

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Ferrara



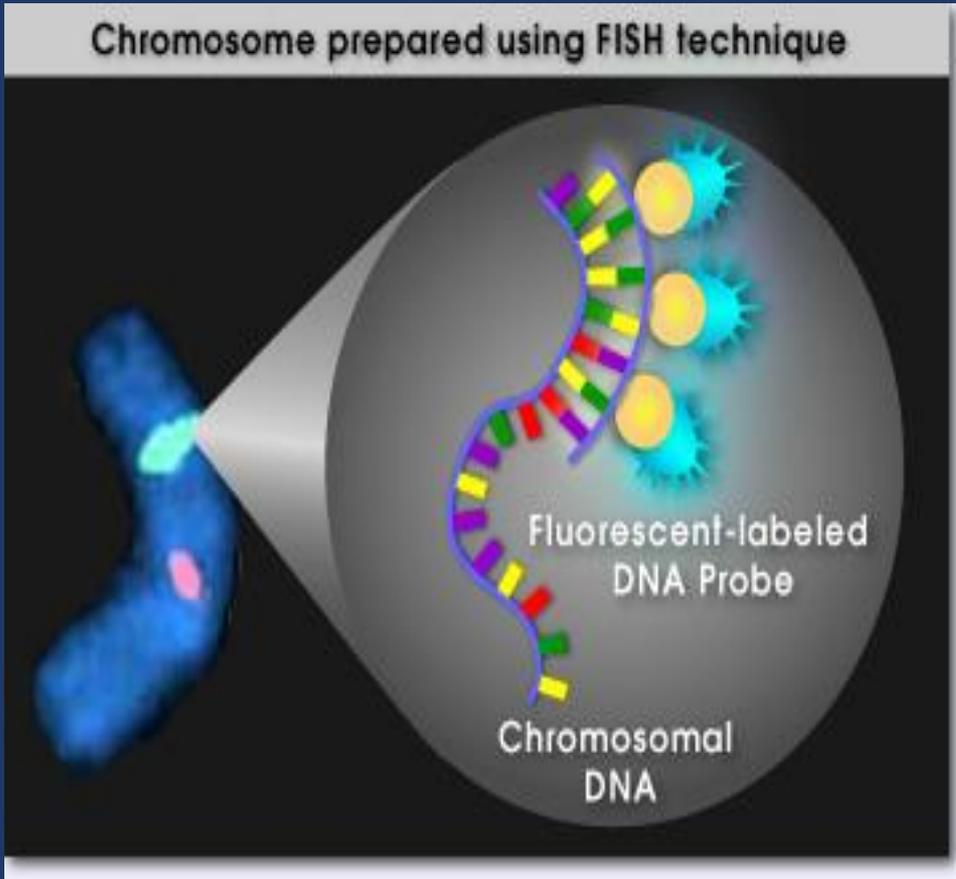
CITOGENETICA MOLECOLARE

Vincenzo Aiello

Ferrara, 2020



CITOGENETICA MOLECOLARE



FISH

Fluorescent
In
Situ
Hybridization



Cariotipo Convenzionale (CC)

Ibridazione in Situ (FISH)

Aspirato midollare
(sangue periferico)

Conteggio nucleate

Semina in terreno

Coltura per 24 - 72 h

Colchicina

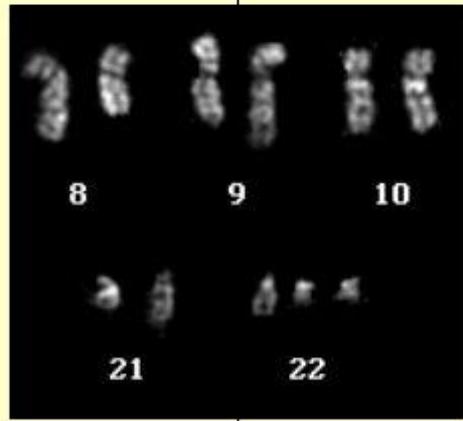
Fissazione e lavaggi

Allestimento
dei vetrini

Colorazione (bandeggio)

Selezione e cattura immagini

Elaborazione e cariotipizzazione



Conclusione diagnostica

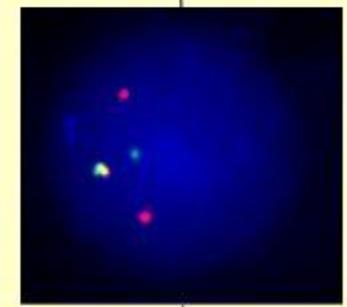
Scelta delle sonde

Denaturazione e
Ibridazione over night

Lavaggi e
controcolorazione

Valutazione immagini

Cattura / elaborazione



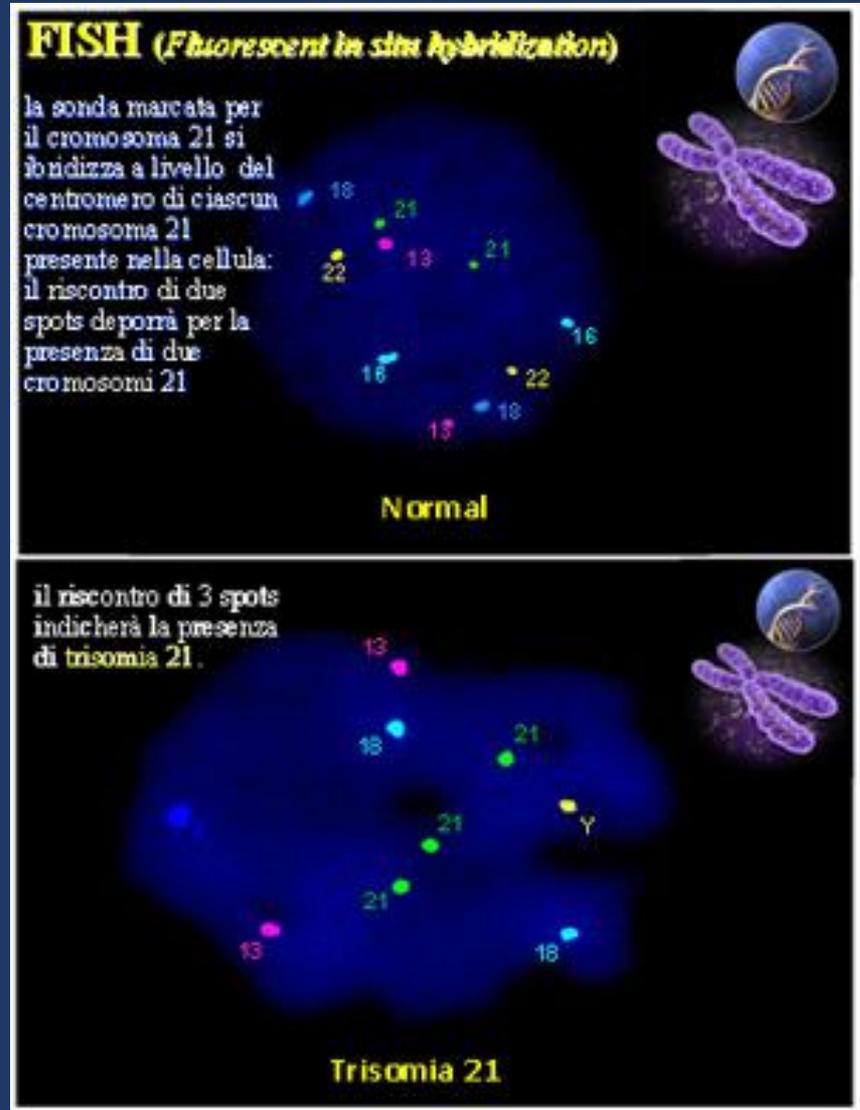
Conclusione diagnostica



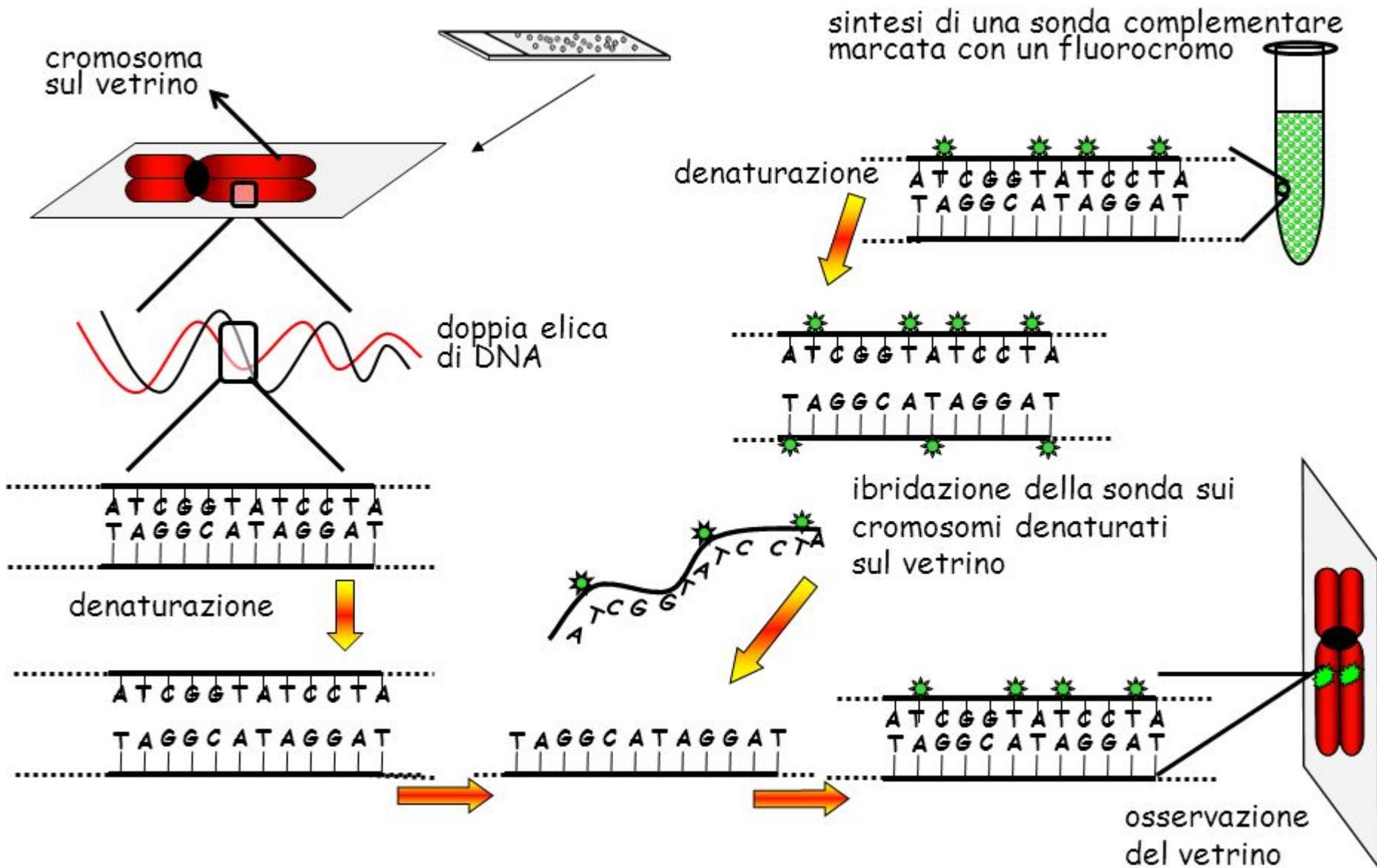
Citogenetica Molecolare FISH

Permette un'analisi mirata di una regione cromosomica consentendo di mettere in evidenza riarrangiamenti di alcune centinaia di chilobasi.

Tale identificazione avviene mediante sonde marcate impiegando fluorocromi che emettono a diverse lunghezze d'onda.

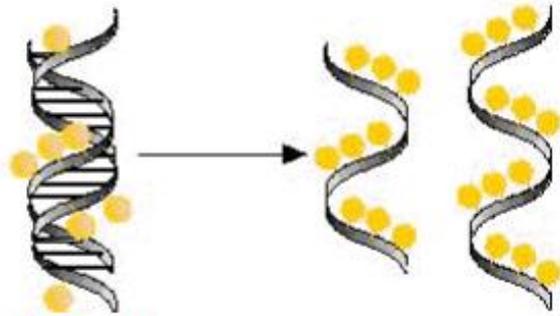


FISH: fluorescent in situ hybridization

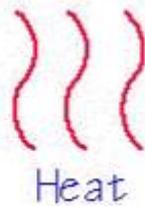


The General Principle of In Situ Hybridisation

Probe

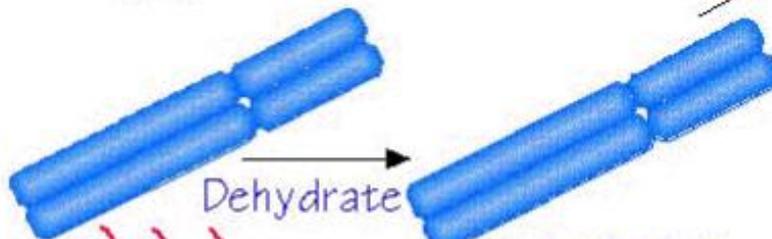


Denatured Probe



Heat

Target



Dehydrate

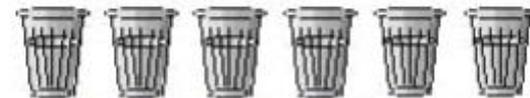
Dehydrated,
Denatured
Target



Heat



Hybridize - in
Hybridisation
solution at
37°C



Post Hybe Stringent
Washes



Spot fixed sample onto clean microscope slide



Spot 10 μ l hybridisation solution onto slide (and probe)

Apply coverslip (and probe)





Wash in 0.4 x
SSC for 2
minutes at 72°C



Wash in 2 x SSC,
0.05% Tween for
30 seconds at
room temperature

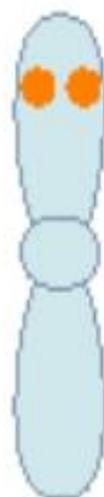


Apply 10 μ l DAPI,
coverslip and view

Tipologia di sonde utilizzate in FISH

- Sonda sequenze ripetute
- Sonda painting
- Sonda sequenza “singola”

a



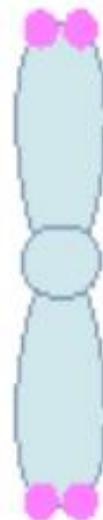
Gene-specific probe

b



Centromeric probe

c



Telomeric probe

d



Chromosome-painting probe

Repetitive-sequence probes

Examples of different types of fluorescence in situ hybridisation (FISH) probes

Expert Reviews in Molecular Medicine © 2000 Cambridge University Press

FISH sonda alfa satellite (10)



