Lab Book

Project: iGEM2026
Author: Brian Lee
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FRIDAY, 7/10/2026

Wet lab plan week by week

iGEM26_Wet_Lab_Plan.xlsx

Masterplan
Golden gate
Transform in DH5a
Pick 3-6 colonies with xgal iptg
Miniprep 5
stock plasmid in -80
run digest
Send all of them through sequencing
transform bl21 DE3
pick single colony, Glycerol stock 0.5
on
large scale culture 50mL in 250mL
grow 0.6OD then split for induction by IPTG 400uM
ON 25 check temp from literature
harvest cells centrifuged
cell lysis via sonication
IMAC for his, ask cameron for strep
Collect fractions of whole cell, soluble etc.
run SDS page
western blot
activity assay turbidity can run for ChiA
check acetic acid readout? for the deacetylation need to talk with raphael here for activity assay in literature check the acetyl group, buy known oligomer and compare nadh turnover potentailly first see if it works at all

gBlock order 21 July 2026:

- Parts must be compatible with the BioBrick standard described in RFC 10 OR Type IIS standards.
- BsaI and SapI are not allowed within part - checked and removed all as followed:

gBlock order 21 July 1

	Name	Source organism	Forbidden sites (BsaI/SapI)	DNA sequence	Amino acids	Ordered via IDT	Action required	Information
1	PpChi_His	Paenibacillus pabuli	yes: SapI removed by changing K61 from AAG to AAA	PpChi	495	yes, 21/07/26		Expected delivery 31/07/26
2	SmChiA_Wu2009_His	Serratia marcescens	no, all fine	SmChiA_Wu2009_His	574	Yes, ordered 23/07/26 as "Genes in Tubes" https://eu.idtdna.com/pages/products/genes-and-gene-fragments/custom-gene-synthesis feature		Expected delivery 04/08/26; is only part that comes in a plasmid
3	SmChiA_BBa_K1913000_ReverseMutation	ChiA Serratia marcescens	Yes, all fine	SmChiA_BBa_K1913000_ReverseMutation	574. pI 6.64	yes, 23/07/26 as gBlock; Brian took Wagingen sequence and did translation as well as adjustment to AA sequence to Wu paper, this is codon optimised version of Wu: https://registry.igem.org/parts/bba-k1913000		Expected delivery 04/08/26
4	SmChiA_BBa_K2718022	Endochitinase Beauveria bassiana	Original part includes a promoter and rbs from https://registry.igem.org/parts/bba-k2718022; only CDS for ChiA is needed, this is part BBa_K2718021: https://registry.igem.org/parts/bba-k2718021	SmChiA_BBa_K2718022	299	no, as unclear why selected, no longer selected accroding to	Team to check why this was suggested; resolved: forget sequence	
5	VcCDA_NC_002505_His	Chitin oligosaccharide deacetylase Vibrio cholerae	no, all fine	VcCDA_NC_002505_His	442, pI 5.75	yes, 21/07/26		From paper: https://doi.org/10.1093/glycob/cwm096; Expected delivery 31/07/26

6	VcCDA_NC_002505_Strep	Chitin oligosaccharide deacetylase Vibrio cholerae	no, all fine	VcCDA_NC_002505_Strep	444	yes, 21/07/26		Expected delivery 31/07/26
7	VcCDA_BBa_K2380044_His	Chitin oligosaccharide deacetylase Vibrio cholerae	no, all fine	VcCDA_BBa_K2380044_His	442	yes, 21/07/26		Expected delivery 31/07/26
8	VcCDA_BBa_K2380044_Strep	Chitin oligosaccharide deacetylase Vibrio cholerae	no, all fine	VcCDA_BBa_K2380044_Strep	444	yes, 21/07/26		Expected delivery 31/07/26

- IDT order summary 21/07/26:

Integrated_DNA_Technologies_-_Order_Confirmation_210726.pdf

- IDT order summary 23/07/26

Integrated_DNA_Technologies_-_Order_Confirmation_230726.pdf

Lab book

Plan for week 20 July

July 20-25

	Task	Output	Chemicals	Equpment	Dates	Supervisors	G
1	LB Agar/Media prep	1.2L LB Media (separated into 300ml in 4 bottles), 10-20 plates worth of LB agar (200mL in 1 bottle)	LB broth powder, MilliQ Water, agar powder	5 x 500ml Duran Bottles			
2	Autoclave Pippette tips, media	Sterile media and pippette tips (1x blue tips, 2x yellow tips, 1x red tips)	-	Autoclave			
3	Make chloranphenicol	3x 2ml tubes	Chloranphenicol stock, 100% ethanol				
4	Stock pSB1C3	Vector stock (might need to amplify if volume is low)					

Lab materials:
communal iptg
miniprep kit from vlad that expires soon
buy strep column if necessary
buy deacetylation activity assay
chitin from shrimp sigma aldrich source get from raphael for ChiA
buy intermediate possible? substrate for CDA

Restriction Digest

	Sample Plasmid	Enzymes	Buffer	Fragment Sizes (bp)
1	GG_BBa_K2380044_his	Sac1+Nde1	NEB rCutSmart	1316 + 8 + 2326
2	GG_BBa_K2380044_strep	Sac1+Nde1	NEB rCutSmart	1316 + 8 + 2323
3	GG_NC002505_his	Sac1+Nde1	NEB rCutSmart	1324 + 2323
4	GG_NC002505_strep	Sac1+Nde1	NEB rCutSmart	1324 + 2320
5	pSSB1C3-PpChi	Sac1+Nhe1	NEB rCutSmart	1515 + 2291

Plan for week 27 July

July 20-1

	Task	Output	Chemicals	Equpment	Dates	Supervisors	G
1	Stock pSB1C3	Vector Stock	N/A	N/A			
2	Store ordered g blocks	gene Stock	N/A	N/A			
3							
4							

Plan for week 3 Aug

July 20-2

	Task	Output	Chemicals	Equpment	Dates	Supervisors	G
1	Perform GG	Golden Gate plasmid	GG Master Mix (BsmBI enzyme), ddH2O, T7 ligase, g blocks	Thermocycler	Tues	Vlad	
2	Transformation onto E. coli	E. coli with plasmid plated	Comp E. coli DH5alpha	Heat block (water bath), BSL cabinet	One day after Golden gate		
3	Overnight culture	Single colony E. coli in media	N/A	BSL cabinet	Afternoon after transformation		
4	Miniprep	Verify plasmid correctly inserted	Miniprep kit	N/A	Morning after overnight		

VcCDA variant gBlocks arrived. Golden Gate was performed, followed by transformation onto Comp E. coli DH5alpha.

VCH colonies appeared blue indicating the transformation was not succesful. Transformation will be repeated next week.

image.png

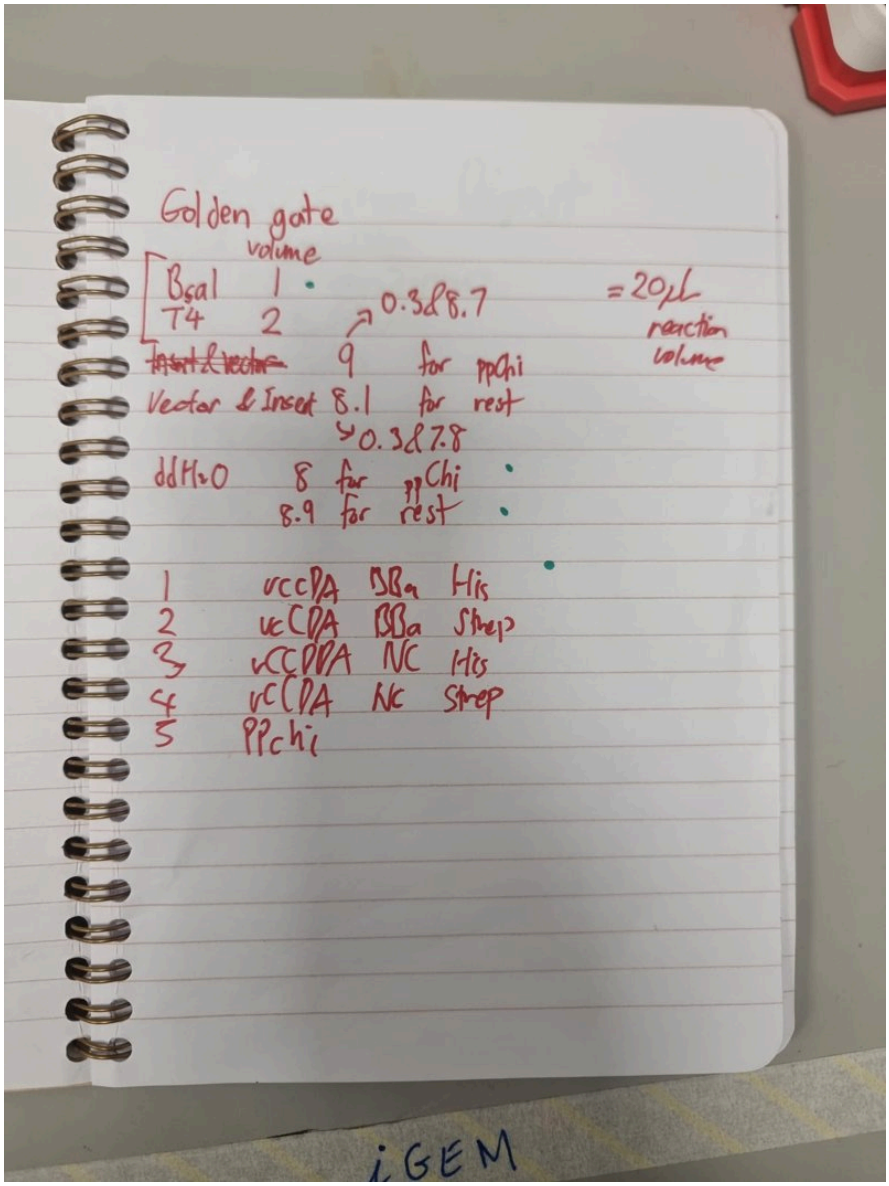
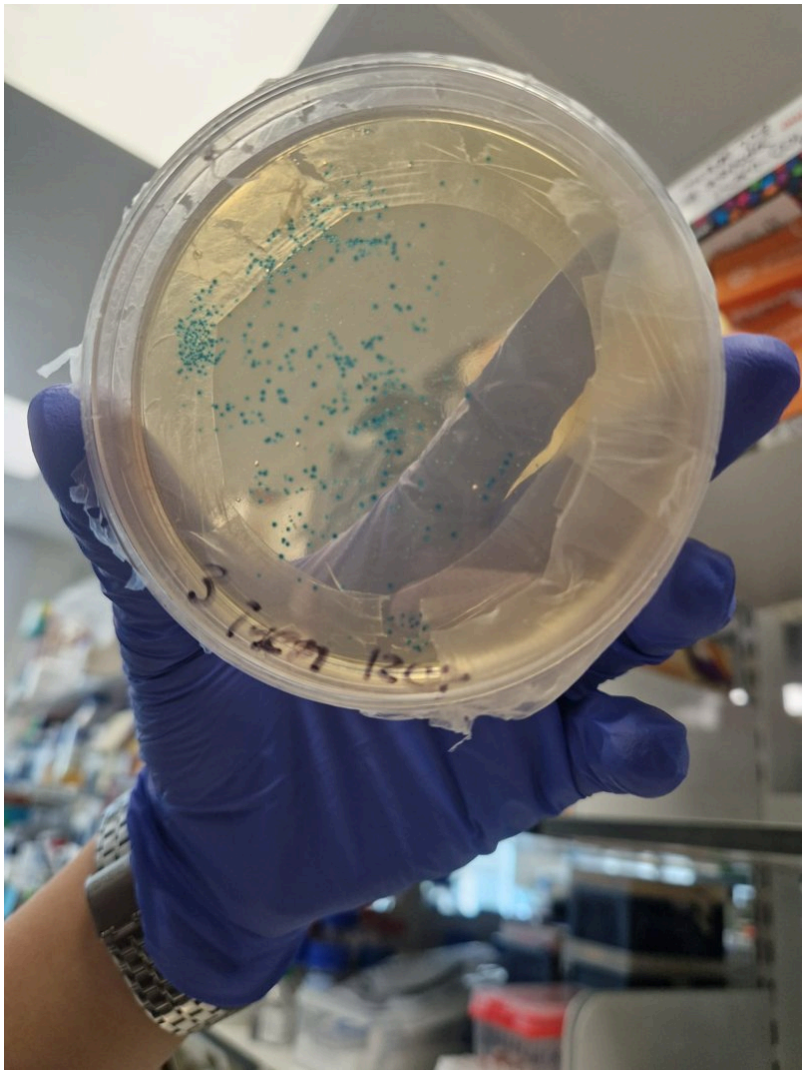

Golden gate
volume
BsaI 1
T4 2
0.3&8.7
=20µL
reaction
volume
~~Insert & vector~~ 9 for ppChi
Vector & Insert 8.1 for rest
0.3&7.8
ddH2O 8 for ppChi
8.9 for rest
1 vccDA BBa His
2 vcCDA BBa Strep
3 vCCDDA NC His
4 vcCDA NC Strep
5 PPchi
iGEM

image.png



Plan for 10th Aug

July 20-6

	Task	Output	Chemicals	Equpment	Dates	Supervisors	G
1	Perform GG	Golden Gate plasmid	GG Master Mix (BsmBI enzyme), ddH2O, T7 ligase, g blocks	Thermocycler	Tues	Vlad	
2	Transformation onto E. coli	E. coli with plasmid plated	Comp E. coli DH5alpha	Heat block (water bath), BSL cabinet	One day after Golden gate		
3	Overnight culture	Single colony E. coli in media	N/A	BSL cabinet	Afternoon after transformation		
4	Miniprep	Verify plasmid correctly inserted	Miniprep kit	N/A	Morning after overnight		

Plan for week 17 Aug

July 20-3

	Task	Output	Chemicals	Equpment	Dates	Supervisors	iGEM lead
1	Run gel electrophoresis of VcCDA & PpChi	Check bands for cut vector & insert	1% Agarose gel need to prep, loading dye	Gel electrophoresis system	Tues 9am	Vlad	Ella & Brian
2	Sample prep for sequencing	Plasmids ready to be sent for sequencing	ddH2O	N/A	Tues 12pm	Vlad	Ella & Brian
3	Perform GG for SmChiA alongside VcCDA non-codon optimized (only blue colonies) + fails	Golden Gate plasmid	GG Master Mix (BsmBI enzyme), ddH2O, T7 ligase, g blocks	Thermocycler	Wed 9am	Kuan	Ella & Brian
4	Transformation onto E. coli	E. coli with plasmid plated	Comp E. coli DH5alpha	Heat block (water bath), BSL cabinet	Wed afternoon	Steffi	Ella & Maria
5	Overnight culture	Single colony E. coli in media	N/A	BSL cabinet	Thurs 4pm after transformation	Vlad	Ella & Maria
6	Miniprep	Verify plasmid correctly inserted	Miniprep kit	N/A	Friday 9am so ready to send sequencing by 2:30pm	Steffi	Brian & Maria

August 17th 2026

Members Attended: Ella, Brian

Supervisor: Kuan

Summary:

1. Gel electrophoresis
2. Nanodrop

1 Gel electrophoresis

Gel run: restriction digest of VcCDA with SacI, NdeI. Restriction digest of PpChi with SacI, NheI.

1. mixed agarose with buffer (1% agarose in 100ml TBE buffer, so added 1g agarose).
2. microwaved for 2 mins, then took out.
3. Added 0.1ul gel red to the mixture in step 1. Cool down mixture by washing flask with water.
4. Added 2ul of purple loading dye to each sample.
5. poured out hot agarose gel into the electrophersis tank, put comb in there
6. when cooled, took out comb so that the wells were made.
7. loaded 1X TAE buffer onto the gel
8. loaded the ladder (8ul) into the last (rightmost) well using pipette.
9. loaded samples 1-16 into the wells, where 1 is on the right and progress to the left until 16 (on the left) next to the ladder next to the ladder.
10. Ran gel at 100V for 4 mins.
11. Liquid from gel removed, gel then slided onto the fluorescence trans-UV plate.
12. Gel inserted into imager, image saved.

Result:

image.png

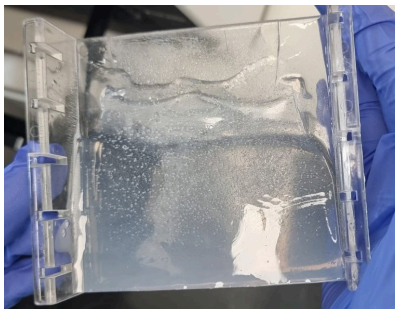
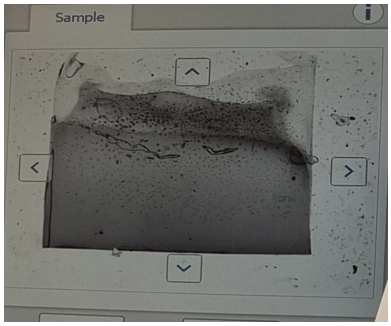


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Gel failed, overheated as run at 300 V instead of 100V.
restriction digest had to be repeated to allow for a new gel run.
During restriction digest, enzyme stock was depleted so only half the amount of enzyme required was added.

New enzymes chosen: Sac1 for both (vector cut), Nde1 for VcCDA (insert cut), Nhe1 for PpChi (insert cut).
A second digest was completed with these enzymes.

2 Nanodrop

Nanodrop results

	A	B
1	Name	Concentration (ng/uL)
2	VcCDA C His 1	11.2
3	VcCDA C His 2	60.9
4	VcCDA C His 3	77.9
5	VcCDA C His 4	55.1
6	VcCDA C Strep 1	96.9
7	VcCDA C Strep 2	191.2
8	VcCDA C Strep 3	189.3
9	VcCDA C Strep 4	50.4
10	VcCDA NC Strep 1	81.5
11	VcCDA NC Strep 2	82
12	VcCDA NC Strep 3	85.3
13	VcCDA NC Strep 4	X
14	PpChi 1	27.5
15	PpChi 2	157.7
16	PpChi 3	40.2
17	PpChi 4	265.4

August 18th 2026

Members Attended: Ella, Brian

Supervisor: Steffi

Summary:

1.1 Gel electrophoresis (Cut sites in vector and insert)

1.2 Gel electrophoresis (Cut sites in vector only)

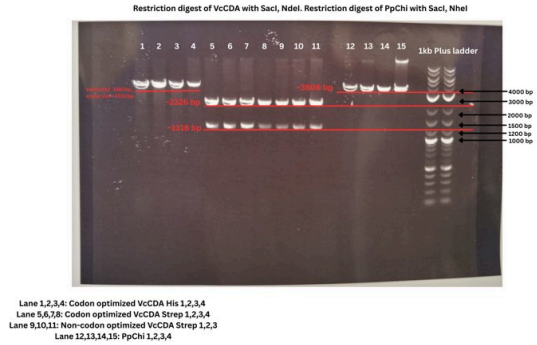
2. Sample Sequencing

1.1 Gel electrophoresis (Cut sites in vector and insert)

Gel run: restriction digest of VcCDA with SacI, NdeI. Restriction digest of PpChi with SacI, NheI.

1. mixed agarose with buffer (1% agarose in 100ml TAE buffer, so added 1g agarose).
2. microwaved for 2 mins, then took out.
3. Added 10ul gel red to the mixture in step 1. Cool down mixture by washing flask with water.
4. Added 2ul of purple loading dye to each sample.
5. poured out hot agarose gel into the electrophersis tank, put comb in there
6. when cooled, took out comb so that the wells were made.
7. loaded 1X TAE buffer onto the gel
8. loaded the ladder (8ul) into the last (rightmost) well using pipette.
9. loaded samples 1-16 into the wells, where 1 is on the right and progress to the left until 16 (on the left) next to the ladder next to the ladder.
10. Ran gel at 100V for 40 mins.
11. Liquid from gel removed, gel then slided onto the fluorescence trans-UV plate.
12. Gel inserted into imager, image saved.

image.png

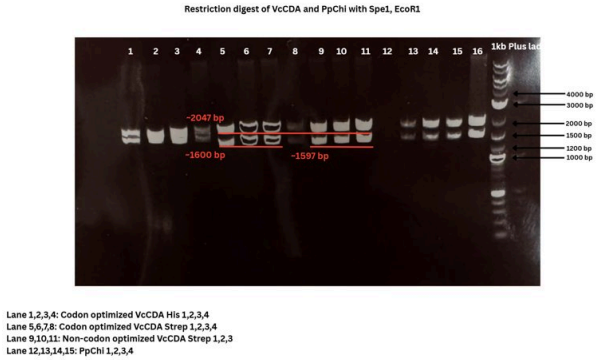


1.2 Gel electrophoresis (Cut sites in vector only)

Gel run: restriction digest of VcCDA and PpChi with SacI, NheI.

1. 1L TAE buffer replenished (20x concentrated TAE diluted in 980ml ddH2O)
2. Electrophoresis process repeated as above

image.png



2 Sample Sequencing

Table1

	A	B	C	D	E
1	Name	Concentration (ng/uL)	Sample add uL	ddH2O add uL	Sequencing
2	VcCDA C His 1	11.2	4.46	5.54	-
3	VcCDA C His 2	60.9	0.82	9.18	Yes
4	VcCDA C His 3	77.9	0.64	9.36	Yes
5	VcCDA C His 4	55.1	0.91	9.09	-
6	VcCDA C Strep 1	96.9	0.52	9.48	-
7	VcCDA C Strep 2	191.2	0.26	9.74	Yes
8	VcCDA C Strep 3	189.3	0.26	9.74	Yes
9	VcCDA C Strep 4	50.4	0.99	9.01	-
10	VcCDA NC Strep 1	81.5	0.61	9.39	-
11	VcCDA NC Strep 2	82	0.61	9.39	Yes
12	VcCDA NC Strep 3	85.3	0.59	9.41	Yes
13	VcCDA NC Strep 4	X	X	X	-
14	PpChi 1	27.5	1.82	8.18	-
15	PpChi 2	157.7	0.32	9.68	Yes
16	PpChi 3	40.2	1.24	8.76	-
17	PpChi 4	265.4	0.19	9.81	Yes

1. For each intert, 2 samples of highest concentration (according to nanodrop) were chosen for sequencing. (highlighted in yellow above).
2. According to the calculations in the table, appropriate volumes of sample and ddH2O were pippeted into pcr tubes.
3. Samples sent to Full Circle Labls for overnight sequencing.

