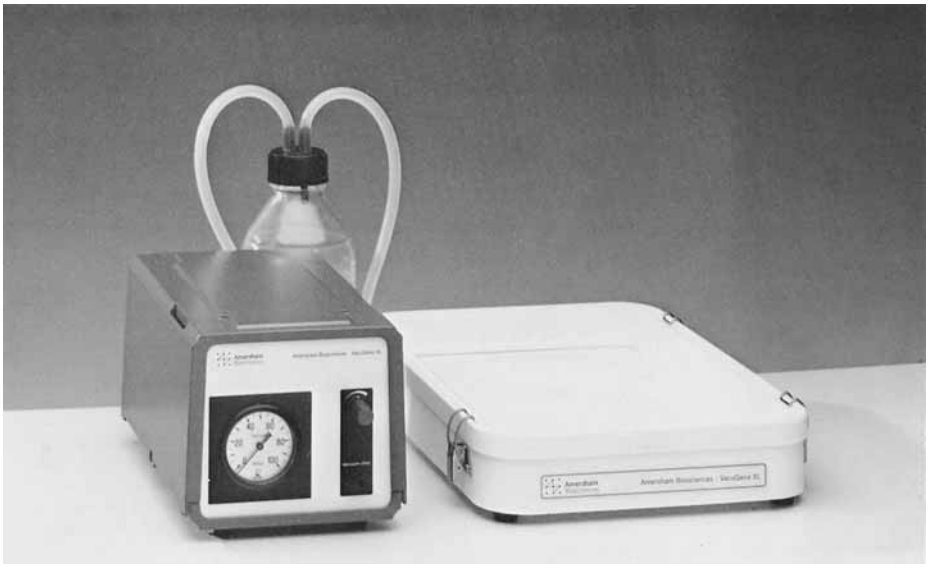
instructions

VacuGene XL Vacuum blotting System



56-1130-80 Edition AF

Amersham Biosciences

Important Information

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Contents

1. Introduction

VacuGene XL Vacuum Blotting System uses a low pressure vacuum to transfer nucleic acids from a gel to a transfer membrane. DNA and RNA fragments separated by agarose gel electrophoresis can be transferred, ensuring that optimal results are obtained even from genomic DNA digests. The procedure is rapid with transfers taking a maximum of one hour.

VacuGene XL unit is convenient and easy to use, with both pre-transfer and transfer steps being carried out in the blotting unit. The gel to be transferred is placed on the transfer membrane which is held within a "window" in the waterproof mask. The mask is supported on the porous screen in the base of VacuGene XL.

After locking the upper frame to the base, the base is evacuated and the gel is flooded sequentially with depurination, denaturation, neutralisation and transfer solutions. By subjecting the gel continuously to a vacuum, transfer of nucleic acids to the transfer membrane occurs in one direction only.

VacuGene XL accepts gels up to 20 cm x 30 cm. The transfer procedure is reproducible and, since no stacks of paper are required and most solutions can be recycled, the unit is inexpensive to run.

The transfer procedure is safe and efficient. No high voltages are used and transfer efficiencies of 95% or better can be obtained.

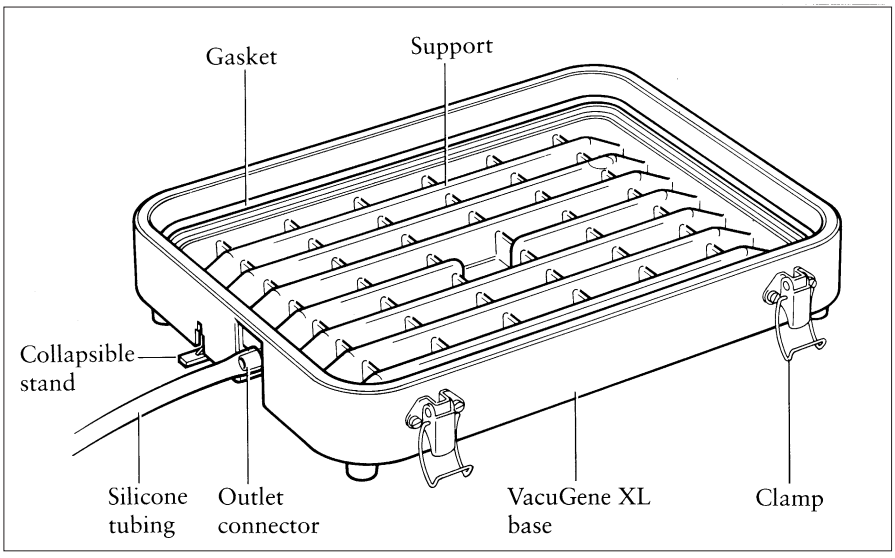
How to use this manual

Section 1 - an introduction to VacuGene XL and its uses
Section 2 - a physical description of each component part of VacuGene XL vacuum blotting system
Section 3 - instructions for installing VacuGene XL vacuum blotting system
Section 4 - step-by-step illustrated instructions on the operation of the system and preparation of recommended solutions and reagents
Section 5 - a guide to maintenance procedures
Section 6 - a trouble-shooting guide for problem solving
Section 7 - a reference list for further reading
Section 8 - a list of accessories, chemicals and consumables used in vacuum blotting
Section 9 - Technical Specifications
Section 10 -Spare Parts

2. Physical description

VacuGene XL vacuum blotting system is comprised of the following components.

2.1 VacuGene XL vacuum blotting unit



Vacuum unit

The vacuum unit, consisting of the base and frame, is moulded in acrylic.

The base is supported on four rubber feet and has a capacity of 940 ml.

The outlet connector on the outer side of the base unit enables connection to the vacuum pump.

The base unit has rubber supports at each corner and four clamps to lock the base and frame together. The rubber gasket, fitted on the inside rim of the base, provides a vacuum seal when the unit is assembled for operation. On one of the short sides there is a collapsable stand which aids the removal of excess liquid.

Note: *Acrylic is not resistant to aromatic or halogenated hydrocarbons, ketones, esters, alcohols over 30%, or concentrated acids over 25%.*

Silicone tube

Silicone tubing connects the blotting unit to the pump via the liquid-trap 1.5 m of tubing is supplied.

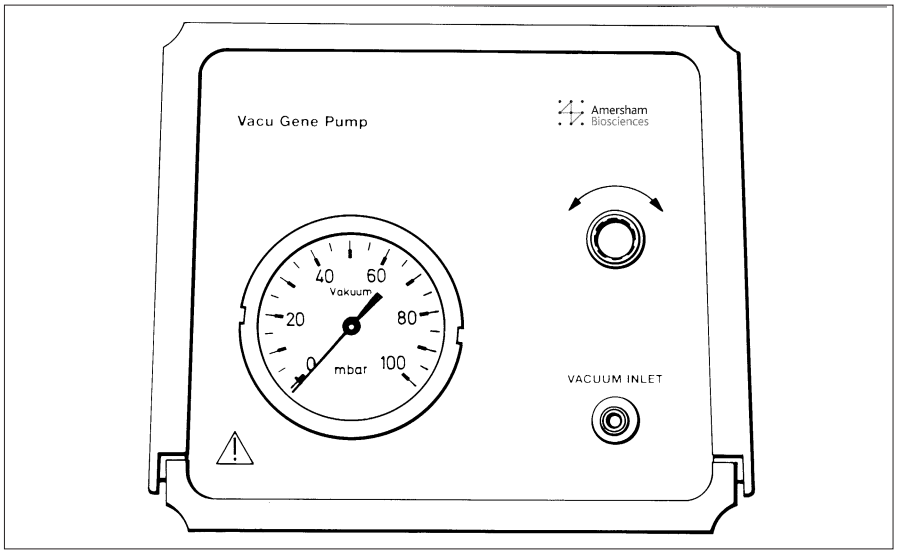
Screen

The screen, manufactured in porous polyethylene, rests on the inner rim of the base unit. The screen acts as a support for the mask, the transfer membrane and the gel.

