## Supplemental Information

### 1. Microfluidic Device Fabrication

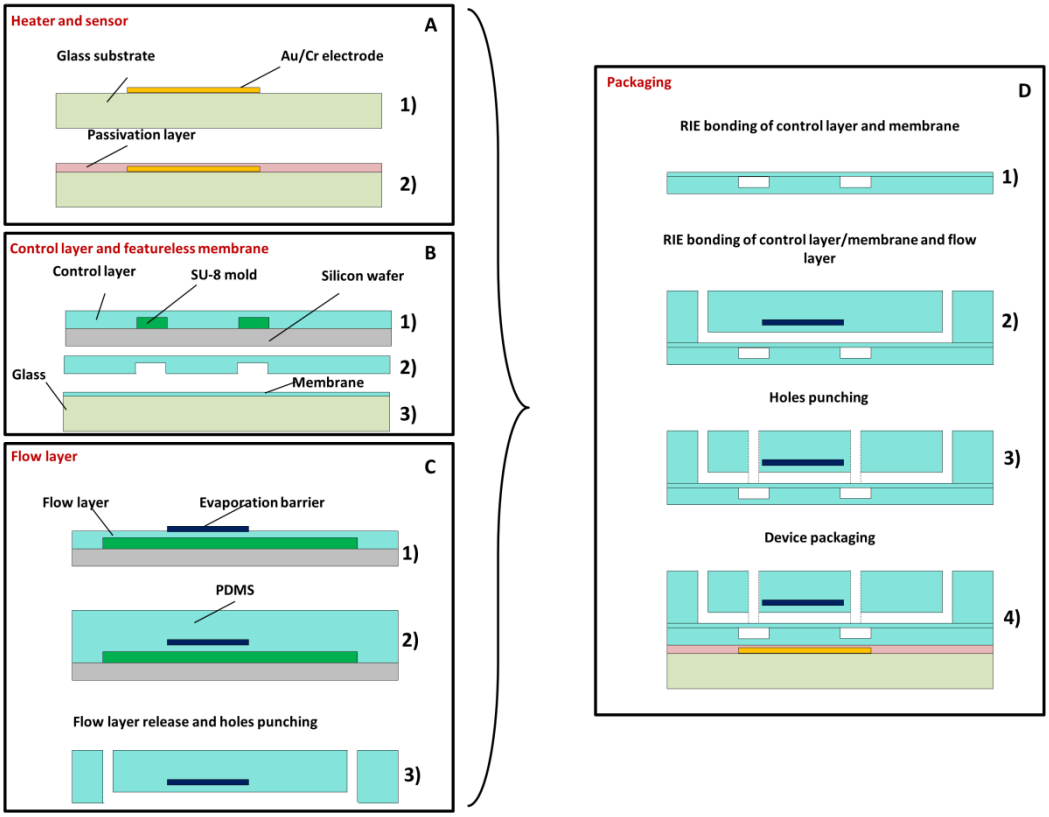


**Figure SI-1.** Fabrication process of the microchip. (A) Heater and sensor. 1) Au/Cr deposition. 2) Passivation. (B) Control layer and membrane. 1) Control layer mold fabrication using standard soft lithorgraphy and PDMS pouring. 2) PDMS baking and peeling off. 3) Featureless PDMS membrane spin coating. (C) Flow layer. 1) Mold fabrication, PDMS baking and evaporation barrier aligning. 2) Evaporation barrier implantation. 3) Flow layer release and holes (Diameter: 1.5 mm) punching. Holes were punched with a 1.5 mm diameter mechanical punch (Harris Uni-Core punch). (D) Device package using RIE method. The thickness of the PDMS was 3±0.1 mm for the flow layer and 240±10 μm for the control layer.

Details of the fabrication protocol have been listed as following.

**Passivation process.** The glass slide bearing the heater and sensor was passivated by sequentially spin coating and curing a 10 μm layer of SU-8 photoresist (MicroChem SU-2010 3500 rpm for 45 seconds, 95 ℃ for 10 min for curing the photoresist), followed by a 10 μm of PDMS (Dow Corning PDMS 5000 rpm for 1 min, 80 ℃ for 20 min for curing PDMS). Oxygen plasma was employed to bind the passivation layer with the PDMS microfluidic device. After each RT-qPCR use, the PDMS microfluidic device was peeled off while the heater and sensor were reused.

