*Supplementary information for*

**Rapid Assembly of Multi-gene Constructs Using Molular Golden Gate Cloning**

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**Table of Contents:**

|  |  |
| --- | --- |
| Table S1. List of primers | 2 |
| Table S2. List of sequences for part plasmids | 4 |
| Figure S1. Optimizing conditions of Golden Gate cloning for a 4-piece assembly | 9 |
| Table S3: Results obtained from different cycle conditions | 10 |
| Figure S2: Decreasing efficiency of Golden Gate Assembly with increasing number of parts | 11 |
| Figure S3**:** Absorption spectra of *β*-carotene and lycopene standards. | 12 |

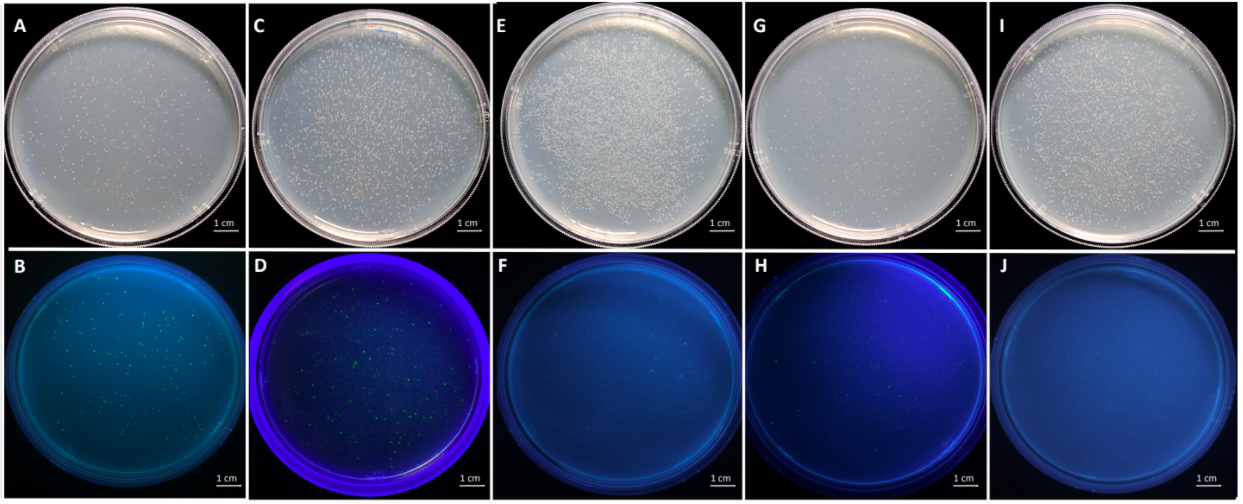
**Table S1:** List of primers

|  |  |  |
| --- | --- | --- |
| **Primers** | **Sequence (5’-3’)** | **Description** |
| BTS1 F | tttcgtctcgtcggggtctcgtatggaggccaagatagatgag | Forward primer to amplify *BTS1* from the yeast genome |
| BTS1-GGGS R | tttcgtctctccatagaaccaccacccaattcggataagtggtc | Reverse primer with a GGGS linker sequence to amplify *BTS1* from the yeast genome |
| ERG20 F | tttcgtctcgatggcttcagaaaaagaaattaggag | Forward primer to amplify *ERG20* from the yeast genome |
| ERG20 R | tttcgtctctggtcggtctccggatctatttgcttctcttgtaaactttgttc | Reverse primer to amplify *ERG20* from the yeast genome |
| crtE F | tttcgtctcgtcggggtctcgtatggattacgcgaacatcctc | Forward primer to amplify *crtE* from pLM494 plasmid |
| crtE R | tttcgtctctggtcggtctccggattcacagagggatatcggctag | Reverse primer to amplify *crtE* from pLM494 plasmid |
| crtI F | tttcgtctcgtcggggtctcgtatgggaaaagaacaagatcagg | Forward primer to amplify *crtI* from pLM494 plasmid |
| crtI R | tttcgtctctggtcggtctccggattcagaaagcaagaacaccaac | Reverse primer to amplify *crtI* from pLM494 plasmid |
| crtYB F | tttcgtctcgtcggggtctcgtatgacggctctcgcatattac | Forward primer to amplify *crtYB* from pLM494 plasmid |
| crtYB R | tttcgtctctggtcggtctccggattcactgcccttcccatc | Reverse primer to amplify *crtYB* from pLM494 plasmid |
| crtYB mut F | tttcgtctctacgctttctttgtcattcaaac | Forward primer to amplify second half of *crtYB* from pLM494 in order to introduce G247A mutation |
| crtYB mut R | tttcgtctcagcgtacttttcatatggaacatc | Reverse primer to amplify the first half of *crtYB* from pLM494 to introduce the G247A mutation |
| pENO2 F | tttcgtctcgtcggggtctcgaacggtgtcgacgctgcg | Forward primer to amplify the *ENO2* promoter sequence from the yeast genome |
| pENO2 R | tttcgtctcgggtcggtctcccatatattattgtatgttatagtattagttgcttgg | Reverse primer to amplify the *ENO2* promoter sequence from the yeast genome |
| pPDC1 F | tttcgtctcgtcggggtctcgaacgcatgcgactgggtgag | Forward primer to amplify the *PDC1* promoter sequence from the yeast genome |
| pPDC1 R | tttcgtctcgggtcggtctcccatatttgattgatttgactgtgttattttgc | Reverse primer to amplify the *PDC1* promoter sequence from the yeast genome |
| pPYK1 F | tttcgtctcgtcggggtctcgaacggaaagtttttccggcaagc | Forward primer to amplify the *PYK1* promoter sequence from the yeast genome |
| pPYK1 R | tttcgtctcgggtcggtctcccatatgtgatgatgttttatttgttttgattg | Reverse primer to amplify the *PYK1* promoter sequence from the yeast genome |
| pTIP1 F | tttcgtctcgtcggggtctcgaacgataagcttgatatcgaattcctgc | Forward primer to amplify the *TIP1* promoter sequence from the yeast genome |
| pTIP1 R | tttcgtctcgggtcggtctcccatattttattttatttagcagagggtatagttatg | Reverse primer to amplify the *TIP1* promoter sequence from the yeast genome |
| tTDH2 F | tttcgtctcgtcggggtctcgatcctaaatttaactccttaagttactttaatg | Forward primer to amplify the *TDH2* terminator sequence from the yeast genome |
| tTDH2 R | tttcgtctcgggtcggtctcccagcgcgaaaagccaattagtg | Reverse primer to amplify the *TDH2* terminator sequence from the yeast genome |
| tHSP26 F | tttcgtctcgtcggggtctcgatccagtgacctggctctatagtg | Forward primer to amplify the *HSP26* terminator sequence from the yeast genome |
| tHSP26 R | tttcgtctcgggtcggtctcccagcacgaggttagattccttcgttaaaac | Reverse primer to amplify the *HSP26* terminator sequence from the yeast genome |
| tADH2 F | tttcgtctcgtcggggtctcgatccgcggatctcttatgtctttacg | Forward primer to amplify the *ADH2* terminator sequence from the yeast genome |
| tADH2 R | tttcgtctcgggtcggtctcccagcatgagaaatatcgagggatacgattc | Reverse primer to amplify the *ADH2* terminator sequence from the yeast genome |
| tACS2 F | tttcgtctcgtcggggtctcgatccaatgagataaaatttcgc | Forward primer to amplify the *ACS2* terminator sequence from the yeast genome |
| tACS2 R | tttcgtctcgggtcggtctcccagccactaagtgcataaagtctttg | Reverse primer to amplify the *ACS2* terminator sequence from the yeast genome |
| ADE2 5’ hom F | tttcgtctcgtcggggtctcacaatgaaacttcatgctcgaaaaagatc | Forward primer to amplify a 500 bp sequence that serves as the 5’ homology arm during integration at the *ADE2* locus |
| ADE2 5’ hom R | tttcgtctcgggtcggtctcaagggtcattactatcattactaaatataccaactgttctagaatccatac | Reverse primer to amplify a 500 bp sequence plus 6 stop codons that would serve as the 5’ homology arm during integration at the *ADE2* locus and stop *ADE2* expression |
| ADE2 3’hom F | tttcgtctcgtcggggtctcagagtgctttggaagtactgaaggatc | Forward primer to amplify a 500 bp sequence that would serve as the 3’ homology arm during integration at the *ADE2* locus |
| ADE2 3’ hom R | tttcgtctcgggtcggtctcatcggctttcgcaagtttctagctc | Forward primer to amplify 500 bp sequence that would serve as the 3’ homology arm during integration at the *ADE2* locus |

All the primers are designed for cloning the parts into the entry vector (pYTK001)

