

8. Sample data - typical results

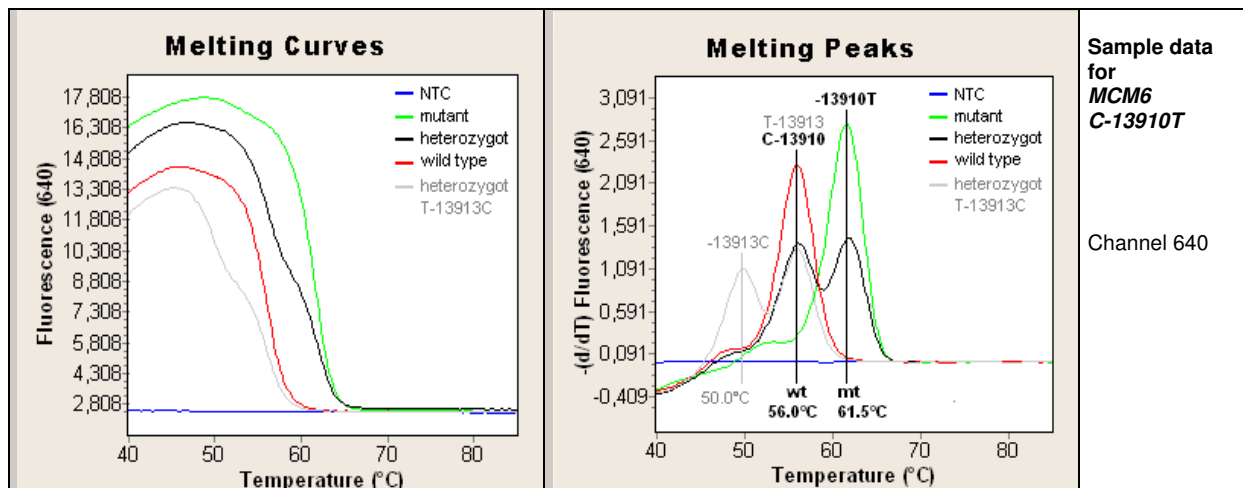


Fig.1. Sample data for the human *MCM6 C-13910T* detection system.

Data from channel 640. Left panel melting curves for human *MCM6 C-13910T*. Right panel melting peaks for the target. Wildtype corresponds with *MCM6 -13910C/C*, heterozygot corresponds with *-13910C/T* and mutant corresponds with *-13910T/T*.

Note: The *MCM6* gene is known to contain other genetic variations. In particular, positions -13915, -13913, -13908, and -13907 are covered by the detection probe and will have an influence on the melting peak temperature. Data for T-13913C are shown (grey curve, phenotype not known). SNP C-13908T is very rare (rs4988236), whereas T-13915G and C-13907G have been published recently by Tishkoff et al., Nat. Genet. 2006, to be responsible for lactose tolerance (African variants, not tested; melting temperatures not known).

Samples with deviating melting curves should be subject for further investigations; the sequence analysis can be provided by TIB MOLBIOL Berlin (contact service@tib-molbiol.de).

Genotype:	wild type homozygot	heterozygot <i>MCM6 -13910C/T</i>	mutant homozygot <i>MCM6 -13910T/T</i>
Number of melting peaks (color)	1 (red)	2 (black)	1 (green)
Melting temperature of peaks	56.0°C	56.0°C and 61.5°C	61.5°C
Temperature difference between peaks	---	5.5°C	---
Phenotype	Intolerant	tolerant	tolerant

Tab. 3. Typical analysis results

Note: The values of the respective melting temperatures (T_M) may vary $\pm 2.5^\circ\text{C}$ between different experiments. The ΔT between the melting peaks for heterozygous genotypes may vary $\pm 1.5^\circ\text{C}$.