Mask
The mask, manufactured in 0.15 mm thick polyethylene, is placed over the gasket on top of the screen in the base of the unit. The transfer membrane and the gel are supported in a window in the mask. The mask ensures that the full effect of the vacuum is concentrated on the gel in order to obtain high transfer efficiencies. A packet of 5 masks is supplied.

Liquid trap
The liquid trap consists of a plastic coated glass bottle, a screw lid with two inlets and a pouring ring to be fitted onto the bottle.

2.2 VacuGene vacuum blotting Pump



Vacuum pump
From the inlet connection on the front panel, the low pressure vacuum pump is connected to the base unit to provide continuous evacuation of the base. The needle valve controls the vacuum pressure and the vacuum gauge gives readings in the range 0-100 mbar with an accuracy of ± 5%.

The main power inlet, fuses and main ON/OFF switch are located on the back panel of the vacuum pump.

Main cables
19-2448-01 - European type
19-2447-01 - US type

Fuses
800 mA (European type) 19-3085-01
1.25 A (US type) 19-3749-01

Tubing “T” connector
A “T” connector is provided for the attachment of a second VacuGene XL blotting unit to the vacuum pump, if required.

3. Installation

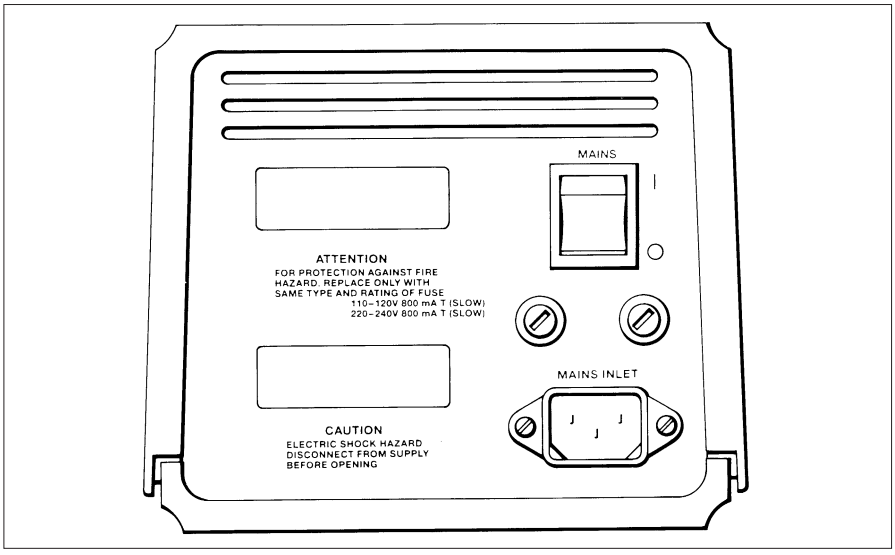
3.1 Unpacking

Unpack the units and accessories. Do not discard any packing materials until each item has been checked against the packing list supplied with the instrument.

Inspect all items for transit damage. If damage has occurred, contact the local Amersham Biosciences office and advise the transport company concerned.

Place VacuGene XL blotting unit together with the liquid trap on a flat, horizontal bench. Place VacuGene XL pump close to the blotting unit within reach of an electrical power point.

