Summary of investigation of A1-like immunity repressor in the F1 phage Coco12

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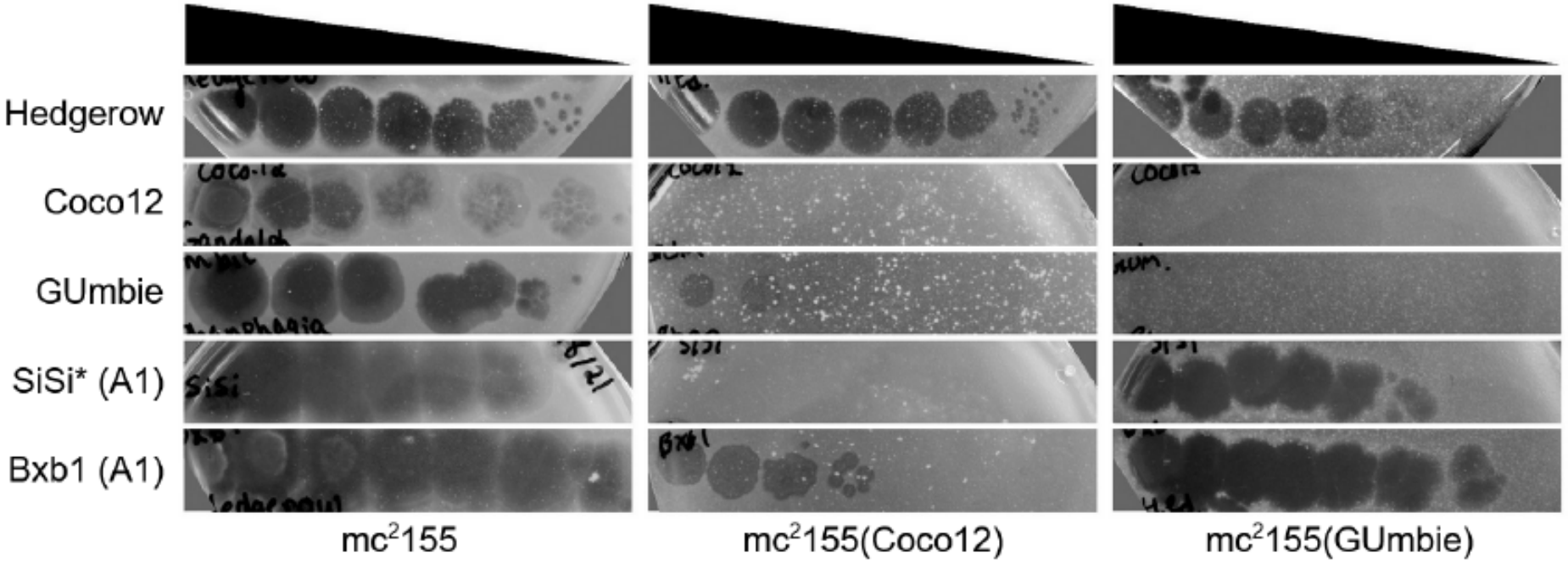
Observation:
Coco12 (F1) is immune to superinfection by Bxb1 (A1) and Sisi* (A1)
Gumbie (F1) is not immune to Bxb1 and Sisi*



Immunity tests of Coco12 and Gumbie lysogens. 10-fold serial dilutions of phage lysates were spotted on lawn of host (*Mycobacterium smegmatis* mc²155), lysogen host with Coco12 prophage (mc²155(Coco12)), and lysogen host with Gumbie (mc²155(Gumbie)). Hedgerow is a Cluster B2 phage. Coco12 and GUmbie are Cluster F1 phages. Sisi* and Bxb1 are Cluster A1 phages. Sisi* is a contaminant that was isolated from our Sisi lysate. Preliminary DNA sequencing shows that it is closely related to Cluster A1 phages.

