

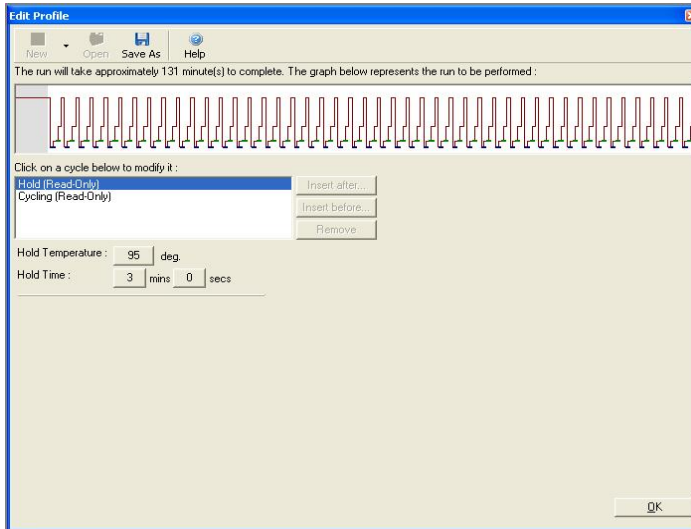
Experimental Protocol and Evaluation for Rotorgene 6000 with Minerva Biolabs real-time PCR Kits

1. Experimental Protocol

Program Step 1: **HOLD**

Hold Temperature: 95 °C

Hold Time: 3 min 0 sec



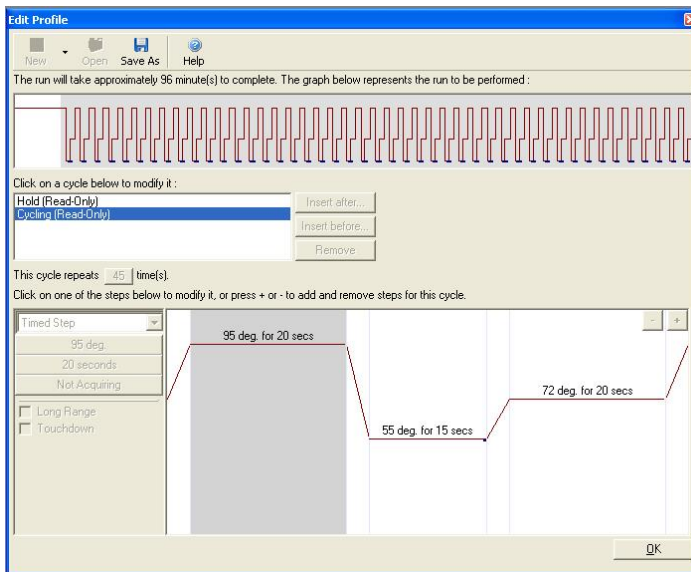
Program Step 2: **CYCLING**

Step 1: 95°C for 20 sec

Step 2: 55°C for 15 sec → **acquiring to Cycling A (Green, Orange)**

Step 3: 72°C for 20 sec

45 cycles



Gain settings:

Green < 5

Orange 9

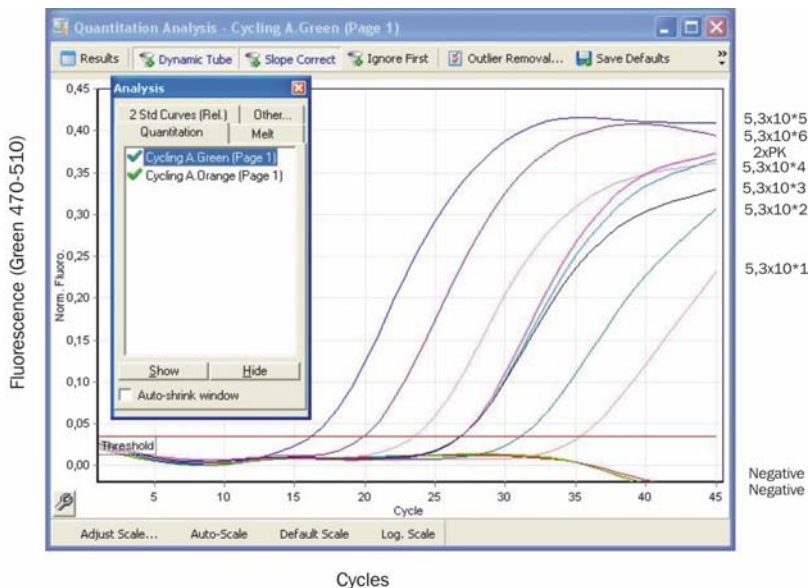
Please check the correct settings for the filter combination of Rotorgene 6000. For target you must choose the filter FAM (Green 470-510) and for the internal control the filter ROX (Orange 585-610).

2. Evaluation and interpretation of the results

- quantitative analysis of DNA in fluorescence channel FAM-specific signal (Green)
- quantitative analysis of internal control DNA in fluorescence channel ROX-specific signal (Orange)

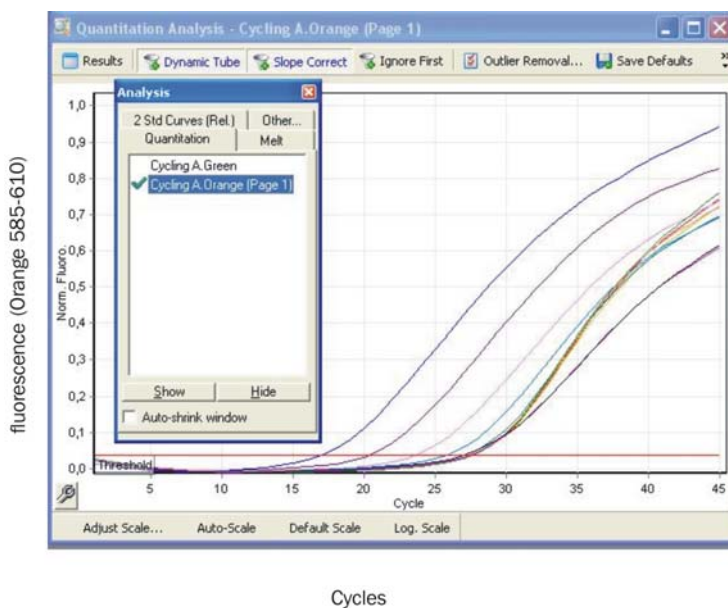
The following amplification curves were obtained by performing the described procedure with a dilution series of a Quantification Standard at the Rotorgene 6000 instrument. The fluorescence values versus cycle number are displayed. In the same run the amplification of internal control DNA was showed in channel ROX (Orange 558-610).

Amplification curves of the FAM-Target „Green 470-510“



Amplified dilution series of of approx. $5,3 \times 10^5$, $5,3 \times 10^4$, $5,3 \times 10^3$, $5,3 \times 10^2$ and 53 genome equivalents of a Quantification standard as starting template.

Amplification curves of the ROX internal control Target “Orange 585-610”



Amplified internal control DNA and a dilution series of a Quantification standard as starting template