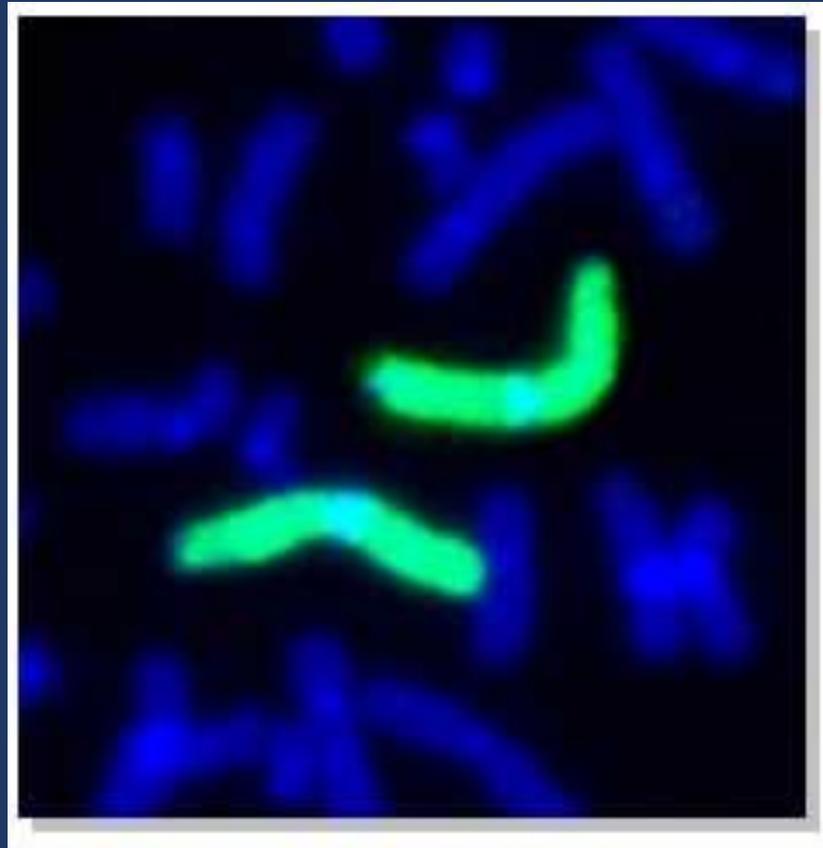
FISH sonda rDNA



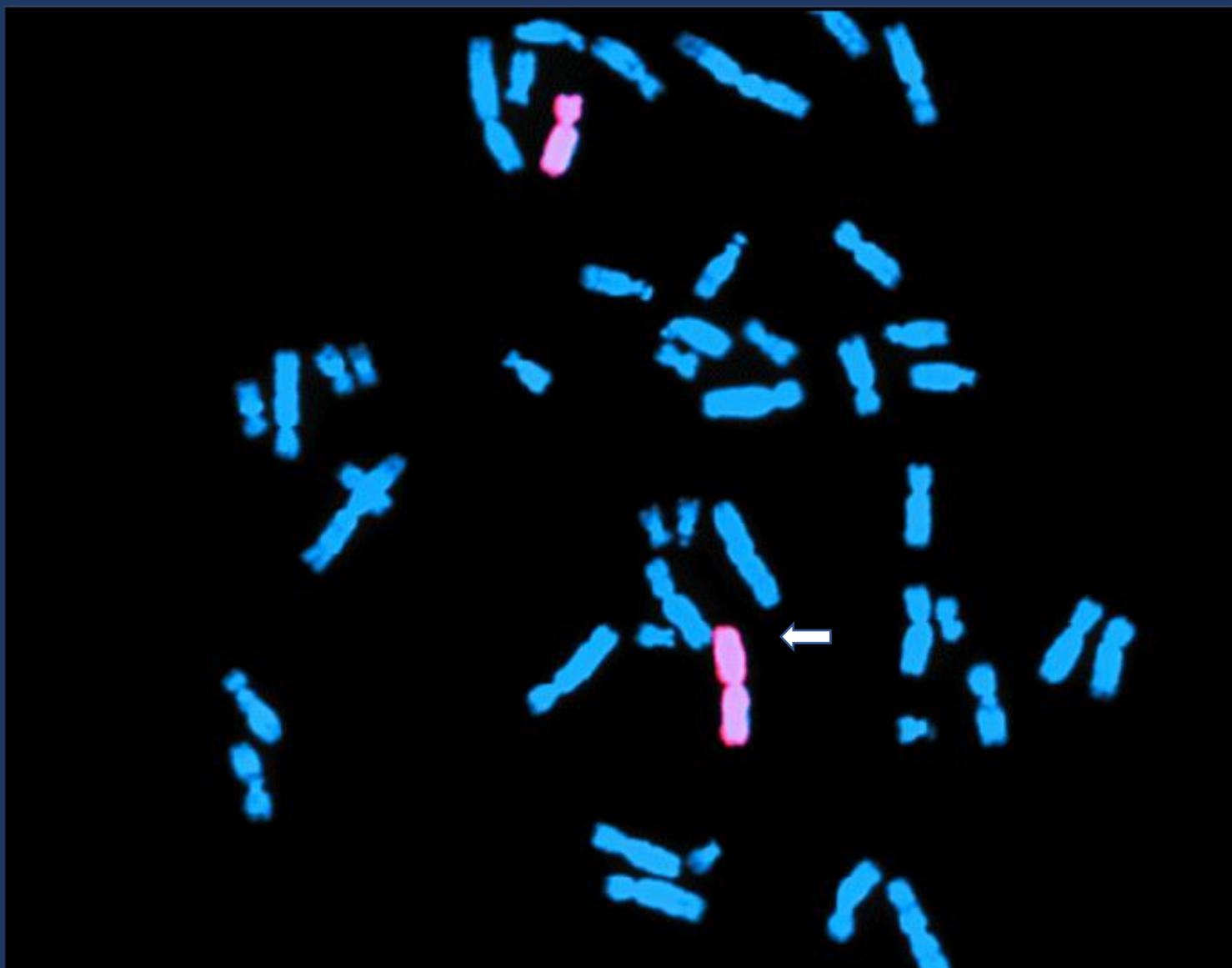
Sonde painting

- Cromosoma specifico
- Braccio specifico
- Regione/banda specifico
- Reverse painting

Painting del cromosoma 1

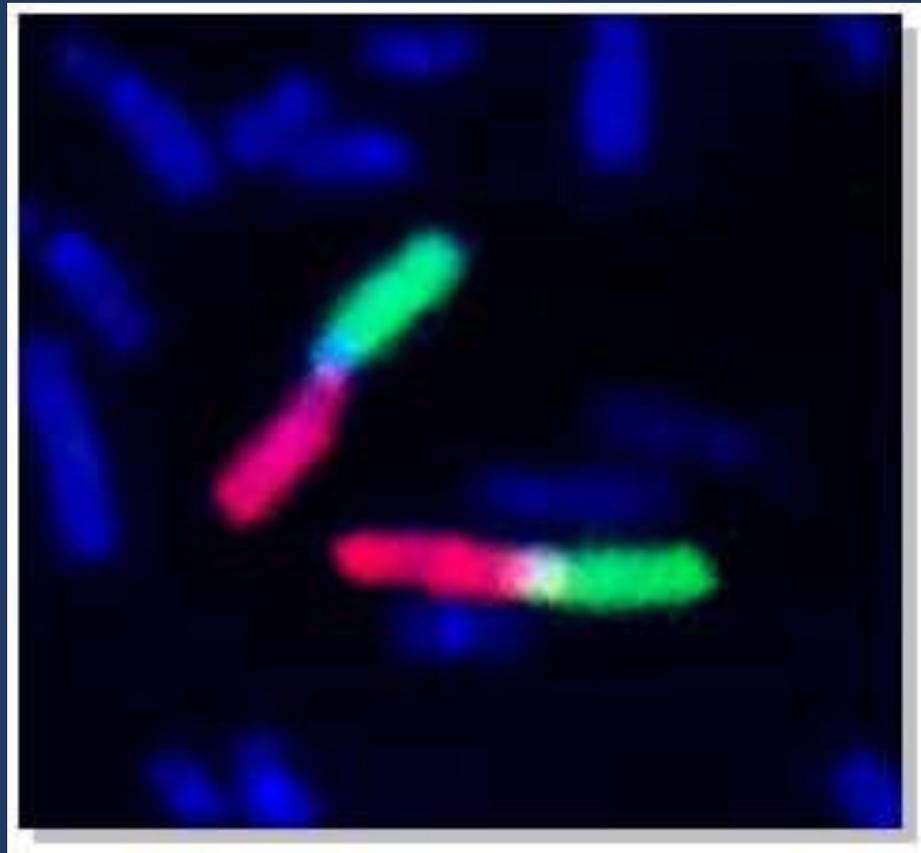


Duplicazione sul braccio corto del cromosoma 8



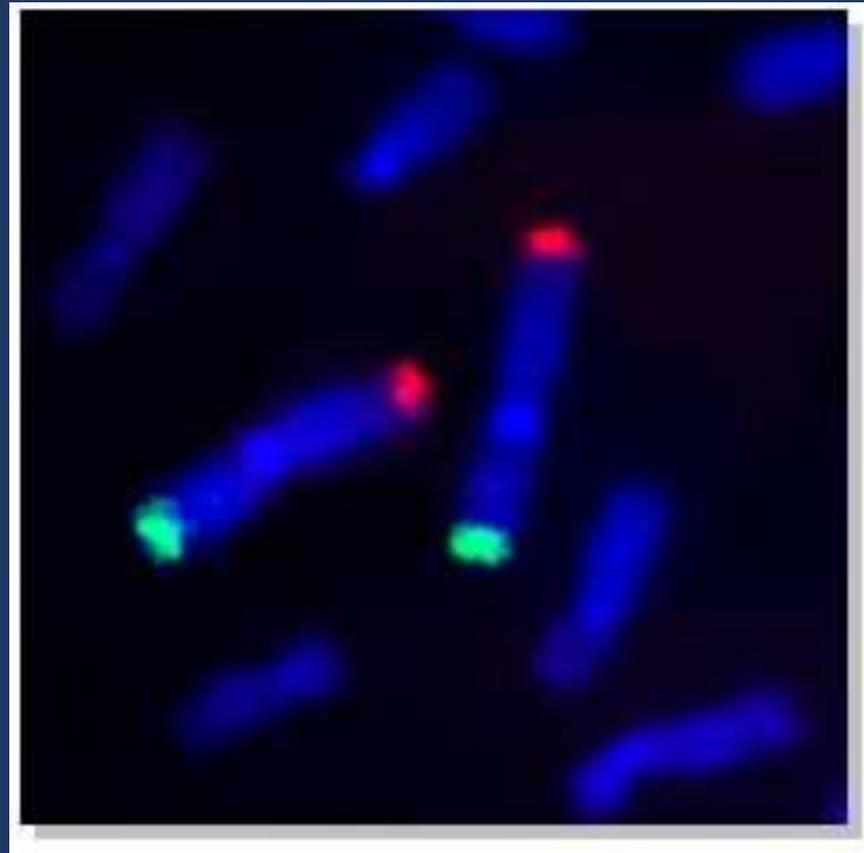
Painting del cromosoma 1

braccio specifico

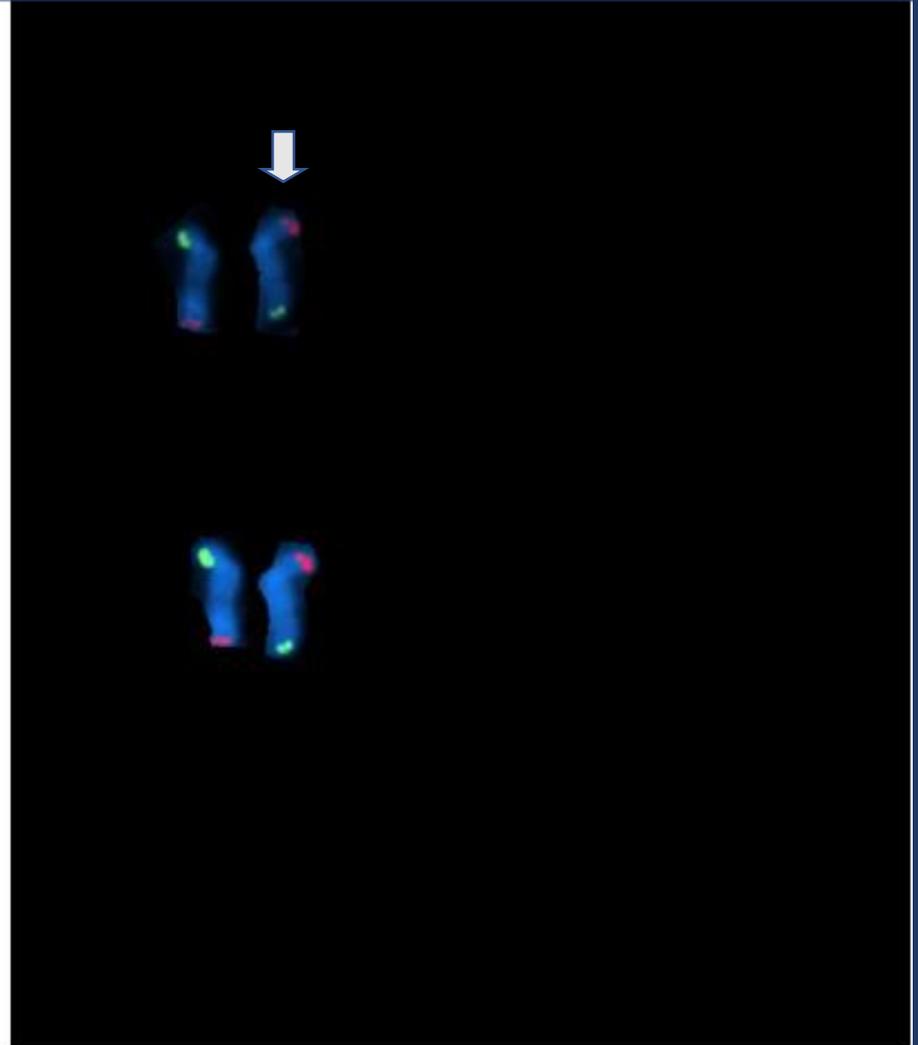
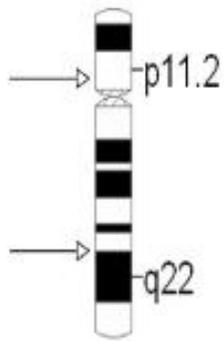


Painting del cromosoma 1

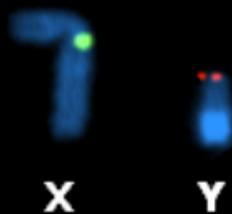
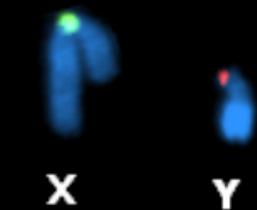
banda specifica



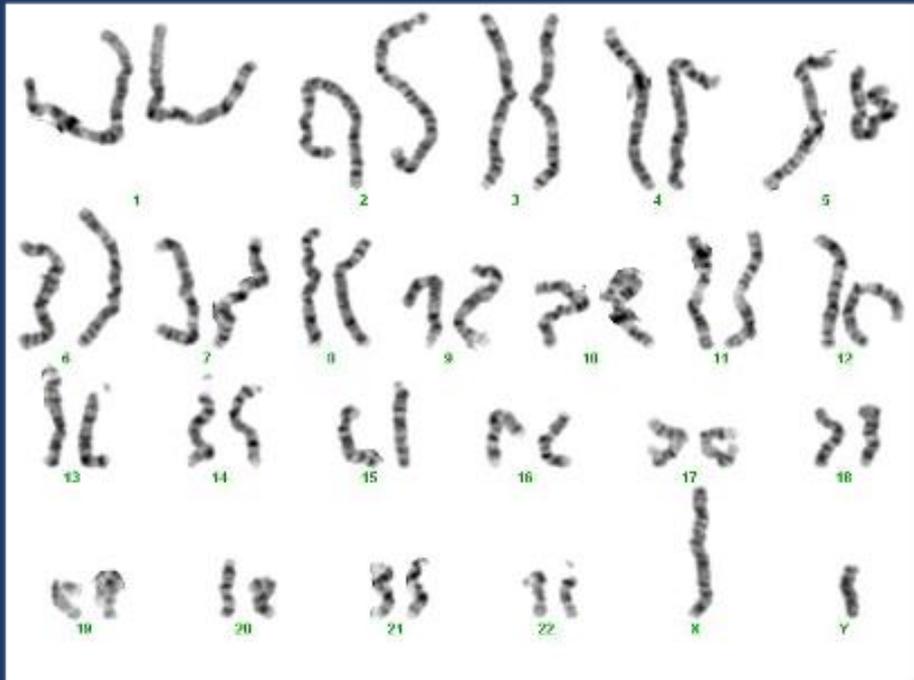
Inversione cromosoma 18



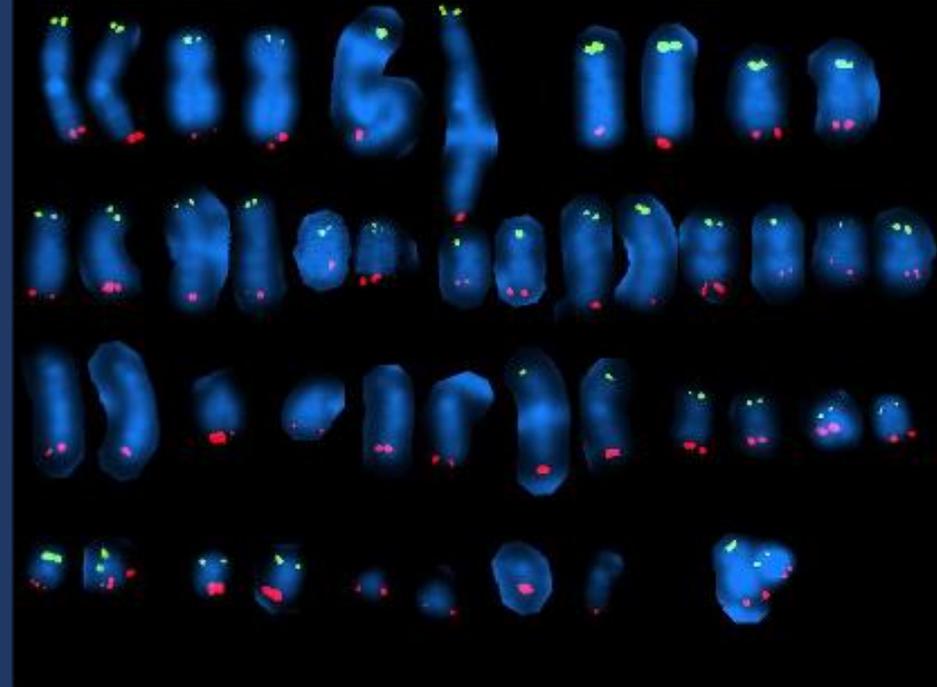
ish Yp11.3 (SRY+)



