Constitutional 9p22q translocation in a patient with melanoma, deafness and DNA repair deficiency disrupts *p14arf* and down-regulates *TBX1*

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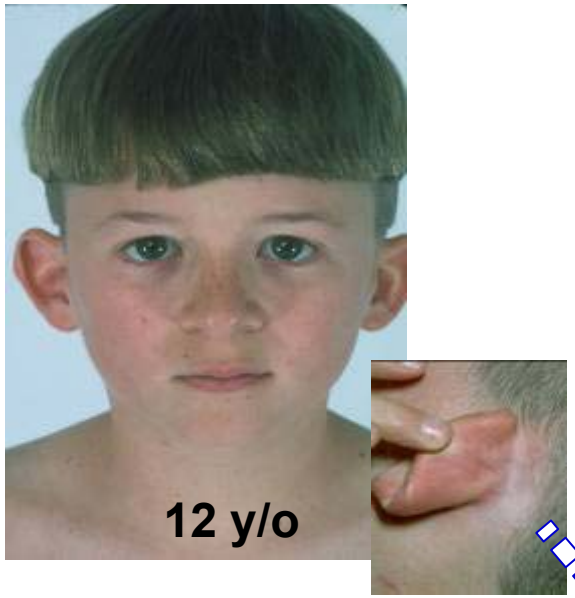
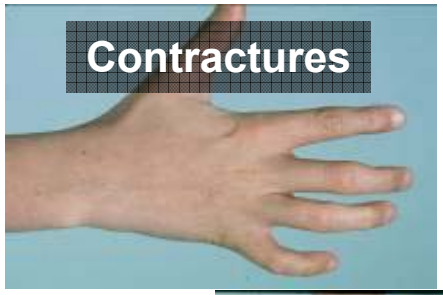
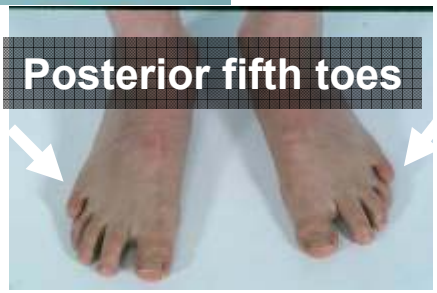
DNA REPAIR DEFICIENCY, MELANOMA, DEAFNESS, AND CHROMOSOME 9p22q TRANSLOCATION – A NEW SYNDROME?



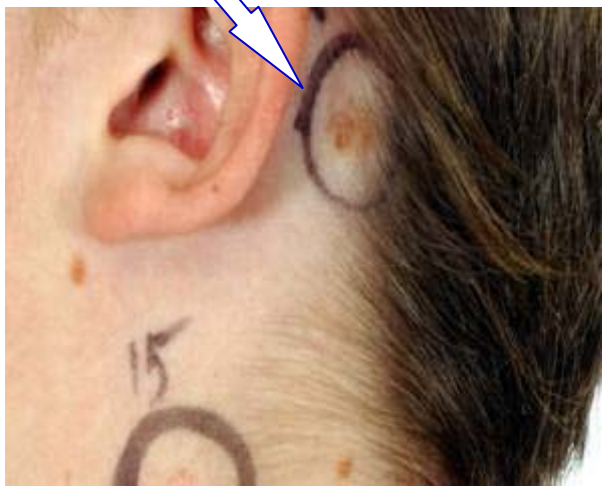


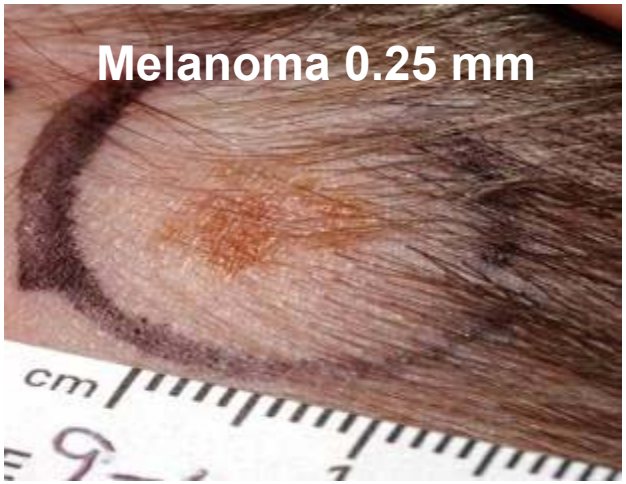


Patient DD129BE

Sensorineural deafness,

DiGeorge Syndrome / Velo-cardio-facial Syndrome like features





CLINICAL EXAMINATION

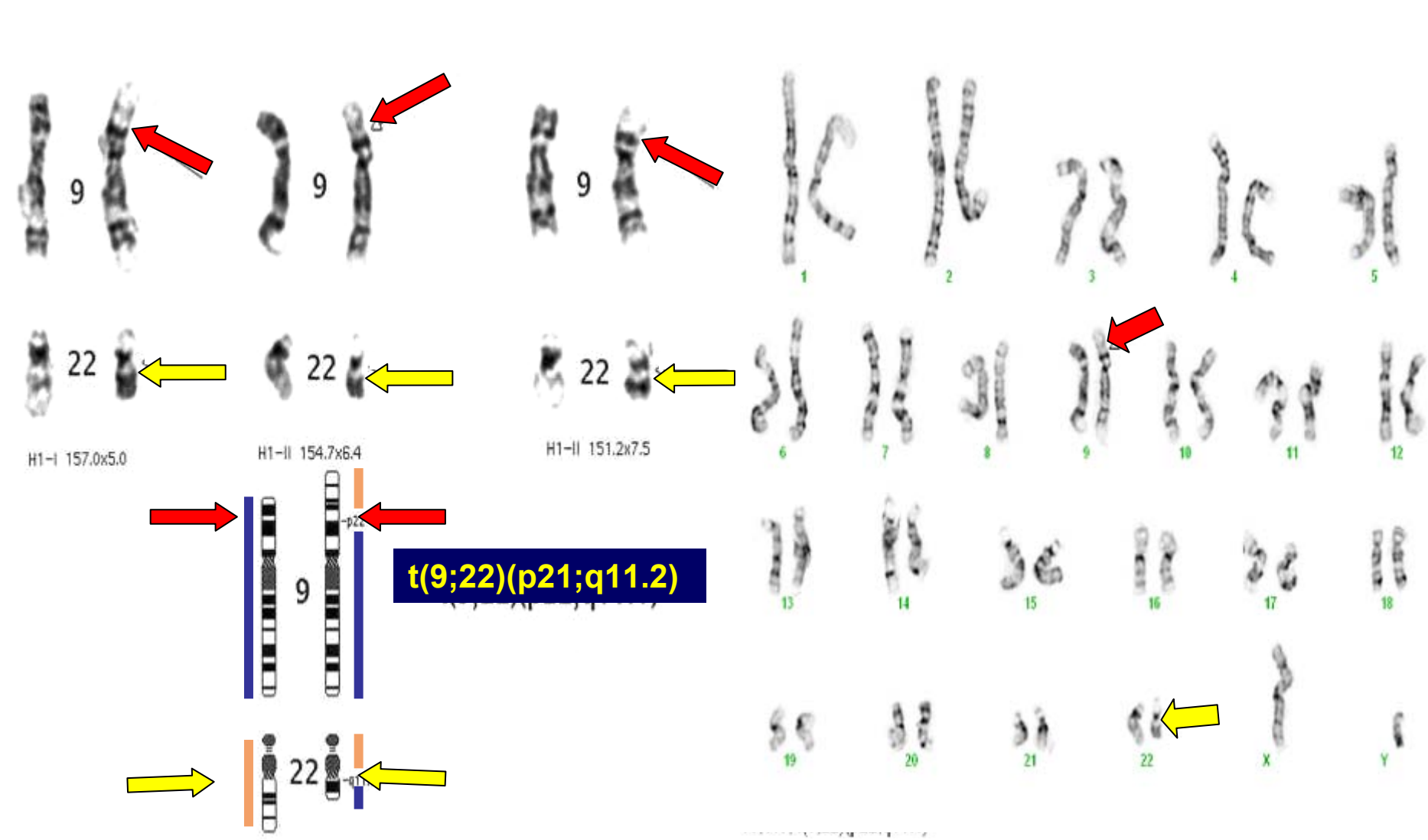
- **Audiologic assessment**

  No measureable hearing bilaterally and absent vestibular reactivity

- **CT and MRI**

  Bilaterally symmetric cochlear hypoplasia and vestibular dysplasia with absence of the cochlear nerve.

KARYOTYPE ANALYSIS

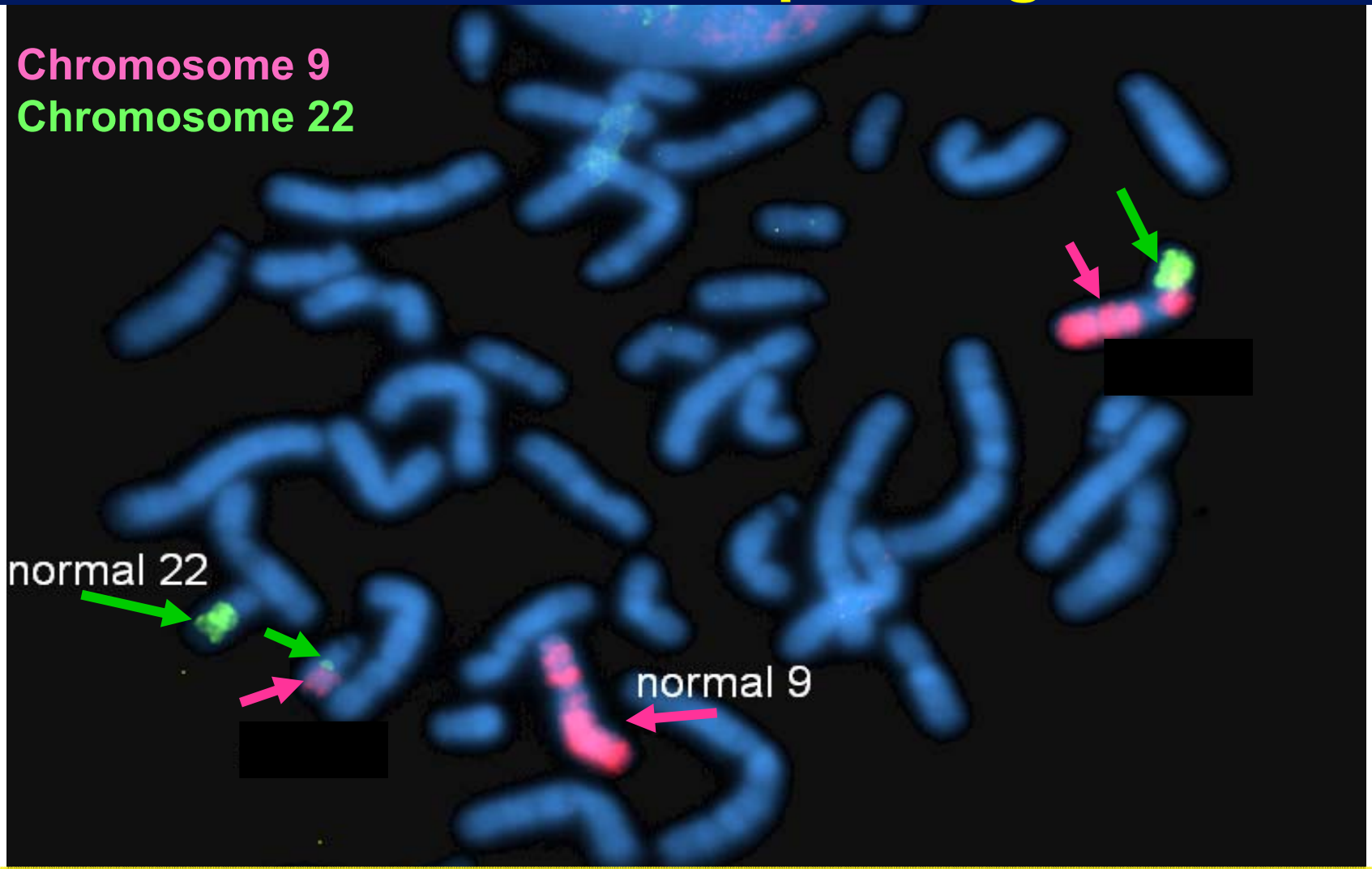


Reciprocal translocation between the short arm of chromosome 9 and the long arm of chromosome 22

