How exactly does DNA synthesis work?

Coupling efficiency?

How do you build genes?

THE SCIENTIFIC MONTHLY

JANUARY, 1939

THE GENESIS OF A CURRICULUM IN BIOLOGICAL ENGINEERING

By President KARL T. COMPTON and Dr. JOHN W. M. BUNKER

THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY

ONE hundred years ago there were but two types of engineers, "military" engineers concerned with the operations of warfare and "civil" engineers whose activities were directed toward problems of civil life. Each utilized many identical techniques in mensuration over the surface of the earth but with different objectives; each was concerned with the building of roads and bridges for which the same scientific data and similar mathematical computations were employed. Neither exercised a monopoly on any particular applications of science; their objectives were different.

For a time, all engineering in civil life was civil engineering, but as some of these engineers became engaged in delving into the earth to secure mineral resources, adaptations of usual procedures in the matter of structures, methods of tunneling, bracing and the like led to the designation of these specialists as "mining" engineers. On the other hand, those engineers who specialized in the harnessing of mechanisms to manufacture, employing the principles of mechanics, came to be known as "mechanical" engineers, the first college curriculum in this field being established at Rensselaer Polytechnic Institute in 1862. Shortly thereafter the increasing applications of electricity in its manifold possibilities to aid the mechanical engineer called for specialization of training and practice in that branch of physics comprised in the field of electricity, as a result of which technical courses in electrical engineering were developed at M.I.T. in 1882.

Meanwhile, the sciences of chemistry and physics and, to a less spectacular degree, the science of biology had been accelerated in their development, and their so-called boundaries expanded until they overlapped. The service of chemistry to biology was obvious, and biochemistry existed at the interphase between the two long before formal recognition of this state came with its definite designation by name.

Chemistry was impressed also into the service of industry, and the utilization of chemistry with a judicious employment of physical and mechanical engineering principles in chemical manufacture was explored systematically and with encouraging results. Alert to the possibilities with which this merger of chemistry and engineering was potent, in 1888 President Francis J. Walker at the Massachusetts Institute of Technology included in its curriculum the first program of training in chemical engineering. Fifteen years later Professor William Walker, teacher of industrial chemistry at M.I.T., conceived the pedagogical plan of instruction in unit processes, such as distillation, dehydration, heat transfer and other processes which are common to many industries, replacing the plan of detailed description of particular processes of manufacture. The art of train-

VII-A. Biophysics and Biological Engineering

First Year

First term			Second term		
Course No.	Subject	Units	Course No.	Subject	Units
5.01	Chemistry, General	7–4	5.02	Chemistry, General	7–4
8.01	Physics	6–5	8.02	Physics	6–5
D11	Engineering Drawing	6–0	D12	Descriptive Geometry	6–0
E11	English Composition	3–5	E12	English Composition	3–5
M11	Calculus	3–6	M12	Calculus	3–6
MS11	Military Science	2–0	MS12	Military Science	2–0
PT1	Physical Training	1–0	PT2	Physical Training	1–0
	Second Year				
5.11	Qual. Analysis	7–2	5.12	Quant. Analysis	7–2
7.01	Biology, Gen.	5–2	7.14	Comp. Anatomy	8–2
8.03	Physics	5–5	8.04	Physics	6–4
E21	Lit. & History	3–5	E22	Lit. & History	3–5
M21	Calculus	3–6	M22	Diff. Equations	3–6
MS21	Mil. Science	2–0	MS22	Mil. Science	3–0
	Gen. Study	2–2			30–19
		28–22			

Summer

5.41	Organic Chem. I	4–3
5.428	Organic Chem. Lab. I	10–0

Third Year

5.61	Phys. Chem. I	4–4	5.62	Phys. Chem. II	4–4
5.611	Phys. Chem. Lab. I	4–0	5.621	Phys. Chem. Lab. II	4–0
7.10T	Invert. Zool.	8–4	6.00	Elec. Eng. Prin.	4–4
7.301	Bacteriology	6–4	6.75	Elec. Eng. Lab.	2–2
Ec11	Political Economy	3–3	7.20	Physiology	6–4
	Language	3–5	Ec12	Political Econ.	3–3
		28–20		Language	3–5
					26–22

