

QIAGEN Supplementary Protocol

PCR and data analysis using the *cador*[®] *T. equigenitalis* PCR Kit on Rotor-Gene[®] Q instruments

This protocol is designed for using the *cador T. equigenitalis* PCR Kit for detection of *T. equigenitalis* DNA by real-time PCR on Rotor-Gene Q, Rotor-Gene 6000, or Rotor-Gene 3000 instruments.

IMPORTANT: Please read the *cador T. equigenitalis* PCR Kit Handbook, paying careful attention to the Safety Information and Important Notes sections, before beginning this procedure. For Research Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease. Analytical and performance characteristics have not been established.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- DNA isolation kit (see "Bacterial DNA isolation" in the *cador T. equigenitalis* PCR Kit Handbook)
- *cador T. equigenitalis* PCR Kit (cat. no. 285003 or 285005)
- Pipets (adjustable)*
- Sterile pipet tips with filters
- Vortexer*
- Rotor-Gene Q or other Rotor-Gene instrument* with fluorescence channels for Cycling Green and Cycling Yellow or with fluorescence channels for Cycling A.FAM and Cycling A.JOE
- Rotor-Gene Q software version 1.7.94 (Rotor-Gene 6000 software version 1.7.65, 1.7.87, 1.7.94; Rotor-Gene 3000 software version 6.0.23)
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)
- Alternatively: PCR Tubes, 0.2 ml, for use with 36-well rotor (cat. no. 981005 or 981008)
- Cooling block (Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901)

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.



Important points before starting

- Before beginning the procedure, read “Important Notes” in the cador *T. equigenitalis* PCR Kit Handbook.
- Take time to familiarize yourself with the Rotor-Gene instrument before starting the protocol. See the instrument user manual.
- Make sure that at least one *T. equigenitalis* Control as well as one negative control (Water, PCR grade) are included per PCR run.

Things to do before starting

- Make sure that the cooling block (accessory of the Rotor-Gene instrument) is precooled to 2–8°C.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

Procedure

1. **If you are using the internal control to monitor the DNA isolation procedure and to check for possible PCR inhibition, follow step 1a. If you are using the internal control exclusively to check for PCR inhibition, follow step 1b.**
- 1a. **The internal control has already been added in the isolation procedure (see “Internal control” in the cador *T. equigenitalis* PCR Kit Handbook). In this case, prepare a master mix, in the cooling block at 2–8°C, according to Table 1.**

The reaction mix typically contains all of the components needed for PCR except the sample. Prepare a volume of reaction mix at least 10% greater than that required for the total number of PCR assays to be performed.

Table 1. Preparation of master mix (internal control already added in the isolation procedure)

Number of samples	1	12
T. equigenitalis Master	33.5 μ l	402 μ l
T. equigenitalis Mg-Sol	6.5 μ l	78 μ l
T. equigenitalis IC	0 μ l	0 μ l
Total volume	40 μl	480 μl

- 1b. The internal control must be added directly to the *T. equigenitalis* Master (no control added in the isolation procedure). In this case, prepare a master mix, in a cooling block at 2–8°C or on ice, according to Table 2.**

The reaction mix contains all of the components needed for PCR except the sample. Prepare a volume of reaction mix at least 10% greater than that required for the total number of PCR assays to be performed.

Table 2. Preparation of master mix with internal control

Number of samples	1	12
T. equigenitalis Master	33.5 μ l	402 μ l
T. equigenitalis Mg-Sol	6.5 μ l	78 μ l
T. equigenitalis IC	2 μ l	24 μ l
Total volume	42 μl*	504 μl*

* The increase in volume caused by adding the internal control is not taken into account when preparing the PCR assay. The sensitivity of the detection system is not impaired.

- 2. Pipet 40 μ l of the master mix into each reaction tube. Then add 10 μ l of the eluate from the DNA isolation (see Table 3). Correspondingly, 10 μ l of the positive control (*T. equigenitalis* Control) must be used as a positive control and 10 μ l of water (Water, PCR grade) as a negative control. Mix the solutions thoroughly by pipetting repeatedly up and down.**

The external positive control (*T. equigenitalis* Control) is treated as previously purified samples and the same volume is used (10 μ l). This positive control provides a positive reference signal.

Table 3. Preparation of PCR assay

Component	Volume
Master mix	40 μ l
Sample	10 μ l
Total volume	50 μ l

- 3. Close the reaction tubes. Make sure that the locking ring (accessory of the Rotor-Gene instrument) is placed on top of the rotor to prevent accidental opening of the tubes during the run.**

4. For the detection of *T. equigenitalis* DNA, create a temperature profile according to the following steps (Table 4). An overview of the cycling protocol is given in Table 5.