RISULTATI



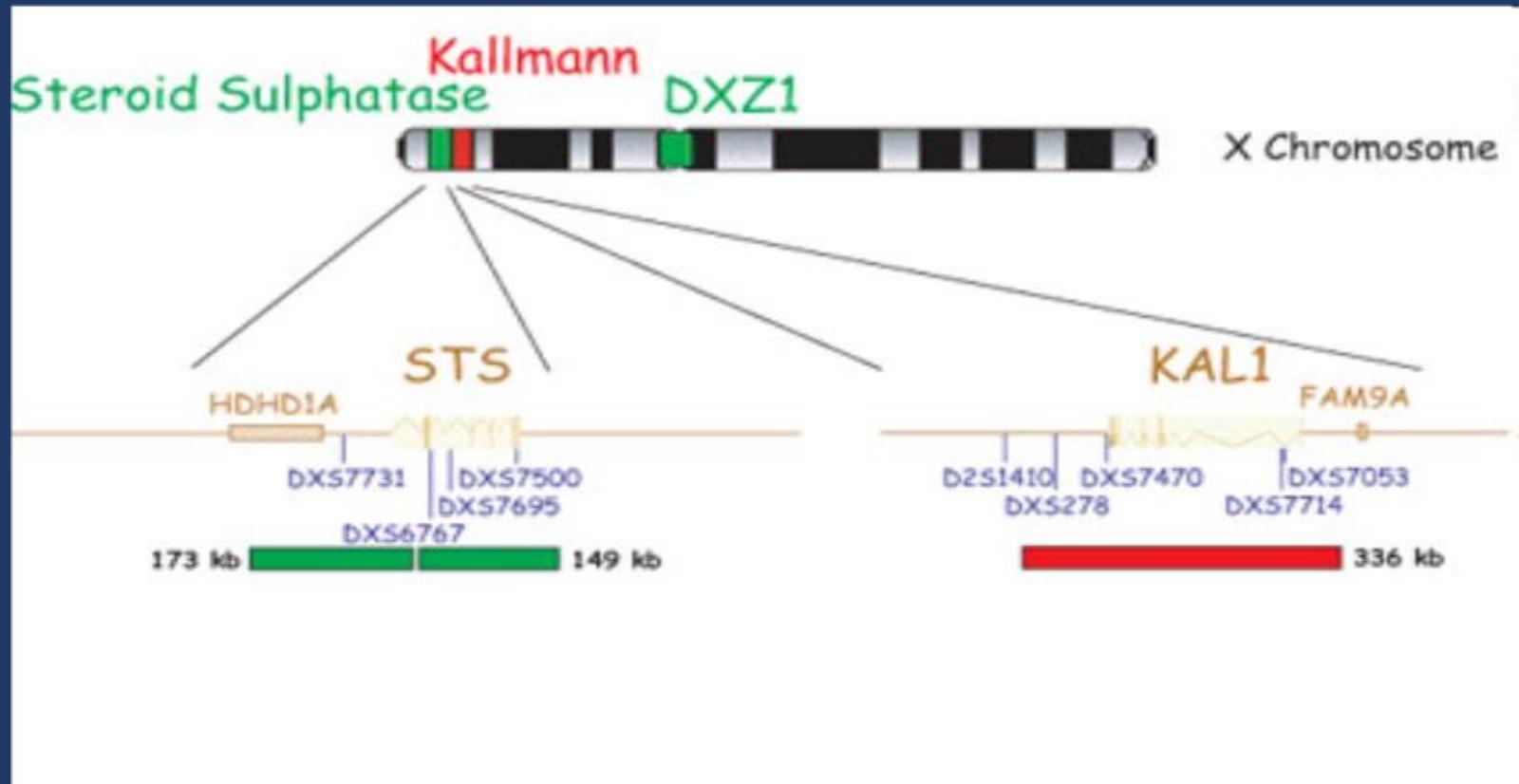
Cariotipo ad alta risoluzione del paziente



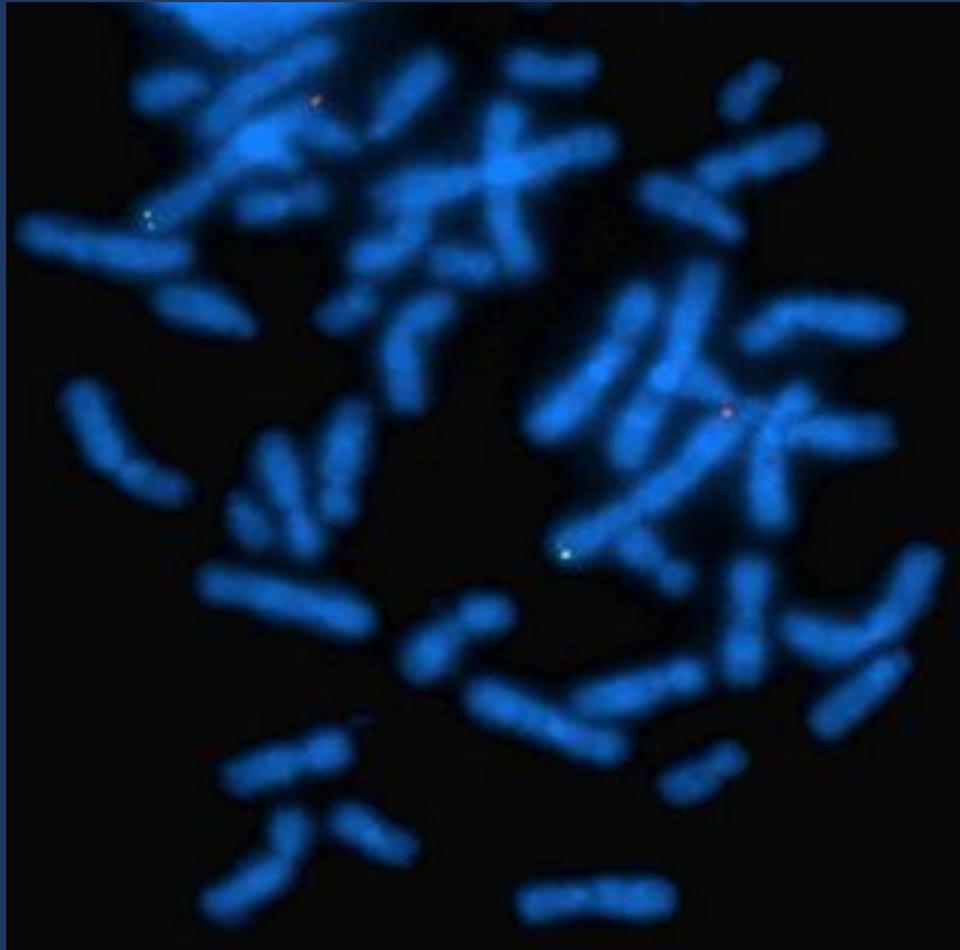
Analisi FISH per lo screening dei riarrangiamenti subtelomerici del paziente

SONDE MICRODELETIVE

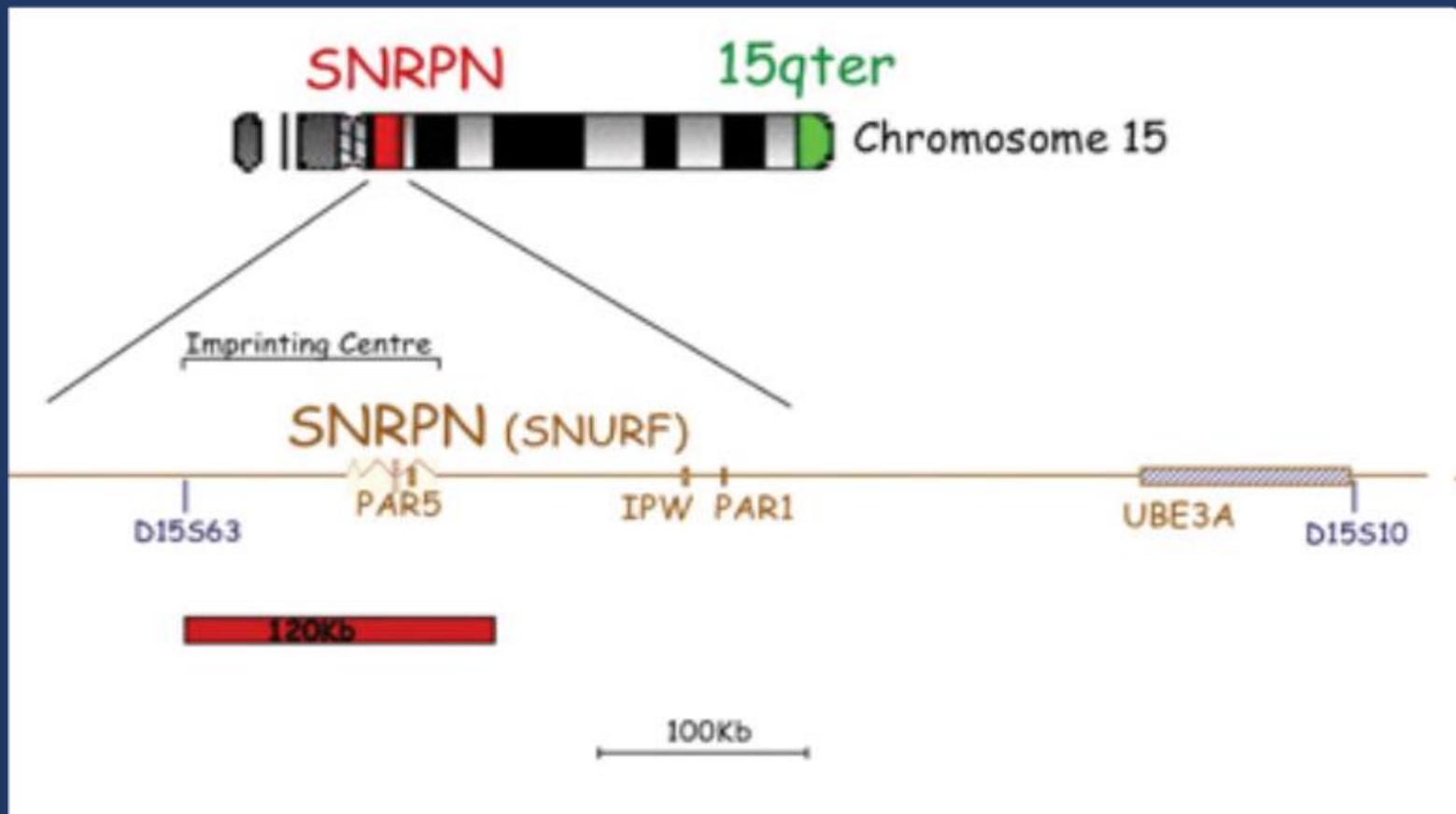
Kallmann (KAL1) and (STS)



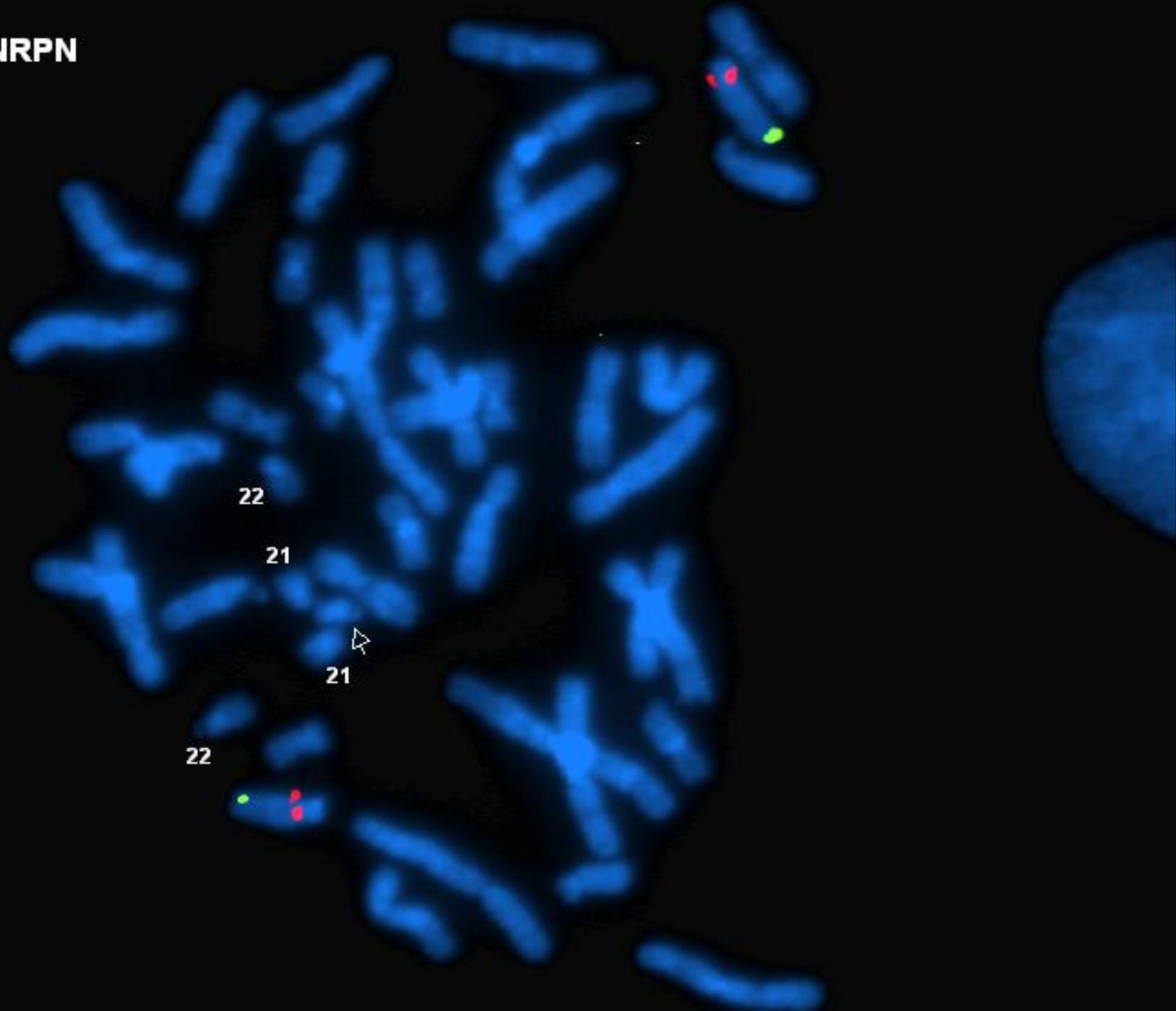
Sindrome di kallmann (KAL1)

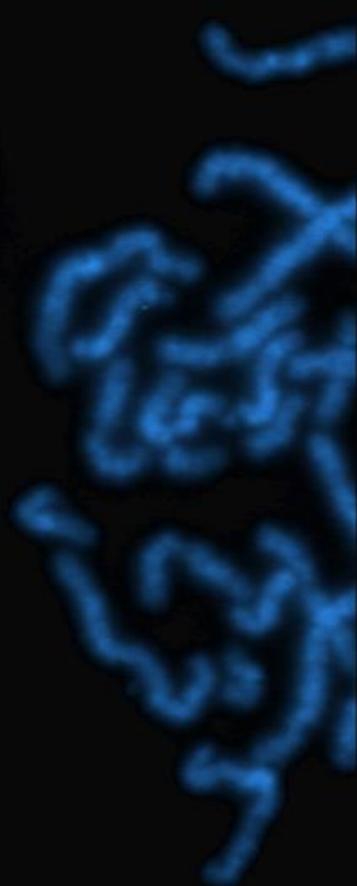
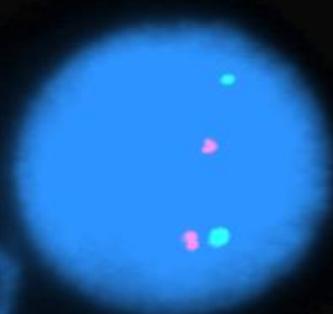
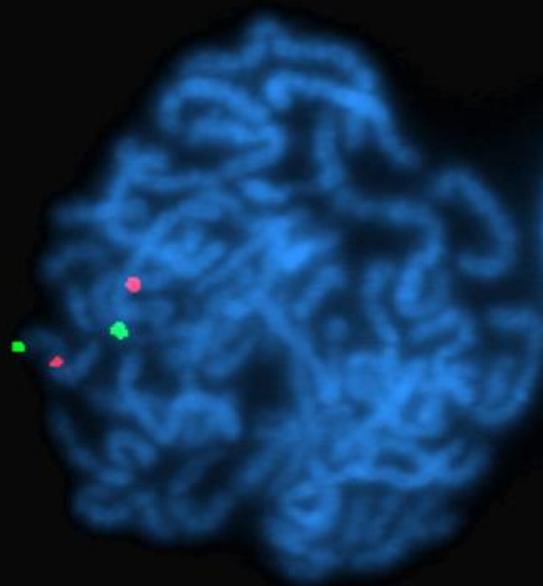
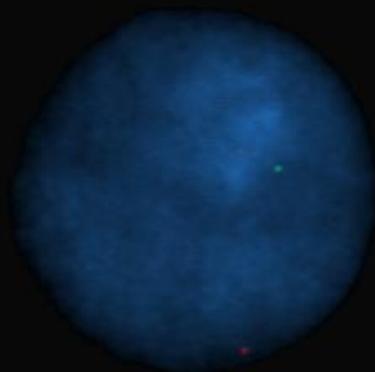


Prader-Willi/Angelman (SNRPN)



SNRPN





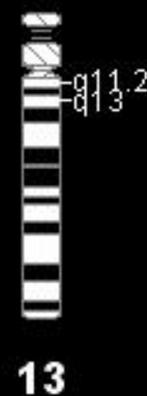
46,XY,del(15)(q12.1q13).ish del(15)(q12.1q12.1)(UBE3A/D15S10-)

UBE3A/D15S10-

QFQ

GTG

QFQ



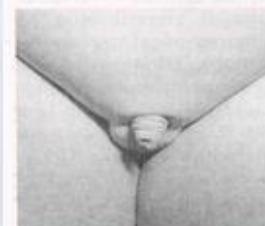
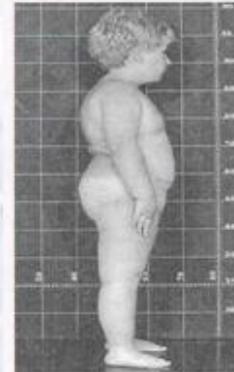
caso 56/10

caso 110/10



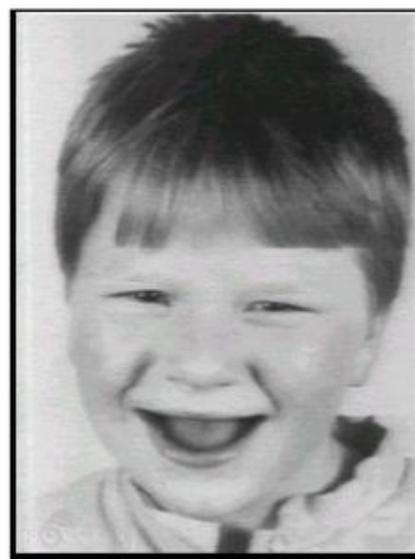
Sindrome di Prader-Willi: Criteri Diagnostici Maggiori

