Instructions For Use

SALSA® MC006 Lactase Persistence Genotyping 2

Version A1

SALSA® Melt Assay for detection of single nucleotide polymorphisms (SNPs), upstream of the lactase gene *LCT*

REF	**MC006-100R, MC006-1000R**
Σ	**100, 1000**
Shipping conditions	**Dry ice or cooling elements**
	-25˚C to -15˚C
	Keep away from heat or direct sunlight
	Read instructions before use
RUO	**All countries**

This product is manufactured by MRC Holland bv in Amsterdam, the Netherlands. The product is provided for use by the end user only and may not be resold, distributed or repackaged without written consent from MRC Holland bv.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

1. Kit Components

Available volumes:

- **MC006-100R:** 100 reactions
- **MC006-1000R:** 1000 reactions

Kit component	Cap colour	Contains	MC006 -100R	MC006-1000R	Ingredients
SALSA MC006 Probemix	brown	2 Melt curve probes+ (1 FAM-labelled, 1 ROX-labelled) 1 Quantity Fragment (Q-fragment) 2 PCR primers	2× 1000 µl	20× 1000 µl	Synthetic oligonucleotides with and without fluorescent FAM/ROX dye, dNTPs, Tris-HCl, Tricine, EDTA, $MgCl_2$, Glycerol, $(NH_4)_2SO_4$
SALSA MC Polymerase	red	Polymerase enzyme	1× 115 µl	5× 230 µl	Glycerol, non-ionic detergents, EDTA, DTT, KCl, Tris-HCl, MC Polymerase enzyme (purified from non-hazardous micro-organisms)

+ Light sensitive.

2. Storage and Shelf Life

A shelf life until the expiry date is guaranteed, also after opening, when stored in the original packaging under recommended conditions. For the exact expiry date, see the outer container. This product should not be exposed to more than 10 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

3. Certificate of Analysis

- MC006 Certificate of Analysis (CoA): quality control specifications; see www.mrcholland.com.

4. Related Products

SALSA® MC005 Lactase Persistence Genotyping 1 – This assay extends the detection capability of MC006 for SNPs located upstream of *LCT*.

5. General Information

The Melt Assay SALSA MC006 Lactase Persistence Genotyping 2 is a research use only (RUO) semi-quantitative assay for the detection or confirmation of single nucleotide polymorphisms (SNPs) -13907 C>G (rs41525747), -13910 C>T (rs4988235), -13915 T>G (rs41380347), -14009 T>G (rs869051967)* and -14010 G>C (rs145946881) within intron 13 of the *MCM6* gene, located upstream of the lactase gene (*LCT*).
*Only detectable when used in combination with SALSA MC005 Lactase Persistence Genotyping 1.

The ability to digest milk depends on the enzyme lactase-phlorizin hydrolase (LPH, commonly referred to as lactase), which hydrolyses lactose, the primary carbohydrate in milk. In most humans, lactase expression declines sharply after the weaning period—a trait known as lactase non-persistence (LNP). While LNP itself is a normal genetic trait, it can lead to gastrointestinal symptoms such as abdominal pain, diarrhoea, and bloating when lactose is consumed; this symptomatic response is commonly referred to as lactose intolerance. In contrast, some individuals retain high levels of lactase activity into adulthood, allowing them to digest lactose efficiently. This heritable autosomal dominant trait is known as lactase persistence (LP).

LP is associated with specific single nucleotide polymorphisms (SNPs) located near the *LCT* gene, which encodes the lactase enzyme. The *LCT* gene is situated on chromosome 2q21, while the relevant SNPs are found approximately 13.9–14.0 kb upstream, within intron 13 of the *MCM6* gene. This region functions as a cis-regulatory element that enhances *LCT* gene expression. Several polymorphisms within this region of *MCM6* are associated with LP (Anguita-Ruiz et al., 2020). The wildtype (WT) sequence refers to the genotype conferring LNP.

More information is available at http://omim.org/entry/601806 and http://omim.org/entry/223100.

