Detection of genetically modified organisms—closing the gaps

To the Editor:

Precise and accurate detection methods are a prerequisite for reliable control of genetically modified organisms (GMOs) on the market. GMO detection laboratories mostly rely on PCR technology or quantitative real-time PCR (qPCR) to detect genetic elements, the presence of which allows discrimination between GMOs and non-GMOs[1]. In general, the testing strategies followed by these laboratories consist of two phases. First, GMO presence is detected by using screening methods that target the most common genetic elements found in genetically modified crops. If GMO presence is confirmed, identification of GMOs is performed using event-specific methods, if necessary followed by quantification to verify compliance with allowed level of adventitious presence of GMOs[2,3]. The event-specific methods validated in the European Union (EU; Brussels) by the Community Reference Laboratory for Genetically Modified Food and Feed assisted by the European Network of GMO Laboratories (ENGL)[4] are being implemented in enforcement laboratories worldwide. In contrast, development and validation of screening methods are not included in this system and they still remain the subject of individual research and choice of testing laboratories. Therefore, this situation results in a tiered approach for GMO testing for which the first tier is less harmonized and which could adversely affect the official control traceability system. Here, we present the case of TC1507 maize (DAS-Ø15Ø7-1, Pioneer Hi-Bred International, Des Moines, IA, USA) which illustrates how a well-recognized and generally used screening approach fails to perform well and we then go on to discuss the implications of the failure in the general context of GMO detection.

While analyzing different methods, we observed differences in performances when assessed on the TC1507 GM maize certified reference material (CRM) produced by the Institute for Reference Materials and Measurements (ERM-BF418d, EC-JRC-IRMM, Geel, Belgium). One of the most commonly used screening methods targeting the 35S promoter (P35S) from Cauliflower Mosaic Virus[5] (qPCR P35S) showed 16-fold lower sensitivity than another screening method targeting a neighboring region of the 35S promoter[6]. Further investigations showed that this low sensitivity was due to the presence of a single nucleotide polymorphism (SNP) located in the target region of the qPCR