Coco12 and Phanphagia display immunity to A1 phages, but Gumbie, Veteran, and Gandalph do not

	mc^{2}155(Coco12)	mc^{2}155(Phanphagia)	mc^{2}155(GUmbie)	mc^{2}155(Veteran)	mc^{2}155(Gandalph)
Coco12	< 10^{-7}	10^{-6}	< 10^{-7}	< 10^{-7}	< 10^{-7}
Phanphagia	< 10^{-7}	< 10^{-7}	< 10^{-7}	< 10^{-7}	10^{-6}
GUmbie	10^{-5}	< 10^{-7}	< 10^{-7}	< 10^{-7}	1
Veteran	10^{-3}	< 10^{-7}	< 10^{-7}	< 10^{-7}	10^{-1}
Gandalph	10^{-5}	10^{-5}	< 10^{-7}	< 10^{-7}	< 10^{-7}
Sisi* (A1)	< 10^{-7}	< 10^{-7}	10^{-1}	10^{-2}	1
Bxb1 (A1)	10^{-3}	10^{-3}	1	1	1
Hedgerow (B12)	1	1	1	1	1

Efficiency of Plating on five Cluster F1 lysogens. 10-fold serial dilutions of phage lysates were spotted on lawns of host (*Mycobacterium smegmatis* mc^{2}155) and lysogens. Efficiency of Plating (EOP) is the ratio of the apparent titer of each phage on a lysogen to the titer on mc^{2}155. EOPs < 10^{-2} are colored gold, and EOPs 10^{-2} or greater are green. Sisi* is a contaminant that was isolated from our Sisi lysate. Preliminary DNA sequencing shows that it is closely related to Cluster A1 phages.

Correlation:

The F1 phages that reduce superinfection by A1 phages carry an A1-like immunity repressor gene, whereas the F1 phages that are not immune to A1 phages do not.

Coco12 gp38 is 92% identical to the immunity repressor of Bxb1 (gp69) by EMBOSS Needle global alignment

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Coco12          1 MRTIHTTPAEFRREQLPRLSLEVIEALKAAGETEADIARMYGVTPQAVSW     50
                  |||        .||||||||||||||||||.||||||||||||||||||||
Bxb1            1 MRT--------TREQLPRLSLEVIEALKATGETEADIARMYGVTPQAVSW     42

Coco12         51 HVHTYGGKLTDRQVIRREYPFKVPEPLSQCAPHKRLRDHGEYIATRGNGM    100
                  ||||||||||.||||||||||||||||||.||||||||||||||||.||
Bxb1           43 HVHTYGGKLTARQVIRREYPFKVPEPLSQCTPHKRLRDHGEYIATRGKGM     92

Coco12        101 KDYKLKRLRSFYRMLRENNWVVEFDPNIPPIPGVSKRGGWAYRERQESDE    150
                  |:||||||||||||||||||||||||||||||||||||||||||||||||||
Bxb1           93 KEYKLKRLRSFYRMLRENNWVVEFDPNIPPIPGVSKRGGWAYRERQESDE    142

