

**GeneProof<sup>®</sup>**

*Factor V Leiden PCR Kit*  
*Factor II Prothrombin PCR Kit*  
*MTHFR A1298C PCR Kit*  
*MTHFR C677T PCR Kit*

**LightCycler<sup>®</sup> 2.0**  
**Operating Instructions**

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## Preparation of the PCR reactions

1. Add **18 µl** of **Master Mix** into a capillary tube.
2. Add **2 µl** of the **DNA template** or **2 µl** of **Positive Control** into the individual capillary tubes.
3. Close the capillary tubes, shortly centrifuge, insert into LightCycler 2.0

*During PCR preparation , all ingredients should be kept on ice (temperature 0 – 6 °C)*

## Programming of the PCR profile

1. Activate **New** in the LightCycler main menu and select **LightCycler Experiment**.
2. Fill following chart according instructions (Fig. 1.):
  - 2.1. Setup parameter entering:
    - I. Enter **530** into **Default channel**. Enter **37** into **Seek Temperature**.
    - II. Enter **Max.Seek Pos.** according to the number of capillary tubes.
    - III. Enter **6** into **Instrument Type** and enter **20 µl** into **Capillary Size**.
  - 2.2. Denaturation setting:
    - I. Enter **Denaturation** into **Program Name**, **1** into **Cycles** and **None** into **Analysis Mode**.
    - II. Enter **95** into **Target (°C)**, **00:10:00** into **Hold (hh:mm:ss)**, **20** into **Ramp Rate (°C/s)** and **None** into **Acquisition Mode**.
  - 2.3. PCR cycling settings:
    - I. Press + to add a new program block in the **Programs** window.
    - II. Enter **PCR cycling** into **Program Name**, **40** into **Cycles** and **None** into **Analysis Mode**.
    - III. Denaturation: Enter **95** into **Target (°C)**, **00:00:10** into **Hold (hh:mm:ss)**, **20** into **Ramp Rate (°C/s)** and **None** into **Acquisition Mode** in the **Program Temperature Targets** window.
    - IV. Annealing: Press + to add a new program block in the **Program Temperature Targets** window. Enter **64** into **Target (°C)**, **00:00:20** into **Hold (hh:mm:ss)**, **20** into **Ramp Rate (°C/s)** and **Single** into **Acquisition Mode**.
    - V. Extension: Press + to add a new program block in the **Program Temperature Targets** window. Enter **72** into **Target (°C)**, **00:00:20** into **Hold (hh:mm:ss)**, **20** into **Ramp Rate (°C/s)** and **None** into **Acquisition Mode**.

