Gender identification of mouse embryos using the Polymerase Chain Reaction (PCR) technique

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Abstract

A multiplex nested PCR technique was used to identify gender from single blastomeres biopsied from 8-cell mouse embryos. The primers amplified sequences of the Sry and Zfy genes on the Y-chromosome, and a polymorphic X chromosome microsatellite locus (DXNds-3). Amplification of male DNA gave three bands, of sizes 217 bp (Zfy), 147 bp (Sry) and 111 bp (Nds). In contrast, amplification of female DNA gave a single band of 111 bp (Nds). Test on blood DNA showed a 98.0% PCR amplification efficiency whereas single blastomeres produced 93.0% PCR amplification efficiency. In conclusion, mouse embryos were successfully sexed within 6 hours by using single blastomeres obtained from 8-cell embryos.

Introduction

Advances in molecular technology have enabled the iden-tification of gender as well as recognition of the genetic make-up in early preimplantation embryos. Consequently, inherited sex-linked disorders of the developing embryo may be determined before implantation occurs. Preim-plantation Genetic Diagnosis (PGD) is extremely useful for the selection of embryos derived from in vitro fertili-zation (IVF). Early PGD trials involved gender identifica-tion of embryos to detect genetically inherited X-linked diseases [1]. Since then, PGD has encompassed the diag-nosis of many genetic disorders such as sickle-cell anaemia [2], Duchenne muscular dystrophy [3] and β-thalassaemia [4]. The objective of this study was to develop a method to identify gender from single blastomeres sexed at the 8-cell preimplantation stage.

Material and Methods

Blood and embryos were collected from 6 to 8-week-old ICR mice. DNA extraction from whole blood was carried out using QIAAmp DNA Mini Kit (QIAGEN, GmbH, Germany). After serial dilution, 0.08 ng/µl was used as template in the PCR. Sry, Zfy and Nds primers [5,6] were used in the PCR. Following PCR optimization with blood DNA, single blastomeres were used as template. Su-perovulation was accomplished by intraperitoneal injec-tion of 10 IU pregnant mare serum gonadotropin (PMSG; Folligon, Intervet, Netherlands) followed by 10 IU human chorionic gonadotropin (hCG; Chorulon, Intervet) at 48 hours apart. Treated females were then cohabited over-night with males of proven fertility at a ratio of 2:1. The following morning, females with vaginal plugs were se-lected as embryo donors. Two-cell embryos were flushed out of the oviducts at 36 hours post coitum, and trans-ferred to individual 10-µl drops of M2 medium. Single blastomeres were recovered following biopsy of 8-cell embryos. They were subjected to digestion with 0.5% pronase in M2 medium (3 minutes) and then moved to 4 µl lysis buffer (20 mM Tris, pH 8, 0.9% Tween 20, 0.9% Nonidet, 0.4 mg/ml proteinase K) in 0.2 ml microfuge tubes. Tubes were placed in thermal cycler to lyse blas-tomeres (at 65°C for 10 minutes and at 94°C for 10 min-utes) before PCR. Lysed blastomeres were then utilised as DNA template.

The PCR utilized autoclaved ultrapure water to make up a total of 15 µl, 10 x PCR buffer (50 mM KCl, 10 mM Tris- HCl, pH 8.3), 1.5 mM $MgCl_2$ (Promega, USA), 200 mM dNTP (Promega, USA), 20 µM primers (Genemed, USA), 1 U Taq polymerase (Taq B, Promega, USA). Denatura-tion was performed at 95°C for 3 minutes, PCR for 30 cycles at 95°C for 1 minute, annealing at 55°C for 1 min-ute, 1 minute at 72°C, followed by final elongation at 72°C for 10 minutes.

Amplicons were run on 3% agarose gel (Promega, USA) at 98 V for 2 hours, stained with ethidium bromide and examined under ultraviolet light.

Results

Female DNA (internal control) produced only one band (111 bp) due to amplification of DXNds3 on chromosome X. Males produced three bands of 111 bp (DXNds3), 147 bp (Sry) and 217 bp (Zfy) sizes (Figure 1). The latter two are sequences on the sex determining regions on the Y chromosome. PCR amplification efficiency was found to be 98% (49/50) with blood DNA and 93% (93/100) with single blastomeres.

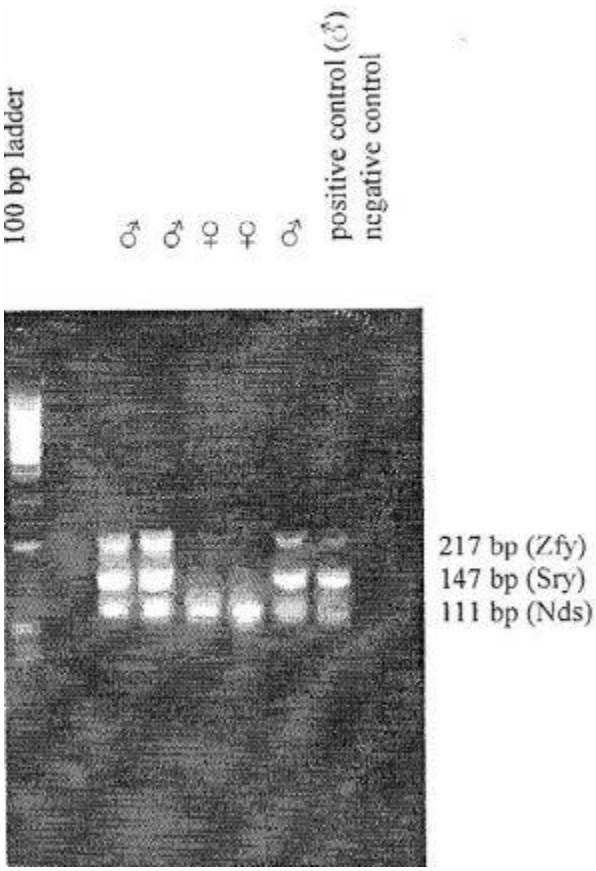

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Fig. 1: Amplicons obtained after PCR

Discussion

The primers used simultaneously amplified two Y-specific primers and a common X-specific primer. Con-current use of the primers minimizes incorrect interpreta-tion of gender. The use of a multiplex PCR in a small re-action volume of 15 µl reduces contamination risk and assay time, while intensifying amplicons on gel [6]. The use of nested PCRs enhances specificity of PCR.

PCR efficiency was found to be very high with blood DNA (98%) as template and slightly less (93%) when single blastomeres were used. Lower efficiency with blas-tomere DNA may be the consequence of various factors such as ineffective lysis procedure, loss of blastomere during transfer to reaction tubes or lack of DNA in anu-cleated blastomeres [6]. To overcome the problem of sel-ecting anucleated blastomeres, some researchers recom-mended visualization of nuclei by staining or microscopy [7].

Apart from the factors mentioned above, the denaturation step in PCR cycles is critical for reliable DNA amplification [8]. Amplification efficiencies were seen to drop drastically from 95% to 60% when denaturation at 97°C for 2 minutes was omitted [6].

This is because the high temperature eliminates allele drop-out (ADO) in single-cell analysis [8]. In the present study, a temperature of 95°C for 3 minutes was used successfully to denaturate DNA template for better binding with primers and more efficient Taq polymerase action. This study also recorded that the lysis buffer containing proteinase K and deter-gents is able to lyse cells and releases DNA efficiently. This lysis buffer seems to perform better and is widely used [9].

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