new and developing technologies for genetics diagnostic
7th and 8th July 2008
Salisbury District Hospital

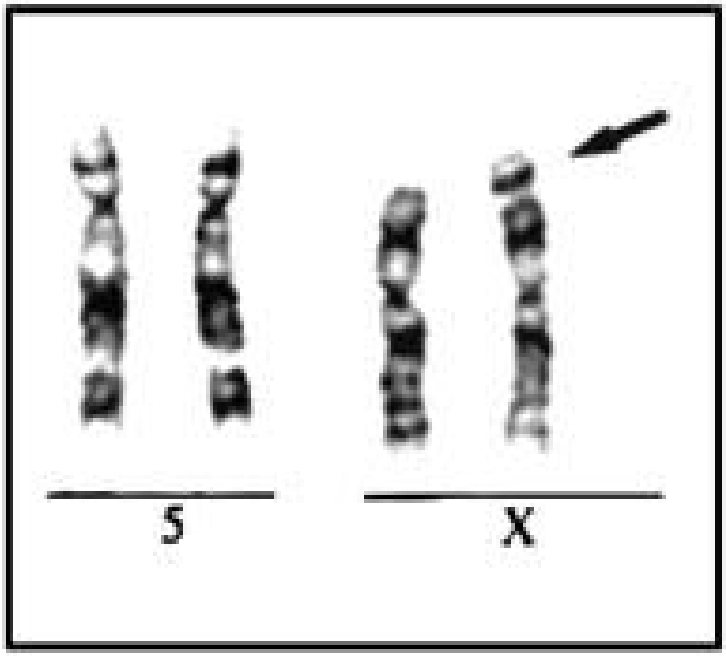
# genome-wide array
# and
# chromosome structural rearrangements

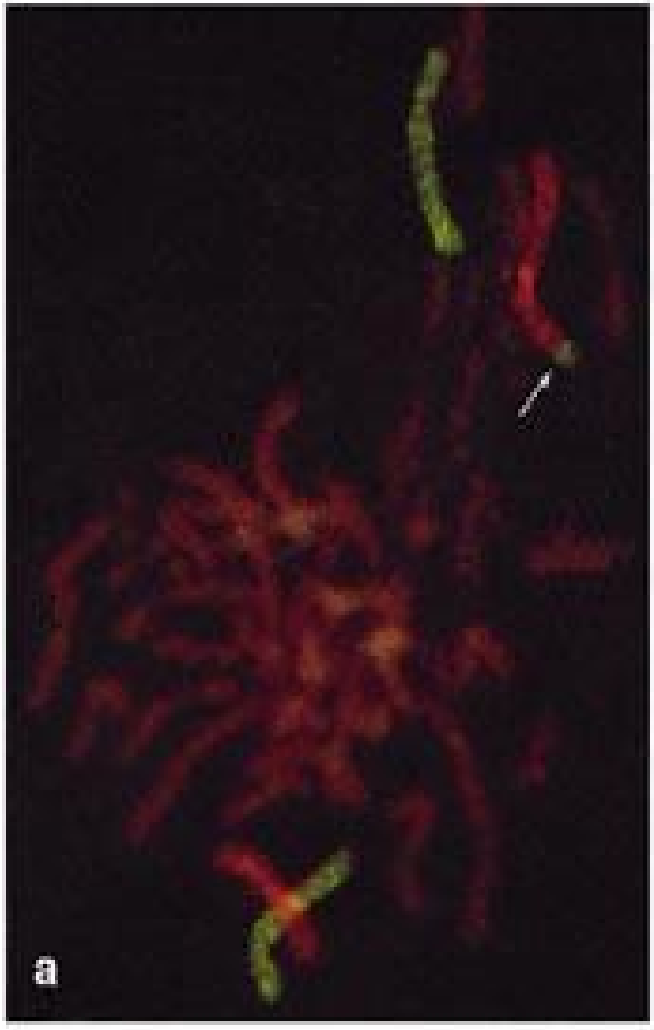
orsetta zuffardi
university of pavia, italy

## Distal 5q Trisomy Resulting From an X;5 Translocation Detected by Chromosome Painting

**Abuelo et al, 2000**

**AJMG**

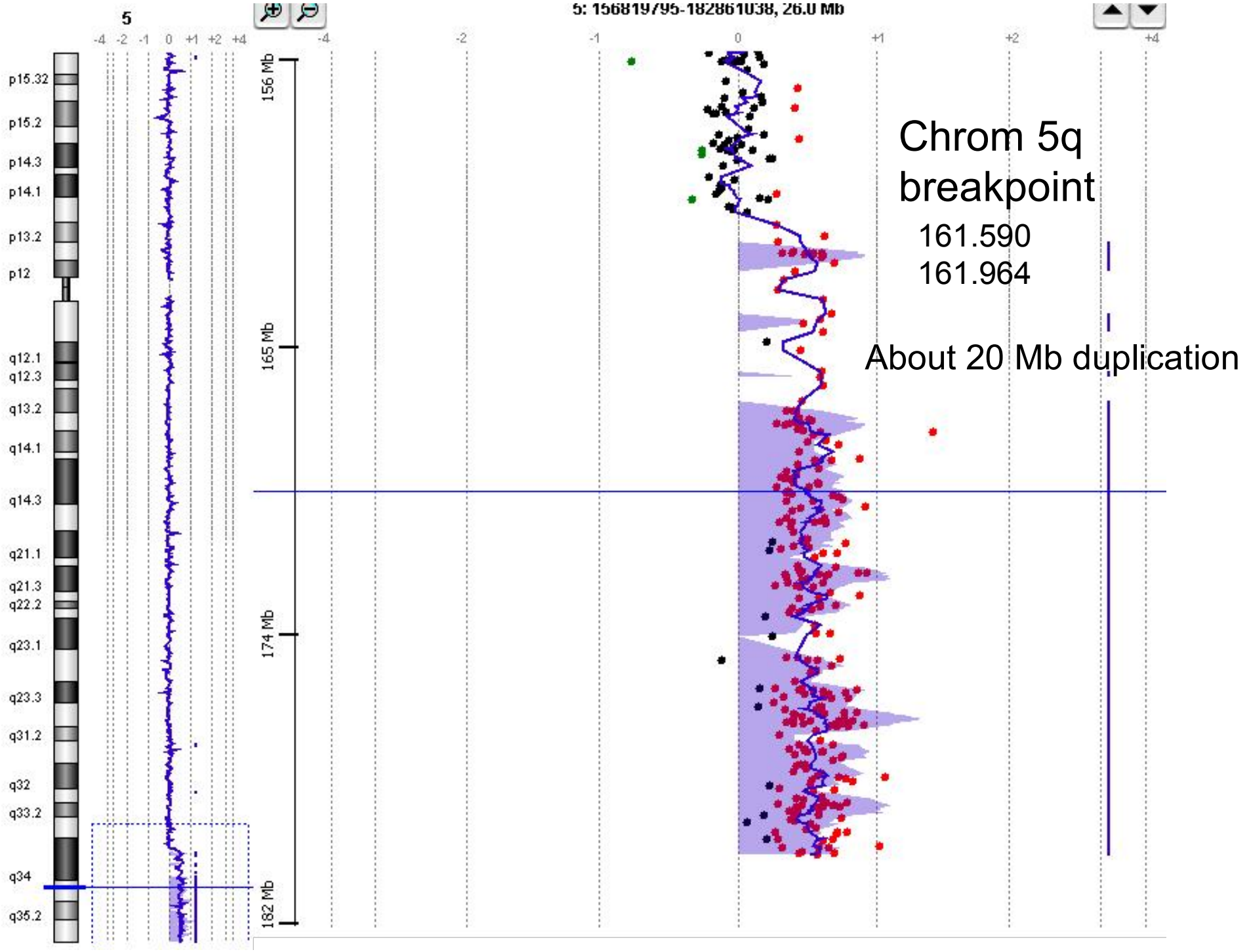


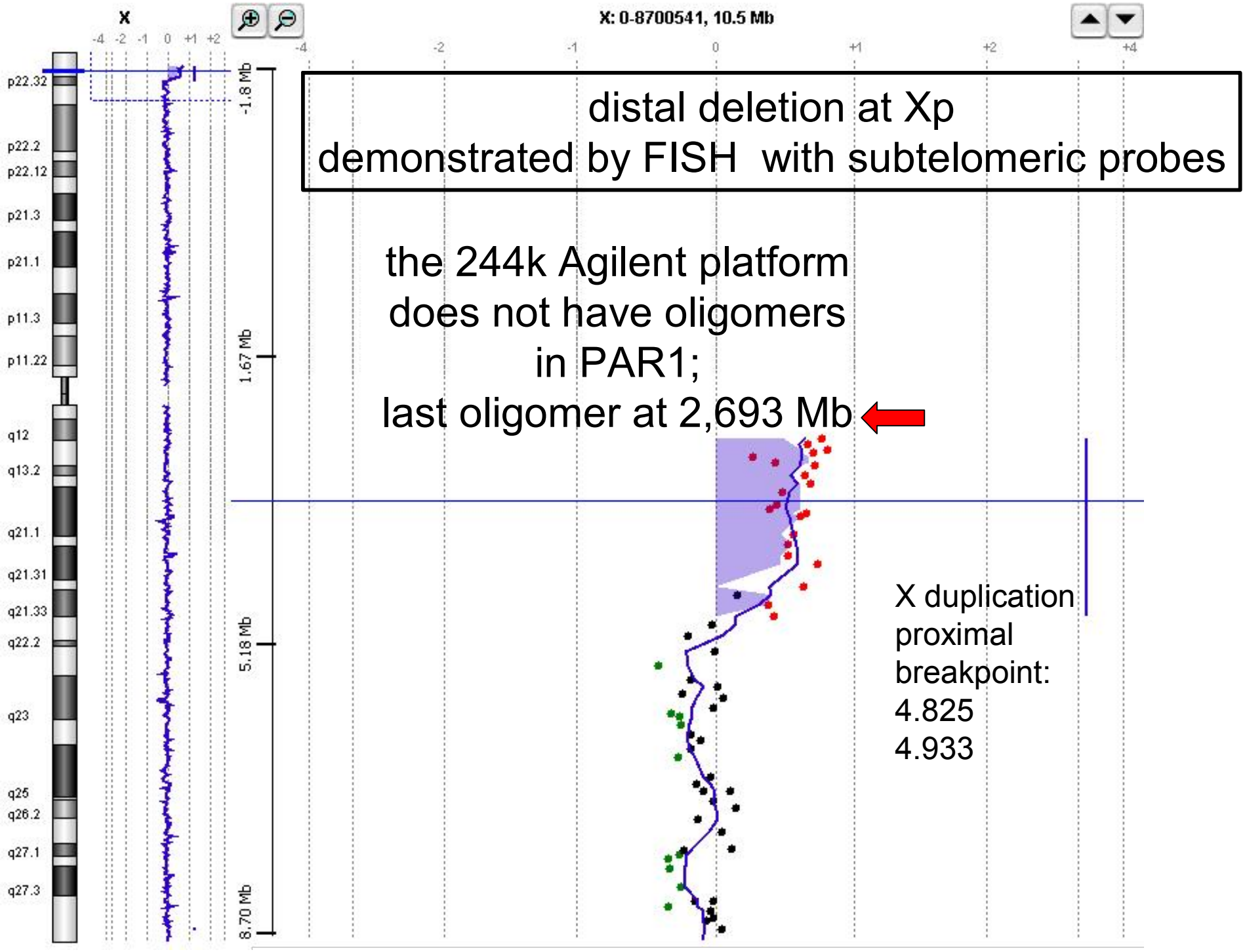




**46,X,der(X)t(X;5)(p22.3;q33)**

***de novo***

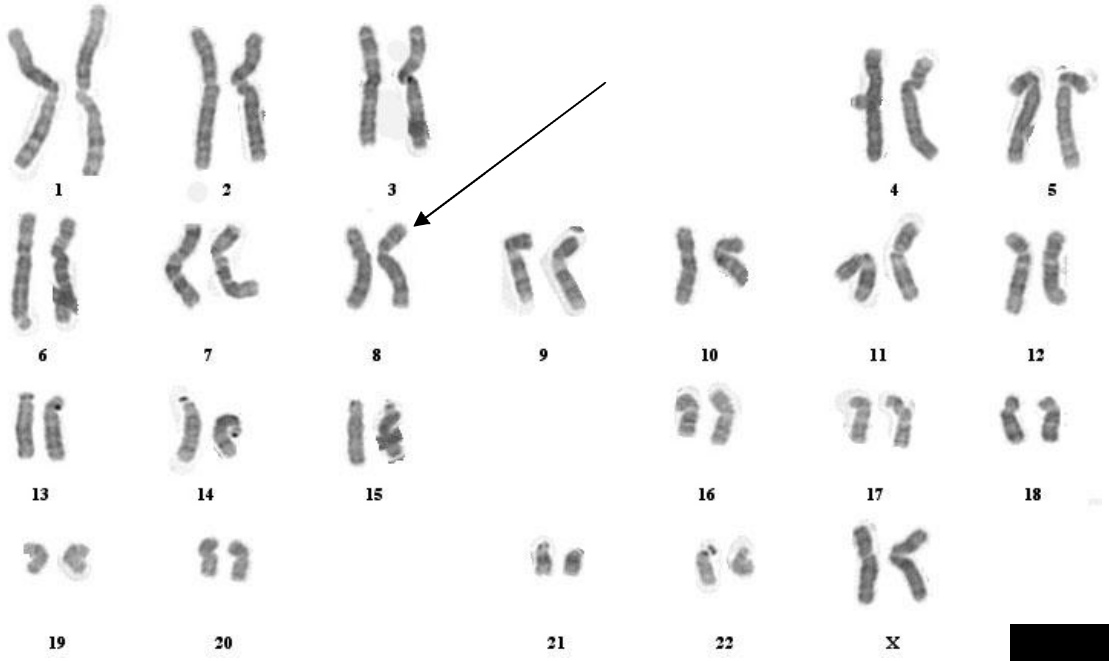
Xp deletion demonstrated through FISH with subtelomeric probes


5
5: 156819795-182861038, 26.0 Mb
Chrom 5q
breakpoint
161.590
161.964
About 20 Mb duplication
156 Mb
165 Mb
174 Mb
182 Mb
p15.32
p15.2
p14.3
p14.1
p13.2
p12
q12.1
q12.3
q13.2
q14.1
q14.3
q21.1
q21.3
q22.2
q23.1
q23.3
q31.2
q32
q33.2
q34
q35.2

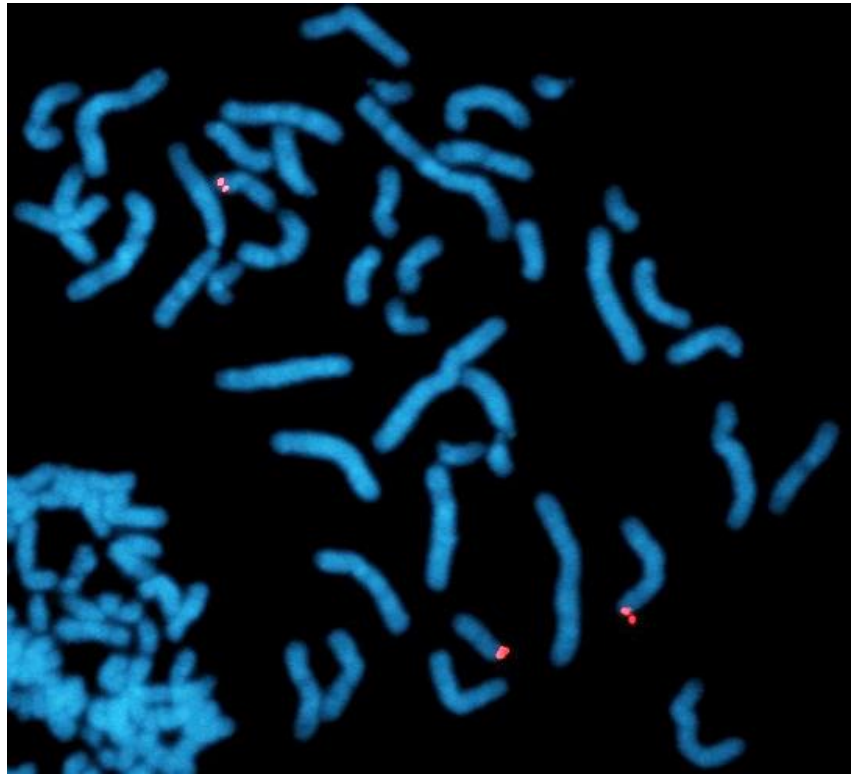

X
X: 0-8700541, 10.5 Mb
distal deletion at Xp
demonstrated by FISH with subtelomeric probes
the 244k Agilent platform
does not have oligomers
in PAR1;
last oligomer at 2,693 Mb
X duplication
proximal
breakpoint:
4.825
4.933
p22.32
p22.2
p22.12
p21.3
p21.1
p11.3
p11.22
q12
q13.2
q21.1
q21.31
q21.33
q22.2
q23
q25
q26.2
q27.1
q27.3
-1.8 Mb
1.67 Mb
5.18 Mb
8.70 Mb

