Validation of the oat PCR system :

- **Specificity test (test for cross reactivity with DNA extracted from animal and plant species).**

I. Specificity tests :

Test for cross reactivity with DNA extracted from animal and plant species :

We have investigated the specificity of the oat-and cereal-specific PCR systems.

Only 2 species (canola and mustard) were tested positive showing amplicons of the expected size (see gel pictures below). Interestingly, these two species were tested negative when analysed with the Real-time PCR system (see LightCycler fluorescnec curves below).

We therefore conclude that the respective species exhibit a high degree of sequence homology with the oat sequence on the primer level. However, the use of the internal hybridisation probe avoids false positive detection of canola and mustard. Interestingly, we observed a very faint signal for sesam in the real-time PCR. However, this signal may be considered as negative by introduction of a cutoff-Ct value. No other plant or animal specie was tested positive with the oat & cereal-specific Real-time PCR system.

Moreover, in a couple of plant and animal species, we observed PCR products of unexpected size (high molecular weight) which may be due to unpecific amplification or PCR artefacts. These side bands were not detected by the hybridisation probe in the Real-time PCR.

DNA was extracted from 16 different plant species and 10 different animal species, and amplified with both the oat- and cereal-specific standard PCR and the Real-time PCR system including a fluorescently labelled hybridisation probe. After revelation of the PCR products on an agarose gel, only 2 species (canola and mustard) were tested positive showing amplicons of the expected size (ca 250 bp). Interestingly, these two species were tested negative when analysed with the Real-time PCR system.

We therefore conclude that the respective species exhibit a high degree of sequence homology with the oat sequence on the primer level. However, the use of the internal hybridisation probe avoids false positive detection of canola and mustard. Interestingly, we observed a very faint signal (Ct > 39) for sesam in the real-time PCR. However, this signal may be considered as negative by introduction of a cutoff-Ct value (eg Ct39). No other plant or animal specie was tested positive with the oat & cereal-specific Real-time PCR system.

Moreover, in a couple of plant and animal species, we observed PCR products of unexpected size (high molecular weight) which may be due to unpecific amplification or PCR artefacts. These side bands were not detected by the hybridisation probe in the Real-time PCR.

The results are presented in the figures below.

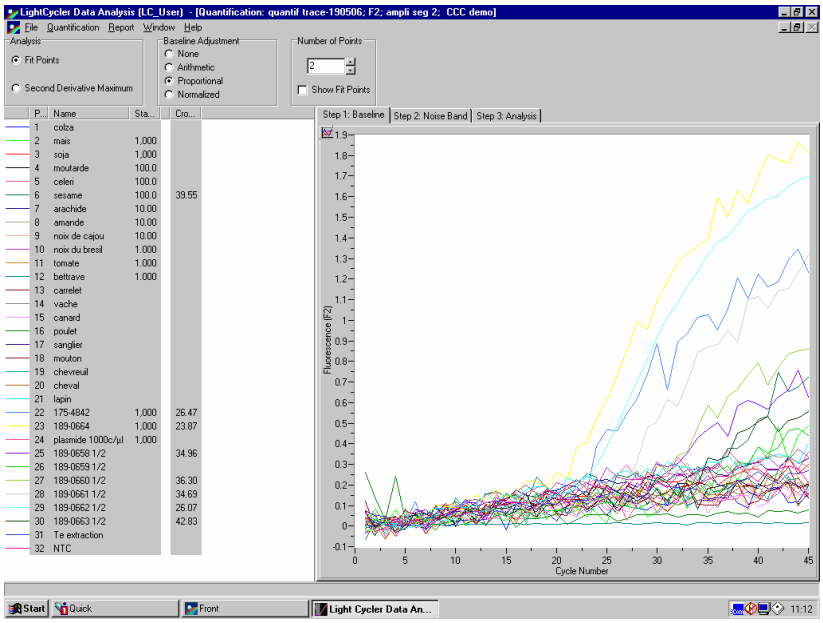
Specificity test- Quantitative LightCycler Real-Time PCR :

Species tested :

Oilrapeseed, corn, mustard, celeri, sesam, peanut, almond, cashew nut, brazil nut, tomato, sugar beet, soya, fish and animal species

