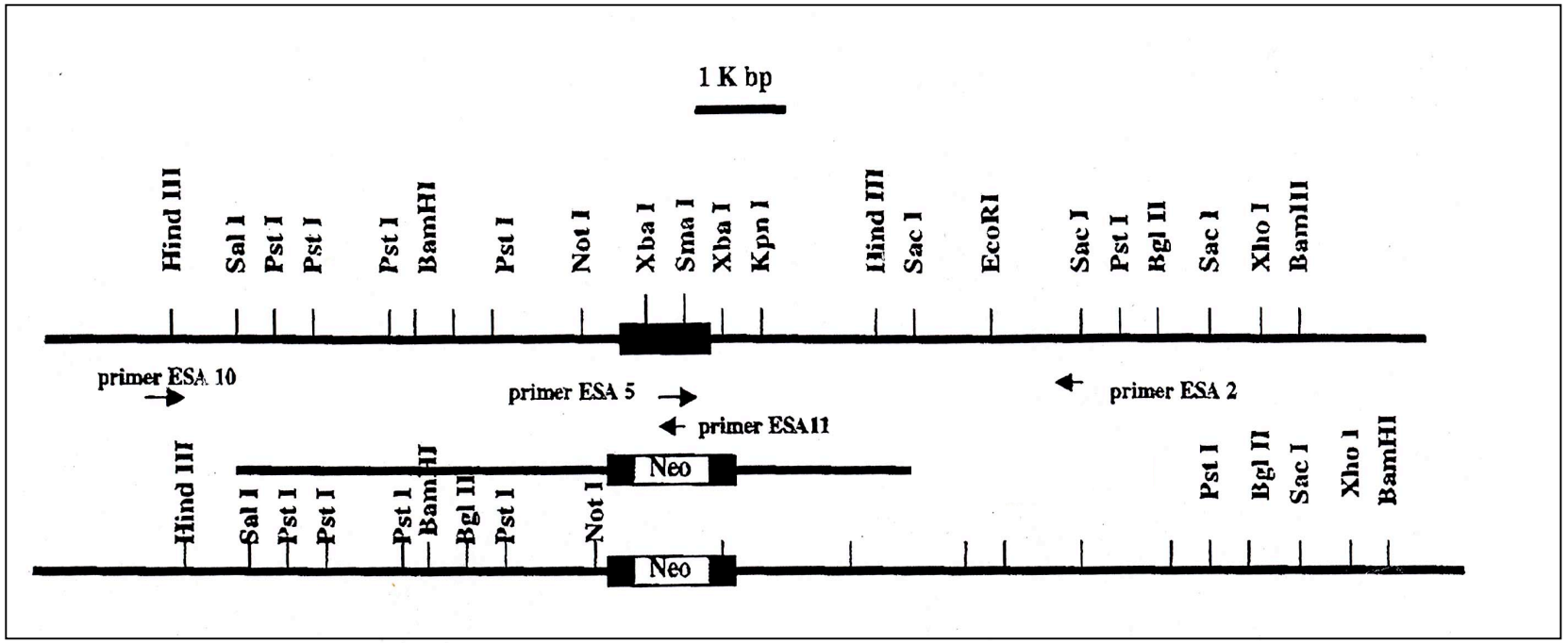
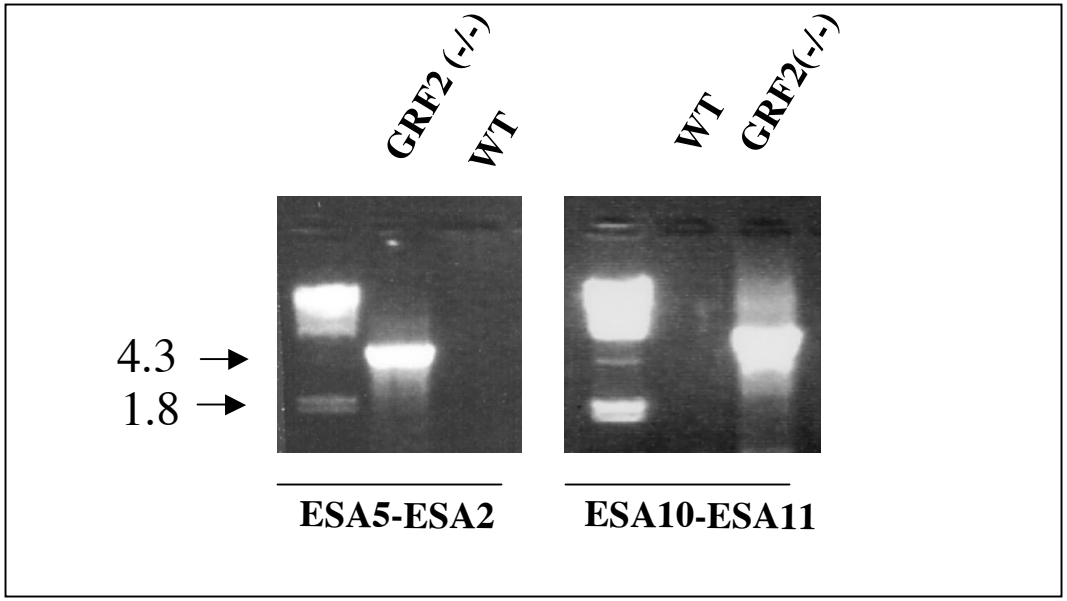
Suppl. Fig 1.

A.



Legend: Targeting vector design. A neomycin gene was inserted into a 10kb Sal/EcoRI fragment containing the first exon of ras-grf2 to generate a targeting vector for homologous recombination. Two pairs of oligonucleotides ESA10/ ESA 11 and ESA2 and ESA5 were used in PCR reactions to confirm that homologous recombination occurred (see Suppl Fig.2). ESA10 is in the region 5' to the recombination site and ESA2 is 3'to this region, while ESA 5 and ESA 11 are in the neo^r cassette.

B.



Legend-Results of PCR on knockout DNA to confirm correct insertion of targeting vector. Left panel-ESA5 and ESA2 primers gave the expected ~4.3Kb band representing knockout DNA beginning outside the targeting vector on the 3' side of the expected recombination site and the DNA in the neocassette, while no band was observed from wild-type DNA because no neo cassette is present. Right panel-An analogous set of results yielding the expected ~4.5Kb band from knockout but not wild-type DNA using a ESA10 on the 5' side of the targeting vector and a
ESA11 within the neo cassette.