

***Ladder: 2-Log**

30.08.17

Parts BBa_K325909 and BBa_K769020 were transformed into E.Coli Pro cells.

31.08.17

Three colonies from both plates were chosen and grown in LB media to be checked by digestion.

2.09.17

Plasmids were extracted from selected colonies and double digested with XbaI/SpeI.

All three colonies of BBa_K325909 indicated succesful transformation but BBa_K769020 failed.

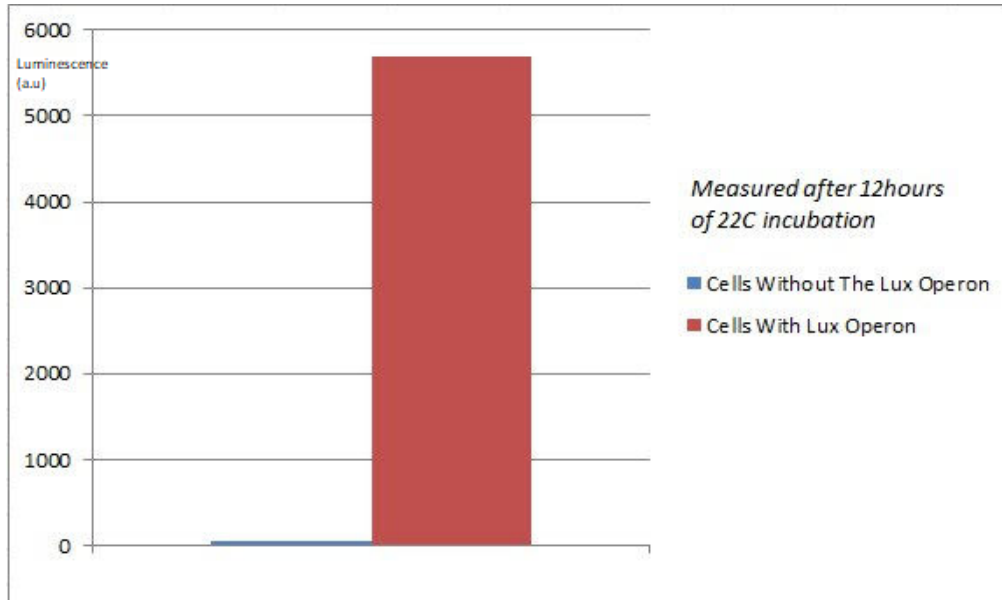
4.09.17

BBa_K325909 culture were grown overnight at 37C. It was diluted to final OD600 of 0.07 and when it reached OD600 of 0.3, 0.015% (w/v) of L-arabinose was added. Culture was sealed with coffee filters to ensure appropriate ventilation to maximize luciferase activity and grown at 22C.



5.09.17

After 12 hours grown culture was measured for luminescence and OD600. Results indicate BBa_K325909 biobrick is functional in E.Coli Pro strain, which is derived from DH5alpha.



27.09.17

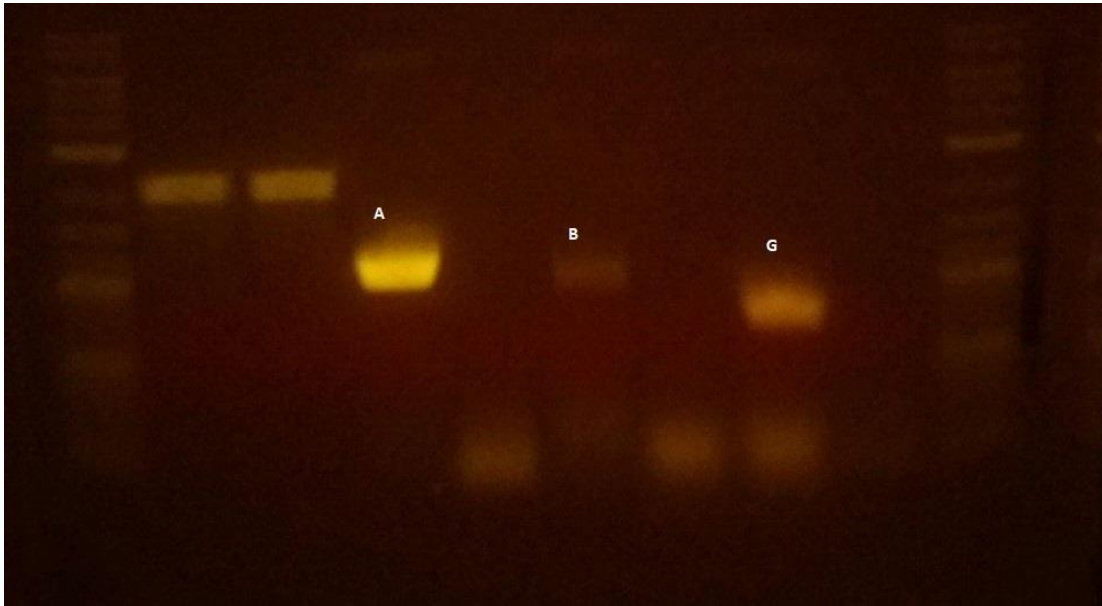
LuxA, LuxB and LuxG was extracted by PCR from K325909 and cloned into pSB1C3.

