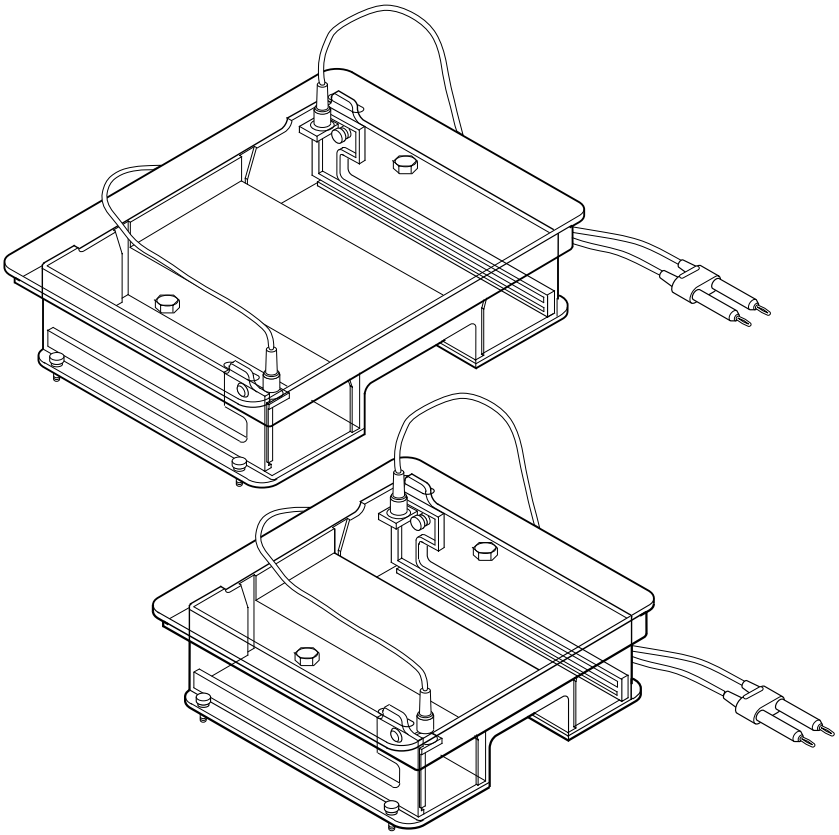

Sub-Cell® Model 96 and Model 192 Agarose Gel Electrophoresis Systems

Instruction Manual

Catalog Numbers
170-4500 through 170-4511

BIO-RAD

Warranty

Bio-Rad Laboratories warrants the Sub-Cell Model 96 and Model 192 electrophoresis systems against defects in materials and workmanship for 1 year. If any defects occur in the instrument during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts free. 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. Use of fittings or other spare parts supplied by anyone other than Bio-Rad Laboratories.
4. Damage caused by accident or misuse.
5. Damage caused by disaster.
6. Corrosion due to use of improper solvent or sample.

This warranty does not apply to parts listed below:

1. Platinum Electrode Wires

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

To insure the best performance from the Sub-Cell electrophoresis systems, become fully acquainted with these operating instructions before use. Bio-Rad recommends that you first read these instructions carefully. Assemble and disassemble the unit completely without casting a gel. After these preliminary steps, you should be ready to cast and run a gel.

Bio-Rad also recommends that all Sub-Cell system components and accessories be inspected for damage, cleaned as recommended in this manual and rinsed thoroughly with distilled water before use. Record the following for your records:

Model _____

Catalog No. _____

Date of Delivery _____

Warranty Period _____

Serial No. _____

Invoice No. _____

Purchase Order No. _____

Table of Contents

Section 1
General Information

1.1 Introduction

The Sub-Cell instruments comprise a comprehensive and versatile gel electrophoresis system that effectively separates nucleic acids using submerged agarose gels. Submarine agarose gels are easy to cast and readily dissipate heat. These gels allow sample underlaying and also prevent electrical field discontinuities caused by wicks or sample well dehydration. Agarose gels are ideal for the separation of DNA restriction digestions, Polymerase Chain Reaction (PCR)* amplified fragments, and genomic DNA and RNA prior to Southern or Northern blotting. If operated correctly, agarose gels can effectively separate nucleic acids from 20 base pairs to 20 kilobase pairs in length.

The Sub-Cell Model 96 and 192 electrophoresis cells have been created specifically for multiple sample analysis. The width of each cell and the analytical combs were designed based on the fixed spacing of multichannel pipets used to transfer samples from standard microplates. Forty eight nucleic acid samples (plus three DNA size standards) can be visualized in one row using the 51-well comb. If two combs are used, samples from all 96 wells of a microplate can be analyzed on the Model 96. Four or more combs can be used on the Model 192 for even higher throughput. The Model 96 can run gels 10 or 15 cm in length, whereas the Model 192 can run gels 10, 15, 20 or 25 cm in length for the analysis of more samples or applications such as genomic DNA separations for Southern blotting.

The Sub-Cell systems give years of rigorous use. These rugged systems incorporate many features that make casting and running agarose gels simple and efficient. The gel caster provides tape-free gel casting in trays and gels can be cast in the Sub-Cell bases using the gel casting gates. Replaceable electrode cassettes provide a simple way to replace electrode wires. A comprehensive assortment of tray sizes and multichannel pipet-compatible combs make these systems ideal for most high throughput agarose gel applications. Recirculation ports are provided to prevent heat and pH effects during high voltage or extended run electrophoresis.

Note: This manual contains instructions for the Sub-Cell Model 96 and Model 192 electrophoresis systems only. Bio-Rad supplies similar but smaller agarose gel electrophoresis cells: the original Sub-Cell, Wide Mini-Sub Cell, and Mini-Sub Cell systems and the Sub-Cell GT, Wide Mini-Sub Cell GT and Mini-Sub Cell GT systems. This manual does not provide information concerning these smaller versions. Contact your local Bio-Rad representative for information concerning the original Sub-Cell and Sub-Cell GT systems.

* The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffmann-LaRoche. Use of the PCR process requires a license.

1.2 Safety

The Sub-Cell electrophoresis systems are designed for maximum user safety. The buffer chambers are made of 1/4-inch (.635 cm) thick cast acrylic to create a leak-free electrophoresis environment. The safety lids surround the entire buffer chamber to protect the user from exposure to electrical currents. Sub-Cell systems were designed for indoor use only.

Before every use, inspect the base for cracks or chips. Cracks or chips may cause the buffer to leak from the base and cause a potential electrical hazard. Additionally, inspect all electrical cables, banana jacks, recirculation port fittings, tubing, and plugs for loose connections, cracks, breaks or corrosion. Do not use any part that is cracked, charred or corroded. These parts may also cause a potential electrical shock. Contact your local Bio-Rad representative before using a part that may be considered hazardous.

During electrophoresis inspect the base and workbench for any signs of buffer leakage. If leaking buffer is detected disconnect the power to the cell immediately and contact your Bio-Rad representative.

Power to Sub-Cell units is to be supplied by an 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. All Bio-Rad power supplies meet this important safety requirement. The recommended power supply for this apparatus is the PowerPac 300. The PowerPac 300 contains several safety features such as no load, overload, rapid resistance change, and ground leak detection capabilities. The maximum specified operating parameters* for the Sub-Cell Model 96 and Model 192 systems are:

200 VDC	Maximum voltage limit
70 Watts	Maximum power limit
50 °C	Maximum buffer temperature
4 °C – 40 °C	Ambient temperature limits

* IEC 1010-1 certification applies to equipment designed to be safe at the operating parameters listed above. Additionally, both Sub-Cell Model 96 and Model 192 have a maximum operating relative humidity of 80% for temperatures up to 31 °C decreasing linearly to 50% relative humidity at 40 °C. Certification is valid when systems are operated at altitudes up to 2000 meters.

