

INSTRUCTION MANUAL

Denaturing Gradient Gel Electrophoresis Systems

DGGE-1001
DGGE-2001
DGGE-2401
DGGE-4001
DGGE-4801

DGGEK-1001
DGGEK-2001
DGGEK-2401
DGGEK-4001
DGGEK-4801

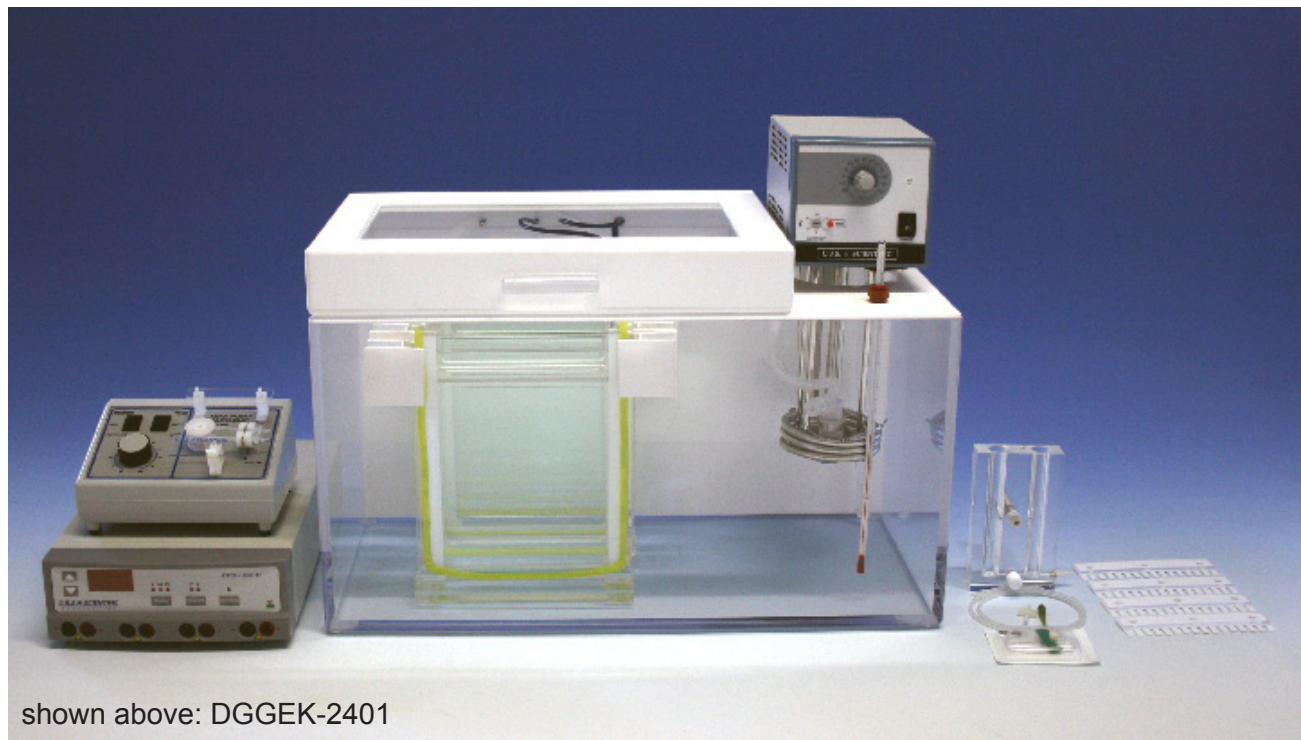


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IMPORTANT USER INFORMATION

This Instruction Manual will explain how to use this product safely and effectively. Please read and carefully follow the instruction manual in its entirety.



The triangle/exclamation mark symbol alerts the user of the product to important operational, maintenance, and/or warranty requirements.



The triangle/lightning bolt symbol alerts the user of the product to potentially hazardous electrical exposure.



Failure to adhere to the instructions could result in personal and/or laboratory hazards, as well as invalidate any warranty. Always turn off the DC power source prior to disconnecting power cords from the product. Disconnect power cords from the power source first, and then from the product. For maximum safety, always operate this system in an isolated, low traffic area, not accessible to unauthorized personnel. Never operate damaged or leaking equipment.

WARRANTY AND LIABILITY

This product was produced utilizing the highest practical standards of materials, workmanship, and design. C.B.S. Scientific warrants that the product has been tested and will meet or exceed published specifications. This warranty is valid only if the product has been operated and maintained according to the instructions provided.

C.B.S. Scientific warrants this product to be free from defects in materials and workmanship under normal service for one year from date of shipment. If the product proves defective during this period, C.B.S. Scientific will repair or replace it at our option, free of charge, if returned to us postage prepaid. This warranty does not cover: damage in transit, damage caused by carelessness, misuse or neglect, normal wear through frequent use, damage caused by solvent corrosion, damage caused by improper handling or user alteration, nor unsatisfactory performance as a result of conditions beyond our control. C.B.S. Scientific shall in no event be liable for incidental nor consequential damages, including without limitation, lost profits, loss of income, loss of business opportunities, loss of use and other related damages, however caused, nor any damage arising from the incorrect use of the product.

<p>FRANÇAIS INFORMATION IMPORTANTE À L'USAGE DES UTILISATEURS</p> <p>Le présent manuel d'utilisation explique la manière de se servir efficacement du produit en conditions de sécurité. Il est recommandé de soigneusement lire la totalité du manuel, avec ses consignes et ses instructions.</p> <p> Le triangle avec point d'exclamation est un symbole destiné à avertir l'utilisateur du produit de l'importance de certaines exigences relatives au fonctionnement, à l'entretien et/ou à la garantie.</p> <p> Le triangle avec flèche en zigzag est un symbole destiné à avertir l'utilisateur du produit de la possibilité d'exposition à des décharges avec danger de secousses électriques.</p> <p> Tout manquement à l'observation des consignes et des instructions peut exposer les personnes et les biens à des dommages corporels et/ou matériels et peut annuler toute garantie. Il faut toujours interrompre l'alimentation de courant continu avant de déconnecter les cordons d'alimentation du produit. Déconnecter d'abord les cordons d'alimentation branchés sur la source de tension (alimentation de secteur) puis ceux branchés sur le produit. Pour une sécurité maximum, il faut toujours faire fonctionner ce système dans un lieu isolé, peu fréquenté, où le personnel non autorisé n'a pas accès. Ne jamais faire fonctionner un matériel endommagé ou affecté par des fuites.</p> <p>GARANTIE ET RESPONSABILITÉ</p> <p>Le produit a été fabriqué conformément aux normes applicables les plus exigeantes en matière de matériaux, de main d'œuvre, de conception et d'ingénierie. C.B.S. Scientific garantit que le produit a subi des essais et que ses performances rempliront les conditions des spécifications publiées ou leur seront même supérieures. La présente garantie n'est valide que si le produit a fonctionné et a été entretenu conformément aux consignes et instructions fournies.</p> <p>C.B.S. Scientific garantit que le produit sera dépourvu de vices de matériaux et de main d'œuvre, en conditions de service normales, pendant un an à compter de la date d'expédition. Au cas où le produit s'avérerait défectueux pendant cette période de garantie, C.B.S. Scientific réparera ou remplacera le produit, à sa discrétion et gratuitement, si le produit lui est retourné port payé d'avance. La garantie ne couvre pas les dommages de transport, les dommages causés par l'imprudence, le manque de soins, l'abus ou la négligence, l'usure normale résultant d'une utilisation fréquente, les dommages causés par la corrosion des solvants; et les dommages causés par la manipulation inadéquate ou des changements apportés par l'utilisateur. La garantie ne couvre pas non plus les performances non satisfaisantes résultant de conditions hors du contrôle de C.B.S. Scientific. C.B.S. 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Sirvase leerlo en su totalidad y seguir detenidamente las indicaciones que contiene.</p> <p> El símbolo del triángulo con exclamación llama la atención del usuario a requisitos importantes para el uso y mantenimiento del producto, así como para la validez de la garantía.</p> <p> El símbolo del triángulo con rayo llama la atención del usuario a la posibilidad de riesgos eléctricos.</p> <p> El incumplimiento de las instrucciones aquí señaladas podría dar lugar a riesgos a la persona, al laboratorio o a ambos y podría anular toda garantía. Siempre apague la fuente de corriente continua antes de desenchufar los cables eléctricos del producto. Primero desconecte los cables de la fuente de energía y después del producto. Para mayor seguridad, siempre use este sistema en un área aislada, de poco movimiento de personas e inaccesible a personal no autorizado. Jamás use equipo que presenta algún daño o fuga.</p> <p>GARANTÍA Y RESPONSABILIDAD</p> <p>Este producto fue fabricado de acuerdo con las normas más estrictas que sean factibles en cuanto a materiales, mano de obra y diseño. C.B.S. Scientific garantiza que se sometió el producto a pruebas y que cumplirá o excederá las especificaciones publicadas. Esta garantía será válida únicamente si se usa y se da servicio de mantenimiento al producto de acuerdo con las instrucciones señaladas.</p> <p>C.B.S. Scientific garantiza que este producto se encontrará libre de defectos de materiales y mano de obra por un periodo de servicio normal de un año a partir de la fecha de embarque. Si el producto resulta defectuoso durante este periodo, C.B.S. Scientific lo reparará o lo repondrá, a criterio de C.B.S., libre de cargos, si se devuelve el producto a C.B.S. porte pagado. 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Um höchste Sicherheit zu gewährleisten sollte dieses System in einem abgesonderten und besonders ruhigen Bereich eingesetzt werden und vor Unbefugten sicher sein.</p> <p>GARANTIE UND HAFTUNG</p> <p>Dieses Produkt wurde unter Anwendung von Produkten mit höchster Qualität und aus Materialien mit bester Verarbeitung und modernstem Design hergestellt. C.B.S. Scientific garantiert, daß das Produkt getestet wurde und alle publizierten Spezifikationen übertrifft. Diese Garantie ist jedoch nur gültig, wenn das Produkt nach der beigefügten Bedienungsanleitung bedient und gewartet wurde.</p> <p>C.B.S. Scientific garantiert, daß dieses Produkt bei normaler Bedienung aus fehlerfreiem Material besteht und fehlerfrei in der Ausführung ist. Diese Garantie gilt für ein Jahr ab Lieferdatum. Sollte das Produkt in diesem Zeitraum fehlerhaft werden, bietet C.B.S. Scientific eine kostenlose Reparatur bzw. kostenlosen Ersatz, einschließlich freiem Rückporto. 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Si preghi di leggere e seguire con cautela le istruzioni di ogni parte di questo manuale.</p> <p> Il triangolo contenete il simbolo di un punto esclamativo avverte l'utente di importanti requisiti relativi al funzionamento, manutenzione e/o garanzia del prodotto.</p> <p> Il triangolo contenete il simbolo di un lampo avverte l'utente del prodotto della possibilità di pericoli dovuti a corrente elettrica.</p> <p> La mancata osservanza delle istruzioni può essere causa di pericolo alla propria persona ed al laboratorio, oltre a poter annullare la garanzia. Prima di distaccare il cordone d'alimentazione dal prodotto, spegnere sempre la sorgente di corrente continua. Distaccare i cordoni d'alimentazione prima dal lato della sorgente di tensione e poi dal lato del prodotto. Per maggior sicurezza, mettere sempre in funzione il prodotto in un'area isolata con poco traffico che non sia accessibile al personale non autorizzato. Non mettere mai in funzione un'apparecchiatura che sia danneggiata o abbia perdite.</p> <p>GARANZIA E RESPONSABILITÀ</p> <p>Questo prodotto è stato fabbricato seguendo gli standard più elevati per i materiali, la manodopera e la progettazione. La C.B.S. Scientific garantisce il prodotto è stato sottoposto a prova e raggiunge o supera i valori pubblicati per i dati tecnici. Questa garanzia è valida solo se il prodotto è messo in esercizio e soggetto a manutenzione secondo le istruzioni fornite.</p> <p>La C.B.S. Scientific garantisce che questo prodotto è libero di difetti di materiali e manodopera, in normali condizioni d'esercizio, per la durata di un anno dalla data di spedizione. Se, in questo periodo, il prodotto si dimostrerà difettoso, la C.B.S. Scientific, a suo giudizio, lo riparerà o sostituirà. 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SECTION 1
General Information
1.1 Introduction

