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

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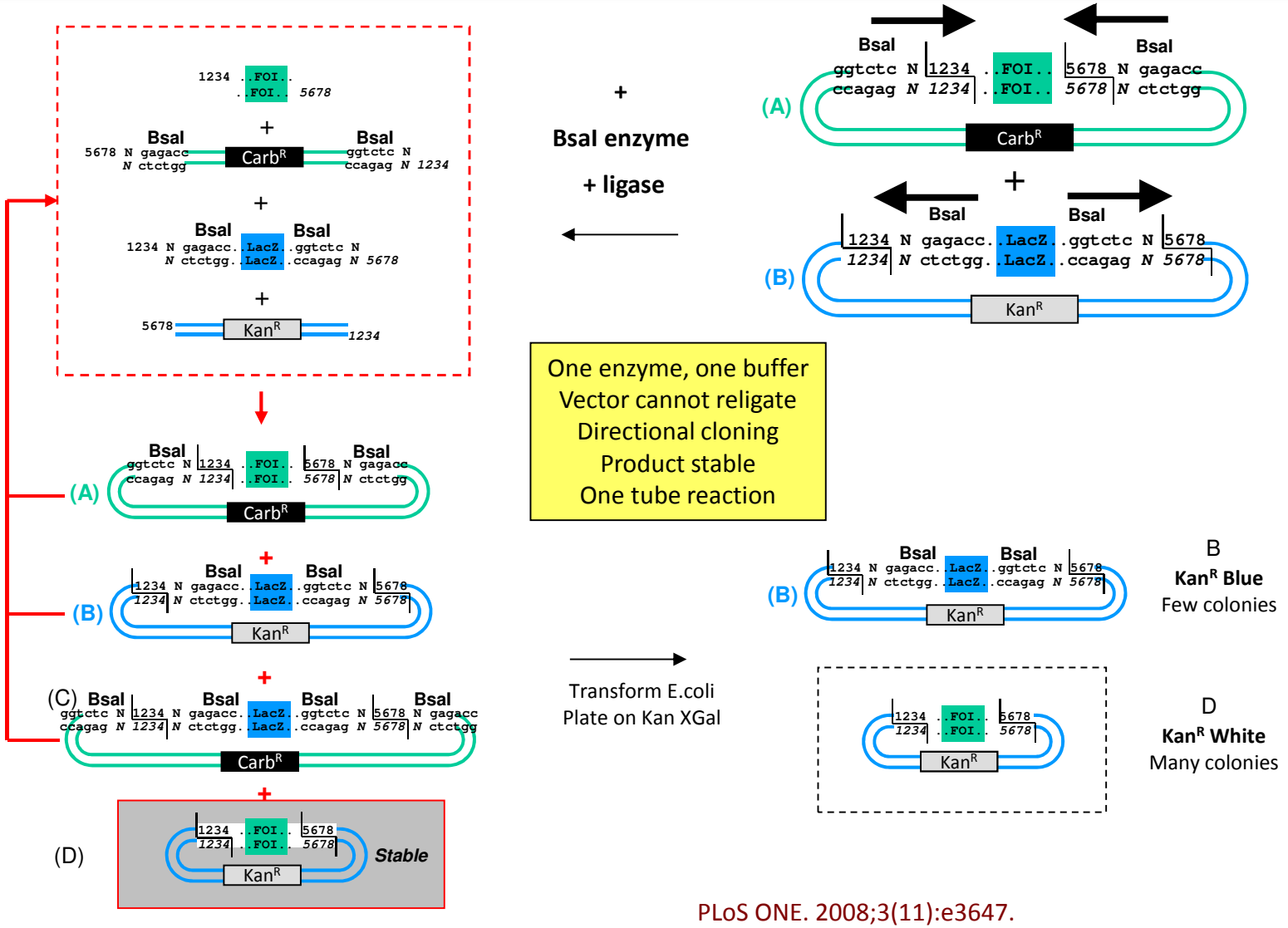
Introduction to Opportunities in Plant Synthetic Biology

Nottingham*, May 2013*

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

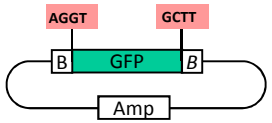


PLoS ONE. 2008;3(11):e3647.

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

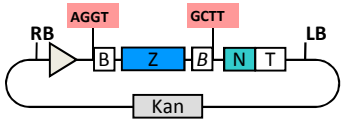
Subcloning a fragment from one entry construct into an expression vector with a one-pot one-step reaction

