



Lethbridge iGEM 2017

JUNE

Thursday June 1st, 2017

Friday June 2nd, 2017

- Ran T7 Transcription assay using PCR amplified DNA fragment
- Urea PAGE of in vitro check of T7 transcription was conducted

Saturday June 3rd, 2017

Sunday June 4th, 2017

Monday June 5th, 2017

- Overnight cultures created for *rrnB* operon in DH5 α and ampicillin resistant plasmid

Tuesday June 6th, 2017

- Minipreped *rrnB* operon and ampicillin resistant plasmid

Wednesday June 7th, 2017

Thursday June 8th, 2017

Friday June 9th, 2017

- PCR amplified *rrnB* operon

Saturday June 10th, 2017

- Agarose gel of the PCR amplification of the *rrnB* operon was run

Sunday June 11th, 2017

Monday June 12th, 2017

Tuesday June 13th, 2017

- Calculated the measurements for PCR amplification of the 16S and 23S ribosomal subunits in the *rrnB* operon

Wednesday June 14th, 2017



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- PCR amplification of 16S and 23S ribosomal sequences
- streak plated colonies for T7 Polymerase testing. colonies are inoculated and put through FACS prep

Thursday June 15th, 2017

- Gel electrophoresis for the 16S and 23S PCR amplifications was conducted

Friday June 16th, 2017

Saturday June 17th 2017

Sunday June 18th, 2017

Monday June 19th, 2017

- pJet cloning and transformation using DH5 α cells of samples AsnRS (pJET), CK (pJET), EF-G (pJET), EF-Ts (pJET), EYFP-Spinach (pJET), IF2 (pJET), IF3 (pJET), HisRS (pJET), MetRS (pJET), MTF (pJET), PPIase (pJET), RRF, and tRNAPhe-MS2 (pJET)
- Preparation of DH5 α competent cells

Tuesday June 20th, 2017

- Overnight cultures made from samples HisRS (pJET), MetRS (pJET), MTF (pJET), IF3 (pJET), EF-G (pJET), EF-Ts (pJET), RF1 (pJET), and CK (pJET)
- Miniprepped pSB1C3 samples and gel confirmed

Wednesday June 21st, 2017

- Miniprepped samples CK (pJET), EF-G (pJET), EF-Ts (pJET), HisRS (pJET) MetRS (pJET), IF3 (pJET), MTF (pJET), RF1 (pJET), and tRNAPhe-MS2 (pJET)
- Overnight cultures made for samples MetRS (pJET), IF3 (pJET), EF-G (pJET), RF1 (pJET), RRF (pJET), CK (pJET), PPIase (pJET), and tRNAPhe-MS2 (pJET)
- Analyzed Agarose gel of the rrnB operon and the 16S and 23S
- Calculated the mutagenesis of the NdeI insert into the 16S and 23S ribosomal subunits
- Blunt end cloning of T7 Polymerase performed

Thursday June 22nd, 2017

- Miniprepped samples MetRS (pJET), IF3 (pJET), EF-G (pJET), RF1 (pJET), RRF (pJET), CK (pJET), PPIase (pJET), and tRNAPhe-MS2 (pJET)
- Ran gel electrophoresis of mutagenesis products
- PCR amplification of the *rrnB* operon

Friday June 23rd, 2017

- Overnight-culture for samples AsnRS (pJET), EYFP-Spinach (pJET), and IF2 (pJET)
- PCR using Taq polymerase for samples AsnRS (pJET), CK (pJET), EF-G (pJET), EYFP-Spinach (pJET), IF3 (pJET), IF2 (pJET), MetRS (pJET), PPIase (pJET), RFI (pJET), RRF (pJET), and tRNA Phe-MS2 (pJET)
- Large scale mutagenesis of the *NdeI* insert into the 16S and 23S ribosomal sequences
- Gel electrophoresis of the the PCR of the *rrnB* operon
- prepared chemically competent DH5α cells

Saturday June 24th 2017

- Miniprepped of samples AsnRS (pJET), EYFP-Spinach (pJET), and IF2 (pJET)
- Gel confirmation for samples AsnRS (pJET), EF-G (pJET), EYFP-Spinach (pJET), IF3 (pJET), PPIase (pJET), and tRNAPhe-MS2 (pJET)
- Overnight cultures for samples CK (pJET) and RFI (pJET)
- Miniprep of PSB1C3
- OD reading of the *rrnB* PCR product

