

JULY

Saturday July 1st, 2017

- Miniprep samples AlaRS (pUC57), ArgRS (pUC57), ProRS (pUC57), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), HisRS (pJET), MS2BP-Histag, ThrRS (pUC57), ProRS (pJET), PheRS beta (pUC57), RF3 (pUC57), T7 Polymerase (pUC57), MetRS (pJET), and AsnRS (pJET)
- PCR amplified samples AlaRS (pUC57), ArgRS (pUC57), ProRS (pUC57), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), MetRS (pJET), MTF (pJET), EF-Ts (pJET), CK (pJET), PPIase (pJET), tRNAPhe-MS2 (pJET), HisRS (pJET), MS2BP-Histag (pJET), ThrRS (pUC57), ProRS (pJET), PheRS beta (pUC57), RF3 (pUC57), 7T Polymerase (pUC57), and AsnRS (pJET)
- Inoculations were done for IF3 (pJET)
- pJET cloning was conducted at 1 μ L and 2 μ L for samples CK, IF2, MetRS, EYFP-Spinach, EF-G, RRF, AsnRS, and RF1

Sunday July 2nd , 2017

- Gel confirmation of samples AlaRS (pUC57), ArgRS (pUC57), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), HisRS (pJET), ProRS (pUC57), MS2BP-Histag, ThrRS (pUC57), ProRS (pJET), PheRS beta (pUC57), RF3 (pUC57), 7T Polymerase (pUC57), and AsnRS (pJET)
- Inoculated samples AlaRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), ThrRS (pUC57), PheRS beta (pUC57), T7 Polymerase (pUC57), MetRS (pJET), IF2 (pJET), RF1 (pJET), RRF (pJET), AsnRS (pJET), EF-G (pJET), and EYFP-Spinach (pJET)
- pSB1C3 plasmid cultures were miniprep for use
- Gel confirmation for Samples MS2BP-Histag (pSb1C3), AsnRS (pSb1C3), AspRS (pSb1C3), and HisRS (pSb1C3)

Monday July 3rd , 2017

- Miniprep samples AlaRS (pUC57), MetRS (pJET), GlyRS beta (pUC57), LeuRS (pUC57), ThrRS (pUC57), PheRS beta (pUC57), and T7 Polymerase (pJET)
- PCR amplification of AlaRS (pUC57), MetRS (pJET), GlyRS beta (pUC57), LeuRS (pUC57), ThrRS (pUC57), PheRS beta (pUC57), and T7 Polymerase (pJET)
- Prepared samples to be sent for sequencing

Tuesday July 4th, 2017

- EF-G (pJET) sample was mini-prepped from overnight culture
- PCR amplification of samples MetRS (pJET), AlaRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), ThrRS (pUC57), PheRS beta (pUC57), EF-G (pJET), AsnRS (pJET), RF3 (pJET), RRF (pJET), AlaRS (pJET), GlyRS beta (pJET), LeuRS (pJET), ThrRS (pJET), T7 Polymerase (pJET), HisRS (pJET), MTF (pJET), EF-Ts (pJET), PPIase (pJET) and T7 Polymerase (pJET)

Wednesday July 5th, 2017

- Miniprepped samples AlaRS (pUC57), AsnRS (pJET), GlyRS beta (pUC57), LeuRS (pUC57), ThrRS (pUC57), RF1 (pJET), T7 Polymerase (pJET)

Thursday July 6th, 2017

- Gel confirmation of Samples EF-G (pJET), AsnRS (pJET), RF1 (pJET), RRF (pJET), AlaRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), ThrRS (pUC57), T7 Polymerase (pJET), HisRS (pJET), MTF (pJET), EF-Ts (pJET), and PPIase (pJET)
- Samples EYFP-Spinach (pJET), IF3 (pJET) and IF2 (pJET) were transformed into chemically competent DH5 α cells
- Ran an agarose gel of all of the pSB1C3 to test the plasmids
- Roto-evaporated the plasmid and took the OD reading
- Ran PCR of the DH5 alpha tRNAPhe-MS2 and MS2BP-Histag Products and ran a gel electrophoresis of the product
- Restriction digest of pSB1C3

