RAPID AIR CYCLING OF D1S80 AMPLIFICATION

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Introduction

In order to obtain a suitable PCR product, the DNA amplification method is currently performed over a period of 2 to 6 hours with metal block PCR machines. The length of the procedure is partly due to the time needed to heat or cool the samples.
A new rapid cycling system "Idaho ATC 1605" is now available. It is based on heat transfer by air to samples contained in thin glass capillary tubes. Air is an ideal heat transfer medium and the temperature changes quickly because of its low density. The glass capillary tubes have a better surface-to-volume ratio and the heat conductivity is higher than with polypropylene microfuge tubes. Thus, the total time required for the polymerase chain reaction is significantly decreased (1, 2, 3). Some authors have already used the air cycler to rapidly amplify DNA from E. Coli (4), from Aeromonas Salmonicida (5), the ΔF508 locus in patients with Cystic Fibrosis (6), and mRNA (7).
Results of assays aimed at the optimization of D1S80 amplification using the air cycler are presented here. Different parameters including temperature and time of denaturation, annealing and elongation were tested. Furthermore, the effects of additives such as Perfect Match polymerase enhancer, Triton, DMSO, PEG, Formamide, Glycerol and Tween 20 on the PCR efficiency were also investigated. The application is illustrated through casework examples.

Materials and Methods

PCR reaction parameters

D1S80 amplification of 20, 40, 60, 80 and 100 ng of K562 genomic DNA was performed with the Idaho air cycler. Each 50 µL sample contained 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatine, 0.4 µM of each primer, 200 µM of each dNTP, 0.016% BSA, and 2U Taq polymerase (Promega). Each sample was placed in 10 cm long, thin-walled capillary tube, the end of which was fused with an oxygen-propane torch.

Time cycling conditions for the metal block cycler (Prem) over 30 cycles were as follows: denaturation 2 min at 94° C, annealing 1 min at 65° C, extension 8 min at 70° C, and final extension 10 min at 70° C.
Time cycling conditions for the air cycler over 40 cycles were as follows : denaturation 10 sec at 94 °C, annealing 10 sec at 65 °C, extension 60 sec at 77 °C, with final extension 10 min at 77 °C.
This standard protocol was used to investigate the effects of certain parameters with respect to the D1S80 PCR product.

PCR parameters investigated

Amplification was performed:
- with and without 2 min template denaturation at 100 °C before PCR
- with 30 or 40 PCR cycles
- with annealing at 60° C or 65 °C
- with 2U or 4 U Taq polymerase (Promega)
- with extension at 72 °C or 77 °C
- with and without final extension for 10 min
- with 1U Perfect Match polymerase enhancer (BRL)
- with 0.2% Triton, 4% DMSO, 4% PEG 8000, 4% Formamide, 8.7 % Glycerol or 2% Tween 20.

Each K562 amplified sample was tested by electrophoresis at 100 V for 45 min on a 2% agarose minigel with ethidium bromide.

Amplification of casework samples

DNA was isolated from blood and semen stains by Chelex extraction after slot-blot quantification : 5-10 ng were used for D1S80 amplification with the air cycler. Five µL of each amplified sample was tested by electrophoresis at 100 V for 45 min on a 2% agarose minigel with ethidium bromide. Genotypes were then determined by vertical hydrolink minigel electrophoresis using a discontinuous glycine/chloride buffer (8).

Results and Discussion

The following parameters were investigated with respect to the D1S80 PCR product :

Pre-denaturation
- a brief nucleic acid pre-denaturation lasting 2-5 min at 100 °C was necessary before rapid cycling if a short 10 sec denaturation time at 94 °C was used (Fig 1a).

Number of cycles
- the utilisation of 40 cycles compared to 30 cycles increased the D1S80 product without any problem of non-specificity or background (Fig 1b).

Annealing temperature
- the optimal annealing temperature was found to be about 64-65 °C, while at 60 °C no PCR product was obtained (Fig 1c).

Taq polymerase quantity
- the use of 2U Taq polymerase gave good results while 4U Taq polymerase increased the PCR product but also the non-specificity and the background. This non-specific priming and a primer dimerization especially occurred with 20 to 60 ng K562 template (Fig 1d).

Extension temperature
- the optimal extension temperature was about 77° C while an extension at 70° C resulted in a decreased PCR product of the larger fragment. Furthermore, a slight background and a dimer polymerization were also present (Fig 1e).

Final extension
- the 10 min final extension was necessary to increase the PCR product about twofold (Fig 1f).

