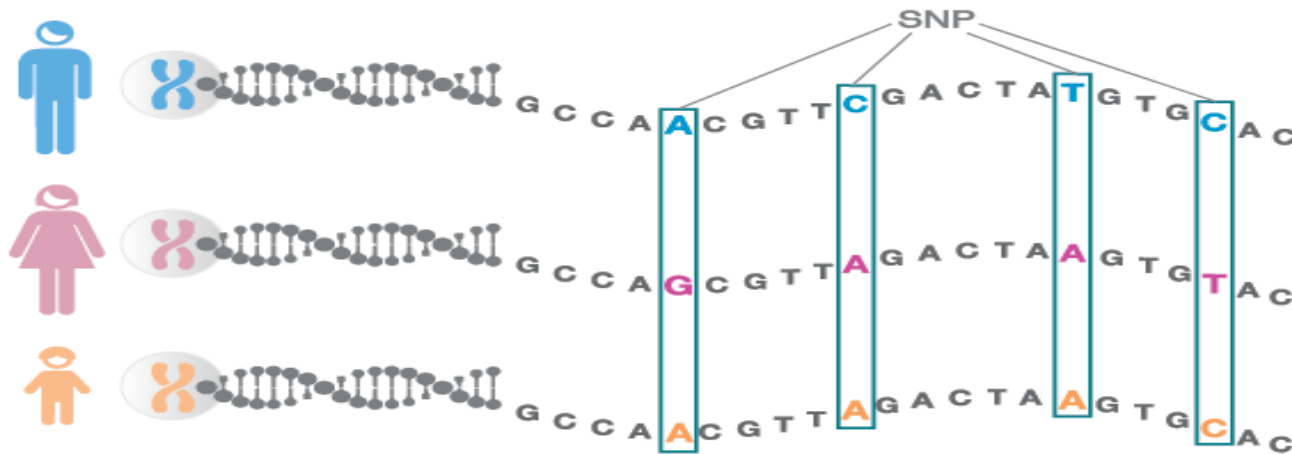
25%

75%



# SNP = Single Nucleotide Polymorphism

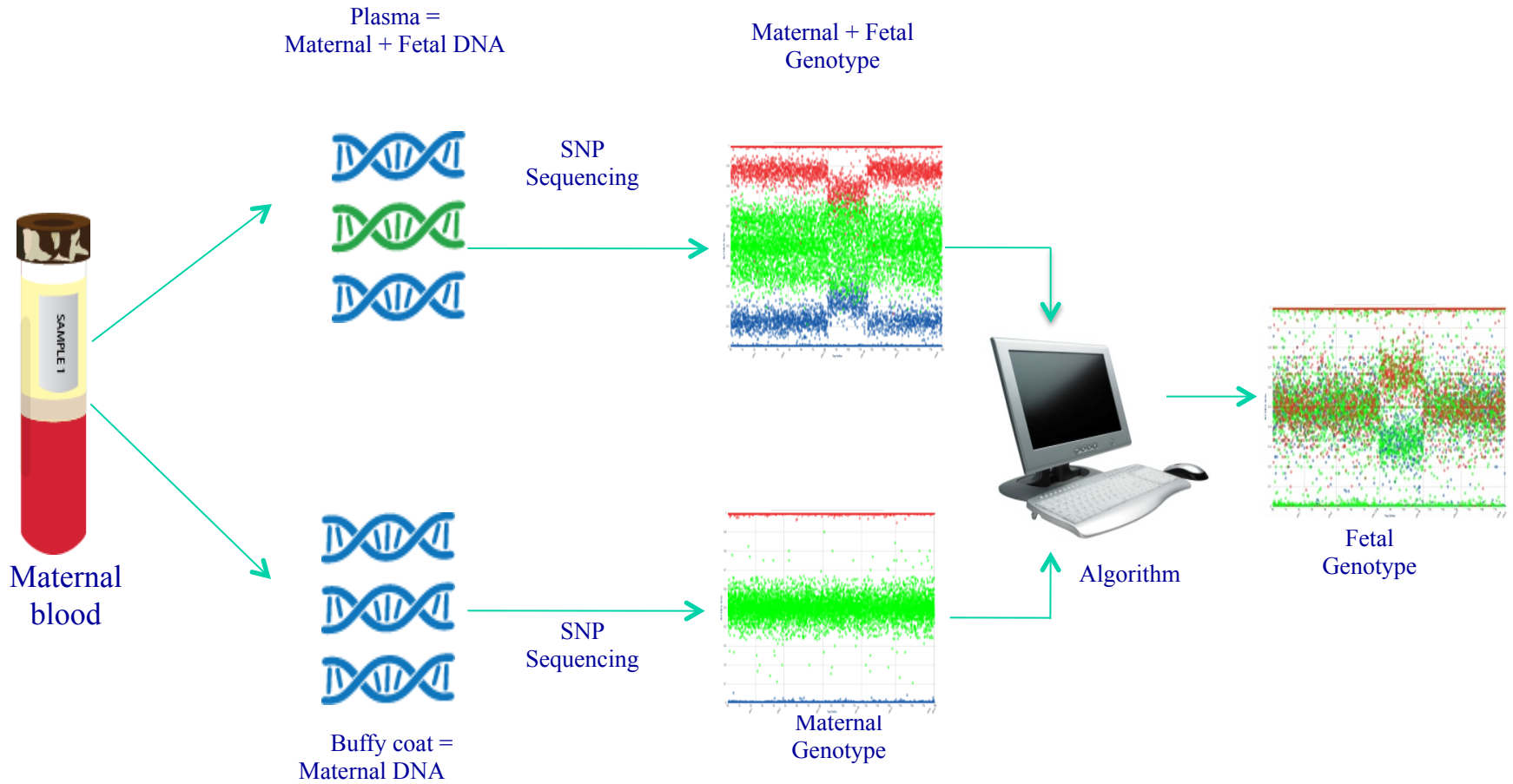
## VERY HIGH SNP DENSITY REQUIRED



- A DNA sequence variation occurring when a single base pair (nucleotide) - A, T, C, or G - is changed.
- These are **normal** genetic changes that occur in every person