Friday July 7th, 2017

- Gel confirmations of PCR/Colony PCR samples T7 polymerase (pUC57), LeuRS (pUC57), GlyRS beta (pUC57), ThrRS (pUC57), AlaRS (pUC57), RF1 (pJET), RRF (pJET), and AsnRS (pUC57)
- Colony PCR of EYFP-Spinach (pJET), IF3 (pJET), and IF2 (pJET) were performed
- Sequencing results were analyzed and ArgRS (pUC57), GlnRS (pUC57), MetRS (pJET), PheRS beta (pUC57), ProRS (pUC57), RF3 (pUC57), and CK (pJET) were all confirmed
- Ran gel electrophoresis of pSB1C3
- Re-attempted to do a PCR amplification pSB1C3

Saturday July 8th, 2017

- Gel confirmation of colony PCR samples EYFP-Spinach (pJET), IF3 (pJET), IF2 (pJET), and RRF (pJET)

- Colony PCR done for samples EYFP-Spinach (pJET), IF3 (pJET), IF2 (pJET), RRF (pJET), AspRS (pUC57), and EF-G (pJET)
- pSB1C3 plasmid samples were miniprepped from overnight cultures.
- Inoculated HisRS (pJET), MetRS (pJET), MTF (pJet), EF-Ts (pJET), CK (pJET), PPIase (pJET), tRNAPhe-MS2 (pJET), MS2BP-Histag (pJET), ArgRS (pUC57), GlnRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), ThrRS (pUC57)
- PCR amplification of 16 and 23S sequences in the rrnB operon

Sunday July 9th, 2017

- Colony PCR of samples EYFP-Spinach (pJET), IF3 (pJET), IF2 (pJET), EF-G (pJET), RRF (pJET), and AspRS (pUC57)
- Gel confirmation of colony PCR samples
- Overnight cultures of RRF (pJET) and EYFP-Spinach were made
- Streak plates of RF3 (pJET), MetRS (pJET), tRNAPhe-MS2 (pJET), and MS2BP-Histag (pJET) were created from DMSO stocks
- PCR amplification of samples EF-Ts (pJET), HisRS (pJET), MTF (pJET), PPIase (pJET), MS2BP-Histag (pJET), tRNAPhe-MS2 (pJET), MetRS (pJET), ArgRS (pUC57), ProRS(pUC57), PheRS beta (pUC57), CK (pJET), GlnRS (pUC57) and RF3 (pUC57). The following samples were also run on a gel for confirmation
- Gel confirmation of the 16S and 23S.PCR products
- Restriction of the 16 and 23S subunits

Monday July 10th, 2017

- Gel confirmation of colony PCR of samples IF3 (pJET), EF-G (pJET), and IF2 (pJET)
- Gel extractions done for samples HisRS (pJET), MTF (pJET), PPIase (pJET), MS2BP-Histag (pJET), tRNAPhe-MS2 (pJET), MetRS (pJET), ArgRS (pUC57), ProRS(pUC57), PheRS beta (pUC57), CK (pJET), GlnRS (pUC57) RF3 (pUC57) and pSB1C3 backbone
- Samples AlaRS (pUC57), AsnRS (pJET), GlyRS beta (pUC57), LeuRS (pUC57), ThrRS (pUC57), RF1 (pJET), and T7 Polymerase (pUC57)
- PCR of the rrnB operon with 16S forward primer/ 23S forward primer and the rrnB terminator reverse primer

Tuesday July 11th, 2017

- Colony PCR reactions of samples IF2 (pJET), EYFP-Spinach (pJET), IF3 (pJET), EF-G (pJET) were run

Wednesday July 12th, 2017

- Overnight cultures of AspRS (pUC57) were miniprepped
- Overnight culture of AspRS (pUC57) was created from DMSO stocks



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- Gel confirmation of colony PCR samples IF2 (pJET), EYFP-Spinach (pJET), IF3 (pJET) and EF-G (pJET)
- Colony PCR of samples IF2 (pJET), EYFP-Spinach (pJET), IF3 (pJET) and EF-G (pJET) were run

Thursday July 13th, 2017

- Gel confirmation of colony PCR samples IF2 (pJET), EYFP-Spinach (pJET), IF3 (pJET) and EF-G (pJET)

Friday July 14th, 2017

- Miniprep samples IF2 (pJET), EF-G (pJET), RRF (pJET), and EYFP-Spinach (pJET).
- Prepared samples IF2 (pJET), EF-G (pJET), RRF (pJET), EYFP-Spinach (pJET) and AspRS (pUC57) for sending for sequence confirmation
- Colony PCR of samples AsnRS (pJET), ThrRS (pUC57), IF3 (pJET), and RF1 (pJET)
- Overnight digests of purified samples EF-Ts (pJET), HisRS (pJET), PPIase (pJET), MTF (pJET), MS2BP-Histag (pJET), tRNAPhe-MS2 (pJET), MetRS (pJET), ArgRS (pUC57), ProRS (pUC57), PheRS beta (pUC57), CK (pJET), GlnRS (pUC57), and RF3 (pUC57)
- PCR amplification of samples RRF (pJET), AspRS (pUC57), IF2 (pJET), EF-G (pJET) and EYFP-Spinach (pJET)

