UNIVERSITEIT GENT

FACULTY OF PHARMACEUTICAL SCIENCES

MPS for translocations

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Lab of Pharmaceutical Biotechnology

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In collaboration with:

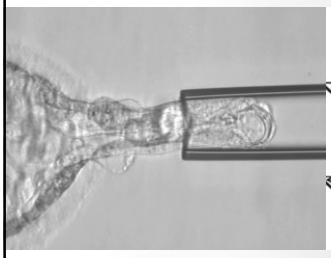
Center for Medical Genetics, Ghent University

Department for Reproductive Medicine , Ghent University

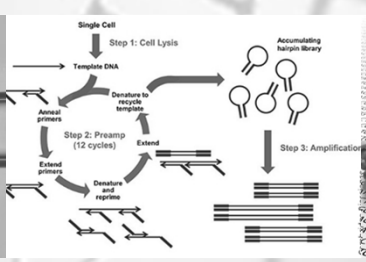
Outline

→ Preimplantation genetic diagnosis (PGD)

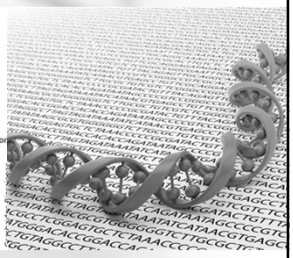
→ Preimplantation genetic screening (PGS)



Trophectoderm Biopsy of Day 5 Blastocyst Embryo



Sureplex Whole Genome Amplification (WGA)



ArrayCGH Shallow MPS ?

Need for PGD / PGS

- Couples can **carry** chromosomal or genetic abnormalities
- Chromosomal abnormalities **arise** during early embryonic development
  - ⇒ congenital anomalies
  - ⇒ implantation failure
  - ⇒ early spontaneous abortion
- **Approaches** for **Embryo selection** by DNA analysis
  - Monogenetic diseases (single-gene mutations): PCR
  - Chromosomal rearrangements:
    - **FISH**
    - **Array Comparative Genomic Hybridization (aCHG)**
    - **NGS?**

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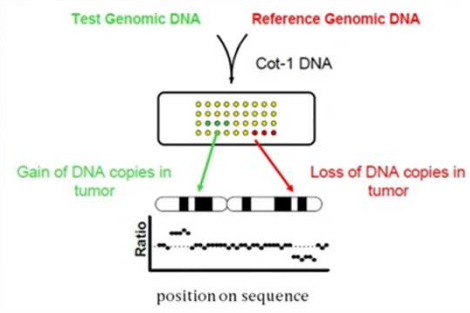
State-of-the-art for PGD / PGS

Array Comparative Genomic Hybridization

- Hypothesis-free genome-wide detection of CNA and aneuploidies
- Resolution of 10-20 Mbp

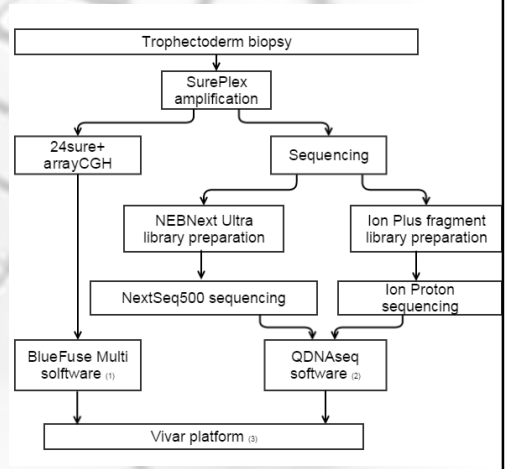


Study objectives

- **Develop** MPS method for CNA detection
- **Validate** that MPS performs equal or better than arrayCGH
- Explore and determine optimal parameters:
  - most appropriate WGA?
  - needed sequencing depth ↔ resolution (needed for translocation)
  - performance of different sequencing technologies
- Study:
  - Limits and limiting factors
  - Cost effectiveness

