

The expression of Tn5 transposase was initially induced in T7 polymerase-expressing strain C3013, following the method described by Picelli *et al.* However, no detectable expression was observed via SDS-PAGE (Figure S8-1).

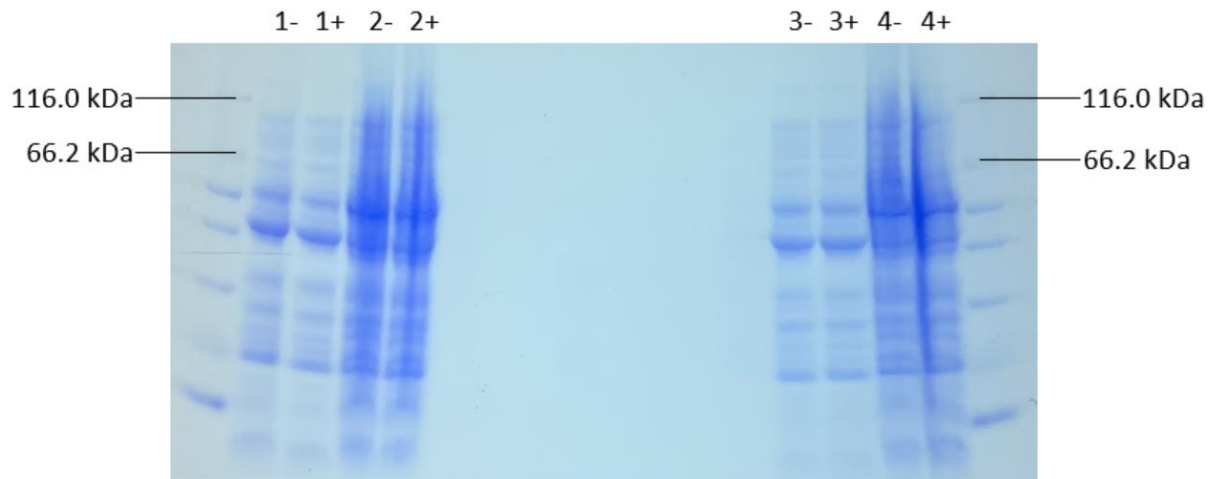


Figure S8-1. Visualization of Tn5 transposase expression in C3013 cells carrying pTXB1-Tn5. Following cultivation in the LB broth at 37°C for 4 hours, proteins from four C3013 colonies harbouring the pTXB1-Tn5 plasmid were separated by SDS-PAGE, with samples from IPTG-induced (lanes 1+ to 4+) and non-induced (lanes 1– to 4–) cultures. No band corresponding to the expected size of the Tn5 transposase–intein–CBD fusion protein (~81.1 kDa) was detected in induced samples. Pierce™ Unstained Protein Molecular Weight Marker (Ladder) was loaded in both sides of the gel.

Subsequently, various modifications were attempted, including raising the incubation temperature from 22°C to 37°C (Figure S8-2), the addition of 1% (w/v) glucose in the LB broth (Figure S8-3), and extending the incubation period from 3 hours to overnight (Figure S8-4). Despite these adjustments, no detectable Tn5 expression was observed.

