

# **Humangenetik für Molekulare Medizin**

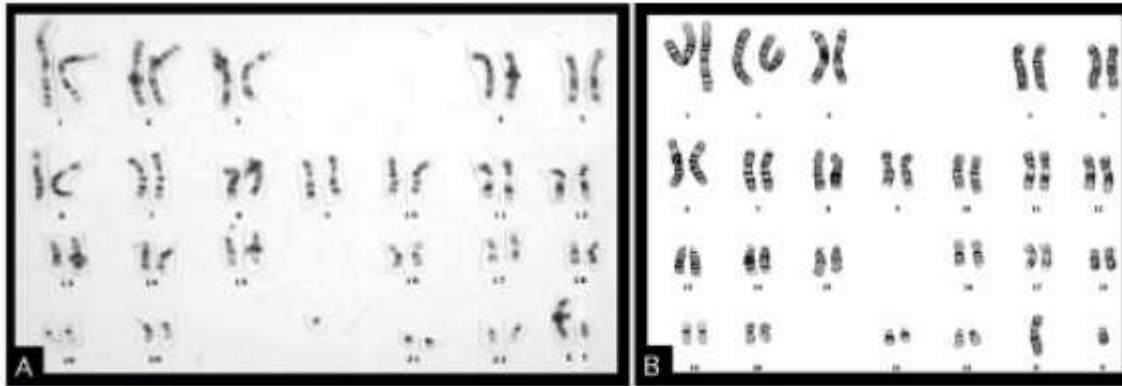
**Wintersemester 2010/2011**

## **Zytogenetik**

**Dr. rer. nat. Michael Leipoldt**

**Institut für Humangenetik, Univ.Klinikum Freiburg**

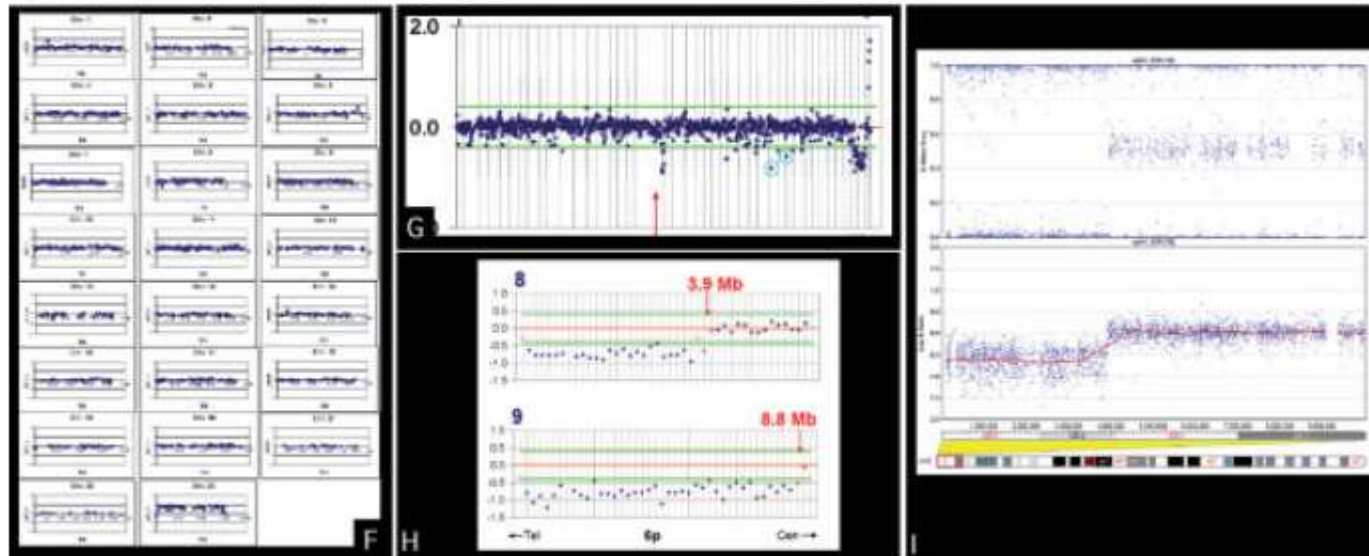
### **3. Molekularzytogenetische Diagnostik**



*Klassisch*



*FISH*



*CGH*

Hanna B., Alter 12 Monate

Schwere allgemeine Retardierung

BNS-artige Krampfanfälle


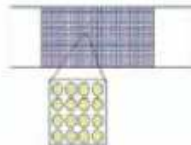






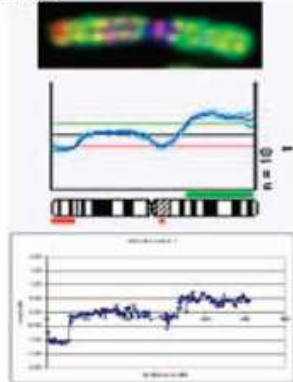
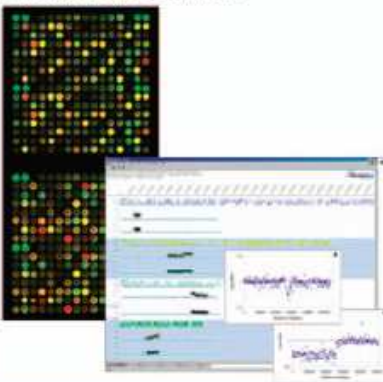
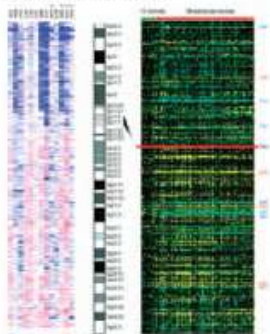
Mikrozephalie

Faziale Dysmorphien

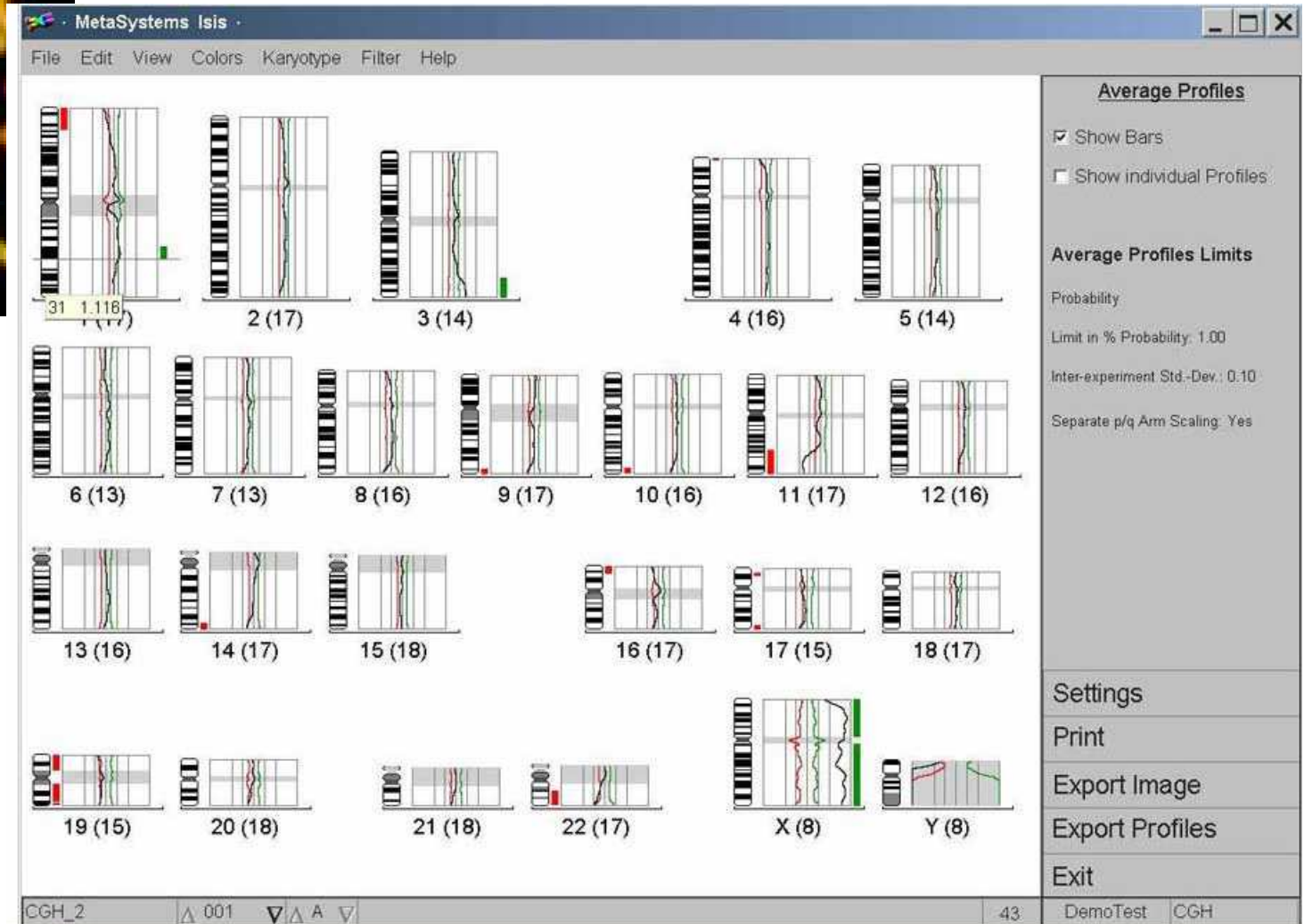
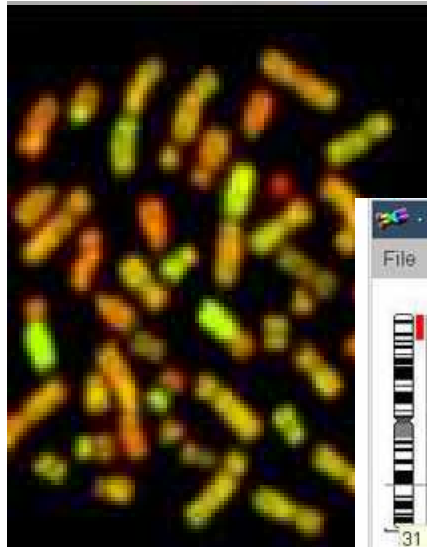
Häufige Atemwegsinfekte

- > Array – CGH
- >> Chromosomenanalyse
- >>> FISH

# Comparative Genome Hybridization (CGH)

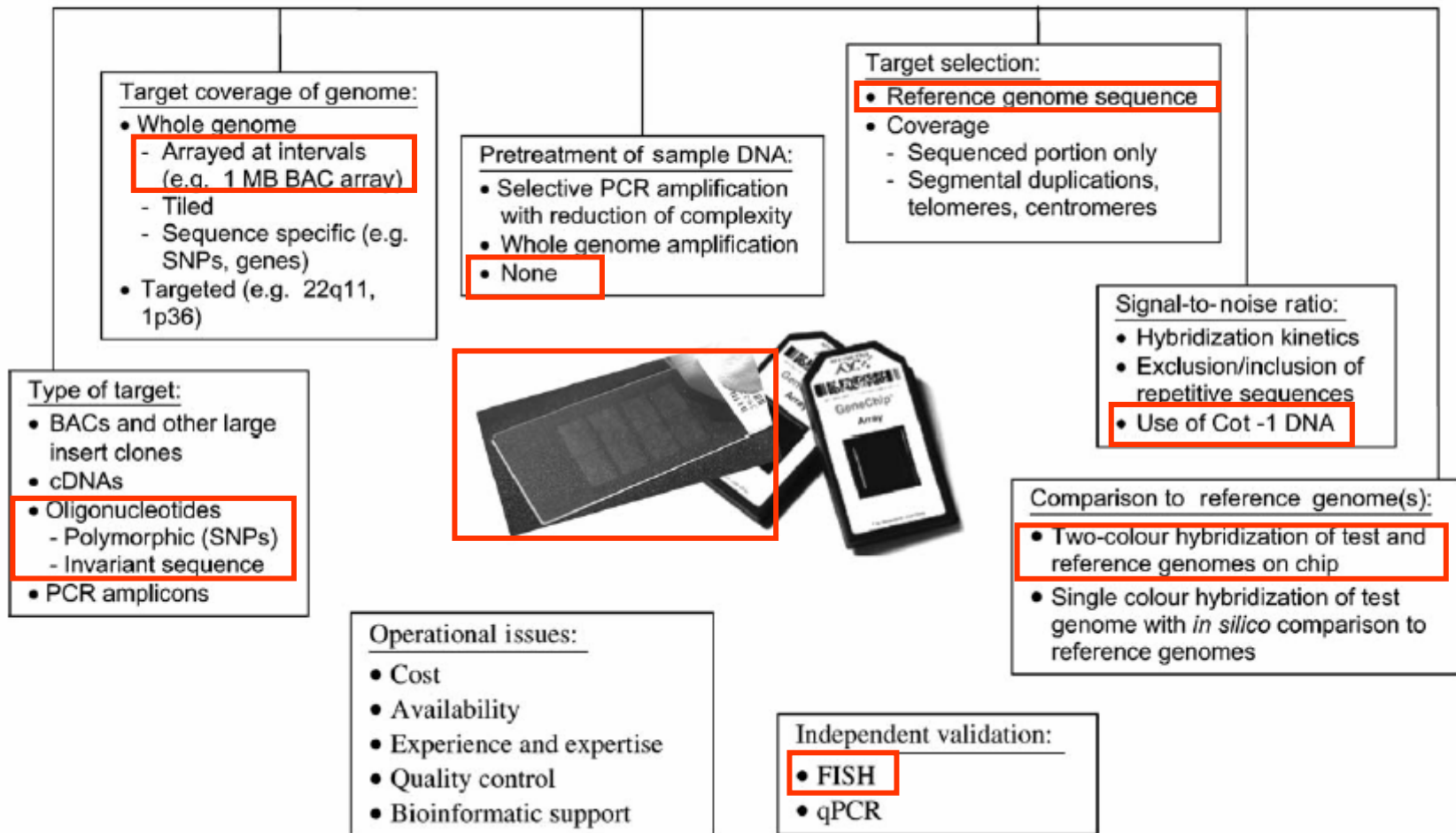
Technology	Cytogenetic CGH	AGH with Sanger 1 Mb BAC array	AGH with Affymetrix GeneChip®
Target	Normal metaphase spread 	3500 BACs at ~1 MB intervals throughout the genome, spotted onto a glass slide. 	100,000 sets of oligonucleotides, synthesized <i>in situ</i> on a chip 
DNA samples, differentially labeled	Test sample and reference sample labeled in two different fluochromes Test  Control 	Test sample and reference sample labeled in two different fluochromes Test  Control 	Test sample labeled with single fluochrome. Test 
Hybridization	Test and reference samples hybridized in equal amounts to targets in presence of Cot-1 DNA	Test and reference samples hybridized in equal amounts to targets in presence of Cot-1 DNA	Test sample hybridized to targets using standard conditions
Data analysis	Relative intensity of test and reference sample fluorescence along chromosome used to detect gains and losses; second hybridization with dye flip may be used to reduce false positives 	Location of each BAC mapped back to genome; relative intensity of test and reference sample fluorescence for each BAC used to detect gains and losses; second hybridization with dye flip used to reduce false positives 	Location of each target mapped back to genome; data normalized and compared <i>in silico</i> to reference standard; statistical evaluation and congruency among adjacent targets used to reduce false positives 