Coco12        151 DLLIRVNEYTTLSEIGRHRIWRFPSVEP    178
                  ||||||||||||||||.|||||||||
Bxb1          143 DLLIRVNEYTTLSEIGRHHIWRFPSVEP    170
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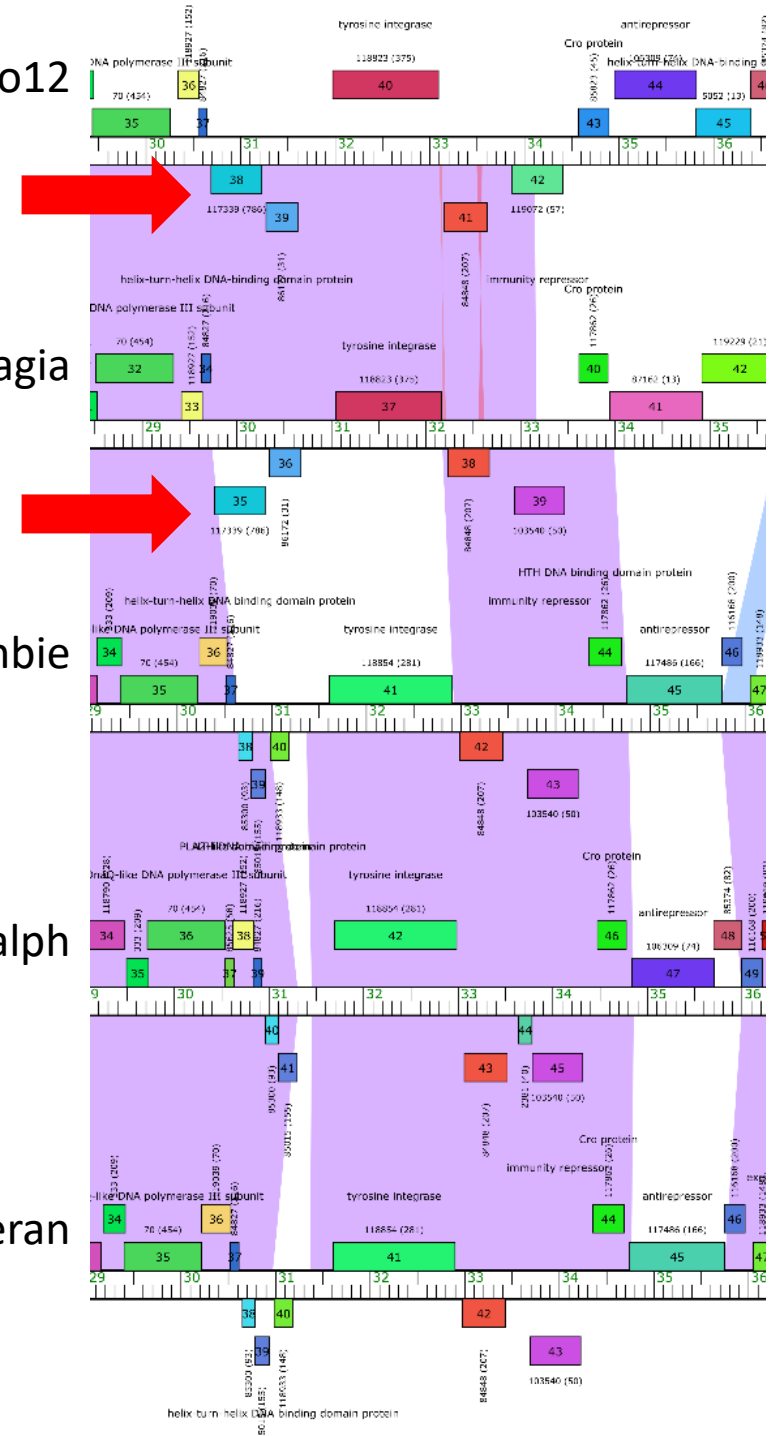
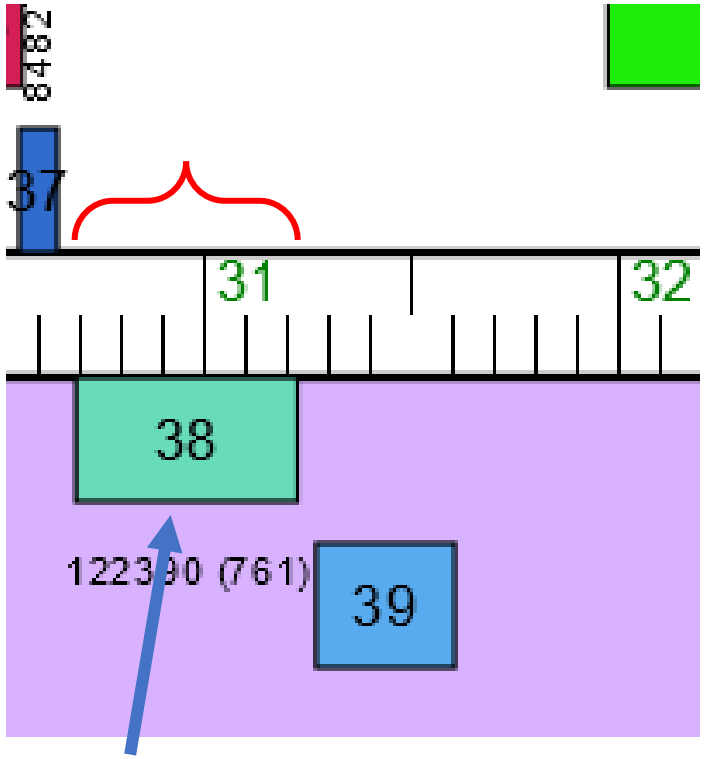