Experimental design

- **15 couples** with balanced structural rearrangements
  - 8 reciprocal translocations
  - 4 Robertsonian translocations
  - 2 inversions
  - 1 insertional translocation
- **47 blastocysts**
- **MPS** vs **arrayCGH**
- **Illumina**
  (24 samples, 75bp)
  vs Ion Torrent
  (6 samples, 158bp)



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Methods: blastocyst biopsy

- **3-6 trophectoderm** cells from **5-day blastocyst**
- Compared to cleavage-stage embryo (**3-day**):
  - < risk of damaging embryo
  - < abnormal embryos: < tests to find normal embryo
  - > cells ⇒ more uniform and robust WGA

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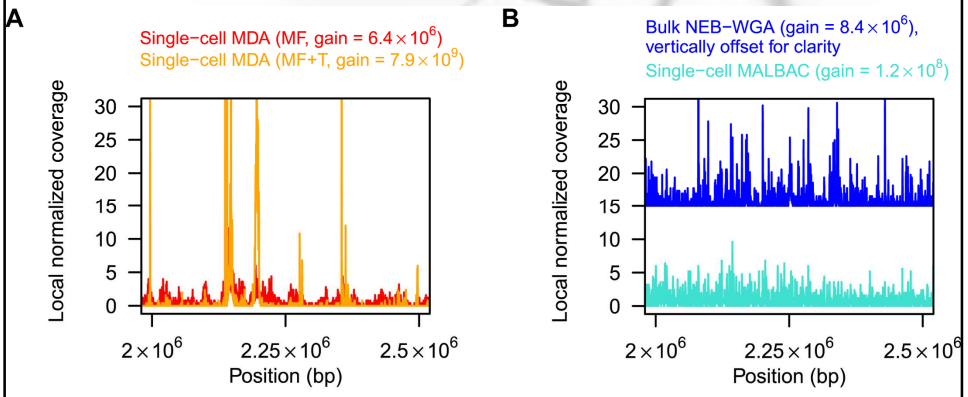
Methods: whole genome amplification (WGA)
→ MDA vs PCR-based



Charles F. A. de Bourcy, Iwijn De Vlaminck, Jad N. Kanbar, Jianbin Wang, Charles Gawad, Stephen R. Quake
A Quantitative Comparison of Single-Cell Whole Genome Amplification Methods
Plos One August 2014 | Volume 9 | Issue 8 | e10558

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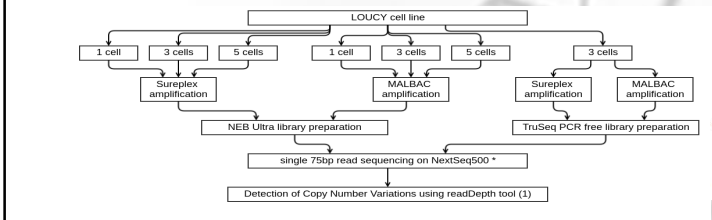
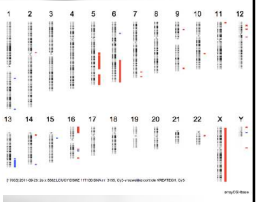
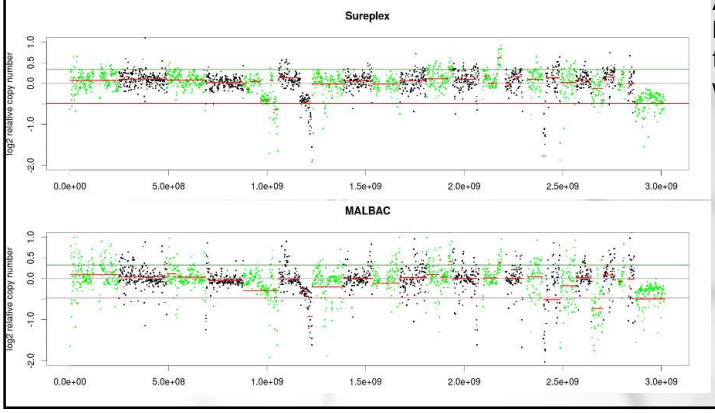
Methods: whole genome amplification (WGA)
→ MALBAC vs Sureplex

