Supplementary Material 5

Protocol for Polyadenylation Status Analysis

(Bernhard Dichtl)

Ligation of a synthetic oligonucleotide linker to 3' hydroxyl groups of RNA:

Each reaction contains:

500 ng RNA,
2 μM Linker Oligo
10 μM ATP,
0.1 μg BSA,
1 x ligase buffer
10 units T4 RNA ligase (Fermentas)
H_20 to 10 μl final volume.

Incubate at 4°C overnight (16 h)

Linker Oligo : (5'-phosphate-GATACTACCTCTATGAATTCTTTGCTAGCTACCTGAACTTATCAGACCTAC-3'-amine),

Reverse transcription:

Two microliters of the ligation reaction is used in a final volume of 28 μl for reverse transcription (RT) with BRevOligo (GTAGGTCTGATAAGTTCAGGTAGCT) and 100 units SuperScript II (Invitrogen).

1st PCR:

0.2 μl of the RT reaction is amplified in 50 μl PCR reactions using UpstreamOligo (ATATGACGTTCCAGATTACGCTGC), BRevOligo and 2.5 units Taq DNA Polymerase (NEB)

with temperature profile: 1 x 95°C, 2 min; 30 x (95°C, 15 sec, 45°C, 45 sec, 72°C, 30 sec); 1 x 72°C, 5 min.

Hot nested PCR:

PCR product from above (0.1 – 2.0 μl) is included in a second, nested PCR amplification using

NestedOligo (TCTTTTCTCTTTCCCCATCCTTTAC), ARevOligo (AGCAAAGAATTCATAGAGGTAGTATC)

a spike of ^{32}P-labeled ARevOligo as described above

with temperature profile: 1x 95°C, 2 min; 25 to 30 (95°C, 15 sec, 45°C, 45 sec, 72°C, 30 sec); 1 x 72°C, 5 min.

PCR reactions are precipitated in the presence of 0.3 M NaOAc and 2.5 volumes 100% ethanol. Pellets are dried with open lid at room temperature and denatured for 5 min at 90°C in the presence of RNA loading buffer. Reaction products are separated on 20 cm 6% polyacrylamide/ 8.3 M urea gels, the gels were dried and exposed to Fujifilm BAS-MS imaging plates. The plates are scanned on a FLA 7000 Imaging System (Fujifilm) and analysed using the ImageGauge V4.22 software.