Current to the cell, provided from the external power supply, enters the unit through the lid assembly, providing a safety interlock to the user. Current to the cell is broken when the lid is removed. Do not attempt to circumvent this safety interlock, and always turn the power supply off before removing the lid or when working with the cell in any way.

Important: These Bio-Rad instruments are designed and certified to meet IEC 1010-1* safety standards. IEC-certified products are safe to use when operated in accordance with this instruction manual. This instrument should not be modified 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 is not responsible for any injury or damage caused either by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or any authorized agent. No user-serviceable parts are contained in this apparatus. To ensure electrical safety, do not attempt to service this apparatus.

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

Definition of Symbols

Caution, risk of electrical shock

Caution (refer to accompanying documents)

1.3 List of System Parts

Each Sub-Cell system comes with the components listed in Table 1.1. Check your instrument to insure all items are present. Note any damage to the unit which may have occurred during shipping. Notify Bio-Rad Laboratories if any items are missing or damaged (see Figure 1.1 for part descriptions, on the following page).

Table 1.1 Sub Cell System Components

Item	Quantity
Base (buffer chamber)	1
Gel Casting Gates	2
Safety Lid and Cables	1
UVTP Gel Tray	1
Comb (51 well, 1.5 mm thick)	1
Comb Holder	1
Leveling Bubble	1
Gel Caster (optional)	1
Instruction Manual	1

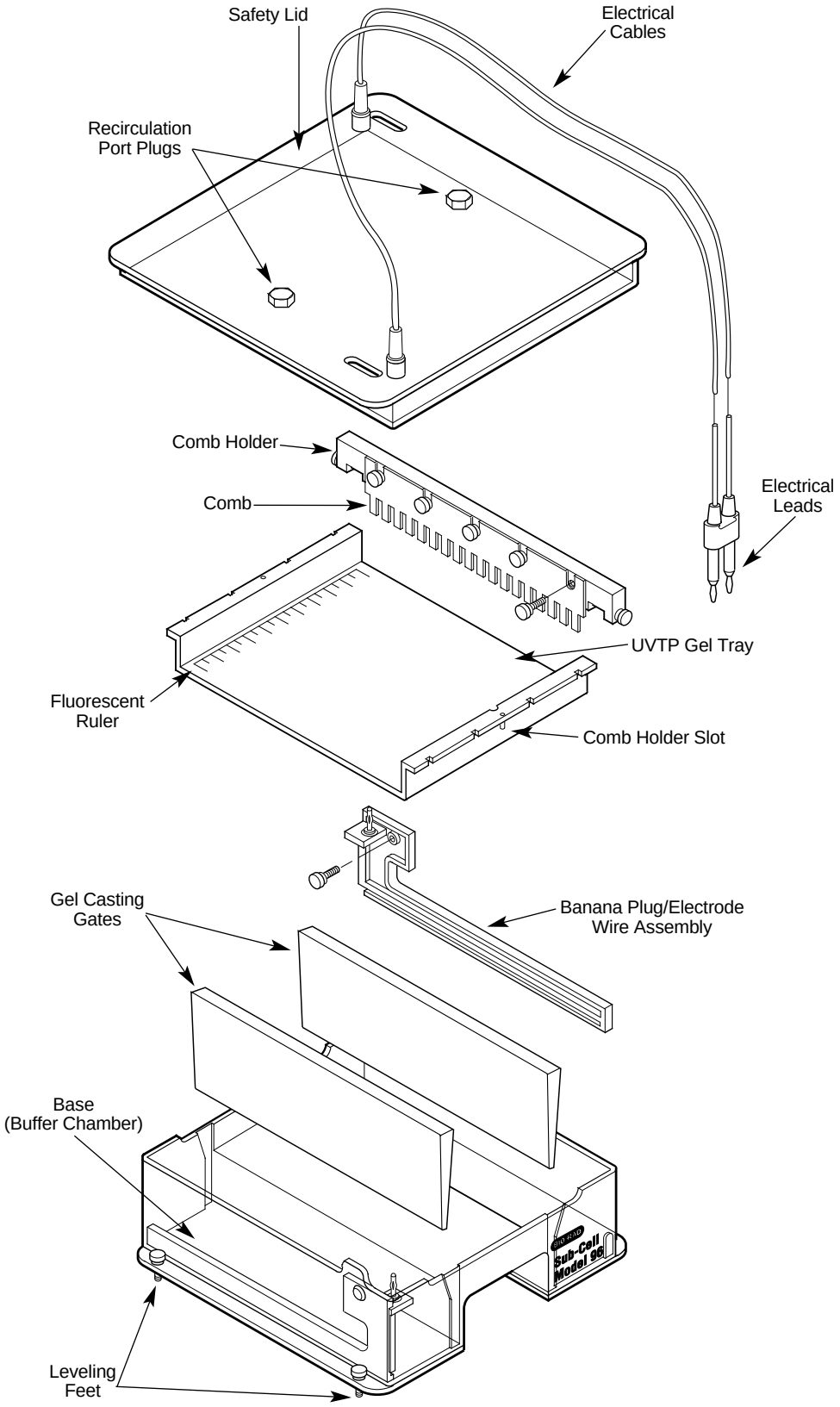

Figure 1.1 Sub-Cell Model 96 and Model 192 Parts

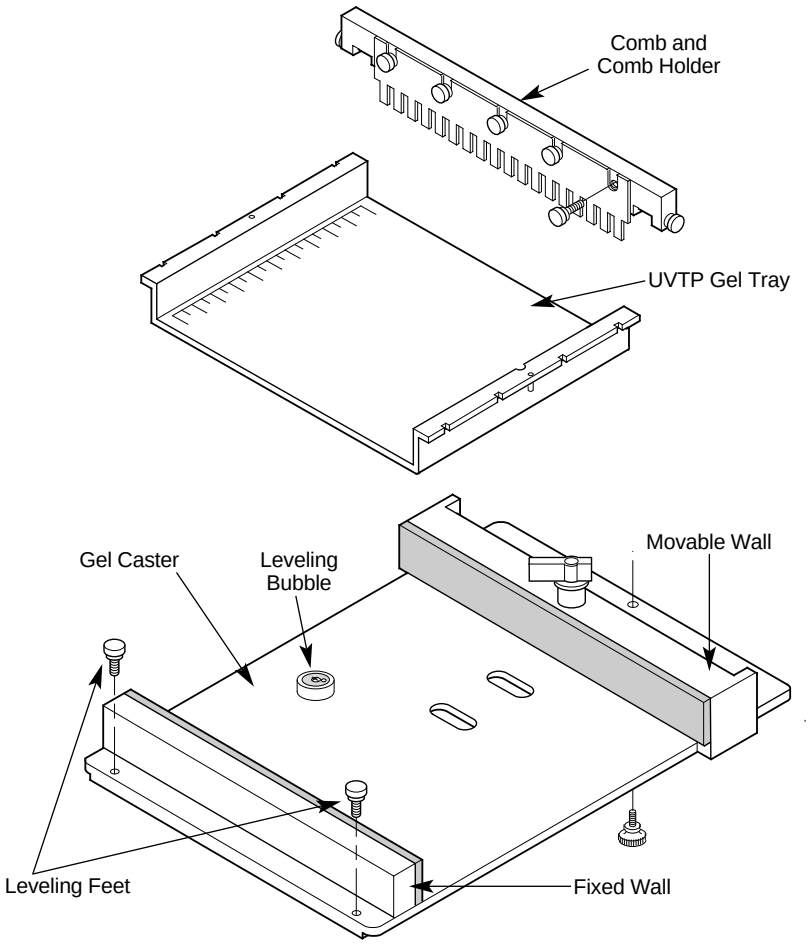

Figure 1.2 Sub-Cell Model 96 and Model 192 Gel Caster Parts

1.4 Specifications

Sub-Cell Model 96

Base Footprint (L x W x H)	29 .5 cm x 29.0 cm x 9.0 cm
Base Buffer Volume*	2.0 L
Base Gel Size	25 x 10 cm
Gel Tray Sizes	25 x 10 cm 25 x 15 cm

