Technical Report

Self-sealing filter tips

Possible inhibition of PCR reactions

Introduction

Pipette tips with filters are often used for PCR-type reactions to prevent cross-contamination of samples. Most filter tips contain pure, polyethylene filter matrices, but self-sealing filters also employ cellulose gum additives which "seal" on contact with liquids. These additives are not bound to the filter and can easily be deposited into samples during regular use.

The purpose of this experiment was to quantify the inhibition of PCR reactions by filters containing cellulose gum additives and those containing only polyethylene. PCR reaction mixtures were prepared using aqueous wash extracts from both self-sealing filters and filters containing pure polyethylene. These results were compared to PCR mixtures prepared using untreated, distilled water.

Materials and methods

Filters from Molecular Bio-Products ART-200 pipette tips (batch #832010) containing their proprietary cellulose-gum sealing additive were washed with 50 µl of distilled de-ionized water and incubated at room temperature for 15 minutes. The same procedure was performed on filters from RAININ aerosol-resistant tips (catalog #RT-200F, lot #4698G) containing no additives.

Each wash extract was used to prepare identical 100 microliter PCR reaction mixtures containing: 1 pg of human genomic DNA (Clontech), 10 µl of 10X PCR Buffer (Perkin-Elmer), 5 µl of 20 mM human primer sets (Research Genetics), 10 µl of 25 mM $MgCl_2$, 8.0 µl of 10 mM dNTP (Perkin-Elmer),and 0.5 µl of Amplitaq Gold (Perkin-Elmer).

Identical PCR mixtures were prepared using distilled, deionized water in place of filter extracts for use as a positive control. A negative control was prepared using the same PCR mixture minus human genomic DNA.

PCR mixtures were subjected to 40 thermal cycles using a GeneAmp PCR System 2400 thermocycler at 94° Celsius for 20 seconds, 50° Celsius for 20 seconds and 72° Celsius for 20 seconds.

100 microliters of each mixture was loaded onto a 4% agarose gel containing ethidium bromide (0.5 µg/µl) and run for 20 minutes at 80 V. Bands were excited at 302 nm, and imaged using a Kodak Digital Science EDAS 120 System.

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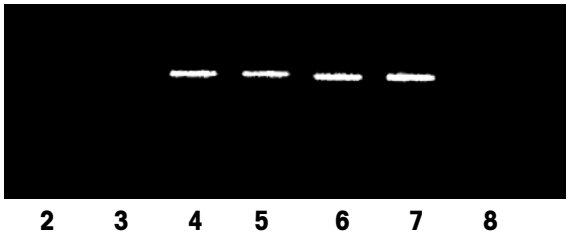
Results

Lane 8 is the negative control – the DNA-free PCR mixture. Lanes 6 and 7 are duplicate positive controls. Lanes 4 and 5 are PCR reaction mixtures composed of extracts from the RAININ pure polyethylene filter. Lanes 2 and 3 represent the PCR mixtures prepared with extracts taken from the ART filter containing cellulose-gum additives.



Lane No.	Content	% of Positive Control
2	Self-sealing filter	0
3	Self-sealing filter	
4	Pure polyethylene filter	94
5	Pure polyethylene filter	
6	Positive Control	N/A
7	Positive Control	

Conclusions

No DNA was detected in lanes 2 or 3. This indicates that the extracts taken from ART filters containing cellulose-gum additives completely inhibited the PCR reaction. This is especially problematic because of the tendency of the filter material to flake from the filter matrix during handling or regular use. Depending on the mechanism of the reaction, cellulose-gum additives may inhibit any reactions that contain DNA or enzymes. Future experiments will determine the extent of other inhibition reactions.