**CRISPR. Adapted from:** J. Altenbuchner, “Editing of the Bacillus subtilis Genome by the CRISPR-Cas9 System.,” *Appl. Environ. Microbiol.*, vol. 82, no. 17, pp. 5421–7, Sep. 2016, doi: 10.1128/AEM.01453-16.

sgRNA sequence used: 5’ - promoter – GGAAAAGAGTATATTAGATA – scaffold – terminator 3’. sgRNA sequence obtained using [3].

Day 1:

1. Start an overnight culture 37°C in minimal medium (MM: 10ml SMM basic salts, 125μl 40% (w/v) glucose, 100μl 2% (w/v) tryptophan, 60μl 1M Mg2SO4\*7H2O, 10μl 20% (w/v) casamino acids, 5μl 2.2mg/ml ferric ammonium citrate).

Day 2:

1. Dilute cells 1:10 in MM and grown for 3h at 37°C. Meanwhile, starvation medium (SMM: 10ml SMM basic salts, 125μl 40% (w/v) glucose, 60μl 1M Mg2SO4\*7H2O) is prepared and prewarmed at 37°C.
2. Dilute in 1:2 SM and make competent cells with a further 2h incubation period at 37°C.
3. Mix 400μl cell with 400 ng pBS-CRISPR-BC. Spin down cells and plate on LB supplemented with 5 μg/mL kanamycin and 0.2% mannose and incubate at 30°C for 1h.

Day 3:

1. Barcode presence is checked by colony-PCR.
2. Cure positive clones from pJOE-BC by restreaking them at 37°C in LB plates

Day 4:

1. Store kanamycin sensitive clones as barcoded.