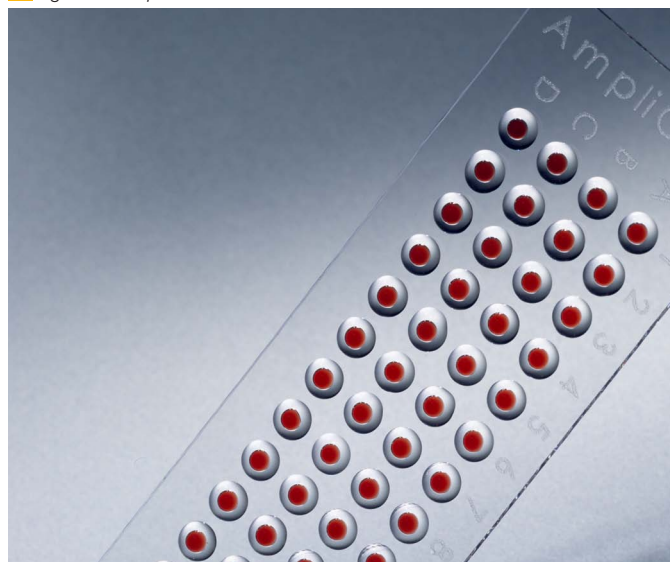


TaqMan® microRNA Assay on single cells using AmpliGrid AG480F

This process instruction describes an experiment using the TaqMan® microRNA Assay (Applied Biosystems) together with the AmpliGrid slide.

1 Figure 1: AmpliGrid AG480F



Material

- Template: single HeLa cells (human HPV18 positive epithelial cells from a cervix carcinoma) deposited on the AmpliGrid AG480F (Figure 1, Advantix) - stained with Hoechst dye
- Positive control RNA (total extracted RNA from HeLa cells)
- TaqMan® MicroRNA Reverse Transcription Kit, Applied Biosystems cat no: PN 4366596
- TaqMan® 2x Universal PCR Master Mix, No AmpErase® UNG (qPCR) Applied Biosystems cat no: PN 4324018
- TaqMan® MicroRNA Assay miR-24, Applied Biosystems cat no: 4373072 (includes 5x primer for RT and 20x primer for qPCR including the FAM-labeled probe)
- Sealing Solution (Advantix, e.g. OAX04207)
- AmpliSpeed slide cycler (Advantix, e.g. OAX04101)
- Electronic multistep pipette

Notes

- Perform all steps on ice.
- Thaw all reagents on ice.
- The miRNA Assay must be carefully thawed on ice and afterwards aliquoted. Aliquots should be stored frozen until use and should be used only once when thawed.

- RT primers are used in a final concentration of 0.5fold.

Protocol

RNA TEMPLATE

- Prepare RNA solution of 10 ng/μL RNA (storable at -80°C)

SINGLE CELL TEMPLATE

- Check Hoechst dye stained cells with cell detection system at the fluorescent microscope in order to verify their presence on the reaction sites

REVERSE TRANSCRIPTION

- Prepare master mix in a fresh PCR-clean tube according to table A when using total RNA as template or to table B when using single cells as template.
- 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 master mix using total RNA as template

Component	1 reaction
dNTP (100 mM)	0.01 μL
10x RT buffer	0.1 μL
RNase inhibitor	0.01 μL
5x RT primer	0.1 μL
Reverse transcriptase	0.07 μL
RNA (10 ng/μL)	0.1 μL
Nuclease free water	0.61 μL
Total Volume	1 μL

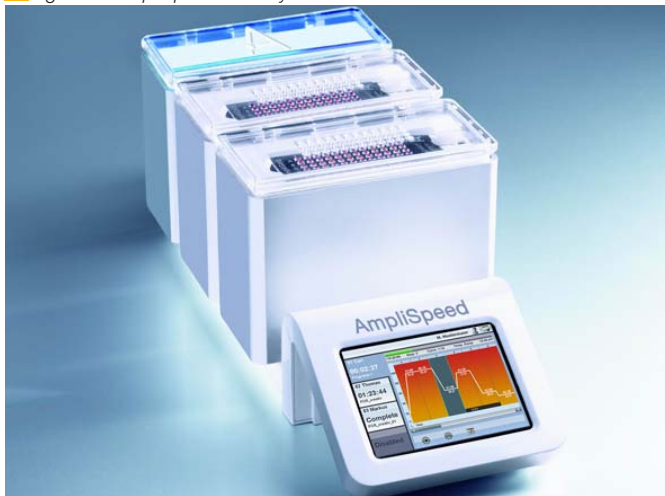
B Table B: reverse transcription master mix using single cells as template

