iDEC Lab Notebook:

End of July 2022

- Designed all DNA parts and primers using Benchling
- Ordered DNA sequences and primers from integrated DNA technologies
- Made the first batch of $AgNO_3$ plates to get the method right and to establish the MIC for untransformed BL21(DE3) cells and TOP10 cells
- Made necessary plates for blue/white colony screen
- Made a stock of chemically competent TOP10 and BL21(DE3) *E. coli* cells

1st Week of August 2022 (started from 01/08/2022)

- Plated untransformed cells on $AgNO_3$ plates (8-10mg/L) in duplicate:

Trial Run Samples:	08 mg/L AgNO3	10 mg/L AgNO3	12 mg/L AgNO3	14 mg/L AgNO3	16 mg/L AgNO3	17 mg/L AgNO3	18 mg/L AgNO3
BL21 DE3 cells	Lawn	Lawn	Lawn	Lawn	Lawn	25	1
BL21 DE3 cells	Lawn	Lawn	Lawn	Lawn	Lawn	Lawn	0
TOP 10 cells	0	0	0	0	0	0	0
TOP 10 cells	0	0	0	0	0	0	0

- Based on the results made all the needed $AgNO_3$ plates (16-30 mg/L) for 8 samples and a negative control

$AgNO_3$ concentration (mg/L)	$AgNO_3$ stock solution volume (µL)
08	1.563
10	1.953
12	2.344
14	2.734
16	3.130
17	3.330
18	3.520
19	3.720
20	3.910
22	4.300
24	4.690
26	5.080
28	5.470
30	5.860

2nd Week of August 2022 (started from 08/08/2022)

- **Some Level 0 parts were received and domesticated into pJUMP18 plasmids:**
  - J23100 promoter
  - Ribosome-binding site (RBS)
  - L2U2H09 terminator

- Received our 6xHis_TEV N part DNA sequence. Because it was a very short sequence, we obtained the forward and reverse sequences separately. We diluted these stock DNA samples with 200 µL TE buffer.
- Annealed the 6xHis_TEV DNA by PCR

Part Type	Name	Stock concentration (fmol/ul)	GC%
N	6xHis_TEV (F)	55000	49.3
	6xHis_TEV (R)	47500	49.3

3rd Week of August 2022 (started from 15/08/2022)

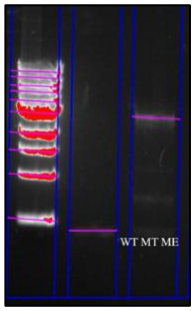
- Received the rest of the DNA sequences we ordered
- List of all Level 0 JUMP parts:

Part Type	Name
P	J23100 promoter
R	Ribosome-binding site (RBS)
T	L2U2H09 terminator
N	6xHis_TEV
O	SUMO_GS
C	ME MT
	MG MT
	SC MT
	DR MT

- Domesticated all remaining Level 0 parts into pJUMP18 plasmid
- A trial Level 1 assembly was done with 30 cycles:

P	Vol of P (µL)	R	Vol of R (µL)	N	Vol of N (µL)	O	Vol of O (µL)	C	Vol of C (µL)	T	Vol of T (µL)	acceptor	Vol of acc (µL)	Vol of water (µL)
J23100	0.997431373	B0034	0.8691873 1	6His TEV	2.19442615 8	SUMO	0.230120625	ME MT	1.086853429	L2U2H09	0.319061728	pJUMP29 LacZ	0.978410359	10.07450902

