

Reference Guide – Designing CRISPR-Cas9 RNA Oligonucleotides for the No-Amp Targeted Sequencing Procedure

Introduction

The purpose of this document is to provide technical guidance on how to generate and select CRISPR RNA oligonucleotides (crRNA) that are compatible with the PacBio *Procedure and Checklist, No-Amp Targeted Sequencing Utilizing the CRISPR-Cas9 System*.

CRISPR-Cas9 RNA oligonucleotides are comprised of CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). When annealed together or synthesized as one continual construct they make up guide RNA (gRNA) or single-guide RNA (sgRNA), respectively. Regardless of the guide RNA type, the tracrRNA enables gRNA to be complexed with Cas9 nuclease. At the same time, the crRNA provides the targeting function of the gRNA-Cas9 complex to enable double-stranded digestion at a specific genomic location.

The PacBio procedure referenced above makes use of gRNA as opposed to sgRNA. The tracrRNA oligo is comprised of a universal sequence that does not require customization. It is the crRNA that requires customization for a specific target. The No-Amp procedure uses two gRNAs to excise the target region from genomic DNA (gDNA).

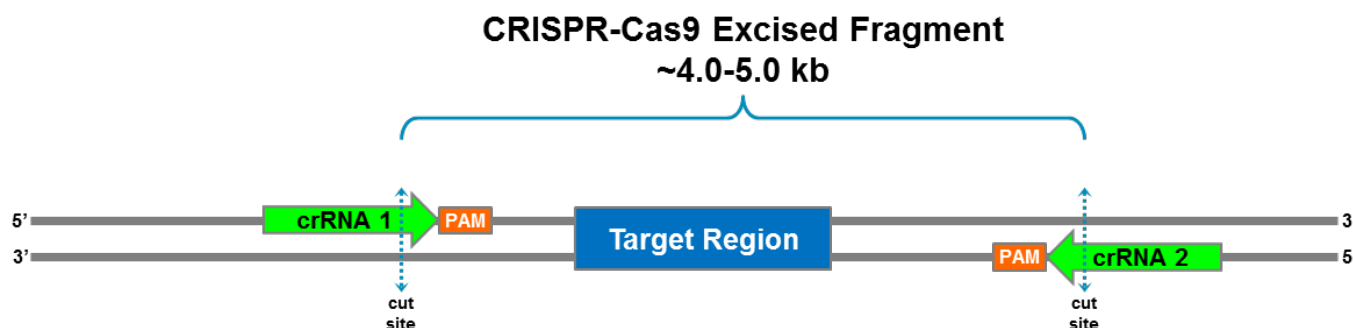


Figure 1. A depiction of a generic No-Amp targeted sequencing assay design. Various aspects of this illustration will be addressed in the design steps that follow. Note that the two flanking crRNA binding sites must be upstream or 5' to the target region with the 3-bp protospacer adjacent motif (PAM) sites closest to the target region necessitating a trans configuration for the crRNAs. The PAM site is a required targeting element located immediately 3' of the crRNA design in the target sequence, not in the crRNA design sequence.

This targeted sequencing method was developed for analysis of HTT, FMR1, ATXN10, and C9orf72 repeat loci but is readily applicable to other targets. The guidance below covers the design process for the ATXN10 repeat locus (as an example).

Design Steps

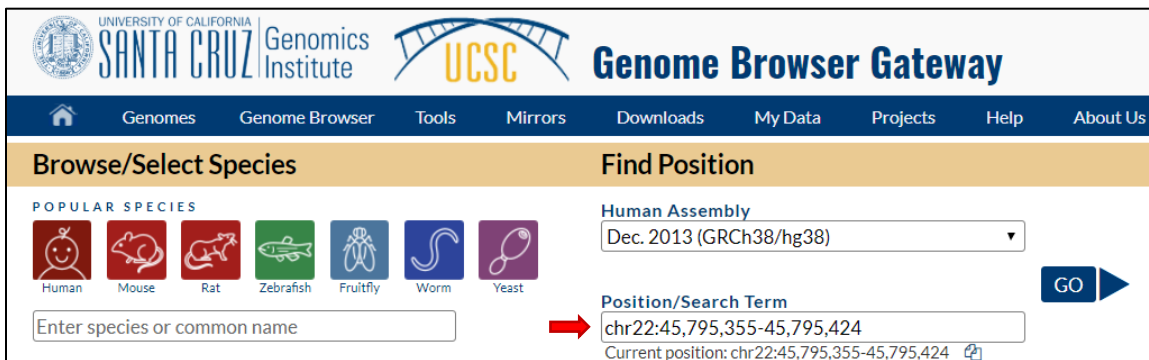
There are 4 steps to designing a pair of crRNAs for a particular target region:

1. Retrieve gDNA sequence with common SNPs identified
2. Generate crRNA designs
3. Select crRNA designs
4. Test crRNA designs

Retrieve gDNA Sequence with Common SNPs Identified

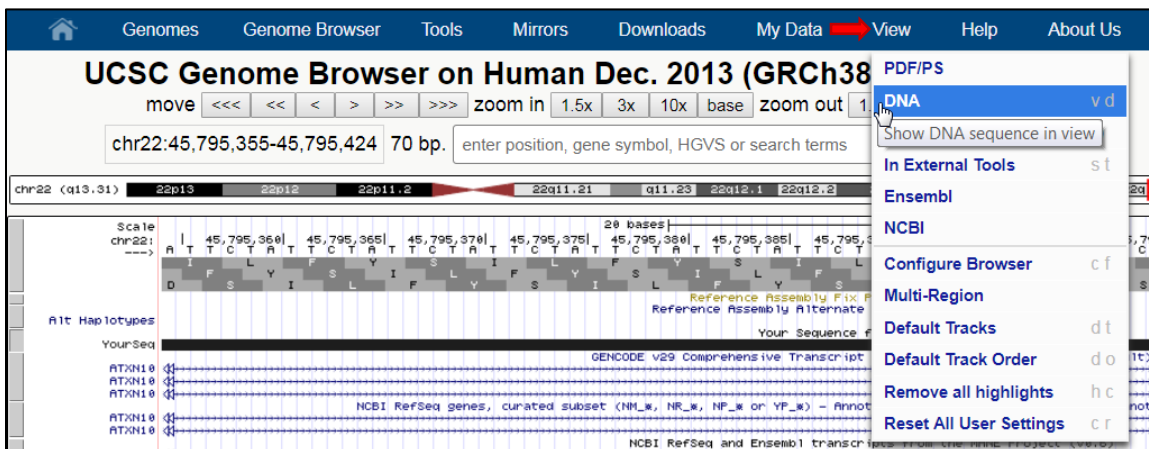
In this step, 5,000 bp of gDNA sequence containing the target region is retrieved in preparation for generating crRNA designs. Use a retrieval tool capable of marking common SNPs in the sequence. Common SNPs located within the crRNA design sequence or PAM site may alter the binding efficiency of the crRNA in the gRNA-Cas9 complex, ultimately reducing the number of on-target sequencing reads. The following instructions use the UCSC Genome Browser website (<https://genome.ucsc.edu/>).

1. Identify the location of the target region. Here the target is the ATXN10 repeat locus in Chromosome 22 with ATTCT repeat motif (chr22:45,795,355-45,795,424; GRCh38/hg38). Use the latest reference assembly build available.
2. On the UCSC Genome Browser website, enter the chromosome and coordinates of the region of interest in the **Position/Search Term** field as shown below and click the **Go** button.



