



KATHOLIEKE UNIVERSITEIT  
**LEUVEN**

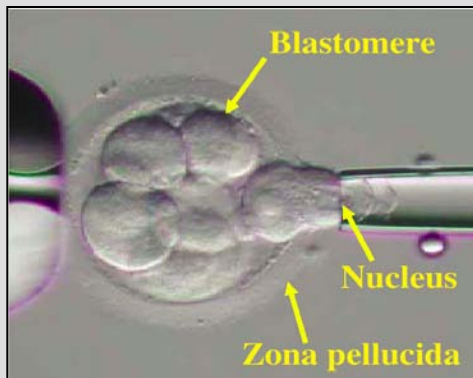
# Genome wide CNV and SNP detection in cleavage stage embryos uncovers chromosomal instability

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K.U.Leuven, Belgium

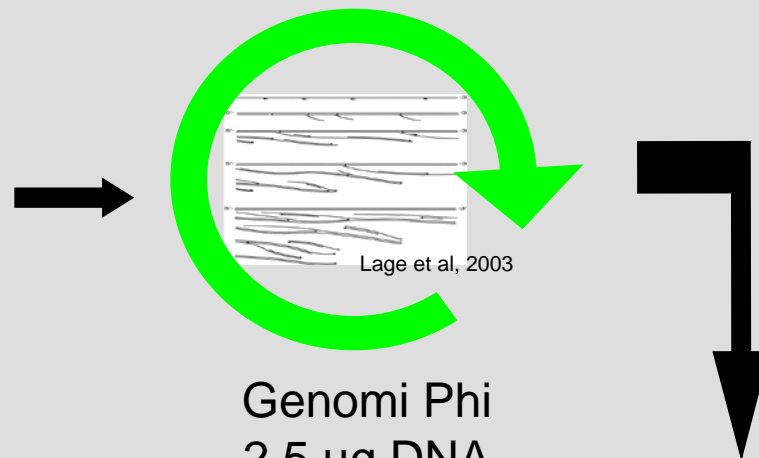
ESHRE

Amsterdam, July 2, 2009

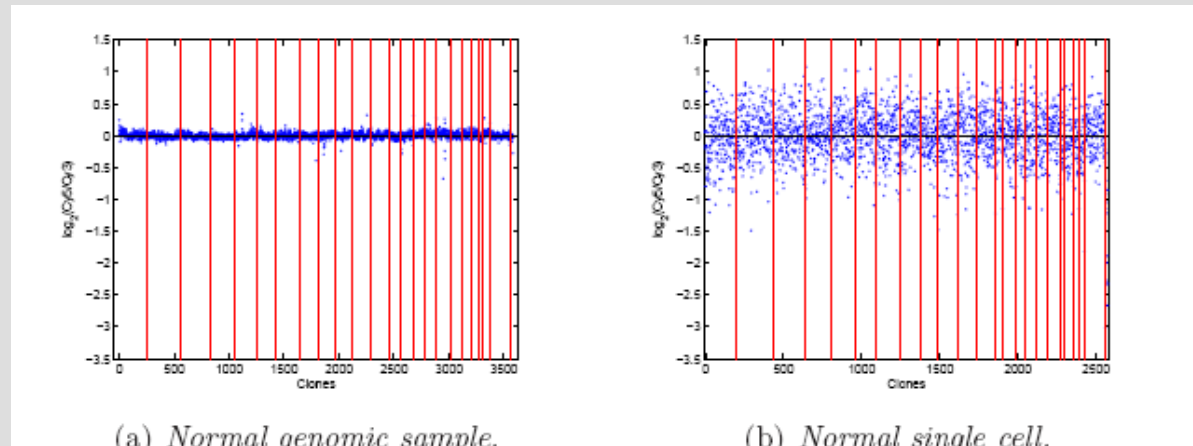
# Single cell array comparative genomic hybridization using arrays



Single blastomere  
7 pg DNA



Genomi Phi  
2.5 µg DNA

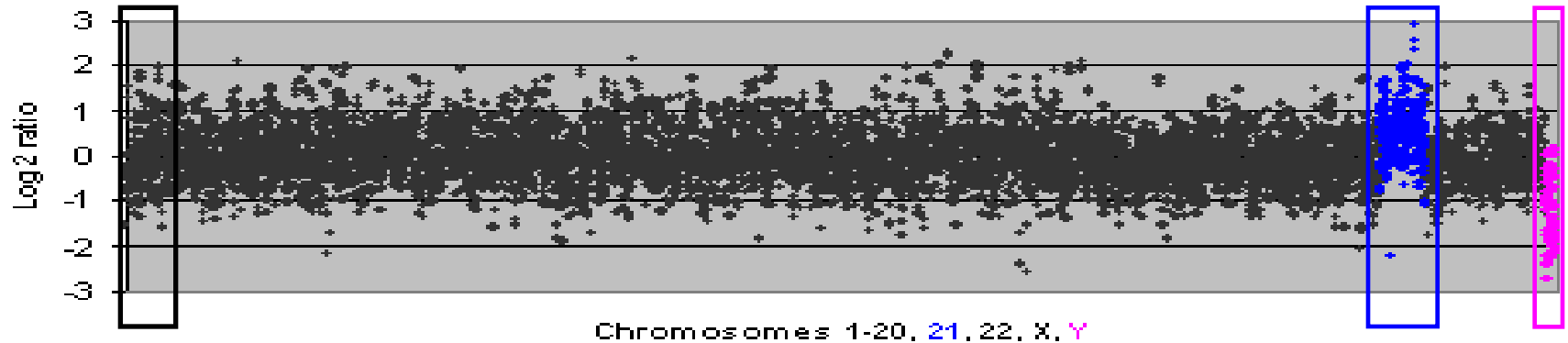


(a) Normal genomic sample.

(b) Normal single cell.

