



Elchrom Scientific

Precast Gels

for Advanced Analysis of Nucleic Acids

by Submerged Gel Electrophoresis

Technical Manual

- Spreadex™ Mini and Wide Mini Gels
- Poly(NAT)® Mini and Wide Mini Gels
- Clearose® BG Mini and Wide Mini Gels
- GMA™ Mini and Wide Mini Gels

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1. INTRODUCTION

Elchrom Scientific offers a complete line of products for nucleic acid analysis by submerged (submarine) gel electrophoresis, including precast gels, the SEA 2000 submarine electrophoresis apparatus, auxiliary equipment, accessories, and DNA markers.

Precast Spreadex™, Poly(NAT)™ and Clearose™ BG gels can be advantageously used for the majority of applications currently performed in the submarine mode. Thanks to unique properties of these new types of matrices, now it is possible to get better results by submarine electrophoresis than by vertical or flat-bed electrophoresis using mini gels.

Most commercial submarine units can be employed for running precast Spreadex, Poly(NAT) and Clearose BG gels, but they perform best in the SEA 2000 apparatus. A combination of the SEA 2000 with Elchrom Scientific's power supply, a thermostated circulating water bath with external temperature probe, and a dual wavelength UV-transilluminator allows the best usage of all benefits of Elchrom's precast gels.

1.1 General Guidelines for Selecting the Appropriate Gel

Elchrom currently manufactures 11 different gel types, and each one of them is available in five gel formats. Spreadex gels are recommended for applications that require the highest resolution in a narrow DNA size range, Poly(NAT) gels provide high resolution in a broad size range, while Clearose BG gels are best suited for fast runs and easy recovery of separated DNA fragments. The table shown on page 29 of Elchrom 1997-98 Product Guide contains the details necessary for choosing a correct gel type. Concerning the choice of gel format, Mini gels are best suited when only a few samples need to be analyzed and when it is necessary to optimize the experimental conditions. Wide Mini gels are made for high throughput applications, and for the applications that require the most precise comparison of many samples that are electrophoresed under identical conditions.

Elchrom's precast gels are produced in three lengths, to satisfy different separation needs. The available running distance is 8.7 cm on the Mini gels, as well as on Wide Mini S-26 and on Wide Mini S-50 gels. The Mini gels have sample wells in two directions, so that 12 samples can be run over a 5.7 cm running distance. The distance is 4 cm on the Wide Mini S-52 and Wide Mini S-100 gels. Cost per sample is the lowest, and runs are the fastest, with the 4 cm long gels. These gels should be selected always when the required resolution can be achieved.

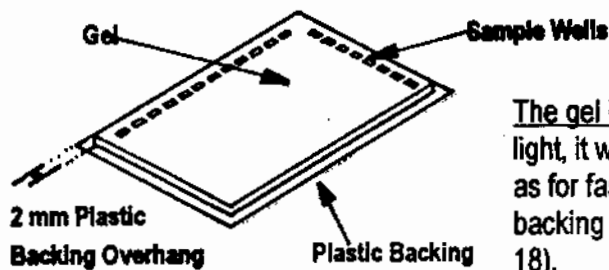
2. PRODUCT SPECIFICATION

2.1 Design of Sample Wells

Sample wells are formed on an elevated gel segment in Wide Mini gels. The wells contain extensions which increase about 2.5-fold the area over which a sample can be released (patent pending). Any sample that is pipetted over the extensions will slide down into the well, making the loading easy. This is particularly the case when using a 12-channel pipette, which is recommend for loading samples on precast Wide Mini gels. All Clearose BG, as well as all S-26 and S-52 Wide Mini gels are currently produced with the new well design.

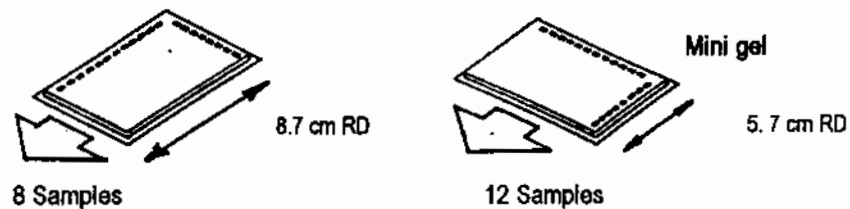
2.2 Design of Precast Gels

The precast gels are covalently bonded to a plastic foil (gel backing) that is 2 mm wider than the gel on each side. This gel backing overhang makes handling and gripping easy and safe. The gels and the backing are flexible, and the gels do not crack upon bending.

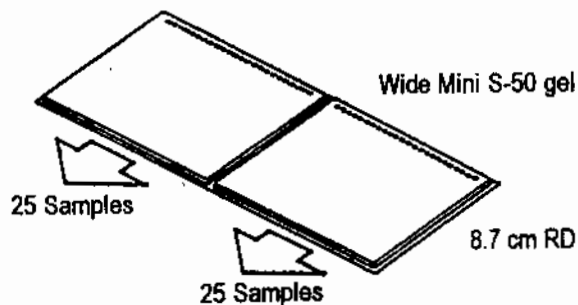


The gel backing is not UV-transparent. Under UV-light, it will fluoresce (page 21). For blotting, as well as for fast staining and the lowest background, the backing should be removed with a nylon string (page 18).

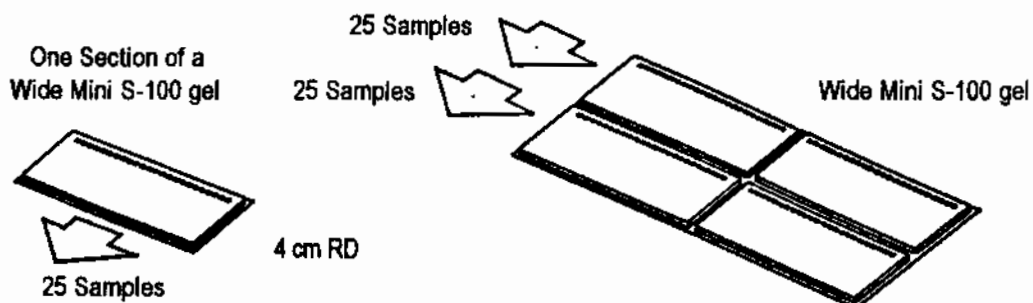
Mini gels: Elchrom Scientific's Mini gels are *gels with sample wells in two directions*[®]. This offers the choice of applying 8 samples into the sample wells made on the short gel side and running them across the distance of 8.7 cm, or applying 12 samples into the wells on the long gel side and running them across the running distance (RD) of 5.7 cm.



Wide Mini S-26 and S-50 gels have two sections, each with a running distance of 8.7 cm. Gel sections of S-26 gels contain 13 wells, and of S-50 gels 25 sample wells.



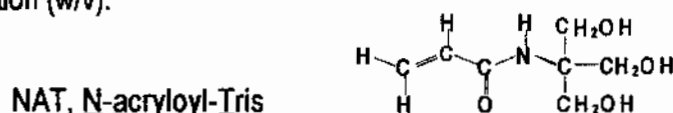
Wide Mini S-52 and S-100 gels have 4 sections, each with a running distance of 4 cm. Gel sections of S-52 gels contain 13 sample wells, and the sections of S-100 gels have 25 wells.



Each section of a Wide Mini gel can be run separately, after cutting the gel backing between the sections.

2.3 Composition of Poly(NAT)[™] Gels

Poly(NAT) gels are prepared by polymerization of the monomer NAT in the presence of a cross-linker (1, 2). The gel concentration, 6%, 9%, and 12%, refers to the total NAT plus Bis concentration (w/v).

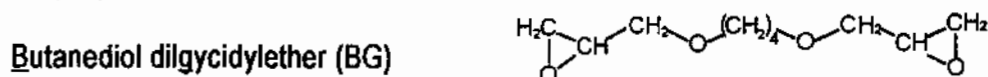


Poly(NAT) gels of each percentage are cast in an optimized gel buffer (3, US Patent 5,458,760), which is different from the running buffer. Precast Poly(NAT) gels should be run only in 30 mM TAE running buffer. POLY(NAT) GELS ARE NOT COMPATIBLE WITH BORATE BUFFERS. The NAT monomer shows no oral toxicity (4).

Poly(NAT) gels are patented (US 5,319,046, European 031 855 B1, Japanese 2 654 681).

2.4 Composition of Clearose[™] BG Gels

Clearose BG gels are composed mainly of agarose cross-linked with 1,4-butanediol diglycidylether (BG).



Gel dry weight is about 1%. The gels are prepared by gelation and simultaneous cross-linking of hydroxyl groups of agarose with oxirane groups of butanediol diglycidylether. After completion of the cross-linking reaction, the gels are equilibrated with 30 mM TAE buffer.

Clearose BG gels are patented (US Patents 5,371,208 and 5,541,255).

2.5 Composition of Spreadex[™] Gels

Spreadex gels are fully synthetic and are prepared by free radical polymerization. The gel polymers are arranged in a unique three-dimensional structure (patent pending), in which they provide increased resistance to migrating DNA molecules, in accordance with new theoretical considerations on gel electrophoresis (5-9). Spreadex gels are characterized by an exclusion limit (EL). DNA fragments with lengths above the limit do not migrate in Spreadex gels. The gels are currently produced in six different concentrations. SPREADEX GELS ARE NOT COMPATIBLE WITH BORATE BUFFERS.

2.6 Dimensions of the Precast Gels of Mini and Wide Mini Formats

Mini gels

Plastic backing size:	6.6 x 9.6 cm
Gel size:	6.2 x 9.2 cm
Sample wells:	1.5 x 5.5 x 2.7 mm (w, l, h)
Gel thickness:	3 mm

Wide Mini

Plastic backing size:	9.6 x 26.1 cm
One gel half:	9.6 x 13.0 cm
Sample wells:	1.5 x 3.5 x 2.7 mm (w, l, h) in Wide Mini S-50 and S-100 gels
Sample wells:	1.5 x 7.0 x 2.7 mm (w, l, h) in Wide Mini S-26 and S-52 gels
Gel thickness:	3 mm

3. IMPORTANT PRODUCT INFORMATION

Please read the information carefully before running a gel in order to assure the best performance. It is simple to run Elchrom's precast Mini and Wide Mini gels in the SEA 2000 apparatus or in other submarine units. Compared to running agarose gels, only a few procedural changes are necessary.