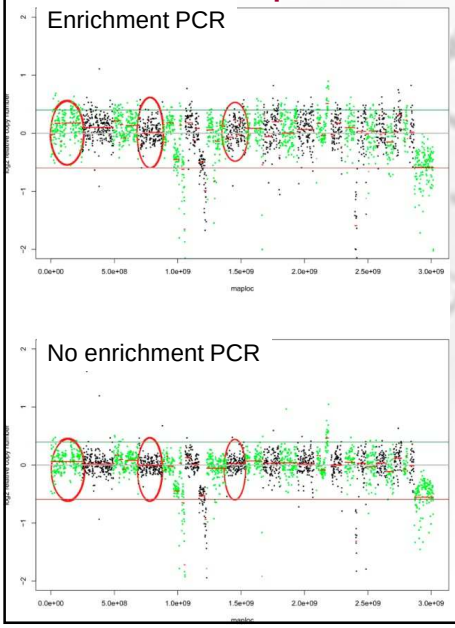






A 180K arrayCGH on bulk genomic DNA from the LOUCY cell was used as reference

Methods
→ Library prep without enrichment PCR



- Read counts per window are more uniform
- Performance in terms of resolution and accuracy stays the same
- Cheaper

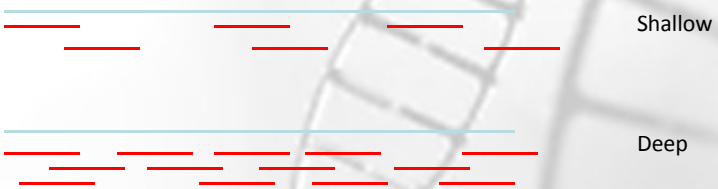
Methods: Summary WGA / library prep

	Mean variance	Standard deviation
MALBAC 1 cell	0.165	0.037
MALBAC 3 cells	0.138	0.012
MALBAC 5 cells	0.146	0.010
MALBAC 3 cells PCR-free lib prep	0.120	0.015
Sureplex 1 cell	0.083	0.013
Sureplex 3 cells	0.077	0.012
Sureplex 5 cells	0.073	0.009
Sureplex 3 cells PCR-free lib prep	0.064	0.004

Lieselot Deleye, Dieter De Coninck, Christodoulos Christodoulou, Tom Sante, Annelies Dheedene, Björn Heindryckx, Van Den Abbeel Etienne, Petra De Sutter, Björn Menten, Dieter Deforce*, Filip Van Nieuwerburgh*
Whole genome amplification with SurePlex results in better copy number variation detection using Massively Parallel Sequencing data compared to Multiple Annealing and Looping Based Amplification Cycles (MALBAC).
Nature Scientific Reports, Volume: 4, Article Number: 5597, Published 30 June 2015

Methods: Shallow sequencing

- ≠ deep sequencing



- Coverage/sample:
  - NextSeq500: 11M reads * 75bp = 825M / 3Mjd = 0.3x
  - Proton: 9.6M reads * 123bp = 1.180Mjd/ 3Mjd = 0.4x

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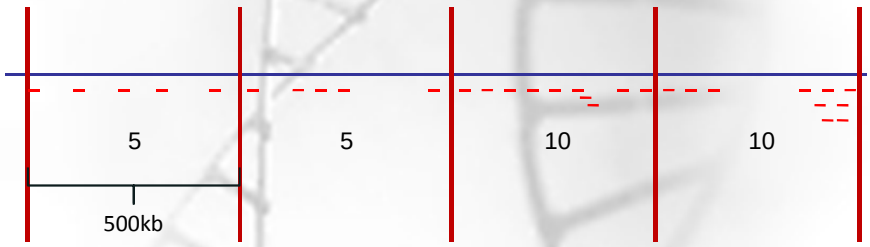
Methods: QDNAseq

STEP 1: Mapping of reads to reference genome
STEP 2: Definition of non-overlapping genomic windows of size *x*
STEP 3: Calculation number of reads in windows
STEP 4: Segmentation: Grouping windows with similar read counts
STEP 5: Estimation of copy number (CN) in each segment
- Under assumption that for diploid genome majority of genome CN = 2



