~~2018-06-01~~
2018-06-0

MIC Culture dilutions

$C_1V_1 = C_2V_2$

	(10' dilution)	Actual	$V_1 = \frac{C_2V_2}{C_1}$	V_1
P1	0.1172	1.172		0.4437 mL
P2	0.1153	1.153		0.4540 mL
P3	0.1030	1.030		0.5048 mL
B1	0.1052	1.052		0.4943mL
B2	0.0996	0.996		0.5221mL
B3	0.1405	1.405		0.3701

7/31/2018

Transforming DH10B w/ PPIX Overproduction Plasmids into BL21

Transform pET-hemA→D and pCDF-hemEF into DH10B for plasmid stock creation ~~and into BL21 for expression~~

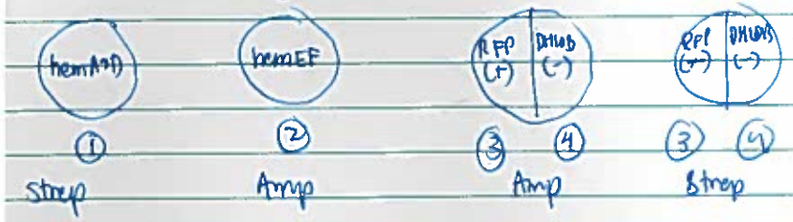
Electroporation of DH10B & BL21

Sample	Volume DNA
① DH10B hemAD	0.5 µL
→ ② DH10B hemEF - C15	0.5 µL
③ (+) ctrl mRFP BL21	1 µL ← obtained from Kit Plate 4 2H
~~④ BL21 hemAD~~	
~~⑤ BL21 hemEF~~	
~~⑥ (-) ctrl mRFP BL21~~	

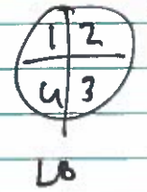
Incubated in 1mL LB+SOC (3mL LB, 1mL SOC) at 31°C & 150rpm for 1hr

Plating Transformants

Streaked out 3 way method ① → ③



Survivor plate



Incubated @ 37°C overnight

Sequencing

C15 (EF) → pET Duet1
↳ use T7 term. primer + pET upstream primer.

Hem A→D → pCDF Duet1
↳ use T7 term primer + ACYC upstream primer.

C16 (EF) → pET Duet
↳ use T7 term primer + pET upstream primer.

* C15 + C16 is too low concentration to follow protocol; added 9µL of sample + 1µL primer for both forwards reverse.

* HemA→D ~~pet~~ sequencing → 2 samples; 1 from plate, 1 from tube
↳ 6µL DNA + 1µL primer (10nM) + 3µL ddH2O → non-calc ~~standardized~~ based on [DNA]
↳ standardized # in C lab

* All primers are used @ [10nM] for sequencing for this part

	Tube HemA→D	Plate HemA→D	C15 (E+F)	C16 (E+F)	
Forward	1µL ACYC Duet	1µL ACYC Duet	1µL pET Duet	1µL pET Duet	← Forward Primer
	6µL DNA	6µL DNA	9µL DNA	9µL DNA	
	3µL ddH2O	3µL DDNH2O	0µL ddH2O	0µL ddH2O	← dilution.
Reverse	1µL ~~T7~~ T7	1µL T7 term	1µL T7 term	1µL T7 term	
	6µL DNA	6µL DNA	9µL DNA	9µL DNA	
	3µL ddH2O	3µL ddH2O	0µL ddH2O	0µL ddH2O	

eing logged by: Anna & Irene

July 25/18

ransformations — pRSET, pET-28A, pRSETB - Sirius.

H5cc transformed on 2 amp. plates & 1 Kan. plate
↳ pRSET, pRSET-Sirius ↳ pET-28A

- 1µL of DNA added to each ~~cuvette~~ m.c. tube
- 250µL of DH5cc added to each ~~cuvette~~ m.c. tube
- Let sit on ice for 5 minutes
- transfered to cuvettes
- electro shocked the cuvettes
  - ↳ 5.60, 5.10, 4.90 → Neither of them arced
- Placed 900µL in the cuvettes (LB)
- Transfered back into m.c. tube after pipette mixing
  - ↳ incubated for an hour

Plating Transformations

- take a heapful of cells, use 3-streak method
  - ↳ labeled + put ~~on~~ in incubator (Red) → Take

-Cleaning Cuvettes

- ↳ 10% bleach added to cuvette; let sit ~ 10 mins → Wash w. H_2O after
- ↳ Add 50/50 HCl & NaOH → let sit for a bit
- ↳ wash each w. H_2O
- ↳ add ETOH → let sit for a while
- ↳ Dump ETOH & invert on paper towel

↳ attempt @ cleaning cuvettes
↳ I failed. Miserably.

July 26: Irene & Anna

put 0.25 g kanamycin into 5 ml sterile H_2O

Syringe filter

-stock made

0.06 mL × 1000 µL = 60 µL

↳ 1000X solution

50 µg/mL ⇒

0,05 g/mL

Aug 1: Irene & Anna

Transforming DH5α w. pCDF w. Heme A - D

① 1µL of vector in 50 µL of DH5α → ON ICE

② Transfer vector + DH5α to cuvette ⇒ SHOCK

③ Transfer 100µL of plain LB into cuvette. Aspirate to mix

④ Transfer contents of cuvette back into labeled microcentrifuge tube.

⑤ Incubate for 1h in shaker

⑥ Plate onto streptomycin + LB agar plate.

↳ Clean cuvette after use following procedural guidelines

Aug 2: Irene & Anna

Transformation of DH5αB
⇒Following previous transformation protocol

Working Concentration of Streptomycin: 50μGr/μL

August 8/18

- Culturing Hem A-D from BL21 in DH5α
  + 6mL LB broth + Spectinomycin

Stock sol'n of spectinomycin : 512mg/mL
working conc : 50μg/my

$$\frac{(50\mu g/mL)(25\mu L)}{50\,000\,\mu g/mL} = 25\mu L$$

→ follow culturing protocols in previous pages for instructions.

