mRNA capture Lab Book

DIENSTAG, 2.6.2026

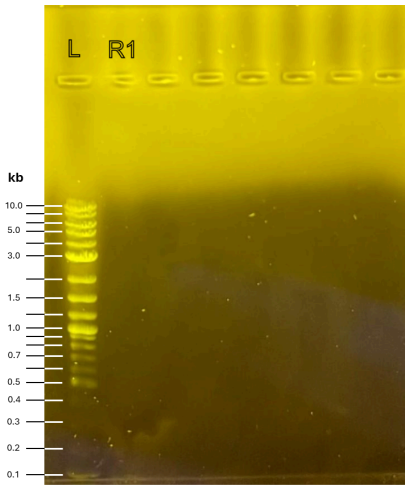
PCR of PAB1 Sequence with truncated Primers (67, 68)

- [ ] PCR of PABP (PAB1) sequence according to Q5 PCR Marina Schreidl Alex John Thottumkal Charlotte Lenné

R1: 67_0.3PAB1_RRM1-2_fw, 68_0.3PAB1_RRM1-2_rv

Tm: 62 °C

PAB1_Q5PCR_1



FREITAG, 5.6.2026

- [ ] Rerun the PAB1 PCR (under the identical conditions) according to Q5 PCR Ziyue Zhou

(R1) Reaction1

primer:

67_0.3PAB1_RRM1-2_fw

68_0.3PAB1_RRM1-2_rv

template PAB1 cds

Tm 62°C

35 cycles

expected length: **548 bp**

(R2) Reaction2

primer:

67_0.3PAB1_RRM1-2_fw

68_0.3PAB1_RRM1-2_rv

template PAB1 cds

Tm 62°C

35 cycles

expected length: **548 bp**

(R3) Reaction3

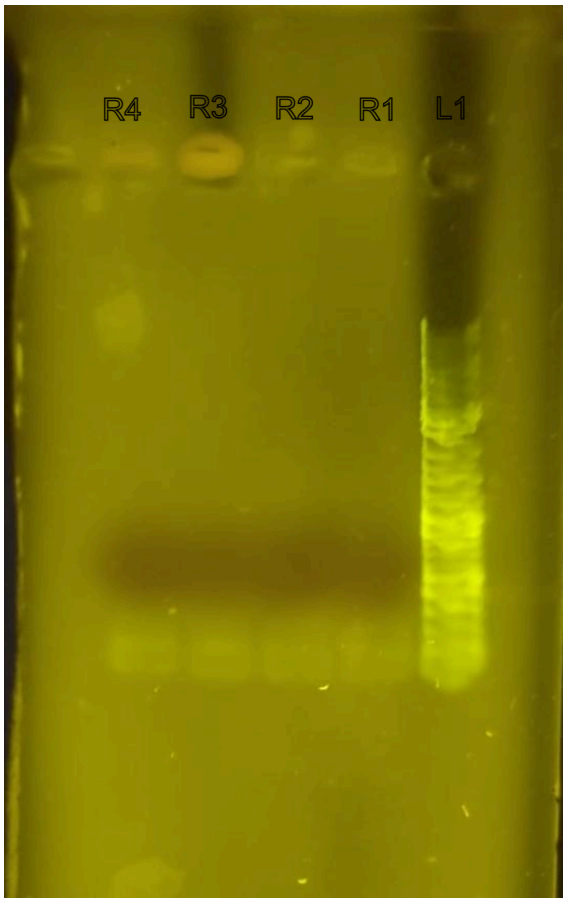
primer:

67_0.3PAB1_RRM1-2_fw
68_0.3PAB1_RRM1-2_rv
template PAB1 cds
Tm 62°C
35 cycles
expected length: **548 bp**

(R4) Reaction4
primer:
67_0.3PAB1_RRM1-2_fw
68_0.3PAB1_RRM1-2_rv
template PAB1 cds
Tm 62°C
35 cycles
expected length: **548 bp**

No band was found.

