Molecular analysis of lactase persistence by melting curve assays

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INTRODUCTION

Adult lactase persistence (LP) is a relatively recent human adaptation where the lactase gene (*LCT*) is still expressed in the small intestine after weaning. In the current human population about 15-25% have lost LNP (lactase non-persistence) due to relatively recent single dominant mutations in the *MCM6* enhancer upstream of *LCT*, which results in adult LP even in heterozygotes. The most common LP variant (-13910C>T) is found in about 60%-70% of the Caucasian population. In addition, 4 other widely studied, closely linked SNPs also generating LP, are found amongst Afro-Beja (-13907G>C), Saudi (-13915T>G), Sudan-Ethiopia (-14009T>C) and Kenia-Tanzania (-14010G>C) ethnic groups[1,2].

Here we describe a test version of a melting curve assay[3] (see **Figure 1**) to detect the 5 most common LP associated SNPs and 4 other rare LP-linked SNPs (see **Table 1**). Until now, identification of nine mutations in a single melting curve assay was challenging due to the clustering and the limited number of fluorophores available. This issue has been addressed with **SALSA® MC001 Lactase Persistence mix-X1**.

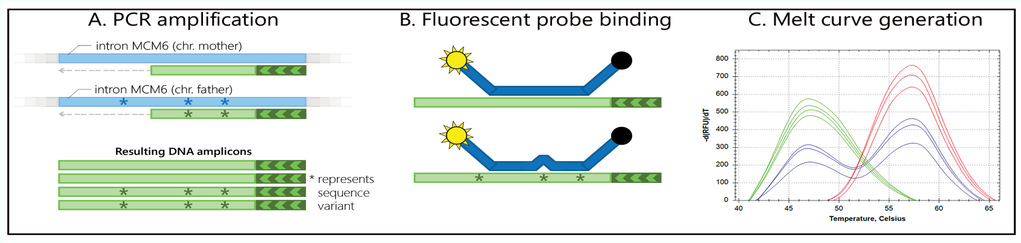

Figure 1. Melt assay workflow. (**A**) Enhancer of the *LCT* gene containing SNPs that may confer lactase persistence is amplified with a single set of primers with one primer in excess. (**B**) 4 different fluorescently (☼) labelled and quenched (●)TEX615 and Cy5 probes are used in 2 separate reactions to generate distinct melting patterns (**C**), which allow the identification of 9 different mutations in the *LCT* region.

MATERIALS AND METHODS

Control DNA samples

This assay was tested with synthetic DNAs (G-Blocks[4]) comprising 268 bp of the *MCM6* enhancer region, each containing a single SNP as indicated in **Table 1**. Subsequently, these G-blocks were cloned individually in pGEM®-T Easy[5] and in tandem repeat configuration with the corresponding wild-type *MCM6* sequence. These plasmids were stably propagated and amplified in *E. coli* NEB 10-beta (*recA1*)[6].

Blood or saliva DNA samples

Presently, more than 25000 human DNA samples from blood or saliva have been analyzed with MC001 Lactase Persistence mix-X1.

Assay description

This melt assay (22µl) requires an asymmetric PCR and two separate reactions with a 2µl sample containing as little as 0.1-1 ng input DNA, whereas a DNA quantity peak (Q-fragment, not shown) will indicate when the input DNA concentration becomes critical. In each of these reactions, two different melting curve profiles are generated using TEX615 and Cy5 fluorescent probes covering distinct regions of the *MCM6* enhancer. The PCR is carried out with a polymerase lacking 5'-3' exonuclease activity, displacing the probe without destroying it. Asymmetric PCR provides single stranded DNA which eventually allow the probes to bind, followed by subsequent melting curve assays.

References

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4. Integrated DNA Technologies, Inc USA.
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SALSA® MC001 Lactase Persistence mix-X1 is for Research Use Only (RUO). Not for use in diagnostic procedures.

The product described concerns a test version that is not available for general purchase.

RESULTS

Accurate SNP detection in plasmids

The mutations in the *MCM6* enhancer that confer LP are clustered within distinct regions between positions -13907 to -13915 and -14009 to -14011, respectively. Hence, probes have been split into 2 different reactions (**A** and **B**) to identify 9 mutations associated with LP.

