

Microscopy

File name: Strains

WT/BY4741 pGFP-HSP104: Wild type strain with GFP-Hsp104

ssa1-2DD/ssa1D ssa2D pGFP-HSP104: SSA1/SSA2 double knockout strain with GFP-Hsp104

File name: Plasmids

pRS415/pRS415-pgpd: Empty vector

pRS415-SSA1/pRS415-PgpdSSA1: Vector with SSA1 allele

pRS415-SSA4/pRS415-PgpdSSA4: Vector with SSA4 allele

pRS415-NBD/pRS415-Pgpdssa4NBDsw: Vector with chimaeric *ssa4* allele, NBD* from SSA1

pRS415-SBD/pRS415-Pgpdssa4SBDsw: Vector with chimaeric *ssa4* allele, SBD** from SSA1

pRS415-CTD/pRS415-Pgpdssa4CTD+RCsw: Vector with chimaeric *ssa4* allele, CTD+RC*** from SSA1

File name: Treatment

ctrl: Control sample taken at mid-exponential growth

HS/Heatshock 30 min: Sample taken after 30 minutes of heatshock at 42 °

Imaging details

Images were obtained using a conventional fluorescence microscope, a Zeiss Axio Observer.Z1 inverted microscope with Apotome and AxioCam 506 camera, and a Plan-Apochromat 100x/1.40 Oil DIC M27 objective. All images were acquired as Z-stacks with 10 slices over a 5 µm range.

Channel 1: EGFP (38 HE Green Fluorescent Protein, λEx.: BP 470/40, Beamsplitter: FT 495, λEm.: BP 525/50), exp. time: 200 ms

Channel 2: Transmitted light DIC, exp. time 10 ms

*NBD: Nucleotide binding domain

**SBD: Substrate binding domain

***CTD+RC: C-terminal domain + Random Coil