# Gibson Assembly

## Material

NEBuilder HiFi DNA Assembly Reaction Master Mix

## Procedure

1. Set up the following reaction on ice:

   Recommended Amount of Fragments Used for Assembly

| | **Recommended Amount of Fragments Used for Assembl** | | |
|---|---|---|---|
| | 2–3 Fragment Assembly | 4–6 Fragment Assembly | Positive Control |
| **Recommended DNA Ratio** | vector:insert = 1:2 | vector:insert = 1:1 | |
| **Total Amount of Fragments** | 0.03–0.2 pmols<br>X µl | 0.2–0.5 pmols<br>X µl | 10 µl |
| **NEBuilder HiFi DNA Assembly Master Mix** | 10 µl | 10 µl | 10 µl |
| **Deionized $H_2O$** | 10-X µl | 10-X µl | 0 |
| **Total Volume** | 20 µl | 20 µl | 20 µl |

2. Incubate samples in a thermocycler at 50°C for 15 minutes (when 2 or 3 fragments are being assembled) or 60 minutes (when 4–6 fragments are being assembled). Following incubation, store samples on ice or at –20°C for subsequent transformation.
3. Transform competent E. coli cells with 2 µ l of the assembled product, following the transformation protocol.