Temporal Temperature Gradient Gel Electrophoresis on the DCode™ System: A Comparison to DGGE and CDGE in Mutation Screening

Anne-Lise Børresen-Dale, Sigrid Lystad, and Anita Langerød, Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, N-0310 Oslo, Norway

Introduction

Temporal temperature gradient electrophoresis (TTGE) exploits the principles upon which denaturing gradient gel electrophoresis (DGGE) and constant denaturing gel electrophoresis (CDGE) are based. TTGE combines some of the advantages of these techniques and eliminates some of the problems. The focusing of bands obtained by DGGE seems to be retained, and in TTGE there is no need for a chemical denaturing gradient, which is the advantage in CDGE. The problem of determining the exact running time and denaturing conditions is eliminated, and since the denaturing conditions in TTGE span a wider range, several fragments with different melting behavior can be analyzed on the same gel. A new electrophoretic instrument, the DCode universal mutation detection system from Bio-Rad Laboratories, Inc., has the ability to perform TTGE.

Improvements to DGGE by CDGE

DGGE, which is based on the discontinuous phenomenon of strand dissociation, allows the resolution of DNA fragments differing by as little as a single nucleotide substitution (Fischer and Lerman 1983). In mutation analysis using parallel DGGE, field strength, temperature, and run time must be strictly controlled to achieve reproducible results. DGGE is complicated by the difficulties of choosing the exact running time and gel conditions to achieve the optimal separation. By running a DGGE gel too long, an achieved separation decreases, and may even be lost. Mutations present in a homo- or hemizygote state, where heteroduplexes are not formed, will be missed. Several of these problems are eliminated by CDGE, in which a single denaturing condition is used to melt a fragment (Børresen 1996, Børresen et al. 1991, Hovig et al. 1991). In CDGE, wild-type and mutant fragments will melt partially into a certain configuration immediately after entering the gel. This configuration will be kept throughout the run, and the fragments will migrate with a constant rate. Therefore, the longer the gel is run, the wider the separation between the mutant and wild-type samples.

Improvements to DGGE and CDGE by TTGE

CDGE detects nearly all mutations within one melting domain, but has the disadvantage of screening only one domain at a time. Fragments with only one low and one high melting domain will often have a short transition state between a double-stranded and a partially melted state. For example, the theoretical melting curve of a GC-clamped exon 2 sequence from the *BRCA1* gene is very flat, containing only one melting domain (Figure 1A). The steep S-shaped curve in the perpendicular DGGE makes it difficult to estimate the optimal denaturing conditions for CDGE (Figure 1B). If the CDGE denaturation conditions are too low or too high, the separation is lost. This is illustrated in Figures 1C and 2C. TTGE is much more robust and solves these problems. In TTGE, a single concentration of urea and formamide is used as in CDGE, but the temperature during the run is gradually increased. The denaturant concentration used in TTGE can be determined either from the theoretical melting curve or experimentally from a perpendicular DGGE. A denaturing concentration of approximately 10 units lower than the one corresponding to the steepest part of the curve is used (Figure 1B and 2B). The temperature is ramped on the DCode system during the run from 63–68°C, giving a temperature ramp rate of 1.7°C/hr. By using these conditions, the problem with determining the exact conditions is eliminated. Another example of the advantage of TTGE over CDGE and DGGE is shown for the *KRAS* exon 1 gene in Figure 2A–D.

Since the denaturing conditions in TTGE span a wider range than CDGE, several fragments with different melting behavior that are analyzed on separate gels by CDGE can be analyzed on the same TTGE gel. This is illustrated in Figure 3, where exons 5–9 and 11 of the *TP53* gene are analyzed. Using two different denaturing conditions and the same temperature ramp allowed all of the exons to be analyzed at one time.

These examples demonstrate that the TTGE technique is more robust and flexible than either DGGE or CDGE in mutation screening and represents a new development with advantages for mutation screening in a diagnostic setting.