THE AIM

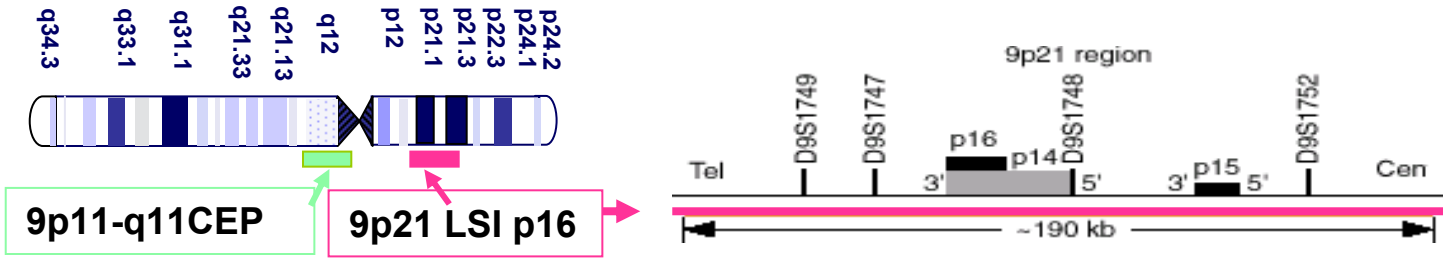
1. To map the chromosome breakpoints in cells from patient DD129BE
2. To develop a detailed molecular characterization of the candidate genes for his clinical syndrome of melanoma, defective DNA repair and deafness.

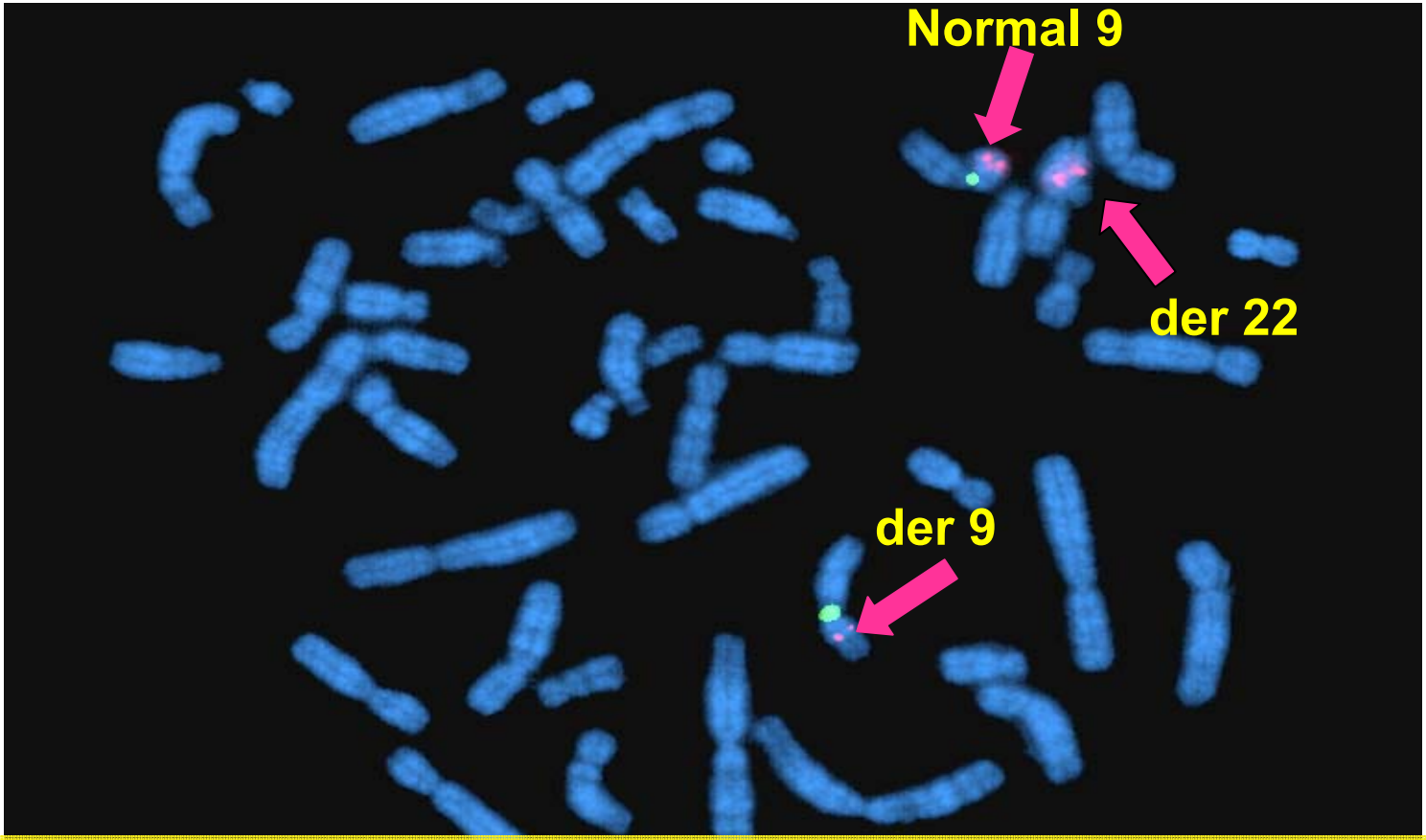
FISH analysis of t(9;22) with whole chromosome painting



CHROMOSOME 9 and 22 PROBES ARE SPLIT BY THE TRANSLOCATION

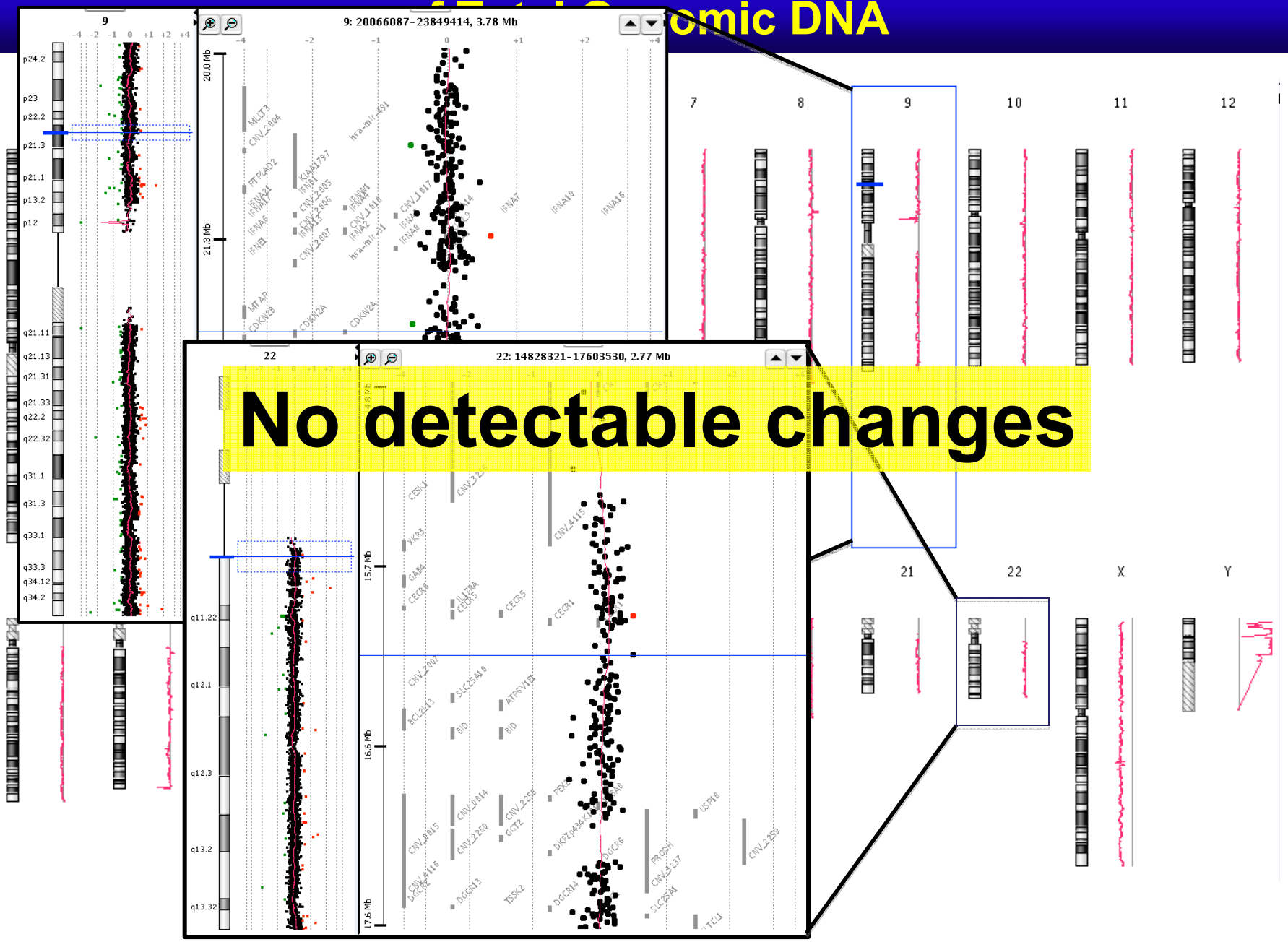
FISH analysis of the translocation on chr9





