SOP Name: gBlock preparation and HiFi assembly
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Source(s): Adapted from Exeter 2015
Time Required: 1 hour

Notes: 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

Procedure:

gBlock preparation:

1. Centrifuge the tube containing dried gBlocks (1000ng) for 35 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 -20°C for long term storage.