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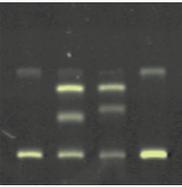
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DCode™ Universal Mutation Detection System



Unravel the Code



Use Any Proven Electrophoretic Method for Universal Mutation Detection

The search for unknown mutations in genomic DNA is important for studying the genetic basis of diseases and disorders, including cancer. Additionally, examining DNA polymorphisms is useful for ecological and evolutionary studies of terrestrial, marine, and microbial organisms, with applications ranging from species identification to delineation of population structure to monitoring genetic diversity.

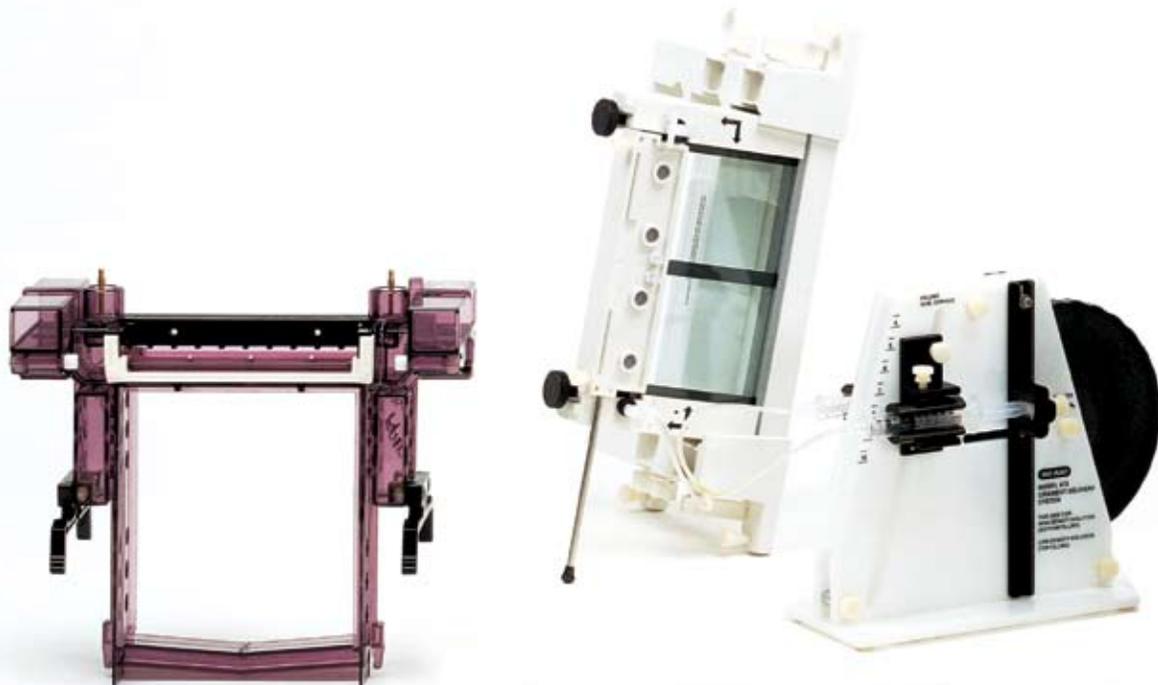
The DCode universal mutation detection system can scan for single-base changes by any of the following electrophoresis techniques:

- Single-stranded conformation polymorphism (SSCP) analysis
- Denaturing gradient gel electrophoresis (DGGE)
- Constant denaturing gel electrophoresis (CDGE)
- Temporal temperature gradient gel electrophoresis (TTGE)

Flexible and powerful, the DCode system is the one electrophoresis system that can perform any combination of these techniques. At the center of the system is the temperature control module, which includes a microprocessor-controlled heater, a buffer-recirculating pump, and a stirrer. For techniques requiring accurate temperature control, such as SSCP and TTGE, the gels are immersed in the buffer, and temperatures are regulated between 5° and 70°C. Any run temperature below ambient can be achieved with the cooling tank used in conjunction with an external laboratory chiller. The DCode system can run 64 samples in as little as 2 hours — a major consideration when you're screening DNA for sequence variations.

You can configure your DCode system to grow as your needs grow. Each system includes a vertical electrophoresis cell and choice of adaptor kits for SSCP, DGGE, and CDGE, as well as for TTGE, a technique codeveloped by Bio-Rad. TTGE has all the benefits of DGGE and CDGE, without chemical denaturant gradients.

