# RNA extraction, reverse transcription and PCR for TMSB4X

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ABSTRACT

Procotol describing RNA extraction from dissected human tissue followed by reverse transcription to cDNA, PCR for detection of TMSB4X and separation of PCR products by agarose gel.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL STATUS

**Working**

1 Following dissection, tissue was placed directly into RNAlater (Ambion). To extract RNA, tissue was removed from the RNAlater, placed in 1 ml TRIzol (Ambion) and ground up using a plastic tissue grinder. Samples were incubated in TRIzol for 5 mins at room temperature and centrifuged down at 12,000 g for 10 mins, transferring the supernatant to a new RNase-free tube, to remove tissue debris.

2 200 µl chloroform was added and samples shaken vigorously for 30 secs, then incubated at room temperature for 15 mins before being centrifuged at 12,000 g for 15 mins. The clear aqueous phase containing the RNA (top layer) was transferred to a new RNase-free tube. 500 µl isopropanol and 1 µl RNA grade glycogen (20 mg/ml, Thermo Scientific) was added and the sample incubated at -80°C for 30 mins.

3 Samples were brought to room temperature and incubated for 15 mins and then centrifuged at 12,000 g for 15 mins at 4°C, to form an RNA pellet.

4 Working on ice, the supernatant was discarded and cell pellets washed twice for 10 mins in pre-chilled (-20°C) 75% ethanol, centrifuging at 7,500 g for 10 mins at 4°C between washes.

5 All the ethanol was removed and pellets left to air dry for 5-10 mins. Pellets were re-suspended in 30 µl DEPC-treated water and treated with rRNasin RNase inhibitor (Promega).

6 RNA concentrations were assessed using a Nanodrop spectrophotometer.

7 RNA samples were treated with RQ1 RNase-free DNase (Promega), according to the manufacturer's instructions. Samples were then reverse transcribed to cDNA using M-MLV Reverse Transcriptase (Promega) with Random Primers (Promega), according to the manufacturer's instructions. 'No reverse transcriptase' controls were created for each RNA sample by using water in place of the reverse transcriptase enzyme. For each set of reverse transcription reactions carried out, a 'no template' negative control was carried out by adding water in place of template RNA. Once complete, reverse transcription products were stored at -20°C.

8 PCR was carried out using GoTaq polymerase (Promega) in combination with dNTPs and specifically designed primers. Primers were

designed using PrimerSelect (Lasergene 8; DNASTAR Inc.).
The primer sequences used to detect TMSB4X transcripts were as follows.
Forward primer: GAAGACAGAGACGCAAGAGAAAAA
Reverse primer: TGCCAGCCAGATAGATAGACAGAT.

9 PCR was carried out using a G-Storm GS1 thermocycler, using standard temperature cycles for amplification of cDNA with an annealing temperature of 58°C.

10 The PCR products obtained were separated on 2% agarose gels containing Nancy-520 (Sigma Aldrich). An in-house 100 bp ladder was run alongside samples. Gels were run in Tris-acetate-EDTA buffer at 70-80 V until samples were resolved.

11 Gels were imaged using a High Performance Ultraviolet Transilluminator (UVP) and the associated DocIT software (UVP).