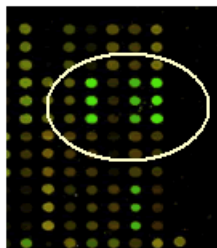
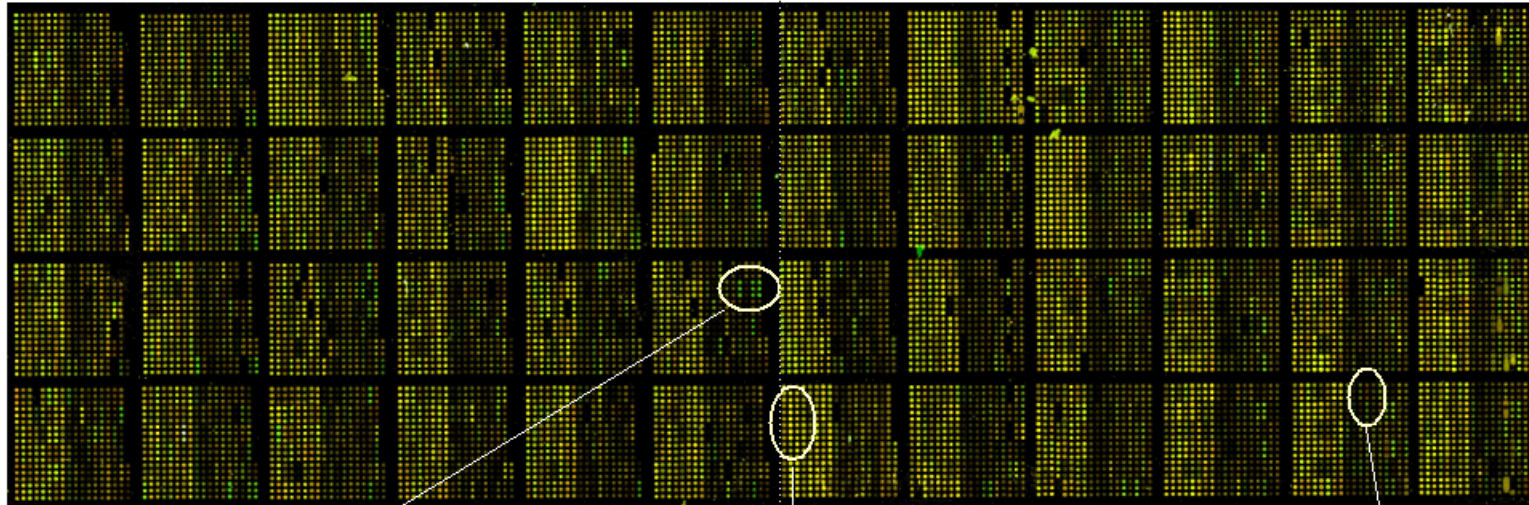
# Chromosomen - CGH



# Array CGH (aCGH)

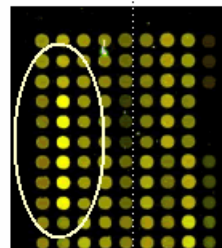
Effective resolution, sensitivity and specificity





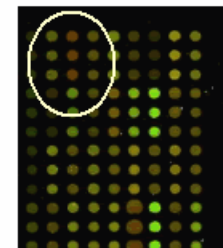
**green signal**

microdeletion  
in patient DNA



**yellow signal**

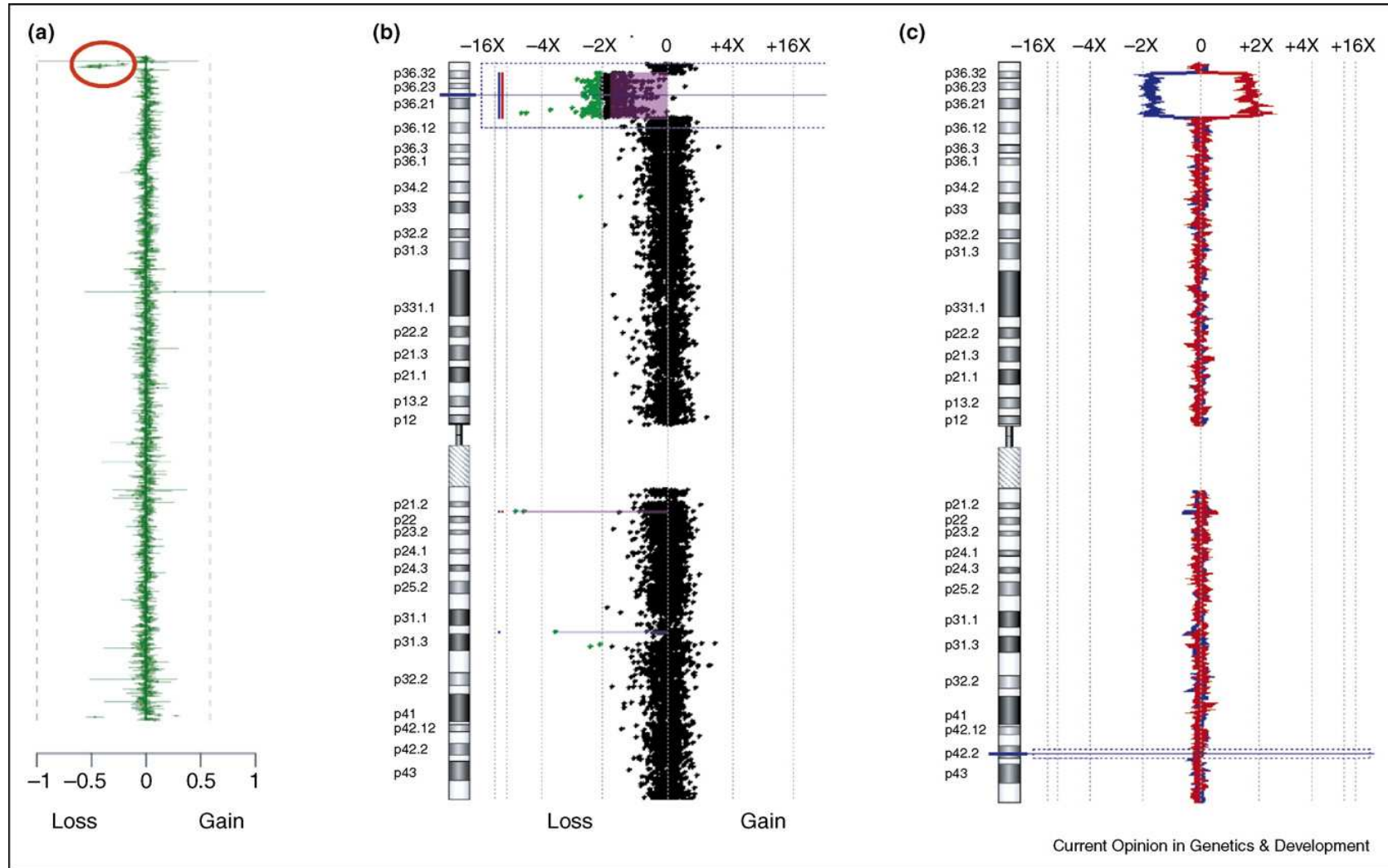
No changes in  
patient DNA



**red signal**

microamplification  
in patient DNA

Scan of an array-CGH slide



**BAC-array**

**Oligo-array**

**Oligo-array / dye-swap**

**1p36 - Mikrodeletion**

CGH Microarray 244A (Agilent)

