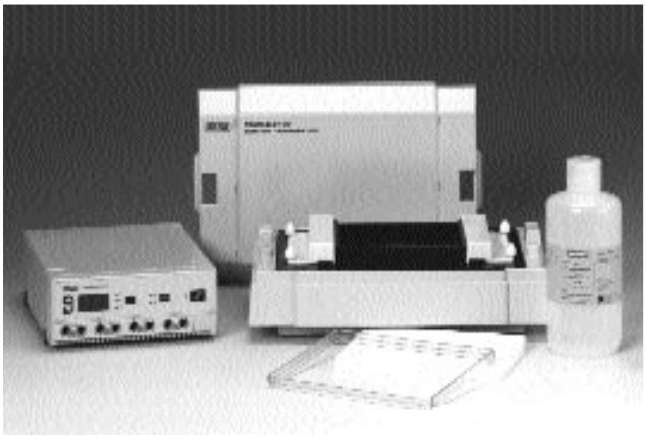
US/EG Bulletin 1714

DNA/RNA Blotting with the Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell



The Trans-Blot SD DNA/RNA agarose gel support frame, used with the Trans-Blot SD semi-dry electrophoretic transfer cell, allows blotting of DNA/RNA from agarose gels to nylon membrane in minutes, without any gel pretreatments. The Trans-Blot SD semi-dry electrophoretic transfer cell generates a high field strength (V/cm^2) which facilitates rapid transfers. The blotting frame allows highly efficient transfers by eliminating crushing of agarose gels.

DNA Transfers in 10 Minutes

PCR* fragments, plasmid, and vector DNA ranging in size from several hundred bases to 15 kilobases can now be quickly transferred in as little as 10 minutes. Set-up time is reduced to just minutes because hydrolyzation of DNA fragments is not required prior to transfer.

RNA Transfers in 30–35 Minutes

The agarose gel support frame can also be used to transfer RNA up to 3.5 kb in 30–35 minutes, although transfer of 28s rRNA is equally efficient as determined by EtBr staining.[1]

Northern and Southern Blotting

The agarose gel support frame will accommodate 6 mm thick agarose gels up to 15 x 20 cm. Table 1 shows the volumes of agarose required for each gel size.

Table 1. Agarose Volumes

Gel Dimensions (cm)	Volume Agarose (ml)
7 x 10	42
10 x 15	90
15 x 15	135
15 x 20	180

For convience, 10x TBE and TAE premixed buffers are available. Extra thick, 2.6 mm, filter paper is necessary to provide enough buffer for transfer. The extra thick blot paper must be identical to the dimensions of the agarose gel. Bio-Rad's extra thick blot paper is available in four sizes: 7.5 x 10 cm, 10 x 15 cm, 15 x 15 cm, and 15 x 20 cm. Use of any other type of blot paper will decrease transfer efficiency.

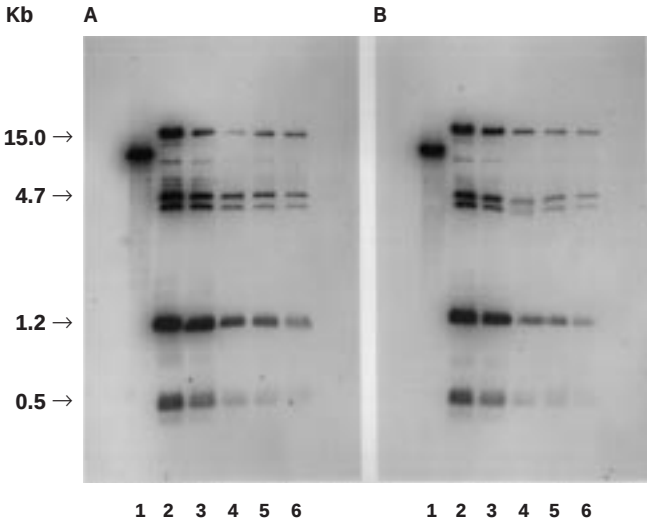


Fig. 1. Trans-Blot SD DNA/RNA blotting kit transfer of lambda DNA fragments. A. 10 minute transfer, **B.** 25 minute transfer of digests of lambda DNA (NEB) and pPL-lambda (left promoter of lambda, Pharmacia). Fragment sizes were 15 kb, 4.7 kb, 1.2 kb, and 0.5 kb. Lanes contain: **1.** DNA high range standard (Bio-Rad); **2.** 100 ng of lambda DNA digest; **3.** 50 ng; **4.** 10 ng; **5.** 1 ng; **6.** 500 pg. DNA was electrophoresed in a 0.7% agarose TBE gel, stained, and blotted in the Trans-Blot SD semi-dry electrophoretic transfer cell. Blots were hybridized (7% SDS, 0.5 M $NaPO_4$, pH 8.0, 1mM EDTA) overnight at 65 °C with labeled probe (1 x 10^6 cpm/ml of buffer). The probe was produced by random primer labeling (Bio-Rad) of lambda PL promoter (GenBlock, Pharmacia) with 50 µCi of alpha ^{32}P dCTP (3,000 Ci/mM, Amersham) having a specific activity of 8.2 x 10^8 dpm/µg.

