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# Temperature gradient capillary electrophoresis (TGCE)–a tool for the high-throughput discovery and mapping of SNPs and IDPs

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**Abstract** Temperature gradient capillary electrophoresis (TGCE) can be used to distinguish heteroduplex from homoduplex DNA molecules and can thus be applied to the detection of various types of DNA polymorphisms. Unlike most single nucleotide polymorphism (SNP) detection technologies, TGCE can be used even in the absence of prior knowledge of the sequences of the underlying polymorphisms. TGCE is both sensitive and reliable in detecting SNPs, small InDel (insertion/deletion) polymorphisms (IDPs) and simple sequence repeats, and using this technique it is possible to detect a single SNP in amplicons of over 800 bp and 1-bp IDPs in amplicons of approximately 500 bp. Genotyping data obtained via TGCE are consistent with data obtained via gel-based detection technologies. For genetic mapping experiments, TGCE has a number of advantages over alternative heteroduplex-detection technologies such as celery endonuclease (CELI) and denaturing high-performance liquid chromatography (dHPLC). Multiplexing can increase TGCE's throughput to 12 markers on 94 recombinant inbreds per day. Given its ability to efficiently and reliably detect a variety of subtle DNA polymorphisms that occur at high frequency in genes, TGCE shows great promise for discovering polymorphisms and conducting genetic mapping and genotyping experiments.

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## Introduction

Gene-based genetic maps are critical tools for functional genomics. To genetically map a gene it is necessary to identify the polymorphism(s) among alleles of that gene and then genotype (i.e. detect) that polymorphism(s) among the members of a mapping population. A variety of techniques can be used to detect the many types of DNA polymorphisms (Kristensen et al. 2001), and the choice of detection technique depends largely on whether the sequences associated with the polymorphism are known in advance. Single nucleotide polymorphisms (SNPs) or small InDel (insertion/deletion) polymorphisms (IDPs) that have been previously identified via sequence analyses can be mapped using such techniques as pyrosequencing, single nucleotide primer extension (SNuPE), 3′-mismatch PCR-mediated detection and others (See et al. 1998; Cato et al. 2001; Lemieux 2001; Martins-Lopes et al. 2001; Prince and Brookes 2001; Qi et al. 2001; Bhattramakki et al. 2002; Brown et al. 2002;

Ching and Rafalski 2002; Nasu et al. 2002; Paris et al. 2002). Each of these methods, however, depends on prior sequence knowledge of the DNA sequence polymorphism being detected.

In many situations, however, the actual sequences of the polymorphisms to be mapped are not known and the techniques mentioned above are not applicable, although it is still possible to identify and map such polymorphisms. For example, primer pairs designed on the basis of gene sequences can be used to survey the parents of a mapping population in order to identify those primer pairs that yield PCR products that exhibit size polymorphisms and that thus can be mapped (Cato et al. 2001; T-J. Wen et al., in preparation). This approach is not, however, suitable for the detection of SNPs and very small IDPs that cannot be detected using agarose gel electrophoresis.

Alternatively, SNPs or small IDPs that exist between the parents of a mapping population can be discovered via heteroduplex analysis. Genic amplicons from the two parents are mixed, denatured and allowed to re-anneal. If the two parents carry the same allele of the amplified gene, only homoduplex molecules will form during renaturation. Alternatively, if the two parents carry different alleles, both homoduplex and heteroduplex molecules will form. Heteroduplex molecules can be detected by means of a number of diverse techniques. For example, a celery endonuclease (CELI) that cleaves heteroduplexes has been used for the detection of SNPs and the detection of other DNA polymorphisms (McCallum et al. 2000; Colbert et al. 2001; Comai et al. 2004).

Both denaturing high-performance liquid chromatography (dHPLC; Kota et al. 2001) and temperature gradient capillary electrophoresis (TGCE) detect SNPs via the formation of heteroduplexes (Li et al. 2002; Murphy et al. 2003; the Reveal system, SpectruMedix, http://www.spectrumedix.com/). Heteroduplex detection is based on the fact that at certain temperatures homo- and heteroduplex DNA molecules exhibit different electrophoretic mobilities. These differences occur because heteroduplex DNA molecules have lower melting temperatures than homoduplex DNA molecules due to the presence of mismatches in the former that results in the formation of secondary structures at lower temperatures. Consequently, heteroduplex DNA molecules have longer retention times during electrophoresis.

