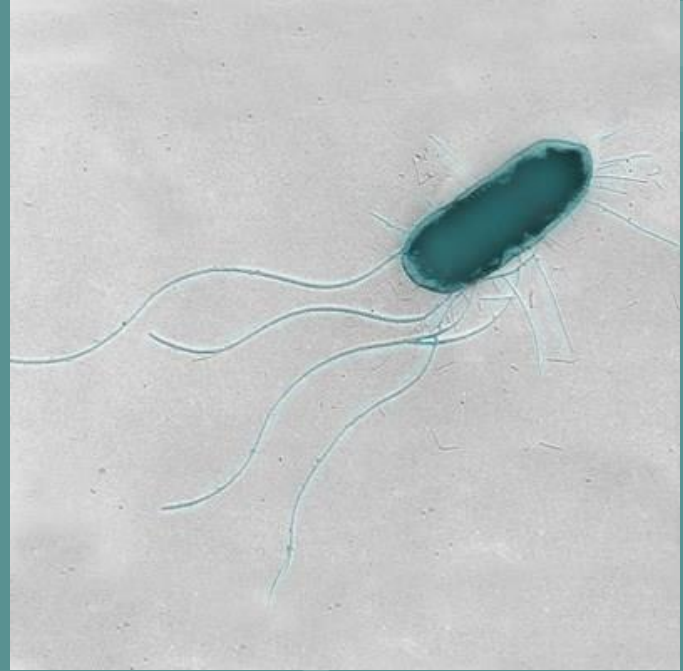
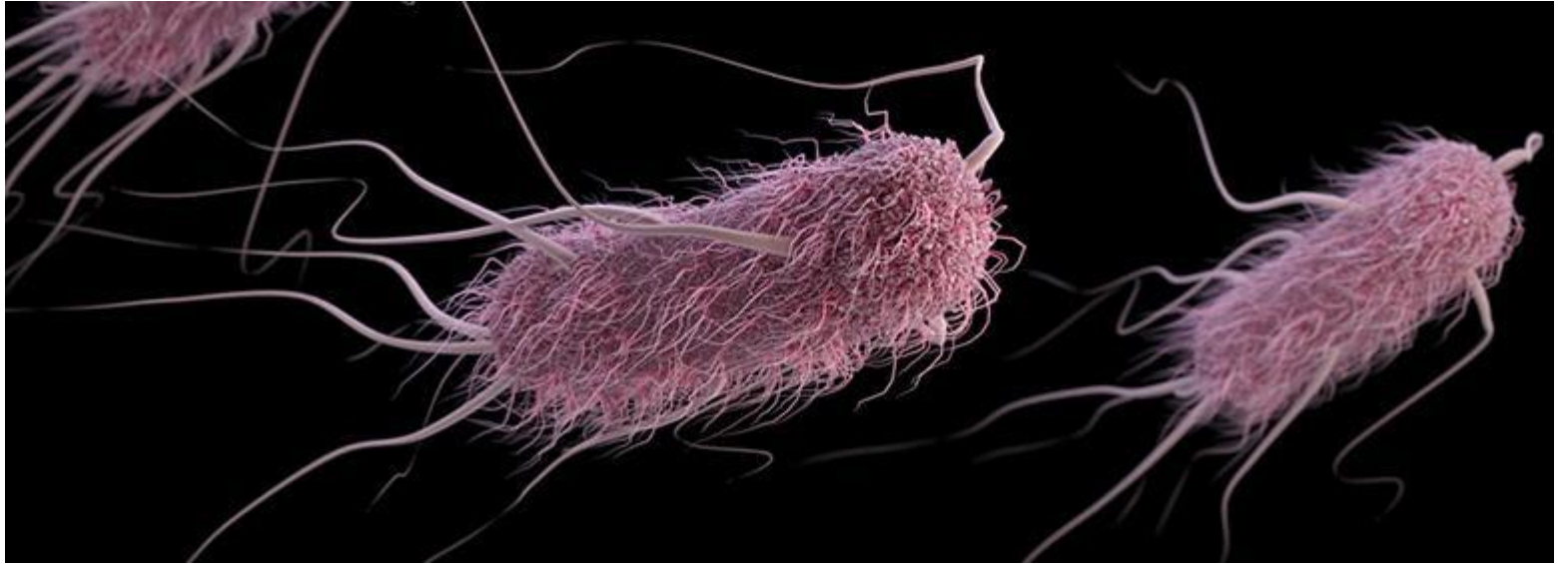


