Microsynth

CRISPR/Cas9 Sequencing

Verify your guide RNA library by amplicon deep sequencing
Validate CRISPR/Cas9 targets and mutation efficiency

Introduction

CRISPR/Cas9 and equivalent gene editing systems are modern and popular genetic screening tools that may for instance be used to facilitate the modification of cell lines or to analyze the function of specific genes. The gene editing systems referred to here work by introducing double strand breaks to activate repair mechanisms and introduce permanent mutations. These mutations might be deleterious or induce new functionality depending on the dominant repair pathway as detailed in Figure 1.

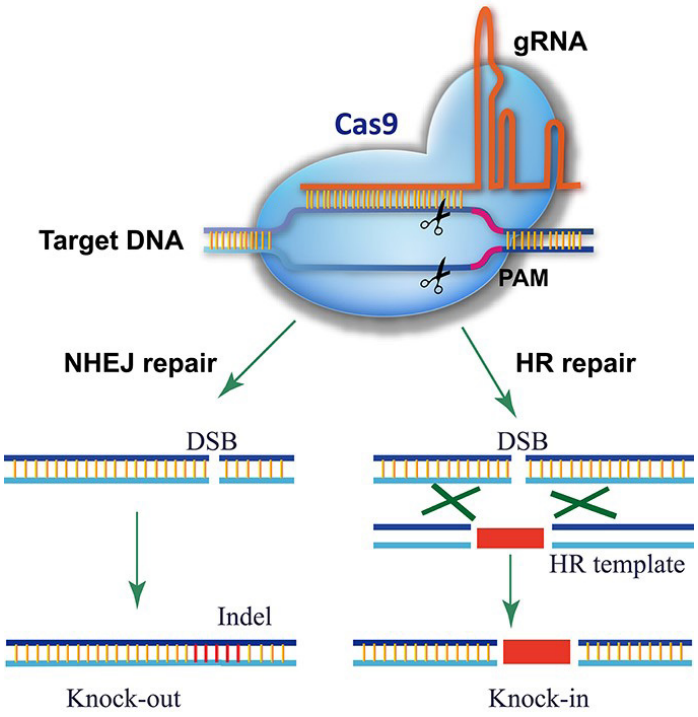

Figure 1: *This figure illustrates the interaction of the guide RNA, the protospacer-adjacent motif and the Cas9 protein to cleave a specific DNA target site, inducing a double strand break which may be repaired by either a non-homologous end join repair pathway or by a homology-directed repair pathway. [1]*

Microsynth Competences and Services

Deep sequencing of PCR products is one of Microsynths core competences. Based on our experience and expertise, we provide a one-stop service from experimental design to bioinformatics analysis. You may either outsource the entire analysis or only single steps to us as illustrated in Figure 2.

Experimental Design

The result and impact of a study depends on its experimental design. Use of replicates and controls are only a couple of many important points to consider. Therefore, make use of our extensive experience to help optimize the setup of your experiment.

DNA Isolation

You may either perform the DNA extraction yourself or outsource this critical step to Microsynth. We have long-standing experience in processing various sample matrices and DNA/RNA sources.

PCR Amplification

PCR amplification follows the established two-step protocol (see our Amplicon Deep Sequencing application note for details). In short: in a first step the target locus sequence is amplified while in a second step sequence-ready libraries are constructed. For projects with very low amounts of starting material we recommend our three-step PCR protocol, which includes two subsequent locus specific PCRs to increase the yield of sequenceable amplicons.

Sequencing

Depending on the requirements of your project, sequencing is performed on one of our Illumina MiSeq or NextSeq 500 NGS platforms which support high read throughput and variable read lengths. [2]

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Bioinformatics Analysis

Microsynth's bioinformatics analysis module is designed to help in different scenarios. [3]

1) In a screening exercise the module may for instance be used to describe the inventory of single guide RNAs (sgRNA) in a plasmid library (see ***Figure 3***). If the experiment was set up having replicates for different conditions to be compared, differential analysis of abundances of sgRNAs is possible (see ***Table 1***).

2) After a gene editing event in a cell line for instance, the analysis module may be used to evaluate modifications of target genes and to determine the efficiency of the employed gene editing system (see ***Table 2*** and ***Figure 4***).

Customized bioinformatics solutions for more specific questions are provided on request.

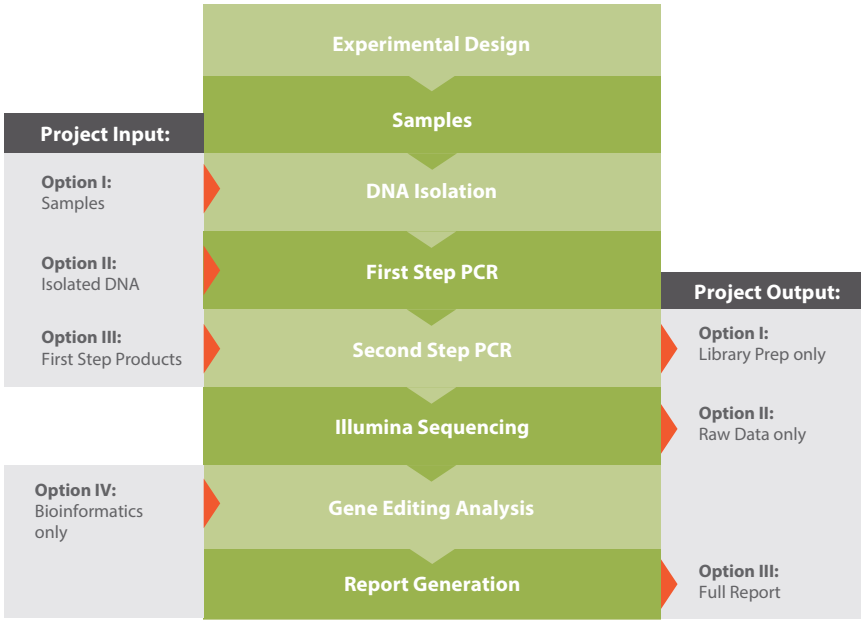

***Figure 2**: This chart depicts the workflow for a gene-editing project at Microsynth and lists all input and output points.*