Denaturing Gradient Gel Electrophoresis (DGGE) is a powerful genetic analysis technique that can be used for detecting single base changes and polymorphisms in genomic (1, 2), cloned, and PCR amplified DNA (3,4). Two of the most valuable uses for DGGE in human genetics are in directly detecting single base changes that cause disease and in detecting polymorphisms with DNA probes for genetic-linkage analysis. Also, DNA fragment melting points can be determined using Perpendicular DGGE (1).

C.B.S. Scientific has designed five different DGGE Systems, which are reliable and easy-to-use. The DGGE-1001 is a 2gel system and features one dual gel cassette. The DGGE-2001 features two single gel cassettes. The DGGE-2401 has the same buffer tank as the DGGE-1001 and DGGE-2001 but features two dual gel cassettes, for a capacity of 4 gels per run. Also available is the DGGE-4001, which has a larger buffer tank and four single cassettes. Finally there is the DGGE-4801, which features the larger buffer tank and four dual cassettes enabling the researcher to run up to 8 gels simultaneously. Improvements to the technique include: a simplified method for casting perpendicular and vertical gels using Gel Wrap®, single or dual gel cassettes have been redesigned eliminating the use of agarose plugs, the need for an external peristaltic pump for buffer cycling has been eliminated, bulky screw clamps have been replaced with polypropylene spring clamps, buffer tank dimensions have been changed to use bench space more efficiently, and a safety cover with an electrical interlock has been added for protection and to help maintain temperature and reduce evaporation.

1.2 Specifications

Constructions

DGGE Tank	Acrylic
DGGE Lid	Sodalime glass, acrylic
Electrodes	Platinum wire .010" diameter
Power cords	FR Silicone, rated 7500V, 200mA, 65° C
DGGE Cassette	Acrylic
Heater/Stirrer Buffer Cycler	Stainless steel
Combs	Teflon
Spacers	Polymer
Glass Plates	Sodalime or Borosilicate
Gradient Maker	Acrylic, teflon, stainless steel

	DGGE-1001	DGGE-2001	DGGE-2401	DGGE-4001	DGGE-4801
Shipping weight	40lbs	58lbs	58lbs	68lbs	108lbs
Gel Size	15x20cm	15x20cm	15x20cm	15x20cm	15x20cm
Buffer Volume	15.2 L**	21 L	21L		
Distance between electrodes	20cm	20cm	20cm	20cm	20cm
Voltage limit	250	250	250	250	250

**requires the use of a buffer displacement chamber

1.3 Safety



Power to the DGGE systems is to be supplied by an external DC voltage power supply that must be ground isolated so that the DC voltage output floats with respect to ground. For any power supply used, the maximum specified operating parameters for the units are:

Maximum Limits

250 VDC voltage

30 watts power

80 mA current

70°C ambient temperature



Current to the unit, provided from the external power supply, must enter the unit through the lid assembly, providing a safety interlock to the user. DC current to the unit is broken when the lid is opened. **Do not attempt to use the unit without the safety lid. Always turn the power supply off before removing the lid, or when working with the unit in any way. Follow safety precautions specified by the power supply manufacturer.**



Input Power: Mains to safety interlock

Depending on country of destination, input voltage from mains electrical supply are as follows: 110-120VAC @ 50/60Hz/5 Amps or 200-240VAC @ 50/60Hz/5 Amps. Country specific power cords or CE approved adapter kits are supplied with each system.

SECTION 2

Description of Parts

2.1 Unpacking

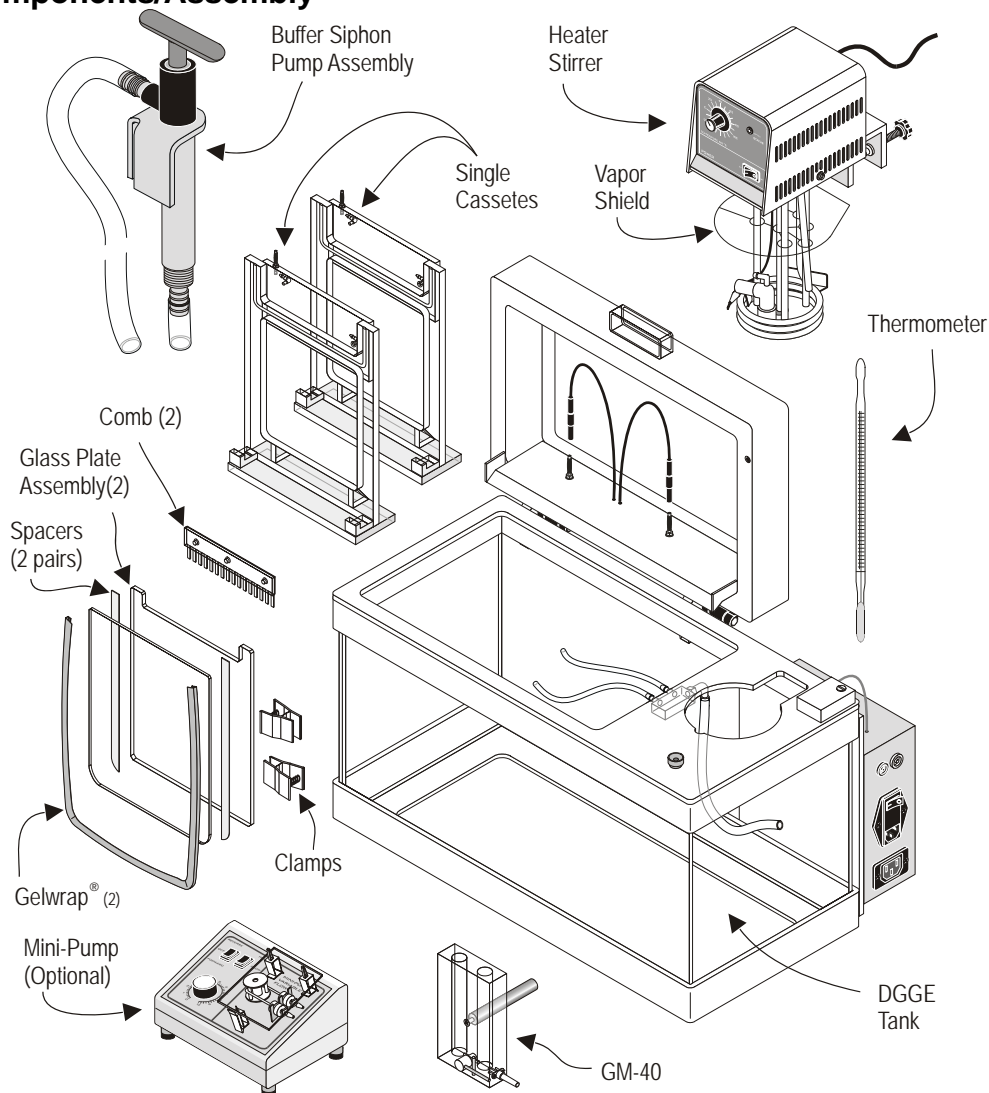
Please verify that your DGGE or DGGEK system comes complete with the following components:

- Lower Reservoir/Glass tank/safety interlock/ with 2 black leads inside lid
- Heater/Stirrer/By-pass pump with clear plastic vapor shield
- Gel cassette(s) as specified (single or dual, single cassettes shown in Figure 2.1)
- 1 Thermometer
- 1 Gradient maker, 20mls per side, Cat. # GM-40
- 2 each combs, 2 sets glass plates, 2 pair spacers and 2 each Gel Wrap® gaskets
- White spring clamps, 12 each GPC-0002-177 and 16 each GPC-0001-177 (Number of clamps received with system will vary depending on format of DGGE)
- Buffer siphon pump with tubing, Cat# BSP-1000

DGGEK Series also includes:

- EPS-300 or EPS-300-II Power supply
- Peristaltic Mini-Pump for gel casting

2.2 Components/Assembly



SECTION 3 Instructions for Use

3.1 Unit Set-up and Unpacking Instructions



1. Unpack lower reservoir and place on level surface in an approved location
2. Install Heater/Stirrer as shown below. Secure in place with thumbscrew. Be certain that the clear plastic vapor shield is between the bottom side of the heater control unit and the TOP surface of the tank lid. Small bore silicone tubing for buffer cycling is already attached to the manifold outlets. Connect large bore tubing from bypass pump to barb fitting on plastic manifold on underside of lid. **WARNING: Do NOT turn on Heater/Stirrer until tank has been filled with buffer.**