Results:

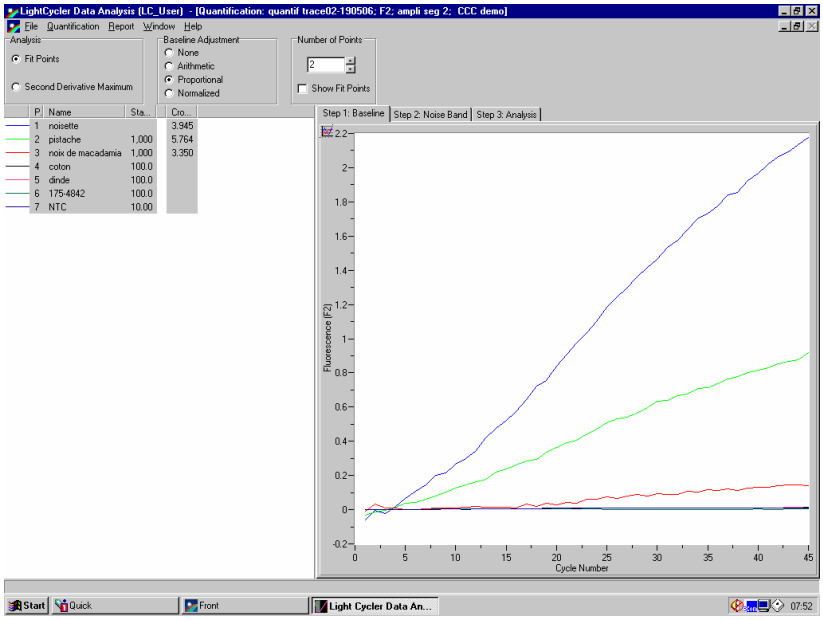
Run 1 :



Species tested :

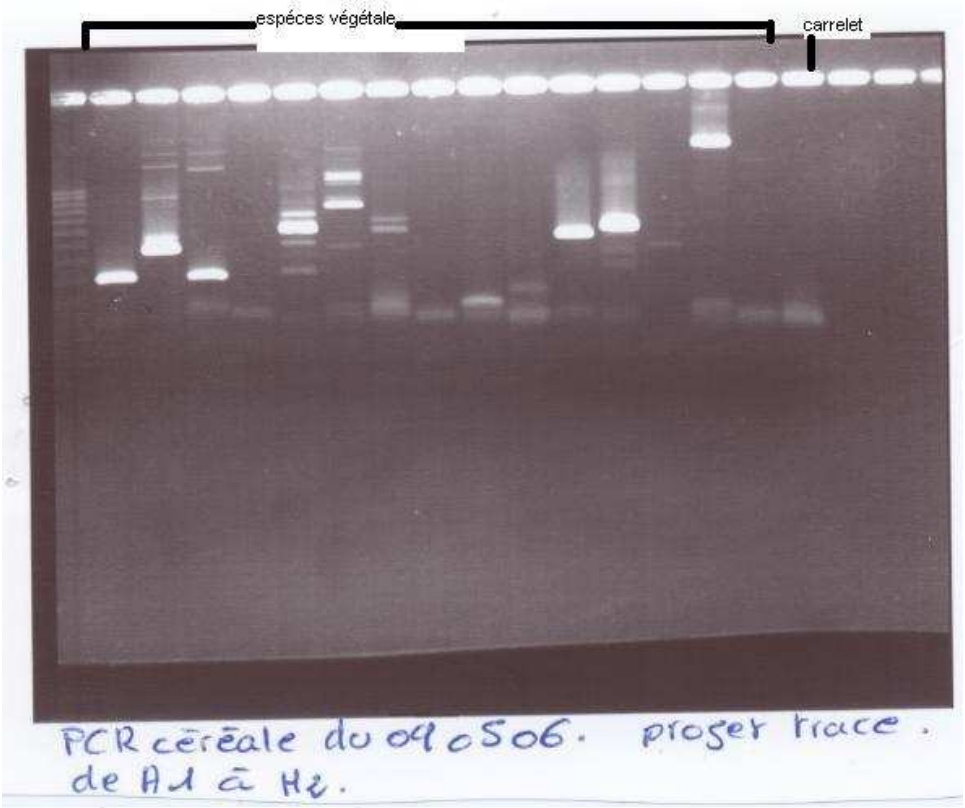
hazelnut, pistachio, macademia nut, cotton

