**Highly reproducible Capillary Gel Electrophoresis (CGE) of DNA fragments
using uncoated columns. Detection of genetically modified maize by PCR-
CGE.**

Virginia García-Cañas, Ramón González, Alejandro Cifuentes*

*Institute of Industrial Fermentations (CSIC)
Juan de la Cierva 3
28006 Madrid, Spain*

Running title: Transgenic maize detected by PCR and capillary gel electrophoresis

Abbreviations: CGE, capillary gel electrophoresis; HEC, 2-hydroxyethyl cellulose; GMOs, genetically modified organisms; PVA, polyvinylalcohol; PEO, polyethylene oxide.

Keywords: dynamic coating, DNA, capillary gel electrophoresis, polymerase chain reaction, transgenic maize, genetically modified organisms.

* Corresponding author: Tel# 34-91-5622900 (Ext 278), Fax# 34-91-5644853

E-mail: acifuentes@ifi.csic.es

ABSTRACT

In this work a Capillary Gel Electrophoresis (CGE) method is presented that allows obtaining reproducible separations of DNA fragments using commercially available polymers together with bare fused silica capillaries. The method combines a washing routine of the column with 0.1 M hydrochloride acid followed by a rinsing step with a dissolution containing 1% polyvinyl alcohol. The use of this procedure together with a running Tris-phosphate-EDTA buffer containing 2-hydroxyethyl cellulose (HEC) at pH 7.3 gives highly resolved separations of DNA fragments ranging from 80 to 500 bp. The separation of these DNA fragments is achieved in ca. 20 minutes with efficiencies up to $1.8 \cdot 10^6$ plates/m. Reproducibility values of migration times (given as %RSD) of the DNA fragments separated by this CGE method are better than 0.86% (n=10) for the same day, 1.61% (n=40) for four different days, and 1.4% (n=15) for three different capillaries. The length up to 500 bp corresponds to the DNA sizes more frequently amplified by PCR for detecting genetically modified organisms (GMOs) in foods. The usefulness of this separation method is demonstrated by detecting genetically modified insect-resistant Bt maize after amplification of a DNA fragment by PCR. Detection of 1% of Bt maize in flour is carried out using this CGE procedure with UV absorbance and laser induced fluorescence (LIF).

1. INTRODUCTION

Capillary gel electrophoresis (CGE) has been demonstrated to be a powerful analytical tool for the separation of charged analytes based on their size. Nowadays, polymer solutions have become the alternative of choice to separate SDS-proteins [1, 2] and DNA fragments [3, 4] by CGE. These polymer solutions provide well-resolved separations of SDS-proteins as well as single- and double-stranded DNA fragments with the well-known advantages related to the use of electrophoresis in capillary format (i.e., speed of analysis, automation, and quantitative analysis).

However, in order to achieve well-resolved and reproducible separations in reasonable times, CGE separations in polymer solutions have to be performed using capillaries with zero electroosmotic flow (EOF). Capillaries internally coated with a neutral polymer are normally used to eliminate the EOF [5-7]. However, the price of these coated columns is too high when compared with bare fused silica columns (ca. 20-fold higher), while to home-make such coated tubing is labor intensive [5-7]. Moreover, coated capillaries can degrade with usage affecting both separation reproducibility and resolution [8].

Different strategies have been proposed to suppress the EOF of fused silica capillaries making them useful for DNA separations in polymer solutions and overcoming the disadvantages mentioned above [9]. Thus, Kleemiß et al. [10] proposed, prior to introduce the separation buffer, to flush the capillary with a solution of a neutral polymer (hydroxyethyl cellulose (HEC) or polyvinylalcohol