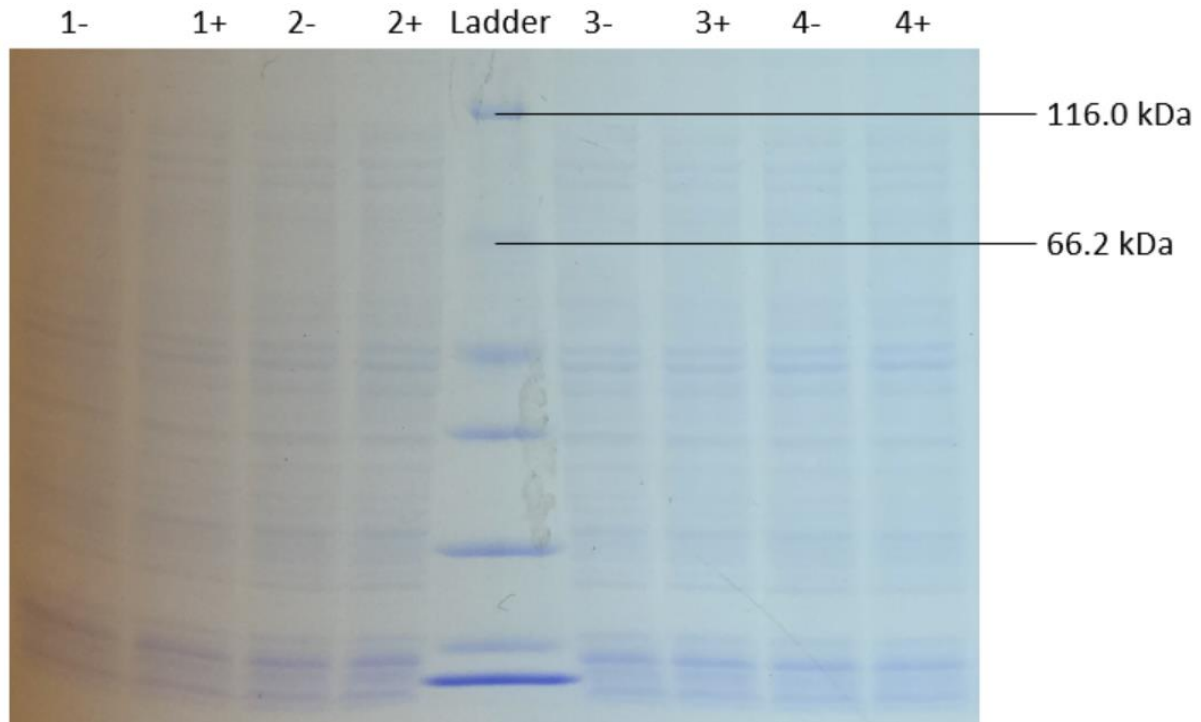


Figure S8-2. Visualization of Tn5 transposase expression in C3013 cells carrying pTXB1-Tn5 incubated at 37°C. Following cultivation at 37°C in LB broth for 4 hours, proteins from four C3013 colonies harbouring the pTXB1-Tn5 plasmid were separated by SDS-PAGE, with samples from IPTG-induced (lanes 1+ to 4+) and non-induced (lanes 1- to 4-) cultures. No band corresponding to the expected size of the Tn5 transposase-intein-CBD fusion protein (~81.1 kDa) was detected in induced samples. Pierce™ Unstained Protein Molecular Weight Marker (Ladder) was loaded in the middle of the gel.

