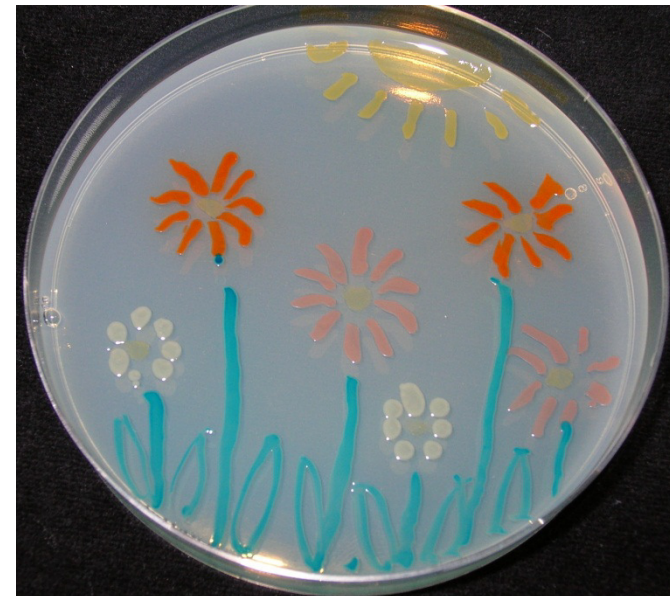


Developing Tools for Synthetic Biology: Golden Gate Cloning and the MoClo System

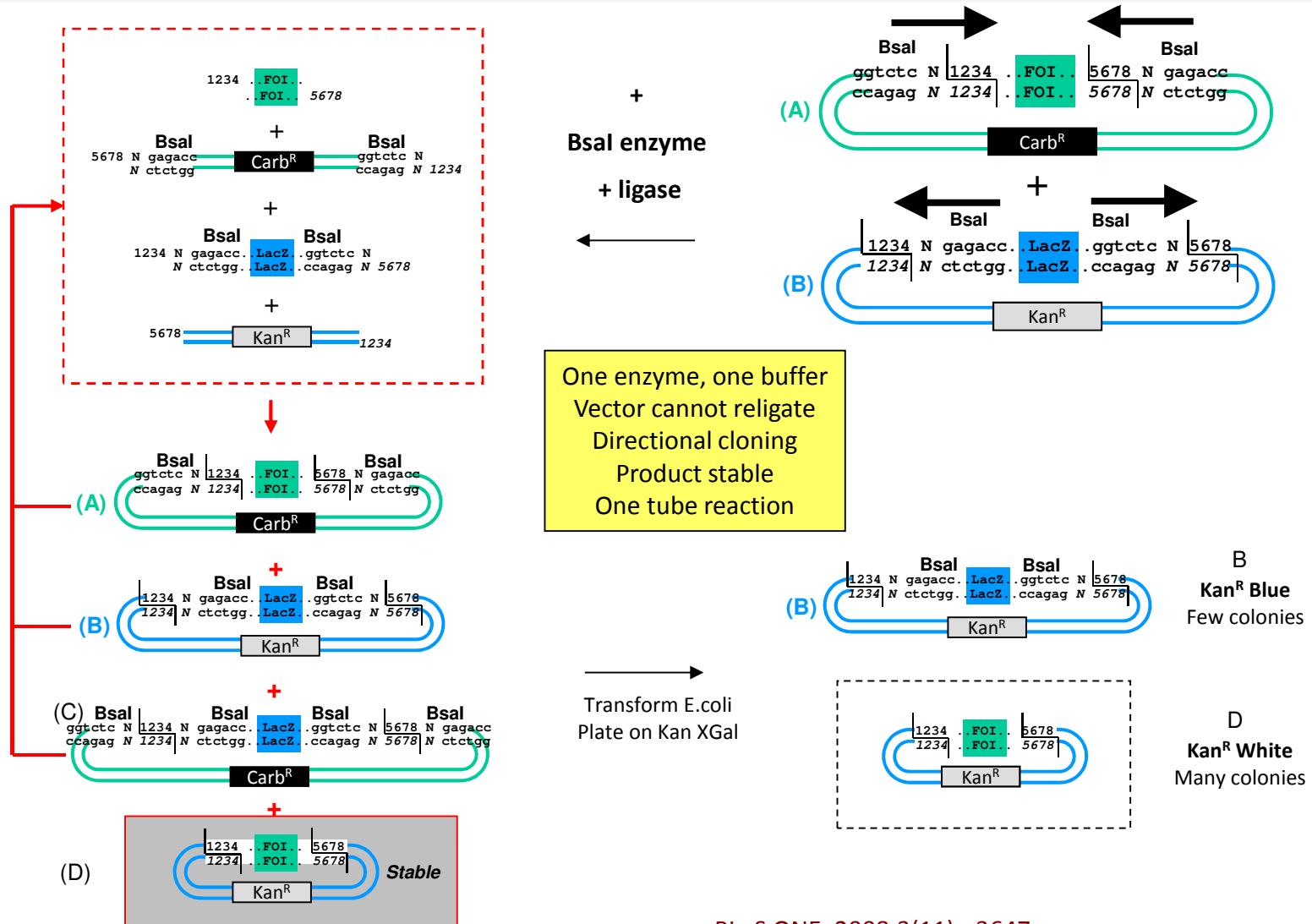
By Sylvestre Marillonnet, IPB Halle

Introduction to Opportunities in Plant Synthetic Biology

Nottingham, May 2013



Golden Gate cloning: use of type IIS enzymes combined with restriction-ligation



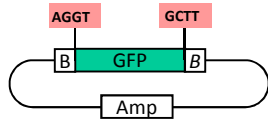
PLoS ONE. 2008;3(11):e3647.

PLoS ONE. 2009;4(5):e5553.



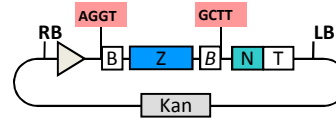
Subcloning a fragment from one entry construct into an expression vector with a one-pot one-step reaction

Entry construct, 50 ng

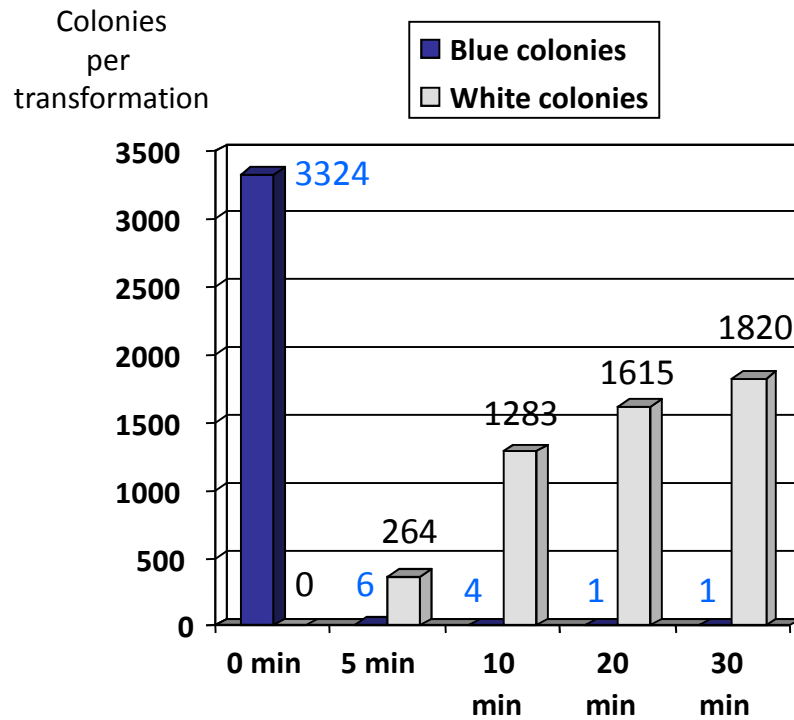
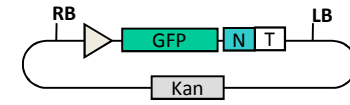


