D GENE™
Denaturing Gel Electrophoresis System

Instruction Manual and Applications Guide

Catalog Numbers
170-9000
through
170-9070

BIO-RAD

Warranty

The *D GENE* lid, tank, casting stand, gradient mixer, and accessories are warranted against defects in materials and workmanship for 1 year. If any defects occur in the instruments or accessories during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Damage caused by substituting alternative parts.
4. Use of fittings or spare parts supplied by anyone other than Bio-Rad Laboratories.
5. Damage caused by accident or misuse.
6. Damage caused by disaster.
7. Corrosion caused by improper solvent† or sample.

This warranty does not apply to parts listed below:

1. Fuses
2. Glass plates
3. Electrodes

For any inquiry or request for repair service, contact Bio-Rad Laboratories. Inform Bio-Rad of the model and serial number of your instrument.

IMPORTANT: This Bio-Rad instrument is designed and certified to meet IEC 1010-1* safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will:

Void the manufacturer's warranty

Void the IEC 1010-1 safety certification

Create a potential safety hazard

Bio-Rad Laboratories is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent.

† The D GENE tank is not compatible with chlorinated hydrocarbons (e.g., chloroform), aromatic hydrocarbons (e.g., toluene, benzene), or acetone. Use of organic solvents voids all warranties.

* IEC 1010-1 is an internationally accepted electrical safety standard for laboratory instruments.

Practice of PCR is covered by U.S. patent numbers 4,683,195, 4,683,202, and 4,899,818 issued to Cetus Corporation which is a subsidiary of Hoffmann-LaRoche Molecular Systems, Inc. Purchase of any of Bio-Rad's PCR-related products does not convey a license to use the PCR process covered by these patents; the user of these products to perform PCR must obtain a license.

First Revision

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Section 1
Equipment Overview

1.1 Safety ⚠

Read the manual before using the *D GENE* system. For technical assistance, contact your local Bio-Rad Office or, in the U.S., call technical services at 1-800-4BIORAD (1-800-424-6723).

This instrument is intended for laboratory use only.

This product conforms to the "Class A" standards for electromagnetic emissions intended for laboratory equipment applications. It is possible that emissions from this product may interfer with some sensitive appliances when placed nearby or in the same circuit as thise appliances. The user should be aware of this potential and take asppropriate measures to avoid interference.

DC power to the *D GENE* system is supplied by an external DC voltage power supply. **This power supply must be ground isolated so that the DC voltage output floats with respect to ground.** All of Bio-Rad's power supplies meet this important safety requirement. Regardless of which power supply is used, the maximum specified operating parameters for the system are

Maximum voltage limit	300 VDC
Maximum power limit	50 watts

AC current for controlling temperature to the system, and DC current for electrophoresis, provided from the external power supply, enter the unit through the lid assembly, which provides a safety interlock. DC current to the cell is broken when the lid is removed. **Do not attempt to circumvent this safety interlock. Always disconnect the AC cord from the unit and the coiled cord from the DC power supply before removing the lid, or when working with the cell.**

Definition of Symbols

⚠ Caution, risk of electric shock

⚠ Caution (refer to accompanying documents)

Important precautions during set up

1. Always inspect *D GENE* system and replace any damaged components before use.
2. Place the *D GENE* system on a steady, level bench top.
3. Do not use near flammable materials.
4. Do not use buffers which are not compatible with construction material of the system.
5. Always place the lid assembly on the buffer tank with the AC and DC power cords disconnected.
6. Always connect the system to correct the AC and DC power sources.

Important precautions during the run

1. Always add buffer to the "Fill" line on the tank when preheating; always keep the buffer below "Max" level during electrophoresis.

2. Do not touch any wet surface before all the electrical sources are disconnected.
3. To allow maximum heat dissipation, do not put anything on the top surface of the cover.

Important precautions after the run ⚠

1. Always turn off power switches and unplug all cables to DC and AC sources. **Allow the heater tube to cool down (more than 15 seconds) before removing it from the tank**. The ceramic tube may be very hot after shut down. Do not touch the ceramic tube for several minutes after turning off the power.
2. Do not cool the hot ceramic tube in cool liquids.
3. Store the lid in the aluminum stand for maximum stability. The heater tube may be hot after use; therefore, support the lid on its own legs, and only on surfaces that can withstand high temperature.

1.2 Specifications

Construction

Core and clamps	Tank: molded polycarbonate Core: molded polysulfone Clamps: molded glass-filled polycarbonate
Gradient former	Cast acrylic and acetyl
Lid	Polycarbonate
Electrodes	0.010" diameter platinum
Electrical leads	Flexible, coiled
Glass plates	16 x 20 cm (16 cm format inner plate) 18.3 x 20 cm (16 cm format outer plate) 10.2 x 20 cm (10 cm format inner plate) 12.4 x 20 cm (10 cm format outer plate)
Gel sizes	16 x 16 cm (max two per run) 16 x 10 cm (max two per run) 7.5 x 10 cm (max four per run)
Spacers available	0.75, 1.0, and 1.5 mm
Combs	16 well comb (compatible with 8 well pipettor), and 1 well comb (Prep comb for perpendicular gradient gels). Optional: Other combs from PROTEAN® II xi system.
Casting stand	Able to cast two 16 x 16 cm, two 16 x 10 cm, or four 7.5 x 10 cm gels per setup.
Heater and control	Temperature control (PID type) ± 0.5 °C variation within gel area, ± 1.5 °C actual in the range 45 to 65 °C.
Maximum set temperature	65.5 °C
DC voltage limit	300 V DC
DC wattage limit	50 W

AC Power Requirement

170-9000/9003/9060/9063	AC power input: 120 VAC 47–63 Hz ,5 A slow blow fuse
170-9001/9004/9061/9064	AC power input: 100 VAC 47–63 Hz , 5 A slow blow fuse
170-9002/9005/9062/9065	AC power input: 220–240 VAC 47–63 Hz 2.5 A slow blow fuse

DC Power Requirement

External DC voltage power supply. This power supply must be ground isolated in such a way that the DC voltage output floats with respect to ground.

Maximum voltage limit	300 VDC
Maximum power limit	50 W

Size and Weight

Overall size	Lid and Tank Assembly: 39 cm (L) x 20 cm (W) x 42 cm (H)
Shipping weight	16 Kg

Environmental Requirements

Storage environment	0–70 °C, humidity 0–95% (non-condensing)
Operating environment	0–35 °C, humidity 0–95%.

Regulatory

Meets the requirements of IEC 1010-1 and FCC, Class A.

1.3 Description of Major Components

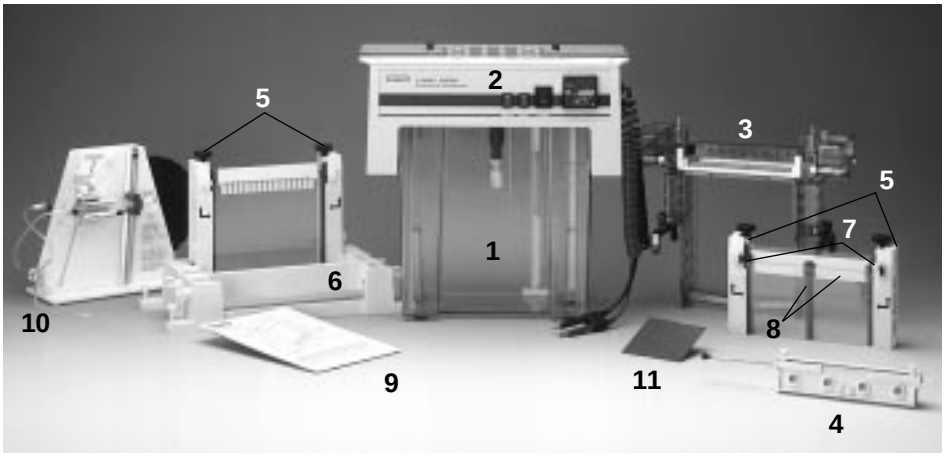

Fig. 1.1. **1.** Lower buffer tank. **2.** Lid with temperature controller. **3.** Core. **4.** Comb gasket holder. **5**. Sandwich clamps. **6.** Casting stand with sponge. **7.** Stopcock. **8.** Combs and spacer set. **9**. Alignment card. **10.** Model 475 Gradient Delivery System. **11.** Filler spacer.

1. Lower Buffer Tank

The lower buffer tank encloses the unit and provides stability during electrophoresis. Always keep the buffer level between 'Fill' and 'Maximum' during a run.

2. Lid with Temperature Controller

Combined with the lower buffer tank, the lid acts to fully enclose the system. The lid should be placed so that the tip of the stirring bar fits inside the support hole at the tank. The loading lid should be left on the system at all times unless you are loading samples.

3. Core

The completed gel sandwich attaches to the core so that the outer plate of sandwich forms the side of the upper buffer chamber. The inner plate is clamped against a rubber gasket on

the core to provide a greaseless, leak-free seal for the upper buffer. Each sandwich forms one side of the cathode chamber. If only one gel is to be run, use a set of glass plates without a spacer to form a buffer dam sandwich. In addition, the core has built-in passage for upper and lower buffer circulation by the pump.

4. Comb Gasket Holder

The comb gasket holder seals the top edge of the glass sandwich for casting perpendicular gradient gels. Align the comb gasket so that the notched steps on both ends of the soft part of the comb gasket is against the notches at the top of the two spacers. A proper alignment is required for a good seal.

5. Sandwich Clamps

The patented sandwich clamps consist of a single screw mechanism which makes assembly, alignment, and disassembly of the gel sandwich an effortless task. The clamps exert an even pressure over the entire length of the glass plates. Each pair of clamps consists of a left clamp and a right clamp. Each sandwich can hold a gel up to 1.5 mm thick.

6. Casting Stand with Sponge

The casting stand is separated from the cell so that gels can be prepared for the next run while others are running.

7. Stopcock and Air Vent Plugs

Stopcocks are used at the inlet ports when casting a gradient perpendicular gel. The air vent plugs are used in the comb gasket to close the gel sandwich after casting.

8. Prep Comb and Spacer Set

The prep comb works with spacer sets to form a single sample well or dual sample wells per gel.

9. Alignment Card

The Alignment Card simplifies sandwich assembly by keeping the spacers upright during sandwich alignment.

10. Model 475 Gradient Delivery System

The Model 475 Gradient Delivery System is an innovative cam-driven module for forming accurate, reproducible gradient gels.

11. Filler spacer (optional)

Allows casting of single mini-gel.

12. Pressure Clamp (not shown in photo)

The pressure clamp provides equal pressure to the comb gasket for a good seal.

Section 2
Introduction to Technology

2.1 Overview of Denaturing Gradient Gel Electrophoresis

There is an increasing need for practical, efficient, and inexpensive ways to identify mutations responsible for genetic diseases and cancer development. PCR has solved the problem of the target limitation. When the precise site of a point mutation is not known, it is necessary to first determine the region harboring the defect. Six methods are currently available: single-strand conformation polymorphism (SSCP), chemical cleavage (CCM), RNase mismatch cleavage, reaction of DNA heteroduplexes with a water-soluble carbodiimide (CDI), direct sequencing, and denaturing gradient gel electrophoresis (DGGE).

RNase cleavage, CDI, CCM, and DGGE use the technique of forming heteroduplex (annealing between wild-type and mutant DNA or RNA) molecules. These molecules are characterized by mismatched nucleotides at the sites of alteration. The first three methods are capable of locating precisely the position of mutation. DGGE depends on electrophoretic shifts of partially denatured molecules caused by differences in the rate of DNA melting; it can not be used to detect the exact site of a mutation, but only identify a region containing one or more mutations. The DGGE method offers the following advantages over the other methods of mutation detection.

- The fragment sizes can be > 500 bp, which is larger than allowed by the other methods and comparable to automated sequencing.
- The efficiency of detection is approximately 100%.
- Labeling with radioactivity is unnecessary.
- DGGE is rapid and easy after the initial set up and is best used for routine screening of small fragments.

