

## Expression and Purification of the Eukaryotic MBP-MOS1 Transposase from *Sf21* Insect Cells

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**[Abstract]** Here, we present the full-length protocol for purifying the recombinant MOS1 transposase from insect cells used in our recent publication (Pflieger *et al.*, 2014), which involved a N-terminal MBP-tag and maltose-affinity chromatography.

Due to their overall basic properties, transposases are often difficult to purify, especially because they tend to aggregate. Since the 90s, we chose a method of purification without a denaturation step. Our first priority was to preserve the 3D structure of the protein in order to maintain its biochemical activities with the highest specific activity. Nevertheless, our production/purification made from bacteria regularly contain truncated products (or degradation products) and their levels increase with concentration of purified transposase. In contrast, production/purification made from eukaryotic cells do not contain such degradation product. We thus developed a protocol involving the pVL1392 baculovirus transfer vector and the BaculoGold™ baculovirus expression system, allowing the expression of recombinant MOS1 from baculovirus-infected *Sf21* cells.

### Materials and Reagents

1. *Sf21* cells (Life Technologies, Gibco®, catalog number: 11497)
2. Insect Express Sf9/S2 medium (PAA, catalog number: E15-875)
3. Fetal bovine serum (Life Technologies, Gibco®)
4. Baculogold baculovirus expression system (BD Biosciences, catalog numbers: 554739 and 560129)
5. Anti-MBP anti-body (Santa Cruz, catalog number: sc-808)
6. Tris·HCl (pH 7.6)
7. NaCl
8. DTT
9. NP40
10. Antiprotease cocktails (like Roche's EDTA-free cocktail tablets, catalog number: 11-873 580-001)
11. Maltose

12. Bovine serum albumin (BSA)
13. Lysis buffer (see Recipes)
14. Binding buffer (see Recipes)
15. Elution buffer (see Recipes)

## **Equipment**

1. 25 cm<sup>2</sup>, 150 cm<sup>2</sup> flask (usual culture-cell equipment)
2. 1 ml prepacked column (GE Healthcare, MBPTrap™ HP) or 1 ml of amylose resin in batch (New England Biolabs)

## **Procedure**

### **A. *Sf21* culture conditions**

1. *Sf21* cells are cultivated in Insect Express Sf9/S2 medium (PAA) containing 5% fetal bovine serum (or FBS, PAA) at 27 °C (herein called “complete media”).
2. Every 4 to 6 days, a new 25 cm<sup>2</sup> flask is inoculated with 1 to 1.25 x 10<sup>6</sup> viable cells in 5 ml of complete medium.

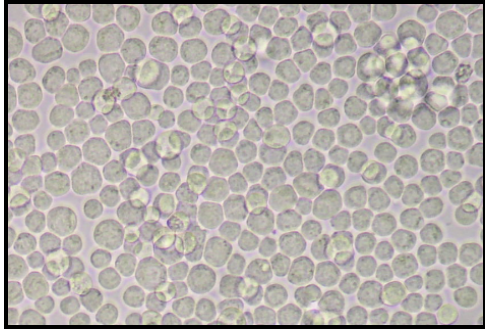
### **B. Generating a recombinant baculovirus that expresses the MBP-MOS1 fusion protein in *Sf21* cells**

1. The MBP-MOS1 coding sequence is cloned into the pVL-1392 at the *Sma*I restriction site.
2. High-titre, low-passage recombinant Baculovirus stocks are obtained in *Sf21* cells following the instructions of the “Baculogold baculovirus expression system” manufacturer (BD Biosciences) ([https://www.bdbiosciences.com/documents/Baculovirus\\_vector\\_system\\_manual.pdf](https://www.bdbiosciences.com/documents/Baculovirus_vector_system_manual.pdf)).
3. 5 to 10 batches of recombinant Baculoviruses are assayed for their ability to produce the recombinant MBP-MOS1 in infected *Sf21* cells by Western blot ([https://www.sciencepub.net/researcher/0102/09\\_1259\\_yang\\_west\\_research0102.pdf](https://www.sciencepub.net/researcher/0102/09_1259_yang_west_research0102.pdf)) with the anti-MBP anti-body (dilution 1/10,000). The most efficient isolate is selected for further protein production processes.

### **C. Producing the MBP-MOS1 fusion protein in *Sf21* cells**

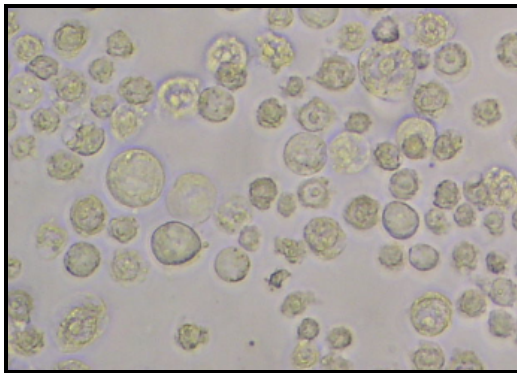
1. The selected baculovirus stock is used to infect *Sf21* cells.
2. Twenty-one 150 cm<sup>2</sup> flasks with 6 x 10<sup>7</sup> viable cells per flask in 30 ml of complete medium are prepared and incubated at 27 °C until the cells reach confluence (see Figure

1).