Saturday July 15th, 2017

- Gel confirmation of colony PCR samples AsnRS (pJET), ThrRS (pUC57), IF3 (pJET) and RF1 (pJET)
- Overnight cultures for samples AlaRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), T7 Polymerase (pUC57) and ThrRS (pUC57) were created

Sunday July 16th, 2017

- Miniprep samples ThrRS (pUC57), AlaRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), and T7 Polymerase (pUC57)
- Gel extractions of samples RRF (pJET), AspRS (pUC57), IF2 (pJET), EF-G (pJET), EYFP-Spinach (pJET) and pSB1C3
- Overnight cultures of pSB1C3, EF-Ts (pJET), HisRS (pJET), MTF (pJET), PPIase (pJET), MS3BP-Histag (pJET), tRNAPhe-MS2 (pJET), MetRS (pJET), ArgRS (pUC57), ProRS (pUC57), PheRS beta (pUC57), CK (pJET), GlnRS (pUC57), and RF3 (pUC57)

Monday July 17th, 2017

- PCR Amplified samples AspRS (pUC57), IF2 (pJET), EF-G (pJET), IF2 (pJET), EYFP-Spinach (pJET), RRF (pJET), EF-Ts (pJET), PPIase (pJET), MetRS (pJET) and GlnRS (pUC57)
- Miniprep samples CK (pJET), MTF (pJET), MS2BP-Histag (pJET) and tRNAPhe-MS2 (pJET)
- Overnight cultures of samples MS2BP-Histag, pSB1C3, EF-Ts (pJET), MetRS (pJET), ArgRS (pUC57), PPIase (pJET), RF3 (pUC57), ProRS (pUC57), HisRS (pJET), PheRS beta (pUC57), GlnRS (pUC57) and RRF (pJET)
- Inoculated BL21 (DE3) gold cells in preparation for chemical competent cell production

Tuesday July 18th, 2017

- Colony PCR was performed of samples AsnRS (pJET) and IF3 (pJET)
- Gel confirmation of samples AsnRS (pJET) and IF3 (pJET)
- Ligation reactions (10µl) into pSB1C3 were created using samples HisRS (pJET), MTF (pJET), MS2BP-Histag (pJET), tRNAPhe-MS2 (pJET), ArgRS (pUC57), ProRS (pUC57), PheRS beta (pUC57), CK (pJET) and RF3 (pUC57)
- PCR amplification (50µL) of sample EYFP-Spinach (pJET) was created and run
- Miniprep pSB1C3 backbone and samples MetRS (pJET), PPIase (pJET), RRF (pJET), Ef-Ts (pJET) and MS2BP-Histag (pJET)
- Prepared chemically competent BL21 (DE3) gold cells
- Electrophoresis of the PCR products from July 10th
- Ran large scale PCR of the rrnB operon
- Transformation into BL21 Gold of samples EYFP-Spinach (pUC57), MTF (pJET), HisRS (pJET), EF-Ts (pJET), PPIase (pJET), and tRNAPhe-MS2 (pJET)
- Transformation into DH5 α cells of samples T7 polymerase (pUC57) and EYFP-Spinach (pJET)

Wednesday July 19th, 2017

- Miniprep pSB1C3 and samples GlnRS (pUC57), HisRS (pJET), PheRS beta (pUC57), ProRS (pUC57), and RF3 (pUC57)
- Ligation reactions into pSB1C3 were created using digested samples AspRS (pUC57), GlnRS (pUC57), MetRS (pJET), IF2 (pJET), EF-G (pJET), RRF (pJET) and PPIase (pJET)
- Gel confirmation for PCR amplified samples EYFP-Spinach (pJET), and enzyme restricted samples HisRS (pJET), MTF (pJET), and EF-Ts (pJET)
- Enzyme restricted pSB1C3 samples were gel extracted for use in ligations
- PCR amplified samples GlnRS (pUC57), HisRS (pJET), PheRS beta (pUC57), ProRS (pUC57) and RF3 (pUC57)

