Nov 03, 2025

Blunt-End Ligation of Synthetic Lethal 7 Gene into pMAL-c2x Vector and Its Protein Expression in E. coli

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6zeyrgqe/v1

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Protocol Citation: Taohsueh Liao 2025. Blunt-End Ligation of Synthetic Lethal 7 Gene into pMAL-c2x Vector and Its Protein Expression in E. coli. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6zeyrgqe/v1

Protocol status: Working
We use this protocol and it's working

Created: November 02, 2025

Last Modified: November 03, 2025

Protocol Integer ID: 231348

Keywords: end ligation of synthetic lethal, gene into pmal, synthetic lethal, end ligation, protein expression, corresponding gene, gene, protein

Abstract

To express the Histone synthetic lethal 7 protein, the corresponding gene was cloned into the pMAL-c2x vector using blunt-end ligation and transformed into competent *E. coli* BL21(DE3) cells.

Image Attribution

Figure 1: Protein expression was confirmed through cell disruption and SDS-PAGE analysis. The second band from the top corresponds to the target gene. 95kDa.

Method

1 Initially, 5 mL of LB medium was inoculated and cultured for 22 hours at 37°C, reaching an OD600 of 2.41.

2 Subsequently, 4.3 mL of the cultured broth was mixed with 95.7 mL of LB medium supplemented with sorbitol and ampicillin (10 mg/mL), resulting in a final volume of 100 mL and an OD600 of 0.6.

3 Protein expression was induced by adding 0.8 mM IPTG, followed by incubation at 30°C for 22 hours.

4 After induction, 1 mL of bacterial culture was transferred to an Eppendorf tube and centrifuged at 5000 rpm for 7 minutes at 4°C using a refrigerated centrifuge.
Protein expression was confirmed by SDS-PAGE analysis (see Figure 1). Lactose was not used for induction due to insufficient protein yield observed in preliminary trials.

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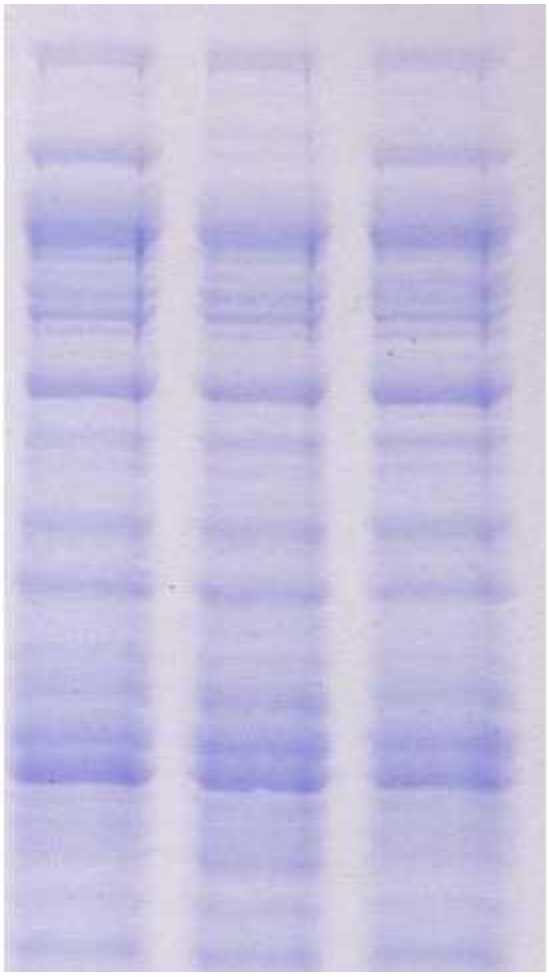

Figure 1: Protein expression was confirmed through cell disruption and SDS-PAGE analysis. The second band from the top corresponds to the target gene. 95kDa.