Heater/Stirrer/Bypass Pump Installation

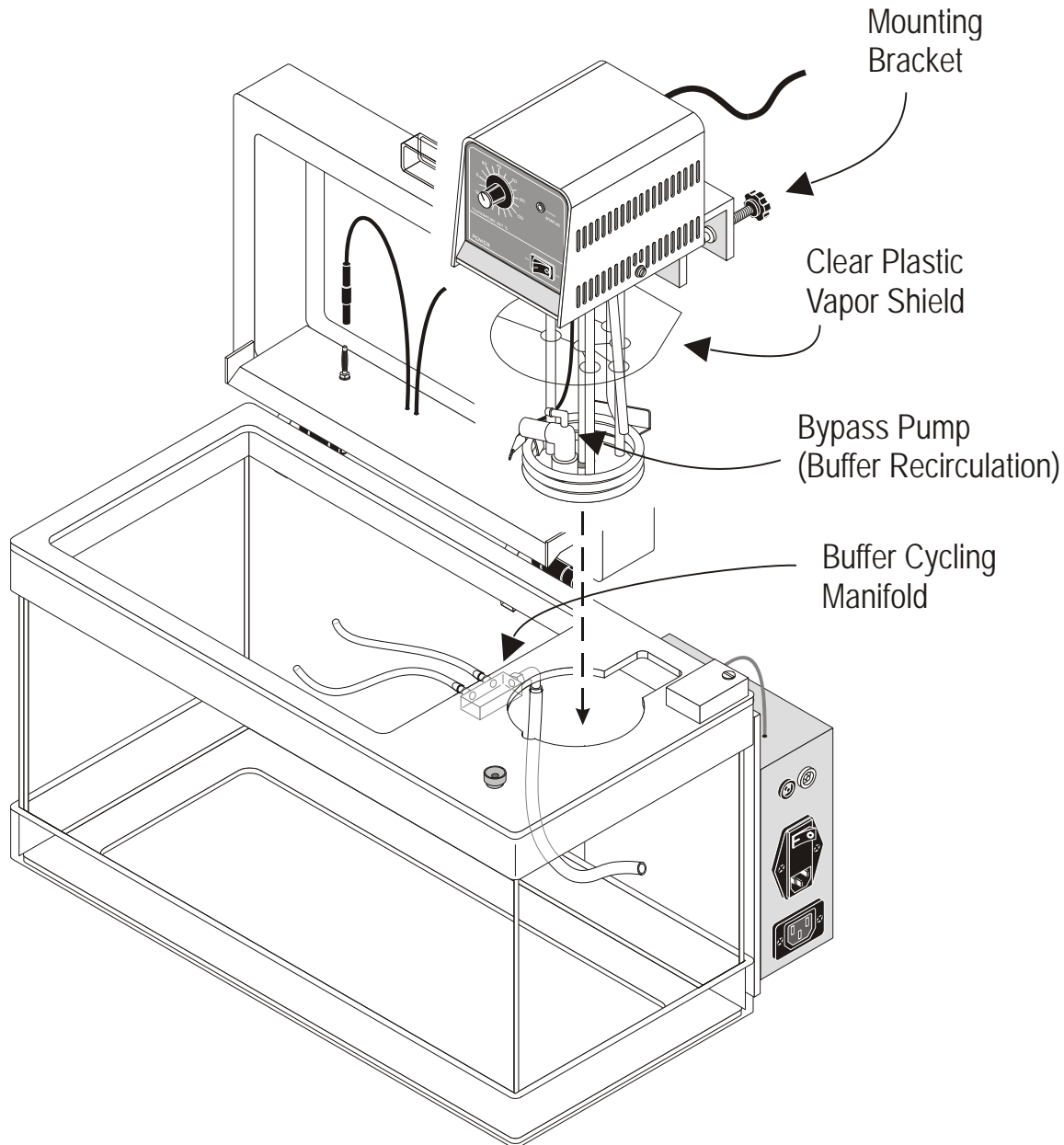


Fig. 3-1

3.1 Unit Set-up and Unpacking Instructions-continued

3. Install thermometer into red silicone stopper on lid. Moisten before inserting. See Figure below.

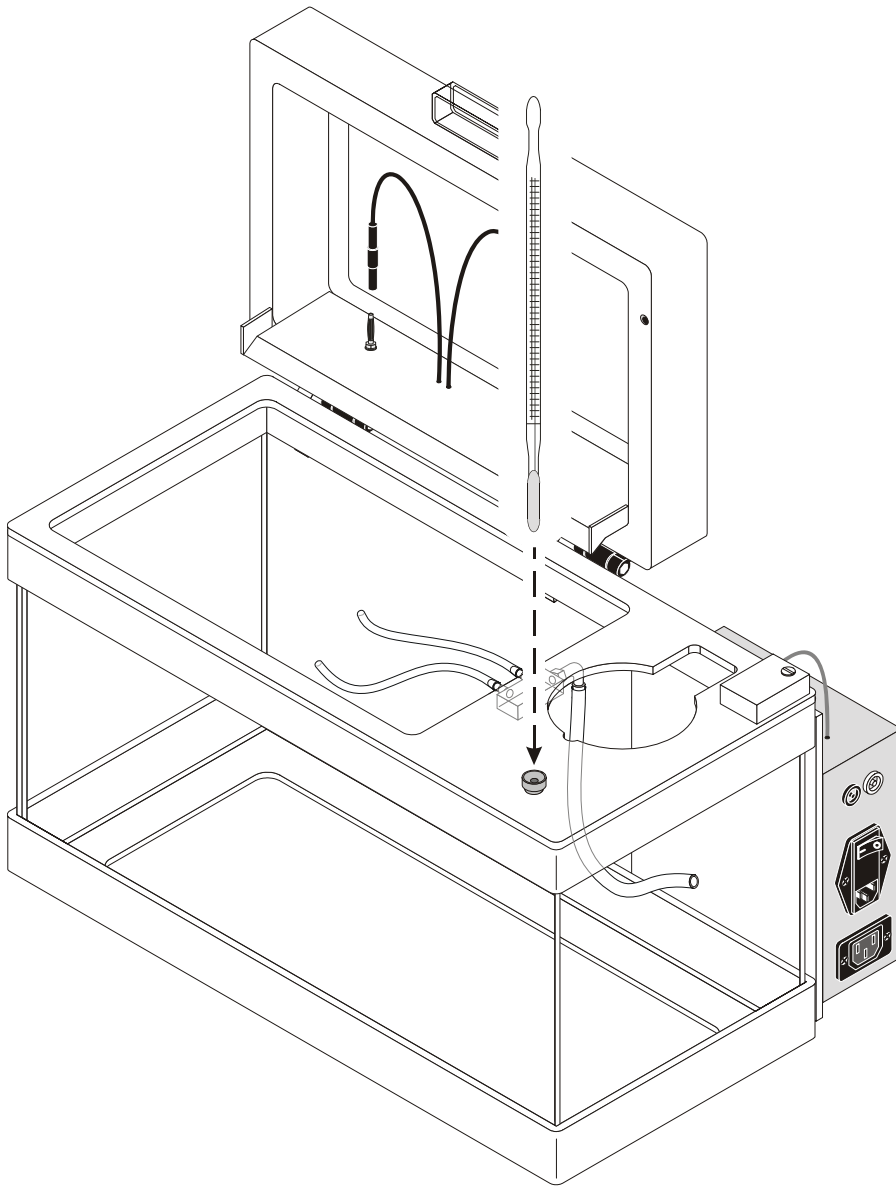


Fig. 3-2

3.1 Unit Set-up and Unpacking Instructions-continued

4. Position an appropriate power supply on a shelf above the tank.
5. Fill the tank with running buffer (see chart page 5) “ To obtain the correct level of buffer, place the cassettes in the tank and fill the tank until the level of buffer reaches the underside of the upper reservoir. The upper reservoir holds only 50mls.
6. Attach the power supply to the safety interlock. Refer to Figure below.
7. Attach the safety interlock to a wall (mains) outlet.
8. Plug the Heater/Stirrer into a wall (mains) outlet.
9. Do NOT attach power leads (from power supply to safety interlock) until gels are loaded and installed in the tank.



Power Supply/Safety Interlock Connections

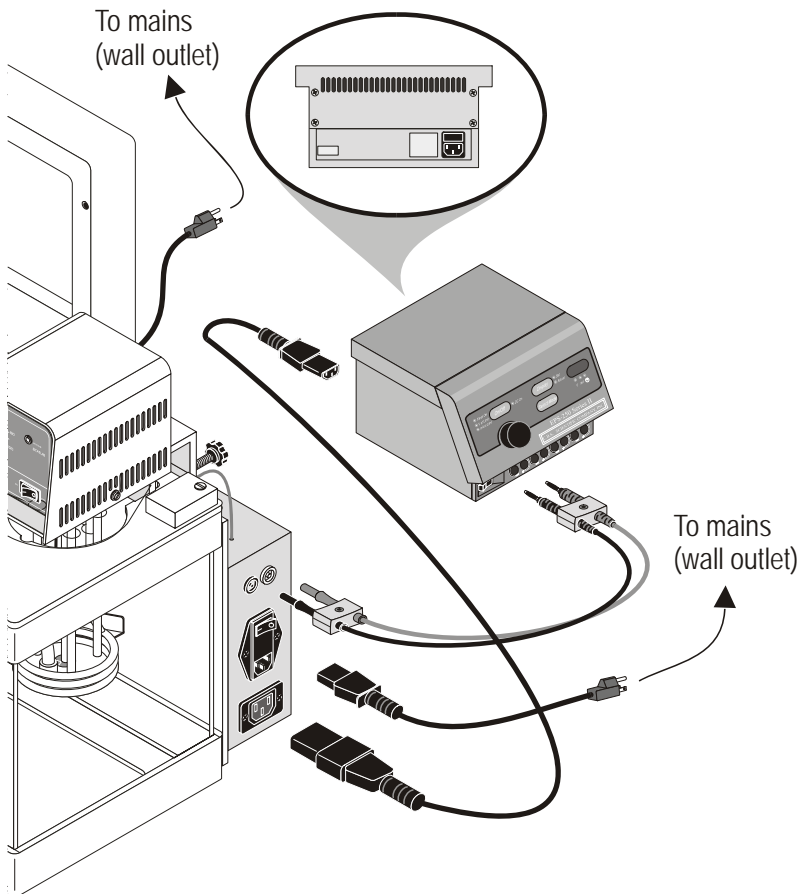


Fig. 3-3

3.1 Unit Set-up and Unpacking Instructions-continued



10. Turn Heater/Stirrer “On” and set temperature to 60°C (Figure 3.2)

Temperature Setting 60°C

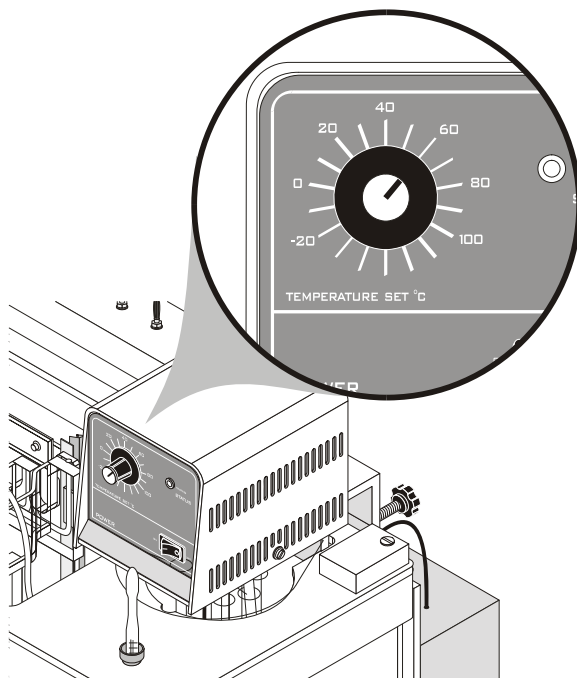


Fig. 3-4

3.2 Preparation/Cleaning of Glass Plate for Gel Casting

Hand wash both plates with a high quality lab detergent followed by a complete rinsing with dH₂O. Air dry or use a lint-free tissue. Spray/wipe the chosen inner surfaces of the plate set with 95% ethanol and dry with lint-free tissue.

