Analysis of CGH and SNP arrays for the detection of chromosomal aberrations in single cells

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Outline

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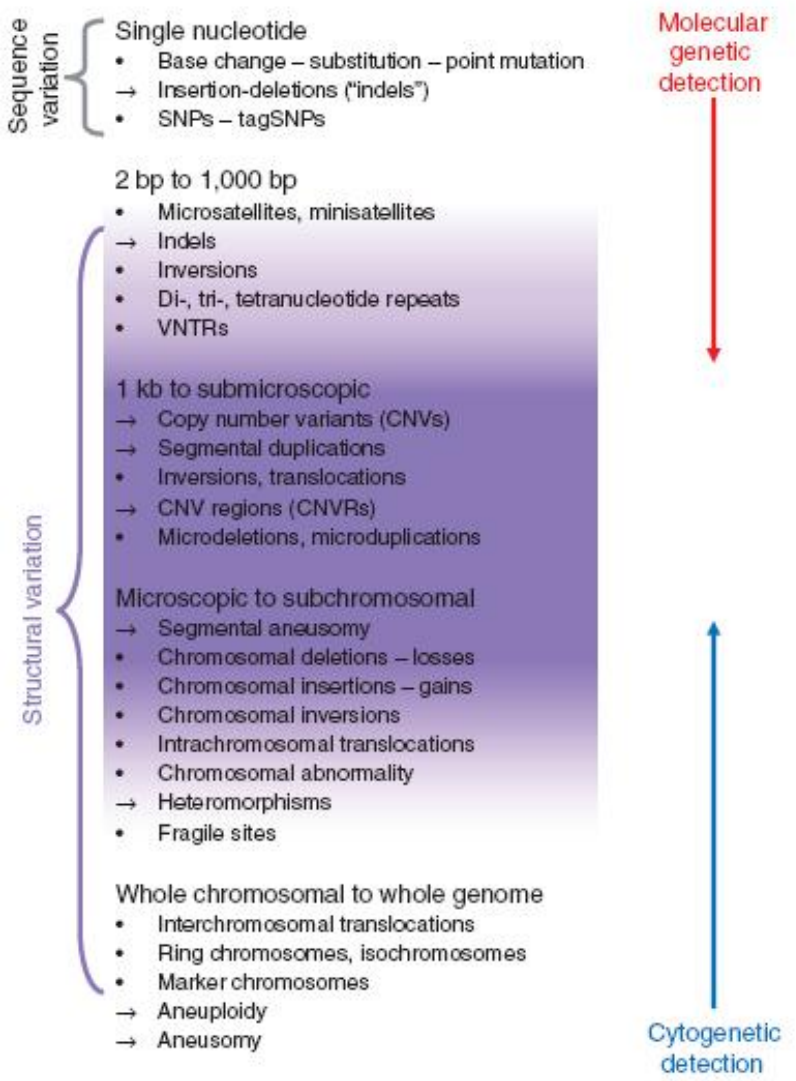
Copy Number Variation (Scherer *et al.*, 2007)



- DNA segments $>$ 1 kb, typically a few 100 kb
- copy number variable compared to a reference segment
- genome-wide measurement through BAC or SNP arrays