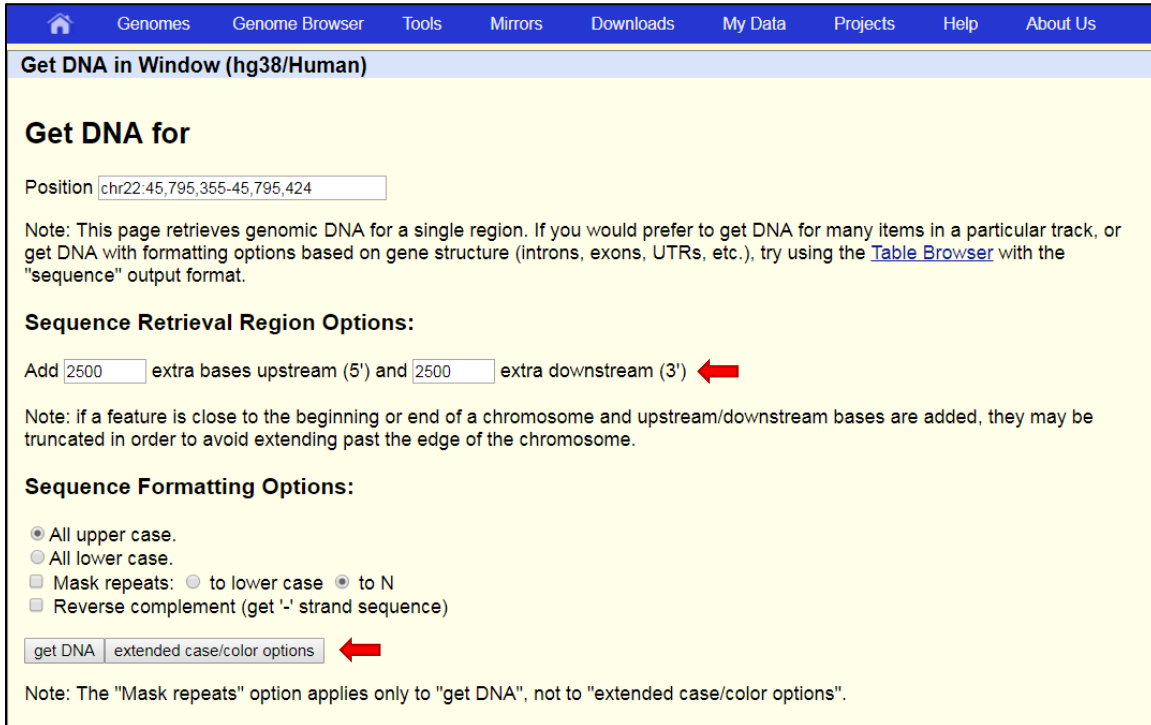
The screenshot shows the UCSC Genome Browser Gateway search interface. The 'Find Position' section has 'Human Assembly' selected with 'Dec. 2013 (GRCh38/hg38)' as the assembly. The 'Position/Search Term' field contains 'chr22:45,795,355-45,795,424'. A red arrow points to this field. The 'GO' button is visible to the right.

3. In the next window, choose **DNA** under the **View** menu at the top of the webpage.



The screenshot shows the UCSC Genome Browser on Human Dec. 2013 (GRCh38) displaying the ATXN10 repeat locus. The 'View' menu is open, and 'DNA' is selected. The sequence viewer shows the ATXN10 repeat motif (ATTCT) and the reference assembly (Fix P and Alternate). The 'View' menu options include PDF/PS, DNA, Show DNA sequence in view, In External Tools, st, Ensembl, NCBI, Configure Browser, c f, Multi-Region, Default Tracks, dt, Default Track Order, do (it), Remove all highlights, h c, and Reset All User Settings, c r.

- Retrieve 5,000 bases surrounding the target region (2,500 bp on each side). In the next window, enter 2,500 bp into the fields for extra upstream and downstream bases under the **Sequence Retrieval Region Options** section.



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Get DNA in Window (hg38/Human)

Get DNA for

Position

Note: This page retrieves genomic DNA for a single region. If you would prefer to get DNA for many items in a particular track, or get DNA with formatting options based on gene structure (introns, exons, UTRs, etc.), try using the [Table Browser](#) with the "sequence" output format.

Sequence Retrieval Region Options:

Add extra bases upstream (5') and extra downstream (3') ←

Note: If a feature is close to the beginning or end of a chromosome and upstream/downstream bases are added, they may be truncated in order to avoid extending past the edge of the chromosome.

