**Assemble reaction**

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

Digested Plasmid (~10-50ng) 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.

For a 10kb plasmid and a 2kb insert, a 1:3 ratio would be 50ng of plasmid and 30ng of insert

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

**Transformation**

Transform up to 5ng of DNA (2ul to 5ul of reaction)

We usually use chemical competent cells (such as JM109 Competent Cells or homemade 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.