JCVI-syn3.0 – A synthetic genome stripped bare!

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“Perfection is finally attained not when there is no longer anything to add but when there is no longer anything to take away.”
Antoine de Saint-Exupery (1900-1944)

While Douglas Adams’ answer to the ultimate question of life is 42, the real answer might be closer to 473 – the minimum number of genes required to sustain life under ideal environmental conditions. The search for the minimum genome, began in earnest in the early 1980s when Morowitz first proposed the mycoplasmas (owing to their parsimonious genomes) as an ideal model for understanding the basic principles of life. Indeed, the smallest known mycoplasma genome (Mycoplasma genitalium; containing 525 genes) provided the most obvious starting point from which to determine the minimal genome. Comparison of the M. genitalium genome to that of H. influenzae (the first bacterial genome sequenced; 1815 genes), revealed a common core of 256 genes; representing a theoretical minimal genome which is less than half that of M. genitalium. Further experimental analysis involving global transposon mutagenesis of the M. genitalium genome predicted a minimal genome of 375 essential genes; significantly more than the theorized minimum, yet less than that found in nature, providing scope for the design and development of a minimal artificial genome.

The first DBT cycle involved the design of a hypothetical minimal genome (HMG), based on existing transposon mutagenesis data and the published literature. The resulting HMG design contained 432 protein encoding genes and 39 RNA genes. Using the modular strategy described previously, the HMG was divided into 8 overlapping segments, each of which has a corresponding syn1.0 segment. This approach allows designed synthetic segments to be mixed and matched with viable syn1.0 segments enabling assembly in any specified combination. However, this initial approach, being based on inadequate transposon mutagenesis data, had only limited success; with only one of the HMG segment designs producing viable cells.

The second DBT cycle involved a refined Tn5 strategy to improve the HMG design; generating ~80,000 clones, each containing a Tn5 chromosomal insertion. Approximately 30,000 unique insertions were tagged in this initial screen and the identified genes were divided into 3 categories, classified as either essential (“e-genes”; of which there were 240), non-essential (“n-genes”; 432) or quasi-essential (“i-genes”; 229). The “i-gene” group was further subdivided as “in-genes” (those exhibiting a minimal growth defect when mutated) and “ie-genes” (those exhibiting severe growth defects). The reduced genome design (RGD1.0) removed ~90% of the “n-genes,” resulting in a ~50% reduction in the syn1.0 genome size. Using the same cloning strategy as before, the 8 segments of RGD1.0 were introduced into the seven-eighths syn1.0 background in yeast, before being transformed into M. capricolum. Interestingly, despite each designed segment supporting cell growth in a seven-eighths syn1.0 background, combining all 8 reduced RGD1.0
segments into a single genome failed to produce a viable cell when transplanted into *M. capricolum*. This limitation is due to the existence of “synthetic lethal pairs”\(^{13}\); the combined loss of redundant genes for essential functions as a consequence of the modular cloning strategy employed. While, 2 segments each containing an “e-gene” will support growth in the context of the seven-eighths syn1.0 background, when the segments are combined both “e-genes” are excluded and the cell fails to grow (or in the case of “i-genese;” grows more slowly). Taking into account the existence of synthetic lethal pairs; 26 genes were added to RGD1.0 to give rise to RGD2.0. A third DBT cycle (with 7 RGD2.0 segments plus a syn1.0 segment with 31 genes deleted) gave rise to JCVI-syn2.0; the first synthetic minimized cell with a genome smaller than *M. genitalium*.

Finally, a fourth round of Tn5 mutagenesis on syn2.0 stripped an additional 42 “n-genes” from RGD2.0, to give RGD3.0. As before, the 8 newly designed RGD3.0 segments were propagated in yeast before being transplanted into *M. capricolum*. One of these resulting viable transplants was chosen as the representative minimal synthetic cell and named JCVI-syn3.0. However, creation of the minimal genome is both a subjective and dynamic process; affected by intrinsic and extrinsic factors. While additional RGB cycles would likely strip the syn3.0 genome even further, growth rate would almost certainly be compromised. Furthermore, environmental factors, such as the nutritional quality of the surrounding medium will dictate the minimum genetic requirement. Therefore, while syn3.0 may not be the smallest possible synthetic construct, it is the most physiologically stable under the environmental conditions prescribed by Hutchison et al.\(^2\)

At the genomic level, all 4 DBT cycles successfully stripped 428 genes from the syn1.0 template, leaving a 531 kbp genome composed of 473 genes. A majority of the identified genes can be grouped into 4 main categories: dedicated to gene expression, genome preservation, membrane structure/function, and cytosolic metabolism. While almost half of the genome (48%; 229 genes) is dedicated to gene expression (195 genes) and genome preservation (34 genes), approximately equal numbers of genes are involved in directing cellular architecture (18%; 84 genes) and metabolism (17%; 81 genes). Significantly, the remaining 79 genes (17% of the genome) cannot easily be assigned to a functional category. Of these, 19 are “e-genese,” 36 are “i- or ie-genese” and 24 are “n- or in-genese.” While most of these genes can be expected to fall into one of the 4 major categories, described above, at least some are likely to direct previously undescribed biological functions.

Phenotypically, syn3.0 has a similar colony morphology to that of its progenitor cell, syn1.0, albeit with a smaller colony size, suggesting a slower growth rate. Indeed, syn3.0 grows approximately 3 times slower than syn1.0 (doubling time of ~180 min as opposed to ~60 min). Furthermore, unlike syn1.0 which grows as predominantly planktonic cells in static liquid culture, syn3.0 forms matted sediments under similar conditions. Additionally, microscopic analysis of syn3.0 under these conditions reveals the existence of long, segmented filamentous structures, together with large vesicular bodies.

Having created the smallest synthetic cell – what next for this branch of synthetic biology\(^{14}\)? Some have questioned the utility of the minimal genome approach, particularly against the backdrop of significant recent advances in CRISPR gene editing.\(^{15}\) Why design synthetic life when it is possible, and in most cases more convenient, to edit existing natural life?

![Figure 1.](image-url)
genomes. Venter’s response to such queries is simple - “if you’re trying to design life, CRISPRs aren’t going to get you there.” A significant aim of the JCVI minimal genome project is not simply to create life, but to improve it, through the design and delivery of safe and effective cellular chassis. These minimal biological frameworks, in which the function of each gene is known, will facilitate the development of improved whole-cell computational models. Such models will enable efficient in silico design and testing (analogous to CAD based systems in traditional engineering), before undertaking wet-lab based development. The potential outputs from this approach include tailored microbes with myriad applications in industry, agriculture and medicine. Moreover, this approach also paves the way for the development of improved whole-cell computational models. Such gene is known, will facilitate the development of biological frameworks, in which the function of each life, but to improve it, through the design and delivery of safe and effective cellular chassis. These minimal biological frameworks, in which the function of each gene is known, will facilitate the development of improved whole-cell computational models. 

The future is bright for synthetic biology, the future is synX.0!

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