3.3 Gel Casting Techniques.

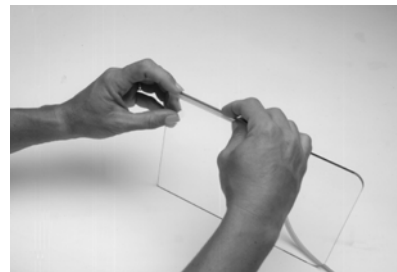
- A. Gel Wrap™ Gasket Casting Method.
- B. Vertical gradient gel casting using GM-40 gradient maker and gravity flow.
- C. Vertical gradient gel casting using GM-40 gradient maker and a Mini-pump.
- D. Vertical gradient gel casting using GM-40 gradient maker, Mini-pump and Multi-gel Caster.

NOTE: Prior to casting, mark the notched glass plate to designate which side is the 'inside' and 'outside'. The same side of the notched plate should always sit against the reservoir gasket.

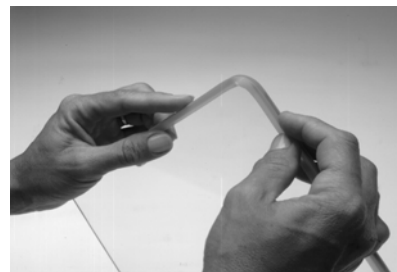
A. Gel Casting using Gel Wrap™ Gasket Casting method

For Vertical DGGE, use the set of spacers which do not have the small hole or channel milled into the lower end, these are for casting perpendicular gels.

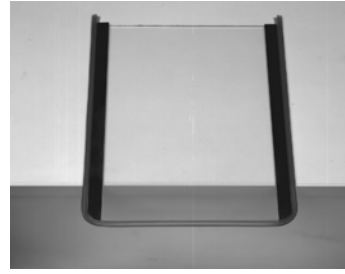
1. Start by holding the rectangular back plate with the rounded bottom corners and start applying the gasket around one side of the glass plate. Note: one side of the "U" shaped gasket is flat, and the other side has tubing that will act as a seal around the spacers.



2. When applying the gasket over the rounded corners of the back glass plate, make sure the notches on the gasket align with the rounded corners of the glass plate. Once the gasket is pushed over the bottom edge and corners, work it down the remaining side.



3. Place the gasketed plate on the lab bench with the tubing side up, and extend the bottom of the plate over the edge of the bench, approximately $\frac{3}{4}$ of an inch. Place the spacers along side the inside edges of the gasket. Be sure the rounded corner end of each spacer is facing the outside bottom of the plate, following the radius of the glass.



3.3 Gel Casting Techniques.

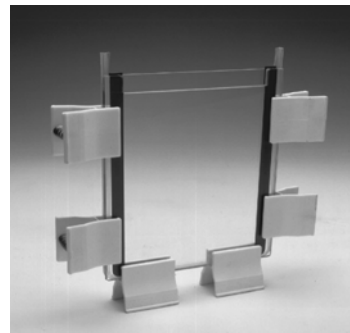
A. Gel Wrap™ Gasket Casting method-continued



4. Place the notched plate on top of the bottom assembly, starting from the bottom edge and gently easing the plate down. Verify the gasket is smooth around the edges and then clamp along the bottom.



5. Lift the assembly and stand it on the base of the clamps. For leveling, push glass plate assembly down until it stops against clamp body. Clamp the sides of the assembly with additional casting clamps on either side. As each clamp is attached, be sure the gasket is aligned between the plates forming a seal.



3.3 Gel Casting Techniques- continued

B. Vertical gradient gel casting using GM-40 gradient maker and gravity flow.

1. Place the GM-40, gradient maker, on an elevated magnetic stirrer with a small “flea” stir bar in the cylinder (C-2) closest to the outlet. The gradient maker should be fitted with a leur valve, (V-2) and a 20ga needle with attached tubing to deliver acrylamide to the gel plate sandwich as shown in figure (3.5). With the height differential between the leur valve (V-2) and the top of the gel plate sandwich between 2-4 inches, it takes approximately 5-9 minutes to cast the gel. Raising the height of the gradient maker can increase flow rate. Flow can be regulated by turning leur valve (V-2). Flow rate appropriate for each gradient should be determined empirically.

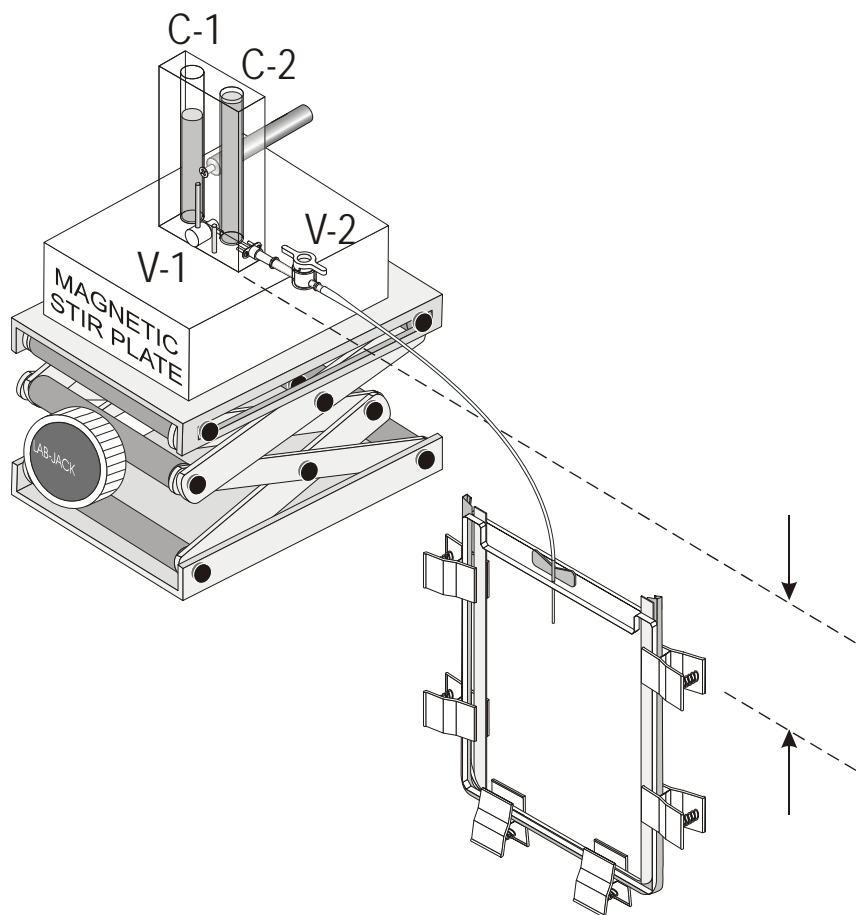


Fig. 3-5

3.3 Gel Casting Techniques- continued

C. Vertical gradient gel casting using using gradient maker (GM-40) and a mini-pump.

1. Alternatively, you may choose to use a “mini-pump” or other peristaltic pump to cast gels as shown in fig (3.6). If so, secure the gradient maker to a ring stand and connect the outlet tubing to the mini-pump tubing adapter. Connect tubing from mini-pump to a 20ga. needle for affixing between glass plates.

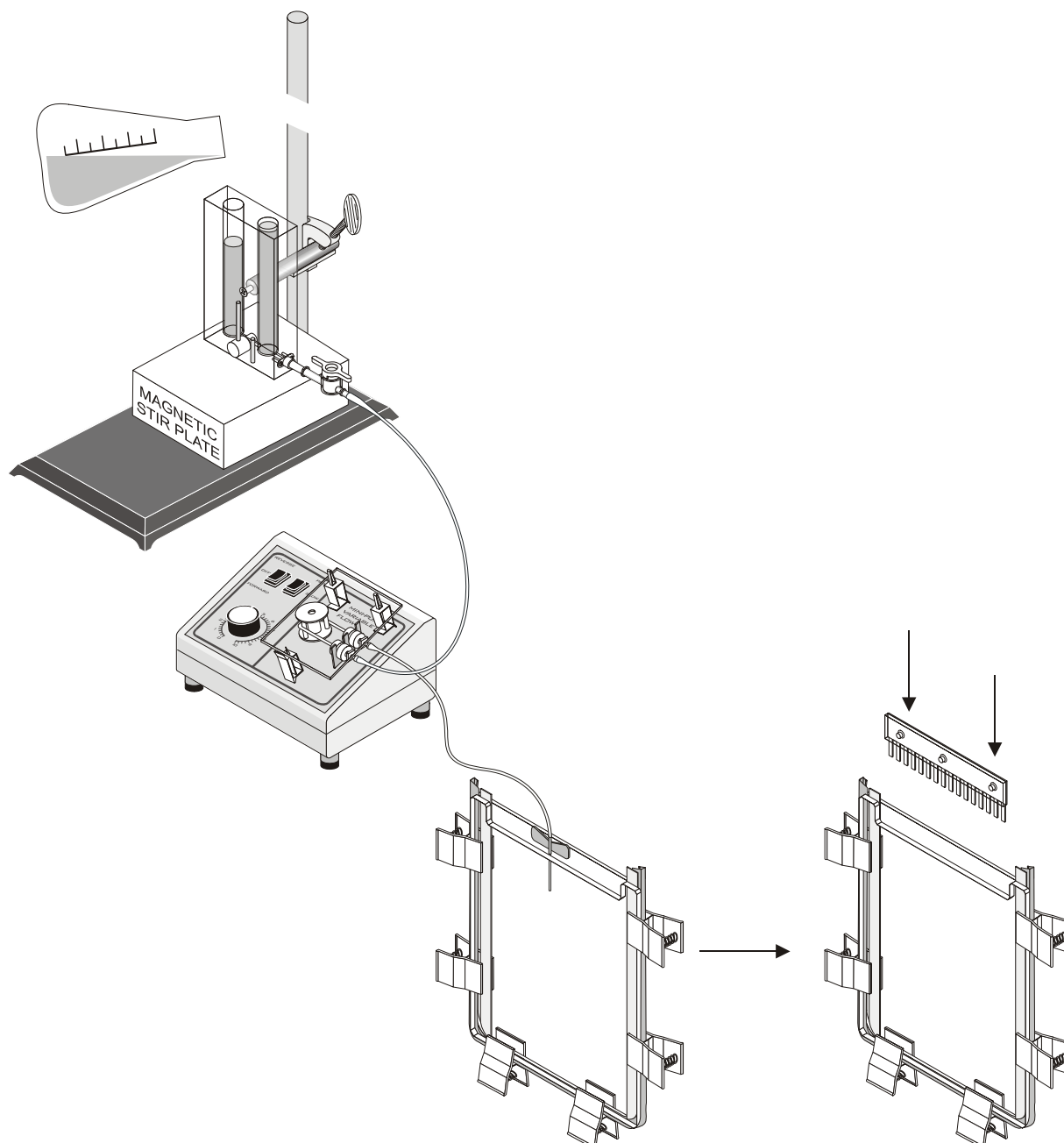


Fig. 3-6

D. Vertical gradient gel casting using GM-40 gradient maker, Mini-Pump and Multi-Gel Caster.

1. Clean the gel caster with soap and water and the glass plates with alcohol.
2. Place a separating sheet in the gel caster.
3. Place the back plate (non-eared) on the sheet.
4. Place the 0.75mm spacers on each side of the back plate.
5. Place the top plate (eared) on the spacers to form 1st sandwich. Repeat as required. Continue to Section 3.4 Vertical Gradient Formation.