- Ipotonia centrale infantile con suzione scarsa, tendente a migliorare nel tempo
- Difficoltà di alimentazione e scarso accrescimento
- Aumento eccessivo di peso dopo 1 a. e prima di 6 a.
- Anomalie facciali:
 - costrizione bitemporale
 - occhi a mandorla
 - bocca piccola con labbro superiore sottile e angoli rivolti in basso
- Ipogonadismo
- Ritardo mentale medio-moderato
- Iperfagia/ossessione per il cibo
- Anomalia cromosomica o molecolare della regione 15q11-q13

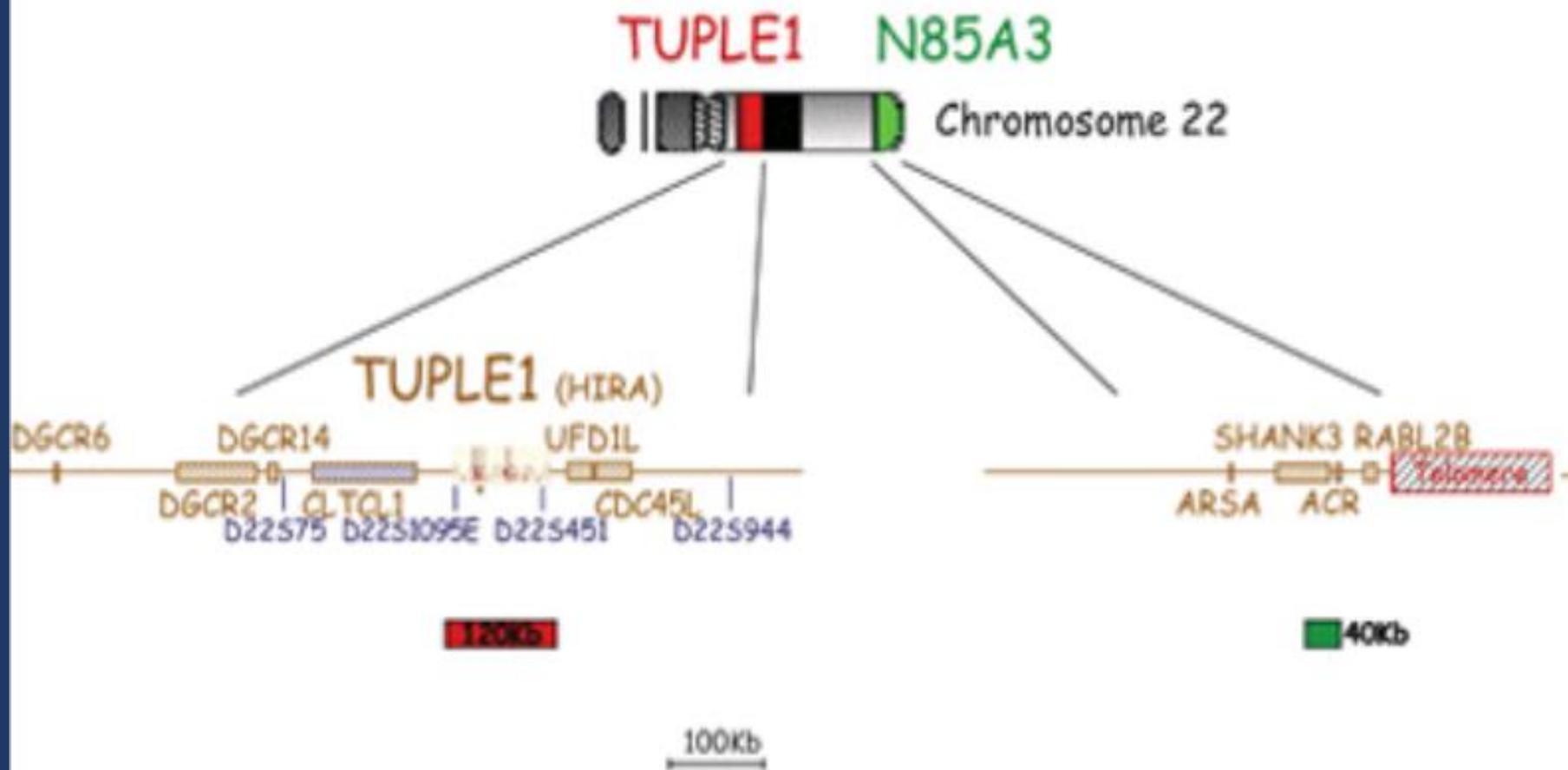


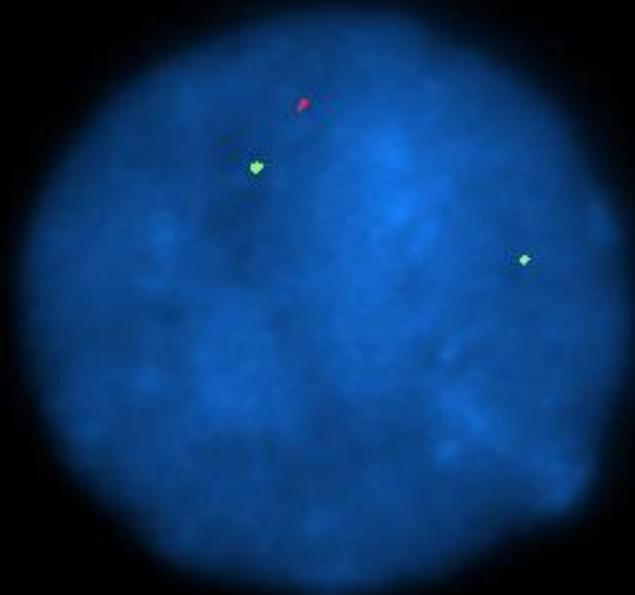
Sindrome di Angelman

- Clinica:
 - Ritardo mentale
 - Comportamento iperattivo
 - Scoppi di risa immotivate
- Basi molecolari
 - ***mancata espressione di geni materni nella regione 15q12***



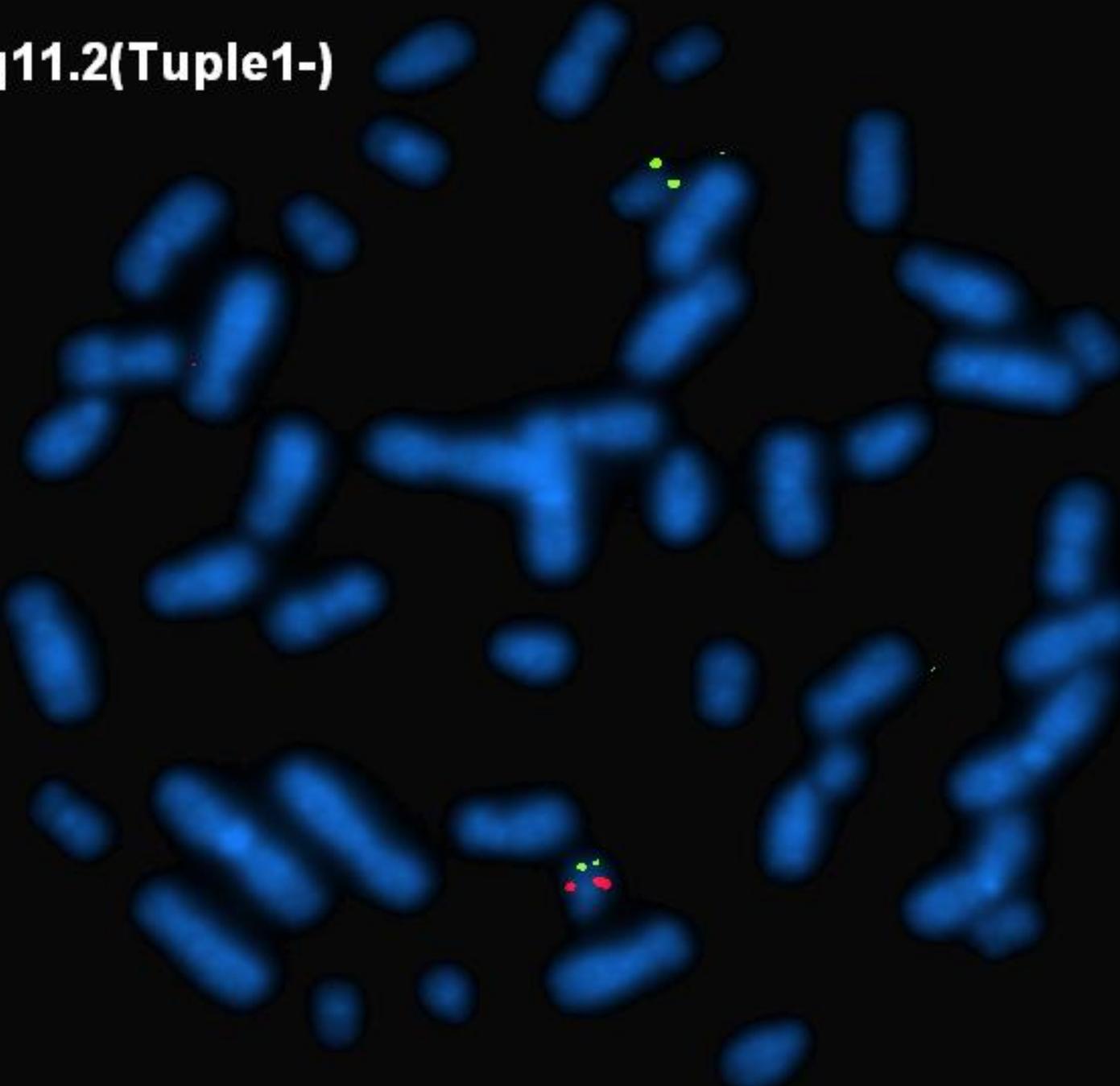
DiGeorge/VCFS TUPLE1 Region 22q13.3





ish 22q11.2(Tuple1-)

ish 22q11.2(Tuple1-)

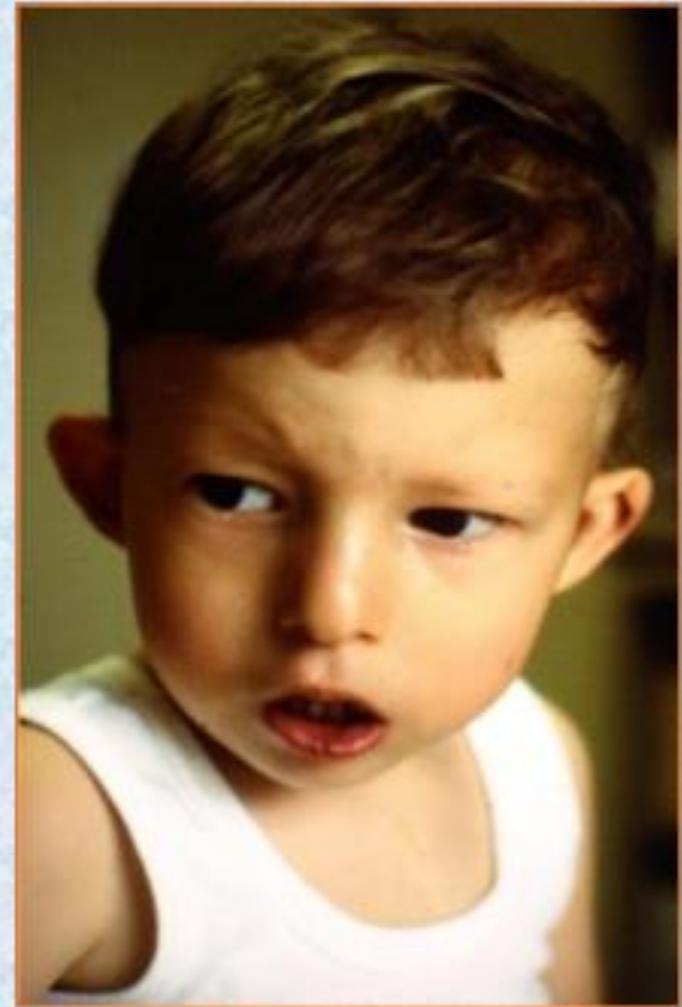


SINDROME da DELEZIONE 22q11.2 *(Sindrome di DiGeorge/Velocardiofaciale)*

Prevalenza: 1:6000 nati vivi

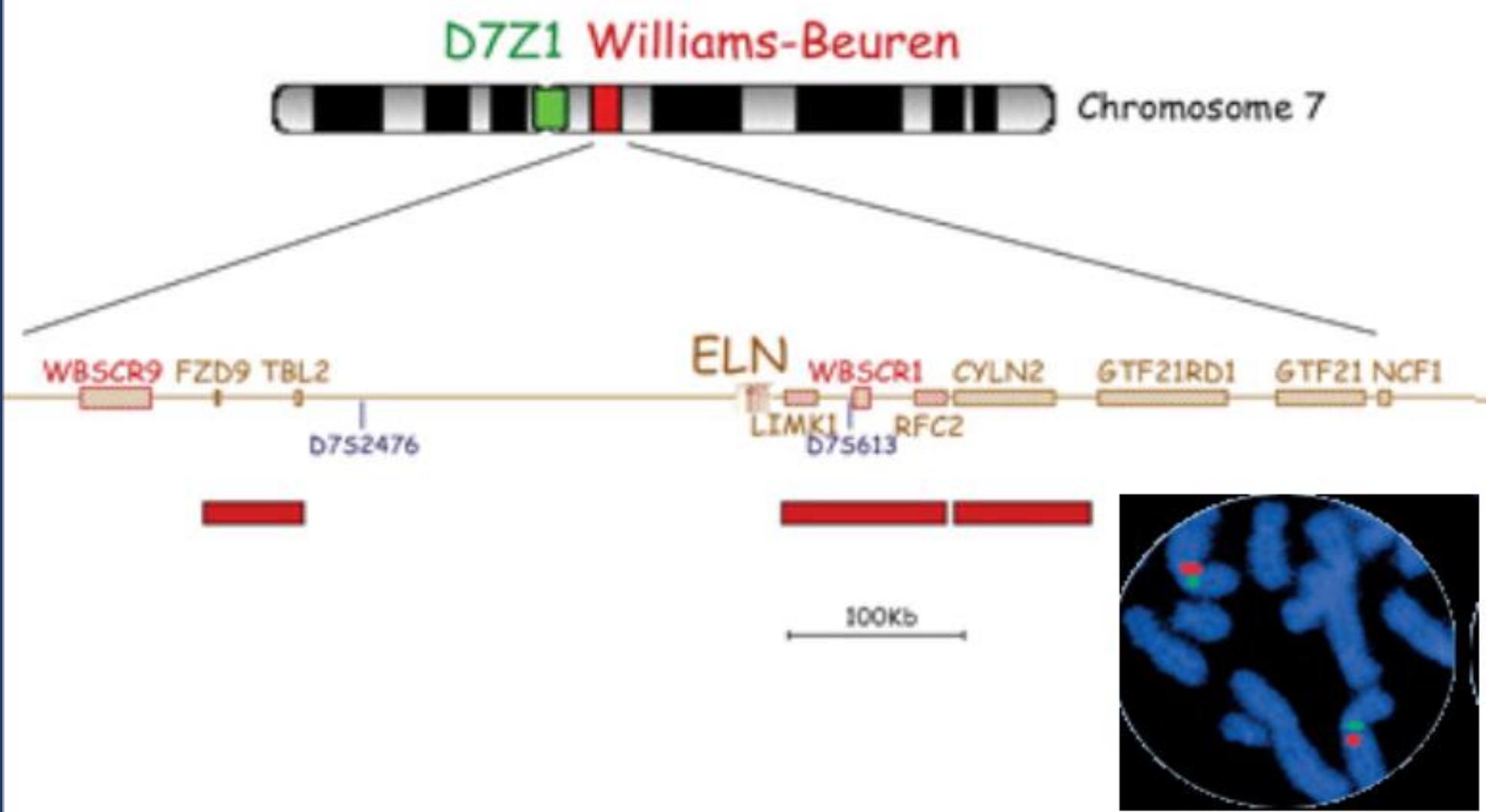
Caratteristiche cliniche:

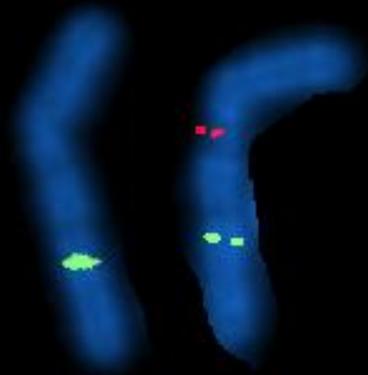
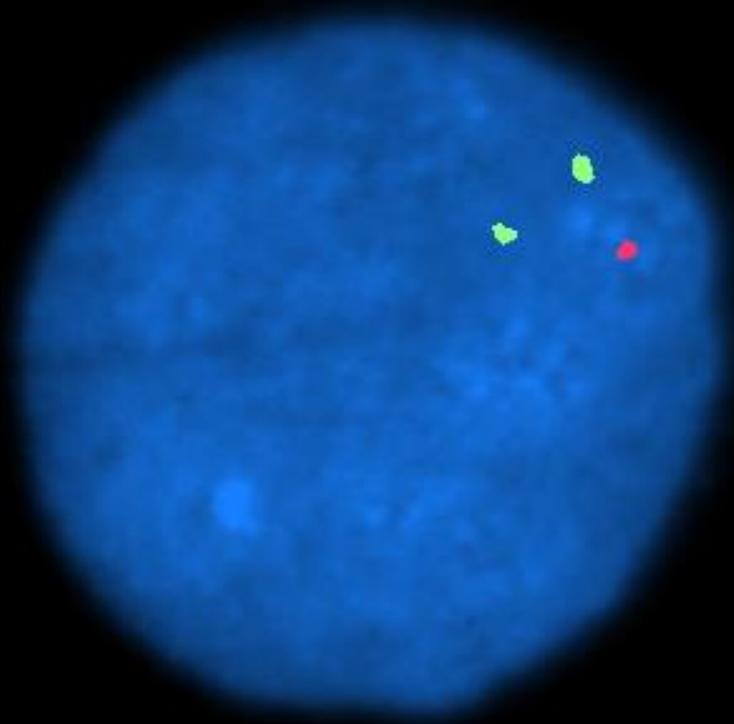
- Anomalie facciali
- Difetti cardiaci (**TRONCOCONALI**)
- Labiopalatoschisi
- Deficit immunitario
- Ipocalcemia neonatale
- Ritardo mentale