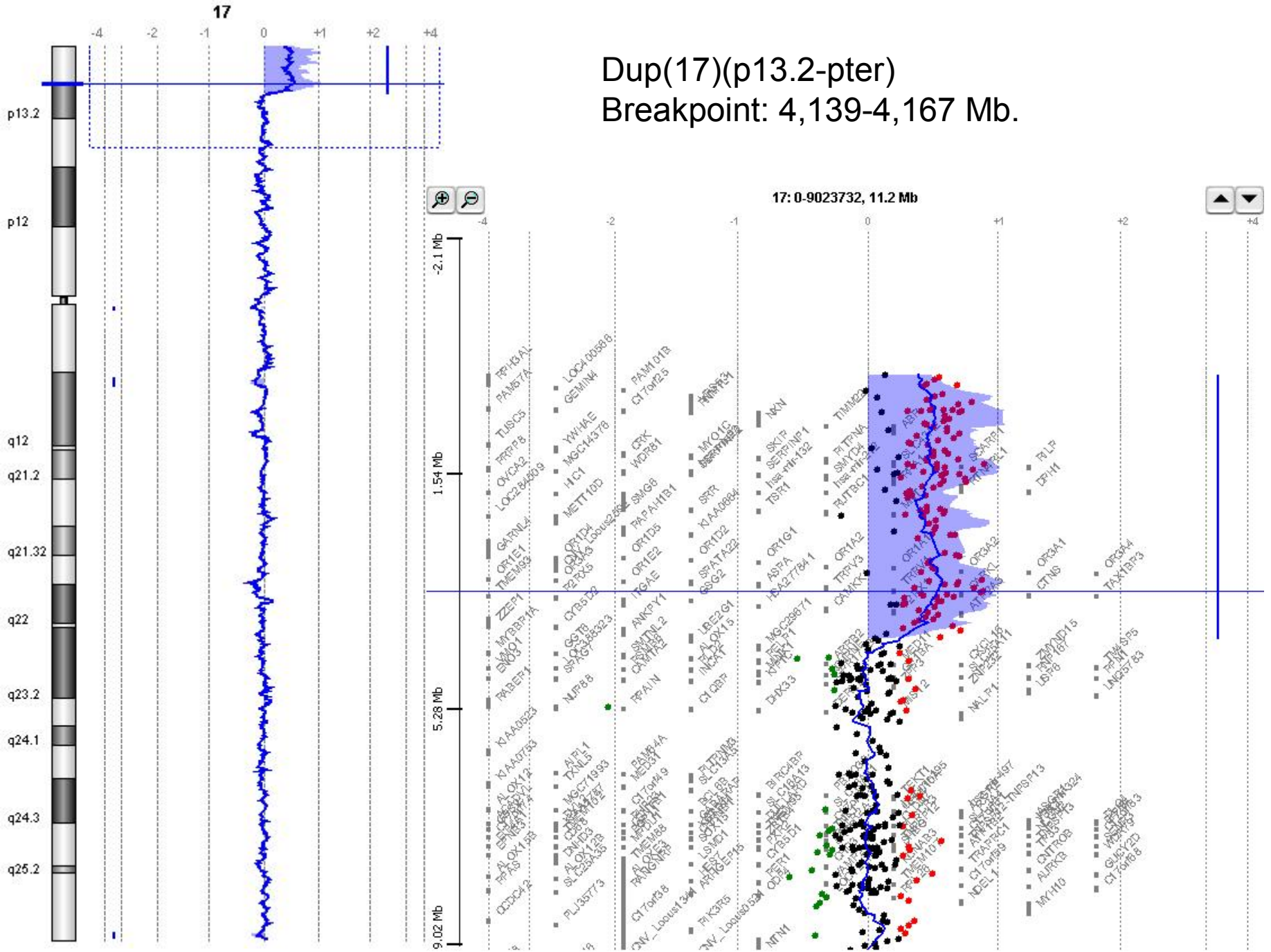
46,X,der(X)t(X;5)(p22.3;q33)
*de novo*

**46,der(X)t(inv dup delX;5)**
**(p22.3;q33)**
***de novo***

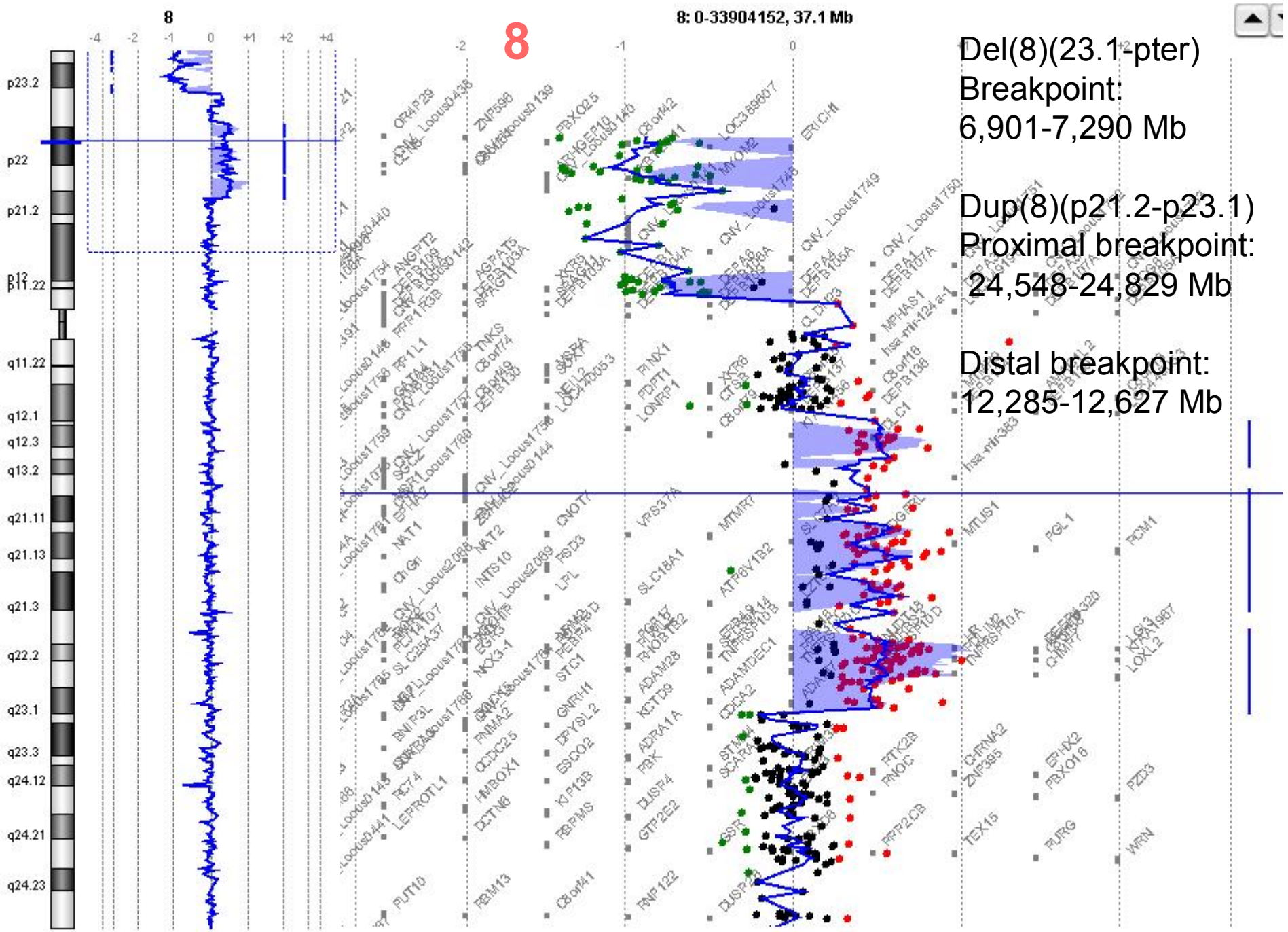



46,XX,der(8)t(8;17)(p22.3;p12)
*de novo*






Dup(17)(p13.2-pter)
Breakpoint: 4,139-4,167 Mb.


8
8: 0-33904152, 37.1 Mb
Del(8)(23.1-pter)
Breakpoint:
6,901-7,290 Mb
Dup(8)(p21.2-p23.1)
Proximal breakpoint:
24,548-24,829 Mb
Distal breakpoint:
12,285-12,627 Mb

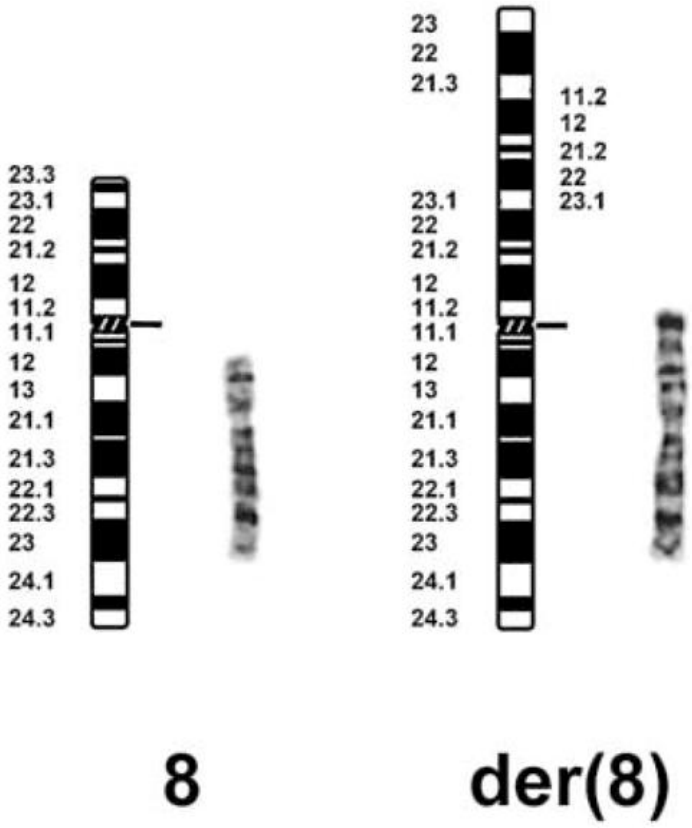
**46,XX,der(8)t(8;17)(p22.3;p12)**
***de novo***

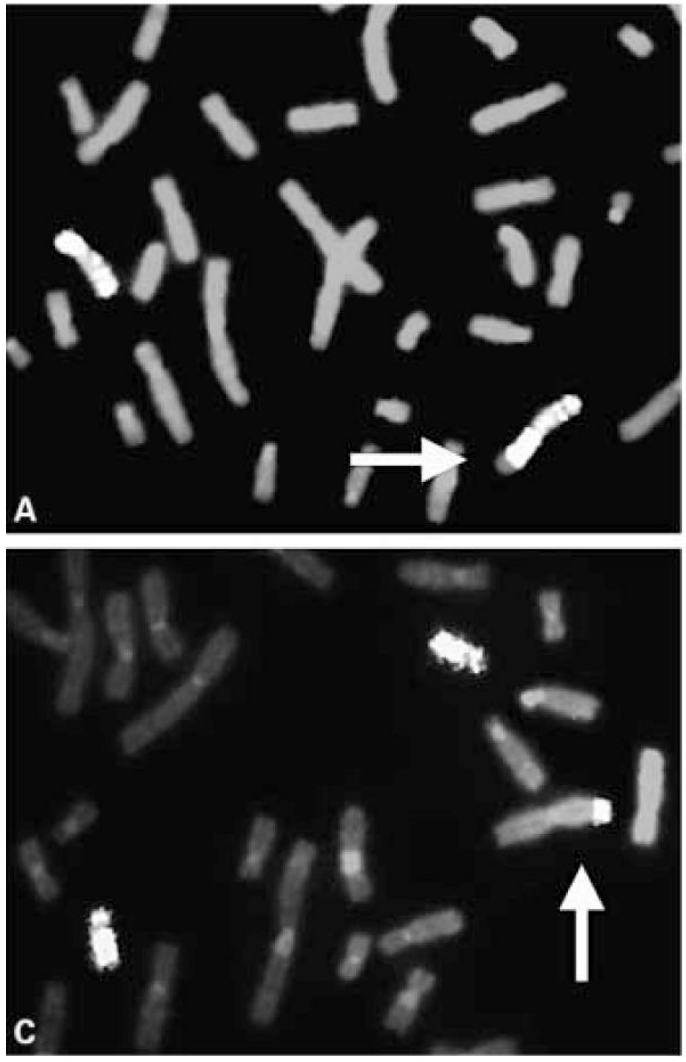
**46,XX,der(8)t(inv dup del8;17)**
**(p22.3;p12)**

***de novo***

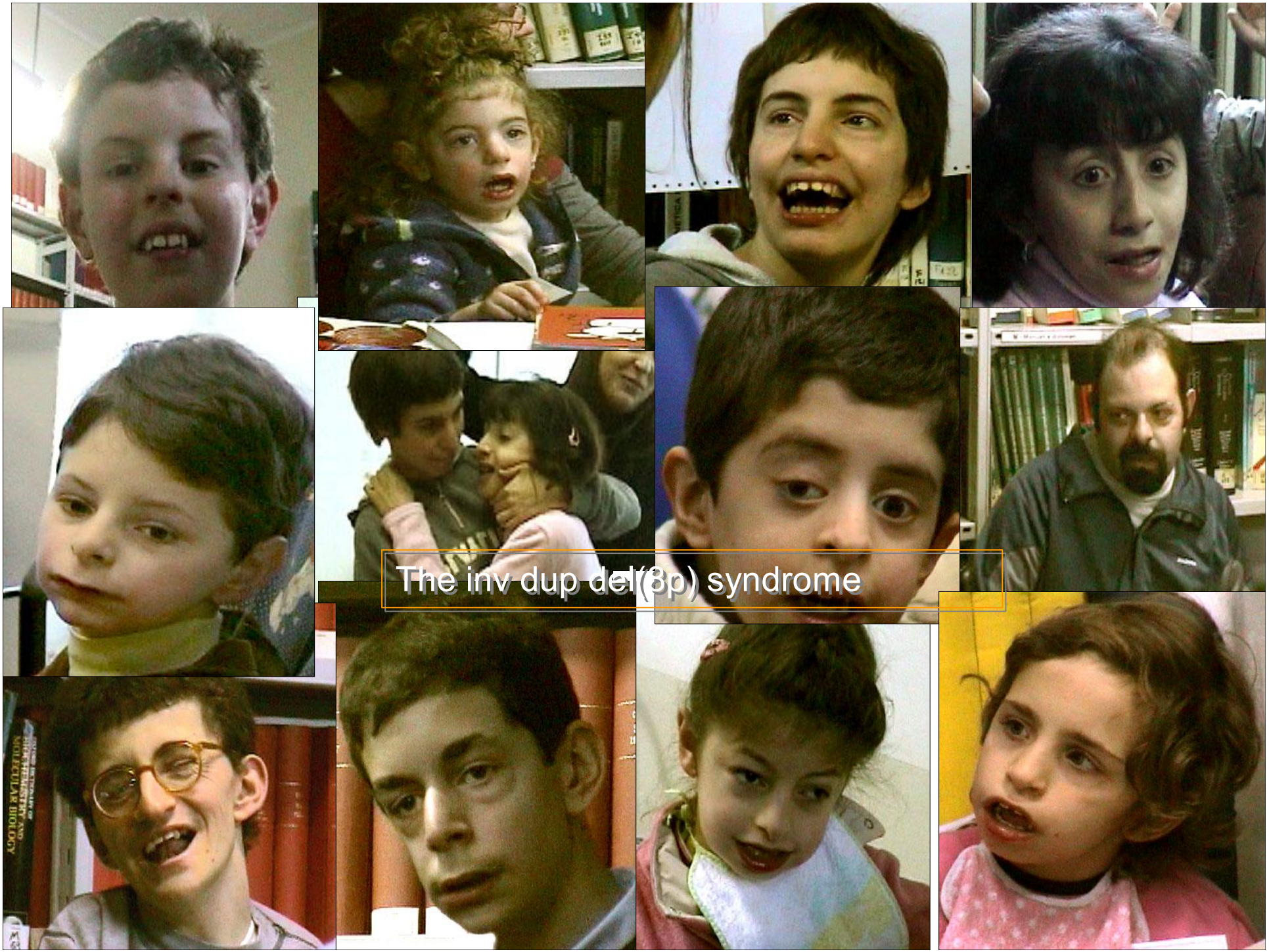
Stabilization of a terminal inversion duplication of 8p by telomere capture from 18q

