

# A simple and reliable PCR-based method for detection and screening of transgenic plants transformed with the same endogenous gene

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## ABSTRACT

To detect integration of a transgene in genetically modified plants that have a native copy of the gene, a simple and reliable PCR-based method has been established. This method is then used to screen for transgenic wheat plants that have been transformed with the high molecular weight glutenin subunit Dy10 gene. Two PCR primers were designed, the forward one matched upstream flanking nucleotide sequences in the pBlueScript KS vector and the reverse primer hybridizes with a Dy10 specific. Use of these primers produced a distinguishable marker only from the transgene with no appreciable signal detected from the endogenous gene.

**Key words:** PCR, glutenin, primers, transgenic plants.

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## INTRODUCTION

Plant transformation has become a very important and widely used tool in biological studies. A technical challenge with this technology is that it often requires screening of a large number of potential candidates to identify the desired transformants. Even though much improvement has been made, however, this is often a laborious work. Most approaches for detecting the transgene in transformed plants are time-consuming and can be quite expensive (Freitas-Astua *et al.*, 2003). Screening transformed plants for gene integration and expression is a process that involves different techniques, including DNA- and RNA-blot hybridization analysis, PCR, ELISA analysis (de Kochko *et al.*, 1994). Also real-time PCR can be used that has distinct advantages such as a wide dynamic range of quantification,

high sensitivity, and high precision, with no post PCR handling (Bustin 2002; Ginzinger 2003; Klein 2002; Mackay *et al.*, 2002). There are, however, critical points and limitations, such as expensive equipments and supplies, time and the feasibility of the technique to screen large number of plants. Among the above mentioned techniques conventional PCR with an analysis of specific PCR products has proved useful in many scientific fields (Kota *et al.*, 1999; Markus *et al.*, 1999; Yongback *et al.*, 2002). However, these assays are not feasible for the detection and differentiation between transgene and the same endogenous gene in transgenic plants. The wheat (*Triticum aestivum*) high molecular weight (HMW) glutenin genes are the most studied wheat genes because they code for wheat gluten protein subunits that critical to the physical and chemical properties of wheat doughs (Shewry *et al.*, 1996). The HMW-

glutenin genes have also been a major focus of efforts in wheat bioengineering (Vasil and Anderson, 1997). In our laboratory, Dy10 gene, coding for glutenin subunit, was introduced into wheat plants (Giza 164) to improve wheat flour quality. After transformation process there was a need to screen transformed plants using PCR and due to the problem of the presence of endogenous gene interfering with the transgene. The objective of this study was to assess the possibility of using simpler and yet reliable method to screen for transgenic plants. Therefore, we demonstrate a simple method that can effectively amplify only the transgene not the same endogenous gene in transgenic plants.

## PRIMER AND METHODS

### Plasmid

PK-Dy10 (Dy10 gene cloned into pBlueScript II KS vector at the *EcoR I* site) was kindly provided by Olin Anderson, USDA, and Albany, CA, USA.

### Plant material and DNA isolation

Putative transgenic wheat (*Triticum aestivum* L. cv. Giza 164) T<sub>0</sub> plants resulted from transformation experiments with Dy10 gene using particle bombardment were used as a plant material. Leaf materials were collected and total genomic DNA isolation was carried out using DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions.

### Primers

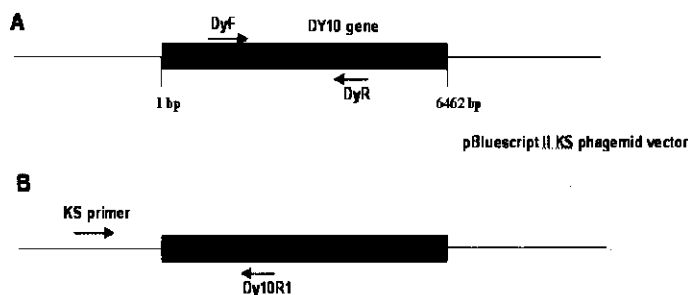
Two sets of primers (Fig. 1) were used in this experiment; the first set was against nucleotide sequences of the Dy10 gene. The forward primer DyF sequence is:

5'-ATGGCTAAGCGGCTGGTCCTCT-3' and the reverse primer DyR sequence is:

5'-ATTGTTGCCCTTGTCTGGTTCTT-3'. The second set, forward primer, KS:

5'-TCGAGGTCGACGGTATC-3 (located upstream of the cloned Dy10 gene at the Blue-Script KS vector) and the reverse primer Dy10R1:

5'-CCGGCCCTGACTCCTAATACACAT-3' (against the nucleotide sequence of the Dy10 gene).



**Fig. (1): Schematic diagram of pK-Dy10 clone showing the locations of the two primer sets. A: locations of DyF and DyR primers; B: locations of KS and Dy10R1 primers.**

### PCR amplification

PCR reactions were performed using 20-100 ng of plant genomic DNA in a 25  $\mu$ l total reaction volume containing 2.5  $\mu$ l 10X buffer,

