Gibson Assembly – SOP

- Calculation of Piece Concentration, similar to Ligation calculations:
  The ratio (X) between the length of the insert and the vector can be related to the amount of DNA added to the reaction mixture as such:

$$\frac{l_i}{l_v} = \frac{n_i}{n_v} = X$$

And rearranged to solve for the molar equivalent of the insert w.r.t. the mass of the vector

$$n_i = X * n_v * k$$

where k is a multiplier for varying the insert concentration (1, 5, 10, etc.). For the assembly of multiple pieces, each piece should be assessed individually and added to the reaction mixture in their appropriate amounts in comparison to the backbone vector, n_v, which is effective at 5 ng.

- The volume of the piece mixture should be 5 uL, so add water if necessary.
- Add 15 uL of the Gibson Assembly Enzyme Master Mix
- Incubate at 50°C for 60 minutes
- Using entire reaction vessel contents, transform TOP10 cells via Heat Shock Transformation Protocol

Gibson Assembly 5X Isothermal Reaction Buffer Stock:

1M Tris-HCl pH 7.5	3 mL
2M $MgCl_2$	150 uL
100 mM dGTP	60 uL
100 mM dATP	60 uL
100 mM dCTP	60 uL
100 mM dTTP	60 uL
1M DTT	300 uL
PEG-8000	1.5 g
50 mM NAD+	600 uL
H_2O	1.7 mL (to 6 mL)

Gibson Assembly Enzyme Master Mix:

5X Isothermal Reaction Buffer	320 uL
10 U/uL T5 Exonuclease	0.64 uL
2 U/uL Phusion Polymerase	20 uL
40 U/uL Taq Ligase	160 uL
H_2O	699 uL (to 1.2 mL)

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