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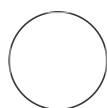
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**Protocol status:** Working  
We use this protocol and it's working

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## 🌐 qPCR and RT-PCR

Ayse Ulusoy<sup>1</sup>, Michael Klinkenberg<sup>1</sup>, Michael Helwig<sup>1</sup>,  
Donato Di Angela Rollar<sup>1</sup>, Shirley Lee<sup>1</sup>, Rita Pinto-Costa<sup>1</sup>, Monte<sup>1</sup>  
<sup>1</sup>DZNE



Ayse Ulusoy

### 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 transcription 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 **ml** cDNA, Power SYBRGreen (Applied Biosystems) and primers.
- 2.6** Run the PCR with a real-time PCR machine (e.g., StepOnePlus™ real-time PCR system, Applied Biosystems) in triplicates.