We report here that TGCE can be used to reliably and sensitively identify SNPs, IDPs and simple sequence repeats (SSRs). TGCE can detect such polymorphisms even in the absence of prior knowledge regarding the specific sequences of these polymorphisms. We additionally demonstrate that multiplexing can be applied to increase the throughput of TGCE-based genetic mapping experiments. Given its ability to efficiently and reliably detect a variety of subtle DNA polymorphisms that occur at high frequency in genes, TGCE has great promise for discovering polymorphisms and conducting genetic mapping and genotyping experiments.

## Materials and methods

### Temperature gradient capillary electrophoresis

TGCE was conducted using the Reveal System, model RVL 9612, rev. 2.0 (SpectruMedix, State College, Pa.). REVELATION analysis software (version 2.4) was used to visualize and score TGCE data.

### PCR primers, sample preparation and TGCE conditions

The sequences of PCR primers are available in the electronic supplementary material (ESM). Final PCR reaction concentrations were 15–25 ng of genomic DNA, 0.2 m*M* dNTP, 0.5 μ*M* each primer, 1× PCR buffer (20 m*M* Tris-HCl, pH 8.4, 50 m*M* KCl), 2 m*M* $MgCl_2$, 0.45 U AmpliTaq Gold (Applied Biosystems, Foster City, Calif.), 0.05 U cloned Pfu DNA polymerase (Stratagene, La Jolla, Calif.). The PCR program consisted of 95°C for 10 min; 94°C for 3 min; 30 cycles of 94°C for 30 s, 60°C for 45 s, 72°C for 1 min 30 s; a final extension at 72°C for 10 min. The resulting PCR products were diluted eight times with 1× PCR buffer (with an estimated final DNA concentration between 0.5 ng/μl and 1.5 ng/μl) before being mixed with a corresponding DNA sample needed for the detection of polymorphisms. Mixed samples were then denatured and re-annealed to enable heteroduplex formation using a Tetrad thermocycler (MJ Research, Waltham, Mass.) and the following program: 95°C for 2 min 40 s; 15 cycles of 95–80°C for 20 s (decrease by 1°C per cycle); 25 cycles of 80–55°C for 1 min (decrease by 1°C per cycle); 55°C for 18 min; 10 cycles of 55–45°C for 1 min (decrease by 1°C per cycle); 10 cycles of 45–35°C for 30 s (decrease by 1°C per cycle); 12°C (no time limitation—until samples are unloaded). The detection of heteroduplex DNA was performed on a Reveal 96 capillary electrophoresis unit (SpectruMedix), and all of the reagents used in the TGCE experiments were purchased from SpectruMedix. Detailed TGCE conditions are listed at http://maize-mapping.plantgenomics.iastate.edu/protocols.html.

### Plant genomic DNA

The maize inbred lines B73 and Mo17 used in this study were originally obtained from Donald Robertson (Iowa State University, Ames, Iowa). Mike Lee (Iowa State University) generously provided seed of the full set of IBM recombinant inbred lines (Lee et al. 2002). Maize and Arabidopsis genomic DNAs were isolated using a 1× CTAB procedure (Saghai-Maroof et al. 1984).

