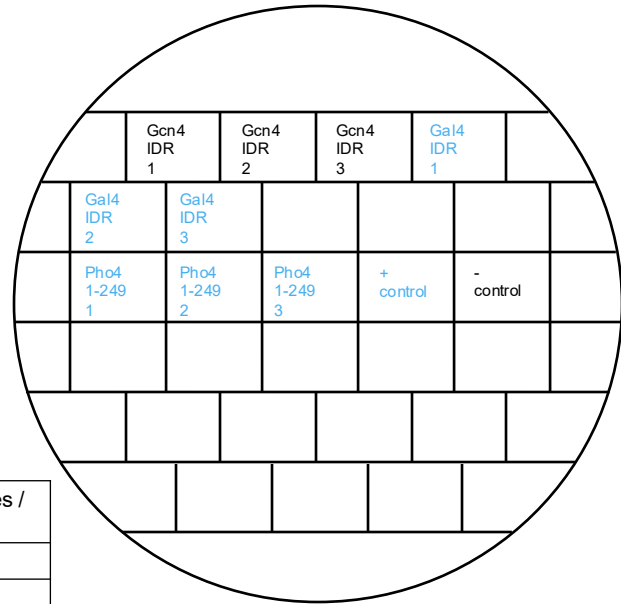
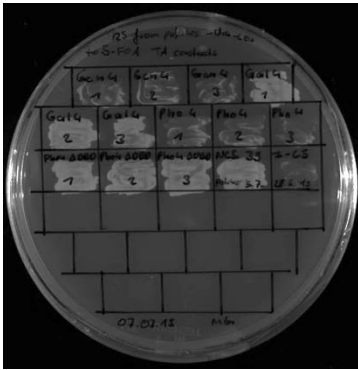


## Supplemental figures

figure S1: Viability of Abf1 constructs assessed by 5-FOA plasmid shuffling assay.

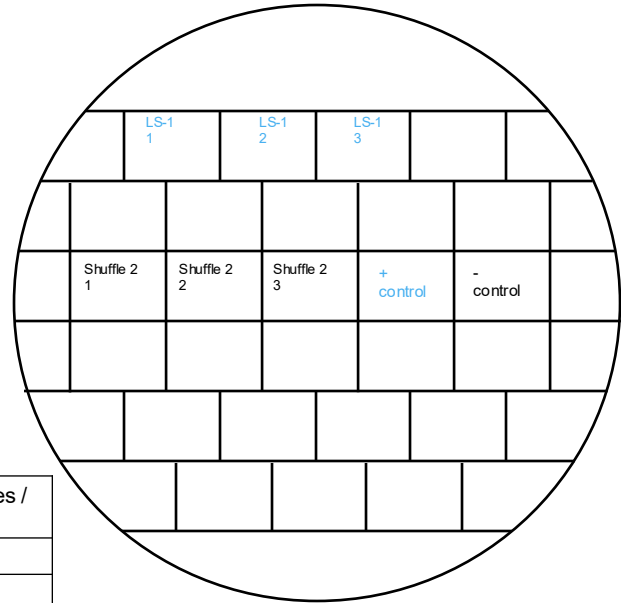
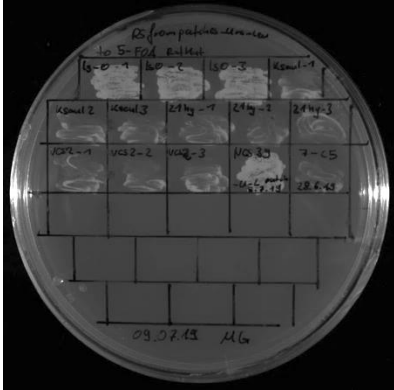
Strains harboring both pRS416-*ABF1* plasmid (*URA3* marker) and pRS315-"*abf1* construct name" (*LEU2* marker) in the strain background with deleted *abf1* chromosomal gene were re-streaked from patches from YNB w/o ura, leu plates onto 5-FOA w/o leu plates. Only constructs discussed in the paper are labeled in plate schemes, where names of viable strains are in blue, names of inviable ones in black. Viability on 5-FOA plates was visually scored by comparison to known viable or inviable strains on the same plate. Inviabile strains were distinguished from viable strains because they did not show growth of a contiguous patch after at most three days of incubation at 30 °C but only a smeary appearance or at most sparse single colonies, whereas viable strains grew as a contiguously dense patch. If few individual colonies grew instead of a contiguous patch, we tested some of them by colony PCR with the primers listed in Table S18. In all cases, these individual colonies contained plasmids of a wrong size or sequence reflecting recombination between pRS416-*ABF1* and pRS315-"*abf1* construct name".

For each construct, independent clones after transformation were tested. On each slide, a table summarizes the result of the shown plate and in brackets the total result obtained for this construct if all tested clones on all plates were considered. If the same clone showed conflicting results in technical replicates, it was not counted as viable or inviable, but included in the total number of tested clones. The final categorization of each construct as viable or inviable followed the majority of tested clones excluding the clones with ambiguous results in technical replicates.



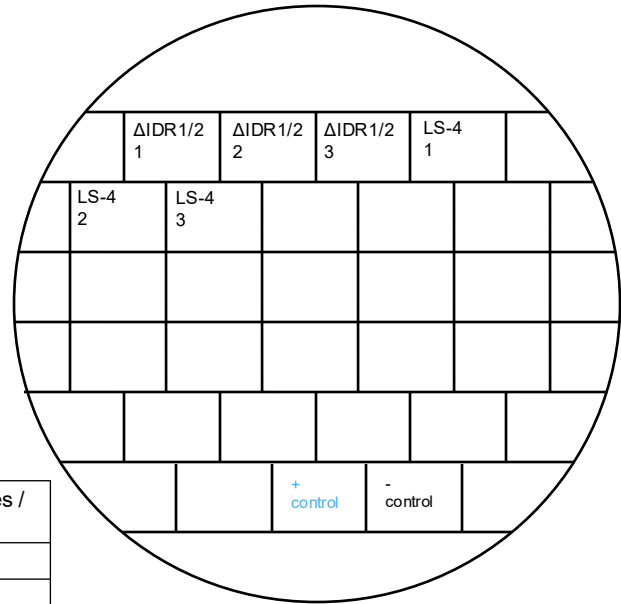
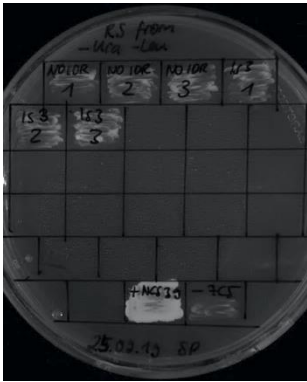
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Gcn4 <sup>17-150</sup>	inviable	3 out of 3
Gal4 <sup>768-881</sup>	viable	3 out of 3
Pho4 <sup>1-249</sup>	viable	3 out of 3



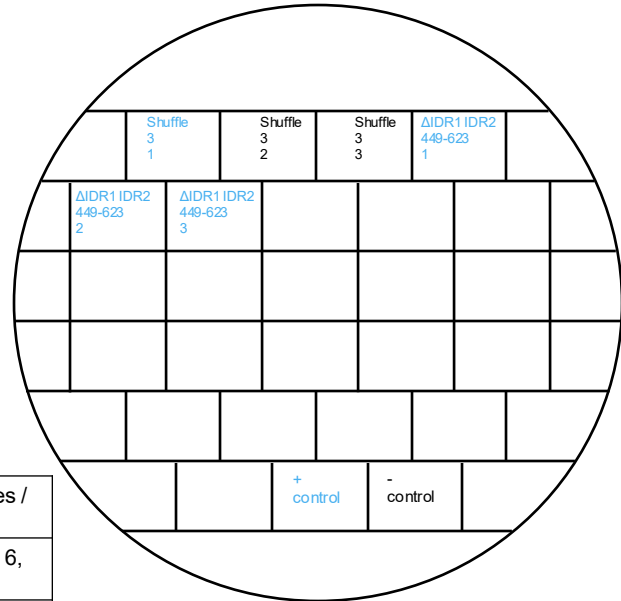
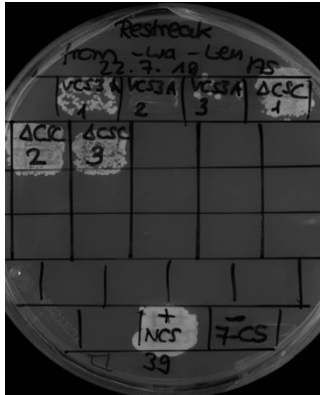
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
LS-1	viable	3 out of 3
Shuffle 2	inviable	3 out of 3



Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
ΔIDR1/2 (NLS-FLAG)	inviable	3 out of 3
LS-4	inviable	3 out of 3



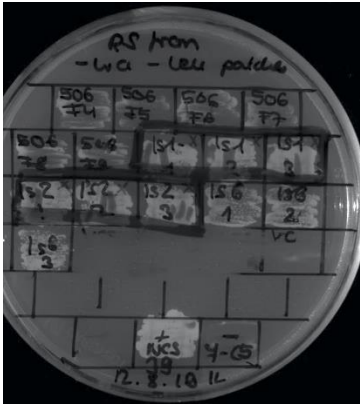
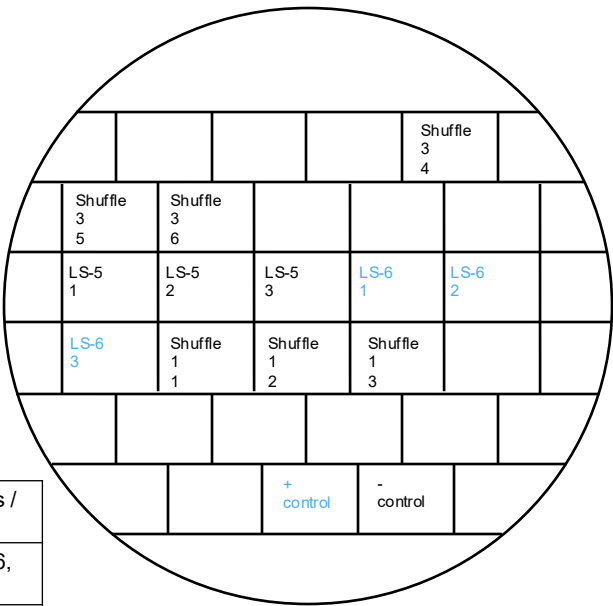
Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Shuffle 3	inviable	2 out of 3 (in total 5 out of 6, see also slide 4)
ΔIDR1 & IDR2 <sup>449-623</sup>	viable	3 out of 3



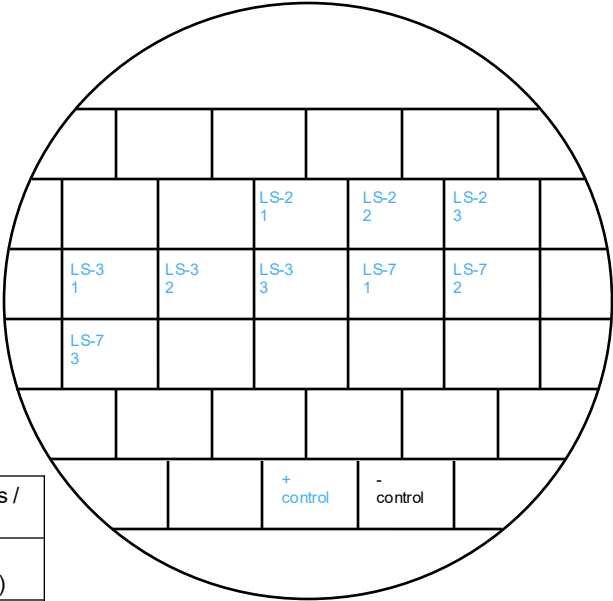
Restreak from YNB –ura –leu to 5-FOA –leu

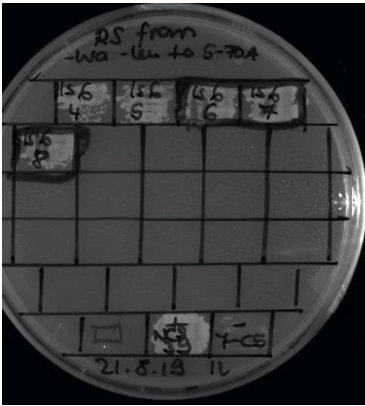
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Shuffle 3	inviable	3 out of 3 (in total 5 out of 6, see also slide 3)
LS-5	inviable	3 out of 3
LS-6	viable	3 out of 3
Shuffle 1	inviable	3 out of 3



Restreak from YNB –ura –leu to 5-FOA –leu

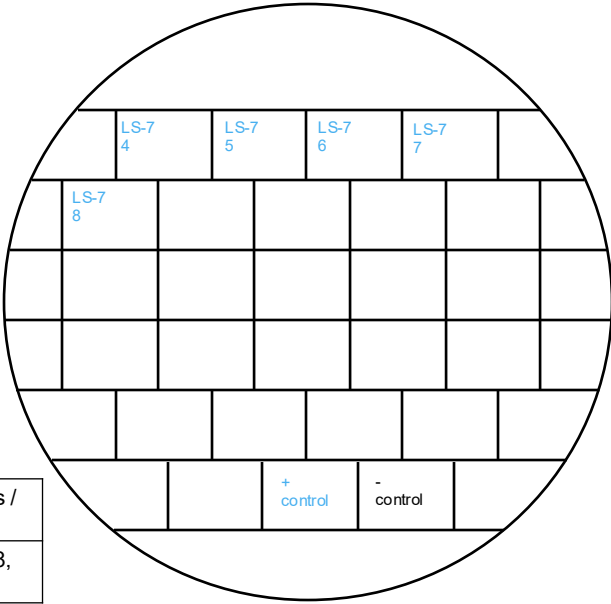
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-2	viable	3 out of 3 (technical replicates, see also slide 6)
LS-3	viable	3 out of 3 (technical replicates, see also slide 6)
LS-7	viable	3 out of 3 (in total 8 out of 8, see also slide 5)

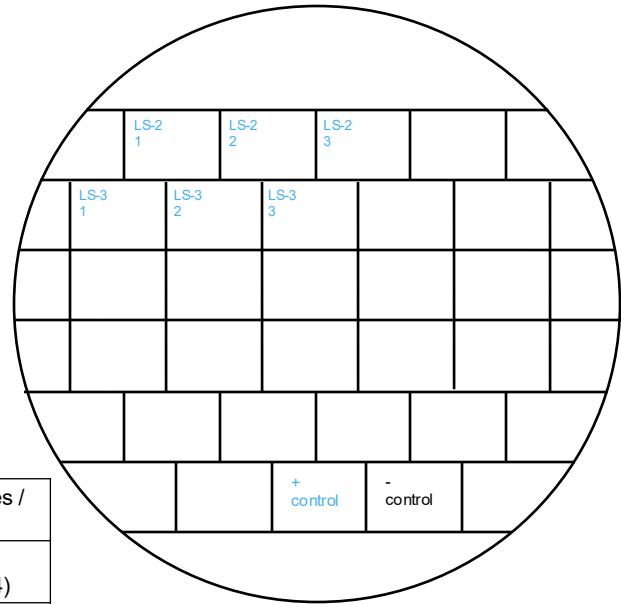
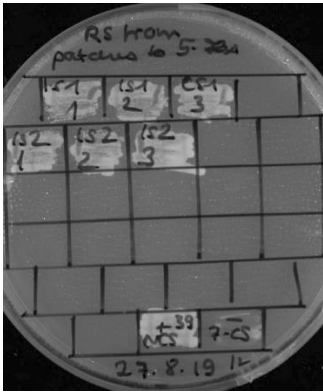