Prepping Primers.
→ dilute to 100 μM (primers) amount ddH_2O added to make
→ multiply # nmol x 10 μL = ~~[illegible]~~ stock solution

pRSET seq F = 17.5nmol x 10μL = 175μL → 10μL

pBADOL-pRSETA-R = 19.5 nmol x 10μL = 195μL → 10 uL

pRSET seq R = 27.4nmol x 10μL = 274μL → 10 μL

pBADOL- pET28a - F = 19.3nmol x 10μL = 193μL → 10 μL

pBADOL- pRSETA - F = 23.3nmol x 10μL = 233μL → 10 μL

pBADOL - pET28a - R = 80.4nmol x 10μL = 204μL → 10μL

10 + 90μL = ~~100~~

Working Conc. = 10 μM

PCR Prep ⇐ pRSET

→ 100μL Q5 PCR

20μL enhancer
20μL Buffer
2μL dNTP
5μL P_F
5μL P_R
1μL template
46μL ddH_2O
1μL Q5 polymerase

[illegible] = pRSET P_F + P_R
[illegible] = pRSET + pBADOL P_F + P_R

→ made PCR ⇐ pBADOL-pRSET F & R

→ Digest template ⇐ DpnI
Gel Extraction
→ Add 15μL loading Dye to sample (6x conc; dilute to 1x theoretically)

Infusion

1 µl Vector
1 µl Inserts
0.5 µl Reaction Mix
} 50°C for 20 minutes

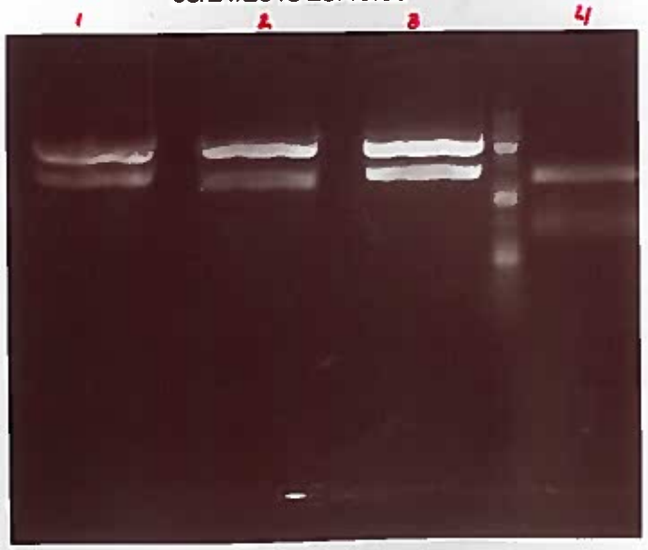
→ Run 2.5 µl for cost effectiveness

→ Then transform

* Library (Rochelin-S) → Insert

→ Rochelin's vector for (+) control (2)
our vector = 1

08/21/2018 23:40:00



- Minipreps of DH5α + pCDF HemA-D from BL21 August 9, 2018

*see protocol in previous pages

- Further action : prep for sequencing + create stock of pCDF HemA-D

- Re-streak pET-28A + DH10B Cells → for creating stock of pET28-A for Gene insertio

- Re-streak RCaMP + BL21 → Feed to bees

Infusion attempt #2

#1 → pRSET + mRuby3 GECO pR 2.1

#2 → pBAD + " " " "

* 3 = pBAD + TSG6 link - GS5GA iGluSnFR

1 = mRuby3 + GECO - pR2-1 + [pRSET + pBAD OL]

2 = " + [pBAD vector]

3 = TSG6 link - GS5GA - iGluSnFR + [pBAD vector]

Infusion into pBAD

Friday

→ gBlocks #1-4 → pBad. ✓

→ gBlocks #1 → pBad pRSET A. ✓

→ Transform ddH, GFP plasmids (chloroform) & RCaMP.

→ Miniprep

→ Digest 00 & ~~00~~ DTU plasmid stocks.

→ diagnostic digestion

gBlocks Aug 10

→ Dilute in 35µl of ddH_2O ②

① Spin down

③ leave them for ~~20~~ 20 minutes after pipette resuspension

For infusion: take 1µl & go

↳ Infusion PCR Tubes:

# 1 →	Hem A + pBAD	→ gBlock:	Hem A + mScarlet in pBAD
# 2 →	Hem B + pBAD	+ "	Hem B + mNeonGreen in pBAD
# 3 →	Hem CD* pBAD	"	Hem C+D + mScarlet in pBAD
# 4 →	Hem E+F + pBAD		Hem E+F ~~#~~ in pBAD
# 5 →	Hem A + pRSET		Hem A + mScarlet in pRSETA
# 6 →	pRSET (1µL) + ddH_2O (1.5µL)	→	2.5µL for -ve control

Culture pETDuet Var 16 from BL21 → DH5α

" pETDuet (empty vector) in DH10B

Transformations

- RCaMP in BL21
- # 4-27 (cjBlue) in ~~DH5α~~ DH10B
- # 1-19E in ~~DH5α~~ DH10B
- Hem A + pBAD in ~~DH5α~~ DH10B
- Hem B + pBAD in ? DH10B
- Hem C+D + pBAD " ~~#~~ DH10B
- Hem E+F + pBAD " ~~#~~ DH10B
- Hem A + pRSET in BL21
- pRSET (-ve control) in BL21

Diagnostic Digest of pCDF-Hem A-D

- 3µL of pCDF-Hem A-D
- Fast Digest buffer ~2µL
- 0.5µL of ~~[illegible]~~ PstI
- 0.5µL of XbaI
- 14µL of ddH_2O

} Run @ 37°C for 30 minutes in thermocycler

August 11/18

-miniprep of pETDuet empty vector, Var1b (Hem E+F), pRSET-insert.
  - protocol found earlier in lab manual

- Diagnostic Digest of pRSET-insert ≈ XhoI, Hind III
  - 3µL of pRSET-insert
  - Fast Digest buffer → 2µL
  - 0.5µL of XhoI
  - 0.5µL of Hind III
  - 14µL of ddH_2O

} 6 total tests; Run @ 37°C for 30 minutes.

→ numbered according to numbering on plasmid tube (light blue)

- Run on Gel

Aug 12

-Diagnostic Digest for pRSET-insert (Rocheline) → XhoI, Hind III
and/or Diagnostic PCR

-miniprep of Var1b, pETDuet Empty vector + pRSET-insert. ✓

Transformations.
- Hem A-pRSET → BL21
- pRSET empty vector → BL21
- Hem C+D - pBAD → DH10B
- Hem E+F → pBAD → DH10B

- Re-Streak RCaMP-PRSET (lawn grew) ✓
- Pick Colonies for
  - Hem A-pBAD (3) #Amp ✓ ~~of pRSET insert vector~~
  - Hem B-pBAD (8) Amp ✓
  - Hem E+F-pBAD (1) Amp ✓
- Re-try infusion for
  - Hem A-pRSET + -ve control
  - Hem C+D -pBAD
  - Hem E+F -pBAD

Diagnostic Digest of Hem A, B, E+F in pBAD

- cut = xhol, Hind III → expect ~4kb, ~2kb

ddH$_2$O → 14µL
plasmid → 3µL
F.D. B → 2µL
XhoI → 0.5µL
Hind III → 0.5µL
} 13 total tests; Run @ 37°C 30min