PAB1_Q5PCR_2_02.06.2026



Golden Gate - Insert into Level 0 Vector

DIENSTAG, 7.7.2026

- [x] Resuspension of the twist sequences Ziyue Zhou

Stock preparation of level 0 modules: 0.1 INT, 0.3 PABP full/PABP RRM 1-4/PABP RRM 1-2/MVP

	A	B	C
1	Stock concentration ng/ul		
2	10	0.1 INT	0.3 PABP RRM 1-2
3	20	0.3 PABP RRM 1-4	0.3 PABP full
4	30	0.3 MVP	

- [x] Adjust the concentration to 15 nM Ziyue Zhou of:
  - 0.1_c_056 INT
  - 0.3_c_057 MVP
  - 0.3_c_058 PABP (full)
  - 0.3_c_059 PABP (RRM1-4)
  - 0.3_c_060 PABP (RRM1-2)

DONNERSTAG, 9.7.2026

- [x] Golden Gate according to Golden Gate Assembly Ziyue Zhou Alex John Thottumkal of:
  - 0.1_c_056 INT
  - 0.3_c_057 MVP
  - 0.3_c_058 PABP (full)
  - 0.3_c_059 PABP (RRM1-4)
  - 0.3_c_060 PABP (RRM1-2)

Master Mix Preparation Golden Gate Assembly. Level 0 (see modules above)

	A	B	C	D	E	F	G
1		reaction oligo	reaction PCR amplified or ordered (15nM stock)	MM 5.2 ordered PCR reactions			
2	DNA Insert	2.5	1				
3	Backbone (1 µl of 15nM stock)	1	1	5.2			
4	10x T4 Ligase Buffer	1	1	5.2			
5	T4 Ligase	0.5	0.5	2.6			
6	BbsI	0.5	0.1	0.52			
7	H2O	4.5	6.4	33.28			
8				add 9 µl each			

FREITAG, 10.7.2026

- [x] Trafo according to E. coli Transformation Ziyue Zhou (but don't incubate at 37 °C like it says in the protocol. instead put it in a dark place like a cupboard since the next day is a Saturday) of:
  - 0.1_c_056 INT
  - 0.3_c_057 MVP

0.3_c_058 PABP (full)
0.3_c_059 PABP (RRM1-4)
0.3_c_060 PABP (RRM1-2)

MONTAG, 13.7.2026

- [x] Incubate the transformant at 37 °C of:
  - 0.1_c_056 INT
  - 0.3_c_057 MVP
  - 0.3_c_058 PABP (full)
  - 0.3_c_059 PABP (RRM1-4)
  - 0.3_c_060 PABP (RRM1-2)

DIENSTAG, 14.7.2026

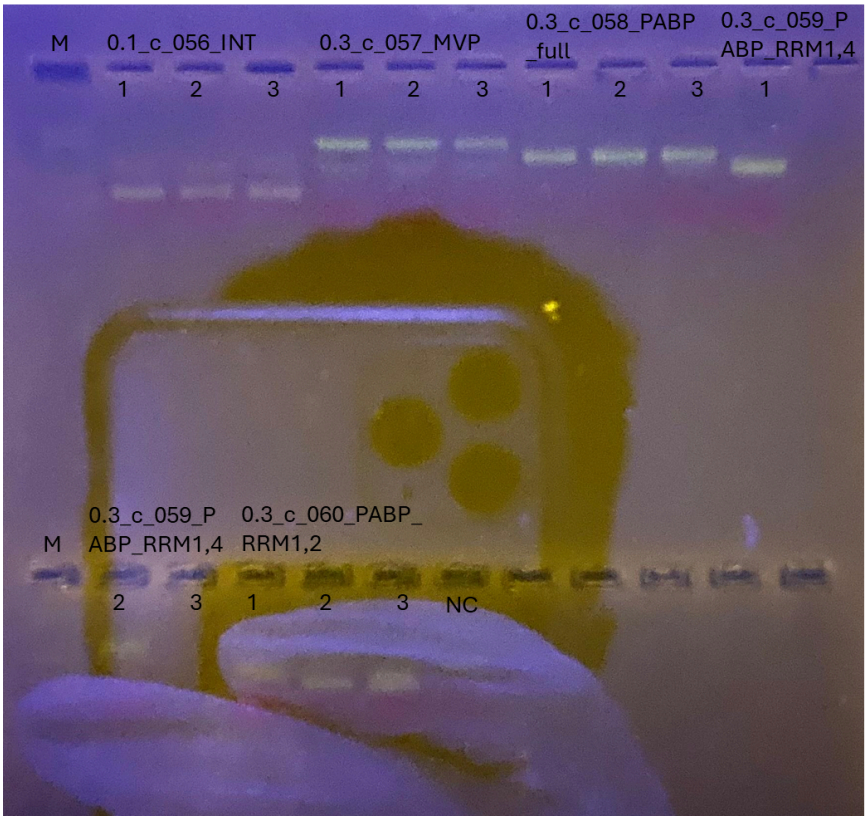
- [x] cPCR according to Taq colony PCR Alex John Thottumkal Ziyue Zhou of:
  - 0.1_c_056 INT
  - 0.3_c_057 MVP
  - 0.3_c_058 PABP (full)
  - 0.3_c_059 PABP (RRM1-4)
  - 0.3_c_060 PABP (RRM1-2)
  - 65 and 66 primer are for taq
  - primer don't have to be thawed on ice, the taq MM SHOULD!!

