Maverick qPCR Quick Guide

For Android® - based software

Version 0.17

Contents

Product overview

Maverick is a portable, yet high performance real time quantitative PCR system. Maverick is equipped with a 4-channel fluorescence optical system, powered by ZheJiangJilong ultra-low-light CMOS bio-imaging sensor. Maverick is optimally suited for applications where portability, minimal space, fast time-to-result is required. Applications of Maverick are: point-of-care molecular diagnostics test (POCT)[1] , food safety and environment testing, agriculture, or research lab use where bench space is limited.

Figure 1.1 Maverick compact qPCR system

Features

- Compact and rugged design. No internal moving parts, and no need for calibration.
- Multi-wavelengths 4-channels fluorescence sensing capability[2].
- Equipped with ultra-low-light sensitive CMOS-based fluorescent imager.
- Cross platform software for Windows® and Android® OS, with cloud-ready connectivity.
- Low power. External supply with ~90W active power.
- Battery-backup option for outdoor use and power loss protection

[1] Clinical clearance maybe required

[2] Support Intercalating dyes, hydroxyls probes and FRET probes

Key parameters

Capacity (# of wells)	16
Channels (# of Fluorophores per well)	4 (1. FAM/SYBR Green; 2. JOE/HEX/VIC/TET; 3. ROX/Texas Red; 4. CY5/LIZ/Cy5.5)
Multiplex capability	Up to 4 targets per well
Minimum detection threshold	Single copies
Dynamic range	＜1.0E9
Signal Interface	USB 2.0
Excitation source	High endurance LED
Detector	Ultra-low-light CMOS Bio-imaging chip
Thermal system	Solid-state, Peltier-based
Tube/plate formats	0.2mL, 8 or 16-tube strip
Reaction volume	20uL – 50uL
Filters:	4 sets exchangeable*, **
Excitation Range:	460nm – 670nm
Emission Range	510nm – 720nm
DNA probes supported:	DNA binding dyes (e.g. SybrGreen), hydrolysis probes (e.g. TaqMan probe) and hybridization probes (e.g. FRET probes).
Temperature accuracy	± 0.25 °C
Temperature Ramp Rate	8°C/s heating; 4°C/s cooling
Size and weight	247mm (L) x 188mm (W) x 133mm (H).
Weight	2.60Kg
Power supply	Adapter provided that accepts 110V/240V AC

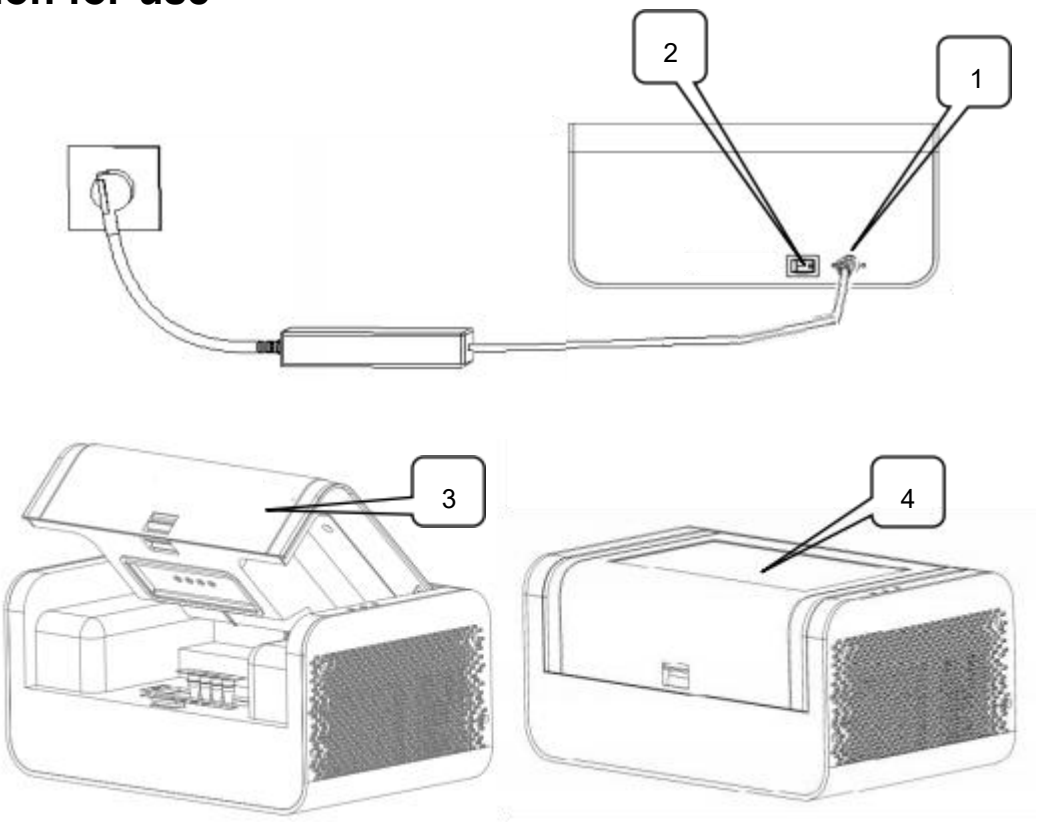
*: Wavelength characteristics can be modified by changing filter modules.