243 504 60-mer Oligonukleotide

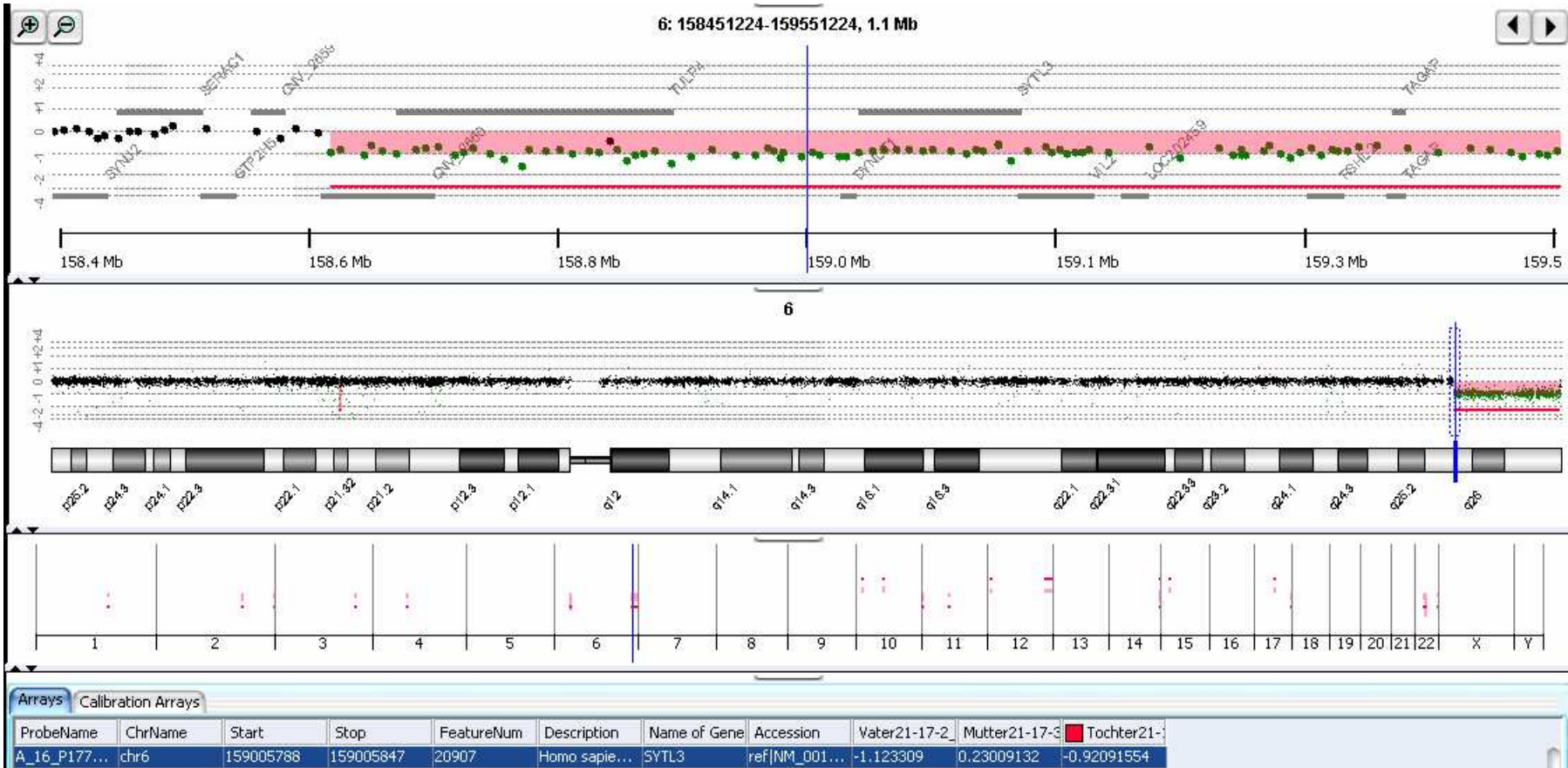
NCBI build 35

70% intragenische Proben, 30% intergenische Proben

Mittlerer Probenabstand 8.9 kb

# DNA Microarray (aCGH) „Oligo-Array“ Chromosom 6

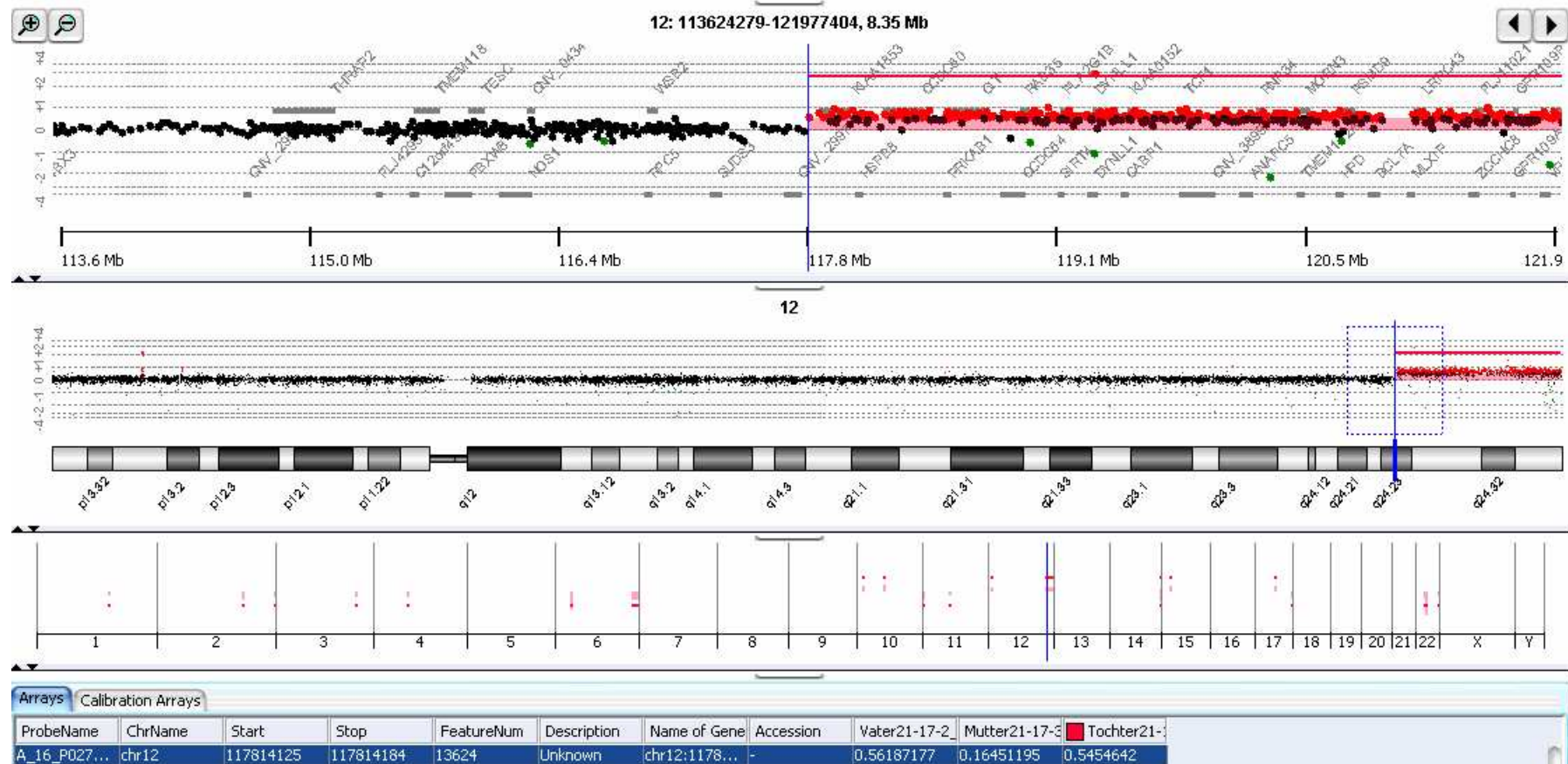
Hanna B.



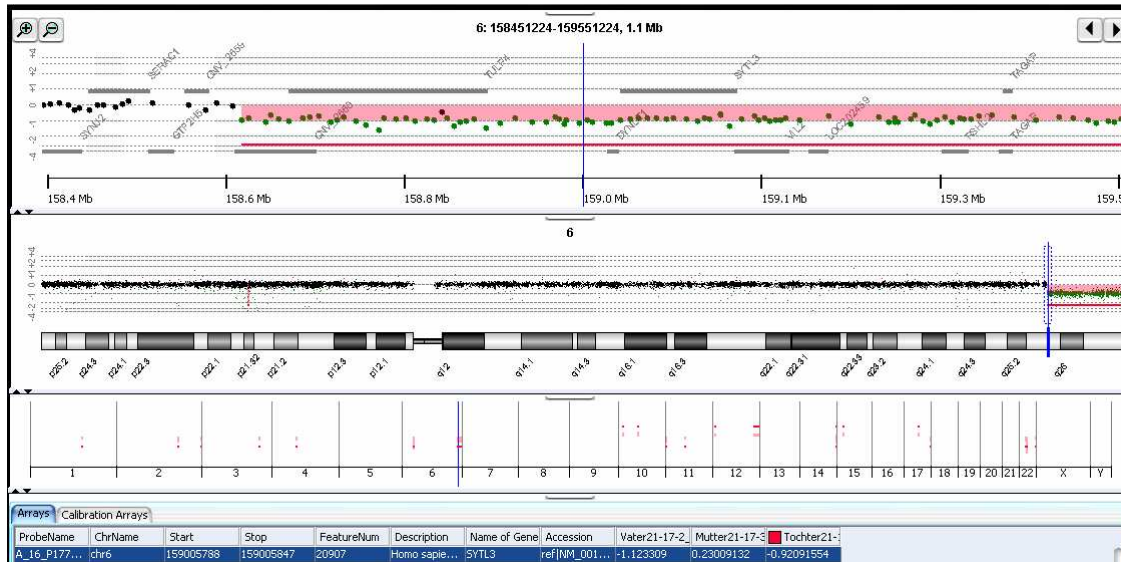
21-17-1 Chr:6 , del 158.654.775 bis tel

# DNA Microarray (aCGH) „Oligo-Array“ Chromosom 12

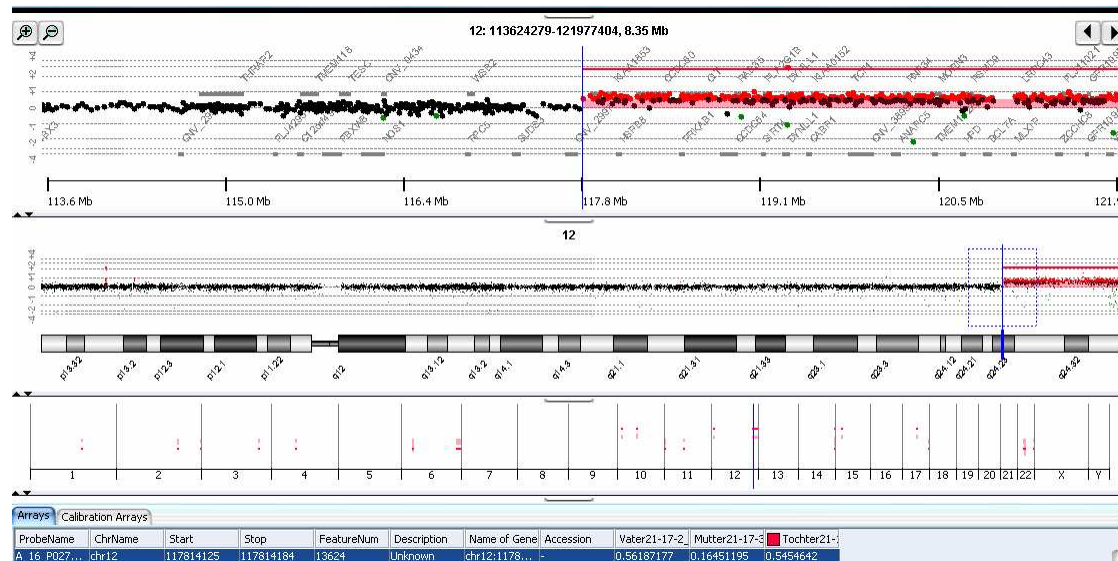
Hanna B.



21-17-1 Chr.12 dup von 117.814.125 bis tel



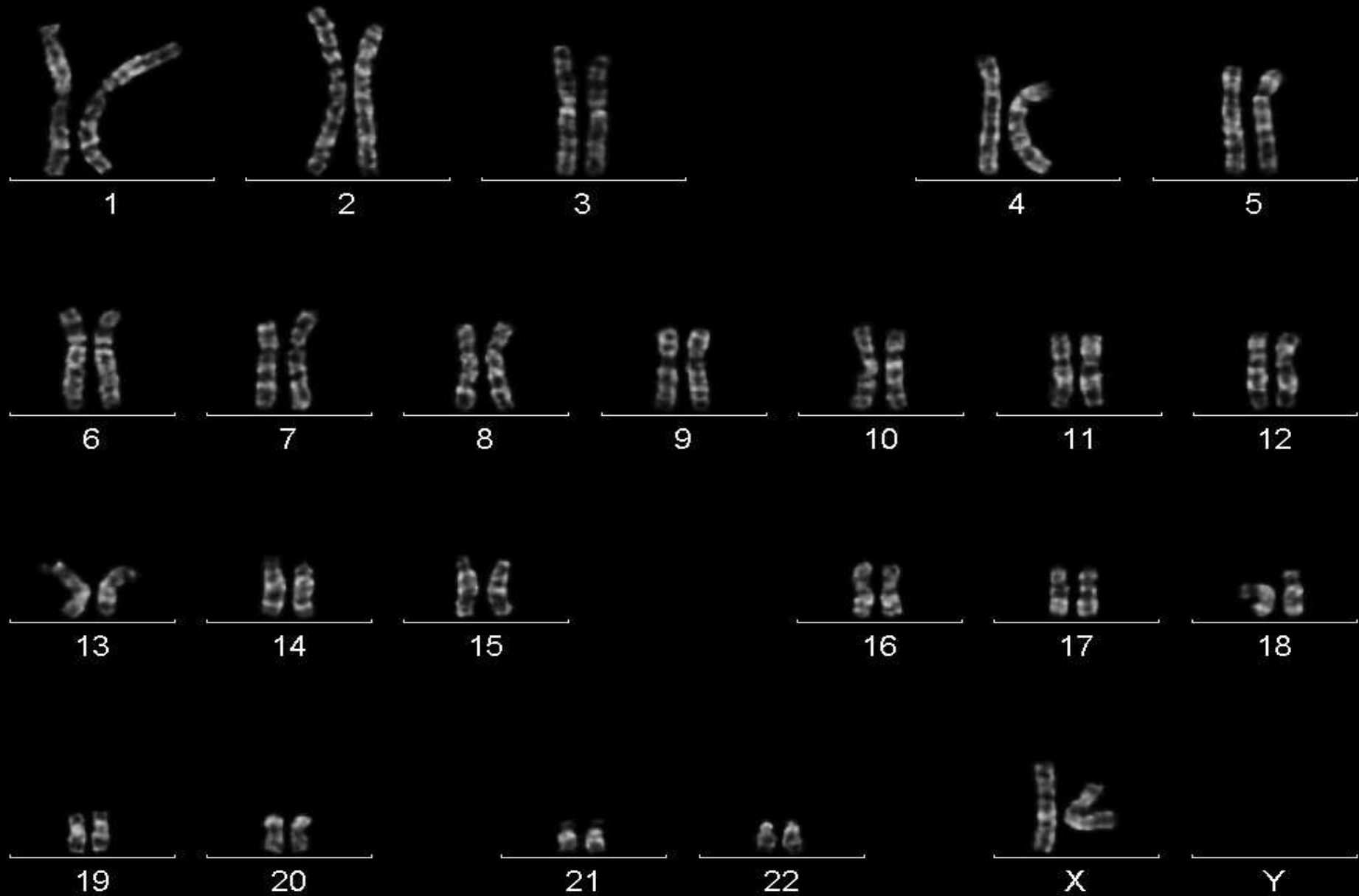
12.3 Mb – Deletion  
im langen Arm von Chr. 6



