Critical Focus

# Who are the parents of *Mycoplasma mycoides* JCVI-syn1.0?

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## Abstract

The rapid advancement of technology is causing people to re-think many ideas that were once considered certainties. During a TED (Technology, Entertainment, Design) conference in May 2010, Dr. Craig Venter stated that his team had created "the first self-replicating species we've had on the planet whose parent is a computer." Their work was published in Science in July 2010. Briefly, the Venter team created a synthetic bacterium, *Mycoplasma mycoides* JCVI-syn1.0, whose genome sequence is composed of the genome sequence of *M. mycoides*, a yeast cloning vector, and some artificial DNA sequence. This paper provides a detailed analysis of their project and several possible indicators that the statement made by Dr. Craig Venter concerning the parents of the synthetic cells might not be altogether reliable, by following the various contributions made by *M. mycoides*, *M. capricolum*, yeast, *E. coli*, and humans.

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For sexually reproducing organisms, the biological parents have long been considered to be the male who supplied the sperm and the female who supplied the egg. For asexually reproducing organisms, the biological parent has long been considered to be the organism that directly gave birth to its offspring by budding or fission. As is now the case in so many areas, the advancement of technology is causing people to re-think what used to be considered absolutes, including the issue, "who are the parents of an organism?" One recent article in particular, *Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome* written by Gibson et al. [1], leads to a discussion of who the parents of *Mycoplasma mycoides* JCVI-syn1.0 actually are, and to a broader discussion of how, if at all, the parents of synthetically created organisms can be described.

Until the recent work conducted by Gibson et al. [1], the majority of artificially created organisms had been created by various types of nuclear transfer, which is the process of removing the nucleus from an oocyte and then injecting the nucleus of a donor cell into the enucleated oocyte. Briggs et al. [2] performed the first successful nuclear transfer when they injected a nucleus from an early tadpole embryo into an enucleated frog egg, and the subsequent cell developed into a viable tadpole. Campbell et al. [3] performed a landmark experiment when they created the first cloned mammal, a famous lamb named Dolly, using the technique of somatic cell nuclear transfer, which is the process by which a viable embryo is created by injecting the nucleus from a somatic cell into an enucleated egg cell.

Although these experiments do not create natural organisms, the parents of these cloned organisms could be fairly simply described as the DNA donor and the DNA recipient or their parents. These experiments are also similar to *in vitro* fertilization in humans, where a donor egg is fertilized by a donor sperm outside of a female, then placed into a different surrogate female for development or back into the female who donated the egg. The parents of these *in vitro* fertilized organisms could be described as either the duo of the sperm donor and the egg donor or the combination of the sperm donor, egg donor, and the gestational carrier if one is necessary. The major difference between the nuclear transfer experiments and the experiment performed by Gibson et al. is that the nuclear transfer experiments involve the transfer of a naturally existing genome into an enucleated egg cell of the same species, while the Gibson et al. experiment involved creating a genome from overlapping synthetic oligonucleotides using several different species of organisms [1].

The creation of *M. mycoides* JCVI-syn 1.0 was led by the J. Craig Venter Institute. The project took more than twenty researchers over ten years of work and cost an estimated forty million dollars [4]. Essentially, the researchers engineered on a

computer a genome that was mostly based on the sequenced genome of *M. mycoides*. Although the researchers technically engineered the genome on a computer, the final genome was largely based on inherent information naturally found in various organisms. The team synthesized the whole genome using a combination of chemical techniques, *E. coli*, and yeast, and then transplanted the synthesized genome, which contained an antibiotic resistance gene, into a *Mycoplasma capricolum* cell that still had its own genomic DNA, but which lacked the antibiotic resistance gene and thus was lost during antibiotic selection. The *M. capricolum* cell containing the synthesized genome was then allowed to reproduce for many generations until no traces of proteins from the original *M. capricolum* cell remained. These resultant new cells were called *M. mycoides* JCVI-syn 1.0 [1].

