

Week 17 (23/4/18 - 29/18)

Wednesday:

- Cultivation of BL21DE3 cells containing adult hemoglobin (HbA) and fetal hemoglobin (HbF) from a stock solution provided by our supervisor

Thursday:

- Plasmid purification of HbA and HbF

Week 18 (30/4/18 - 6/5/18)

HbA and HbF amplification

Sunday:

- Cultivation of BL21DE3 cell containing HbA and HbF

Week 19 (7/5/18 - 13/5/18)

Cloning of HbA gene

Monday:

- Gel electrophoresis was done to confirm HbA and HbF in pET-Duet1

Thursday:

- Digestion of HbA and HbF in pET-Duet1 with NcoI and NdeI restriction enzymes

Note: The digestion was not successful. We decided to knock out α subunit from the HbA

Friday:

- Digestion of HbA and HbF in pET-Duet1 with NcoI and NdeI restriction enzymes

Note: The digestion was not successful, all the genes was destructed

Sunday:

- Cultivation repetition of HbA and HbF

Week 20 (14/5/18 - 20/5/18)

Wednesday:

- Digestion of HbA plasmid with NcoI and NdeI restriction enzymes

Note: The digestion was not successful, all the genes was destructed

Thursday:

- Digestion of HbA plasmid was repeated. DNA concentration was to low to continue.

Note: We discovered mistake in our digestion strategy.

1. The enzymes used for digestion was wrong. NcoI and NdeI have different restriction site so they couldn't be ligated.
2. The promoter that was removed is the wrong promoter since we want to keep the beta-subunit in the plasmid.

Week 21, 22, 23, 24 (21/5/18 - 17/6/18)

- NiC and preparations

Week 25 (18/6/18 -24/6/18)

Monday:

- Propagation of HbA, HbF and pET-Duet1

Note: A1 & F1 grew but not with the pET-Duet1 plate

Tuesday:

- Cultivation of BL21DE3 with HbA, HbF and pET-Duet1

Wednesday:

- Culture purification
- Gel electrophoresis confirmed the insert

Thursday:

- Efficiency test of BL21 and TG1 competent cells according to the iGEM protocol

Note: The TG1 cells was efficient but the BL21 test need to be repeated

Friday:

- Amplification of VHb (BBa_K1321200) and RFP into TG1

Note: The cells was not growing in the liquid media, probably wrong antibiotic was used

- Repeat efficiency test of BL21 competent cell

Note: Low efficiency was accomplished

Saturday:

- Cultivation of VHb (K1321200) and RFP in TG1 for the glycerol stock

Week 26 (25/6/18 - 1/7/18)

Monday:

- Transformation of: BBa_J23100, BBa_R0010, BBa_R0011, BBa_K173003, BBa_K1465302 and pSB1C3 backbone into TG1. Plating of the TG1 cells.
- Cultivation of VHb and RFP in TG1 from the glycerol stock

Tuesday:

- Plasmid purification of VHb (BBa_K1321200) and RFP followed by plasmid purification
- 3A assembly of VHb (BBa_K1321200) and RFP into pSB1A3
Note: The 3A assembly was not successful

Wednesday:

- Plasmid purification of GFP (BBa_E0040), BBa_J23100, BBa_R0010, BBa_R0011, BBa_K173003, BBa_K1465302 according to manufacturer's protocol

Friday: OUR BIOBRICKS arrived and DH5alpha from chalmers

- Resuspension of biobricks: BBa_K2602010, BBa_K2602011, BBa_K2602012, BBa_K2602013, BBa_K2602014, BBa_K2602015, BBa_K2602016, BBa_K2602017
- Trial digestion of BBa_K2602010, BBa_K2602012 with XbaI and SpeI restriction enzyme. **Note:** the digestion was not successful

Saturday:

- Digestion of BBa_K2602014 and BBa_K2602016 with XbaI and SpeI restriction enzyme. Digestion was confirmed by gel electrophoresis
- 3A assembly of VHb biobricks (BBa_K2602014 and BBa_K2602016) and RFP/GFP into pSB1A3 backbone
- Transformation of the ligated genes into TG1 competent cells and plating of the cells
Note: The 3A assembly was successful and all colonies was growing

