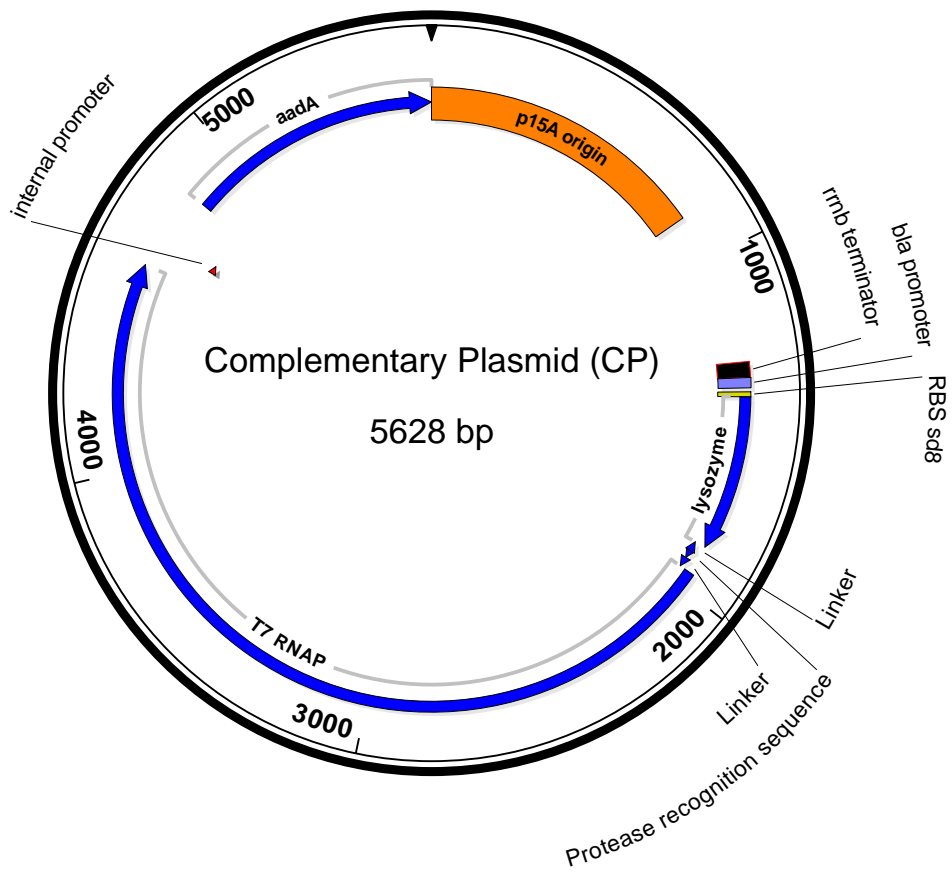
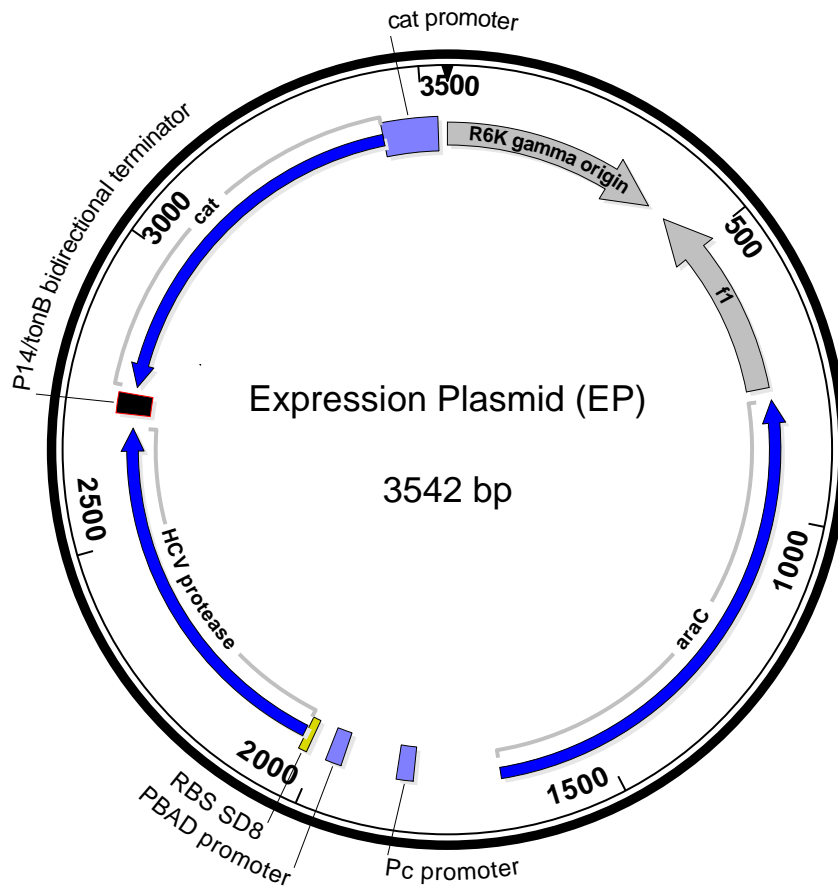


