Arrayed synthetic gRNA libraries for high-throughput knockout screens in immortalized cell lines and iPSCs

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Introduction

IDT is launching a custom CRISPR gRNA library solution designed to address the need for a robust and consistent design space. This method can be used to screen phenotypic outcomes, with user-defined parameters such as transcript location, distance between guides, and off-target scores. The Library Design workflow integrates seamlessly with IDT's rhAmpSeq™ analysis system, which provides robust and uniform assessment of CRISPR editing, streamlining the process from experimental design to analysis.

Here we demonstrate some of the experimental approaches we took to understand the best practices for design and delivery of these libraries, including considerations for cell types and number of guides. We are beginning to explore ways of looking at phenotypic outcomes, which includes a novel multiplexed ELISA system. This will further link editing outcomes to measured protein changes and provide further evidence of knockout efficiency. Finally, IDT has developed various gRNA synthesis and purification methods, including single channel capillary electrophoresis (SCCE) and ultra-performance liquid chromatography (UPLC), to ensure high purity and full-length product (FLP) of gRNAs. This demonstrates that gRNA synthesis for libraries has the best possible outcome regarding your preferred application.

Library design tool and integration with rhAmpSeq for CRISPR knockout and analysis

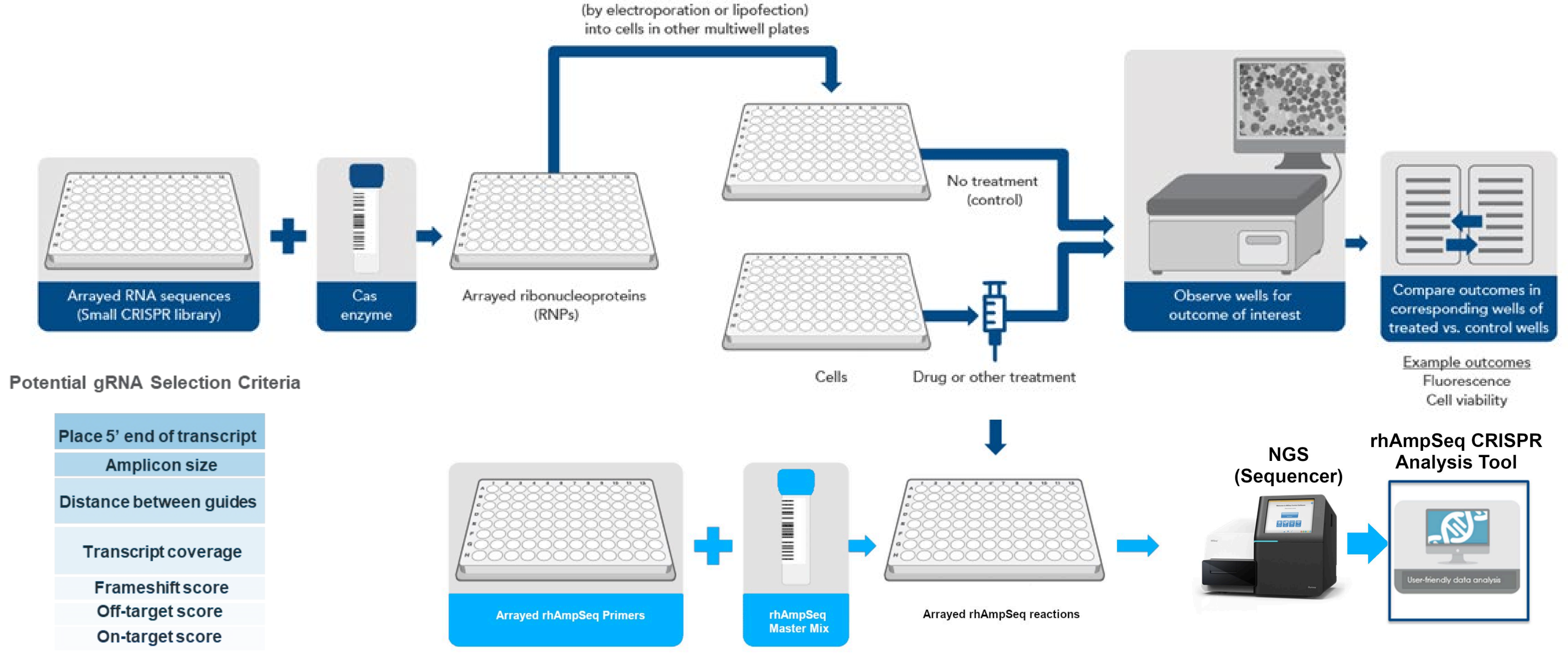
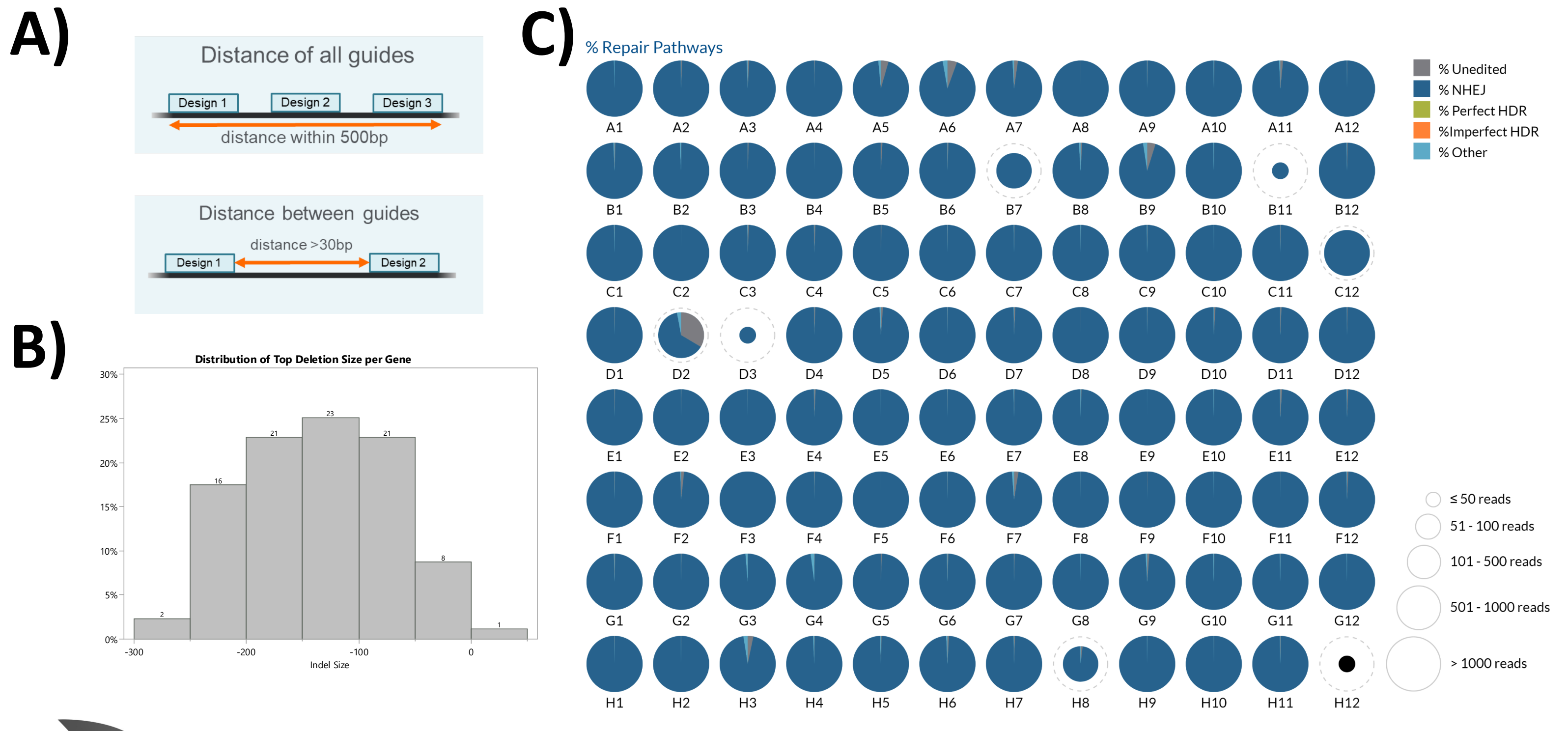