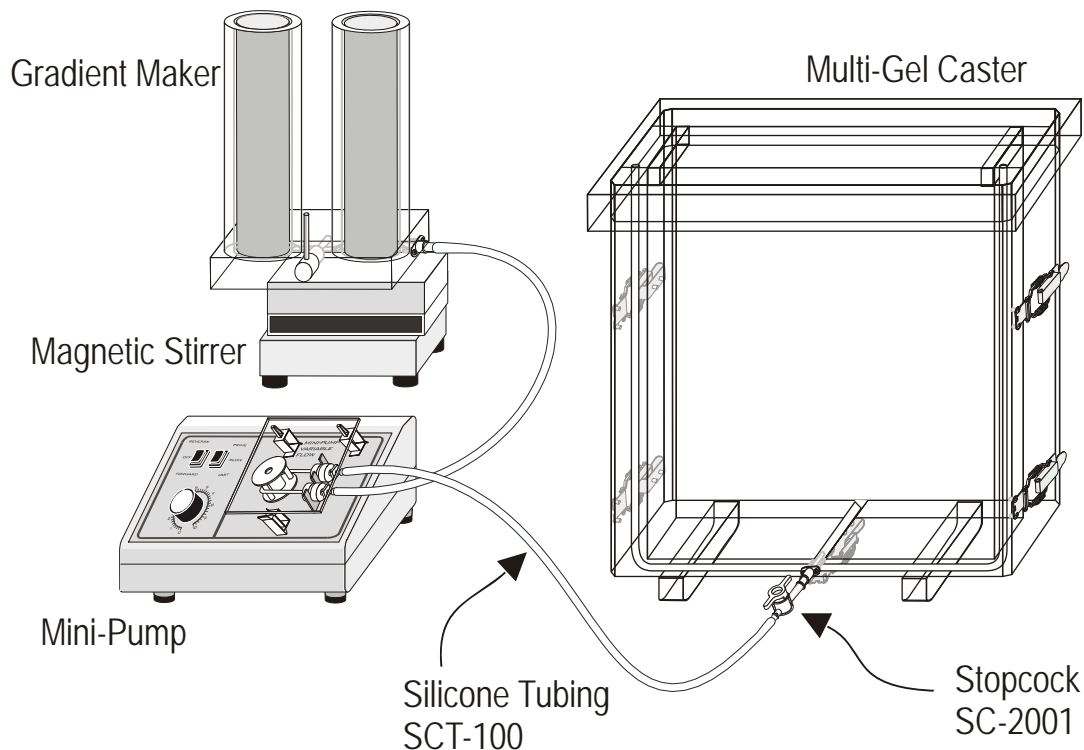


Fig. 3-7

3.4 Vertical Gradient Formation

To determine the range of gradient appropriate for your fragment analysis, please read the enclosed paper by Myers, Sheffield and Cox, especially section 6.1.3 through 6.2.1 (pages 124 to 126). This gives you an excellent overview of the determination of melting behavior of your fragments.

1. The following is a typical protocol for casting a 40%-60% gradient gel. Refer to Section 3.4 Vertical Gradient Formation for apparatus assembly. In an ice bucket, place two 50ml conical tubes labeled "A" and "B". Add to tube "A"
 - 11.5ml of 40%/7.5%
 - 80 μ l (10%) APS
 - 5 μ l TEMED
2. Add to tube "B":
 - 11.5ml of 60%/7.5%
 - 80 μ l (10%) APS
 - 5 μ l TEMED
3. Pour solution "B" into right side of gradient maker (C-2),(GM-40) (fig. 3.5), and open interior valve to allow air bubble to escape. Let as much as 1 ml "B" solution BACKFLOW into left side of gradient maker. Decant the 1 ml back into right side with pasteur pipette. Remove any residual solution from (C-1) with absorbent paper.
4. Add solution "A" to left side of gradient maker.
5. Turn on magnetic stirrer.
6. Exit tube with needle should be secured to gel plate notch with tape.
7. Open inside valve first (V-1), then outside (V-2) valve to start gravity flow. Optionally, Turn on mini-pump and pump acrylamide at medium speed. Determine optimal speed empirically.
8. Gel volume is 23ml, using the 0.75mm spacers. If gel volume is not enough to fill sandwich, use 0% to "top-off". If using water saturated butanol for overlay, leave 0.5cm void to create flat interface.
9. Insert the 16-well rectangular tooth comb. Allow the gel to polymerize for 20-30 minutes.

3.5 Preparation of the Cassettes

1. After polymerization rinse gel plate assembly with D.I. water to remove excess acrylamide or denaturants from plate exterior.
2. Remove comb and quickly transfer gel sandwich to cassette by removing all #2 clamps. **LEAVE GEL WRAP IN PLACE.** The Gel Wrap acts as a barrier and prevents perpendicular electrical fields from interfering with outside lanes. Re-clamp to cassette using #1 clamps/4 per side. Dislodge by pulling the bottom section of the Gel Wrap from the plates to allow buffer contact with the gel. See figure 3.8.
3. Immerse cassette into pre-heated buffer tank (fig.3.8), and attach recirculating tubing to each upper reservoir (fig. 3.9 a,b,c). Rinse loading interface of gel with tank buffer to remove non-polymerized acrylamide or excess urea. Rinse each well with buffer and fill upper reservoir

