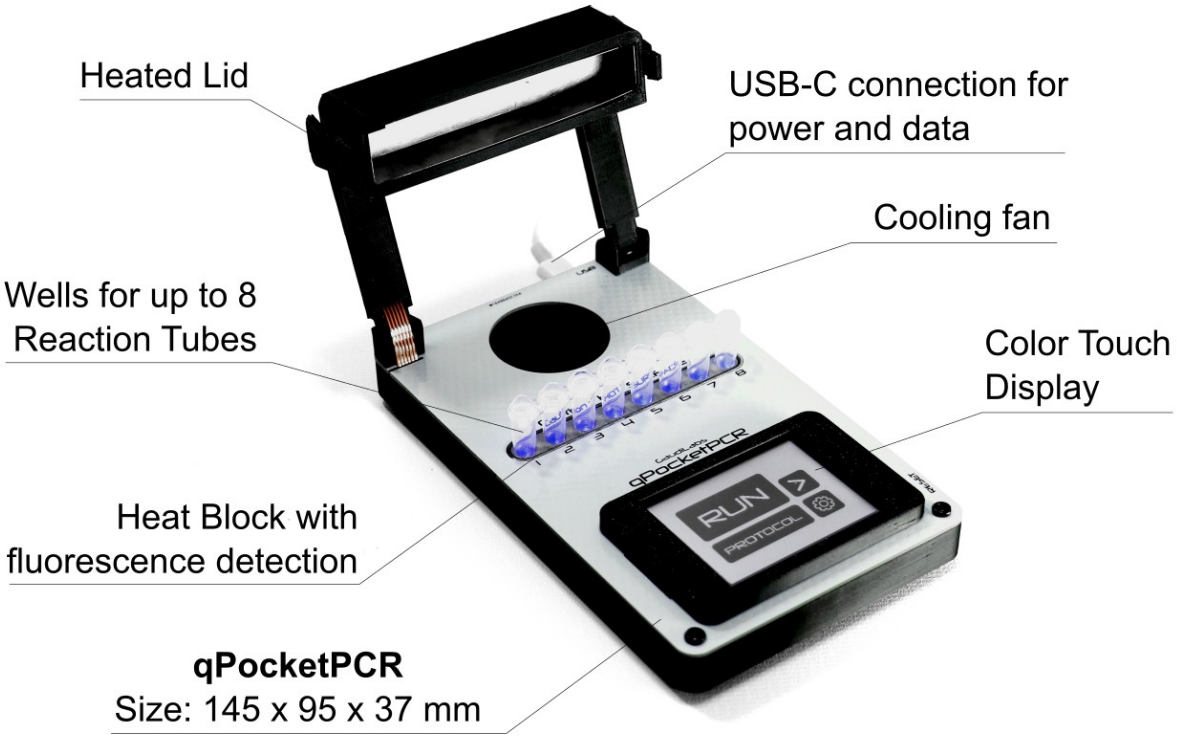
# Instructions for qPocketPCR V1.1

The qPocketPCR is an ultra-compact, open-source Real-Time PCR thermal cycler designed for portability, accessibility, and versatility. About the size of a smartphone and powered via USB-C (PD), it performs Polymerase Chain Reaction (PCR) on up to 8 standard sample tubes, bringing advanced molecular techniques to the field, classroom, or community lab.

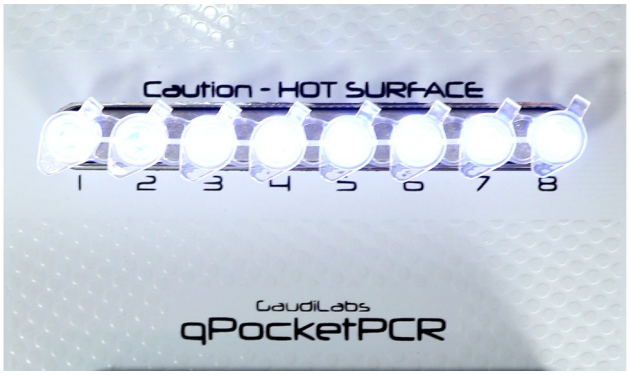
## Device description

As a compact thermocycler with integrated fluorescence detection, the **qPocketPCR** provides all basic functions required for PCR amplification in an easy-to-use form factor. The main elements of the qPocketPCR include:




**Color Touch Display**: An intuitive color touchscreen provides access to basic functionalities, displays protocols, and shows real-time status information. All operations with the device are performed through the touch display.