Table 4. Creating the temperature profile

Setting the general assay parameters	Figures 1, 2, 3
Initial activation of the hot-start enzyme	Figure 4
Amplification of the DNA	Figure 5
Adjusting the fluorescence channel sensitivity	Figure 6, 7
Starting the run	Figures 8, 9

All specifications refer to the Rotor-Gene Q software version 1.7.94, Rotor-Gene 6000 software versions 1.7.65, 1.7.87, 1.7.94, and Rotor-Gene 3000 software version 6.0.23. Please find further information on programming Rotor-Gene Instruments in the instrument user manual. In the illustrations these settings are framed in bold black. Illustrations are included for Rotor-Gene Q Instruments. Where different values are required for the Rotor-Gene 3000, these differences are described in the text.

Table 5. Cycling protocol

			Comments
Initial PCR activation step:	5 min	95°C	Initial denaturation of template DNA.
3-step cycling:			
Denaturation	15 s	95°C	Data acquisition
Annealing	45 s	60°C	
Extension	20 s	72°C	
Number of cycles	45		

5. First, open the "New Run Wizard" dialog box (Figure 1). Select the rotor used. Check the "Locking Ring Attached" box and click "Next".

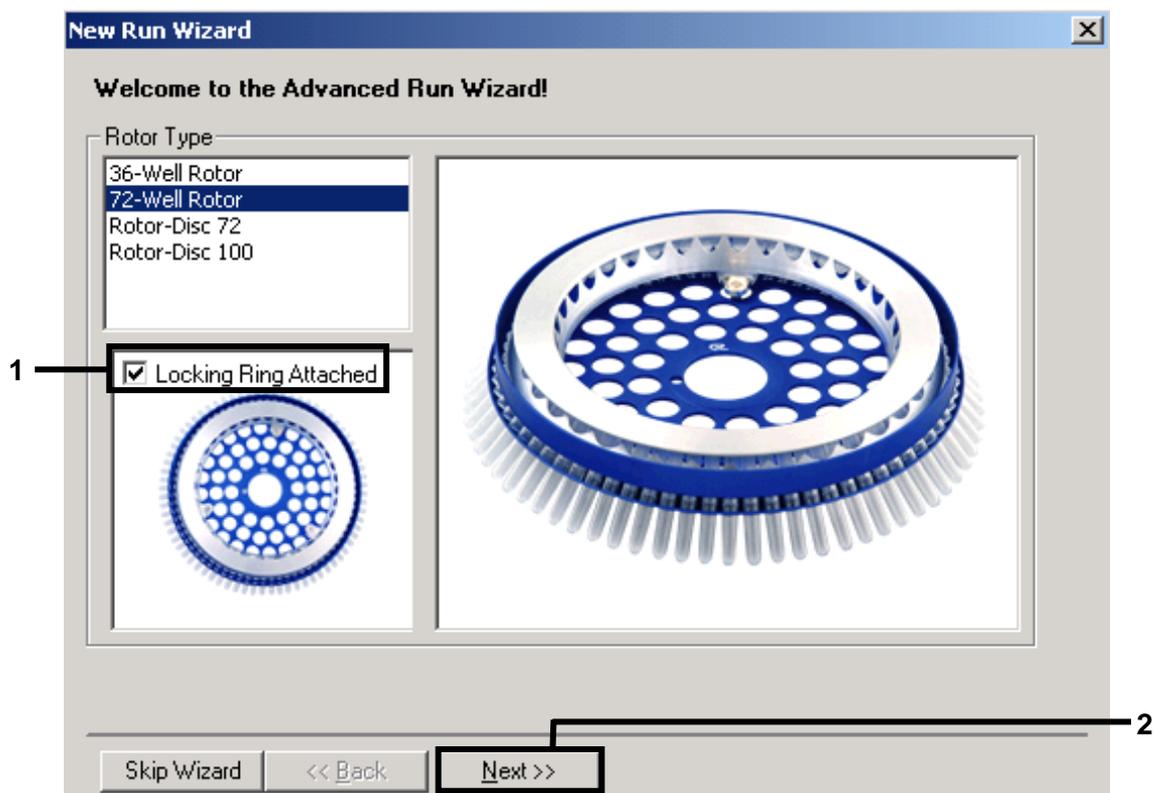


Figure 1. The "New Run Wizard" dialog box.