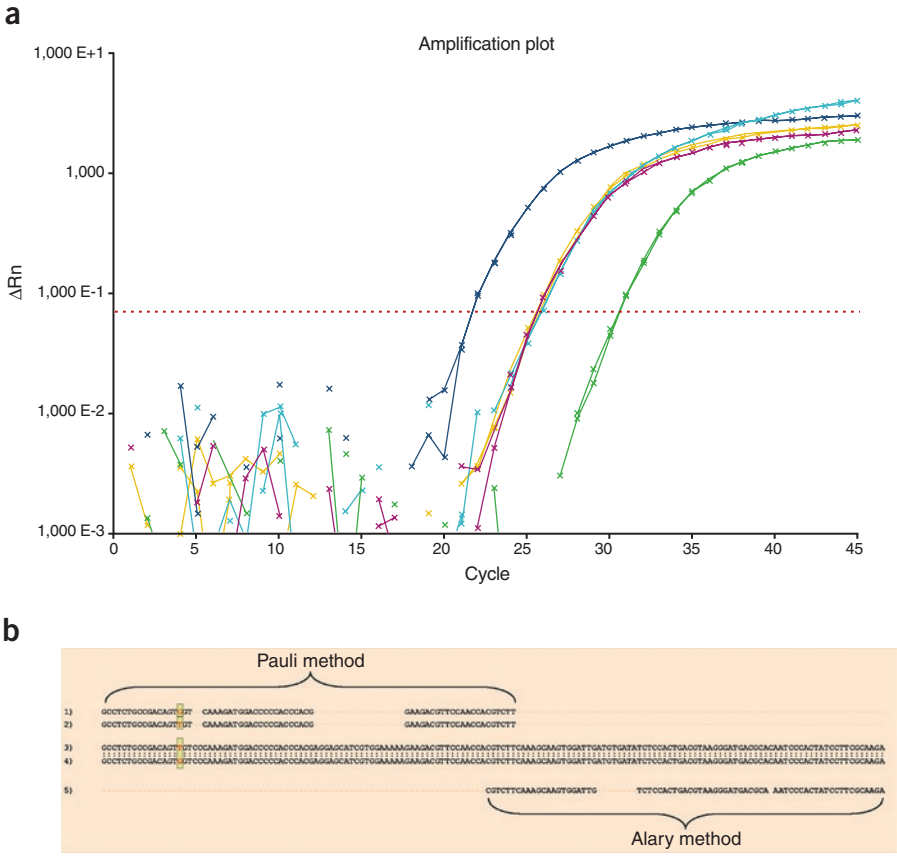

Figure 1 qPCR analysis of TC1507 GM maize line DNA. (**a**) Amplification curves for the maize endogenous invertase gene (dark blue), TC1507 event–specific target (magenta), P35S screening element using the Pauli-P35S method[5] (green), P35S screening element using a modified Pauli-P35S method (orange) and the Alary-P35S method[6] (turquoise blue) were compared. The modified Pauli-P35S method differs from the original Pauli-P35S method by the use of a forward primer in which the substitution G-T found in TC1507 GM maize line DNA is included. The same DNA sample was used in all four analyses; it contained ~50,000 copies of invertase and 2,400 copies of the transgene. The threshold used for determining Ct values is indicated by the dotted line. The Alary-P35S and the modified Pauli-P35S methods give similar Ct values as the TC1507 event–specific method, whereas Ct value with Pauli-P35S method is four units higher. (**b**) DNA sequence of the CaMV 35S promoter introduced in the TC1507 GM maize. The DNA sequence obtained from the CRM TC1507 GM maize was compared with the DNA sequences described in patents and with the sequences of the primers and probes from both P35S screening methods (Pauli-P35S (ref. 5), and Alary-P35S (ref. 6)). A SNP (substitution G-T) is located on the sequence targeted by the forward primer in the Pauli-P35S screening method. Only a part of the sequence is shown. (1) Forward primer, probe and reverse primer DNA sequences described by Pauli *et al.*[5], (2) forward primer, probe and reverse primer DNA sequences from the modified Pauli-P35S method, (3) DNA sequence described in this study, (4) DNA sequence integrated in maize genome described in patents for TC1507 maize (US 7288643, JP 2006525028-A 57 and WO2004099447), (5) forward primer, probe and reverse primer DNA sequences described by Alary *et al.*[6]. Pauli method, P35S sequence targeted by the original and the modified Pauli-P35S screening methods; Alary method, P35S sequence targeted by the Alary-P35S screening method. The position of the SNP is shown by the box, the mutation in the DNA sequence is shown in red.

method (Genbank acc. no. FJ605509). This SNP was not identified in the sequences available for TC1507 maize (as specified in patents and application dossier to the European Commission (EC; Brussels)), or in other available P35S sequences inserted in different transgenic plants (**Fig. 1**; see **Supplementary Notes** for further details). The lower sensitivity of the commonly applied screening method may affect the efficiency of GMO traceability because at low target-DNA copy number levels, as is often found in processed food and feed, the presence of GMOs would not be detected. Our finding of a SNP in the TC1507 CRM maize thus raises additional challenges regarding the efficiency of current and future analytical traceability.

A possible reason for the discrepancy between the actual DNA sequence of TC1507 CRM and the data from the application dossier submitted to the EC (and patents) may also be that the SNP was introduced after the applicant had verified the insertion sequence and during the breeding process between the elite event (the transformation event that is used to produce commercial lines) and the nongenetically modified seed lines to produce commercial seeds. Even so, seeds from the TC1507 line are not yet commercialized for cultivation in the EU and therefore we were not able to test different genetically modified varieties to verify this assumption. Such variation of a transgenic line genome is not an isolated case. In two recent publications, it has been reported that two commercial seed varieties of the MON810 maize genetically modified event (ARISTIS BT and CGS4540) present genetic variation thus hampering the detection by several methods available for MON810 (Monsanto, St. Louis)[7,8].

Reliable screening methods are important both for detection of unauthorized GMOs and as a first step in labeling control. The qPCR P35S screening method that showed low sensitivity toward the TC1507 genetically modified line is the most widely used one by the members of the ENGL[9] and it was validated in an interlaboratory study following the criteria specified in ISO 5725-2. However, the validation was performed for the detection of P35S in the Roundup Ready soybean (Monsanto) genetically modified line only. Therefore, our finding suggests that even when fully validated, a screening method should be systematically assessed for its accuracy and sensitivity against all genetically modified lines present on the market. Moreover, there is no consistency in the way GMO enforcement laboratories in the EU and other countries detect the presence of transgenic elements in the first step of GMO analysis: in the case of the P35S methods used by the ENGL members, 7 quantitative PCR and 12 qualitative PCR methods targeting different regions of the P35S sequence are reported[9]. At an international scale, this may lead to even larger heterogeneity of approaches and consequently test results during the screening phase for GMO presence. The procedure to validate screening methods is not available in the context of international standardization effort. As a consequence, the possible occurrence of SNPs or genetic rearrangements within the inserted DNA, the heterogeneity of the screening methods and the fact that their reliability is not verified on all genetically modified varieties found on the market increase the chances that some laboratories do not detect properly the presence of GMO during the screening phase. In addition, it is very important that staff in the testing laboratories and those interpreting the tests have a good understanding of molecular biology. Deficiency in molecular biology knowledge may lead to irrelevant use of methods that do not meet rigorous testing standards.

In conclusion, we believe that the use of harmonized testing strategies in the screening phase would lead to equivalent results of GMO detection on the international scale and consequently fewer problems in international trade. Another prerequisite for reliable detection would be well-designed validation of screening methods, including careful verification of the performance of methods in terms of robustness and reproducibility (e.g., small changes in the laboratory protocol, material or reagents used); thus far, such validation procedures have not been incorporated into legislation relating to GMOs. If they were implemented, such validation dossiers could be constantly updated with data on specificity and sensitivity for GMOs entering the market as well as previously commercialized varieties of approved genetically modified events.

Note: Supplementary information is available on the Nature Biotechnology website.

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