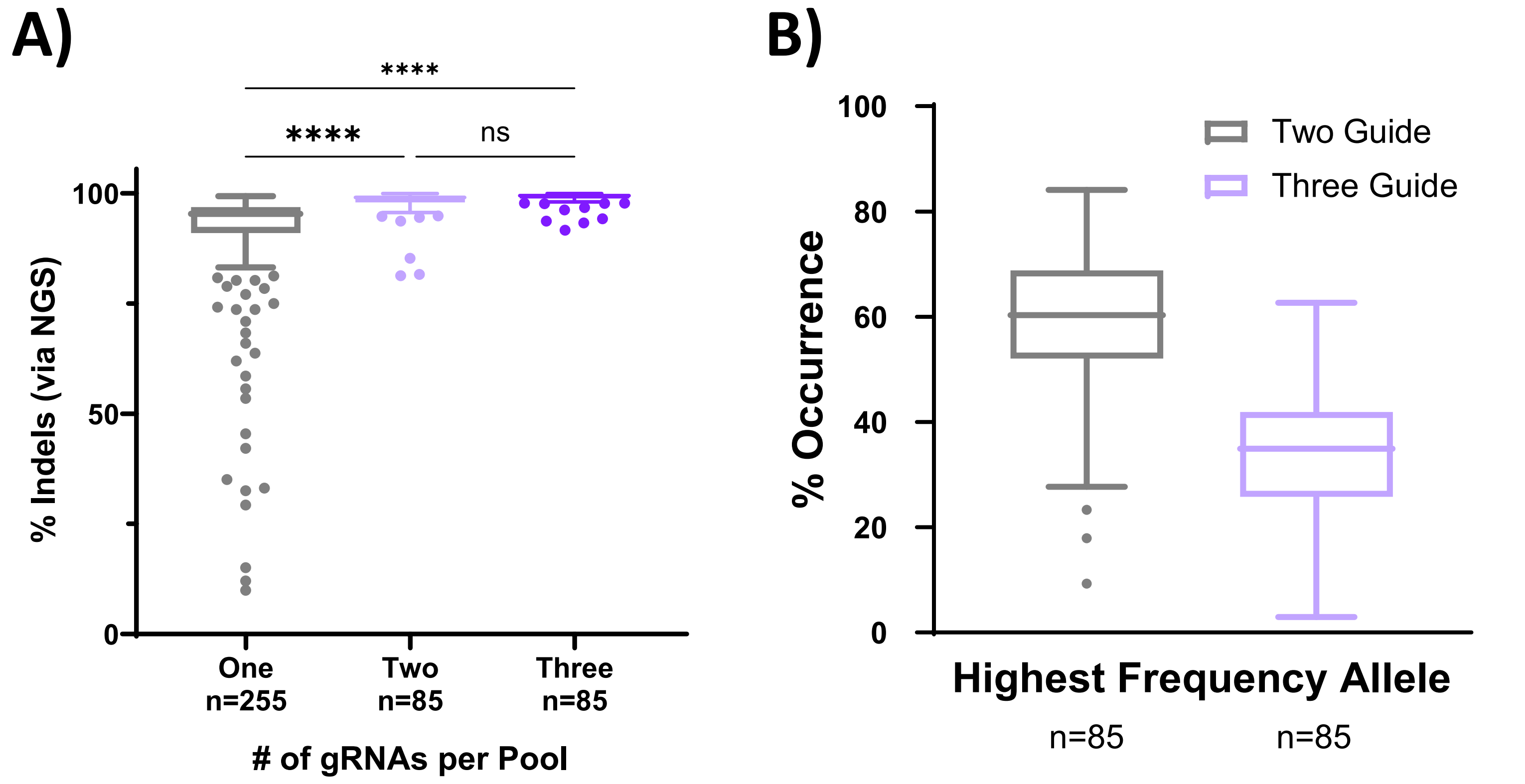
Figure 1. To serve current research workflows IDT is launching custom CRISPR gRNA libraries. Leveraging our full workflow going from experimental design to analysis, we are providing a solution for design, reagents and analysis for this application. The goal is to provide synthetic arrayed gRNA, with specific design parameters defined by the user, in order to screen a large number of targets for phenotypic outcomes in a high throughput fashion. Libraries are highly customizable depending on the downstream application. Many factors must be considered when designing a CRISPR library. A weighted selection criteria is applied based on characteristics such as location within the transcript, total distance, distance between guides, transcript coverage, and off-target score. Currently we support hg38 genome but take customer requests on a case-by-case basis. To complete your workflow, we offer our robust CRISPR analysis pipeline by using our rhAmpSeq™ analysis system to analyze CRSISPR edits. rhAmpSeq™ allows for simultaneous and uniform assessment of editing efficiency, while integrating seamlessly with other IDT solutions.

Editing outcomes when using the CRISPR library design tool

Figure 2. A gRNA library was designed (95 gene targets; 3 gRNAs/gene) to fit within a single sequence-able amplicon and evaluated for editing efficacy. A) Definition of library spacing requirements (<500 bp total; >30 bp between gRNAs) B) Deletion size distribution of gRNA library show that when using pooled guides deletion sizes are most often between 100–150 bases. C) Example rhAmpSeq™ Analysis Tool 96-well plate output of the three sgRNA experiment described above.