Sunday:

- Gel extraction of the biobricks (BBa_K2602014 and BBa_K2602016) and pSB1C3
- Ligation of BBa_K2602014 and BBa_K2602016 respectively in to pSB1C3

Week 27 (2/7/18 - 8/7/18)

Monday:

- Transformation of the biobricks (BBa_K2602014 and BBa_K2602016) into TG1
Note: Only the BBa_K2602016 transformation was successful
- Digestion of BBa_K2602010, BBa_K2602011, BBa_K2602012, BBa_K2602013, BBa_K2602015 and pSB1C3
Note: Gel electrophoresis confirmed the digestion

Tuesday:

- Clean up of biobricks (BBa_K2602010, BBa_K2602011, BBa_K2602012, BBa_K2602013, BBa_K2602017) according to PCR clean-up protocol of Macherey-Nagel
- Ligation of the biobricks into pSB1C3
- Gel purification of BBa_K2602015 and pSB1C3
- Plasmid purification of VHb + RFP/GFP in pSB1A3

Wednesday:

- Transformation of BBa_K2602010, BBa_K2602011, BBa_K2602012, BBa_K2602013, BBa_K2602017 into TG1 according to IDT, gBlocks fragment protocol
- Efficiency test of DH5 α and BL21
- Transformation of:
 - K608008 (GFP) in pSB1C3 in to DH5 α
 - K608008 (GFP) in pSB1C3 \rightarrow BL21
 - VHb + GFP in pSB1A3 \rightarrow BL21
 - VHb + GFP in pSB1A3 \rightarrow BL21
 - VHb + GFP in pSB1A3 \rightarrow BL21
 - BBa_R0011 \rightarrow BL21

Thursday:

- Resuspension of primers for the sequencing of standard gene from plate kit
- Plasmid purification of BBa_K2602016

Note: We found that the cleaning procedure of the biobrick was done improperly

- Cultivation of the biobricks (BBa_K2602010, BBa_K2602011, BBa_K2602012, BBa_K2602013, BBa_K2602017) was repeated

Friday:

- Plasmid purification of BBa_K2602010, BBa_K2602011, BBa_K2602012, BBa_K2602013, BBa_K2602017

- Growth curve measurements of BL21, BL21 + GFP and BL21 + VHb +GFP
Note: All measurements was done in replica of three

Sunday:

- Cultivation of: GFP in DH5 α , GFP and VHb+GFP in BL21 for glycerol stock

Week 28 (9/7/18 -15/7/18)

Monday:

- Biobrick digestion (BBa_K2602010, BBa_K2602011, BBa_K2602012, BBa_K2602013, BBa_K2602014, BBa_K2602015, BBa_K2602016, BBa_K2602017)
- Transformation of VHb and VHb + RFP into BL21 and cell plating
Note: all colonies grow
- Plasmid purification of BBa_K2602016
- Gel electrophoresis of pSB1C3

Tuesday:

- Digestion purification
- Plasmid purification of BBa_K2602011 and BBa_K2602013
- Transformation of BBa_R0010 and BBa_R0011 into BL21
- PCR amplification of K6808008 (GFP), K1321200 (VHb), RFP, BBa_R0011, GFP + VHb, GFP + RFP, K26020161, K2602013 and K2602016

Wednesday:

- Gel electrophoresis of PCR products
Note: The result was positive for all amplification beside VHb+RFP and VHb+GFP
- Inoculation of R011 and R0010, VHb+RFP, VHb(K1321200)

Thursday:

- Repetition of PCR amplification
Note: Gel electrophoresis of PCR products confirmed GFP, RFP, VHb and BBa_R0016
- Transformation of ligated BBa_K2602010, BBa_K2602013, BBa_K2602016 in sPB1C3 into TG1

Friday:

- Gel electrophoresis of PCR amplification productions
Note: Failed
- Colony PCR
- Transformation of BBa_R0040, BBa_I20270, BBa_J364000, BBa_J364001, BBa_J364002, BBa_J364007, BBa_J364008 and BBa_J364009
Note: This done for the interlab measurements
- Growth curve of BL21, BL21+VHb, BL21 + VHb + GFP and BL21 + BBa_R0010
Note: OD of each starter culture was adjusted to same value