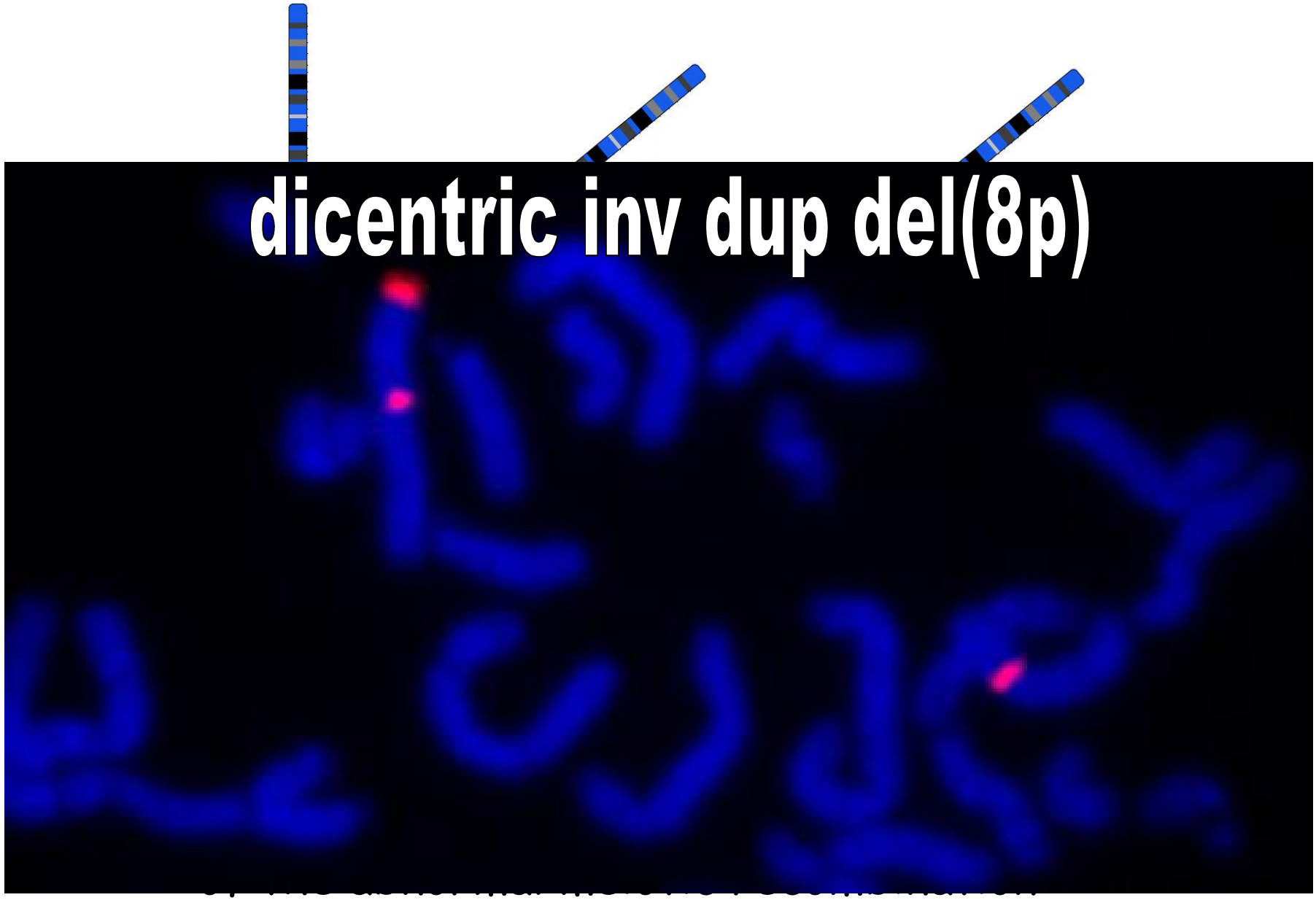
**D.R. Kostiner, 2002**



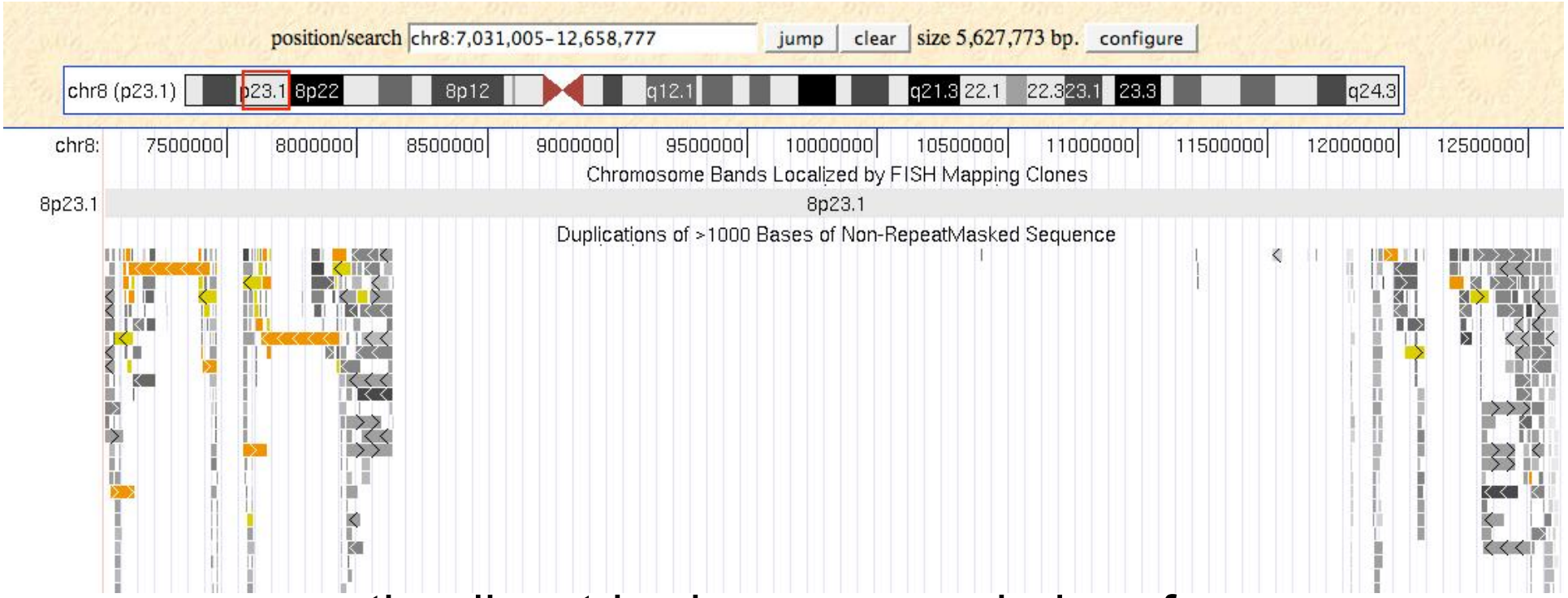



two cases of inv dup del(8p)
stabilized by the distal region
of another chromosome


The inv dup del(8p) syndrome

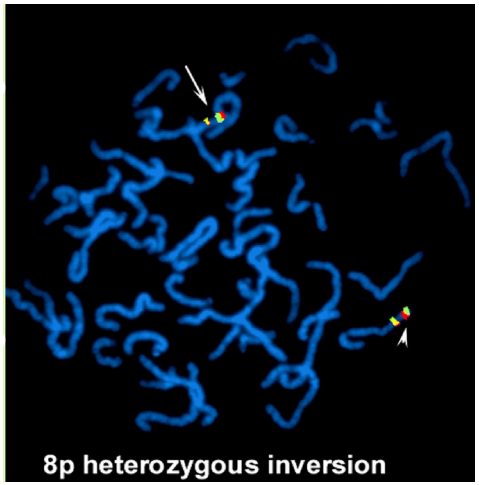



Floridia et al 1996

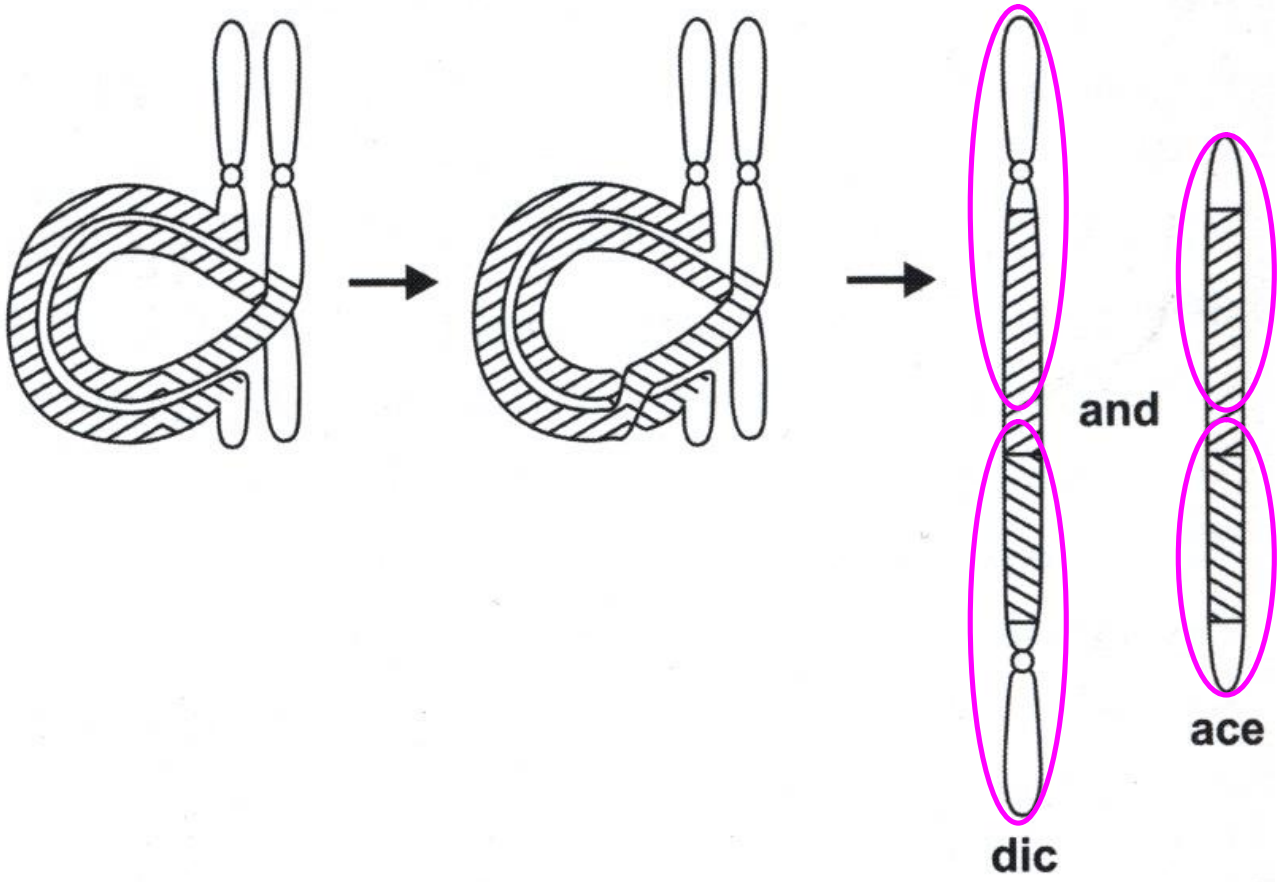



the dicentric chromosome derives from
non-allelic homologous recombination at the maternal meiosis
on the background of a cryptic paracentric inversion

**Giglio et al, 2001**

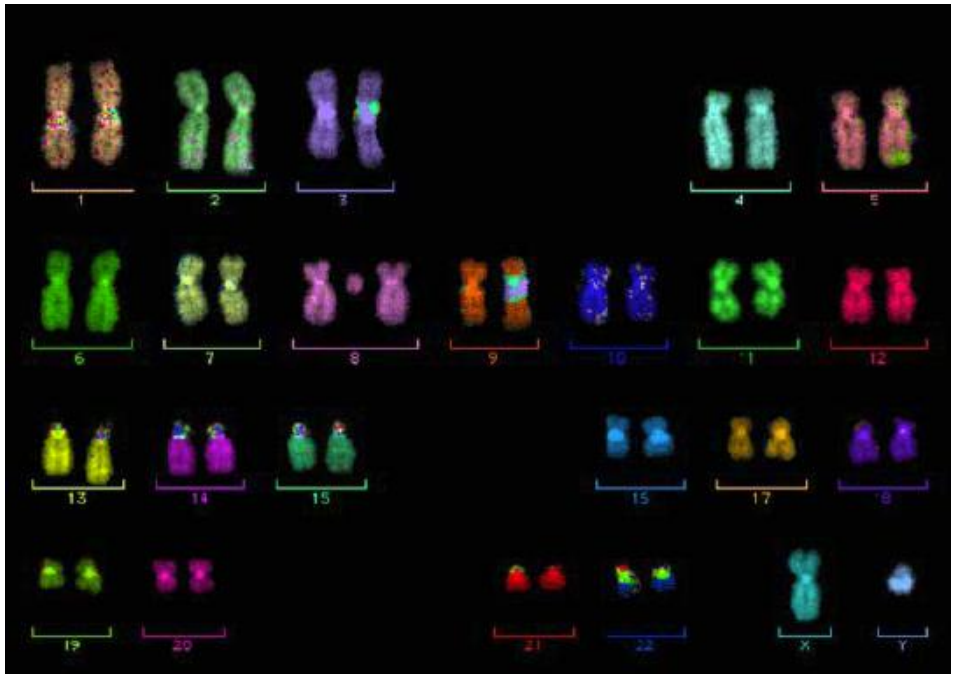



**Metaphase from a mother transmitting “de novo” inv dup del(8p)**



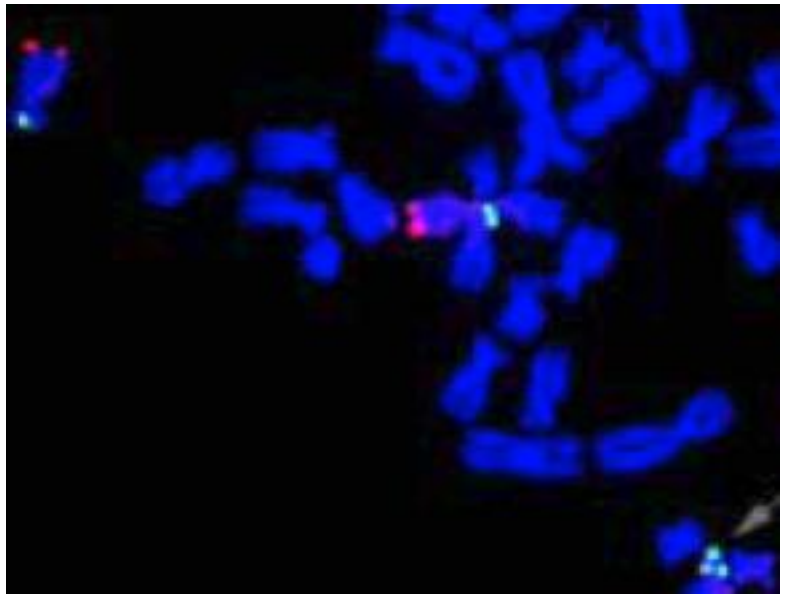

Theoretical recombinant products
from classical crossover in paracentric inversion

Does the reciprocal product
of the dicentric chromosome exist??