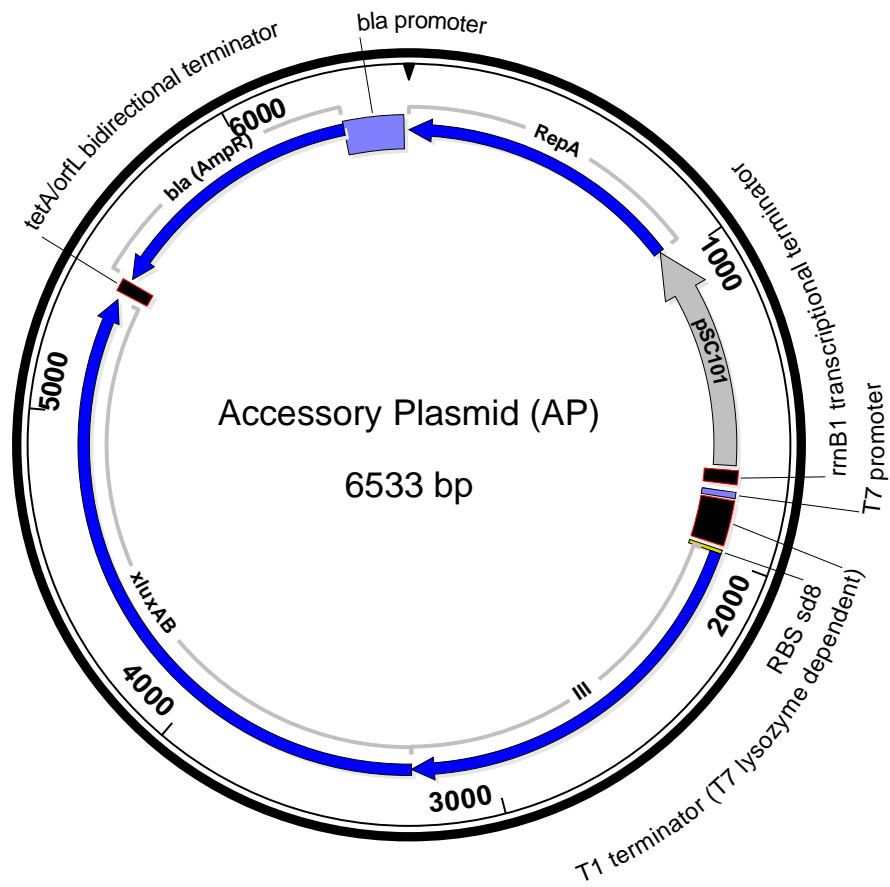
**Supplementary Figure 1.** Crystal structure of T7 lysozyme bound to T7 RNAP. Generated from PDB-1ARO<sup>1</sup>.



