

Rainin Aerosol-Resistant Tips

Prevent DNA Contamination

Abstract

The purpose of this experiment was to validate the effectiveness of Rainin aerosol-resistant tips for protecting against cross-contamination by DNA aerosols. Tests are based on primers specific for human genomic DNA. The primer set will amplify a 268 bp fragment.

Test 1

First, a new P-200 mechanical pipette was tested to determine that the instrument was DNA free. The bottom 1 cm of the pipette barrel and inside of the shaft were washed completely with 200 µL sterile water to extract any possible DNA contamination. The extract was tested for the presence of DNA.

Test 2

The P-200 pipette was equipped with a Rainin FinePoint™ aerosol resistant tip (RT-200F) and set at maximum volume. 200 µL of an aqueous solution containing 20 ng/µL of human genomic DNA was aspirated and dispensed 25 times. The top of the filter was washed with 50 µL of sterile water. In addition, the bottom 1 cm of the pipette barrel and inside of the shaft was washed with 200 µL sterile water. Each extract was separately tested for DNA.

Test 3

Same as test 2 but using a Rainin tip without filter (RT-250) certified free of DNA.

Procedure

Reaction conditions (30 µL total volume)
 20 µL sample
 5.4 µL buffer
 3.0 µL dNTP mix
 0.5 µL human primer set
 0.5 µL Taq DNA Polymerase
 0.6 µL water
 Prepare 70 µL of Master mix

| Master mix | per rxn | Volume |
|----------------------|---------|---------|
| Buffer | 5.4 µL | 37.8 µL |
| dNTP's | 3.0 µL | 21.0 µL |
| Human primer set HBG | 0.5 µL | 3.5 µL |
| Taq DNA Polymerase | 0.5 µL | 3.5 µL |
| H ₂ O | 0.6 µL | 4.2 µL |

Add 10 µL Master mix to tubes 1-6
 Add mineral oil overlay to all tubes
 For negative control tube 5, add 20 µL sterile water and seal. For positive control tube 6, make a solution containing 30 pg of DNA in a final volume of 20 µL sterile water. Add mineral oil overlay.
 For remaining tubes, add 20 µL of wash (extract) from test 1, 2, and 3.

Total number of reactions = 6

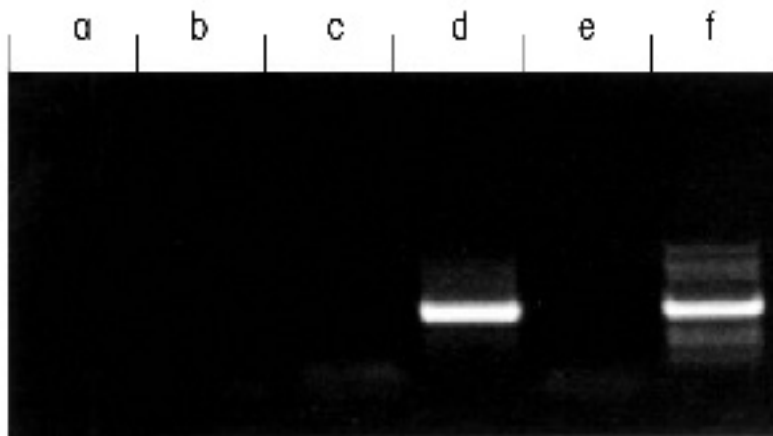
- Tube 1 Reaction 1 = Extract from Test 1 (Cleaned pipette extract)
- Tube 2 Reaction 2 = Filter extract from Test 2 (Tip with filter)
- Tube 3 Reaction 3 = Pipette barrel and shaft extract from Test 2 (Tip with filter)
- Tube 4 Reaction 4 = Pipette barrel and shaft extract from Test 3 (Tip without filter)
- Tube 5 Reaction 5 = Negative control. Sterile water
- Tube 6 Reaction 6 = Positive control. 30 pg human genomic DNA

PCR details

40 cycles of 94°C (1 min.), 56°C (1 min.), 72°C (1 min.).
 Mineral oil overlay

Results

Gel conditions 1.2% Agarose gel, in 1/2 X TAE, 10 µL of each reaction



- Lane (a) Tube 1 Reaction 1 = Extract from Test 1 (Cleaned pipette extract)
- Lane (b) Tube 2 Reaction 2 = Filter extract from Test 2 (Tip with filter)
- Lane (c) Tube 3 Reaction 3 = Pipette barrel and shaft extract from Test 2 (Tip with filter)
- Lane (d) Tube 4 Reaction 4 = Pipette barrel and shaft extract from Test 3 (Tip without filter)
- Lane (e) Tube 5 Reaction 5 = Negative control. Sterile water
- Lane (f) Tube 6 Reaction 6 = Positive control 30 pg human genomic DNA

Conclusions

The results show that Rainin aerosol resistant tips protect against cross-contamination of mechanical pipettes by DNA aerosols. Lane b shows that the filter prevents DNA aerosols from passing through. Lane c shows that the pipette was protected from DNA aerosols by the filter. Lane d shows that a pipette will become contaminated with DNA if pipet tips without filters are used when pipetting DNA samples.