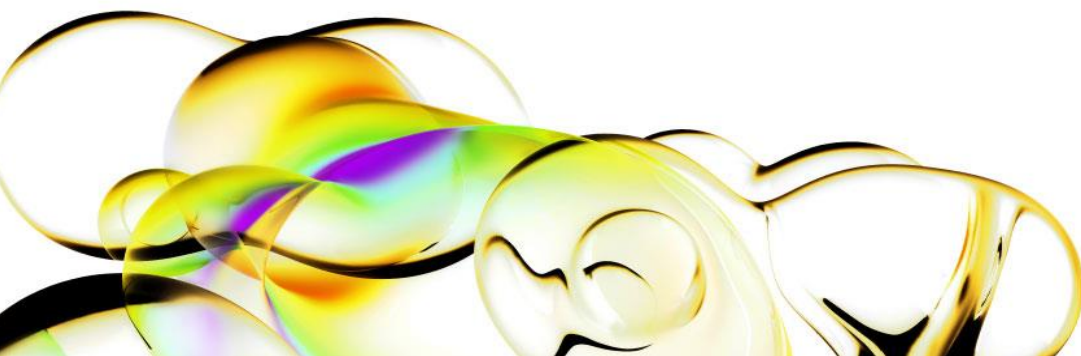# revvity

Designing custom single guide RNAs (sgRNAs) for use with the Pin-point™ base editing platform: guidelines for selection of sgRNAs for protein knockout experiments based on nCas9 (NGG PAM requirements) and rat APOBEC deaminase.

1) Using manual methods or publicly available base editing guide RNA design tools (see Additional External Resources section, or appendix A), generate lists of candidate sgRNA protospacer (targeting) sequences for your purpose based on the transcript of interest:

   a. Generate premature stop codons for protein knockout by designing sgRNAs with NGG PAMs that allow the conversion of CAA, CAG, CGA, or TGG codons into STOP codons (Figure 1) when the target C(s) are located within position 3 to 8 of the spacer sequence in the 5'-3' direction (Figure 2)[1].

   b. Generate splice site disruptions for protein knockout:

      i. Design sgRNAs with an NGG PAM that locate the target C within position 3 to 8 of the spacer sequence in the 5'-3' direction (Figure 2)[1];

      ii. Splice acceptor site is generally: intron-AG|exon;

      iii. Splice donor site is generally exon|GT-intron;

      iv. Converting the GC base pair to an AT base pair will lead to splice site disruption[2].

2) Further refine candidate lists by the following criteria, if necessary:

   a. Consider your gene structure to select a subset of gRNAs with highest probability to result in protein knockout:

      i. Avoid gRNAs that target the earliest and/or latest exons;

      ii. Give higher priority to gRNAs that targets the middle part of the protein, with preference for gRNAs located within the middle 10-60% of your transcript – if necessary to find additional gRNA candidates, look beyond 60% into the CDS until sufficient guides are found;

      iii. Give preference to gRNAs that target exon coding for essential domains in the protein (i.e transmembrane domain for receptors);

      iv. Target non-symmetric exons. If a symmetric exon is targeted, and the targeted exon is skipped, the following exons will be in-frame[3];

      v. Ensure the sgRNA targets all required isoforms.

   b. Prioritize guides based on the 2 nt 5' of the target C[3]:

      i. Give preference where the sequence context is NTC and TCC;

      ii. Avoid where the sequence context is NGC and GCC.

To order custom sgRNAs for the Pin-point platform, visit our website: https://horizondiscovery.com/en/gene-editing/pin-point-base-editing-platform/base-editing-reagents/sgrna-custom-control-form

Need more help? Contact us at technical@horizondiscovery.com

CAA (Gln) → TAA (STOP)
CAG (Gln) → TAG (STOP)

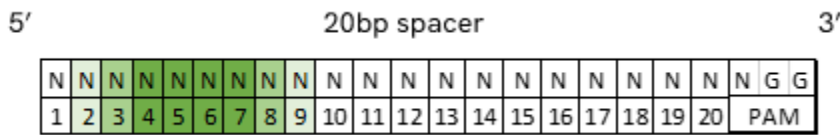CGA (Arg) → TGA (STOP)

TGG (Trp) → TAG (STOP)
TGG (Trp) → TGA (STOP)
TGG (Trp) → TAA (STOP)

Figure 1. Stop codons that can be generated from a cytidine deamination[1].

Figure 2. Base editing window. Dark green= potential for highest efficiency editing. Light green= potential for lower efficiency editing. White= potential for bystander editing.

References
1) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5610906/ (see figure 1)
2) https://www.nature.com/articles/s41467-021-22009-2 (see figure 1)
3) https://horizondiscovery.com/-/media/Files/Horizon/resources/Posters/performance-modularity-pin-point-base-editing-system-arrayed-pooled-screening-poster.pdf?sc_lang=en

Additional External Resources
- BE-Designer http://www.rgenome.net/be-designer/
- iSTOP https://www.ciccialab-database.com/istop/#/
- PnB Designer https://fgcz-shiny.uzh.ch/PnBDesigner/
- beditor https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6553823/ (requires the use of Python)
- SpliceR https://pubmed.ncbi.nlm.nih.gov/33893286/
- CRISPR-BETS https://zhangtaolab.org/software/crisprbets

How to manually design a gRNA for the Pin-point platform configured with a nickase Cas9 and rat APOBEC deaminase.

