

How exactly does  
DNA synthesis work?

Coupling efficiency?

How do you build genes?

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Excerpts from: Compton, K. T., and J. W. M. Bunker (MIT). "The Genesis of a Curriculum in Biological Engineering." *The Scientific Monthly* (January 1939).

# Refactoring bacteriophage T7

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Natural biological systems are selected by evolution to continue to exist and evolve. Evolution likely gives rise to complicated systems that are difficult to understand and manipulate. Here, we redesign the genome of a natural biological system, bacteriophage T7, in order to specify an engineered surrogate that, if viable, would be easier to study and extend. Our initial design goals were to physically separate and enable unique manipulation of primary genetic elements. Implicit in our design are the hypotheses that overlapping genetic elements are, in aggregate, nonessential for T7 viability and that our models for the functions encoded by elements are sufficient. To test our initial design, we replaced the left 11 515 base pairs (bp) of the 39 937 bp wild-type genome with 12 179 bp of engineered DNA. The resulting chimeric genome encodes a viable bacteriophage that appears to maintain key features of the original while being simpler to model and easier to manipulate. The viability of our initial design suggests that the genomes encoding natural biological systems can be systematically redesigned and built anew in service of scientific understanding or human intention.

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*Subject Categories:* synthetic biology

*Keywords:* bacteriophage T7; synthetic biology; refactor

To build section alpha, we first cloned parts 5, 6, 7, 8, 12, 13, 14, 15, 16, 18, 20, 22, and 24 into pSBI04. We cloned part 11 into pSB2K3. We cloned each part with its part-specific bracketing restriction sites surrounded by additional BioBrick restriction sites. We used site-directed mutagenesis on parts 6, 7, 14, and 20 to introduce the sites U1, U2, U3, and U4, respectively. Our site-directed mutagenesis of part 20 failed. We used site-directed mutagenesis to remove a single Eco0109I restriction site from the vector pUBI19BHB carrying the scaffold Fragment 4. We cloned part 15 into this modified vector. We then cloned scaffold Fragment 4 into pREB and used serial cloning to add the following parts: 7, 8, 12, 13, 14, 16, 18, 20, 22, and 23. We digested the now-populated scaffold Fragment 4 with NheI and BclI and purified the resulting DNA. Next, we cloned parts 5 and 6 into pUBI19BHB carrying scaffold Fragment 3. We used the resulting DNA for in vitro assembly of a construct spanning from the left end of T7 to part 7. To do this, we cut wild-type T7 genomic DNA with AseI, isolated the 388 bp left-end fragment, and ligated this DNA to scaffold Fragment 2. We selected the correct ligation product by PCR. We fixed the mutation in part 3 (A1) via a two-step process. First, PCR primers with the corrected sequence for part 3 were used to amplify the two halves of the construct to the left and right ends of part 3. Second, a PCR ligation joined the two constructs. We added scaffold Fragment 3 to the above left-end construct once again by PCR ligation as described above. We repaired the mutation in part 4 (A2, A3, and R0.3) following the same procedure as with part 3. We used a right-end primer containing an MluI site to amplify the entire construct, and used the MluI site to add part 7. We used PCR to select the ligation product, digested the product with NheI, and purified the resulting DNA. We isolated the right arm of a BclI digestion of wild-type T7 genomic DNA and used ligation to add the populated left-end construct and the populated Scaffold Fragment 4. We transfected the three-way ligation product into IJ1127. We purified DNA from liquid culture lysates inoculated from single plaques. We used restriction enzymes to digest the DNA and isolate the correct clones. Next, we added part 11 via three-way ligation and transfection. Because the restriction sites that bracket part 9 (RsrII) also cut wild-type T7 DNA, we needed to use in vitro assembly to add this part to a subsection of section alpha. To do this, we used PCR to amplify the region spanning parts 5–12 from the refactored genome. We cut the PCR product with RsrII and ligated part 9. We used PCR to select the correct ligation product; this PCR reaction also added a SacII site to the fragment. We digested the PCR product with SacI and SacII and cloned onto the otherwise wild-type phage. Lastly, we used the SacII site to clone part 10 onto the phage.

