

## AUGUST

### Tuesday August 1st, 2017

- Overnight ligations for samples AlaRS (pUC57), ArgRS (pUC57), AspRS (pUC57), GlnRS (pUC57), IleRS (pUC57), ProRS (pUC57), RF3 (pUC57), ThrRS (pUC57), T7 polymerase (pUC57), CK (pJET), EF-G (pJET), EF-Ts (pJET), EYFP-Spinach (pJET), HisRS (pJET), IF3 (pJET), MS2BP-Histag (pJET), MTF (pJET), and tRNA Phe-MS2 (pJET)
- PCR amplification for samples HisRS (pJET), IF2 (pJET), MetRS (pJET), PheRS beta (pJET), PPiase (pJET), and RRF (pJET)
- PCR amplification of rrnB operon
- Overnight cultures of pKK3535 and pSB1C3
- A 3.5% urea page was run with tRNA Phe-MS2 samples

### Wednesday August 2nd, 2017

- Gel confirmation of HisRS (pJET), IF2 (pJET), MetRS (pJET), PheRS beta (pJET), PPiase (pJET), and RRF (pJET)
- pSB1C3 was digested with EcoR1 and *pst*1
- Transformed AlaRS (pUC57), ArgRS (pUC57), AspRS (pUC57), GlnRS (pUC57), LeuRS (pUC57), ProRS (pUC57), RF3 (pUC57), ThrRS (pUC57), T7 polymerase (pUC57), CK (pJET), EF-G (pJET), EF-Ts (pJET), EYFP-Spinach (pJET), HisRS (pJET), IF3 (pJET), MS2BP-Histag (pJET), MTF (pJET), and tRNA Phe-MS2 (pJET)
- PCR products of samples HisRS (pJET), IF2 (pJET), MetRS (pJET), PheRS beta (pJET), PPiase (pJET), and RRF (pJET) were cleaned up with the enzymatic reaction clean up protocol
- Overnight cultures of AsnRS (pJET), RF1 (pJET) and RRF (pJET)
- PCR clean up of rrnB operon

### Thursday August 3rd, 2017

- Transformed tRNA Phe-MS2 (pJET) and T7 polymerase (pJET) into DH5 $\alpha$
- Digest performed on pSB1C3 and rrnB
- A urea page was run with tRNA Phe-MS2 samples

### Friday August 4th, 2017

- Overnight digestion for samples HisRS (pJET), IF2 (pJET), MetRS (pJET), PheRS beta (pJET), PPiase (pJET), and RRF (pJET)
- Overnight ligations was carried out for samples HisRS (pSB1C3), IF2 (pSB1C3), MetRS (pSB1C3), PheRS beta (pSB1C3), and PPiase (pSB1C3)

### Saturday August 5th, 2017

- RRF was digested using *pst1* and *EcoR1* and was set for overnight ligations using pJET
- Heat kill ligated products HisRS, IF2, MetRS, and PPiase in pSB1C
- Streak plates of samples AlaRS (pSB1C3), ArgRS (pSB1C3), AspRS (pSB1C3), CK (pSB1C3), EF-G (pSB1C3), EF-Ts (pSB1C3), EYFP-spinach (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), HisRS (pSB1C3), IF3 (pSB1C3), LeuRS (pSB1C3), MS2BP-Histag (pSB1C3), MTF (pSB1C3), ProRS (pSB1C3), RF3 (pSB1C3), tRNAPhe-MS2 (pSB1C3), and T7 polymerase (pSB1C3) in DH5 $\alpha$  cells