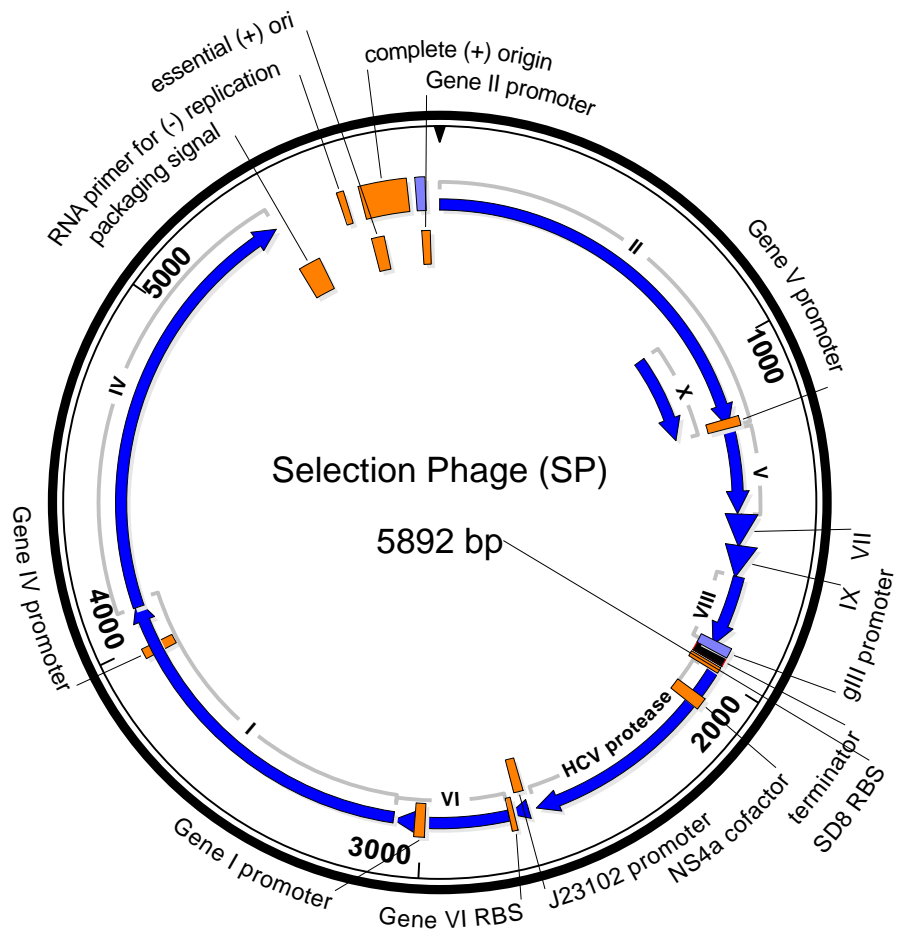
**Supplementary Figure 2.** Vector map of complementary plasmid (CP) used in this study.



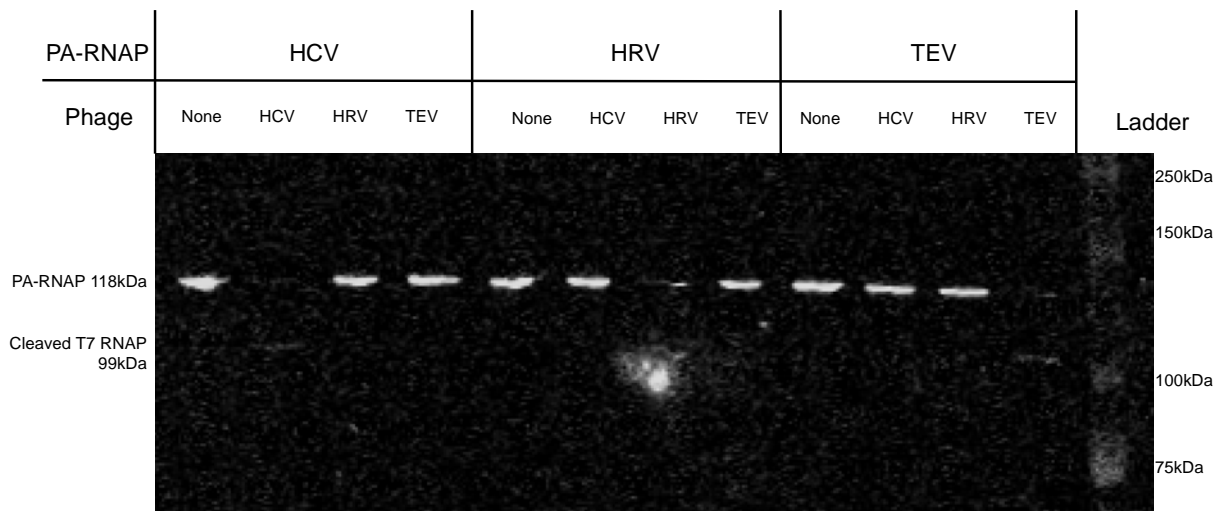
**Supplementary Figure 3.** Vector map of expression plasmid (EP) used in this study.



