Ligate glyoxylate shunt into plasmid

1) Cut pBS1C with XbaI, SpeI (BcuI) and FastAP (prevents recircularization).

	Plasmid
Water, nuclease -free	Up to 20 µL
10X FastDigest Green Buffer	2 µL
pBS1C with RFP	7 µL (~ 1 µg)
XbaI	1 µL
SpeI (BcuI)	1 µL
FastAP	1 µL
Total	20 µL

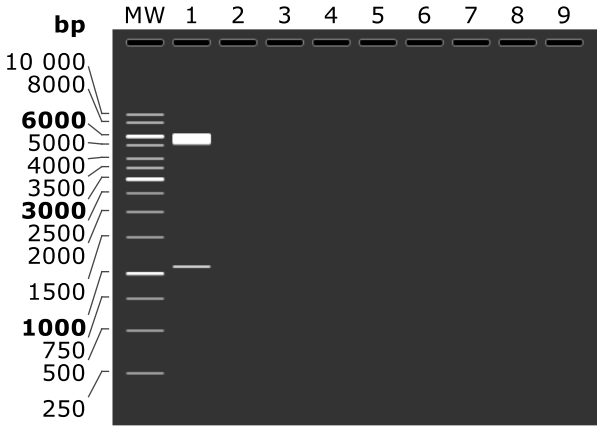
30 min 37 degrees

Purify band **6097** band from gel, post-stain GelGreen, using gel extraction kit:

MW: GeneRuler™ 1 kb DNA Ladder

1: pBS1C with RFP
XbaI + SpeI
1**. 6097 bp**
2. 1077 bp

Measure concentration



2) Cut purified 3.1 glyoxylate shunt with XbaI and SpeI (BcuI)

	Plasmid
Water, nuclease -free	Up to 20 µL
10X FastDigest Buffer	2 µL
3.1 Glyxolate shunt PCR	9 µL (~ 1µg)
XbaI	1 µL
SpeI (BcuI)	1 µL
Total	20 µL

30 min 37 degrees

Purify 3.1 using PCR clean kit (SpeI is not inactivated by heat)
Measure conc

3) **Ligation**

	Plasmid
Water, nuclease -free	Up to 20 µL
10X ligation buffer	2 µL
Cut glyoxylate shunt	1:1 to 5:1 molar ratio over vector
Gel extracted cut pBS1C	20-100ng
Ligase	1 U (1 µL)
Total	20 µL

Ligate (will result in a 9464 bp plasmid), **don't measure conc**

Save some of the ligation mixture in case the plasmid cannot be propagated in Ecoli -> PCR amplify instead

4) **Transformation**

Use 5 µL for transformation into *E. coli*
Negative control: transform cut plasmid without insert (from gel purification). Use the same amount of DNA as in the ligation.
Ex if you used 100 ng of cut plasmid for ligation and 5 µL for transformation: use (100ng / 20µL) * 5 µL = 25 ng of cut plasmid as negative control.
Plate on LB+amp
37 degrees' o/n **(max 16 hours)**

5) **Restreak**
Restreak **white** colonies on LB+amp plates and inoculate in LB+amp liquid media o/n **(max 16 hours)** for miniprep the next day.

6) **Miniprep**

7) **Restriction verify with XbaI and SpeI (BcuI) of the glyoxylate shunt**

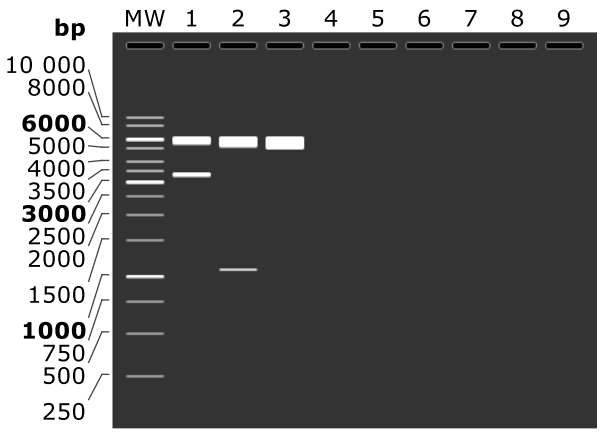
	Plasmid
Water, nuclease -free	Up to 20 µL
10X FastDigest Green Buffer	2 µL
DNA	Up to 1000 ng
XbaI	1 µL
SpeI (BcuI)	1 µL
Total	20 µL

30 minutes 37 degrees.

Load 3 µL on GelRed gel

should look like **lane 1**

MW: GeneRuler™ 1 kb DNA Ladder



1: circular shunt in pBS1C

XbaI + SpeI

1. 6097 bp
2. 3367 bp

2: pBS1C with RFP XbaI + SpeI

1. 6097 bp
2. 1077 bp

3: pBs1C

XbaI + SpeI

1. 6097 bp
2. 20 bp