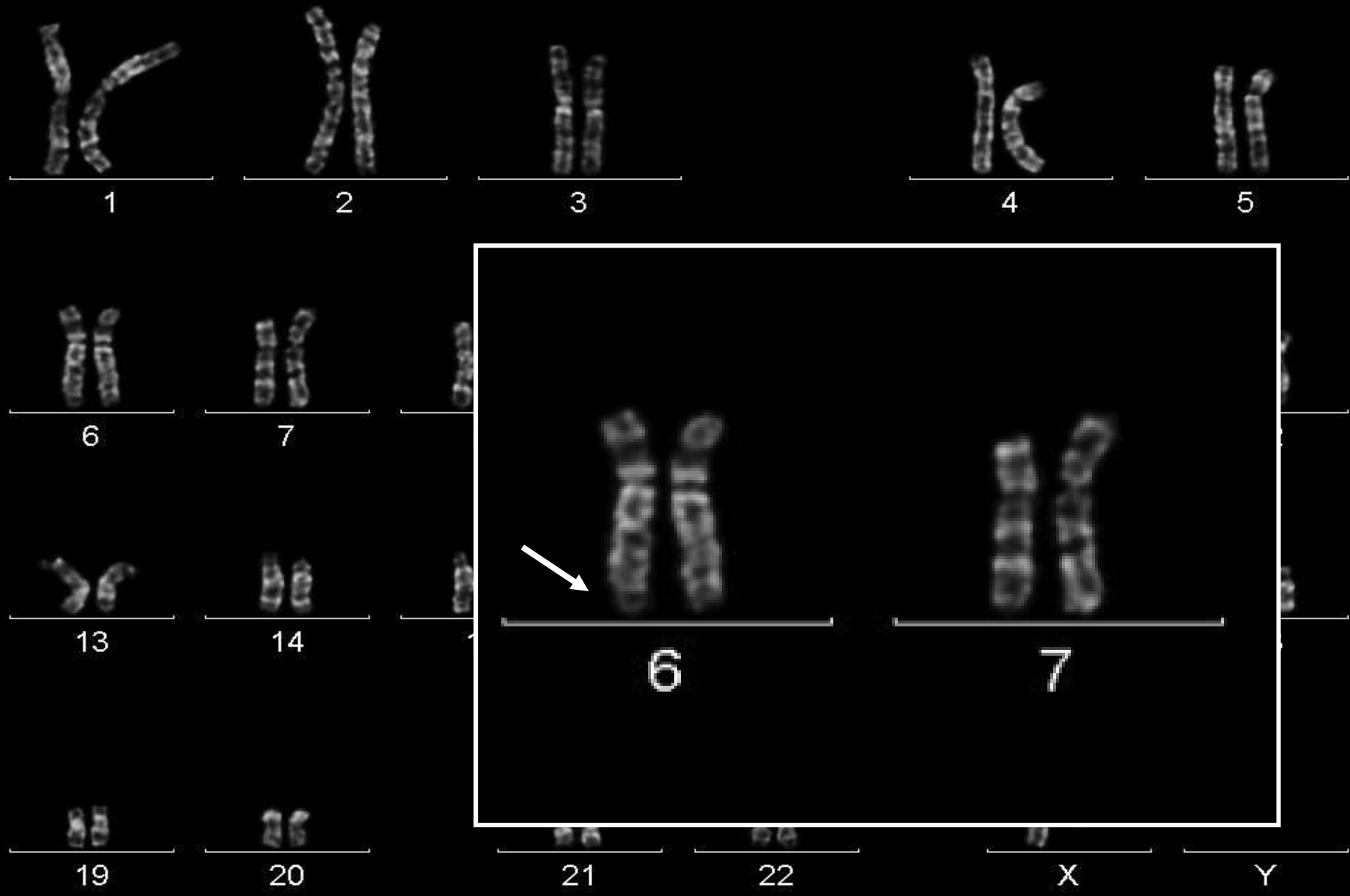
14.5 Mb – Duplikation  
im langen Arm von Chr. 12

**Unbalanzierte kombinierte Deletionen – Duplikationen – Aberration**

46,XX?

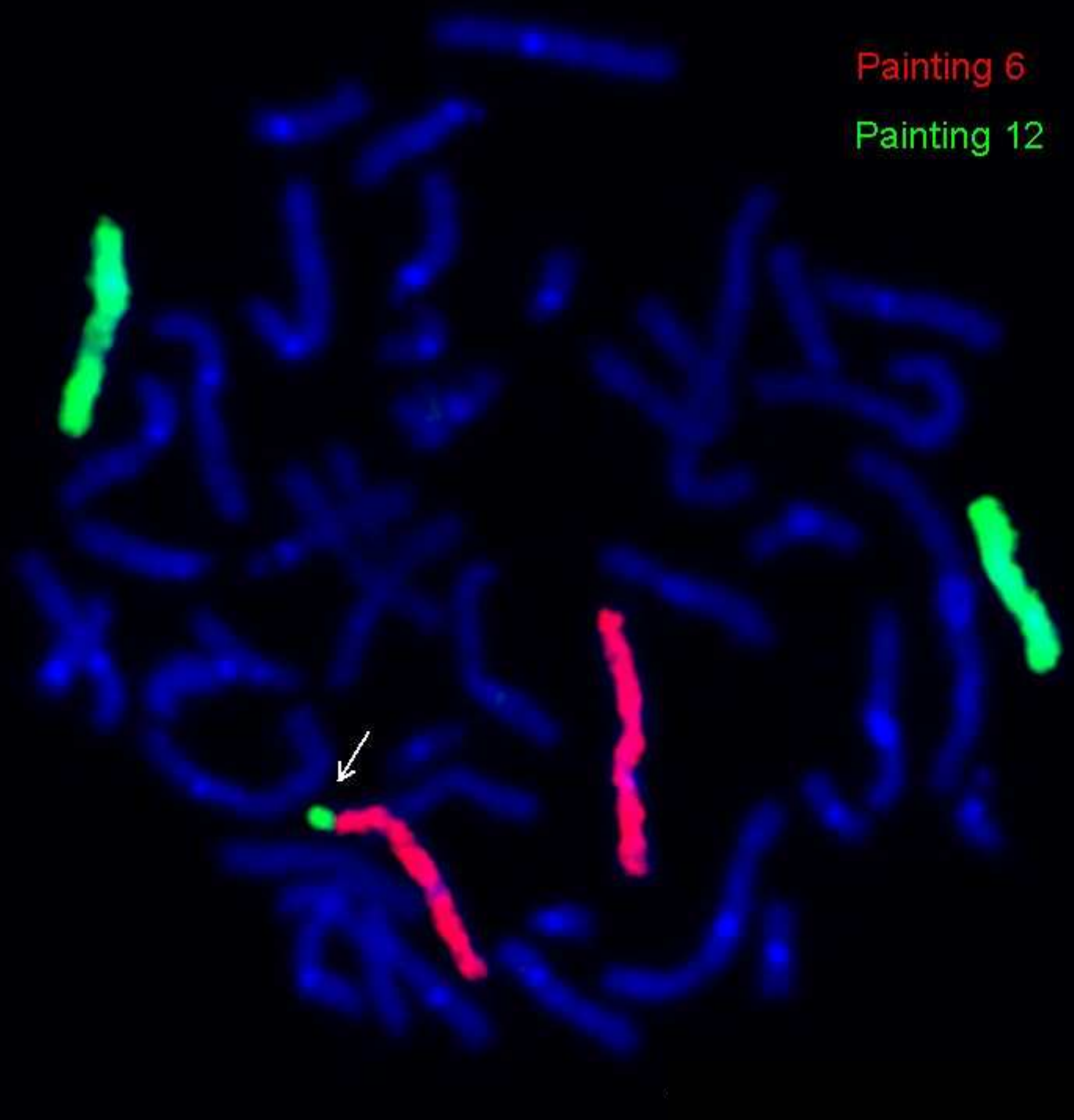


46,XX?



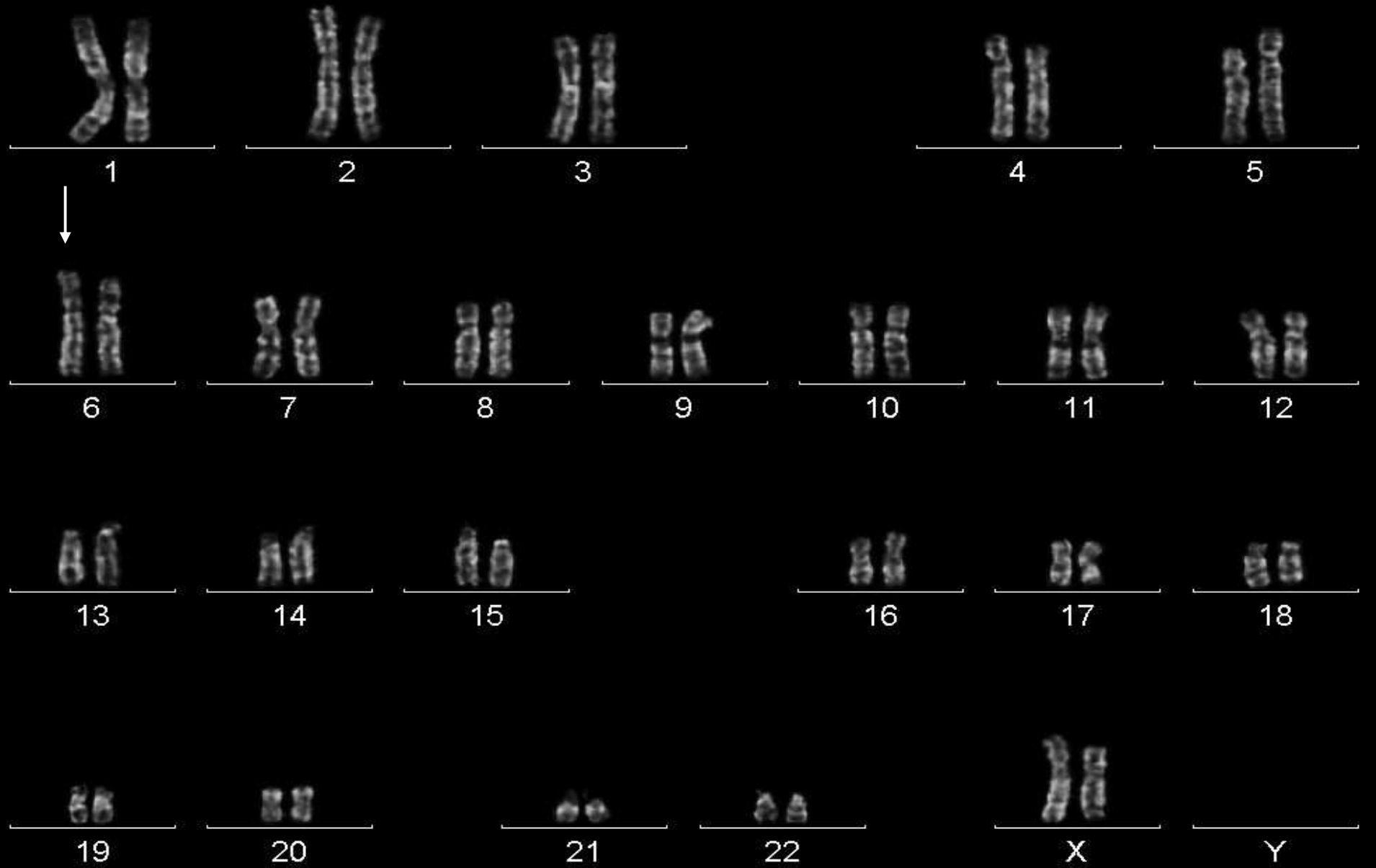
Painting 6

Painting 12

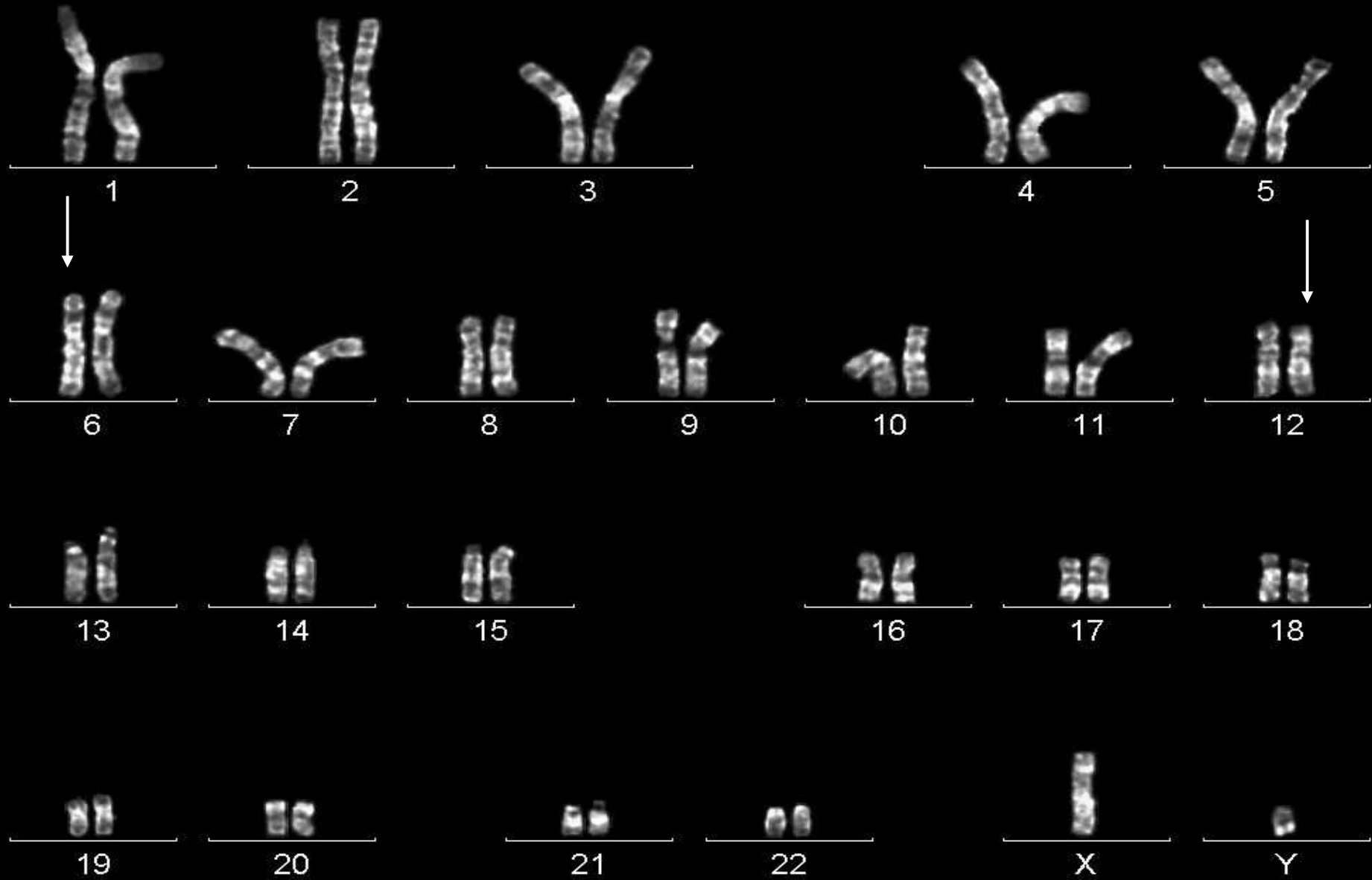


46,XX, der(6)