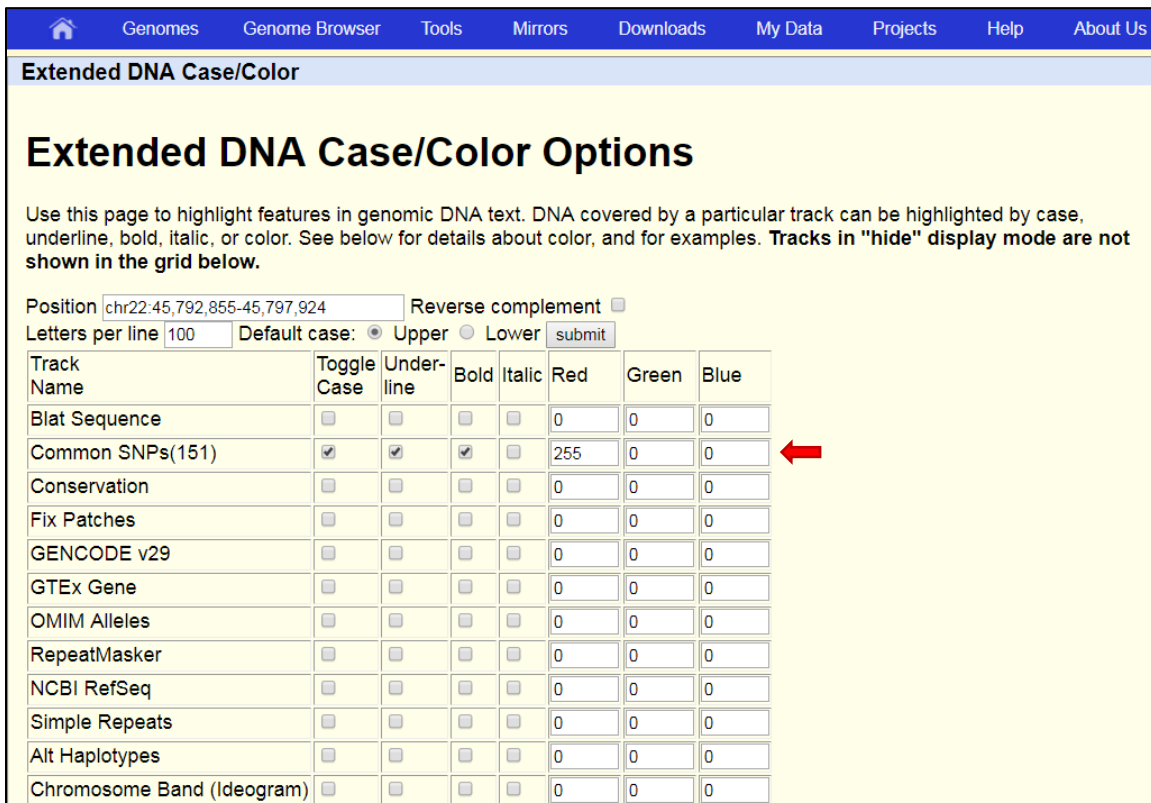
Sequence Formatting Options:

All upper case.
 All lower case.
 Mask repeats: to lower case to N
 Reverse complement (get '-' strand sequence)

←

Note: The "Mask repeats" option applies only to "get DNA", not to "extended case/color options".

- Click the **Extended Case/Color Options** button to advance to the next webpage and enable marking of SNPs in the sequence.
- Select the desired SNP marking attributes and retrieve the sequence by clicking the **Submit** button.



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Extended DNA Case/Color

Extended DNA Case/Color Options

Use this page to highlight features in genomic DNA text. DNA covered by a particular track can be highlighted by case, underline, bold, italic, or color. See below for details about color, and for examples. **Tracks in "hide" display mode are not shown in the grid below.**

Position Reverse complement

Letters per line Default case: Upper Lower

Track Name	Toggle Case	Under-line	Bold	Italic	Red	Green	Blue
Blat Sequence	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
Common SNPs(151)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="255"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
Conservation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
Fix Patches	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
GENCODE v29	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
GTEX Gene	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
OMIM Alleles	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
RepeatMasker	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
NCBI RefSeq	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
Simple Repeats	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
Alt Haplotypes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>
Chromosome Band (Ideogram)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>

