



LEIDS UNIVERSITAIR MEDISCH CENTRUM

Determination of Genomic Imbalances by Genome-wide Screening Approaches

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Overview

Introduction/Methodologies

Applications/Results

Conclusion



Introduction/Methodologies

Chromosome based

DNA Based

Applications/Results

Conclusions



Approaches

Chromosome based

*screening for numerical/structural alterations
without previous knowledge*

DNA based

*screening for well defined alterations
screening for unknown genomic imbalances*

L U
M C

Approaches

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screening for unknown genomic imbalances

L U
M C

Whole Genomic Analysis

L U
M C

Karyotyping

- Karyotype provides overview of chromosome constitution (4 - 10 Mb)
- Informative both for numerical and for structural changes
- Highly relevant in pre and postnatal diagnosis (especially in cases with “chromosome phenotype”)
- Used often in hematological malignancies but not in solid tumors (simple *versus* complex rearrangements)

L U
M C

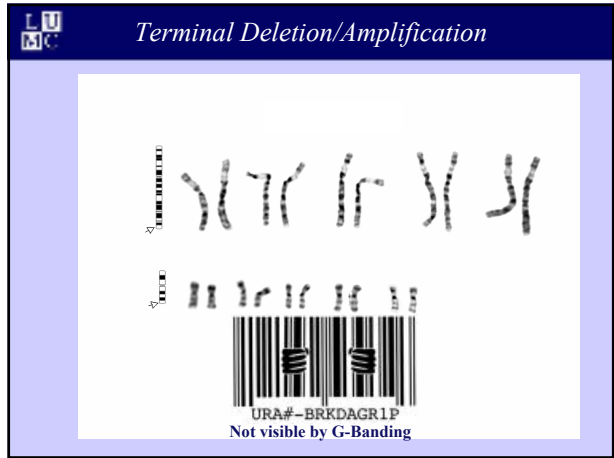
Metaphase Study

Metaphases (cell division):

- no paraffin fixed material
- no frozen material
- fresh material: often hard to cultivate
- cell cultivation from heterogeneous population >>>> selection

Are the karyotyped cells representative of the original population?

Change in banding pattern (barcodes) or length is necessary

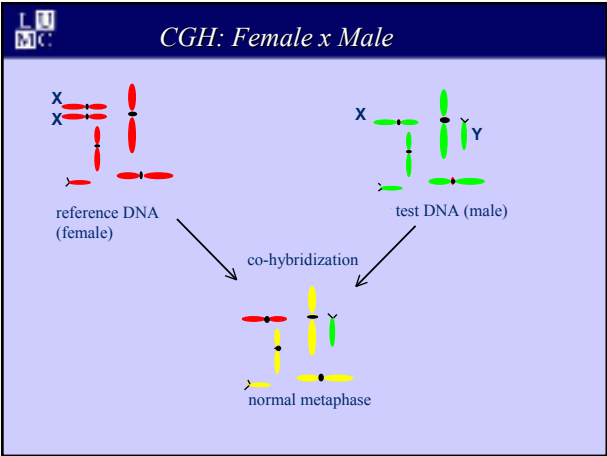
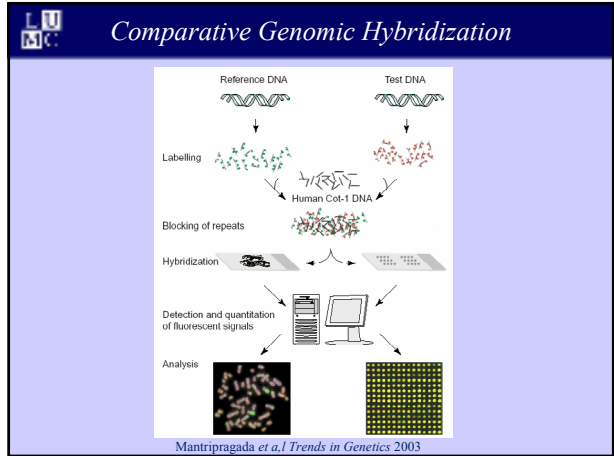