This product is not CE/FDA registered for use in diagnostic procedures.

6. Assay

In the MC006 assay, PCR amplification of part of intron 13 of the *MCM6* gene –a regulatory region of the *LCT* gene – is performed, followed by fluorescent probe binding to the amplicons and generation of a melt curve (Figure 1). Fluorescence is only measured during melt curve generation.

A single PCR primer pair is used to amplify a 218 nt fragment which covers part of the human *MCM6* gene. (Figure 1A). In this PCR reaction, one primer is used in much larger amount than the other PCR primer. As a result, one strand is formed in excess (asymmetric PCR).
In each reaction, two fluorescent probes are present, one labelled at its 5' end with FAM and the other with a ROX molecule. When free in solution, the fluorescence of these probes is quenched because they have a specific quencher moiety at their 3' end. After the PCR reaction, the fluorescent probes hybridise to the amplicon strand that was produced in excess. This separates the quencher and the fluorescent label, resulting in a high fluorescent signal (Figure 1B). When the reaction mixture is slowly heated, probes detach from the amplicon strand at their respective melting temperature. Depending on the presence and zygosity of a SNP, a specific melt profile is produced (Figure 1C). The melting temperature depends on the amplicon sequence and can be used to identify specific sequence changes. A sequence difference between the amplicon and the fluorescent probe results in a hybrid with a mismatch and consequently in a lower melting temperature.

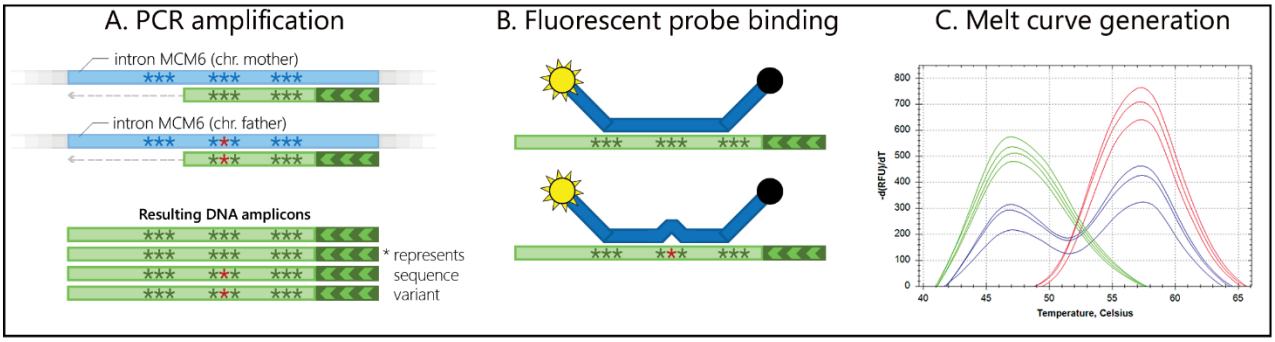

Figure 1. Summary of assay steps. (A) The intron 13 region of *MCM6* is amplified with a single set of primers, with one primer in excess. (B) A fluorescently-labelled probe binds to the amplicons. (C) The resulting melt curve indicates the presence and zygosity of a SNP causing LP and if sufficient DNA was used.

MC006 can be used in conjunction with MC005. MC005 contains the same primer pair as MC006, but different probes, resulting in different peak patterns for the same genomic sequence. This allows for the confirmation/co-detection of SNPs detected by MC006.

6.1. **Overview**

SNPs associated with LP are indicated by their position upstream of the *LCT* gene (e.g. the -13910 SNP is located 13910 base pairs upstream of *LCT*). Figure 2 shows a schematic overview of the probes in MC006, the targeted region which includes SNPs with a strong association to LP (bold) and others with varying evidence for their relation to LP. For clarity, an arbitrary distinction is made in the targeted genomic sequence defining two regions (Region 1 and Region 2) corresponding to the binding locations of the two probes.