7. Copy the resulting target sequence into a text editing program (e.g. Microsoft Word) and mark the region of interest (highlighted yellow below). Note that common SNPs are marked as instructed above.

```
>chr22:45792855-45797924
TCGACATTTTTACTGACTTTGTAAATCTTCTAAACTTGCATTGGCTGTTATACCTACATAgGGAGCCACATGAAGATCATAGATAAAGGTGAAT
CAAATGATTTACATGTTTACTGACAAGAGCTTTTTTAGTTCCACCCAAATGCTTTCTCCTAATAGCCAGCCAGGCCTTTTGCAGGCTCTGCAGT
TGCTTCTCAGGACCGTGGTCACCAGGTTTGTCTACAGTTCTCCACAAAACCTGACATATCTTAAATTCAAAAACAATGTATTGTACTCTGTT
GATGTCCAACAGACTTTTCTATTTTTAAAAATCTCCATAACCATTAAATTAGATAAAGTACCATCAAACCCAAAGACATAGAATTGTTTGCTA
GAGAGCTTTGTGTTGAGCATCTCCTAGTCAGTTGTGAATTTGTCTCAGCATGTCTCCATCAGTGACAACATCAgTCCAGAGTGTAGTAAATTA
AAAATTGTCTTTCACGAAGCCTGGCAGTAATTTGTCTGTGCTtTTTCTGTGTCCATCATCCAAATACCAGCTGACGGAAACATCTACTGCATTTCA
ATAAGGGTAAACAATGGTAGAGTCCACATCTTGAGGCTTTATCCATTTAATAAATCTCTCAGTCTCTCTGCTATTTCTGAATTCAGGAT
CTCTTTAACAAATAATTTCTGTCAAGCTCTTCAATCTTTCTGTGACAAAATGACTAGAATTTCTGGAGCCTGACCTTCATTTTAGGCAGCTATTGCT
TCAGAAGTCAACAGTCTGTTCCACCAGCCTTTGCCAAGGATGCAGGTGCCACAGCATCTCAAGAGAAGTCCCAATCACACAGCTCCATGCTGAGG
CCCTCTTGCTGCTACTGAGGAGCTTTGCCACCCCGATTCCTGCCTTTCCCCAGTGTGAGGACAGGTAAGCCCCAAATTTGGGACTTAGCC
TGGGAAGGTTCTTGCTTTGCCCGGAAATAATCAAGGGTGAAGCTGGTGGTAAACAGCACTTTGATTGAAGCAGCAGTGTATAGCAGCAG
CAGAGGTGCTGCTCTTGCAGAGCAGGGCTGCCCTATAGGCAGTGTGCCAGAATAGCAGCTCAGAGCAGGCTGCACATATTTATACCAC
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GCGGTAACCTTCTGGGTGTTGCCATGGCAATGGTAAACTGACATGACATATTGGTGGGTATGTCTTATaGAAAAATGGCTTCCACCCTGACCTACC
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TTGTTCTAATTCAGTACTTTCTTGCTGTGAGTTTATAAGAACAAAGATAAAAATGATAACAGGCTTTCTTCCAGAAATGATGCAAGACAGGAA
TCAATGGAATGACATCTTAAAAATACTAAAGGAAAAGTGTAAATCTAGAATTATGTATCCAGCCAAAATATCTTCAAATAAAGGTCaAAAAA
AAGAAGGTAATAATGCTCTGTAAGAAATGTTAAATGAAGTCAACAGGTAAGGTAAGTATAGACATGGAAACCTGACCTATACAAATATATA
CAAAGGATCAGAATCCCTGGAAAAGTAAATATATGGGTAAAAAGAAAAGATTTCTATTCTCACTTTTAAATTTTCTTGAATGTAATTTGGCTCT
TTAAAGTAATCATAATGGCAGTATATAGGGGATTTTAAATGTGTATATAAGAAGAAAATATATAGAGTAATTTGCCAAAGATTAACAGAGTA
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TTATAATTTCTATGCCTGTCTTCTTTAATATCTTAATCTCGTCATCTTCATAAGCTGAGGATGTATGTCACCTCAGGATCCCGTGATGATCA
CGTTATCTGCACAAATGTTTGTAAAGCATGTGTGTTtGAACAATATGAAATTTGGGCACCTTGAAAAAGAACTGGGTAACAGCGATTTTCAAG
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GCTGAATTTCTTTCTCAGCAAGGAATAACCTGGGAAACGAATGCATTTCCAGGGGGAGgTCTCTAAAATGGCCGCTCAGGGAGTGTCTGTCTTA
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AGTTTCGCCCTGGCCTTGATCTCACTCTGCCTCTCTGCCTTTGTGATATTTTCATTTGCCCTTTGAGCATGTGATCTCTGTGACCCACACCCCTGT
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CAGATAAATCTgTCTACCTGTCAAGAAAATGACACAAAATATAAATGACCTCTATCAGCAGTGAAGAGGAGACATCATTaTAGATATTACAGAC
ATTA AAAAGATAATAAGAGAATAGCATAAACCT
```

Generate crRNA Designs

In this step, the retrieved sequence is submitted to an online CRISPR-Cas9 design tool to generate several crRNA designs. Note that many websites use the terms crRNA and gRNA interchangeably, but, as explained in the introduction, crRNA is the targeting component of gRNA. Also, at times a distinction isn't made between gRNA and sgRNA. As pointed out in the introduction, gRNA is constructed by annealing separate crRNA and tracrRNA oligos together and sgRNA is formed by synthesizing the crRNA and tracrRNA as one continuous oligonucleotide.

