SYNTHEGO

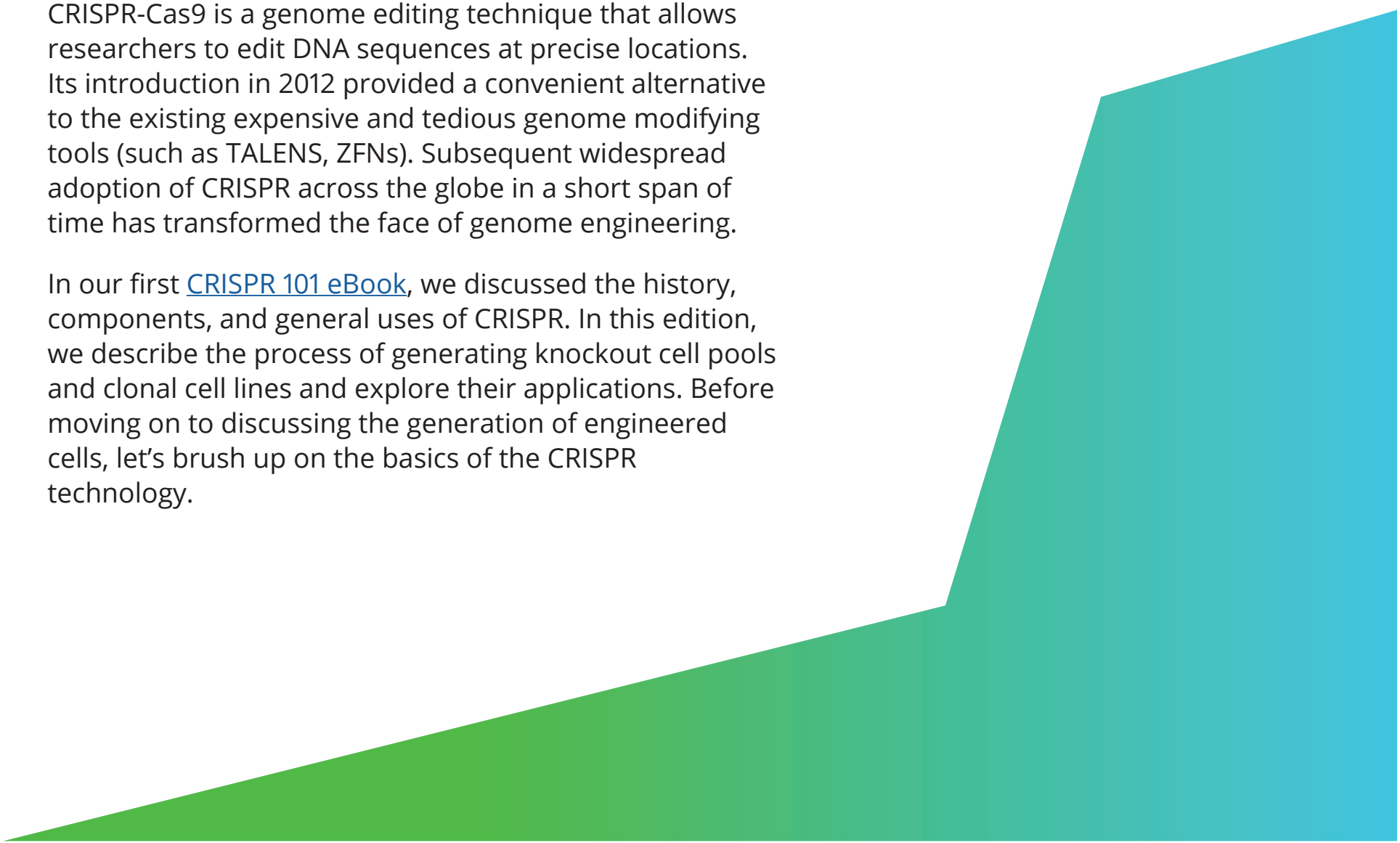
Cell Engineering 101 //

CRISPR Knockouts

GENOME ENGINEERING WITH CRISPR

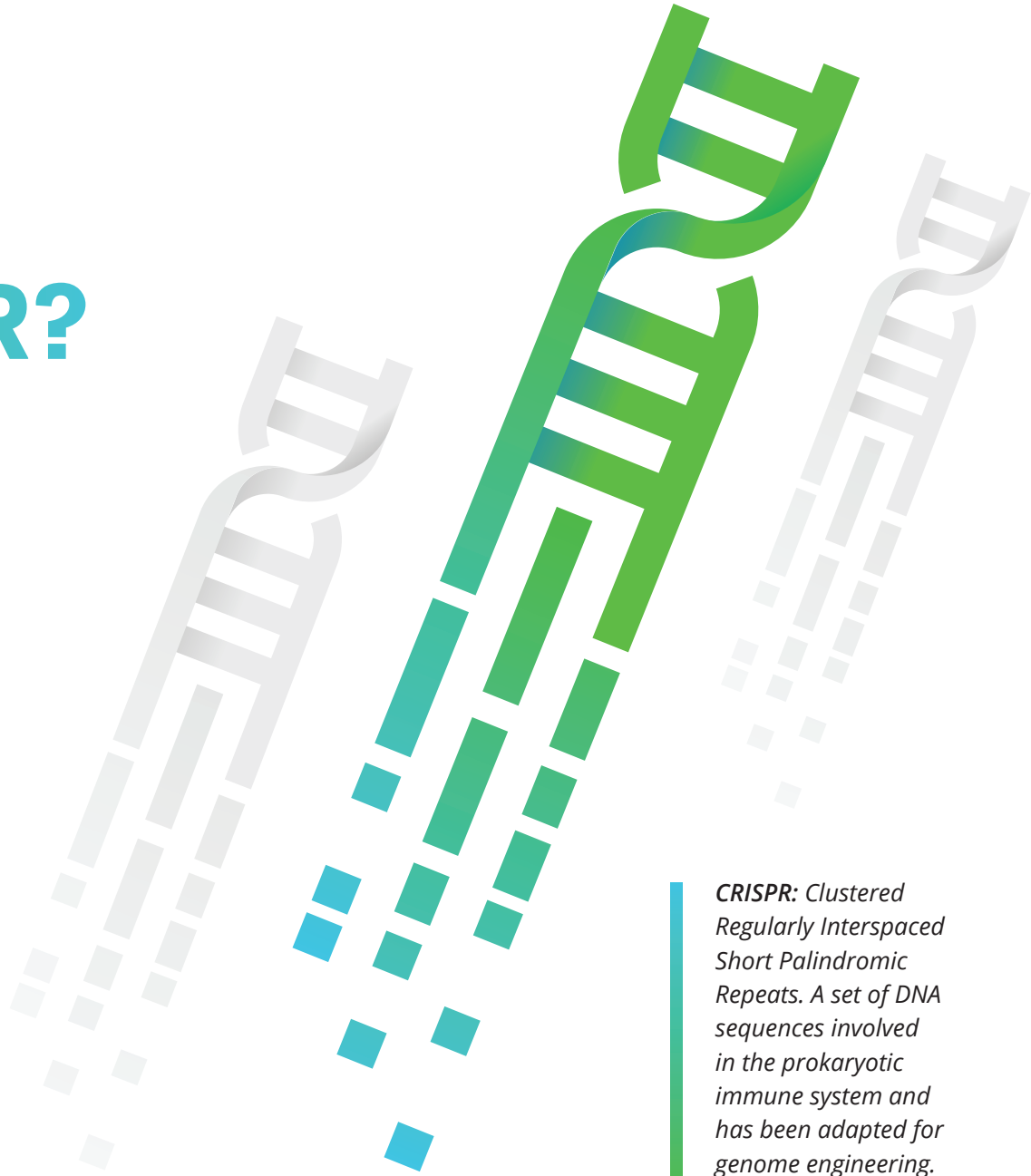
CRISPR-Cas9 is a genome editing technique that allows researchers to edit DNA sequences at precise locations. Its introduction in 2012 provided a convenient alternative to the existing expensive and tedious genome modifying tools (such as TALENS, ZFNs). Subsequent widespread adoption of CRISPR across the globe in a short span of time has transformed the face of genome engineering.

In our first CRISPR 101 eBook, we discussed the history, components, and general uses of CRISPR. In this edition, we describe the process of generating knockout cell pools and clonal cell lines and explore their applications. Before moving on to discussing the generation of engineered cells, let's brush up on the basics of the CRISPR technology.



What is **CRISPR?**