+

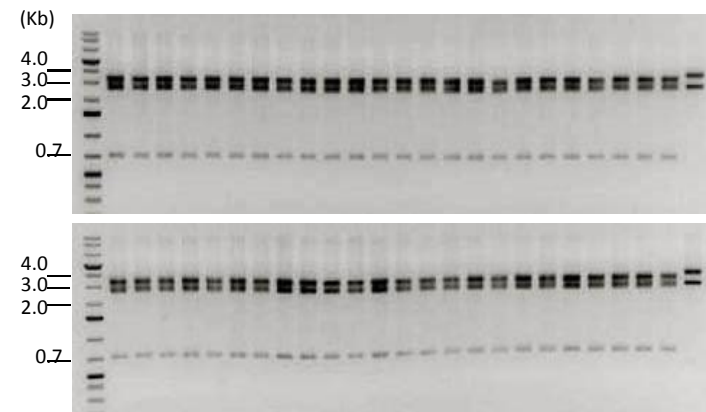
Recipient vector, 50 ng



Final construct

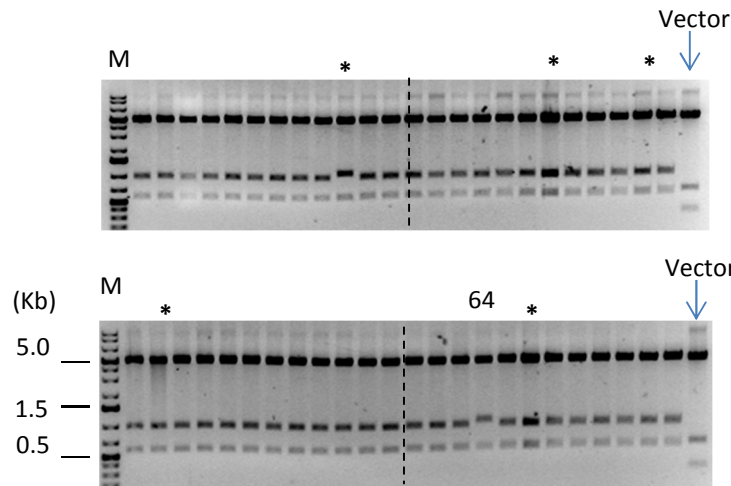
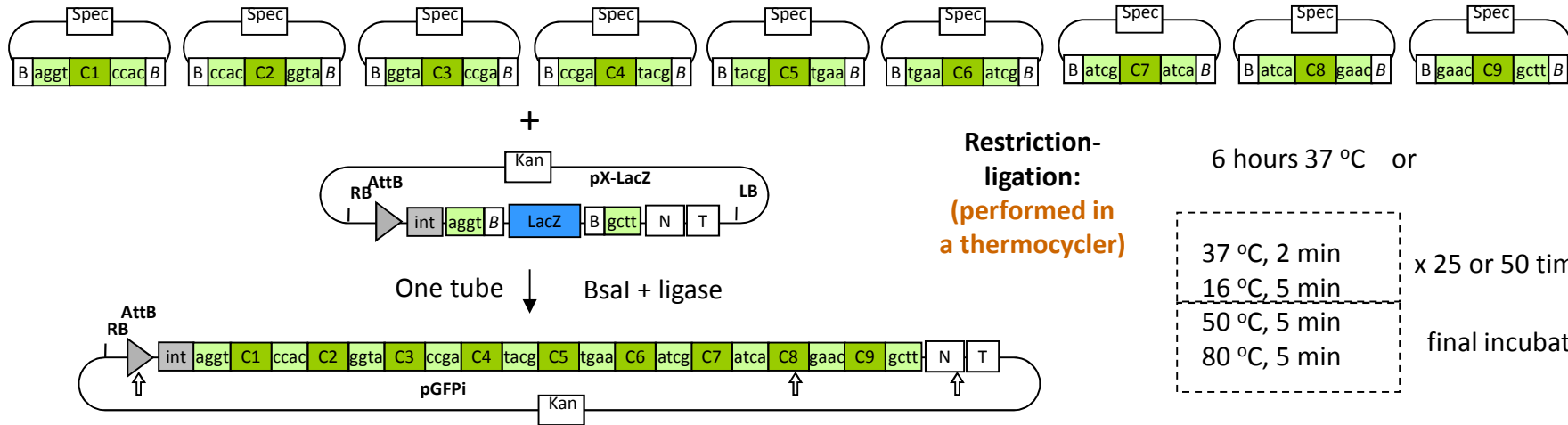


