**Team3ispA/back, ispA#: Golden Gate Assembly**

*Master Mix:*

33 uL ddH2O

5.5 uL 10x T4 DNA Ligase Buffer

5.5 uL frag2 (back1)

2.75 uL BsaI

*Reaction:*

8.5 uL Master Mix

1 uL frag1 (ispA# PCR)

0.5 uL T4 DNA ligase

**source**

*dna location*

back1 Box\_Team3ispA/F6

ispA1 Box\_Team3ispA/A6

ispA2 Box\_Team3ispA/B6

ispA3 Box\_Team3ispA/C6

ispA4 Box\_Team3ispA/D6

ispA5 Box\_Team3ispA/E6

**samples**

*label frag1 frag2 product*

a1 ispA1 back1 pLYC73S-ispA1

a2 ispA2 back1 pLYC73S-ispA2

a3 ispA3 back1 pLYC73S-ispA3

a4 ispA4 back1 pLYC73S-ispA4

a5 ispA5 back1 pLYC73S-ispA5

destination: thermocycler\_\_

program: main/GG1

note:

Never let enzymes warm up! Only take the enzyme cooler out of the freezer when you are actively using it, and only take the tubes out of it when actively dispensing.