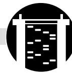

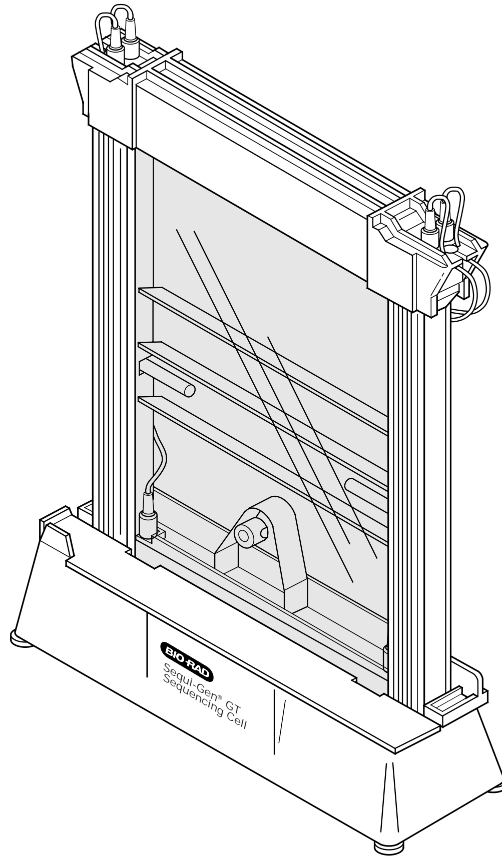

Sequi-Gen® GT Nucleic Acid Electrophoresis Cell

Instruction Manual

Catalog Numbers
165-3860, 165-3861, 165-3862 and 165-3863

Note

To insure best performance from the Sequi-Gen GT electrophoresis system, become fully acquainted with these operating instructions before using the cell. Bio-Rad recommends that you first read these instructions carefully. Then assemble and disassemble the cell 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 Sequi-Gen GT 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 you records:

Model ________________________________

Catalog No. ____________________________

Date of Delivery _________________________

Warranty Period _________________________

Serial No. ______________________________

Invoice No. _____________________________

Purchase Order No. ______________________

Warranty

Bio-Rad Laboratories warrants the Sequi-Gen GT electrophoresis system 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 warrant does not apply to parts listed below:

1. Platinum wire
2. Glass plates

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

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Section 1
General Information

1.1 Introduction to the Sequi-Gen* GT Nucleic Acid Sequencing Cell

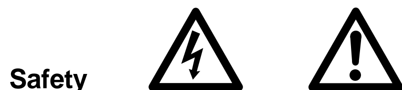
The Sequi-Gen GT cell is a modular electrophoresis cell capable of separating nucleic acids with single base-pair resolution, using a vertical slab gel format. This manual tells you how to operate and care for your new Sequi-Gen GT cell. Read Sections 1 through 3 before attempting to assemble the cell. The remainder of the manual gives you detailed procedures, a troubleshooting guide, and parts lists.

The Sequi-Gen GT cell employs a simple design that provides maximum resolution with high reproducibility, while eliminating the temperature artifacts which often occur in sequencing gels. Some of the unique features of this sequencing cell are the gel casting method, durable construction, modular components, and ease of operation, which make this the most advanced DNA sequencing cell available.

Note:
This manual contains instructions for the Sequi-Gen GT electrophoresis systems only. Prior to the release of the Sequi-Gen GT systems, Bio-Rad supplied two similar sequencing electrophoresis cell systems: the original Sequi-Gen cell and the Sequi-Gen II cell. This manual does not provide information on these systems. Contact your Bio-Rad representatives for information on the original Sequi-Gen and the Sequi-Gen II systems.

* US Patent number 4,663,015 issued to Bio-Rad Laboratories.

Safety



The Sequi-Gen GT cell has safety features to protect the operator from injury. These features include:

- Interlocking safety lids to prevent high voltage buffer shock
- Permanently sealed upper buffer chamber to prevent leaks and arcing
- Plastic components made from self-extinguishing material
- Full-length clamps to shield user from edges of glass plates
- Chemically tempered glass plates that significantly reduce glass plate breaking due to overheating and routine heating and cooling
- No exposed metallic parts
- Pour spout in lower buffer chamber allows radioactive buffer to be easily and safely poured for disposal

Important:
This apparatus meets I.E.C. 1010-1† safety standards. Sequi-Gen GT systems are safe to use when operated in accordance with the instructions. This instrument should not be modified in any way. Alteration of this instrument will:

- Void the manufacturer's warranty
- Void the IEC1010-1 safety certification
- Create a potential safety hazard

† IEC1010-1 is an internationally accepted electrical safety standard for laboratory instruments.

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

Power to the Sequi-Gen GT cell is supplied by an external DC power supply. This power supply must be ground isolated in such a way that the DC voltage output floats with respect to ground. The recommended power supply for this apparatus is the PowerPac 3000 power supply. The maximum specified operating parameters for the Sequi-Gen GT cell are:

Maximum operating voltage – 3,000 VDC
Maximum operating power – 100 Watts

Electrical current to the Sequi-Gen GT cell enters the unit through the top and bottom safety covers, providing a safety interlock to the user. Current flow to the cell is broken when either safety cover is removed. Do not attempt to circumvent this safety interlock. Always turn the power supply off while working with the sequencing cell when the safety covers are not connected.

No user-serviceable parts are contained in this apparatus. To insure electrical safety, do not attempt to service this apparatus.

Caution — Arcing

Arcing within an electrophoresis cell is represented by sparks, smoke, or charred surfaces created when an electrical short has developed. Arcing can occur if the buffer level drops below the recommended height, if there is buffer leakage, or if loose electrical connections exists. If arcing is detected during electrophoresis, immediately remove the source of electrical current (i.e., turn off the power supply).

Always use a power supply that is capable of detecting electrical conditions that may cause accidental electrical shock or damage to the apparatus. The PowerPac 3000 power supply contains safety features such as arc, no load, overload, rapid change in resistance, and ground leak detection capabilities, that will reduce the chance of accidental electrical shock and damage to the electrophoresis cell.

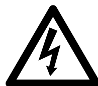
Before every use, inspect all plastic parts, glass plates, all electrical cables, jacks, and receptors for loose connections, cracks, charring, or corrosion. Do not use any part that is cracked, chipped, charred, or corroded. These parts may cause arcing. Contact your Bio-Rad representative before using a part that may cause buffer leaking or arcing.

Warning:
Never allow the gel to exceed 60 °C. Excessive heat may crack the plates or cause the silicon bond of the IPC to deteriorate.

Make sure that the upper and lower buffer chambers are filled with buffer during electrophoresis. Do not allow the buffer level to drop below the level of the short glass plate of the upper buffer chamber or below the bottom of the IPC assembly in the lower buffer chamber at any time.

Certain solvents and cleaning agents should be avoided with this unit. Refer to Section 3.2 for compatible solvents, reagents and cleaning agents.

Definition of Symbols



Caution, risk of electrical shock

Caution (refer to accompanying documents)

System Components

Each Sequi-Gen GT system comes with the components listed in Table 1.1. Check your unit to be sure 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.

Table 1.1. Sequi-Gen GT System Components

Item	Quantity
GT Universal Base	1
Stabilizer Bar	1
GT Safety Covers[1]	1
GT IPC, with bonded inner (short) glass plate[2]	1
Outer (long) Glass Plate[2]	1
GT Clamp Set (left and right clamp)[3]	1
Precision Caster Base[1]	1
Precision Caster Gasket[1]	1
Precision Caster Syringe[4]	1
Precision Caster Tubing, 60 cm	1
Precision Caster Luer Tapers	4
IPC Drain Port/Tubing Connector	1
Gel Temperature Indicator	1
Vinyl Spacers, 0.4 mm thick[3]	2
Vinyl Sharkstooth Comb, 0.4 mm thick[5]	1
Leveling Bubble	1
Instruction Manual	1

1 Parts come in 21 cm or 38 cm widths
2 GT IPC and Glass Plates are 21 x 40, 21 x 50, 38 x 30 cm or 38 x 50 cm sizes
3 GT Clamp Sets and Vinyl Spacers are either 30 cm, 40 cm or 50 cm lengths
4 Syringe sizes are 60 cc for 21 cm systems and 140 cc for 38 cm systems
5 Vinyl Sharkstooth combs are 24 well (25 teeth) for 21 cm units or 49 well (50 teeth) for 38 cm units

See Section 6 for information on accessories and replacement parts.

General Description

The Sequi-Gen GT DNA sequencing cell uses several innovative design features that are especially useful for DNA/RNA sequencing or other nucleic acid separation applications. Sequi-Gen GT DNA sequencing cell features and benefits include:

Features	Benefits
Unique, horizontal, syringe injected gel casting method	Easy gel casting without tape, grease, and acrylamide spills and waste
Upper buffer chamber heat distribution system	Provides uniform gel temperature that prevents smiling
Permanently sealed upper buffer chamber	No gaskets or grease needed to provide leak-free electrophoresis

(continued on the next page)

Features	Benefits
A universal base accepts all gel dimensions, including wide and narrow gel formats of various lengths	Modular system allows different sized gels to be used with the same lower buffer chamber
Injection molded parts	Provides years of rigorous use
Chemically tempered glass plates	Resists cracking due to overheating and rough handling
One-piece, lever-operated clamps	Conveniently and easily slides onto gel sandwich, and shields edges of glass plates from operator contact
Molded chambers with pour spouts or drain ports	Easy and safe radioactive buffer disposal
Machined vinyl spacers and sharkstooth combs	Uniform thickness of combs and spacers reduces well-to-well leakage during sample loading

Sequi-Gen GT Buffer Heat Dissipation

Uneven dissipation of the Joule heat produced by the gel during electrophoresis causes electrophoresis artifacts. “Smiling” is a common artifact that develops when a gel sandwich loses heat more efficiently at the edges than in the center. When a gel runs hotter in the center, the electrical resistance decreases, and more current flows down the center. As the current flow increases, the gel heats even more. Thus a positive feedback loop is set up which results in the lanes near the center of the gel running hotter, and therefore faster, than the lanes near the edges. Smiling can lead to ambiguity in reading the sequence.

The Sequi-Gen GT cell employs natural convection and conduction of the upper buffer to distribute heat evenly. The problems of uneven heat dissipation are avoided. Complicated, expensive thermostatic plates are not necessary.

A thin, transparent, upper buffer chamber, called an IPC (Integral Plate Chamber), acts as a heat sink across the full area of the gel. Convection occurs any time a slight temperature gradient develops, mixing the buffer (and heat) to prevent smile patterns from developing. Convection is the most effective way to distribute heat evenly. The upper buffer dampens temperature fluctuations in the gel, and adds to the reproducibility of each run. The contact between the buffer and the gel plate is direct and uniform. Thermal and physical stresses are reduced. The sample loading wells are always at the same temperature as the gel, resulting in fewer re-annealing problems. Bubbles of gas, generated by electrolysis along the cathode, rise through the buffer. These bubbles also help to prevent temperature gradients from forming by stirring the upper buffer while rising to the top of the IPC chamber.