Screening of 48 white colonies
Digested plasmid run an agarose gel



48/48 positive

Assembly of nine fragments into an expression vector



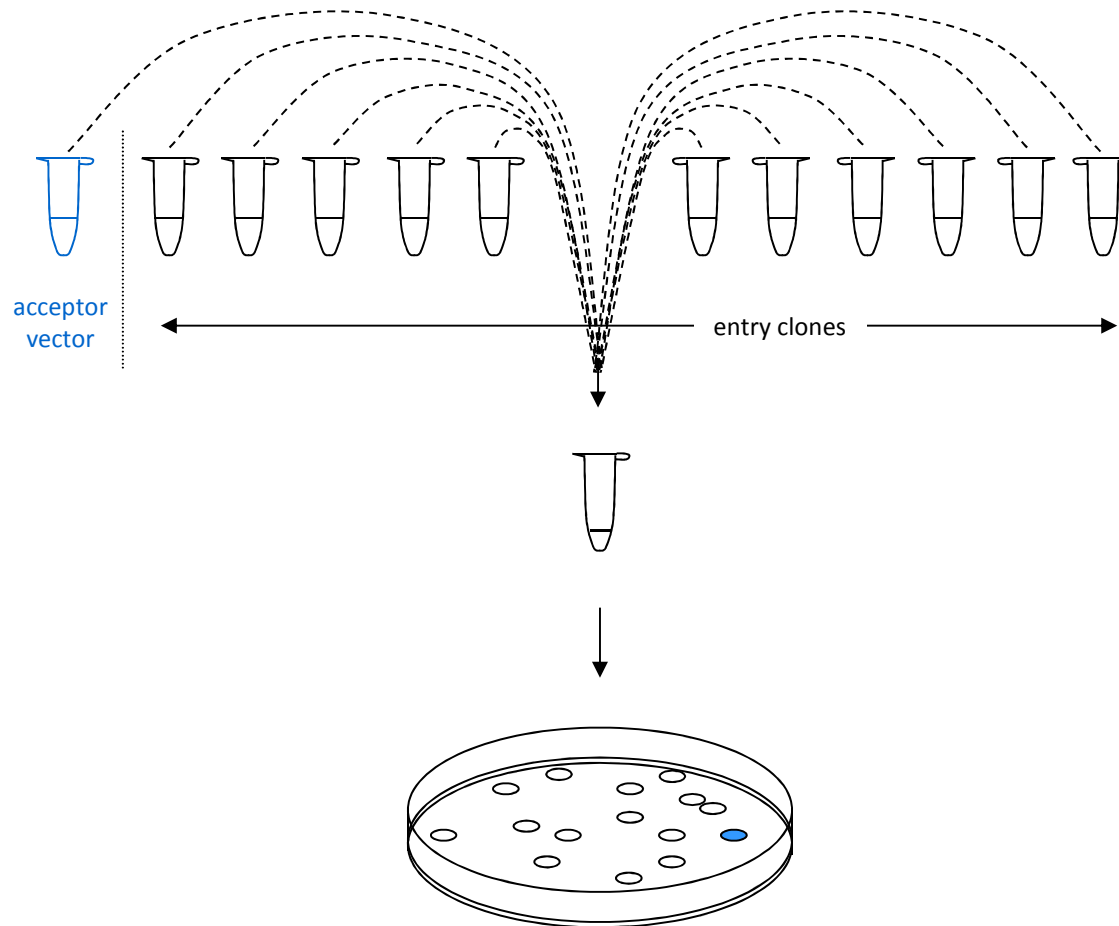
64 / 72
correct

Cloning Efficiency:

6,000-14,000 white colonies
(< 100 blue colonies)
per transformation
(~ 150 ng vector)

with 84 to 100% of
white colonies with
correct restriction
pattern

Golden Gate Cloning Overview



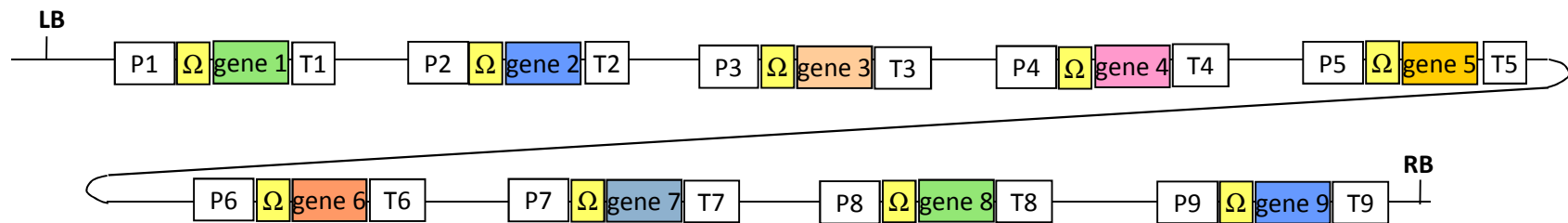
Pipet:
entry clones
recipient vector
buffer
Bsal
ligase

Incubate:
37°C 30 min to 5 hours
50°C 10 min
80°C x 5 min
or other program

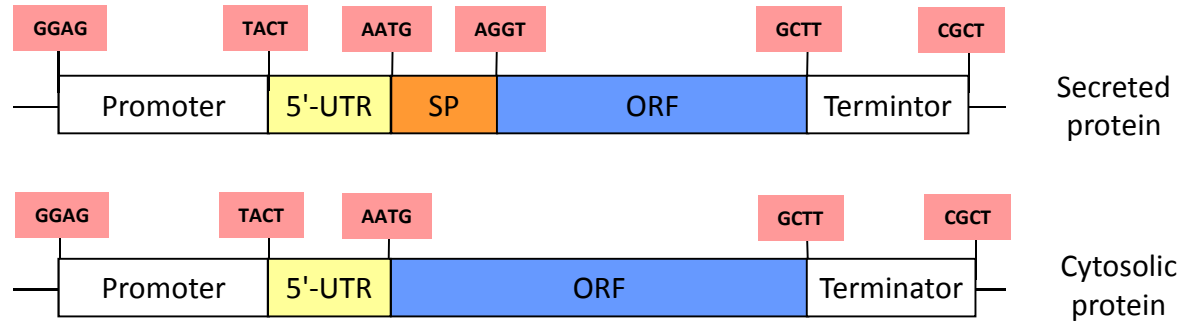
Plate:
on selectable
medium,
pick white colony
analyze by
restriction digest

Assembly of multigene constructs

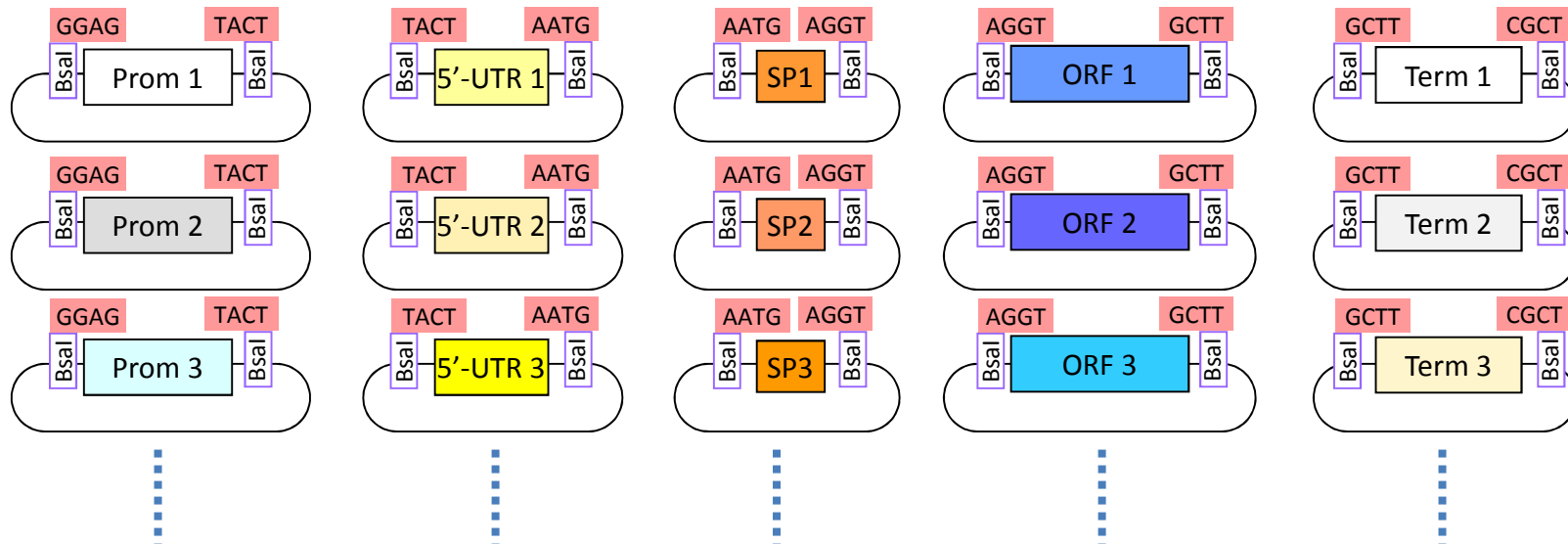
How can we build a standardized cloning system for assembly of any construct of choice?



