New Tools for Targeted Cloning and Over Expression of Biosynthetic Gene Clusters

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Introduction

The genome sequencing revolution and corresponding development of biosynthetic gene cluster (BGC) prediction and analysis tools such as antiSMASH (1) have resulted in a wealth of new biosynthetic potential for further examination. Once a BGC of interest is identified, isolating a physical DNA clone for expression, refactoring, and other analyses can be a slow, expensive process. Classical methods of cloning can take months to complete, and gene synthesis is expensive and can be stymied by GC-rich and/or repetitive sequences (2).

The development of CRISPR-Cas has revolutionized many fields within biology and medicine since it allows for precise, site-specific restriction with few off-target cuts. We developed a technique using CRISPR-Cas to directly clone large BGCs from genomic DNA without using gels or agarose plugs, to aid in the characterization of new BGCs and accelerate the pace of drug discovery.

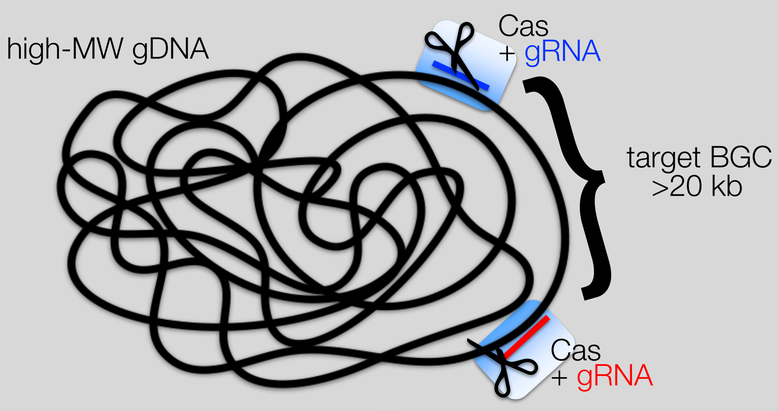
In order to facilitate the chemical analyses of the biosynthetic potential of gene clusters from genomic or metagenomic sources, we developed a method for heterologous expression of BGCs in hosts such as *Streptomyces, Bacillus,* and *Aspergillus*.

Here, we conducted experiments to determine:

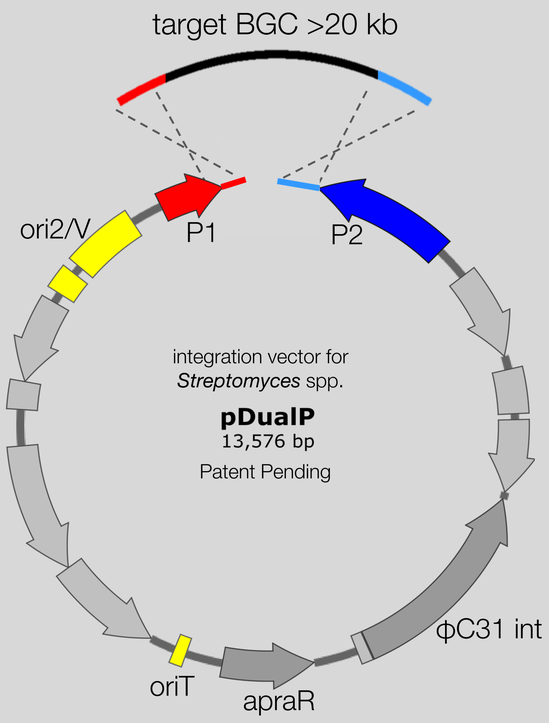
Can CRISPR-Cas be used to rapidly cut and assemble BGCs directly into a new vector that enables [inducible] heterologous expression?