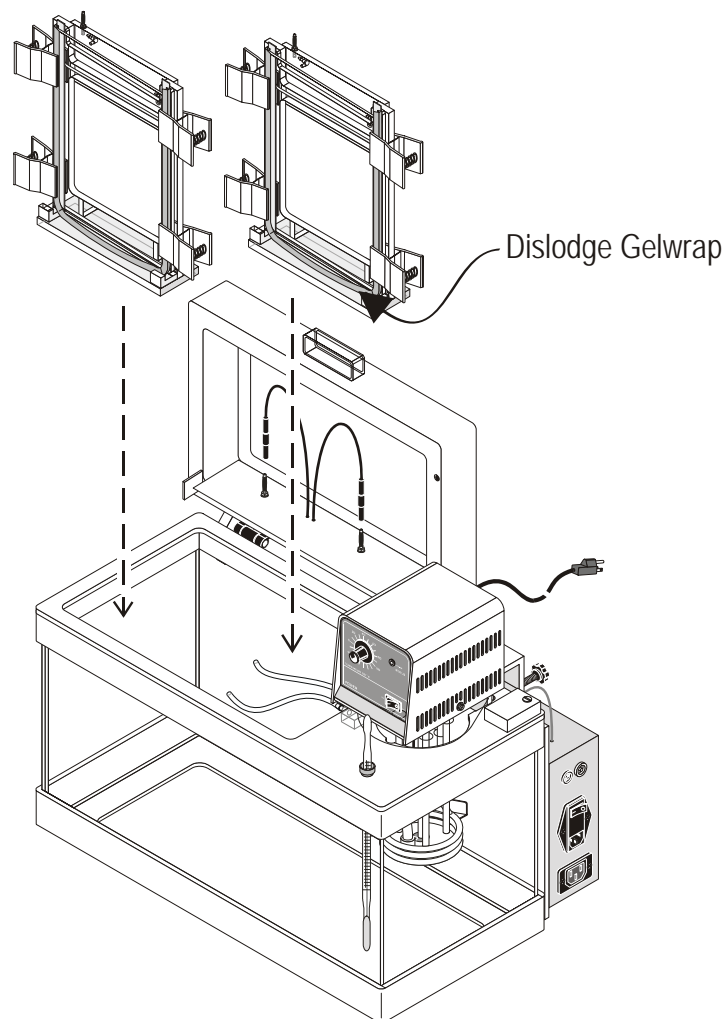


Fig. 3-8

3.6 Buffer Cycling Connections

Buffer Cycling Connections - Single

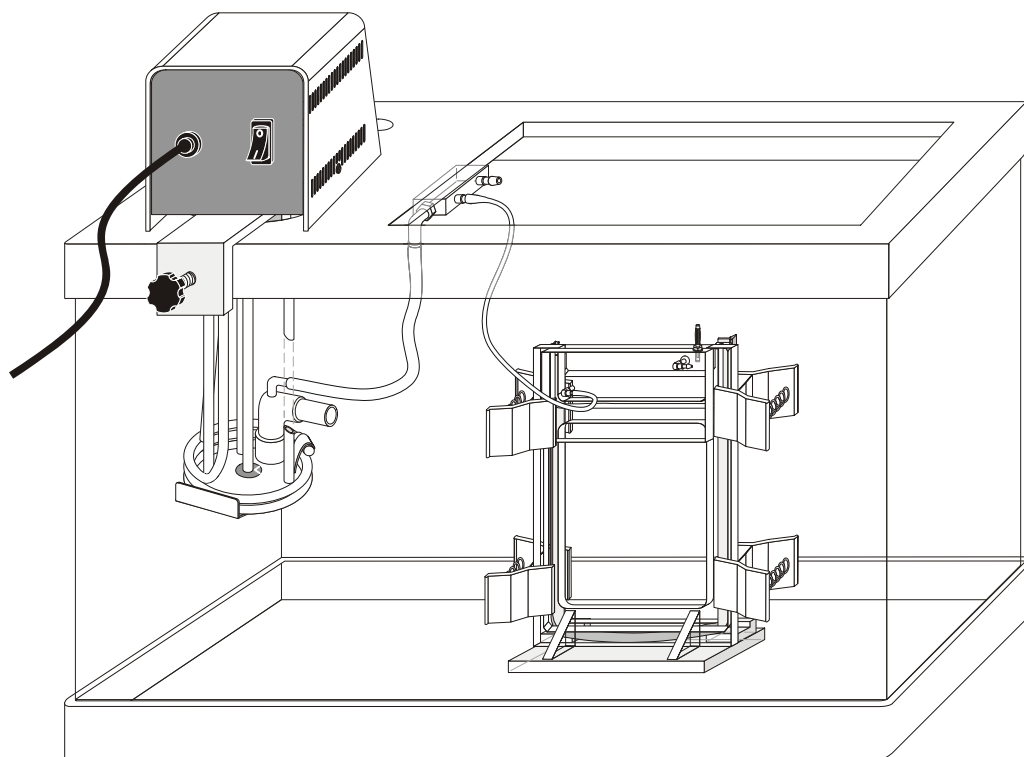


Fig. 3-9a

Buffer Cycling Connections – Two Single Cassettes

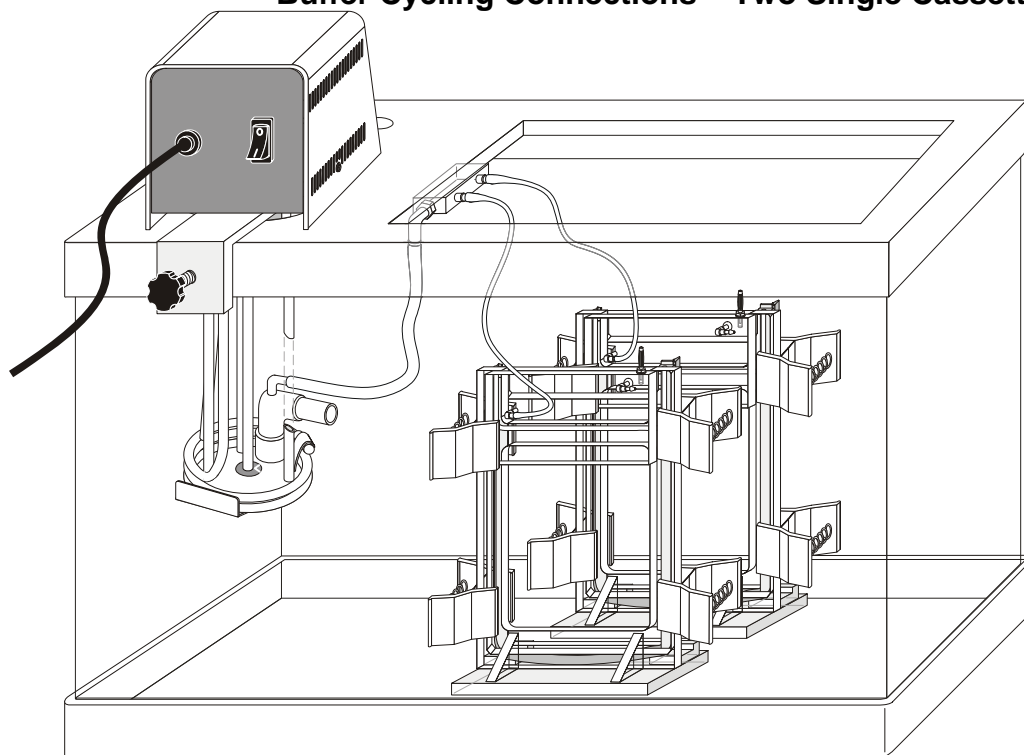


Fig. 3-9b

3.6 Buffer Cycling Connections-continued.

Buffer Cycling Connections- Dual Cassette

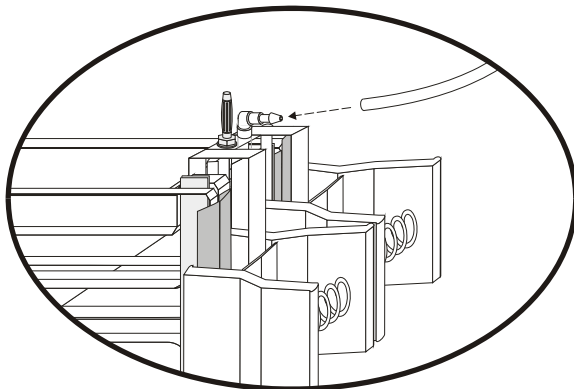


Fig. 3-9c



1. Attach small bore tubing to barb fitting on cassettes.
2. Turn heater/stirrer on to fill upper reservoir; begin buffer cycling.

3.7 Running the Gels

1. Load samples at 1:1 with neutral dye. Load 5-10 ug Genomic DNA/well or 1-2 ug cloned (B-globin)/well. Determine concentration by O.D. 260.
2. Attach black power leads to cassettes
3. Close lid to engage safety interlock. Turn on power supply to 150V (40mA) constant Volts for 5-7 hours.

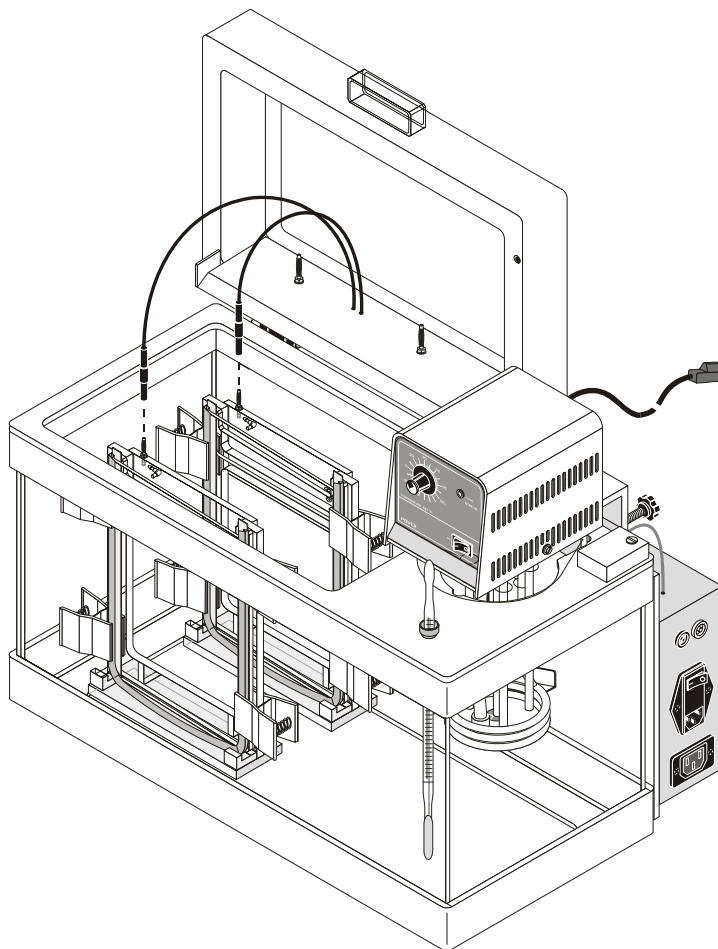


Fig. 3-10

3.8 Removing the Gels



1. When run is completed, turn off power supply, disconnect recirculating tubes and power leads, remove cassettes, remove glass sandwiches from cassettes.
2. Using a wedge plate separator, cat. # WPS-100, pry plates apart and immerse gel/plate in buffer tray. Stain with EtBr (.5ug/ml) for 5-10 minutes. Lift floating gel on plate out of tray and rinse with ddH₂O. Flip over onto saran wrap and peel off glass plate. View bands on transilluminator. Electro-Blot or photograph.

3.9 Perpendicular Gel Casting

NOTE: Prior to casting, mark the notched glass plate to designate which side is the 'inside' and 'outside'. The same side of the notched plate should always sit against the reservoir gasket.

1. For perpendicular gel casting, locate the spacer which has a channel machined into it on one end which will be referred to as the "channel spacer". The gel sandwich will be cast on its side, with the channel spacer on top and the channel further away from the single well comb (see figure 3-11 top for casting gels 1.0mm and thicker, see figure 3-11 bottom for casting gels .75mm or thinner).
2. Follow the Gel Wrap instructions 1-5 (pages 12-13), **making sure to use the channel spacer**.
3. Insert single well comb into glass notch. A small amount of agarose is needed in two places once the gel sandwich is placed on its side, both places are located on the lower **corner** where the single well comb interfaces the spacer, and on the other side of the spacer where it interfaces the gel wrap.
4. Once the agarose has set, insert the gradient maker outflow tube tipped with a 20ga. through the Gel Wrap and into the "channel" (figure 1), or space created between the horizontal spacer and the comb (figure 2). You may use the same piece of Gel Wrap many times (20-30) for the perpendicular gels: do not use a piece of Gel Wrap Gasket for Vertical casting after you have poked a hole in it for perpendicular casting—it will leak.

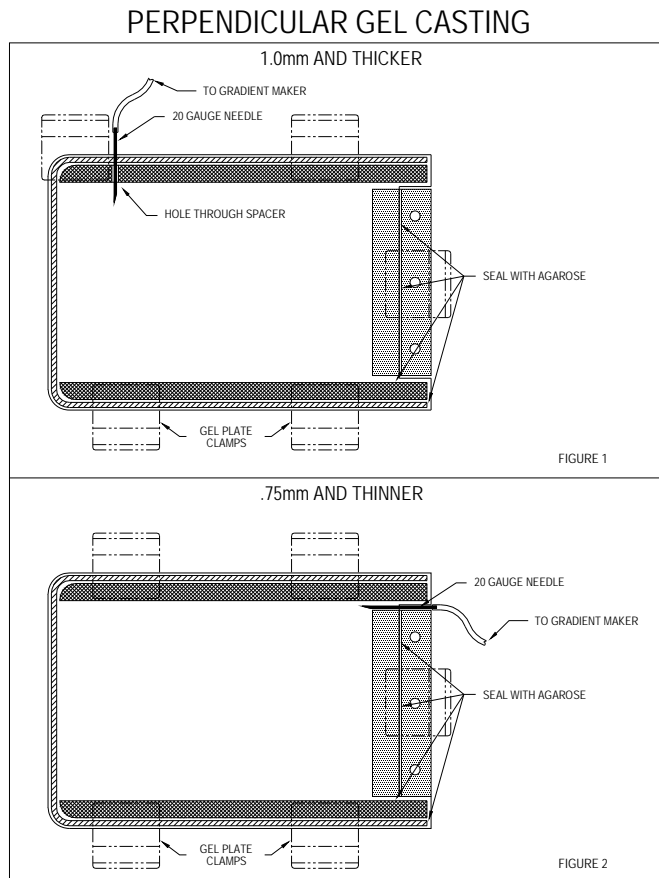


Fig. 3-11 Top

Fig. 3-11 Bottom

3.10 Perpendicular Gradient Formation

To determine the range of perpendicular gradient appropriate for your fragment analysis, please read the enclosed paper by Myers, Sheffield and Cox, as well as the Methods and Enzymology V. 212 paper by Abrams and Stanton. This gives you an excellent overview of the determination of melting behavior of your fragments.

