Polymerase Chain Reaction (PCR)

4- PCR Troubleshooting

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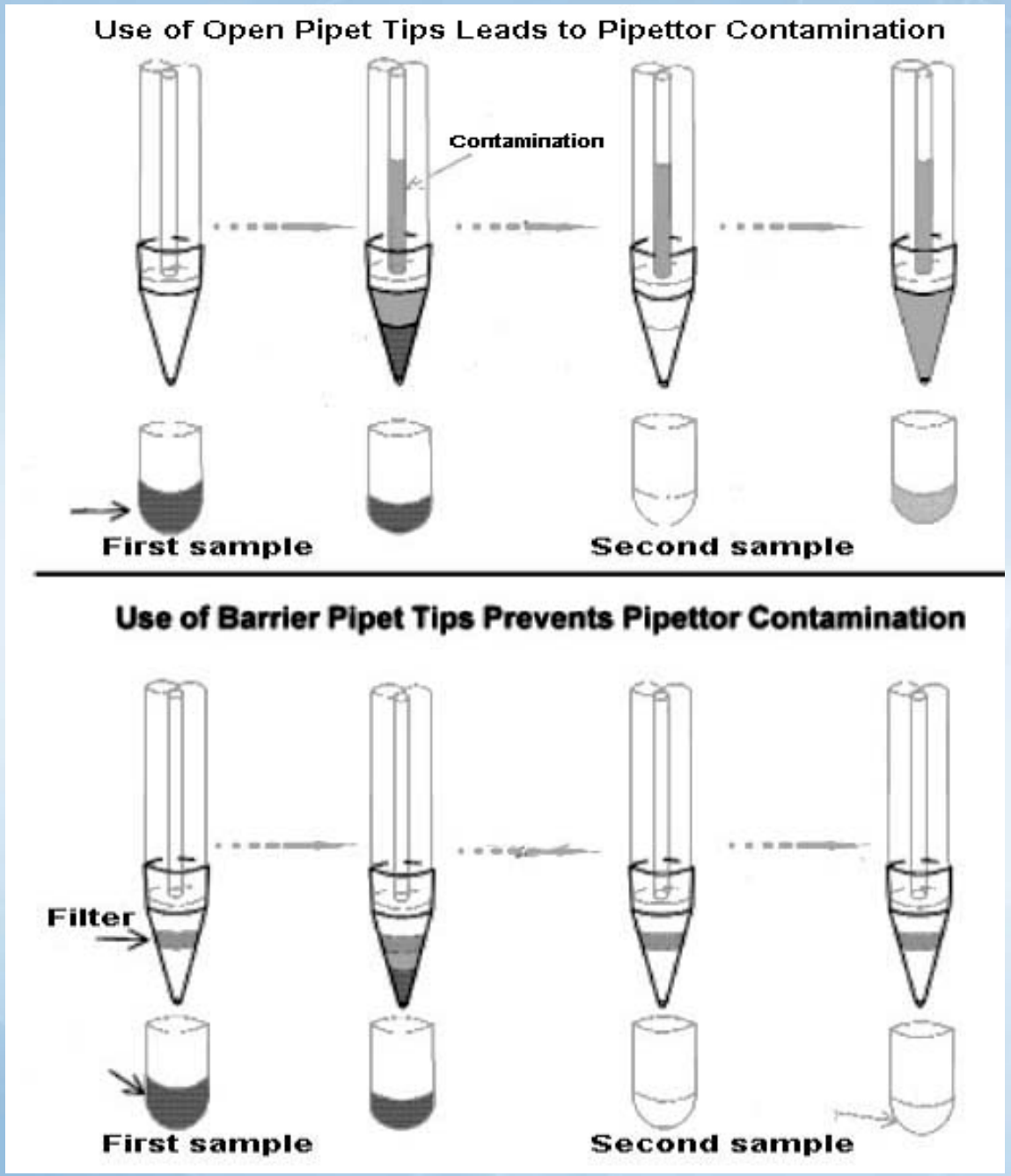
Avoiding Contamination

- DNA sample preparation, reaction mixture assemblage should be performed in separate areas.
- A Laminar Flow Cabinet with a UV lamp is recommended for preparing the reaction mixture.
- New gloves should be used for DNA purification and each reaction set-up.

The use of tips with aerosol filters for both DNA sample and reaction mixture preparation, is strongly recommended.



- Autoclaving of all solutions, except dNTPs, primers and *Taq* DNA Polymerase is recommended.

- A control reaction, omitting template DNA, should always be performed, to confirm the absence of contamination.

Common problem during PCR

- **Template DNA:**

  Larger template DNA amounts usually increase the yield of non-specific PCR products.

- **Primers.**

  - The primer should not be self-complementary or complementary to any other primer in the reaction mixture, to prevent primer-dimer and hairpin formation.

  - The melting temp. estimated as follows:

    $$Tm = 4\ (G + C) + 2\ (A + T).$$

  - The annealing temp. ~ 5°C lower than the Tm

MgCl concentration.

* It forms complexes with dNTPs, primers and DNA templates

- Too few Mg2+ ions result in a low yield of PCR product
- Too many will increase the yield of non-specific products.

Taq DNA polymerase.

- Higher Taq polymerase concentrations than needed may cause synthesis of non-specific products.

dNTPs.

The concentration of 4 dNTPs (dATP, dCTP, dGTP, dTTP) should be equal in the reaction mixture.


Discussion
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