Building a diverse CRISPR gRNA library using arrayed-synthesized oligo pools

Application Note

GenScript
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Introduction

CRISPR-Cas9 is a powerful tool for genome editing due to its simplicity, specificity and versatility. Originally identified in the *Escherichia coli* (*E. coli*) genome, CRISPR (clustered regularly interspaced short palindromic repeats) sequences were found to function as part of an RNA-based adaptive immune system to target and destroy genetic parasites at the DNA level. CRISPR-associated protein 9 (Cas9) is an endonuclease that uses these CRISPR sequences as a guide to recognize and cleave specific portions of DNA. In order for the Cas9 protein to cleave parts of the target genome, RNA oligos called guide RNAs (gRNA) are needed to identify the target genomic sequence and simultaneously complex with the Cas protein to edit specific genes. Together, these two components are being utilized for various applications, including gene editing within organisms to study molecular targets, building CRISPR libraries for target screening, using modified CRISPR-Cas9 versions for RNA modification or transcriptional regulations, epigenetic editing, live imaging of DNA/mRNA, and, more recently, as a therapeutic tool for gene and cell therapy.

This application note will focus on how to build a reliable, pooled CRISPR gRNA library for rapid, high-throughput screening of molecular targets. Use of these CRISPR gRNA libraries provide a great tool for genome-wide and gene-focused applications.

To construct a CRISPR gRNA library, gRNAs are typically cloned into lentiviral vectors for efficient transduction into host cells. Following cloning, libraries are amplified, packaged into viruses, and transduced into host cells to generate mutant cell clones. These mutants are then screened under specific conditions for a phenotype of interest (**Figure 1**). When designing a CRISPR gRNA library, certain factors need to be considered, including the type of genetic modification desired, how much of the genome is being targeted, and the species that the library will be screened in as gRNAs are unique to the genome being targeted.

To optimize gRNA design, we recommend using an online tool such as the Broad Institute Genetic Perturbation Platform (GPP) sgRNA Designer. It is important to pick 3-6 highest scoring gRNAs for each target gene. Each gRNA should (1) target the consensus sequence of all transcriptional isoforms, (2) target the 5' exon, and (3) target the functional domain.

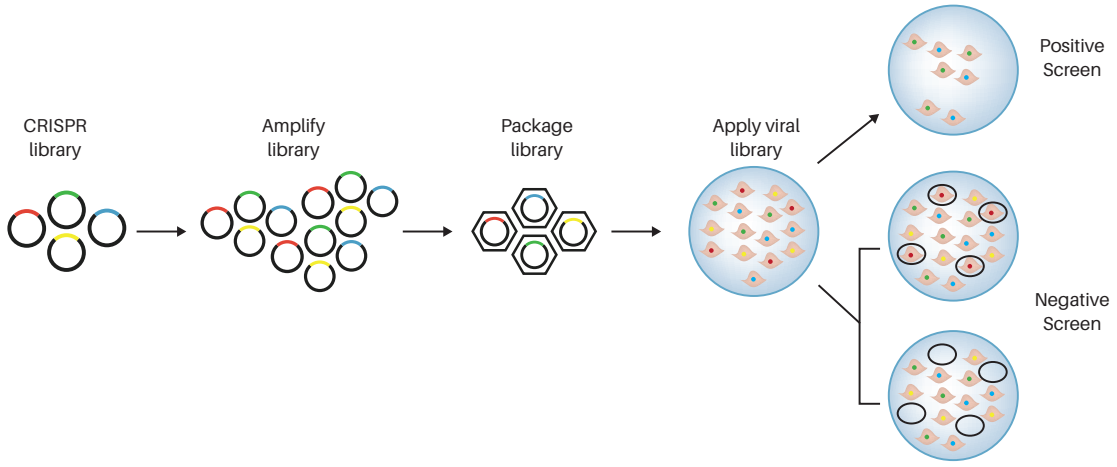

Figure 1: Typical workflow of using CRISPR gRNA libraries for high-throughput screening.

To have a successful CRISPR gRNA library, the quality of the gRNAs in the pool is important and requires ultimate control over the synthesis process. In this application note, we provide an example of how our array-synthesized oligonucleotides can be utilized in the construction of a customized gRNA library for genome-scale knockout. Individually designed gRNAs are synthesized simultaneously using our semiconductor chip platform. Each gRNA is electrochemically synthesized and seamlessly cloned into a lentiviral vector to create a perfect customized gRNA library. Using GenScript's arrayed, semiconductor-based oligonucleotide synthesis platform for synthesis of the gRNAs allows easy construction of CRISPR gRNA libraries with complete coverage and uniform distribution, maximizing your screening efficiency.