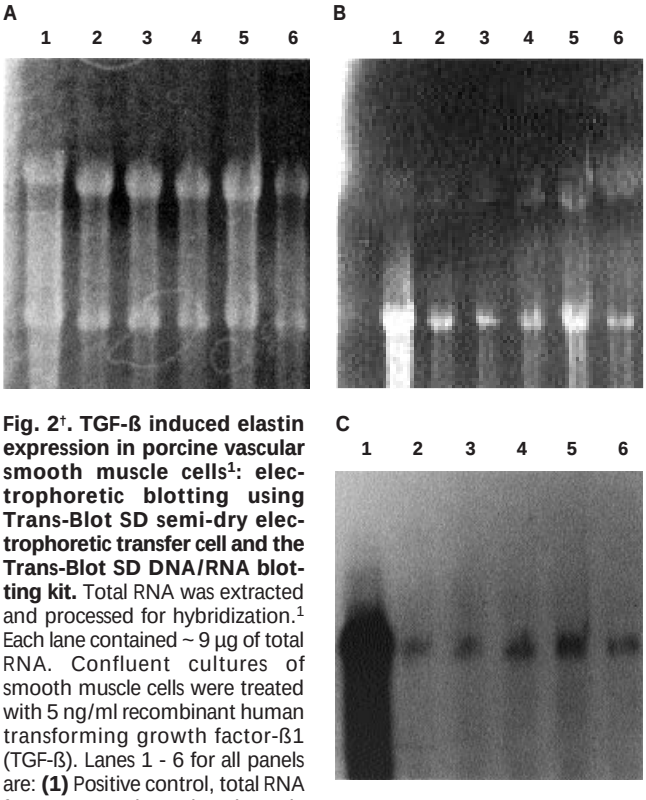


Fig. 2†. TGF-ß induced elastin expression in porcine vascular smooth muscle cells[1]: electrophoretic blotting using Trans-Blot SD semi-dry electrophoretic transfer cell and the Trans-Blot SD DNA/RNA blotting kit. Total RNA was extracted and processed for hybridization.[1] Each lane contained ~ 9 µg of total RNA. Confluent cultures of smooth muscle cells were treated with 5 ng/ml recombinant human transforming growth factor-ß1 (TGF-ß). Lanes 1 - 6 for all panels are: **(1)** Positive control, total RNA from neonatal porcine thoracic aorta; **(2)** 0 hr after TGF-ß; **(3)** 2 hr after TGF-ß; **(4)** 6 hr after TGF-ß; **(5)** 8 hr after TGF-ß; **(6)** 12 hr after TGF-ß. **A.** Gel after EtBr staining and 0.5x TBE equilibration. **B.** Membrane after 35 minute electrophoretic blotting at 3 mA/cm^2. The differential transfer seen between the 28s and 18s ribosomal bands can be lessened by extending the run 5–6 min. **C.** Autoradiograph of membrane filter hybridized with a [^{32}P]-cDNA probe for elastin. Numbers indicate arbitrary densitometric units.

References

1. Lane, K., Zoia, O. and Davidson, J. M., *Biotechniques,* **12**, 3, 340–346 (1992).
2. Trans-Blot Instruction Manual.

Ordering Information

Catalog Number	Product Description
Support Frame	
170-4019	**Agarose Gel Support Frame,** 16 x 21 cm
DNA/RNA Blotting Kit Accessories	
170-3965	**Extra Thick Blot Paper,** 7.5 x 10 cm, 60
170-3958	**Extra Thick Blot Paper,** 10 x 15 cm, 30
170-3959	**Extra Thick Blot Paper,** 15 x 15 cm, 30
170-3960	**Extra Thick Blot Paper,** 15 x 20 cm, 30
Trans-Blot SD Electrophoretic Transfer Cells	
170-3940	**Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell,** complete unit
170-3948	**Trans-Blot SD System,** 100/120 V, includes Trans-Blot SD semi-dry electrophoretic transfer cell and PowerPac 200 Power Supply
170-3949	**Trans-Blot SD System,** 220/240 V
Power Supplies	
165-5052	**PowerPac 200 Power Supply,** 100/120 V
165-5053	**PowerPac 200 Power Supply,** 220/240 V
Zeta-Probe® GT Blotting Membrane	
162-0191	**Sheets,** 10 x 15 cm, 15
162-0192	**Sheets,** 15 x 15 cm, 15
162-0193	**Sheets,** 15 x 20 cm, 15
Premixed Buffers	
161-0733	**10x Tris/Boric Acid/EDTA,** 1 L
161-0756	**10x Tris/Boric Acid/EDTA,** 6 x 1 L
161-0743	**50x TBS/Acetic Acid/EDTA,** 1 L
161-0759	**50x Tris/Acetic Acid/EDTA,** 6 x 1 L

For more information call your local Bio-Rad representative.

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* Practice of PCR is covered by U.S. patent numbers 4,683,195, 4,683,202, and 4,899,818 issued to Cetus Corporation which is a subsidiary of Hoffmann-LaRoche Molecular Systems, Inc. Purchase of any of Bio-Rad's PCR-related products does not convey a license to use the PCR process covered by these patents; the user of these products to perform PCR must obtain a license.

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