

Western blot**WT ssa12DD and ssa12DDSSA4OE**

Primary antibody 1: mouse anti-Hsp70 [C92F3A-5] (Abcam Cat# ab47455, RRID:AB_881520) (abcam)

Primary antibody 2: mouse anti-Pgk1 (Thermo Fisher Scientific Cat# 459250, RRID:AB_2532235)

Secondary antibody 1: goat800LT anti-mouse (LI-COR Biosciences Cat# 926-68020, RRID:AB_10706161)

Secondary antibody 2: goat680CW anti-mouse (LI-COR Biosciences Cat# 926-32210, RRID:AB_621842)

Load order (from left to right)

Lane	Strain /Sample	Growth temperature
1	Size ladder	-
2	Empty	-
3	BY4741	22 °C
4	Ssa4 OE	22 °C
5	<i>ssa1</i> Δ <i>ssa2</i> Δ	22 °C
6	<i>ssa1</i> Δ <i>ssa2</i> Δ Ssa4 OE	22 °C
7	BY4741	30 °C
8	Ssa4 OE	30 °C
9	<i>ssa1</i> Δ <i>ssa2</i> Δ	30 °C
10	<i>ssa1</i> Δ <i>ssa2</i> Δ Ssa4 OE	30 °C
11	Empty	-
12	Size ladder	-

Ssa4 OE: Ssa4 over expression through the GPD-promoter

WT ssa12DD w chimaera plasmids

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Load order (from left to right)

Lane	Strain /Sample	Plasmid	Base growth temperature	Treatment
1	Size ladder	-	-	-
2	Empty	-	-	-
3	WT	Empty vector	30 °C	Control
4	ssa1Δ ssa2Δ	Empty vector	30 °C	Control
5	ssa1Δ ssa2Δ	pSSA1	30 °C	Control
6	ssa1Δ ssa2Δ	pSSA4	30 °C	Control
7	ssa1Δ ssa2Δ	pNBDsw	30 °C	Control
8	ssa1Δ ssa2Δ	pSBDsw	30 °C	Control
9	ssa1Δ ssa2Δ	pCTDsw	30 °C	Control
10	WT	Empty vector	30 °C	42 °C 30 min
11	ssa1Δ ssa2Δ	Empty vector	30 °C	42 °C 30 min
12	ssa1Δ ssa2Δ	pSSA1	30 °C	42 °C 30 min
13	ssa1Δ ssa2Δ	pSSA4	30 °C	42 °C 30 min
14	ssa1Δ ssa2Δ	pNBDsw	30 °C	42 °C 30 min
15	ssa1Δ ssa2Δ	pSBDsw	30 °C	42 °C 30 min
16	ssa1Δ ssa2Δ	pCTDsw	30 °C	42 °C 30 min
17	Empty	-	-	-
18	Size ladder	-	-	-

pRS415: Empty vector, vector backbone

pNBDsw: Nucleotide binding domain swapped Ssa4-Ssa1 chimaera

pSBDsw: Substrate binding domain swapped Ssa4-Ssa1 chimaera

pCTDsw/pCTD+RCsw: C-terminal and random coil domain swapped Ssa4-Ssa1 chimaera