

## PROTOCOL 1A

### TALE/N assembly into module A, B and C plasmids

Time needed to complete this protocol: **3 days (1 cloning step)**

Time needed to obtain the plant transformation vector with complete TALE/Ns: **9 days (3 cloning steps)**

Vectors compatible with this protocol: **pMOD\_A1001, pMOD\_A1002, pMOD\_A1010, pMOD\_A3101, pMOD\_A3301, pMOD\_B2000, pMOD\_B3200, pMOD\_B3400, pMOD\_C3106, pMOD\_C3306**

**Summary:** The TALE will be cloned into the Esp3I sites of the pMOD vector, replacing the ccdB gene. Correct clones are ready for assembly into transformation backbones (PROTOCOL 5). See also PROTOCOL 1 description.

Enzymes:

- **Esp3I**
- **T4 DNA ligase + buffer**
- **DNA polymerase (for colony PCR)**

1. Start with the repeat arrays cloned into pFUS\_A and pFUS\_B plasmids (Day 3 in Cermak et al. (2015)).
2. For each TALE/N, prepare the following reaction:
  - a) 150 ng of each array vector (pFUS\_A with ten repeats cloned and pFUS\_B with ten or fewer)
  - b) 150 ng of the last repeat vector
  - c) 75 ng of the module vector pMOD\_A1001, pMOD\_A1010 or pMOD\_B2000
  - d) 1 µl Esp3I
  - e) 1 µl T4 DNA ligase
  - f) 2 µl 10X T4 DNA ligase buffer
  - g) H<sub>2</sub>O up to 20 µl total reaction volume
3. Place the Golden Gate reactions in a PCR machine and run the following cycle: 10x (37°C/5min + 16°C/10min) + 37°C/15min + 80°C/5min.
4. Transform 5 µl of each Golden Gate reaction into *E. coli* (DH5α or similar, but sensitive to the presence of ccdB gene) and plate on LB + 50mg/L ampicillin/carbenicillin
5. Correct clones can be identified via colony PCR, using primers TAL2Aseq\_F1 and TAL\_R2 for module A and TALseq\_5-1 and TAL2Aseq\_R2 for module B (see the table below for primer sequences)
6. Isolate plasmid DNA for one correct clone (can be sequenced using primers TAL2Aseq\_F1 and TAL\_R2 for module A and TALseq\_5-1 and TAL2Aseq\_R2 for module B).

7. Assemble TALE/Ns in modules A and B into selected transformation backbone using **PROTOCOL 5**.

**Primers for colony PCR and sequencing (5' to 3')**

MODULE A	TAL2Aseq_F1	TGCCAGCAGGAATGCGTTA
	TAL_R2	GGCGACGAGGTGGTCGTTGG
MODULE B	TALseq_5-1	CATCGCGCAATGCACTGAC
	TAL2Aseq_R2	CGCGAGTGCCACCAGATGA