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Cloned Sequences as Positive Control Samples for Qualitative PCR

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INTRODUCTION

The integrated European project TRACE ("Tracing the origin of food") aims to improve the quality of food and the control of food production. In Work Package 3, methods to trace the origin of species based on DNA will be established. This will allow supporting the PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication) quality labels of the European Union for selected food products.

Farro della Garfagnana, a land race of the species *Triticum turgidum* subsp. *dicoccum* is cultivated in Tuscany (Italy) and holds a PGI quality label. In order to detect adulteration of Farro della Garfagnana, specific markers for wheat, barley, rye, oat, maize, and the so-called Q locus were selected and tested. The Q locus determines the free-threshing character of bread wheat and hard wheat, which *Triticum turgidum* subsp. *dicoccum* lacks. For each marker a primer pair suitable for its identification by Polymerase Chain Reaction (PCR) was defined. In order to ensure the correct application of these PCR methods a set of positive control samples was developed by the Institute for Reference Materials and Measurements (IRMM). Here we present the plasmid that was established for the detection of barley (*Hordeum vulgare* L.) sequence.

STRATEGY

A part of the barley (*Hordeum vulgare* L.) sequence was amplified by Biolytix AG using conventional PCR. Subsequently, the 68 bp PCR fragment was purified and cloned in the commercial vector pCR®2.1 (Invitrogen). The *EcoRI* fragment of this intermediate vector was then cloned into the vector pUC18 to obtain the plasmid pIRMM-0046 (Fig. 1). Restriction digests, PCR analysis with sequence-specific primers (Fig. 2) and sequencing were performed as quality control and proved that the inserted DNA sequence is identical to the sequence targeted by the PCR method. Additionally the plasmid has been tested for its specificity. DNA extracted from seed powders from different species served as a control in the PCR reaction.

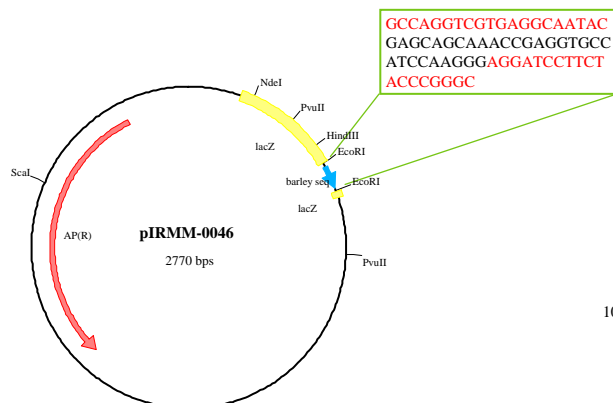


Fig. 1: Plasmid pIRMM-0046 containing the barley sequence (see box, primer sequences for the qualitative PCR indicated in red)

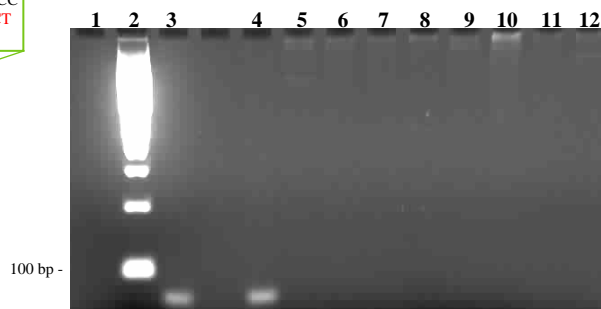


Fig. 2: Quality control via PCR analysis (using 100 ng per PCR reaction)
Lane 1: sterile water, lane 2: molecular weight marker (100 bp), lane 3: pIRMM-0046, lanes 4-12: DNA from barley, buck wheat, rye, wheat, cotton, maize, soybean, sugar beet, potato, respectively.

CONCLUSION

A plasmid has been developed containing a specific barley sequence. The plasmid proved to be a suitable quality assurance tool and can be used as positive control in PCR methods targeting the barley sequence. Hence, using this plasmid laboratories can prove the correct application of their PCR method. This is of major importance when wishing to demonstrate that PCR results on unknown samples are true negatives and no false negatives.

For this reason it was shown that the chosen primer pair can specifically identify the barley sequence. This enables to detect contamination of Farro della Garfagnana products with barley. Similar plasmids have been produced for several other markers.