- Restriction digest samples AspRS (pUC57), GlnRS (pUC57), MetRS (pJET), IF2 (pJET), EF-G (pJET), EF-Ts (pJET), RRF (pJET) and PPIase (pJET) were enzymatically cleaned
- Transformations were done with purified plasmid samples CK (pJET), RF3 (pUC57), HisRS (pJET), ProRS (pUC57), PheRS beta (pUC57), ArgRS (pUC57), tRNAPhe- MS2 (pJET), MS2BP-Histag (pJET) and MTF (pJET)
- Ran large scale 16S and 23S ribosomal subunits PCR amplification
- Gel confirmation of the PCR products

Thursday July 20th, 2017

- Colony PCR was performed of samples RF1 (pJET), MS2BP-Histag (pSB1C3), MTF (pSB1C3), RF3 (pSB1C3) and AsnRS (pJET)
- Enzymatic digests of samples GlnRS (pUC57), PheRS beta (pUC57), ProRS (pUC57) and RF3 (pUC57)
- Miniprep samples IF3 (pJET), ArgRS (pUC57), and plasmid backbone pSB1C3.
- Samples ThrRS (pUC57), IF3 (pJET), EF-G (pJET) and EYFP-Spinach were sent for sequencing
- PCR amplification of samples ArgRS (pUC57), HisRS (pJET), PheRS beta (pUC57), ProRS (pUC57), CK (pJET), tRNAPhe-MS2 (pJET), AlaRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), and T7 Polymerase (pUC57)
- Agarose gel confirmation of colony PCR samples RF1 (pJET), AsnRS (pJET), MTF (pJET), RF3 (pSB1C3) and MS2BP-Histag (pSB1C3)
- Prepared new competent BL21 Gold and DH5 α cells
- PCR amplification of the rrnB operon
- Gel confirmation was done for the rrnB operon
- Transformations into DH5 α of samples AspRS (pUC57), GlnRS (pUC57), MetRS (pJet), IF2 (pJET), EF-G (pJET), EF-Ts (pJET), RRF (pJET), PPIase (pJET), and pUC19 (pJET- control)

Friday July 21st, 2017

- Gel confirmation of samples AlaRS (pUC57), ArgRS (pUC57), tRNAPhe-MS2 (pJET), PheRS beat (pUC57), ProRS (pUC57), HisRS (pJET), CK (pJET), LeuRS (pUC57) and T7 Polymerase.
- Digested samples GlnRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), RF3 (pUC57), and HisRS (pJET) were enzymatically cleaned for use in ligations
- Restriction digested samples GlyRS beta (pUC57), LeuRS (pUC57) and T7 Polymerase (pUC57)

- Overnight cultures of samples EYFP-Spinach (pJET), ArgRS (pUC57), HisRS (pJET), PheRS beta (pUC57), ProRS (pUC57), CK (pJET), tRNAPhe-MS2 (pJET) and AlaRS (pUC57)
- Restricted pSB1C3 and the rrnB PCR products
- Ran agarose Gel electrophoresis of the restriction samples and gel extracted

Saturday July 22nd, 2017

- pSB1C3 was gel extracted
- Restriction digest samples GlyRS beta (pUC57), LeuRS (pUC57), and T7 Polymerase (pUC57) were enzymatically cleaned for use in ligation reactions
- pSB1C3 samples were digested
- Overnight cultures were created for samples EYFP-Spinach (pJET), ArgRS (pUC57), HisRS (pJET), PheRS beta (pUC75), ProRS (pUC57), CK (pJET), tRNAPhe-MS2 (pJET) and AlaRS (pUC57).

Sunday July 23rd, 2017

- Performed transformations of 6 samples into BL21 Gold cells

Monday July 24th, 2017

- Miniprep samples EYFP-Spinach (pJET), AlaRS (pUC57), ArgRS (pUC57), CK (pJET), PheRS beta (pUC57), ProRS (pUC57), tRNAPhe-MS2 (pJET) and HisRS (pJET)
- Enzyme restricted pSB1C3 samples were gel extracted for use in ligations
- Colony PCR samples were confirmed on an agarose gel for samples RF3 (pUC57), MS2BP-Histag (pJET), RF1 (pJET), AsnRS (pJET), MTF (pJET), and ProRS (pSB1C3)

Tuesday July 25th, 2017

- Ligation reactions into pSB1C3 were performed with samples RF3 (pUC57), HisRS (pJET), GlnRS (pUC57), PheRS beta (pUC57), GlyRS beta (pUC57), ProRS (pUC57), T7 Polymerase (pUC57) and LeuRS (pUC57)
- Miniprep samples EYFP-Spinach (pJET), IF3 (pJET), ThrRS (pUC57) and EF-G (pJET).
- PCR amplifications of samples EYFP-Spinach (pJET)
- In vitro transcription of RNA spinach was performed

