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

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Analysis of CNV in Single Cells

Outline

Copy Number Variation in Single Cells
 Copy Number Variation
 CNV in Human Embryos

2 Methodology

- Isolation and Amplification
- SNP arrays
- BAC arrays
- BAC and SNP arrays combined
- 8 Results and Discussion
 - Results
 - Discussion

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



c Genes that flank a structural variant

Position effect alters expression or regulation of dosage-sensitive gene





polymorphism







b Genes that overlap a structural variant



d Genes that are involved in complex disorders



No phenotype









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



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Other methods

- qPCR
- sequencing
- FISH
- ...



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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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Isolation and Amplification SNP arrays BAC arrays BAC and SNP arrays combined

Single Cells: wet lab

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



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Isolation and Amplification SNP arrays BAC arrays BAC and SNP arrays combined

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



Isolation and Amplification SNP arrays BAC arrays BAC and SNP arrays combined

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

Copy Number Variation in Single Cells Isolation Methodology SNP arm Results and Discussion BAC arre Acknowledgements BAC and

Isolation and Amplification SNP arrays BAC arrays BAC and SNP arrays combined

Typical Result



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Isolation and Amplification SNP arrays BAC arrays BAC and SNP arrays combined

CNV detection on BAC arrays

BAC arrays: 2 color array with about 3000 BAC clones, each spotted twice

- o preprocessing:
 - background subtraction
 - If iter weak spots (intensity < 2 imes median autosomal background)
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 - averaging of duplicates



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CNV detection on BAC arrays (2)



After Normalisation



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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

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

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

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

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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

Typical result



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BAC and SNP arrays combined (2)





Results Discussion

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Results Discussion

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 Discussion

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

Results Discussion

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Results Discussion

Discussion: biological and clinical consequences

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



Copy Number Variation in Single Cells Methodology Results and Discussion

Results Discussion

Discussion: array variability





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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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Thank you for your attention Questions?



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