

Golden Gate TALE Assembly Protocol

DAY1

- 1 Enter the RVD sequence of your TALE on the Golden Gate TAL Assembly form, remember to enter the Last Repeat RVD in the separate box and the desired s pTAL backbone (1, 2, 3 or 4). Print the form.
- 2 Mix the 1st Cycle Reactions accordingly to the form. Label each tube with the TAL number/name and the reaction letter (e.g. 35A, 35B, 35C) and date
- 3 Run cycle: 10x(37°C/5min + 16°C/10min) + 50°C/5min + 80°C/5min
- 4 Plasmid-Safe nuclease treatment: To each of your 1st cycle reactions add:
 - a)1ul 25mM ATP
 - b)1ul Plasmid-Safe nucleaseIncubate at 37°C/1h
- 5 Transform DH5alpha chemically competent cells with 5ul of the reactions.
- 6 Plate on LB+Sp50+X-Gal+IPTG

DAY2

- 7 Inoculate 1-3 white colonies from each reaction into 5ml LB+Sp50

DAY3

- 8 Miniprep and nanodrop the plasmids
- 9 Diagnostic restriction digestion with AflIII + XbaI to check clones:
AflIII + XbaI

Vector	Backbone	Repeats*
pFUS A	2447	572
pFUS A + 10 Repeats	2447	1048
pFUSA-30A	2447	571
pFUSA-30A + 10 Repeats	2447	1048
pFUSA-30B	2447	559
pFUSA-30B + 10 Repeats	2447	1035
pFUS B	2447	530 to 590
pFUS B + 1 Repeat	2447	147
pFUS B + 2 Repeat	2447	245
pFUS B + 3 Repeat	2447	344
pFUS B + 4 Repeat	2447	430
pFUS B + 5 Repeat	2447	523
pFUS B + 6 Repeat	2447	622
pFUS B + 7 Repeat	2447	720
pFUS B + 8 Repeat	2447	820
pFUS B + 9 Repeat	2447	920
pFUS B + 10 Repeats	2447	1008

* Approximated sizes

- 10 Send clones to sequence with the following primers
pCR8-F1 ttgatgcctggcagttccct
pCR8-R1 cgaaccgaacaggcttatgt
- 11 Mix 2nd Cycle Reactions accordingly to the form

- 12 Run cycle: 10x(37°C/5min + 16°C/10min) + 37°C/15min + 80°C/5min
 13 Transform DH5alpha chemically competent cells with 5ul of the reactions.
 14 Plate on LB+Ap100+X-Gal+IPTG

DAY4

- 15 Inoculate 1-3 white colonies from each reaction into 5ml LB+Ap100

DAY5

- 16 Miniprep the pTAL vectors containing your final full-length TALE.
 17 Diagnostic restriction digestion with AatII + StuI to check clones:

AatII + StuI

Original Vector	Backbone	Fragment*
pTAL1	4141	636
pTAL2	4138	636
pTAL3	6894	636
pTAL4	7831	636

Final number of Repeat	Backbone	Fragment
11	See above	1316
12	See above	1418
13	See above	1520
14	See above	1622
15	See above	1724
16	See above	1826
17	See above	1928
18	See above	2030
19	See above	2132
20	See above	2234
21	See above	2336
22	See above	2438
23	See above	2540
24	See above	2642
25	See above	2744
26	See above	2846
27	See above	2948
28	See above	3050
29	See above	3152
30	See above	3254
31	See above	3356

* Approximated sizes

- 18 Send clones to sequence with the following primers

TAL-F1 ttggcgtcggcaaacagtgg
 TAL-R2 ggcgacgaggtggtcgttg