To do all this, the researchers first sequenced the genome of *M. mycoides* and stored the sequence on a computer. They then added four different human-engineered watermarks to the *M. mycoides* genome sequence so that the *M. mycoides* JCVI-syn 1.0 genome would be distinguishable from the natural *M. mycoides* genome once the experiment was complete. The researchers created an artificial code with DNA sequences that allowed them to use the entire English alphabet, punctuation, and numbers in order to create the watermarks, which describe the artificial code, the names of 46 different authors and key contributors of the project, a website address for *M. mycoides* JCVI-syn 1.0, and three quotations. The watermarks do not encode any genes or any functional information for the cells. The *M. mycoides* JCVI-syn 1.0 genome to be synthesized was then divided into 1078 overlapping DNA cassettes; each cassette was 1080 bp (base pair) long with 80 bp overlaps to each of its two adjacent cassettes.

The process of creating the real full length *M. mycoides* JCVI-syn 1.0 genome was initiated by chemically synthesizing the 1080 bp cassettes using overlapping synthetic oligonucleotides [1] (Figure 1 and Table 1). These cassettes were then ligated to an *Escherichia coli* cloning vector so that they could be amplified in *E. coli* using its DNA replication machinery. For the next part of the experiment, the researchers isolated the cassettes and transferred them, in sets of ten, each set with its unique *E. coli*-yeast shuttle vector, which had terminals that matched the two outer ends of that set, into *Saccharomyces cerevisiae* so that the cassettes could be assembled into 10 kb intermediates using *S. cerevisiae* homologous recombination machinery in a process named *transformation-associated recombination* (TAR). The 10 kb intermediates were transferred back into *E. coli* so that they could be amplified using the *E. coli* DNA replication machinery. After the 10 kb intermediates were isolated from *E. coli*, they were assembled into 100 kb intermediates in yeast using TAR, also in sets of ten and together with an *E. coli*-yeast shuttle vector unique to each set.

Unexpectedly, the 100 kb intermediates were too large to be amplified in *E. coli*, so they were amplified in yeast using the *S. cerevisiae* DNA replication machinery. Once the 100 kb intermediates were purified from yeast, they were assembled into the full-length JCVI-syn1.0 genome in yeast using TAR.

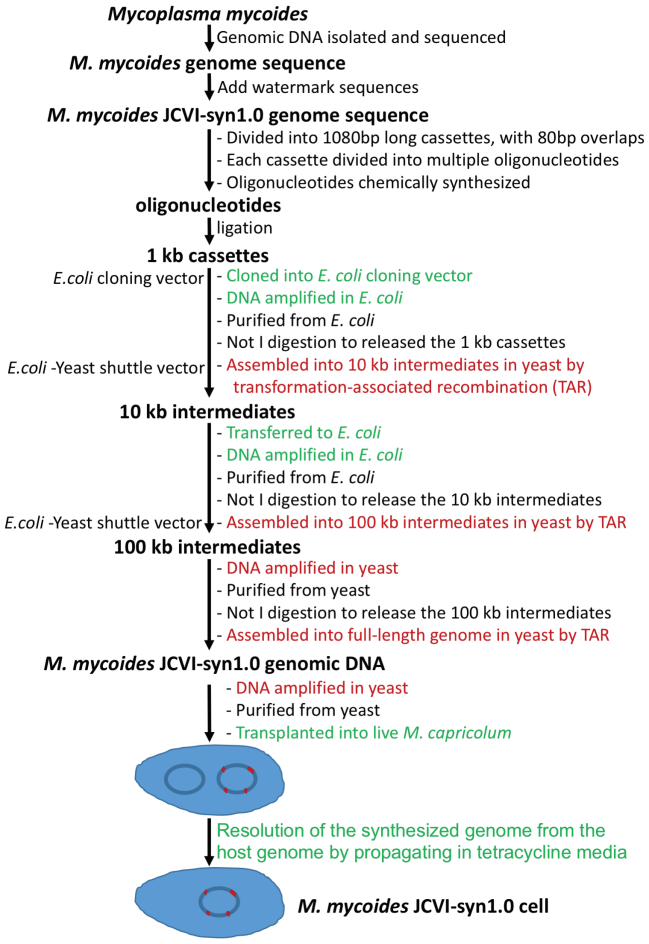


**Figure 1: A flow chart displaying the basic procedure followed to create *M. mycoides JCVI-syn 1.0*.** The steps performed by humans are in black. The steps performed using bacteria (*E. coli* or *M. capricolum*) are in green. The steps performed using yeast are in red. The *M. mycoides* JCVI-syn1.0 genome is marked with red insertions indicating the human engineered features. **doi:**10.5048/BIO-C.2016.2.f1