Sunday June 25th, 2017

- Miniprepped samples CK (pJET) and RFI (pJET)

Monday June 26th, 2017

- Transformed samples CK (pJET), IF2 (pJET), MetRS (pJET), RF1 (pJET), and RRF (pJET). pUC 19 was transformed into pJET as a positive control
- SOC broth prepared
- Streak plated samples AsnRS (pJET), EF-G (pJET), EF-Ts (pJET), EYFP-Spinach (pJET), HisRS (pJET), IF3 (pJET), MTF (pJET), PPIase (pJET) and tRNAPhe-MS2 (pJET) from the DMSO stocks
- Overnight cultures for pSB1C3

- Overnight cultures for DH5 α in LB media
- Inoculated MetRS (pJET), IF2 (pJET), RF3 (pJET), RRF (pJET), CK (pJET), MTF (pJET), EF-G (pJET), PPIase (pJET), tRNAPhe-MS2 (pJET), EYFP-Spinach (pJET)

Tuesday June 27th, 2017

- Gel extraction and overnight ligations for samples AsnRS (pJET), EF-G (pJET), EYFP-Spinach (pJET), HisRS (pJET), IF3 (pJET), MTF (pJET), PPIase (pJET), and tRNAPhe-MS2 (pJET)
- Samples AsnRS (pJET), EF-G (pJET), EF-Ts (pJET), EYFP-Spinach (pJET), IF3 (pJET), MS2BP-Histag (pJET), PPIase (pJET), and tRNAPhe-MS2 (pJET) were sent for sequencing
- Miniprepped samples CK (pJET), RF1 (pJET), and plasmid pSB1C3
- Inoculated samples CK (pJET), IF2 (pJET), MetRS (pJET), RF1 (pJET), and RRF (pJET)
- Restriction of pSB1C3 and the *rrnB* operon using XbaI and SpeI

Wednesday June 28th, 2017

- Miniprepped samples CK (pJET), IF2 (pJET), MetRS (pJET), RF1 (pJET), and RRF (pJET)
- Inoculated samples AsnRS (pJET), EF-G (pJET), EF-Ts (pJET), EYFP-Spinach (pJET), HisRS (pJET), IF3 (pJET), MTF (pJET), and PPIase (pJET)
- Gel electrophoresis to confirm restriction sample

Thursday June 29th, 2017

- Miniprepped samples AsnRS (pJET), EF-G (pJET), EF-Ts (pJET), EYFP-Spinach (pJET), HisRS (pJET), IF3 (pJET), MTF (pJET), and PPIase (pJET)
- PCR amplification of samples CK (pJET), IF2 (pJET), MetRS (pJET), and RF1 (pJET)
- Gel confirmation of the PCR products was carried out
- Samples AlaRS (pUC57), ArgRS (pUC57), AspRS (pUC57), GlnRS (pUC57), GlyRS beta (pUC57), LeuRS (pUC57), PheRS beta (pUC57), ProRS (pUC57), RF3 (pUC57), ThrRS (pUC57), and T7 polymerase (pUC57) were transformed into DH5 α cells

- Restriction of the pSB1C3 and *rrnB* operon was done
- OD reading of the miniprepped plasmids of DH5 α tRNA phe(pJET) and the MS2BP(pJET)
- Gel electrophoresis of the restriction of pSB1C3 and the *rrnB* operon
- Overnight cultures of pSB1C3 were made
- Miniprepped MetRS (pJET), IF2 (pJET), RF3 (pJET), RRF (pJET), and CK (pJET)

Friday June 30th, 2017

- PCR amplified samples AsnRS (pJET), EF-G (pJET), and EYFP-Spinach (pJET).
- Gel confirmation of the PCR was carried out
- Inoculated samples IF3 (pJET), AsnRS (pJET), HisRS (pJET), MS2BP-Histag (pSB1C3), AlaRS (pUC57), ArgRS (pUC57), AspRS (pUC57), GlnRS (pUC57), GlyRS alpha (pUC57), LeuRS (pUC57), PheRS beta (pUC57), RF3 (pUC57), ThrRS (pUC57), and T7 polymerase
- Miniprepped pSB1C3 plasmid
- Restricted pSB1C3 plasmid with *x*Bal and *S*peI
- Gel electrophoresis of the restricted pSB1C3 sample