## Sunday August 6th, 2017

- Ligated product of RRF in pSB1C3 was heat killed and transformed
- Overnight cultures of samples AlaRS (pSB1C3), ArgRS (pSB1C3), AspRS (pSB1C3), CK (pSB1C3), EF-G (pSB1C3), EF-Ts (pSB1C3), EYFP-spinach (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), HisRS (pSB1C3), IF3 (pSB1C3), LeuRS (pSB1C3), MS2BP-Histag (pSB1C3), MTF (pSB1C3), ProRS (pSB1C3), RF3 (pSB1C3), tRNAPhe-MS2 (pSB1C3), and T7 polymerase (pSB1C3) were made
- Streak plates of samples AlaRS (pSB1C3), AspRS (pSB1C3), EYFP-spinach (pSB1C3), GlnRS (pSB1C3), GlyRS (pSB1C3), HisRS (pSB1C3), IF3 (pSB1C3), MS2BP-Histag (pSB1C3), MTF (pSB1C3), ProRS (pSB1C3), RF3 (pSB1C3), ThrRS (pSB1C3), and tRNAPhe-MS2 (pSB1C3)
- Transformed samples CK (pSB1C3), EF-G (pSB1C3), EF-Ts (pSB1C3), EYFP-spinach (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), IF2 (pSB1C3), IF3 (pSB1C3), LeuRS (pSB1C3), MetRS (pSB1C3), MTF (pSB1C3), PheRS beta (pSB1C3), PPiase (pSB1C3), ProRS (pSB1C3), RF3 (pSB1C3), RRF (pSB1C3), T7-polymerase (pSB1C3), ThrRS (pSB1C3), and tRNAPhe-MS2 (pSB1C3) in DH5 $\alpha$  cells

## Monday August 7th, 2017

- Transformed CK using DH5 $\alpha$  competent cells to check cell validity
- Streak plated samples AlaRS (pSB1C3), ArgRS (pSB1C3), AspRS (pSB1C3), CK (pSB1C3), EF-G (pSB1C3), EF-Ts (pSB1C3), GlnRS (pSB1C3), IF3 (pSB1C3), MS2BP-Histag (pSB1C3), LeuRS (pSB1C3), MTF (pSB1C3), ProRS (pSB1C3), RF3 (pSB1C3), T7-polymerase (pSB1C3), ThrRS (pSB1C3), and tRNAPhe-MS2 (pSB1C3)
- Inoculated samples AlaRS (pSB1C3), AsnRS (pSB1C3), HisRS (pSB1C3), PheRS beta (pSB1C3), PPiase (pSB1C3), RF1 (pSB1C3), ThrRS (pSB1C3), and tRNAPhe-MS2 (pSB1C3)
- Miniprepped samples AsnRS (pSB1C3) and EF-G (pSB1C3)

## Tuesday August 8th, 2017

- Miniprepped samples AlaRS (pSB1C3), AsnRS (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), RF1 (pSB1C3), PheRS beta (pSB1C3) and PPiase (pSB1C3)
- Inoculated samples ArgRS (pSB1C3), LeuRS (pSB1C3), MS2BP-Histag (pSB1C3), and RF3 (pSB1C3)
- Inoculated samples AsnRS (pJET) and RF1 (pJET)
- Transformed samples AlaRS (pSB1C3), AspRS (pSB1C3), CK (pSB1C3), GlnRS (pSB1C3), HisRS (pSB1C3), EF-Ts (pSB1C3), EYFP-Spinach (pSB1C3), IF2 (pSB1C3), IF3 (pSB1C3), LeuRS (pSB1C3), MetRS (pSB1C3), MS2BP-Histag (pSB1C3), MTF (pSB1C3), ProRS (pSB1C3), RF3 (pSB1C3), tRNAPhe-MS2 (pSB1C3), and T7-polymerase (pSB1C3) into DH5 $\alpha$  competent cells
- Ligation of tRNAPhe-MS2 into pSb1C3
- PCR amplification of EYFP-Spinach

## **Wednesday August 9th, 2017**

- PCR amplification of AsnRS (pJET) , GlyRS beta (pSB1C3), PheRS beta (pSb1C3), ThrRS (pSb1C3), EF-G (pSb1C3), RF1 (pSb1C3), RRF (pSb1C3), and PPiase (pSb1C3)
- PCR clean up of EYFP-Spinach was performed and gel confirmed

## **Thursday August 10th, 2017**

- Digestions of AlaRS (pJET), AsnRS (pJET), EF-G (pJET), GlnRS (pJET), GlyRS beta (pJET), PheRS beta (pJET), PPiase (pJET), RF1 (pJET), and ThrRS (pJET) EF-G (pJET), PPiase (pJET), and RRF (pJET) was digested with EcoR1 and *pst1* overnight
- Overnight cultures of samples AsnRS (pJET) and RF1 (pJET) was made
- Transformed MTF using DH5 $\alpha$  competent cells to check the validity of the competent cells
- Transformed tRNAPhe-MS2 (pSb1C3)
- In vitro transcription of RNA Spinach using PCR cleaned-up and gel confirmed EYFP-Spinach
- Transformation into BL21(DE3) Gold of tRNAPhe-MS2(pJET)