Saturday:

- Gel electrophoresis of colony PCR

Week 29 (16/7/18 - 22/7/18)**Monday:**

- Transformation of K2602016 into BL21
- Cultivation of K2602010, K2602013, J364008, RFP (BBa_J04450), J364000 and GFP (K608008)

Tuesday:

- Preparation of samples for sequencing
- Plasmid purification of:
 - K1321200 (VHb)+K608008 (GFP)
 - K1321200 (VHb)+J04450 (RFP)
 - K1321200 (VHb)
 - J04450 (RFP)
 - BBa_R0010
 - K608008
 - BBa_R0011
 - K2602016

Note: It can be seen from the plate that the J364000, J364008, J364007 have the highest intensity of the green colour

- Transformation of K2602010, K2602013 & K2602016 into BL21. Cell plating.
Note: No culture of K2602016 was found

Wednesday:

- Preparation of cultures for the interLab
- Growth curve measurements of BL21, VHb, VHb+GFP, empty plasmid

Thursday:

- Biobricks (K2602010, K2602013, BBa_J364000, BBa_J364008) was send for second sequencing
- Repeated cultivation of K2602016

Friday:

- Plasmid purification of K2602016
- Calibrations and cells samples for Interlab

Week 30 (23/7/18 - 29/7/18)**Monday:**

- Digestion of pSB1C3 from J04450(RPF)
Note: Gel electrophoresis confirmed extraction
- Ligation of K260200, K2602011, K2602012, K2602013, K2602014, K2602015, K2602016 and K2602017 in to pSB1C3
- Transformation of ligation production into BL21 & DH5 α
- Plating of Interlab cell samples from 96-well plates

Tuesday:

- Colony PCR.
Note page 52: The gene insert was not successful
- Repetition of Colony PCR
- Expression of VHb (K132000) in fresh BL21, old BL21, TG1 and DH5 α
- Counting the colonies from the plating of cell samples from Interlab

Wednesday:

- Plasmid purification of ligated biobricks(K2602011, K2602012, K2602014 and K2602015) in pSB1C3
- PCR amplification

- 3A assembly (K2602010 + GFP, K2602013 + GFP, VHB + GFP, pSB1C3) and transformation into DH5 α

Note: only the K2602010 + GFP and K2602013 + GFP was positive

Thursday:

- Colony PCR of DH5 α
- Sending samples (K2602010, K2602011, K2602012, K2602014 and K2602015 K2602016) for sequencing
- Repeat transformation of K2602017 into DH5 α

Friday:

- Plasmid purification of 3A assembly products (K2602010 + GFP and K2602013 + GFP)
 - Colony PCR of K2602017, K2602010+GFP, K2602013+GFP and VHB+GFP
- Note:** Only K2602010+GFP and VHB+GFP gave positive result

Week 31 (30/7/18 - 5/8/18)

Monday:

- Cultivation of K2602010+GFP, K2602013+GFP, K132000+GFP
- Preparation of starter culture (K2602010, K2602013, K132000 in DH5 α and in BL21)

Tuesday:

- Plasmid purification of 3A assembly product
- Starter culture of R0011, K132000, K2602010, K2602013
- Expression of VHB biobricks:
 - K2602010 + ALA + CAM
 - K2602010 + ALA + CO + CAM
 - K2602013 + ALA + CAM
 - K2602013 + ALA + CO + CAM
 - K2602013 NO ALA & NO CAM
 - R0011 NO ALA, NOCAM + AMP
 - R0011 + ALA + AMP
 - R0011 + ALA + CO + AMP

Wednesday:

- Harvested pellet from expression of VHB
- pH measurements of the cell

- Send samples (K2602010+GFP, K2602013 + GFP, K1321200+GFP) for sequencing

Thursday:

- Prepared starter culture of BL21, R0010+BL21, VHb+BL21, K2602010+BL21, K2602013+BL21
- Expression of VHb biobricks:
 - BL21
 - R0010 + ALA + CO
 - R0010 + ALA,
 - R0010
 - VHb+ALA+CO
 - VHb+ALA+CO, no buffer
 - VHb + ALA
 - VHb
 - K2602010 + ALA + CO
 - K2602010 + ALA + CO, no buffer
 - K2602010 + ALA,
 - K2602010
 - K2602013 + ALA + CO
 - K2602013 + ALA + CO, no buffer
 - K2602013 + ALA
 - K2602013