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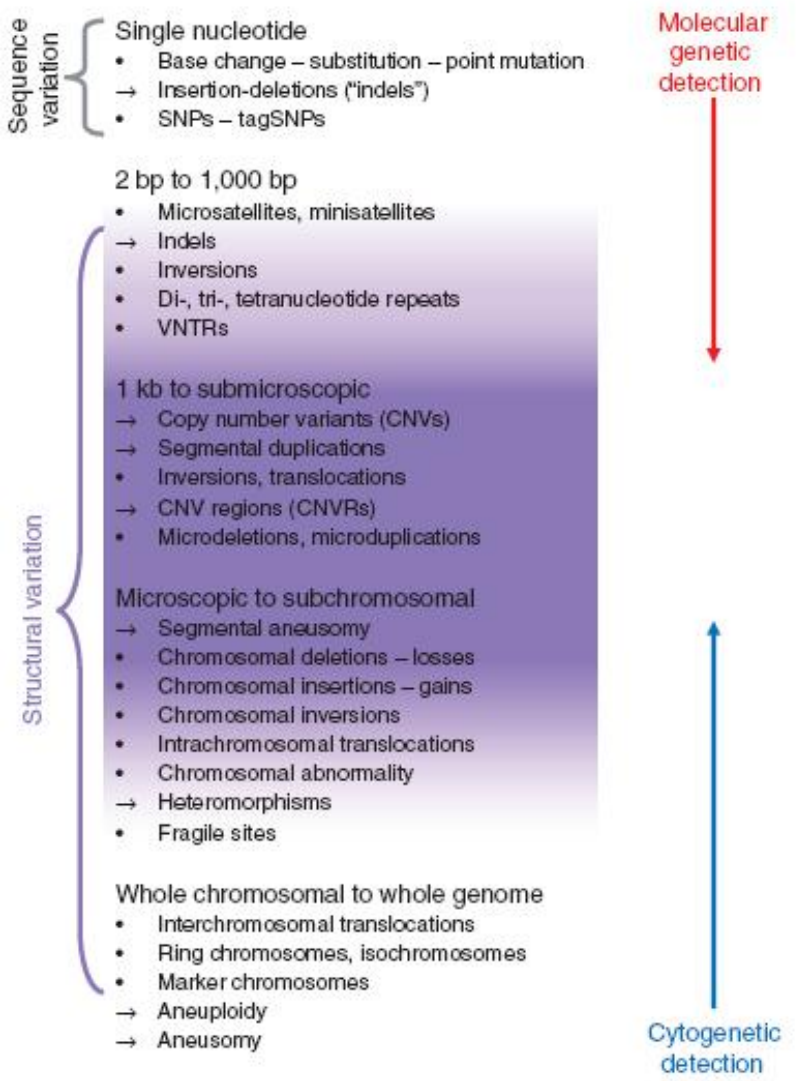

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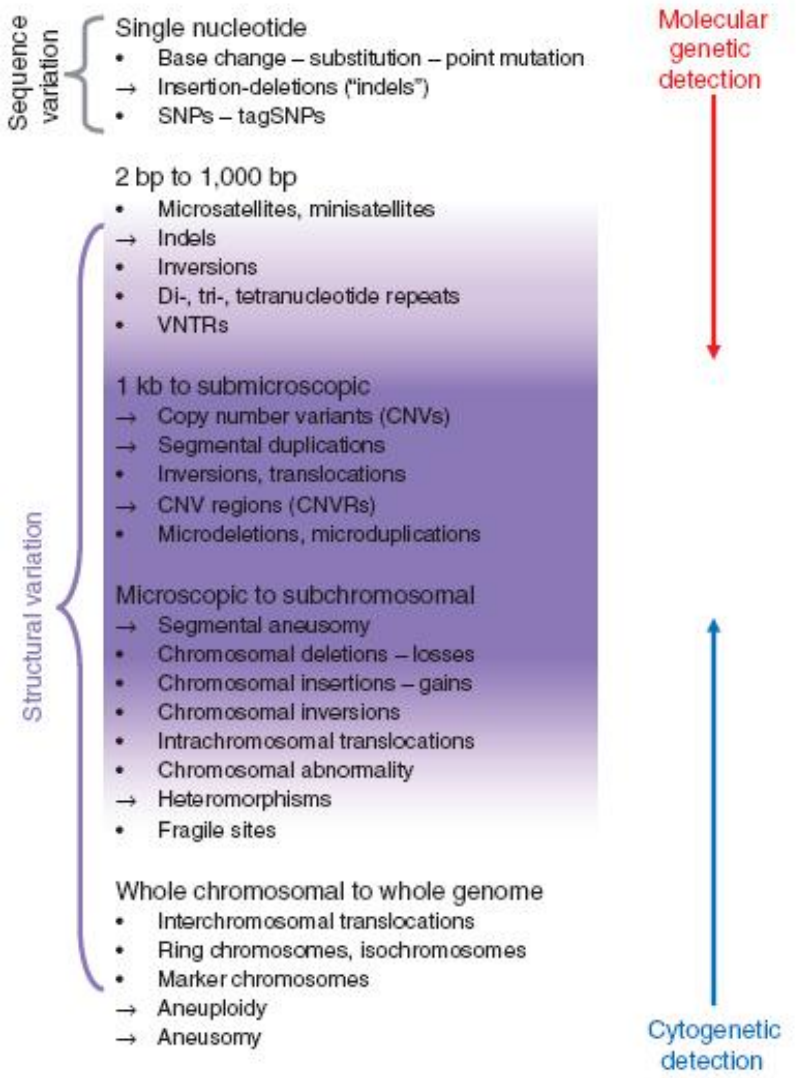

