PROTOCOL EXCHANGE | *COMMUNITY CONTRIBUTED*

mRNA expression analysis by real-time PCR

Ivan Zanoni & Renato Ostuni

Granucci Lab (University of Milano-Bicocca)

Introduction

Relative mRNA expression of the indicated genes in the indicated conditions was quantified using a 7500 Fast Real-Time PCR System (Applied Biosystems). To adjust for possible differences in the amount of template added to the reaction, 18s RNA served as an endogenous control (expression levels of the endogenous control were subtracted from expression levels of target genes). Each sample was assessed in replicate for both the target gene and the endogenous control. Each experiment was repeated at least three times to ensure statistical significance.
Real-Time PCR primers (18 – 20 nucleotides) for the indicated genes were designed with Primer 3 http://frodo.wi.mit.edu/ according to stringent product size (75 – 150 nucleotides) and annealing temperature (58 – 63°C) specifications. Predicted unspecific primer binding and secondary structure formation were excluded using the BLAST and IDT tools http://eu.idtdna.com/Home/Home.aspx

Subject terms: Cell culture Tissue culture Immunological techniques Cell biology Developmental biology

Keywords: dendritic cells macrophages real-time PCR

Procedure

1.Extract total RNA from at least 2×10^6 cells using TRIZOL reagent (Ambion) and the appropriate RNeasy RNA extraction kit (Qiagen).
2.Reverse-transcribe 60 ng of total RNA with a High-capacity cDNA Reverse Transcription kit (Applied Biosystems) in a reaction volume of 200 microl.
3.Prepare a reaction mix (25 µl) according to the following recipe:
2X Sybr Green PCR Master Mix – 12,5 microl
1 µM primer mix – 2,5 microl
cDNA (5 ng/microl) – 2 microl
water – 8 microl

4.Perform Real-Time PCR on 10 ng of cDNA using a Power Sybr Green PCR Master MIX (Applied Biosystems).
5.Start the amplification procedure according to the following standard 7500 run protocol: 50°C (2min) – 95°C (10min) – 95°C (15sec), 60°C (1min), 40 cycles – 95°C (15sec), 60°C.
6.Perform a dissociation assay to evaluate any problem related to primer unspecific annealing or secondary structure formation.
7.Analyze data with built-in SDS Analysis Software.

Associated Publications

This protocol is related to the following articles:

- CD14 regulates the dendritic cell life cycle after LPS exposure through NFAT activation
  Ivan Zanoni, Renato Ostuni, Giusy Capuano, Maddalena Collini, Michele Caccia, Antonella Ellena Ronchi, Marcella Rocchetti, Francesca Mingozzi, Maria Foti, Giuseppe Chirico, Barbara Costa, Antonio Zaza, Paola Ricciardi-Castagnoli, and Francesca Granucci

Author information

Affiliations

. **University of Milano-Bicocca**
Ivan Zanoni & Renato Ostuni

Competing financial interests

The authors declare no competing financial interests.

Readers' Comments

Comments on this thread are vetted after posting.

Protocol Exchange ISSN 2043-0116