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Performance Certificate

Zeta-Probe® GT Membrane

The basis of the Zeta-Probe GT membrane quality control procedure is to detect the presence of a single-copy gene in 6 µg of *Hin*dIII-digested human genomic DNA. The DNA is transferred to membranes by Southern or alkaline blotting. The membranes are hybridized with a labeled probe generated from a 1.5 kb cDNA insert from the 3' untranslated region (UTR) of the N-*ras* proto-oncogene. This labeled probe hybridizes to a 9.8 kb *Hin*dIII restriction fragment in human genomic DNA.

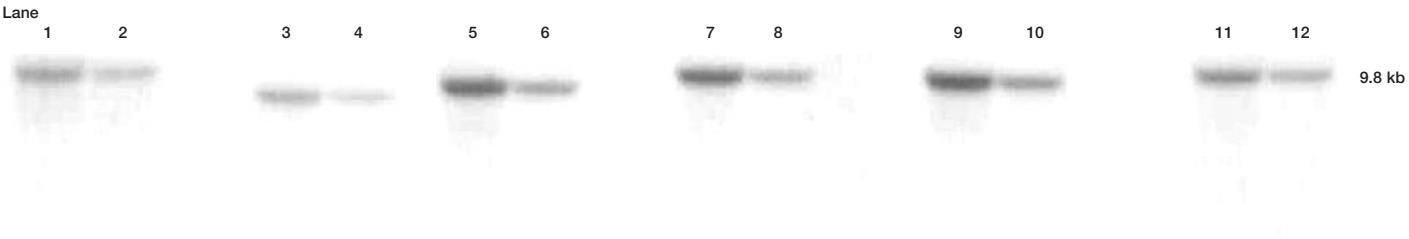
Specifications

The 9.8 kb *Hin*dIII fragment must be visible in the 6 µg genomic DNA load (3 pg of target DNA) after an 18-hour exposure, with no nonspecific binding to the membrane.

Methods

Human genomic DNA (>50 kb) is digested to completion with *Hin*dIII. Two separate 1% agarose/TAE gels are electrophoresed, one for Southern transfer and the other for alkaline transfer. Each gel is loaded with 6 µg and 3 µg of genomic DNA/*Hin*dIII. This represents 3.0 pg and 1.5 pg, respectively, of the target 1.5 kb single-copy sequence contained within the 9.8 kb *Hin*dIII fragment. After electrophoresis, the DNA is depurinated by soaking both gels in 0.25 N HCl for 15 minutes. The Southern transfer gel is soaked for 30 minutes in 0.5 N NaOH, 1.5 M NaCl.

After denaturation, the gel is neutralized in 0.5 M Tris-HCl, 1.5 M NaCl, pH 7.5 for 30 minutes. Once neutralized, the DNA is transferred from the gel to the membranes by capillary action in 10x SSC for 16 hours. The alkaline transfer gel is directly transferred by capillary action in 0.4 N NaOH for 4 hours. The membranes are rinsed briefly in 2x SSC and blotted dry between two sheets of Whatman 3MM paper. The dried membranes are exposed to UV irradiation (5,000 µJ/cm^2) to crosslink the DNA to the membrane. The probe is prepared by random primer labeling of the N-*ras* fragment. The incorporated nucleotides are removed using a preequilibrated spin column. The membranes are prehybridized in 7% SDS, 0.5 M Na_2PO_4, pH 7.2 at 68°C for a minimum of 30 minutes. Labeled probe is added at 1 x 10^6 cpm/ml, and the membranes are hybridized for 16–18 hours. The hybridized membranes are washed in 1x SSC, 0.1% SDS for 10 minutes at room temperature. This solution is replaced with a prewarmed solution of 1x SSC, 0.1% SDS at 68°C, and membranes are washed for 10 minutes at 68°C. This solution is replaced with a prewarmed solution of 0.1x SSC, 0.1% SDS at 68°C, and membranes are washed for 10 minutes at 68°C. After washing, the membranes are sandwiched between plastic wrap and exposed overnight to Kodak BioMax film in cassettes containing intensifying screens.



Alternating 6 µg and 3 µg human genomic DNA digested with *Hin*dIII. Lanes 1 and 2, Southern blot control; lanes 3 and 4, Southern transfer blot 1; lanes 5 and 6, Southern transfer blot 2; lanes 7 and 8, alkaline blot control; lanes 9 and 10, alkaline transfer blot 1; lanes 11 and 12, alkaline transfer blot 2.

Comments: This blot demonstrates hybridization to the 9.8 kb *Hin*dIII restriction fragment from the 6 µg and 3 µg DNA load, representing 3.0 pg and 1.5 pg single-copy DNA sequence.
Probe Data: Specific activity: 1.63 x 10^9 cpm/µg cpm in hybridization solution: 1 x 10^6 cpm/ml

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