Applications

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Technical Report

Inhibitory effects of filter tips concerning PCR and real-time PCR

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Abstract

The use of various filter tips for preparation of PCR experiments can lead to significant decreases in the efficiency of DNA amplification. With a minimal effect on PCR reactions, ep Dualfilter T.I.P.S. are particularly well suited for preparing PCR experiments; this is evident with both classic PCR and real-time PCR.

Introduction

The polymerase chain reaction is a common application in molecular biology research. Various filter tips are well suited for the preparation of these types of experiments. However, it cannot be excluded that the filter material may come into contact with the PCR sample, which can negatively affect the efficiency of classic PCR and quantitative real-time PCR.

Filter tips are vitally important for preventing contamination during PCR experiments and other molecular biology applications such as bacteriology and work in radioactive areas. Primarily, they prevent the sample solution from contaminating the pipette cone during aspiration. They also prevent that contaminated aerosols from the pipette cone come into contact with, for example, a PCR sample if a contaminated pipette is used.

Figure 1: Incubation of a real-time PCR sample on a filter

Due to filter contact, general adhesive effects can cause specific sample agents to bind to the filter material, which means that these agents will no longer be available for the DNA amplification process.
Moreover, many filter tips contain self-sealing additives. These substances, which contain cellulose rubber, swell and close up when they come into contact with liquids. This causes sample loss because the sample material is encased in the filter and cannot be recovered. Furthermore, some self-sealing additives may dissolve when they come into contact with an aqueous phase, thus contaminating the sample. These factors are another source of potential losses in efficiency which can occur during a PCR experiment.
Non-self-sealing filter tips, such as the Eppendorf ep Dualfilter T.I.P.S., contain a pure PE filter. If improper pipetting and subsequent filter contamination occurs, the sample material is not aspirated and is therefore retained. Moreover, contamination due to self-sealing additives is impossible.

Here, it was investigated how the efficiency of classic and real-time PCR is affected by possible contamination due to the use of various filter tips.

Materials and methods

The inhibition of classic PCR and real-time PCR using ep Dualfilter T.I.P.S. and various other filter tips was tested.

Classic PCR

1. Filter contact with the PCR sample

The examination of the inhibition of classic PCR was carried out after the PCR sample came into contact with pipette tip filters under defined incubation conditions. A sterile needle was used to push the filters out of the tips into a 1.5 mL tube. 99 µL PCR mix (without dNTPs) were added to each filter and the samples were incubated in an Eppendorf Thermomixer (500 rpm, 1 min). 3 tips were investigated per supplier.

2. PCR

The PCR sample for the incubation contained the following components:

	Volume [µL]	Final concentration
HotMaster *Taq* buffer (5 Prime)	10.0	1 x
HotMaster *Taq* polymerase (5 Prime)	0.5	2.5 U/100 µL
Globin primer	4.0	0.4 µM each
Human DNA (Roche)	1.0	80 pg/µL
H_2O	83.5	

The dNTPs (0.1 mM final concentration) were added after the incubation. The PCR was then carried out using a Mastercycler pro (Eppendorf) with 20 µL as sample volume.
The following temperature profile was used:

1 min, 95 °C → 30 x (15 sec, 95 °C; 15 sec, 56 °C; 30 sec, 68 °C) → 4 °C

A 536 bp fragment of the human beta globin gene was amplified. The primers had the following sequences:

Primer forward: 5’ GCTCACTCAGTGTGGCAAAG 3’
Primer reverse: 5’ GGTTGGCCAATCTACTCCCAG 3’.

5 µL of the samples were electrophoretically separated in a 2.5 % agarose gel/1 x TBE. The gels were stained in an EtBr solution containing 50 µL 1.5 % EtBr in 1 L water.

real-time PCR

1. Filter contact with the PCR sample

The real-time PCR examination was also conducted after the PCR sample came into contact with the pipette tip filters. In the first step, the tips were cut off a bit on the lower end using scissors. Then, 30 µL PCR samples were inserted into the filter tips from below to cover the filters (Fig.1). After incubation in an Eppendorf Thermomixer (500 rpm, 1 min), the PCR samples were carefully resuspended, then removed from the filter and transferred to a 1.5 mL tube. 5 tips were examined per supplier; two technical replicates were inspected for each tip.

2. Real-time PCR

The PCR sample for the incubation contained the following components:

	Volume [µL]	Final concentration
SYBR kit (Eurogentec)	15	1 x
GAPDH primer	1.8	0.3 µM each
Human DNA (Roche)	12	3.2 ng/µL
H_2O	1.2	

The real-time PCR was carried out with a Mastercycler ep realplex (Eppendorf). The following temperature profile was used:

10 min, 95 °C → 40 x (15 sec, 95 °C; 30 sec, 60 °C) → 15 sec, 95 °C → 15 sec, 60 °C

A melting curve was then created by heating from 60 °C to 95 °C over 10 min.