Induced expression of FLIC In non-motile bacteria

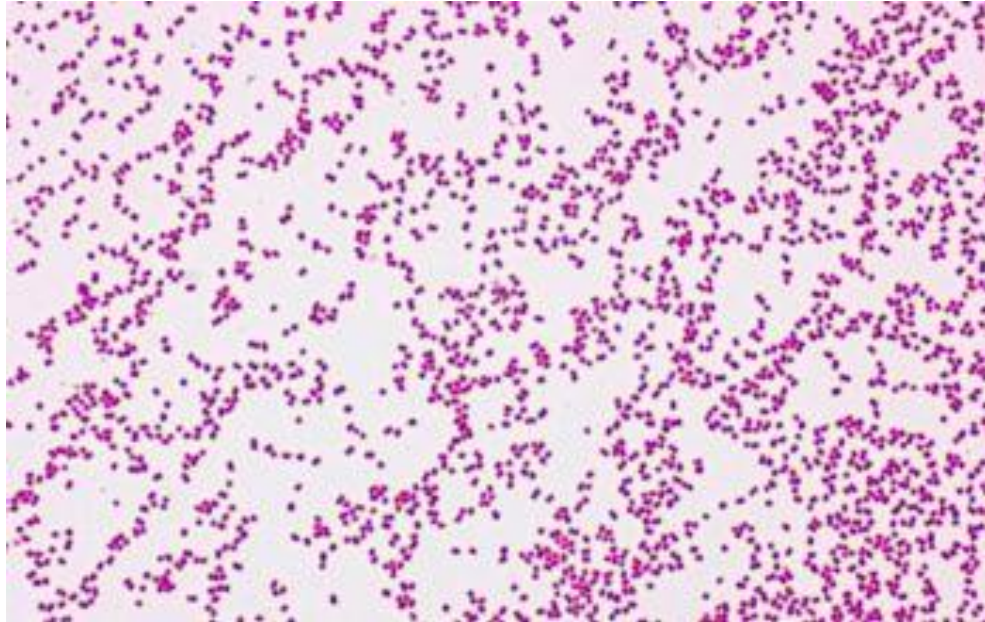
Cameron Harper
Tyler Jones



E Coli

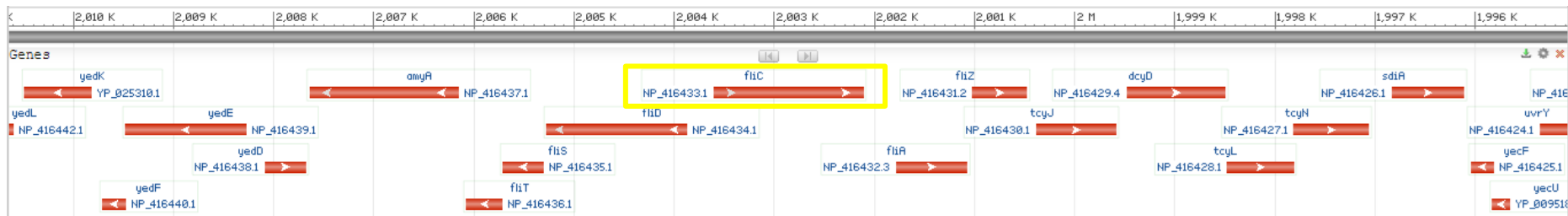


Neisseria Subflavia



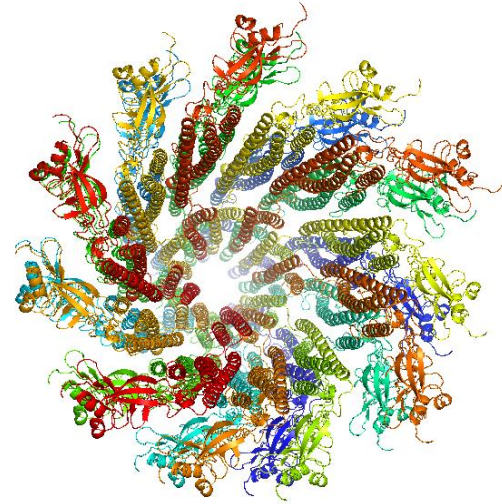
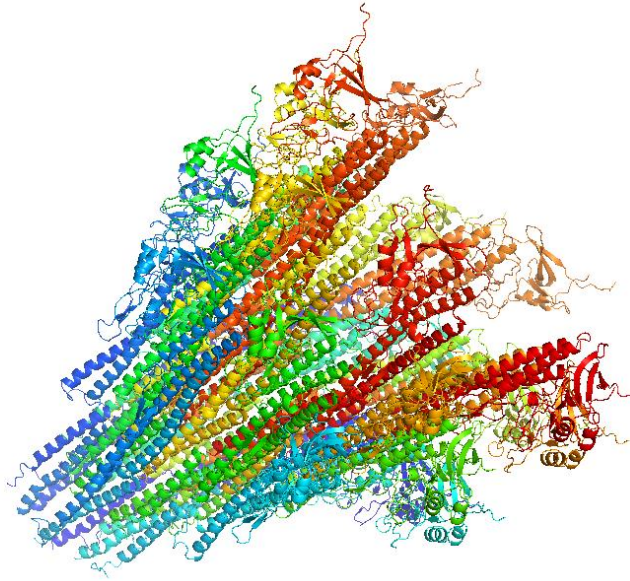


