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 In Project: Journal MoClo  
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## 17.03.2020 – 22.03.2020: MoClo for pAR-BaLac-3xHA-cyt and pAR-marLac-3xHA-cyt (Lv 1 and Lv 2)

### Lv 1: 17.03 – 18.03.:

- see protocol MoClo Lv 1

used parts:

- pICH47742 (destination)	#20	4968 bp
- L0pAR-AA_A1_B2	#96	2899 bp
A)		<ul style="list-style-type: none"> <li>• 4044 bp</li> <li>• 3915 bp</li> </ul>
<ul style="list-style-type: none"> <li>• L0-pUC57-kan-BaLac-up</li> <li>• L0-pUC57-kan-BaLac-down</li> </ul>		
B)		4576 bp
<ul style="list-style-type: none"> <li>• L0-pUC57-kan-marLac</li> </ul>		
- L03xHA B5	#140	2423 bp
- tRPL23: B6-C1	#101	2963 bp

Plasmid-name:

- A) L1-pAR-BaLac-3xHA-cyt  
 B) L1-pAR-marLac-3xHA-cyt

Test-Digest: **HindIII**:

- A) L1-pAR-BaLac-3xHA-cyt 5084 + 3512 bp
- B) L1-pAR-marLac-3xHA-cyt 5084 + 2755 bp

Digest L1



**Lv 2: 21.03. – 22.03.:**

- see protocol MoClo Lv 2

used parts:

- pAGM4673 (destination)                      #20                      4968 bp

Test-Digest: **HindIII**:

- A) L2-pAR-BaLac-3xHA-cyt                      5084 + 3512 bp
- B) L2-pAR-marLac-3xHA-cyt                      5084 + 2755 bp

Digest L2



**25.05.2020 – 10.06.2020 MoClo for pAR-cCA-BaLac-HA-RGS-8His, pAR-cCA-BaLac-SP20-HA-RGS-8His, pAR-cCA-marLac-HA-RGS-8His and pAR-cCA-marLac-SP20-HA-RGS-8His (Lv 1 and Lv 2)**

**Lv 1: 25.05.2020 – 28.05.2020**

- see protocol MoClo Lv 1

used parts:

- pICH47742 (destination)	#20	4968 bp
- L0pAR-AA_A1_B2	#96	2899 bp
- L0-cCA_B2	#157	2314
A)		
• L0-pUC57-kan-BaLac-up		
• L0-pUC57-kan-BaLac-down		• 4044 bp
		• 3915 bp
B)		
• L0-pUC57-kan-marLac		4576 bp
1) L01xHA-RGS-8His2)	SP20-HA-RGS- #140	2423 bp
8His		
- tRPL23: B6-C1	#101	2963 bp

Plasmid-name:

A1) L1-pAR-cCA-BaLac-HA-RGS-8His

A2) L1-pAR-cCA-BaLac-SP20-HA-RGS-8His

B1) L1-pAR-cCA-marLac-HA-RGS-8His

B2) L1-pAR-cCA-marLac-SP20-HA-RGS-8His

Test-Digest: **NdeI** and **BamHI**:

- A1) L1-pAR-cCA-BaLac-HA-RGS-8His 4086 bp + 2582 bp + 1961 bp
- A2) L1-pAR-cCA-BaLac-SP20-HA-RGS-8His 4086 bp + 2708 bp + 1961 bp

Test-Digest: **HindIII**:

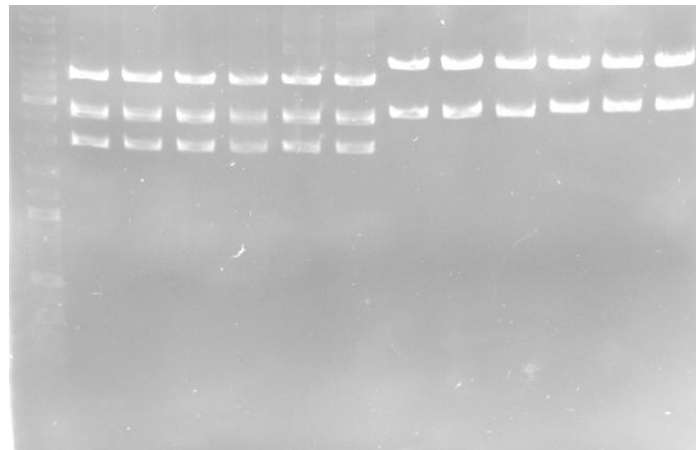
- B1) L1-pAR-cCA-marLac-HA-RGS-8His 5084 bp + 2784 bp
- B2) L1-pAR-cCA-marLac-SP20-HA-RGS-8His 5084 bp + 2910 bp

