

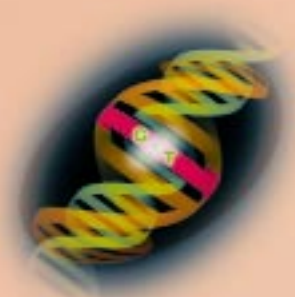
Unravel the Code



WITH THE DCODE™ UNIVERSAL MUTATION DETECTION SYSTEM



BIO-RAD



With DCode, Use Any Proven Method for Universal Mutation Detection

In molecular genetics, the detection of single-base mutations and polymorphisms is of fundamental importance. Many electrophoretic techniques rapidly screen DNA for sequence variations. Most labs use a combination of methods to maximize detection efficiency.

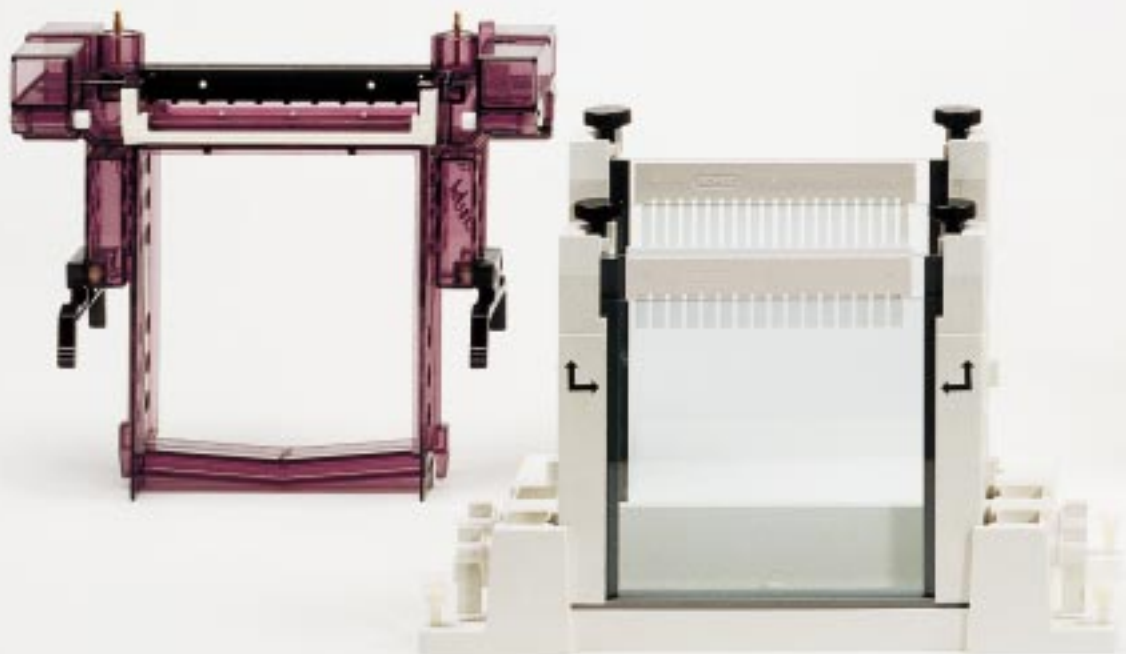
The most popular techniques are Single Strand Conformation Polymorphism (**SSCP**), Denaturing Gradient Gel Electrophoresis (**DGGE**), Constant Denaturing Gel Electrophoresis (**CDGE**), Heteroduplex Analysis (**HA**), and Protein Truncation Tests (**PTT**). All require gel electrophoresis. Optimal run temperatures may vary between 5 and 70 °C and more powerful techniques, like DGGE, need reproducible denaturing gradients and software for primer optimization.

Flexible and powerful, DCode is the one system that can do any combination of 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 temperature control, like SSCP, 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.