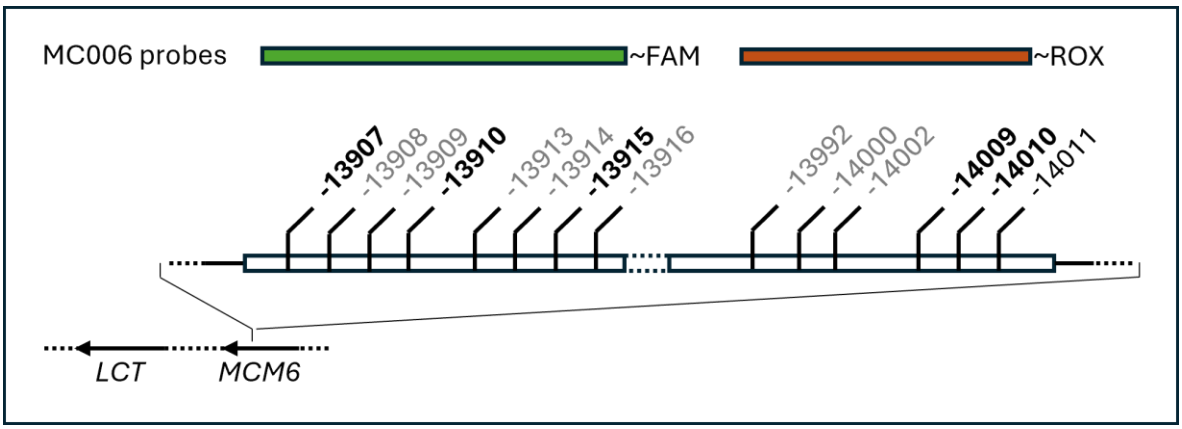

Figure 2. Schematic representation of the probes in MC006 and the SNPs in the *MCM6* intron 13. Bold SNPs are targeted by MC006. Grey SNPs are other rare SNPs not associated with LP. For -14011 *some* evidence for association with LP has been found (Anguita-Ruiz et al., 2020).

6.2. **MC006 Target SNPs**

The MC006 can be used to identify the presence of the following SNPs in the *MCM6* sequence:
1. The -13910 C>T SNP (rs4988235). In European lineages, LP is strongly associated with the T variant of this SNP. This variant is widely distributed in Europe, and it can be present in up to 70% of certain European populations (Guimarães Alves et al, 2021).
2. The -13915 T>G SNP (rs41380347). Found in several Arab populations (up to 50%) (Ingram et al. 2007), LP is strongly associated with the G variant of this SNP.
3. The -13907 C>G polymorphism (rs41525747). Present in Afro-Asiatic Beja populations (~20%) (Tishkoff et al., 2007), LP is strongly associated with the G variant of this SNP. **Primary detection with MC005 is strongly recommended.**
4. The -14010 G>C SNP (rs145946881). Found in several east African populations (~27%) (Tishkoff et al., 2007), LP is strongly associated with the C variant of this SNP. **Primary detection with MC005 is strongly recommended.**
5. The -14009 T>G polymorphism (rs869051967). This variant has been identified in some individuals of Sudanese, Ethiopian, and Somali descent (variable frequencies) (Ranciaro et al., 2014). LP is associated with the G variant of this SNP. **Co-detection with MC005 is required.**

The table below summarises when MC006 can or should be used in combination with MC005.

SNP	rs ID	major>minor	MC005	MC006
-13907	rs41525747	C>G	Detection	Confirmation
-13910	rs4988235	C>T	Detection	Detection
-13915	rs41380347	T>G	Outside detection range	Detection
-14009	rs869051967	T>G	Co-detection with MC006 required	Co-detection with MC005 required
-14010	rs145946881	G>C	Detection	Confirmation

6.3. **Other SNPs**

Several other rare SNPs have been described in literature with varying evidence for their relation to LP. **Please note that some of these rare SNPs may affect MC006 results and, in certain populations, their frequency might be significantly high.**

7. Warnings and Precautions

- The level of evidence supporting functional regulation of *LCT* expression by the SNPs detected with this assay varies.
- For professional use only. Assay performance is dependent on operator proficiency and adherence to procedural directions. The assay should be performed by professionals trained in molecular techniques.
- Follow good laboratory practice and safety guidelines.

