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Expression and Purification of the Rice Clock protein, GIGANTEA

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Isabel A. Abreu: Revised and approved final version of the protocol



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Protocol status: Working

We use this protocol and it's working

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Abstract

GIGANTEA is an essential component of the plant circadian oscillator. Although its precise molecular function remains to be fully elucidated, GIGANTEA is known to interact with and modulate multiple protein partners [1, 2]. This protocol describes the heterologous expression of *Oryza sativa* L. GIGANTEA (OsGIGANTEA, Q9AWL7) in *Escherichia coli* and the subsequent purification of the recombinant protein using affinity and size-exclusion chromatography. It provides detailed procedures for gene cloning, protein expression in *E. coli* Rosetta (DE3) pLysS, MBP-tag affinity purification, and preparative SEC to obtain highly purified protein suitable for downstream biochemical analyses.

Guidelines

Columns should be cleaned according to the manufacturer's instructions and stored in 20% ethanol when not in use.

All buffers must be filtered (0.22 µm) or degassed prior to use.

All chromatography steps were carried out at 4 °C.

Materials

Table 1. Primer list.

	Primer name	Sequence 5' to 3'
	OsGI- -GW-Fwd	GGGGACAAGTT TGTACAAAAAAG CAGGCTTAATGT CAGCTTCAAATG AGAAGTG
	OsGI- -GW-Rv	GGGGACCACTT TGTACAAGAAAG CTGGGTAGCAA GTGAGTGGGCA GCCGAG

Plasmids:

pMAL-c2x

E. coli Strain:

Rosetta(DE3) pLysS (invitrogen)

Lysogeny broth (LB) Medium: per 1 L: 10 g tryptone, 5 g yeast extract, 10 g NaCl

Antibiotics:

Ampicillin (Amp) : stock concentration 100 mg/mL

Chloramphenicol (Cam): stock concentration 30 mg/mL

Buffers:

- **Lysis buffer:** 20 mM Tris HCl, pH 7.5; 200 mM NaCl; 1 mM DTT; 1 mM EDTA; 5 mM PMSF; 2 mM MgCl₂; and DNase (the tip of a small weighing spoon, approximately)
- **Buffer A:** 20 mM Tris-HCl, pH 7.5; 200 mM NaCl; and 1 mM EDTA
- **Buffer B:** 20 mM Tris-HCl, pH 7.5; 200 mM NaCl; 1 mM EDTA; and 10 mM maltose
- **Running buffer (preparative SEC column):** 20 mM Tris-HCl, pH 7.5; 200 mM NaCl
- **Final buffer:** 10 mM Tris-HCl, pH 7.5; 150 mM NaCl

Purification equipment:

Equipment

ÄKTA pure

NAME

Protein purification system

TYPE

Cytiva

BRAND

29046665

SKU

<https://www.cytivalifesciences.com/en/us/support/products/akta-pure-150-l-29046665>^{LINK}

Columns:

Equipment

MBPTrap™ HP

NAME

Protein Purification Columns

TYPE

Cytiva

BRAND

28918779

SKU

<https://www.cytivalifesciences.com/en/us/products/items/mbptrap-hp-p-00306>^{LINK}

Equipment		
HiLoad Superdex 200 pg preparative SEC column	NAME	
Protein Purification column	TYPE	
Cytiva	BRAND	
28989336	SKU	
https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/size-exclusion/hiload-superdex-200-pg-preparative-size-exclusion-chromatography-columns-p-06283	LINK	
16/60	SPECIFICATIONS	

Equipment		
PD-10 desalting columns packed with Sephadex G-25 resin	NAME	
Desalting column	TYPE	
Cytiva	BRAND	
17085101	SKU	
https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/desalting-and-buffer-exchange/sephadex-g-25-in-pd-10-desalting-columns-p-05778?srsId=AfmBOooJv9gajWJJ0zO9u63gpc4O6iqrFBAJp3ig50ykPYVk4dqle0Ob	LINK	

Before start

Users should be familiar with bacterial culture techniques and standard protein purification procedures. All buffers should be freshly prepared, filtered (0.22 μm), and degassed to ensure optimal chromatographic performance. Chromatography steps should be carried out at 4 °C to maintain protein stability. The complete workflow for protein expression and purification typically requires 3–4 days, depending on the production scale and equipment availability.

