**Design of polyvalent guide RNAs**

Description of each step of the algorithm are as follows:

*Step 1: Identification of Targets (‘protospacers’).* The first step in the algorithm is to isolate potential target sites in the reference viral genome sequence. At first, every (20-27) nucleotides sequences along with the reference sequence were selected. The CRISPR activity scores of their complementary (sgRNA/crRNA) were then calculated using sgRNA designer tools which are available online. Target sequences with relatively high CRISPR activity scores were then picked to ensure that there is significant CRISPR activity at the selected target sites. For example, for RfxCas13d, every 23 nucleotides sequence along the Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1 complete genome was considered as a CRISPR target. The crRNA activity score was then evaluated at these target sites by Cas13 design algorithm developed by Wessels et al. [1]. Only those targets were then selected which were in top quartile sorted according to their respective crRNA activity score. For spyoCas9, every 20 nucleotides sequences in the reference viral genome which were located immediately downstream of a “Tier 1” protospacer adjacent motif (‘PAM’) (NGG) was considered. The respective sgRNAs activity scores were evaluated by GPP sgRNA Designer online tool [2]. The target sites were then sorted based on these activity scores to pick top candidates for further consideration.

*Step 2: Identification of Targetable Pairs with high homology.* In this step, every potential target for each virus was matched to every other potential target. Only the pairs with >75% sequence similarity (≥ 17 nt identity for Cas13d targets and ≥ 15 nt identity for spyoCas9 targets) were considered as suitable candidates. Further the pairs which are overlapping the SARS-CoV-2 poly(rA)-tail were removed from the list of potential pairs. For targeting the HIV provirus, exact target matches between pairs of sequences on the two long terminal repeat (LTR) regions were not considered (for reasons discussed below) unless they also formed a “target pair” with a segment between the two regions.

*Step 3: Optimization of pgRNA activity at pair sequences.* For a given target pair, pgRNA spacer templates complementary to the target pairs was generated. Different ‘candidate pgRNA’ spacers were then designed with all four potential nucleotides (rA, rU, rC, rG) at each of the sites of sequence divergence between the target pairs, *i.e.,* candidates for target pairs with n differences between sequence. A mismatch penalty (CFD score) between the prospective candidates and the template pgRNA spacers was calculated using the multiplicative approach (Figure 2 right). For Cas13d, those with predicted relative activity (vs. the pgRNA candidate spacer’s “on-target” or antisense sequence) greater than minimum activity score of top quartiles pgRNA spacers candidates (the threshold value) at both sites in the pair were kept for further evaluation. For spyoCas9, the protospacer adjacent motif (‘PAM’) scores were also considered while calculating the mismatch penalty (CFD score) for the potential pgRNA candidates. The filtering criteria based on CFD score was kept same as for Cas13d. In addition, the candidate pgRNAs with homopolymer repeats (≥4 consecutive ‘rU’ or ≥5 consecutive ‘rG’, ‘rC’, or ‘rA’) were removed. Also, candidates with GC% <30% or >70% were eliminated. The respective ‘direct repeat’ sequence for each crRNA (5’-ACCCCUACCAACUGGUCGGGGUUUGAAAC-3’ for RfxCas13d sequence was appended 5’- to their pgRNA candidate spacers and the pgRNA secondary structures was evaluated using the RNAfold function from MATLAB’s Bioinformatic Toolbox and ViennaRNA package in python. If the secondary structure of the direct repeat was perturbed by presence of the candidate spacer from its canonical structure, it was removed from consideration, as those with secondary structure free energy in the spacer region lower than -5 kcal/mol. For spyoCas9, candidate pgRNAs with secondary structure free energy lower than -5kcal/mol in the spacer were removed. The lower value of secondary structure free energy is a measure of the likelihood that pgRNAs will have strong affinity for the target sites in the viral genome.

*Step 4: Estimate relative CRISPR activity across viral strain variants (SARS-CoV-2).* Sequences of 942 SARS-CoV-2 clinical strain variants were downloaded from the Severe acute respiratory syndrome coronavirus 2 data hub (NCBI Virus, accessed April 23, 2020) (48) as all the “complete” nucleotide sequences available at the time. The sequences were then each individually aligned to the Wuhan-1 reference strain using a Needleman-Wunsch global alignment, and for each potential target site (27 nt region) across the genome, the number and prevalence of unique variants were counted. In evaluating pgRNA candidates, if the minimum relative activity across variants (MRAV) for the candidate pgRNAs across all the sequenced SARS-CoV-2 strains was <95% at either target site, the candidates were flagged. Sequences with ambiguous sites or indels (because their effect on Cas13d and Cas12a are less well defined) were removed from the calculation. To evaluate sequence conservation and “conservation of targets” across the SARS-CoV-2 genome in general (*i.e.*, Figure 3B and Figure 3C, resepctively), the most common target sequence was considered the “consensus” variant. The relative activity at each other unique variant was calculated using a gRNA for the consensus variant.