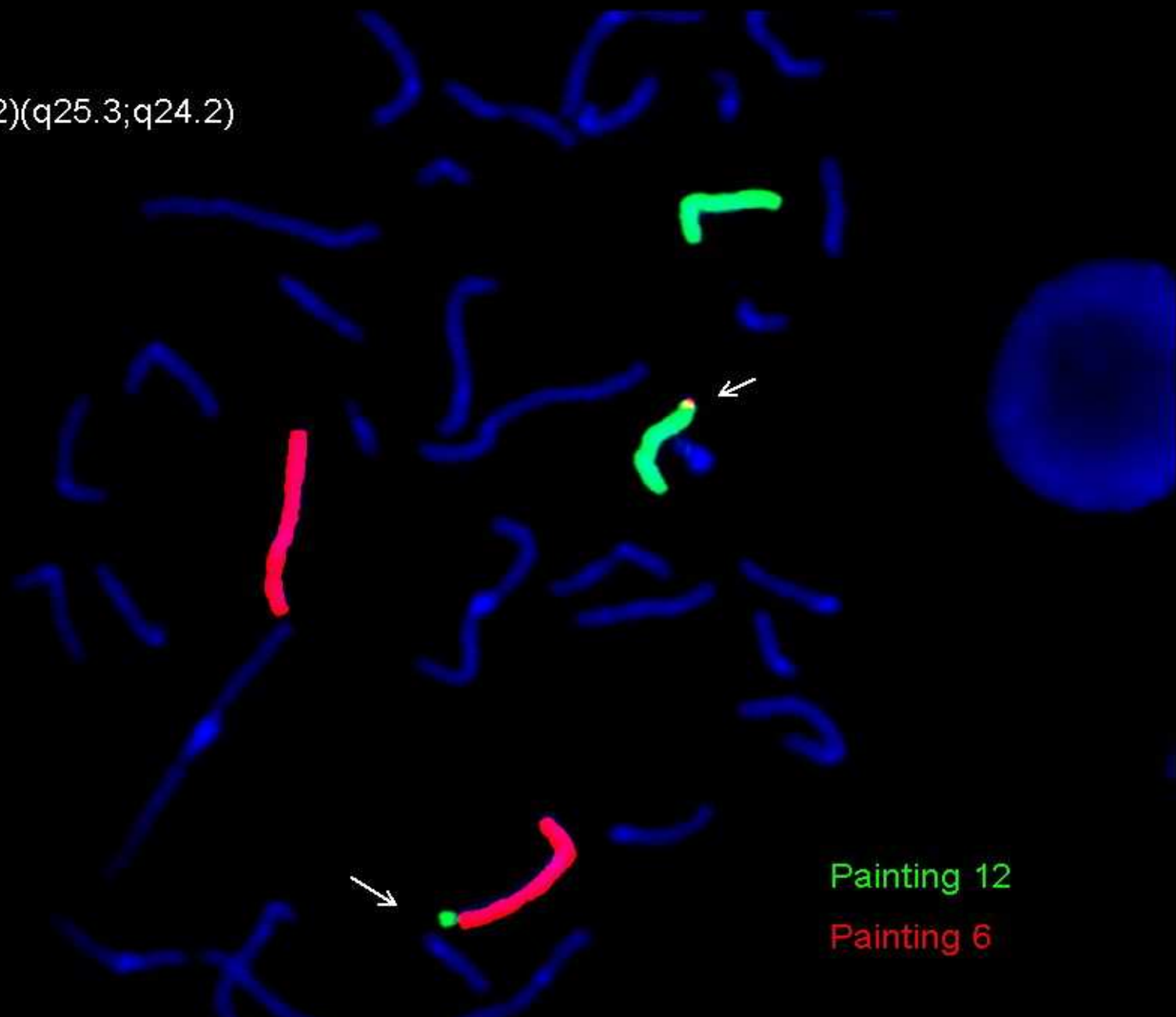
Translokation 6 ↔ 12?



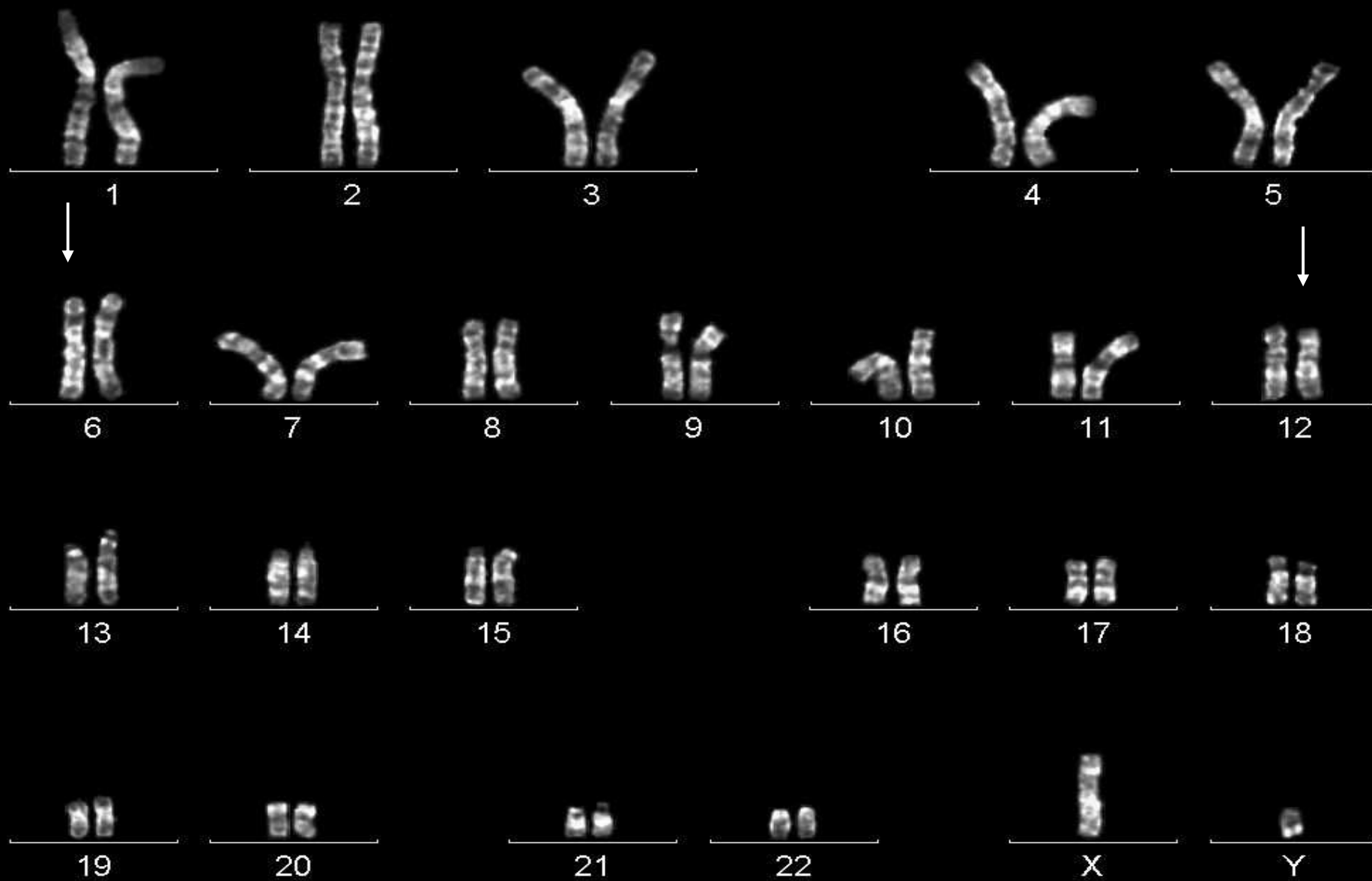
Vater von Hanna B. : 46,XY,t(6;12) ?



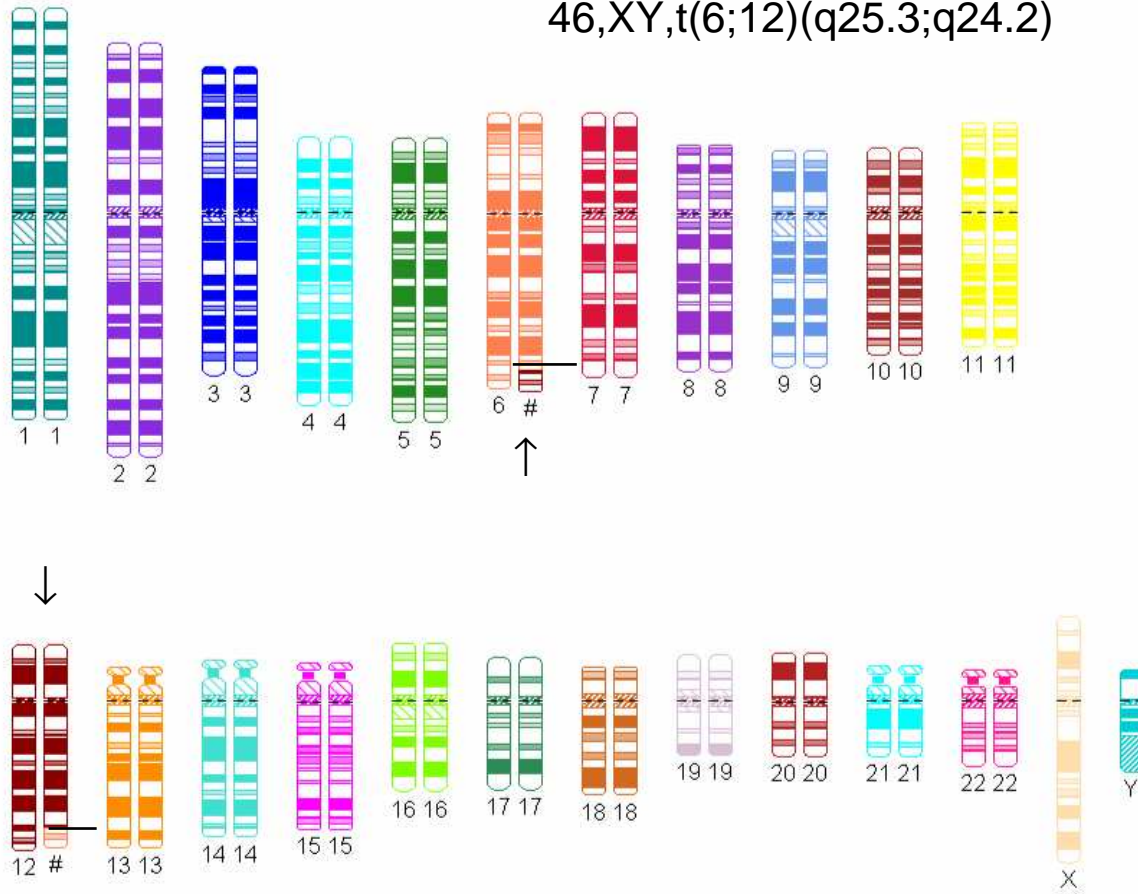
t(6;12)(q25.3;q24.2)



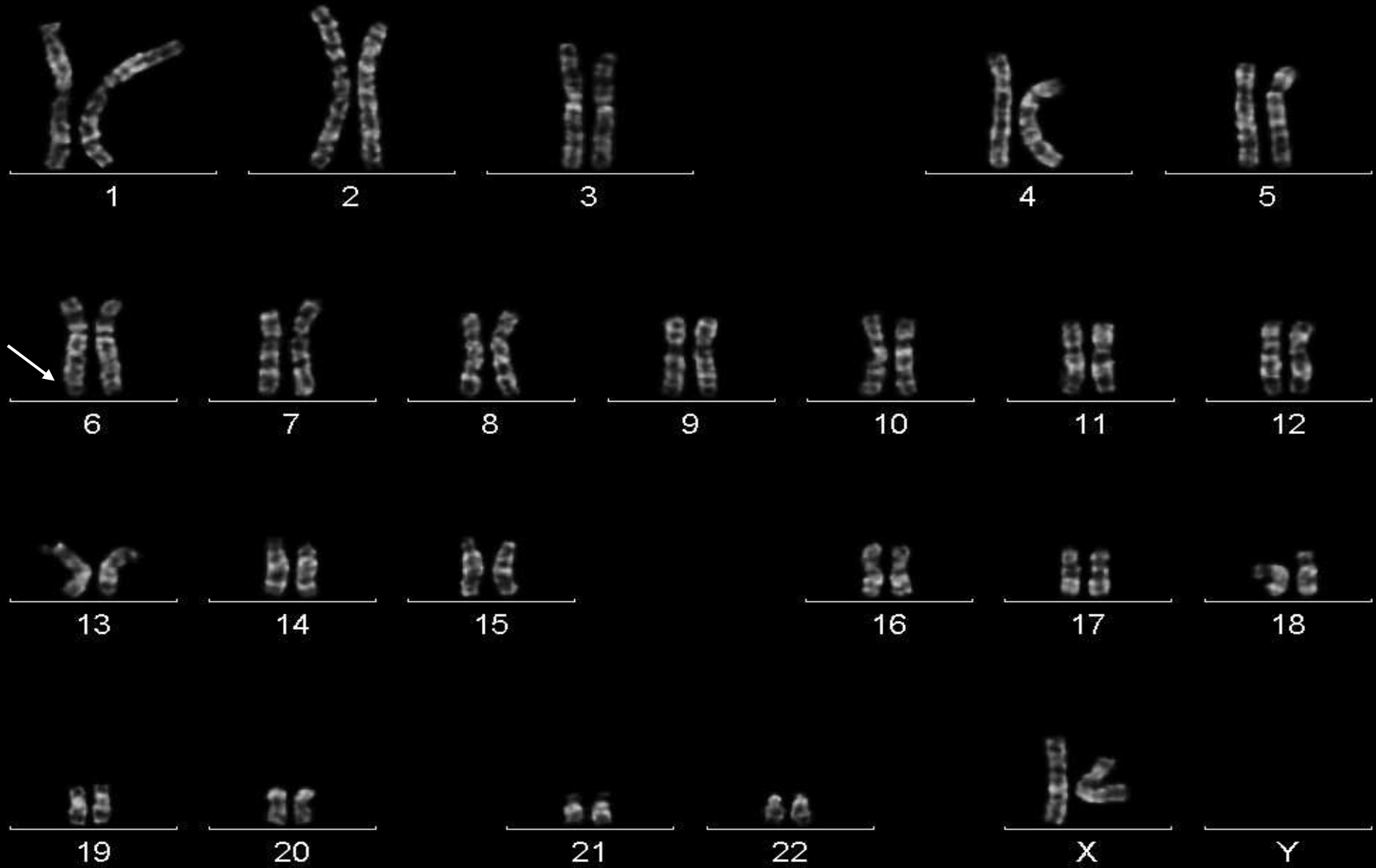
Vater B., 46,XY,t(6;12)(q25.3;q24.2)