- Re-plate Hem A-pRSET (120µl instead of 60µL)
  → 3 plates

- Miniprep
  → HemA+pBAD in DH10B (3)
  → " B + pBAD " " (8)
  → " E+F + pBAD " " (2)

- Culture RCaMP-pRSET in BL21, (Amp), #1-19E (Chlor), #4-2I (chlor)

DH10B.
↓
8/16/18
→ no promotor; coding only
Chose #6-MII instead

A_2, A_3 B_3, B_4 → send off for sequencing on monday
→ bright bands @

August 13

CD #5 → flourese
CD 1-4, 6 → x flourescence

Miniprep of Cultures Hem C+D 1-6, Hem E+F 1-6

No Growth on RCAMP-PRSET
→ Re-culture 3 tubes, from flourescent areas

Amy

3 reactions of 6 µL ~~of plasmid~~ ⇒ Heme A2
→ 6µL plasmid
→ 42 µL H_2O
→ 6 µL Buffer
→ 3µL enzymes → 6 total (Kpn I, Hind III)
→ ~~40µL 10X Buffer~~
2 reactions of 10µL of Heme B4
→ 10µL plasmid
→ 6µL Buffer
→ 3µL enzymes → 6µL total (Kpn I, Hind III)
→ 38µL ddH_2O

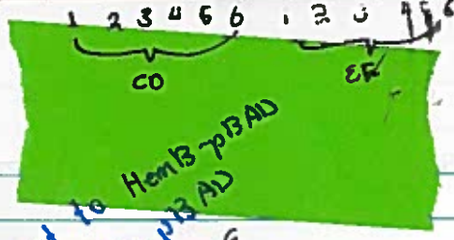
08/14/2018 00:32:04

A, A_2 A_3 B_1 B_2 B_3 B_4 B_5 B_6 B_7 B_8 B_9 B_{10} 1kb Ladder

Ligation Protocol – Aug 14th
→ 20 µL total rxn volume.
→ 1 µL vector — 22°C - 15-20 min to 1 hr.
→ 16 µL insert — 70°C - 5 min
→ 2 µL buffer — 4°C - ∞.
→ 1 µL T4 ligase.

08/15/2018 03:00:09

1 2 3 4 5 6 1 2 3 4 5 6
CD ER



- C+D → insert to HemB-pBAD
- Incubation → CD into pBAD
- Digestion → 60 minutes

*Elution of C+D insert to HemB-pBAD
↳ Elute in 35µL

Aug 15/18

#1 → Hem A-pRSET 1
#2 → " " 2
#3 → " -pRSET 3
#4 → " - " 4
#6 → " - " 6

} Diagnostic Digest → BglII, HindIII

#6, #4 & #2 sent for sequencing

-pick colonies from A2 in pBAD → 3
B4 " " → 3
" " " Hem CD + pBAD infusion →
" " " Hem B+C+D in pBAD

* Transform pRSET in BL21 → plated + streaked

Aug 16/18

Miniprep
- Hem A in pBAD #R → had success in sequencing, extra stock
- Hem B " " #4 → " " " " " "
- iGEM vector 6-MII → see blue colonies
- Hem CD infusion → send off for sequencing
- Hem CD in pBAD-Hem B →

0.6g of Agar } Microwave for 60s
50ml of TAE 1x }
↓
Add 2µL of Et Br
↓
add in combs (4 well for extraction)
20 (small) for diagnostic
↓
let set for ~30 min
to ensure it set,
↓
cut

Diagnostic Digest of heme BCD in pBAD
1, 2, 3, 4, 5, 6

Aug 17

Hemocytometer protocol

Prepare cytometer
1. decon glass coverslip with alcohol before use
2. moisten coverslip with water and affix to hemocytometer

Prepare cell suspention
1. swirl to ensure even distribution
2. Take 0.5 mL out and pipette into Eppendorf tube
3. Take 100µL of cells into new Eppendorf and add 400 µL of 0.4% Trypan Blue (final concentration 0.32%)

Counting
1. ~~10~~ µL of suspention to cytometer
2. Focus on grid lines with 10x objective
3. Count cells in one set of 16 squares
4. Count all four sets of 16 squares

Calculation
1. Average cell count ~~from~~ from four sets of 16 corner squares
2. Multiply by 100,000 (10^5)
3. Multiply by 5 to correct dilution

Miniprep of BCD + A_2 + B_4

1. A_2 in pBAD #1
2. A_2 in pBAD #2
3. A_2 in pBAD #3
4. B_4 #1
5. B_4 #2
6. B_4 #3
7. BCD in pBAD #1
8. BCD in pBAD #2
9. BCD in pBAD #3
10. BCD in pBAD #4
11. BCD in pBAD #5
12. BCD in pBAD #6
13. 6-M11 #1
14. 6-M11 #2

18µL hem BCD
6µL Buffer
3µL SalI
3µL HindIII
30µL ddH_2O

10µL hem EF
6µL buffer
3µL SalI
3µL HindIII
38µL ddH_2O

The Inoculating RCamp+PRSET BL21 8/18/2018
Objective Generate a culture to infect bees for LD50
Reference Experiment #0

Procedure - Inoculated 250ml LB + Amp w/ tip scrapped on BL21 lawn
1:20AM - Incubated at 37°C and 250rpm → check in a few hours

The Retransforming BL21 w/ RCamp+PRSET
Objective Generate fresh transformants of RCamp+PRSET 8/18/2018
Literature

Procedure - 50µL DH10B comp cells + 1µL RCamp+PRSET
- Electroporated @ 2kV → Time = ms
- Plated on Amp LB plates, incubated @ 37°C overnight

Observations After overnight incubation → 8/19/2018

Spore
~~Cell~~ Counts: Cage 4

8/18/2018

Preparing Samples
- 100 µL homogenized, filtered bees + 400 µL Trypan Blue → try counting on 20x
- loaded 10µL on each well of the hemocytometer
- 20x objective works, 40x should work → cannot focus

Sample 1	Count	Average
Square 1.1	42	
1.2	55	
1.3	51	43
1.4	35	
1.5	32	
1.6	40	
1.7	44	
1.8	47	42.4
1.9	35	
1.10	46	
Square 2.1	50	
2.2	~~60~~	
2.3	62	52.6
2.4	~~50~~	
2.5	41	
2.6	49	
2.7	48	
2.8	47	52.6
2.9	~~69~~	
2.10	55	

47.65 × $10^{5.5}$ = 4,765,000 spores

Ligation: BCD + EF

16µL of EF	22°C → 20min → 1hr
1µL of BCD	70°C → 5min
2µL of buffer	4°C → ∞
1µL of T4 ligase	