Restreak from YNB –ura –leu to 5-FOA –leu

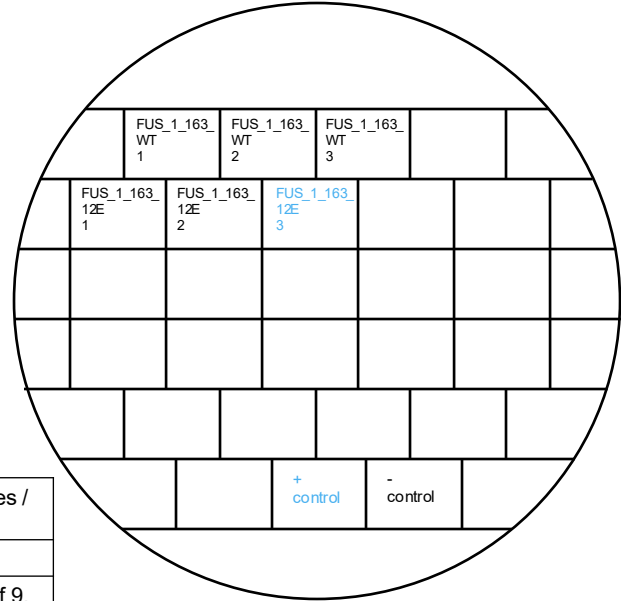
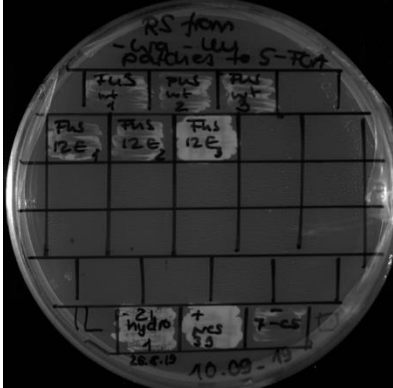
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-7	viable	5 out of 5 (in total 8 out of 8, see also slide 4)





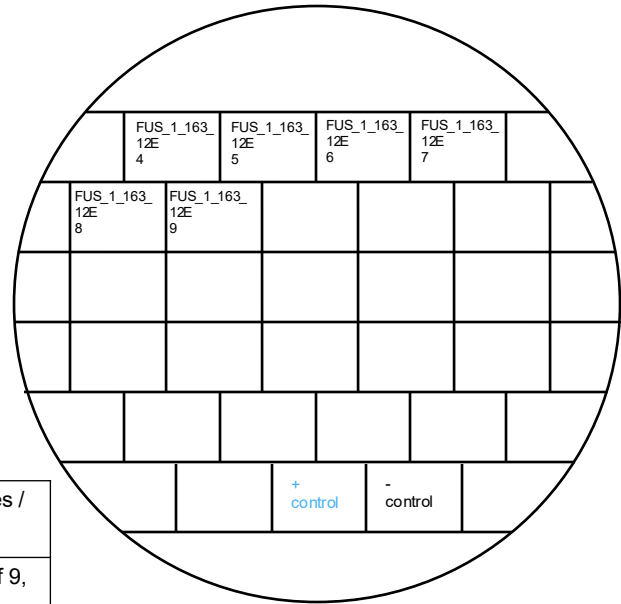
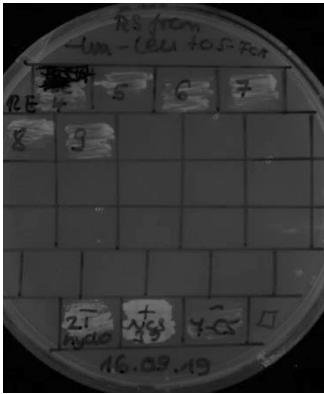
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-2	viable	3 out of 3 (technical replicates, see also slide 4)
LS-3	viable	3 out of 3 (technical replicates, see also slide 4)



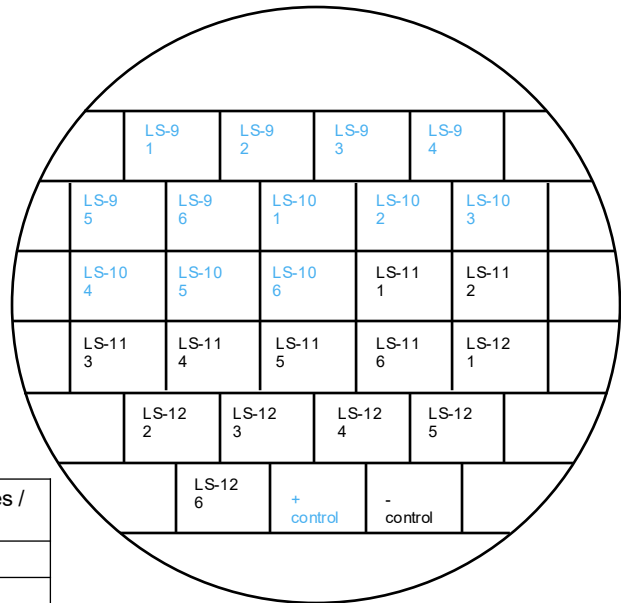
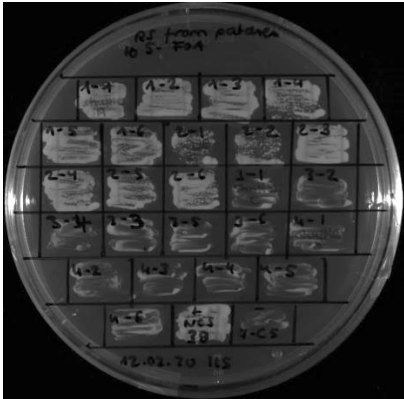
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Construct	Number of (in)viable clones / total number of clones
FUS <sup>1-163</sup>	inviabile	3 out of 3
FUS <sup>1-163</sup> 12E	inviabile	2 out of 3 (in total: 8 out of 9, see also slides 7 and 14)



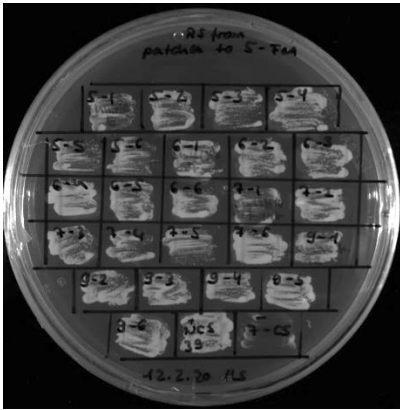
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS1-16312E	Invisible	6 out of 6 ((in total 8 out of 9, see also slides 6 and 14)



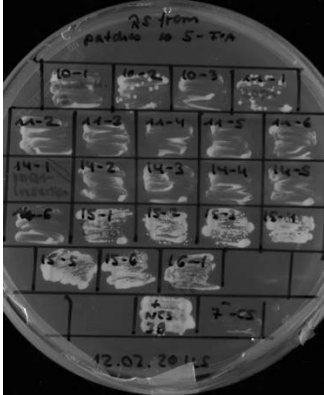
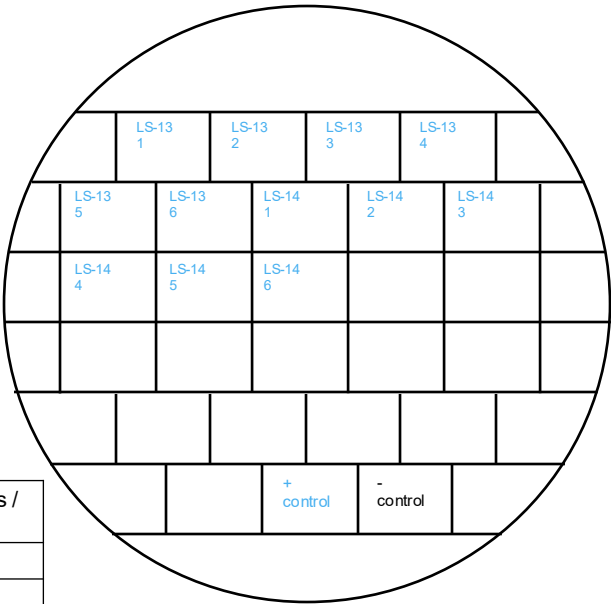
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-9	viable	6 out of 6
LS-10	viable	6 out of 6
LS-11	inviabile	6 out of 6
LS-12	inviabile	6 out of 6



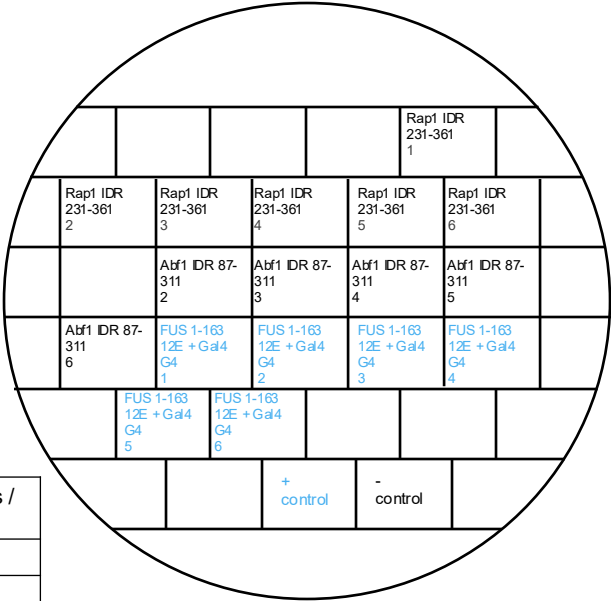
Restreak from YNB –ura –leu to 5-FOA –leu

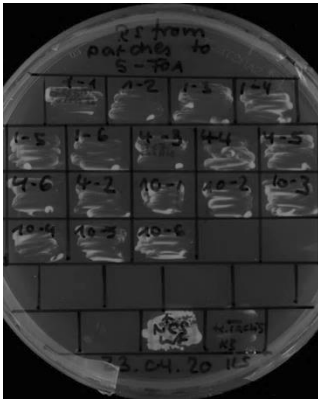
Construct	Construct	Number of (in)viabile clones / total number of clones
LS-13	viable	6 out of 6
LS-14	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

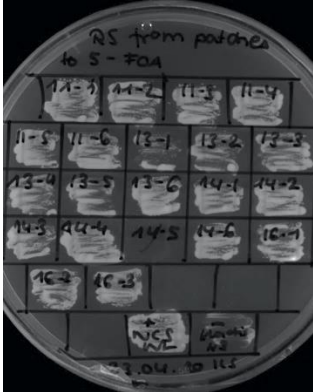
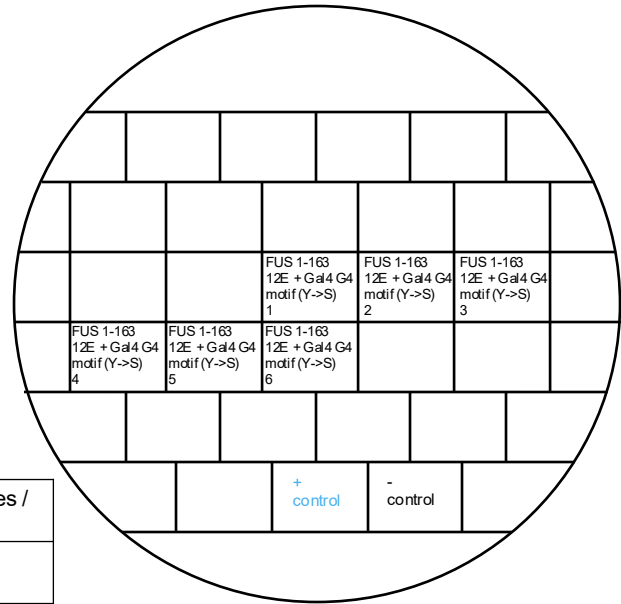
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Rap1 <sup>231-361</sup>	inviable	6 out of 6
Abf1 IDR <sup>187-311</sup>	inviable	6 out of 6
FUS1-163 <sup>12E + Gal4<sup>G4</sup></sup>	viable	6 out of 6





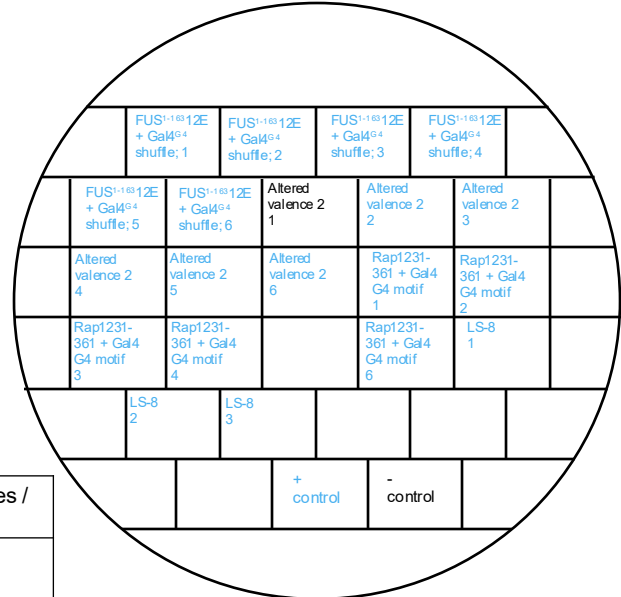
Restreak from YNB –ura –leu to 5-FOA –leu

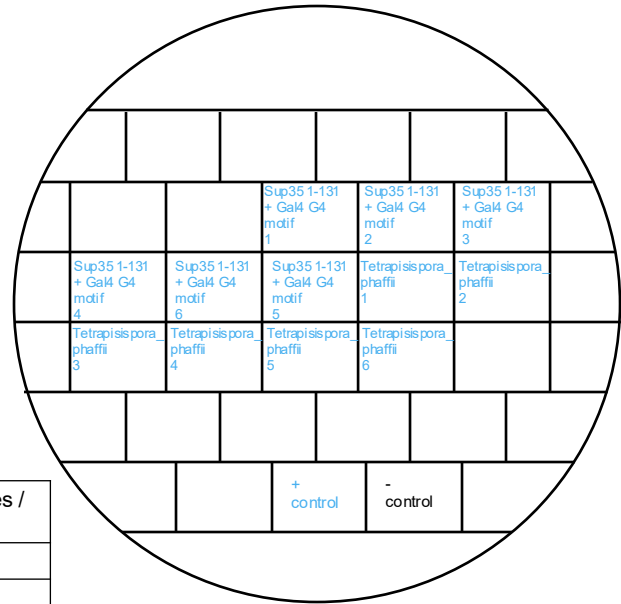
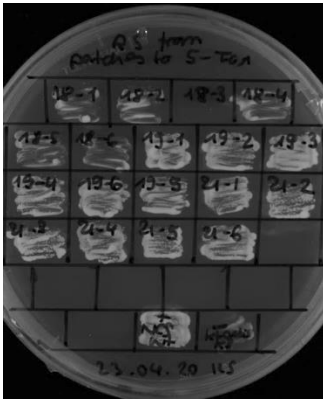
Construct	Construct	Number of (in)viable clones / total number of clones
FUS1-163 12E + Gal4 <sup>G4</sup> Y→S in context	inviabile	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