**DNA concentrations:**

Plasmid	Concentration [ng/μL]
• A1,	
• 1	
• 2	• 745
• 3	• 590
	• 590
• A2,	
• 1	
• 2	• 640
• 3	• 665
	• 580
• B1,	
• 1	
• 2	• 715
• 3	• 600
	• 490
• B2,	
• 1	
• 2	• 800
• 3	• 790
	• 735

Scheme: Marker, A1 (1 – 3), A2 (1 – 3), B1 (1 – 3), B2 (1 – 3)

Digest Lv1



**Lv 2: 29.05.2020 – 03.06.2020**

- see protocol MoClo Lv 2

used parts:

- pAGM4673 (destination)

#20

4968 bp

Test-Digest: **EcoRV** and **Nde1**:

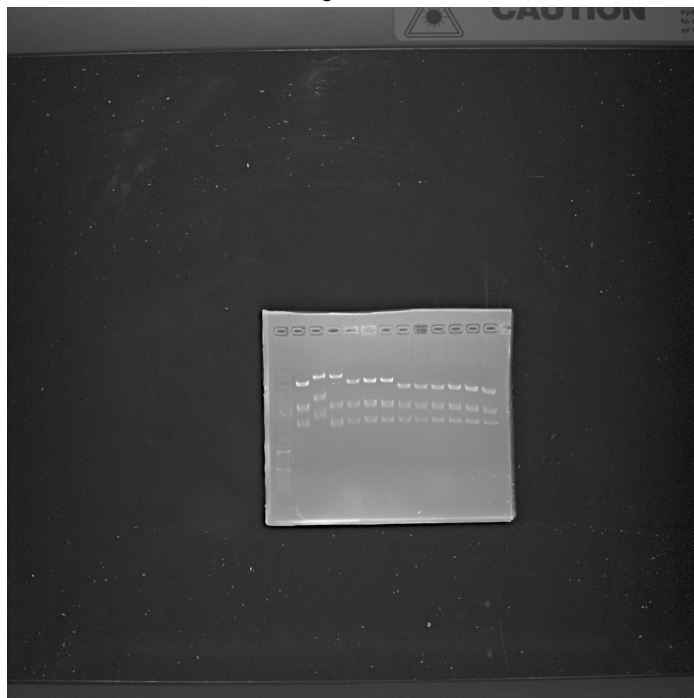
- A1) L2-pAR-cCA-BaLac-HA-RGS-8His 5787 bp + 3079 bp + 2110 bp
- A2) L2-pAR-cCA-BaLac-SP20-HA-RGS-8His 5913 bp + 3079 bp + 2110 bp
- B1) L2-pAR-cCA-marLac-HA-RGS-8His 5029 bp + 3079 bp + 2110 bp
- B2) L2-pAR-cCA-marLac-SP20-HA-RGS-8His 5152 bp + 3079 bp + 2110 bp

## DNA concentrations:

Plasmid	Concentration [ng/μL]
• A1,	
• 1	
• 2	• 444
• 3	• 169
	• 438
• A2,	
• 1	
• 2	• 465
• 3	• 318
	• 376
• B1,	
• 1	
• 2	• 360
• 3	• 370
	• 373
• B2,	
• 1	
• 2	• 353
• 3	• 329
	• 396

Scheme: Marker, A1 (1 – 3), A2 (1 – 3), B1 (1 – 3), B2 (1 – 3)

Digest Lv2



**Transformation in *C. reinhardtii*: 04.06.2020 – 10.06.2020**

Constructs:

L2-pAR-cCA-BaLac-HA-RGS-8His, L2-pAR-cCA-BaLac-SP20-HA-RGS-8His, L2-pAR-cCA-marLac-HA-RGS-8His and L2-pAR-cCA-marLac-SP20-HA-RGS-8His

See protocol "Transformation Chlamydomonas reinhardtii with NEPAGENE-Electroporation"

Pick random colonies

**05.08.2020 – 26.08.2020 MoClo for pAR-GLE/ARS-BaLac-SP20-HA-RGS-8His and pAR-GLE/ARS-marLac-SP20-HA-RGS-8His (Lv 1 and Lv 2)**

**Lv 1: 05.08.2020 – 12.08.2020**

- see protocol MoClo Lv 1



used parts:

- pICH47742 (destination)	#20	4968 bp
- L0pAR-AA_A1_B2	#96	2899 bp
1) L0-ARS_B2		
2) L0-GLE_B2		
A)		<ul style="list-style-type: none"> <li>• 4044 bp</li> <li>• 3915 bp</li> </ul>
• L0-pUC57-kan-BaLac-up		
• L0-pUC57-kan-BaLac-down		
B)		4576 bp
• L0-pUC57-kan-marLac		
SP20-HA-RGS-8His	#140	2423 bp
- tRPL23: B6-C1	#101	2963 bp