August 19th 2026

Members Attended: Ella, Brian

Supervisor: Kuan, Steffi

Summary:

1 Golden Gate (SmChia, failed VcCDA)

2 Gel electrophoresis (Correction of the labelling error)

August 20th 2026

Members Attended: Ella, Brian

Supervisor: Kuan, Steffi

Summary:

1.1 Gel electrophoresis (Cut sites in vector and insert)

1.2 Gel electrophoresis (Cut sites in vector only)

2. Sample prep for sequencing

3. Samples sent out for sequencing

image.png

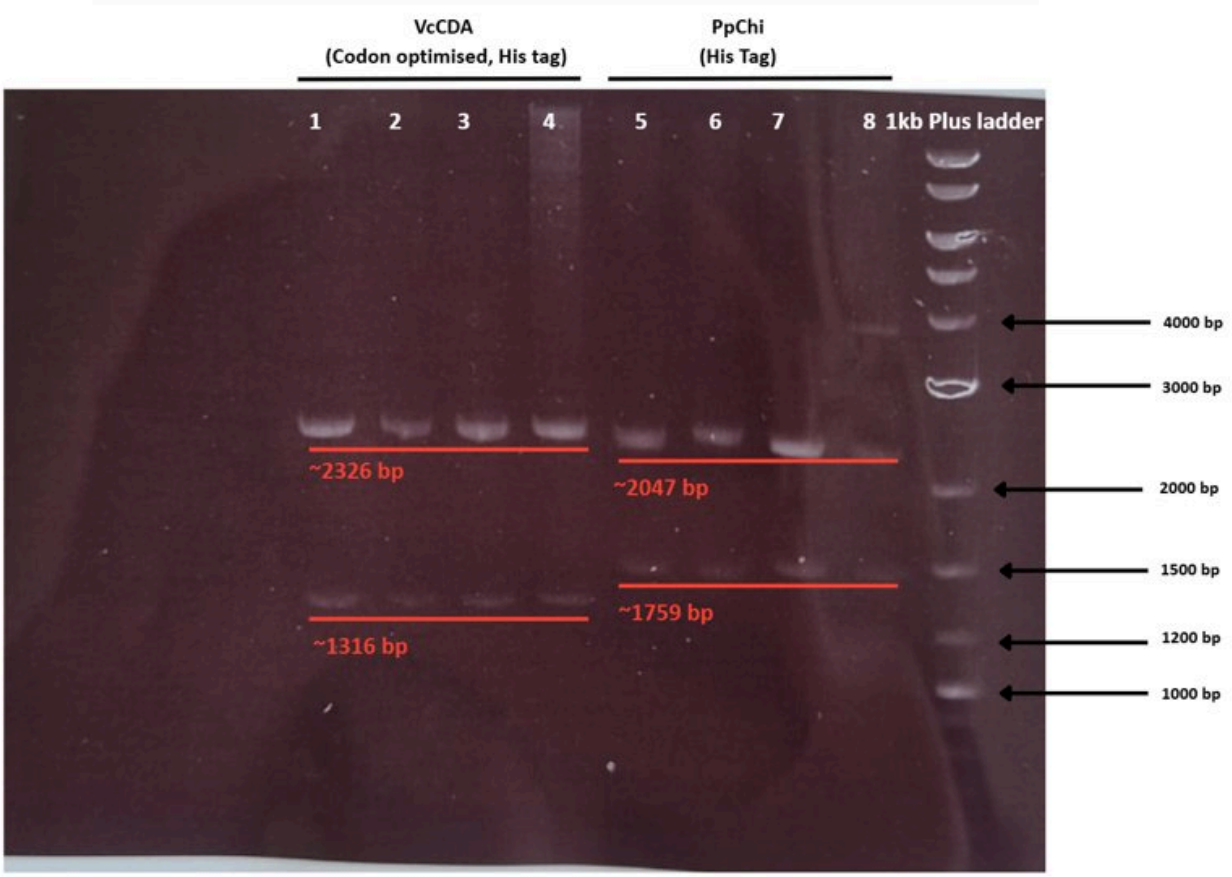


Figure 1. Image of Gel electrophoresis analysis for restriction digest of VcCDA (Codon optimised, His tag) and PpChi expressed in Competent *E. coli* DH5alpha. Four colonies of each strain were digested using two restriction enzymes SacI , NdeI (VcCDA) and SacII, NheI (PpChi). Samples ran on the gel as follows: lanes 1-4: VcCDA (Codon-optimised, His tag), lanes 5-8: PpChi.

Both SmChia variants succesfully transformed, as well as VcCda non codon optimised His repeat transformation.

image.png

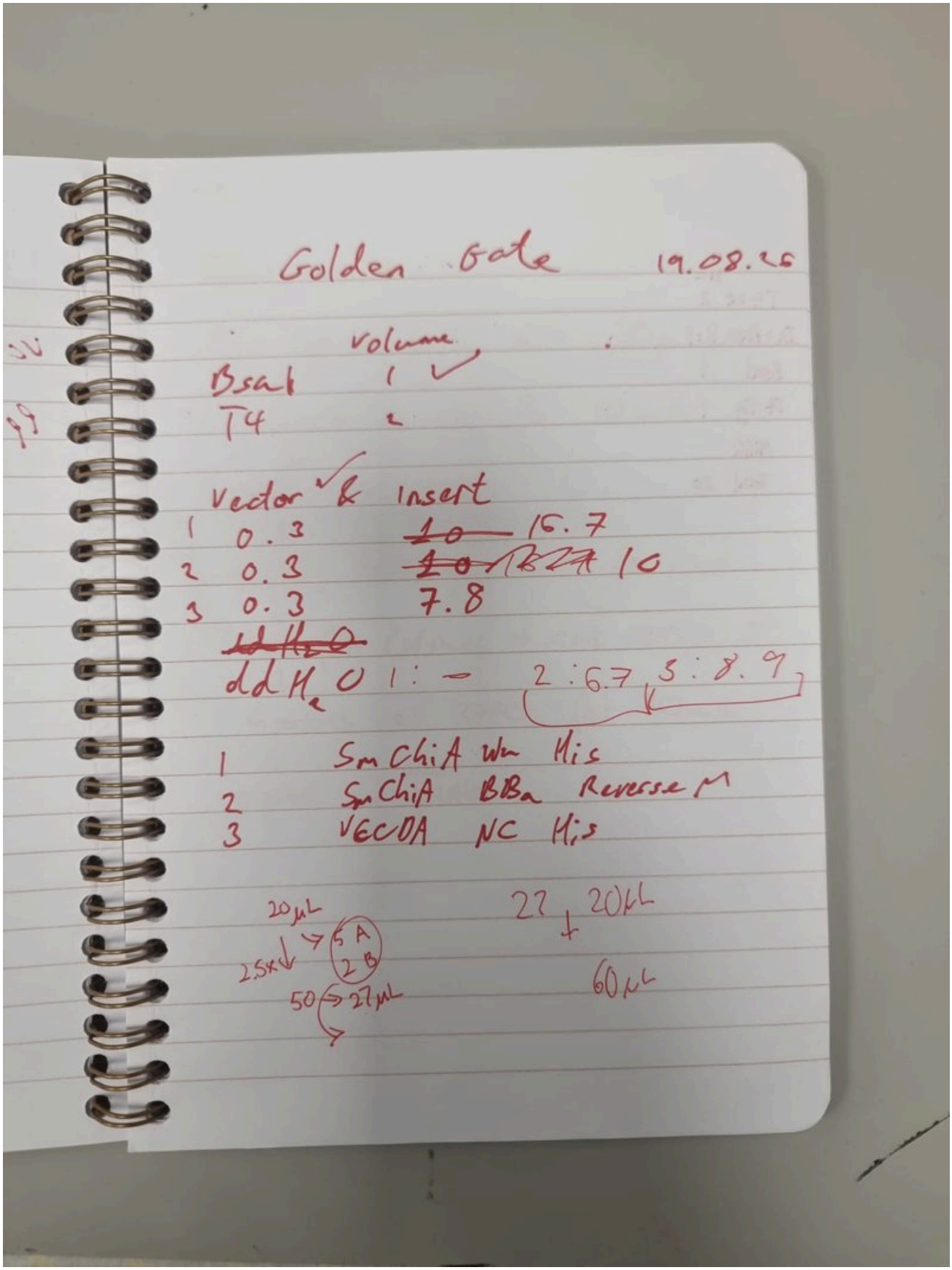

Golden Gate 19.08.26
Volume
BsaI 1
T4 2
Vector & Insert
1 0.3 16.7
2 0.3 10
3 0.3 7.8
ddH2O 1: - 2: 6.7 3: 8.9
1 SmChiA Wt His
2 SmChiA BBa Reverse M
3 VECOA NC His
20µL
2.5x
5 A
2 B
50 27µL
27 + 20µL
60µL

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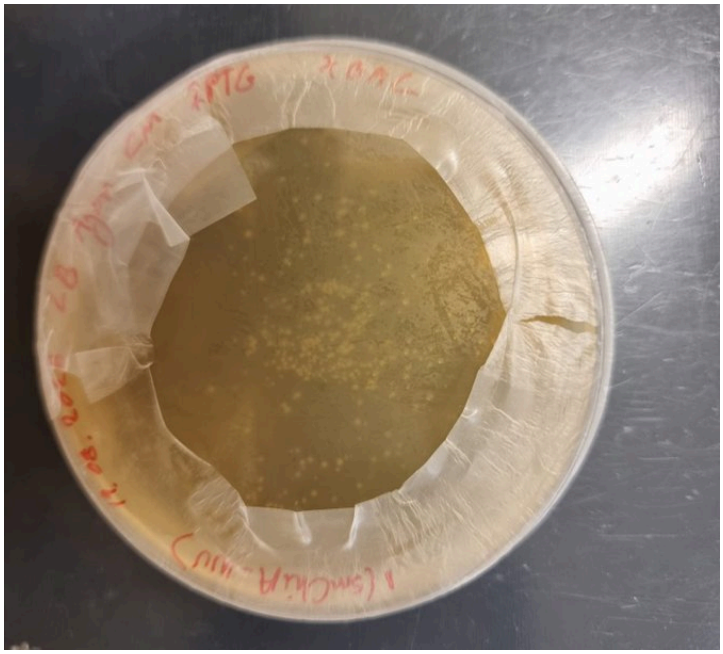


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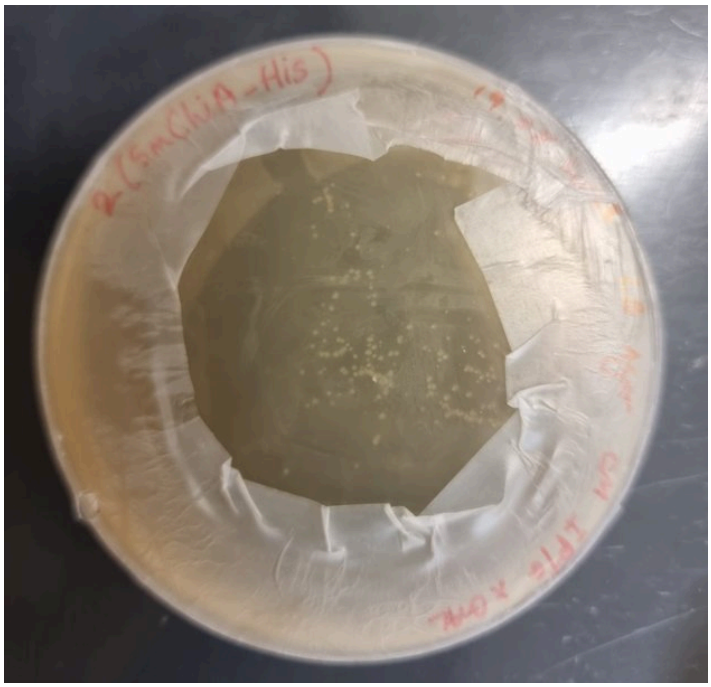
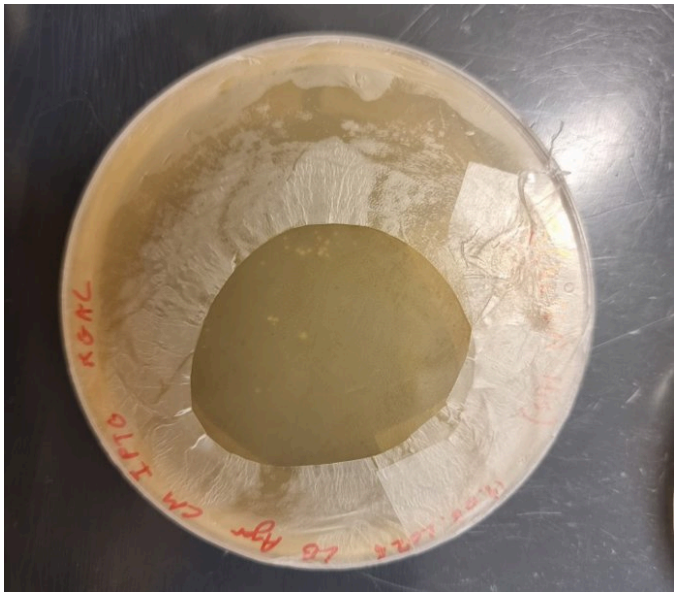


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Plan for week 24 Aug = Brian

July 20-4

	Task	Output	Chemicals	Equpment	Dates	Supervisors	iGEM lead
1	Transformation onto E. coli, autoclave LB agar (we need 2 more plates!), autoclave cotton, autoclave 250mL flask	E. coli with plasmid plated	Comp E. coli BL21	Heat block (water bath), BSL cabinet, autoclave	Tues 9am (autoclave stuff), 11am transformation, plates ready by 2pm		Brian & Raphael
2					Wednesday 9am put plates in fridge		Brian
3	Pick single colony, overnight culture	ON culture		BSL cabinet	Wednesday 4pm		Brian & Maria
4	Glycerol stock & put ON culture in 4degC until use	Long term storage in -80	50% glycerol	2mL cryotube	Thursday 9am		Brian & Raphael
5	Protein expression (start OD at 0.1) usually ~2hrs	Get OD 0.6		Shaking incubator	Prepare Thursday 2pm so ready by 3pm		Brian (not sure) Raphael & Maria
6	Protein expression induction phase	2x SmChiA (16 hours), 1x PpChi (18 hours)	IPTG		Thursday 5pm		Brian (not sure) Raphael & Maria
7	Harvest cell pellet and store in -20				16 hours ends at 9am, 18 hours ends at 11am		Brian & Raphael

Consumables order placed by Steffi 24 Aug 2026:

iGEM_Consumables_order_240826.PNG



Line	Description	Need-By	Date-From	Date-To	Offset Days	Deliver-To	Unit	Quantity	Qty Delivered	Qty Cancelled	Open Quantity	Price	Amount (GBP)	Details
1	1Kb PLUS DNA ladder, 250-10Kp, 1x500ul vial	26-Aug-2026 00:00:00	28-Aug-2026	28-Aug-2026	2 (Punchout)	F47 - Elaine Briggs	Each	1	0.0		1	64 GBP	64.00	
2	Chitin from shrimp shells practical grade, powder- Research Use Only	26-Aug-2026 00:00:00	28-Aug-2026	28-Aug-2026	2 (Punchout)	F47 - Elaine Briggs	Each	1	0.0		1	91.9 GBP	91.90	
3	Chitosan from shrimp shells, 775% (deacetylated)- Research Use Only	26-Aug-2026 00:00:00	28-Aug-2026	28-Aug-2026	2 (Punchout)	F47 - Elaine Briggs	Each	1	0.0		1	63.6 GBP	63.60	
4	Gel Loading Dye Purple (6X)	26-Aug-2026 00:00:00	28-Aug-2026	28-Aug-2026	2 (Punchout)	F47 - Elaine Briggs	Each	1	0.0		1	43.2 GBP	43.20	
5	GELRED NUCLEIC ACID STAIN10.000X IN WATE	26-Aug-2026 00:00:00	28-Aug-2026	28-Aug-2026	2 (Punchout)	F47 - Elaine Briggs	Each	1	0.0		1	36.67 GBP	36.67	
												Total	299.37	

Enzyme conditions disussed in Wetlab meeting:

Enzyme Variations to Week 24 August Plan

	Step	SmChiA	VcCDA	PpChi
1	**Overnight Starter Culture**	37c 250rpm	37c 200rpm	37c 200rpm
2	**Pre-Induction Culture**	37c 130rpm	37c 180rpm	37c 200rpm
3	**Post-Induction Culture**	20c 180rpm 16h	16c 180rpm 16-18h	16c 180rpm 18h
4	**Centrifugation**	6000g 10min	2500g 30min	4000g 15min

Enzyme Variations to Week 24 August REVISITED

	Step	SmChiA	PpChi
1	**Overnight Starter Culture**	37c 250rpm	37c 250rpm
2	**Pre-Induction Culture**	37c 180rpm	37c 180rpm
3	**Post-Induction Culture**	20c 180rpm 16h	16c 180rpm 18h
4	**Centrifugation**	4000g xmin	4000g 15min

Check starter culture volume - 10mL recommended (LB) + 1 colony.

Run 2 colonies for each - 6 total.

Start with 50mL LB culture in a flask baffled.

C1V1 = C2V2 (start with 0.1 OD600). Grow at 37c 180rpm. Increase to 0.6 OD600 (2-4h estimate).

Check after an hour. Once it becomes cloudy start taking optical density reading.

Take a sample before induction to see leaky protein production. Spin down and store cells.

0..5mM IPTG concentration. Always take samples before leaving lab (2-3h after induction).

SmChiA 180rpm 16h-18h. PpChi 180rpm 16c and one at 20c.

Don't worry too much about induction duration (UP TO 24 HOURS).

Harvest ~50mL. 4000g x 15min? Retain cell pellet and supernatant samples (SDS PAGE, in the event of protein secretion).
Storer pellet in **-20c** or -80c.
Monday or tuesday - resuspension double check buffers.

Buy SDS-PAGE equipment for next week.
Run sonication beforehand.

Need to find buffer for storage over weekend.
Tris 20mM, pH 8.0, 500mM sodium chloride
Need to make wash buffers (imadazole conc)
Initial = 5mM
Wash 1 = 50mM
Wash 2 = 100mM
Elute = 400mM
Consider only doing 1 wash at 60mM to reduce risk of loss of protein at wash 2.

Note: Benchling or Protein Param - calculate theoretical isoelectric point for general buffer pH - uses amino sequences.

Buy resin and charge with nickel - IMAC.

August 25st 2026

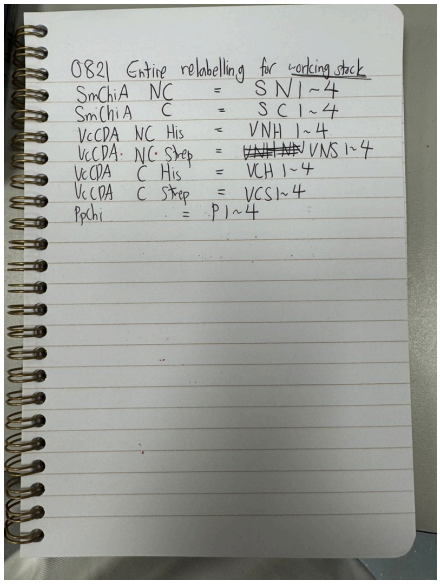
Members Attended: Brian, Raphael
Supervisor:

Summary:

1 Relabelling tubes and working stock inventory
2 Transformation
3 Autoclave materials

Tubes relabelled:

image.png



Working stock:

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Name	Concentration (ng/uL)
PpChi 1	11.2
PpChi 2	60.9
PpChi 3	77.9
PpChi 4	55.1
VcCDA NC Strep 1	96.9
VcCDA NC Strep 2	191.2
VcCDA NC Strep 3	189.3
VcCDA NC Strep 4	50.4
VcCDA C Strep 1	81.5
VcCDA C Strep 2	82
VcCDA C Strep 3	85.3
VcCDA C Strep 4	X
VcCDA C His 1	27.5
VcCDA C His 2	157.7
VcCDA C His 3	40.2
VcCDA C His 4	265.4
SmChiA NC 1	127.45
SmChiA NC 2	129.7
SmChiA NC 3	140.15
SmChiA NC 4	84.75
SmChiA C 1	64.56
SmChiA C 2	202.07
SmChiA C 3	238.13
SmChiA C 4	135.92
VcCDA NC His 1	104.45
VcCDA NC His 2	88.86
VcCDA NC His 3	103.47
VcCDA NC His 4	154.93

August 27th 2026

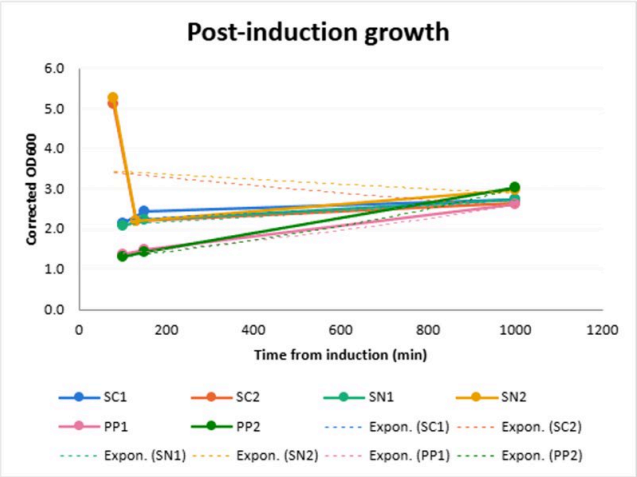
Members Attended: Brian, Raphael

Supervisor:

Summary:

1 Protein Expression

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Plan for week 31 Aug

July 20-5

	Task	Output	Chemicals	Equpment	Dates	Supervisors	iGEM lead
1	Transformation onto E. coli for VcCDA	E. coli with plasmid plated	Comp E. coli BL21	Heat block (water bath), BSL cabinet, autoclave	Tues 9am (autoclave stuff), 11am transformation, plates ready by 2pm		Brian Raphael
2					Wednesday 10am put plates in fridge		Brian
3	SDS PAGE?						TBC
4	Pick single colony, overnight culture. ALSO OVERNIGHT single colonies of SmChiA & PpChi so we can glycerol stock ONLY no expression	ON culture		BSL cabinet	Wednesday 4pm		Ella Maria (Brian)
5	Glycerol stock & put ON culture in 4degC until use	Long term storage in -80	50% glycerol	2mL cryotube	Thursday 9am		Ella Raphael
6	Protein expression (start OD at 0.1) estimated 3hrs	Get OD 0.6		Shaking incubator	Prepare Thursday 2pm so ready by 3pm		Ella Raphael
7	Protein expression induction phase	2x SmChiA (16 hours), 1x PpChi (18 hours)	IPTG		Thursday 5pm		Ella Raphael
8	Harvest cell pellet and store in -20				Friday 9am		Brian Maria
9	SDS PAGE?						TBC
10							

September 1st 2026

Members Attended: Brian, Raphael

VcCDA Transformation

VNH, VCH, VCS, VNS transformed onto Comp E. coli BL21 according to protocol
4x plates in total.

