Model 785 Vacuum Blotter

Instruction Manual

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Section 1
General Information

1.1 Safety/Definition of Symbols

Caution

This instrument is intended for laboratory use only.

This product conforms to the “Class A” standards for electromagnetic emissions intended for laboratory equipment applications. It is possible that emissions form this product may interfere with some sensitive appliances when placed nearby or in the some circuit as those applicances. The user should be aware of this potential and take appropriate measures to avoid interference.

1.2 Introduction to the Model 785 Vacuum Blotter

The Model 785 Vacuum Blotter is a nucleic acid transfer apparatus which rapidly transfers DNA or RNA from agarose gels onto nylon membranes by using low vacuum. The Model 785 Vacuum Blotter simplifies every step of the blotting process. In addition, the Model 785 Vacuum Blotter is constructed for long term heavy use. The following protocols have been thoroughly tested and have the continuing technical support of Bio-Rad Laboratories.

1.3 List of Parts

Each Model 785 Vacuum Blotter unit comes with the items listed below. Check to make sure that all components are present. Also note any damage to the instrument that may have occurred during shipping. Inform Bio-Rad Laboratories if any items are missing or damaged.

Item	Quantity
Base Unit	1
Vacuum Stage	1
Porous Vacuum Plate	1
Window Gasket	
– precut 15 x 15 cm	2
– precut 15 x 20 cm	2
– blank	2
Sealing Frame	1
Lid	1

Offered Accessories	
Vacuum Regulator	1
Vacuum Station	1

1.4 General Description

The Vacuum Blotter has several innovative features which simplify the nucleic acid blotting process and facilitate complete transfer. The Model 785 Vacuum Blotter features include:

- Easy-to-use spring latches which make assembly and disassembly quick. No screws or nuts to tighten.
- Vacuum Stage and Porous Vacuum Plate create an even vacuum pressure throughout the transfer area, providing even transfers and highly reproducible results.
- Large Vacuum Stage format permits large or small gel transfer using the blank Window

Gasket or standard gel transfer using the precut Window Gasket.
- Green Window Gasket allows you to easily see the gel/gasket overlap for easy alignment.
- Vacuum Regulator facilitates accurate vacuum pressure using Bio-Rad's Vacuum Pump or almost any vacuum source.
- Durable molded construction for long term heavy use.
- Base Unit and Sealing Frame designed for easy alignment of gel and membrane.

1.5 Specifications

Dimensions

Overall outer size	21" (L) x 12" (W) x 5" (H)
Gel capacity (maximum size)	20 cm x 25 cm

Unit weights

Complete assembly	8.5 lb
Lid weight	1.5 lb
Sealing frame assembly	1.5 lb
Porous vacuum support	0.25 lb

Materials

Base	Mindel-S plastic
Springs	Stainless steel
Posts	Delrin plastic
Vacuum stage	Mindel-S plastic
Window gasket	Flexible green vinyl
Porous vacuum plate	Polypropylene
Sealing frame	Glass-filled Mindel-S
O-ring	Silicone rubber
Lid	Acrylic
Tubing	Tygon (1/2" OD x 1/4" ID)

Vacuum regulator pump

Dimensions

Overall outer size	16" (L) x 9" (W) x 8" (H)

Weights

With pump	9.5 lb
Without pump	3.9 lb

Materials

Valve	Polyvinylchloride
Fittings	Nylon
Housing	Acrylic
Interior pump components	PTFE

Ratings

Vacuum pump	20 inches Hg (max)/250 Torr
Regulator	0-25 inches Hg (max)/125 Torr

Electrical

Input voltage range	100–120 VAC/50-60 Hz/1.5 A 220–240 VAC/50 Hz/0.75 A

Fuses

Internal Thermal Fuse

Environmental

Operating	50 °F (10 °C) to 90 °F (32 °C) temperature 30–80% humidity
Storage	32 °F (0 °C) to 140 °F (60 °C) temperature 10–90% humidity

Cautions

1. Not designed to handle liquids or heavy vapors. If pump does get liquid inside, open valve to max position and let run for 15 minutes to allow interior components to dry.
2. If valve knob loosens up during operation, wrap threads with PTFE tape to increase thread engagement.
3. Pump will become hot after a few hours of continuous use. This is normal and should not be a cause for concern.