2. The following is a typical protocol for casting a 40%-60% gradient gel. Refer to Section 3.4 Vertical Gradient Formation for apparatus assembly. In an ice bucket, place two 50ml conical tubes labeled "A" and "B". Add to tube "A"
 - 11.5ml of 40%/7.5%
 - 80 μ l (10%) APS
 - 5 μ l TEMED
2. Add to tube "B":
 - 11.5ml of 60%/7.5%
 - 80 μ l (10%) APS
 - 5 μ l TEMED
3. Pour solution "B" into right side of gradient maker,(GM-40), and open interior valve to allow air bubble to escape. Let as much as 1 ml "B" solution BACKFLOW into left side of gradient maker. Decant the 1ml back into right side with pasteur pipette. Remove any residual solution with absorbent paper.
4. Add solution "A" to left side of gradient maker.
5. Turn on magnetic stirrer.
6. Exit tube should be attached to near side of gel plate with tape.
- 7 Open inside (V-1) valve first, then outside (V-2) valve to start flow.
8. Gel volume is 23ml, using the 0.75mm spacers. Allow 20-30 minutes for gel polymeraztion. If gel volume is not enough to fill gel sandwich, use 0% to "top-off".
9. After polymerization rinse gel plate assembly with D.I. water to remove excess acrylamide or denaturants from plate exterior.

3.10 Perpendicular Gradient Formation-continued

10. Remove comb and quickly transfer gel sandwich to cassette by removing all #2 clamps. **LEAVE GEL WRAP IN PLACE.** The Gel Wrap acts as a barrier and prevents perpendicular electrical fields from interfering with outside lanes. Re-clamp to cassette using #1 clamps/4 per side. Dislodge the bottom section of the Gel Wrap from the plates to allow buffer contact with the gel. See **figure 3.8** (page 18).

11. Immerse cassette into pre-heated buffer tank, and attach recirculating tubes to each upper reservoir (**fig. 3.8, 3.9a,b,c**). Rinse loading interface of gel with tank buffer to remove non-polymerized acrylamide or excess urea. Rinse each well with buffer and fill upper reservoir

12. Load samples.



13. Turn on power supply to 150V (40mA) constant Volts for 5-7 hours.

14. When run is completed, turn off power supply, disconnect recirculating tubes and power leads, remove cassettes, remove glass sandwiches from cassettes.

15. Using a wedge plate separator, cat. # WPS-100, pry plates apart and immerse gel/plate in buffer tray. Stain with EtBr (1-5ug/ml) for 5-10 minutes. Lift floating gel on plate out of tray and rinse with DD H₂O. Flip over onto saran wrap and peel off glass plate. View bands on transilluminator. Electro-Blot or photograph.

3.11 Instructions for Use Buffer Siphon Pump

Dispose of solutions in accordance with the safety regulations of your institution.

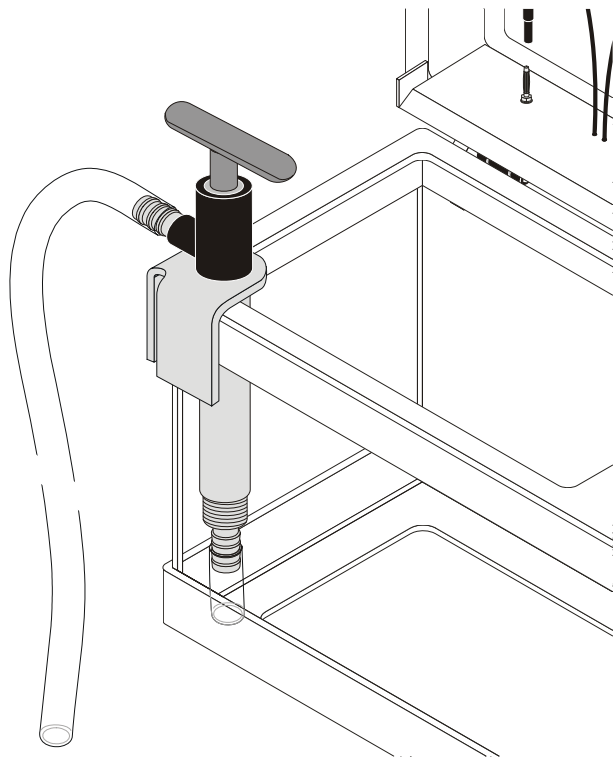


Fig. 3-12

1. Attach pump assembly to corner of tank as shown above.
2. Place the discharge tubing into a waste carboy located **LOWER** than the pump inlet.
3. Begin siphon with half strokes until liquid starts to flow. Leave shaft, fully extended to allow siphon to operate.
4. To stop siphon, depress handle and remove pump from tank.

3.12 Maximum Well/Comb Volumes

NOTE: To calculate sample well volume expressed in millimeters (mm) of height, divide maximum volume by tooth depth. VGC-xxxx-177

# of wells	Tooth width (mm)	Spacing between teeth (mm)	Overall length (mm)	Tooth depth (mm)	0.75mm thickness volume per tooth microliters (ul)	1.0mm thickness volume per tooth microliters (ul)	1.5mm thickness volume per tooth microliters (ul)	2.0mm thickness volume per tooth microliters (ul)
16	5.2	3.2	130	10	39	52	78	104
20	4.2	2.4	130	10	31	42	63	84
22	4.35	1.6	130	10	33	43	65	86
29	2.9	1.6	130	10	22	29	44	58
30	2.8	1.6	130	10	21	28	42	56
VGC-XXXX	Larger	Volume Combs						
# of wells	Tooth width (mm)	Spacing between teeth (mm)	Overall length (mm)	Tooth depth (mm)	0.75mm thickness volume per tooth microliters (ul)	1.0mm thickness volume per tooth microliters (ul)	1.5mm thickness volume per tooth microliters (ul)	2.0mm thickness volume per tooth microliters (ul)
16	5.2	3.2	130	19.1	74	52	111	134
20	4.2	2.4	130	17.7	56	74	63	84
22	4.35	1.6	130	17.7	33	44	66	88
29	2.9	1.6	130	17.7	38	51	77	102
30	2.8	1.6	130	17.7	37	49	74	96

SECTION 4

Running Conditions

4.1 Recommended Power

1. 150V (40mA) CV for 5-7 hours

4.2 Recommended Buffers



Warning: Do not mix buffer in the DGGE tank.

STOCK SOLUTIONS

Acrylamide Stock Solution 40% Acrylamide/Bis (37.5:1)

For 100ml use the following:

38.93 g of acrylamide

1.07 g of Bis-acrylamide

Add dH₂O to 100ml. Do not autoclave

50x TAE Gel Running Buffer

For 1 liter use the following:

242g Tris Base

57.1ml Glacial acetic acid

100ml .5 M EDTA, pH 8.0

Add dH₂O to 1 liter

Denaturant Stock Solution (7.5% gel)

For 100ml use:

	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
40% Acrylamide/Bis (ml)	18.8	18.8	18.8	18.8	18.8	18.8	18.8	18.8	18.8	18.8
50x TAE Buffer (ml)	2	2	2	2	2	2	2	2	2	2
Formamide (ml)	4	8	12	16	20	24	28	32	36	40
Urea (g)	4.2	8.4	12.6	16.8	21	25.2	29.4	33.6	37.8	42

Add dH₂O to 100ml

Store denaturant stock solutions at 4° C in amber bottles

Polymerization Catalysts:

1. TEMED (10ul/ml gel solution)
2. APS Stock Solution (.6ul/ml gel solution)

For 50 ml of APS stock (10%) use 5 g of ammonium persulphate and water to 50ml. Store at 4 ° C.

Neutral Loading Solution

For preparation of 10ml:

20% Ficoll or sucrose, 2 g of Ficoll 400 or sucrose
10mM Tris-HCl, pH 7.8, 100ul of 1.0 M Tris-HCl, pH 7.8
1mM EDTA, 20 ul of 0.5 M EDTA, pH 8.0
0.1% dye (BPB), 10 mg of dye
Water to 10ml

4.3 References

1. S.G. Fischer and L.S. Lerman, (1983) *PNAS* 80:1579.
2. R.M. Myers, T. Maniatis, L.S. Lerman, (1987) *Methods in Enzymology*, 155: 501-529.
3. R.M. Meyers, V.C. Sheffield, D.R. Cox (1988) "Genome Analysis: A Practical Approach," Ed. K Davies, *IRL Press*, Oxford. PP. 95-13
4. V.C. Sheffield, D.R. Cox, L.S. Lerman and R.M. Meyers, (1989) *PNAS*, 86:232-236
5. Guldberg, P., Henriksen, K.F., and Guttler, F. (1993). "Molecular analysis of phenylketonia in Denmark: 99% of the mutations detected by denaturing gradient gel electrophoresis." *Genomics* 17:141-146
6. Moyret, C., Thellet, C., Puig, P.L., Moles, J-P., Thomas, G. and Hamelin, R. (1994). "Relative efficiency of denaturing gradient gel electrophoresis and single-strand conformation polymorphisms in the detection of mutations in exons 5 to 8 of the p53 gene." *Oncogene* 9:1739-1743
7. Sheffield, V.C., Beck, J.S., Kwitek, A.E., Sandstrom, D.W., and Stone, E.M. (1993). "The sensitivity of single-strand conformation polymorphism analysis for the detection of single bas substitutions." *Genomics* 16: 325-332.
8. Abrams, E.S. Stanton, V.P. Jr., (1992) "Use of Denaturing Gradient Gel Electrophoresis to Study Conformational Transitions in Nucleic Acids" *Methods in Enzymology* 212:71-104.

SECTION 5

Maintenance of Equipment



5.1 Care and Handling

The plastic components of the DGGE units are fabricated from acrylic and polycarbonate. Electrodes and connectors are made from pure platinum, stainless steel, and chrome plated brass. As with any laboratory instrument, adequate care ensures consistent and reliable performance.

After each use, rinse buffer chamber, gel tray and combs with de-ionized water. Wipe dry with a soft cloth or paper towel, or allow to air dry. Whenever necessary, all components may be washed gently with water and a non-abrasive detergent, and rinsed and dried as above. *Never* use abrasive cleaners, glass cleaning sprays or scouring pads to clean the components, as these will damage the unit and components.

Additional precautions:

- Do not autoclave or dry-heat sterilize the apparatus or components.
- Do not expose the apparatus or components to phenol, acetone, benzene, halogenated hydrocarbon solvents or undiluted alcohol's.
- Avoid prolonged exposure of the apparatus or components to UV light.
- Do NOT treat with diethylpyrocarbonate (DEPC)-treated water for extended periods at 37°C. A brief rinse with DEPC-water is sufficient after a thorough wash.

5.2 Maintenance



The following inspection and maintenance procedures will help maintain the safety and reliable performance of the Mini-Horizontal systems. Replacement parts can be ordered by calling 858-755-4959 or by contacting your local distributor.