Hypothesis:
Coco12 gp38 (A1-like repressor) protects *M. smeg* from superinfection by Cluster A1 phages

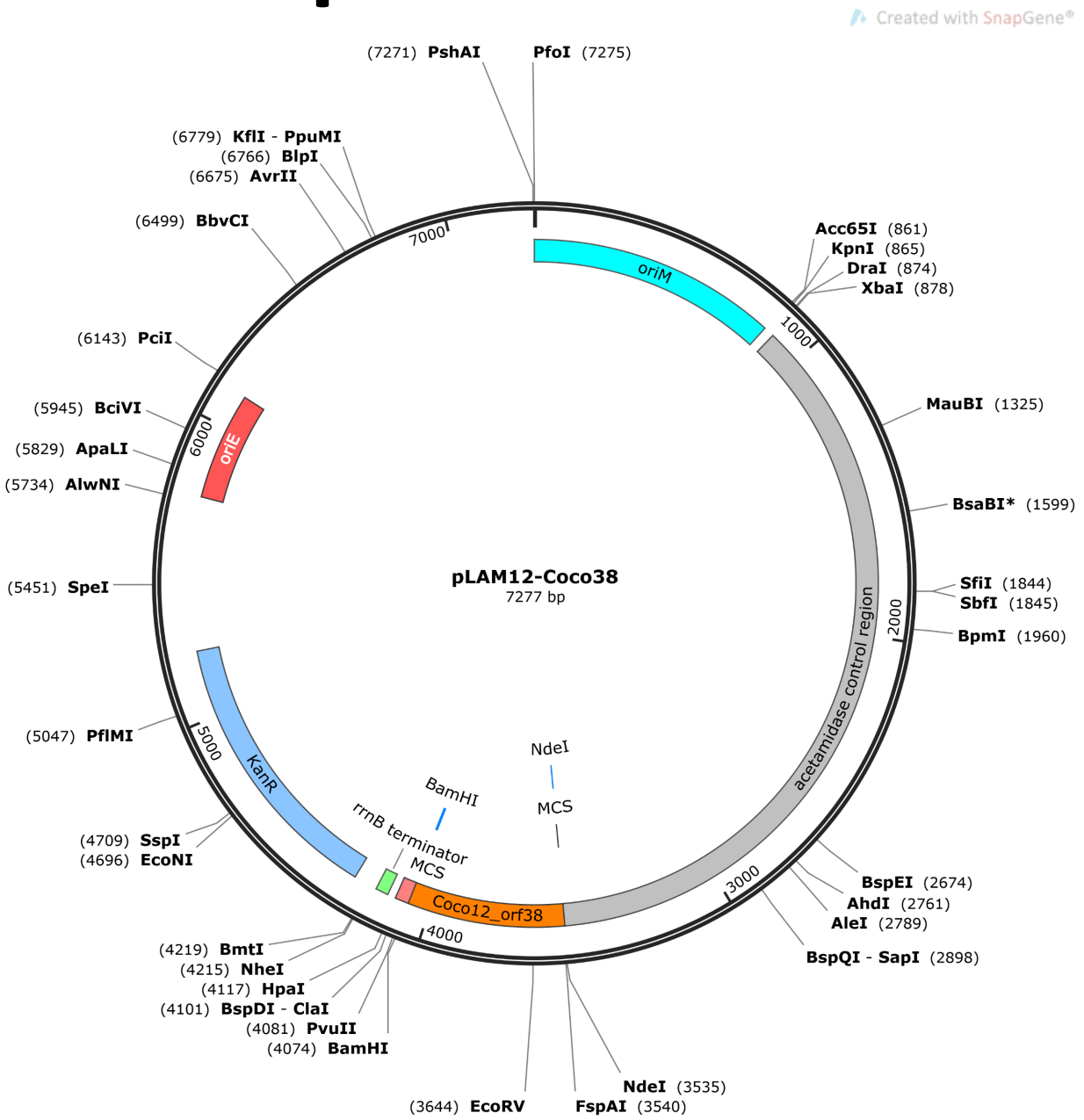
Experiment:

- Clone Coco12 gene *38* into two different plasmids, pLAM12 and pMH94.
- Transform mc^2155 with the plasmids.
- Test transformants for immunity to Bxb1 (A1) infection.