The full-length genome was then amplified in yeast, isolated, and transplanted into live *M. capricolum* cells. These *M. capricolum* cells still had their own *M. capricolum* genomic DNA, so the transferred *M. mycoides* JCVI-syn1.0 genomic DNA was replicated initially alongside the *M. capricolum* genome using the DNA replication machinery provided by the *M. capricolum* host cell. The *M. mycoides* JCVI-syn1.0 genes were transcribed and translated using the transcription and translation machineries provided by the *M. capricolum* host cells, along with the *M. capricolum* genes.

Cell division caused the separation of the two genomes, resulting in the formation of cells with only the synthetic genome, cells with only the *M. capricolum* genome, and cells with both. The cells containing only the *M. capricolum* genome were killed off when all of the cells were placed in tetracycline medium because their genome did not contain the antibiotic resistance gene. The cells with both genomes were out-competed by those with only the synthetic genome, probably because it takes twice as much energy to duplicate two genomes. Consequently, the

**Table 1: Steps of creating *M. mycoides* JCVI-syn1.0, and the responsibilities of different agents.**

| Task* | Bacteria | Yeast | Humans | Computer |
|---|---|---|---|---|
| **Design the *M. Mycoides* syn1.0 genome sequence** | 1. *M. mycoides* makes the genomic DNA. | | 2. Isolate and sequence the *M. mycoides* genomic DNA<br>3. Add watermark sequences into the *M. Mycoides* genome sequence, generating the syn1.0 genome sequence<br>4. Store the sequence in the computer | 3′. Help to assemble the sequence.<br>4′. Store the sequence. |
| **Make the 1kb cassettes** | 6. *E. coli* takes in the vectors containing the cassettes.<br>7. *E. coli* multiplies these vectors. | | 1. Divide the syn1.0 genome sequence into 1080 bp long cassettes, with 80 bp overlaps.<br>2. Divide each cassette into multiple oligonucleotides.<br>3. Synthesize the oligonucleotides.<br>4. Ligate the oligos with *E.coli* cloning vector.<br>5. Transfer the ligations into *E. coli.* | |
| **Make the 10 kb intermediates** | 1. *E. coli* provides the vectors containing the cassettes.<br>9. *E. coli* multiplies the shuttle vectors containing the 10 kb intermediates. | 5. Assemble the cassettes into 10 kb intermediates.<br>6. Multiply the vectors containing the intermediates. | 2. Isolate the vectors containing the cassettes from *E. coli.*<br>3. Release the cassettes by enzyme digestion.<br>4. Transfer the cassettes together with the *E.coli* -Yeast shuttle vector designed for each intermediate into yeast.<br>7. Isolate the shuttle vectors containing the intermediates from yeast.<br>8. Transfer the isolates into *E. coli.* | |
| **Make the 100 kb intermediates** | 1. *E. coli* provides the shuttle vectors containing the 10 kb intermediates. | 5. Assemble the 10 kb intermediates into 100 kb intermediates.<br>6. Multiply the vectors containing the 100 kb intermediates. | 2. Isolate the vectors containing the 10 kb intermediates from *E. coli.*<br>3. Release the intermediates by enzyme digestion.<br>4. Transfer the intermediates together with the *E.coli* -yeast shuttle vector designed for each intermediate into yeast. | |
| **Make the full length genomic DNA of *M. mycoides* JCVI-syn1.0** | | 1. Provide the shuttle vectors containing the 100 kb intermediates.<br>5. Assemble the 100 kb intermediates into full length genome.<br>6. Multiply the genomic DNA. | 2. Isolate the shuttle vectors containing the 100 kb intermediates from yeast.<br>3. Release the intermediates by enzyme digestion.<br>4. Transfer the intermediates into yeast. An *E.coli* -yeast shuttle vector was not needed at this step because one of the 100 kb intermediates contains yeast vector sequence. | |
| **Make the self-replicating *M. mycoides* JCVI-syn1.0 cells** | 4. *M. capricolum* receives the *M. mycoides* JCVI-syn1.0 genomic DNA.<br>5. *M. capricolum* proteins and RNAs replicate the *M. mycoides* JCVI-syn1.0 genomic DNA, transcribe and translate *M. mycoides* JCVI-syn1.0 genes.<br>6. *M. mycoides* JCVI-syn1.0 proteins and RNAs replicate its own genomic DNA, transcribe and translate its own genes. | 1. Provide the full length genomic DNA. | 2. Isolate the full length genomic DNA from yeast.<br>3. Transfer the DNA into *M. capricolum.*<br>7. Selectively kill the *M. capricolum* cells that do not contain the fostered *M. mycoides* JCVI-syn1.0 genomic DNA. | |