Sandwich core, cam-operated manual gradient former, gel caster

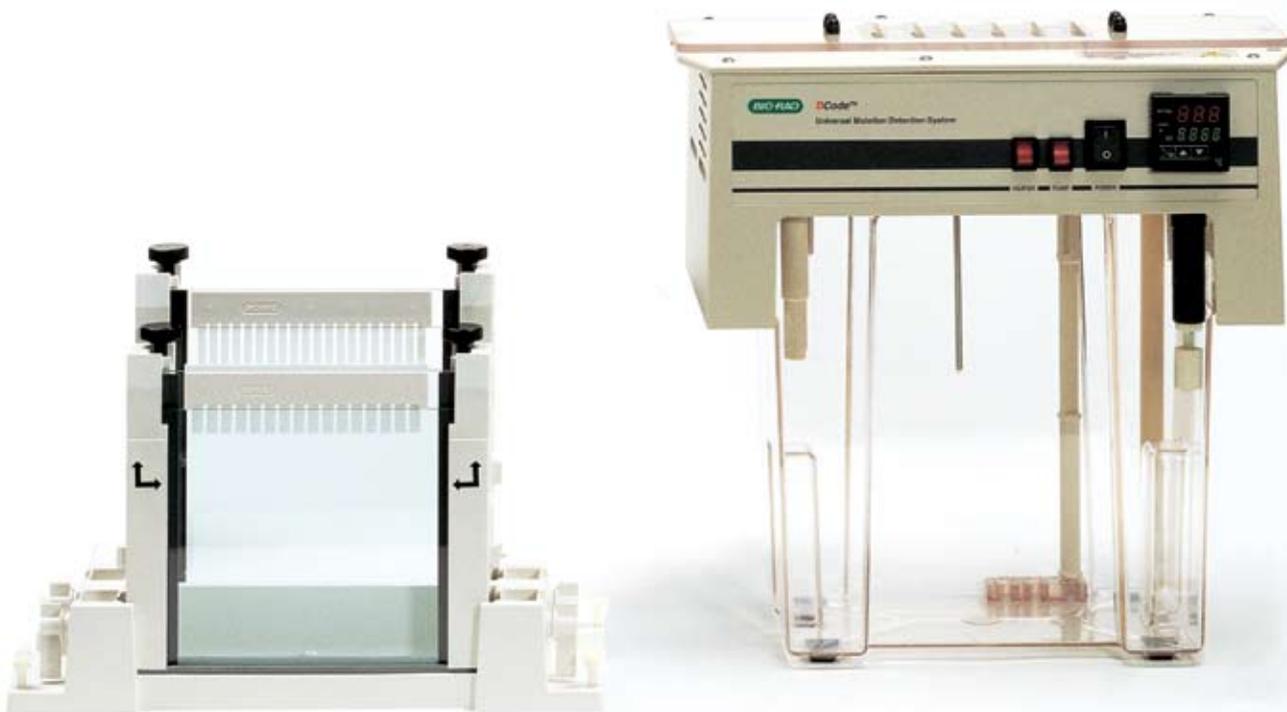


Combine Detection Methods for Ultimate Sensitivity

To achieve the highest throughput and efficiency in mutation screening, you may want to use multiple electrophoresis techniques. Being able to rapidly switch between methods, or to convert to a new method, is critical in a fast-paced research environment. The DCode system meets the demands of all major mutation detection techniques. The DCode system's versatility offers a number of powerful research tools at an affordable price. Key features and benefits include:

- Accurate temperature control options for electrophoresis runs between 5° and 70°C
- Modular design for customizing current and future laboratory demands
- Specialized accessory kits that simplify startup
- Technique-specific reagents and controls that are optimized for DGGE, CDGE, SSCP, or TTGE
- Application notes with proven run conditions
- Ability to perform powerful TTGE

with gel sandwiches, electrophoresis temperature control module.



