## **QIAGEN Supplementary Protocol**

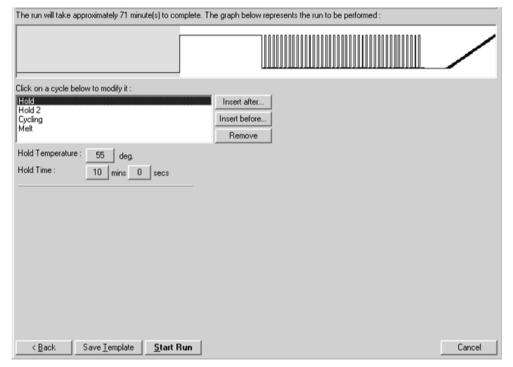
# Rotor-Gene® software setup for the Rotor-Gene SYBR® Green RT-PCR Kit

This protocol shows the necessary parameters that need to be entered into the Rotor-Gene software (version 6.0) when using the Rotor-Gene SYBR Green RT-PCR Kit.

**IMPORTANT**: Please read the Rotor-Gene SYBR Green Handbook, paying careful attention to the "Safety Information" section, before beginning this procedure.

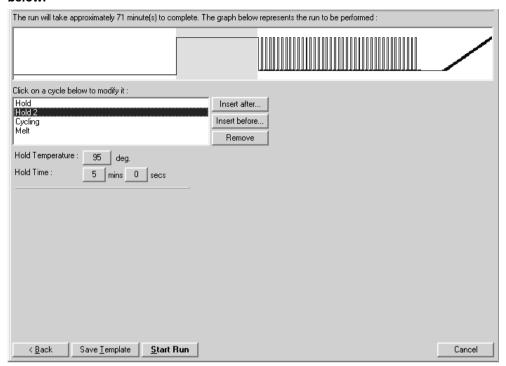
### **Procedure**

- 1. Launch the software and set up the program as described in the next few steps.
- Select "Hold" and set up the parameters for the reverse-transcription step as shown below.

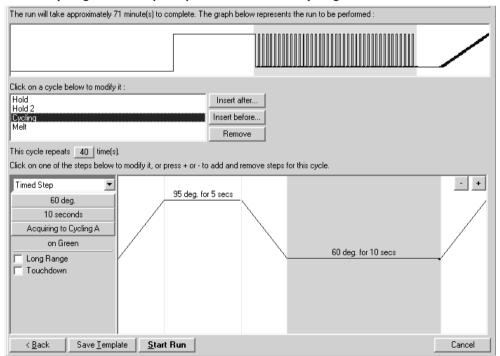




3. Select "Hold 2" and set up the parameters for the initial activation step as shown below.



4. Select "Cycling" and set up the parameters for PCR cycling as shown below.



### 5. Select "Melt" and set up the parameters for melting curve analysis as shown below. The run will take approximately 71 minute(s) to complete. The graph below represents the run to be performed:

Click on a cycle below to modify it Hold Hold 2 Insert after... Insert before. Remove Ramp from 60 degrees to 95 Rising by degree(s) each step, seconds of pre-melt conditioning on first step, seconds for each step afterwards. on Green Melt A Acquire to Gain Optimisation Optimise gain before melt on all tubes The gain giving the highest fluorescence less than 70 will be selected. < Back Save <u>T</u>emplate Start Run Cancel

Load your PCR tubes and start the program.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.giagen.com/Support/MSDS.aspx.

The Rotor-Gene SYBR Green RT-PCR Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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#### NOTICE TO PURCHASER:

The purchase of this product (Rotor-Gene Q, Rotor-Disc) includes a limited, non-transferable license to certain patents (see details below) surrounding rapid polymerase chain reaction (PCR) methods and instrumentation, the use of SYBR Green I in PCR reactions, melting curve analysis, analysis methods of DNA melting data, specifically high resolution melting (HRM) and others.

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