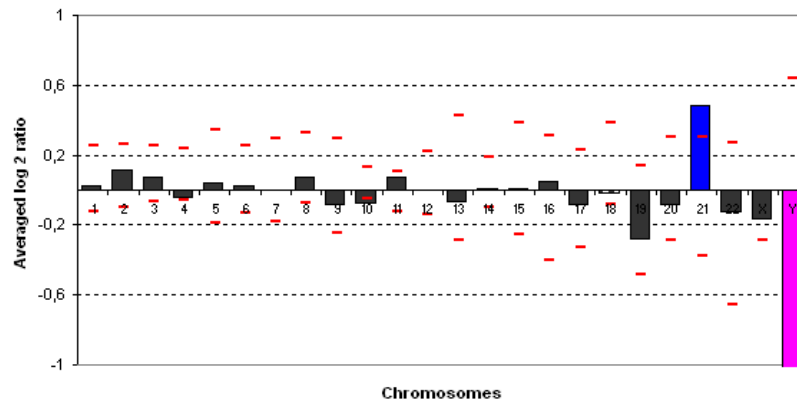
# Single cell array CGH: Large segments

47,XX,+21 vs 47,XXY

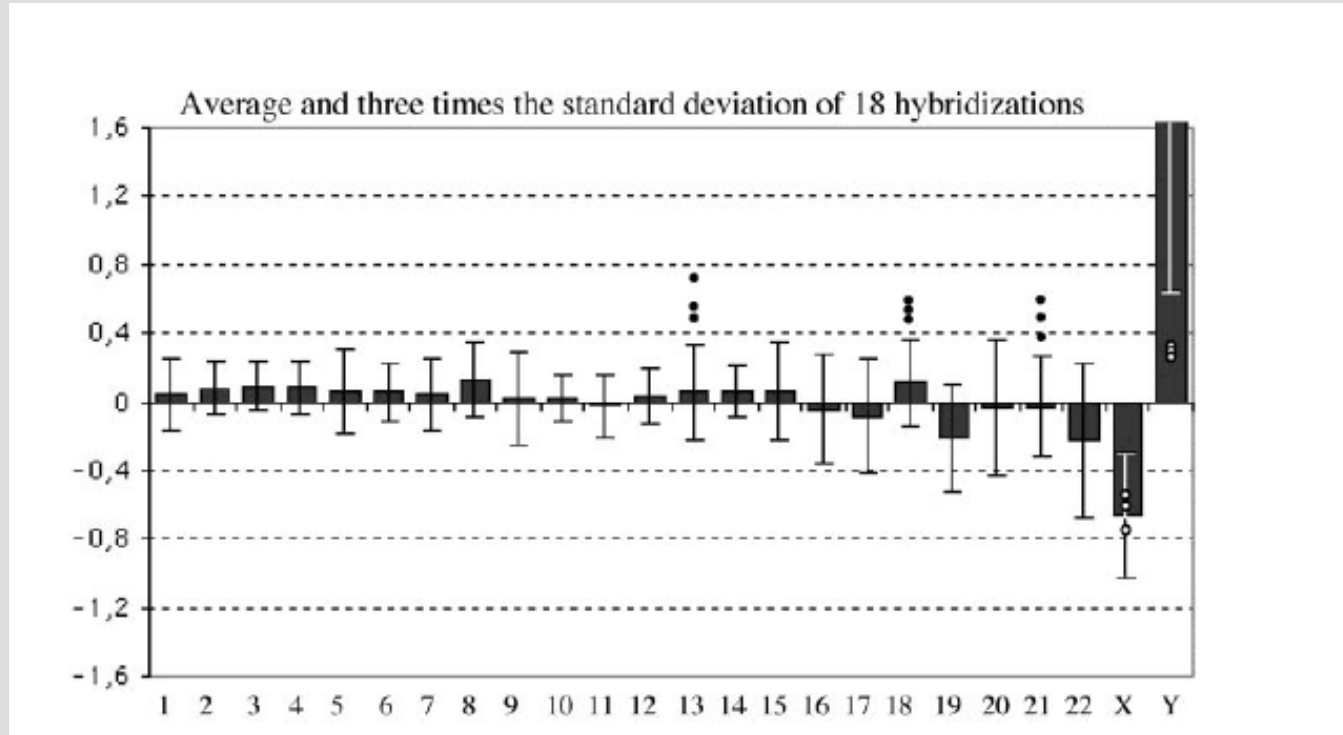


Chromosome 1

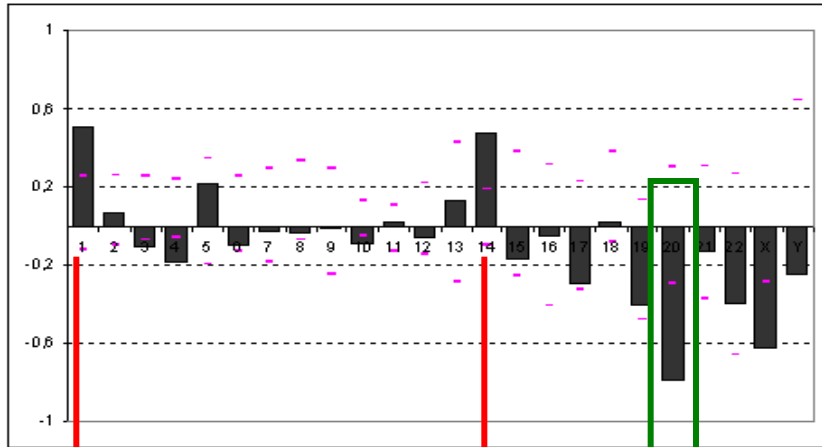
47,XX,+21 vs 47,XXY



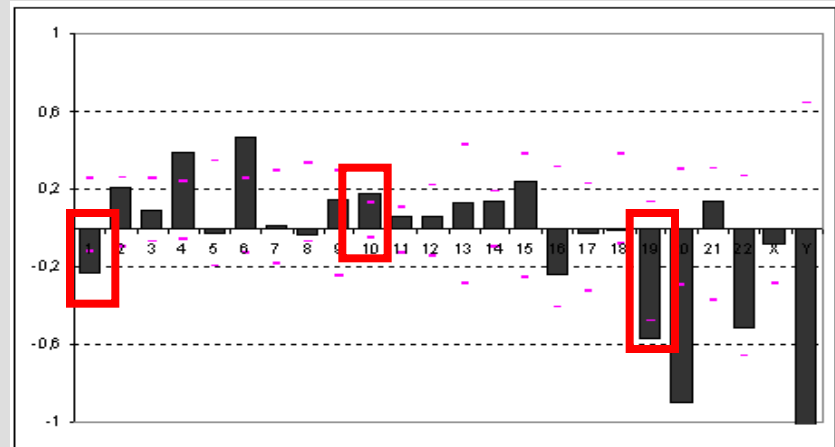
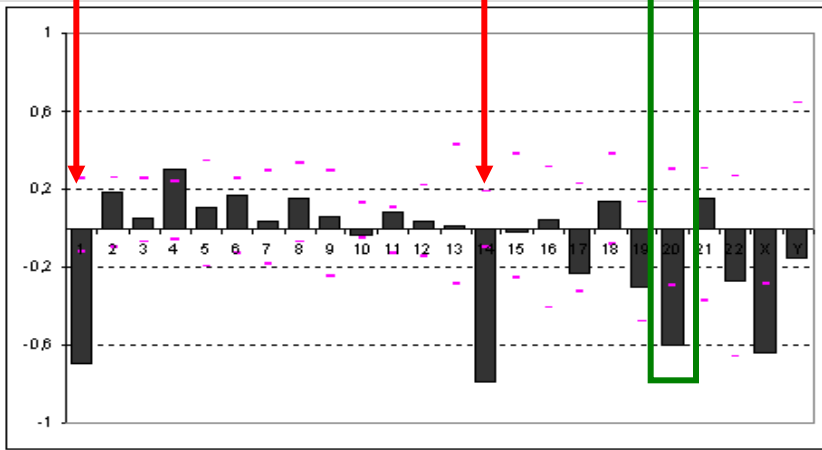
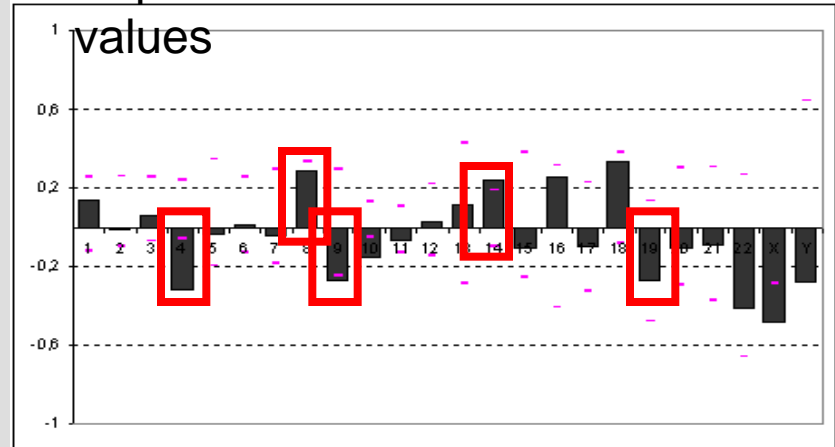
# Single cell array CGH: aneuploidy



# What is the accuracy?



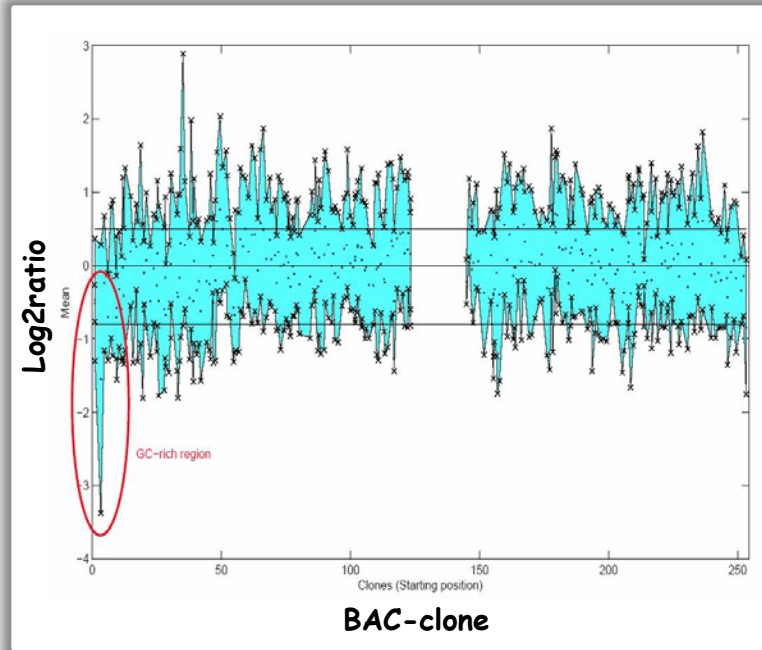
Reproducible but intermediate



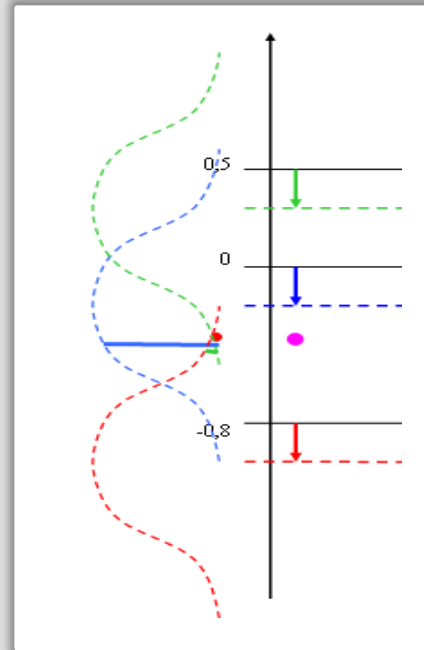
Non disjunction ?

Anaphase lag ?

