

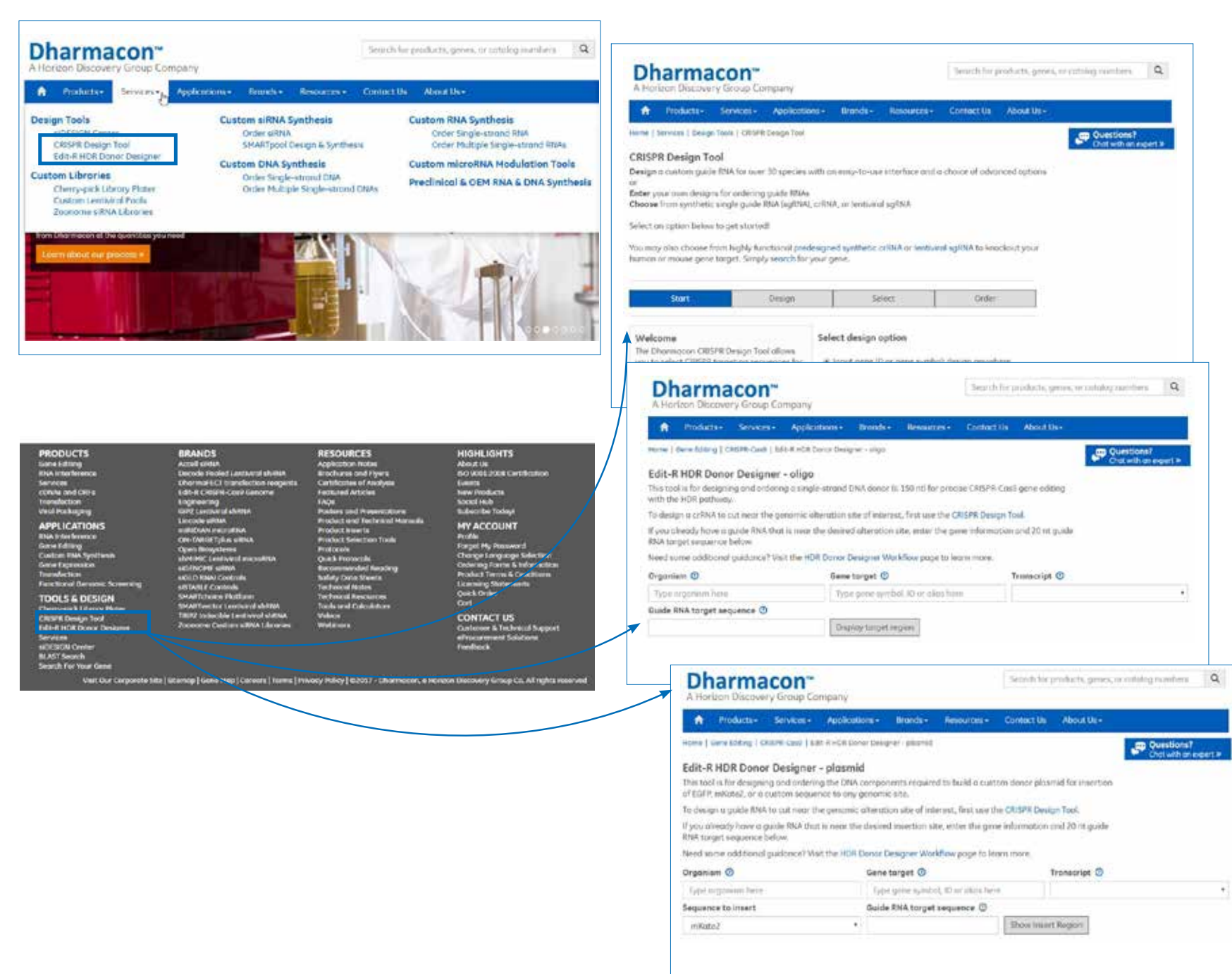
Advances in tools for designing guide RNAs and repair templates for efficient CRISPR-Cas9-induced homology-directed repair