Mastermix Preparation Taq cPCR. Level 0 (see modules above)

	A	B	C
1		**25ul Reaction**	17,2 MM
2	2x Taq MM	12.5	215
3	10uM P fw	0.5	8.6
4	10um P rv	0.5	8.6
5	Template	colony	
6	H20	11.5	197.8
7			

- [x] Gel electrophoresis of cPCR products Alex John Thottumkal :

0.1_c_056 INT; 0.3_c_057 MVP; 0.3_c_058 PABP (full); 0.3_c_059 PABP (RRM1-4); 0.3_c_060 PABP (RRM1-2)



No ladder bands observed due to unknown reason. The sample band sizes appear approximately correct.

- [x] Set up overnight cultures of Alex John Thottumkal :
  0.1_c_056_INT_1
  0.3_c_057_MVP_1
  0.3_c_058_PABP_full_2
  0.3_c_059_PABP_RRM1-4_1
  0.3_c_060_PABP_RRM1-2_3

MITTWOCH, 15.7.2026

- [x] Miniprep according to instructions of the overnights Alex John Thottumkal :
  0.1_c_056_INT_1
  0.3_c_057_MVP_1
  0.3_c_058_PABP_full_2
  0.3_c_059_PABP_RRM1-4_1
  0.3_c_060_PABP_RRM1-2_3
- [x] Prep for Sequencing Lvl 0 Send to Sequencing (Mycrosynth) Alex John Thottumkal :
  12µl of plasmid eluated containing 40-100 ng/µl
  3 µl of 20uM 69_level0_seq_fw
  Economy run in 1.5 Eppis

Sequencing 15.07. Level 0

	A	B	C	D	E	F	G	H	I
1		seq number	construct	ng/µL	A260/A280	A260/A230	plasmid ul	plasmid amount	Water amount
2	1	98902325	0.1_c_056_INT	331.6	1.88	2.24	2	55.2666666667	10
3	2	98902326	0.3_c_057_MVP	313.9	1.88	2.26	2	52.3166666667	10
4	3	98902327	0.3_c_058_PABP_full	402.4	1.89	2.22	2	67.0666666667	10
5	4	98902328	0.3_c_059_PABP_RRM1,4	166.7	1.85	2.02	4	55.5666666667	8
6	5	98902329	0.3_c_060_PABP_RRM1,2	492.2	1.89	2.29	2	82.0333333333	10

after preparing and sending to sequencing, the plasmid tubes of 0.1_c_056_INT_1 and 0.3_c_057_MVP_1 were accidently diluted with 10 µL miliQ water each
therefore, both were nanodropped again. new concentrations are:

Nanodrop 15.07

	A	B	C	D	E
1	construct	ng/µL	A260/A280	A260/A230	
2	0.1_c_056_INT	215.2	1.90	2.26	
3	0.3_c_057_MVP	195.5	1.89	2.26	

FREITAG, 17.7.2026

Sequencing results were good Alex John Thottumkal

0.1_c_056_INT_1
0.3_c_057_MVP_1
0.3_c_058_PABP_full_2
0.3_c_059_PABP_RRM1-4_1
0.3_c_060_PABP_RRM1-2_3

Only 0.1_c_056_INT_1 and 0.3_c_060_PABP_RRM1-2_3 were fully sequenced, the other sequences were too big but the sequenced parts were good. moving forward with them

MONTAG, 20.7.2026

Overnight cultures Ahmed Haj Kacem

1:1000 SpecR
3 mL Medium, 3 µL of antibiotic

0.1_c_056_INT_1
0.3_c_057_MVP_1
0.3_c_058_PABP_full_2
0.3_c_059_PABP_RRM1-4_1
0.3_c_060_PABP_RRM1-2_3

- [ ] Aliquot working stocks of Ahmed Haj Kacem Alex John Thottumkal :

  0.1_c_056_INT_1
  0.3_c_057_MVP_1
  0.3_c_058_PABP_full_2
  0.3_c_059_PABP_RRM1-4_1
  0.3_c_060_PABP_RRM1-2_3

Calculation Table for 15nM working stocks:

	Fragment	Size [bp]	Mass conc. [ng/µl]	Mol. conc. [nM]	Target Mol. [nM]	Target Vol. [µL]	G - Vol. DNA [µL]	H- Vol. H2O MQ [µL]	I
1	calculation:			(C2*10^-9)/((B2*617.96)+36.04)*10^15			E2/D2*F2	F2-G2	
2	0.1 INT	2568	215.2	135.605411240 7	15	25	2.77	22.23	
3	0.3 MVP	4756	195.5	66.5180065775	15	25	5.64	19.36	
4	0.3 PABP	3806	402.4	171.089031858	15	25	2.19	22.81	
5	0.3 PABP RRM 1-4	3272	166.7	82.4430783215	15	25	4.55	20.45	
6	0.3 PABP RRM 1-2	2684	492.2	296.7490222 174	15	25	1.26	23.74	
7	0.3 Enc_Qt	2924	10	5.5341841352	15	25	67.76	-42.76	
8	1.2 GAL1/10	705	95.6	219.4181178 406	15	25	1.71	23.29	
9	0.1TPQt* plasmid	2112	220	168.5607325 392	15	25	2.22	22.78	
10	0.2 L1 plasmid	2093	257.4	199.0063106 185	15	25	1.88	23.12	
11	0.4 NCSrc plasmid	2114	244.3	187.001951342	15	25	2.01	22.99	
12	0.5 NCSrc plasmid	2118	299.9	229.128011979	15	25	1.64	23.36	
13	Level 0 backbone	2659	327.8	199.4898124 119	15	50	3.76	46.24	
14	level 1bb	2621	285	175.9575113 854	15	25	2.13	22.87	

DIENSTAG, 21.7.2026

- [ ] Glycerol stocks of positive level 0 constructs

  500 µL of overnight culture with 500 µL glycerol in cryo-vial Ahmed Haj Kacem Alex John Thottumkal

  0.1_c_056_INT_1
  0.3_c_057_MVP_1
  0.3_c_058_PABP_full_2
  0.3_c_059_PABP_RRM1-4_1
  0.3_c_060_PABP_RRM1-2_3

Golden Gate - Level 0 into Level 1

- [ ] Golden Gate according to Golden Gate Assembly Alex John Thottumkal

  two Mastermixes:

- MM01: one for Lvl1.1_c_080, Lvl1.1_c_081, Lvl1.1_c_082
- MM02: one for Lvl1.1_c_083, Lvl1.1_c_084, Lvl1.1_c_085

no MM for Lvl1.3_c_086

keep in mind, that 1 µL of each Level 0 plasmid gets added to the MM with all other reactants and then Level 1 backbone get added up to 10 µL

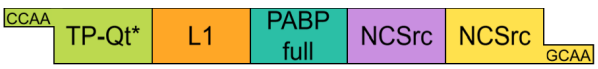
- **e. g. for MM01 10 - (4+1+0.5+0.5) = 4 µL of backbone gets added (NOT 1 µL like it says in the protocol !!)**

Master mix Preparation Golden Gate Assembly. Level 1 (see the following modules)

	A	B	C	D	E
1		reaction PCR amplified or ordered (15nM stock)	4x MM		
2	each Level 0 plasmid 15nM	1	4		
3	10x T4 Ligase Buffer	1	4		
4	T4 Ligase	0.5	2		
5	BsaI (10 U)	0.5	2		
6	Backbone (1 µl of 15nM stock)	Make up to 10 µl with 15nM Backbone			
7					

Construct Lvl1.1_c_01 / Lvl1.1_c_080

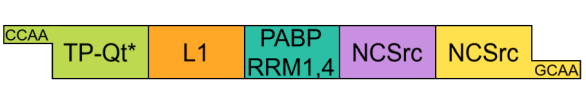
Construct Lvl1.1_c_01



Lvl0.1_a_008 Tp-Qt
Lvl0.2_a_010 L1
Lvl0.3_c_058 PABP full
Lvl0.4_a_021 NCSrc
Lvl0.5rc_a_023 NCSrc

Construct Lvl1.1_c_02 / Lvl1.1_c_081

Construct Lvl1.1_c_02

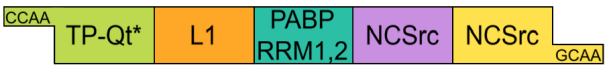


Lvl0.1_a_008 Tp-Qt
Lvl0.2_a_010 L1
Lvl0.3_c_059 PABP RRM 1,4
Lvl0.4_a_021 NCSrc

Lvl0.5rc_a_023 NCSrc

Construct Lvl1.1_c_03 / Lvl1.1_c_082

image.png



Lvl0.1_a_008 Tp-Qt
Lvl0.2_a_010 L1
Lvl0.3_c_060 PABP RRM 1,2
Lvl0.4_a_021 NCSrc
Lvl0.5rc_a_023 NCSrc