Fourth Year

6.01T	Elec. Eng. Prin.	5–7	7.84	Biophysics	6–3
6.761	Elec. Eng. Lab.	2–3	8.311	Atomic Struct.	3–5
7.80	Biochemistry	8–5	8.312	Atomic St. Lab.	3–2
8.09	Physical Meas.	3–2		General Study	2–2
8.161	Optics	3–6		Electives	20
8.162	Optical Meas.	3–2			49
		24–25			

Fifth Year

7.321	Adv. Bact.	3–4	7.82	Adv. Biochem.	6–3
7.81	Zymology	6–3	7.92	Biol. Eng. II	4–2
7.91	Biol. Eng. I	6–3	10.662	Colloid Chem.	2–4
10.661	Int. Colloid Chem.	2–4		Elective	9
	Elective	9		Thesis	20
	Thesis	10			50
		50			

Molecular Systems Biology (2005) doi:10.1038/msb4100025

www.molecularsystemsbiology.com

molecular systems biology

Refactoring bacteriophage T7

Leon Y Chan[1,3], Sriram Kosuri[2,3] and Drew Endy[2,*]

[1] Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA and [2] Division of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA
[3] These authors contributed equally to this work
* Corresponding author. Division of Biological Engineering, Massachusetts Institute of Technology, 68-580, 77 Massachusetts Avenue, Cambridge, MA 02139, USA. Tel.: +1 617 258 5152; Fax: +1 617 253 5865; E-mail: endy@mit.edu

