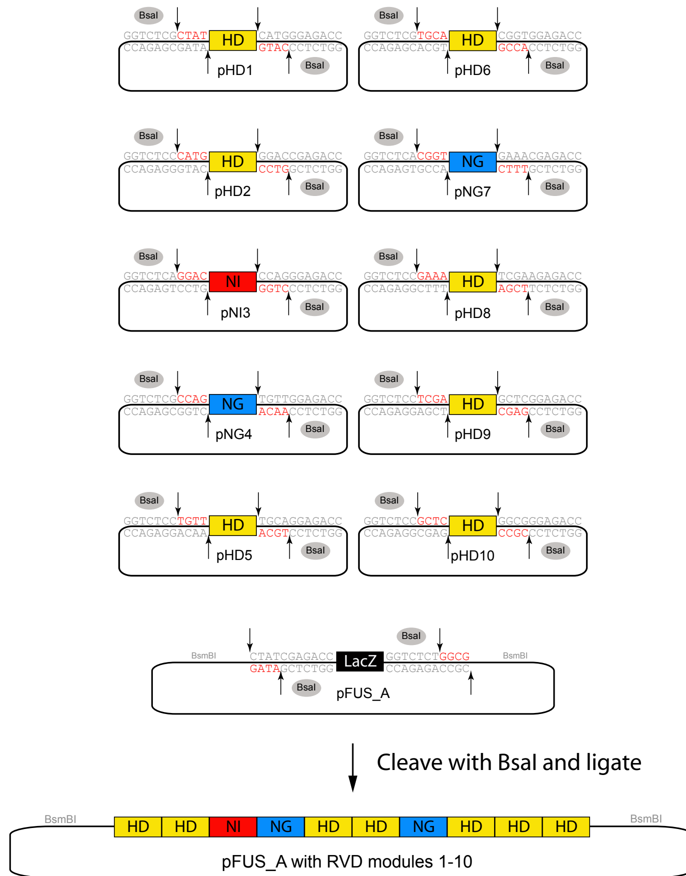
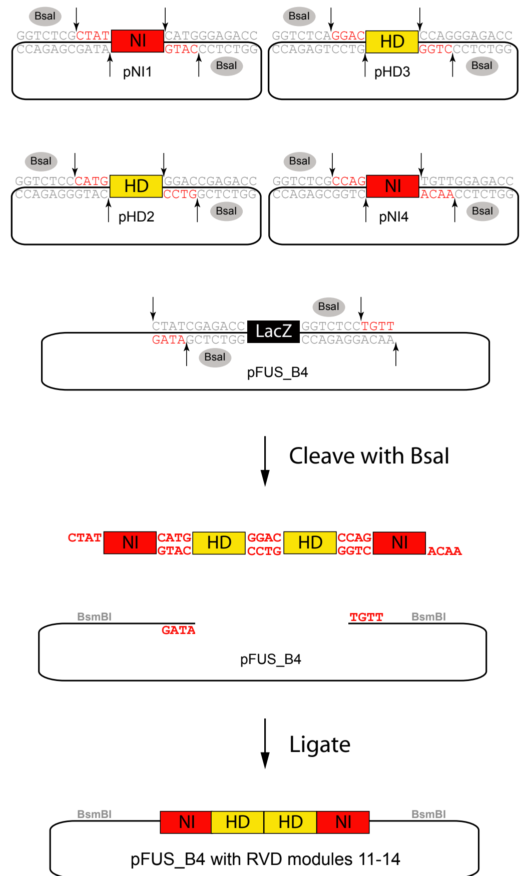
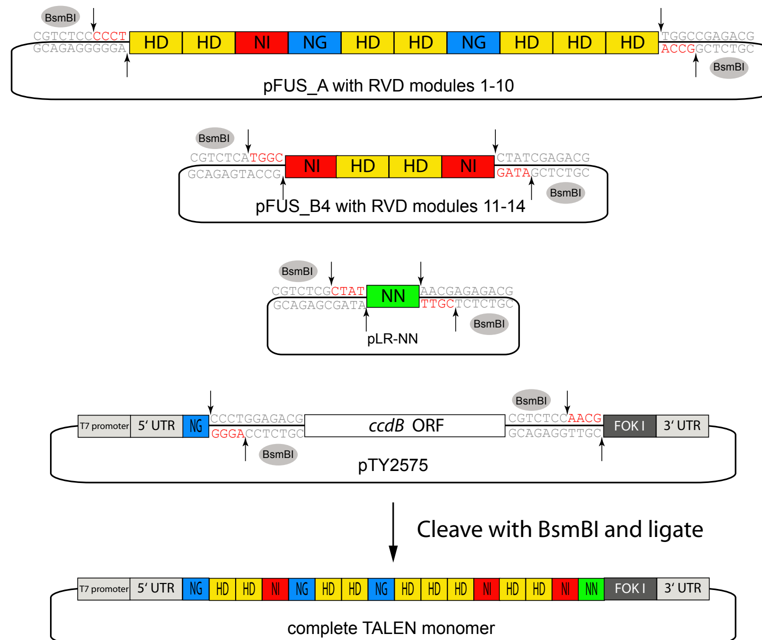


