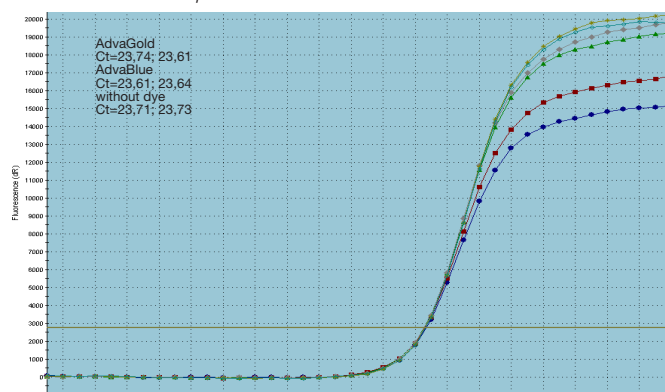


Quantitative RT-PCR on AmpliGrid AG480F

This process instruction describes the quantitative analysis of the housekeeping gene *Calm*. The reverse transcription is done on the AmpliGrid system using the Qiagen OneStep RT-PCR Kit – for a better visualization of the sample the master mixes are stained with either AdvaGold or AdvaBlue. Afterwards the quantitative amplification is done on a conventional real-time instrument (Stratagene) using SYBR green.

1 Figure 1: Quantitative RT-PCR results using AdvaBlue or AdvaGold for the reverse transcription



Material

- OneStep RT-PCR Kit (Qiagen)
- RNasin® Ribonuclease Inhibitor (Promega)
- Nuclease free water
- 2x SYBR® Premix Ex Taq (TaKaRa)
- AmpliGrid slides incl. Sealing Solution (Advalytix; e.g. OAX04503)
- Primer for reverse transcription: *Calm* antisense (0.6 µM)
- Primer for qPCR: *Calm* sense+antisense (6 µM)
- 0.1% AdvaGold (Advalytix, OAX04227)
- 0,1% AdvaBlue (Advalytix, OAX04243)
- human total RNA (Promega); 1 ng/µL (store at -80°C)
- AmpliSpeed slide cyclers (Advalytix; e.g. OAX04101)
- Electronic multistep pipette
- 96 well microtiter plate (for qPCR applications)
- Real-time cyclers (e.g. Stratagene Mx3005P®)

Protocol

- Deposit 1 µL of reverse transcription primer (only antisense primer) on each reaction site and let air-dry
- Prepare master mix in a fresh PCR-clean tube according to table A (pipette always on 4°C)

- Make sure to prepare enough master mix for all the reactions, taking pipetting errors into account (dead volume of electronic multistep pipette about 10 µL)

A Table A: reverse transcription setup with Qiagen reagents

Component	1 reaction
Qiagen OneStep RT-PCR Buffer, 5x	0.2 µL
Q-Solution, 5x	0.16 µL
dNTP Mix, 10 mM each	0.04 µL
Enzyme Mix	0.04 µL
RNasin® 40 U/µL	0.02 µL
RNA (10 ng/µL)	0.1 µL
AdvaGold or AdvaBlue (0.1%)	0.1 µL
Nuclease free water	0.34 µL
Total Volume	1 µL

- Mix gently and spin down shortly
- Distribute 1 µL of master mix on each reaction site
- Immediately cover with 5 µL sealing solution
- Transfer AmpliGrid slide onto the preheated AmpliSpeed slide cycler
- Incubate AmpliGrid slide for 10 min at 42°C and 30 min at 58°C
- Meanwhile prepare qPCR master mix according to table B

B Table B: real-time amplification master mix

Component	1 reaction
2x SYBR® Premix Ex Taq	5 µL
qPCR primer mix (sense and antisense)	1 µL
Nuclease free water	3 µL

- Pipette 9 µL qPCR master mix into each well of a microtiter plate (four reactions per reaction site of the AmpliGrid)
- Add 4 µL of nuclease free water to each reaction site of the AmpliGrid by pipetting on the sealing solution and mix carefully by pipetting up and down slowly
- Transfer 4 times 1 µL from the reaction sites of the AmpliGrid into the microtiter plate wells; close the plate with optical lid
- Run the program described in table C on a real-time instrument and analyse the data afterwards using the software

C Table C: Amplification program

Temperature	Time	Cycle
95°C	10 min	
94°C	30 sec	
60°C	60 sec	45 cycles
72°C	60 sec	
72°C	10 min	

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