Optimized NGS based protocol for the detection of small duplications/deletions in preimplantation embryos from carriers of balanced translocations and inversions

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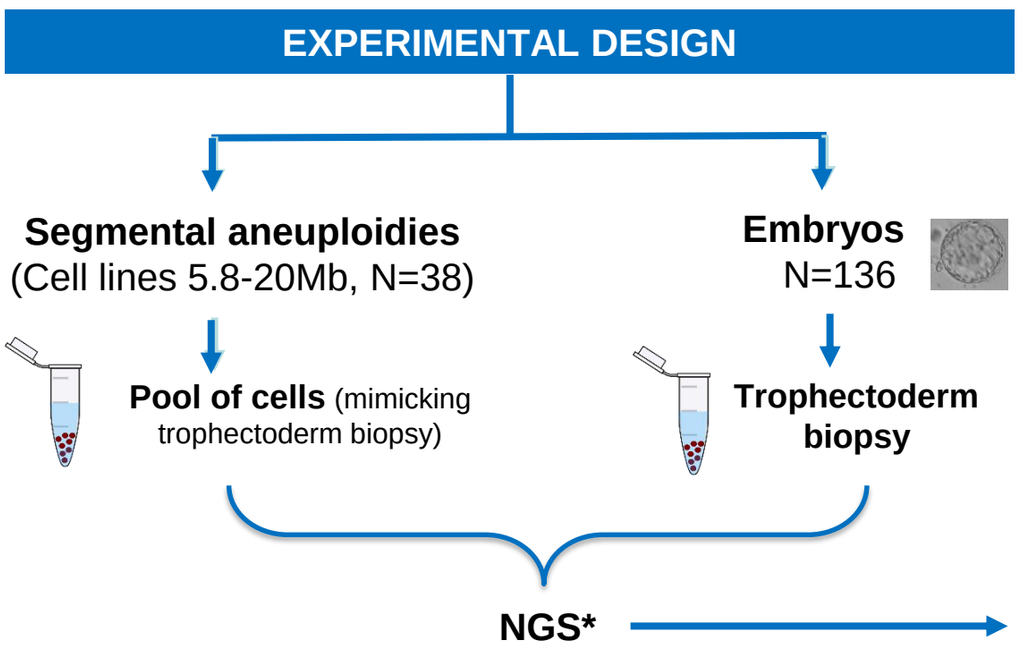
Is it possible to develop an accurate, automated and fast Next Generation Sequencing (NGS) based Preimplantation Genetic Testing for structural rearrangements (PGT-SR) protocol?

Introduction: Chromosome translocations are the most frequent structural chromosomal abnormalities in humans. In carriers of balanced translocations and inversions, small duplications and deletions can arise because of adjacent meiotic segregation. Array-CGH and FISH have been used to detect these alterations. Currently, NGS is widely applied in Preimplantation Genetic Testing for Aneuploidy (PGT-A). However, improved NGS protocols are needed to increase resolution for **PGT-SR** in order to detect deletions/duplications ≥6Mb.

M&M: An **optimized NGS protocol to detect unbalances ≥6Mb** was developed and validated.



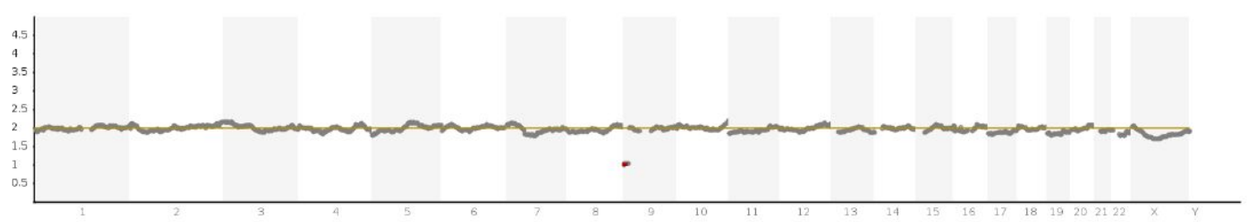
NGS* analysis general steps

Library prep	Template prep: Ion Chef System	Sequencing: Ion GeneStudio S5 System	Analysis: Ion Reporter Software v5.4 or higher
Whole-genome amplification and fragment library construction with Ion SingleSeq™ Kit	Isothermal amplification with Ion Chef System	Sequence up to 96 preimplantation embryos with Ion 530 Chip	Data analysis and storage with Torrent Suite™ Software v5.4 or higher and Ion Reporter Software and server

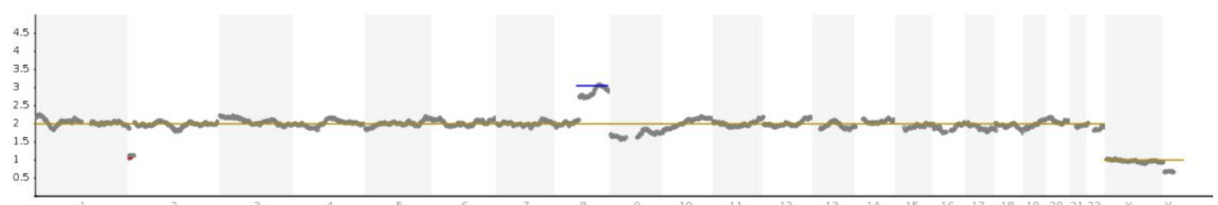
A modified Reproseq protocol was employed for genome amplification, library preparation and purification, using Ion Chef and S5 sequencer (Thermofisher). A customized workflow was developed using the Ion Reporter software version 5.4 for analysis and interpretation of the sequencing data.

Results: Using the pools of cells with deletions of different sizes, we set the detection limit of our protocol in 6Mb. **The percentage of detection was 100%.** In trophoblast biopsies from 35 patients, we analysed 136 embryos and 99.3% of them were informative. The percentage of unbalanced embryos was 44.8% (61/136). Full chromosome aneuploidies for chromosomes not involved in the rearrangements were observed in 38.9% (53/136) of the biopsies: 16.9% (23/136) of them in unbalanced biopsies and 22.1% (30/136) in balanced biopsies. Additionally, **this protocol can be completed in 12 hours**, from the preparation of the sample (lysis, preamplification, amplification, purification, pooling and quantification) to the release of the results.

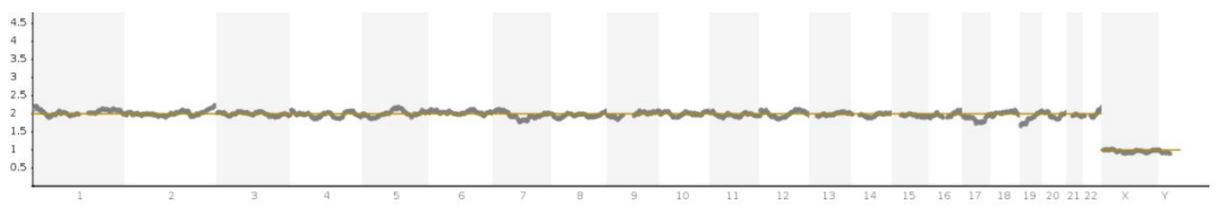
Deletion, 16 Mb



Abnormal unbalanced embryo (paternal Karyotype: 46,XY,t(2;8)(p23;q13))



Normal embryo



Wider implications of the findings: Formerly, FISH or array-CGH were the techniques used for PGT-SR. Here in, we describe an improved, mostly automated, fast, and accurate protocol for detecting small del/dup up to 6Mb. An additional advantage is that PGT-A and PGT-SR could be performed with similar equipment.

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