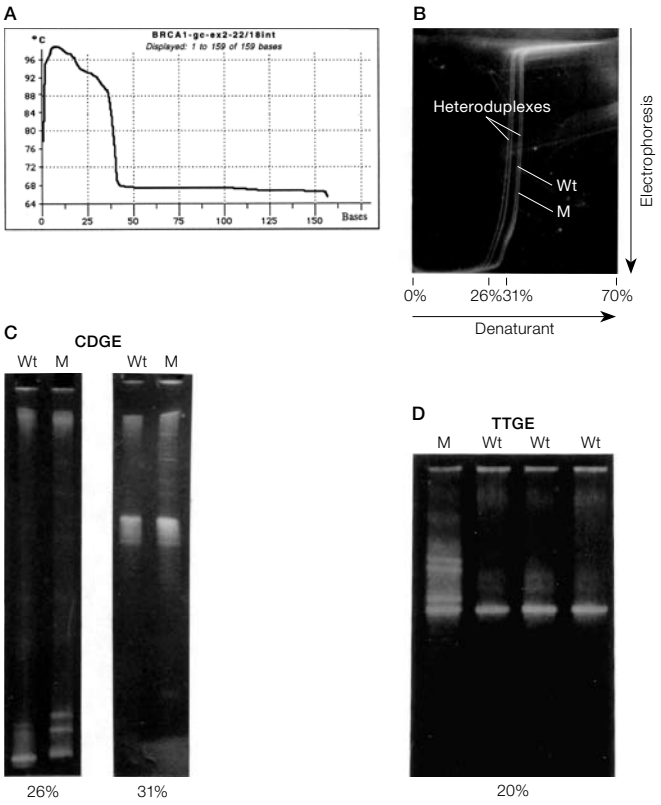

Fig. 1. Analyses of *BRCA1* exon 2. A, theoretical melting curve of a GC-clamped exon 2 of the *BRCA1* gene, containing one melting domain; **B**, perpendicular DGGE shows a very steep S-shaped curve. Wild-type (Wt) and mutant (M) DNA fragments from exon 2 of the *BRCA1* gene were mixed after PCR, followed by denaturation and reannealing. The gel was run for 2 hr at 56°C and 130 V; **C**, mutant and wild-type sample of *BRCA1* exon 2 run on CDGE, using 26% and 31% denaturant in the gels, respectively. Note that the separation on CDGE is lost if denaturation conditions are too low or too high. The gels were run for 2 and 2.5 hr, respectively, at 56°C and 130 V; **D**, mutant and wild-type sample of *BRCA1* exon 2 run on TTGE, using 20% denaturant in the gel. The temperature was ramped during the 3 hr run from 63–68°C at 130 V.

Conclusions

With TTGE, the high reproducibility in casting a constant denaturant gel and the rapid and high throughput of the analyses obtained by CDGE are achieved. The problems in determining the exact denaturing conditions for fragments with only one melting domain are eliminated. The focusing of bands obtained by DGGE seems to be achieved, and since the denaturing conditions in TTGE span a wider range, several fragments with different melting behavior can be analyzed on the same gel.

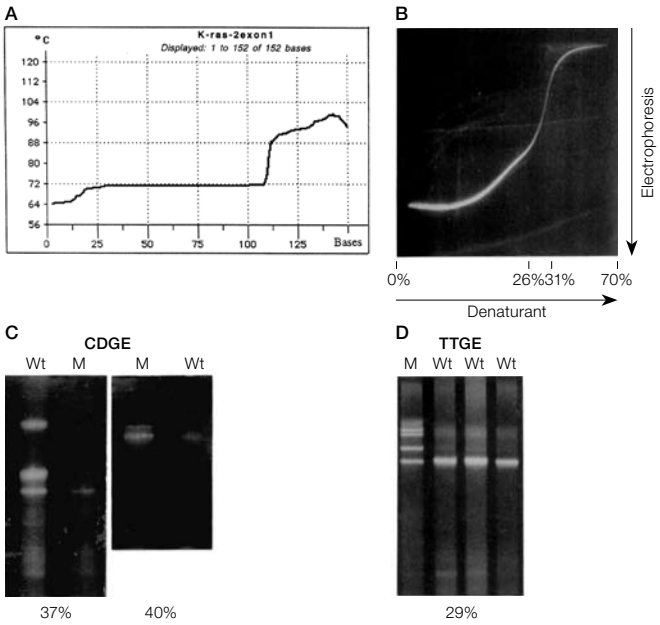

Fig. 2. Analyses of *KRAS2* exon 1. A, theoretical melting curve of GC-clamped exon 1 of the *KRAS2* gene, containing one melting domain; **B**, perpendicular DGGE shows a very steep S-shaped curve of wild-type (Wt) fragment of *KRAS2* exon 1; **C**, mutant (M) and wild-type samples of *KRAS2* exon 1 run on CDGE, using 37% and 40% denaturant in the gels, respectively. Note that the separation on CDGE is lost if denaturation conditions are too low or too high. The gels were run for 2.5 hr at 56°C and 130 V; **D**, mutant and wild-type sample of *KRAS2* exon 1 run on TTGE, using 29% denaturant in the gel. The temperature was ramped during the 3 hr run from 63–68°C at 130 V.

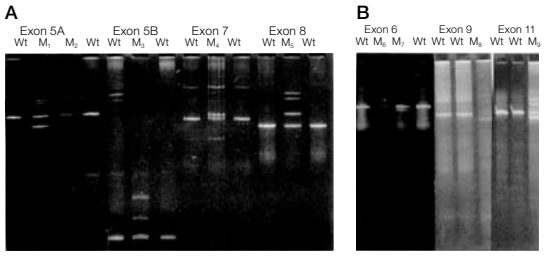

Fig. 3. TTGE analyses of exons 5–9 and 11 of the *TP53* gene using two separate gels in one run. The temperature was ramped during the 3 hr run from 63–68°C at 130 V. Denaturant in the gels was 37% (**A**) and 31% (**B**), respectively.

References

Borresen AL, Constant denaturant gel electrophoresis (CDGE) in mutation screening, pp 267–279 in (Pfeifer GP ed), Technologies for Detection of DNA Damage and Mutation, Plenum Press, New York (1996)

Borresen AL et al., Constant denaturant gel electrophoresis as a rapid screening technique for *p53* mutations, Proc Natl Acad Sci USA 88, 8405–8409 (1991)

Fischer SG and Lerman LS, DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory, Proc Nat Acad Sci USA 80, 1579–1583 (1983)

Hovig E et al., Constant denaturant gel electrophoresis, a modification of denaturing gradient gel electrophoresis, in mutation detection, Muta Res 262, 63–71 (1991)

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