**Heated Lid:** The hinged cover shields the reaction tubes from light during fluorescence reading and holds the heated lid in place to reduce evaporation. The lid temperature is regulated to 110 °C. Caution: The lid becomes hot on the inside during operation and warm on the outside. To open the lid, press the latches on both sides while lifting the cover.



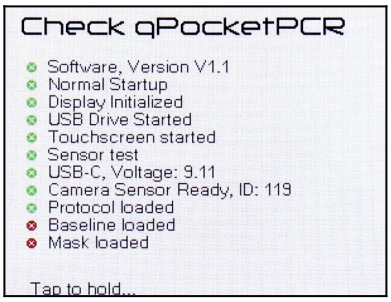

**Wells for up to 8 Reaction Tubes:** The qPocketPCR can accommodate up to eight standard 200 µL reaction tubes or one PCR tube strip. It is recommended to use flat-cap tubes for optimal contact and thermal performance. For best amplification and fluorescence reading, use a reaction volume between 25 µL and 35 µL. If evaporation is too high despite the heated lid, add approximately 15 µL of mineral oil on top of each reaction to prevent evaporation. After inserting the reaction tubes into the wells, close the lid until the latches snap into place.

**Heat Block with Fluorescence Detection:** The 3D-printed aluminum heat block is precisely temperature-regulated to perform the thermocycling reaction. During the run, the qPocketPCR detects **Cybr® Green** fluorescence in real time. Each tube is illuminated from below with 497 nm blue excitation light, and the emitted fluorescence at 520 nm (green) is recorded by a built-in, highly sensitive camera. To maintain optimal performance, keep the wells clean by closing the lid when not in use. For the best signal-to-background ratio, use a Cybr® Green master mix without ROX.

**Cooling Fan:** The device uses resistive heating and fan-assisted cooling to regulate the temperature of the heat block. The lowest attainable temperature is therefore the ambient room temperature. Ensure that the fan inlet on top of the device and the air outlets on both sides are not obstructed.

**USB-C Connection for power and data:** The qPocketPCR is entirely powered via USB-C. The same USB-C connection is used to transfer data to and from your computer or phone. The device is recognized as a USB storage device, allowing protocol files to be easily copied, edited, or retrieved directly on the device. During a PCR run, the device must be powered using a 20 W USB Power Delivery (USB-PD) charger or a compatible computer port, and connected with a USB-C cable (charger and cable are included). For mobile research or in situations with unstable power, the device can be powered using a USB-C Power Delivery battery pack with a capacity of 20,000 mAh.

# Powering up and device checks

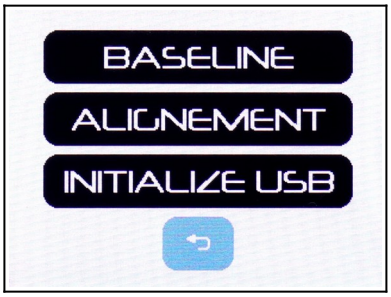



To start using your qPocketPCR, connect it to your computer using the USB-C port. You can also view and edit the files on your qPocketPCR using a phone with an OTG (On-The-Go) adapter. The device display will turn on, showing the device checkup page. To keep the information on the screen visible, simply hold your finger on the display.

On the checkup page, you can see the software version and other device status information. The USB-C voltage indicator shows the voltage your current connection can deliver. For preparing a run, around 5 V is sufficient. To perform a PCR run, the voltage must be approximately 12 V. If your computer cannot provide 12 V via USB Power Delivery (PD), use the included power adapter for the PCR run.

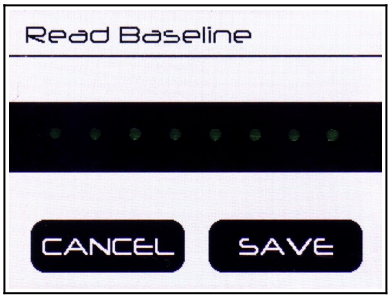
The checkup page also indicates whether the sensor baseline and mask are loaded. If either point is shown in red, perform the calibration (described below) before starting a run.

# Calibration



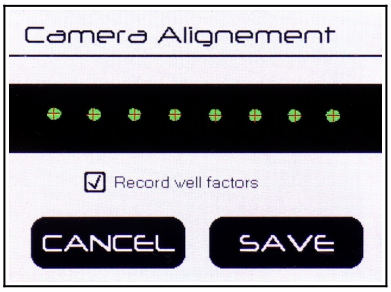

For accurate detection, the fluorescence camera of the qPocketPCR must be calibrated before use. Once calibration is complete, the data is stored on the device. Repeat the calibration periodically or whenever you suspect the detection is not working properly. The calibration tools can be found in the settings menu.