The following instructions use the GPP sgRNA Designer webtool on the Broad Institute GPP Web Portal website (<https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design>). Designs generated by this webtool are compatible with the No-Amp targeted sequencing procedure.

1. Select the first 500 bp from the beginning of the retrieved target sequence and submit to the GPP sgRNA Designer webtool. Despite the “sgRNA” reference in the website screen capture below, it is crRNA designs that are generated.

Make sure the **CRISPRko** tab is active and that **SpyoCas9 (NGG)** is selected in the **CRISPR Enzyme** pull-down field. The No-Amp procedure uses Cas9 nuclease from *S. pyogenes* and is associated with the specific PAM sequence, NGG.

Under the **Target Genome** pull-down field choose the latest build. Enter a value of “15” in the **Pick Quota** field and make sure the **Report Unpicked Sequences?** box is unchecked. Click the **Submit** button to generate results after verifying you are not a bot.

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Home | Search by Gene | Search by Clone

GPP sgRNA Designer

CRISPRko CRISPRa CRISPRi

CRISPR Enzyme: **SpyoCas9 (NGG)**

Target Genome: **Human GRCh38 (NCBI Mar. 2019)**

Specify Target(s):

Input Transcript IDs, Gene IDs/Symbols, or raw DNA sequence:

```
TCGACATTTTACTGACTTTGTTAAATCTTCTAAACTTGCATTGGCTGTTATACC
TACATAgGGAGCCACATGAAGATCATAGATAAGGTGAATCAAATGATTTACATGTT
TACTGACAAGAGCTTTTTAGTTCCACCCAAATGCTTCTCCTAATAGCCAGCC
AGGCCTTTTGCAGGCTCTGCAGTTGCTTCTCAGGACCGTGGTCACCAGGTTTG
TCTACAGTTCTCCACAAAACCTGACATATCTTAAATTCAAAAACAATTGTATTG
TACTCTGTTGATGTCCAACAGACTTTTCTATTTAAAAAATCTCCATAACCATTA
AATTAGATAAAGTACCATCAAACCCAAAGACATAGAATTGTTGCTAGAGAGCT
TTGTGTTCAAGCATCTCCTAGTCAGTTGTGAATTTGTCCTCAGCATGTCTCCATC
```

Enter up to 200 Transcript IDs (e.g., NM_014911.3, ENST00000456328, etc.), Gene IDs or Symbols (e.g., 988, CDC5L, ENSG00000223972, etc.), or a **single** DNA sequence.

File inputs must be smaller than 20kb in size, and any sequences submitted via file *must* be in FASTA format.

Please refer to our [sgRNA Designer Help Page](#) for details on how a transcript is chosen for a gene input.

-OR-

Upload a list of Transcript IDs, Gene IDs/Symbols, or a FASTA file of DNA sequences:

Choose File No file chosen

Pick Quota: **15**

Report Unpicked Sequences?

I'm not a robot

reCAPTCHA Privacy - Terms

Submit >>

2. Download the “sgRNA Picking Results” file in the next window by clicking the link.

The screenshot shows the GPP Web Portal interface. At the top left is the Broad Institute logo and 'GENETIC PERTURBATION PLATFORM'. The main title is 'GPP Web Portal'. On the top right, it says 'Public User | [Help](#)'. Below the title is a navigation bar with 'Home | Search by Gene | Search by Clone'. The main content area is titled 'sgRNA Design Submission Completed'. Under 'Submission Information', it shows 'Job ID' as 'bc3e1e6e-feca-4415-b94c-6aa0afa31c60' and 'Status' as 'Complete'. Under 'Download Results', there are three links: 'sgRNA Picking Results' (highlighted with a red arrow), 'sgRNA Picking Summary', and 'Input Target Sequences'. Each link has a description: 'Picked sgRNA candidate sequences (tab-delimited text file)', 'Score statistics by pick order (tab-delimited text file)', and 'DNA sequences provided as inputs to the sgRNA designer (FASTA file)'. There is also a '(download all)' link and a 'Return to the [Analysis Submission Page](#)' link. At the bottom, there are 'Contact Us | Broad Home' links on the left, '© 2019 Broad Institute' on the right, and '0.0478 sec.' in the bottom right corner.

