Material & Method

1. PCR

   PCR was performed to amplify the target DNA fragment.

   We used PrimeSTAR® HS (Premix) (Takara Bio,JAPAN).

   1) Template DNA, 2x master mix, forward and reverse primer were mixed in a PCR tube.
   2) Reaction cycles: [98 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 30 seconds] x 35 cycles

DW	8.2 (μL)
Premix	10 (μL)
forward primer (10 μM)	0.4 (μL)
reverse primer (10 μM)	0.4 (μL)
template	1.0 (μL)

2. Restriction enzyme digestion

   Restriction enzyme digestion was performed following the manufacturer's instructions.

3. Electrophoresis

   To confirm the length of DNA chains, agarose gel electrophoresis was used.

   1) 1% agarose gel containing trace amount ethidium bromide was prepared.
   2) For each sample, loading buffer (Takara Bio, JAPAN) 1 μL and sample DNAs (typically 2μL) were mixed. As a size marker, λ-StyI ladder was used.
   3) The gel images were photographed with a gel imager.

1×TAE	50 ml
agarose	50 μg
ethidium bromide	1 μl

4. Miniprep
   We used the Wizard® Plus Minipreps DNA Purification System kit (Promega).

5. Gel extraction and PCR purification
   We used Wizard® SV Gel and PCR Clean-Up System (Promega).

6. Transformation
   1) Competent cells are stored at -80℃.
   2) 10μL competent cells and 2.5μL plasmid DNA were mixed and incubated for 15 minutes on ice.
   3) Cells were heat shocked at 42℃ for 45 seconds.
   4) For each tube, 100μL SOC medium was added.
   5) Cells were incubated for 40 minutes at 37℃.
   6) Cells were spread on LB plates and incubated for 24 hours at 37℃

7. Sequencing
   DNA sequencing was performed by AB 3030xl Genetic Analyzers using standard protocol.

8. *E. coli* culture
   E. coli overnight culture was grown in LB medium (5g Bacto Tryptone, 1.25g Bacto Yeast Extract, 1.25g NaCl in 500 mL) at 37℃. For miniprep, usually 3mL culture was prepared by 14-16 hours shaking (180 rpm) at 37℃.

9. Protein expression and purification
   For protein expression, we made 200mL culture of BL21(DE3) strain with expression plasmids. Cells were grown to OD=0.5 at 37℃ and were induced by adding IPTG at 0.5 mM (final). 2 hours later, cells were harvested and frozen at -80℃ freezer.

   To purify recombinant protein, Ni-NTA was used as the following protocol.

   1.1. 2 mL of Lysis buffer were added to the cell pellets.
   2. Cells were resuspended thoroughly.
   2.1. Lysis cell by Sonification (BRANSON Sonifier 2) 50% 1min x 4 on ice.
   2. Cell debris were collected by centrifugation at 5000 rpm for 5 minutes.
   3.1. 1.5mL of Supernatant was collected and added to 400μL of washed Ni-NTA agarose beads.
   2. Rotate at 4℃ for 1 hour.
   4.1. Remove 1.5 mL of supernatant.
   2. Add 1.5 mL of Lysis buffer.
   3. Mix by inverting.
   4. Centrifuge at 15,000 rpm FLASH.

repeat this step for 6 times.

5.1. Remove 1.5 mL of supernatant.
2. Add 400 µL of Elution buffer (Lysis buffer containing 0.25M Imidazole).
3. Mix by inverting.
4. Centrifuge at 15,000 rpm FLASH.
5. Take 400 µL of supernatant as eluted fraction for a new tube
   repeat this step for 6 times (Get 6 eluted fractions).

10. SDS-PAGE

To visualize purified proteins, SDS-PAGE was performed.

1. make gel by using the recipe indicated below.
2. prepare 2× SDS Sample buffer and add it to the sample.
3. Vortex & Flash, Boil at 98 °C (5min), Vortex & Flash.
4. apply marker and sample to gel.
5. electrophoresis at 200V for about 35 minutes.

	separation gel	concentration gel
DW	5.3 ml	3.4 ml
acrylamide	2.0 ml	0.83 ml
Tris-HCl	2.5 ml	0.63 ml
10%APS	0.1 ml	0.05 ml
10%SDS	0.1 ml	0.05 ml
TEMED	0.008 ml	0.005 ml

total	10 ml	10 ml

11. CBB stain

CBB stain was used to visualize protein bands.

1. wash the gel 3 times (5 minutes) by DW.
2. stain it by CBB for 1 hour with shaking.
3. wash it by DW and decolorizing it by deionized water with shaking
4. keep it in DW.

12. Centrifugal Filter Devices

We used the Amicon® Ultra-0.5 Centrifugal Filter Devices bought from Millipore Corporation.

13. Film blotting

To visualize our protein bound on a PET film, the protocol below was used.

1. Film was washed by detergent containing water.
2. protein samples were spotted to the surface of PET film and incubated for 20 minutes at room temperature.
3. PET film was washed by TBST (below).
4. New PVDF membrane was immersed into a solution for AP.
5. The soaked PVDF membrane was placed on the film. The reaction was stopped by washing with TBST.
6. The membrane was scanned and visualized.

https://link.springer.com/article/10.1007/s00253-019-09760-9

TBST

50 mM Tris/HCl pH7.4,140mM NaCl,0.1%Tween20

14. Fiber binding assay

PET fibers were immersed into GFP-containing solution (control) and solution containing plastic binding protein. After a short time incubation, fibers were washed with buffer three times by TBST.

1. 20 cm of PET fiber was soaked in 200 μL of protein solution (200 ng/μL) for 20 min at RT.
2. Protein solution was removed and fibers were then washed by adding 500 μL of TBST for 5 minutes. This step was repeated 3 times.
3. 50μL of 2x SDS sample buffer was added to fibers to elute proteins.