Sequi-Gen GT Gel Casting

Because of their large size, casting sequencing gels has traditionally been extremely problematic. Taping the bottom or sides of the glass plate sandwich is time consuming and does not always result in a perfect seal. Thus, vacuum grease is required to seal corners and edges. The user must then “wrestle” with the gel mold in order to pour the gel correctly. Sliding glass plates, or plate dropping methods always result in acrylamide spills and waste. Cleaning the hazardous neurotoxin after the spills is also time consuming.

The precision caster allows quick and easy gel casting without acrylamide spills or waste. By casting the gel with a syringe through the precision caster base, gels can be poured in less than 1 minute. The gel is cast with the glass plate assembly in the horizontal position. Two full-length clamps secure the assembly and allow attachment of the precision caster base to the bottom of the glass plate sandwich. A seal between the caster gasket and the plates is created without tape or grease. The gel is injected from the bottom of the glass plate sandwich (via the injection port of the precision caster base) and moves to the top of the glass plates as a dome-shaped gel front. Acrylamide spills and waste can be eliminated by controlling the flow of the gel front at the top of the glass plates.

Modular Assembly

There are four IPC dimensions to choose from, as shown in Figure 1.1. One universal base functions as the lower buffer chamber for all IPC sizes.

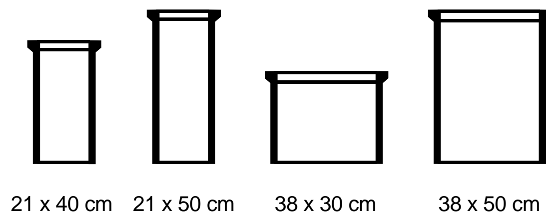


Fig. 1.1. Interchangeable sizes.

1.2 Specifications

General Specifications	
Base footprint	16 x 48 cm
Maximum unit height	65 cm (50 cm cells); 55 cm (40 cm cells); 45 cm (30 cm cells)
IPC sizes	21 x 40, 21 x 50, 38 x 30 cm and 38 x 50 cm (width x length)
Actual gel sizes	17 x 40, 17 x 50, 34 x 30 cm, 34 x 50 cm
Gel thickness range	0.25 – 0.75 mm
Nominal gel volumes (0.25 mm)	17 ml (21 x 40 cm); 21 ml (21 x 50 cm); 40 ml (38 x 30 cm); 43 ml (38 x 50 cm)
Nominal gel volumes (0.40 mm)	27 ml (21 x 40 cm); 34 ml (21 x 50 cm); 50 ml (38 x 30 cm); 68 ml (38 x 50 cm)
Minimum upper buffer volumes	500 ml (21 x 40 cm); 575 ml (21 x 50 cm); 650 ml (38 x 30 cm); 1,400 ml (38 x 50 cm)
Minimum lower buffer volume	350 ml
Maximum lower buffer volume	500 ml
Electrical Specifications	
Electrical Safety Certification	IEC 1010-1
Rated voltage limit	3,000 volts
Rated power limit	100 watts
Rated temperature limit	60 °C
Electrical cables	Rated to 3,000 volts (VDC)
Electrical leads	Rated to 3,000 volts (VDC)
Banana plugs	Rated to 3,000 volts (VDC)

Construction Specifications

GT IPC panel	Injected molded polycarbonate
GT safety covers	Injected molded polycarbonate
Universal base	Injected molded polycarbonate
Stabilizer bar	Injected molded polycarbonate
GT clamp set	PVC clamp body Protruded G10 polyester/glass cam shaft Polycarbonate insulated stainless steel rod
Glass plates	Chemically tempered 4.8 mm float glass
Combs and spacers	Plastic or machined vinyl (see Sections 2.7 and 6.1)
Electrodes (IPC and base)	Platinum, 0.25 mm diameter
Banana plugs (IPC and base)	Gold plated stainless steel, 5.08 cm length
Electrical cables	Dual, 20 AWG, tinned copper wire cable Flame retardant polyurethane insulation jacket
Electrical leads	Polyurethane insulated nickel silver, 2.95 cm length
Precision caster base	Injection Molded Polycarbonate
Tubing	Polyurethane, 3.2 mm internal diameter, 4.8 mm outer diameter
Luer taper	Polypropylene, 3.2 mm internal diameter
Gasket	Silicon Foam Sponge
Syringe	Polypropylene, 60 cc or 140 cc
Drain port connector	Polypropylene (quick coupling assembly) 3.2 mm internal flow diameter

Section 2
Description of Major Parts

2.1 Sequi-Gen GT Parts

See Figures 2.1 and 2.2 for Sequi-Gen GT part identification.

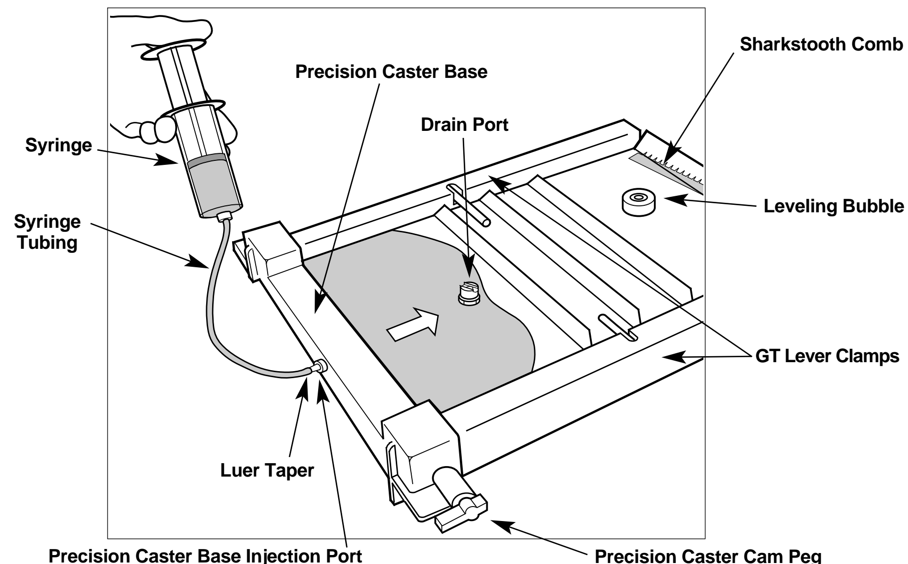


Fig. 2.1. Sequi-Gen GT gel casting parts.

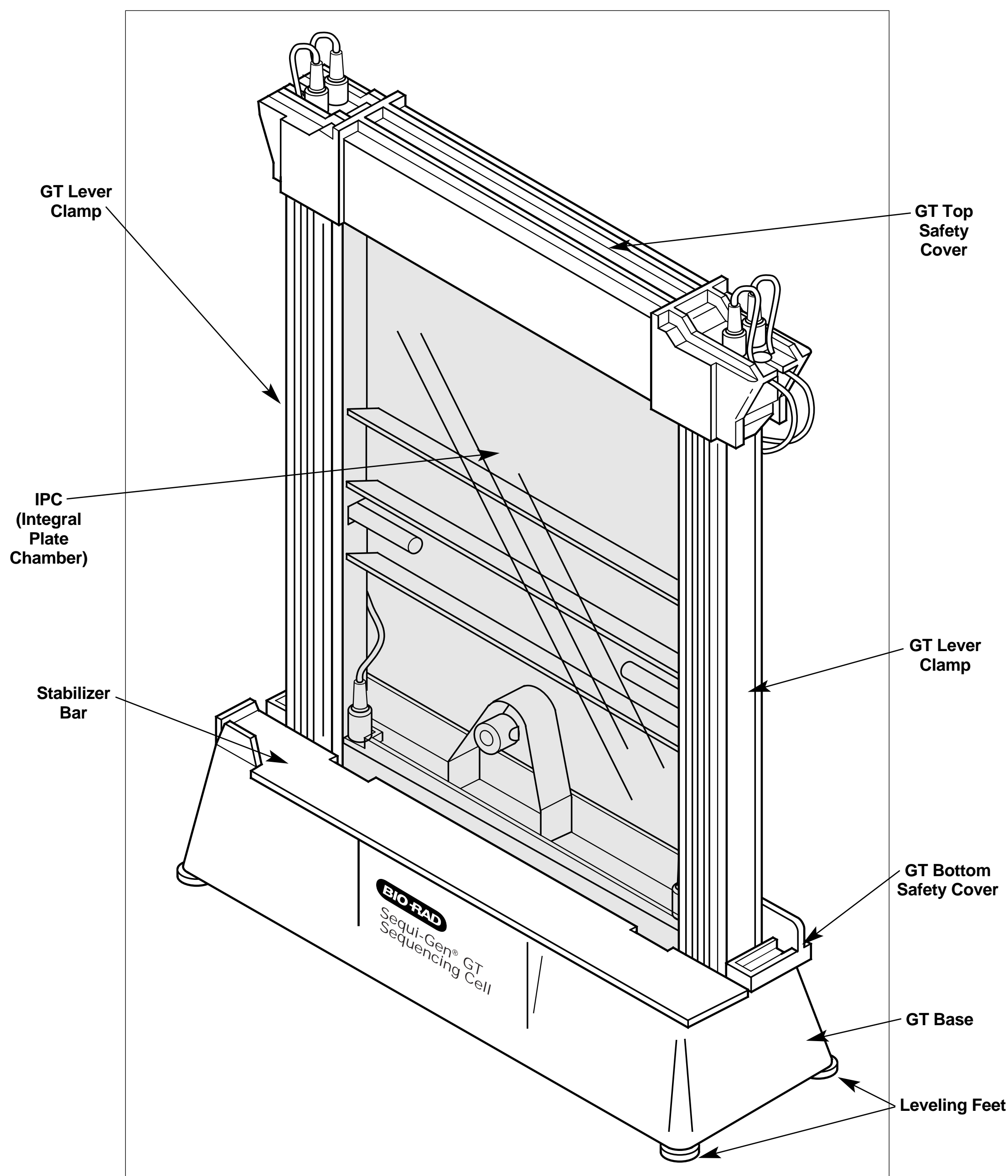


Fig. 2.2. Sequi-Gen GT nucleic acid electrophoresis cell.

2.2 Gel Reagents and Electrophoresis Buffers

For most DNA sequencing or nucleic acid separations, a 19:1 acrylamide:bis solution is required. A 1x TBE (Tris, boric acid and EDTA) solution is the preferred electrophoresis buffer. Reproducibility is affected by the quality of the gel and buffer reagents. A full line of high quality polyacrylamide gel reagents and nucleic acid electrophoresis buffers is available from Bio-Rad. Premixed reagents and buffers are also available and offer convenience, time savings, and reproducible results. Each reagent and buffer is purified to meet rigorous quality control standards. See Section 6.2 for ordering information.

2.3 Electrical Path

Both electrode wires are positioned near the bottom of the gel. The upper buffer carries the current from the cathode up to the top of the plates near the fill spout, where the gel is exposed. The lower buffer contacts the gel at the bottom edge of the plates in the standard fashion (See Figure 2.3).