- None of the ingredients are derived from humans, animals, or pathogenic bacteria. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments). If spills occur, clean with water and follow appropriate site procedures.
- False positive or negative results can be caused by various factors, including:
  - Sequence changes. See the section 13. Limitations of the Procedure.
  - Impurities in test samples that adversely affect the DNA denaturation, the PCR reaction or the melting curve procedure, including blood-derived fluorescence quenchers, salts, phenol, ethanol, heparin, EDTA and Iron (Fe^{3+}).
  - Use of incorrect DNA quantities. See section 8. Required Specimens.
  - Improper mixing of enzyme solutions, e.g. by mixing insufficiently or too vigorously.
  - Evaporation during the MC006 reaction, causing abnormal results due to increased salt concentration and strong sample DNA secondary structures.
  - Contamination of reactions with amplicons generated in earlier experiments.
  - Problems during melting curve analysis, resulting in peaks that are too low for reliable interpretation.

8. Required Specimens

- MC006 can be used with DNA (concentration 0.5-15 ng/µl; 5-15 ng is optimal) from:
  - Peripheral blood (purified)
  - Saliva (purified)
  - Buccal swabs (crude lysates)
- For purified DNA, the following extraction method has been validated:
  - Salting out (manual)
- The extraction method should be similar for all samples used. Heparinised blood may only be used if the sample has been purified by methods that remove heparin (e.g. Nucleospin gDNA Clean-up XS).
- EDTA concentration of the samples should not be higher than 5 mM. Sample DNA should not be concentrated by evaporation or SpeedVac as this can lead to a very high EDTA concentration.
- In case of doubts about DNA quality: a) use only 1-5 ng of sample DNA; b) clean all samples by ethanol precipitation or clean-up kits (for instance silica-based).

9. Positive samples

See the product page of SALSA MC006 Lactase Persistence Genotyping 2; see www.mrcholland.com.

10. SALSA Melt Assay Technical Validation

10.1. **Technical Validation**

Internal validation of this product is essential before implementation and subsequently when changing DNA extraction method or thermocycler type. Use samples with a known genotype for validation (See MC006 product page on the MRC Holland website for a list of positive samples). As melting temperatures (Tm) between machines differ, determining the relevant Tm in these positive samples should be part of the internal validation of MC006.

10.2. **Expected Results Technical Validation**

- SNP-positive and WT samples should show the correct peak pattern and Tm (Tm depends on which probe/fluorophore is viewed, see section 12. Interpretation of Results).
- No DNA reaction: Quantity Fragment (Q-fragment) specific melt peak at ~45.5°C should be the highest peak.
  - The MC006 Q-fragment measures whether sufficient DNA was used. The Q-fragment is present in a very low amount in the MC006 probemix and is amplified by the same PCR primers that amplify part of the *MCM6* gene. In MC006 only the ROX probe will bind to the amplified Q-fragment. In “No DNA” reactions, the Q-fragment will be the only sequence amplified. The more sample DNA is added, the lower the Q-fragment melting curve peaks are. Reactions in which the Q-fragment peak is the highest melting curve peak should be regarded as failed due to an insufficient amount of sample DNA.

11. SALSA Melt Assay Procedure

11.1. **Materials Required but Not Provided**

- Standard laboratory equipment, such as micropipettes and microcentrifuges.
- 0.2 mL PCR tubes, PCR strips or PCR plates and optical-grade adhesive seals, suitable for the instrument used.
- Centrifuge with swing-out buckets for multiwell plates.
- Calibrated thermocycler meeting the following specifications:
  - melt curve option available;
  - heated lid (99-105°C) present;
  - capable of recording at least 2.5 datapoints every 1°C during melt curve (or 1 datapoint every 0.4°C);
  - compatible with 96-well plates;
  - a temperature range of 35°C-99°C.

