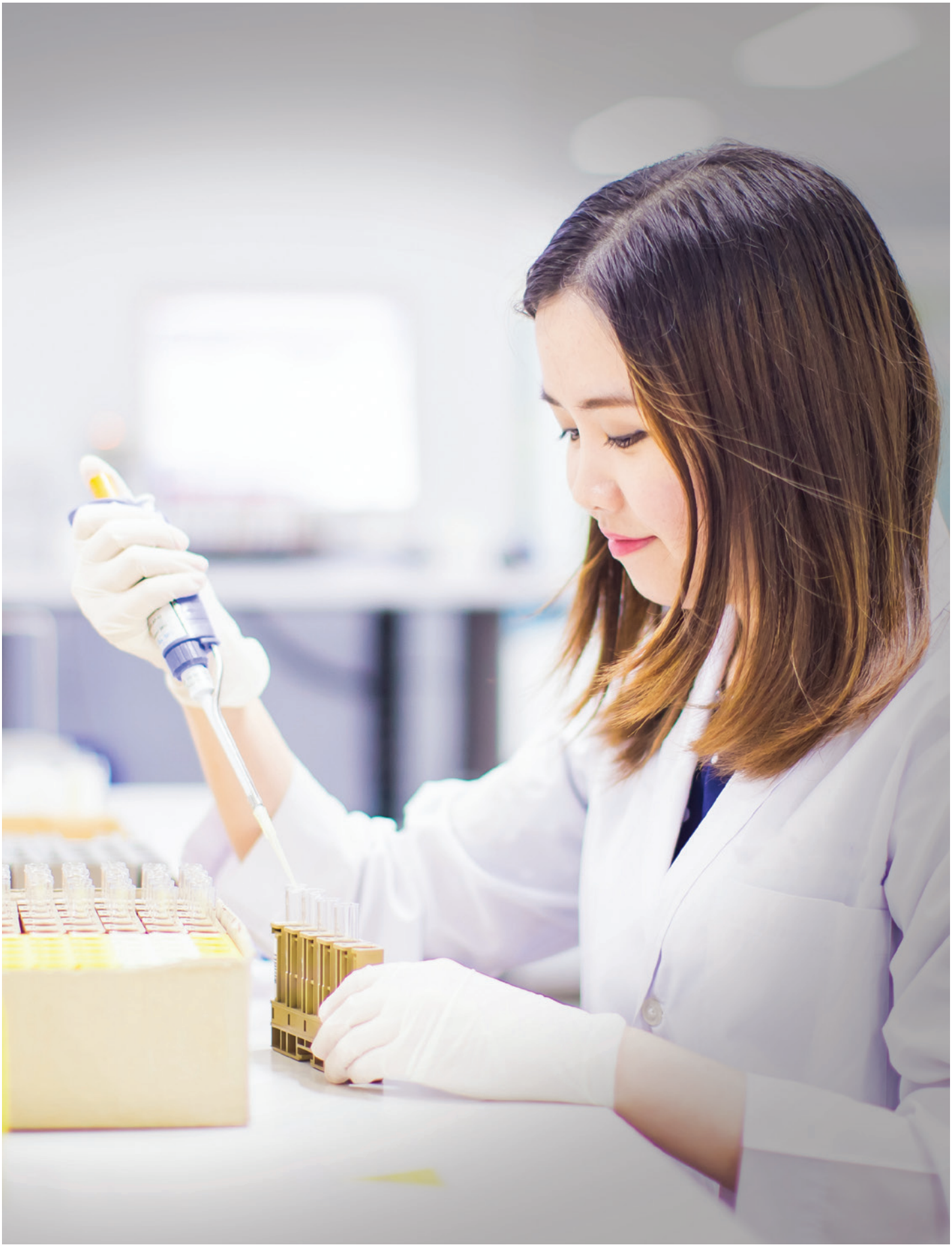
Agilent
Trusted Answers

SureGuide
CRISPR Library Solutions

Build A Better Future



Advanced Nucleic Acid Technology for Better CRISPR Libraries.

Agilent Technologies has a long history of building nucleic acids. Originating through Agilent's spin-off from Hewlett-Packard Company, our DNA oligonucleotide synthesis technology is based on the concepts that enable ink-jet printing. This platform empowers the printing of up to a million oligo features on a single chip, creating highly complex oligo pools with each oligo present in precise ratios. The advanced chemistries developed at Agilent also enables the printing of longer oligos than are typically available from commercial suppliers. We have harnessed this technology to develop a wide range of market-leading products including SurePrint custom microarrays, SureSelect for NGS target enrichment, SureFISH probes, and the QuikChange HT mutagenesis kit for protein engineering. Whether you are looking for the highest quality pre-defined content or you need to create your oligo libraries from the ground-up, Agilent has a solution for your CRISPR needs.

Contents

Pooled Libraries

Pooled Screens

Genetic screens using pooled libraries are typically performed to locate and identify genes that are involved in cellular response, such as in signaling pathways, or to discover the function of novel genes. Pooled screening libraries can vary in scale from as few as 100 constructs, up to hundreds of thousands. In contrast to an arrayed approach, pooled screens do not normally require extensive and costly automation equipment. Instead, with the appropriate experimental design, a pooled approach allows researchers to screen a population of modified cells with just a few culture plates. Cloning, trans formation, transfection, packaging, transduction and screening are all done in a single, pooled sample requiring little in the way of specialized equipment and offering a more user-friendly approach to functional, phenotypic screens.

CRISPR Screening

While pooled screens have been a functional genomics tool for over a decade previous technologies, like RNAi, have a number of restrictions which may limit their use for some applications. The introduction of CRISPR-based tools has provided an opportunity to overcome many of these limitations. CRISPR utilizes a ribonucleoprotein with a single guide RNAs (sgRNAs) that targets an enzyme (typically a Cas9 nuclease or a modified version of Cas9) to a genetic locus where it can modify the DNA. To generate knock-outs for functional screening, the Cas9 nuclease induces a double-strand break at the target site. Error prone repair of this break by non-homologous end joining results in modification of the target site, and often causes a loss-of function phenotype by inserting or deleting bases to cause a frameshift mutation. Unlike other engineered nucleases that require extensive protein engineering, CRISPR systems rely on simple base pairing to target genomic loci making the system easily adaptable to a wide-range of applications and targets.

