**K. phaffi barcoding:** Adapted from “Thermo Fisher Scientific’s pPICZ A, B, and C transformation protocol (Manual part no. 25-0148)”

Day 1:

1. Digest ~5–10 μg of barcoded plasmid DNA with SacI
2. Check linearization in agarose gel
3. Column purify the digestion reaction
4. Grow 5 mL of *Pichia pastoris* strain in YPD at 30°C overnight.

Day 2:

1. Inoculate 50 mL of fresh medium with the overnight culture.
2. Grow overnight again to an OD600 = 1.3–1.5.

Day 3:

1. Centrifuge the cells at 1,500 × g for 5 minutes at 4°C. Resuspend the pellet with 500 ml of ice-cold, sterile water.
2. Centrifuge again, then resuspend the pellet with 250 ml of ice cold, sterile water.
3. Centrifuge again, then resuspend the pellet in 20 ml of ice-cold 1 M sorbitol.
4. Centrifuge again, then resuspend the pellet in 1 ml of ice-cold 1 M sorbitol for a final volume of approximately 1.5 ml. Keep the cells on ice and use that day. Do not store cells.
5. Mix 80 μL of the cells from Step 6 (previous page) with 5–10 μg of linearized DNA (in 5–10 μL sterile water) and transfer them to an ice-cold 0.2 cm electroporation cuvette.
6. Incubate the cuvette with the cells on ice for 5 minutes.
7. Pulse the cells using the manufacturer’s instructions for *Saccharomyces cerevisiae*.
8. Immediately add 1 ml of ice-cold 1 M sorbitol to the cuvette. Transfer the cuvette contents to a sterile 15-ml tube and incubate at 30°C without shaking for 1 to 2 hours.
9. Spread 10, 25, 50, 100, and 200 μl each on separate, labeled YPDS plates containing 100 μg/ml Zeocin. Plating at low cell densities favors efficient Zeocin™ selection.
10. Incubate plates from 3–10 days at 30°C until colonies form.

Day 4:

1. Pick 10–20 colonies and purify (streak for single colonies) on fresh YPD or YPDS plates containing 100 μg/ml Zeocin.

Day 5:

1. Colony PCR to check barcode presence.