The Great Journey to Lux-embourg

WEDNESDAY, 7/12/2017

My plan is as follows to synthesize both the Lux-LysR/PmmsA and Lux-PmmsA constructs.

For the Lux vector: K823025
- Restriction digest with EcoRI/PstI
- Gel purify

For the Insert: LysR/PmmsA or PmmsA
- Restriction digest with EcorI/PstI
- PCR Miniprep Purify

Ligate the digested parts using NEB Calculator overnight with a heat inactivation step the following morning.

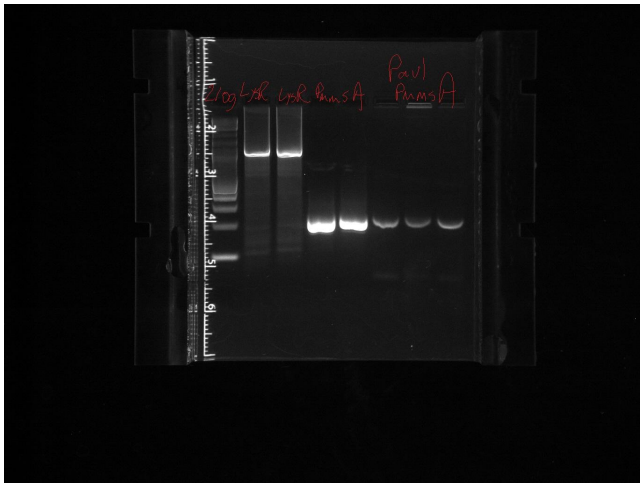
Tfx into comp cells:
+ control K823025
- control Comp cells w h20
Vector/Insert/Ligase: The dream
Vector/Insert/No ligase: little to no
Vector/ligase: No colonies

White colonies will then be subject to colony PCR. If a 1.1 kb (LysR-PmmsA) or .2 kb (PmmsA) is present, miniprep and confirm by restriction digest the presence of the insert before sending for sequencing. This must work or I may lose my mind.

THURSDAY, 7/13/2017

PCR + gel Purified LysR-Pmmsa and Pmmsa. Restriction Digest with EcorI PstI... K823025 was digested with EcoRI PstI yesterday.

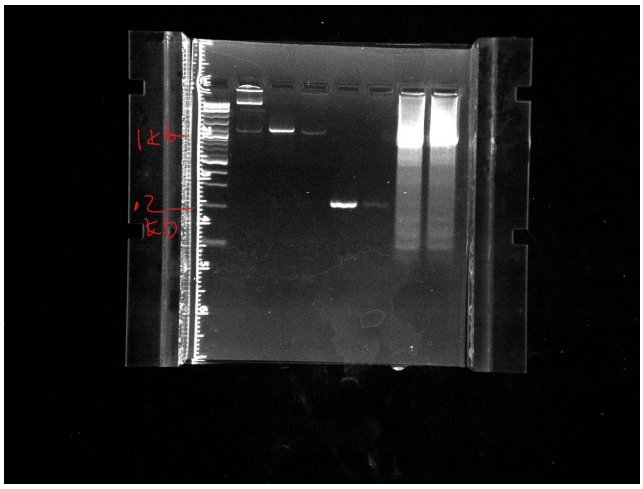
7-12 Amplification and Paul's Colony.jpg



Ran a 1.5% gel for digested materials
1: 2 log ladder
2: Digested k823025 lux vector
3: Digested LysR-PmmsA
4: Control LysR-PmmsA G block
5: Digested PmmsA
6: Control PmmsA G block

7+8: LysR for purification

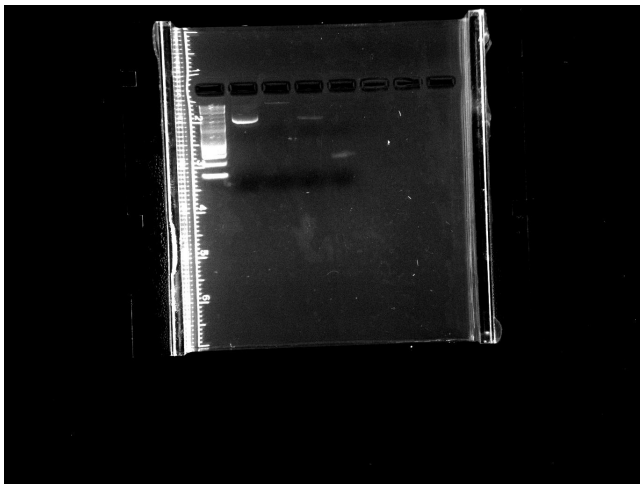
7-12ResDigest_LI.jpg



FRIDAY, 7/14/2017

Ran gel concentrations
Well 1: 2 log
Well 2: LysR 25 ng
Well 3: Vector Digested expected 13 ng (2.6 ng/ul)- 5 ul
Well 4: LysR digested expected 6 ng (1.2 ng/ul)- 5 ul
Well 5: Pmmsa Digested expected 5.5 ng (1.1 ng/ul)- 5 ul

7-14 Gel Concentration.jpg



MONDAY, 7/17/2017

Tfx on 7/15, low results on 7/16 so began ligation to retransform with fresh digested plasmid. Colony PCR on 7/16 which failed so reran DNA on 7/17. Will talk with Paul to verify PCR was done correctly. If failed, will re-tfx cells and reperform colony pcr.

TUESDAY, 7/18/2017

May have LysR construct... confirming PmmsA and LysR from 7/17 tmrw.

Inked7-18 LysR Nina and Mason_LI.jpg