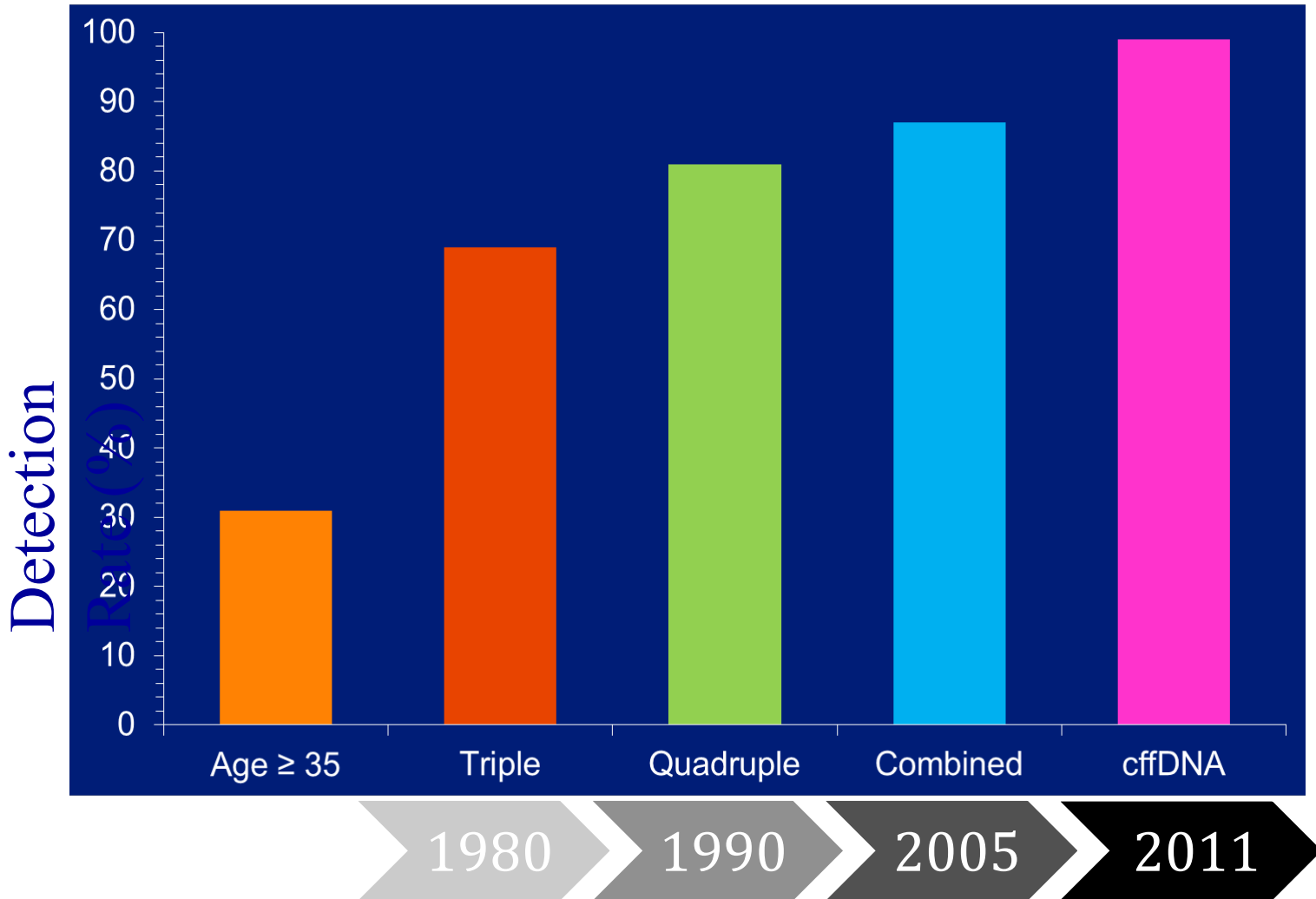


# SNP approach Using the Buffy Coat to Sequence Maternal Genotype



**(cffDNA + cfmDNA) FF >4%**

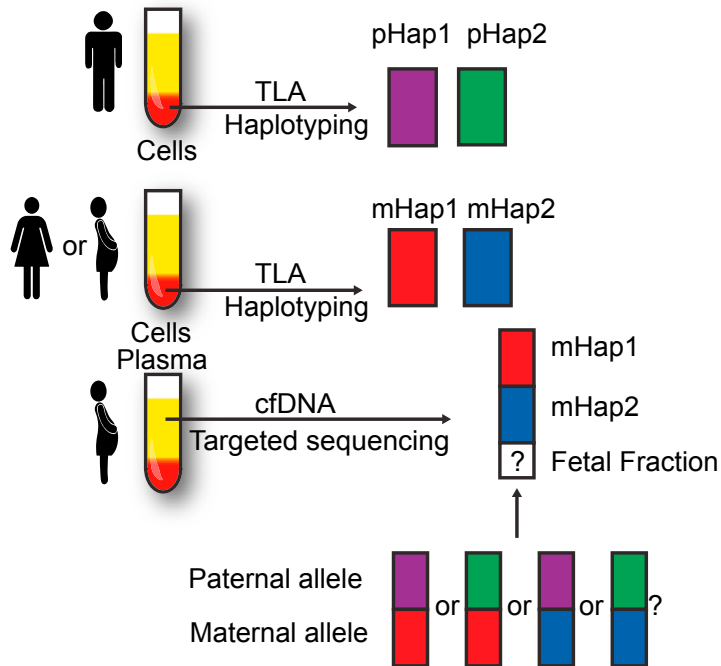
# Aneuploidy Screening Approach: Observed Detection Rates