Plasmid-name:

- A1) L1-pAR-ARS-BaLac-SP20-HA-RGS-8His
- A2) L1-pAR-GLE-BaLac-SP20-HA-RGS-8His
- B1) L1-pAR-ARS-marLac-SP20-HA-RGS-8His
- B2) L1-pAR-GLE-marLac-SP20-HA-RGS-8His

Test-Digest: **HindIII**:

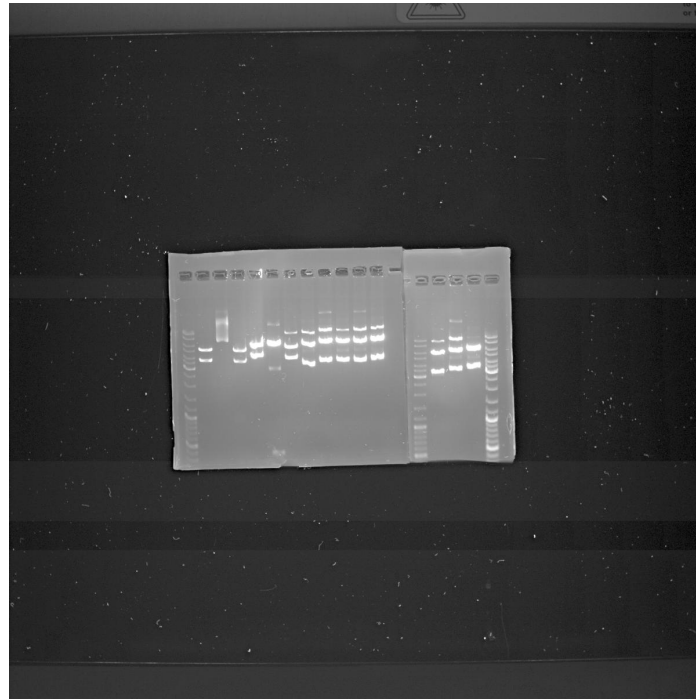
- A1) L1-pAR-ARS-BaLac-SP20-HA-RGS-8His 5084 bp + 3677 bp
- A2) L1-pAR-GLE-BaLac-SP20-HA-RGS-8His 5084 bp + 3689 bp
- B1) L1-pAR-ARS-marLac-SP20-HA-RGS-8His 5084 bp + 2916 bp
- B2) L1-pAR-GLE-marLac-SP20-HA-RGS-8His 5084 bp + 2928 bp

**DNA concentrations:**

Plasmid	Concentration [ng/ $\mu$ L]
• A1,	
• 1	
• 2	• 425
• 3	• 451
	• 391
	• 437
• A2,	
• 1	
• 2	• 82
• 3	• 83
	• 267
	• 374
• B1,	
• 1	
• 2	• 384
• 3	• 366
	• 393
	• 338
• B2,	
• 1	
• 2	• 512
• 3	• 509
	• 487
	• 401

Scheme: Marker, A1 (1 – 4), A2 (1 – 4), B1 (1 – 4), B2 (1 – 4)

Digest Lv1

**Lv 2: 12.08.2020 – 26.08.2020**

- see protocol MoClo Lv 2

used parts:

- pAGM4673 (destination)                      #20                      4968 bp

Test-Digest: **HindIII**:

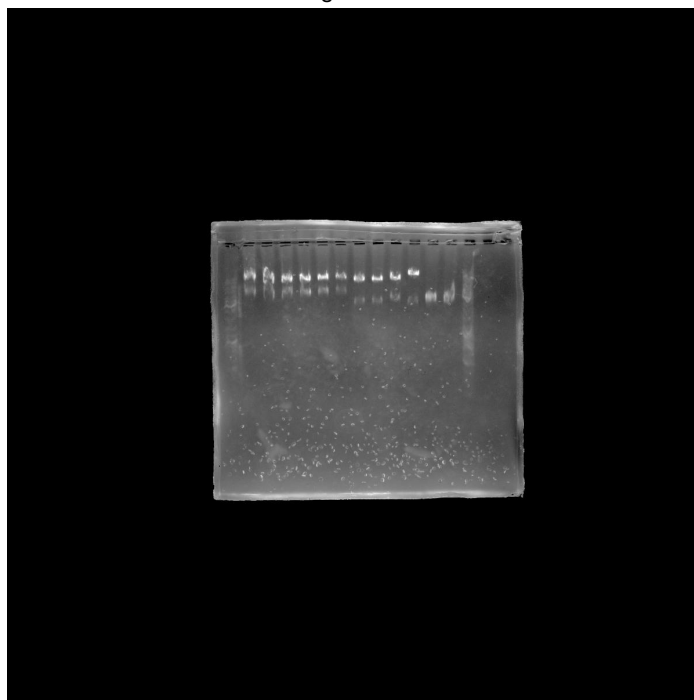
- A1) L2-pAR-ARS-BaLac-SP20-HA-RGS-8His                      5084 bp + 3677 bp
- A2) L2-pAR-GLE-BaLac-SP20-HA-RGS-8His                      5084 bp + 3689 bp
- B1) L2-pAR-ARS-marLac-SP20-HA-RGS-8His                      5084 bp + 2916 bp
- B2) L2-pAR-GLE-marLac-SP20-HA-RGS-8His                      5084 bp + 2928 bp

