For general laboratory use.

LightCycler® 480 Genotyping Master

Version: 09

Content Version: December 2022

Ready-to-use hot start reaction mix for PCR, followed by melting curve analysis for genotyping using the LightCycler® 480 System.

Cat. No. 04 707 524 001

4 × 384 µl
5x conc.
384 reactions of 20 µl final volume each

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function/Description	Content
1	yellow	LightCycler® 480 Genotyping Master, 5x conc.	Ready-to-use hot start PCR reaction mix. Contains a modified Taq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), and 15 mM $MgCl_2$.	4 vials, 384 µl each
2	colorless	LightCycler® 480 Genotyping Master, Water, PCR Grade	To adjust the final reaction volume.	4 vials, 1 ml each
3	blue	LightCycler® 480 Genotyping Master, $MgCl_2$, 25 mM	To adjust $MgCl_2$ concentration, if necessary.	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.
When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label.
Once the kit is opened, store the kit components as described in the following table:

Vial / Bottle	Cap	Label	Storage
1	yellow	Master; 5x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	colorless	Water, PCR Grade	Store at −15 to −25°C.
3	blue	$MgCl_2$, 25 mM	

1.3. Additional Equipment and Reagent required

Instruments and consumables

- LightCycler® 480 Instrument II*
- LightCycler® 480 Multiwell Plate 384* or LightCycler® 480 Multiwell Plate 96*, white
- LightCycler® 8-Tube Strip Adapter Plate*
- LightCycler® 8-Tube Strips (white)*
- Standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors
- Nuclease free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile 1.5 ml reaction tubes for preparing master mixes and dilutions

Reagents for the LightCycler® 480 Instrument II*

- LightCycler® Uracil-DNA Glycosylase* (optional)

ⓘ *For details about prevention of carryover contamination, see the* **Prevention of Carryover Contamination** *section.*

1.4. Application

The LightCycler® 480 Genotyping Master is designed for research studies on the LightCycler® 480 System. The LightCycler® 480 Genotyping Master is a ready-to-use hot start reaction mix designed specifically for genotyping (SNP analysis with melting curves). It is optimized for use with HybProbe probes but can also be used with SimpleProbe probes.

The kit can also help prevent carryover contamination during PCR when used with LightCycler® Uracil-DNA Glycosylase or to perform the second step of a two-step RT-PCR.

1.5. Preparation Time

Assay Time

Variable, depending on the number of cycles, annealing time, and melting curve program. For example, a LightCycler® 480 PCR run will last about 50 minutes if the program specifies 10 minutes pre-incubation, followed by 40 cycles amplification (each with 5 s denaturation, 5 s annealing, and 5 s elongation), and a monocolor melting curve analysis (see **Results** section).

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template DNA, such as genomic or plasmid DNA, cDNA suitable for PCR, as long as it is sufficiently pure, concentrated, and free of PCR inhibitors.
For reproducible isolation of nucleic acids, use:

- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation kit (for manual isolation).
- Use up to 50 ng complex genomic DNA or 1 to 10^8 copies plasmid DNA for a 20 µl reaction. For larger volumes, the amount of template can be increased equivalently.

When using unpurified cDNA from a reverse transcription reaction, especially when it contains high concentrations of RNA and oligonucleotides, you can improve your results by using 2 µl (or less) of that sample in the reaction.

Control Reactions

Always run a negative control with the samples. To prepare a negative control:

- Replace the template DNA with Water, PCR Grade (Vial 2). This will reveal whether a contamination problem exists.
- For a 2-step RT-PCR setup, omit the addition of reverse transcriptase to the cDNA synthesis reaction; this will indicate whether DNA in RNA samples causes false-positive results.

Primers

Suitable concentrations of PCR primers range from 0.1 to 5 µM (final concentration in reaction). The recommended starting concentration is 0.5 µM each. For some applications, such as SimpleProbe format or multiplexing, it is advantageous to run asymmetric PCR. In this case, you will need to titrate your primer ratio. The recommended starting ratio is 1:5.

Probe

- A suitable concentration for HybProbe probes is 0.2 µM each (final concentration in reaction).
- A suitable concentration for SimpleProbe probes is 0.2 µM (final concentration in reaction).