# NIPD IN MENDELIAN

- **Non invasive prenatal **D**agnosis:**  
EARLY STAGE
  - -CFTR
  - -BETA Thalassemia
  - -metabolic diseases
  - -DMD
- **Bottleneck:**
    - **Discriminate parents and fetus genotypes**
    - **TARGET LOCUS AMPLIFICATION (TLA) or TARGETED REGION CAPTURE (TRC) NEEDED**

# NIPD IN mendelian diseases



## CONCEPT

-targeted amplification of the locus (TLA) containing the Known mutation

-massive SNP sequencing of parents' DNA  
-building HAPLOTYPE AND PHASING

-allele-specific SNP haplotype

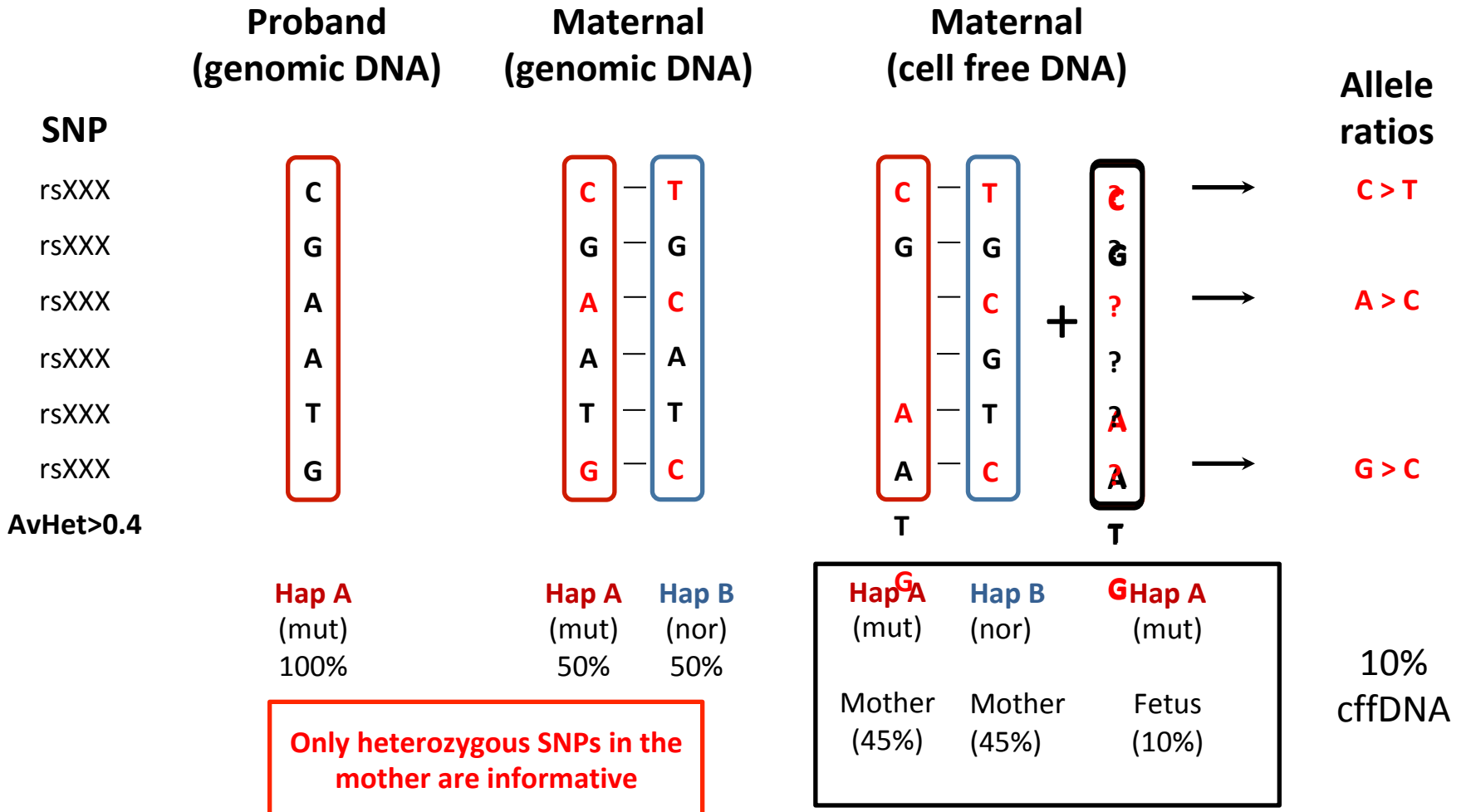
-simulating expected haplotypes in the fetus

## ARTICLE

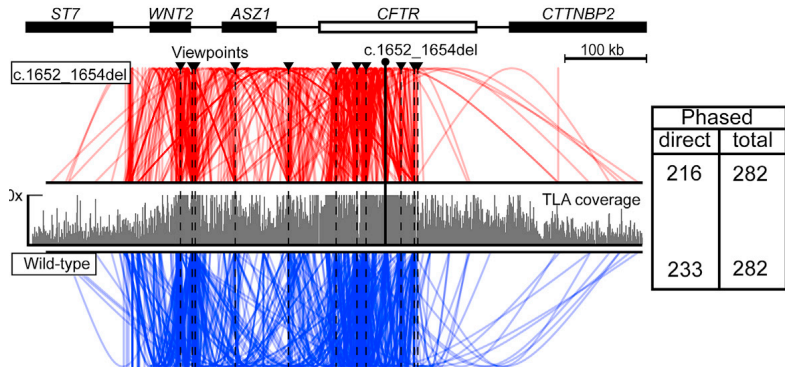
### Sensitive Monogenic Noninvasive Prenatal Diagnosis by Targeted Haplotyping