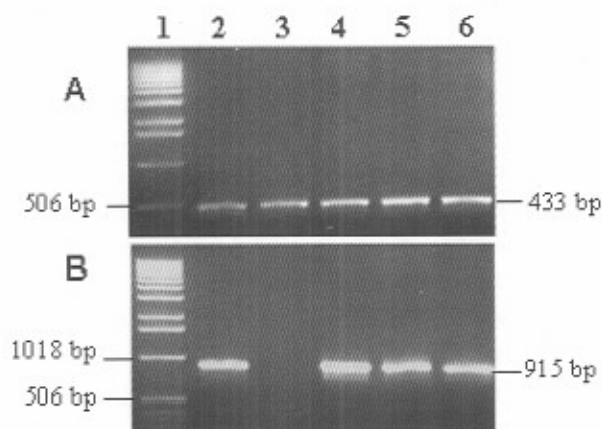
2 units HotStart Taq polymerase (Qiagen, Germany), and 0.5  $\mu$ M of each primer. For the positive control reaction 10 ng of plasmid (pK-Dy10) was used as a template. PCR was

carried out in DNA Thermal Cycler 9600 (Applied Biosystems, USA) by an initial 10 min denaturing at 94 °C followed by 35 cycles of PCR. Each cycle included 1 min at 94 °C followed by 1 min at 55 °C, and 1 min at 72 °C. The last cycle of the PCR included an additional 5 min extension at 72 °C. The reaction was analyzed by electrophoresis on 1.2 % Agarose gel.

## RESULTS AND DISCUSSION

In recent years a different screening approaches of transgenic plants have been successfully used. Although the information derived from those studies undoubtedly provided a powerful means for screening methods, it is not a common approach in the transformation processes to transform plants

with the same endogenous gene. Therefore, most of the available techniques are directed to the screening of transgenic plants that had transformed with a foreign gene. In this report, we demonstrate here that a simple PCR technique can be used to screen putatively transgenic plants transformed with the same endogenous gene. In our laboratory, we have been transformed wheat using one of the high molecular weight glutenin subunit (HMW) Dy10 gene. Our first attempt was to design two specific primers (DyF and DyR) against nucleotide sequences of the Dy10 gene. Interestingly when applying those specific primers to the control plants (non-transformed plants) it produced a PCR product of the same expected size obtained from putatively transgenic plants as shown in Fig. (2A).



**Fig. (2):** PCR screening of some transgenic wheat plants using different PCR primers. Lane 1: 1 Kb DNA Ladder; lane 2: positive control (plasmid); lane 3: negative control (non-transgenic plant); lanes 4-6: transgenic wheat plants; A: PCR amplifications using DyF and DyR primers; B: PCR amplifications using KS and Dy10R1 primers.

This finding suggests that wheat plant cultivar Giza 164 contains a native copy of the Dy10 gene. At the same time those specific primers have been used successfully before in screening of Maize plants transformed with the same gene (Abdallah *et al.*, 2004). It revealed

reliable results that clearly detect the introduced Dy10 gene in transformed Maize and can differentiate between transformed and non transformed Maize plants. In this regard the results were expected due to the absence of Dy10 gene in Maize plants. Previous studies

(Blechl and Anderson, 1996) have shown that when applying genomic southern analysis it was complicated by the presence of cross-reacting bands derived from the endogenous HMW genes. Also southern analysis is not a method of choice that can be applicable in screening large number of putatively transgenic plants. Besides, the same authors reported the using of a unique coding region created by the fusion of two native genes in order to distinguish the products of transgene from the native endosperm protein. However, these results have been achieved through protein analysis, using SDS-PAGE. Other methods used to quantitate the levels of individual proteins in flour, such as HPLC and capillary electrophoresis (Bietz and Simpson, 1992) may also be applied in order to assign more precise numerical values to the apparent increases. Therefore, it was important to find a simple method to analyze putatively transgenic wheat plants. The PCR was chosen but with a simple modification in locating the PCR primers with the possibility of producing amplified PCR product that is distinguishable from those of the endogenous genes. Dy10 gene was cloned into pBlueScript KS phagemid vector, therefore, the idea was to use the flanking upstream DNA sequences of the vector as a forward primer (KS primer) while the reverse primer against Dy10 nucleotide sequence is Dy10R1 as indicated by Figure 1. Interestingly, as shown in Fig. (2B), the PCR reaction revealed distinguish fragment that only could be produced in case of the integration of transgene into plant genome. This finding suggests the feasibility of applying such method in screening of any transformed plants with a native gene present in the target plants as endogenous gene. Such modification will enhance and extend the screening of large number of putatively transgenic plants before proceeding to the other techniques in the screening process.

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### الملخص العربي

طريقة مبسطة وفعالة لفحص النباتات المحورة وراثيا بأحد جينات المحتوى الوراثي الاصلى للنباتات تعتمد على استخدام التفاعل التسلسلي البوليميريزي (PCR)

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للتأكد من اندماج جينات التحور في النباتات المحورة وراثيا والتي تحتوي على نسخة أخرى من جين التحور داخل محتواها الوراثي ، فقد تم استخدام طريقة سهلة وفعالة معتمدة على طريقة التفاعل التسلسلي البوليميريزي تلك الطريقة تم استخدامها لاختبار نباتات قمح محورة التركيب الوراثي بأحد جينات الجلوتين ذات الوزن الجزيئي العالي والذي يوجد منه نسخ أخرى داخل المحتوى الوراثي لهذه النباتات. وتعتمد هذه الطريقة على تصميم زوج من البادئات أحدهما يهجن مع التسايع النيوكليوتيدي للبلازميد المحتوى على جين التحور قبل بدايته بالاتجاه الأمامي، والآخر يهجن مع التسايع النيوكليوتيدي لجين التحور بالاتجاه العكسي. وبهذه الطريقة قد تم التمييز بين النباتات المحورة وراثيا وتلك غير المحورة وراثيا (الشواهد) باستخدام التفاعل التسلسلي البوليميريزي (PCR).