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Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):
Tong, Y. (2017). *New developments on actinomyces CRISPR tools*. Abstract from DTU Biosustain Annual Seminar 2017, Elsinore, Denmark.

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New developments on actinomyces CRISPR tools

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Keywords: CRISPR-Cas9, actinomycetes, sgRNA finder, DNA free genome editing, multiplex sgRNA

Actinomycetes are one of the most important sources of pharmacological and industrial relevant natural products (Weber, T., et al, 2015). Unfortunately, many of the wild-type strains are recalcitrant to efficient genetic manipulation approaches, which is a severe bottleneck for systematic metabolic engineering. We developed a genome editing toolkit based on CRISPR-Cas9, which includes three sub-systems, pCRISPR-Cas9, pCRISPR-Cas9-ScaligD, and pCRISPR-dCas9 (CRISPRi), for genome editing and modulating transcription of target genes (Tong, Y., et al, 2015; Tong, Y., et al, 2016). The system is widely used in our section and the actinomycete community. Although the toolkit significantly speeds up genetic engineering for many strains, there are still limitations: for instance, often non-model actinomycetes cannot be transformed using the standard protocols, are resistant to the standard antibiotics used for plasmid selection, or cannot replicate commonly used plasmids; there is no very good spacer finder for non-model organisms; the current system cannot meet the high throughput and automation genome editing purpose. In order to address these limitations, we extended our actinomycete CRISPR-Cas9 toolkit: a spacer finder, CRISPy-web (Blin, K., et al, 2016) was released to facilitate the sgRNA design for non-model organisms, which can design sgRNAs for any microbial genome. A prototype of a “DNA-free” genome editing system for actinomycetes was demonstrated by inactivating actinorhodin production in the model strain *S. coelicolor*. An USER-based multiple sgRNA assembly strategy (Tong, Y., et al, 2017) was developed and validated for automated high-throughput cloning. This update of our CRISPR-Cas9 toolkit will further extended its applicability for actinomycetal genome editing.

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