*Step 5: Estimate relative activity at potential host/human off-targets.* Candidate pgRNA spacers were aligned to the host genome. For example, for spyoCas9 spacers were aligned to the human genome (Genome Reference Consortium Human Build 38, GRCh38 human reference genome) and human transcriptome for Cas13d (GRCh38 human RefSeq transcripts) using a local nucleotide BLAST optimized for short sequences <30 nt (blastn-short). The region surrounding each hit to the human genome or transcriptome, to a total of 23 nt (the 23 nt protospacer for Cas13d and a 4 nt PAM + 20 nt protospacer for Cas9) was then extracted and evaluated for a mismatch penalty score with its respective pgRNA candidates. While “off-target” interactions with the host transcriptome by Cas13d is not expected to have too detrimental of consequences compared to off-target genomic mutations by spyoCas9, these unwanted interactions may titrate or dilute the activities of the Cas13d against the desired targets. Initially we took only spacer candidates which has no hits/off target effect to the host genome. However, for some cases few other candidates were also considered. For example, for Cas13d in some cases, we also took the pgRNA spacer candidates with maximum predicted relative activity at any human transcript <10% and, for spyoCas9, those with maximum predicted relative activity at any site in the human genome < 1%.

*Step 6: Selection of pgRNA based on additional functional criteria.* At this stage, the RNA candidates have been screened for high relative activity at multiple viral targets and across clinical strains, low predicted activity at host “off-target” sites, and biophysical characteristics that suggest high overall CRISPR activity (35,41). The candidates can then be further refined by considering pgRNA targets located within specific genes or regions of interest (ROIs) that may be of clinical or functional significance, conservation of the targets / viral intolerance to mutations, and on-target activity prediction, which can be performed using several bioinformatic tools and methods available, prior to experimental validation.

Calculation of mismatch penalties and relative CRISPR activities

Estimates of the relative CRISPR activity at sites not perfectly targeted by the gRNA/pgRNA spacer sequence were generated by calculating the Cutting Frequency Determination (CFD) score [,]. To calculate the CFD score, the penalty (relative reduction in CRISPR activities) that result from each site with a mismatch was drawn from a CFD matrix, the table of position-specific reductions of activity that occur as a result of mispairing between specific nucleotides in the spacer and target. For RfxCas13d the CFD matrix was generated by the Sanjana lab [] using massively parallel screens of gRNA libraries for CRISPR activity. They recovered the penalty scores by taking the value of the reported log2(Fold-Change in expression) to the second power, vs. a perfectly complementary targeted mRNA reporter in their massively parallel screen for gRNA activity in the presence of mismatches []. In the event of a missing value, they used interpolation. For example, a missing rA-rC mismatch penalty at position 15 was interpolated from the penalties of the rA-rC mismatches at positions 14 and 16. For multiple sequential mismatches (two-in-a-row, three-in-a-row, etc.), the position-specific penalties for double- and triple- mismatches were used to calculate the CFD scores at those sites. For SpyCas9, CFD matrix was developed by Doench lab [,] using the data from the “dropout” experiments. These datasets are publicly available []. The effect of different protospacer adjacent motif, PAMs (PAM strength) on spyoCas9 activity at different sites was recovered using data from similar large-scale screens of PAM libraries []. The individual penalty scores thus obtained for each mismatch sites were then multiplied to calculate the CFD score for a given target and gRNA spacer. The position-specific penalties (average over all possible mismatched nucleotides) are summarized in Figure S1. For SpyCas9 the product of individual mismatch penalty scores was further multiplied with respective protospacer adjacent motif (‘PAM’) score to obtain the final CFD score. This approach is fast to implement and has been successfully used as a reasonable approximation for CRISPR activity at off-target sites by a number of different CRISPR effectors 36, 38. In general, if the off-target sites had <15 nt identity as the intended target (<55% identity for RfxCas13d or <65% identity for SpyCas9), the CRISPR effectors were considered effectively inactive at those sites.