Section 2
Description of Major Parts

2.1 Base Unit

The Base Unit is the foundation of the Model 785 Vacuum Blotter. The plastic used to make the Base Unit is an off-white color with a slight texture. The Base Unit is made of a durable and chemically resistant mindel-S plastic. In low concentrations, acids and bases 0.25 N HCl or 0.5 N NaOH will not damage the Base Unit or the Vacuum Stage. The Base Unit supports the Vacuum Stage on springs and catches the transfer solution during disassembly.

2.2 Vacuum Stage

The Vacuum Stage is supported by springs on the Base Unit. The spring action on the Vacuum Stage is used to lock and unlock the Sealing Frame. The center portion of the Vacuum Stage has a grooved grid pattern. This grid pattern helps distribute the vacuum pressure evenly across the Porous Vacuum Plate. In the center of the grid pattern is a port. This port is the vacuum inlet and is connected to vacuum tubing underneath the Vacuum Stage. Surrounding the grid area is a soft silicon Reservoir Seal O-ring that provides a water-tight seal to the upper buffer chamber.

2.3 Porous Vacuum Plate

The Porous Vacuum Plate is a white polypropylene sheet. The Porous Vacuum Plate is used to support the transfer membrane and the gel. Along with the grid pattern on the Vacuum Stage, the Porous Vacuum Plate helps distribute the vacuum pressure evenly.

2.4 Window Gasket

The Precut Window Gasket is a green vinyl sheet with a window cut out of the center. Refer to Section 3.2 for instructions on how to cut the Blank Window Gasket. The Window Gasket is used to mask off the remaining Porous Vacuum Plate not covered by the gel. The Window Gasket forces the vacuum through the gel/membrane complex by making a watertight seal where the gel overlaps the Window Gasket. The Window Gasket also seals in the vacuum along the Reservoir Seal O-ring when the Sealing Frame is compressed onto the Vacuum Stage.

2.5 Sealing Frame

The Sealing Frame is made of the same mindel-S plastic as the Base Unit and has the same off-white color as the Base Unit. The Sealing Frame features easy-to-use latches. The latches will lock on to the post when the Sealing Frame is pressed onto the Vacuum Stage. Simply press the latches inward to unlock the Sealing Frame. The Sealing Frame compresses the Reservoir Seal O-ring onto the Window Gasket, creating an air-tight seal between the Porous Vacuum Plate and the Window Gasket. The Sealing Frame also holds the transfer solution over the gel during the transfer.

2.6 Vacuum Regulator

The Vacuum Regulator is used to control the vacuum pressure. There is a post on either end of the Vacuum Regulator. One post is used for the vacuum source inlet and the other post is used to connect the Model 785 Vacuum Blotter. The Vacuum Regulator has a vacuum gauge attached to the center portion. Because the vacuum gauge is sensitive to liquids, it is very important to connect a side-arm flask between the Vacuum Blotter and the Vacuum Regulator. There is an adjustment valve in front of the pressure gauge. Unscrewing the outer-most adjustment nut causes the vacuum pressure to decrease. Screwing the adjustment nut clockwise increases the pressure.

2.7 Vacuum Pump

The Vacuum Pump is used to create a vacuum underneath the gel/Window Gasket complex. The Vacuum Pump is connected directly to the Vacuum Regulator with vacuum tubing. The Vacuum Pump is either 100–120 VAC or 220–240 VAC. The voltage is not user settable and is configured at the factory. Verify that the voltage of the pump is appropriate for your country.

2.8 Lid

The Vacuum Blotter Lid is made of a clear polycarbonate. The Lid shields the gel from falling objects during transfer. It also protects the instrument during storage. The Model 785 Vacuum Blotter Lid allows you to stack vacuum blotting units on top of each other without damaging the units.

Section 3
Operating Instructions

3.1 Vacuum Blotter Setup

1. Set the instrument up as illustrated in the diagram below.

Fig. 3.1. Model 785 Vacuum Blotter with liquid trap, Vacuum Regulator, and pump.

2. To start, make a liquid-trap from a side-arm flask 1,500–2,000 ml. Connect the tube from the Base Unit to the arm of the side-arm flask. Then, find a stopper for the side-arm flask with a single hole that tightly fits a 5 ml pipet. Push half of pipet through the stopper. Place the stopper on the side-arm flask.
3. Connect a vacuum tubing between the 5 ml pipet of the side-arm flask to the Vacuum Regulator port.
4. Connect vacuum tubing from the Vacuum Regulator port to the Vacuum Pump. Refer to Figure 1.

   Note: Setting up for an alternative vacuum source and for two Vacuum Blotters using the same vacuum source are discussed in Section 7.2B.