Received 15.7.05; accepted 23.7.05

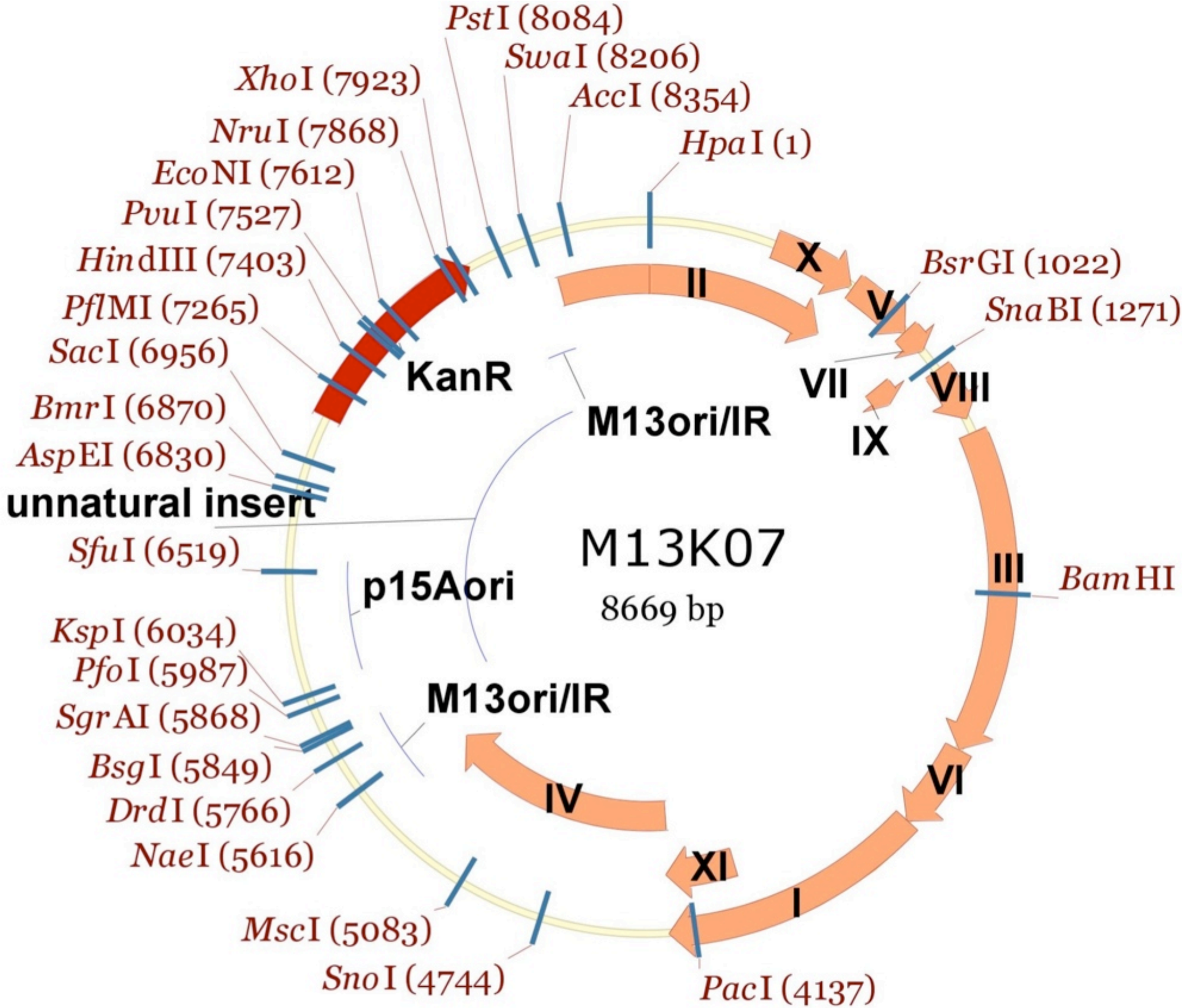
Natural biological systems are selected by evolution to continue to exist and evolve. Evolution likely gives rise to complicated systems that are difficult to understand and manipulate. Here, we redesign the genome of a natural biological system, bacteriophage T7, in order to specify an engineered surrogate 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.
Molecular Systems Biology 13 September 2005; doi:10.1038/msb4100025
Subject Categories: synthetic biology
Keywords: bacteriophage T7; synthetic biology; refactor

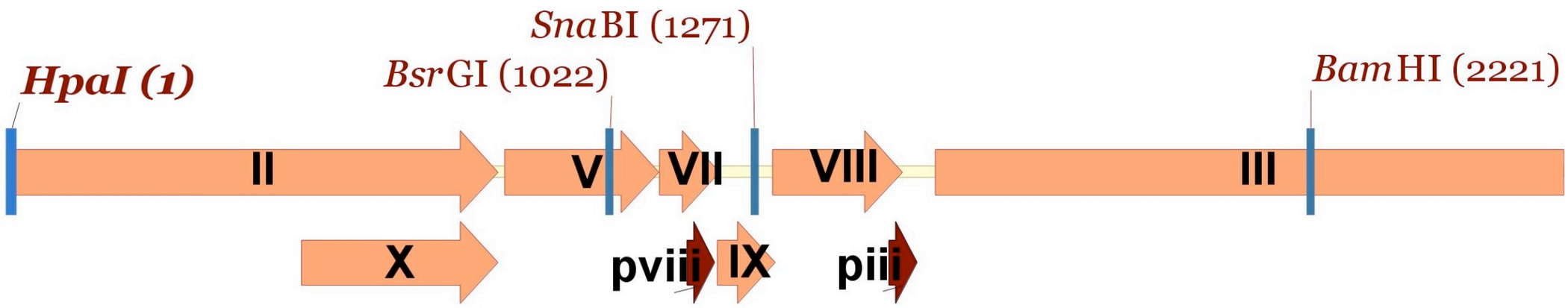
To build section alpha, we first cloned parts 5, 6, 7, 8, 12, 13, 14, 15, 16, 18, 20, 22, and 24 into pSB104. We cloned part 11 into pSB2K3. We cloned each part with its part-specific bracketing restriction sites surrounded by additional BioBrick restriction sites. We used site-directed mutagenesis on 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 pUB119BHB 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 pUB119BHB 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?

