

Protocol: Reconstruction of plant biosynthetic pathway in *Saccharomyces cerevisiae* (Smolke Lab)

1. Gene fragment amplification
  - a. Design and order Gibson primers for insert that overlap by 30 bp with the plasmid backbones
  - b. Amplify backbone plasmid as below:
    - 19.6 uL water
    - 2.5 uL Expand buffer
    - 0.5 uL dNTPs
    - 0.75 uL primer 1
    - 0.75 uL primer 2
    - 0.5 uL template
    - 0.4 uL Expand
  - c. Amplify insert
  - d. DpnI treat backbone and insert, clean up with Zymo columns
  
2. Gibson isothermal assembly for heterologous gene expression cassette construction
  - a. Making the mix (Adapted from Gibson Nature Protocols April 2009)
    - 320ul 5X ISO buffer (made identically as listed in the paper)
    - 0.64 ul T5 Exonuclease
    - 20 ul Phusion
    - 40 ul Taq ligase
    - 820 ul water
  - b. Aliquoting the mix
    - 7.5 ul aliquots per 10 ul reaction system in PCR tubes
  - c. Gibson isothermal assembly
    - The 1x concentration of the mix is 10 ul. So to carry out an assembly, add a total volume of 2.5 ul of prepped, linear DNA to the mix and incubate at 50C for 30-60 minutes and then directly transform the mix into heat-shock competent E. coli and plate onto the appropriate selective solid medium.
  - d. Miniprep colonies and send for sequencing
  
3. Yeast artificial chromosome construction
  - a. Use <https://www.lifetechnologies.com/order/oligoDesigner2/multifileUpload.do?assemblytype=invivo&vectortype=circular&formType=assembly> to design stitching oligonucleotides for YAC assembly
  - b. Amplify cassettes for assembly
    - 20.3 uL water
    - 2.5 uL Ultra buffer
    - 0.5 uL dNTPs
    - 0.5 uL primer 1
    - 0.5 uL primer 2

0.2 uL template ( ng)

0.5 uL Hotstart PfuUltra

50 °C annealing temperature, 2.5 min extension time (or based on fragment length)

- c. DpnI treat fragments and clean up with Zymo columns
- d. Transform yeast by electroporation with 100 ng of each fragment and 100 ng of digested pYES1L vector
- e. Functionally assay 4-8 transformants per strain.