15. Ultracentrifuge

To analyze the size of recombinant encapsulin, we used sucrose density gradient sedimentation assay.

1) 10%-60% linear sucrose gradient in 20 mM Tris 7.5, 50 mM NaCl was prepared.
2) *E. coli* lysates or imidazole eluates of encapsulin were loaded onto the top of the gradients.
3) Ultracentrifugation was performed at 100,000 × g for 18 hours at 4℃ with SW41 (Beckman).
4) Fractions were collected by BioComp and A260 was monitored and recorded.

16. Quantification of protein concentration

After purification, we quantified each protein's concentration by SDS-PAGE and CBB stain (Table 1). The intensity of each band was compared with BSA standards. The image quantification was done with ImageJ. In Fig. 2, the example of the BSA calibration line is shown.

As a result, each protein concentration was calculated, as shown in Table.1 below.

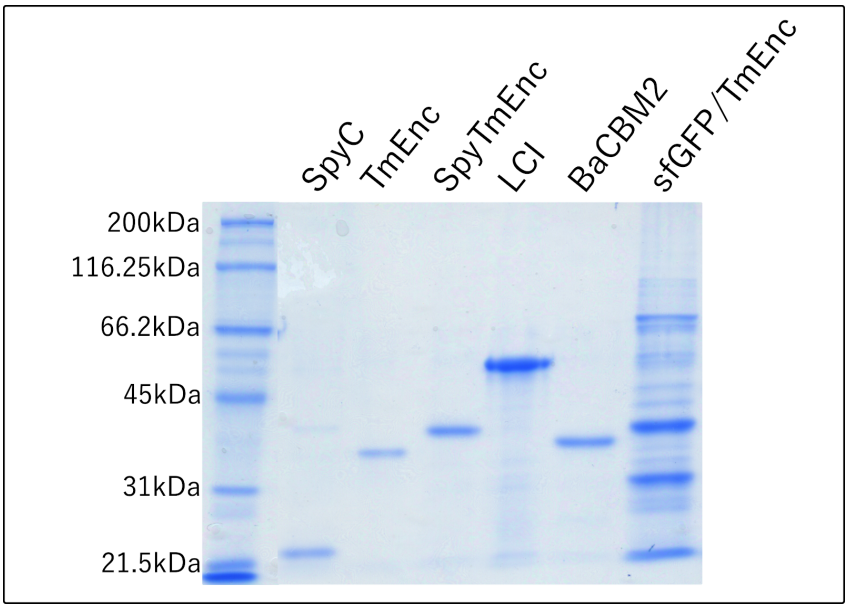

Fig. 1a SDS-PAGE gel of purified protein

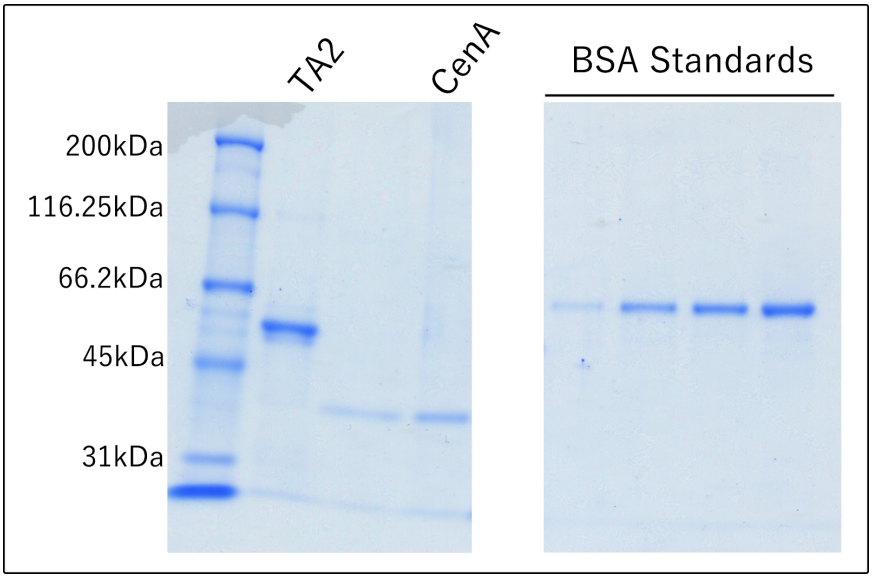

Fig. 2 SDS-PAGE gel for quantification of protein concentrations

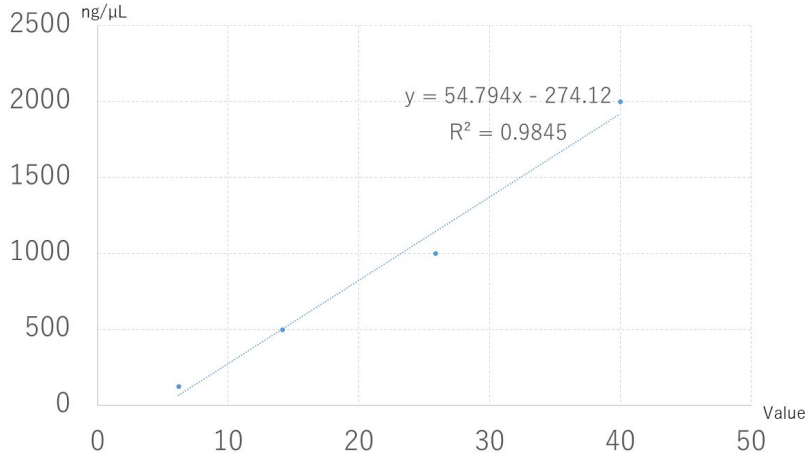

Fig. 3 Example of BSA calibration line