A cost effective screening method for various single gene defects in single cells by using high magnesium and total ionic strength and restriction enzymes

蔡郁惠
Tsai YH

Abstract

A reliable cost-effective protocol for the diagnosis of various defective genes in single blastomeres from preimplantation embryos has been established. Single cells were lysed in alkali buffer followed by neutralization and addition of a solution containing a high concentration of sulfhydryl reducing agents and MgCl2 in relatively high ionic strength (0.45) (solution M) with or without restriction enzyme(s). The reaction mixture was incubated at 37°C for 15 min followed by heat denaturation at 95°C for 10 min. Respective polymerase chain reaction (PCR) mixture was then added to amplify each designated DNA region. The treatment of neutralized single cell lysate with adequate restriction enzyme(s) which do not cleave the target DNA sequences but shortens the genomic template DNA strands. This may facilitate primer-template annealing. The subsequent heat denaturation of the cell lysate in solution M indeed gave better signals of amplified DNA fragments on polyacrylamide gels. Defects in Tay Sachs exons 11 and 12, CF-AF508 and CF-N1303K, and genomic sequences of ZFX/ZFY were successfully detected on gels after one-step PCR amplification, especially those cell lysates treated with restriction enzymes. In conclusion, a cost-effective one-step PCR method for amplifying various specific genomic regions containing a single gene defect in single cells has been established. This protocol may be applied to genetic screening for many single defective genes of biopsied single blastomeres from preimplantation in vitro fertilization (IVF) embryos.