

September

Friday September 1st, 2017

- Gel confirmed PCR samples RF3 (pSB1C3), IF1 (pUC57), EF-G (pSB1C3), T7 Polymerase (pUC57), HisRS (pJET), LeuRS (pUC57), GlyRS beta (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), AsnRs (pSB1C3), ArgRS (pSB1C3), IF3 (pSB1C3) and RF1 (pSB1C3)
- Inoculated samples T7 Polymerase (pUC57), LeuRS (pSB1C3), EF-G (pSB1C3), HisRS (pSB1C3), PPIase (pJET), tRNAPhe-MS2 (pSB1C3), AlaRS (pSB1C3), EF-TS (pSB1C3), AspRS (pSB1C3), IF1 (pUC57), RF1 (pSB1C3), RF3 (pSB1C3), ArgRS (pSB1C3), GlnRS (pSB1C3), and GlyRS beta (pSB1C3)

Saturday September 2nd, 2017

- Miniprep samples AsnRS (pSB1C3), AspRS (pSB1C3), IF3 (pSB1C3), IF1 (pUC57), RF1 (pSB1C3), Ef-Ts (pSB1C3), ArgRS (pSB1C3), PPIase (pJET), RF3 (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), and EF-G (pSB1C3)

Tuesday September 5th, 2017

- PCR amplified samples IF3 (pSB1C3), RF1 (pSB1C3), EF-TS (pSB1C3), IF1 (pUC57), PPIase (pSB1C3), EF-G (pJET), ArgRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), LeuRS (pUC57), RF3 (pSB1C3)
- Transformed samples RRF (pSB1C3), MS2BP-Histag (pSB1C3), tRNAPhe-MS2 (pSB1C3), and AlaRS (pSB1C3)

Wednesday September 6th, 2017

- Digested samples PPIase (pJET), LeuRS (pUC57), and EF-G (pJET)
- Inoculated BL21 (DE3) gold and DH5 α strains for making chemically competent cells
- Gel confirmed PCR amplification samples IF3 (pSB1C3), RF1 (pSB1C3), EF-TS (pSB1C3), IF1 (pUC57), PPIase (pSB1C3), and EF-G (pSB1C3)
- Samples ArgRS (pSB1C3), AsnRS (pSB1C3), AspRS (pSB1C3), GlyRS beta (pSB1C3), IF1 (pUC57), and RF3 (pSB1C3) were sent for sequencing
- Phenol/Chloroform extraction and ethanol precipitation for RNA from in-vitro was performed

Thursday September 7th, 2017

- Made chemically competent cells using both DH5alpha and BL21 (DE3) gold E. coli Strains
- Transformed tRNAPhe-MS2 (pJET), EYFP-Spinach (pJET), MS2BP-Histag (pJET), RRF (pJET), RF3 (pUC57), IF2 (pJET), ProRS (pUC57) and LeuRS (pUC57) into BL21 (DE3) gold

Friday September 8th, 2017

- Colony PCR of samples ArgRS (pSB1C3), AsnRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), MetRS (pSB1C3), MTF (pSB1C3), IF2 (pSB1C3), IF3 (pSB1C3), and RF3 (pSB1C3)
- Gel confirmed samples ArgRS (pSB1C3), AsnRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), LeuRS (pSB1C3), MetRS (pSB1C3), MTF (pSB1C3), IF2 (pSB1C3), RF1 (pSB1C3), RF3 (pSB1C3), CK (pSB1C3), PPIase (pSB1C3), EYFP-Spinach (pSB1C3), GluRS (pSB1C3), PheRS alpha (pUC57), SerRS (pUC57), TrpRS (pUC57), ValRS (pUC57), MK (pUC57), NDK (pUC57), T7 Polymerase (pJET) and HisRS (pJET)

Saturday September 9th, 2017

- Test streaked plates containing antibiotic
- digested samples HisRS (pJET) and T7 Polymerase (pJET)
- Transformed HisRS (pSB1C3), EF-G (pSB1C3), EF-Ts (pSB1C3), RF1 (pSB1C3), RRF (pSB1C3), PPIase (pSB1C3), EYFP-Spinach (pSB1C3), MS2BP-Histag (pSB1C3), AlaRS (pSB1C3), LeuRS (pSB1C3), PheRS beta (pSB1C3), ProRS (pSB1C3), ThrRS (pSB1C3), T7 Polymerase (pSB1C3), tRNA-Phe-MS2 (pSB1C3), IF2 (pSB1C3), RF3 (pSB1C3), and ProRS (pSB1C3)
- Gel confirmed samples ArgRS (pSB1C3), AsnRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), MetRS (pSB1C3), MTF (pUC57), IF2 (pSB1C3), tRNAPhe-MS2 (pJET), GluRS (pSB1C3), PheRS alpha (pUC57), SerRS (pUC57), TrpRS (pUC57), ValRS (pUC57), IF1 (pUC57), IF3 (pSB1C3), RF3 (pSB1C3), CK(pSB1C3), and PPIase (pSB1C3)
- Ran PCR samples on a gel