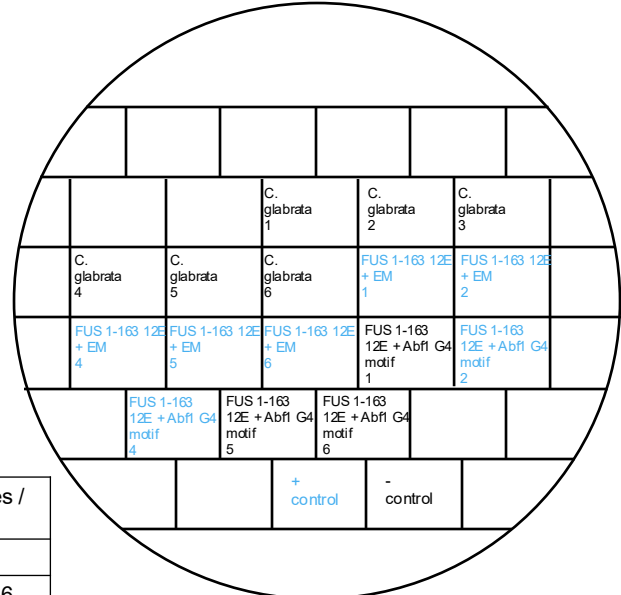
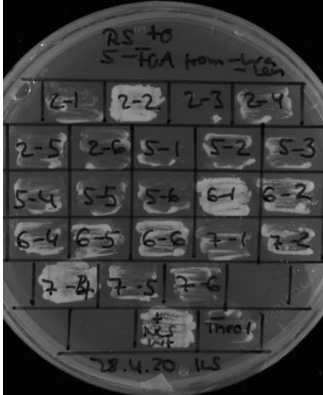
Construct	Construct	Number of (in)viable clones / total number of clones
FUS1-163 12E + Gal4 <sup>G4</sup> shuffle	viable	6 out of 6
Altered valence 2	viable	5 out of 6 (in total 5 out of 6, see also slide 22)
Rap1231-361 + Gal4 G4	viable	5 out of 5
LS-8	viable	3 out of 3





Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Sup35 <sup>1-131</sup> + Gal4 <sup>G4</sup>	viable	6 out of 6
T. phaffii	viable	6 out of 6



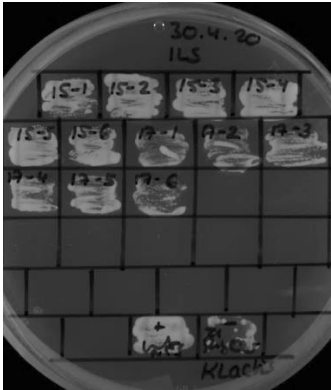
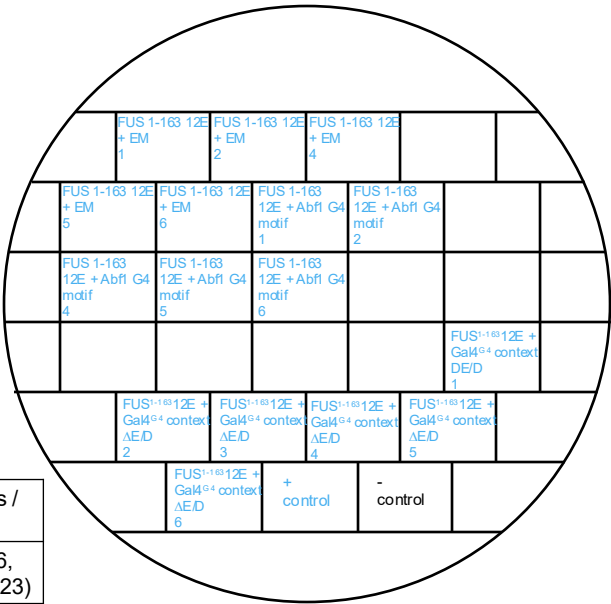
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
C. glabrata	inviable	6 out of 6
FUS 1-163 12E + EM	viable	5 out of 5 (in total 6 out of 6, see also slides 11, 22, 23)
FUS 1-163 12E + Abf1 <sup>G4</sup>	viable	2 out of 5 (in total 2 out of 2, technical replicate, see also slide 11)



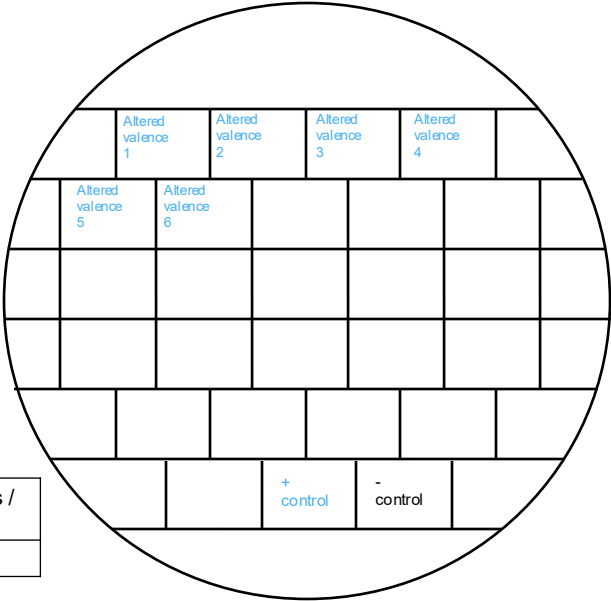
Restreak from YNB –ura –leu to 5-FOA –leu

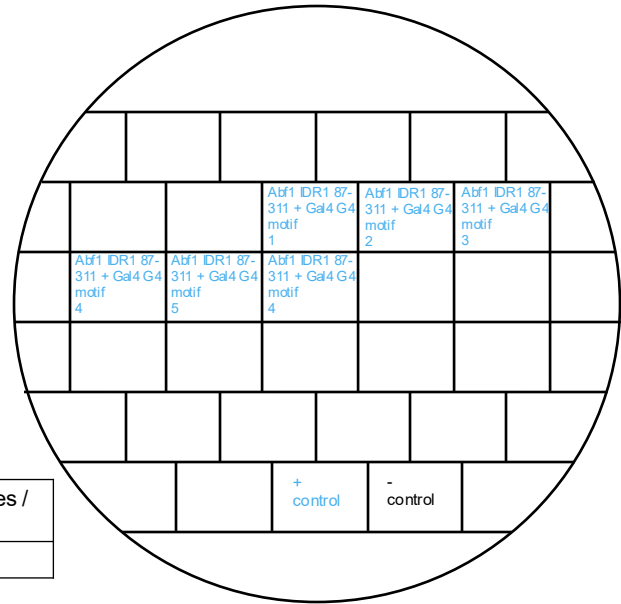
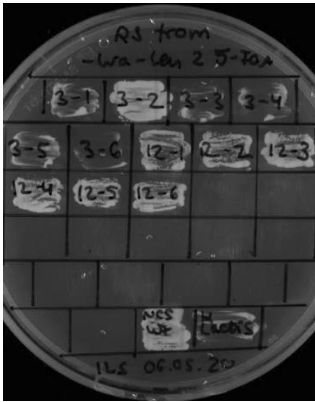
Construct	Construct	Number of (in)viable clones / total number of clones
FUS1-163 12E + EM	viable	5 out of 5 (in total 6 out of 6, see also slides 10, 22 and 23)
FUS1-163 12E + Abf1 <sup>G4</sup>	viable	5 out of 5 (in total 2 out of 2, technical replicate, see also slide 10)
FUS1-163 12E + Gal4 <sup>G4</sup> context ΔE/D	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

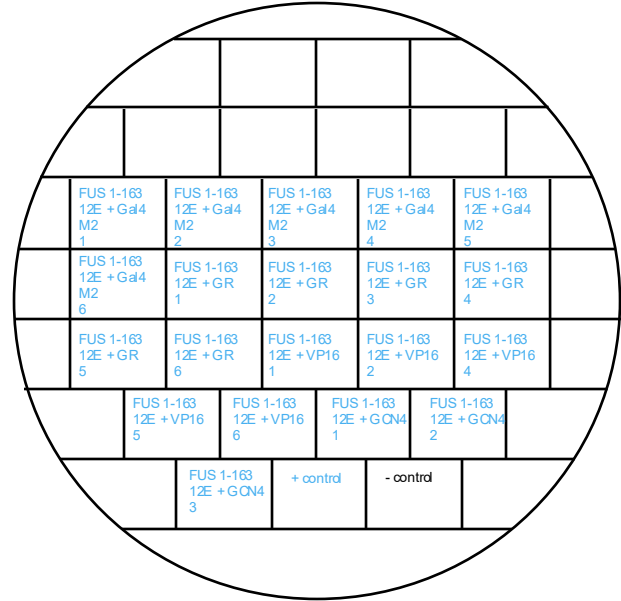
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Altered valence 1	viable	6 out of 6





Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Construct	Number of (in)viable clones / total number of clones
Abf1 IDR1 <sup>87-311</sup> + Gal4 <sup>G4</sup>	viable	6 out of 6



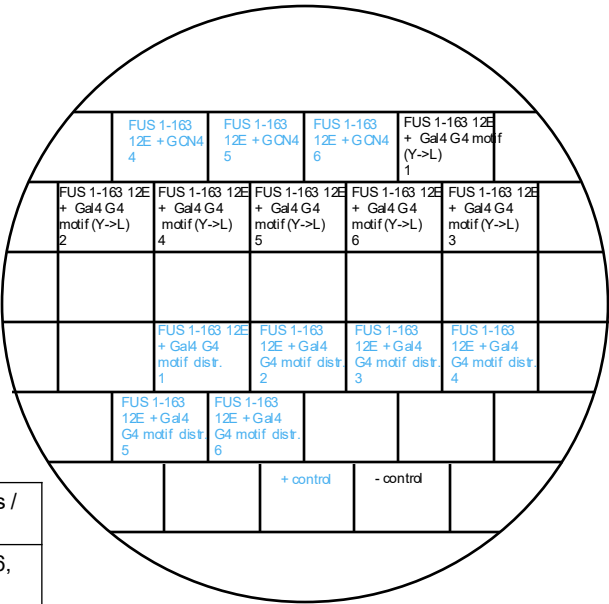
Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS <sup>1-163</sup> 12E+ Gal4 <sup>M2</sup>	viable	6 out of 6
FUS <sup>1-163</sup> 12E+ GR	viable	6 out of 6
FUS <sup>1-163</sup> 12E+ VP16	viable	5 out of 5
FUS <sup>1-163</sup> 12E+ Gcn4	viable	3 out of 3 (in total 6 out of 6, see also slide 13)



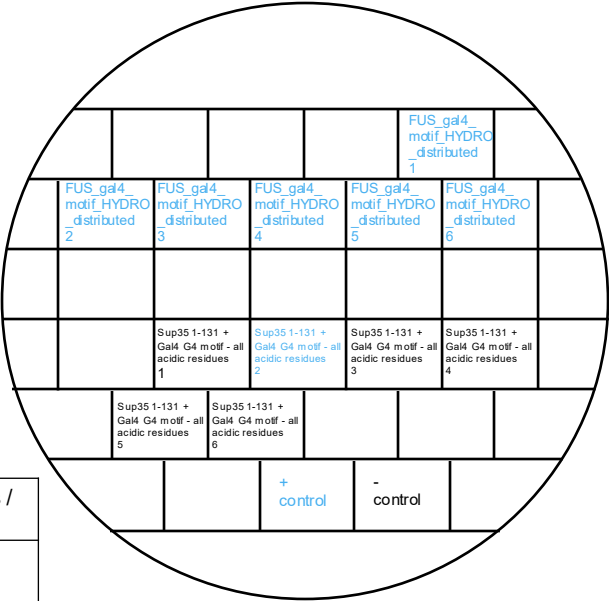
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
<b>FUS<sup>1-163</sup>12E + Gcn4</b>	viable	3 out of 3 (in total 6 out of 6, see also slide 12)
<b>FUS<sup>1-163</sup>12E + Gal4<sup>G4</sup> Y→L in context</b>	inviable	6 out of 6
<b>FUS<sup>1-163</sup>12E + Gal4<sup>G4</sup> distr.</b>	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

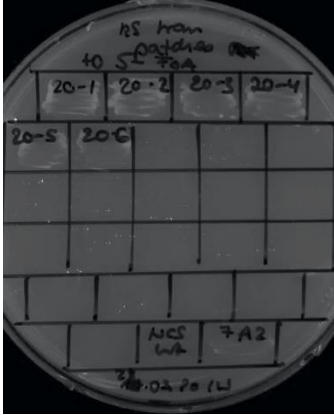
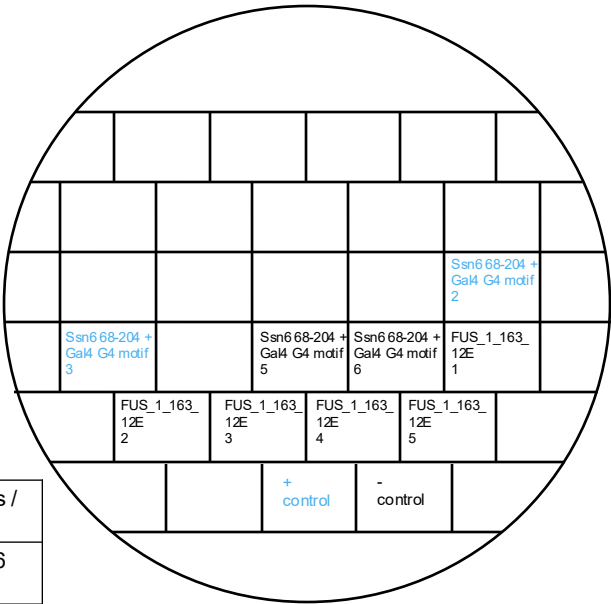
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
<b>FUS<sup>1-163</sup>12E + Gal4<sup>G4</sup> motif hydro distr.</b>	viable	6 out of 6
<b>Sup35<sup>1-131</sup> + Gal4<sup>G4</sup> - all acidic residues</b>	inviable	5 out of 6