3.1 Running Buffer for Precast Poly(NAT) and Spreadex Gels

Ionic composition inside the gels has been optimized to give sharp bands with 30 mM TAE as the running buffer. This is a 0.75X dilution of the standard TAE buffer (10). The exact amounts for a 40X stock solution are provided in the table below.

Components	Amount for 1 liter (40 X)
Tris(hydroxymethyl) aminomethane	145.37 g
Na ₂ EDTA · 2 H ₂ O	11.16 g
Acetic Acid (glacial)	34.4 ml

The 40X stock solution of 30 mM TAE buffer, 1 l packed in 20 centrifuge tubes of 50 ml each, is available from Elchrom (P/N 30 31). The use of any other running buffer will generate inferior results. POLY(NAT) AND SPREADEX GELS ARE NOT COMPATIBLE WITH BORATE BUFFERS.

A running buffer can be reused about five times, depending on electrophoresis time and amperage. If you are concerned about DNA contamination, use fresh buffer for each experiment.

3.2 Running Buffer for Clearose BG Gels

Clearose BG gels contain 30 mM TAE buffer. We recommend running them in the same buffer. Alternatively, Clearose BG gels may be equilibrated against other running buffers (equilibration time is at least two hours, overnight is better).

Example: To analyze RNA with Clearose BG gels, equilibrate the gel against the MOPS formaldehyde buffer. If the TAE inside the gel is of concern, first wash the gel with water.

3.3 Ethidium Bromide Compatibility

Poly(NAT) and Spreadex gels need to be stained after electrophoresis. Never add ethidium bromide (EtBr) or another intercalating dye to the running buffer or to DNA samples. DNA-EtBr complexes are not stable during electrophoresis in these gels, and they appear as diffuse bands or as a smear.

Clearose BG gels contain a low polymer content, and DNA-EtBr complexes are stable during electrophoresis. This allows fast analysis, because electrophoresis typically takes only 20-30 min, and because no additional staining step is required. Clearose BG gels that are pre-equilibrated with ethidium bromide are available from Elchrom.

Important: Ethidium bromide at 0.5 µg/ml should be present in the running buffer as well as in the gel, as recommended in Current Proocols in Molecular Biology (11). Ethidium ion is positively charged and during electrophoresis it migrates in the opposite direction to DNA. (This can be easily checked by mixing 5 µl of an EtBr stock solution with loading buffer, loading the mixture onto a gel and running for 5 min. The red EtBr band migrates in the opposite direction to

bromphenolblue). When a running buffer does not contain EtBr, the dye gradually migrates out of the gel. The depletion of EtBr in the lower gel part results in less sharp bands. We do not recommend adding EtBr to DNA samples, as this often leads to streaking of DNA bands.

Note: Bind-ET™, an ethidium removal system able to adsorb over 1 g of ethidium, is available from Elchrom (P/N 2350).

3.4 Gel Reuse

Only Clearose BG gels can be reused, and for that they need to remain bound to their backing. It is more convenient to reuse the gels with ethidium bromide than the gels without it, since no separate staining and equilibration steps are required. To "clean" a gel from DNA fragments, reverse electrophoresis is performed at least 10 min longer than previous analysis time. For practical reasons, it is better to turn the gel by 180° than to change polarity of the electrodes.

3.5 Storage of Precast Gels

Poly(NAT) and Spreadex gels

The gels should be stored in flat position at 4°C. At room temperature, their shelf life would be shortened. The resolving power of gels that are stored longer than 6 months decreases very gradually. Expired gels tend to swell when released from their plastic backing. Excessive swelling is an indication that the gel is old or that it has not been stored properly.

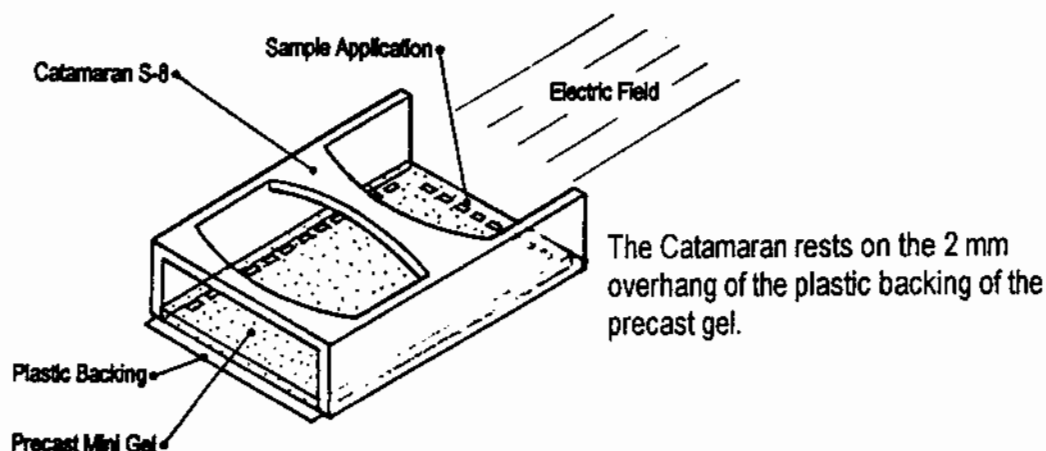
Clearose BG gels

Clearose BG gels should be stored in flat position at 4°C. Keeping the gels in vertical position would cause a loss of liquid from the upper gel part. Clearose BG gels are stable at room temperature for at least 6 months, but storage at 4°C is recommended.

Caution! Never freeze precast gels! Ice crystal would destroy gel structure.

3.6 Catamaran Frames

The use of an Elchrom's precast gel requires a Catamaran frame. The frame is positioned on the overhang of gel backing, and its weight prevents gel floating during electrophoresis. Different Catamaran frames are available for running precast gels of varying formats. Electrophoresis in Elchrom's SEA 2000 apparatus requires a Catamaran with slots in its sides to allow buffer circulation. Catamaran frames without slots are better for running the gels in regular submarine units. The frames are designated according to the number of samples that can be run on the gel fixed with the Catamaran. For example, Catamaran S-8 is used for running 8 samples on a Mini gel.



4. PREPARATION OF PRECAST GELS FOR ELECTROPHORESIS

1. The gels are ready to use. Take a chosen gel out of its box from a refrigerator. Cut the aluminum bag with scissors on one side and take out the gel in its polyethylene bag.
2. Cut carefully the polyethylene bag with scissors on three sides and peel away the polyethylene foil from the gel. Hold the gel, best with Elchrom Scientific's forceps, on the plastic backing overhang. The gels are considered non toxic. Always wear gloves when handling Clearose BG gels with ethidium bromide.
3. For running a Poly(NAT) or a Spreadex gel at 55°C, place the gel in its polyethylene bag on the lid of the SEA 2000 during warming up of the running buffer, but not longer than 30 min.

Note: If you wish to use only a section of a Wide Mini gel, carefully cut the gel backing between the gel sections while the gel is still in the polyethylene bag. About 2 mm of the backing should remain on each side of the cut gel sections. Wrap the non-used gel section in a polyethylene foil and put it back into the aluminum bag. Close the bag with a tape and refrigerate. Any remaining gel sections should be used within one week.

5. PREPARATION OF DNA SAMPLES

Usually, 3-4 parts of a sample are mixed with 1 part of Elchrom Scientific's sample loading buffer (LB) provided in each gel box. When DNA concentration is high, or when SYBR Green is used to stain the gel, it is convenient to dilute the loading buffer with water (1 volume of LB and 3 volumes of water), and then take 1 part of sample plus 4 parts of the diluted LB. A final loading volume of 5 μ l is adequate for all Elchrom's precast gels. It should be noted that less DNA, and a smaller sample volume, is required with Elchrom's precast gels than with typical lab made agarose gels. The loading buffer should not be diluted with DNA sample more than 1:4, since then the final sample density would be too low for proper loading. This is especially critical when using multichannel pipettes, in which case the LB should be diluted 1:3 for best results.

Important: Band distortion may occur when adjacent lanes contain samples of significantly different salt concentration. To reduce salt concentration, dilute all samples and use SYBR Green for staining. A less good alternative is to add appropriate buffer concentrate to all samples including the markers. For example, to adjust salt concentration in a DNA marker to that of PCR* samples, mix two parts of the marker with 2 parts of a 2X PCR buffer, and then add 1 part of the sample loading buffer.

Important: Overloading impairs resolution. The optimal DNA amount per band depends on the dye used for staining. With SYBR Green I staining, the optimum is between 0.4 and 4 ng, and for EtBr staining it is between 2 and 20 ng. Loading of more than 50 ng per band may result in broad bands and poor resolution.
SDS in sample (>0.01%) may impair resolution.

5.1 Elchrom's DNA Markers

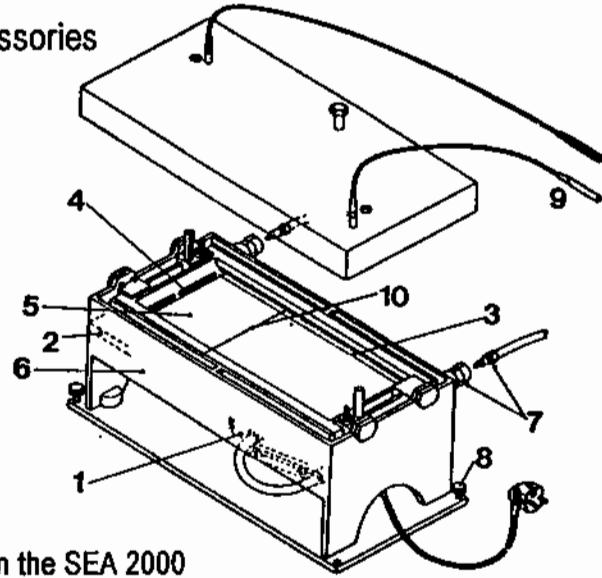
Three DNA markers, 20 bp ladder, 100 bp ladder, and the quantification markers that are manufactured by GenSura Laboratories can be purchased from Elchrom only together with a box of precast gels. The M3 marker, which is a mixture of three pBR322 digests, can be ordered separately. The amount of DNA in the 20 and 100 bp ladders is sufficient for 25 loadings when EtBr is used for staining, or for 125 loadings when SYBR Green I is used. The quantification marker consists of three DNA fragments with the length of 200 bp, 500 bp, and 1,000 bp, each supplied in a separate vial at the concentration of 50 ng/ μ l. The amount of M3 marker is sufficient for 50 loadings with EtBr stained gels.

