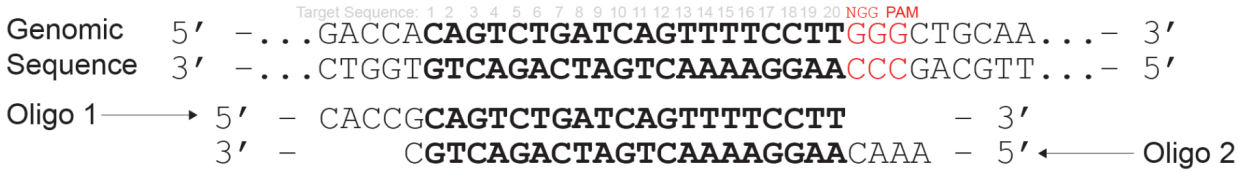
sgRNA cloning protocol – Modified version of S. Konermann's Zhang lab protocol, 2014

In order to clone the guide target sequence into the pLentiCRISPRv2 backbone, synthesize two oligos of the following form. **Both plasmids have the same overhangs**:

*NOTE: The 5' "**G**" (in CACC**G** on Oligo 1) and complementary "**C**" (at 3' end of Oligo 2) only need to be added if sgRNA sequence doesn't already start with "G". If it does, only append 5'CACC to Oligo 1 and 5'AAAC to Oligo 2.*

Example oligo design: Note that the NGG PAM is **not** included in the designed oligos.



Oligonucleotide ordering tips: Standard de-salted oligos (usually the most inexpensive synthesis) are sufficient for cloning. If not already resuspended, dilute each oligo to 100 **µ**M in sterile water or TE.

PROTOCOL: Clone sgRNA into lentiCRISPR vectors using Golden Gate cloning

Step 1: Anneal Oligos

reagent	Volume [in *µ*l]
Oligo 1 (100*µ*M)	1
Oligo 2 (100*µ*M)	1
10X T4 Ligation buffer	1
ddH2O	6.5
T4 PNK	0.5
	10

PCR Program: Anneal

1 37°C - 30min
2 95°C - 5min
3 Ramp to 25°C at rate 6°C/min

- Add 90uL of ddH2O to **product** from **Step 1** to dilute it 1:10, then proceed to **Step 2.**

Step 2: Golden Gate reaction (modified from original Zhang lab protocol)

reagent	1x reaction Volume [in μl]
10x T4 DNA Ligation buffer	2.5
BSA [10mg/ml]	0.25
BsmBI-v2 (*aka* **Esp3i** from NEB)	1
T4-ligase	1
1:10 diluted annealed oligo (in ddH2O)	1
backbone vector [25ng/μl]	1
ddH2O	18.25
	25

PCR Program: GoldenGate

1	42°C - 5min
2	16°C - 5min
	> 15 cycles

Transform Stabl3 E.coli with 2μl of Golden Gate product. Plate onto **Ampicillin** plates. In general, picking 2-3 colonies per guides should be sufficient to ensure a correct clone.

Note: it is not necessary to perform a negative control golden-gate reaction (without insert) as it will always contain colonies and is not a good indicator of cloning success.