**: We support 1 – 4 channels in different configurations.

Applications

- Point-of-care molecular diagnostics[3]
- Food safety test
- Environmental microbial-threat monitoring
- Agriculture DNA testing
- Forensic testing
- Research and educational lab use
- Drug quality assurance testing

Instruction for use



[3] Clinical clearance required.

1. Insert DC interface into input hole of instrument power supply;
2. Turn on the power supply;
3. Open the cover, Put the samples to be examined
4. Close the cover and open the software to for use.

Step-by-step guide of the software (Android® version)

Launching the software

When the Maverick PC software is launched, after a brief delay for device initialization, the front page will display as shown in Figure 1. The first page is a log in page. Just use the default log in ID that is already filled in and click “Log In”, when asked about whether to allow USB connection, click OK, and go to settings of the Android ® device and allow USB connection.

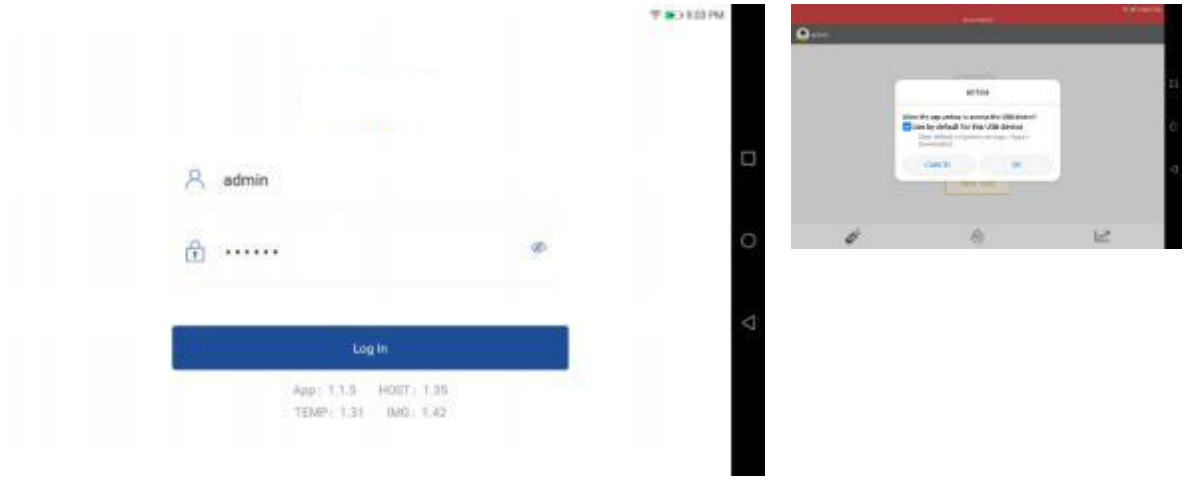