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Introduction

Recent studies have resulted in elucidation of many of the key requirements for achieving precise CRISPR-Cas9 gene engineering using homology-directed repair (HDR). Combining these advances with our experimental observations has enabled the development of three tools that significantly speed up and simplify the process of 1) selecting appropriate CRISPR RNAs (crRNAs), 2) designing single-stranded DNA oligo donors (ssDNA), and 3) generating plasmid DNA donor templates to enable scientists to leverage the power of HDR in their research. Successful HDR applications begin with the selection of crRNAs near a desired genomic-sequence alteration site and requires high functionality and specificity. The Dharmacon CRISPR Design Tool incorporates both a functional algorithm for evaluating DNA cleavage efficacy and a novel approach for rapidly and thoroughly evaluating guide RNA specificity. The Edit-R HDR Donor Designer enables a researcher to select the specific bases to be either removed or inserted, define the length of each donor homology arm, and automatically creates silent mutations within the donor template to ensure that the genomic target site of HDR-based editing will not be susceptible to further cleavage by Cas9. Similarly, the plasmid donor tool enables researchers to design the necessary components for rapid assembly of donor plasmids for insertion of longer sequences, such as a fluorescent reporter, and automatically determines which silent mutations would be most appropriate for the crRNA being employed.

Locating the CRISPR Design Tool and HDR Donor Designers



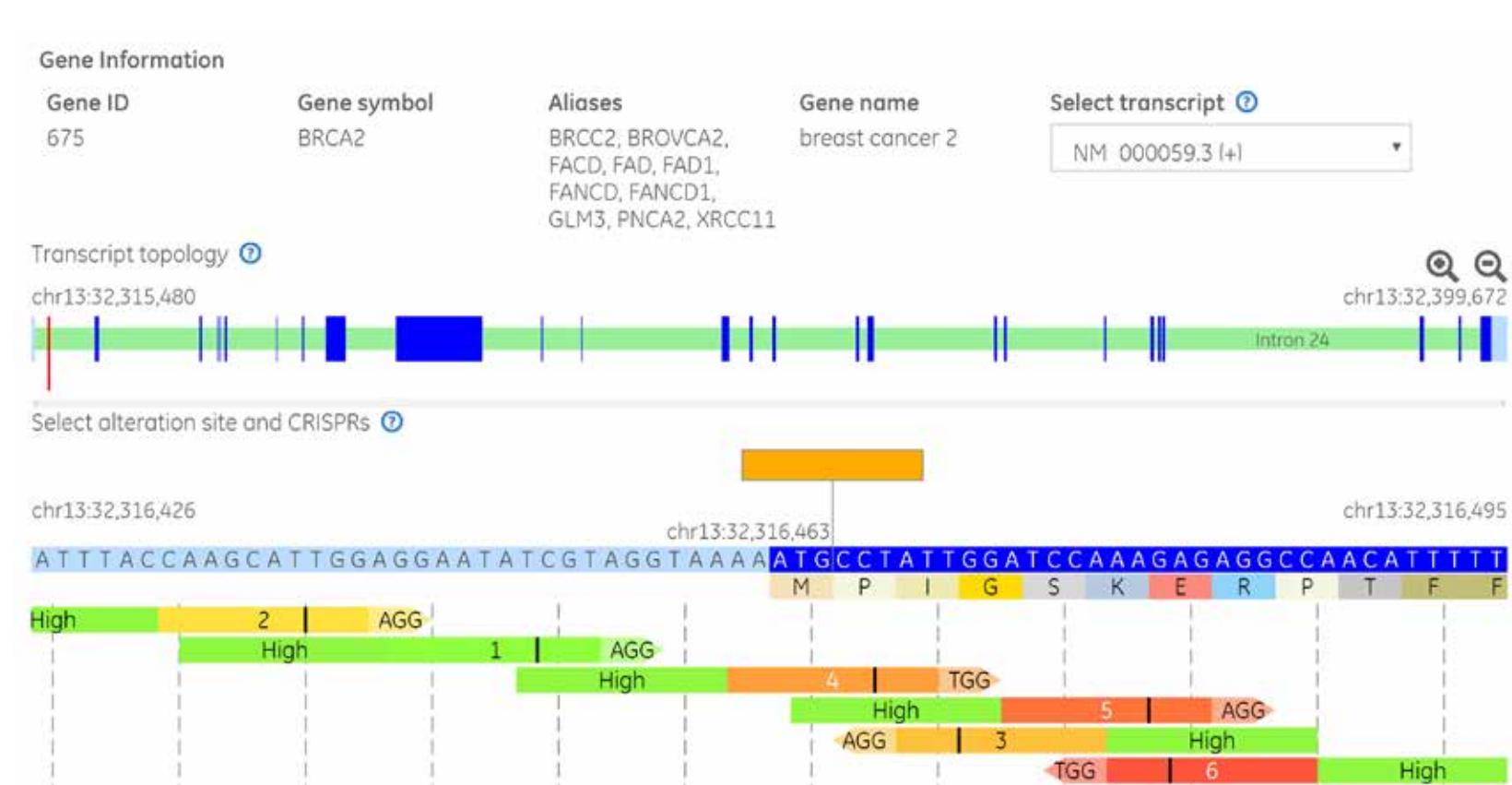
Selecting guide RNAs to use for HDR

Key selection factors:

- Proximity of crRNA cut site to location of insertion/replacement/removal
 - Ideally cutting is within 10 bases, although larger separations can work
- Predicted functionality
 - Functionality scores and ranking assigned using the Dharmacon Edit-R algorithm
- Specificity
 - Specificity determined using the comprehensive alignment methodology behind the Dharmacon CRISPR Specificity Analysis tool

Using the CRISPR Design Tool to select crRNAs for HDR

- Select a species
- Indicate a target gene
- Verify the transcript to be targeted (primary principal transcript select by default)



