A versatile one-step CRISPR-Cas9 based approach to plasmid-curing

**Background**
Plasmids are widely used and essential tools in molecular biology. However, plasmids often impose a metabolic burden and are only temporarily useful for genetic engineering, bio-sensing and characterization purposes. While numerous techniques for genetic manipulation exist, a universal tool enabling rapid removal of plasmids from bacterial cells is lacking.

**Results**
Based on replicon abundance and sequence conservation analysis, we show that the vast majority of bacterial cloning and expression vectors share sequence similarities that allow for broad CRISPR-Cas9 targeting. We have constructed a universal plasmid-curing system (pFREE) and developed a one-step protocol and PCR procedure that allow for identification of plasmid-free clones within 24 h. While the context of the targeted replicons affects efficiency, we obtained curing efficiencies between 40 and 100% for the plasmids most widely used for expression and engineering purposes. By virtue of the CRISPR-Cas9 targeting, our platform is highly expandable and can be applied in a broad host context. We exemplify the wide applicability of our system in Gram-negative bacteria by demonstrating the successful application in both Escherichia coli and the promising cell factory chassis Pseudomonas putida.

**Conclusion**
As a fast and freely available plasmid-curing system, targeting virtually all vectors used for cloning and expression purposes, we believe that pFREE has the potential to eliminate the need for individualized vector suicide solutions in molecular biology. We envision the application of pFREE to be especially useful in methodologies involving multiple plasmids, used sequentially or simultaneously, which are becoming increasingly popular for genome editing or combinatorial pathway engineering.

**General information**
Publication status: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Research Groups, Microbial Evolution and Synthetic Biology, Bacterial Synthetic Biology
Contributors: Lauritsen, I., Porse, A., Sommer, M. O. A., Nørholm, M.
Number of pages: 10
Publication date: 2017
Peer-reviewed: Yes

**Publication information**
Journal: Microbial Cell Factories
Volume: 16
Article number: 135
ISSN (Print): 1475-2859
Ratings:
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 4.2 SJR 1.443 SNIP 1.252
Web of Science (2017): Impact factor 3.831
Web of Science (2017): Indexed yes
Original language: English
Keywords: CRISPR-Cas9, Plasmid-curing, pFREE, Replicon analysis, Pseudomonas putida, Genome engineering

**Electronic versions:**
Lauritsen_et_al_2017_Microbial_Cell_Factories_1_.pdf
DOIs:
10.1186/s12934-017-0748-z

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Source: PublicationPreSubmission
Source ID: 134332358
Research output: Contribution to journal › Journal article – Annual report year: 2017 › Research › peer-review