Methods

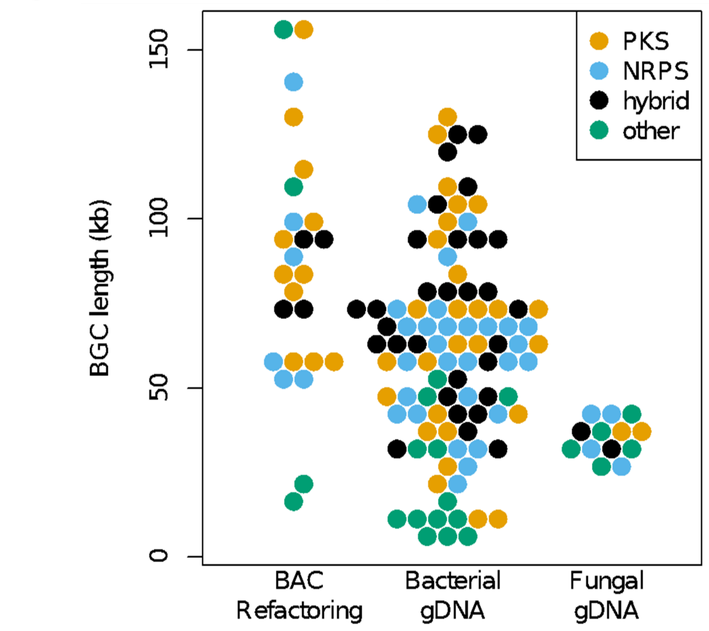
CRISPR-Cas restriction

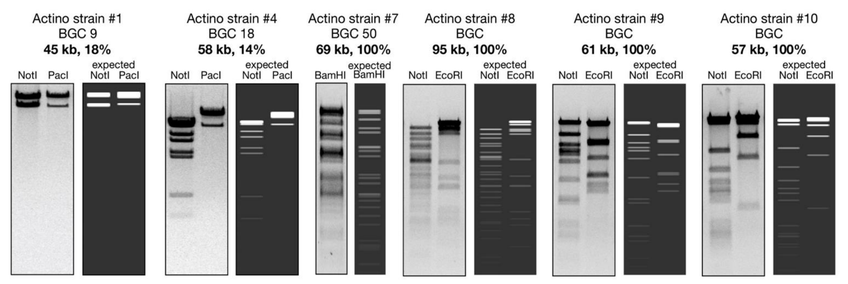


DNA assembly to BAC vector

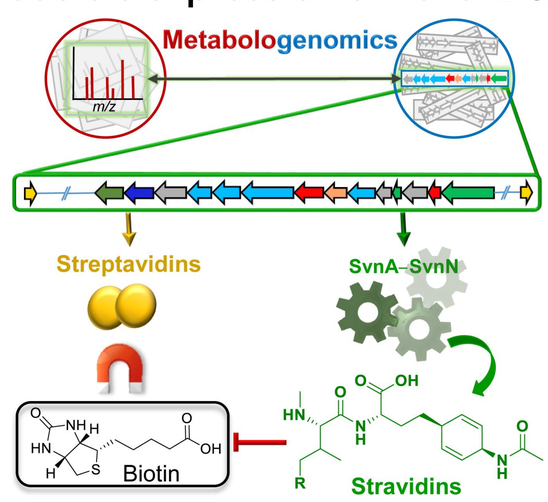


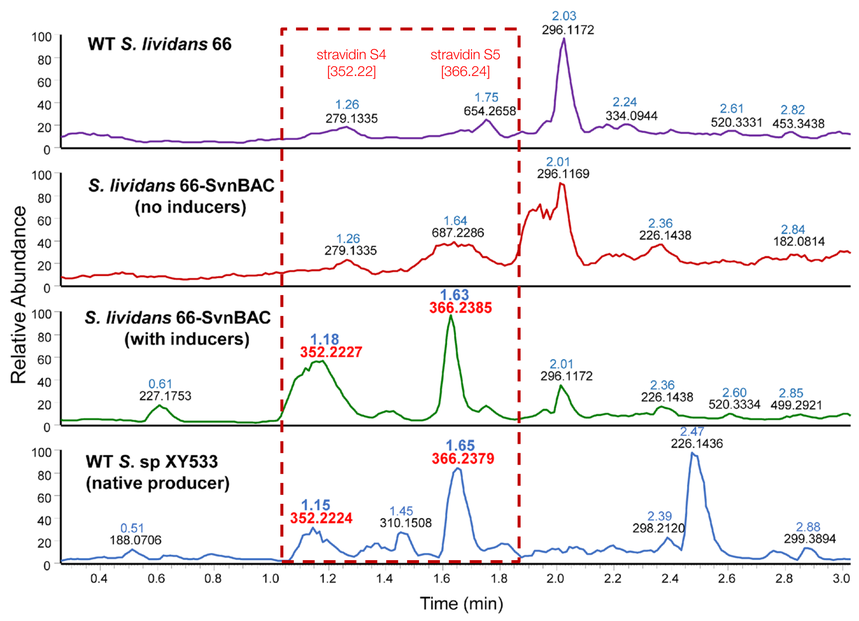
1 Successful cloning of many clusters



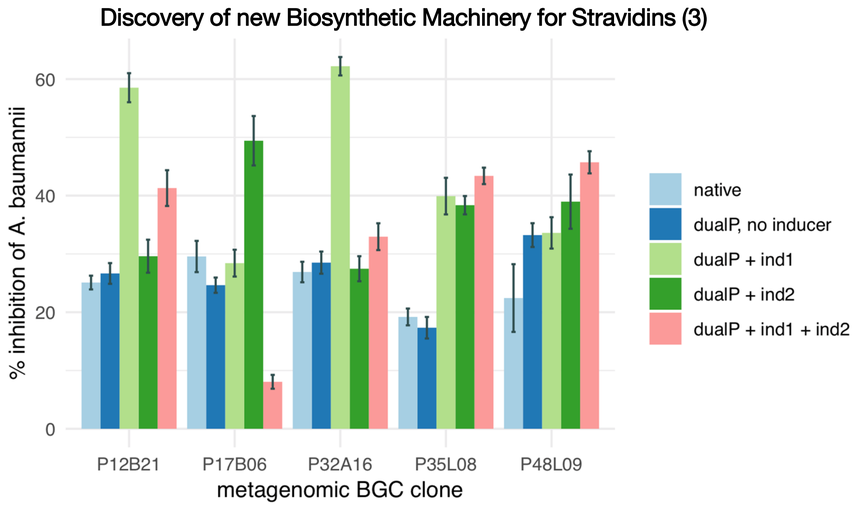


2 Inducible expression of novel BGCs



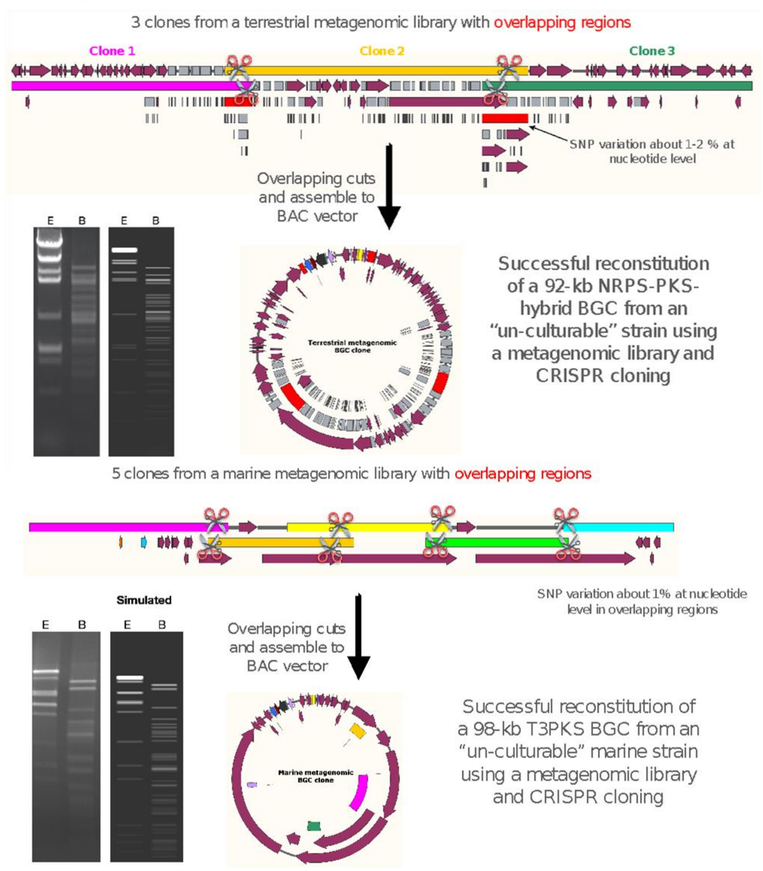


Discovery of new Biosynthetic Machinery for Stravidins (3)

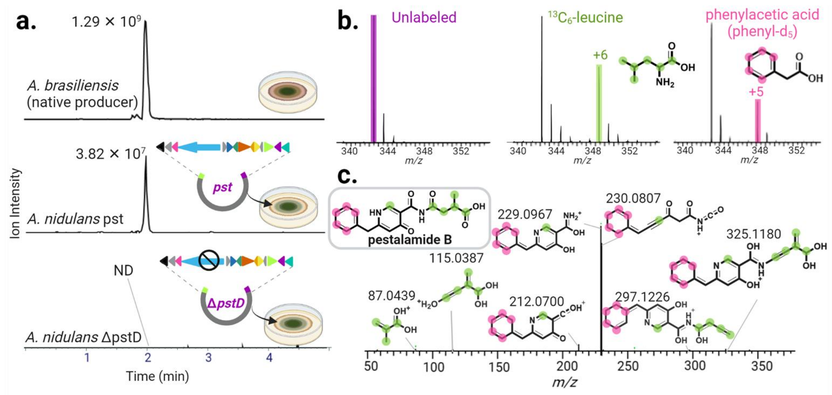


Metagenomic BGC Heterologous Expression in *S. coelicolor* M1154

3 Rebuilding metagenomic BGCs



4 Capture and express a novel fungal BGC



Conclusions

- 130+ large pathways ranging in size from 12–150 kb (median = 60) were cloned directly from genomic DNA, including BGCs from GC-rich ***Streptomyces***, ***Bacillus,*** and other diverse bacteria and fungi
- BGCs are cloned directly into an integrative vector ready for heterologous expression testing
- pDualP dual-inducible vector allows for controlled heterologous expression of BGCs
- New stravidin analogues and biosynthetic machinery were uncovered in only 2 months through inducible expression (3)
- Metagenomic BGCs can be rebuilt from fosmid parts
- New biosynthetic machinery for pestalimide B was uncovered through fungal direct cloning and heterologous expression in *Aspergillus*
- These results indicate that any sequenced biosynthetic gene cluster can be cloned intact from complex genomes and heterologously expressed to produce secondary metabolites, thereby expanding our available resources for natural product discovery

References

1. Blin, K., Wolf, T., Chevrette, M. G., Lu, X., Schwalen, C. J., Kautsar, S. A., et al. (2017). antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Research.
2. Zhang, M. M., Wang, Y., Ang, E. L., & Zhao, H. (2016). Engineering microbial hosts for production of bacterial natural products. Natural Product Reports, 33(8), 963–987.
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