

Real-Time PCR Protocol – $\Delta F508$ Mutation Detection (Generalized Version)

This sample demonstrates my ability to document complex laboratory procedures in a clear and professional format. The protocol is adapted from routine work with PCR-based genetic testing and is anonymized for portfolio use.

Purpose:

To detect the $\Delta F508$ mutation in the CFTR gene using Real-Time PCR with a LightCycler 480 system.

Intended Users:

Laboratory technicians trained in molecular diagnostics

Laboratory Preparation

- Clean lab surfaces with soap and water regularly.
 - Clean outside the PCR area as needed.
 - Clean inside the PCR hood approximately once a month.
- Disinfect with 70% ethanol (commercial grade).
 - Do not use 100% ethanol, as it can cause fixation of DNA or contaminants onto surfaces, making them harder to remove. Use 70% ethanol for effective decontamination.
 - Do not clean the PCR base plate with ethanol to avoid corrosion.
 - The PCR workstation must remain DNA-free at all times.
- Open both centrifuges for air exchange and readiness.

Instrument Startup

1. Power on the real-time PCR machine and allow it to complete its internal checks.

2. Once the loading tray opens and closes automatically, turn on the monitor and tower.
3. On the main screen:
 - Select User Defined Workflow → press Enter.
 - Choose Instrument Operator → enter corresponding password/code.
4. Launch the real-time PCR machine's Software. Log in with credentials.
5. Select:
 - New Experiment → Apply Template → Run Template → choose deltaF508 template.
 - Ensure Reaction Volume is set to 10 µL.

Reagents & Master Mix

- Use an Excel file or lab notebook to document reagent volumes and sample layout.
- Place reagents in order of use on a clean tray. After use, move them to a separate position.
- Retrieve the Reagent Mix from the top shelf of the freezer.
 - It is light-sensitive and must remain in its black protective pouch when not in use.

Master Mix Recipe (per reaction):

Component	Volume
PCR-grade H ₂ O	11.4 µl
FastStart DNA Master	2.0 µl
MgCl ₂ (25 mM)	1.6 µl
Reagent Mix (light-sensitive)	1.0 µl
Total	16.0 µl (Master Mix)

Tips:

- Vortex only the MgCl₂ (~8 seconds). No centrifugation required.
- Do not vortex enzymes to avoid activity loss.

- After each addition, mix gently by pipetting up and down.
- Once complete, gently tap the mix and spin briefly (~11,500 rpm).

Sample Preparation

- Return all reagents to the fridge or freezer immediately after use.
- Bring DNA samples and PCR-grade water to bench temperature (outside PCR hood).
- Add 16 μ L Master Mix to each well of your PCR plate or strip.
 - Optional: Mark each loaded well, immediately after loading it, with a permanent marker to prevent errors.
- Add 4 μ L of sample DNA per well, or 4 μ L PCR-grade water for NTCs.
 - Mix gently (2–3 pipette cycles per well).

Plate Sealing and Centrifugation

- Seal wells:
 - Strips: Ensure tight cap fit with slight pressure.
 - Plates: Apply sealing film with no air bubbles.
- Briefly centrifuge (~40 seconds) with appropriate counterbalance.

PCR Run

1. Load the sealed plate into the machine (tray opens with front-right button).
2. Click Start Run.
3. In the run dialog:
 - Enter the experiment name (e.g., 2025-07-21_deltaF508).
 - Select: Experiments > [Year] > deltaF508 → confirm with .
4. In the Subset Editor, click + to create a new subset.
 - Name it with the date and experiment (e.g., 2025-07-21 deltaF508).
 - Assign the correct wells (Control + click).
5. In the Sample Editor, assign names and colors to each sample in Latin characters.
6. In the Data tab:
 - Right-click on the graph > switch X-axis to Cycles instead of Time.
 - Monitor amplification progress (signal may appear late in run).

7. When run is complete, status changes to Run Completed.

 Disclaimer:

This protocol is a generalized example based on standard commercial DNA amplification workflows. It has been anonymized and adapted for portfolio purposes only. Always consult your laboratory's official SOPs and reagent manuals.