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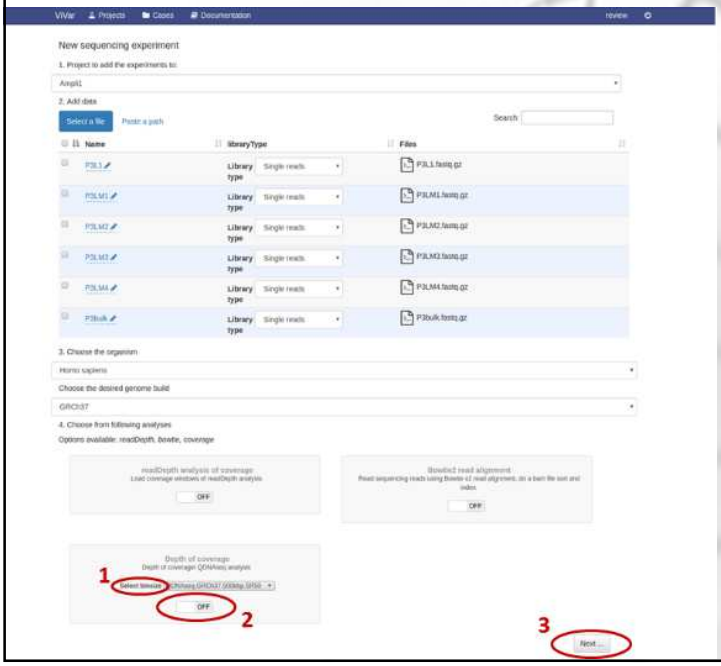
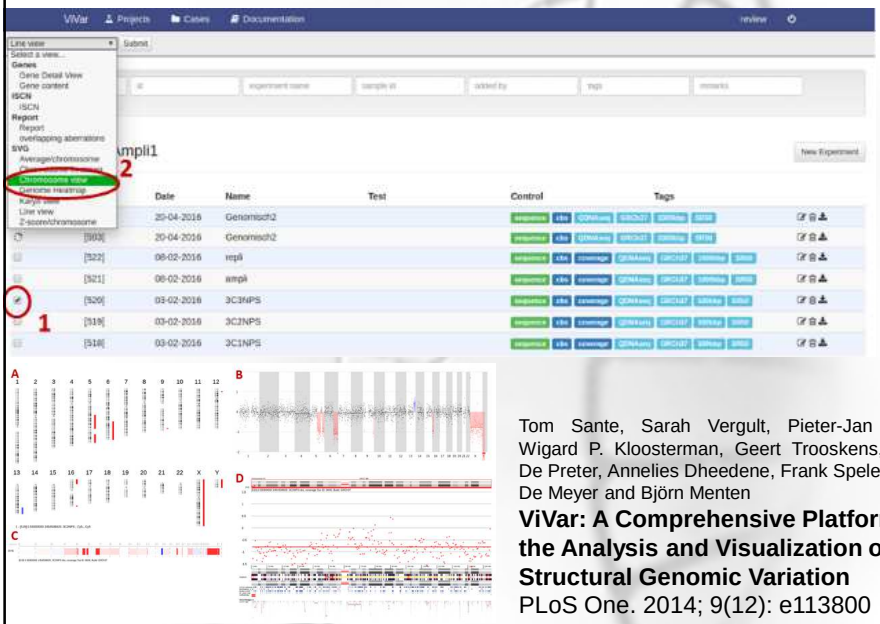
Methods: Vivar



Methods: Vivar



Tom Sante, Sarah Vergult, Pieter-Jan Volders, Wigard P. Kloosterman, Geert Trooskens, Katleen De Preter, Annelies Dheedene, Frank Speleman, Tim De Meyer and Björn Menten

ViVar: A Comprehensive Platform for the Analysis and Visualization of Structural Genomic Variation

PLoS One. 2014; 9(12): e113800

Filip Van Nieuwerburgh, NXTGNT sequencing facility, Lab of Pharmaceutical Biotechnology, Ghent University, Belgium

Results

- 47 embryos
  - 5 normal embryos
  - 42 abnormal embryos
    - Structural abnormalities
      - 31 deletions
      - 23 duplications
    - 17 monosomies
    - 16 trisomies

