Supplementary Files for

Characterization of Met25 as a Color Associated Genetic Marker in *Yarrowia lipolytica*

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Supplementary Table 1

Primers used in this study

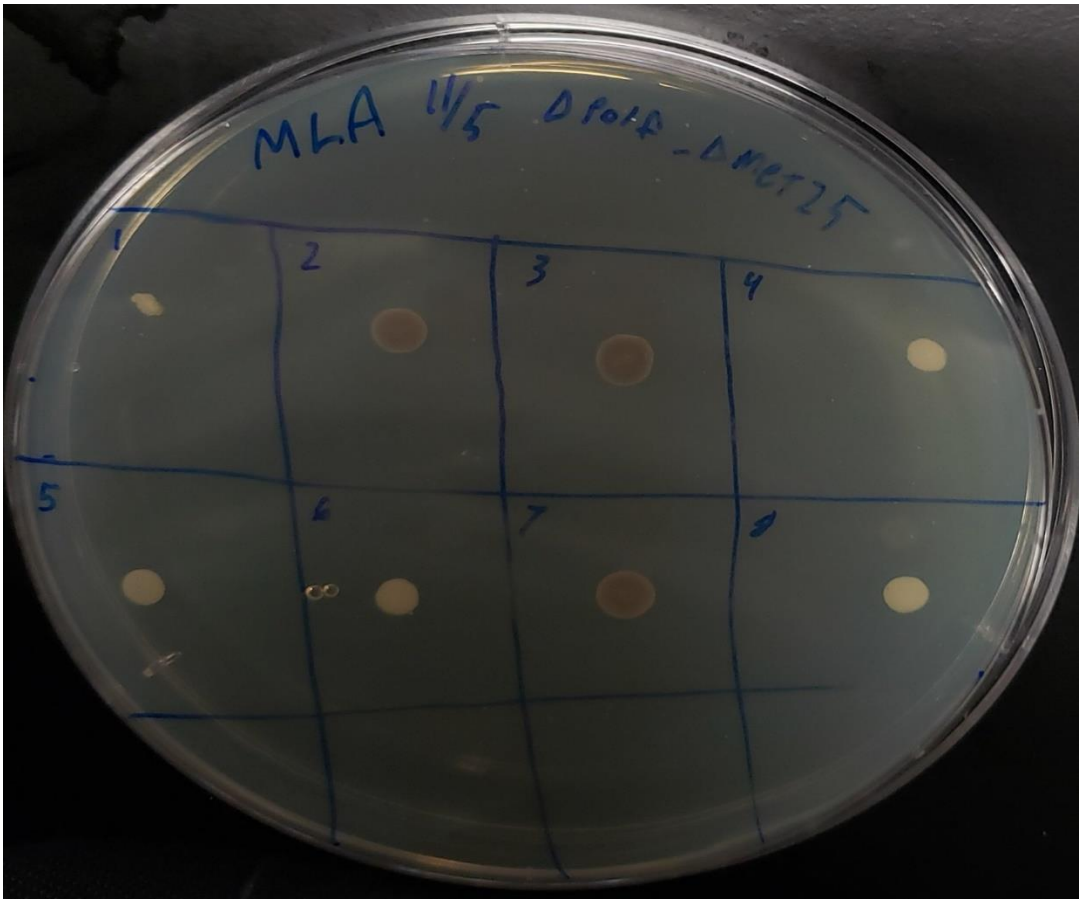
Primers	Sequence (5'-3')
MET25upfw	ggaattctcatgtttgacagcttatcCTAGCTTCTGAATCGCTACACAGTTC
MET25uprv	gtattccattatctacgaaaagcatcgatTGTGGATGTTAGTCTGAGGTGAGGTGG
MET25dwfw	ctagcgagacaataacggaggagtcgacTATCTTCATAACCACAACAGAGGACG
MET25dwrv	ctatgttacatccttttatcagacataGAGAGTGCTCACTGCGCTCTACAAG
MET25upchk	GTCACTATCCACGTGACCTAGCCACGC
MET25dwchk	GTTCCTAGACGCTACACGAAAGTAC
MET25cassfw	GCTTCTGAATCGCTACACAGTTCC
MET25cassrv	GAGTGCTCACTGCGCTCTACAAGG
MET25fw	cagcacttttgcagtactaaccgcagCCCTCGCACTTTGACACTCTGCAGCTC
MET25rv	ggggacaggccatggaactagtcggtaccCTAGTACACCGCCTTGAAAGCCTGC
ylMET25-crRR	GACGTTGATGCCGTAGGTCTTatctacaagagtagaaatta
Avr2_fw	gcatccctaaatttgatgaaagc
ylMET25-crRF	gtagatAAGACCTACGGCATCAACGTCggccggcatggtcccagcctc
Sal1_rv	GTTACATCCTTTTATCAGACATAGTC
Tef_fw	GGGTATAAAAGACCACCGTCCCC
Xpr2_rv	CCGTTGTAGGCAACAGCGTTGGG
MetC_Upfw	ggcgacgacggaattctcatgtttgacagcttatcACACCGAAATATCACTCTACAAG

