

samples used are Kanakin 3 [8]. Fabric swatches were prepared as 1.0 cm × 1.0 cm squares, wrapped with aluminum foil, autoclaved at 121°C for 15 min, and dried under sterile conditions.

#### Fluorescent glucose derivative treatments

Synthesis of 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy glucose (2-NBDG) was conducted following the protocol described elsewhere [18].

A 200 µl inoculum of the seed culture containing about  $5 \times 10^6$  cells of *C. albicans* was inoculated onto a swatch, and the swatch was placed on Trypticase Soy Agar (TSA) plates. After incubation at 33°C for 1 h, the remaining aqueous liquid on the swatch was removed by Ultrafree-MC centrifuging treatment (6,000 rpm × 30 s). To obtain individual components amenable to microscopic observations, the centrifuged swatch was untied and dissected to individual strings with pre-sterilized tweezers. The individual fabric strings were set on a glass slide upon which a square grid has been imprinted. Approximately 2.0 ml of 0.2% agarose solution containing 12 µM 2-NBDG was prepared in molten condition at 46°C, and poured onto the reassembled fabric strings on the slide glass. The glass slide was kept in a petri-dish and incubated for 1 h at 33°C to facilitate the uptake of 2-NBDG by *C. albicans*.

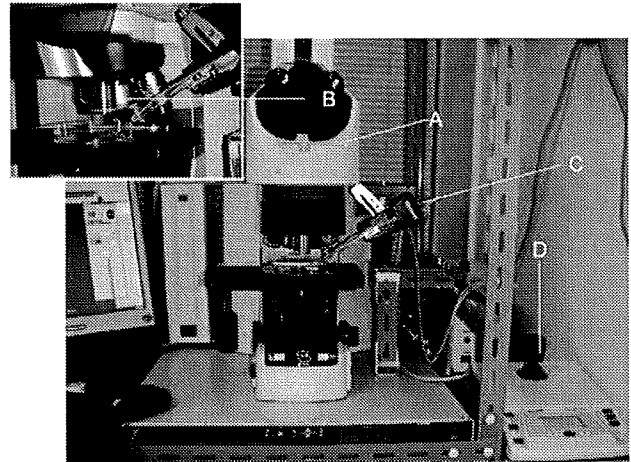
#### Microscope and manipulation systems

After incubation, the glass slide was examined with a UDF fluorescent microscope system. The detailed components of the UDF fluorescent system are described previously [4, 5]. Ultra long distance industrial optical lenses (CFI Plan EPI SLWD 50 and 30, NIKON Co., working distance 17.0 and 24.0 mm, respectively) and a semi-automatic cell injection manipulator (InjectMan NI2, Eppendorf Co.) were integrated to assemble a cell manipulation system (Fig. 1). InjectMan NI2 was fixed on the UDF system stage with steel frames.

To prepare glass capillaries for the yeast cell manipulation, borosilicate glass tubes (BF100-78-10, diameter 1.0–0.78 mm, Sutter Instrument Co.) were pulled with a laser puller (P-2000, Sutter Instrument Co.), sterilized with anhydrous ethyl alcohol and dried in a dry oven at 50°C for 48 h. For cell manipulation, a sterile silicone tube 2 mm in diameter was connected to the capillary and to the InjectMan NI2.

#### DNA sequence analyses

Isolated cells were incubated on TSA plates at 33°C for 48 h to prepare sufficient cell mass for DNA sequence analyses. The harvested cells were transferred to a 1.8 mL



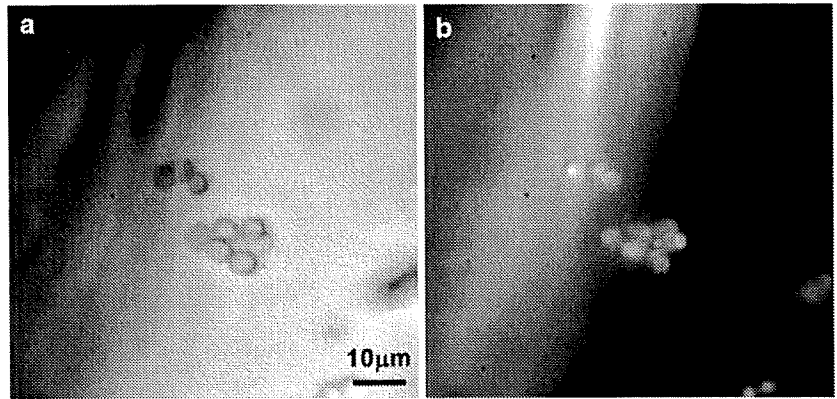
**Fig. 1** A UDF fluorescent microscope system with a cell manipulation system. A microscope, B ultra long distance industrial optical lens, C manipulator, D operation module of manipulator

serum tube and frozen in liquid nitrogen. Frozen cells were treated with homogenization pestle to break the cell wall. This process was repeated two times. DNA was extracted from the homogenate with E.N.Z.A. Fungal DNA Kit (Omega Bio-tek, Inc.). PCR amplification was conducted on the 26S rDNA D1/D2 regions [9] with primers NL-1(5'-CGATATCAATAAGCGGAGGAAAAG) and NL-4(5'-GGTCCGTGTTTCAAGACGG) [12] with a thermal cycler (PTC-200 Peltier Thermal Cycler, MJ Research Co.) under the conditions of 95°C × 10 s + 50°C × 60 s + 70°C × 60 s (denaturation, annealing, and extension, 30 cycles), and 72°C × 10 min (extension). The PCR products obtained were purified with QIA quick PCR Purification Kit (QIAGEN Co.) and DNA sequence analyses were done with PRISM3100 genetic Analyzer (ABI Co.).