OD 700 Measurements

Aug 19

1. Spec. can only run on windows XP mode
2. > USB Attach
3. DU 800 Spectrophotometer
4. → Set to "visible"
5. → Force settings ⇒ "Methods" ⇒ Set your parameters
6. ⇒ Put in blank as prompted (800 µl) to (1 ml)
7. → Narrower side must face light
   ↳ of cuvette
8. Run Blank → *Arrow must face light*
9. Save all reads a CSV. files
   ↳ In "C" Drive of the computer

Experiment O implementation

Aug 19

→ Found lower & higher thresholds for OD ⇒ Relationship: Non-linear
↳ Using OD to inform intermediate concentrations → unadvised

⇒ Mass & concentration will inform feeding within the experimental OD_{600} threshold

Digestion of - Heme A - pBAD
Heme B - pBAD
Heme EF - pBAD
6-M11
} 10 µl DNA + 3 µl enzymes (6 µl Buffer, xbal & pst I)

Heme A - pBSET
Heme A - pBAD
} BglII & HindIII
{15 µl DNA, 3 µl enzymes, 6 µl Buffer}

1. Heme A - pBaD
2. Heme B - pBAD
3. Heme EF - pBAD
4. 6 - M11
} xbal + pst I → 41 µl H_2O — smaller, smaller, smaller

5. Heme A - pBSET
6. Heme A - pBAD
} BglII + HindIII → 34 µl H_2O — larger, larger, larger.

P1 pw's Aug 20 → Miniprep.

1. Heme BCDEF in pBAD - DH10B
2. Heme BCDEF in pBAD - DH10B
3. Heme BCDEF in pBAD - DH10B
4. Heme BCDEF in pBAD - DH10B
5. Heme BCDEF in pBAD - DH10B
6. Heme BCDEF in pBAD - DH10B
7. Heme BCDEF in pBAD - DH10B
8. Heme BCDEF in pBAD - DH10B

Diagnostic Digest of miniprep 1-8

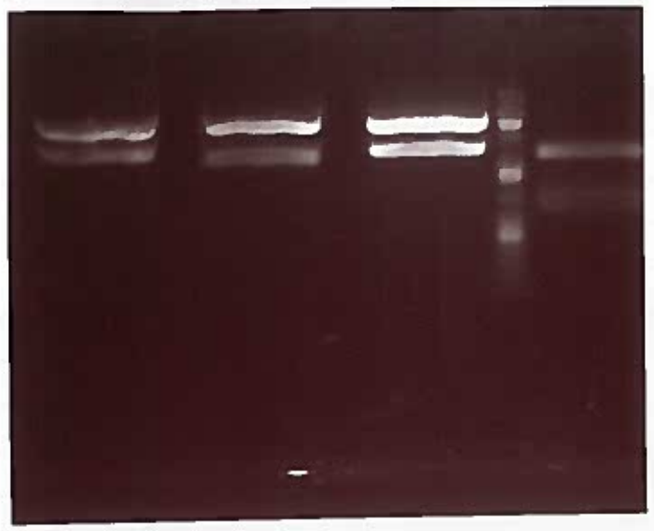
13.5μL ddH₂O
2μL FastDigest buffer
3μL DNA
0.5μL BglII
0.5μL HindIII
0.5μL KpnI (other RE)

84.73g → Clear bottle 86.18g RCaMP-pRSET 1 = 1.45g

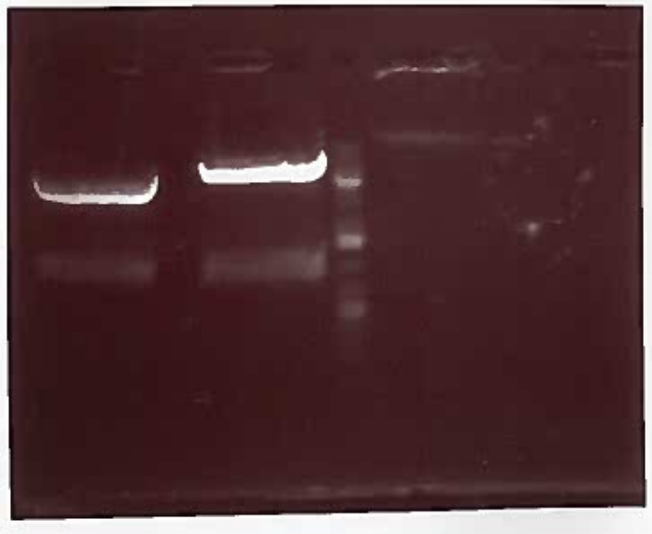
77.11g → translucent bottle. 78.94 = 1.83g

86.18
84.73
1.45

08/21/2018 23:39:33



08/21/2018 23:38:22



Diagnostic Digestion Results:

Aug 20

ChemRoutes CORPORATION

making drug discovery affordable

20 → 10 mL. #1

10 mL → 20 mL $C_{12}H_{22}O_{11}$

→ 10 mL (2x) #2

10 mL → 20 mL

→ 10 mL (4x)

10 mL → 20 mL

→ 10 mL (8x) #3

10 mL → 20 mL (16x)

→ 10 mL (16x) #4

10 mL → 20 mL

→ 10 mL (32x) #5

10 mL → 20 mL (64x) #6

→ 10 mL (64x) #6

10 mL → 20 mL

→ 10 mL (128x) #7

10 mL.

10 100% sugar H2O – #8

E. coli dilution

Experiment #1

9719 – 42 Avenue, Edmonton, AB T6E 5P8 CANADA
P: (780) 485-8836 F: (780) 485-9914
E: info@chemroutes.com W: www.chemroutes.com

08/21/18

Digestion of BCDEF

↳ #4, #7 ; use Bgl II Hind III

Digestion conc.

- 6μL buffer
- 3μL Bgl II
- 3μL Hind III
- 8μL DNA
- 40μL ddH_2O

} 4hr digestion →

Ligation of gBlocks into iGEM Vectors.

Hem A	Hem B	Hem EF.
1μL vector	1μL vector	1μL vector
16μL insert	16μL insert	16μL insert
2μL buffer	2μL buffer	2μL buffer
1μL T4 ligase	1μL T4 ligase	1μL T4 ligase

Transformations of iGEM vector - gBlocks into DH10B.

↳ use 4 μL of resulting conc.

- plated

ion + Transformation of Heme A & BCDEF

Aug 22 2018

→ Picked colonies for Heme ABCDEF in pBAD x3

→ Helped Bachelin w. her stuff

↳ Digestion – XhoI & HindIII → 3 μL each

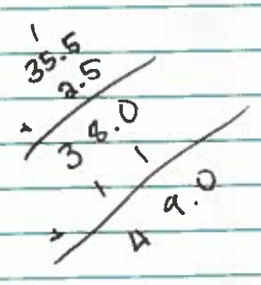
16 μL DNA
6 μL Buffer
32 μL H_2O

2 hour digestion

↳ ① jRCamp - pBad
② mRuby - GECO

buffer primer dNTP.

