PROTOCOL 1 - DESCRIPTION

TALE/N assembly

Protocols for TALE and TALEN assembly are based on the Golden Gate cloning approach described earlier (Cermak et al., 2011) and require the Golden Gate TALEN and TAL Effector Kit 2.0 (Addgene, Cambridge, MA, USA) (http://www.addgene.org/TALeffector/goldengateV2/). For a more detailed, updated protocol, see also Cermak et al. (2015). All TALE/N vectors in the vector set described here are fully compatible with the cloning approach described in these two publications and should be used as the final expression vectors instead of pTAL1, pTAL2, pTAL3, pTAL4, pZHY500 and pZHY501. The following two protocols describe the cloning process to assemble TALE repeats into the module A, B and C and the direct cloning backbones, and start on the Day 3 of TALE/N assembly (Cermak et al., 2015). The time required to assemble a complete TALE/N pair in a transformation vector is the same for the modular and direct protocols, and the final expression vectors are functionally equivalent. However, the modular protocol should be used when additional elements (such as a donor template for gene targeting, TREX2 or GFP expression cassettes) are required in the final vector, as these are included in module C vectors that are easily combined with the TALE/Ns in modules A and B.

References

Cermak, T., Doyle, E., Christian, M., Wang, L., Zhang, Y., Schmidt, C., Baller, J. A., Somia, N. V., Bogdanove, A.J., and Voytas, D.F. (2011). Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res. **39**: e82.

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.