The term ***CRISPR***, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, refers to a set of DNA sequences involved in the prokaryotic immune system. The sequences contain palindromic repeats, each of which is followed by a piece of DNA from past viral invaders (called spacers). The stored viral fragments are used to target and destroy similar viruses in future attacks. Once the underlying mechanism of CRISPR was decoded in 2012 (1), researchers extended its potential applications far beyond bacteria. In 2013, CRISPR was used to edit eukaryotic cells for the first time (2). Since then, this technique has been used to modify many cell types and organisms across a broad range of research areas.



CRISPR: *Clustered Regularly Interspaced Short Palindromic Repeats. A set of DNA sequences involved in the prokaryotic immune system and has been adapted for genome engineering.*

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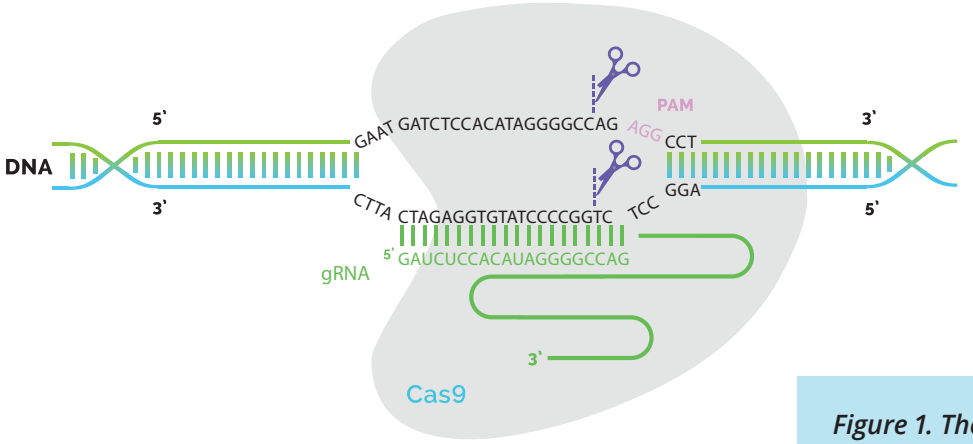
Mechanism of CRISPR

