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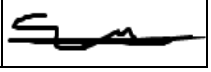
	Signature authorizer:	

1. Real-time PCR machine TRP89

2. Objectives & Scope

This protocol describes how to perform a real-time PCR on the TRP89 PCR detection systems. This SOP is applicable to all staff members working with this machine.

3. Abbreviations and definitions

For general abbreviations, definitions and terminology see QM 1 “General”.

TRP	TRP89 Real-Time PCR detection system
Ct	Threshold Cycle
LM	Laboratory Manager
LT	Laboratory Technologist
RT	Room temperature
Tm	Melting Temperature
EO	Technical Officer

4. Tasks, responsibilities and accountabilities

For general authorizations refer to the Authorization Matrix.

Task	Authorized	Responsible
Operating the machine according to chapter 8 “Operation”	LT	LM
Troubleshooting basic problems	LT/External technical expert	EO
Contacting supplier about errors (after consulting with LM)	EO	LM
Updating agenda	LT	LM
Updating logbook “Genetic Analyzer/PCR machines”	EO	LM

5. Description of the piece of equipment

The TRP89™ detection system is a six-channel real-time PCR system. It includes a X1090 thermal cycler chassis, TRP89 optical reaction module and PCR analysis software. See the manual for more information.

6. Safety and Environment

See the general safety instructions in the Biosafety Manual.

7. Startup procedure (calibration and controls) and maintenance

7.1 Calibration

The device tests itself before the measurement begins (see manual, http://www.TRP89.com).

7.2 Maintenance

It is not necessary to service the device on a regular basis. EO notes all malfunctions and actions undertaken in the logbook.

8. Operation

1. Sign up in time in the REAL TIME PCR TRP 1 + 2 (and sequencer) agenda with the addition TRP 1 or 2 and your name.
2. Turn on the computer (if off) and fill in username (RtPCR) and Password.
3. Turn on TRP machine No. 1 or 2. If under remote control appears on the TRP then the TRP is connected with the computer.
4. Go to Start, Specific systems, and click on PCR Analysis 2.0 software.
5. On the Startup wizard window, select Create a new run, then click OK.
6. If the PCR Analysis 2.0 software is already started then select under the tab “file”; “new”; “run”. The Run Setup window will appear, click on open lid or open the lid manually by using the Open button on the TRP machine 1 or 2, then place your plate or strips in the correct orientation in the machine, then click close lid (by computer or manual).
7. Click on Protocol, then click Select Existing for an existing protocol.
8. For creating a new protocol see manual.
9. On the “select protocol” window, the folder “admin” opens and then click on appropriate folder: If one of the TRP machines is already running, select “Desktop”, select “Shortcut admin” and select the appropriate folder.
   The following protocol will appear:
   1: 95,0°C for 10:00,
   2: 95,0°C for 0:10,
   3: 54,0°C for 0:05,
   4: 72,0°C for 0:15, Plate Read,
   5: GOTO 2, 39 more times,
   6: 95,0°C for 2:00,
   7: 20,0°C for 1:00,
   8: Melt Curve 70°C to 95°C: Increment 0,5°C for 0:10, Plate Read, End.
   * Sample volume 25µl, Run time approximately 1:35:00.
10. Click “next”. On the “plate tab”, select appropriate “express load” (e.g. Quick Plate 96 wells all channels or Quick Plate 96 wells only SYBR), click on “edit” to add or remove fluorophores, to change sample volume and add IDs (only one fluorophore per channel) or click “Select Existing” for a fixed plate design.
11. Click “next”. On the “Start Run” tab, select the machine: TRP1 OR TRP2.
12. Click on “start run” at the bottom of the window.
13. “Save optical data file” window will appear. Select the appropriate file to save the data.
14. The machine will automatically start.
15. When the machine finishes the experiment, open the lid, take out the plate or strips then close the lid, discard the plate or strips in the blue container with black lid near the machine according to P09 “Waste disposal”.
16. Leave machine and computer on. Close only your data analysis file.

9. Problem solving

Check the manual for more information in case of problems, or refer to the responsible person of the device and/ or EO. When necessary, contact the supplier (after consulting HGA/LM). Communicate all problems with the EO, who will record the actions undertaken in the logbook “Genetic Analyser/PCR machines”.

10. Related Documents

- QM 1 “General”
- P09 “Waste disposal”
- REAL TIME TRP 1 + 2 (and sequencer) agenda, next to the device
- TRP89 Real-Time PCR Detection Systems. Instruction manual. Online at: http://www.TRP89.com Paper manual: next to the device in room A3.
- Logbook “Genetic Analyser/PCR machines”, room A3
- Biosafety Manual in room B3 (office Biosafety Officer) B2 (secretary), B1 (LM) and A4 (weighing room).

11. Related Forms

- A12 form 01 “Worksheet SYBR Green real time PCR”
- P43 form 01 “Induction of New Personnel”

12. References

N/A

13. Attachments

- QM 2 annex 1 “Authorization Matrix”