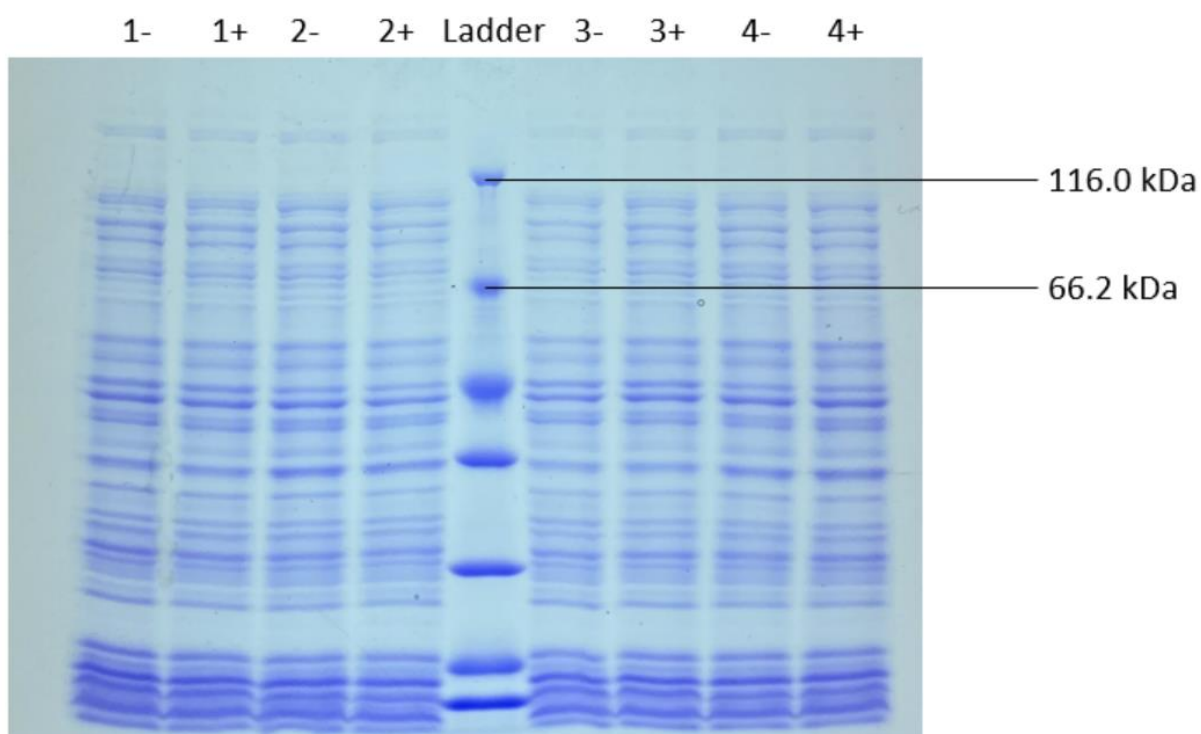


Figure S8-3. Visualization of Tn5 transposase expression in C3013 cells carrying pTXB1-Tn5 incubated in LB broth supplemented with glucose. Following cultivation in the LB broth supplemented with 1% (w/v) glucose at 37°C in LB broth for 4 hours, proteins from four C3013 colonies harbouring the pTXB1-Tn5 plasmid were separated by SDS-PAGE, with samples from IPTG-induced (lanes 1+ to 4+) and non-induced (lanes 1- to 4-) cultures. No band corresponding to the expected size of the Tn5 transposase-intein-CBD fusion protein (~81.1 kDa) was detected in induced samples. Pierce™ Unstained Protein Molecular Weight Marker (Ladder) was loaded in the middle of the gel.

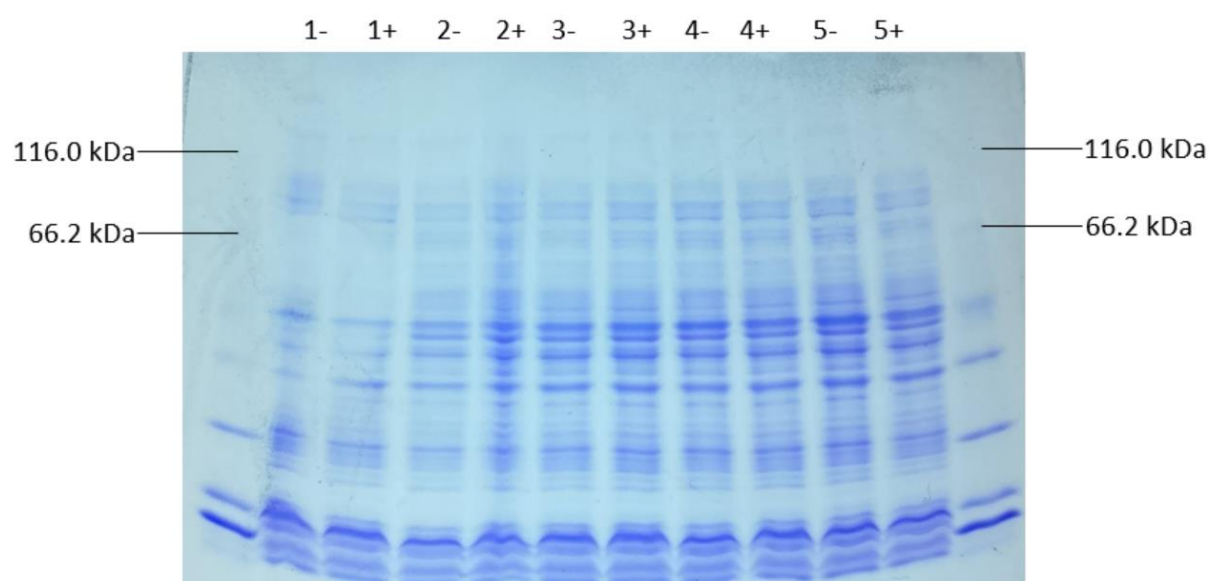


Figure S8-4. Visualization of Tn5 transposase expression in C3013 cells carrying pTXB1-Tn5 incubated overnight. Following overnight cultivation in the LB broth at 37°C, proteins from four C3013 colonies harbouring the pTXB1-Tn5 plasmid were separated by SDS-PAGE, with samples from IPTG-induced (lanes 1+ to 4+) and non-induced (lanes 1- to 4-) cultures. No band corresponding to the expected size of the Tn5 transposase-intein-CBD fusion protein (~81.1 kDa) was detected in induced samples. Pierce™ Unstained Protein Molecular Weight Marker (Ladder) was loaded in the middle of the gel.