# Supplementary methods

## Chip fabrication

One micrometer low-stress MF silicon-nitride was deposited on a Pyrex wafer using Plasma Enhanced Chemical Vapor Deposition (PECVD), followed by the lift-off patterning of Titanium/Palladium/Titanium (Ti/Pd/Ti) with respective layer thickness of 800 Å/1200 Å/300 Å. A second, 800 nm thick, MF nitride layer was deposited and patterned by reactive ion etching (RIE).

Microfluidic structures were patterned in Ordyl SY330 dry film resist (Elga Europe, Italy) [1]. A first layer of Ordyl is laminated and exposed with 240 mJ $cm^{-2}$ to create phaseguides. Three additional layers of Ordyl were laminated and exposed with the same amount of energy, followed by a one-minute post-exposure bake (PEB) at 85°C. Resist was then developed in three baths of BMR developer (Elga Europe) of increasing cleanliness followed by rinsing and drying.

Holes were drilled in the top wafer using a CNC platform (ISEL CPM 3020, Techno, NY, USA) and a diamond bit (Proxxon, RS Components GmbH, Germany). The drilled and cleaned wafer was bonded to the bottom wafer under a pressure of 60 N $cm^{-2}$ and a temperature of 95 °C for 30 minutes in a Süss Microtec SB6 waferbonder. After wafer bonding, the cross-linking was finalized by a one-minute UV flood exposure followed by a 2-hour postbake at 150°C.

Chips were diced with the top substrate upwards. Fluidic access holes were therefore sealed with dicing foil, to prevent cooling water from entering the chips. First, contact pads were diced free by dicing the chip half through, then the chips were fully diced.

## Chip operation

Chips were filled with 5% polyacrylamide gel consisting of 625 µl 40% acrylamide solution (acrylamide:bisacrylamide, 29:1, Sigma, Germany), 250 µL 10x TBE (Fermentas, Lithuania) 4092.5 µL water, 5 µl N,N,N',N'-tetramethylethylenediamine (TEMED, Sigma), and 27.5 µL 10% w/v ammonium persulfate (APS, Sigma). To assure a complete gelling and prevent drying out, gel-filled chips were incubated in a nitrogen-flooded humidity chamber. After gelling, the elution chamber was filled with 1x TBE buffer and the sample chamber was filled with a cell sample. The chip is placed in a PCB slide holder. Thermo-electric lysis is driven by a Hewlett Packard 33120A function generator that is amplified 100 times by an A600 voltage amplifier (FLC Electronics, Sweden). Electrophoresis is controlled by a HVS448 high voltage programmable power source (Labsmith, USA). The AC voltage is applied to the electrophoresis cathode, while the passivated lysis electrode is grounded. A DC constant current is then applied to the electrophoresis anode, while putting the cathode to ground. After RNA extraction, the eluted sample is recovered from the elution chamber with a pipette.

Visualization of phaseguide behavior was done using 4% NuSieve Agarose (Cambrex Bio Science Rockland, USA.) colored with Xylene Cyanol and 0.5x TBE buffer colored with Neutral Red.

Filling and emptying of the chip was done with pipette pressure. Pipette tips were adjusted to the size of the chip-holes, so that a sealed connection could be created.

**Cell samples**

*Escherichia coli K12 (E. coli),* DSM strain 1077 (DSMZ, Germany) were grown overnight in Luria-Bertani (LB) medium (Merck, Germany) in a shaking incubator at 37°C. For counting of colony forming units (CFUs) and storage, *E. coli* was plated on LB agar plates using the droplet method [2]. *Streptococcus thermophilus (S. thermophilus)* DSM strain number 20617 were grown in M17 medium according to Terzaghi (Merck) in a shaking incubator at 37°C in tightly closed falcon tubes. For counting and storage *S. thermophilus* was plated on M17 agar plates that were tightly sealed with parafilm.