**Table S2:** List of sequences for part plasmids

|  |  |
| --- | --- |
| Name | Sequence |
| pENO2 (promoter) | GTGTCGACGCTGCGGGTATAGAAAGGGTTCTTTACTCTATAGTACCTCCTCGCTCAGCATCTGCTTCTTCCCAAAGATGAACGCGGCGTTATGTCACTAACGACGTGCACCAACTTGCGGAAAGTGGAATCCCGTTCCAAAACTGGCATCCACTAATTGATACATCTACACACCGCACGCCTTTTTTCTGAAGCCCACTTTCGTGGACTTTGCCATATGCAAAATTCATGAAGTGTGATACCAAGTCAGCATACACCTCACTAGGGTAGTTTCTTTGGTTGTATTGATCATTTGGTTCATCGTGGTTCATTAATTTTTTTTCTCCATTGCTTTCTGGCTTTGATCTTACTATCATTTGGATTTTTGTCGAAGGTTGTAGAATTGTATGTGACAAGTGGCACCAAGCATATATAAAAAAAAAAAGCATTATCTTCCTACCAGAGTTGATTGTTAAAAACGTATTTATAGCAAACGCAATTGTAATTAATTCTTATTTTGTATCTTTTCTTCCCTTGTCTCAATCTTTTATTTTTATTTTATTTTTCTTTTCTTAGTTTCTTTCATAACACCAAGCAACTAATACTATAACATACAATAATA |
| pTIP1 (promoter) | ATAAGCTTGATATCGAATTCCTGCAGCCCCCAGTATAACTTCGTATAATGTACATTATACGAAGTTATTATTAGCGGCGCCGGGAAATCCAGCATATTCTCGCGGCCCTGAGCAGTAGGTGTCTCGGGCAGTCAATATTCCCAAAAAGAGCATCAGACGATCTGGTTATGGTTTTTCTTGACTATAACCTTAATTATGAGACTAATGTCTTCGGGAGGTCCCTTTTCCGATTTTCCGACTCTTTTCCGTTGAAGAATGTACTTGTGGTTTTGAATCCTACGGCAGTTATTGCGGCGGTTTGGCCCTTTCTTTCAAAGATTGTGATGGAAATAATTGATTGTTCCGGGAAATGTGTCTTATTTTCTAAAAGCATCTTTTTTTCTCTCCAATTCTTCGAGCTATTTCCAGTAAAGGAAAAAAAAGGTTTGCTGTAAGGGTGAATATGTCTCCAACCTCTTTGAGGTACTGCGTTGCTTCATTCACCATTTAATATAAATAGTACATTGGCAGCCCTCTTTCAAACGTCAATTATTCTCGCTTGCCTAACTTTGTTCGGACCGAAATTATAAAGGCATTCAATCAGTAACAATAATTGCTATTGCATAACTATACCCTCTGCTAAATAAAATAAAA |
| pPDC1 (promoter) | CATGCGACTGGGTGAGCATATGTTCCGCTGATGTGATGTGCAAGATAAACAAGCAAGGCAGAAACTAACTTCTTCTTCATGTAATAAACACACCCCGCGTTTATTTACCTATCTCTAAACTTCAACACCTTATATCATAACTAATATTTCTTGAGATAAGCACACTGCACCCATACCTTCCTTAAAAACGTAGCTTCCAGTTTTTGGTGGTTCCGGCTTCCTTCCCGATTCCGCCCGCTAAACGCATATTTTTGTTGCCTGGTGGCATTTGCAAAATGCATAACCTATGCATTTAAAAGATTATGTATGCTCTTCTGACTTTTCGTGTGATGAGGCTCGTGGAAAAAATGAATAATTTATGAATTTGAGAACAATTTTGTGTTGTTACGGTATTTTACTATGGAATAATCAATCAATTGAGGATTTTATGCAAATATCGTTTGAATATTTTTCCGACCCTTTGAGTACTTTTCTTCATAATTGCATAATATTGTCCGCTGCCCCTTTTTCTGTTAGACGGTGTCTTGATCTACTTGCTATCGTTCAACACCACCTTATTTTCTAACTATTTTTTTTTTAGCTCATTTGAATCAGCTTATGGTGATGGCACATTTTTGCATAAACCTAGCTGTCCTCGTTGAACATAGGAAAAAAAAATATATAAACAAGGCTCTTTCACTCTCCTTGCAATCAGATTTGGGTTTGTTCCCTTTATTTTCATATTTCTTGTCATATTCCTTTCTCAATTATTATTTTCTACTCATAACCTCACGCAAAATAACACAGTCAAATCAATCAAA |