Entry construct, 50 ng

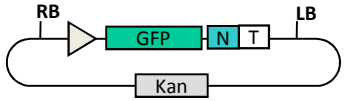


+

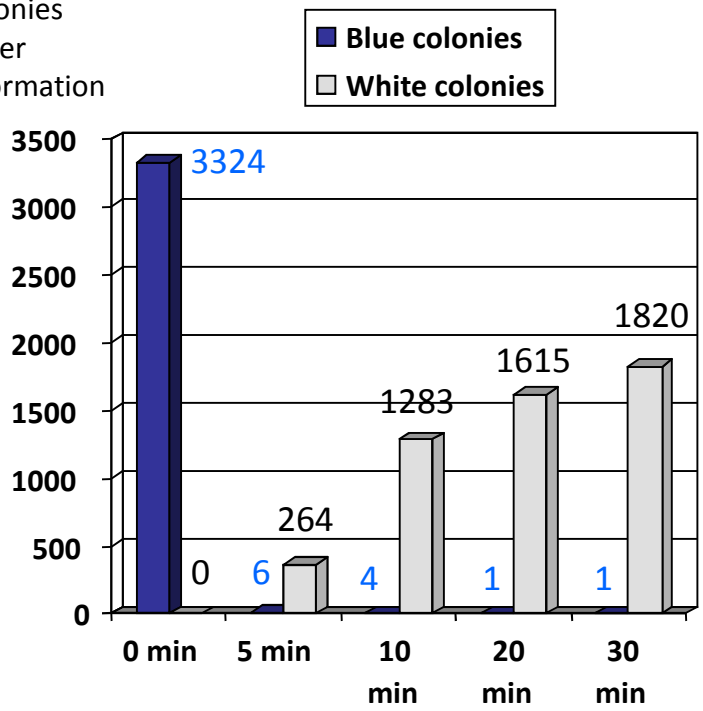
Recipient vector, 50 ng



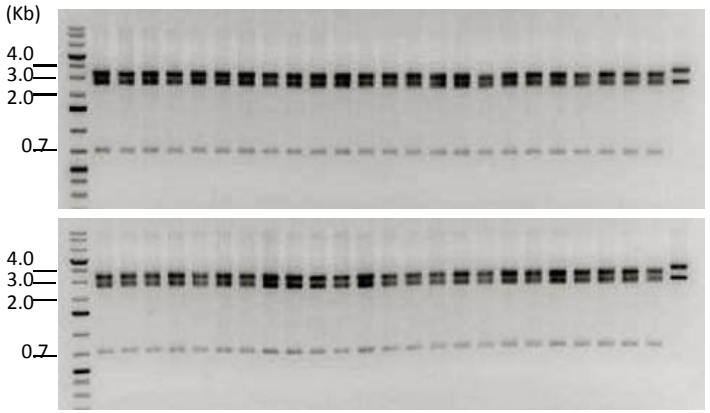
Final construct



Colonies per transformation

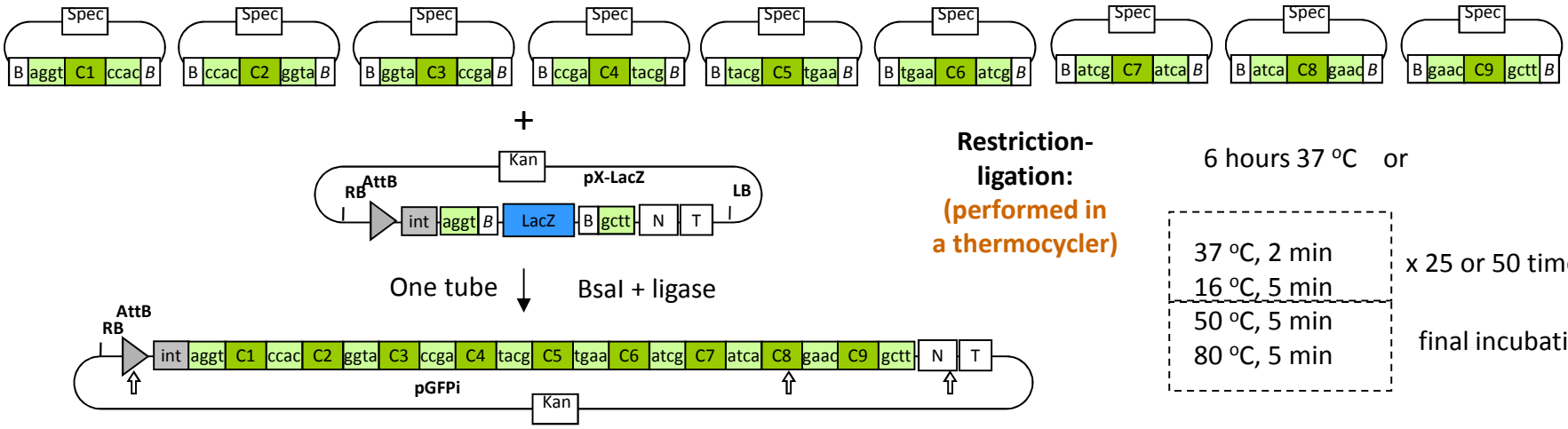


Screening of 48 white colonies
Digested plasmid run an agarose gel



48/48 positive

Assembly of nine fragments into an expression vector



Restriction-ligation: (performed in a thermocycler)