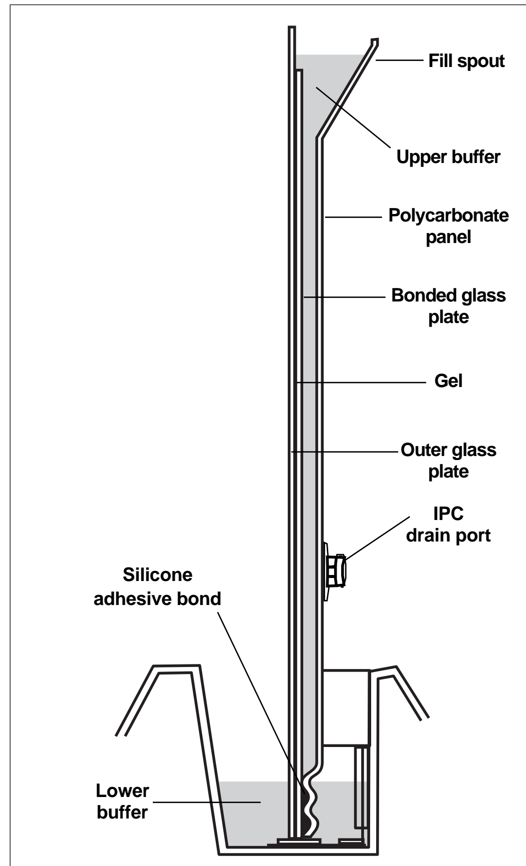


Fig. 2.3. Electrical path through IPC (Integral Plate Chamber) to lower buffer reservoir.

Section 3
Cleaning and Maintenance

3.1 Cleaning and Siliconizing Plates

Important:
To insure "bubble-free" gels using the Sequi-Gen GT precision caster, the glass plates must be thoroughly cleaned and the outer (long) glass plate siliconized or coated before each use.

1. Clean both Sequi-Gen GT glass plates (IPC and outer plates) thoroughly before each use.
    - Carefully place the plate into the sink and rinse with warm water.
    - Pour powdered lab detergent (Alconox [Alconox, Inc.] or Micro [International Products]) into a gloved hand and add sufficient water to make a paste.
    - Apply the paste and scrub the entire glass surface with a gloved hand, using circular motions.

- Rinse off all of the detergent with warm water.
- Rinse with deionized water.
- Wipe the cleaned plate with a large lint free tissue to dry.

2. Inspect the plates carefully for pieces of detergent, dried polyacrylamide, or other particles. Rewash if necessary.
3. Perform siliconizing under a fume hood, to reduce the hazard from breathing silanizing reagent. Alternatively, several non-toxic, non-corrosive glass plate coating solutions are commercially available. We recommend siliconizing or coating only the outer (long) plate, so that when the plates are separated, the gel sticks to the IPC-bound glass plate.

   - Use a glass Pasteur pipette to dispense 2 ml of the silanizing reagent onto the front plate. Coat the plate completely and evenly by spreading the silanizing reagent on the plate surface with a large lint free tissue, using a motion that travels from the top to the bottom of the plate.

   Caution: Do not siliconize the IPC plate unless hexane, heptane, or water is used as a solvent in the silanizing reagent. Other organic solvents will craze or damage the IPC plastic and weaken the adhesive bond.

   - Never heat an IPC in an oven. Severe damage will result to the adhesive bond. Use siliconizing compounds that react, or cure, at room temperature.

   Note: If the gels will be fixed or stained, the IPC (short) plate should be siliconized or coated, since its immersion into fixing or staining solutions is not recommended.

4. Prior to assembling the plates, apply a small amount of ethanol to each plate and rub to dryness with a tissue. Using the same tissue, clean the spacers.

3.2 Cleaning Sequi-Gen GT Components

1. Rinse the universal base buffer chamber, stabilizer bar, combs, spacers and precision caster base, gasket, syringe and tubing assembly with a mild detergent solution in warm water. Use a soft-bristled brush or sponge to remove polyacrylamide gel pieces.

   Note: Do not snag or break the electrode wire in the universal base while cleaning.

2. Rinse thoroughly with warm water and air dry.

Compatible Cleaning Agents for Polycarbonate Parts

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

- Aqueous solutions of soaps and mild detergents
- Organic solvents:
- Hexane
- Aliphatic hydrocarbons
- Alcohols
- Methanol
- Ethanol
- Isopropyl alcohol
- Dilute acids

Caution: Do not touch plastic molded parts with solvents that contain chlorinated hydrocarbons or aromatic hydrocarbons (*e.g.*, carbon tetrachloride, toluene, methyl ethyl ketone, acetone).

Do not use abrasive or highly alkaline cleaners on the polycarbonate plastic IPC panel. (The glass may be cleaned with abrasive or strong alkaline detergents, if adequate care is taken to avoid contact with the plastic panel.)

Do not soak plastic parts in detergents more than 30 minutes.

Cleaning the Clamps

Rinse the clamps with warm water, and wipe any polymerized acrylamide off the clamping surfaces. Drain the banana plug mounts at the top of the clamps, and wipe the clamping surfaces dry before each use. Do not use organic solvents to clean the clamps.

Section 4
Operating Instructions

4.1 Before Assembly

1. Thoroughly clean all parts as described in Section 3.

   Caution: Certain solvents and cleaning agents should be avoided. Refer to Section 3.2 for compatible cleaning agents.

2. Depending on the size of the Sequi-Gen GT IPC, make up the appropriate amount of electrode buffer (typically 1x TBE) from Table 4.1.

Table 4.1. Electrode Buffer Volumes

IPC Size	Total Buffer Required	Upper	Lower
21 x 40 cm	850 ml	500 ml	350 ml
21 x 50 cm	925 ml	575 ml	350 ml
38 x 30 cm	1,000 ml	650 ml	350 ml
38 x 50 cm	1,750 ml	1,400 ml	350 ml

4.2 Assembling the Glass Plate Sandwich

After the Sequi-Gen GT components have been washed and the glass plates siliconized or coated, assemble the Sequi-Gen GT apparatus. Always wear gloves while handling the glass plates during assembly to avoid fingerprints on the glass plates. Fingerprints will cause bubbles to form during gel casting.

Important: Before assembling the Sequi-Gen GT cell, inspect all plastic parts, glass plates, electrical cables, jacks, and receptors for loose connections, cracks, chips, charring, or corrosion. Do not use any part that is damaged. These parts may cause buffer leaks or arcing.

1. Clean and siliconize the glass plates as instructed in Section 3.1.
2. Place the IPC flat on the bench with glass plate facing upward (Figure 4.1).
   - Position one spacer along each long edge of the IPC glass plate. The bottom edges of the spacer and the glass plate should be flush and the long edge of the spacer should be next to the plastic lip of the IPC panel.

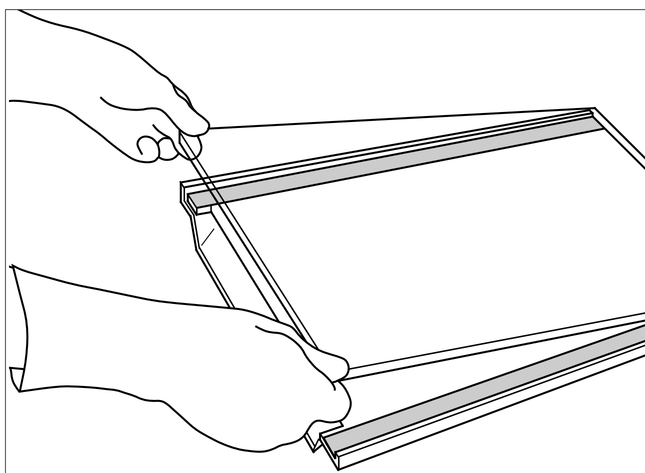


Fig. 4.1. Assembling glass plate sandwich.

3. Place the front (outer, long) glass plate onto the IPC and spacers with the siliconized or coated surface facing down.
   - With both hands, stand the IPC/glass plate sandwich on the benchtop with the outer glass plate facing away from you.
   - Allow the glass plates and spacers to touch the benchtop, to temporarily align the assembly for gel casting.
4. Slide the clamps over the IPC assembly.
   - The levers of the clamps should be on the IPC panel side of the assembly and need to be facing away from the unit (perpendicular to the IPC panel) for the clamps to slide easily onto the assembly. Secure the clamps to the IPC/glass plate sandwich by moving the levers toward the IPC panel (Figure 4.2).

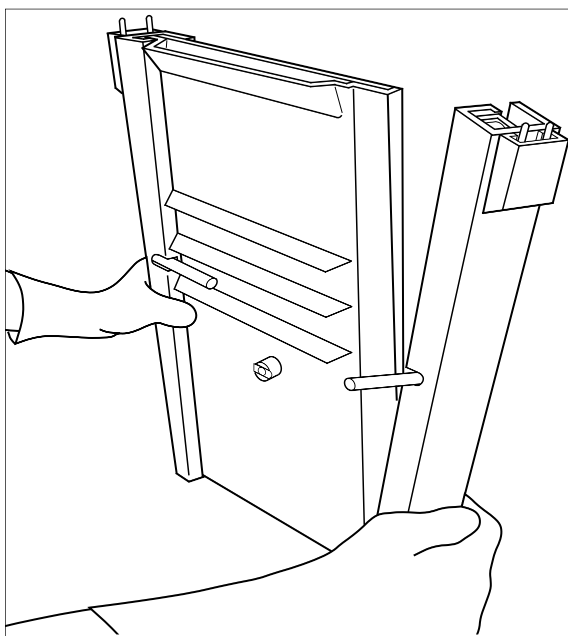


Fig. 4.2. Attaching full-length lever clamps.

5. Lay the IPC assembly on the benchtop with the IPC panel (drain port side) facing up.
   - Check the alignment of the glass plates, spacers and clamps. The bottom of the glass plates, spacers and clamps should be flush. If either glass plate, spacer, or clamp is not properly aligned or flush, adjust the alignment by loosening the clamps and move clamps, glass plates and spacers into alignment (Figure 4.3).
   - Tighten the clamps by moving the levers back down towards the IPC after the assembly is flush.

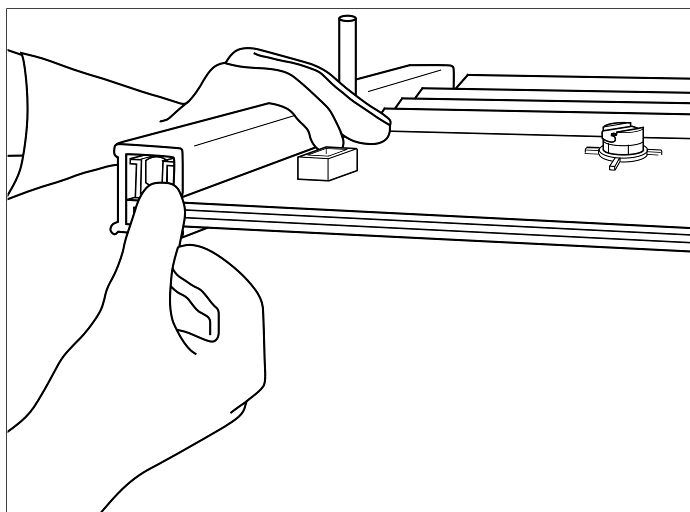


Fig. 4.3. Alignment of glass plate sandwich.