Sub-Cell Model 192

Base Footprint (L x W x H)	39.5 cm x 29.0 cm x 9.0 cm
Base Buffer Volume*	3.0 L
Base Gel Size	25 x 15 cm
Gel Tray Sizes	25 x 10 cm 25 x 15 cm 25 x 20 cm 25 x 25 cm

Contruction

Base	Cast Acrylic
Gel Casting Gates	Anodized Aluminum
Safety Cover	Cast Acrylic
Banana Plug/Electrode Cassette	Polycarbonate
Banana Plugs	Gold-Plated Brass, 4.4 cm Length
Electrodes	Platinum, 0.25 mm Diameter
Electrical Cables	Dual, 20 AWG, Tinned Copper Wire Cable Flame-Retardant Polyurethane Insulation jacket
Electrical Leads	Nickel Silver
Gel Tray	UV-Transparent Acrylic Plastic (UVTP)
Combs	Machined Acrylic
Comb Holder	Polycarbonate
Gel Casting Device	Polycarbonate 0.64 cm Silicon Foam

* Base buffer volumes will vary depending on the size and thickness of gel used.

Section 2
Operating Instructions

Note: Refer to Section 3 for information on preparation of RNA gels. See References 1 and 2 for more information on DNA and RNA electrophoresis.

2.1 DNA Gel Preparation

DNA agarose gels can be used to separate and visualize DNA of various sizes. Before casting an agarose gel, consult Table 2.1 to determine the appropriate percent agarose gel to use based on the size of DNA to be separated.

Procedure

1. Determine the amount of agarose (grams) and volume needed. Use Tables 2.1 and 2.2 as a guide for agarose concentration and gel volume requirements.

   Example: For a 1% agarose gel, add 1 gram of agarose to 100 ml of electrophoresis buffer.

Table 2.1 Gel Concentration Required for DNA Separation[1-2]

Gel Concentration %	DNA Size (Kbp)
0.50	1 – 30
0.75	0.8 – 12
1.00	0.5 – 10
1.25	0.4 – 7
1.50	0.2 – 3
2-5*	0.01 – 0.5

* Sieving agarose such as Bio-Rad AmpliSize® agarose.

Table 2.2 Gel Volume Requirements

Gel Size	0.5 cm thick	0.75 cm thick	1.0 cm thick
Bases			
25 x 10 cm (Model 96)	125 ml	185 ml	250 ml
25 x 15 cm (Model 192)	185 ml	280 ml	375 ml
Trays			
25 x 10 cm	125 ml	185 ml	250 ml
25 x 15 cm	185 ml	280 ml	375 ml
25 x 20 cm	250 ml	375 ml	500 ml
25 x 25 cm	310 ml	465 ml	625 ml

2. Add the agarose to a suitable container (e.g., 500-ml Erlenmeyer flask, Wheaton bottle, etc.). Add the appropriate amount of electrophoresis buffer (see Section 3) and swirl to suspend the agarose powder in the buffer. If using an Erlenmeyer flask, invert a 50 ml Erlenmeyer flask into the open end of the 500-ml Erlenmeyer flask containing the agarose. The small flask acts as a reflux chamber, thus allowing long or vigorous boiling without much evaporation.

   Note: Place a mark on the flask at the liquid level. If evaporation occurs, water can be added to bring the volume back to the original liquid level.

3. The agarose can be melted by boiling on a magnetic hot plate or in a microwave oven.

   Caution: Always wear protective gloves, goggles, and a lab coat while preparing and casting agarose gels. Boiling molten agarose or the vessels containing hot agarose can cause severe burns if allowed to contact skin. Molten agarose can become super-heated and boil over vessels when swirled which can also cause severe burns.

Magnetic Hot Plate Method

4a. Add a stir bar to the undissolved agarose solution. Heat the solution to boiling while stirring on a magnetic hot plate. Use the appropriate size container to allow bubbles or foam to disrupt before rising to the neck of the container.

5a. Boil the solution until all of the small translucent agarose particles are dissolved. Set aside to cool to 50-60 °C before pouring.

Microwave Oven Method

4b. Place the gel solution into the microwave. Using a low to medium setting, set the timer for a minimum of 5 minutes, stopping the microwave oven every 30 seconds and swirling the container gently to suspend the undissolved agarose. This technique is the fastest and safest way to dissolve agarose.

5b. Boil and swirl the solution until all of the small translucent agarose particles are dissolved. Set aside to cool to 50-60 °C before pouring.

2.2 Comb Set-up

Comb and Comb Holder Set-up

The comb holder used for the Model 96 and 192 was designed to incorporate all the necessary features required for any agarose gel application. The comb holder allows for adjustable comb height and can be adjusted so that the comb can be placed anywhere on the

base stage or UVTP tray. The following instructions describe how to manipulate the comb and comb holder for obtaining comb height and comb holder position on a UVTP tray or base stage.

Adjusting and Setting Comb Height

1. Loosen the five thumbscrews from the front plate of the comb holder.
2. Align the slots of the well-forming comb with the thumbscrews on the comb holder. Insert the slots over the shaft (threaded portion) of the thumbscrews and tighten until the flat head (shoulder) of the screws come in contact with the comb.
3. Place the comb holder assembly on the cell base or UVTP tray and adjust the height of the comb to the desired distance from the surface of the base stage or tray (typically 1-2 mm). Tighten all five screws once the full-length of the comb is at a uniform distance from the base stage or tray.

Adjusting and Setting Comb Position on UVTP Tray or Base Stage

1a. Turn the two thumbscrews clockwise on the sides of the comb holder until resistance is felt. With the screws in this position, the comb holder can be placed into the comb slots of the base and UVTP tray for gel casting.

OR

1b. Turn the two thumbscrews counterclockwise on the sides of the comb holder until the shaft (threaded portion) of the thumbscrews can no longer be seen in the comb holder notches. With the screws in this position, this will allow the comb holder assembly to be placed anywhere on the base or UVTP tray. The comb can be secured to the tray or base by turning the thumbscrews clockwise until resistance is felt.

2.3 Procedures for Casting Agarose Gel Slabs

There are several ways to cast agarose submarine gels for the Model 96 and Model 192. Gels may be cast with or without UV-transparent plastic (UVTP) trays directly on the stage of the Sub-Cell bases using the gel casting gates. Gels may also be cast on UVTP trays with the aid of the gel caster or with standard laboratory tape.

Casting Gels on the Base Stages

1. Level the Sub-Cell base using the leveling bubble provided.
2. Slide the gel casting gates into the slots at opposite ends of the gel stage.
3. Place the comb(s) into the appropriate slot(s) of the base so that the sample wells are near the cathode (black) (refer to Section 2.2 for comb adjustments). DNA samples will migrate towards the anode (red) during electrophoresis.
4. When the solution of agarose has cooled to 60 °C (Section 2.1), pour the molten agarose between the gates.

