**Cre-Lox. Adapted from:** F. Fang *et al.*, “A vector set for systematic metabolic engineering in Saccharomyces cerevisiae,” *Yeast*, 2011, doi: 10.1002/yea.1824.

Day 1:

1. Start an overnight culture of *S. cerevisiae* cells in YPD.

Day 2:

1. Inoculate 5mL of YPD with 500uL of culture at 30°C.
2. Incubate until OD=0.2-0.3.
3. Prepare competent cells by the LiAc method.
4. Transform with the PCR product coming from the cassette amplification of pSC-CreLox-BC.
5. Incubate in YPD for 2 hours (if the selected marker is URA3, this step can be skipped).
6. Plate on YPD supplemented with 200 μg/mL of G418 at 30°C.

Day 3:

1. Wait until colonies appear.
2. Check by PCR barcode presence.
3. Inoculate one positive clone in YPD.

Day 4:

1. Repeat protocol of Day 1 to transform pBF3060\_NatMX (cre recombinase expression).
2. Plate on YPD supplemented with 100 μg/mL Neurothreocin.
3. Plate at 30°C.

Day 5:

1. Inoculate 2 mL of appropriate media to maintain selection but with 2% Raffinose /0.1% Glucose as the carbon source.
2. Grow overnight at 30°C.

Day 6:

1. Make a 1/10 dilution using the appropriate selection media supplemented with 2% Galactose and 0.1% Glucose.
2. Plate cells in YPD/Neurothreocin.

Day 7:

1. Check selection cassette removal by colony PCR
2. Inoculate 2 mL of YPD without antibiotic with a positive clone (this step may need to be repeated 2 or 3 days to allow the plasmid curation).
3. Grow overnight at 30°C.

Day 8:

1. Plate on YPD after dilution looking for single colonies.
2. Check the single colonies for Neurothreocin sensitivity (pSC-Crelox-BC curation).