6. To avoid incompatibility problems between combs and spacers after the gel is cast, check the fit of the combs in the assembled Sequi-Gen GT cell by trying to place them between the plates.
   - If the combs clearly will not fit between the plates without damaging the comb, try a different comb. Optimally, combs should demonstrate slight resistance to being placed between the glass plates.

4.3 Casting the Gel

Section 7.1 contains a checklist of required items for DNA sequencing. Polyacrylamide is a hazardous chemical and neurotoxin. Always wear gloves, lab coat, and safety glasses while working with polyacrylamide.

1. Prepare the gel solution described in Section 7.1 and 7.2.
   - Degas the gel solution for 5-15 minutes under a strong vacuum (≥ 26 in./Hg) to insure reproducible gel porosity.

Table 4.2 Required Gel Volumes Using the Precision Caster Assembly

IPC Size	0.25 mm spacers	0.4 mm spacers	0.75 mm spacers	0.25 – 0.75 mm wedge spacer	0.40 – 1.2 mm wedge spacer
21 x 40 cm	25 ml	35 ml	70 ml	50 ml	60 ml
21 x 50 cm	30 ml	45 ml	90 ml	65 ml	85 ml
38 x 30 cm	40 ml	50 ml	90 ml	—	—
38 x 50 cm	55 ml	85 ml	170 ml	120 ml	140 ml

2. Place the precision caster base on the bench with its open cavity facing up. Place the gray precision caster gasket into the base. The cam pegs in the precision caster must be pulled out to accommodate the apparatus.

   Note: If the gasket is wet, remove any remaining water from the gasket by squeezing it with a paper towel.

3. Place the bottom edge of the IPC assembly into the precision caster base with the bottom edge of the assembly resting against the gray gasket of the precision caster base.

4. When the IPC assembly is seated in the caster base, use the cam pegs to connect the base to the clamps (Figure 4.4).

   - Push each cam peg into the corresponding hole on the clamp with the lever in the up position. Slight downward pressure applied to the top of the IPC assembly may be required to engage each cam peg.

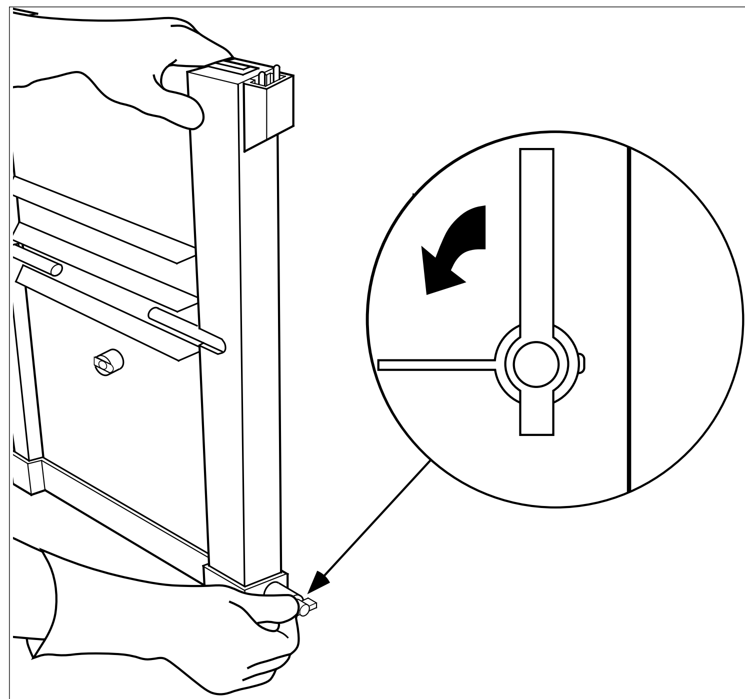


Fig. 4.4. Attaching precision caster base to IPC assembly.

5. When both pegs are engaged, turn them evenly until moderate resistance is felt or the handles of the cam pegs are perpendicular to the benchtop. This action causes the precision caster base to fit tightly against the plate assembly.

   - Lay the IPC assembly flat on the benchtop with the precision caster base facing toward you.
   - Look through the injection port of the base. If the precision caster has been attached properly, a space should be seen between the two green glass plates (Figure 4.5).
   - If the space cannot be seen, loosen the caster base by rotating the cam pegs upward. Adjust the caster base either up or down until the space between the green glass plates can be seen and is in the middle of the injection port hole.
   - While securing the precision caster base in place with one hand, turn the cam pegs back to their original position to secure the base to the bottom of the IPC assembly.

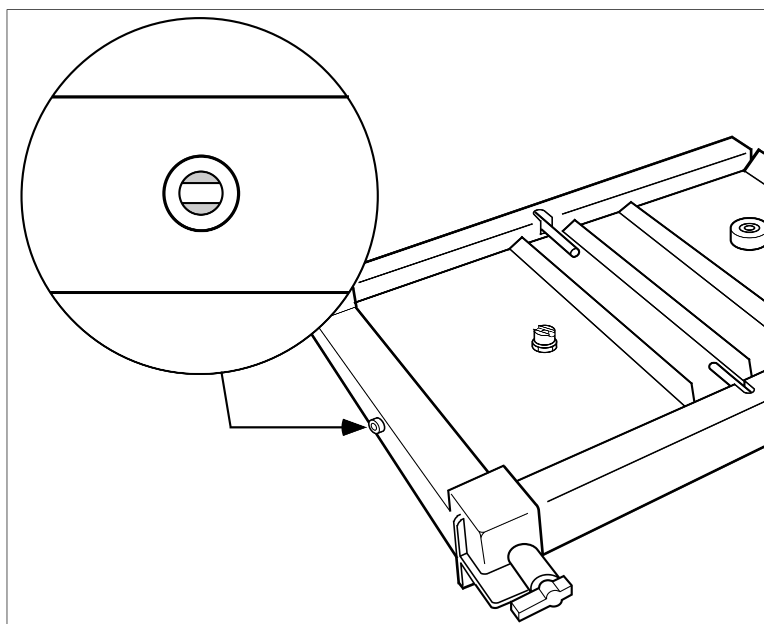


Fig. 4.5. Correct alignment of precision caster base with glass plate sandwich.

6. Lay the IPC assembly and attached precision caster base flat on a bench with the IPC panel (drain port) facing up and the long edges of the clamps running parallel with the edge of the benchtop.

   - The most even pouring can be obtained by insuring that the assembly is level on the benchtop. Failure to level the assembly may result in gel leakage. A leveling bubble is provided to facilitate leveling the IPC assembly. Props (approximately 2 cm) will be required at the top of the IPC to level the unit for casting. The unit is now ready for gel casting. An alternative to the use of props is to cast the gel with the precision caster positioned off the edge of the lab bench.

   Note: If casting a 38 x 50 cm IPC, place the 38 x 50 cm IPC assembly at an incline, with the top of the apparatus approximately 4-5 cm higher than the bottom. (The bottom of the apparatus contains the attached precision caster base). After the gel is cast, level the assembly for gel polymerization.

7. While the gel solution is degassing, prepare a fresh 25% ammonium persulfate solution (catalog number 161-0700).

   - Choose the appropriate syringe and tubing assembly (tubing and luer taper) provided with the precision caster. Insert the luer taper into the one end of the tubing. Secure the other end of the tubing onto the luer end of the syringe.

8. When the gel solution has degassed, add 25% APS and TEMED (catalog number 161-0800) in the recommended amounts (see Section 7.2).
   - Swirl the solution gently to mix.
   - **Slowly** pull the required gel volume into the syringe (see Table 4.1).
   - Tap air bubbles to the top of the syringe (luer end) and gently force them out. If bubbles are inadvertently introduced into the tubing, pinch the portion of the tubing where the bubbles exist while forcing some of the gel solution out. This should allow the bubble to exit the tubing with the gel solution.

9. When all air bubbles are removed from the tubing, place the luer taper into the injection port of the precision caster base (Figure 4.6). Tighten the luer taper fitting in place on the injection port of the precision caster base and begin to **slowly** inject the gel solution. Slow and even pressure on the syringe plunger will insure uniform gel casting with no bubbles (Figure 4.7).

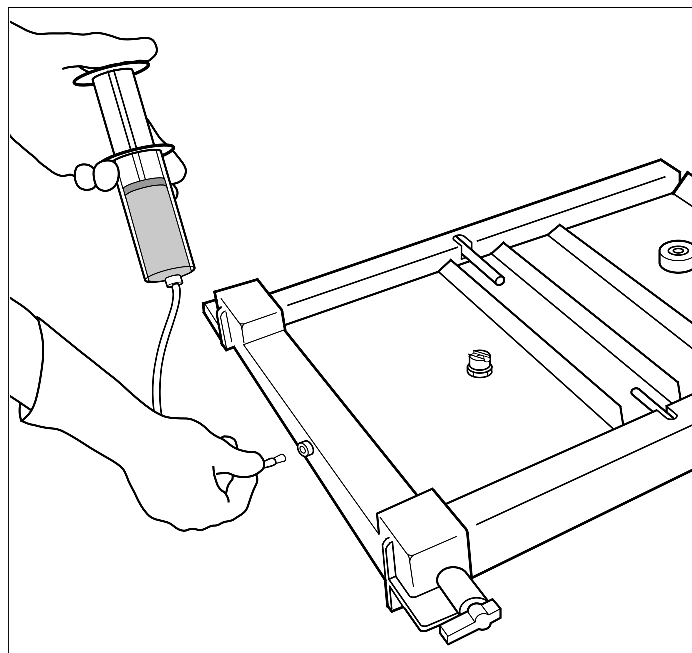


Fig. 4.6. Inserting syringe tubing into precision caster base.

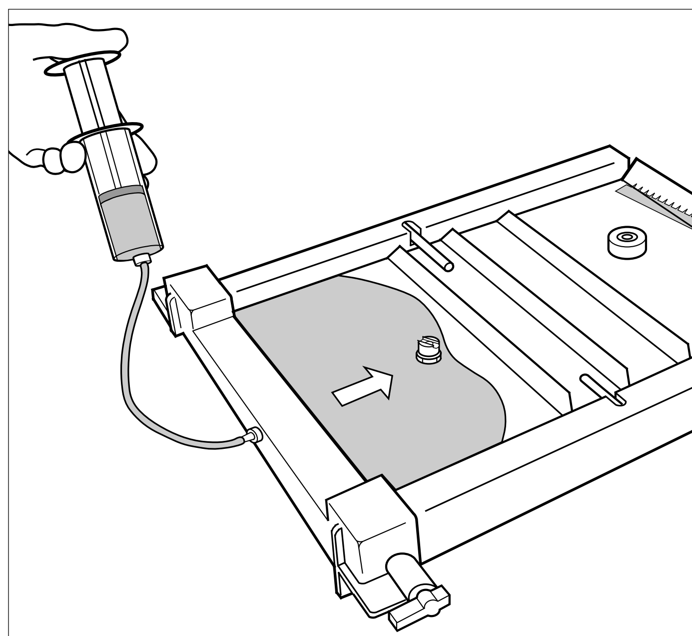


Fig. 4.7. Injecting gel solution into glass plate sandwich.

