Custom CRISPR Guide Libraries to Suit Any Screen

The use of CRISPR screening technology enables the interrogation of gene function at virtually any scale, especially in pooled screening applications. While genome-wide CRISPR screen libraries are easily accessible off-the-shelf, their lack of design flexibility means it is difficult to cost-effectively address specific research questions, thus limiting the applicability of these libraries. Twist Bioscience's Oligo Pools enable the creation of custom CRISPR guide libraries that give you the flexibility to tailor your screens to your species, strain, and research question, all with the confidence that guides in your library will be precisely synthesized and equally represented.

NOT ALL sgRNA LIBRARIES ARE CREATED EQUAL

Off-the-shelf sgRNA libraries are a convenient source for CRISPR screening experiments. However, rigorous QC and additional validations are often required to ensure unbiased results. Alternatively, custom CRISPR guide libraries can be tailored to your research questions without the additional burden.

Important features to keep in mind when designing a custom guide RNA library include:

- Accurately synthesizing sgRNAs to maximize Cas9 targeting
- Avoiding dropout and biased results by maintaining a uniform representation of sgRNAs from synthesis to experiment
- Flexibility of sgRNA designs to keep costs low when scaling CRISPR screens focusing on only what you want to target

Twist synthesizes highly uniform oligonucleotide pools wherein >90% of unique sequences are represented within 2.5x of the mean. With the ability to synthesize oligos up to 300nt in length, researchers can design more complex elements into oligo libraries. This flexibility at both the sgRNA and library levels means that custom libraries can be built with unique identifiers, capture sequences, amplifcation sites, dual-guide designs, CRISPR arrays, and other custom elements to match each experiment's unique needs.

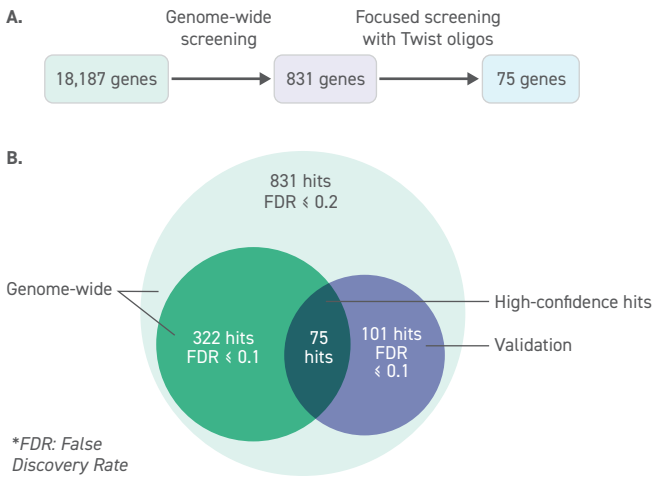
	OFF-THE-SHELF sgRNA LIBRARIES	TWIST CUSTOM sgRNA LIBRARIES
Validated and quality controlled?	No	Yes
Targeted to your regions of interests?	No	Yes
Flexibility to be used with targeted secondary, multi-plexed, or target capture single-cell screens?	No	Yes
Design space to enhance your CRISPR screen	0 nt	Up to 300 nt

Comparison of key features of off-the-shelf and Twist custom sgRNA libraries

HOMING IN ON TARGETS WITH FOCUSED SECONDARY SCREENS

Genome-wide CRISPR screens provide a powerful first pass at identifying potential genes of interest. However, the possibility of finding hundreds to thousands of hits make hit validation both difficult and costly to scale. Focused secondary screens with Twist custom CRISPR guide libraries are a useful tool for narrowing in on true hits of interest, enabling more efficient characterization of genotype-phenotype relationships.

For example, scientists from Boehringer Ingelheim used a Twist custom guide library to address the time and expense of examining more than 800 hits from their genome-wide screen[1]. Combining the results of the genome-wide and focused screens, a total of 75 high confidence hits were identified for follow up, reducing the number of candidate genes to be individually tested by 90%.



Successive elimination of genes not involved in biological process of interest through a genome-wide screen and focused screen.

COMBINING TARGET CAPTURE AND SINGLE-CELL CRISPR SCREENING

More efficient, insightful data through target capture and single-cell CRISPR screening allows researchers to analyze complex phenotypes across a spectrum of transcriptomic profiles. However, linking sgRNA's to specific transcriptomic outcomes can be challenging because sgRNAs are not polyadenylated. Without the ability to directly capture and sequence sgRNAs, the scale of single-cell CRISPR screening is severely limited.