September 2nd 2026

Members Attended: Brian, Ella, Maria

Supervisors: Tamas, Steffi

Summary:

1 Consumables order (Columns and gels)
2 Plates removed from incubation,
2. Buffers
4 Overnight culture

1 Consumables order (Columns and gels)

Order SFK 02/09/2026

iGEM_2026_Consumables_SFK020926.PNG

Details

Line	Description	Need-By	Date-From	Date-To	Offset Days	Deliver-To	Unit	Quantity	Qty Delivered	Qty Cancelled	Open Quantity	Price	Amount (GBP)	Details	Order Number
1	Chelating Sepharose™ Fast Flow Cytiva 17-0575-01, pack of 50 mL-Research Use Only	04-Sep-2026 00:00:00	03-Sep-2026	08-Sep-2026	2 (Punchout)	F47 - Elaine Briggs	Each	1	0	0	1	274.62 GBP	274.62		
2	COLUMN PD-10 EMPTY DISPOSABLE	04-Sep-2026 00:00:00	03-Sep-2026	08-Sep-2026	2 (Punchout)	F47 - Elaine Briggs	Each	1	0	0	1	216.9 GBP	216.90		
3	NuPAGE™ 4 to 12%, Bis-Tris, 1.5 mm, Mini Protein Gel, 15-well, 10 gels (1 box)-Each	04-Sep-2026 00:00:00	03-Sep-2026	08-Sep-2026	2 (Punchout)	F47 - Elaine Briggs	Each	1	0	0	1	88.9 GBP	88.90		
4	Invitrogen NuPAGE MOPS SDS Buffer Kit (for Bis-Tris Gels), Quantity: 1 kit	04-Sep-2026 00:00:00	03-Sep-2026	08-Sep-2026	2 (Punchout)	F47 - Elaine Briggs	Each	1	0	0	1	98.55 GBP	98.55		
												Total	678.97		

2 Plates collected from incubation (VNH, VCH, VCS, VNS)

- VNH had very few colonies and was left to incubate for a further 4.5 hours. No new colonies grew.

WhatsApp_Image_2026-09-02_at_22.31.50.jpeg

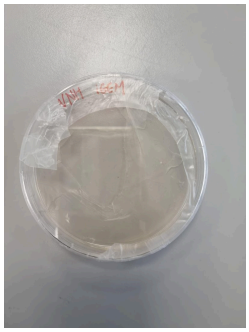


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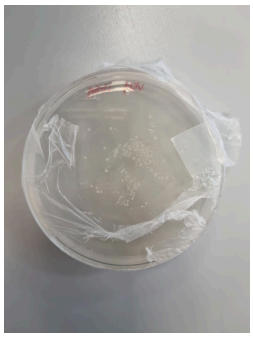


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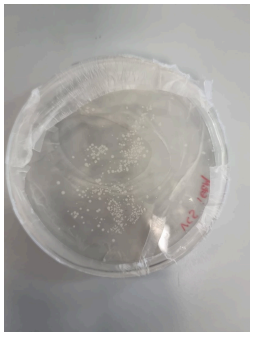


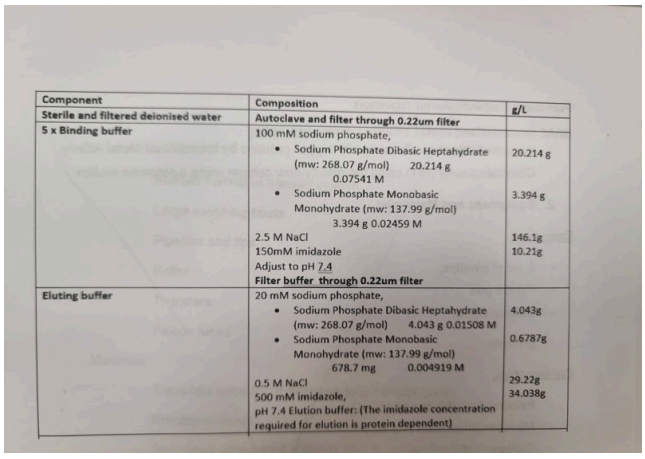
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3 Buffers

Column Binding and Elution buffers (1L each) prepared according to the following protocol and stored in the fridge:

image.png



Component	Composition	g/L
Sterile and filtered deionised water	**Autoclave and filter through 0.22um filter**	
5 x Binding buffer	100 mM sodium phosphate, • Sodium Phosphate Dibasic Heptahydrate (mw: 268.07 g/mol) 20.214 g 0.07541 M • Sodium Phosphate Monobasic Monohydrate (mw: 137.99 g/mol) 3.394 g 0.02459 M 2.5 M NaCl 150mM imidazole Adjust to pH 7.4 **Filter buffer through 0.22um filter**	20.214 g 3.394 g 146.1g 10.21g
Eluting buffer	20 mM sodium phosphate, • Sodium Phosphate Dibasic Heptahydrate (mw: 268.07 g/mol) 4.043 g 0.01508 M • Sodium Phosphate Monobasic Monohydrate (mw: 137.99 g/mol) 678.7 mg 0.004919 M 0.5 M NaCl 500 mM imidazole, pH 7.4 Elution buffer: (The imidazole concentration required for elution is protein dependent)	4.043g 0.6787g 29.22g 34.038g

- Both buffers adjusted to PH 7.4 (Binding buffer initial PH 9.16, Elution buffer initial PH 7.95).

4 Overnight Culture

14 cultures made - 2 colonies per plate

PpChi, SmChiA NC, SmChiA C
VNH, VCH, VCS, VNS

- VNH 2 is a small colony culture.

These steps are carried out in the hood

1. pipette 5 mL of LB medium into each falcon tube
2. pipette 5 uL of chloramphenicol into each falcon tube
3. place the dishes upside down, pick 1 colony with a hoop
4. insert the hoop into the falcon tube and swirl it until the colony dissolves in the solution
5. put the falcon tubes in incubator in 37C 180 rpm overnight (to be collected Thursday 9:20am)

September 3rd 2026

Members Attended: Ella, Raphael

Supervisor: Vlad

Summary:

1 Glycerol Stock
2 Protein Expression
3 Protein expression Induction

1 Glycerol Stock

x14 - 2 per plate
PpChi, SmChiA NC, SmChiA C
VNH, VCH, VCS, VNS

Total volume: 500ul = 250ul sample, 250ul 50% glycerol (Carried out in hood)

2 Protein Expression

Total volume 50ml (volumes of sample and LB added per C1V1=C2V2 calculation in excel)

+50ul chloramphenicol

3 Protein expression Induction

25ul iptg added to all vials

Overnight incubation at 16C

49kDa for VcCDA
53kDa for PpChi
62kDa for SmChia

image.png

Sample	OD (600nm)	df	C2	V2 (ml)	V1 (ml)	LB volume	OD (17:20pm)
VCS 1	0.126	10	0.1	50	3.968254	46.03175	0.021
VCS 2	0.103	10	0.1	50	4.854369	45.14563	0.074
VNS 1	0.144	10	0.1	50	3.472222	46.52778	0.027
VNS 2	0.122	10	0.1	50	4.098361	45.90164	0.059
VCH1	0.059	10	0.1	50	8.474576	41.52542	0.031
VCH2	0.111	10	0.1	50	4.504505	45.4955	0.02
VNH1	0.111	10	0.1	50	4.504505	45.4955	0.021
VNH2	0.118	10	0.1	50	4.237288	45.76271	0.035

September 4rd 2026

Members Attended: Brian, Raphael
Supervisor: Steffi

1. Harvest of cell cultures
Centrifuge at 2500rpm for 30 minutes at 4c (low rpm due to VcCDA fragility).
1mL supernatant and cell pellets both taken.

2. SDS-PAGE Sample Prep
Resuspension of cell pellets from post induction VcCDA, SmChiA, and PpChi as well as pre induction VcCDA (note: preinduction pellets not taken for SmChiA and PpChi unfortunately).
Resuspension performed using calculated volumes (10mL/g cell weight) of 1X Binding Buffer (stored in 4c). Exact resuspension volumes found in physical lab notebook.
If the concentration is too high, perform 1:1 dilution of cell resuspensions also performed (and stored at -20c) - 500uL:500uL resuspension:binding buffer.
Mix 50uL resuspension with 50uL Laemmli buffer and heat for 10 minutes at 95c.

3. SDS-PAGE
Loading dye (5uL) and prepared samples (10uL) loaded onto 2 gels - one with just VcCDA (post induction only; pellet and media), one with SmChiA and PpChi (preinduction and post induction; pellet and media).

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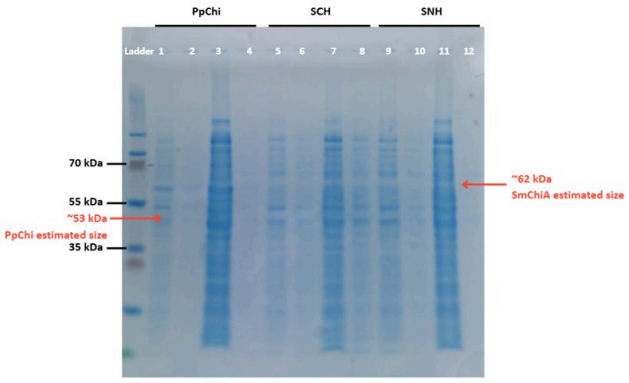


Figure 4. SDS PAGE, Expression study of PpChi, SmChiA (Codon-optimised, His tag), SmChiA (Non Codon-optimised, His tag) expressed in BL21*(DE3). Cell pellet and media were both collected for each variant pre and post-induction with IPTG. Samples for each strain appear as follows: [1] Pre- induction Cell pellet, [2] Pre- induction Media, [3] Post-induction Cell pellet, [4] Post-induction Media.

On the other hand, the SmChiA bands do not show significant band formation at the expected point, implying that the protein has not been expressed. On the other hand, it could just be hidden as a result of the smearing from host cell proteins.
Note: lane 8 shows some proteins, but it is most likely due to some of the sample from lane 7 falling into it.
All three enzymes will be selected for large-scale protein-expression.

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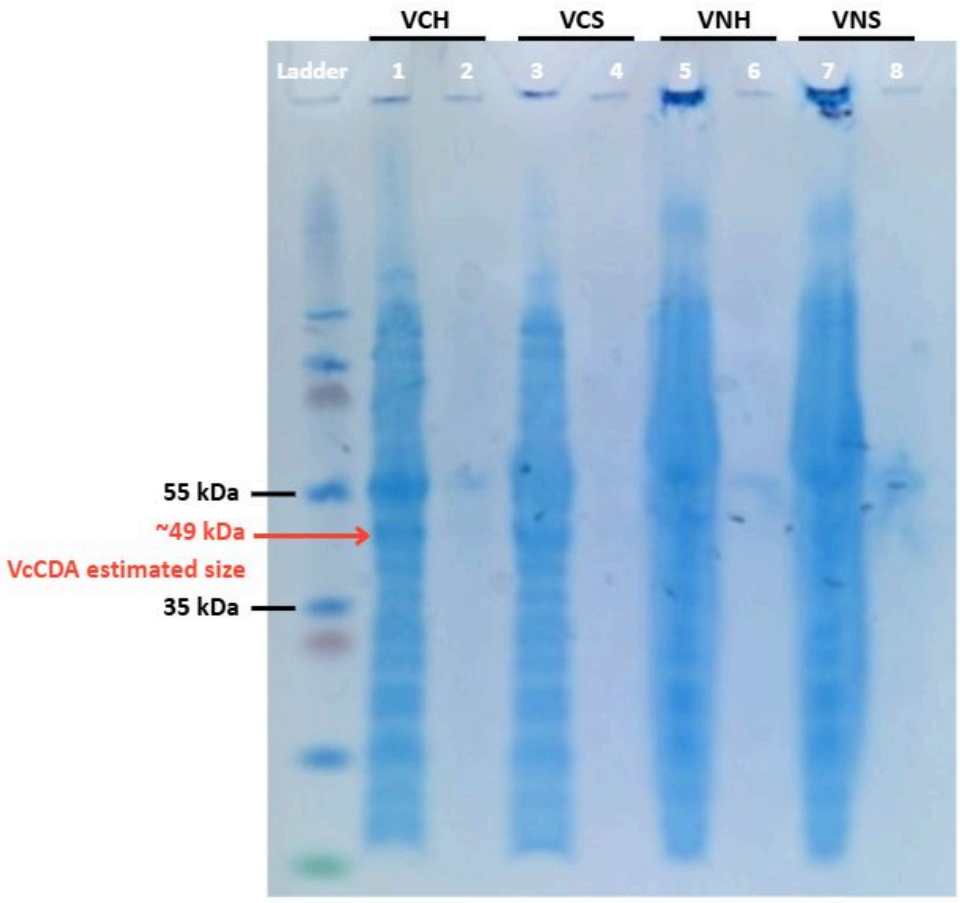


Figure 2: SDS PAGE, Expression study of four VcCDA variants expressed in BL21*(DE3). Variants VCH, VCS, VNH and VNS are VcCDA (Codon-optimised, His tag), VcCDA (Codon-optimised, Strep tag), VcCDA (non Codon-optimised, His tag), and VcCDA (non Codon-optimised, Strep tag) respectively. Cell pellet and media were collected for each variant post-induction with IPTG. Samples for each strain appear as follows: (1) Cell pellet, (2) Media.

As seen above, no significant band formation occurs at the expected point, though lane 1 has a notable band at around 55kDa that is not visibly seen in any other lanes. This could potentially be the successful expression of VcCDA Codon-Optimised His-tag with the band being in the wrong location as a result of interference - notably, the large amount of crystals formed within the gel.

Additionally, it can be seen that the protein is not expressed extracellularly.

VcCDA Codon-Optimised His-Tag will be selected for large-scale protein-expression.

All visual annotations can be justified using density analysis from ImageJ software. These have been pasted below, the first being for PpChi/SmChIA, the second being for VcCDA.

Plan for week 7 Sep

July 20-7

	Task	Output	Chemicals	Equpment	Dates	Team members	Supervisors	H	I	J	K	L	M	N
1	autoclave everything (on our bench + 5 1L baffled flask)				monday 9am	Brian Maria								
2	collect deliveries (SDS PAGE stuff, 1kb ladder)				monday 10am	Brian Maria								
3	sample prep & imaging SDS-PAGE				monday morning	Brian Maria								
4	sonication	Supernatant, as is, pellet resuspend in 2mL 1X binding buffer		Sonicator level 6 ask Steffi lab group, 2mL centrifuge 18000rpm 20 mins	monday 4pm	Brian Maria								
5	run SDS PAGE (rerun of the full 20 samples + soluble/insoluble)				tuesday 9am	Raphael Maria								
6	overnight culture with glycerol stock	VcCDA C His, NC His, PpChi, SmChiA C, NC	10mL LB		tuesday 4pm	Ella Maria								
7	Protein expression (start OD at 0.1) estimated 3hrs	Get OD 0.6		Shaking incubator	Thursday 9am	Ella Raphael								
8	Protein expression induction phase for 16-18 hours	make sure to take samples before induction	IPTG		Thursday 1pm?	Ella Raphael								
9	Pack Chelating Sepharose column				Thursday 1pm	Ella Raphael	Steffi							
10	Harvest cell pellet and store in -20 (resuspend inn binding buffer and store in -20)				Friday 9am	Maria Raphael								
11	Sonicate + purify in column				Friday	Maria Raphael								
12	run SDS PAGE (either the stuff that wasn't done in time on tuesday, and big culture if possible) possibly move to monday				Fri/next Monday	probably not possible this week								

Aim for the week: Larger scale (200mL) protein expression, and immobilised metal affinity chromatography (IMAC) for protein purification.

September 7th 2026

1 . Protein Expression

aim/ note on the procedure:
To generate larger quantities of protein for later purification by IMAC.
This was done for all his-tagged enzymes - VCH, VNH, SCH, SNH, P.

1. Prepare all reagents needed: LB media.
2. Autoclave all required components: LB Media, 1L flasks, cotton, pipette tips.

September 8th 2026

Members Attended: Ella, Maria, Raphael

Supervisor:

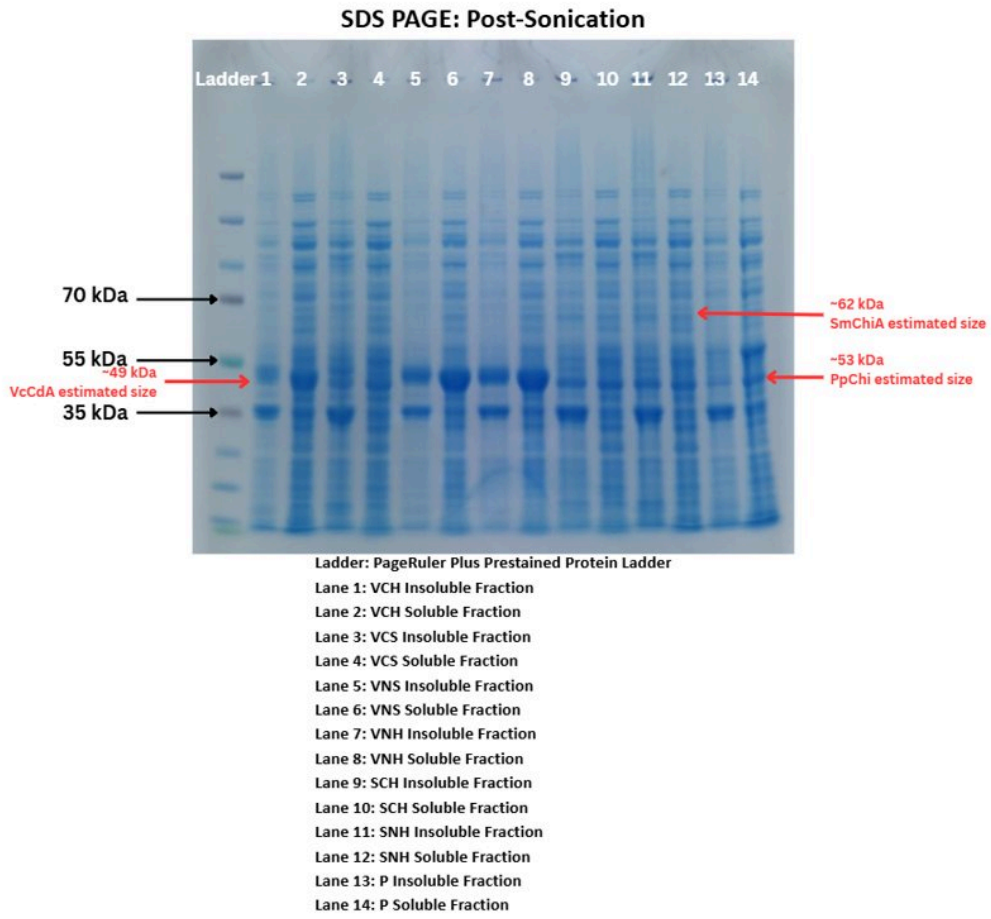
Summary:

1 SDS PAGE

2 Overnight Culture

1 SDS PAGE

image.png



Conclusion: VcCDA and PpChi appear expressed, SmChiA doesn't.

2 Overnight Culture

VCH1, VNH1, SmChiA C 1, SmChiA NC 1, PpChi

These steps are carried out in the hood

1. pipette 10 mL of LB medium into each falcon tube
2. pipette 10 uL of chloramphenicol into each falcon tube
3. place the dishes upside down, pick 1 colony with a hoop
4. insert the hoop into the falcon tube and swirl it until the colony dissolves in the solution
5. put the falcon tubes in incubator in **37C 180rpm (*incubator was in use so ideal conditions not met, actual conditions 30C 170rpm) overnight (to be collected Thursday 9:30am)

OD values measured at 9:30am (Too low for

September 9th 2026

Members Attended: Ella, Brian
Supervisor: Vlad

Summary:

1 Protein expression
2 Overnight culture

1 Protein Expression

OD values measured at 9:30am (Too low for induction)

11:15am samples moved to 37C incubator at 180rpm.

12:15 OD re-measured, values too low.

13:30 OD re-measured, values were similar to previous measurement so we decided to induce with IPTG.

image.png

9:30am 09.09					
sample	OD (600nm)	df	C2	V2 (ml)	V1 (ml)
VNH	0.068	10	0.1	200	29.41176
VCH	0.093	10	0.1	200	21.50538
SmChiaC1	0.04	10	0.1	200	50
SmChiaNC1	0.092	10	0.1	200	21.73913
ppchi	0.129	10	0.1	200	15.50388
12:15pm 09.09					
sample	OD (600nm)	df	C2	V2 (ml)	V1 (ml)
VNH	0.133	10	0.1	200	15.03759
VCH	0.143	10	0.1	200	13.98601
SmChiaNC1	0.131	10	0.1	200	15.26718
SmChiaC1	0.116	10	0.1	200	17.24138
ppchi	0.168	10	0.1	200	11.90476
13:30pm 09.09					
sample	OD (600nm)	df	C2	V2 (ml)	V1 (ml)
VNH	0.136	10	0.1	200	14.70588
VCH	0.145	10	0.1	200	13.7931
SmChiaNC1	0.131	10	0.1	200	15.26718
SmChiaC1	0.119	10	0.1	200	16.80672
ppchi	0.189	10	0.1	200	10.58201

2 IPTG Induction

IPTG Induction at 2pm

100ul IPTG added to 200ml flasks. Overnight incubation at 20C 180rpm

Total volume 200ml - 290ml LB broth, 200ul chloramphenicol, ~11ml sample

IDT order by Steffi 07/09/2026:

Fragments for fusion proteins

fragment 1 ChiA CDA His

fragment 2 ChiA CDA His

fragment 1 CDA ChiA His

fragment 2 CDA ChiA His

Expected delivery date 21/09/2026

Integrated_DNA_Technologies_-_Order_Confirmation_Fusions_SFK070926.pdf

3:40 bring everything to mezzanine - ice, glycerol stock

Make overnight at 16:30 pm ready by 5 (2x10ml each) from glycerol, bring iptg up, image gel

use lab 6.01 37degree shaker

September 10th 2026

Members Attended: Ella, Raphael

Supervisor: Tamas

Summary:

1 Harvest

2 LB broth and autoclave

3 Column loading

1 Harvest

1. Harvest of cell cultures

Aliquot culture into Falcon tubes for centrifugation at 4C and 2500rpm for 30 minutes (low rpm due to the fragility of VcCDA).

Pellets were weighed and resuspended in 1X binding buffer at 10mL/g.

Resuspension was placed in -20C.

2. SDS-PAGE Sample Prep

Resuspension of cell pellets from post induction VcCDA C, VcCDA NC, SmChiA C, SmChiA NC and PpChi

Resuspension performed using calculated volumes (10mL/g cell weight) of 1X Binding Buffer (stored in 4c).
1:1 dilution of cell resuspensions also performed (and stored at -20c).
Dilutions mixed with Laemmli buffer and heated at 100c for 10 minutes.