Cloning of OsGIGANTEA

- 1 *OsGIGANTEA* was amplified and cloned into pMAL-c2x to generate an N-terminal MBP-tagged fusion protein (Figure 1). The gene was PCR-amplified using the primers OsGI-GW-Fwd and OsGI-GW-Rv (see primer list). The resulting PCR product was first cloned into pDONR221 (Invitrogen) via a Gateway BP reaction. The entry clone (pDONR221-*OsGIGANTEA*) was then recombined with pMAL-c2x in a subsequent Gateway LR reaction to obtain the final expression plasmid.

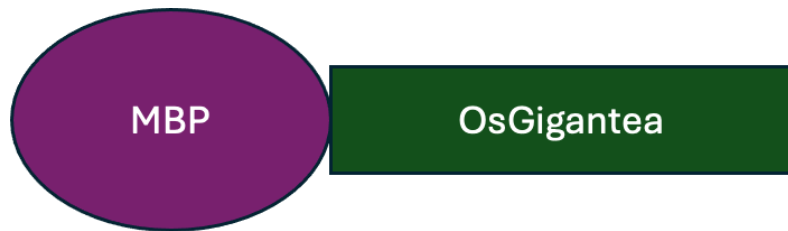


Figure 1. Schematic representation of *OsGIGANTEA* fused to an N-terminal MBP tag.

Bacterial cell growth

- 2 *E. coli* Rosetta (DE3) pLysS cells were transformed with the pMAL-c2x-*OsGI* construct. Liquid cultures were grown in 2.5 L of LB medium (per 1 L: 10 g tryptone, 5 g yeast extract, 10 g NaCl) supplemented with 100 µg/mL ampicillin and 30 µg/mL chloramphenicol. Cultures were incubated in 5 L baffled Erlenmeyer flasks at 37 °C with shaking at 180 rpm until reaching an OD₆₀₀ of 0.8–1.0.
- 3 Protein expression was induced with 500 µM IPTG, and cultures were incubated for 16 h at 28 °C with shaking at 180 rpm before harvesting.
- 4 Cells were collected by centrifugation at 16,000 × *g* for 1 h at 4 °C.

Cell lysis

- 5 The pellet obtained from 2.5 L of culture (approximately 7 g wet weight) was resuspended in 70 mL of lysis buffer containing: 20 mM Tris-HCl, pH 7.5; 200 mM NaCl; 1 mM DTT; 1 mM EDTA; 5 mM PMSF; 2 mM MgCl₂; and DNase I (5–10 µg/mL of lysate).
- 6 Cells were lysed using a French press, applying three passes at 15,000 psi.

- 7 The lysate was centrifuged at $20,000 \times g$ for 1 h and 30 min at 4 °C. The supernatant was collected, and the pellet discarded.
- 8 The collected supernatant was passed through a 0.22 µm cellulose nitrate syringe filter to obtain the clarified protein extract for subsequent purification steps.

Protein Purification - Step 1: MBPTrap

9

Note

All chromatography steps were performed at 4 °C.

10

Column preparation:

The column was regenerated by washing (Flow rate: 5 mL/min) with the following solutions:

- 3 column volumes (CV) of ddH₂O
- 3 CV of 0.5 M NaOH
- 3 CV of ddH₂O
- 5 CV of buffer A (20 mM Tris-HCl, pH 7.5; 200 mM NaCl; 1 mM EDTA)

11

Column loading: The protein extract (70 mL) was loaded onto the column by recirculating it 2–3 times to maximize binding. The flow rate was maintained at 2.5 mL/min.

12

Column washing: The loaded column was connected to the ÄKTA Pure system (pressure limit set to 0.5 MPa at all times), and non-bound proteins were removed by washing with Buffer A (20 mM Tris-HCl, pH 7.5; 200 mM NaCl; 1 mM EDTA) until a stable baseline was achieved.

13

Elution: Proteins were eluted with 100% Buffer B (20 mM Tris-HCl, pH 7.5; 200 mM NaCl; 1 mM EDTA; 10 mM maltose). Fractions were collected in 1 mL volumes, and fractions 4–12 were selected for further purification.

