# PROTOCOL 1B

**TALE/N assembly into DIRECT vectors**

Time needed to complete this protocol: **6 days (2 cloning steps)**

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

Vectors compatible with this protocol: **pDIRECT_21J, pDIRECT_22J, pDIRECT_23J, pDIRECT_25K, pDIRECT_26K, pDIRECT_37J, pDIRECT_38J, pDIRECT_39J**

**Summary: The first TALE will be cloned into the Esp3I sites of the pDIRECT vector, replacing the ccdB gene. After clones containing the first TALE are identified and DNA prepared, the second TALE is cloned into the BsaI sites of the pDIRECT-derived vector, replacing the LacZ gene. Correct clones are ready for plant transformation after sequencing. See also PROTOCOL 1 description.**

Enzymes:

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

1. Start with the repeat arrays for TALE/N 1 cloned into pFUS_A and pFUS_B plasmids (Day 3 in Cermak et al. (2015). TALE/N 2 will be assembled in another cloning step.

2. Prepare the following reaction:
   a) 150 ng of each array vector for TALE/N 1 (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 pDIRECT vector
   d) 1 µl Esp3I
   e) 1 µl T4 DNA ligase
   f) 2 µl 10X T4 DNA ligase buffer
   g) $H_2O$ 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 the ccdB gene) and plate on LB + 50mg/L kanamycin. Note, if X-gal is included in this step, all colonies (correct and incorrect) should be blue.

5. Correct clones can be identified via colony PCR, using primers TAL2Aseq_F1 and TAL_R2 (see the table below for primer sequences)

6. Isolate plasmid DNA for one correct clone.

7. Prepare the following reaction:
   d) 150 ng of each array vector for TALE/N 2 (pFUS_A with ten repeats cloned and pFUS_B with ten or fewer)
   e) 150 ng of the last repeat vector
   f) 75 ng of the vector from step 6
   d) 0.5 µl BsaI
   e) 0.5 µl Esp3I
   f) 1 µl T4 DNA ligase
   g) 2 µl 10X T4 DNA ligase buffer
   h) $H_2O$ up to 20 µl total reaction volume

8. 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.

9. Transform 5 µl of each Golden Gate reaction into *E. coli* (DH5$\alpha$ or similar) and plate on LB + 50mg/L kanamycin + 32mg/L X-gal.

10. Correct clones can be identified via PCR on white colonies, using primers TALSeq_5-1 and TAL2A_SeqR2 (see the table below for primer sequences)

11. Isolate the plasmid DNA for one correct clone (can be sequenced using primers TAL2Aseq_F1 and TAL_R2 for TALEN 1 and TALseq_5-1 and TAL2Aseq_R2 for TALEN 2).

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

| | | |
|---|---|---|
| TALE/N 1 | TAL2Aseq_F1 | TGCCAGCAGGAATGCGTTA |
| | TAL_R2 | GGCGACGAGGTGGTCGTTGG |
| TALE/N 2 | TALseq_5-1 | CATCGCGCAATGCACTGAC |
| | TAL2Aseq_R2 | CGCGAGTGCCACCAGATGA |

**REFERENCES**

**Cermak, T., Starker, C.G., and Voytas, D.F.** (2015). Efficient design and assembly of custom TALENS using the golden gate platform. Methods Mol. Biol. **1239**: 133–159.