3.2 Installing VacuGene XL System

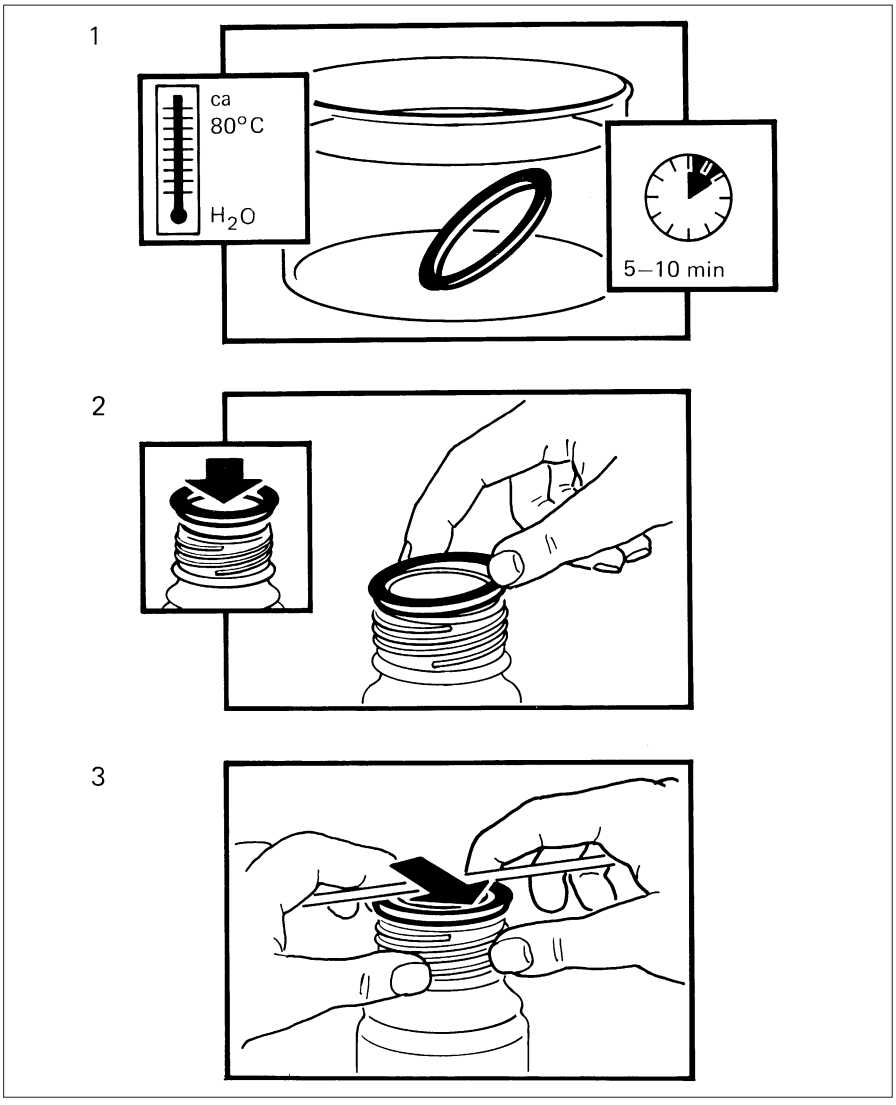


1. Check that the main power switch on the back panel of the pump is OFF, in the 0 position.

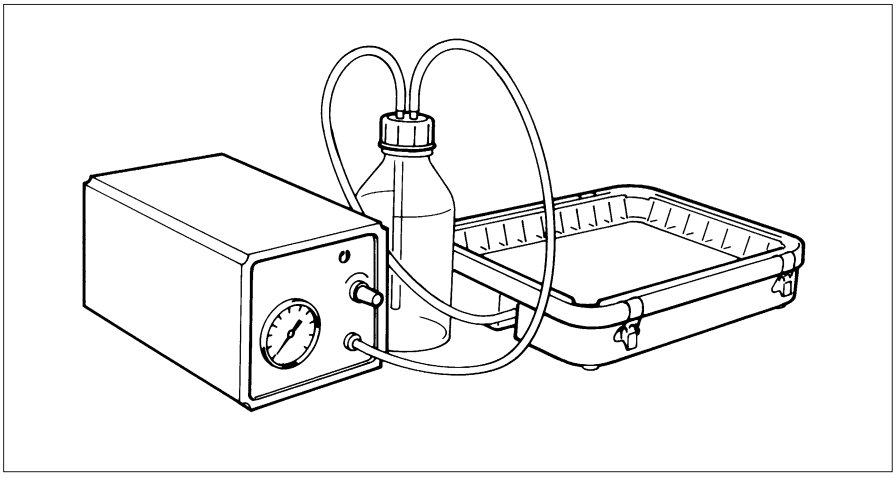
2. Locate the main cable for use with the pump. If necessary, fit a main plug appropriate to the main electricity supply in the laboratory. Follow the colour coding on the cable. Connect the GREEN/YELLOW to the EARTH terminal, the BROWN to the LIVE terminal and the BLUE to the NEUTRAL terminal.

3. Fit the main cable into the main power inlet and connect to the main power supply.

4. Fit the pouring ring on the liquid trap bottle with the sharp edge up as shown in the following figures.



5. Using the silicone tubing supplied, connect the pump to one of the inlets on the liquid trap and connect the blotting unit to the other inlet (on the liquid trap). This will ensure that no liquid will reach the pump. If the T-connector is placed in between the liquid trap and the blotting unit the vacuum can be used to remove excess liquid from the blotting unit.



4. Operation

The protocol below is given as an example. It has been optimised for the transfer of λ-DNA digested with Hind III separated as follows:

Electrophoresis

Gel:	0.7% Agarose NA in 1xTBE, 4 mm thick
Sample:	0.1–0.01 µg λ-DNA digested with Hind III to give fragments in the size range 125-23130 base pairs, and labelled with ^{32}P using Klenow fragment.
Electrophoresis:	HE 33 Mini Submarine, 2 hours at 80 V

Transfer

Membrane:	GeneBind 45 nylon membrane
Transfer efficiency:	>95% for fragments in the size range 2027-23130 bp, according to evaluation of autoradiograms by UltroScan XL and GelScan XL.

Transfer parameters must be optimised for specific applications depending on the sample, gel type etc. (see section 4.5).

Note: *Always use gloves when handling gels, transfer membranes and the screen and mask of VacuGene XL blotting unit.*

4.1 Preparing the mask

Before use, the mask should be cut to make an opening or window for the gel. To achieve adequate sealing of the gel, it is important that the window is slightly smaller than the gel. The gel should overlap the window by between 3 - 10 mm. It is recommended that the overlap should not exceed 10 mm since the gel may float upwards when the solutions are applied, giving a poor seal.

When using gels with open sample wells, such as vertically run gels or horizontally run gels where the wells completely penetrate the gel, it is important that the wells lie over the mask, or poor sealing will result.

For vertical gels, draw a line on the plastic mask 2 mm from the edge of the window. The origin of the samples can then be placed on top of this line when assembling the gel for transfer. The line should be parallel to the edge of the window.

For horizontal gels, the wells can be placed along the edge of the window provided that they are intact. However, if the sample wells are broken, use the same method as above for vertical gels.

4.2 Preparing the transfer membrane

For vacuum blotting, either a nylon, PVDF or a nitrocellulose transfer membrane, with a pore size of 0.45 µm or smaller, can be used.

Pretreat the membrane according to the instructions supplied with the membrane. GeneBind 45 nylon membrane is pretreated by wetting with water and then floating on 20xSSC (transfer solution 4) for 20 minutes.

4.3 Solutions for vacuum blotting

Note: *All chemicals should be UltroGrade or of the highest purity. Double distilled water should be used. All solutions can be recycled.*

1. Depurination solution
   0.25 M HCl

2. Denaturation solution

1.5 M NaCl	87.6 g NaCl
0.5 M NaOH	20.0 g NaOH
	Add distilled water to a final volume of 1 litre. Filter through a 0.45 µm filter before use.

3. Neutralising solution

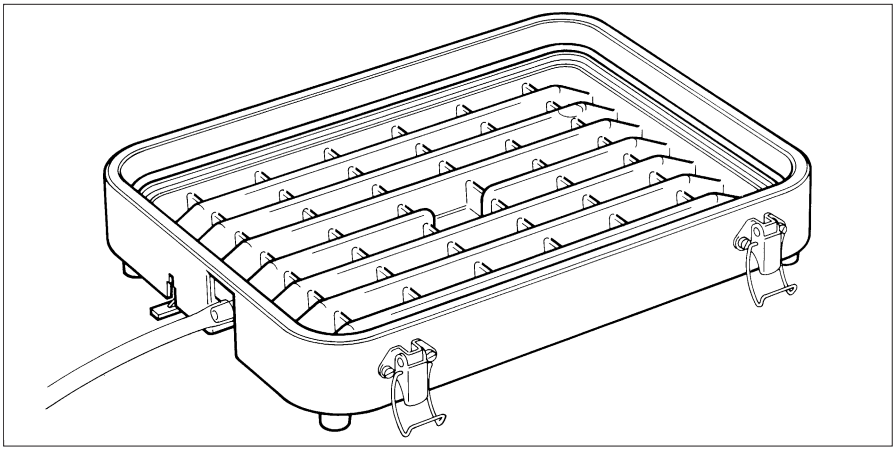
1.0 M Tris	121.1 g Tris
1.5 M NaCl	87 g NaCl
pH 7.5	
	Add to 800 ml distilled water and mix to dissolve. Adjust to pH 7.5 with HCl. Make up to a final volume of 1 litre with distilled water. Filter through a 0.45 µm filter before use.

4. Transfer solution

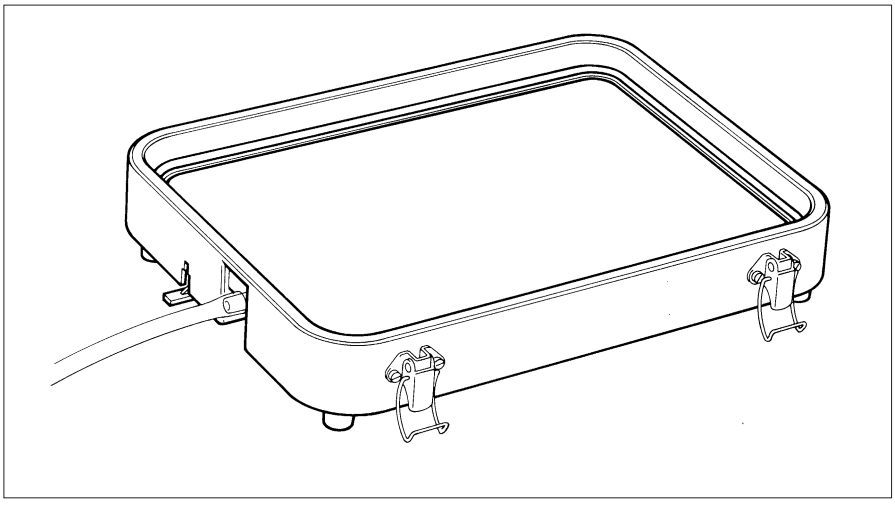
20 x SSC	175 g NaCl
	88.2 g Trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)
	Add distilled water to a final volume of 1 litre. If necessary, adjust to pH 7.0 - 7.2 with concentrated citric acid. Filter through a 0.45 µm filter before use.