   Warning: Hot agarose (>60 °C) may cause the cell to warp or craze and will decrease the lifetime of the cell. Warping may also result in sample wells of uneven depth.
5. Allow 30 – 60 minutes for the gel to solidify at room temperature.
6. Carefully remove the comb and then remove the gel casting gates from the gate slots of the base.
7. Submerge the gel beneath 4 to 6 mm of electrophoresis buffer (Section 3.1).

Removable Tray (UVTP) Gel Casting

Casting gels on the base stage with UVTP tray

1. Level the cell using the leveling bubble provided.
2. Place the UVTP tray on the cell base stage.

   Note: The Sub-Cell Model 96 system requires the 25 x 10 cm UVTP tray for casting in the base. Sub-Cell Model 192 system requires the 25 x 15 cm UVTP tray for casting in the base.
3. Slide the gel casting gates into the slots at opposite ends of the base stage. Ensure the gates are evenly seated in the slots and the gates uniformly contact all edges of the UVTP tray. The weight of the gates provide a tight seal to avoid any leakage problems during gel casting.
4. Place the comb(s) into the appropriate slot(s) of the trays so that the sample wells are near the cathode (black). DNA samples will migrate towards the anode (red) during electrophoresis.
5. Prepare the desired concentration and amount of agarose in 1x electrophoresis buffer (see section 2.1). When the agarose solution has cooled to 50-60˚ C pour the molten agarose between the gates.

   Warning: Hot agarose (>60 ˚C) may cause the tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.
6. Allow 30 – 60 minutes for the gel to solidify at room temperature.
7. Carefully remove the comb from the solidified gel. Remove the gel casting gates.
8. Submerge the gel beneath 2 to 6 mm of 1x electrophoresis buffer (see Section 3, Gel and Electrophoresis Reagent Preparation). Use greater depth overlay (more buffer) with increasing voltages to avoid pH and heat effects.

Gel Caster Method

1. Level the gel caster on the lab bench using the leveling bubble provided.
2. Disengage and slide the movable wall to the open end of the gel caster by turning and lifting the cam peg upward.
3. Place the UVTP tray against the fixed wall of the gel caster.
4. Slide the movable wall against the edge of the UVTP tray (Figure 2.1).
5. To seal the open tray ends, engage the cam peg by turning and pressing downward simultaneously.
6. Once the cam peg has dropped down into the appropriate slot, turn the peg in either direction until resistance is felt. This action seals the ends of the tray for casting.

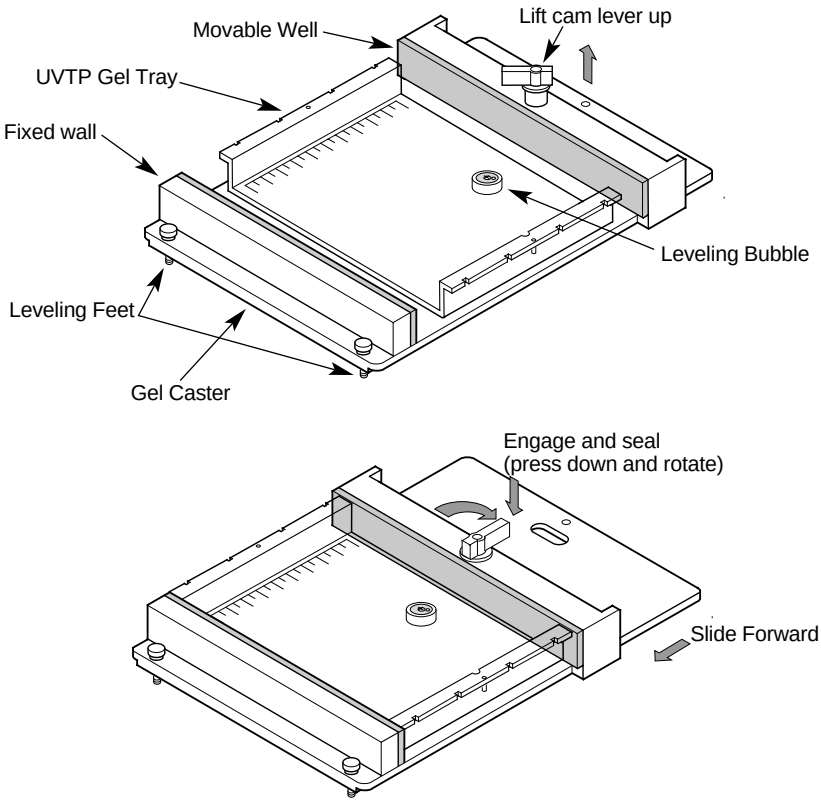

Figure 2.1 Sealing the UVTP tray for gel casting.

7. Place the comb(s) into the appropriate slot(s) of the tray (refer to Section 2.2 for comb adjustments).
8. When the solution of agarose has cooled to 60 °C (Section 2.1), pour the molten agarose onto the tray.

   Warning: Hot agarose (>60 °C) may cause the tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.
9. Allow 30 – 60 minutes for the gel to solidify at room temperature.
10. Carefully remove the comb from the solidified gel.
11. Disengage the cam peg by turning and lifting upward. Slide the movable wall away from the tray. Remove the tray from the gel caster.

    Note: While the gel is solidifying, a light seal is formed between the gasket and the gel (especially for low percentage agarose gels (<0.8%). Carefully lift the tray on one side to release the seal.
12. Place the tray onto the leveled Sub-Cell base so that the sample wells are near the cathode (black). DNA samples will migrate towards the anode (red) during electrophoresis.
13. Submerge the gel beneath 4 to 6 mm of electrophoresis buffer (Section 3.1).

Tape Method

1. Seal the ends of the UVTP gel tray securely with strips of standard laboratory tape. Press the tape firmly to the edges of the gel tray to form a fluid-tight seal.
2. Level the gel tray on a leveling table or workbench using the leveling bubble provided with the instrument.
3. Place the comb(s) into the appropriate slot(s) of the tray (refer to Section 2.2 for comb adjustments).
4. When the solution of agarose has cooled to 60 °C (Section 2.1), pour the molten agarose onto the tray.

   Warning: Hot agarose (>60 °C) may cause the tray to warp or craze and will decrease the lifetime of the tray. Warping may also result in sample wells of uneven depth.
5. Allow the gel to solidify at room temperature for 30 – 60 minutes.
6. Carefully remove the comb from the solidified gel.
7. Remove the tape from the edges of the gel tray. Be careful when removing tape so the gel does not slide off the tray.
8. Place the tray onto the leveled Sub-Cell base so that the sample wells are near the cathode (black). DNA samples will migrate towards the anode (red) during electrophoresis.
9. Submerge the gel under 4 to 6 mm of electrophoresis buffer.

2.4 Recirculation Ports

Buffer recirculation is not required for most run conditions on the Sub-Cell systems. We recommend buffer recirculation for extended run times (over 2 hours) or for high voltage run conditions (150-200 volts). This will prevent lane distortion that can arise from uneven heating or buffer pH gradients. If recirculation is desired, the buffer recirculation kit (Bio-Rad catalog number 170-4537) contains the adapters required to connect the pump tubing to the Sub-Cell lid.

1. Carefully remove the port plugs from the safety lid.
2. Turn clockwise and tighten the elbow-shaped recirculation port fitting into the threaded port holes (Figure 2.2).

   Note: There should be at least three threads extending below the bottom surface of the safety lid.