- **TEX615 probe (reaction A)** clearly distinguishes between wild-type (**wt**) sequence from those with -13910CC and -13910TC genotypes. The presence of the SNPs at -13907 and -13909 also results in distinct patterns that are recognized in a background with **wt** samples (**Figure 2A**).
- **Cy5 probe (reaction A)** differentiates samples with -14010CC and -14010GC from **wt** individuals. Individuals with SNPs at -14009 and -14011 have Cy5 patterns that are also clearly differentiated in a **wt** background (**Figure 2B**).
- **TEX615 probe (reaction B)** allows identification of known genotypes between -13913 and -13915 (**Figure 3A**).
- **Cy5 probe (reaction B)** was designed to distinguish SNPs at position -14009 and -14011 in combination with the Cy5 probe pattern in reaction A (**Figure 3B**).

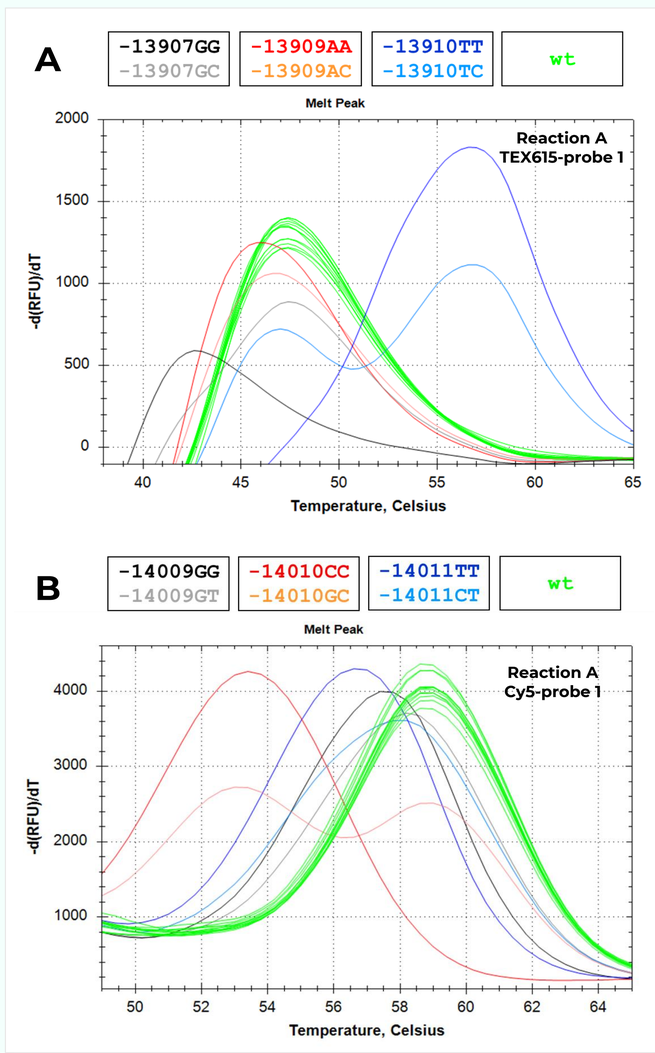

Figure 2. Reaction A results. In **2A**, 7 distinct control plasmids were used to mimic homozygous, heterozygous or the wt mutations located between -13907 and -13910, which were detected using **TEX615 probe 1**. In **2B**, results of wt and 6 other control plasmids that are homozygous and/or heterozygous for SNPs between -14009 and -149011 are displayed using **Cy5 probe 1**. For simplicity only one specific probe, mutation and resulting melt curves are shown in **Figure 2A** and **2B**.