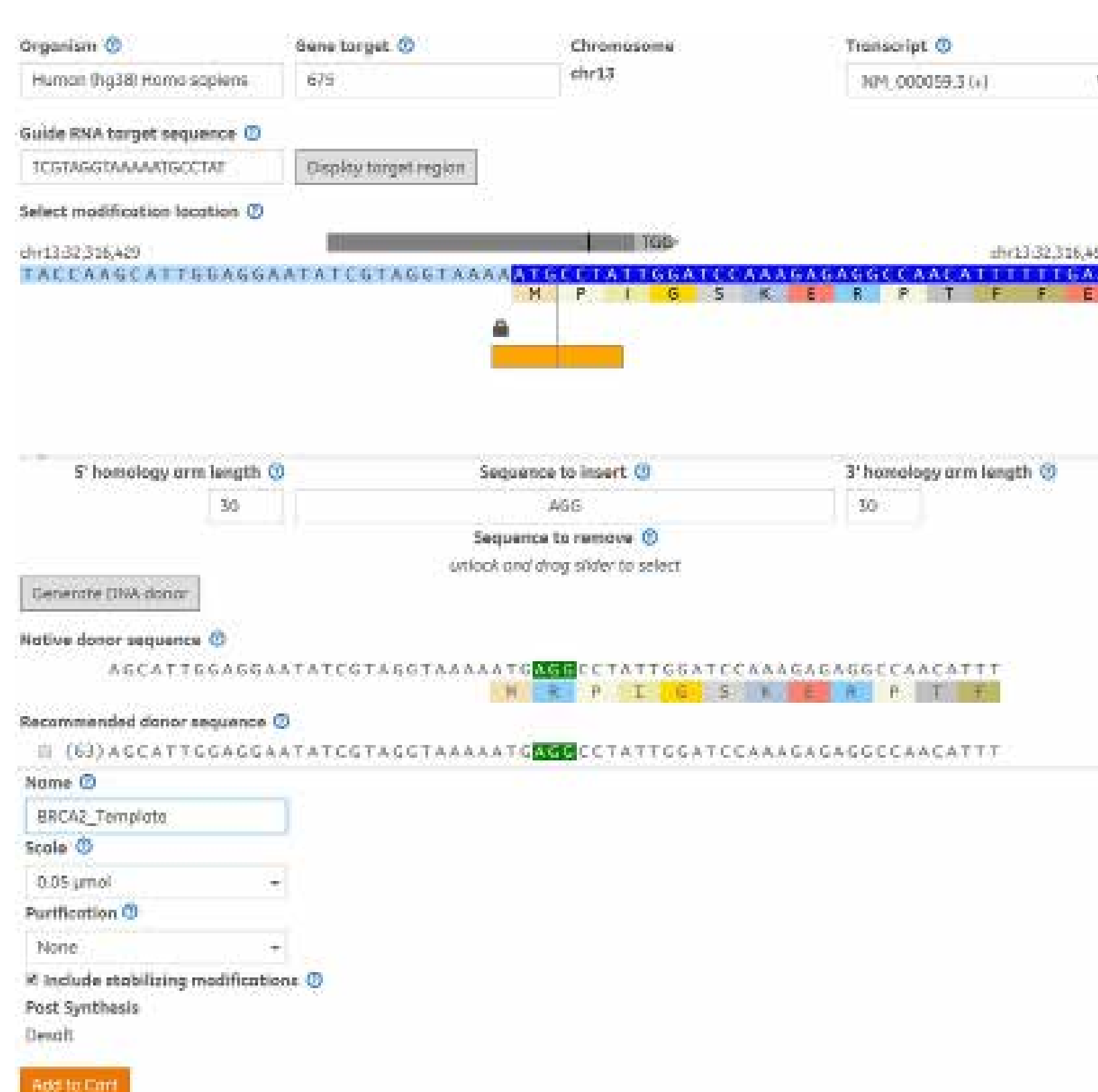
Designing ssDNA donor templates for HDR

Key design factors:

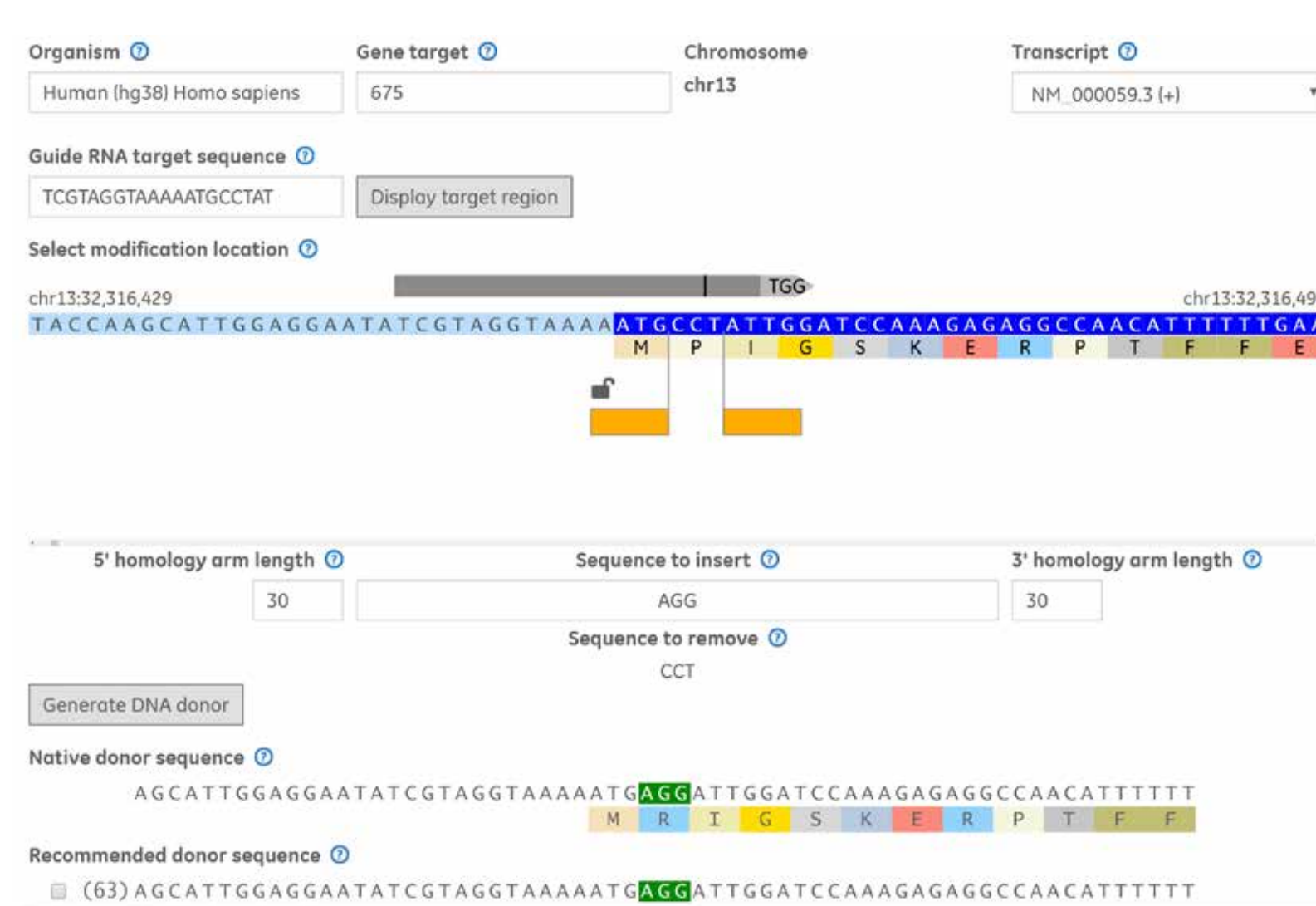
- The targeting crRNA sequence cannot occur adjacent to a valid PAM in the edited sequence
- Must support use of variable (and not necessarily equal) homology arm lengths
- All modes of alteration (insertion, replacement, removal) supported

Using the Edit-R HDR Donor Designer

- Select a species and indicate a target gene
- Verify the transcript to be targeted (primary principal transcript select by default)
- Enter the sequence of the guide RNA being used to create the double-strand break
- Specify the desired genomic alteration position