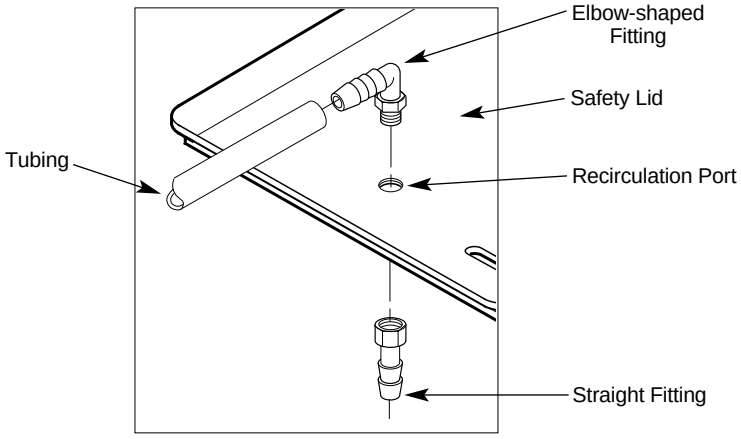

Figure 2.2. Connecting the recirculation ports and tubing.

3. Attach and tighten (10 lb.-in. torque) the straight fitting to the elbow-shaped fitting.

4. Connect tubing to the elbow-shaped fittings on the safety lid. Connect the other end of the tubing to a suitable buffer recirculation pump (Section 6.3). Attach the tubing clips at all tubing/fitting connections to insure that tubing does not disengage during electrophoresis.

5. Recirculate the buffer at a rate of 300-500 ml/min. Pumping at a higher rate will cause the gel to float or slide off the tray causing variable sample migration rates during electrophoresis. Check for any leaking in the fitting, tubing, and pump connections before turning on the power supply and starting electrophoresis.

   Note: If recirculation port fittings are to be removed, always cover the port holes by replacing the port plugs (use 5 lb.-in. torque to tighten).

2.5 Electrophoresis

Once the agarose gel has solidified, sample loading and electrophoresis can begin. Agarose gels can be run in many different types of electrophoresis buffers. Nucleic acid agarose gel electrophoresis is usually conducted with either Tris-Acetate-EDTA (TAE) buffer or Tris-Borate-EDTA (TBE) buffer. While TAE buffer provides faster electrophoretic migration of linear DNA and better resolution of supercoiled DNA, TBE buffers have a stronger buffering capacity and are less conductive than TAE buffers and therefore are used for longer or higher voltage electrophoresis runs.

Note: Because of the higher voltages and resulting higher currents often used with the Model 96 and Model 192, it is strongly recommended that only TBE buffers be used for electrophoresis. TBE buffers have a stronger buffering capacity and are less conductive. Thus, pH or temperature gradient formation during extended electrophoresis will be reduced. If pH or temperature gradients cause uneven sample migration reduce the voltage, add more buffer or recirculate the buffer during electrophoresis to eliminate these effects (Section 2.4). Bio-Rad offers premixed 10x TBE buffers as well as individual buffer reagents for use with the Sub-Cell systems (Section 6.3).

1. When placing the gel tray into the base, make sure that the sample wells are at the cathode (black). DNA samples will migrate towards the anode (red) during electrophoresis.

2. Prepare the desired concentration of electrophoresis buffer (the electrophoresis buffer used should be identical to the type used for gel preparation).

3. Submerge the gel under 4 to 6 mm of electrophoresis buffer. Do not fill buffer above the max. buffer mark on the Sub-Cell base.

4. Prepare samples for gel loading. The maximum sample loading volume for Bio-Rad combs is listed in Section 6.2. Loading volume is dependent upon the type of comb used (i.e., well thickness and length) and thickness of the gel.

5. Once loading volume is determined, samples are made dense for underlaying into sample wells by using standard nucleic acid sample loading dyes (refer to Section 3.4 for sample loading dye preparation). Add loading dye to a final 1x concentration.

6. Load the samples into the wells using standard pipets or multichannel pipets.

   Note: Sample wells are often difficult to see. Well visualization can be enhanced by placing black paper or tape under the base or tray where comb placement or well formation is common.

7. Place the lid on the DNA cell carefully. Do not disturb the samples. The Sub-Cell systems lid attaches to the base in one orientation only. To attach the lid correctly, match the red and black banana jacks on the lid with the red and black banana plugs of the base.

8. Power requirements vary depending on gel thickness, length and concentration, and type of electrophoresis buffer used. Refer to Table 2.3 for relative sample migration rate for the Sub-Cell Model 96 and Model 192 systems. Also, review Table 2.4 for DNA size migration with sample loading dyes.

   Note: Buffer recirculation is not required for most standard DNA and RNA agarose gel electrophoresis. For most electrophoresis, TBE buffer is recommended. If buffer recirculation is required, use the recirculating ports (Section 2.4).

Table 2.3 Relative Sample Migration Rates*

Cell Type	Voltage	Bromophenol Blue migration rate
Sub-Cell Model 96	200 V	5.15 cm/hr
Sub Cell Model 192	200 V	6.20 cm/hr

* **Note:** These sample migration rates were determined based on a 0.5 cm thick 1.0% agarose gel using Bio-Rad Molecular Biology Certified Agarose in 1x TBE buffer diluted from Bio-Rad Premixed 10x TBE Buffer). Migration rates will vary depending on the voltage, current, and type of agarose or buffer used.

Table 2.4 DNA Size Migration with Sample Loading Dyes

Agarose Concentration (%)	Xylene Cyanol	Bromophenol Blue
0.5 – 1.5	4-5 Kbp	400-500 bp
2.0 – 3.0 *	750 bp	100 bp
4.0 – 5.0*	125 bp	25 bp

* Sieving agarose such as Bio-Rad AmpliSize agarose.

9. With the desired power requirements, begin electrophoresis. If using buffer recirculation, electrophorese for 15 minutes before turning the pump ON.

   Note: Buffer recirculation is optional for gels that require short run times. Gels run at higher voltages (200 volts) may require recirculation to prevent heat or pH effects. Recirculate the buffer at a rate of 300-500 ml/min. Do not pump at a higher rate, it will cause the gel to float or slide off the tray causing variable sample migration rates during electrophoresis.

10. After electrophoresis is complete, turn off the power. If using buffer recirculation, do not turn the pump OFF and do not disconnect the tubing from the safety lid. Lift the safety lid with the pump still ON and empty the buffer contained in the tubing and pump into the base buffer chamber. When the tubing is empty, turn the pump OFF and disconnect the tubing if desired.

2.6 Nucleic Acid Staining and Visualization

Gels can be removed from the base or gel tray for nucleic acid staining. The gel can also remain on the UVTP gel tray for staining.

Ethidium Bromide Staining Procedure

1. Place the gel into the appropriate volume of 0.5 µg/ml ethidium bromide (EtBr) and stain for 15–30 minutes. Use enough staining solution to cover the entire gel.

   Caution: Ethidium bromide is a suspected carcinogen and should be handled with extreme care. Always wear gloves, eye glasses and a laboratory coat. Dispose of used EtBr solutions

and gels appropriately (Review EtBr Material Safety Data Sheet [MSDS] for proper disposal methods).

2. Destain the gel for 10-30 minutes in dH_2O using the same volume used for staining.

   Note: Ethidium Bromide can be removed from the DNA with extended destaining. This will cause lower sensitivity of detection. However, insufficient destaining will create higher background fluorescence.

3. Rinse the gel briefly with dH_2O once to remove any residual staining solution.

4. Place the gel on a UV transilluminator for nucleic acid visualization and analysis. DNA-Ethidium Bromide complexes may be illuminated with UV light of 254, 302, or 366 nm. Sensitivity decreases with illumination at higher wavelength. However, nicking of DNA will increase below 302 nm. Table 2.5 indicates the percentage of transmittance of UV light through 1/4″ (.635 cm) UV-transparent plastic.

