Microarray technology in PGD

Thierry Voet (Thierry.Voet@med.kuleuven.be)
Laboratory of Reproductive Genomics
Department of Human Genetics, KULeuven, Belgium

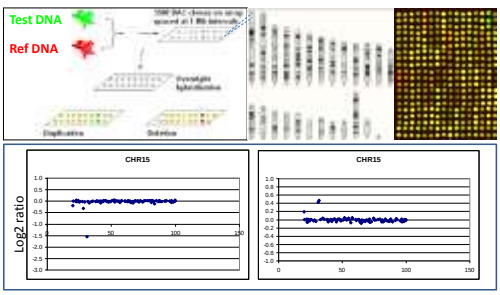
SNP-, CNV- and Haplo-typing of single human cells by microarray methodology

Part 1: Chromosome instability is common in human cleavage stage embryos
-> implications for PGS/PGD

Part 2: Microarrays and PGD for complex chromosomal rearrangements

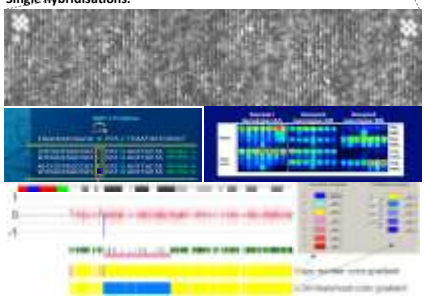
Part 3: Microarrays and genome-wide single cell haplotyping as a generic method for PGD

arrayCGH



SNP-arrays

Single hybridisations:



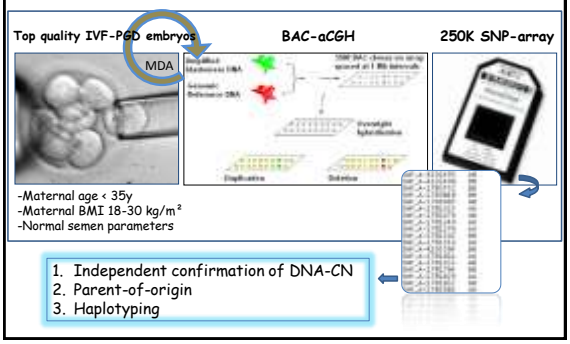
SNP-, CNV- and Haplo-typing of single human cells by microarray methodology

Part 1: **Chromosome instability is common in human cleavage stage embryos**
-> implications for PGS/PGD

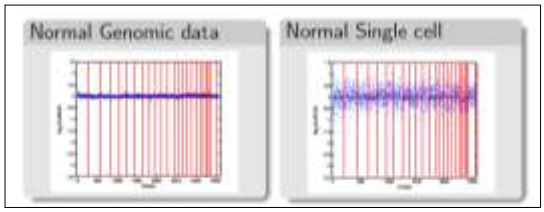
Part 2: Microarrays and PGD for complex chromosomal rearrangements

Part 3: Microarrays and genome-wide single cell haplotyping as a generic method for PGD

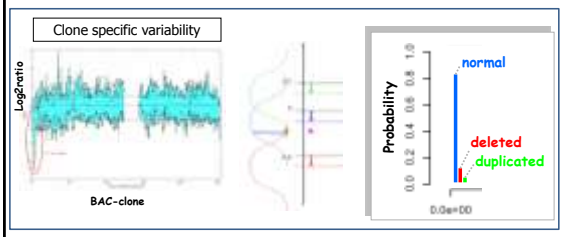
General workflow



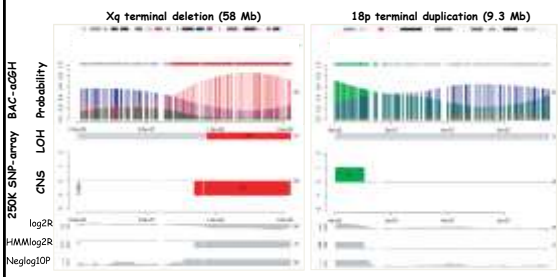
Single cell DNA amplification introduces systematic and stochastic noise in the aCGH data



A novel statistical method that calculates likelihood estimates on imbalances detected with single cell BAC-arrayCGH