9. Version history

V110330 Change of volumes for the control reagent
 V130424 Revised version, reference to CE-IVD kit

Notice to Purchaser

A license under U.S. Patents 4,683,202, 4,683,195 and 4,965,188 or their foreign counterparts, owned by Hoffmann-La Roche Inc. and F. Hoffmann-La Roche Ltd ("Roche"), has an up-front fee component and a running-royalty component. The purchase price of this product includes limited, nontransferable rights under the running-royalty component to use only this amount of the product to practice the Polymerase Chain Reaction ("PCR") and related processes described in said patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. Rights to the up-front fee component must be obtained by the end user in order to have a complete license. These rights under the upfront fee component may be purchased from Perkin-Elmer or obtained by purchasing an authorized thermal cycler. No right to perform or offer commercial services of any kind using PCR, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted by implication or estoppel. Further information on purchasing licenses to practice the PCR process for research applications may be obtained by contacting the Director of Licensing at The Perkin-Elmer Corporation, 850 Lincoln Center Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501. The purchase of this product does not convey any right for its use in clinical diagnostic applications. No rights for TaqMan technology under U.S. Patents 5,210,015 and 5,487,972 are hereby conveyed.

These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany. LightCycler[®] hybridization probes produced under license from Roche Diagnostics.



LightMix[®] Kit Lactose Intolerance (*MCM6 C-13910T*)

Cat.-No. 40-0307-32

Roche n° 05945577001

Reagents for detection of the human gene *MCM6 C-13910T* polymorphism using Roche Diagnostics LightCycler[®] 1.x / 2.0 / 480 II / Cobas[®] Z480 Instruments.

Lyophilized mix of primers and probes (3 tubes with 32 rxns each) for a total of 96 reactions with a final volume of 20 µl each. **Store protected from light at room temperature (18-25°C), do NOT freeze!**

Additional reagents required (Roche Diagnostics):

LightCycler[®] FastStart DNA Master Hybridization Probe

Cat.-No.03 003 248 001

Note : Alternative Product Version (Diagnostic Use)

LightMix[®] Kit Lactose Intolerance CE-IVD (64 reactions)

Cat.-No. 40-0307-64

1. Introduction

Lactose intolerance (hypolactasia) is the inability to digest lactose, the major sugar found in milk and other dairy products, caused by a shortage of the enzyme lactase. Lactose intolerance results in gastrointestinal symptoms such as abdominal pain, bloating and diarrhea.

The polymorphisms G-22018A and C-13910T, located in the introns 9 and 13 of the helicase encoding minichromosome maintenance 6 (*MCM6*) gene were shown to have a correlation with hypolactasia. The *MCM6* gene is located upstream from the lactase gene (*LCT*), encoding for lactose phlorizin hydrolase (*LPH*), converting lactose into glucose and galactose. For review see Campbell et al.¹.

A significant percentage of the human population is affected by hypolactasia (80-100% in Asia, Africa). The mutation C-13910T must have developed in Europe, where only 2-10% hypolactasia are found.

The dbSNP at NCBI reports a frequency of 23% for the T allele in position -13910 (rs4988235).

This LightMix[®] Kit provides a fast and accurate system to identify this polymorphism. Two neighbored polymorphisms C-13908T (rs4988236, frequency 0.6%, data not shown) and T-13913C (for melting curve data see results) are also known. There are no reports about an association with hypolactasia.

This LightMix[®] Kit is tested with the Roche Diagnostics "LightCycler[®] FastStart DNA Master Hybridization Probe" ready-to-use reaction mix in the LightCycler[®] 2.0 Instrument.

¹ The molecular basis of lactose intolerance. Campbell AK, Waud JP, Matthews SB. Sci Prog. 2005;88(Pt 3):157-202

2. Description

This LightMix[®] detects the genotype of human *MCM6 C-13910T* in a nucleic acid extract.

A 156 bp fragment of the human *MCM6* gene is amplified with specific primers and detected with probes labeled with LightCycler[®] Red 640 (detected in channel 640). The genotype is identified by running a melting curve with specific melting points (*T_m*) of 56.0°C for the wildtype and 61.5°C for the mutant in channel 640.

The supplied control DNAs allow for accurate comparison with the unknown samples.

For use in LightCycler[®] 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler[®] 1.x Instruments to software version 4.1.

For use in LightCycler[®] 480 Instruments use filter combination 483-640 instead of channel 640.

For use with the Cobas[®] Z480 Instrument use filter combination 483-645 instead of channel 640.