- Banana plugs and power cords should be inspected regularly. If the banana plugs become loose or do not feel friction tight replace the plugs or power cords.
- Should power cord assemblies (connectors, wire or shrouds) show any signs of wear or damage (e.g. cracks, nicks, abrasions, or melted insulation), replace them immediately.
- The platinum wire is secured to the banana jack by compression between a stainless washer and the jack nut. The nut/washer interface should be tight and free of corrosion.

SECTION 6

Denaturing Gradient Gel Electrophoresis systems and accessories

Cat. #	Item
DGGE-1001	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 1 Dual Cassette, (2 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 1 dual cassette, heater/stirrer/buffer cycler, gradient maker, 4 combs (2 each 1 well and 2 ea 16 well combs), 4 sets of spacers, (2 sets for vertical DGGE and 2 sets for perpendicular DGGE), 2 glass plate sets, 4 pieces gel wrap gasket, 12ea #2 clamps, and 18ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, and thermometer. Please specify thickness of combs and spacers.
DGGE-2001	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 2 single cassettes, (2 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 2 single cassettes, heater/stirrer/buffer cycler, gradient maker, 4 combs (2 each 1 well and 2 ea 16 well combs), 4 sets of spacers, (2 sets for vertical DGGE and 2 sets for perpendicular DGGE), 2 glass plate sets, 4 pieces gel wrap gasket, 12ea #2 clamps, and 18ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, and thermometer. Please specify thickness of combs and spacers.
DGGE-2401	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 2 Dual Cassettes, (4 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 2 dual cassettes, heater/stirrer/buffer cycler, gradient maker, 8 combs (4 each 1 well and 4 ea 16 well combs), 8 sets of spacers, (4 sets for vertical DGGE and 4 sets for perpendicular DGGE), 4 glass plate sets, 8 pieces gel wrap gasket, 24ea #2 clamps, and 36ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, and thermometer. Please specify thickness of combs and spacers.
DGGE-4001	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 4 single cassettes, (4 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 4 single cassettes, heater/stirrer/buffer cycler, gradient maker, 8 combs (4 each 1 well and 4 ea 16 well combs), 8 sets of spacers, (4 sets for vertical DGGE and 4 sets for perpendicular DGGE), 4 glass plate sets, 8 pieces gel wrap gasket, 24ea #2 clamps, and 36ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, and thermometer. Please specify thickness of combs and spacers.
DGGE-4801	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 4 Dual Cassettes, (8 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 4 dual cassettes, heater/stirrer/buffer cycler, gradient maker, 16 combs (8 each 1 well and 8 ea 16 well combs), 16 sets of spacers, (8 sets for vertical DGGE and 8 sets for perpendicular DGGE), 8 glass plate sets, 16 pieces gel wrap gasket, 48ea #2 clamps, and 72ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, and thermometer. Please specify thickness of combs and spacers.
DGGEK-1001	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 1 Dual Cassette, (2 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 1 dual cassette, heater/stirrer/buffer cycler, gradient maker, 4 combs (2 each 1 well and 2 ea 16 well combs), 4 sets of spacers, (2 sets for vertical DGGE and 2 sets for perpendicular DGGE), 2 glass plate sets, 4 pieces gel wrap gasket, 12ea #2 clamps, and 18ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, EPS-300-II, 300V power supply, MPP-100 Mini-peristaltic pump and thermometer. Please specify thickness of combs and spacers.
DGGEK-2001	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 2 single cassettes, (2 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 2 single cassettes, heater/stirrer/buffer cycler, gradient maker, 4 combs (2 each 1 well and 2 ea 16 well combs), 4 sets of spacers, (2 sets for vertical DGGE and 2 sets for perpendicular DGGE), 2 glass plate sets, 4 pieces gel wrap gasket, 12ea #2 clamps, and 18ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, EPS-300-II, 300V power supply, MPP-100 Mini-peristaltic pump and thermometer. Please specify thickness of combs and spacers.
DGGEK-2401	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 2 Dual Cassettes, (4 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 2 dual cassettes, heater/stirrer/buffer cycler, gradient maker, 8 combs (4 each 1 well and 4 ea 16 well combs), 8 sets of spacers, (4 sets for vertical DGGE and 4 sets for perpendicular DGGE), 4 glass plate sets, 8 pieces gel wrap gasket, 24ea #2 clamps, and 36ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, EPS-300-II, 300V power supply, MPP-100 Mini-peristaltic pump and thermometer. Please specify thickness of combs and spacers.
DGGEK-4001	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 4 single cassettes, (4 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 4 single cassettes, heater/stirrer/buffer cycler, gradient maker, 8 combs (4 each 1 well and 4 ea 16 well combs), 8 sets of spacers, (4 sets for vertical DGGE and 4 sets for perpendicular DGGE), 4 glass plate sets, 8 pieces gel wrap gasket, 24ea #2 clamps, and 36ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, EPS-300-II, 300V power supply, MPP-100 Mini-peristaltic pump and thermometer. Please specify thickness of combs and spacers.
DGGEK-4801	DENATURING GRADIENT GEL ELECTROPHORESIS SYSTEM, 4 Dual Cassettes, (8 place). Gel size 17.7cm (w) x 22cm (l). Includes Electrophoresis tank, 4 dual cassettes, heater/stirrer/buffer cycler, gradient maker, 16 combs (8 each 1 well and 8 ea 16 well combs), 16 sets of spacers, (8 sets for vertical DGGE and 8 sets for perpendicular DGGE), 8 glass plate sets, 16 pieces gel wrap gasket, 48ea #2 clamps, and 72ea #1 clamps, glass safety cover, buffer tank drain valve, power leads, gel tape, EPS-300-II, 300V power supply, MPP-100 Mini-peristaltic pump and thermometer. Please specify thickness of combs and spacers.

DGGE ACCESSORIES

SPACER SETS

Spacer Sets

Vertical DGGE

(Heat resistant polycarbonate)

<u>Cat.#</u>	<u>Spacer Dimensions</u>
VGS-0420R-177	0.4mm x 22cm
VGS-0520R-177	0.5mm x 22cm
VGS-7520R-177	0.75mm x 22cm
VGS-1020R-177	1.0mm x 22cm
VGS-1520R-177	1.5mm x 22cm
VGS-2020R-177	2.0mm x 22cm

Gel Wrap Gasket

<u>Cat.#</u>	<u>Spacer Thickness x Plate Height</u>
VGE-0520-177	0.5mm x 22cm
VGE-7520-177	0.75mm x 22cm
VGE-1020-177	1.0mm x 22cm
VGE-1520-177	1.5mm x 22cm
VGE-2020-177	2.0mm x 22cm

Combs for DGGE

<u>Cat.#</u>	<u>Comb Description</u>
VGC-0716-177	0.75mm x 16 well
VGC-0720-177	0.75mm x 20 well
VGC-0722-177	0.75mm x 22 well
VGC-0729-177	0.75mm x 29 well
VGC-0730-177	0.75mm x 30 well
VGC-1016-177	1mm x 16 well
VGC-1020-177	1mm x 20 well
VGC-1022-177	1mm x 16 well
VGC-1029-177	1mm x 29 well
VGC-1030-177	1mm x 30 well
VGC-1516-177	1.5mm x 16 well
VGC-1520-177	1.5mm x 20 well
VGC-1522-177	1.5mm x 22 well
VGC-1529-177	1.5mm x 29 well
VGC-1530-177	1.5mm x 30 well
VGC-2016-177	2mm x 16 well
VGC-2020-177	2mm x 20 well
VGC-2022-177	2mm x 16 well
VGC-2029-177	2mm x 29 well
VGC-2030-177	2mm x 30 well

Bulk Gel Wrap Gasket

<u>Cat.#</u>	<u>Gel Thickness</u>
VGE-05XX	0.5mm thick-yellow
VGE-75XX	0.75mm thick-yellow
VGE-10XX	1.0mm thick-red
VGE-15XX	1.5mm thick-blue
VGE-20XX	2.0mm thick-purple
VGE-30XX	3.0mm thick-white

DGGE Glass Plate Sets, notched

(1/8" back plate with rounded corners)

<u>Cat.#</u>	<u>Glass Plate Dimensions</u>
NGP-17720-NR	17.7cm (w) x 22cm (h)
NGP-17720-B	17.7cm (w) x 22cm (h) borosilicate

<u>Cat.#</u>	<u>Description</u>
EPS-300-II	Mini Power Supply, 110V/60Hz
EPS-300-IIV	Mini Power Supply, 220V/50Hz

Perpendicular DGGE

(Spacer sets made from heat resistant polycarbonate: 1 standard spacer & 1 spacer with injection port.)

<u>Cat.#</u>	<u>Spacer Dimensions</u>
VGS7520RC-177	0.75mm x 22cm
VGS1020RC-177	1.0mm x 22cm
VGS1520RC-177	1.5mm x 22cm
VGS2020RC-177	2.0mm x 22cm

Combs for Perpendicular DGGE

<u>Cat.#</u>	<u>Comb Description</u>
VGC-7501-177	0.75mm x 1 well
VGC-1001-177	1.0mm x 1 well
VGC-1501-177	1.5mm x 1 well
VGC-2001-177	2.0mm x 1 well

Combs for DGGE – Larger Volume

<u>Cat.#</u>	<u>Comb Description</u>
VGC-0716	0.75mm x 16 well
VGC-0720	0.75mm x 20 well
VGC-0722	0.75mm x 22 well
VGC-0729	0.75mm x 29 well
VGC-0730	0.75mm x 30 well
VGC-1016	1mm x 16 well
VGC-1020	1mm x 20 well
VGC-1022	1mm x 22 well
VGC-1029	1mm x 29 well
VGC-1030	1mm x 30 well
VGC-1516	1.5mm x 16 well
VGC-1520	1.5mm x 20 well
VGC-1522	1.5mm x 22 well
VGC-1529	1.5mm x 29 well
VGC-1530	1.5mm x 30 well
VGC-2016	2mm x 16 well
VGC-2020	2mm x 20 well
VGC-2022	2mm x 22 well
VGC-2029	2mm x 29 well
VGC-2030	2mm x 30 well

Gel Cassettes

<u>Cat.#</u>	<u>Item</u>
DGC-177-201	Additional single gel cassette
DDGC-177-201	Additional dual gel cassette

Buffer Siphon Pump

<u>Cat.#</u>	<u>Item</u>
BSP-1000	Buffer Siphon Pump

White Clamps

<u>Cat.#</u>	<u>Item</u>
GPC-0001-177	White clamp for holding glass plate sandwich to upper reservoir. (Jaws slightly open in resting position)
GPC-0002-177	White clamp for gel casting (Jaws closed in resting position) (Jaws slightly open in resting position)
GPC-0002-177	White clamp for gel casting (Jaws closed in resting position)

CONTACT INFORMATION



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