Warburton, 2004

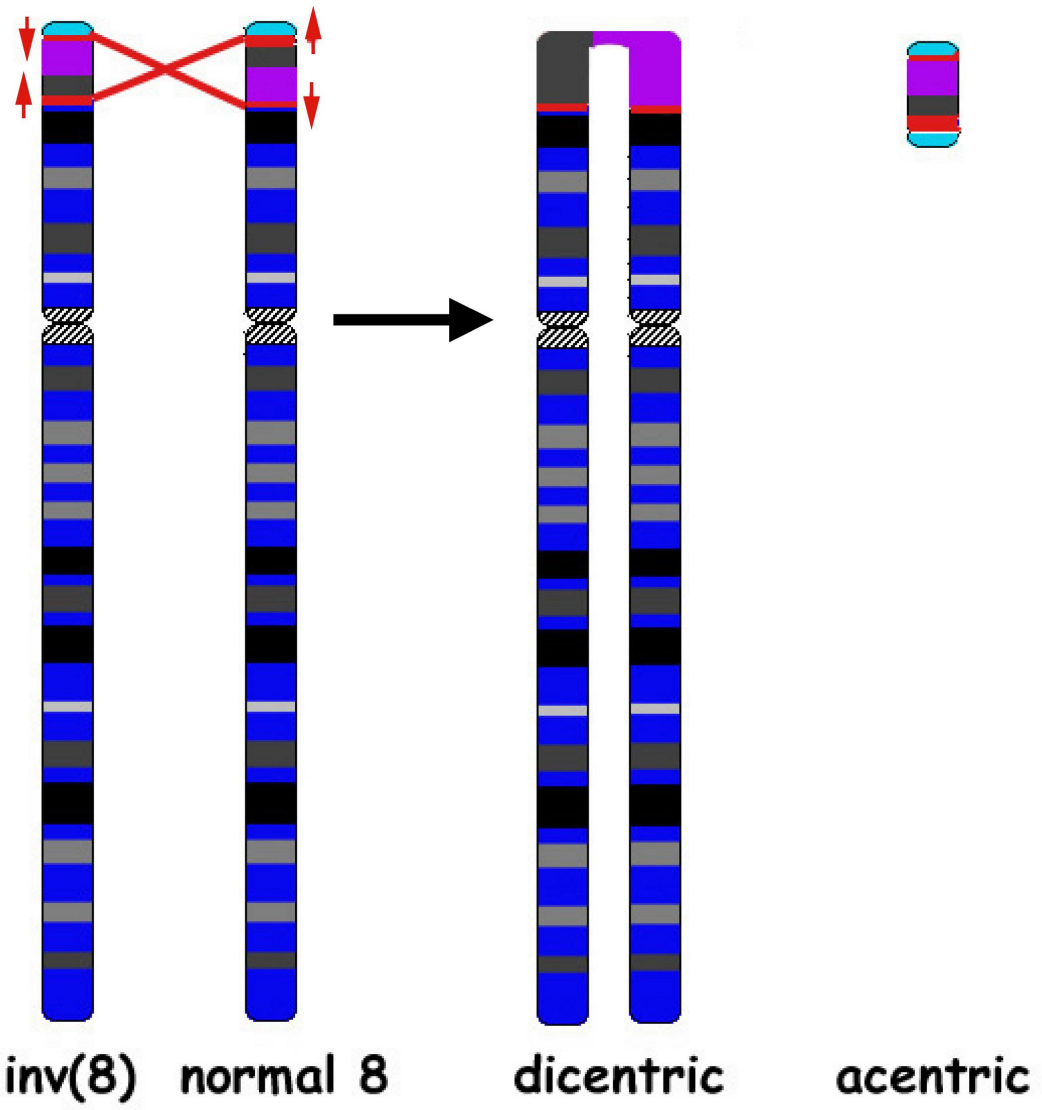
FISH with chrom. 8
subtelomeric specific probes



Nonallelic homologous recombination between two olfactory receptor genes clusters results in a dicentric and an acentric recombinant chromosome

**The inv dup del (8p) derives from the breakage of a dicentric chromosome**

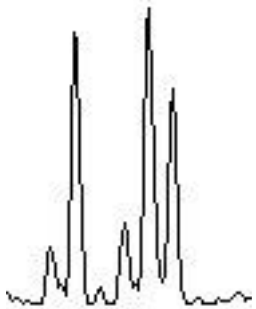
**The acentric chromosome can acquire a neocentromere thus being rescued**

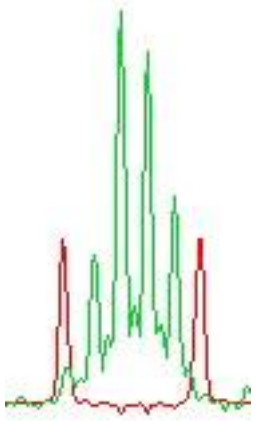


another demonstration that the dicentric originates at MI and it is not postzygotic

The duplication region of the inv dup del(8p) contains two maternal and one paternal allele

| Marker | Position | Alleles | Status |
|---|---|---|---|
| D8S504 | 1004 | 1 | D |
| D8S264 | 2117 | 1 | D |
| D8S201 | 3027 | | |
| D8S1824 | 3540 | | |
| D8S262 | 3664 | | |
| D8S518 | 4475 | | |
| D8S277 | 6504 | | |
| D8S1819 | 6737 | 1 | D |
| MSAT3 | 7750 | | |
| OR-REPD | | | |
| D8S503 | 9270 | 1, 2 | N |
| D8S1721 | 10178 | 1, 2 | N |
| D8S520 | 10593 | 1, 2 | N |
| D8S550 | 10990 | | |
| D8S265 | 11317 | | |
| OR-REPP | | | |
| D8S552 | 12752 | 1, 2, 3 | Dup |
| D8S511 | 14690 | 1, 2, 3 | Dup |
| D8S549 | 15660 | | |
| D8S254 | 16618 | 1, 2, 3 | Dup |
| D8S261 | 17836 | 1, 2, 3 | Dup |
| D8S258 | 20377 | 1, 2, 3 | Dup |
| D8S282 | 21425 | 1, 2, 3 | Dup |
| D8S1734 | 22817 | 1, 2, 3 | Dup |
| D8S1771 | 25463 | | |
| D8S1809 | 28213 | 1, 2, 3 | Dup |
| D8S278 | 32606 | 1, 2 | N |
| D8S513 | 33727 | | |
| D8S1750 | 35470 | 1, 2 | N |
| D8S1821 | 38369 | 1, 2 | N |
| D8S255 | 39902 | 1, 2 | N |
| D8S268 | 41264 | 1, 2 | N |
| D8S1115 | 42554 | | |
| D8S531 | 49074 | 1, 2 | N |

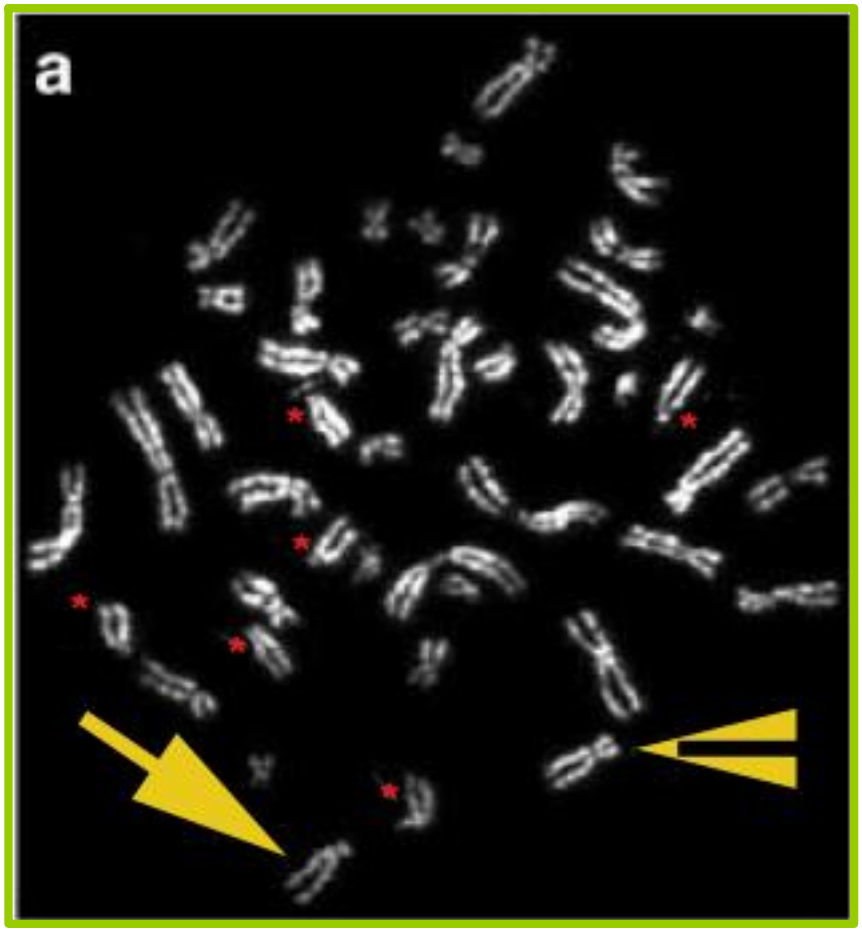




The rearrangement occurs at MI through NAHR;
the **dicentric** chromosome can be
either immediately broken at MII
or
be inherited as such in the zygote

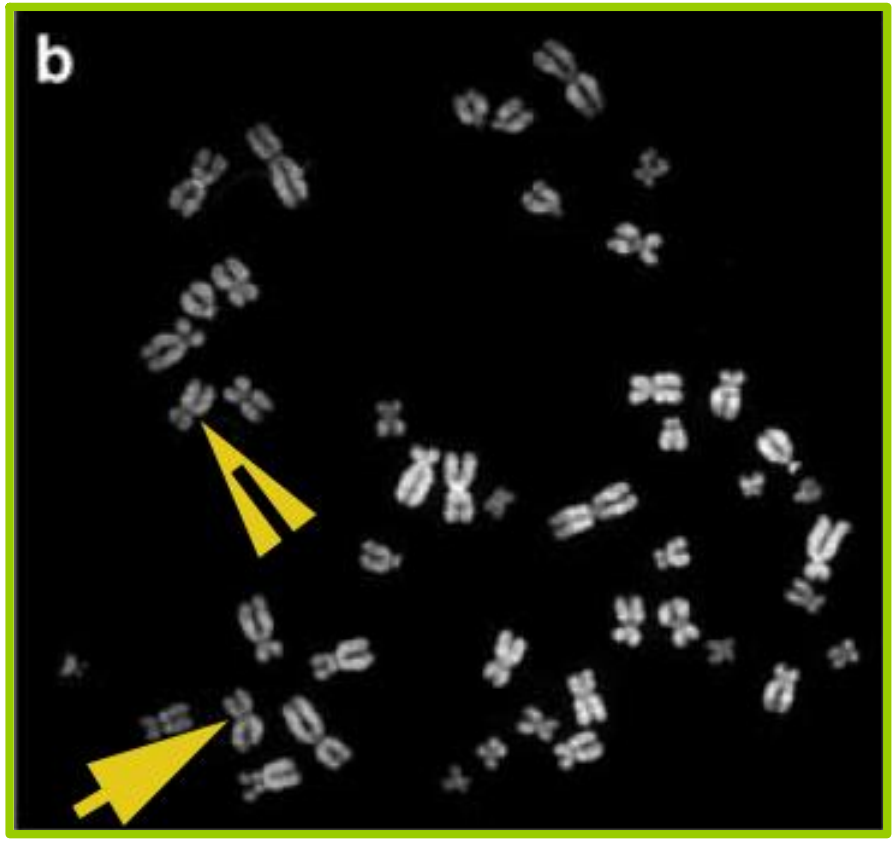
some inv dup del (8p) cases in mosaic with different derivatives of the original dicentric:

- ✓presence of the dicentric in the zygote;
- ✓the occurrence of different cell lines in the early embryogenesis;
- ✓the most viable one(s) will be detected at birth



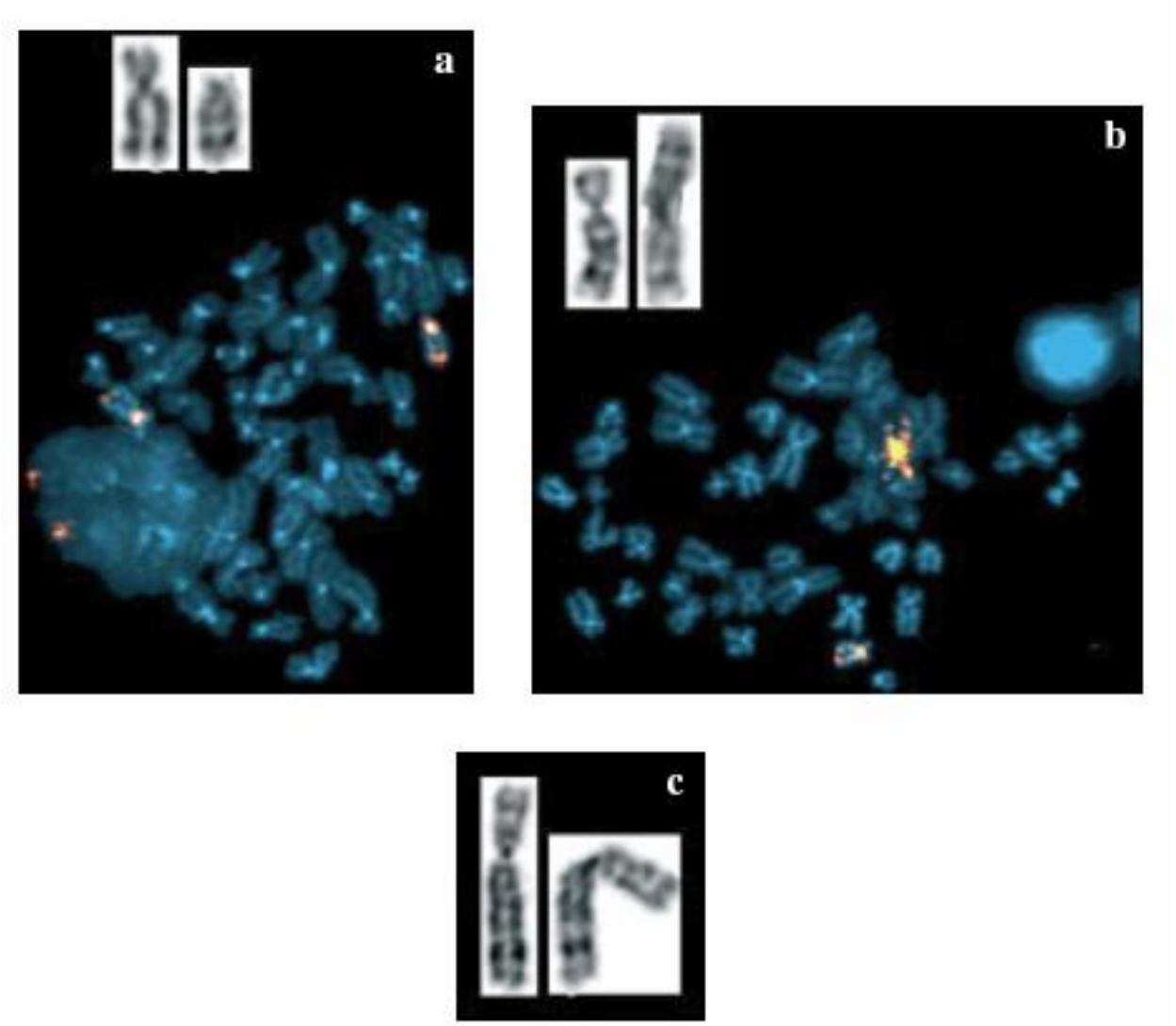

direct chorion villi: del(8)(p11.1)

cultured chorion villi:
inv dup del(8p)