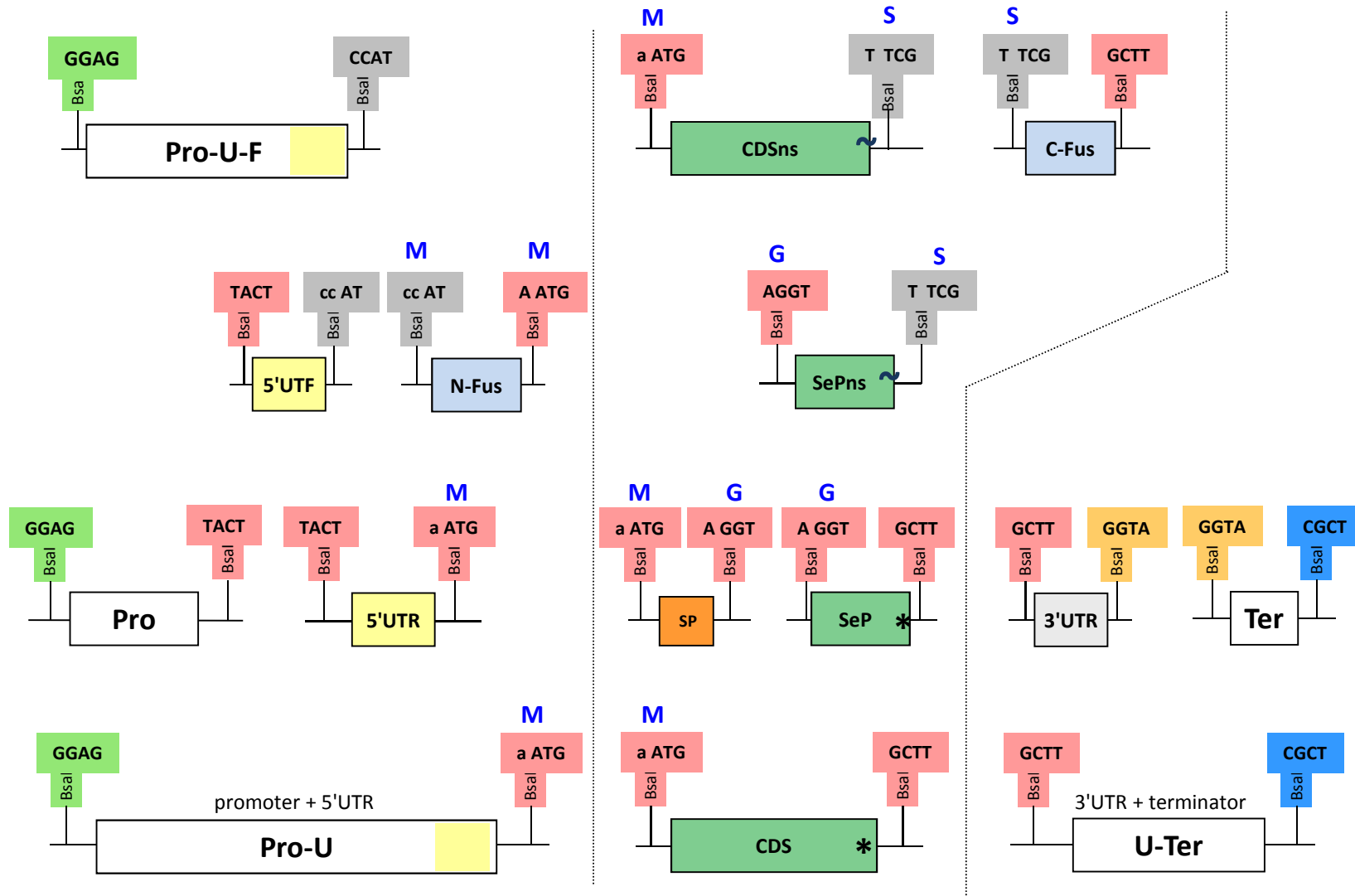
Any expression cassette can be divided into universal elementary modules



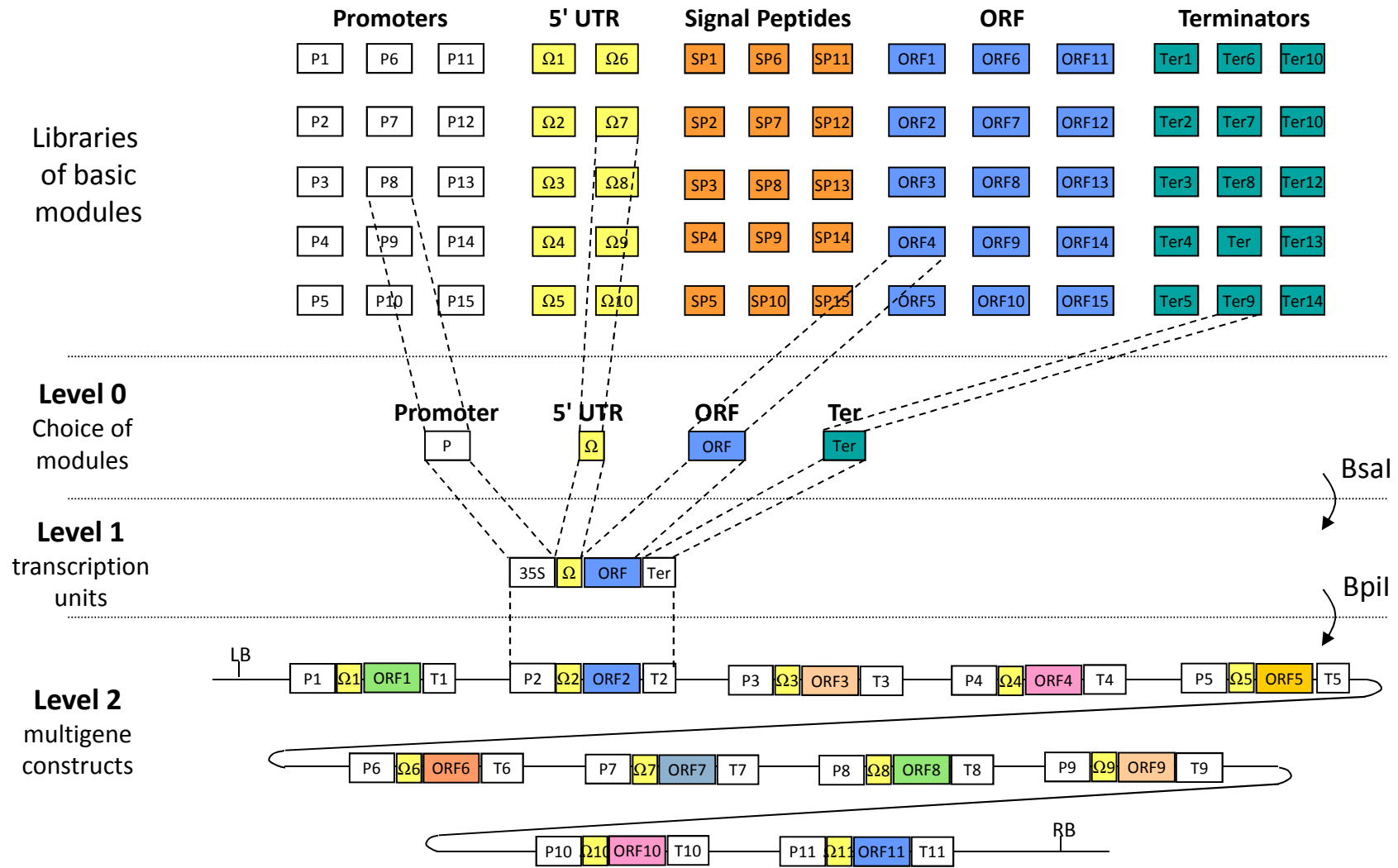
Elementary modules are prepared as level 0 modules *standard biological parts*



