

The prospect of multi-purpose single cell genetic analysis using whole genome amplification and comparative genomic hybridization

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Genetic analysis using whole genome amplification (WGA) and comparative genomic hybridization (CGH) allows a comprehensive analysis of chromosomal imbalances across the whole genome from a single cell. The aim of this study is to verify the feasibility of applying these techniques to a single cell and investigate the prospect of a novel strategy using whole genome amplification (WGA), nested PCR and CGH to increase the scope and capacity of single cell genetic analysis.

The amplification power of DOP-PCR from a single cell (5 pg) resulted in 10 µg yield with the bulk of DOP-PCR products between 200-2000 bp. Thus, numerous specific loci and the imbalance of every chromosome can be assessed in a single cell. To investigate the feasibility of locus detection from WGA products, two sets of nested PCR aiming at sex determination and CF $\Delta F508$ detection were optimised and tested on a range of DNA sources. The results proved that a strategy of nested PCR could be used to determine the sex and CF status on DOP-PCR-amplified DNA derived from single cells.

The feasibility of CGH in the diagnosis of major chromosome abnormalities, such as trisomy 21 and 18, from a single cell was established in this study. To produce successful CGH experiments, the normal reference DNA could be made from either amplified DNA or non-amplified DNA. However, this study demonstrated that a reliable non-amplified reference DNA could increase the success rate of single cell CGH and help to identify the underlying causes of failed CGH experiments. For quantitative analysis of single cell CGH experiments, the average number of false positives was 5 when threshold of 1.2/0.8 was used.

The sensitivity and reliability of CGH was further tested on 10-20 stained cells scraped from slides in order to simulate the current strategy used in the non-invasive prenatal diagnosis. This part also involved firstly, the correct diagnosis of trisomy 18 and 21 and secondly, several coded samples of known sex were tested for the performance of single cell CGH. The results showed that chromosomal aberrations such as isochromosome X and segmental aneuploidy could be reliably detected. Thirdly, a manufactured mosaicism designed by mixing trisomy 18 male cells with normal female cells was tested to see if at 50-70 % mosaic level the trisomy could be detected by CGH. The results illustrated that trisomy 18 could not be detected at the 70% mosaic level using the current protocol. This may illustrate that accurate identification of cells of fetal origin is mandatory if current DOP-PCR/CGH techniques are to be applied in the field of non-invasive prenatal diagnosis.

The success of overnight CGH illustrates that, experimentally, further reduction of time is possible. This may allow an expansion of its future application in preimplantation genetic diagnosis (PGD).

Overall, this study demonstrates that DOP-PCR/nested PCR/CGH has the potential to serve as a powerful supplement to the present genetic analysis from a single cell. Concomitant detailed chromosome analysis and specific locus detection may become feasible in the field of PGD and non-invasive prenatal diagnosis in the near future.