Denaturing gradient gel electrophoresis (DGGE) identifies single base changes in a segment of DNA. The separation techniques on which DGGE is based were first described by Fischer and Lerman.[212] The separation principle of DGGE involves differences in melting behavior of DNA fragments which are identical in sequence except for a single base pair. This melting behavior is detected as a reduction in the mobility of the DNA fragment as it moves through an acrylamide gel containing denaturing agents, as a consequence of partial strand separation. In a denaturing gradient polyacrylamide gel, DNA fragments migrate according to size until they reach the gradient where the molecule begins to denature. When the DNA denatures, it opens in a domain which is anchored by a higher melting domain or domains that have not melted. At this point the migration rate is slowed down. When the fragment completely denatures, then migration again becomes a function of size (see Figure 2.1).

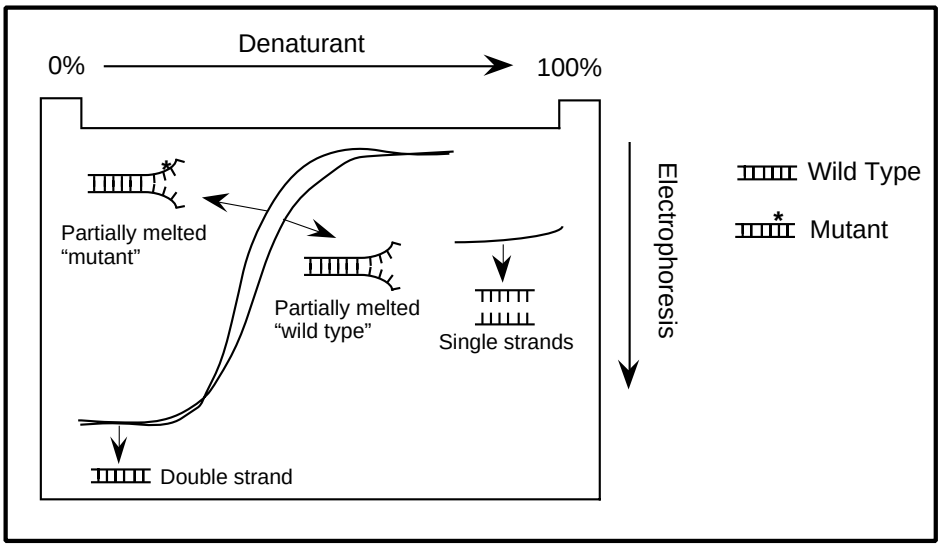

Fig. 2.1. An example of DNA melting properties in a denaturing gradient gel. At a low concentration of denaturants the DNA fragment remains double stranded, but as the concentration of denaturants increases the DNA fragment begins to melt. Then, at very high concentrations of denaturants, the DNA fragment can completely melt, creating two single strands.

The thermodynamics of the transition of double stranded DNA to single stranded DNA have been described by a computer program developed by Lerman,[232] based on the statistical mechanical principles and algorithms developed by Poland[233] and the nearest-neighbor base-pair doublet parameters introduced by Gotoh and Tagashira.[234] There are programs available which calculate the theoretical DNA melting profile of a given sequence. Bio-Rad offers Macintosh® computer program, MacMelt™ software, which calculates and graphs theoretical DNA melting profiles.

Applications of the *D GENE* system are varied, but its primary purposes are screening for mutations after *in vitro* or *in vivo* mutagenesis, screening for tumor material or acquired mutations, analysis of candidate genes for possible mutation, analysis of inherited disease, analysis of new genes, analysis of polymorphism, and marker screening. Other applications include regulatory protein-nucleic acid complex formation, confirmation of the accuracy of PCR amplified reaction mixtures, and assay of PCR induced mutations in cloned sequences without sequencing.

Section 3
DGE Background Information

3.1 Introduction

In DGGE, DNA is melted by using chemicals denaturants and increased temperature. A solution of 100% chemical denaturant consists of 7 M urea and 40% formamide. Denaturing gels are typically run at temperatures between 50 and 65 °C. The size of the DNA fragments run on a denaturing gel can be as large as 1 kb in length, but only the lower melting domains will be available for mutation analysis. For complete analysis of fragments over 1 kb in length, more than one PCR reaction should be performed.[231]

When running a denaturing gel, both the mutant and wild-type DNA fragments are run on the same gel. This way one can detect a mutation by seeing a band shift on the gel. In some cases, it is possible to detect a mutation by running just homoduplex DNA, for example, if the mutation is a base change from A to G (G-C pairing has a higher melting temperature than A-T pairing).[201] The method of heteroduplex analysis helps in resolving wild-type and mutated fragments when it is not possible to detect a mutation by running homoduplex fragments, for example, if the mutation is a base change from A to T. Heteroduplexes can be formed by adding the wild-type and mutant template sequence in the same PCR reaction or by adding separate PCR products together, then denaturing and allowing them to re-anneal. A heteroduplex has a mismatch in the double strand causing a distortion in its usual shape; this has a destabilizing effect and causes the DNA strands to separate at a lower concentration of denaturant (Figure 3.1). The heteroduplex bands always migrate more slowly than the corresponding homoduplex bands.[231]

Three types of DGGE gels may be run to detect mutations in DNA. The first gel type is a perpendicular gradient gel, in which the gradient is perpendicular to the electric field and uses a broad denaturing gradient range, such as 0–100% or 20–70%.[212] From this gel, one can determine the concentration of denaturants in which the wild-type and mutant fragments can be separated. The other types of gels are parallel DGGE and constant denaturing gradient gel electrophoresis (CDGE). In parallel DGGE, the denaturing gradient is parallel to the electric field, and the range of denaturants is narrowed to allow better separation of fragments.[201] In CDGE there is no gradient. Instead the optimum denaturing concentration which allowed separation in perpendicular DGGE is used. These three types of gels are discussed in more detail later in this section.

Starting a DGGE experiment depends on the sequence information available. If the sequence is known, then PCR can be used to amplify the region of interest. Computer programs that can calculate the theoretical melting profiles, such as MacMelt software, can help determine what PCR primers should be used. The theoretical melting profile will also tell you what part of a gene can be screened for mutations by the system; the primers should be chosen to flank these regions. A 30-40 base-pair GC clamp (Section 4.1) may be added to one of the primers to produce a high melting region, so that the rest of the sequence can be screened for mutations. If the sequence is not known, then a perpendicular DGGE gel with a large denaturation range (0–100%) should be run to visualize the melting profile of a given fragment. This will enable one to determine suitable denaturation conditions for subsequent analysis.

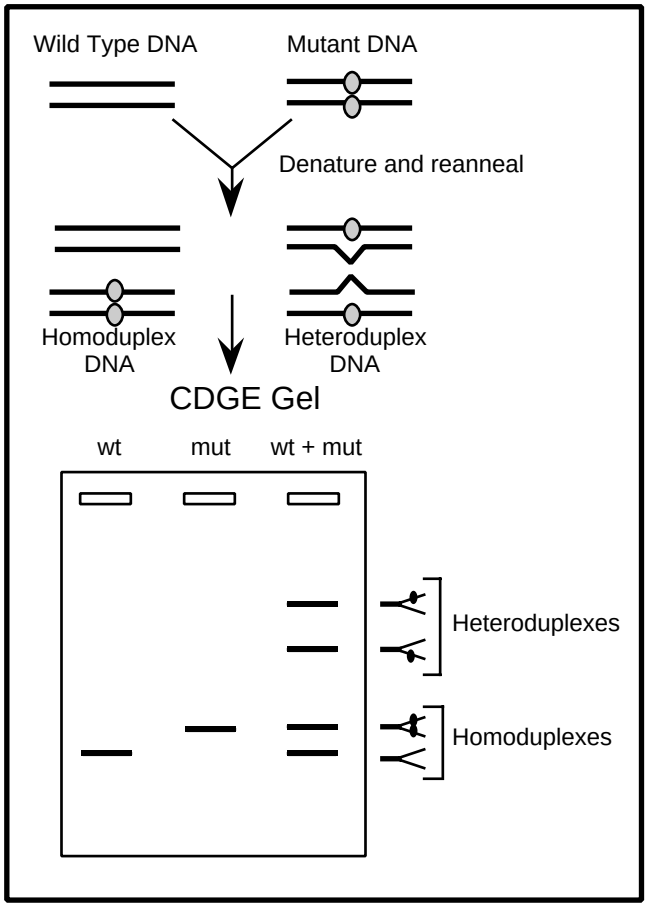

Fig. 3.1. An example of wild-type and mutant DNA fragments that were denatured and re-annealed to generate four fragments, two heteroduplexes and two homoduplexes. The melting behavior of the heteroduplexes is altered so that they melt at a lower denaturant concentration than the homoduplexes, and can be visualized on a denaturing gel, for example a CDGE gel.

3.2 Perpendicular DGGE

Perpendicular DGGE a useful first step in the analysis of any DNA fragment. This technique is used to determine the number of melting domains and the optimum denaturing conditions. If nothing is known about the sequence of interest, a 0–100% gradient gel should be run. If the sequence information is known, the range of denaturants may be narrowed (*e.g.* 20–70%) to improve resolution between the wild-type and mutant fragments.

The perpendicular gel is cast with a preparative comb (1 wide well) and the wild type and mutant DNA are both loaded into this well. One can run the homoduplex form of the wild-type and mutant fragments or one can create heteroduplexes. Heteroduplexes can be formed during PCR or by mixing the mutant and wild-type PCR fragments in a microfuge tube and denaturing the DNA by heating at 95 °C for 2 minutes, followed by an incubation at 65 °C for 1 hour, and finally incubation at room temperature for 2–20 hours.[231] An example of a perpendicular denaturing gradient gel with homoduplex and heteroduplex fragments is shown in Figure 3.2.

Perpendicular gels are usually run at 100–165 volts for 2–3 hours or until the xylene cyanol dye in the loading buffer reaches the bottom of the gel. In some cases, only one melting domain may be seen on the gel, even if the theoretical melting calculations indicate the

presence of two or more melting domains. This is caused by the melting of the first domain which leads to an almost complete stop in the gel migration. This can be resolved by increasing the run time so that higher melting domains can be seen.

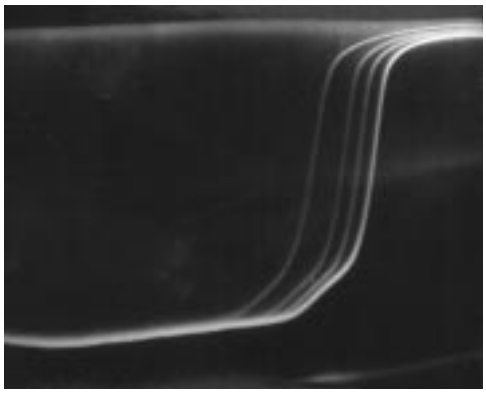

Fig. 3.2. Perpendicular denaturing gradient gel in which the denaturing gradient is perpendicular to the electrophoresis direction. Mutant and wild-type alleles of exon 6 from the TP53 gene amplified from primary breast carcinomas and separated by perpendicular DGGE (0-70% denaturant) run at 80 V for 2 hours at 56°C. The first two bands on the left are heteroduplexes and the other two bands are the homoduplexes.

3.3 Parallel DGGE

For parallel DGGE, the boundary of denaturant concentrations are determined to be above and below the melting of a given domain as seen in the perpendicular denaturing gel. Examples for determining the denaturing concentrations are shown in Figure 3.3. Typically, a difference of 25–30% stock denaturant from top to bottom, which is centered at the melting point, is used.[201] If the melting of two or more domains is seen in the perpendicular gel, then two different gels with different gradients can be used to maximize separation.

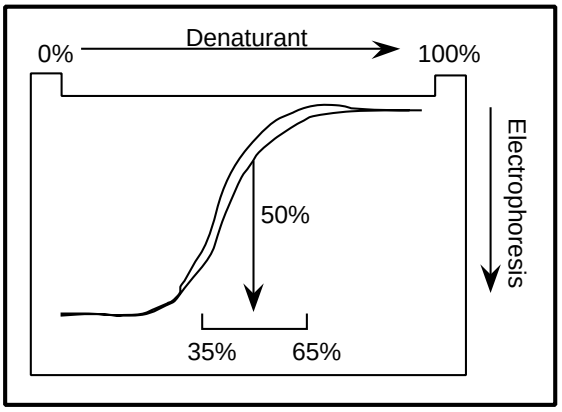

Fig. 3.3. Example of a perpendicular denaturing gradient gels used for determining the denaturant concentration range in a parallel DGGE gel. The DNA fragment melts at a denaturing concentration of 50% and a range of 35–65% denaturants can be used.