The popularity of CRISPR stems from its simplicity and ease of use. The system consists of two components: a ***guide RNA*** and an endonuclease, which together form a ribonucleoprotein complex (RNP, Fig. 1). ***Cas9*** from *Streptococcus pyogenes* (SpCas9) is a commonly-used endonuclease for CRISPR editing. The guide RNA contains a sequence complementary to the target site. Once bound, Cas9 makes a double strand break (DSB) in the targeted DNA. However, the cleavage of the target is contingent upon the initial binding of the nuclease to a ***protospacer adjacent motif (PAM)*** 3-4 nucleotides downstream of the cut site. The PAM motif for SpCas9 is 5'-NGG-3' (with N signifying any nucleotide). Different types of CRISPR nucleases recognize different PAM sequences. Once a DSB is made, the cell will activate machinery to repair the cut site. »



Guide RNA (gRNA): *the RNA component of the CRISPR-Cas9 genome engineering tool. Contains a guide sequence that is complimentary to the genomic target.*

Cas9: *a common endonuclease used in the CRISPR-Cas9 genome engineering tool. Makes a double strand break on the DNA at the targeted genomic region.*

PAM (protospacer adjacent motif) *A short sequence of nucleotides that must be present downstream of the target site in order for the nuclease to make a double strand break.*

Figure 1. The CRISPR-Cas9 System. *The CRISPR-Cas9 system comprises a guide RNA (gRNA) and Cas9 nuclease, which together form a ribonucleoprotein (RNP) complex. The presence of a specific protospacer adjacent motif (PAM) in the genomic DNA is required for the gRNA to bind to the target sequence. The Cas9 nuclease then makes a double strand break in the DNA (denoted by the scissors). Endogenous repair mechanisms triggered by the double strand break may result in gene knockout via a frameshift mutation or knock-in of a desired sequence if a DNA template is present.*

The cell has two natural repair mechanisms for mending a DSB: ***non-homologous end joining (NHEJ)*** and ***homology-directed repair (HDR)***. NHEJ is a quick-fix repair mechanism that involves ligating the ends of each DNA strand together. Prone to error, NHEJ often results in the insertion or deletion of nucleotides (called ***indels***) at the break site. Indels in protein-coding regions that are not multiples of three induce frameshift mutations. Because the reading frame of the gene is altered, these mutations lead to loss of gene function (i.e., no functional protein is made). This type of alteration, called a ***knockout (KO)***, can be used for a variety of loss-of-function applications.

Homology-directed repair uses a homologous DNA template inside the cell to repair DSBs. Unlike NHEJ, the HDR mechanism is highly accurate. Scientists can thus utilize this mechanism to insert desired genetic sequences by introducing donor DNA into the cell. The insertion of a genetic sequence, called a ***knock-in (KI)***, can be used for a variety of purposes, such as replacing a missing nucleotide or specifically altering gene.

Other applications of CRISPR include CRISPRa, CRISPRi, genome labeling, CRISPR screens, etc. However, in the rest of this eBook, we will focus on generation of the one of the most commonly desired experimental outcome: gene knockouts.

Non-homologous end joining repair (NHEJ): *an error-prone cellular mechanism that repairs double strand breaks in DNA, but often inserts or deletes nucleotides (indels) in the process. Can be used to induce knockouts.*

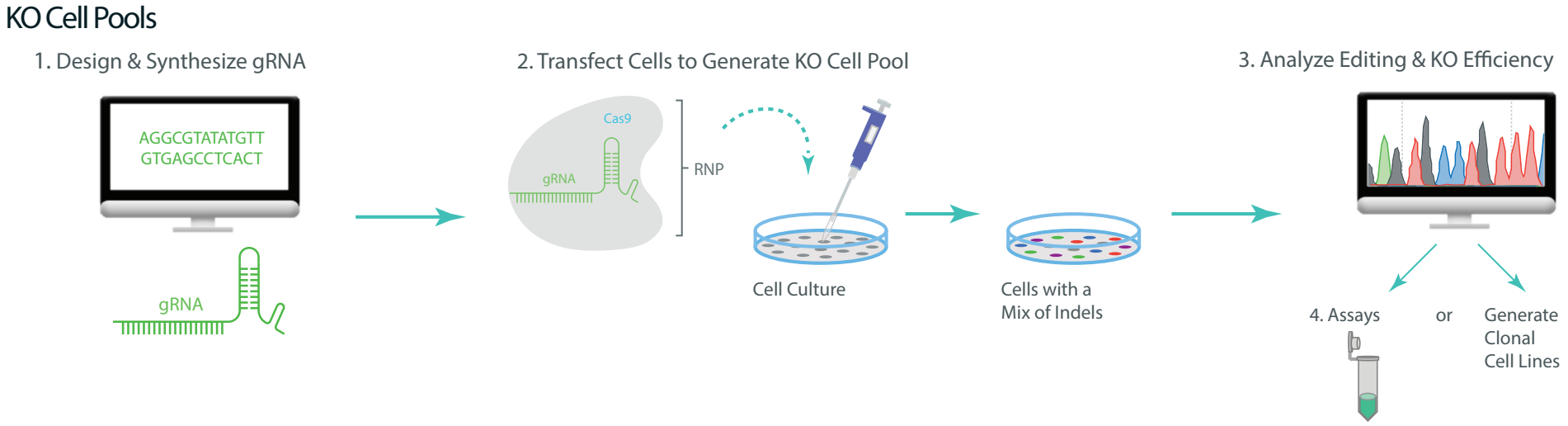
Homology-directed repair (HDR): *a cellular mechanism that repairs double strand breaks in DNA by using a homologous DNA template. Can be used to induce knock-ins.*

Indel: *the insertion or deletion of nucleotides in the genome. Often caused by NHEJ repair following a double strand break in DNA.*

Knockout (KO): *a mutation in a genetic sequence that causes it to be inoperative (i.e., no functional protein is made).*

Knock-in (KI): *the integration of a foreign genetic sequence into a cell's genome.*

KNOCKOUT CELL POOLS



1. Design and Synthesize gRNA

The first step in generating a knockout cell pool is to design and synthesize a guide RNA for the target gene (Fig. 2, step 1). Each guide RNA is composed of two components, a CRISPR RNA (crRNA) and a trans-activating RNA (tracrRNA). These components may be linked to form a continuous molecule called a single guide RNA (sgRNA) or annealed to form a two-piece guide (cr:tracrRNA). The latter option is less expensive but requires extra lab time and may not be as reliable.