### Base Line Calibration




The baseline calibration removes any signal detected by the camera when no fluorescence is present. To perform the calibration, insert tubes of the same type you plan to use in your experiment, each filled with 35 µL of pure water. Close the lid and press the **“BASELINE”** button. You will see the camera image showing the eight tubes. The image may appear completely dark, but the device still records small background signals to subtract from the actual fluorescence signal. Press **“SAVE”** to store the background image.

## Camera Alignement




To align the camera capture regions with the wells, a camera alignment must be performed. Prepare a strip of eight tubes filled with a 35ul of fluorescent sample medium. The simplest way is to prepare a diluted **Cybr® Green** solution (e.g., 1/5 of the working concentration) or a similar DNA-binding dye that emits in the green range. To ensure the correct fluorescence intensity, use the **Fluroescence Measurement Function** (described below) and aim for a reading between 50 and 150. Insert the tubes into the qPocketPCR, close the lid, and select **“ALIGNMENT.”** You should see eight green fluorescent dots, each centered with a red cross. If not all dots appear clearly, the fluorescence of your sample may not be strong enough. If your samples have uniform fluorescence quality, you can select **“Record Well Factors”** to equalize the wells. Otherwise, the well factors will default to 1. Press **“SAVE”** to store the alignement and well factor data.

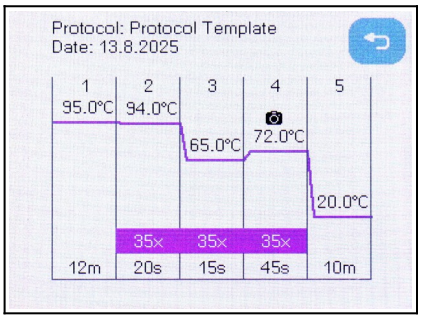
## Initialize USB

Calibration, protocol, and measurement data are stored in the device’s non-volatile internal memory. If you encounter problems with USB data transfer or calibration data storage, you can select **“INITIALIZE USB”** to reformat the memory to its original state and restore the default template protocol. The formatting process takes about 30 seconds. Caution: All stored data will be deleted during this process.

## Fluorescence Measurement Function


Using the angle bracket symbol in the main menu, you can activate the qPocketPCR’s fluorescence camera. The display shows the image captured by the camera of the eight tubes, along with the current fluorescence readings. A graph illustrates the signal variation over time. This function allows you to verify proper measurement and calibration performance, as well as measure fluorescence from samples and calibration standards. Press the back button in the top right corner to return to the main menu. No data is stored in this mode.

# Loading a Protocol




The qPocketPCR offers simple yet flexible ways to load a thermocycling protocol. To see the currently loaded protocol, press the **PROTOCOL** button on the main menu. A graph of the temperature profile will be displayed. If no protocol appears, re-initialize the device memory using the **“Initialize USB”** function described in the previous chapter to restore the default protocol template.

The display shows the different steps, including temperatures and durations, as well as the repeat sections and the capture step (camera symbol). If the protocol contains more than five steps, use the arrows on the side of the diagram to navigate between pages.

Note: Times shown in minutes may be rounded on the display, but the device will execute each step precisely as defined in your protocol.

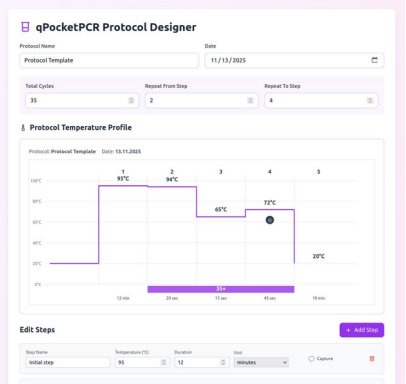
To change the protocol, connect the device to your computer or phone. The qPocketPCR will appear as a USB drive named **qPocketPCR**. On the drive, you will find two files: **DATAQPCR.TXT** and **PROTOCOL.TXT**. The data file contains the readings from a previous run, while the protocol file describes the current thermocycling protocol. You can open the protocol file with any text editor to view its contents. The default protocol looks as follows:

```
NAME: Protocol Template
 DATE: 13.8.2025

 PROTOCOL:

 REPEAT: 2-4
 CYCLES: 35


  STEP 1: Initial step
    TEMPERATURE: 95C
    DURATION: 12 min

  STEP 2: Denaturation
    TEMPERATURE: 94°C
    DURATION: 20 sec

  STEP 3: Annealing
    TEMPERATURE: 65°C
    DURATION: 15s

  STEP 4: Extension
    TEMPERATURE: 72°C
    DURATION: 45s
    CAPTURE: yes

  STEP 5: Final Step
    TEMPERATURE: 20°C
    DURATION: 10 min
```

You can modify the names of the protocol and its steps, as well as the temperatures and durations. Steps can be added or deleted, and the cycle count and repeat sections can be changed. While editing, ensure that the basic structure and keywords, such as “TEMPERATURE:” or “DURATION:”, remain unchanged. Once you save the file in your text editor, the updated protocol will be stored on the device, and the changes will be reflected on the protocol display.