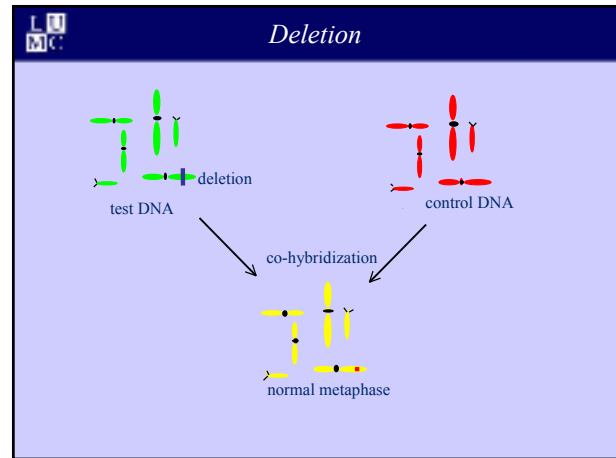
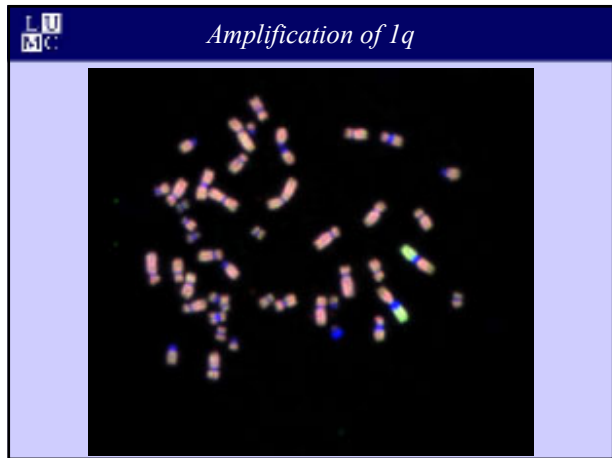
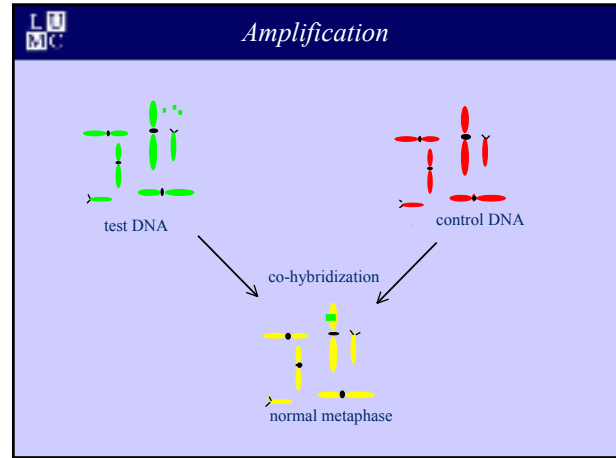
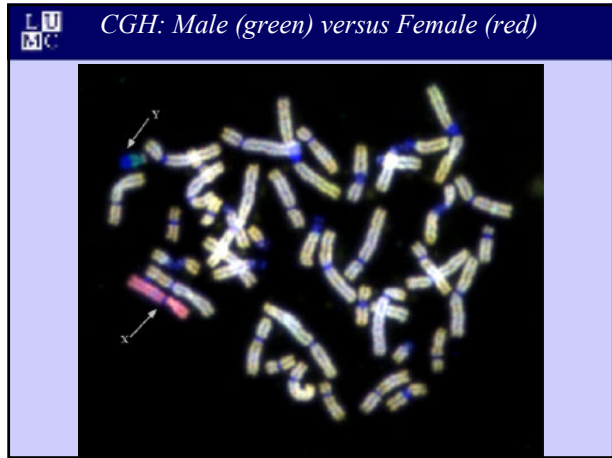
LU
MC

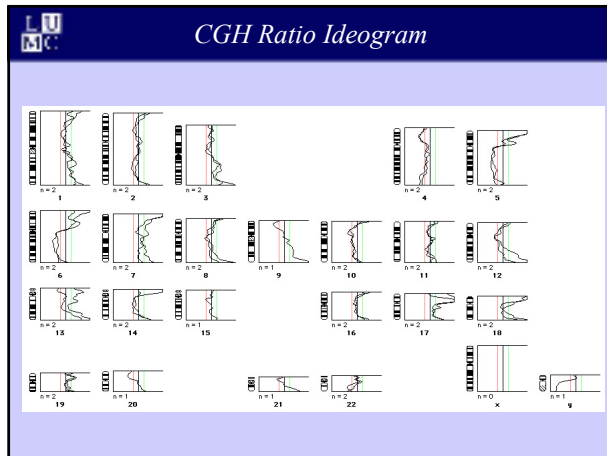
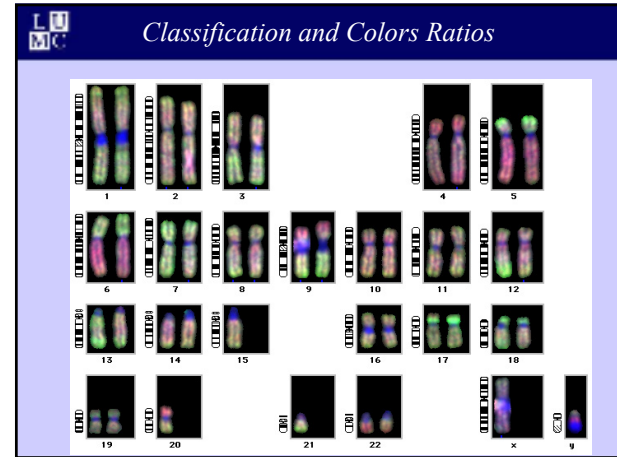
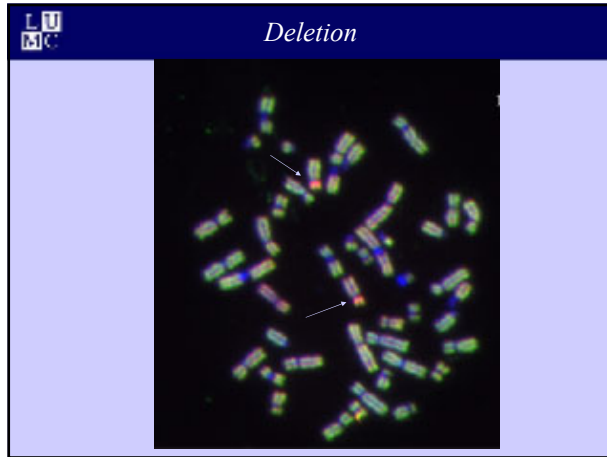
Approaches