Note on Gel Bubble Formation

- The following injection times (from the bottom of IPC to the top) were found to result in bubble-free gels: for 50 cm gels with 0.4 mm spacers, between 40–45 seconds; for 50 cm gels with 0.25 spacers, between 50–65 seconds. Injection times of 10 seconds or less can result in bubble formation in the gel.
- Bubbles can form at the gel front because of soiled areas or uneven siliconization or coating of the glass plates.
- To achieve bubble free gels, thoroughly clean both plates and siliconize the outer glass plate before each use.
- If bubbles begin to form at the gel front, hard tapping on top of the IPC assembly (above the bubble formation) while slowly injecting the gel solution should eliminate the bubble. Alternatively, the comb end of the IPC assembly can be momentarily lifted at an angle to facilitate elimination.

10. Continue to **slowly** inject the gel solution until the gel solution emerges a few centimeters from the top of the notched (shorter) glass plate (across the entire width of the gel).

    Important: If pouring a 38 x 50 cm IPC, remove the support that created an incline and lay the unit level on the benchtop (use the Leveling Bubble provided). An additional 2 cm support will be needed to level the IPC assembly. Some users find it convenient to use two 1.5 ml tube racks as props.

    When the gel is past the short plate, lay the syringe on top of IPC assembly until gel polymerization is complete. **Do not** remove the luer taper from the precision caster base injection port, or the gel solution will drain out of the plates. Do not adjust the syringe plunger after the gel has been cast (Figure 4.8).

11. Insert the comb(s) between the plates to the desired depth.

    - If a sharkstooth comb is used, insert the flat edge of the comb no more than 5 mm past the short glass plate.
    - Clamp the comb(s) in place with three large metal binder clamps.

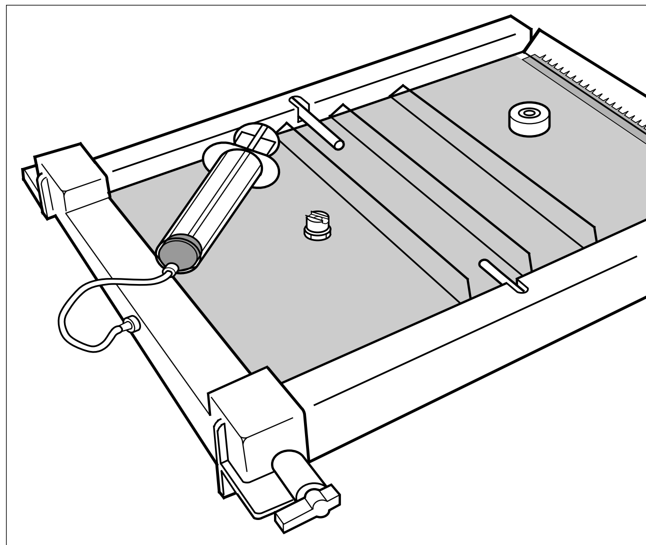


Fig. 4.8. Syringe position for gel polymerization.

- Alternatively, prior to injecting the gel solution, insert the corner of the comb to facilitate comb placement and insertion after gel casting.

12. Let the gel polymerize for 30–60 minutes.
    - After gel polymerization, remove the luer taper from the precision caster base.
    - The syringe, tubing, and luer taper can be cleaned of any remaining polymerized gel solution by rinsing with hot tap water, followed by a distilled water rinse.
13. Remove the precision caster base from the IPC assembly and clean the caster base and gasket of polymerized gel solution with tap water, followed by a distilled water rinse.

4.4 Preparing for Operation

1. Adhere a gel temperature indicator onto the outside of the outer plate, somewhere near the center, to monitor the gel temperature during electrophoresis.
    - Place the IPC assembly into the universal base, against the back wall, between the alignment tabs.
2. Insert the stabilizer bar (Figure 4.9).
    - The stabilizer bar should slide into place with a snug fit, locking the IPC to the base in a vertical position.
    - The heads of the screws on the stabilizer bar should push against the front wall of the base to press the IPC clamps against the back wall of the universal base.

   Note: When first setting up your Sequi-Gen GT cell, adjust the screws on the stabilizer bar if the fit seems too loose or too tight (turning the screws counterclockwise makes the stabilizer bar fit more tightly). Too much pressure will make it difficult to insert and remove the stabilizer bar. Too little pressure will result in the stabilizer bar sliding in and out of position without pressing the IPC against the back wall of the base.

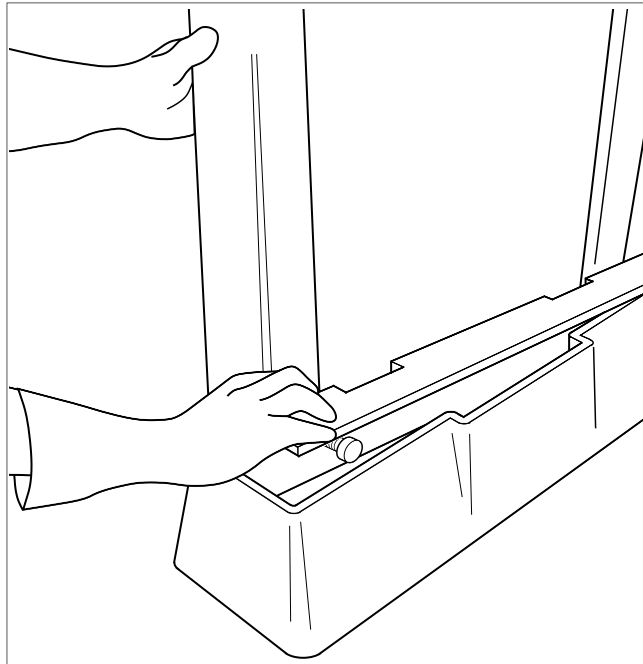


Fig. 4.9. Inserting the stabilizer bar into the universal base.

3. To avoid buffer spills and cell tipping accidents, adjust the leveling screws on the universal base, as necessary.

   - To insure that the unit will not tip over during electrophoresis, make sure the leveling feet threaded rods are at least 1 cm deep into the threaded boss of the base.

   - At this time, test whether the IPC assembly is properly aligned in the universal base by attaching the top and bottom safety covers. The IPC assembly may have to be shifted to the right or the left to properly attach the safety covers. After this final alignment is complete, remove the safety covers.

4. Fill the upper buffer chamber (the IPC) with running buffer (1x TBE) using the flared portion of the panel as a fill spout.

   - The level of the buffer should be about 1 cm from the top of the fill spout at all times during the run.

   - Remove the comb(s) from between the glass plates.

   - Thoroughly rinse the resulting well(s) or gel front using a syringe with a needle, or disposable plastic transfer pipet (catalog number 223-9911).

   - If using a sharkstooth comb, insert the comb with the teeth facing the gel front. Lower the comb toward the gel surface until the teeth of the comb just touch the gel surface.

5. Fill the lower buffer chamber with 350-500 ml of the running buffer. Refer to Appendix 7.1 for running buffer recipes.

   Caution: Do not fill the lower chamber with more than 500 ml of buffer. The lower buffer chamber holds the entire volume of the upper buffer chamber should a leak develop in the IPC. Buffer levels over 500 ml will not allow the entire volume of the upper buffer chamber to be contained in the universal base.

6. Attach the top and bottom safety covers and pre-electrophorese the gel at normal operating voltage or power (see Section 4.7), if desired, to increase the gel temperature.

   - Pre-electrophoresis prior to sample loading will create a uniform gel temperature and bring the gel temperature to the recommended run temperature. This will help eliminate any smile patterns from developing early in the run.

   Note: Gel electrophoresis buffers can be heated to 50 ˚C in a microwave before adding buffer into the upper buffer chamber. This will reduce the time needed to bring the gel to the appropriate run temperature before sample loading, and will greatly reduce pre-electrophoresis time.

   Warning: The upper buffer level may drop slightly due to evaporation as the system becomes warmer. Make sure that the upper chamber is always filled with buffer during electrophoresis. Do not allow the buffer level to drop below the level of the notched (shorter) IPC glass plate at any time during electrophoresis, as this may cause arcing and cell damage. Additionally, never allow the gel to exceed 60 °C under any circumstance. This excessive heat may crack the plates or cause the IPC/glass bond to deteriorate.

4.5 Loading the Gel

1. Turn off the power supply, and remove the top safety cover.

   - Rinse the well(s) with a syringe with needle, or disposable plastic transfer pipet (catalog number 223-9911), (to remove urea) before applying the samples to the gel.

2. Load samples on the gel (see Table 2.1 for recommended sample loading volumes for all Bio-Rad combs).
    - Samples may be applied with a 5 µl Hamilton syringe, or a pipettor fitted with gel loading tips (use Bio-Rad catalog number 223-9911).
    - Syringe loading requires rinsing the needle between samples.
    - Be sure to reconnect the upper safety cover before turning on the power supply.

    Note: Sample loading is the key to high resolution gels.

    - Rinse wells thoroughly before sample loading begins.
    - Deposit samples directly on the gel surface.
    - Electrophorese the samples into the gel soon after loading (every 4 lane sets) to reduce sample diffusion and enhance band resolution.

4.6 Gel Electrophoresis

1. Make sure both safety covers are in place.
    - Apply the voltage by pressing the Start or Run button on the power supply.
    - Verify that current is flowing (note bubbles forming at the cathode wire in the IPC), and that all electrical connections are solid.

    Running the gel with constant power (watts) will result in a constant gel temperature during the run and reproducible gel electrophoresis.

    Power conditions for DNA sequencing gels are usually dictated by gel running temperature. Run sequencing gels at 50 °C for best results. Refer to the following table for typical power (watts) settings that result in 50 °C runs.

    These settings are only guidelines–optimal settings for gels should be determined empirically. Use a temperature indicator (one is included with this unit) to monitor running temperatures. If the temperature goes above 55 °C, reduce the power output of the supply. Alternatively, use a power supply with temperature control functions (PowerPac 3000 with temperature probe) to monitor and control gel temperature.

Table 4.3 Approximate Power (Watts) Settings for Operating Sequi-Gen GT Cells

Sequi-Gen GT Cell Size	Gel Thickness	Recommended Power Setting
21 x 40 cm	0.25 mm	35-45 W
21 x 40 cm	0.40 mm	40-50 W
21 x 40 cm	0.75 mm	45-55 W
21 x 40 cm	0.25-0.75 mm wedge	45-55 W
21 x 40 cm	0.4-1.2 mm wedge	45-55 W
21 x 50 cm	0.25 mm	45-55 W
21 x 50 cm	0.40 mm	50-60 W
21 x 50 cm	0.75 mm	55-65 W
21 x 50 cm	0.25-0.75 mm wedge	55-65 W
21 x 50 cm	0.4-1.2 mm wedge	55-65 W

(continued on the next page)

Table 4.3 *(continued)*

Sequi-Gen GT Cell Size	Gel Thickness	Recommended Power Setting
38 x 30 cm	0.25 mm	70-75 W
38 x 30 cm	0.40 mm	70-75 W
38 x 30 cm	0.75 mm	70-75 W
38 x 30 cm	0.25-0.75 mm	70-75 W
38 x 30 cm	0.40-1.20 mm	70-75 W
38 x 50 cm	0.25 mm	70-80 W
38 x 50 cm	0.40 mm	75-85 W
38 x 50 cm	0.75 mm	75-85 W
38 x 50 cm	0.25-0.75 mm wedge	75-85 W
38 x 50 cm	0.40-1.2 mm wedge	75-85 W

Important: Never allow the gel temperature to exceed 60 °C. Severe damage to the glass or adhesive bond may result.