   Note: Nucleic acids in the gel can be visualized through the UVTP trays. If a UVTP tray is not used, place household plastic wrap between the UV transilluminator and the gel to avoid contaminating the transilluminator with nucleic acids or EtBr.

Table 2.5 Percent UV Transmittance through 1/4" (.635 cm) UV Transparent Plastic

Wavelength (nm)	Approximate % Transmittance
254	0
302	80
360	90

5. Photograph the gel using standard cameras and film (e.g., Bio-Rad Standard Polaroid Gel Documentation System) or with CCD-based digitized image analysis systems (e.g., Bio-Rad Gel Doc™ 1000). Gels are generally photographed with a yellow, orange, or red interference filter. Red filters generally give the cleanest background. Bio-Rad offers a full-line of standard photography and CCD-based imaging systems for nucleic acid gel analysis.

2.7 Note on Blotting

Nucleic acids within the gel can be transferred to membranes using the techniques of Southern and Northern blotting. It is beyond the scope of this instruction manual to include blotting procedures. Consult References 1 and 2 for blotting techniques. Bio-Rad offers a full-line of nitrocellulose and positively-charged nylon membranes, as well as vacuum and electrophoretic blotting apparatus for Southern and Northern blotting (Section 6.3).

Section 3
Gel and Electrophoresis Reagents Preparation

3.1 Electrophoresis Buffer Preparation

DNA agarose gel electrophoresis is usually conducted with either Tris-Acetate-EDTA (TAE) or Tris-Boric Acid-EDTA (TBE). While TAE provides faster electrophoretic migration of linear DNA and better resolution of supercoiled DNA, TBE buffers have a stronger buffering capacity for longer- or higher-voltage electrophoresis runs. Bio-Rad offers premixed 50x TAE and 10x TBE buffers for use with the Sub-Cell systems. RNA formaldehyde gels require a MOPS [3-(N-morpholino)-propanesulfonic acid] electrophoresis buffer.

1x Tris-Acetate-EDTA (TAE) — 40 mM Tris (pH 7.6), 20 mM Acetic Acid, and 1 mM EDTA.

50x Stock (1 liter): dissolve in 600 ml distilled water:

Tris Base (FW = 121)	242.0 g
Glacial acetic acid	57.1 ml
0.5 M EDTA (pH 8.0)	100.0 ml

Fill to a final volume of one liter with distilled water.

1x Tris-Boric Acid-EDTA (TBE) — 89 mM Tris (pH 7.6), 89 mM Boric Acid, 2 mM EDTA

10x Stock (1 liter): dissolve in 600 ml distilled water:

Tris Base (FW = 121)	108 g
Boric Acid (FW = 61.8)	55 g
0.5 M EDTA (pH 8.0)	40 ml

Fill to a final volume of one liter with distilled water.

1x MOPS Buffer (RNA Gels) — 0.02 M MOPS [3-(N-morpholino)-propanesulfonic acid] (pH 7.0), 8 mM Sodium Acetate, 1 mM EDTA (pH 8.0)

5x Stock (1 liter): dissolve in 600 ml DEPC-treated distilled water:

MOPS	20.6 g
3 M Sodium Acetate (DEPC treated) pH 7.4	13.3 ml
0.5 M EDTA (DEPC-treated) pH 8.0	10.0 ml

Fill to a final volume of one liter with DEPC-treated distilled water.

Caution: DEPC is a suspected carcinogen. Always wear gloves, eye glasses and a laboratory coat. Use caution when handling DEPC containing solutions. Consult the DEPC MSDS (Material Safety Data Sheet) for more information.

3.2 DNA and RNA Gel Preparation

DNA Agarose Gels

(See Section 2.1)

RNA Agarose Formaldehyde Gels[1-2]

For 100 ml of a 1% agarose formaldehyde gel prepare as follows:

1.6% melted agarose	62 ml
5x MOPS Electrophoresis Buffer (1x final concentration)	20 ml
12.3 M (37.5%) Formaldehyde (2.2 M final concentration)	18 ml

Caution: Formaldehyde solutions and formaldehyde vapors are toxic. When handling solutions or gels that contain formaldehyde use a chemical hood. Always wear gloves, eye glasses and a laboratory coat while using formaldehyde. See the formaldehyde MSDS for more safety information.

3.3 RNA Sample Preparation[1-2]

Prior to loading RNA onto an agarose formaldehyde gel prepare each RNA sample as follows:

3.0 µl RNA in DEPC-treated water
5.0 µl 5x MOPS Buffer (final concentration 1.67x)
4.5 µl 12.3 M Formaldehyde (final concentration 3.7 M)
12.5 µl Formamide (final concentration 50% v/v)

Caution: Formamide is a teratogen. Always wear gloves, eye glasses and a laboratory coat. Use caution when handling formamide. Consult the formamide MSDS for more information.

3.4 DNA and RNA Sample Loading Dye[1-2]

A convenient 10x sample buffer stock consists of 50% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanole FF in 1x electrophoresis buffer. Prepare only 1-10 ml of the 10x loading dye.

3.5 Gel Staining Solution

Add 10 mg of ethidium bromide to 1 ml distilled water. Bio-Rad offers pre-mixed EtBr solutions (10 mg/ml). Store reagent in the dark.

Section 4
Care and Maintenance

4.1 Cleaning Sub-Cell System Components

1. All Sub-Cell systems parts should be washed with a mild detergent solution in warm water. If necessary, use a soft-bristled brush or sponge to remove dried buffer salts or agarose.

   Note: Be careful not to snag or break the electrode wire in the base while cleaning.

2. Rinse all parts thoroughly with warm water or distilled water and air dry, if possible.

3. To clean recirculation ports and tubing, simply pump distilled water into the tubing to rinse. Thoroughly empty tubing of liquid before use.

4.2 Compatible Cleaning Agents

Chemically compatible cleaners must be used to ensure long life of parts. These include:

- Aqueous solutions of soaps and mild detergents:
  Bio-Rad Cleaning Concentrate (catalog number 161-0722)
  Dishwashing Liquid
- Organic Solvents:
  Hexane
  Aliphatic Hydrocarbons

Do not leave plastic parts to soak in detergents more than 30 minutes. A short detergent rinse typically is all that is required.

Caution: Do not use the following chemicals to clean Sub-Cell parts. Exposure to these chemicals may cause the plastic parts to crack, craze, etch or warp.

- Chlorinated Hydrocarbons
  Carbon Tetrachloride
  Chloroform
- Aromatic Hydrocarbons
  Benzene
  Phenol
  Toluene
  Methyl Ethyl Ketone
  Acetone
- Alcohols
  Methanol
  Ethanol
  Isopropyl Alcohol

Do not use abrasive or highly alkaline cleaners on Sub-Cell parts. Do not expose Sub-Cell parts to temperatures >60 °C. Do not sterilize Sub-Cell parts by autoclaving or dry heat.

4.3 Maintenance Schedule

Item	Look For	Frequency	Action
All Parts	Dried salts, agarose, grease, and dirt	Each Use	Clean parts as described in Section 4.1
Electrical cables	Breaks or fraying	Each Use	Replace Cables
Trays	Chips or cracks	Each Use	Replace Tray
Electrode Wires	Breaks	Each Use	See Section 4.4 (Electrode Cassette Replacement)
Cable Connections (Banana Jacks and Plugs)	Looseness	Weekly	Replace Banana Jacks or Banana Plug Holders
Base	Crazing, cracks, or leaks	Monthly	Replace Base
Recirculation Tubing	Looseness or cracks	Each Use	Tighten or Replace

4.4 Electrode Replacement

The Sub-Cell systems allow easy, hassle-free replacement of broken electrode wires by simply removing the banana plug/electrode wire assembly and ordering a new assembly from Bio-Rad (Figure 4.1). See Ordering Information (Section 6.2) for catalog numbers and part descriptions.