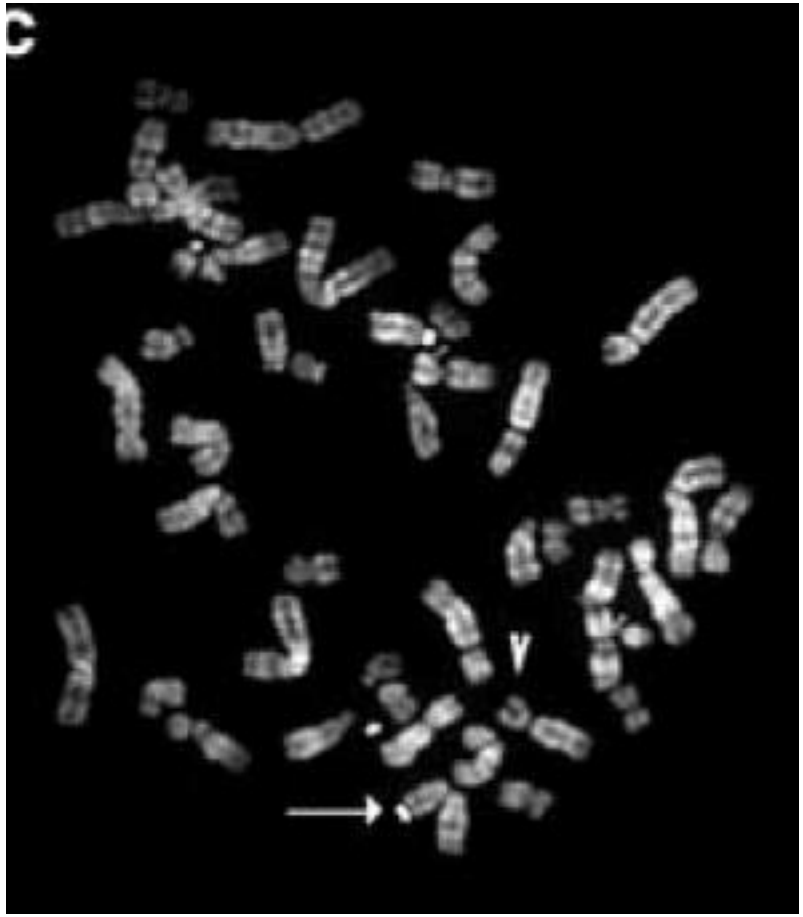



Pramparo et al, EJHG,2004

Soler et al, 2003; both cell lines found in direct CVS

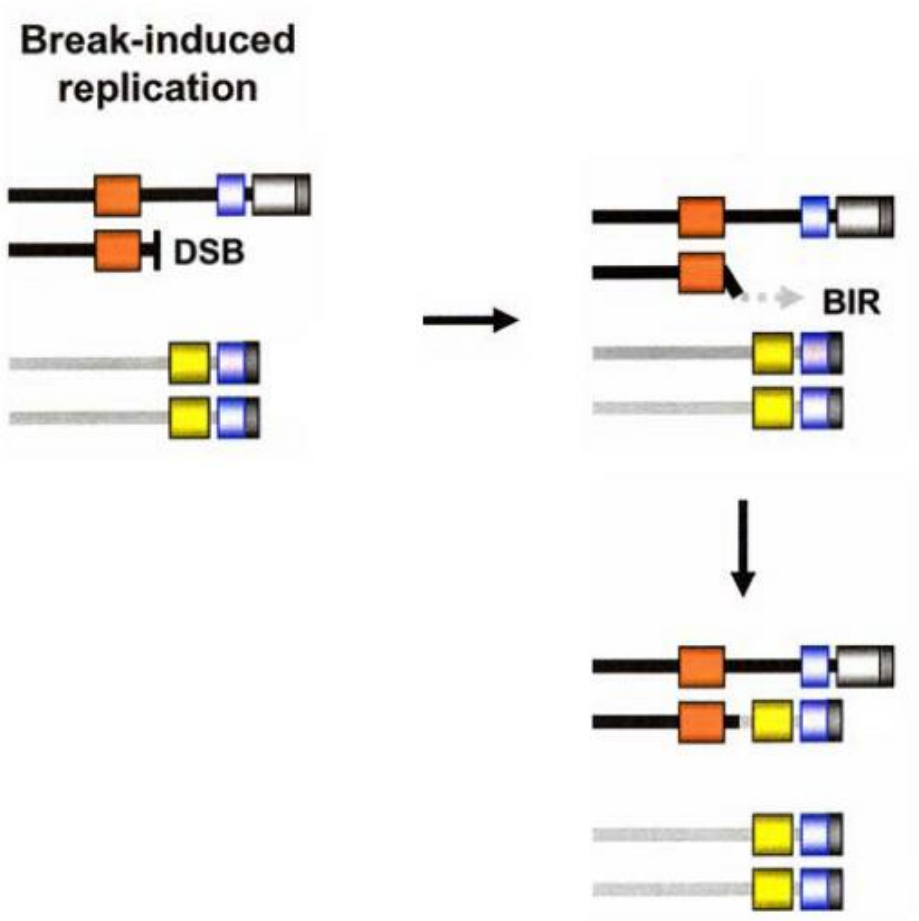


A third cell line found in the cultured CV showed the transposition of satellites to the short arm of the inv dup del(8p): telomere capture




Pramparo et al, EJHG, 2004

# Translocations and telomere capture




…… a broken chromosome that is repaired by invading and replicating sequences from another chromosome end.

This results in a nonreciprocal translocation

From L. Shaffer, 2004

the zygote may receive the dicentric chromosome that undergoes different breakages in the different cells leading to different cell lines

**the absence of telomerase during early embryogenesis may cause telomere capture**

Telomerase activity is low or absent in cleavage stage embryos
and then high again in blastocyst

How early embryos reset telomere length remains poorly understood

We suggest that telomeres lengthen during the early cleavage cycles following fertilization through a recombination-based mechanism

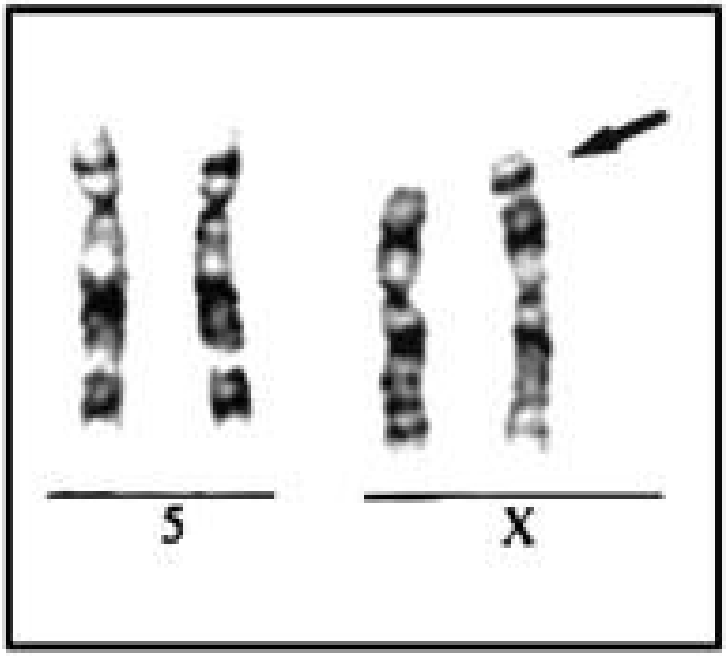
Telomere lengthening early in development recombination-based mechanism

L. Liu, Nature Cell Biology December 2007

## Distal 5q Trisomy Resulting From an X;5 Translocation Detected by Chromosome Painting

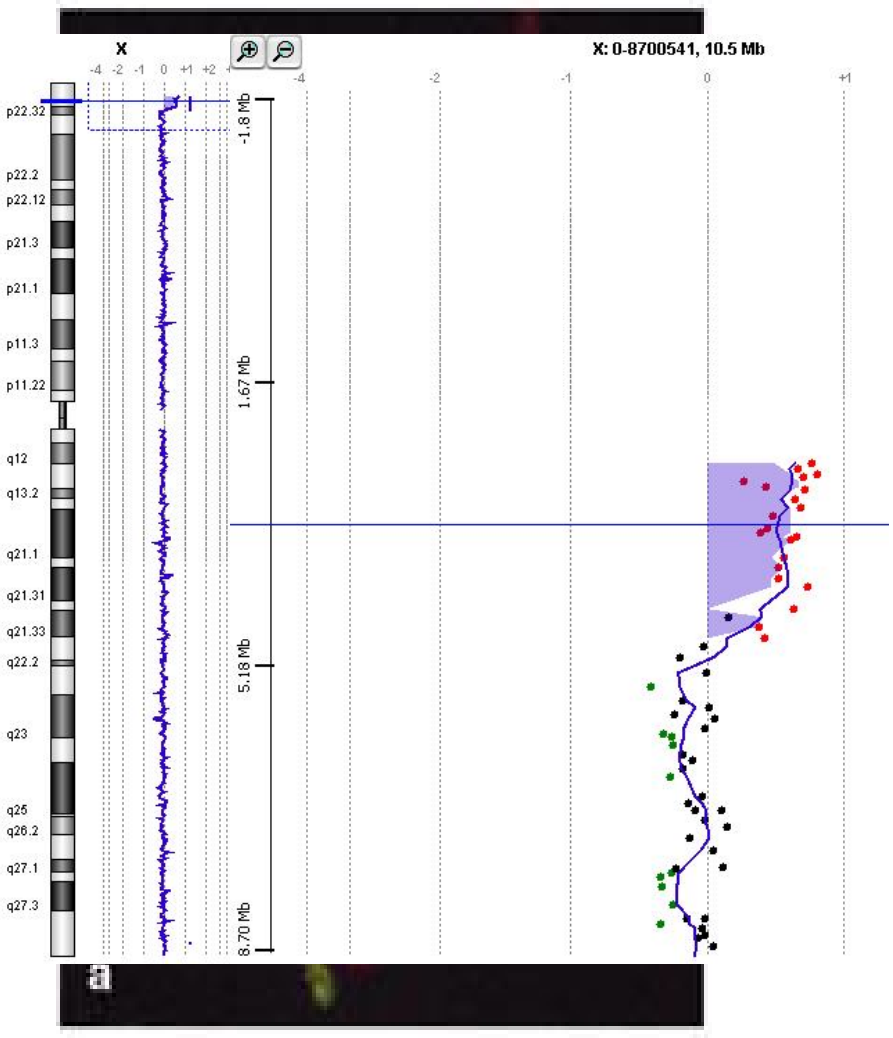
**Abuelo et al, 2000**

AJMG

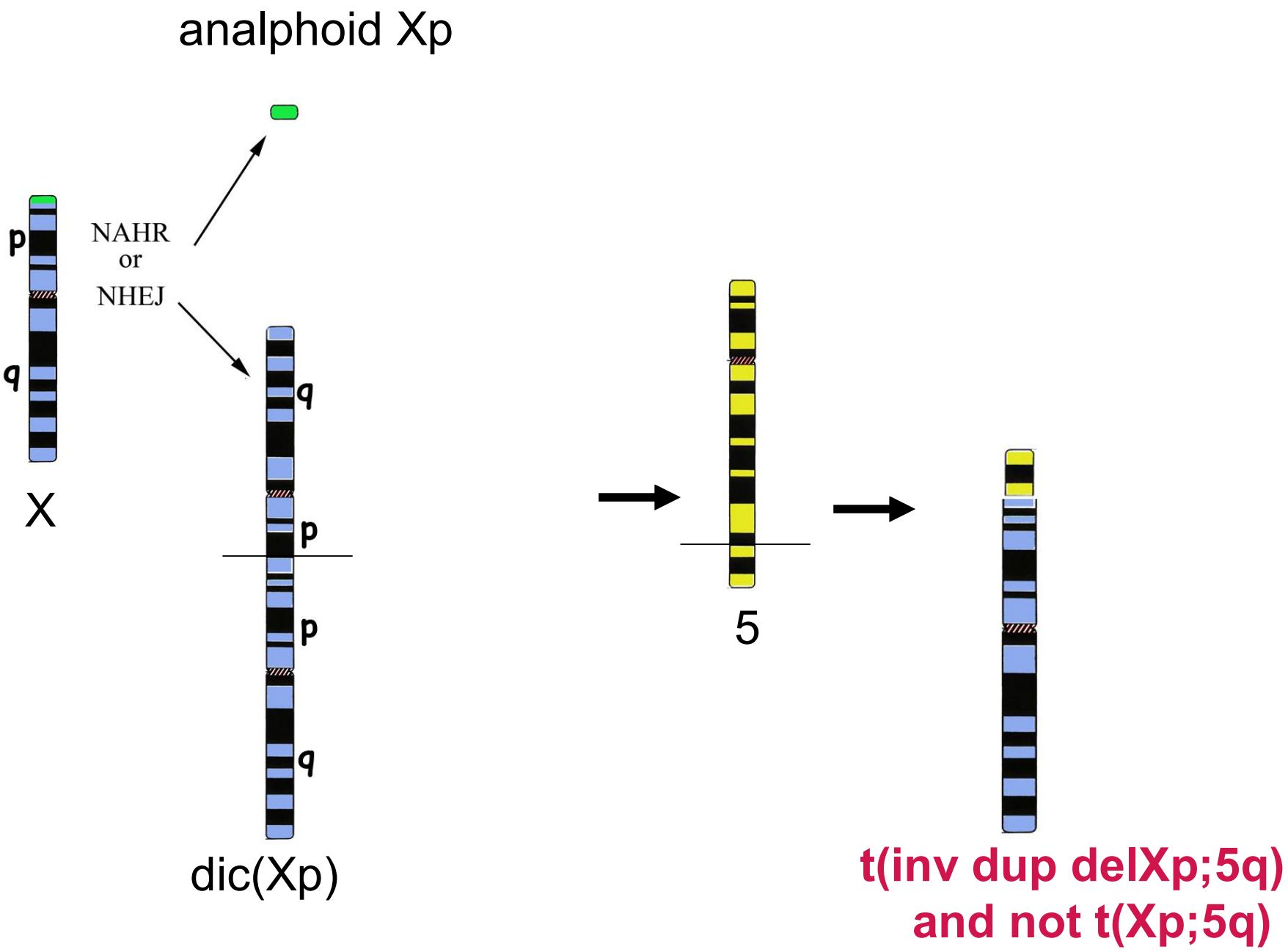



**46,X,der(X)t(X;5)(p22.3;q33)**

***de novo***




Xp deletion demonstrated through FISH with subtelomeric probes

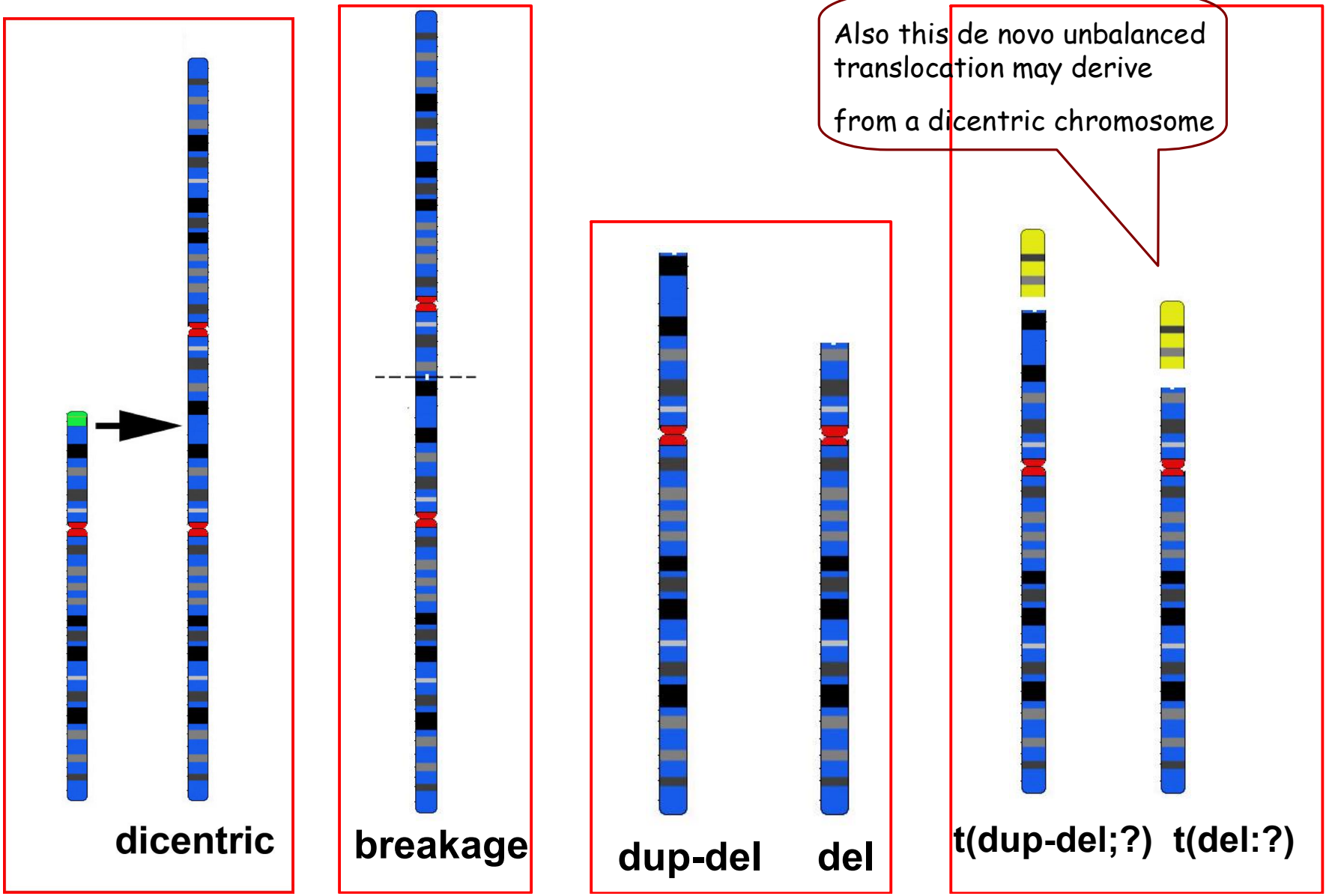

analphoid Xp
p
q
X
NAHR
or
NHEJ
q
p
p
q
dic(Xp)
5
t(inv dup delXp;5q)
and not t(Xp;5q)

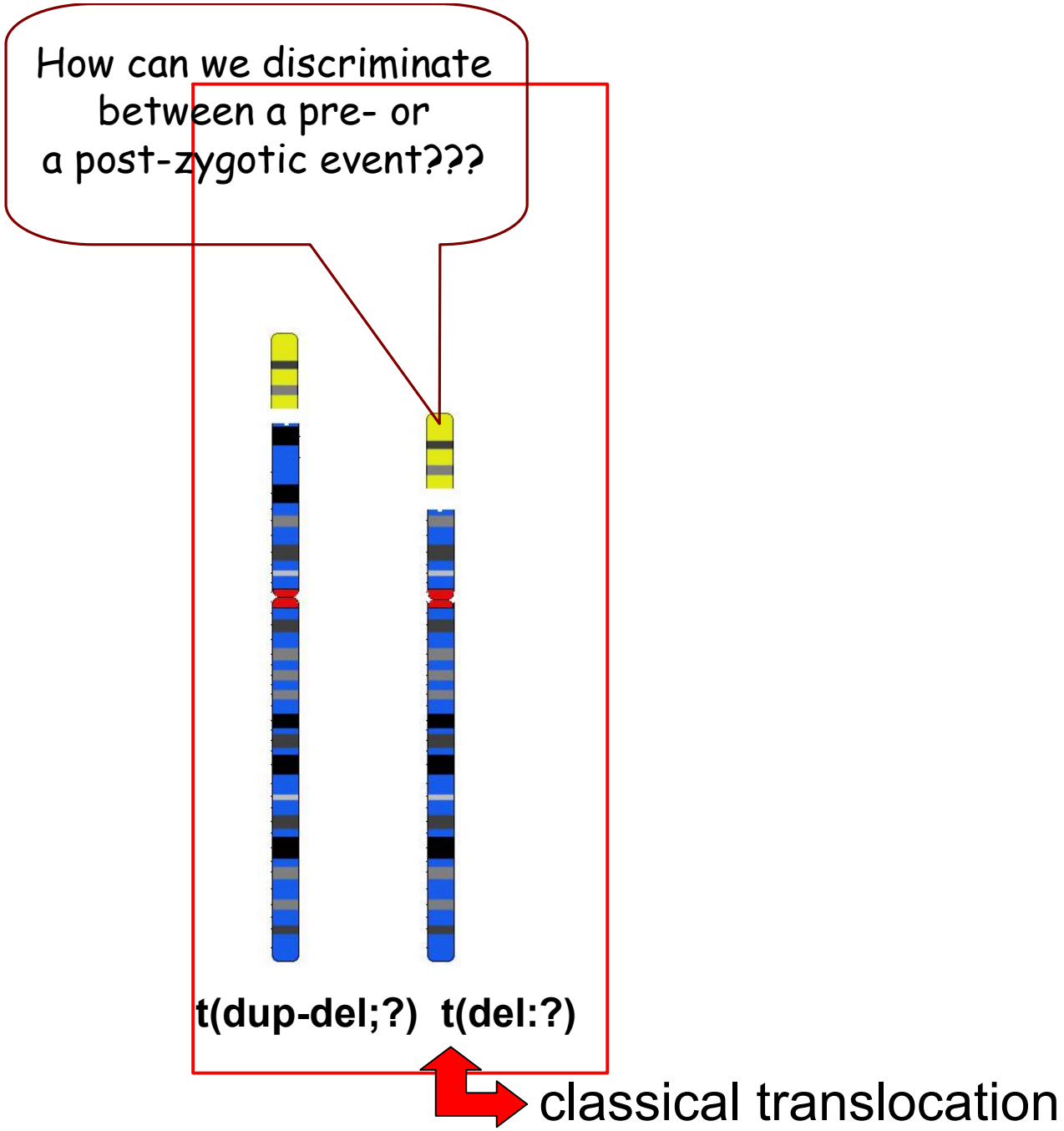
how many times in *de novo* unbalanced translocations
one of the two derivative chromosomes arises
postzygotically from
an inv dup del
stabilized through telomere capture???

are all de novo unbalaced translocations
formed through this mechanism?????

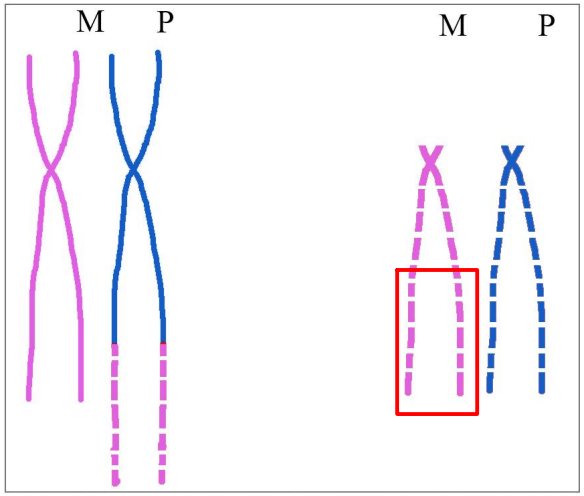
# Take care

Terminally deleted chromosome can also result
post-zygotically
from the breakage of the dicentric chromosome

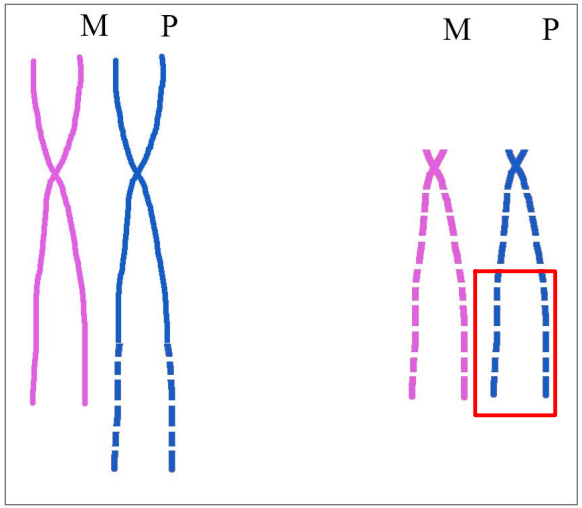



How can we discriminate between a pre- or a post-zygotic event???
t(dup-del;?)
t(del:?)
classical translocation

If de novo unbalanced translocations were post-zygotic events,
there is a 50% probability that the deletion arises on the paternal chromosome and the duplication on the maternal one or vice-versa




Postzygotic
(pat. deletion;
mat. duplication)

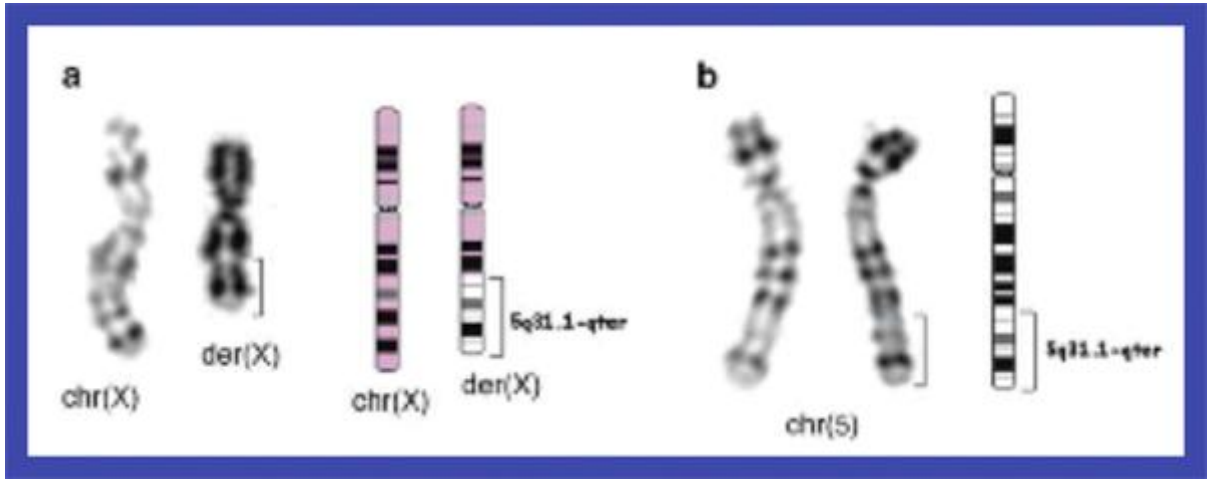



either paternal
gametogenesis or
postzygotic
(pat. deletion;
pat. duplication)

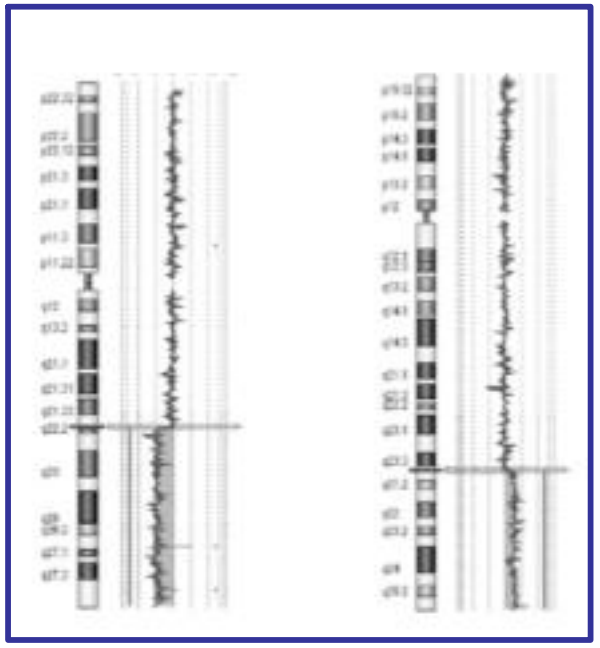
# Molecular and cytogenetic analysis of the spreading of X inactivation in a girl with microcephaly, mild dysmorphic features and t(X;5)(q22.1;q31.1

Giorda et al, 2008 EJHG

transcriptional activity of a number of chromosome 5 loci by RT-PCR analysis




*de novo*



Xq deletion 5q duplication

**Table 1** Typing with chromosome X polymorphic markers

| *Marker* | *Position (Kb)* | *Proband* | *Mother* | *Father* | *Chr X* | *Chr X/der(X)* |
|---|---|---|---|---|---|---|
| *DXS 1060* | 5.420 | 237/243 | 237/245 | 243 | 243 | 237/243 |
| *DXS 1226* | 22.857 | 349/351 | 349/351 | 351 | 351 | 349/351 |

Xq deletion occurred on the mat chrom. X
5q duplication occurred on the pat chrom. 5:
a postzygotic rearrangement

was the der(X) formed by
the breakage of a dicentric chromosome X???

**Table 2** Typing w

| *Marker* | *Po* | *Chr der(X)* |
|---|---|---|
| *D5S 2002* | 1 | — |
| *D5S 2117* | 1 | — |
| *D5S393* | 1 | 165 |
| *D5S399* | 1 | 123 |

Molecular typing performed on proband and parents +
two somatic cell hybrids containing the pat and the mat chromosome 5

* : duplicated alleles

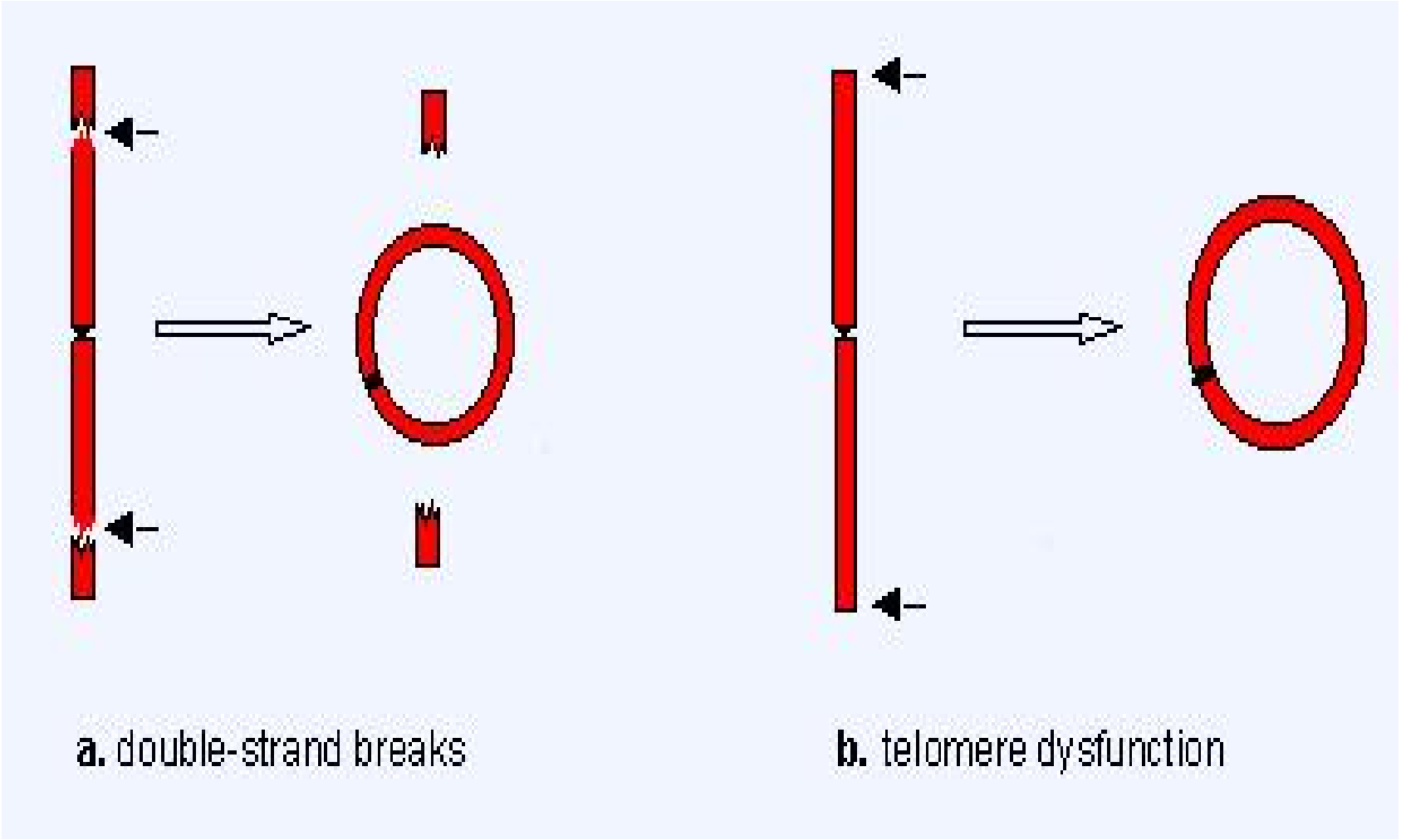
postzygotic formation of translocations is considered
a rare event in absence of mosaicism

the parental origin of non-mosaic unbalanced translocations
is usually stated by testing microsatellites for the deletion region