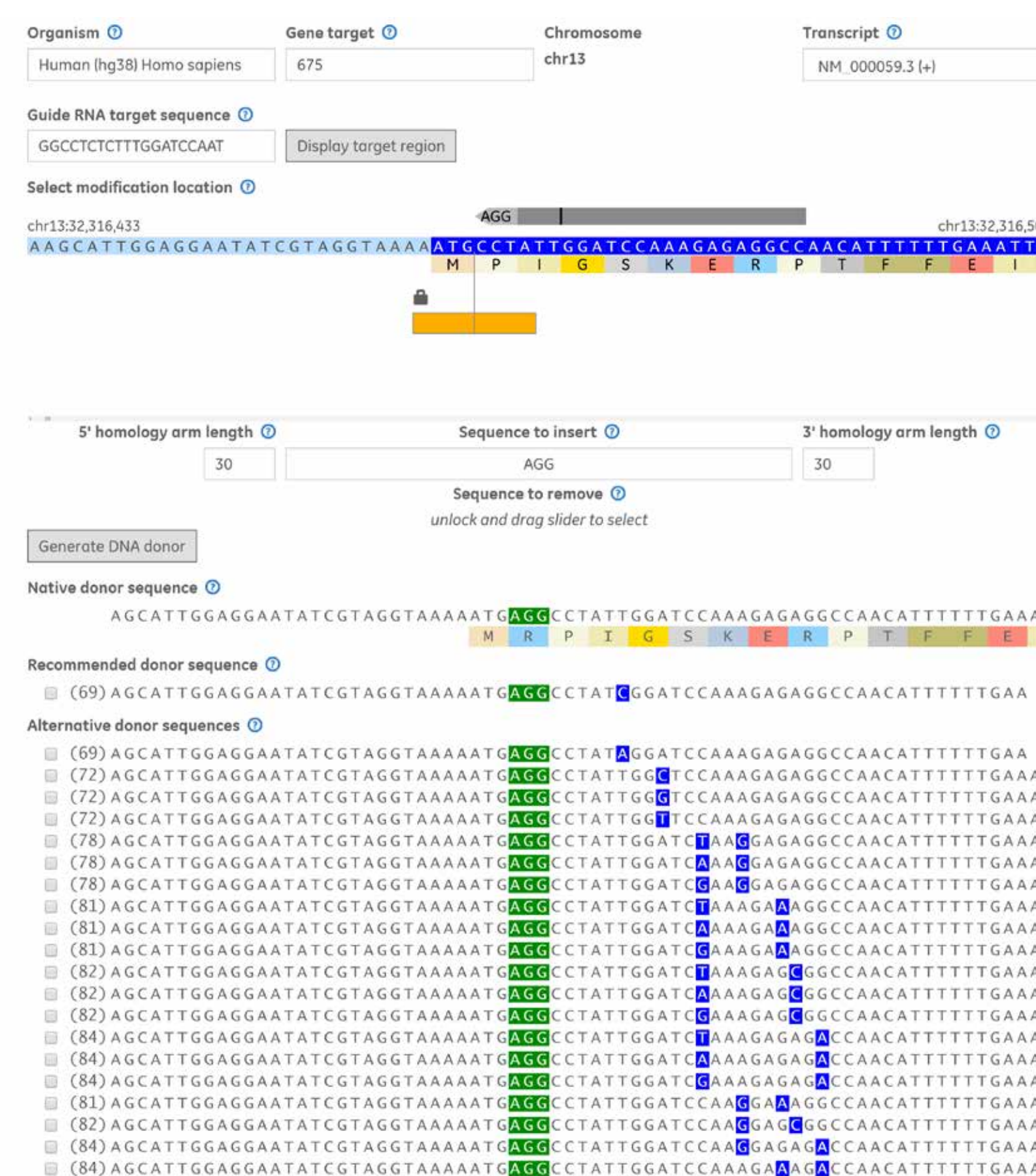


Designing a donor template for sequence replacement



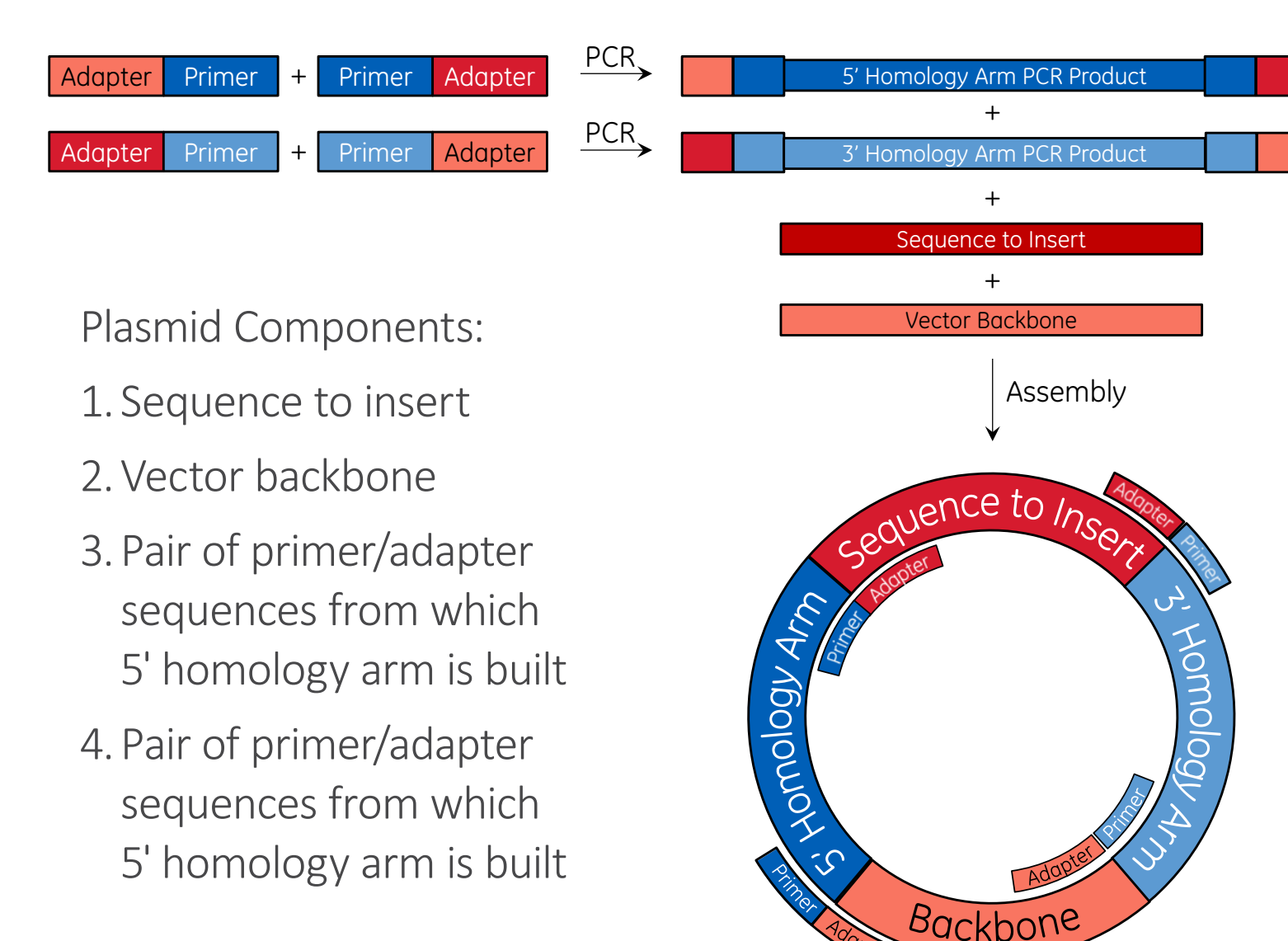
Unlocking the position slider allows easy selection of bases to be excised.

What if Cas9 targets the coding region of the edited sequence?



Building a plasmid HDR donor template

To insert longer sequences, such as those coding for fluorescent reporters, plasmids with homology arms of length 500-1000 perform well as donors. Desired homology arms are constructed by PCR using primers containing an adapter sequence, and the finished plasmid can be assembled from these arms, the sequence to insert, and the plasmid backbone.



Plasmid Components:

- Sequence to insert
- Vector backbone
- Pair of primer/adaptor sequences from which 5' homology arm is built
- Pair of primer/adaptor sequences from which 3' homology arm is built

Building a plasmid HDR donor template for insertion of fluorescent tag

To insert longer sequences, such as those coding for fluorescent reporters, plasmids with homology arms of length 500-1000 perform well as donors. Desired homology arms are constructed by PCR using primers containing an adapter sequence, and the finished plasmid can be assembled from these arms, the sequence to insert, and the plasmid backbone.

- Select a species
- Indicate a target gene
- Verify the transcript to be targeted (primary principal transcript select by default)
- Enter the sequence of the guide RNA being used to create the double-strand break
- Specify the position at which to insert mKate2 fluorescent tag
- Select primers to construct homology arms of the desired length



Simplifying and streamlining homology-directed repair

- Visualize the crRNA options near a desired HDR alteration site, with functionality and specificity evaluated for each design
- Quickly assemble ssDNA donor templates to guide double-strand break repair, allowing for insertion, replacement, or removal of genomic sequence and ensuring that the edited sequence is not susceptible to further Cas9 cleavage
- Generate target-specific primer/adaptor sequences to enable construction of plasmids suitable for controlled insertion of large fluorescent tags