- Simple, automation-free workflow
- Easily enable high-throughput functional genomics in your lab
- Flexibility to screen hundreds to hundreds of thousands of guides

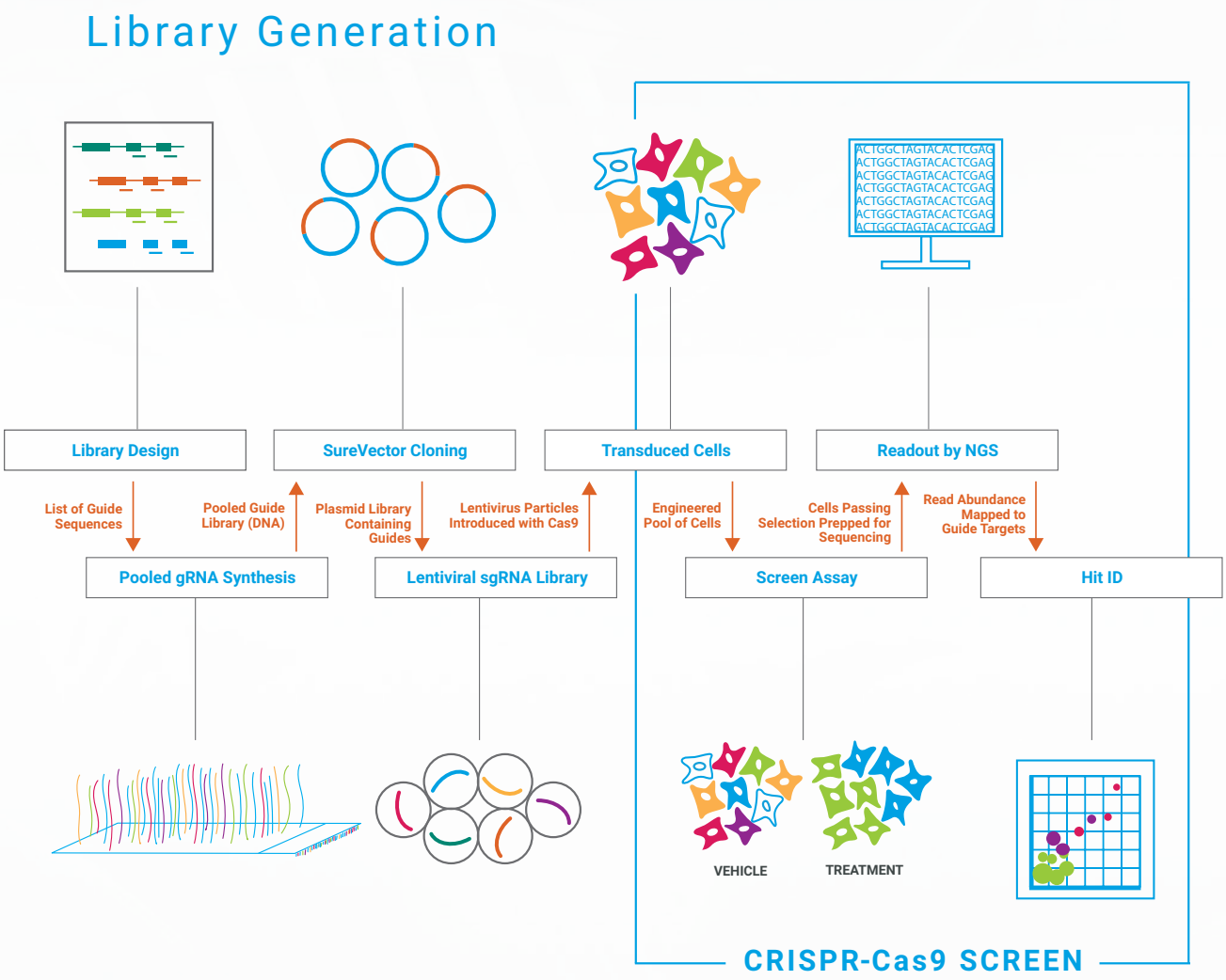


Figure 1. CRISPR screening begins with the *in silico* design of guides. These guides are then synthesized, cloned and delivered via virus to a pool of cells. Cells are exposed to a treatment or selection after which they are sequenced to identify guides which induced a phenotypic change.

Working with Pooled Libraries

Pooled Libraries in Functional Genomics Workflow

CRISPR screens in mammalian cells typically begin with the design of a set of guides targeting genomic regions of interest that are defined by the user. This can be a small set of genes involved in a signaling pathway, up to all of the exons in an organism. After designing these guides, they are synthesized as DNA oligos and cloned into a viral delivery plasmid.

From Library to Cells

After packaging into viruses, the guides are delivered alongside Cas9 to a pool of cells. Under the correct conditions, approximately one guide will be active per cell, providing a pool of cells each with a single gene knocked-out. This pool is then exposed to a specific treatment, such as a drug or other external stimulus, and cells passing the selection are sequenced to determine which guide was present in the selected cell.

One of the key inputs into this workflow is the CRISPR guide library. Library quality and composition can affect all of the downstream segments of the workflow including screening effort, sequencing cost, and false positive/negative identification.

- Agilent's SureGuide CRISPR Libraries are printed on our proprietary, high-fidelity oligo synthesis platform
- SureGuide products support the pooled screening workflow all the way through analysis and hit verification
- CRISPR libraries are designed to allow the maximum amount of flexibility in downstream screens
- Rapid customization at ultra-high quality means you can design your library around your workflow