### Ethyl methanesulfonate (EMS)-induced alleles

To generate point mutations in *a1*, EMS mutagenesis was conducted as described by Neuffer (1994) with minor modifications. Pollen with the genotype *A1-LC* from Line C, a color-converted W22 line, was mutagenized and used to pollinate plants homozygous for the mutant allele, *a1::rdt* (Brown et al. 1989). *A1* confers reddish pigmentation to the coleoptile, and seedlings derived from gametes that carry EMS-induced point mutations that disrupt *A1* function can be identified because, unlike most progeny from this cross, these mutant seedlings (*a1*-EMS /*a1::rdt*) will have green coleoptiles. M1 seeds were germinated in a sand bench, and seedlings with green coleoptiles were identified. DNA was extracted (Dellaporta 1994) from these candidate mutants, and the *a1* coding region PCR amplified using primers that span the coding region. Gel-purified (QiaQuick Gel Purification kit; Qiagen, Valencia, Calif.) PCR products were sequenced to identify EMS-induced lesions. Plants carrying confirmed *a1* mutants were self-pollinated to purify the mutant alleles. A total of ten mutants were isolated from this mutagenesis experiment. Three mutants (GenBank nos. AY535012, DQ012661 and DQ012665) were derived from *A1-LC* (GenBank no. X05068) and seven (GenBank nos. DQ012660, DQ012662-DQ012666, DQ017580 and DQ017581) were derived from *A1-U46055* (GenBank no. U46055). One of the alleles derived from *A1-LC*, *a1-3150-1(LC)* (GenBank no. AY535012), was selected for testing the sensitivity of TGCE. The *a1-3150-1(LC)* allele contains a G-to-A transition in exon 3 at site 2,496 relative to the sequence of *A1-LC*. This mutation changes a tryptophan codon to a stop codon.

## Results

### Detection of SNPs by means of TGCE

The use of temperature gradient capillaries during electrophoresis, TGCE, enables the efficient detection of SNPs without prior knowledge of the actual sequence of the SNP (Li et al. 2002; Murphy et al. 2003). To test for the presence of a SNP, two alleles are PCR-amplified and the resulting amplicons mixed in a 1:1 ratio. Following denaturation and re-annealing, the resulting duplex DNA is subjected to TGCE. If the two amplicons have the same sequence, only homoduplex molecules will form during re-annealing. Such molecules migrate as a single major electrophoretic peak during TGCE. In contrast, if the two alleles exhibit a SNP relative to each other, re-annealing will generate both homoduplex and heteroduplex DNA molecules. Because heteroduplex