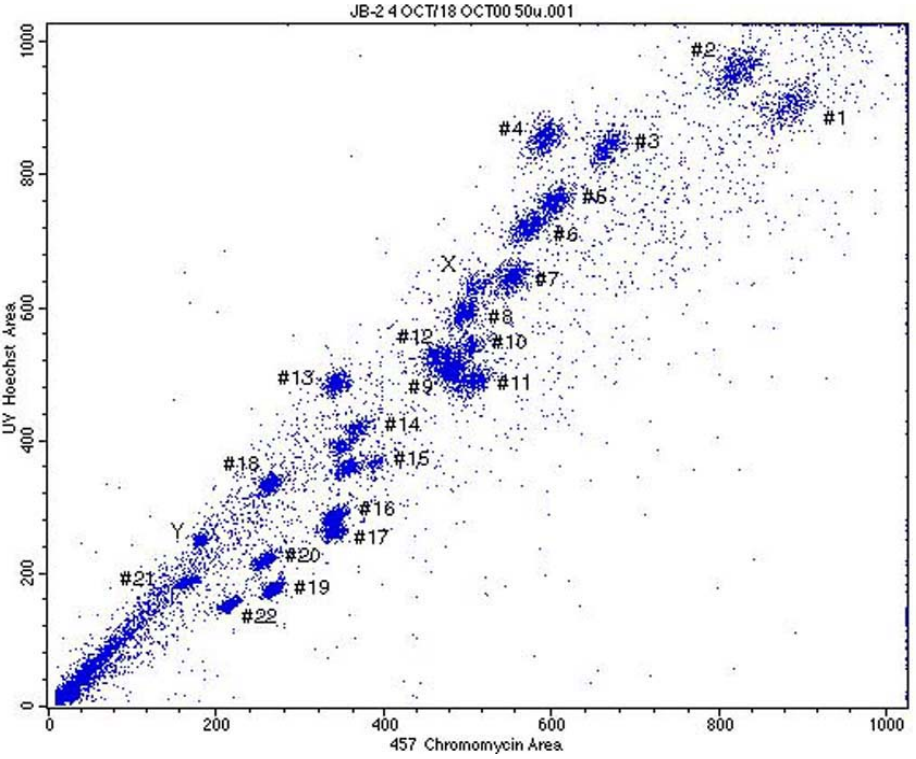
CHROMOSOME 9 p16 PROBE IS SPLIT BY THE TRANSLOCATION

Array Comparative Genome Hybridization (aCGH)

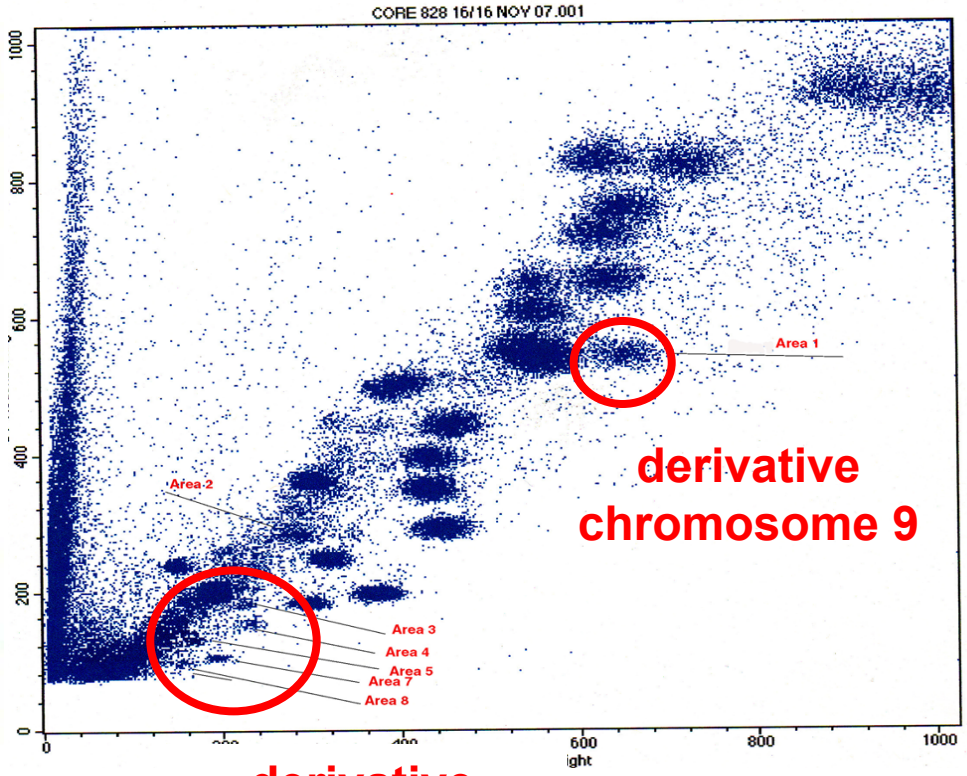


BIVARIATE FLOW SORTING TO ISOLATE DERIVATIVE CHROMOSOMES

Normal chromosomes



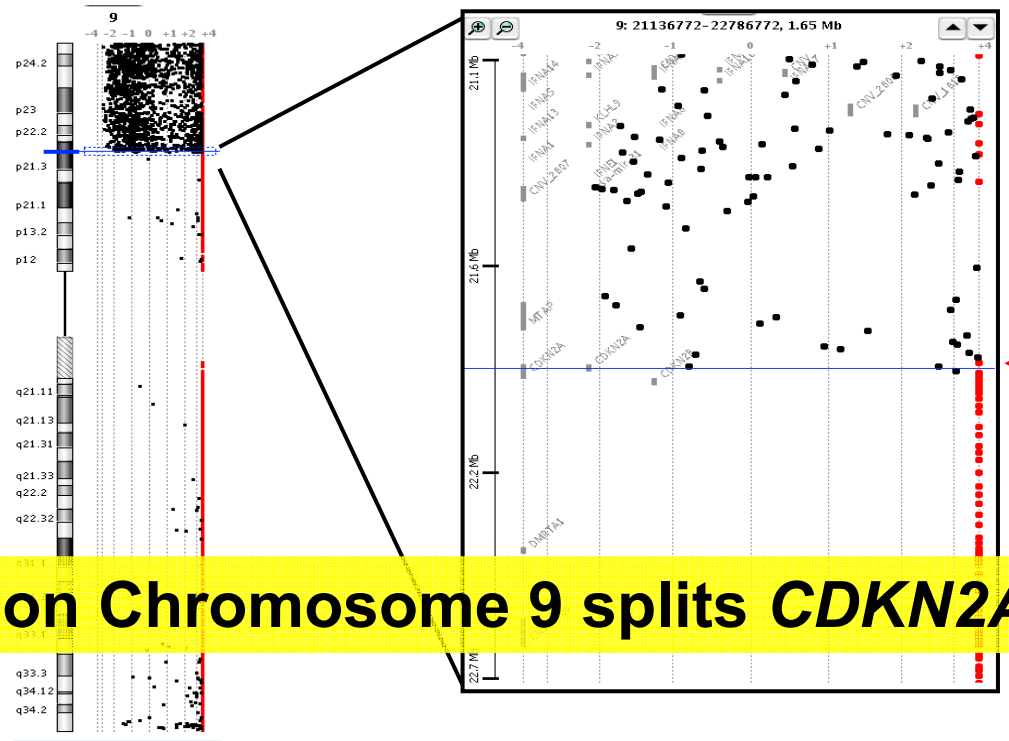
Patient chromosomes



Derivative Chromosomes can be Separated By Bivariate Flow Sorting

Breakpoint Located Within CDKN2A Gene Using aCGH On Flow Sorted Derivative Chromosome 9

