Testing of matrix effects : Inclusiveness test : testing of different samples containing oat :

The objective of this part of the study is to demonstrate, whether the qualitative cereal-specific PCR system is able to detect oat in complex food samples containing oat and other species.

1- Purchase and traceability of test samples containing oat

- Seven food samples containing oat have been purchased in local supermarkets and retailer shops. All samples have been enregistered in our internal Eurodat LIMS.
- Sample 0175-4842 is an oat reference sample used as a positive control

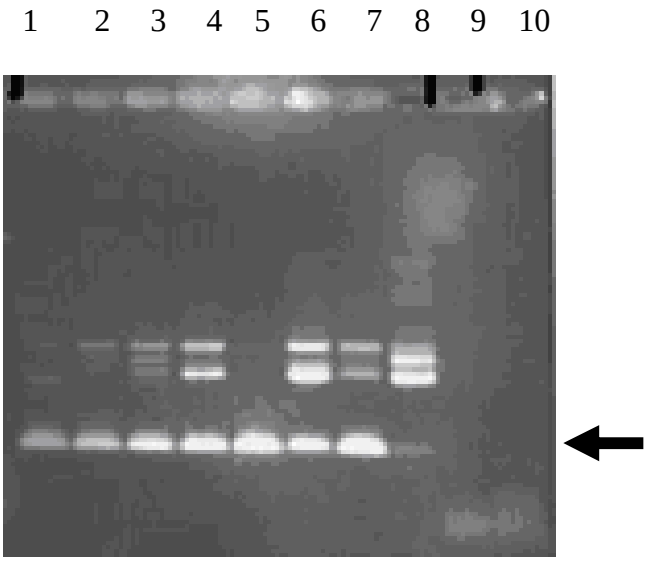
Sample list :

Food sample :	Internal Eurodat sample identification code
Gruau d'avoine	0175-4842
6 Barres céréaliéres	0189-0658
Quaker Oats crunchy	0189-0659
Quaker Oats flocon d'avoine	0189-0660
Petit déjeuner LU	0189-0661
Muesli ECO+	0189-0662
Pain de mie 7 Céréales	0189-0663
L'aliment Grain d'avoine	0189-0654

4- Qualitative PCR with analysis of the amplicons on an agarose gel

In a first step, all food samples have been analysed by means of the qualitative standard PCR method (**SOP see annex**) using a cereal-specific primer set. With this PCR system, oat can be identified and distinguished by a different amplicon size from other cereals, such as rye, barley and wheat. After the qamplification step, the PCR products were loaded on an ethidium bromide stained agarose gel.

Lane :	Internal Eurodat sample identification code
1	0175-4842
2	Positive control
3	0189-0658
4	0189-0659
5	0189-0660
6	0189-0661
7	0189-0662
8	0189-0663
9	Extraction blank
10	PCR negative control