Chromosome based
screening for numerical/structural alterations
without previous knowledge

DNA based
screening for well defined alterations
screening for unknown genomic imbalances
CGH and array-CGH



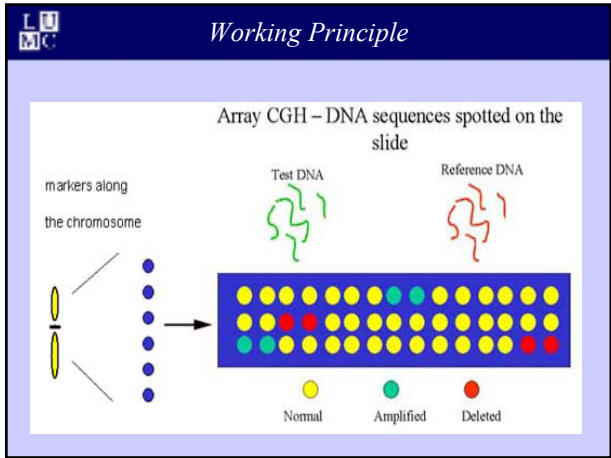
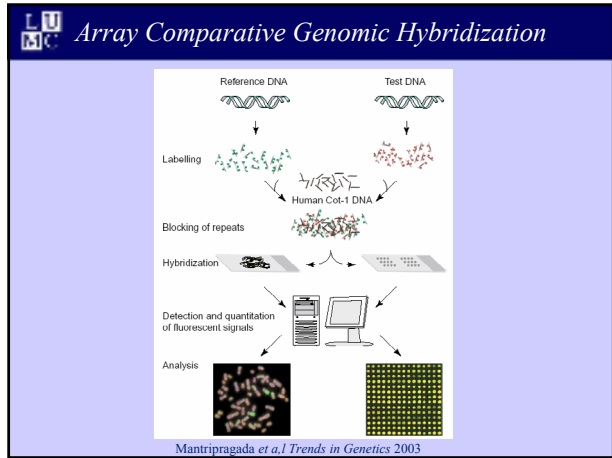




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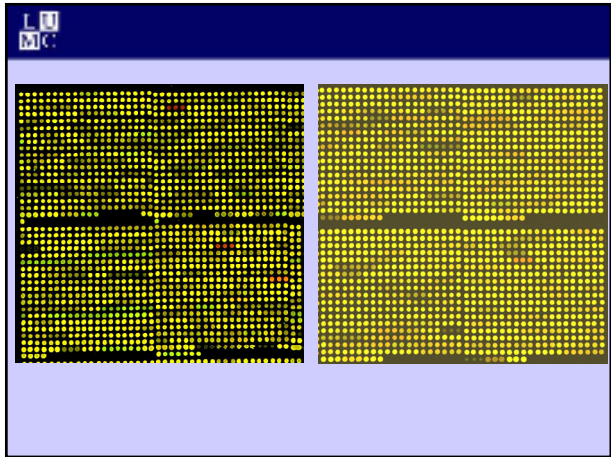
CGH: No information on absolute copy number

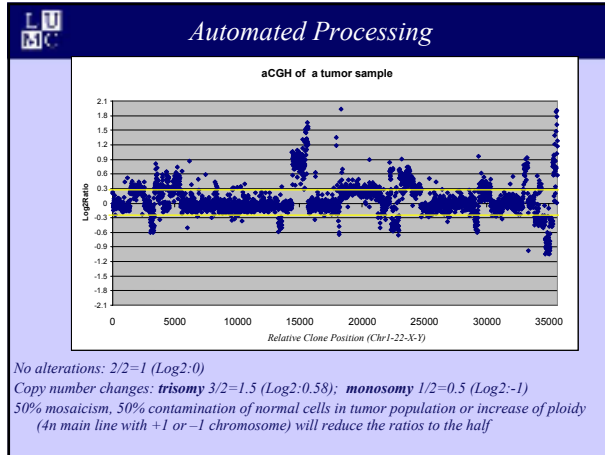
*Comparative information:
What is deleted or amplified compared to the total genome (red/green ratio on a metaphase)*