Difetto genetico: **microdelezione 22q11.2**

Williams-Beuren Syndrome





ish 7q11.23(ELN-)

Sindrome di Williams-Beuren (WBS) Sindrome di Williams (WS)

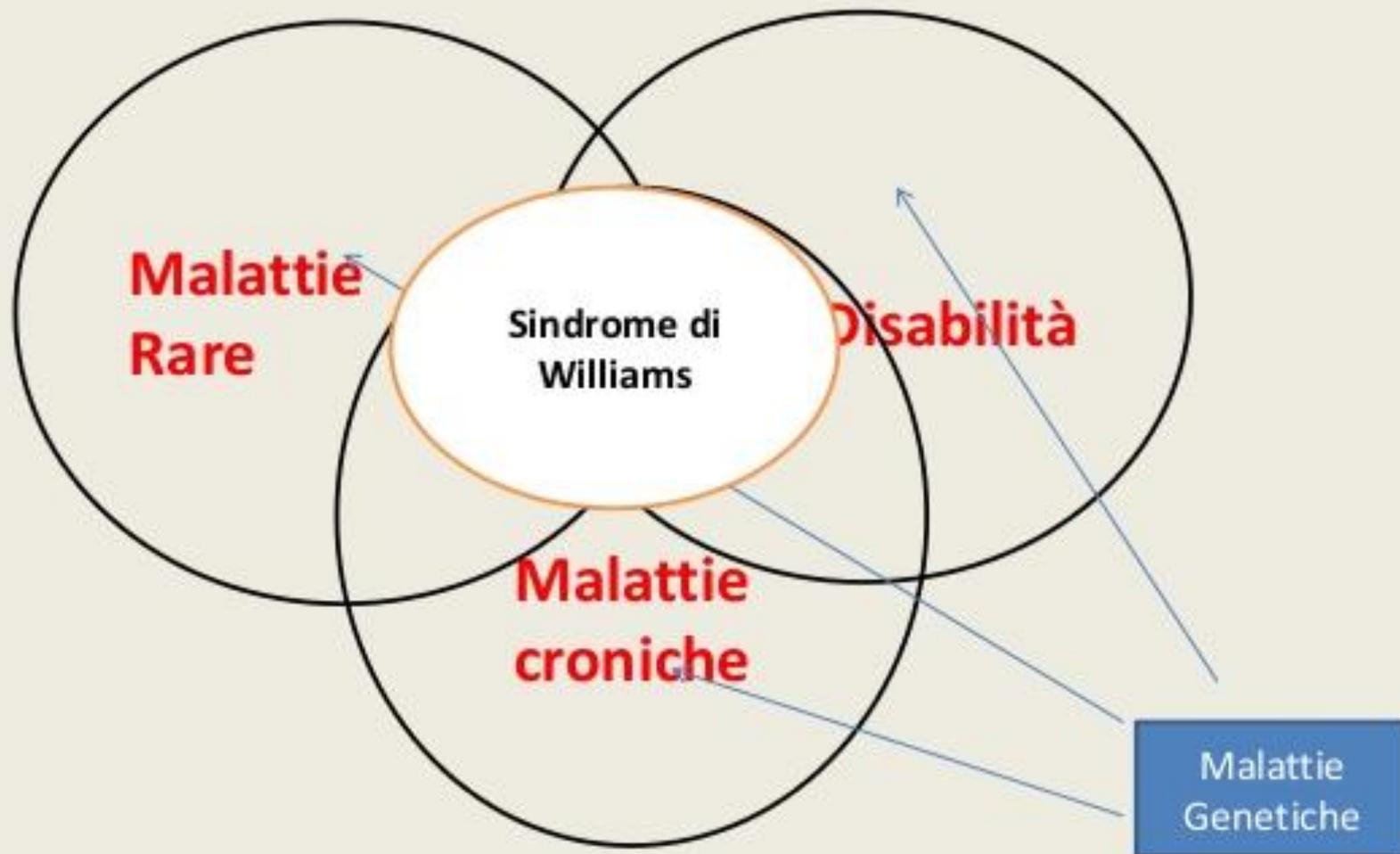
Disordine multisistemico, causato da una delezione della regione cromosomica 7q11.23, che consiste in circa 1.5 - 1.8 Mb e contenente 25-28 geni.

Incidenza di circa 1 su 20000.

1961-2 Descrizione dei primi casi (Williams –Beuren)

1993 riconoscimento della traslocazione di ELN (Morris et al)

1995 Ibridazione fluorescente in Situ (FISH) (Lowery et al.)

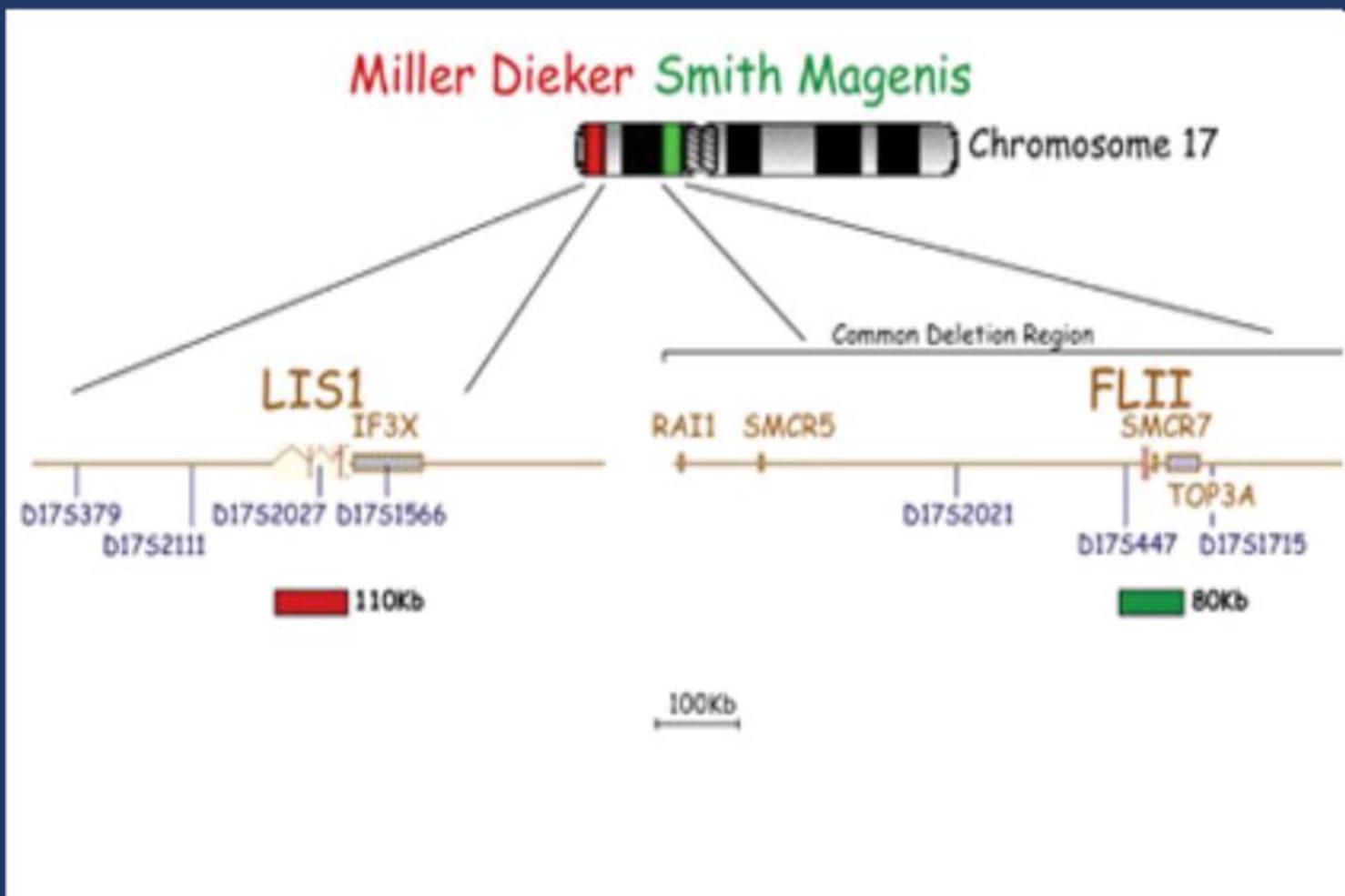


malattie rare ad alta complessità assistenziale



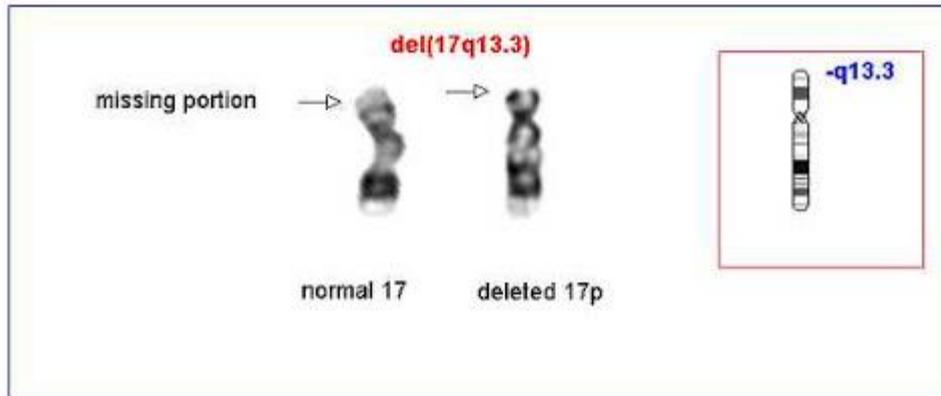
- Disturbi della sfera psichiatrica. ansia
- Fobia (ossessivo –compulsiva)
- Difficoltà di concentrazione e/o deficit di attenzione ed iperattività (ADHD)
- Incapacità di stabilire rapporti sociali soprattutto con i coetanei , comportamenti stereotipati , ripetitivi
- se associata ad limitazioni motorie, cognitive ed intellettve - > Vulnerabilità sociale

Smith-Magenis/Miller-Dieker



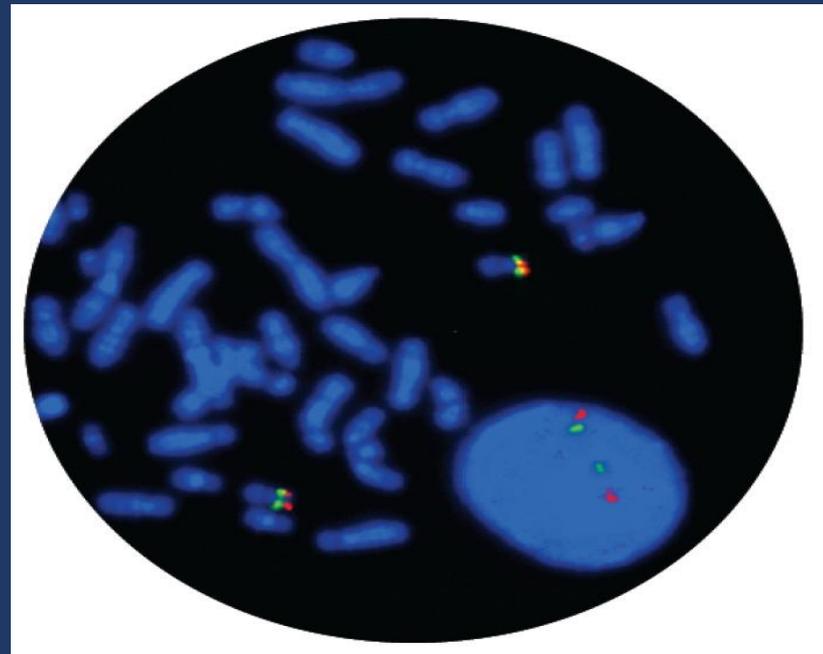
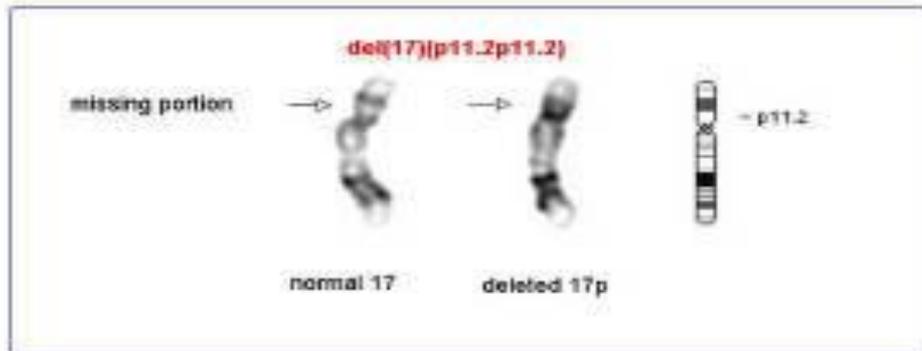
MILLER DIEKER SYNDROME