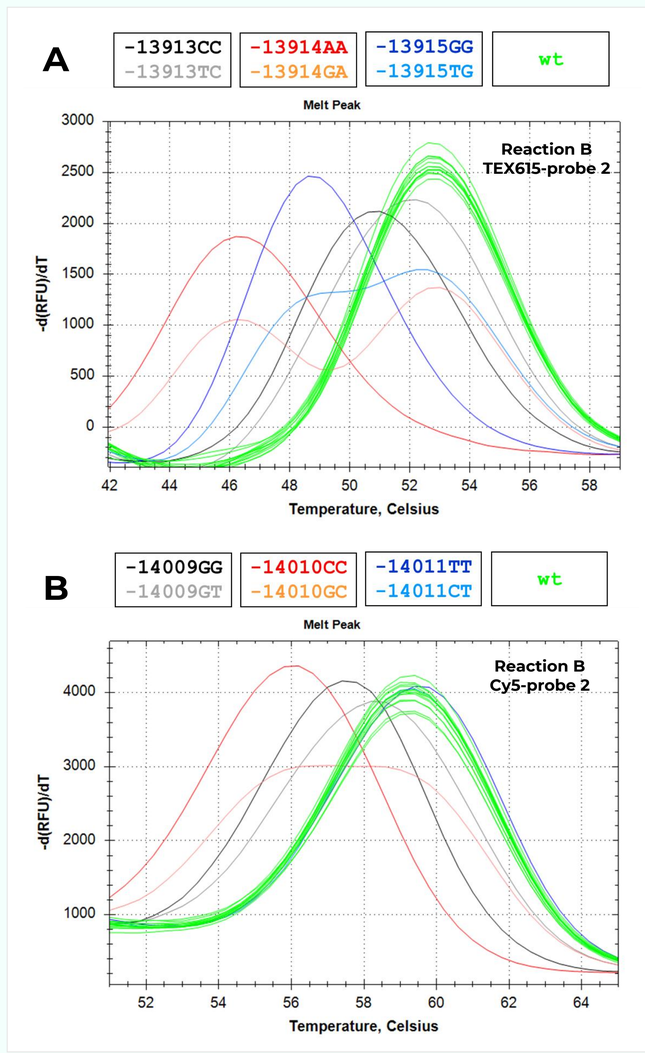

Figure 3. Reaction B results. In **3A**, 6 different control plasmids were used to mimic the mutations between -13913 and -13915, which were detected using **TEX615 probe 2**. In **3B**, the same control plasmids used in 2B were screened with **Cy5 probe 2** to unambiguously distinguish the mutation at -14009 from that at -14011. For simplicity only one specific probe, mutation and resulting melt curves are shown in **Figure 3A** and **3B**.

LP-related SNP detection in human DNA samples

MC001 Lactase Persistence mix-X1 has been extensively tested in the Netherlands and Ethiopia. In the Netherlands, research mainly focused on individuals with prominent bowel complications, possibly caused by lactose intolerance. Approximately, 75% of these individuals were genotypically either -13810TT or -13910TC instead of -13910CC. Only a very small percentage (< 1.5%) of other mutations besides at -13910 were found. In Ethiopia, random sampling showed that more than 40% of samples presented mutations (see **Table 1**).

Table 1. SNPs with confirmed or unknown clinical significance found in the Ethiopian samples.

Mutation	rs number	major>minor	% Literature	% Ethiopia	Population	Tolerance
-13907	**rs41525747**	**C>G**	**21% Afro-Beja[1]**	**11%**	**Sudan/Ethiopia**	**Clinvar[7] confirmed**
-13909	rs768790493	C>A	0.03% Europe[?]	0%	European	no Clinvar[7]
-13910	**rs4988235**	**C>T**	**70% Europe[1]**	**0%**	**European**	**Clinvar[7] confirmed**
-13913	rs41456145	T>C	0.01% Europe[?]	3.75%	European	Clinvar[7] uncertain
-13914	rs773131166	G>A	1.1% Askenazi[?]	0%	Jewish	no Clinvar[7]
-13915	**rs41380347**	**T>G**	**50% Saudi[1]**	**24%**	**Middle East**	**Clinvar[7] confirmed**
-14009	**rs869051967**	**T>G**	**1-6% Sudan[?]**	**2.5%**	**Camel Herders**	**Clinvar[7] confirmed**
-14010	**rs145946881**	**G>C**	**30% Kenia/Tanzania[1]**	**1.5%**	**Massai**	**Clinvar[7] confirmed**
-14011	rs4988233	C>T	0.4% South Asian[?]	0%	South Asian	no Clinvar[7]

CONCLUSIONS

- SALSA® MC001 Lactase Persistence mix-X1 is a reliable and quick lactase persistence detection assay that can be used in a large quantity of samples simultaneously.
- This assay detected 9 lactose persistence-related SNPs in both synthetic and human DNA samples.
- In Europe, genotypes other than at location -13910 were rarely found (<1.5%), however, in Ethiopia mutations at -13907, -13913, -13915 and -14009 and -14011 were common in the population.