**SU-8 photolithography process.** The SU-8 mold features were 15 μm high for the flow layer and 80 μm high for the control layer. The protocol for the 15 μm high flow layer was adapted from the manufacturer (MicroChem Corp., Newton, MA, USA) guidelines. First, at room temperature, permanent epoxy negative photoresist SU-8 was spin-coated on a cleaned 4-in silicon substrate at a speed of 3200 rpm for 45-60 seconds with an acceleration of 300 rpm/second. Then, the coated SU-8 photoresist was placed on a level hotplate for 10-15 min at 95 °C. Next, the baked photoresist was exposed under UV light at a dose of 130-150 $mJ/cm^2$ using a mask aligner (Süss MicroTec MA6 Mask Aligner). After exposure, the patterned SU-8 was placed on a hotplate for 4 minutes at 95 °C. Then, the exposed SU-8 2015 photoresist was sprayed with MicroChem's SU-8 developer for 2-3 min. At the end of the development, the exposed photoresist was sprayed and washed with fresh SU-8 developer for approximately 10 seconds, followed by a second spray/wash with Isopropyl Alcohol (IPA) for another 10 seconds. The mold was dried with pressurized nitrogen. Finally, the silicon substrate bearing the SU-8 features was baked (150-250 °C) for 5 to 30 min to ensure that SU-8 properties do not change with thermal cycling.

Similarly, according to the manufacturer's guidelines, we built an 80 μm high mold using SU-8 2075 photoresist.

**Evaporation barrier implantation.** From the manufacturer, the vapor barrier (optical adhesive film) is composed of polypropylene and was designed for creating a secure seal across a microplate to prevent evaporation. The thickness of the film was 0.1 mm as measured by a vernier caliper. A two-step PDMS casting process was employed to embed the vapor barrier above the reaction chamber. Initially, base and agent of PDMS were mixed in a 10:1 ratio. The mixture was degassed for 45 minutes and then was spin-coated on the mold at a speed of 4000 rpm for 45 second with an acceleration of 300 rpm/second followed by baking at 72 °C for 15 minutes. Next, a piece of adhesive film was stamped on PDMS at the region of the reaction chamber. After that, 15 mL uncured PDMS (10:1) was poured on the solid-state PDMS layer and baked to finalize the barrier implantation.

**RIE bonding process.** The mechanism is related to the breaking of bonds on each surface of PDMS during treatment followed by the formation of Si–O–Si bonds when the two surfaces are brought into contact. The PDMS layers or substrates were first installed in the process chamber of the Technics Series 800 RIE (Oxygen Plasma Asher). The samples were treated with oxygen plasma at 250 mTorr pressure by a power of 50 Watt for 4 seconds.

**2. Flow Control Procedure**

**Experimental procedure for on-chip bead-based single-cell RT-qPCR:**

**Step 1: Beads preparation and incubation**

The beads were first washed using binding buffer and then resuspended in binding buffer using a 30 s vortex. Then we transferred a specific volume of beads to an RNase-free PCR tube, added 10 μL binding buffer, resuspended the beads in binding buffer for 10 s, placed the tube in a magnet for 1 min and discarded the supernatant. The beads were then

suspended in 2 μL binding buffer and the bead density was calculated by ImageJ. The bead solution was finally introduced to device using a microcapillary pipette.

**Step 2: On-chip cell processing**

During the on-chip cell processing, we sealed the cell washing outlet using a plastic plug, closed the downstream valve to cut off cell carrier flow to chamber, and opened the upstream valve and the cell trapping outlet. We diluted the loading cell concentration and introduced cells to the chip using a syringe pump. Once a single cell was immobilized at the trap, we closed the upstream valve and opened the cell washing outlet, directing subsequent cells away from the trap. Then, we sealed the cell trapping outlet and opened the upstream valve to introduce lysis buffer through the cell inlet. Meanwhile, we opened the downstream valve and moved the beads to the lysate using a magnet. The beads and lysate were mixed for 10 min to capture the released mRNA onto the beads' surface. The beads with bound mRNA were then moved to reaction chamber and extracellular RNA and debris were removed by buffer washing.

**Step 3: Integrated on-chip RT-qPCR**

Each RT was performed in the device using the TaqMan® reverse transcription reagents. RT reagent was first pipetted into the device while the beads were immobilized in the chamber by placing the device on a magnet. Meanwhile, the cell trapping and washing outlets were closed and the upstream and downstream valves were opened. Once the reaction chamber was fully filled with RT reagent, we closed all the inlets and outlets. Then a typical pulsed temperature RT protocol was carried out (10 min at 25°C and 50 min at 42°C). The TaqMan® reverse transcription reagents kit includes 1.5 mM magnesium chloride. The proportion of each reagent was consistent with the manufacturer's protocol.

