

MoClo

Level 1

Level 1 Destination Vector	40 fmol
Level 0 part #1	40 fmol
Level 0 part #2	40 fmol
Level 0 part #3	40 fmol
...	
CutSmart Buffer	2 μ l
ATP	2 μ l
Bsal	0.5 μ l
T4 Ligase	0.5 μ l
H ₂ O	To 20 μ l

Thermocycler protocol:

- 5 hours 37 °C
 - 5 min 5 °C
 - 10 min 80 °C
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- Perform heat shock transformation in *E. coli* (see protocol "Heat-Shock Transformation")
 - Select positive transformants with LB + Ampicillin
 - white colonies are positive

Level 2

Level 2 Destination Vector	40 fmol
Level 1 part #1	40 fmol
Level 1 part #2	40 fmol
Level 1 part #3	40 fmol
...	
End Linker	40 fmol
CutSmart Buffer	2 μ l
ATP	2 μ l
BbsI	0.5 μ l
T4 Ligase	0.5 μ l
H ₂ O	To 20 μ l

Thermocycler protocol:

- 5 hours 37 °C
- 5 min 5 °C
- 10 min 80 °C

- Perform heat shock transformation in *E. coli* (see protocol "Heat-Shock Transformation")
- Select positive transformants with LB + Kanamycin
- white colonies are positive