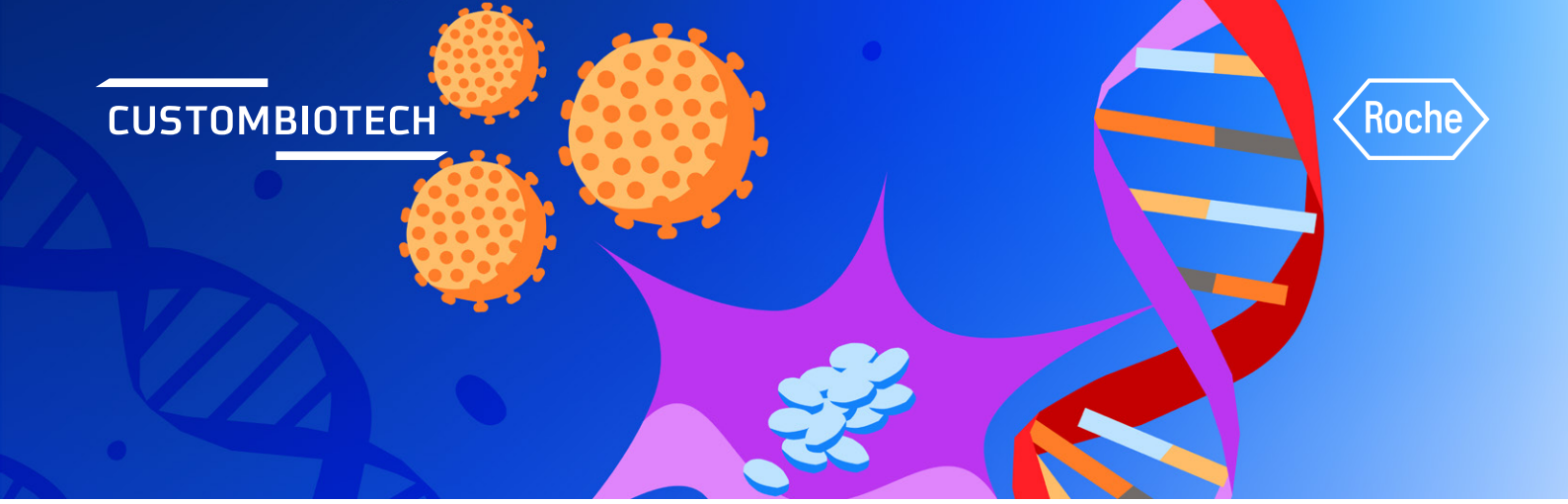

Avoiding contamination during PCR setup

General guidelines

The ability of the Polymerase Chain Reaction to amplify a single molecule means that even trace amounts of foreign DNA could potentially serve as template, resulting in amplification of the wrong target DNA.

Possible sources for DNA contamination

- Laboratory benches, equipment and pipetting devices that can be contaminated by previous DNA preparations, by plasmid DNA, or purified restriction fragments
- Products/amplicons from previous PCR reactions
- Cross-contamination between samples

Laboratory facilities, equipment and consumables

At a minimum, set up physically separated working areas and equipment for:

- **DNA template preparation before PCR**
- **Setting up PCR reactions**
- **Post-PCR analysis**

- Workflow should be unidirectional.
- Avoid setting up work areas where there is a high volume of foot traffic. This is especially important when working on an open bench, which is not recommended.
- Ensure all working areas are clean, especially for aliquoting PCR reagents.
- Use dedicated necessary laboratory equipment (pipettes, vortex mixer, microcentrifuges), consumables (disposable gloves, tips, tubes, pens), and laboratory coats for each area. **If this is not possible** due to the laboratory being a shared space, then the laboratory coat should be worn from clean to dirty area and then sent for cleaning. Also, color code the pipettes, pens, etc. with colored tape as a visual indicator of which area they belong to.
- Use aerosol resistant pipette tips and dedicated positive displacement pipettes.
- Use pipette tips that properly fit the pipettes. Improperlyfitting tips can lead to droplets that can contaminate the work area.
- If possible, set up PCR reaction under a PCR hood equipped with a UV lamp for decontamination.
  Wipe pipetting tools with 10% bleach followed by 70% ethanol or other disinfectants.
  Wipe down all other items with 70% ethanol before bringing into the hood.

Sample handling

- Use sterile techniques and always wear fresh gloves. Change gloves frequently.
- Always use new or sterilized glassware, plastic ware, and pipettes to prepare PCR reagents and template DNA.
- Sterilize all reagents and solutions by 0.22 µm filter or autoclaving. All reagents should be certified “nuclease free”.

Reaction setup

- To avoid carry-over contamination, do not pipette directly from a reagent stock to a sample tube. Generate a reaction master mix by transferring required components to a separate, new tube then aliqouting to the individual sample tubes.
- Place the reagents in a laminar flow hood, and let them thaw at room temperature (+15 to +25°C).
- Always include the following PCR control reactions.
  Negative Control – contains all reaction components except template DNA.
  Positive Control – contains all reaction components plus a DNA template that has amplified successfully in previous PCR reactions.
- Spin all reagent and reaction tubes briefly in the centrifuge before opening to bring down any liquid in the cap of the tube and minimize bubbles.
- Take care when opening tubes (especially snap cap tubes) not to create aerosols.
- Use care when pipetting (aspirating and dispensing) not to create aerosols.
- Discard pipette tips immediately after use. Do not rest a pipette on the bench with a pipette tip attached as this can lead to contamination of the work area.
- Only have one tube open at a time.
- Close or cap each reaction vessel after the addition of PCR template. Add the positive control template last after all other control and sample reactions have been closed.

MycoTOOL method hints

- For successful and consistent DNA recovery during manual extraction, it is helpful to mark the outside of the tubes and to align the tubes carefully in the centrifuge to assist in locating the pellet.
- After steps 7* and 15*, briefly spin the tubes to remove any liquid in the cap that could contaminate the technologist's gloves.
- To reduce the possibility of losing the pelleted DNA, after centrifugation steps 9* & 12*, carefully remove the supernatant using a pipette tip, taking care to avoid disturbing the pellet, which may be difficult to see.
- When conducting a limit of detection study, the mycoplasma standard spikes (gDNA or other standards) should be added to the sample prior to DNA extraction, not to the PCR master mix.

* Steps refer to instructions for use, MycoTOOL Mycoplasma Real-Time PCR Kit (06 495 605 001), August 2020, version 10.
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