Additives
- the use of 1 µL Perfect Match Polymerase Enhancer (BRL) produced a preferential and non-specific amplification with a three bands product (Fig 1g).
- Formamide and Glycerol inhibited the reaction while 0.2% Triton seemed to increase the D1S80 product. Tween 20, DMSO and PEG had little effect on the result (Fig 2).

Air cycler D1S80 amplification of routine cases
Casework examples involving sexual assault and burglary were successfully amplified with the air cycler after Chelex extraction. The denaturation step before the PCR was not necessary since the template was already single strand DNA (Fig 3). The genotypes were easily determined by vertical hydrolink minigel electrophoresis using a glycine/chloride buffer system.

Conclusion

With 40 cycles of 1 min 20 sec using the Idaho air cycler, D1S80 products were increased compared to the products obtained with 30 cycles of 11 min on a metal block cycler. Furthermore, in contrast to the conventional cycler, the air cycler amplification was more efficient with less template : the D1S80 product was higher with 20, 40 and 60 ng compared to 80 and 100 ng K562 DNA (Fig 4).

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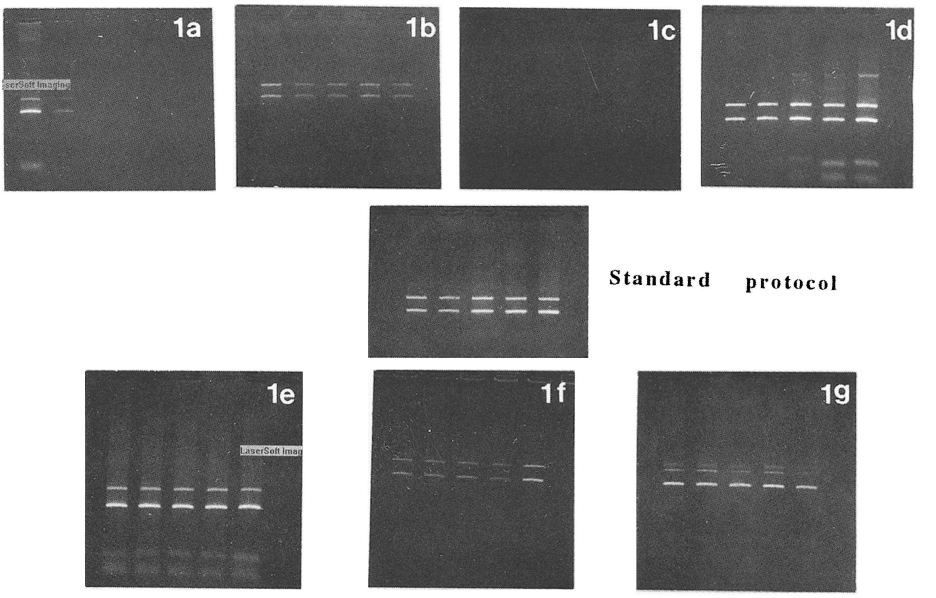


Fig 1. PCR parameters investigated. From left to right : 100, 80, 60, 40 and 20 ng of K562 genomic DNA compared with the standard protocol.

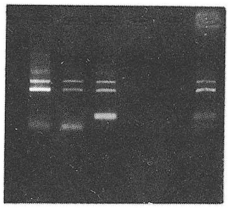
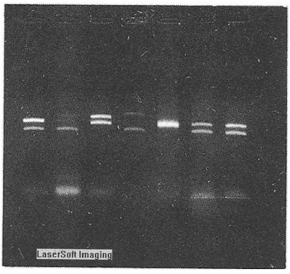


Fig 2. D1S80 amplification with additives. From left to right : 0.2% Triton, 4% DMSO, 4% PEG 8000, 4% Formamide, 0.7% Glycerol, 2% Tween 20.

Fig 3. Air cycler D1S80 products from two sexual assaults and one burglary obtained with the standard protocol.
From left to right : **Rape case** : victim blood, semen stain, suspect blood; **Burglary case** : blood stain; **Rape case** : victim blood, semen stain, suspect blood.



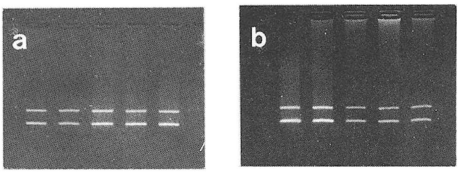


Fig 4. Comparison between the air cycler and the metal block cycler with 100, 80, 60, 40 and 20 ng K562 genomic DNA : a) D1S80 products obtained with the air cycler, b) D1S80 products obtained with the metal block cycler.