Example Results of the Gene Editing Analysis Module

>Plasmid-1.1;size=4292;
TGGGAATGGCCGCCGCCGCCCTCGCCGTGG
>Plasmid-1.2;size=308;
GCATGTGGGTGACAGCCAGGGGCTGGATCG

***Figure 3**: This cut-out is a fragment of a sequence file in fasta format listing the inventory of guides (sequence and abundance) amplified from a plasmid library.*

***Table 1**: Differential analysis results*

ID	baseMean	log2FoldChange	pvalue	padj
3759	16.81677	5.718399	1.41E-05	0.002115
11384	4.476830	0.000391	0.999761	0.999908
1320	55.52621	-6.233259	3.33E-08	1.73E-05

This cut-out presents details of a differential analysis results table listing sequence IDs, abundance fold changes and their statistical significance (corrected p-values: padj) between the compared conditions.

***Table 2**: Detected modifications*

Sample	InDelType	InDelSize	InDelCoord	Frequency	Percent
S1-10_merged	WT	0	NA	7622	41.01
S1-10_merged	D	18	220-237	1689	9.09
S1-10_merged	I	4	229-232	27	0.15

This is a cut-out of a results table listing detected modifications of a target gene and giving information on type (insertions or deletions: InDels), size, location and portion of the modifications.

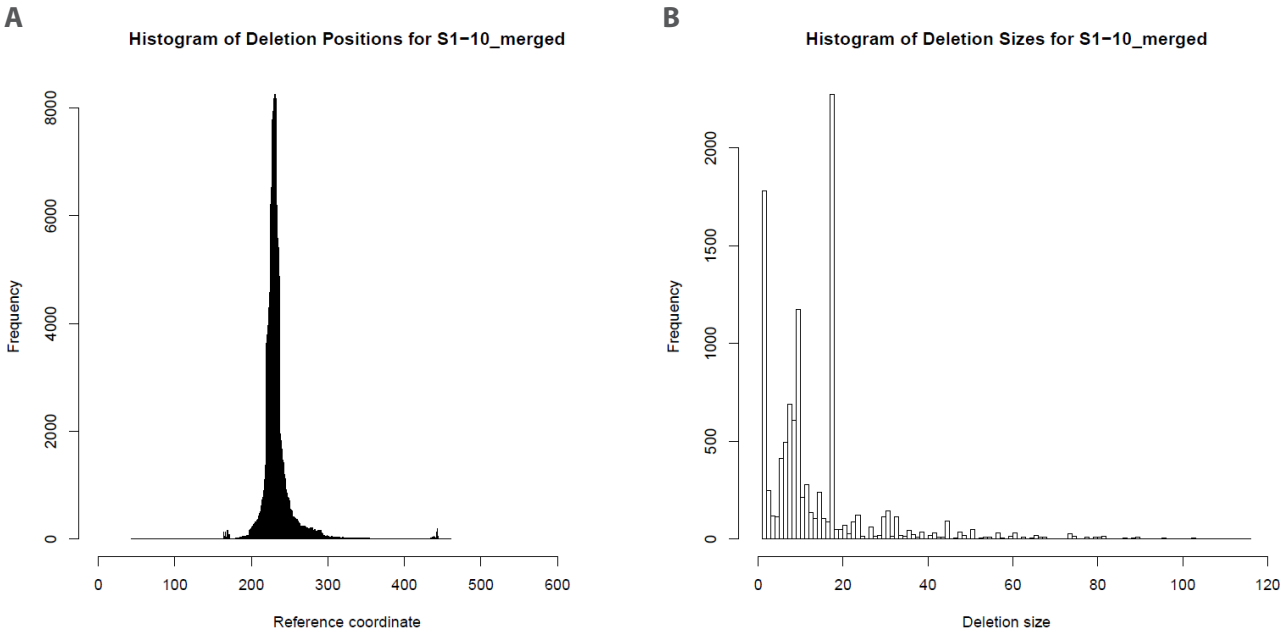

***Figure 4**: The analysis also includes visualization of results such as the two presented histograms depicting (A) the location of deleted bases and (B) the size of deletions introduced into a target gene.*

Further Analysis Possibilities

Further interesting analysis avenues related to gene editing experiments available in the portfolio of Microsynth include whole genome/exome sequencing (for instance, to find off-target effects) and RNA sequencing (for instance, to analyze the influence of a knock-out on entire gene pathways). For more details on the mentioned services, please refer to the respective application notes published on our website.

References

1. *Ding Yuduan et al., Recent Advances in Genome Editing Using CRISPR/Cas9, Frontiers in Plant Science, 7, 2016, 703, https://doi.org/10.3389/fpls.2016.00703*
2. *Benedict C. S. Cross et al., Increasing the performance of pooled CRISPR–Cas9 drop-out screening, Scientific Reports, 6, 2016, 31782, http://dx.doi.org/10.1038/srep31782*
3. *Ulrike Mock et al., mRNA transfection of a novel TAL effector nuclease (TALEN) facilitates efficient knockout of HIV co-receptor CCR5, Nucleic Acids Research, 43, 2015, 5560–5571, https://doi.org/10.1093/nar/gkv469*

Related Topics

• Optimized crRNA and tracrRNA from Microsynth
• Amplicon Deep Sequencing at Microsynth

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