  MC006 is compatible with machines that meet the specifications above, but please note that instrument implementation always requires in-house validation.

  MC006 was developed with:
    - Bio-Rad CFX96 Touch — with Bio-Rad HSP9655 white/white plates and Bio-Rad MSB1001 MicroSeal Plate Sealing film.
    - Thermo Fisher QuantStudio 5 — with Thermo Fisher N8010560 MicroAmp Optical 96 well plates and Thermo Fisher 4360954 MicroAmp Optical Adhesive Film.

  These instruments are used throughout this document to illustrate important settings.

11.2. **Procedure Notes**

- MC Polymerase solution contains 50% glycerol and remains liquid at the recommended storage temperature.
- Start the PCR reaction within 3 hours of preparing the master mix.
- Never vortex MC Polymerase or MC006 master mix, as this may cause enzyme inactivation.
- Fluorescence is only measured during the melting curve generation. Never open post-PCR tubes, strips, or plates in the room where PCR reactions are prepared to prevent contamination.
- PCR products can be stored at 4°C for 1 week in case the melt curve generation needs to be performed later, or repeated. As fluorescent dyes are light-sensitive, store PCR products in the dark.
- After use, store all remaining reagents between -25°C and -15°C.

11.3. **Experimental Set-up**

Each MC006 experiment/plate should include the following:

- At least one no-DNA control reaction: this control is intended to check for contamination of e.g., MC006 reagents, pipettes, and thermocycler.
  - Use 2 µl TE (10 mM Tris-HCl pH 8; 1 mM EDTA).

11.4. **Experimental Protocol**

1. Thaw the MC006 probemix tube and vortex the thawed solution. It is essential that the tube is completely thawed. Centrifuge the probemix tube for a few seconds before opening, as drops may have adhered to the lid.
2. Warm the MC Polymerase tube for 10 seconds in your hand to reduce viscosity and centrifuge for a few seconds before use, as drops may have adhered to the lid.
3. Prepare an MC006 master mix by mixing 19 µl probemix and 1 µl MC Polymerase for each reaction.
   - When preparing master mix, include a 5-10% volume surplus to allow for pipetting errors.
   - A master mix for 50 reactions can be prepared by adding 55 µl MC Polymerase to a complete tube of MC006 probemix.
   - Master mix can be prepared and dispensed at room temperature.
   - Mix the master mix well by repeatedly pipetting up and down until the viscous MC Polymerase and the MC006 probemix are completely mixed. **Never vortex solutions containing enzymes.**
   - Dispense 20 µl of the master mix in each well.
   - Start the PCR reaction within 3 hrs of preparing the master mix.
4. Add 2 µl DNA sample to each reaction.
5. Seal the plate and centrifuge briefly.

6. Place the plate in the thermocycler. Start the MC006 thermocycler program.
   - When the PCR is performed on an instrument with melt curve function, proceed with the melt curve generation immediately after the MC Polymerase heat inactivation step.
   - When the PCR is performed on an instrument without melt curve function, transfer the plate to the melt curve instrument. When the transfer is not completed within 2 hours and/or the samples have not been kept at RT during the transfer period, incubate for 120 seconds at 99°C before starting the melt curve generation.

11.5. **Thermocycler Program**

DNA denaturation			
1.	95°C		*60 seconds*
PCR reaction			
2.	45 cycles:	• 95°C	*20 seconds*
		• 63°C	*30 seconds*
		• 68°C	*40 seconds*
MC Polymerase heat inactivation			
3.	99°C		*120 seconds*

Melt curve generation, fluorescence detection*

4. Cool rapidly to 35°C and hold at this temperature for 20 minutes.
5. Slowly increase temperature to 85°C with at least 2.5 datapoints / 1°C (1 datapoint / 0.4°C).**

* When the melt curve generation (step 4+5) is not performed within 2 hours after the last PCR cycle and/or the samples have not been kept at RT during the transfer period, the reactions should be heated again for 120 seconds at 99°C prior to continuing with steps 4 and 5.