Caution: Periodically check the level of the upper buffer to make sure that it is at least 1 cm above the short glass plate.

2. Continue gel electrophoresis until the desired fragment size separation is achieved. Typically, gel electrophoresis times are monitored by observing the dye front mobility of either the bromophenol blue ("fast blue") or xylene cyanol ("slow blue") during the course of electrophoresis. Fragment and dye front mobility as a function of polyacrylamide percentage are shown in Table 4.4 below, and should be used as a guide for gel electrophoresis monitoring.

Table 4.4 Migration of Single-stranded DNA in Denaturing Polyacrylamide Gels in Relation to Dye Marker Gel Migration*

Polyacrylamide Gel Percentage	Bromophenol Blue	Xylene Cyanol
5%	35 bases	130 bases
6%	26 bases	106 bases
8%	19 bases	75 bases
10%	12 bases	55 bases

* From Ausubel, F. M. et. al., Current Protocols in Molecular Biology, Greene and Wiley, 1993.

4.7 Disassembly

1. When the desired dye front mobility has been achieved, turn off the power supply, and remove both safety covers.
   - The upper buffer chamber can be partially emptied by inserting the drain port connector (and any attached tubing) into the drain port on the IPC. A "click" will be heard when the drain port/tubing connector has been properly inserted (Figure 4.10).
   - Buffer will begin to drain from the IPC immediately after the connector is inserted into the drain port.

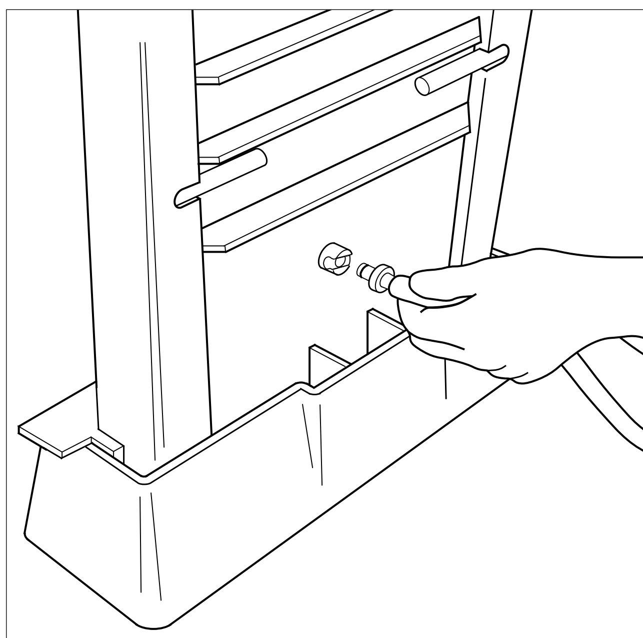


Fig. 4.10. Inserting the drain port connector for upper buffer chamber drainage.

2. After the upper buffer chamber is emptied to the level of the drain port, pull out the stabilizer bar, and remove the IPC assembly. Blot the bottom edge of the IPC assembly onto absorbent paper before removing it to a nearby sink.

3. Carefully pour the remaining upper buffer out of the IPC assembly into a sink. Slowly and carefully pour the lower buffer contained in the universal base into the appropriate sink or container.

   Caution: Never store buffers in an IPC. Never add buffer to an IPC unless the clamps are in place. The lever clamps provide the necessary force to keep the static head pressure of the upper buffer from straining the adhesive bond.

4. Remove the clamps from the IPC assembly by first pulling the levers away from the IPC and then sliding the clamps off the IPC assembly.

   - Place the Sequi-Gen GT cell flat on a bench with the outer glass plate facing up.
   - Carefully separate the glass plates by pulling up gently near the top of the outer plate.
   - After the plates begin separating, carefully determine to which plate the gel is sticking (the gel should stick to the short (inner, bonded) glass plate on the IPC).

5. When the gel is secured onto one glass plate, carefully place a piece of filter paper (catalog number 165-0921) onto the gel surface. Press firmly on the filter paper to make the gel adhere.

6. Trim around the filter paper with a razor blade or scissors to remove any excess gel.

   - Remove the filter paper and gel by lifting up one end of the filter paper and carefully peeling the gel off the plate.
   - Place the gel, filter paper side down, on the bench and cover it with a piece of plastic wrap. Use a razor blade or scissors to trim away any excess plastic wrap.

7. The gel is now ready for drying, autoradiography, and interpretation of results. Refer to Section 7.3 for gel drying and autoradiography procedures.

Section 5
Troubleshooting Guide

5.1 Operational Troubleshooter

The following table summarizes possible Sequi-Gen GT operational difficulties, probable causes, and solutions. Refer to Sections 3 and 4 for recommended procedures.

Problem	Probable Causes	Solution
No gel solution entering gel mold	• Luer taper, tubing or syringe orifices blocked.	• Thoroughly clean syringe, tubing and tapers of dried gel solution with warm water and mild detergent (see Section 3)
	• Precision Caster Base injection port misaligned with gap between the glass plates	• Readjust precision caster base so that white slit can be seen between the green glass plates while looking through the injection port (see section 4)
	• Precision caster gasket missing	• Insert gasket into base
	• Gasket hole not aligned with the injection port of the precision caster base	• Disassemble precision caster base from the IPC assembly, adjust the gasket and reassemble
	• Glass plates and gasket are not in contact	• Disassemble and make sure the bottom edges of the clamps, glass plates and spacers are flush
Gel solution is leaking into the precision caster base	• Glass plates, spacers and clamps are not flush at their bottom edge	• Disassemble and make sure the bottom edges of the clamps, glass plates and spacers are flush
	• Entire casting assembly (precision caster assembly and IPC assembly) at too high of an incline	• Lower the casting assembly or completely level the assembly
Gel solution is receding from the top of the gel	• Glass plates, spacers and clamps are not flush	• Disassemble and make sure the bottom edges of the clamps, glass plates and spacers are flush
	• Entire casting assembly (precision caster assembly and IPC assembly) at an incline	• Level the casting assembly
Bubbles in gel	• Air bubbles injected into the mold because gel was injected too quickly	• Refer to injection rate recommendations under Note on Gel Bubble Formation, Section 4.3
	• Gel solution not degassed	• Degas gel before casting
	• Air trapped in tubing	• Remove tubing before drawing gel solution into the syringe, then attach tubing and gently push gel solution through tubing
	• Air bubbles trapped in syringe	• Draw gel solution into the syringe barrel slowly to avoid introducing bubbles on the side of the barrel
	• Air injected into the gel mold because gel volume was inadequate	• See Section 4.4 (Table 4.2) for suggested gel volumes

Condition	Probable Causes	Solutions/Preventions
Upper buffer level drops too fast during run	• Normal consequence of IPC plastic bowing slightly as it heats up • Spacers leaking out the sides of the gel • Buffer leaks down between gel and spacers • Bond failure. Chamber leaking. Sparks or burn marks in adhesive.	• Refill upper buffer chamber • Caution. Monitor run. Refill upper buffer chamber. • Polymerization problem • Stop the run. Electrical hazard. IPC needs replacement.
Sparks at the top of the gel	• Upper buffer level dropped below minimum level	• Refill upper buffer chamber
Sparks in lower chamber	• Lower buffer level too low or too high	• 350 ml is minimum, 500 ml is maximum
Well-forming loading wells deform when comb pulled out	• Comb inserted too far • Gel polymerized too long, dried out • Comb pulled out too quickly • Gel not polymerized	• Insert comb minimum distance • Rinse comb/gel with buffer before pulling out comb • Pull comb out slowly • Refer to Bulletin 1156
Unexpected power conditions	• Buffers made incorrectly • Gel hydrolyzed, more conductive • Gel too hot or cold	• Check buffers • Remake gel, run gel cooler • Run gel at 50 °C
Gel sticks to both plates when opening sandwich	• Neither plate siliconized • Both plates siliconized • Plates unclean • Outer plate pried off too quickly	• Siliconize outer plate according to Section 3.1 • Separate plates slowly

5.2 DNA Sequencing Artifacts

Electrophoretic artifacts are described below. A DNA sequencing artifact may be defined as any non-ideal graphic pattern on the X-ray film that reduces your confidence in reading, or interpreting, a sequence from that film. There are three types of DNA sequencing artifacts:

- Template-dependent artifacts
- Electrophoretic artifacts
- Autoradiographic (or data acquisition) artifacts

Template-specific artifacts are caused by biological or chemical phenomena, and relate to issues beyond the scope of this manual. Each sequencing method has its own set of potential sequence-specific artifacts. Section 7.5 contains references that discuss sequence-specific artifacts.

The basic premise for reading a DNA sequence is that each band on the film exists in a vertical register that corresponds to one base in the sequence. Non-ideal patterns, caused by problems in the three categories above, may interfere with the accurate determination of DNA sequences. The following is a guideline for description and analysis of artifacts in DNA sequencing gels, especially electrophoretic ones.

Electrophoretic Artifacts

There are many sources of electrophoretic artifacts. To simplify the task of defining an artifact, we use a systematic description of electrophoretic artifacts, dividing all of the possible patterns into three hierarchical sub-categories:

- Lane-local artifacts
- Set (template)-local artifacts

- Total signal artifacts

Many artifacts appear in more than one sub-category. For example, smile patterns can exist in lane-local or in total signal situations (or both), but rarely appear in set-local. It is important to distinguish the extent and location of each artifact before trying to analyze or troubleshoot the anomalous pattern.

