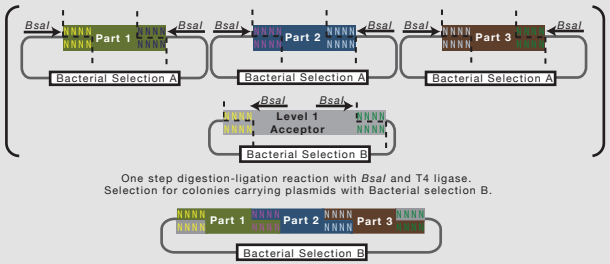
QUICK GUIDE PART 1: TYPE IIS CLONING WITH THE STANDARD PLANT SYNTAX AND THE GOLDEN GATE MoClo PLASMIDS

NOTE: THIS IS QUICK REFERENCE GUIDE - IF YOU ARE A NOVICE PLEASE START BY WATCHING THE VIDEOS AT http://synbio.tsl.ac.uk/golden-gate/
TO LEARN HOW TO DESIGN PRIMERS FOR CONSTRUCTING NEW STANDARD PARTS (LEVEL 0 PARTS) PLEASE SEE PART 2

HOW THE ONE-POT REACTION WORKS

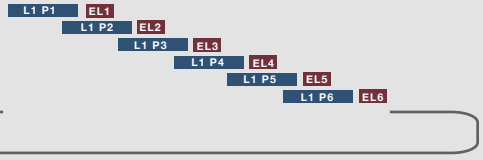
Type IIS enzymes cut outside of their recognition site. This allows you you to assemble multiple parts at the same time. This is what happens when you assemble three level 0 parts (Part 1, 2 and 3) into a Level 1 acceptor:



One step digestion-ligation reaction with *BsaI* and T4 ligase.
Selection for colonies carrying plasmids with Bacterial selection B.

Providing that you put level 0 parts with compatible ends into the tube at a 2:1 molar ratio to the level 1 acceptor plasmid they will assemble in order. To enable this we use standard overhangs for each type of part. See the common syntax at the top of the next column.

MAKING MULTIGENE CONSTRUCTS



There are seven Level 1 acceptors. All are binary plasmids. You can assemble up to six in a level 2 or M acceptor. These will assemble in order using BpiI. The BpiI overhangs are different to the BsaI overhangs. They are not defined in the standard syntax.The Level 1 acceptor that you choose to assemble your standard parts into will determine the position of that transcriptional unit in your multigene assembly.

To use the MoClo Lveel M and 2 acceptor plasmids your first transcriptional unit must be in position 1. You also need to use the correct End Linker (EL) to join to the last Level 1 transcriptional unit to the acceptor (see diagram above).

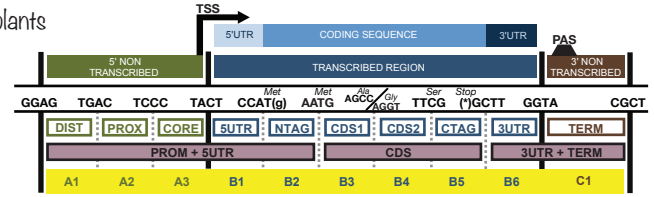
Any Level 1 transcriptional unit can be substituted for a dummy.

The COMMON SYNTAX for plants defines the 4 base-pair overhangs or fusion sites that join basic, standard parts.

These sites allow a multitude of standard parts to be generated.

Standard parts comprise any portion of a gene cloned into a plasmid flanked by a convergent pair of BsaI recognition sequences.

Parts can comprise the region between an adjacent pair of fusion sites. Alternatively, to reduce complexity or when a particular functional element is not required, parts can span multiple fusion sites (examples in pink boxes).



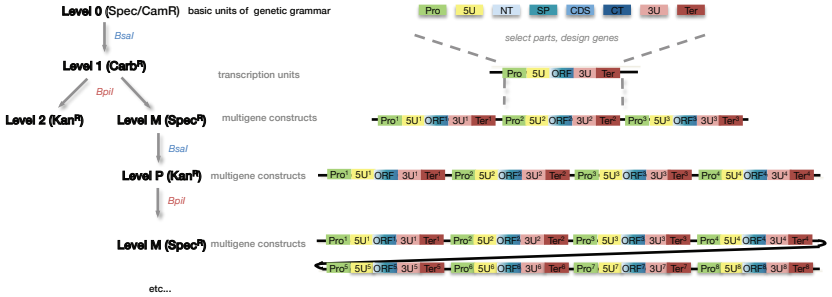
POSITION	NAME	FUNCTION	5' OVERHANG	3' OVERHANG
A1	DIST	Distal promoter region, *cis* regulator or transcriptional enhancer	GGAG	TGAC
A2	PROX	Proximal promoter region, *cis* regulator or transcriptional enhancer	TGAC	TCCC
A3	CORE	Minimal promoter region, including transcription start site (TSS)	TCCC	TACT
A4	5UTR	5' untranslated region	TACT	CCAT
B2	NTAG	N terminal coding region	CCAT	AATG
B3	CDS1	Coding region - optional N terminal coding region	AATG	AGCC /AGGT
B4	CDS2	Coding region - no start or stop codon	AGCC /AGGT	TTCG
B5	CTAG	C terminal coding region	TTCG	GCTT
B6	3UTR	3' untranslated region	GCTT	GGTA
C1	TERM	Transcription terminator including polyadenylation signal (PAS)	GGTA	CGCT

