

## PROTOCOL 5 - DESCRIPTION

### Assembly of A, B and C modules into transformation backbones

Construction of transformation vectors from the intermediate module plasmids A, B and C is based on a simple Golden Gate protocol using the AarI enzyme. Functional elements in each of the vector types are flanked by two AarI sites producing specific 4 bp overhangs after digestion with the AarI enzyme. The specific 4 bp overhangs allow for assembly of the DNA fragments released from the module plasmids in a specified order (A → B → C). All additional AarI sites have been removed from all plasmid backbones, regulatory elements and genes in the four vector sets (A, B, C and transformation backbones) in order to adapt them to this protocol. One plasmid from each group A, B and C is mixed with a selected transformation backbone in a single tube and functional elements are assembled into a final transformation vector as shown below. All three modules must be supplied in the reaction mix for successful assembly. However, empty modules A, B and C (containing ~50-100 bp of sequence with unique restriction sites) are available as placeholders to fill in any position in the assembly, if a coding/gRNA sequence is not required.