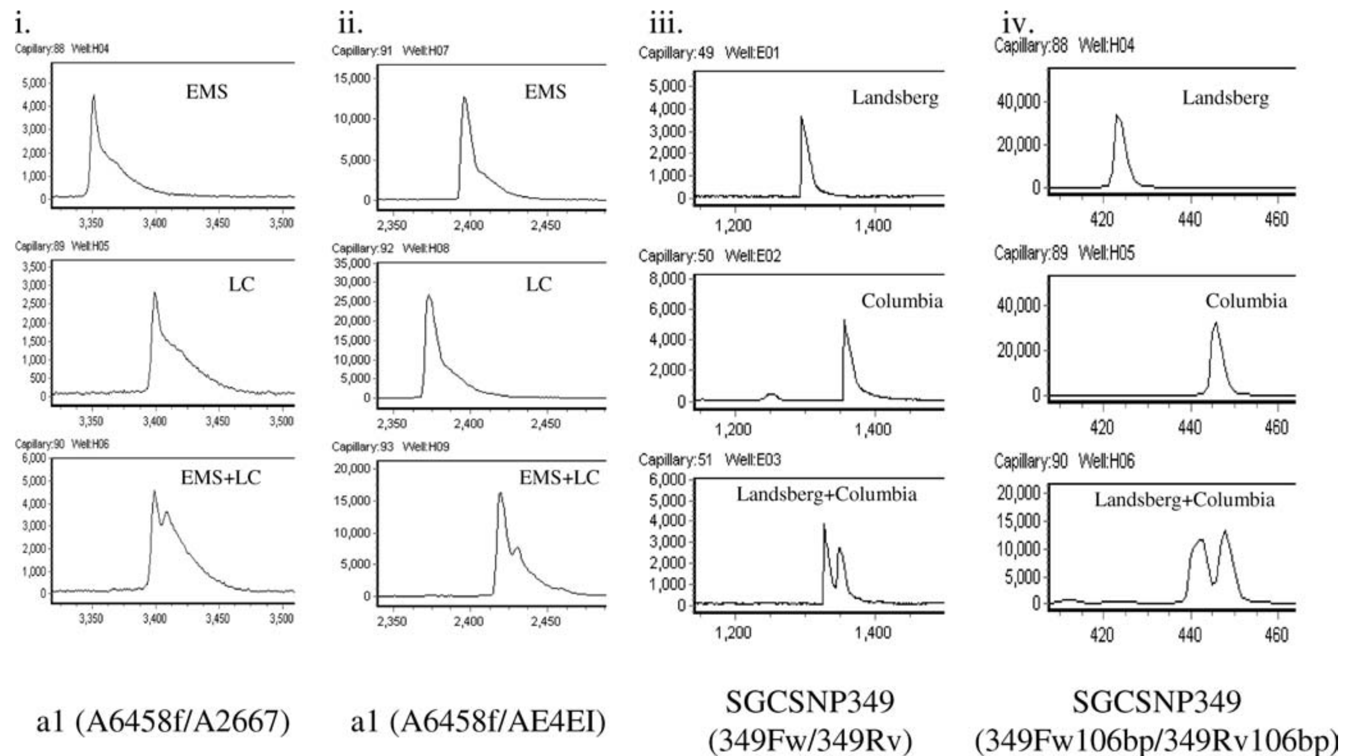


**Fig. 1** Detection of SNPs and IDPs using TGCE. The *top* and *middle panels* in each column contain the electropherograms obtained using unmixed amplicons from the indicated genotypes; the *bottom panel* contains the electropherograms of mixed amplicons. The regions detected and primer pairs used are indicated under each column. *EMS* and *LC* represent the EMS-induced allele of *a1-3150-1(LC)* and the wild-type progenitor *A1-LC*, respectively

**Table 1** Tested 1-bp IDPs in Arabidopsis

| Designation/IDP[a] | PCR primers[b] | Size (bp) of amplicon from Columbia | Position of IDP[c] | TGCE detection[d] |
|---|---|---|---|---|
| SGCSNP349/A | 349Fw/349Rv106 bp | 299 | 251 | Y |
| | 349Fw/349Rv209 bp | 348 | 251 | Y |
| | 349Fw/349Rv299 bp | 384 | 251 | Y |
| | 349Fw/349Rv∼400 bp | 437 | 251 | Y |
| | 349Fw/349Rv | 499[e] | 251 | Y |
| | 349Fw299 bp/349Rv106 bp | 215 | 167 | Y |
| | 349Fw299 bp/349Rv209 bp | 264 | 167 | Y |
| | 349Fw299 bp/349Rv299 bp | 300 | 167 | Y |
| | 349Fw299 bp/349Rv∼400 bp | 353 | 167 | Y |
| | 349Fw209 bp/349Rv106 bp | 163 | 115 | Y |
| | 349Fw209 bp/349Rv209 bp | 212 | 115 | Y |
| | 349Fw209 bp/349Rv299 bp | 248 | 115 | Y |
| | 349Fw209 bp/349Rv∼400 bp | 301 | 115 | Y |
| | 349Fw209 bp/349Rv | 363 | 115 | Y |
| | 349Fw106 bp/349Rv106 bp | 107 | 59 | Y |
| | 349Fw106 bp/349Rv209 bp | 156 | 59 | Y |
| | 349Fw106 bp/349Rv299 bp | 192 | 59 | Y |
| | 349Fw106 bp/349Rv∼400 bp | 245 | 59 | Y |
| | 349Fw106 bp/349Rv | 307 | 59 | Y |
| SGCSNP357/C | 357Fw/357Rv139 bp | 290 | 204 | Y |
| | 357Fw139 bp/357Rv139 bp | 140 | 54 | Y |
| | 357Fw139 bp/363Rv | 347[e] | 234[f] | Y |
| SGCSNP363/T | 363Fw141 bp/363Rv141 bp | 142 | 108 | Y |

[a]Designation of polymorphism in the TAIR database/nucleotide that is present in the Columbia ecotype and absent in the Landsberg ecotype
[b]Primer sequences are available in the ESM
[c]The position of the 1-bp IDP relative to the left end of the amplicon
[d]Y, Polymorphism detected via TGCE
[e]These amplicons were sequenced to determine whether they contain any polymorphisms other than the indicated SNPs
[f]This amplicon contains an additional A (Landsberg)/C (Columbia) SNP at position 279

molecules have lower melting temperatures than the corresponding homoduplex DNA molecules, heteroduplex molecules exhibit slower electrophoretic mobilities than their homoduplex counterparts. As such, re-natured samples from two amplicons that exhibit a SNP relative to each will yield multiple electrophoretic peaks during TGCE.

