**08.08.14**

## Gradient PCR of - RLuc + addition of hog1 flanking sequence H1
## - RLuc + addition of pbs2 flanking sequence P1
## - split RLucN + pbs2 flanking sequence P2

Since we had some trouble with our PCRs and our primers are quite long we decided to try a gradient PCR.

Master Mix
GC buffer 250µL
dNTPs 25µL
Phusion 12,5µL

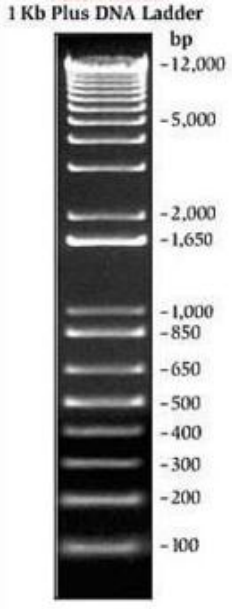
Reaction

| | H1 | P1 | P2 |
|---|---|---|---|
| Master Mix | 11,5 | 11,5 | 11,5 |
| Forward primers 10mM | 2,5 | 2,5 | 2,5 |
| Reverse primers 10mM | 2,5 | 2,5 | 2,5 |
| Template pRLuc | 0,6 | 0,6 | 0,6 |
| Water | 33 | 33 | 33 |

Temperature gradient:

H1 64→72°C
P1, P2 55→63°C

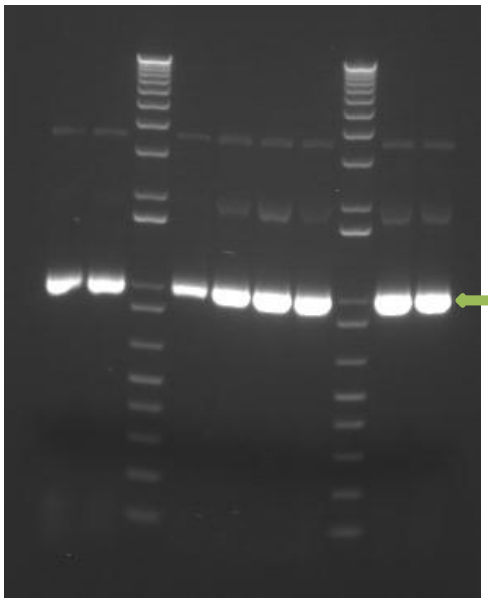
We also increase the extension time: 30secondes→1min30seconds!



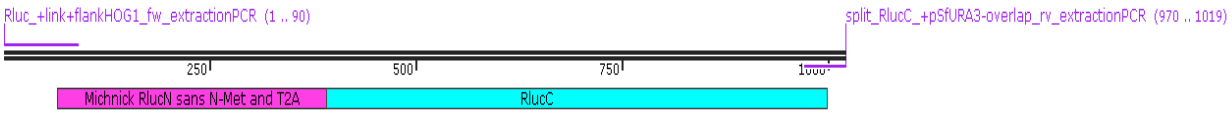

We used the Invitrogen 1kb Plus DNA Ladder. In each well of a 1% agarose gel, we loaded 8.8ul Nuclease free Water, 2ul of PCR products or Ladder and 1.2ul 10X Loading Blue Dye. The electrophoresis ran 40 minutes at 120V.

Results:

H1

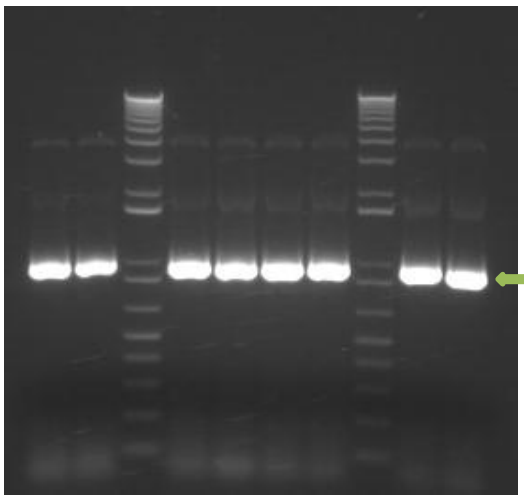



RLuc+hog1 flank
1019bp

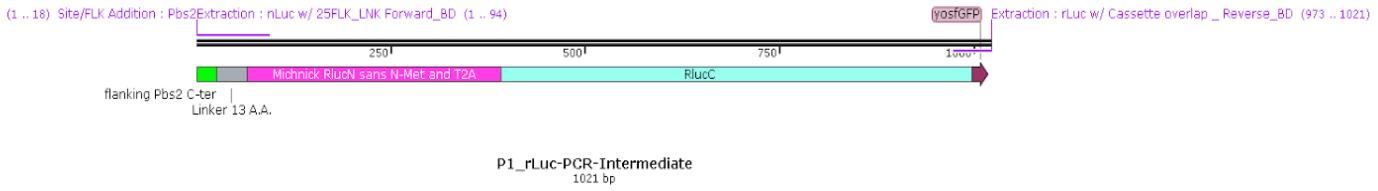



RLuc+hog1flank
1019 bp

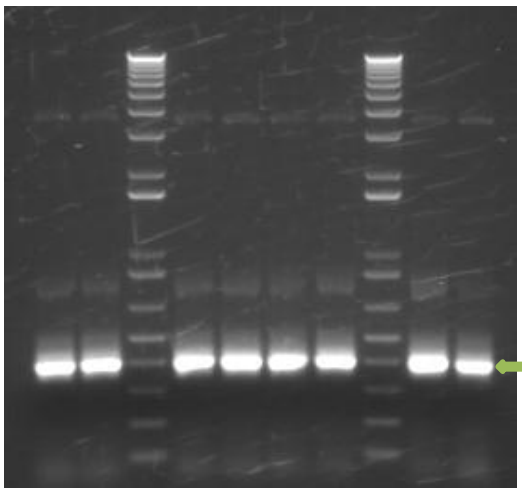
P1




RLuc+Pbs2 flank
1021bp

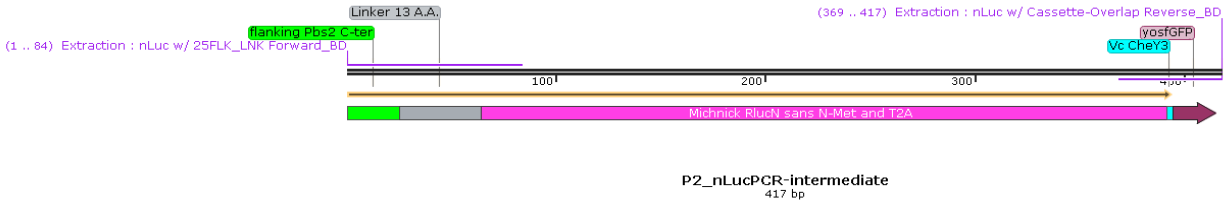



P2



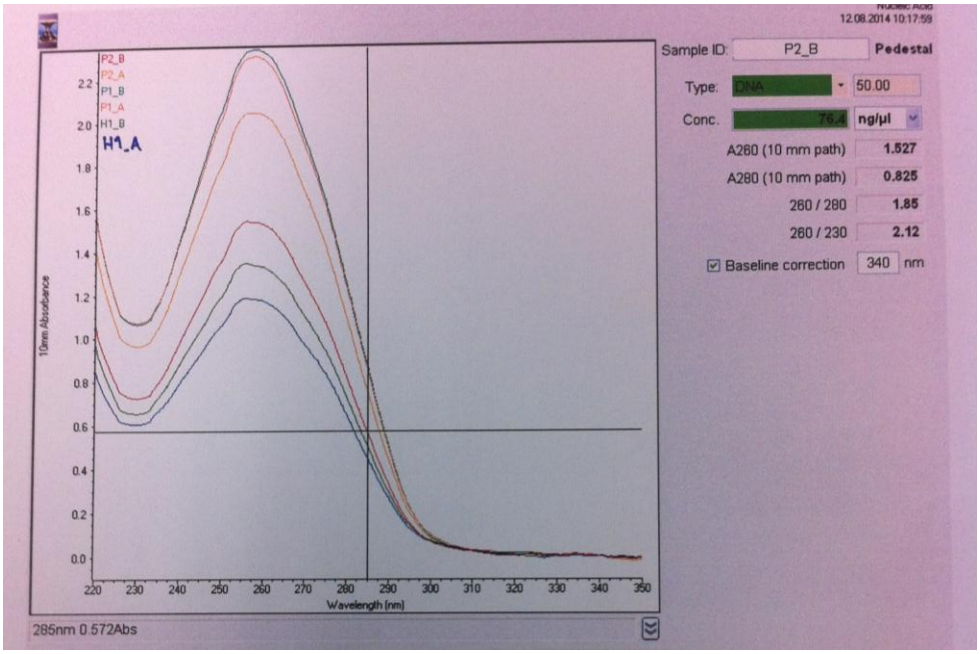
400
300

Split RLucN+Pbs2 flank
417 bp




We purified two PCR samples for each experiment and measured their concentration and purity with the Nanodrop.

| # | Sample ID | User name | Date and Time | Nucleic Acid Conc. | Unit | A260 | A280 | 260/280 | 260/230 | Sample Ty |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | H1_A | tp-ssv | 12.08.2014 10:10 | 58.7 | ng/µl | 1.174 | 0.652 | 1.80 | 1.94 | DNA |
| 2 | H1_B | tp-ssv | 12.08.2014 10:12 | 66.4 | ng/µl | 1.329 | 0.732 | 1.82 | 2.04 | DNA |
| 3 | P1_A | tp-ssv | 12.08.2014 10:13 | 114.7 | ng/µl | 2.295 | 1.245 | 1.84 | 2.15 | DNA |
| 4 | P1_B | tp-ssv | 12.08.2014 10:15 | 116.4 | ng/µl | 2.328 | 1.261 | 1.85 | 2.19 | DNA |
| 5 | P2_A | tp-ssv | 12.08.2014 10:16 | 101.8 | ng/µl | 2.037 | 1.100 | 1.85 | 2.12 | DNA |
| 6 | P2_B | tp-ssv | 12.08.2014 10:17 | 76.4 | ng/µl | 1.527 | 0.825 | 1.85 | 2.12 | DNA |


12.08.2014 10:17:59
Sample ID: P2_B
Pedestal
Type: DNA 50.00
Conc. 76.4 ng/µl
A260 (10 mm path) 1.527
A280 (10 mm path) 0.825
260 / 280 1.85
260 / 230 2.12
Baseline correction 340 nm
P2_B
P2_A
P1_B
P1_A
H1_B
H9_A
10mm Absorbance
Wavelength (nm)
285nm 0.572Abs