6. RUNNING OF PRECAST GELS IN ELCHROM'S SEA 2000 APPARATUS

Thanks to advanced features of this apparatus (12), running of Elchrom's precast gels in the SEA 2000 will give the best results. The SEA 2000 is the most important part of Elchrom Scientific's submerged gel electrophoresis system, which also includes a power supply with integrated timer, a pump start delay controller, a thermostated circulating water bath with a temperature probe, and direct screen camera with two hoods and filters. The system allows full control of all relevant electrophoresis parameters and ensures optimal, reproducible, and predictable results, gel after gel.

6.1 Short Specifications of the SEA 2000 and Accessories

1. Integrated pump for circulation of running buffer
2. Temperature control with integrated cooling/heating plate
3. Linear electric field due to improved electrode and apparatus design
4. Even buffer flow with specially designed barriers and dams
5. Gel compartment: Length 10.8 cm, Width 27.8 cm
6. Buffer volume: 1.9 liters
7. Quick couplings with valves for connecting the tubing from the bath to the cooling/heating plate
8. Three leveling screws
9. High safety plugs
10. Electrode distance of 12 cm



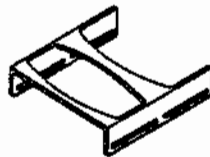
Catamaran plastic frames for running precast gels in the SEA 2000

Catamaran S-8
P/N 20 08



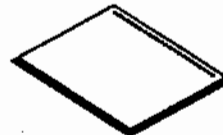
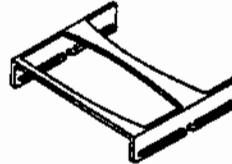
Mini gel
8 samples

Catamaran S-12
P/N 20 12



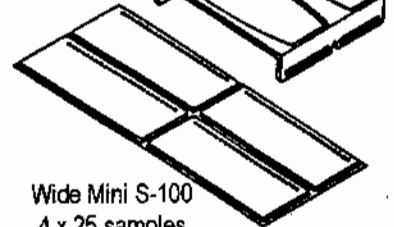
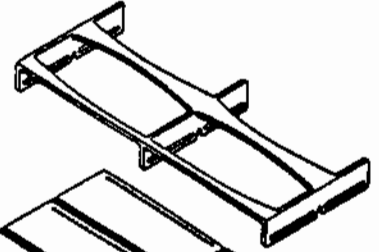
Mini gel
12 samples

Catamaran S-13/50
P/N 20 15



1/2 Wide Mini S-50
25 samples

Catamaran S-26/100
P/N 20 20



Wide Mini S-100
4 x 25 samples

Electrophoresis Power Supply PSE

Specifications:
200 Volts 998 mA.
One digit increments.
Timer for automatic stop (0-9999 min).
Automatic restart in the case of mains failure.
Optional: Pump Start Delay interface box for automatic control of the SEA 2000 pump.

Pump Start Delay Controller, PDC

Specifications:
For manual control of the SEA 2000 pump.
Two delay switches with the delay time of 1.5 min and 4.5 min. The delay time is selected such that buffer circulation begins after DNA fragments enter the gel.

Thermostated Water Bath, TCB

Specifications:
-25....120 °C, digital display, 0.1°C increments.
Internal or external temperature control. With a special temperature probe it is possible to maintain running buffer temperature within 0.5°C.
Pressure and suction pump.
Protection against overheating, and automatic stop when water level falls below a minimum.
Weight: 18 kg.

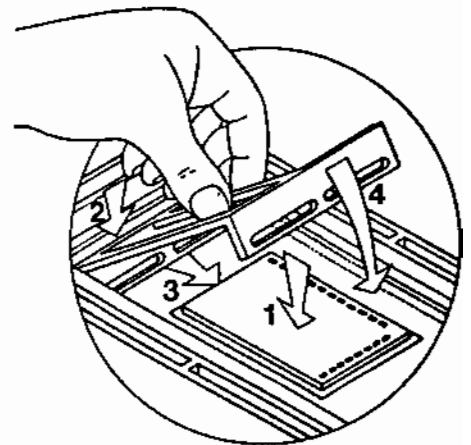
6.2 Electrophoresis

1. Prepare 2 liters of running buffer. Dilute 50 ml of the 40X stock solution (page 7) to 2 l with double distilled (dd) water, or with water of equivalent quality. Pour about 1.9 l in the SEA 2000, carefully to avoid buffer spillage. A slight tilting of the apparatus will help the air to escape from the lower buffer compartment. Buffer level should be 2-3 mm above the upper platinum wire. Excess buffer can be removed with a 50 ml syringe. When reusing running buffer, check the buffer level and if necessary add ddH₂O to bring the level 2-3 mm above the upper wire.
2. Set-up of a temperature control system. For runs at a constant temperature, connect a thermostated circulating water bath to the SEA 2000 using the quick couplings provided. With Elchrom's TCB, set first the temperature limits and then the desired temperature, as described in the TCB manual. When using a water bath without an external temperature control, set the temperature of the circulating water below the desired buffer temperature by 5-10 degrees, if that temperature is 20°C. Exact settings depend also on the applied electric field strength, since more heat is produced at higher voltages. A correct setting needs to be found empirically when external temperature control is not available. Turn on the SEA 2000 pump for temperature equilibration. It will take about 30 min until the buffer temperature reaches 50°C, with the power on (120 V) to speed up warming of the running buffer. When working without Elchrom's external temperature probe, buffer temperature can be monitored with an ordinary thermometer inserted in the opening made in the lid of the SEA 2000.

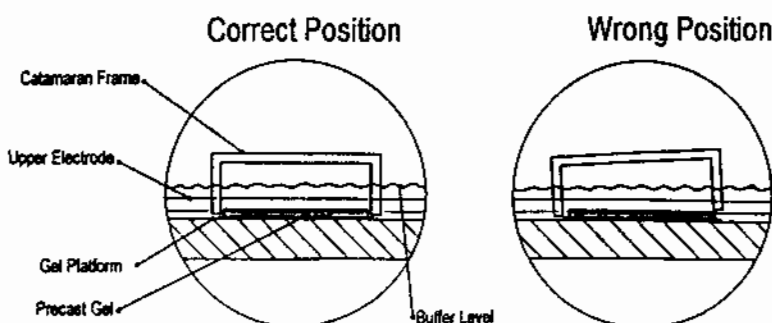
Technical Tip To achieve steady-state conditions, pre-run the SEA 2000 at the desired voltage and temperature for at least 10 min. The amperage readings stay constant (+/- 2%) at steady-state conditions.

3. Prepare the gel for electrophoresis (page 9). Turn off the pump. Place the gel in the middle of the platform [1]. Make sure that no air bubbles are trapped under the gel backing or in the sample wells. It is a good practice to quickly rinse all sample wells with a Pasteur pipette. To ensure that a Mini gel rests flat, gently place an S-8 Catamaran with one of its legs on the gel for 5-10 seconds.
4. Position the Catamaran frame [2], which was selected according to gel format (page 5). The gel can be moved left-right using the Catamaran [3]. To move it up or down, use the forceps, or a needle, and press it on gel backing, never on the gel. Lower the Catamaran until it rests on the gel backing overhang [4].

Important! Wide Mini S-52 and S-100 gels. These gel should be positioned such that the horizontal channel which separates gel sections comes in line with the round openings in the sides of the Catamaran S-26/100. That is necessary to ensure optimal buffer flow through the channel, essential for washing away the DNA that exits the upper gel section.



5. Check that the Catamaran is positioned properly. Its sides should rest on the gel backing, not on the gel. For best visualization, have a look through the front side of the SEA 2000.



6. **Loading of samples. Mini gels.** Optimal loading volume is 5 μ l. Samples can be loaded with a 1-20 μ l pipette using regular pipette tips. **Wide Mini gels:** Samples are best loaded with a 12-channel pipette. Alternate wells are filled on Wide Mini S-50 and S-100 gels with one pipetting step, while each well is filled on the S-26 and S-52 gels. Mixing of samples with loading buffer is most conveniently done in a 96-well or 384-well microtiter plate. Optimal loading volume is 4 μ l for the S-50 and S-100 gels, and it is 5 to 8 μ l for the S-26 and S-52 gels.

Technical Tip

If you have difficulties in seeing sample wells, pipette some loading buffer over the area where sample wells are, wait 1 min and then rinse the wells with a Pasteur pipette. The dye that has diffused from the sample wells into the gel makes the wells readily visible. Loading buffer may be pipetted into the wells before placing the gel in running buffer.

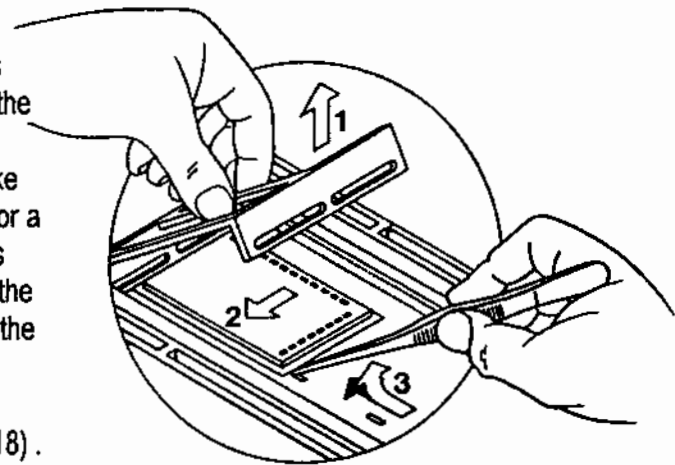
7. **Close the lid and select a pump start delay interval.** The pump should start with some delay after the voltage is turned on in order to allow DNA molecules to enter the gel undisturbed by circulating buffer. The interval depends on applied voltage, gel type and buffer temperature. In general, buffer circulation may start after bromphenol blue has migrated 0.5-1 mm in the gel. The 1.5 min interval is usually sufficient when running Clearose BG gels, Spreadex or Poly(NAT) gels at 55°C, and for all gels when the electric field strength is above 10 V/cm.

Important:

FAILURE TO START THE PUMP WITHIN 10 MINUTES AFTER APPLYING VOLTAGE WILL RUIN THE EXPERIMENT. When using manual start, always check that the pump is working before doing something else. Then you can leave the run unattended.

8. **Start electrophoresis.** Select the appropriate running time and voltage prior to loading of samples. Leaving the loaded samples for several minutes without applying voltage may result in broad bands. The electric field strength in submarine electrophoresis is determined by the distance between the electrodes, not by gel length as in vertical electrophoresis. The distance between electrodes in the SEA 2000 is 12 cm, so that applying 120 V gives the electric field strength of 10 V/cm. When electrophoresis is carried out at a constant temperature, it is simple to calculate the running time necessary for separating any DNA fragments (page 14).

