**Cre-Lox. Adapted from:** B. M. Koo *et al.*, “Construction and Analysis of Two Genome-Scale Deletion Libraries for Bacillus subtilis,” *Cell Syst.*, vol. 22, pp. 291–305, 2017, doi: 10.1016/j.cels.2016.12.013.

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 (SM: 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. 400μl cell aliquots are mixed with 1μg recombinant DNA PCR (coming from the amplification of pBS-CreLox-BC). Incubate 1 h at 37°C.
4. Cells are spun down, concentrated and plated on NA/Zeocin (20 μg/mL) and incubated at 37°C.

Day 3:

1. Colonies are tested for the integration of the recombinant DNA by PCR and sequencing.
2. A positive clone is grown overnight at 37°C in MM.

Day 5:

1. Dilute cells 1:10 in MM and grown for 3h at 37°C. Meanwhile, starvation medium (SM: 10ml SMM basic salts, 125μl 40% (w/v) glucose, 60μl 1M Mg2SO4\*7H2O) is prepared and prewarmed at 37°C.
2. The culture is diluted 1:2 in SMM and cells are made competent with a further 3h incubation period at 37°C.
3. 400ul cell aliquots are mixed with 100 ng pDR244.
4. Cells are spun down, concentrated and plated on LB supplemented with spectinomycin (100 μg/mL) and incubated at 30°C.

Day 6:

1. Selection cassette removal is checked by colony PCR.
2. Positive clones are plated on NA/Zeocin (to check ABR loss) and on LB at 37°C to cure pDR244.

Day 7:

1. pDR244 curation is checked by plating cells on LB/Spec.