- Results **arrayCGH** and **MPS** were **concordant:**
  = diagnoses
  = aberrations detected
- **Ion Torrent** and **NextSeq500** are **interchangeable**
- **Higher resolution** for MPS: abnormalities < 3Mb detected
- **Better dynamic range in MPS**

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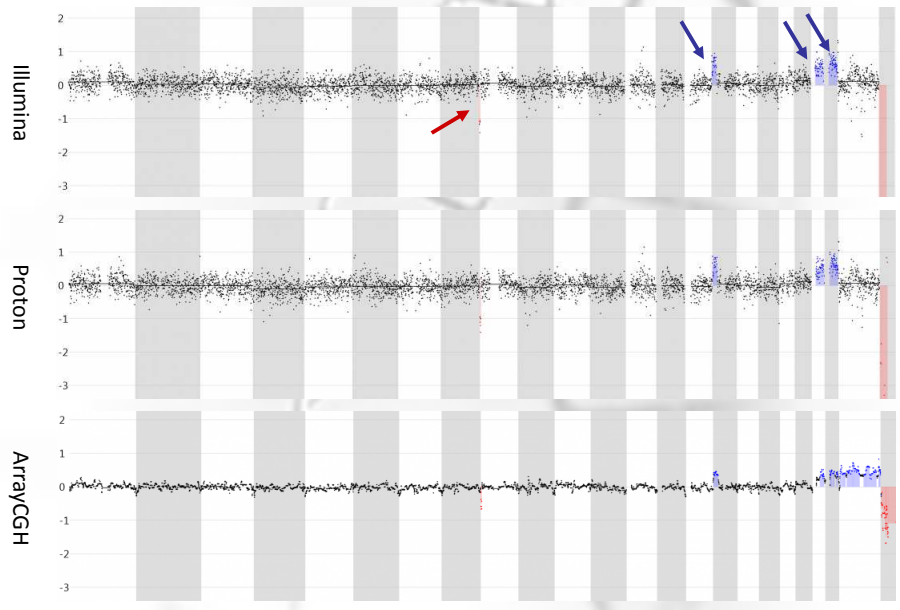
Results

→ 3 methods, same result



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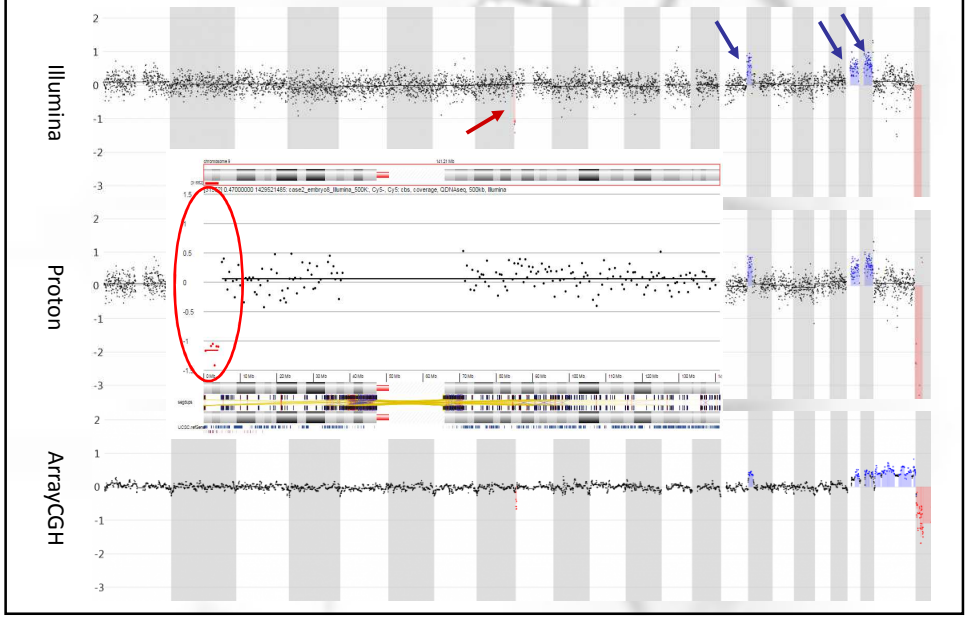