In parallel DGGE, the denaturant concentration increases from the top of the gel to the bottom of the gel. With the parallel gel, it is possible to run more samples under the optimal conditions. Gel combs are used to form wells in the gel and depending on the number of samples needed to run, different combs with different number of wells can be used. Parallel gradient

denaturant gels usually take about 2–6 hours to reach good resolution between mutant and wild-type DNA fragments. An example of parallel DGGE is shown in Figure 3.4.

In parallel DGGE, separation is not time dependent for optimal separation. This is due to the fact that a molecule will migrate with a constant velocity until it reaches the position in the gel where the denaturant concentration causes the molecule to begin to melt. At this point, the molecule will migrate more slowly as it moves into higher denaturing conditions.

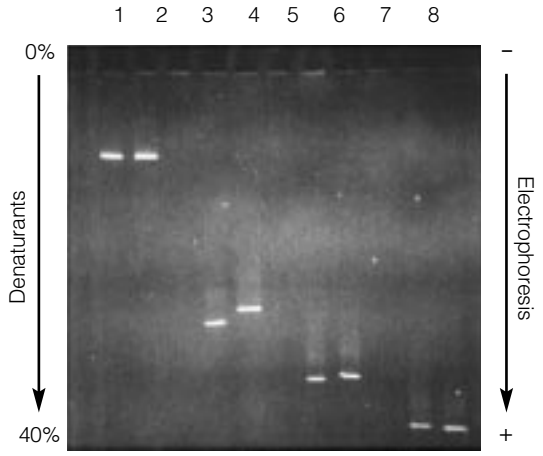

Fig. 3.4. Parallel denaturing gradient gel. Time course of wild-type mouse and hamster exon 3 HPRT fragments in a 0-40% parallel DGGE gel. Samples 1, 3, 5, and 7 contains the wild-type mouse fragment. Samples 2, 4, 6, and 8 contain the wild-type hamster fragment.

3.4 CDGE

For a constant denaturing gel, only one denaturing condition is used to melt the fragment.[218] The concentration of denaturant to use for a CDGE is determined at the steepest increase in gel retardation or the maximum split between wild-type and mutant DNA, as seen in the perpendicular denaturing gel. An example for determining the denaturing concentrations are shown in Figure 3.5. If the melting of two or more domains is seen in the perpendicular gel, two different gels with optimized denaturants are used to maximize separation.

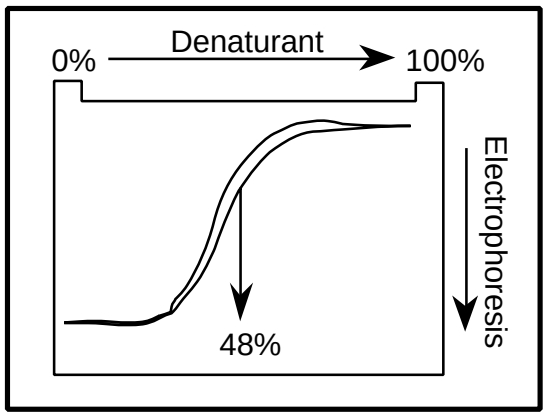

Fig. 3.5. Example of perpendicular denaturing gradient gel used for determining the optimum denaturant concentration used in a constant denaturing gradient gel. The DNA fragment melts at a denaturing concentration of 48% and this concentration of denaturants can be used.

In constant denaturing gels, only a single denaturing condition is used in the gel. With the constant denaturing gels, it is possible to run more samples under the optimal conditions. Gel combs are used to form wells in the gel and depending on the number of samples being run, different combs with different numbers of wells can be used. Constant denaturing gels usually take about 2–4 hours to reach good resolution between mutant and wild-type DNA fragments. An example of a constant denaturing gel is shown in Figure 3.6.

In CDGE, separation is time dependent for optimal separation, because wild-type and mutant fragments immediately begin to partially melt and migrate at a constant rate through the constant denaturant. Therefore, the running time needed will depend on the resolution required. A wide separation between wild-type and mutant DNA can be achieved by longer run times.

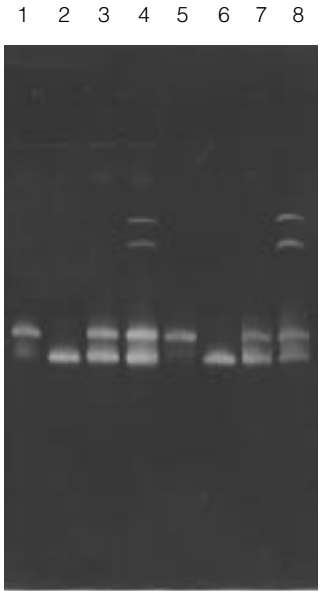

Fig. 3.6. Constant denaturing gel. Amplified mutant and wild-type alleles of exon 8 from the TP53 gene. Separation by CDGE run at 130 V for 2.5 hours on a 10% acrylamide gel in 48% denaturant at 56 °C. Lanes 1 and 5 mutant alleles, lanes 2 and 6 wild-type alleles, lanes 3 and 7 mutant and wild-type alleles, and lanes 4 and 8 homoduplexes and heteroduplexes of the mutant and wild-type alleles.

Section 4
Sample and Reagent Preparation

4.1 Sample Preparation

In some cases, adding a high melting domain to the DNA fragment allows one to analyze mutations that normally are not seen.[210, 211] The addition of a 30–40 base pair GC clamp to the DNA fragment during PCR creates a high melting domain and will influence the other melting domains.[196] As a result, the sequence of interest should be in the first (low) melting domain and therefore, can be analyzed. A GC clamp is not needed if the sequence of interest resides in a low melting domain and has one end of the fragment containing a natural high melting domain. The use of melting profile programs, such as MacMelt software, can show regions of theoretical high and low melting domains of a known sequence. These programs can help determine if a GC clamp will allow better detection of mutations.

If GC clamps are not used, this might cause missing of the transition zone and show a strong single strand band in a perpendicular DGGE gel. In this case, it might be a good idea to add a GC clamp to one end of the fragment.

As mentioned above, the use of GC clamps helps in the ability to detect mutations. An alternative to GC clamps is using psoralen derivative PCR primers called ChemiClamp primers.[28] Psoralens are photoreagents that form covalent bonds with pyrimidine bases of nucleic acids. Psoralen-oligonucleotide conjugates allow crosslinking of DNA fragments at one end by photoinduction with a UV source. Because ChemiClamps covalently link the two DNA strands at one end, they should not be used when isolating a DNA fragment which is going to be sequenced from a gel.

Samples run in the DGGE gel are typically prepared by PCR. The PCR samples can be loaded onto the gel after PCR without any other manipulations. The size of the DNA fragments run on DGGE should be in the 100–800 bp range, although DNA fragments as long as 1,000 bp can also be analyzed.

4.2 Reagent Preparation

The concentration of denaturants varies for samples analyzed in the *D GENE* system. The concentration of acrylamide can vary as well, depending on the size of the fragment that is being analyzed. Both 0% and 100% denaturants should be made as stock solutions. A 100% denaturant is a mixture of 40% deionized formamide and 7 M urea. The following reagents are included in the *D GENE* Electrophoresis Reagent Kit, catalog number 170-9032.

40% Acrylamide/Bis (37.5:1)*

Acrylamide	38.0 g
Bis-acrylamide	2.0 g

Add dH_2O to 100 ml. Filter through a Whatman No. 1 and store at 4 °C.

* Polyacrylamide gels are described by reference to two characteristics:

1) The total monomer concentration (%T)

2) The crosslinking monomer concentration (%C)

$$\%T = \frac{\text{gm acrylamide + gm Bis-acrylamide}}{\text{Total Volume}} \times 100$$

$$\%C = \frac{\text{gm Bis-acrylamide}}{\text{gm acrylamide + gm Bis-acrylamide}} \times 100$$

50x TAE Buffer (1 L)

Tris base	242.0 g
Acetic acid, glacial	57.1 ml
0.5 M EDTA, pH 8.0	100.0 ml

Mix and add dH_2O to 1 L. Autoclave for 20–30 minutes. Store at room temperature.

0% Denaturing Solution (100 ml)

	7.5% Gel	10% Gel	12.0% Gel
40% Acrylamide/Bis (37.5:1)	18.8 ml	25.0 ml	30.0 ml
50x TAE buffer	2.0 ml	2.0 ml	2.0 ml
dH_2O	to 100 ml	to 100 ml	to 100 ml

Degas for about 10–15 minutes. Store at 4 °C in a brown bottle for about 1 month.

100% Denaturing Solution (100 ml)

	7.5% Gel	10% Gel	12.0% Gel
40% Acrylamide/Bis (37.5:1)	18.8 ml	25.0 ml	30.0 ml
50x TAE buffer	2.0 ml	2.0 ml	2.0 ml
Formamide (deionized)	40.0 ml	40.0 ml	40.0 ml
Urea	42.0 g	42.0 g	42.0 g
dH_2O	to 100 ml	to 100 ml	to 100 ml

Degas for about 10–15 minutes. Store at 4 °C in a brown bottle for about 1 month.

For other denaturing solutions, use the volumes in the 100% Denaturing Solution, with the exception of the formamide and urea. For these reagents, use the volumes indicated below.

Denaturing Solution

	10%	20%	30%	40%	50%	60%	70%	80%	90%
Formamide (ml)	4	8	12	16	20	24	28	32	36
Urea (g)	4.2	8.4	12.6	16.8	21	25.2	29.4	33.6	37.8

10% Ammonium Persulfate (1 ml)

Ammonium persulfate	0.1 g
dH_2O	1.0 ml

Mix and store at -20 °C for about a month.

D GENE Dye Solution (10 ml)

Bromophenol blue	0.05 g
Xylene cyanol	0.05 g
1x TAE buffer	10.0 ml

Store at room temperature.

1x TAE Running Buffer (7 L)

50x TAE buffer	140.0 ml

dH_2O	6860.0 ml

4.3 Gel Volumes

The final gel volumes to use for the three different gel sizes are listed below.

Spacer Thickness	16 x 16 cm gel	16 x 10 cm gel	7.5 x 10 cm gel*
0.75 mm	25 ml	15 ml	8 ml (16 ml)
1.00 mm	30 ml	20 ml	10 ml (20 ml)
1.50 mm	45 ml	26 ml	14 ml (24 ml)

* The first volume is for one gel. The volume in parenthesis is for two gels.

Linear Denaturing Gradient Gels

For casting a linear gradient perpendicular and parallel gel, use half the volume of the low density denaturing solution and half the volume of the high density denaturing solution. For example, if the total gel volume is 25 ml, use 12.5 ml low density solution and 12.5 ml high density denaturing solution.

Constant Denaturing Gradient Gels

For casting constant denaturing gradient gels, use the formula below to determine the volume of 0% and 100% denaturing solutions needed to achieve the desired denaturant concentration.

1. (% desired denaturant)(total gel volume needed) = ml of 100% denaturant solution
2. (total gel volume needed) - (ml 100% denaturant) = ml of 0% denaturant solution

Example: To cast a 52% constant denaturing gel, using 30 ml total volume for a 16 x 16 cm gel with a 1.0 mm spacer.

1. (0.52)(30 ml) = 15.6 ml 100% denaturing solution needed
2. (30 ml) - (15.6 ml) = 14.4 ml 0% denaturing solution needed

Section 5
Buffer Temperature

5.1 Temperature Controller

The temperature controller maintains the desired buffer temperature in the *D GENE* system (Figure 5.1). The actual and set buffer temperatures are displayed in °C. The set temperature can be adjusted by using the raise and lower buttons. The maximum buffer temperature that can be set is 65 °C. The heater light will come on when the heater is on.

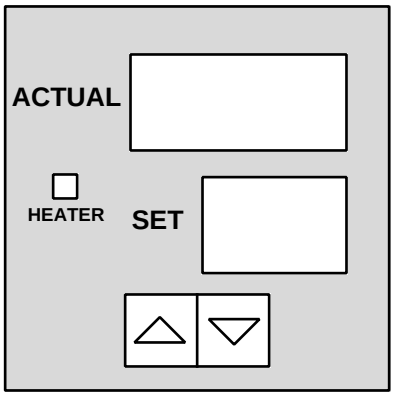

Fig. 5.1. The Temperature Controller displays the actual and set temperatures.