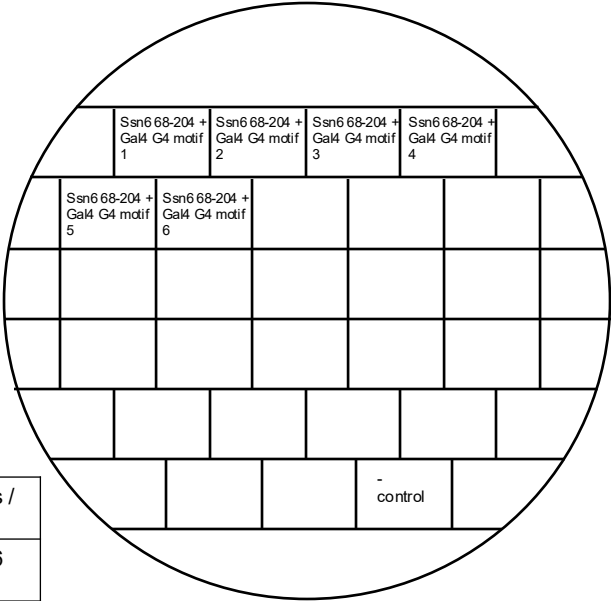
Restreak from YNB –ura –leu to 5-FOA –leu

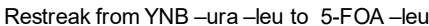
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Ssn6 <sup>68-204</sup> + Gal4 <sup>G4</sup>	inviable	2 out of 4 (in total 4 out of 6 see plate below)
FUS <sup>1-163</sup> 12E	inviable	5 out of 5 (in total 8 out of 9, see also slides 6 and 7)



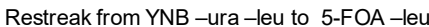
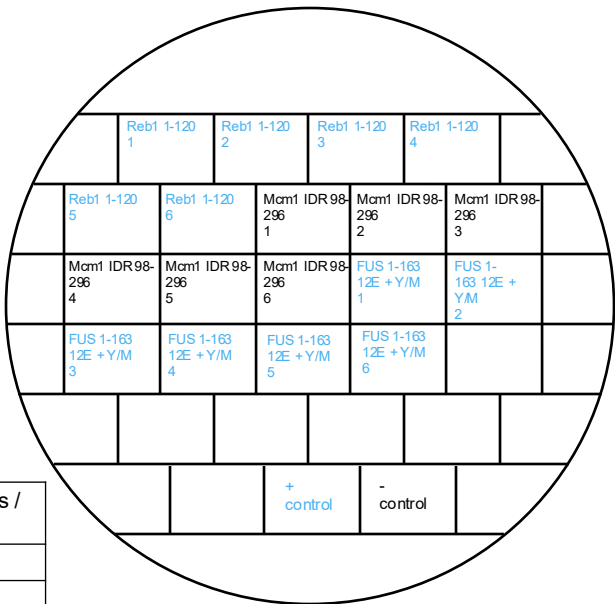
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Ssn6 <sup>68-204</sup> + Gal4 <sup>G4</sup>	inviable	6 out of 6 (in total 4 out of 6 see plate above)

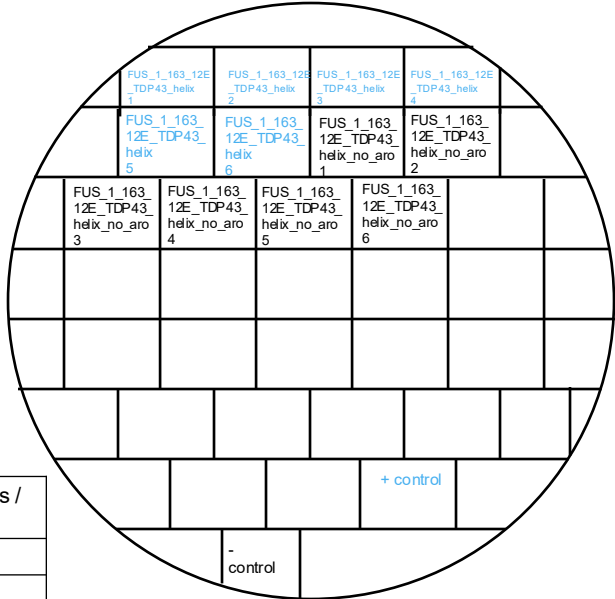


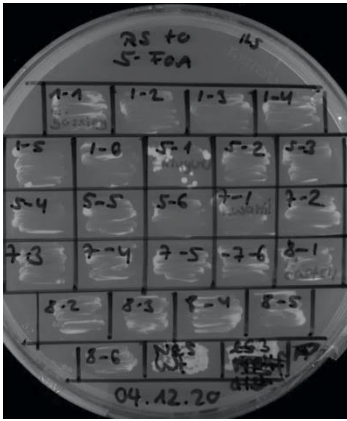


Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
Reb1 <sup>1-120</sup> (+PKEEEEGL)	viable	6 out of 6
Mcm1 <sup>98-296</sup>	inviable	6 out of 6
FUS <sup>1-163</sup> 12E+ Y/M	viable	6 out of 6



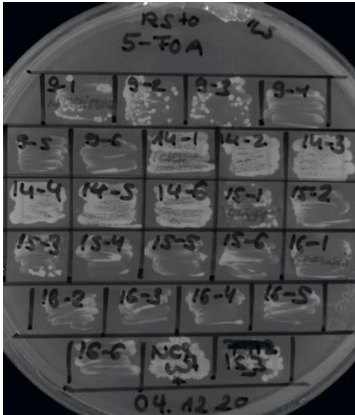
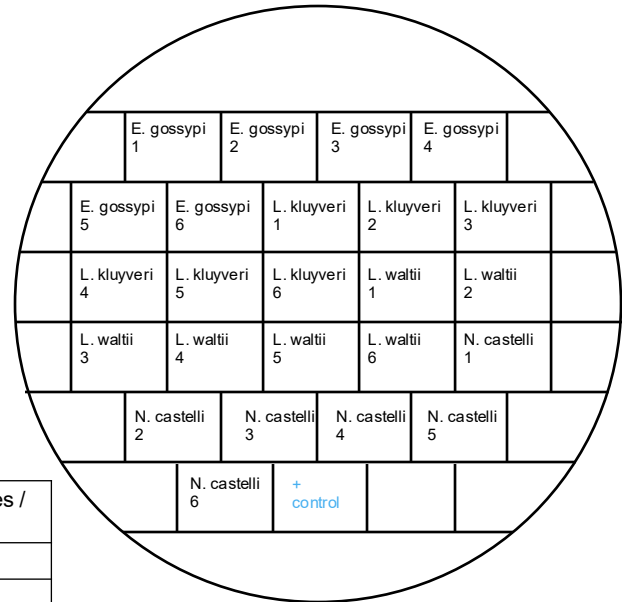
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS <sup>1-163</sup> 12E + TDP-43	viable	6 out of 6
FUS <sup>1-163</sup> 12E + TDP-43 Y→S in context	inviable	6 out of 6





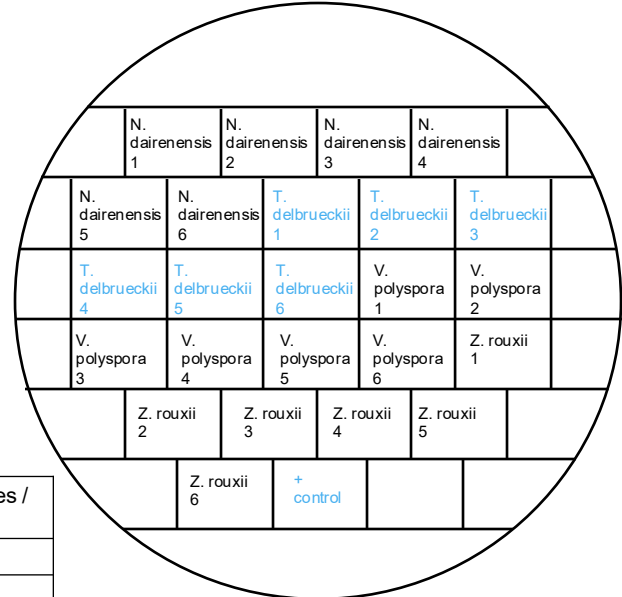
Restreak from YNB –ura –leu to 5-FOA –leu

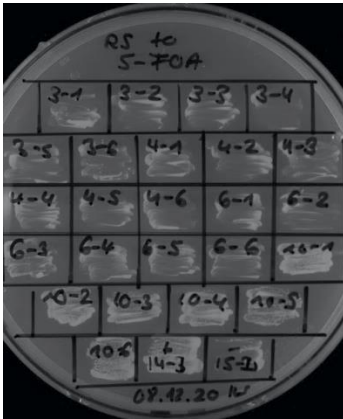
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
<i>E. gossypii</i>	inviabile	6 out of 6
<i>L. kluyveri</i>	inviabile	6 out of 6
<i>L. waltii</i>	inviabile	6 out of 6
<i>N. castellii</i>	inviabile	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

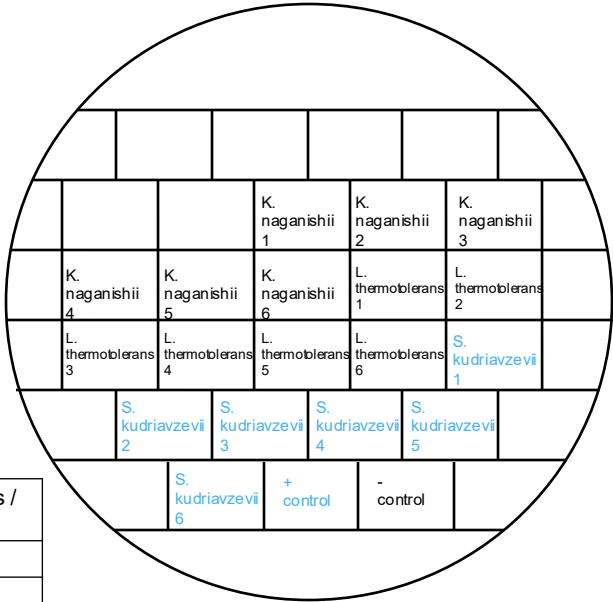
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
<i>N. dairenensis</i>	inviabile	6 out of 6
<i>T. delbrueckii</i>	viable	6 out of 6
<i>V. polyspora</i>	inviabile	6 out of 6 (in total 14 out of 14, see also slide 36)
<i>Z. rouxii</i>	inviabile	6 out of 6





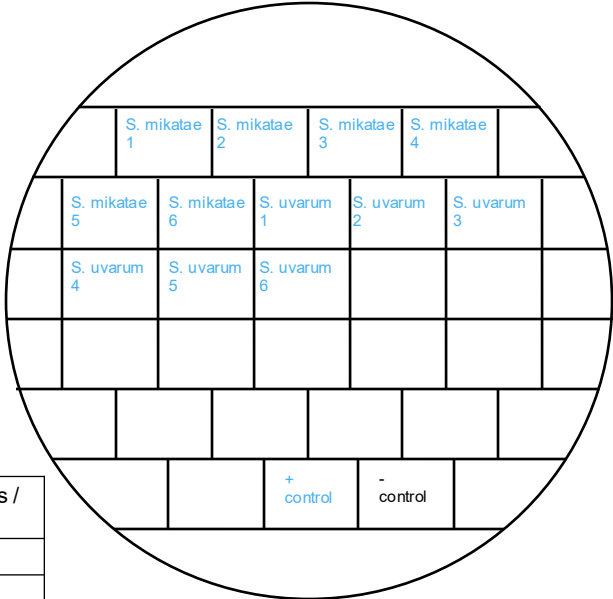
Restreak from YNB –ura –leu to 5-FOA –leu

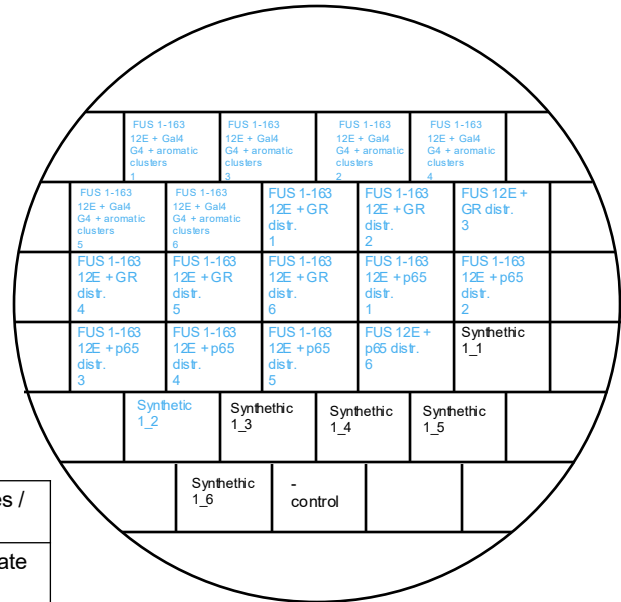
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
K. naganishii	inviable	6 out of 6
L. thermotolerans	inviable	6 out of 6
S. kudriavzevii	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

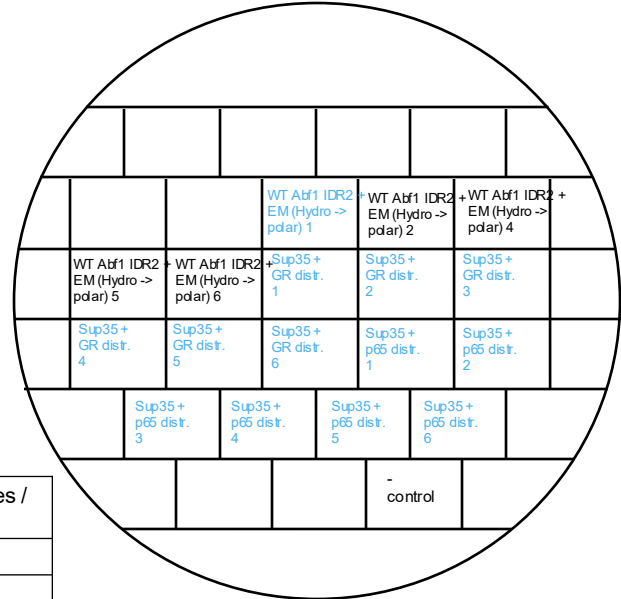
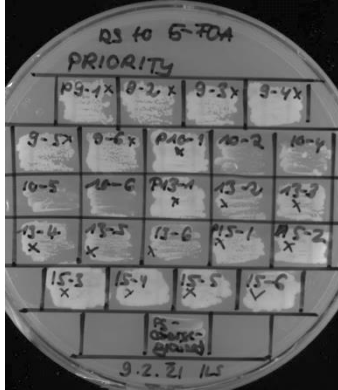
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
S. mikatae	viable	6 out of 6
S. uvarum	viable	6 out of 6





Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
<a href="#">FUS1-163 12E + Gal4 G4 aromatic clusters</a>	viable	6 out of 6 (technical replicate see slide 22)
<a href="#">FUS1-163 12E + GR distr.</a>	viable	6 out of 6
<a href="#">FUS1-163 12E + p65 distr.</a>	viable	6 out of 6
Synthetic 1	inviable	5 out of 6