9. **Stop electrophoresis.** The run stops automatically when using a power supply with a timer, such as Elchrom's PSE. Remove the lid by pressing with thumbs onto the blue and red rods near the jacks while pulling the lid upwards. Take the gel out of the SEA 2000. A groove (or wells in older SEA 2000 model) has been drilled in the gel platform to make gel lifting easy. Position the gel over the groove (or a well) such that the edge of the gel backing comes over it. This can be done by sliding the gel using the Catamaran [1] and the forceps provided [2]. Grip the gel firmly at its backing with forceps [3], lift it, and transfer to an "Easy-stain" tray (P/N 23 44) for staining, or onto a bottle for backing removal (p. 18).



10. **Stain (page 19), and photograph (page 22) the gel.**

6.3 Running of Spreadex and Poly(NAT) Gels at Elevated Temperatures

Electrophoresis at 55°C reduces the running time by a factor of two, and also greatly suppresses anomalous, sequence-dependent mobilities of dsDNA fragments. Prior to electrophoresis, it is necessary to warm up the gel which was stored at 4°C. When this is omitted, band patterns may be disturbed by bubbles which sometimes form as dissolved air tries to escape from a cold gel placed in the running buffer of 55°C. Gel warming can be done by placing it, still in its polyethylene bag, on the lid of the SEA 2000 during heating up of the running buffer, but not longer than 30 min. Prolonged gel heating may cause a significant reduction of DNA mobilities, especially with Spreadex gels. A better alternative is to take the gel out of refrigerator 2-3 h prior to electrophoresis and leave it at room temperature. Keeping the gel at room temperature overnight is also good. For runs at or below 30°C, there is no need for temperature equilibration of precast gels. Running buffer will faster reach a desired high temperature if power is switched on (120 V) during warming up period.

Note: Clearose BG gels should not be run at temperatures above 35°C.

7. ABSOLUTE MOBILITIES OF DNA FRAGMENTS IN PRECAST GELS

Based on full control of the production process, Elchrom is able to provide tables with absolute mobilities of DNA fragments in precast gels. For a given gel, the mobility (μ) of a DNA fragment of defined size represents a physical constant, so that running time for any required separation can be easily calculated from the equation $d = \mu \cdot t \cdot E$, where d is running distance, t is electrophoresis time, and E is electric field strength.

- Notes:**
- DNA mobility depends strongly on temperature. The values tabulated below can be used only for the specified temperatures. A desired constant temperature is easily maintained in the SEA 2000 apparatus.
 - Certain DNA fragments show anomalous, sequence-dependent mobilities, which may differ by more than 10% from those expected. Anomalous mobilities are suppressed during electrophoresis at 55°C, especially with Spreadex gels.
 - Electrophoretic mobility of DNA fragments longer than about 1,000 bp depends on electric field strength, increasing at higher strengths. This needs to be taken into account when using 6% Poly(NAT) and Clearose BG gels.

Technical Tip For best resolution, the running distance should be a few millimeters less than the length of a gel. In many cases, the 4 cm long Wide Mini S-52 and S-100 gels will provide adequate resolution.

Examples: Two closely spaced bands of about 400 bp will be best resolved in a 6% Poly(NAT) Mini gel run at 8 V/cm and 20°C after a running time of:

$$t = \frac{d}{\mu \cdot E} = \frac{8.5 \text{ (cm)}}{4.4 \cdot 10^{-3} \text{ (cm}^2 \text{ V}^{-1} \text{ min}^{-1}) \cdot 8 \text{ (V cm}^{-1})} = 241 \text{ min}$$

In a Spreadex EL 600 Wide Mini S-52 gel, whose length is 4 cm, at 10 V/cm and 55°C, a 180 bp fragment will migrate close to the gel end after a running time of:

$$t = \frac{d}{\mu \cdot E} = \frac{3.8 \text{ (cm)}}{3.6 \cdot 10^{-3} \text{ (cm}^2 \text{ V}^{-1} \text{ min}^{-1}) \cdot 10 \text{ (V cm}^{-1})} = 106 \text{ min}$$

7.1 Tables with absolute mobilities* of DNA fragments in precast gels,

cm² · V⁻¹ · min⁻¹

DNA Size (bp)	6% POLY(NAT)		9% POLY(NAT)		12% POLY(NAT)		CLEAROSE BG	
	20° C	55° C	20° C	55° C	20° C	55° C	20° C	20° C
4000	0.88							
3000	1.00							
2000	1.40							
1000	2.40	4.61	0.94	1.88	0.43	1.02	3.85	2.89
900	2.62	4.97	1.04	2.06	0.47	1.15	4.11	3.13
800	2.86	5.41	1.15	2.31	0.52	1.28	4.43	3.43
700	3.12	5.93	1.32	2.64	0.61	1.64	4.81	3.79
600	3.45		1.53	3.07	0.72	1.76	5.29	4.27
500	3.86		1.83	3.63	0.88	2.11	5.87	4.91
460						2.30		
420						2.52		
380		9.13				2.76		
340		9.67				3.07		
300	4.95	10.31	2.78	5.50	1.44	3.42	7.58	6.76
260		11.04				3.81		
220		11.87				4.28		
180	6.06	12.92				4.83		
140	6.65	14.15				5.56		
100	7.35	16.02	4.57	9.59	2.84	6.62	10.74	10.30
60	8.40					8.37		

DNA Size (bp)	Spreadex EL 1200		Spreadex EL 800		Spreadex EL 600		Spreadex EL 500		Spreadex EL400		Spreadex EL 300	
	20° C	55° C	20° C	55° C	20° C	55° C	20° C	55° C	20° C	55° C	20° C	55° C
1000	0.13	0.37										
900	0.19	0.52										
800	0.28	0.73										
700	0.42	1.03	0.12	0.40								
600	0.64	1.52	0.18	0.57								
580	0.69	1.66	0.20	0.65								
560	0.75	1.78	0.23	0.74								
540	0.83	1.96	0.26	0.84								
520	0.90	2.13	0.30	0.93								
500	0.99	2.34	0.34	1.05								
480	1.08	2.52	0.37	1.18								
460	1.18	2.76	0.43	1.34								
440	1.31	3.02	0.50	1.42	0.16							
420	1.43	3.27	0.57	1.59	0.18							
400	1.58	3.50	0.66	1.81	0.24	0.45	0.13	0.27				
380	1.72	3.93	0.75	2.06	0.27	0.51	0.15	0.34				
360	1.90	4.27	0.87	2.35	0.33	0.63	0.18	0.40	0.08	0.20		
340	2.08	4.67	0.99	2.64	0.40	0.78	0.22	0.52	0.09	0.22		
320	2.28	5.09	1.15	3.00	0.48	0.96	0.27	0.64	0.11	0.27		
300	2.50	5.56	1.32	3.42	0.59	1.23	0.33	0.80	0.15	0.35	0.06	
280	2.75	6.08	1.51	3.86	0.71	1.46	0.41	1.05	0.20	0.46	0.08	
260	3.00	6.62	1.73	4.36	0.87	1.83	0.52	1.33	0.28	0.61	0.11	0.21
240	3.28	7.23	1.98	4.92	1.05	2.24	0.65	1.69	0.36	0.83	0.16	0.32
220	3.58	7.90	2.25	5.53	1.26	2.76	0.82	2.13	0.47	1.12	0.21	0.43
200	3.88	8.58	2.56	6.27	1.52	3.42	1.03	2.67	0.65	1.53	0.30	0.62
180	4.19	9.33	2.90	6.98	1.82	4.10	1.29	3.33	0.85	2.04	0.43	0.93
160	4.55	10.13	3.28	7.86	2.13	4.96	1.60	4.21	1.13	2.71	0.62	1.36
140	4.90	10.90	3.60	8.73	2.54	6.02	1.95	5.07	1.47	3.53	0.88	2.00
120	5.27	11.90	4.02	9.63	2.97	7.12	2.36	6.12	1.86	4.51	1.23	2.87
100	5.77	12.87	4.52	10.63	3.49	8.27	2.83	7.16	2.32	5.58	1.68	3.96
80	6.25	14.06	5.02	11.86	4.11	9.76	3.40	8.60	2.83	6.98	2.19	5.34
60			5.63	13.32	4.77	11.43	4.02	10.12	3.42	8.57	2.81	6.95
40						13.46	4.82	12.05	4.16	10.53	3.55	8.99
20									5.11	13.71	4.57	12.44

* DNA mobilities in expired gels, in the gels stored at room temperature, and in the gels exposed to elevated temperatures may be considerably lower (especially with Spreadex gels).

7.2. RUNNING OF PRECAST GMA™ GELS IN THE SEA 2000 APPARATUS

The GMA™ (Gene Mutation Analysis) gels are optimized for single strand conformational polymorphism (SSCP) method, which is widely used for the detection of mutations in DNA. The method relies on different migration rates of single stranded DNA fragments in a non-denaturing gel run at a low temperature. The conformation of a ssDNA is thought to be the major factor governing its electrophoretic mobility. Two single stranded DNA fragments whose sequence differs in just one base often migrate at sufficiently different rates to allow complete separation of the two bands.

At present the mechanism of SSCP is poorly understood, and there is no theory that is able to correlate the sequence of a ssDNA fragment with its conformation or with its electrophoretic mobility. Two major factors are known to determine the success of SSCP. First, the length of the DNA fragment should be in the range of 150 to 300 bp. Second, gel electrophoresis should be performed at a low constant temperature favorable for the maintenance of a particular conformation. Since most DNA fragments that are subjected to SSCP analysis are obtained by PCR, it is simple to get DNA fragments of the optimal length by choosing appropriate primers. To keep a low constant temperature, the best is to use an electrophoresis apparatus equipped with a heat exchange element. Elchrom's SEA 2000 apparatus is perfectly suited, not only because it has a heat exchange element, but also because it features laminar buffer flow that maintains even temperature over the whole gel surface.

Elchrom's precast GMA™ gels were optimized by monitoring the separation of many ssDNA fragments in the gels made of different acrylamide type monomers. We have observed that the separation is sensitive to small changes of temperature during electrophoresis, as well as to the electric field strength, in accordance with literature reports. In most instances the best results were obtained when the running buffer temperature was 7-9°C and the electric field strength from 5 to 6 V/cm in overnight runs. Due to uncertainties inherent to the SSCP method, Elchrom is unable to guarantee a successful separation of a particular pair of ssDNA fragments, or to give absolute values for electrophoretic mobilities of ssDNA fragments. On the other hand, the protocol which gave the most consistent results in our lab is described below.