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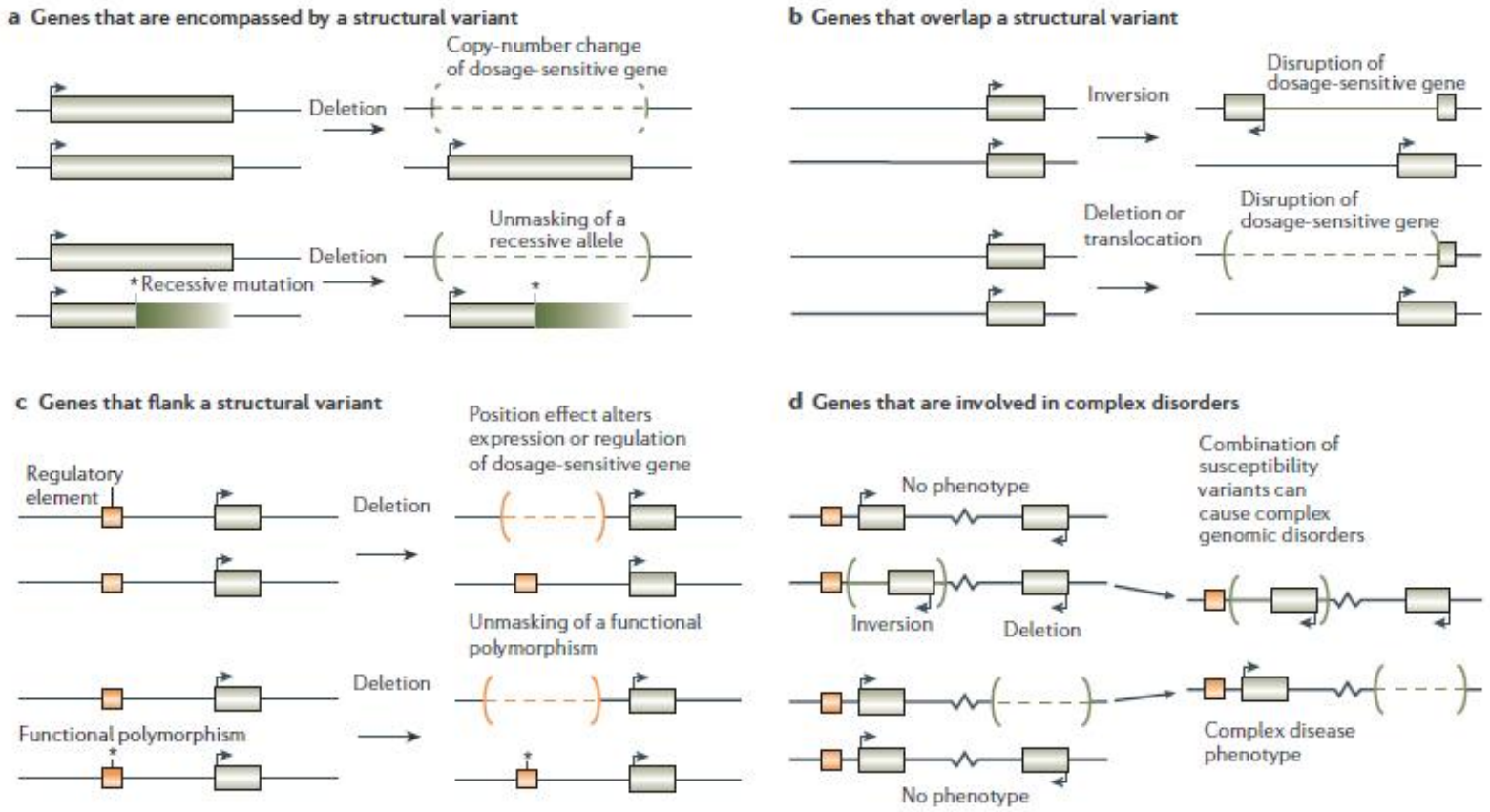