Figure 1 Maverick software front page

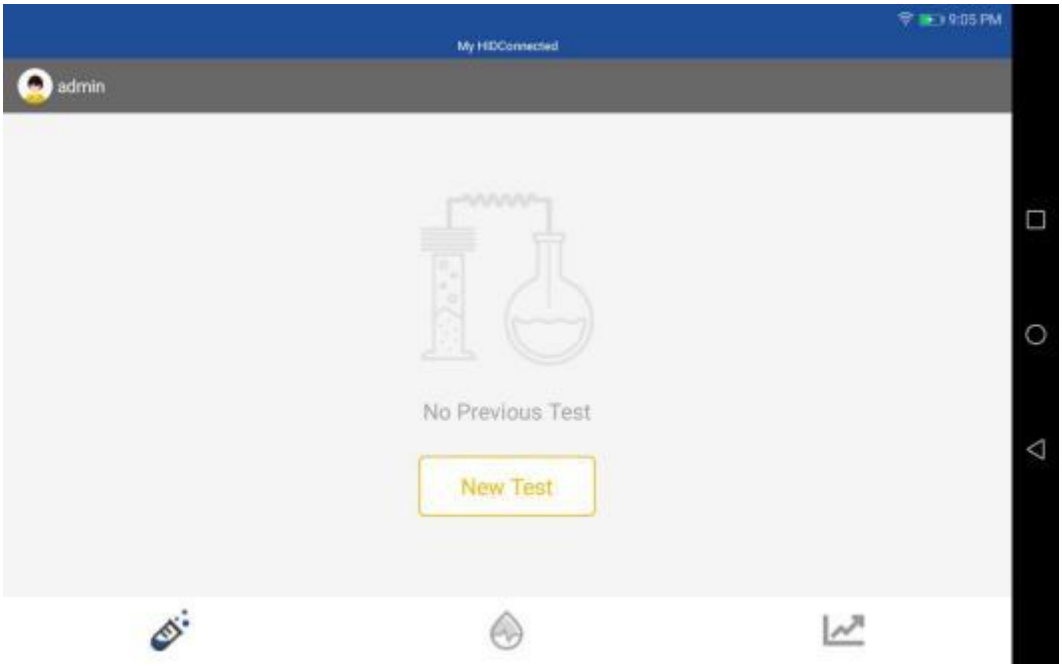
The setup page – set up new test

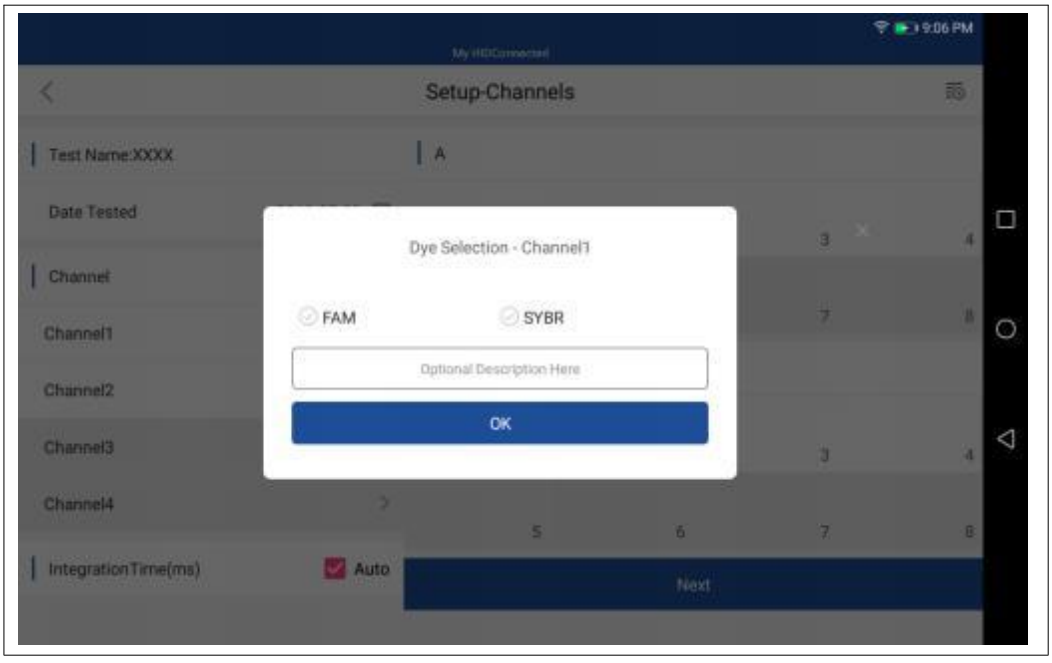
Click on the “New Test” button will take us to the setup page (Figure 2). Here we can set up the experiment. Here we can name the experiment and all the samples in the wells. We can also name and select the fluorescence channels for qPCR fluorescence reading.

Samples and fluorescence channels set up

The software automatically detects the number of reaction wells the connected instrument can support. Each reaction well can be associated with a sample. In this page, the user can enter a name for each sample. It a sample well name field is left empty (white spaces), it is considered that the sample does not exist for that well.

It is important to select the fluorescence channels we need to use for the experiment. The selection of the channels depends on the type of fluorescence dyes used in the assay design. We can also give a name for each selected channel