3. Repeat Steps 1 through 2 in this section for the last 500 bp at the end of the retrieved target sequence. In the next section, crRNA designs flanking the target region will be selected from the first 500 bp of sequence and last 500 bp of sequence. This will generate a 4-5 kb fragment containing the target region after CRISPR-Cas9 digestion.

Select crRNA Designs

In this step, crRNA designs are selected from the output of a CRISPR-Cas9 design tool based on orientation and uniqueness. They are then mapped to the gDNA target sequence obtained earlier in the design process to determine the presence of SNPs in the designs. Final design selections are then made for testing. Following the previous section, the “sgRNA Picking Results” files generated separately for the first and last 500 bp of the 5 kb target sequence are used below.

1. Open the “sgRNA Picking Results” files (tab-delimited text file) in Microsoft Excel and save each as an Excel Workbook (.xlsx file extension).
2. Sort the tables by the “Pick Order” column to order the crRNA design picks as shown in Tables 1 and 2 below in the next step. Only the columns to aid in mapping the designs to the 5 kb target sequence are shown, and other columns have been hidden. Again, “sgRNA Sequence” as appears in the table is synonymous with “crRNA Sequence”.
3. Identify crRNA designs compatible with the No-Amp procedure.

Orientation of the crRNA design relative to the target sequence is **critically** important to the No-Amp targeted sequencing procedure. The gRNA-Cas9 complex cuts 3-bp upstream of the associated PAM site. After cleavage, the gRNA-Cas9 complex remains associated with the DNA on the 5' side of the cut site, resulting in low or no read coverage of the associated DNA.

Compatible crRNA designs must have 3' ends directed toward the target region as shown in the figure below. Fragments generated with the correct crRNA design orientation will not be encumbered by the presence of the gRNA-Cas9 complex in the ligation step following CRISPR-Cas9 digestion.



Figure 2. Illustration of four possible locations for crRNA designs for a generic target region. The two flanking crRNA designs for any target region must be located on opposite strands with the 3' end of each design closest to the target region. This will also place the PAM site in-between the crRNA design and the target region. Designs crRNA1 and crRNA2 are compatible with the No-Amp targeted sequencing procedure whereas designs crRNA 3 and crRNA 4 are not (shown crossed-out) as the 5' ends of these designs are closest to the target region with the PAM sites not adjacent to the target region.

In both tables below, the rows containing designs with compatible orientations are labeled in italicized, bolded text. Compatible “forward” designs generated for the first 500 bp of sequence before the target region are located on the sense strand (Table 1). Compatible “reverse” designs generated for the last 500 bp after the target region are located on the antisense strand (Table 2).

Table 1. Forward crRNA designs from the first 500 bp of the 5 kb ATXN10 sequence.

Orientation	sgRNA Cut Position (1-based)	sgRNA Sequence	PAM Sequence	Pick Order
<i>sense</i>	85	CCACATGAAGATCATAGATA	AGG	1
antisense	218	GGGAGAACTGTAGACAAACC	TGG	2
<i>sense</i>	59	ATTGGCTGTTATACCTACAT	AGG	3
<i>sense</i>	165	TTTCTCCTAATAGCCCAGCC	AGG	4
antisense	297	TTAAAATAAGAAAAGTCTGT	TGG	5
antisense	209	GTAGACAAACCTGGTGACCA	CGG	6
<i>sense</i>	203	GCAGTTGCTTCTCAGGACCG	TGG	7
antisense	74	CCTTATCTATGATCTTCATG	TGG	8
<i>sense</i>	60	TTGGCTGTTATACCTACATA	GGG	9
<i>sense</i>	41	AAATTCTTCTAAACTTGCAT	TGG	10
antisense	61	CTTCATGTGGCTCCCTATGT	AGG	11
antisense	167	AGCCTGCAAAAGGCCTGGCT	GGG	12
antisense	142	ATTAGGAGAAAGCATTTGGG	TGG	13
antisense	177	GCAACTGCAGAGCCTGCAAA	AGG	14
antisense	159	AAAGGCTGGCTGGGCTATT	AGG	15

Table 2. Reverse crRNA designs from the last 500 bp of the 5 kb ATXN10 sequence.