A 146 bp fragment of the human GAPDH gene was amplified. The primers had the following sequences:

Primer forward: 5' TGCCTTCTTGCCTCTTGTCT 3'
Primer reverse: 5' GGCTCACCATGTAGCACTCA 3'

Results

Classic PCR

The incubation of the filter from manufacturers B and A in the PCR reaction mix leads to a nearly complete inhibition of the subsequently completed PCR. In a following gel electrophoresis DNA bands could not be detected. In contrast, no significant inhibitory effect was detected with the use of ep Dualfilter T.I.P.S. (E) (Fig. 2).
No PCR could be completed with a different filter used in the experiment (manufacturer G) because the entire PCR sample was aspirated by the filter during the incubation.

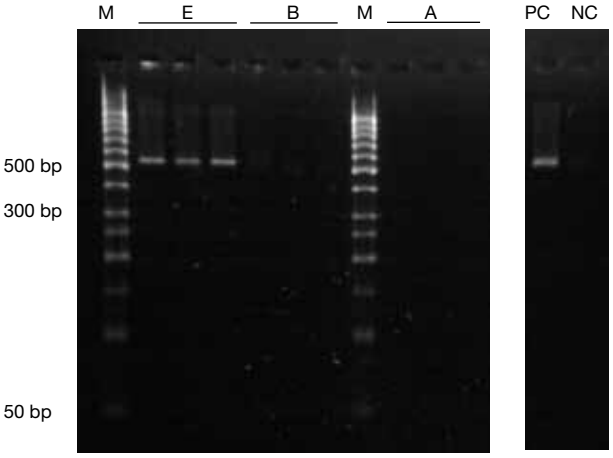


Figure 2: Electrophoretic separation of various PCR samples.

E: ep Dualfilter T.I.P.S.
B: filter from manufacturer B
A: filter from manufacturer A
M: marker (Fermentas 50 bp ladder)
PC: positive control (PCR sample with no contact with filters)
NC: negative control (PCR sample without dNTPs)

real-time PCR

The influences of all filter tips on the results of DNA amplification were determined using real-time PCR, a highly sensitive quantitative method. The average C_q values of all samples deviate from the positive control (PC) values. However, the extent of the deviation varies significantly according to the filter tip used (Fig. 3). The upward shift of the C_q value was at its lowest when ep Dualfilter T.I.P.S were used. The highest C_q value was measured with the samples incubated on filter G. The largest difference between the highest and lowest measured C_q value was observed with this sample (as depicted in the error bar in Fig. 3).

The detailed real-time PCR results are shown in Figure 4, which illustrates the positive controls (green), negative controls (black) and the samples used with ep Dualfilter T.I.P.S. (blue) and filter tips of manufacturer G (red). The difference among the C_q values of the tested replicates increases in tandem with the increasing inhibitory effect of the filters. When using ep Dualfilter T.I.P.S. (E), the deviations among the replicates are very low and also their gradients in the amplification plot do not differ significantly from those of the positive controls. When other filter tips are tested both gradients and deviations differ particularly to a significant extent from those of the positive controls.

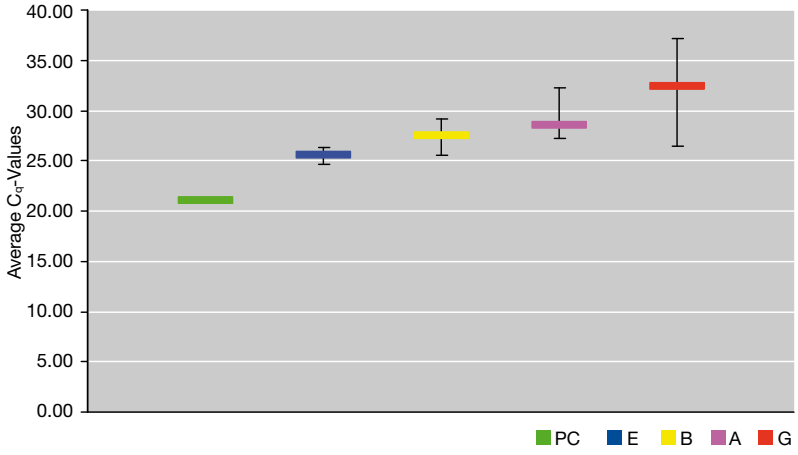
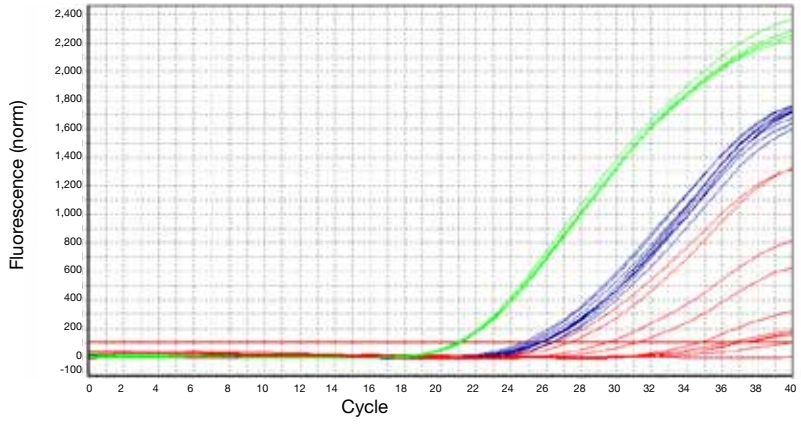


