

# GIBSON ASSEMBLY MIX PREPARATION

ADAPTED FROM THE BONNET TEAM PROTOCOL REPOSITORY

## MATERIALS:

- 1,5 mL Microtubes
- PCR tubes

### To prepare the 5X Isothermal solution (ISO 5X):

- Tris-HCl pH 7.5 solution 1M
- PEG-8000
- MgCl<sub>2</sub> solution 1M (on bench)
- DTT 1 M (-20°C)
- dNTP Mix 10mM (-20°C)
- NAD 100 mM (-20°C)
- dd H<sub>2</sub>O

### To prepare the Gibson Assembly Mix 2X solution:

- ISO 5x (-20°C)
- T5 exonuclease (10 U/uL) (-20°C)
- Taq DNA ligase (40 U/uL) (-20°C)
- Phusion DNA polymerase (2U/uL) (-20°C)
- dd H<sub>2</sub>O

## PROTOCOL:

5X isothermal reaction buffer.

Preparation for 10mL final solution

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- Incubate at 37°C overnight

	10mL final	Final Conc
Tris-HCl pH 7.5	5mL	500mM
PEG-8000	2,5g	50%
Vortex		
MgCl <sub>2</sub>	500µL	50mM
DTT	500µL	50mM
dNTP Mix	1mL	1mM
NAD	500µL	5mM
ddH <sub>2</sub> O	Qsp 10mL	

The 5X isothermal reaction buffer has to be aliquot in 1mL, in 1,5mL tubes.

### 2X Gibson Assembly mix.

	x2	x2	
Final volume (µL)	1000	1500	
ISO 5x (µL)	300,8	451,2	
T5 exonucelase (10 U/µL)	0,6	0,9	Or do a dilution 1/10 of the solution 10U/µL and add 9µL for 1500µL final
Taq DNA ligase (40 U/µL)	150,4	225,6	
Phusion DNA polymerase (2U/µL)	18,8	28,2	
dd H <sub>2</sub> O	529,4	794,1	

The Gibson Assembly 2X mix has to be aliquot in 10µl in PCR tubes on a rack.

Put the rack at -20°C. When it's freeze put all tubes in a "tips" box.