Wednesday July 26th, 2017

- PCR amplification samples AlaRS (pUC57), ArgRS (pUC57), AspRS (pUC57), MetRS (pJET), PheRS beta (pUC57), ProRS (pUC57), ThrRS (pUC57), MTF (pJET), IF2 (pJET), IF3 (pJET), EF-G (pJET), EF-Ts (pJET), RF3 (pUC57), RRF

(pJET), CK (pJET), PPIase (pJET), tRNAPhe-MS2 (pJET), EYFP-Spinach (pJET), and MS2BP-Histag (pJET) were gel confirmed.

- AlaRS (pUC57), ProRS (pUC57), MTF (pJET), and MS2BP-Histag (pJET) were digested with SpeI and XbaI
- Overnight cultures were done for pSB1C3 backbone samples
- PCR amplification of samples MTF (pJET), RF3 (pUC57), MS2BP-Histag (pJET) and ProRS (pUC57)
- Transformations into pSB1C3 were done with samples GlyRS beta (pUC57), PheRS beta (pUC57), LeuRS (pUC57), ProRS (pUC57), T7 Polymerase (pUC57), RF3 (pUC57), GlnRS (pUC57), HisRS (pJET), AsnRS (pJET), and RF1 (pJET)
- Performed test overexpression of the 6 samples transformed into BL21 (DE3) gold
- Test Over expression of HisRS (pJET), MTF (pJET), EF-Ts (pJET), PPIase (pJET), tRNAPhe-MS2 (pJET), and MS2BP-Histag (pJET)

Thursday July 27th, 2017

- pSB1C3 backbone samples were digested overnight and cut with SpeI and XbaI
- Ligation reactions into pSB1C3 were done with digested samples AlaRS (pUC57), ProRS (pUC57), MTF (pJET) and MS2BP-Histag (pJET)

Friday July 28th, 2017

- pSB1C3 backbone samples were restriction digested overnight and was cut with EcoRI and PstI
- DH5α cells were inoculated for use as competent cells

Saturday July 29th, 2017

- Transformations into pSb1C3 were done with samples AlaRS (pUC57), ArgRS (pUC57), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), RF3 (pUC57), LeuRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), ThrRS (pUC57), and T7 Polymerase (pUC57)
- Colony PCR was performed and then gel confirmed on samples MetRS (pJET), IF2 (pJET), RF1 (pJET), RRF (pJET), and CK (pJET). CK (pJET) was confirmed for insert

Sunday July 30th, 2017

- pSB1C3 digests were enzymatically cleaned and gel extracted
- Colony PCR was performed on samples RF3 (pUC57), ProRS (pUC57), PheRS beta (pUC57), RF1 (pJET), T7 Polymerase (pUC57), LeuRS (pUC57), GlyRS beta (pUC57), HisRS (pJET), and GlnRS (pUC57)

- Inoculated samples IF3 (pJET), AsnRS (pSB1C3), HisRS (pSB1C3), MS2BP-Histag (pSB1C3), AlaRS (pUC57), ArgRS (pUC57), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), RF3 (pUC57), LeuRS (pUC57), PheRS beta (pUC57), ThrRS (pUC57), and T7 Polymerase (pUC57)

Monday July 31st, 2017

- Gel confirmation of colony PCR samples RF3 (pUC57), ProRS (pUC57), PheRS beta (pUC57), RF1 (pJET), AsnRS (pJET), T7 Polymerase (pUC57), LeuRS (pUC57), GlyRS beta (pUC57), HisRS (pJET), and GlnRS (pUC57)
- Gel confirmation of digested samples AlaRS (pUC57), ArgRS (pUC57), AsnRS (pJET), AspRS (pUC57), HisRS (pJET), ProRS (pUC57), ThrRS (pUC57), MTF (pJET), IF3 (pJET), EF-G (pJET), EF-Ts (pJET), RF3 (pUC57), RRF (pJET), CK (pJET), tRNAPhe-MS2 (pJET), EYFP-Spinach (pJET), and MS2BP-Histag
- Colony PCR of samples RF1 (pJET) and AsnRS (pJET)
- PCR amplification (50µL) of samples MetRS (pJET), HisRS (pJET), PheRS beta (pUC57), PPIase (pJET), IF2 (pJET), and RRF (pJET)
- pSB1C3 backbone samples were digested Overnight with EcoRI and PstI restriction enzymes