** For example, for instruments QuantStudio 5 and Bio-Rad CFX96 Touch, step-and-hold settings (+0.4°C / 5 seconds) are recommended.

11.6. **Instrument Filter Settings**

The excitation peak of the FAM (6-Carboxyfluorescein) fluorophore is at ~495 nm and the emission peak is at ~520 nm. For ROX (carboxy-X-rhodamine) the excitation peak is ~575 nm and the emission peak is at ~602 nm. Use the instrument manufacturer's recommendations for the detection of FAM and ROX.

11.7. **Data analysis**

Data analysis and quality control are done by visual examination of the melt curve profiles obtained by the standard instrument software. No separate High-Resolution Melting (HRM) program is required. The following table shows the settings for the example instruments presented in Section 11.1 Materials Required but Not Provided.

Instrument	**Melt program data analysed using**
Bio-Rad CFX96 Touch	Melt curve tab of the CFX Manager software
QuantStudio 5	Melt curve analysis option of the standard instrument software

Examples of melting curve patterns and the melting temperatures expected for various SNPs are shown in Section 12. Interpretation of Results.

12. Interpretation of Results

Visual confirmation of results is mandatory. Do not rely on the automatic calling by the PCR platform software.

12.1. **Quality Control**

Examine the following:

1. The no DNA control reactions: is the Q-fragment specific melt peak (at ~45.5˚C) at least twice as high as any other peak? If not, contamination of reactions with amplicons of previous experiments may have occurred.
2. The peak profile of each sample: If the Q-fragment peak is higher than any other peak, insufficient sample DNA was present in that reaction and data cannot be interpreted.

For troubleshooting help, see additional resources offered on our support portal (www.mrcholland.com).

12.2. **Interpretation of Results**

Figure 3A-E below shows example results for the different SNPs detected by MC006. It should be noted that the melting temperatures (Tm) between machines differ. Determining the relevant Tm should be part of the internal validation of MC006.

1. First, through visual examination of the results, determine which samples do not show a homozygous wildtype (WT) peak. Presence of this peak indicates the absence of LP causing SNPs. For the MC006 Region 1 (FAM) probe, the WT Tm is ~51.5°C. For the Region 2 (ROX) probe, the WT Tm is ~58.5°C.
2. Then for the remaining samples, compare the melt profile of each sample with that of the example reaction in the figure below. Per SNP, a sample picture is provided. In each picture, the blue line represents the wildtype result (absence of a SNP), the red line represents a homozygous SNP. The orange line represents the presence of a heterozygous SNP in the sample. Both the wildtype (WT) and the SNP Tm are provided.

Region 1 (FAM probe)

3. The Tm for SNP rs41525747 (-13907) is ~45°C and can be found using filter settings for FAM (Figure 3A). MC006 results for this SNP should be used to confirm MC005 results.
4. The Tm for SNP rs4988235 (-13910) is ~55.5°C and can be found using filter settings for FAM (Figure 3B).
5. The Tm for SNP rs41380347 (-13915) is ~42.5°C and can be found using filter settings for FAM (Figure 3C).

Region 2 (ROX probe)

6. The Tm for SNP rs145946881 (-14010) is ~54°C and can be found using filter settings for ROX (Figure 3D). MC006 results for this SNP should be used to confirm MC005 results.
7. The Tm for SNP rs869051967 (-14009) is ~53°C and can be found using filter settings for ROX (Figure 3E). The Tm of this SNP and the Tm of other SNPs in this region are relatively close together. The presence of this SNP should therefore be determined using the combined results of MC005 and MC006.

8. In case of a heterozygous sample, a hybrid peak will be generated (orange lines in Figure 3).
9. Other SNPs in the sequence covered by a probe will also lead to distinct peak patterns. See the document *Interpretation Aid - Melt patterns and Tm per SNP detected by MC006* on the product page (www.mrcholland.com) of MC006.
10. Positive samples, like the ones listed on the MC006 product page (www.mrcholland.com), can be used to aid in identifying peak patterns and their underlying SNPs.