Case Study

1. Library Construction

In this study, a customized CRISPR gRNA library containing 58,028 unique gRNAs for gene knock-out in the human genome was constructed. All gRNAs were synthesized simultaneously as short, single-stranded oligonucleotides using our arrayed chip platform. Conserved sequences were added to the end of each gRNA to facilitate PCR amplification. Following synthesis, gRNAs were amplified to double-stranded PCR products and cloned into lentiviral vectors to create the library. Overall, quality of the CRISPR gRNA library was determined by assessing library diversity and sequence distribution using next-generation sequencing (NGS) (**Figure 2**).

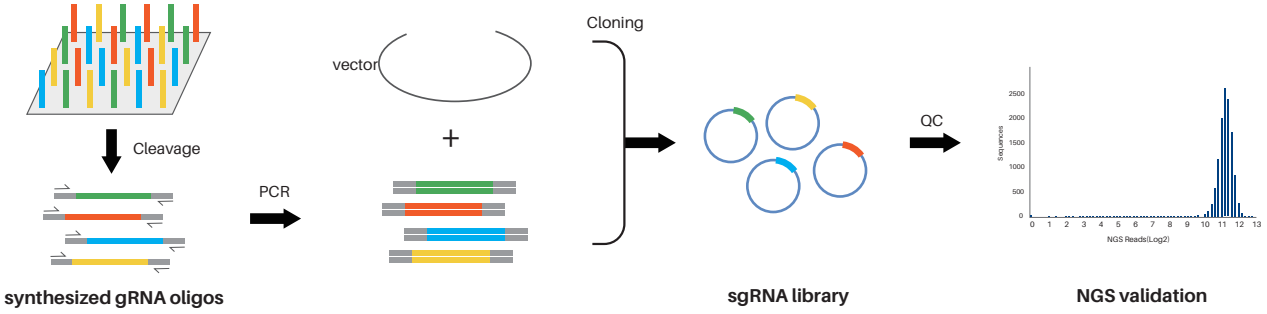

Figure 2: Workflow for constructing gRNA libraries using arrayed, semiconductor-based oligonucleotides. gRNAs are precisely synthesized using our advanced semiconductor technology as single-stranded oligonucleotides. Following PCR amplification, double-stranded gRNAs are cloned into vector of choice to create a screening library. Typically, a lentiviral vector optimized to produce high-titer virus is used for efficient transduction.

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We recommend using a two vector format when selecting a cloning vector, one that expresses the gRNA and one expressing the Cas9 protein. For example, using LentiGuide-Puro for gRNA expression and the Cas9 vector LentiCas9-Blast. Using a two vector format has a higher advantage over an all-in-one vector, such as LentiCRISPR v2, because they produce a higher titer of virus ensuring efficient transduction of CRISPR-Cas9.

2. QC tests

➤ CRISPR gRNA library diversity validated by Sanger sequencing

In order to determine the diversity of the CRISPR gRNA library, 30 clones were randomly picked and sequenced using Sanger sequencing. Sequencing results showed that all the gRNA sequences were unique with 100% diversity among the randomly picked clones. Additionally, each gRNA sequence exactly matches a desired gRNA sequence, indicating accurate oligonucleotide synthesis and successful PCR amplification of the gRNAs following synthesis (**Figure 3**).