**Step 4: Quantitative real-time PCR**

The PCR reagent was prepared with TaqMan® Gene Expression master mix and template specific primer. The PCR reagent was introduced to device which simultaneously flushed away the RT reagent while the chip was situated on a magnet to immobilize the beads. After PCR reagent completely filled the reaction chamber, all the outlets and inlets were sealed with plugs. Then the platform was placed on the stage of a fluorescent microscope. Each PCR process was initialized and thermocycled with the following protocols: 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Fluorescent images of the beads were taken in two different colors (one reference dye and one reporter dye) after each PCR cycle.

All of the experimental procedures were demonstrated in Figure SI-2.

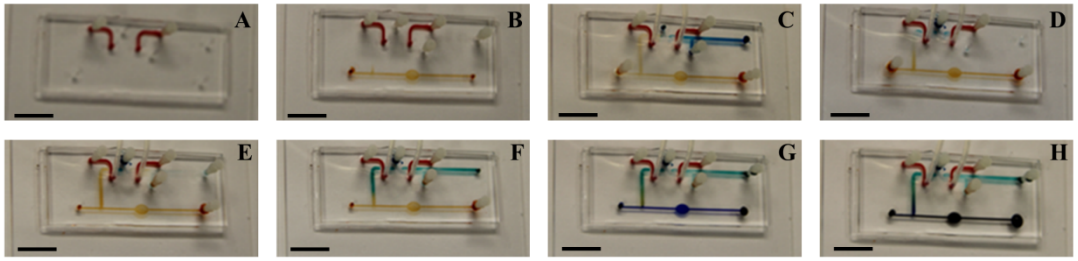


**Figure SI-2.** Demonstration of the on-chip flow control. (A) Before fluid is introduced into the chamber. (B) Bead introduction. (C) Cell trapping. (D) Cell washing. (E) Bead and cell mixing. (F) Cell lysis. (G) RT reagent introduction. (H) PCR reagent introduction. Scale bar: 1cm.

## 3. On-chip Heater Calibration and Thermal Characterization

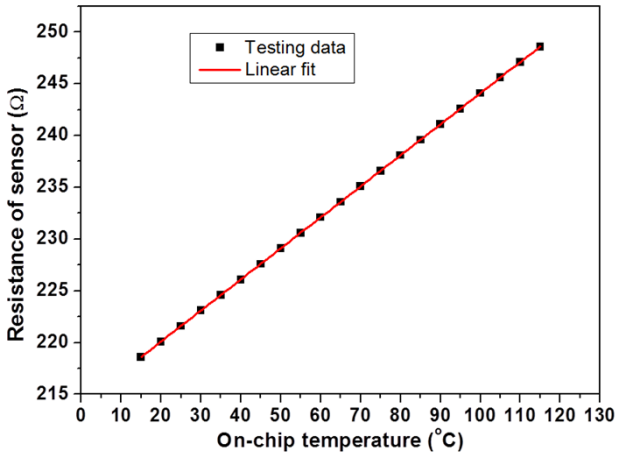


**Figure SI-3.** Resistance of temperature sensor (R) shows highly linear dependence on temperature (T). The solid line represents a linear fit to the experimental data with a regression equation: $R=R_0\,[1+1.36\times10^{-3}(T-T_0)]$, (coefficient of determination $R^2$=0.998)

