Exploring a Novel Solution to Antibiotic Pollution in Daily Life through Synthetic Biology

Zilin Hong

Shanghai Guanghua Cambridge International School, Shanghai, China

jolinhong42@gmail.com

Keywords: Synthetic biology, tetracyline degradation, engineered E.coli, environmental pollution control, antibiotic resistance

Abstract: Tetracycline is a common antibiotic, however, its residues are really harmful to both human health and the environment. To solve this problem, this study developed a bioremediation method based on synthetic biology and put it into use. The goal was to break down tetracycline in the environment, so it tried to make modified E. coli produce CotA laccase on their surface. Firstly, this paper used molecular cloning techniques to successfully make a recombinant plasmid. This plasmid can produce the pGAS-CotA fusion protein, and this project later confirmed the plasmid's sequence was correct. After making sure the protein was being produced, this paper developed a tetracycline detection method based on ELISA, and it checked that this method worked. In the final degradation test, the bacterial strain the paper built showed a stable ability to break down low levels of tetracycline in the culture medium. This study shows a new possibility for using biology to clean up antibiotic pollution, and it also proves that synthetic biology could help solve environmental problems.

1. Introduction

Tetracycline is a commonly used broad-spectrum antibiotic in both medicine and animal farming [1]. However, its overuse and improper disposal can lead to environmental pollution and the rise of antibiotic-resistant bacteria, posing serious risks to both ecosystems and human health.

Synthetic biology is an emerging field that combines biology, engineering, and genetics to design and build new biological systems [2]. One promising application is using engineered microbes to break down environmental pollutants, including antibiotics [3].

Motivated by the growing problem of antibiotic pollution, this paper explored the use of synthetic biology to degrade tetracycline. Through this project, I aimed to find an innovative and practical solution to help protect our environment.

2. Project Design

CotA is a very powerful enzyme that can break down tetracycline, which is a kind of antibiotic[4]. By using some synthetic biology methods to make *E. coli* display this enzyme on its surface, if that works, then the *E. coli* wouldn't just be a normal bacterium—it could actually help break down tetracycline in the environment. From this perspective, it could be a useful way to explore how biology can be used to solve problems like antibiotic pollution.

3. Knowledge Learning before Lab Work

Since this is my first time working on this kind of project, this paper started by researching more about this area's knowledge involved. That included getting a better understanding of synthetic biology and also the specific steps of the experiment.

First of all, get a deeper understanding of synthetic biology. It came from genetic engineering, but compared to that, synthetic biology focuses more on carefully controlling gene expression—for example, making sure a gene only turns on under certain conditions [5]. It's kind of like how you can only unlock your phone (gene expression) when you put in the right password (the specific condition).

  DOI: 10.25236/iwmecs.2025.034

Secondly, get a better idea of how a synthetic biology project is built. It's kind of like how we use raw materials like iron and steel to design tiny parts such as diodes and resistors, then put them together into small circuits, and later connect many small circuits into a big system. Finally, we wrap it all in a case, and it becomes a phone. In synthetic biology, DNA is the raw material, while things like promoters, ribosome binding sites, and genes are the small parts [6]. When we put them together, they form a "small circuit," which is the plasmid. After putting the plasmid into *E. coli*, we actually get an artificial biological system (like the phone), and it can start working and be tested. It can be seen in Figure 1.

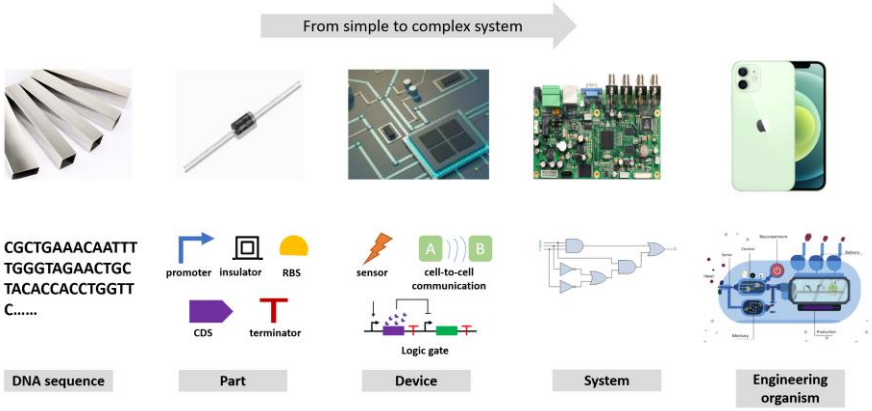

Figure 1 From DNA sequence to engineering organism in synthetic biology.