10 + 0.5 + 1 + 35.5



Aug 23 2019

ABCDEF ⇒ No growth in pBAD or pRSET

⇒ Miniprepping & D. Digesting Heme ABCDEF cultures

① #2a pBAD Heme ABCDEF in DH10B
② #2b pBAD Heme ABCDEF in DH10B
③ #4* pBAD Heme ABCDEF in DH10B

↳ 3µl DNA
2µl Buffer
0.5µl KpnI
0.5µl XhoI
14µl ddH₂O

#1 : Abs: 2.182
260/280: 1.97
260/230: 1.93

#3: Abs: 1.772
260/280: 1.90
260/230: 1.95

50µL Q5 PCR
- 20µL enhancer
20µL buffer
2µL dNTP
5µL PF
5µL PR
1µL template
46µL ddH₂O
1µL Q5 polymerase

50µL Q5 PCR
- 10µL enhancer ✓
10µL buffer ✓
1µL dNTP ✓
2.5µL P_F
2.5µL P_R
0.5µL template ✓
23µL ddH₂O
0.5µL Q5 polymerase

→ pBAD HemA
pBAD HemB
pBAD HemC
pBAD HemD
pBAD HemE
pBAD HemF
BB HemA
BB HemC
BB HemD

08/24/18

- Digestions
  - All 48µL of A, B, C, D, E ≈ XhoI, HindIII
    - X run gel.

- PCR on E, F
  - dilute 3µL → 5µL (add 2µL ddH_2O), run. ≈ gblock

  - run ≈ end sequenced EF.

Miniprep
- pSB1C3 ≈ Hem A-mScarlet
- pSB1C3 ≈ Hem B-MNG
- pSB1C3 ≈ Hem EF
- CD, Re-cultured (stock)
- BD, Re-cultured (stock)

2 PCR for EF each → dilute w. H_2O from 3µL → 6µL
↳ Run @ 1° ↑ than yesterday's temp → 62° for ① E F
↳ Run PCR ② w. plasmid. E F
↳ (↑ temp. makes it harder to bind)

PCR Protocol

10 µL buffer	
10 µL primer	T_a : 62°C
1 µL dNTPs	t_e : 40 s.
2.5 µL Fp	t_{ef} : 3 min
2.5 µL Rp	
1 µL template	
22.5 µL ddH_2O	
0.5 µL Q5	

Aug 24

Ligations: ABCDE into pBAD

↓

Infusion

↓

Digestion

Ligation of ABCDE – pBAD
Ligation of j13CaH 17h0 – pBAD
Digestion of Hem E+F
Infusion of Hem E+F ≅ pBAD *
Infusion of pBAD ≅ BM 40CFP
" " GSSGA link pBAD ≅ BM 40CFP

Aug 28th

Miniprepped

ABCDEF$_1$ → *
ABCDEF$_3$ → O
Heme b infusion in pBAD → :(
Picked colonies

08/29/2018 03:38:03

29 → 33

Tris Base Sol'n prep.

MW = 121.14g/mol

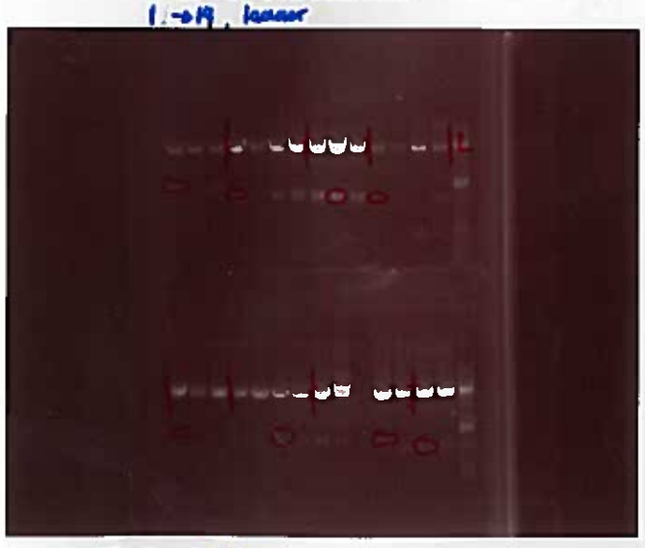
1mol = 121.14g/L
0.1mol = 12.11g/L

500mL of 0.1mol tris base = 6.055g/L

2	260/280: 1.05	260/230: 3.67
1	260/280: 1.06	260/230: -0.90
3	260/280: 2.14	260/230: -1.67
	260/280: 0.72	260/230: -0.98
21	260/280: 1.33	260/230: -3.52
13	260/280: 1.18	260/230: -1.39

08/29/2018 03:50:20

1 → 14, ladder



15 → 28, ladder.

Aug 29 th

Digestion

→ Heme A-F$_3$ #1 & CD

Digestion of A3CDEF, Hem CD+mScarlet

↳ Sca I, Hind III as Restriction Enzymes

60µL total

↳ 6µL buffer
↳ 3µL Sca I
↳ 3µL Hind III
↳ ~~24µL~~ ddH$_2$O
↳ ~~24µL~~ DNA

CD →

A-F
9µL DNA
39µL ddH$_2$O

Changed from 24µL ddH$_2$O & 24µL DNA to
10µL DNA + 38µL ddH$_2$O
↳ initial run = fail; re-run w another duplicate of Hem CD+pl3AD mScarlet

August 30, 2018

- Gel Extraction of mScarlet + ligation.

Ligation
1µL vector
1µL insert
2µL buffer
1µL T4 ligase

Run @ 22°C →
70°C → 5 min
4°C → ∞

Transformation into DH10B → Plate onto Amp+Ara plates.

BB PCR → Delegated to friday, August 31
↳ sitting in 4°C fridge.