Construct Lvl1.1_c_04 / Lvl1.1_c_083

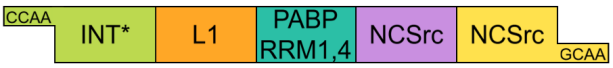
Construct Lvl1.1_c_04



Lvl0.1_a_056 INT
Lvl0.2_a_010 L1
Lvl0.3_c_058 PABP full
Lvl0.4_a_021 NCSrc
Lvl0.5rc_a_023 NCSrc

Construct Lvl1.1_c_05 / Lvl1.1_c_084

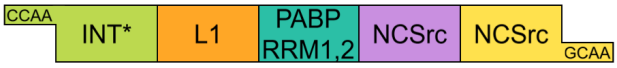
Construct Lvl1.1_c_05



Lvl0.1_a_056 INT
Lvl0.2_a_010 L1
Lvl0.3_c_059 PABP RRM 1,4
Lvl0.4_a_021 NCSrc
Lvl0.5rc_a_023 NCSrc

Construct Lvl1.1_c_06 / Lvl1.1_c_085

Construct Lvl1.1_c_06



Lvl0.1_a_056 INT
Lvl0.2_a_010 L1

Lvl0.3_c_060 PABP RRM 1,2
Lvl0.4_a_021 NCSrc
Lvl0.5rc_a_023 NCSrc

Golden Gate Lv 1.3 constructs

Golden Gate according to Golden Gate Assembly Alex John Thottumkal

Construct Lvl1.3_c_07 / Lvl1.3_c_086

Construct Lvl1.3_c_08



Lvl0.1_a_006 NCS
Lvl0.2_a_011 NCS
Lvl0.3_c_057 MVP
Lvl0.4_a_022 NCS*
Lvl0.5_a_030 NCS

DONNERSTAG, 23.7.2026

cPCR of Level 1.1 and 1.3 constructs

cPCR according to Taq colony PCR Alex John Thottumkal

Lvl1.1_c_080
Lvl1.1_c_081
Lvl1.1_c_082
Lvl1.1_c_083
Lvl1.1_c_084
Lvl1.1_c_085
Lvl1.3_c_086

- for level1: oSN_17 and oSN_18 primer are for taq, in the pirmer marina box
- primer don't have to be thawed on ice, the taq MM SHOULD!!
- 5 colonies per construct were picked ´, only 2 colonies of Lvl1.1_c_083

