



Background

MultiGreen is a cloning technique to assemble multiple GreenGate expression units. It is an alternative to our Golden Gibson and Gateway methods that can be used to assemble six full expression cassettes into one destination vector. It is a two-step cloning process, cycling between two different Golden Gate assembly reactions.

The original MultiGreen idea was developed by Vincent Pennetti from the Parrott lab:

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0306008>

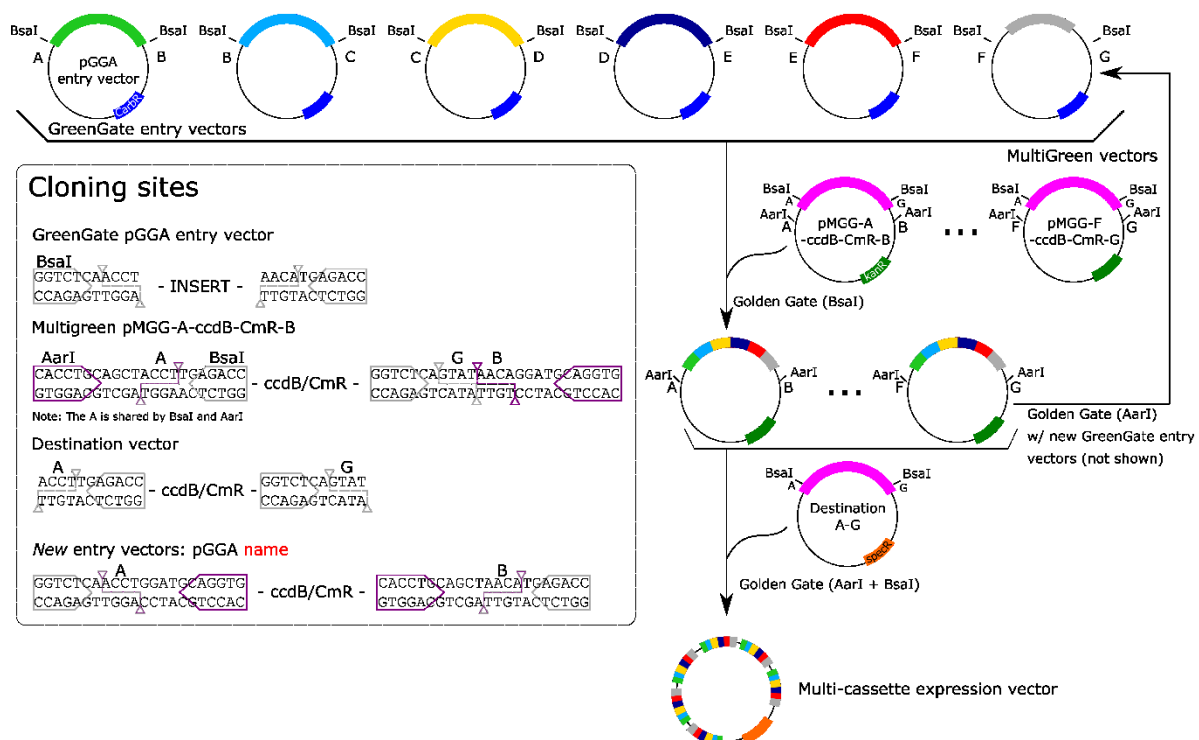
Their system uses the type IIS restriction enzyme BsmBI, but since BsmBI has a cleavage site frequency comparable to that of BsaI (i.e. a lot), it was opted to replace these restriction sites by AarI sites instead, similar to GreenGate2.0 by the Maizel lab:

<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0290097>

However, GreenGate2.0 is similar to Goldenbraid where only 2 expression cassettes are combined at a time. This is why we opt for the 6-cassette design of MultiGreen. The best of both worlds.

Method

The first step creates the initial cassettes that are assembled into one of six MultiGreen vectors (pMGG-A-ccdB-CmR-B, pMGG-B-ccdB-CmR-C,...) through a regular Golden Gate reaction with BsaI. The MultiGreen vectors all contain the GreenGate overhangs with outwards-oriented BsaI restriction sites. Flanking these are a second set of Golden Gate overhangs with inwards-oriented AarI restriction sites (A-B, B-C,...). These six vectors (or fewer when combined with linkers) are then assembled into a destination vector via a Golden Gate reaction with AarI.



Importantly, BsaI and AarI can be used in the same Golden Gate reaction. Thus we can mix GreenGate and MultiGreen vectors in the same reaction and use the exact same Destination vectors.



There is an optional step where MultiGreen cassettes can be cloned back into an entry vector. This creates a cycle where one can ultimately clone >6 expression cassettes. To facilitate this, we have made new GreenGate entry vectors that contain both Bsal and AarI sites.

Protocol

Follow the regular Golden Gate protocol, using one of the MultiGreen intermediaries as destination vector.

The MultiGreen intermediaries are transformed into *E. coli* and selected on Kanamycin.

The intermediaries are assembled in a destination vector through a second Golden Gate reaction with AarI (if you use a specific destination vector with AarI flanks) or a combination of Bsal and AarI (if you use a regular “A-G” Golden Gate destination vector). For AarI, we use the PaqCI enzyme from NEB, as it seems to work better than the AarI enzyme from ThermoFisher.

GoldenGate with AarI:

Component	Amount	Volume (μL)
pMGG-A-ccdB-CmR-B	100 ng	1
pMGG-B-ccdB-CmR-C	100 ng	1
pMGG-C-ccdB-CmR-D	100 ng	1
pMGG-D-ccdB-CmR-E	100 ng	1
pMGG-E-ccdB-CmR-F	100 ng	1
pMGG-F-ccdB-CmR-G	100 ng	1
Destination vector	100 ng	1
T4 Buffer		1.5
PaqCI activator		0.25
PaqCI		0.75
T4 DNA ligase		0.75
H ₂ O		4.75
Total		15



GoldenGate with AarI + BsaI:

Component	Amount	Volume (μL)
pMGG-A-ccdB-CmR-B	100 ng	1
pMGG-B-ccdB-CmR-C	100 ng	1
pMGG-C-ccdB-CmR-D	100 ng	1
pMGG-D-ccdB-CmR-E	100 ng	1
pMGG-E-ccdB-CmR-F	100 ng	1
pMGG-F-ccdB-CmR-G	100 ng	1
Destination vector	100 ng	1
T4 Buffer		1.5
PaqCI activator		0.25
PaqCI		0.75
BsaI-HF [®] v2		0.75
T4 DNA ligase		0.75
H ₂ O		4
Total		15

Reaction cycles:

37°C	15 min	30-50 X
37°C	3 min	
16°C	3 min	
37°C	5 min	
60°C	5 min	
16°C	∞	

Vector	Vector Vault ID
pMGG-A-ccdB-CmR-B	22_77
pMGG-B-ccdB-CmR-C	22_78
pMGG-C-ccdB-CmR-D	22_79
pMGG-D-ccdB-CmR-E	22_80
pMGG-E-ccdB-CmR-F	22_81
pMGG-F-ccdB-CmR-G	23_01
pGGA000-AarI	25_59
pGGB000-AarI	25_60
pGGC000-AarI	25_61
pGGD000-AarI	25_62
pGGE000-AarI	25_63
pGGF000-AarI	25_64