A Simplified Ribonuclease Protection Assay For Embryos

(Source: G.M. Kelly and R.T. Moon from Zebrashif Book 5th Edition)

This is a ribonuclease protection assay based on the methods of Thompson and Gillespie (Anal. Biochem. 163:281-291. 1987) and Haines and Gillespie (Biotechniques 12:736-740, 1992). This rapid technique, which eliminates the need to isolate purified RNA, is extremely sensitive such that an overnight exposure is sufficient to detect a wnt1 signal from ten 12-hr embryos (Kelly and Moon, in preparation). In addition, with the generation of a standard curve, this assay can also be used to determine the abundance of a specific transcript.

1. Collect embryos at particular developmental stages in 1.5 ml micro-centrifuge tubes, and remove the medium. Add 45 l of lysis buffer (4 M guanidine thiocyanate, 25 mM sodium citrate and 0.5% sarcosyl) for every ten embryos.

2. Draw embryos and lysis buffer through a 22 gauge needle into a syringe, expel and repeat three times, then vortex and store at -20°C.

3. Centrifuge the embryonic lysates for 1 minute to pellet the broken chorions. For hybridization, transfer 45 l of each sample to tubes containing 1x10^5 cpm of 32P-UTP-labeled antisense probe diluted in 5 l of lysis buffer. Varying the amount of labeled probe may be necessary to optimize signal to background. We prefer the Maxiscript kit available from Ambion, Inc. (Austin, TX) for making the probes.

4. As a positive control and to generate a standard curve, hybridize 45 l of lysis buffer containing either 1.0, 0.1, 0.01, or 0.001 ng of synthetic sense strand RNA with the radioactive antisense probe. Similarly, hybridize the antisense probe with 45 l of lysis buffer containing 10 l of torula tRNA to ensure that the RNase will digest any unprotected single-stranded RNA molecules.

5. After an overnight hybridization at 55°C, mix the sample with 500 l of RNase cocktail (20 l RNase A, Sigma R-5503, previously boiled for 5 minutes, aliquoted and stored at -20°C plus 500 U of RNase T1, Sigma R-1003, in 10 mM Tris-HCl, pH 7.5, 300 mM NaCl and 5 mM EDTA), and incubate at 37°C for 1 hour. Varying the hybridization temperature may have an effect on the sensitivity of the assay, we begin at 55°C and then, if necessary, alter the temperature in 5°C intervals.

To isolate nucleic acids after hybridization, digest the lysate with 10 µl of 20% SDS and 5 l of 20 mg/ml proteinase K, for 45 minutes at 37°C. Add isopropanol (500 µl) and 3 ng of torula RNA to each sample before centrifuging. Air dry the pellets, resuspend in 10 µl of loading buffer (88% formamide, 10 mM EDTA, 1 mg/ml each of xylene, cyanol, and bromophenol blue), heat at 75°C for 4 minutes, then place on ice before loading onto a 5% polyacrylamide/urea/taurine gel (details in the Sequenase kit, US Biochemical). Electrophorese samples next to 2000 cpm of undigested probe and DNA sequencing reactions to provide size markers, and process the gel for standard autoradiography.