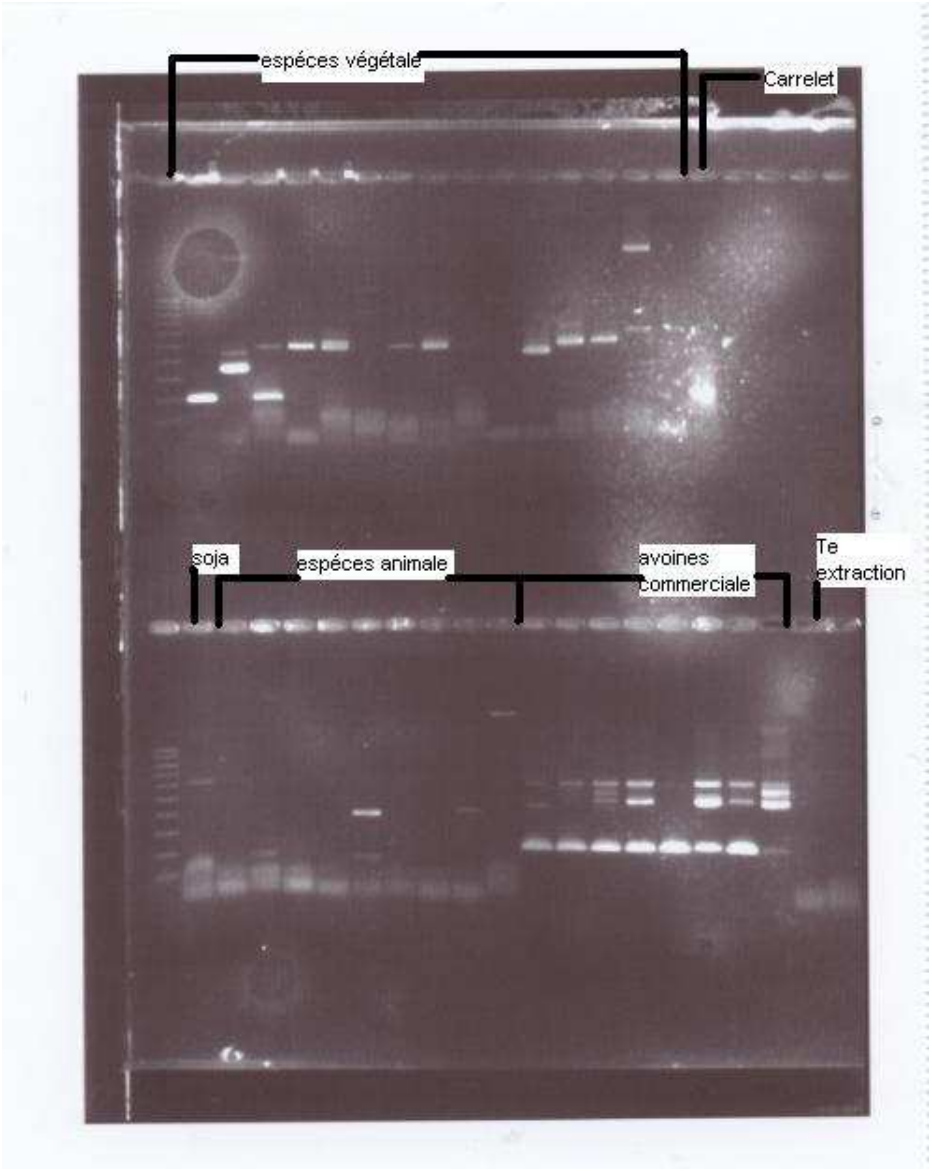
Run2 :



Specificity test- qualitative cereal-specific PCR :

Gel pictures specificity tests carried out on plant and animal species :




espéces végétale
Carrelet
soja
espéces animale
avoines commerciale
Te extraction