6. Select 50 for the PCR reaction volume and click "Next" (Figure 2).

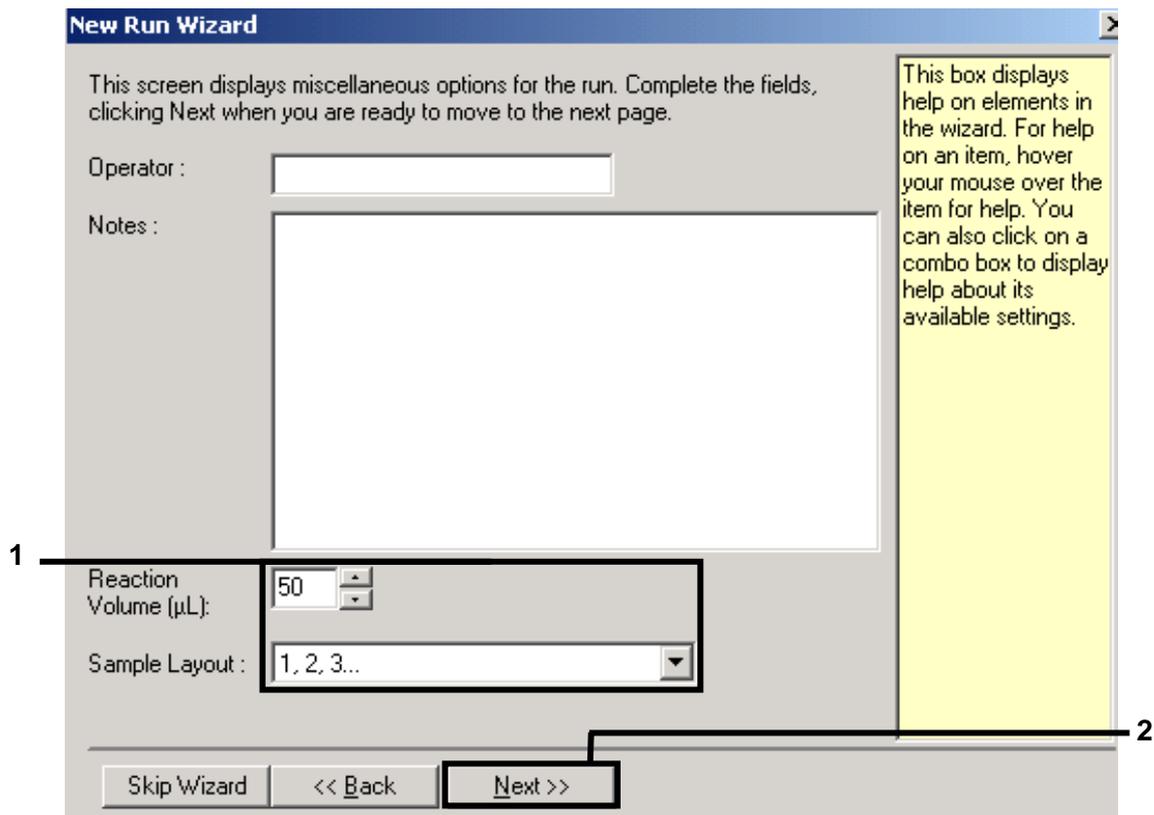


Figure 2. Setting the general assay parameters.

- Click the "Edit Profile" button in the next "New Run Wizard" dialog box (Figure 3), and program the temperature profile as shown in Figures 3–5).

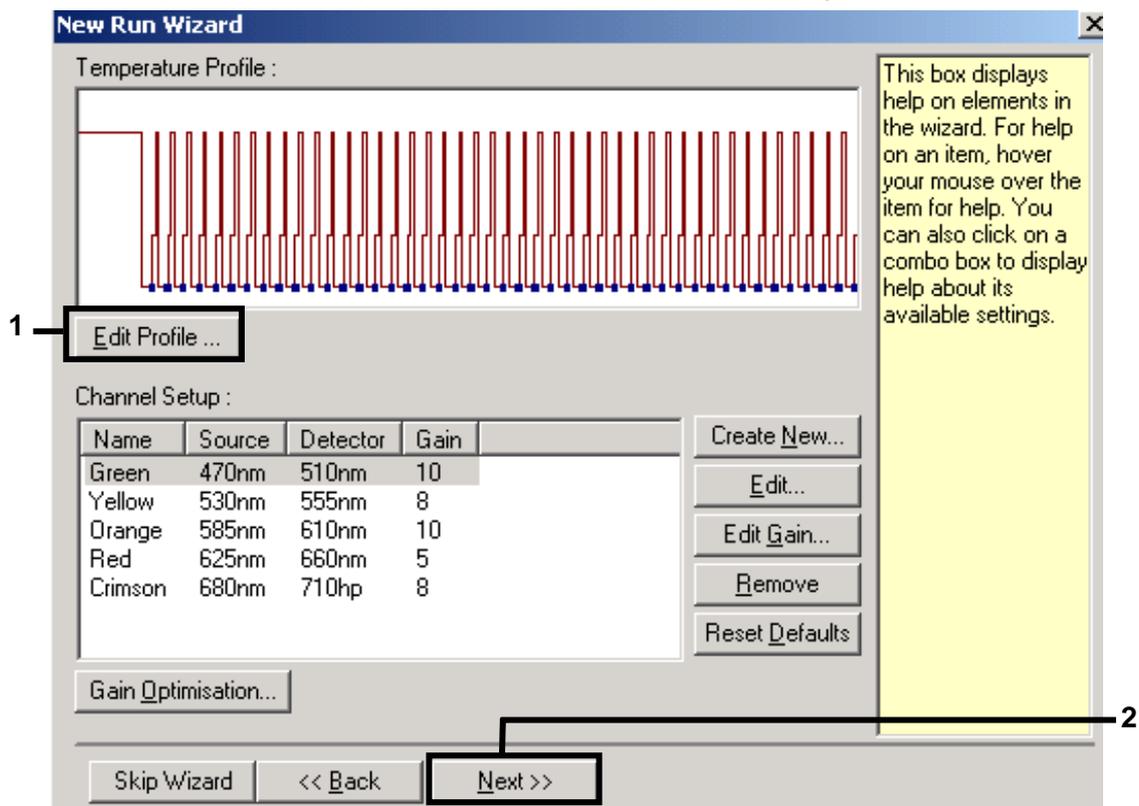


Figure 3. Editing the profile. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