Tuesday September 12th, 2017

- Chemically competent cells of BL21 (DE3) gold were made and transform tested

- pSB1C3 sample was cut with PstI aliquot to test enzyme viability
- Samples SerRS (pUC57), AlaRS (pUC57), ArgRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3) and TrpRS (pUC57) were sent for sequencing

Wednesday September 13th, 2017

- pSB1C3 sample cut with PstI was heat killed at 80 degrees for 20 minutes

Thursday September 14th, 2017

- pSB1C3 sample cut with PstI was gel confirmed
- Colony PCR of samples HisRS (pSB1C3), MetRS (pSB1C3), RF3 (pSB1C3), EF-Ts (pSB1C3), GlnRS (pSB1C3), ArgRS (pSB1C3), ValRS (pUC57), SerRS (pUC57), IF3 (pSB1C3), MetRS (pSB1C3), MTF (pUC57), PheRS alpha (pUC57), and IF2 (pSB1C3)
- Test overexpression of LeuRS (pUC57), ProRS (pUC57), RF3 (pUC57), RRF (pJET), tRNAPhe-MS2 (pJET), EYFP-Spinach (pJET), and MS2BP-HIstag (pJET)
- Fluorescence test for EYFP-Spinach was performed

Friday September 15th, 2017

- Two pSB1C3 plasmid aliquots predigested were dephosphorylated and heat killed
- Ligations were done with the dephosphorylated vector and samples PPIase (pJET), RRF (pJET), LeuRS (pUC57), EF-G (pJET), T7 Polymerase (pUC57), HisRS (pJET), ProRs (pUC57), EYFP-Spinach (pJET), CK (pJET), IF3 (pJET), IF2 (pJET), and MTF (pJET)
- Run SDS-PAGE gel of overexpression samples LeuRS (pUC57), ProRS (pUC57), RF3 (pUC57), RRF (pJET), tRNAPhe-MS2 (pJET), EYFP-Spinach (pJET), and MS2BP-Histag (pJET)

Saturday September 16th, 2017

- Chemical competent cells were made with the DH5alpha strain
- Gel confirmed samples MK (pUC57), NDK (pUC57), TrpRS (pUC57), and IF1 (pUC57)

- Overnight Digestions were done with samples MK (pUC57), NDK (pUC57), TrpRS (pUC57), and IF1 (pUC57) with enzymes EcoRI and PstI
- Inoculated samples HisRS (pSB1C3), RRF (pSB1C3), ProRS (pSB1C3), LeuRS (pSB1C3), TyrRS (pUC57), IleRS (pUC57), LysRS (pUC57), and ValRS (pSB1C3)
- Heat killed ligation samples
- Transformed sample IF2 (pJET) into BL21 (DE3) Gold
- Transformed samples PPIase (pSB1C3), RRF (pSB1C3), LeuRS (pSB1C3), EF-G (pSB1C3), T7 Polymerase (pSB1C3), HisRS (pSB1C3), ProRS (pSB1C3), EYFP-Spinach (pSB1C3), CK (pSB1C3), IF2 (pSB1C3)
- PCR amplified samples MK (pUC57), NDK (pUC57), IF1 (pUC57), and TrpRS (pUC57)

Monday September 18th, 2017

- Miniprep samples ValRS (pUC57), LeuRS (pSB1C3), TyrRS (pUC57), IleRS (pUC57), LysRS (pUC57), ProRS (pSB1C3), PPIase (pSB1C3), RRF (pSB1C3), and HisRS (pSB1C3)

Tuesday September 19th, 2017

- Inoculated samples MetRS (pSB1C3), IF2 (pSB1C3), IF3 (pSB1C3), EF-G (pSB1C3), CK (pSB1C3) and HisRS (pSB1C3) was done
- Removed incorrect stop codon from MetRS via site-directed mutagenesis
- Inoculated samples GluRS (pUC57), IleRS (1)(pUC57), LysRS (pUC57), PheRS alpha (pUC57), SerRS (pUC57), TyrRS (pUC57), ValRS (1)(pUC57), and ValRS (2)(pUC57)

