Icahn School of Medicine at Mount Sinai LINCS Center for Drug Toxicity Signatures

Standard Operating Procedure:
Quantitative RT-PCRPromoCellCardiomyocytes

DToxS SOP Index:A-3.0
Last Revision:June 29, 2015
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Quality Assurance/Control (QA/QC) steps are indicated with green highlight.

Metadata recording is highlighted with yellow highlight and superscript indices.

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1. In order to assess the redifferentiation status of PromoCell cardiomyocytes (PCM), we compare the expression of the following cardiac differentiation markers using quantitative RT-PCR with mRNA isolated from undifferentiated PCM and differentiated PCM that were cultured at the same time. The cardiac markers are:
    a. α-sarcomeric actinin (PCR primer from Qiagen, Cat: QT00082131)
    b. Cardiac troponin T2 (PCR primer from Qiagen, Cat: QT00089782)
    c. HERG channel (PCR primer from Qiagen, Cat: QT01003254)
    d. Mef 2C (PCR primer from Qiagen, Cat: QT00053368)
    e. Gata4 (PCR primer from Qiagen, Cat: QT00031997)
    f. Nkx2.5 (PCR primer from Qiagen, Cat: QT00010619)
    g. GAPDH, loading control (PCR primer from Qiagen, Cat: QT00079247)
2. Measure the total RNA concentration for all the samples that are going to be assayed as collected by DToxS SOP A-1.0
    a. We use a NanoDrop ND-1000 Spectrophotometer
3. For each sample, mix the following contents from Superscript III First Strand Synthesis Kit (Invitrogen, Cat: 18080-051) in a thin walled 0.6 mL microfuge tube, ordered by descending volume:
    a. 1 µg of RNA (x µL)
    b. RNase-free water (8-x µL)
    c. Oligo(dT) (1 µL)
    d. dNTP mix (1 µL)
4. After each addition, mix by pipetting up and down, and quick spin on a tabletop mini centrifuge

5. Incubate mixture at 65ºC for 5 minutes and move onto ice
6. Prepare the following cDNA synthesis mix by mixing the contents from the Superscript III Kit in descending volume order:
    a. 10 µL mix for each sample made up of
        i. 25 mM $MgCl_2$ (4 µL)
        ii. 10x RT Buffer (2 µL)
        iii. 0.1 M DTT (2 µL)
        iv. RNase OUT (1 µL)
        v. SuperScript III RT (1 µL)
7. Add the synthesis mix into the RNA solution, mix by pipetting up and down, and quick spin on a tabletop mini centrifuge
8. Incubate at 50ºC for 50 minutes
9. Terminate reaction at 85ºC for 5 minutes
10. Add 1 µL of RNase H and incubate at 37ºC for 20 minutes to remove any leftover RNA
    a. cDNA is stable at -20ºC for up to six months
11. We use the Applied Biosystems 7500 Real-time PCR System with SYBR Green PCR Kit (Qiagen, Cat: 204143) and the primers listed in Step 1
    a. Dilute each primer to 10 µM by adding RNase-free water according to the manufacturer's label
    b. For each sample, add 6 µL of SYBR Green Master Mix, 1 µL primer, 1 µL cDNA, and 4 µL RNase-free water into a single well of a 96-well plate (Applied Biosystems, Cat: 4306737)
    c. Using 7500 Software bundled with the instrument, quantify message levels using "standard experiment" setting via the crossing threshold metric.