An EMS-induced *a1* allele that contains a SNP relative to its wild-type progenitor was used to test the applicability of TGCE for SNP detection and to determine the sizes of amplicons in which TGCE can resolve single SNPs. The EMS-induced allele [*a1-3150-1(LC)*] differs from its wild-type progenitor allele (*A1-LC*) by a single SNP at position 2,496 (in exon 3) relative to the sequence of *A1-LC* (GenBank no. X05068). Two DNA fragments of 651 bp and 841 bp, each of which contains this single SNP, were PCR amplified from stocks homozygous for *a1-3150-1(LC)* and *A1-LC* using primer pairs A6458f/A2667 and A6458f/AE4EI (see ESM for primer sequences), respectively. When

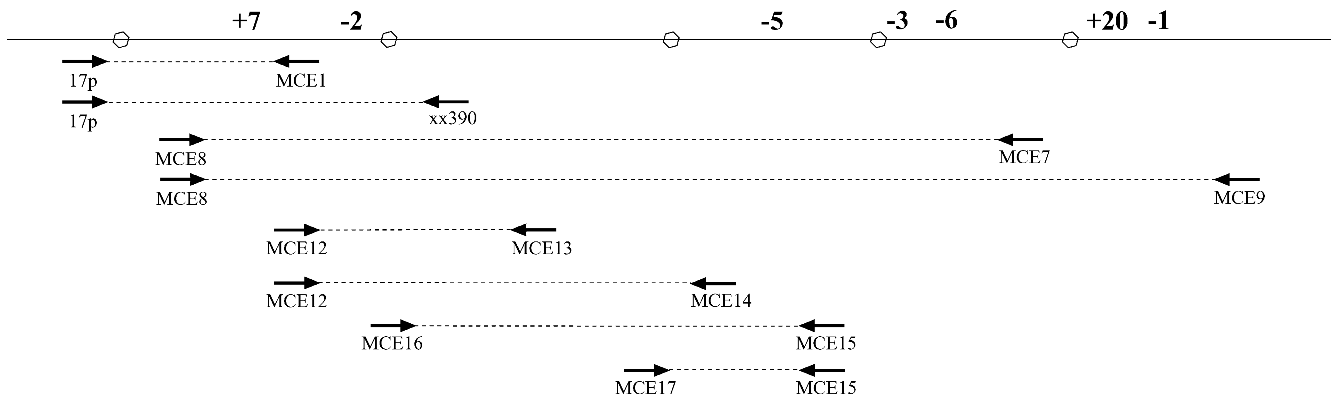


**Fig. 2** Primer positions relative to IDPs and SNPs in the *a1* gene (not to scale). Primers are depicted as *arrows* underneath the *a1* gene, amplicons are depicted as *dotted lines* between primer pairs. *Numbers* above the *a1* gene represent insertions (+*numbers*) and deletions (−*numbers*) in the B73 allele relative to the Mo17 allele. The *hexagons* designate the positions of SNPs

**Table 2** Tested IDPs and SNPs in the maize *a1* gene

| PCR primers[a] | Number of IDPs[b] | Number of SNPs[c] | Amplicon sizes (bp)[d] | TGCE detection[e] |
|---|---|---|---|---|
| 17p/MCE1 | 1 | 1 | 448/441 | Y |
| 17p/xx390 | 2 | 2 | 516/511 | Y |
| MCE8/MCE7 | 5 | 3 | 487/496 | Y |
| MCE8/MCE9 | 7 | 4 | 563/553 | Y |
| MCE12/MCE13 | 1 | 1 | 139/141 | Y |
| MCE12/MCE14 | 1 | 2 | 290/292 | Y |
| MCE16/MCE15 | 1 | 2 | 296/301 | Y |
| MCE17/MCE15 | 1 | 1 | 142/147 | Y |

[a]Primer sequences are available in the ESM
[b]Number of IDPs between the B73 and Mo17 amplicons
[c]Number of SNPs between the B73 and Mo17 amplicons
[d]Sizes of the B73/Mo17 amplicons
[e]Y, Polymorphism detected via TGCE

mixed together, denatured, re-annealed and subjected to TGCE analysis, each of these amplicons yielded TGCE peaks that differed from the single peaks detected in the unmixed controls (Fig. 1i, ii), demonstrating that TGCE can detect the SNP present in each pair of amplicons. Because the largest of these *a1* amplicons is over 840 bp, these results substantially extend the size of amplicons in which a single SNP can be detected by means of TGCE relative to earlier reports (493 bp by Li et al. 2002).