Module types

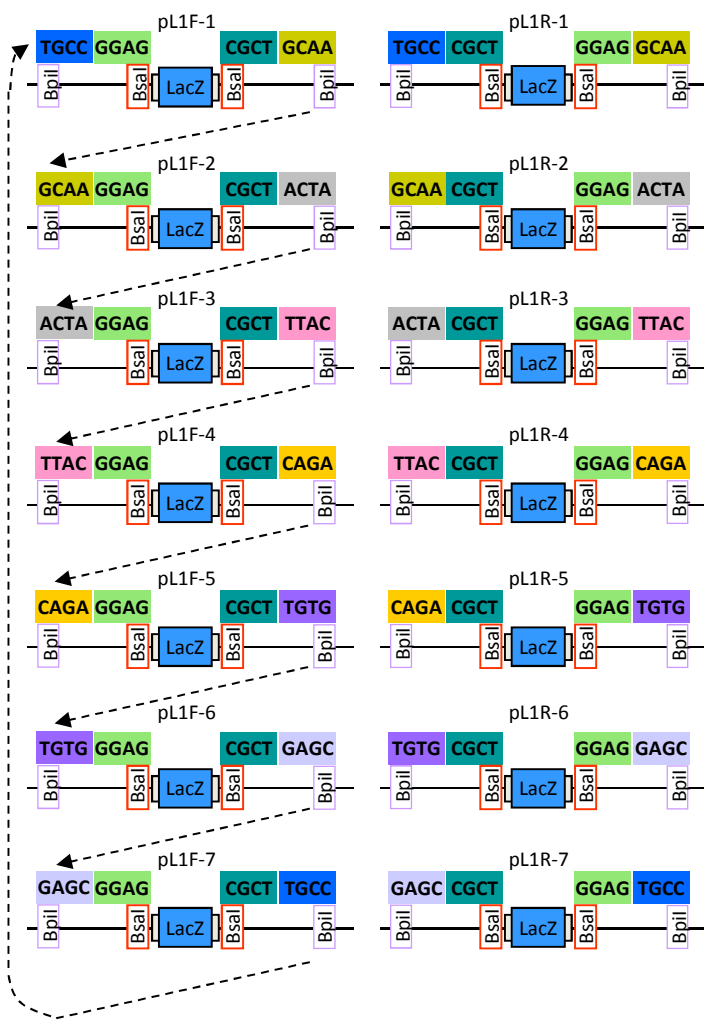


A modular cloning system (MoClo), modular and hierarchical

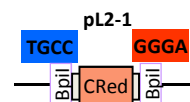


MoClo cloning vectors

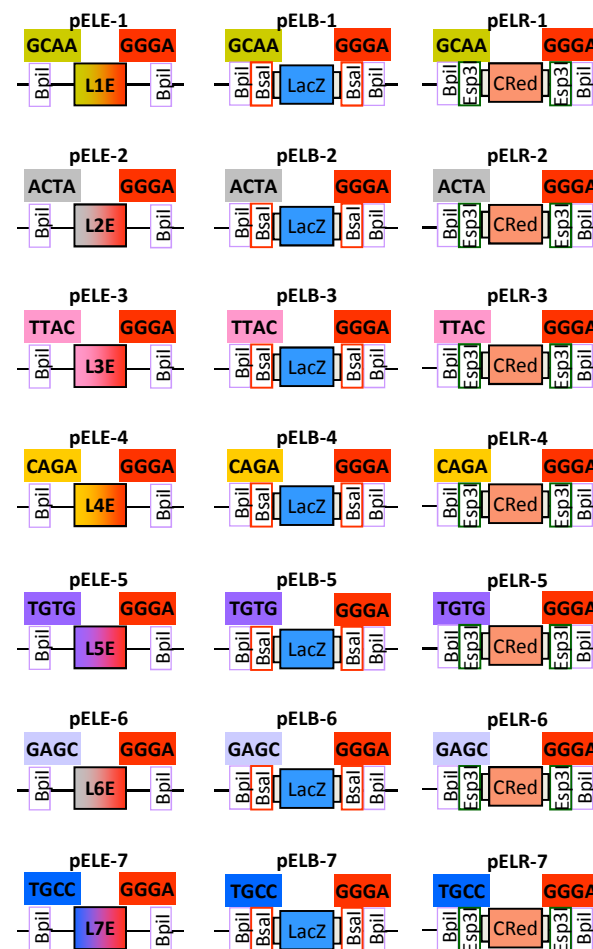
Level 1 destination vectors (Ap^R)