Carlo Vermeulen,<sup>1</sup> Geert Geeven,<sup>1</sup> Elzo de Wit,<sup>1,12</sup> Marjon J.A.M. Versteegen,<sup>1</sup> Rumo P.M. Jansen,<sup>2</sup> Melissa van Kranenburg,<sup>1</sup> Ewart de Bruijn,<sup>2</sup> Sara L. Pulit,<sup>2</sup> Evelien Kruisselbrink,<sup>3</sup> Zahra Shahsavari,<sup>4</sup> Davood Omrani,<sup>5</sup> Fatemeh Zeinali,<sup>6</sup> Hossein Najmabadi,<sup>6</sup> Theodora Katsila,<sup>7</sup> Christina Vrettou,<sup>8</sup> George P. Patrinos,<sup>7</sup> Joanne Traeger-Synodinos,<sup>8</sup> Erik Splinter,<sup>9</sup> Jeffrey M. Beekman,<sup>3</sup> Sima Kheradmand Kia,<sup>10</sup> Gerard J. te Meerman,<sup>11</sup> Hans Kristian Ploos van Amstel,<sup>2</sup> and Wouter de Laat<sup>1,\*</sup>

# EXAMPLE



# SNP TLA Heat Map



TLA haplotyping a CF carrier mother.

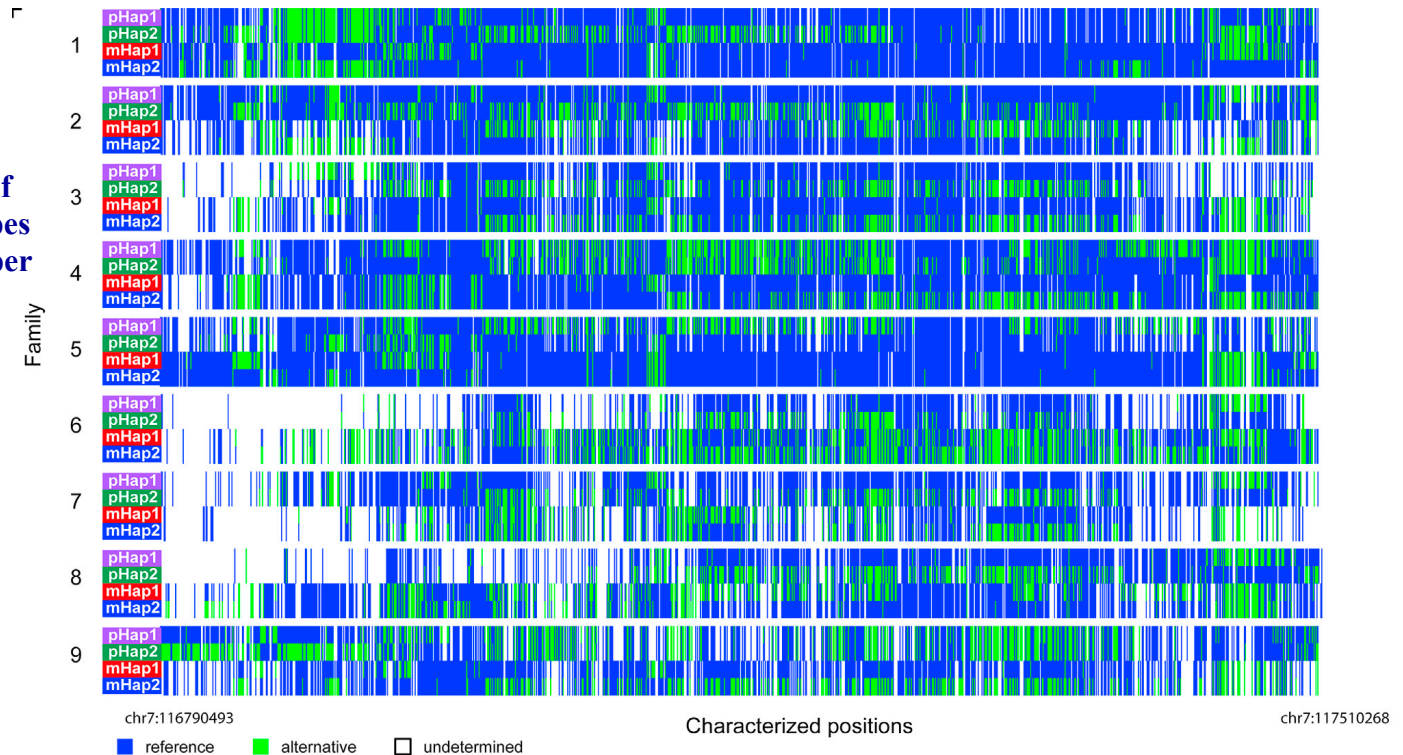
TLA: 710 kb chromosomal interval around the CFTR gene

c.1652\_1654del (top, in red) is DISEASE Phase

Schematic representation of the 36 CFTR LTA haplotypes in the 9 families clustered per family.