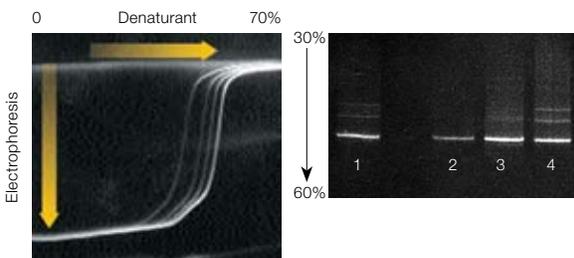
Use the Most Powerful Techniques for Maximum Efficiency

DGGE Quickly Hunts for the Unknowns

DGGE is based on the principle that increasing denaturant concentrations will melt double-stranded DNA in distinct domains. When the domain with the lowest melting temperature reaches the corresponding denaturant concentration in the gel, the DNA will partially melt, creating branched molecules with reduced mobility (Fischer and Lerman 1983). The denaturing environment is created by a uniform run temperature (between 50° and 65°C) and a linear denaturant gradient formed with urea and formamide. The gradient may be formed perpendicular or parallel to the direction of electrophoresis. DGGE is one of the most sensitive mutation detection methods, providing efficiency up to 99% (Grompe 1993).

The following features of the DCode system simplify DGGE:

- Patented* cam-operated Model 475 gradient former simplifies gradient gel casting
- MacMelt™ and WinMelt™ software streamline GC-clamp and primer placement
- Temperature control module provides consistent run temperatures between 45° and 70°C
- Up to two 16 x 16 cm gels or four 7.5 x 10 cm gels can be run simultaneously



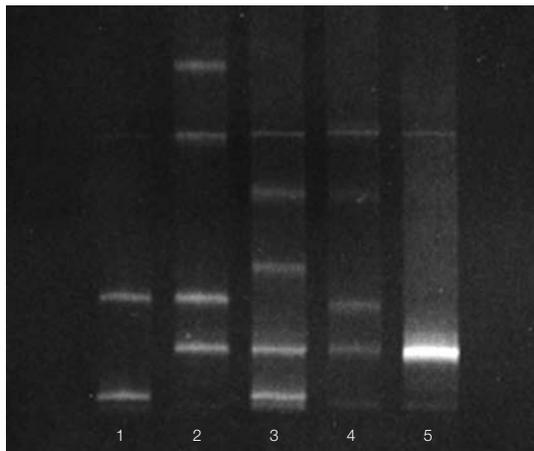
Perpendicular and parallel DGGE separation run on the DCode system. Left, alleles amplified from exon 6 of the *p53* gene were separated by DGGE, in which the denaturing gradient (0–70%) was perpendicular to the direction of electrophoresis. These data can be used to determine the gradient range that produces maximal separation of alleles. (Data courtesy of AL Børresen, Radium Hospital, Oslo, Norway.) Right, alleles amplified from *K-ras* exon 1 were separated by DGGE in which the denaturing gradient (30–60%) was parallel to the direction of electrophoresis. Different band patterns indicated different alleles. A large number of samples can be screened in this way. Lane 1, positive control; lane 2, negative control; lane 3, patient sample 1; lane 4, patient sample 2 (see bulletin 2293).

CDGE Rapidly Screens for Mutations

After a mutation has been identified, CDGE rapidly screens multiple samples for the presence of the mutation (Børresen et al. 1991). The optimal concentration of denaturant to use for a CDGE gel is determined from a perpendicular gradient gel. Once the optimal concentration for a particular mutation is determined, multiple samples can be screened on a constant denaturant gel. Thus, with CDGE, no chemical gradient is required and rapid high-throughput screening is made simple.

The following features of the DCode system simplify CDGE:

- The DCode system simplifies optimization of denaturant concentration for CDGE
- Optimized electrophoresis reagents and controls ensure quality results for high-throughput screening
- Convenient 16 x 16 cm and 16 x 10 cm formats and simple tape-free gel casting facilitate rapid screening



CDGE separation run on the DCode system. Amplified alleles from the β -globin gene were separated by CDGE on an 8% acrylamide gel in 45% denaturant at 56°C. Lane 1, a compound mutant sample IVS1-1 + IVS1-6; lane 2, mutant sample IVS1-1; lane 3, mutant sample IVS1-6; lane 4, mutant sample IVS1-110; lane 5, wild-type DNA. For more information, see bulletin 2107.

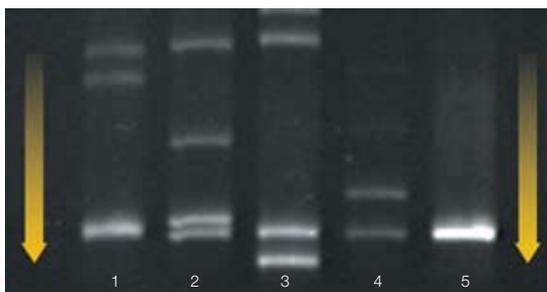
* U.S. patent 5,540,498.