Results

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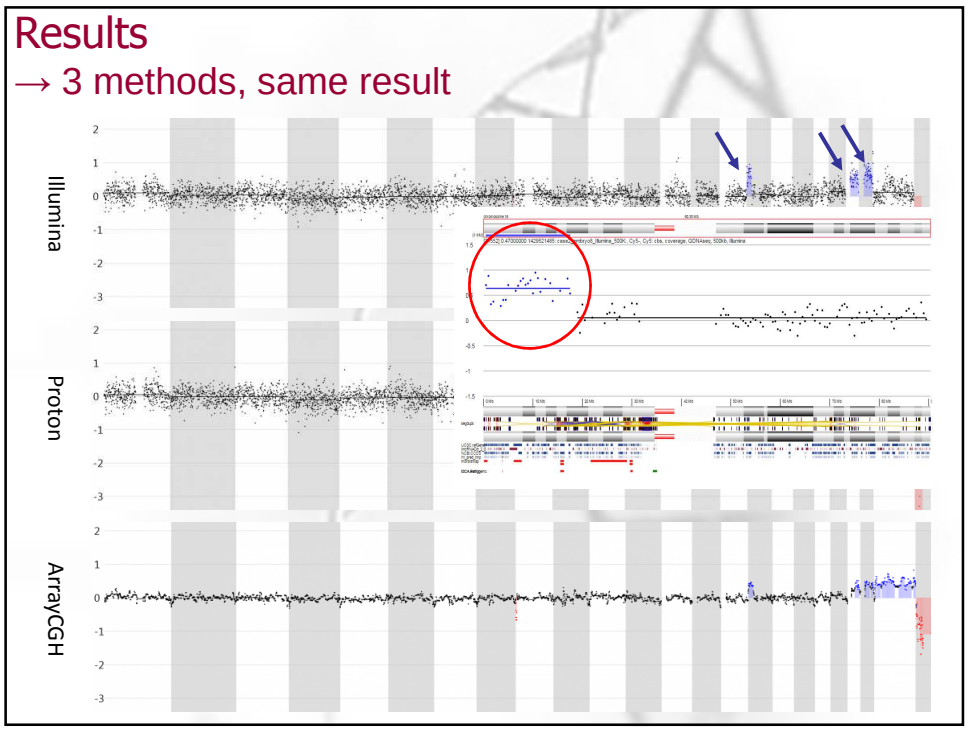
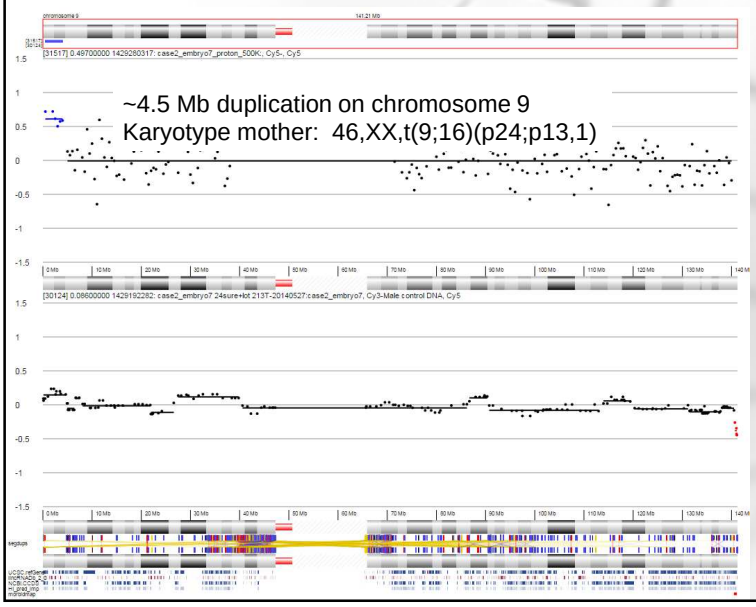
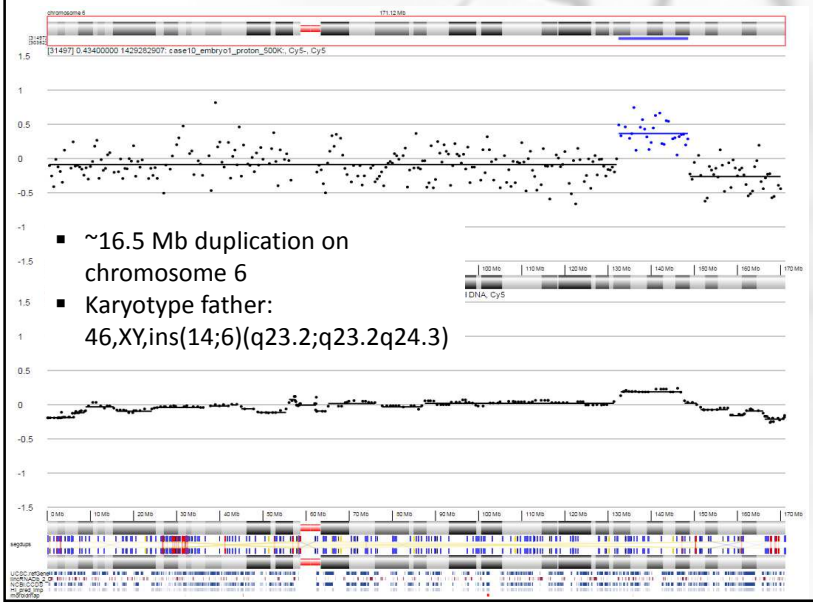


