**CRISPR. Adapted from:** M. M. Jessop-Fabre *et al.*, “EasyClone-MarkerFree: A vector toolkit for marker-less integration of genes into Saccharomyces cerevisiae via CRISPR-Cas9,” *Biotechnol. J*, vol. 11, pp. 1110–1117, 2016, doi: 10.1002/biot.201600147.

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 pCfBf2312 (Cas9).
5. Incubate in YPD for 2 hours.
6. Plate on YPD supplemented with 200 μg/mL G418 at 30°C.

Day 3:

1. Wait until colonies appear.
2. Inoculate a positive clone into YPD/G418 at 30°C.

Day 4:

1. Repeat protocol of Day 1 to co-transform pCfBf3020 (gRNA) and the PCR product coming from pSC-CRISPR-BC.
2. Incubate in YPD for 2 hours.
3. Plate on YPD supplemented with Neurothreocin (100 μg/mL) and G418 (200 μg/mL) at 30°C

Day 5:

1. Wait until colonies appear.
2. Check by PCR barcode presence
3. Inoculate a positive clone in YPD without antibiotic (this step may need to be repeated 2 or 3 days to allow the plasmids curation).

Day 6:

1. Plate on YPD after dilution looking for single colonies.
2. Check the single colonies for Neurothreocin and G418.