## **Friday August 11th, 2017**

- Transformed samples ArgRS (pSB1C3), AspRS (pSB1C3), CK (pSB1C3), IF3 (pSB1C3), MTF (pSB1C3), ProRS (pSB1C3), RF3 (pSB1C3), ThrRS (pSB1C3), and tRNAPhe-MS2 (pSB1C3) in DH5 $\alpha$  cells

## **Saturday August 12th, 2017**

- Gel extraction of pSB1C3
- Inoculated samples ArgRS (pSB1C3), MTF (pSB1C3), ProRS (pSB1C3), and IF3 (pSB1C3)

- Overnight ligations for samples AlaRS, AspRS, tRNAPhe-MS2, EF-Ts, and EYFP-Spinach into pSB1C3
- Transformed samples GlnRS (pSB1C3), HisRS (pSB1C3), IF2 (pSB1C3), LeuRS (pSB1C3), MetRS (pSB1C3), MS2BP-Histag (pSB1C3), RF3 (pSB1C3), T7 Polymerase (pSB1C3), and ThrRS (pSB1C3) into DH5 $\alpha$  cells

## Sunday August 13th, 2017

- Miniprepped samples AlaRS (pJET), ArgRS (pSB1C3), AspRS (pSB1C3), MTF (pSB1C3), ProRS (pSB1C3), ThrRS (pSB1C3), and EYFP-Spinach (pJET)

## Monday August 14th, 2017

- PCR amplified samples AlaRS (pJET), ArgRS (pSB1C3), AspRS (pSB1C3), MTF (pSB1C3), ProRS (pSB1C3), ThrRS (pSB1C3), and EYFP-Spinach (pJET)

## Tuesday August 15th, 2017

- Transformed samples AlaRS (pSB1C3), ArgRS (pSB1C3), AspRS (pSB1C3), EF-Ts (pSB1C3), HisRS (pSB1C3), MS2BP-Histag, PheRS beta (pSB1C3), ProRS (pSB1C3), and ThrRS (pSB1C3) in DH5 $\alpha$  cells

## Wednesday August 16th, 2017

- RRF was digested using EcoR1 and Pst1
- Transformed samples HisRS (pSB1C3), MetRS (pSB1C3), ProRS (pSB1C3), and PheRS beta (pSB1C3) into DH5 $\alpha$  cells
- Overnight cultures of pSB1C3 RFP

## Thursday August 17th, 2017

- RRF was digested with EcoR1 and Pst1, heat killed and then cleaned up with the enzymatic cleaned
- Inoculated AspRS (pSB1C3), and EF-Ts (pSB1C3)
- pSB1C3 was miniprepped and then digested with EcoR1 and Pst1

## Friday August 18th, 2017

- Digested pSB1C3 was cleaned up using enzymatic reaction clean-up kit
- Miniprepped samples HisRS (pSB1C3), MetRS (pSB1C3), PheRS beta (pSB1C3), and ProRS (pSB1C3)

## Saturday August 19th, 2017

- Samples HisRS (pSB1C3), MetRS (pSB1C3), ProRS (pSB1C3), and PheRS Beta (pSB1C3) were PCR amplified and gel confirmed

- pSB1C3 was digested using EcoR1 and Pst1 and then gel extracted
- Overnight ligations for samples CK, IF3, IF2, RF3, MS2BP-Histag, MTF, and ThrRS into pSB1C3

## Sunday August 20th, 2017

- Overnight ligated samples CK (pSB1C3), IF3 (pSB1C3), IF2 (pSB1C3), RF3 (pSB1C3), MS2BP-Histag (pSB1C3), MTF (pSB1C3), and ThrRS (pSB1C3) was heat killed
- ProRS was PCR amplified and then gel confirmed
- Overnight culture for samples MetRS (pSB1C3) and HisRS (pSB1C3)

## Monday August 21st, 2017

- Samples GlnRS (pSB1C3), GlyRS alpha (pSB1C3), HisRS (pSB1C3), LeuRS (pSB1C3), T7 Polymerase (pSB1C3), RRF (pSB1C3), and MetRS (pSB1C3) were PCR amplified
- Transformed samples AlaRS (pSB1C3), CK (pSB1C3), EF-Ts (pSB1C3), HisRS (pSB1C3), IF3 (pSB1C3), ProRS (pSB1C3), RF3 (pSB1C3), MetRS (pSB1C3), MS2BP-Histag (pSB1C3), ThrRS (pSB1C3), and tRNAPhe-MS2 (pSB1C3) into DH5 $\alpha$  cells
- Streak plated samples RF1 (pJET), ProRS (pUC57), and MTF (pSB1C3), AlaRS (pUC57), ArgRS (pUC57), AsnRS (pJET), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), HisRS (pJET), LeuRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), ThrRS (pUC57), MTF (pJET), IF2 (pJET), IF3 (pJET), EF-G (pJET), EF-Ts (pJET), RF1 (pJET), RF3 (pUC57), RRF (pJET), CK (pJET), PPiase (pJET), T7 Polymerase (pUC57), and MS2BP-Histag (pJET)