4.4 Vacuum blotting

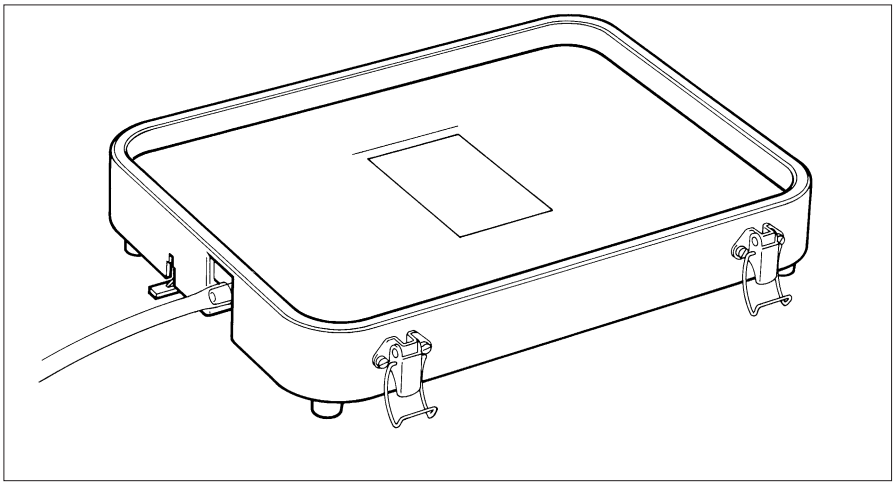
Note: *Always use gloves when handling gels, transfer membranes and the screen and mask of VacuGene XL blotting unit. Care should be taken when handling gels stained with ethidium bromide which is mutagenic.*



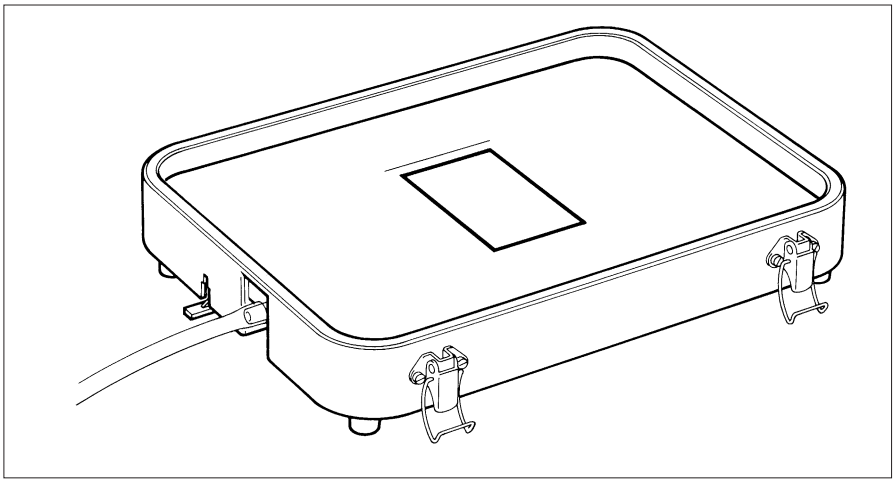
1. The Vacuum blotting unit should be clean, dry and ready for use, with the Vacuum pump and in-line liquid trap connected. Ensure that the rubber sealing gasket is clean and dry. Insert it into the well around the inner rim of the base unit.



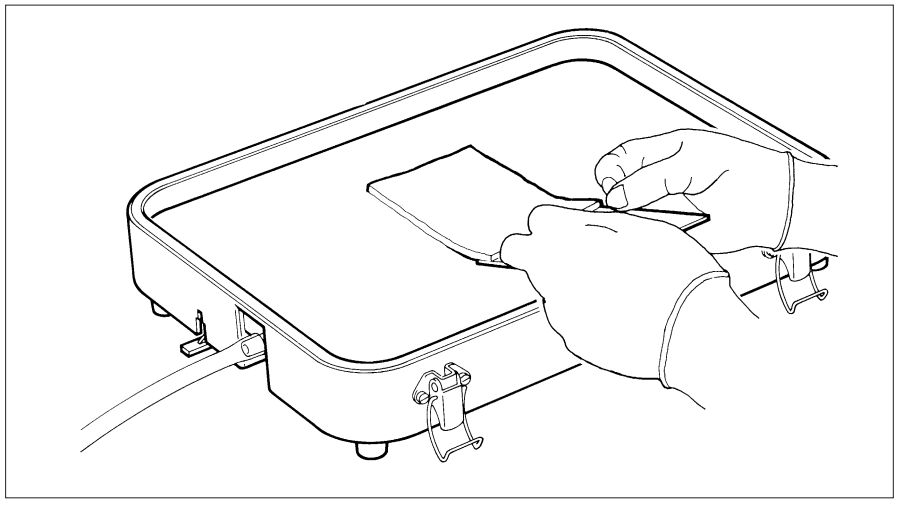
2. Prewet the porous support screen in distilled water and place it on the inner rim of the base unit with the shiny side up.



3. Place the plastic mask with the window on the support screen.



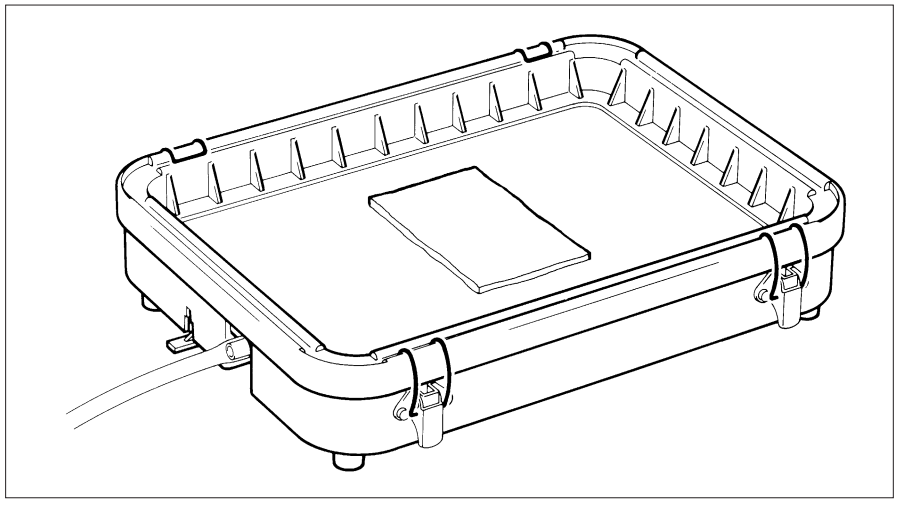
4. Position the pretreated transfer membrane under the mask so that it covers the window in the plastic mask completely. Make sure no air bubbles are trapped in between the membrane and the porous support screen.