(PVA)) which adsorbs onto the capillary wall suppressing the EOF. However, they could observe that HEC was more weakly adsorbed to the wall than PVA, and that capillaries dynamically coated with PVA prior to each run, tend to decrease in separation performance after 10 separations. Besides, the same authors mentioned later [11] that at $\text{pH} > 5$ this dynamic coating is not effective due to insufficient adsorption on the negatively charged capillary wall. Fung and Yeung [8] have proposed the use of poly(ethylene oxide) (PEO) added to the separation buffer together with a washing step with HCl prior to each injection to suppress the EOF. However, too long treatments are required between runs (up to 33 min) in order to achieve reproducible separations. Besides, the viscosity of the PEO solution used for separations was too high (1200 cp at room temperature) what required the use of too high pressures (up to 400 psi) to recondition the capillary between runs within reasonable times. Moreover, these high pressures are out of the capabilities of many commercial CE instruments. Also, Gao and Yeung have proposed to use poly(vinylpyrrolidone) for EOF suppression and DNA separation [12]. Following this idea, some new polymers have been described in literature (e.g., polydimethylacrylamide [13], poly(ethylene oxide)-poly(propylene oxide) copolymer [14, 15], poly(N-isopropylacrylamide)-g-poly(ethyleneoxide) [16], N,N-diethylacrylamide-N,N-dimethylacrylamide copolymer [17]) which provide EOF suppression together with good DNA separations. However, many of these polymers are lab-synthesized [13, 16, 17] and, to our knowledge, not commercially available [13-17] what reduces in a large extent their usefulness.

Recently, according to a new European regulation (49/2000/CEE), any foodstuff containing more than 1% of genetically modified maize or soya must be labeled

as transgenic. To carry out such detection, the usual procedure is to amplify a DNA fragment specific of the genetically modified organism by Polymerase Chain Reaction (PCR) and next to detect it after agarose gel electrophoresis and ethidium bromide staining. The main problem is that both procedures, i.e. PCR and conventional electrophoresis, are essentially semiquantitative. Several procedures have been proposed to overcome the PCR semiquantitative character, e.g., competitive PCR, real time-PCR [18-20]. However, the subsequent use of conventional electrophoresis in competitive quantitative PCR introduces a new source of error. Real time-PCR has proven to be a very powerful tool for quantitative analysis of nucleic acids, however, it has limitations for multiplex analysis of several DNA targets due to limitations in the number of different specific probes than can be detected in a single PCR. Therefore, the use of CGE can be a good alternative in order to improve the quantitative capabilities of competitive quantitative PCR. This includes the possibility of multiplex analysis of several DNA targets, thanks to the better resolution obtained with CGE related to conventional electrophoresis [21], avoiding the need to use and design specific and expensive fluorescent amplification probes.

The goals of this work are, first, to develop a CGE method able to provide reproducible DNA separations using commercially available polymers together with bare fused silica capillaries. Secondly, to apply it for the detection of transgenic maize in foods by analyzing a specific DNA target previously amplified by PCR.

2. EXPERIMENTAL

2.1. Chemicals

All chemicals were of analytical reagent grade and used as received. Tris and EDTA (ethylenediamine tetraacetic acid) from Sigma (St. Louis, MO), 2-hydroxyethyl cellulose (HEC) ($M_{w_{av}}$ 90000), polyvinyl alcohol (PVA) ($M_{w_{av}}$ 50000), orthophosphoric acid, boric acid and sodium tetraborate hydrate from Aldrich (Milwaukee, WI) were used for the CE running buffers at the different concentrations and pHs indicated. LIF Enhance (Beckman Instruments, Fullerton, CA, USA, concentration not supplied) was added as intercalating dye to the CE running buffers (250 nL of Enhance per mL of buffer) when CGE-LIF detection was used. The buffers were stored at 4°C and warmed at room temperature before being used. N-Cetyl-N,N,N-trimethyl-ammonium bromide (CTAB) and sodium hydroxide were from Merck (Darmstadt, Germany). Distilled water was deionized by using a Milli-Q system (Millipore, Bedford, MA).

The test sample Φ X174RF DNA *Hae*III was from Beckman, the 50-bp ladder was from Roche Diagnostics (Barcelona, Spain) and the 100-bp ladder from Biotools (Madrid, Spain). These samples were diluted till a total concentration of ca. 400 μ g/ml in PCR reaction buffer (see below) containing Orange G as CGE marker.

Certified reference maize powder MZ0 (conventional, i.e., containing 0% transgenic maize) and MZ2 (containing 2% insect-resistant Bt-176 transgenic maize) produced by the Institute of Reference Materials and Measurements were

purchased from Fluka Chemie GmbH (Buchs, Switzerland). Oligonucleotides were synthesized at Centro de Investigaciones Biológicas (Spanish Council for Scientific Research, Madrid, Spain). AmpliTaq DNA polymerase, including reaction buffer and $MgCl_2$ was from Perkin Elmer (Madrid, Spain). Deoxynucleotides were from Amersham Pharmacia Biotech Europe GmbH (Barcelona, Spain).