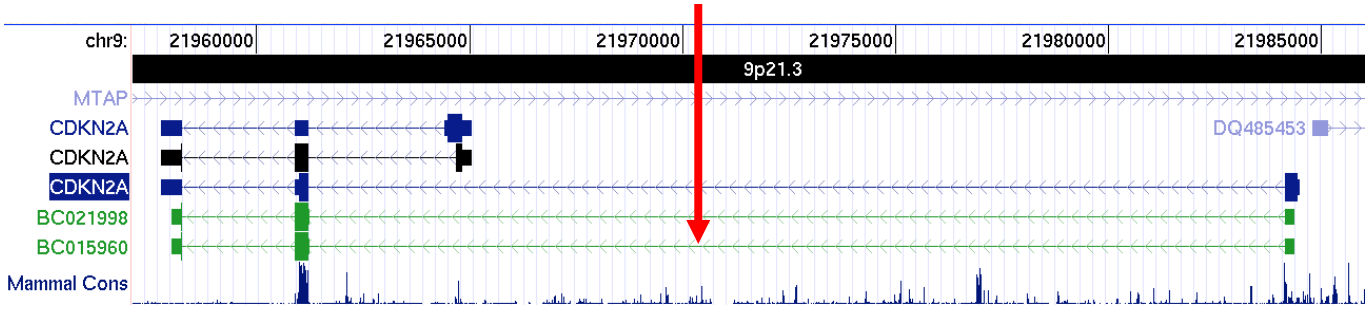
Area 1 derivative chromosome 9



BREAKPOINT

Break on Chromosome 9 splits *CDKN2A* gene

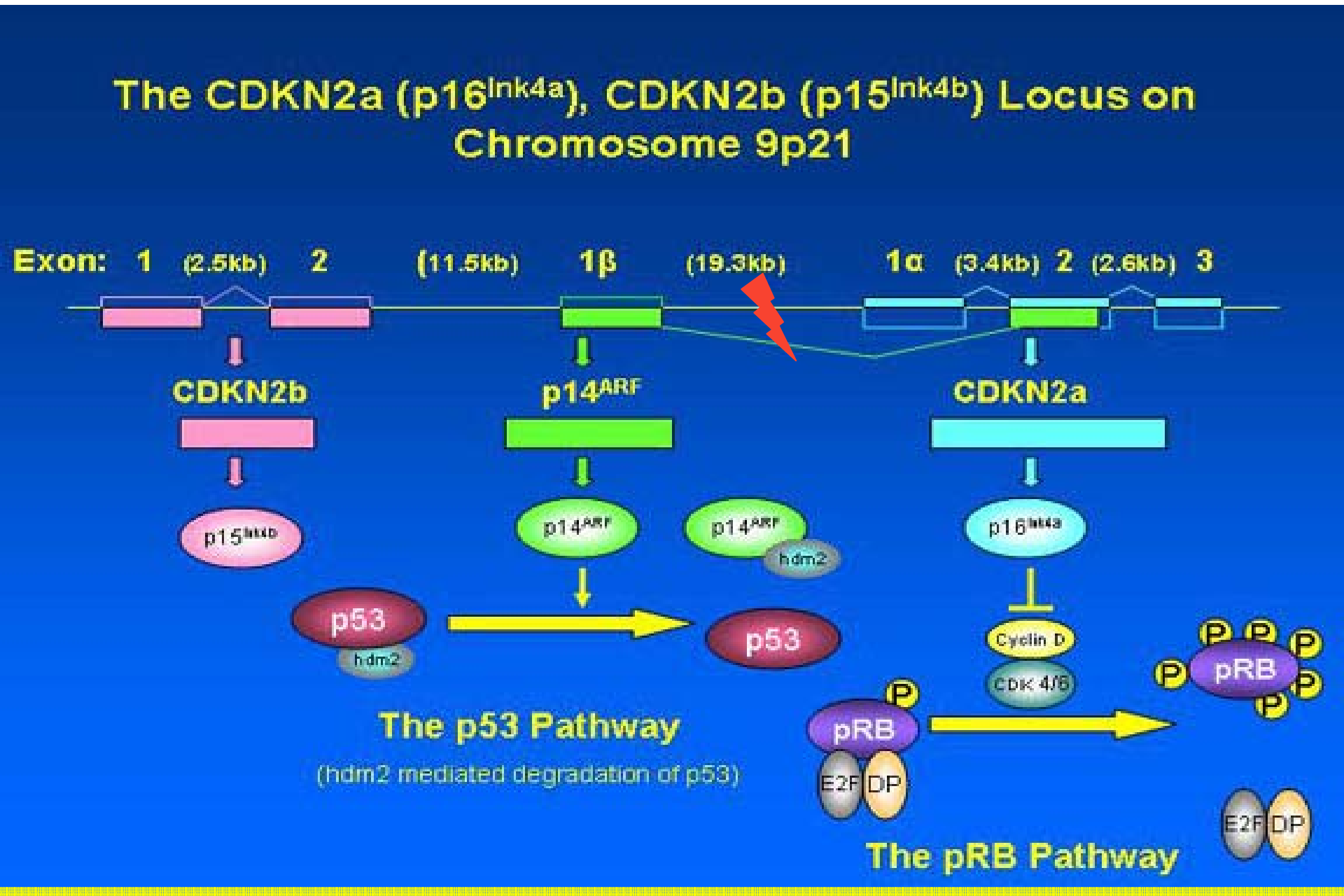
BREAKPOINT



TEL

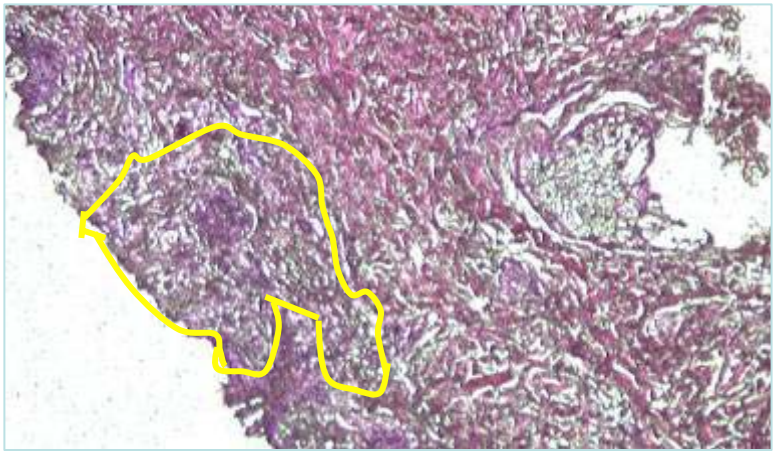
CEN

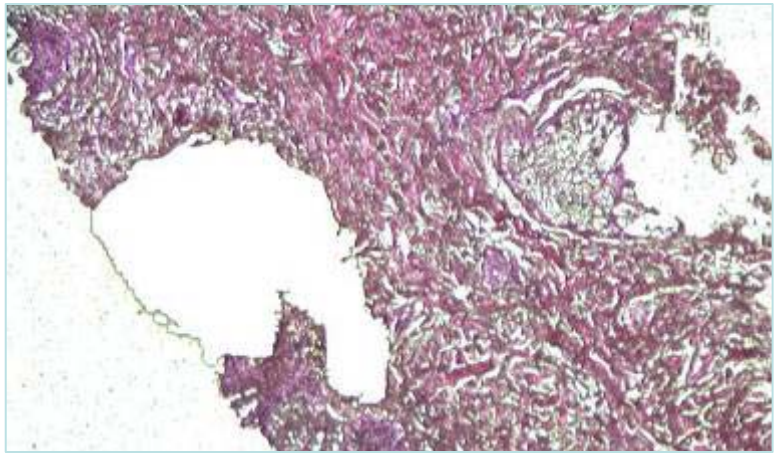
Intron 1



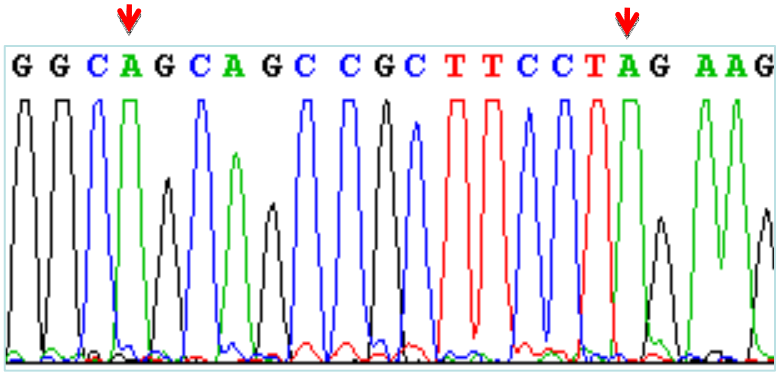
Breakpoint on Chromosome 9 Interrupts p14/ARF Protein Leading to Melanoma

Laser Capture Microdissected Melanoma Tissue from patient DD129BE

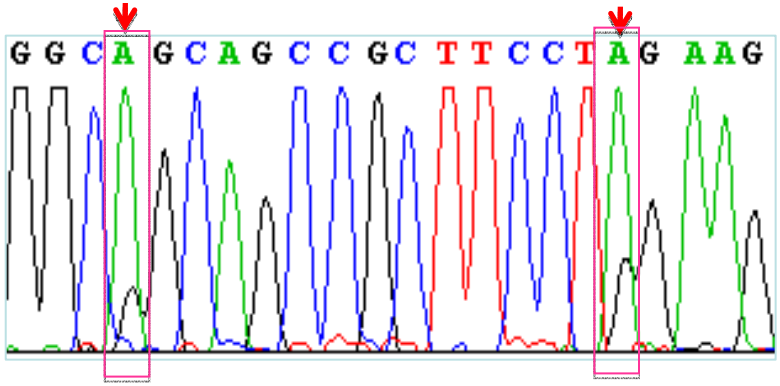




Two UV-type Missense Mutations in Exon 1 β of p14arf Gene



Wild type

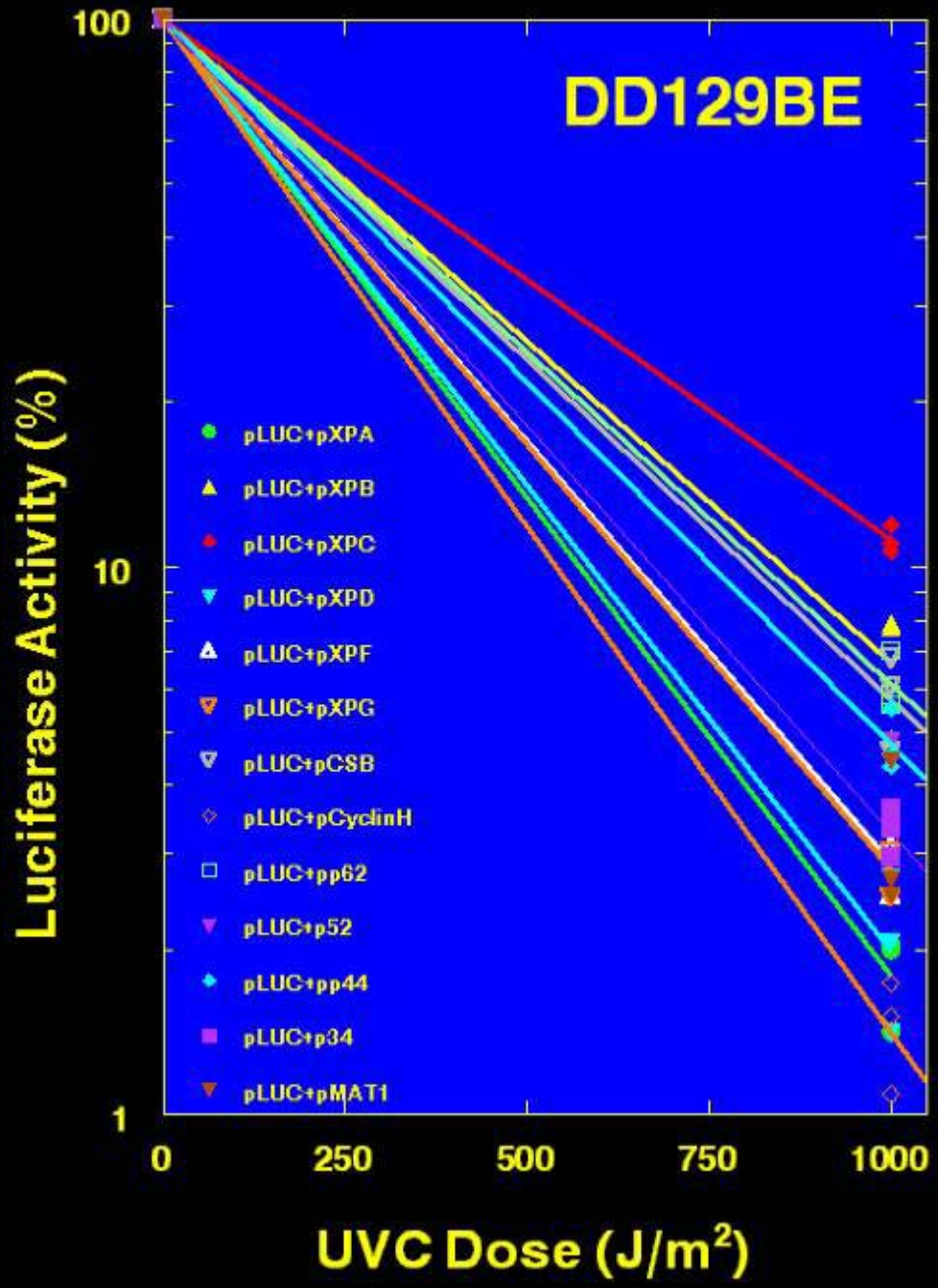


gCAG>CGG pQ57R
gAGA>GGA pR62G

Knudson Model for Cancer

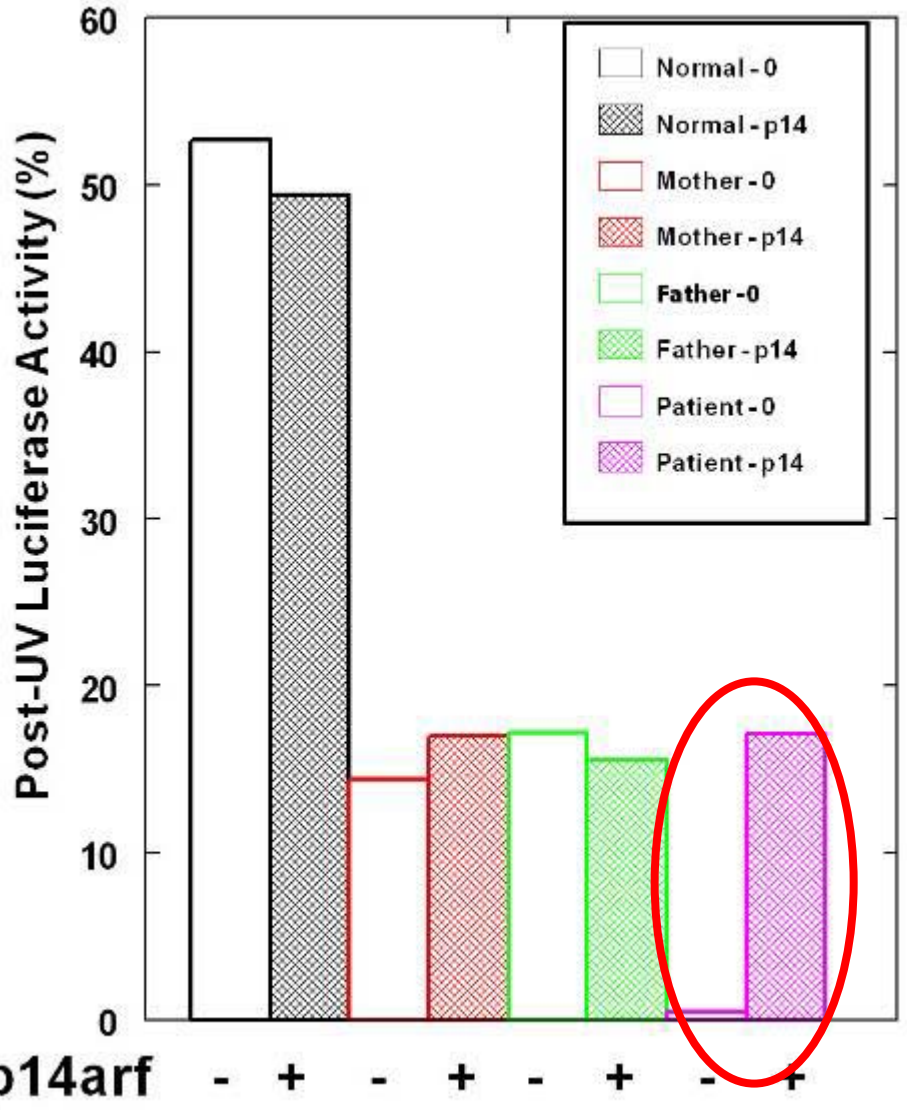
The melanoma in patient DD129BE may have resulted when one copy of *p14arf* was disrupted by the translocation followed by somatic mutations in the other copy.