Instructions

1. Remove the thumb screw from the side wall of the base to release the banana plug/electrode wire assembly. Do not discard this thumb screw (keep this screw with the base).
2. Remove the broken wire assembly from the base and discard the broken assembly.
3. Insert the new electrode assembly ensuring the electrode wire guard is properly seated into the electrode wire guard slot in the bottom of the base.
4. Replace and tighten the thumb screw to secure the assembly in the base.

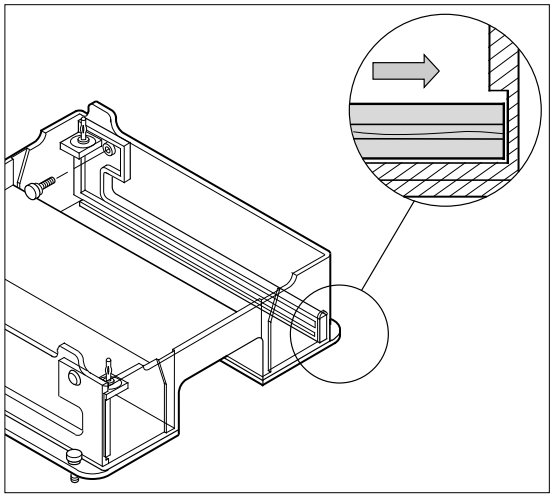

Figure 4.1 Replacement of banana plug/electrode wire assembly.

4.5 RNase Decontamination

Sub-Cell parts can be cleaned with a mild detergent and treated for 10 minutes with 3% hydrogen peroxide (H_2O_2) and then rinsed with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water to eliminate RNases prior to using the Sub-Cell systems for RNA gels[1-2]. Do not soak Sub-Cell parts in DEPC water. Consult references [1-2] for other suggestions regarding the use of DEPC in RNase decontamination.

Caution: DEPC is a suspected carcinogen. Always wear gloves, eye glasses and a laboratory coat. Use caution when handling DEPC-containing solutions. Consult the DEPC MSDS for more information.

Do not attempt to RNase decontaminate Sub-Cell parts using extreme dry heat.

Note: Several commercial products are also available for eliminating RNase contamination. RNaseZAP™ (Ambion) or RNase AWAY™ (Molecular Bio-Products) are safe, simple and effective methods that if used properly do not craze or fog the Sub-Cell parts. See manufacturer instructions for proper use.

Section 5
Troubleshooting

Symptoms	Probable Causes	Solutions
Slanted lanes (bands)	• Gel not fully solidified.	• Let gel solidify for at least 30-60 minutes.
	• Comb warped or at an angle.	• Check alignment of comb.
Curved line or distortion of lanes (bands)	• Bubbles in sample wells.	• Remove bubbles prior to electrophoresis.
Differential relative mobilities	• Sample spilled out of wells.	• Samples should have proper density. Apply carefully.
	• Unit not leveled.	• Level unit. Place on steady work bench.
	• Gel floated or slid off tray.	• Recirculate at a rate of 300-500 ml/min.
Curved bands, smiles	• Sample overload.	• Reduce the amount of sample loaded.
	• Temperature or pH buffer gradients	• Reduce load. • Add more buffer. • Recirculate buffer.
Ragged bands	• Sample density incorrect.	• See sample application instructions.
	• Sample well deformed.	• Carefully remove comb, especially from soft gels. Be sure gel has solidified. Cooling soft gels aids in comb removal. Add buffer to help lubricate removal of the comb.
	• Excessive power or heating.	• Reduce voltage. See electrophoresis instructions.
Band smearing and streaking	• Agarose has improper endosmosis ($-m_r$).	• Consult Bio-Rad about agarose.
	• Salt concentration in sample too high.	• Reduce salt concentration to ≤ 0.1 M.
	• Excessive power and heating.	• Reduce voltage. See electrophoresis instructions
	• Sample spilled out of well.	• Take care in applying sample. Increase gel thickness for large sample volumes.
	• Incomplete digest, nuclease contamination, bad enzyme.	• Heat sample. Check enzyme activity. Digest sample further.
	• Sample wells cast through the gel. Sample leaks along bottom of running surface.	• Comb should be placed 1 to 2 mm above the base of the running surface. Add buffer to help lubricate removal of the comb.
	• Sample overload.	• Dilute sample.
Bands sharp but too few bands seem	• Too high gel percentage.	• Lower gel percentage.
	• Incomplete digest.	• Check enzyme activity, digest further.
High MW bands sharp/Low MW bands smeared	• Gel percentage too low.	• Increase gel percentage. • Switch to polyacrylamide.
Gels crack	• Too high voltage gradient especially with low melting temperature agarose or low gel strength gels.	• Reduce voltage. Run gel at lower temperature.

Section 6
Ordering Information

6.1 Sub-Cell Model 96 and Model 192 Systems

Catalog Number	Product Description
	Sub-Cell Model 96 Systems
170-4540	**Sub-Cell Model 96/PowerPac 300 System,** 100/120 V
170-4542	**Sub-Cell Model 96/PowerPac 300 System,** 220/240 V
170-4500	**Sub-Cell Model 96,** with 25 x 10 cm tray and Gel Caster
170-4501	**Sub-Cell Model 96,** with 25 x 15 cm tray and Gel Caster
170-4502	**Sub-Cell Model 96,** with 25 x 10 cm tray
170-4503	**Sub-Cell Model 96,** with 25 x 15 cm tray
	Sub-Cell Model 192 Systems
170-4541	**Sub-Cell Model 192/PowerPac 300 System,** 100/120 V
170-4543	**Sub-Cell Model 192/PowerPac 300 System**, 220/240 V
170-4504	**Sub-Cell Model 192,** with 25 x 10 cm tray and Gel Caster
170-4505	**Sub-Cell Model 192,** with 25 x 15 cm tray and Gel Caster
170-4506	**Sub-Cell Model 192,** with 25 x 20 cm tray and Gel Caster
170-4507	**Sub-Cell Model 192,** with 25 x 25 cm tray and Gel Caster
170-4508	**Sub-Cell Model 192,** with 25 x 10 cm tray
170-4509	**Sub-Cell Model 192,** with 25 x 15 cm tray
170-4510	**Sub-Cell Model 192,** with 25 x 20 cm tray
170-4511	**Sub-Cell Model 192**, with 25 x 25 cm tray

6.2 Sub-Cell Model 96 and Model 192 Systems Accessories

Catalog Number	Product Description
	Sub-Cell Model 96 Accessories
170-4512	**Sub-Cell Model 96 Base**
170-4513	**Sub-Cell Model 96 Safety Lid**, with cables
170-4514	**Model 96 Gel Caster**
170-4518	**Electrode Assembly (Anode) – Red**
170-4519	**Electrode Assembly (Cathode) – Black**
170-4520	**Gel Casting Gates**
170-4521	**UV Transparent Tray,** 25 x 10 cm
170-4522	**UV Transparent Tray,** 25 x 15 cm
170-4525	**Comb Holder**
170-4537	**Buffer Recirculation Kit**