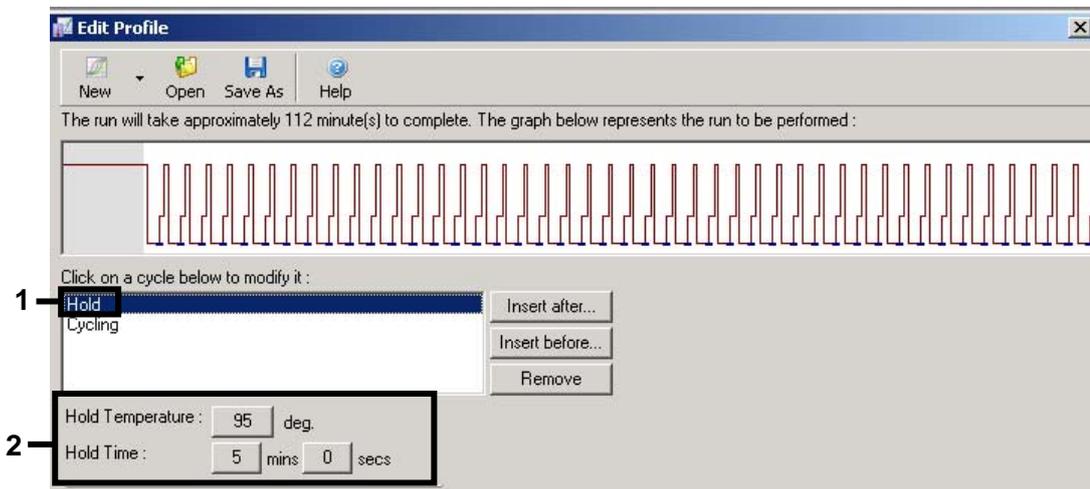


Figure 4. Initial activation of the hot-start enzyme.

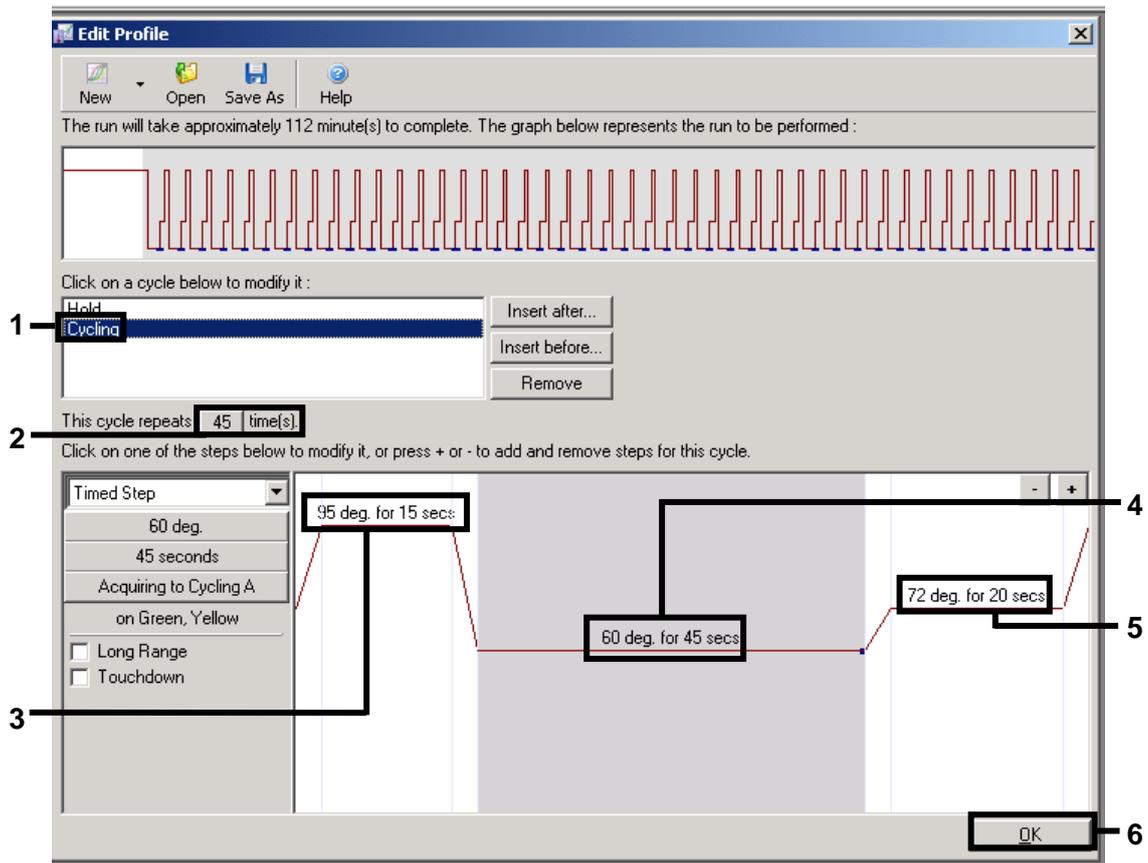


Figure 5. Amplification of the DNA. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr, JOE".

