



THE PERKS OF GOING TARGETED: SAMPLE CONTAMINATION, DNA FINGERPRINTING AND CHROMOSOMAL MOSAICISM ACCURATELY PREDICTED BY TARGETED NGS-BASED COMPREHENSIVE CHROMOSOME SCREENING

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OBJECTIVE: Most current NGS-based CCS platforms are limited to chromosome copy number analysis due to shallow sequencing resulting from an initial step of random DNA amplification. Using a targeted amplification approach capable of genotyping targeted loci, this study applies a model of a trophoctoderm (TE) biopsy in order to validate the detection of sample contamination and chromosomal mosaicism. In addition, it evaluates if genotyping data can be reliably used to perform DNA fingerprinting, correctly identifying the transferred embryo and its parents, all in parallel to CCS.

DESIGN: Retrospective and prospective blinded.

MATERIALS AND METHODS: TE biopsy model: 6-cell mixtures of 2 different cell lines were prepared in the following ratios (0:6, 1:5, 2:4, 3:3, 4:2, 5:1 and 0:6). First mixture had cells with trisomy 13 and 15, the second one cells with trisomy 18 and a normal female karyotype. Samples were blindly sequenced by targeted NGS (TNGS). Mosaicism predictions were performed and a contamination score (CS) estimating the deviation from 0.5 of heterozygous allele frequencies was calculated for each sample. Fingerprinting: 32 single embryo transfer cases with sequenced data from both parents, all embryos and the newborn were analysed for validation. Genotype calls were retrieved and identity by state (IBS) scores were blindly calculated among all samples. Self, sibship and parentage predictions were made and compared to actual relationships.

RESULTS: TNGS sensitivity to detect aneuploidy was 100% for mixture levels of 100, 83 and 67%. It decreased to 93.5, 69.6 and 10.0% for 50, 33 and 17% mixtures respectively. Overall specificity was 100%. 6-cell aliquots of same cell line had the lowest CS (0.078 ± 0.003), whereas samples with at least one different cell presented significantly higher CS (1:5, 0.157 ± 0.004 ; 2:4, 0.190 ± 0.003 and 3:3, 0.190 ± 0.005 , $p < 0.0001$). In all cases the newborn's genotype matched the transferred embryo's (IBS1 and IBS0=0). All 188 embryos analysed were correctly assigned to their respective parents (IBS0=0).

CONCLUSIONS: To our knowledge this is the first time that detection of sample contamination, chromosomal mosaicism and DNA fingerprinting are validated as concomitant applications of NGS-based CCS. Further studies addressing if contamination may result in misdiagnosis and impact outcomes should be carried out, similarly with putative mosaics. The ability to correctly identify which embryo was transferred and its parentage comes to aid in solving cases of alleged gamete/embryo mix-up, reveal cases of spontaneous conceptions and the discovery of biomarkers for reproductive potential.