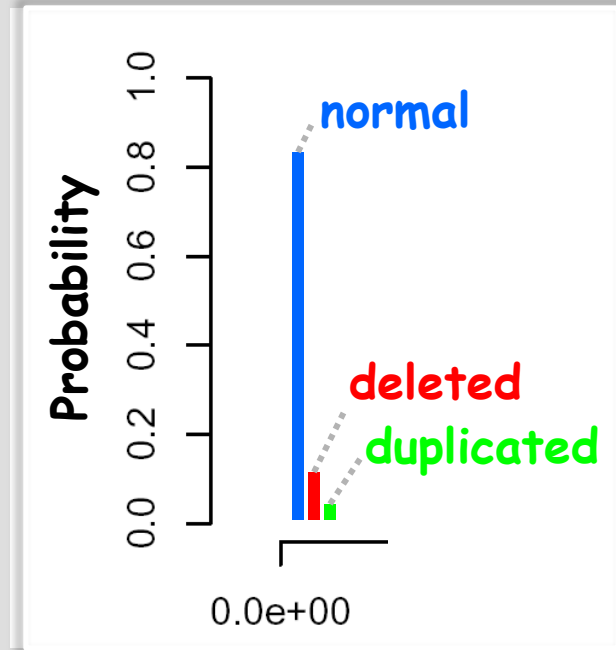
# A novel statistical method that calculates likelihood estimates on imbalances detected with SC BAC-arrayCGH



Clone specific variability



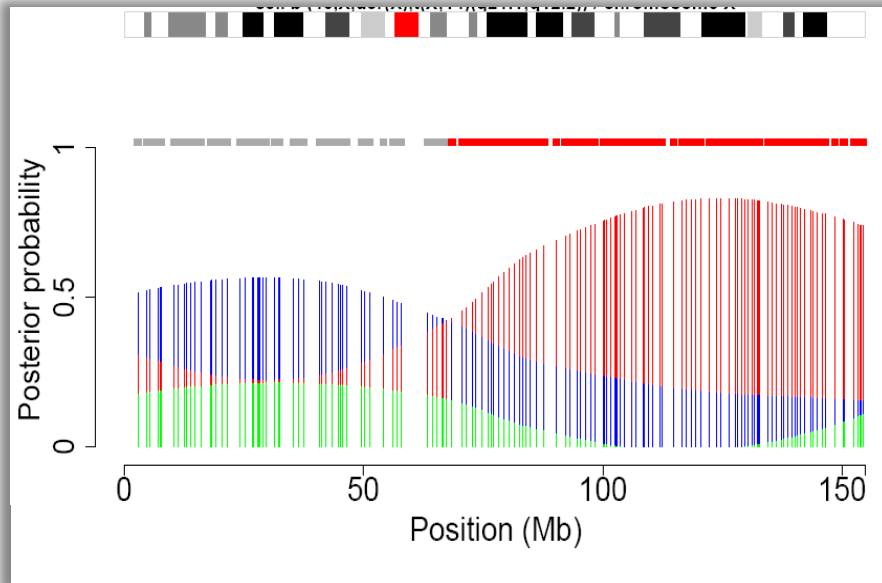
Clone specific correction



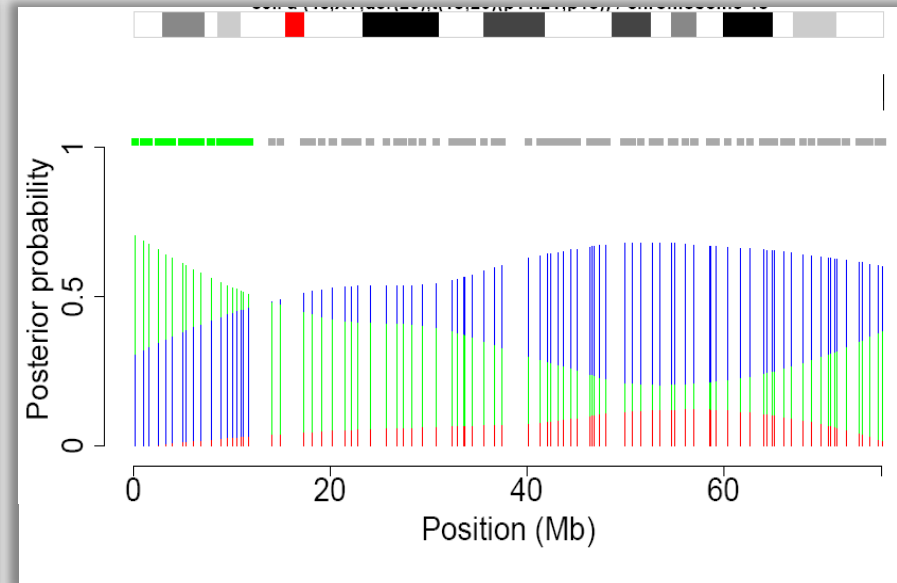
Clone specific likelihood estimate

# Validation using single-cells with known imbalances

Xq terminal deletion (58 Mb)

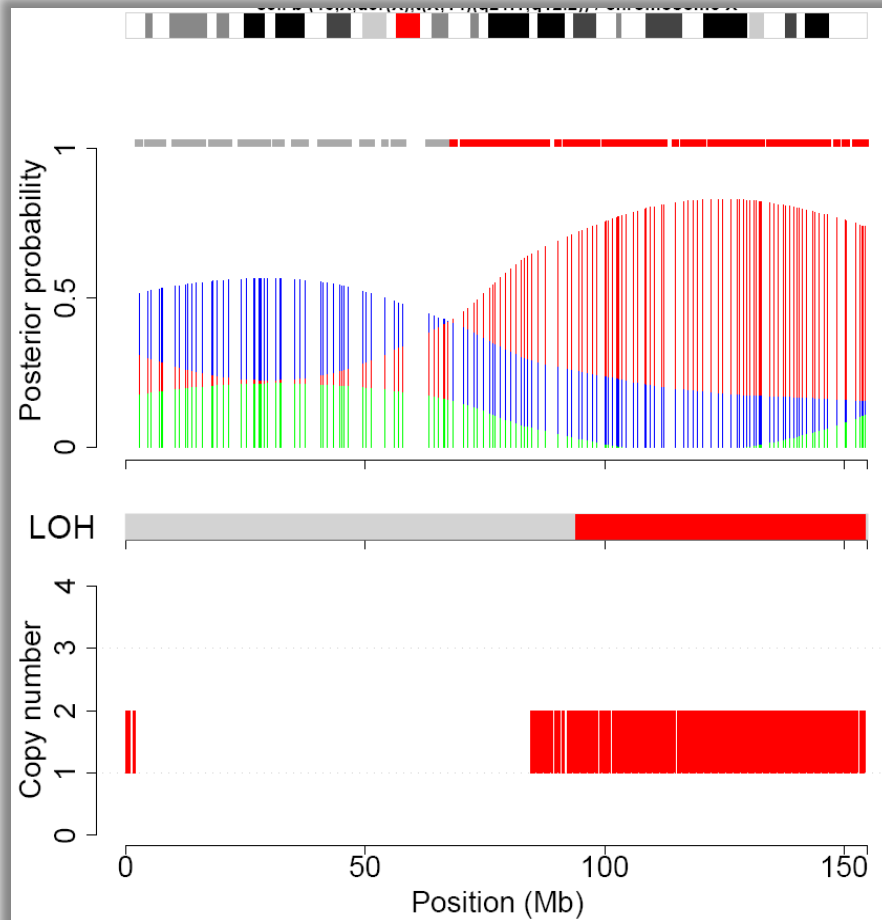


18p terminal duplication (9.3 Mb)