8. The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click "Gain Optimisation" in the "New Run Wizard" dialog box (Figure 6) to open the "Auto-Gain Optimisation Setup" dialog box. Tick the box "Perform Optimization Before 1st Acquisition" (Figure 7).

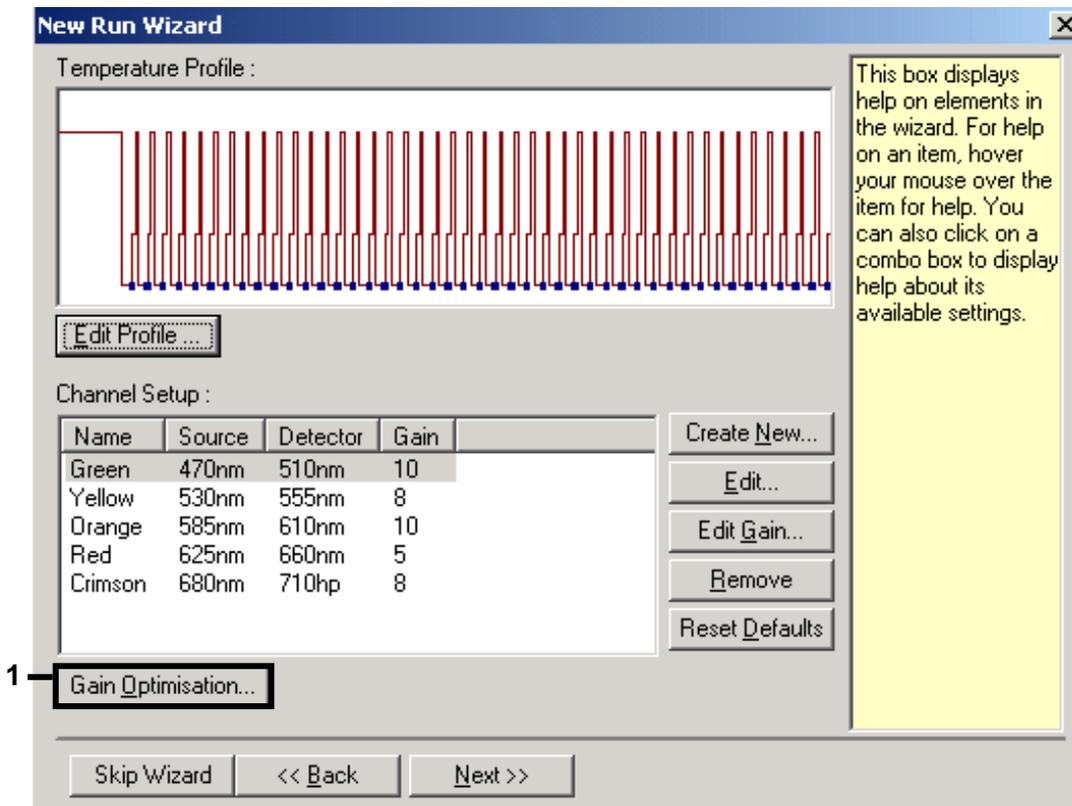


Figure 6. Gain Optimisation. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