$del(17q13.3)$

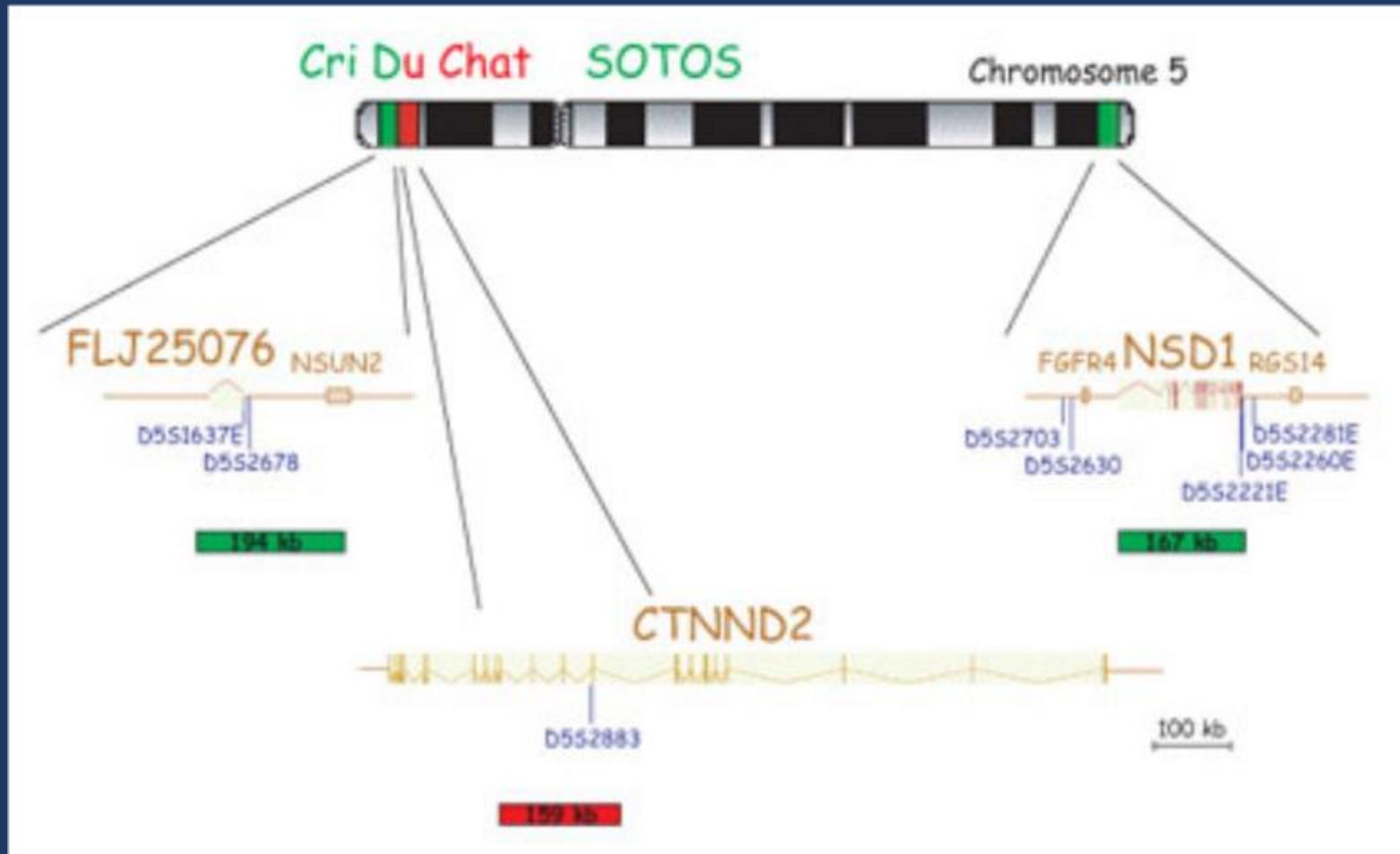


SMITH MAGENIS SYNDROME

$del(17p11.2)$



Cri-Du-Chat and SOTOS



del5p : CRI DU CHAT SYNDROME

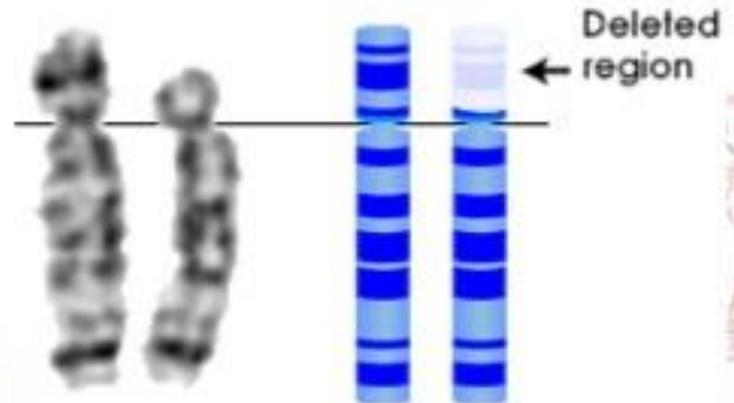


5p-

❖ Incidenza: 1:20.000-50.000

❖ 85% de novo

❖ 15% ereditata



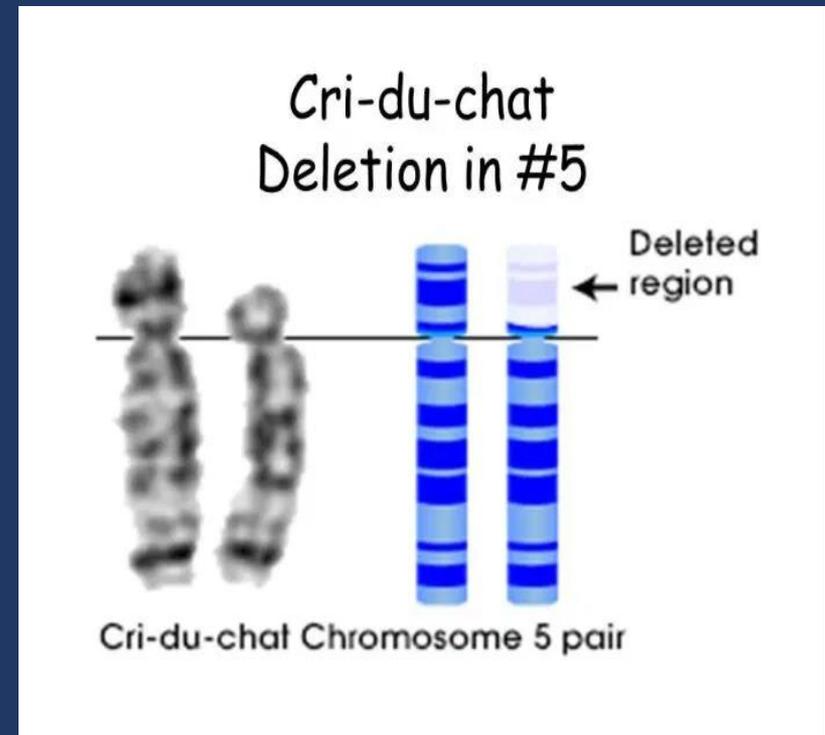
Cri-du-chat Chromosome 5 pair



Sindrome di Cri-Du-Chat

Quadro clinico:

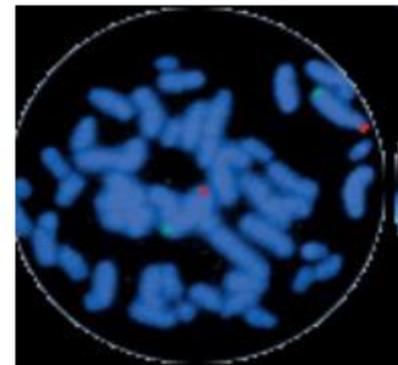
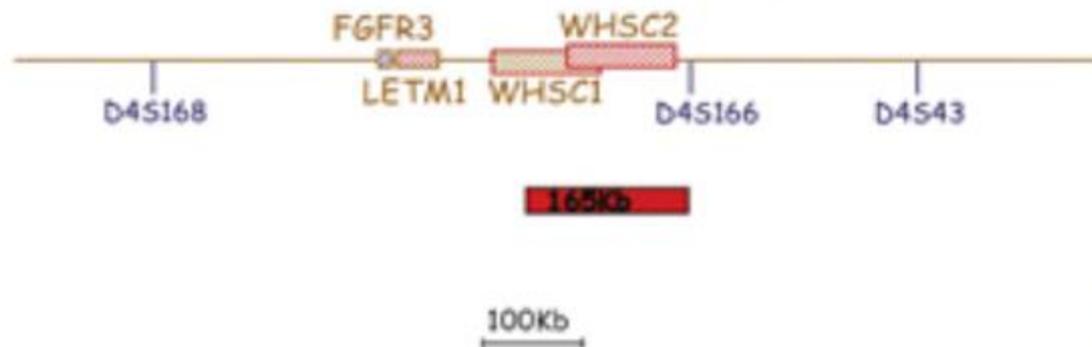
- Tipico pianto, causato Dall'ipoplasia delle cartilagini del laringe.
- Ritardo mentale grave (con tendenza all'automutilazione).
- Microcefalia.
- Ipertelorismo marcato.
- Epicanto,
- Sella nasale slargata,
- Micrognazia
- Orecchie a basso impianto.
- Nel 15% dei casi è presente una cardiopatia congenita

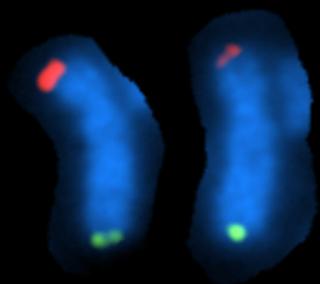


Wolf-Hirschhorn

Wolf Hirshhorn

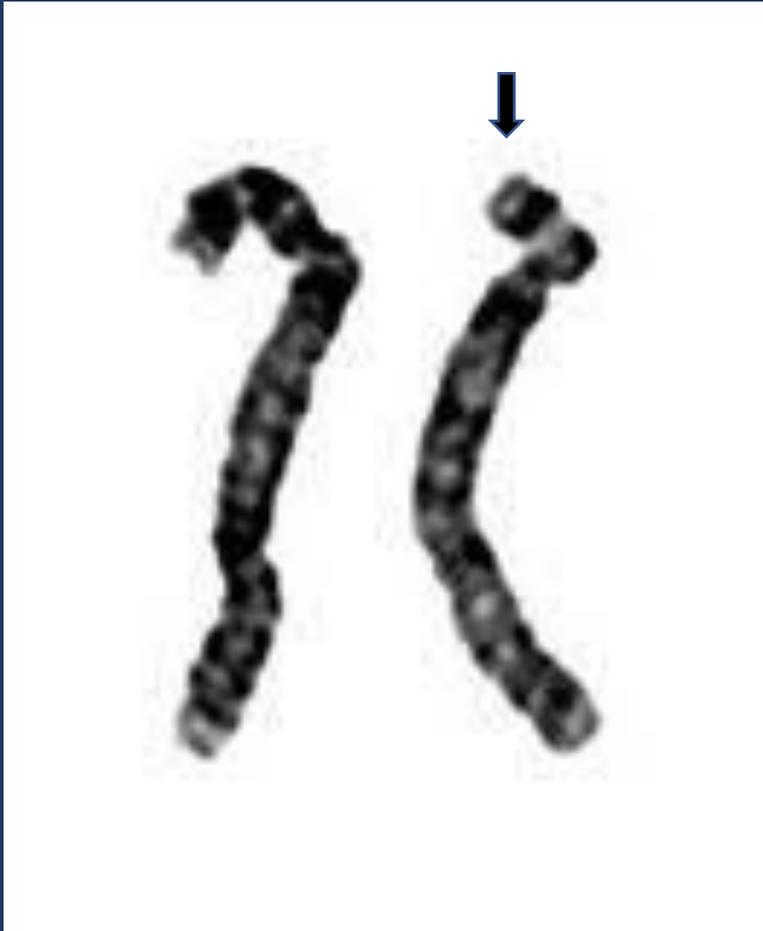
4qter





ish 4p(WHSCRX2)(qterX2)

GTG



FISH



- Wolf-Hirschhorn syndrome

- deletion on chromosome 4
- characterized by mental retardation, heart defects, poor muscle tone, seizures and other problems
- affects about 1 in 50,000 babies

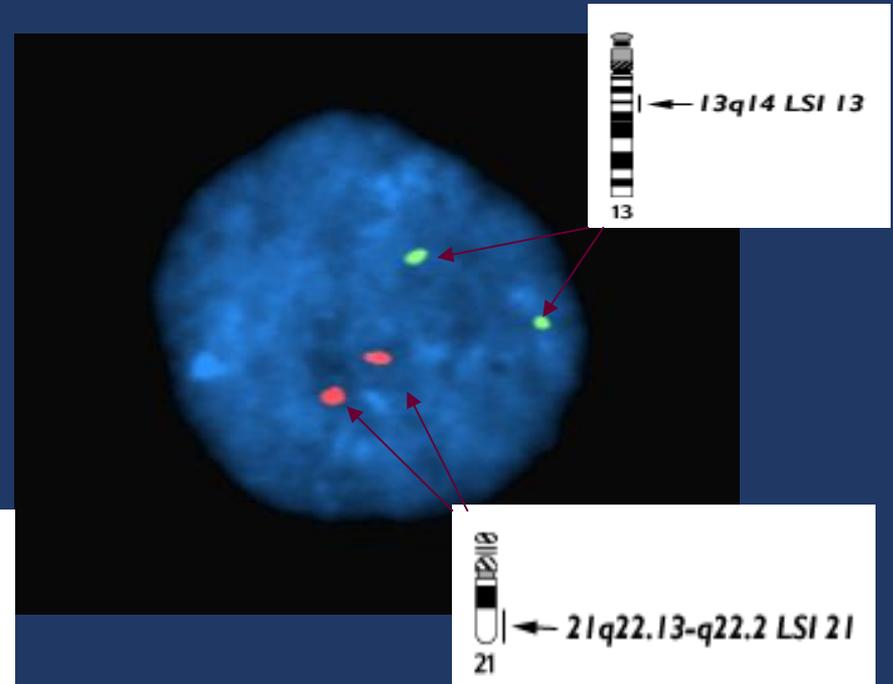
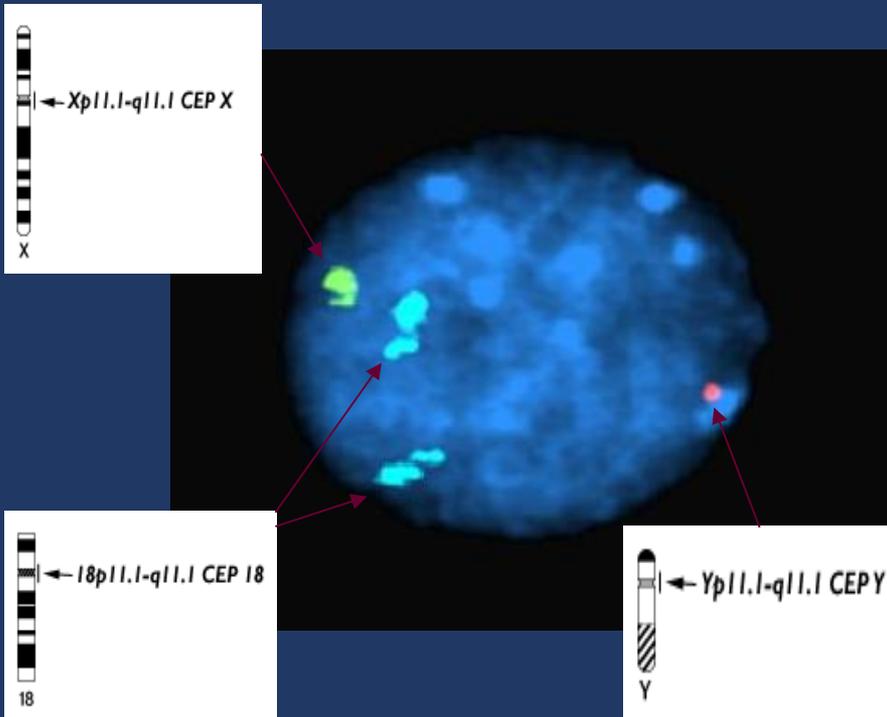


FISH INTERFASICA

Aneuvysion Vysis

CEP Probe centromeriche

LSI Probe locus-specifiche



Sono utilizzabili anche altre sonde intese per diagnosi su metafase

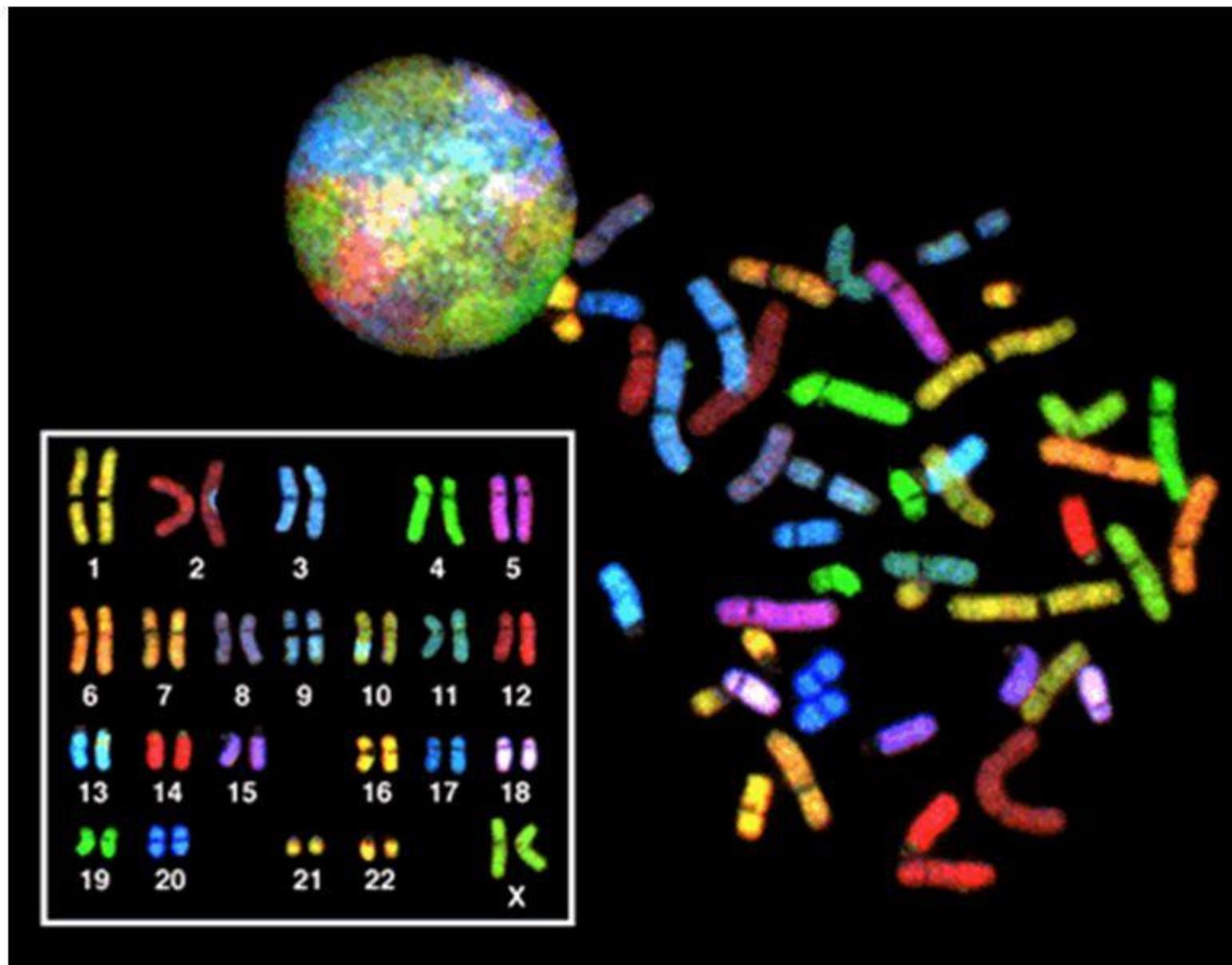
Visualizzazione simultanea di target genomici in molti colori: M-FISH e SKY

M-FISH: e SKY sono sviluppi della FISH con sonde di painting che permettono la visualizzazione simultanea dei 22 autosomi e dei 2 cromosomi sessuali in 24 colori;