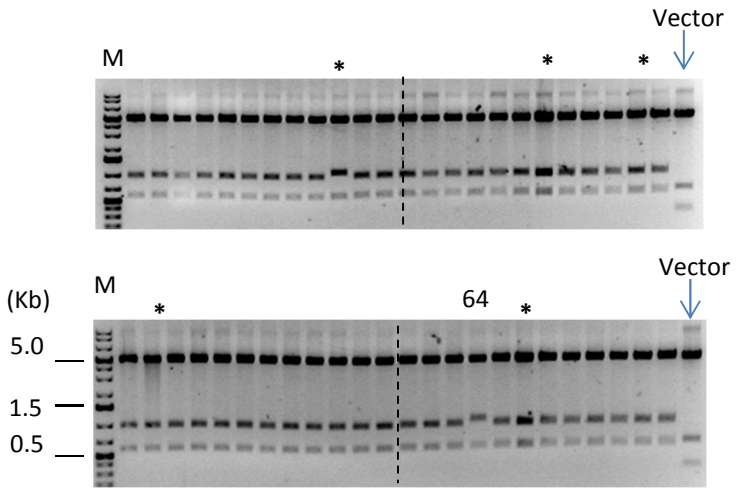
6 hours 37 °C or

37 °C, 2 min
16 °C, 5 min

x 25 or 50 times

50 °C, 5 min
80 °C, 5 min

final incubation



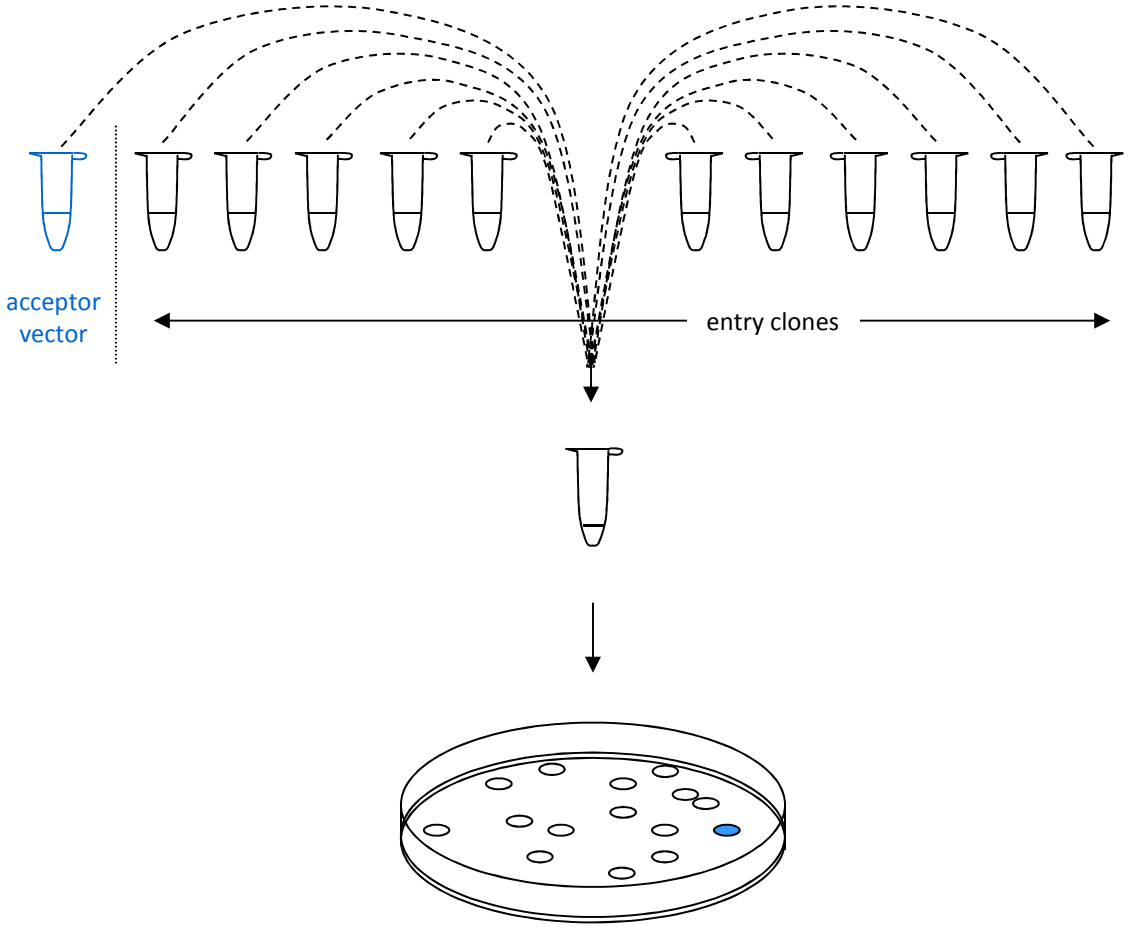
64 / 72 correct

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



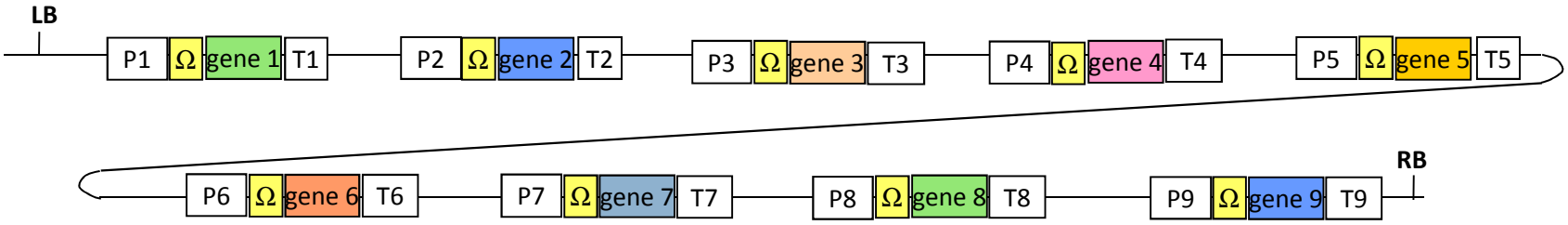
Pipet:
entry clones
recipient vector
buffer
BsaI
ligase

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

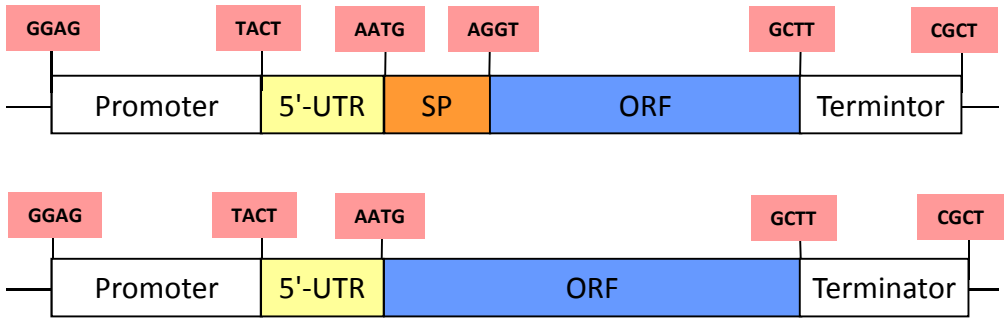
Plate:
on selectable
medium,
pick white colony
analyze by
restriction digest

Assembly of multigene constructs

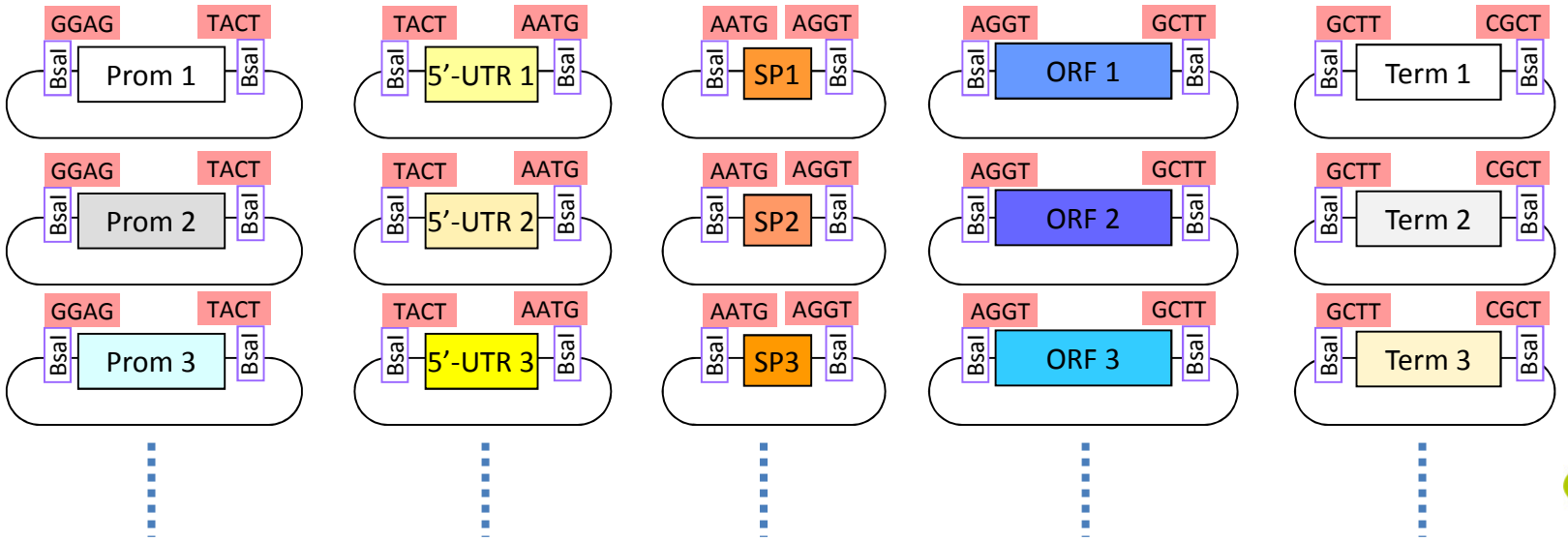
How can we build a standardized cloning system for assembly of any construct of choice?