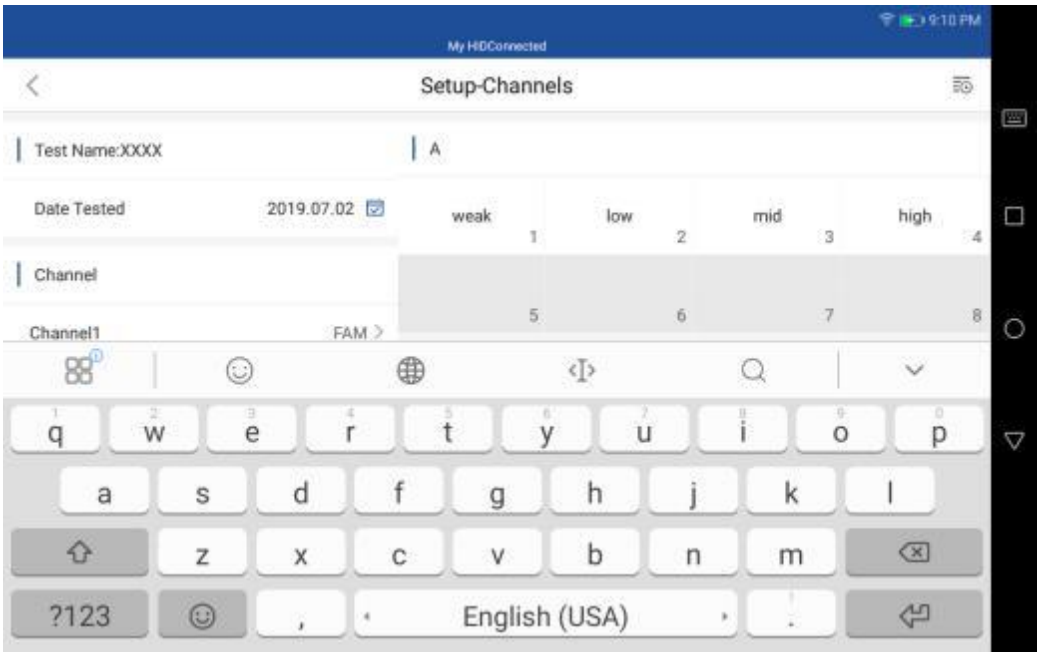


Figure 2 The setup page – channel and sample name setup

Open existing experiment for template file

TBD

When this step is done, we can click on the “Next step” button to continue setting the thermal cycling program.

The setup page – cycler setup

Here we can setup the thermal cycler program. We could choose the type and number of thermal periods and steps, assign time period and temperatures. We could have up to two Pre-Denature periods. The main thermal cycling period can have 2, 3, or 4 steps. We can also define at which step the fluorescence capture is done. Lastly, we have a hold period whose temperature and time period are configurable.

A cycler program can have several periods; and each period can have one or more steps. For example, we could have pre-denature period, cycle period and hold period. In the cycle period, we can have many repeated cycles consists of denature, annealing, and extension steps.

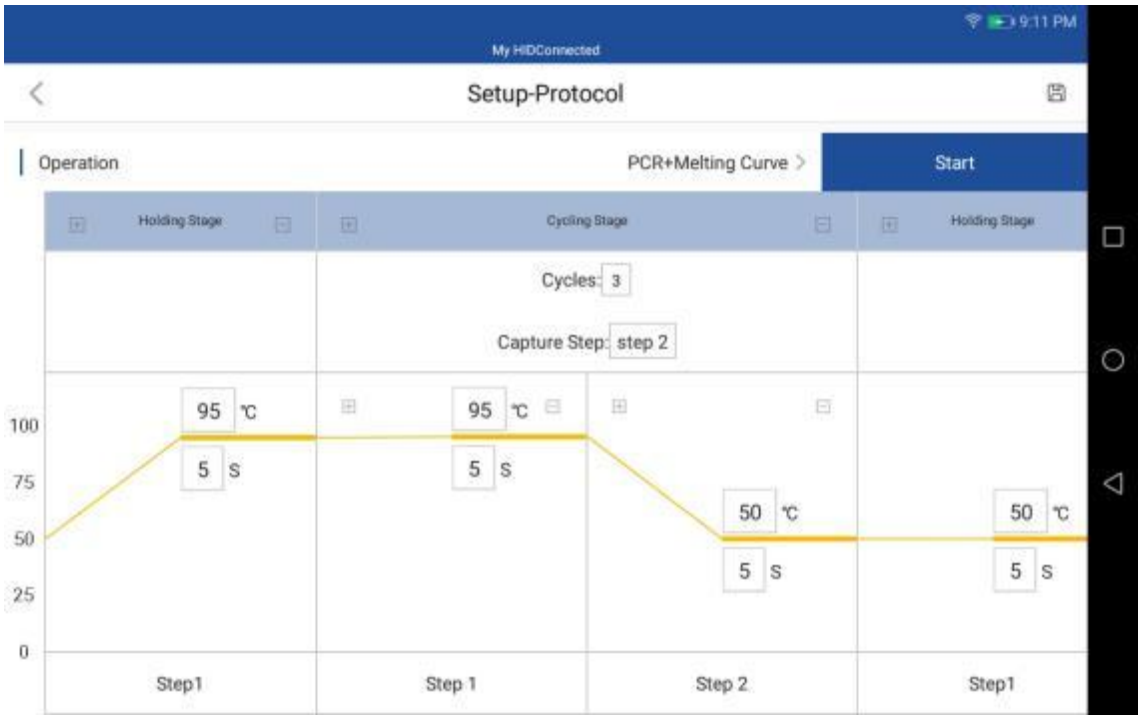


Figure 3a Thermal cycler setup

qPCR amplification thermal cycling program starts when the user clicks on the “Start” button.