3.2 Preparation For Vacuum Transfer

1. Cut a nylon membrane 0.5 cm bigger than each border of the precut window on the Window Gasket. Cut a sheet of filter paper the same size as the nylon membrane.

   Note: The larger the membrane/filter paper, the easier the task of centering the Window Gasket on top of the membrane.

2. When cutting a customized window from the blank Window Gasket, make sure the window dimensions are at least 0.5 cm smaller than the gel dimensions, *i.e.*, if the gel is 15 x 10 cm, then the maximum window size should be 14 x 9 cm.

3.3 Gel Pretreatment

1. Fill the wells of the agarose gel with melted agarose gel of equal concentration. Allow agarose to dry before continuing.

2. There are three transfer procedures listed: the Standard Transfer Procedure, the Rapid Transfer Procedure, and the RNA Transfer Procedure. The Standard Transfer Procedure is for detection of single copy gene in genomic DNA. The Rapid Transfer Procedure is for fast identification of DNA inserts from various cloned vectors.

Standard Procedure:

1. Depurinate the gel in 0.25 N HCl for 15 minutes in a tray. Cover the gel with 0.25 N HCl and shake gently.
2. Remove the 0.25 N HCl solution. Rinse the gel twice with deionized distilled water.
3. Denature the gel in 0.5 N NaOH for 30 minutes. Cover the gel with 0.5 N NaOH and shake gently.
4. Transfer the gel in 10x SSC for 90 minutes at 5 inches Hg. Follow the instructions in Section 3.4 for the transfer procedures.

Rapid Transfer Procedure:

1. Depurinate the gel in 0.25 N HCl for 15 minutes in a tray. Cover the gel with 0.25 N HCl and shake gently.
2. Remove the 0.25 N HCl solution. Rinse the gel twice with deionized distilled water.
3. Immediately, transfer in 0.5 N NaOH, 0.6 N NaCl for 90 minutes at 5 inches Hg. Follow the instructions in Section 3.4 for the transfer procedures.

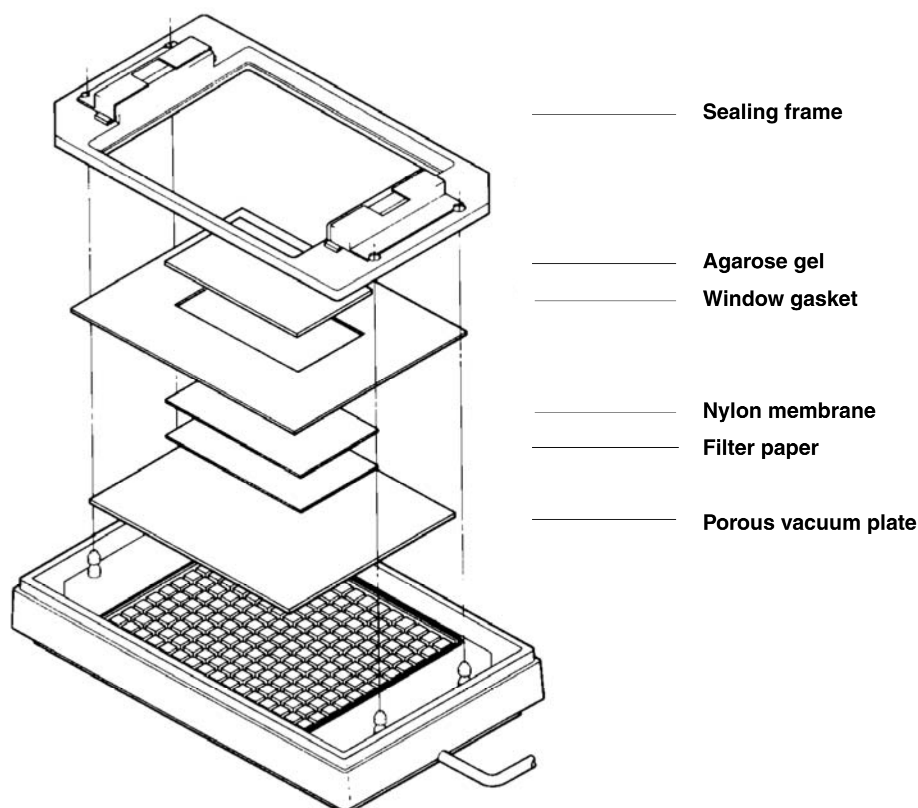


Fig. 3.2. Exploded view of vacuum transfer set-up.