Orientation	sgRNA Cut Position (1-based)	sgRNA Sequence	PAM Sequence	Pick Order
<i>antisense</i>	224	ACATTGGTTTAATCTACTCT	TGG	1
<i>antisense</i>	40	ATGGTTGTGTCTATGACATG	TGG	2
sense	119	CATACCTAAATTGTAAGATG	GGG	3
sense	270	AGAATTAACAAAGATAAGAA	TGG	4
sense	308	AAAATAAAATAGACATAGAG	TGG	5
sense	204	TCTTAGTTTCCAACCTAAGA	AGG	6
<i>antisense</i>	39	TGGTTGTGTCTATGACATGT	GGG	7
sense	118	GCATACCTAAATTGTAAGAT	GGG	8
<i>antisense</i>	112	TTATCCCCATCTTACAATTT	AGG	9
sense	117	GGCATACCTAAATTGTAAGA	TGG	10
sense	136	ATGGGGATAAAGTAGTGTTT	AGG	11
<i>antisense</i>	327	GCATCCAGCTTCTGATTACA	TGG	12
<i>antisense</i>	376	GACAGGTAGACAGATTTATC	TGG	13
sense	334	AAGTCCATGTAATCAGAAGC	TGG	14
<i>antisense</i>	425	TCCTTTTCACTGCTGATAG	AGG	15

In addition to being located on the appropriate strand, designs are further selected by their uniqueness following the pick order (green shaded cells in Tables 1 and 2 above). For example, in Table 1, designs with pick orders 1, 3, 4 and 7 (Designs 1, 3, 4 and 7) all occupy unique 20-bp positions on the sense strand and are all highlighted as potential designs. However, Design 9 almost entirely overlaps with Design 3, therefore, preference is given to Design 3. Lower ranked designs with minimal overlap with higher ranked designs are considered as a potential design as is the case with Design 10 which overlaps with Design 3 by 2 bp.

4. Map the selected crRNA designs that are both properly oriented and unique to the 5 kb target sequence in order to determine the presence of common SNPs in crRNA and downstream PAM sequences.

The sequence below shows the five forward designs highlighted in green in Table 1 and the 6 reverse designs highlighted in green in Table 2. PAM sites are enclosed within a box.

```

>chr22:45792855-4579792
TCGACATTTTTACTGACTTTGTTAAATTCTTCTAAACTTGCATGGCTGTATACCTACATAgGGAGCCACATGAAGATCATAGATAAGGTTGAAT
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- Select a final set of designs for testing which do not contain common SNPs. Carefully inspect all designs for the presence of common SNPs in the crRNA sequence and downstream PAM sequence. Of the forward designs for ATXN10, Design 3 has a SNP in the PAM site. Of the reverse designs for ATXN10, Designs 9 and 13 have a single SNP in the crRNA sequence. All other designs present valid selections. Because not all crRNAs are equivalent in practice, choose several crRNA designs to be tested.

We recommend testing the top 3 compatible designs as ranked by pick order from the forward (sense) and reverse (antisense) flanking orientations. For the ATXN10 target above, this would be Designs 1, 4 and 7 from the forward flanking design set and Designs 1, 2 and 12 from the reverse flanking design set.

Test crRNA Designs

It is advised to order and test several designs before choosing a final crRNA design set for a particular target. The crRNA designs can be tested with the No-Amp procedure or with a plasmid construct containing the flanking crRNA design sequences. This is a more efficient and economical testing model for a small number of targets as CRISPR-Cas9 digestion products are analyzed directly by agarose gel electrophoresis instead of sequencing.

We recommend ordering crRNA oligos from [Integrated DNA Technologies](#) (IDT) under the product offering “Alt-R® CRISPR-Cas9 crRNA”. The universal 67mer tracrRNA used in conjunction with crRNA to form gRNA is also ordered from IDT under the product offering “Alt-R® CRISPR-Cas9 tracrRNA”. These RNA oligos contain chemical modifications to protect from degradation by cellular RNases.

Revision History (Description)	Version	Date
Initial Release.	01	August 2019

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