Platinum Gate TALEN construction protocol (Yamamoto lab)

Ver. 1.0

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This is a protocol for the construction of highly-active **Platinum TALEN** described in Sakuma *et al.*, Sci Rep, 2013 (http://dx.doi.org/10.1038/srep03379). Since the assembly method is based on the "Golden Gate TALEN and TAL Effector Kit" (Addgene; cat#1000000024) and the "Yamamoto Lab TALEN Accessory Pack" (Addgene; cat#1000000030), NAR paper by Cermak *et al.* (http://dx.doi.org/10.1093/nar/gkr218) and Genes to Cells paper by Sakuma *et al.* (http://dx.doi.org/10.1111/gtc.12037) may be of good help to understand more about the principle of the Platinum Gate system.

Note that this kit is NOT compatible with the Golden Gate TALEN and TAL Effector Kit, and does NOT contain the reporter vector for single-strand annealing assay. If you need the vector for the evaluation of TALEN activities, you should separately obtain the pGL4-SSA vector (Addgene plasmid ID: 42962) aside from the Platinum Gate TALEN kit.

■Table of Contents

■Materials

○Plasmids

Platinum Gate TALEN kit (Addgene)

Module plasmids: **p1-4HD, p1-4NG, p1-4NI, p1-4NN**
(16 plasmids, Ampicillin resistant)

Intermediate array plasmids: **pFUS2_a1a, a2a, a2b, a3a, a3b, a4a, a4b, b1, b2, b3, b4**
(11 plasmids, Spectinomycin resistant)

Destination vectors: **ptCMV-136/63-VR-HD, NG, NI, NN**
ptCMV-153/47-VR-HD, NG, NI, NN
(8 plasmids, Ampicillin resistant)

○Reagents (excluding standard PCR enzymes and reagents for bacterial culture)

Maker	Product name	Cat. No.	Size/Unit
NEB	Quick Ligation Kit	M2200S	30 reactions
		M2200L	150 reactions
NEB	BsaI-HF	R3535S	1000 units
		R3535L	5000 units
Thermo Scientific	Esp3I	ER0452	1000 units
NEB	10×T4 DNA Ligase Reaction Buffer	B0202S	6 ml
Life Technologies	ChargeSwitch-Pro Plasmid Mini Kit	CS30250	250 reactions

○Primers

pCR8_F1: TTGATGCCTGGCAGTTCCCT

pCR8_R1: CGAACCGAACAGGCTTATGT

TALE-F: GAGCACCCCTCAACCTGACCCCAG

TALE-R: CTCGAAAGCTGGGCCACGATTG

■Methods

1. TALEN design

Design TALEN target sequence using “TALEN Targeter”.