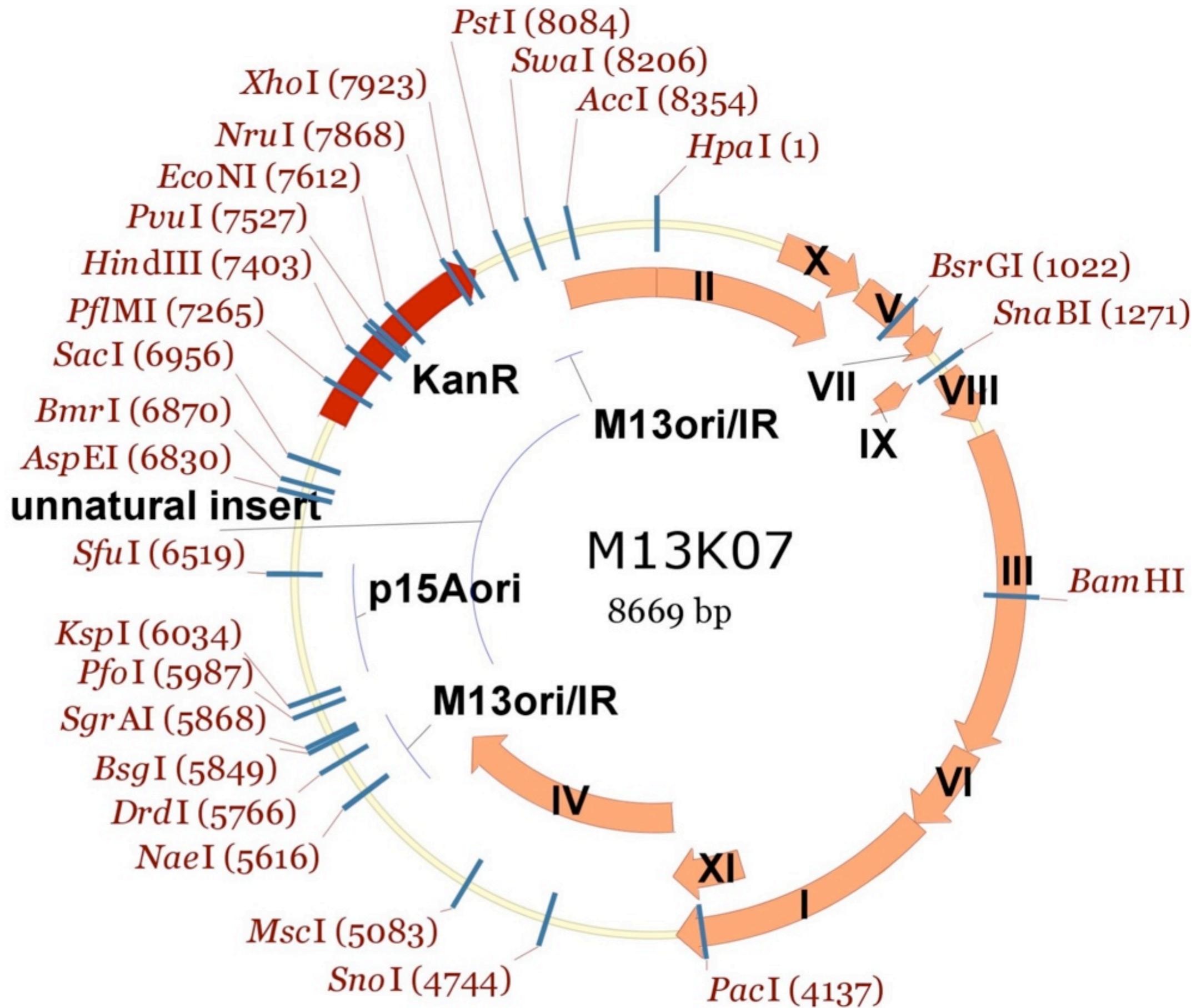
Get me this DNA!