To grow with your laboratory, each DCode system includes a vertical electrophoresis cell and a choice of adaptor kits for SSCP, DGGE, CDGE, PTT, HA and a new technique that Bio-Rad helped to develop called Temporal Temperature Gradient Electrophoresis (**TTGE**). TTGE has all the benefits of DGGE and CDGE without the chemical denaturants.

Left to right:

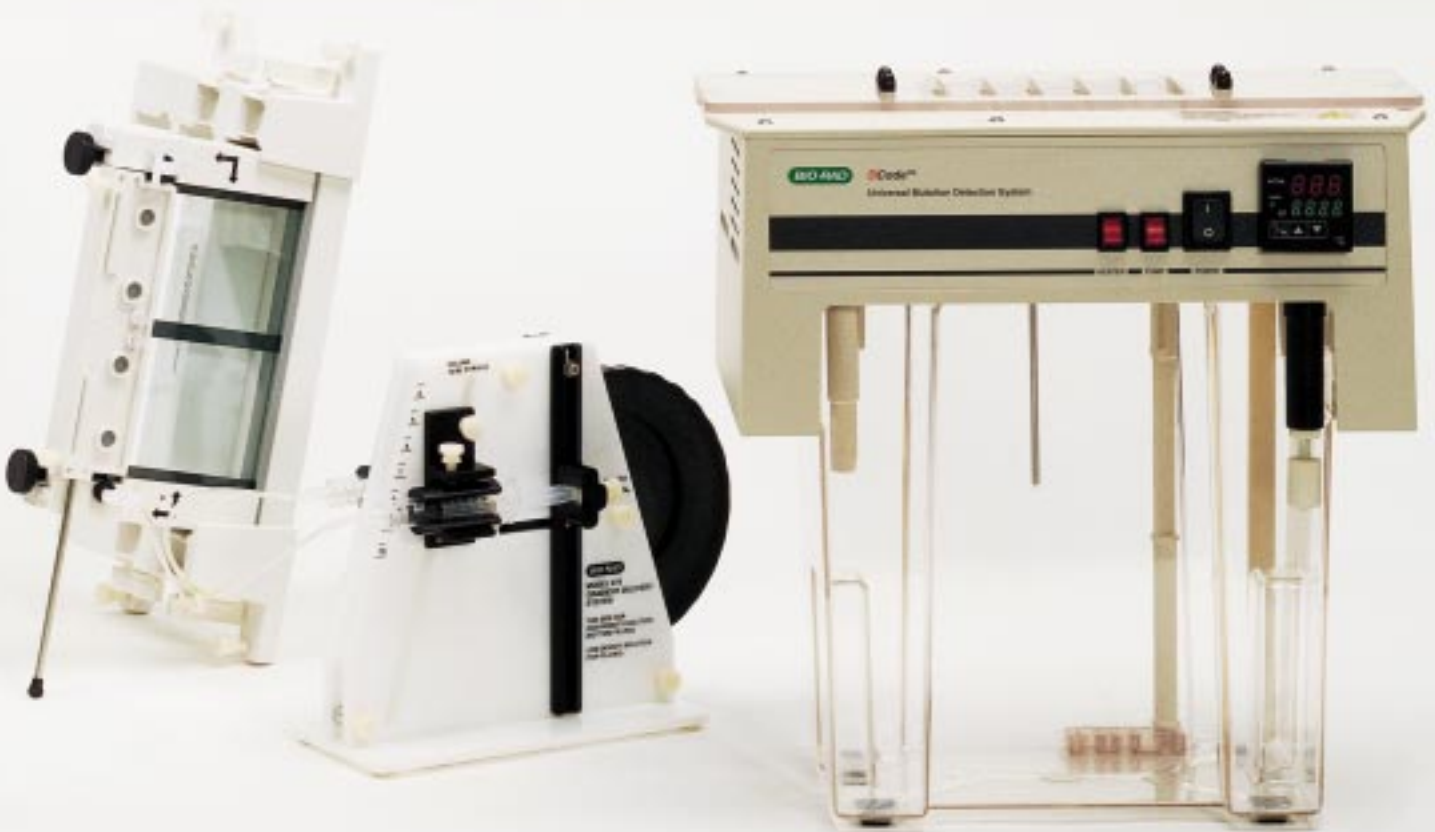
Sandwich core, gel caster with gel sandwiches, cam-operated manual gradient former; electrophoresis temperature control module