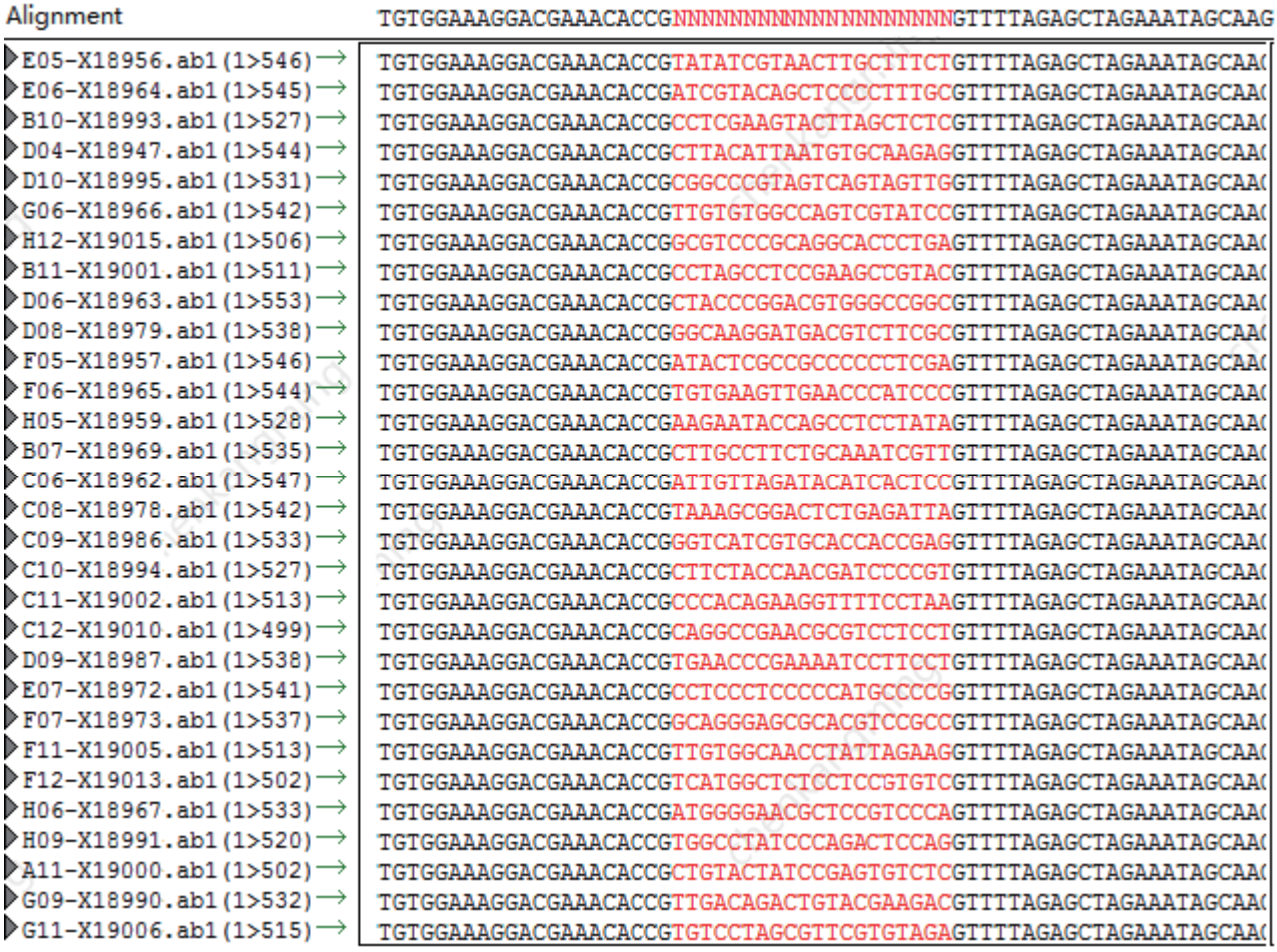

Figure 3: Sanger sequencing result of randomly picked clones. Clones from the library were picked and Sanger sequencing was performed to check library diversity. All gRNA sequences are unique and exactly match the expected sequences.

➤ Library coverage and distribution of gRNAs determined by NGS

In order to determine the coverage and distribution of the CRISPR gRNA library, NGS was performed. The NGS results show that all desired gRNAs were detected, indicating 100% coverage of the gRNA library. In addition, the 10x coverage is 99.95%, indicating almost all of the gRNAs in the library are still detected at deep sequencing depth (**Table 1**).

Another important indicator to evaluate the quality of library is to determine gRNA distribution. In an ideal situation, all gRNAs should be evenly distributed within the library. However, after PCR and cloning processes, the quantity of some gRNAs are changed, resulting in the presence of some gRNAs being very low (~10 reads) while other gRNAs are in more abundance (~2,000 reads). The ratio between these two extremes should be less than 8, indicating an acceptable library. Our NGS analysis reveals the 90/10 ratio is 5.1 indicating equal distribution of desired gRNAs within the library, ensuring maximum screening efficiency and identification of a hit (**Figure 4**).

Reference	52,959
Yield (Gbases)	10
Number of Reads (M)	33.34
% of ≥ Q30 Bases (PF)	95.88
1x Coverage	100%
10x Coverage	99.95%
10%qual	204
90%qual	1054

Table 1: Analysis of NGS raw data.

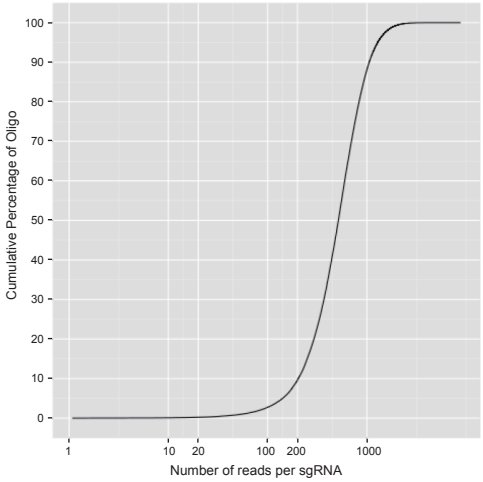

Figure 4: Number of reads per gRNA in the library.

