**mazF toxin-mediated barcoding. Adapted from:** Z. Lin *et al.*, “A versatile mini-mazF-cassette for marker-free targeted genetic modification in Bacillus subtilis,” *J. Microbiol. Methods*, vol. 95, pp. 207–214, 2013, doi: 10.1016/j.mimet.2013.07.020.

Day 1:

1. Inoculate *B. subtilis* cells from a single colony overnight at 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. In the morning, dilute cells 1:100 in MM and incubate for 3h at 37°C.
2. Dilute 1:2 in SM (SM: 10ml SMM basic salts, 125μl 40% (w/v) glucose, 60μl 1M Mg2SO4\*7H2O). Incubate 2h at 37°C.
3. Mix 400 μL of cells with 1μg of barcode DNA cassette coming from the PCR amplification of the HiFi assembly of all the parts. Incubate 1h at 37°C.
4. Plate cells on NA supplemented Zeocin (20 µg/mL) plates. Incubate overnight at 37°C.

Day 3:

1. Check integration by colony PCR.
2. Incubate overnight one positive clone at 37°C in LB/0.4% (w/v) glucose/zeocin.

Day 4:

1. Dilute the culture to OD600=0.1 in fresh LB/O.4% (w/v) glucose without antibiotics and grow to OD600=0.4.
2. Add 1% (w/v) xylose. Incubate 8h at 37°C.

Day 5:

1. Plate cells on NA supplemented with 1% (w/v) xylose.

Day 6:

Restreak individual colonies on NA and NA/Zeocin and colony-PCR to test for cassette removal.