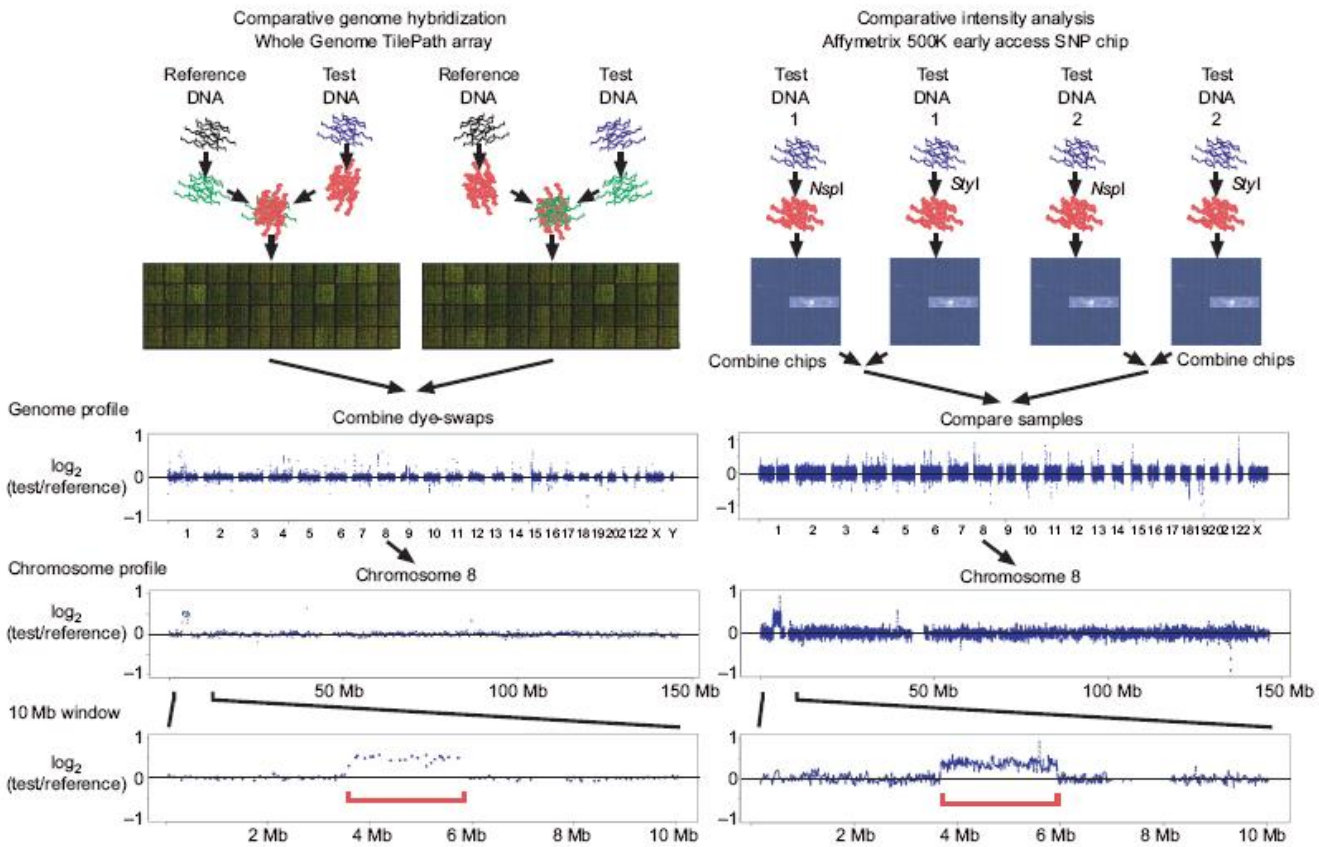
CNV: biological consequences (Feuk *et al.*, 2006)