5.2 Preheating the Running Buffer

1. Pour 1x TAE buffer into the *D GENE* chamber up to the Fill line. It takes approximately 7 liters of 1x TAE buffer to fill the chamber.
2. Place the *D GENE* lid onto the chamber. Attach the power cord. Turn the power and heater switch on. The loading lid should be on the *D GENE* lid during preheating.
3. Set the temperature 2–4 °C above the desired temperature. During sample loading the buffer temperature can drop 2–4 °C.
4. It can take about 1 to 1.5 hours for the *D GENE* system to heat the buffer up to the desired temperature (56–60 °C). Heating the buffer in a microwave can reduce the time it takes to get to the desired temperature.

Section 6
Gel Casting

6.1 Assembling the Glass Plate Sandwiches

To insure proper alignment, make sure all plates and spacers are clean and dry before assembly. **Use caution when assembling the glass plate sandwiches. Wear gloves and eye protection.**

1. Assemble the gel sandwich on a clean surface. Lay the long rectangular plate down first, then place the left and right spacers of equal thickness along the short edges of the rectangular plate. For casting perpendicular gradient gels, place the spacers so that the holes on the spacers are at the top of the plates with the grooved side of the spacer facing the glass plate and the notched ends facing inwards. For parallel and constant gradient gels, place the spacers so that the holes in the spacers are at the top of the plate, facing inward and the grooved side of the spacer facing upward or against the short glass plate.
2. Place a short glass on top of the spacers so that it is flush with one end of the long plate.
3. Locate both the right and left sandwich clamps and loosen the single screw of each clamp by turning counterclockwise. Place each clamp by the appropriate side of the glass plate stack with the locating arrows facing up and toward the glass plates (Figure 6.1).

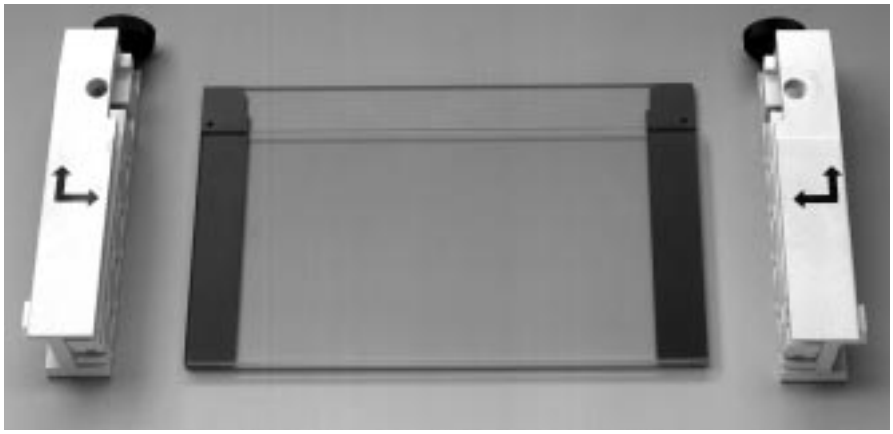

Fig. 6.1. Positioning glass plates, spacers, and clamps.

4. Grasp the whole glass plate sandwich firmly. Guide the left and right clamps onto the sandwich so that the long and short plates fit the appropriate notches in the clamp. Tighten the screws enough to hold the plates in place (Figure 6.2).

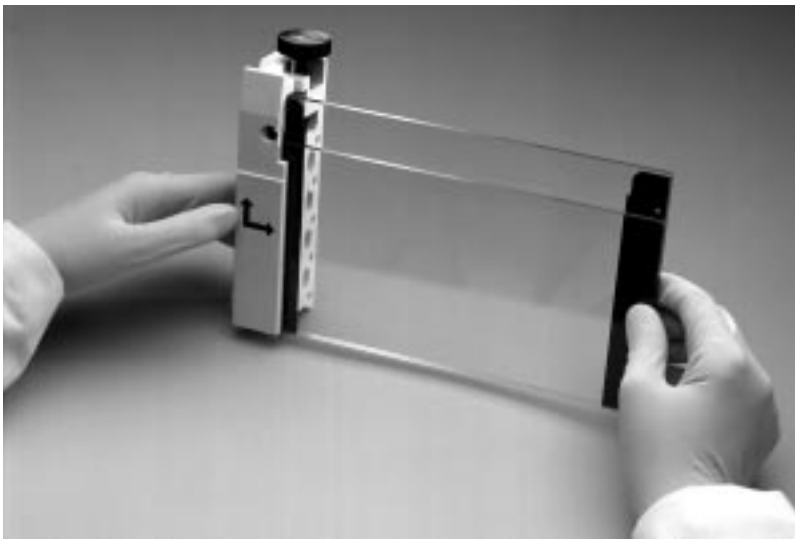

Fig. 6.2. Adapting the clamps to the glass plate assembly.

5. Place the sandwich assembly in the alignment slot of the casting stand (the alignment slot is the slot without cams) with the short glass plate forward (Figure 6.3). Loosen the sandwich clamps and insert a *D GENE* alignment card to keep the spacers upright.

   Note: Always use the alignment slot and alignment card to set the spacers in place. Failure to use this slot for alignment can result in gel leakage while casting or buffer leakage during the run.

6. Flush the plates and spacers by simultaneously pushing inward on both clamps at the locating arrows while at the same time pushing down on the spacers with a thumb; tighten both clamps just enough to hold the sandwich in place. Pushing inward on both clamps at the locating arrows will insure that the spacers and glass plates are flush against the sides of the clamps(Figure 6.3).

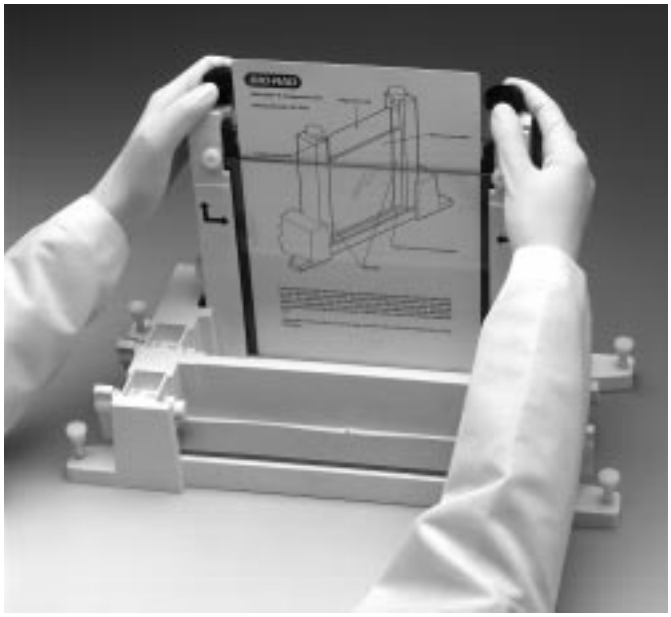

Fig. 6.3. Aligning spacers in the sandwich assembly.

7. Remove the alignment card. Remove the sandwich assembly from the casting stand and check that the plates and spacers are flush at the bottom. If the spacers and glass plates are not flushed, realign the sandwich and spacers to obtain a good seal (Repeat steps 5-7).

8. Once a good alignment and seal are obtained, tighten the clamp screw until it is finger-tight. Do not over tighten, plates may crack.

9. For assembling a 16 x 16 cm or 16 x 10 cm CDGE or parallel gel, go to step 16. If you are assembling a 16 x 16 cm or 7.5 x 10 cm perpendicular gradient gel, go to step 10.

10. Place the proper comb in the sandwich and set it against the special notches provided on the spacers. For the 7.5 x 10 cm perpendicular gel, insert the middle spacer on the center of the sandwich until it touches the middle notch on the comb and straighten the spacer. The bottom of the middle spacer should also be flushed against the glass plates without any snags (Figure 6.4).

    Note: If casting a single 7.5 x 10 cm gel, use the filler spacer, which fills the glass plate on the side that is not being used.

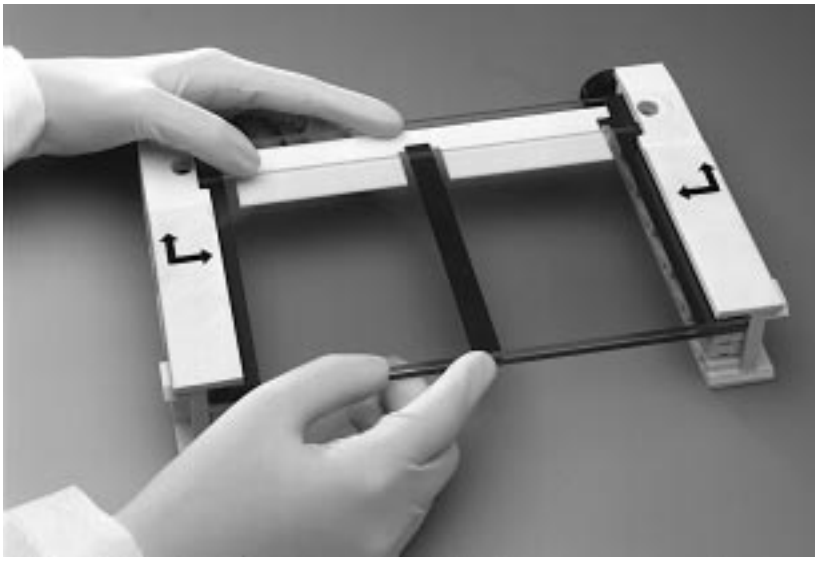

Fig. 6.4. Positioning the middle spacer in the sandwich assembly.

11. Set the sandwich standing upright on a flat surface. Loosen the comb gasket screws (located on the back, Figure 6.5). Mark a straight line on the middle of the screw head using a permanent marker (this will be the marker for the turns needed for the screws). Carefully place the comb gasket on top of the comb with the screws facing towards the large plate. Position the comb gasket so that the notched steps at the top of both ends of the soft part of the comb gasket is against the notches at the top of the spacers. Twist the screws until it makes contact with the glass plate. Twist the screws an additional ¼ turn.

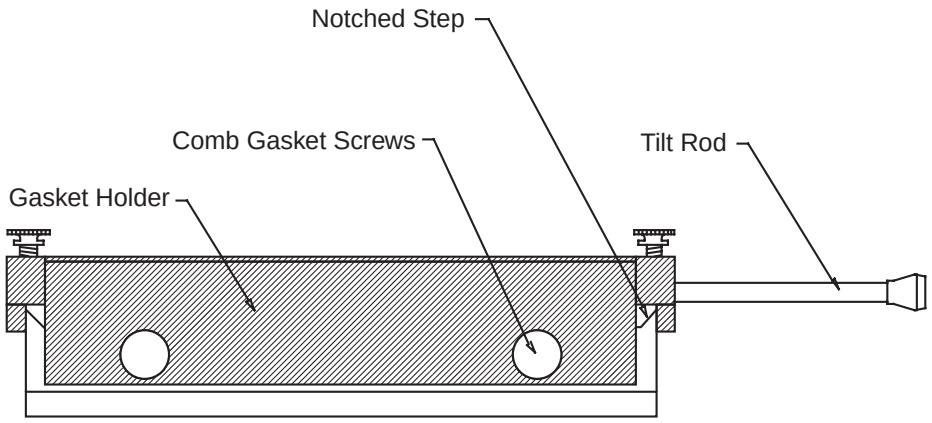

Fig. 6.5 Comb gasket and holder.

Note: Ensure that the comb gasket and it components are free of any gel material. Remove any polymerized gel material in the comb gasket air vents. The soft part of the comb gasket should be snugged within the comb gasket holder. There should be no "wavy" areas on the soft gasket. Use a sturdy, flat spatula to press the soft gasket straight down into the holder.

12. Loosen the Pressure Clamp screw to be used for the appropriate gel sandwich size (Figure 6.6). Mark a straight line on the middle of the screw head using a permanent marker (this will be the marker for the turns needed for the screws). Lay the Pressure Clamp on a flat surface with the screw upwards.