Also, to better research this area, it is necessary to continue learning about one of the core techniques in synthetic biology—molecular cloning, and study PCR technology. In terms of PCR, it stands for Polymerase Chain Reaction [7], which is really cool. It's like a "DNA copy machine." Even if you only start with a super tiny amount of DNA, PCR can make millions of copies in just a few hours. As shown in Figure 2, the PCR amplification process proceeds through repeated cycles of denaturation, annealing, and extension. The process goes through cycles of heating and cooling: first the DNA strands split apart, then primers attach, and finally an enzyme makes the new DNA [8]. What amazes people is that with PCR, scientists can do so many things—like testing for viruses, studying genes, or even solving crimes. In other words, it makes people feel that biology is not only about cells and organisms, but also about powerful tools that let us explore the invisible world.

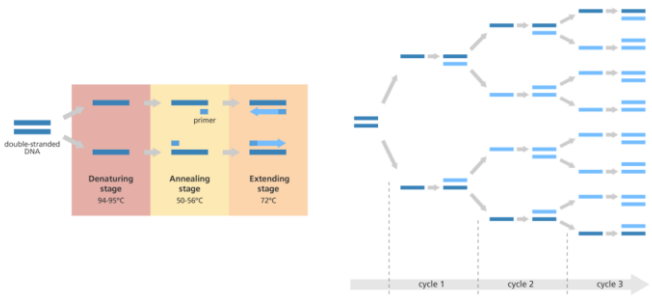

Figure 2 PCR amplification process with cycles of denaturation, annealing, and extension.

This paper also digs into a lot of molecular cloning strategies. Molecular cloning is like "cutting and pasting" pieces of DNA to build something new. Among the different methods, Gibson Assembly is special. As shown in Figure 3, unlike the older ways that need restriction enzymes to cut DNA at certain spots, Gibson Assembly can join several DNA fragments together in one step, almost like snapping Lego blocks into place [9]. What makes it cool is that the overlapping ends of the DNA fragments help them fit together, and then enzymes finish the job. This method feels faster and more flexible, because you can design the DNA pieces the way you want and combine them without leaving extra "scars." It demonstrates how powerful modern biology tools are for creating new genetic systems.

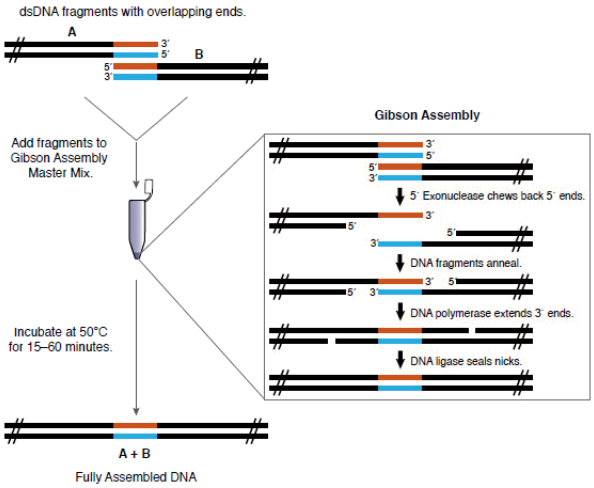

Figure 3 Gibson Assembly process for joining DNA fragments with overlapping ends.

In addition, it is significant to learned about gene expression and knowledge related to bacterial plasmids. After learning these, this paper has built up enough knowledge and have started working on project design and execution.

4. A Small Project Training before Lab Work

To prepare for later laboratory projects, the paper first tried a simple case: using molecular cloning techniques to make *E. coli* express the tspurple gene, which produces a purple protein. As a result, the successfully cloned *E. coli* would turn purple. The plasmid map is shown as follows in Figure 4:

Figure 4 Plasmid map of pTspurple showing major functional elements.

Table 1 presents the PCR process steps. By utilizing the reaction system shown in Table 2, the reaction process depicted in Table 3 is ultimately obtained.

Table 1. PCR process steps.