FLIC Gene





FLIC Protein flagellar filament structural protein



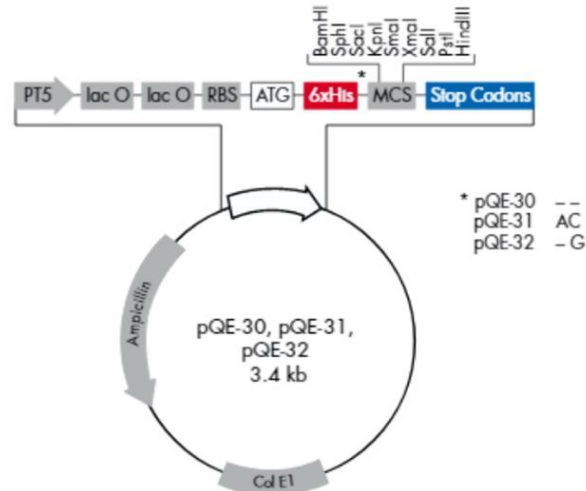
Design Primers

Forward

A*GAT CTG ACC TGA CCC GAC TCC CA
BglIII Restriction site

Reverse

GCC GTC AGT CTC AGT TAT CAA GCT*T
HindIII Restriction site





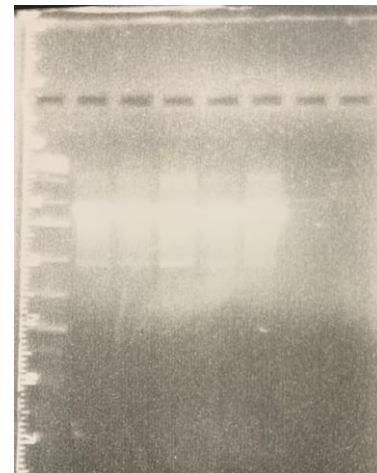
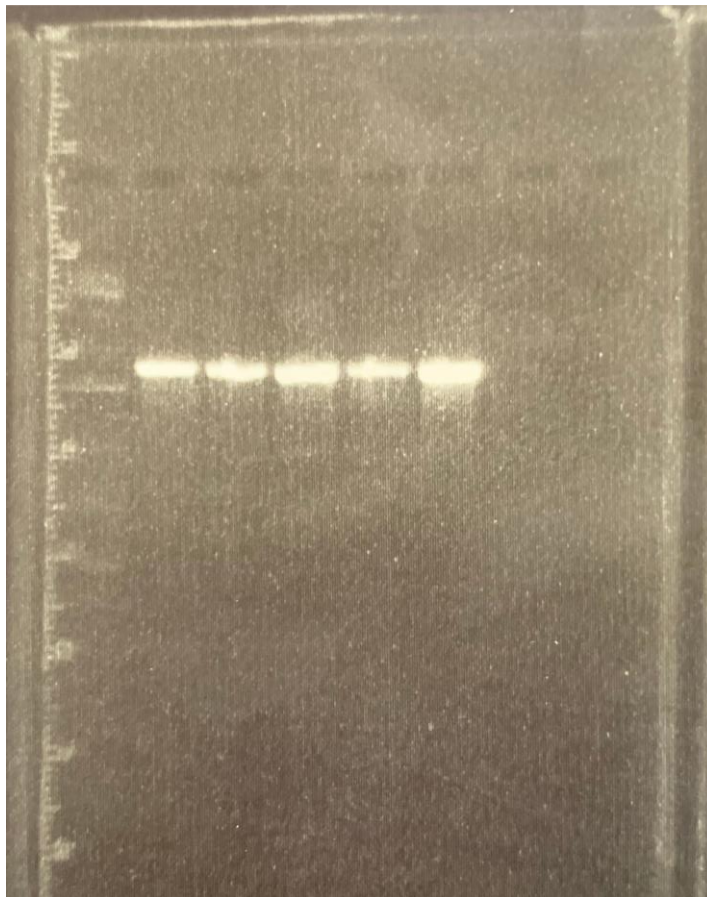
Waiting for Primers

Things we could have done while waiting for primers...

- Go to Hawaii
- Watch all of the Harry Potter and Lord of the Rings movies
- Invested time into watching march madness
- Ordered primers and received them
- Finished our project with week to spare
- Done another IRP
- Learned a new talent
- Built a full size lego millennium falcon