RNA Transfer Procedure:

1. After electrophoresis rinse the RNA gel in deionized distilled water.
2. Transfer in 10x SSC for 90 minutes at 5 inches of Hg. Follow the instructions in Section 3.4 for the transfer procedures.

3.4 Vacuum Transfer

1. Wet the precut nylon membrane from Section 3.2 in double distilled water by slowly lowering the membrane at a 45 degree angle to the water. Then, wet the membrane and the filter paper in the appropriate transfer solution.
2. Make sure that the Porous Vacuum Plate lies flush on the Vacuum Stage. Place the wetted filter paper on the Porous Vacuum Plate. Lay the filter paper in the area where the cut window of the Gasket will be. Place the wetted membrane on top of the filter paper. Remove the bubbles by rolling a 10 ml glass pipet over the membrane.
3. Wet the Reservoir Seal O-ring with water.
4. Place the Window Gasket on top of the membrane/filter paper. Make sure the Window Gasket covers the entire O-ring on the Vacuum Stage. At the same time, make sure that the filter/membrane is overlapping the Window Gasket. Realign as necessary. Refer to Figure 2.

   Alternatively, place the Window Gasket on the Porous Vacuum Plate first. Adjust the Window Gasket to cover the entire O-ring. Then, hold one end of the Window Gasket and slowly peel the other end back until the window area is up off the Porous Vacuum Plate. Place the wet membrane/filter on the Porous Vacuum Plate under the window area and under the Window Gasket. Finally, replace the partially peeled back Window Gasket over the membrane/filter paper. Make sure the Window Gasket covers the entire O-ring and overlaps the membrane/filter paper on all sides.
5. Gently place the gel, well side up, on top of the Window Gasket. The gel must overlap the window. Remove bubbles by using a 10 ml glass pipet. As a final check, make sure that the gel edges overlap the Window Gasket by at least 5 mm.
6. Place the Sealing Frame on top of the Vacuum Stage. Lock the Sealing Frame onto the four latch posts. The spring latch handle of the Sealing Frame has a precut region in the middle. Push down on this exposed area of the Sealing Frame with your thumb until the latches snap onto all four latch posts.
7. Unscrew (counterclockwise) the Vacuum Regulator bleeder valve several turns to prevent strong initial vacuum.
8. Start the vacuum source and slowly turn the bleeder valve clockwise until the gauge reads at 5 in. of Hg. If the Bio-Rad Vacuum Pump is used, prewarm the pump for 10 minutes before blotting. Without prewarming, the vacuum pump will slowly increase the pressure and then stabilize after 10 minutes. Adjust the pressure to 5 inches of Hg.
9. With a finger, apply gentle pressure on top of the gel along the window border. The pressure helps to form a tight vacuum seal between the gel and the Window Gasket.
10. Gently pour 1,000–1,500 ml of the appropriate transfer solution (refer to Section 3.3) into the upper reservoir. Check to see if the gel is displaced. The gel should stick to the Window Gasket. If the gel floats, simply disassemble the Sealing Frame to drain the transfer solution and repeat Steps 5 to 10 again.
11. Place the lid on top of the Vacuum Blotter. Occasionally check the buffer level. It should be higher than the gel. Check the vacuum pressure and adjust the vacuum pressure to 5 inches Hg as needed.
12. Transfer the gel for 90 minutes at 5 inches Hg.

3.5 Post-Transfer

1. After 90 minutes, turn off the vacuum source.
2. Squeeze the Sealing Frame spring handle inward to release it from the latch posts. Remove the Sealing Frame. Let the upper buffer drain into the Base Unit.
3. Remove the transferred gel. The gel area inside the window should be half as thick as the gel area outside the window. To check for any remaining DNA, stain the gel with 1.0 μg/ml of Ethidium Bromide.
4. Remove Window Gasket and nylon membrane. Soak the membrane in 2x SSC for 5 minutes and air-dry between two sheets of filter paper. Dry the membrane between two sheets of filter paper in a vacuum oven at 80 degrees for 30 minutes. The blotted membrane is now ready for hybridization.
5. Remove the filter paper on the Porous Vacuum Plate and dump the transfer solution from the Base Unit. Clean the Model 785 Vacuum Blotter according to Section 4.

Section 4
Instrument Maintenance

4.1 Cleaning

1. Rinse all Vacuum Blotter components with warm water. Remove any salts by rinsing the Vacuum Stage and the Reservoir Seal O-ring with water. Clear all vacuum tubing of any liquids before transferring.

   Caution: Do NOT rinse or wet the Vacuum Regulator or the Vacuum Pump.