Blue reference allele

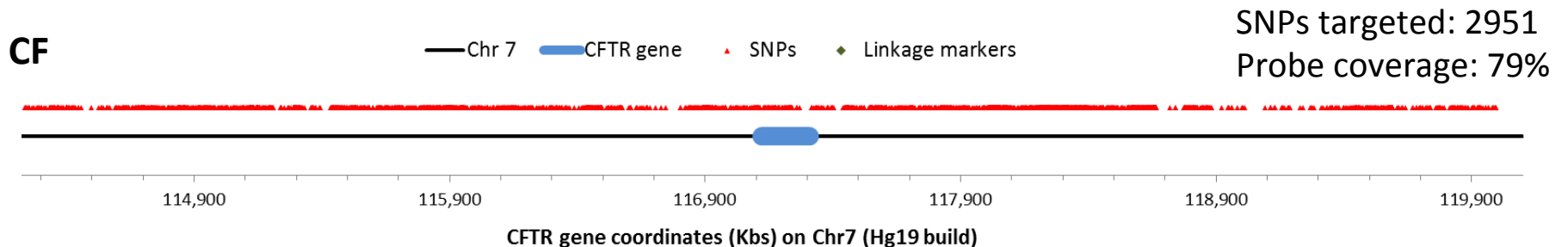
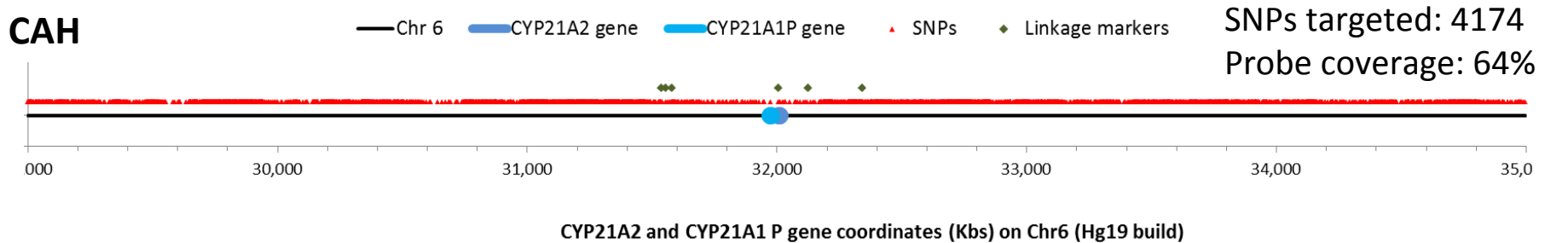
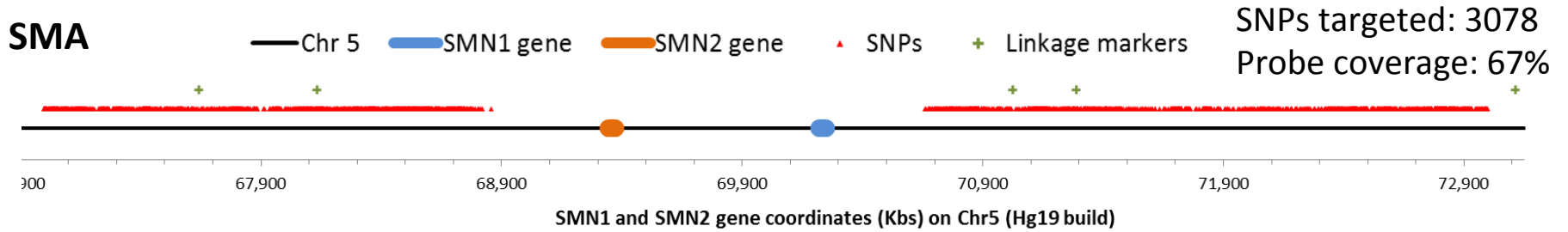
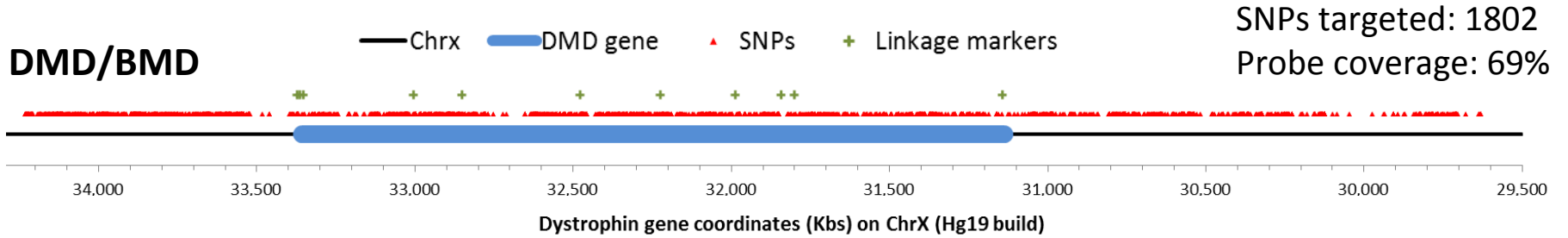
Green variant



# Relative Haplotype Dosage (RHDO)

- **Linkage based assay using SNPs**
- **Tracks inheritance of the mutated genes**
- **“phasing the SNPs”**
- **TLA or capture regions of interest using probe libraries**
- **Multiplexing of different disorders to reduce costs**

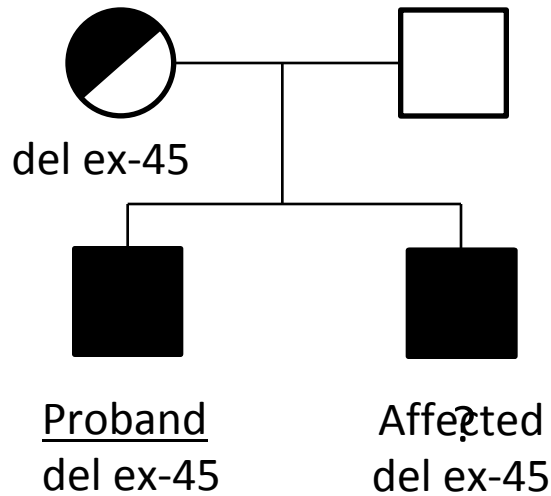
# Custom built SNP libraries





# Non-invasive prenatal diagnosis of Duchenne and Becker muscular dystrophies by relative haplotype dosage<sup>†</sup>

Michael Parks\*, Samantha Court, Siobhan Cleary, Samuel Clokie, Julie Hewitt, Denise Williams, Trevor Cole, Fiona MacDonald, Mike Griffiths and Stephanie Allen