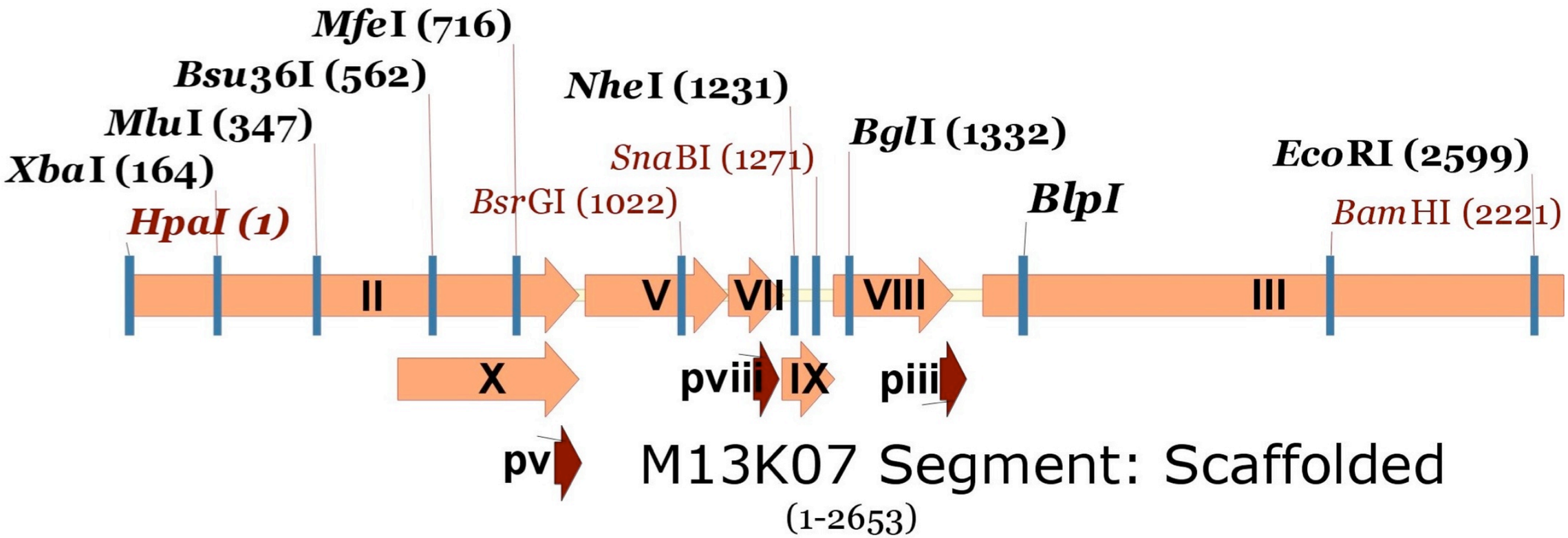
Design?
Construction?
Other?


PstI (8084)
SwaI (8206)
XhoI (7923)
AccI (8354)
NruI (7868)
HpaI (1)
EcoNI (7612)
PvuI (7527)
HindIII (7403)
BsrGI (1022)
PflMI (7265)
SnaBI (1271)
SacI (6956)
KanR
II
X
V
VII
VIII
IX
BmrI (6870)
M13ori/IR
AspEI (6830)
unnatural insert
M13K07
8669 bp
SfuI (6519)
p15Aori
III
BamHI
KspI (6034)
PfoI (5987)
M13ori/IR
SgrAI (5868)
BsgI (5849)
VI
IV
DrdI (5766)
XI
NaeI (5616)
I
MscI (5083)
SnoI (4744)
PacI (4137)



M13K07 Segment: Wild Type

(1-2653)



M13K07 Segment: Scaffolded

(1-2653)

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, complexity)