2. Remove any agarose particles or filter paper fiber from the Porous Vacuum Plate by rinsing it with a strong jet of water. Be careful not to smash any agarose into the Porous Vacuum Plate. These cleaning instructions will prevent the fine pores of the Porous Vacuum Plate from clotting due to salts, filter paper fibers, or agarose.

4.2 Replacement Parts

The parts listed below will wear out over time. These parts have a definite lifetime, depending on use and maintenance. Please examine these parts carefully before each use. Replace these parts as needed:

Catalog Number	Product Description
165-5007	**Reservoir Seal O-ring**, 1
165-5008	**Blank Window Gasket**, 6
165-5012	**Porous Vacuum Plate**, 1

Section 5
Troubleshooting Guide

5.1 Operational Troubleshooting

The following table summarizes possible Vacuum Blotter operational difficulties, probable causes, and remedies. Refer to Sections 3 and 4 for recommended procedures.

Condition	Probable Causes	Solutions/ Preventions
1. Upper buffer level drops too fast during run.	• No water-tight seal formed between O-ring and Window Gasket.	• Make sure that gasket covers the entire O-ring before placing the reservoir frame. • Prewet O-ring with H_2O.
	• Incorrect window size for the transferred gel.	• Refer to Section 3.2, Step 2 on how to determine correct window dimensions.
2. Gel does not attach to window or float during transfer.	• No vacuum in the window area.	• Check tubings, connections, and regulator bleeder for vacuum leaks. • Check vacuum pump by placing finger at vacuum port.
	• Too low vacuum + violent disturbance during transfer.	• Maintain a constant 5 inches Hg throughout the run. Minimize bench disturbance.
	• Incorrect window dimensions for a gel.	• Refer to Section 3.2, Step 2 on determination of window dimensions.
3. Difficult to get accurate reading from the regulator gauge.	• Too strong vacuum pump used.	• Bleed some pressure off by employing a three-way valve.
	• Violent shaking of pump when placed near regulator.	• Stop the shaking or place pump on another bench.
	• If using Bio-Rad pump.	• Prewarm pump for 10 minutes before transfer.
4. Vacuum pressure changes during run.	• Pump was not prewarmed.	• Prewarm pump before transfer.
5. Moisture in regulator.	• Liquid trap container is too small.	• Use a 1,500-2,000 ml side-arm flask
	• Too much liquid in the trap.	• Remove liquids from the trap every run.

5.2 Southern Hybridization Troubleshooting

The performance of the vacuum transfer will reflect in Southern hybridization results. The table below summarizes the problems, the probable causes, and the solutions. The term "signal" refers to the band on the Southern hybridization autoradiograph. Refer to the Zeta-Probe® GT manual also for specific troubleshooting.

Condition	Probable Causes	Solutions/ Preventions
1. No signal/ weak signal.	• Insufficient DNA loaded.	• Load between 5-10 µg of DNA.
	• Poor or no DNA transferred.	• Stain gel after it is transferred. Have positive control lane. Both precautions are for checking if DNA has transferred.
	• ^{32}P-labeled probe is faulty.	• Make sure probe concentrations and specific activity is correct.
	• Prolonged vacuum transfer.	• Vacuum transfer no longer than 90 minutes.
	• Nylon membrane was not baked.	• Bake membrane in 80 degree oven for 30 minutes.
2. Partial signal.	• Porous membrane is clotted.with agarose or salt at signal.	• Use new porous gel support or clean the clotted spot.
3. High background.	• Uncleaned probe.	• Remove all radio-isotopic nucleotides in labeling reaction. Can use Bio-Rad P-30 desalting column.
	• Dirty nylon membrane.	• Soak nylon membrane in 2x SSC for 5 minutes before air drying.
	• Hybridization conditions.	• For Zeta-Probe membrane use hybridization buffer with 7% SDS + 0.5 M NaH_2PO_4 pH 7.2 + 1 mM EDTA. • Check hybridization temperature.

5.3 Repairing Cracked Gels

A slightly damaged gel can be transferred with the Model 785 Vacuum Blotter. The gel can be successfully transferred if it is partially torn on the side. However, if the gel is completely cracked in two, or if it cracks in four, the chance of a successful transfer will vary from case to case. The following instructions can help you piece together and transfer a damaged gel.

