Cost-effective one step pcr amplification of cystic fibrosis d f508 fragment in a single cell for preimplantation genetic diagnosis.

蔡郁惠
Tsai YH

Abstract

The combination of in vitro fertilization (IVF) with PCR technologies enables diagnosis of single gene defects for preimplantation genetic diagnosis. This has been accomplished by two-step nested PCR, or PEP-PCR followed by nested PCR processes. To improve the detection of single cell genetic defects, the lysate of a single lymphocyte, with or without cystic fibrosis F508 mutation (CFF508), was incubated in a higher ionic strength solution containing mercaptoethanol prior to the addition of primers to the denatured cellular DNA. A single cell in 5 µl lysis buffer was incubated at 65°C for 15 min, cooled, and neutralized with an equal volume of neutralizing buffer. A 5 µl aliquot of a solution X containing 50 mM MgCl2, 1 M NaCl, and 10 mM mercaptoethanol was added to the neutralized cell lysate, followed by incubation at 93°C for 15 min. The step was crucial to the successful amplification of CFF508 DNA fragment. The incubation of cell lysate in solution with the high level of sulphydryl reducing agent and a high ionic strength of about 0.45, at 93°C for 15 min, might denature many chromatin-binding proteins and also ensure the complete dissociation of dsDNA. After the addition of PCR mix, the resulting reaction mixture still contained a sufficient level of sulphydryl reducing agent and 0.135 total ionic strength. This might reduce significantly the interference of various protein factors with DNA, and favour the primer-template annealing. The efficient initial annealing of the primers to target DNA sequences would facilitate PCR amplification efficacy. In conclusion, in more than 80 single cells tested (apart from one) the CFF508 defect was successfully demonstrated with the present protocol (>99 per cent), without using fluorescent primers and expensive automatic instrumentation.
Copyright © 1999 John Wiley & Sons, Ltd.