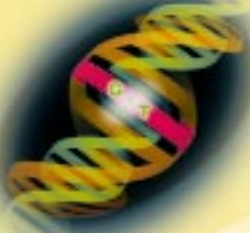


For Ultimate Sensitivity, DCode Allows You to Combine Detection Methods

For the highest throughput and efficiency, many labs use multiple techniques. The ability to rapidly switch between methods or convert to a new technique is critical in a fast-paced research environment. DCode is the only system that meets the demands of all major mutation detection techniques. Versatile features and distinct benefits include:

- Temperature control options for electrophoresis runs between 5 and 70 °C
- Modular design allowing labs to customize for current and future lab demands
- Specialized accessory kits which simplify start-up
- Research tools which can be added to the lab without investing in expensive instrumentation
- Technique-specific reagents and controls that are optimized for DGGE/CDGE, SSCP, HA, PTT, TTGE
- Application notes outlining proven run conditions for all the major techniques
- The exclusive ability to perform powerful Temporal Temperature Gradient Electrophoresis





With DCode, Use The Most Powerful Techniques for Maximum Efficiency

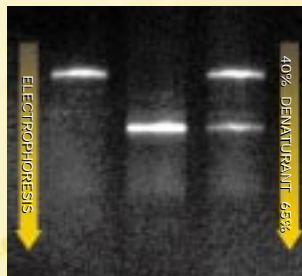
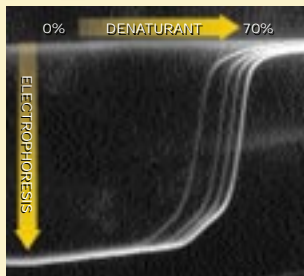
Denaturing Gradient Gel Electrophoresis Quickly Hunts for the Unknowns

DGGE is based on the principle that increasing denaturants will melt double-stranded DNA in distinct domains. When the melting temperature (T_m) of the lowest domain is reached, the DNA will partially melt, creating branched molecules and reducing its mobility in a polyacrylamide gel.¹

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%.² DCode optimizes DGGE in the following ways:

- Gradient gel casting is simple with patented cam-operated Model 475 Gradient Former
- MacMelt™ software streamlines GC clamp and primer placement
- Temperature control module provides consistent run temperatures between 45 and 70 °C
- Run up to two 16 x 16 cm gels or four 7.5 x 10 cm gels



Denaturing Gradient Gel Electrophoresis

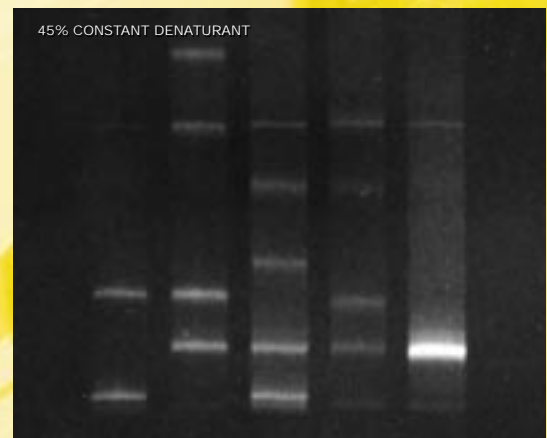
Above left: Perpendicular denaturing gradient gel in which the denaturing gradient is perpendicular to the electrophoresis direction. Mutant and wild-type alleles of exon 6 from the p53 gene amplified from primary breast carcinomas and separated by perpendicular DGGE (0-70% denaturant) run at 80 volts for 2 hours at 56 °C (data courtesy of A.L. Børresen. Radium Hospital, Oslo, Norway).

