pIK86 T7-Cas9-2xNLS

This plasmid is associated with the paper:
Iskra Katic and Helge Großhans (2013): Targeted heritable mutation and gene conversion by Cas9-CRISPR in *Caenorhabditis elegans*. Genetics (accepted for publication).

To use pIK86, first digest with HaeII. This will release the T7-Cas9-2xNLS-3'UTR-polyA sequence. We *in vitro* transcribe using NEB # E2040S and cap with ARCA (NEB # S1411S), LiCl-precipitate and inject at a concentration of 1400 ng/µl together with an sgRNA at a concentration of 15-50 ng/µl. sgRNAs cloned into plasmid pDR274 (HWANG *et al.* 2013, Addgene # 42250) are similarly *in vitro* transcribed and capped (NEB # S1407S), column purified (Ambion # AM10070) and boiled for 1 minute before assembling the injection mix.

HWANG W. Y., FU Y., REYON D., MAEDER M. L., TSAI S. Q., SANDER J. D., PETERSON R. T., YEH J.-R. J., JOUNG J. K., 2013 Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol **31**: 227–229.