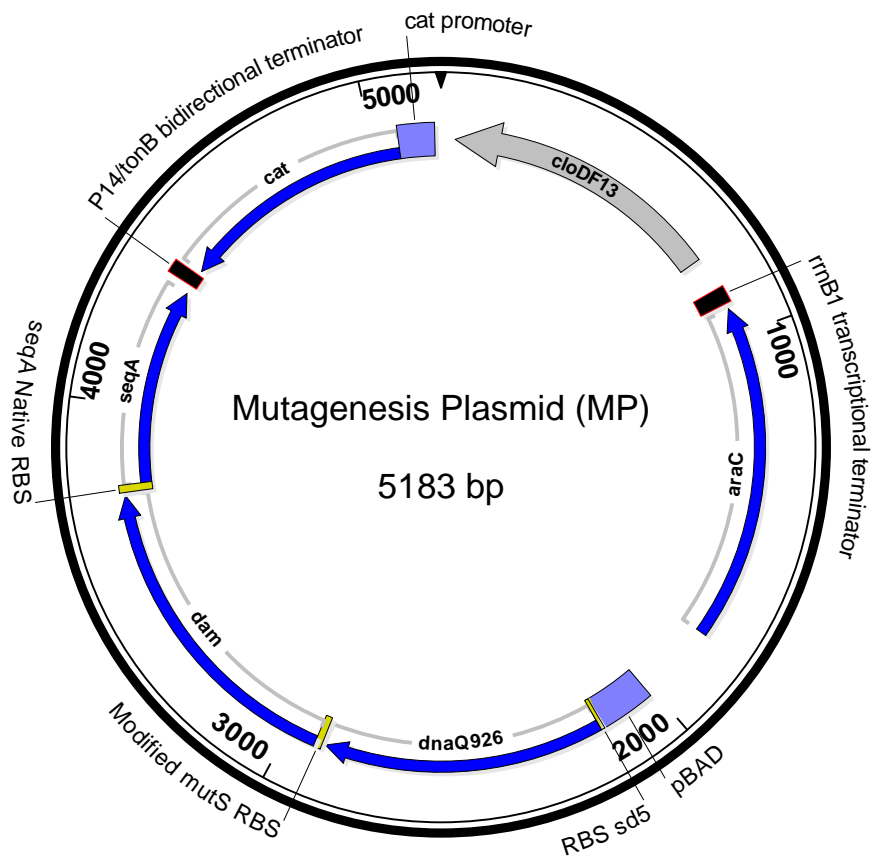
**Supplementary Figure 4.** Vector map of accessory plasmid (AP) used in this study.