## Detection of SSRs by means of TGCE

TGCE has not previously been used to detect SSRs. Four existing SSR markers (umc1604, umc1268, umc1350 and umc1608) were selected for this experiment. These SSRs are between 100 bp and 150 bp in length and have been genetically mapped via an agarose gel-based method (Sharopova et al. 2002). The SSRs were analyzed by means of TGCE using 93 recombinant inbreds (RIs) from the IBM population (see Materials and methods) and the resulting scores compared to gel-based scores available in a public database (http://www.maizegdb.org). Among all four SSR markers tested, nine data points could not be evaluated due to missing gel-based or TGCE data (data not shown). More than 99% (361/363) of the remaining data points were consistent between gel-based and TGCE scores. The two data points that were inconsistent were generated from the same RI using primer pairs for umc1350 and umc1608. This result demonstrates that SSR markers can be reliably detected via TGCE.

## Detection of IDPs by means of TGCE

TGCE has not previously been used to detect IDPs. To test the suitability of TGCE for detecting small IDPs, three 1-bp IDPs between the Columbia and Landsberg Arabidopsis ecotypes were identified from The Arabidopsis Information Resource (TAIR) database (http://arabidopsis.org/servlets/Search?action = new_search & type = polyallele). A series of PCR primers was designed to generate amplicons of various sizes that would contain these IDPs (Table 1). Sequence analysis of the longest amplicon designed to include each IDP confirmed the presence of each 1-bp IDP and that these Columbia and Landsberg amplicons do not contain any other polymorphisms (data not shown). Analysis of these amplicons by means of TGCE established that it was possible to detect all three 1-bp IDPs in amplicons that ranged in size from 107 bp to as large as 499 bp (for examples, see Fig. 1iii, iv).

To test whether SNPs and IDPs interact in ways that might confound analysis via TGCE, we identified in silico a series of IDPs and SNPs between the B73 and Mo17 alleles of the *a1* locus that we had sequenced (GenBank nos. U46052 and U46062). By using eight PCR primer pair combinations (Fig. 2), we were able to generate amplicons of various lengths (Table 2) from these two alleles that contained one or more of the seven IDPs and five SNPs. Ten RI lines from the IBM population were used to test the reliability and reproducibility of TGCE detection of these polymorphisms. The PCR products amplified from these RIs were mixed with the corresponding PCR products amplified from B73 or Mo17 genomic DNA templates and subjected to TGCE. All eight pairs of polymorphisms were detected via TGCE (see Fig. 3 for an example), and the genotypes of RIs obtained via TGCE were consistent with *a1* genotyping data obtained previously for these RIs using an independent genotyping technology similar to that described by Cato et al. (2001) and T-J. Wen et al. (in preparation). Hence, we conclude that it is possible to use TGCE to reliably distinguish amplicons as small as 140 bp and that differ by a single 2-bp IDP and one SNP (i.e. the amplicon obtained using primers MCE12 and MCE13) or over 500 bp and that contain seven IDPs and four SNPs (i.e. the amplicon obtained using primers MCE8 and MCE9).

## Genetic mapping of IDPs using TGCE

To test whether TGCE can be used to map genes without access to the sequences of polymorphisms, we developed primers for two genes, *Mha1* (GenBank no. U09989) and *P* (GenBank no. Z11879), that we had mapped using the IBM RIs and a technology similar to that described by Cato et al. (2001) and T-J. Wen et al. (in preparation). For each gene, two pairs of primers were identified to amplify regions of the B73 and Mo17 alleles that contain TGCE-detectable polymorphisms. Both primer pairs for each gene were used in conjunction with TGCE to genotype the *Mha1* and *P* alleles carried by 93 IBM RIs (data not shown). All of the resulting data points were internally consistent (i.e. the results with both primer pairs involving a single gene gave identical results). All but one (371/372) of the data