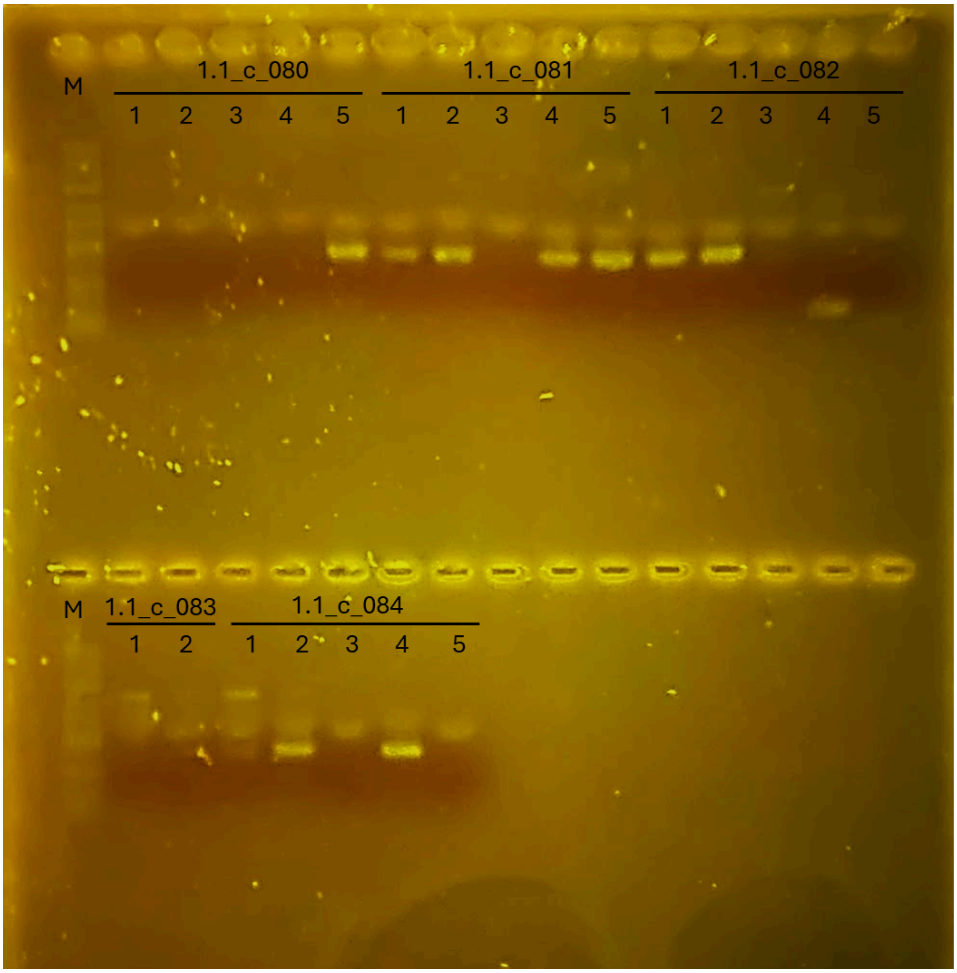
Calculations for Level 1 Taq cPCR

	A	B	C	D
1		50ul Reaction	**25ul Reaction**	34x MM
2	2x Taq MM	25ul	12.5	425
3	10uM P fw	1ul	0.5	17
4	10um P rv	1ul	0.5	17
5	Template	colony	colony	colony
6	H20	to 50ul	11.5	391
7				

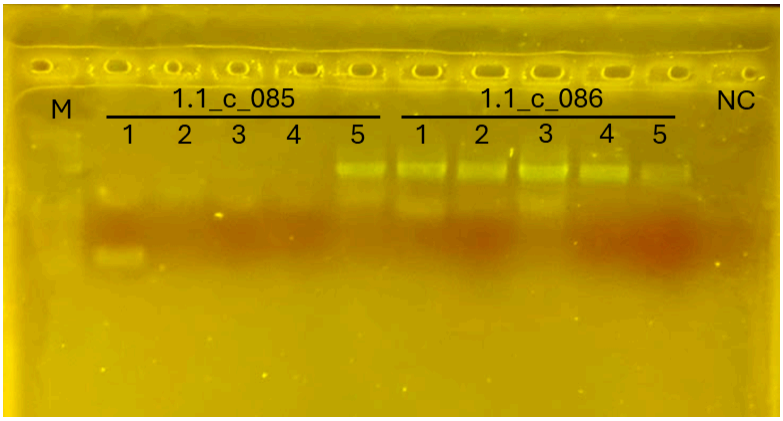
gel electrophoresis of cPCR of Alex John Thottumkal :

Lvl1.1_c_080
Lvl1.1_c_081
Lvl1.1_c_082
Lvl1.1_c_083
Lvl1.1_c_084
Lvl1.1_c_085
Lvl1.3_c_086

Lvl1.1_c_080; Lvl1.1_c_081; Lvl1.1_c_082; Lvl1.1_c_083; Lvl1.1_c_084



Lvl1.1_c_085; Lvl1.3_c_086



- 1 kb plus ladder was used, next time only 1 kb ladder
- stain was forgotten to be added in the agarose-TAE Mix, therefore Post-staining was done:
  - 400 mL TAE
  - 24 µLstain
  - but gel after electophoresis is done in the TAE-stain mixtue for 1 h, slightly stir the gel every 15 min to guarantee sufficent diffusion but only every 15 min to not interrupt the diffusion
  - ROTI Gel stain is NOT suited for post-staining according to manufacture's notice.

- [x] set up overnight cultures of:
  Lvl1.1_c_080_5
  Lvl1.1_c_081_4
  Lvl1.1_c_082_2
  Lvl1.1_c_083 no colony was picked
  Lvl1.1_c_084_4
  Lvl1.1_c_085 no colony was picked
  Lvl1.3_c_086_3