Mg^{2+} Concentration

The reaction mix in this kit already contains an optimal concentration of $MgCl_2$, which works well for HybProbe analysis with nearly all primer combinations.
If using SimpleProbe probes, use a 1:10 dilution of the Master and adjust the $MgCl_2$ accordingly, for example, add additional $MgCl_2$ to the PCR mix to make the final concentration 3 mM. For additional information, see the **Protocol** section.

General Considerations

In principle, the LightCycler® 480 Genotyping Master can be used to amplify and detect any DNA target. However, you would need to adapt your detection protocol to the reaction conditions of the LightCycler® 480 Instrument, and design specific PCR primers and probes for each target. See the LightCycler® 480 Operator's Manual for general recommendations.

⚠ ***The amplicon size should not exceed 700 bp in length. For optimal results, select a product length of 100 to 500 bp for monoplex and ≤350 bp for multiplex assays.***

⚠ ***The performance of the kit described in this Instructions for Use is warranted only when it is used with the LightCycler® 480 System.***

Detection formats

You can use this kit for both the HybProbe and SimpleProbe detection format.

Use a 1:5 dilution of the LightCycler® 480 Genotyping Master to prepare the PCR Mix for HybProbe probes. The $MgCl_2$ concentration in this mix gives optimal results with nearly all primer combinations.

Use a 1:10 dilution of the LightCycler® 480 Genotyping Master to prepare the PCR Mix for SimpleProbe probes. For this type of analysis, it might be helpful to add additional $MgCl_2$ (Vial 3). Melting curve results will improve if you dilute the mix even further, for example, using a 1:20 dilution. If you dilute the mix further, you must also add additional $MgCl_2$ to the reaction. The final concentration must be 3 mM, therefore, add an extra 1.8 µl $MgCl_2$ from Vial 3 if you dilute 1:20.

Two-Step RT-PCR

LightCycler® 480 Genotyping Master can also be used to perform two-step RT-PCR. In two-step RT-PCR, the first step, reverse transcription of RNA into cDNA, is performed outside the LightCycler® 480 System. Subsequent amplification and online monitoring is performed according to the standard LightCycler® 480 System procedure, using cDNA as starting sample material.
One of the following reagents is required for reverse transcription of RNA into cDNA (see **Ordering Information** section for details):

- Transcriptor Reverse Transcriptase*
- Transcriptor First Strand cDNA Synthesis Kit*

Synthesis is performed according to the detailed instructions provided with the cDNA synthesis reagent.

⚠ ***For initial experiments, run undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel to determine the optimum template amount.***

Prevention of Carryover Contamination

Uracil DNA-Glycosylase (UNG) can help prevent carryover contamination in PCR. The prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the Master Mix in this kit) into amplification products, then pretreating later PCR mixtures with UNG. If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the pre-incubation step; it will not serve as a PCR template.

⚠ ***Use only LightCycler® Uracil-DNA Glycosylase* in combination with the LightCycler® 480 Genotyping Master.***

Since your target DNA template contains thymidine rather than uridine, it is not affected by this procedure.

The use of UNG might influence the melting temperature (Tm) in melting curve analysis.

2.2. Protocols

LightCycler® 480 Instrument Protocol

The following procedure is optimized for use with the LightCycler® 480 System.

⚠ ***If the instruments type is not stated, "LightCycler® 480 Instrument" stands for LightCycler® 480 Instrument I and II.***

⚠ ***Program the LightCycler® 480 Instrument before preparing the reaction mixes.***

A LightCycler® 480 Instrument protocol that uses the LightCycler® 480 Genotyping Master should contain the following programs:

- **Pre-Incubation** for activation of modified Taq DNA Polymerase and denaturation of the DNA.
- **Amplification** of the target DNA.
- **Melting Curve** for amplicon analysis.
- **Cooling** of the thermal block.

For details on how to program the experimental protocol, see the LightCycler® 480 Operator's Manual.

Protocol for use with the LightCycler® 480 Multiwell Plate 96 and 384

The following table shows the PCR parameters that must be programmed for a LightCycler® 480 System PCR Run with the LightCycler® 480 Genotyping Master using a LightCycler® 480 Multiwell Plate 96 or 384.