Detecting CNV: typical array set-up (Redon *et al.*, 2006)



Other methods

- qPCR
- sequencing
- FISH
- ...

Single Cells: Motivation

- pre-implantation diagnostics for *in-vitro* fertilization
- traditional cytogenetics tools have a relatively low resolution
- diagnosis of *de novo* CNV

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Single Cells: wet lab

- disection of 3 day old human embryos with a pipette
- amplification of DNA with GenomiPhi V2

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CNV detection on SNP arrays

- SNP arrays have higher resolution, can detect loss of heterozygosity
- we used Affymetrix GeneChip 250k NSP I
- quantile normalization, compared to HapMap pool of 41 females
- analysis using CNAG and CNAT

CNV detection on SNP arrays

- Copy Number Analysis Tool
  - 5 state Hidden Markov Model
  - parameters tuned for detection of known imbalances on EBV transformed cells
- Copy Number Analyzer for GeneChip (Nannya *et al.*, 2005)
  - based on log ratio of sample to reference
  - parameters tuned for detection of known imbalances on EBV transformed cells

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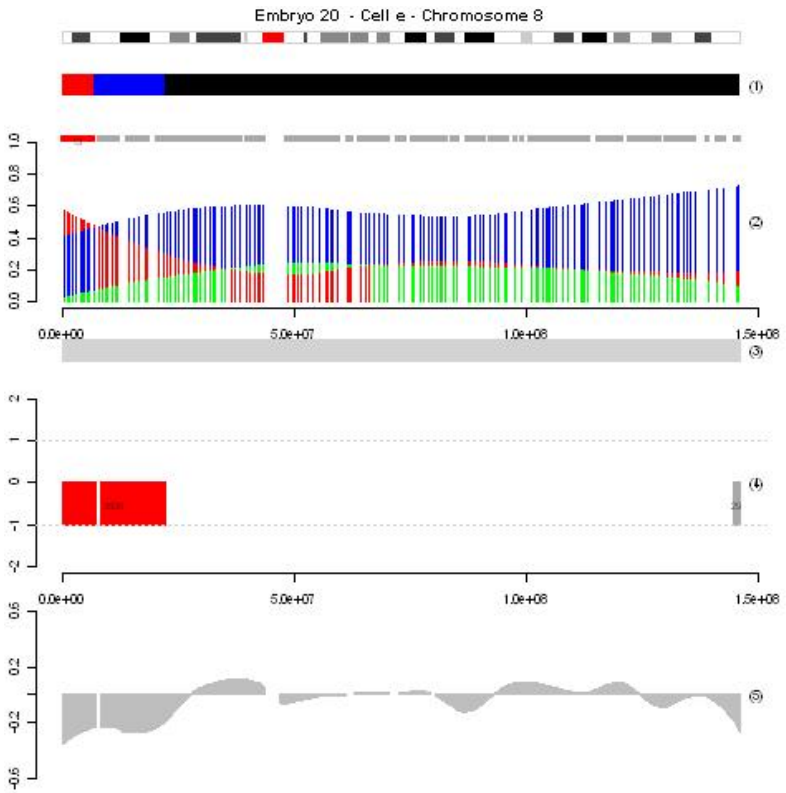
Typical Result