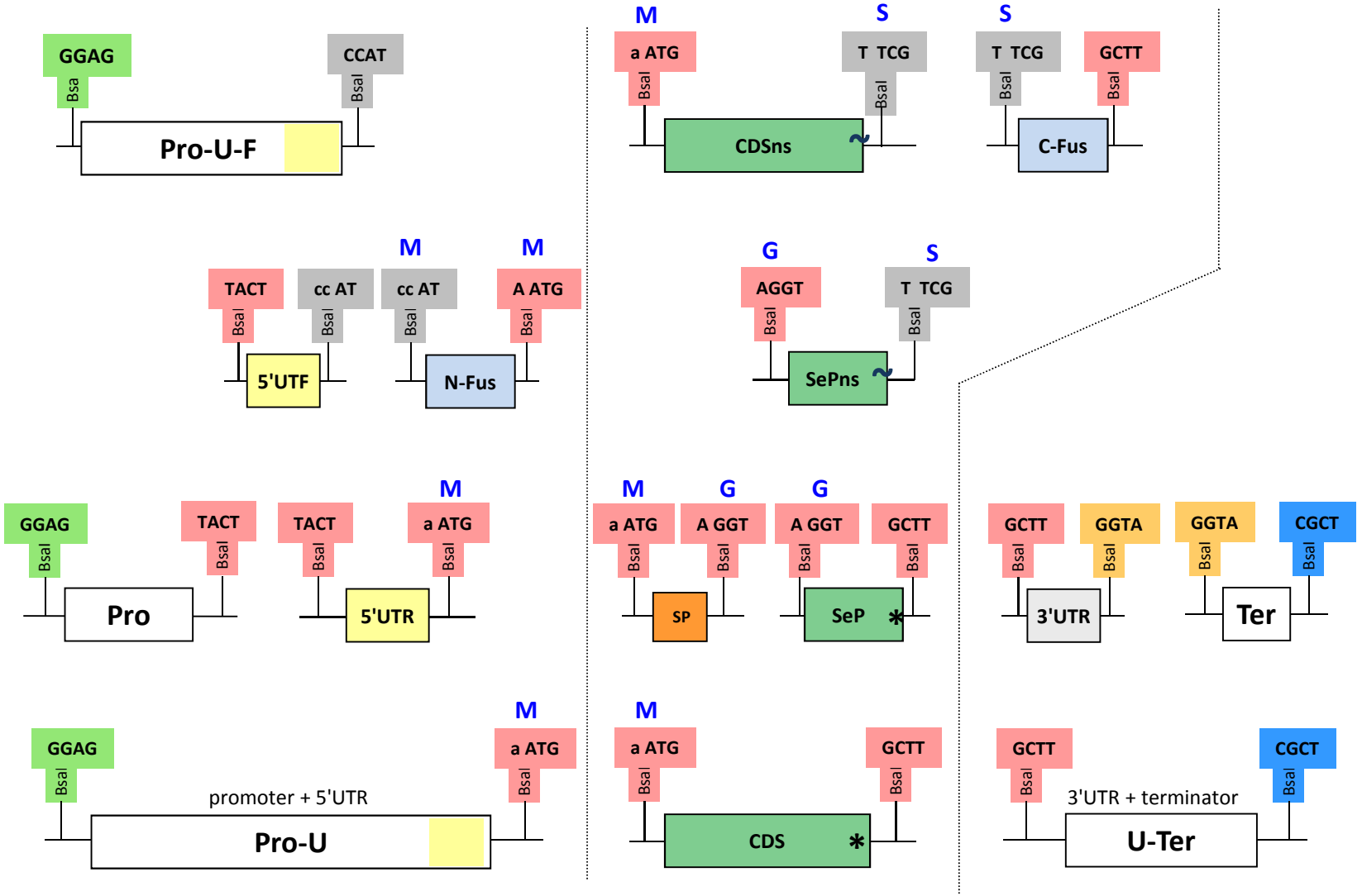
Any expression cassette can be divided into universal elementary modules



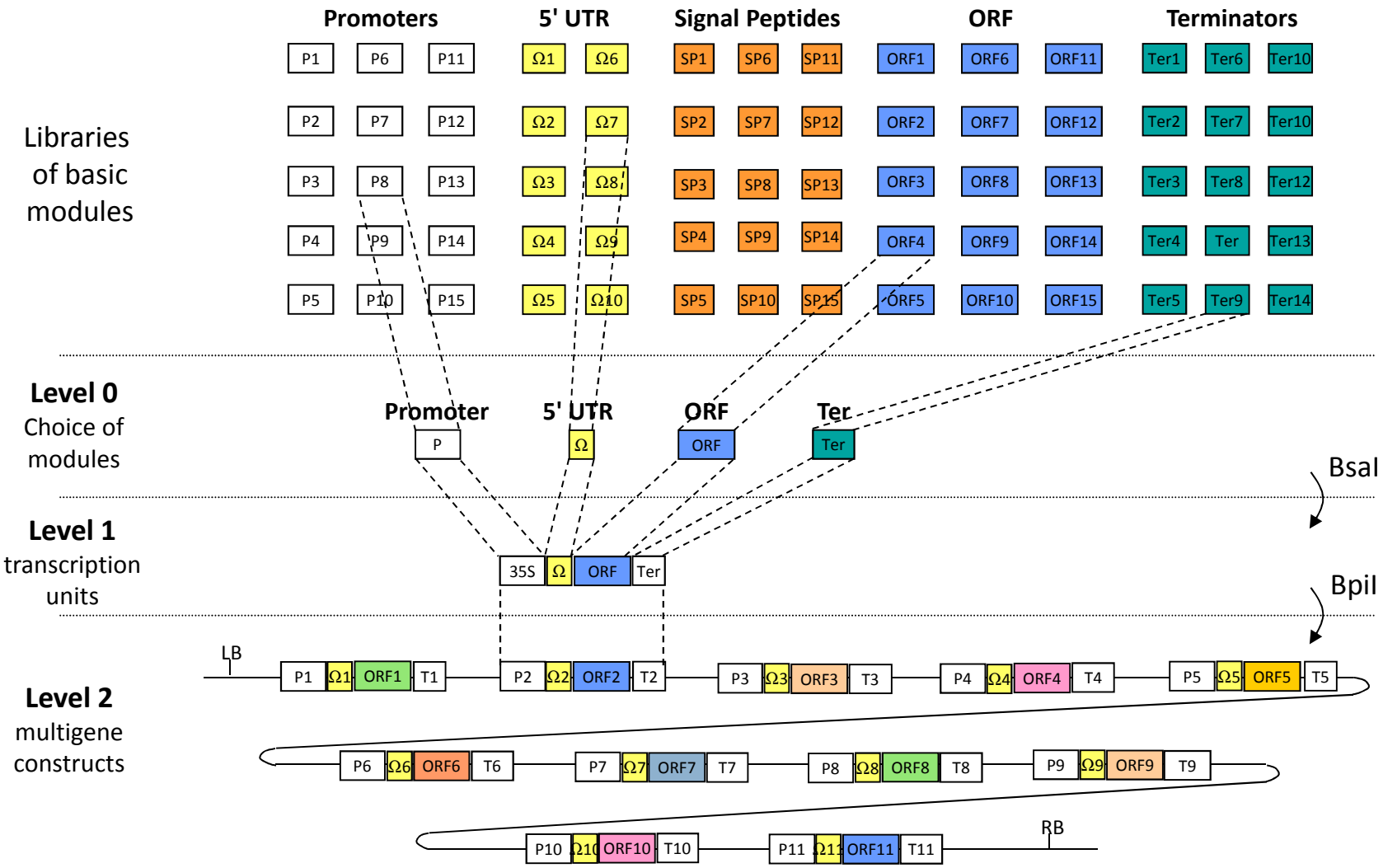
Elementary modules are prepared as level 0 modules *standard biological parts*