1. Pretreat the gel as described in Section 3.3. Do not use the Rapid Transfer Procedure, Section 3.3 for cracked gels. Carry out Steps 1–4 of Section 3.4.
2. Assemble the gel pieces on the casting tray. Dry the top and the cracked side of the gel with filter paper or absorbance paper.
3. Tape both ends of the casting tray to hold the gel in place.
4. Use a pasteur pipet to pipet melted agarose along the cracked area. Allow the agarose to dry.
5. Remove the tape and squirt distilled water under the gel with a squirt bottle. The water allows the gel to slide out of the tray easily.
6. Carefully slide gel onto the Gasket Window at a low angle. Try to minimize manipulation of gel.
7. Carefully remove bubbles underneath the gel by gently pressing them out to the sides.
8. Carry out Steps 6–12 of Section 3.4.

Section 6
Equipment and Accessories

6.1 Model 785 Vacuum Blotter and Accessories

Catalog Number	Product Description
165-5000	**Model 785 Vacuum Blotter with Regulator,** includes Vacuum Regulator, Base Unit with Vacuum Stage, Porous Vacuum Plate, Reservoir Seal O-ring, Sealing Frame, Assortment Window Gaskets, Vacuum Blotter Lid, Instruction Manual
165-5001	**Model 785 Vacuum Blotter System,** includes Vacuum Pump (120 V), Vacuum Regulator, Base Unit with Vacuum Stage, Porous Vacuum Plate, Reservoir Seal O-ring, Sealing Frame Assortment Window Gaskets, Vacuum Blotter Lid, Instruction Manual
165-5002	**Model 785 Vacuum Blotter System,** includes Vacuum Pump (220/240 V), Vacuum Regulator, Base Unit with Vacuum Stage, Porous Vacuum Plate, Reservoir Seal O-ring, Sealing Frame, Assortment Window Gaskets, Vacuum Blotter Lid, Instruction Manual
165-5003	**Model 785 Vacuum Blotter Basic Unit,** includes Base Unit with Vacuum Stage, Porous Vacuum Plate, Reservoir Seal O-ring, Sealing Frame, Assortment Window Gaskets, Vacuum Blotter Lid, Instruction Manual
165-5004	**Vacuum Station,** includes Vacuum Pump (120 V), Regulator, Instruction Manual
165-5005	**Vacuum Station,** includes Vacuum Pump (220/240 V), Regulator, Instruction Manual
165-5006	**Vacuum Regulator,** with instruction manual
165-5007	**Reservoir Seal O-ring**, 1
165-5008	**Blank Window Gasket**, 6
165-5012	**Porous Vacuum Plate**, 1

6.2 Electrophoresis Reagents

Ultra Pure DNA Agarose

Catalog Number	Product Description
161-0716	**Tris Base**, 500 g
161-0719	**Tris Base**, 1 kg
161-0729	**EDTA**, 500 g
161-0733	**10x TBE Buffer**,1 L
161-0404	**Bromophenol Blue**, 10 g
161-0423	**Xylene Cyanole FF**, 25 g

6.3 Blotting Reagents

Catalog Number	Product Description
162-0153	**Zeta-Probe Membrane**, sheets, 9 x 12 cm, 15
162-0154	**Zeta-Probe Membrane**, sheets, 10 x 15 cm, 15
162-0155	**Zeta-Probe Membrane**, sheets, 15 x 15 cm, 15
162-0156	**Zeta-Probe Membrane**, sheets, 15 x 20 cm, 15
162-0157	**Zeta-Probe Membrane**, sheets, 20 x 20 cm, 15
162-0158	**Zeta-Probe Membrane**, sheets, 20 x 25 cm, 3
162-0159	**Zeta-Probe Membrane**, roll 30 cm x 3.3 m, 1
162-0165	**Zeta-Probe Membrane**, roll 20 cm x 3.3 m, 1
162-0190	**Zeta-Probe GT Membrane,** 9 x 12 cm, 15
162-0191	**Zeta-Probe GT Membrane,** 10 x 15 cm, 15
162-0192	**Zeta-Probe GT Membrane,** 15 x 15 cm, 15
162-0193	**Zeta-Probe GT Membrane,** 20 x 15 cm, 15
162-0194	**Zeta-Probe GT Membrane,** 20 x 20 cm, 15
162-0195	**Zeta-Probe GT Membrane,** 20 x 25 cm, 3
162-0196	**Zeta-Probe GT Membrane,** 30 cm x 3.3 m, 1
162-0197	**Zeta-Probe GT Membrane,** 20 cm x 3.3 m, 1
161-0302	**Sodium Dodecyl Sulfate** (SDS), 1 kg
165-0962	**Filter Paper Backing**, 35 x 45 cm, 50 sheets
165-0921	**Filter Paper Backing**, 18 x 34 cm, 25 sheets

For more information on related products from Bio-Rad Laboratories see Bulletin 1900. For a complete list of molecular biology products refer to the Bio-Rad general catalog.