3. Set contents

- 3 Vials with red cap containing premixed and lyophilized primers and probes for 32 reactions each
- 3 Vials with colorless caps with control DNA (*wt*, *hetero*, *mt*), 10⁵ target equivalents per reaction

4. Programming

The protocol consists of four program steps

- 1: Denaturation: samples denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Programm LC 1.x/2.0

	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification Mode			Melting Curves Mode			None
Cycles	1	45			1			1
Segment	1	1	2	3	1	2	3	1
Target [°C]	95	95	60	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]	20	20	20	20	20	20	0.2	20
Acquisition Mode	None	None	Single	None	None	None	Continu.	None

Programm: LightCycler® 480 / Cobas® Z480 Instruments

	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification Mode			Melting Curves Mode			None
Cycles	1	45			1			1
Segment	1	1	2	3	1	2	3	1
Target [°C]	95	95	60	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:15	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate [°C/s] 96	4.4	4.4	2.2	4.4	4.4	1.5	-	1.5
Ramp Rate [°C/s] 384	4.6	4.6	2.4	4.6	4.6	2.0	-	2.0
Acquisition Mode	None	None	Single	None	None	None	Continu.	None
Acquisitions [per °C]							3	

5. Data analysis

Perform data analysis, as described in the LightCycler® operator's manual.

View human *MCM6 C-13910T* data in channel 640, Melting Curves mode.
The negative control (NTC) must show no signal.

6. Product characteristics

PCR results are obtained within 1 hour (LightCycler® 1.x / 2.0 Instruments)

Sensitivity

These reagents detect 1 ng of genomic DNA.

Measuring range

The measuring range of the assay is 5 ng to 100 ng of genomic DNA.

Storage and Stability

- Lyophilized reagents are stable for at least six months after shipment if stored protected from light at room temperature (18-25°C). See expiry on the product label.
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 10 days if stored light-protected and refrigerated (4°C).

7. Experimental protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 / 480 Instruments. Start programming before preparing the solutions. See the LightCycler® operator's manual for details.

Sample: Use aqueous nucleic acid preparations (e.g. High Pure PCR Template Preparation Kit).

Negative control: Always run at least one negative control - replace the template DNA with water.

Positive control: Run a positive control - replace the template DNA with the provided control DNA.

7.1 Preparation of parameter-specific reagents (32 reactions):

One reagent vial with a **red** cap contains all primers and probes to run 32 reactions, Add 66 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

► Use 2 µl **reagent** for a 20 µl PCR reaction.

| This solution is stable for at least three days when stored refrigerated at 4°C. Avoid prolonged exposure to light.

7.2 Preparation of the control DNA

Add 80 µl PCR-grade water to each vial (16×10^5 target molecules) with a colorless cap. Mix the target DNA by pipetting the solution up and down 10 times (final concentration: 10^5 target molecules in 5 µl).

Notice: Control DNA can be dissolved up to 160 µl to achieve 32 control reactions.

► Use 5 µl control DNA for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C, for long term storage freeze at -20°C. Avoid repeated freezing thawing cycles. For the heterozygote control DNA provided with the kit please note that the relative amounts of wild type DNA and mutant DNA may change during time.

| Please note that opening these vials may cause contaminations of the work-space (aerosol).

7.3 Preparation of the LightCycler® reaction mix

In a cooled reaction tube, prepare the reaction mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

For use with the Roche FastStart kit	
Single reaction	Component
9.4 µl	water, PCR-grade (colorless cap, provided with the Roche FastStart kit)
1.6 µl	Mg ²⁺ solution 25 mM (blue cap, provided with the Roche FastStart kit)
2.0 µl	reagent mix (parameter specific reagents containing primers and probes, see 7.1)
2.0 µl	FastStart mix (vial 1 (red cap), combined from vials 1a and 1b, see Roche manual)

15.0 µl

Volume of reaction mix

Mix gently, spin down and transfer 15 µl each of the reaction mix to a LightCycler® capillary (LightCycler® 1.x / 2.0 Instrument) or to a multiwell plate (LightCycler® 480 Instrument).

Add 5 µl of sample or control DNA (see 7.2) to each capillary or multiwell plate to give a final reaction volume of **20 µl**.

Start run.