Region 1
(FAM-labelled probe)

-13907

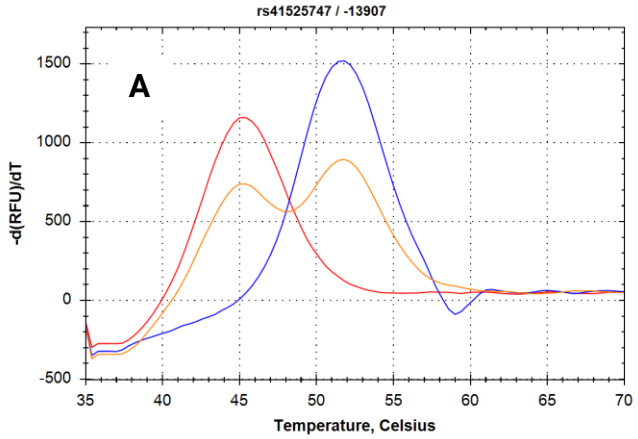

Figure 3A.
This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs41525747 (-13907) (red) and a heterozygous sample (orange). The WT peak in this region has a Tm of ~51.5°C. The SNP peak has a Tm of ~45°C. *MC006 results for this SNP should be used to confirm MC005 results.*

-13910

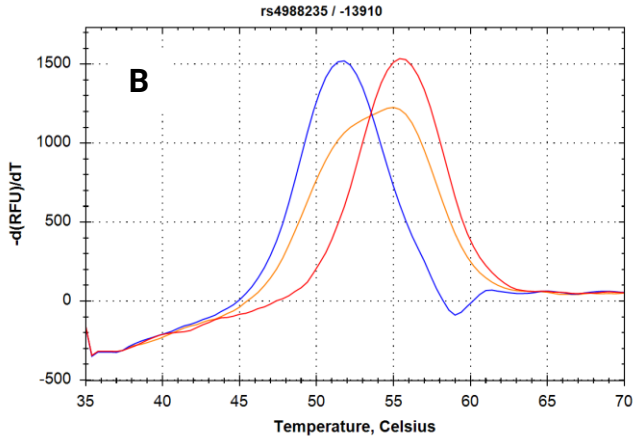

Figure 3B.
This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs4988235 (-13910) (red) and a heterozygous sample (orange). The WT peak in this region has a Tm of ~51.5°C. The SNP peak has a Tm of ~55.5°C.

-13915

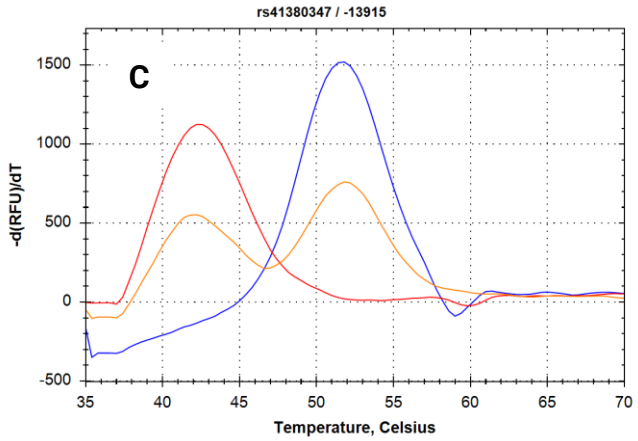

Figure 3C.
This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs41380347 (-13915) (red) and a heterozygous sample (orange). The WT peak in this region has a Tm of ~51.5°C. The SNP peak has a Tm of ~42.5°C.