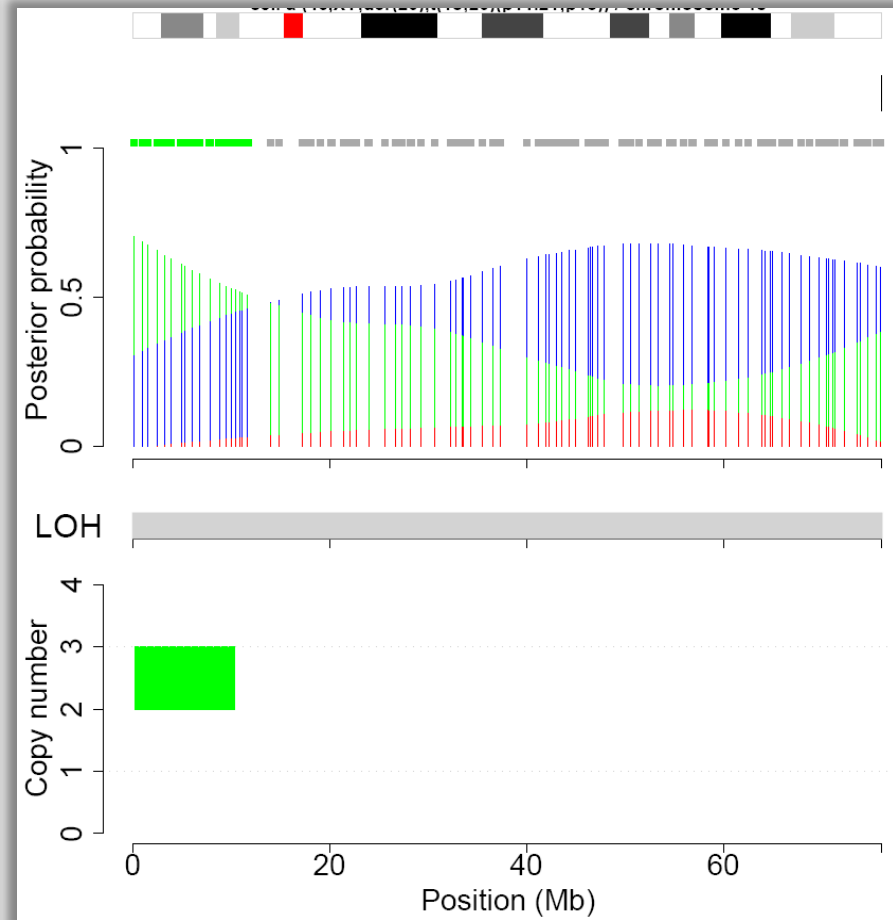


# Validation using single-cells with known imbalances

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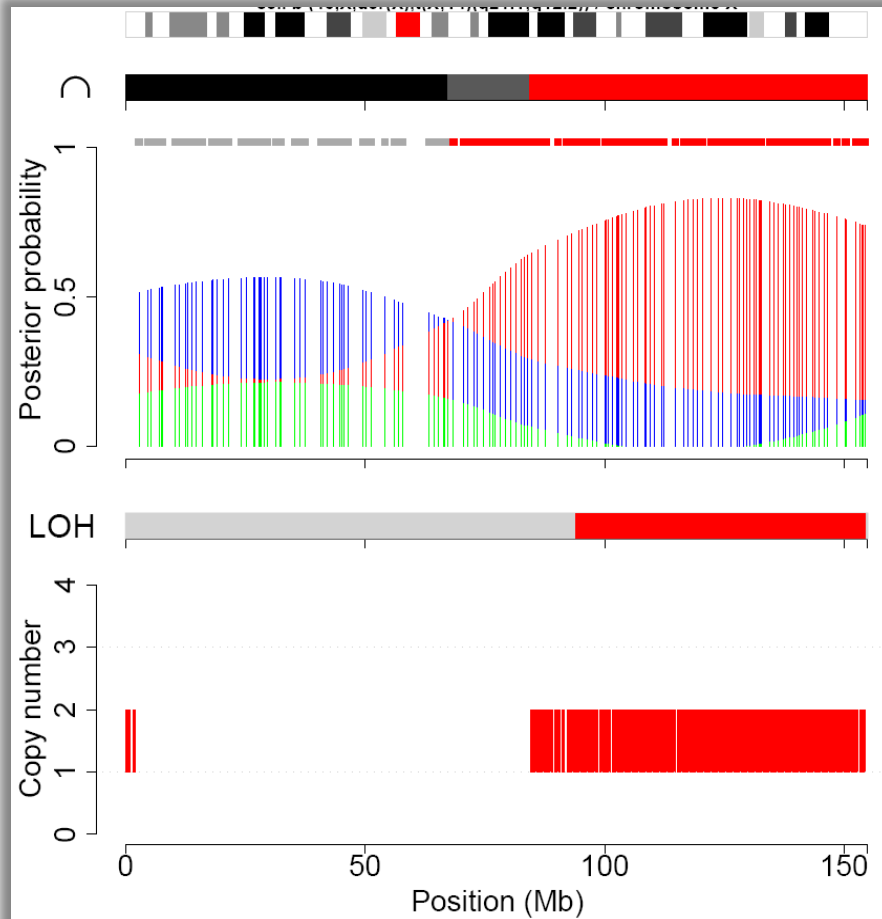
18p terminal duplication (9.3 Mb)



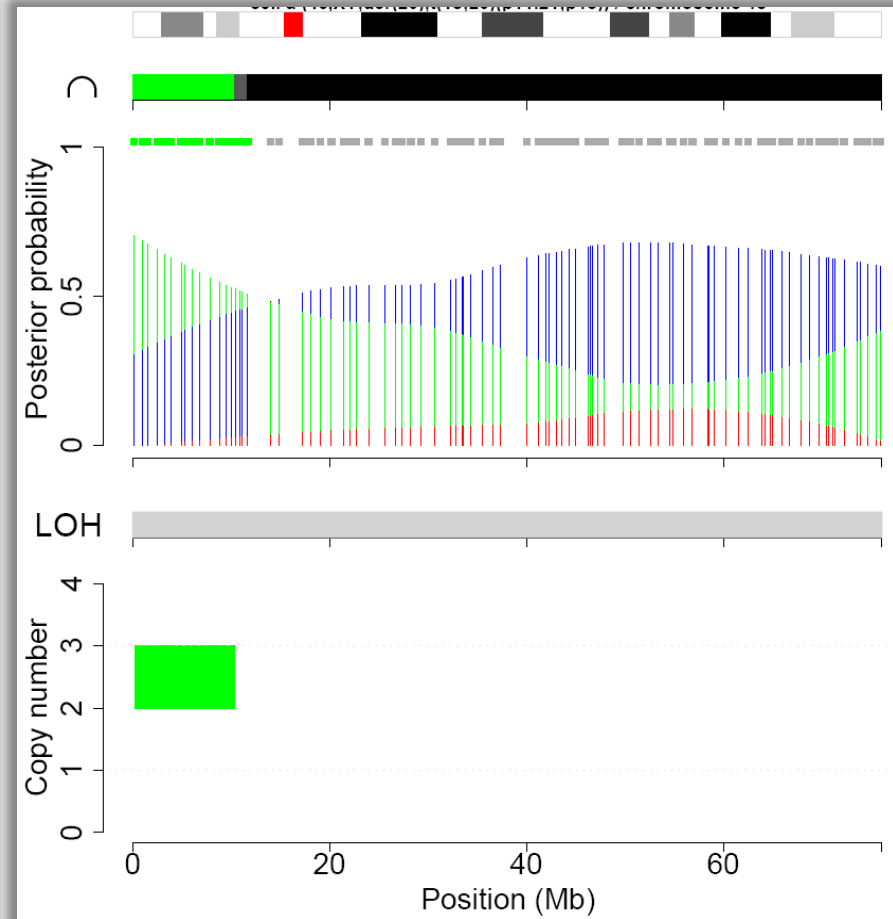


# Validation using single-cells with known imbalances

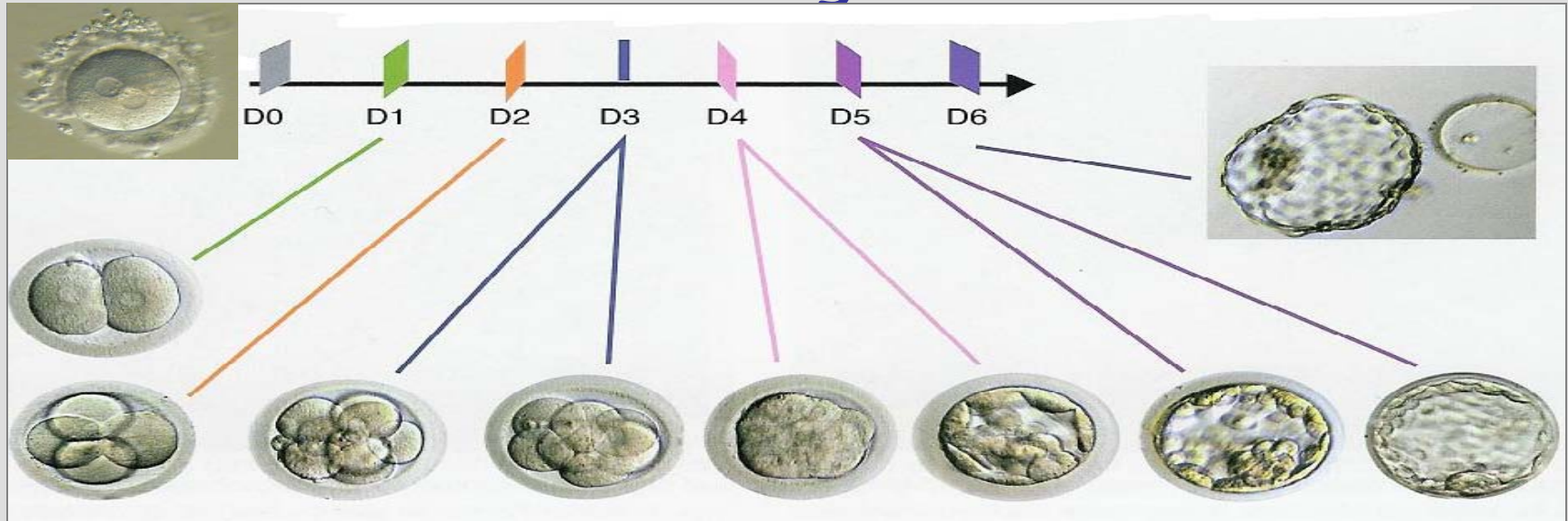
Xq terminal deletion (58 Mb)



18p terminal duplication (9.3 Mb)