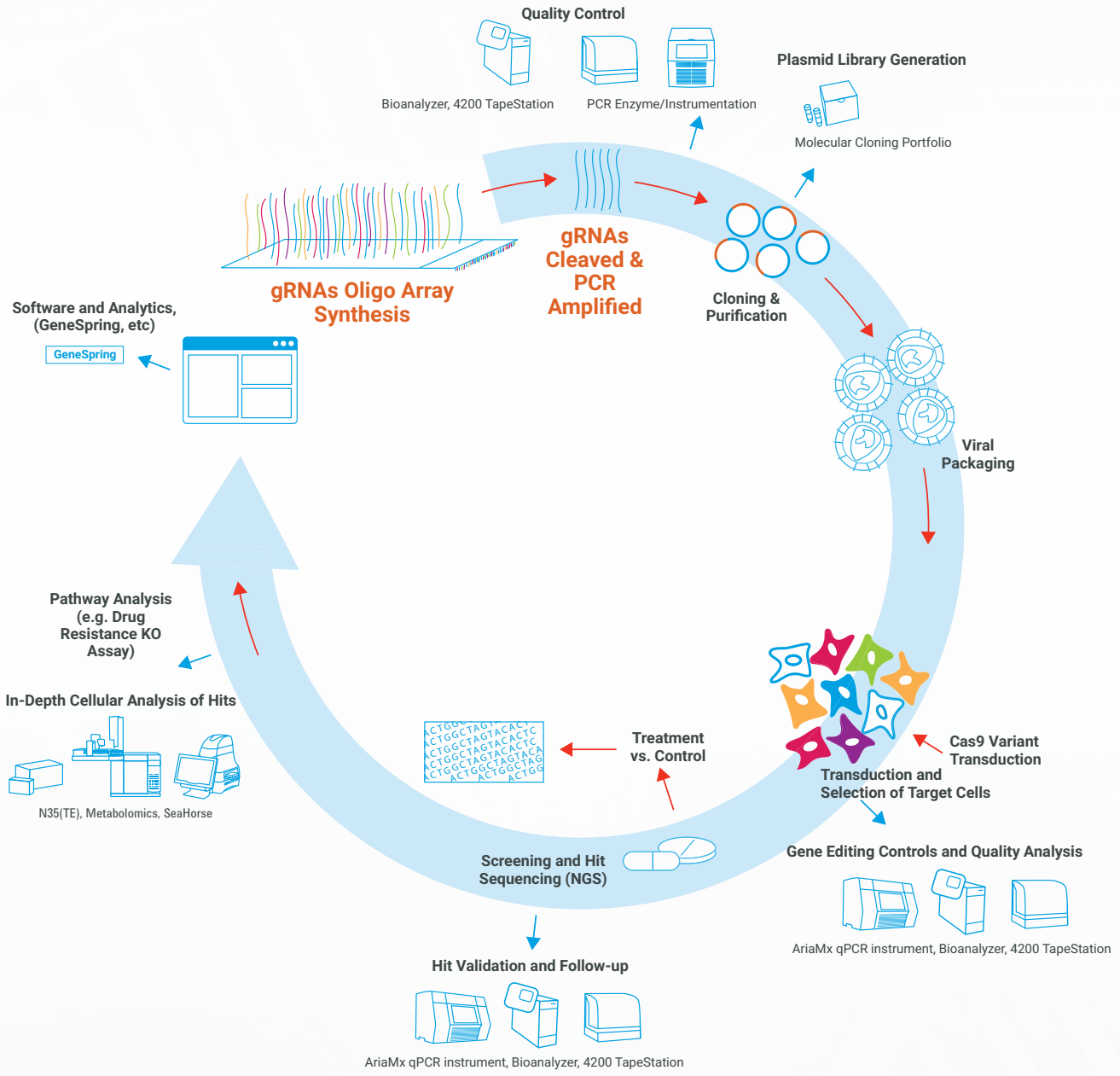


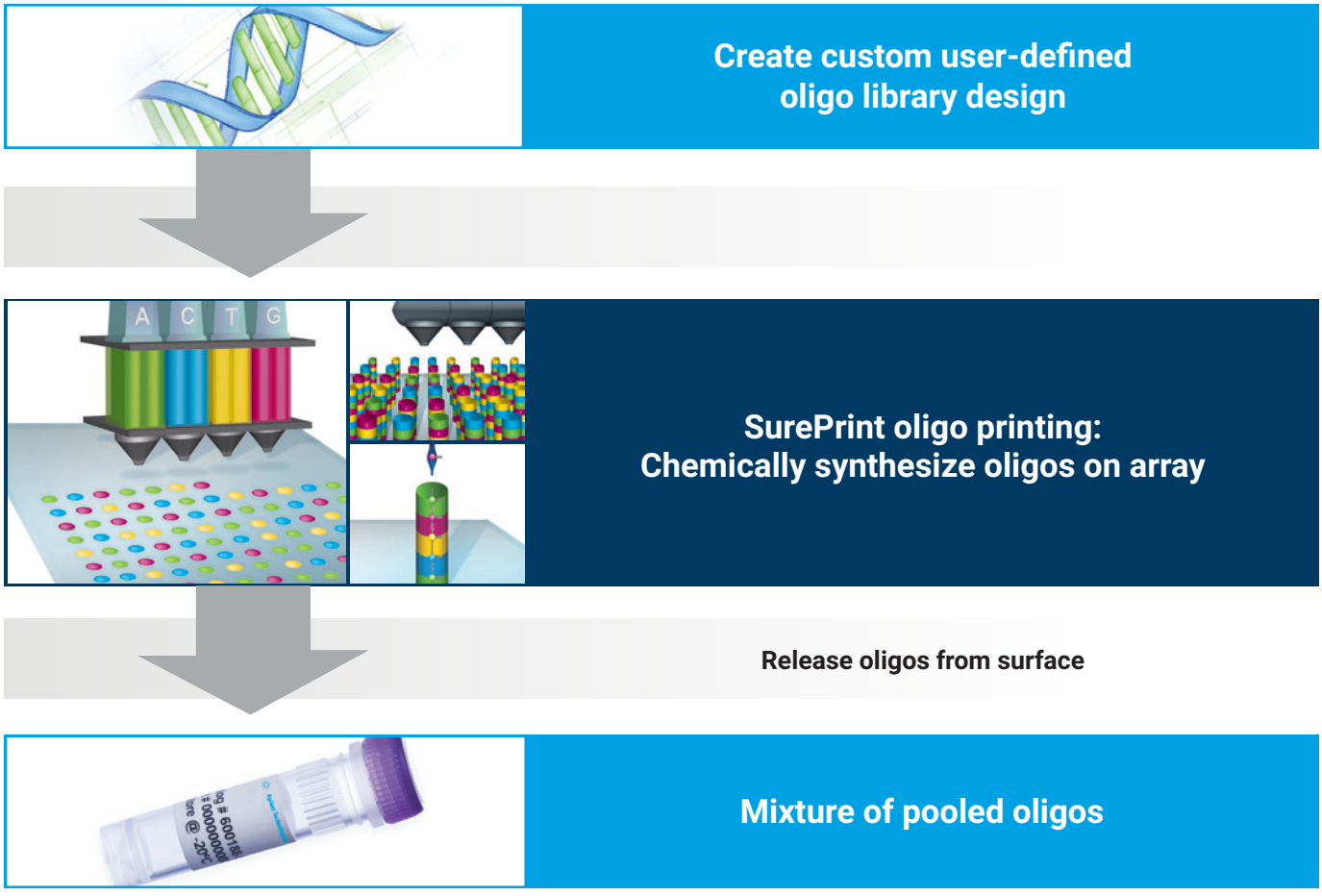
Figure 2. Agilent's massively parallel oligo synthesis platform is the basis for our offering in CRISPR libraries. However, we offer an extensive range of instrumentation and reagents that can help you get the best possible results from your CRISPR screens.