Clone: Coco12 gene *38* ORF in pLAM12 expression plasmid

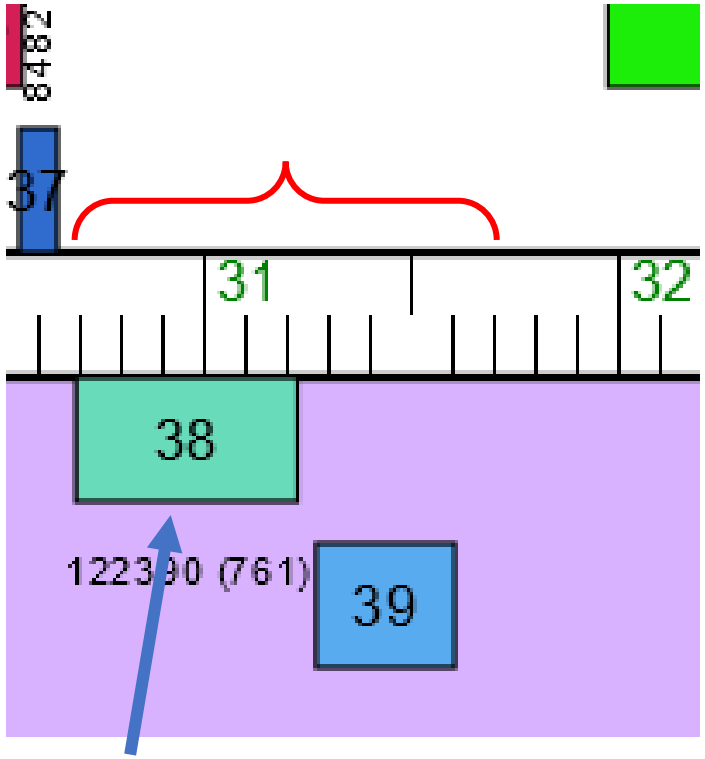


A1-like repressor

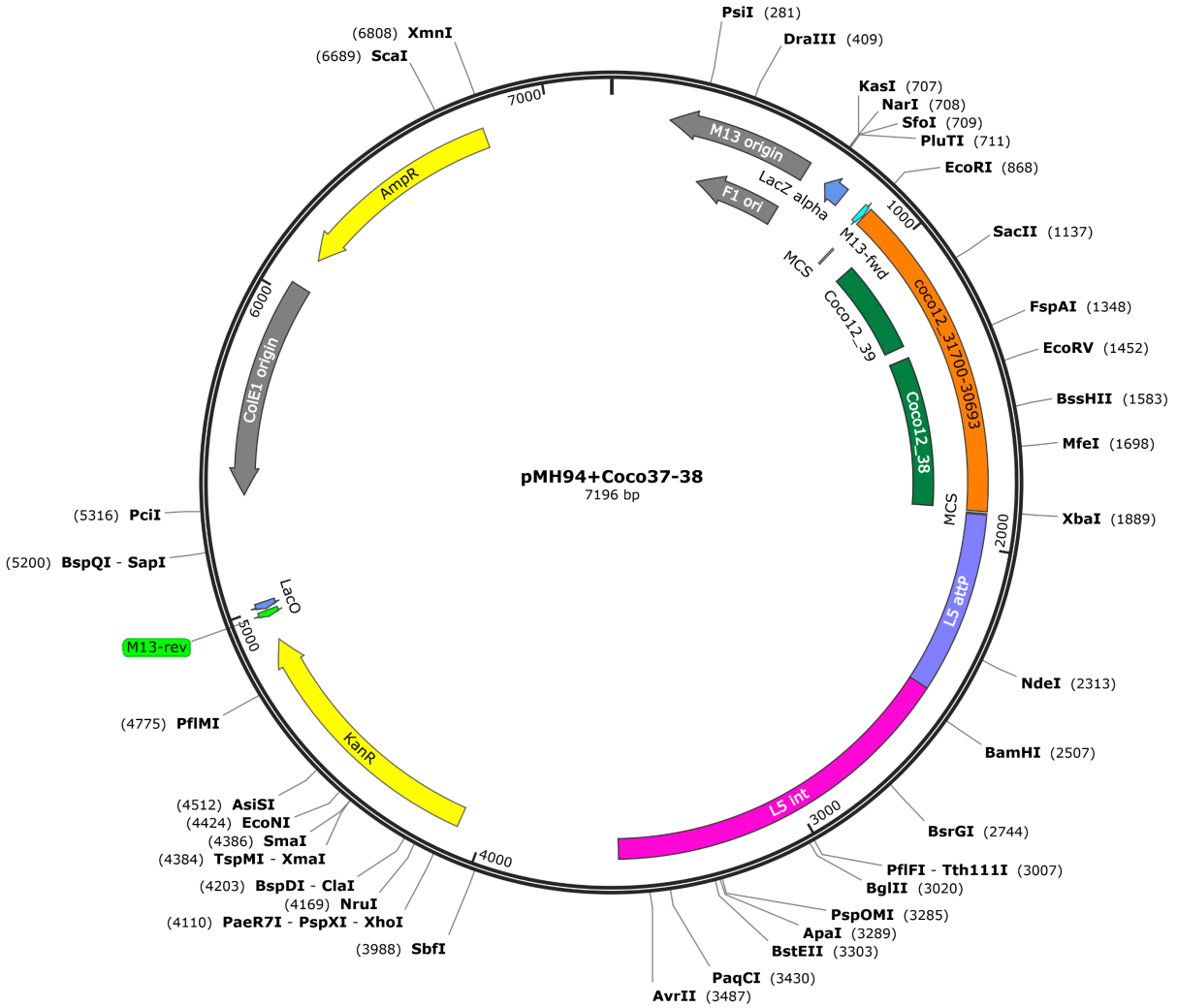