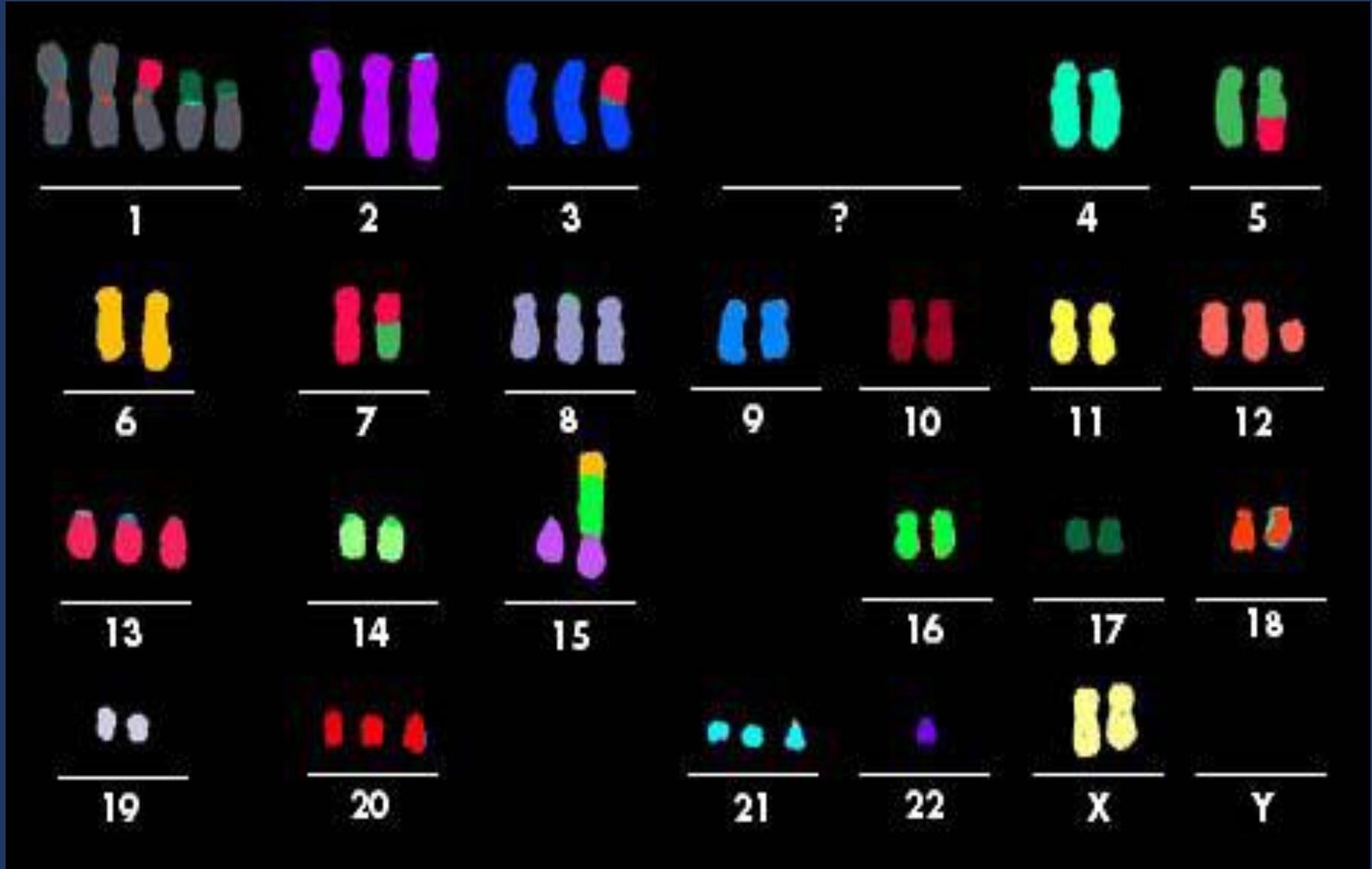
M-FISH (Speicher et al Nature Genet. 12,368-375, 1996)

SKY (Schrock et al. Science 273:494-497, 1996)

SKY e M-FISH

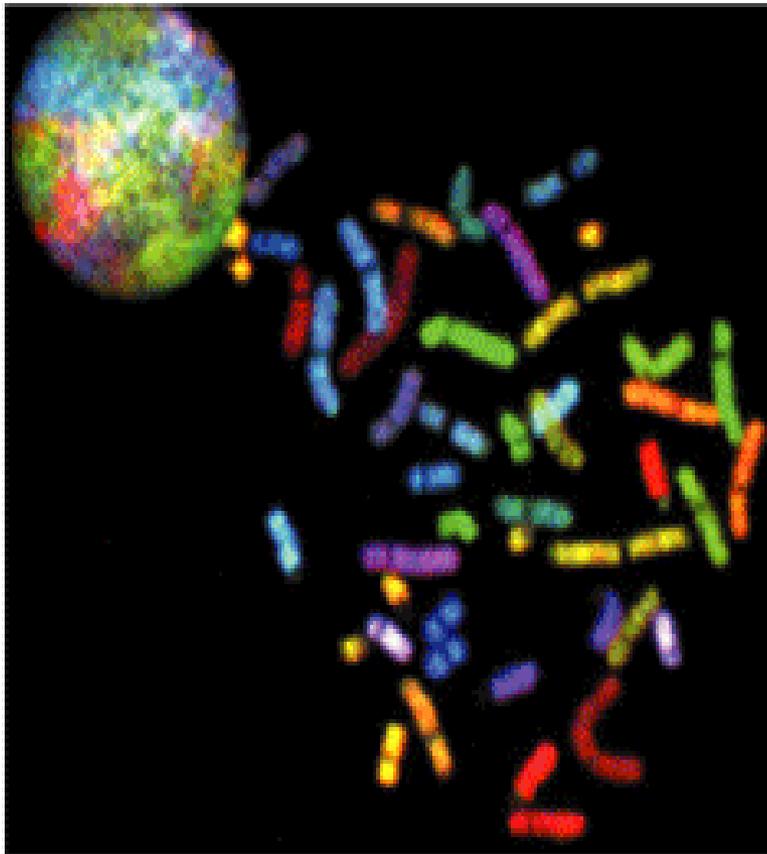


M-FISH: applicazione alla citogenetica dei tumori

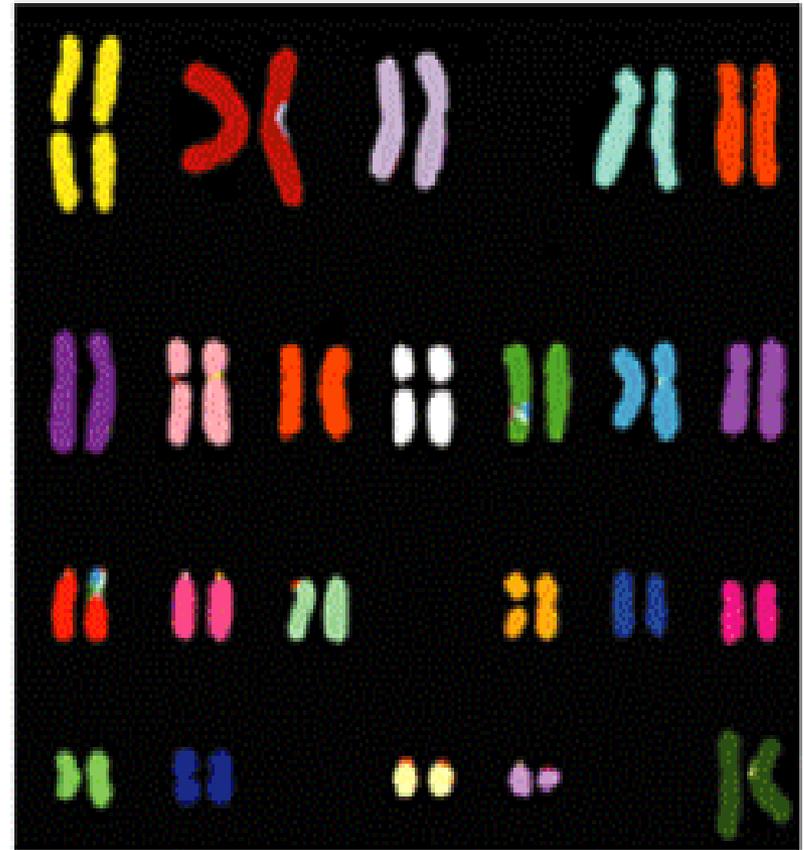


SKY

(1) Display colours

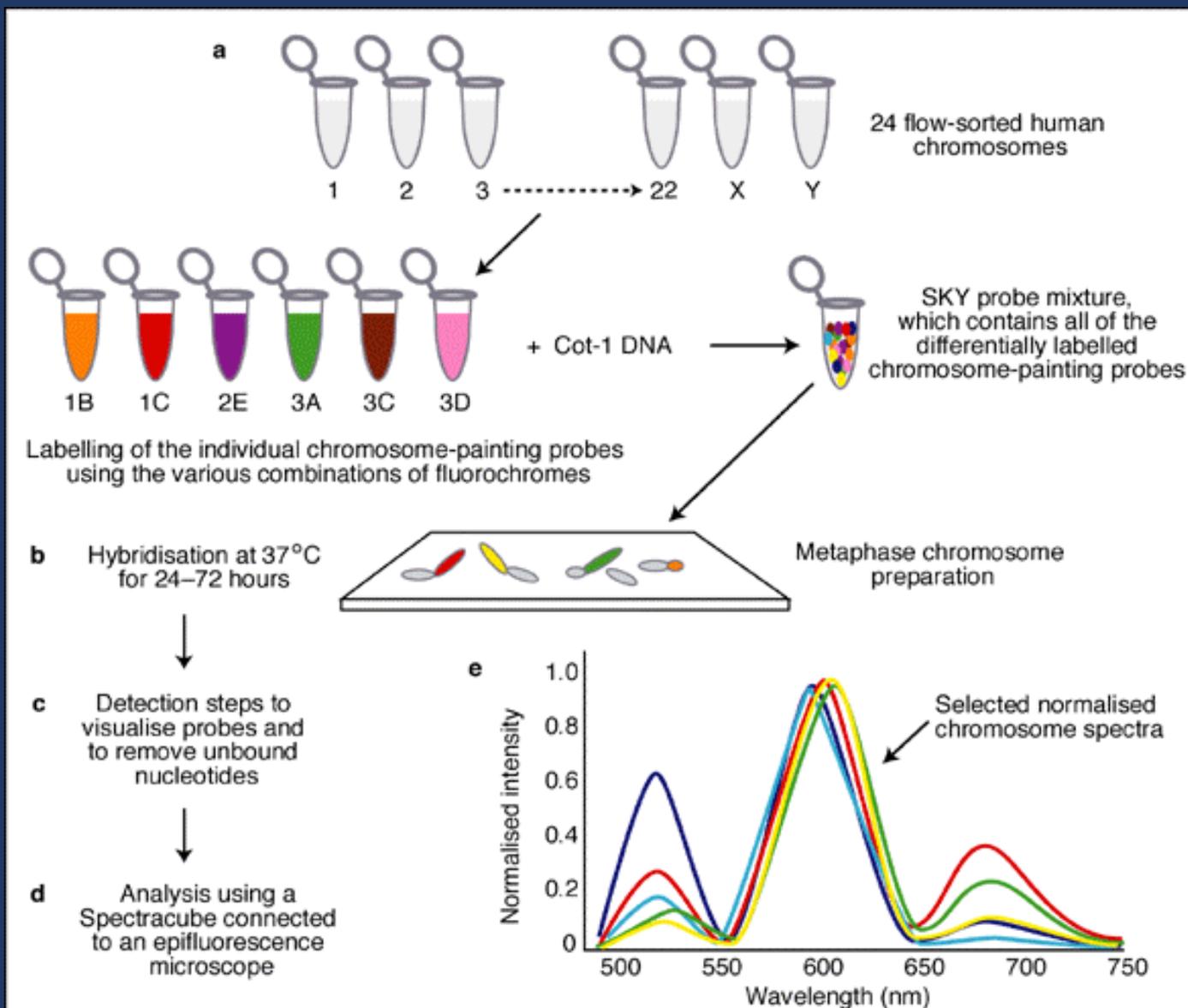


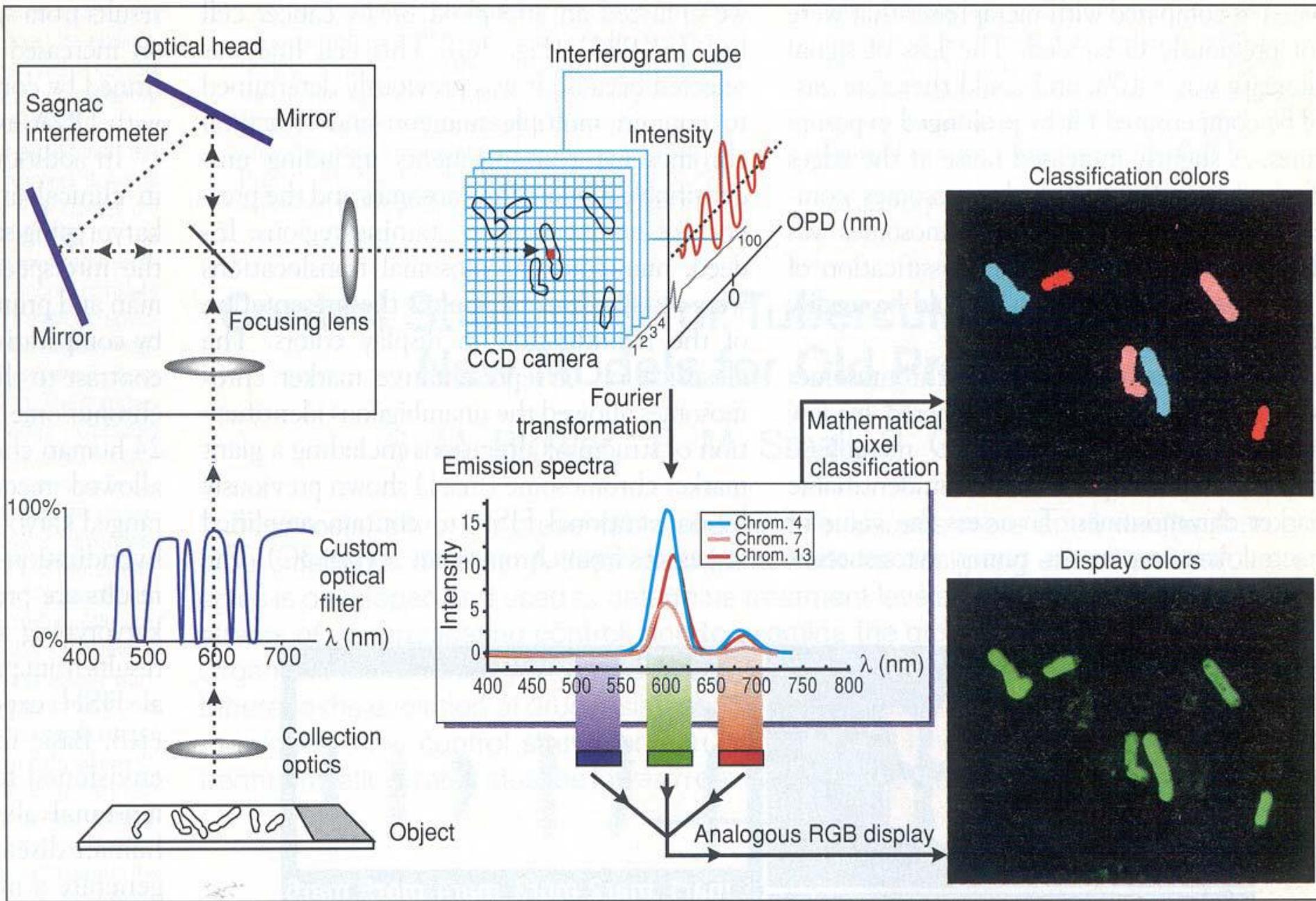
(2) Classification colours



Schematic of cytogenetic analysis using spectral karyotyping (SKY)

Spectral karyotyping: metodo

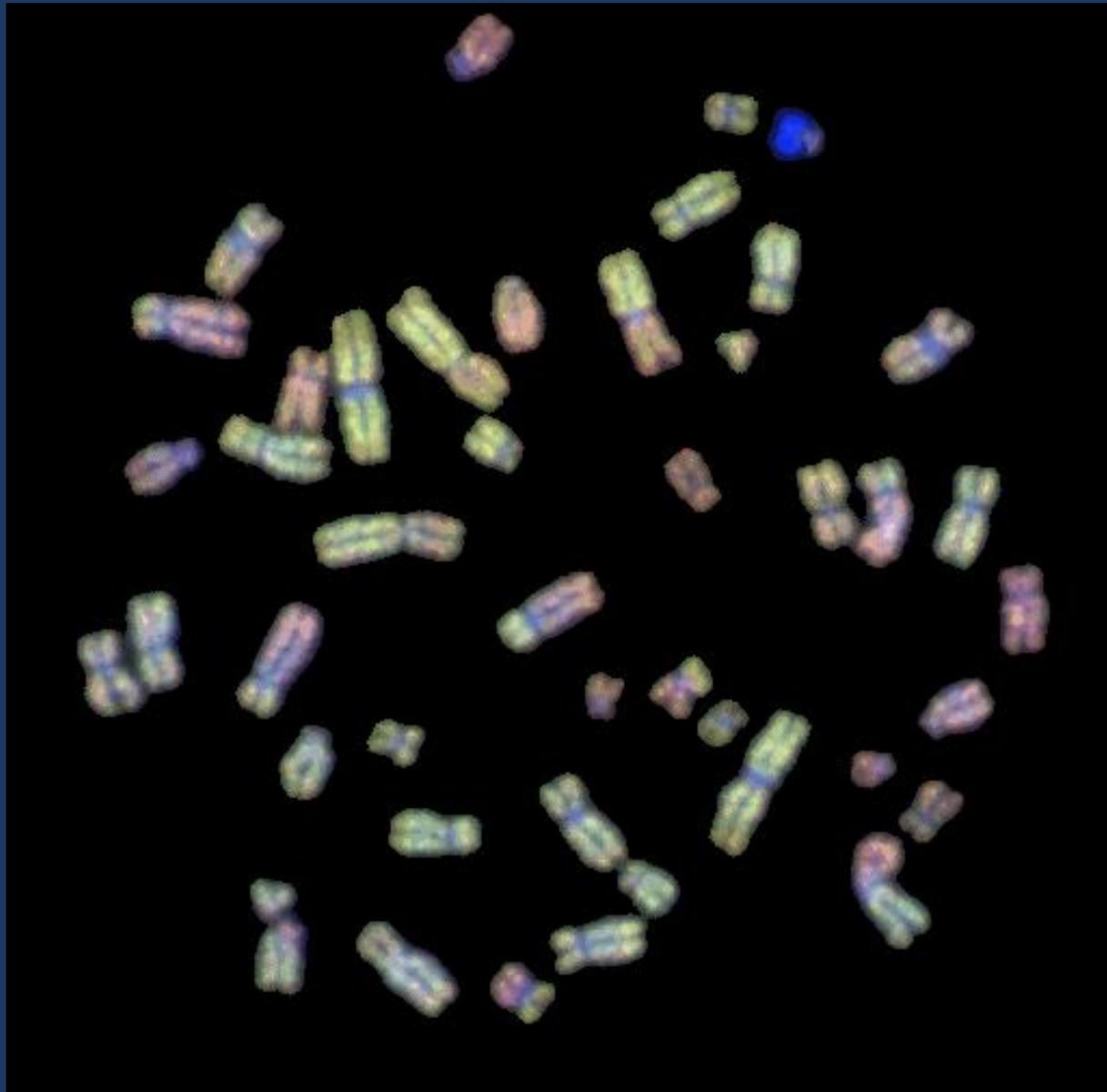


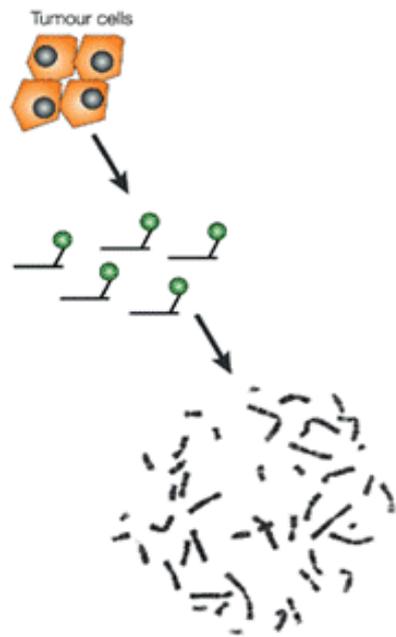


Red-labeled chromosome painting probes are sufficient to enable the discrimination of the chromosomes after spectral interference.