| pPYK1 (promoter) | GAAAGTTTTTCCGGCAAGCTAAATGGAAAAAGGAAAGATTATTGAAAGAGAAAGAAAGAAAAAAAAAAAATGTACACCCAGACATCGGGCTTCCACAATTTCGGCTCTATTGTTTTCCATCTCTCGCAACGGCGGGATTCCTCTATGGCGTGTGATGTCTGTATCTGTTACTTAATCCAGAAACTGGCACTTGACCCAACTCTGCCACGTGGGTCGTTTTGCCATCGACAGATTGGGAGATTTTCATAGTAGAATTCAGCATGATAGCTACGTAAATGTGTTCCGCACCGTCACAAAGTGTTTTCTACTGTTCTTTCTTCTTTCGTTCATTCAGTTGAGTTGAGTGAGTGCTTTGTTCAATGGATCTTAGCTAAAATGCATATTTTTTCTCTTGGTAAATGAATGCTTGTGATGTCTTCCAAGTGATTTCCTTTCCTTCCCATATGATGCTAGGTACCTTTAGTGTCTTCCTAAAAAAAAAAAAAGGCTCGCCATCAAAACGATATTCGTTGGCTTTTTTTTCTGAATTATAAATACTCTTTGGTAACTTTTCATTTCCAAGAACCTCTTTTTTCCAGTTATATCATGGTCCCCTTTCAAAGTTATTCTCTACTCTTTTTCATATTCATTCTTTTTCATCCTTTGGTTTTTTATTCTTAACTTGTTTATTATTCTCTCTTGTTTCTATTTACAAGACACCAATCAAAACAAATAAAACATCATCACA |
| ERG20 (CDS) | ATGGCTTCAGAAAAAGAAATTAGGAGAGAGAGATTCTTGAACGTTTTCCCTAAATTAGTAGAGGAATTGAACGCATCGCTTTTGGCTTACGGTATGCCTAAGGAAGCATGTGACTGGTATGCCCACTCATTGAACTACAACACTCCAGGCGGTAAGCTAAATAGAGGTTTGTCCGTTGTGGACACGTATGCTATTCTCTCCAACAAGACCGTTGAACAATTGGGGCAAGAAGAATACGAAAAGGTTGCCATTCTAGGTTGGTGCATTGAGTTGTTGCAGGCTTACTTCTTGGTCGCCGATGATATGATGGACAAGTCCATTACCAGAAGAGGCCAACCATGTTGGTACAAGGTTCCTGAAGTTGGGGAAATTGCCATCAATGACGCATTCATGTTAGAGGCTGCTATCTACAAGCTTTTGAAATCTCACTTCAGAAACGAAAAATACTACATAGATATCACCGAATTGTTCCATGAGGTCACCTTCCAAACCGAATTGGGCCAATTGATGGACTTAATCACTGCACCTGAAGACAAAGTCGACTTGAGTAAGTTCTCCCTAAAGAAGCACTCCTTCATAGTTACTTTCAAGACTGCTTACTATTCTTTCTACTTGCCTGTCGCATTGGCCATGTACGTTGCCGGTATCACGGATGAAAAGGATTTGAAACAAGCCAGAGATGTCTTGATTCCATTGGGTGAATACTTCCAAATTCAAGATGACTACTTAGACTGCTTCGGTACCCCAGAACAGATCGGTAAGATCGGTACAGATATCCAAGATAACAAATGTTCTTGGGTAATCAACAAGGCATTGGAACTTGCTTCCGCAGAACAAAGAAAGACTTTAGACGAAAATTACGGTAAGAAGGACTCAGTCGCAGAAGCCAAATGCAAAAAGATTTTCAATGACTTGAAAATTGAACAGCTATACCACGAATATGAAGAGTCTATTGCCAAGGATTTGAAGGCCAAAATTTCTCAGGTCGATGAGTCTCGTGGCTTCAAAGCTGATGTCTTAACTGCGTTCTTGAACAAAGTTTACAAGAGAAGCAAATAG |
| BTS1- ERG20 (CDS) | ATGGAGGCCAAGATAGATGAGCTGATCAATAATGATCCTGTTTGGTCCAGCCAAAATGAAAGCTTGATTTCAAAACCTTATAATCACATCCTTTTGAAACCTGGCAAGAACTTTAGACTAAATTTAATAGTTCAAATTAACAGAGTTATGAATTTGCCCAAAGACCAGCTGGCCATAGTTTCGCAAATTGTTGAGCTCTTGCATAATTCCAGCCTTTTAATCGACGATATAGAAGATAATGCTCCCTTGAGAAGGGGACAGACCACTTCTCACTTAATCTTCGGTGTACCCTCCACTATAAACACCGCAAATTATATGTATTTCAGAGCCATGCAACTTGTATCGCAGCTAACCACAAAAGAGCCTTTGTATCATAATTTGATTACGATTTTCAACGAAGAATTGATCAATCTACATAGGGGACAAGGCTTGGATATATACTGGAGAGACTTTCTGCCTGAAATCATACCTACTCAGGAGATGTATTTGAATATGGTTATGAATAAAACAGGCGGCCTTTTCAGATTAACGTTGAGACTCATGGAAGCGCTGTCTCCTTCCTCACACCACGGCCATTCGTTGGTTCCTTTCATAAATCTTCTGGGTATTATTTATCAGATTAGAGATGATTACTTGAATTTGAAAGATTTCCAAATGTCCAGCGAAAAAGGCTTTGCTGAGGACATTACAGAGGGGAAGTTATCTTTTCCCATCGTCCACGCCCTTAACTTCACTAAAACGAAAGGTCAAACTGAGCAACACAATGAAATTCTAAGAATTCTCCTGTTGAGGACAAGTGATAAAGATATAAAACTAAAGCTGATTCAAATACTGGAATTCGACACCAATTCATTGGCCTACACCAAAAATTTTATTAATCAATTAGTGAATATGATAAAAAATGATAATGAAAATAAGTATTTACCTGATTTGGCTTCGCATTCCGACACCGCCACCAATTTACATGACGAATTGTTATATATAATAGACCACTTATCCGAATTGggtggtggttctATGGCTTCAGAAAAAGAAATTAGGAGAGAGAGATTCTTGAACGTTTTCCCTAAATTAGTAGAGGAATTGAACGCATCGCTTTTGGCTTACGGTATGCCTAAGGAAGCATGTGACTGGTATGCCCACTCATTGAACTACAACACTCCAGGCGGTAAGCTAAATAGAGGTTTGTCCGTTGTGGACACGTATGCTATTCTCTCCAACAAGACCGTTGAACAATTGGGGCAAGAAGAATACGAAAAGGTTGCCATTCTAGGTTGGTGCATTGAGTTGTTGCAGGCTTACTTCTTGGTCGCCGATGATATGATGGACAAGTCCATTACCAGAAGAGGCCAACCATGTTGGTACAAGGTTCCTGAAGTTGGGGAAATTGCCATCAATGACGCATTCATGTTAGAGGCTGCTATCTACAAGCTTTTGAAATCTCACTTCAGAAACGAAAAATACTACATAGATATCACCGAATTGTTCCATGAGGTCACCTTCCAAACCGAATTGGGCCAATTGATGGACTTAATCACTGCACCTGAAGACAAAGTCGACTTGAGTAAGTTCTCCCTAAAGAAGCACTCCTTCATAGTTACTTTCAAGACTGCTTACTATTCTTTCTACTTGCCTGTCGCATTGGCCATGTACGTTGCCGGTATCACGGATGAAAAGGATTTGAAACAAGCCAGAGATGTCTTGATTCCATTGGGTGAATACTTCCAAATTCAAGATGACTACTTAGACTGCTTCGGTACCCCAGAACAGATCGGTAAGATCGGTACAGATATCCAAGATAACAAATGTTCTTGGGTAATCAACAAGGCATTGGAACTTGCTTCCGCAGAACAAAGAAAGACTTTAGACGAAAATTACGGTAAGAAGGACTCAGTCGCAGAAGCCAAATGCAAAAAGATTTTCAATGACTTGAAAATTGAACAGCTATACCACGAATATGAAGAGTCTATTGCCAAGGATTTGAAGGCCAAAATTTCTCAGGTCGATGAGTCTCGTGGCTTCAAAGCTGATGTCTTAACTGCGTTCTTGAACAAAGTTTACAAGAGAAGCAAATAG |
| crtE (CDS) | ATGGATTACGCGAACATCCTCACAGCAATTCCACTCGAGTTTACTCCTCAGGATGATATCGTGCTCCTTGAACCGTATCACTACCTAGGAAAGAACCCTGGAAAAGAAATTCGATCACAACTCATCGAGGCTTTCAACTATTGGTTGGATGTCAAGAAGGAGGATCTCGAGGTCATCCAGAACGTTGTTGGCATGCTACATACCGCTAGCTTATTAATGGACGATGTGGAGGATTCATCGGTCCTCAGGCGTGGGTCGCCTGTGGCCCATCTAATTTACGGGATTCCGCAGACAATAAACACTGCAAACTACGTCTACTTTCTGGCTTATCAAGAGATCTTCAAGCTTCGCCCAACACCGATACCCATGCCTGTAATTCCTCCTTCATCTGCTTCGCTTCAATCATCAGTCTCCTCTGCATCCTCCTCCTCCTCGGCCTCGTCTGAAAACGGGGGCACGTCAACTCCTAATTCGCAGATTCCGTTCTCGAAAGATACGTATCTTGATAAAGTGATCACAGACGAGATACTTTCCCTCCATAGAGGGCAGGGCCTGGAGCTATTCTGGAGAGATAGTCTGACGTGTCCTAGCGAAGAGGAATATGTGAAAATGGTTCTTGGAAAGACGGGAGGTTTGTTCCGTATAGCGGTCAGATTGATGATGGCAAAGTCAGAATGTGACATAGACTTTGTCCAGCTTGTCAACTTGATCTCAATATACTTCCAGATCAGGGATGACTATATGAACCTTCAGTCTTCTGAGTATGCCCATAATAAGAATTTTGCAGAGGACCTCACAGAAGGGAAATTCAGTTTTCCCACTATCCACTCGATTCATGCCAACCCCTCATCGAGACTCGTCATCAATACGTTGCAGAAGAAATCGACCTCTCCTGAGATCCTTCACCACTGTGTAAACTACATGCGCACAGAAACCCACTCATTCGAATATACTCAGGAAGTCCTCAACACCTTGTCAGGTGCACTCGAGAGAGAACTAGGAAGGCTTCAAGGAGAGTTCGCAGAAGCTAACTCAAGGATGGATCTTGGTGACGTAGATTCGGAAGGAAGAACGGGGAAGAACGTCAAATTGGAAGCGATCCTGAAAAAGCTAGCCGATATCCCTCTGTGA |
