

hCas9-pSB1A3
6215 bp

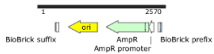
**GIBSON
ASSEMBLY**

Insert between DNA ends
1 .. 2570

Overlap and insert
1 .. 670

Overlap and insert
1 .. 793

Overlap and insert
1 .. 4265



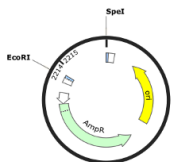
Lin-hCas9backbone
2570 bp

PCR
Amplify 2215 .. 2214 using:
G17
G18

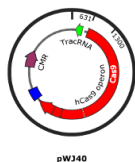
PCR
Amplify 631 .. 1300 using:
G19
G20

PCR
Amplify 1 .. 793 using:
G21
G22

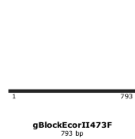
PCR
Amplify 2054 .. 6318 using:
G23
G24



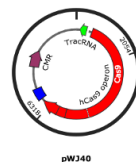
pSB1C3+gBlock
2570 bp



pWJ40
10,349 bp



gBlockEcorI1473F
793 bp

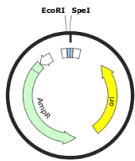


pWJ40
10,349 bp

**INSERT
FRAGMENT**

Replace
EcorI (2134) ~ SpeI (2)

Insert
EcorI (4) ~ SpeI (442)



pSB1A3
2155 bp



AmplifiedgBlockPrefix-XbaI-Suffix
468 bp

PCR
Amplify 5 .. 468 using:
G15
G16



gBlockPrefix-XbaI-Suffix
472 bp