

## Synthetic tools for the genetic engineering of *Methylomonas* sp. DH-1

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Genetic engineering is a fundamental technology to engineer living systems. There are no such technologies for newly identified bacterial species, which dampers down the advance of bacterial cell factory construction. Here, we developed several technologies to support the engineering of *Methylomonas* sp. DH-1, that has been recently identified from soil. For the introduction of bacterial plasmids, it is essential to develop a transformation method. For rapid and easy use, we developed a physicochemical transformation method that is applicable to a wide variety of bacterial species including Gram positive bacteria. Optimization of the method showed over  $1e5$  CFU/ $\mu$ g of DNA in *Methylomonas* sp. DH-1 and comparable efficiency in other bacterial species. For the fine-optimization of protein expression, here we identified  $> 100$  promoters showing a different strength in *Methylomonas* sp. DH-1.