An overnight culture of *E. coli* (typically 1 mL) was spun down at 9,000 g for 4 minutes. The supernatant was discarded and cells were resuspended in 1 mL 0.5x TBE buffer. The procedure was repeated once. The same procedure was also used for *S. thermophilus.* For chip experiments with *S. thermophilus*, the final buffer contained 0.2 % Triton X-100 (Sigma).

**RNA isolation**

RNA was extracted using the commercial RNA extraction kit Ambion RiboPure-Yeast (Ambion, Germany, as recommended by [3]). DNA was removed by TURBO DNA-*free*™, DNase treatment (Ambion). For *E. coli,* 500 µL cell sample volume was used and the purified RNA volume was adjusted to 500 µL for a direct comparison with chip-extracted RNA. For *S. thermophilus* 50 µL cell sample volumes were used that were eluted in the same volume.

**Plasmid preparation and cRNA transcription**

*E. coli* transfected with a pCR II TA cloning Vector (Invitrogen, USA) containing *E. coli* tmRNA was used for quantification in non-RT PCR assays. The plasmid length was 3971 basepairs with a tmRNA insert of 330 bp. Transfected *E. coli* were grown on LB agar supplemented with the antibiotic Ampicilline (50 µg/mL). Plasmids were isolated from 1 mL overnight culture with the QIAprep Miniprep kit (Qiagen, Germany).

Complementary RNA (cRNA) was constructed for quantification of the RT PCR assay. The plasmid was linearized using the enzymes *Xho* I and *Hin*d III (Fermentas, USA) and reverse transcribed with the SP6 enzyme from MAXIscript (Ambion) followed by DNase treatment (TURBO DNA-*free*). Free nucleotides were removed by addition of 5 µL 5 M ammonium-acetate and 3 volumes 100% ethanol. The solution was chilled for 30 min. at –20°C followed by a 15-minute centrifugation step at 14,000 g at 4°C. The pellet was washed once with cold 70% ethanol. Optical density was

measured using NanoDrop-ND-1000-spektrophotometer and cRNA aliquots were stored at –80°C.

**Real-time PCR**

LightCycler RNA Master HybProbe kit (Roche Diagnostics, Germany) was used for RT qPCR. 1.4 µL 50mM $Mn(OAc)_2$, 1 µL 10µM primers and 0.4 µL 10µM probe solution was added on a final reagent volume of 20 µL. LightCycler FastStart DNA Master HybProbe kit (Roche Diagnostics, Germany) was used for a PCR without RT. The 20 µL reaction volume contained 0.8 µL 25mM $Mg(Ca)_2$ and the same amount of primers and probe as the RNA Master kit. Probes and primers (TIB MolBiol, Germany ) are shown in Table 1. Amplicons were made as short as possible to enable a good quantification.

All assays used a two-temperature step PCR. The denaturing step was performed at 95°C for 1 s for all assays followed by a 50 s step for primer binding and extension. The temperature for the latter was 59°C for the *E. coli* tmRNA assays and 60°C for the *S. thermophilus* assay. The reverse transcription step was run for 50 minutes at 61°C. For PCR assays with the Faststart kit, a 10 min hotstart of 95°C anticipated the thermal cycling. Assays were run on a Lightcycler 1.2. 10-fold dilution series of cRNA, plasmid and *S. thermophilus* total RNA, were run in order to determine the efficiencies of the PCR and RT-PCR assays. Real-time PCR analysis was done with the Fit Point method as available in Lightcycler 3.5 software. For *E. coli*, a noiseband was set at a fluorescence level (F1/F2) of 0.02 and a threshold value for a fluorescence of 0.02 was used. For *S. thermophilus*, a noiseband was set at a fluorescence level (F1/F2) of 0.01 and a threshold value for a fluorescence of 0.02 was used. Proportional quantification was used for all experiments.