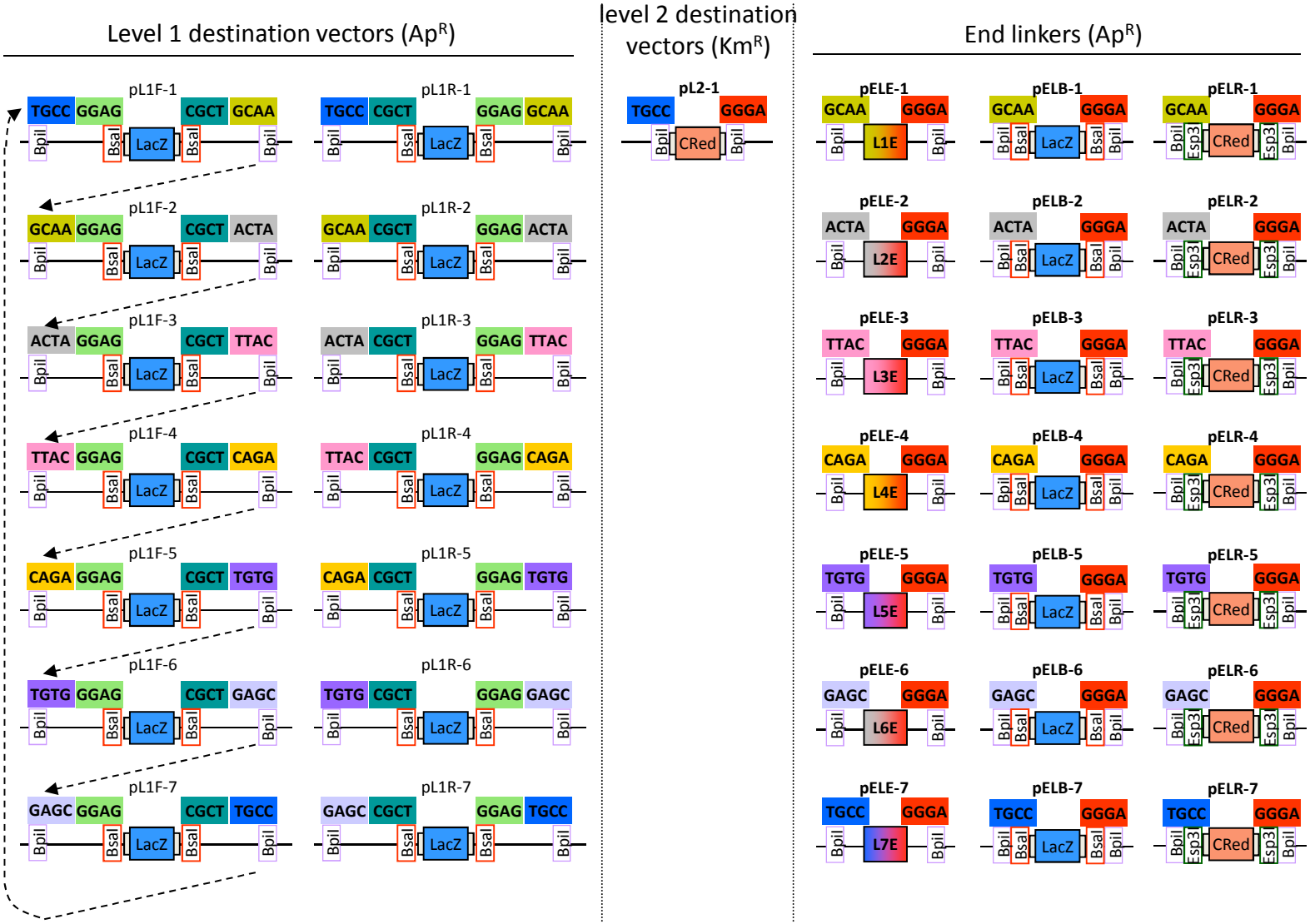
Module types



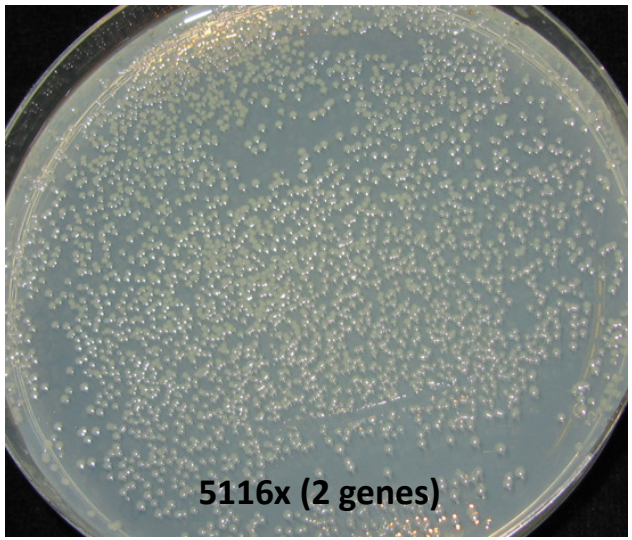
A modular cloning system (MoClo), modular and hierarchical