Region 2
(ROX-labelled probe)

-14010

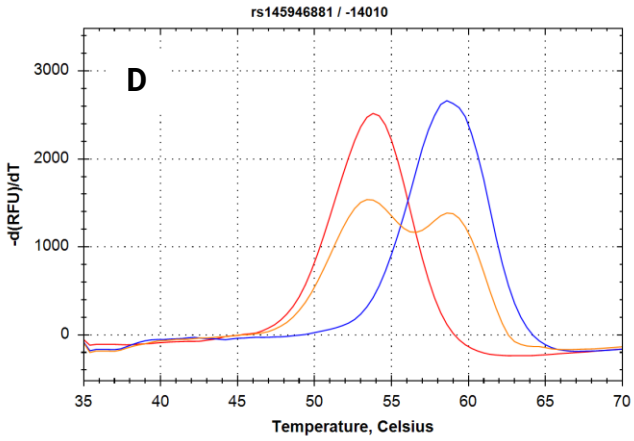

Figure 3D.
This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs145946881 (-14010) (red) and a heterozygous sample (orange). The WT peak in this region has a Tm of ~58.5°C. The SNP peak has a Tm of ~54°C. *MC006 results for this SNP should be used to confirm MC005 results.*

-14009

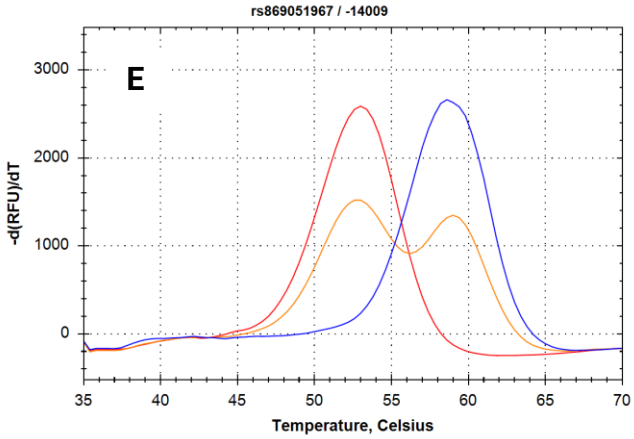

Figure 3E.
This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs869051967 (-14009) (red) and a heterozygous sample (orange). The WT peak in this region has a Tm of ~58.5°C. The SNP peak has a Tm of ~53°C. *Consult MC005 results for co-detection of this SNP.*

13. Limitations of the Procedure

- Sequence changes (e.g., SNPs, point mutations, small indels) in the target sequence detected by a probe can potentially cause false results.
- Rare polymorphisms have been described in the sequences targeted by the MC006 PCR primers. These can result in false results by reducing or eliminating the amplification of the allele with such a rare polymorphism.

Please report false positive results due to SNPs, as well as any unusual results, to MRC Holland: info@mrcholland.com.

False positive or negative results can also be caused by experimental factors, including:
- Contamination of reactions with amplicons generated in earlier experiments.

Assay failure can be caused by:
- Impurities in test samples that strongly affect sample DNA denaturation and/or the PCR reaction or melt curve procedure, including fluorescence quenchers, salts, phenol, ethanol, heparin, EDTA, and iron (Fe^{3+}).
- Improper mixing of the master mix, e.g., by mixing insufficiently or too vigorously.
- Excessive evaporation during the MC006 PCR reaction.

14. References

- Anguita-Ruiz et al (2020). Genetics of Lactose Intolerance: An Updated Review and Online Interactive World Maps of Phenotype and Genotype Frequencies. *Nutrients* 12(9):2689.
- Guimarães Alves et al (2021). Tracing the Distribution of European Lactase Persistence Genotypes Along the Americas. *Frontiers in Genetics* 12:671079.
- Ingram et al (2007). A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? *Human Genetics* 120(6):779-88.

- Ranciaro et al (2014). Genetic origins of lactase persistence and the spread of pastoralism in Africa. *American Journal of Human Genetics* 3;94(4):496-510.
- Tishkoff et al (2007). Convergent adaptation of human lactase persistence in Africa and Europe. *Nature Genetics* 39(1):31-40.

MC006 Product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description and instructions for use
Version 01 – 04 August 2026 - First version of the MC006 Instructions For Use.