GE Healthcare

Change of material and Corrections to the Instruction Manual

The Support Screen used with VacuGene™ XL has been changed to a more porous version with improved performance. The information below gives guidelines for the use of the new screen, based on the existing manual (80-1300-50). In addition, corrections for a number of errors in the manual are given.

Section 4. Operations

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

Sample:	0.01-0.1 µg λ DNA digested with Hind III to give fragments in the size range 125-23130 base pairs, and labelled with 32p by an end-fill reaction using Klenow fragment.	
Electrophoresis		
Gel:	0.7 % Agarose NA in 1 x TBE, 4 mm thick	
Electrophoresis:	GNA-100, 2 hours at 80 V	
Transfer		
Membrane:	GeneBind 45 nylon membrane	
Vacuum pressure:	50 mbar	
Blotting times:	Depurination	7 mins
	Denaturation	7 mins
	Neutralisation	7 mins
	Transfer	30 mins
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 of the manual).

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WARNING: When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

The instructions for making up neutralising solutions are as follows:

1.0 M Tris	121.1 g Tris
1.5 M NaCl	87.6 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 before use through a 0.45 μm filter.

page 11. Replace “see Section 5. A” by “see Section 4.1”

Section 6. Trouble-shooting guide

page 16. Replace “see Section 6. Maintenance” with “see Section 5 Care and Maintenance”.
Replace “see Section 5A” with “see Section 4.4”
Replace “see Section 5C” with “see Section 4.3”
Replace “see Section 5D” with “see Section 4.4”

Section 11. VacuGene XL Protocol No. 1

The protocol below is given as an example. It has been optimised for the transfer of Guide Line ΦX-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 0.5 x TBE, 4 mm thick
Sample:	0.3 µg Guide Line ΦX-174 Ladder labelled with 32p by an end-fill reaction 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	
Vacuum pressure:	50 mbar	
Blotting times:	Depurination	20 mins
	Denaturation	20 mins
	Neutralisation	20 mins
	Transfer	60 mins

Transfer efficency:	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 efficency.

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

Sections 12, 13 and 14: Protocols Nos. 2,3 and 4.

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

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