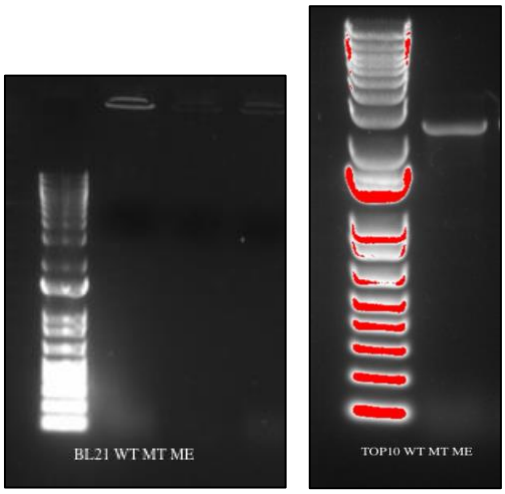
- The assembly results were transformed into BL21 (DE3) cells and plated.
- It was noted that no colonies grew
- Already functioning plasmids were transformed into our competent BL21(DE3) cells as positive control to see if our cells were competent, while another assembly was done with 60 cycles and transformed into both competent BL21(DE3) cells and TOP10 cells.
    - The positive control plasmids plate showed no growth
    - The Level 1 assembly in BL21(DE3) cells showed no growth
    - The Level 1 assembly in TOP10 cells showed growth, a colony was picked for colony PCR



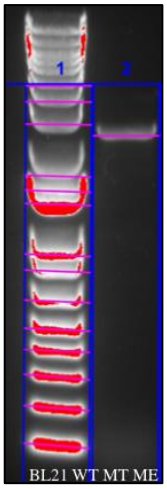
- It was inferred, that BL21(DE3) cells were the cause of the unsuccessful transformation, and another competent batch should be made while TOP10 cells were taking up the plasmid which was of the correct molecular weight.

4th Week of August 2022 (started from 22/08/2022)

- A new batch of chemically competent BL21(DE3) cells were prepared with a freshly sourced strain and frozen.
- A trial of the same Level 1 construct performed previously was done with 60 cycles.
- The assemblies were transformed into both TOP10 cells and BL21(DE3) cells.
    - The transformed BL21(DE3) cells were plated on Kanamycin containing plates while, TOP10 cells where plated on plates prepared for blue/white screening.
    - A single white colony from TOP10 cells was picked for colony PCR and it showed a band of the correct molecular weight.
    - BL21(DE3) cells showed growth but upon colony PCR showed no insert.



- **The colony confirmed via PCR in TOP10 cells was cultured, miniprepped and transformed into BL21(DE3) cells**



- Following this, all Level 1 assemblies were first transformed in TOP10 cells, confirmed, cultured, and re-transformed into BL21(DE3) cells. This means that mutants won't be screened instead a random MT expressing mutant will picked for the negative selection.

4th Week of August + 1st Week of September 2022 (started from 29/08/2022)

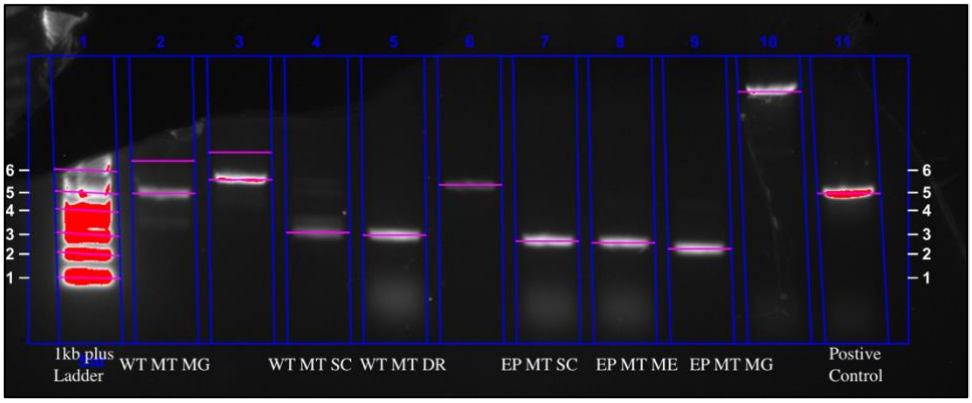
- Error prone PCR on the undomestic C-part g-blocks of the following MTs was carried out:

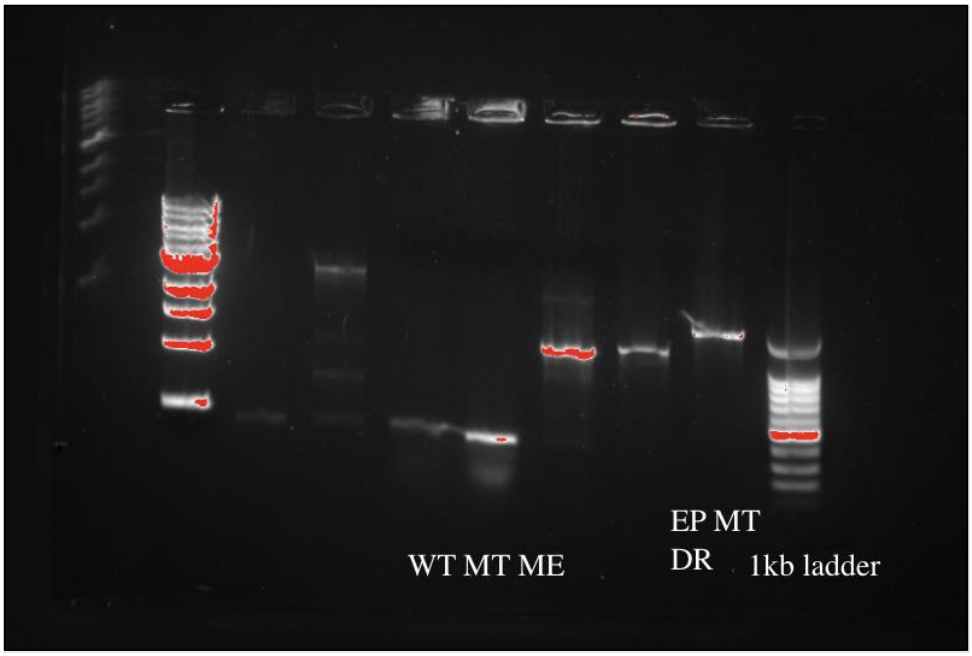
MTs:	Primers to use	Annealing temp (°C)	Total reaction (µL)	pol buffer (µL)	dNTP mix (µL)	MgCl2 (µL)	forward primer (µL)	reverse primer (µL)	Taq pol (µL)	DNA template (µL)	water (µL)
ME MT	ME 72C	72	50	10	5	1.475	3.610108303	6.289308176	0.5	1.650165017	21.4754185
MG MT	PS1/PS2	72	50	10	5	1.475	1	1	0.5	3.943217666	27.08178233
DR MT	PS1/PS2	72	50	10	5	1.475	1	1	0.5	5.263157895	25.76184211
SC MT	PS1/PS2	72	50	10	5	1.475	1	1	0.5	4.456327986	26.56867201

- The PCR products were used as C-parts for Level 1 assemblies.
- All Level 1 Assemblies (WT and EP mutated) were conducted:

P	P (µL)	R	R (µL)	N	N (µL)	O	O (µL)	C	C (µL)	T	T (µL)	acceptor	acceptor (µL)	water (µL)
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.230120625	ME MT	1.086853429	L2U2H09	0.319061728	pJUMP29LacZ	0.978410359	10.07450902
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.230120625	MG MT	0.337503395	L2U2H09	0.319061728	pJUMP29LacZ	0.978410359	10.82385905
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.230120625	SC MT	0.462871599	L2U2H09	0.319061728	pJUMP29LacZ	0.978410359	10.69849085
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.230120625	DR MT	0.443048203	L2U2H09	0.319061728	pJUMP29LacZ	0.978410359	10.71831424
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.264790687	EP ME MT	1.086853429	L2U2H09	0.319061728	pJUMP29LacZ	0.978410359	10.03983896
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.230120625	EP MG MT	0.513742615	L2U2H09	0.487622642	pJUMP29LacZ	0.978410359	10.47905892
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.230120625	EP CS MT	0.456309506	L2U2H09	0.487622642	pJUMP29LacZ	0.978410359	10.53649203
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.230120625	EP SC MT	0.795544948	L2U2H09	0.487622642	pJUMP29LacZ	0.978410359	10.19725659
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.230120625	EP PF MT	0.945313608	L2U2H09	0.487622642	pJUMP29LacZ	0.978410359	10.04748793
J23100	0.997431373	B0034	0.869187311	6His TEV	2.194426158	SUMO	0.230120625	EP DR MT	0.651253663	L2U2H09	0.487622642	pJUMP29LacZ	1.0	10.34154787