1. Lane-local artifacts

Condition	Probable Causes	Solutions/Preventions
Smiling bands	• Loading wells not straight	• Allow gel to polymerize more completely before removing comb
	• Sample molarity too high relative to gel	• Check sample and gel buffers
	• Sample overloaded	• Reduce sample load volume
	• Sample was allowed to diffuse into gel too long before electrophoresis	• Load a set or two at a time
Frowning bands	• Loading wells not straight	• Pour new gel with better lanes
	• Sample molarity too low relative to gel	• Check sample and gel buffers
	• Sharkstooth comb inserted too far into the gel	• Touch the top of the gel with the teeth
Complex (curvy) bands	• Loading wells not straight or clean	• Allow gel to polymerize more completely before removing comb
	• Bubble in gel	• Pour new gel without bubbles
Funneling or lane narrowing	• Sample molarity too high	• Check sample and gel buffers
Lane widening	• Sample molarity too low	• Check sample and gel buffers
Variations in signal intensity along the lane(s)	• Sample dependent artifact • Check sample and gel buffers	
Band spacing compressed or stretched	• Sample dependent artifact	

2. Set-local artifacts

Condition	Probable Causes	Solutions/Preventions
Smiling within sets	• Loading wells not straight within set	• Pour new gel with better lanes
	• Molarity problems in the samples of the set	• Check sample and gel buffers
Frowning within sets	• Loading wells not straight	• Pour new gel with better lanes
	• Molarity problems in the samples of the set	• Check sample and gel buffers
Funneling within sets	• Sample molarity too high, or contamination	• Check sample and gel buffers
Non-continuous vertical register	• Sample dependent artifact • Autoradiographic artifact	
Band spacing compressed within a set	• Sample dependent artifact	
Film exposure differences within a set	• Sample dependent artifact	

3. Total Signal Artifacts

Condition	Probable Causes	Solutions/Preventions
Large horizontal exposed areas of film	• Buffer contamination with label	• Clean IPC and buffer containers, remake buffers
High molecular weight area distorted on film	• Molarity or pH anomaly in gel causing dehydration (gel shrinking and bubbles) located near the top of the gel	• Gel run too hot, gel buffer hydrolyzed, gel buffer not made up correctly, or upper buffer degraded
Fuzzy bands, bands smeared, resolution problems	• Improper sample loading • Hydrolyzed gel matrix • Ionic contaminants in gel	• Refer to Section 3.5 • Don't run gel above 55 °C • Use only electrophoresis grade reagents, check purity • TEMED or APS concentration too high • Pre-running gel may result in better resolution
	• Polymerization problem	• Refer to Section 7.1 and 7.2 for protocols • Refer to bulletin 1156
	• Improper gel temperature	• Use Gel Temperature Indicator; 50 °C is usually high enough
Autoradiogram shows large black spots or radiating patterns	• Wet plastic wrap or wet gel • Static electricity sparks exposed film during handling	• Re-expose with dry Saran Wrap • Do not rub film prior to placing or removing film
Film sticks to dried gel	• Gel not completely dried • Hygroscopic urea has bound water	• Dry gels longer. Remove urea by soaking gel in methanol-acetic acid before drying.
Blank autoradiogram	• Sample dependent problem • Autoradiography problem	

Section 6
Equipment and Accessories

6.1 Sequi-Gen GT Nucleic Acid Electrophoresis Cells and Accessories

Catalog Number	Product Description
165-3860	**Sequi-Gen GT System***, 21 x 40 cm
165-3861	**Sequi-Gen GT System***, 21 x 50 cm
165-3862	**Sequi-Gen GT System***, 38 x 30 cm
165-3863	**Sequi-Gen GT System***, 38 x 50 cm
165-3802	**Sequi-Gen GT/PowerPac 3000 System**, 21 x 40 cm, 100/120 V†
165-3805	**Sequi-Gen GT/PowerPac 3000 System**, 21 x 40 cm, 220/240 V†
165-3803	**Sequi-Gen GT/PowerPac 3000 System**, 21 x 50 cm, 100/120 V†
165-3806	**Sequi-Gen GT/PowerPac 3000 System**, 21 x 50 cm, 220/240 V†
165-3810	**Sequi-Gen GT/PowerPac 3000 System**, 38 x 30 cm, 100/120 V†
165-3811	**Sequi-Gen GT/PowerPac 3000 System**, 38 x 30 cm, 220/240 V†
165-3804	**Sequi-Gen GT/PowerPac 3000 System**, 38 x 50 cm, 100/120 V†
165-3807	**Sequi-Gen GT/PowerPac 3000 System**, 38 x 50 cm, 220/240 V†
165-3870	**GT IPC Assembly**, 21 x 40 cm
165-3871	**GT IPC Assembly**, 21 x 50 cm
165-3872	**GT IPC Assembly**, 38 x 30 cm
165-3873	**GT IPC Assembly**, 38 x 50 cm
165-3880	**GT IPC**, 21 x 40 cm
165-3881	**GT IPC**, 21 x 50 cm
165-3882	**GT IPC**, 38 x 30 cm
165-3883	**GT IPC**, 38 x 50 cm
165-3644	**Outer Glass Plates**, 38 x 30 cm, 2
165-3882	**Outer Glass Plates**, 21 x 40 cm, 2
165-3646	**Outer Glass Plates**, 21 x 50 cm, 2
165-3649	**Outer Glass Plates**, 38 x 50 cm, 2
165-3866	**GT Universal Base**
165-3801	**Stabilizer Bar**
165-3867	**GT Safety Covers**, with cables, 21 cm
165-3868	**GT Safety Covers**, with cables, 38 cm
165-3875	**GT Clamp Set**, 30 cm
165-3876	**GT Clamp Set**, 40 cm
165-3877	**GT Clamp Set**, 50 cm
165-3878	**Precision Caster Assembly**, 21 cm

165-3879 **Precision Caster Assembly**, 38 cm
165-3886 **Precision Caster Base**, 21 cm
165-3887 **Precision Caster Base**, 38 cm
165-3888 **Precision Caster Gasket**, 21 cm
165-3889 **Precision Caster Gasket**, 38 cm
165-3891 **Precision Caster Syringe**, 60 cc
165-3892 **Precision Caster Syringe**, 140 cc
165-3893 **Precision Caster Tubing**, 60 cm
165-3894 **Precision Caster Luer Taper**, 4
165-3895 **Drain Port Connector**, 2
165-3720 **Gel Temperature Indicator**, 5

Vinyl Spacers

165-3812 **Machined Vinyl Spacers,** 30 cm, 0.4 mm, red
165-3813 **Machined Vinyl Spacers,** 30 cm, 0.25 mm, blue
165-3814 **Machined Vinyl Spacers,** 40 cm, 0.4 mm, red
165-3815 **Machined Vinyl Spacers,** 40 cm, 0.25 mm, blue
165-3816 **Machined Vinyl Spacers,** 50 cm, 0.4 mm, red
165-3817 **Machined Vinyl Spacers,** 50 cm, 0.25 mm, blue
165-3818 **Machined Vinyl Spacers,** 30 cm, 0.75 mm, grey
165-3819 **Machined Vinyl Spacers,** 40 cm, 0.75 mm, grey
165-3828 **Machined Vinyl Spacers,** 50 cm, 0.75 mm, grey
165-3820 **Machined Vinyl Wedge Spacers,** 40cm, 0.25-0.75mm, blue
165-3821 **Machined Vinyl Wedge Spacers,** 40cm, 0.4-0.1.2mm, red
165-3822 **Machined Vinyl Wedge Spacers,** 50cm, 0.25-0.75mm, blue
165-3823 **Machined Vinyl Wedge Spacers,** 50cm, 0.4-1.2mm, red

Clear Plastic Spacers

165-3710 **Plastic Spacers,** 40cm, 0.4mm, 10
165-3711 **Plastic Spacers,** 40cm, 0.25mm, 10
165-3712 **Plastic Spacers,** 50cm, 0.4mm 10
165-3713 **Plastic Spacers,** 50cm, 0.25mm, 10
165-3714 **Plastic Spacers,** 80cm, 0.4mm, 10
165-3715 **Plastic Spacers,** 80cm, 0.25mm, 10
165-3716 **Plastic Spacers,** 100cm, 0.4mm, 10
165-3717 **Plastic Spacers,** 100cm, 0.25mm, 10

* All Sequi-Gen GT systems include: GT IPC assembly (IPC and bonded inner glass plate, outer glass plate, and clamp set), GT universal base, GT safety covers with cables, stabilizer bar, precision caster assembly (precision caster base, gasket, tubing, luer tapers, tubing, and syringe), 0.40 mm vinyl sharkstooth comb and spacers, gel temperature indicator, leveling bubble, drain port connector, and instruction manual.

† All Sequi-Gen GT/PowerPac 3000 systems include the appropriate Sequi-Gen GT system (described above), PowerPac 3000 power supply, PowerPac temperature probe and PowerPac instruction manual.

Machined Vinyl Combs

Vinyl sharkstooth combs and spacers are machined to maintain a uniform and precise thickness throughout the length of each spacer and comb and between all vinyl spacers and combs. All spacers and sharkstooth combs are color-coded based on thickness. Blue (0.25 mm), red (0.4 mm), and gray (0.75 mm) spacers are available in 30, 40 and 50 cm lengths. Blue and red sharkstooth combs are available in 15 and 30 cm lengths and a wide range of well formats including multichannel pipet/microplate-compatible (MP) combs for high throughput applications.

Catalog Number	Comb Thickness (mm)	Comb Length (cm)	Number of Wells	Well Width (mm)	Well Separation (mm)	Maximum Well Volume* (µl)
Machined Vinyl Sharkstooth Comb						
165-3830	0.25	15	24	6.1	None	4.5
165-3831	0.25	15	36	4.1	None	3.0
165-3832	0.25	15	48	3.1	None	2.3
165-3833	0.25	30	48	6.1	None	4.5
165-3834	0.25	30	72	4.1	None	3.0
165-3835	0.25	30	96	3.1	None	2.3
165-3836	0.40	15	24	6.1	None	7.3
165-3837	0.40	15	36	4.1	None	5.0
165-3838	0.40	15	48	3.1	None	3.7
165-3839	0.40	30	48	6.1	None	7.3
165-3840	0.40	30	72	4.1	None	5.0
165-3841	0.40	30	96	3.1	None	3.7
MP Vinyl Sharkstooth Combs						
165-3842	0.25	15	34		None	
165-3843	0.25	30	68		None	
165-3844	0.25	30	100		None	
165-3845	0.40	15	34		None	
165-3846	0.40	30	68		None	
165-3847	0.40	30	100		None	

* Maximum well volumes are calculated values based on an assumed well height. Well height will vary with each user. The well volumes indicated will vary from run to run and user to user.

Clear Plastic Combs and Spacers

Well forming combs are 14 cm and 31 cm wide. All plastic well-forming combs, sharkstooth combs, and spacers are made from inert plastic which does not catalyze or inhibit polymerization. Thus, the combs are easy to remove without damaging the sample loading wells.

Catalog Number	Comb Thickness (mm)	Comb Length (cm)	Number of Wells	Well Width (mm)	Well Separation (mm)	Maximum Well Volume* (µl)
Plastic Sharkstooth Comb						
165-3700	0.40	15	24	6.1	None	7.3
165-3702	0.40	15	48	3.1	None	3.7
165-3701	0.25	15	24	6.1	None	4.5
165-3703	0.25	15	48	3.1	None	2.3
Plastic Well-forming Comb						
165-3684	0.40	14	16	6.7	2.4	42.5
165-3685	0.25	14	16	6.7	2.4	26.5
165-3686	0.40	14	20	4.9	2.4	31.0
165-3687	0.25	14	20	4.9	2.4	19.5
165-3688	0.40	14	36	2.4	1.6	3.6
165-3689	0.25	14	36	2.4	1.6	2.3
165-3692	0.40	31	32	7.4	2.4	47.0
165-3693	0.25	31	32	7.4	2.4	29.5
165-3694	0.40	31	44	4.8	2.4	30.0
165-3695	0.25	31	44	4.8	2.4	18.5
165-3696	0.40	31	60	3.6	1.6	5.5
165-3697	0.25	31	60	3.6	1.6	3.4
165-3698	0.40	31	80	2.3	1.6	3.5
165-3699	0.25	31	80	2.3	1.6	2.2
MP Plastic Well-forming Combs						
165-3848	0.40	15	34			
165-3849	0.40	30	68			
165-3850	0.75	15	34			
165-3851	0.75	30	68			

* Maximum well volumes are calculated values based on an assumed well height. Well height will vary with each user. The well volumes indicated will vary from run to run and user to user.

