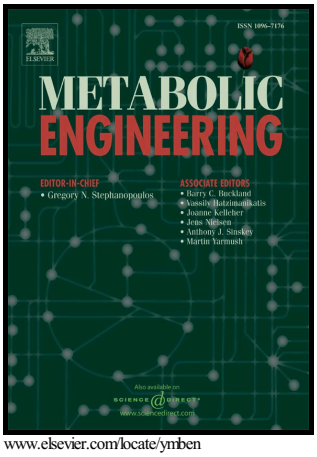

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Direct Pathway Cloning (DiPaC) to Unlock Natural Product Biosynthetic Potential

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C.G., E.R.D., P.M.D., A.G., K.L. and T.A.M.G. developed the DiPaC method and experimental design. C.G., E.R.D. and P.M.D. performed experiments; C.G., E.R.D., P.M.D. and T.A.M.G. wrote the manuscript.

The authors declare no conflict of interest.

Abstract.

Specialized metabolites from bacteria are an important source of inspiration for drug development. The genes required for the biosynthesis of such metabolites in bacteria are usually organized in so-called biosynthetic gene clusters (BGCs). Using modern bioinformatic tools, the wealth of genomic data can be scanned for such BGCs and the expected products can often structurally be predicted *in silico*. This facilitates the directed discovery of putatively novel bacterial metabolites. However, the production of these molecules often requires genetic manipulation of the BGC for activation or the expression of the pathway in a heterologous host. The latter necessitates the transplantation of the BGC into a suitable expression system. To achieve this goal, powerful cloning strategies based on *in vivo* homologous recombination have recently been developed. This includes LCHR and LLHR in *E. coli* as well as TAR cloning in yeast. Here, we present Direct Pathway Cloning (DiPaC) as an efficient complimentary BGC capturing strategy that relies on long-amplicon PCR and *in vitro* DNA assembly. This straightforward approach facilitates full pathway assembly, BGC refactoring and direct transfer into any vector backbone *in vitro*. The broad applicability and efficiency of DiPaC is demonstrated by the discovery of a new phenazine from *Serratia fonticola*, the first heterologous production of anabaenopeptins from *Nostoc punctiforme* and the transfer of the native erythromycin BGC from *Saccharopolyspora erythraea* into a *Streptomyces*. Due to its simplicity, we envisage DiPaC to become an essential method