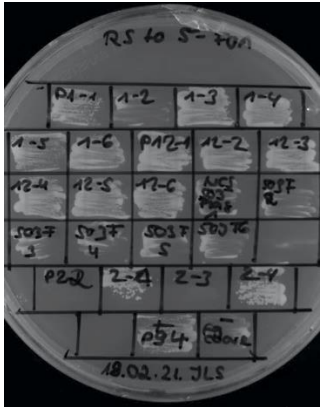
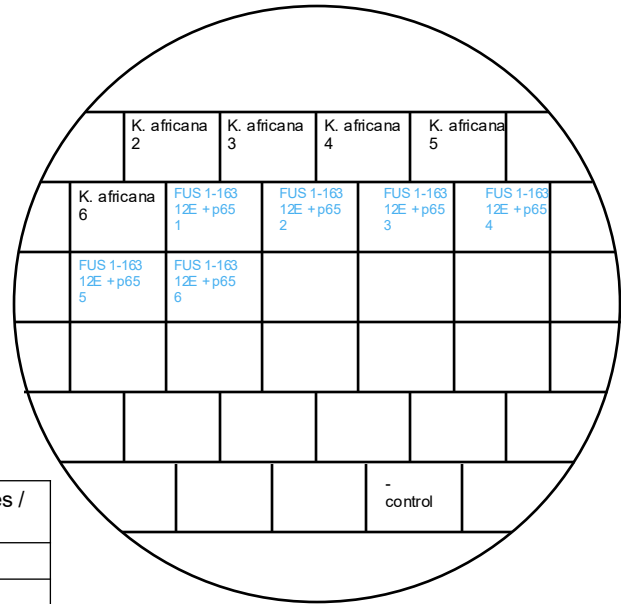
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
IDR2 WT hydro→polar	inviable	4 out of 5
<a href="#">Sup35<sup>1-131</sup> + GR distr.</a>	viable	6 out of 6
<a href="#">Sup35<sup>1-131</sup> + p65 distr.</a>	viable	6 out of 6



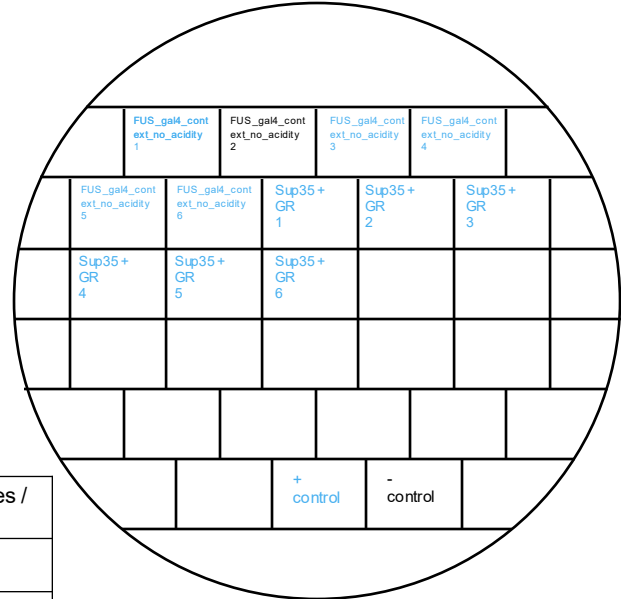
Restreak from YNB –ura –leu to 5-FOA –leu

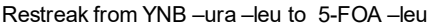
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
K. africana	inviable	5 out of 5
FUS <sup>1-163</sup> 12E + p65	viable	6 out of 6



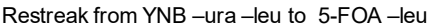
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
FUS <sup>1-163</sup> 12E + Gal4 <sup>G4</sup> context ΔE/D	viable	5 out of 6
Sup35 <sup>1-131</sup> + GR	viable	6 out of 6



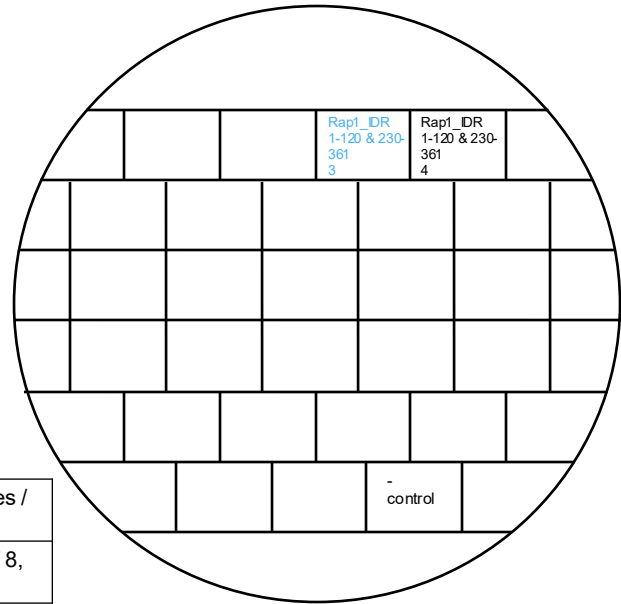


Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Synthetic 2	inviable	4 out of 6
Sup35 <sup>1-131</sup> + p65	viable	3 out of 3



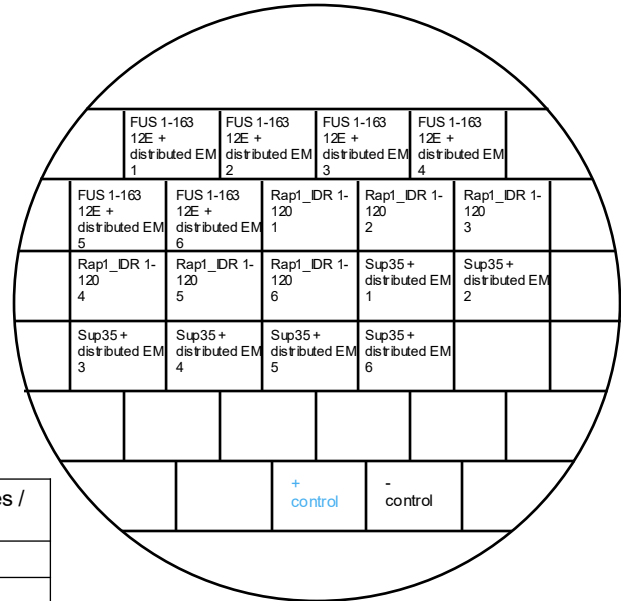
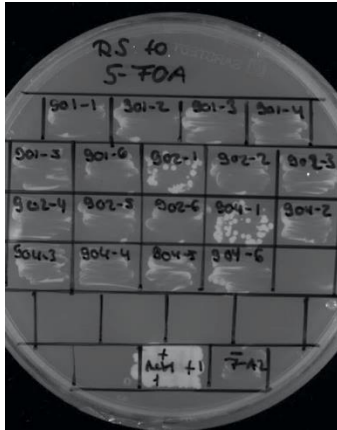
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Reb1 <sup>1-420</sup>	viable	6 out of 6
T. blattae	inviable	6 out of 6





Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Rap1 <sup>1-120 &amp; 230-361</sup>	inviabile	1 out of 2 (in total 7 out of 8, see also slide 39)



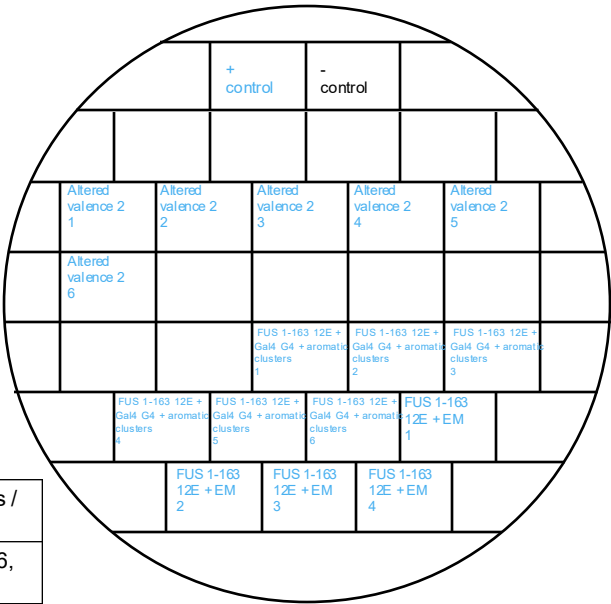
Restreak from YNB –ura –leu to 5-FOA –leu

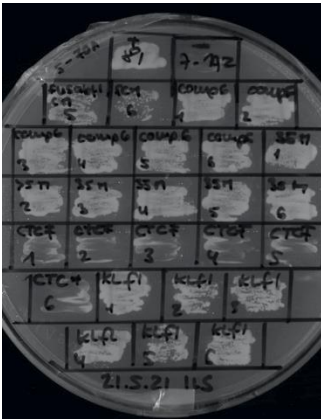
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS <sup>1-163</sup> 12E + EM distr.	inviabile	6 out of 6
Rap1 <sup>1-120</sup>	inviabile	6 out of 6
Sup35 <sup>1-131</sup> + EM distr.	inviabile	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

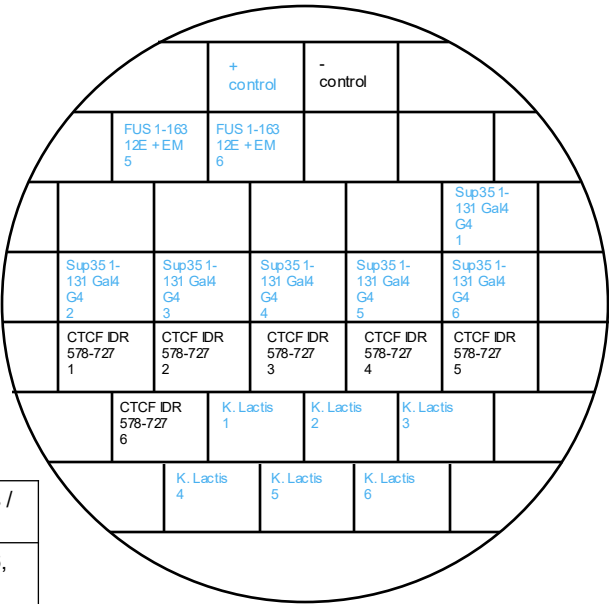
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Altered valence 2	viable	6 out of 6 (in total 5 out of 6, see also slide 9)
FUS <sup>1-163</sup> 12E + Gal4 <sup>G4</sup> aromatic clusters	viable	6 out of 6 (technical replicate see slide 18)
FUS <sup>1-163</sup> 12E + EM	viable	4 out of 4 (in total 6 out of 6, see also slides 10, 11, 23)





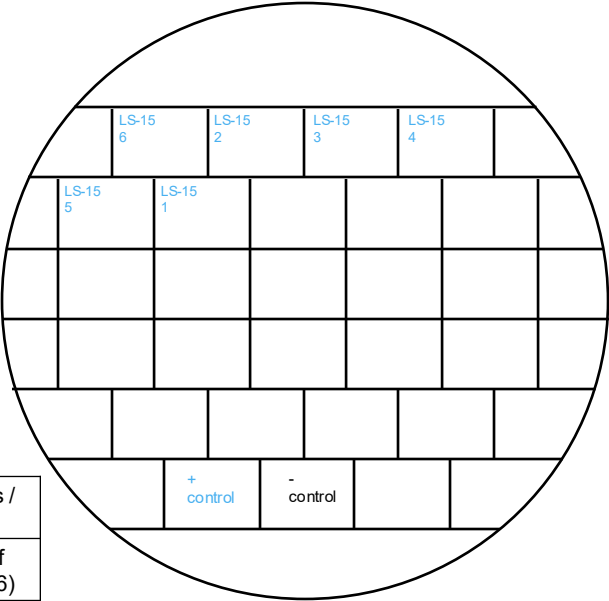
Restreak from YNB –ura –leu to 5-FOA –leu

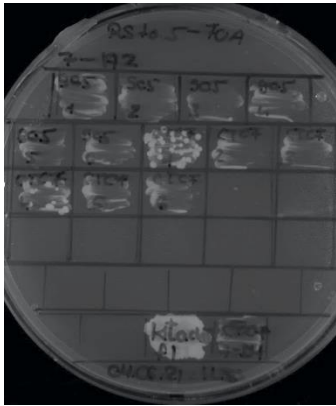
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS1-16312E + EM	viable	2 out of 2 (in total 6 out of 6, see also slides 10, 11, 22)
Sup351-131 + Gal4 <sup>G4</sup>	viable	6 out of 6
CTCF <sup>578-727</sup>	inviable	6 out of 6 (technical repliate, see slide 24)
K. Lactis	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

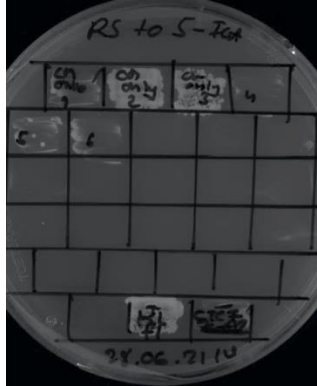
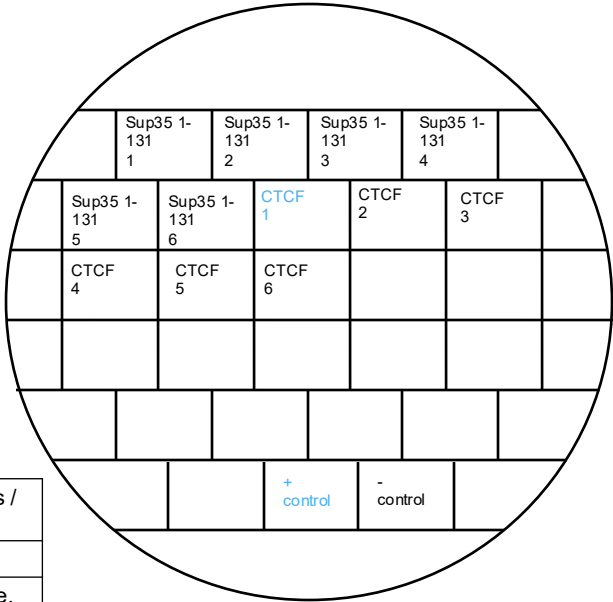
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-15	viable	6 out of 6 (in total 14 out of 14 clones, see also slide 36)





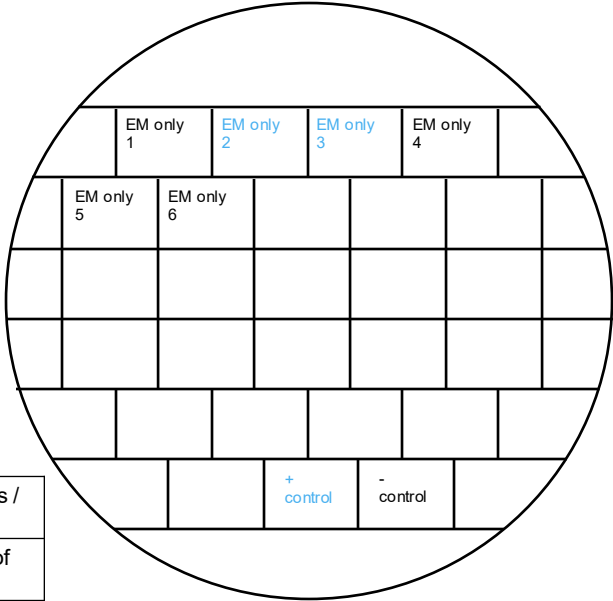
Restreak from YNB –ura –leu to 5-FOA –leu