Summary

GenScript has developed a method for creating diverse, well-designed CRISPR gRNA libraries using our advanced oligonucleotide synthesis platform. Our proprietary arrayed, semiconductor-based electrochemical oligonucleotide synthesis technology allows precise synthesis of each individual gRNA. Accurate synthesis of gRNAs ensures each library contains complete coverage and equal distribution of all desired gRNAs, thus minimizing screening burden and saving the researcher valuable time and effort.

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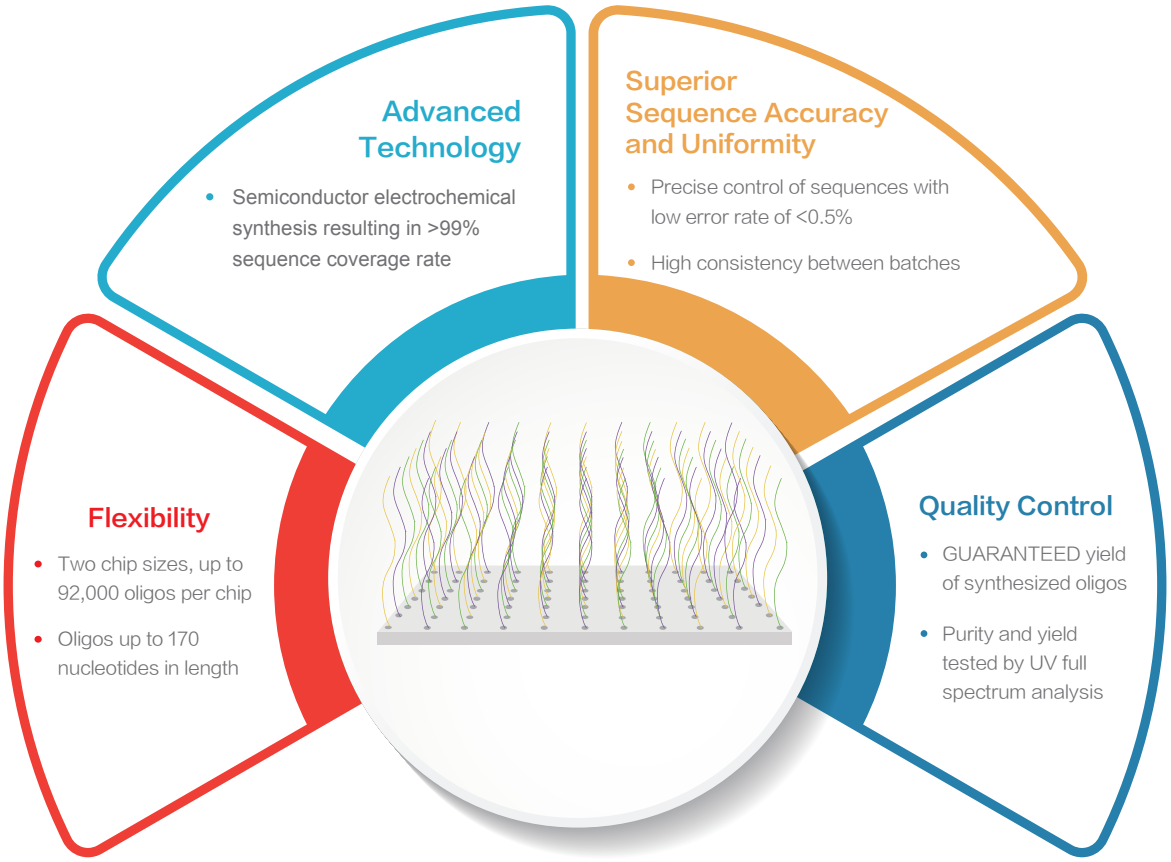
GenScript's Oligo Pool Service



With unbeatable accuracy and uniformity, GenScript's oligo pools provide faster identification of your hits with minimal screening

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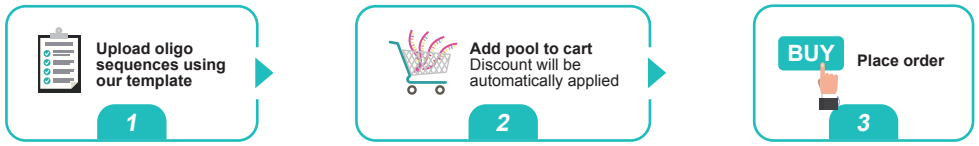

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