VIVLION'S OFF-THE-SHELF LIBRARY PORTFOLIO

A GOOD SCREEN STARTS WITH A VIVLION LIBRARY

Library	No.	Genes	Library size*	Selection	Fluoro-phore	Skew#
Alexandria						
Single-targeting human genome-wide CRISPRko	LGW101	20,817	83,547	Puromycin	NA	1.62
Fixed-pair human genome-wide CRISPRko	LGW201	20,908	41,949	Puromycin	NA	2.22
Fixed-pair mouse genome-wide CRISPRko	LGW202	22,314	44,672	Puromycin	NA	1.70
Fixed-pair human genome-wide CRISPRko	LGW203	20,908	41,798	Puromycin	mCherry	2.35
Fixed-pair mouse genome-wide CRISPRko	LGW204	22,314	44,649	Puromycin	mCherry	1.66
Pergamon						
Single-targeting human genome-wide CRISPRko	LGW103	20,256	40,494	Puromycin	NA	1.61
Single-targeting human genome-wide CRISPRko	LGW104	20,256	40,492	Blasticidin	NA	1.66
Targeted protein families						
Single-targeting ubiqui-tin-proteasome system (UPS) CRISPRko library	LPF103	853	3,416	Puromycin	NA	2.41

* number of sgRNAs for single libraries, number of sgRNA pairs for fixed-pair libraries
\# skew always depends on lot number

We are working on expanding our off-the-shelf portfolio. Let us know what you would like to see next at info@vivlion.com!

Customized libraries can be based on one of our standard plasmid backbones (see page 4), adapted according to your needs, or on customer-specific plasmids.

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PRCISR® CRISPR: ACCELERATING DISCOVERIES

A good screen starts with a good library.

Our proprietary PRCISR® platform delivers CRISPR libraries with unmatched uniformity, boosting resolution and expanding the search space while requiring less cell material. Combinatorial PRCISR® libraries enable unbiased screening of genetic interactions, helping you to map functional pathways, identify synthetic lethality, and uncover novel biological insights.

Vivlion is your reliable partner for CRISPR libraries, screening, and computational analysis.

Please reach out to one of our scientific project managers at info@vivlion.com.

Vivlion services at a glance

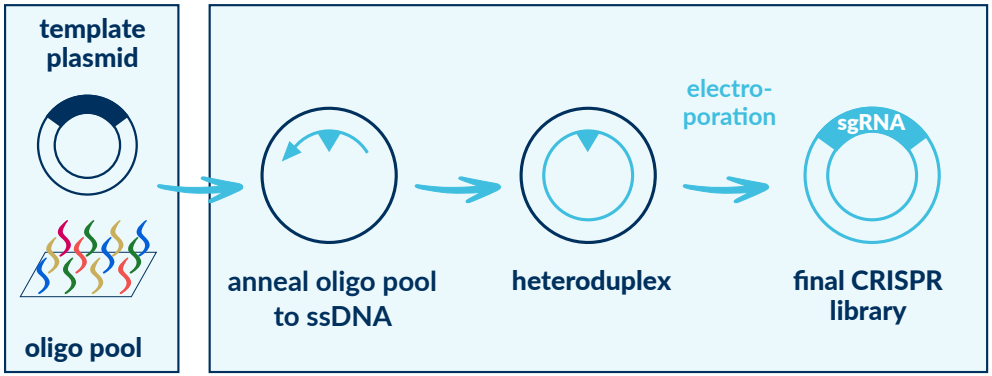
Vivlion provides highly uniform CRISPR libraries with sgRNAs meticulously designed for maximum functionality. Libraries are manufactured using our proprietary 3Cs technology. 3Cs technology enables the creation of libraries in a variety of formats, with a unique capability for combinatorial CRISPR library assembly.

Beyond library generation, we support you at every step of your CRISPR screen. We help you design a screening experiment tailored to your scientific question. Starting with a custom CRISPR library and cell engineering, performance of the CRISPR screen, all the way to data analysis and interpretation.

3Cs creates uniform CRISPR libraries

Conventional cloning methods use PCR to amplify sgRNAs, resulting in preferential enrichment of certain guides. This leads to libraries with uneven sgRNA representation, confounding your screening results.

Our 3Cs technology eliminates PCR bias, ensuring the consistent production of highly uniform libraries. When screening with such a PRCISR® CRISPR library, each sgRNA's contribution to a phenotype can be accurately captured (Wegner *et al*, eLife 2018, Diehl *et al*, Nucleic Acids Res 2021).



How uniformity is measured?

Library uniformity, i.e. the distribution of sgRNAs in a library, can be shown in a histogram of sgRNA frequencies and quantified by the skew.

