**CRISPR. Adapted from:** R. E. Cobb, Y. Wang, and H. Zhao, “High-Efficiency Multiplex Genome Editing of Streptomyces Species Using an Engineered CRISPR/Cas System,” vol. 18, p. 3, 2018, doi: 10.1021/sb500351f.

**And** T. Kieser, M. J. Bibb, M. J. Buttner, K. F. Chater, and D. A. Hopwood, *Practical Streptomyces Genetics*. The John Innes Foundation, 2000.

Note: Two different sgRNA sequence used in different experiments:

* sgRNA1: 5’ - promoter – TCATCGTTCTCAATACACCG– scaffold – terminator 3’
* sgRNA2: 5’ - promoter – TGCAACCTCCGTGATCATTC– scaffold – terminator 3’

Day 1:

1. Start an overnight culture of *E. coli* ET12567 at 37°C in LB supplemented with Chloramphenicol and Kanamycin.

Day 2:

1. Prepare competent cells of *E. coli* ET12567.
2. Transform using your favourite protocol with pSA-CRISPR-BC.
3. Plate in LB supplemented with chloramphenicol (25 μg/mL), kanamycin (50 μg/mL) and apramycin (50 μg/mL).

Day 3:

1. Pick one colony and inoculate 5 mL of LB supplemented with Chloramphenicol, Kanamycin and Apramycin.

Day 4:

1. Pellet down cells.
2. Wash cells 3 times with 1 mL 2xYT media.
3. Heat shock ~108 *S. albidoflavus* spores in 100 uL of 2xYT.
4. Use the 100uL spores to resuspend the *E. coli* pellet.
5. Plate on MS-agar supplemented with 20mM MgCl2.
6. Incubate overnight at 30°C.

Day 5:

1. Dissolve Apramycin and Nalidixic Acid in 1mL H2O.
2. Overlay the antibiotic mixture on the plates. Let them dry.
3. Incubate at 30°C for 1 week.

Day 6:

1. Restreak 10 *S. albidoflavus* colonies on MS/Nal at 37°C to cure the plasmids.
2. Grow until colonies appear.
3. Repeat three times.

Day 7:

1. Restreak on MS-agar/Apra to check plasmid curing.

Day 8:

1. Extract genomic DNA of cells using Sigma’s Genomic extraction Kit.
2. Check barcode presence by PCR.