Pellet weight and resuspension volumes

	A	B	C
1	Pellet	weight (g)	BB (ml)
2	VNH 1	0.5	5
3	VNH 2	0.46	4.6
4	VNH 3	0.7	7
5	VNH 4	0.4	4
6	VCH 1	0.47	4.7
7	VCH 2	0.57	5.7
8	VCH 3	0.7	7
9	VCH 4	0.56	5.6
10	SmChiA NC 1	0.5	5
11	SmChiA NC 2	0.55	5.5
12	SmChiA NC 3	0.67	6.7
13	SmChiA NC 4	0.67	6.7
14	SmChiA C 1	0.7	7
15	SmChiA C 2	0.5	5
16	SmChiA C 3	0.8	8
17	SmChiA C 4	0.5	5
18	PpChi 1	0.65	6.5
19	PpChi 2	0.6	6
20	PpChi 3	0.6	6
21	PpChi 4	0.2	2

3. column packing
    1. Prepare nickel sulfate solution in a fume hood.
    2. Prepare IMAC columns using the following workflow:
        a. Place filter with rough side facing upwards
        b. Secure the column, making it vertical using a clamp stand with a liquid receptacle below it.
        c. Wash through once with 20% ethanol
        d. Add 5mL of nickel chelating sepharose, making sure to mix it well beforehand to dissolve the resin
        e. Add 2.5 column volumes of MilliQ water to rinse out the ethanol and pack the resin

f. Add 2.5 column volumes of nickel sulfate to charge the resin
g. Add 5 column volumes of MilliQ water to rinse out the excess nickel sulfate
h. Add 2.5 column volumes of 20% ethanol (long-term use, not done) or 1X binding buffer (short-term use)
i. Place the cap and lid onto the column, ensuring a small reservoir of liquid sits above the resin - never allow it to dry out
j. Place the column into the fridge (4C), keeping it vertical

September 11th 2026

Members Attended: Raphael, Maria
Supervisor: Vlad

Summary:

1. Made W60 buffer (see paper lab book).
2. Performed sonication of VNH, SCH, and P samples made on September 10th [30 sec on, 30 sec off, 5 cycles].
3. Took a 1mL sample of lysate "L".
4. Centrifuged 17mL of lysate at 13,000g x 30 min (reduced from 18,000g x 20 min for safety reasons) and removed supernatant.
5. Resuspended pellets in 10mL 1X Binding Buffer.
6. Took 1mL samples of supernatant "S" and resuspension "I". Put resuspension in the freezer.
7. Ran all remaining supernatant through IMAC columns and washed through with 25mL Binding Buffer, 25mL W60 Buffer, and 14mL of Elution Buffer.
8. Took 1mL samples of all flowthroughs (supernatant "FT", Binding Buffer "BB", W60 Buffer "W60") and 2mL samples of elutions ("E1" to "E7").
9. All eppendorf samples ("L", "S", "I", "FT", "BB", "W60", "E1-E7") placed in freezer whilst all Falcon collections (resuspension, flowthrough, binding buffer, w60) placed in fridge.
10. Washed columns with MilliQ and 20% EtOH before storing in fridge.

Note: IMAC took around 5.5 hours for some reason. As a result, some changes to the protocol were made:

1. Used 25mL BB and W60 rather than the full 50mL.
2. Threw out the VNH column. It was the slowest one and would've taken an additional 30-60 minutes to rinse and store whilst the other two columns had already been in the fridge for half an hour.
3. Attempted to use positive pressure to speed up the flow rate.

results: See SDS PAGE performed 14th September.

Conclusions: See SDS PAGE performed 14th September. In summary: all three proteins expressed and were eluted sucessfully with notable purity.

Plan for week 14 Sep

Table2

	A	B	C	D	E	F	G
1	Task	Output	Chemicals	Equiment	Dates	iGEM lead	Supervisors
2	run SDS PAGE (200ml culture and IMAC VNH, SCH, PpChi) + imaging	3x Gels (L", "S", "I", "FT", "BB", "W60", "E2-E4")			Monday	Ella	Brandon Alex
3	Sample prep VCH, SNH for IMAC (sonication) + make buffers if needed, load 3 columns			Sonicator	Tuesday 9am	Brian Raphael	Tamas/ Brandon
4	IMAC (VCH, SNH)	Get OD 0.6			Wednesday 9am	Ella Maria	Brandon Alex
5	SDS Page IMAC purified VCH, SNH			Centrifuge	Wednesday 2:30pm	Ella Maria	Brandon Alex
6	Buffer exchange elutions (imidazole free)				Wednesday 5pm	Ella Maria	Brandon Alex
7	Bradford assay	+standard curve need to make		Microplate Reader, Centrifuge	Thursday 9am	Brian Ella	Alex
8							
9							
10							
11							

September 14th 2026

Members Attended: Ella

Supervisor: Kuan, Alex

Summary:

1 SDS Page

2 Gel imaging

1 SDS Page for IMAC purified VCH, SCH, PpChi

1. SDS Page sample prep:

Total volume of all loading samples 20ul

Loading dye used: 2x Laemmli

Samples:

Diluted 1:5 for clearer gel bands: 2ul sample + 8ul BB + 10ul loading dye:

Lysate after sonication

Supernatant (after sonication and spinning)

Pellet (after sonication and spinning)

10ul sample + 10ul loading dye:
Flow Through (supernatant)
Binding Buffer
Wash 60
Elution samples 2-4

2. Samples boiled at 95C for 10mins

3. Run SDS Page

Running buffer made: 5x running buffer diluted: 50ml buffer + 950ml MilliQ

SDS page run at 150volts for 1hr 10min

ladder: stored in the fridge until use - Primestep prestained protein ladder

2 Gel imaging

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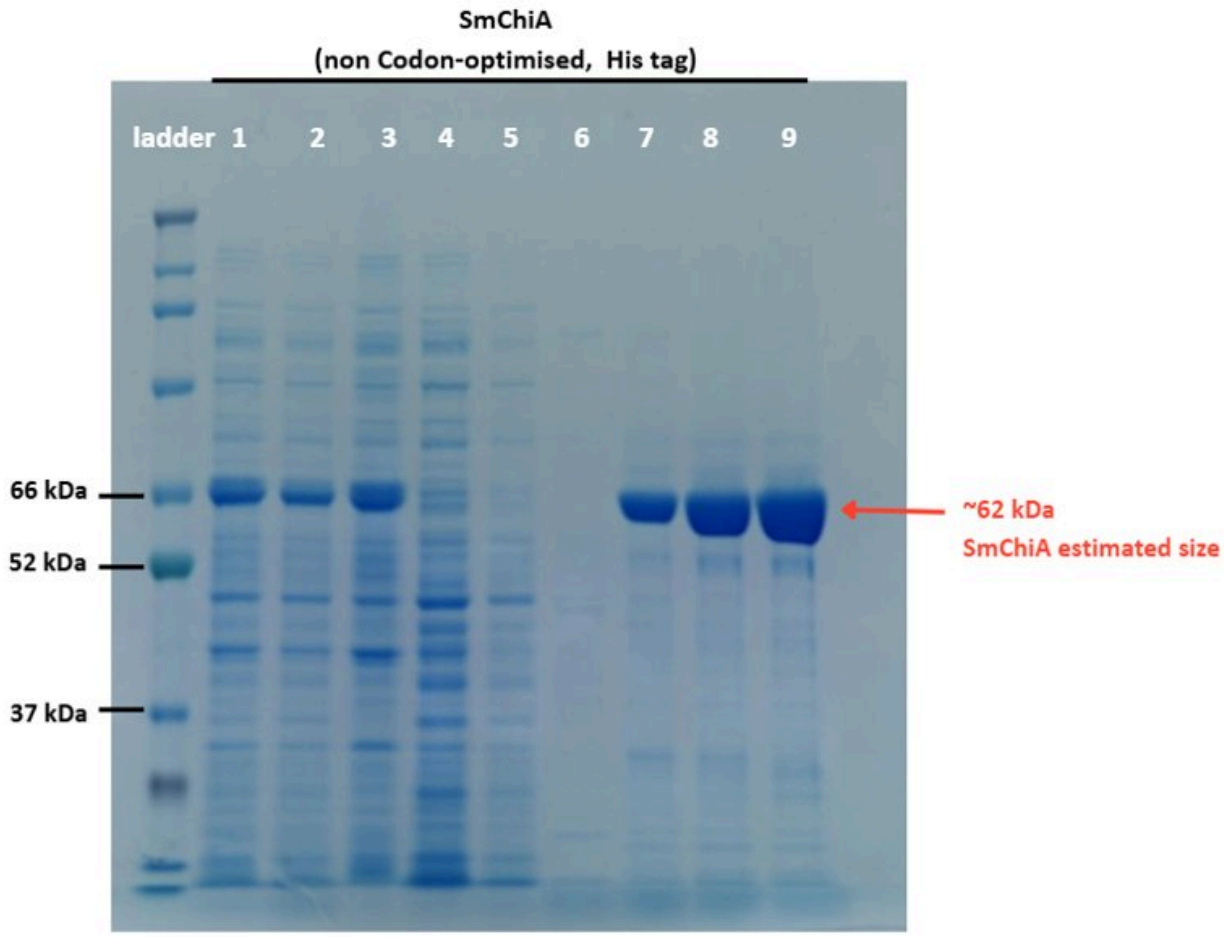
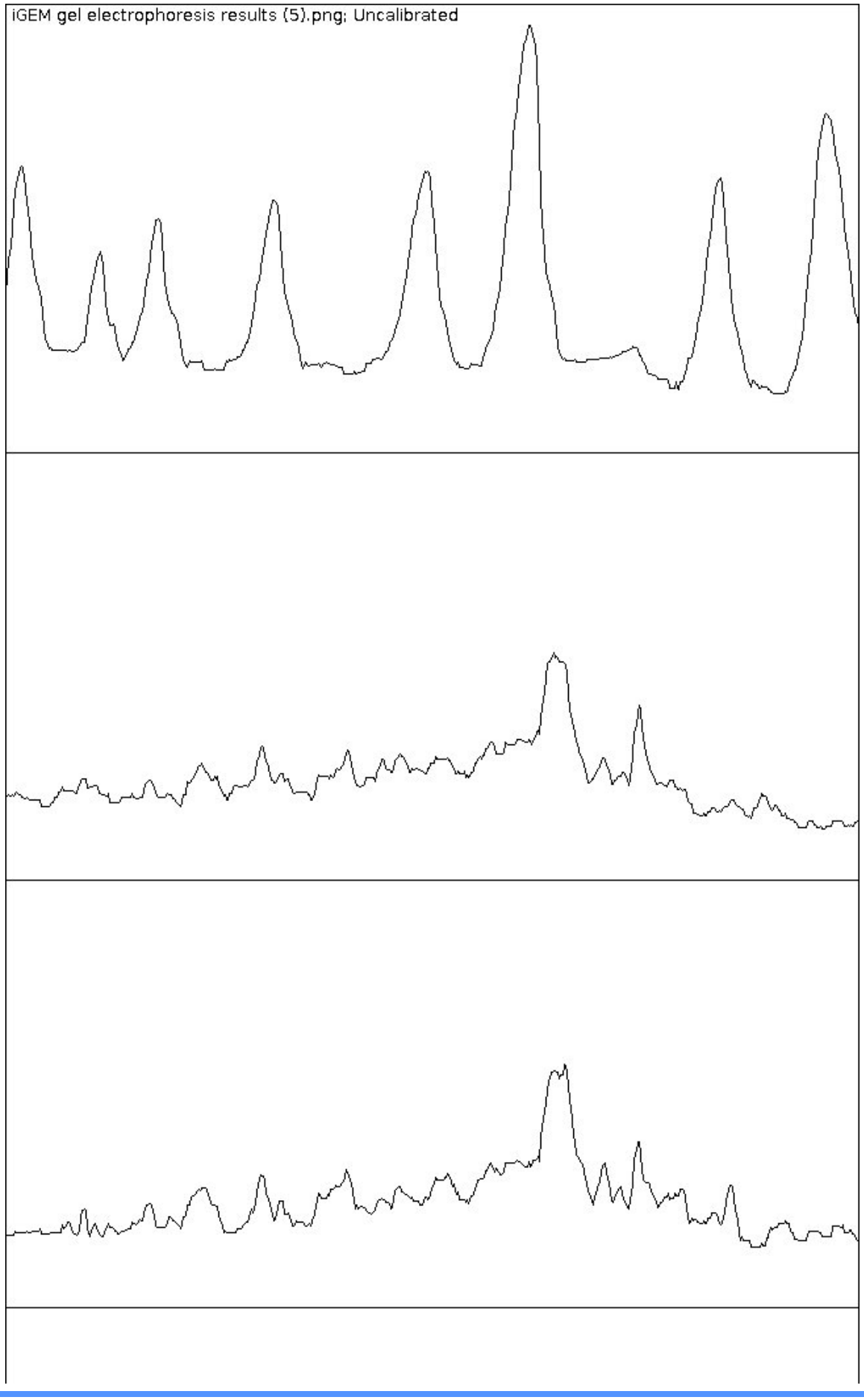
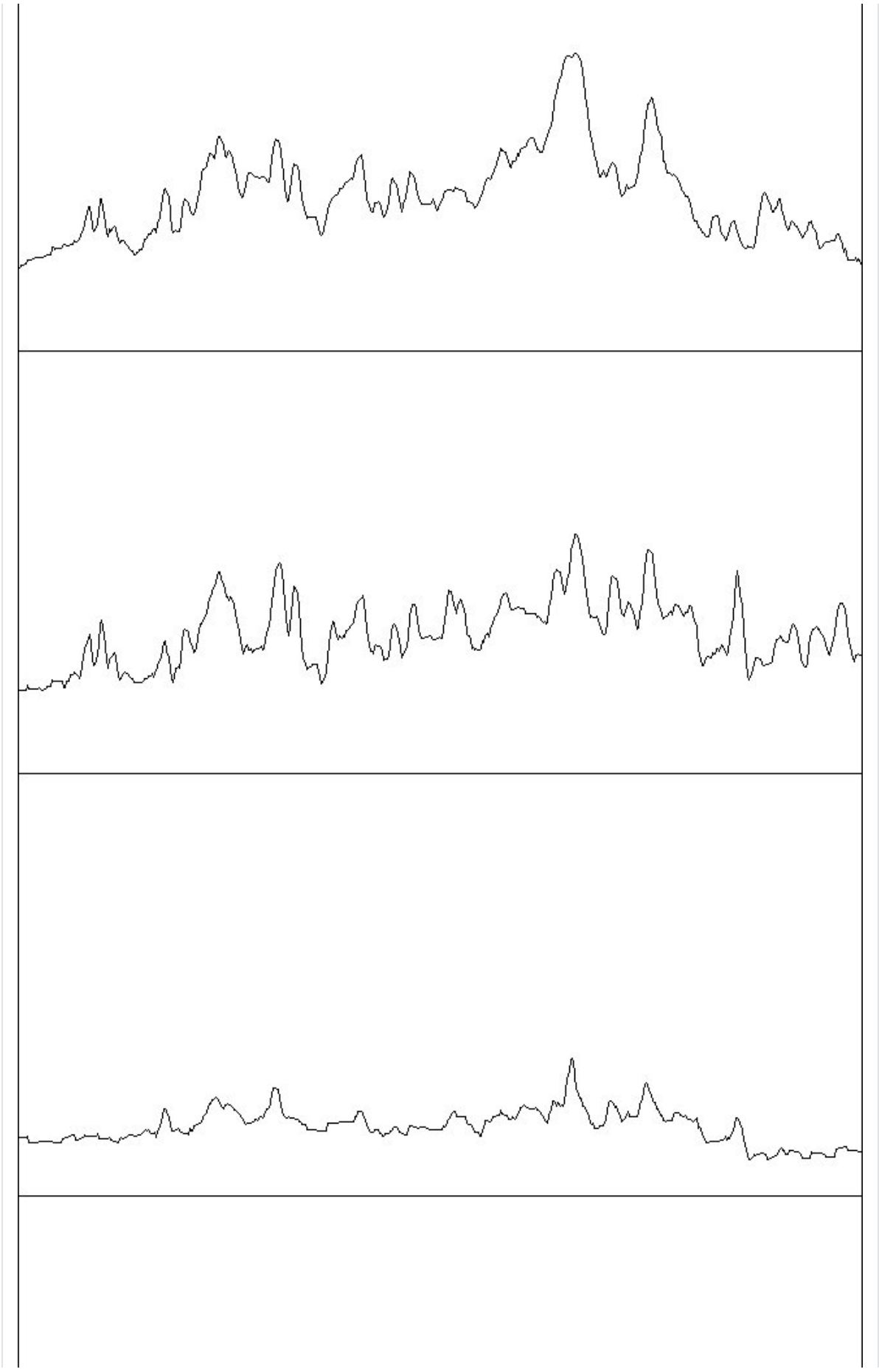


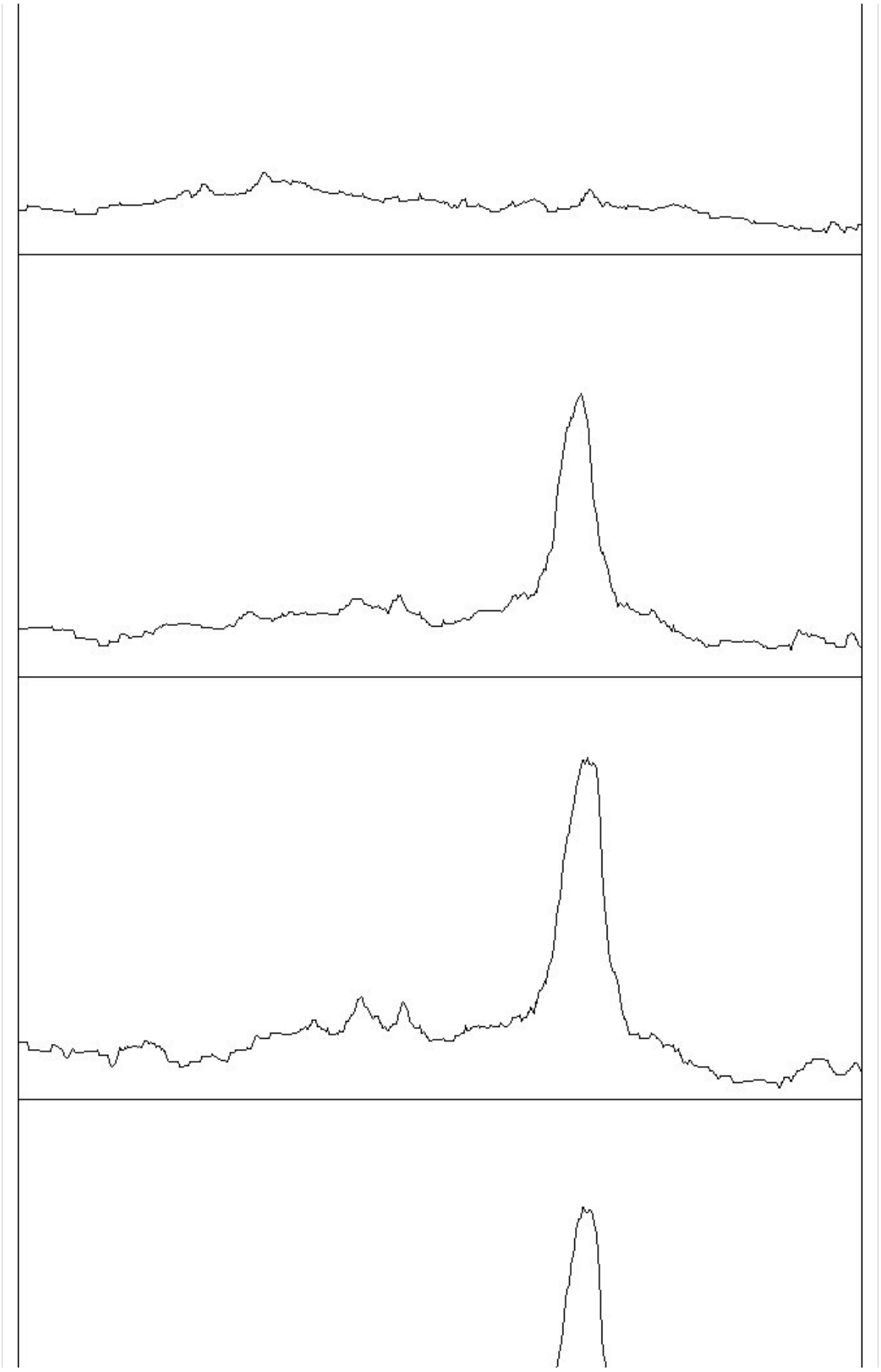
Figure 4. SDS Page, His-Trap IMAC Purification study of SmChiA (non Codon-optimised, His tag) (lanes 1-9). Nine samples collected after IMAC purification were loaded in the following order: [1] Lysate after sonication (5x dilution), [2] FSupernatant after sonication and spinning (5x dilution), [3] Pellet after sonication and spinning (5x dilution), [4] Flow Through Supernatant [5] 5x Binding Buffer Wash, [6] 60mM Imidazole Wash, [7] Elution fraction 2, [8] Elution fraction 3, [9] Elution fraction 4.

