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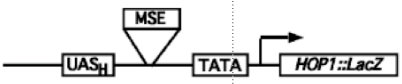
101 Homework Week 2

You may work in groups or consult other members of the class to answer the questions.

1. In Figure 1 is an experiment in which MSEs from different genes (*SMK1*, *NDT80*, *SPR3* etc) were cloned into the *HOP1-LacZ* fusion. *LacZ* expression was measured by β-galactosidase assays during vegetative growth to measure the ability of sites to repress transcription. *LacZ* expression was measured by β-galactosidase assays during the middle stages of meiosis to measure the ability of sites to activate transcription. The vector alone is the *HOP1-lacZ* fusion without an MSE.



Site	Position	Sequence	Mitotic repression units	Mitotic repression fold	Meiotic activation units	Meiotic activation fold
Vector		———————	23		27	
SMK1	-69	ccactaATTTGTGACacttt	0.3	77	75	2.7
NDT80	-78	cctccaTTTTGTGTCacct	0.7	33	272	10
NDT80	-221	ctactcTTTTGTGTCatac	14	1.6	170	6.3
SPS4	-191	atacgtTTTTGTGGCgcgc	21	1.1	306	11
SPR3	-14	ggtctcTTTTGCGTCgcta	5.0	4.6	203	7.5
SPR3	-289	ggtctcTTTTGTGTCgcta	3.6	6.4	313	12
DIT1	-342	acccttATTTGTGAGgagt	39	0.6	57	2.1
DIT1	-555	accttttTTTTGCGACgcgc	35	0.7	157	5.8
BBP1	-131	acccgtTTTTGTGTCgctc	0.2	115	280	10
CLB6	-345	ttttttTTTTGCGACggta	27	0.9	182	6.7

Fig 1 *Tests for activity of different MSEs*

Do all MSEs function the same way? If not, how are they different and how many different types (classes) are there? Indicate what examples to support your conclusions.

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2. A screen was performed to screen for mutants that fail to activate transcription from a reporter with an MSE (Fig 2). A yeast strain was transformed with a plasmid containing a *LacZ* fusion to gene that is activated by a transcription factor binding to the UAS site. The transformed yeast cells were mutated and the cells spread on plates containing X-gal. Mutants were screened for failure to activate expression by appearing white instead of blue. These mutants can be in the genes that code for the activator protein(s) that bind to the MSE (mutA) or in the MSE in the promoter fusion (mutB).

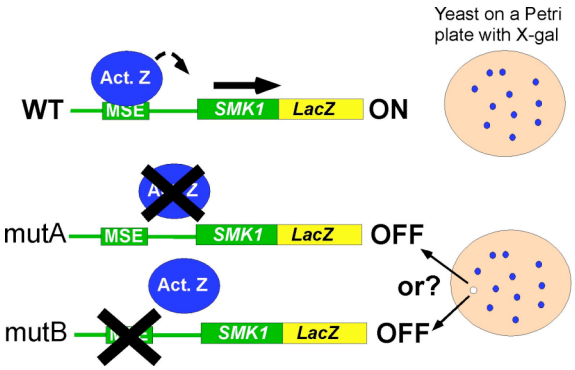

Fig 2 *Screen for mutants that fail to activate transcription.*

How would you determine if mutation was in the activator gene (mutA) or in the MSE site on the *SMK1-lacZ* fusion (mutB)? (Hint: Yeast tend to lose plasmids easily if the selection for the marker on the plasmid is not maintained)

3. You are given a plasmid clone that contains a *SMK1-lacZ* reporter fusion. The MSE represses expression of the fusion during vegetative growth (Fig 3). Explain how you would do a genetic screen to isolated mutants in the repressor protein(s). What conditions would you look under? What phenotype would you look for?

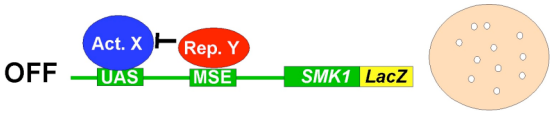

Fig 3 *The SMK1-lacZ fusion is repressed by the MSE during vegetative growth.*