| crtI (CDS) | ATGGGAAAAGAACAAGATCAGGATAAACCCACAGCTATCATCGTGGGATGTGGTATCGGTGGAATCGCCACTGCCGCTCGTCTTGCTAAAGAAGGTTTCCAGGTCACGGTGTTCGAGAAGAACGACTACTCCGGAGGTCGATGCTCTTTAATCGAGCGAGATGGTTATCGATTCGATCAGGGGCCCAGTTTGCTGCTCTTGCCAGATCTCTTCAAGCAGACATTCGAAGATTTGGGGGAGAAGATGGAAGATTGGGTCGATCTCATCAAGTGTGAACCCAACTATGTTTGCCACTTCCACGATGAAGAGACTTTCACTTTTTCAACCGACATGGCGTTGCTCAAGCGGGAAGTCGAGCGTTTTGAAGGCAAAGATGGATTTGATCGGTTCTTGTCGTTTATCCAAGAAGCCCACAGACATTACGAGCTTGCTGTCGTTCACGTCCTGCAGAAGAACTTCCCTGGCTTCGCAGCATTCTTACGGCTACAGTTCATTGGCCAAATCCTGGCTCTTCACCCCTTCGAGTCTATCTGGACAAGAGTTTGTCGATATTTCAAGACCGACAGATTACGAAGAGTCTTCTCGTTTGCAGTGATGTACATGGGTCAAAGCCCATACAGTGCGCCCGGAACATATTCCTTGCTCCAATACACCGAATTGACCGAGGGCATCTGGTATCCGAGAGGAGGCTTTTGGCAGGTTCCTAATACTCTTCTTCAGATCGTCAAGCGCAACAATCCCTCAGCCAAGTTCAATTTCAACGCTCCAGTTTCCCAGGTTCTTCTCTCTCCTGCCAAGGACCGAGCGACTGGTGTTCGACTTGAATCCGGCGAGGAACATCACGCCGATGTTGTGATTGTCAATGCTGACCTCGTTTACGCCTCCGAGCACTTGATTCCTGACGATGCCAGAAACAAGATTGGCCAACTGGGTGAAGTCAAGAGAAGTTGGTGGGCTGACTTAGTTGGTGGAAAGAAGCTCAAGGGAAGTTGCAGTAGTTTGAGCTTCTACTGGAGCATGGACCGAATCGTGGACGGTCTGGGCGGACACAATATCTTCTTGGCCGAGGACTTCAAGGGATCATTCGACACAATCTTCGAGGAGTTGGGACTCCCAGCCGATCCTTCCTTTTACGTGAACGTTCCCTCGCGAATCGATCCTTCTGCCGCTCCCGAAGGCAAAGATGCTATCGTCATTCTTGTGCCGTGTGGCCATATCGACGCTTCGAACCCTCAAGATTACAACAAGCTTGTTGCTCGGGCAAGGAAGTTTGTGATCCAAACGCTTTCCGCCAAGCTTGGACTTCCCGACTTTGAAAAAATGATTGTGGCAGAGAAGGTTCACGATGCTCCCTCTTGGGAGAAAGAATTTAACCTCAAGGACGGAAGCATCTTGGGACTGGCTCACAACTTTATGCAAGTTCTTGGTTTCAGGCCGAGCACCAGACATCCCAAGTATGACAAGTTGTTCTTTGTCGGGGCTTCGACTCATCCCGGAACTGGGGTTCCCATCGTCTTGGCTGGAGCCAAGTTAACTGCCAACCAAGTTCTCGAATCCTTTGACCGATCCCCAGCTCCAGATCCCAATATGTCACTCTCCGTACCATATGGAAAACCTCTCAAATCAAATGGAACGGGTATCGATTCTCAGGTCCAGCTGAAGTTCATGGATTTGGAGAGATGGGTATACCTTTTGGTGTTGTTGATTGGGGCCGTGATCGCTCGATCCGTTGGTGTTCTTGCTTTCTGA |
| crtYB (CDS) | ATGACGGCTCTCGCATATTACCAGATCCATCTGATCTATACTCTCCCAATTCTTGGTCTTCTCGGCCTGCTCACTTCCCCGATTTTGACAAAATTTGACATCTACAAAATATCGATCCTCGTATTTATTGCGTTTAGTGCAACCACACCATGGGACTCATGGATCATCAGAAATGGCGCATGGACATATCCATCAGCGGAGAGTGGCCAAGGCGTGTTTGGAACGTTTCTAGATGTTCCATATGAAGAGTACGCTTTCTTTGTCATTCAAACCGTAATCACCGGCTTGGTCTACGTCTTGGCAACTAGGCACCTTCTCCCATCTCTCGCGCTTCCCAAGACTAGATCGTCCGCCCTTTCTCTCGCGCTCAAGGCGCTCATCCCTCTGCCCATTATCTACCTATTTACCGCTCACCCCAGCCCATCGCCCGACCCGCTCGTGACAGATCACTACTTCTACATGCGGGCACTCTCCTTACTCATCACCCCACCTACCATGCTCTTGGCAGCATTATCAGGCGAATATGCTTTCGATTGGAAAAGTGGCCGAGCAAAGTCAACTATTGCAACAATCATGATCCCGACGGTGTATCTGATTTGGGTAGATTATGTTGCTGTCGGTCAAGACTCTTGGTCGATCAACGATGAGAAGATTGTAGGGTGGAGGCTTGGAGGTGTACTACCCATTGAGGAAGCTATGTTCTTCTTACTGACGAATCTAATGATTGTTCTGGGTCTGTCTGCCTGCGATCATACTCAGGCCCTATACCTGCTACACGGTCGAACTATTTATGGCAACAAAAAGATGCCATCTTCATTTCCCCTCATTACACCGCCTGTGCTCTCCCTGTTTTTTAGCAGCCGACCATACTCTTCTCAGCCAAAACGTGACTTGGAACTGGCAGTCAAGTTGTTGGAGAAAAAGAGCCGGAGCTTTTTTGTTGCCTCGGCTGGATTTCCTAGCGAAGTTAGGGAGAGGCTGGTTGGACTATACGCATTCTGCCGGGTGACTGATGATCTTATCGACTCTCCTGAAGTATCTTCCAACCCGCATGCCACAATTGACATGGTATCCGATTTTCTTACCCTACTATTTGGGCCCCCGCTACACCCTTCGCAACCTGACAAGATCCTTTCTTCGCCTTTACTTCCTCCTTCGCACCCTTCCCGACCCACGGGAATGTATCCCCTCCCGCCTCCTCCTTCGCTCTCGCCTGCCGAGCTCGTTCAATTCCTTACCGAAAGGGTTCCCGTTCAATACCATTTCGCCTTCAGGTTGCTCGCTAAGTTGCAAGGGCTGATCCCTCGATACCCACTCGACGAACTCCTTAGAGGATACACCACTGATCTTATCTTTCCCTTATCGACAGAGGCAGTCCAGGCTCGGAAGACGCCTATCGAGACAACAGCTGACTTGCTGGACTATGGTCTATGTGTAGCAGGCTCAGTCGCCGAGCTATTGGTCTATGTCTCTTGGGCAAGTGCACCAAGTCAGGTCCCTGCCACCATAGAAGAAAGAGAAGCTGTGTTAGTGGCAAGCCGAGAGATGGGAACTGCCCTTCAGTTGGTGAACATTGCTAGGGACATTAAAGGGGACGCAACAGAAGGGAGATTTTACCTACCACTCTCATTCTTTGGTCTTCGGGATGAATCAAAGCTTGCGATCCCGACTGATTGGACGGAACCTCGGCCTCAAGATTTCGACAAACTCCTCAGTCTATCTCCTTCGTCCACATTACCATCTTCAAACGCCTCAGAAAGCTTCCGGTTCGAATGGAAGACGTACTCGCTTCCATTAGTCGCCTACGCAGAGGATCTTGCCAAACATTCTTATAAGGGAATTGACCGACTTCCTACCGAGGTTCAAGCGGGAATGCGAGCGGCTTGCGCGAGCTACCTACTGATCGGCCGAGAGATCAAAGTCGTTTGGAAAGGAGATGTCGGAGAGAGAAGGACAGTTGCCGGATGGAGGAGAGTACGGAAAGTCTTGAGTGTGGTCATGAGCGGATGGGAAGGGCAGTGA |
| tTDH2 (terminator) | TAAATTTAACTCCTTAAGTTACTTTAATGATTTAGTTTTTATTATTAATAATTCATGCTCATGACATCTCATATACACGTTTATAAAACTTAAATAGATTGAAAATGTATTAAAGATTCCTCAGGGATTCGATTTTTTTGGAAGTTTTTGTTTTTTTTTCCTTGAGATGCTGTAGTATTTGGGAACAATTATACAATCGAAAGATATATGCTTACATTCGACCGTTTTAGCCGTGATCATTATCCTATAGTAACATAACCTGAAGCATAACTGACACTACTATCATCAATACTTGTCACATGAGAACTCTGTGAATAATTAGGCCACTGAAATTTGATGCCTGAAGGACCGGCATCACGGATTTTCGATAAAGCACTTAGTATCACACTAATTGGCTTTTCGC |
| tHSP26 (terminator) | AGTGACCTGGCTCTATAGTGTTGTCCCTCTCGCGAGGACCATTGTTGCTTGCATATGGGCTTGAAACATATGGTCATCACATCTGAGCGATTTTACCTCTTAGAATTAGTTTAGATATATATGAGTTGATGAATAAATAGTTATAAAAACTTGCTTTGGCTTCGATATATGACCGTTATTTTTGACTAAGTTTTAACGAAGGAATCTAACCTCGT |
| tADH2 (terminator) | GCGGATCTCTTATGTCTTTACGATTTATAGTTTTCATTATCAAGTATGCCTATATTAGTATATAGCATCTTTAGATGACAGTGTTCGAAGTTTCACGAATAAAAGATAATATTCTACTTTTTGCTCCCACCGCGTTTGCTAGCACGAGTGAACACCATCCCTCGCCTGTGAGTTGTACCCATTCCTCTAAACTGTAGACATGGTAGCTTCAGCAGTGTTCGTTATGTACGGCATCCTCCAACAAACAGTCGGTTATAGTTTGTCCTGCTCCTCTGAATCGTATCCCTCGATATTTCTCAT |
| tACS2 (terminator) | AATGAGATAAAATTTCGCTCCTTTTCTGTTTTCTATTTTCTATTTTCCCAACTTTTGCTCTATTCAGTTATAAATTACTATTTATCCATCAGTTAAAAAACAAGATCTTTTACTGGTCAGCTAGGAAAGCGAAAATACAAAGACTTTATGCACTTAGTG |



