**Golden Gate Cloning, QVEU**

*Walker Orr, 03/15/2023*

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| **Steps** |
| 1. Prepare ice and preheat PCR block:   For BsaI: 37°C  For BsmBI: 42°C  Thaw, vortex spin buffer. Mix DNA stocks by pipetting up and down.  Check concentration of insert DNA if there’s concern; adjust volumes as necessary. |
| 1. Reaction setup:  |  |  | | --- | --- | | **Reagent** | **Assembly Reaction** | | Water | To 20 µL | | T4 DNA Ligase Buffer (10x) | 2 µL | | Backbone: ~.055 pmol |  | | Insert: ~.11 pmol | Usually 1 µL at 20 ng/µL | | NEB GG Enzyme Mix  \*BsaI or BsmBI, make sure to use the correct one! | For single inserts: 1 µL  For library preps: 2 µL |   Mix by pipetting up and down with a multichannel pipette; use a submaximal volume to avoid introducing air bubbles. |
| 1. For:   BsaI-HF:  Single Insert:   1. 30x [37°C, 1 minute 🡪 16°C, 1 minute] 2. 60°C, 5 minutes 3. Hold at 4°C   Library prep:   1. 60x [37°C, 5 minute 🡪 16°C, 5 minute] 2. 60°C, 5 minutes 3. Hold at 4°C   BsmBI:  Single Insert:   1. 30x [42°C, 1 minute 🡪 16°C, 1 minute] 2. 60°C, 5 minutes 3. Hold at 4°C   Library prep:   1. 60x [42°C, 5 minute 🡪 16°C, 5 minute] 2. 60°C, 5 minutes 3. Hold at 4°C |