Grow at 32°C, 180 rpm several hours

Inoculate fresh biomass to 5 mL YES medium

Grow at 32°C, 180 rpm to desired OD

Dilute culture to 10 mL YES medium

1 mL cells + 1 µL lipid staining solution

Stain LDs

Vortex briefly

+ 10 µL solution for cell boundary visualization

Stain medium

Vortex briefly and centrifuge 3 min, 1000 x g

Remove supernatant

Mount onto a microscope slide

Image cell boundaries in blue channel

Image LDs in green channel

Save as 16-bit z-stack TIFF

Automated analysis in MATLAB

Segmented images

CSV files