PCR





Nanodrop

Plasmid

Sample ID: Pedestal

Type: DNA 50.00

Conc. 15.3 ng/μl

A260 (10 mm path) 0.306

A280 (10 mm path) 0.176

260 / 280 1.74

260 / 230 1.19

☒ Baseline correction 340 nm

PCR Product

Sample ID: Pedestal

Type: DNA 50.00

Conc. 35.9 ng/μl

A260 (10 mm path) 0.717

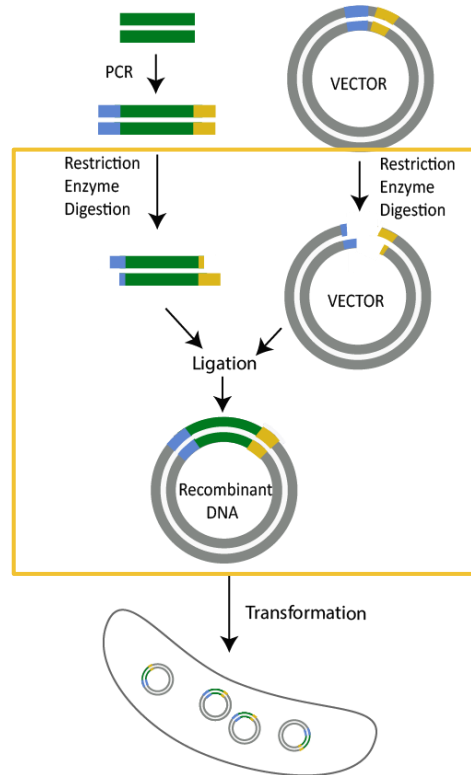
A280 (10 mm path) 0.393

260 / 280 1.82

