

Quantitative gene expression on single cells with the GenomeLab™ GeXP Start Kit on the AmpliGrid AG480F

Using the GeXP reagents on the AmpliGrid AG480F a cost-effective, quantitative gene expression analysis from multiple genes on single cells can be done.

Material

- Single MCF-7 cells deposited on the AG 480F
- Template RNA (if available), 10ng/μL
- Hoechst Bisbenzimid H 33342 (Sigma, order no. 14533)
- Fluorescence microscope for detection of deposited stained cells
- AmpliGrid AG480F incl. Sealing Solution (Advalytix, e.g., order no. OAX04505)
- GenomeLab™ GeXP Start Kit (Beckman Coulter, A21019)
- Custom RT Rev Primer Plex (0.125μM housekeeping gene 1, 0.0625μM housekeeping gene 2, 0.5μM housekeeping gene 3)
- Custom PCR Fwd Primer Plex (1μM housekeeping gene 1 + 2, 2μM housekeeping gene 3)
- AmpliSpeed slide cycler (Advalytix, e.g. order no. OAX04102)
- Electronic multistep pipette
- RNase-free water (e.g. Qiagen, 129112)

Protocol

SINGLE CELL TEMPLATE

- Cells are stained with the DNA stain Hoechst Bisbenzimid H 33342 with a concentration of 1mg/mL (100x staining solution). Hoechst dye is pipetted to the cell suspension (1:100) and incubated for 5-10 min at room temperature.
- Single cells are deposited on the AmpliGrid slide using flow cytometry and air dried.
- Hoechst dye stained cells are checked at the fluorescent microscope in order to verify their presence on the reaction sites.

REVERSE TRANSCRIPTION

- Work done on ice at all steps.
- All RT reagents are thawed completely but slowly on ice, mixed gently.
- Dilute KANr RNA 1:50 with water, use dilution for master mixes.
- Components are combined in a sterile, nuclease-free tube according to the following table:

Component	Volume (RNA mix)	Volume (cell mix)
DNase/RNase Free H2O	4.5 µL	5.5 µL
RT Buffer	2 µL	2 µL
Custom RT Rev Primer Plex	1 µL	1 µL
Reverse Transcriptase	0.5 µL	0.5 µL
KANr RNA with RI (1:50)	1 µL	1 µL
Sample RNA (10 ng/µL)	1 µL	---

- The mix is gently vortexed and spun down shortly.
- The AmpliGrid slide is positioned on a cooling device to keep the reagents cold during the pipetting process.
- 1 µL of the reverse transcription mix with RNA is pipetted on several empty reaction sites of the AmpliGrid and immediately covered with 5 µL of sealing solution.
- 1 µL of the reverse transcription mix for cells is pipetted on the reaction sites with deposited single cells on the AmpliGrid and immediately covered with 5 µL of sealing solution. For no template controls (NTC) 1µL of the cell mix are added to several empty reaction sites and covered as described.
- The AmpliGrid slide is placed on the AmpliSpeed slide cycler and the following programme is started:

Temperature [°C]	Time
52°C	2 min
42°C	60 min
95°C	5 min
ambient	hold

AMPLIFICATION

- Reagents for the PCR are thawed completely, shortly vortexed and the components are combined in a sterile, nuclease free tube according to the following table:
Note: protect PCR buffer from light.

Component	Volume 1 reaction	Volume 20 reactions
PCR Buffer 5X	0.2 µL	4 µL
25 mM MgCl ₂	0.2 µL	4 µL
Custom PCR Fwd Primer Plex	0.1 µL	2 µL
Thermo-Start® DNA Polymerase	0.035 µL	0.7 µL
DNase/RNase Free H2O	0.465 µL	9.3 µL

- The master mix is gently vortexed and spinned down shortly.
- 1 μ L of the master mix is pipetted on top of each of the AmpliGrid reaction sites.
NOTE: Aqueous solution that is pipetted on top of each reaction site will move through the sealing solution due to physical reasons and will automatically merge with the sample.
- The following programme on the AmpliSpeed slide cycler is started:

Temperature [°C]	Time	
95°C	10 min	
94°C	30 sec	
55°C	60 sec	45 cycles
70°C	90 sec	
ambient	hold	

- After amplification samples are prepared for the analysis.

Analysis

- Using the CEQ 2000 XL or 8000 according to the instrument manual.
- For each sample 38 μ L Sample Loading Solution + 0.5 μ L DNA standard + 1.5 μ L cDNA are mixed and transferred into a fresh well of the sample plate.
- Usage of the default frag-3 protocol.
- Fragment lengths:
 - housekeeping 1: 210bp
 - housekeeping 2: 227bp
 - housekeeping 3: 334bp
 - Kanamycin control: 325bp

Referenzen

<http://www.beckmancoulter.com/literature/Bioresearch/A-10295A.pdf>