Library Quality

Our Platform

There are multiple parameters that one needs to consider in both choosing an oligo library and designing an experiment. The first is library fidelity, which refers to the error rate (errors per kilobase) in a sequenced library. Agilent's CRISPR libraries are all synthesized on its technologically advanced SurePrint platform with error rates well below the industry average. While fidelity is important to generating a high quality library the second parameter, representation, is even more critical. Representation refers to the relative abundance of each guide in the pool and can be measured by comparing the number of under-represented guides to the number of over-represented guides. If too many over-represented guides are present, they will dominate the results and increase the number of cells that need to be screened to get full coverage of the members in the pool. Similarly, if too many guides are under-represented then the amount of screening that needs to be done rapidly becomes prohibitive, especially for genome-wide approaches or experiments of a similar scale.

Agilent's DNA synthesis technology allows rational design of parallel synthesis that permits tailoring of library representation to achieve the most uniform distribution of guides across even the most complex libraries.



Agilent's CRISPR libraries harness the SurePrint platform to provide more uniform guide representation and better fidelity than other commercially available solutions. Whether pre-defined or fully custom, every SureGuide CRISPR library is made with the same exceptional quality.

Superior Fidelity and Representation in Agilent CRISPR Libraries

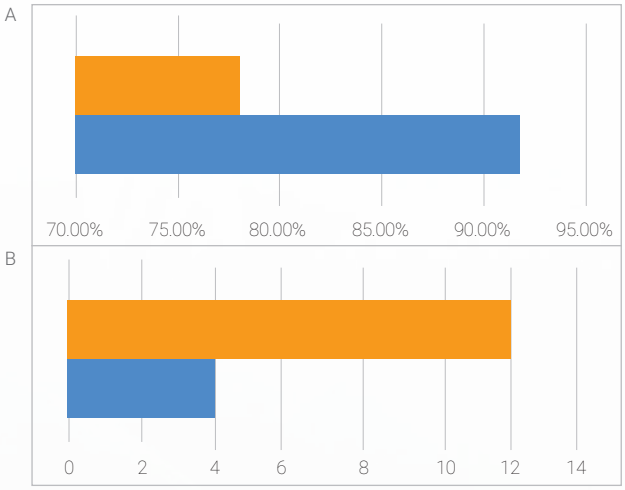


Figure 3. A) A comparison of the % of sequence perfect constructs in a SureGuide CRISPR library (blue) constructed with the SureVector library cloning kit (92%) and the same library from a competitor (orange, 78%). **B)** A comparison of the number of errors/kb in the same library for Agilent (blue, 4) and a competitor (orange, 12). SureGuide CRISPR libraries contain less than half the number of errors as competitors leading to clearer results and reduced screening times.

Libraries	Missed Guides	90th/10th percentile	95th/5th percentile	99.5th/0.5th percentile
Agilent option 1	1	2.32	3.04	6.72
Agilent option 2	1	2.47	3.38	11.06
Agilent option 3	1	2.38	3.19	9.83
Agilent option 4	1	1.99	2.64	8.30
GeCKO (Broad)	?	8.73	16.00	NA
GeCKO (Competitor)	39	5.29	9.83	68.40
GeCKO (Competitor) expanded	204	6.00	11.95	333.00

Table 1. With <.01% missed guides, you can be sure the guides in your library will be present in your delivered library. The uniformity of our libraries has an average 90/10 ratio of 2.29, which is less than half that of our competitor's libraries (5.29).

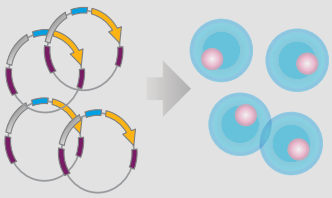
SureGuide CRISPR Solutions

Any Format, Any Site, Any Species

Agilent's CRISPR libraries are available in three formats. **Ready-to-Package** plasmid libraries consist of pre-defined, licensed content. These genome-wide knock-out libraries, or GeCKO libraries, target all exons in the human or mouse genome and have been pre-validated by researchers around the world who are studying functional gene interactions at an -omics scale. SureGuide **Ready-to-Clone** libraries offer the added flexibility of designing your own guide sequences, and arrive as an amplified pool of linear DNA compatible with our SureVector library cloning kit. With ready-to-clone libraries you can fully specify the sequence of each guide in the library. **Ready-to-Amplify** libraries offer the ultimate flexibility in customization. Synthesized using the same ultra-high quality process as our standard libraries, you can fully design every aspect of your CRISPR protocol allowing for the use of alternative delivery systems, cloning approaches, and targeting in any organism of your choice.

SureGuide CRISPR Libraries

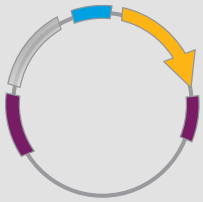
Ready-to-Package



Catalog Libraries

- Plasmid Library
- GeCKOv2
- Human and Mouse
- Cloned into lentivirus vector with hU6 promoter

Ready-to-Clone



Custom Libraries

- Pre-amplified OLS library
- User defined subset or designed
- Mammalian
- Compatible with SureVector cloning

Ready-to-Amplify

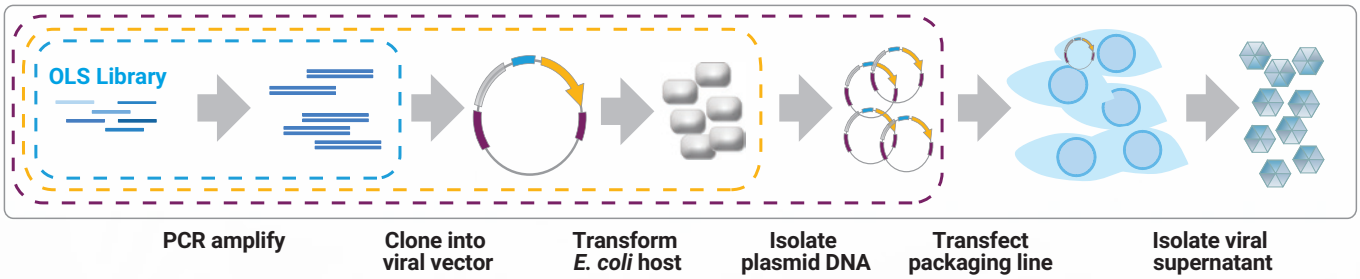
OLS Library

Custom Libraries

- Unamplified oligo pool
- Any species, any cloning method
- Entirely custom by user design

The key input into this workflow is the CRISPR guide library. Both library quality and composition can affect all of the downstream segments of your experiment including screening effort, sequencing cost, and false positive/negative identification.