Array Design

- 3500 BACs/PACs: ~ 1Mb spaced on the genome including:
 - Cancer,
 - Microdeletion syndrome,
 - Subtelomeric probes
- Triplicates of 3500 probes (10500 spots/slide)
- PCR in house
- Arraying using LGTC facility (joint effort Rosenberg/Fodde)
- Clones were obtained from the Sanger Center





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Conclude aCGH

aCGH: No information on absolute copy number

Comparative information:

- What is deleted or amplified compared to the total genome (red/green ratio over a set of BAC clones)
- High spatial resolution regarding clone position
- Suited for semi-automated analysis
- Direct link to *in silico* data mining

LUMC

Introduction/Methodologies

Applications/Results

- Mental retardation**
- Tumor cytogenetics**

Conclusion

LUMC

Diagnosis of Mental Retardation

- **Mental impairment affects ~ 3% of the general population**
- **Genetic abnormalities are diagnosed in less than half of all mental cases**
 - No detection of carriers
 - No risk of recurrence (empirical)
 - No pre-natal diagnosis

Supported by LUMC "Doelmatigheidsgrant"



Alterations Associated with MR

Genomic imbalance inherited from parents carrying balanced rearrangement

Interstitial duplication

Interstitial deletion

Balanced rearrangements (inversions, translocations) via gene disruption

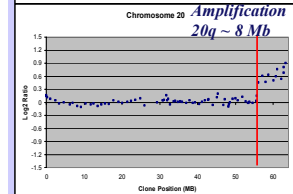
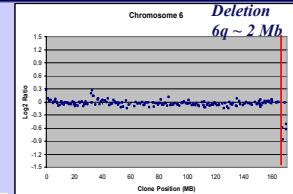
Microdeletions, point mutations

Other/Unknown



Terminal Deletion/Amplification Pattern

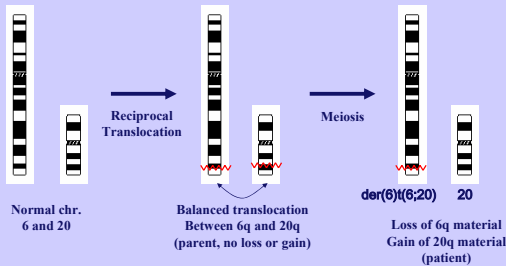
The pattern indicates that one of the parents must be a carrier of a balanced translocation (6;20).



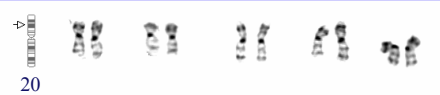
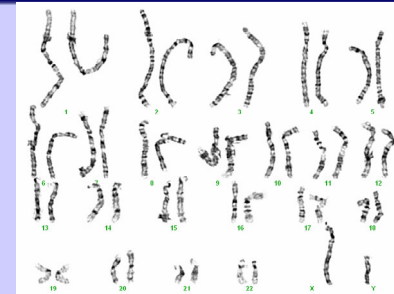
Not visible by G-Banding

