



once the user has designed a pegrna, they will be presented with the list of potential nicking guides according to the design suggestions above. they will be able to select and print them as desired. to change the nick site, they can change the guanine nucleotide that is connected to the 3-prime end of the rna guide sequence to a cytidine or uridine, a process which can be achieved by the "fix nicking" tab. alternatively, they can select the "don't fix" option and leave the guanine nucleotide the same as it was in the original pegrna. this is useful to keep the original pegrna sequence unchanged, for instance if the correct base is found in the genomic position where the pegrna binds. as a result, novaya gazeta has received numerous threats and actual physical attacks. the journalists of the newspaper have been repeatedly attacked by law enforcement and anti-extremist forces, which have also used the newspaper's offices as a base for attacks on the newspaper's employees. the attacks have included the assault of the newspaper's chief editor and the arrest of a number of editors and reporters. in 2002, the newspaper was the target of a special operation, in which the offices and the homes of numerous employees were raided, and in 2003 the newspaper's director was arrested. after a court ruling in 2014, andreyevich muratov was sentenced to five years in prison. the police used his criminal record and his political opinion as grounds to arrest him. in a separate trial, the court sentenced him to an additional two-and-a-half years in prison for economic crimes. in the past few years, novaya gazeta has been under constant police surveillance. police have raided the newspaper's offices on several occasions, sometimes with the collaboration of the federal security service (fsb), which also uses the newspaper's offices as a base for attacks on the newspaper's employees. these attacks include the assault of the newspaper's chief editor and the arrest of a number of editors and reporters. novaya gazeta's editors and employees have also been subject to political pressure. in 2015, police detained a number of novaya gazeta journalists. in 2017, they searched the offices of the newspaper and confiscated computers and cellphones. in october 2018, police raided the newspaper's office and the homes of several employees. in march 2019, the newspaper was awarded the 2018 international press freedom award.

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after all input parameters have been set, pnb designer will generate a table containing the grnas that can be exported by pressing the export button. to check the preconfigured grnas, the tab pre-selected grnas can be opened by pressing the open button. the grna sequences generated by the program are shown in the textbox. to modify the guide sequence, the user needs to open the tab edit grna by pressing the open button. there, a specific grna can be edited or removed from the grnas list by pressing the delete or reduce button respectively. to add and remove grnas from the list, the add grna or remove grna buttons can be pressed. the grna sequences for the grnas that can be removed are shown in the textbox for visual reference. as only the spry, be3 and ube2t base editors can be used with >21 nt grna sequences, it is advised to remove all grnas of this length. however, pnb designer includes the possibility to easily use longer grnas, which can be used by cbes and any base editors. the output table consists of the grna sequence, the position and the 5'-3'-sequence of the target site, the pam sequence and the location where the pam will be recognized. base editors have a preference to recognize their pams in the +1 nt location before the target site, which makes it advantageous to include the pam in the first position in the pegrna. however, pnb designer will always include the pam in the +1 nt position. this is done because users who edit off-target bystander adenines or cytosines that could also be modified, by mistake. hence, the location of the pam is highlighted in blue to indicate the possibility of off-targets of the deaminases at the on-target site. to prevent the user from selecting a possibly damaging grna, the pnb designer highlights grnas with a potential off-targeting of the deaminase at the on-target site, like in example in fig. 4 c (first row). 5ec8ef588b

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