Flexibility and Customization



Catalog Ready-to-Package Library

Custom Ready-to-Clone Library

Custom Ready-to-Amplify Library

Figure 4. All CRISPR libraries begin with the synthesis of a high-quality oligo pool. For full flexibility, these ready-to-amplify pools are directly available for order by fully specifying the sequences of all library members. Ready-to-clone libraries take you one step further in the workflow, allowing customization but requiring a cloning step while ready-to-package libraries are only available in predefined content but require no cloning, only viral packaging and delivery.

Catalog GeCKOv2 Libraries

Genome-Wide Screens

Genome-wide CRISPR screens are designed to provide functional knock-outs of all the genes within a cell. Agilent offers two genome-wide plasmid libraries in a pooled format. Both are designed, validated, and licensed, with one targeting all the exons in human cells and the other targeting all the exons in mouse cells. GeCKO libraries are delivered in an optimized lentivirus plasmid in a **ready-to-package** format.

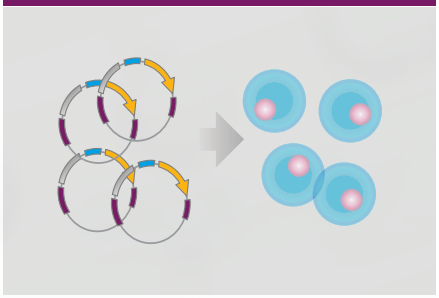
GeCKOv2 Libraries

	Wang *et al.* 2 library	Shalem *et al.*1 GeCKOv1 library	Koike-Yusa *et al.* 3 library	GeCKOv2 human library	GeCKOv2 mouse library
Species	Human	Human	Mouse	Human	Mouse
Genes targeted	7,114	18,080	19,150	19,050	20,611
Targeting constructs per gene	10	variable (typically 3 or 4)	variable (typically 4 or 5)	6	6
miRNA targeted	None	None	None	1,864	1,175
Targeting constructs per miRNA	n/a	n/a	n/a	4	4
Control (nontargeting) sgRNA	100	None	None	1,000	1,000
Total sgRNA constructs	73,151	64,751	87,897	123,411	130,209
Viral plasmid vector	Dual Vector: sgRNA only	Single Vector: Cas9 & sgRNA (lentiCRISPRv1)	Dual Vector: sgRNA only	Dual Vector: sgRNA only	Dual Vector: sgRNA only

Table 2. The GeCKOv2 library contains experimentally validated CRISPR guides targeting all human or mouse genes.

- Highest quality supplier of fully licensed GeCKO libraries
- Fully cloned and sequence verified plasmid library
- Advanced lentiviral vector delivery for optimal sgRNA expression

Ready-to-Package



Genome-wide screens require considerable time and resources to complete. Agilent's GeCKO libraries assure that you can focus on your experimental design, leaving the library quality to us.

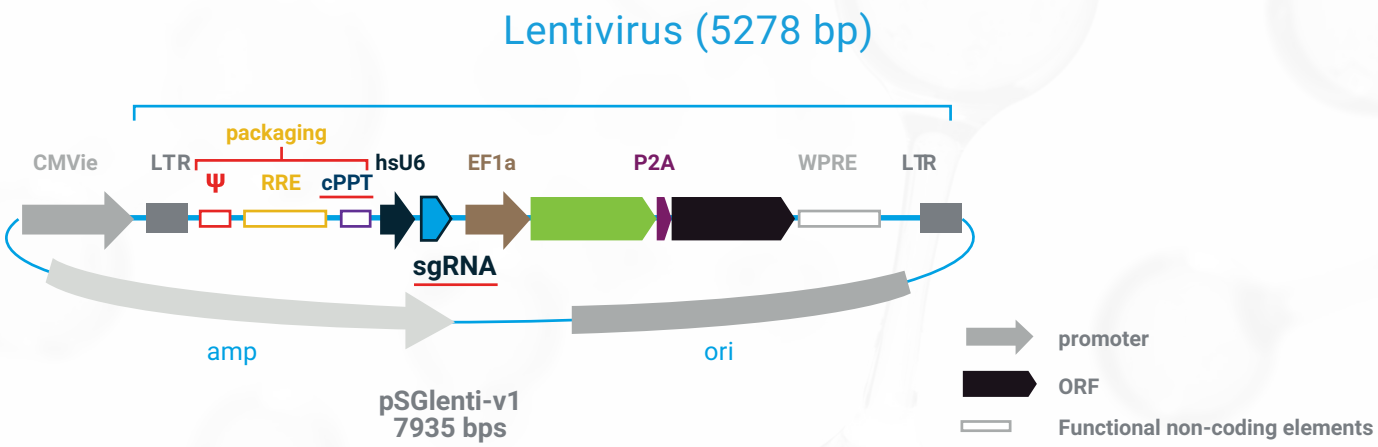


Figure 5. GeCKO libraries from Agilent are delivered in a plasmid format, ready-to-package into lentiviral particles. The state-of-the-art vector construct includes a U6 promoter, puromycin selection marker, and GFP reporter.

Product	Catalog	Quantity
SureGuide GeCKOv2 Catalog CRISPR Libraries	G7553A - Human	123,411 guides
SureGuide GeCKOv2 Catalog CRISPR Libraries	G7554A - Mouse	130,209 guides

Custom Amplified Libraries

Build Your Own Library

Agilent's platform is highly customizable, allowing the synthesis of custom CRISPR libraries with the same ease and quality of our GeCKO libraries. For researchers working in mammalian cells, we offer a cloning system combined with pre-amplified oligo libraries in a **ready-to-clone** format that makes it simple to obtain catalog quality plasmid libraries with your customized guide designs.

Custom Amplified Library Configuration

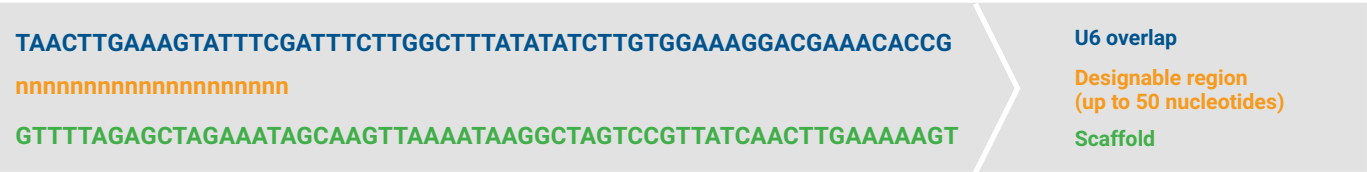


Figure 6. Designable construct for custom, ready-to-clone CRISPR libraries that can be used in mammalian cells compatible with the U6 promoter. The first 60 bases and last 60 bases are fixed and designed to be compatible with Agilent's pSGLenti vector (figure 5). The designable region typically contains only the CRISPR guide (17-20 nucleotides) but can contain up to 50 nucelotides specified at the time of order.