Above right: Parallel denaturing gradient gel in which gradient is parallel to the electrophoresis direction. Mutant and wild-type alleles of exon 8 from the p53 gene electrophoresed in an 8% acrylamide:bis (37.5:1) gel with a parallel gradient of 40-65% denaturant. Gel was run at 150 volts for 2.5 hours at 60 °C in 1x TAE buffer. Lane 1, mutant fragment; lane 2, wild-type fragment; lane 3, mutant and wild-type fragments.

Constant Denaturing Gel Electrophoresis Rapidly Screens for Mutations

After a mutation has been identified, CDGE rapidly screens multiple samples for its presence.³ The denaturant concentration that gives optimal resolution on the gradient gel is held constant. With no gradients required, high throughput screening is simpler. The denaturing concentration found to give optimal resolution in DGGE is held constant in CDGE.

- DCode system for CDGE makes optimization of denaturant concentration easy
- 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 facilitates rapid screening



Constant Denaturing Gel Electrophoresis

Amplified mutant and wild-type alleles from β -globin gene. Separation by CDGE run at 130 volts for 3 hours 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.

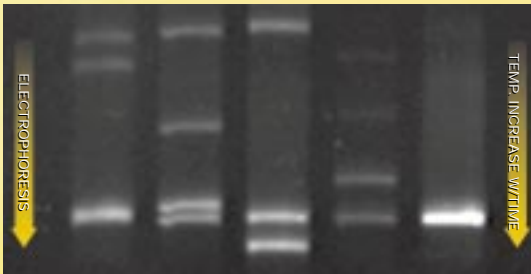
Software You Can Count On

Temporal Temperature Gradient Electrophoresis Has the Power of DGGE without Gradient Gels

TTGE exploits the principles of DGGE without using chemical denaturing agent gels, making it simpler, faster and easily reproducible.⁴

Amplified DNA is loaded onto a polyacrylamide gel containing urea. During electrophoresis, the temperature is increased gradually and uniformly. The result is a linear temperature gradient over the time of the electrophoresis run. The denaturing environment is formed by a constant concentration of urea in the gel combined with the temporal temperature gradient. The DCode system reduces TTGE to simple, reproducible practice.

- Temperature controller and heater allow reproducible temperature ramps as low as 0.1 °C per hour
- Elimination of gradient gels makes set-up and gel casting easy
- MacMelt software helps determine optimal temperature ranges
- Control reagents and application notes with proven protocols help get you started



Temporal Temperature Gradient Electrophoresis

Amplified mutant and wild-type alleles of exon 7 from cystic fibrosis gene. Separation by TTGE run at 130 volts for 5 hours in 1.25x TAE buffer on 6 M urea/6% acrylamide gel (37.5:1) using a temperature range of 50-60 °C and ramp rate of 2 °C/hr. Lane 1, mutant allele (1154 insTC); lane 2, mutant allele (G330X); lane 3, mutant allele (ΔF311); 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).

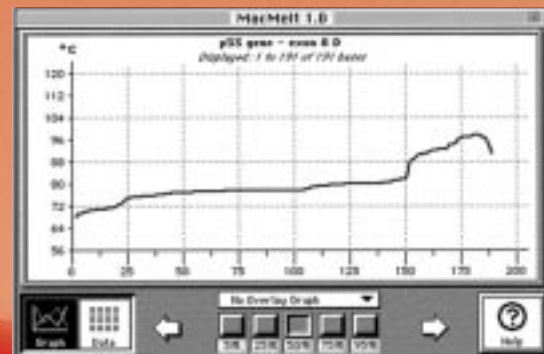
Use Macintosh® and PC Software With DCode to Take Out the Guesswork.