TTGE Has the Power of DGGE Without Gradient Gels

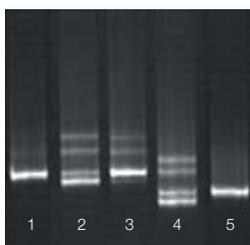
TTGE uses the principles of DGGE, but without chemical denaturing gradient gels, making TTGE simpler, faster, and more easily reproduced (Yoshino et al. 1991). During the electrophoresis run, the temperature is increased linearly. The denaturing environment is formed by the temporal temperature gradient in the presence of a constant concentration of urea in the gel.

The following features of the DCode system help reduce TTGE to a simple, reproducible practice:

- Temperature controller and heater allow reproducible temperature ramps as low as 0.1°C/hr
- Elimination of gradient gels simplifies setup and gel casting
- MacMelt and WinMelt software help determine optimal temperature ranges
- Control reagents and application notes with proven protocols help you get started



TTGE separation run on the DCode system. Amplified alleles of exon 7 from the cystic fibrosis gene were separated by TTGE on a 6 M urea/6% acrylamide/bis gel (37.5:1) in 1.25x TAE buffer, using a temperature range of 50–60°C and a ramp rate of 2°C/hr. Lane 1, mutant allele (1154 insTC); lane 2, mutant allele (G330X); lane 3, mutant allele (DF311); lane 4, mutant allele (R334W); lane 5, wild-type allele. (Samples courtesy of L Silverman, Division of Molecular Pathology, University of North Carolina School of Medicine.)



Detection of homoplasmic and heteroplasmic mutations.

PCR product of 643 bp containing the tRNA^{Leu}(UUR) and part of the ND1 region was analyzed by TTGE.

Lane 1, normal individual with no sequence variation in this region; lane 2, an individual with 62% heteroplasmic A3243G mutation; lane 3, an individual with 11% heteroplasmic A3243G mutation;

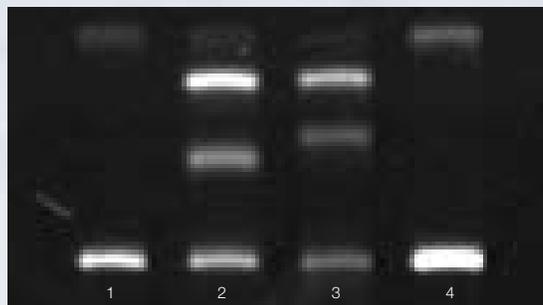
lane 4, an individual with homoplasmic T3197C polymorphism and 47% of A3243G mutant mtDNA; lane 5, homoplasmic T3197C only. For more information, see bulletin 2450.

Perform SSCP at Your Bench

Consistent Results With SSCP and the DCode System

SSCP is a widely used mutation screening method because of its simplicity. Experimental conditions cannot be predicted for a particular sample, so it is important to optimize gel electrophoresis conditions including run temperature to ensure the highest sensitivity (Orita et al. 1989, Hayashi 1991) The temperature-controlled buffer bath makes the DCode system ideal for SSCP.

- Electrophoresis cooling tank with ceramic cooling fingers connects to external standard laboratory recirculating chillers
- Temperature control module with stirrer, heater, and buffer-recirculating pump maintains uniform temperatures between 5° and 25°C
- Reproducible run temperatures give consistent results
- 16 x 16 cm and 16 x 20 cm gel sizes simplify nonisotopic detection with silver or fluorescent stains



SSCP separation run on the DCode system. Amplified alleles of exon 8 of the *p53* gene were separated by SSCP run in 1x TBE on an 8% acrylamide/bis gel (37.5:1) with 3.5% glycerol at 8°C. Lane 1, undenatured mutant allele; lane 2, mutant allele; lane 3, wild-type allele; lane 4, undenatured wild-type allele.