MetC_UpRv	gtattccattatctacgaaaagcatcgatTTTTGTAGGGATCGCTCGTACTGTAG
MetC_DwFw	gctagcgagacaataacggaggagtcgacACCAATCCACAAAAATCCAATAAATT
MetC_DwRv	cttgcctatgttacatccttttatcagacataGTATATATGGGACCTCTCCCCCG
MetC_CassFw	CACCGAAATATCACTCTACAAGTATAG
MetC_CassRv	ATATATGGGACCTCTCCCCCGTTC
MetC_upChk	CCATTCACATTGAACAGTGTACAG
MetC_DwChk	CAATCCTCAATCCTAAACAAACC
ylMET2(A)-crRF	gtagatCTGGTCCGAGACCAGTCCCGAggccggcatggtcccagcctc
ylMET2(A)-crRR	GACGTTGATGCCGTAGGTCTTatctacaagagtagaaatta
ylMET2(B)-crRF	gtagatCCACGCTCTTACCGGTTCCGCggccggcatggtcccagcctc
ylMET2(B)-crRR	TCGGGACTGGTCTCGGACCAGatctacaagagtagaaatta
ylMET2_checkFW	GCAAGGAGAAGCCGAACCGTAGGG
ylMET2_checkRV	CACATCTTTTCATCTTCTTCTACAACCA

Supplementary Table S2. Plasmids and Strain genotype used in this study.

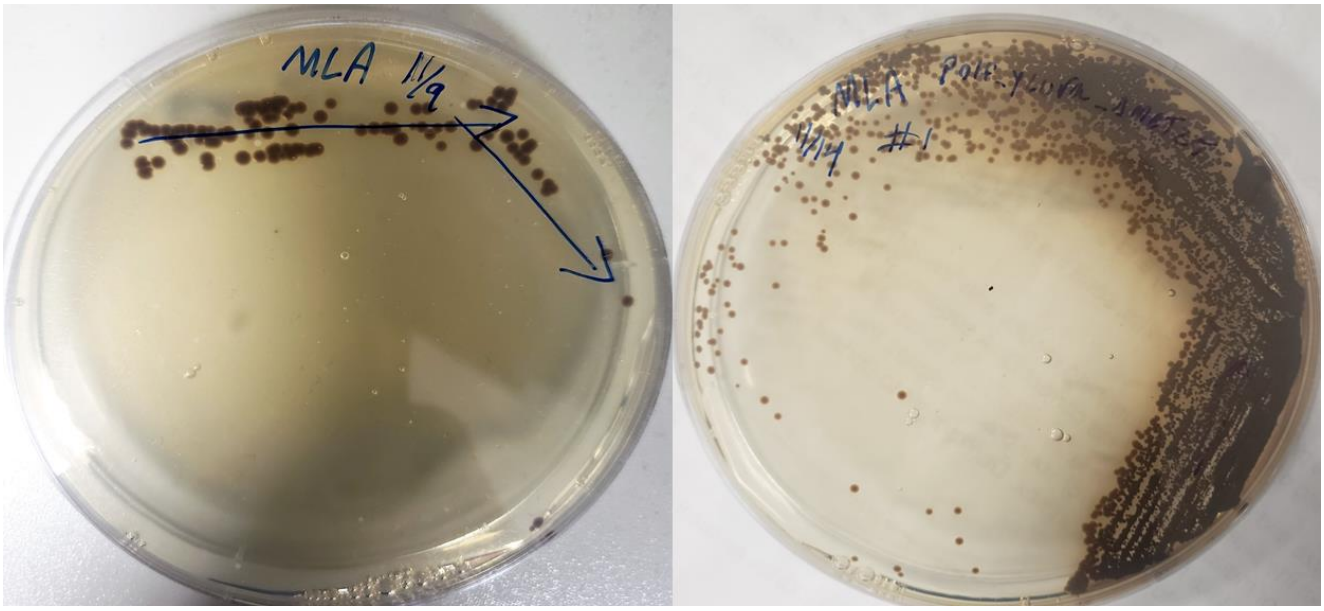
Strains or plasmids	Description	Reference
Strains		
E. coli NEB 5α	*fhuA2* Δ(*argF-lacZ*)*U169 phoA glnV44 Φ80 Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	New England Biolabs
Y. lipolytica po1g	MATa, *leu2*-270, *ura3*-302::URA3, *xpr2*-3	Lab stock
Y. lipolytica po1f	MATa *ura3*-302 *leu2*-270 *xpr2*-322 *axp2*-deltaNU49 *XPR2*::*SUC2*	Lab stock
Y. lipolytica	po1f+*ylUra3-Met25*	This work
Y. lipolytica	po1f+*ylUra3-MetC*	This work
Y. lipolytica	po1fΔ*Met25*+*ylUra3-MetC*	This work
Y. lipolytica	po1fΔ*Met25*	This work
Y. lipolytica	po1fΔ*Met6*	This work
Y. lipolytica	po1fΔ*Met2*	This work
Plasmids		
pYLXP'	Cloning vector	Lab stock
pYLXP'+ -*Met25*	MET25 restoration plasmid	This work
pYLXP'+*ylUra3-Met25*	Homologous recombination MET25 knockout cassette construction plasmid	This work
pYLXP'-*ylUra3-MetC*	Homologous recombination METC knockout cassette construction plasmid	This work
pYLXP'-*AsCpf1-AsCrRNA-Met25-*	CRISPR/Cas12 nuclease expression plasmid with gRNA targeting MET25 for indel knockout	This work
pYLXP'-*AsCpf1-AsCrRNA-Met2-*	CRISPR/Cas12 nuclease expression plasmid with gRNA targeting MET2 for indel knockout	This work
pYLXP'-*AsCpf1-AsCrRNA-Met6-*	CRISPR/Cas12 nuclease expression plasmid with gRNA targeting MET6 for indel knockout	This work

