

AMB Express

Efficient production of secretory *Streptomyces clavuligerus* β -lactamase inhibitory protein (BLIP) in *Pichia pastoris* for restoring resistant Gram-positive bacterial susceptibility to β -lactam antibiotics

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Methods. Production of TEM-1 β -lactamase

A single colony of *E. coli* BL21(DE3) harboring pRSET-K/TEM-1 β -lactamase was inoculated into 5 mL of 2 \times TY medium with 50 μ g/mL kanamycin and cultivated at 37°C for 14 – 16 h with shaking at 250 rpm. After that, 1 mL of the pre-culture was transferred into 100 mL of 2 \times TY medium and incubated at 37°C with shaking at 250 rpm. When OD₆₀₀ of the culture was 0.8, 0.3 mM IPTG was added to the culture to induce the expression of TEM-1 β -lactamase. The culture was then incubated at 37°C with shaking at 250 rpm for an additional of 4 h. Afterward, the cells were collected by centrifugation at 10,000g at 4°C for 30 min. The cells were resuspended in 10 mL of solubilization buffer (20 mM Tris-HCl, 0.2 M sodium chloride, pH 7.4) with 75 μ g/mL lysozyme and incubated at 30°C for 45 min. Then the cells were lysed by sonication. Bacterial cell lysate was then subjected to centrifugation at 18,000g for 30 min at 4°C. The cell lysate was loaded onto a 5 mL of HiTrap Chelating column charged with nickel for purification. Histidine-tagged TEM-1 β -lactamase was eluted with a linear gradient elution from 0 – 0.5 M imidazole in 20 mM sodium phosphate buffer (pH 7.4). Fractions containing TEM-1 β -lactamase were pooled, dialyzed against 10 mM ammonium bicarbonate and lyophilized.

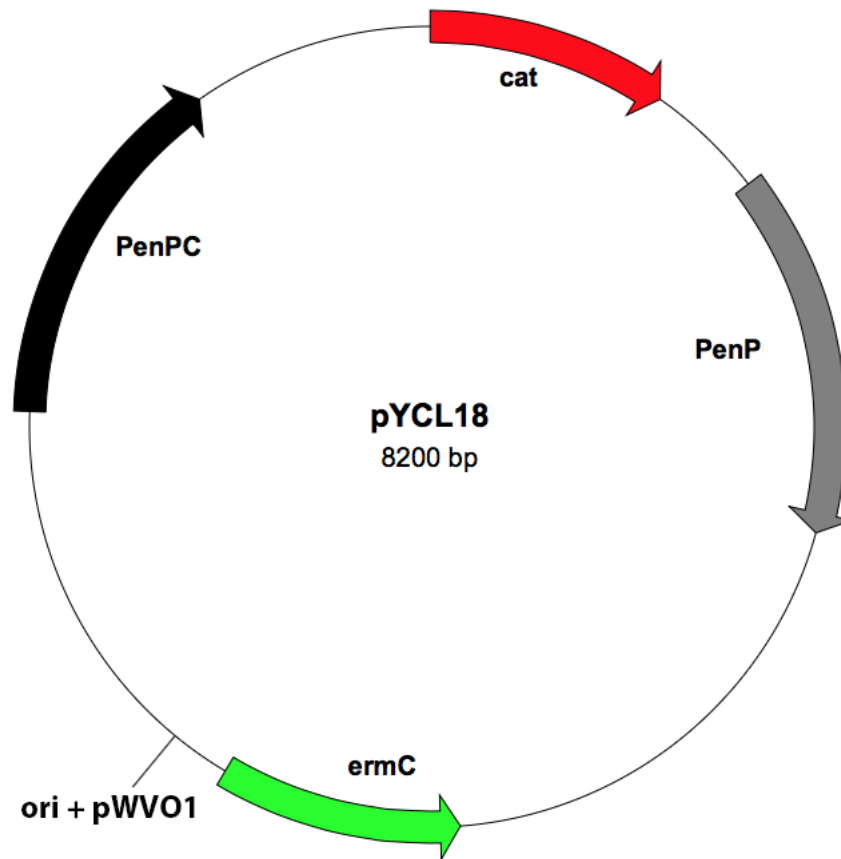


Figure. S1. Plasmid map of the *E. coli*/*B. subtilis* shuttle plasmid pYCL18. *cat* chloramphenicol acetyltransferase gene, *ermC* Erythromycin resistant marker, *PenP* β -lactamase gene from *Bacillus licheniformis*, *PenPC* β -lactamase gene from *Bacillus cereus*, and *ori + pWVO1* The plus origin of replication of pWVO1, a plasmid that replicates via a rolling-circle mode of replication.