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Gene Expression

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michela.deleidi^{1,2}, Bianca Marchetti^{3,4}, Federico Bertoli^{1,2}, Carmela Giachino³

¹Mitochondria and Inflammation in Neurodegenerative Diseases, DZNE, Tübingen-Germany;

²Hertie Institute for Clinical Brain Research, University of Tübingen;

³Neuropharmacology Laboratory, Oasi Research Institute-IRCCS, Troina, Italy;

⁴Biomedical and Biotechnological Sciences, Pharmacology Section, University of Catania-Italy



carmela giachino

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Protocol status: Working

We use this protocol and it's working

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Abstract

RT-PCR is a technique to quantify gene expression in samples.

Attachments



[k4drb48if.docx](#)

14KB

Materials

Materials

⊗ QIAzol Lysis Reagent (200ml) **Qiagen Catalog #79306**

⊗ RNeasy Lipid Tissue Mini Kit (50) **Qiagen Catalog #74804**

- Nanodrop-ND 1000
- Retroscript Kit (Ambion #1710, Austin, Texas)
- 2X TaqMan Universal PCR Master Mix (Applied Biosystems)
- Step One Detection System (Applied Biosystems)
- lysis buffer (Qiagen)
- DNase I, RNase-free (Qiagen)
- NanoDrop ND-100 (Nano Drop Technologies)
- the Retroscript Kit (Ambion)
- QIAquick PCR Purification kit (Qiagen)



Troubleshooting





Gene Expression

- 1 Perform gene expression on tissue samples of the ventral midbrain (containing the substantia nigra pars compacta, SNpc).

RNA Extraction


- 2 At due time points, homogenize tissue samples in  1 mL of QIAzol Lysis Reagent (Qiagen, #79306) using a rotor-stator homogenizer.
- 3 Isolate the total RNA from homogenized tissue samples using RNeasy Lipid Tissue Kit (Qiagen, #74804) including Dnase digestion.
- 4 At the end, re-dissolve RNA samples in  30 μ L of RNase-free water and determine their concentrations spectrophotometrically by A₂₆₀ (Nanodrop-ND 1000).

Reverse Transcription

- 5 Perform cDNA synthesis using Retroscript Kit (Ambion #1710, Austin, Texas) according the manufacturer's instructions.
- 6 Add  50 μ L of water solution containing  0.5 μ g of each pool to an equal volume of 2X TaqMan Universal PCR Mastrer Mix (Applied Biosystems).





Real Time PCR

- 7 Perform real-time quantitative PCR using TaqmanTM Assay Reagents on an Step One Detection System (Applied Biosystems) according to manufactures protocol.
- 8 Process tissue samples as above.
- 9 Lyse the cell samples in lysis buffer (Qiagen) and store at  -80 °C until the RNA extraction following manufacture's instructions.
- 10 Remove residual genomic DNA by incubating with DNase I, RNase-free (Qiagen) and elute from the RNeasy mini columns with RNase-free water.





- 11 Quantify the amount of total RNA was using a NanoDrop ND-100 (Nano Drop Technologies) and synthesize the cDNA from  2 μg of total RNA using the Retroscript Kit (Ambion).
 - 12 After purification using QIAquick PCR Purification kit (Qiagen), use  250 ng of cDNA for Real-time PCR using pre-developed Taqman Assay Reagents (Applied Biosystems).
 - 13 Perform real-time quantitative PCR with Step One Detection System (Applied Biosystems) according to manufacturer protocol.
- Note**
- We used the housekeeping gene, β -actin, as normalizer and embryonic mouse brain as calibrator.
- 14 Using the delta delta C_t ($2^{-\Delta\Delta C_t}$) comparative method, quantification of the abundance of target gene expression was determined relative to β -actin with respect to the control group, express the results as arbitrary units (AU).
 - 15 Indicate relative fold changes over WT in the respective treatment groups.