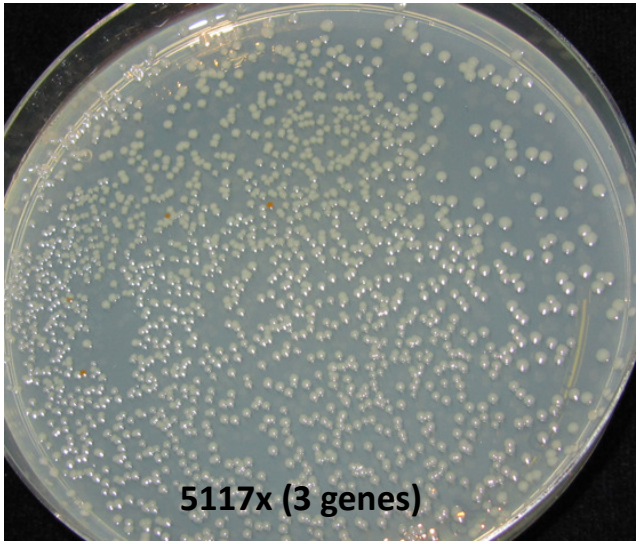


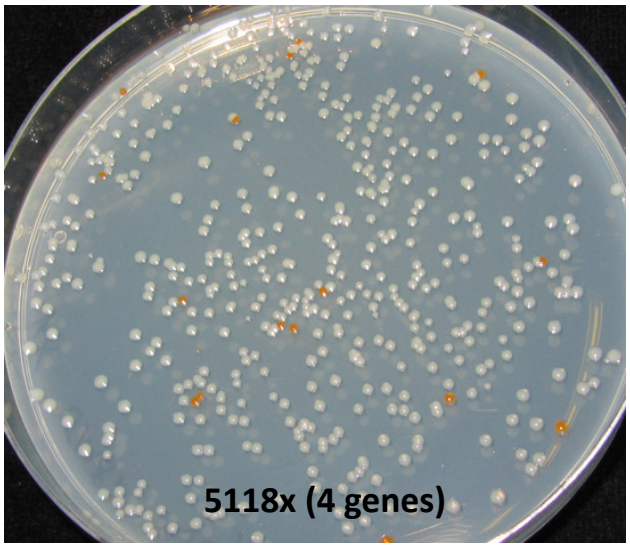
MoClo cloning vectors

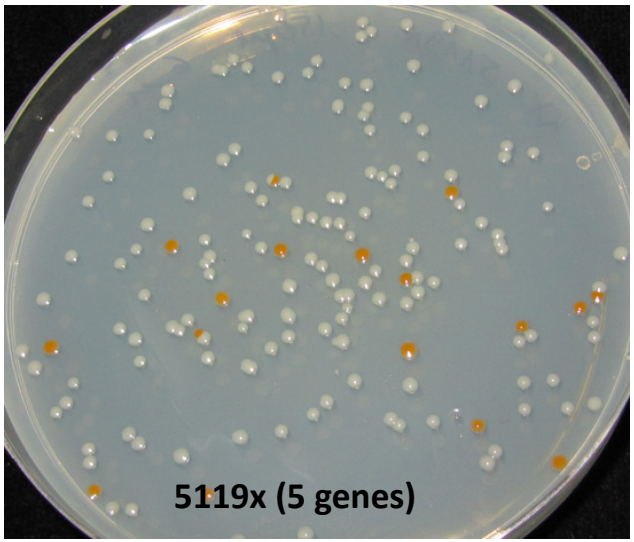


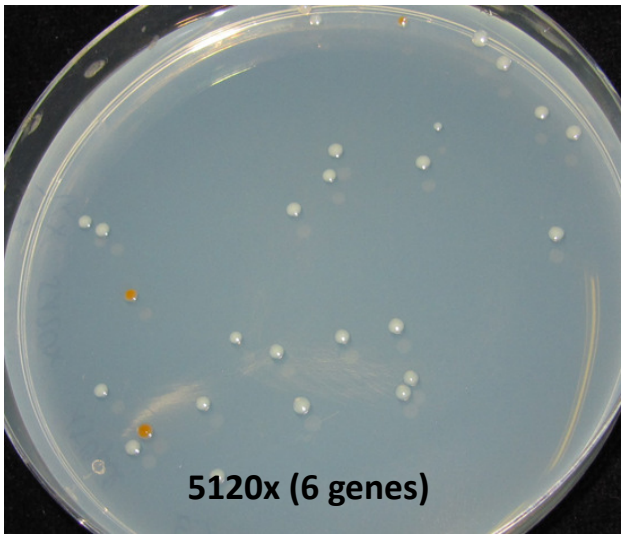
Level 2-1 cloning of 2 to 6 genes
The majority of white colonies contain correct constructs