Imagej of purification results:

Plots_of_Vc.tif


iGEM gel electrophoresis results (5).png; Uncalibrated





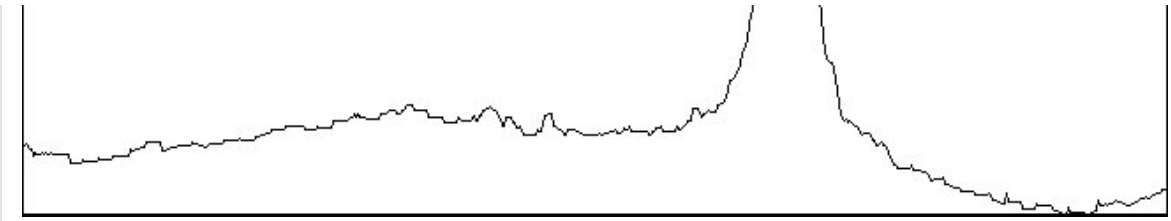


image.png

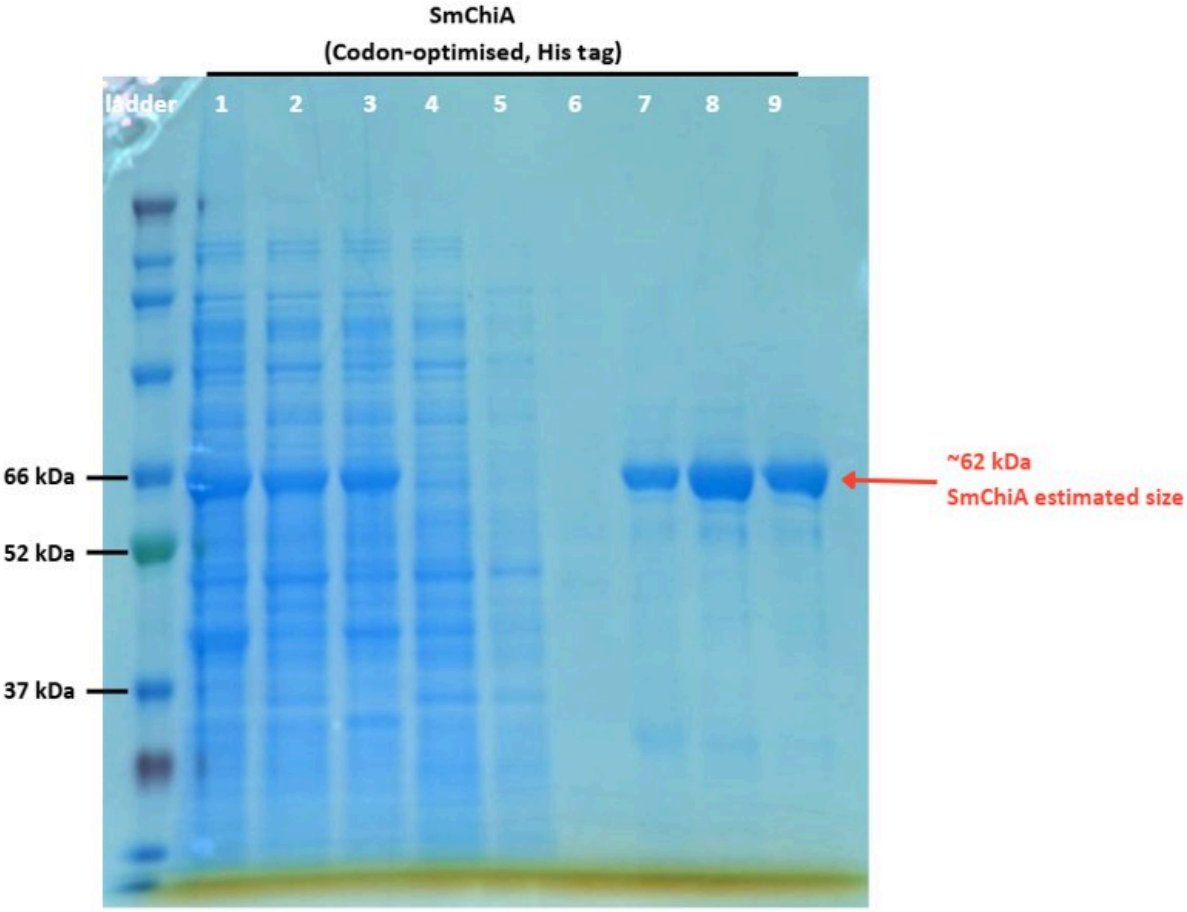
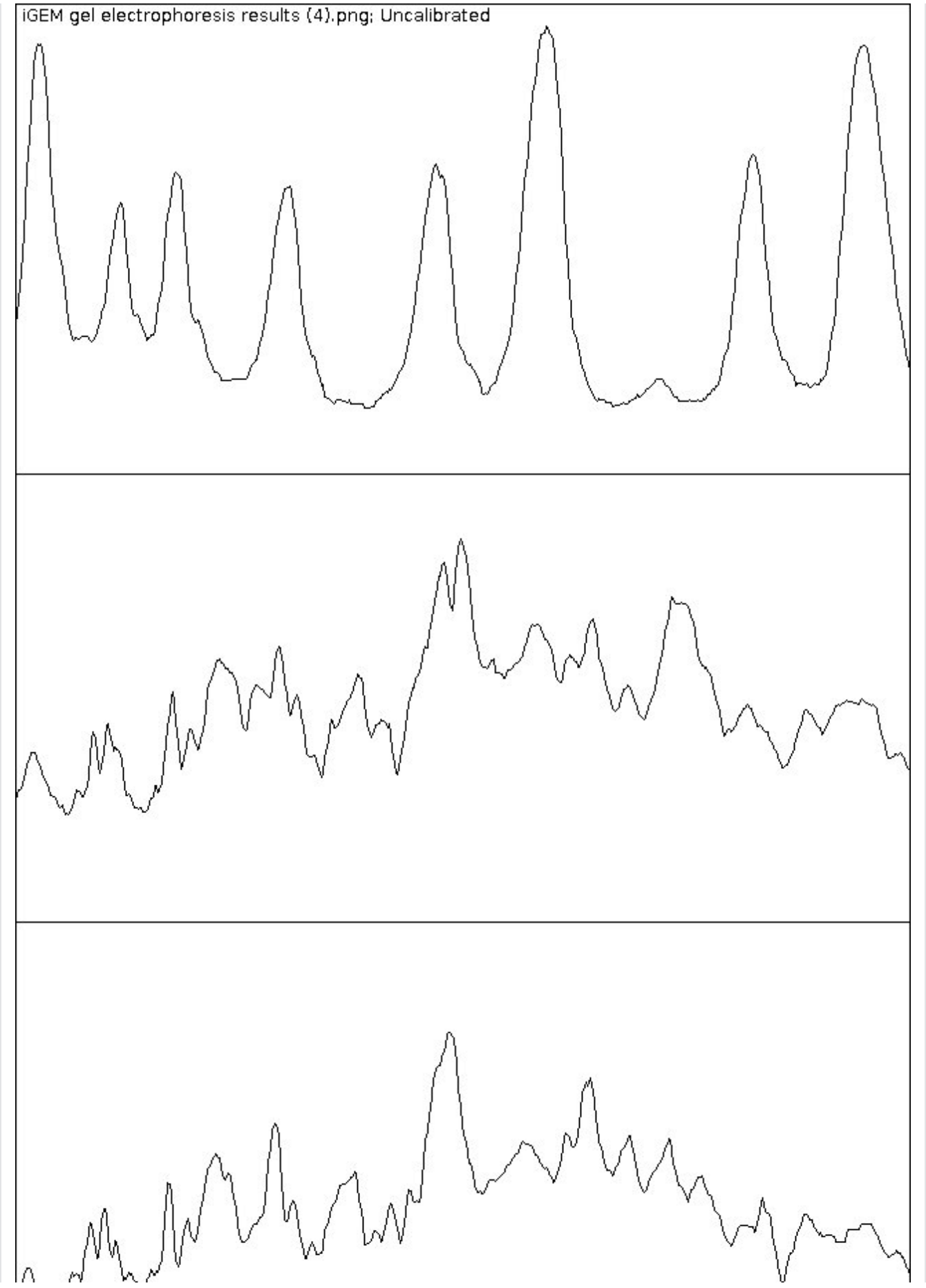
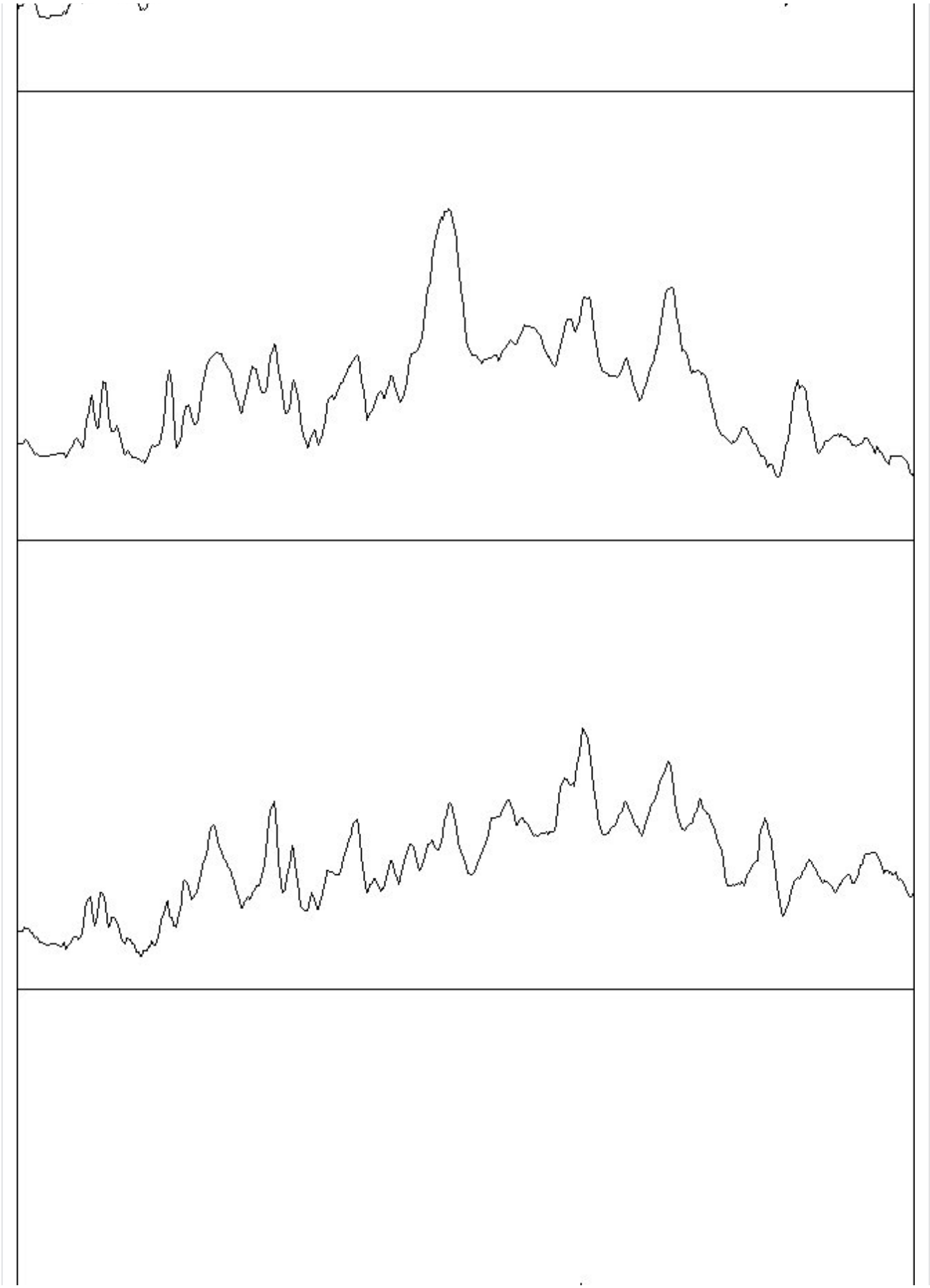
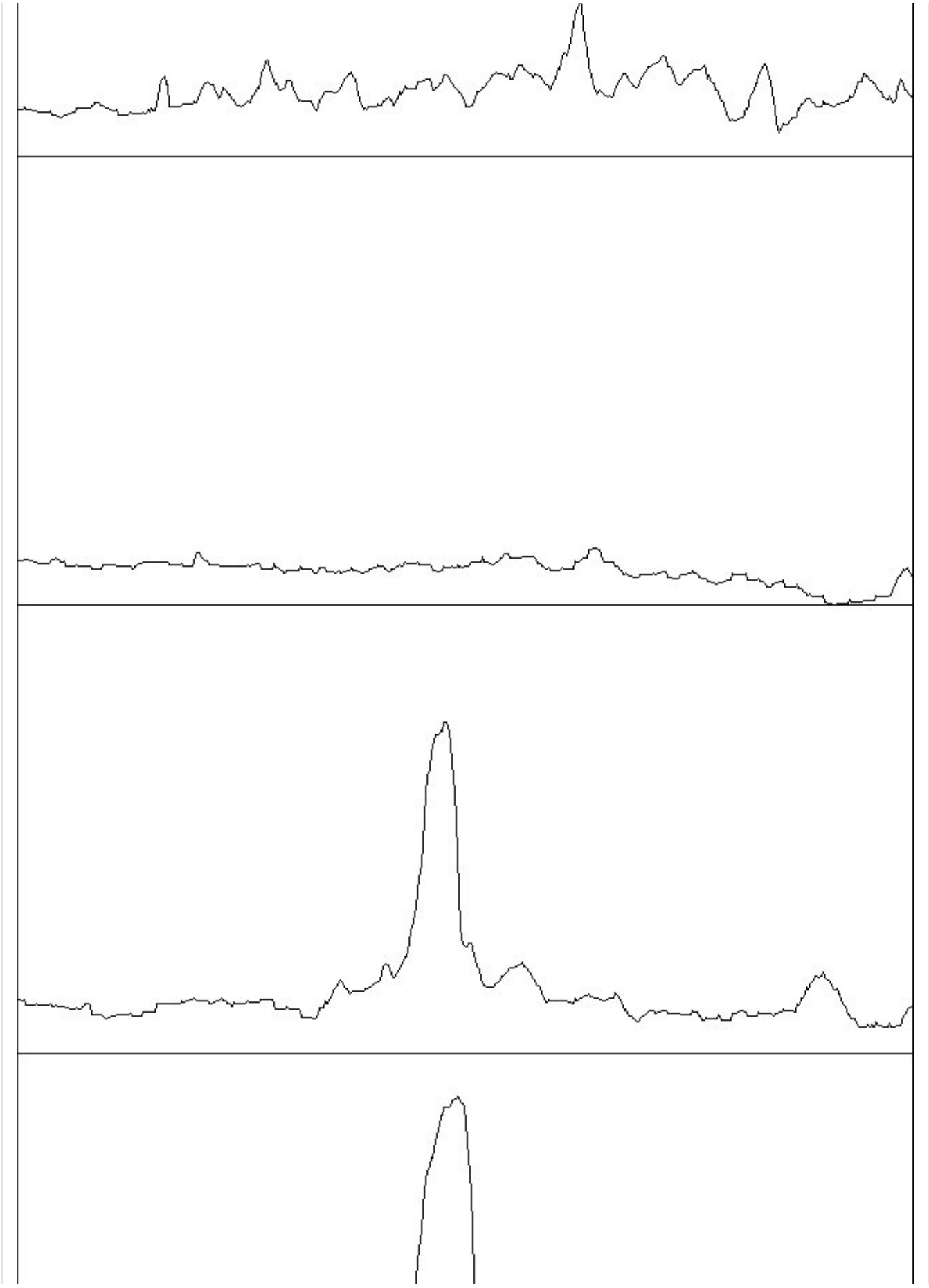


Figure 4: SDS Page, His-Trap IMAC Purification study of SmChiA (Codon-optimised, His tag) (lanes 1-9). Nine samples collected after IMAC purification were loaded in the following order: [1] Lysate after sonication (5x dilution), [2] FSupernatant after sonication and spinning (5x dilution), [3] Pellet after sonication and spinning (5x dilution), [4] Flow Through Supernatant [5] 5x Binding Buffer Wash, [6] 60mM Imidazole Wash, [7] Elution fraction 2, [8] Elution fraction 3, [9] Elution fraction 4.