Friday:

- Harvested pellet from expression of VHb
- pH measurements of the cell

Week 32 (6/8/18 - 12/8/18)

Monday:

3A assembly of VHb/K1321200/K262010/K262013 (upstream) and GFP/J36400 (downstream) into psb1A3 backbone:

- Digestion of VHb with EcoRI and SpeI, GFP with XbaI and PstI, backbone with EcoRI and PstI
- Gel electrophoresis confirmed correct digestion of the genes except for own biobrick K262013
- Digestion of K262013 was attempted again but purification still showed negative results (however sequencing showed positive results)
- Ligation of K1321200 and K262010 into backbone and GFP

Tuesday:

- PCR amplification of digested biobricks K2602011, K2602014, K2602015, K2602016

- Gel electrophoresis of PCR amplification with positive results for all
- PCR product cleaning

Wednesday:

- Colony PCR of 3A assembly product (inserted biobricks K1321200 and K2602010)
- Gel electrophoresis
- Digestion of K2602011, K2602014, K2602015, K2602016 as well as psB1C3 plasmid containing RFP, with XbaI and SpeI.
- Gel electrophoresis and extraction
- Digestion of K2602013 with EcoRI and SpeI, with use of a different buffer (CutSmart)
- Gel extraction for biobrick ligation and 3A assembly of K2602011, K2602014, K2602015, K2602016, psBIA3 and psB1C3.

Thursday:

- Ligation of vector VHb K2602011 and K2602014 with psB1C3
- Transformation in TG1, and remaining DH5 α
- Gel extraction for the biobrick assembly
- Plasmid purification of the 3A assembly of K1321200 and K2602010 with VHb J364000

Friday:

- Colony PCR of K2602011 and K2602014 conducted with two different primers
- Ligation of biobrick K2602015 and K2602016 into psB1C3

Saturday:

- Colony PCR of K2602015 and K2602016 conducted with two different primers
- Gel electrophoresis only positive result for K2602016
- Plasmid purification of K260211 and K260214

Sunday:

- Colony PCR of K2602015
- Gel electrophoresis of K2602015
- Plasmid purification of K2602016

Week 33 (13/8/18 - 19/8/18)

Monday:

- Plasmid purification of K2602015 which showed bad results and were thus disregarded
- Digestion of K2602016, J36400 and R0011 for the 3A assembly
- Running of gel in order to extract plasmid and insert
Note: The expected band for K2602016 was not visible, therefore the electrophoresis was continued for J364000 and R0011
- Plasmid purification of K2602011, K2602014, K2602015, K2602016, R0011 and J364000
- Samples were sent for sequencing on the following thursday
Note: no conclusions could be drawn about successful insert of the biobricks from the results
- Gel extraction of psB1A3 and J364000
- Digestion of biobrick K2602011, K2602014, K2602016 and K2602015 for 3A assembly
- Electrophoresis, no inserted gene found

Wednesday:

- Transformation of K2602011, K2602013, K2602014, K2602015, K2602016 into BL21
- Digestion of K2602013, K2602015 and VHB with EcoRI and SpeI
- Gel electrophoresis of digestion products showed negative results for K2602013 and K2602015

Thursday:

- Amplification PCR for purified plasmids containing K2602011, K2602014, K2602015, K2602016 (different Tms) → sequencing
- Colony PCR for BL21 transformations with K2602011, K2602013, K2602014, K2602015 and K2602016
- Gel electrophoresis
- Glycerol stock was made of BL21 containing K2602011

Friday:

- Sent sample for sequencing
- Electrophoresis for repeating the colony PCR of K2602014, K2602015 and K2602016
- Starter culture was prepared for expression
- Expression of VHB biobricks:
 - K2602010 + ALA + CO + CAM
 - K2602011 + CAM

- K2602011 + ALA+ CAM
- K2602011 + ALA + CO+ CAM
- K2602013 + ALA + CO+ CAM
- K2602014 + CAM
- K2602014 + ALA + CAM
- K2602014 + ALA + CO + CAM
- K2602015 + CAM
- K2602015 + ALA + CAM
- K2602015 + ALA + CO + CAM
- K2602016 + CAM
- K2602016 + ALA + CAM
- K2602016 + ALA + CO + CAM
- Harvesting of the cells and pH measurements. Red cells were present in almost all variables of + ALA + CO but with varying intensity for different promoters.