As the specificity of CRISPR depends on the guide RNA, designing an optimum guide RNA sequence is a key factor for a successful CRISPR experiment. Several design tools are available to help researchers design highly specific guide RNAs with low off-target effects. Synthego's free

Figure 2. Schematic showing stepwise generation of knockout cell pools. *1) Design and synthesize gRNA. 2) Transfect cells: introduce ribonucleoprotein complexes (gRNA and Cas9 protein) into a cell culture. After CRISPR editing takes place, the culture will contain a mix cells with different indels as well as unedited cells (a KO cell pool). 3) Analyze editing & KO efficiency: assess the indel frequency & KO score of the pool. 4) Conduct assays and/or generate clonal cell lines.*

and easy-to-use CRISPR Design Tool enables you to design guides within 5 minutes. In addition to good design, site-specific chemical modifications that protect guide RNAs from degradation can be incorporated to enhance their performance.

The design of a gRNA also depends on the nuclease that one wants to use in an experiment. As described above, a nuclease will only cut genomic DNA if a specific PAM sequence is present. Therefore, one must choose a guide sequence that also has a correct PAM sequence downstream of the intended cut site. Please refer to our first CRISPR 101 eBook for more details on designing guide RNAs and choosing a nuclease. You can also get help choosing a nuclease on Synthego's blog post.

Format of CRISPR components

CRISPR components (gRNA and Cas9) can be delivered to cells in one of the three formats: DNA, RNA, or ribonucleoprotein (RNP) complex.

DNA

The DNA format involves cloning gRNA and Cas9 sequences into a plasmid, which is then introduced into cells. If permanent expression of gRNA and/or Cas9 is desired, then the DNA can be inserted into the host cell's genome using a lentivirus.

RNA

Guide RNAs can be produced either enzymatically (via *in vitro* transcription) or synthetically. Synthetic RNAs are typically more pure than IVT-derived RNAs and can be chemically modified to resist degradation. Cas 9 can also be delivered as RNA.

Ribonucleoproteins (RNP)

The ribonucleoproteins (RNP) format consists of gRNA and Cas9 protein. The RNPs are pre-complexed together and then introduced into cells. This format is easy to use and has been shown to be highly effective in many cell types (3).

The decision of which format to use for gRNA and Cas9 may ultimately depend on the experimental goals, time, cost, and whether transient or permanent expression of the components is desired. Please see CRISPR 101 for more details on the benefits and drawbacks of each option.

Marker Genes

Markers genes are used to indicate whether a desired genetic sequence has entered a cell or been integrated into a cell's genome. Screenable markers (a type of marker gene) are sometimes co-transfected when introducing CRISPR components via a plasmid or lentivirus). For instance, when a GFP or luciferase gene is introduced into cells along with CRISPR components, a fluorescence signal allows one to select cells that have been successfully transfected. However, the presence of such a signal does not indicate that cells have been edited. Verification of a knockout requires additional analyses, such as genotyping or a functional assay. If a lentivirus is used to insert gRNA or Cas9 DNA directly into the genome, one may also insert a selectable marker (e.g., antibiotic resistance gene) so that colonies that successfully integrated the foreign DNA can be identified.

In most cases, marker genes are used when transfecting CRISPR components using plasmid or lentivirus, as these methods generally do not result in high editing efficiencies. However, newer transfection formats such as ribonucleoprotein complexes (RNPs) typically achieve high editing efficiencies. Because there is a high probability of obtaining knocked out cells when using RNPs, using a marker gene may not be necessary. Avoiding the insertion of this extra DNA results in a cleaner edit.

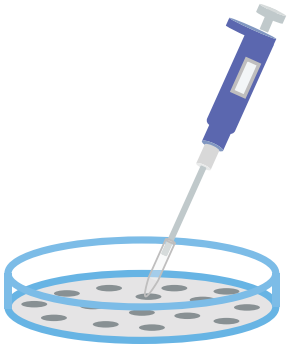
2. Transfect Cells

After designing and generating the guide RNA, the next step is to introduce the CRISPR components into cells via one of several possible transfection methods, such as lipofection, electroporation, nucleofection, or microinjection. The choice of the transfection method depends on the cell type and experimental goals. Lipofection for instance requires no external equipment, but may not work as effectively with some cell types. Electroporation and nucleofection (a type of electroporation) are often effective for transfecting challenging cell types and microinjection is commonly used for injecting the CRISPR machinery into embryos. Learn more about the different transfection methods in our CRISPR 101 eBook. Check out Synthego's protocols using different transfection methods.

After a guide RNA and Cas9 are introduced into a cell culture, they produce a DSB at the the target site within some of the cells (Fig. 2, step 2). The NHEJ pathway then repairs the break, potentially inserting or deleting nucleotides (indels) in the process.