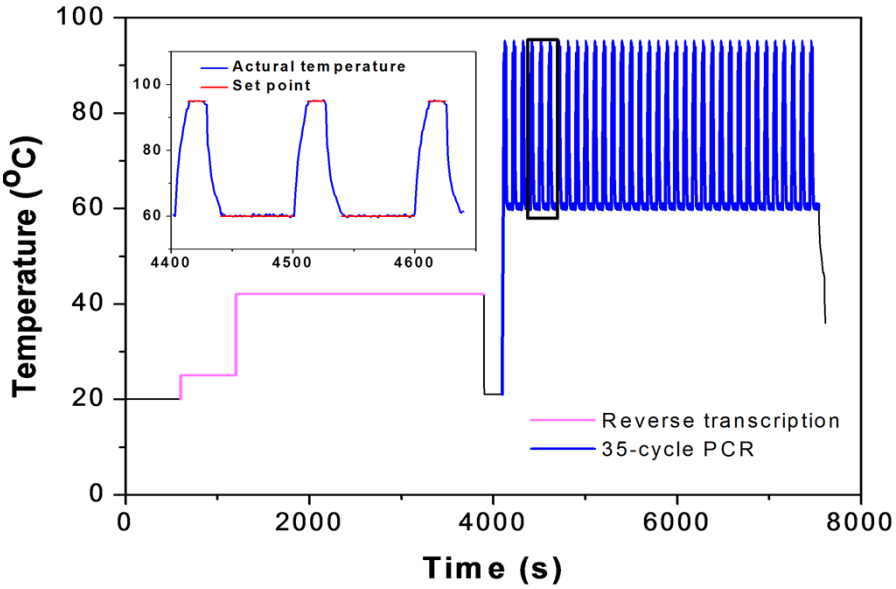


**Figure SI-4.** Time-resolved tracking of the chamber temperature. A typical RT (10 min at 25 °C and 50 min at 42 °C) and a 35-cycle PCR (15 s at 95 °C and 1 min at 60 °C) processes are fully integrated in the platform.

The temperature sensor was characterized to enable accurate on-chip temperature control. The chip was placed in a temperature-controlled environmental chamber (9023, Delta Design Inc., CA). Using platinum resistance temperature detector probes (Hart Scientific 5628), the temperature of the chamber was measured and the corresponding on-chip resistance was measured by a digital multimeter (Agilent 34420A). The measured resistance (R) of the gold temperature sensor was observed to vary linearly with temperature (T). The dependence could be represented by the relationship $R=R_0\,[1+\alpha(T-T_0)]$, where $R_0$ is the sensor resistance at a reference temperature $T_0$, and α is the temperature coefficient of resistance (TCR) of the sensor. Fitting this relationship to the measurement data determined the values of the parameters, which were used to determine the chamber temperature from the measured sensor resistance during single-cell RT-qPCR experiments. The temperature sensor had a measured resistance of 217.2 Ω at a reference temperature of 14.9 °C with a TCR of $1.363\times10^{-3}$ 1/°C, as shown in Figure SI 4. Based on this, we evaluated the accuracy and precision of the system over the course of RT and 35 consecutive cycles PCR. The accuracy was computed as difference between the set point and measured average temperature. The precision was defined as the average of the measured standard deviation of temperature variation at set point. For the RT step, with set points of 25 °C and 42 °C, we measured the temperature accuracy of 0.11 °C and 0.16 °C and the precision was 0.08 °C and 0.1 °C. In parallel, for the PCR step, the accuracy of the two set points (denaturing at 95 °C, annealing/ extension at 60 °C) was 0.53 and 0.21 and the precision was 0.16 °C and 0.14 °C. The chip achieved target temperatures with minimal overshoot (<10s). All these results indicate that the chamber temperature can be controlled to produce accurate and rapid amplification reactions. The time course of temperature during this control test is shown in the Fig. SI-4.

**4. Microscope Imaging Calibration**

The fluorescent images were acquired by Olympus IX81 motorized inverted microscope at the end of each PCR cycle. A 10X objective magnification was used. The microscope leveraged a halogen light source powered by an external power supply/control box to remove a significant source of electrical noise and heat from the microscope frame. The software IX2-BSW Ver.01.07 was used to acquire images and the fluorescent intensity of the images were quantified using Image J.

Background subtraction and flat-filed compensation were used to minimize the effect of background noise on the qPCR images. Prior to image processing FAM filter and ROX filter background images were recorded of the empty reaction chamber using the FAM filter and the ROX filter. The average fluorescent intensities from these images were respectively subtracted, using Image J, from the FAM and ROX images of the reaction chamber recorded during qPCR. Flat-field correction was achieved by capturing images after closing the camera shutter and then subtracting these dark-current images from the images acquired during qPCR.

In addition, we confirmed the linear response of the camera using a series of fluorophore dilutions. We diluted the DIO dye to a range of concentrations (20-80 μL/mL) and calculated the fluorescent intensity using Image-J. The Hamamatsu CCD camera has a 12 bit digital output dynamic range and the results of the camera response testing have been included in Figure-SI 5.