Section 7
Appendix

7.1 Facts About Vacuum Transfer

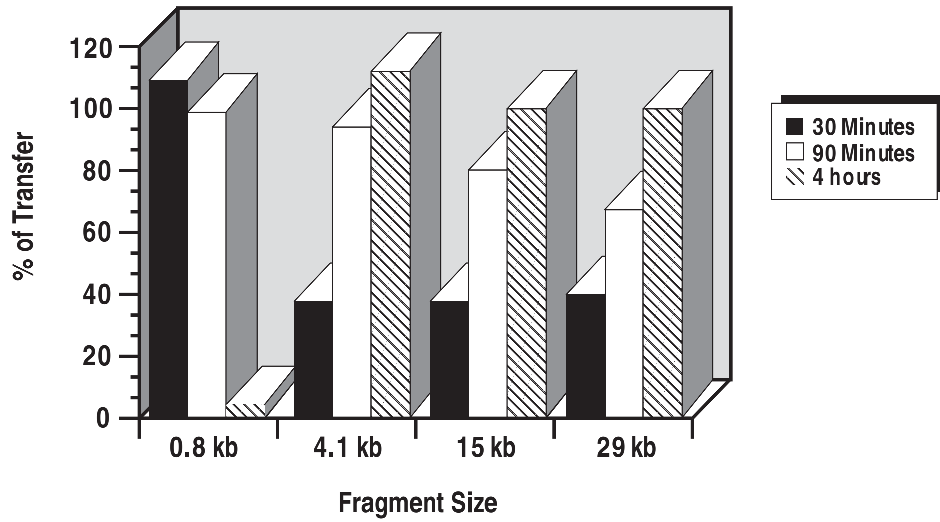
Quantitative vacuum transfer mainly depends on three factors: transfer time, vacuum pressure, and agarose concentration.

We studied the effect these three factors have on quantitive vacuum transfer. In our study, we used four different sized target fragments: 0.8 kb, 4.1 kb, 15 kb, and 29 kb. We electrophoresed 10 pg, 1 pg, and 0.5 pg of the target DNA fragments with a total background DNA of 5 µg. Our experimental control was an overnight capillary Southern. The densitometer readings of the overnight Southern on the 1 pg and 0.5 pg lanes were our 100% transfer standard. Therefore, 100% vacuum transfer means the vacuum transfer is equal to the overnight Southern transfer. Greater than a 100% vacuum transfer means the vacuum transfer exceeded the Southern transfer. Efficiency of vacuum transfer is calculated as follows:

$$\text{\% of Transfer} = 100 \times \frac{\text{O.D. of vacuum transfer}}{\text{O.D. of overnight capillary transfer}}$$

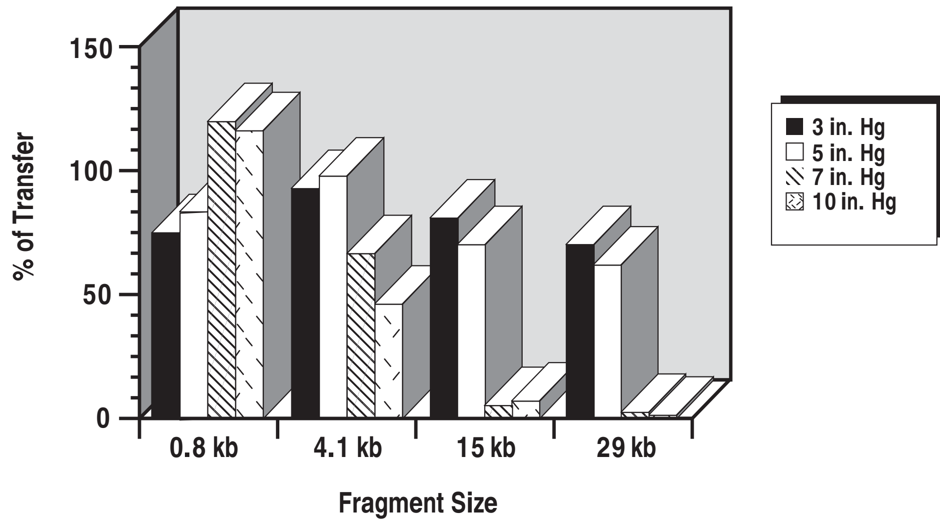
We found the optimum vacuum transfer at 90 minutes using 5 inches of Hg with a gel concentration of 0.7% agarose. We have summarized our work in the following charts. The three essential factors in vacuum transfer are varied: time of transfer, vacuum pressure, and gel concentration. The following charts illustrate the effect these factors have on vacuum transfer.