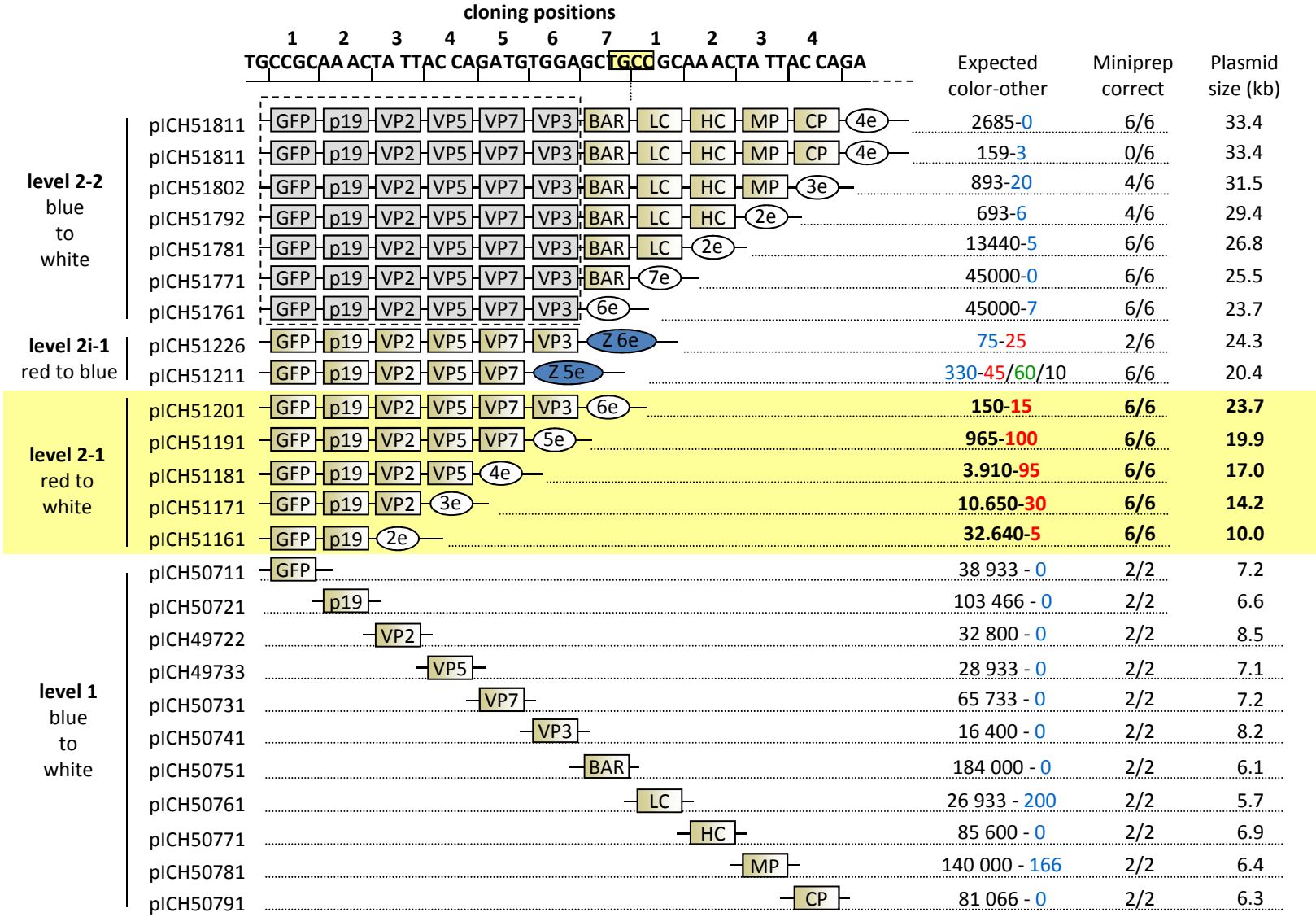




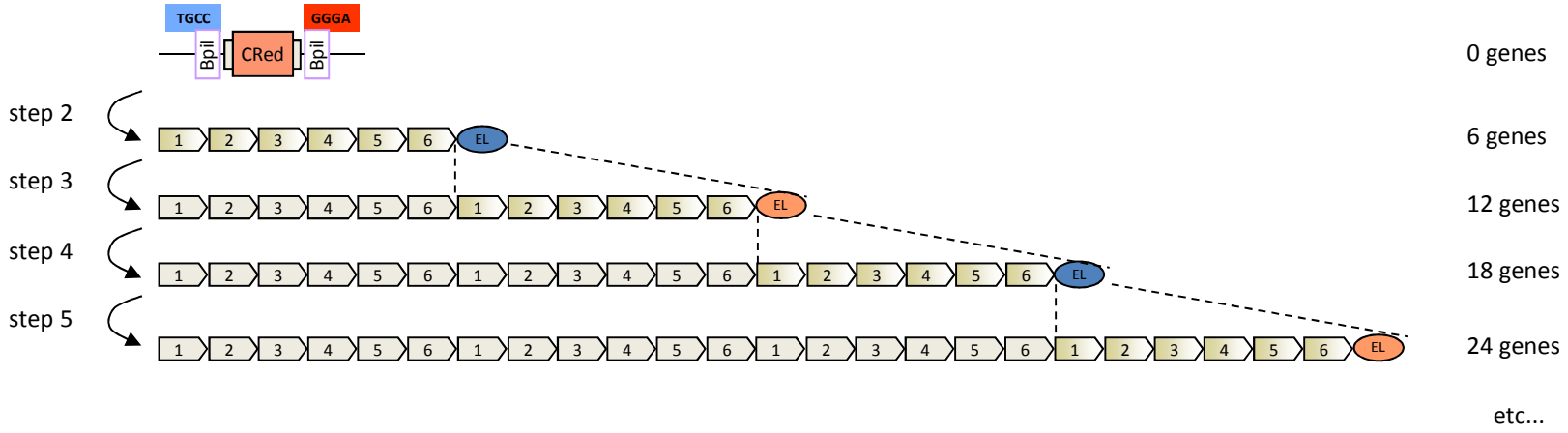


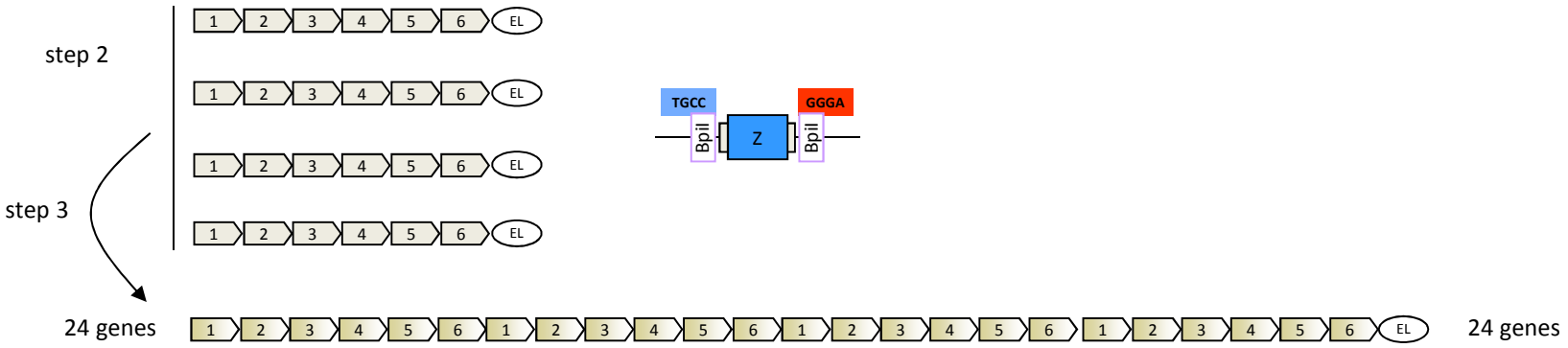


Cloning efficiency of multigene constructs

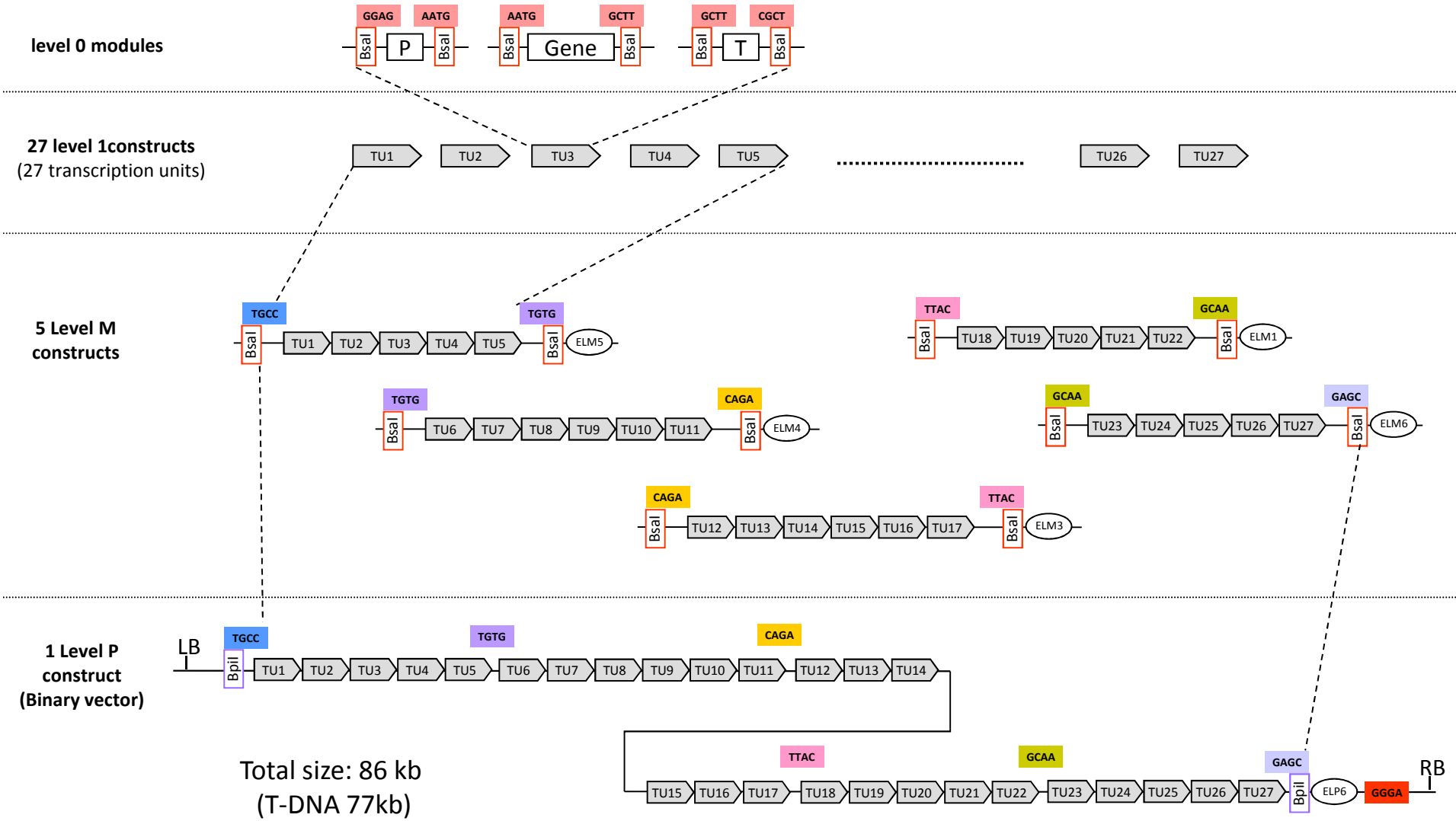


Additive or multiplicative assembly of transcription units





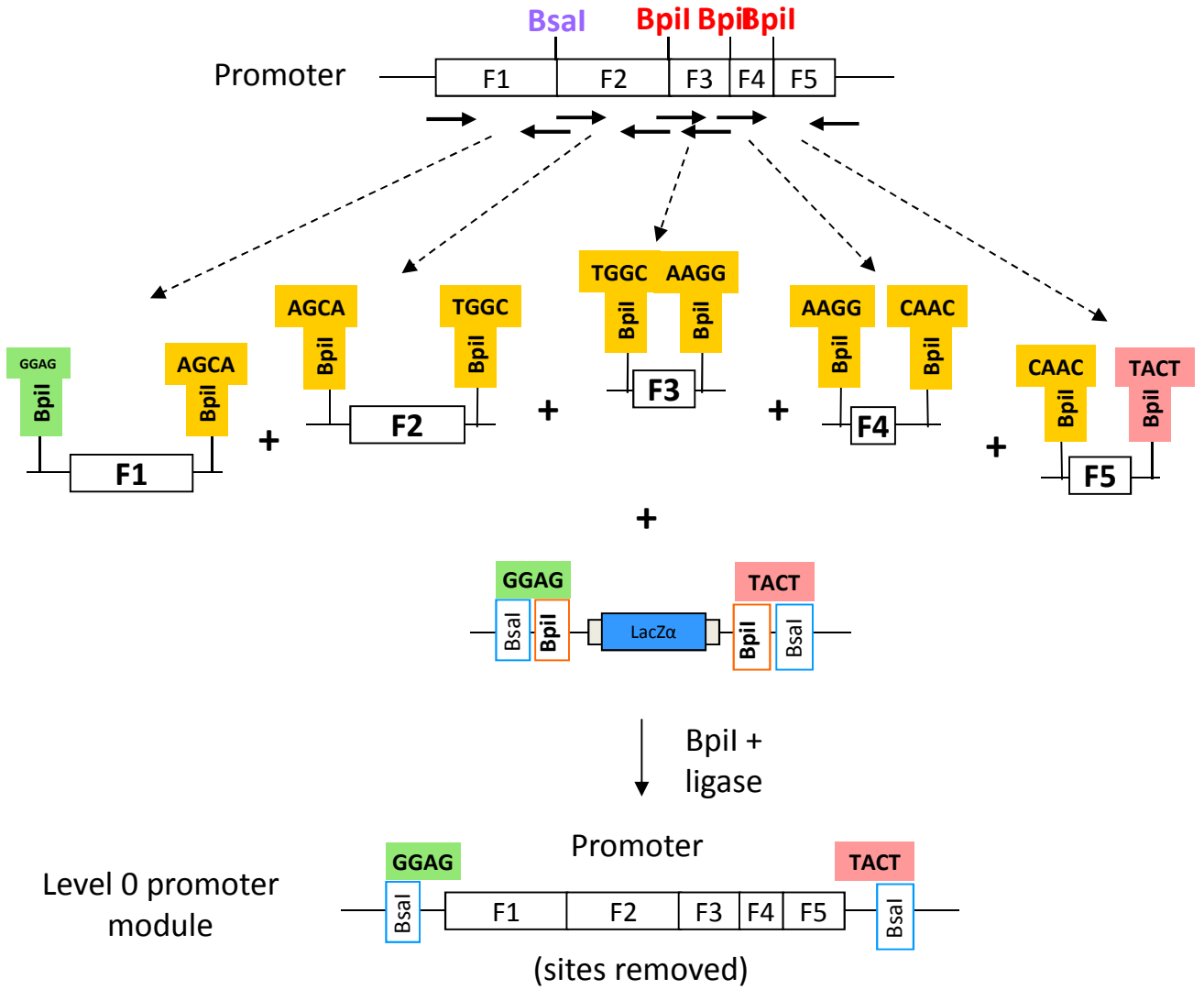
A construct with 27 transcription units was made in 3 cloning steps



Building libraries of basic genetic elements

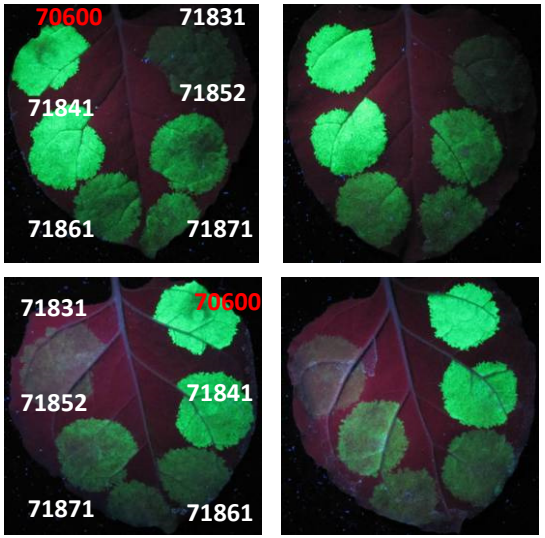
- Use of the MoClo system requires libraries of basic elements (promoters, UTRs, terminators, etc...)