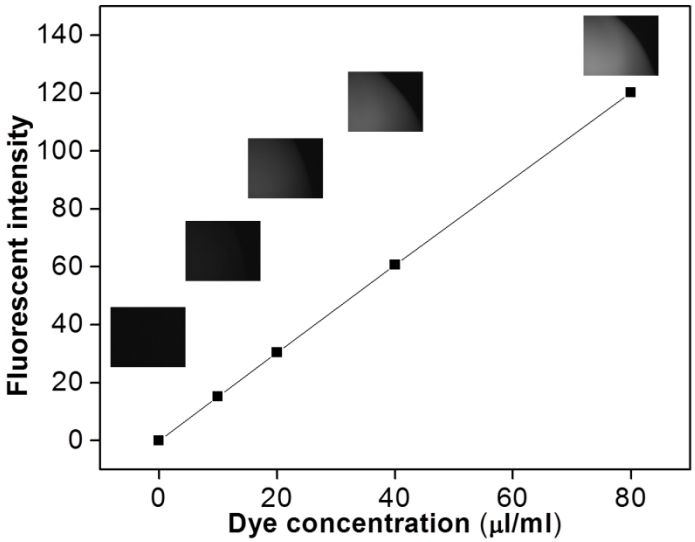


**Figure SI-5.** Camera response testing

## 5. ROX Intensity Detection During the Entirety of On-chip RT-qPCR

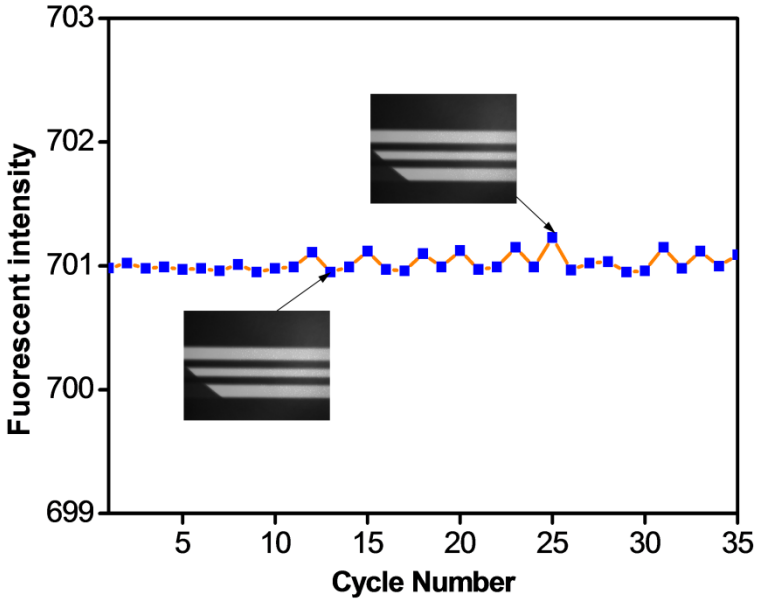


**Figure SI-6** ROX intensity during the real-time PCR process

From Figure SI-6, the steady ROX fluorescent intensity, in addition to the constant pathlength during the qPCR process indicates stable reagent concentrations.

