

gBlock preparation and HiFi assembly

*Note; HiFi assembly is equivalent to Gibson assembly except that it utilises a high fidelity polymerase

Materials:

- gBlocks
- Plasmid backbone (50 ng)
- TE resuspension buffer (50 µl)
- HiFi DNA assembly master mix (10 µl)
- Nuclease-free water

gBlock preparation:

1. Centrifuge the tube containing dried gBlocks (1000ng) for 3-5 seconds to ensure all DNA is at the bottom of the tube.
2. Add 50µl resuspension buffer solution for a final concentration of 20ng/µl and vortex briefly.
3. Incubate at 50C for 20 mins in a water bath.
4. Briefly vortex and centrifuge. Keep on ice, store at -20C for long term storage.

HiFi assembly:

1. Prepare the following assembly mix on ice:

HiFi DNA assembly Master Mix	10 µl
Linear plasmid	50 ng
Resuspended gBlock	2 fold increase from plasmid
Nuclease-free water	n µl
Total volume	20µl

Use the equation below to determine the amount of gBlock required (pmols should be 2x that of the linear plasmid):

$$pmols = \frac{ng\ DNA \times 1000}{bp \times 650\ Da}$$

2. Incubate at 50°C for 15 minutes. Keep on ice, store at -20C for long term storage.