The rat APOBEC deaminase can either be used to convert a C to T or a G to A. Examples of both are provided below.
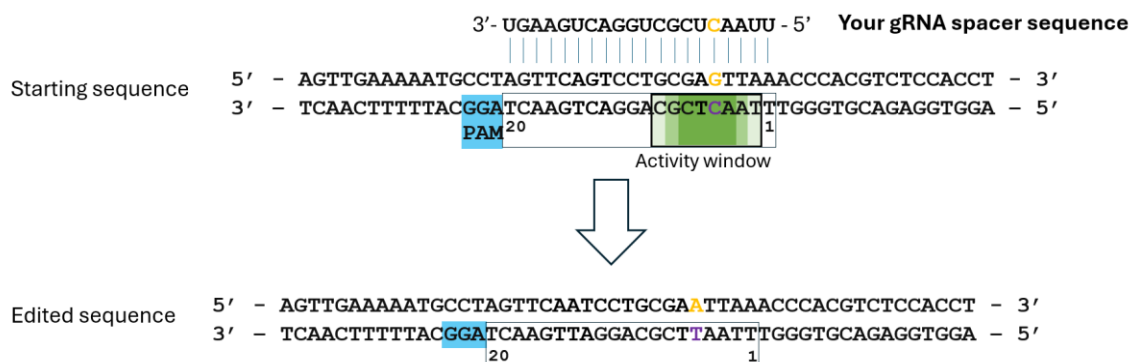
Example 1: Targeting a C

Activity window

Starting sequence
5' – AGTTGAAAAATCCTTAGTTCAGGCCTGCGAATTAATGGCACGTCTCCACCT – 3'
3' – TCAACTTTTTAGGAATCAAGTGCGGACGCTTAATTACCGTGCAGAGGTGGA – 5'

5'- AGUUCAGGCCUGCGAAUUAA - 3' **Your gRNA spacer sequence**

Edited sequence
5' – AGTTGAAAAATCCTTAGTTTAGGCCTGCGAATTAATGGCACGTCTCCACCT – 3'
3' – TCAACTTTTTAGGAATCAAATGCGGACGCTTAATTACCGTGCAGAGGTGGA – 5'
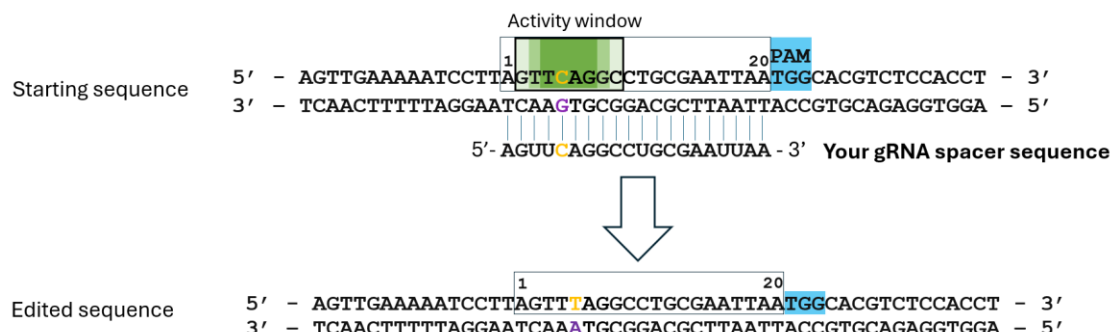
1. Identify the nucleotide to edit (in orange); in this example CAG is converted to a STOP codon (TAG). Consider that the NGG PAM is always located at the 3' of the spacer on the targeted strand*.
2. Ensure it is positioned in the activity window (darker green = potential for highest efficiency editing).
3. Your spacer sequence will bind the non-targeted sequence and should be complementary to it.
4. Note that sequences are input form 5' to 3' and as DNA sequences (T instead of U).
   In this example your input sequence would be: 5'- AGTTCAGGCCTGCGAATTAA - 3'

Example 2: Targeting a G

3'- UGAAGUCAGGUCGCUCAAUU - 5' **Your gRNA spacer sequence**

Starting sequence
5' – AGTTGAAAAATGCCTAGTTCAGTCCTGCGAGTTAAACCCACGTCTCCACCT – 3'
3' – TCAACTTTTTACGGATCAAGTCAGGACGCTCAATTTGGGTGCAGAGGTGGA – 5'

PAM 20   Activity window

Edited sequence
5' – AGTTGAAAAATGCCTAGTTCAATCCTGCGAATTAAACCCACGTCTCCACCT – 3'
3' – TCAACTTTTTACGGATCAAGTTAGGACGCTTAATTTGGGTGCAGAGGTGGA – 5'
20   1

1. Identify the nucleotide to edit (in orange); in this example a splice acceptor site (AG) is mutated. Consider that the NGG PAM is always located at the 3' of the spacer on the targeted strand*.
2. Ensure it is positioned in the activity window (darker green = potential for highest efficiency editing).
3. Your spacer sequence will bind the non-targeted sequence and should be complementary to it.
4. Note that sequences are input form 5' to 3' and as DNA sequences (T instead of U).
   In this example your input sequence would be: 5'-TTAACTCGCTGGACTGAAGT-3'

*targeted stand = the strand where the C substrate of the deaminase is located