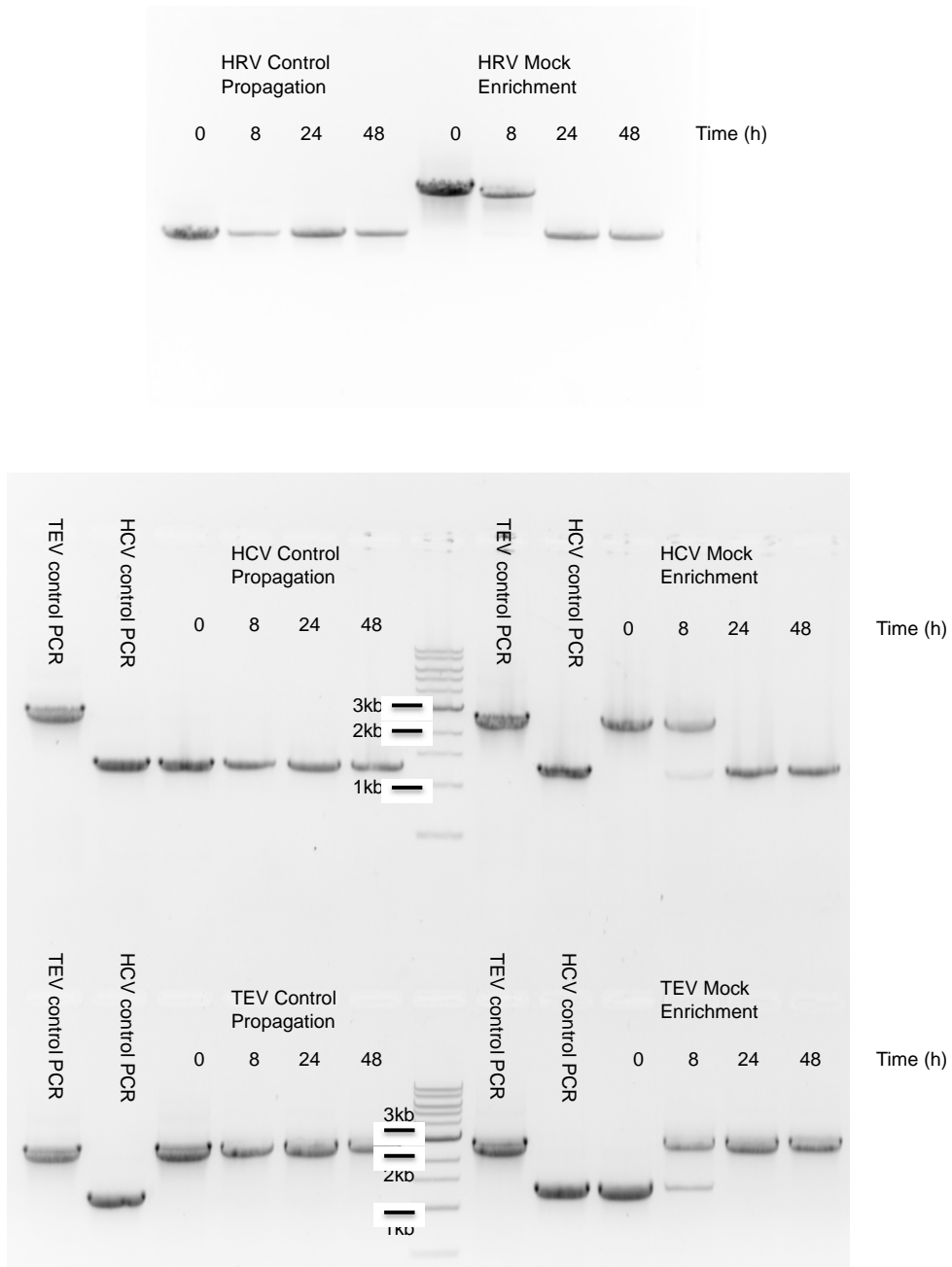
**Supplementary Figure 5.** Vector map of selection phage (SP) used in this study.



**Supplementary Figure 6.** Western blot showing PA-RNAP is cleaved only by the protease that is known to recognize the target sequence. Band sizes of ~120 kDa and ~100 kDa correspond to the full PA-RNAP construct and the cleaved RNAP, respectively.



**Supplementary Figure 7.** Vector map of mutagenesis plasmid (MP) used in this study.



**Supplementary Figure 8. Gel images from Figure 2 without cropping.**



	Glucose	Arabinose	Fold above pJC184
None	1.21 ± 1.25	0.26 ± 0.13	-
pJC184	29.6 ± 32.5	2146 ± 2062	1
pAB086k8	10.4 ± 18.5	10330 ± 13862	4.8

**Supplementary Table 1.** Frequency of rifampin resistant colonies using the improved MP.

### Supplementary References

- 1 Jeruzalmi, D. & Steitz, T. A. Structure of T7 RNA polymerase complexed to the transcriptional inhibitor T7 lysozyme. *EMBO J* **17**, 4101-4113, (1998).