

Microscopy

Filename: Strains

WT pguk1-7-GFP: Wild type strain with guk1-7-GFP

ssa1-2DD pguk1-7-GFP: *SSA1/SSA2* double knockout strain with guk1-7-GFP

ssa1-2DD SSA4OE pguk1-7-GFP: *SSA1/SSA2* double knockout strain with over expression of *SSA4* through the GPD promoter and with guk1-7-GFP

wt/WT hsp104D pguk1-7-GFP: *HSP104* knockout strain with guk1-7-GFP

Filename: Treatment

recovery timelapse: Cells imaged during recovery at 30 °C after 30 minutes of heat shock at 42 °C.

Imaging details

Cells were grown in YPD medium to an OD₆₀₀ between 0.3 and 0.5 cell units/ml at 30 °C after which they were heat shocked at 42 °C for 30 minutes. The heat shocked samples were washed with PBS and then placed onto agar pads with complete synthetic media supplemented with YNB and 2 % (w/v) glucose mounted on microscope slides. The samples were imaged at 30 °C on a Zeiss Axio Observer.Z1 inverted microscope with Apotome, DefiniteFocus 2 and AxioCam 506 camera, and a Plan-Apochromat 100x/1.40 Oil DIC M27 objective, with the following incubation equipment to maintain temperature: TempModule S1 (Zeiss), Y module S1 (Zeiss), Temperable insert S1 (Zeiss), Temperable objective ring S1 (Zeiss), and Incubator S1 230 V (Zeiss). All images were acquired as Z-stacks with 10 slices over a 5 µm range at four distinct coordinates in the sample every 10 minutes for as long as the imaging position was stable.

Channel 1: Transmitted light DIC, exp. time 20 ms

Channel 2: EGFP (λEx.: BP 470/40, Beamsplitter: FT 495, λEm.: BP 525/50), exp. time: 80 ms