

## **PROTOCOL 2 - DESCRIPTION**

### **Assembly of a single gRNA spacer into Pol III cassettes**

This protocol describes cloning of a single gRNA spacer under the control of an RNA Pol III promoter in either module or DIRECT vectors. Spacer sequences are added via Golden Gate assembly of annealed oligonucleotides containing the gRNA spacer sequence. The approach does not require a separate plasmid digestion and purification step. While cloning into the DIRECT vectors (**PROTOCOL 2B**) provides the fastest way to build constructs ready for transformation, the modular protocol (**PROTOCOL 2A**) should be used when additional elements (such as a gene targeting donor, TREX2 or GFP expression cassettes) are required in the final vector.