2.2. DNA extraction

DNA was extracted using a modified CTAB method, 100 mg of MZ0 or MZ2 transgenic maize powder standard were incubated with 300 μ l of (2% CTAB, 1.4 M NaCl, 20mM EDTA, 100 mM Tris·HCl pH 8.0, 0.2% β -mercaptoethanol) for 30 minutes at 60°C, then extracted with 300 μ l of cloroform:isoamyl alcohol (24:1). The nucleic acids on the aqueous phase were recovered by precipitation with 1 volume of isopropanol, washed with 70% ethanol and dissolved in 50 μ l of deionized water. MZ0 DNA was used directly (hereinafter: conventional maize DNA), while MZ2 DNA was diluted 1:1 in distilled water in order to obtain 1% transgenic containing sample (hereinafter: transgenic maize DNA).

2.3. PCR conditions

A test fragment of the modified *cryIA(b)* gene (GenBank accession number I41419) was amplified using primers *cryIA(b)-V3* and *cryIA(b)-V4* (Table 1). Amplification of a fragment of the maize starch synthase gene, used as a control for DNA quality and amplificability, was performed with primers MSS-S and MSS-A (Table 1). Reaction mixtures contained 1x AmpliTaq reaction buffer, 2

mM MgCl₂, 0.25 mM dATP, 0.25 mM dCTP, 0.25 mM dGTP, 0.25 mM dTTP, 2.5 μM each primer, 10 μl template DNA and 2.5 U of AmpliTaq DNA polymerase. The following thermal parameters were used for each amplification, *cryIA(b)*: first denaturation: 12 min at 95°C, 40 cycles (1 min at 95°C, 30 sec at 58°C, 30 sec at 72°C), terminal elongation 10 min at 72°C; starch synthase: first denaturation: 12 min at 95°C, 40 cycles (1 min at 95°C, 30 sec at 58°C, 30 sec at 72°C), terminal elongation 10 min at 72°C. AmpliTaq DNA polymerase was added after the first denaturation step (manual Hot-start).

2.4. CE conditions

The analyses were carried out in a P/ACE 5500 CE apparatus, equipped with an UV-Vis detector working at 254 nm and in a PACE-MDQ equipped with an Ar⁺ laser working at 488 nm (excitation wavelength) and 520 nm (emission wavelength), both instruments from Beckman Instruments (Fullerton, CA, USA). Bare fused-silica capillaries with 75 μm i.d. were purchased from Composite Metal Services (Worcester, England). For the initial studies on running buffer optimization coated capillaries with 100 μm i.d. (from Beckman) were used. Injections were made at the cathodic end using N₂ pressure of 0.5 or 1 p.s.i. for a given time (1 p.s.i.=6894.76 Pa). The P/ACE 5500 CE instrument was controlled by a PC running the System GOLD software and the PACE-MDQ was controlled by a PC running the 32 Karat Software both from Beckman.

Before first use, any uncoated capillary was preconditioned by rinsing with 0.1 M HCl for 30 min. Between injections, capillaries were rinsed using 0.1 M HCl for 4 min, 1% PVA for 2 min and separation buffer for 4 min. Phosphate buffer instead

of tetraborate buffer was used together with these capillaries, in order to prevent any possible interaction with PVA coating [10]. At the end of the day, the capillary was rinsed with deionized water for 5 min and stored overnight with water inside. During the reproducibility study (*vide infra*) the first injection of each day was used as stabilization time for the system.

3. RESULTS AND DISCUSSION

3.1. CGE separation conditions and reproducibility study.

Adequate CGE conditions that can provide fast and highly resolved separations of DNA fragments using commercially available polymers were first investigated. Coated capillaries were initially used in order to make easier this preliminary study. Also, an interval of interest of DNA sizes up to 500 bp was defined since it is the range most frequently used to detect GMOs in foods via their amplification by PCR techniques. DNA fragments with sizes shorter than 500 bp are usually chosen because longer DNA fragments have more chances to degrade under any heat-treatment in processed foods.

Several parameters affecting the resolution and speed of analysis of these DNA fragments were tested. Namely, separation buffer (89 mM Tris, 89 mM boric acid at pH 8.4; 89 mM Tris, 200 mM boric acid at pH 7.9; 89 mM Tris, 300 mM boric acid at pH 7.6; 89 mM Tris, 400 mM boric acid at pH 7.3), polymer concentration (5%, 4% and 3% of HEC), temperature (20, 30 and 40°C) and EDTA concentration (1, 2 and 3 mM) were studied.