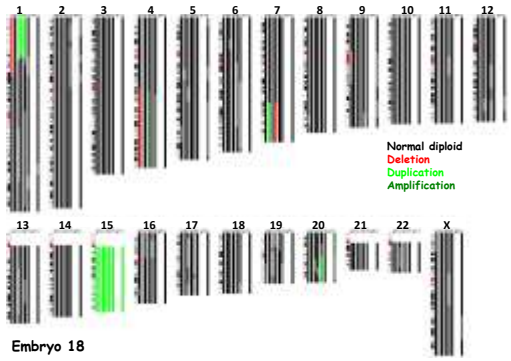


Validation using single cells with known imbalances



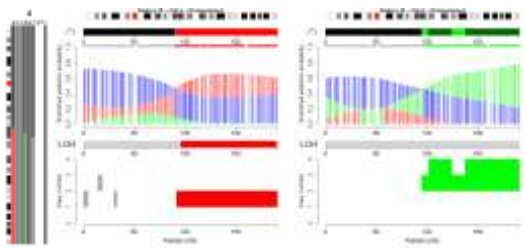
Towards an embryo-wide image of chromosome aberrations

70% of the embryos contain segmental chromosomal imbalances (simple – complex – arm)

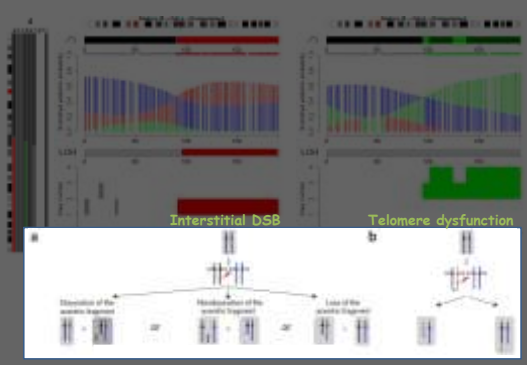


Embryo 18

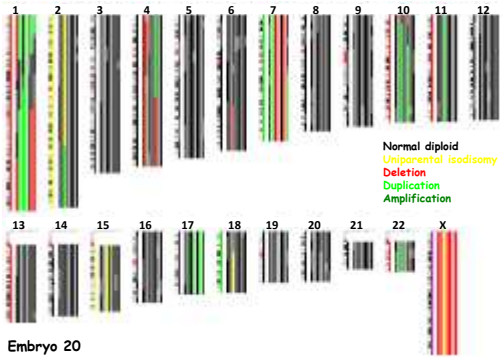
Simple segmental imbalances were noted in 50% of the embryos



Simple segmental imbalances were noted in 50% of the embryos

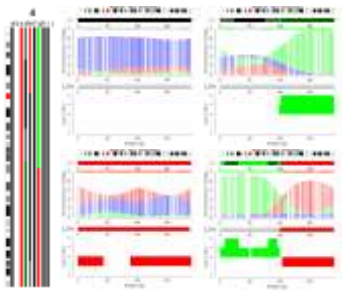


Complex terminal imbalances are terminal imbalances accompanied by aneuploidies for the same chromosome

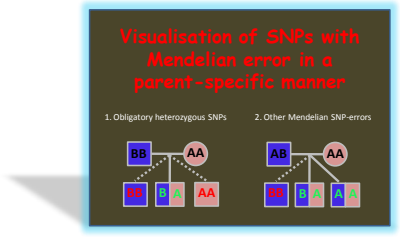


Embryo 20

Complex segmental imbalances were observed in 33% of the embryos

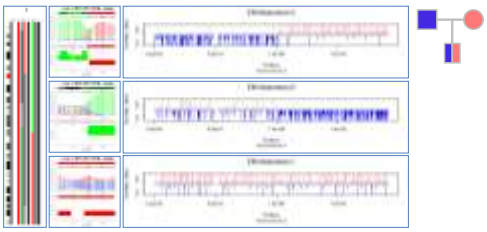


To gain insight in the chromosome segregation patterns and to unriddle the origin of chromosome instability