**Disorder:** Duchenne muscular dystrophy

**Mutation:** deletion of exon 45

**Reference haplotype:** affected male sibling

**Gestation:** 8 w + 4 d

**Fetal fraction:** 9.24%

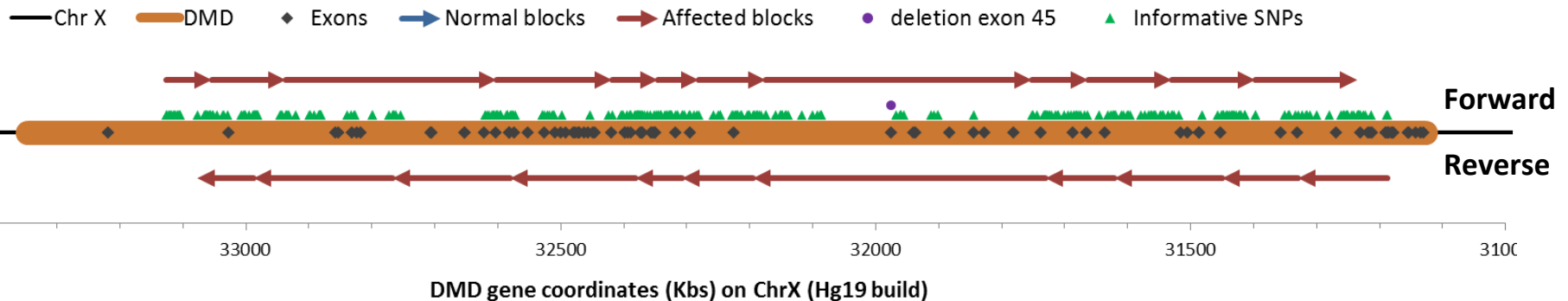
**SNPs sequencing depth:** 48

**Informative SNPs used:** 325

**SPRT classifications:** 12 for / 11 rev

**Outcome:** **AFFECTED**

## RHDO analysis



# NIPD fetal sexing (X-linked RDs)

- Fully validated and into routine testing
- Maternal blood sample from 7/9\* weeks gestation
- Real Time PCR – SRY and CCR5 ( Y markers)
- Identifies the fetal sex only

# NON INVASIVE PRENATAL TEST

## Sensitivity NIPT for T21, T18, T13

### Sensitivity

T21 : 99.5 %

T18 : 98 %

T13 : 90 %

### False-negatives

If NIPT is normal, the residual risk for

trisomy 21, 18, 13 :  $< 1 / 10.000$

# NON INVASIVE PRENATAL TEST

## Specitivity NIPT for T21, T18, T13

### Specitivity

T21 > 99.9 %

T18 > 99.9 %

T13 > 99.9 %

### False-positives

If NIPT is abnormal, the risk that the fetus  
has no trisomy 21, 18, 13 :

small (high risk population)

? (low risk population)

# NON INVASIVE PRENATAL TEST

NIPT is the test of choice when there is :

- Increased maternal age
- Increased risk on Combination or triple test
- Anxiety for invasive procedure (AC / CVS)



# NON INVASIVE PRENATAL TEST

## NIPT Contra indications

NIPT is NOT the test of choice when there is :

- Fetal anomalies on ultrasound
- A triplet pregnancy
- Vanished twin
- Known genetic anomalies that cannot be diagnosed by NIPT



# NON INVASIVE PRENATAL TEST

## NIPT Advantages versus combi test with AC / CVS

- High sensitivity (few false-negatives)
- High specificity (few false-positives)
- More than T21
- Non-invasive : no fetal risk
  - CVS : Risk of miscarriage : 1-2 %
  - AC : Risk of miscarriage : 0.5 %

# NON INVASIVE PRENATAL TEST

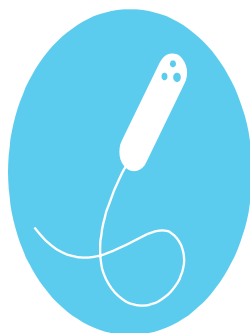
## NIPT Disadvantages

- Expensive (690 Euro)
  - Combi test : 150 Euro
  - Combitest + AC + karyotype : 1000 Euro ?
- Only testing 3 chromosomes, and gender
- Failure rate (after 1 or 2 tests): < 1 %
- Specific kits
- Not available everywhere



# INFERTILITA'

La WHO definisce infertilità l'assenza di concepimento dopo 12/24 mesi di rapporti mirati non protetti in una donna con meno di 35 anni; i mesi si riducono a 6 qualora l'età del partner femminile superi quella prima citata.