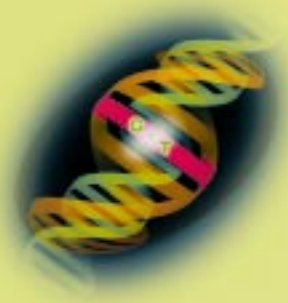
Mutations are most reliably detected when the sequence difference occurs in the lowest melting domain, before the molecules are completely denatured. Adding a GC clamp to one end of the DNA ensures that the region screened is in a lower melting domain and that the DNA will remain partially double-stranded.

MacMelt is a Macintosh-based application used with DCode for DGGE, CDGE and TTGE that predicts the melting profile of a DNA sequence, up to 3,200 bases. Primer placement and GC clamp positioning are precise through analysis of a melting profile. Using a melt map, software theory is used to predict results that can then be used in actual comparisons.

Operating MacMelt is simple. First, the DNA sequence is imported from a text file, and 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 for use into other software programs.

Inquire about our new Microsoft Windows®-based PC-compatible software which has similar features to MacMelt.



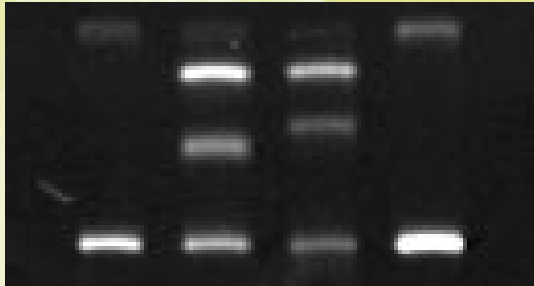


With DCode, SSCP, HA and PTT Are Used to Their Full Advantage

Consistent Results with DCode and Single-Strand Conformation Polymorphism

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.^{5,6} Our temperature-controlled buffer bath makes DCode ideal for SSCP, with these benefits:

- Electrophoresis cooling tank with ceramic cooling fingers connects to standard external lab recirculating chillers
- Temperature control module with stirrer, heater and buffer recirculating pump controls and maintains uniform temperatures between 5 and 25 °C
- Reproducible run temperatures for consistent results
- In place of cooling tank, DCode may be put in a cold room set to any temperature above ambient
- 16 x 16 cm or 16 x 20 cm gel sizes make nonisotopic detection with silver or fluorescent stains easy



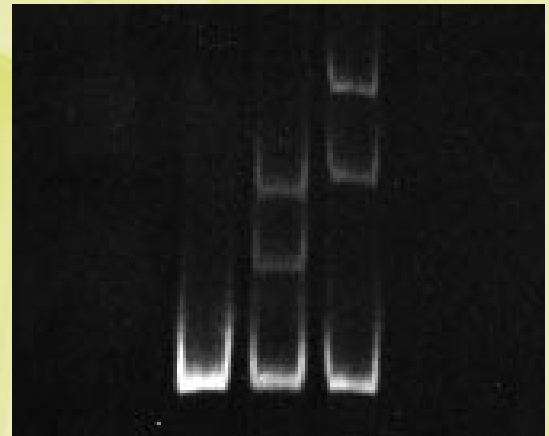
Single-Strand Conformation Polymorphism

Amplified mutant and wild-type alleles of exon 8 from the p53 gene. Separation by SSCP run at a constant 30 W for 3.5 hours in 1x TBE on an 8% acrylamide 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.

The DCode System Offers Benefits for Heteroduplex Analysis

Heteroduplex molecules form when wild-type and mutant DNA are mixed during PCR or denatured together and allowed to reanneal. Heteroduplex molecules with as little as one mismatch can show different mobility from homoduplex molecules in a polyacrylamide gel.⁷ Heteroduplex analysis (HA) is often used in conjunction with SSCP to improve sensitivity.⁸ The DCode system offers these advantages to HA experiments:

- Optional temperature control for reproducibility of results
- Enhanced gel matrix, DEM™ (Detection Enhancing Matrix) for increased resolution
- Control reagents and proven run conditions for simplified electrophoresis



Heteroduplex Analysis

Heteroduplex analysis of amplified mutant and wild-type alleles of exon 7 from cystic fibrosis gene. Lane 1, wild-type allele; lane 2, mutant allele (1154insTC); lane 3, mutant allele (Δ F311). Separation run at constant 120 volts for 20 hours in 0.6x TBE on a 1x DEM gel at room temperature (samples courtesy of L. Silverman, Division of Molecular Pathology, University of North Carolina School of Medicine).