Supplementary Figure 1



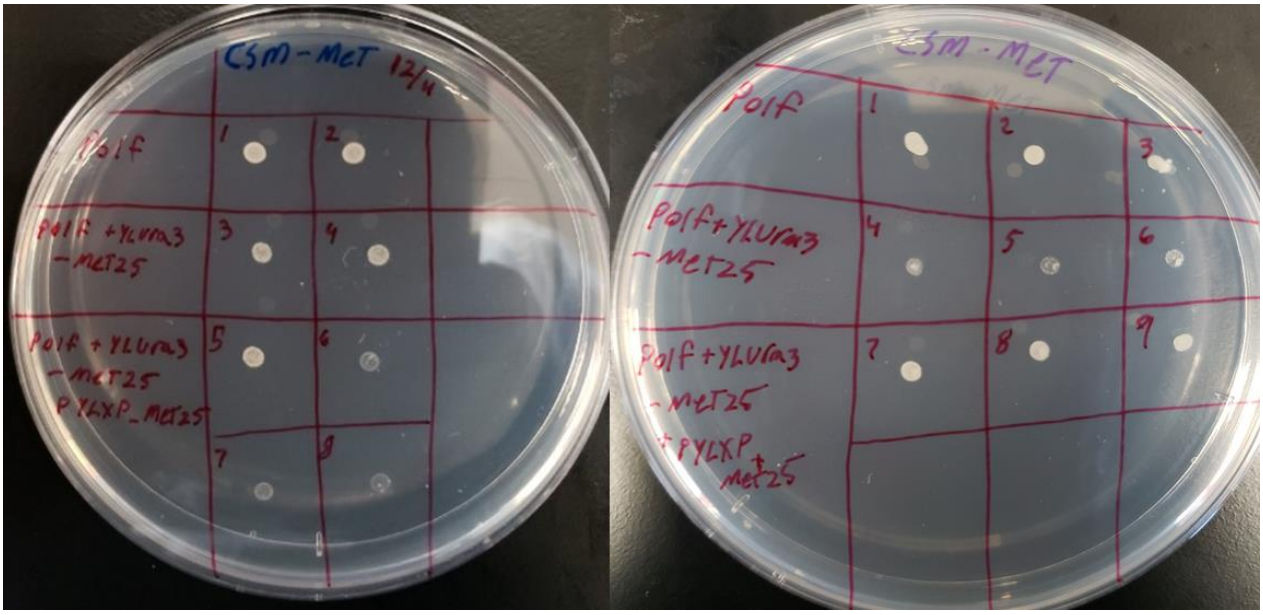
Supplementary Figure 1. Preliminarily positive transformants on MLA media, selected immediately after cassette transformation and growth on CSM-Ura plates.