Phanphagia gene 35 is identical to Coco12 gene 38.

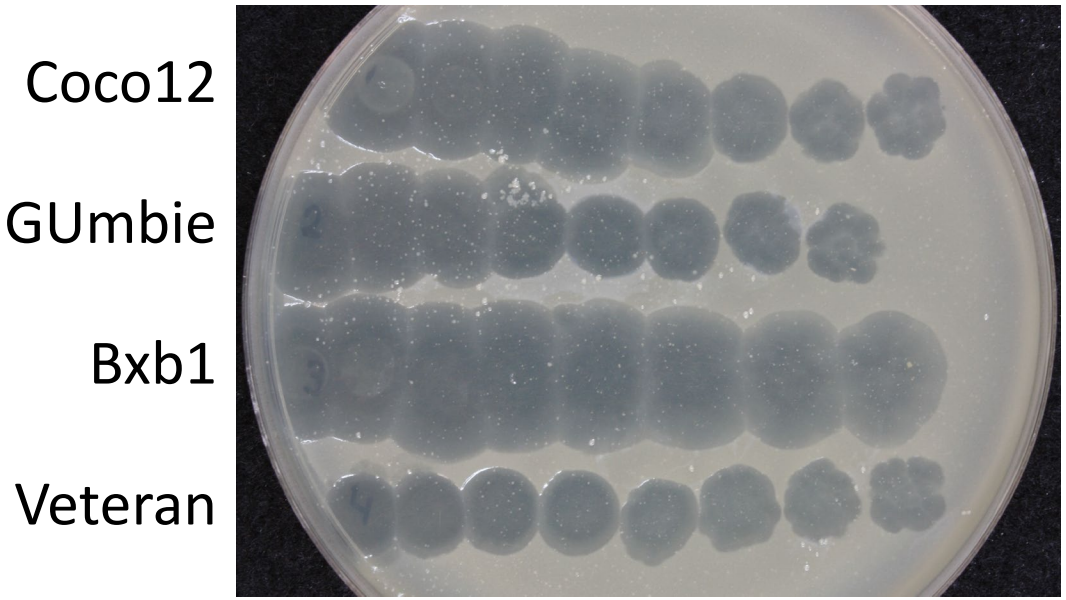
Clone: Coco12 genes *38,39*, and native promoter in pMH94 integrative plasmid