- Each module needs to be made lacking restriction sites for the type IIS enzymes used.
- Each basic module needs to be evaluated, for example promoter strength

Cloning of level 0 modules, removal of BsaI and BpiI in promoter



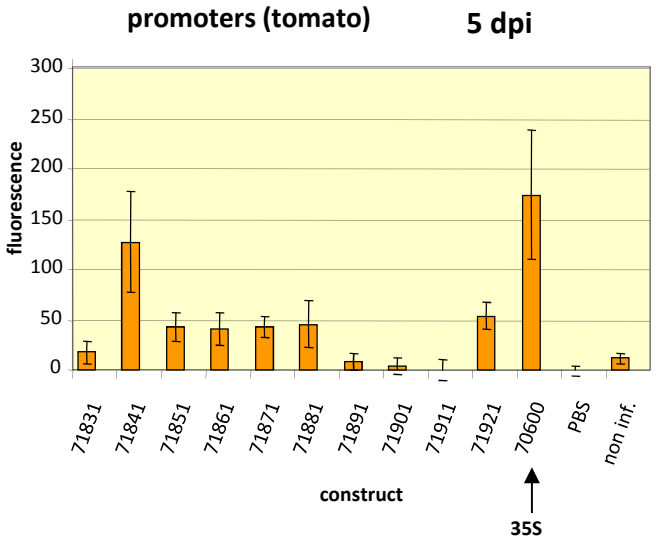
Evaluation of level 0 modules

Transient expression in *Nicotiana benthamiana* leaves



Leaf 1 Leaf 2

or GUS
or eGFP



GV3101 OD[600]=0,2

Acknowlegements

Icon Genetics

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