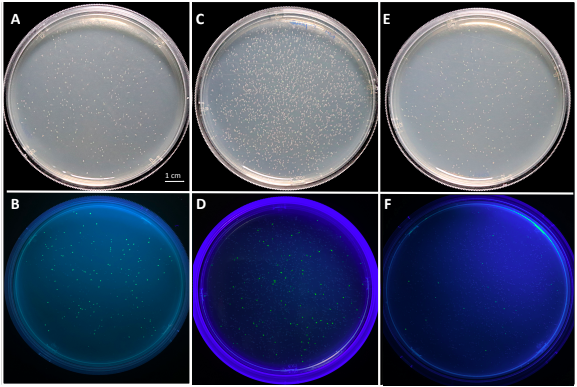
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Condition 1 | Condition 2 | Condition 3 | Condition 4 | Condition 5 |
| X | X | X | X | X |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

**Figure S1:** Optimizing conditions of Golden Gate cloning for a 4-piece assembly: Different temperatures were tested for ligation by T4 ligase (25 °C and 16 °C) and final digestion by BsaI-v2 (37 °C and 50 °C). Different time durations (2 min vs 5 min) were tested for the initial digestion step. One representative plate from each cycle was shown in the figure. White colonies contained potentially correct constructs. The first panel was taken under visible light and the second panel was taken under the UV light.