6.2 Electrophoresis Reagents

Catalog Number	Product Description
Electrophoresis Buffers and Gel Reagents	
161-5100	**PAGE Reagent Starter Kit**,1 includes Acrylamide, 100 g; Bis, 5 g; TEMED, 5 ml; Ammonium Persulfate, 10 g
Premixed Acrylamide Solutions[1,2]	
161-0154	**30% Acrylamide/Bis Solution**, 19:1, 500 ml
161-0155	**30% Acrylamide/Bis Solution**, 19:1, 2 x 500 ml
161-0144	**40% Acrylamide/Bis Solution**, 19:1, 500 ml
161-0145	**40% Acrylamide/Bis Solution**, 19:1, 500 ml
Premixed Acrylamide/Bis Powders[1]	
161-0120	**Acrylamide/Bis**, 19:1, 30 g
161-0123	**Acrylamide/Bis**, 19:1, 150 g
Crosslinkers and Catalysts	
161-0200	**Bis**,[1] 5 g
161-0201	**Bis**,[1] 50 g
161-0800	**TEMED**,[1,3] 5 ml
161-0801	**TEMED**,[1,3] 50 ml
161-0700	**Ammonium Persulfate**,[1,3] 10 g
Premixed Buffers	
161-0741	**Premixed 10x TBE Extended Range**, 1 L
161-0758	**Premixed 10x TBE Extended Range**, 6 x 1 L
161-0733	**Premixed 10x Tris/Boric Acid/EDTA (TBE)**, 1 L
161-0756	**Premixed 10x Tris/Boric Acid/EDTA (TBE)**, 6 x 1 L
Powders and Reagents[1]	
161-0100	**Acrylamide**, 99.9%, 100 g
161-0101	**Acrylamide**, 99.9%, 500 g
161-0107	**Acrylamide**, 99.9%, 1 kg
161-0103	**Acrylamide**, 99.9%, 2 kg
161-0108	**Acrylamide**, 99.9%, 5 kg
161-0730	**Urea**, 250 g
161-0731	**Urea**, 1 kg
161-0716	**Tris**, 500 g
161-0719	**Tris**, 1 kg
161-0750	**Boric Acid**, 500 g
161-0751	**Boric Acid**, 1 kg
161-0728	**EDTA**, 100 g
161-0729	**EDTA**, 500 g

1. Hazardous shipping charges may apply.
2. Store at 4 °C.
3. For a longer shelf life, store desiccated at room temperature.

6.3 Power Supplies and Slab Gel Dryers

Catalog Number	Product Description
Power Supplies	
165-5056	**PowerPac 3000 Power Supply**, 110/120 V
165-5057	**PowerPac 3000 Power Supply**, 220/240 V
165-5059	**PowerPac 3000 Power Supply with Temperature Probe**, 110/120 V
165-5060	**PowerPac 3000 Power Supply with Temperature Probe**, 220/240 V
Slab Gel Dryers	
165-1752	**Model 583 Gel Drying System**, 110/120 V; includes Model 583 Gel Dryer, Vacuum Pump, Trap, tubing and connectors
165-1753	**Model 583 Gel Drying System**, 220/240 V; includes Model 583 Gel Dryer, Vacuum Pump, Trap, tubing and connectors
165-1745	**Model 583 Slab Gel Dryer**, 100/120 V
165-1746	**Model 583 Slab Gel Dryer**, 220/240 V
165-0959	**Sequencing Gel Filter Paper**, 35 x 45 cm, 25 sheets
165-0962	**Filter Paper Backing**, 35 x 45 cm, 25 sheets
165-0963	**Cellophane Membrane Backing**, 35 x 45, 50 sheets

6.4 DNA Template Purification, Sequencing and Cloning Products

Catalog Number	Product Description
DNA Template Purification	
732-6100	**Quantum Prep™ Plasmid Miniprep Kit**, 100 preps
DNA Template Sequencing	
170-3407	**Bst® Premixed Standard Sequencing Kit**, 50 reactions
170-3414	**Bst Premixed 7-deaza-dGTP Sequencing Kit**, 50 reactions
170-3409	**Bst adjustable Ratio Sequencing Kit**, 50 reactions
DNA Mutagenesis	
170-3580	**Muta-Gene® M13 In Vitro Mutagenesis Kit**, 25 reactions
170-3581	**Muta-Gene Phagemid In Vitro Mutagenesis Kit**, 25 reactions

6.5 Liquid Handling

Catalog Number	Product Description
223-9911	**Seque/Pro Capillary Tips**, 200/Box
223-9912	**Seque/Pro Capillary Tips**, 200/Box, autoclaved
223-9314	**MTP-39 Pipet Tips**, 960/Box
223-9319	**MTP-39-S Pipet Tips**, 960/Box, Sterilized
211-2001	**Xcluda Aerosol Barrier Pipet Tips**, 0.5–10 µl, 960/Box, Sterilized
211-2006	**Xcluda Aerosol Barrier Pipet Tips**, 5–20 µl, 960/Box, Sterilized
211-2016	**Xcluda Aerosol Barrier Pipet Tips**, 20–200µl, 960/Box, Sterilized
223-9480	**EZ Micro Test Tube**, 1.5 ml, 500/Box
223-9503	**EZ Micro Test Tube**, 0.5 ml, 500/Box

Section 7 Appendix

7.1 DNA Sequencing Checklist

For DNA sequencing, you will need the following buffers, reagents, and equipment:

1. DNA sequencing samples, suitably labeled. (see Section 6.4)
2. 10x TBE buffer: 108 g Tris base, 55 g boric acid, 9.3 g $Na_2EDTA•H_2O$, in 1 liter deionized H_2O, autoclave. The pH of this solution should be 8.3, without adjustment (see Section 6.2).
3. Acrylamide stock solution, 30%:

   A. For low percent gels (4%-10%):

   Prepare a 30% stock solution, 19:1 Acrylamide/Bis:
   28.5 g Acrylamide
   1.5 g Bis-Acrylamide
   30.0 g Total up to 100 ml deionized H_2O
   (see Section 6.2)

   B. For medium percent gels (8%-16%):

   Prepare a 30% stock solution, 29:1 Acrylamide/Bis:
   29.0 g Acrylamide
   1.0 g Bis-Acrylamide
   30.0 g Total up to 100 ml deionized H_2O
   (see Section 6.2)

   C. For high percent gels (12%-20%):

   Prepare a 40% stock solution, 37.5:1 Acrylamide/Bis:
   38.96 g Acrylamide
   1.04 g Bis-Acrylamide
   40.0 g Total up to 100 ml deionized H_2O
   (see Section 6.2)

4. TEMED (see section 6.2)

5. Ammonium Persulfate, 25% stock solution: 0.25 g in 1 ml distilled H2O (in a microfuge tube). Make fresh daily (see Section 6.2).
6. A constant power (or constant voltage) power supply (see Section 6.3).
7. Slab gel dryer(see Section 6.3).
8. Table top micro-centrifuge
9. Gel loading syringe (e.g. Hamilton 701-SN, 28 Gauge, 1.25 inch needle)
10. 1.5 ml microcentrifuge tubes (see Section 6.5)
11. Adjustable pipettors (e.g. Pipetman P-20, P-200, P-1000)
12. Balance
13. Plastic wrap
14. Pipette tips, autoclaved (see Section 6.5)
15. Waterbath or Temp-Block at 95 °C.
16. X-ray film and cassettes (dark-room facilities)
17. Filter Paper (see Section 6.3)
18. Siliconizing solution or glass coating solution
19. Geiger Counter
20. Ice bucket

7.2 Standard Gel Protocol

The following protocol is for a standard 7 M urea, 5% polyacrylamide gel for DNA sequencing. See Section 4 for additional information on gel casting, sample loading, and gel electrophoresis. For ordering information on gel reagent and electrophoresis buffers see Section 6.

1. Combine 63 g of urea, 15 ml of 10x TBE, and 25 ml of 30% acrylamide stock solution. Bring the volume to 150 ml with distilled water (low heat may be required to dissolve the urea, but do not boil).
2. Filter the solution through a 0.45 micron mesh filter (optional). Then, degas under strong vacuum 5–15 minutes to remove dissolved oxygen.
3. Add 150 µl TEMED and 150 µl 25% ammonium persulfate (or one microliter of each reagent for every milliliter of gel solution) prior to gel casting.
4. Cast the gel according to procedures in Section 4.

   Note: Wedge spacers (see Section 6.1) increase the number of readable bases per lane in a sequencing gel. The use of wedge spacers results in a gel which becomes gradually thicker toward the bottom. As thickness increases, resistance, voltage, and DNA mobility decrease. The resulting gel has bands more closely spaced at the bottom. Wedge spacers allow the use of standard polyacrylamide solution and buffers. No alterations to the gel solution, gel casting or electrophoresis protocols are required to run DNA sequencing wedge gels.

7.3 Gel Drying and Autoradiography

The radiolabeled oligonucleotides may be visualized by a variety of techniques involving autoradiography. For the best resolution and signal intensity, dry DNA sequencing gels with a slab gel dryer.

1. Transfer sequencing gels to a fresh sheet of filter paper. Wet the gel slightly by misting the gel with deionized H_2O. Lay the dry filter paper on top of the gel, and press firmly. The gel will stick to the paper. Pick up the gel by lifting the filter paper carefully from one end.
2. Cover the sequencing gel with plastic wrap. Smooth out air bubbles and folds by rubbing with a paper towel, and trim the edges to fit the slab gel dryer.
3. Set Model 583 Gel Dryer to sequencing cycle. 30 minutes at 80 °C should suffice for drying thin low percent gels, if the applied vacuum is above 28 inches of mercury or 125 torr. Refer to the dryer's instruction manual for details.
4. Autoradiograph the gel with high speed X-ray film (such as Kodak XAR) and a suitable film cassette. Intensifying screens are optional. If ^{35}S radiolabel is used, the gel can be left on the outer glass plate and fixed in 1 liter of 10% acetic acid, 10% methanol for 15 minutes. This removes hygroscopic urea. The gel may then be dried on filter paper. Removal of plastic wrap before autoradiography is important because ^{35}S is a weak beta emitter. Autoradiography of ^{35}S labeled fragments typically requires 1–3 days. (However, we have found the fixative step unnecessary, even when sequencing with ^{35}S.)

7.4 Nucleic Acid Separation Applications for the Sequi-Gen GT Electrophoresis System

Several other nucleic acid separation techniques requiring single nucleotide resolution can be conducted using the Sequi-Gen GT systems. Below is a comprehensive list. Refer to Sambrook, J., Fritsch, E. F., and Maniatas, T., Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989, or Ausubel, F. M., et al., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience, 1987, for more information and protocols.

- Microsatellite Analysis
- Single-Strand Conformational Polymorphism (SSCP) studies
- Heteroduplex analysis
- DNA footprinting
- DNA fingerprinting
- RNase protection assays
- S1 nuclease mapping
- Primer extension studies
- DNA/Protein binding studies (gel shift assays)
- Oligonucleotide analysis

7.5 Suggested Reading

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