X-axis: shows how abundant each sgRNA is in the library
Y-axis: shows how many sgRNAs fall in a certain abundance range

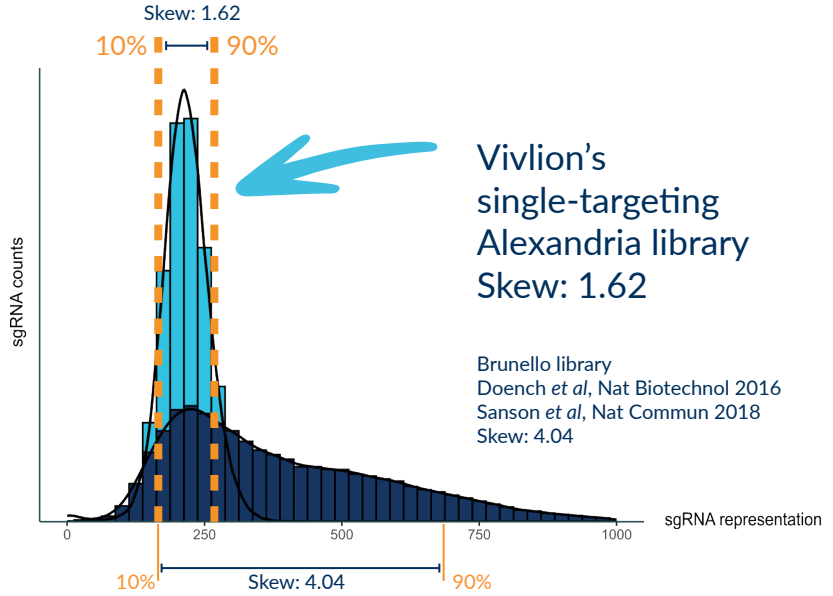
The skew is calculated as follows:

$$\frac{\text{Representation at 90th percentile}}{\text{Representation at 10th percentile}}$$

The more symmetric and narrow the distribution within the histogram, the lower is the library skew.

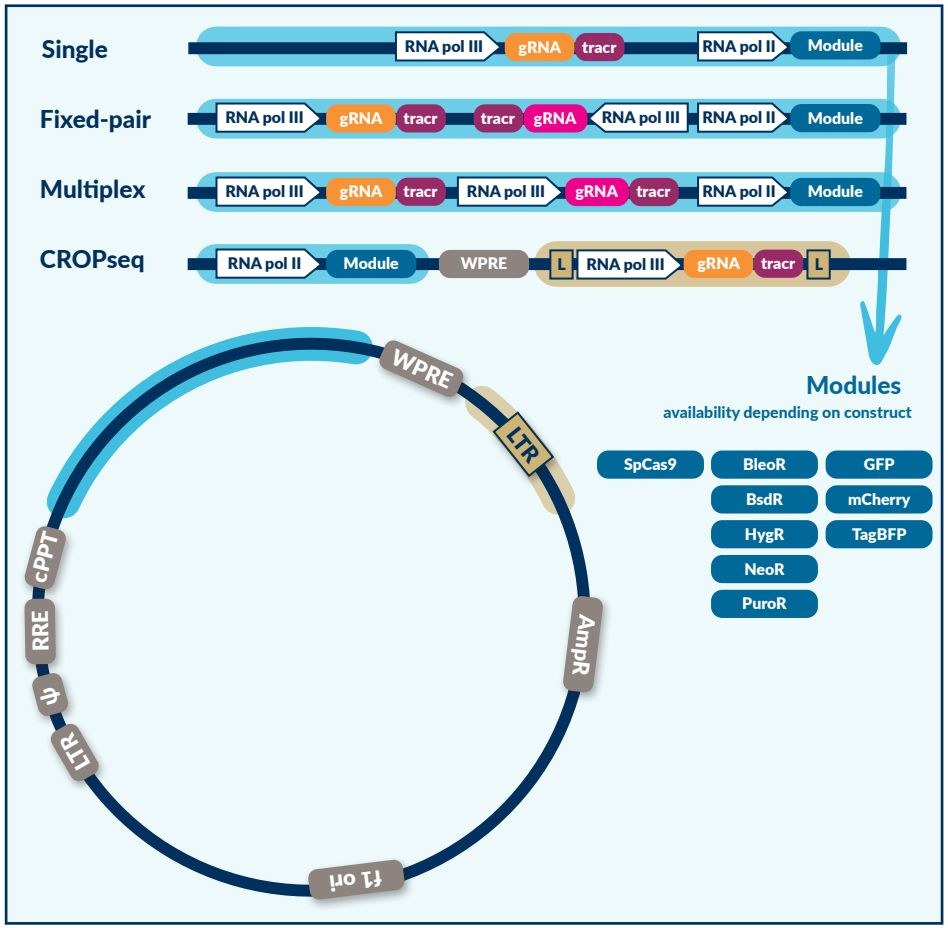
Why uniformity matters?

A high level of uniformity in a CRISPR library (skew of 2.5 or lower) significantly enhances the efficiency and reliability of the screening experiment. With a uniform library, you can reduce screening coverage from approximately 500 to just 100, resulting in substantial savings in both reagents and time. In addition, lower coverage enables experiments with limited cell numbers, as well as high-resolution read-outs such as fluorescent-activated cell sorting or single-cell RNA sequencing.