46,XY,t(6;12)(q25.3;q24.2)



Hanna B., 46,XX,der(6)t(6;12)(q25.3;q24.2)pat



**Del 6q25.3→ter \***  
(n=16)

**Dup 12q24.2→ter \*\***  
(n=9)

**Hanna B.**  
del6q25.3dup12q24.2

**Del6q25.3dup12q24.2 \*\*\***  
(Einzelfallbeschreibung)

**IUGR**

**IUGR**

**Mikrozephalie**

**Mikrozephalie**

**Mikrozephalie**

**Faziale Dysmorphien**

**Faziale Dysmorph.**

**Faziale Dysmorph.**

**Faziale Dysmorph.**

**Ment. Retardierung**

**Schwere glob. Retard.**

**Schwere glob. Ret**

**Schwere glob. Ret.**

**Zerebrale Anfälle**

**BNS-Krämpfe**

**Zerebrale Anfälle**

**Hirnfehlbildungen**

**Hirnfehlbildungen**

**Schädeldeformation**

**Herzfehler**

**Atemwegsinfekte**

**Infektanfällig**

**sehr ähnlich KMS**

\* Valtat et al. (1992) ClinGen 41:159-166

\*\* Tengström et al. (1985) ClinGen 28: 112-117

\*\*\* Jardine et al. (1993) ClinDysmorph 2: 269-273

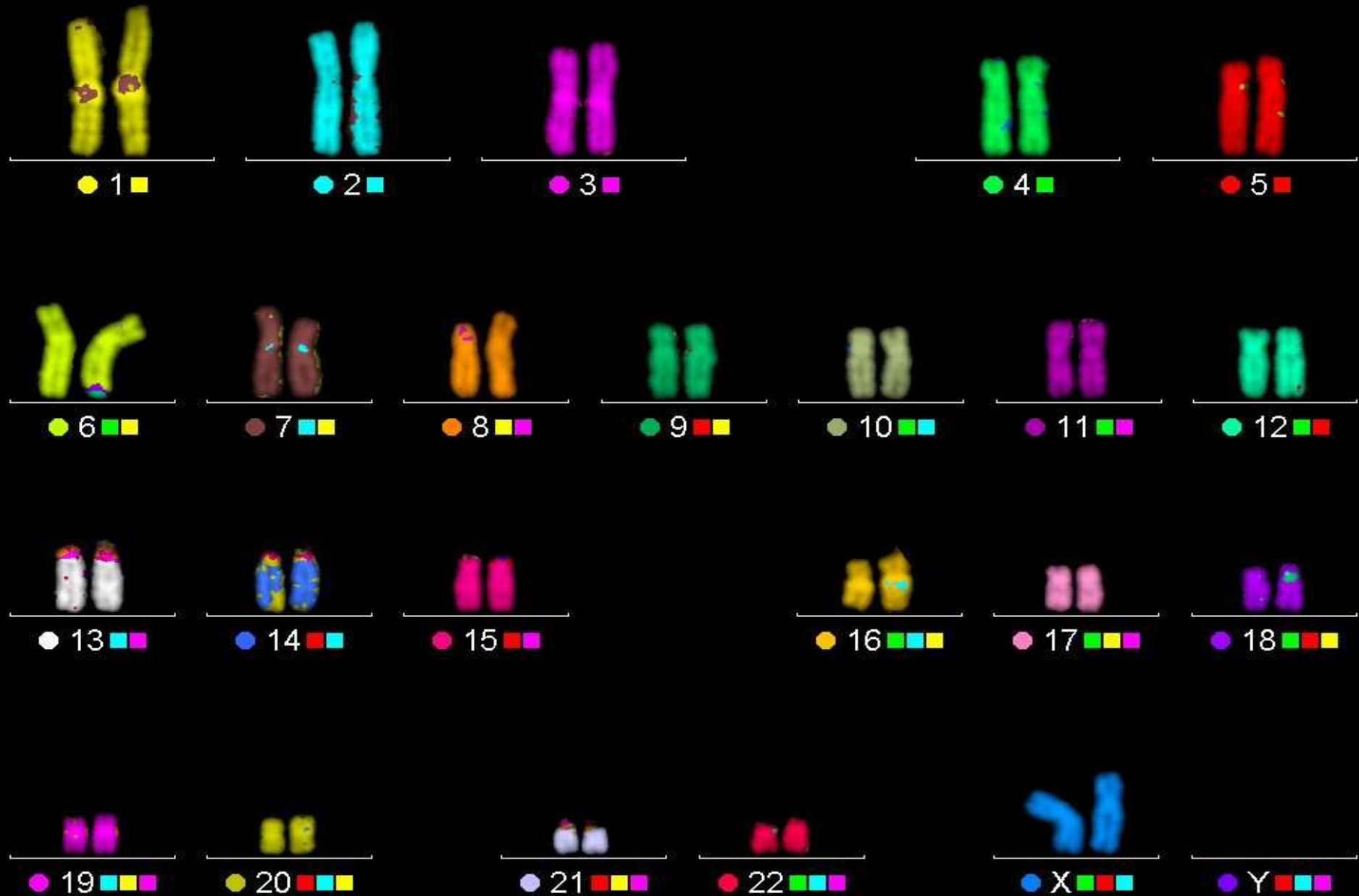


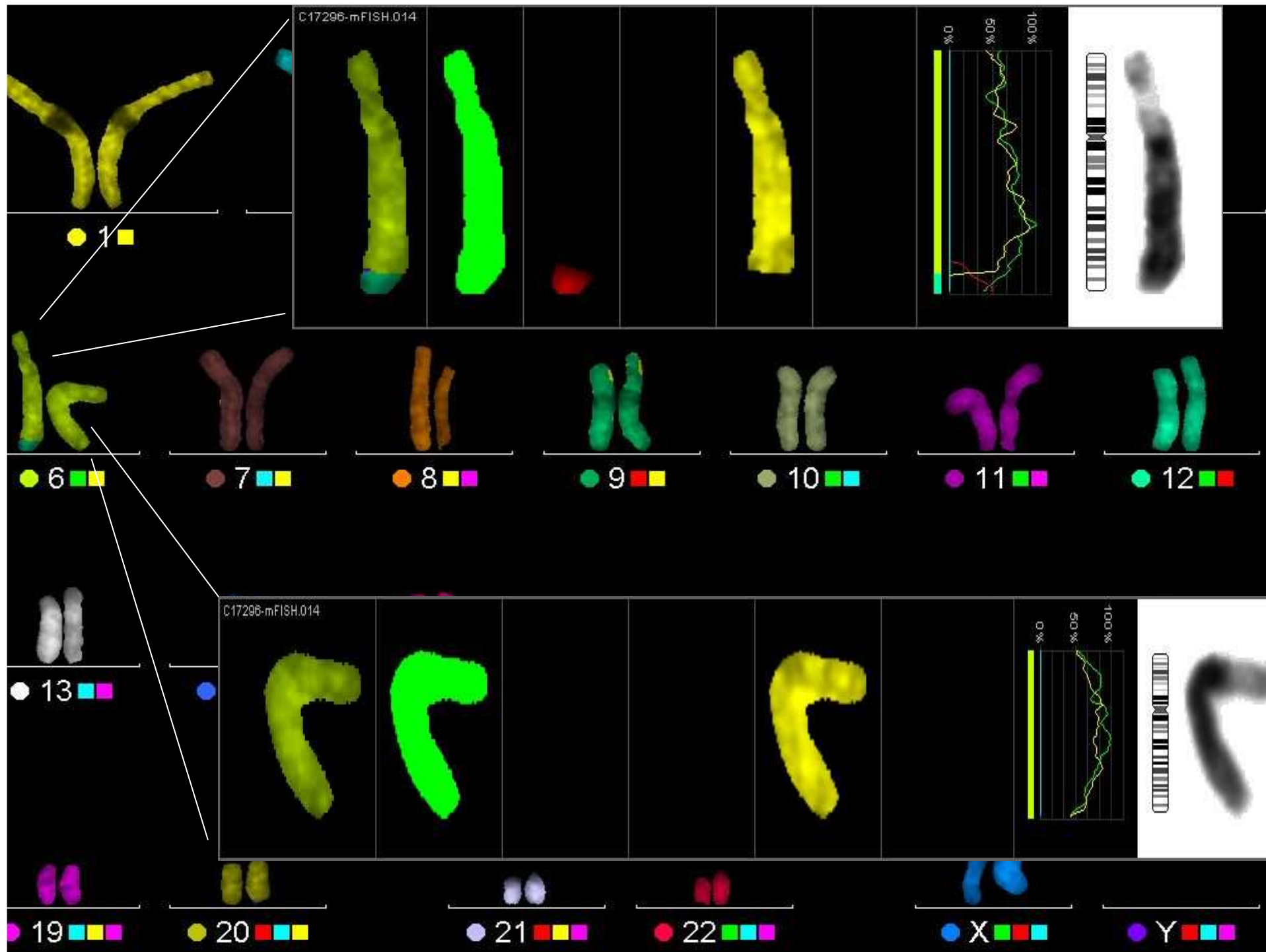


Multicolor-FISH (mFISH)  
24 chromosomen-spezifische Paintings  
Metasystems

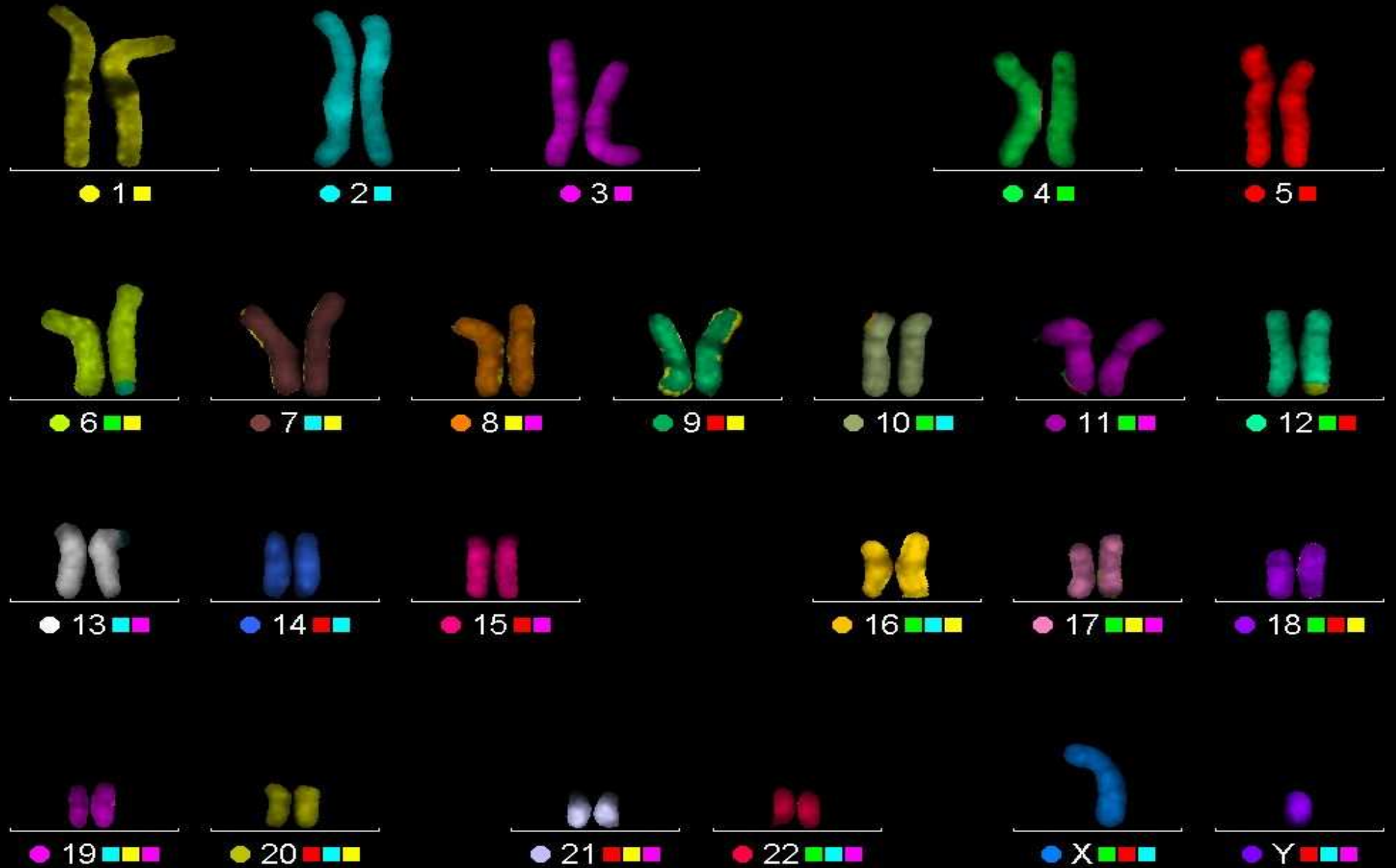
#	DEAC Ex 426nm/ Em 480nm	FITC Ex 502nm/ Em 530nm	Spectrum Orange™ Ex 559nm/ Em 588nm	Texas Red® Ex 595nm/ Em 615nm	Cy™5 Ex 649nm/ Em 670nm
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
X					
Y					