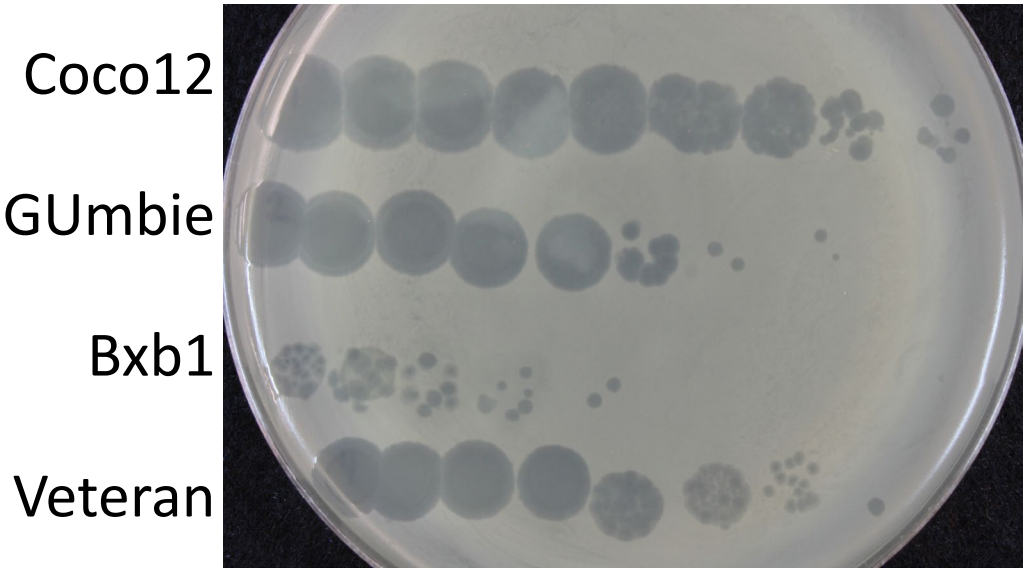
A1-like repressor



Coco12 gene *38* ORF (A1-like repressor) is sufficient to reduce Bxb1 plaque formation



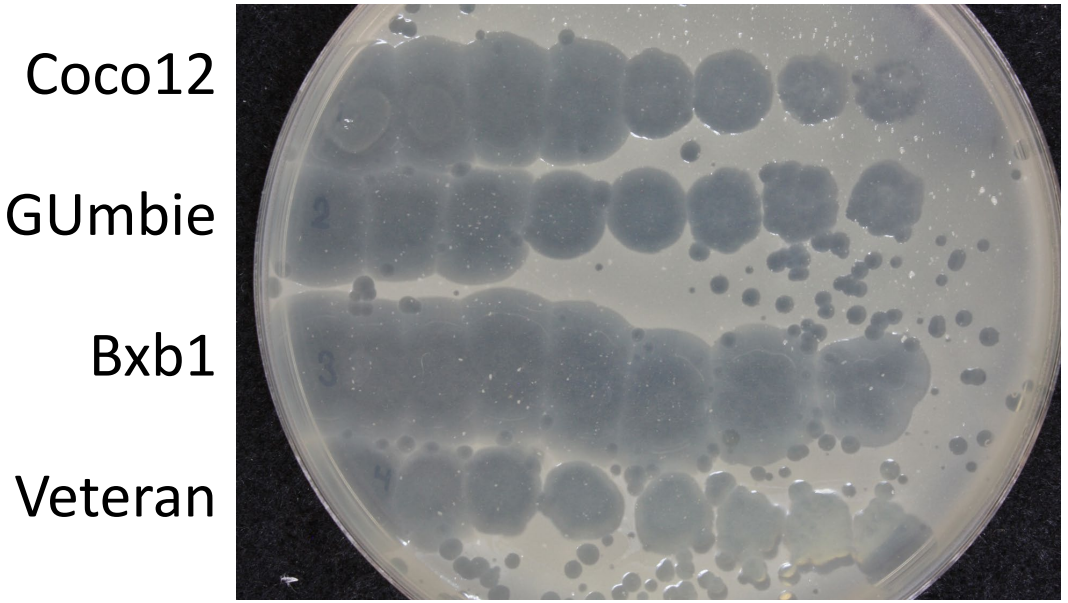
mc^{2}155(pLAM12)