Researchers from UCSF and Princeton University devised a way to add specific capture sequences to sgRNAs, enabling direct capture of sgRNA transcripts and genome-wide single-cell CRISPR screening[3].

The team partnered with Twist for precise sgRNA synthesis to insert capture sequences without disrupting guide activity. With this approach, they demonstrated numerous applications in CRISPR screening, including analysis of gene epistatic interactions and genome-wide single-cell CRISPR screening[4].

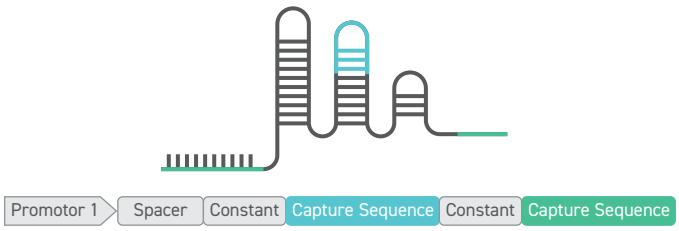
What's more, through the use of Twist custom target capture panels, the team were able to focus RNA sequencing resources on the transcripts of interests, improving target transcript representation from just 6% to 87%. Combining Twist Oligo Pools and Twist Custom Target Enrichment Panels in this single workflow enables highly efficient and sensitive single-cell functional genomic screening.

CREATING MULTIPLE FOCUSED LIBRARIES FROM A SINGLE OLIGO POOL

The flexibility to create multiplexed focused libraries and allow multiple experiments to be performed from a single oligo pool is a useful way to efficiently and cost-effectively conduct small CRISPR screens.

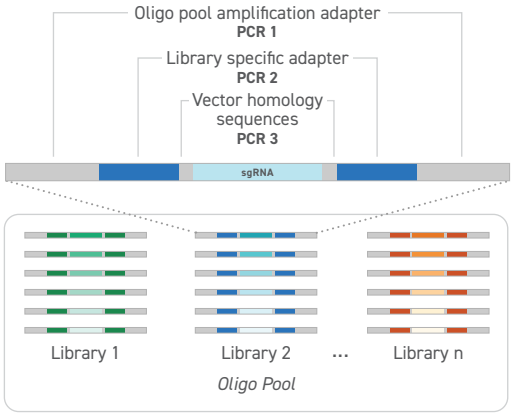
Researchers from two leading German research institutions designed an sgRNA library to contain multiple unique amplification adapters, enabling the single library to be sub-divided into several smaller screens[2].

Because of the high-fidelity of the oligo pools synthesized by Twist, sgRNA representation in PCR-amplified and cloned sub-libraries was maintained, providing a high-quality, end-to-end pipeline for generating multiplexed, custom sgRNA libraries.



Single guide RNA design with two potential capture sequence positions highlighted

SgRNA design for direct capture Perturb-seq



CLUE Oligo Pool design. Each library within the pool is assigned a specific adaptor which can be used for PCR amplification of that specific library. NGS-based quality assessment of 10 pooled sgRNA libraries produced from a single oligo pool.

REFERENCES

1. Lindner, Benjamin et al. "A genome-wide CRISPR/Cas9 screen to identify phagocytosis modulators in monocytic THP-1 cells." Sci Rep 11, 12973 (2021).
2. Becker, Martin et al. "CLUE: a bioinformatic and wet-lab pipeline for multiplexed cloning of custom sgRNA libraries." Nucleic Acids Res 48, e78 (2020).
3. Replogle, Joseph M et al. "Combinatorial Single-Cell CRISPR Screens by Direct Guide RNA Capture and Targeted Sequencing." Nature Biotechnology, vol. 38, no. 8 (2020).
4. Replogle, Joseph M et al. "Mapping information-rich genotype-phenotype landscapes with genome-scale Perturb-seq." BioRxiv, (2021).

TALK TO US ABOUT TOOLS AND STRATEGIES TO TAKE YOUR CRISPR SCREENS TO THE NEXT LEVEL

Contact the Twist Bioscience team at **support@twistbioscience.com** or visit **twistbioscience.com**