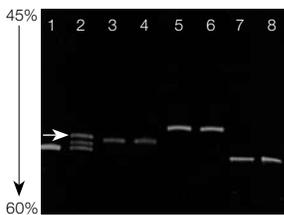
DCode System Applications

Screening for Mutations That Cause Cancer

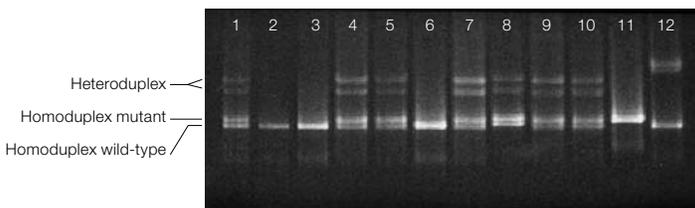
Identification of mutations within specific genes is an increasingly important strategy in determining the diagnosis and prognosis for many diseases, including cancer. Several techniques, including DGGE, CDGE, TTGE, and SSCP, have been developed to examine heterogeneous tissue samples for specific mutations. These techniques are useful for rapid screening, and each technique has its individual strength.

By using any of the screening techniques listed, you can identify known polymorphisms. This reduces the number of bands that must be fully sequenced, thus reducing the cost of analysis and increasing the overall efficiency of genetic screening.

The following examples illustrate the use of the DCode system to screen mutations, such as *p53*, *K-ras*, and mutations in the Fas antigen of lymphoma tumors.



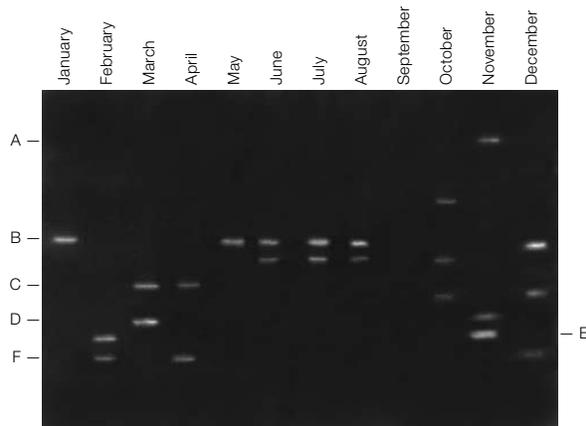
Parallel DGGE analysis of the human *p53* gene. Lane 1, wild-type DNA, exon 5; lane 2, sample (breast cancer) DNA, exon 5; lane 3, wild-type DNA, exon 6; lane 4, sample DNA, exon 6; lane 5, wild-type DNA, exon 7; lane 6, sample DNA, exon 7; lane 7, wild-type DNA, exon 8; lane 8, sample DNA, exon 8. The arrow in lane 2 shows a mutation in exon 5. For more information, see bulletin 2415.



DGGE analysis of the Fas III domain in lymphoma patients. Lane 1, CEM (polymorphic); lane 2, 8226 (wild-type); lanes 3–12, lymphoma patients' specimens. For more information, see bulletin 2295.

Monitoring Microbial Diversity

DGGE, CDGE, or TTGE can be used in a powerful culture-independent approach for assessing bacterial genetic diversity in the natural environment. Many researchers use the ubiquitous prokaryotic 16S ribosomal (r)RNA gene for species identification in these studies (Avaniss-Aghajani et al. 1996). A mixture of amplified variable regions can be separated by DGGE, CDGE, or TTGE, and the resulting diversity patterns analyzed and compared. The major advantages of these techniques compared to cloning and subsequent sequencing are their direct determination of bacterial genetic diversity, lower cost, and relative simplicity. This culture-independent approach has greatly enhanced the ability to assess bacterial genetic diversity in natural ecosystems and in mouth and intestinal microflora.



TTGE gel of *Nitrosospira* 16S rRNA PCR products amplified from monthly freshwater samples. DNA bands representing positions A to F were excised and sequenced for the determination of phylogenetic relatedness. For more information, see bulletin 2427.

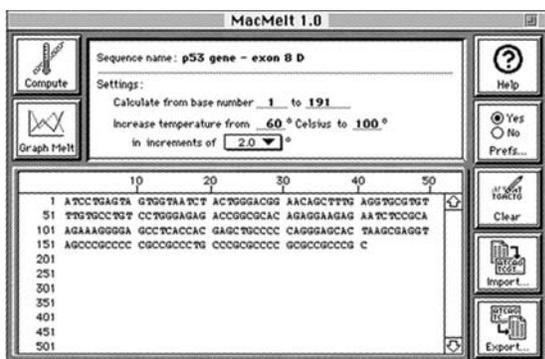
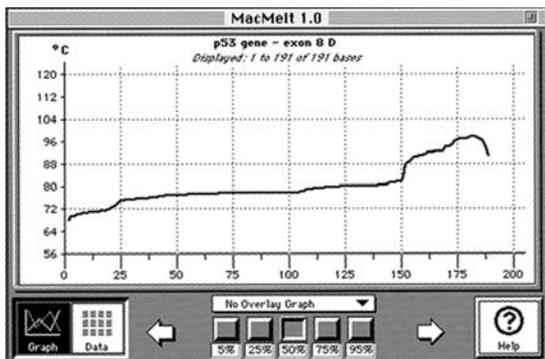
Software You Can Count On