Component	1 reaction
dNTP (100 mM)	0.01 μL
10x RT buffer	0.1 μL
RNase inhibitor	0.01 μL
5x RT primer	0.1 μL
Reverse transcriptase	0.07 μL
Nuclease free water	0.71 μL
Total Volume	1 μL

- Incubate the RT master mix on ice for 3 min.
- Add 1 μL of RT mix onto one reaction site of the AmpliGrid (on ice/cooled) and immediately cover with 5 μL sealing solution.
- Incubate the slide for 30 min at room temperature.

- Transfer the AmpliGrid onto the AmpliSpeed slide cycler (Figure 2) and incubate further 30 min at 50°C.

2 Figure 2: AmpliSpeed slide cycler ASC200D



qPCR MASTER MIX

- Prepare the qPCR master mix as described in table C on ice.

C Table C: qPCR master mix

Reagent	1 reaction
20x TaqMan® MicroRNA Assay	1 µL
2x TaqMan® Universal PCR master mix	10 µL
Nuclease free water	8 µL
Total Volume	19 µL

- Pipette 19 µL qPCR master mix in each qPCR tube.
- In each well add 1 µL sample out of reverse transcription to the qPCR mix.
- Vortex gently and centrifuge the plate shortly to collect the solution at the bottom.
- Start qPCR program according to table D (use FAM filter for quantitative measurement)

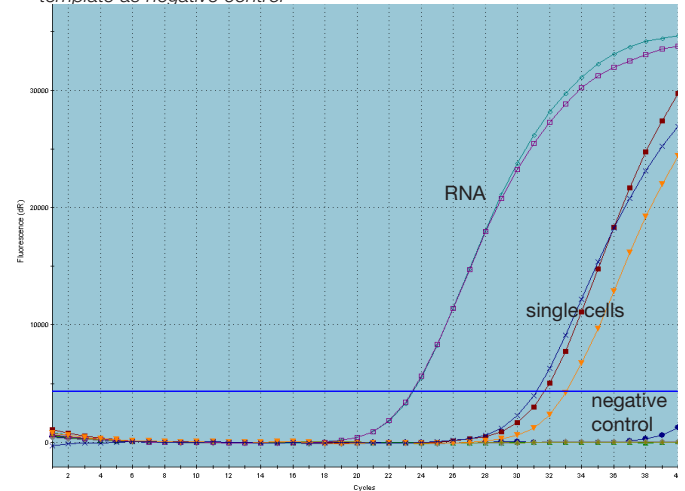
D Table D: qPCR program

Temperature	Time	Cycle
95°C	10 min	
95°C	15 sec	40 cycles
60°C	1 min	

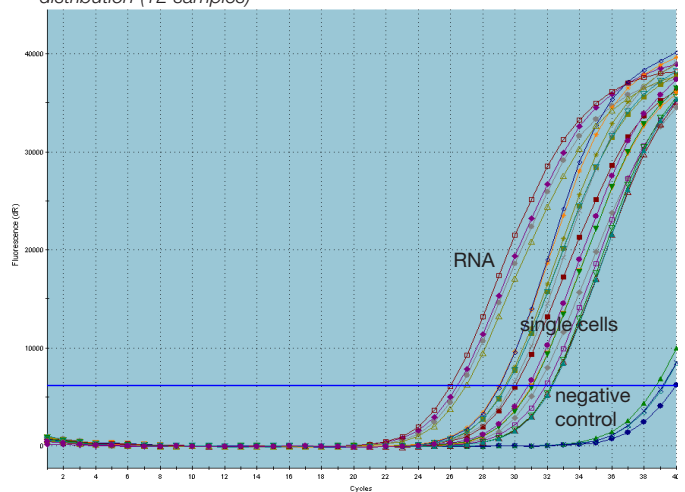
Analysis

- Analyse data using the real-time cycler software

3 Figure 3: Example 1: experiment performed on single cells (three independent single cell reactions); extracted RNA as positive and no template as negative control



4 Figure 4: Example 2: experiment performed on single cells; curves of samples with single cells (dependent on cell cycle) show normal distribution (12 samples)



As shown in Figure 3 and 4, the microRNA “miR-24” can be amplified in single HeLa cells using a TaqMan® microRNA assay on the AmpliGrid system.

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