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We use this protocol and it's working

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qPCR and RT-PCR

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ABSTRACT

The protocol describes the methodology and materials we use in the Di Monte lab for mRNA extraction and q-PCR/or RT-PCR analyses from fixed tissue samples.

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1 Dissect the dorsal medulla oblongata from paraformaldehyde-fixed coronal sections of the medulla oblongata.

2 Extract total RNA using Nucleic Acid Isolation Kit (Ambion), and measure yield using a nanodrop.

Conventional reverse trancription polymerase chain reactio...

2.1 Synthesize cDNA from 100 ng template RNA using SuperScriptVILO Master Mix (Life Technologies) and the suitable primers (20 µL final volume).

2.2 Run a 30-cycle PCR reaction.

2.3 Mix the PCR products with 6x sample buffer (New England Biolabs) and 5% DMSO and load on 2.0% SeaKem agarose gel (Lonza Bioscience) pre-stained with RedSafe dye (1:20,000, Intron Biotechnology)

real-time PCR

2.4 If necessary, extract RNA from human brain (Agilent Technologies) as a reference sample.

2.5 Prepare a 20 µL reaction mix containing 1 **m**l cDNA, Power SYBRGreen (Applied Biosystems) and primers.

2.6 Run the PCR with a real-time PCR machine (e.g., StepOnePlusTM real-time PCR system, Applied
Biosystems) in triplicates.