Because NHEJ may repair the target site on each chromosome differently, each cell may have a different set of indels or a combination of indels and unedited sequences.

The cells within a CRISPR-edited culture are thus a mix of unedited (wild type) cells and edited cells with different indels, some of which induce knockouts of the target gene or sequence. We call these cultures of heterogeneous cells ***knockout (KO) cell pools*** (Fig. 2, step 2). Note that the use of the word "pool" here is different from pooled library screens generated by introducing sgRNAs targeting multiple genes into cell culture (discussed in *high-throughput* screens).



Knockout (KO) cell pool: *a CRISPR-edited cell culture containing a mix of edited and unedited cells. Edited cells have different indel profiles at the target site, some of which induce a knockout of the targeted gene or genetic sequence.*

3. Analyze Editing & Knockout Efficiency

After transfection, the editing efficiency (indicated by indel frequency) is analyzed by sequencing the genomic DNA (Fig. 2., step 3). As next-generation sequencing is expensive, researchers often use Sanger sequencing for their analysis. Synthego's Inference of CRISPR Edits (ICE) software tool efficiently analyzes CRISPR editing data with NGS-level quality. From the results, one can determine the editing efficiency (indel frequency) and the contribution of each type of indel generated in the pool. Learn how to use ICE with our step-by-step guide.

It is important to remember efficiency does not equate to KO efficiency. Each KO cell pool contains a mix of cells with different indels, only some of which cause knockouts. Synthego's ICE tool calculates a ***Knockout (KO) Score***, the percentage of knockout-inducing sequences in a CRISPR-edited pool. Pools with high KO scores have a high likelihood of containing indels that cause knockouts. Therefore, highly efficient pools are suitable for direct use in many types of assays.

4. Conduct Assays

Loss-of-function assays

Cell pools with efficient knockouts can be used to identify gene function. One can assess the function of a gene by inducing a genotypic aberration that directly leads to an observable phenotypic effect. For instance, if the knocked out gene encodes for a protein responsible for cell proliferation, then cell abundance can be measured using proliferation assays. Similar methods can be applied to test any genotype-phenotype relationship including, but not limited to, signaling pathway involvement, differentiation ability, and drug response.

One particularly useful application of KO pools is identifying the contributions of specific genes to disease states. A gene thought to be associated with disease can be knocked out in a cell pool. If the knockout results in a disease phenotype, then further analyses can be conducted to validate the findings. Ultimately, this information may be used to develop a disease model.

Knockout (KO) Score: *A metric of knockout efficiency determined by Synthego ICE analysis. KO Score is defined as the percentage of sequences in a CRISPR-edited pool that lead to a putative knockout, including frameshift-inducing indels and indels that are 21+nt in length or larger.*

High-throughput screens

High-throughput screens are used to investigate genotype-phenotype relationships on a genome-wide scale. For each screen, a gRNA library is applied to cells to systematically knock out hundreds of genes. The phenotypic effects of each knockout can then be assessed. Loss-of-function screens can be used to identify how genes function in entire biological pathways or disease states. They have also been critical to the process of drug discovery. For instance, a knockout that confers resistance or sensitivity to a drug may provide information about the drug's target.

Pooled screens

Pooled screens utilize libraries of gRNAs targeting different genes in a communal pool of cells. The gRNAs are first introduced into the genomes of cells through lentiviral transduction. Cas9 is also applied and edits are made to the targeted genes. A selective pressure (e.g., drug) is then applied such that knockout cells either survive/proliferate (positive selection) or die (negative selection). The knockouts responsible for cell survival or death are then identified by assessing the relative proportion of gRNAs integrated into the genomes of the resulting cells. Although useful for screening large gRNA libraries, pooled screens are best suited to assessing viability phenotypes and require NGS and complex data deconvolution.

Arrayed screens

In arrayed screens, one gene is targeted per well of a multiwell plate (i.e., each well is an individual KO pool for a particular gene. Guide RNAs and Cas9 may be introduced via transfection or lentiviral transduction for editing to take place.

A treatment (e.g., drug) is then applied to the cells and the resulting phenotypes are analyzed. Unlike pooled screens, arrayed screens are compatible with a wide variety of phenotypes that can be measured through various methods, such as fluorescence and high-content image analysis. Check out Synthego's screening libraries.

Small molecule screens

CRISPR KO cell pools can also be used to screen small molecule libraries in order to identify potential therapeutic compounds. For instance, cell pools with a gene KO that induces a disease can be generated and a library of compounds can be systematically applied. Resulting phenotypes can be assessed to determine if any of the compounds affected the disease state of the cells.

Antibody validation

All immunoassays utilize antibodies that are specific for cellular proteins. For each assay, the specificity of an antibody to a particular protein must be validated in order to avoid non-specific results. To test specificity, a cell pool can be generated with a knockout of the antibody's protein target. Because less target protein is made in the pool, subsequent immunoblot or immunofluorescence analyses (which use the antibody to detect and visualize the protein) should have a proportionally lower signal. If this is observed, it is a good indication that the protein is indeed the antibody's target.

Are KO Pools Enough or **Do I Need Clones?**

As discussed above, knockout cell pools with high knockout efficiencies may be an economical and time-efficient option for loss-of-function assays. In most cases, the gene knockout does not impact cell viability and the total population of edited cells does not change. However, because KO cell pools contain a mix of edited and wild type cells, the knockout efficiency may vary over time. For instance, if wild type cells proliferate faster or if the knockout induces cell death, the percentage of KO cells in the pool may gradually decrease (and the protein knockout could be lost).