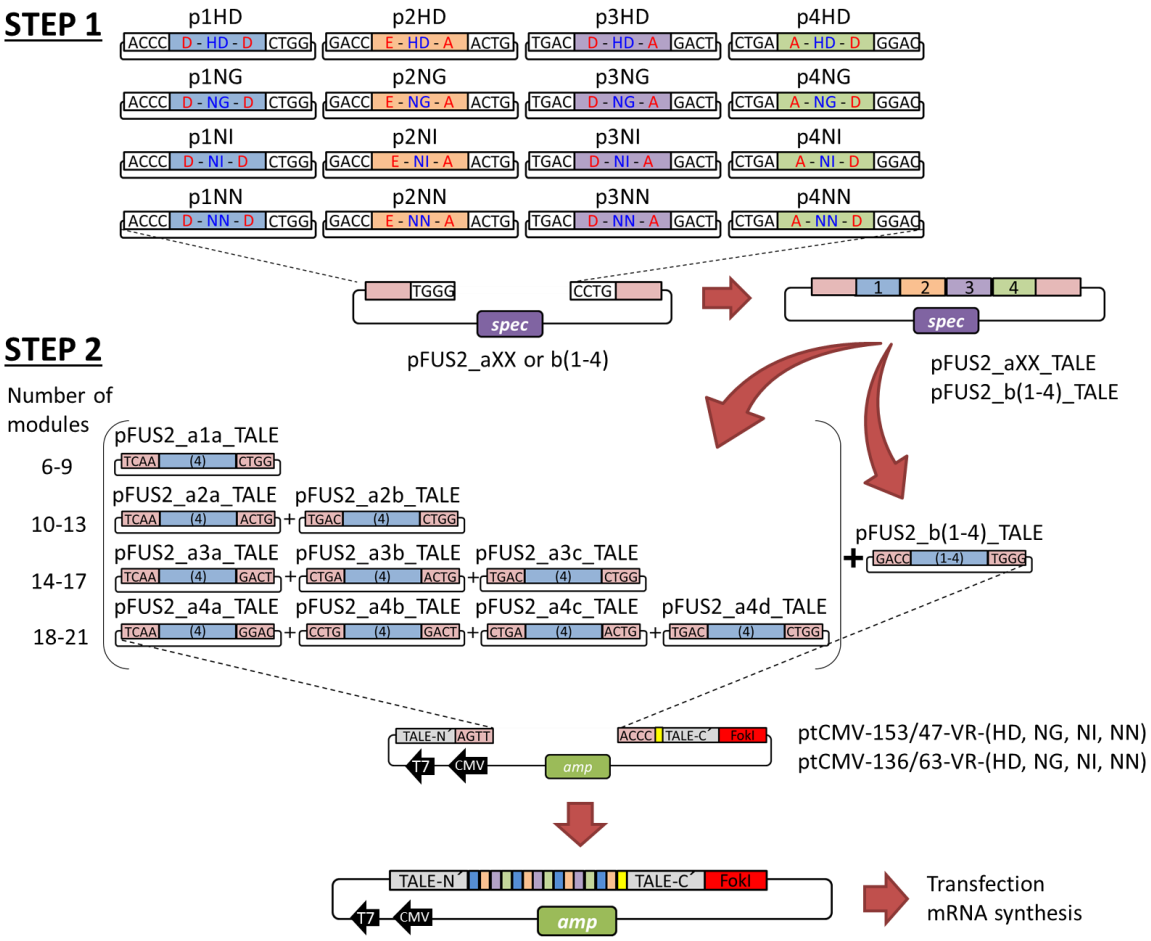
TALEN Targeter

https://tale-nt.cac.cornell.edu/

According to our past results, it is preferable that spacer length is around 15 bp (12-16 bp) and 17 bp (15-19 bp) for 153/47 and 136/63 scaffolds, respectively. Optimal number of repeat array is 15-20. Set “Upstream Base” as “T only”.

2. Platinum Gate assembly -STEP 1-

Assemble RVD repeats for the chosen target sequences. Using our methods, you can make TALENs bearing 6-21 repeats. It is necessary that we choose appropriate combinations of vectors for the desired module numbers.



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Note that a3c, a4c and a4d are just expediential names. Use a2b as a3c/a4d, and a3b as a4c, respectively.

To construct each pFUS2_axx_TALE plasmid harboring four modules, perform Golden Gate assembly (see Appendix for detailed reaction conditions).

Transform 0.5-1 μl of the reaction product directly to chemical competent *E. coli* such as XL1-Blue and streak the transformant on Spectinomycin/X-gal/IPTG LB plate as described in Appendix. Culture O/N at 37°C.

Select the desired clones using colony PCR. Detailed reaction condition is described in Appendix.

Culture the desired clones with 100 ng/μl Spectinomycin/LB media. Purify the plasmids using Miniprep kit.

3. Platinum Gate assembly -STEP 2-

Perform 2nd-step assembly using the 4-module ligated plasmids constructed in the section 2, following a protocol described in Appendix.
Transform the reaction product to XL1-Blue and streak the transformant on Ampicillin/X-gal/IPTG LB plate. Culture O/N at 37°C.

Screen for correctly assembled clones by colony PCR. You can use TALE-F/TALE-R primers both for ptCMV-153/47-VR and ptCMV-136/63-VR vectors. The detailed reaction condition is described in Appendix.

Culture the desired clones and purify the plasmids using Miniprep kit.

Platinum Gate TALEN construction protocol

-Appendix-
Bench protocol for STEP1 & 2 (Yamamoto lab)

STEP1 (μl)

■Golden Gate reaction	1 module	2 modules	3 modules	4 modules
25ng/μl pFUS2 vector	0.3			
50ng/μl module	0.3 × 1	0.3 × 2	0.3 × 3	0.3 × 4
10×T4 DNA ligase buf.	0.2			
BsaI-HF	0.1	premix → 0.4μl each		
Quick ligase	0.1			
SDW	1	0.7	0.4	(0.1)
Total	2			

37℃	16℃	4℃
5min.	10min.	∞
×3		

→ Additional digestion:
add 10×NEBuf.4 0.25 μl, 10×BSA 0.25 μl, BsaI-HF 0.1 μl.
premix → 0.6μl each 50℃, 30min. / 80℃, 5min. / 4℃, ∞

■Transformation

Product	0.5	
XL1-Blue (homemade)	10	on ice >5min. → 42℃, 30 sec. → on ice → plating (100μg/ml Spec plate w/ X-gal+IPTG)

■Colony PCR

10X buf. for Hybripol	0.8						
dNTPs	0.64						
10μM primer	0.16+0.16	(pCR8_F1/R1)					
Hybripol DNA polymerase (BIOLINE)*	0.04						
$MgCl_2$	0.24	95℃	95℃	55℃	72℃	72℃	4℃
SDW	5.96	30s.	15s.	15s.	15s.	15s.	∞
Total	8			×27			

*Any other standard Taq polymerase can be used.

STEP2

■Golden Gate reaction		a1a	a2a-a2b	a3a-a3c	a4a-a4d
50ng/μl	pFUS2_a	0.6	0.6×2	0.6×3	0.6×4
50ng/μl	pFUS2_b	0.6			
50ng/μl	ptCMV vector	0.3			
10×T4 DNA ligase buf.		0.4			
Esp3I		0.2	premix → 0.8μl each		
Quick ligase		0.2			
SDW		1.7	1.1	0.5	0
Total		4			

37℃	16℃	4℃
5min.	10min.	∞

×6

→ Additional digestion: add 10×Tango buf. 0.5 μl, 10mM DTT 0.5 μl, Esp3I 0.2 μl.
premix → 1.2μl each 37℃, 1hr / 80℃, 5min. / 4℃, ∞

■Transformation

Product	2	
XL1-Blue (homemade)	20	on ice >5min. → 42℃, 30 sec. → on ice → plating (100μg/ml Amp plate w/ X-gal+IPTG)

■Colony PCR

10X buf. for Hybripol	0.8	
dNTPs	0.64	
10μM primer	0.16+0.16	(TALE-F/R)
Hybripol DNA polymerase (BIOLINE)*	0.04	
$MgCl_2$	0.24	
SDW	5.96	
Total	8	

95℃	95℃	66℃	72℃	72℃	4℃
30s.	15s.	15s.	50s.	50s.	∞

×27

*Any other standard Taq polymerase can be used.