- The assemblies were transformed into TOP10 cells and plated for blue/white screening.
- The white colonies for all constructs were confirmed with colony PCR:



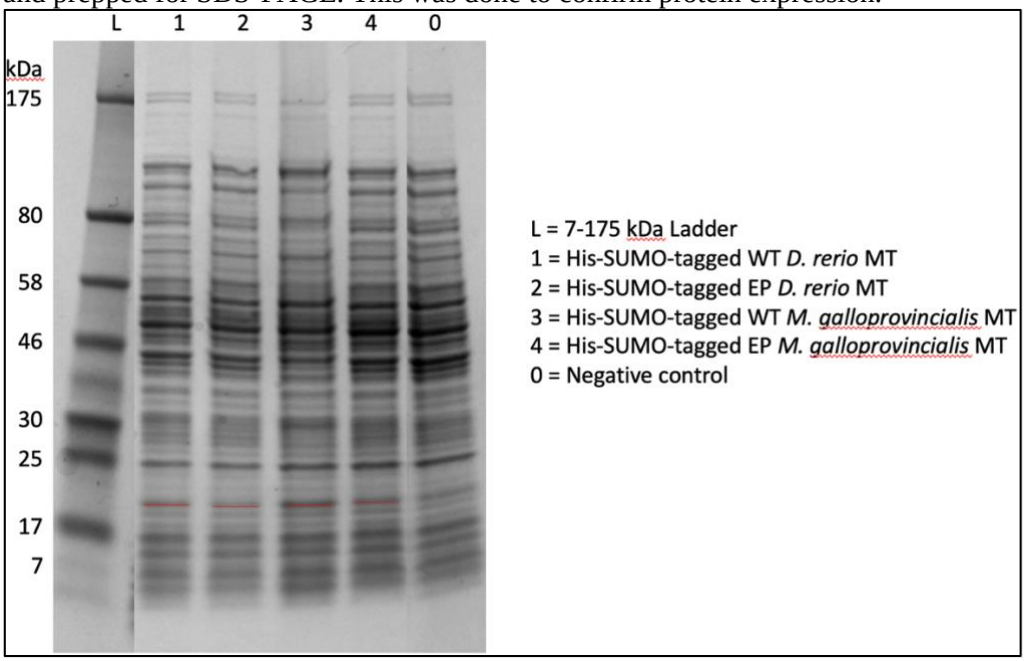


2nd Week of September 2022 (started from 05/09/2022)

- All confirmed assemblies were cultured from TOP10 cells, miniprepped and transformed into BL21(DE3) cells.
- These cultures were plated onto the $AgNO_3$ plates made in the first week of August and incubated for 50 hours, with the following results:

MT constructs:	16 mg/L AgNO3	17 mg/L AgNO3	18 mg/L AgNO3	19 mg/L AgNO3	20 mg/L AgNO3	22 mg/L AgNO3	24 mg/L AgNO3	26 mg/L AgNO3	28 mg/L AgNO3	30 mg/L AgNO3
Negative Control BL21 DE3	Lawn	Lawn	0	1	1	0	0	0	0	0
WT MT ME	Lawn	Lawn	2	0	5	0	0	0	0	0
WT MT MG	Lawn	1	Lawn	1	Lawn	0	0	1	0	2
WT MT DR	Lawn	Lawn	3	Lawn	Lawn	0	0	1	0	0
WT MT SC	Lawn	0	1	Lawn	2	0	0	0	0	0
EP MT ME	Lawn	Lawn	0	0	1	3	0	0	0	0
EP MT MG	Lawn	Lawn	2	1	0	3	0	0	0	0
EP MT DR	Lawn	Lawn	0	3	0	0	0	1	0	0
EP MT SC	Lawn	Lawn	2	0	1	3	0	0	0	0

- A colony from 2 species of MTs (WT and EP mutant) were cultured, lysed via sonication and prepped for SDS-PAGE. This was done to confirm protein expression.



  - The results show bands at the current molecular weight suggesting that the BL21(DE3) cells were expressing their respective MTs.