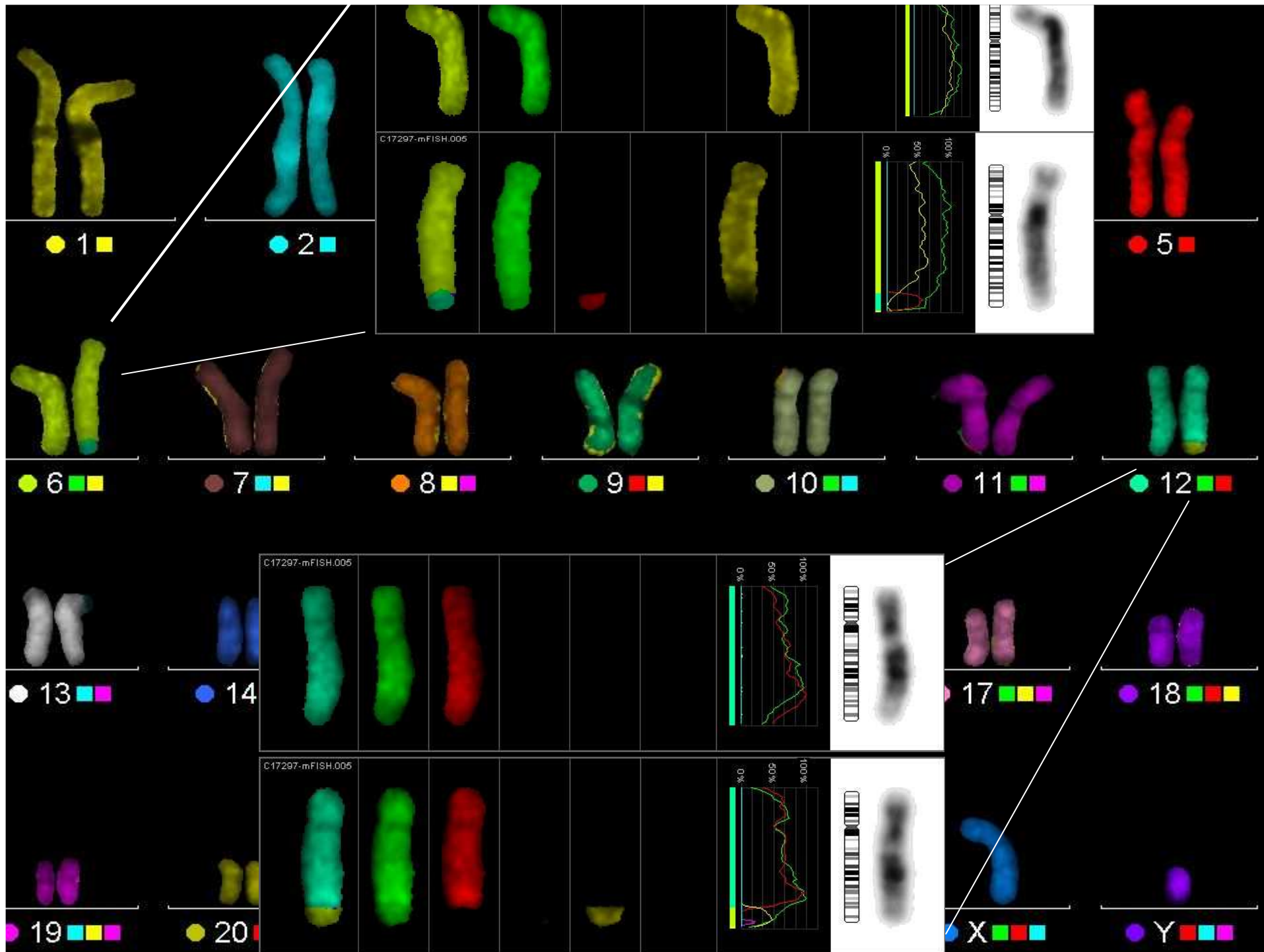
Hanna B., 46,XX,der(6)t(6;12)(q25.3;q24.2)pat



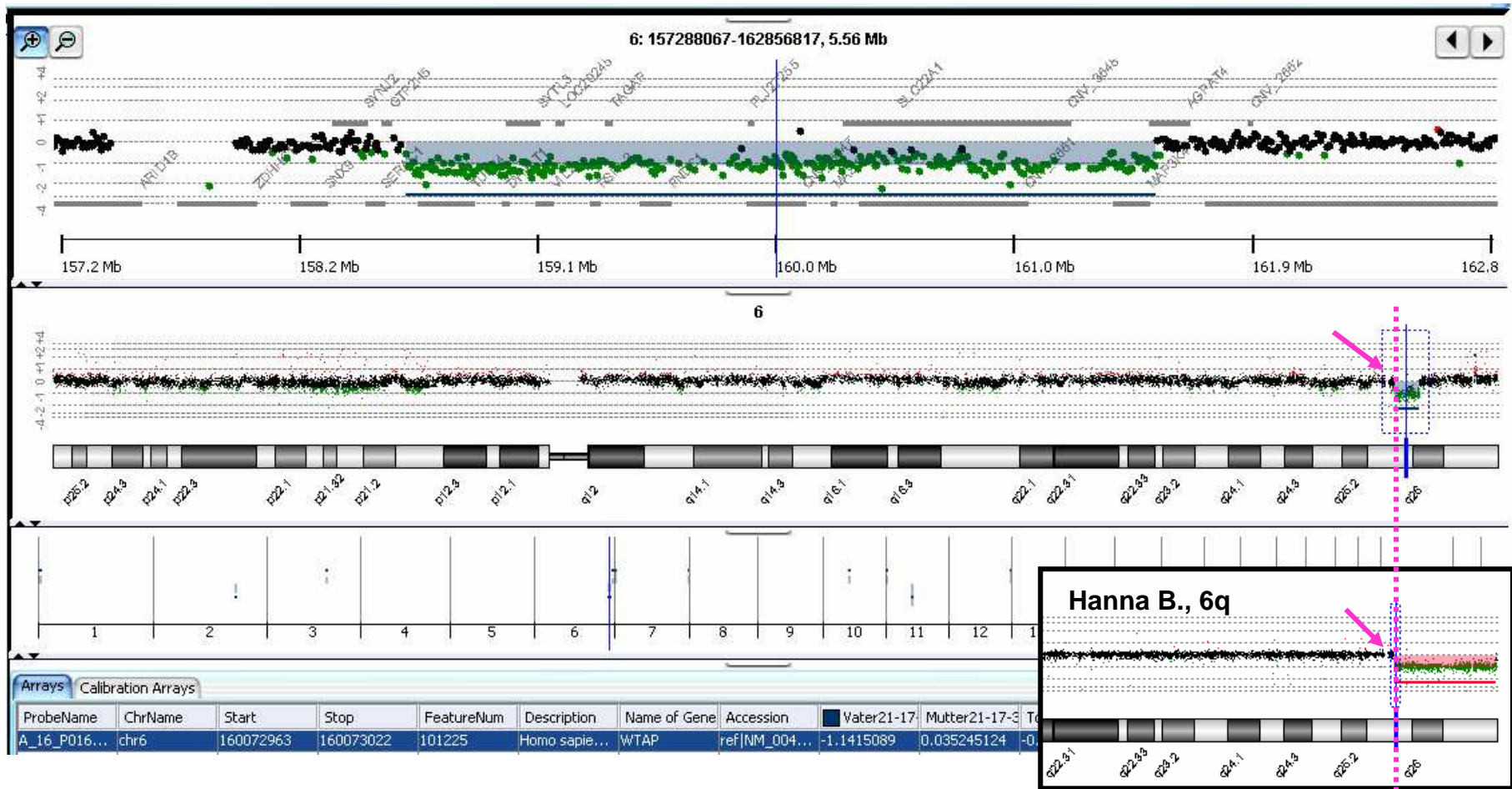


Vater B., 46,XY,t(6;12)(q25.3;q24.2)



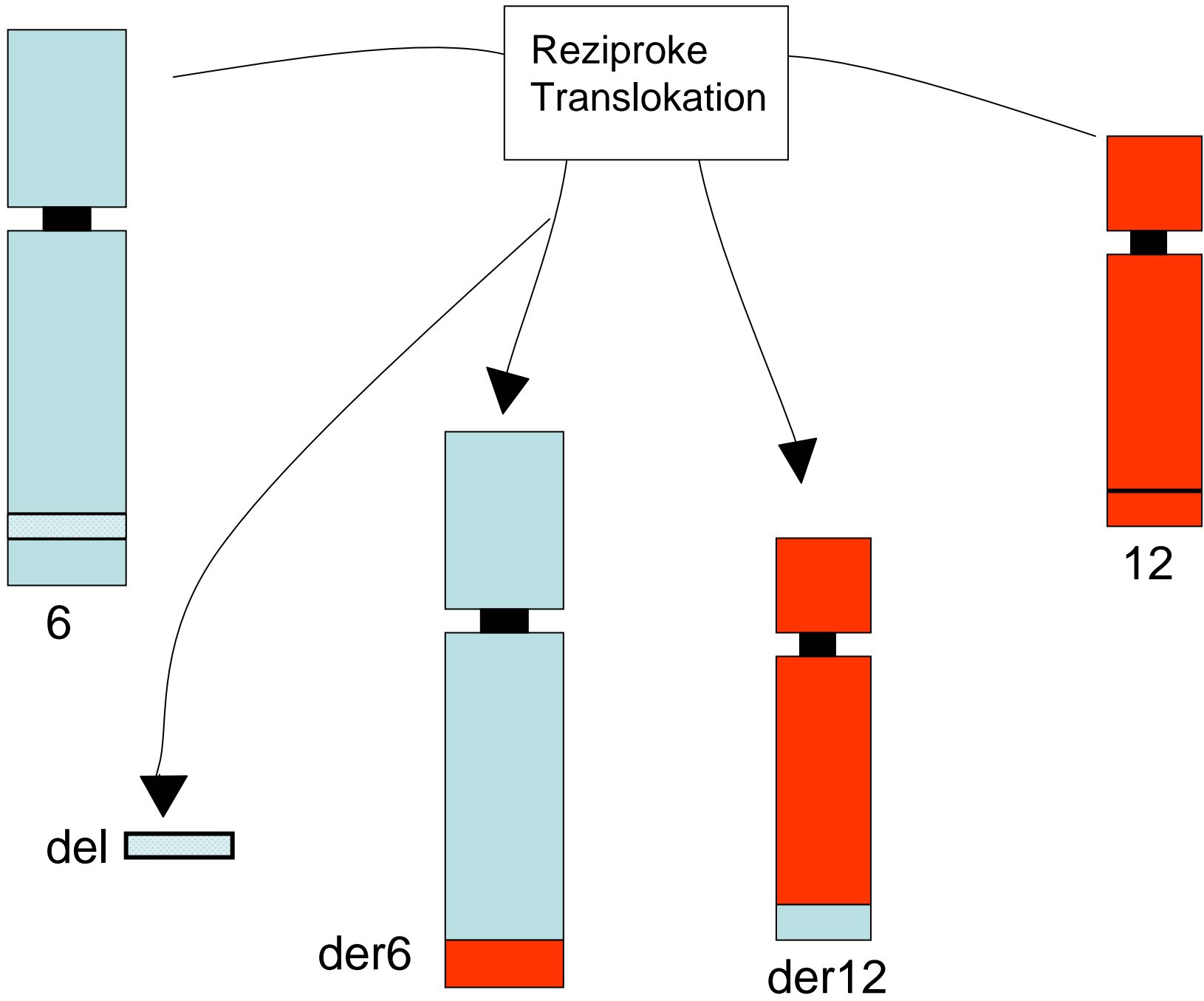


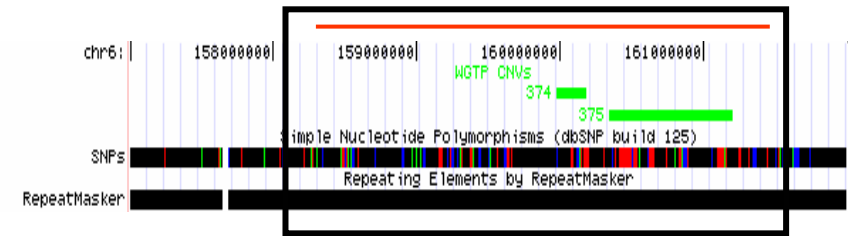
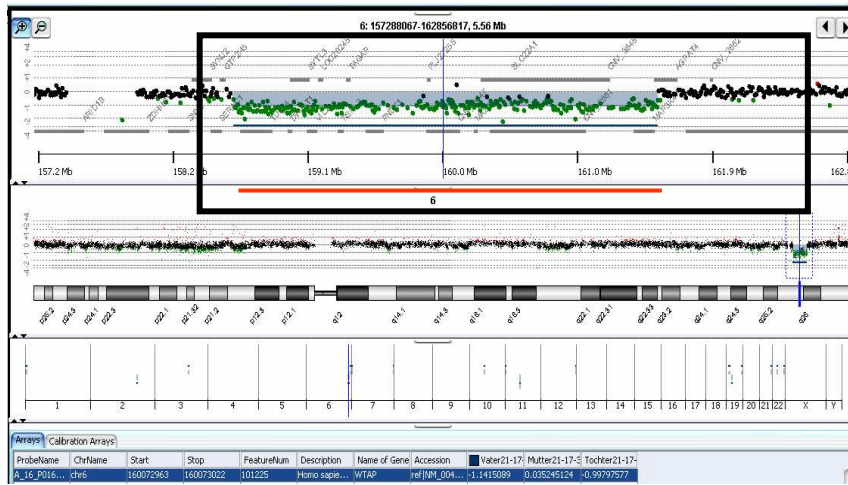
# DNA Microarray (aCGH) „Oligo-Array“ Vater B.