Setup					
Block Type			Reaction Volume [µl]		
96 (384)			10 – 100 (3 – 20)		
Detection Format	Excitation Filter		Emission Filter		
SimpleProbe	465		510		
or Monocolor HybProbe Red 640	498		640		
or Multi Color HybProbe Fluos Red 610 Red 640 Cy 5/Cy 5.5	 465 498 498 498		 510 610 640 660		
Programs					
Program Name	Cycles		Analysis Mode		
Pre-Incubation	1		None		
Amplification	45[1]		Quantification		
Melting Curve	1		Melting Curve		
Cooling	1		None		
Temperature Targets					
	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisitions [per °C]
Pre-Incubation	95	None	00:10:00[2]	4.4 (4.8)	–
Amplification	95	None	00:00:05 – 00:00:10	4.4 (4.8)	–
	primer dependent[3]	Single	00:00:05 – 00:00:20[4]	2.2 (Target °C ≥50°C) or 1.5 (Target °C <50°C[5]) (2.5 (Target °C ≥50°C) or 2.0 (Target °C <50°C)[5])	–
	72	None	00:00:05 – 00:00:20[4] [6]	4.4 (4.8)	–
Melting Curve	95	None	00:01:00	4.4 (4.8)	–
	40	None	00:00:30 – 00:04:00[7]	1.5[5] (2.0)[5]	–
	75[8]	Continuous	–	–	1 – 10[9]
Cooling	40	None	00:00:30	1.5 (2.0)[5]	–

[1] 45 cycles is suitable for most assays. If the assay is optimized and has steep amplification curves and early crossing points (even when target concentrations are low), 40 cycles should be sufficient. Reducing the number of cycles will reduce the time required for the assay.

[2] If you expect your PCR product in early cycles, you can reduce the pre-incubation time to 5 minutes.

[3] For initial experiments, set the target temperature, that is the primer annealing temperature, 5°C below the calculated primer Tm. Annealing usually occurs at 60°C.

[4] For greater precision in target quantification experiments, it can be advantageous (in some cases) to choose longer annealing and extension times for the amplification cycles.

[5] **For the 96-multiwell plate:** For target temperatures of 50°C and above, set the Ramp Rate for Cooling to 2.2°C/s. For target temperatures below 50°C, set the Ramp Rate to 1.5°C/s.
For the 384-multiwell plate: For target temperatures of 50°C and above, set the Ramp Rate for Cooling to 2.5°C/s. For target temperatures below 50°C, set the Ramp Rate to 2.0°C/s.

(6) Calculate the hold time for the PCR elongation step by dividing the amplicon length over 25, for example, a 500 bp amplicon requires 20 second elongation time.
(7) For initial experiments, set the Hold to 1 minute (00:01:00).
(8) You can shorten the time required for the melting curve run by setting the target temperature to less than 75°C. The target temperature must be at least 5°C higher than the highest Tm you want to determine.
(9) For HybProbe probes: 1 - 5 acquisitions/°C; for SimpleProbe probes: 10 acquisitions/°C. If you expect more than one melting peak, use 1 acquisition/°C.

Choosing the right temperature and time for the melting curve analysis

If you expect more than one peak, you can improve melting curve results by setting up a multi-step melting curve program that includes each of the expected or calculated annealing temperatures (from high to low) as a separate step in the program (that is, perform Step 2 below repeatedly, at different annealing temperatures, each with its own hold time). After all different annealing steps, program one final annealing step (Step 3 below) and a reheating step (Step 4 below).

1. Denature your double-stranded PCR product at 95°C.

2. Set the annealing temperature for the melting curve, for example, 40°C to at least 5°C below the annealing temperature chosen for the amplification cycles, and program a hold at that temperature. The hold time will depend on the parameter analyzed; typical values range from 15 to 30 seconds.