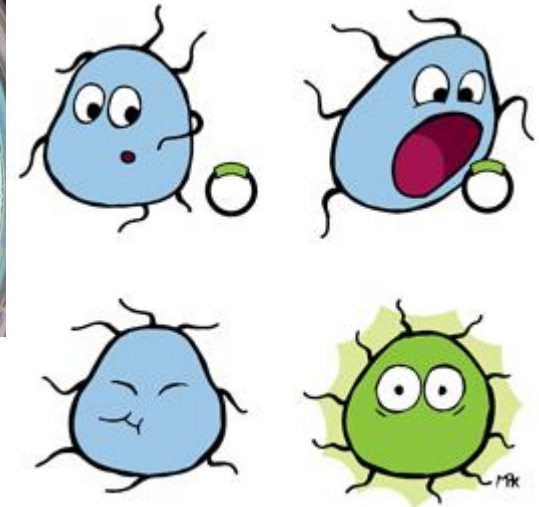
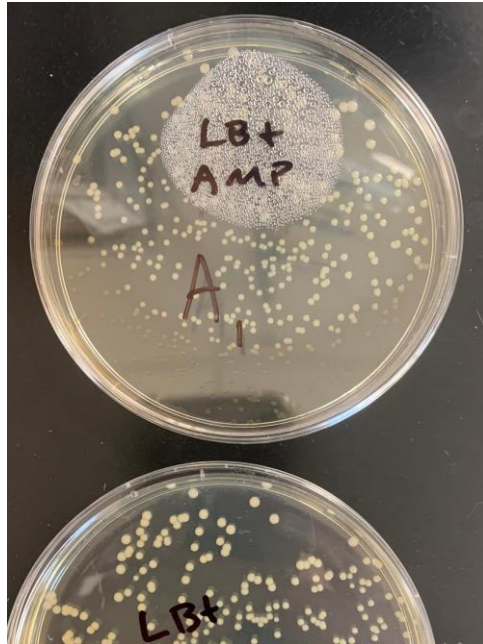
260 / 230 1.41

☒ Baseline correction 340 nm

Restriction Digest & Ligation



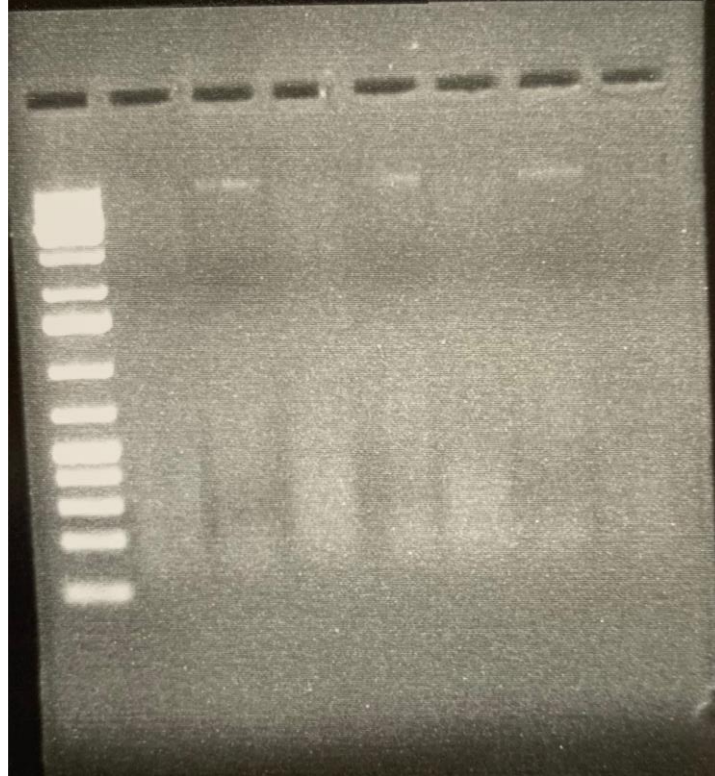
Transformation





Verification Digest

Not very good but a reasonable explanation





Sequence and MW of flagellin

Compute pI/Mw

Theoretical pI/Mw (average) for the user-entered sequence:

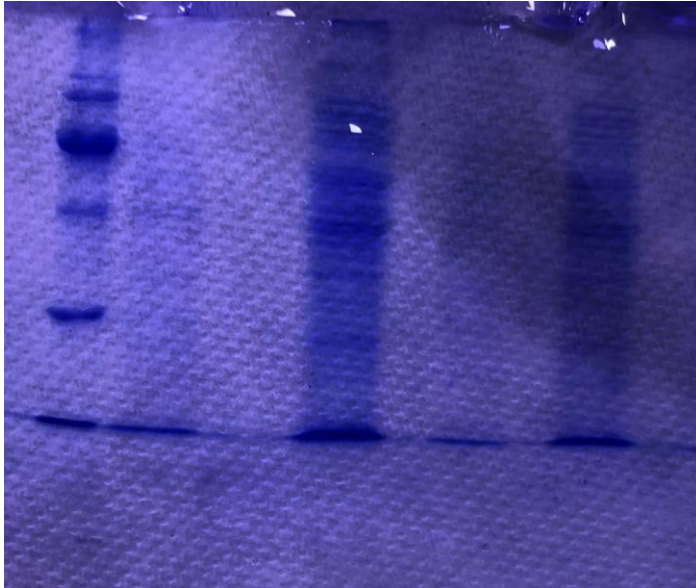
<u>10</u>	<u>20</u>	<u>30</u>	<u>40</u>	<u>50</u>	<u>60</u>
MAQVINTNSL	SLITQNNINK	NQSALSSSIE	RLSSGLRINS	AKDDAAGQAI	ANRFTSNIKG
<u>70</u>	<u>80</u>	<u>90</u>	<u>100</u>	<u>110</u>	<u>120</u>
LTQAARNAND	GISVAQTTEG	ALSEINNNLQ	RVRELTVQAT	TGTNSESDLS	SIQDEIKSRL
<u>130</u>	<u>140</u>	<u>150</u>	<u>160</u>	<u>170</u>	<u>180</u>
DEIDRVSGQT	QFNGVNVLAK	NGSMKIQVGA	NDNQITITIDL	KQIDAKTLGL	DGFSVKNNDT
<u>190</u>	<u>200</u>	<u>210</u>	<u>220</u>	<u>230</u>	<u>240</u>
VTTSAPVTAF	GATTTNNIKL	TGITLSTEAA	TDGGGTPAS	IEGVYTDNGN	DYYAKITGGD
<u>250</u>	<u>260</u>	<u>270</u>	<u>280</u>	<u>290</u>	<u>300</u>
NDGKYAVTV	ANDGVTMAT	GATANATVTD	ANTTKATTIT	SGGTPVQIDN	TAGSATANLG
<u>310</u>	<u>320</u>	<u>330</u>	<u>340</u>	<u>350</u>	<u>360</u>
AVSLVKLQDS	KGNDTDYAL	KDTNGNLYAA	DVNETTGAVS	VKTITYTDSS	GAASSPTAVK
<u>370</u>	<u>380</u>	<u>390</u>	<u>400</u>	<u>410</u>	<u>420</u>
LGGDDGKTEV	VDIDGKTYDS	ADLNGGNLQT	GLTAGGEALT	AVANGKTTDP	LKALDDAIAS
<u>430</u>	<u>440</u>	<u>450</u>	<u>460</u>	<u>470</u>	<u>480</u>
VDKFRSSLGA	VQNRLDASVT	NLNNTTTNLS	EAQSRIQDAD	YATEVSNMSK	AQIIQQAGNS
<u>490</u>					
VLAKANQVPQ	QVLSLLQG				

