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DNA extraction from mixtures of body fluid using mild preferential lysis

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Summary. A modification to the DNA extraction method "preferential lysis" (Gill et al. 1985) is proposed which can be applied to DNA mixtures of vaginal cells and spermatozoa. In mixtures with a low sperm content the further loss of sperm DNA caused by the extraction can be avoided by using "mild preferential lysis". Amplification by PCR (polymerase chain reaction) then yields sufficient DNA to be able to identify both components in the mixture.

Key words: DNA extraction – Mild preferential lysis – PCR

Zusammenfassung. Vorgestellt wird eine Modifikation der DNA-Extraktionsmethode „Preferentielle Lyse" (Gill et al. 1985) die üblicherweise bei Vorliegen von Vaginalzell/Sperma-Mischungen angewendet wird. Bei Mischungen mit nur geringem Spermienanteil sollen extraktionsbedingte Verluste von Sperma-DNA durch Anwendung einer „milden preferentiellen Lyse" vermieden werden. Der Nachweis erfolgte durch PCR-Amplifikation der extrahierten DNA-Proben.

Schlüsselwörter: DNA Extraktion – Milde preferentielle Lyse – PCR

Introduction

In stain work it is quite common that the DNA yield is low and the quality poor. In these cases it is unlikely that sufficient high molecular weight DNA can be obtained for RFLP analysis but PCR offers a realistic alternative. However with mixed samples, such as vaginal swabs contaminated with semen, vastly different DNA concentrations can be present and this can lead to an amplification of the major component (vaginal cell DNA) whereas the minor component (sperm DNA) does not amplify. In our experience the use of preferential lysis can result in a further loss of sperm DNA and subsequent loss of valuable evidential material.

A modified "mild" preferential lysis is proposed in which the male and female component are not separated but the female DNA is reduced while the amount of male DNA remains constant.

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Materials and methods

Vaginal swabs were taken 1–2 days after sexual intercourse and dried overnight at room temperature. Part of the stain extract was stained (haematoxilin and eosin) and the density of sperm heads/cells quantified microscopically into 4 groups: "1+" = isolated heads in few microscopic fields, up to "4+" = many sperm heads in every field.

Swab extraction. The cotton wool swabs were teased apart and extracted in a reaction tube (1.5 ml) for 15 min with 338 µl lysis buffer I (10 m*M Tris*-HCl pH 8.0, 0.4 *M* NaCl, 2 m*M* EDTA). The amount of proteinase K (2 mg/ml) added varied depending on the spermatozoa concentration and ranged from 20 µl (sperm count 1+) to 50 µl where spermatozoa were abundant (4+). To this solution 12 µl SDS (20%) was added and thoroughly mixed. Incubation was carried out for 40 min at 37°C in a shaking water bath. The swab material and the extract were transferred to a pierced reaction tube (0.5 ml) which was placed in a larger tube (1.5 ml) and centrifuged for 5 min at 13,000 rpm to separate the extract from the substrate (piggy back method; Kimes and Tahir 1985). The extract was then centrifuged for a further 15 min at 13,000 rpm. The supernatant was extracted in phenol/chloroform/isoamyl alcohol (24:24:1) as described previously (Sambrook et al. 1989; Brinkmann et al. 1991). The main alterations to the preferential lysis described by Gill et al. (1985) were to avoid washing stages and reduce DNA transfer steps to minimise sperm loss. Furthermore the conditions of lysis were less stringent to reduce the probability of sperm lysis during the first stage.

DNA extraction. The sediment together with the original stain substrate was placed in a 0.5 ml tube and mixed with 313 µl lysis buffer II (50 µl proteinase K, 12 µl SDS and 25 µl DTT). After thorough mixing, the contents were incubated for 1 h at 56°C in a shaking water bath. The tube was pierced, placed in a larger tube (1.5 ml) and centrifuged for 5 min at 13,000 rpm to remove the liquid phase

from the stain substrate. DNA was purified using the phenol/chloroform/isoamyl alcohol extraction procedure described previously.

Precipitation of DNA was carried out with 0.1 vol 3*M* Na acetate (pH 5.2) and 2.5 vol ice-cold absolute ethanol, air dried and resuspended in 20–50 µl bidestilled water. The concentration of DNA was measured fluorimetrically and degree of fragment degradation of the DNA was assessed by electrophoretical separation in a 1% agarose gel by comparison with known standards (DRIgest III, Pharmacia).