3. After all annealing steps, include a final annealing step (to 40°C from 30 seconds to 4 minutes).

4. After the final annealing step, raise the temperature to 75°C.

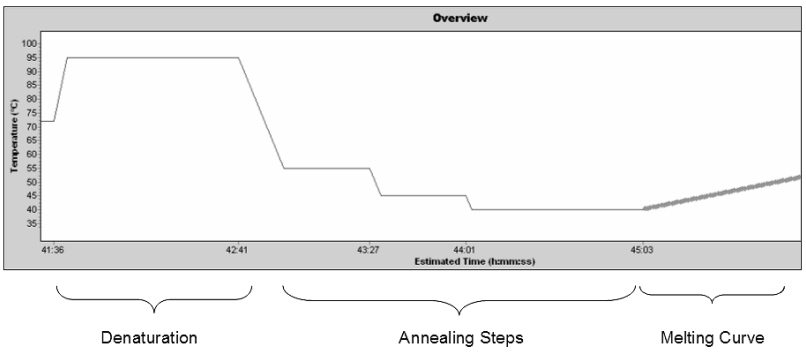

Fig. 1: Example of a multi-step melting curve program.

Preparation of the PCR mix

Thaw the LightCycler® 480 Genotyping Master (Vial 1), Water, PCR Grade (Vial 2), and if necessary, $MgCl_2$ (Vial 3). Follow the procedure below to prepare one 20 µl standard reaction.

⚠ ***Do not touch the surface of the LightCycler® 480 Multiwell Plate when handling the plate.***

1. Thaw the solutions and, to ensure recovery of all the contents, briefly spin vials in a microcentrifuge before opening.
   – Mix carefully and store on ice; avoid foam formation.

2. Prepare a 10x conc. solution that contains PCR primers and probes.

3. In a 1.5 ml reaction tube on ice, prepare the PCR mix for one 20 µl reaction by adding the following components in the order mentioned below:

Reagent	Volume HybProbe Probes [µl]	Volume SimpleProbe Probes [µl]
Water, PCR Grade (Vial 2)	9.0	9.8
Primer probe mix(1), 10x conc.	2.0	2.0
LightCycler® 480 Genotyping Master, 5x conc. (Vial 1)	4.0	2.0
$MgCl_2$ solution (Vial 3)	–	1.2
Total Volume	**15.0**	**15.0**

ⓘ *To prepare the PCR Mix for more than one reaction, multiply the amount in the "Volume" column above by z, where z = the number of reactions to be run plus two additional reactions.*

4. Mix carefully and avoid foam formation; do not vortex.
   – Pipette 15 µl PCR mix into each LightCycler® 480 Multiwell Plate.
   – Add 5 µl of the DNA template.
   – Seal Multiwell Plate with a LightCycler® 480 Sealing Foil.

5. Place the Multiwell Plate in a centrifuge and balance it with a suitable counterweight, such as another Multiwell Plate.
   – Centrifuge for 2 minutes at 1,500 × *g* in a standard swinging-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.

6. Load the Multiwell Plate into the LightCycler® 480 Instrument.

7. Start the PCR program described above.

⚠ ***If you use reaction volumes different from 20 µl, be sure to adapt the right volume in the running protocol. As a starting condition, we recommend to use the same hold times as for the 20 µl volume.***

(1) Due to possible primer/primer interactions that occur during storage, it may be necessary to preheat the PCR primer-probe mix for 1 minute at 95°C before starting the reaction. This extra step will ensure optimum sensitivity.

Color compensation

For more information on generating and using a color compensation file, see the LightCycler® 480 Operator's Manual.

3. Results

Quantification analysis

The following amplification and melting curves were obtained using the LightCycler® 480 Genotyping Master in combination with a set of primers and HybProbe probes, specific for a fragment of 207 bp of the ApoB gene. The fluorescence values versus cycle numbers are displayed.