DNA REPAIR DEFICIENCY IN PATIENT DD129BE FIBROBLASTS



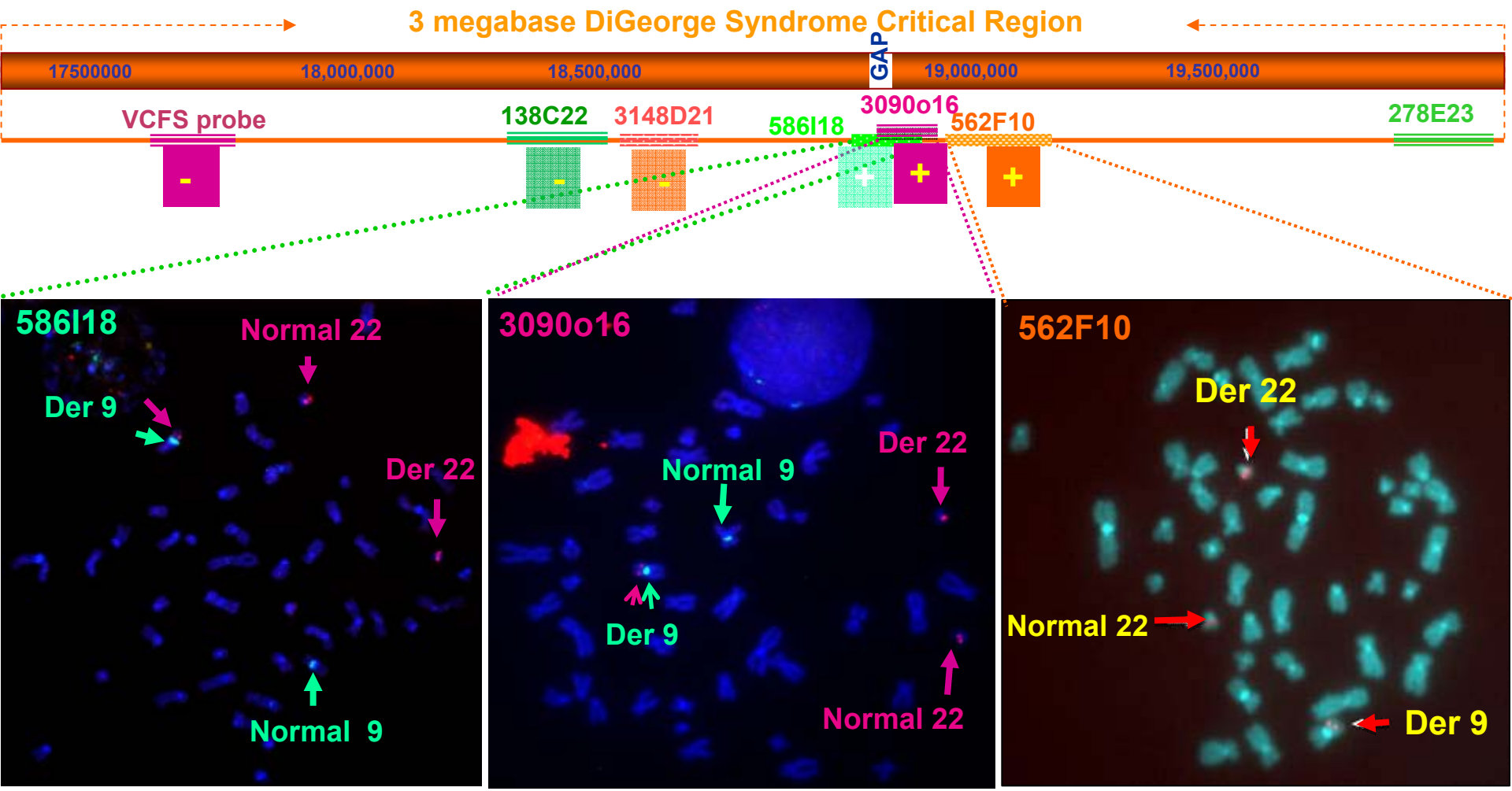
Reduced HCR is not corrected by any of the known XP complementation groups

Increased HCR by Co-transfection with p14



DNA repair defect improved by transfection with p14 cDNA vector

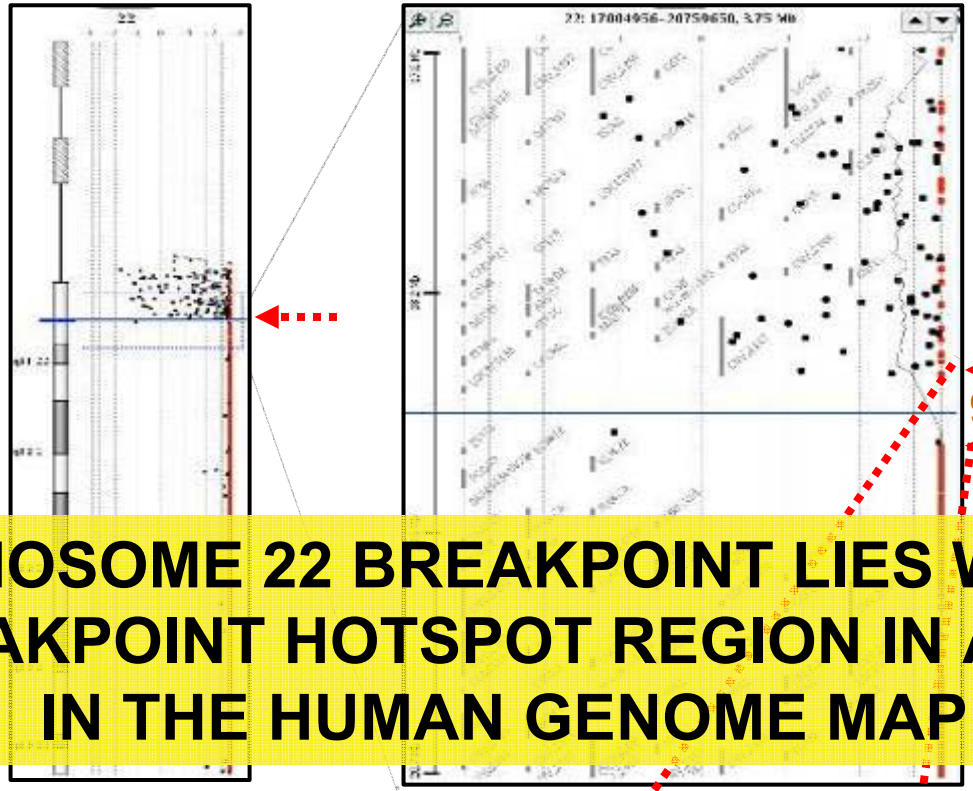
FISH Analysis of DiGeorge Syndrome Critical Region on Chr22



562F10, 3090o16 and 586I18 PROBES ARE SPLIT BY TRANSLOCATION

Array CGH On Flow Sorted Derivative 22

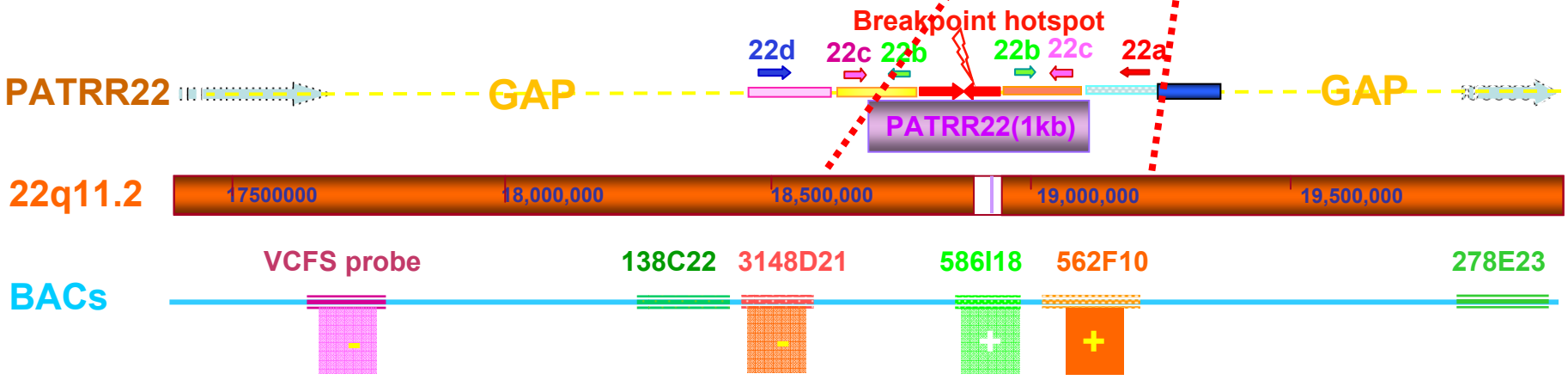
Areas 3-8 pooled derivative chromosome 22



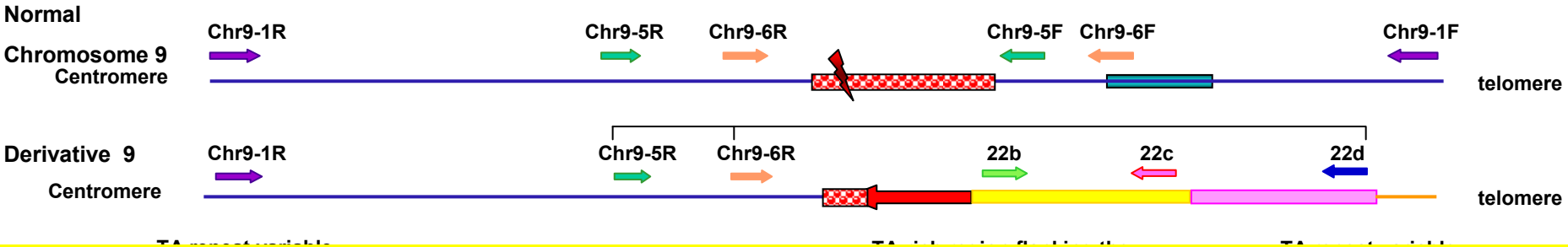
BREAKPOINT

980kb

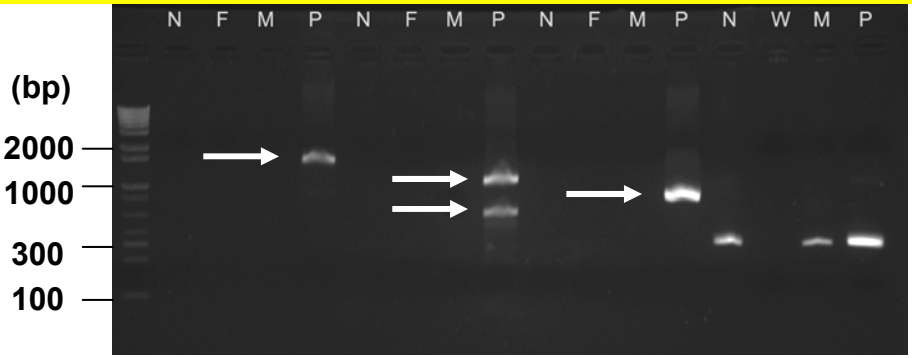
CHROMOSOME 22 BREAKPOINT LIES WITHIN A BREAKPOINT HOTSPOT REGION IN A GAP IN THE HUMAN GENOME MAP

