

Table S6. Size of *SIR3* mutagenesis pools during selection and sequencing

Colony counts, as plated after recovery during transformation, prior to drug selection in pool. Cells were diluted appropriately to prevent a lawn of growth and plated onto media as indicated. Numbers in the table are calculated to represent the number of colonies in the full transformation resistant to the given drug. nd = not determined. Fold coverage was calculated by comparing the number of colonies from the indicated drug selection to the size of the DNA available for mutagenesis (Supplemental Note 1). * The Sir3-myc pool was treated differently than other pools. After transformation of the Sir3-myc pool into *C. glabrata*, the pooled culture was outgrown in YPD+NAT for ~24 hours prior to plating on YPD+NAT. All other tagged Sir3 pools were plated directly onto YPD+NAT plates following transformation and recovery.

Step of processing	Fold coverage based on which drugs?	Tn used to mutagenize <i>C. glabrata</i> <i>SIR3</i>							
		myc		bio		mEOS2		GFP	
		# colonies	fold coverage	# colonies	fold coverage	# colonies	fold coverage	# colonies	fold coverage
Mutagenesis of entry vector	Tmp Kan	5.4E+05	67x	2.9E+05	36x	3.2E+05	39x	6.46E+05	79.99
xpC = Gateway LR to create expression pool	Tmp Car	6.0E+06	920x	6.0E+06	920x	6.0E+06	920x	6.00E+06	920x
xpT	Tmp Car	5.7E+07	8700x	8.2E+07	13000x	1.8E+07	2800x	2.70E+07	4100x
xpT-Tn7L	Tmp Car	1.2E+07	1800x	3.6E+07	5500x	4.1E+07	6200x	2.34E+07	3600x
xpT-Tn7L-Tn7R	Car	1.6E+07	2400x	5.8E+07	8900x	6.2E+07	9500x	3.50E+07	5400x
Transformation into <i>C. glabrata</i>	Nat	7992*	1.2x	2172	0.33x	2432	0.37x	1895	0.29x
Screening for functional isolates	5-FOA	906	n/a	190	n/a	162	n/a	105	n/a
% functional clones		11%	n/a	8.7%	n/a	6.7%	n/a	5.5%	n/a
Sent for sequencing		95		63		52		48	
# mappable sequences		90		53		47		48	
# isolates with inframe insertions in <i>SIR3</i> orf		88		52		46		47	
# in-frame unique insertions sites		21		42		33		30	
Max # insertions at a single site		27		4		6		6	