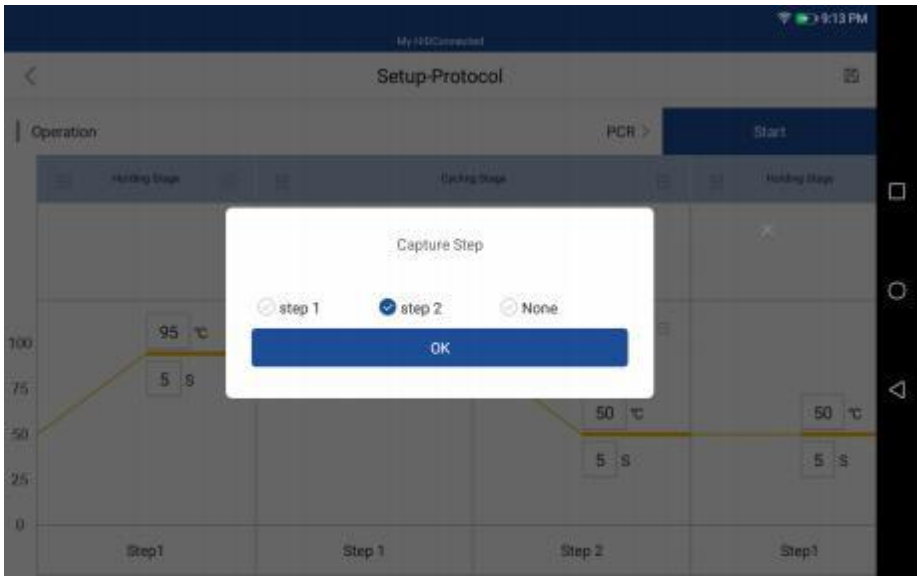


Figure 3b Set up the step to capture fluorescence image

Melting curve analysis period

We can also add a Melt Curve analysis period at the end of the main thermal cycling program. The Melt curve analysis can be triggered either manually by clicking the start melt analysis button, or automatically by checking “(tbd)” check box.

Hot lid temperature and reaction volume

In this page, we can also set the desired hot lid temperature and reaction volume. The latter (reaction volume setting) is used for data analysis only. It will not affect how the experiment is run.

Saving template

TBD

Auto Integration Time

This is an option to let the instrument automatically choose the optical sensor integration time (i.e. Exposure time of the fluorescence camera) according to the fluorescence strength of the assay (Marker (3)). The goal is to adjust the sensitivity of the fluorescence camera so that the signals from the chemistry fall into the appropriate dynamic range of the sensors and yield the best signal to noise ratio (signal quality). We recommend leaving this check box checked.

Running the experiment and monitor status

In the setup page – cycler setup, when we are satisfied with all the settings and loaded the samples, we can start running the experiment by click the “Start…” button.

Before the instrument starts to execute thermal cycling program, it will automatically check to ensure the lid is closed and the main power is applied. If either of these conditions is not met, the software will show popup windows with warnings. We could correct these programs and start again by simply press the “Start…” button again.

When the qPCR cycler starts to run, the software will automatically switch to Run pages. The first Run page will allow us to monitor hot lid and reaction well temperature. We can easily see if the temperature is going through the cycling programs or not.

Running amplification program

When running normal qPCR amplification program, the amplification window will real time monitor the fluorescence signal from the samples. In addition, the Run-Amplification Curve page will also show the current run status and estimated remaining run time.

Running melt analysis

When running Melt analysis, the “Run-Melt curve” will show the fluorescence change rate, i.e. fluorescence level derivative, as function of temperature. Note that negative change in fluorescence level will result in positive peaks in the derivative curve.

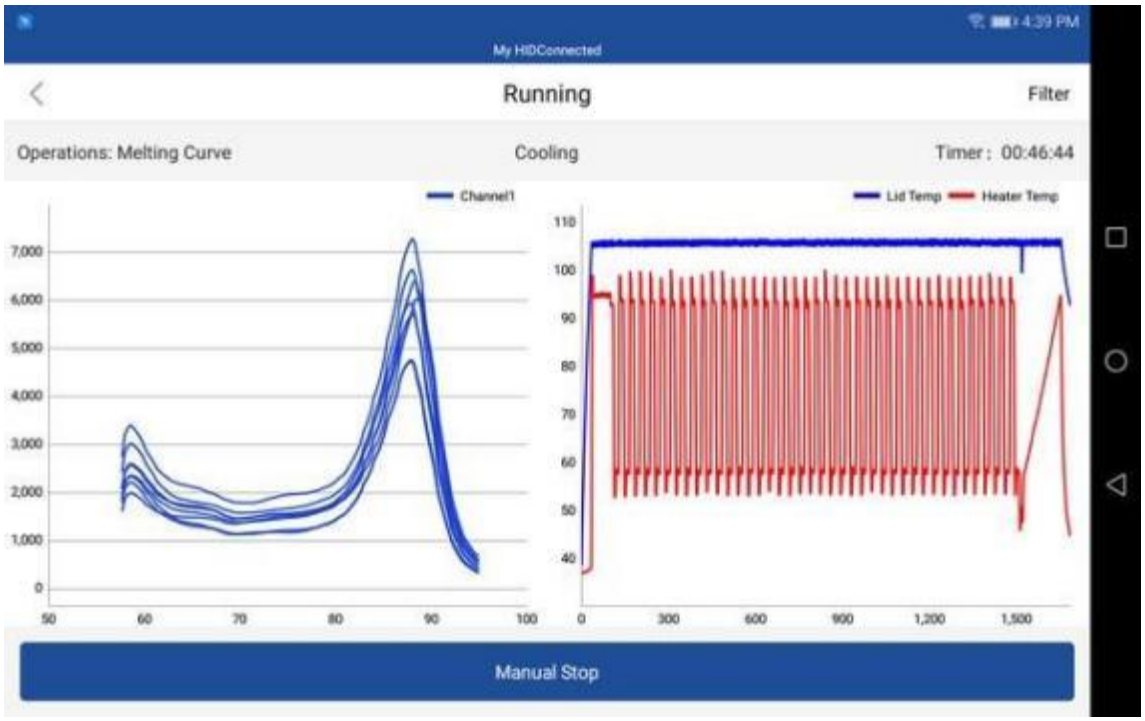


Figure 4. Melt running

Analysis

There are two analysis pages: Amplification analysis and Standard curve analysis. The latter is used for determining the quantity of the DNA targets using standard curve analysis method.