## Tuesday August 22th, 2017

- Samples GlyRS beta( pUC57), LeuRS (pUC57), MetRS (pJET), T7 Polymerase (pUC57) gel confirmed and then digested using EcoR1 and Pst1
- Inoculated samples RRF (pJET), HisRS (pJET), and RF1 (pJET)
- Inoculated samples MTF (pSB1C3), ProRS (pSB1C3), ThrRS (pSB1C3), MetRS (pSB1C3), AlaRS (pSB1C3), IF3 (pSB1C3), tRNAPhe-MS2 (pSB1C3), EF-Ts (pSB1C3), RF3 (pSB1C3), CK (pSB1C3), EYFP-Spinach (pSB1C3), HisRS (pSB1C3) and AsnRS (pJET)
- GlyRS alpha, MetRS, LeuRS, and T7 polymerase samples were digested overnight using EcoR1 and Pst1
- Samples GlyRS alpha (pSB1C3), MetRS (pSB1C3), and GlnRS (pUC57) were PCR amplified

- Inoculated samples AlaRS (pUC57), ArgRS (pUC57), AsnRS (pJET), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), HisRS (pJET), LeuRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), ThrRS (pUC57), MTF (pJET), IF2 (pJET), IF3 (pJET), EF-G (pJET), EF-Ts (pJET), RF1 (pJET), RF3 (pUC57), RRF (pJET), CK (pJET), PPiase (pJET), T7 Polymerase (pUC57), pSB1C3 RFP, and MS2BP-Histag (pJET)

## Wednesday August 23rd, 2017

- Miniprepped samples HisRS (pSB1C3), MetRS (pSB1C3), ProRS (pSB1C3), MTF (pSB1C3), RF1 (pSB1C3), RF3 (pSB1C3), CK (pSB1C3)
- Samples HisRS (pSB1C3), MetRS (pSB1C3), ProRS (pSB1C3), MTF (pSB1C3), RF1 (pSB1C3), RF3 (pSB1C3), CK (pSB1C3), and GlnRS (pSB1C3) were PCR amplified
- Digested and enzymatically cleaned samples GlyRS (pUC57), LeuRS (pUC57), MetRS (pUC57)
- pSB1C3 was digested using EcoR1 and Pst1
- Inoculated samples RRF (pSB1C3), HisRS (pSB1C3), ThrRS (pSB1C3), AlaRS (pSB1C3), IF3 (pSB1C3), tRNAPhe-MS2 (pSB1C3), EF-Ts (pSB1C3), and EYFP-Spinach (pSB1C3)
- Sample MetRS (pSB1C3) was PCR amplified and gel confirmed
- Miniprepped samples AlaRS (pUC57), ArgRS (pUC57), AsnRS (pJET), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), HisRS (pJET), LeuRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), ThrRS (pUC57), MTF (pJET), IF2 (pJET), IF3 (pJET), EF-G (pJET), EF-Ts (pJET), RF1 (pJET), RF3 (pUC57), RRF (pJET), CK (pJET), PPiase (pJET), T7 Polymerase (pUC57), pSB1C3 RFP, and MS2BP-Histag (pJET)
- Samples AlaRS (pUC57), ArgRS (pUC57), AsnRS (pJET), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), HisRS (pJET), LeuRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), ThrRS (pUC57), MTF (pJET), IF2 (pJET), IF3 (pJET), EF-G (pJET), EF-Ts (pJET), RF1 (pJET), RF3 (pUC57), RRF (pJET), CK (pJET), PPiase (pJET), T7 Polymerase (pUC57), pSB1C3 RFP, and MS2BP-Histag (pJET) was PCR amplified