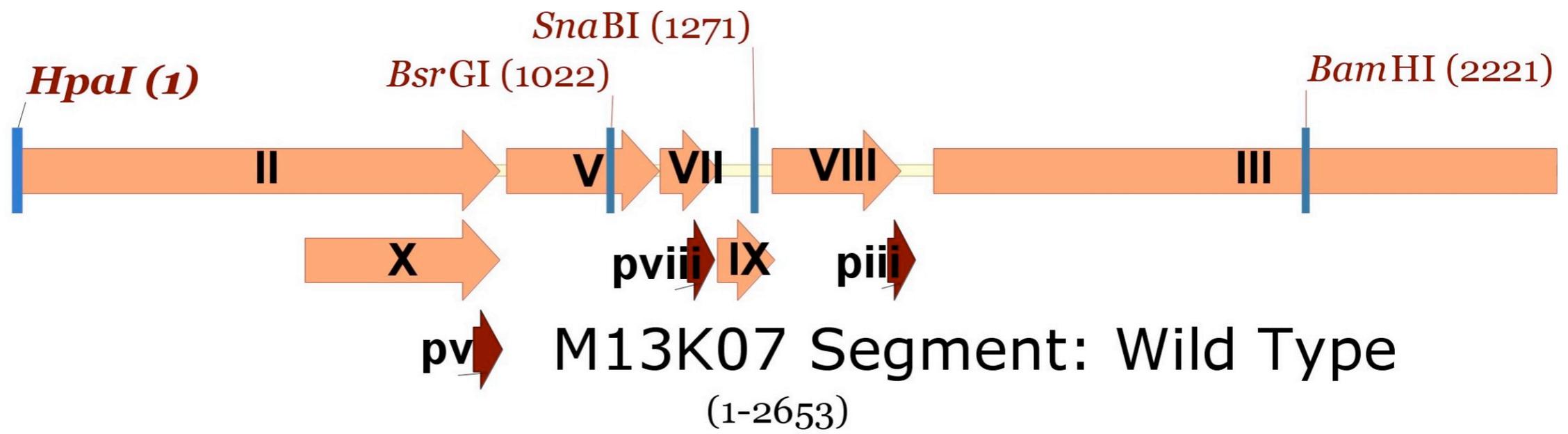
# Questions about T7.1?

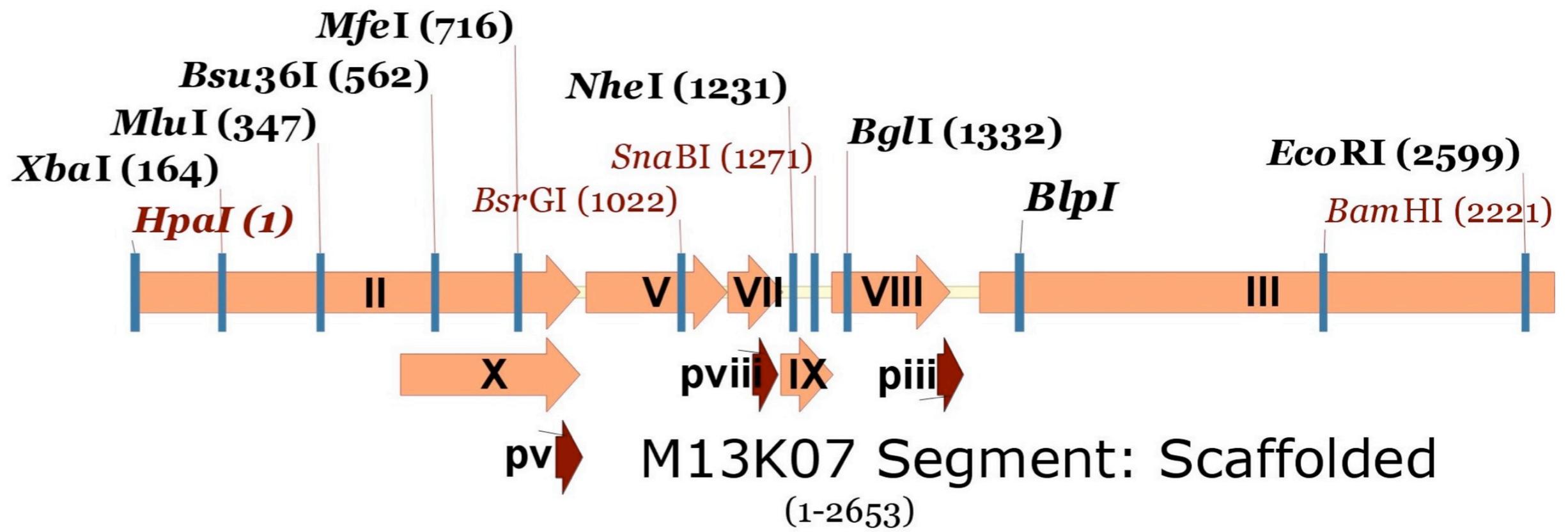
Design?

Construction?

Other?







# Part 2 of your portfolio:

1. Choose a section (site-site)
2. Design DNA for your section (must include terminal RE sites)
3. Annotate sequence (follow scheme)
4. Write summary paragraph (include \$ estimate and GO/NO-GO)
5. 4-8 week fab time,  $f(L, \text{complexity})$