1. After the PCR, take 3 µl and add it to 7 µl of formamide which contains 10 mM NaOH. (Mix 1 ml of formamide and 10 µl of 1 M NaOH just before use. Upon standing, formamide reacts with NaOH to produce sodium formate and ammonia. Bromphenolblue may be added for better visualization). Make sure that the reagents are properly mixed.
2. Heat at 95°C for 4-5 min. Immediately place the hot tubes on ice.
3. Load 4 to 8 µl aliquotes. (There is no need for the addition of loading buffer.) The temperature of the running buffer should be about 9°C at the time when the gel is placed in it. Cooling of the buffer in the SEA 2000 will take about 30 min with the pump switched on. Therefore, start cooling the buffer before starting with sample preparation. When using the external temperature probe, set the lower temperature limit at 4°C and the upper limit at 9.1°C. Without the external temperature probe, set the temperature of the circulating water at 5°C. Run the gel at 6 V/cm for 15 h.
4. Release the gel from its backing and stain it with SYBR Green II or SYBR Gold (1 : 10,000 in 10 mM TAE) for 40 min. Destain in double distilled water for at least 30 min. Photography at 254 nm, f-stop 4.5, 1 s, with Polaroid 667 film will give strong ssDNA bands in most cases. No background should be visible. If the bands are weak, increase the number of PCR cycles, or the concentration of primers, or the concentration of the DNA template.

8. RUNNING OF PRECAST GELS IN REGULAR SUBMARINE UNITS

Elchrom Scientific's precast gels can be electrophoresed in many medium and large size submarine units, provided that a gel is fixed with an appropriate Catamaran frame. It should be noted that electrophoresis in the units with a non-linear electric field, without temperature control, and without buffer circulation will produce inferior results compared to those obtained in the SEA 2000.

Elchrom's Wide Mini gels do not fit into the majority of existing submarine units, which are usually wide 20 cm or less. However, after cutting the gel backing with scissors between sections of a Wide Mini gel, it is possible to use one gel half, which is 13 cm wide.

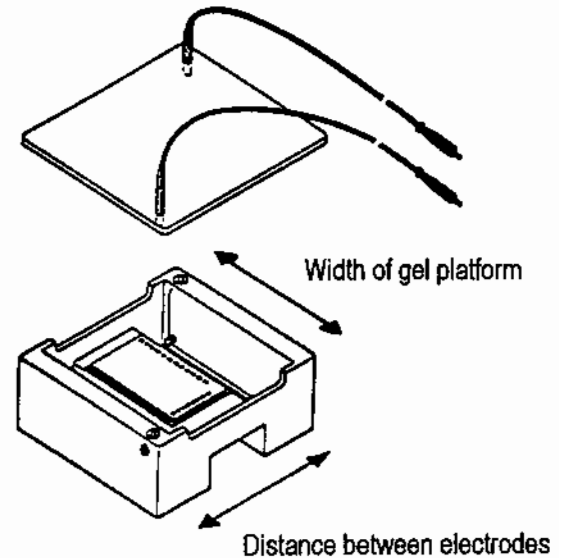
8.1 Equipment Requirements

1. A submarine unit

A unit with a wide gel platform (≥ 15 cm) and a buffer volume of at least 600 ml is recommended. Most commercially available Midi and Maxi units will work well.

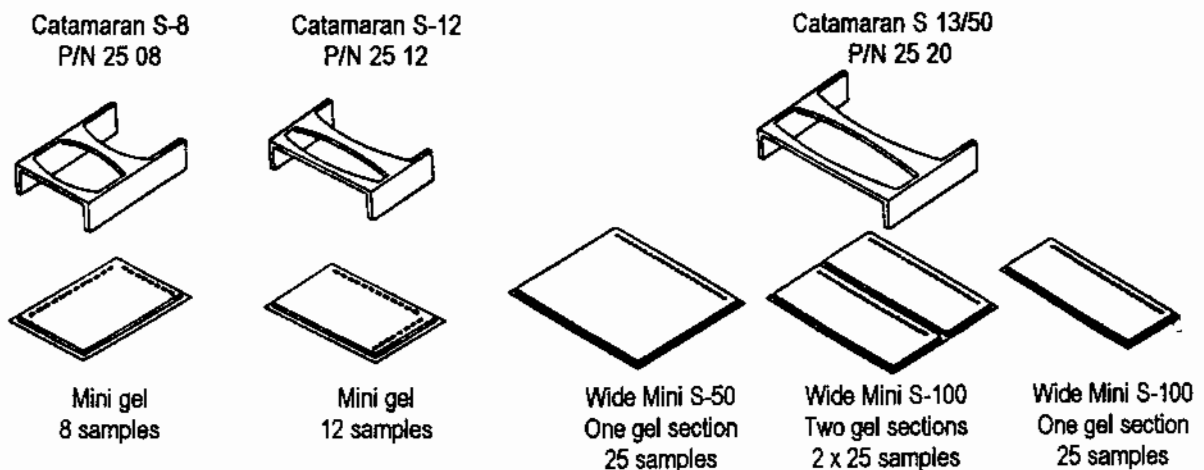
NOT recommended are the following units:

- BioRad Mini-Sub™ Cell
- Hoefer HE 33
- Life Technologies Horizon™ 58.
- Other Mini units with a low buffer volume (<300 ml) and a narrow gel chamber (<10 cm).



2. Catamaran plastic frames for running gels in regular submarine units

Note: The Catamaran frames are without slots in their side, in contrast to the Catamarans used with the SEA 2000 apparatus.



3. Electrophoresis power supply

Minimum requirements:

Voltage: ~ 250 V

Amperage: 250 mA minimum

One digit increments for precision.

8.2 Electrophoresis

1. Prepare the running buffer.

The running buffer (RB) is 30 mM TAE. To prepare 1 liter of RB, dilute 25 ml of the 40X stock solution (page 8) with double distilled (dd) water or with water of equivalent quality.

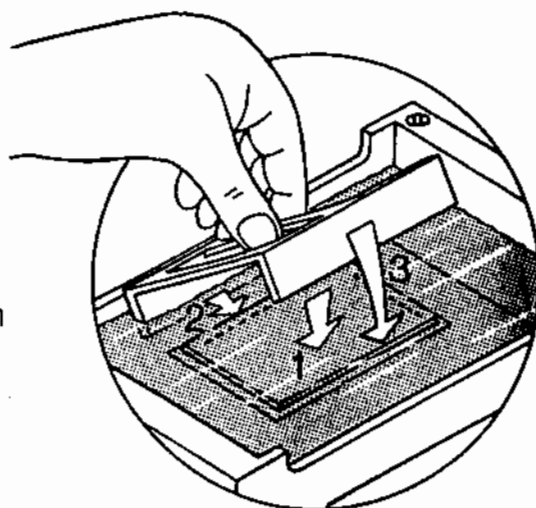
2. Pour the buffer in your electrophoresis apparatus. The buffer level should be 6-8 mm above the gel platform, or 3-5 mm above the gel.

3. Place the gel into the unit.

Hold the gel with forceps (P/N 23 66) at the plastic backing. Make sure that no air bubbles are trapped under the plastic backing. The gel should be in the center of the gel platform of the submarine unit. If the platform is shorter than the gel, place the gel on an agarose casting tray supplied with the submarine unit. Two or three Mini gels can be run simultaneously, if the space allows. Work quickly once a Poly(NAT) or a Spreadex gel is placed in the running buffer.

4. Position the gel

Using the appropriate Catamaran frame (page 15), position the gel parallel to side walls of the apparatus, so that the row of sample wells is parallel to the cathode. Should the gel resist being moved with the Catamaran, use the forceps by pressing on the gel backing. Carefully place the Catamaran on the overhang of the gel backing.



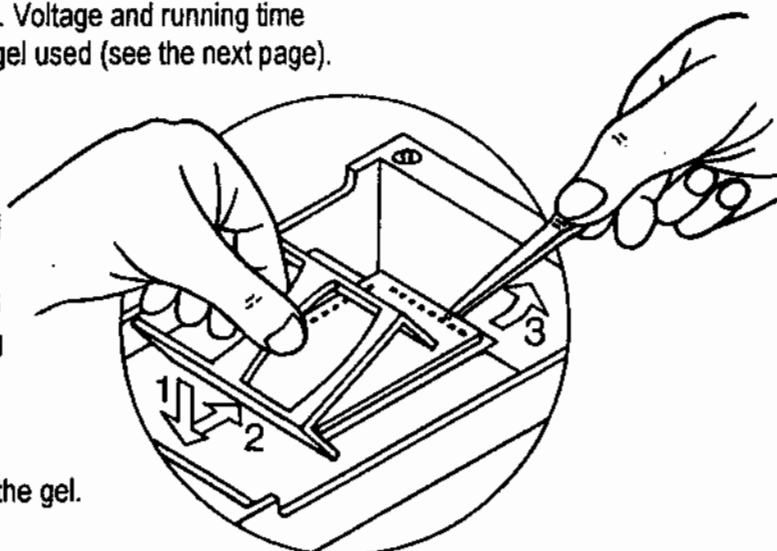
5. Sample loading

See page 9 for sample preparation. Load the samples with a 1-20 μ l pipette on Mini gels. Loading of samples on Wide Mini gels is best done with a 12-channel pipette. Sample well spacing of Wide Mini S-26 and of S-52 gels is identical to the spacing of 96-well plates, and well spacing of Wide Mini S-50 and S-100 gels is identical to that of 384-well plates. It is a good practice to rinse the wells before sample loading.

6. Close the lid and start electrophoresis. Voltage and running time depend on specific application and the gel used (see the next page).

7. Stop the run and take out the gel.

Use the Catamaran frame to slide the gel to the edge of the platform (Steps 1 and 2). Step 3: Grasp the gel at the plastic backing with the forceps (P/N 23 66) and transfer it into a tray for staining or onto a bottle for removal of the gel backing (page 18).



Stain (page 19) and photograph (page 22) the gel.

8.3. Examples of Electrophoretic Settings

In submarine electrophoresis, the distance between electrodes determines the electric field strength, in contrast to vertical electrophoresis where the field strength is determined by gel length. When the distance between electrodes in a submarine apparatus is 15 cm, then applying 150 V will correspond to the electric field strength of 10 V/cm.