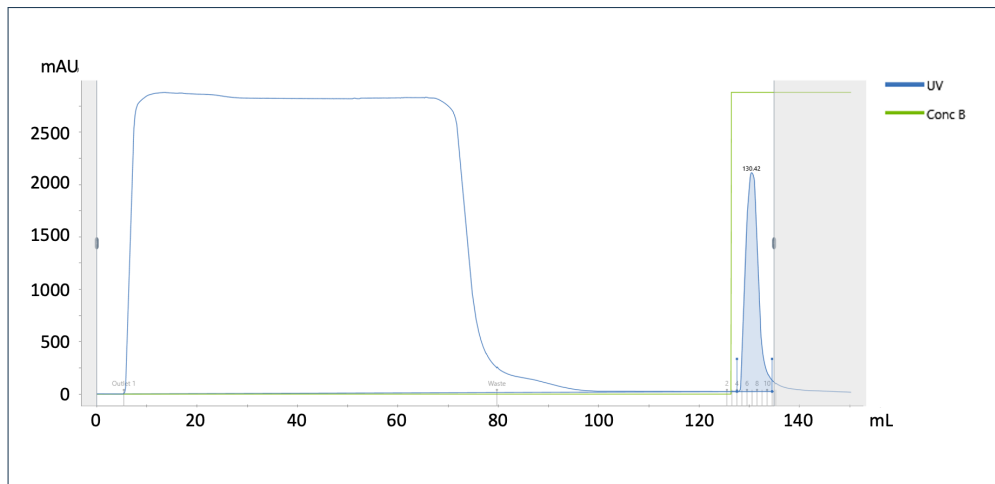


Figure 2. Purification of MBP-tagged OsGIGANTEA using an MBPTrap HP column (Cytiva). Protein extract from *E. coli* Rosetta (DE3) pLysS expressing MBP–OsGIGANTEA was loaded onto an MBPTrap HP column and purified on an ÄKTA Pure system at a flow rate of 2.5 mL/min. The column was washed with Buffer A (20 mM Tris-HCl, pH 7.5; 200 mM NaCl; 1 mM EDTA), and proteins were eluted with 100% Buffer B (20 mM Tris-HCl, pH 7.5; 200 mM NaCl; 1 mM EDTA; 10 mM maltose).

- 14 **Analysis of obtained fractions:** The injected sample, flow-through, and eluted fractions were analyzed by SDS-PAGE on a 10% gel.

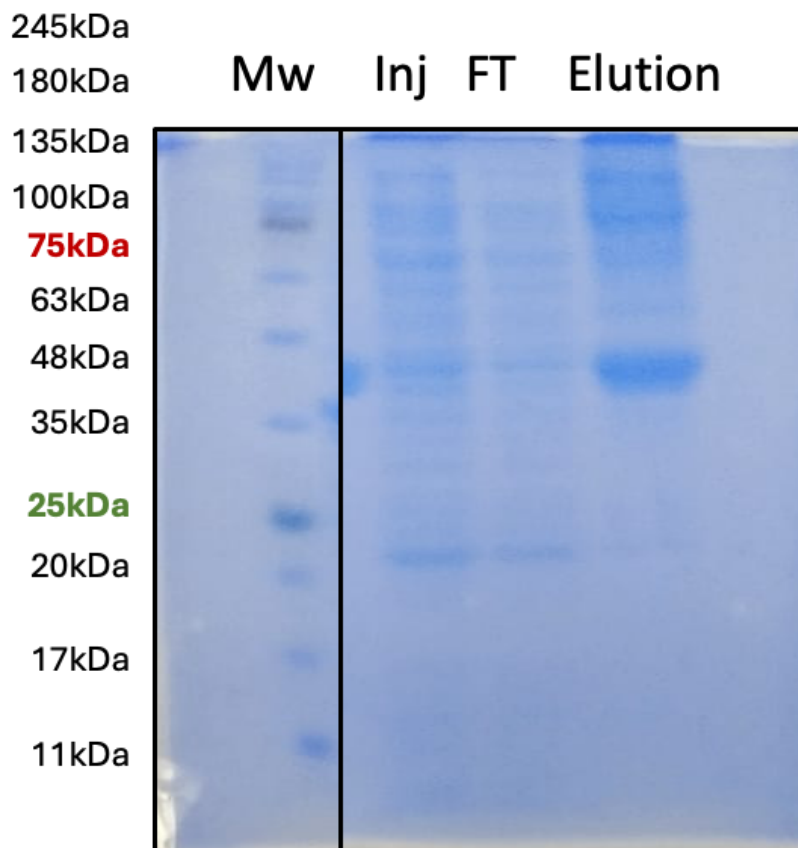


Figure 3. SDS–PAGE analysis of eluted fractions containing MBP-tagged OsGIGANTEA.

