**Gibson Assemble Homemade mix**

[**http://miller-lab.net/MillerLab/protocols/molecular-biology-and-cloning/gibson-assembly/**](http://miller-lab.net/MillerLab/protocols/molecular-biology-and-cloning/gibson-assembly/)

**Reagent**

Taq DNA Ligase (NEB; M0208L)

T5 Exonuxlease (NEB; M0363S)

Phusion HF DNA Polymerase (NEB; M0530S)

β-Nicotinamide adenine dinucleotide (NAD+) (NEB; B9007S)

Single-Use JM109 Competent Cells (Promega; L2005)

10-beta Electrocompetent *E. coli* (NEB; C3020K)

ElectroMAX™ Stbl4™ Competent Cells (Invitrogen; 11635-018)

**5x ISO reaction Buffer**

3 ml of 1 M Tris-HCl pH 7.5

150 μl of 2 M MgCl2

60 μl of 100 mM dGTP

60 μl of 100 mM dATP

60 μl of 100 mM dTTP

60 μl of 100 mM dCTP

300 μl of 1 M DTT

1.5 g PEG-8000

300 μl of 100 mM NAD

Add water to 6 ml

Aliquot 350 μl and store at -20 °C for one year

**2xGibson Assemble Master Mix**

Dilute 2ul T5 exo 1:10 in 1x NEB Buffer 4 to make 1U/ul stock

320 μl 5X ISO buffer

6.4 μl of 1U/μl T5 exonuclease

20 μl of 2 U/μl Phusion polymerase

160 μl of 40 U/μl Taq ligase

Add 693.6ul of water to 1.2 ml

Aliquot 50 μl and store at -20 °C for one year.

**Assemble reaction**

**2xGibson Assemble Master Mix 10ul 5ul**

Digested Plasmid x ul x ul

Insert (as many as you want) y ul y ul

H2O z ul z ul

20ul 10ul

Make the molar ration between plasmid and insert as 1:2-10.

**Incubate at 50C for 15 min to 2h (usually 2h)**

**Transformation**

We usually use chemical competent cells (such as JM109 Competent Cells) when the insert is less than 10 kb.

We use electrocompetent cells (such as 10-beta Electrocompetent *E. coli* orElectroMAX™ Stbl4™ Competent Cells) when the insert is more than 10 kb.

For electrocompetent cells, assembled DNA is diluted 3 times with H2O to avoid the effect of PEG8000.