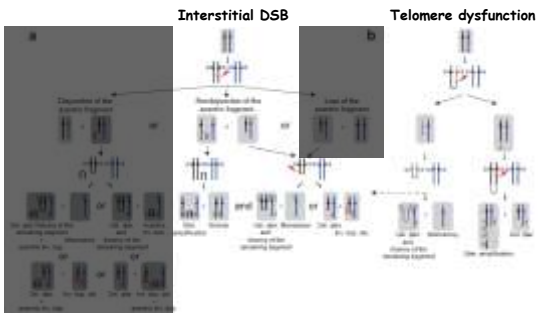


(Voet et al., in prep)

Confirmation of segmental amplifications
&
Mechanistic insight in the creation of structural and numerical chromosome aberrations

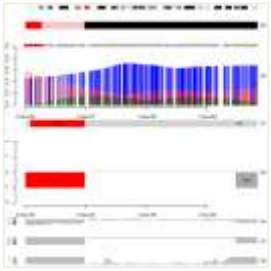


Mechanics behind terminal imbalances

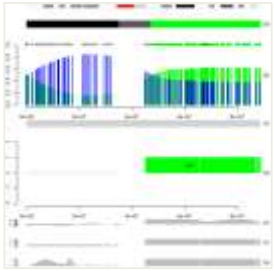


p- or q-arm imbalances in 40% of the embryos suggesting centric fission

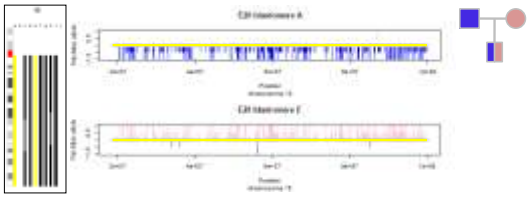
Loss of 4p-arm in blastomere 4 (E20)



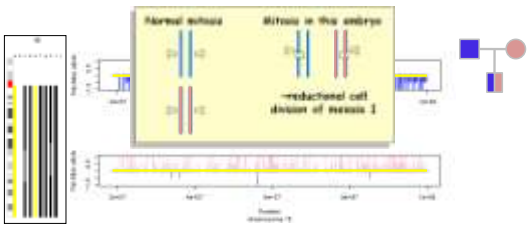
Loss of 16q-arm in blastomere 9 (E10)



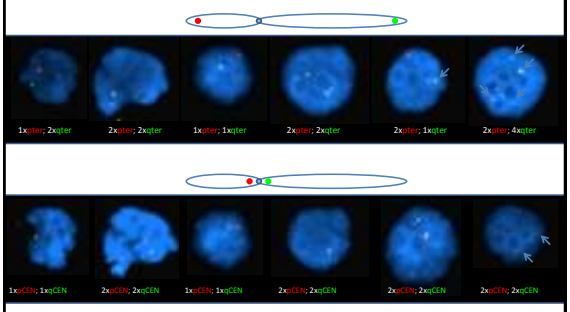
De novo Uniparental isodisomies (UPIDs) are of different parental origin, revealing a novel mechanism of UPID formation



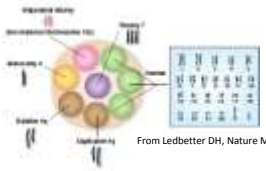
De novo Uniparental isodisomies (UPIDs) are of different parental origin, revealing a novel mechanism of UPID formation



FISH confirmation of segmental imbalances



Embryos are chromosomally unstable

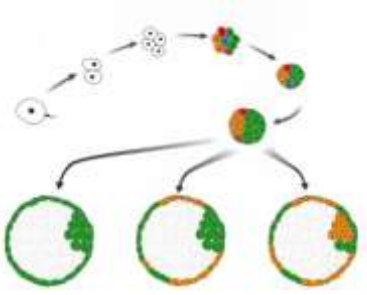


From Ledbetter DH, Nature Medicine (2009)

PGD for aneuploidy screening at cleavage-stage is useless because
- **Mitotic error rate is high(er than meiotic error rate)**
- **One cell is not representative for the remaining cells in the embryo**

Why are we (most of us) healthy?

Survival of the normal (cell)?