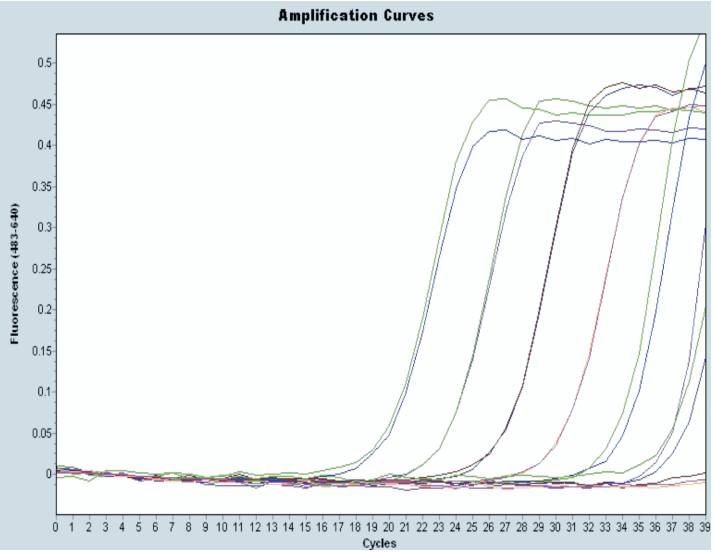

Fig. 2: Amplification curves were obtained from serial dilutions of 106 (far left), 105, 104, 103, 102, 101, 1 (far right) copies of ApoB transcript per well. A specific set of primers and a HybProbe probe that recognizes a 207 bp fragment of the ApoB gene was used. As a negative control, template DNA was replaced by Water, PCR Grade.

Melting curve analysis

Specificity of the amplified PCR product was assessed by performing a melting curve analysis. The resulting melting curves allow discrimination between wild type, mutant, and heterozygote products. The specific ApoB wild type product melts at a higher temperature than the mutant product. The melting curves display the specific amplification of the ApoB sequence, when starting from 10^6, 10^5, 10^4, 10^3, 10^2, 10^1, 1 copies of DNA.

Melting temperatures might vary depending on the reaction volume used.

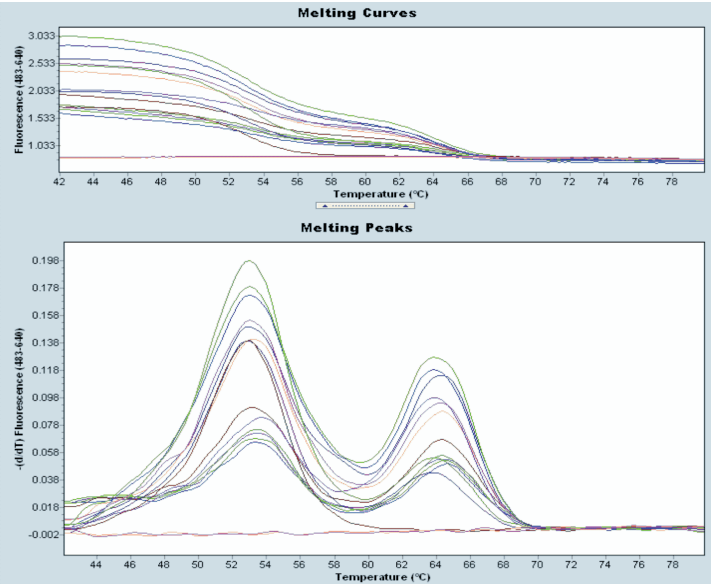

Fig. 3: Melting curve analysis of amplified samples containing 10^6, 10^5, 10^4, 10^3, 10^2, 10^1, 1 copies of ApoB gene as starting template. As a negative control, template DNA was replaced by Water, PCR Grade (flat line).

