Golden Gate Digestion/Ligation Protocol

Level 1 reaction using a C-terminal tag

Background
Golden Gate cloning utilizes 4bp overhangs to ligate genes into expression cassettes. Multiple 4bp overhang systems are in use within the plant science community, with some overlap between systems.

Here, we are using the system offered by ensa, Engineering Nitrogen Symbiosis for Africa. The ensa system uses a modified C-terminal tag 5'overhang, **ggtg**, which is very similar to the promoter 5' overhang, **ggag**. As a consequence, reaction efficiency for creating correct clones of C-terminal tagged genes can be very low. To avoid the problem of having the C-terminal fragment ligating at the promoter position, the protocol has been optimized as follows.

Modifications to assemble fragments into a level 1 plasmid:

1. Pre-linearize the acceptor plasmid with *Bsa*I-HF according to the manufacter's protocol. Run on a gel and excise the backbone band. Clean up the DNA and elute in water.

2. Combine:

200 ng of pre-linearized acceptor backbone
2:1 molar ratio of insertion fragments
1 µl *Bsa*I-HF
2 µl CutSmart Buffer
1 µl T4 DNA Ligase (M0202M or M0202T from NEB)
2 µl 10 mM dATP
water to 20 µl

5 cycles of 10 min at 37°C / 10 min at 16°C
Add Cter tag
5 cycles of 10 min at 37°C / 10 min at 16°C
10 min at 37°C
20 min at 80°C
4°C infinite

3. Transform bacteria and plate on appropriate antibiotic. (Blue/white selection is not necessary because no contaminating destination vector is present.)

4. Screen 10 colonies by PCR and verify.