Wednesday September 20th, 2017

- Miniprep and PCR amplified samples HisRS (pSB1C3), MetRS (pSB1C3), EF-G (pSB1C3), CK (pSB1C3), IF3 (pSB1C3), IF2 (pSB1C3), RRF (pSB1C3) and PPIase (pSB1C3)
- Overnight Dpn1 digest of MetRS
- Make DMSO stocks of GluRS (pUC57), IleRS (1)(pUC57), Lys RS (pUC57), PheRS alpha (pUC57), SerRS (pUC57), TyrRS (pUC57), ValRS (1)(pUC57), ValRS (2) (pUC57) in DH5alpha

- Miniprep samples GluRS (pUC57), IleRS (1)(pUC57), LysRS(pUC57), PheRS alpha (pUC57), SerRS (pUC57), TyrRS (pUC57), ValRS (1)(pUC57), ValRS (2)(pUC57)
- PCR amplified GluRS (pUC57), IleRS (1)(pUC57), LysRS (pUC57), PheRS alpha (pUC57), SerRS (pUC57), TyrRS (pUC57), ValRS (1)(pUC57), ValRS (2)(pUC57)
- Inoculations for test expressions of ProRS (pUC57), RF3 (pUC57), EYFP-Spinach (pJET), and MS2BP-Histag (pJET)
- Overexpression of tRNA construct and MS2
- Copurification of tRNA and MS2

Thursday September 21th, 2017

- Transformed PPIase (pSB1C3), CK (pSB1C3), RRF (pSB1C3), RF1 (pSB1C3), EF-Ts (pSB1C3), EF-G (pSB1C3), MTF (pSB1C3), and AsnRS (pSB1C3)
- Gel confirmed PCR product samples HisRS (pSB1C3), MetRS (pSB1C3), EF-G (pSB1C3), CK (pSB1C3), IF3 (pSB1C3), IF2 (pSB1C3), RRF (pSB1C3) and PPIase (pSB1C3)
- Heat killed Dpn1 and confirmed mutagenesis by running a 1% agarose gel
- Transformed MetRS with stop codon removed into DH5alpha
- Sent GluRS, IleRS (1), LysRS, PheRS alpha, SerRS, TyrRS, ValRS (1), ValRS (2) in pUC57 for sequencing
- Transformed GlyRS alpha (pUC57) and EF-Tu (pUC57) into DH5alpha
- Transformed ArgRS (pSB1C3), AspRS (pSB1C3), and RF3 (pSB1C3) into BL21 (DE3) Gold
- Test overexpression of ProRS (pUC57), RF3 (pUC57), EYFP-Spinach (pJET), and MS2BP-Histag (pJET) and ran on SDS-PAGE gel
- Gel confirmation of MS2/tRNA purification samples

Sunday September 24th, 2017

- Overnight culture was prepared for sample T7-polymerase (pUC57)

Monday September 25th, 2017

- Ligation reactions for samples HisRS (pSB1C3), MetRS (pSB1C3), MTF (pSB1C3), IF2 (pSB1C3), IF3 (pSB1C3), EF-G (pSB1C3), EF-Ts (pSB1C3), CK (pSB1C3), and PPIase (pSB1C3)
- Transformed IleRS (2) into DH5alpha
- Transformed HisRS (pJET), MTF (pJET), IF2 (pJET), IF3 (pJET), EF-G (pJET), EF-Ts (pJET), RRF (pJET), CK (pJET), PPIase (pJET), EYFP-Spinach (pJET), TrpRS (pUC57), IF1 (pUC57), MK (pUC57), NDK (pUC57), AlaRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), ThrRS (pUC57), T7 Polymerase (pUC57) into BL21 (DE3) Gold
- Set up 5 mL overnight cultures in triplicate of GlyRS alpha (pUC57), EF-Tu (pUC57) and Met RS stop codon removed
- Miniprepmed T7-polymerase (pUC57) sample