13. Grasp the gel sandwich with the short glass plate facing up. **Do not touch the comb gasket.** Lay the sandwich on top of the Pressure Clamp with the short plate facing upward and the middle spacer aligned with the Pressure Clamp. The base of the large plate should be against the "notched" portion of the Pressure Clamp and the Pressure Clamp screw against the top of the comb gasket and above and between the two air vent plugs/holes. Ensure that the pressure clamp is positioned in the middle of the comb gasket. This provides equal force onto the comb gasket for a good seal. Twist the Pressure Clamp screw until it makes contact with the comb gasket. Tighten the Pressure Clamp screw one and a half turns.

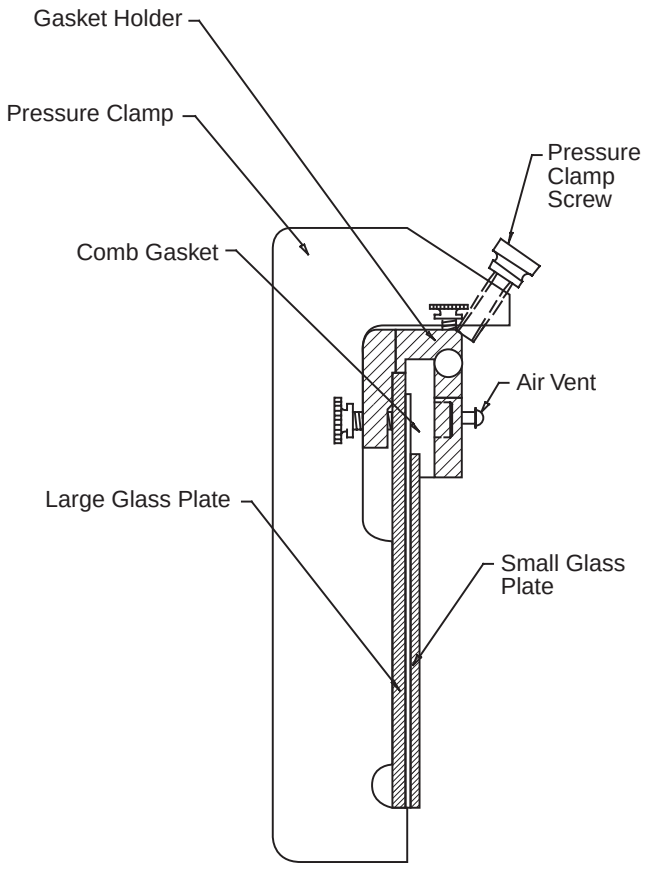

Fig. 6.6 Pressure clamp assembly.

14. Tighten the comb gasket screws an additional one to one and a quarter turns. If it is tightened more, the glass plate may crack. For a proper seal, check to see that the notches on both the comb gasket and spacers are butted/scrunched against each other. It is important that the gasket is placed properly to prevent leakage while casting. **Remove the Pressure Clamp.**

15. Twist a stopcock into each of the injection ports until it is finger-tight. A loose stopcock may cause leakage during gel casting. Screw the injection port into the holes located on the sandwich clamps. Do not tighten with the stopcock, it may loosen. Do not overtighten the injection port, it will damage the O-ring and cause a leakage. A snug fit is all that is needed to place the injection port against the glass plate/spacer.

    a. For the 7.5 x 10 cm dual gel sandwich, only one half of the sandwich is cast at a time. Open the stopcock on the gel sandwich and be sure the air port is unplugged. Be sure that the ports on the half of the sandwich that is not being cast are closed. This helps to lessen leakage.

    b. For assembling a 16 x 16 cm perpendicular gradient gel sandwich, plug or tape the two middle holes on the comb gasket to prevent leakage upon casting.

16. The camshafts on the casting stand should have the handles pointing up and pulled out. Place the gray sponge into the front casting slot. Place the sandwich on the sponge with the shorter plate facing forward. When the sandwich is placed correctly, press down on the sandwich clamp screw and turn the handles of the camshaft pointing downwards so that the cams lock the sandwich in place.

6.2 Model 475 Gradient Delivery System

The Model 475 Gradient Delivery System is used with the *D GENE* system to construct reproducible linear polyacrylamide gradient gels. Refer to the Model 475 Gradient Delivery System instructions for information on its set-up and use. The Model 475 Gradient Delivery System has a 7–50 ml capacity, making it ideal for the construction of 16 x 16 cm or 7.5 x 10 cm polyacrylamide gradient gels. The mixing of the high and low density solutons occurs in the Y-fitting and a single outlet leads to the gel sandwich. The gradient may be cast from the top for parallel gels or from the side for perpendicular gels.

6.3 Casting Perpendicular DGGE Gels

1. Position the gel assembly tilted to about a 20° angle by adjusting the rod on the comb gasket. This is to insure that any air bubbles that form while casting the gel escapes to the air vent (Figure 6.7).

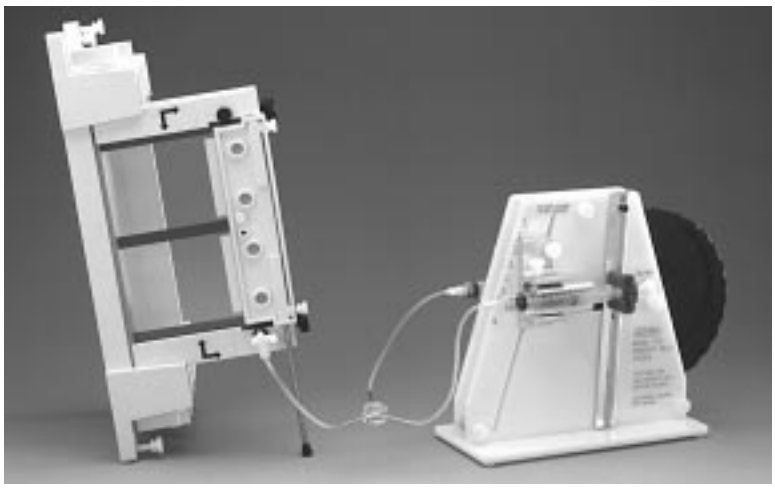

Fig. 6.7. Pouring a perpendicular gradient gel using the Model 475 Gradient Delivery System.

2. Refer to the Model 475 Gradient Delivery System manual for details on set-up and operation of the gradient former.

3. Insert and fasten all three metal fittings into the Y-fitting. Connect the free end of the 9 cm Tygon tubing to one of the metal fittings in the Y-fitting and connect to the other end a luer coupling. Connect each of the luer fittings on the two long pieces of tubing, then connect the luer fitted ends to same size syringes. Do not connect the long Tygon tubings to the Y-fitting.

4. Label one of the syringes LO (for the low density solution) and one HI (for the high density solution). Attach a plunger cap onto each syringe plunger "head." Tighten enough to hold the plunger in place. Slide each syringe into a syringe sleeve. Twist and position the sleeve to the middle of the syringe with the volume gradations visible. Make sure that the lever attachment screw is in the same plane as the flat or back side of the sleeve. This is extremely important for proper attachment of the syringe to the lever.

   Note: Ensure that the tubing pieces are free of any gel material by simply pushing water through the tubing with the syringe. The tubings should be free of any materials before casting.

5. Rotate the cam wheel counterclockwise to the start position. At the start point, the lever is in the vertical position. Adjust the volume adjustment screw to the desired setting. Refer to the Model 475 Gradient Delivery System instructions, Section 4.1, for the correct setting to use.

6. Prepare the high and low density gel solution by pipeting the desired amounts into two disposable culture tubes (refer to the Model 475 Gradient Delivery System Instructions, Section 4.2). For visually checking the formation of the gradient, add 100 µl of *D GENE* dye solution per 5 ml high density solution.

7. Add 1/100 of the total gel volume of 10% ammonium persulfate and 1/1000 of the total gel volume of TEMED to the tubes containing the gel solutions (these ratios allow about 3–5 minutes to finish casting the gel before polymerization). Cap and mix by inverting several times. With the syringe connected to the tubing, withdraw all of the high density solution using the high density solution syringe. (Excess air is not a problem at this time). Do the same for the low density solution syringe.

   Note: Acrylamide is a very hazardous substance. Use caution: wear gloves and eye protection. Avoid skin contact.

8. Carefully remove any air bubbles by tapping the syringe. Completely fill the tubing with gel solution. Do not displace the gel solution, loss of gel solution will result in an incompletely cast gel.

   Note: The volume of gel solution should be greater (about 1 ml total volume for both syringe volumes) than the volume set on the volume adjustment screw (See Section 4.1). For example, if the volume adjustment screw is set at 4.5, the volume of each gel solution should be ≥ 5 ml in the syringe in order to have enough gel solution for casting the gel. The excess gel solution will not affect formation of the desired gradient.

9. Place the high density syringe into the syringe holder (high density side for bottom filling). Make sure to place the syringe in the correct syringe holder, i.e. the placement of the low and high density syringes depends on whether the gel is being poured from the top or bottom. **Note: Perpendicular gradient gels are bottom filled. Check for proper high and low syringe position on the gradient delivery system.** Hold the syringe **by the plunger** and insert the lever attachment screw into the lever groove. Try not to handle the syringe, it may dispense the gel solution out of the syringe. Carefully position the lever attachment screw by placing it in the groove and sliding the syringe back towards the cam side. Tighten the syringe holder screw against the syringe sleeve. Do the same for the low density solution (low density side for bottom filling).

10. Slide the tubing from the high density syringe over a metal tubing fitting on the Y-fitting (about 0.3-0.5 cm of the tip of the tubing). Do the same for the low density syringe. This sequence of inserting the tubings is extremely important to cast the desired gradients.

11. Connect the Y-fitting to the gel sandwich stopcock fitting. Make sure the stopcock on the gel sandwich is open and the air vent port on the comb gasket is unplugged.

12. Rotate the cam wheel slowly and steadily to deliver the gel solution. It is important to cast the gel solution at a steady pace to avoid mixing of the gradients within the gel sandwich.

13. Close the air vent port and stopcock on the gel sandwich when the cam wheel has reached the stop position. Carefully level the gel sandwich by adjusting the gasket tilt rod. Make sure that you loosen the gasket tilt rod screw and not the sandwich clamp screw.

14. Remove the tubing from the gel sandwich stopcock. Remove both syringes from the syringe holder on the gradient delivery system. Detach the syringe tubings from the metal tubings on the Y-fitting. Run or push water out through the tubings and Y-fitting. It is extremely important that this is done quickly after casting to avoid any gel polymerization (See Gradient Delivery System manual for information on cleaning).

15. Let the gel polymerize for about 30-45 minutes. Remove the gasket tilt rod and place it on the second gel side of the comb gasket. Repeat steps 6 through 15.

6.4 Casting Parallel DGGE Gels

1. Position the gel assembly by standing it upright (Figure 6.8).

   Note: Place a comb into the sandwich before casting the gel. Placing a comb after casting the gel will disturb the gradient.

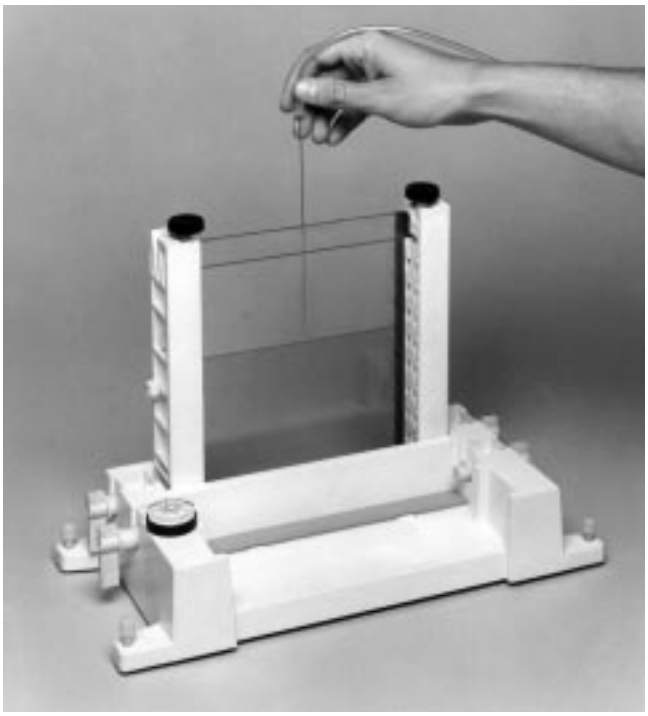

Fig. 6.8. Pouring a parallel gradient gel.