Catalog Number	Product Description

Sub-Cell Model 192 Accessories

170-4515 **Sub-Cell Model 192 Base**

170-4516 **Sub-Cell Model 192 Safety Lid**, with cables

170-4517 **Model 192 Gel Caster**

170-4518 **Electrode Assembly (Anode) – Red**

170-4519 **Electrode Assembly (Cathode) – Black**

170-4520 **Gel Casting Gates**

170-4521 **UV Transparent Tray,** 25 x 10 cm

170-4522 **UV Transparent Tray,** 25 x 15 cm

170-4523 **UV Transparent Tray,** 25 x 20 cm

170-4524 **UV Transparent Tray,** 25 x 25 cm

170-4525 **Comb Holder**

170-4537 **Buffer Recirculation Kit**

Sub-Cell Model 96 and 192 Comb Specifications

Catalog Number	Well Number	Thickness (mm)	Well Width (mm)	Well Volume Capacity* (µl)
170-4526	26	0.75	6.0	22.50
170-4527	26	1.5	6.0	45.00
170-4528	51	0.75	3.0	11.25
170-4529	51	1.5	3.0	22.50
170-4530	2	0.75	97	364.0
170-4530	4	0.75	46	172.5
170-4531	2	1.5	97	727.5
170-4531	4	1.5	46	345.0

All Sub-cell Model 96 and Model 192 combs require a comb holder (170-4525)

* Well volume capacity determined based on 0.5 cm thick gel

6.3 Related Bio-Rad Products

Contact your local Bio-Rad representative concerning the following products for nucleic acid electrophoresis and blotting.

Catalog Number	Product Description

Sub-Cell GT Systems

170-4400 **Sub-Cell GT System**

170-4401 **Sub-Cell GT System,** with 15 x 10 cm tray

170-4402 **Sub-Cell GT System,** with 15 x 15 cm tray

170-4403 **Sub-Cell GT System,** with 15 x 20 cm tray

Catalog Number	Product Description

Sub-Cell GT Systems (continued)

170-4404	**Sub-Cell GT System,** 15 x 25 cm tray
170-4405	**Wide Mini-Sub Cell GT System**
170-4406	**Mini-Sub Cell GT System**
170-4481	**Sub-Cell GT System,** with 15 x 10 cm tray and Gel Caster
170-4482	**Sub-Cell GT System,** with 15 x 15 cm tray and Gel Caster
170-4483	**Sub-Cell GT System,** with 15 x 20 cm tray and Gel Caster
170-4484	**Sub-Cell GT System,** with 15 x 25 cm tray and Gel Caster
170-4485	**Wide Mini-Sub Cell GT System and Gel Caster**
170-4486	**Mini-Sub Cell GT System and Gel Caster**

Power Supplies

165-5050	**PowerPac 300 Power Supply,** 100/120 V
165-5051	**PowerPac 300 Power Supply,** 220/240 V

Buffer Recirculation Pump Systems

170-2929	**Buffer Recirculating Pump,** 120/100 V
170-2930	**Buffer Recirculating Pump,** 220/240 V

Zeta-Probe® Positively-Charged Nylon Blotting Membranes

161-0153	**Sheets,** 9 x 12 cm, 15
161-0154	**Sheets,** 10 x 15 cm, 15
161-0155	**Sheets,** 15 x 15 cm, 15
161-0156	**Sheets,** 15 x 20 cm, 15
161-0157	**Sheets,** 20 x 20 cm, 15
161-0158	**Sheets,** 20 x 25 cm, 3
161-0159	**Roll,** 30 cm x 3.3 m, 1
161-0165	**Roll,** 20 cm x 3.3 m, 1

Zeta-Probe GT (Genomic Tested) Positively Charged Nylon Blotting Membranes

161-0190	**Sheets,** 9 x 12 cm, 15
161-0191	**Sheets,** 10 x 15 cm, 15
161-0192	**Sheets,** 15 x 15 cm, 15
161-0193	**Sheets,** 15 x 20 cm, 15
161-0194	**Sheets,** 20 x 20 cm, 15
161-0195	**Sheets,** 20 x 25 cm, 3
161-0196	**Roll,** 30 cm x 3.3 m, 1
161-0197	**Roll,** 20 cm x 3.3 m, 1

Catalog Number	Product Description

Supported Nitrocellulose Membrane (0.45 micron)

161-0090 **Sheets,** 7 x 8.4 cm, 10

161-0091 **Sheets,** 10 x 15 cm, 10

161-0092 **Sheets,** 15 x 15 cm, 10

161-0093 **Sheets,** 20 x 20 cm, 10

161-0094 **Roll,** 30 cm x 3 m, 1

Supported Nitrocellulose Membrane (0.20 micron)

161-0095 **Sheets,** 7 x 8.4 cm, 10

161-0096 **Sheets,** 15 x 15 cm, 10

161-0097 **Roll,** 30 cm x 3 m, 1

Vacuum Blotting Apparatus

165-5000 **Model 785 Vacuum Blotter**

165-5001 **Model 785 Vacuum Blotter System,** 120 VAC

165-5002 **Model 785 Vacuum Blotter System,** 220/240 VAC

Semi-Dry Transfer Cells

170-3940 **Trans-Blot® SD Semi-Dry Electrophoresis Transfer Cell**

170-3948 **Trans-Blot SD System,** 100/120 VAC

170-3949 **Trans-Blot SD System,** 220/240 VAC

UV Crosslinking Chamber

165-5031 **GS Gene Linker® UV Chamber,** 120 VAC

165-5032 **GS Gene Linker UV Chamber,** 220 VAC

165-5033 **GS Gene Linker UV Chamber,** 240 VAC

165-5034 **GS Gene Linker UV Chamber,** 100 VAC

Gel Reagents

162-0019 **Low Melt Preparative Grade Agarose,** 100 g

162-0133 **Molecular Biology Certified Agarose,** 500 g

162-0126 **High Strength Analytical Grade Agarose,** 500 g

162-0144 **Amplisize Agarose,** 50 g

170-8200 **AmpliSize® DNA Size Standard,** 50-2,000 bp

170-8210 **DNA Size Standard,** 1-4.2 Kb ladder

170-8220 **DNA Size Standard,** 0.7-8.4 Kb

161-0404 **Bromophenol Blue,** 10 g

161-0423 **Xylene Cyanole FF,** 25 g

161-0433 **Ethidium Bromide Solution,** 10 ml, 10 mg/ml

Catalog Number	Product Description
Electrophoresis Buffers	
161-0733	**10x Tris/Boric Acid/EDTA (TBE),** 1 L
161-0743	**50x Tris/Acetic Acid/EDTA (TAE),** 1 L
161-0719	**Tris,** 1 kg
161-0751	**Boric Acid,** 1 kg
161-0729	**EDTA,** 500 g
DNA Gel Image Analysis and Documentation Systems	
170-3742	**Standard Polaroid® Documentation System,** 120 VAC
170-3746	**Standard Polaroid Documentation System,** 100 VAC
170-3747	**Standard Polaroid Documentation System,** 220/240 VAC
170-7520	**Gel Doc® 1000 UV Gel Documentaion System-PC,** 100 VAC
170-7521	**Gel Doc 1000 UV Gel Documentaion System-PC,** 120 VAC
170-7522	**Gel Doc 1000 UV Gel Documentaion System-PC,** 220/240 VAC
170-7525	**Gel Doc 1000 UV Gel Documentaion System-Mac,** 100 VAC
170-7522	**Gel Doc 1000 UV Gel Documentaion System-Mac,** 120 VAC
170-7522	**Gel Doc 1000 UV Gel Documentaion System-Mac,** 220/240 VAC

Section 7
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