Design parameter assessment for CRISPR libraries demonstrates optimal variables.



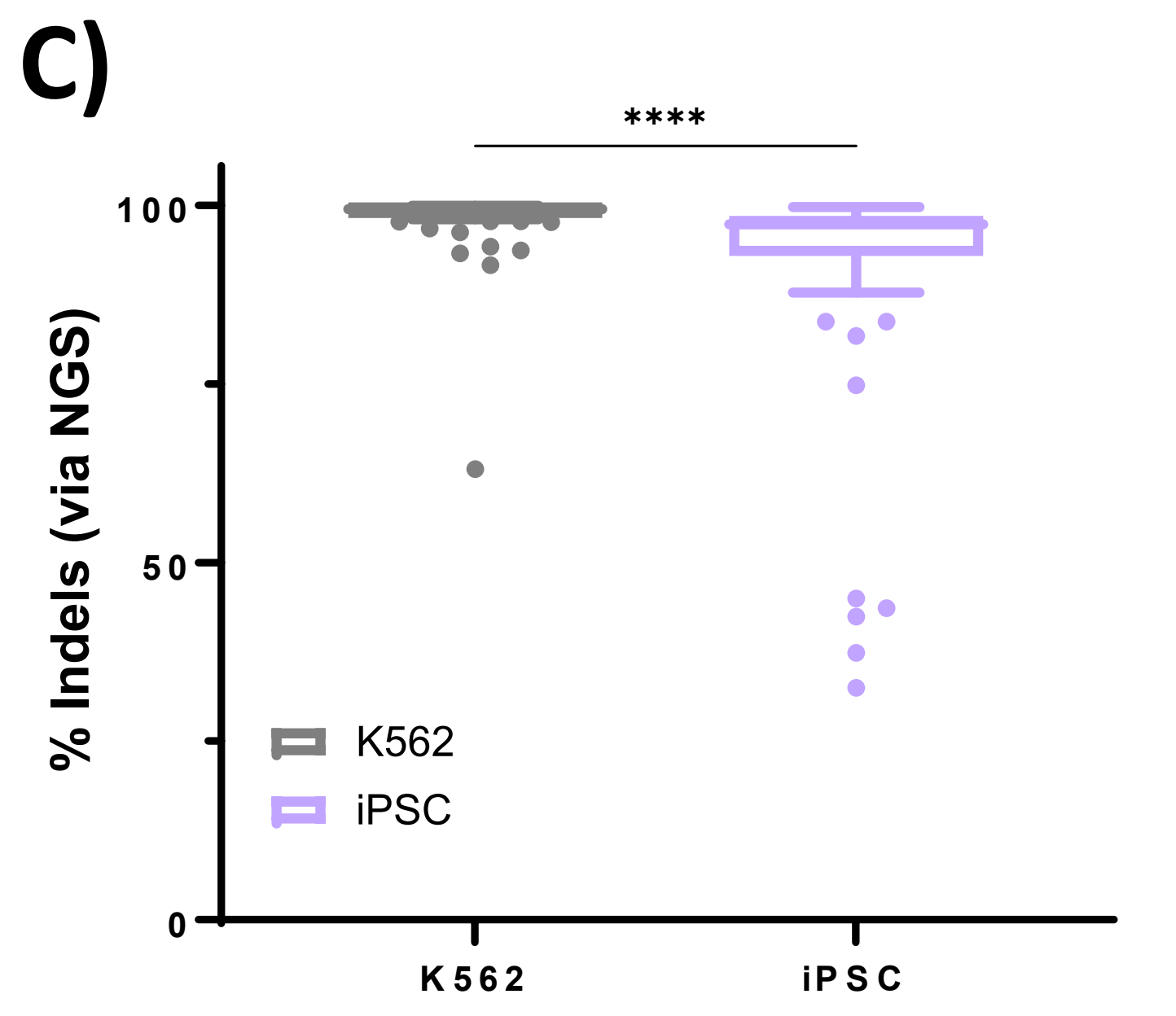


Figure 3. Multiple variables were experimentally evaluated to deliver a balance of best outcome vs design constraints for gRNA libraries. K562 cells were transfected via the Lonza 4D-Nucleofector™ System with 3 sgRNAs each targeting 95 unique genes. The sgRNAs were transfected individually or pooled together with 2 or 3 gRNAs for each target, alongside Alt-R® S.p. Cas9 Nuclease V3, at a final concentration of 1 µM per gRNA. A) Using this gRNA libraries, sgRNAs were nucleofected individually, all together (three), or with the middle guide dropped out (two). Plotted is the average editing frequency. B) Indel profiles were characterized by NGS + CRISPAltRations. Two and three sgRNAs induced the highest frequency of indels. Indel profiles show that three sgRNAs produce a wider variety of alleles compared to two sgRNAs. C) Pooled sgRNAs efficiently induce indels in iPSCs. %Indels were measured after nucleofection of pooled gRNAs in either K562 cells or iPSCs. Though efficiency is significantly lower in iPSCs, the average across all genes remains high.