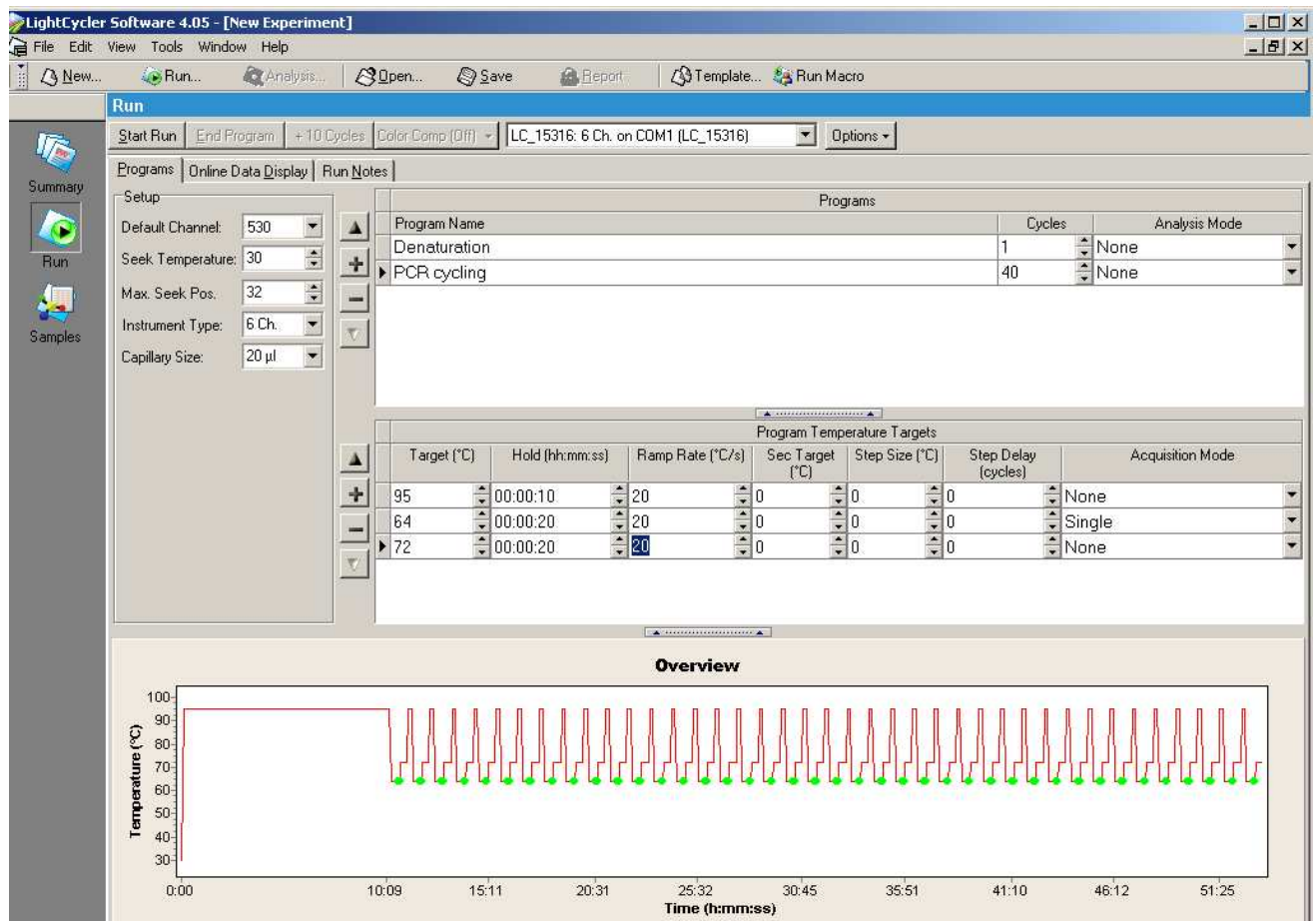


Fig. 1. PCR profile settings.

3. Switch into the **Samples** menu and describe the samples.
4. Save the program.
5. Press the **Run** button to switch into the main window. Press **Start Run** to run the PCR program. When the program is finished, switch to result evaluation.

## Analysis of the results

1. Open the **Analysis** tab and enter the **Qualitative Detection**.
2. **Qualitative Detection** window will open. (Fig. 2)
3. **Wild Type** allele detection.
  - 3.1. Enter the **Channel 530**.
  - 3.2. Open the **Color Compensation** tab and enter the 530 versus 560 channel color-compensation protocol
  - 3.3. Click **Advance** button.
  - 3.4. The sample is positive for **Wild Type** allele presence if:
    - I. In the column **Calls Positive** is written
    - II. The **Score** of sample is **5.0**

**Note:** LightCycler software is not able automatically distinguish between real positivity and nonspecific background. That's why samples marked as a Positive with Score 5.0 should be considered Positive only. If the sample marked as a Positive has Score lesser value than 5.0, should be considered as a Negative with nonspecific background (Fig. 2)

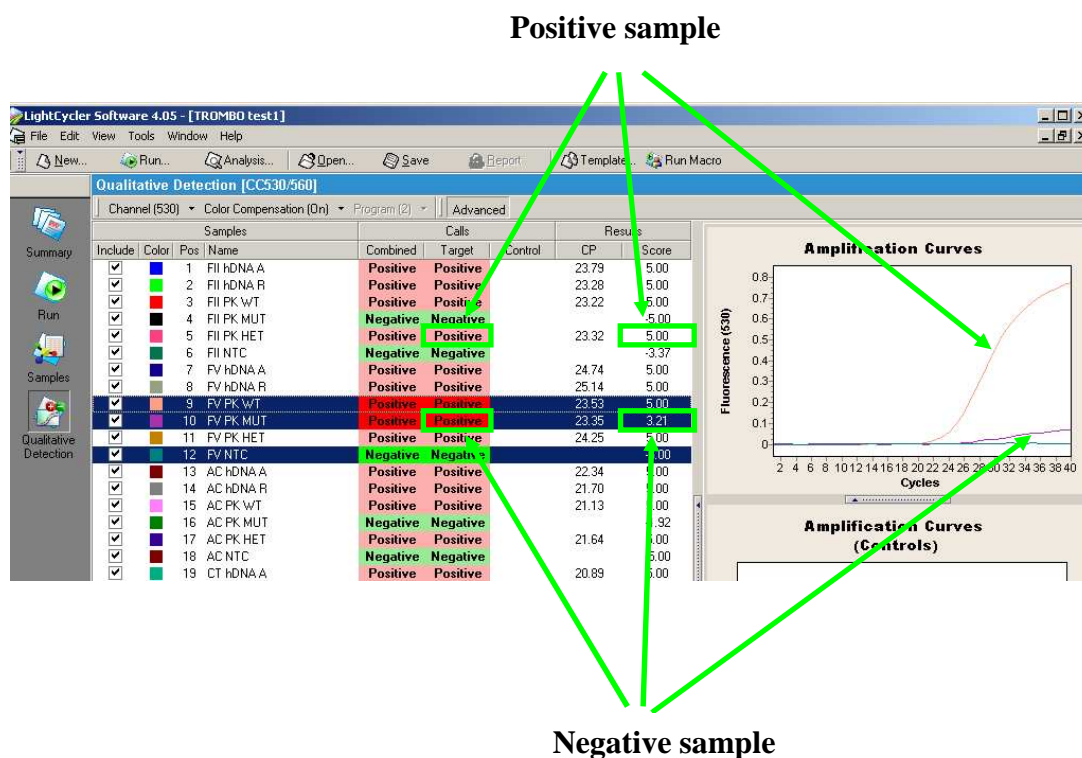


Fig. 2. PCR profile settings.

4. **Mutant** allele detection.
  - 4.1. Enter the **Channel 560**.
  - 4.2. Open the **Color Compensation** tab and enter the 530 versus 560 channel color-compensation protocol
  - 4.3. Click **Advance** button.
  - 4.4. The sample is positive for **Mutant** allele presence if:
    - I. In the column **Calls Positive** is written
    - II. The **Score** of sample is **5.0**

## Analysis evaluation

Wild type allele detection Channel 530	Positive	Positive	Negative
Mutant allele detection Channel 560	Negative	Positive	Positive
<b>RESULTS</b>	<b>Homozygote WILD TYPE</b>	<b>Heterozygote</b>	<b>Homozygote MUTANT</b>