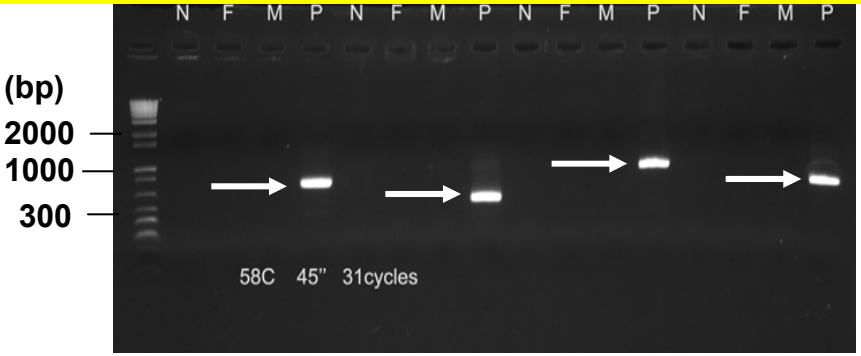


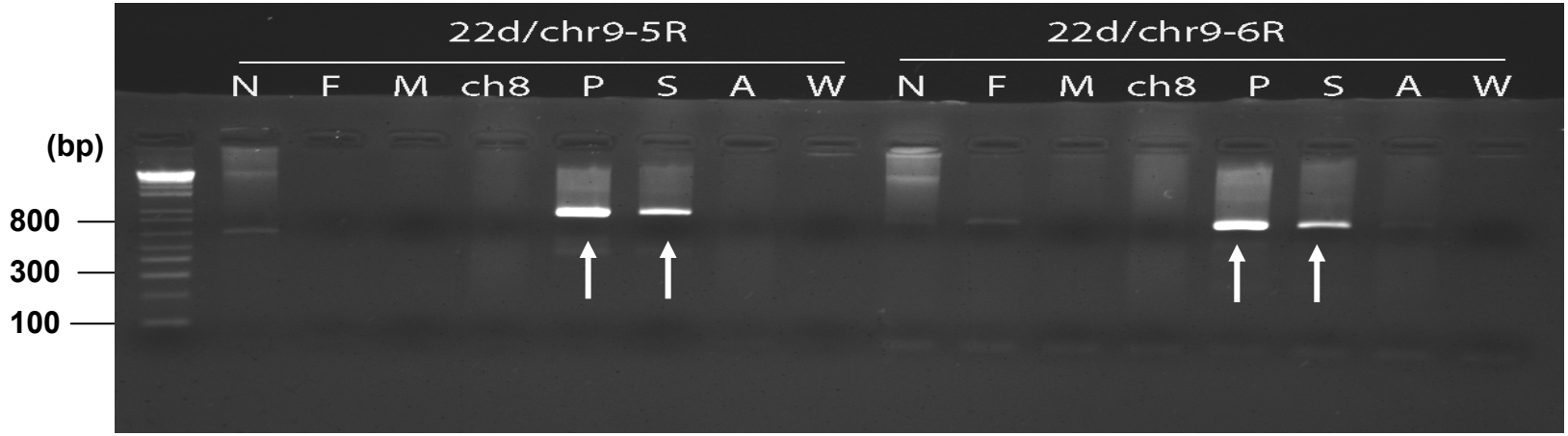
ISOLATION OF TRANSLOCATION JUNCTION FROM Derivative 9 BY PCR



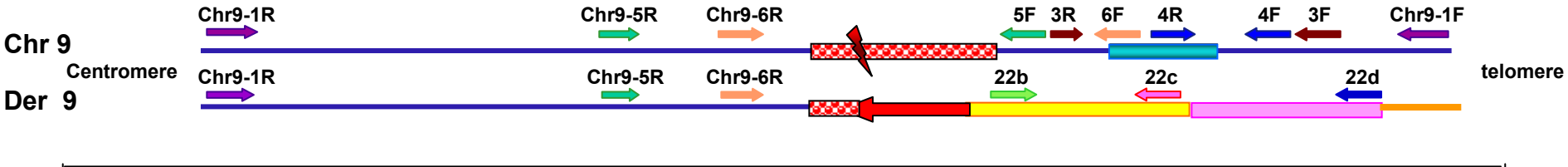
Breakpoint Junction is present only in cells from patient DD129BE







Junction Fragment Sequences of Der9



TRANSLOCATION BREAKPOINT LIES WITHIN AT-RICH REGIONS ON CHR 9 AND CHR 22

TCCTTACATTATTGCTTTTCTAAATTAAAGGGATGCATGGAATTATTCCT
CCATTGCCTTTGCCTTCAAATAATTATCTATTGCACCCAACATCCTATTC
TAGAACTCATCTATGAAGGCTTAACACAGCTGTACCTGGGAGCTCCATTA
CAGGGCATATATCTCGCTCTCATAAGCTACTTCCTAAGGAATTCTCTTTA
ATTATGGGAGCTTTTCCAGACTCTGAAATCTTTTTTTCCTGGTAACACAA
GTGTGAGGTGTCATTTATCAGAATGCATCACCCCAGTCTTCCCTCCTCAA
ATGATTACTGTAGGCTCCACTCAAGAGCTCATCCCAGTTCAAGACCACCT
TCCTCCTCCAGAGAAGCAAATATATATATACACGTATATATATATATACA
CGTATATATATATACGTATATATATATATATATATACACGTATATATATA
CACGTATATATATATATACACGTATATATATATACGTGTATATATATATA
TACGTGTATATATATATACGTGTATATATATATACGTGTATATATATA
TACGTGTATATATATATACGTGTATATATATATATACGTGTATATATATA
TATATACGTGTATATATATATTTGCTTTCTCTGGAGGAGGAAGGTGGTCTT
GAACTGGGATGAGCTCTTGAGTGGAGCCTACAGTAATCATTTGAGGAGGG
AAGACTGGGGTGATGCATTCTGATAAATGACACCTCACACTTGTGTTACC
AGGAAAAAAAGATTTCAGAGTCTGGAAAAGCTCCCATAATTAAAGAGAAT
TCCTTAGGAAGTAGCTTATGAGAGCGAGATATAT GCCCTGTAATGGAGC
TCCCAGGTACAGCTGTGTTAAGCCTTCATAGATGAGTTCTAGAATAGGAT
GTTGGGTGCAATAGATAATTATTTGAAGGCAAAGGCAATGGAGGAATAAT
TCCATGCATCCCTTTAATTTAGAAAAGCAATAATGTAAGGAAATTAGAGT

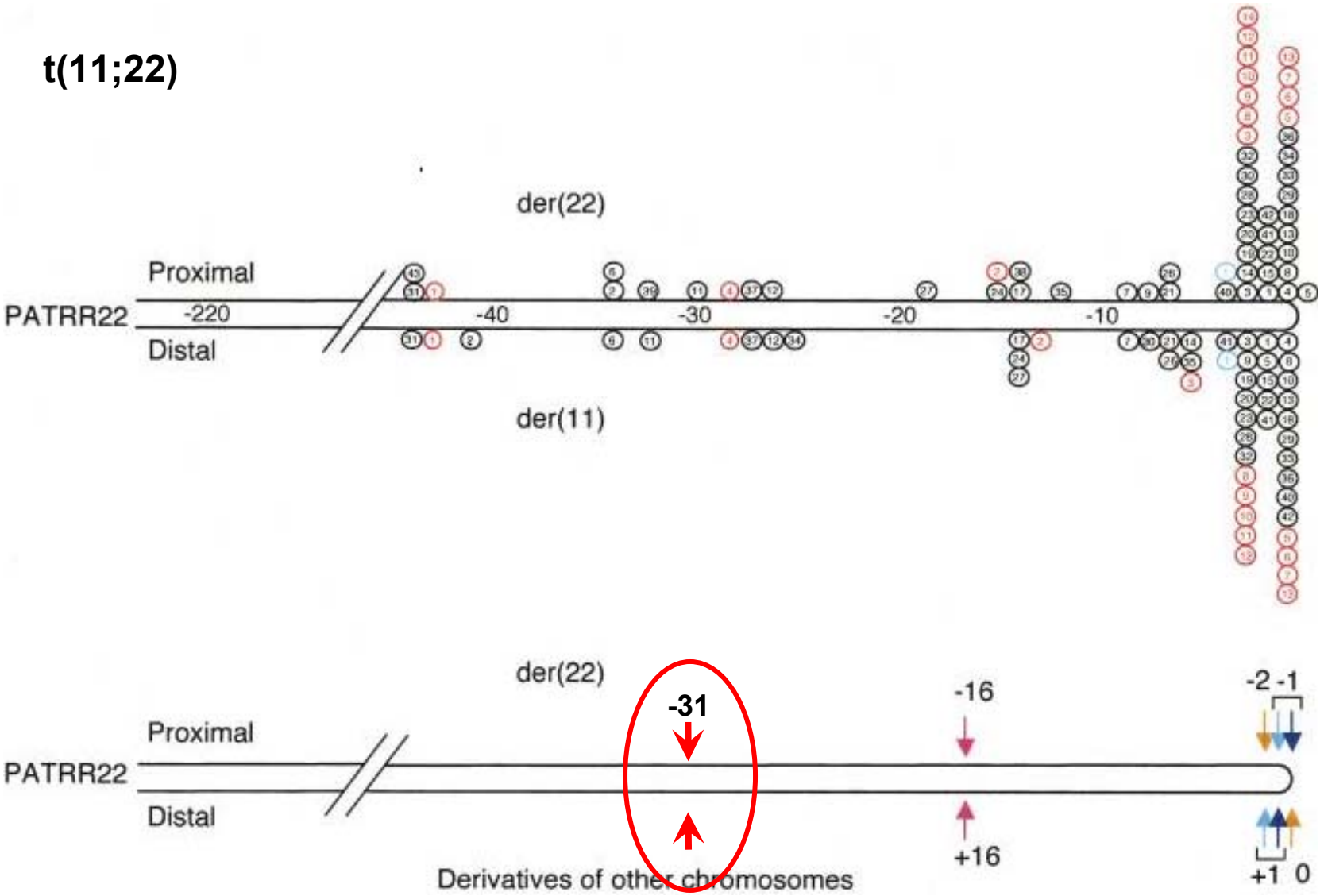
Sequence of normal chromosome 9

TCCTTACATTATTGCTTTTCTAAATTAAAGGGATGCATGGAATTATTCCT
CCATTGCCTTTGCCTTCAAATAATTATCTATTGCACCCAACATCCTATTC
TAGAACTCATCTATGAAGGCTTAACACAGCTGTACCTGGGAGCTCCATTA
CAGGGCATATATCTCGCTCTCATAAGCTACTTCCTAAGGAATTCTCTTTA
ATTATGGGAGCTTTTCCAGACTCTGAAATCTTTTTTTCCTGGTAACACAA
GTGTGAGGTGTCATTTATCAGAATGCATCACCCCAGTCTTCCCTCCTCAA
ATGATTACTGTAGGCTCCACTCAAGAGCTCATCCCAGTTCAAGACCACCT
TCCTCCTCCAGAGAAGCAAATATATATATACACGTATATATATATATACA
CGTATATATATATACGTATATATATATATATATATACACGTATATATATA
CACGTATATATATATATACACGTATATATATATACACACGTGTAATTAAATAT
ATAATATATGATATATAAAATATATATAATATATAATATATAATATATAT
AATATATATATAATTTCCTTTTACATCCTGCATCCTTCAACGTTCCATCC
CCCACCCCACAGTTTAAGTTATTCCCCAGGGGAGAATATGGCAAAGTCTA
TTTTAATGCAGTTTTTAATCCAATTAAGAACCTATGAAATCATTACTTTC
CAAAACTTTGGAACAAAGCCACAGTAGTATGGATCCGTTGGAGGCTTTTC
ACACAATAAAATGTACCTCTCTTTGTTTTTAACATGTTTTTCCCTTCCTC
TCTTCTTTTTTTGTGAAATGTGTATTTACTTTAATATATTTGTAGTAAGT
CACTTCCATGCACATATTAATTTTTTAAAGTAATAAGTATGTGTATTGTC
TACGTGTGAAATAAAACACACATTTATTTTTATGCTTTGGAAGTTATCCA
GAATCATGGAATTGTCAATCACAGTCAATCACCCAAC