Important: Due to non-linear electric field inherent to regular submarine units, and also due to varying heat capacity related to buffer volume and apparatus construction, DNA migration rates differ from one apparatus to another even at an equal nominal electric field strength.

Table 8.3.1 Running of samples on a 8.7 cm long Mini gel.

Type of Mini Gel	Voltage Setting *	Resulting Current (mA) #	Running Time	Temp. of RB	DNA Size range spanning 80% of the Mini gel length **	Bromophenol Blue Migration
Clearose BG	150 V 10 V/cm	130 mA	50 min	20 °C start 38 °C end	50 - 2,000 bp	To the end of the gel
6% Poly(NAT)	105 V 7 V/cm	115 mA	150 min	20 °C start 30 °C end	100 - 2,000 bp	To the end of the gel
9% Poly(NAT)	120 V 8 V/cm	120 mA	135 min	20 °C start 35 °C end	60 - 1,200 bp	To the end of the gel
12% Poly(NAT)	150 V 10 V/cm	130 mA	130 min	20 °C start 53 °C end	60 - 600 bp	Out of the gel

(*) The distance between electrodes in the unit was 15 cm.

(#) The current (mA) depends on the level of buffer in the unit (5 mm above gel). The current will be higher at the end of the run due to increased temperature of the running buffer.

(**) Measured from the end of Mini gels upwards, i. e. 7 cm from the end of the gel towards sample wells.

In regular submarine units, temperature of the running buffer increases during a run, and heat dissipation is uneven. That causes the following problems:

- Lanes are not parallel, making difficult a precise estimation of DNA size.
- Smiling effect is pronounced, as the middle of the gel becomes warmer than its edges.
- Runs are difficult to reproduce, unless room temperature and starting temperature of the running buffer are identical between runs.

In the absence of temperature control, tracking dyes are frequently used to estimate when to stop a run. The table below shows which DNA fragments migrate at the same velocity as the two most commonly used dyes. Generally, with Poly(NAT) and Clearose BG gels, bromphenolblue should be allowed to migrate to the end or out of the gel, whereas with Spreadex gels xylene cyanol should migrate at least to the gel bottom.

Table 8.3.2. Relative migration of two dyes and DNA fragments in Elchrom's precast gels.

DYE	SPREADEX						POLY(NAT)			CLEAROSE BG
	EL 1200	EL 800	EL 600	EL 500	EL 400	EL 300	6%	9%	12%	
Bromphenolblue	40 bp	40 bp	40 bp	30 bp	30 bp	20 bp	100 bp	60 bp	40 bp	80 bp
Xylene Cyanol	160 bp	140 bp	120 bp	90 bp	80 bp	75 bp	440 bp	220 bp	120 bp	600 bp

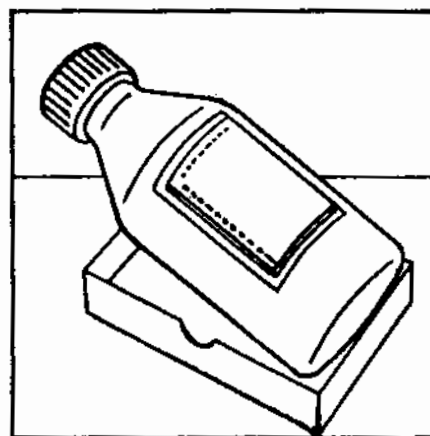
9. REMOVAL OF THE BACKING OF PRECAST GELS

The gel backing needs to be removed to ensure rapid staining, to allow blotting, to obtain a clear background for photographic recording at 312 nm, and generally for the best background using other methods of recording. The procedure is easy and does not require any special equipment. In fact, removal of the backing takes less than 30 seconds using the nylon string provided in every gel box.

1. Place an inverted cardboard or Styrofoam box on a flat surface. Lay a cylindrical glass bottle into the box so that the top of the bottle is angled away from you. Any other way of fixing the bottle to the flat surface can also be used.

A 1 liter bottle is recommended for the Mini gels and a larger size bottle (2-3 liter) is ideal for the Wide Mini gels. Filling the bottle with water will make it more stable.

Place the gel, while still wet, with plastic backing down on the curved side of the bottle. The shorter side of the gel should be parallel to the bottom of the bottle.

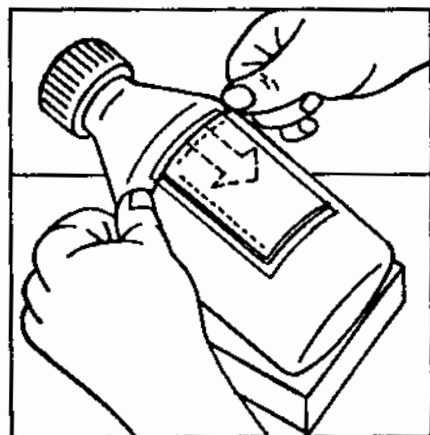


2. If the gel starts to slide, remove it and take away the excess of water with a paper towel. Too little water will also cause gel sliding, since it is kept in place by capillary forces. Make sure that there are no particles on the glass surface, in particular dried gel pieces from previous usage.

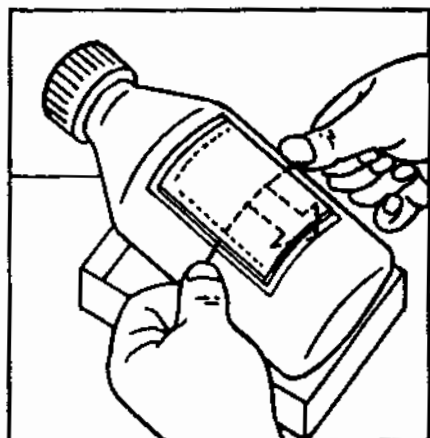
Next, take the nylon string with both hands and position it on the plastic backing overhang in front of the gel.

Press the string down onto the bottle.

Pull the string carefully and slowly down the gel towards you while pressing downward.



3. The detached gel usually expands a little. It remains loosely attached to the plastic backing. Using forceps (P/N 23 66), grip the plastic backing at a corner and carefully lift it together with the gel. Place it, plastic backing up, in a tray, onto a transilluminator, or on a flat surface for further processing. When placed in a tray containing a staining solution, the backing is easily removed from the gel by bending it with forceps at a corner and gently moving up and down. On a flat surface, inserting a needle in the corner which is lifted helps the detachment.

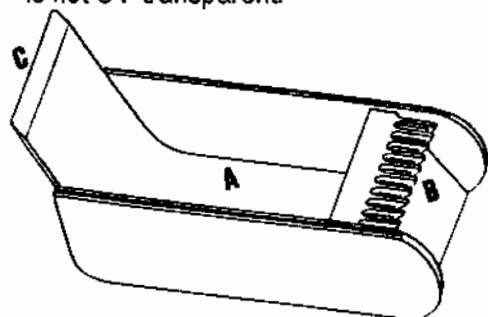


Elchrom Scientific's 'Easy-stain' gel tray (P/N 23 44) is strongly recommended for staining of the gels released from their backing.

10. STAINING OF PRECAST GELS

10.1 'Easy-Stain' Gel Tray

Gel staining may be difficult when an ordinary box is used, due to the tendency of gels to slip out of the box during the exchange of staining solutions. When pressed by hand to prevent the slippage, gels often break. Staining is greatly facilitated by using Elchrom Scientific's 'Easy-Stain' gel tray (P/N 23 44). There is no need to touch the gel by hand when exchanging solutions. The tray can accommodate two precast Mini gels, or one half of a Wide Mini gel. When gel backing is left during staining, only one gel, or a gel section, per tray should be stained, since otherwise the backing from one gel may damage the other. If viewing is desired while the gel is being stained with EtBr, gel backing should be up, since the backing is not UV-transparent.



Special features:

- UV-transparent bottom (at 312 nm) (A).
- Special barrier to safeguard the gel during exchange of solutions (B).
- Scoop to slide the gel onto a transilluminator and back into the tray (C).

10.2 Ethidium Bromide Staining

Caution! Ethidium bromide is a powerful mutagen. Always wear gloves when handling EtBr solutions and stained gels. Ethidium bromide solutions should be properly disposed of, for example by using Elchrom's Bind-ET™ ethidium removal system.

Poly(NAT) and Spreadex gels are always stained after electrophoresis. Staining can be done with the gel backing in place or removed. Without the backing, the staining and destaining process are considerably faster. In most cases it is advantageous to remove the backing (see page 18). Possible exceptions include: i) when it is essential that the gel keeps its original size and shape during recording, and ii) when handling of released gels represents a problem. For details on staining conditions, refer to Table 10.3.

Technical Tip The extremely low diffusion of DNA bands in Poly(NAT) and Spreadex gels is advantageous in that the gels do not need to be stained and photographed immediately after electrophoresis. Long destaining times (overnight at room temperature) cause no detectable band broadening. When gel background is clear and DNA bands sharp, it is possible to achieve a high detection sensitivity. About 0.2 ng of a 500 bp DNA can be detected in precast Poly(NAT) gels under optimal conditions.

In Clearose BG gels, DNA can be electrophoresed in the presence of EtBr. Destaining may not be necessary in many cases, so that the analysis time for a quick checking is as short as 5 to 10 min. Gel backing should not be removed, i) when recovering DNA from Clearose BG-EtBr gels, and ii) when gel reuse is planned. Clearose BG gels can also be stained after electrophoresis, and then destained in water in order to get a completely clear background.

Important: Background staining and detection sensitivity critically depend on quality of the EtBr solution. Old EtBr stock solutions, or those stored at room temperature or exposed to light, invariably give a high background. A stock solution (usually 1 mg/ml in water) should be stored refrigerated in a dark bottle or in a bottle wrapped in aluminium foil. Working solutions of EtBr (typically 0.3 - 0.5 µg/ml in water) should be prepared fresh in a measuring flask. Do not pipette an aliquot of the stock solution into the staining tray, as this leads to a high background staining. Staining should be done on a shaker, away from direct light.

