IMPROVED DNA AMPLIFICATION USED FOR HUMAN KARYOMAPPING TO PHASE SINGLE GENE DEFECTS

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Karyomapping allows to phase single gene defects in embryos and is performed when a couple has a family history of a genetic disorder. In most cases, the method is applied on a single cell or small number of cells from an embryo during preimplantation genetic diagnosis. One obstacle when performing karyomapping on single or few cells is the small amount of sample DNA. Whole genome amplification (WGA) can be used to overcome this limitation. The performance of WGA is essential as the quality of amplified DNA affects karyomapping results. To improve reliability and reproducibility of WGA and subsequent karyomapping experiments we developed an advanced version of the Phi-29 amplification system and added a single cell storage buffer optimized for collecting and storing eukaryotic single cells.In this study we have validated the performance and robustness of the new WGA components and protocol (REPLI-g Advanced DNA Single Cell Kit, QIAGEN) and its compatibility with subsequent karyomapping experiments. For karyomapping we performed SNP genotyping of 300.000 SNP markers across human genome using the Human KaryoMap-12 SNP array (Illumina) and used the corresponding QC metrics for validation of WGA performance. These QC metrics comprised overall call rate, AB rate, Allelic drop out rate and Miscall rate.We analyzed numerous cases (on average 2 - 9 embryos per case) with family history of different genetic disorders e.g. fragile X syndrome, cystic fibrosis or nephronophthisis. Overall, the QC metrics of all cases displayed good quality of the amplified sample DNA from parental origin as well as from embryo biopsies. In addition, the karyomapping could be used to identify embryos free of chromosomal aneuploidies, which frequently occur in human embryos.Our study confirms that the improved DNA amplification is highly suitable for human karyomapping and gives reliable and reproducible results for phasing of single gene defects.