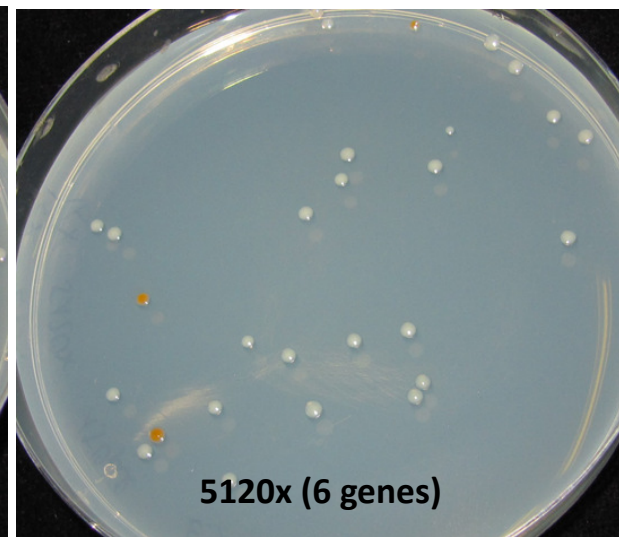
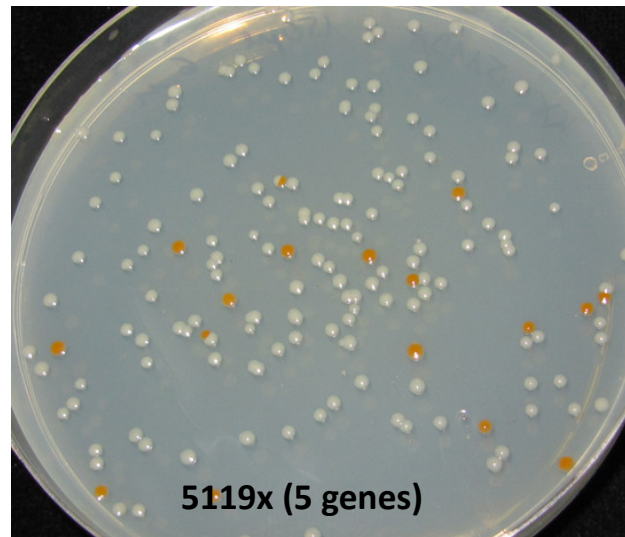
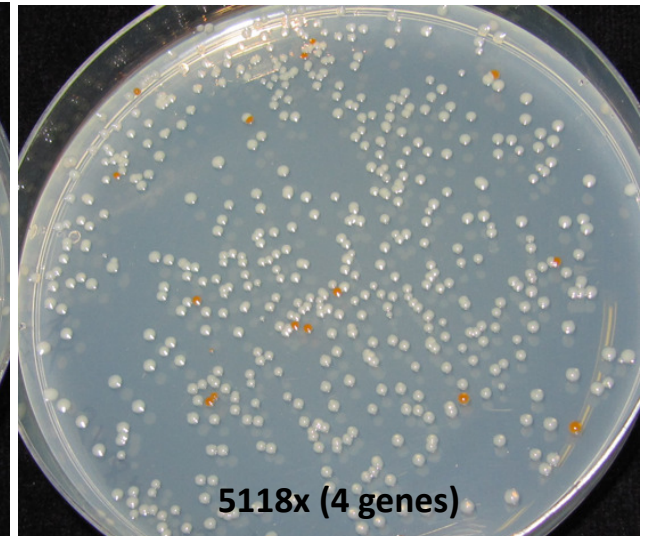
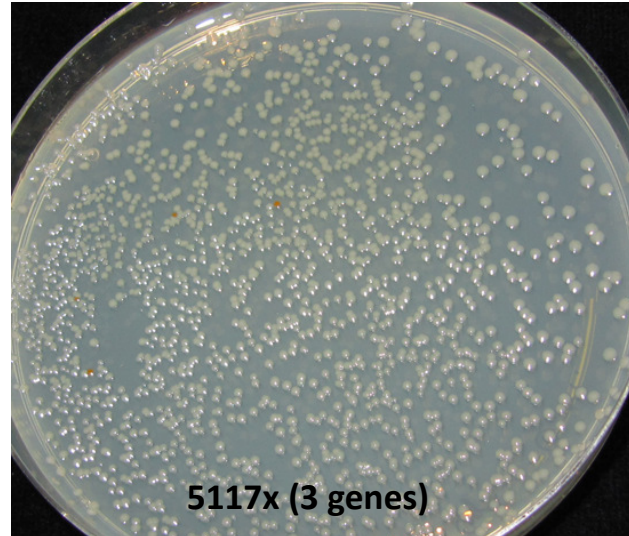
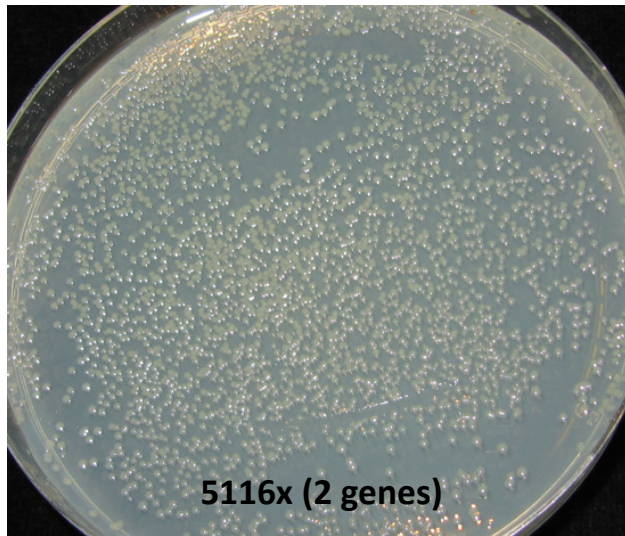
level 2 destination vectors (Km^R)



End linkers (Ap^R)



Level 2-1 cloning of 2 to 6 genes
The majority of white colonies contain correct constructs

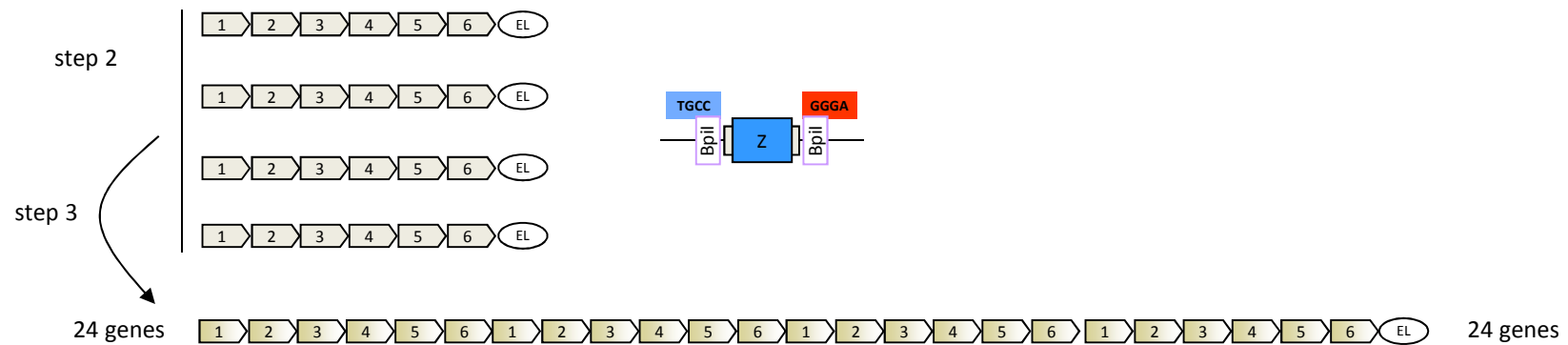
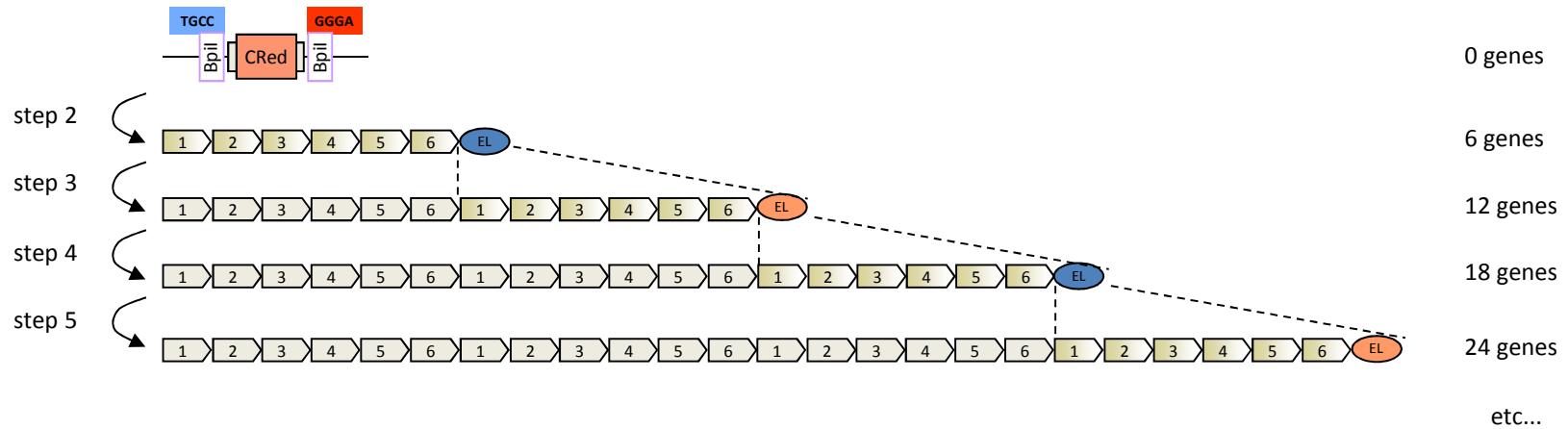