4. Troubleshooting

Observation	Possible cause	Recommendation
Amplification curves reach plateau phase before cycling is completed.	Starting amount of nucleic acid is very high.	Stop the cycling program by clicking the *End Program* button. The next cycle program will start automatically.
	The number of cycles is too high.	Reduce the number of cycles in the program *Amplification*.
Log-linear phase of amplification just starts as the amplification program finishes.	Starting amount of nucleic acid is very low.	Improve PCR conditions, such as primer and probe design.
		Use more starting DNA template.
		Repeat the run.
	The number of cycles is too low.	Increase the number of cycles in the cycle program.
No amplification detectable.	Wrong filter combination was used to display amplification on screen.	Select appropriate filter combination for your assay on the analysis screen and start again.
	Wrong detection format was chosen for experimental protocol.	Select appropriate detection format for your assay and start again.
	Impure sample material inhibits reaction.	Try a 1:10 dilution of your sample.
		Purify the nucleic acids from your sample material to ensure removal of inhibitory agents.
		Do not use more than 5 µl of DNA per 20 µl PCR reaction mixture.
	Modified Taq DNA polymerase is not sufficiently activated.	Make sure PCR includes an initial 5 to 10 minutes pre-incubation at 95°C.
		Make sure denaturation time during cycles is at least 10 seconds.
	Pipetting errors or omitted reagents.	Check for missing or defective reagents.
	Scale of axes on graph are unsuitable for analysis.	Change the values for the x- and y-axis; right-click on the chart and select Chart Preferences from the context menu. In the Chart Preference window, reset the maximum and/or minimum axis values.
	Measurements do not occur.	Check the temperatures in the experimental protocol. For probe detection formats, choose “Single” as the acquisition mode at the end of the annealing phase.
	Amplicon is too long.	Do not design primers that produce amplicons >400 bp in multiplex and >700 bp in monoplex assays. Amplification efficiency is reduced with amplicons of this size. Optimal results are obtained for amplicons ≤350 bp.
	Difficult templates, such as GC-rich sequence.	Optimize temperatures and times in the amplification cycles.
		Optimize primer/probe sequences.
		Repeat PCR under same conditions and add increasing amounts of DMSO, up to 10% final concentration. ⚠ ***Melting peaks will be lower with increasing DMSO concentrations.***
	Unsuitable HybProbe probes.	Check sequence and location of the HybProbe probes.

Fluorescence intensity is too low.	Low concentration or deterioration of dyes in the reaction mixtures due to unsuitable storage conditions.	Store the dye-containing reagents at −15 to −25°C, protected from light.
		Avoid repeated freezing and thawing.
		After thawing, store the Genotyping Master at +2 to +8°C for a maximum of 1 week.
		Low hybridization probe signals can be improved by using a two times higher concentration of the LC Red-labeled probe than of the fluorescein-labeled probe.
	Reaction conditions are not optimized, leading to poor PCR efficiency.	Primer concentration should be between 0.2 and 1.0 µM. HybProbe concentration should be between 0.2 and 0.4 µM.
		Check annealing temperature of primers and probes.
		Check experimental protocol.
		Always run a positive control along with your samples.
		Titrate $MgCl_2$ concentration.
	Mutation analysis using HybProbe probes; the melting temperature of the mismatch strand-HybProbe probe hybrid is lower than the annealing temperature. Thus, the HybProbe probes cannot bind and do not create a signal.	This will not affect amplification efficiency.
		Ensure that the melting curve will start at a temperature below the annealing temperature used for PCR. You will then get a clear signal after melting-curve analysis and will be able to interpret the data.
Fluorescence intensity varies.	Skin oils on the surface of the sealing foil.	Always wear gloves when handling the multiwell plate and the sealing foil.
	Some of the reagent is still in the upper part of the microwell, or an air bubble is trapped in the microwell.	Repeat centrifugation, but allow sufficient centrifugation time, for example, 2 minutes at 1,500 × *g* for all reagents to reach the bottom of the microwell and/or to expel air bubbles.
Negative control samples give a positive signal.	Contamination	Exchange all critical solutions.
		Pipette on a clean bench.
		Use heat-labile UNG to eliminate carryover contamination.