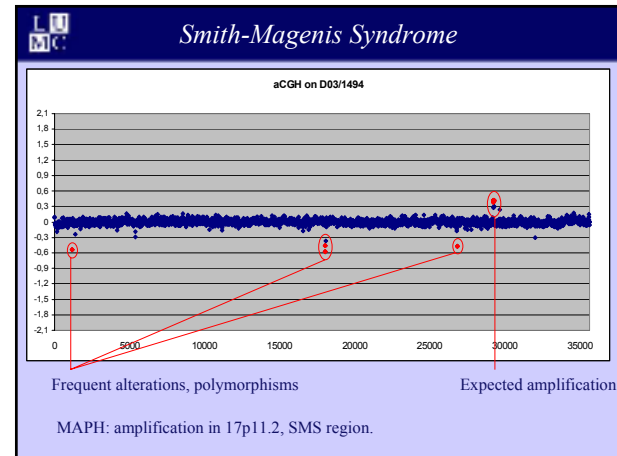
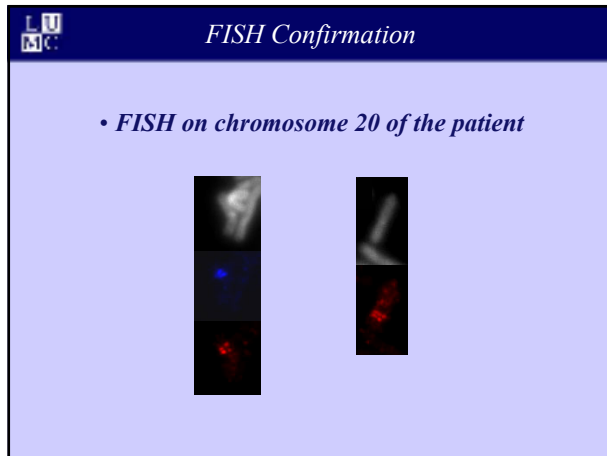
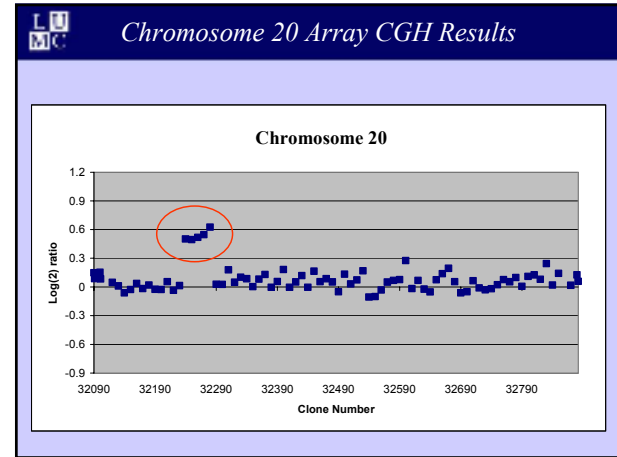
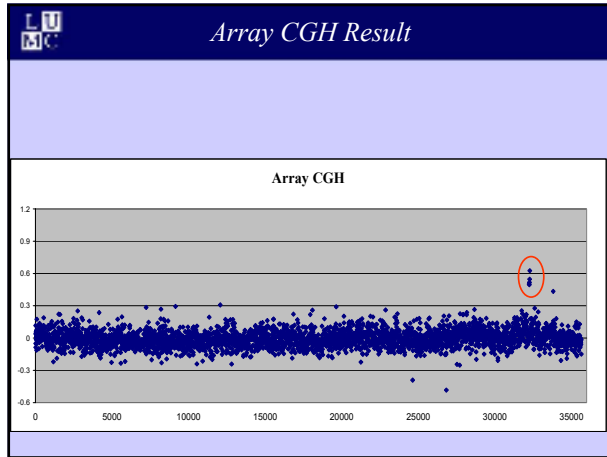


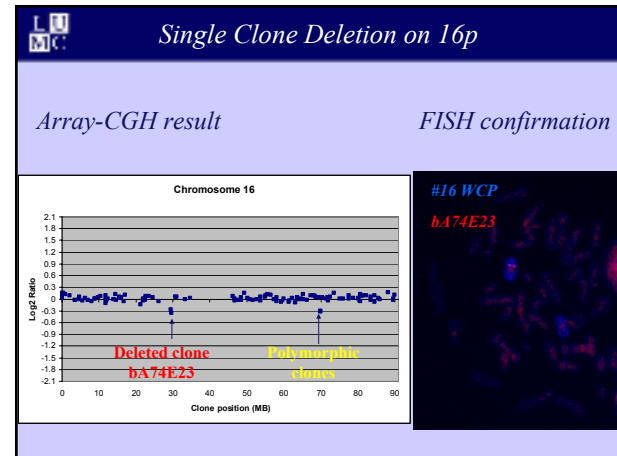
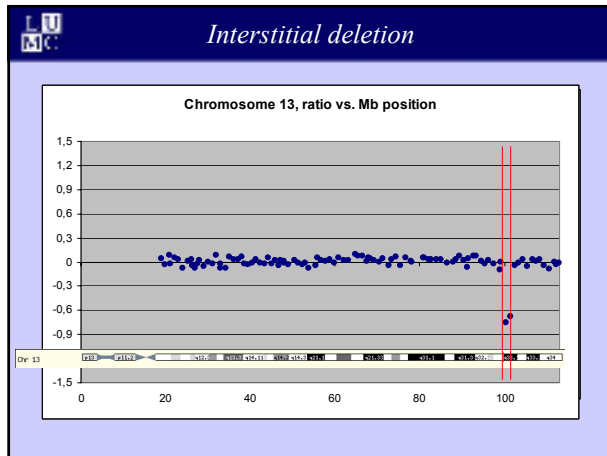
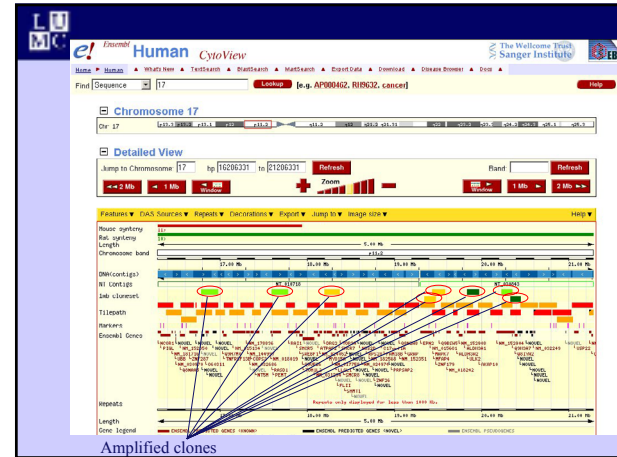
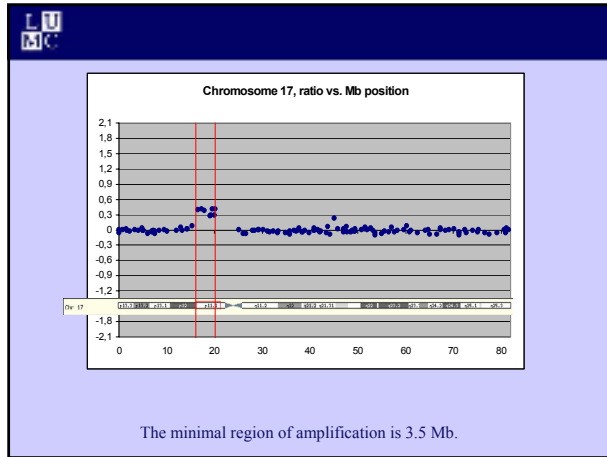
The deletion/amplification pattern can be inherited from a balanced carrier.



