Supporting Information

Single-Cell Pick-up System

A glass capillary tube (O.D.: 1.0 mm; I.D.: 0.58 mm; Harvard Apparatus) was pulled using a CO_2 laser puller (model P-2000, Sutter Instrument, CA, USA). The outer diameter of the glass capillaries was within the range of 3–10 μm. The inner and outer surface of the microcapillary probe was silanized with fluorosilane LS-912 (3,3,4,4,5,5,6,6,6-non-afluorohexyltrichlorosilane; Shin-etsu Chem. Ind. Co., Ltd.) by gas-phase silanization under a nitrogen atmosphere for 2 h at room temperature. The fluorosilane coating process was essential in preventing the adsorption of the cytosol and nucleic acids to the inner/outer glass surface of the probe. Before use, the microcapillary probe was washed with 99.5% ethanol, dried, and exposed to UV light in a clean bench (Oriental Giken, Inc.) for at least 30 min. The microcapillary probe was used to pick-up single cells while these were observed under an optical microscope. The position of the probe was controlled using an oil-derived three-axis manual micromanipulator (MNO-203; Narishige). The single-cell pick-up operation was accomplished within 10 min after electrochemical evaluation.

Single-Cell mRNA Analysis

The solution (less than 1 μL) containing a single or lysed cell was collected using a pick-up capillary and transferred into a 0.2-mL PCR tube. Lysis buffer (Lysis Buffer RLT from RNeasy Micro Kit; Qiagen) solution (75 μL) was added to the PCR tube and vortexed. The RLT buffer deactivates RNase and stabilizes RNA. Carrier RNA (Qiagen) (5 μL) was added to prevent the loss of the target mRNA. Total RNA purification was performed according to the protocol for the RNeasy Micro Kit (Qiagen). The RT reaction was carried out to synthesize first-strand cDNA, following the protocol of the QuantiTect reverse transcription kit (Qiagen) at 42°C for 30 min (RT reaction), followed by 95°C for 3 min (deactivation of RTase). Synthesized cDNA sample solutions with a final volume of 20 μL were stored at -30°C. Quantitative PCR (qPCR) was performed using the LightCycler 1.5 System (Roche) and the Light-Cycler Fast Start DNA Master kit (Roche) using a total volume of 20 μL in the glass capillaries. Approximately 2 μL of the cDNA sample, 1.6 μL of 25 mM $MgCl_2$, 1 μL of the 10 μM forward (Fw) primer, 1 μL of the 10 μM reverse (Rv) primer, 2 μL of SYBR Green, and 12.4 μL of H_2O were added. After initial denaturation at 95°C for 10 min, 45 PCR cycles were performed with denaturation at 95°C for 10 s, annealing at the annealing temperature of the individual primer pair as mentioned below for 10 s, and extension at 72°C for 13 s. Primers for the

glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and POU domain transcription factor (Oct4) were designed and synthesized by Nihon Gene Research Laboratories, Inc. The actual sequences and annealing temperatures of the primers were as follows: Gapdh (Fw) 5′-AAA TGG TGA AGG TCG GTG TG-3′, (Rv) 5′-AGG TCA ATG AAG GGG TCG TT-3′; Oct4 (Fw) 5′-TGT GGA CCT CAG GTT GGA CT -3′; and (Rv) 5′-AGG TTC CCT CTG AGT TGC TT -3′. The expression level of each targeted gene was normalized to the expression level of the housekeeping gene Gapdh. Real-time PCR was performed for at least two genes using the 2 μL of the cDNA samples obtained from the source solution (20 μL).

Bulk mRNA Analysis

We also performed bulk measurements using cells at a density of 5×10^5. In this case, the medium of the suspension of ES cells was replaced with the RLT lysis buffer (Qiagen). Lysis buffer (Lysis Buffer RLT from RNeasy Micro Kit; Qiagen) solution (350 μL) containing 5×10^5 ES cells were added to the PCR tube and vortexed. The condition of total RNA purification, RT reaction and qPCR were same as the condition in single-cell mRNA analysis. The expression level of each targeted gene was normalized to the expression level of the housekeeping gene Gapdh. Real-time PCR was performed for at least two genes using the 2 μL of the cDNA samples obtained from the source solution (20 μL).