CNV detection on BAC arrays

- BAC arrays: 2 color array with about 3000 BAC clones, each spotted twice
- preprocessing:
  1. background subtraction
  2. filter weak spots (intensity $< 2\times$ median autosomal background)
  3. loess normalization
  4. averaging of duplicates

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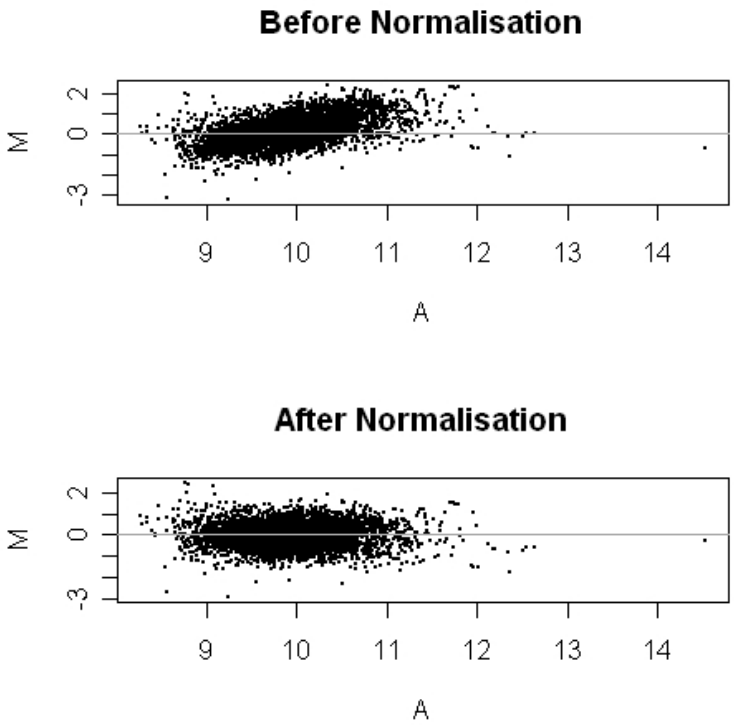
CNV detection on BAC arrays (2)



CNV detection on BAC arrays (3)

- large chromosomal abberations: chromosomal averages (Le Caignec *et al.*, 2006)
- higher resolution: mixture model (Ampe *et al.*, submitted)

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CNV detection on BAC arrays: mixture model

$$y_{jk} = \pi_{j(dup)} N(0.5 - \hat{\mu}_{jk}, \sigma^2_{jk}/n_{jk}) + \quad (1)$$

$$\pi_{j(norm)} N(\hat{\mu}_{jk}, \sigma^2_{jk}/n_{jk})) + \quad (2)$$

$$\pi_{j(del)} N(-0.8 - \hat{\mu}_{jk}, \sigma^2_{jk}/n_{jk}) \quad (3)$$

with clone k on chromosome j.
estimate μ and σ from reference cells
obtain estimates for posterior probability using EM algoritm
loess normalize posterior probabilities over chromosome, call as highest posterior probability