Take-home messages: part 1

Chromosome instability (CIN) = common to human (IVF) embryogenesis

- not confined to tumorigenesis
- not confined to couples considered at risk for aneuploidy (advanced maternal age, recurrent implantation failure, recurrent miscarriage,…)

CIN = contributor of reduced human fertility

Frequency of normal diploid embryos < IVF success-rate

- Mosaic diploid(/aneuploid) embryos may yield healthy offspring

CIN is likely a major contributor of non-recurrent genomic disorders & human variation

- Inv dup del often observed in terminal deletion syndromes
- Isochromosomes & chromosome-arm imbalances due to centric fissions are observed in birth defects and spontaneous abortions
- Neocentromeric markers in the form of inv dup ter segments have been detected postnatally
- Mosaicisms in placenta and foetuses are due to embryonic CIN

(Vanneste* and Voet* *et al*., Nature Medicine 2009: *joint first authors)

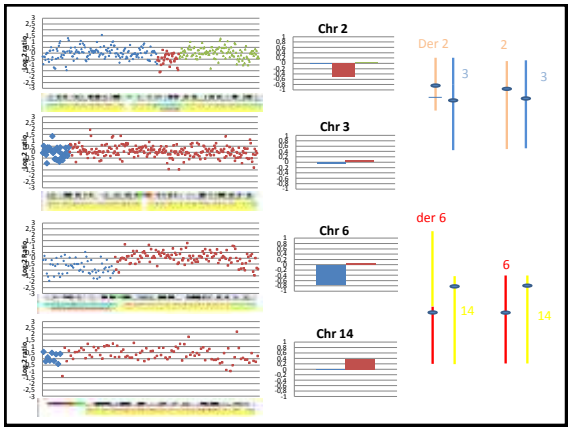
SNP-, CNV- and Haplo-typing of single human cells by microarray methodology

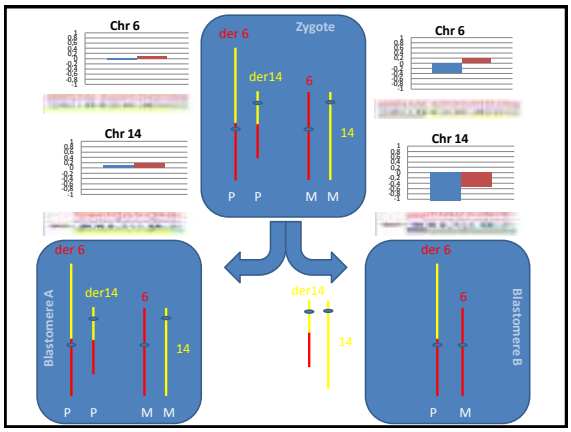
Part 1: Chromosome instability is common in human cleavage stage embryos
-> implications for PGS/PGD

Part 2: Microarrays and PGD for complex chromosomal rearrangements

Part 3: Microarrays and genome-wide single cell haplotyping as a generic method for PGD

PGD: Paternal karyotype
46, XY, ins(3;2)(p23;q23q14.2),t(6;14)(p12.2;q13)





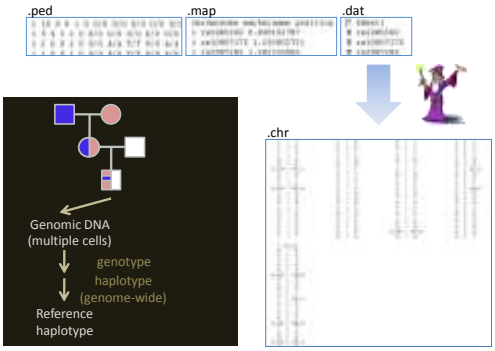
SNP-, CNV- and Haplo-typing of single human cells by microarray methodology

Part 1: Chromosome instability is common in human cleavage stage embryos
-> implications for PGS/PGD

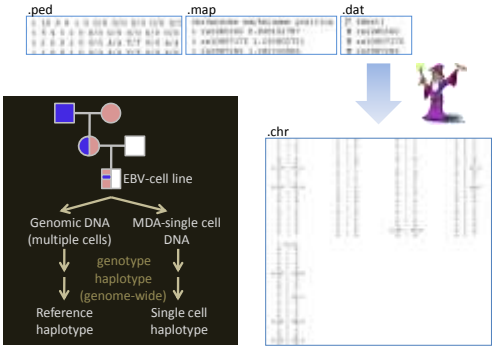
Part 2: Microarrays and PGD for complex chromosomal rearrangements