Gel Results: no second band @ ~ 700 kbp observed.
↳ Transform + Streak CD$_2$-mScarlet in DH10B on Amp+Ara plates + plate 60µL
↳ pick 2 cultures from old CD$_2$ plates + culture @ 37°C

* Also Run digestions on previous constructs. (BCD+mScarlet) → FAILED
↳ DNA conc. can't exceed 3µg;

BCD$_6$ (original) → 398.3 ng
- 6µL buffer
- 3µL Sal I
- 3µL Hind III
- 4µL ddH$_2$O
- 7µL DNA

BCD$_3$ (re) → 359 pg
- 6µL buffer
- 3µL Sal I
- 3µL Hind III
- 40µL ddH$_2$O
- 8µL DNA

BCD$_{21}$ (re) → 338 ng
- 6µL buffer
- 3µL Sal I
- 3µL Hind 3
- 40µL ddH$_2$O
- 8µL DNA

$\frac{3000\mu g}{398.3} \doteq 7\mu L$ rounded up

$\frac{3000ng}{359ng} \approx \sim 8\mu L$

$\frac{3000ng}{338ng} \doteq \sim 8\mu L$

August 31

→ Running a digestion for the "Missing Gel band incident of 2018".

➢ Going to run a "mini-digestion" w. less enzyme to determine why our band isn't showing in the gel.

↳ Don't know if salt is the issue
↳ Trying to figure out what's going on

→ Miniprep → Diagnostic digest → Run @ 150 → Show Landon?
→ Use 1 µl Enzyme
↓
Nano-drop

SalI + HindIII
XhoI + HindIII
SalI + XhoI

-Qs Polymerase on Plem CD (50µL) gblock → also for hopeful PP extraction

10µL enhancer
10µL buffer
1µL dNTP
2.5µL P_F
2.5µL P_R
0.5µL template
23µL ddH_2O
0.5µL Qs polymerase

~~24µL of Plem CD+mScarlet~~

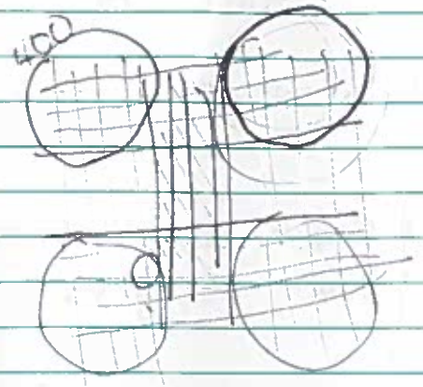
~~9µL of ABCDEF~~

ALL COLONIES ON PLATES ARE RED
-see red flouresence in both
Colony picking results
↳ MScarlet is in there.
↳ do we have a mutation in cut sites?

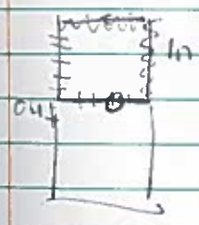
Sept 3/18

Continuation of "finding the missing mScarlet DNA
1) Miniprep + Digestion
2) PCR → run to check for success → PCR clean up + Digestion

Sept 3 2018



→ dilution ratio: 1 to 5
↳ 100 µL spores, 400 µL Trypan Blue



Top right (Major) #(x)

↳ Top left 4x4: ~39 spores
↳ Bottom left 4x4: ~40 spores
↳ Bottom right 4x4: ~111 spores
↳ Top right 4x4: ~126 spores
} Total: 316 spores

~~Bottom right (Major)~~

↳ ~~Top left: ~70~~ spores
↳ ~~Bottom left: ~~

Total 906 spores

Bottom right (Major)

↳ Top left 4x4: ~164 spores
↳ Bottom left: ~165 spores
↳ Bottom right: ~282 spores
↳ Top right: ~290 spores

Sept 3

Bottom left (Major)

↳ Top left 4x4: ~151 spores
↳ Bottom left 4x4: ~130 spores
↳ Bottom right 4x4: ~62 spores
↳ Top right 4x4: ~13 spores
} Total: 356 spores

Top left (Major)

↳ Top left 4x4: ~311 spores
↳ Bottom left 4x4: ~260 spores
↳ Bottom right 4x4: ~105 spores
↳ Top right 4x4: ~72 spores
} Total: 748 spores

Grand Total (All four major quadrants): 2326 spores

Average per quadrant: 581 spores

~~581/10μl x 1.5 x 10^4~~ → 2,905

→ 2,905

$\left[\frac{581}{10\mu l} \times 5 \times 10^4\right] = 9.68 \text{ million}$

3

CD-mscarlet digestion → Successful; ligated into pBAD-ABCD

Tube 1)
6μL buffer
3μL SalI
3μL HindIII
26μL DNA
22μL ddH_2O

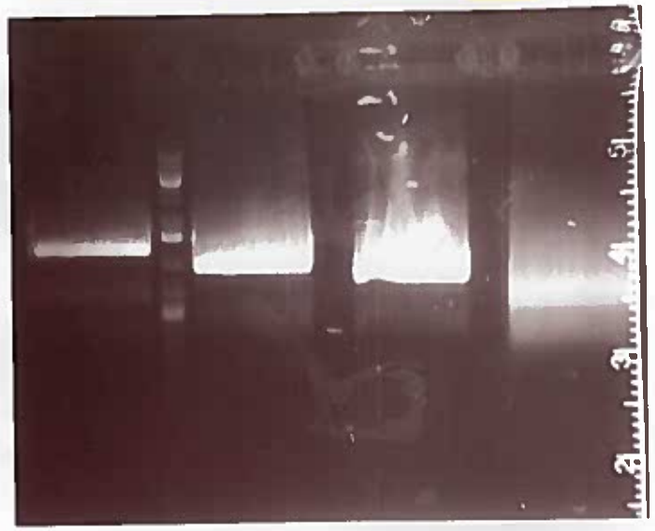
Tube 2)
6μL buffer
3μL SalI
3μL HindIII
25μL DNA
23μL ddH_2O

Rangel on Q5 ~~Q5~~ PCR on Hem CD-mscarlet → band size ~1000bp
↳ failed PCR.

Diagnostic Digest of psB1C3 ← Hem A-mscarlet
13 + mNG
Hem E + Hem F

16μL ddH_2O
2μL buffer
1μL DNA
0.5μL XbaI
0.5μL pst1

Biobrick Hem A Hem B C. Hem D pBAD-BBOL



A, B, C, D PCR successful ; gel extracted.

Ligat

Digestion of pSBC13 for Hem A, B C, D insert. 09/04/18

Digestion

1) $\frac{3000}{58.3} = 51\mu L$ 4over 2) $\frac{3000}{112.2} = 26\mu L$ 3) $\frac{3000}{128.8} = 23\mu L$

6µL buffer	6µL buffer	6µL buffer.
3µL xbal	3µL xbal	3µL xbal
3µL pstI	3µL pstI	3µL pstI
48µL DNA.	26µL DNA	23µL DNA
	22µL ddH_2O	25µL ddH_2O.

Digestion of BBOL Hem A, C, D

A)	C)	D)
6µL buffer	6µL buffer	6µL buffer
3µL xbal	3µL xbal	3µL xbal
3µL pstI	3µL pstI	3µL pstI
48µL DNA	48µL DNA	48µL DNA

Extracted in 50µL EB

36µL buffer.
3µL xbal
3µL pstI

09/05/18

Ran PsB1C3 on Gel → Correct sizes
↳ extracted ≈ 50µL

Ligation of A B + C ≈ pSB1C3.

