

Gibson Assembling

1. We gain the Gibson Cloning Master Mix from Luo's lab, and it consists of three different enzymes:
 - 1) T5 Exonuclease - creates single-strand DNA 3' overhangs by chewing back from the DNA 5' end. Complementary DNA fragments can subsequently anneal to each other.
 - 2) Phusion DNA Polymerase - incorporates nucleotides to "fill in" the gaps in the annealed DNA fragments.
 - 3) Taq DNA Ligase - covalently joins the annealed complementary DNA fragments, removing any nicks and creating a contiguous DNA fragment.

Gibson Cloning Master Mix	5 μ l
Insert DNA	1:1 to 5:1 molar ratio over vector
Water, nuclease-free	To 10 μ l

2. Preparing the following reaction mixture:
3. Incubate the mix for 30 mins at 50°C or follow manufacturer's instructions.
4. Transform the DNA into bacteria and screen for the correct plasmid product by Restriction Digest.
5. Sequence the important regions of the final plasmid