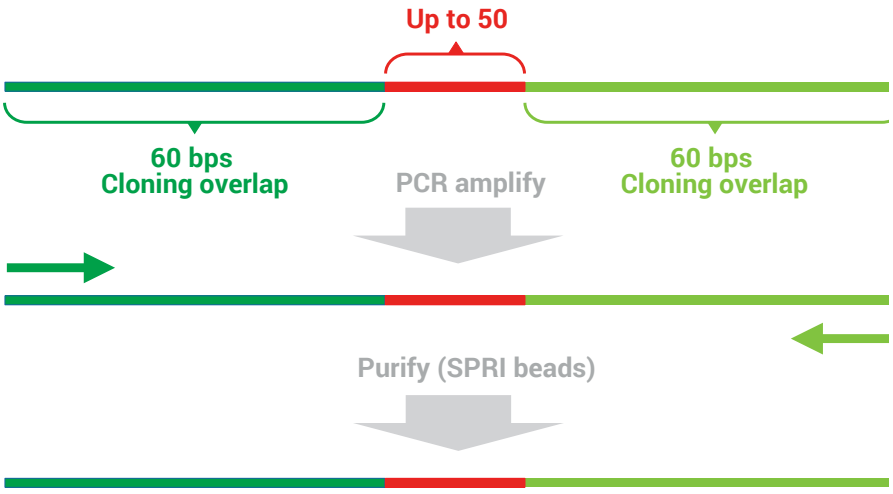
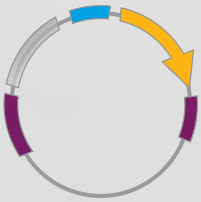


Figure 7A. The construct shown in figure 6 is amplified and purified prior to shipping and comes in a single tube, ready-to-clone in the SureVector library cloning kit.

- Fully amplified custom library compatible with SureVector cloning kit
- Full control of guide design including alternate Cas9 variants
- Constructs contain U6 promoter for compatibility with mammalian systems

Ready-to-Clone



Take control of your CRISPR experiments by designing your own libraries for mammalian applications. Design guides for any target or compatibility with any CRISPR-associated protein, all with the assurance of the same quality and cost as our catalog libraries.

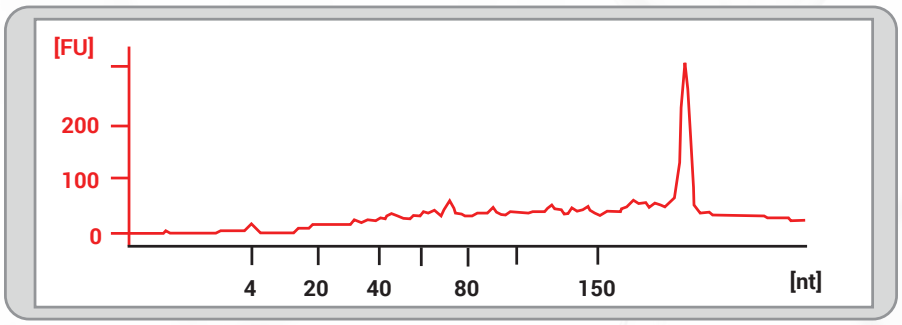


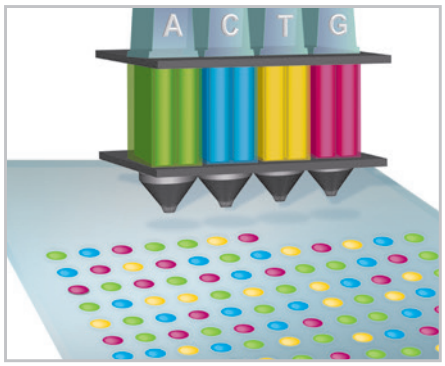
Figure 7B. A typical bioanlyzer trace of an amplified library. The strong peak represents the full length product following amplification.

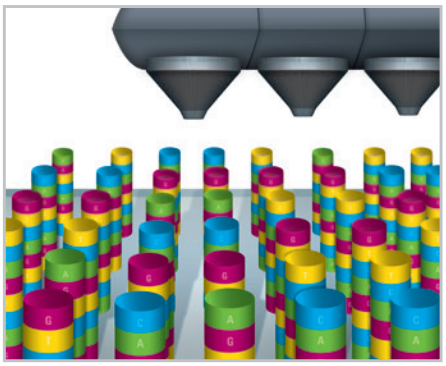
Product	Catalog	Quantity
SureGuide Custom Amplified Library – 10k	G7555A (Option #010)	Up to 10,000 guides, 125 ng total DNA
SureGuide Custom Amplified Library – 30k	G7555A (Option #030)	Up to 30,000 guides, 125 ng total DNA
SureGuide Custom Amplified Library – 60k	G7555A (Option #060)	Up to 60,000 guides, 125 ng total DNA

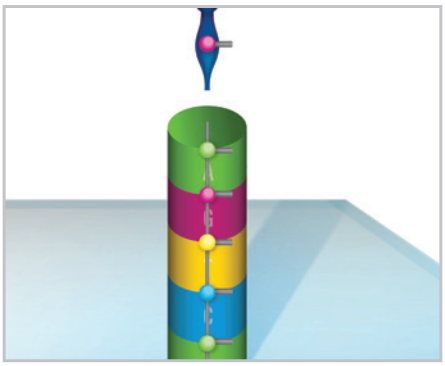
Custom Ready-to-Amplify Libraries

Unlimited Flexibility

In addition to the ready-to-clone libraries, Agilent offers fully customizable guide libraries where the user can define their own promoter sequence and cloning strategy, as required. These libraries have sequences that are fully specified for your application and come in a **ready-to-amplify** format along with all of the necessary reagents to maintain library quality and representation when generating a CRISPR library for any screening set-up you can imagine.







- Unamplified oligo pool, fully licensed for CRISPR applications
- Design every aspect of your CRISPR library, from cloning strategy to guide structure and targets
- Complete freedom to customize at catalog quality and pricing

At Agilent we utilize our advanced DNA synthesis platform to offer fully custom CRISPR libraries compatible with any application or experimental approach.