SUMMARY

Basic, standard parts are known as "Level 0 parts".

Level 0 modules are assembled into transcriptional units in "Level 1 acceptors".

Level 1 transcriptional units are assembled into multigene constructs in either "Level 2 acceptors " or Level M acceptors. Level M constructs can be assembled with other level Level M constructs into Level P acceptors to make very large multi-gene constructs:



THE DIGESTION-LIGATION PROTOCOL

This protocol assumes that:
(a) All parts being assembled are free of internal BsaI and BpiI recognition sequences
(b) That the junctions of all parts being assembled have a unique set of compatible overhangs
(c) Your acceptor plasmid has a different antibiotic resistance to all of the modules being assembled into it

The restriction buffer protocol is shorter and works sufficiently well for short/easy assemblies. We find that the longer ligase buffer protocol is generally more efficient, especially for large (Level 2+) assemblies.
We have had many problems with NEB restriction enzymes and do not use or recommend them for these reactions.
If your B.S.A. is shipped as a 100X stock this should be diluted in water to make a 10X working stock. Add the following to a PCR tube, make the reaction volume up to 20 µl with sterile distilled water and cycle as shown.

To assemble fragments in Level 0, Level 2 and Level M acceptors BpiI is required.

Short protocol in restriction buffer

- 100-200 ng of acceptor plasmid
- Plasmids containing each module/ part to be inserted. Use a 2:1 molar ratio of insert:acceptor.
- 10 units *BpiI* (1µl of 10U/µl *BpiI*, ThermoFisher)
- 2 µl Buffer G (ThermoFisher)
- 400 units T4 DNA Ligase (1µl of 400U/µl, NEB)
- 2 µl 10mM ATP (*not dATP!!!!*)

10 minutes 37°C
10 minutes 16°C
10 minutes 37°C
20 minutes 65°C
16°C
x3

Long protocol in ligase buffer

- 100-200 ng of acceptor plasmid
- Plasmids containing each module/ part to be inserted. Use a 2:1 molar ratio of insert:acceptor.
- 1.5µl T4 Ligase Buffer (NEB)
- 1.5 µl Bovine Serum Albumin (10x)
- 200 units T4 DNA Ligase (0.5µl of 400U/µl, NEB)
- 5 units *BpiI* (0.5µl of 10U/µl *BpiI*, ThermoFisher)

20 seconds 37°C
3 minutes 37°C
4 minutes 16°C
5 minutes 50°C
5 minutes 80°C 5:00
16°C
x26

To assemble fragments in Level -1, Level 1 and Level P acceptors BsaI is required.

Short protocol in restriction buffer

- 100-200 ng of acceptor plasmid
- Plasmids containing each module/ part to be inserted. Use a 2:1 molar ratio of insert:acceptor.
- 10 units *BsaI* (Eco31I) (1µl of 10U/µl *BsaI*, ThermoFisher)
- 2 µl buffer G
- 400 units T4 DNA Ligase (1µl of 400U/µl, NEB)
- 2 µl 10mM ATP

10 minutes 40°C
10 minutes 16°C
10 minutes 50°C
20 minutes 80°C
16°C
x3

Long protocol in ligase buffer

- 100-200 ng of acceptor plasmid
- Plasmids containing each module/ part to be inserted. Use a 2:1 molar ratio of insert:acceptor.
- 1.5µl T4 Ligase Buffer (NEB)
- 1.5µl Bovine Serum Albumin (10x)
- 200 units T4 DNA Ligase (0.5µl of 400U/µl NEB)
- 5 units *BsaI* (Eco31I) (0.5µl of 10U/µl Thermo Fisher)

20 seconds 37°C
3 minutes 37°C
4 minutes 16°C
5 minutes 50°C
5 minutes 80°C 5:00
16°C
x26