The soluble fraction from *E. coli* Rosetta (DE3) pLysS expressing MBP–OsGIGANTEA was loaded onto the ÄKTA Pure system (injected sample, Inj). Non-bound proteins were collected in the flow-through (FT) after washing with Buffer A (20 mM Tris-HCl, pH 7.5; 200 mM NaCl; 1 mM EDTA). Elution was performed with 100% Buffer B (20 mM Tris-HCl, pH 7.5; 200 mM NaCl; 1 mM EDTA; 10 mM maltose). Eluted fractions were pooled and analyzed by SDS–PAGE (10%) and stained with Coomassie Blue (Blue Safe, NZYTech). MBP-tagged OsGIGANTEA has a predicted molecular mass of 171 kDa. The molecular weight marker (MW) and corresponding sizes are indicated on the left. The gel was cropped to remove sections not relevant to this figure.

15 Fractions 4–10 were pooled and injected into the HiLoad Superdex 200 pg preparative SEC column in two separate runs.

16 **Column and Equipment used:**



Equipment

MBPTrap™ HP

NAME

Protein Purification Columns

TYPE

Cytiva

BRAND

28918779

SKU

<https://www.cytivalifesciences.com/en/us/products/items/mbptrap-hp-p-00306>

LINK

Equipment

ÄKTA pure

NAME

Protein purification system

TYPE

Cytiva

BRAND

29046665

SKU

<https://www.cytivalifesciences.com/en/us/support/products/akta-pure-150-l-29046665>

LINK

Protein Purification - Step 2: HiLoad Superdex 200 pg preparative SEC column

- 17 All chromatography steps were performed on an ÄKTA pure system (see Materials) at 1 mL/min, with a pressure limit of 0.5 MPa, and maintained at 4 °C. A HiLoad Superdex 200 pg preparative SEC column (Cytiva) was used.



Equipment

HiLoad Superdex 200 pg preparative SEC column	NAME
Protein Purification column	TYPE
Cytiva	BRAND
28989336	SKU
https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/size-exclusion/hiload-superdex-200-pg-preparative-size-exclusion-chromatography-columns-p-06283	LINK
16/60	SPECIFICATIONS

- 18 **Column preparation:** The column was washed with 5 column volumes (620 mL) of ultrapure water to remove storage buffer and residual contaminants. It was then equilibrated with 5 column volumes of running buffer (20 mM Tris-HCl, pH 7.5; 200 mM NaCl) to establish the appropriate ionic environment prior to sample loading.

Note

To minimize non-specific ionic interactions between the proteins and the column matrix, the running buffer should contain at least 0.15 M salt (e.g., ≥ 150 mM NaCl). This ensures proper shielding of charged groups and improves the specificity of the chromatographic separation.

- 19 **Column loading:** The pooled fractions (fractions 4 to 10) from the MBPTrap purification were loaded onto the column using the ÄKTA Pure system, in two sequential injections of 3 mL each.

Note

The sample volume should be carefully controlled, as overloading the column can significantly reduce separation resolution. In SEC, the recommended maximum injection volume is typically **1–5% of the total column volume (CV)**, with **1–2% CV** for high-resolution analytical runs and up to **3–5% CV** for preparative purposes. Using larger volumes leads to band broadening and peak overlap, compromising separation quality.

20 Elution: Proteins were eluted with 120 mL of running buffer (20 mM Tris-HCl, pH 7.5; 200 mM NaCl), and 1 mL fractions were collected throughout the elution.