**DNA concentrations:**

<b>Plasmid</b>	<b>Concentration [ng/<math>\mu</math>L]</b>
• A1,	
• 1	
• 2	• 570
• 3	• 539
	• 562
• A2,	
• 1	
• 2	• 604
• 3	• 598
	• 531
• B1,	
• 1	
• 2	• 531
• 3	• 553
	• 739
• B2,	
• 1	
• 2	• 802
• 3	• 247
	• 217

Scheme: Marker, A1 (1 – 3), A2 (1 – 3), B1 (1 – 3), B2 (1 – 3)

Digest Lv2

**Transformation in *C. reinhardtii*: 08.09.2020**

Constructs:

L2-pAR-GLE-BaLac-SP20-HA-RGS-8His, L2-pAR-ARS-BaLac-SP20-HA-RGS-8His, L2-pAR-GLE-marLac-SP20-HA-RGS-8His and L2-pAR-ARS-marLac-SP20-HA-RGS-8His

See protocol "Transformation Chlamydomonas reinhardtii with NEPAGENE-Electroporation"

Didn't work

**05.08.2020 – 24.09.2020 MoClo for pAR-cCA/GLE/ARS-BaLac-SP20-3xHA and pAR-cCA/GLE/ARS-marLac-SP20-3xHA (Lv 1 and Lv 2)****Lv 1: 05.08.2020 – 12.08.2020**

- see protocol MoClo Lv 1

used parts:

- pICH47742 (destination)	#20	4968 bp
- L0pAR-AA_A1_B2	#96	2899 bp
1) L0-cCA_B2) L0-ARS_B2	#157	2314 bp
3) L0-GLE_B2		
A)		<ul style="list-style-type: none"> <li>• 4044 bp</li> <li>• 3915 bp</li> </ul>
<ul style="list-style-type: none"> <li>• L0-pUC57-kan-BaLac-up</li> <li>• L0-pUC57-kan-BaLac-down</li> </ul>		
B)		4576 bp
<ul style="list-style-type: none"> <li>• L0-pUC57-kan-marLac</li> </ul>		
SP20-3HA	#514	XXX
- tRPL23: B6-C1	#101	2963 bp

#### Plasmid-name:

- A1) L1-pAR-cCA-BaLac-SP20-3HA
- A2) L1-pAR-GLE-BaLac-SP20-3HA
- A3) L1-pAR-ARS-BaLac-SP20-3HA
- B1) L1-pAR-cCA-marLac-SP20-3HA
- B2) L1-pAR-GLE-marLac-SP20-3HA
- B3) L1-pAR-ARS-marLac-SP20-3HA