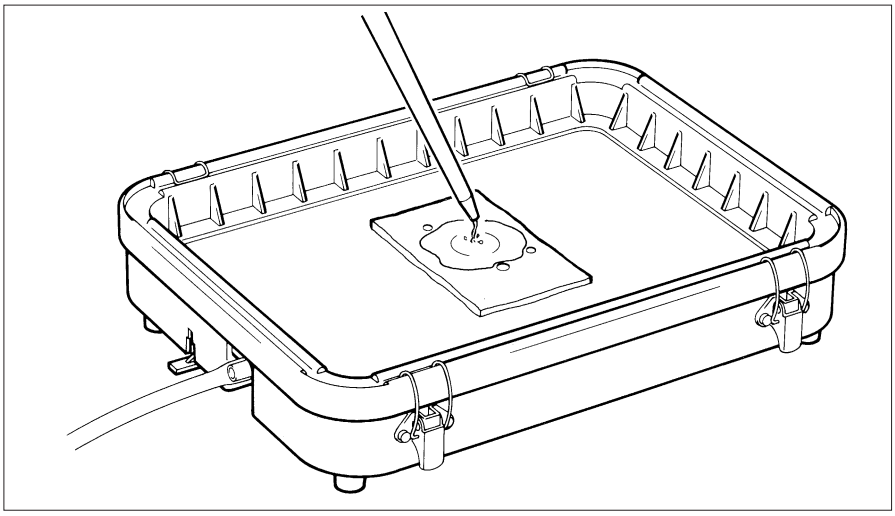
5. Starting with one of the gel edges, gradually slide the gel from the support plate onto the membrane to fill the window. As necessary, position the part of the gel carrying the sample wells accurately on the line drawn on the mask (see Section 4.1).

   Avoid trapping air bubbles between the gel and the membrane.

Note: *With high concentration agarose gels, it is possible to remove trapped air bubbles by gently pushing them outwards with a gloved finger.*



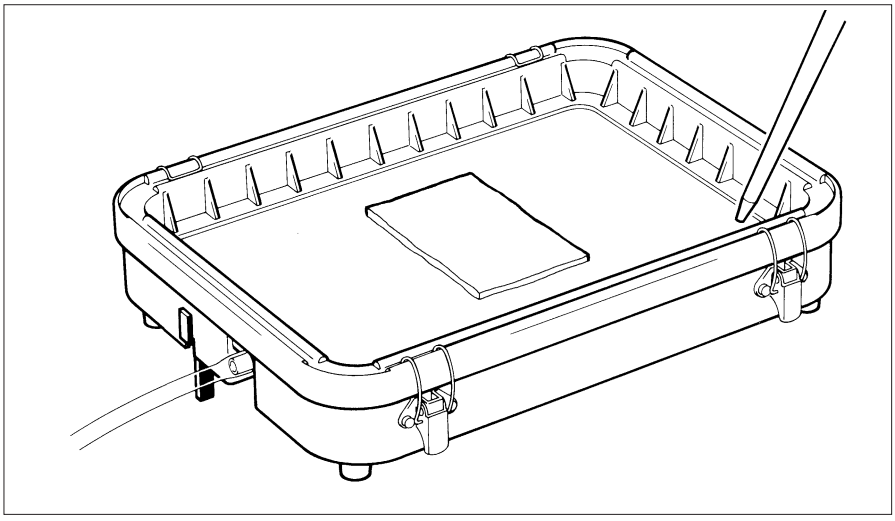
6. Fit the top frame and secure it by tightening the four clamps. Switch on the vacuum pump to immobilize the gel.



7. Without waiting for the vacuum to reach the working level, pour Solution 1, Depurination Solution, onto the centre of the gel. Use enough solution to cover the gel. Leave for 7 minutes. Ensure that the gel remains covered with solution during the treatment.

Note: *To prevent the gel from lifting from the membrane while solutions are being added, it is recommended that solutions are poured gently onto the centre of the gel. If the solutions are poured onto the mask, liquid can easily flood under the gel and cause it to lift upwards. Avoid pouring solution on the fragile sample well area of the gel.*

8. During the depurination step, stabilise the vacuum at 50 mbar. Note: All further steps should be performed at a vacuum pressure of 50 mbar. Depending on the application, transfer may be performed at a vacum pressure in the range 30–50 mbar. If the vacuum is allowed to fall below this range, the transfer efficiency may be impaired; if the vacuum is allowed to increase above this range, the gel can collapse, again resulting in low transfer efficiency. If the recommended vacuum cannot be reached, the rubber sealing ring in the base can be lightly coated with Silicone grease.



9. After 7 minutes tilt the blotting unit using the collapsible stand. Remove residual liquid on the gel by gently wiping over the surface with a gloved finger. Remove liquid from the base unit either by pipette or by using vacuum suction. The vacuum pump can be utilized for this purpose by coupling a t-connector to the tubing between the blotting unit and the liquid trap. Make sure the liquid suction outlet can be closed when not in use.

Note: *All solutions used in vacuum blotting can be re-used since transfer is in one direction only. Check the pH of recycled solutions regularly and discard them when a pH change is observed.*

10. Pour on Solution 2, Denaturation Solution, using enough solution to cover the gel surface. Ensure that the gel remains covered with solution during the treatment. Leave for 7 minutes and then remove completely as before.

Note: *During each treatment of the gel, examine the base unit and the liquid trap for the presence of liquid. There should be little leakage of liquid through the porous screen, providing the gel and membrane have been correctly assembled on the mask. If liquid is present, the resulting transfer efficiency will be greatly reduced.*

11. Pour on Solution 3, Neutralising Solution, as before. Ensure that the gel remains covered with solution during the treatment. Leave for 7 minutes and then remove completely as before.

12. Pour on Solution 4, Transfer Solution to cover the gel to about twice its depth. Leave for 30 minutes. Ensure that the gel remains immersed during this time.

13. Remove the Transfer Solution as before. With the vacuum still on, lift up a corner of the gel and peel it off, leaving the membrane in place. Switch off the vacuum. Remove the membrane and blot it between filter papers.
    Wash the support screen thoroughly to remove salt which can crystallise and block the pores.

Note: *If it is necessary to mark the positions of the tracks, this should be done with a sharp scalpel before removing the gel. Alternatively, if the gel has been stained with ethidium bromide, this can be done later by examining the membrane under UV light.*

4.5 Transfer parameters

The recommendations are given for the separation defined in the introduction to Section 4. The following factors must be considered when optimising transfer parameters for other separations:

- gel thickness
- gel concentration
- substances being transferred
- level of vacuum

As a general rule, transfer from thin gels and from gels of low concentration is fast, while the transfer will be slower from thicker gels and from gels of higher concentration.

The transfer time is also dependent on the molecular weight of the DNA /RNA fragments being transferred, as higher molecular weight molecules take a longer time to move out of the gel. For higher molecular weight fragments, the depurination time can be increased slightly to produce smaller fragments which will transfer more rapidly. Too small fragments might also cause problems due to penetration through the transfer membrane and/or reduction in hybridisation signal. The depurination step can be omitted if the sample contains DNA fragments of less than 10,000 bp.

5. Care and maintenance

After use, dismantle the Vacuum blotting unit.

Rinse the mask and screen in distilled water and dry them with a lint-free towel or tissue. Regularly inspect the mask for damage, especially around the window. Replace the mask when it is torn or damaged in any way.

If necessary, wash the base unit and frame in a mild solution of domestic detergent and then rinse in distilled water. Dry with a paper towel, especially around the rubber gasket.

Note: *The acrylic base and frame is NOT resistant to aromatic or halogenated hydrocarbons, ketones, esters, alcohols over 30% or concentrated acids over 25%.*

Regularly inspect the rubber gasket to ensure that it is correctly seated within the inner rim of the base unit. If necessary, after cleaning apply a thin layer of silicone grease to ensure good sealing.

