QIAGEN Supplementary Protocol

Rotor-Gene[®] software setup for the Rotor-Gene SYBR[®] Green 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 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 initial activation step as shown below.

The run will take approximately 61 minute(s) to complete. The	ne graph below represents the run to be performed :
Click on a cycle below to modify it :	
Hold Cycling Melt	Insert after Insert before Remove
, Hold Temperature : 95 deg.	
Hold Time : 5 mins 0 secs	
< <u>B</u> ack Save <u>T</u> emplate <u>S</u> tart Run	Cancel



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

The run will take approximately 61 minute(s) to complete. The graph below represents the run to be performed :		
Click on a cycle below to modify it :		
Hold Insert after		
Melt Insert before		
Remove		
This cycle repeats 40 time(s).		
Click on one of the steps below to modify it, or press + or - to add and remove steps for this cycle.		
Timed Step 95 deg. for 5 secs 60 deg. 95 deg. for 5 secs 10 seconds Acquiring to Cycling A on Green 60 deg. for 10 secs Long Range 60 deg. for 10 secs	-	
< <u>Back</u> Save <u>T</u> emplate <u>Start Run</u>	Cancel	

4. Select "Melt" and set up the parameters for melting curve analysis as shown below.

The run will take approximately 61 minute(s) to complete. The graph below represents the run to be performed :
Click on a cycle below to modify it :
Hold Insert after
Melt Insert before
Remove
Ramp from 60 🕂 degrees to 95 📫 degrees,
Rising by 1 each step,
Wait for 90 seconds of pre-melt conditioning on first step,
Wait for 5 seconds for each step afterwards.
Acquire to Melt A on Green
- Gain Optimisation
✓ Optimise gain before melt on all tubes.
The gain giving the highest fluorescence less than 70
will be selected.
< Back Save Template Start Run Cancel

5. 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 <u>www.qiagen.com/literature</u>.

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

The Rotor-Gene SYBR Green 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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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.

The purchase of this product (Rotor-Gene Q, Rotor-Disc) includes a limited, non-transferable license to one or more of US Patents Nos 6,787,338; 7,238,321; 7,081,226; 6,174,670; 6,245,514; 6,569,627; 6,303,305; 6,503,720; 5,871,908; 6,691,041; 7,387,887; and U.S. Patent Applications Nos. 2003-0224434 and 2006-0019253 and all continuations and divisionals, and corresponding claims in patents and patent applications outside the United States, owned by the University of Utah Research Foundation, Idaho Technology, Inc., and/or Roche Diagnostics GmbH, for internal research use or for non-in vitro diagnostics applications. No right is conveyed, expressly, by implication or estoppel, for any reagent or kit, or under any other patent or patent claims owned by the University of Utah Research Foundation, Idaho Technology, Inc., and/or Roche Diagnostics GmbH, or by any other Party. For information on purchasing licences for in-vitro diagnostics applications or reagents, contact Roche Molecular Systems, 4300 Hacienda Drive, Pleasanton, CA 94588, USA.

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Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA and/or Roche Molecular Systems, 4300 Hacienda Drive, Pleasanton, CA 94588, USA. No right is conveyed expressly, by implication or by estoppel under any other patent claim, such as claims to apparatus, reagents, kits, or methods such as 5' nuclease methods.

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