Modified Yeast Transformation for KO, pCORE Integration

Inoculate 100 ml YPD with 1 single (reasonably fat) yeast colony at about 5 pm.
Incubate over night at 30 C with shaking.

Check OD600 next day, and also check cells under microscope for contamination.
Culture should be at about OD600=1 at 9-10 am.

When OD600 is about 1, harvest cells by centrifugation. Pellet cells 5 minutes at 5000 rpm in clinical centrifuge in each of 2 sterile 50 ml conical tubes.

Decant supernatant, wash cells in 10 ml sterile water. Pellet again as above.

Wash cells in 5 ml TE/LiOAc.

Resuspend cells in 1 ml TE/LiOAc.

Boil single-stranded carrier DNA for 5 minutes and cool on ice.

Mix **150 µl** yeast cells with **5-20 µl PCR fragment** and **150 µg** freshly boiled single-stranded DNA (e.g. **15 µl** 10 µg/µl SS DNA) in eppendorf. Mix by tapping with finger.

Add **400 µl** TE/LiOAc/PEG solution to each tube. Vortex immediately after solution is added to each tube.

Incubate **60 minutes** at 30 C.

Heat shock 15 minutes at 42 C.

Pellet cells 5 seconds in microfuge, discard supernatant.

Resuspend cells in 0.2 ml TE.

Plate 200 µl of each transformation on **YPD plate**.

Wrap plates and incubate at 30 C.

Next day: replica plate to selective media.

Solutions

sterile water

10 ml TE/LiOAc per strain	1 ml 10 X TE 1 ml 10 X LiOAc 8 ml water
5 ml TE/LiOAc/PEG	4 ml 50% PEG 4000 0.5 ml 10 X TE 0.5 ml 10 X LiOAc
10 X TE	10 ml 1 M Tris-Cl pH 7.5 4 ml 250 mM EDTA 86 ml water sterilize by autoclaving
10 X LiOAc	1 M LiOAc in water, pH 7.5 (adjust with dilute acetic acid) sterilize by autoclaving