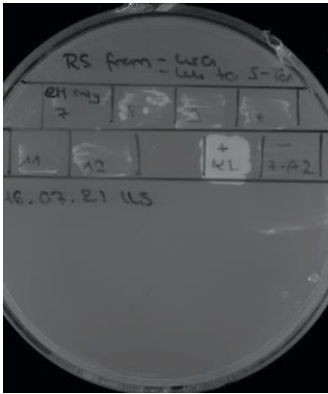
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Sup35 <sup>1-131</sup>	inviable	6 out of 6
CTCF <sup>578-727</sup>	inviable	5 out of 6 (technical repliate, see slide 23)



Restreak from YNB –ura –leu to 5-FOA –leu

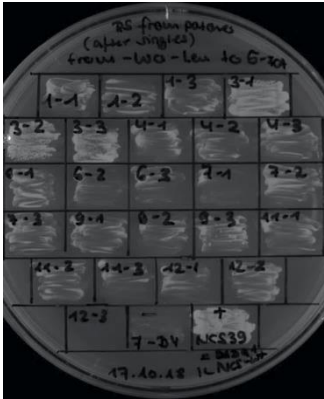
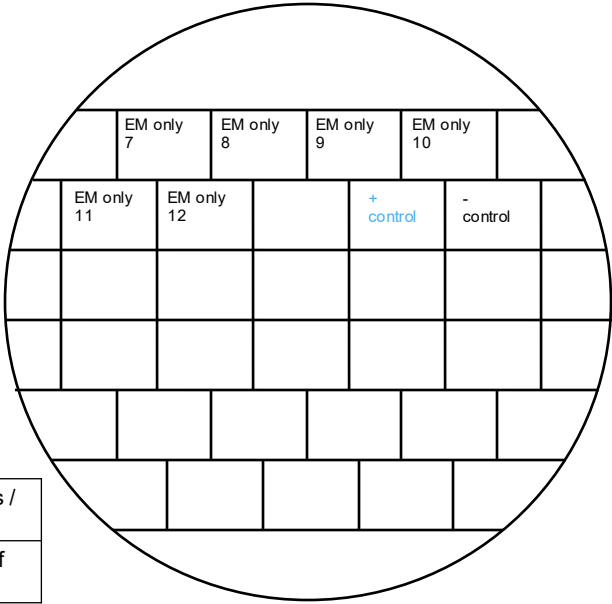
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
EM only	inviable	4 out of 6 (in total: 10 out of 12, see also slide 25)





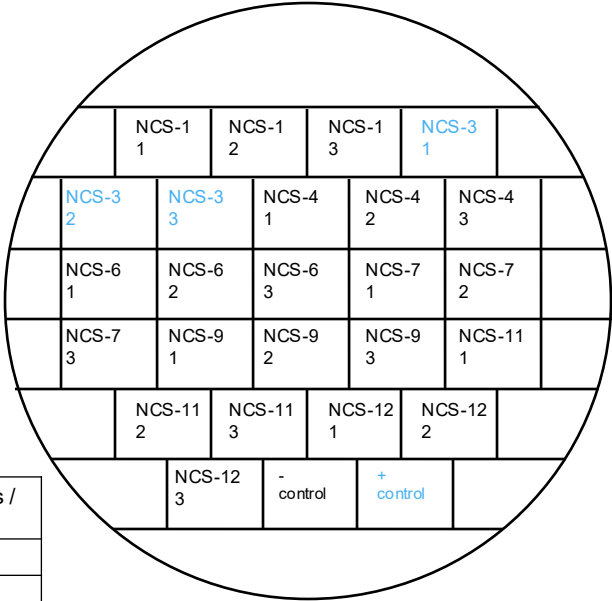
Restreak from YNB –ura –leu to 5-FOA –leu

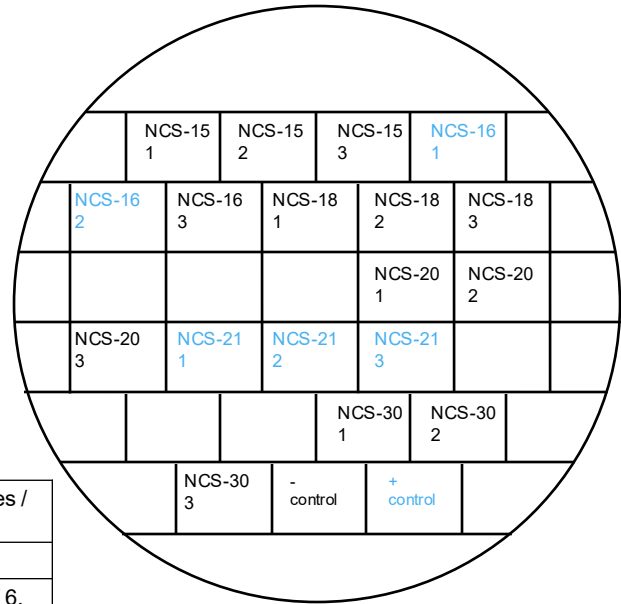
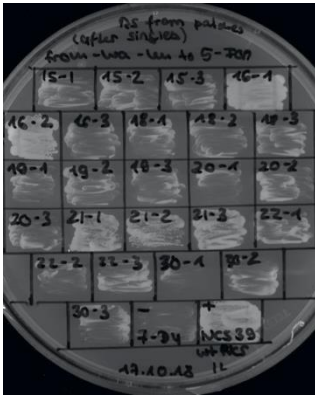
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
EM only	Invisible	6 out of 6 (in total: 10 out of 12, see also slide 24)



Restreak from YNB –ura –leu to 5-FOA –leu

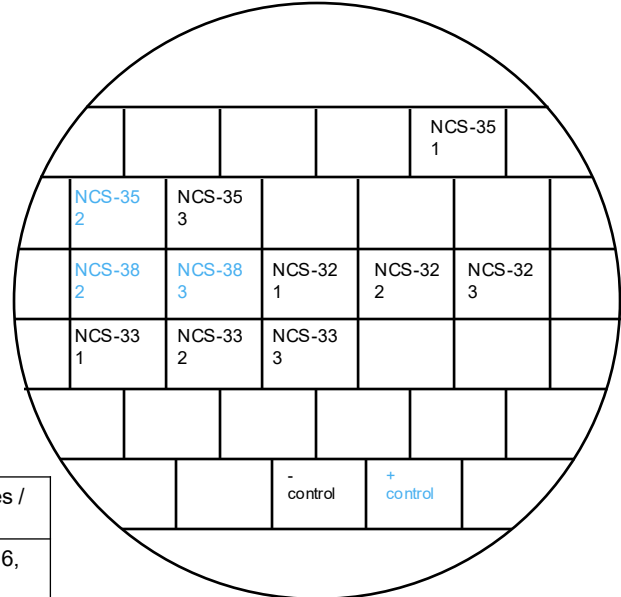
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-1	inviabile	3 out of 3
NCS-3	viable	3 out of 3
NCS-4	inviabile	3 out of 3
NCS-6	inviabile	3 out of 3
NCS-7	inviabile	3 out of 3
NCS-9	inviabile	3 out of 3
NCS-11	inviabile	3 out of 3
NCS-12	inviabile	3 out of 3





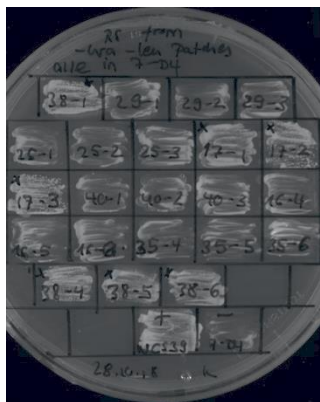
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-15	inviable	3 out of 3
NCS-16	inviable	1 out of 3 (in total 4 out of 6, see slide 27)
NCS-18	inviable	3 out of 3
NCS-20	inviable	3 out of 3
NCS-21	viable	3 out of 3
NCS-30	inviable	3 out of 3



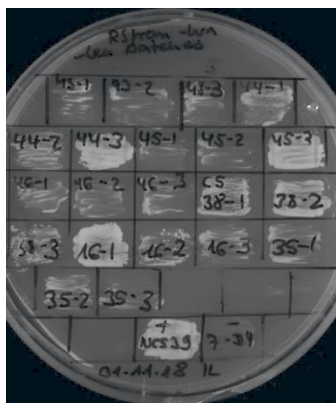
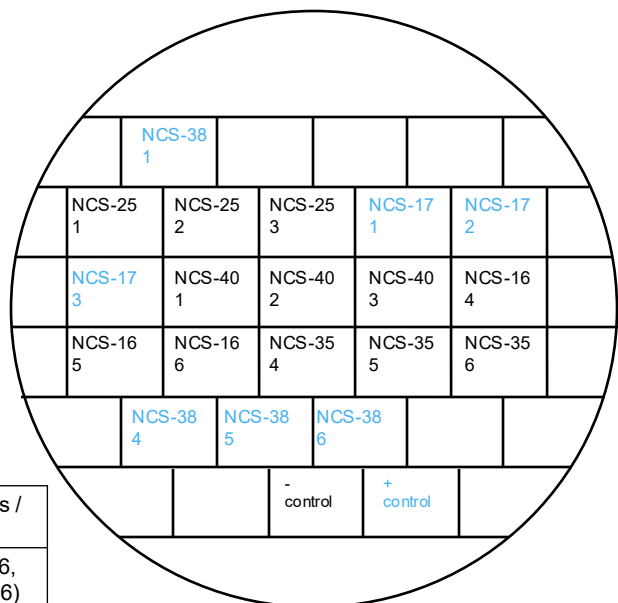
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-35	inviable	2 out of 3 (in total 5 out of 6, see also slide 26)
NCS-38	viable	2 out of 2 (in total 5 out of 6, see also slide 26)
NCS-32	inviable	3 out of 3
NCS-33	inviable	3 out of 3



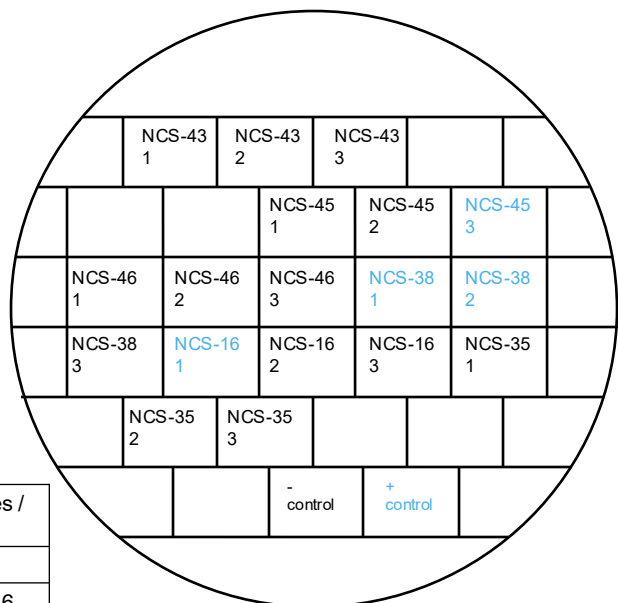
Restreak from YNB –ura –leu to 5-FOA –leu

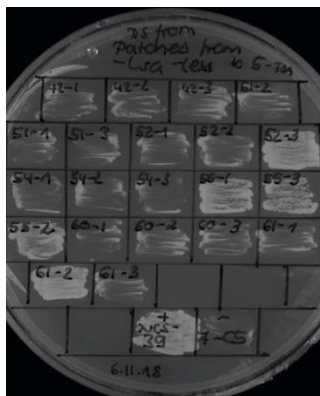
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-38	viable	4 out of 4 (in total 5 out of 6, see also below and slide 26)
NCS-25	inviable	3 out of 3
NCS-17	viable	3 out of 3
NCS-40	inviable	3 out of 3
NCS-16	inviable	3 out of 3 (in total 4 out of 6, see below and slide 26)
NCS-35	inviable	3 out of 3 (in total 5 out of 6, see also below and slide 26)



Restreak from YNB –ura –leu to 5-FOA –leu

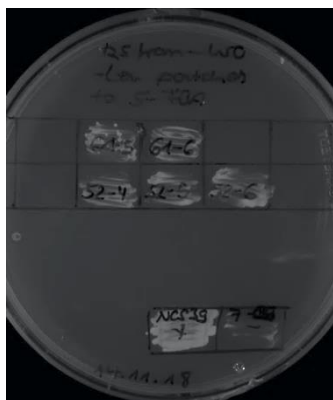
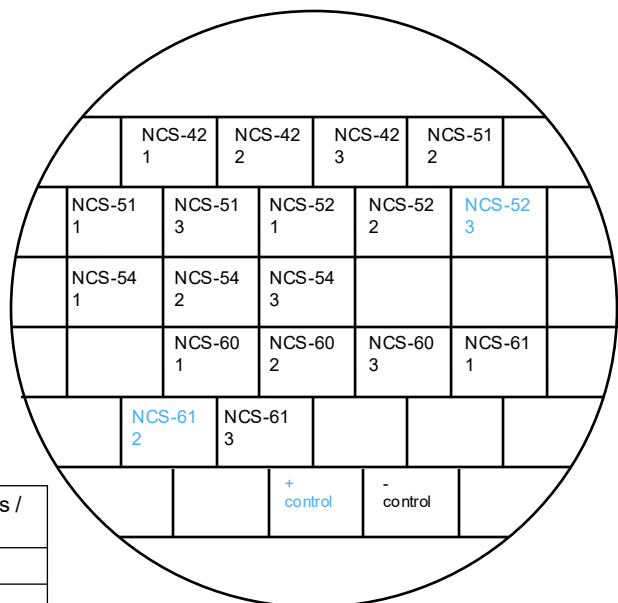
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-43	inviable	3 out of 3
NCS-45	inviable	2 out of 3 (in total 5 out of 6, see also slide 29)
NCS-46	inviable	3 out of 3
NCS-38	viable	2 out of 3 (in total 5 out of 6, see also above and slide 26)
NCS-16	inviable	2 out of 3 (in total 4 out of 6, see above and slide 26)
NCS-35	inviable	3 out of 3 (in total 5 out of 6, see also above and slide 26)





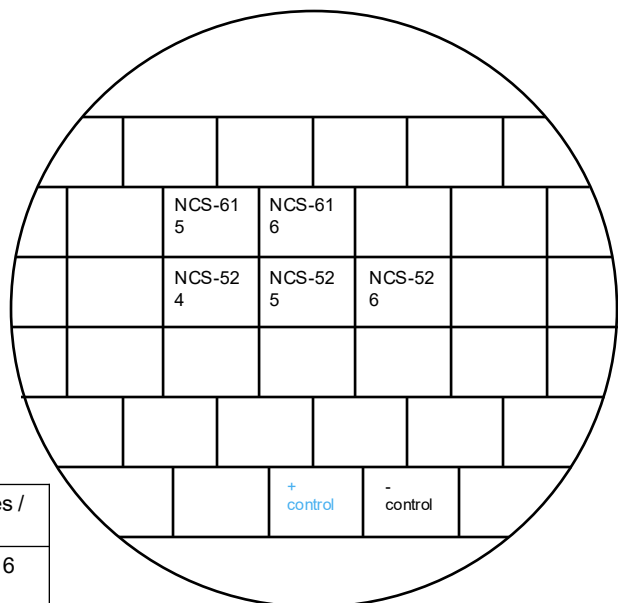
Restreak from YNB –ura –leu to 5-FOA –leu