Use MacMelt or WinMelt Software to Take the Guesswork Out of Experimental Design

Mutations are most reliably detected when they occur in the lowest melting-temperature domain, which melts before the DNA is completely denatured. Adding a GC clamp to one end of the DNA ensures that the region screened is in a lower melting-temperature domain and that the DNA will remain partially double-stranded.

You can use MacMelt (Mac-based) or WinMelt (PC-based) software with the DCode system for DGGE, CDGE, and TTGE to predict the melting profile of a DNA sequence of up to 3,200 bases. Optimal primer placement and GC-clamp position are determined by analyzing the melting profile. Using a melt map, the software predicts results that can then be used in actual comparisons.

Using MacMelt and WinMelt software is simple. First, a DNA sequence is imported from a text file, then the melting profile is computed. The data appear on screen and can be graphed according to user preference. Sequences and melt data may also be exported to other software programs.



Reagents, Applications, and Training Guide

Electrophoresis and Control Reagents

Bio-Rad electrophoresis reagent kits are customized for each application to ensure the highest quality buffers and acrylamide. Our control reagents for DGGE, CDGE, TTGE, and SSCP help you quickly master any new techniques.

Applications

Technical notes with proven DCode system run conditions are available on our web site at discover.bio-rad.com. To obtain all DCode application notes, request literature package 1720J. To obtain DCode application notes on microbial diversity studies, request literature package 1720K.

Training Guide

This self-training guide is an interactive CD-ROM that helps you learn about the techniques used to screen mutations using the DCode system. It includes the following:

- Principles of DGGE, CDGE, TTGE, and SSCP
- Videos on setting up and using the DCode system
- WinMelt software tutorial program, DCode application notes, instruction manual, and other literature



To obtain your self-training guide, request catalog #170-9241. The CD-ROM is PC-compatible only.

References

- Avaniss-Aghajani E et al., Molecular technique for rapid identification of mycobacteria, *J Clin Microbiol* 34, 98-102 (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 Natl Acad Sci USA* 80, 1579-1583 (1983)
- Grompe M, The rapid detection of unknown mutations in nucleic acids, *Nat Genet* 5, 111-117 (1993)
- Hayashi K, PCR-SSCP: a simple and sensitive method for detection of mutations in the genomic DNA, *PCR Methods Appl* 1, 34-38 (1991)
- Orita M et al., Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms, *Proc Natl Acad Sci USA* 86, 2766-2770 (1989)
- Yoshino K et al., Temperature sweep gel electrophoresis: a simple method to detect point mutations, *Nucleic Acids Res* 19, 3153 (1991)