* The numbering in each row corresponds to the order of steps taken to accomplish the task in the leftmost column.

cells containing the synthetic genome, which has the antibiotic resistance gene, were isolated.

Note that the computer was only used, passively, to store genome sequence information. It did not generate a single molecule necessary for the survival or arrival of *M. mycoides* JCVI-syn 1.0 cells. Therefore, Dr. J. Craig Venter's claim that his group had created "the first self-replicating species we've had on the planet whose parent is a computer"[1] is misleading.

There are a few additional arguments that can be made against his statement that the *M. mycoides* JCVI-syn1.0 has a computer for its parent. The first argument concerns the composition and origin of the DNA sequence of the complete synthetic genome. In the final complete genome, 98.55% of the genome sequence was based on the natural *M. mycoides* genome sequence, 0.94% was the yeast cloning vector sequence, and 0.08% came from bacterial insertions (Figure 2). The last 0.43% was designed by humans in the form of watermarks, using a computer as a tool to convert the letters, numbers, and punctuation into DNA sequences. If one were to classify the parents of an organism on the basis of the providers of the genetic sequence, then we should consider the parent of *M. mycoides* JCVI-syn1.0 to be *M. mycoides* because it provides almost 99% of the genome sequence of *M. mycoides* JCVI-syn1.0.

It should also be noted that the human-engineered watermark sequences do not produce any functional products within the cell, so even the small percentage of sequences that were actually designed by humans using computers do not affect the cell with respect to function (except perhaps as a burden to maintain those sequences). The *M. mycoides* JCVI-syn1.0 cell is completely controlled by the *M. mycoides* DNA sequence. Phenotypically, the final synthetic *M. mycoides* JCVI-syn1.0 cells and the initial non-synthetic *M.* mycoides (with a yeast cloning vector inserted into its chromosome) are not distinguishable, so the watermarks were designed and placed into the final *M. mycoides* JCVI-syn1.0 synthetic genome to make it unique. Thus, an argument can be made that the fact that the starting natural cells and the final synthetic cells could not be distinguished *without* the inclusion of the watermarks demonstrates that the cells do not actually have computers for parents. The phenotypes of the synthetic *M. mycoides* JCVI-syn1.0 cells are virtually identical to that of natural *M. mycoides* cells—both phenotypes are derived from the unengineered genome, not the computer.