Results
→ Higher resolution in MPS

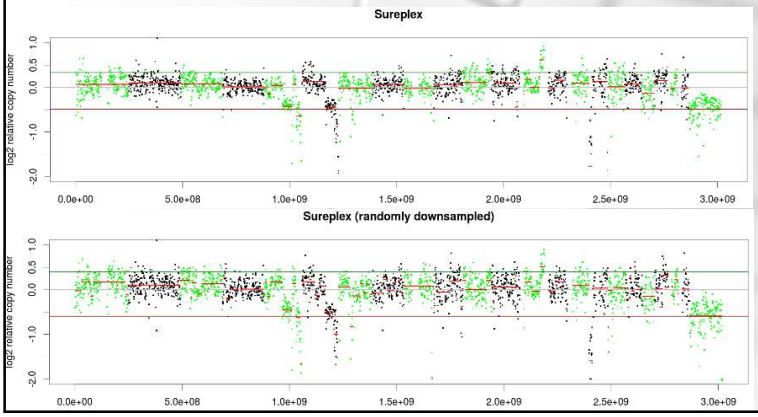


Results
→ Higher dynamic range in MPS



WGA is limiting resolution

- Parameters with minor effect:
  - Number of input cells
  - Library preparation
  - Sequencing technology
- Random downsampling of the reads, reducing data 10-fold
  → similar results



Conclusion

- Validated MPS in **47** trophectoderm samples
  - **Concordance** between **arrayCGH** and **MPS**
  - **Concordance** between **MPS technologies**
- **Higher resolution** and **better signal/noise** with MPS
- **WGA** is currently **limiting resolution**

Deleye L, De Coninck D, Christodoulou C, Sante T, Dheedene A, Heindryckx B, Van den Abbeel E, De Sutter P, Menten B, Deforce D, Van Nieuwerburgh F

Shallow whole genome sequencing is well suited for the detection of chromosomal aberrations in human blastocysts.

Fertil Steril. 2015 Nov;104(5):1276-85.e1.

Acknowledgements

- **NXTGNT,** Ghent University, Belgium

Dieter De Coninck, Filip Van Nieuwerburgh, Dieter Deforce, Sarah De Keulenaer, Ellen De Meester, Karoline Kimpe, Lieselot Deleye

- **Center for Medical Genetics,** Ghent University, Belgium

Annelies Dheedene, Tom Sante, Björn Menten

- **Department for Reproductive Medicine,** Ghent University, Belgium

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