10.3 Summary of Recommended Staining Procedures

Type of gel	Plastic Backing removed (see page 18)	[EtBr] (Fresh Solution in dd water)	Staining time*	Destaining time (with dd water)
Clearose BG gels	No	0.5 µg/ml	20 min	15 min
Clearose BG gels	Yes	0.5 µg/ml	15 min	10 min
6% Poly(NAT) gels	No	0.5 µg/ml	60 min	60 min
6% Poly(NAT) gels	Yes	0.4 µg/ml	30 min	30 min
9% Poly(NAT) gels	Yes	0.4 µg/ml	40 min	30 min
12% Poly(NAT) gels	Yes	0.3 µg/ml	50 min	30 min
All Poly(NAT) gels **	Yes and No	0.2 µg/ml	Overnight at 4 °C or at RT	Usually not necessary
Spreadex EL 1200 gels	Yes	0.4 µg/ml	35 min	30 min
All other Spreadex gels	Yes	0.4 µg/ml	45 min	30 min

(*) The gels should be stained in the Easy-Stain tray on a shaker, away from direct light.

(**) At the end of a busy day, there is no need to allow for extra time for staining and photographing. Poly(NAT) and Spreadex gels can be stained, or better destained, overnight and photographed the next day. Band sharpness will not change thanks to negligible diffusion of DNA fragments. Water of a good quality needs to be used for destaining. The presence of magnesium or calcium ions may diminish the sensitivity or cause a complete loss of DNA bands. In such a case, the gel can be re-stained.

10.4 SYBR Green Staining

SYBR Green I and II and SYBR Gold (Molecular Probes, Inc.) are fluorescent dyes that are about 5 times more sensitive than EtBr. With SYBR Green I, it is possible to detect less than 0.1 ng of dsDNA per band. It is essential to use a transilluminator with 254 nm UV light, since at 312 nm the detection sensitivity is only slightly better compared to EtBr. The precast gels should be incubated in a dilute solution (1:10,000 in 10 mM TAE) of SYBR Green for the time periods indicated in Table 10.3.

Important: SYBR Green I is extremely light sensitive. The fully thawed stock solution should be divided in dim light into 5 µl aliquots (sufficient for one staining), and stored at -20°C. Immediately after taking a test tube with 5 µl of SYBR Green out of the refrigerator, add 1 ml of 10 mM TAE and transfer the whole volume into a beaker with 50 ml of 10 mM TAE. Mix and pour in the staining tray. Stain in the absence of direct light. If a SYBR Green I solution has intense red colour, it will give a strong background and diminished sensitivity. To reduce the cost, two gels can be stained simultaneously with 50 ml of the dye in one tray. Four gels can be also stained in one tray, with slightly inferior results. Reuse of the staining solution is not recommended.

Technical Tips Do not try to view the gel on a transilluminator while it is being stained with SYBR Green I. The UV light would cause decomposition of the dye, leading to a significantly reduced sensitivity. Once the destaining step is completed, it is safe to view the gel. DNA-SYBR Green complexes appear to be more stable than DNA-EtBr complexes to repeated exposure to UV light, so that multiple photos of a stained gel can be taken without a loss of sensitivity.

Note: Under optimal conditions, SYBR Green I staining of dsDNA is equally sensitive as silver staining. On the other hand, SYBR Green II staining of ssDNA is less sensitive than silver staining. Silver staining of 3 mm thick precast gels is not recommended.

10.5 Destaining solution

After incubation in a solution containing an intercalating dye, DNA gels are usually destained in water. Work in Elchrom labs has shown that water solutions of certain polymers, such as PVP, speed up the destaining process, and also improve gel background. This is particularly the case with Spreadex EL 300-500 gels, as well as with 12% Poly(NAT) gels stained with SYBR Green or Gold. The destaining solution is available as a 100X concentrate. Typical destaining time is 20-30 min. A gel should not be left in the destaining solution overnight, since band intensity may be reduced. After exchanging the destaining solution for water, the gel can be left in it for prolonged time periods without any reduction of band intensities.

10.6 Viewing of Stained Gels on a Transilluminator

Caution: UV-light, especially of 254 nm, is dangerous for the skin and eyes. Wear protective glasses and close the protective shield, if available on the transilluminator.

- Gels with the gel backing

1. The gels should be placed on a transilluminator with the backing upward, since the plastic backing is not transparent to UV-light.
2. The plastic backing fluoresces at 312 nm (greenish), impairing somewhat visual inspection of stained gels. This fluorescence is largely eliminated by the orange filter used in front of camera lens.
3. View the gels at 254 nm whenever no DNA recovery is planned. At this wavelength, the fluorescence due to the plastic backing is greatly reduced. For preparative work, view the gel at 312 nm and mark the band of interest with a waterproof pen on the gel backing. Band cutting can be done later at the bench.
4. When gels are not destained, for example Clearose BG gels run in the presence of EtBr, there is additional background fluorescence coming from the dye. This fluorescence is also much less pronounced at 254 nm than at 312 nm.

- Gels without the backing

1. For analytical work, viewing at 254 nm is better as the sensitivity is 2-4 times higher than at 312 nm.
2. For preparative work, use 312 nm, never 254 nm. Band cutting needs to be done on the transilluminator. For your protection, cover all the surface not occupied by the gel with Elchrom's black rubber pads and/or aluminum foil. Wear protective face shield.
3. Without the gel backing, Elchrom's precast gels show negligible fluorescence when properly stained and destained.
4. When using Easy-Stain tray, the progress of gel staining may be checked without taking the gel out of an EtBr staining solution. The bottom of the tray is transparent to 312 nm UV light, so that one can see DNA bands just by placing the tray on a transilluminator and switching on the light. This kind of checking is not recommended for SYBR Green stained gels.
5. With EtBr staining, most DNA bands become visible within 10 min of staining. However, only the top and bottom of the bands are stained during this time period. Interrupting the staining at this point would result in a greatly reduced detection sensitivity.

Important: SYBR Green stained gels should not be viewed during staining. After staining and destaining, the gels should be viewed at 254 nm to benefit from the enhanced sensitivity of this dye.

Note: Elchrom Scientific's dual wavelength transilluminator, DWT (P/N 20 38), features 254 and 312 nm UV light, variable light intensities (70 and 100%), a UV-protective shield and a cooling fan.

11. PHOTOGRAPHIC RECORDING OF GELS

Various documentation systems, such as video/CCD and Polaroid cameras, can be used to record or photograph the gels. We have mostly used Polaroid® DS-34 camera and direct screen hoods. The set-up is fast, it requires no dark room, and the hoods eliminate the need for focusing of the lens. A complete system which includes the direct screen camera, hoods and filters is available from Elchrom Scientific (P/N 20 34). The hood DS-H.5 (8 x 10 cm) is perfect for recording Mini gels, and the hood DS-H.7 (13 x 14 cm) for recording one half of a Wide Mini gel. Polaroid 667 film is recommended for routine use, and Polaroid 665 film for best resolution. The 665 film is about 20 times less sensitive, so that the exposure time needs to be prolonged accordingly.

11.1 Photographic filters

EtBr stained gels. For excellent contrast and maximal reduction of background fluorescence, we recommend the use of Kodak's Wratten 22 filter (orange). Place it behind a UV filter (Wratten 2A), so that UV light passes first through the UV filter.

SYBR Green I stained gels. Replace the Wratten 22 filter with Wratten 15 filter (yellow).

Technical Tip With direct screen camera, the hood should rest at the same height as the top of the gel to get optimal sharpness, i. e. 3 mm above the transilluminator surface. Elchrom Scientific offers 3 mm thick black foam pads (four pads, 20 x 5 cm) that are positioned around the gel to form a frame. The hood is then placed on the frame, so that the light reaching the camera passes only through the gel.

11.2 Recommended Settings for the Polaroid DS-34 Camera* and the UV-Transilluminator DWT

Type of film	Recommended application	Type of hood	Wave-length	f-stop	Exposure time [#]	Detection limit (EtBr)
Polaroid 667	Routine analytical documentation	H.5 (for Mini gels)	254 nm	5.6 - 8	1 s	0.5 ng
Polaroid 667	Routine preparative documentation	H.5	312 nm	5.6 - 8	1 s	2.0 ng
Polaroid 667	Routine analytical documentation	H.7 (WM gels)	254 nm	5.6 - 8	1 s	0.5 ng
Polaroid 665 **	Publication quality images	H.5	254 nm	4.5	20 s	0.5 ng
Polaroid 665 **	Publication quality images	H.5	312 nm	4.5	30 s	0.5 ng
Polaroid 665 **	Publication quality images	H.7	254 nm	4.5	20 s	0.5 ng
Polaroid 665 **	Highest sensitivity (thorough destaining recommended)	H.5	254 nm	4.5	40 s or more	less than 0.2 ng of DNA

(*) In front of camera lens, a Kodak Wratten 22 filter was placed behind a Wratten 2A UV filter.

(#) The settings may need some adjustment depending on the age of the UV-filter and UV-lamps in the transilluminator.

(**) On the film negative, one can usually see the DNA bands that are hardly visible on the positive. For prolonged exposure, it is advantageous to use a transilluminator with a cooling fan, since DNA-EtBr complexes are degraded faster when the transilluminator surface becomes warm due to long exposure.

12. DNA RECOVERY

12.1 Poly(NAT) and Spreadex gels

DNA can be recovered using all methods that are currently in use for recovering DNA from polyacrylamide gels (13), including "crush and soak" (14), and electroelution (15-17). Electroelution is more quantitative than are other methods. "Crush and soak" procedure is simple and does not require special equipment, but takes more time and the yields are variable. The increased band spacing on Spreadex gels makes them particularly well suited for DNA recovery. Many PCR fragments differing by only 4 bp have been recovered. The DNA recovered from Poly(NAT) and Spreadex gels is of excellent quality for cloning.

Recovery of PCR fragments is easy. A small gel piece containing the band of interest is excised and transferred into a fresh tube. After the addition of PCR reagents and 10-15 cycles, ample amounts of the desired fragment are produced. Band excision from a gel released from its backing can be done with an ordinary 1 ml pipette tip, after enlarging its opening to about 1.5 mm. Making the inner surface of the tip rough with Elchrom's forceps prevents slippage of the cut gel piece as the tip is lifted. The gel piece is pushed out by another, thinner and longer tip. Cutting the 1 ml tip in half allows the use of an ordinary 0.2 ml tip for pushing the gel out. Gel cutting can be done also with a scalpel, but precision is lower.

12.2 Clearose BG gels

Clearose BG gels are unique in that they contain only about 1% of polymers and that the gels are insoluble in boiling water. These features allow easy recovery of DNA fragments simply by incubating a gel block in a suitable buffer at elevated temperatures (60 - 75°C). DNA molecules diffuse quickly into the incubation buffer. No gel crushing is necessary.