Sequence of der 9

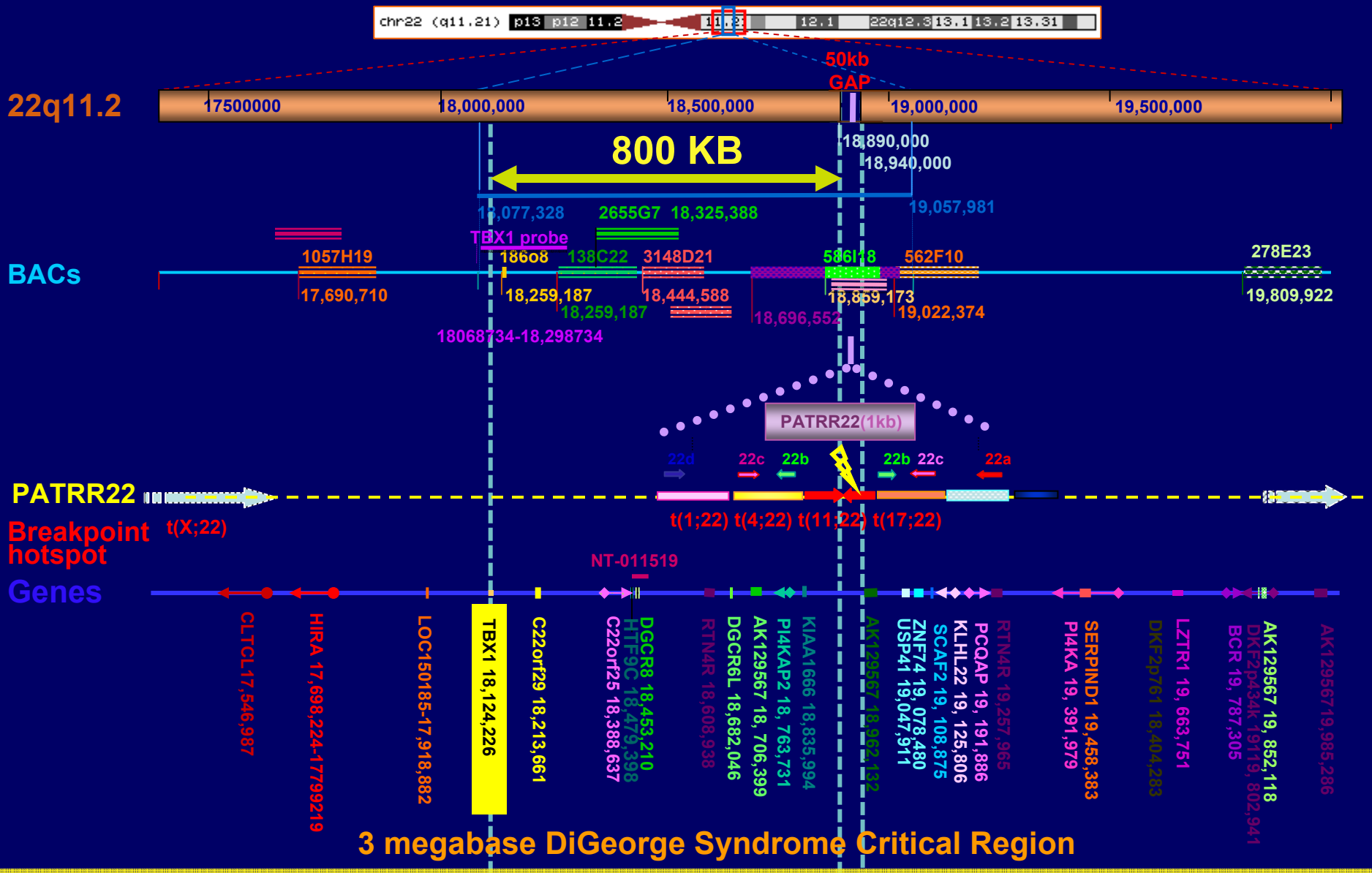
Bold lettering indicates the sequence on der(9)

Localization of Translocation Breakpoints Within PATRR22



9,22 TRANSLOCATION BREAKPOINT IS IN A BREAKPOINT HOTSPOT REGION WITHIN PATRR22

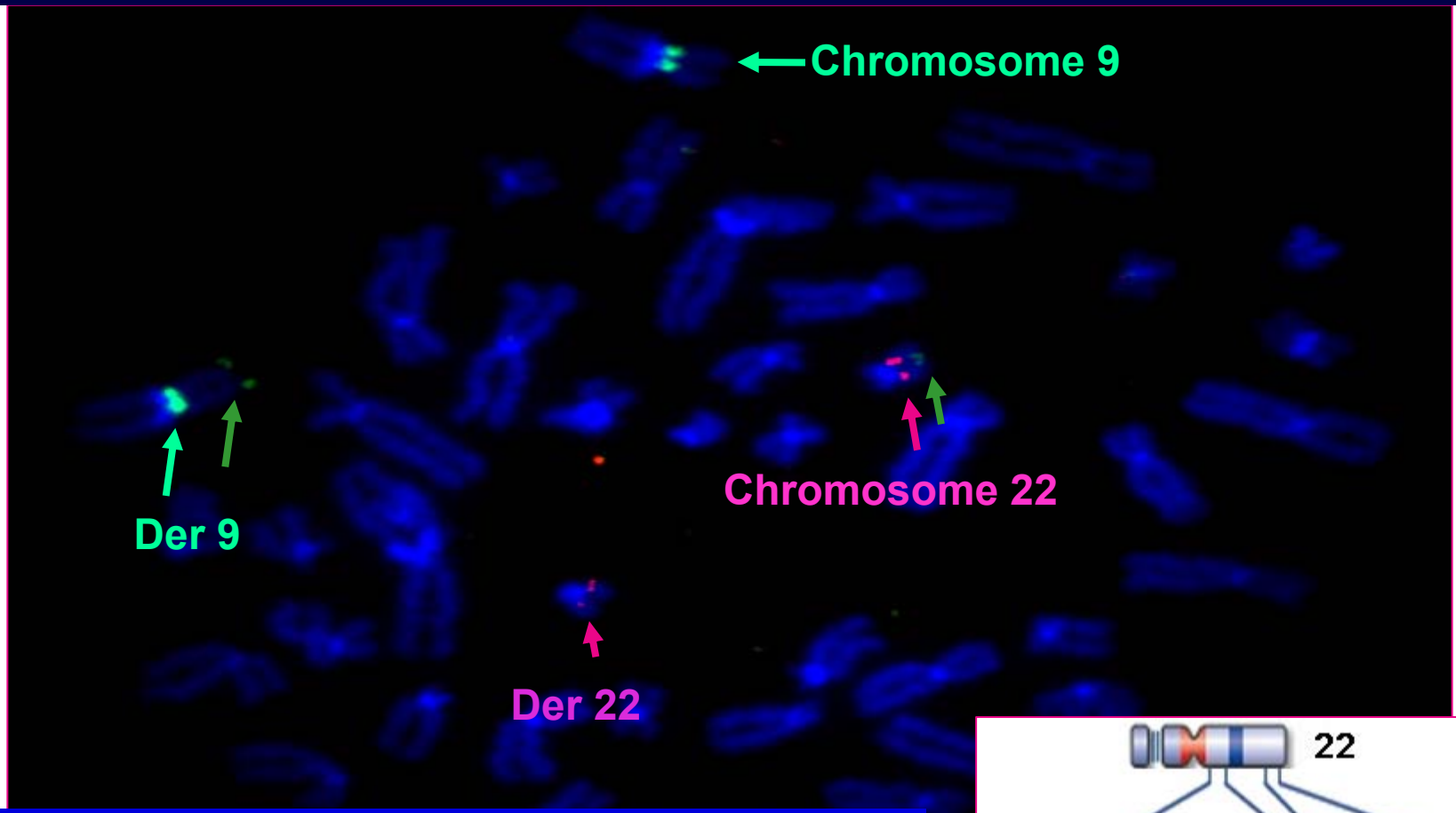
22q11.2



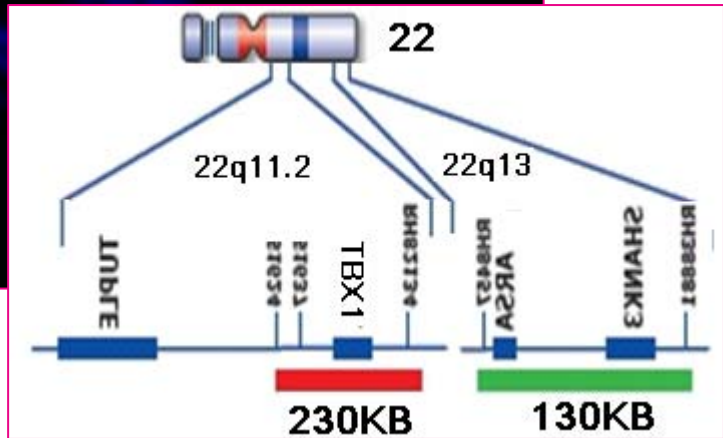
3 megabase DiGeorge Syndrome Critical Region