Functional evaluation of CRISPR editing using multiplexed high throughput ELISA

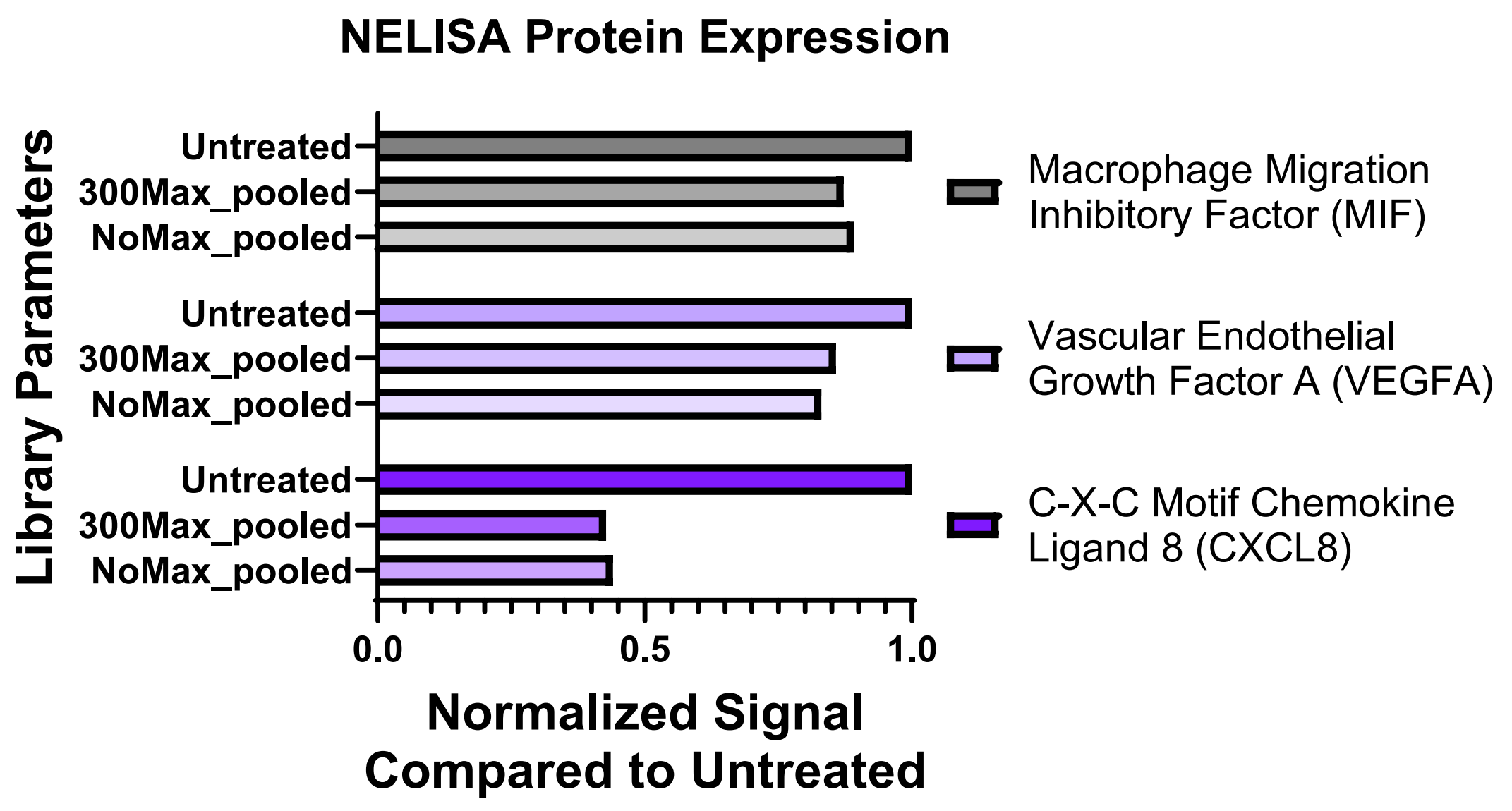


Figure 4. To determine a functional outcome for our gRNA libraries, we designed a library and used a novel fluorescent multiplexing ELISA from Nomic[2] to assess proof-of-concept functional knock down. Libraries were designed using 3 guides per target. 2 designes per target were used, either constraining the design space to 300 bp (300 max) or allowing for no constraint (NoMax). Tranfections were performed using Lonza 4D-Nucleofector™ System with 3 sgRNAs each targeting 11 genes. Here we display 3 representative genes. All ELISA values were normalized to the input amount and compared to the untreated control.