Important: The incubation buffer needs to contain magnesium ion at a rather high concentration (10 mM). DNA recovery will be extremely poor without it. A suitable buffer is 10 mM Tris-HCl pH 7.9, 50 mM KCl, 10 mM MgCl₂ and 1 mM DTT (New England Biolabs restriction buffer 2).

1. After staining the gel with EtBr and thorough destaining, place the gel, with its backing up, on a transilluminator (312 nm). Mark the band(s) of interest with a waterproof pen.
2. Place the gel on a bottle as for removal of the gel backing (page 18). Use a scalpel to make a small cut between sample wells at the gel edge. Take the nylon string to cut the gel between lanes. The best is to load samples in every second well when recovery is planned. Removing first the gel strips with empty lanes makes subsequent band cutting easier. Do not release the marked gel lane from the backing before cutting a gel block containing the band(s) of interest.
3. Transfer the gel block, which will typically weigh about 50 mg, into a 0.5 ml test tube. The gel will stick to tube walls. Take 60 µl of the incubation buffer, pipette a portion of it on the gel block, and push it down to the bottom of the well while pipetting the remaining buffer. Centrifuge briefly to recover all the liquid. The gel block should be completely submerged in the incubation buffer.
4. Incubate the tube at 60 to 75°C for 30 - 45 min. The lower temperature and shorter time are sufficient for small DNA fragments (below 200 bp). The yield of recovered DNA is typically 20 - 50%, largely because the volume of the gel block is comparable to the volume of incubation buffer, and the DNA is equilibrated between them. Second incubation of the same gel block with a fresh buffer will increase the yield.
5. The recovered DNA is ready for cloning. A high number of positive colonies was observed using small amounts of DNA. In contrast to agarose, the recovered DNA is not contaminated with soluble polymers, since in Clearose BG gels the polymers are covalently cross-linked.

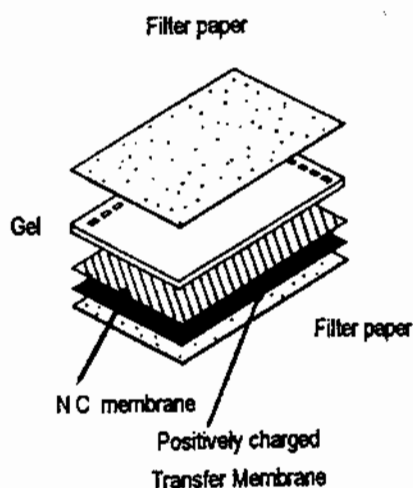
13. BLOTTING

Poly(NAT) and Spreadex gels are not stable in strongly alkaline solutions used for blotting of ssDNA from agarose gels (18, 19). It is best to electrotransfer dsDNA from Elchrom's precast gels to a positively charged membrane such as Zeta Probe (BioRad), Immobilon N (Millipore), or equivalent. The dsDNA is then quickly denatured on the membrane and is ready for further processing.

Note: Capillary and vacuum blotting will not work with Poly(NAT) or Spreadex gels. We do not recommend blotting of DNA from Clearose BG gels.

13.1 Preparation of the gel - membrane "sandwich".

1. After electrophoresis, release the gel from the backing (see page 18). Do not remove the plastic backing yet. Lay the gel, backing side down, on a flat glass or plastic surface.
2. Cover the gel with a dry filter paper 2 mm larger than the gel on all sides. This paper helps to preserve the gel's original size and shape. Turn the gel over and place it on the filter paper.
3. Carefully lift the plastic backing off the gel.
4. **Important:** A nitrocellulose membrane (NC, 0.45 μm), wetted with 30 mM TAE, should be placed on the gel surface that was attached to gel backing. The NC membrane will not bind dsDNA. Its role is to prevent sticking of the gel to positively charged nylon membrane, which sometimes occurs with low percentage synthetic gels, such as 6% Poly(NAT) gels.
5. Place a positively charged nylon or PVDF membrane on top of the NC membrane. Be careful to avoid entrapment of air bubbles between the membrane layers.
6. On the positively charged nylon membrane, place a filter paper wetted with 30 mM TAE. The final arrangement of the membranes and filter paper is shown below.



13.2 Tank blotting

The Mighty Small™ Transphor Tank (Hoefer Scientific Instruments) or a similar transfer unit is convenient for blotting of precast Mini gels. A larger unit is necessary for blotting of Wide Mini gels, which may be cut into sections to facilitate handling.

The 30 mM TAE running buffer is also used as the transfer buffer, so that equilibration of gels prior to blotting is not necessary.

1. Place the gel-membrane sandwich onto a porous pad provided by the manufacturer of the tank blotter. Cover it with a second pad and place it in the center of the gel cassette. Make sure that the gel is not squeezed too much by the cassette after closing it. If the sandwich is too thick, replace the porous pads by filter papers.
2. Fill the tank with running buffer and insert the gel cassette.
3. Add a magnetic stirring bar and place the blotter on a magnetic stirrer. Close the lid and turn power on. In a unit with about 4 liters of buffer and an electrode distance of about 10 cm, blotting at 50 V for 120-180 min will transfer most DNA fragments with a high yield.

13.3 Semi-Dry Blotting

Semi-dry electrotransfer offers a rapid alternative to tank blotting (20, 21). The procedure described below has proven successful in Elchrom Scientific's laboratories. However, the transfer yields were somewhat lower than with conventional tank blotting.

1. Cut eight 1 mm thick filter papers to a size slightly bigger than the gel (2 mm on all sides). Wet the filter papers with RB and place 4 of them in the center of the semi-dry blotter.
2. Take the gel-membrane sandwich and place it on the filter stack. Add a few drops of buffer to the filter paper on top of the gel stack. Place the four remaining pre-wetted filter papers on it.
3. Close the semi-dry blotter and apply voltage (10 V). Initially, the amperage will be quite high (280 mA), but it will decrease rapidly. It should not be lower than 80 mA when blotting Mini gels, otherwise the voltage should be set to a higher value. Blotting for 90 min at 80 mA constant amperage is usually sufficient.

13.4 Denaturation of blotted double stranded DNA

After the transfer, DNA is denatured on the membrane by a 10 min incubation of the membrane in a solution of 0.5 M sodium hydroxide and 1.5 M sodium chloride (22).

This method of electroblotting and denaturation of dsDNA on a membrane reduces the time from the end of electrophoresis to beginning of hybridization to under 2 hours.

14. PRESERVING OF STAINED GELS

In some instances, it is desirable to preserve a stained gel for future analysis of separated DNA bands. Precast Poly(NAT) and Spreadex gels are best preserved sealed in polyethylene bags. Suitable bags, and sealing devices, are available in many food stores, for storage of food at -20°C. DNA bands with the lengths in the optimal separation range, or above it, show negligible diffusion in Poly(NAT) and Spreadex gels when kept at 4°C for several months. For even a longer preservation, sealed gels can be placed at -20°C. Ice crystals tend to leave bubbles, and freezing causes a loss of water so that gels shrink, but DNA bands remain sharp. Preliminary data, with 6% Poly(NAT) and Spreadex EL 300 gels, indicate that the gels can be preserved for a year or longer. Stained Clearose BG gels cannot be preserved.

15. TROUBLESHOOTING

PROBLEM	CAUSE	ACTION
Gel slips when lifted with forceps	Incorrect forceps	Use the forceps provided with the SEA 2000 or starter kits
Removal of gel backing	The bottle moves The gel slides	Fix the bottle Add a drop of water under the gel backing, or wipe away the excess of running buffer
Gel handling difficult	A simple box used for staining	Use Elchrom's Easy Stain Tray
Lanes are not straight	Non-linear electric field Depletion of buffer ions	Use the SEA 2000 submarine electrophoresis apparatus
"Smiling effect" (The bands migrate further in the middle of the gel)	Temperature is higher in the middle lanes than in side lanes	Use Elchrom's SEA 2000 apparatus
Bands are distorted in some lanes	High salt concentration	Dilute the samples and use SYBR Green for staining
Bands are severely distorted, especially in the upper gel part	Borate in the loading buffer or in the sample	Take another buffer, borate-free
All bands are diffuse	Incorrect buffer, or old buffer	Make a fresh buffer
No bands are visible, or Weak bands	DNA amount too low Staining time too short Gel not destained Gel destained in water of poor quality overnight Gel exposed to strong light Illumination at 312 nm	Check DNA concentration Increase staining time Destain the gel Use double distilled water Keep it away from direct light Use 254 nm illumination
High background	Old ethidium bromide solution Concentration of EtBr too high SYBR Green exposed to light Staining too long Destaining too short Incorrect photography filters Illumination at 312 nm Contaminated buffer or staining tray Concentrated dye pipetted into tray Staining without shaking	Make a fresh stock solution Lower the concentration to 0.3 µg/ml Perform staining in the dark Stain for 1.5 h maximum Prolong destaining, up to overnight Check the filters Switch to 254 nm Change the buffer and clean the tray Dilute the dye in a beaker and then pour it in the staining tray Place the tray on a shaker
Expected resolution is not achieved	Bands wide, gel overloaded Migration distance too short Incorrect gel type Anomalous DNA mobilities	Load less DNA Increase the running time Check the optimal separation range Run the gel in Elchrom's SEA 2000 at 55°C
Runs are not reproducible	Lack of temperature control, depletion of buffer ions, non-linear electric field	Use Elchrom's SEA 2000 apparatus
Throughput is low	The Mini gels have only 8/12 sample wells	Use Wide Mini gels with 26, 50, 52 or 100 sample wells

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17. PRODUCT USE LIMITATIONS

All Guest Elchrom Scientific products are for research use only.

18. WARRANTY

If stored at 4°C, the gels will perform without loss in quality up to the date specified on each gel pack. The Polaroid picture attached with Quality Control Certificate shows a typical band pattern. Should any precast gel give unsatisfactory results, we will promptly replace the whole box, under the condition that the results are documented by showing one of our DNA markers, and that detailed running conditions are described.

No other warranties are expressed or implied, including but not limited to, the implied warranties of merchantability and fitness for a particular purpose. Guest Elchrom Scientific is not liable for incidental or consequential damages.

19. PATENTS AND TRADEMARKS

Poly(NAT) gels, Clearose BG gels and the SEA 2000 apparatus are patented.

The following is a list of the US Patents owned by GUEST ELCHROM SCIENTIFIC AG:

5,185,466, 5,202,007, 5,258,501, 5,259,943, 5,278,270, 5,316,912, 5,319,046,
5,371,208, 5,438,092, 5,458,760, 5,541,255

Other patents are pending. Patents in other countries.

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