Gibson assembly

Ligation of several DNA segments into a vector to create a circular vector. This exonuclease-based method does not require digestion by restriction enzymes for the assembly. Instead, all adjacent segments must have 15-30 bp of overlapping ends to each other.

Pre-calculations

Calculate how much DNA fragments are needed for the reaction.
Assemble of 1-3 inserts: a total of 0.02 – 0.5 pmol of DNA fragments.
Assemble of 4-6 inserts: a total of 0.2-1.0 pmol of DNA fragments.
For optimal cloning efficiency, a 2-3 fold excess of inserts is required.

Formula: $pmol = \frac{weight\ (ng) * 1000}{bp * 650Da}$

Total reaction volume: 15µL Gibson mix + 5µL DNA fragments.
For optimal cloning efficiency, a 2-3-fold excess of inserts-to-vector is required.

Protocol

1. **Work under sterile conditions, in an ice cooler.**
2. Set the reaction in a PCR tube on ice.
3. Add vector and inserts volumes according to the pre-calculations.
4. Incubate the samples in a thermocycler at 50 °C for 1 hour.
5. After incubation, store the samples on ice or at -20 °C for subsequent transformation.