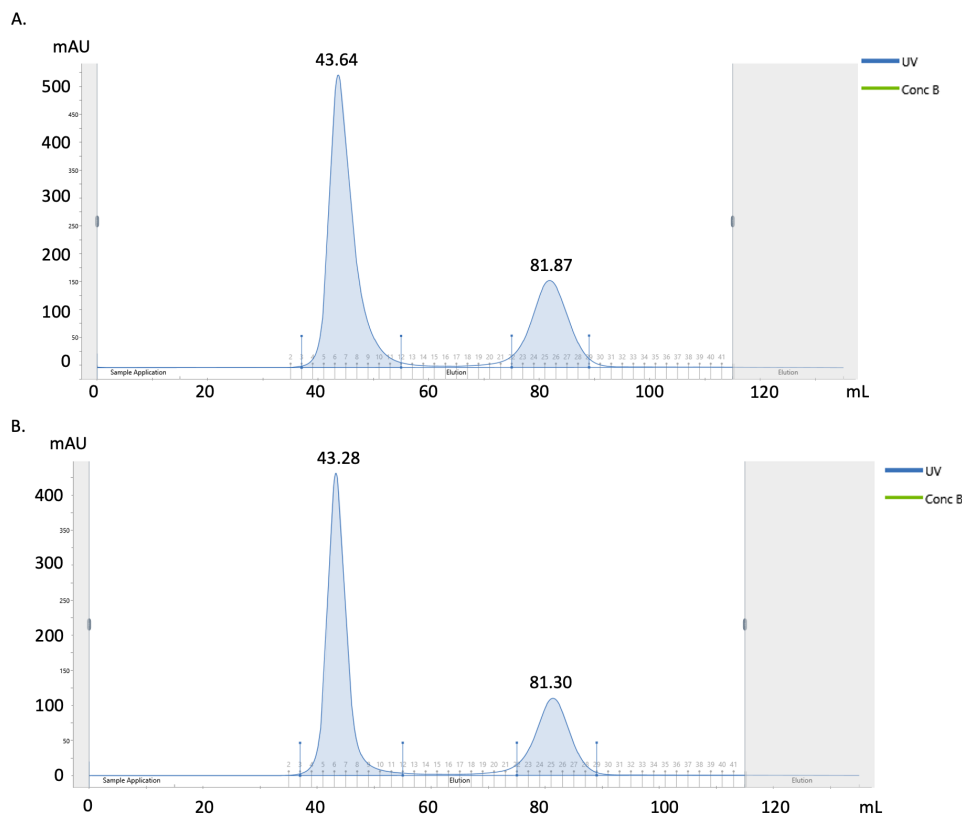


Figure 4. Purification of MBP-tagged *OsGigantea* using preparative size-exclusion chromatography (SEC).

Pooled fractions from the MBPTrap purification were loaded onto a HiLoad Superdex 200 pg preparative SEC column (Cytiva) in two separate 3 mL injections. The running buffer was 20 mM Tris-HCl, pH 7.5, with 200 mM NaCl. Eluted proteins were collected in 1 mL fractions. **A.** Chromatogram of the first injection. **B.** Chromatogram of the second injection.

- 21 The eluted fractions 4 to 12 from both the first and second injections were pooled, concentrated, and subjected to buffer exchange. Separately, fractions 22 to 28 from both injections, corresponding to free MBP, were pooled and labeled as "Free MBP". Free MBP sample was at a concentration of 0.6 mg/mL.

Protein Purification - Step 3: Protein concentration and Buffer exchange

22 Buffer exchange – Column preparation:

A PD-10 desalting column was equilibrated with 25 mL of buffer (10 mM Tris-HCl, pH 7.5; 150 mM NaCl) before sample loading.

Equipment

PD-10 desalting columns packed with Sephadex G-25 resin NAME

Desalting column TYPE

Cytiva BRAND

17085101 SKU

<https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/desalting-and-buffer-exchange/sephadex-g-25-in-pd-10-desalting-columns-p-05778?srsId=AfmBOooJv9gajWJJ0zO9u63gpc4O6iqrFBAJp3ig50ykPYVk4dqle0Ob> LINK

- 23 **Column loading:** A total of 16 mL of the selected, concentrated fractions (from Step 19) was gently and directly loaded onto the PD-10 column to ensure proper interaction between the resin and the sample.

- 24 **Elution:** Proteins were eluted by centrifuging the column at 1,000 × *g* for 2 minutes at 4 °C, yielding a final elution volume of approximately 5.5 mL at a concentration of 2.17 mg/mL.

Note

No precipitate was observed during the concentration process.

25 Analysis of purified proteins:

The final purified proteins were analyzed by SDS-PAGE using a 10% polyacrylamide gel.