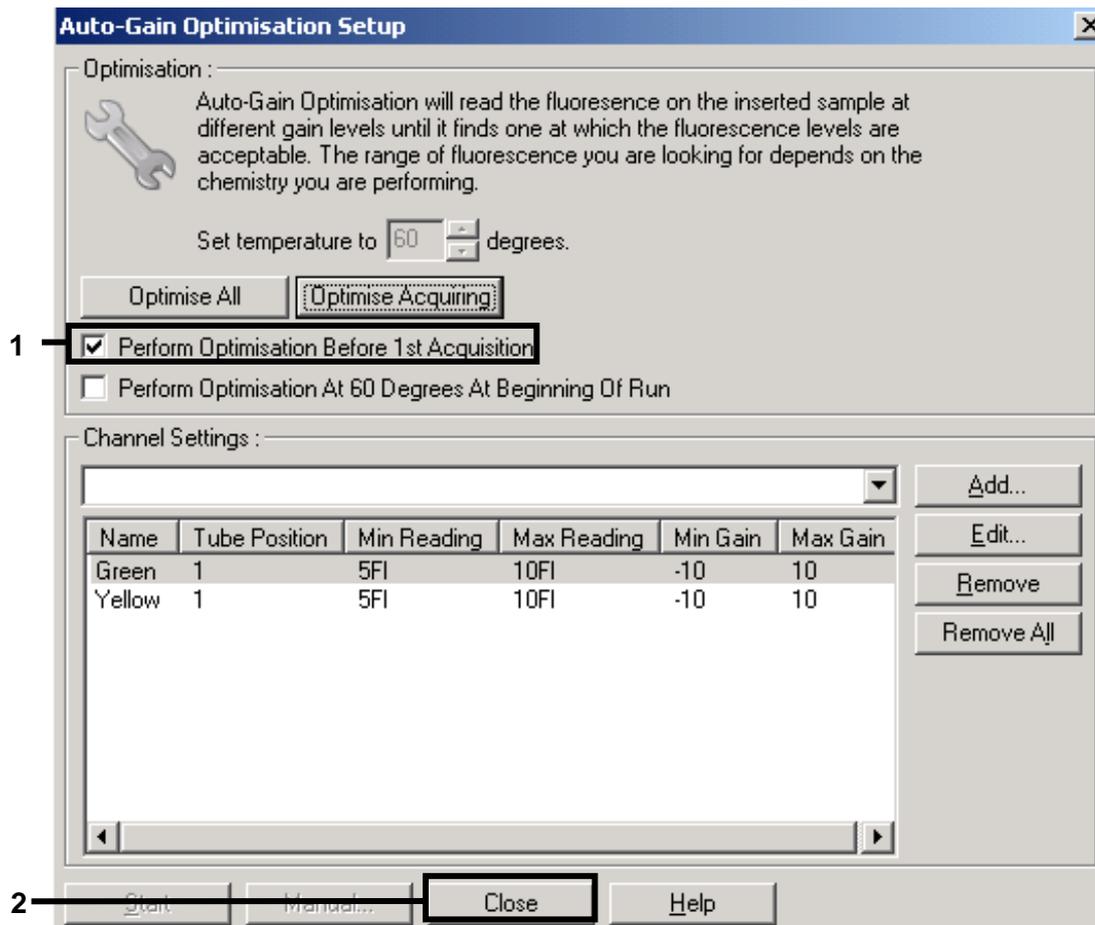


Figure 7. Adjusting the fluorescence channel sensitivity. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

9. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (Figure 8). Click "Start Run". A dialog box will appear for you to name the run and store it (Figure 9).

It is recommended to store the run on the computer initially and not on the network drive.

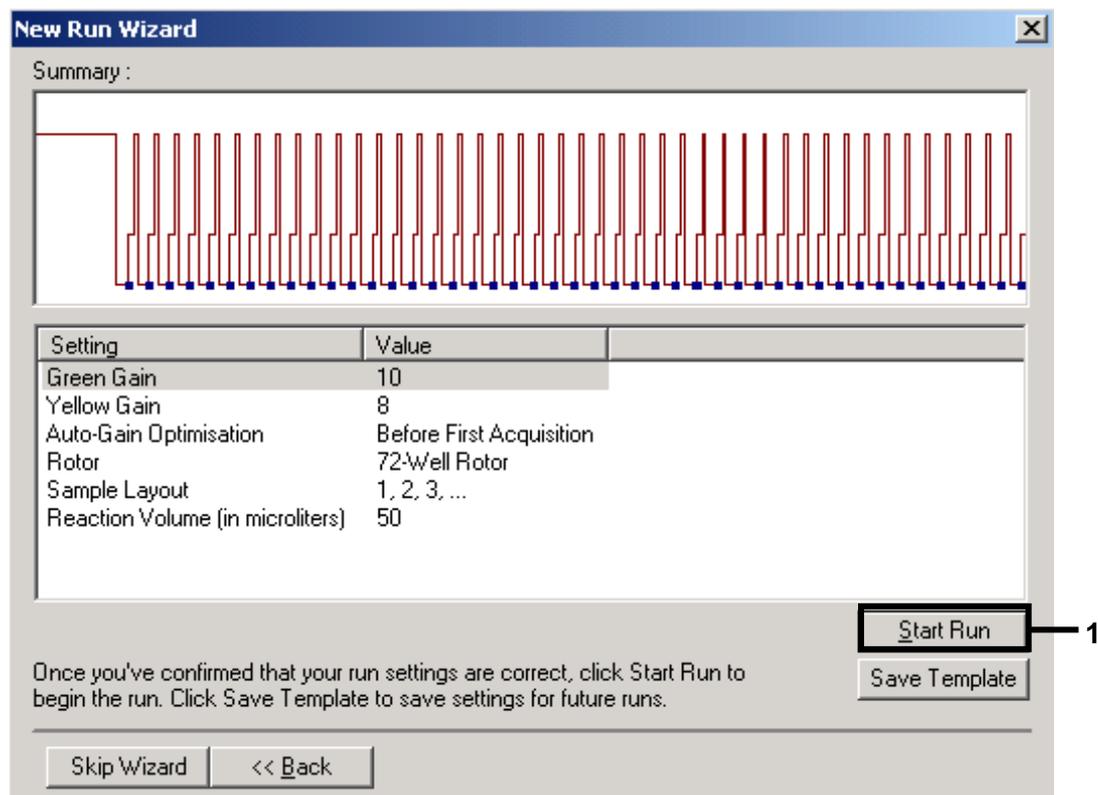


Figure 8. Starting the run. Note that, on the Rotor-Gene 3000, the software will define the fluorescence dyes as "FAM/Sybr" and "JOE".

