# Protocol for qPCR reaction set up

## 1.) Reverse Transcription

~ 2 µg total-RNA

adjust to 13.25 µL with RNase-free water

add 1 µL random primers (50ng/µL)

➔ incubate 5 min at 65°C, 5 min at 4°C

add master mix:

4 µl 5xRT-Puffer

1 µl dNTPs (10mM)

0.25 µl RNasin

0.5 µl M-MLV reverse transcriptase (Promega, M1705)

➔ incubate 2 h at 42°C, 15 min at 75°C

storage at -20°C

## 2.) real-time PCR reaction set up (96well for Biorad MyIQ5):

master mix per 1 reaction:

7.5 µl OneTaq™ 2X Master Mix with Standard Buffer (NEB, M0482 L)

0.3 µl SYBRgreen (SIGMA, 86205, 1:1000)

0.3 µL Flourescein (Biorad) [750nM]

1.9 µL water

0.03 µl each primer (100µM)

use 10 µL master mix for each reaction

add 5µl cDNA (1:5 dilution or even lower concentrated)

PCR program:

3’ 95°C

30’’95°C ⎫

30’’60°C ⎬ 40-50x

30’’72°C ⎭

melting curve

## 3.) real-time PCR reaction set up (384well for Applied 7900HT):

master mix per 1 reaction:

5 µl 2xPCR-Mix (SYBR Select Master Mix, Life Technologies, 4472908)

0.02 µl each primer (100µM)

use 5 µL master mix for each reaction

add 5µl cDNA (1:5 dilution or even lower concentrated)

PCR program:

2‘ 50°C

2’95°C

15‘‘ 95°C ┐

1’60°C ┘ 40-50x

melting curve

(for TaqMan probes there is no need to perform a melting curve. Please refer to the manual)