Step	**Description**
Denaturation	Heat to 98 ℃, the DNA unwinds and becomes single-stranded.
Denaturation	Cool down to 60–65 ℃, allowing primers to specifically bind to the DNA template.
Extension	Raise the temperature to 72 ℃, where DNA polymerase synthesizes double-stranded DNA using dNTPs as substrates.
Cycling	DNA is exponentially amplified, doubling with each cycle.

Table 2. Reaction system.

Name	**Volume (μL)**
ddH_2O	19
2 ×Phanta Mix	25
DNA Template	2
8.6 J23-ts-BB-R	2
8.6 J23-ts-BB-F	2

Table 3. PCR process.

Step	**Temperature (℃)**	**Time (s)**	**Cycles**
Pre-denaturation	98	30	1
Denaturation	98	10	35
Annealing	60	5	
Extension	72	60	
Final Extension	72	60	1
Hold	16	∞	1

Through the agarose gel electrophoresis experiment, it successfully amplified the plasmid backbone and the tspurple gene.

Agarose gel is like a "jelly" used to separate DNA. It is made by dissolving agarose powder in buffer and then letting it cool down to form a clear, jelly-like block. Since DNA molecules carry a negative charge, they move toward the positive side when electricity is applied. The agarose gel works like a "sieve": big DNA fragments move more slowly, while small ones move faster. This way, we can separate DNA by size.

In experiments, agarose gel electrophoresis is often used to check DNA—for example, to see if a PCR worked or if the DNA is the right size. When the gel is placed in an imaging system, the DNA shows up as glowing bands, kind of like bright little "roads," which makes it easy (and fun) to analyze.

The electrophoresis results are in Figure 5, and it successfully amplified two gene fragments:

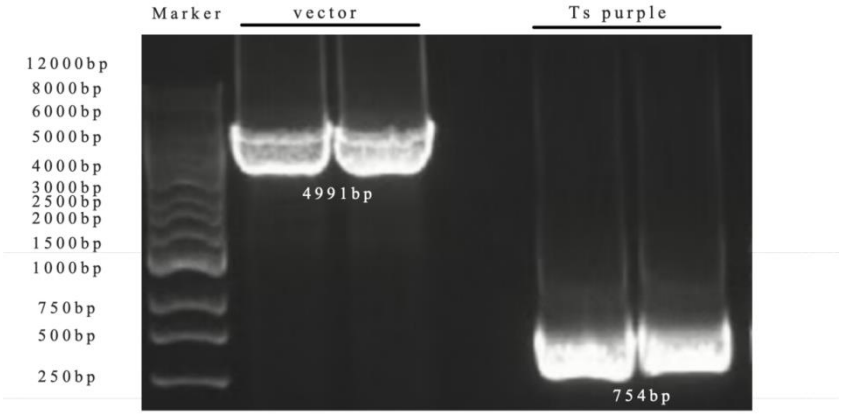

Figure 5. Agarose gel electrophoresis results show amplified vector and tsPurple gene fragments.

Since the DNA was embedded in the agarose gel at this stage, it is necessary to release it for further use. Therefore, it used an agarose gel DNA recovery kit to extract the DNA. After recovering the DNA, it performed fragment assembly using a Gibson-based homologous recombination kit, and then transformed the recombinant DNA into *E. coli*, as shown in Figure 6. The result showed that the *E. coli* colonies turned purple, indicating that it has successfully mastered the technique of molecular cloning.

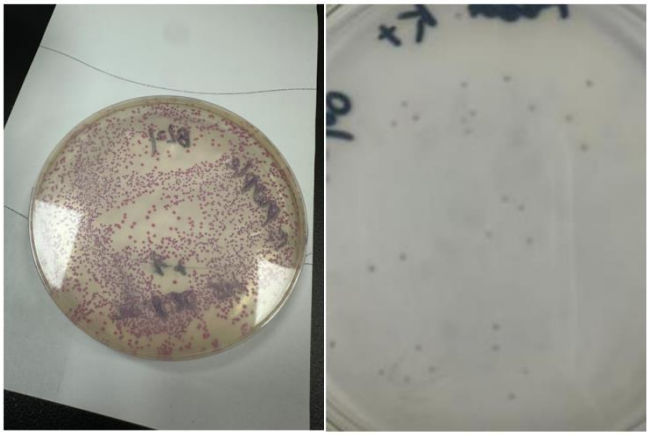

Figure 6. Colony morphology of transformed E. coli.