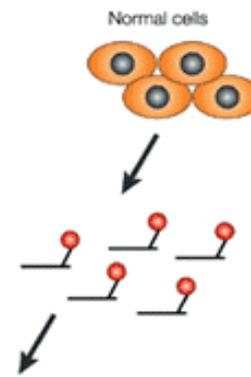
CGH-array

Comparative Genome Hybridization

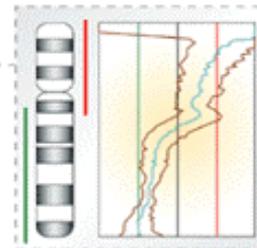
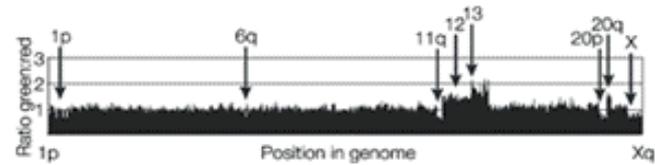
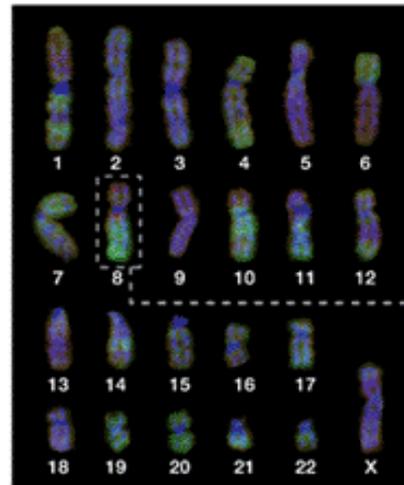
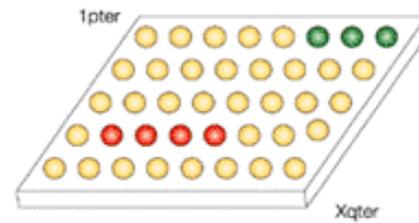




Extract, amplify and label DNA

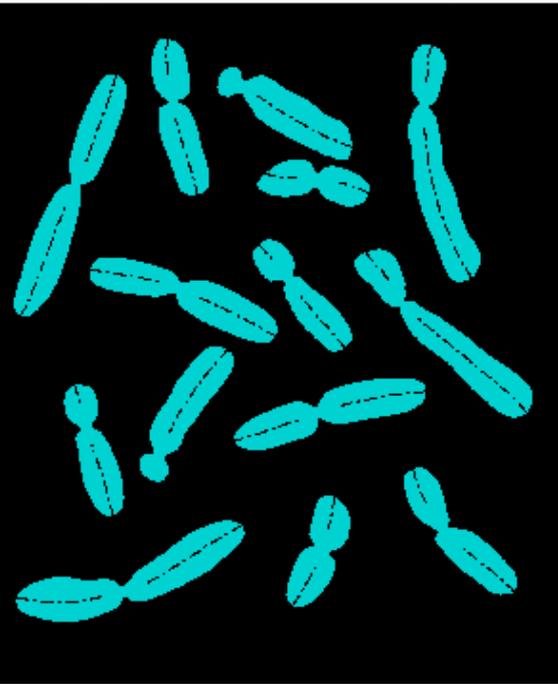


Hybridize to metaphase spread or BAC array



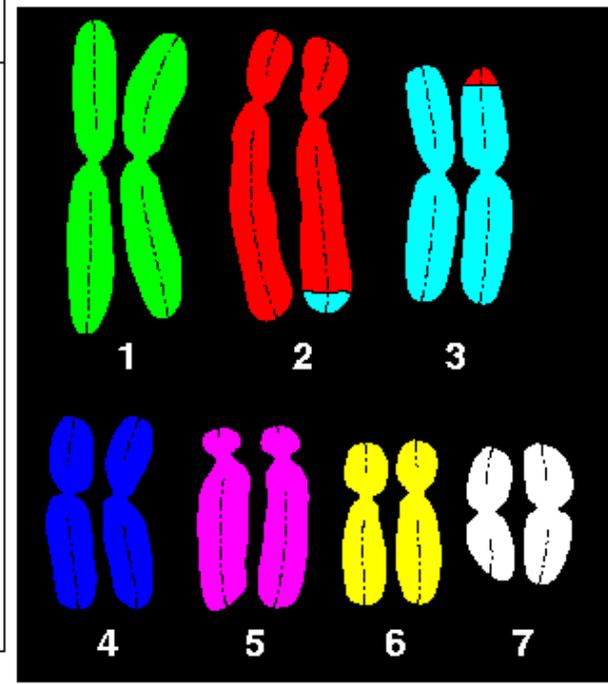
Marcatura con diversi fluorocromi

metaphase chromosome spread



chrom. #	probe fluor composition	chromosome paint
1	FITC	
2	Cy3	
3	Cy5 FITC	
4	Cy5	
5	Cy3 Cy5	
6	FITC Cy3	
7	FITC Cy3 Cy5	

completed karyotype



...Uno sguardo al CGH-array...

DNA controllo



Estrazione DNA



DNA test

CY5

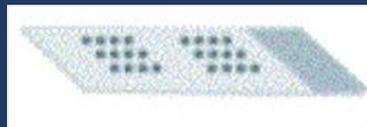
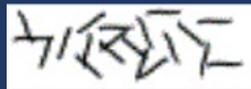


Marchatura con diversi fluorocromi



CY3

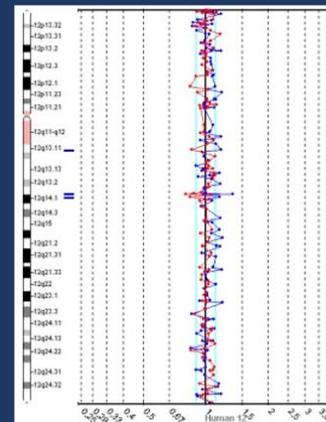
+human cot-1

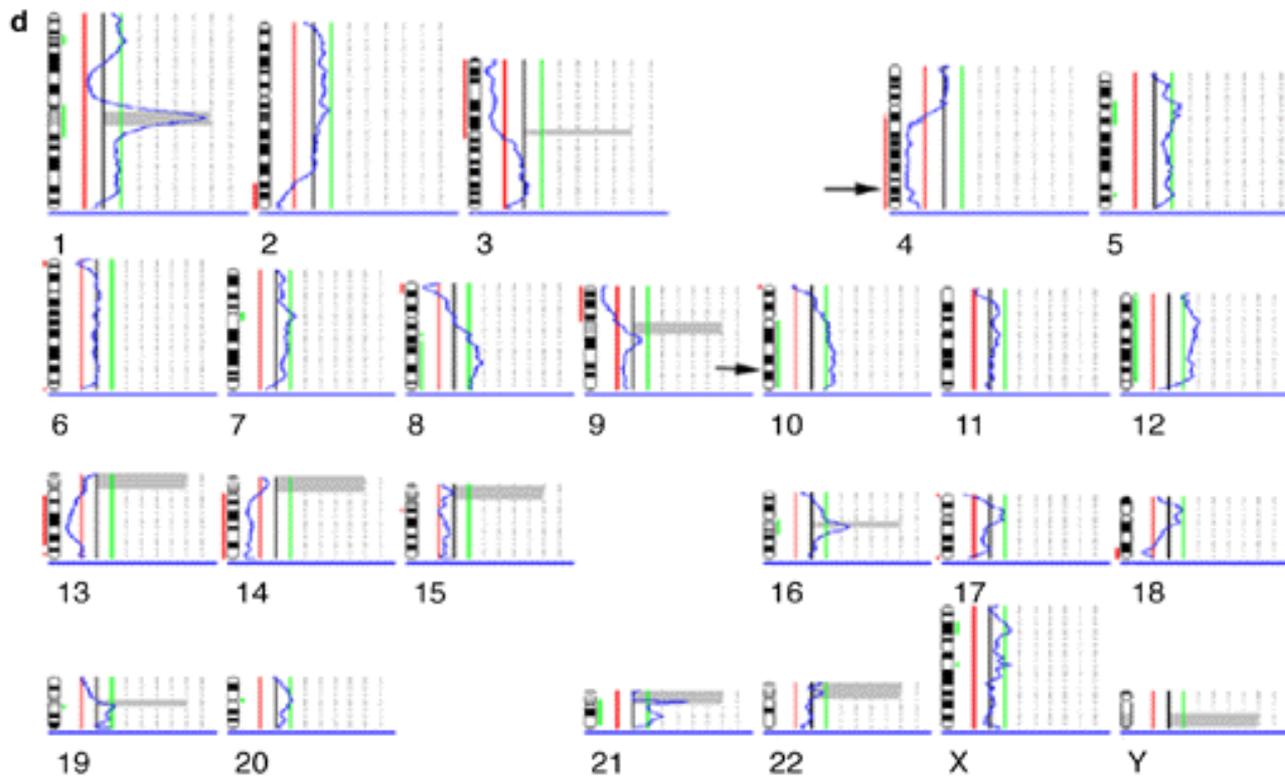
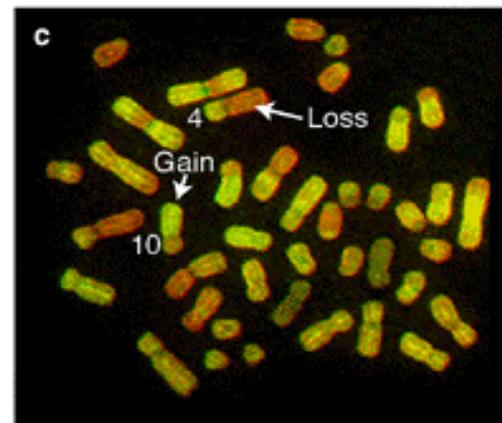
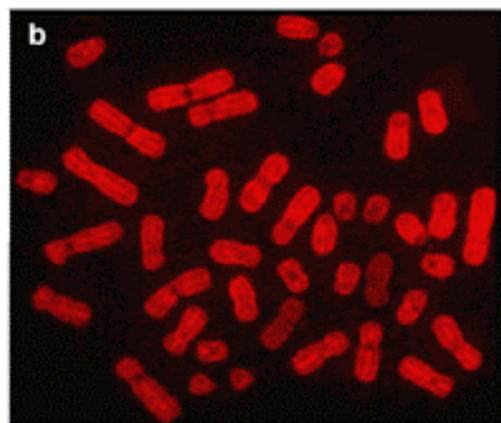
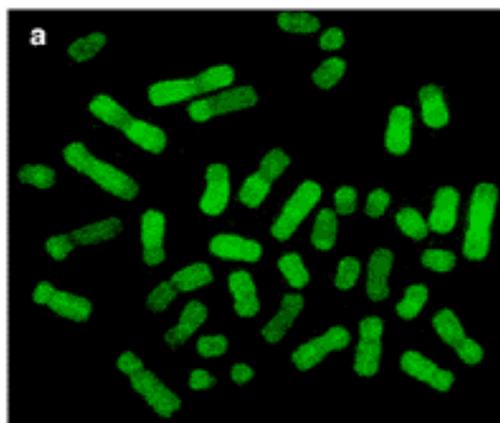


Co-ibridazione su chip



Scannerizzazione e quantificazione della fluorescenza





Comparative genomic hybridisation (CGH) analysis of a lymph node metastasis from a renal cell carcinoma

CGH ARRAY

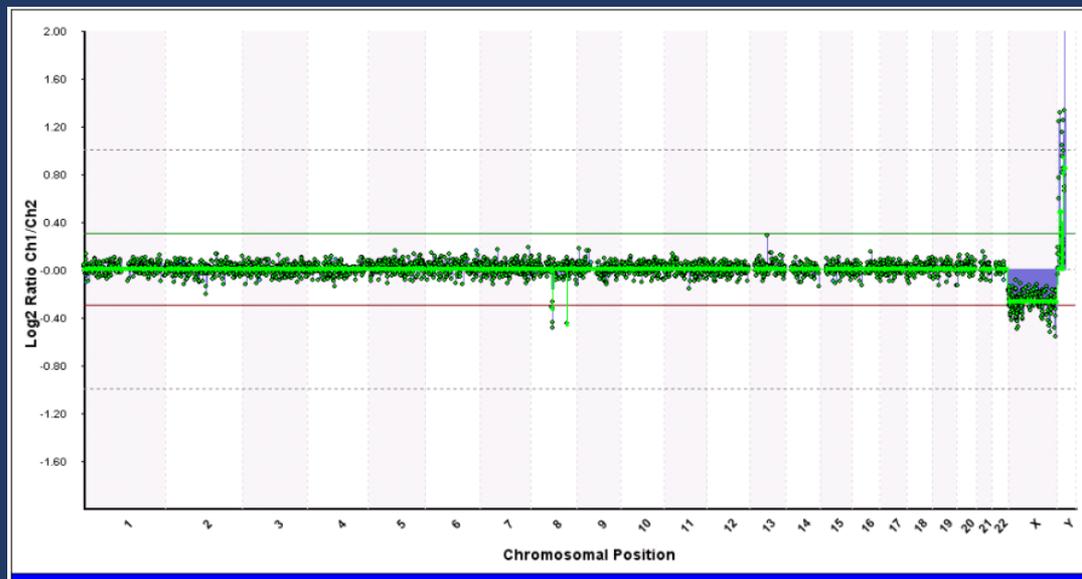
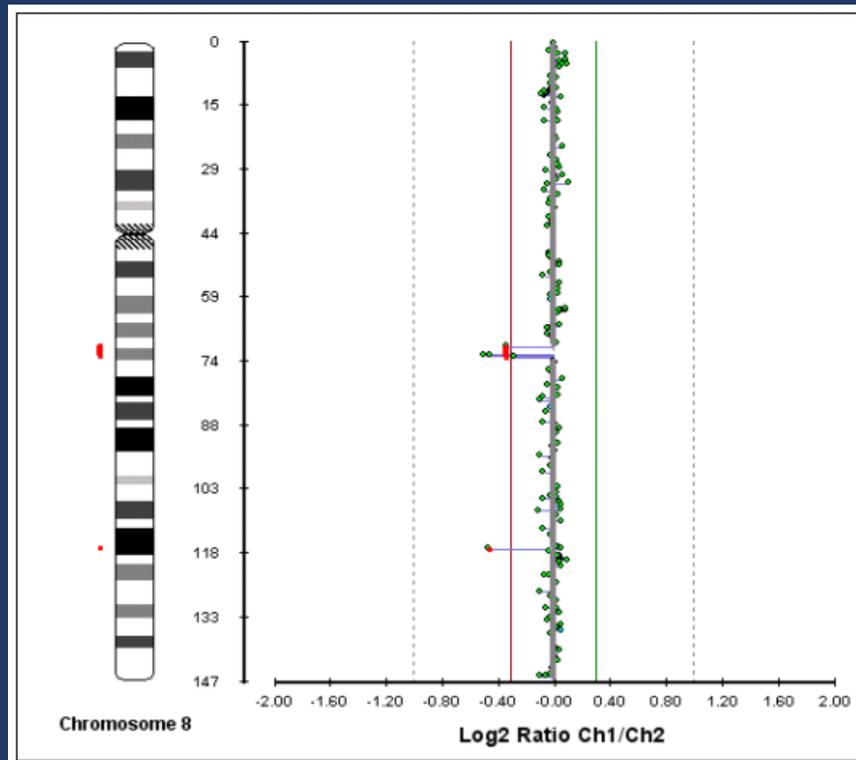


Grafico sintetico del risultato dell'analisi per tutti i cromosomi



Risultato CGH array cromosoma 8

Grazie

