Gene Expression

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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

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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 Mastrer 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)

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 µL of RNase-free water and determine their concentrations spectrophotometrically by A_{260} (Nanodrop-ND 1000).

Reverse Transcription

5 Perform cDNA synthesis using Retroscript Kit (Ambion #1710, Austin, Texas) according the manufacturer's instructions.

6 Add 50 µL of water solution containing 0.5 µg 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 Taqman^{TM} 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 ($2^{-\Delta\Delta Ct}$) 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.