Background information

Jena Bioscience
Building Blocks of Life

Target-specific oligonucleotide design
for HighYield T7 sgRNA Synthesis Kit (*Sp*Cas9), #RNT-105

1. Introduction

The following guidelines are intended to design a target-specific oligonucleotide to be used for PCR assembly of a sgRNA-encoding T7 DNA template with the HighYield T7 sgRNA Synthesis Kit (*Sp*Cas9) (#RNT-105). The kit contains a *Streptococcus pyogenes* Cas9 (*Sp*Cas9) scaffold as well as two oligonucleotide primer (T7fwd sgRNA & T7rev sgRNA) for PCR amplification.[1,2] A target-specific oligonucleotide (Fig. 1A) needs to be provided by the customer. PCR assembly results in a 127 bp sgRNA encoding T7 DNA template (Fig. 1A) that is subsequently *in vitro* transcribed into the sgRNA transcript (Fig. 1B).

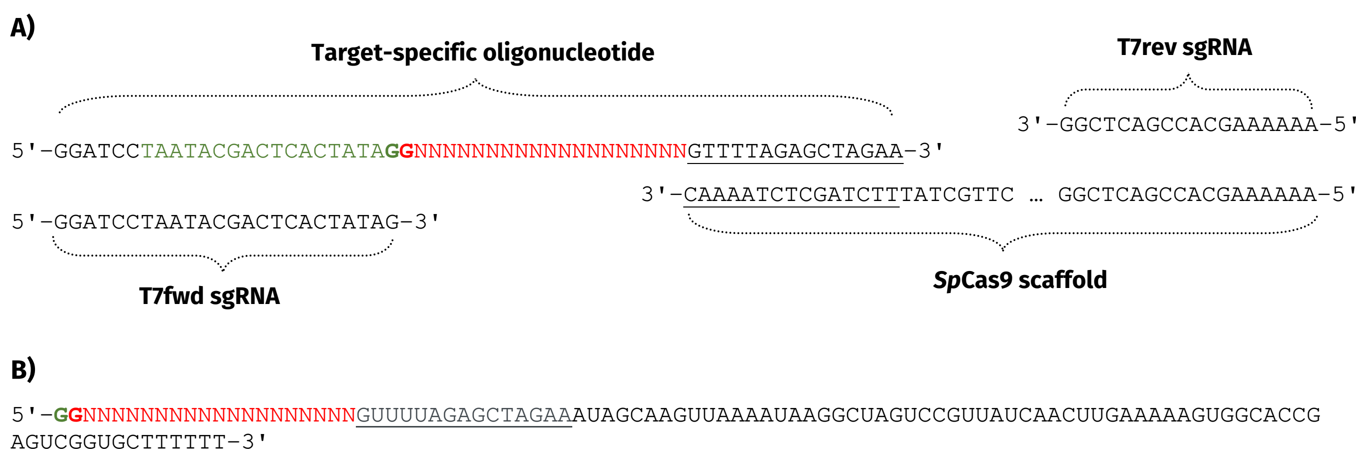

Figure 1 Schematic presentation of PCR assembly reaction (A) as well as final sgRNA transcript (B). N=any nucleotide base, Bold: GG (two G) for efficient *in vitro* transcription, underlined: overlap sequence.

2. Selection of target-specific DNA sequence

We recommend to use a DNA target selection program e.g. Benchling, ChopChop or Deskgen. Several programs have been compared by Cui *et. al.* (2018).[3]

- Select a target-specific DNA sequence that must end with the protospacer adjacent motif **(PAM) sequence NGG** on its 3' end:

  Example: 5'-CTAGCTAGCATTTCTCAGTCCTAAACA**CGG**-3'

- Select 20 nts upstream of the PAM sequence as target-specific DNA sequence (the PAM sequence is not a part of sgRNA sequence):

  Example: 5'-GCATTTCTCAGTCCTAAACA-3'

- Check 5'-end of target-specific DNA sequence for the presence of a "G":

  ➢ *Example 1: Target-specific DNA sequence contains a natural "G" at its 5'-end*

  5'-**G**CATTTCTCAGTCCTAAACA-3'→ proceed with 3.

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➢ *Example 2: Target-specific DNA sequence contains another base at its 5'-end (e.g. A)*

5'-**A**CATTTCTCAGTCCTAAACA-3' → remove 5'-end base and replace with a "G"
→ 5'-**G**CATTTCTCAGTCCTAAACA-3' -> proceed with 3.

3. Design of target-specific oligonucleotide

- Add the following T7 promoter sequence to the 5' end of target-specific DNA sequence:

  5'-GGATCCTAATACGACTCACTATA**G**-3'

  At least one "G" is required for efficient *in vitro* transcription by T7 RNA Polymerase however, two "G" will increase transcription efficiency in most cases. The selected target-specific DNA sequence already contains a "G" (see 2.). A second "G" will be introduced by the T7 promoter sequence above.

- Add the following *Sp*Cas9 scaffold overlap sequence to the 3' end of target-specific DNA sequence

  5'-GTTTTAGAGCTAGAA-3'

- Check final sequence and order the target-specific oligonucleotide at your favourite oligonucleotide supplier (HPLC purification is recommended, but a desalted oligonucleotide works as well).

  General: 5'-GGATCCTAATACGACTCACTATA**GG**NNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAA-3'-

  Example: 5'-GGATCCTAATACGACTCACTATA**GG**CATTTCTCAGTCCTAAACAGTTTTAGAGCTAGAA-3'

- We recommend to design 2-3 target-specific oligonucleotides per cut site.

Selected References

[1] Modzelewski *et al.* (2018) Efficient mouse genome engineering by CRISPR-EZ technology. *Nature Protocols* **13(6)**:1253.

[2] Jinek *et al.* (2012) A programmable dual-RNA guided DNA Endonuclease in adaptive bacterial immunity. *Science* **337**:816.

[3] Cui *et al.* (2018) Review of CRISPR/Cas9 sgRNA Design Tools. *Interdiscip. Sci* **10(2)**:455.