Supplemental Information

Characterization of a highly efficient antibiotic-degrading metallo-β-lactamase obtained from an uncultured member of a permafrost community

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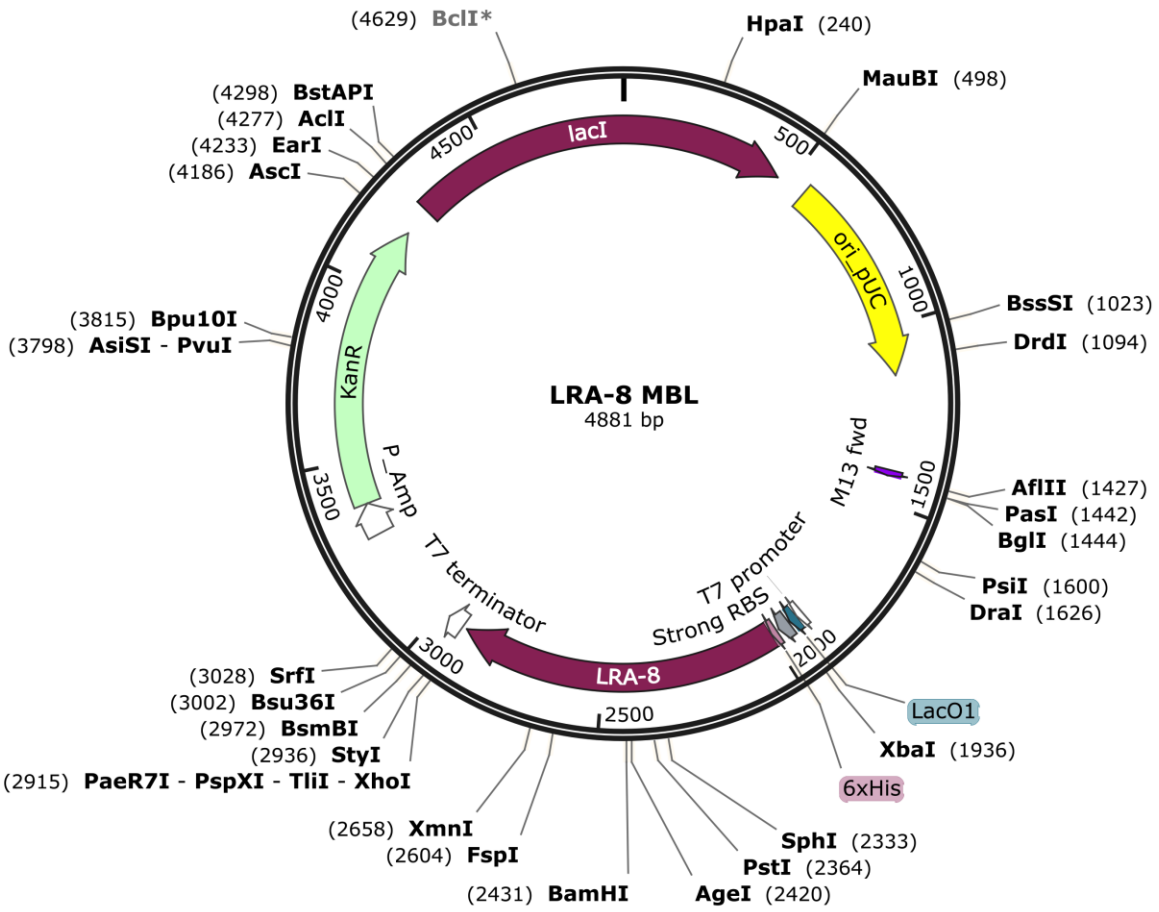

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CGGGTCTTGAGGGGTTTTTTG

Figure S1. Map of the entire recombinant expression system for LRA-8 (top) and the relevant DNA sequence (bottom). In yellow is shown the T7 promoter, in green the Lac promoter, in pink the 6xHis tag and in red the LRA-8-encoding sequence.

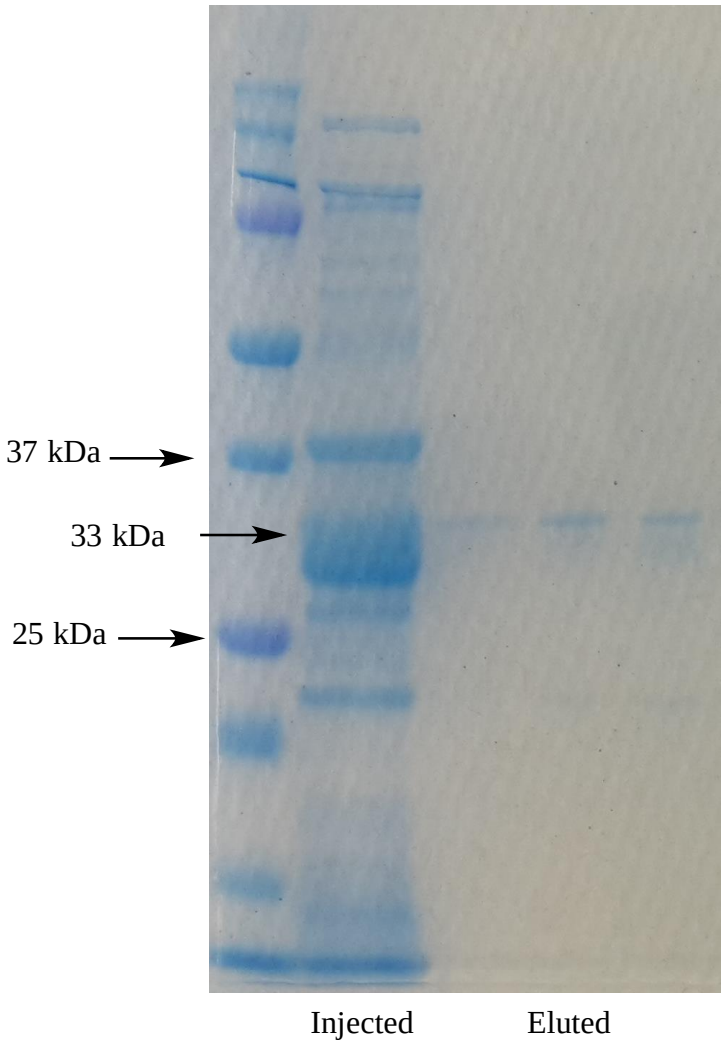

Figure S2. SDS-PAGE gel from a routine LRA-8 purification using an IMAC column. Most of the protein eluted with the flow-through ("Injected"), while several fractions ("Eluted") contained virtually pure recombinant LRA-8 with an estimated molecular weight of ~34 kDa, in good agreement with both the calculated size for the full-length protein (33,824.51 Da; note that the addition of two Zn(II) increases the calculated weight to 33,955.27 Da) and the value obtained by non-denaturing ESI mass spectrometry (33,970 ± 20 Da). A routine preparation resulted in approximately 10 – 15 mg of pure LRA-8 per litre of culture medium.