Patient with Suspected Alteration on chromosome 20p









Applications for Tumor Cytogenetics

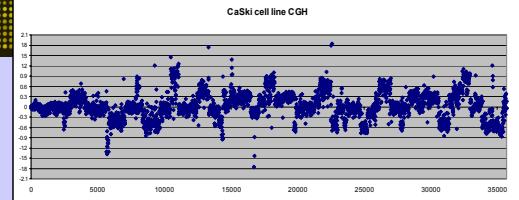
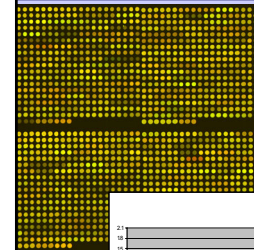
- *Suited for the identification of breakpoints in tumors with complex rearrangements*
- *Detection of small deletions/amplifications*
- *Suited for archived materials*
- *Not suited for truly balanced rearrangements*



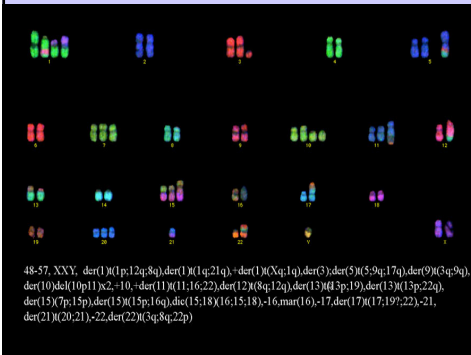
CaSki Cell Line,

Cervical cancer cell line:

70-76 3n, -X,-X,der(X)(X;22),+(?(X)x2,-1,+(?(1)x2,-2,-2,+(?(2)x5, del(3)x2, der(3)(3;5), +der(7)(7;5;3;5;3;5;3), -4,-4, +(7;4)x2,-5, del(5), +(7;5)x3, +del(6)x2,+(7;6), -7,-7,-7, +(7;7)x8,-8,-8, +(7;8)x3, +(7;9), der(10)(10;20)(p11.2;q2), +der(10)(10;11)(p11.2;q7), der(10)(7;10)(q11.2), -11,-11, del(11), +(7;11)x3,-12,+(7;12)x2,-13,-13, der(13)(7;13), +(7;13;15), +(7;13)x2,-14,+(7;14)x3,-15,-15,-15, +(7;15)x2, +(7;16)x2, del(17),+(7;17)x2,-18,+(7;18), -19, +(7;19)x2, +del(20), +(20;22), +(7;20)x2,-21,-21, +(7;21)x3,-22,-22, t(7;22), inc



