Gibson Assembly

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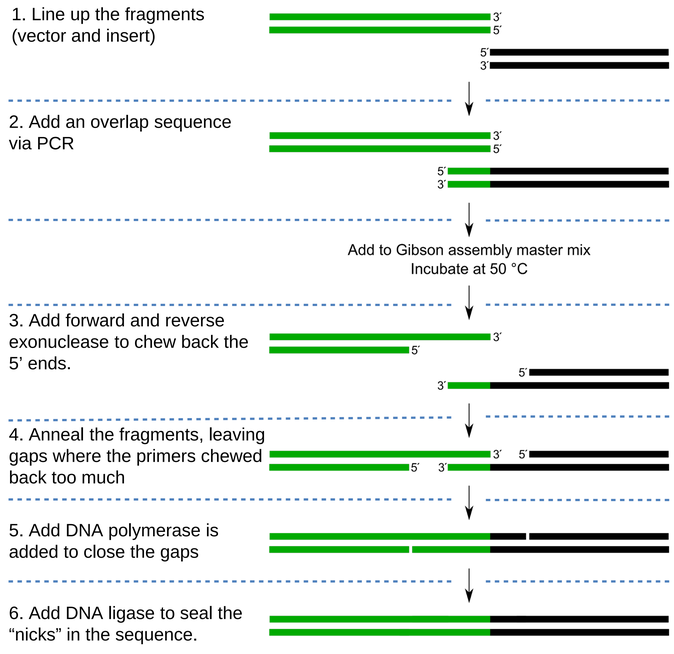
ABSTRACT

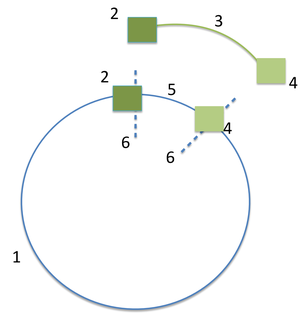
For many years now, biologists have been cloning recombinant DNA using older, more conventional restriction enzyme/ligation methods, but recently, Dr. Daniel G. Gibson discovered a new method for replicating segments of DNA by using a small number of components and just a few manipulations.

In his paper, entitled “Enzymatic assembly of DNA molecules up to several hundred kilobases”, Dr. Gibson describes his discovery as an “isothermal, single-reaction method for assembling multiple overlapping DNA molecules by the concerted action of a 5’ exonuclease, a DNA polymerase and a DNA ligase. In layman’s terms, that means that he discovered a single-step reaction that is only performed at one temperature (50º C) and only requires 5’ exonuclease (forward and reverse primers to provide overlap during the replication of the DNA strand) as well as DNA polymerase and DNA ligase, two molecules used in every DNA replication process to add bases and smooth over kinks in the strands

GIBSON ASSEMBLY



SCHEMATIC



Step 1: Mix the Vector (1 in the schematic) and Insert (3) in a tube with water, DNTP and buffer, as well as the forward and reverse primers (2 and 4).

Step 2: Add DNA Polymerase to break the vector DNA at the spot where you want the primers to begin adhering (6)

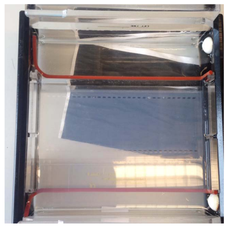
Step 3: Incubate in the PCR Machine, to allow the primers to correctly combine the vector and insert.

Step 4: Run a gel electrophoresis to separate the DNA strands by size to see if the primers successfully annealed the insert to the vector.

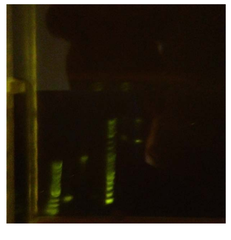
Process

Combine all the ingredients of step 1 while still at my station.

Incubate the mixtures in the PCR machine for 3-4 hours



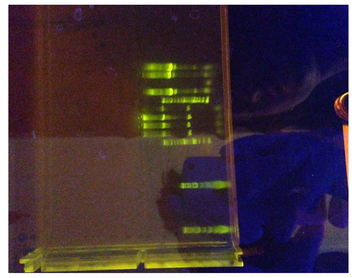
Run the incubated solutions through a gel, to sort them by size (relative length in kilobases)



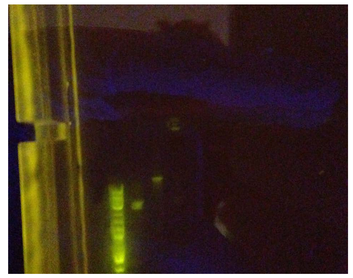
Chck the results under UV light and determine whether the cloning was successful

Effectiveness of the primers

While conducting these experiments, my mentor and I realized that there is a very narrow temperature margin where the primers were able to effectively anneal to the vector in the little time it could spend in the incubator. We were forced to conduct several small scale reactions and incubate them all at different temperatures in the incubator while still staying in the bounds of the range that the primers were designed to work in.



After running 8 different temperatures side by side, we compared the results



We then took the three best temperatures and ran them again to determine which we would choose for the final, real reaction

CONCLUSIONS

Scientists have been cloning recombinant DNA for over 40 years, but only recently have they started doing so in an efficient and quick manner. Dr. Daniel G. Gibson revolutionized this field by discovering a new method that uses only one reaction, and very few reactants to reach the same products. His discovery has greatly revolutionized this field of biology and he will most likely win a Nobel Prize in the years to come.

In our own applications we combined KanR with JDE131 by using Gibson assembly and we came to the conclusion that our methods successfully combined the two sequences.

FUTURE RESEARCH

I hope to be able to pursue this field of biology in my undergraduate studies, potentially at UCSD and at the same lab. I think that this field is only getting bigger and it will eventually provide ground-breaking information on the human genome and other moral-biological clashes.

WORKS CITED

“Enzymatic assembly of DNA molecules up to several hundred kilobases” by Daniel G. Gibson (among others)

“From cell differentiation to cell collectives: bacillus subtilis uses division of labor to migrate” by Jordi van Gestel (among others)