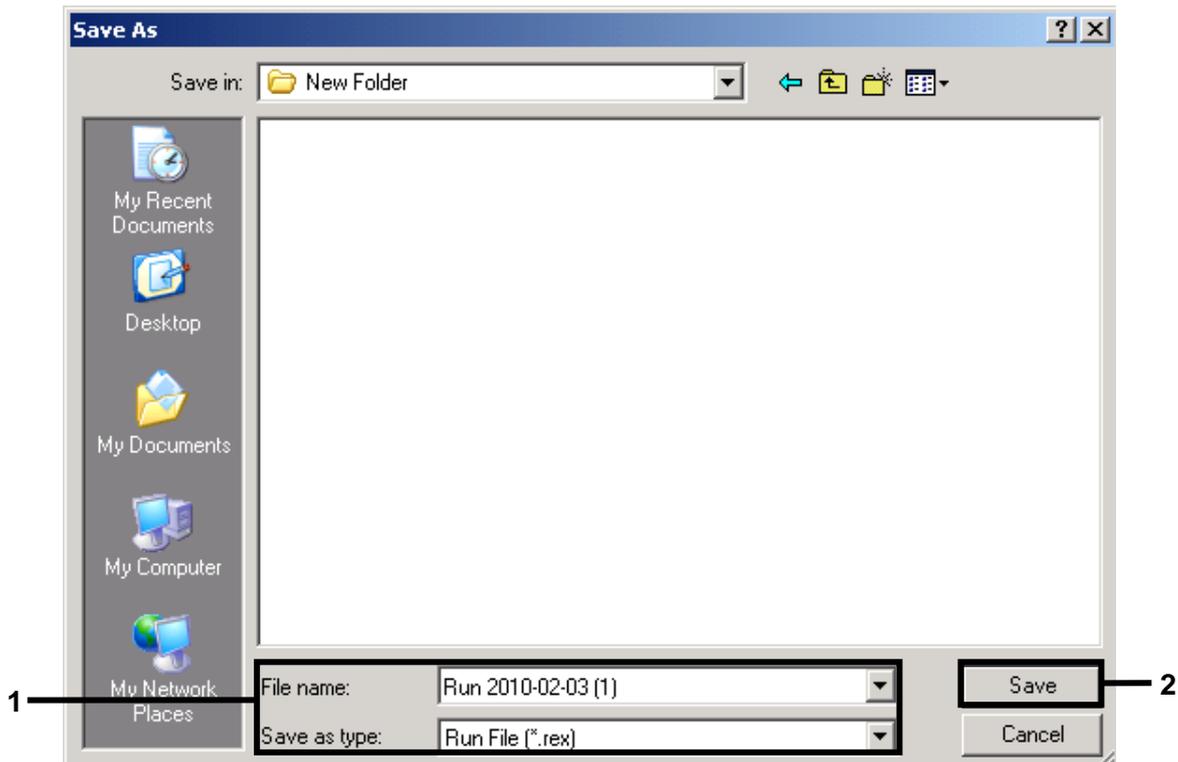


Figure 9. Naming and saving the run.

- 10. After the run is finished, analyze the data. Click the “Analysis” button, mark the channel to be analyzed, and click “Show”. The following results (10a, 10b, and 10c) are possible.**

Channels used are Cycling Green or A.FAM (for target) and Cycling Yellow or A.JOE (for internal control). Make sure to switch “Dynamic tube” on and activate linear scale.

Examples of positive and negative PCR reactions are given in Figure 10 and Figure 11.

- 10a. A signal is detected in fluorescence channel Cycling Green. The result of the analysis is positive: the sample contains *T. equigenitalis* DNA.**

In this case, the detection of a signal in the Cycling Yellow channel is dispensable, since high initial concentrations of *T. equigenitalis* DNA (positive signal in the Cycling Green channel) can lead to a reduced or absent fluorescence signal of the internal control in the Cycling Yellow channel (competition).

Note: Note that, on the Rotor-Gene 3000, the relevant channels are Cycling A.FAM for the positive signal and Cycling A.JOE for the internal control.

10b. In fluorescence channel Cycling Green no signal is detected. At the same time, a signal from the internal control appears in the Cycling Yellow channel. In the sample no *T. equigenitalis* DNA is detectable. It can be considered negative.

In the case of a negative *T. equigenitalis* PCR, the detected signal of the internal control rules out the possibility of PCR inhibition, which could lead to false negative results.

Note: Note that, on the Rotor-Gene 3000, the relevant channels are Cycling A.JOE for the internal control and lack of a signal for Cycling A.FAM.

10c. No signal is detected in the Cycling Green channel, and no signal is detected in the Cycling Yellow channel. No result can be concluded.

Information regarding error sources and their solution can be found in "Troubleshooting Guide" in the cador *T. equigenitalis* PCR Kit Handbook.

Note: Note that, on the Rotor-Gene 3000, the relevant channels are Cycling A.FAM and Cycling A.JOE.

