



**Application Notes:** For application of Cas9 for site-specific genome editing in eukaryotic cells and organisms, we have computationally identified suitable target sites for the *S. pyogenes* Cas9. These sites are viewable as [UCSC Genome Browser tracks](#) for the human, mouse, rat, zebrafish, *C. elegans*, and *D. melanogaster* genomes. Sites are selected such that the seed sequence for each SpCas9 target site, 5'-NNNNNNNNNNNN-NGG-3', is specific to the relevant genome. A protocol for oligo cloning is included here and the plasmid sequences and additional information can be found at Zhang Lab's website ([www.genome-engineering.org](http://www.genome-engineering.org)).

**Citation Information:** please reference the following publication for the use of these material.

*Multiplex Genome Engineering using CRISPR/Cas Systems*

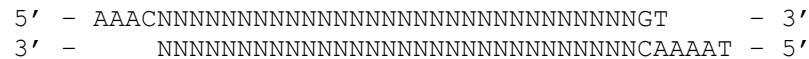
Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Science. 2013 Jan 3. DOI: 10.1126/science.1231143

## Target Sequence Cloning Protocol

(standard de-salted oligos are sufficient)

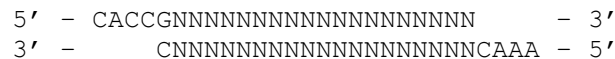
### pX260 (or pX334) – hSpCas9 (or hSpas9n nickase) + CRISPR array + tracrRNA:

In order to clone the target sequence into the pX260 backbone, synthesize two oligos of the form:



### pX330 (or pX335) – hSpCas9 (or hSpCas9n nickase) + chimeric guideRNA:

In order to clone the target sequence into the pX260 backbone, synthesize two oligos of the form:



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### Oligo annealing and cloning into backbone vectors:

1. Digest 1ug of pX260 or pX330 with *BbsI* for 30 min at 37C:

1 ug	pX260 or pX330
1 ul	FastDigest <i>BbsI</i> (Fermentas)
1 ul	FastAP (Fermentas)
2 ul	10X FastDigest Buffer
X ul	ddH <sub>2</sub> O
20 ul	total

2. Gel purify digested pX260 or pX330 using QIAquick Gel Extraction Kit and elute in EB.

3. Phosphorylate and anneal each pair of oligos:

1 ul	oligo 1 (100mM)
1 ul	oligo 2 (100mM)
1 ul	10X T4 Ligation Buffer (NEB)
6.5 ul	ddH <sub>2</sub> O
0.5 ul	T4 PNK (NEB)
10 ul	total

Anneal in a thermocycler using the following parameters:

37°C	30 min
95°C	5 min and then ramp down to 25°C at 5°C/min

4. Set up ligation reaction and incubate at room temperature for 10 min:

X ul	<i>BbsI</i> digested pX260 or pX330 from <b>step 2</b> (50ng)
1 ul	phosphorylated and annealed oligo duplex from <b>step 3</b> (1:200 dilution)
5 ul	2X Quickligation Buffer (NEB)
X ul	ddH <sub>2</sub> O
10 ul	subtotal
1 ul	Quick Ligase (NEB)
11 ul	total

5. (optional but highly recommended) Treat ligation reaction with PlasmidSafe exonuclease to prevent unwanted recombination products:

11 ul	ligation reaction from <b>step 4</b>
1.5 ul	10X PlasmidSafe Buffer
1.5 ul	10mM ATP
1 ul	exonuclease
15 ul	total

Incubate reaction at 37C for 30 min.

6. Transformation