Library design is key to screening success

Designing a library is a critical step for achieving high-quality screening results. Careful selection of appropriate controls and highly functional sgRNA sequences directly impacts the resolution and reliability of your data. At Vivlion, we support you throughout the library design process, helping you maximize your screening success. We offer flexible library formats, including single- and dual-targeting designs. We can incorporate custom features, such as fluorophores or selection markers, tailored to your experimental needs.



Expanding research horizons with dual-targeting libraries

Combinatorial libraries enable the investigation of gene interactions without the need for prior engineering of cells to introduce specific gene knockouts. This approach allows simultaneous analysis of multiple interactions, ideal for large-scale screens to uncover genetic interactions and gene relationships.

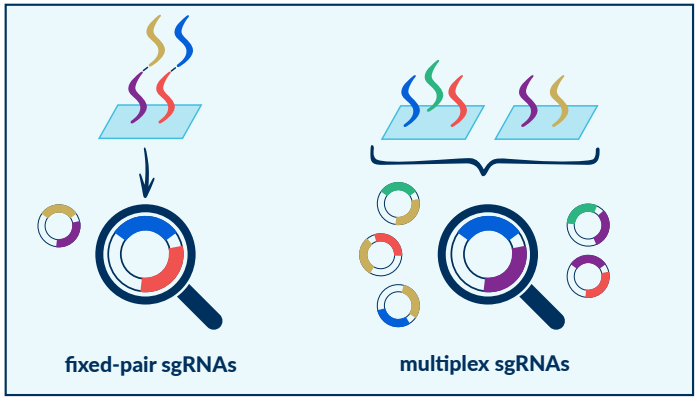
Fixed-pair libraries are designed to target defined gene pairs, making them ideal for testing hypotheses on gene interactions or functional dependencies. They are particularly useful for studying paralogous genes or synthetic lethal interactions, where precise pairing enhances the focus on predefined genetic relationships, leading to higher resolution insights.

Boosting editing efficiency with fixed-pair libraries. In addition to targeting specific gene pairs, fixed-pair libraries can deliver two sgRNAs targeting the same gene to each cell, reducing library complexity by half while enhancing the likelihood of gene loss-of-function. This strategy is exemplified in our Alexandria fixed-pair libraries for human and mouse models (LGW201 – LGW204).

Unlocking genetic interactions. In contrast, **multiplex libraries** combine two independent sgRNA pools, systematically pairing each guide from one pool with each from the other. This unbiased method is well-suited for discovering unknown gene interactions and supports flexible screening strategies, such as all-by-all, few-by-all, or few-by-few analyses, to map complex genetic networks.

Single-targeting

Dual-targeting



Outsource your screening experiments

At Vivlion, we offer end-to-end CRISPR screening services, covering everything from experimental design to data analysis. One of our dedicated scientific managers will support you throughout the entire project.

Phase 1 – From concept to library design

- Strategic planning of all critical experimental parameters, including cellular context, treatment conditions, control strategy, read-out, and screening duration.
- Customizable library design: Select from our PRCISR® CRISPR off-the-shelf libraries or create a tailored library to fit your experimental needs.

Phase 2 – Preparing for the screening experiment

- If needed, production of a tailored PRCISR® CRISPR library.
- Transfer of materials, including client cell lines or compounds.
- Cell line quality control (QC) and characterization, such as doubling times and antibiotic susceptibility.
- Engineering and testing of Cas9 in your cell line(s) of choice.

Phase 3 – Screening experiment and data collection

- Cell expansion to the required scale.
- Screen execution, including optional treatments.
- Diverse read-outs: viability, FACS sorting, scRNA-seq (in collaboration with partners).
- Sample preparation for next-generation sequencing (NGS) and subsequent NGS analysis.

Phase 4 – Bioinformatic data analysis and reporting

- Processing of NGS and screening samples.
- Hit-calling using the pre-agreed analysis pipelines.
- Data transfer and delivery of the final report.

Discover potential read-outs for your PRCISR® CRISPR screen

Choosing the appropriate assay for your CRISPR screen is essential to accurately measure both the qualitative and quantitative impact of your knockouts. Each assay can be customized to address the biological questions you aim to explore.

Cellular viability analysis. The classic CRISPR screening approach analyzing the bulk population of cells for gene enrichment or depletion.

\+ Positive selection: Identifies cells with knockouts that confer a growth or survival advantage.

\− Negative selection: Identifies genes whose loss results in reduced growth or survival.

Flow cytometry-based analysis. Fluorescence-activated cell sorting (FACS) allows the sorting of edited cells into subpopulations, making it ideal for phenotypic screening.

Gene expression analysis by single-cell RNA sequencing (scRNA-seq). In contrast to the relatively basic output of viability CRISPR screening, scRNA-seq profiles transcriptomic changes across individual cells in response to gene perturbations induced by specific sgRNAs. This provides a high-resolution view of gene expression changes and helps identify gene networks and pathways influenced by individual knockouts.

Typical timeline for a PRCISR® CRISPR screen