Supplementary Figure 2



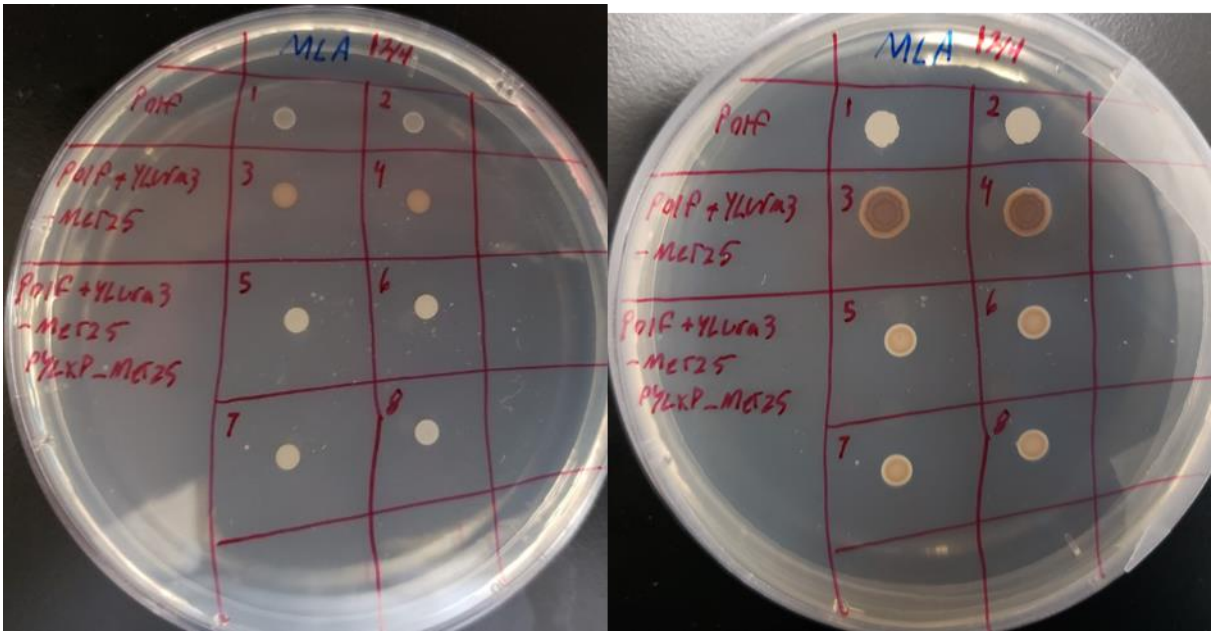
Supplementary Figure 2. 2.A On the left is the result of growth from a culture of positive ΔMET25 mutants from Figure 1, after MLA and subsequent 24 hour incubation in CSM-Ura liquid media. Two white colonies can be observed indicating cells with functional copies of MET25. 2.B was the result of a single colony from the plate in 2.A, inoculated into CSM-Ura, cultured for 24 hours, diluted, and re-plated on MLA.

Supplementary Figure 3



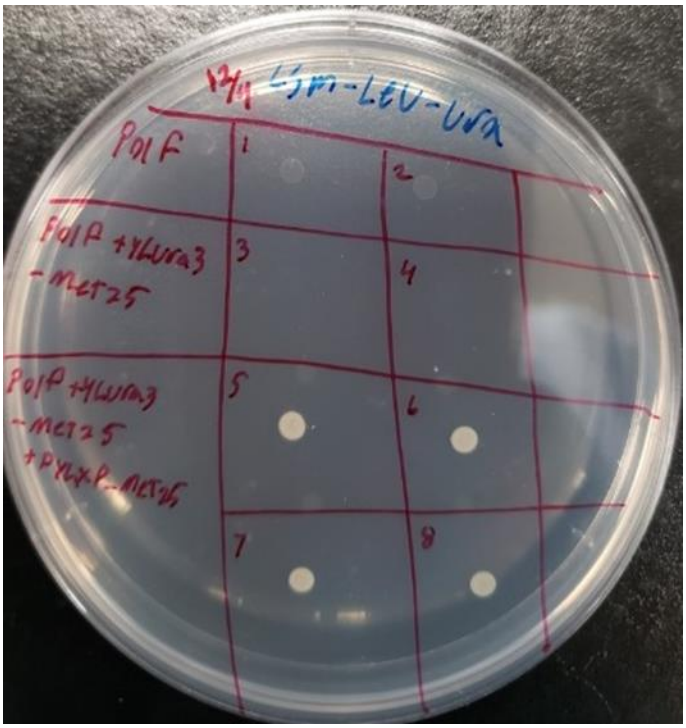
Supplementary Figure 3. Top to bottom there is the wildtype, ΔMET25 mutant, and the mutant with the restorative plasmid. On the left are cells spotted directly from culture solution. On the right, the cells were first pelleted, and resuspended in PBS, twice.