Under these conditions, almost no difference could be observed on the separation of a standard mixture of DNA fragments at the different pHs studied, except for a slight increase of analysis times obtained at the lower pH. However, it has been repeatedly mentioned that the more basic the buffer pH the faster the capillary coating can degrade [22]. Therefore, a separation pH equal to 7.3 was selected as more adequate.

HEC concentration was varied from 3 to 5% and its effect on the separation of the DNA fragments analyzed. As expected the higher the polymer concentration the higher the analysis time. Moreover, it could be observed a slight deformation (i.e. fronting) of the peak shapes of DNA fragments at 3% of HEC that disappeared at 4% and 5% HEC (data not shown). Since, for similar resolution values, longer separation times were obtained with buffers containing 5% HEC compared to 4% HEC, a percentage of HEC equal to 4% was selected as optimum.

The effect of EDTA concentration on DNA separation was tested using separation buffers at pH 7.3 containing 1, 2 and 3 mM of EDTA. No substantial differences were observed and, therefore, an intermediate value of 2 mM was selected.

To test the effect of temperature on separation a dissolution containing a standard mixture of DNA fragments plus the PCR reaction mixture described above was injected. It could be seen that the speed of analysis at 40°C was higher than the speed at 30°C and 20°C. However, the resolution obtained between the shorter DNA fragments and PCR products was better at 20°C. Therefore, a separation temperature of 20°C was selected.

Under these optimum conditions (i.e., 89 mM Tris, 400 mM boric acid, 2 mM EDTA and 4% HEC at pH 7.3 and 20 °C) good separations of the DNA fragments could be obtained in a coated capillary (see below). In spite of these good results, it could be observed that after ca. 80 injections done during the optimization procedure the separation resolution was lost. It has been frequently mentioned in the literature the degradation of capillary coating with usage [8]. This degradation can be of the whole capillary length or just of a small fragment that was not adequately coated [6]. However, also a degraded fragment of a coated capillary can bring about very low separation performance [23]. Thus, a fragment of the coated capillary was cut out and the resulting shorter capillary tested under identical conditions (keeping the electric field constant). The separation of the DNA fragments could be recovered after cutting a segment of 10 cm, what seems to corroborate that only a portion of the capillary was responsible of the degradation of separation performance. However, under these conditions the 800 bp and 900 bp DNA fragments comigrate. Degradation of the DNA sample was discarded as a possible reason since similar results were obtained for several fresh DNA samples. Unfortunately, we have not been able to find an adequate explanation for this effect. Interestingly, the separation up to 500 bp (in which we are mostly interested) is achieved with high resolution in analysis times shorter than 20 min. This result encouraged us to go on with the second part of the method optimization, i.e., the use of bare fused silica capillaries instead of coated tubing.

Due to the mentioned degradation problems (together with the high cost of coated tubing), we intended to develop a new, cheaper and more robust CGE method using bare fused silica capillaries. The new method should be able to provide good and reproducible DNA separations overcoming some of the disadvantages mentioned above linked to the use of uncoated columns (e.g., the use of non-commercial polymers, too long washing routines between runs, low reproducibility, etc). To do this, different approaches were tested and the best results were obtained using a treatment of the silica wall of the capillary with a strong acid, followed by a treatment with PVA and the use of the above running buffer at nearly neutral pH containing HEC. The rinsing times were also optimized trying to obtain a high reconditioning speed between runs together with adequate and reproducible DNA separations. Our final reconditioning conditions were: 0.1 M HCl for 4 min, 1% PVA for 2 min and separation buffer for 4 min, i.e., requiring only 10 minutes of total reconditioning time. Under these conditions, good separations of the fragments up to 500 bp are typically achieved in ca. 20 minutes as shown in Figure 1. As with coated capillaries comigration of two DNA fragments is also observed, namely, 700 bp and 800 bp fragments in this case.