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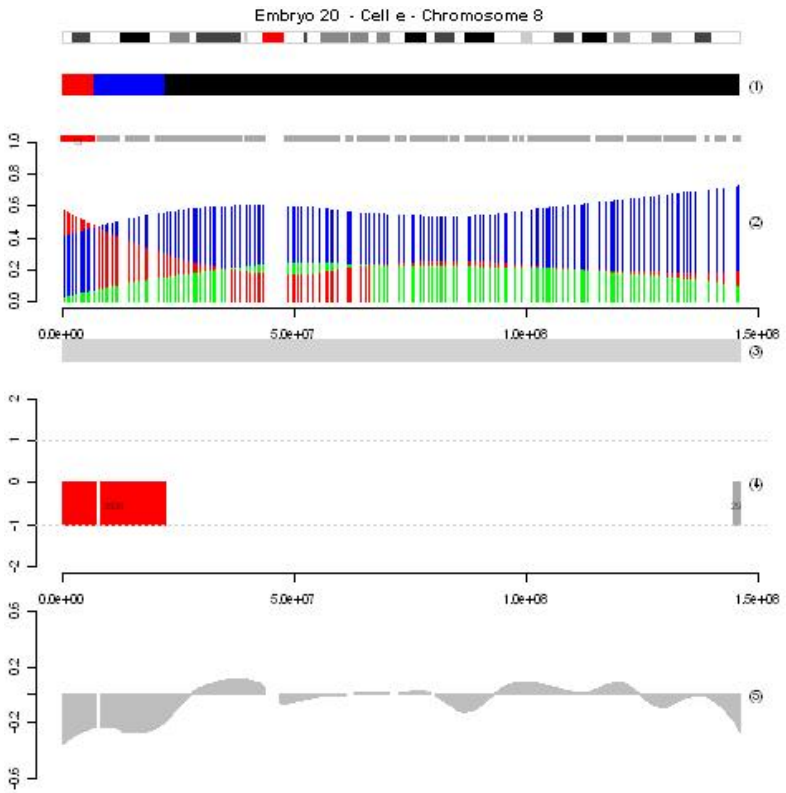
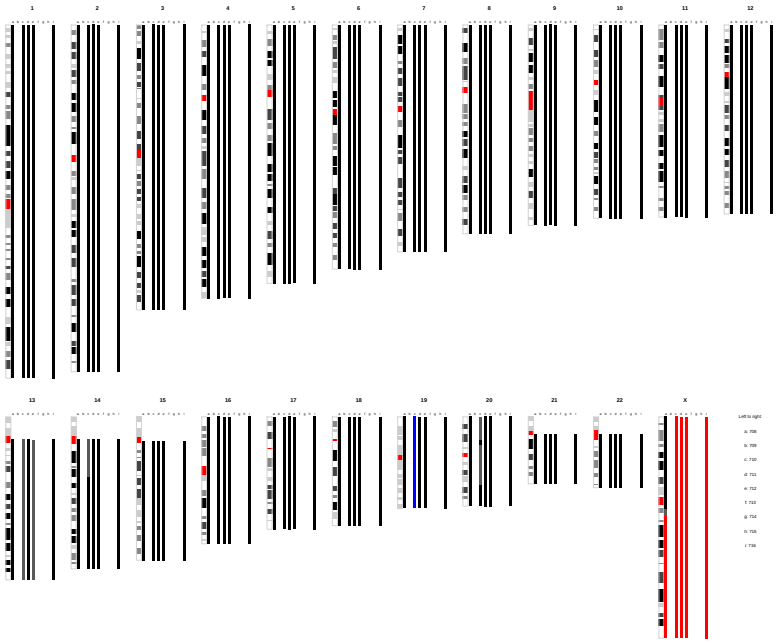
Typical result



BAC and SNP arrays combined (2)



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Results

- 23 embryos were picked
- 146 cells were succesfully amplified
- 50 of those excluded after QC, the others were analyzed on BAC array
- 86 of those were also analyzed on SNP array

Results(2)

- only 2/23 embryos were completely normal
- all others contained one or more abnormalities
- only three of those were the effect of meiotic problems
- all others show mosaicism due to mitotic problems