Plots_of_Sm.tif


iGEM gel electrophoresis results (4).png; Uncalibrated





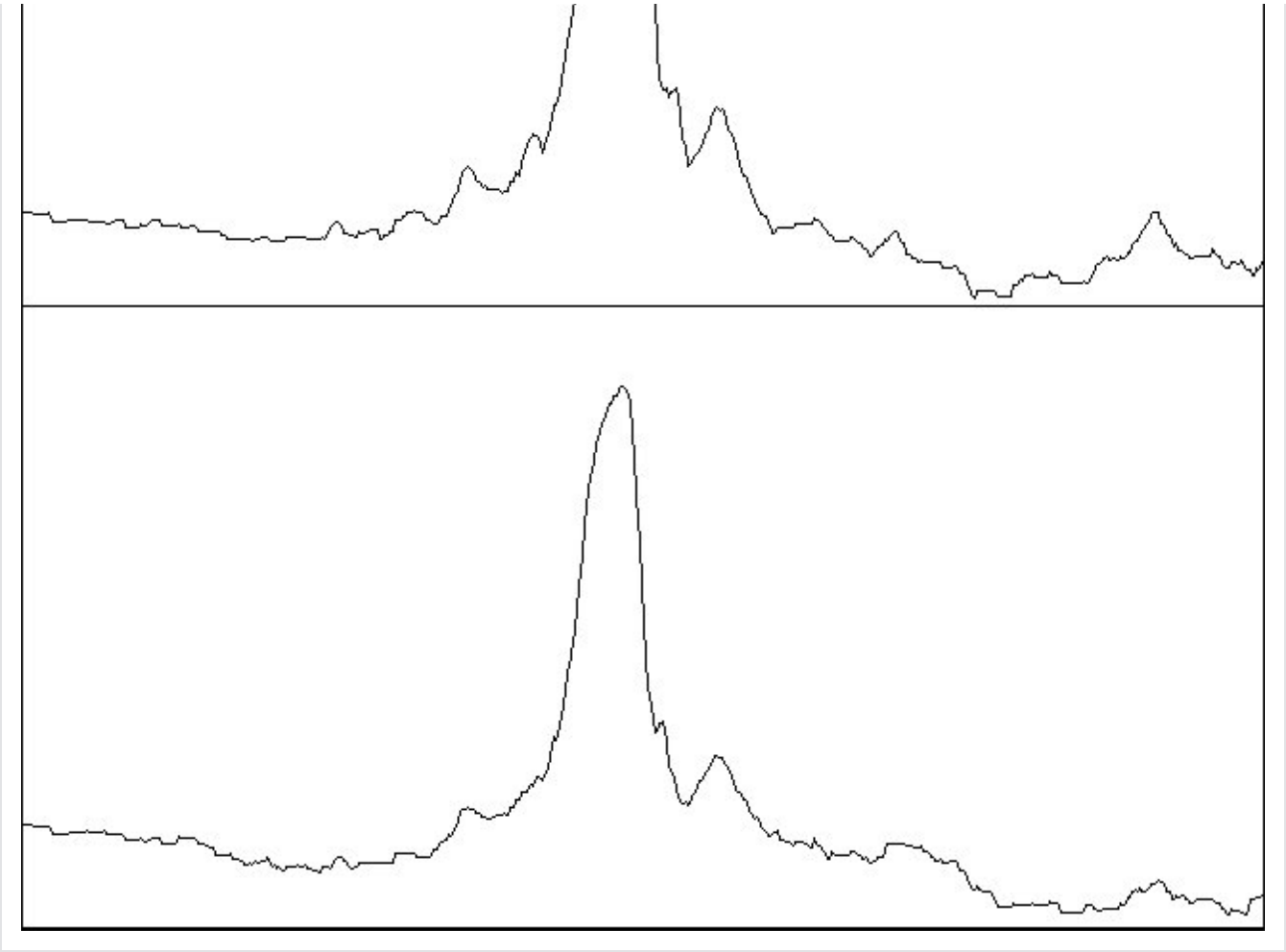


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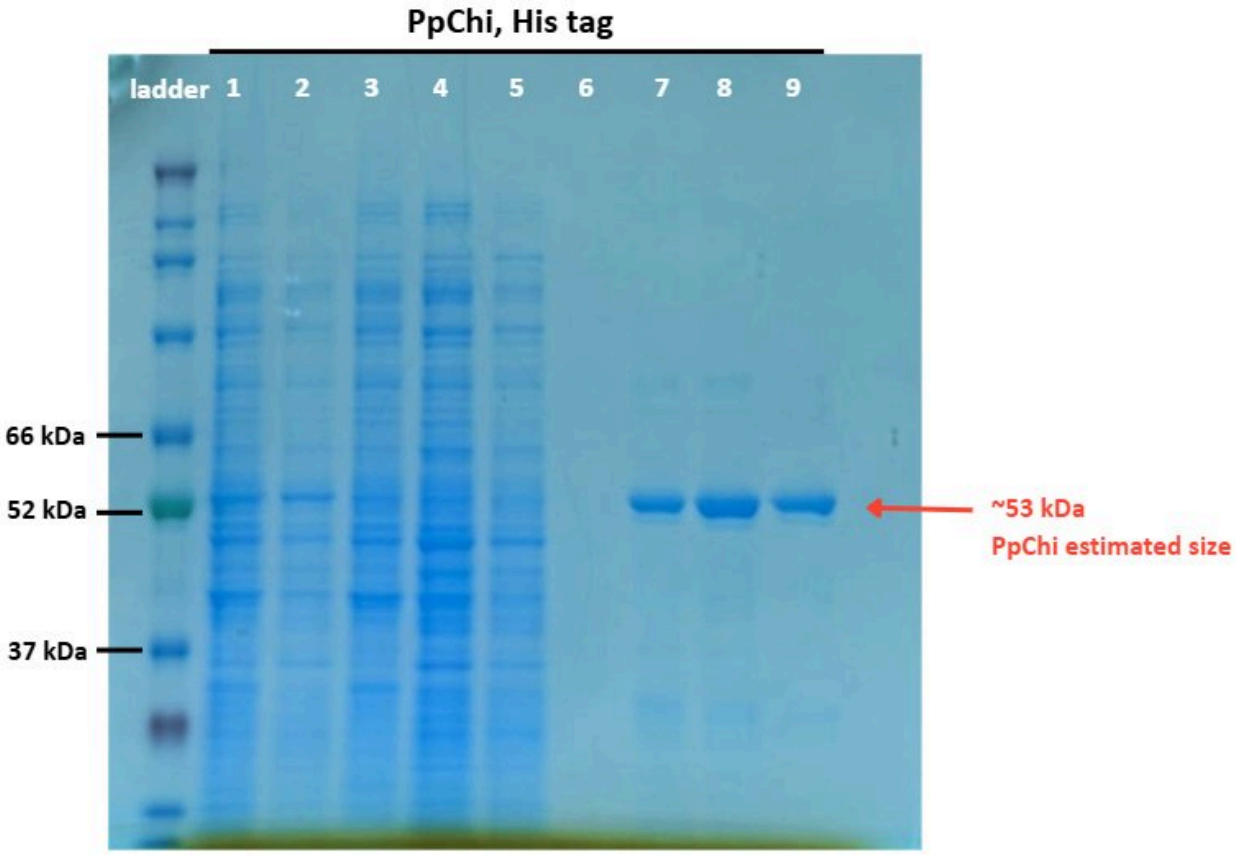
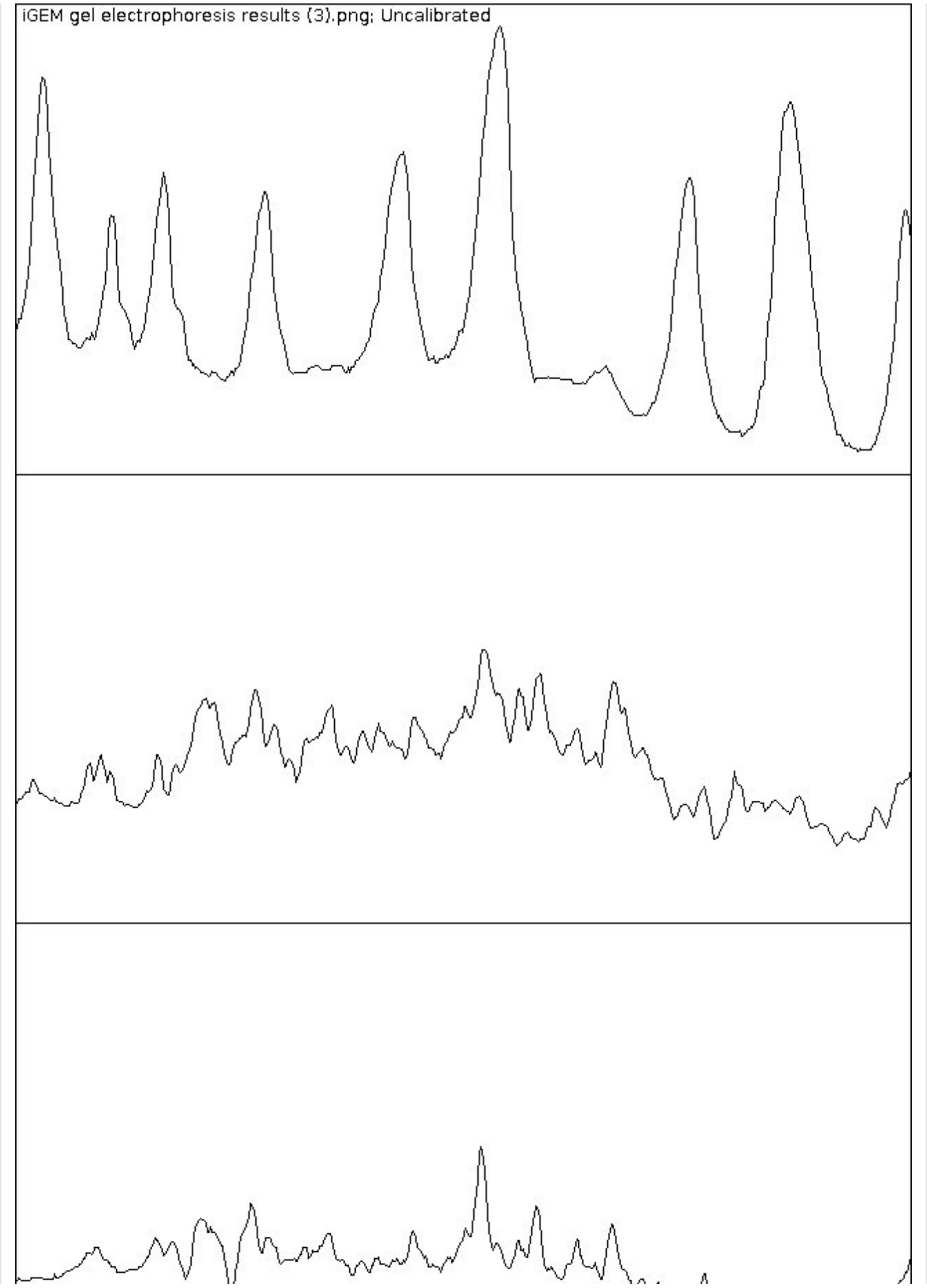
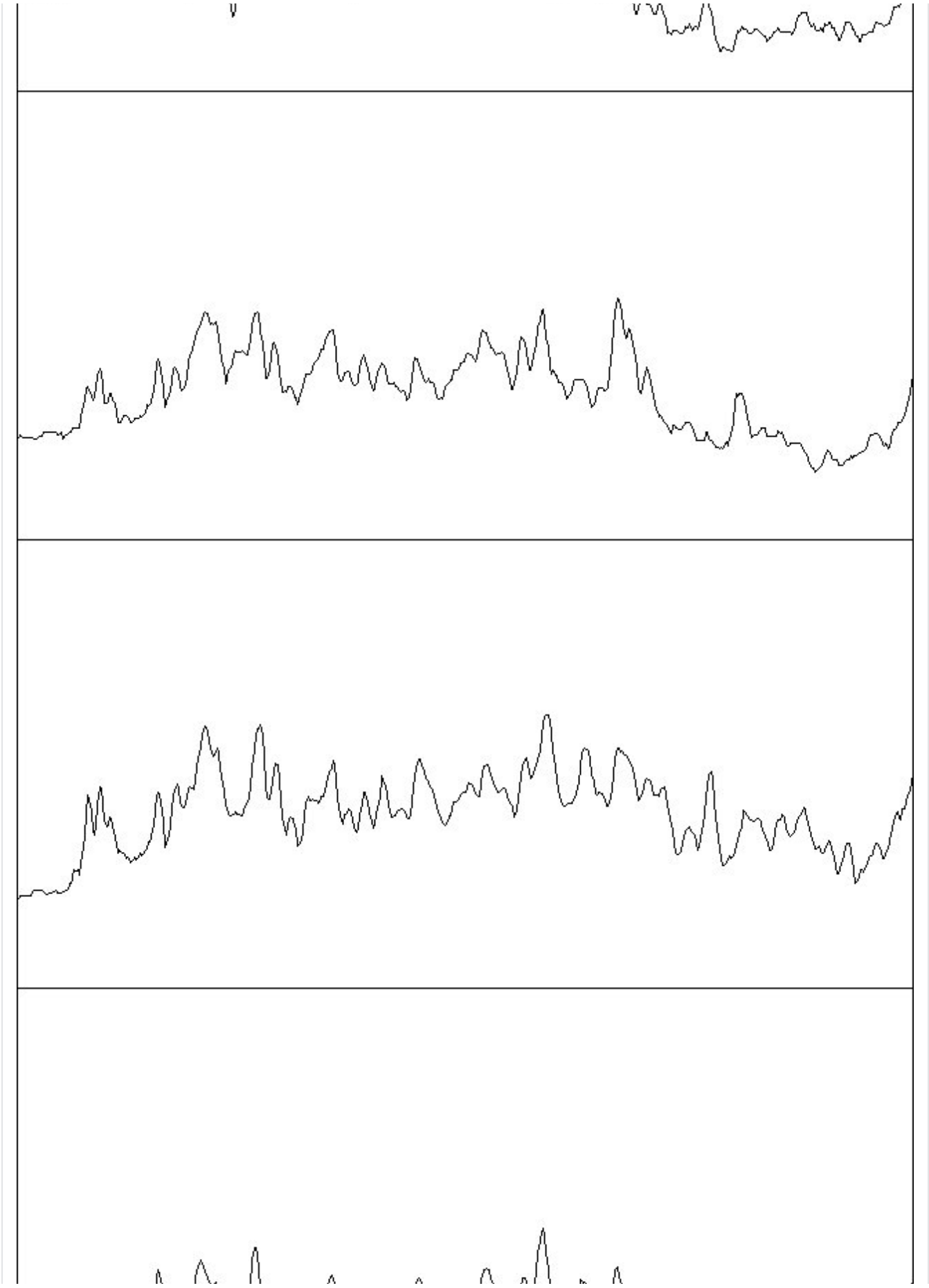
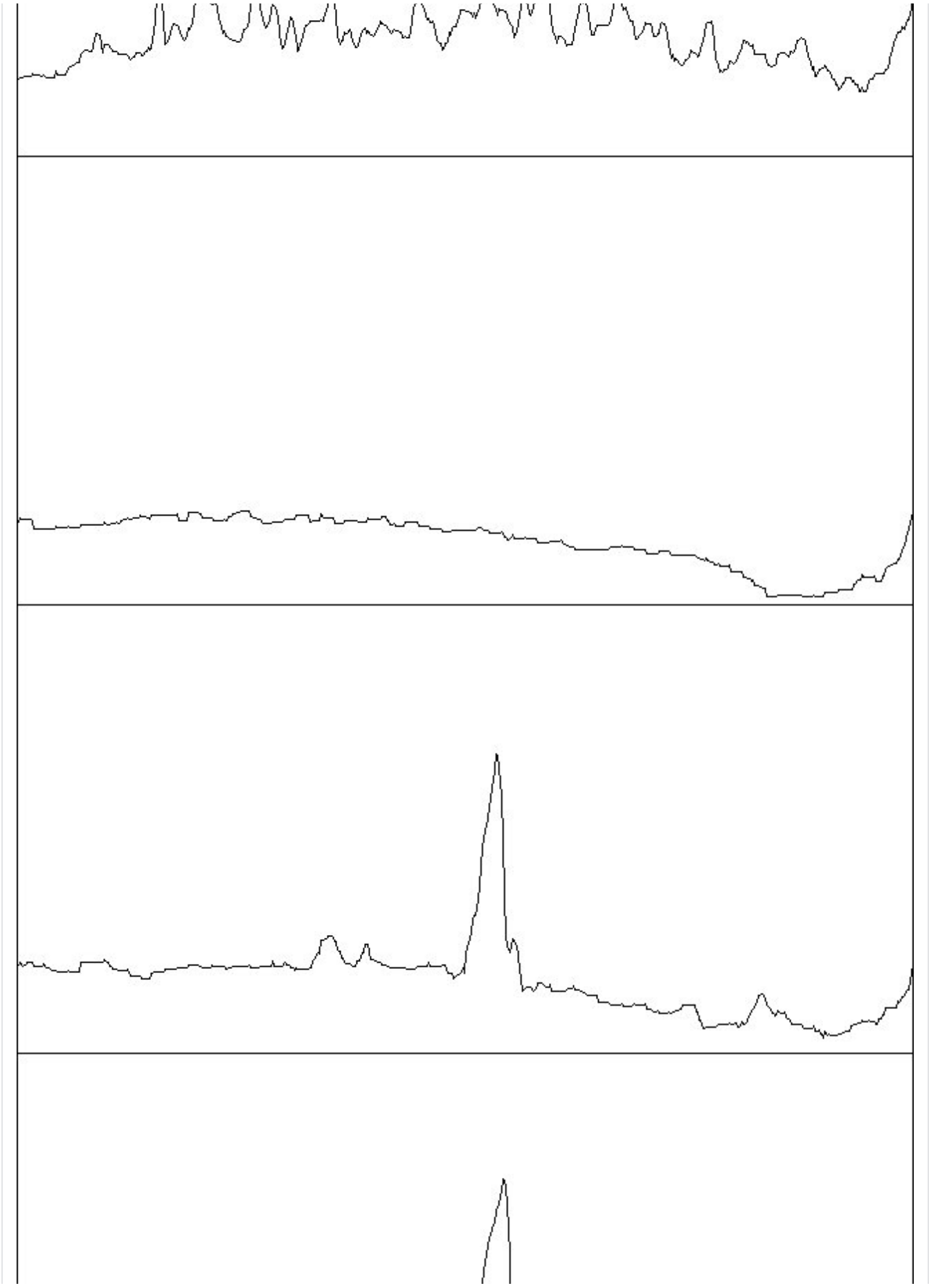


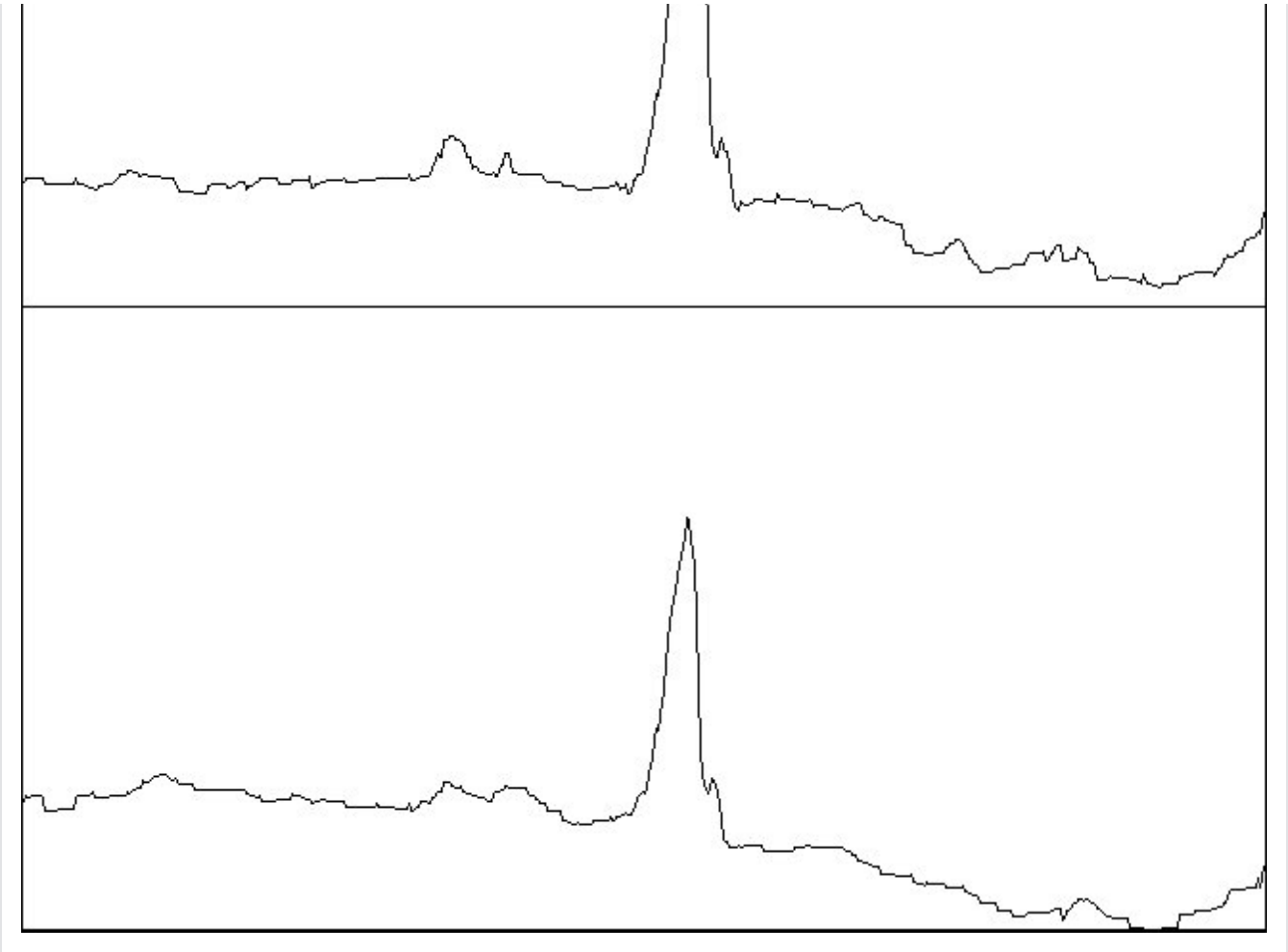
Figure 5. SDS Page, His-Trap IMAC Purification study of PpChi with a His tag (lanes 1-9). Nine samples collected after IMAC purification were loaded in the following order: [1] Lysate after sonication (5x dilution), [2] FSupernatant after sonication and spinning (5x dilution), [3] Pellet after sonication and spinning (5x dilution), [4] Flow Through Supernatant [5] 5x Binding Buffer Wash, [6] 60mM Imidazole Wash, [7] Elution fraction 2, [8] Elution fraction 3, [9] Elution fraction 4.

Plots_of_Pp.tif


iGEM gel electrophoresis results (3).png; Uncalibrated







All three (VCH, SCH, PpChi) show clear bands at estimated protein size.

September 16th 2026

Members Attended: Ella, Maria
Supervisor: Alex, Kuan

1 IMAC VCH, SNH

Summary:
11.15 start

1. Ran 3ml Binding Buffer to start flow in the columns.
2. Ran all remaining supernatant for sonicated and spun samples of VCH and SNH (20ml each) through IMAC columns and washed through with 25mL Binding Buffer, 25mL W60 Buffer, and 14mL of Elution Buffer.

3. Took 1mL samples of all flowthroughs (supernatant "FT", Binding Buffer "BB", W60 Buffer "W60") and 2mL samples of elutions ("E1" to "E7").
4. All eppendorf samples ("L", "S", "I", "FT", "BB", "W60", "E1-E7") placed in freezer whilst all Falcon collections (resuspension, flowthrough, binding buffer, w60) placed in fridge.
5. Washed columns with MilliQ and 20% EtOH before storing in fridge.

VCH - concentrated protein in earlier elution 2-7
SNH - concentrated protein in later elution 2-7

2 SDS Page for IMAC purified VCH, SNH

1. SDS Page sample prep:

Total volume of all loading samples 20ul
Loading dye used: 2x Laemmli

Samples:

Diluted 1:5 for clearer gel bands: 2ul sample + 8ul BB + 10ul loading dye:
Lysate after sonication
Supernatant (after sonication and spinning)
Pellet (after sonication and spinning)

10ul sample + 10ul loading dye:
Flow Through (supernatant)
Binding Buffer
Wash 60
Elution samples 2-4

2. Samples boiled at 95C for 10mins

3. Run SDS Page

Running buffer made: 5x running buffer diluted: 50ml buffer + 950ml MilliQ

SDS page run at 200volts for 40min

ladder: stored in the fridge until use - Primestep prestained protein ladder

image.png

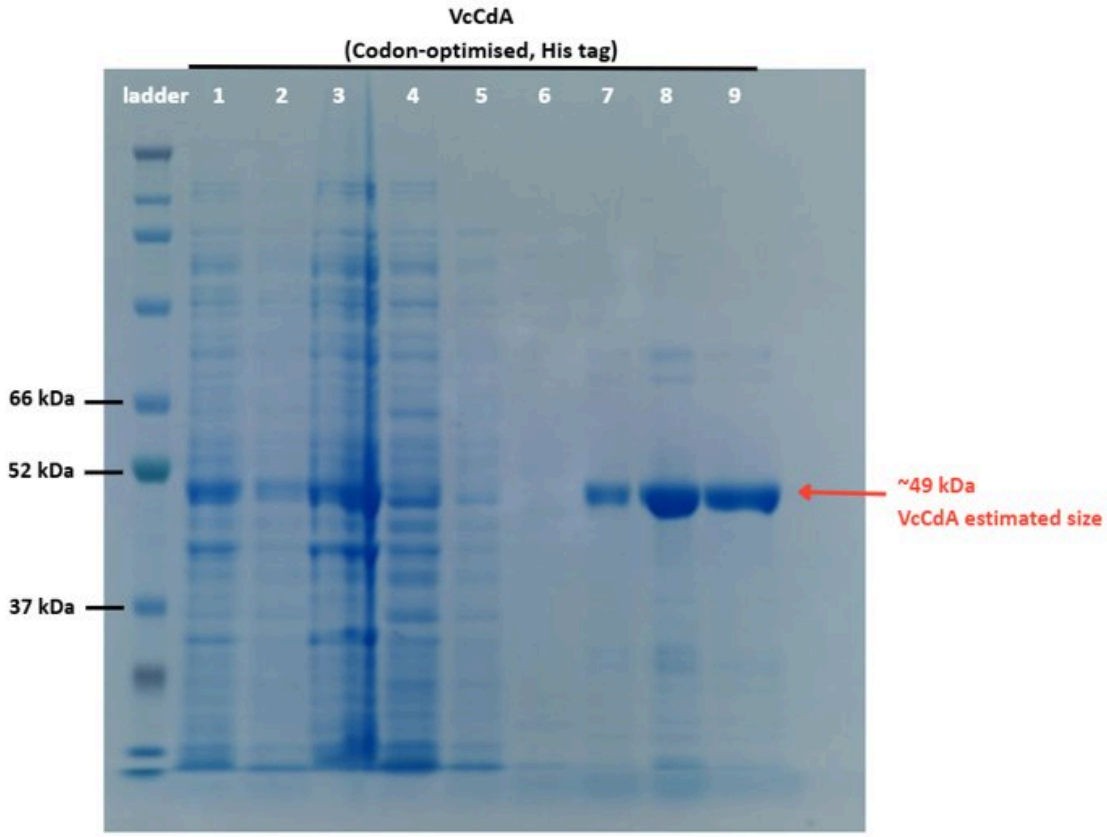


Figure 3. SDS Page, His-Trap IMAC Purification study of VcCDA (Codon-optimised, His tag) (lanes 1-9). Nine samples collected after IMAC purification were loaded in the following order: [1] Lysate after sonication (5x dilution), [2] FSupernatant after sonication and spinning (5x dilution), [3] Pellet after sonication and spinning (5x dilution), [4] Flow Through Supernatant [5] 5x Binding Buffer Wash, [6] 60mM Imidazole Wash, [7] Elution fraction 2, [8] Elution fraction 3, [9] Elution fraction 4.

3 Buffer exchange

Previous elution samples from IMAC of VNH, SCH, PpChi show signs of preotein precipitation. Therefore, Elutions of VCH, SNH need buffer exchange into imidazole-free buffer to avoid precipitation.

Prepare 250ml Sodium Phosphate buffer PH 7.4:

1. Weigh out 0.58g sodium phosphate monobasic and 1.08g sodium phosphate dibasic anhydrous.
2. Combine in a 500ml glass bottle
3. Add 230ml MilliQ and stir using magnetic stirrer and plate

4. PHing: use PH probe to measure PH of the solution and add acid or base drop-wise until PH 7 is achieved. (7.5ml acid/base added)
5. Add MilliQ to complete 250ml volime (12.5ml added)

PD10 column buffer exchange:

1. Centrifuge set to 2min, 4C, 700rcf

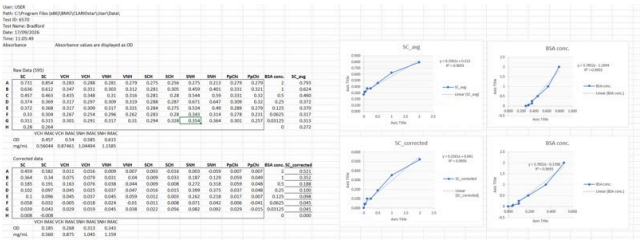
September 17th 2026

Members Attended: Ella, Brian

Supervisor: Alex

Performed Bradford Assay

image.png



Plan for week 21 Sep

Purple: Fusion Enzyme Black: Individual enzymes

	A	B	C	D	E	F	G
1	Task	Output	Chemicals	Equiment	Dates	iGEM lead	Supervisors
2	Perform GG	Golden Gate plasmid	GG Master Mix (BsmBI enzyme), ddH2O, T7 ligase, g blocks	Thermocycler	Monday 9am	Brian & Raphael	
3	Transformation into DH5a E. coli	E. coli with plasmid plated	Comp E. coli DH5alpha	Heat block (water bath), BSL cabinet	Monday afternoon	Brian & Raphael	
4	Overnight culture	Single colony E. coli in media	N/A	BSL cabinet	Tuesday 4pm	Brian	
5	Miniprep	Verify plasmid correctly inserted	Miniprep kit	N/A	Wednesday 9am	Ella & Maria	
6	Send it off for sequencing	FCL			Wednesday before 3:30pm	Ella & Maria	
7	Overnight culture	Single colony E. coli in media	N/A	BSL cabinet	Thursday 4pm	Brian & Ella	
8	Miniprep	Verify plasmid correctly inserted	Miniprep kit	N/A	Friday	Maria & Raphael	
9	Send it off for sequencing	FCL			Friday	Maria & Raphael	
10	transfer to redilute OD to 0.1				tuesday 9am	Ella & Maria	
11	0.5-0.6 OD IPTG induction				tuesday 12-1pm	Ella & Maria	
12	harvest cell pellet and sonicate				wednesday 9am	Ella & Maria	
13	Gel Electrophoresis				wednesday afternoon	Ella & Maria	
14	run IMAC				thursday 9am	Brian & Ella	
15	buffer exchange				thursday afternoon	Brian & Ella	
16	Bradford Assay, Flash freeze				Friday	Maria & Raphael	
17							
18							
19							

Aim for the week: Parallel Fusions and protein expression for VNH, SCH, PpChi

September 21st 2026

1.1 Golden Gate

aim/ note on the procedure: Insert Fusion Gblocks into PSB1C3 vector.

1.2 Transformation into DH5a E.coli

protocol including any changes:

Before Starting
Equilibrate a water bath to 42°C
Warm the vial of SOC medium to room temperature
Place the plates in a 37°C incubator to remove excess moisture (use two plates for each transformation)
Defrost chemically competent cells on ice. One vial of cells per transformation

Cell Transformation
Add 5 µL of plasmid DNA to 50 µL of competent cells (1:10 Dilution). Mix by tapping not pipetting.
Incubate the vial(s) on ice for 30 minutes.
Heat shock: Incubate for exactly 45 seconds in the 42°C water bath. Do not mix or shake.
Remove vial(s) from the 42°C bath and quickly place on ice for 30s.
Add 250 µl of pre-warmed SOC medium to the vial(s).
Note: SOC is a rich medium; practice good sterile technique to avoid contamination. (LB broth can also be used).
Incubate the vials for 1hr at 37C, 250rpm.