1µL vector
16µL insert
2µL buffer
1µL T4 ligase
} Run @ 22°C
@ 85°C
@ 4°

Transformed into DH10B + plated on Amp-Ara plates.

- Pick ~~Colony~~ 3 colonies/plate for pBAD-ABCD-mScarlet ligation + transformation
  ↳ 8 & 2 Bright Flor. were also picked for miniprep ↑ higher

1:1 dilution of trypan blue: "bee juice"

1:1 dilution of trypan blue (0.4%) : bee aliquot.

Spore Counts

Nosema Control 2 VQJP → Count of all cell matter, not just spores.

top right

1	2
3	4

4x4 Area 1: 1892 spores
4x4 Area 2: 1658 spores
4x4 Area 3: 1560 spores
4x4 Area 4: 1637 spores

OD

bottom right

1	2
3	4

4x4 Area 1: 1362 spores
4x4 Area 2: 1345 spores
4x4 Area 3: 1577 spores
4x4 Area 4: 1465 spores

bottom left

1	2
3	4

4x4 Area 1: 1532 spores
4x4 Area 2: 1523 spores
4x4 Area 3: 1370 spores
4x4 Area 4: 1500 spores

1	2
3	4

4x4 Area 1: 1786 spores
4x4 Area 2: 1720 spores
4x4 Area 3:
4x4 Area 4:

09/06/18

↳ Miniprep of ABCD-mScarlet

↳ BF = brighter flourescence → contamination?

-Diagnostic Digest-

↳ Run = SalI / HindIII

14μL ddH_2O
2μL buffer
0.5μL SalI
0.5μL HindIII
3μL DNA

} Success on everything except ABCD-mScar BF#1

Digestions

1-B1 → 20μL DNA, 28μL ddH_2O
1BF2 → 11μL " 37μL "
2-1 → 11μL " 37μL "
2-2 → 11μL " 37μL "
2-3 → 12μL " 36μL "
2BF2 → 16μL " 32μL "
2-1 → 10μL " 38μL "
2-2 → 11 " 37μL "
2-3 → 10μL " 38μL "

Quikchange of Hem E+F

↳ primers diluted according to previous protocol

Quikchange Multi Protocol Total volume = 25μL

-18.3 ddH_2O
-QC buffer = 2.5μL
Quick soln = 0.75μL
Primer E = 0.5 μL
Primer F = 0.5 μL
dNTP = 1μL
Template = 0.5μL
QCM enzymes = 1μL

95° - 2min
95° - 20s
85° - 30s
65° - 30s / kbp (6.4kb ~ 3m20
Rep 30X
65° 5min.

Re-shipped Hem EF

↳ diluted [illegible] in 35μL

↳ 1μL → Infusion = pBAD

↳ 9μL → Digation = SalI / HindIII

Sept 8

→ Miniprep + Digest (diagnostic)
of
- Heme A in pBAD
- Heme C in pBAD
- Heme D in pBAD

} (digested w. pstI & xbaI)

Pick Colonies for Hem EF-pBAD infusion
↑ new synth.

Transform DH10B ≅ Hem-EF-pBAD quikchange plasmid
↳ 2µL

Digest 7µL glukxk Hem EF-pBAD, ≅ SalI / HindIII

↳ Ligate ≅ Hem ABCD -pBAD

↳ Transform in DH10B.

PPIX Treatment 9/8/2018

2M sucrose → sucrose 684.593g in liter of H_2O

342.2965 g in 500mL

$80\,\mu mol_{PPIX} = 80\cdot10^{-6}\,mol \cdot \frac{562.658\,g}{mol} = \quad g/L$

$\frac{\mu mol}{L} = 10^{-6}\frac{mol}{L}\cdot\frac{g}{mol} = 0.045\frac{g}{L}$ or $0.0225\,g_{PPIX}/500mL$

$\frac{20mg}{} = 20\cdot10^{-3}g\,\frac{mol}{562.658\,g} \Big/ 50mL = \frac{\quad mol}{L}$

$20mg\cdot\frac{10^{-3}g}{mg}\cdot\frac{1\,mol}{562.688g}\cdot\frac{1}{0.05L} = 7.12\cdot10^{-4}\frac{mol}{L}\cdot\frac{\mu mol}{10^{-6}mol}$

$\left(\frac{20mg}{50mL}\right)\left(\frac{1000mL}{L}\right)\left(\frac{1\,mol}{562.658g}\right)\left(\frac{\mu mol}{10^{-6}mol}\right)$ $\frac{71.2\,\mu mol}{L} = 71.2\,\mu M$

$\frac{\mu mol}{L} = \mu M$

50 $\frac{45mg}{50mL}\cdot\frac{1000mL}{L} = \frac{45000mg}{50L} = \frac{900mg}{L}$

$\frac{45mg}{100mL}\cdot\frac{1000mL}{L} = \frac{45000}{100L} = \frac{450mg}{L}$

$\frac{45mg}{200mL} = \frac{1000mL}{L}\quad\frac{45000mg}{400L} = \frac{225mg}{L}$

Sept 10 2018

→ Mini preped: [redacted] A- pBIC3, C- pBIC3, D- pBIC3, EF- pBAD

↳ Heme EF quickchange in pBAD - DH10B ⇒ Picked colonies

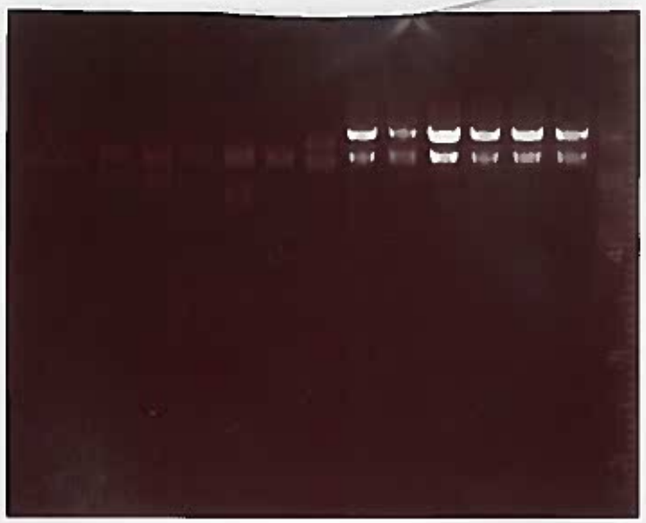
Heme ABCDEF ligation in pBAD DH10B → picked colonies