***TBX1* is 800kb away from the breakpoint in the gap**

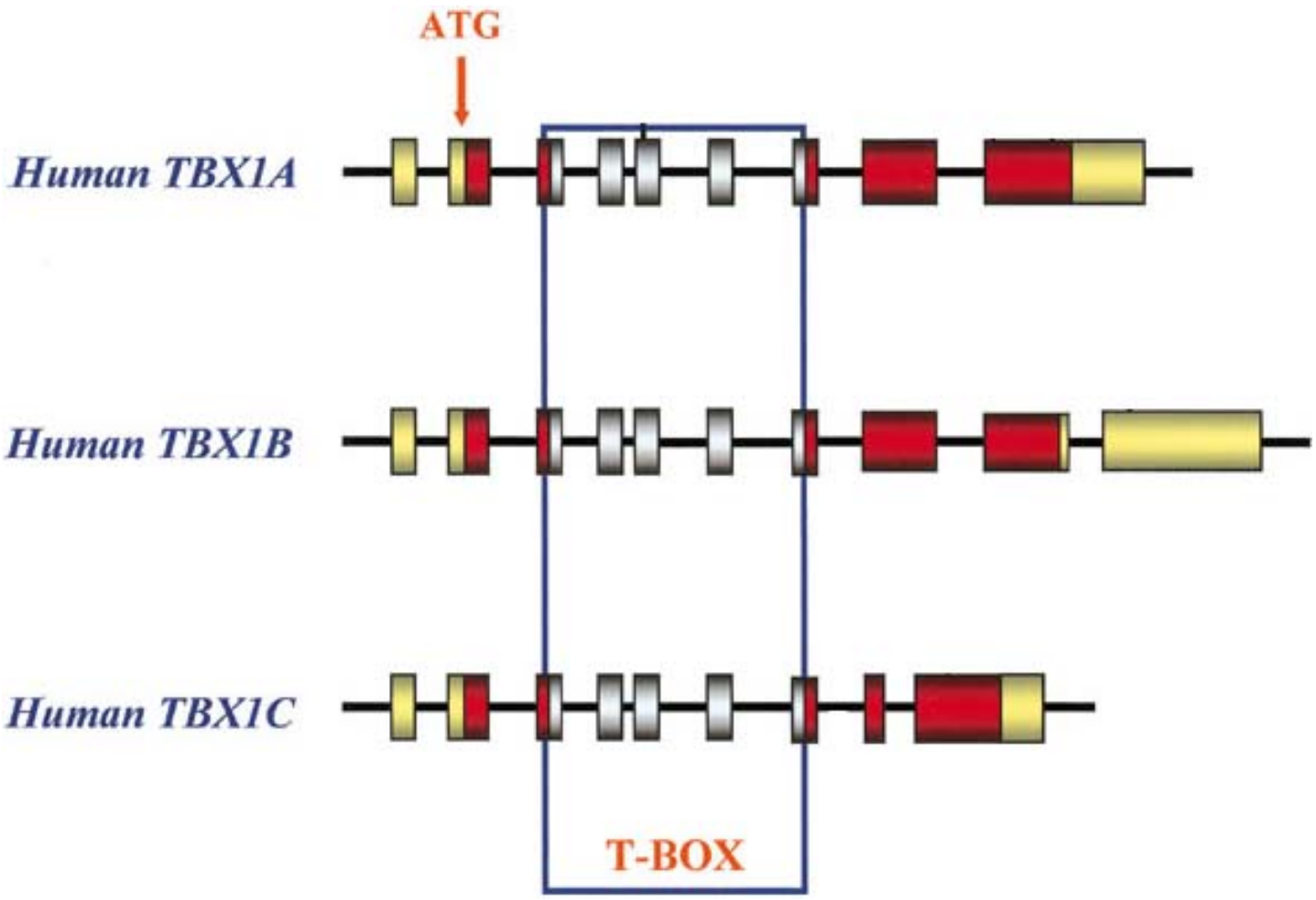
FISH analysis of the translocation on chr22 with TBX1 probe



TBX1 PROBE IS NOT SPLIT BY THE TRANSLOCATION

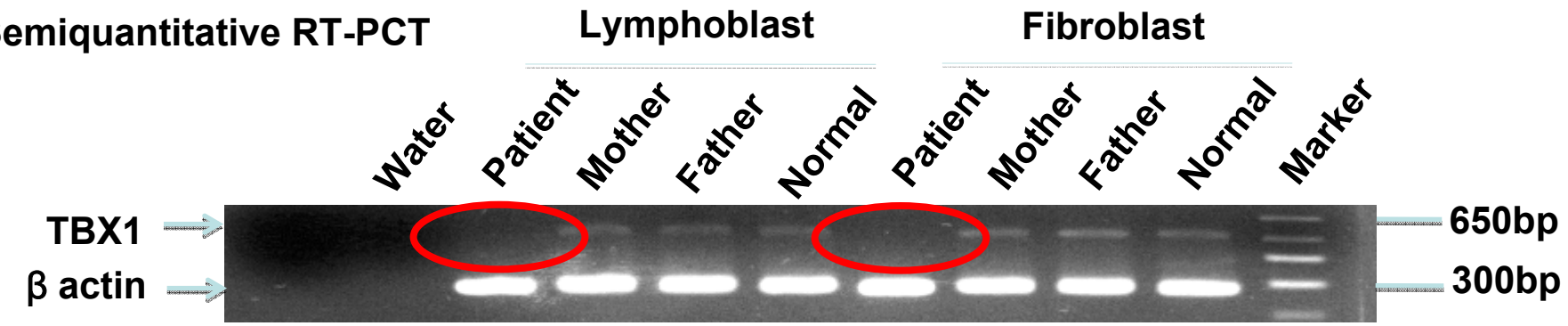


No mutations in promoter or exons in three isoforms of TBX1



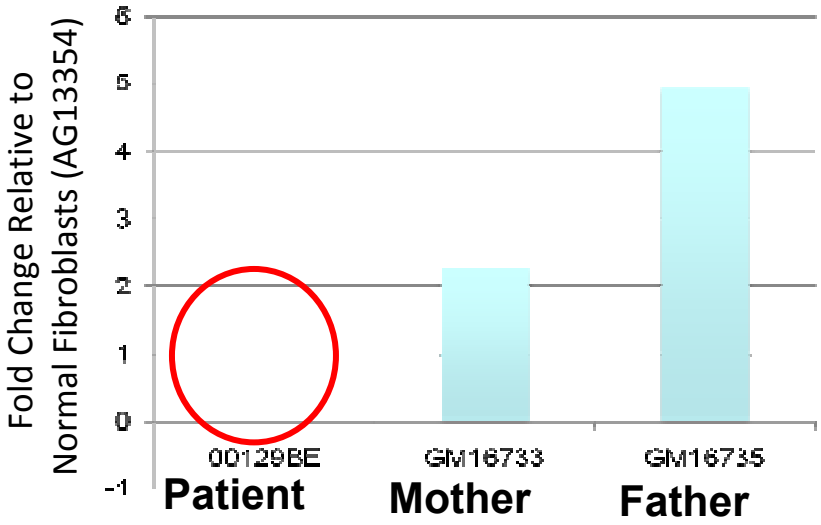
Dramatically reduced TBX1 Expression in Patient's cells

Semiquantitative RT-PCT

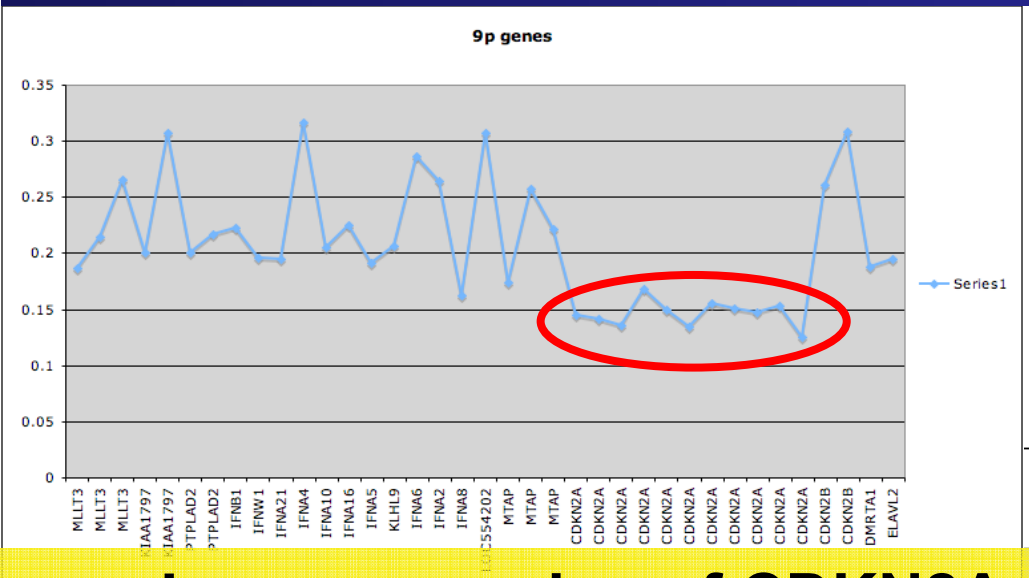


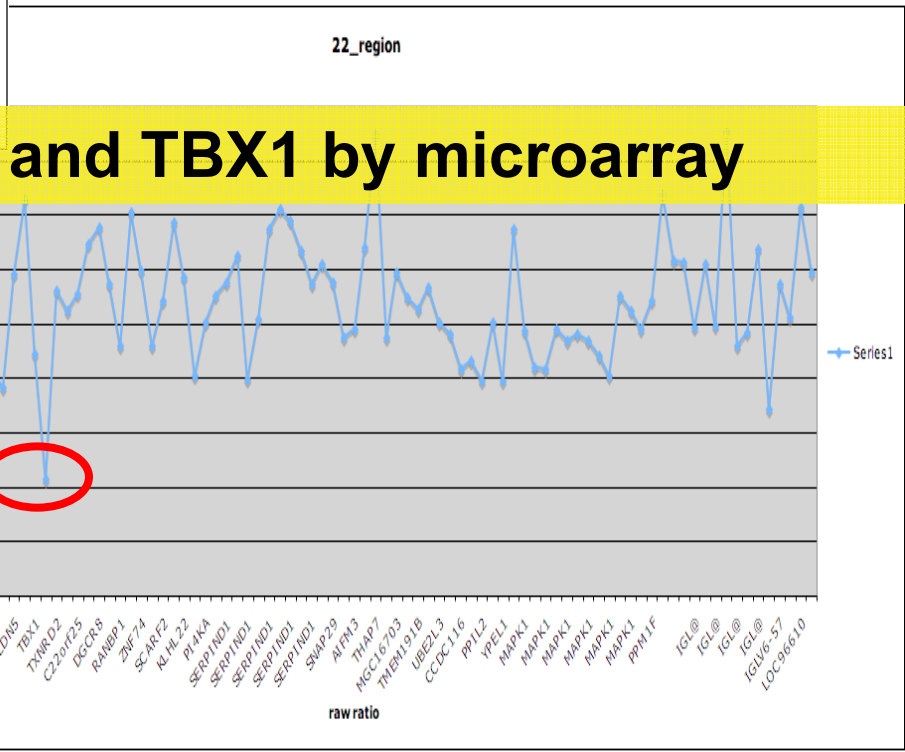
No detectable *TBX1* expression in DD129BE cells by PCR or qRT-PCR

Real Time T-PCT



Microarray Analysis of Expression of the Genes Around the Translocation





Low expression of CDKN2A and TBX1 by microarray

SUMMARY

- **We have demonstrated that the constitutional t(9;22) translocation occurs in AT-rich repeat intervals on both chromosomes 9 and 22**

- **The rearrangement was mediated by a 6 bp CACGTG palindromic sequence between the breakpoints. Chromosome 9 had a deletion of 71 bp in an AT-rich repeat region in intron 1 of *CDKN2A* gene, and chromosome 22 had a deletion of 62 bp in PATRR22 in an uncloned gap.**

- **The melanoma in this patient may have arisen according to the Knudson model in which one copy of *p14arf* is disrupted by the translocation followed by a somatic mutations in the other copy**

CONCLUSIONS

These rearrangements on chromosomes 9 and 22 in patient DD129BE resulted in:

1. down regulation of *CDKN2A* on chr 9 that contributes to the melanoma susceptibility and reduced DNA repair and

2. down regulation of *TBX1* on chr 22 that contributes to his deafness.