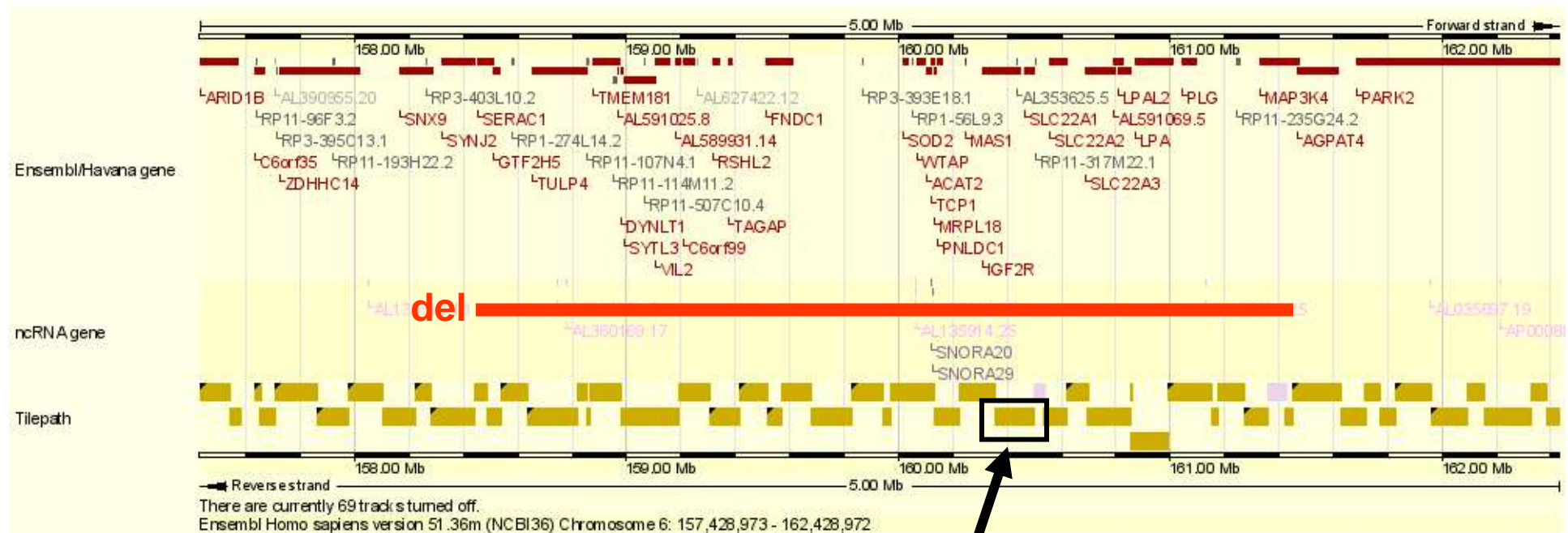
Chromosom 6q25.3:

Interstitielle Mikrodeletion von 2.89 Mb (158.645.291 bis 161.537.538)  
 (Proximaler Bruchpunkt entspricht Translokationsbruchpunkt)



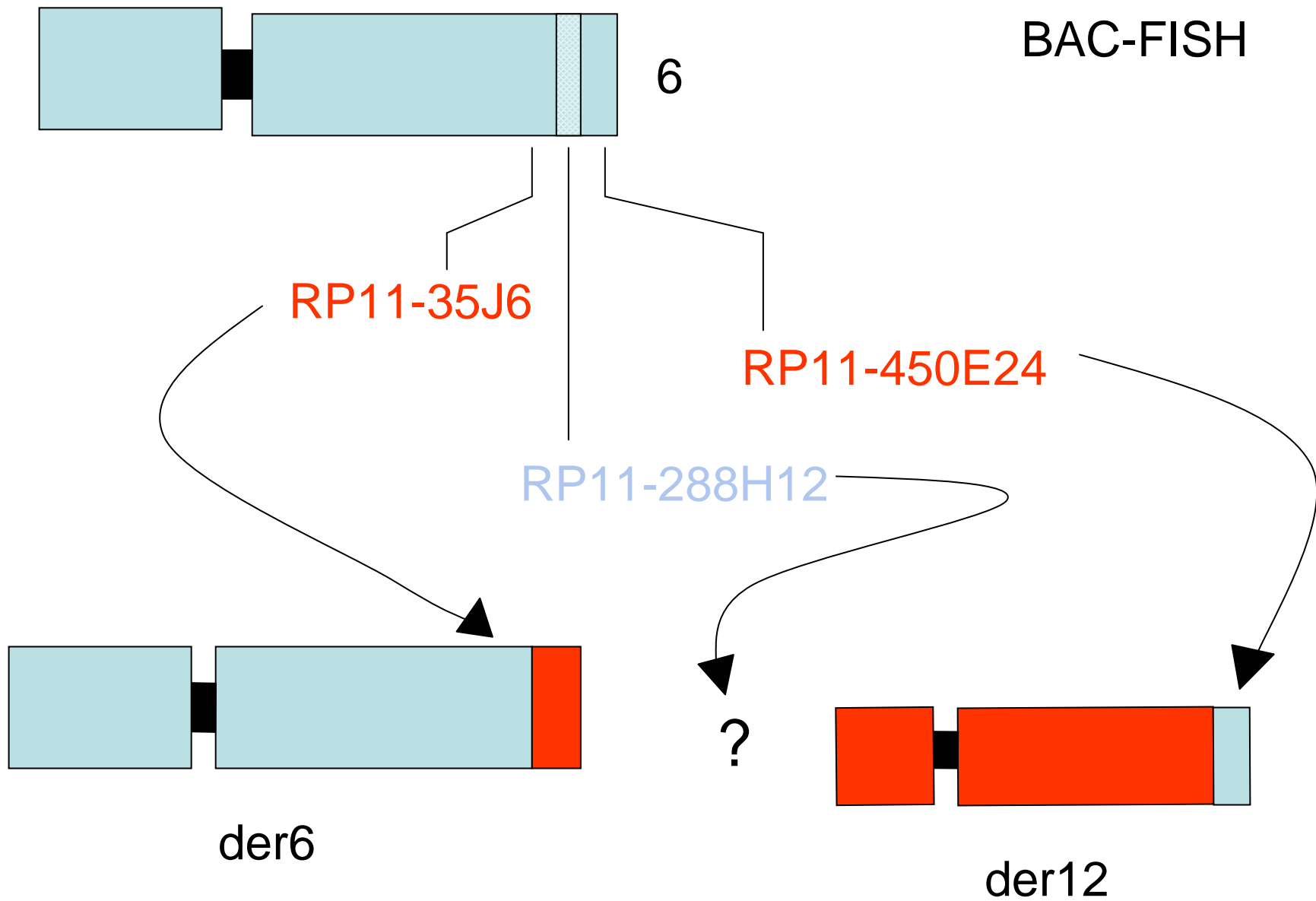


UCSC Genome Browser  
<http://genome.ucsc.edu/cgi-bin/hgGateway>



BAC RP11-288H12

Ensembl Release 51 (Nov 2008) <http://www.sanger.ac.uk/HGP/>



BACs aus dem BAC/PAC Resources Center, Oakland (P. de Jong)

RP11-35J6

RP11-288H12

RP11-450E24

450E24

der12

6

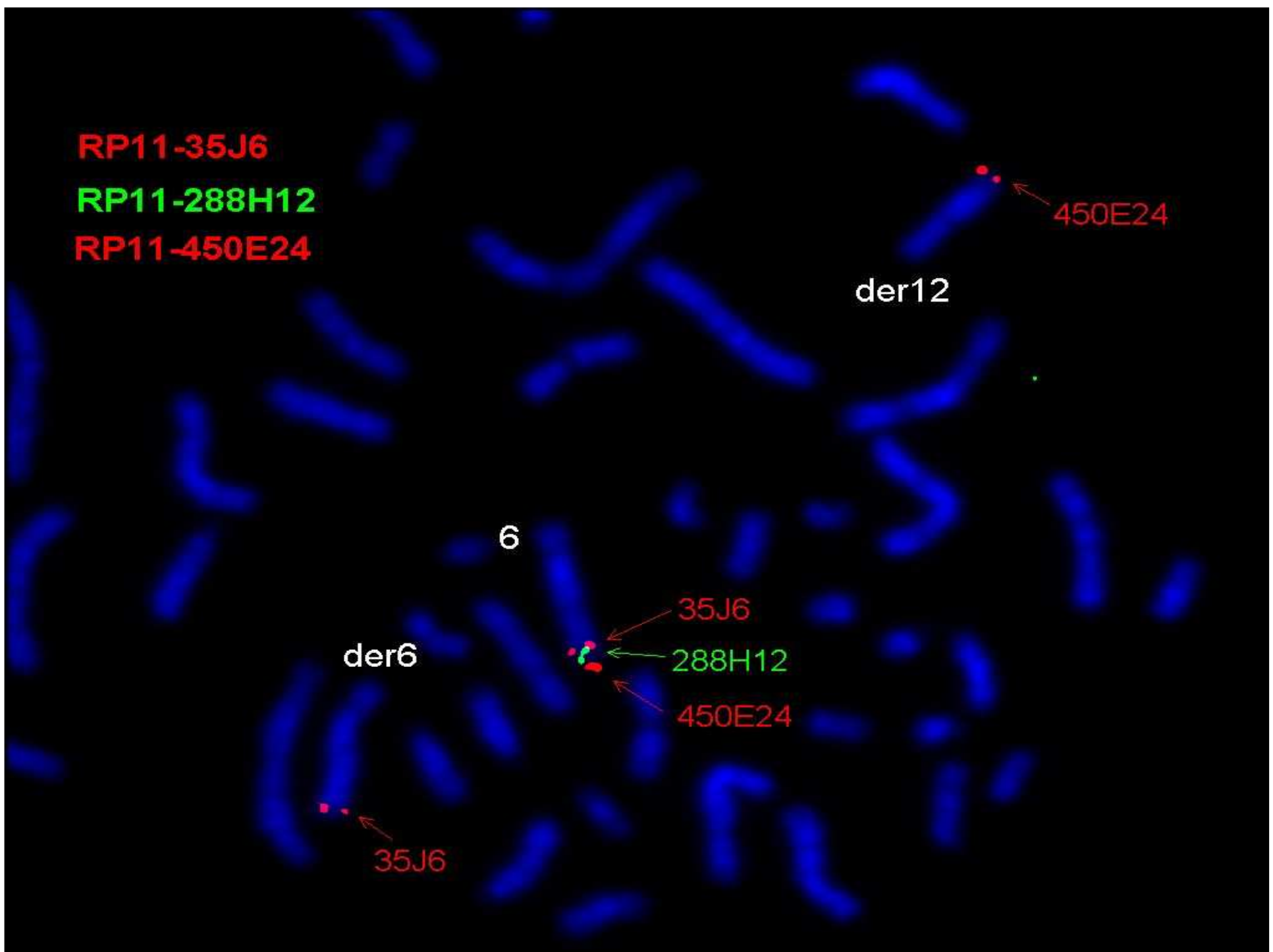
der6

35J6

288H12

450E24

35J6



## Karyotyp des Vaters:

Zytogenetisch:

46,XY,t(6;12)(q25.3;q24.2)

*Erstes bzw. letztes  
Deletiertes Oligo*

Molekularzytogenetisch:

46,XY,t(6;12)(q25.3;q24.2).

*Deletierter BAC*

arr cgh 6q25.3(202545-90440)x1.

ish del(6)(q25.3q25.3)(RP11-288H12 -)

[arr cgh = array CGH, ish = in-situ Hybridisierung]

# Zusammenfassung

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Die konventionelle (lichtmikroskopische) Chromosomenanalyse liefert ein Gesamtbild des Genoms bei relativ niedriger Auflösung ( $\geq 3$  Mb) und wird eingesetzt zum Nachweis gonosomaler und autosomaler Aneuploidien (Trisomien) bei entsprechendem klinischem Verdacht sowie als Einstiegsdiagnostik zur Charakterisierung unklarer syndromaler Krankheitsbilder.

Molekularzytogenetische Verfahren arbeiten zielgerichtet mit mittelhoher Auflösung (50 - 100kb) bei FISH (target DNA!) bzw. mit hoher Auflösungskraft (bis zu  $\geq 2$  kb) bei aCGH. Mit Ausnahme von multicolour – FISH wird mit FISH ein fest – umschriebener, begrenzter Ausschnitt des Genoms betrachtet (Locus, Bande, Einzelchromosom). Array CGH erkennt keine Chromosomen – Rearrangements (Translokationen, Inversionen), sondern Verlust oder Zugewinn (Deletionen, Duplikationen). Dennoch wird aCGH im klinischen Kontext die konventionelle Chromosomenanalyse mehr und mehr ersetzen, während FISH einen ergänzenden und weiterführenden Ansatz zur konventionellen Chromosomenanalyse darstellt.