Cloning efficiency of multigene constructs

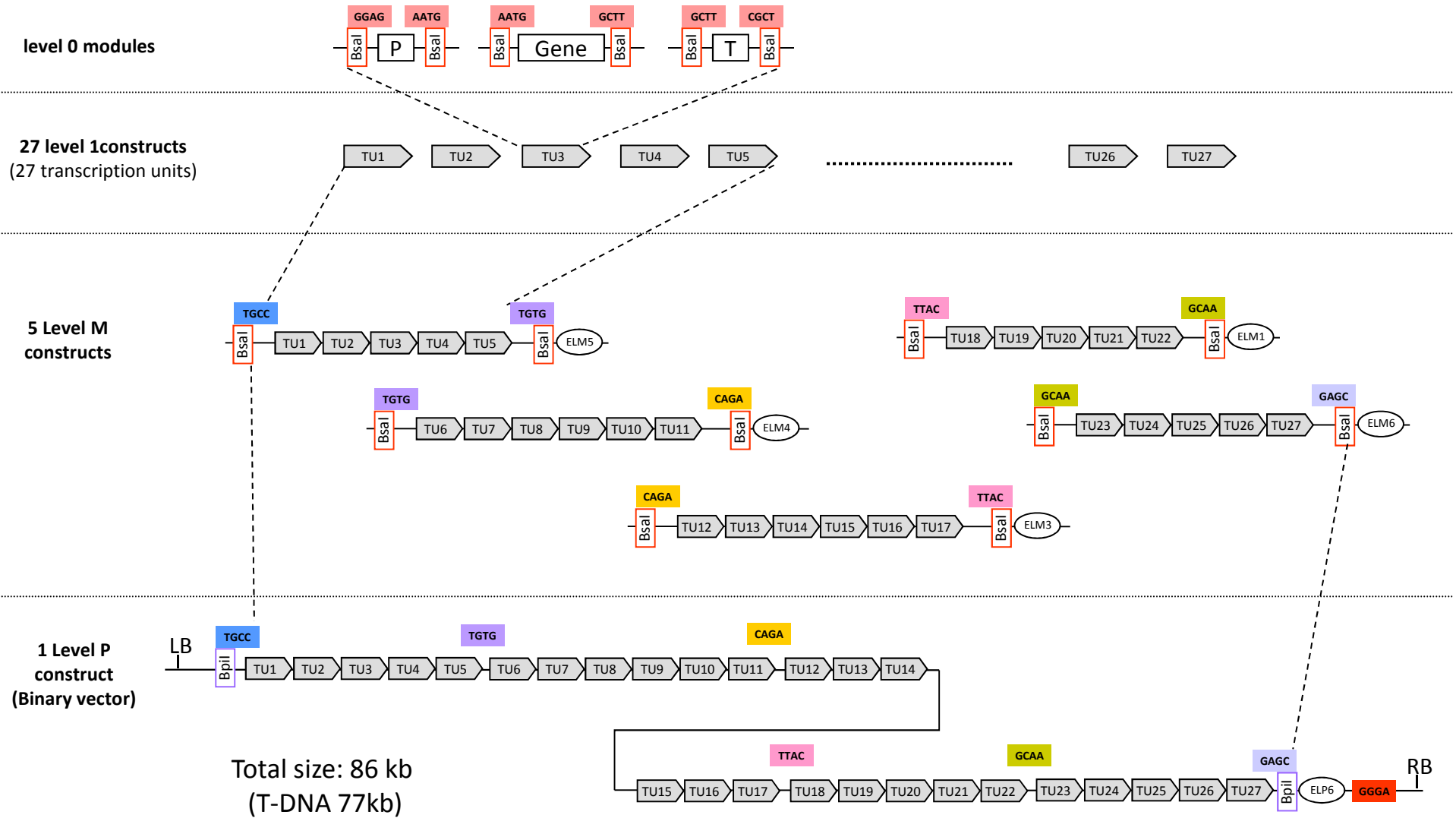
		cloning positions														Expected color-other	Miniprep correct	Plasmid size (kb)
		1	2	3	4	5	6	7	1	2	3	4						
		TGCCGCAA ACTA TTAC CAGATGTGGAGCTGCCGCAA ACTA TTAC CAGA																
level 2-2 blue to white	piCH51811	GFP	p19	VP2	VP5	VP7	VP3	BAR	LC	HC	MP	CP	4e	2685-0	6/6	33.4		
	piCH51811	GFP	p19	VP2	VP5	VP7	VP3	BAR	LC	HC	MP	CP	4e	159-3	0/6	33.4		
	piCH51802	GFP	p19	VP2	VP5	VP7	VP3	BAR	LC	HC	MP	3e	893-20	4/6	31.5			
	piCH51792	GFP	p19	VP2	VP5	VP7	VP3	BAR	LC	HC	2e	693-6	4/6	29.4				
	piCH51781	GFP	p19	VP2	VP5	VP7	VP3	BAR	LC	2e	13440-5	6/6	26.8					
	piCH51771	GFP	p19	VP2	VP5	VP7	VP3	BAR	7e	45000-0	6/6	25.5						
	piCH51761	GFP	p19	VP2	VP5	VP7	VP3	6e	45000-7	6/6	23.7							
level 2i-1 red to blue	piCH51226	GFP	p19	VP2	VP5	VP7	VP3	2 6e	75-25	2/6	24.3							
	piCH51211	GFP	p19	VP2	VP5	VP7	2 5e	330-45/60/10	6/6	20.4								
level 2-1 red to white	piCH51201	GFP	p19	VP2	VP5	VP7	VP3	6e	150-15	6/6	23.7							
	piCH51191	GFP	p19	VP2	VP5	VP7	5e	965-100	6/6	19.9								
	piCH51181	GFP	p19	VP2	VP5	4e	3.910-95	6/6	17.0									
	piCH51171	GFP	p19	VP2	3e	10.650-30	6/6	14.2										
	piCH51161	GFP	p19	2e	32.640-5	6/6	10.0											
level 1 blue to white	piCH50711	GFP	38 933 - 0	2/2	7.2													
	piCH50721	p19	103 466 - 0	2/2	6.6													
	piCH49722	VP2	32 800 - 0	2/2	8.5													
	piCH49733	VP5	28 933 - 0	2/2	7.1													
	piCH50731	VP7	65 733 - 0	2/2	7.2													
	piCH50741	VP3	16 400 - 0	2/2	8.2													
	piCH50751	BAR	184 000 - 0	2/2	6.1													
	piCH50761	LC	26 933 - 200	2/2	5.7													
	piCH50771	HC	85 600 - 0	2/2	6.9													
	piCH50781	MP	140 000 - 166	2/2	6.4													
	piCH50791	CP	81 066 - 0	2/2	6.3													