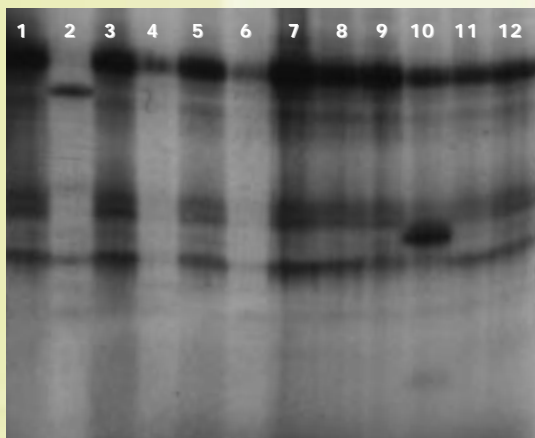
Reagents, Power and References

DCode™

The DCode System Easily Adapts to Protein Gel Electrophoresis

An increasing number of genes with translation-terminating mutations are being identified. PTT is a mutation screening method that detects truncated proteins.⁹ Since the method is able to scan larger regions of DNA than SSCP or even DGGE, it is becoming useful as an initial screen before moving to methods with higher detection efficiencies. With the following features, the DCode system easily adapts to protein gel electrophoresis:

- PTT adaptor kit makes conversion from DNA-based to protein-based screening methods simple
- Modular design facilitates staged screening strategies
- Electrophoresis reagent kit optimized for PTT
- Proven run conditions make set-up easy



Protein Truncation Tests

12 PTT samples from region b of the ataxia-telangiectasia gene run on an SDS-PAGE gel. Separation by PTT run at 200 volts for 3 hours in 1x Tris/glycine/SDS buffer on a 14% SDS-PAGE acrylamide gel at room temperature. Lanes 2 and 10 contain samples having premature termination mutations (data courtesy of R. Gatti, Department of Pathology, UCLA School of Medicine).

Electrophoresis and Control Reagents

Bio-Rad electrophoresis reagent kits are customized for each application to ensure that you use the highest quality buffers and acrylamide. Our control reagents for DGGE/CDGE, TTGE, SSCP and HA serve as learning tools to help get new techniques up-and-running quickly.

The Power Source for DCode

Ideal for DCode, the PowerPac 300 power supply is the most versatile, economical laboratory power supply available. It is lightweight and easy to program, with four sets of output terminals.

Helpful Applications and Bibliographies

An extensive reference list will assist you in choosing and optimizing the method best suited for your laboratory. Technical bulletins with examples of proven run conditions on the DCode for each application will help you get fast results.

To order technique-specific technical bulletins for applications using DCode, request the following:

- Bulletin #2102 for DGGE
- Bulletin #2104 for SSCP
- Bulletin #2105 for HA
- Bulletin #2106 for PTT
- Bulletin #2107 for CDGE

For a bibliography of mutation detection techniques, request Bulletin #2099.

References:

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2. Grompe, M., *Nature Genetics*, **5**, 111 (1993)
3. Børresen, A. L., et al., *Proc. Natl. Acad. Sci. USA*, **88**, 8405 (1991)
4. Yoshino, K., et al., *Nucleic Acid Research*, **19**, 3153 (1991)
5. Orrita M., et al., *Proc. Natl. Acad. Sci. USA*, **86**, 2766-2770 (1989)
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8. Ravnik-Glavac, M., et al., *Hum. Molec. Genet.*, **3**, 801-801 (1994)
9. Roest, P.A.M., et al., *Hum Molec. Genet.*, **2**, 1719-1721 (1993)