# Analysis fertilised oocytes in pronuclear stage

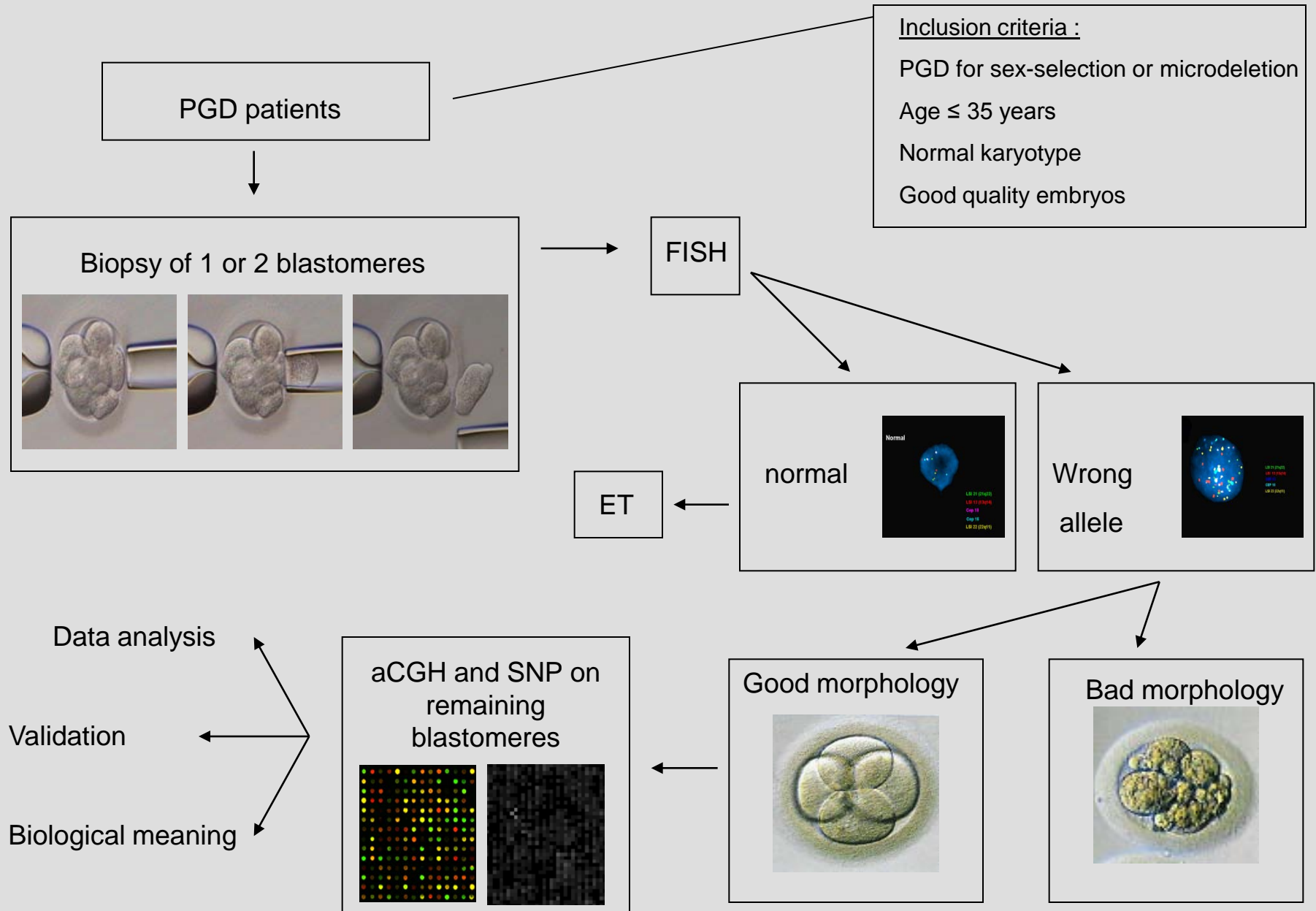


31 frozen pronuclear oocytes -> 17 survived -> 15 well amplified -> 7 not informative

8 analysed on BAC and SNP

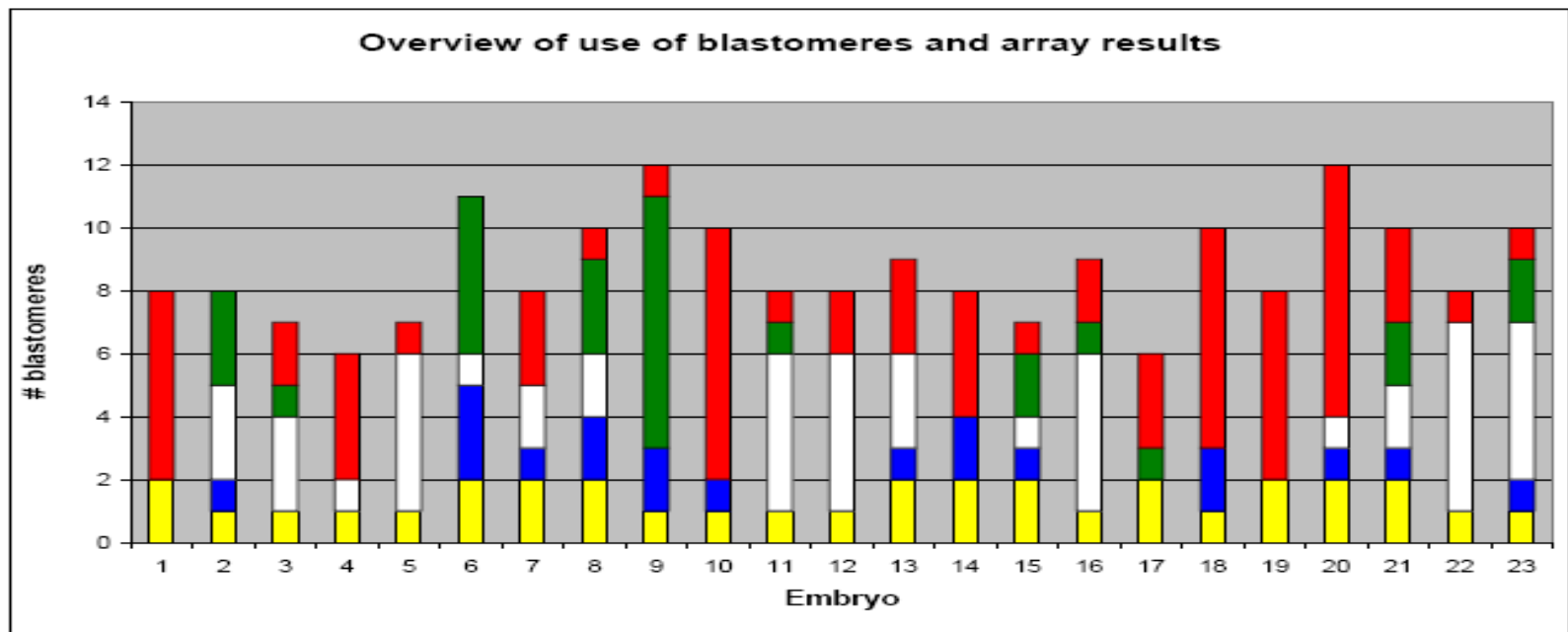
- 7/8 (88%) : normal diploid
- 1/8 (12%) : terminal segmental imbalances (Chr. breakage during preceding meiotic cell division ?)

# Analysis of human embryos : study design

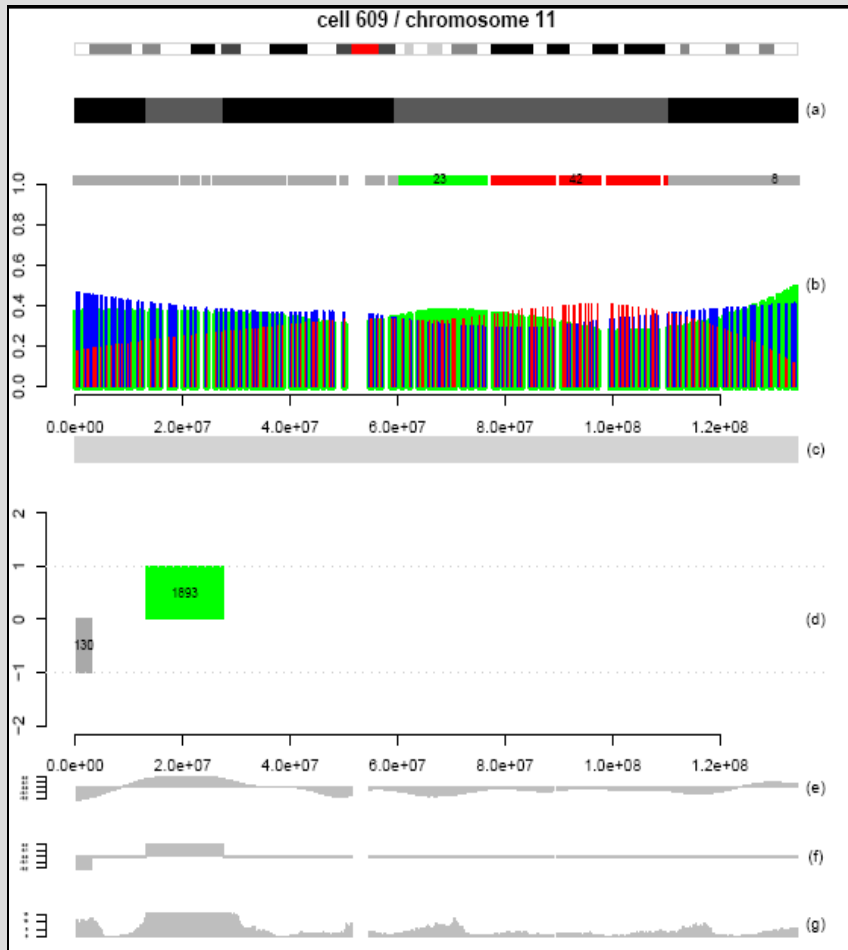


# The majority of human cleavage stage embryos contain chromosomally imbalanced blastomeres

- 2/23 (9%) : normal diploid in all cells
- 1/23 (4%) : diploid, but UPID
- 8/23 (35%) : mosaic diploid/aneuploid (4 embryos : ratio diploid/aneuploid > 1
- 12/23 (52%) : mosaic aneuploid (3 embryos : meiotic (same aberration in all cells)