Chart 1: Time Effect on Transferring Efficiency



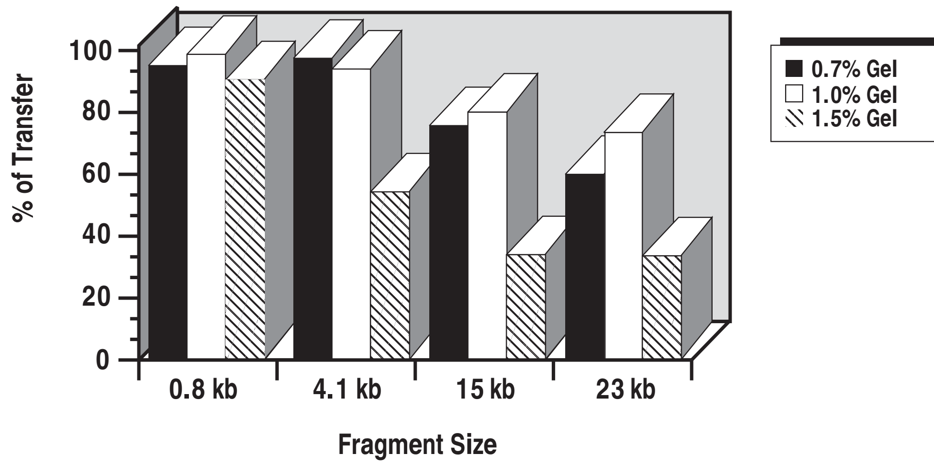
Vacuum Pressure - 5 inches Hg.; Time of Transfer - **varied**; Gel Percentage - 0.7%.

Chart 2: Vacuum Effect on Transferring Efficiency



Vacuum Pressure – **varied**; Time of Transfer - 90 minutes; Gel percentage - 0.7%.

Chart 3: Gel Concentration Effect On Transferring Efficiency



Vacuum Pressure - 5 inches Hg; Time of Transfer - 90 minutes; Gel percentage – **varied**.

7.2 Required Solutions and Equipment

The check list below consists of stock solutions and equipment that are necessary for the vacuum transfer process.

A. Stock Solutions:	To Make 1,000 ml Solution
1. 20x SSC	175.3 g of NaCl + 88.2 g of sodium citrate in 800 ml of double distilled water. Adjust to pH 7.0 with a few drops of concentrated HCl. Adjust to 1,000 ml.
2. 5 M NaCl	292.2 g of NaCl in 800 ml of double distilled water. Adjust to 1,000 ml.
3. 5 N NaOH	200 g NaOH pellets in 800 ml of double distilled water. Adjust to 1,000 ml. **Caution:** Highly exothermic reaction.
4. HCl	Purchase concentrated liquid stock.

B. Equipment:	Comments/Setup
1. Model 785 Vacuum Blotter Unit	Two or three units can be operated from the same vacuum source. A three or four-way splitter can be installed between the vacuum units and the liquid trap. Each connector port from the splitter is connected to the vacuum unit by vacuum tubing. One port from the splitter is connected to the liquid trap which in turn is connected to the Vacuum Regulator. The Vacuum Regulator is connected to the vacuum source.
2. Vacuum Regulator	Only one regulator is needed to run two to three Vacuum Blotter units. Will not work efficiently with too strong a vacuum pressure (≥25 inches of Hg).
3. Vacuum sources	Bio-Rad's Vacuum Pump, house vacuum, or vacuum pumps can be used. A strong pump may require a three-way valve to reduce the vacuum pressure. This valve should be connected between the pump and the Vacuum Regulator. **Caution**: Do not apply ≥25 inches of Hg to the Vacuum Regulator.
4. Tygon tubing	1/4" inner diameter and 1/2" outer diameter. Offered by Fisher Scientifics.
5. Liquid trap	1,500 ml-2,000 ml side-arm flask or commercially available liquid trap.
6. Three or four-way splitter	Offered by the following venders: Fisher Scientific, VWR, Baxter, and others.
7. Three-way valve	Offered by Fisher Scientific.
8. Plug	For side-arm flask. It should have a single hole that fits a 5 ml pipet.