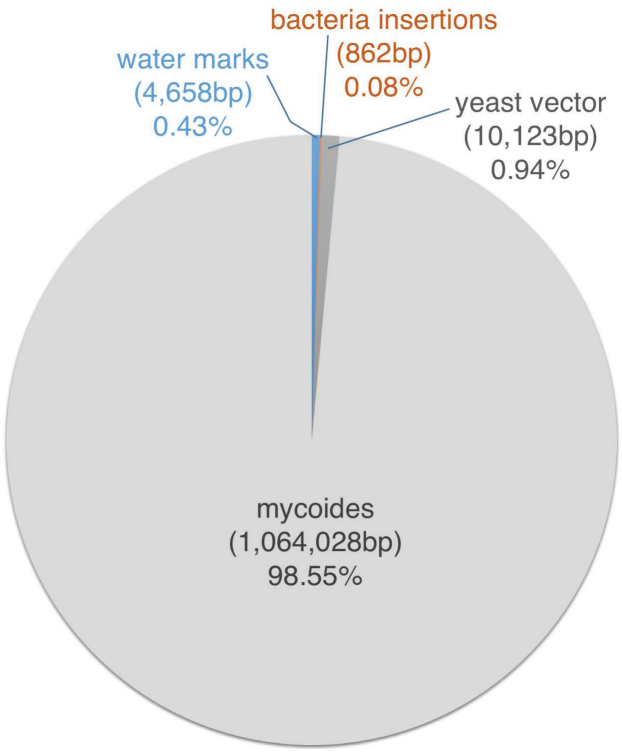


**Figure 2: A pie chart displaying the makeup of the *M. mycoides JCVI-syn1.0* genome.** **doi:**10.5048/BIO-C.2016.2.f2

The second argument against the idea that the *M. mycoides* JCVI-syn1.0 cells' parent was a computer is the fact that the cells could not be created without the help of four different organisms: humans, *E. coli,* yeast, and *M. capricolum* (Table 1). The real *M. mycoides* JCVI-syn1.0 genomic DNA was synthesized by *E. coli*, yeast, and *M. capricolum*, each using its own existing DNA replication machinery, except the initial 1080bp cassettes, which were chemically synthesized by humans. Of course, humans did all of the transfer of DNA to and from *E. coli*, yeast, and *M. capricolum*. In fact, computers were only involved in one step of the whole experiment (Table 1). Thus, the final genome could not be physically synthesized without the help of *E. coli*, yeast, or humans because they all played essential roles in different phases in the process of going from a DNA sequence on a computer to a complete physical strand of DNA. By itself, the *M. mycoides* JCVI-syn1.0 genomic information stored in the computer is completely incapable of producing any DNA, RNA, proteins, or any living cells.

The third argument against the idea that the *M. mycoides* JCVI-syn1.0 cells' parent was a computer concerns the activation of the *M. mycoides* JCVI-syn1.0 genome, the last essential step of generating an *M. mycoides* JCVI-syn1.0 cell. To activate the *M. mycoides* JCVI-syn1.0 genome, its genomic DNA, completed in yeast cells, had to be transferred into a live *M. capricolum* host cell. The host cell has a cell membrane and a full set of functional RNAs and proteins, including hundreds of kinds of *M. capricolum* proteins and multitudes of RNAs necessary to activate the *M. mycoides* JCVI-syn1.0 genome, *i.e.* to transcribe and to translate *M. mycoides* JCVI-syn1.0 genes. The *M. capricolum* RNAs and proteins are very similar to those encoded in the *M. mycoides* JCVI-syn1.0 genome because the genome donor *M. mycoides* is very similar to the *M. capricolum* genome host—on average at 91.5% nucleotide identity, except for those *M. mycoides* specific insertions [5]. *M. mycoides* and *M. capricolum* share more than 99% identity on their 16S ribosomal DNA (rDNA) as well as on their core proteome [6]. Consequently, the *M. capricolum* RNAs and proteins are not only able to replicate the synthesized *M. mycoides* JCVI-syn1.0 genome, but also to interpret and execute the

[1] http://www.ted.com/talks/craig_venter_unveils_synthetic_life.html

instructions encoded in this genome. Note that the yeast RNAs and proteins are not able to read these instructions; therefore, the *M. mycoides* JCVI-syn1.0 cells cannot be generated by the yeast cells, even though the yeast cells did assemble the *M. mycoides* JCVI-syn1.0 genome and were able to replicate and to pass it to their daughter cells. Indeed, yeast cells have a multitude of yeast RNAs and yeast proteins–many times more than the number of RNAs and proteins in the *M. capricolum* cells. However, it is not the number of the RNAs and proteins that matters but their identities, structures and functions. In fact, the only reason that the yeast cells could replicate the *M. mycoides* JCVI-syn1.0 genome was that a yeast origin of replication had been inserted in the *M. mycoides* JCVI-syn1.0 genome. Therefore, to make a *M. mycoides* JCVI-syn1.0 cell capable of replicating itself, the pre-existing cytosol of the *M. capricolum* recipient cells, including their RNAs and proteins, were essential, both to replicate the *M. mycoides* JCVI-syn1.0 genome and to read, interpret, and execute its coded instructions. And, of course, the recipient cell had to be alive. Nothing would have happened if the *M. mycoides* JCVI-syn1.0 genome was transferred into a dead cell.

