**Lycopene6/Eback,Eori: Golden Gate Assembly**

*Master Mix:*

24 uL ddH2O

4 uL 10x T4 DNA Ligase Buffer

2 uL frag2 (Eback1pcr)

2 uL frag3 (Eback2pcr)

2 uL BsaI

*Reaction:*

8.5 uL Master Mix

1 uL frag1 (the E# ori PCR)

0.5 uL T4 DNA ligase

**source**

*dna location*

Eback1 Box\_Lyc6/A5

Eback2 Box\_Lyc6/B5

Eori0 Box\_Lyc6/C5

Eori1 Box\_Lyc6/D5

Eori2 Box\_Lyc6/E5

**samples**

*label frag1 frag2 frag3 product*

E0 Eori0 Eback1 Eback2 pLYC31E

E1 Eori1 Eback1 Eback2 pLYC32E

E2 Eori2 Eback1 Eback2 pLYC33E

destination: thermocycler2A

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.

**Lycopene6/Kback,Kori: Golden Gate Assembly**

*Master Mix:*

24 uL ddH2O

4 uL 10x T4 DNA Ligase Buffer

2 uL frag2 (Kback1pcr)

2 uL frag3 (Kback2pcr)

2 uL BsaI

*Reaction:*

8.5 uL Master Mix

1 uL frag1 (the K# ori PCR)

0.5 uL T4 DNA ligase

**source**

*dna location*

Kback1 Box\_Lyc6/A6

Kback2 Box\_Lyc6/B6

KP0 Box\_Lyc6/C6

KP1 Box\_Lyc6/D6

KP2 Box\_Lyc6/E6

**samples**

*label frag1 frag2 frag3 product*

K0 KP0 Kback1 Kback2 pLYC31K

K1 KP1 Kback1 Kback2 pLYC32K

K2 KP2 Kback1 Kback2 pLYC33K

destination: thermocycler2A

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.