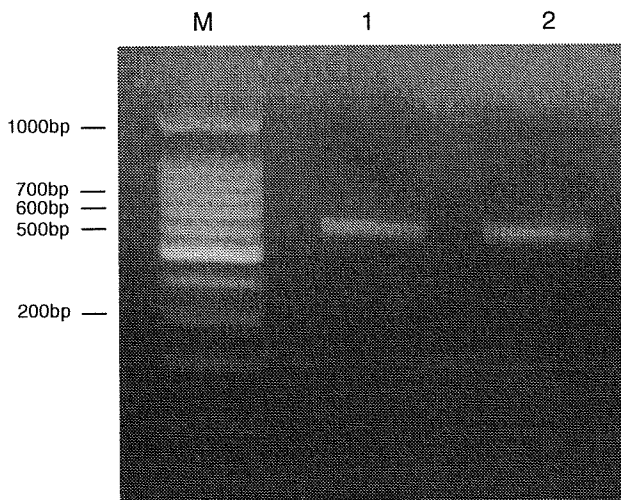
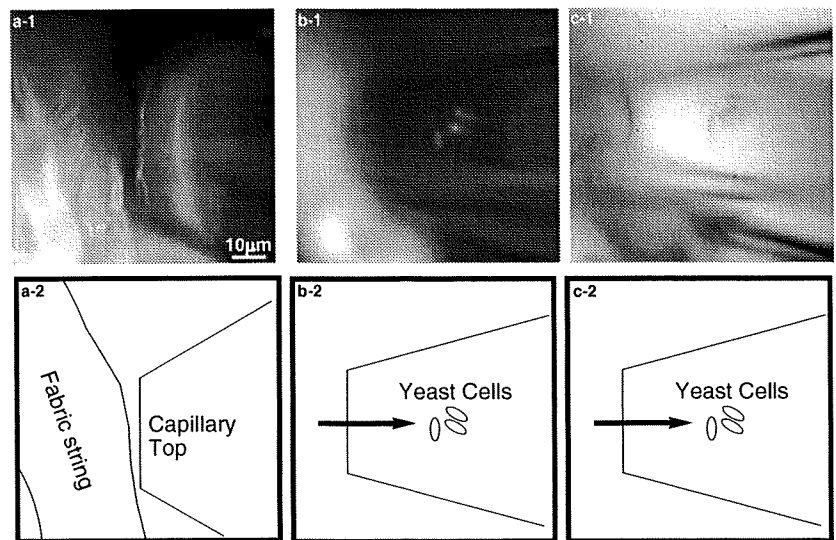
#### Results and discussion

The fluorescent images indicate viable cells attaching on the surface of fabrics and their morphologies can be recognized (Fig. 2). Following this cell detection, a glass capillary was inserted in molten agar and placed adjacent to cells (Fig. 3a, fluorescent image). Then the cells were sucked into the capillary (Fig. 3b, c). The isolated cells were cultured as described and subjected to DNA sequence analysis at D1/D2 domain in 26S rDNA to certify that the originally inoculated strain was recovered. The gel electrophoresis band picture of the PCR product is shown in Fig. 4. A single band appeared at the same position as that obtained from the originally inoculated cells. The DNA sequence analyses data of 572 bp indicated 100% sequence matching. These indicate the clear traceability of the inoculated strain.

**Fig. 2** Microscopic images of *Candida albicans* observed on fabric before cell isolation. **a** Optical image, **b** fluorescent image



**Fig. 3** Cell isolation procedure. **a** Glass capillary inserted to molten agar coating the fabric (optical image). **b, c** *C. albicans* cells sucked in a capillary stored in the glass capillary [optical image (**b**) and fluorescent image (**c**)]



**Fig. 4** Gel electrophoresis band picture of the PCR products. A total of 2% agarose, TAE Buffer, *M* Takara 100 bp ladder as a marker, *1* DNA derived from the inoculated strain, *2* DNA derived from the manipulated/isolated strain

Successful visualization and manipulation demonstrate the applicability of the present system to the detection of very low numbers of microbial cells and for their successive

culture. The first is to detect viable cells rapidly and the next is to investigate them carefully depending upon the necessity.

Single-cell manipulation supporting technologies have recently gained marked progress [19, 20] and therefore the present system may be advanced to a higher throughput system in response to practical needs.

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