2. Refer to the Model 475 Gradient Delivery System manual for details on set-up and operation of the gradient former.

3. Insert and fasten all three metal fittings into the Y-fitting. Connect the free end of the 9 cm Tygon tubing to one of the metal fittings in the Y-fitting and connect to the other end a luer coupling. Connect each of the luer fittings on the two long pieces of tubing, then connect the luer fitted ends to same size syringes. Do not connect the long Tygon tubings to the Y-fitting.

   Note: Ensure that the tubings are free of any gel material by simply pushing water through the tubing with the syringe. The tubing should be free of any material before casting.

4. Repeat steps 4 through 8 in Section 6.3.

5. Place the high density syringe into the syringe holder (high density side for top filling). Make sure to place the syringe in the correct syringe holder, i.e., the placement of the low and high density syringes depends on whether the gel is being poured from the top or bottom. **Note: Parallel gradient gels are top filled. Check for proper high and low syringe position on the gradient delivery system.** Hold the syringe **by the plunger** and insert the lever attachment screw into the lever groove. Try not to handle the syringe, it may dispense the gel solution out of the syringe. Carefully position the lever attachment screw by placing it in the groove and sliding the syringe back towards the cam side. Tighten the syringe holder screw against the syirnge sleeve. Do the same for the low density solution (low density side for top filling).

6. Slide the tubing from the high density syringe over a metal tube fitting on the Y-fitting (about 0.3-0.5 cm of the tip of the tubing). Do the same for the low density syringe. This sequence of inserting the tubing is extremely important to cast the desired gradients.
7. Hold the needle with the beveled edge against the glass plate in the middle of the sandwich to create a uniform flow.
8. Rotate the cam wheel slowly and steadily to deliver the gel solution. It is important to cast the gel solutions at a steady pace to avoid mixing of the gradients within the gel sandwich.
9. Remove the tubing from the gel sandwich stopcock. Remove both syringes from the syringe holder on the gradient delivery system. Detach the syringe tubings from the metal tube fitting on the Y-fitting. Run or push water out through the tubings and Y-fitting. It is extremely important that this is done quickly after casting to avoid any gel polymerization (See Gradient Delivery System manual for information of cleaning).
10. Let the gel polymerize for about 30–45 minutes.

6.5 Casting CDGE Gels

A constant denaturant gel contains the same chemicals as both the perpendicular and parallel gradient gels. The only difference is CDGE has a uniform concentration of denaturant.

1. Assemble a gel sandwich as in Section 6.1 steps 1–12. The gel sandwich is cast in an upright position.
2. Into a 50 ml tube, add the required amounts of 0% and 100% gel solutions needed to get the desired percent of denaturant (see Section 4). Add the ⅟₁₀₀ the total gel volume of ammonium persulfate and ⅟₁₀₀₀ the total gel volume of TEMED. Cap the tubes and mix.
3. Place a comb in the gel sandwich and tilt it so that the teeth are at a slight (~10°) angle. This will prevent air from being trapped under the comb teeth while pouring the gel solutions (Figure 6.9).

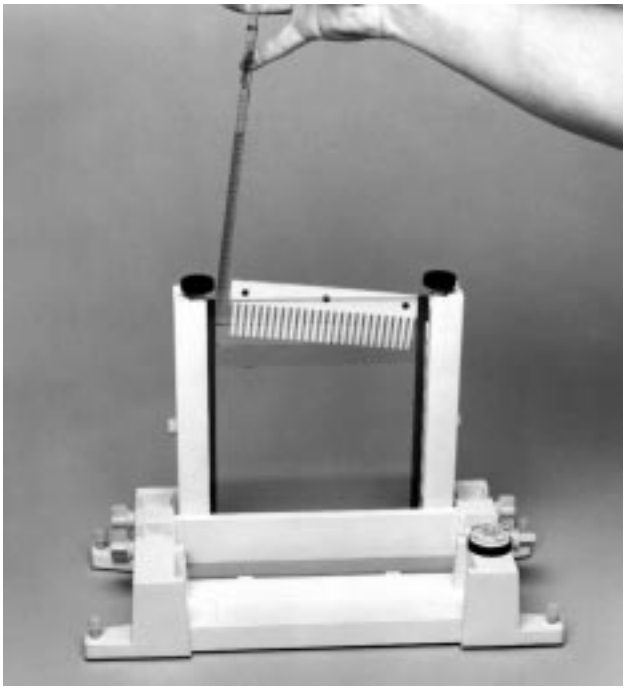

Fig. 6.9. Pouring a CDGE gel.

4. Pour or pipette the gel solution into the sandwich until the gel covers the comb teeth. Properly align the comb in the sandwich. Add more gel solution if needed.
5. Allow the gel to polymerize for about 30–45 minutes.

Section 7
Electrophoresis

7.1 Assembling the Upper Buffer Chamber

1. Lay the inner core down flat on a bench. Seat the white U-shaped gasket onto the core with the flat (non-stepped) side down (Figure 7.1). Make sure the U-shaped gasket is clear of any particles, such as residual gel material, that may cause leakage.

   Note: To help insure a good buffer seal with the gaskets for the *D GENE* cell, lubricate the entire front of the gaskets (the shaded portion in Figure 7.1) with water or upper buffer prior to attaching the gel sandwich to the central cooling core. This will allow the glass plate sandwich to slide onto the gasket properly.

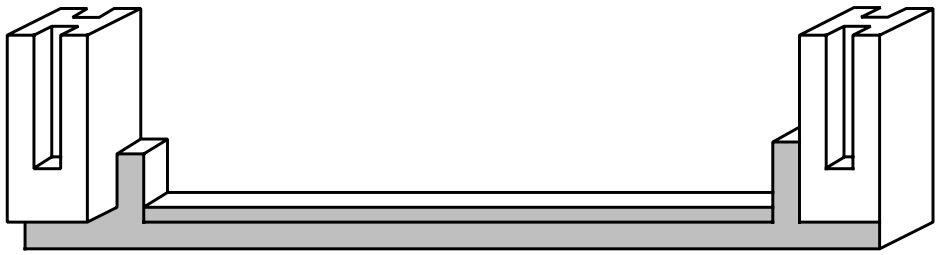

Fig. 7.1. Core gasket.

2. After the gel has polymerized, release the gel sandwich from the casting stand by turning the camshafts 180°, to the up position and pulling them outward. Remove the gel sandwich. Remove any stopcocks and comb gaskets. Remove the comb.
3. With the short glass plate facing the core, position the gel sandwich so that the grooves in the upper portion of the clamps are fitted onto the locating pins on the core. The gel sandwich should be positioned at an angle of ≤ 20° with the core. Keeping this angle to a minimum will prevent distortion of the gasket while the sandwich slides into place.
4. With your fingers below the latch on the core and your thumbs resting on the sandwich clamps, gently push the gel sandwich onto the core with one simple motion. You should be able to hear a click. The upper edge of the short inner glass plate should be butted against the notches of the U-shaped gasket and the tabs of each clamp should be held securely against the latch assemblies on both sides of the core (Figure 7.2).

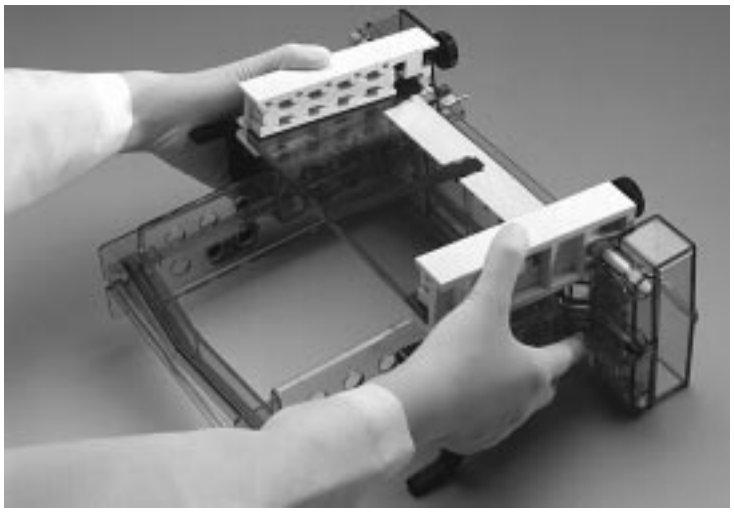

Fig. 7.2. Adapting the sandwich assembley to the core.

5. Turn the core to its other side and repeat steps 1–4 to attach the second gel sandwich.

   Note: When the gel sandwich has been properly installed, the shorter inside glass plate will be forced against the notch in the U-shaped gasket to create a leak proof seal. Always inspect the contact between the gasket and glass plate to make sure the glass plate is butted against the notch in the gasket and is not resting above or below this notch. Improper installation of the gel sandwich can result in buffer leakage during the run. As a standard procedure, stand the core and the attached gel sandwiches, pour buffer into the upper buffer chamber, and check for leaks prior to a run. Pour enough buffer to immerse the cathode wires connected in the core. If no leaks are present, it is safe to run the gel.

6. If only one gel is to be run, assemble the other set of glass plates, without the spacers. Place the short glass plate on top of the long glass plate. Guide the left and right clamps onto the sandwich so that the plates fit appropriate notches in the clamp. Tighten the screws enough to hold the plates in place. No further alignment is necessary. Attach it to the other side of the core to form an upper chamber dam.

   Note: Failure to slide the dam up completely to the top of the clamp will result in buffer leaking from the upper chamber.

7.2 Loading Samples

1. When the running buffer has reached the correct temperature in the *D GENE* chamber, the samples are ready to be loaded onto the gel. Turn the *D GENE* system off. Disconnect the power cord. Place the core and the attached gel assemblies into the lower buffer chamber. The core goes in the chamber in only one direction. Add approximately 350 ml of 1x TAE buffer to the upper buffer chamber.

   Note: If you want to prerun to equilibrate the system, place the lid on the *D GENE* system, attach cords, and adjust the voltage setting on the power supply.

2. Wash the wells using a 19 gauge needle and syringe with the running buffer to remove any unpolymerized gel material in the wells before loading samples.

3. Place the *D GENE* lid back onto the buffer chamber. Turn the power and heater on. Adjust the temperature setting to the desired temperature (see Section 5.1).

Note: The lid can be attached to the buffer chamber in only one orientation, so that the anode and cathode connections cannot be reversed.

4. Remove the *D GENE* loading lid from the *D GENE* lid.
5. Load the samples into the wells using a pipet and a sequencing loading tip. Be careful not to not pierce the gel during sample delivery. A multichannel pipet with 8 tips can be used.

   Note: The loading volume for the single well comb that is used with the perpendicular gels is about 200–400 µl.

6. Place the *D GENE* loading lid back onto the *D GENE* lid. Turn the pump on. Adjust to the correct temperature.

7.3 Running the Gel

1. Attach the electrical leads to a suitable DC power supply with the proper polarity (this connection could accidentally be reversed).
2. Apply power to the *D GENE* system and begin electrophoresis. You can also run the samples into the gel before turning on the pump. As a safety precaution always set voltage, current, and power limits when possible.

7.4 Removing the Gel

1. After electrophoresis is complete, turn the power supply and *D GENE* system (heater, pump, and main power) off. Disconnect the power cord and electrical leads. Let the heater cool for about 15 seconds in the buffer.
2. Remove the *D GENE* electrophoresis system and place it on the holder. **CAUTION: The heater is still hot. Do not touch**. Carefully pull the core out of the lower buffer chamber. Pour off the upper buffer into the lower buffer chamber by tilting the core into the chamber.
3. Lay the core and gel sandwiches on a padded surface.
   a. For 16 x 10 cm and 7.5 x 10 cm gels, remove the sandwich assembly with your index fingers on the sandwich clamps and your thumbs resting on the latches on the core. Gently remove the assembly by pushing the latch away from the gel sandwich. Pry the sandwich upward and pull (Figure 7.3).
   b. For 16 x 16 cm gels, remove the sandwich assembly with your index fingers below the sandwich clamps and your thumbs resting on the latches on the core. Gently remove the assembly by pulling up toward you (in a manner opposite to the way it was attached). Pull the sandwich assembly off the locating pins on the top of the core.