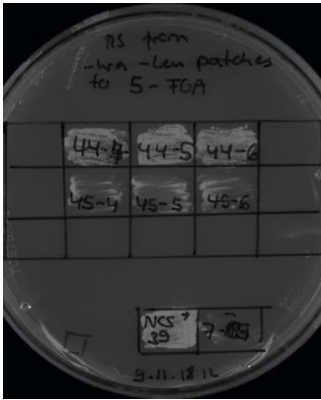
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-42	inviable	3 out of 3
NCS-51	inviable	3 out of 3
NCS-52	inviable	2 out of 3 (in total 5 out of 6 see also below)
NCS-54	inviable	3 out of 3
NCS-60	inviable	3 out of 3
NCS-61	inviable	2 out of 3 (in total 5 out of 6 see also below)



Restreak from YNB –ura –leu to 5-FOA –leu

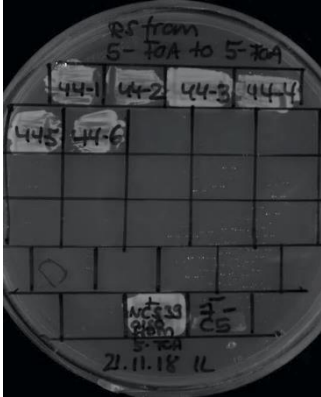
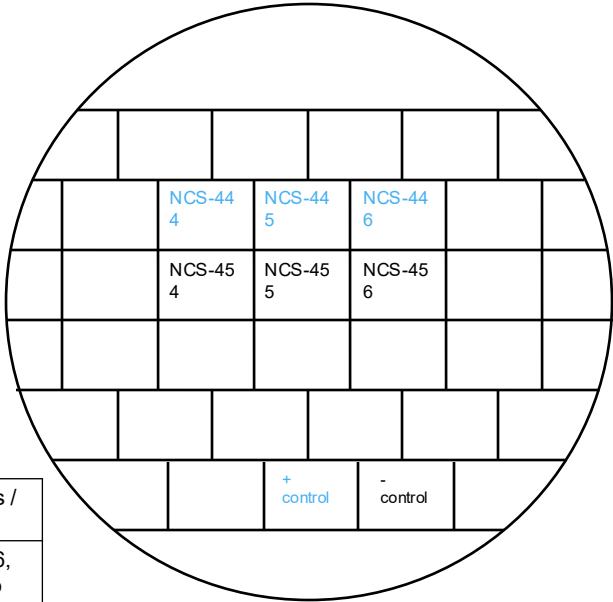
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-61	inviable	2 out of 2 (in total 5 out of 6 see also above)
NCS-52	inviable	3 out of 3 (in total 5 out of 6 see also above)





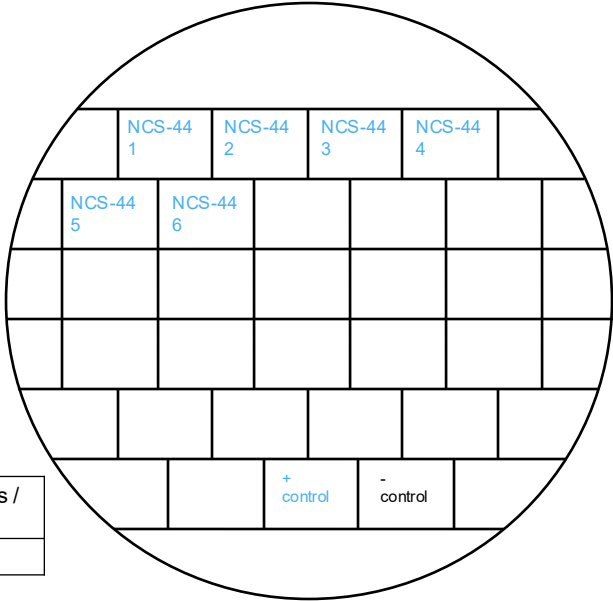
Restreak from YNB -ura -leu to 5-FOA -leu

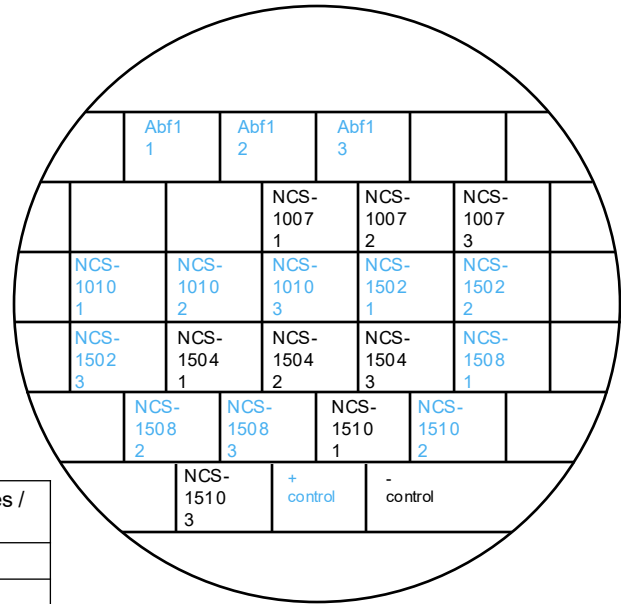
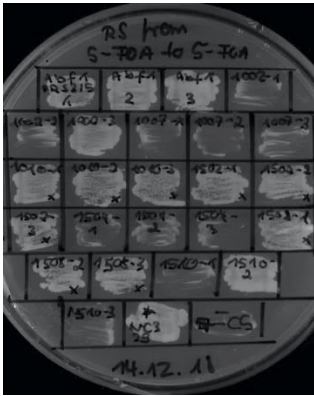
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-44	viable	3 out of 3 (in total 6 out of 6, technical replicate see also below)
NCS-45	inviable	3 out of 3 (in total 5 out of 6, see also slide 27)



Restreak from 5-FOA -leu to 5-FOA -leu

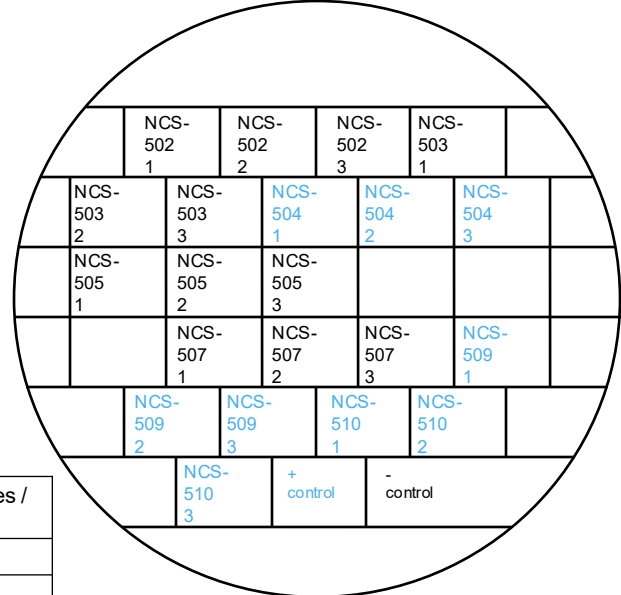
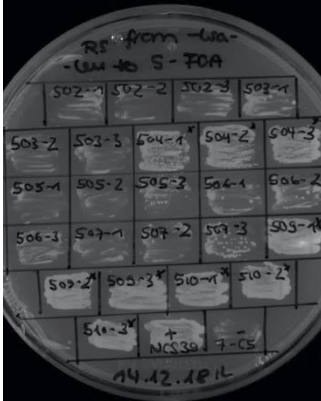
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-44	viable	6 out of 6





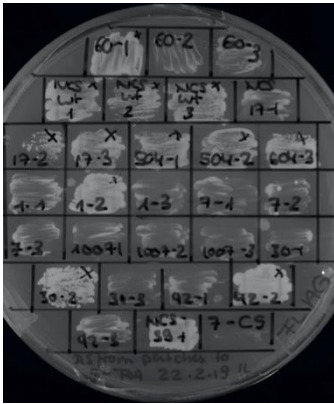
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
WT Abf1	viable	3 out of 3
NCS-1007	inviable	3 out of 3
NCS-1010	viable	3 out of 3
NCS-1502	viable	3 out of 3
NCS-1504	inviable	3 out of 3
NCS-1508	viable	3 out of 3
NCS-1510	inviable	2 out of 3 (in total 12 out of 15, see also slides 31 and 32)



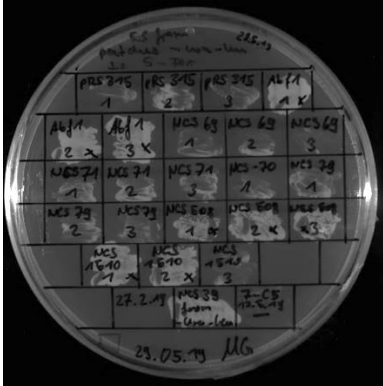
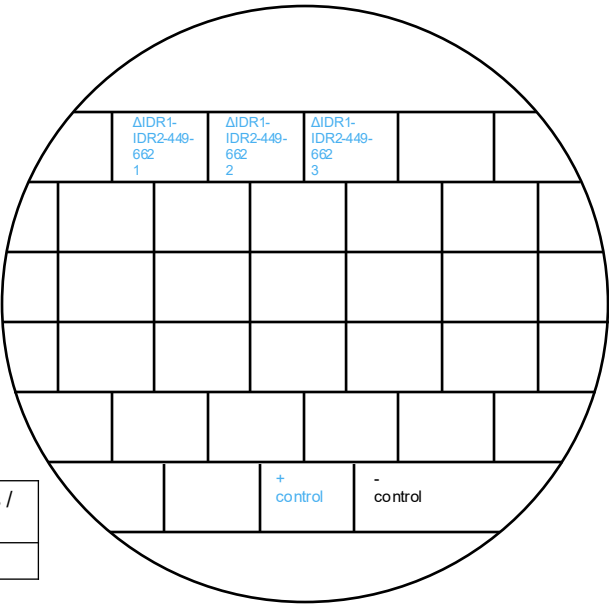
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-502	inviable	3 out of 3
NCS-503	inviable	3 out of 3
NCS-504	viable	3 out of 3
NCS-505	inviable	3 out of 3
NCS-507	inviable	3 out of 3
NCS-509	viable	3 out of 3
NCS-510	viable	3 out of 3



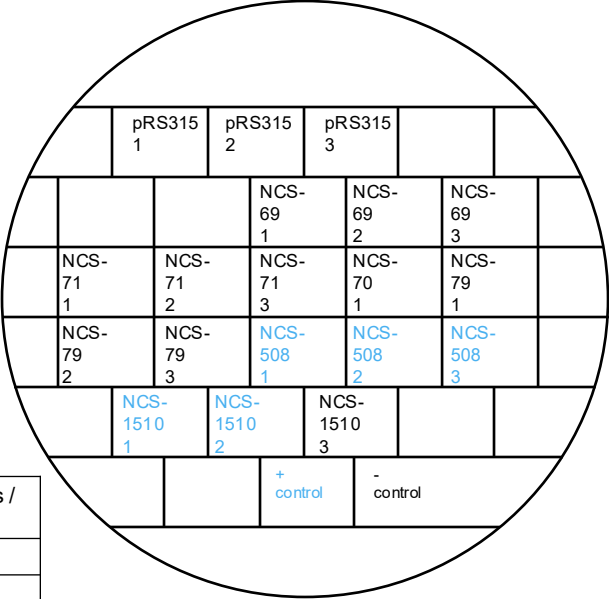
Restreak from YNB –ura –leu to 5-FOA –leu

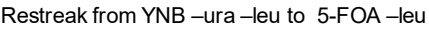
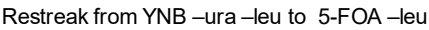
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
<a href="#">ΔIDR1 &amp; IDR2<sup>449-662</sup></a>	viable	3 out of 3



Restreak from YNB –ura –leu to 5-FOA –leu

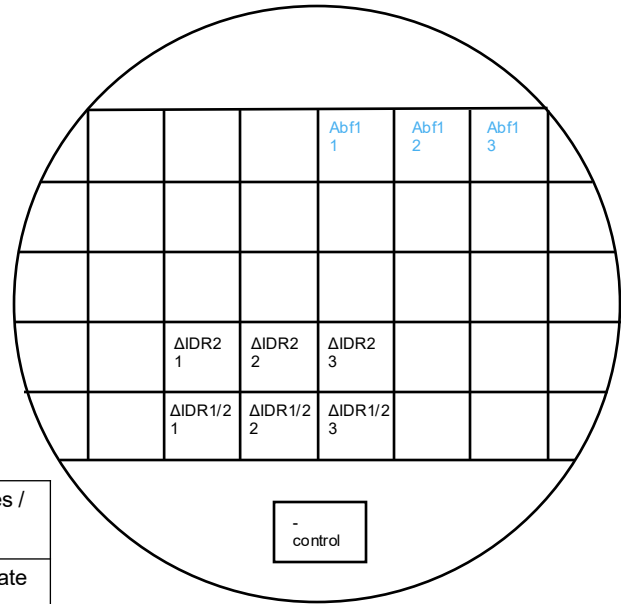
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
pRS315 empty	inviable	3 out of 3
NCS-69	inviable	3 out of 3
NCS-71	inviable	3 out of 3
NCS-70	inviable	1 out of 1 (in total 3 out of 3, see slide 32)
NCS-79	inviable	3 out of 3
<a href="#">NCS-508</a>	viable	3 out of 3
NCS-1510	inviable	1 out of 3 (in total: 12 out of 15, see also slides 30 and 32)





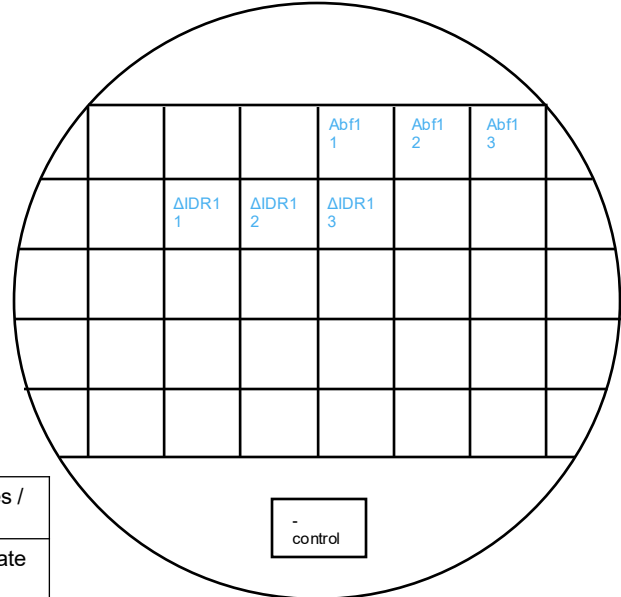
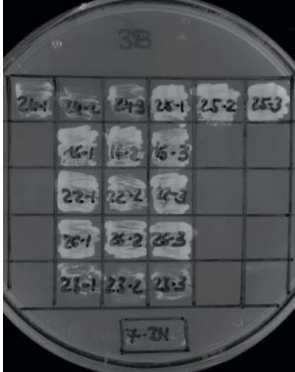
The diagram shows a circular arrangement of 36 NCS-1510 units in a 6x6 grid. The top row is designated as 'w/o Flag'. The units are numbered sequentially from 7 to 15 in the first five rows. The bottom row contains control units, specifically '+ control' and '- control'.