CRISPR Guide Synthesis

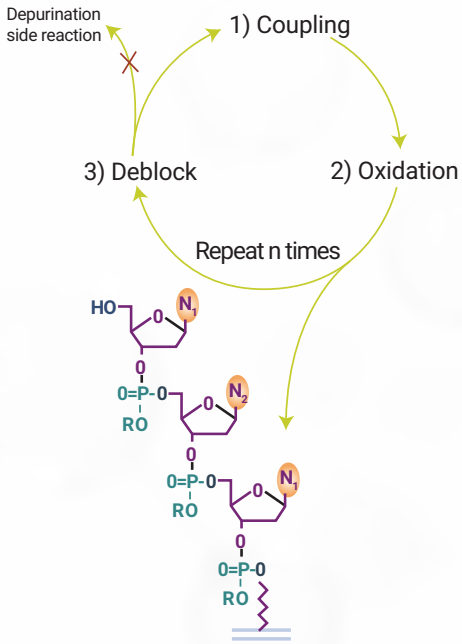


Figure 8. The general cycle of oligo synthesis via phosphoramidite chemistry. Agilent's real time quality control inspection system verifies chemical deposition at each step in the process to minimize guide drop-out and premature truncation.

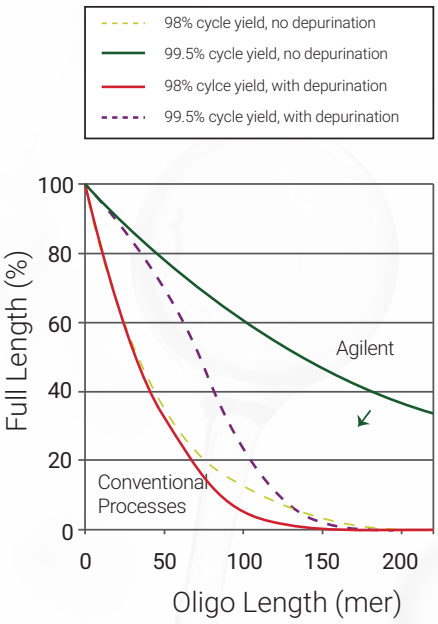


Figure 9. Calculated yield of full-length oligonucleotides as a function of length, at two different cycle yields, with and without depurination. Convectional processesused by competitors see a large fall-off in fidelity compared to Agilent's platform.

Product	Catalog	Quantity
SureGuide Unamplified Custom CRISPR Library – 5k	G7555B (Option #005)	Up to 5,000 guides
SureGuide Unamplified Custom CRISPR Library – 25k	G7555B (Option #025)	Up to 25,000 guides
SureGuide Unamplified Custom CRISPR Library – 50k	G7555B (Option #050)	Up to 50,000 guides
SureGuide Unamplified Custom CRISPR Library – 100k	G7555B (Option #100)	Up to 100,000 guides

SureVector Library Cloning

Reliable Library Construction

Agilent has developed a proprietary method for cloning complex oligo libraries. This approach, using our SureVector technology, uses a single step, homology-based DNA assembly to generate a plasmid library from an amplified oligo pool. The SureVector library cloning kit includes the enzymes and buffers required for assembly, a human or mouse specific control fragment, and a lentivirus backbone. For fully custom applications, designing your constructs to be compatible with SureVector is simple, as it only requires 30-60 base-pair overlaps with your choice of vector.

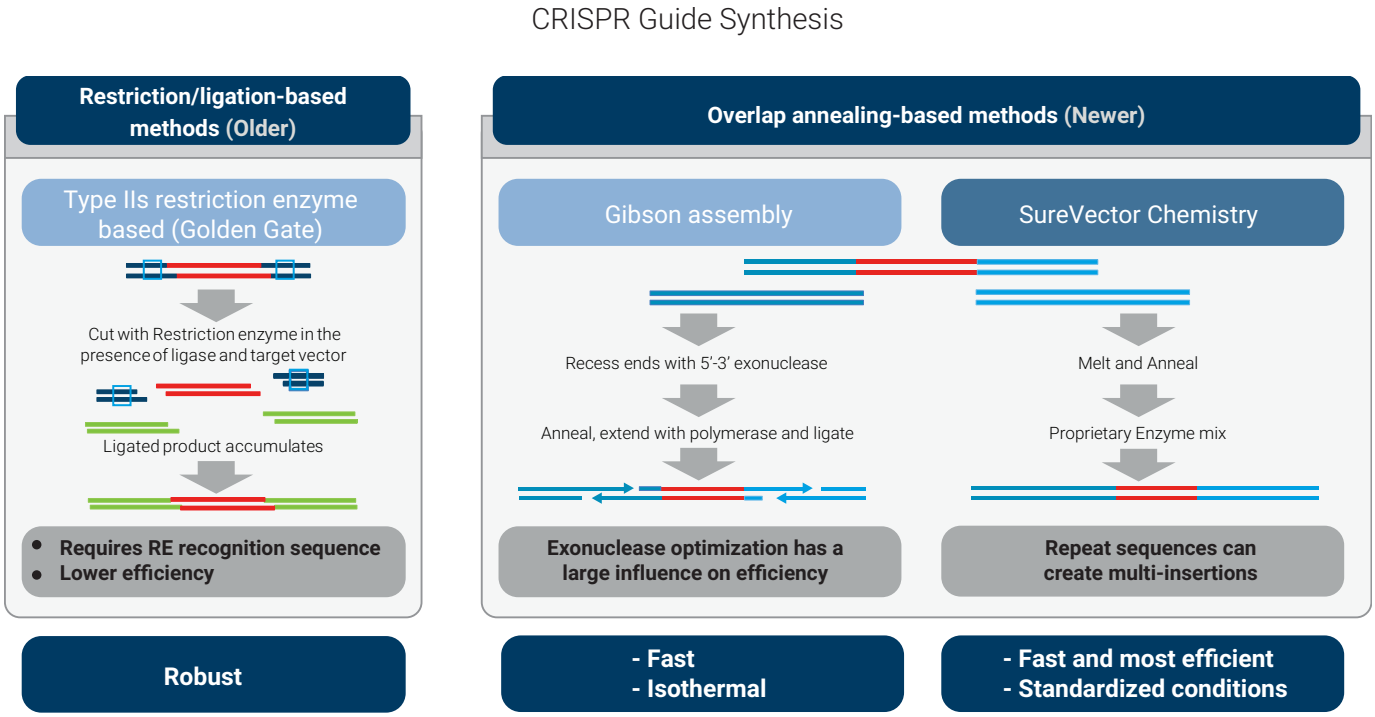


Figure 10. Traditional cloning technologies rely on restriction enzymes to generate clonable DNA fragments. Overlap based methods (right) include both Gibson assembly and SureVector. These methods use a blend of enzymes to combine homologous ends into a seamless strand of DNA.

Cloning	OLS	Gibson plasmid	SureClone plasmid
Total clones	N/A	1.17×10^6	4.13×10^6
Fraction empty	N/A	0.100	0.043
Projected total complete clones	N/A	1.05×10^6	3.95×10^6
Average clones/guide	N/A	16.3	61.2

Table 3. The SureVector library cloning gives over 3x more colonies than plasmid libraries generated using Gibson assembly. This translates to nearly 6x more clones per guide in a genome-wide scale library (~60,000 total guides).