Ordering Information

<i>Catalog No.</i>	<i>Product Description</i>	<i>Catalog No.</i>	<i>Product Description</i>
170-9102	DCODE UNIVERSAL MUTATION DETECTION SYSTEMS Complete DCode System, 120 V , includes electrophoresis/temperature control module with cooling tank, sandwich core, Model 475 Gradient Former with all accessories required to cast gradient gels, MacMelt software, control reagents for DGGE/CDGE/TTGE, SSCP and HA, plates, combs and spacers to cast 1 mm and 0.75 mm thick 10, 16 and 20 cm gels	170-9125	ADAPTOR KITS AND ACCESSORIES DGGE Kit, 16 cm , includes two sets of 16 cm plates, two sets of 1 mm spacers, two 1-well 1 mm prep combs, sandwich clamps, pressure clamp, comb gasket and holder, required fittings
170-9103	Complete DCode System, 220/240 V	170-9126	DGGE Kit, 10 cm , includes two sets of 10 cm plates, two sets of 1 mm spacers, two 2-well 1 mm prep combs, sandwich clamps, pressure clamp, comb gasket and holder, required fittings
170-9104	Complete DCode System, 100 V	170-9127	CDGE/TTGE Kit , includes two sets of 16 cm plates, two sets of 1 mm spacers, two 20-well 1 mm combs, sandwich clamps
170-9080	DCode System for DGGE, 16 cm, 120 V , includes electrophoresis/temperature control module, sandwich core, DGGE kit for 16 cm gel casting (two sets of plates, two sets of clamps and 1 mm spacers, two 1 mm prep combs, comb gasket), all parts required to cast gradient gels, Model 475 Gradient Former, control reagents	170-9128	Complete SSCP Kit , includes electrophoresis cooling tank for use with external chiller, two sets of 20 cm plates, two sets of 0.75 mm spacers, two 20-well 0.75 mm combs, sandwich clamps
170-9081	DCode System for DGGE, 16 cm, 220/240 V	170-9129	Basic SSCP Kit , includes two sets of 20 cm plates, two sets of 0.75 mm spacers, two 20-well 0.75 mm combs, sandwich clamps
170-9082	DCode System for DGGE, 16 cm, 100 V	170-9130	HA Kit , includes two sets of 20 cm plates, two sets of 0.75 mm spacers, two 20-well 0.75 mm combs, sandwich clamps
170-9083	DCode System for DGGE, 10 cm, 120 V , includes electrophoresis/temperature control module, sandwich core, DGGE kit for 10 cm gel casting (two sets of plates, two sets of clamps and 1 mm spacers, two 2-well 1 mm prep combs, comb gasket), all parts required to cast gradient gels, Model 475 Gradient Former, control reagents	170-9131	PTT Kit , includes two sets of 20 cm plates, two sets of 1 mm spacers, two 20-well 1 mm thick combs, sandwich clamps
170-9084	DCode System for DGGE, 10 cm, 220/240 V	170-9034	MacMelt Software, Macintosh
170-9085	DCode System for DGGE, 10 cm, 100 V	170-9042	Model 475 Gradient Former , includes cam-operated manual gradient former, 2 each of 10 and 30 ml syringes, accessories
170-9086	DCode System for CDGE, 120 V , includes electrophoresis/temperature control module, sandwich core, CDGE kit for 16 cm gel casting (two sets of 16 cm plates, two sets of 1 mm spacers, two 20-well 1 mm combs), control reagents	170-9140	Electrophoresis Cooling Tank , cooling adaptor for hook-ups to laboratory recirculating chiller
170-9087	DCode System for CDGE, 220/240 V	170-9150	DCODE CONTROL AND ELECTROPHORESIS REAGENTS DCode Control Reagent Kit for DGGE/CDGE/TTGE , includes primers (one GC-clamped) and DNA templates for production of wild-type and mutant DNA