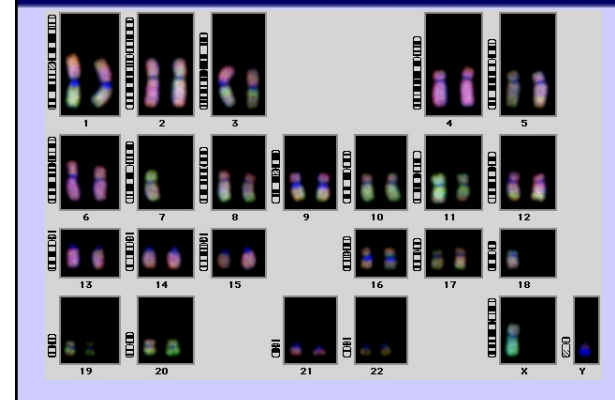
Multicolor-FISH of a Sarcoma Case

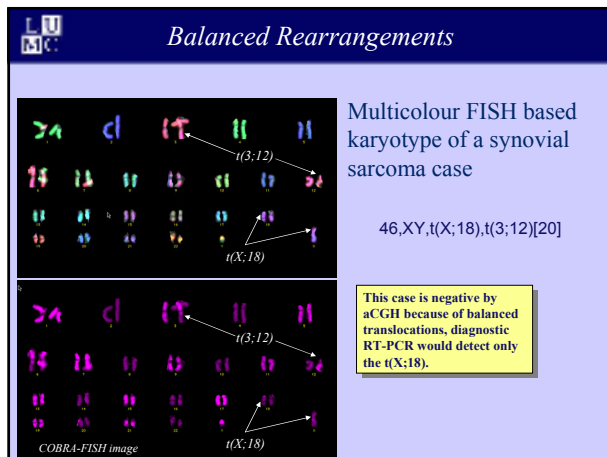
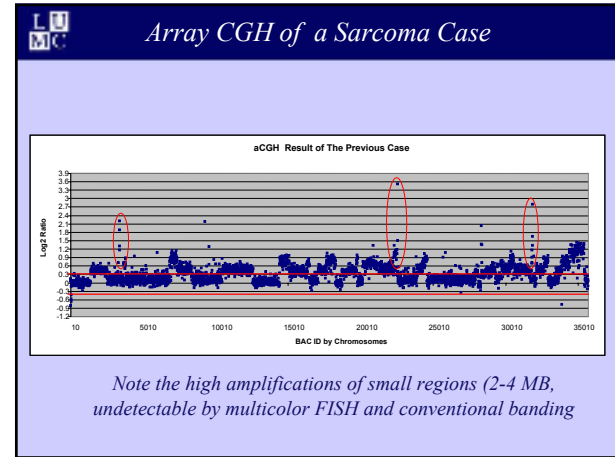
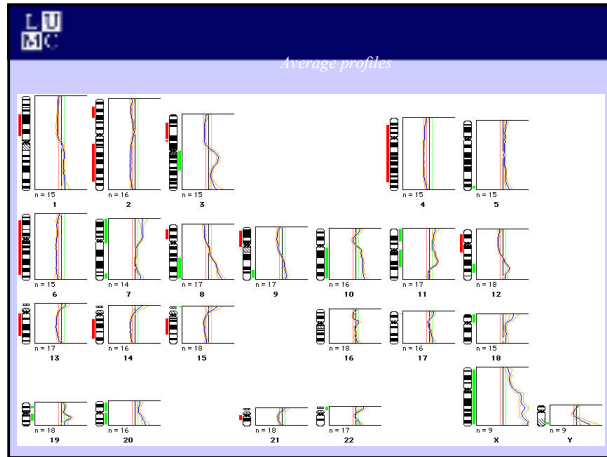


48-57, XXY, der(1)(p13q;8q), der(1)(q;21q), +der(1)(Xq;1q), der(3)der(5)(5;9q;17q), der(9)(9;9q;9q), der(10)del(10)(p11)x2,-10,-der(11)(11;16;22), der(12)(8q;12q), der(13)(d13p19), der(13)(13p;22q), der(15)(7p;15p), der(15)(15p;16q), del(15;18)(16;15;18), -16, mar(16), -17, der(17)(17;19;22), -21, der(21)(20;21), -22, der(22)(3q;8q;22p)