**Table S3:** Results obtained from different cycle conditions:

|  |  |
| --- | --- |
| **Condition used** | **Approximate percentage of white (potentially correct) colonies\*** |
| Condition 1 | % |
| Condition 2 | % |
| Condition 3 | % |
| Condition 4 | % |
| Condition 5 | % |

\* (Calculated from 3 biological replicates)

**

|  |  |  |  |
| --- | --- | --- | --- |
| **Number of parts assembled** | **No. of green colonies** | **No. of total colonies** | **percentage of potentially correct (green) colonies** |
| 6 | 80 | 300 | 26.6% |
| 7 | 45 | 2658 | 1.7% |
| 8 | 15 | 2275 | 0.65% |

**Figure S2:** Decreasing efficiency of Golden Gate Assembly with increasing number of parts: Golden Gate assembly of 6 parts (A and B), 7 parts (C and D), and 8 parts (E and F) showed a progressive decrease in the ratio of potentially correct green colonies. The first panel shows plates under visible light and the second panel shows plats under the UV light.



**Figure S3:** Absorption spectra of *β*-carotene and lycopene standards. *β*-carotene and lycopene standards were dissolved in hexane at 0.7 μg/mL and 0.65 μg/mL respectively. The absorption spectra between 300 nm and 550 nm was recorded using a UV-vis spectrometer. Red: lycopene. Orange: *β*-carotene.