If a pool has low KO efficiency, this effect is likely to have a larger impact on the cell population, making it difficult to directly assay the cells. For research in which changes in cell populations may be a concern, including work that requires long time spans and cells with confirmed knockouts, using clonal cell lines may be more appropriate. The following sections describe generation, maintenance, and the applications of clonal cell lines.

KNOCKOUT CLONAL CELL LINES

Knockout clonal cell lines are generated by separating individual cells from a KO cell pool and expanding each of them into a monoclonal population. Because each clonal cell line is composed of genetically identical cells, there is no risk of some cells (i.e., wild type) outnumbering others over time. Thus, clonal populations may be more stable than cell pools. There are several other benefits of clonal cell lines. As each cell of a clonal population has an identical indel profile, it is possible to determine which particular mutation(s) lead to a knockout phenotype. Also, isogenic cell lines comprising a parental (wild type) cell line and an engineered cell line can be developed. Because both lines share genetic backgrounds, the parental cells can serve as a control for the KO cells.

KO clonal cell line: *a monoclonal population of cells containing the same KO-inducing indel profile.*

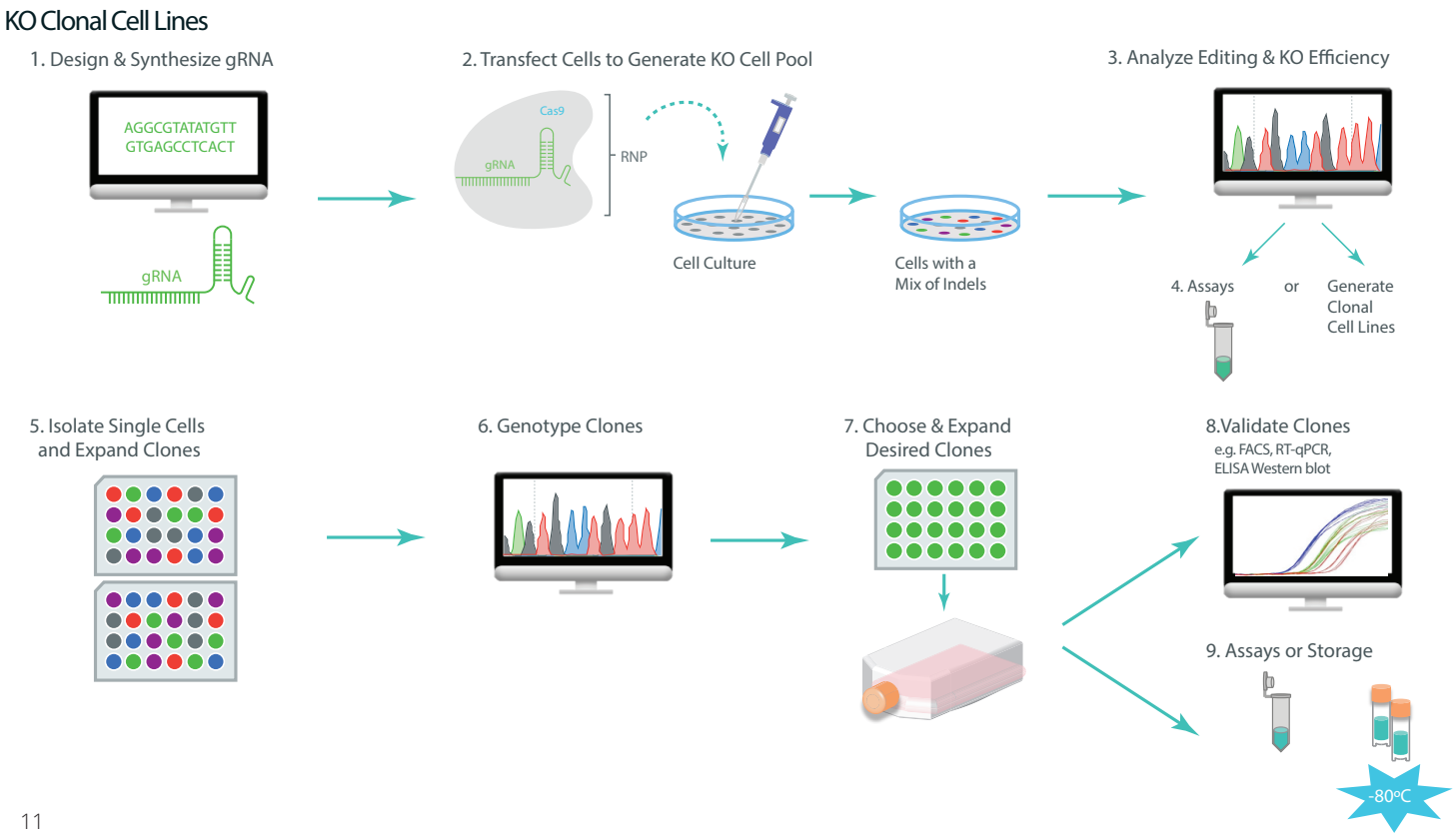
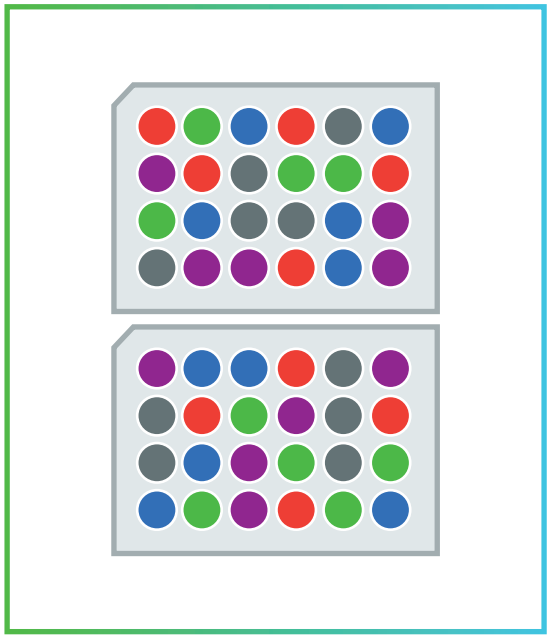