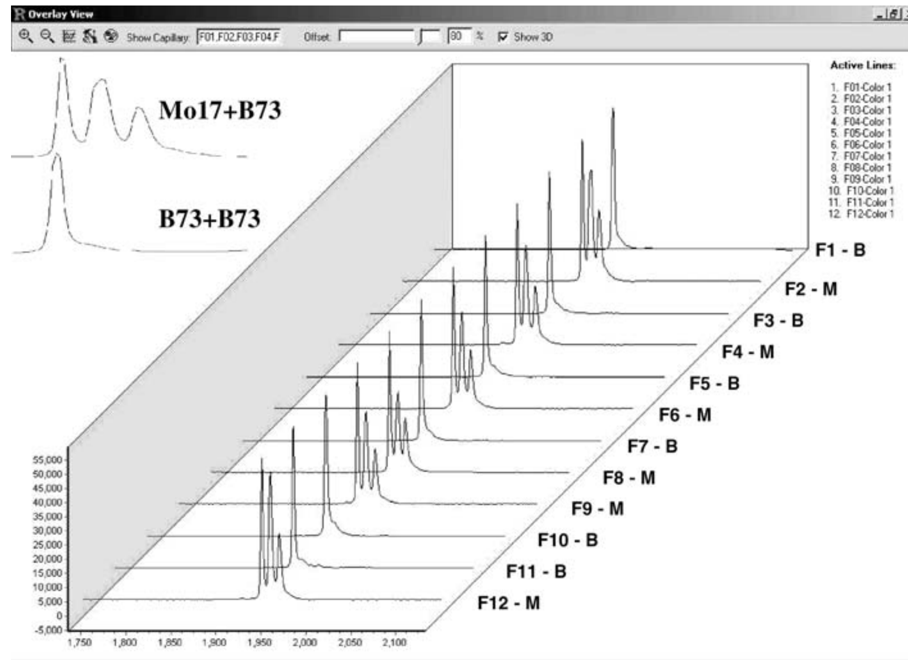


**Fig. 3** Genotyping the *a1* gene by means of TGCE. PCR products obtained through the amplification of the *A1-B73* and *A1-Mo17* alleles with the primer pair MCE12 and MCE14 differ by a 2-bp IDP and two SNPs. Microtiter dish-well positions *F1* and *F2* contained amplification products from the inbred lines B73 (coded here as *B*) and Mo17 (*M*), respectively, mixed with amplification products obtained from B73 (*B*). Note evidence of heteroduplex molecules (*multiple peaks*) in well position *F2* (*A1-Mo17* + *A1-B73* amplicons), but not in well *F1* (only *A1-B73* amplicon). Well positions *F3–F12* contained amplicons from RIs M0066, M0067, M0074, M0075, M0076, M0077, M0079, M0262, M0264 and M0265 mixed with the amplicons from the inbred line B73. Electropherograms that exhibit evidence of heteroduplexes (*multiple peaks*) are derived from RIs that carry the *A1-Mo17* allele; those with *single peaks* carry the *A1-B73* allele. The genotype scoring (B vs. M) of each RI (as labeled to the *right* of each well's electropherogram) is automatically generated by the REVELATION software. The sequences of the *A1-B73* and *A1-Mo17* alleles are available as GenBank nos. U46052 and U46062. The sequences of the PCR primers are available in the ESM

points were consistent with prior genotyping scores. The single exception is due to residual heterozygosity at the *Mha1* locus in one RI. Consequently, we conclude that TGCE is both sensitive and reliable enough for mapping purposes.

## Multiplexed TGCE

To further increase the throughput of TGCE, we developed a multiplexing strategy. We reasoned that two duplex DNA species associated with two different markers could be distinguished and thus separately scored in a single electrophoresis capillary if their peak profiles are "spaced out" far enough in the electropherogram. The camera in the TGCE unit captures DNA duplexes at different frames determined by their mobility during electrophoresis. Pairs of IDP markers with peak profiles appearing at different frame times during the initial survey screening were combined and assayed in single capillary runs. IDP pairs with zero and 100 frame differences could be scored manually but not by the REVELATION software (data not shown). IDP pairs with more than 200 frame differences could be scored both manually and by means of REVELATION (data not shown). Assigning two markers in one capillary run increases the throughput of TGCE by twofold.