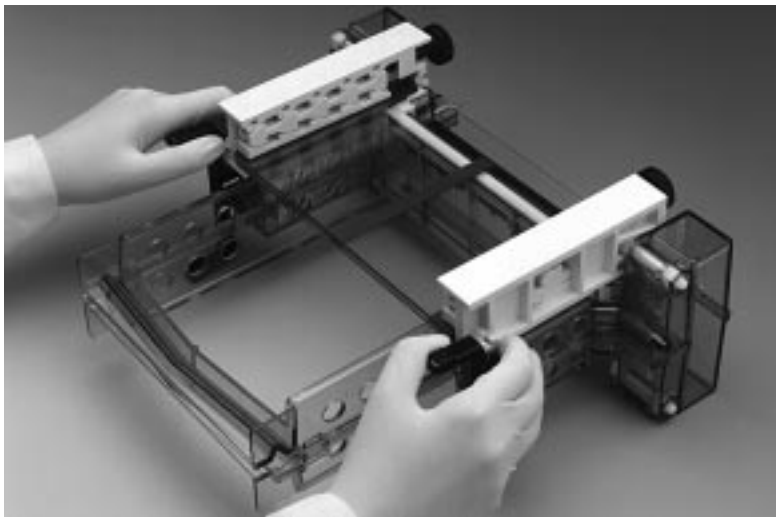

Fig. 7.3. Removing the sandwich assembly from the core.

4. Loosen the single screw of each clamp and remove the clamps from the sandwich. With a spatula or an unused spacer, carefully pry off the shorter glass plate.

5. With a razor blade, gently cut the gel along the spacers. This insures that the gel does not tear when the spacers are removed. Remove the spacers and mark one corner of the gel to distinguish between gels.

7.5 Staining and Photographing the Gel

1. Remove the gel from the glass plate by gently grasping two corners and lifting off. Place the gel into a dish containing 250 ml of 1x TAE buffer and 100 µl of 1 mg/ml ethidium bromide. Stain for about 1–2 minutes. Other dyes such as SYBR-Green I (Molecular Probes) can be used.

2. After staining, carefully transfer the gel into a dish containing 250 ml of 1x TAE buffer. Destain for 5-20 minutes.

3. Place the gel on a UV transilluminator and photograph gel (Bio-Rad's Gel Documentation System, catalog numbers 170-3742 through 170-3749).

   Note: If your are photographing a perpendicular gel, place a ruler on the bottom of the gel with the 0 mark starting on the low density portion of the gel. This will help determine the denaturant concentration at which the wild-type and mutant DNA fragments separate.

4. Gels that have been labeled with radioisotopes must be autoradiographed. Carefully place a 3MM Whatman paper on top of the gel. Gently slide your hand across the paper to adhere the gel to the paper and to remove any air bubbles. Flip the gel over and place Saran Wrap™ plastic wrap evenly on top of the gel without creating any bubbles. This helps to keep the gel intact as well as to prevent any contamination to the gel dryer. Dry in a gel dryer for about 30–60 minutes at 60 °C.

5. Expose the gel to film and develop film.

Section 8
Troubleshooting

Always confirm that the line voltage is correct for the *D GENE* system.

8.1 Equipment

Problem	Cause	Solution
Controller		
No display on power up	Burnt fuse	Replace fuse
	Faulty main switch	Replace main switch
	Broken wire	Replace wire
	Controller malfunction	Replace controller
Buffer Circulation		
No buffer circulation	Buffer level too low	Add buffer up to fill level
	Pump failure	Replace pump
	Faulty pump switch	Replace pump switch
	Clogged tubing or fitting	Replace tubing or fitting
	Broken inlet tube	Confirm that the inlet tube extends about 4.5” from cover
Buffer Heating		
Can not preheat buffer	Buffer level too low	Add buffer to ‘Fill’ level
	Level switch malfunction	Replace level switch
	Cartridge heater burnt	Replace cartridge heater
	Thermal fuse burnt	Replace thermal fuse
	Solid state relay damaged	Replace solid state relay
	Wrong controller setting	Set controller parameter according to controller instruction
	Controller malfunction	Replace controller
	Loose wiring	Repair wiring
Warm-up time too long	Buffer level too low	Add buffer to ‘Fill ‘ level
	Cartridge heater burnt	Replace cartridge heater
	Solid state relay damaged	Replace solid state relay
	Wrong controller setting	Set controller parameter according to controller instruction
	Loading lid not on system	Place Loading lid on system
Non-uniform Temperature		
Stir bar not rotating	Damaged motor	Replace motor
	Loose set screws at stir bar	Tighten set screws
	Stir bar interferes with gel sandwiches	Maximum thickness of gel is 1.5 mm
	Bent stir bar	Replace stir bar
	Buffer level too low	Fill to recommended level
	Broken belt	Replace belt

Problem	Cause	Solution
Excessive noise		
Excessive noise during run	Worn fan	Replace fan
	Damaged pump	Replace pump
	Pump touches cell cover	Move pump by shortening tubing
	Broken inlet tube for buffer	Replace inlet tube assembly circulation
	Stir bar interferes with gel sandwiches	Maximum thickness of gel is 0.5 mm
	Misaligned core or cover	Align cover
	Stir bar not engaged in support at tank properly	Align stir bar in support tank
Casting gels		
Glass plate cracked	Excessive force at thumb screws	Apply about one turn to thumb screw after it touches glass
Perpendicular gradient		
Gel solution leaks	Not sufficient pressure on comb gasket	Make sure Pressure Clamp screws are turned one and one half turns
Perpendicular gradient		
	Bad or wrong comb gasket	Make sure correct comb gasket is used
	Misalignment of spacers and glass plates	Check alignment at bottom of glass sandwiches, use alignment slot at casting stand
	Different thickness of spacers and comb	Use spacers and comb of same thickness
	Poor, dirty inlet fitting or missing O-ring	Replace fitting
	Loose stopcock	Tighten stopcock/injection connection
	No air vent plug	Use plug at vent and close it during gel polymerization
	Misaligned comb gasket	Ensure comb gasket notches are against spacer notches
	Damaged or non Bio-Rad glass plates	Replace with Bio-Rad glass plates only
	Damaged or dirty spacers or combs	Replace spacers or combs
Poor result		
No electrophoresis	Bad cables	Replace cables
	Corroded banana plugs	Repair banana plugs
	Broken electrodes	Repair electrodes

8.2 Applications

Problem	Solution
Perpendicular DGGE	
Only a single band is seen in the "S" curve when at least two bands are expected.	1. Mix normal and mutant DNA prior to the run. 2. Check PCR reaction products for mutant and normal DNA by sequencing or restriction digestion.
Hard to visualize hetero-duplex and homoduplex DNA bands.	1. Increase DNA loading.
Unknown faint bands	1. Impurity or contamination of PCR product.
Poor Gradient. "S" curve not fully seen.	1. Make sure gradient delivery system is working properly. See instructions. 2. Increase upper gradient concentrations.
Parallel DGGE	
Normal and mutant DNA unresolved.	1. Increase or decrease run time. 2. Poor gradient made. Re-cast gradient gel. 3. Recalculate from perpendicular gel or run a time course gel.
CDGE	
Curved band on side lane.	1. Leaking current due to gel spacer problem. Check that side clamps are fully tightened and correct spacer thickness are used.
Air bubbles in gel.	1 Clean glass plates.
Fuzzy DNA bands.	1. Gel in well. Clean wells before use. Make sure clamp over comb in tightened correctly. Check for correct comb thickness with spacer thickness.
Smear at top of gel.	1. Probably genomic DNA, this is OK.
Bands don't migrate far into gel.	1. Increase run time. 2. Decrease acrylamide concentration. 3. Decrease denaturant concentration.
DNA leaks between wells.	1. Acrylamide not polymerized. Add more TEMED and ammonium persulfate. 2. Degas acrylamide solution before casting gel.
Streaking or DNA spikes in gel.	1. Impurities in acrylamide. Filter before use. Check shelf life date of acrylamide solution.

Section 9
Maintenance

Maintenance of Equipment

D GENE system with lid	Remove core and clamps from tank. Replace buffer inside tank with distilled water, turn pump on for 1–2 minutes to rinse pump. Remove water from tank.
Core, tank, clamps	Rinse thoroughly with distilled water after use.
Glass plates, spacer, combs	Wash with a laboratory detergent (catalog number is 161-0722), then rinse with distilled water.

Always inspect the *D GENE* system and replace any damaged components before use. Repair damaged parts by Bio-Rad trained personnel with Bio-Rad approved components only.

The controller retains its tuning parameters in non-volatile memory for 10 years without power. Use unit at least once every 10 years to retain the setting.

Section 10
References

For updated references, please request Bio-Rad's bulletin 1934.

10.1 Applications in Mutation Detection Electrophoresis

Application	Reference numbers
DGGE	
Genes	
16S rRNA gene	52
ABO blood group polymorphism	36
Adenine phosphoribosyltransferase gene (APRT)	186
Adenomatous polyposis coli gene (APC)	85, 95, 119
Alpha-1-antitrypsin gene (AAT)	132, 138
Amyloid precursor protein gene	21
Androgen receptor gene	1, 16, 74, 100, 136
Angiotensinogen gene	80
Apolipoprotein B gene	167
Apolipoprotein E gene	42, 44
Beta-globin gene	49, 86, 94, 151, 153, 160, 184, 196, 210, 211
Blue-sensitive opsin gene	124
Cardiac beta-myosin heavy-chain gene (MHC)	110
CF chromosomes	53, 56
CF transmembrane conductance regulator gene	11, 47, 38, 53, 55, 98, 121, 161, 172
cHa-ras 1 proto-oncogene	155
Clonal antigen receptor gene	169
Collagen gene (COL3A1)	89
Collagen gene (COL4A5)	58, 66, 107
Collagenase gene	140
Cytochrome P-450 21-hydroxylase genes (CYP21)	131
Diabetic nephropathy	80
Dopamine D2 receptor gene	76
F9 gene	174
Factor IX gene	54, 67, 194
Factor VIII gene	34, 48, 63, 90, 109, 142, 148, 182, 183, 187, 190
FAU gene	29
Gamma globin gene	84, 113
Growth hormone receptor gene	41, 51, 189
Gs alpha-gene	9, 31, 32, 81, 108, 139, 159, 170
H2kb DNA	154
HEXA gene	37
HOX2B gene	75

Application	Reference numbers
HPRT gene	10, 19, 24, 25, 72, 79, 91, 92, 111, 136, 152, 166, 176, 178, 192, 200
Human acid beta-glucosidase gene	188
Human hypoxanthine guanine phosphoribosyltransferase gene	112
Human KRAS2 gene	60
Human serotonin receptor gene	39
Human thyroid hormone receptor-beta gene (hTR beta)	120
Hypertension	135
Insulin receptor gene	80, 104, 105
K-ras gene	6, 13, 64, 73, 82
Low density lipoprotein receptor gene (LDLR)	26, 99
Mitochondrial DNA	57, 127
Na+/H+ antiporter gene (APNH)	135, 168, 173
N-ras gene	13, 68, 69, 73
NS gene	208
Ornithine aminotransferase gene (OAT)	71, 102, 103
p53	8, 15, 17, 25, 40, 59, 60, 88, 122, 141, 163, 171
Phenylalanine hydroxylase gene	5, 50
Phosphofructokinase (PFK) gene	65
Pneumocystis carinii gene	106
Porphobilinogen deaminase gene (PBG)	97, 130
Prion protein gene (PrP)	2, 68, 143
Protein C gene	20, 46, 133
Proteoglycan core protein	117
PTH gene	125
RB1 gene	12, 22, 116
Rhodopsin gene	145, 150
Scalloped gene	165
Serotonin receptor gene	33
Thyrotropin receptor gene	32
Type II procollagen gene (COL2A1)	4, 23, 43, 62, 197
von Willebrand factor gene	87, 144
Disease	
Acute intermittent porphyria (AIP)	97, 130
Albright hereditary osteodystrophy	31, 170
Alport syndrome	58, 107
Alport syndrome	66
Alzheimer's disease	21
Androgen insensitivity syndrome (AIS)	74
Autosomal dominant retinitis pigmentosa	145, 150
Beta-thalassemias	49, 86, 151, 160, 184

