

Overlap extension PCR of
- Spit LucC-Hog1+Ura3 H7
-split GFP-N+hog1+Kan P8
-rLuc-pbs2+Kan P9
-split rLucN-pbs2+Kan P10
Extension PCR of
Split GFP-C H55 (repeat previous experiment)

30µL reactions

Equimolar template ratios: we used 100 ng of the longest template, and estimated the necessary amount of the shortest template with its size.

In µL	Master Mix	Template 1	Template 2 (100ng)	Water	Primers*
P8: P4+P7	5.75	P4: 0.83	P7: 2	16.4	2.5 each (p12/p2)
P9: P1+P5	5.75	P1: 0.53	P5: 1	17.7	2.5 each (p12/p2)
P10: P2+P6	5.75	P2: 0.4	P6: 2	16.9	2.5 each (p12/p2)
H7: H3+H2	5.75	H1: 1	H3: 1.7	15.4	2.5 each (h7/h1.Rv)

*Add primers after the first program (after fusion of the templates).

Thermocycler programs :

P8,9,10 : 15 cycles at 60°C for overlap, add primers, then 15 cycles at 72°C annealing. Always 2min30 of extension time at 72°C.

H7: 15 cycles at 62°C for overlap, add primers, then 15 cycles at 62°C annealing. Always 2min30 of extension time at 72°C.

Results :