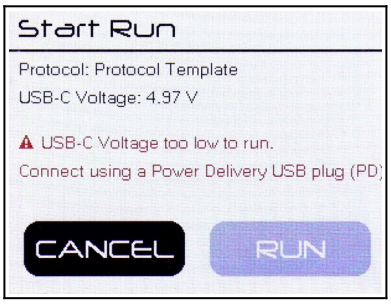
You can also copy the protocol file to your computer for editing or backup and then copy it back to the qPocketPCR USB drive. Always use the exact file name **PROTOCOL.TXT**, and avoid storing other files on the device memory, as space is limited.

Finally, we also provide a web app called **“Protocol Builder”** on the qPocketPCR website, where you can edit the protocol using a graphical user interface and then save the automatically generated **PROTOCOL.TXT** file to the qPocketPCR USB drive.



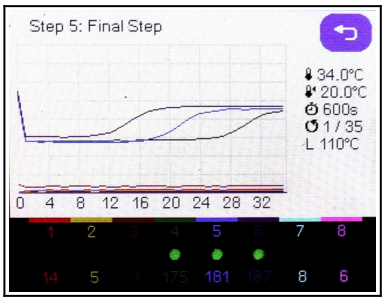

# Starting a qPCR Run

To start a qPCR run, connect the device to the included USB Power Delivery (PD) adapter or any other compatible USB PD port. Load the samples, close the lid, verify that the protocol is correct, and press the **“RUN”** button. The **Start Run** page will display the USB voltage supplied to the device and notify you if the voltage is too low, prompting you to use an appropriate USB adapter.




**Note:** All data from previous runs will be deleted when a new run is started. Press **RUN** to begin the thermocycling process.

The display will now show the **Run Display**, providing information on the current step in the title bar, the thermoblock temperature, the set temperature, the step timer, the cycle count, and the lid temperature on the right side of the screen. The fluorescence reading is shown at the bottom, with the real-time diagram displayed in the central graph. Run data is continuously stored in the device memory. To abort the run before it finishes, press the back button in the top left corner. **Note:** Data can only be retrieved from the device after the run has been stopped.



# Data collection

After completing a PCR run, the data can be retrieved from the qPocketPCR USB storage. Connect the qPocketPCR to your computer and open or copy the “DATAQPCR.TXT” file. The file is a .TXT file containing comma-separated values (CSV).

```
Protocol name: Protocol Template
Cycle, Time, Sample1, Sample2, Sample3, Sample4, Sample5, Sample6, Sample7, Sample8
1, 701, -3.21, -20.82, -0.94, 117.14, 108.81, 111.60, -12.34, -16.71
1, 820, -6.58, -23.28, -4.95, -37.62, -13.33, -15.48, -15.01, -16.23
2, 1072, -2.48, -20.91, -3.37, 119.13, 110.66, 113.82, -13.04, -16.04
2, 1191, -5.61, -23.22, -5.26, -42.84, -15.48, -17.96, -15.35, -17.02
3, 1446, -3.30, -21.66, -3.91, 118.41, 109.77, 112.85, -13.46, -15.33
3, 1565, -6.85, -23.95, -6.05, -44.05, -15.95, -18.41, -16.00, -16.78
```

*Comma-Separated Values in DATAQPCR.TXT*

The file can be imported into spreadsheet software such as LibreOffice Calc or Excel, or into any other bioinformatics software for further processing.

We also provide a web app called **“File Reader”** to read and analyze the data. Click to select the data file from the device or your computer. The web app lets you normalize the data and automatically calculate the threshold cycles based on a chosen CT threshold value. Finally, a report can be copied to the clipboard and pasted into a text program for documentation.


qPocketPCR by GaudiLabs is an open source hardware and software project.
Project Website: https://gaudi.ch/PocketPCR
GitHub Page: https://github.com/GaudiLabs/qPocketPCR

Buy a qPocketPCR in our shop: https://gaudishop.ch/index.php/product-category/pocketpcr/