Produce *E. coli gcvR* Promoter with Oligos

The goal is to have the workshop make this new promoter with BioBrick ends.
Will use oligo assembly method.

Desired sequence:

Need biobrick prefix end with **EcoRI sticky end**

```
                                                  -35
GGCATTAAAAGCAAGCAGACAGAACCGTTCTGATTGTTGTATGCATGTTTTTTTTATGC
 -10         +1                RBS
TTTCCTTAAGAACAACTCACCCCTTAAAGGAATAA
```

Need biobrick suffix end with **Spe I sticky end.**

```
5'GGCATTAAAAGCAAGCAGACAGAACCGTTCTGATTGTTGTATGCATGTTTTTTTTAT
GCTTTCCTTAAGAACAACTCACCCCTTAAAGGAATAA 3'
```

```
32-mer 5'-AATTCGCGGCCGCTTCTAGAGGGCATTAAAAG
56a-mer 5'-CAAGCAGACAGAACCGTTCTGATTGTTGTATGCATGTTTTTTTTATGCTTTCCTTA
28-mer 5'-AGAACAACTCACCCCTTAAAGGAATAAA
56b-mer 5'-CAACAATCAGAACGGTTCTGTCTGCTTGCTTTTAATGCCCTCTAGAAGCGGCCGCG
60-mer 5'-CTAGTTTATTCCTTTAAGGGGTGAGTTGTTCTTAAGGAAAGCATAAAAAAAACATGCATA
```

Analysis and Results

BioBrick Restriction Site Analysis

Sequence contains no BioBrick restriction enzymes.

Oligator Results

The 4 overlap areas have melting temperatures of 68.72°C, 64.33°C, 55.54°C, 59.94°C respectively.

Oligonucleotide Hybridization Map:

```
AATTCGCGGCCGCTTCTAGAG GGCATTAAAAGCAAGCAGACAGAACCGTTCTGATTGTTGTATGCATGTTTTTTTTATGCTTTCCTTAAGAACAACTCACCCCTTAAAGGAATAA A
    GCGCCGGCGAAGATCTC CCGTAATTTTCGTTCGTCTGTCTTGGCAAGACTAACAACATACGTACAAAAAAAATACGAAAGGAATTCTTGTTGAGTGGGGAATTTCCTTATT TGATC
```

Individual Oligonucleotides: DOWNLOAD

```
32-mer 5'-AATTCGCGGCCGCTTCTAGAGGGCATTAAAAG
56-mer 5'-CAAGCAGACAGAACCGTTCTGATTGTTGTATGCATGTTTTTTTTATGCTTTCCTTA
28-mer 5'-AGAACAACTCACCCCTTAAAGGAATAAA
56-mer 5'-CAACAATCAGAACGGTTCTGTCTGCTTGCTTTTAATGCCCTCTAGAAGCGGCCGCG
60-mer 5'-CTAGTTTATTCCTTTAAGGGGTGAGTTGTTCTTAAGGAAAGCATAAAAAAAACATGCATA
```

PCR Amplify from *E. coli* colony
developed by Mike Waters '10

One forward primer and three reverse primers specific for the ampicillin resistance gene present on all pSB1A2 plasmids were designed. Annealing temperature is 42° C.

The forward primer sequence chosen was: (5′ CAGTTACCAATGCTTAATCA ′3) box1, #65
Reverse primer 1 sequence was chosen to be (5′ GGCCAGATGGTAAGCCCTCC ′3) box1, #64
Reverse primer 2 sequence was chosen to be (5′ GCAGGACCACTTCTGCGCTC ′3) box1, #63
Reverse primer 3 sequence was chosen to be (5′ CTGTAGCAATGGCAACAAGTT ′3) box1, #62

The PCR of each pair of forward and reverse primer will yield PCR products of known dsDNA length: 100 bps, 200 bps, and 300 bps (Figure 10).