**pCrU6.4-SpCas9 cloning (pPH160)**

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Cloning vector for *Streptococcus pyogenes* Cas9 (SpCas9) single guide RNA transcription, controlled by the U6 snRNA promoter #4 (3’ of [Cre15.g640800](https://phytozome.jgi.doe.gov/pz/portal.html#!gene?search=1&crown=1&detail=1&method=0&searchText=transcriptid:30783541)). The 20 bp target site can be inserted in a cut-ligation reaction as annealed oligos into an *Esp*3I cloning site following the protocol of Ann Ran ([Ran et al. 2013](https://doi.org/10.1038/nprot.2013.143)). The immediate 4-bp sequence upstream of the transcriptional start site was changed to ACTT in all U6 constructs to simplify the cloning procedures.

*Hind*III and *Kpn*I restriction sites can be used for *C. reinhardtii* antibiotic resistance gene insertion (*aphVII*, *aphVIII*, *ble*).

Selection in *C. reinhardtii*: -

Selection in *E.coli*: ampicillin

host strain: DH5α

Sequence map: <https://benchling.com/s/OPkVaRUz>

**Reference**

Greiner, A., Kelterborn, S., Evers, H., Kreimer, G., Sizova, I., and Hegemann, P.Targeting of photoreceptor genes via zinc-finger nucleases and CRISPR/Cas9 in *Chlamydomonas reinhardtii.* Plant Cell 30 (2017). <https://doi.org/10.1105/tpc.17.00659>

**Overview of all CRISPR/Cas9 plasmids from the Hegemann lab**

<https://www.chlamycollection.org/hegemann_lab>

Visit [www.chlamy.de](http://www.chlamy.de) for more info or contact CRISPR@chlamy.de