VacuGene XL vacuum blotting pump requires no regular maintenance.

6. Trouble-shooting guide

Symptom	Possible Cause	Remedy
Low vacuum pressure	Poor sealing	Check that the tubing connections are secure.
		Make sure the clamps are correctly tightened.
		Check that the rubber gasket on the inner ring is seated correctly. If necessary, clean it and apply a light coating of silicone grease (see Section 5, Care and maintenance)
		Ensure that the gel overlaps the mask correctly (see section 4.4) and is in contact with the mask and the transfer membrane
		Check that the mask is not dama ged or torn. If so, replace the mask
	Leaking gel	Inspect gel for cracks or tears, especially around the sample wells
Poor transfer efficiency	Incorrect pretreatment	Check that the gel is correctly seated on the mask
		Check that the four solutions required have been correctly prepared (see Section 4.3)
		Check that the four solutions
	Air bubbles trapped between gel and membrane and/or membrane and porous screen	When positioning membrane and gel, take care to avoid trapping air bubbles (see Section 4.4)
	Incorrect membrane in use or treated incorrectly	Use nylon or nitrocellulose membranes. Follow the manufacturers instructions for membrane pretreatment
	Vacuum too low to draw DNA/RNA out of the gel	Ensure that the vacuum is maintained at a constant 50 mbar
	Vacuum too high causing the pores of the gel to collapse and trap DNA/RNA within the gel	Ensure that the vacuum is maintained at a constant 50 mbar
	Salt in the blotting solutions has blocked the pores of the support screen	Wash the support screen thoroughly

7. Further reading

Current protocols in Molecular Biology. Eds. F.M. Ausubel et al. John Wiley and Sons, New York. 1990.

Genomic Sequencing, Proc. Natl. Acad. Sci. *USA, 81, 1991, 1984,* Church, G. M. and Gilbert, W.

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Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose, *Gene Anal. Techn, 1, 3, 1984,* Johnson, D. A. , Gautsch, J. W. , Sportsman, J. R. , Elder, J. H.

Optimised hybridization of DNA blotted and fixed to nitrocellulose and nylon membranes, *Biotechnology, 5, 165, 1987,* Khandjian, E. W.

Detection of specific sequences - the Southern Transfer, in *"Methods in Molecular Biology", Vol. 2, 55,* ed J. M. Walker Humana Press, Clifton, New Jersey, 1984, Mathew, C. G. P.

Hybridisation of nucleic acids immobilised on solid supports, *Anal. Biochem. ,138, 267, 1984,* Meinkoth, J. and Wahl, G.

Molecular cloning. A laboratory manual. Sambrook, J., Fritsch, E.F., and Maniatis, T. Cold Spring Harbour Laboratory Press, USA. 1989.

Gel electrophoresis of DNA, in "Gel electrophoresis of nucleic acids: a practical approach", eds D. Rickwood and B. D. Holmes, *IRL Press, Oxford, Washington DC, 1982,* Sealey, P. G. and Southern, E. M.

Detection of specific sequences among DNA fragments separated by gel electrophoresis, *J. Mol. Biol. , 98, 503, 1975,* Southern, E. M.

8. Accessories, chemicals and consumables

The following items used in vacuum blotting are available from Pharmacia LKB Biotechnology AB.

Designation	Code No.	Pieces
Accessories		
Gel supporting screen	18-1010-43	
Mask	18-1010-44	pkg/5
Silicone O-ring	80-1300-51	
Silicone tubing	18-1010-45	
Liquid trap	18-1010-96	
Chemicals and Consumables		
Nitrocellulose membrane 45 µm, 150x200 mm	80-1098-90	pkg/15
Nitrocellulose membrane 0.20 µm, 150x200 mm	80-1098-91	pkg/15
ProBind 45, Nitrocellulose membrane roll, 0.45 µm, 20x300 cm	80-1247-86	
GeneBind 45, nylon membrane roll, 0.45 µm 20x300 cm	80-1247-87	
Agarose NA	17-0554-02	100g
Agarose NA	17-0554-03	1000g
Agarose Prep, low melting agarose	80-1130-07	50g
Tris	17-1321-01	500g
EDTA	17-1324-01	100g
Boric acid	17-1322-01	500g
Ethidium bromide	17-1328-01	10ml
Bromophenol Blue	80-1129-15	10g

9. Technical specifications

VacuGene XL vacuum blotting unit	
Liquid trap volume	940ml
Dimensions (WxDxH)	280 x 380 x 60mm
VacuGene vacuum blotting pump	
Vacuum range	0-100 mbar
Ambient operating temperature	40° C maximum
Mains voltage	100 - 120V, 220-240V, 50/60Hz
Power consumption	46W
Dimensions (WxDxH)	160 x 290 x 150mm