## 6. No-template Control Testing of On-chip RT-qPCR

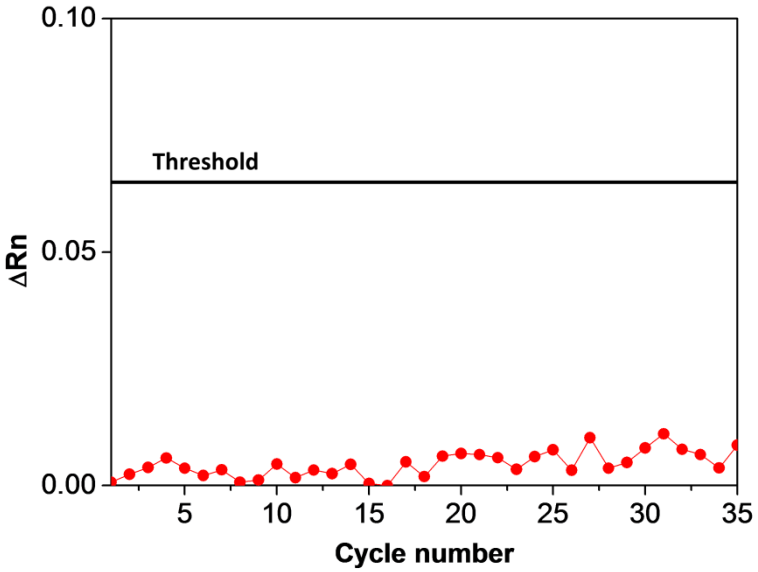


**Figure SI-7.** NTC testing of the fully integrated on-chip single-cell RT-qPCR.

## 7. Solution-Phase In-tube RT-qPCR

First, we added 10 μL 10 mM Tris-HCl to the tubes containing different copy numbers of bead-bound mRNA. Incubated the tubes at 75 °C to 80 °C for 2 min, then placed the tubes on a magnet and quickly transfer the supernatant containing the mRNA to new RNase-free tubes. Finally, solution-phase RT-qPCRs were performed using freely diffused oligo(dT) primer..

## Table SI-1. On-chip Experimental Validation

| **Protocol 1: Two-step RT-qPCR for validation testing** | | | | |
|---|---|---|---|---|
| Pipette 0.2 µL XenoRNA to 0.5 µL bead pre-incubated chip and mix them for 10 min | | | | |
| | Reaction component | Volume (µL) | | |
| | $MgCl_2$ (25 mM) | 0.22 | | |
| | dNTP (10 mM, 2.5 mM each) | 0.2 | | |
| | RT buffer (10X) | 0.1 | | |
| | Reverse Transcriptase (50 U/µL) | 0.025 | | |
| | RNase inhibitor (20 U/µL) | 0.02 | | |
| | RNase free water | 0.435 | | |
| | **Total** | 1.0 | | |
| Pipette the RT master mix to the chip and flush through the reaction chamber while immobilize bead/mRNA by a magnet. | | | | |
| Place the chip on the heating stage and follow the conditions: | | | | |
| 25°C | | 10 min | | |
| 42°C | | 50 min | | |
| Place the chip on ice and pipette PCR reagents to the chip: | | | | |
| Reaction component | Volume (µL) | | | |
| PCR master mix (2X) | 0.5 | | | |
| XenoRNA assay (20X) | 0.05 | | | |
| PCR grade water | 0.45 | | | |
| **Total** | 1 | | | |
| Place the chip on the heating stage and follow the thermal cycling conditions: | | | | |
| Step | UDG Incubation | Enzyme activation | PCR | |
| | Hold | Hold | Cycle (35 cycles) | |
| | | | Denature | Anneal/Extend |
| Temperature | 50°C | 95°C | 95°C | 60°C |
| Time | 2 min | 10 min | 15 s | 1 min |
| Test the fluorescent intensity of the PCR products at the end of PCR. | | | | |

## Table SI-2. Bead Quantity Optimization

| Protocol 2: Two-step RT-PCR for bead quantity optimization | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reaction component | Volume (μL) | | | | | | | | | |
| | XenoRNA (100,000 copies/μL) | 1.0 | | | | | | | | | |
| | Oligo$(dT)_{25}$ bead ($10^6$) | 0.75 | 1.5 | 2.25 | 3 | 3.75 | 4.5 | 5.25 | 6 | 6.75 | 7.5 |
| | $MgCl_2$ (25 mM) | 1.1 | | | | | | | | | |
| | dNTP (10 mM, 2.5 mM each) | 1.0 | | | | | | | | | |
| | RT buffer (10X) | 0.5 | | | | | | | | | |
| | Reverse Transcriptase (50 U/μL) | 0.125 | | | | | | | | | |
| | RNase inhibitor (20 U/μL) | 0.1 | | | | | | | | | |
| | RNase free water | 1.075 | 0.975 | 0.815 | 0.775 | 0.675 | 0.575 | 0.475 | 0.375 | 0.275 | 0.175 |
| | **Total** | 5.0 | | | | | | | | | |

| Place the tubes in the thermal cycler and follow the conditions: | |
|---|---|
| 25℃ | 10 min |
| 42℃ | 50 min |

| Place the 5 μL-RT products on ice and pipette PCR reagents: | |
|---|---|
| Reaction component | Volume (μL) |
| PCR master mix (2X) | 10.0 |
| XenoRNA assay (20X) | 1.0 |
| PCR grade water | 4.0 |
| **Total** | 20.0 |

| Place the tubes in the thermal cycler and follow the thermal cycling conditions: | | | | |
|---|---|---|---|---|
| Step | UDG Incubation | Enzyme activation | PCR | |
| | Hold | Hold | Cycle (35 cycles) | |
| | | | Denature | Anneal/Extend |
| Temperature | 50℃ | 95℃ | 95℃ | 60℃ |
| Time | 2 min | 10 min | 15 s | 1 min |
| Test the fluorescent intensity of the PCR products. | | | | |