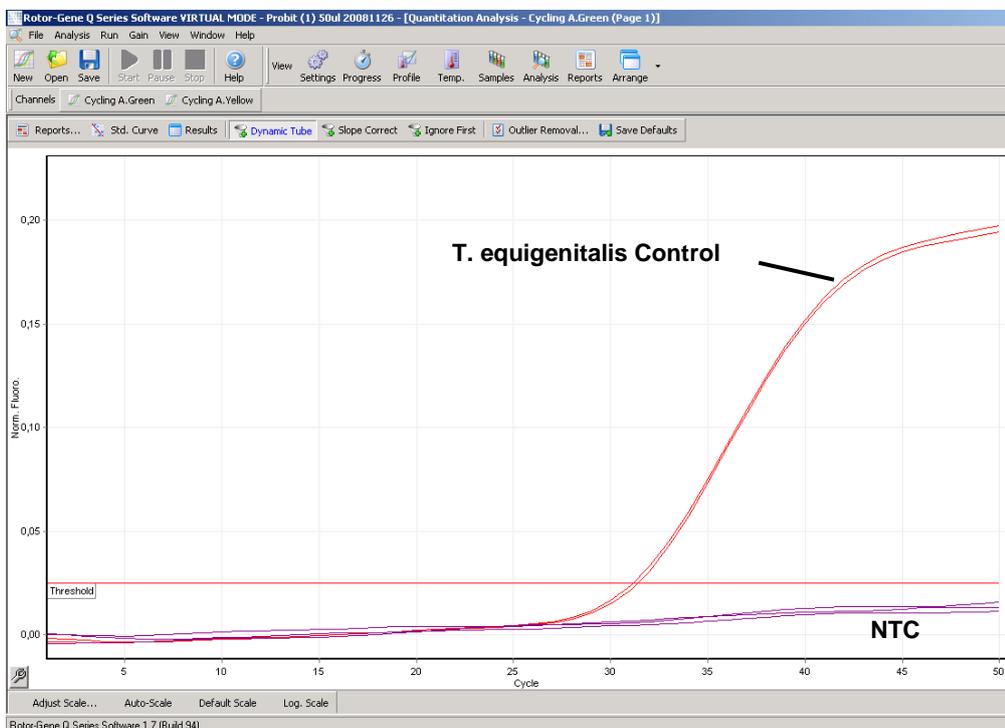


Figure 10. Detection of the positive control (*T. equigenitalis* Control) in fluorescence channel Cycling Green. NTC: No template control (negative control).

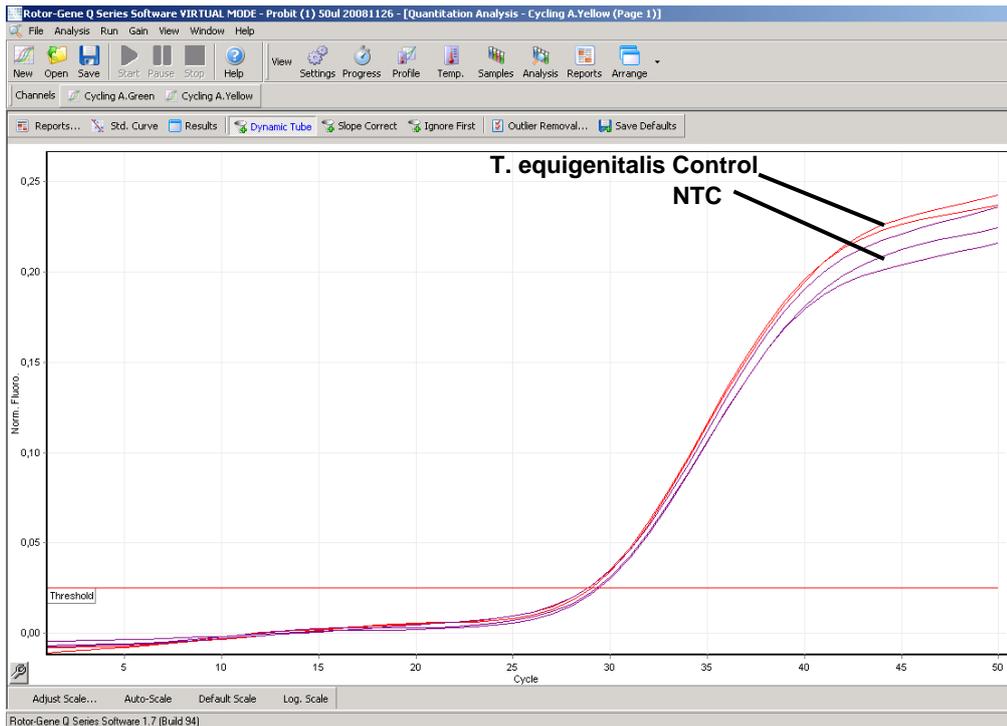


Figure 11. Detection of the internal control (IC) in fluorescence channel Cycling Yellow with simultaneous amplification of the positive control (*T. equigenitalis* Control). NTC: No template control (negative control); amplification plot shows amplification of the internal control in a *T. equigenitalis* negative sample.

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the *cador* T. equigenitalis PCR Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

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