Figure 3. Schematic showing stepwise generation of knockout clonal cell lines. *1) Design and synthesize gRNA. 2) Transfect cells: introduce ribonucleoprotein complexes (gRNA and Cas9 protein) into a cell culture. After CRISPR editing takes place, the culture contains a mix of cells with different indels as well as unedited cells (a KO cell pool). 3) Analyze editing & KO efficiency: assess of the indel frequency & KO score of the pool. 4) Conduct assays and/or generate clonal cell lines (proceed to step 5). 5) Isolate single cells & expand clones. 6) Genotype clones: determine indels of each clone. 7) Choose & expand desired clones. 8) Validate clones. 9) Conduct assays and/or store clones.*

5. Isolate Single Cells and Expand Clones

In order to produce a clonal cell line, one must first isolate individual cells from a pool (Fig. 3, step 5). There are several sophisticated techniques for isolating cells, including flow cytometry, fluorescence activated cell sorting (FACS), magnetic-activated cell sorting (MACS), and use of microfluidics, among others (4, 5).



The separation of individual cells can also be accomplished using a simple limiting dilution method that only requires pipettes and multiwell plates. The procedure involves diluting a cell suspension to a concentration that maximizes the probability of obtaining a single cell per well. The technique is of moderate throughput, as it can be performed manually or by using automation. As the isolation of a single cell per well is based on statistical probability (Poisson distribution), downstream verification of clonality is required. Still, limiting dilution is a gentle, cost-effective, and relatively simple way of sorting cells.

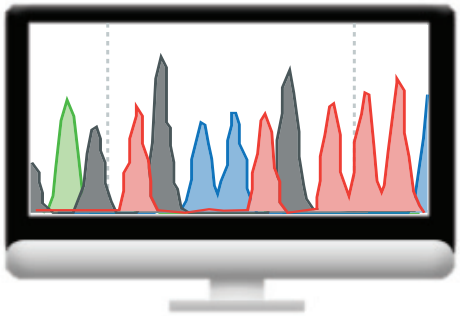
Once single cells are plated, they must be expanded in order to generate monoclonal populations. Plates should be imaged under a microscope a few hours after cells are deposited to identify the wells containing only one cell, and then every week thereafter. This practice enables one to track cell growth over time and confirm that the final clonal populations originated from a single cell. When colonies are about 70% confluent, they should be transferred to a plate with larger wells to allow more space for cell division. During the transfer, cells should be sampled from each colony for genotyping (see section below). As different cell types divide at different rates, the time required for this expansion may vary.

6. Genotype Clones

Genotyping is performed to identify which clones have desired edits (i.e., ones predicted to cause a knockout) so that they can be expanded further (Fig. 3, step 6). Edited regions of genomic DNA is extracted from clones, PCR- amplified, and submitted for Sanger sequencing. Sequencing data is then analyzed using indel analysis software (e.g., Inference of CRISPR Edits (ICE)) The sequencing data can reveal whether a clone is wild type (unedited), contains a ***homozygous edit***, or contains ***heterozygous edit***.

A clone has a homozygous edit if all alleles of a chromosomal pair have the same mutation. These edits can be identified by non-overlapping peaks around the cut site.

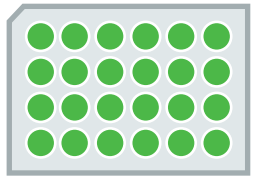
Alternatively, a clone has a heterozygous edit if each allele of a chromosomal pair is different. One allele may be unedited (wild type) and the other edited. Or, each allele may contain a different mutation (compound heterozygous). Given that the NHEJ pathway repairs each allele independently, heterozygous edits are common and can be easily identified by the overlapping peaks in the chromatogram centered around the cut site.



Homozygous edit: *both alleles of a chromosomal pair have the same mutation.*

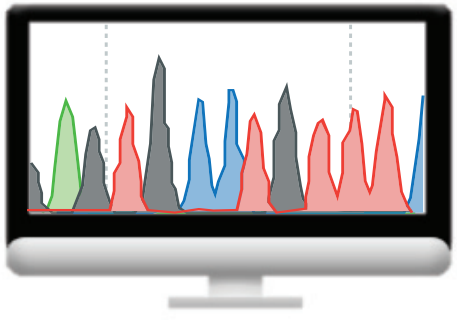
Heterozygous edit: *each allele of a chromosomal pair either has a different mutation, or one allele has a mutation and one is unedited.*

7. Choose & Expand Desired Clones



Next, clonal cell lines with putative knockouts should be identified for further expansion (Fig. 3, step 7). Populations with either homozygous or compound heterozygous edits may result in complete functional knockouts of the protein. Colonies containing indels that give rise to a frameshift mutation on each allele (ideally those that generate a premature stop codon) should be chosen for continued expansion. Indels that maintain the gene's reading frame or alter the coding of only a few amino acids towards the 3' end of the exon may not lead to the loss of the protein's function.