The SureVector library cloning kit allows you to reliably generate plasmid libraries, maintaining maximum uniformity of representation in your unamplified or pre-amplified custom CRISPR libraries.

Comparison of Cloning Technologies

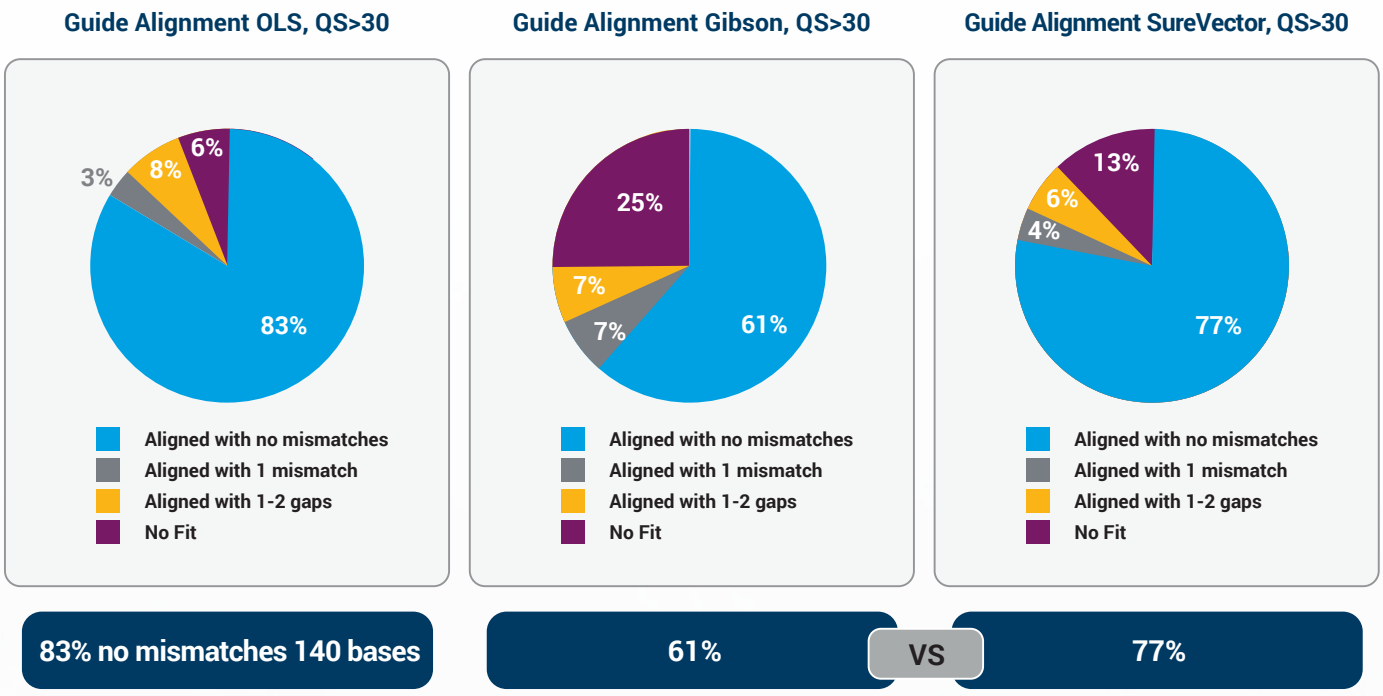


Figure 11. Agilent's SureVector technology uses a proprietary enzyme blend to assemble DNA in a single step. We've tested this process alongside both Gibson assembly and traditional techniques and demonstrated that this approach leads to a significantly higher assembly fidelity compared to existing techniques. The percentage of correct clones from a genome-wide custom library is 16% higher with SureVector than it is with Gibson assembly.

Product	Catalog	Quantity
SureVector Library Cloning Kit	G7556A	Sufficient to build 4 libraries

Instructions to Order GeCKOv2 Libraries

GeCKOv2 libraries are available for both mouse (G7554A) and human (G7555A) applications. The table below provides a summary of the libraries including the number of guides per gene, non-coding targets, and control sequences. Kits also include a control appropriate for the organism the library is designed for. If you require additional information, including the sequences of each component of the library please contact your local Agilent representative or technical support.

Instructions to Order Custom Libraries

Custom ready-to-clone libraries (G7555A) are available in three sizes, up to 10,000 guides, up to 30,000 guides and up to 60,000 guides. Promoter elements are included in the construct and are a U6 promoter optimized for human applications (alternative promoters can be ordered in an unamplified format). You will provide to your Agilent representative either: (1) a text file containing the variable sequence of the guide corresponding to your targets, (2) a list of genomic regions preferably in a .bed format or (3) a list of target gene names using the 'NCBI gene' nomenclature. Options 2 and 3 are only available for *S. Pyogenes* Cas9. Custom ready-to-amplify libraries (G7555B) are available in four sizes, up to 5,000 guides, up to 25,000 guides, up to 50,000 guides and up to 100,000 guides. To order a custom library you will provide the full sequence of the construct you will be cloning.

	SureGuide GeCKOv2 CRISPR Libraries	SureGuide Amplified Custom CRISPR Libraries	SureGuide Unamplified Custom CRISPR Libraries
Part Numbers	G7553A - Human G7554A - Mouse	G7555A - Human (hU6 promoter)	G7555B - Any species
Library Format	Ready-to-package plasmid library	Ready-to-clone amplified DNA library	Ready-to-amplify DNA library
Delivery	Lentivirus	Designed for lentivirus, user choice	Users choice
Number of Guides	Human - 123,411; Mouse - 130,209	Up to 60,000	Up to 100,000
User Supplies	Lentivirus packaging, delivery, screening	Cloning, viral packaging, delivery screening	Amplification, cloning, viral packaging, delivery, screening
Sequence Verified	Yes	No	No
Quantity	200 ug of plasmid DNA split into A and B libraries	125 ng of linear, amplified, pooled library	10 pmol of linear, unamplified, pooled library
Also Included	Competent Cells	N/A	Amplification kit
Custom Design Input	None	Up to 50 nt sequence for each guide	Complete sequence up to 200 nt
Control	Mal1-C	User designed	User designed

Learn more:
www.agilent.com/genomics

U.S. and Canada
1-800-227-9770
agilent_inquiries@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

For Research Use Only. Not for use in diagnostic procedures.

This information is subject to change without notice

PR7000-0246
© Agilent Technologies, Inc. 2017
Published in the USA, September, 2017
5991-9158EN

Agilent
Trusted Answers