**PROTOCOL FOR: High-yield expression of periplasmic scFvs by solid *Escherichia coli* cultures**

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**Preparation of expression vectors**

1. All vectors were driven by a T7 promoter. An scFv that recognizes the constant region of rabbit IgG was constructed from the sequence of a monoclonal IgG1 antibody generated from an A10B hybridoma cell line [1]. scFv(A10B) consists of VH and VL sequences joined by the linker sequence GGGGSGGGGSGGGGS. DNA for scFv(A10B) was synthesized by GenScript (Piscataway, NJ ), digested with the EcoRI and NotI restriction enzymes, and inserted into the pET22b(+) (Merck Millipore, Darmstadt, Germany) vector for periplasmic protein expression.
2. scFv against rabbit IgG [2], human IgG [3], *Gaussia* luciferase [4] and scFv against rabbit IgG from the chicken IgY library [5] have been described previously. These scFvs were digested with EcoRI and NotI and cloned into pET22b(+) vectors to create anti-rabbit IgG scFv, anti-human IgG scFv and anti-*Gaussia* luciferase scFv.
3. After the nucleotide sequences were confirmed, the resulting expression plasmids were transformed in *E. coli* BL21 (DE3) (Merck Millipore). The transformants were cultured at 37°C in Luria-Bertani (LB) medium containing 50 μg/mL of ampicillin. The glycerol stock was made.

**Expression of scFvs**

1. Inoculi were prepared by adding 100 µL high-cell-density glycerol stocks (optical density at 600 nm, OD600 of 20–30) containing scFv-expressing clones to 3 mL LB medium containing 50 μg/mL of ampicillin and incubating for 4 h at 37 °C and 200 rpm in a shake flask.
2. For solid cultivation, a hydrophilic membrane was placed on filter paper soaked with 20 mL TB medium containing 50 μg/mL of ampicillin, and fitted in a 90-mm dish. The membranes and filters were autoclaved (15 min at 121 °C ) for sterilization. Inoculum (100 ml) was suspended in 100 ml of an auto-induction solution containing 0.05% glucose and 0.1% lactose, spread on the membrane, and grown at 26 °C in a humidified incubator (MIR-154S, PHCbi, Japan). The humidity in the incubator was kept saturated. Then, the filter was transferred to a new dish. All cells were scraped from the filter and suspended in 20 mL LB.
3. For shake-flask cultivation, the overnight culture was inoculated in a 200-mL flask containing 20mL TB medium containing 50 μg/mL of ampicillin and incubated at 37 °C and 200 rpm. At OD600 of 0.5, expression was initiated by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM. The cells were incubated overnight at 26 °C, harvested by centrifugation at 6,000 × *g* and 4 °C, and washed with with phosphate-buffered saline (PBS).

**Extraction of periplasmic scFvs**

1. Harvested cells were suspended in 2 ml of Spheroplasting buffer (10 mM Tris-HCl, 20% sucrose pH 8.0). EDTA was added at a final concentration of 1.0 mM, and the suspension was rotated at 4°C for 30 min.
2. The cells were centrifuged at 23000 × g at 4°C for 10 min. The supernatants were discarded. The cells were suspended in 2 ml of ice-cold sterile water. The suspension was incubated on ice for 10 min.
3. The cells were centrifuged at 23000 × g at 4°C for 10 min. The supernatants were collected as the periplasmic extract.

**Purification of recombinant scFvs from the periplasmic extracts**

1. The periplasmic supernatants for scFvs were collected and diluted with binding buffer (20 mM phosphate, 0.5 M NaCl, and 20 mM imidazole [pH 7.6]).
2. The diluents were filtered through a 0.22-μm filter and loaded onto a 1-mL HisTrap-FF column (GE Healthcare, Wauwatosa, WI) that was equilibrated with binding buffer.
3. The His-tagged fragments were eluted with a linear gradient of 20- 500 mM imidazole in binding buffer. The purified scFvs were dialyzed against phosphate buffered saline (PBS) (pH 7.4) and stored at −30°C.

**ELISA**

1. Each well of 96-well ELISA plates was coated with 100 μL of 5 μg/mL human IgG, and then a blocking solution (Blocking Reagent for ELISA; Roche Diagnostics, Swiss) was applied and the plates were incubated for 2 h.
2. The plates were washed with PBS-T, after which 100 μL of the periplasmic extract of bacterial cultures was added to each well. The wells were washed and then HRP-labeled anti-His antibody was added.
3. The amount of the antigen-specific antibody present was measured using an HRP substrate kit (Sigma-Aldrich, St. Louis, MO), and the plates were read using a microplate reader (Model 680; Bio-Rad Laboratories, Hercules, CA), at a wavelength of 405 nm.

**References**

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