PCR amplification analysis

This will show the amplification curves for all samples. We can select the samples and study, set several parameters to this analysis.

Ct Threshold

The threshold for determining Ct is expressed as the percentage of the saturating level of the amplification levels after normalization. The default Ct threshold is 10%. The bigger this percentage, the more delayed the Ct values are.

Ct Low limit

The minimum value of Ct is set through this parameter. This setting is helpful in determining the base fluorescence value for amplification curve analysis. In most qPCR experiments, the concentration of the analytes prior to amplification is low, such that the Ct value cannot be less than 13 or so.

Normalize

When displaying amplification curve, we usually normalize the fluorescence values. This will allow us to visualize Ct threshold value. This choice will not affect the result of the analysis.

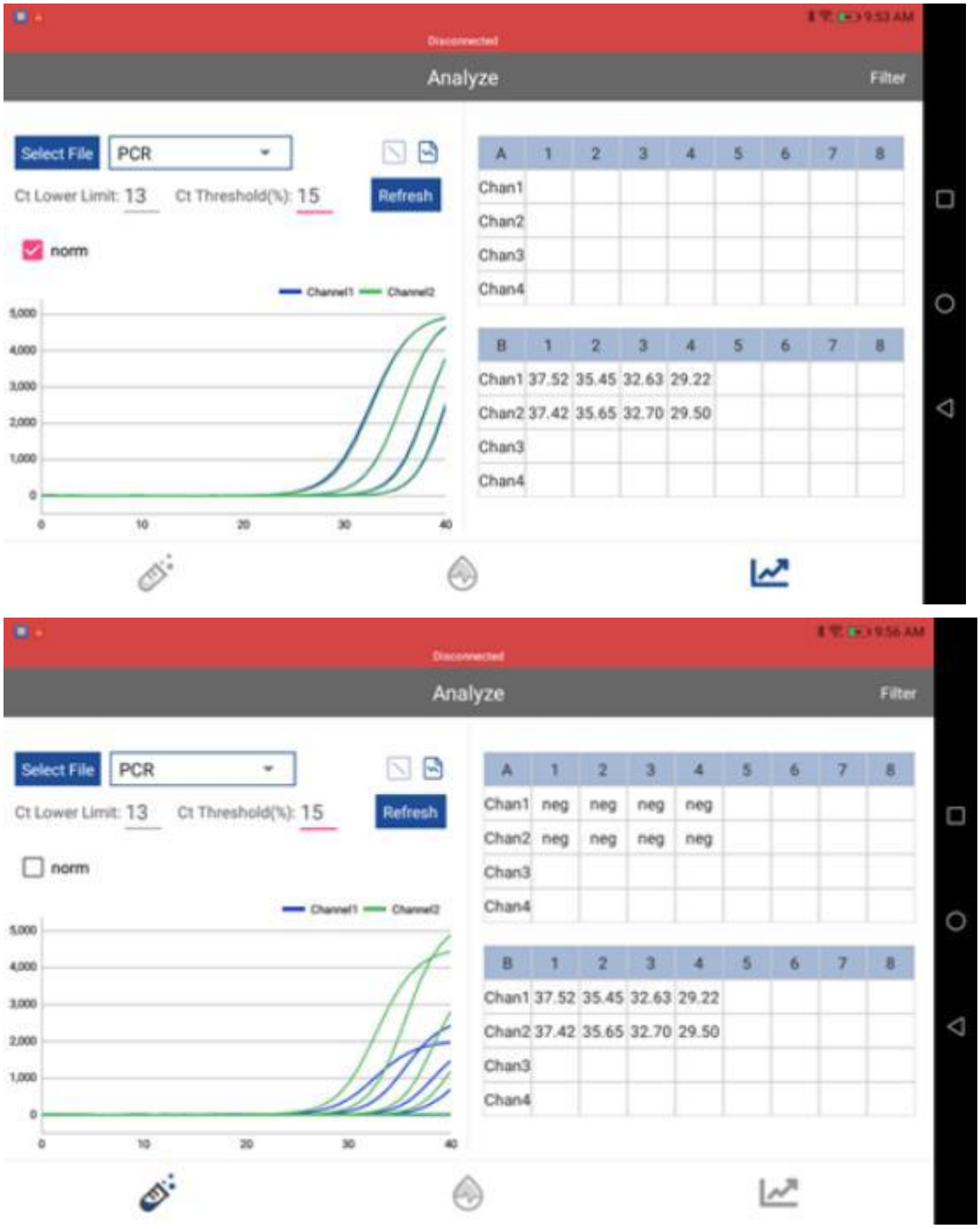


Figure 5. Amplification analysis

Standard Curve Analysis

Standard curve analysis is used to determine the absolute concentration of a sample by comparing to other samples with known amount of analyte concentration. We called the samples with known concentration "standards" and the ones with concentration that needs to be determined "unknowns".