Part 3: Microarrays and genome-wide single cell haplotyping as a generic method for PGD

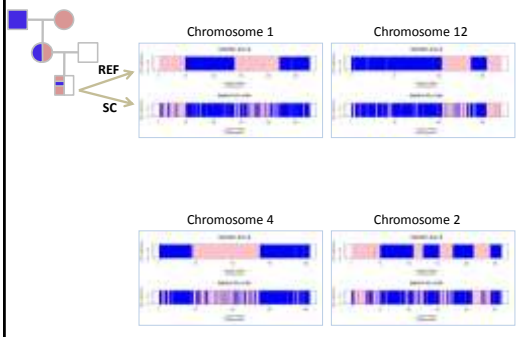
Haplotype reconstruction in single cells: proof-of-principle



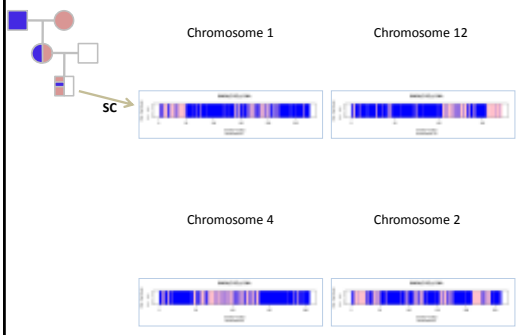
Haplotype reconstruction in single cells: proof-of-principle



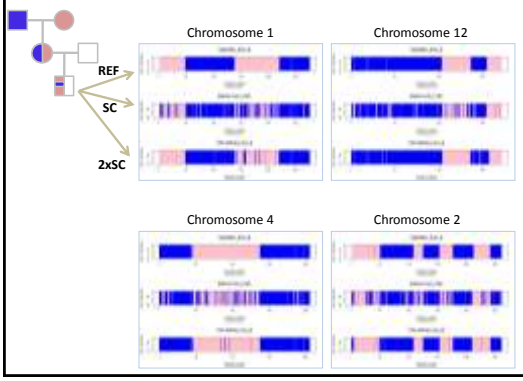
Single cell haplotyping: false-positive homologous recombinations due to genotyping errors



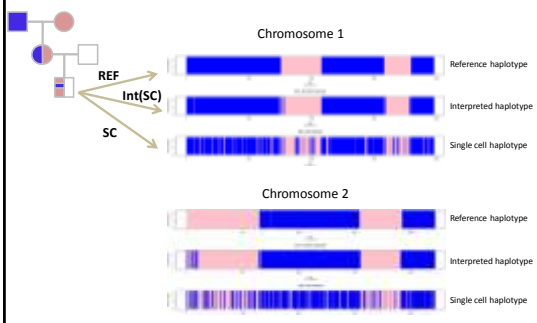
Single cell haplotyping: difficult to discern the true homologous recombination events



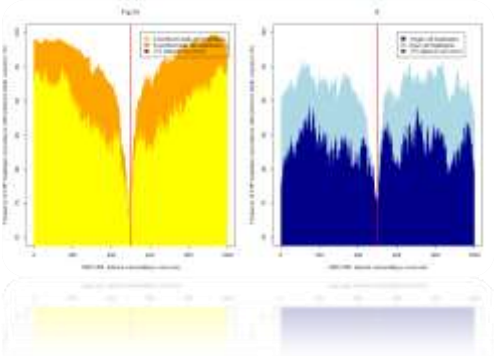
Dual cell haplotyping: virtual genotypes (more reliable SNP calls at the cost of SNP call rate)



