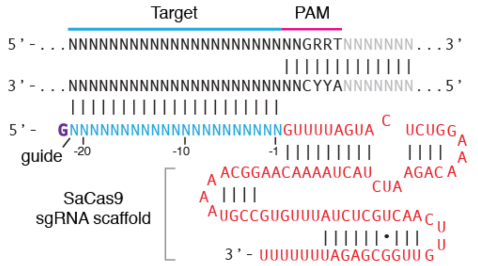
SaCas9 User Manual

PAM sequence

The SaCas9 PAM sequence for optimal on-target cutting is NNGRRT. Nevertheless, targets with PAMs of the form NNGRR(N) also have appreciable cutting and should be considered in evaluating off target activity.

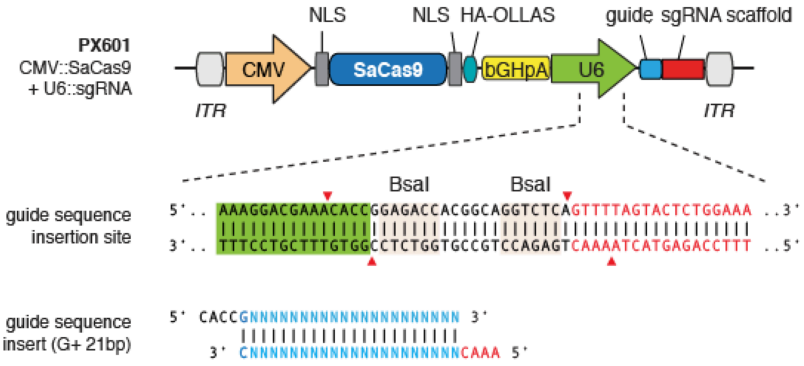


Optimal guide length

The optimal guide length for SaCas9 is 21- or 22-nt. If the guide does not begin with a "G," it is advisable to add a G to the 5' to enhance transcription from the U6 promoter.

Cloning of Guides

Cloning the guide into the vector can be done using the Type IIs restriction site BsaI, which allows scarless insertion of the 21- or 22-bp guide.



Guide sequences can be ordered as standard oligos with 5'-CACC and 5'-AAAC overhangs and "G" addition as illustrated. For details with PNK-treatment, annealing of oligos, and digestion of vector, please see:

http://www.genome-engineering.org/crispr/wp-content/uploads/2014/05/CRISPR-Reagent-Description-Rev20140509.pdf.

Note that the Type IIs sites for SpCas9 plasmids are **BbsI** and those for SaCas9 are **BsaI**, with cloning instructions otherwise identical.

Alternatively, the entire U6::sgRNA region can be replaced by restriction site-flanked PCR amplicon of the appropriate guide. PCR amplicons allow high-throughput screening of guides when co-transfected with SaCas9, and the highest efficiency U6::sgRNA can be cloned into a single vector with SaCas9. See Ran et al., Nature Protocols 2013 for details:

(http://www.nature.com/nprot/journal/v8/n11/abs/nprot.2013.143.html).

Guide testing workflow

Guides can be tested in a cell line from the relevant species. In general, we have found that guides that work in cell lines will also work when delivered to *in vivo* tissue, though efficiencies may differ.

Additionally, AAV purity and accurate titration is essential to getting efficient cutting *in vivo*.