## Table SI-3. On-chip mRNA Capture Efficiency Testing

| **Protocol 3: Two-step RT-qPCR for mRNA capture efficiency testing** | | | | | | |
|---|---|---|---|---|---|---|
| | Reaction component | Volume (μL) | | | | |
| | XenoRNA binding waste | 0.1 | 0.2 | 0.5 | 1.0 | |
| | Oligo$(dT)_{25}$ bead | $3.75\times10^6$ | | | | |
| | $MgCl_2$ (25 mM) | 1.1 | | | | |
| | dNTP (10 mM, 2.5 mM each) | 1.0 | | | | |
| | RT buffer (10X) | 0.5 | | | | |
| | Reverse Transcriptase (50 U/μL) | 0.125 | | | | |
| | RNase inhibitor (20 U/μL) | 0.1 | | | | |
| | RNase free water | 1.575 | 1.475 | 1.175 | 0.675 | |
| | **Total** | 5.0 | | | | |
| Place the tubes in the thermal cycler and follow the conditions: | | | | | | |
| 25℃ | | 10 min | | | | |
| 42℃ | | 50 min | | | | |
| Place the 5 μL-RT products on ice and pipette PCR reagents: | | | | | | |
| Reaction component | Volume (μL) | | | | | |
| PCR master mix (2X) | 10.0 | | | | | |
| XenoRNA assay (20X) | 1.0 | | | | | |
| PCR grade water | 4.0 | | | | | |
| **Total** | 20.0 | | | | | |
| Transfer the reagents to the real-time PCR system and follow the thermal cycling conditions: | | | | | | |
| Step | UDG Incubation | Enzyme activation | PCR | | | |
| | Hold | Hold | Cycle (40 cycles) | | | |
| | | | Denature | Anneal/Extend | | |
| Temperature | 50℃ | 95℃ | 95℃ | 60℃ | | |
| Time | 2 min | 10 min | 15 s | 1 min | | |
| Fluorescent intensity was tested by the ABI system (HT 7900 real-time PCR) | | | | | | |

## Table SI-4. PCR Efficiency, Sensitivity and Repeatability testing

| **Protocol 4: Two-step RT-qPCR for efficiency, sensitivity and repeatability testing** | | | | | | |
|---|---|---|---|---|---|---|
| | Reaction component | Volume (μL) | | | | |
| | XenoRNA (100,000 copies/μL) | 0.1 | 0.2 | 0.5 | 1.0 | |
| | $Oligo(dT)_{25}$ bead | $3.75\times10^6$ | | | | |
| | $MgCl_2$ (25 mM) | 0.22 | | | | |
| | dNTP (10 mM, 2.5 mM each) | 0.2 | | | | |
| | RT buffer (10X) | 0.5 | | | | |
| | Reverse Transcriptase( 50 U/μL) | 0.025 | | | | |
| | RNase inhibitor (20 U/μL) | 0.02 | | | | |
| | RNase free water | 0.035 | | | | |
| | **Total** | 1.0 | | | | |
| Pipette the RT master mix to the chip and flush through the reaction chamber while immobilize bead/mRNA by a magnet. Then perform on-chip RT follow the thermal conditions: | | | | | | |
| 25℃ | | 10 min | | | | |
| 42℃ | | 50 min | | | | |
| Prepare PCR reagents: | | | | | | |
| Reaction component | Volume (μL) | | | | | |
| PCR master mix (2X) | 1 | | | | | |
| XenoRNA assay (20X) | 0.1 | | | | | |
| PCR grade water | 0.9 | | | | | |
| **Total** | 2.0 | | | | | |
| Pipette the PCR reagents to the chip and follow the thermal cycling conditions: | | | | | | |
| Step | UDG Incubation | Enzyme activation | PCR | | | |
| | Hold | Hold | Cycle (35 cycles) | | | |
| | | | Denature | Anneal/Extend | | |
| Temperature | 50℃ | 95℃ | 95℃ | 60℃ | | |
| Time | 2 min | 10 min | 15 s | 1 min | | |
| Test the fluorescent intensity of the PCR products at the end of each cycle. | | | | | | |
| For the in-tube testing, using the same volume, qPCR were performed by ABI system. | | | | | | |