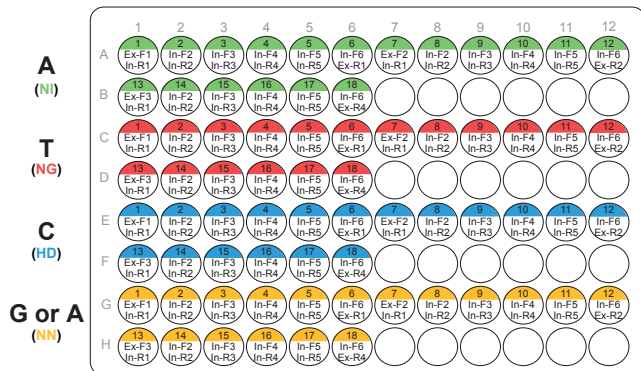


The following protocol is adapted from Sanjana *et al.*, Nature Protocols, 2011. Additional details and troubleshooting steps can be found in the full protocol manuscript and www.taleffectors.com.

- 1 I Set up two 96-well monomer library PCR plates following the primer organization below.

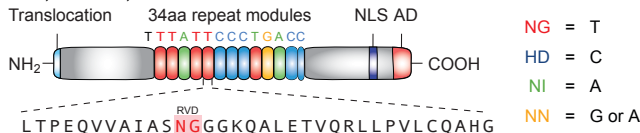


- 2 I Prepare two 100 ul reactions for each monomer as follows:
- | | |
|--------------------------------|--------------------------------|
| Monomer template (5 ng/ul) | 2 ul |
| 100mM dNTP (25 mM each) | 2 ul |
| 5X Herculase II PCR buffer | 40 ul |
| 20uM primer mix (10 uM each) | 4 ul |
| Herculase II Fusion polymerase | 2 ul |
| Distilled water | 150 ul |
| Total | 200 ul (100 ul per rxn) |

Perform PCR reactions using the following cycle parameters:

95 °C	2 min	30 times
95 °C	20 sec	
60 °C	20 sec	
72 °C	10 sec	
72 °C	3 min	

- 3 I Purify PCR products and normalize the concentration of each monomer using gel electrophoresis. Adjust monomers 1, 6, 7, 12, 13, and 18 to 18 ng/ul and the other monomers to 15 ng/ul.
- 4 I TALE recognition sequences are identified in the 5' to 3' direction and begin with a 5' thymine. The procedure below describes the construction of TALEs that bind a 20 bp target sequence (5'-T₀N₁N₂N₃N₄N₅N₆N₇N₈N₉N₁₀N₁₁N₁₂N₁₃N₁₄N₁₅N₁₆N₁₇N₁₈N₁₉-3', where N = A, G, T, or C), where the first base (typically a thymine) and the last base are specified by sequences within the TALE backbone. The middle 18 bp are specified by the RVDs in 18 tandem repeat monomers in the middle of TALE according to the cipher NI = A, HD = C, NG = T, NN = G or A.



Divide target sequences into three hexamers: N₁N₂N₃N₄N₅N₆, N₇N₈N₉N₁₀N₁₁N₁₂, and N₁₃N₁₄N₁₅N₁₆N₁₇N₁₈.

- 5 I Assemble hexamers using Golden Gate digestion-ligation as follows:
- | | |
|--------------------------------|--------------|
| Esp3I (<i>Bsm</i> BI, 5 U/ul) | 0.75 ul |
| Tango Buffer (10 X) | 1 ul |
| Dithiothreitol (DTT, 10 mM) | 1 ul |
| T7 Ligase (3000 U/ul) | 0.25 ul |
| ATP (10 mM) | 1 ul |
| 6 monomers (1 ul each) | 6 ul |
| Total | 10 ul |

Incubate the reaction using the following cycling parameters:

37 °C	5 min	15 times
20 °C	5 min	

- 6 I Treat Golden-Gate reaction from previous step using exonuclease to remove incomplete ligation products.
- | | |
|-------------------------------------|--------------|
| Golden Gate rxn from Step 5 | 7 ul |
| PlasmidSafe DNase (10 U/ul) | 1 ul |
| Plasmid-Safe Reaction Buffer (10 X) | 1 ul |
| ATP (10 mM) | 1 ul |
| Total | 10 ul |

Incubate the reaction at 37 °C for 30 min, followed by inactivation at 70 °C for 30 min.

- 7 I PCR amplify successfully assembled hexamers:
- | | |
|---|--------------|
| PlasmidSafe-treated hexamer from Step 6 | 1 ul |
| 100mM dNTP (25 mM each) | 0.5 ul |
| 5 X Herculase II PCR buffer | 10 ul |
| 20 uM Hex-F & Hex-R primer mix (10 uM each) | 1 ul |
| Herculase II Fusion polymerase | 0.5 ul |
| Distilled water | 37 ul |
| Total | 50 ul |

Incubate the PCR reactions using the following cycling parameters:

95 °C	2 min	30 times
95 °C	20 sec	
60 °C	20 sec	
72 °C	30 sec	
72 °C	3 min	

- 8 I Purify each hexamer PCR product (~700bp) using gel extraction and adjust concentration to 20ng/ul.
- 9 I Golden Gate assembly of hexamers into TALE backbone: Combine the hexamers and the appropriate TALE backbone vector (transcription factor or nuclease) in a Golden Gate digestion-ligation. Make sure to select the vector with the correct 0.5 repeat (determined by the 20th base N₂₀ in the targeting site).

	TALE	Neg Ctrl
TALE backbone vector (100 ng/ul)	1 ul	1 ul
<i>Bsal</i> -HF (20 U/ul)	0.75 ul	0.75 ul
10 X NEBuffer 4	1 ul	1 ul
10 X Bovine Serum Albumin (BSA)	1 ul	1 ul
ATP (10 mM)	1 ul	1 ul
T7 Ligase (3000 U/ul)	0.25 ul	0.25 ul
3 purified hexamers (1ul each)	3 ul	-
Distilled H ₂ O	2 ul	5 ul
Total	10 ul	10 ul

Incubate the reaction using the following cycling parameters:

37 °C	5 min	20 times
20 °C	5 min	

- 10 I Transform 5 ul of the Golden Gate reaction from Step 9 into competent cells.
- 11 I Screen successful clones using colony PCR.
- | | |
|---|--------------|
| Bacterial colony suspension (single colony diluted in 100 uL distilled water) | 1 ul |
| 100mM dNTP (25 mM each) | 0.25 ul |
| 10 X <i>Taq</i> -B PCR Buffer | 2.5 ul |
| 20 uM TALE-Seq-F1 & TALE-Seq-R1 primer mix (10 uM each) | 0.25 ul |
| <i>Taq</i> -B polymerase | 0.1 ul |
| Distilled water | 20.9 ul |
| Total | 25 ul |

Incubate the PCR reactions using the following cycling parameters:

94 °C	3 min	30 times
94 °C	30 sec	
60 °C	30 sec	
68 °C	2 min	
68 °C	5 min	

- 12 I Sequence PCR-positive colonies and verify TALE sequences. To generate a reference sequence, see www.taleffectors.com/tools/.