09118

- D.D of → A-pSB1C3 (3)
  C-pSB1C3 (3)
  D-pSB1C3 (3)
  Hem EF- ligation (6)

- miniprep of Hem A-F (1)
  EF-QC (11)
  ↓
  failed

09/13/2018 03:55:45



D.D on Hem EF

09/21/8
↳ Sequencing — insertion of globocks to BBs confirmed

- Miniprep Hem A-F (5) 09/13/18
- Infusion of Ju5119-cjblue + J23100-cj Blue → Transformation.
  ↳ Chlor. resd. (1 hr incubation)
  09/14/18
- EFQC$_3$, EFQC$_4$ → no mid.
  ↳ digestion + ligation(?)
- Diagnostic Digest. of A-F

Digestion
↳ EFQC$_4$ $\frac{3000}{44.4}$ = 48µL EFQC$_3$ $\frac{3000}{49.8}$ = 48µL

EFQC$_3$
25µL DNA
23µL ddH$_2$O

EFQC$_4$
30µL DNA
18µL ddH$_2$O

→Transformation of EFQC$_4$ & EFQC$_3$

Ligation: insertion to ABC0828-pBAD

011518

- Colony Picking from ABCD-EFGC$_3$
  → no growth on ABCD-EFGC$_4$

- Re-Stock of EGGC$_3$, EFGC$_4$ (Colony Picking)

J23100-cjBlue, J23119-cjBlue → failure to turn blue after 24h
→ use/find another construct to improve

13C plate!

Bbar_K554001

↳GFP

→ transform in DH10B.

04/15/18

Digest HemEF$_3$ pBAD, eGFP generator

$\frac{3000}{176} = 17.04\mu L$ $\frac{3000}{92.6} = 32\mu L$

HemEF$_4$ pBAD,

$\rightarrow 48\mu L$

6µL buffer
3µL XbaI
3µL pstI
32µL DNA
16µL ddH_2O

- Qs Pol on E, F

- Digestion of J23100-gBlock, J23119-gBlock gBlocks 57 TA
  $t_{ex} = 40$
  $t_{ef} = 30s$

Qs Pol on J23100-gBlock J23119 gBlock.

Transformation of HemF-pBAD + HemE pBAD-

01/2018

Transformations
- J23160, 119, HemEF → pSB1C3

-Picked Colonies For E(3) & F(3)

Protein Prep Concentrations.

10/2/18

Hem B = 2.91 mg/mL
= ~~2.91 g/L~~ / ~~35.652 g/mol~~
2.91 g/L

δ-ALA dehydratase.
↳ 35 625 Da.
= 35 652 g/mol

~~0.1252 mol/L~~

8.2×10^{-5} g/mol

Hem C = 0.88 mg/mL

Porphobilinogen deaminase
↳ 33 852 Da

~~0.88 g/L~~

2.6×10^{-5} mol/L

Hem D = 0.06 mg/mL

Uroporphyrinogen III synthase

27 798 Da.

2.2×10^{-6} mol/L.

04/28/16

GRAD Q5 PCR on mCherry (50µL)

↳ 1-8 ↳ ~~confocal~~ BBdL PF, 9-16 ↳ ~~Ara promoter PF~~ AraC pF

↓ 30s ~~1:20~~ ext

↓ ~~30s~~ ext 1:20

Temp range: 57°C — 62°C

34x repeat

Final Ext. 2:00

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16

57°

62°

10/18

1 } A_2 in BCDEF4
2
3
4

5 } A_3 in BCDEF4
6
7

8 } A_2 in BCDEF5
9
10
11

12 Para-mCh 1-3
13 Para - mCh 1-7
14 araC+mCh 1-4
15 araC+mCh 2-1
16 Para-eGFP 4
17 araC eGFP1
18 araC eGFP2
19 araC eGFP4
20 araC eGFPb
21 [illegible] + mRFP #1
22 [illegible] + mRFP2
23 K584000 eGFP1
24 K584000 eGFP2

Oct 13

SDS-PAGE

Loading previously made SDS PAGE Gel (12%)

Lane 1 ~~[illegible]~~ T7 mut
± 2 Hem A mut
3 ~~2~~ Protein mw marker (20 µl)
4 ~~3~~ Hem A
5 ~~4~~ Hem B
6 ~~5~~ Hem C
7 ~~6~~ Hem D
8 ~~7~~ Hem E
9 ~~8~~ Hem F
10 ~~9~~ Empty
~~10~~

Running in 1% SDS

25 µl sample/well (20 µl protein : 5 µl loading dye)

Running at 250 V

KF Value Calc.

No Substitution

Standard = DCF	Extract DCF	Standard x DCF	Extracted x DCF
$\frac{1cm}{3.5cm} = 0.286$	$\frac{0.9cm}{3.5cm} = 0.257$	$\frac{1.5cm}{3.5cm} = 0.514$	$\frac{1.9cm}{3.5cm} = 0.543$

TCL substitution

Std = DCF	Ext. = DCF	Std x DCF	Ext. x DCF
0.297	- 0.216	+ 0.405	+ 0.378

Anti-Chemicals.

10/15/18

Hem A = 1.33mg/mL / 44 374 Da

$= 2.30 \times 10^{-5}$ mol/L

Hem B = 2.91 / 35 652 Da

$= 8.2 \times 10^{-5}$ mol/L

Hem C = 0.88mg/mL

2.6×10^{-5} mol/L

Hem D = 0.06mg/mL or 0.43mg/mL

2.2×10^{-6} mol/L ↓ 1.55×10^{-5} mol/L

Hem E = 6.63mg/mL / 39 248 Da

$= 1.69 \times 10^{-4}$ m/L

Hem F = 0.87mg/mL / 34 323 Da.

$= 2.53 \times 10^{-5}$ mol/L

1.55×10^{-5} mol/L x 1mL = 2.30×10^{-5} mol/L x

$\frac{1.55 \times 10^{-5} \text{ mol/L}}{8.2 \times 10^{-5}} =$

$\frac{1.55 \times 10^{-5} \text{ mol/L}}{2.6 \times 10^{-5} \text{ mol/L}}$

$\frac{1.55 \times 10^{-5} \text{ mol/L}}{1.69 \times 10^{-4} \text{ mol/L}}$

$\frac{2.2 \times 10^{-6}}{2.3 \times 10^{-5}}$

= 0.674mL Hem A
= 0.189mL Hem B
= 0.596mL Hem C
= 1mL Hem D
= 91.7µL Hem E
= 0.613mL Hem F

= 95.7µL Hem A
= 26.8µL Hem B
84.6µL Hem C
= 13.0µL Hem E
= Hem F. 87.0µL Hem F