Tuesday September 26th, 2017

- Inoculated samples IF2 (pSB1C3), IF3 (pSB1C3), EF-G (pSB1C3), RRF (pSB1C3), CK (pSB1C3), PPIase (pSB1C3) and AsnRS (pSB1C3)
- Heat killed ligation reactions
- Miniprepmed GIRS alpha (pUC57), EF-Tu (pUC57) and MetRS (pJET) stop codon removed
- Sent GlyRS alpha (pUC57), EF-Tu (pUC57) and MetRS stop codon removed (pJET) for sequencing
- PCR amplified samples GlnRS (pSB1C3), GlyRS beta (pSB1C3), AlaRS (pSB1C3), LeuRS (pSB1C3), PheRS beta (pSB1C3), ProRS (pSB1C3), ThrRS (pSB1C3), T7 Polymerase (pSB1C3), EYFP-Spinach (pSB1C3), MS2BP-Histag (pSB1C3), and the pSB1C3 vector
- GlnRS PCR products were gel confirmed

Wednesday September 27th, 2017

- Miniprepmed and PCR amplified samples RRF (pSB1C3), PPIase (pSB1C3), CK (pSB1C3), EF-G (pSB1C3), IF3 (pSB1C3), IF2 (pSB1C3), and AsnRS (pSB1C3), GlnRS (pSB1C3) and GlyRS beta (pSB1C3)
- PCR clean up of AlaRS (pSB1C3), LeuRS (pSB1C3), PheRS beta (pSB1C3), ProRS (pSB1C3), ThrRS (pSB1C3), T7 Polymerase (pSB1C3), EYFP-Spinach (pSB1C3), MS2BP-Histag (pSB1C3) and pSB1C3 vector

- Ligations of AlaRS (pSB1C3), LeuRS (pSB1C3), PheRS beta (pSB1C3), ProRS (pSB1C3), ThrRS (pSB1C3), T7 Polymerase (pSB1C3), EYFP-Spinach (pSB1C3), and MS2BP-Histag (pSB1C3)
- Gel confirmation of GlnRS (pSB1C3) and GlyRS beta (pSB1C3) samples

Thursday September 28th, 2017

- Gel confirmed PCR samples RRF (pSB1C3), PPIase (pSB1C3), CK (pSB1C3), EF-G (pSB1C3), IF3 (pSB1C3), IF2 (pSB1C3), and AsnRS (pSB1C3)
- Transformed samples AsnRS (pSB1C3), MTF (pSB1C3), EF-TS (pSB1C3), and RF1 (pSB1C3)
- Inoculated samples HisRS (pSB1C3), MetRS (pSB1C3), IF2 (pSB1C3), IF3 (pSB1C3), EF-G (pSB1C3), RRF (pSB1C3), and CK (pSB1C3)
- Transformed AlaRS (pSB1C3), LeuRS (pSB1C3), PheRS beta (pSB1C3), ProRS (pSB1C3), ThrRS (pSB1C3), T7 Polymerase (pSB1C3), EYFP-Spinach (pSB1C3), and MS2BP-Histag (pSB1C3)

Friday September 29th, 2017

- Miniprep and PCR amplified samples HisRS (pSB1C3), RRF (pSB1C3), EF-G (pSB1C3), MetRS (pSB1C3), IF3 (pSB1C3), IF2 (pSB1C3), and CK (pSB1C3)
- Gel confirmed sample T7-polymerase (pUC57) on 1% agarose gel
- Overnight culture of T7-polymerase (pUC57)

Saturday September 30th, 2017

- Chemically competent cells of the DH5alpha strain were made
- Inoculated samples HisRS (pSB1C3), MTF (pSB1C3), IF3 (pSB1C3), EF-TS(pSB1C3), EF1 (pSB1C3), and RRF (pSB1C3)
- Gel confirmed samples HisRS (pSB1C3), RRF (pSB1C3), EF-G (pSB1C3), MetRS (pSB1C3), IF3 (pSB1C3), IF2 (pSB1C3), and CK (pSB1C3)
- Test overexpression of AlaRS (pUC57), GlnRS (pUC57), LeuRS (pUC57), PheRS beta(pUC57), ProRS (pUC57), ThrRS (pUC57), TrpRS (pUC57), SerRS (pJET), IF1 (pUC57), MK (pUC57), Arg RS (pSB1C3), AspRS (pSB1C3), and RF3 (pSB1C3)



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- Inoculated HisRS (pJET), MetRS (pJET), AsnRS (pJET), IF2 (pJET), IF3 (pJET), EF-G (pJET), Ef-TS (pJET), CK (pJET), PPIase (pJET), and EYFP-Spinach (pJET) for test over expressions
- Overexpression of T7-polymerase(pUC57) was performed