Application	Reference numbers
Breast cancer	141
Cancer, general	22, 59
Cataracts/retinal detatchment	23
Colorectal adenomas/tumors	8, 17, 95, 163
Coronary heart disease	167
Creutzfeldt-Jakob disease	2, 143
Cystic Fibrosis	3, 11, 35, 47, 53, 55, 98, 121, 121, 161, 172
Ehlers-Danlos syndrome	89
Familial adenomatous polyposis (FAP)	95, 119
Familial hypercholesterolemia (FH)	26, 99
Familial isolated hypoparathyroidism (FIH)	125
Fetal haemoglobin (HPFH)	113
Gaucher disease (GD)	188
Generalized resistance to thyroid hormone syndrome (GRTH)	120
Generalized recessive dystrophic epidermolysis bullosa (RDEB)	140
Gout	200
Gyrate atrophy (GA)	71, 102, 103
Haemoglobinopathies	14
Hemoglobin cocody variant	27
Hemophilia A	45, 48, 63, 90, 109, 128, 142, 148, 182, 183, 187
Hemophilia B Leyden	54, 194
Hepatocellular carcinoma	15, 88, 64
HIV-1	18
Hypertrophic cardiomyopathy	110
Inherited hemoglobinopathies	101
Laron syndrome	41, 51, 189
Lung tumour	13, 73, 171
Lymphoid neoplasia	169
Male Pseudohermaphroditism	16
McCune-Albright syndrome (MAS)	108, 139
Medulloblastoma	122
Mitochondrial disease	127
Myoclonic Epilepsy and Ragged-Red Fibres (MEFFR)	57
Nanomelia	117
Non-insulin-dependent diabetes mellikus (NIDDM)	104, 147
Ornithine transcrbamylase deficiency (OTC)	179
Osteoarthritis	62
Osteochondrodysplasia	43
Pancreatic tumors	29, 82
Parathyroid tumors	29
Phenylketonuria	5, 50

10.2 Mutation Detection Electrophoresis References

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Section 11
Systems, Accessories, and Reagents for Mutation Detection Electrophoresis

For updated prices in the U.S., please request Bio-Rad's bulletin 1935. For specifications, please request Bio-Rad's bulletin 1936.

Catalog Number	Product Description
170-9060	***D GENE* Complete System,** 16 cm, 120 V
170-9061	***D GENE* Complete System,** 16 cm, 100 V
170-9062	***D GENE* Complete System,** 16 cm, 220/240 V
170-9063	***D GENE* Complete Mini System,** 10 cm, 120 V
170-9064	***D GENE* Complete Mini System,** 10 cm, 100 V
170-9065	***D GENE* Complete Mini System,** 10 cm, 220/240 V
170-9006	**Converter Kit to 16 cm,** *D GENE* system
170-9007	**Converter Kit to 10 cm,** *D GENE* system
170-9008	**Comb,** 16 well, 0.75 mm, *D GENE* system
170-9009	**Comb,** 16 well, 1.00 mm, *D GENE* system
170-9010	**Comb,** 16 well, 1.50 mm, *D GENE* system
170-9011	**Glass Inner,** 10 cm, 2, *D GENE* system
170-9012	**Glass Outer,** 10 cm, 2, *D GENE* system
170-9013	**Glass Inner,** 16 cm, 2, *D GENE* system
170-9014	**Glass Outer,** 16 cm, 2, *D GENE* system
170-9016	**Tubing Kit,** luer inlet fitting, 2, *D GENE* system
170-9017	**Comb,** 2 well, 0.75 mm, 10 cm, *D GENE* system
170-9018	**Comb,** 2 well, 1.00 mm, 10 cm, *D GENE* system
170-9019	**Comb,** 2 well, 1.50 mm, 10 cm, *D GENE* system
170-9020	**Spacer Kit,** 16 cm, 0.75 mm, 1 pair, *D GENE* system
170-9021	**Spacer Kit,** 16 cm, 1.00 mm, 1 pair, *D GENE* system
170-9022	**Spacer Kit,** 16 cm, 1.50 mm, 1 pair, *D GENE* system
170-9023	**Spacer Kit,** 10 cm, 0.75 mm, 3 piece set, *D GENE* system
170-9024	**Spacer Kit,** 10 cm, 1.00 mm, 3 piece set, *D GENE* system
170-9025	**Spacer Kit,** 10 cm, 1.50 mm, 3 piece set, *D GENE* system
170-9026	**Casting Stand Sponge,** *D GENE* system
170-9027	**Stand,** *D GENE* system
170-9028	**Tank,** *D GENE* system
170-9029	**Top Gasket Holder,** *D GENE* system
170-9030	**Top Gasket,** 1.0 mm, *D GENE* system

Catalog Number	Product Description
170-9031	**Top Gasket,** 1.5 mm, *D GENE* system
170-9032	***D GENE* Electrophoresis Reagent Kit**
170-9033	**Replacement Core Gasket,** 2
170-9034	**MacMelt Software**
170-9038	***D GENE* Control Reagent Kit**
170-9039	**Casting Stand,** *D GENE* system
170-9040	**Clamp Assembly,** 10 cm, 1 pair, *D GENE* system
170-9041	**Clamp Assembly,** 16 cm, 1 pair, *D GENE* system
170-9042	***D GENE* Model 475 Gradient Delivery System**
170-9043	**Core,** *D GENE* system
170-9044	**Comb,** 1 well, 0.75 mm, 16 cm, *D GENE* system
170-9045	**Comb,** 1 well, 1.00 mm, 16 cm, *D GENE* system
170-9046	**Comb,** 1 well, 1.50 mm, 16 cm, *D GENE* system
170-9047	**Filler spacer,** for single 7.5 x 10 cm gel, 0.75 mm
170-9048	**Filler spacer,** for single 7.5 x 10 cm gel, 1.00 mm
170-9049	**Filler spacer,** for single 7.5 x 10 cm gel, 1.50 mm
170-9053	**Replacement belt for stirring bar**
170-9054	**Tubing kit,** Model 475 Gradient Delivery System
170-9055	**Pressure Clamp,** 10 cm
170-9056	**Pressure Clamp,** 16 cm
170-- 057	**Comb,** 32 well, 1.0 mm, 1⅜ cm, *D GENE* System

Bio-Rad Laboratories

Life Science Group

2000 Alfred Nobel Drive
Hercules, California 94547
Telephone (510) 741-1000
Fax: (510) 741-5800
www.bio-rad.com

Australia, *Bio-Rad Laboratories Pty. Ltd., Block Y, Unit 1, Regents Park Industrial Estate, 391 Park Road, Regents Park, NSW 2143 Phone 02 9914 2800 • Fax 02 9914 2889*
Austria, *Bio-Rad Laboratories Ges.m.b.H., Auhofstraße 78D, A-1130 Wien • Phone (01)-877 89 01 • Fax (01)-876 56 29*
Belgium, *Bio-Rad Laboratories S.A.-N.V., Begoniastraat 5, B-9810 Nazareth • Phone 09-385 55 11 • Free Phone 0800/97032 • Fax 09-385 65 54*
Brazil, *Bio-Rad Laboratories (Brazil), Rua dos Invalidos 212 - 5 andar, Lapa - Rio de Janeiro - RJ, CEP 20331-020 • Phone 55 21 507 6191*
Canada, *Bio-Rad Laboratories (Canada) Ltd., 5671 McAdam Road, Mississauga, Ontario L4Z 1N9 • Phone (905) 712-2771 • Fax (905) 712-2990*
China, *Bio-Rad China (Beijing), Rm 615, Shang Fang Plaza, No. 27, North Third Round Center Road, West District, Beijing 100029 Phone 86-10-8201-1366/68 • Fax 86-10-8201-1367*
Denmark, *Bio-Rad Laboratories, Generatorvej 8 C, 2730 Herlev • Phone 45 44 52-1000 • Fax 45 44 52-1001*
Finland, *Bio-Rad Laboratories, Pihatörmä 1A, FIN-02240 Espoo • Phone 358 (0)9 804 2200 • Fax 358 (0)9 804 1110*
France, *Bio-Rad Laboratories, 3, Boulevard Raymond Poincaré, 92430 Marnes-la-Coquette • Phone 01 47 95 69 65 • Fax 01 47 41 9133*
Germany, *Bio-Rad Laboratories GmbH, Heidemannstraße 164, D-80939 München, Postfach 45 01 33, D-80901 München Phone 089 318 84-177 • Fax 089 318 84-123*
Hong Kong, *Bio-Rad Pacific Ltd., Unit 1111, 11/F, New Kowloon Plaza, 38 Tai Kok Tsui Road, Tai Kok Tsui, Kowloon Phone 852-2789-3300 • Fax 852-2789-1257*
India, *Bio-Rad Laboratories (India) Pvt. Ltd., B&B1, Enkay Towers Vanijyanikunj, Udhyog Vihar Phase V, Gurgaon, Haryana 122016 Phone (91-124)-6398112/113/114 • Fax (91-124)-6398115*
Israel, *Bio-Rad Laboratories, Ltd., 14 Homa Street, P.O. Box 5044, Rishon Le Zion 75150 • Phone 03 951 4124 • Fax 03 951 4129*
Italy, *Bio-Rad Laboratories S.r.l., Via M. Peroglio 23, 00144 Rome • Phone 34 91 590 5200 • Fax 34 91 590 5211*
Japan, *Nippon Bio-Rad Laboratories KK, 7-18 Higashi-Nippori 5-chome, Arakawa-ku Tokyo 116-0014 • Phone 03-5811-6270 • Fax 03-5811-6272*
Korea, *Bio-Rad Korea Ltd., Cambridge Building, 1461-15 Seocho-Dong Seocho-Ku, Seoul 137-070 • Phone 82-2-3473-4460 • Fax 82-2-3472-7003*
Latin America, *Bio-Rad Latin America, 14100 Palmetto Frontage Road, Suite 101, Miami Lakes, Florida USA 33016 • Phone 305-894-5950 • Fax 305-894-5960*
Mexico, *Bio-Rad Laboratorios Mexico, Adolfo Prieto No. 1653, Col. De Valle, CP. 03100, Mexico D.F. • Phone 52 5 534 2552 to 54 • Fax 52 5 524 5971*
The Netherlands, *Bio-Rad Laboratories B.V., Fokkerstraat 10, 3905 KV Veenendaal • Phone 0318-540666 • Fax 0318-542216*
New Zealand, *Bio-Rad Laboratories Pty Ltd., PO Box 300-571, Albany, Auckland • Phone 64-9-4152280 • Fax 64-9-443 3097*
Norway, *Bio-Rad Laboratories, Johan Scharffenbergs vei 91, N-0694 Oslo • Phone 47-23-38-41-30 • Fax 47-23-38-41-39*
Russia, *Bio-Rad Laboratorii, ul. Butirskaya 79 "B", office 156 RF-125015 Moscow • Phone 7 095 979 98 00 • Fax 7 095 979 98 56*
Singapore, *Bio-Rad Laboratories, Singapore, 211 Henderson Rd. #03-02, Henderson Industrial Park, 159552 • Phone 65-2729877 • Fax 65-2734835*
Spain, *Bio-Rad Laboratories, S.A., Lopez de Hoyos, 245-247, 28043 Madrid • Phone 34-91-590-5200 • Fax 34-91-590-5211*
Sweden, *Bio-Rad Laboratories AB, Vintergatan 1, Box 1097, S-172 22 Sundbyberg • Phone 46 (0)8-55 51 27 00 • Fax 46 (0)8-55 51 27 80*
Switzerland, *Bio-Rad Laboratories AG, Nenzlingerweg 2, CH-4153 Reinach • Phone 061-717-9555 • Fax 061-717-9550*
United Kingdom, *Bio-Rad Laboratories Ltd., Bio-Rad House, Maylands Avenue, Hemel Hempstead, Hertfordshire HP2 7TD Phone 0181 328 2000 • Free Phone 0800-181134 • Fax 01442-259118*

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