Modular cloning strategy - Moclo
- uses the Type IIS restriction enzymes Bsal and BpiI/BbsI to efficiently assemble up to six DNA fragments at a time.
- this methods exploits the ability of Type IIS enzymes to cut outside their recognition site, and permits DNA fragments with compatible overhangs to be efficiently assembled.
- engineer unique enzyme recognition sites that flank a DNA module in an inverse orientation, so that multiple DNA components can directionally assemble in a single reaction, while retaining only a defined 4bp fusion site in between

Steps:
1. insert fragments of DNA containing basic parts (promoters, UTRs, coding sequences, terminators, etc) into individual Level 0 plasmids, or choose from a growing number of libraries containing pre-constructed standardized modules.
2. compatible Level 0 vectors are directionally assembled into a Level 1 vector creating a single transcriptional unit (Ex: a promoter, 5’UTR, coding region, and terminator)
3. Joining up to six Level 1 modules —> Level 2 vector (functional genetic circuit). Flexibility has been built into the Level 2 vectors to allow for additional iterations of Level 1 assembly if necessary.
4. Combining multiple Level 2 vectors in the final assembly —> complex constructs constrained only by the ability of E. coli to maintain the final plasmid after transformation.
5.
MoClo Plasmid Kits Available from Addgene

1. **EcoFlex MoClo Toolkit** (deposited by Paul Freemont's lab)
   - The EcoFlex Toolkit is a collection of plasmids that features a library of constitutive promoters, T7 expression, RBS strength variants, synthetic terminators, protein purification tags and fluorescence proteins for use in *E. coli* for a variety of applications.

2. **MoClo-YTK** (deposited by John Dueber's lab)
   - A set of 96 standardized and characterized parts that can be used for bottom-up hierarchical assembly of single and multi-gene constructs for *S. cerevisiae* expression.

3. **CIDAR MoClo Parts Kit** (deposited by Douglas Densmore's lab)
   - Collection of modular DNA parts and enhanced MoClo protocols to enable rapid one-pot, multipart assembly, combinatorial design and expression tuning in *E. coli*. 