An important parameter to be tested for this type of CGE procedures involving uncoated capillaries is the reproducibility between separations. Although in general authors determine the reproducibility between consecutive runs (see e.g. references [12, 13, 16]), it is difficult to find reproducibility studies carried out for different days and different bare capillaries. In Table 2, the %RSD values obtained using our procedure for the same day, four different days and three

different capillaries calculated for the DNA fragments of 80, 300 and 1000 bp (corresponding to the first, an intermediate and last migrating peak of the DNA test mixture used) are given. As can be seen, high reproducibility was obtained for all cases, with %RSD values up to 0.86 within the same day, 1.61 for four different days (i.e., the worst case) and 1.40 for three different capillaries. Moreover, the efficiency achieved was up to $1.8 \cdot 10^6$ plates/m calculated for the 80 bp fragment, although it decreased for the longer DNA fragments as can be deduced from Figure 1. It is interesting to mention that the resolution of separation between injections was also reproducible, in good agreement with the results mentioned of Table 2. These values demonstrate that the method proposed is reproducible and efficient, and, therefore, can be used with confidence for analyzing GMOs in foods. To demonstrate that, detection of transgenic maize addition in conventional maize powder was carried out via the amplification of a DNA fragment corresponding to the *cryIA(b)* gene by PCR and subsequent analysis by this CGE method.

3.2. Detection of genetically modified Bt maize.

Figure 2 shows the electrophoregrams obtained for the direct injection of the PCR amplification reactions of the *cryIA(b)* gene fragment from the transgenic (Figure 2B) and conventional (Figure 2C) maize DNA. Figure 2A corresponds to the starch synthase gene fragment amplified from transgenic maize DNA, used to check that the DNA preparation is suitable for PCR amplification and detection. Similar results were obtained for the starch synthase gene amplified from conventional maize DNA (data not shown). Thus, MSS-DNA peak in Figure 2A

corresponds to the amplicon obtained with the primer pair *MSS-S/MSS-A* of Table 1, and Bt-DNA peak in Figure 2B corresponds to the amplicon obtained with the primer pair *cryIA(b)-V3/cryIA(b)-V4* both from transgenic maize DNA. A control amplification reaction without template DNA is also shown in Figure 2D.

In the absence of template DNA, no peak could be observed for the PCR control reaction (Figure 2D) in the region where the amplicon used for transgenic DNA detection should come out (about 16 min), indicating that no interferences have to be expected from the PCR reaction mixture. Amplification using the primers of Table 1 corresponding to the maize starch synthase gene (Figure 2A) gave similar results for both types of maize, thus confirming the suitability of both DNA preparations for PCR amplification and detection. By using the *cryIA(b)* primer pair a single peak could be observed for the transgenic maize (Figure 2B) that could not be detected for the conventional one (Figure 2C). This method is, therefore, able to specifically detect 1% of transgenic maize in conventional maize fulfilling the requirements imposed by the European regulation (49/2000/CEE). Moreover, this is done via the direct injection of the PCR products in the CGE system without further purification steps.

Using this CGE method, the agreement between the experimental and theoretical size of the *cryIA(b)* amplicon obtained under our PCR conditions can be also checked. To do this, the data of migration times (t_m) corresponding to DNA fragments of 80, 100, 200, 300, 400 and 500 bp under the separation conditions of Figure 1 were employed. After least square fitting of the plot $\log(\text{bp})$ versus $1/t_m$, the equation: $\log(\text{bp}) = 4.33 - 31.87/t_m$ was obtained ($r=0.998$, $n=6$). This

equation was used to determine the number of base pairs of the *cryIA(b)* amplicon based on its t_m . The calculated value was 240 bp, which is in good agreement with the theoretical value (i.e. 244 bp).

Although the sensitivity of the PCR-CGE procedure is enough to detect 1% of transgenic maize in food samples (Figure 2B), it would be convenient to have higher sensitivity since the peak obtained is too close to the detection limit. In order to overcome this limitation, and based on the well-known better sensitivity provided by LIF compared to UV detection [24], LIF detection was used. The intercalating dye Enhance was added to the running buffer in order to obtain fluorescence signal from the dsDNA-Enhance complex [25, 26]. A sample containing 1% transgenic maize was then injected in the CGE-LIF instrument and the electrophoregrams shown in Figure 3A were obtained (as in Figure 4, MSS-DNA refers to the maize starch synthase gene and Bt-DNA to the *CryIA(b)* gene amplified by PCR). As can be seen, slightly larger analysis times were obtained for the Bt-DNA and MSS-DNA fragments when the CGE-LIF was used (Figure 3A) compared to the migration times with CGE-UV (Figure 2). This effect has been already observed and attributed to the effect of the intercalating dye onto the electrophoretic mobility of DNA fragments [27]. In good agreement, this increase in migration times was also observed for the DNA ladder injected under the same conditions as demonstrated in Figure 3B where, moreover, all the DNA fragments are adequately separated. As expected, by comparing Figure 2B and Figure 3A, an important improvement in sensitivity (ca. 1000 fold) was obtained by using CGE-LIF compared with CGE-UV, what corroborates the usefulness of this procedure to detect minute quantities of transgenic maize in foods.

