Synergistic Activation Mediators

ZhangLab

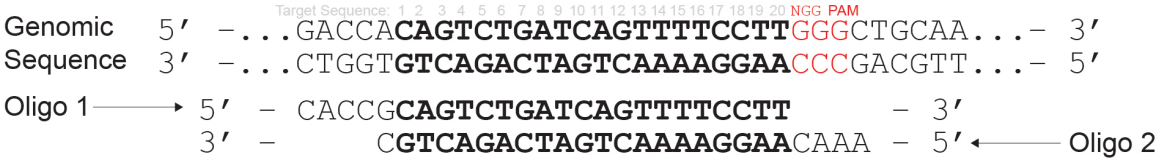
SAM target sgRNA cloning protocol – S. Konermann, Zhang lab, 2014

Optimized sgRNAs for any coding human gene can be found using our SAM Cas9 activator design tool:

http://sam.genome-engineering.org/database/

In order to clone the guide target sequence into the sgRNA(MS2) cloning backbone (addgene #61424) or lenti sgRNA(MS2)_zeo backbone (addgene #61427), synthesize two oligos of the following form. **Both plasmids have the same overhangs**:

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.

Golden-Gate sgRNA cloning protocol

1. **Oligo anneal**

Component	Amount [ul]
Each oligo [100uM]	1
10X T4 ligase buffer (NEB)	1
T4 PNK (NEB)	0.5
H_20	6.5

Mix the components above and anneal in a thermal cycler with the following conditions:

37ºC for 30min
95ºC for 5 min
Ramp to 25ºC at 5ºC/min

2. Golden Gate reaction

Add 90ul of PCR clean H_2O to the finished oligo anneal from above to dilute it 1:10. Then mix the following components:

Note: use **BbsI enzyme** for the non-lentiviral SAM sgRNA backbone (addgene **#61424**) and **BsmBI enzyme** for the lenti SAM sgRNA (zeo) backbone (addgene **#61427**).

Component	**Amount [ul]**
2X rapid ligase buffer (*Enzymatics*)	12.5
BSA [20mg/ml] (*NEB*)	0.125
Restriction enzyme (BbsI for **61424** or BsmBI for **61427**) (*Fermentas FD*)	1
T7 ligase (*Enzymatics*)	0.125
Diluted oligo anneal (1:10) from above	1
Backbone Vector [25ng/ul]	1
H_20	9.25

Run the following program on a thermal cycler:
37C for 5 min
20C for 5 min
→ repeat for 15 cycles total

Transform 2ul of the golden gate reaction in Stbl3 (or other recombination deficient) competent cells. 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.