#### Test-Digest: HindIII:

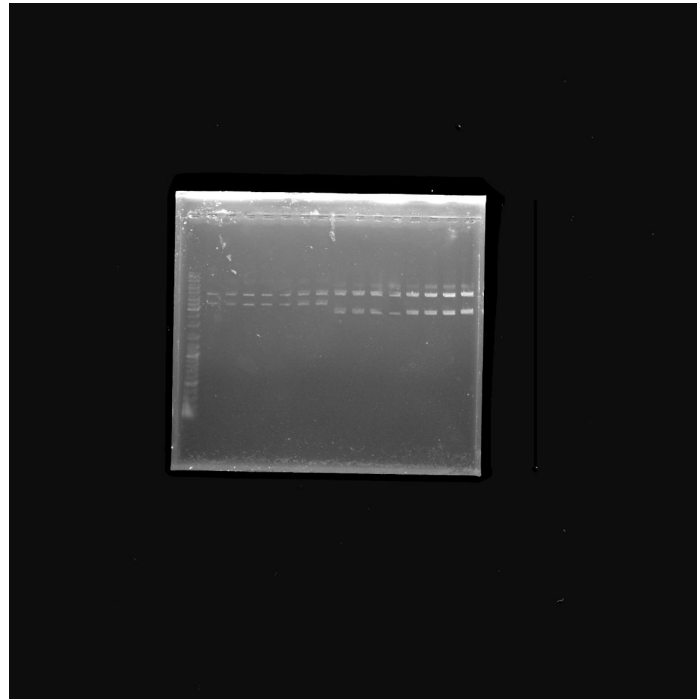
- A1) L1-pAR-cCA-BaLac-SP20-3HA 5084 bp + 3698 bp
- A2) L1-pAR-GLE-BaLac-SP20-3HA 5084 bp + 3716 bp
- A3) L1-pAR-ARS-BaLac-SP20-3HA 5084 bp + 3704 bp
- B1) L1-pAR-cCA-marLac-SP20-3HA 5084 bp + 2937 bp
- B2) L1-pAR-GLE-marLac-SP20-3HA 5084 bp + 2955 bp
- B3) L1-pAR-ARS-marLac-SP20-3HA 5084 bp + 2943 bp

#### DNA concentrations:

Plasmid	Concentration [ng/ $\mu$ L]
• A1,	
• 1	
• 2	• 392
• 3	• 469
	• 405
• A2,	
• 1	
	• 399
• A3,	
• 1	
• 2	• 375
• 3	• 371
	• 400
• B1,	
• 1	
• 2	• 413
• 3	• 335
	• 318
• B2,	
• 1	
• 2	• 362
• 3	• 295
	• 334
• B3,	
• 1	
• 2	• 333
• 3	• 363
	• 383

Scheme: Marker, A1 (1 – 3), A2 (1), A3 (1 – 3), B1 (1 – 3), B2 (1 – 3), B3 (1 – 2)

Digest Lv1

**Lv 2: 08.09.2020 – 24.09.2020**

- see protocol MoClo Lv 2

used parts:

- pAGM4673 (destination)

#20

4968 bp



Test-Digest: **HindIII**:

- A1) L2-pAR-cCA-BaLac-SP20-3HA 5084 bp + 3698 bp
- A2) L2-pAR-GLE-BaLac-SP20-3HA 5084 bp + 3716 bp
- A3) L2-pAR-ARS-BaLac-SP20-3HA 5084 bp + 3704 bp
- B1) L2-pAR-cCA-marLac-SP20-3HA 5084 bp + 2937 bp
- ~~B2) L2-pAR-GLE-marLac-SP20-3HA 5084 bp + 2955 bp~~
- B3) L2-pAR-ARS-marLac-SP20-3HA 5084 bp + 2943 bp

L2-pAR-GLE-marLac-SP20-3HA didn't work.

## DNA concentrations:

Plasmid	Concentration [ng/μL]
• A1,	
• 1	
• 2	• 440
• 3	• 392
	• 389
• A2,	
• 1	
• 2	• 352
• 3	• 396
	• 432
• A3,	
• 1	
• 2	• 323
• 3	• 448
	• 466
• B1,	
• 1	
• 2	• 342
• 3	• 332
	• 380
• B3	
• 1	
• 2	• 375
• 3	• 332
	• 596

Scheme: Marker, A1 (1 – 3), A2 (1 – 3), A3 (1 – 3), B1 (1 – 3), B3 (1 – 3)

### **Transformation in *C. reinhardtii*: 22.09.2020**

Constructs:

L2-pAR-GLE-BaLac-SP20-HA-RGS-8His, L2-pAR-ARS-BaLac-SP20-HA-RGS-8His, L2-pAR-GLE-marLac-SP20-HA-RGS-8His and L2-pAR-ARS-marLac-SP20-HA-RGS-8His

See protocol "Transformation Chlamydomonas reinhardtii with NEPAGENE-Electroporation"

### **Transformation in *C. reinhardtii*: 24.09.2020**

Constructs:

L2-pAR-cCA-BaLac-SP20-3xHA- L2-pAR-cCA-marLac-SP20-3xHA, pAR-GLE-BaLac-SP20-3xHA, pAR-ARS-BaLac-SP20-3xHA and pAR-ARS-marLac-SP20-3xHA

See protocol "Transformation Chlamydomonas reinhardtii with NEPAGENE-Electroporation"

## **24.09.2020: Digestion for L2-pAR-GLE-marLac-SP20-3HA**

Digest Lv2



Digest didn't work.

Author: Allyssa Hinkle  
Entry 2/2: No entry title yet  
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No tags associated

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