Guide synthesis and evaluation of contaminants are important considerations for gRNA Libraries

Evaluation of full-length product by advancing detection methods

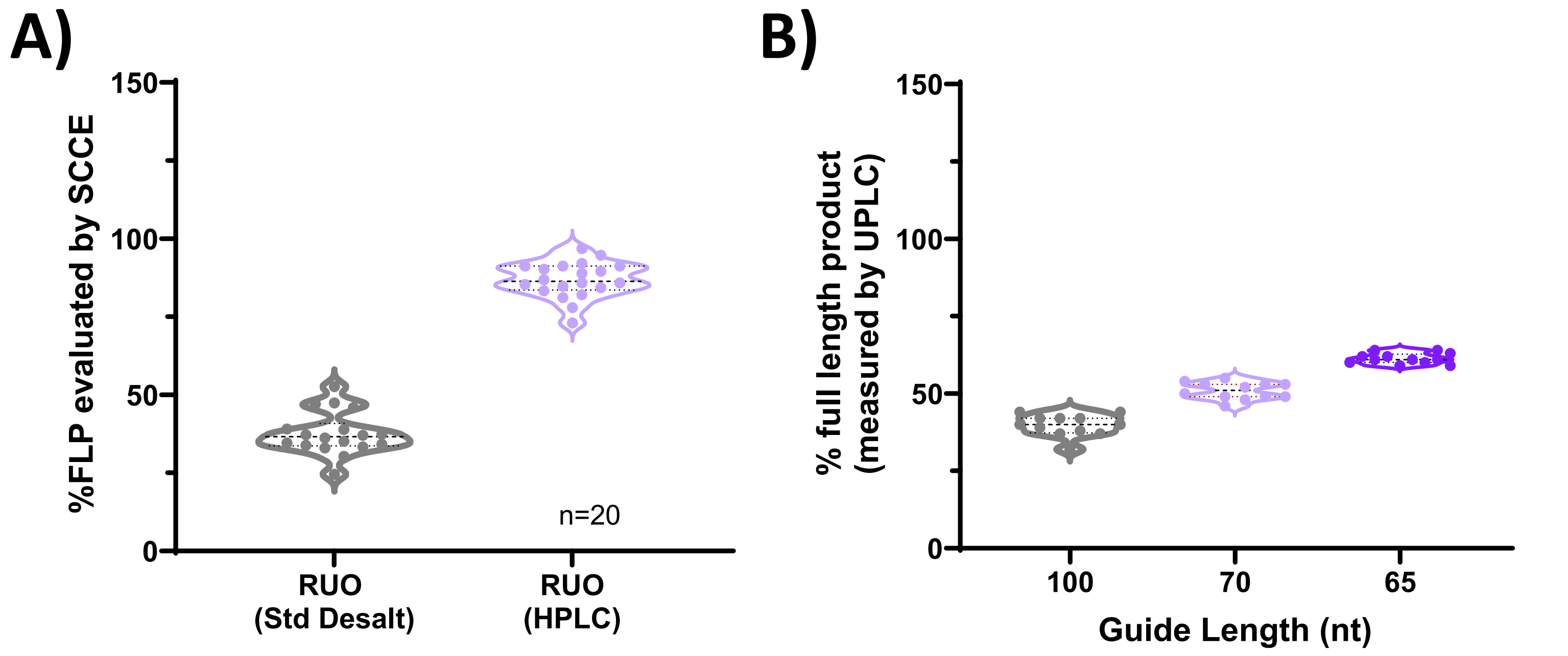
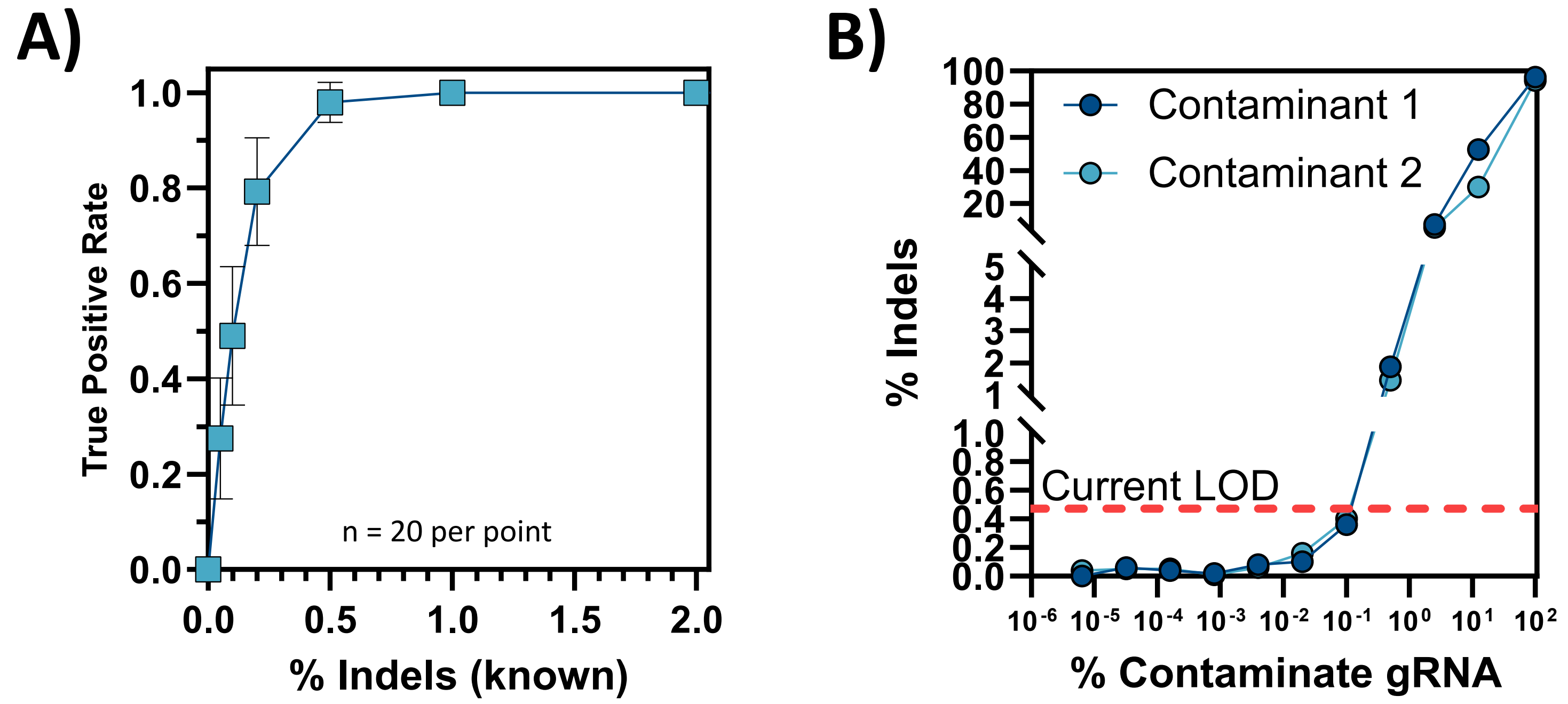
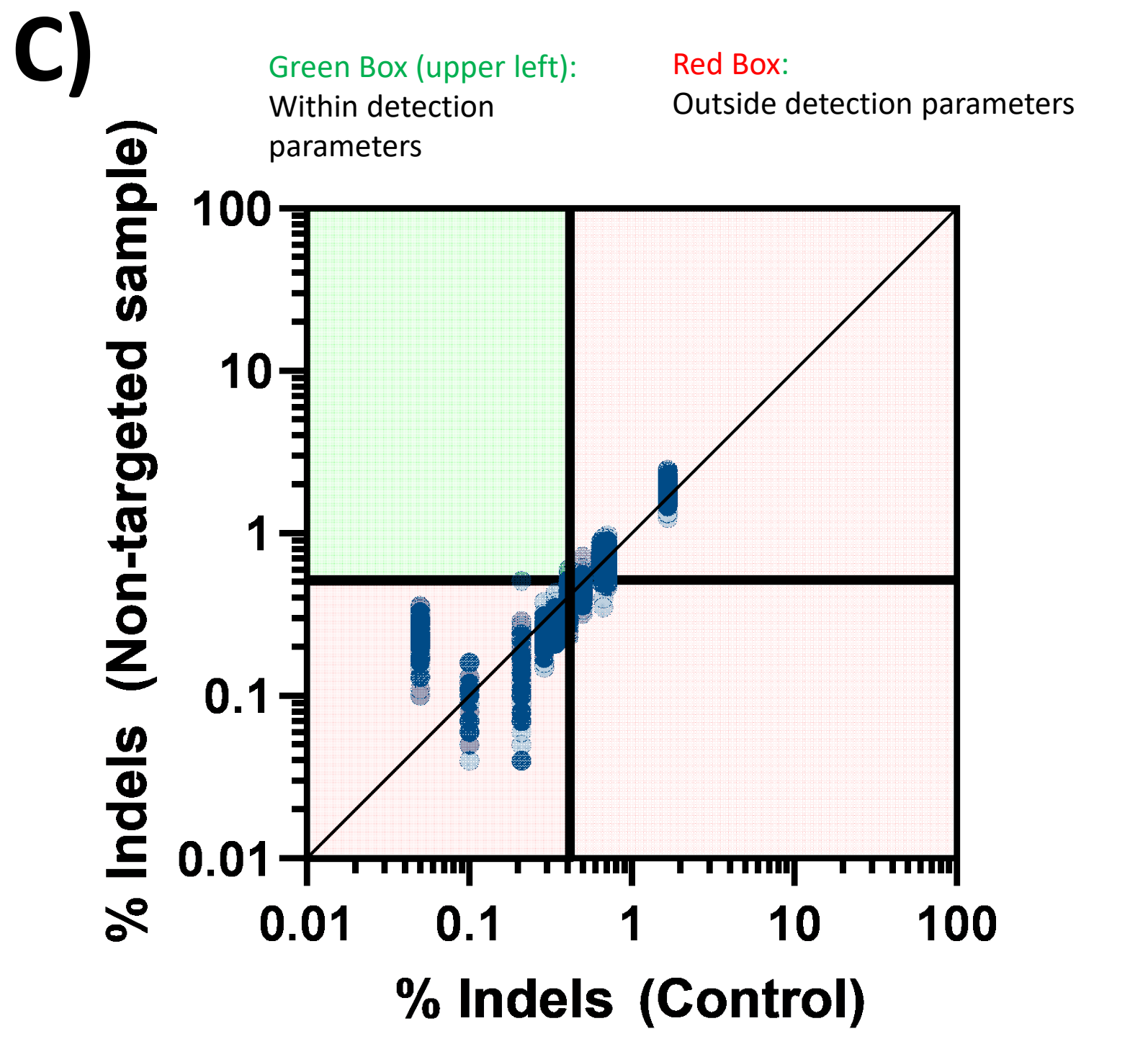