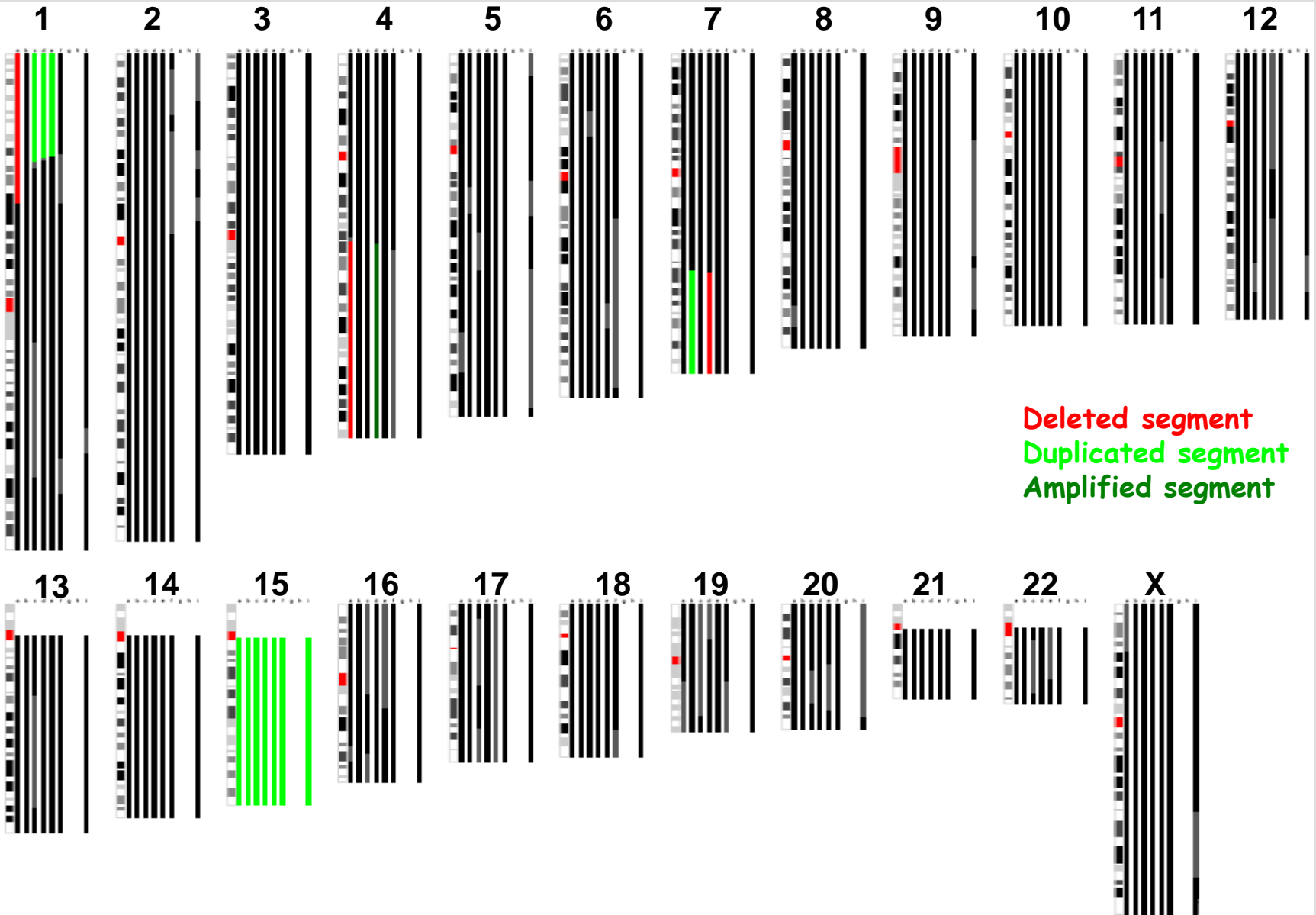


# Genome-wide equal probabilities for all copy numbers states

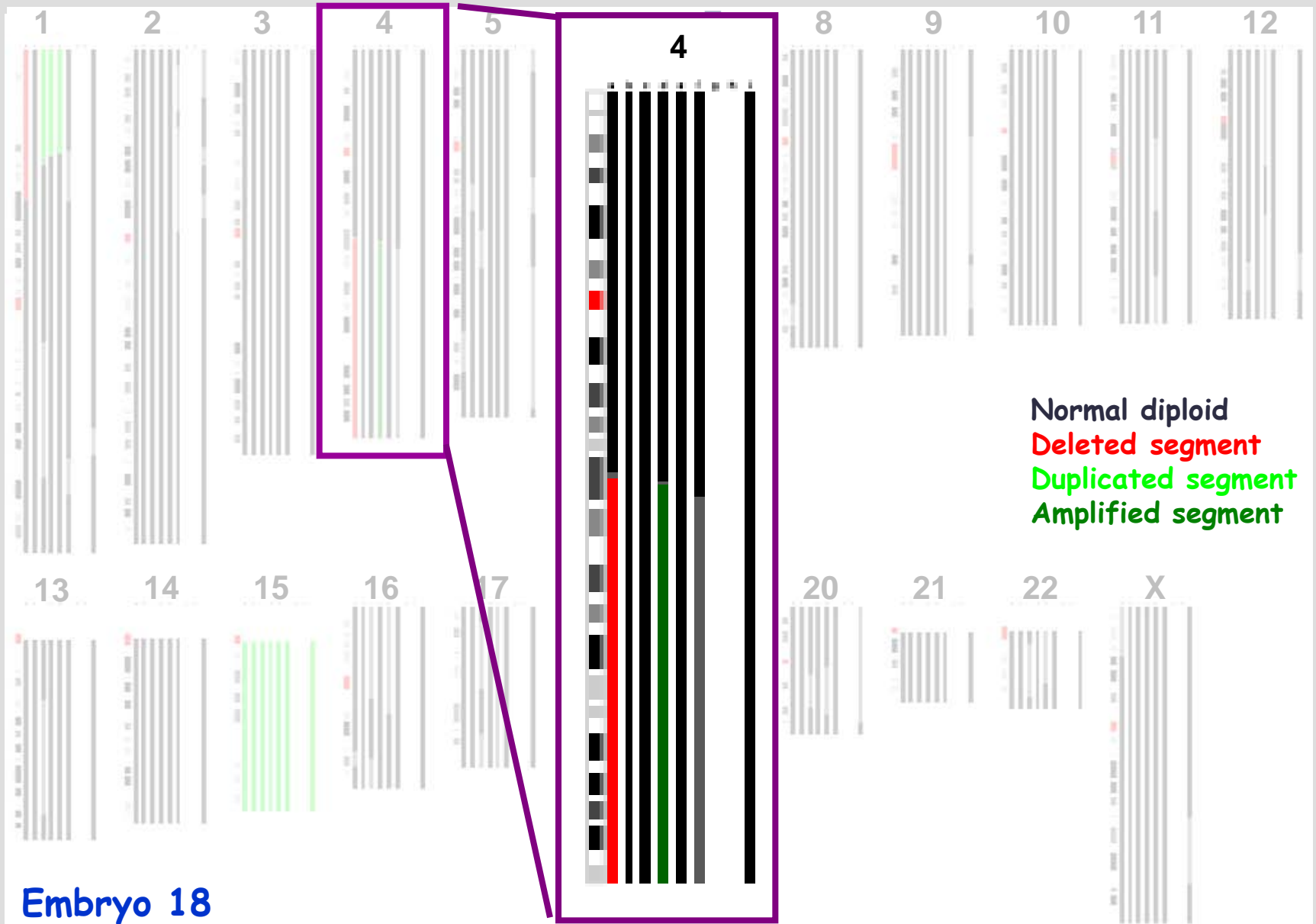


- Origin : High SD between intensity ratios of consecutive BAC-clones ?
- Technical or biological ?
- Hypothesis : relation with cell cycle
  - S-phase : more scatter because different loci have different copy number state