## TGCE data analysis software

With multiplexing, the TGCE system can generate mapping data for 12 markers (2,304 data points) per day. To maintain the high throughput of the pipeline an efficient and accurate method of data analysis is essential. Although Spectrumedix provides an analysis program (REVELATION), this program is not optimized for the analysis of mapping data. A new software package called the GENETIC RECOMBINANT ANALYSIS MAPPING ASSISTANT (GRAMA) has been created and implemented (Maher et al., in preparation) to facilitate and validate the analysis of genetic mapping data. GRAMA presents mapping data in intuitive layouts and stores TGCE data in a database without manual processing. In addition, GRAMA applies a different algorithm to assign mapping scores from captured images generated by REVELATION and cross-checks the REVELATION output to validate mapping scores. The combined use of REVELATION and GRAMA not only improves the efficiency of mapping experiments but also the quality of the resulting mapping data.

## Discussion

There are relatively few technologies that permit the detection of SNPs and small IDPs without prior knowledge of the sequences of the polymorphisms. We demonstrate here that TGCE can reliably detect both SNPs and IDPs in amplicons without prior knowledge of the sequences of the polymorphisms that exist in these amplicons. It has been shown previously that TGCE is sensitive enough to detect SNPs in fragments of between 200 bp and 493 bp (Li et al. 2002). We have extended these studies by demonstrating that TGCE can detect a single SNP in amplicons of 800 bp. We have, in addition, demonstrated for the first time that TGCE can detect IDPs as small as 1 bp in amplicons that are over 400 bp in length. We have also demonstrated that TGCE can detect SSRs. We thus conclude that TGCE can probably be used to detect virtually any type of polymorphism that will allow for the formation of heteroduplexes.

One of the most significant advantages of TGCE is that it is not necessary to determine the sequence of the DNA polymorphism prior to the detection or mapping of that polymorphism. Of course, if so desired, amplicons that contain TGCE-detectable polymorphisms can be further analyzed and converted into markers that can be detected using alternative technologies that require prior knowledge of the sequence of polymorphism—for example, primer extension.

For genetic mapping experiments, TGCE has a number of advantages relative to alternative technologies for detecting heteroduplex molecules. Unlike CELI-based detection, TGCE does not rely on an enzyme that is not commercially available nor does it require that samples be fluorescently labeled or purified after PCR amplification, thereby reducing both labor and reagent costs. Reagent costs are further reduced because, compared to CELI-based detection, TGCE requires less PCR product for detection purposes.

Although dHPLC can be used for reverse genetic experiments (where thousands of samples are analyzed for each primer pair), it is not an efficient approach for genetic mapping experiments that involve thousands of different primer pairs, each of which is used to genotype at most only a few hundred individuals. This is because dHPLC detection requires that the denaturing conditions of each pair of mapping primers be optimized prior to detection. In contrast, TGCE employs a temperature gradient during electrophoresis that eliminates the need to optimize denaturing conditions for each pair of mapping primers.

As part of NSF Plant Genome project DBI-9975868, we generated over 35,000 3′-ESTs (expressed sequence tags) from the inbred line B73 (T-J. Wen et al., in preparation) and a much smaller number from the inbred line Mo17. To estimate the rate of IDPs in 3′-untranslated regions (UTRs) of maize, we compared 353 unique alignments between the B73 and Mo17 ESTs. Consistent with published reports (Bhatrammakki et al. 2002) regarding the frequency of IDPs in B73 and Mo17 3′-ESTs, 24% (85/353) of these alignments exhibited an IDP equal to or greater than 2 bp and 23% contained 1-bp IDPs. Based on these data and the high frequency of maize SNPs (Ching and Rafalski 2002), we estimate that more than 50% of the 3′-UTRs from B73 and Mo17 contain a polymorphism that can be detected via TGCE.

Although this report demonstrates the detection of polymorphisms only in organisms that are expected to be homozygous for the gene under analysis ( RIs and Arabidopsis ecotypes), it is possible to use TGCE to detect polymorphisms in other types of mapping populations (data not shown). For example, to map a gene in an $F_1$BC population, the amplicons from the mapping population can be analyzed directly without mixing with parental amplicons. Those $F_1$BC individuals that yield a single peak carry the allele from the backcross parent; those that yield multiple peaks carry the other allele.

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