Southern hybridization

Genomic DNA (5 µg) was digested at 37°C for 3 hours with restriction enzymes (50 unit of Bbu I, Bgl II, or EcoR I, Promega, Madison, WI). The digested DNA and DNA markers (0.2 µg/lane High Weigh DNA Markers and 0.5 µg/lane 1 kb DNA Markers, Ambion, Austin, Texas) was electrophoresed through agarose gel (1% Seakem Gold, FMC, Rockland, ME). The digested DNA on the gel was transferred to a nylon membrane (Immobion-NY+, Millipore, Bedford, MA). The DNA was downwardly transferred for 1.5 hours by mean of a stack assembly (TurboBlotter, Schleicher & Schuell, Keene, NH) and transfer buffer (20 x SSC). The blotted DNA was crosslinked with UV light (5 mJ/cm^2), and hybridized at 42°C overnight with Digoxigenin(Dig)-labeled probe (0.1 ng/ml) in a commercial hybridization solution (Ultrahyb, Ambion, Austin, Texas). The membrane with hybridized DNA and probe was washed twice at 42°C for 15 minutes in a low stringency solution (2 x SSC, 0.1% SDS) and twice in a high stringency solution (0.1 x SSC, 0.1% SDS).

DIG-labeled DNA probes were synthesized according to the manufacturer's protocol (PCR DIG Probe Synthesis Kit, Boehringer Mannheim, Indianapolis, IN). DIG was incorporated into the probes by PCR. The PCR mixture (50 µl) contained PCR buffer (with MgCl$_2$), enzyme mix (2.6 U), PCR DIG probe synthesis mix (200 µM dATP, 200 µM dCTP, 200 µM dGTP, 130 µM dTTP and 7 µM DIG-11-dUTP), template (0.1 pg) and primer (200 nM each).

The probe targeting the large subunit genes was synthesized according to a template, the conserved coding region of the cDNA encoding Large Subunit A (Table 6). Therefore, the probe detected not only the gene encoding Large Subunit A, but also genes encoding other homologous RNR large subunits.

The probe targeting the small subunit gene was synthesized according to a template, the conserved coding region of the cDNA encoding the small subunit (Table 6). Therefore, the probe could detect not only the gene encoding the small subunit, but also genes encoding other homologous RNR small subunits, if they existed.