5. Project Design and Carryout

CotA is a very powerful enzyme that can break down tetracycline, which is a kind of antibiotic. The approach the paper used is designed to enable the engineered bacteria to function as a live biocatalyst for environmental remediation of antibiotic pollution. If that works, then the *E. coli* wouldn't just be a normal bacterium—it could actually help break down tetracycline in the environment. This could be an effective way to explore how biology can be used to solve problems like antibiotic pollution.

First, get the genes for CotA and pGAS and put them into an E. coli expression vector, pET28A. After inducing expression, it checked whether the proteins were made using gel electrophoresis, and here's what the study did:

Mixed 200 μL Tris-HCl (pH 8.0) with 200 μL 2× SDS-PAGE loading buffer; Took 20 μL of the bacteria mixture, boiled it in hot water for 5 minutes; Spun it down at 13,000 g for 30 minutes, used the supernatant, loaded 20 μL, and ran the gel (90 V for 90 minutes + 120 V for 20 minutes); Stained with Coomassie blue for 15 minutes, then washed in destaining solution and left overnight.

Basically, SDS makes proteins lose their shape and gives them a negative charge. The gel works like a sieve: smaller proteins move faster, bigger ones move slower, so you can tell them apart by size. DTT helps break disulfide bonds so you can see the protein subunits.

In the end, the study found that CotA didn't express much (on the left side), while pGAS expressed a lot more (on the right side). Figure 7 shows the SDS-PAGE analysis.

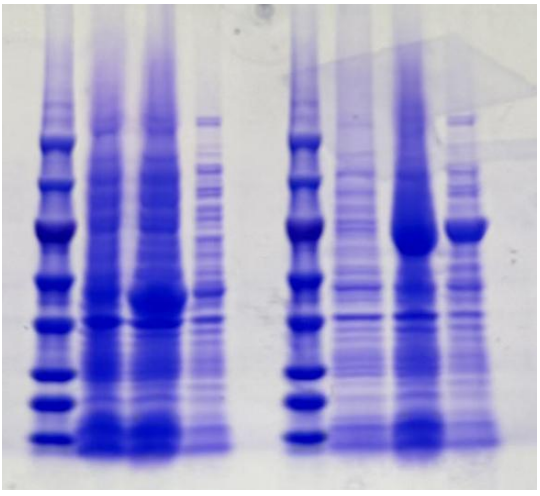

Figure 7. SDS-PAGE analysis.

Although CotA shows poor expression, the advisor noted that the strong expression of pGAS may enhance it after fusion with CotA, so we can consider proceeding to the next step. It designed these

primers to construct plasmid expressing this system. The specific primers used for plasmid construction are summarized in Table 4. After plasmid construction, the correctness of the recombinant plasmid pET-pGSA-G4S-2x-product was verified by sequencing, as illustrated in Figure 8.

Table 4. Primers used for plasmid construction.

Primer name	**Sequence (5′–3′)**
pGSA-BB-R	GCATCCACAAATTTTTCCAGGGTCATGCTACTGCTACCGCTACT GCTACCTTTACTCTTTAATTTATCACTATGATCAATGTCAAACG
CotA-F	TGACCCTGGAAAAATTTGTGGATGC
CotA-R	TTTATGCGGATCGGTAATATCCATCGG
pGSA-BB-F	ATTACCGATCCGCATAAACACCACCACCACCACCACTGAGATC CGGCTGCTAACAAAGC

Figure 8. Sequencing confirmation of the recombinant plasmid pET-pGSA-G4S-2x-product.

And after molecular cloning, the study confirmed the success of the construct by sequencing. The blue part shows the sequencing result, which matched the expected map, shown as Figure 9:

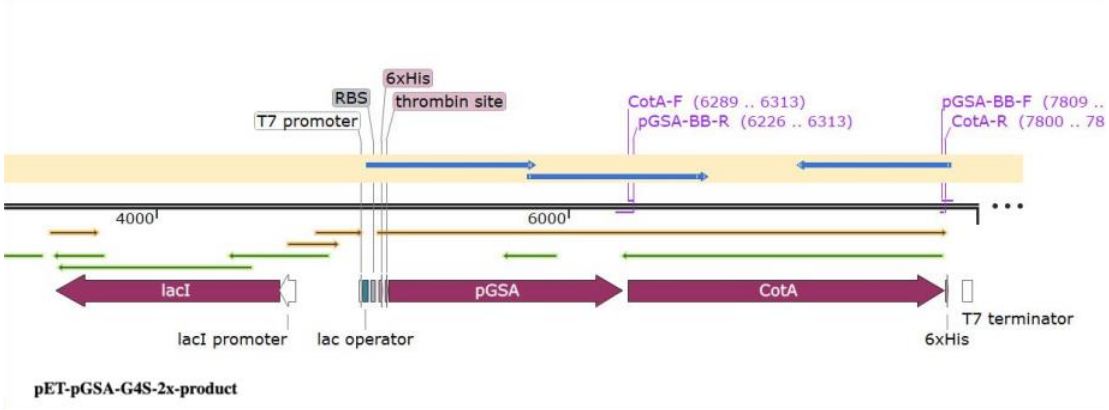

Figure 9. Linear schematic representation of sequencing results.

After that, this paper induced the expression of this fusion protein and tested its ability to degrade

tetracycline. Once the samples were prepared, it began looking for suitable downstream detection methods. Although physical and chemical methods such as mass spectrometry can provide very precise results, I thought that since my focus is on an environmental problem, it would be best to use a fast and convenient method for direct detection. This would not only be useful for my project, but also practical for detecting actual tetracycline residues in the environment. After doing some research, I decided to use ELISA for testing.

ELISA, which stands for Enzyme-Linked Immunosorbent Assay, is a popular method for detecting proteins, antigens, or antibodies. What makes it really useful is not just its accuracy, but also how convenient and practical it is compared to other lab techniques. Unlike heavy equipment like mass spectrometers that require specialized labs and trained technicians, ELISA can be done with a simple plastic plate, some reagents, and basic lab tools. It doesn't take a huge setup, and results come fairly quickly. Because the reaction produces a visible color change, the readout is easy to understand—sometimes even with portable plate readers that can be used outside big labs. This makes ELISA especially valuable in fieldwork and environmental testing, where scientists need to check samples on-site, or in hospitals where quick diagnostic tests are necessary. Its combination of speed, portability, and user-friendliness has made it one of the most widely used methods for real-world applications.

But since ELISA has a lot of steps, this study wanted to make sure it could test samples correctly. So, as practice, it first used pure milk with a known concentration of tetracycline (added tetracycline into store-bought milk) and ran it with a commercial ELISA kit.

The ELISA experiment was performed as follows. First, the sample dilution buffer was prepared by diluting the stock buffer 1:19 with deionized water. Next, the wash buffer was prepared in the same way. Then, 100 μL of fresh milk was taken into a 2 mL tube, mixed with 900 μL sample dilution buffer, and vortexed for 20 seconds. From this mixture, 20 μL was taken for testing. The strips were inserted into the plate holder, and duplicates were set for accuracy. Unused strips were sealed and stored at 2–8 °C. Subsequently, 20 μL of each standard/sample solution was added to the wells, followed by 50 μL of enzyme conjugate solution and 50 μL of antibody solution. The plate was covered, shaken gently for 10 seconds, and incubated at 25 ℃ in the dark for 30 minutes. After incubation, the liquid was removed, and each well was washed four times with 260 μL wash buffer, soaking for 15–30 seconds each time. After washing, the wells were dried on absorbent paper. Then, 100 μL substrate AB mix (A and B mixed 1:1) was added to each well within 5 minutes. The plate was covered again, shaken gently for 10 seconds, and incubated at 25 ℃ in the dark for 15–20 minutes. Finally, 50 μL stop solution was added to each well, shaken gently for 10 seconds, and the result was recorded.

This kit comes with standards, and the manual shows their corresponding sensitivities (ppb). I first ran ELISA with the standards and found that there were some abnormal results at 1.35 ppb and 4.05 ppb (as shown in blue). For the rest, the data showed a trend where absorbance went down as ppb increased. After thinking about it, I realized the problem might have been from mistakes during my operation—like adding reagents twice or forgetting to add the antibody solution. The detailed results are summarized in Table 5.

Table 5. ELISA results.

Kit Sensitivity (ppb)	**Absorbance**
0	2.958
0.15	2.740
0.45	1.297
1.35	4.345
4.05	3.330
12.15	0.238

After learning from those mistakes, it tested the pure milk samples that was prepared with gradient dilutions of tetracycline. This time, the trend looked normal, which shows that I've already gotten the hang of this technique. The ELISA results of tetracycline detection are shown in Table 6.

Table 6. ELISA results of tetracycline detection.