170-9088	DCode System for CDGE, 100 V	170-9151	DCode Control Reagent Kit for SSCP , includes primers and DNA templates for production of wild-type and mutant DNA
170-9089	DCode System for TTGE, 120 V , includes electrophoresis/temperature control module, sandwich core, TTGE kit for 16 cm gel casting (two sets of 16 cm plates, two sets of 1 mm spacers, two 20-well 1 mm combs), control reagents	170-9152	DCode Control Reagent Kit for HA , includes homoduplexed and heteroduplexed DNA in 1x loading buffer
170-9090	DCode System for TTGE, 220/240 V	170-9170	DCode Electrophoresis Reagent Kit for DGGE/CDGE , includes 500 ml of 40% acrylamide/bis solution 5(37.5:1), 250 g of urea, 225 ml of 100% formamide (deionized), 2 x 1 liter of 50x TAE buffer, 10 ml of 10 mg/ml EtBr, 1 ml of 2x gel loading dye, 10 ml of DCode dye solution, 5 ml of TEMED, 10 g of ammonium persulfate
170-9091	DCode System for TTGE, 100 V	170-9171	DCode Electrophoresis Reagent Kit for TTGE , includes 500 ml of 40% acrylamide/bis solution (37.3:1), 1 kg of urea, 2 x 1 liter of 50x TAE buffer, 10 ml of 10 mg/ml EtBr, 1 ml of 2x gel loading dye, 10 ml of DCode dye solution, 5 ml of TEMED, 10 g of ammonium persulfate
170-9092	DCode System for SSCP, 120 V , includes electrophoresis/temperature control module, sandwich core, SSCP kit for gel casting (two sets of 20 cm plates, two sets of 0.75 mm spacers, two 20-well 0.75 mm thick combs), electrophoresis cooling tank adaptor for use with external cooling bath, control reagents	170-9172	DCode Electrophoresis Reagent Kit for SSCP , includes 500 ml of 40% acrylamide solution, 500 ml of 2% bis solution, 100 ml of 100% glycerol, 6 x 1 liter of 10x TBE buffer, 2x SSCP gel loading dye, 5 ml of TEMED, 10 g of ammonium persulfate
170-9093	DCode System for SSCP, 220/240 V	170-9173	DCode Electrophoresis Reagent Kit for HA , includes 250 ml of 2x DEM solution, 250 g of urea, 6 x 1 liter of 10x TBE buffer, 2x gel loading dye, 10 ml of 10 mg/ml of EtBr solution, 5 ml of TEMED, 10 g of ammonium persulfate
170-9094	DCode System for SSCP, 100 V	170-9174	DCode Electrophoresis Reagent Kit for PTT , includes 500 ml of 40% acrylamide/bis (37.5:1), 2 x 1 liter of 10x Tris/glycine/SDS buffer, 30 ml Laemmli sample buffer, 500 g Tris base, 25 ml of 2-mercaptoethanol, 250 ml of 10% SDS, 10 g of ammonia persulfate, 5 ml TEMED
170-9095	DCode System for Heteroduplex Analysis, 120 V , includes electrophoresis/temperature control module, sandwich core, heteroduplex kit for gel casting (two sets of 20 cm plates, two sets of 0.75 mm spacers, two 20-well 0.75 mm thick combs), control reagents	165-5050	PowerPac 300 Power Supply, 100/120 V
170-9096	DCode System for Heteroduplex Analysis, 220/240 V	165-5051	PowerPac 300 Power Supply, 220/240 V
170-9097	DCode System for Heteroduplex Analysis, 100 V		
170-9098	DCode System for PTT, 120 V , includes electrophoresis/temperature control module, sandwich core, PTT kit for gel casting (two sets of 20 cm plates, two sets of 1 mm spacers, two 20-well 1 mm thick combs)		
170-9099	DCode System for PTT, 220/240 V		
170-9100	DCode System for PTT, 100 V		

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For complete ordering information, request Bulletin #2100.
For DCode system and accessories specifications, request Bulletin #2101.



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