Plate pouring and cell seeding (for two plates, conducted in the hood)
Add 50mL of LB agar into a 50mL falcon tube; add 500uL of Xgal (20 mg/mL); add 50uL IPTG (1 M); add 50uL of chloramphenicol (1:1000 dilution) (34 mg/mL).
Pour 25 mL of solution per plate and wait 1hr for it to cool down: leave the lid half open to avoid condensation.
pipette 100uL of transformed cell onto each plate; spead the cell evenly with the spreader.
Place the LB agar plate overnight at 37˚C. Make sure the plates are placed upside down in the incubator to prevent water disturbing the colonies, store in fridge if not using so colonies won't grow (4C), not freezer.

results: Transformation succesful. Many colonies present on both plates.

1.3 Overnight Culture

aim/ note on the procedure:

protocol including any changes:

These steps are carried out in the hood

1. pipette 5 mL of LB medium into each falcon tube
2. pipette 5 uL of chloramphenicol into each falcon tube
3. place the dishes upside down, pick 1 colony with a hoop
4. insert the hoop into the falcon tube and swirl it until the colony dissolves in the solution
5. put the falcon tubes in incubator in 37C 180 rpm overnight (16hours)

1.4 MiniPrep

1.5 Sequencing

aim/ note on the procedure: Verify that fusion gblock bases are accurate after insertion into plasmid.

protocol including any changes:

1. Nanodrop sample t o obtain protein concentration
2. Sample prep for sequencing: dilute samples in ddH2O to achieve concentration of 50ng/ul with a final volume of 10ul.
3. Send to FullCircle labs for overnight sequencing

results: Sequencing showed succesful transformation.

September 22nd 2026

Members Attended: Ella, Maria
Supervisor: Alex

1 Protein Expression

10:30am

Steps carried out in laminar flow hood:

1. 900ul sample and 100ul LB broth pippeted into cuvettes for each 10ml sample (VNH 1, VNH 2, SCH 1, SCH 2, PpChi 1, PpChi 2).
2. OD measured in spectrophotometer at 600nm and blanked with 1ml LB broth cuvette.
3. Average OD values for each enzyme is calculated and samples 1 and 2 of each enzyme are combined.
4. Sample and LB Broth volumes added to 1L flasks according to C1V1=C2V2 calculations in the table below. Total volume 200ml, 3x flasks)
5. 200ul Chloramphenicol pipetted into eah flask.
6. Flasks sealed with cotton and aluminium foil.
7. Flasks placed in incubator at 37C 180rpm.

OD600 22.09

	A	B	C	D	E	F	G	H
1	Sample	OD (600nm)	df	C2	V2	V1	V1 (avg)	LB volume (ml)
2	SCH 1	0.236	10	0.1	200	8.474576	9.391927	190.6081
3	SCH 2	0.194	10	0.1	200	10.30928		
4	VNH 1	0.271	10	0.1	200	7.380074	7.788398	192.2116
5	VNH 2	0.244	10	0.1	200	8.196721		
6	PpChi 1	0.315	10	0.1	200	6.349206	6.86464	193.1354
7	PpChi 2	0.271	10	0.1	200	7.380074		

2 Protein Expression Induction

aim/ note on the procedure: IPTG induction for expression of proteins in DH5a E.coli

Steps carried out in laminar flow hood:

1. 100ul IPTG added to 200ml flasks. Overnight incubation at 20C 180rpm
2. Flasks placed in incubator at 16C 180rpm overnight.

September 23rd 2026

Members Attended: Ella, Maria
Supervisor: Kuan

1 Harvest
2 Mini Prep
3 nanodrop + send for sequencing
4 sonication
5 gel electrophoresis

September 25th 2026

Members Attended: Raphael, Maria
Supervisor: Francesca

1. Miniprep the overnight cultures with CDA-ChiA-His and ChiA-CDA-His
2. Linearise with HindIII
3. Gel electrophoresis
4. Send to FCL for sequencing

5. 2.1 Buffer Exchange

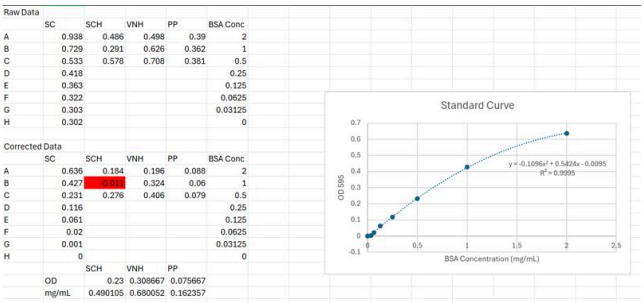
aim/ note on the procedure: Storing samples in Imidazole free buffer to avoid protein precipitation. Storage in Sodium Phosphate buffer allows for protein storage for activity assays.

6. Bradford assay on IMAC elutions of VCH, SCH, P
7. Flash freeze remaining elution for long-term storage

Gel electrophoresis results showed that all CDA-ChiA-His minipreps and the first 2 of 3 ChiA-CDA-His minipreps were the right sides (around 5.3kbps).
As such, 7 of the 8 minipreps were sent to Full Circle Labs for sequencing.

Some proteins precipitated, therefore results showed low protein concentration. Expression will be re-attempted including buffer exchange and analysed using bradford.

image.png



Raw Data

	SC	SCH	VNH	PP	BSA Conc
A	0.938	0.486	0.498	0.39	2
B	0.729	0.291	0.626	0.362	1
C	0.533	0.578	0.708	0.381	0.5
D	0.418				0.25
E	0.363				0.125
F	0.322				0.0625
G	0.303				0.03125
H	0.302				0

Corrected Data

	SC	SCH	VNH	PP	BSA Conc
A	0.636	0.184	0.196	0.088	2
B	0.427	-0.011	0.324	0.06	1
C	0.231	0.276	0.406	0.079	0.5
D	0.116				0.25
E	0.061				0.125
F	0.02				0.0625
G	0.001				0.03125
H	0				0

	SCH	VNH	PP
OD	0.23	0.308667	0.075667
mg/mL	0.490105	0.680052	0.162357

[Plan for week 28 Sep]

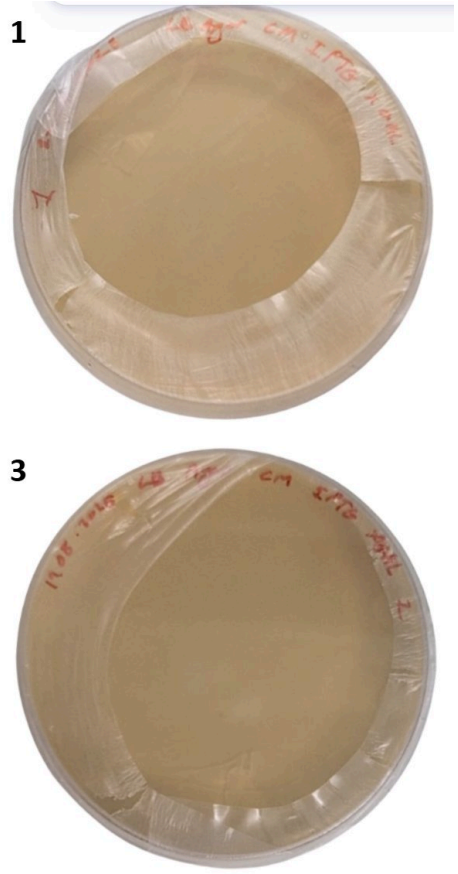
Original Plan:

July 20-8

	Task	Output	Chemicals	Equipment	Dates	Team members	Supervisors	H	I	J	K	L	M	N
1	Transformation into BL21 E. coli	E. coli with plasmid plated		Heat block (water bath), BSL cabinet	Monday	Raphael Brian								
2	Overnight culture	fusions	2x10mL LB	BSL cabinet	wed 4pm	Brian								
3	Protein expression in 200mL culture (start OD at 0.1) estimated 3hrs	Get OD 0.6		Shaking incubator	thurs 9am	Ella Maria								
4	Protein expression induction phase for 16-18 hours	make sure to take samples before induction	IPTG		thurs 2pm	Raphael								
5	Harvest				fri 9am	Brian Raphael								
6	sonicate sample & run SDS page to see expression				fri 10am	Brian Raphael								
7														
8														
9														
10														
11														
12														

Fusion transformation failed, no colonies on plates.

image.png



Due to transformation failiure, the original plan was revised. The new plan is below:

Table3

	Task	Output	Chemicals	Equipment	Dates	Team Members	Supervisors
1	Transformation into BL21 E. coli	E. coli with plasmid plated		Heat block (water bath), BSL cabinet	Monday	Raphael Brian	
2	Transformation into BL21 E. coli	fusions		Heat block (water bath), BSL cabinet	Tuesday 4pm	Ella Raphael	
3	Pour plates without IPTG		Agarose, D+ Glucose		Thursday	Ella Brian	
4	Transformation into BL21 E. coli			Heat block (water bath), BSL cabinet	thurs 2pm	Raphael Brian	
5							
6							
7							
8							
9							
10							
11							
12							

New pagar plates:

300ml LB Agar made:

0.2% D+ glucose (0.6g)

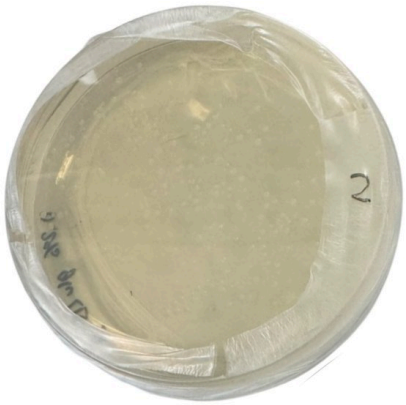
4g Agarose (40g/L)

Filled to 300ml with ddH2O

Tranformation was succesful on the new plates:

image.png





Plan for week 05 Oct

July 20-9

	Task	Output	Chemicals	Equpment	Dates	Team members	Supervisors	H	I	J	K	L	M	N
1	VcCDA assay (pNPA)		pNP, pNPA, DMSO, sodium phosphate	Microplate reader (booking needed)	monday 9am-11am, 1pm-3pm, 4pm-6pm	Isaiah, Raphael (until 3pm), Maria (after 4pm)								
2	Overnight culture	fusions	2x10mL LB	BSL cabinet	monday 4pm	Brian								
3	Protein expression in 200mL culture (start OD at 0.1) estimated 4-5hrs	Get OD 0.6		Shaking incubator (booking needed)	tues 9am	Brian								
4	Protein expression induction phase for 16-18 hours		IPTG		tues 1pm	Ella								
5	Harvest				wednesday 9am	Brian Ella								
6	sonicate sample				wed 11am	Brian Raphael								
7	purification				Wed	Brian, Raphael								
8	buffer exchange				Wed	Brian Raphael								
9	SDS-PAGE & Bradford			microplate reader (booking needed)	Thurs 9am	Brian Ella								
10	MBTH assay prep					Isaiah, Raphael								
11														
12														

Members Attended: Brian, Ella, Raphael

Supervisor: Alex

Week 11 28.09 - 04.10

Aim for the week: Transform Fusion constructs into BL21 E.coli

Procedures that week

1 . Transformation

aim/ note on the procedure: Both fusion constructs (CDA-Chia-His, ChiA-CDA-His) transformed onto BL21 E.coli.

protocol including any changes:

Before Starting
Equilibrate a water bath to 42°C
Warm the vial of SOC medium to room temperature
Place the plates in a 37°C incubator to remove excess moisture (use two plates for each transformation)
Defrost chemically competent cells on ice. One vial of cells per transformation

Cell Transformation
Add 1 µL of plasmid DNA to 25 µL of competent cells (1:25 Dilution). Mix by tapping not pipetting. (2ul and 50ul)
Incubate the vial(s) on ice for 30 minutes.
Heat shock: Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
Remove vial(s) from the 42°C bath and quickly place on ice for 30s.
Add 250 µl of pre-warmed SOC medium to the vial(s).
Note: SOC is a rich medium; practice good sterile technique to avoid contamination. (LB broth can also be used).
Incubate the vials for 1hr at 37C, 250rpm.

cell seeding (for two plates, conducted in the hood)

1. pipette 100uL of transformed cell onto each plate; spead the cell evenly with the spreader.
2. Place the LB agar plate overnight at 37˚C. Make sure the plates are placed upside down in the incubator to prevent water disturbing the colonies, store in fridge if not using so colonies won't grow (4C), not freezer.

results: The initial transformation failed and no colonies were seen on eiher plateThe transformation and cell seeding was repeated, with double the volume of transformed cells seeded on the plates, and again no colonies were seen.

Plates remade without IPTG or XGAL. Instead, D+ Glucose is used.

Protocol:
Make300ml LB Agar:

1. Weigh out 0.6g 0.2% D+ glucose, add to 500ml bottle.
2. Weigh out 4g Agarose (40g/L), add to 500ml bottle.

3. Fill to 300ml with ddH20.
4. Autocalave

Plate pouring and cell seeding (for two plates, conducted in the hood)

1. Add 50mL of LB agar into a 50mL falcon tube; add 50uL of chloramphenicol (1:1000 dilution) (34 mg/mL).
2. Pour 25 mL of solution per plate and wait 1hr for it to cool down: leave the lid half open to avoid condensation.
3. pipette 100uL of transformed cell onto each plate; spead the cell evenly with the spreader.
4. Place the LB agar plate overnight at 37˚C. Make sure the plates are placed upside down in the incubator to prevent water disturbing the colonies, store in fridge if not using so colonies won't grow (4C), not freezer.

results: Transformation onto plates without IPTG was succesful, many colonies present on the plates for both fusions.

1 Harvest

1. Harvest of cell cultures

1. Aloquot 200ml culture into 4 40ml falcon tubes.
2. Centrifuge at 2500rpm for 30 minutes at 4c (low rpm due to VcCDA fragility).
3. Take 1mL supernatant and cell pellet samples.

2. SDS-PAGE Sample Prep

Resuspension of cell pellets from post induction fusions.

Resuspension performed using calculated volumes (10mL/g cell weight) of 1X Binding Buffer (stored in 4c). Total volume ~16ml for each fusion.

Plan for week 12 Oct

July 20-10

	Task	Output	Chemicals	Equpment	Dates	Team members	Supervisors	H	I	J	K	L	M	N
1	reattempt sds-page	fusion data	We need to borrow 1 bis-tris gel		monday 11am-2pm	Ella								
2	MBTH first attempt	All the chitinase	need to make DTT on the day, need to mix with MBTH	need to use microplate reader very quick (10 minutes) to measure	tuesday 11am-4pm	Raphael, Isaiah								
3	MBTH second attempt (if necessary)	All the chitinase	need to make DTT on the day	need to use microplate reader very quick (10 minutes) to measure	wednesday 11am-4pm	Raphael, Isaiah								
4	MBTH third attempt (if necessary)	All the chitinase	need to make DTT on the day	need to use microplate reader very quick (10 minutes) to measure	friday 11am-4pm	Raphael, Isaiah								
5														
6														
7														
8														
9														
10														
11														
12														

SDS-Page of Purified fusion proteins new attempt was succesful. Image below:

image.png

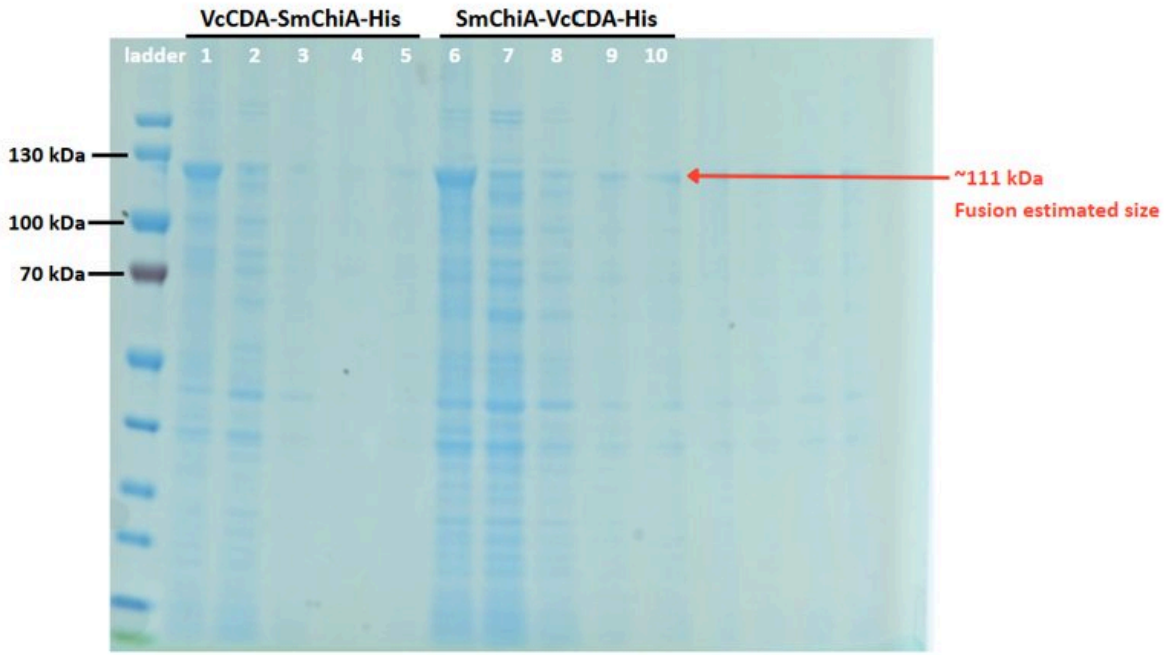


Figure 3. Image of SDS-Page analysis for HisTrap IMAC Purification study of VcCDA-SmChiA Fusion Proteins expressed in BL21*(DE3). Two fusion variants appear: VcCDA-SmChiA-His (lanes 1-5) and SmChiA-VcCDA-His (lanes 6-10). Each variant had a cell pellet sample taken before purification and four IMAC fractions loaded in the following order: (1) Cell pellet after sonication and spinning (5x dilution), (2) Flow Through Supernatant, (3) 5x Binding Buffer Wash, (4) 60mM Imidazole Wash and (5) Elution.

Week 12 05.10 - 09.10

Aim for the week: Characterise VcCDA kinetics using a pNPA assay

Refer to the protocol on the "Experiments" page of the Wiki. Note that the protocol was modified from 16th September from 10 µL of Enzyme to 30 µL of Enzyme.

Before Starting: Preparing 50 mM sodium phosphate buffer (pH 7.4)

1. Prepare 500mL of 50mM Sodium Phosphate Buffer by weighing out 1.16g of anhydrous monobasic (NaH2PO4) sodium phosphate and 2.17g of dibasic (Na2HPO4) sodium phosphate in ddH2O.
2. Utilising a calibrated pH probe, verify the pH of the buffer system. Store in a refrigerator (4C) until required.

Preparing the pNP Standard Curve

1. Dilute the 10 mM pNP stock tenfold in buffer.
2. The pNP Standard Curve was prepared to the the varying concentrations listed in the Table below.

Dilutions: pNP standard curve

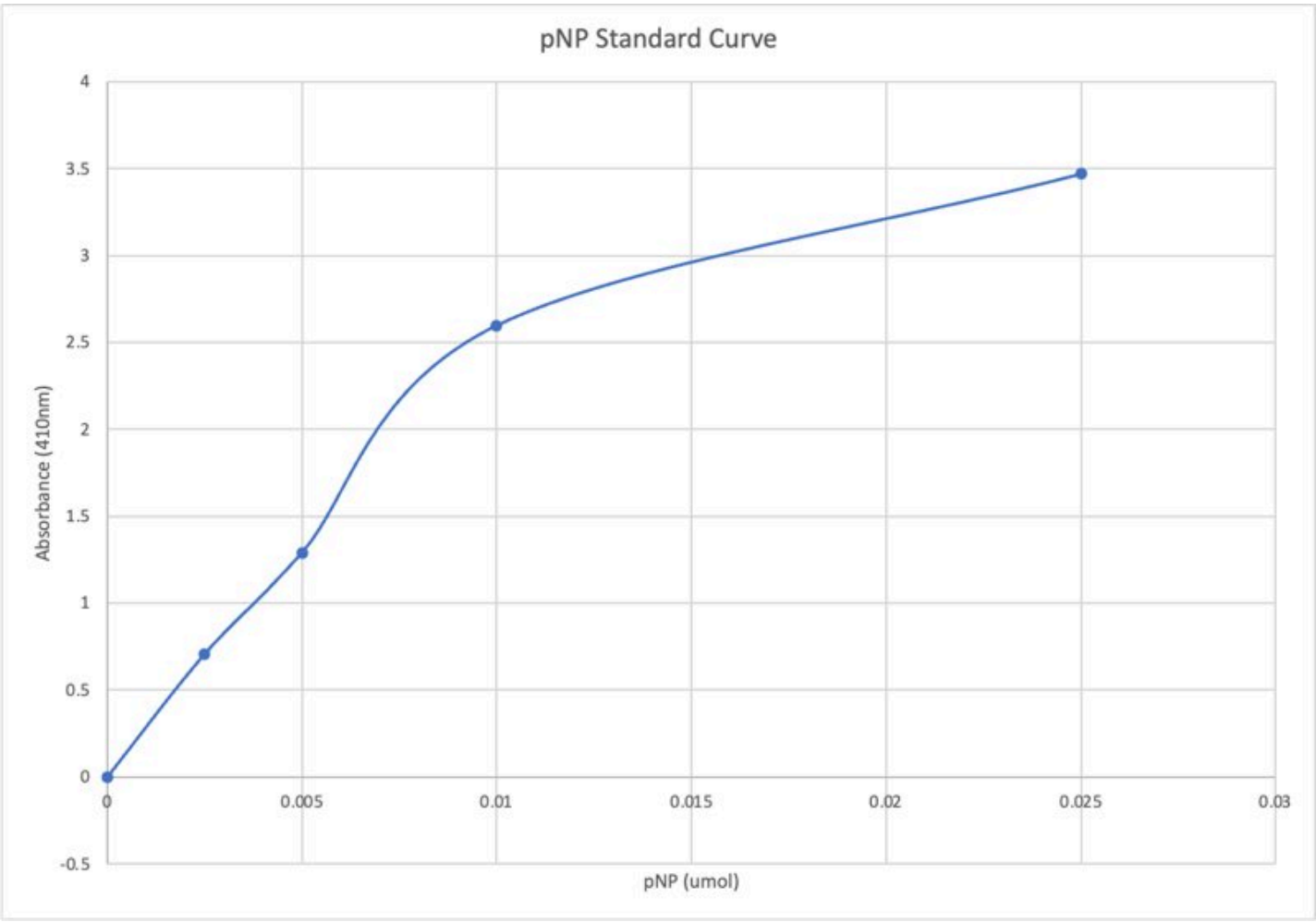
	A	B	C	D	E
1	pNP (µmol)	1 mM stock (µL)	Buffer (µL)	DMSO (µL)	Final pNP (µM)
2	0 (blank)	0	228	12	0
3	0.0025	2.5	225.5	12	10.4
4	0.005	5	223	12	20.8
5	0.01	10	218	12	41.7
6	0.025	25	203	12	104
7	0.05	50	178	12	208
8	0.1	100	128	12	417

The corresponding optical density measurements were correlated to the differing pNP concentrations as shown below and a standard curve was plotted using linear regression.