For such analysis, the "standards" and "unknowns" should be in the same fluorescence channel to ensure they are comparable. Thus standard curve analysis is always performed on one specific channel.[4] We have to first the select the specific channel and perform such analysis.

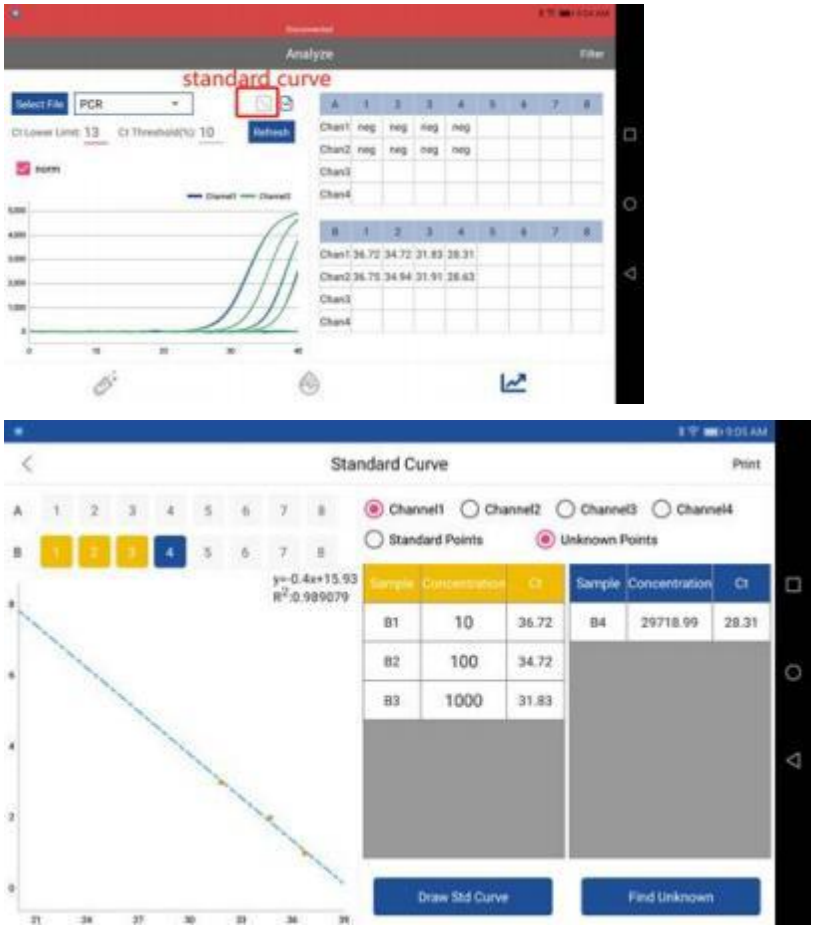


Figure 7. Standard curve analysis page

[4] We usually also make sure no other competing DNA targets and primer/probe sets are present in standard curve analysis samples, unless in very low concentration.

Steps to perform standard curve analysis:

Step 1:

Select the fluorescence channel for such analysis

Step 2:

Add standard samples – we can select the samples that are standard and click “Add the standard” button (Marker (1)) to add to standard table. We can then assign the concentration numerical values to these standard samples by typing into the table.

Step 3:

Draw the curve by clicking “Draw Std Curve”.

Step 4:

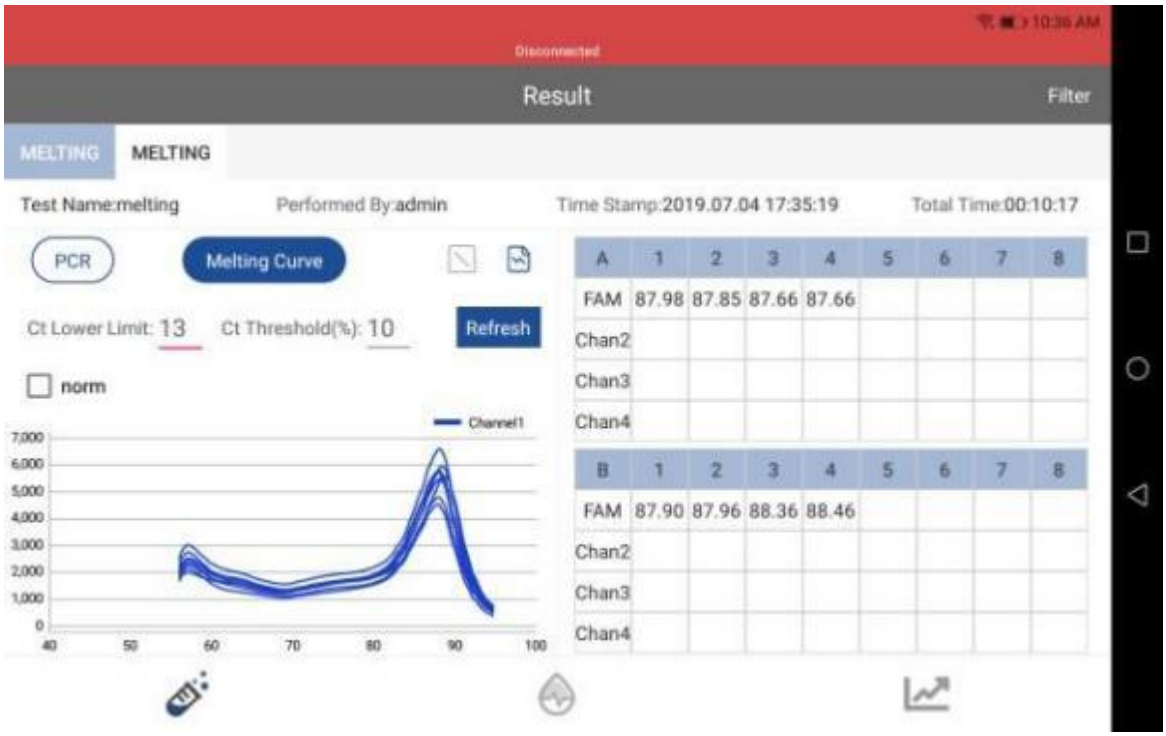
Add unknown samples to unknown table.

Step 5:

Calculate concentration for unknown samples.

Melt curve analysis

Melt curve analysis will calculate the peak of the melting curve – defined as the melting temperature. The melting temperature is calculated per sample/channel and the value is shown in the data table of the analysis page.



Save experiment and Print Report

Open report page to print report and save experiment. The experiment file will contain all the settings and experiment results.

To print report, click the report button shown. It will cause a print preview page to show. Click on “Print” option button to print a PDF report.

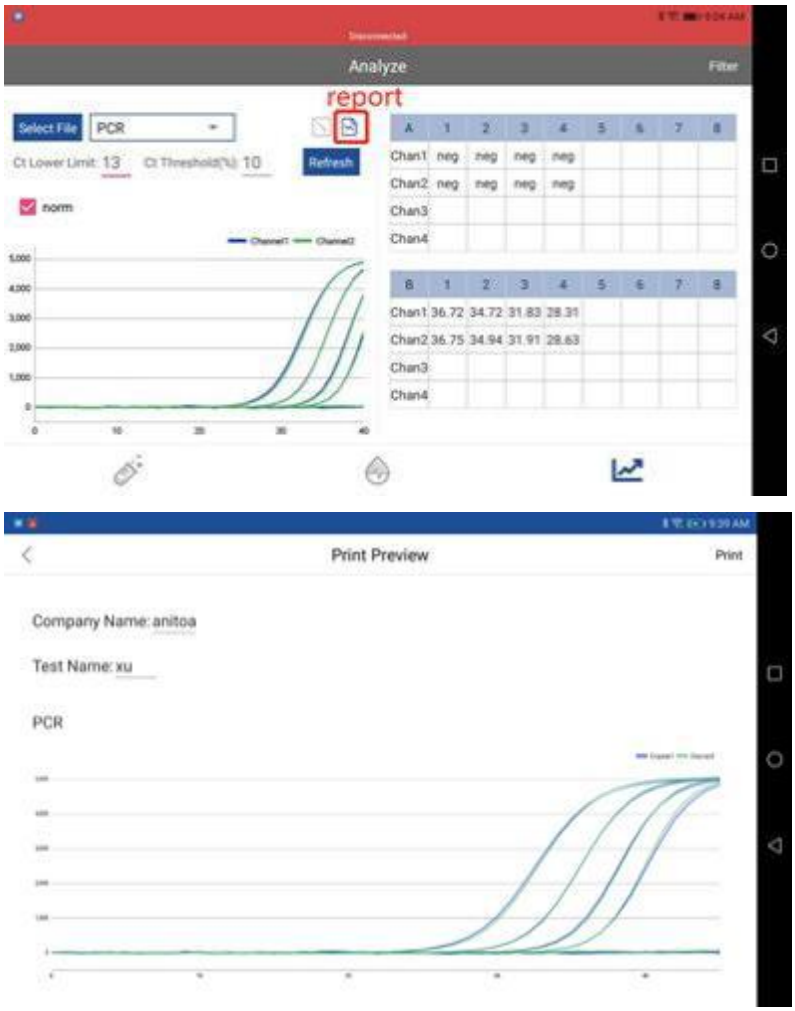


Figure 8. Save and print page