# Gibson Assembly

For assembly, the following was found to be highly reliable

1. Prepare the Assembly Mix on ice
2. Incubate for one hour at 50°C
3. Transform

| Assembly Mix | Volume (µl) |
|---|---|
| Gibson Master Mix | 15 |
| Total DNA | 5 |
| Total | 20 |

## Master Mix recipe

| Gibson Master Mix | Volume (µl) |
|---|---|
| Taq ligase (40u/µl) | 50 |
| 5x isothermal buffer | 100 |
| T5 exonuclease (1u/µl) | 2 |
| Phusion polymerase (2u/µl) | 6.25 |
| Nuclease-free water | 216.75 |
| Total | 375 |

Whilst the mixture can be refrozen, for best performance you should aliquot it into 25x15µl.

| 5x isothermal buffer | Volume (µl) |
|---|---|
| 25% PEG-8000 | 0.75g |
| 500 mM Tris-HCl pH 7.5 | 1500 |
| 50mM MgCl2 | 75 |
| 50mM DTT | 150 |
| 1mM dATP | 30 |
| 1mM dTTP | 30 |
| 1mM dCTP | 30 |
| 1mM dGTP | 30 |
| 5mM NAD | 300 |
| Nuclease-free water | ... |
| Total | 3000 |

Use nuclease-free water to make up to 3000µl. In this case, very little water is needed as the volume is 2145µl + 0.75g.