No precise melting peak can be identified.	HybProbe probes are nonhomogeneous, and/or contain secondary structures. Or Pseudogenes lead to multiple PCR products.	Redesign HybProbe probes.
		Check PCR products on an agarose gel.
		Melting Peaks can be optimized by performing an asymmetric PCR, by favoring the amplification of the DNA strand that the HybProbe probes bind to.
	Primer-dimers have outcompeted specific PCR product for available primers.	Keep all samples at +2 to +8°C until the run is started.
		Keep the time between preparing the reaction mixture and starting the run as short as possible.
		Increase starting amount of DNA template.
		Increase annealing temperature in order to enhance stringency.
	Annealing temperature not adapted.	Lower the annealing temperature used for melting curve analysis by an additional 3°C, and/or increase the hold time up to 4 minutes.
		If you expected more than one melting peak, include each of the different annealing temperatures (from high to low) as a separate step in the program, each with its own hold time (from 30 seconds to 4 minutes). After all steps, program a “cool down” step (to 40°C) and another hold step (for 2 minutes), followed by a reheating step (to 80°C).
Melting temperature of a product varies from experiment to experiment.	Variations in reaction mixture, such as salt concentration.	Check purity of template solution.
		Reduce variations in parameters, such as heat-labile UNG, primer preparation, program settings, and $MgCl_2$.
	Different intensity of lamp due to aging or exchange of lamp.	Run positive control.
One peak of the same height occurs in all samples.	Contamination in all samples.	Use fresh solutions.
High background	Very low fluorescence signals, therefore the background seems relatively high.	Follow general optimization strategies for the LightCycler® PCR.
	HybProbe probe concentration is too high.	HybProbe probe concentration should be in a range of 0.2 to 0.4 µM.
	Different intensity of lamp due to aging or exchange of lamp.	Run positive control.
	Insufficient quality of HybProbe probes.	Prepare a new pair of HybProbe probes.
Amplification curve decreases after reaching the plateau in late cycles.	“Hook effect”: competition between binding of the HybProbe probes pair and re-annealing of the PCR product.	This does not affect interpretation of the results. It can be avoided by performing an asymmetric PCR, by favoring the amplification of the DNA strand that the HybProbe probes bind to.

Fluorescence values in real-time quantification drop below initial background fluorescence in late cycles. This might cause wrong Cp calls in the range of decreasing fluorescence.	High DNA/amplicon concentrations influence buffer conditions, reflected by a decrease of the fluorescence in the Fluos channel 530.	This does not affect interpretation of general results.
		Dilution of your template decreases the effect.
		Repeat the assay while using Pyrophosphatase*. Add 0.05 units/µl of Pyrophosphatase (final concentration) to each master mix before starting the PCR.
Run error (or program abort) in melting curve program for the 96-well format.	Ramp rate for cooling in the annealing step(s) of the melting program is not correct.	Set the right ramp rate for cooling, for example, 1.5°C/s for reaction volumes less than 50 µl. Inspite of this error, you can still analyze the data captured during the amplification program. Confirm the error message; then wait at least 5 seconds to be sure that all data are saved. The amplification data can be analyzed immediately or later. Create and start a separate melting protocol if required.

5. Additional Information on this Product

5.1. Test Principle

HybProbe probes consist of two different oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of PCR cycles. One probe is labeled at the 5' end with a suitable acceptor fluorophore (LightCycler® Red 610, LightCycler® Red 640, or Cy5), and to avoid extension, modified at the 3' end by phosphorylation. The second probe is labeled at the 3' end with the donor dye fluorescein (Fluos). Only after hybridization, the two probes are in close proximity, resulting in fluorescence resonance energy transfer (FRET) between the two fluorophores. During FRET, fluorescein, the donor fluorophore, is excited by the light source of the LightCycler® 480 Instrument, and part of the excitation energy is transferred to LightCycler® Red, the acceptor fluorophore. The emitted fluorescence of the acceptor fluorophore is measured. LightCycler® Red 610, LightCycler® Red 640, or Cy5-labeled HybProbe probes can be used separately or in combination, therefore allowing single- or multiple-color detection. Refer to the LightCycler® 480 Operator`s Manual for additional information.

The SimpleProbe format uses only one oligonucleotide probe. This single probe is designed to specifically hybridize to a target sequence that contains the SNP of interest. Once hybridized, the SimpleProbe probe emits much more fluorescence than it does when it is not hybridized. As a result, fluorescent signal changes are based solely on the hybridization status of the probe. SimpleProbe assays can distinguish between wild type, mutant, and heterozygous samples.