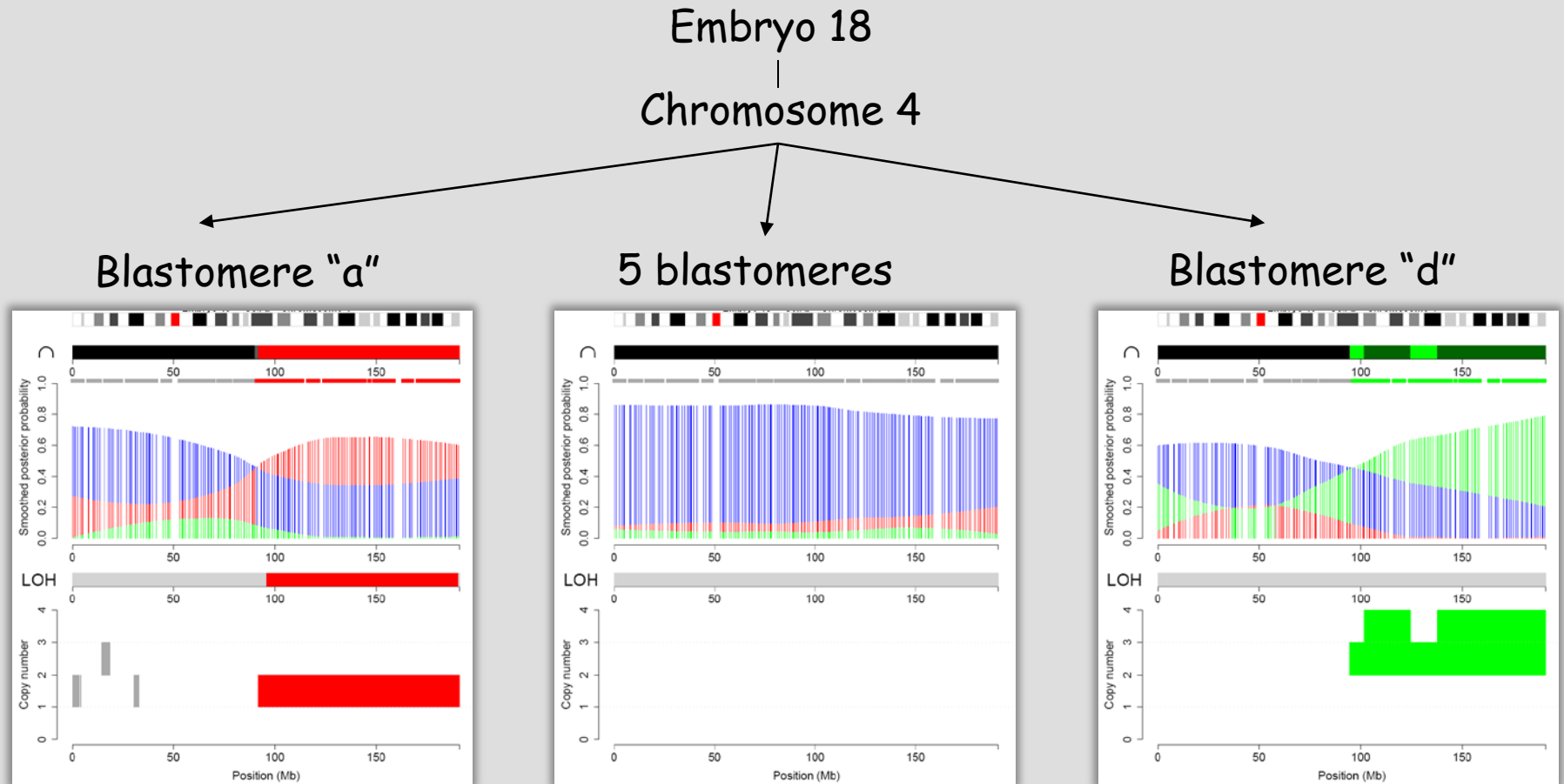
# Overview of the chromosomal status of all blastomeres of embryo 18



# Simple terminal imbalances are terminal deletions, duplications or amplifications



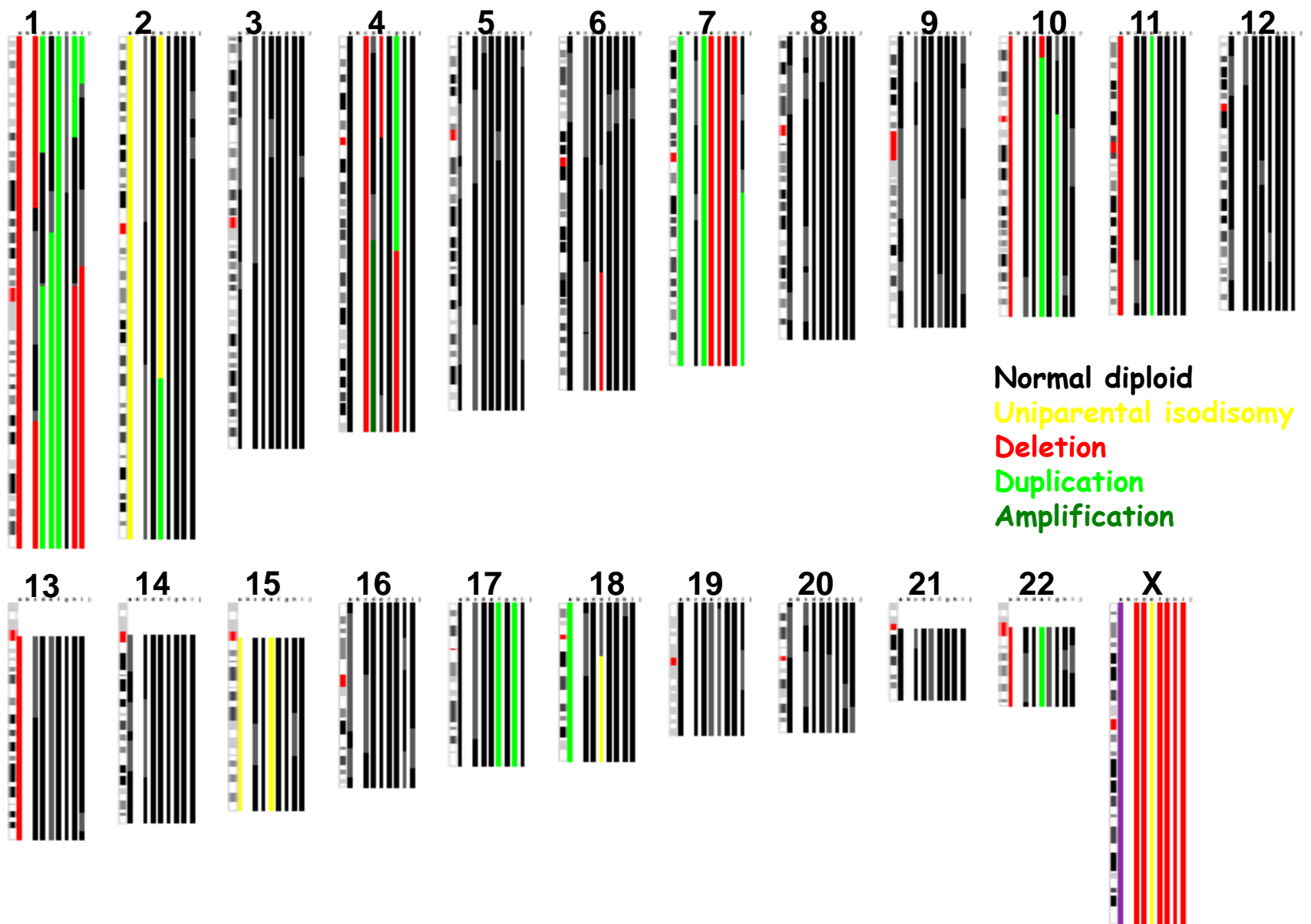
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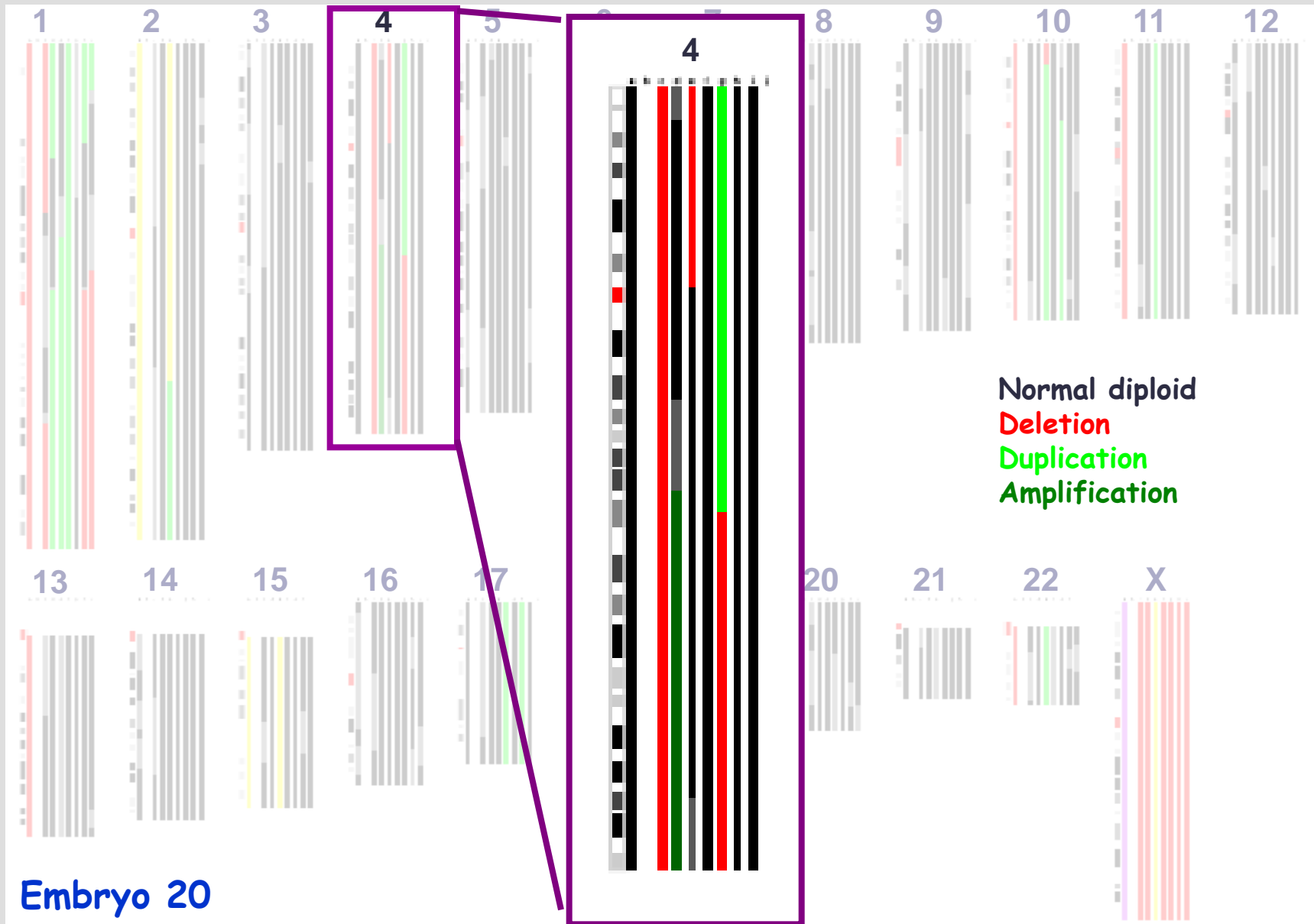
9/23 (39%) carried 'simple' terminal imbalances