Additive or multiplicative assembly of transcription units



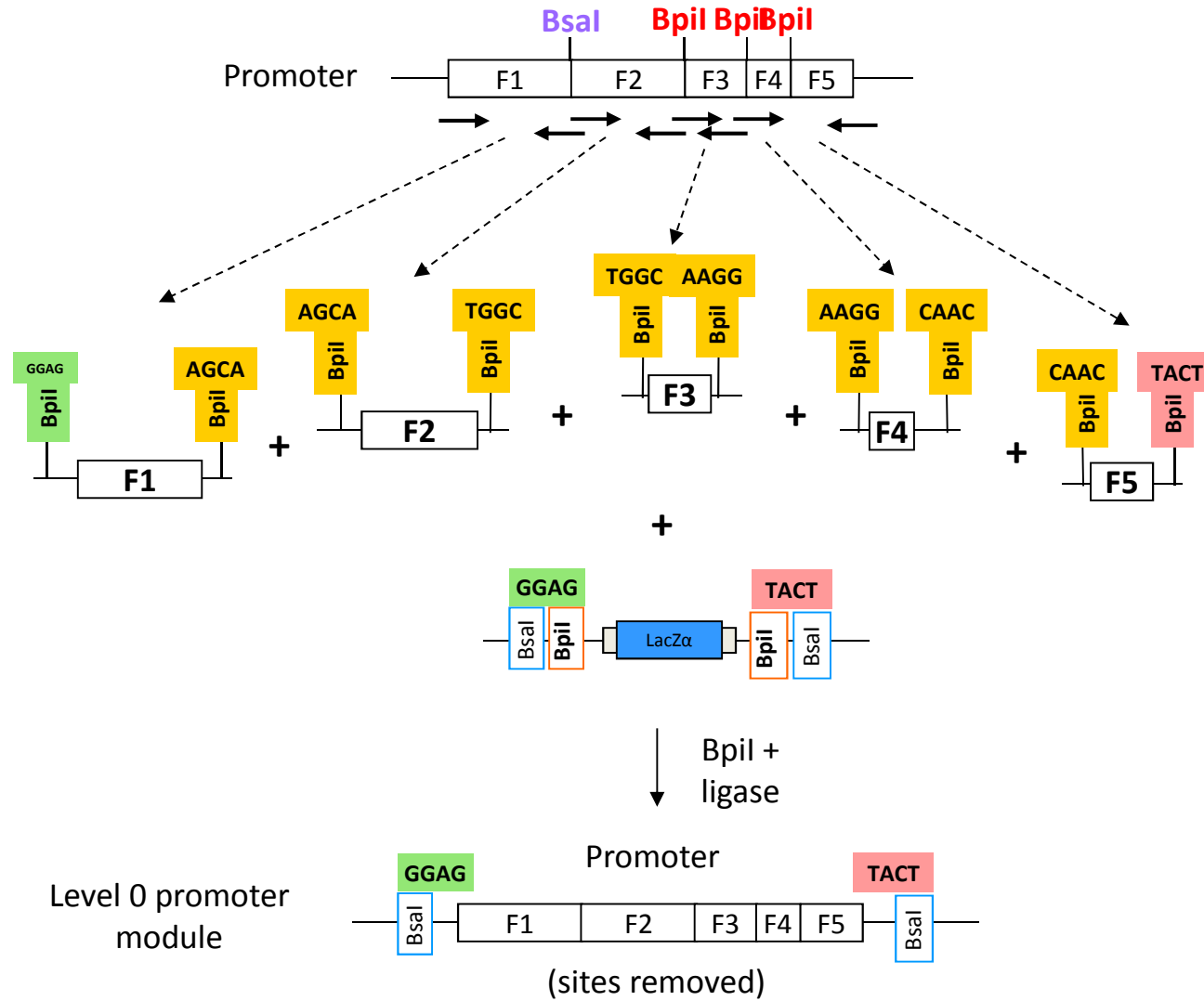
A construct with 27 transcription units was made in 3 cloning steps



Building libraries of basic genetic elements

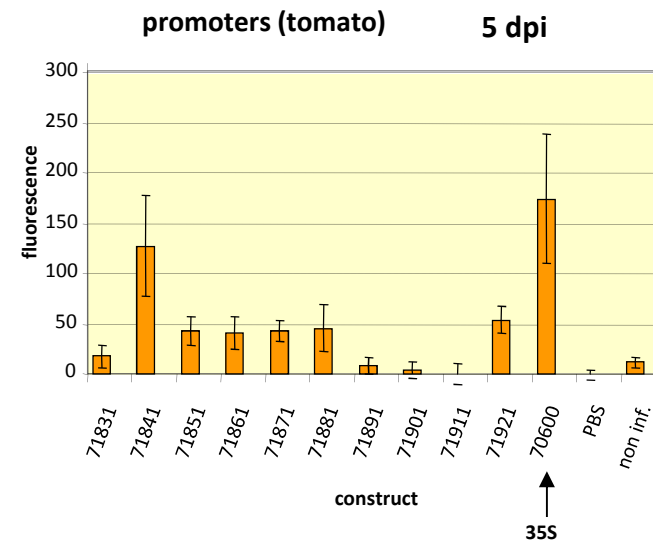
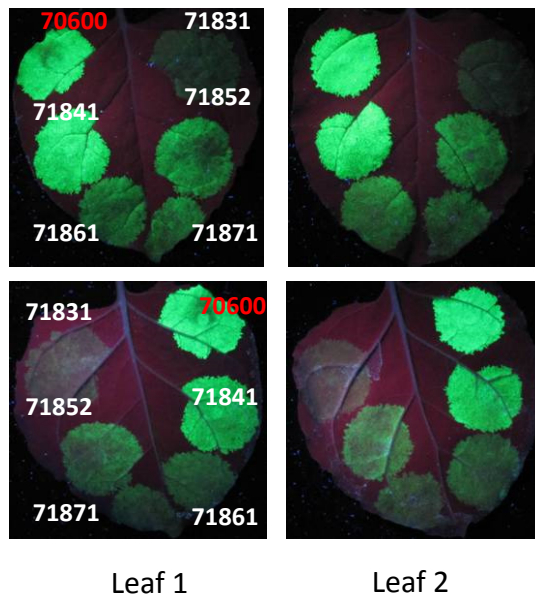
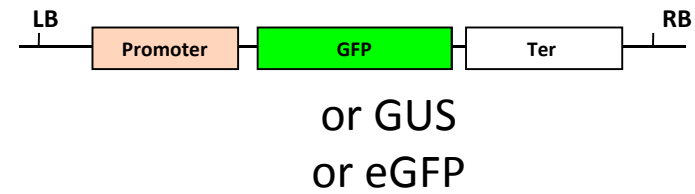
- Use of the MoClo system requires libraries of basic elements (promoters, UTRs, terminators, etc...)
- Each module needs to be made lacking restriction sites for the type IIS enzymes used.
- Each basic module needs to be evaluated, for example promoter strength

Cloning of level 0 modules, removal of Bsal and Bpil in promoter



Evaluation of level 0 modules

Transient expression in *Nicotiana benthamiana* leaves



GV3101 OD[600]=0,2

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Icon Genetics

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**Leibniz Institute of
Plant Biochemistry
(IPB)**

Ramona Grützner