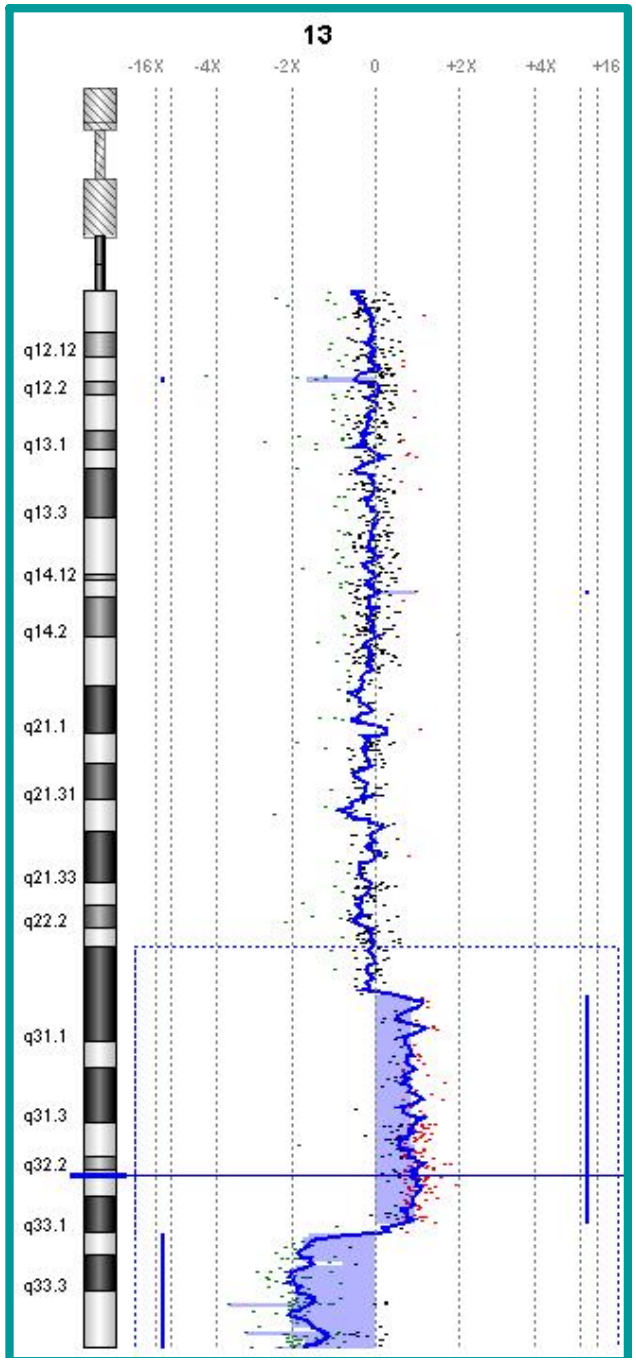
making impossible to detect any disagreement
with the parental origin of the duplicated chromosome

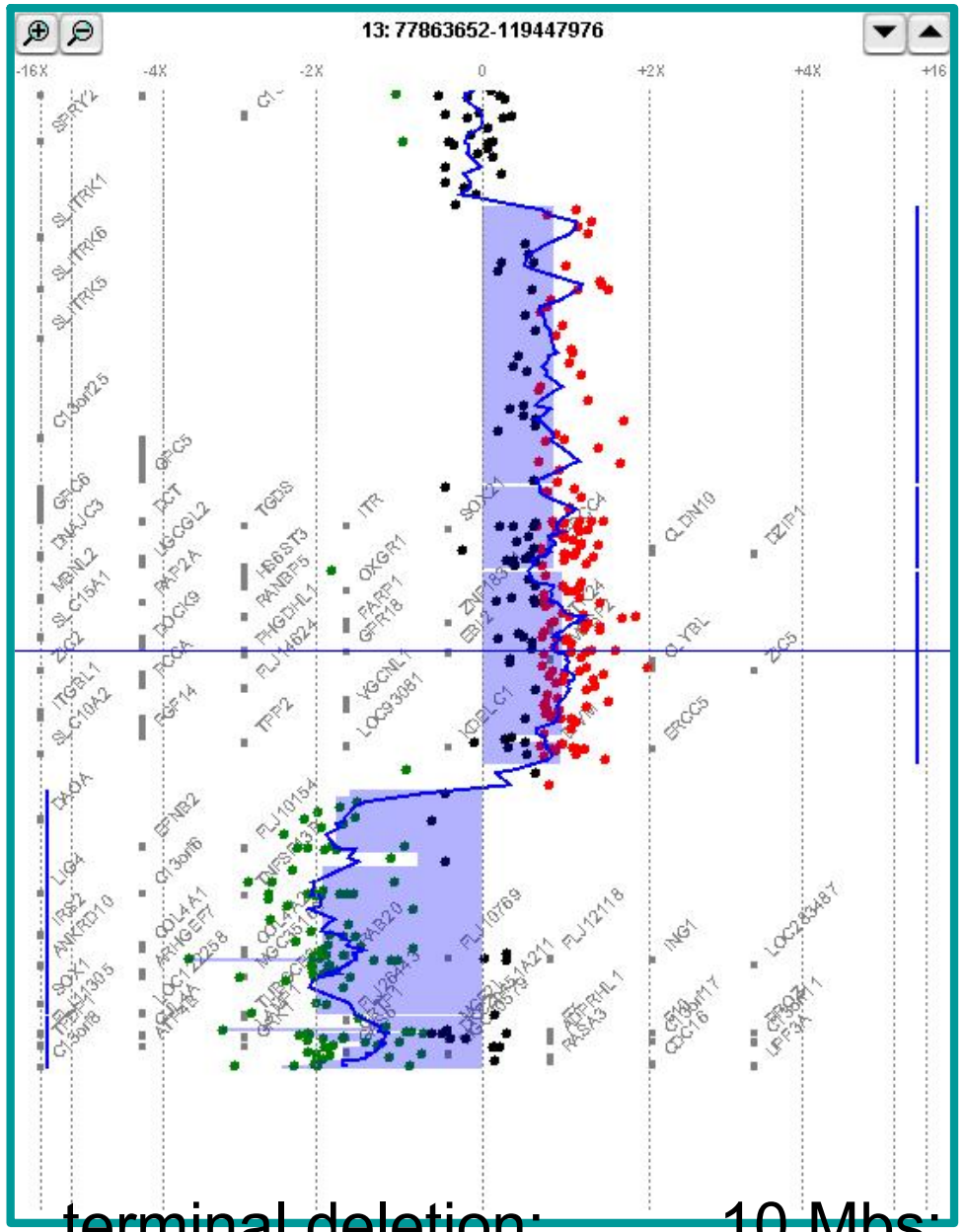
postzygotic translocations may be more frequent
than ever suspected

# Mechanisms of ring formation



# Array-CGH prophile (100 kb resolution) of a r(13)

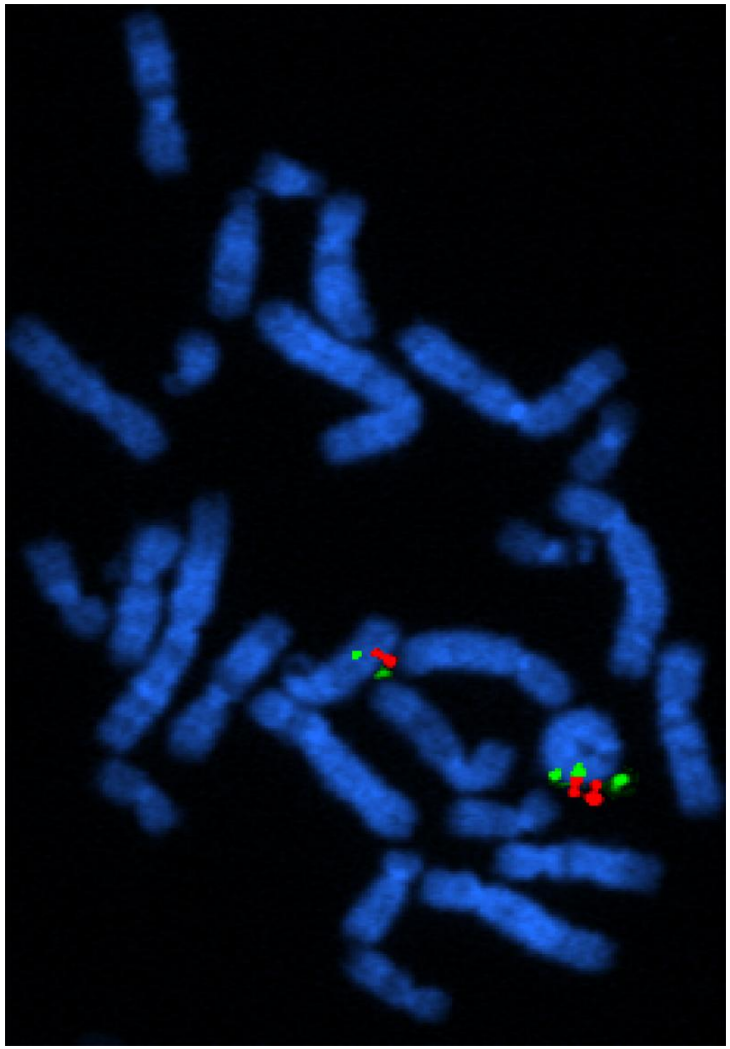



terminal deletion: 10 Mbs;
contiguous duplication: 21 Mbs

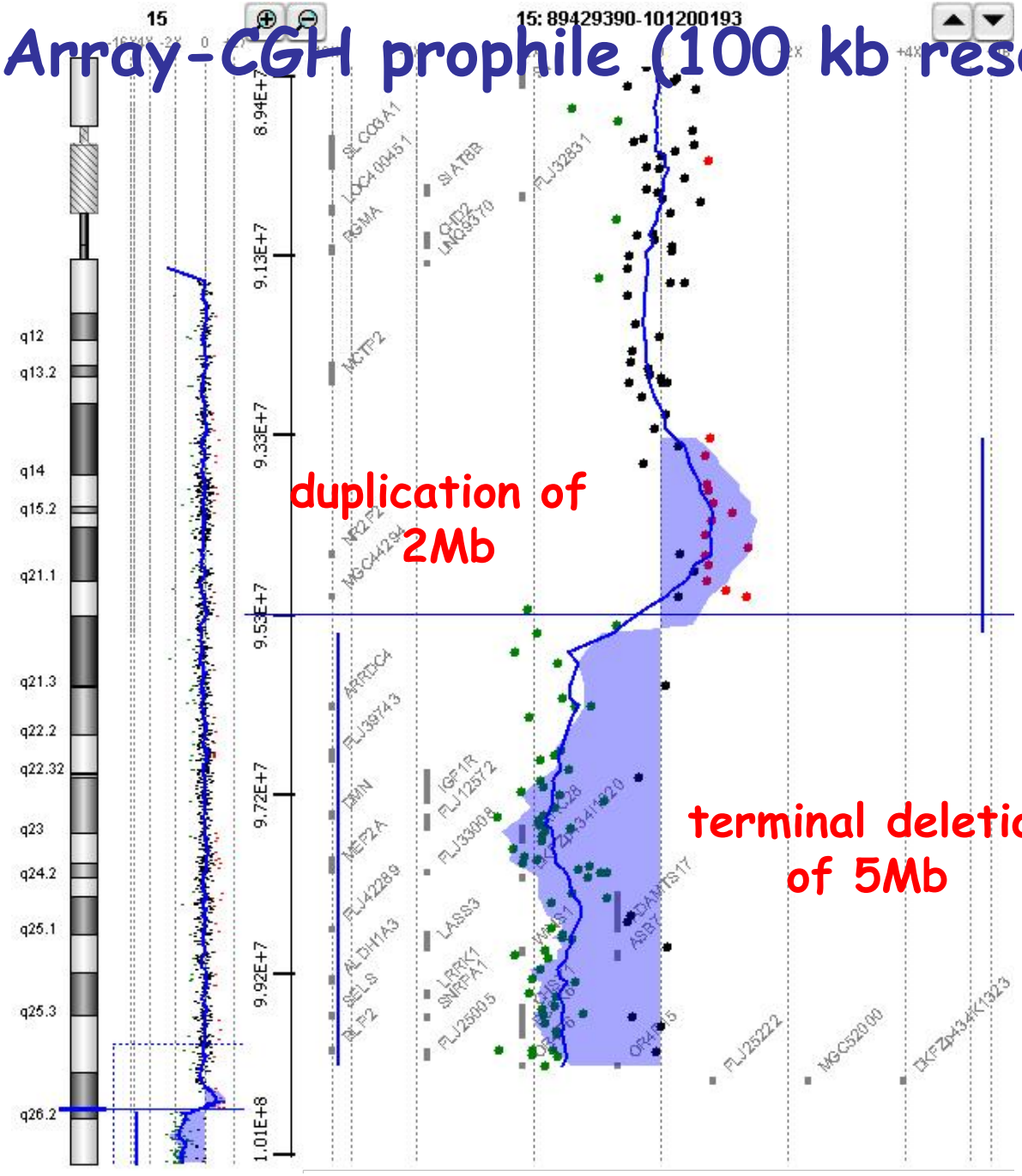
## FISH analysis:

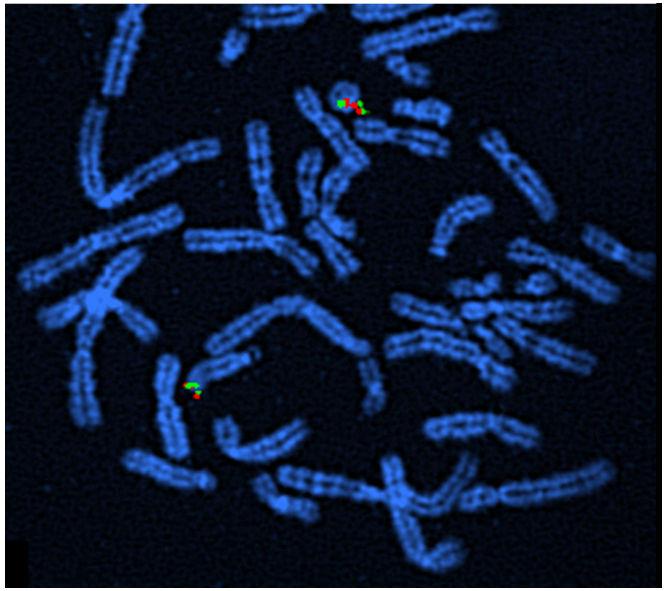
Inv dup del r(13)



**The duplication region is inverted**

# Array-CGH prophile (100 kb resolution) of a r(15)



The duplication region is inverted

# dup del rings

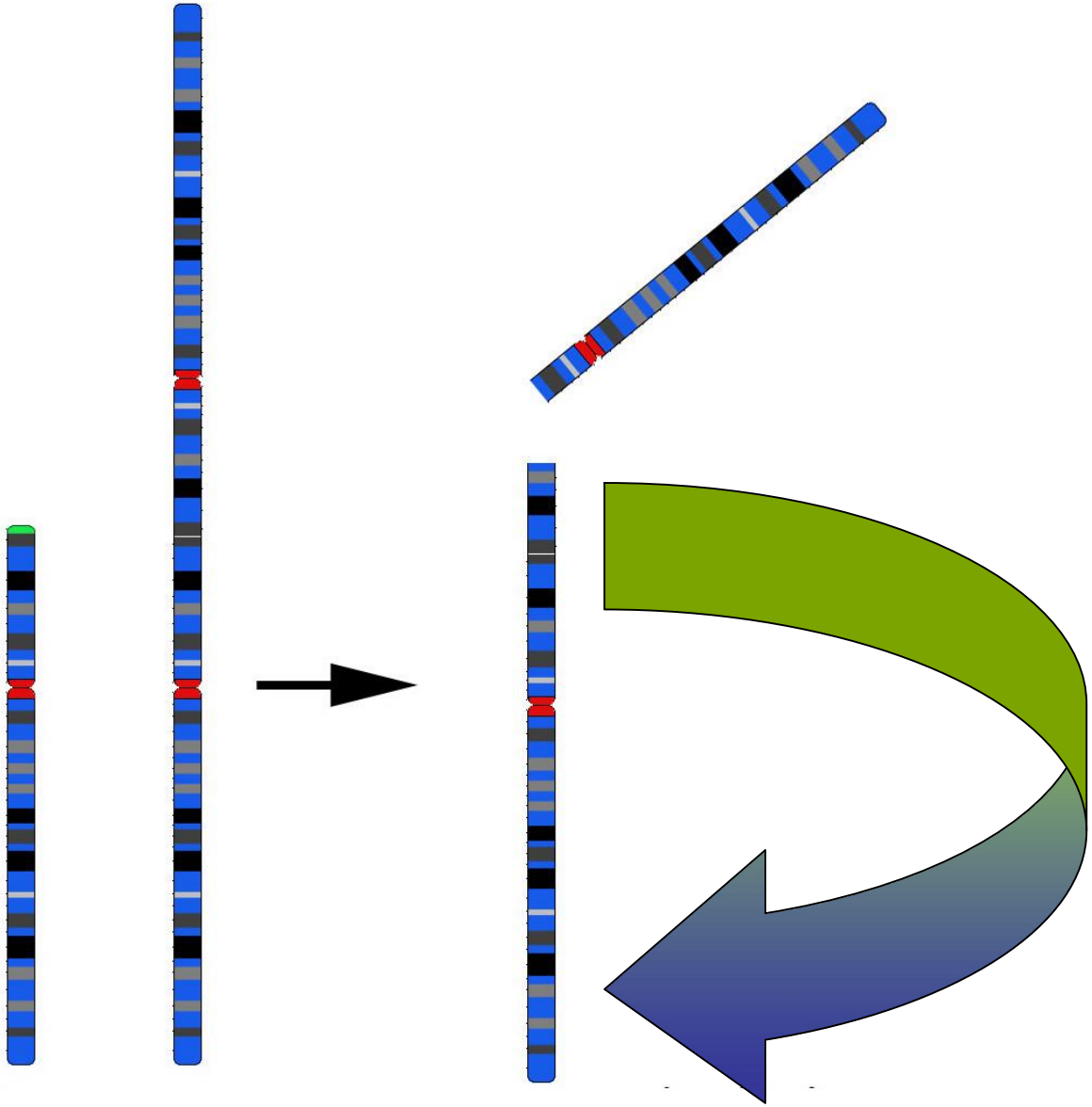
Important in genotype/phenotype correlations!!!