Ordering Information

Catalog # Description

DCode Systems*

170-9080	DCode System for DGGE, 120 V, for 16 cm gels with single prep well (1 mm), includes comb gasket, 2 sets of clamps, Model 475 gradient former, all parts required to cast gradient gels
170-9081	DCode System for DGGE, 220/240 V, for 16 cm gels with single prep well (1 mm)
170-9082	DCode System for DGGE, 100 V, for 16 cm gels with single prep well (1 mm)
170-9083	DCode System for DGGE, 120 V, for 10 cm gels with 2 prep wells (1 mm)
170-9084	DCode System for DGGE, 220/240 V, for 10 cm gels with 2 prep wells (1 mm)
170-9085	DCode System for DGGE, 100 V, for 10 cm gels with 2 prep wells (1 mm)
170-9086	DCode System for CDGE, 120 V, for 16 cm gels with 20 wells (1 mm)
170-9087	DCode System for CDGE, 220/240 V, for 16 cm gels with 20 wells (1 mm)
170-9088	DCode System for CDGE, 100 V, for 16 cm gels with 20 wells (1 mm)
170-9089	DCode System for TTGE, 120 V, for 16 cm gels with 20 wells (1 mm)
170-9090	DCode System for TTGE, 220/240 V, for 16 cm gels with 20 wells (1 mm)
170-9091	DCode System for TTGE, 100 V, for 16 cm gels with 20 wells (1 mm)
170-9092	DCode System for SSCP, 120 V, for 20 cm gels with 20 wells (0.75 mm), includes cooling tank adaptor for use with external cooling bath, control reagents for SSCP
170-9093	DCode System for SSCP, 220/240 V, for 20 cm gels with 20 wells (0.75 mm)
170-9094	DCode System for SSCP, 100 V, for 20 cm gels with 20 wells (0.75 mm)
170-9105**	Complete DCode System, 120 V, PC, for all gel sizes and types described above, includes software, standard and cooling tanks, Model 475 gradient former, sandwich clamps, pressure clamp, comb gasket and holder, fittings required for gradient gels
170-9106**	Complete DCode System, 220/240 V, PC
170-9107**	Complete DCode System, 100 V, PC
170-9102**	Complete DCode System, 120 V, Mac
170-9103**	Complete DCode System, 220/240 V, Mac
170-9104**	Complete DCode System, 100 V, Mac

DCode Adaptor Kits***

170-9125	DGGE Kit, for 16 cm gels with single prep well (1 mm), includes sandwich clamps, pressure clamp, comb gasket and holder, fittings required for gradient gel casting
170-9126	DGGE Kit, for 10 cm gels with 2 prep wells (1 mm)
170-9127	CDGE/TTGE Kit, for 16 cm gels with 20 wells (1 mm)
170-9128	Complete SSCP Kit, for 20 cm gels with 20 wells (0.75 mm), includes sandwich clamps, cooling finger adaptor for use with external chiller
170-9129	Basic SSCP Kit, for 20 cm gels with 20 wells (0.75 mm), includes sandwich clamps

* Each system includes electrophoresis/temperature control module, sandwich core, kit to cast gels of indicated size and type (2 sets of plates, 2 sets of clamps and spacers, 2 combs), control reagents for indicated application(s).

** For PC, includes WinMelt software; for Mac, includes MacMelt software.

*** Each kit includes 2 sets of plates, 2 sets of spacers, 2 combs.

Catalog # Description

DCode Accessories

170-9240	WinMelt Software, PC/Windows
170-9034	MacMelt Software, Mac
170-9241	Interactive CD-ROM Training Guide
170-9042	Model 475 Gradient Delivery System, includes cam-operated manual gradient former, 2 each of 10 and 30 ml syringes, all accessories required to cast gradient gels
170-9140	Electrophoresis Cooling Tank, with cooling adaptor for hookup to laboratory recirculating chiller

Electrophoresis Reagents and DNA Control Reagents

170-9150	DCode Control Reagent Kit for DGGE/CDGE/TTGE, includes primers (one GC-clamped) and DNA templates for production of wild-type and mutant DNA
170-9151	DCode Control Reagent Kit for SSCP, includes primers and DNA templates for production of wild-type and mutant DNA
170-9170	DCode Electrophoresis Reagent Kit for DGGE, includes 500 ml 40% acrylamide/bis (37.5:1), 2 x 1 L 50x TAE buffer, 225 ml 100% deionized formamide, 10 ml 10 mg/ml ethidium bromide, 10 ml DCode dye solution, 5 ml TEMED, 1 ml 2x gel loading dye, 10 g ammonium persulfate
170-9171	DCode Electrophoresis Reagent Kit for TTGE, includes 500 ml 40% acrylamide/bis (37.5:1), 1 kg urea, 2 x 1 L 50x TAE buffer, 10 ml 10 mg/ml ethidium bromide, 1 ml 2x gel loading dye, 5 ml TEMED, 10 g ammonium persulfate
170-9172	DCode Electrophoresis Reagent Kit for SSCP, includes 500 ml 40% acrylamide solution, 500 ml 2% bis-acrylamide solution, 100 ml glycerol, 6 x 1 L 10x TBE buffer, 2x SSCP gel loading dye, 5 ml TEMED, 10 g ammonium persulfate

For complete ordering information, request bulletin 2100. For DCode system and accessories specifications, request bulletin 2101. For additional information, contact your local Bio-Rad representative, or visit us on the Web at discover.bio-rad.com

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