pFUS_A Reaction
step 1
pHD1
pHD6
pHD2
pNG7
pNI3
pHD8
pNG4
pHD9
pHD5
pHD10
LacZ
pFUS_A
Cleave with BsaI and ligate
pFUS_A with RVD modules 1-10



pFUS_B Reaction
pNI1
pHD3
pHD2
pNI4
LacZ
pFUS_B4
Cleave with BsaI
pFUS_B4
Ligate
pFUS_B4 with RVD modules 11-14



step 2
pFUS_A with RVD modules 1-10
pFUS_B4 with RVD modules 11-14
pLR-NN
T7 promoter
5′ UTR
ccdB ORF
FOK I
3′ UTR
pTY2575
Cleave with BsmBI and ligate
complete TALEN monomer

Figure S2 Diagram for the construction of a TALEN. TALENs were constructed using the Golden Gate TALEN Kit. See http://www.addgene.org/TALefector/goldengate/ voytas/. This kit includes numerous RVD-encoding plasmids that permit the efficient assembly of RVD domains in the desired order for DNA recognition. The assembly principle is based on the ability of Type IIS restriction enzymes to cleave outside of their recognition sequence, specifically to one side. The nucleotides between the recognition site and the cleavage site of each RVD has a unique end once cleaved from its plasmid so that the cleaved RVD can only ligate with an RVD having a complementary sequence. Thus, RVDs can assemble in only one order. The kit is organized to permit 10 RVDs to be assembled in one reaction (A reaction) and the next 10 RVDs to be assembled in a second reaction (B reaction) (see step 1). The products of the two reactions are then assembled into the final TALEN (step 2). A more extended explanation for the RVD assembly in the first reaction is the following: For each RVD position in the final assembly, plasmids are provided for all possible RVDs so that after BsaI digestion, any RVD for that position will have the same overhanging sequences. Using the first position as an example, the desired RVD can be retrieved from any one of the plasmids pHD-1, pNN-1, pNG-1 pNI-1, and either pNK-1 or pNH-1. After digestion with BsaI each RVD will have overhangs of 5' CTAT 3' at one end and 3' GTAC 5' at the other end (red bases). Similarly, for the second position, plasmids are provided for all possible RVDs such that after BsaI digestion, all RVDs have a 3' GTAC 5' overhang that uniquely complements the 5' CATG 3' overhang of the first-position RVD.

Step 1. The plasmids for the first 10 RVDs (Reaction A) are mixed with the plasmid pFUS_A, T4DNA ligase, and the Type IIS restriction enzyme BsaI. The mixture is treated to 10 cycles of 37°C for BsaI digestion and 16°C for ligation. The plasmids for RVDs 11 - 14 and pFUS_B4 are treated in the same manner. Ligated DNAs from the two reactions are transformed independently into DH5α chemically competent cells and plated on LB agar with spectinomycin, IPTG, and X-gal. For each transformation, white colonies are selected and checked by colony PCR or by restriction digestion of isolated plasmid DNA with ApaI and BamHI. Plasmids can be sequenced to insure that they have the correct RVDs.

Step 2. The two plasmids with the correct RVDs from step 1 are mixed with a plasmid containing the last RVD (pLR-NN in this example), and our customized C. elegans vector (pTY2575) that contains a T7 promoter, a 5' UTR, the initial RVD NG, a ccdB gene for selection, a FokI nuclease monomer, and 3' UTR. The type IIS restriction enzyme BsmBI and T4 ligase are added to this mix and treated to 10 cycles of 37°C and 16°C as above. The resulting ligation is transformed into DH5α cells and plated on LB agar with Ampicillin. The ccdB ORF targets DNA gyrase and kills bacteria that receive a plasmid containing this DNA. In a correctly assembled TALEN clone, the ccdB ORF will have been replaced by TALEN sequences, permitting the bacteria to live. Resulting colonies are screened by colony PCR or by digestion with diagnostic restriction enzymes and sequenced to confirm that the RDVs are correct.