Theoretical pI/Mw: 4.50 / 51294.99



SDS Page Attempt 1

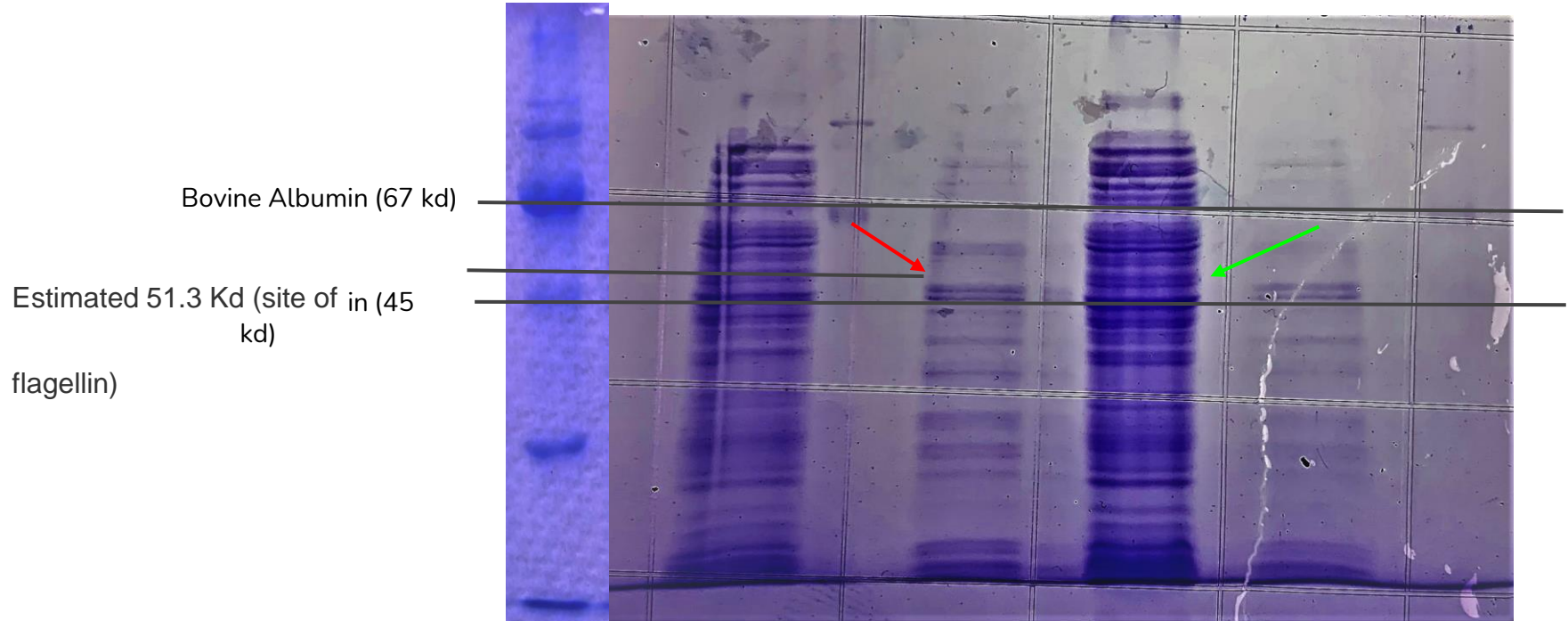
A sad excuse for a gel but a great ladder





SDS Page Attempt 2

WT N. Subflava Transformed N. Subflava





What we would do with more time and money

1. Order anti-flagellar filament structural protein antibodies and perform a western blot to confirm the presence of the specific protein we desired.
2. Continue transforming the rest of the flagella genes into the bacterium and try to express fully functioning flagellum.
3. Perform a flagellar stain to allow flagellum to strut their stuff
4. Kill all remaining bacteria to prevent another pandemic...