**Golden Gate Cloning of PCC01**

1. Measure inserted phosphorylated (1/10 diluted) oligo by nanodrop, adjust to 7.5ng/uL
2. Mix the following 1x reaction.

|  |  |
| --- | --- |
|  | 1x (uL) |
| PCC01 (75 ng/uL) | 1 |
| Insert oligo (7.5ng/uL) | 1 |
| 10x T4 ligase buffer | 2 |
| NEB GG Enzyme mix | 1 |
| dW | 15 |

\* NEB GG Enzyme mix = BsmbI v2 NEB golden gate kit

1. Thermocycler O/N (10hr)

|  |  |  |
| --- | --- | --- |
| 42\*c | 5 mins | X60 cycles |
| 16\*c | 5 mins |
| 60\*c | 5 mins |  |

1. Bead purification

* Add dW 30 uL to total 50 uL
* Add 1.0x Ampure bead (50 uL)
* Wash 80% EtOH x2
* Elute in 12 uL dW

1. Heat-shock transform 5 uL in 50 uL competent cells.