CGH hybridization of L948



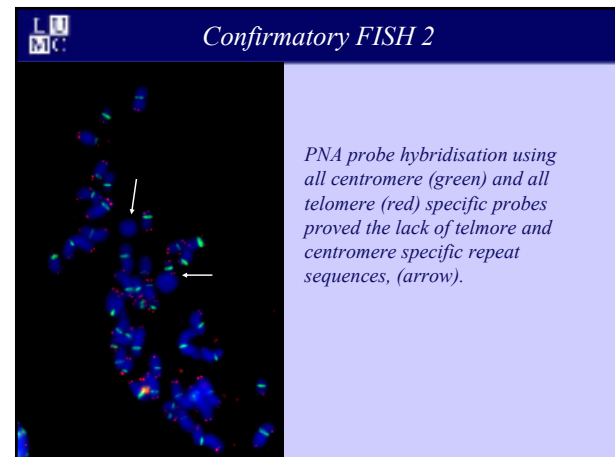
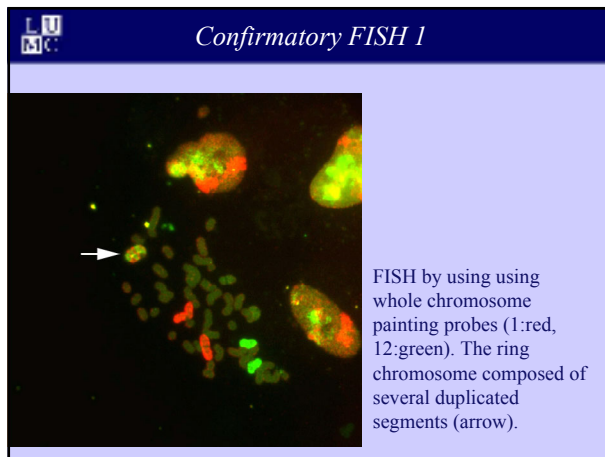
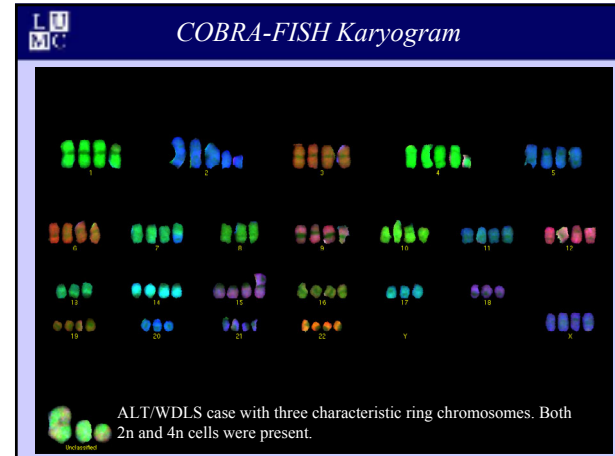
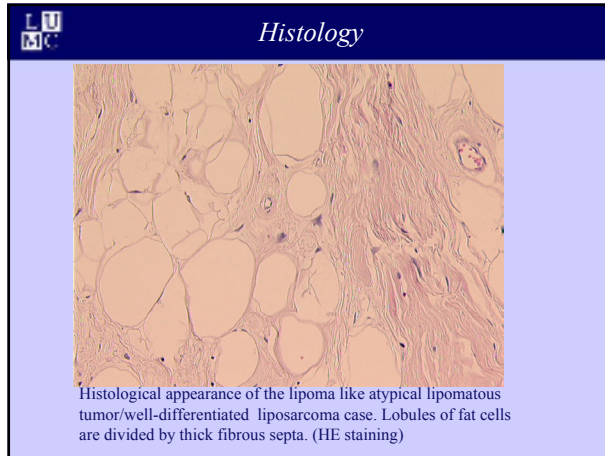


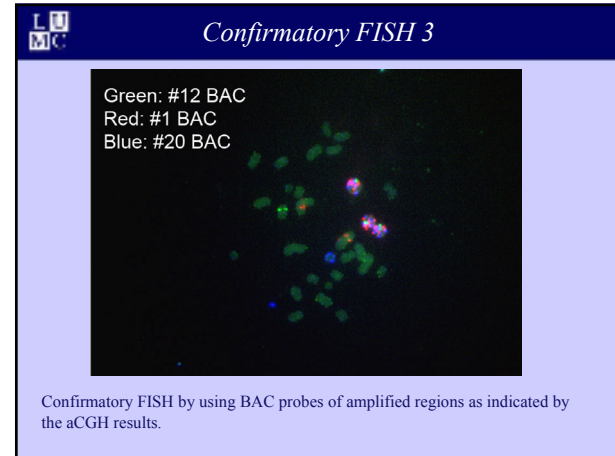
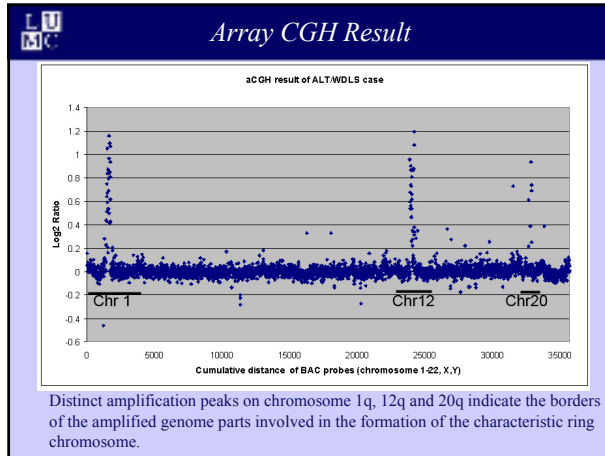
L U
M C

aCGH as Diagnostic Aid in Histopathology

A differential diagnostic problem is the separation of lipoma (especially necrotic) from atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDLs).

The later is characterized by the presence of a supernumerary ring chromosome or giant marker consists of segments of 12q and other chromosome such as 1q.





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Conclusion

Conclusion

Array CGH is a powerful tool for semi-automated screening of genomic imbalances both in patient with mental retardation and in tumors.

The use of aCGH in diagnosis of MR+DM was conclusive in 30% of the analyzed 80 samples.



MCB, Cytochemistry
& Cytometry

KGCL

Pathology

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