Who are the parents of *M. mycoides* JCVI-syn1.0 cells? How are we to judge? What criteria shall we use? Shall we judge based on the genome sequence? Then the parents should be *M. mycoides* cells; they provided the template for 99% of the total JCVI-syn1.0 genomic DNA sequence and 100% of the functional DNA sequences. Shall we judge based on the source of the physical genome? Then the parents should be *E. coli* and yeast; since they generated the first genomic DNA of JCVI-syn1.0. Shall we judge based on the molecular machinery that created the first JCVI-syn1.0 cell? Then the parents should be the *M. capricolum* cells; since they provided all of the molecules, including the RNAs, proteins, and lipids, that not only made the functional JCVI-syn1.0 genome, but also interpreted and executed the instructions encoded in it and eventually made a JCVI-syn1.0 cell that was capable of self-replicating. Shall we judge based on the designer of the JCVI-syn1.0 genome? Then the parents should be the human intellects of the Venter team. They designed the sequence of the genome of JCVI-syn1.0 and all the experimental steps to synthesize the JCVI-syn1.0 cells and carried out the experiments, yes, with the help of computers, as well as *M. mycoides*, *E. coli*, *S. cerevisiae*, and *M. capricolum*.

Indeed, the Venter team did design and did carry out all the experimental steps. Without their direct and consistent intervention, none of these steps could have occurred naturally. No cloned DNA could have been reproduced in *E. coli* without an *E. coli* origin of replication being inserted by humans; no cloned DNA could have been generated within the yeast without the artificially introduced origin of replication of *S. cerevisiae*. In addition, DNA could not have been isolated from the large number of cells without the artificially introduced bacterial selectable genes or the yeast selectable genes.

Nonetheless, the Venter team is responsible for only 0.43% of the JCVI-syn1.0 genome sequence in the form of those four watermarks. Furthermore, none of the watermarks provide any functional benefit for the survival and propagation of the JCVI-syn1.0 cells; all of the functional DNA sequences, gene-coding or not gene-coding, came from inherent information present in existing natural organisms.

All things considered, regardless of which criteria one chooses to use in order to define what constitutes the actual parent for the *M. mycoides* JCVI-syn1.0 cells, the computer would be the least plausible candidate. It was just a place that was used by humans to store the sequences in transit. The sequence on a computer will not give birth to even a single DNA, RNA, or protein molecule of any cell.

Who are the parents of *M. mycoides* JCVI-syn1.0 cells? How can we know? How shall we judge? What criteria shall we use? Note that two parts are necessary to make a self-replicating *M. mycoides* JCVI-syn1.0 cell, not only a functional genome, but also appropriate proteins and RNAs to activate the genome. In fact, the experiments of the Venter team demonstrate that only a live, functional cell can self-replicate; its genome, proteins, or RNAs cannot do so by themselves. Genetically and phenotypically speaking, *M. mycoides* are the parents of *M. mycoides* JCVI-syn1.0 cells since *M. mycoides* provided the template for 99% of the total JCVI-syn1.0 genomic DNA sequence and 100% of its functional DNA sequences, and *M. mycoides* and *M. mycoides* JCVI-syn1.0 are phenotypically identical. However, it is the *M. capricolum* cells that synthesized both the genome and the cytoplasm of JCVI-syn1.0 and made the first living JCVI-syn1.0 cells that were then able to self-replicate and, thus, were the immediate birth parents of the first JCVI-syn1.0 cells. Yet at the end of the cloning experiment, no trace of *M. capricolum* can be found in the JCVI-syn1.0 cells.

Based on the information provided above, it can be stated that humans, *M. mycoides*, *E. coli*, *S. cerevisiae*, and *M. capricolum* are *all* parents of the *M. mycoides* JCVI-syn1.0 cells since they were all vital in the creation of the first *M. mycoides* JCVI-syn1.0 cells.


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