Your facebook link goes to the 2016 iGEM team page

What is the Gibson Assembly?

Gibson assembly is a useful molecular cloning technique published in 2009 by Dr. Daniel Gibson and his colleagues. This technique allows for the possibility of assembling up to 15 different DNA fragments in a single in vitro reaction, which allows for more efficient use of time and resources when compared with conventional cloning techniques. Despite its efficient process, the Gibson Assembly deemed to be expensive because it requires the use of a costly enzyme, DNA ligase.

What is ~~the~~ Gibson Assembly?

… and his colleagues (Gibson, D.G., et al. (2009) *Nat.* Methods 6, 343-345).

This technique allows for the assembly of up to 15 different DNA fragments in a single *in vitro* reaction.

… Gibson assembly is an expensive cloning procedure because it requires high amounts of Taq DNA ligase.

What are we aiming to do?

Escherichia coli is a model organism that has been developed into a useful tool for researchers to express genes of interest due to their ease of genetic manipulation and high replication rate. We believe that we can use engineered E. coli to overcome the high cost of using Gibson assembly method. Theoretically, two of the three genes required to encode for the proteins required in Gibson Assembly could be inserted into a duet vector plasmid and the third into a single plasmid vector to be transformed into E. coli. These E. coli strains harboring the Gibson Assembly machinery will then naturally replicate and the plasmids that contain the genes for the 5'-exonuclease, DNA polymerase, and DNA ligase will be expressed. Afterwards, cell lysate can be mixed with a plasmid backbone and DNA fragments of interest. In theory, this should produce the same combination effect as the traditional Gibson assembly method.

** Remember that E. coli is written in italics, as *E. coli*

Also, you didn't put the genes in a duet vector, and you didn't pursue the exonuclease. Can you instead describe what you actually aimed to do?

You should name the proteins accurately, as their source organisms are important. T5 Exonuclease. Phusion DNA polymerase. Taq DNA ligase.

Background

Gibson Assembly is a revolutionary method for assembling multiple linear DNA fragments (original paper link here) by Dr. Daniel Gibson at the J. Craig Venter Institute in 2009. The multiple overlapping DNA fragments can be joined by a single reaction regardless of the fragment length, which adds to the versatility of the method. By adding the three different enzymes (5' exonuclease, DNA polymerase, and DNA ligase), a fully ligated double-stranded DNA molecule is acquired. This method is proven to be efficient due to the ease of the reaction – needing only one tube of reaction – and the effectiveness of the reaction: no scars on the ligated DNA, non-selective compatibility of DNA fragments, no specific restriction sites needed.

(original paper link here) should be fixed
Again call the enzymes by their proper full names. There is a reason those enzymes are used.

… of DNA fragments, and no specific restriction sites are needed.

Preparation before undergoing Gibson Assembly reaction

1. Primer Design In designing the primer, adjacent segments in the plasmid should have identical sequences on the ends; the insert sequence and the vector sequence should be compatible with each other. These identical sequences can be created via PCR with primers containing a 5' end identical to the adjacent segment and a 3' that would anneal to the target sequence. Also, an effective amount of 60 bp primers might be more effective due to the more targeted approach by the enzymes. Once a preferred primer design has been achieved, amplify the amount of primer DNA by PCR.
2. Check size and yield from the PCR product. When the product is found to be impure, consider using gel purification protocol to rinse the impurities.

Undergoing the Gibson Assembly Reaction

For our method, we used NEB's Gibson Assembly Master Mix. The Master Mix consists of three enzymes in a single buffer:

1. T5 exonuclease: chews back the 5' end of the DNA to create a 3' overhang, so the complementary strand would anneal to each other.
2. Phusion DNA Polymerase: incorporates nucleotides to fill in the gaps in the annealed DNA fragment
3. Taq DNA ligase: joining the annealed DNA fragments and removing the 'nicks' and 'scars'.

Preparation before performing Gibson Assembly ~~reaction~~

Primer Design:

"Also, an effective amount of 60 bp primers might be more effective due to the more targeted approach by the enzymes" I don't know what that is supposed to mean. Are you trying to describe the efficiency of the reaction due to the size of the overlap? "Classic" cloning relied on the sticky ends created by restriction sites, which normally provide 4 bp overhang. Gibson Assembly improves this by providing a much larger overhang, where we generally design primers so a 20-30 bp overhang can be generated.

"Once a preferred primer design has been achieved" This language is odd. You should mention that your DNA fragments need to be prepared through restriction digest or PCR amplification using primers that generate compatible ends, which create these 20-30 bp overhang.

Check the purity and concentration of the PCR amplification and/or restriction digest. If the product is found to be impure, perform a gel purification to remove impurities.