Figure 3: Average C_q values of the samples. The error bars show the highest or lowest measured C_q values.
PC: positive control (n=5), E: ep Dualfilter T.I.P.S (n=10), B: filter from manufacturer B (n=10), A: filter from manufacturer A (n=10), G: filter from manufacturer G (n=8, two values below the threshold value and therefore not included in the calculation)



Threshold: 99 (Noiseband)
Baseline settings: automatic, Drift correction OFF

Figure 4: Amplification plots for selected samples.
Green: positive controls (n=5), black: negative controls (n=5),
Blue: ep Dualfilter T.I.P.S. (n=10), red: filter from manufacturer G (n=10)

Discussion

Classic PCR

The results show that inhibitory effects caused by PCR samples coming into contact with various filter materials lead to the full suppression of DNA amplification in many cases (Fig. 2, samples B or A). Figure 2 illustrates that ep Dualfilter T.I.P.S. are particularly well suited for conducting PCR experiments because no significant influence on the PCR reaction was observed even if the PCR mix came into contact with the filter (Fig. 2, sample E).

Real-time PCR

With regard to real-time PCR, the goal is to prove that any filter contact decreases the efficiency of DNA amplification, which leads to an increase in the C_q value compared to the positive control. However, the scale of the negative effect on the reaction is highly dependent on the type of filter. In the test, the ep Dualfilter T.I.P.S. exhibited the smallest influence on real-time PCR, whereas filter G, for example, had a significant effect on the PCR efficiency (Figure 3). For the real-time PCR a more robust system was selected as for the classic PCR in order to investigate quantitative differences among the inhibitory effects of various filter tips. The DNA concentration used was significantly higher than the concentration of the PCR samples used for the classic PCR experiments. Therefore, the inhibition in this experiment did not turn out to be as extreme as with the classic PCR, for which DNA amplification was almost completely avoided due to the inhibition of filters from manufacturers B and A. Moreover, with real-time PCR, the contact surface between the PCR sample and filter was significantly lower during the incubation because the sample was directly inserted into the tip from below, and the entire filter was not incubated in the sample. Therefore, the inhibitory effects may be weaker with real-time PCR than with classic PCR.

Figure 3 shows that, as the inhibitory effect of the filter increases (higher C_q values), deviations among the replicates also markedly increase, thus limiting the reproducibility of PCR experiments. The reason for this may be the non-uniform inhibitory effects of the filters. Furthermore, the possibility that the PCR samples were not contaminated with inhibitory agents to the exact same degree cannot be ruled out, because, for example, the resuspending processes of the PCR samples on the filter may slightly deviate. The differences are very low when ep Dualfilter T.I.P.S. are used, which is directly correlated to a low inhibitory effect. Because of the abovementioned reasons, the deviations increased significantly when the filter from manufacturer G was tested.

In conclusion, it was established that all filters have a certain inhibitory effect on PCR after coming into contact with the PCR sample, either due to general adhesive effects or because of the self-sealing adhesives in the filter. These inhibitory effects vary considerably from filter to filter. ep Dualfilter T.I.P.S. are especially well suited for preparing PCR applications because they exhibit a very low effect on PCR compared to other filter tips.

Ordering information

ep Dualfilter T.I.P.S.® PCR clean, sterile and pyrogen-free	Order no. International	Order no. North America
Volume range/pipette tip		
0.1–10 µL S	0030 077.504	022491202
0.1–10 µL M	0030 077.512	022491211
0.5–20 µL L	0030 077.520	022491229
2–20 µL	0030 077.539	022491270
2–100 µL	0030 077.547	022491237
2–200 µL	0030 077.555	022491296
20–300 µL	0030 077.563	022491245
50–1,000 µL	0030 077.571	022491253
50–1,250 µL L	0030 077.750	022494002
100–5,000 µL	0030 077.580	022491261
1–10 mL L	0030 077.598	022491288
Thermomixer® comfort	5355 000.011	022670107
Mastercycler® pro S	6325 000.510	950040025
Mastercycler® ep realplex⁴ S	6302 000.601	please inquire

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