w/o Flag					
	NCS-1510 7	NCS-1510 8	NCS-1510 9	NCS-1510 10	
NCS-1510 11	NCS-1510 12	NCS-1510 13	NCS-1510 14	NCS-1510 15	
		+ control	- control		



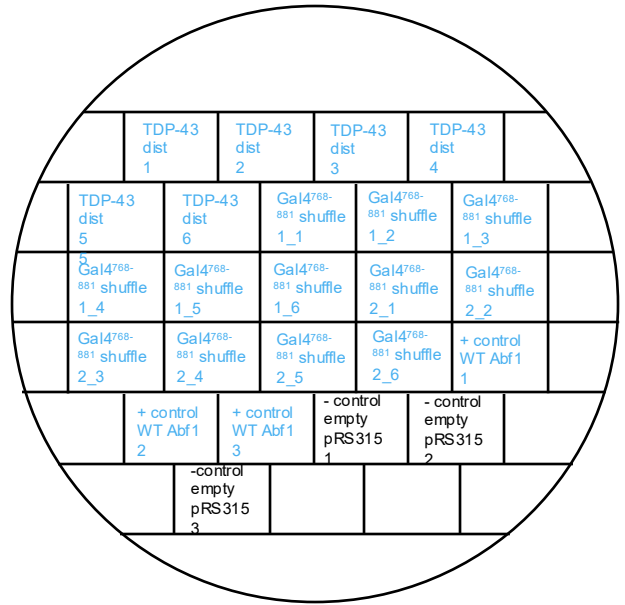
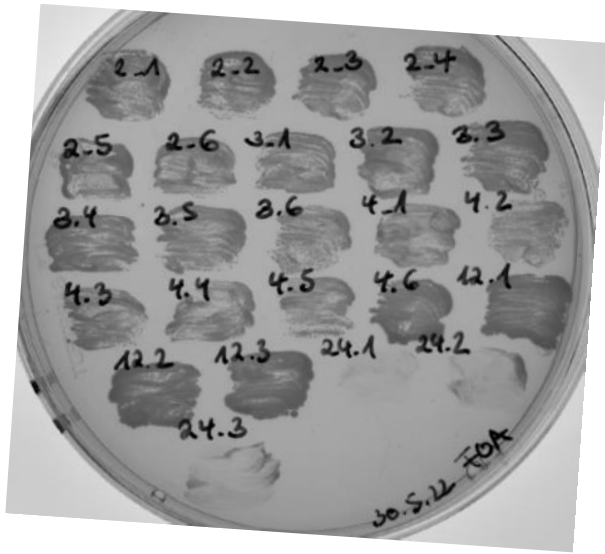
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Abf1 (NLS)	viable	3 out of 3 (technical replicate see below)
ΔIDR2	inviable	3 out of 3
ΔIDR1/2	inviable	3 out of 3



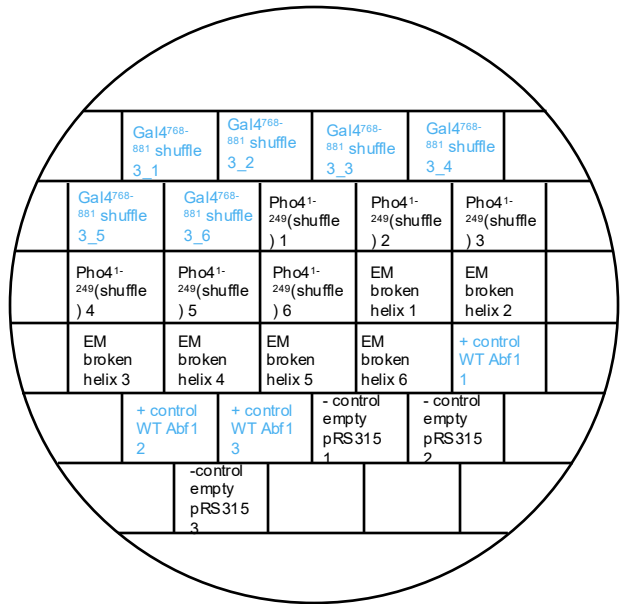
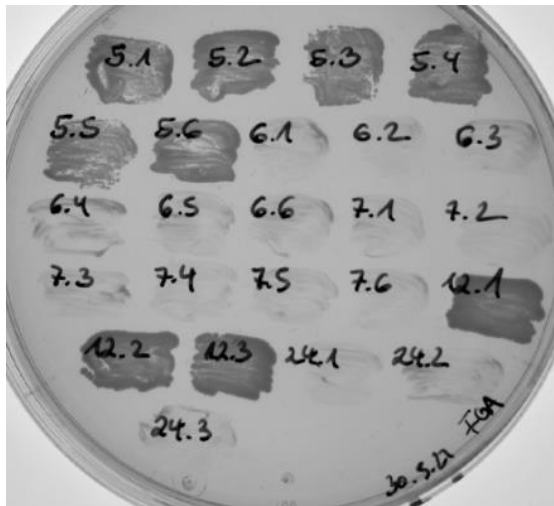
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Abf1 (NLS)	viable	3 out of 3 (technical replicate see above)
ΔIDR1	viable	3 out of 3



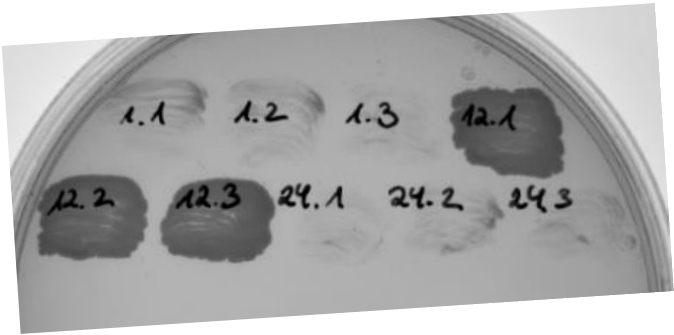
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
TDP-43 distr.	viable	6 out of 6
Gal4 <sup>768-881</sup> shuffle 1	viable	6 out of 6
Gal4 <sup>768-881</sup> shuffle 2	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

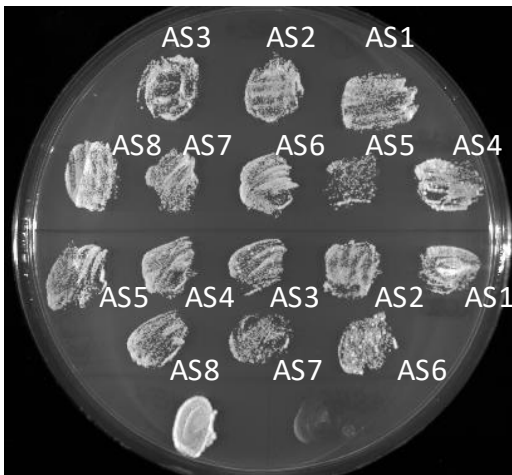
Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
Gal4 <sup>768-881</sup> shuffle 3	viable	6 out of 6
Pho4 <sup>1-249</sup> shuffle	inviable	6 out of 6
IDR2 WT broken helix	inviable	6 out of 6



	$\Delta$ IDR2-Flag 1	$\Delta$ IDR2-Flag 2	$\Delta$ IDR2-Flag 3	+ control WT Abf1 1	
	+ control WT Abf1 2	+ control WT Abf1 3	- control empty pRS315 1	- control empty pRS315 2	-control empty pRS315 3

Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
$\Delta$ IDR2-Flag	inviable	3 out of 3



NCS-506-Flag (clones AS1-AS8)

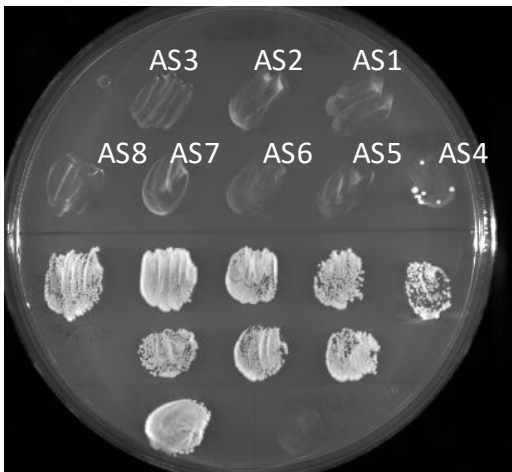
LS-15 (clones AS1-AS8)

+ control WT Abf1

- control empty pRS315

Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-506-Flag	viable	8 out of 8
LS-15	viable	8 out of 8 (in total 14 out of 14, see also slide 23)



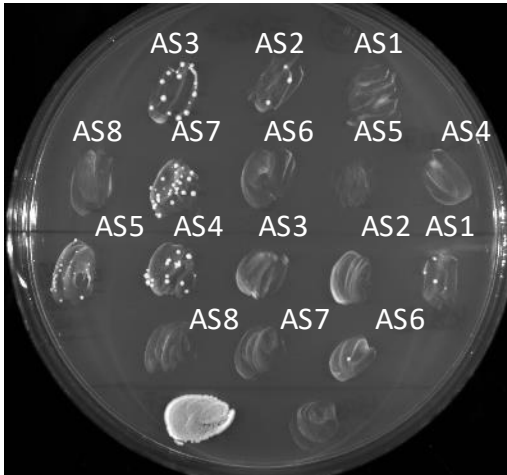
V. polyspora (clones AS1-AS8)

+ control WT Abf1

- control empty pRS315

Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
V. polyspora	inviable	8 out of 8 (in total 14 out of 14, see also slide 16)



**FUS<sup>1-163</sup>12E + Abf1<sup>G4</sup> distr.**  
(clones AS1-AS8)

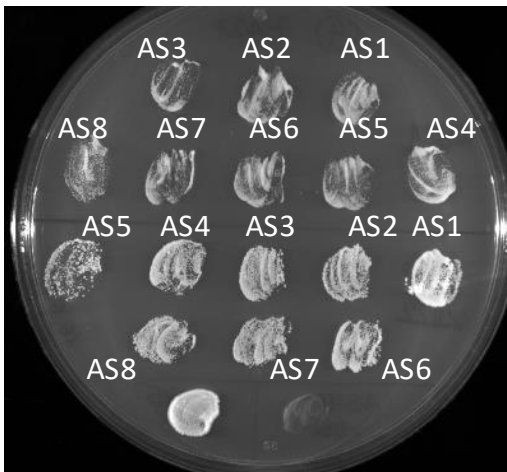
**FUS<sup>1-163</sup>12E + EM shuffle**  
(clones AS1-AS8)

+ control WT Abf1

- control empty pRS315

Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
FUS <sup>1-163</sup> 12E + Abf1 <sup>G4</sup> distr.	inviable	8 out of 8 (in total 14 out of 14, see also slide 39)
FUS <sup>1-163</sup> 12E + EM shuffle	inviable	8 out of 8 (in total 14 out of 14, see also slide 39)



**FUS<sup>1-163</sup>12E + Gal4<sup>G4</sup> all ΔE/D**  
(clones AS1-AS8)

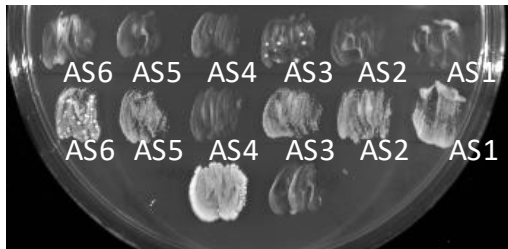
**NCS-21 hydro→polar**  
(clones AS1-AS8)

+ control WT Abf1

- control empty pRS315

Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
FUS <sup>1-163</sup> 12E + Gal4 <sup>G4</sup> all ΔE/D	viable	8 out of 8
NCS-21 hydro→polar	viable	8 out of 8



Pho4<sup>1-249</sup> segmental shuffle (clones AS1-AS6)

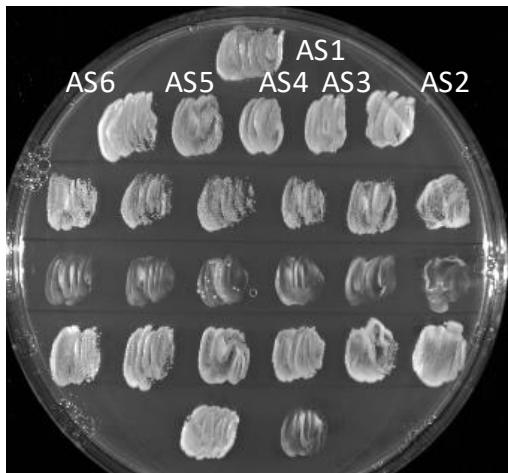
Sup35<sup>1-131</sup> + EM (clones AS1-AS6)

+ control WT Abf1

- control empty pRS315

Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Pho4 <sup>1-249</sup> segmental shuffle	inviabile	6 out of 6
Sup35 <sup>1-131</sup> + EM	viable	5 out of 6



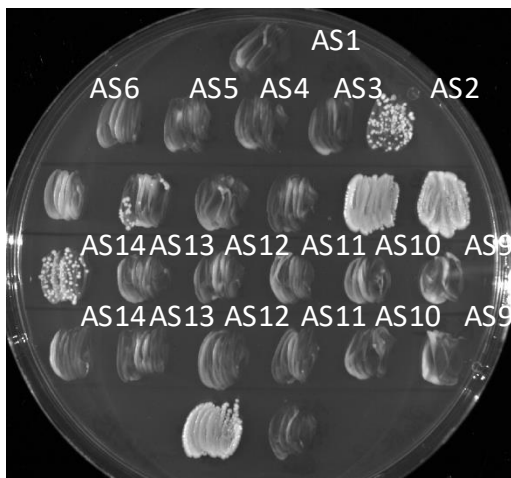
FUS<sup>1-163</sup>12E + Gal4<sup>G4</sup> distr. II (clones AS1-AS6)

+ control WT Abf1

- control empty pRS315

Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
FUS <sup>1-163</sup> 12E Gal4 <sup>G4</sup> distr. II	viable	6 out of 6



Rap1 IDR<sup>1-120</sup> & IDR<sup>230-361</sup> (clones AS1-AS6)

FUS<sup>1-163</sup>12E + Abf1<sup>G4</sup> distr. (clones AS9-AS14)

FUS<sup>1-163</sup>12E + EM shuffle (clones AS9-AS14)

+ control WT Abf1

- control empty pRS315

Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Rap1 <sup>1-120</sup> & 230-361	inviable	6 out of 6 (in total 7 out of 8, see also slide 21)
FUS <sup>1-163</sup> 12E + Abf1 <sup>G4</sup> distr.	inviable	6 out of 6 (in total 14 out of 14, see also slide 37)
FUS <sup>1-163</sup> 12E + EM shuffle	inviable	6 out of 6 (in total 14 out of 14, see also slide 37)