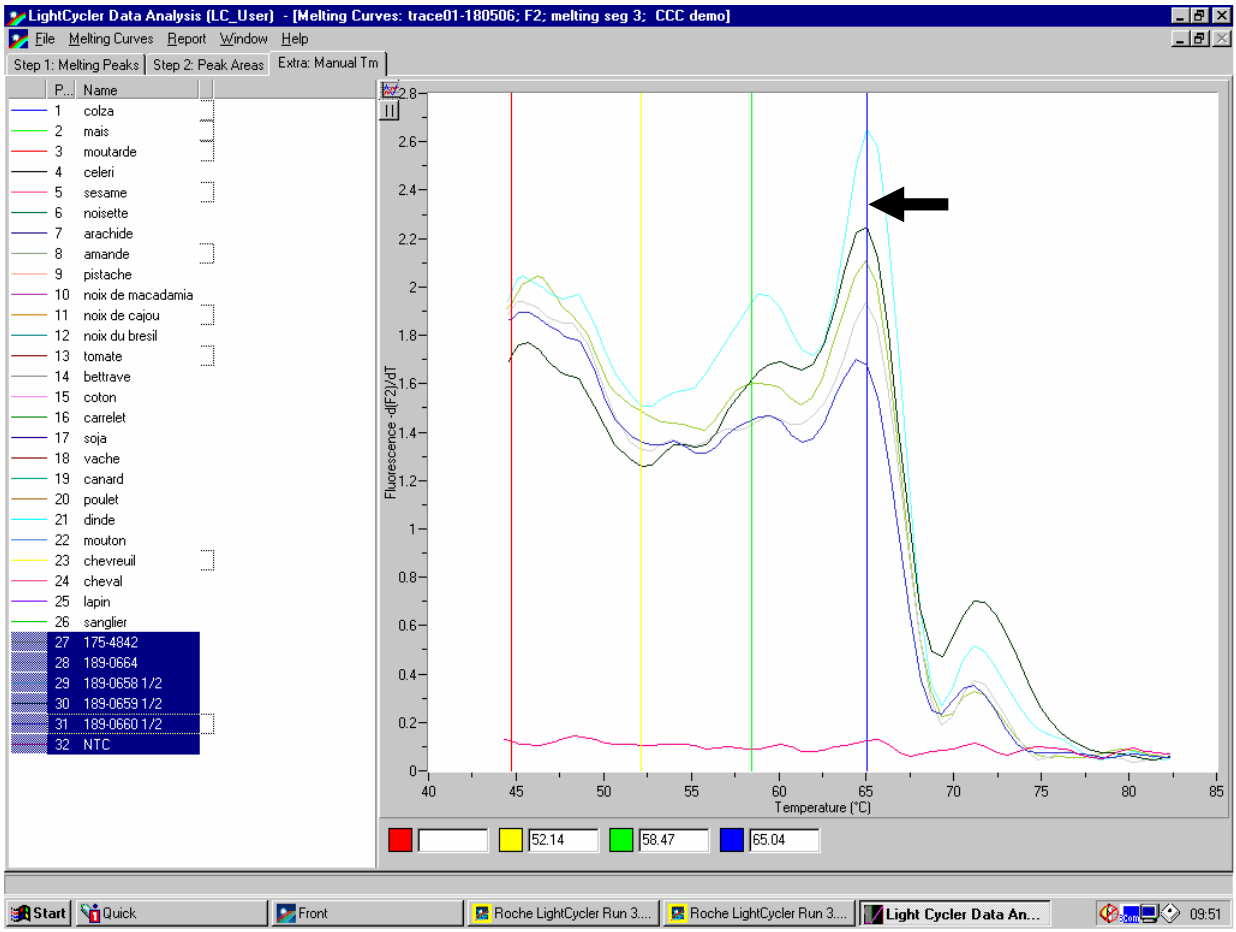
The arrow indicates the oat-specific amplicon (217bp). PCR products of higher molecular weight may correspond to other cereal species present in the respective samples, amplified by the primer set.

The result demonstrates, that the oat fragment could be amplified in all food samples containing oat. This indicates, that the PCR system is suitable for the use in complex products.

5- Analysis of the PCR ampification product using the LightCycler melting curve technology

In a second step, 15µL of the PCR amplification product have been transferred into a LightCycler capillary containing 5µL of a cereal-specific FRET hybridisation probe mix (protocol see SOP annex)

Résult of the LightCycler melting curve analysis:



Résult of the LightCycler melting curve analysis:

Sample code	Amplicon size (bp) on an agarose gel	result Ligthcycler melting curve (℃)
175-4842	≅ 217 bp	65.04 ℃
189-0664	≅ 217 bp	65.04 ℃
189-0658	≅ 217 bp	65.04 ℃
189-0659	≅ 217 bp	65.04 ℃
189-0660	≅ 217 bp	65.04 ℃
189-0661	≅ 217 bp	66.55 ℃
189-0662	≅ 217 bp	66.55 ℃
189-0663	≅ 217 bp	66.55 ℃

The results show, that all samples containing oat as well as the positive control are characterized by a melting curve peak of ≅ 65°C. This peak does not occur in the negative control. However, the same peak is obtained in other cereal species such as barley, wheat and rye. Therefore, this latter technique is not considered to be discriminative for oat. For qualitative detection of oat, the standard PCR with revelation of the amplicons on an agarose gel is the method of choice.