Sample	Absorbance
Original	0.017
1/2×	0.109
1/10×	0.113
1/100×	0.210
1/1000×	0.224

Next, we added a low concentration of tetracycline (2 ng/mL) into LB medium and used the engineered bacteria I constructed earlier for degradation. After that, we centrifuged the culture to collect the bacterial pellet, took the supernatant, and tested for tetracycline. We found that it had a certain effect, and the results are shown in Table 7.

Table 7. ELISA results of tetracycline degradation.

Sample	Absorbance
No treat-1	0.210
No treat-2	0.189
No treat-3	0.197
Treat-1	0.234
Treat-2	0.256
Treat-3	0.223

6. Limitation of this Project

In this study, I successfully achieved the degradation of tetracycline using *E. coli*. However, in the protein expression experiments, it was observed that when pGSA and CotA were expressed separately, pGSA showed relatively strong expression, whereas CotA exhibited weak expression. I hypothesize that the fusion expression of pGSA-CotA stabilized the CotA protein, thereby enhancing its expression and ultimately enabling the achievement of the intended goal. In other words, further engineering and optimization may improve CotA expression, leading to even more effective outcomes.

In addition, later on I might need some extra supporting hardware, either to keep my engineered *E. coli* inside the device, or to add an extra "suicide switch" to prevent the bacteria from escaping into environments outside of what we designed. This would help improve its potential for real-world applications.

7. Conclusion

Overall, in this research project, I tried my best to address the increasingly serious issue of antibiotic pollution by proposing a synthetic biology-based solution for antibiotic degradation. I successfully demonstrated the degradation of antibiotics using this method. This approach offers several key advantages.

Firstly, use of E. coli as a host organism. I employed Escherichia coli (E. coli) as the chassis organism due to its high replication rate, ease of cultivation, and reusability. These characteristics make it a cost-effective choice for antibiotic degradation on a larger scale.

Secondly, efficient detection through ELISA. To monitor antibiotic levels, I proposed using the ELISA (Enzyme-Linked Immunosorbent Assay) method. This technique is not only efficient but also suitable for on-site detection, making it highly practical in real-world scenarios.

Throughout this research journey, I gained hands-on experience with various laboratory techniques and deepened my understanding of synthetic biology and its transformative impact on our world. More importantly, I was able to take initiative and actively contribute—albeit modestly—to both scientific progress and the improvement of our daily lives.

References

[1] Daghrir, R. and Drogui, P. (2013) Tetracycline Antibiotics in The Environment: A Review. Environmental Chemistry Letters, 11(3), 209–227.

[2] Danchin, A. and de Lorenzo, V. (2008) Synthetic Biology: Discovering New Worlds and New Words. EMBO Reports, 9(9), 822–827.

[3] Singh, J.S., Abhilash, P.C., Singh, H.B., Singh, R.P. and Singh, D.P. (2011) Genetically Engineered Bacteria: An Emerging Tool for Environmental Remediation and Future Research Perspectives. Gene, 480(1–2), 1–9.

[4] Zhang, C., You, S., Zhang, J., Qi, W., Su, R. and He, Z. (2020) An Effective In-Situ Method for Laccase Immobilization: Excellent Activity, Effective Antibiotic Removal Rate and Low Potential Ecological Risk for Degradation Products. Bioresource Technology, 308, 123271.

[5] Engstrom, M.D. and Pfleger, B.F. (2017) Transcription Control Engineering and Applications in Synthetic Biology. Synthetic and Systems Biotechnology, 2(3), 176–191.

[6] Genewiz (2025) A Beginner's Guide to Artificial Dna Synthesis. Genewiz.Com. Available at: https://blog.genewiz.com/a-beginners-guide-to-artificial-dna-synthesis.

[7] Nikoomanzar, A., Chim, N., Yik, E.J. and Chaput, J.C. (2020) Engineering Polymerases for Applications in Synthetic Biology. Quarterly Reviews of Biophysics, 53, e6.

[8] Avantor (2025) Avantor is Setting Science in Motion for a Better World. Avantorsciences.Com. https://www.avantorsciences.com/uk/en/support/knowledge-center/pcr-cycling-process.

[9] Oswald, N. (2018) Cloning Methods: Five Different Ways to Assemble. Bitesize Bio. https://bitesizebio.com/26961/cloning-methods-5-different-ways-to-assemble/.