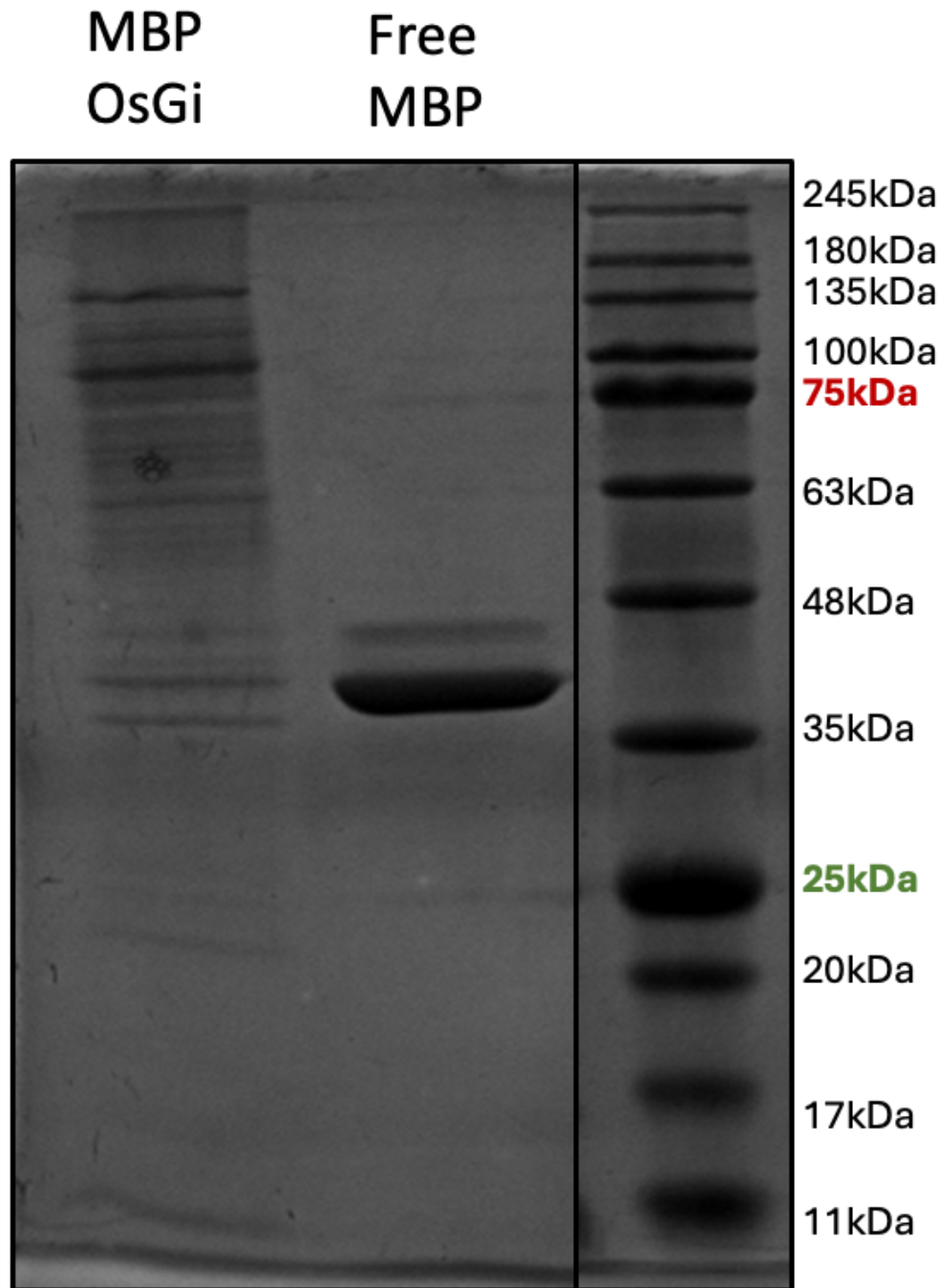


Figure 5. SDS-PAGE analysis of purified MBP-tagged OsGigantea and free MBP. Three micrograms of each purified protein were resolved by SDS-PAGE and stained with Coomassie Blue (BlueSafe, NZYTech). MBP-tagged OsGigantea has an expected molecular weight of ~171 kDa, whereas free MBP is ~42 kDa. The molecular weight marker (MW) and corresponding sizes are indicated on the left. The gel was cropped to remove regions not relevant to this figure.



Protocol references

- [1] Zheng, T., Sun, J., Zhou, S., Chen, S., Lu, J., Cui, S., Tian, Y., Zhang, H., Cai, M., Zhu, S., Wu, M., Wang, Y., Jiang, L., Zhai, H., Wang, H., & Wan, J. (2019). Post-transcriptional regulation of Ghd7 protein stability by phytochrome and OsGI in photoperiodic control of flowering in rice. *New Phytologist*, 224(1), 306–320. <https://doi.org/10.1111/nph.16010>
- [2] Nohales, M. A., & Kay, S. A. (2019). GIGANTEA gates gibberellin signaling through stabilization of the DELLA proteins in Arabidopsis. *PNAS*, 116(43), 21893–21899. <https://doi.org/10.1073/pnas.1913532116>

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