Undergoing the Gibson Assembly Reaction: for this part, I would stay consistent with your verb tense "chews back … incorporates nucleotides… joining the annealed DNA" and I would remove the colons, and instead say something like "T5 exonuclease, which chews back…"

The method can simultaneously combine up to 15 DNA fragments based on sequence identity. It requires that the DNA fragments contain ~20-40 base pair overlap with adjacent DNA fragments. The appropriate amount for a 2-3 fragments reaction is a 0.02 – 0.5 pmol of fragments, as cloning efficiency is at its peak at 50-100 ng vectors with 2-3 times excess insert. Sample should then be incubated in a thermocycler at 500C for 15 minutes, then sample should be saved in –200C until further examination is made.

The appropriate amount of DNA, when combining 2-3 fragments in a Gibson Assembly reaction, is 0.02 – 0.5 pmol of total DNA. Cloning efficiency is best when 50 -100 ng of vector is used, with 2-3 equivalents of insert in a 20 µl reaction.

Please incubate samples at 50 ºC and -20 ºC, not 500C and -200C. Also, you shouldn't mention how to store DNA. Only mention that the assembly should be complete after 15 min.

Experiment Description

Once of the biggest obstacles to scientific advancement and discovery is the exorbitant monetary price for conducting small experiments. To put this in perspective, the purchase of one Gibson Assembly Kit for ten reactions is currently $185.00. For research that heavily depends on assembling different DNA fragments, the price can rise very quickly.

The aim of creating a homemade Gibson Assembly recipe was to drastically lower the prices of performing Gibson Assembly. The price could be lowered if the same outcome could be performed using unpurified enzymes. The unpurified enzymes would replace the purified enzymes commonly used in the Gibson Assembly master mix.

The inspiration for this project stems from Genentech's 1978 success story when the first successful production of human insulin in the laboratory was announced[1].

Scientists at Genentech used recombinant DNA techniques to produce a hybrid DNA molecule that contained the gene to produce human insulin. This recombinant plasmid was inserted into E. Coli cells for mass production of the human insulin protein hormone. Thus, the underlying process of our experiment is the same . However, we used a common recombinant DNA technique to our advantage.

The idea of our project can be summarized in one sentence "using Gibson assembly to make enzymes for Gibson Assembly."

To expand on this statement, the Gibson assembly cloning method was used to create constructs that contained the genes for DNA polymerase and DNA ligase. These constructs were created so that when they are transformed into DH5α cells, the local cell machinery would be used for high expression of the gene and produce an abundant number of DNA ligase and DNA polymerase enzymes.

Once colonies of the transformed cells grow, cell lysate will be made in buffer similar to the Gibson assembly buffer. Two tubes of cell lysate, one containing DNA ligase and the other containing DNA polymerase, will be added in ratios relative to each other to the reaction tubes. The ratio that yields results will be determined empirically. The background exonuclease naturally present in the lysate would account for the exonuclease needed for the reaction.

To verify that the isothermal reaction using unpurified enzyme worked, we transformed the allegedly assembled construct and then purify the DNA from the bacterial colonies. This purified DNA is sent for sequencing. If the returned matches the known sequence, we conclude that the isothermal reaction was successful.

[1] Press Releases. (1978, September 6). Retrieved October 29, 2017, from https://www.gene.com/media/press-releases/4160/1978-09-06/first-successful-laboratory-production-o

Same edits here as we talked about yesterday during your talk. *E. coli* and "Using Gibson Assembly to make enzymes for Gibson Assembly" Use the exact organism names.

… mass production of human insulin.

Your "Team" page has some of the text overlapping on your bio page

This years' Interlab Study directly addresses the one of the sources of imprecision indicated in the paper published in PLOS using data from the 2016 Interlab Study1. A source of imprecision identified while analyzing last year's data was the lack of use of standard protocols across teams for usage of instruments and analysis of data.

This year's Interlab … 2016 Interlab Study [1]. (your reference at the bottom of the page needs a 1) in front of it. I would change it to this:

1) Beal J, Haddock-Angelli T, Gershater M, de Mora K, Lizarazo M, Hollenhorst J, Rettbug R, iGEM Interlab Study Contributers. (2016) Correction: Reproducibility of Fluorescent Expression from Engineered Biological Constructs in *E. coli*. PLoS ONE 11(3): e0150182. pmid:26937966

Figure 1. The above fluorescein standard curve was constructed using the fluorescein measurements made from the TecanM1000 Pro. To achieve the linearity of the log scale standard curve, the fluorescein measurements at 50 and 25 were omitted. Although the optimal gain of the Tecan M1000 Pro was determined to be 62, the standard curve did not show a 1:1 slope behavior using the data points at 50 and 25 microMolar.

… the fluorescein measurements at 50 µM and 25 µM were omitted.

… data points at 50 µM and 25 µM.