Supplementary Figure 4



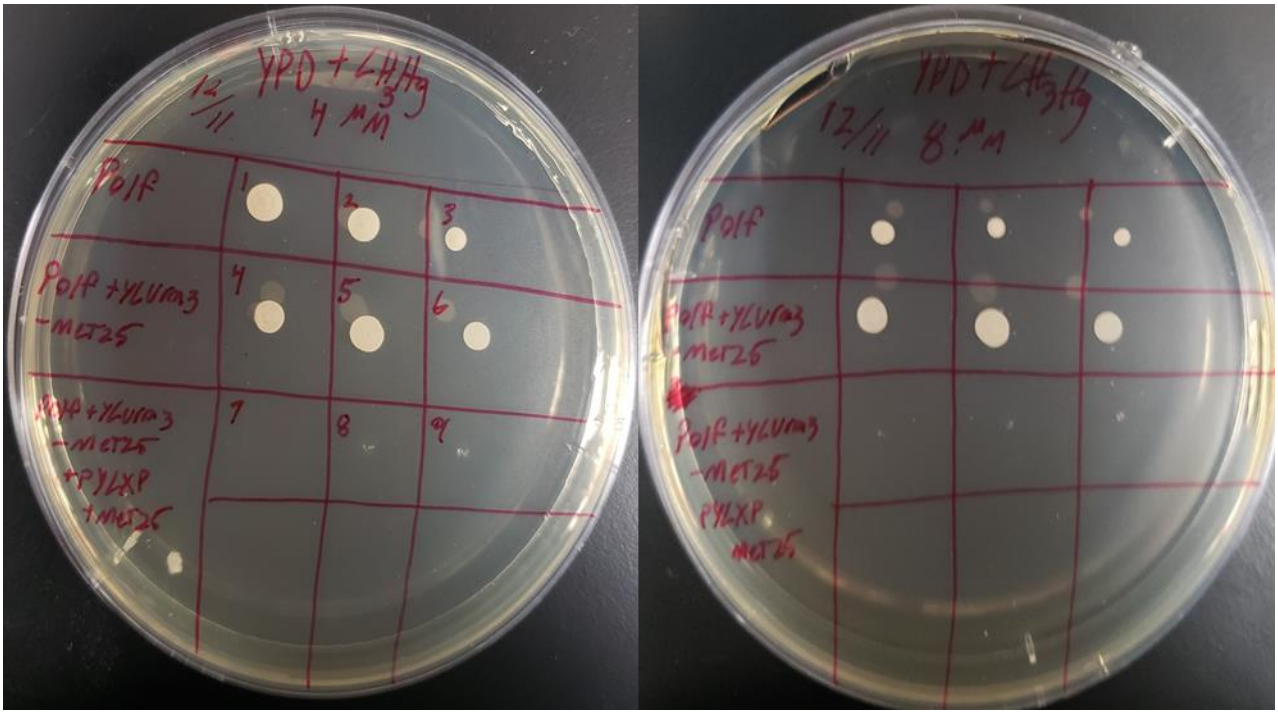
Supplementary Figure 4. Here are two images of growth on rich media containing divalent lead. In the top section the wildtype is white. In the second row, the MET25 mutant, and on the third and fourth row, the plasmid restoration of MET25 activity. On the left is 24 hours after spotting on the plate, on the right 48 hours.

Supplementary Figure 5



Supplementary Figure 5. This is the control plate to ensure both markers were functionally expressed in our testing. The first row is the wildtype, second is the MET25 mutant, third is the mutant with the restorative plasmid.

Supplementary Figure 6



Supplementary Figure 6. On the left, the concentration is 4μM methyl mercury in YPD, on the right, 8μM. Under 8μM, we can see a slight advantage afforded to the MET25 mutant cells.