Week 34 (20/8/18 - 26/8/18)

Monday:

- Digestion of K2602011, K2602013 and J364000 for 2A assembly
- Gibson assembly
- Repeated amplification of J364000

Tuesday:

- Gel electrophoresis of Gibson products
Note: K2602010, K2602011, K2602012, K2602013, K2602014, K2602015 was positive
- Clean PCR products and Gel extraction of digestion products
- Repeated PCR amplification of J364000

Wednesday:

- Gel electrophoresis of amplification product
- Ligation of K2602013 and J264000 (GFP)
- Transfer of ligation product into TG1
- Repeat amplification of K2602012, K2602014, K2602015 and pSB1C3 for Gibson assembly
- Gibson Assembly of K2602015 and K2602016 and J364000

- Transformation into DH5α

Thursday:

- Colony check of ligation products
- Colony PCR
Note: Colony PCR was not successful
- PCR of K2602023 and K2602026

Friday:

- Repeat transformation of the GA products
Note: Transformation didn't work for K2602020, K2602021, K2602022, K2602023, K2602024 and K2602025

Sunday:

- Plasmid purification of K2602010, K2602023, K2602026
- PCR amplification

Week 35 (27/8/18 - 2/9/18)

Monday:

- Gel electrophoresis of PCR amplification
- Digestion of K2602010, K2602011, K2602014 and K2602015 for 2A assembly
- Gel electrophoresis
- Digestion of Biobrick for confirmation (K2602010, K2602013, K2602014, K2602015, K2602016, K2602023, K2602026)
Note: K2602010 was successful
- Transformation of K2602023 and K2602026 in BL21
Note: Transformation was successful
- Expression of biobricks in BL21 with +ALA, no CO & +ALA, + CO

Tuesday:

- Digestion of K2602011, K2602014, K2602015, K2602016
- Gel purification of K2602010.
- Ligation of K2602010 with J364000 → K2602020
- Transformation of ligated K2602020 into DH5α

- Cultivation of BL21 colonies containing K2602023 and K2602026
- Run the gel electrophoresis for Gibson assembly products
Note: The Gibson assembly was not successful

Wednesday:

- Gel purification of K2602011 K2602014, K2602015, K2602016
- Repeat Gibson assembly of K2602011, K2602012 K2602014, K2602015 and K2602016 with J264000 (GFP)
Note: Positive result for all ligations without K2602016 and K2602012
- Transformation of ligated products into DH5 α
- Cultivation of K2602020, K2602021, K2602024, K2602025 and K2602026 for plasmid purification

Thursday:

- Digestion of K2602020, K2602021, K2602023, K2602024, K2602025, K2602026 with NotI
Note: All digestions was positive

Week 36 (3/9/18 - 9/9/18)

Monday:

- Transformation of K2602020, K2602021, K2602024 and K2602025
- Cultivation of all double insert in DH5 α
- Preparation of biobricks for sending to iGEM HQ (BBa_K2602010 to BBa_K2602016 and BBa_K2602020 to K2602026)

Tuesday:

- Pick the dried samples from biotech department
- Collection of all pellet from the cultivation
- Cultivation of transformed products
- Prepare starter culture for VHB-GFP measurements

Wednesday:

- GFP and OD measurements of Vhb-GFP biobricks

Thursday:

- Repeat transformation of K2602020 and K2602025

Friday:

- Prepare the cultivation of K2602020, K2602024, K2602025 and K2602012 for glycerol stock
- Cell dry weight measurements
- Prepare starter culture for K2602010, K2602011, K2602013, K2602014, K2602015, K2602016, R0011, BL21 for the growth curve measurements

Saturday:

- Growth curve measurements

Sunday:

- Prepare starter culture of K2602020 to K2602026, BL21 and R0010
- Prepare starter culture of K2602011, K2602013 and K2602017 for flow cytometry measurements

Week 37 (10/9/18 - 16/9/18)

Monday:

- Expression of K2602011, K2602013 and K2602016
- Cell pellet storage for later measurements
- Transformation of K2602011, K2602014 and K2602016 + J364000

Tuesday:

- Repeat the transformation of J364000

Wednesday:

- Colony PCR.
- Cultivation of positive samples + J36400
- Prepared starter culture of K2602011, K2602014 and K2602021, K2602024, R0010, J36400 and BL21

Thursday:

- Expression of starter culture from the day before

- Digestion of HbA
- Gel extraction and purification
- Lysis of the starter culture cells for SDS-page

Sunday:

- Digested K2602020 to K2602026 for new protein test
Note: the digestion was not successful for K2602024, K2602024 and K2602026

Week 38 (17/9/18 - 23/9/18)

Monday:

- Repeat digestion of K2602024, K2602024 and K2602026
- Prepare starter culture of K2602010, K2602011 and K2602013, K2602014, K2602020, K2602021, K2602023, K2602024, K2602025, K2602026, R0011 and BL21
- Expression of the cultures above

Tuesday:

- Preparation of new gBlocks
- Harvested the cells (K2602010, K2602011 and K2602013, K2602014, K2602020, K2602021, K2602023, K2602024, K2602025, K2602026, R0011 and BL21) for OD and cell dry weight measurements

Wednesday:

- Transformation of K2602012, K2602015 and K2602016, into BL21
- Cell plating

Thursday:

- Preparation of starter culture of K2602012, K2602015 and K2602016
- Digestion of K26021012

Friday:

- Harvested the cells (K2602012, K2602015 and K2602016) for OD and cell dry weight measurements
- Preparation of starter culture (for 80% media)
- Ligation of K2602012 + J364000.
Note: Ligation was not successful.

Saturday:

- Cell harvesting for OD and cell dry weight measurements
- PCR amplification of mutants and protein A

Week 39 (24/9/18 - 30/9/18)**Monday:**

- Prepared started culture of all biobrick

Tuesday:

- Expression of K2602010, K2602013, K2602020, K2602021, K2602023, K2602024, K2602025, K2602026

Wednesday:

- Cell harvesting for OD and dry cell weight measurements
- Digestion of protein A and Hb mutants
- Gel extraction of the Hb mutants
- Plasmid purification of K2602012
- Expression of K2602010, K2602011, K2602012, K2602015 and K2602016 + CO

Thursday:

- PCR amplification Hb mutants
- Ligation of Hb mutants

Friday:

- Resuspended the pellet from harvested cells (from 18 and 21 of September)
- Cells was expressed with 20% and 25% medium
- OD mesurments and dry cell weight

Saturday:

- Plasmid purification of HbA mutants (W37H and D78K)
- Digestion of K2602004
- Transformation of E6D and D73K, K63F in to TG1

Sunday:

- PCR amplification of K2602004, JP2013059242 (antibody)

- Run the gel electrophoresis

Week 40 (1/10/18 - 7/10/18)

Monday:

- Plasmid purification of Hb mutants (E6D, D731 and K65D)
- PCR cleaning

Tuesday:

- Digestion of K2602004 and K2602007 (Ab)
- PCR amplification
- PCR cell lysis and measurements
- Gel screening of the mutants
- Resuspension of cells harvested on 21/9

Wednesday:

- Ligation of K2602004 into pSB1C3 and VHb
- Ligation of K2602007 (Ab) into pSB1C3
- Transformation of the mutants into BL21-DE3
- Transformation of K2602004+K2602010, K2602014+K2602011, K2602014+K2602014, K2602014 in psB1C3 into DH5 α
- SDS-page of R0011, K2602010, K2602011, K2602012, K2602013, K2602014, K2602015, K2602016, K2602020, K2602021, K2602023, K2602024, K2602025 and K2602026
- Cell measurements

Thursday:

- Colony PCR of HbA and K2602004
- Measurement of harvested sample on 28th and 23rd of September, which were K2602015, K2602016 and K2602025
- Digestions:
 - K2602020, K2602021 and K2602026 with SpeI and PstI
 - K2602007 (Ab) and RFP (in psB1C3 with EcoRI and SpeI)
- Expression of J364000 + ALA + CAM

