

Control of Contamination Associated with PCR and Other Amplification Reactions

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Key Words

- **Thermo Scientific™ ART™ Barrier pipette tips** – The first choice with a 100% protection guarantee against aerosol and liquid contamination of your pipette.
- **Carryover-contamination** – Occurs when aerosols or liquids from one sample are inadvertently carried to the next sample.
- **Cross-contamination** – Occurs when successive samples are corrupted due to use of a pipette that has been contaminated from aerosols or liquids from another sample.
- **PCR (polymerase chain reaction)** – A scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA generating thousands to millions of copies of a particular DNA sequence. *In-vitro* DNA amplification.



Goal

The control of in-vitro amplification reaction products is becoming an increasing concern for molecular genetics laboratories, diagnostic molecular pathology laboratories and all laboratories in general working with amplified reactions. The reason behind ART Barrier pipette tip's incredible success is the interactive barrier whose porous structure actually closes and seals when in contact with airborne aerosols or aqueous solutions. The ART Barrier pipette tip protects the pipette from sample carryover that is usually the cause of contamination in PCR experiments.

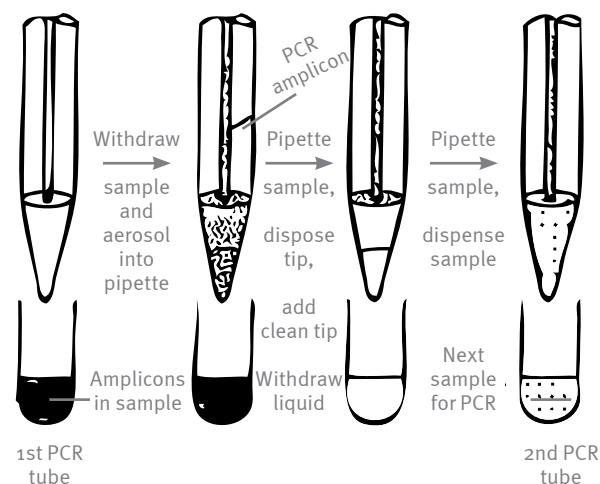
Contamination Control Procedures

Described as being to genes what Gutenberg's printing press was to the written word, PCR can amplify a desired DNA sequence of any origin (virus, bacteria, plant or human) hundreds of millions of times in a matter of hours, a task that would have required several days with recombinant technology. PCR is especially valuable because the reaction is highly specific, easily automated and capable of amplifying minute amounts of sample.

For these reasons, PCR has also had a major impact on clinical medicine, genetic disease diagnostics, forensic science and evolutionary biology. Unfortunately, the PCR's capacity for amplification is accompanied by its extreme sensitivity to the presence of its own product as a feedback contaminant. Many laboratories routinely detect less than 100 copies of certain target templates, for example viruses, and the experiment is susceptible to trace amounts of its own product. It is the amplicons in the aerosol which, if deposited inside the pipette, could contaminate the next sample during subsequent pipetting steps. When the plunger button of the pipette is released, air flows up inside the pipette tip and pipette body. The principal feature of the ART Barrier pipette tip is a porous, self-sealing, physical barrier that is located between the pipette tip's upper section and the tip's orifice. Using ART Barrier pipette tips, air flows up through the porous barrier, leaving any aerosols trapped inside the barrier. Researchers the world over have made ART Barrier pipette tips their first choice for contamination control.

Ordinary Filters Allow Contamination to Pass Through

Use of Standard Pipette Tips



Use of ART Self-sealing Barrier Pipette Tips

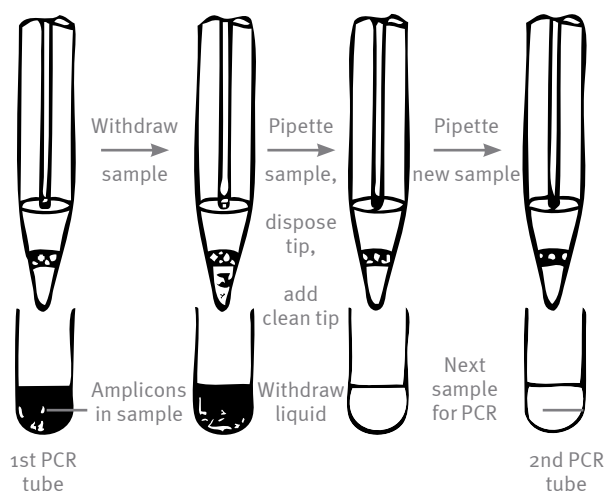


Figure 1. Use of ART self-sealing Barrier pipette tips illustrates that aerosols generated during initial pipetting steps are restricted to the lower portion of the tip.

Manufactured according to the industry's highest quality standards, ART Barrier pipette tips are certified as RNase, DNase and Pyrogen free, and are ideal for the pipetting of radioactive samples, nucleic amplification procedures or any application where critical sample handling is required.

Prior to certification, each lot of ART Barrier pipette tips undergoes 13 inspection points on 25 parameters and is sent for final certification at an independent testing laboratory using standard statistical procedures.

Additional Contamination Control Suggestions

1. Prepare your master mix in a separate room from your current location, somewhere PCR is not the main practice. Be sure to have a separate lab coat, gloves, tubes and pipette tips to be used only in that clean room.
2. Use a separate aliquot of DEPC water stock for each round of PCR.
3. Prepare your mix in a hood with laminar flow. Decontaminate it with bleach, alcohol, RNase, DNase, etc. Be sure to UV-irradiate pipettes, pipette tips, tubes, racks, gloves, and also your aliquots of water and PCR buffer before the procedure.
4. Use a different pipette tip when pipetting all your reagents, even in the same master mix to each tube.
5. Keep your tubes closed during the procedure, even your master mix tube. Be sure your tubes are closed when discarding the pipette tip. Open the tubes only when necessary.
6. Schedule your PCR when not handling plasmids.

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