Results: pNP Standard Curve

	A	B	C	D
1	pNP (µmol)	OD (410nm)	Final pNP (µM)	Gradient (OD/umol)
2	0	-0.002	0	132.6164557
3	0.0025	0.706	10.4	
4	0.005	1.29	20.8	
5	0.01	2.597	41.7	
6	0.025	3.468	104	
7	0.05	3.468	208	
8	0.1	3.468	417	

image.png



Preparing the pNPA Substrate

1. Weigh 36.2 mg of pNPA and dissolve into 10mL of DMSO to formulate a 20mM stock of pNPA substrate. Make fresh and store cooled in a refrigerator (4C)
2. Pefrome dilutions to prepare different substrate concentration of pNPA according to the Table below.

Dilutions: pNPA Substrate for pNPA Assay

	A	B	C	D	E
1	**Final pNPA (mM)**	**Stock needed (mM)**	**20 mM master (µL)**	**DMSO (µL)**	**Batch total (µL)**
2	0.1	2	50	450	500
3	0.2	4	100	400	500
4	0.3	6	150	350	500
5	0.5	10	250	250	500
6	0.75	15	375	125	500
7	1.0	20	500	0	500

The pNPA protocol was carried out in accordance with the **"Experiments" page of the Wiki.** A Plate Reader was utilised to obtain the optical density results below for 10µL of VcCDA enzyme at 0.58µM for different pNPA substrate concentrations. The 96-well microwell plate layout is displayed as shown below:

Experiment 1: pNPA Assay (10uL Enzyme)

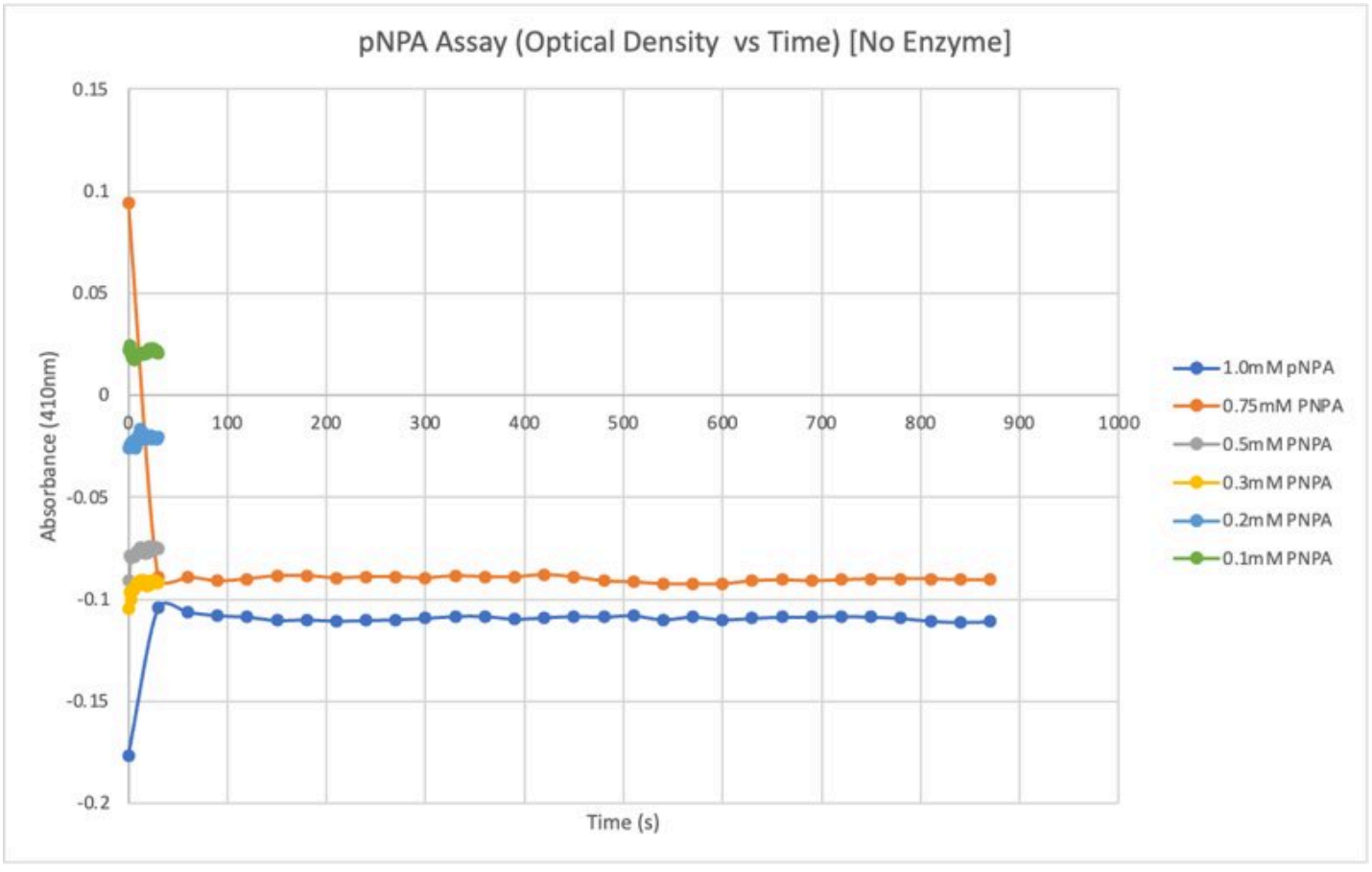
	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard	Measured	Measured	Blank	Negative Control							
B	Standard	Measured	Measured	Positive Control								
C	Standard	Measured	Measured									
D	Standard	Measured	Measured									
E	Standard	Measured	Measured									
F	Standard	Measured	Measured									
G	Standard											
H												

***Note: The plate reader injectors were not functional for the Clariostar plate reader utilised. Hence, manual injection with differing concentrations of pNPA substrate was performed swiftly using a multi-channel pipette in duplex. The 96-well plate was subsequently placed in the plate reader, and the measurement was carried out. It was estimated that the initial 25 seconds of the VcCDA Kinetic reaction with pNPA were not recorded as a result.**

Optical Density Results (410nm) for 10uL of Enzyme

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	10uL ENZYME (Negligible Enzyme)																					
2	1.0mM pNPA				0.75mM PNPA				0.5mM PNPA				0.3mM PNPA				0.2mM PNPA				0.1mM PNPA	
3	Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)
4	0	-0.1766667	0.000747349		0	0.0945	-0.002510345		0	-0.091	0.000455172		0	-0.1045	0.000444049		0	-0.026	0.000231368		0	0.022
5	30	-0.1043333			30	-0.089			30	-0.0785			30	-0.0965			30	-0.0245			30	0.0243333
6	60	-0.1063333	R2		60	-0.089	R2		60	-0.08	R2		60	-0.1005	R2		60	-0.0235	R2		60	0.02
7	90	-0.108	0.070149286		90	-0.091	0.107441588		90	-0.079	0.413242261		90	-0.0955	0.427486346		90	-0.0225	0.214586428		90	0.01833333
8	120	-0.1086667			120	-0.09			120	-0.0785			120	-0.096			120	-0.0225			120	0.01833333
9	150	-0.1103333	Velocity (umol/min)		150	-0.0885	Velocity (umol/min)		150	-0.079	Velocity (umol/min)		150	-0.0945	Velocity (umol/min)		150	-0.025	Velocity (umol/min)		150	0.017
10	180	-0.11	5.63542e-6		180	-0.0885	-1.89294e-5		180	-0.078	3.43225e-6		180	-0.0935	3.34837e-6		180	-0.026	1.74464e-6		180	0.01866667
11	210	-0.1106667			210	-0.0895			210	-0.0775			210	-0.0935			210	-0.024			210	0.01966667
12	240	-0.1103333			240	-0.089			240	-0.077			240	-0.092			240	-0.021			240	0.02
13	270	-0.11			270	-0.089			270	-0.0765			270	-0.0925			270	-0.0205			270	0.02033333
14	300	-0.1093333			300	-0.0895			300	-0.077			300	-0.0925			300	-0.0195			300	0.01966667
15	330	-0.1083333			330	-0.0885			330	-0.075			330	-0.091			330	-0.0165			330	0.02033333
16	360	-0.1083333			360	-0.089			360	-0.075			360	-0.091			360	-0.018			360	0.02033333
17	390	-0.1096667			390	-0.089			390	-0.0755			390	-0.0915			390	-0.0185			390	0.02066667
18	420	-0.109			420	-0.088			420	-0.0755			420	-0.091			420	-0.0195			420	0.02066667
19	450	-0.1083333			450	-0.089			450	-0.077			450	-0.091			450	-0.02			450	0.02033333
20	480	-0.1086667			480	-0.091			480	-0.0775			480	-0.0925			480	-0.0215			480	0.02
21	510	-0.108			510	-0.0915			510	-0.077			510	-0.092			510	-0.02			510	0.021
22	540	-0.11			540	-0.0925			540	-0.0775			540	-0.0935			540	-0.0215			540	0.02033333
23	570	-0.1086667			570	-0.0925			570	-0.075			570	-0.0925			570	-0.021			570	0.02133333
24	600	-0.11			600	-0.0925			600	-0.074			600	-0.093			600	-0.021			600	0.02266667
25	630	-0.1093333			630	-0.091			630	-0.074			630	-0.0925			630	-0.02			630	0.02233333
26	660	-0.1086667			660	-0.0905			660	-0.0745			660	-0.0915			660	-0.02			660	0.023
27	690	-0.1086667			690	-0.091			690	-0.0765			690	-0.0915			690	-0.021			690	0.02166667
28	720	-0.1083333			720	-0.0905			720	-0.076			720	-0.091			720	-0.0215			720	0.023
29	750	-0.1086667			750	-0.09			750	-0.075			750	-0.091			750	-0.021			750	0.023
30	780	-0.1093333			780	-0.09			780	-0.0745			780	-0.091			780	-0.021			780	0.02133333
31	810	-0.1106667			810	-0.09			810	-0.075			810	-0.092			810	-0.021			810	0.02166667
32	840	-0.1113333			840	-0.0905			840	-0.075			840	-0.092			840	-0.0215			840	0.02166667
33	870	-0.111			870	-0.0905			870	-0.0755			870	-0.092			870	-0.0205			870	0.02066667

image.png



There was a negligible increase in optical density throughout the 870 seconds the kinetic reaction was carried out. Given the positve readout of the positive and negative control, this was attributed to negligable enzymatic esterase side chain reaction between the pNPA and the VcCDA substrate due to the low concentration of enzyme added.

According to Aragunde, Biarnés and Planas (2018), who worked on Vibrio cholerae Deacetylase, the enzyme was predicted to work in the range of 0.05–0.8μM enzyme for the substrate chitobiose. This aligned with the enzyme concentration of 0.58 μM we utilised for the experiment. The enzymatic process for both substrates results in the hydrolysis of an acetyl group and the corresponding release of acetate. However, for the pNPA assay, an ester bond is broken in comparison to the chitobiose substrate, where an amide bond is broken. Hence, it was hypothesised that the enzymatic reaction of VcCDA on pNPA was due to poor side-chain reactivity and therefore a greater concentration or volume of enzyme was required to observe a measurable increase in acetate ions. This was troubleshot, as shown below, by tripling the enzyme concentration to identify a measurable kinetic reading on the plate reader.

Troubleshooting Enzyme Concentration

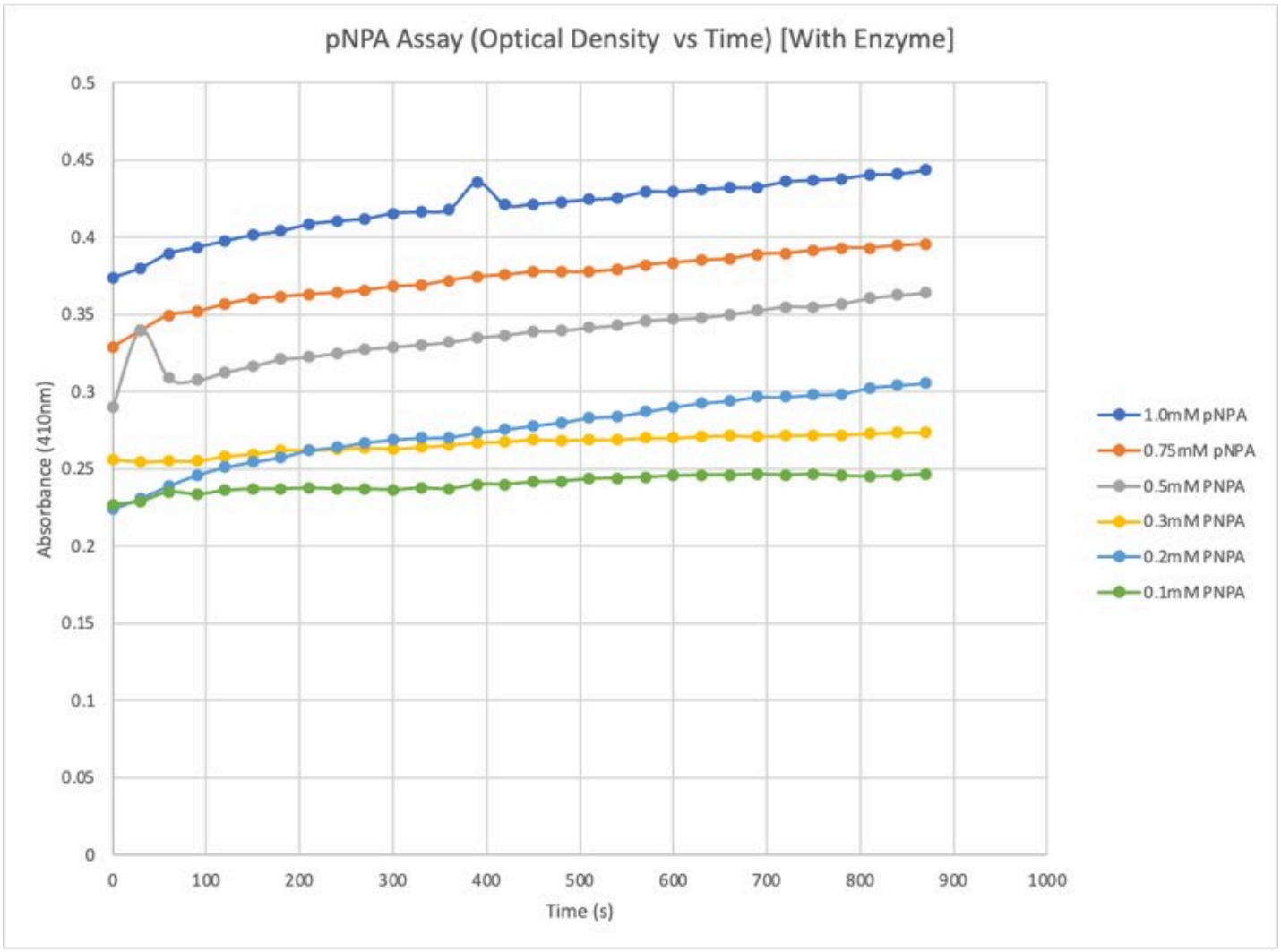
	A	B	C	D
1	Enzyme added (μL)	Buffer (μL)	Substrate (μL)	Enzyme Concentration (μM)
2	10	218	12	0.58
3	30	198	12	1.74

The experiment was rerun on the Clariostar Plate reader with 30 μL of VcCDA enzyme, and the optical density results at 410nm over the 870-second period. The experimental results are shown below:

Experiment 2: Optical Density Results (410nm) for 30uL of Enzyme

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	ENZYME																						
2	1.0mM pNPA				0.75mM pNPA				0.5mM PNPA				0.3mM PNPA				0.2mM PNPA				0.1mM PNPA		
3	Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)	Rate (OD/min)		Time (s)	OD (410nm)	Rate (OD/min)
4	0	0.374	0.003971746		0	0.329	0.003674082		0	0.29	0.003818687		0	0.256	0.00132703		0	0.224	0.004905673		0	0.227	0.001150389
5	30	0.38			30	0.3395			30	0.3395			30	0.2545			30	0.231			30	0.229	
6	60	0.3895	R2		60	0.3495	R2		60	0.309	R2		60	0.255	R2		60	0.239	R2		60	0.235	R2
7	90	0.3935	0.893819417		90	0.352	0.930368719		90	0.3075	0.864724247		90	0.255	0.936337423		90	0.246	0.954817186		90	0.2335	0.8811877
8	120	0.3975			120	0.3565			120	0.3125			120	0.258			120	0.251			120	0.236	
9	150	0.4015	Velocity (umol/min)		150	0.36	Velocity (umol/min)		150	0.3165	Velocity (umol/min)		150	0.2595	Velocity (umol/min)		150	0.2545	Velocity (umol/min)		150	0.237	Velocity (umol/min)
10	180	0.404	2.99491e-5		180	0.3615	2.77046e-5		180	0.321	2.8795e-5		180	0.262	1.00065e-5		180	0.2575	3.69914e-5		180	0.237	8.67456e-6
11	210	0.4085			210	0.363			210	0.3225			210	0.262			210	0.262			210	0.2375	
12	240	0.4105			240	0.364			240	0.325			240	0.2625			240	0.264			240	0.237	
13	270	0.412			270	0.3655			270	0.3275			270	0.2635			270	0.267			270	0.237	
14	300	0.4155			300	0.368			300	0.329			300	0.263			300	0.269			300	0.2365	
15	330	0.4165			330	0.369			330	0.3305			330	0.264			330	0.27			330	0.2375	
16	360	0.418			360	0.372			360	0.332			360	0.2655			360	0.2705			360	0.237	
17	390	0.4355			390	0.3745			390	0.335			390	0.267			390	0.2735			390	0.24	
18	420	0.421			420	0.3755			420	0.3365			420	0.2675			420	0.2755			420	0.24	
19	450	0.4215			450	0.3775			450	0.339			450	0.269			450	0.278			450	0.2415	
20	480	0.423			480	0.3775			480	0.3395			480	0.2685			480	0.28			480	0.242	
21	510	0.4245			510	0.3775			510	0.3415			510	0.269			510	0.283			510	0.2435	
22	540	0.4255			540	0.379			540	0.343			540	0.269			540	0.284			540	0.244	
23	570	0.4295			570	0.382			570	0.346			570	0.27			570	0.287			570	0.2445	
24	600	0.4295			600	0.3835			600	0.347			600	0.27			600	0.29			600	0.2455	
25	630	0.431			630	0.385			630	0.348			630	0.271			630	0.2925			630	0.246	
26	660	0.432			660	0.386			660	0.35			660	0.2715			660	0.294			660	0.246	
27	690	0.4325			690	0.389			690	0.3525			690	0.271			690	0.2965			690	0.2465	
28	720	0.436			720	0.3895			720	0.355			720	0.2715			720	0.2965			720	0.246	
29	750	0.437			750	0.3915			750	0.355			750	0.272			750	0.298			750	0.2465	
30	780	0.438			780	0.393			780	0.357			780	0.272			780	0.2985			780	0.2455	
31	810	0.4405			810	0.393			810	0.3605			810	0.273			810	0.3025			810	0.245	
32	840	0.441			840	0.3945			840	0.3625			840	0.2735			840	0.304			840	0.2455	
33	870	0.4435			870	0.3955			870	0.364			870	0.2735			870	0.3055			870	0.2465	

image.png



The initial reaction window (0-25seconds) was not captured due to manual injection of the pNPA substrate. However, a measurable increase in optical density was observed at differing pNPA substrate concentrations over the 870-second kinetic window during which the experiment was carried out. Kinetic Data analysis was performed on these results to obtain the reaction velocity and, subsequently, the relevant kinetic parameters of VcCDA (Vmax, Km, Kcat) for comparison with our predictive kinetic model.

Week 13 12.10 - 17.10

Aim for the week: Characterise SmChiA and PpChi kinetics using an MBTH assay

Procedures that week: MBTH Assay
Refer to the protocol in the "Experiments" page of the Wiki.

Results: The MBTH assay did not produce good results, with both the standard curve and the time-course.

image.png

	Standard	PpChi 1	PpChi 2	SmChiA 1	SmChiA 2	Time (minutes)
A	BLANK	-0.003	0	0.711	0.035	0
B	S1 | 20mM | 0.032	0.035	0.019	0.079	0.043	2
C	S2 | 40mM | 0.029	0.068	0.018	0.069	0.006	5
D	S3 | 60mM | 0.019	0.038	0.022	0.041	0.09	10
E	S4 | 80mM | 0.012	0.018	0.1	0.075	0.047	15
F	S5 | 100mM | 0.025	0.013	0.005	0.055	-0.015	30
G		-0.019	0.012	0.06	-0.019	60

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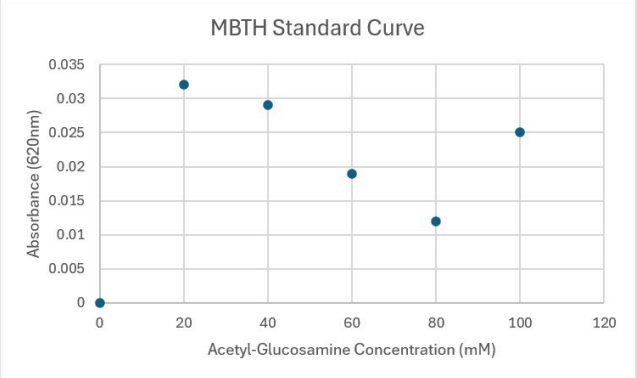
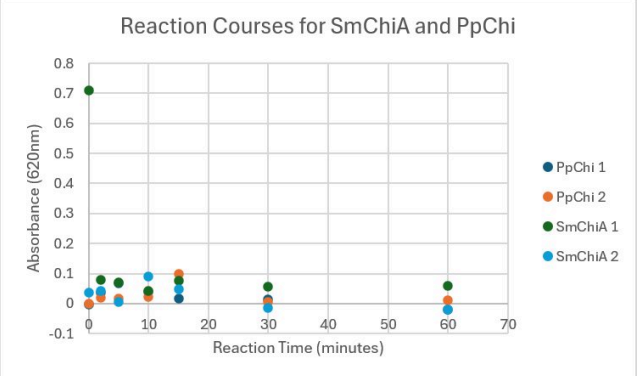


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The cause of these results was not identified and is presumed to be down to the reagents (eg: DTT) having degraded.

Similar problems were encountered by Wageningen_UR (2016).

Without enough materials left to rerun the assay, nor the time to generate more of these materials, the MBTH assay will not be re-attempted.