luxAR	AAAAAACTGCAGCGCCGCTACTAGTATTA	ttatttaggttcttttaagaaaggag
luxAF	AAAAAAGAATTCGCGGCCGCTTCTAG	atgaagtttgaaatatttgttttc
luxBR	AAAAAACTGCAGCGCCGCTACTAGTATTA	ttatggtaaattcatttcgatttttg
luxBF	AAAAAAGAATTCGCGGCCGCTTCTAG	atgaaatttgattatTTTTTctaac
luxGR	AAAAAACTGCAGCGCCGCTACTAGTATTA	ttatacgtagtgcAAAagcatcgg
luxGF	AAAAAAGAATTCGCGGCCGCTTCTAG	atgattgttgatggtagagtttcaaaaatag

LuxA-Tannealing=59C

LuxB-Tannealing=57C

LuxG-Tannealing=64C

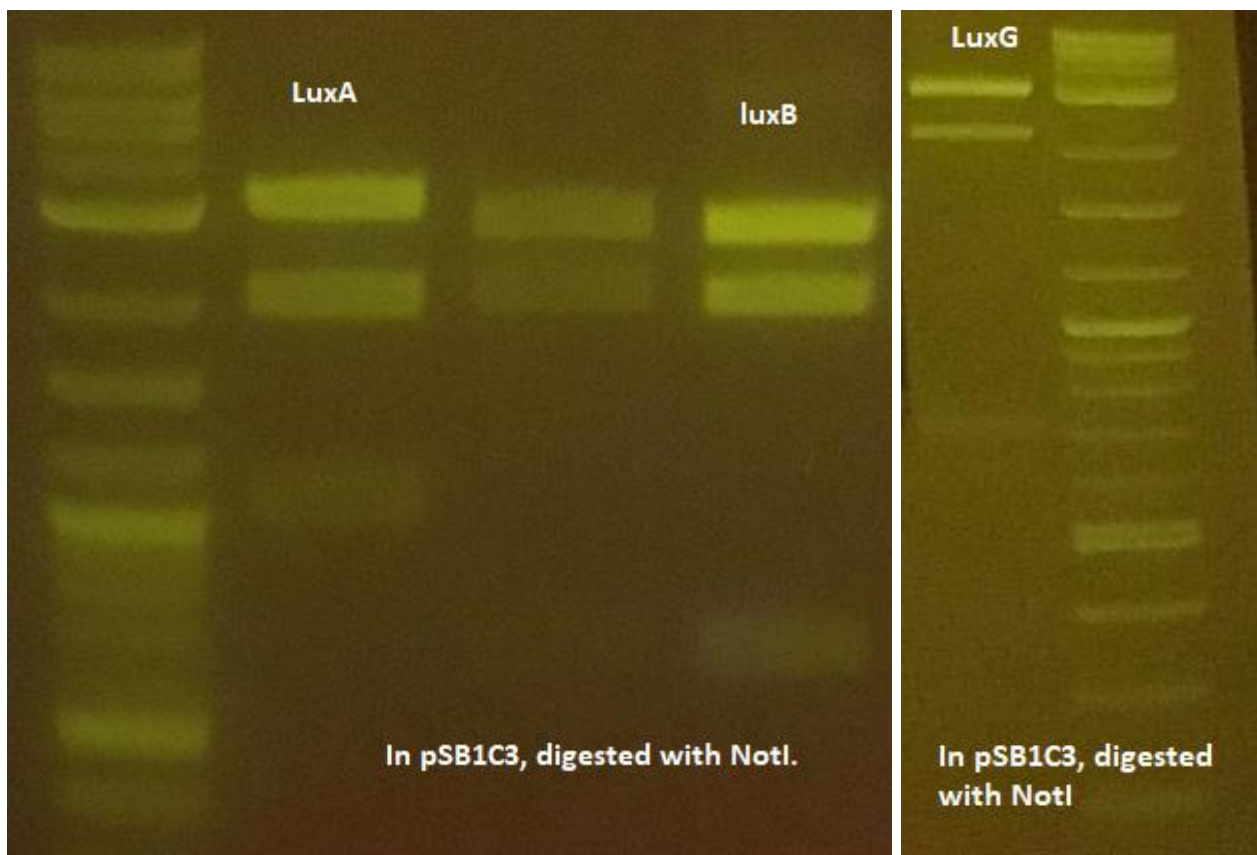


30.09.17

LuxABG, each with added iGEM prefix and suffix, were cut by EcoRI/PstI. EcoRI/PstI cut linear pSB1C3 backbone was also prepared. Each CDS was ligated with the backbone and ligation mixture was transformed to E.Coli Pro cells.

1.10.17

Colonies were selected from plates. Minipreps were digested with NotI to confirm succesful ligation.



Unfortunately luciferase work ends here. We had some unlucky moments while constructing the final construct which delayed it further and further and it was never finished due to time constraints.