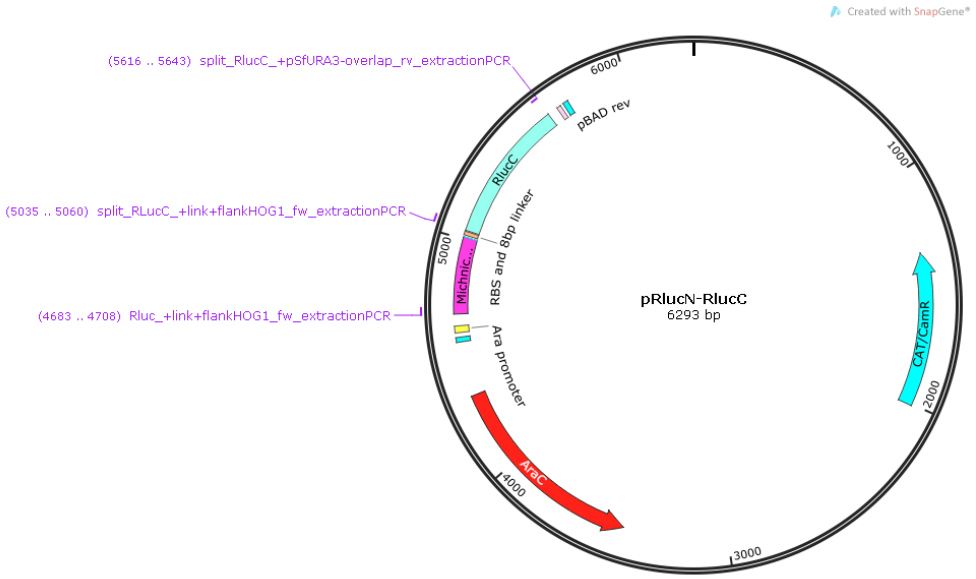
07.08.14

PCR of - RLuc + addition of hog1 flanking sequence H1
- RLuc + addition of pbs2 flanking sequence P1
- split RLucN + pbs2 flanking sequence P2
- split RLucC + hog1 flanking sequence H2



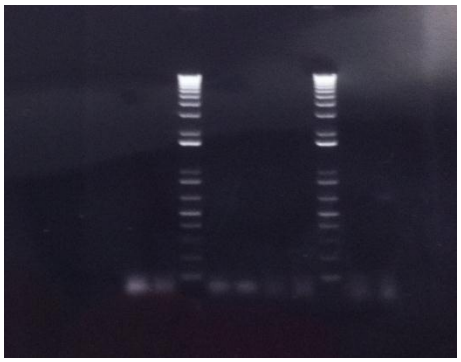
Master Mix
5x phusion GC buffer 85 µL
10mM dNTPs 8,5 µL
Phusion polymerase 4,25 µL

Reaction

	H1	H2	P1	P2
Master Mix	11,5	11,5	11,5	11,5
Forward primer 10mM	2.5	2,5	2,5	2,5
Reverse primer 10mM	2,5	2,5	2,5	2,5
Template pRLuc ou pRLucNRLucC	0.21	0,2	0,21	0,2
Nuclease Free Water	33.32	33,33	33,33	33,33

PCR programs
H1, H2 HLuc1
P1, P2 PLuc1

First try result

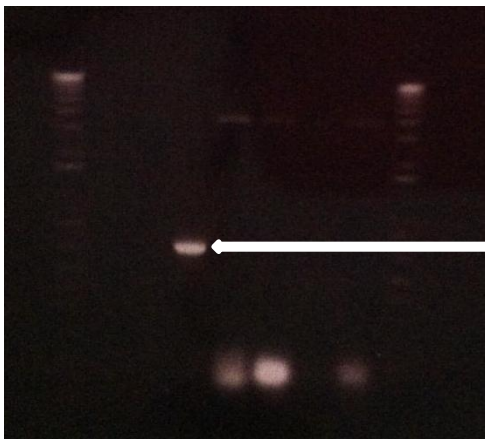


Second try

We added more template to the reaction: 3 µL (60ng/µL solution).

	H1	H2	P1	P2
Master Mix	11,5	11,5	11,5	11,5
Forward primer 10mM	2.5	2,5	2,5	2,5
Reverse primer 10mM	2,5	2,5	2,5	2,5
Template pRLuc ou pRLucNRLucC	3	3	3	3
Nuclease Free Water	30,75	30,75	30,75	30,75

Results



Only one PCR worked: the C terminal part of the split renilla luciferase was successfully amplified.
Expected amplicons size: 695 bp

Nanodrop after purification: 89,2 ng/µL

Note: three tubes exploded

What we have: