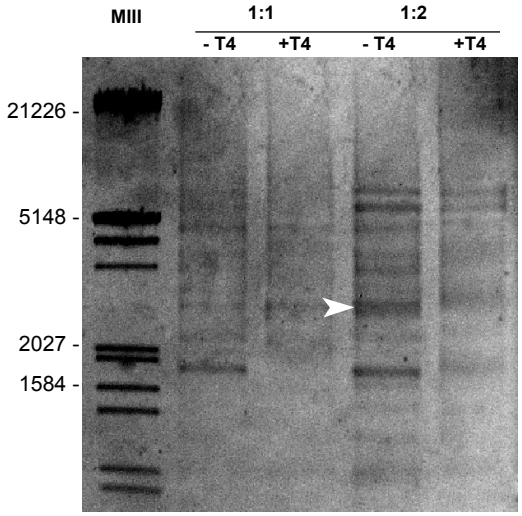
Electronic Supplementary Material (ESI) for RSC Advances.



Supplementary Data 1. Comparison of SLIC recombinations with (+T4) or without T4 DNA ligase (-T4) in different molarity ratios of vector and inserts (1:1 and 1:2). The white arrow demonstrates the correct assembly band (3387 bp) of a four-way assembly containing GW-RFA, GFP, 3'-UTR and eNOS fragments. MIII (Roche) represents the molecular size marker. The highest amount of assembly is shown in 1:2 molar ratio and without T4 DNA ligase treatment (-T4).

Supplementary Data 2. Comparison of the mutation rate estimated for different DNA polymerases used in the study. We sequenced a total of around 30 kb comprising all constructs. When KAPA HiFi HotStart was used to amplify the fragments, we found only one mutation (a silent mutation in the vector backbone) introduced into the final construct by DNA polymerase. The polymerase error rate was higher for Qiagen and Phusion (4.4×10^{-7}) in the preliminary experiments and therefore we employed KAPA HiFi HotStart as the preferred DNA polymerase for all cloning studies. On the other hand, gel purification of the fragments was carried out in a minimum exposure time under UV to avoid DNA damage by UV.

DNA Polymerase	Mutation rate*	SD**
KAPA HiFi HotStart	2.8	0.20
Phusion	4.4	0.17
Qiagen HotStar HiFidelity	12	2.65

* The mutation rate is presented as the number of point mutations in 10 Mb of the amplified sequence.

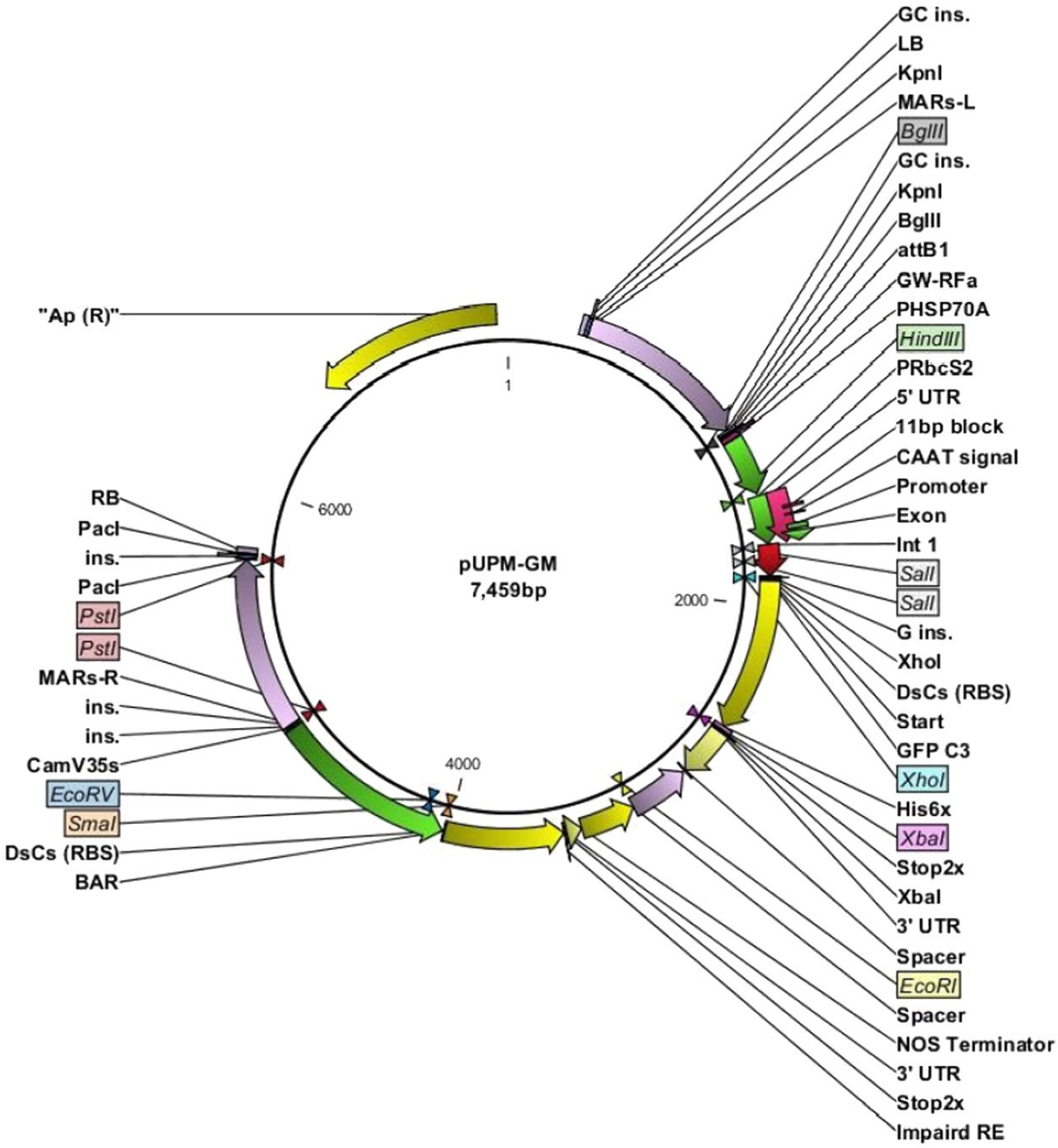
** SD: standard deviation

Supplementary Data 3. The efficiency of an eight-way DNA fragment assembly using the same amount of DNA through SLIC and MOE-PCR, as presented in the number of colonies obtained after transformation. Two different experiments were assessed in each method; the number of cycles (15/25) in MOE-PCR and presence/absence (+/-) of T4 DNA ligase in SLIC. There were no colonies obtained for the SHA technique.

Method	Experiment	1 : 1*		1 : 2	
		TC**	PC	TC	PC
MOE-PCR	15 cycles	19	4	26	5
	25 cycles	51	10	38	8
SLIC	- T4 DNA ligase	90	5	60	3
	+ T4 DNA ligase	85	4	58	4

* Molar ratio of the vector : inserts

** TC: total colonies, PC: positive colonies according to the restriction enzyme analysis



Supplementary Data 3. Virtual map of a circular recombinant construct containing all required elements. This vector represents a series of expression vectors constructed though MOE-PCR.