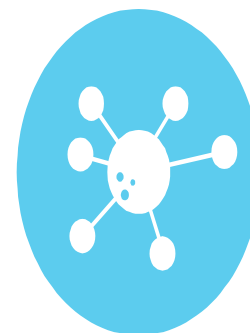
FATTORE  
ORGANICO-  
FUNZIONALE



FATTORE  
GENETICO

Patologie  
D o R

Autosomi o  
cromosomi  
sex



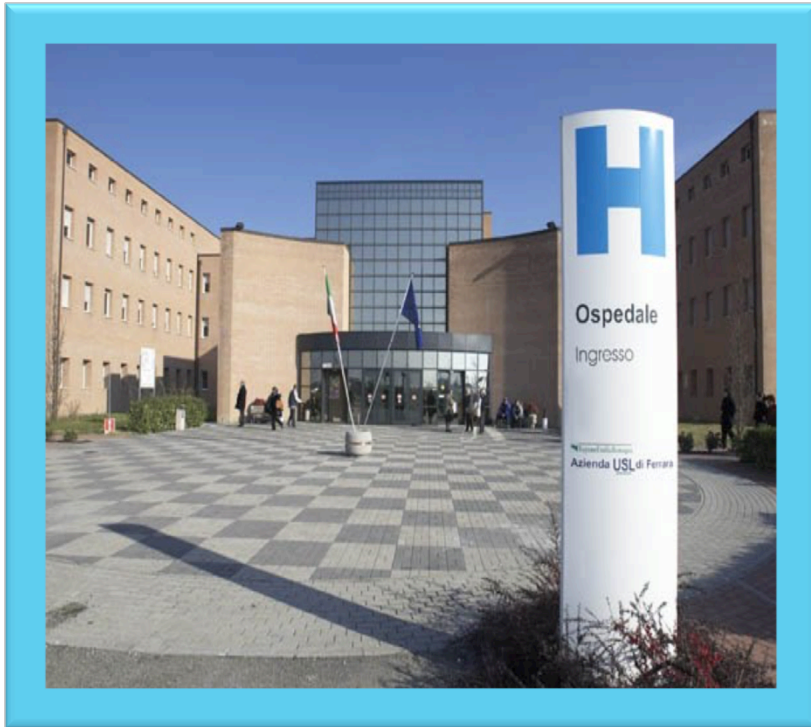
FATTORE  
EPIDEMIOLOGI  
CO

# Preimplantation Genetic Testing (PGT)

Procedura che permette di identificare in vitro un'embrione “sano” da trasferire in utero

- PGS, cioè Preimplantation Genetic Screening, punta in un ciclo di **Procreazione Medicalmente Assistita (PMA)** a minimizzare il rischio di insuccesso procreativo dato dallo sviluppo di anomalie cromosomiche nell'embrione, un evento de novo, non prevedibile ma stimabile in relazione alla “vecchiaia” dei gameti (e quindi dei partner) → **IMPIANTO DI EMBRIONI CON CARIOTIPO NORMALE**
- PGD, cioè Preimplantation Genetic Diagnosis, è un'analisi ideata per identificare e trasferire, tra gli embrioni prodotti in un ciclo di Procreazione Medicalmente Assistita, quelli non affetti da una patologia a trasmissione ereditaria, in coppie a elevato rischio riproduttivo.

# Preimplantation Genetic Testing (PGT)



Centro di Procreazione Medicalmente Assistita dell'ospedale del Delta, presso Lugo (FE)

- Centro di PMA accreditato anche come II livello
- Collaborazione per l'analisi del cariotipo (aneuploidie) dell'embrione con la UO di Genetica Medica dell'Università degli studi di Ferrara

# GENETICA e INFERTILITA' MASCHILE

1

ABERRAZIONI CROMOSOMICHE

di numero (47, XXY)

di struttura (microriarrangiamenti  
del cromosoma Y)

2

Mutazioni del gene CFTR

mutazioni esoniche o polimorfismi  
intronic

# FIBROSI CISTICA E FENOTIPI

La fibrosi cistica è una patologia a trasmissione autosomica recessiva, definibile borderline come malattia rara, causata da mutazioni del gene CFTR (P.Phe508del la più comune).



- 7q
- 27 esoni

**FENOTIPO CLASSICO DI FC**  
(genotipo omozigote/eterozigote composto)

- Patologia polmonare,
- Patologia del tratto gastroenterico
- **INFERTILITÀ MASCHILE**

**CFTR RELATED DISEASE**  
(genotipo omozigote/eterozigote composto per le ALTRE varianti)

- In genere compromissione di un solo organo
- Agenesia congenita bilaterale dei vasi deferenti (CBAVD) la più comune → **INFERTILITÀ MASCHILE**
- **Eterozigosi composta (mutazione del gene CFTR associata a due polimorfismi intronici STR *in trans*)**

# CORRELAZIONE GENOTIPO-FENOTIPO (CBAVD)

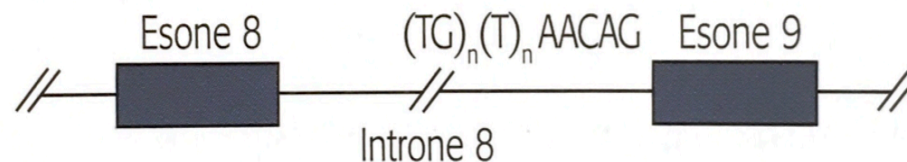
Nell'introne 8 sono presenti due unità ripetute

- dinucleotide TG (che contiene dalle 10 alle 13 ripetizioni)
- mononucleotide T (che contiene dalle 5 alle 9 ripetizioni)