FREITAG, 24.7.2026

Nanodrop of Level 1 constructs from 23.7.2026

Viktor Lukanow

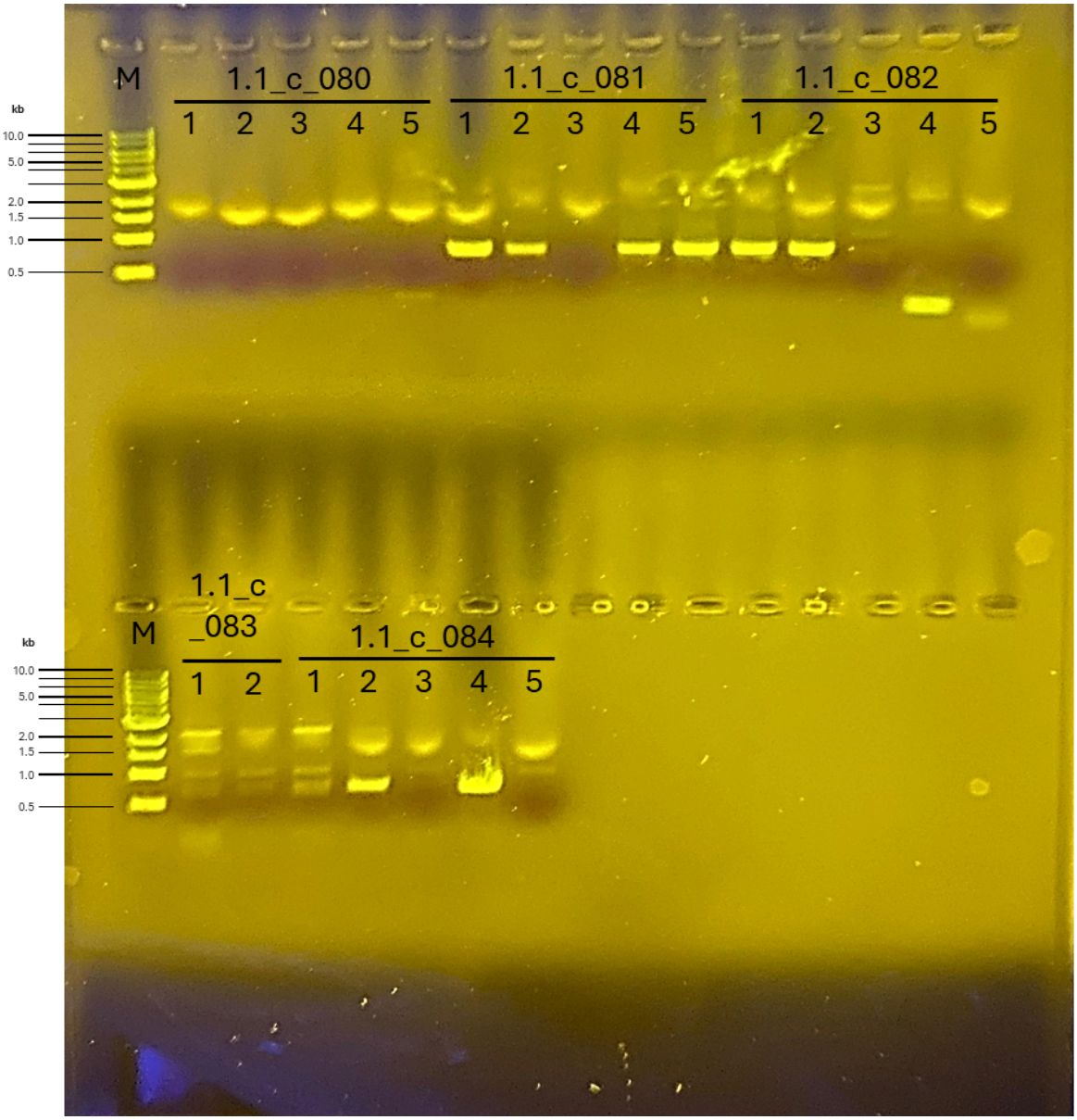
Nanodrop 24.07

	A	B	C	D	E
1	construct	ng/µL	A260/A280	A260/A230	
2	1.1_c_084	56.6	1.90	1.39	
3	1.1_c_082	89.5	1.74	0.94	
4	1.1_c_081	64.2	1.89	2.17	
5	1.1_c_080	45.8	1.91	1.42	
6	1.3_c_086	22.2	1.81	2.15	