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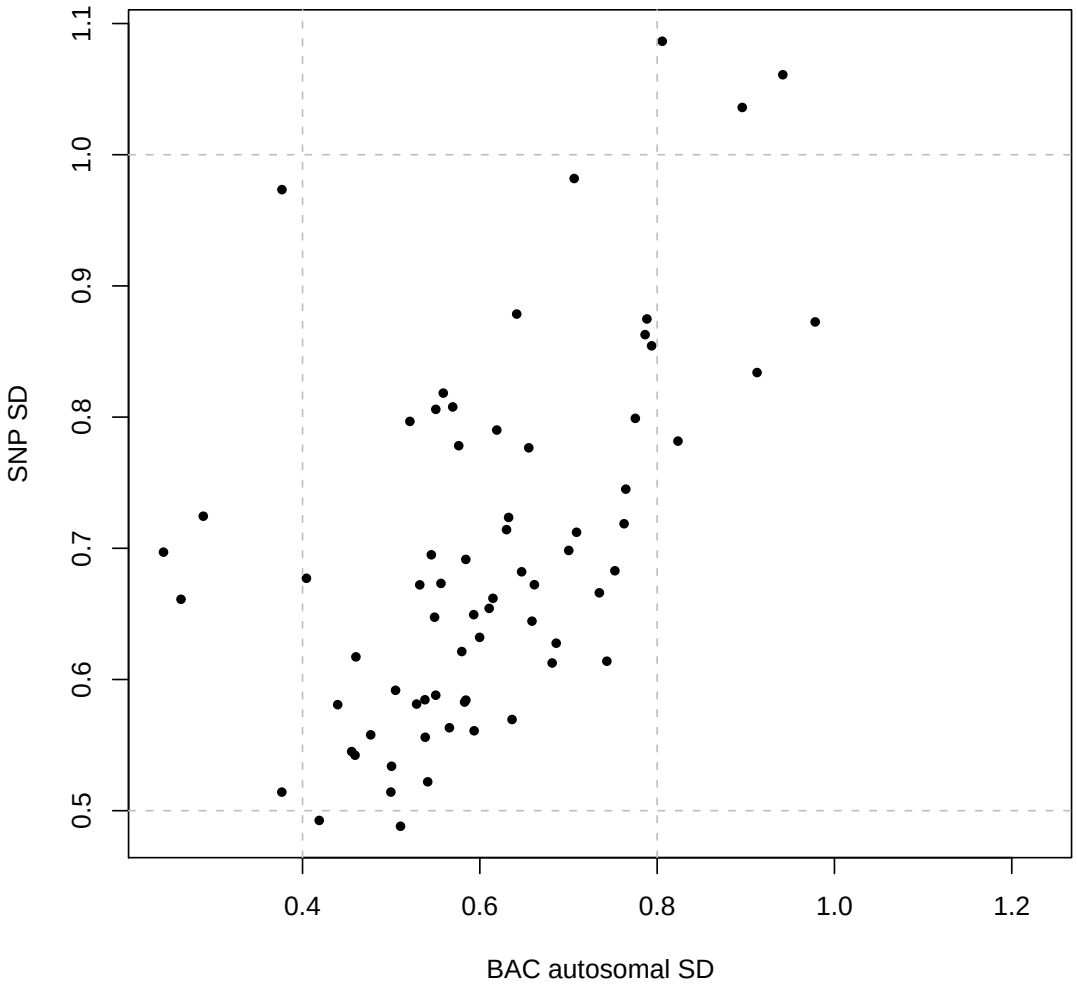
Discussion: biological and clinical consequences

- mosaicism makes it difficult to do pre-implantation diagnosis
- a lot of different mechanisms might be involved

Discussion: array variability



- families who donated embryos
- my supervisors: Yves Moreau, Joris Vermeesch (K.U.Leuven) and Kristel Van Steen (ULg)
- biologists and technicians at Gasthuisberg University Hospital

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