## Thursday August 24th, 2017

- Miniprepped HisRS (pSB1C3) sample
- pSB1C3 was digested with EcoR1 and Pst1 and enzymatically cleaned
- Samples AlaRS (pUC57), ArgRS (pUC57), AsnRS (pJET), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), HisRS (pJET), LeuRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), ThrRS (pUC57), MTF (pJET), IF2 (pJET), IF3 (pJET), EF-G (pJET), EF-Ts (pJET), RF1 (pJET), RF3 (pUC57), RRF (pJET), CK (pJET), PPiase (pJET), T7 Polymerase (pUC57), pSB1C3 RFP, and MS2BP-Histag (pJET)

(pJET), EF-G (pJET), EF-Ts (pJET), RF1 (pJET), RF3 (pUC57), RRF (pJET), CK (pJET), PPiase (pJET), T7 polymerase (pUC57), pSB1C3 RFP, and MS2BP-Histag (pJET) were digested using EcoRI and PstI, and enzymatically cleaned

- The above samples were ligated into PSB1C3 and then transformed into DH5 $\alpha$  cells

## **Friday August 25th, 2017**

- Miniprepped samples ArgRS (pSB1C3), AsnRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), HisRS (pSB1C3), LeuRS (pSB1C3), MetRS (pSB1C3), MTF (pSB1C3), IF2 (pSB1C3), RF1 (pSB1C3), RF3 (pSB1C3), CK (pSB1C3), PPiase (pSB1C3), EYFP-Spinach (pSB1C3), GluRS (pUC57), PheRS alpha (pUC57), SerRS (pUC57), TrpRS (pUC57), ValRS (pUC57), IF1 (pUC57), MK (pUC57), and NDK (pUC57)

## **Sunday August 27th, 2017**

- Samples ArgRS (pSB1C3), AsnRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), GlyRS beta (pSB1C3), HisRS (pSB1C3), LeuRS (pSB1C3), MetRS (pSB1C3), MTF (pSB1C3), IF2 (pSB1C3), RF1 (pSB1C3), RF3 (pSB1C3), CK (pSB1C3), PPiase (pSB1C3), EYFP-Spinach (pSB1C3), GluRS (pUC57), PheRS alpha (pUC57), SerRS (pUC57), TrpRS (pUC57), ValRS (pUC57), IF1 (pUC57), MK (pUC57), and NDK (pUC57) were PCR amplified

## **Monday August 28th, 2017**

- Samples HisRS (pSB1C3), LeuRS (pSB1C3), MetRS (pSB1C3), MTF (pSB1C3), IF2 (pSB1C3), CK (pSB1C3), PPiase (pSB1C3), EYFP-spinach (pSB1C3), HisRS (pSB1C3), GluRS (pUC57), PheRS alpha (pUC57), SerRS (pUC57), TrpRS (pUC57), ValRS (pUC57), MK (pUC57), and NDK (pUC57) was gel confirmed and then sent for sequencing
- Phenol/chloroform extraction of RNA from in-vitro was performed and then gel confirmed

## **Tuesday August 29th, 2017**

- Inoculated samples ArgRS (pSB1C3), AsnRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), LeuRS (pSB1C3), IF3 (pJET), RF1 (pSB1C3), RF3 (pSB1C3), and IF1 (pUC57)

## **Wednesday August 30th, 2017**

- Samples tRNAPhe-MS2 (pSB1C3), MS2BP-Histag (pSB1C3), ThrRS (pSB1C3), AlaRS (pSB1C3), and EF-Ts (pSB1C3) was transformed into DH5 $\alpha$  cells
- Miniprepped samples ArgRS (pSB1C3), AsnRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), LeuRS (pSB1C3), IF3 (pJET), RF1 (pSB1C3), RF3 (pSB1C3), and IF1 (pUC57)
- PCR amplified EYFP-Spinach

## Thursday August 31st, 2017

- PCR amplified samples ArgRS (pSB1C3), AsnRS (pSB1C3), AspRS (pSB1C3), GlnRS (pSB1C3), LeuRS (pSB1C3), IF3 (pJET), RF1 (pSB1C3), RF3 (pSB1C3), T7 Polymerase (pUC57), PPiase (pJET), HisRS (pJET), LeuRS (pUC57), EF-G (pJET) and IF1 (pUC57)
- Transformed RRF (pSB1C3), tRNAPhe-MS2 (pSB1C3), MS2BP-Histag (pSB1C3), ThrRS (pSB1C3), AlaRS (pSB1C3), and EF-Ts (pSB1C3) into DH5 $\alpha$  cells