The extracted DNA was used for PCR amplification with the AMPFLP (amplified fragment length polymorphism) system YNZ22 (Wolff et al. 1988; Horn et al. 1989). The conditions of PCR were as follows (B. Budowle, pers. com.):

- 100 ng template DNA
- 2.5 U Taq DNA polymerase (Promega corporation, USA)
- 0.5 µ*M* primer 1 (5′-AAACTGCAGAGAGAAAGGTCGA-AGAGTGAAGTG-3′)
- 0.5 µ*M* primer 2 (5′-AAAGGATCCCCCACATCCGCTCCC-CAAGTT-3′)
- 5 µl 10 × Taq DNA Polymerase buffer (500 m*M* KCl, 100 m*M* *Tris*-HCl pH 9.0, 15 m*M* $MgCl_2$, 0.1% gelatin, 1% Triton X-100)
- 200 µ*M* each dNTP
- addition of Aqua bidest to a final volume of 50 µl
- 30 µl oil overlay
- Temperature: 1 min – 94°C (denaturing)
  1 min – 63°C (annealing)
  6 min – 72°C (extension)
- Cycles: 27
- Thermo-cycler: Triothermoblock (Biometra, Göttingen, FRG)

The amplified DNA fragments were separted in 6% PAG (prepared after Sambrook et al. 1989 with the exception that 0.35 *M* *Tris* sulfate pH 9.0 was used in place of TBE) and visualised by silver staining (Budowle et al. 1991).

Results and discussion

After single lysis extraction (1-step extraction with proteinase K, SDS and DTT) of vaginal swabs containing few spermatozoa (microscopically: isolated spermatozoa in most fields) both male and female bands were visible (Fig. 1a, lane 4) but the male bands were much weaker. After mild preferential lysis of the same extract both band patterns were of approximately equal intensity (Fig. 1a, lane 3). The female proportion was strongly reduced while the male bands remained constant. In a further example with a very low proportion of spermatozoa in the vaginal smear only the female fragments could be seen after single lysis (Fig. 1b, lane 3). After mild preferential lysis 2 additional fragments could be seen (Fig. 1b, lane 4) which corresponded with the band pattern of the blood DNA from the male in question.

Using this method, it was also possible to amplify larger fragments, which were not visible after amplification of DNA extracted with single lysis.

If many spermatozoa are present in a vaginal swab preferential lysis can be used, because relatively small amout of sperm lost will not be disadvantageous.

In our preliminary experience a standard procedure for the swab extraction step using 30 µl proteinase K

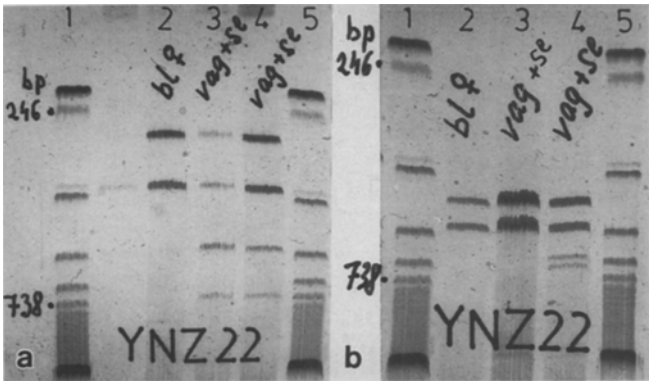

Fig. 1a, b. Amplified DNA fragments from the system YNZ22 after separation in PAG and silver staining. **a** lane 1 = size marker (123 bp ladder, Gibco-BRL); lane 2 = female blood control DNA; lane 3 = swab extraction, mild preferential lysis; lane 4 = swab extraction, single lysis; lane 5 = size marker. **b** lane 1 = size marker; lane 2 = female blood control DNA; lane 3 = swab extraction, single lysis; lane 4 = swab extraction, mild preferential lysis; lane 5 = size marker

(2 mg/ml) and 40 min incubation time at 37°C should be successful.

These are at present only observations based on a few examples and must therefore be considered as preliminary results but indicate that the use of a milder extraction method prior to PCR analysis can substantially improve the yield and quality of amplifiable DNA from mixtures. This mild extraction method is obviously not exclusive to PCR techniques and can also be applied prior to RFLP analysis.

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