## Frequency??

## 7/31 rings

**Rossi et al, JMG 2008**

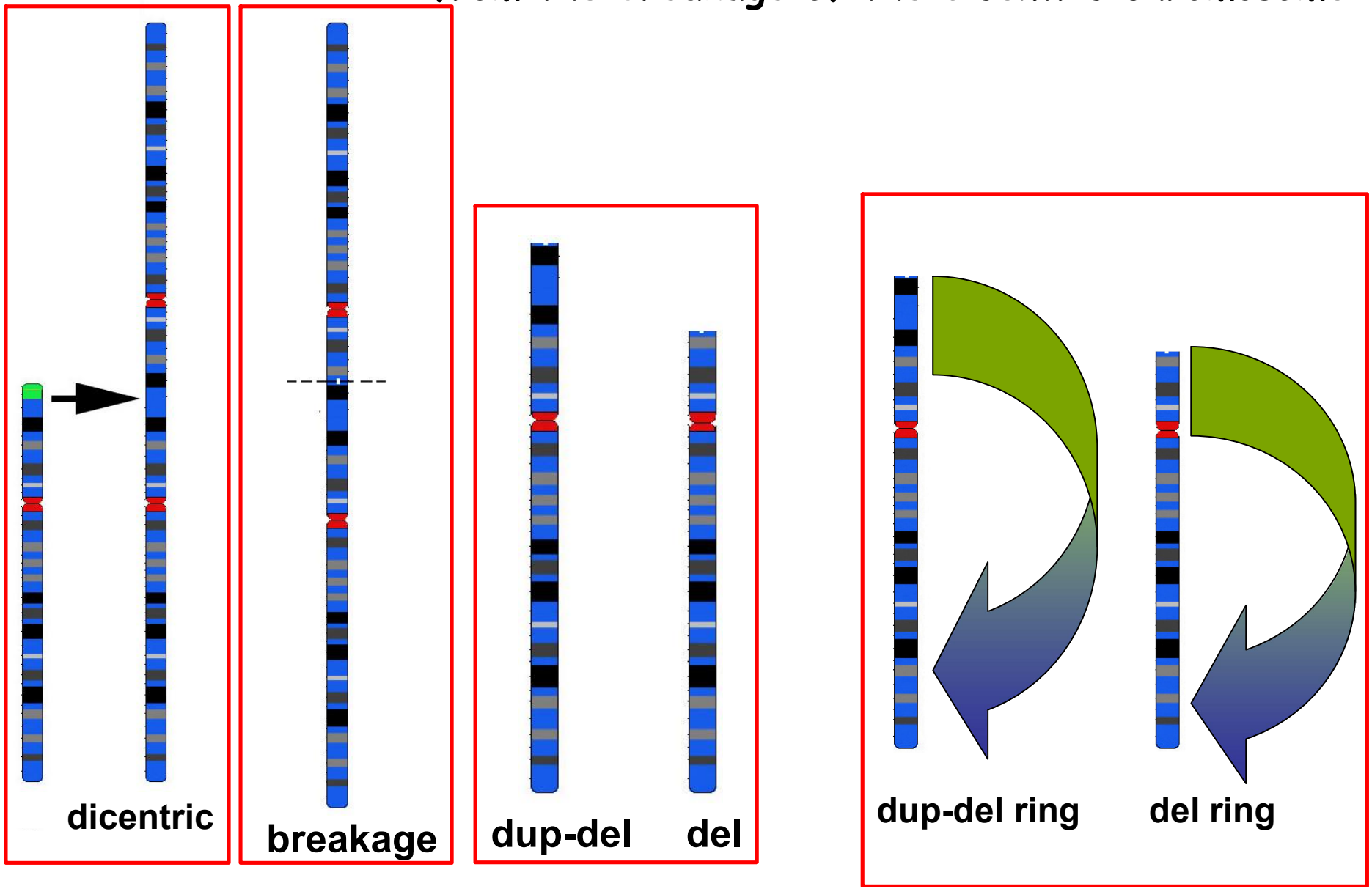
# Ring chromosomes may have the same origin of inv dup del rearrangements



Telomere healing, in absence of telomerase, might be obtained through circularization instead than through telomere capture

# Take care

Some "normal"ring chromosomes can also result post-zygotically from the breakage of the dicentric chromosome



**Dicentric chromosomes, both from NAHR and NHEJ, may generate different post-zygotic unbalanced rearrangements**

Inv dup dels

Dels??

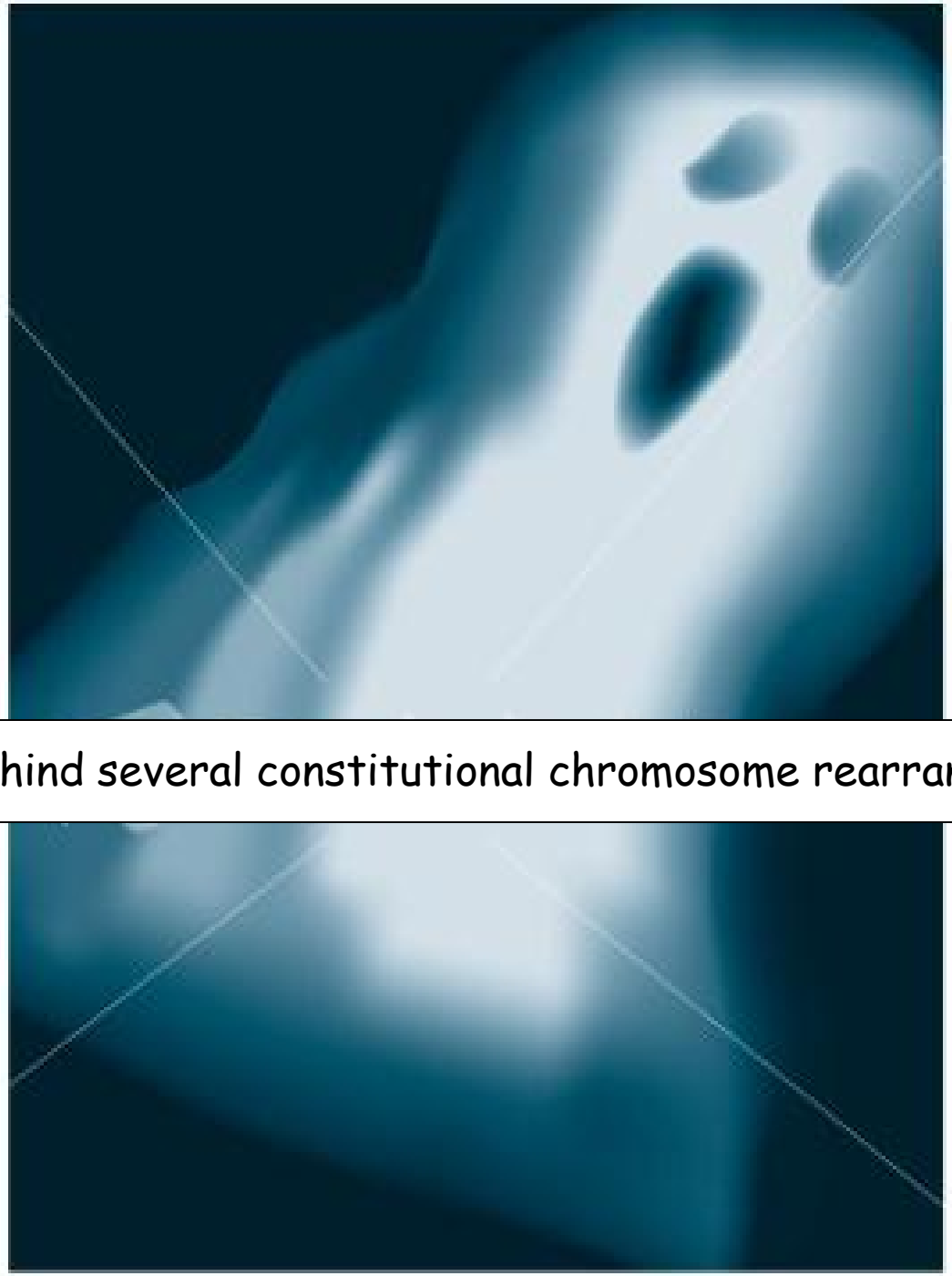
Acentric supernumerary marker chromosomes

Unbalanced translocations (Inv dup del; second chromosome)

Unbalanced translocations (del; second chromosome)??

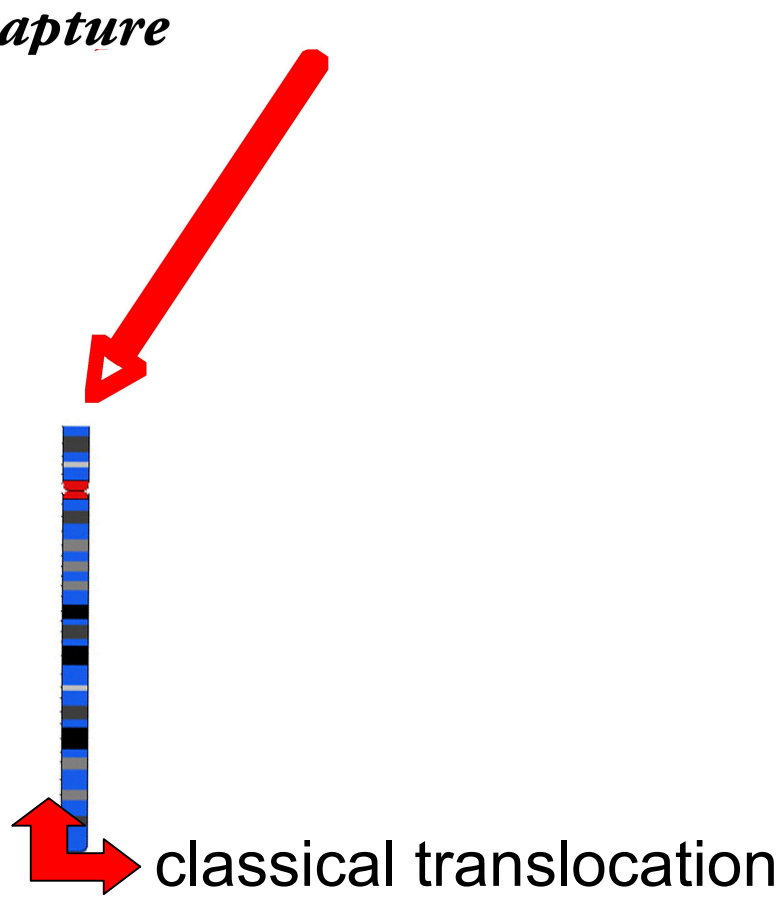
Rings (Inv dup del)

Rings (del)??

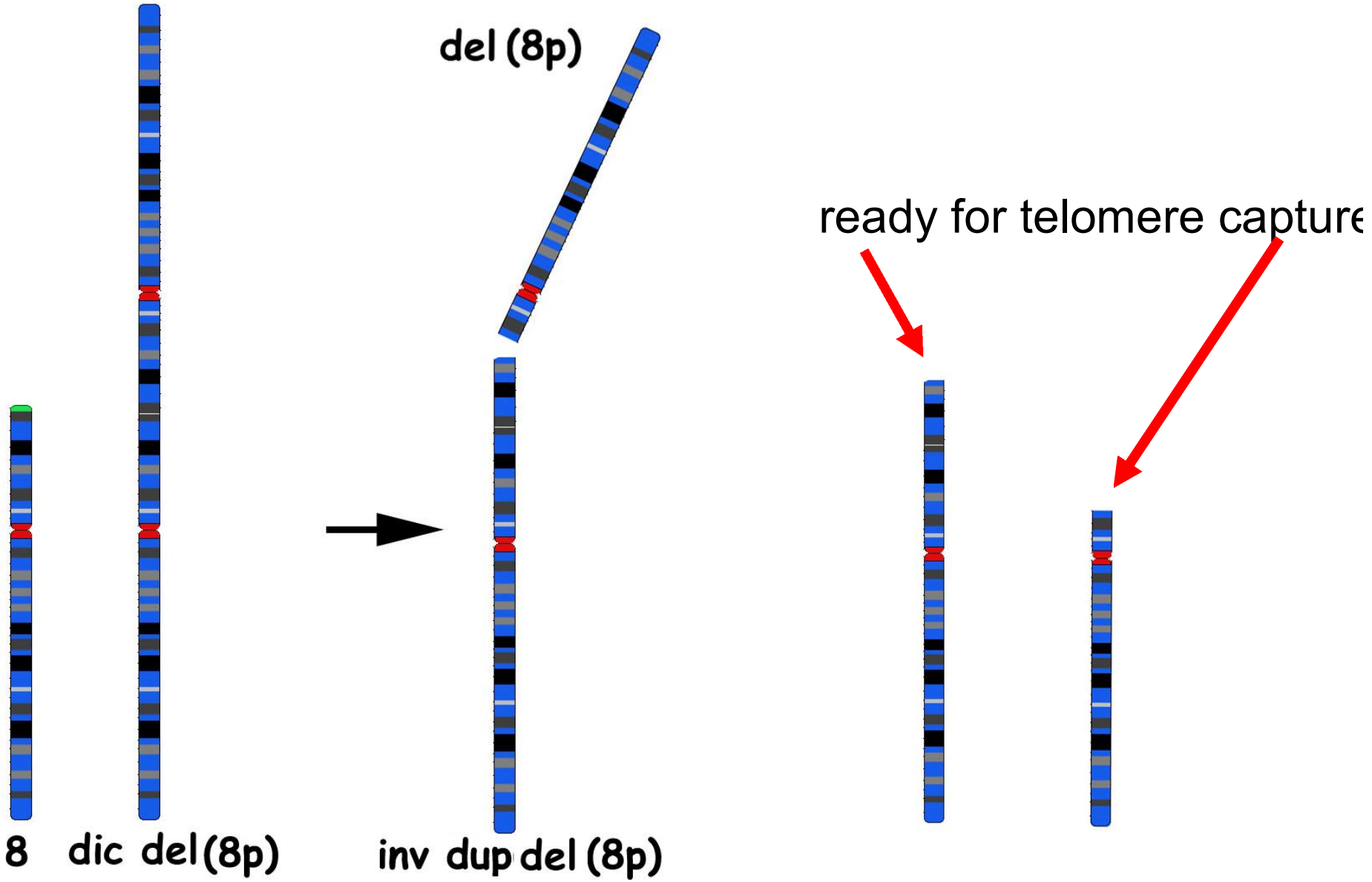


a dicentric ghost behind several constitutional chromosome rearrangements???

How can we discriminate
between a pre- or
a post-zygotic event???



post zygotic translocations due to telomere capture
of a broken dicentric chromosome



To prove that de novo unbalanced translocations are post-zygotic:

Test a number of de novo unbalanced translocations for the parental origin of both the deleted and the duplicated portion

If postzygotic origin: in 50% of the cases deletion paternal and duplication maternal or viceversa

Perform genome-wide array to see if one of the derivative is an inv-dup-del

a translocation with a derivative from one parent
and the second derivative from the other one demonstrates
a post-zygotic event and is in favor of the hypothesis of a zygote
in which one of the derivative chromosome was originally
a dicentric chromosome