**Figure 1. Confluent non-infected Sf21 cells**

3. The medium is then replaced by 10 ml of medium without FBS and cells are infected with 100  $\mu$ l of high titre recombinant Baculoviruses stock solution (virus titer should be 1 to 2 x 10<sup>8</sup> pfu/ml).
4. Cells are incubated for 3 h at 27 °C and then 5 ml of complete medium (with 5% FBS) is added. The cells are incubated for 3 days at 27 °C, in the dark.
5. Check for signs of infection 2-3 days after inoculation. Cells should be enlarged in size (about 2 fold) and a large nucleus should be visible (see Figure 2).



**Figure 2. Infected Sf21 cells**

6. To pellet cells, gently dislodge cells from monolayers by smacking and transfer the cell suspension to a sterile centrifuge tube of appropriate size. Spin down cells at 10,000 x g for 5 min. The MBP-MOS1 protein will be found in the cell pellet, which can be stored at -80 °C or immediately processed.

#### D. MBP-MOS1 fusion protein extraction in crude extracts

1. Pellets are washed in 1x PBS and pooled so that one pellet contains the cells coming from three 150 cm<sup>2</sup> flasks.
2. Cytoplasmic crude extract (CCE).

- a. Each pellet is resuspended in 3.5 ml of lysis buffer.
    - b. Cells are lysed on ice for 30 min and then centrifuged at 15,000 x g and 4 °C for 30 min. The supernatant (≈30 ml) corresponds to the cytoplasmic crude extract (CCE).
    - c. Protein concentration is usually around 5 to 10 µg/µl (*i.e.* a total amount of about 125 to 250 mg), as determined by Bradford assay (<http://web.mnstate.edu/provost/bradfordproteinassayprotocol.pdf>) using BSA as a standard. Keep the CCE on ice.
  3. Nuclear crude extract (NCE)
    - a. Each pellet is resuspended in 0.75 ml of lysis buffer without NP40.
    - b. Sonicated on ice (Ultrasonic Processor, output power at 100%, 3 pulses of 15 sec each) and centrifuged at 10,000 x g and 4 °C for 10 min. The supernatant (≈ 6 ml) corresponds to the nuclear crude extract (NCE).
    - c. Protein concentration is usually around 5 to 10 µg/µl (*i.e.* a total amount of about 30 to 50 mg). Keep the NCE on ice.
- E. MBP-MOS1 fusion protein purification
- CCE and NCE previously obtained can be processed either separately or pooled. In the following, CCE and NCE are pooled together, giving a whole cellular crude extract. MBP-MOS1 can be purified by two ways: Using a 1 ml prepacked column or 1 ml of amylose resin in batch.
1. Prepacked column
    - a. Equilibrate the column with at least 5 ml of binding buffer at 0.5 ml/min.
    - b. Adjust the crude extract concentration to 1 mg/ml with the NCE lysis buffer. Apply the sample.
    - c. Wash with at least 10 ml of binding buffer (until no material appears in the flow-through). Elute with 5 ml of elution buffer, and collect into 0.5 ml fractions.
    - d. After one column volume (about 1 ml), the peak of purified MBP-MOS1 is eluted within two to three fractions, corresponding to those having the maximum UV absorbance at 280 nm.
    - e. Pool the positive fractions and store as 50 µl aliquots at -80 °C.
  2. Amylose resin in batch
    - a. Wash the resin with at least 5 ml of binding buffer. Add 1 ml of resin to the crude extract.
    - b. Incubate for 1 h at 4 °C with gentle mixing. Centrifuge at 800 x g and 4 °C for 5 min. Discard the supernatant.
    - c. Wash the resin pellet with at least 10 ml of binding buffer. Centrifuge at 800 x g and 4 °C for 5 min. Discard the supernatant. This is to be repeated five times.

- d. Elute the MPB-MOS1 with 1 ml of elution buffer; Centrifuge at 800 x *g* and 4 °C for 5 min. Recover the supernatant (E1 fraction).
- e. Elution can be repeated twice, giving E2 and E3 fractions.
- f. Highly concentrated fractions (E1 and often E2) are stored as 50 µl aliquots at -80 °C.
- g. MBP-MOS1 concentration in positive fractions (from both methods) is determined by Bradford assay using BSA as a standard.
- h. From twenty F1 50 cm<sup>2</sup> infected flasks, 1-2 ml at about 0.5 to 1 µg/µl is expected, depending on the efficiency of the infection.

### **Recipes**

1. Lysis buffer
  - 20 mM Tris·HCl (pH 7.6)
  - 100 mM NaCl
  - 1 mM DTT
  - 1% NP40
  - Antiprotease cocktails (according to the manufacturer's recommendations)
2. Binding buffer
  - 20 mM Tris·HCl (pH 9)
  - 100 mM NaCl
  - 1 mM DTT
3. Elution buffer
  - Binding buffer containing 10 mM maltose

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### **References**

1. Pflieger, A., Jaillet, J., Petit, A., Auge-Gouillou, C. and Renault, S. (2014). [Target capture during Mos1 transposition](#). *J Biol Chem* 289(1): 100-111.