Single cell 'raw' haplotype interpretation algorithms

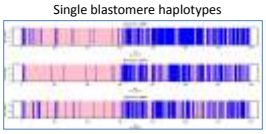


Accuracy of homologous recombination site pinpointing by single-cell haplotyping

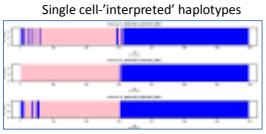


Single cell haplotyping on blastomeres

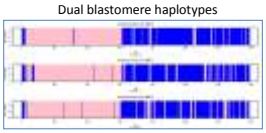
Single blastomere haplotypes



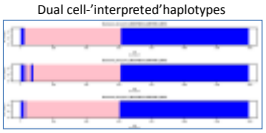
Single cell-'interpreted' haplotypes



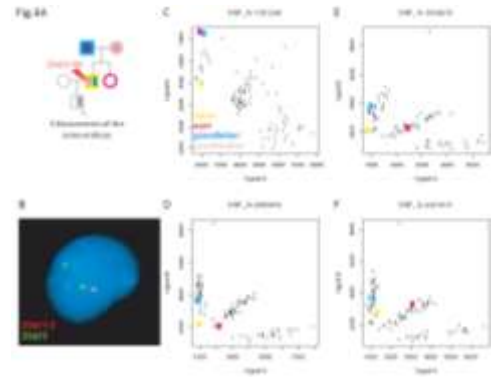
Dual blastomere haplotypes



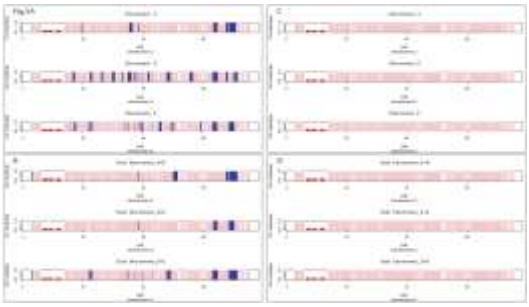
Dual cell-'interpreted'haplotypes



Single cell haplotyping detects disease-alleles in IVF embryos



Single cell haplotyping detects disease-alleles in IVF embryos



Take-home messages: part 2 and 3

ArrayCGH as well as SNP-arrays enable PGD for complex chromosomal rearrangements

- Keep cleavage stage chromosomal instability in mind
- Time issue

SNP-arrays enable generic PGD methodology

- Accurate detection of chromosome imbalances
- Accurate detection of homologous recombination sites
- Follow the inheritance of risk haplotypes
- Discern balanced, but rearranged genome from normal genome

Advantages of SNP-arrays in single cell genomics:

1. **Independent confirmation of (segmental) chromosomal imbalances**
   -> allele-specific copy number in single cells!!!
   - deletions
   - duplications
   - amplifications

2. Detection of uniparental disomies

3. **Parent-of-origin analyses of chromosomal aberrations**
   - insight in chromosome segregation mechanisms
   - origin of chromosome instability in cleavage-stage embryos

4. **Haplotyping of single human cells**
   - applicative value for preimplantation genetic diagnosis (PGD)

Center for Human Genetics

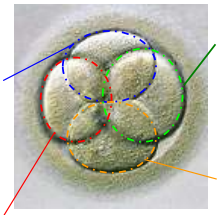
K.U.Leuven

Laboratory of Reproductive Genomics

Ph.D. position available

Center for Human Genetics
K.U.Leuven
Joris R. Vermeesch
Evelyne Vanneste
Hilde Peeters
Sigrun Jackmaert
Cindy Melotte
Jean-Pierre Fryns

de Duve Institute, UCL
Viikkula Mikka
Centre Hospitalier Universitaire, Nantes
Le Caignec Cédric



Biostatistics / Bioinformatics
K.U.Leuven
Yves Moreau
Peter Konings
Geert Verbeke
Michèle Ampe

Leuven University Fertility Center
K.U.Leuven
Thomas D'Hooghe
Sophie Debrock

VUB / UZ-Brussel

References

- Vanneste E*, Voet T*, Le Caignec C, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F, Fryns JP, Verbeke G, D'Hooghe T, Moreau Y, Vermeesch JR. Chromosome instability is common in human cleavage-stage embryos. Nat Med. 2009 May;15(5):577-83. * Joint first authors
- Vanneste E*, Voet T*, Melotte C, Debrock S, Sermon K, Staessen C, Liebaers I, Fryns JP, D'Hooghe T, Vermeesch JR. What next for preimplantation genetic screening? High mitotic chromosome instability rate provides the biological basis for the low success rate. Hum Reprod. 2009 Nov;24(11):2679-82. * Joint first authors
- Ledbetter DH. Chaos in the embryo. Nat Med. 2009 May;15(5):490-1.