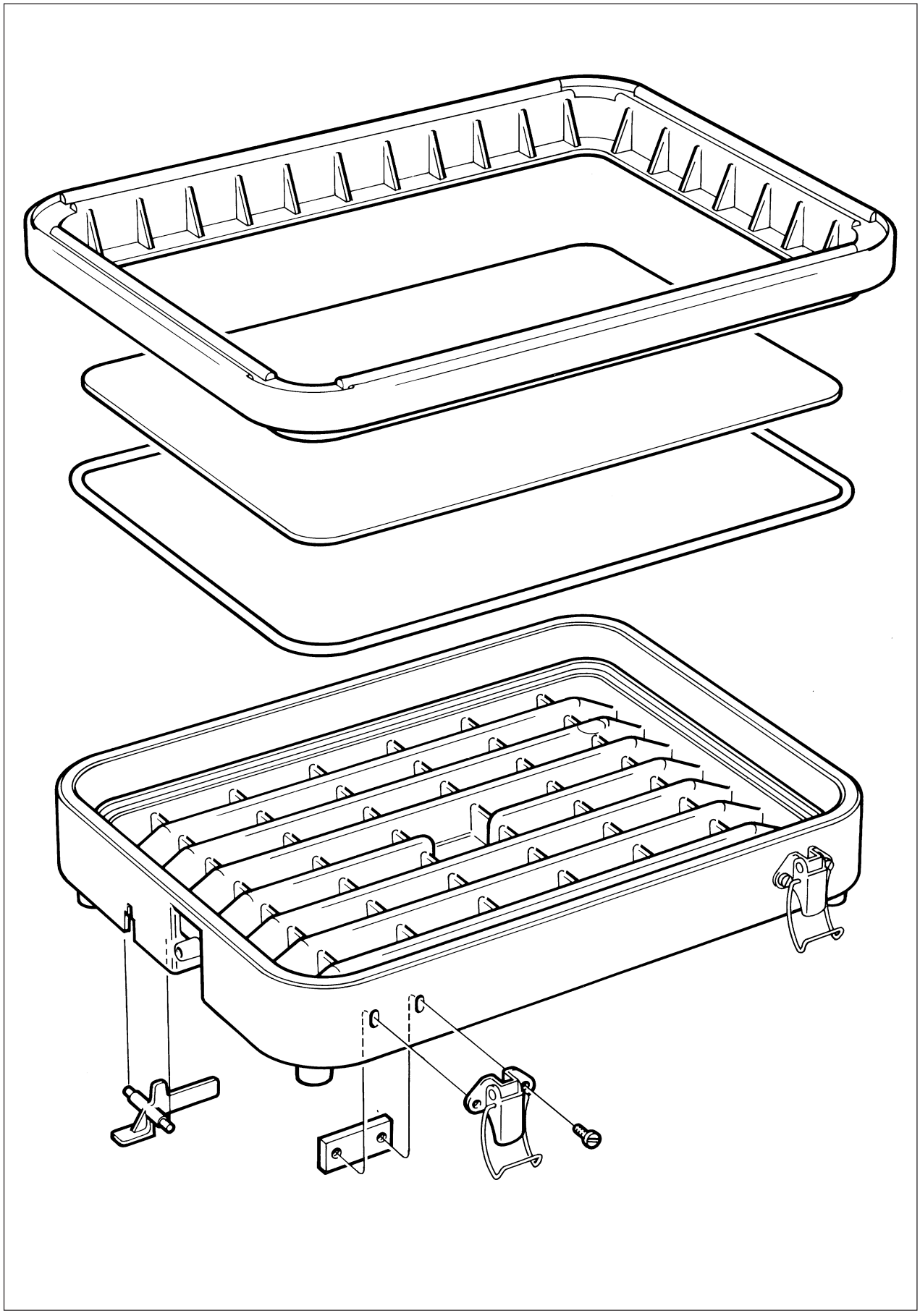
10. Spare parts

VacuGene XL blotting unit

Designation	Code No.
Frame	18-1004-87
Collapsible Stand	18-1004-88
Clamp	18-1004-89

VacuGene pump unit

Designation	Code No.
Fuse 800 mA (European type)	19-3085-01
Fuse 1.25 A (US type)	19-3749-01



11. VacuGene XL Protocol No. 1.

Hydrochloric acid Depurination and Vacuum Transfer of High Molecular Weight DNA

Introduction

The protocol below is given as an example. It has been optimised for the transfer of Guide Line ϕ×-174 Ladder (in the size range 5.4–134.7 kb) separated by pulsed-field gel electrophoresis as follows:

Electrophoresis

Gel:	1.2% Agarose NA in 0.5xTBE, 4 mm thick
Sample:	0.3 µg Guide Line ϕ×-174 Ladder labelled with ^{32}P using Klenow fragment.
Electrophoresis:	Gene Navigator, separation parameters according to Application Note "Preparation and separation of *Borrelia burgdorferi* plasmid DNA", product no. 18-1030-06

Transfer

Membrane:	GeneBind 45 nylon membrane
Transfer efficiency:	Average of 80% for fragments in the size range 5–70 kb, according to evaluation of autoradiograms by UltroScan XL and GelScan XL. The transfer of larger fragments could not be measured due to poor labelling efficiency.

The transfer achieved is sufficient for most applications but can be improved by increasing depurination and transfer times. Transfer parameters must be optimised for specific applications depending on the sample, gel type etc. (see section 4.5).

Procedure

1. Connect the pump inlet on the front panel to the liquid trap and connect the trap to the base of VacuGene XL blotting unit.

2. Pre-treat the nylon membrane as recommended and place it on the porous screen (nitrocellulose membranes can be sensitive to the NaOH solution). Place the plastic mask on the membrane in a way that it overlaps on each side of the membrane by approximately 5 mm.

b. Fit the top frame and secure it by tightening the four locking clamps.

3. Load the gel on VacuGene XL: Gels larger than VacuGene XL blotting unit should be cut to the right size. (Cut the top of the gel above the sample wells.)

a. Starting with one of the gel edges, let it gradually slide onto the membrane. Avoid trapping air bubbles. Make sure that gel and mask overlap by at least 2 mm.

b. Small cracks in the gel must be filled with melted agarose to get a good seal. (All leakages can be sealed with agarose, preferably low melting point agarose).

4. Switch on VacuGene XL pump. Stabilize the vacuum at 50 mbar. The gel must always be covered with solution when the vacuum is on.

Note: *During Depurination Denaturation and Neutralization, only the gel is covered with solution - the blotting chamber is not flooded.*

5. Depurination: Immediately pour about 50ml (depending on gel size) of solution I onto the gel with a pipette. Leave for 20 minutes.

   Tilt the blotting unit using the collapsible stand.

   Remove residual liquid on the gel by gently wiping over the surface with a gloved finger. The excess liquid is removed by using a pipette or by vacuum aspiration.

6. Denaturation: Immediately pour about 50 ml of solution II onto the gel with a pipette. Leave it for 20 minutes. Remove solution II by using the technique described above.

7. Neutralization: Pour about 50 ml of solution III onto the gel with a pipette. Leave it for 20 minutes. Remove solution III.

8. Transfer: Pour solution IV onto the gel, enough to cover the gel to about twice its depth (the blotting chamber is flooded).

   Transfer for 60 minutes. Ensure that the gel remains immersed during this time. Remove the transfer solution.

Note: *If you pour solution beside the gel it may lift from the membrane.*

9. Turn off the pump. Mark the wells. Remove the gel.

10. Wash the filter in solution IV for 10 min. to eliminate agarose. Air dry the filter for 30 minutes. GeneBind 45 nylon membrane does not require baking in a vacuum oven. If another membrane is used then heat the membrane or expose to UV light according to the manufacturers instructions.

Solutions

I Depurination	0.2 N HCl
II Denaturation	0.5 M NaOH, 1.5 M NaCl
III Neutralization	1 M Tris pH 7.5, 1.5 M NaCl
IV Transfer	20 x SSC

12. VacuGene XL Protocol No. 2

UV Pre-treatment and Vacuum Transfer of High Molecular Weight DNA

Introduction

VacuGene XL has greatly reduced the amount of time required for the transfer of DNA and RNA to different membranes. The transfer time of high molecular weight DNA can also be aided by pre-treatment with UV irradiation. The following describes the steps involved in this transfer.

The protocol outlined below can be applied to any high molecular weight DNA. Modification will be necessary due to variability of UV light sources and thickness of gels. Please note whether you have a 254 nm or a 302 nm UV table. The times for UV pretreatment and transfer are given as a guide only and should be optimised for the particular application in question (see Section4.5).

Procedure

1. Connect the pump inlet on the front panel to the liquid trap and connect the trap to the base of VacuGene XL blotting unit. Place the porous support screen on the inner rim of the base unit.

2a. Pre-treat the nylon membrane as recommended and place it on the porous screen (nitrocellulose membranes can be sensitive to the NaOH solution). Place the plastic mask on the membrane in a way that it overlaps on each side of the membrane by approximately 5 mm.

b. Fit the top frame and secure it by evenly tightening the four locking clamps.

3. Nick the DNA before the transfer by exposing the gel to UV light at 302 nm for 10 minutes.

Note: *Each UV table has to be calibrated! Light intensity decreases with age of light tubes. For a 254 nm UV table the exposure is approximately half the time compared to 302 nm.*

4. Load the gel on VacuGene XL: Gels larger than VacuGene XL blotting unit should be cut to a suitable size. (Cut the top of the gel above the sample wells.)

a. Starting with one of the gel edges, let it gradually slide onto the membrane. Avoid trapping air bubbles. Make sure that the gel and mask overlap by at least 2 mm.

b. Small cracks in the gel must be filled with melted agarose to get a good seal.

5. Switch on VacuGene XL pump. Stabilize the vacuum pressure between 50 and 55 mbar. The gel must always be covered with solution when the vacuum is on. Add more solution if necessary.