# Overview of the chromosomal status of all blastomeres of embryo 20

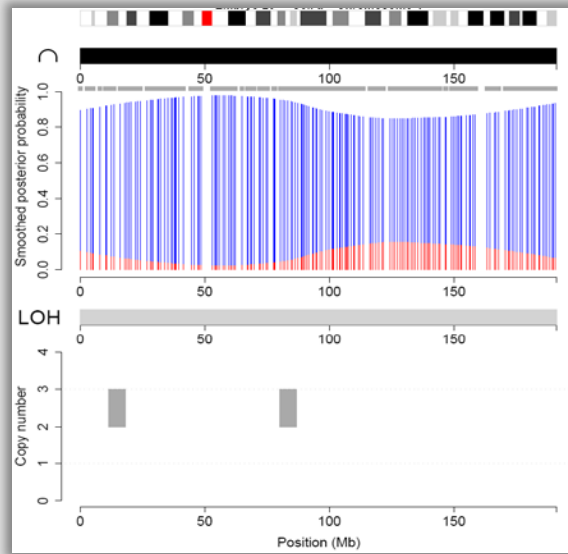


# Complex terminal imbalances are terminal imbalances accompanied by aneuploidies for the same chromosome

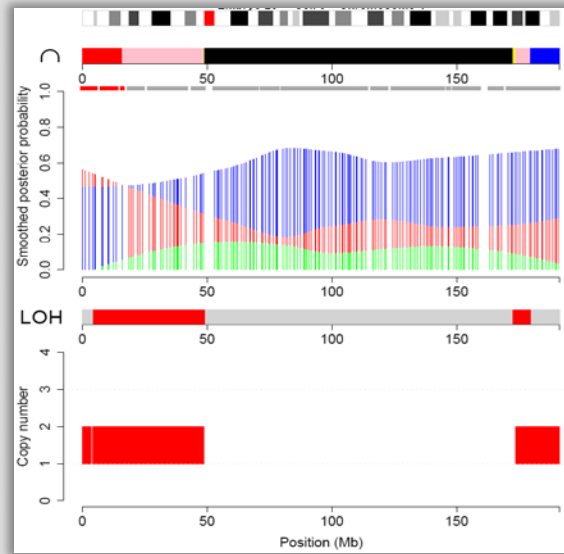


# Complex terminal imbalances are terminal imbalances accompanied by aneuploidies for the same chromosome

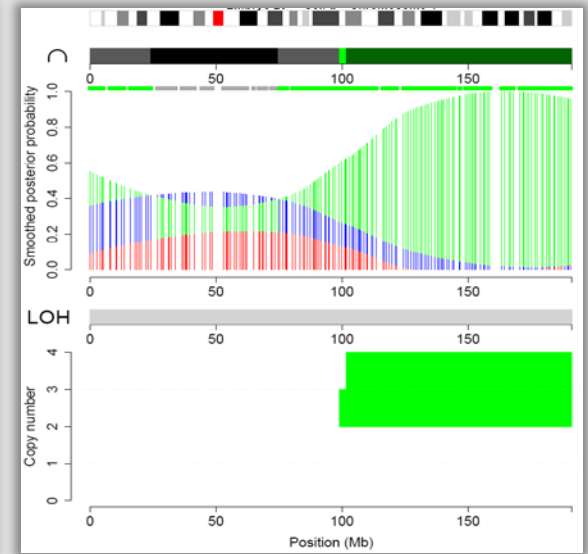
## Blastomere "a and f"



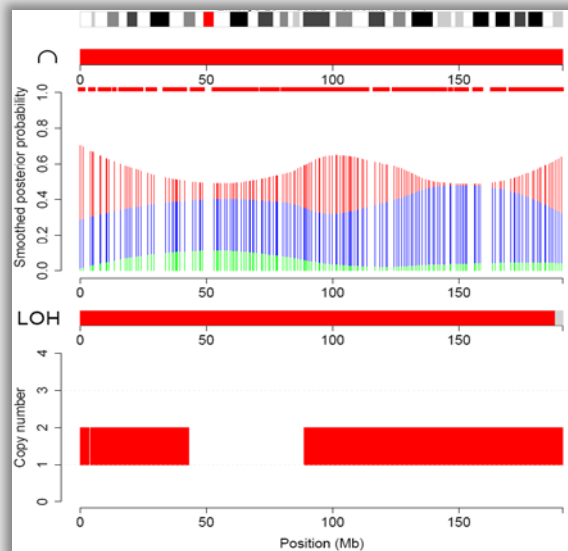
## Blastomere "e"



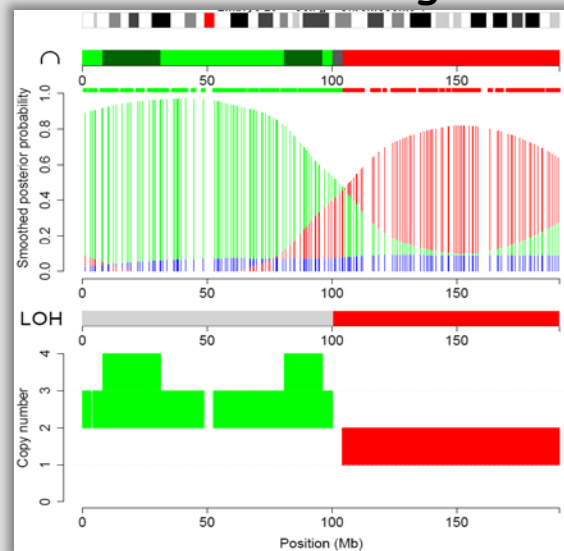
## Blastomere "d"



## Blastomere "c"



## Blastomere "g"

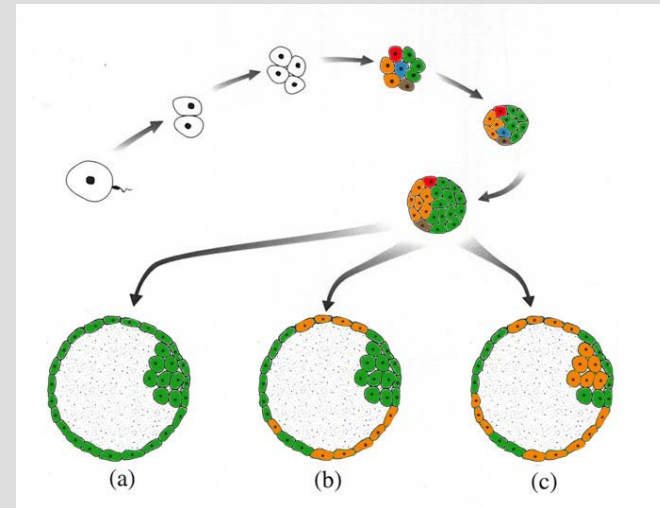
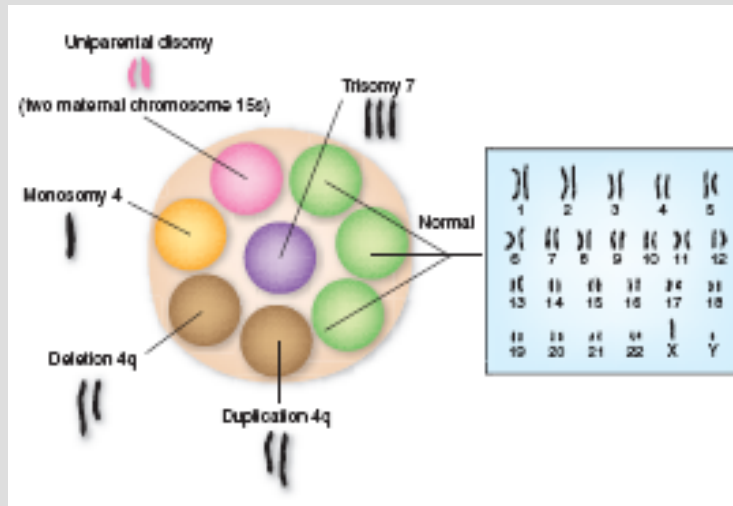


4/23 (17 %) embryos carried

'complex' terminal segmental imbalances

# Conclusions

**CHROMOSOME INSTABILITY = common to human IVF embryogenesis**  
91% of early human IVF embryos are chromosomally abnormal



**PGD for aneuploidy screening is useless because**

- mitotic error rate is higher than meiotic error rate
- One cell is not representative of whole embryo

Vanneste et al., Nature Medicine, 2009

Vanneste et al., Human Reproduction, in press

# Acknowledgements...

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Michèle Ampe

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