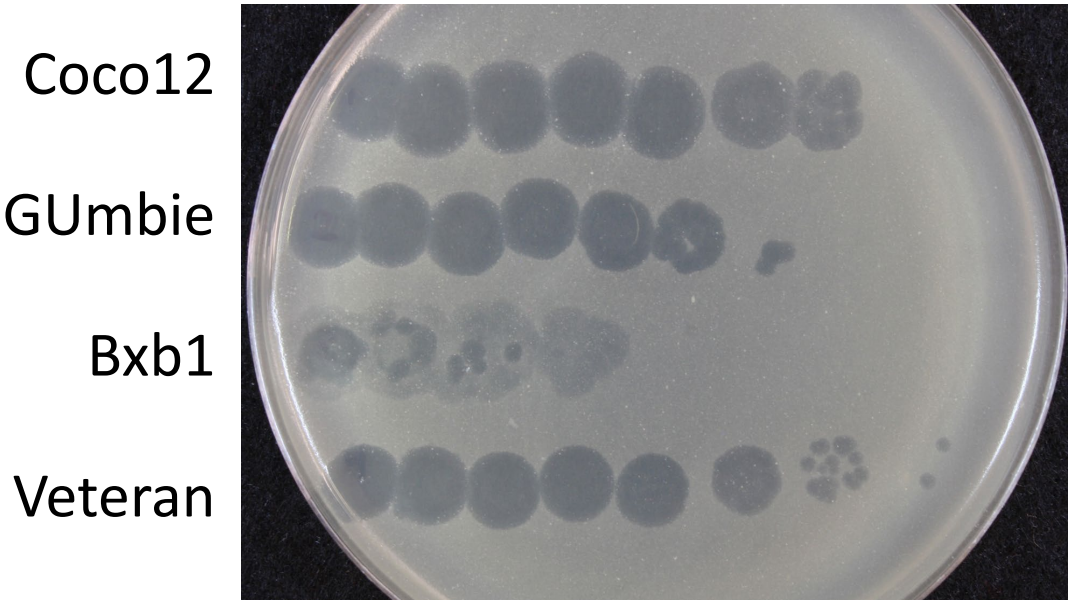
mc^{2}155(pLAM12+Coco12_38)

Immunity test of Coco12 gene *38* ORF in *M. smegmatis* mc^{2}155. 10-fold serial dilutions of phage lysates were spotted onto lawns of mc^{2}155 carrying the empty pLAM12 plasmid or the clone with the Coco12 gene 38 ORF under the control of the acetamidase promoter. The bacteria were grown in non-inducing conditions. Perhaps the promoter is leaky and expressing the cloned ORF. We suspect the rare, clear plaques in Bxb1 are caused by a contaminating phage or by defense-escape mutants of Bxb1. We will plaque-purify Bxb1 and repeat the tests.

Coco12 genes *38* and *39* are sufficient to reduce Bxb1 plaque formation



mc^{2}155(pMH94)



mc^{2}155(pMH94+Coco12_38,39)

Immunity test of Coco12 genes *38, 39* in *M. smegmatis* mc^{2}155. 10-fold serial dilutions of phage lysates were spotted onto lawns of mc^{2}155 carrying the empty pMH94 plasmid or the clone with the Coco12 genes *38,39* and presumably a native promoter. We suspect the rare, clear plaques in Bxb1 are caused by a contaminating phage or by defense-escape mutants of Bxb1. We will plaque-purify Bxb1 and repeat the tests.