6. Denaturation: Immediately pour about 50 mL of solution II onto the gel with a pipette. Leave it for 20 minutes. Tilt the blotting unit using the collapsible stand.

   Remove residual liquid on the gel by gently wiping over the surface with a gloved finger. The excess liquid is then removed by using a pipette or by vacuum aspiration.

7. Neutralization: Pour about 50ml of solution III onto the gel with a pipette. Leave it for 20 minutes. Remove solution III by using the technique described above.

8. Transfer: Pour Solution IV onto the gel, enough to cover the gel to about twice its depth (the blotting chamber is flooded). Transfer for 1 hour. Ensure that the gel remains immersed during this time. Remove the transfer solution by using a pipette or vacuum aspiration.

Note: *If you pour solution beside the gel it may lift from the membrane.*

9. Turn off the pump. Mark the wells. Remove the gel.

10. Wash the filter in Solution IV for 10 min. to eliminate agarose. Air dry the filter for 30 minutes. GeneBind 45 nylon membrane does not require baking in a vacuum oven. If another membrane is used then heat the membrane or expose to UV light according to the manufacturers instructions.

Solutions-Contents

II Denaturation	0.5 M NaOH, 0.5 M NaCl
III Neutralisation	1 M Tris pH 7.5, 1.5 M NaCl
IV Transfer	20 x SSC

13. VacuGene XL Protocol No. 3

Alkaline Vacuum transfer of DNA

Introduction

VacuGene XL blotting system uses a low pressure vacuum to transfer DNA and RNA to membranes. This protocol describes an alkaline method for the transfer of DNA using VacuGene XL.

The following protocol describes the transfer of DNA previously separated by submarine electrophoresis. In this protocol we recommend using an alkaline solution for the transfer. This means that no separate denaturation step and no baking or UV cross-linking is needed. The times are given as a guide only and should be optimised for the particular application in question (see Section 4.5).

Procedure

Setting up

1. Connect the pump inlet on the front panel to the liquid trap and connect the trap to the base of VacuGene XL blotting unit.

2 a. Pre-treat the nylon membrane as recommended and place it on the porous screen (Note: nitrocellulose membranes are sensitive to the NaOH solution). Place the plastic mask on the membrane in such a way that it overlaps each side of the membrane by approximately 5 mm.

b. Place the frame on top of the unit and tighten the four locking clamps.

Loading the unit

3. Gels larger than VacuGene XL blotting unit should be cut to a suitable size. The cut should be made at the top of the gel above the sample wells.

a. Starting with one of the gel edges, gradually slide the gel onto the membrane. Avoid entrapping air bubbles. Make sure that the gel and mask overlap by at least 2 mm.

b. Small cracks in the gel must be filled with melted agarose to ensure a good seal. (All leakages can be sealed with agarose - preferably low melting point agarose).

4. Switch on VacuGene XL pump. Stabilize the vacuum between 50 and 55 mbar. The gel must always be covered with solution whenever the vacuum is on.

5. Depurination: Immediately pour about 50 ml (depending on gel size) of Solution I onto the gel with a pipette. Leave until the bromophenol blue turns yellow (about 20 min) or UV illuminate the gel for 5 min using a low energy UV table (302 nm)
   e. g. MacroVue transillumination. The blotting unit can be tilted using the collapsible stand when removing liquid. Wipe the gel over the surface with a gloved finger and remove the excess liquid by using a pipette or by vacuum aspiration.

6. Transfer: Immediately pour enough Solution II onto the gel to cover it to about twice its depth (the blotting chamber is flooded). Transfer for 1-1,5 hours, depending on the size of DNA.

   Make sure that the gel remains immersed all the time. Remove transfer solution by using a pipette or vacuum aspiration.

Note: *If you pour solution alongside the gel, the gel may lift from the membrane!*

7. Turn off the pump. Mark the wells. Remove the gel.

8. Wash the filter in Solution III for 10 min to eliminate agarose. Air dry the filter for 30 minutes.

Solutions-Content

I Depurination solution	0.2 M HCl
II Alkaline buffer	1 M NaOH
III Wash solution	2 x SSC

14. VacuGene XL Protocol No.4

Vacuum transfer of RNA

Introduction

VacuGene XL blotting system uses a low pressure vacuum to transfer RNA and DNA to membranes. This protocol describes a method optimized for transfer of RNA. Previously separated by electrophoresis in a formalin or a glyoxal buffer. Here we recommend using 20 x SSC for the transfer. Researchers have also had good results with phosphate buffer.

The times are given as a guide only and should be optimised for the particular application in question (see Section 4.5).

Procedure

Setting up

1. Connect the pump inlet on the front panel to the liquid trap and connect the trap to the base of VacuGene XL blotting pump.

2 a. Soak the nylon membrane in transfer buffer and place it on the porous screen (nitrocellulose membranes are sensitive to the NaOH solution). Place the plastic mask on the membrane in such a way that it overlaps each side of the membrane by approximately 5 mm.

b. Place the frame on top of the unit and tighten the four locking clamps.

Loading the unit

3. Gels larger than VacuGene XL blotting unit should be cut to a suitable size. The cut should be made at the top of the gel above the sample wells.

a. Starting with one of the gel edges, gradually slide the gel onto the membrane. Avoid entrapping air bubbles. Make sure that the gel and mask overlap by at least 2 mm.

b. Small cracks in the gel must be filled with melted agarose to ensure a good seal. (All leakages can be sealed with agarose - preferably low melting point agarose).

4. Switch on VacuGene XL pump. Stabilize the vacuum between 50 and 55 mbar. The gel must always be covered with solution whenever the vacuum is on.

5.* Immediately pour enough water onto the gel to cover the gel (the chamber is not flooded). Leave for 5 minutes. Tilt the blotting unit using the collapsible stand. Remove residual liquid on the gel by gently wiping over the gel surface with a gloved finger. The excess liquid is then removed by using a pipette or by vacuum aspiration.

6.* To improve the transfer the RNA can be partially hydrolysed by alkaline hydrolysis. Pour the alkaline solution II onto the gel and leave it for 5 minutes. Remove the excess liquid by using the technique described above.

7.* Neutralize the gel by adding solution III onto the gel. Leave it for 5 minutes. Remove the excess liquid as described above.

8. Immediately pour the transfer solution IV (or V) onto the gel to cover it to about twice its depth (the blotting chamber is flooded). Transfer for 30 minutes or longer depending on type of RNA and if partial hydrolysis was used. Make sure that the gel remains immersed all the time. Remove the remaining liquid by using a pipette or vacuum aspiration.

Note: *If you pour solution alongside the gel, the gel may lift from the membrane.*

9. Turn off the pump. Mark the wells. Remove the gel.

10. Wash the filter in transfer buffer for a maximum of 5 minutes.

Note: *RNA may be lost from the membrane if the wash is to long.*

11. Dry the filter in room temperature 30 minutes. Bake the filter for 1 to 2 hours or treat it with UV light depending on which membrane that is used.

* these steps are not needed for glyoxylated RNA.

Solutions-Recipe

I	Distilled water	
II	50 mM NaOH, 10 mM NaCl	
III	0.1 M Tris-Cl pH 7.4	
IV	20 x SSC	175 g NaCl 88.2 g Tri-sodium citrate in 800 ml. Adjust pH to 7.0 and volume to 1 liter.
V	Phosphate buffer	Mix 64.8 ml of 1M NaH_2PO_4 with 35.2 ml of 1M NaH_2PO_4 and dilute to 1 litre. The pH should be 6.6.

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