4. CONCLUDING REMARKS

A new CGE method for DNA separation that uses uncoated capillaries together with commercially available polymers was developed. The separation method showed a good reproducibility for consecutive runs, as well as for different days and capillaries. The usefulness of this method was demonstrated by detecting GMOs in foods by using CGE-UV and CGE-LIF. In a future work, the use of competitive PCR to accurately estimate the percentage of transgenic maize in commercial samples will be addressed by PCR-CGE-LIF.

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Table 1. Sequence of the primers used in PCR reactions.

PRIMER	SEQUENCE	ACCESSION NUMBER	POSITION
<i>CryIA(b)-V3</i>	5'-CCTGACCAAGAGCACCAACCTGG-3'	I41419	1425-1447
<i>CryIA(b)-V4</i>	5'-GCTCATGGTGGCGCTGAAGTTGC-3'	I41419	1668-1646
<i>MSS-S</i>	5'-TCAACATCCGTGGATTGCATC-3'	AF023159	933-954
<i>MSS-A</i>	5'-TTCAGGGAAATCATCAGTTAATTGC-3'	AF023159	1166-1142

Table 2. Reproducibility of migration times of the DNA fragments of 80, 300 and 1000 bp using uncoated capillaries for the same day, four different days and three different capillaries. All the conditions as in Figure 3.

	80 bp		300 bp		1000 bp	
	t _{av} (min)	%RSD	t _{av} (min)	%RSD	t _{av} (min)	%RSD
Same day (n=10) ^a	13.49	0.66	17.60	0.82	22.57	0.20
Four days (n=40) ^a	13.43	0.99	17.51	1.29	22.40	1.61
Three capillaries (n=15)	13.58	0.99	17.68	1.30	22.55	1.40

^a same capillary.

FIGURE LEGENDS

Figure 1. Separation of a 100 bp DNA ladder using an uncoated fused silica capillary with 47 cm of total length, 40 cm of effective length and 75 μ m i.d.. Separation voltage: -14 kV. Running buffer: 20 mM Tris, 10 mM orthophosphoric acid, 2 mM EDTA, 4% HEC at pH 7.3, 20 °C. Detection at 254 nm. Injection for 50 s using N₂ pressure (0.5 psi) of: 1) 80 bp; 2) 100 bp; 3) 200 bp; 4) 300 bp; 5) 400 bp; 6) 500 bp; 7) 600 bp; 8) 700 bp and 800 bp; 9) 900 bp; 10) 1000 bp.

Figure 2. Electrophoregrams obtained for the PCR amplification reactions using: A) transgenic maize DNA and the primer pair *MSS-S/MSS-A* (see Table 1); B) transgenic maize DNA and the primer pair *cryIA(b)-V3/cryIA(b)-V4*; C) conventional maize DNA and the primer pair *cryIA(b)-V3/cryIA(b)-V4*; and D) control amplification reaction without template DNA (i.e., blank) and the primer pair *cryIA(b)-V3/cryIA(b)-V4*. Samples injected for 75 s using N₂ pressure (0.5 psi). Other conditions as in Figure 1.

Figure 3. CGE-LIF electrophoregrams obtained for A) the PCR amplification reactions using transgenic maize DNA and the primer pairs *cryIA(b)-V3/cryIA(b)-V4* and *MSS-A/MSS-S*; and B) a 100 bp DNA ladder. Separation conditions: uncoated fused silica capillary with 50 cm of total length, 40 cm of effective length and 75 μ m i.d.. Separation voltage: -15 kV. LIF Enhance used as intercalating. The transgenic samples were injected for 38 s using N₂ pressure (1 psi). The 100 bp ladder was injected for 25 s using N₂ pressure (1 psi). Peak identification: 1) 80 bp; 2) 100 bp; 3) 200 bp; 4) 300 bp; 5) 400 bp; 6) 500 bp; 7)

600 bp; 8) 700 bp; 9) 800 bp; 10) 900 bp; 11) 1000 bp. LIF detection
($\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$).