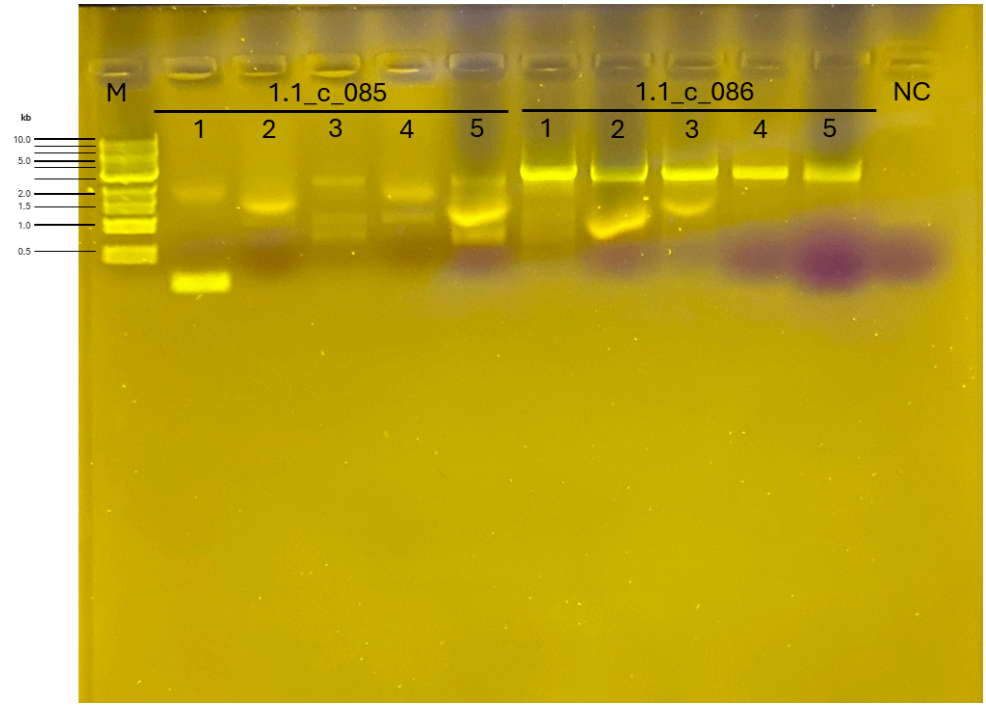
Gel electrophoresis of Level 1 constructs

Gel from 22.7.2026 was done again, 1kb ladder was used Alex John Thottumkal

Lvl1.1_c_080; Lvl1.1_c_081; Lvl1.1_c_082; Lvl1.1_c_083; Lvl1.1_c_084



Lvl1.1_c_085; Lvl1.3_c_086



Expected nt length of the PCR products of the Level 1 Inserts (of the Golden Gate from 22.7.2026)
length starting from the primer oSN17 until oSN18 (and in between both primers the five level0 inserts)

- Lvl1.1_c_080 - 2108 bp
- Lvl1.1_c_081 - 1574 bp
- Lvl1.1_c_082 - 986 bp
- Lvl1.1_c_083 - 2564 bp
- Lvl1.1_c_084 - 2030 bp
- Lvl1.1_c_085 - 1442 bp
- Lvl1.3_c_086 - 3086 bp

MONTAG, 27.7.2026

- [ ] Golden Gate again according to Golden Gate Assembly of: Ahmed Haj Kacem
  - Lvl1.1_c_080
  - Lvl1.1_c_081
  - Lvl1.1_c_082
  - Lvl1.1_c_083

DIENSTAG, 28.7.2026

- [ ] Trafo of Golden Gate according to E. coli Transformation Ahmed Haj Kacem
  - Lvl1.1_c_080 27.7.
  - Lvl1.1_c_081 27.7.
  - Lvl1.1_c_082 27.7.
  - Lvl1.1_c_083 27.7.
- [ ] Golden Gate according to Golden Gate Assembly Ziyue Zhou
  - Lvl1.1_c_084 28.7.

Lvl1.1_c_085 28.7.

Golden Gate Lv1 Mix Preparation1

	A	B	C	D	E
1		reaction PCR amplified or ordered (15nM stock)	4x MM	2x MM	
2	each Level 0 plasmid 15nM	1	4	8	
3	10x T4 Ligase Buffer	1	4	2	
4	T4 Ligase	0.5	2	1	
5	BsaI (10 U)	0.5	2	1	
6	Backbone (1 µl of 15nM stock)	Make up to 10 µl with 15nM Backbone		6	
7	PABP			2	

MITTWOCH, 29.7.2026

- [x] cPCR of Golden Gate reactions from 27.7.2026 according to Taq colony PCR Alex John Thottumkal

  Lvl1.1_c_080 27.7.
  Lvl1.1_c_081 27.7.
  Lvl1.1_c_082 27.7.
  Lvl1.1_c_083 27.7.
  five colonies were picked per construct (20 reactions + 1 NC)

  Master Mix Preparation Taq cPCR. Level 1.1 (see modules above)

	A	B	C
1		**25ul Reaction**	19.2x MM
2	2x Taq MM	12.5	240
3	10uM P fw	0.5	9.6
4	10um P rv	0.5	9.6
5	Template	colony	
6	H20	11.5	220.8
7			

- [x] gel electrophoresis of cPCR samples from 27.7.2026 Alex John Thottumkal

  Lvl1.1_c_080 27.7.
  Lvl1.1_c_081 27.7.
  Lvl1.1_c_082 27.7.
  Lvl1.1_c_083 27.7.