Genotyping can also be used to identify unwanted polyclonal populations. The presence of many indel species may indicate that the clone did not originate from a single cell. However, if a hyperploid cell line is used, multiple indels may reflect the presence of multiple alleles. In these cases deep sequencing may be necessary to verify monoclonality.



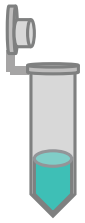
8. Validate Clones

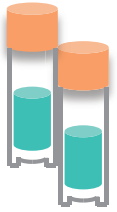
Clones that are identified as putative knockouts by genotype can be expanded further and then split into two groups, one used for validation of the KO and one for assays or banking (Fig. 3, step 8). The subculture used for validation may be analyzed using an appropriate assay, such as ELISA, flow cytometry, qRT-PCR, western blot, or a functional assay. Ideally an assay that can establish whether the effect of the CRISPR-induced mutation terminates protein production or causes the protein to be non-functional should be conducted.

9. Assays and Storage

The other subculture of clones can be used for various loss-of-function assays, or cryopreserved for future use.

For a more detailed instructions, see Synthego's limiting dilution and clonal expansion protocol.





CONCLUSION

Knockout cell pools and clones are both robust tools for functional genomics and biomedical research. Pools are a relatively economical option and can be just as effective as clones for many types of assays. However, efficient knockout pool generation requires optimization because editing efficiencies typically vary based on many variables, such as the cell type, the target gene, and the guide RNA used. It is critical that only knockout cell pools with high KO efficiencies be used directly for loss-of-function assays, as low-efficiency pools will not have a robust knockout of the target gene. Additionally, because KO cell pools contain a mix of edited and wild type cells, there is a risk of wild type cells outgrowing edited cells over time (or vice versa). Therefore, it is often recommended that KO cell pools be only used for assays that can be conducted in the short-term.

Clonal cell lines are generated by isolating and expanding individual cells from a KO cell pool. Although clones require more time and skill to produce, they have consistent genetic backgrounds and are stable over time. Clones are thus commonly used in research labs that require edited cells for extended time periods. When using knockout cell clones, it is important to test multiple different clones with different indels in experiments to ensure any results obtained can be attributed to the knockout in general, rather than something specific about the one particular indel. It should be noted that not all cell types are amenable to cloning. Single cells of some cell types cannot be easily propagated, as they require physical contact with other cells and/or high concentrations of secreted growth factors. The stress associated with separating and expanding cells can also reduce cell viability or change cellular biology. In such cases, working with pools may be required.

Whether one plans to use knockout cell pools or clones, both options require time and labor to produce in the lab, with no guarantee of success. As an alternative, Synthego offers Engineered Cells products, including KO cell pools and clones. Not only do these products allow scientists to bypass lab work associated with pool and clone generation, but they also eliminate the risk of obtaining a poor result. The Knockout Cell Pools are guaranteed to contain 50% or greater knockout at the protein level. Knockout Cell Clones have 100% sequence verification and can be produced in essentially any human cell line. For knock-in options and non-human cell lines, Advanced Cell pools and clones are also available. Visit Synthego's Engineered Cells for more information. ◆

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References

1. Jinek, M, et al. (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science* 1225829.
2. Cong, L, et al. (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science* 1231143.
3. Farboud, B., et al. (2018) Enhanced genome editing with Cas9 ribonucleoprotein in diverse cells and organisms. *Journal of visualized experiments: JoVE* 135.
4. Hu P, Zhang W, et al. (2016) Single cell isolation and analysis. *Front Cell Dev Biol*, 4:116.
5. Gross A, et al. Technologies for single-cell isolation. *Int. J. Mol. Sci.* 16:16897-16919.

Additional Information

For an up-to-date list of all Synthego Application Notes and other resources, please visit synthego.com/resources

For technical assistance, contact our Scientific Support Team:

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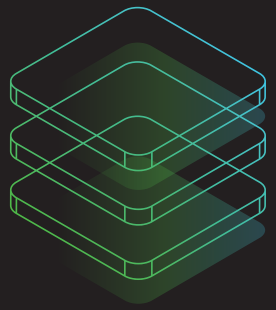
About Synthego

Synthego is the leading genome engineering innovation company. The company's automated, full stack genome engineering platform enables broader access to CRISPR to accelerate basic scientific discovery, uncover cures for diseases, and develop novel synthetic biology applications. Headquartered in Silicon Valley, Synthego is used by scientists from the largest global biotechnology companies and global biology universities to unlock the potential of gene editing.

Engineered Cells



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Full Stack Genome Engineering

Synthego's Engineered Cells take the complexity out of CRISPR by delivering essentially any edit in any cell, with guaranteed results. Spend your time on discovery, not optimizing CRISPR.

Knockout Cell Pool

Pick your cell line and gene and we'll do the rest! We guarantee at least 50% protein knockout in as few as 4 weeks or your money back.

Knockout Cell Clone

100% verified knockout clonal cell lines in as few as 8 weeks. We'll generate your knockout clonal cell lines, so you can focus on results, not methods.

Advanced Cells

Your edit, your cells. Tailor-made cells built on our genome engineering platform. Realize the full power of CRISPR without limitations.

CRISPR with Confidence

Synthego.com/cells