Figure 5. IDT has leveraged its experience in gRNA synthesis to enable a wide selection of gRNA formats and purification options. Although not all of them are compatible with gRNA libraries, we are working on development of analytical methods and expanding the variables for library synthesis. A) Single channel capillary electrophoresis (SCCE) was used to assess purity of 20 different RUO gRNAs, undergoing standard desalt purification or HPLC purification. The mean %FLP for each grade of RUO gRNA is presented. IDT manufactures various grades of gRNAs. 100-nt Cas9 sgRNAs targeting HPRT1 was performed. B) Using ultra-performance liquid chromatography (UPLC) we were able to determine the percent full length product. Various guide RNA lengths can be used to of the needs of your specific gRNA library applications. Custom guide RNAs targeting HPRT1 consisting of 100, 70, or 65 nucleotides (12 guides for each design) were ordered from IDT with standard desalt purification. UPLC methods reflect our consistent gRNA synthesis.

IDT synthesized gRNA libraries have no measurable cross-well contamination as measured by gRNA editing activity





A) A subset of 20 loci within gene bodies were chosen (ctrl: <= 0.4% indels; tx: 0.05– 3.5% indels) and serial diluted into unedited gDNA in triplicate to 0.05, 0.1, 0.2, 0.5, 1, 2, 3.5% indels, amplified using rhAmpSeq library prep, and analyzed using the rhAmpSeq CRISPR Analysis Tool + a Fishers Exact test for classification ($p < 0.05$) to determine limits of detection (LOD). B) Measurement of the editing activity of gRNAs at different contamination fractions. C) Using the defined limit of detection of 0.5% indels, a plate of sgRNAs from a library were evaluated and displayed no detectable levels of “contaminate” sgRNA editing. This LOD may be of interest to researchers searching for very low on- or off-target events.

References

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