- Running the fermentor sample K2602020

Friday:

- Gel electrophoresis of K2602004, D73K, E6D, D73A, K65D and digestion products
- Gel extraction
- Made glycerolstock of K2602004 (3 tubes) and D73K (2 tubes)
- Cultivation of K2602004 for plasmid purification
- Cultivation of K65D and D73A (4 tubes each)
- SDS-page:
 - 1st round (no dilution)**
 - First Harvested sample:
 - R0010, K2602010, K2602011, K2602012, K2602013, K2602014, K2602015, K2602016, K2602020, K2602021, K2602023, K2602024, K2602025, K2602026
 - 2nd round (4x diluted)**
 - First Harvested sample:
 - R0010
 - Harvested on 26/9:
 - K2602013, K2602014, K2602020, K2602023, K2602024, K2602026, R0010
 - Harvested on 27/9:
 - K2602011, K2602012, K2602015, K2602016
 - Harvested on 28/9:
 - K2602015, K2602014
- Ligations
 - K2602007 (Ab) into psb1C3
 - K2602004 and K2602020 into psb1C3
- Transformation of the ligated samples
- Measurements of K2602004 in psB1C3 of DH5α

Saturday:

- Checked the plates, no colonies

Sunday:

- Plasmid purification of K2602004 from DH5α.
- Digestion of K2602004, K2602010, K2602013 and mutants
- Gel electrophoresis of digested K2602004 and mutants
- Streaked all mutants from glycerol stocks onto new plates + AMP

- Digestions of K2602004 with EcoRI and XbaI, and K2602010 with EcoRI and SpeI
- Electrophoresis of digestion products
- Ligations of K2602004 and K2602010

Week 41 (8/10/18 - 14/10/18)

Tuesday

- Transformations:
 - K2602004 + K2602010 in psb1C3 → DH5α (+CAM)
 - K2602004 in psb1C3 → BL21 (+CAM)
- Preparation of starter culture

Wednesday

- Made starter culture of J364000 for fermentor
- Start of expression of VHb-GFP with 2013 and J364000 as control
- Growth curve
- Harvesting of expressed mutants
- OD measurements
- Colony PCR of K2602004, K2602004+K2602010
- Electrophoresis of colony PCR
- Sampling of VHb-GFP for growth curve and flow cytometry
- Kept samples for plasmid purification

Thursday

- Flow cytometry measurements of VHb-GFP samples

Friday

- Spectrophotometry analysis of lysed samples:
 - Hba-K65D
 - HbA-D73K
 - HbA-E6D
 - HbA-D73D
 - Fermentor
- Made starter culture of 4 mutants + K2602004 in BL21

Saturday:

- Expression of protein A (+ ALA + CAM) and VHb-protein A (+ ALA + CO)
- Measurement of mutant D73A, K65D, D73K and E6D cell weight after lysis
- Digestion of K2602010+K2602004 with NotI

Sunday:

- Cell harvesting
- SDS-PAGE of supernatant
 - 1st round (VHb protein A)**
 - 1, 2, 3, 4, 6
 - 2nd round**
 - K2602004, K2602011, K2602012, K2602013, K2602020, K2602021, K2602023, K2602025, K2602026
- Expression of mutants
- SDS-PAGE:
 - 1st round**
 - Samples from fermentor (5 wells for each):
 - K2602020, J364000
 - 2nd round (4x diluted)**
 - Harvested on 12/10 (2 wells for each):
 - D73A, E6D, D73K, K65D
 - Harvested on 13/10 (2 wells for each):
 - D73A, E6D, D73K, K65D
- Preparing samples (all mutants) for sequencing
- Preparing the starter culture of protein A and VHb-protein A.

Monday:

- Check the SDS-PAGE result of the protein A.
- Harvesting the expressed mutants
- Analysed the functional mutant using spectrophotometer
- Expressed the protein A

Note: realized that was something wrong with the Protein A construct

Tuesday

- Prepared the lysed mutants for purification
- Note: no further analysis of the mutants due to very low concentration of the protein.