**Agilent Bioanalyzer**

Caliper nanoRNA LabChips (Caliper Technologies Corp., USA) were used to obtain a full electrophoretic profile of extracted RNA. An RNA 6000 ladder (Ambion) was used as a reference. Chip results were analysed using the Agilent Bioanalyzer 2100 and 2100 Expert Software (Agilent Technologies, USA).

**Statistical analyses and data manipulation**

Pictures in Figures 3 and 4 were extracted from .avi movies followed by automatic contrast optimization in Corel Photopaint (Corel corp.). The original .avi files have been elaborated in windows moviemaker and are available as supplementary information (Supplementary Video 1.swf and Supplementary Video 2.swf). The Agilent Bioanalyzer curves in Figure 6 of the article have been recorded in separate runs. They have been normalized and aligned according to the 20 bp marker size. The Ambion RNA curve has been time-shifted for 0.5 s with respect to the Chip RNA curve. Normalization data was provided by the 2100 Expert Software and was a factor 0.625 for Ambion RNA and a factor 0.417 for Chip RNA.

Quantification of the *E. coli* RT qPCR curves was done with an external cRNA standard curve. Two different researchers recorded three standard curves each, yielding a total number of 20 and 14 measurement points respectively. A linear regression analysis was performed for each researcher's data set and a 95% confidence interval was calculated.

The resulting slope, crossing-points and confidence interval were used to quantify unknown RNA samples and obtain their error values. A linear regression analysis yielded the slope of an experimental series. The maximum and minimum slope values of the standard curves according to the 95% confidence interval were used to obtain maximum and minimum values for the slope of the unknown data. To obtain the full error, the 95% confidence error of the second fit was added to this value.

The three extraction experiments were performed on three different days and RT qPCR experiments were performed by two different researchers, each using his own standard curves. The error of the obtained mean value is represented by the range of the three experiment series.

Quantification of the *S. thermophilus* RT qPCR largely followed the same strategy. This time however, DNase treated total RNA was used to obtain a relative standard curve. 23 measurement points were obtained by one researcher only. Six cell concentrations were measured in duplex, yielding 12 measurement points. A linear regression analysis was executed over ten points for the chip (excluding measurement points below 1 CFU/μL) and eight points for the Ambion kit (excluding measurement points below 10 CFU/μL). Errors for the cell counts represent the standard deviations for the droplet method (*E. coli*) or plating method (*S. thermophilus*).

## References

1. Vulto, P.*, et al.* Microfluidic channel fabrication in dry film resist for production and prototyping of hybrid chips. *Lab Chip* **5**, 158-162 (2005).

2. Pfeltz, R.F., Schmidt, J.L. & Wilkinson, B.J. A microdilution plating method for population analysis of antibiotic-resistant Staphylococci. *Microb Drug Resist* **7**, 289-295 (2001).

3. Glynn, B.*, et al.* Quantification of bacterial tmRNA using *in vitro* transcribed RNA standards and two-step qRT-PCR. *Res. J. Biol. Sci.* **2**, 564-570 (2007).

Table 1 Various probe/primer sets. The amplicon lengths were 65 nt for the E.coli tmRNA system and 68 nt for the S. thermophilus tmRNA system.

| **Name** | **Sequence** |
|---|---|
| *E. coli* tmRNA Forward | 5' – gggATCAAgAgAggTCAAACCC – 3' |
| *E. coli* tmRNA reverse | 5' – TTAACgCTTCAACCCCA – 3' |
| *E. coli* tmRNA Probe | 5' FAM - AAAgAgATCgCgTggAAGCCCTgC – TAMRA 3' |
| *S. thermophilus* tmRNA Forward | 5' – ggCCgTTACggATTCgAC – 3' |
| *S. thermophilus* tmRNA Reverse | 5' – CTgAgCgTTAACgTCgCCACA – 3' |
| *S. thermophilus* tmRNA Probe | 5' FAM – CATTATgAggCATATTCTgCgACTCg – TAMRA 3' |