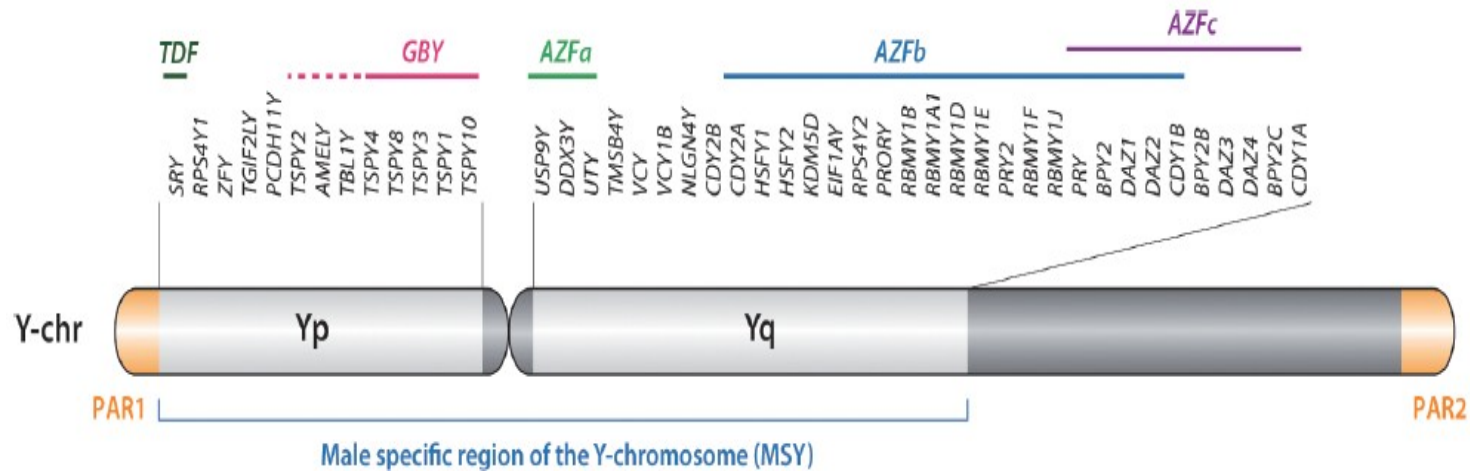
Entrambe fanno parte della regione di consensus di splicing dell'esone 9

In particolare, il numero di ripetizioni  $(TG)^{12}(T)^5$  influenza lo splicing determinando una riduzione dell'incorporazione dell'esone 9 nel trascritto maturo.

Si ottiene così una proteina incompleta e con un dominio non funzionale che rende in particolare le cellule epiteliali dei dotti deferenti “patologiche”, spiegando l'agenesia degli stessi.



# CROMOSOMA Y e MICRODELEZIONI



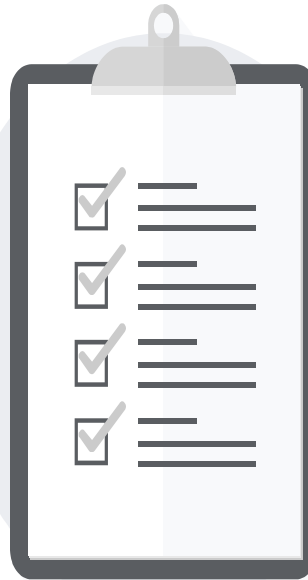
# STATISTICHE

SESSO  
MASCHI  
LE

1

Dato  
laboratoristico  
di azoospermia  
o oligospermia

2



3

Pazienti in  
percorso di  
PMA

ANALISI per  
mutazioni CFTR  
e/o MICRO-  
RIARRANGIAME  
NTI DELL'Y



# RISULTATI DELL'ANALISI DELLE MICRODELEZIONI CROMOSOMA Y

		AZFa	AZFb	AZFc	% di microdeleti
Azoospermia	70	-	-	6	8,5%
Oligoastenosp ermia	2	-	-	1	50%
Oligospermia	12	-	-	1	8,3%
Criptozoosper mia	2	-	-	-	-
Generico <i>infertilità</i>	27	-	-	1	3,7%
<b>TOT.</b>	<b>113</b>			<b>9</b>	<b>7,9%</b>

# RISULTATI DELL'ANALISI DELLE DUPLICAZIONI CROMOSOMA Y

		AZFa	AZFb	AZFc	% di duplicati
Azoospermia	70	-	-	5	7,1%
Oligoastenosp ermia	2	-	-	-	-
Oligospermia	12	-	-	2	16,6%
Criptozoosper mia	2	-	-	-	-
Generico infertilità	27	-	-	4	14,8%
<b>TOT.</b>	<b>113</b>			<b>11</b>	<b>9,7%</b>

# STATISTICHE DATI: GENE CFTR

	*	FIELD et al.	STUPPIA et al.	RICCABONI et al.
<b>FREQUENZA ETEROZIGOTI</b>	1 su 13,1	1 su 21,5	1 su 19,6	1 su 26,6

Frequenza portatore sano nella popolazione italiana **1 su 25-32**

# CONCLUSIONI: GENE CFTR

- ✓ Il rischio residuo di FC nel nascituro in pazienti che intraprendono percorsi di PMA non risulta essere maggiore rispetto a quello atteso nella popolazione generale; non è giustificato quindi uno screening a tappeto su tale popolazione.
- ✓ L'analisi del gene CFTR (mutazioni e polimorfismo (TG)<sup>12</sup>(T)<sup>5</sup>) nel partner di sesso maschile può essere considerato uno strumento utile per la pratica clinica quando associato ad un referto ecografico di CBAVD; qualora questo manchi non ci sono dati a supporto che confermino la necessità di indagine del gene come emerso da questo studio;
- ✓ Dal 2017 l'analisi del gene CFTR in percorsi di PMA in assenza di un dato ecografico di agenesia dei dotti è stata esclusa dai Livelli Essenziali di Assistenza (LEA).

# CONCLUSIONI: MICRO-RIARRANGIAMENTI DEL CROMOSOMA Y

- ✓ Conferma in modo inequivocabile della letteratura, che mostra come la maggior parte delle microdelezioni mappino nella regione AZFc e come queste siano principalmente associate ad un dato laboratoristico di azoospermia;
- ✓ Si è potuta apprezzare anche una percentuale non trascurabile di duplicazioni (9,7%);
- ✓ Utile per il futuro indagare sia in popolazioni di PMA che in quella generale il ruolo delle duplicazioni in tema di fisiopatologia della riproduzione.