The LightCycler® 480 Genotyping Master is a ready-to-use reaction mix designed for the HybProbe probe detection format and SimpleProbe probe detection format using the LightCycler® 480 System. It is used to perform hot start PCR in LightCycler® 480 Multiwell Plates.
Hot start PCR has been shown to significantly improve the specificity and sensitivity of PCR (Chou, Q., et al., 1992, Kellogg D. E., et al., 1994, Birch, D. E., 1996) by minimizing the formation of nonspecific amplification products at the beginning of the reaction.
The polymerase in this master is a 5'-3'-exo-minus, N-terminal deletion of thermostable recombinant Taq DNA polymerase and furthermore, is chemically modified. It shows no activity up to 75°C because of the heat-labile blocking groups on some of the amino acid residues of the enzyme. Therefore, there is no elongation during nonspecific primer binding. The modified enzyme is "activated" by removing the blocking groups at a high temperature (that is, pre-incubation at 95°C for a maximum of 10 minutes).
The LightCycler® 480 Genotyping Master provides convenience, excellent performance, reproducibility, and minimal contamination risk. All you have to supply is template DNA, PCR primers, HybProbe or SimpleProbe probes, and additional $MgCl_2$ (if necessary).

5.2. References

- Birch DE, Kolmodin L, Wong J, Zangenberg GA, Zoccoli MA, McKinney N, Young KKY. Simplified hot start PCR. Nature. 1996;381(6581):445-446.
- Chou Q, Russell M, Birch DE, Raymond J, Bloch W. Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. Nucleic Acids Research. 1992;7:1717-1723.
- Kellogg DE, Rybalkin I, Chen S, Mukhamedova N, Vlasik T, Siebert PD, Chenchika A. TaqStart antibody : hot start PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. BioTechniques. 1994;16(6):1134-1137.

5.3. Quality Control

The LightCycler® 480 Genotyping Master is function tested using the LightCycler® System.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Editorial changes.
Regulatory Disclaimer has been changed to: “For general laboratory use”.
Quality Control has been changed to LightCycler® System.

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a full overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LightCycler® 8-Tube Strip Adapter Plate	1 piece, adapter plate The adapter plate can be used multiple times	06 612 598 001
LightCycler® 480 Block Kit 96 Silver	1 block kit	05 015 219 001
LightCycler® 480 Block Kit 384 Silver	1 block kit	05 015 197 001
Accessories software		
LightCycler® 480 LIMS Interface Module		09 262 091 001
Consumables		
LightCycler® 8-Tube Strips (white)	10 x 12 white strips and clear caps	06 612 601 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 480 Multiwell Plate 96, white	5 x 10 plates	04 729 692 001
LightCycler® 480 Multiwell Plate 384, white	5 x 10 plates	04 729 749 001
Instruments		
LightCycler® 480 Instrument	1 instrument, 96-well version	05 015 278 001
	1 instrument, 384-well version	05 015 243 001
Reagents, kits		
LightCycler® 480 Probes Master	5 × 1 ml, 2x conc. 5 x 100 reactions of 20 µl final volume each	04 707 494 001
	10 x 5 ml, 2x conc. 10 x 500 reactions of 20 µl final volume each	04 887 301 001
	1 × 50 ml, 2x conc. 5,000 reactions of 20 µl final volume each	04 902 343 001
LightCycler® 480 High Resolution Melting Master	5 × 1 ml, 2x conc. 5 x 100 reactions of 20 µl final volume each	04 909 631 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit, 50 reactions, including 10 control reactions	04 379 012 001
	1 kit, 100 reactions	04 896 866 001
	1 kit, 200 reactions	04 897 030 001
LightCycler® Uracil-DNA Glycosylase	50 µL, 100 U, (2 U/µl)	03 539 806 001
LightCycler® 480 SYBR Green I Master	5 × 1 ml, 2x conc. 5 x 100 reactions of 20 µl final volume each	04 707 516 001
	10 x 5 ml, 2x conc. 10 x 500 reactions of 20 µl final volume each	04 887 352 001
Transcriptor Reverse Transcriptase	250 U, 25 reactions of 20 µl final volume	03 531 317 001
	500 U, 50 reactions of 20 µl final volume	03 531 295 001
	2,000 U, 4 x 500 U 200 reactions of 20 µl final volume	03 531 287 001

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6.5. License Disclaimer

For additional documentation such as certificates and safety data sheets, please visit:
documentation.roche.com.

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.
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please visit our **Online Technical Support** Site.

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