APPLICATION NOTE

TRANSFORMING LIFE SCIENCE: CRISPR-CAS9

HIGH QUALITY OLIGONUCLEOTIDES FOR GENOME EDITING

An Introduction to CRISPR

The capability to carry out targeted modifications straightforward and with high accuracy to the genome is transforming life science research.

In just a few short years the fast evolving technique CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), has gained huge popularity and opens doors to a vast array of applications. Many consider this technique as a major breakthrough in the field of synthetic biology and the rate of publications emerging around CRISPR has reflected this and increased dramatically within a short period of time [1].

WE ♥ YOUR DNA

SYNTHEGO

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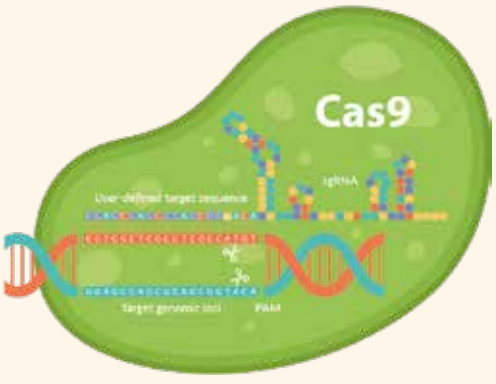
Initially discovered as an "adaptive immune system" in prokaryotes [2] CRISPR has now been extensively used as a genome editing tool. This is mainly due to the relatively straightforward and high accuracy targeting of nucleic acid strands in addition to it's speed, ease of use and relatively low cost [3].

This is made possible by the use of guide RNA associated with CAS (CRISPR Associated protein) nuclease systems. Cas9 catalyzes site-specific cleavage of (double stranded) DNA when guided by two short RNA sequences – crRNA (CRISPR RNA), which is complementary to the target DNA or protospacer, and tracrRNA (transactivating RNA) which, fused with the crRNA, complexes with the Cas9 nuclease to direct and facilitate cleavage of the target DNA [4]. The RiboNucleoProtein (RNP) complex of crRNA/tracrRNA and Cas9 nuclease anneal to the target sequence next to a PAM (Protospacer Adjacent Motif) sequence.

Once the complex is bound to the target, a cut is achieved leaving several options for editing the target such as "Non-Homologous End Joining" (NHEJ) and "Homology Direct Repair" (HR) (figure 1).

Figure 1 | schematic overview of CRISPR-Cas9 Gene Editing Tool



Although challenging aspects remain, such as off-target effects, indels and discrepancies in expected gRNA specificity [5], the results are astonishing and the further development of this technique will continue to impact the field of genome engineering.

Synthetic RNA – For Improved CRISPR Editing

Since October 2016 Biolegio started to distribute Synthego CRISPRevolution RNA. We are confident that CRISPRevolution RNA's will bring you the best quality for your CRISPR applications! The CRISPRevolution range offers improved editing efficiency up to 90%, lower Off Target effects, consistent quality, improved speed, ease of use and a cost effective solution.

The wide range high quality synthetic oligonecleotides for CRISPR application include:

crRNA

17-20 nts variable RNA sequences complementary to the genomic DNA target site, or protospacer, followed by the required S. pyogenes repeat sequence that interacts with the tracrRNA.

tracrRNA

Long RNA based on the published S. pyogenes tracrRNA sequence, hybridizes to crRNA and acts as a bridge between the crRNA and the Cas9 nuclease.

sgRNA

A single RNA chimera of tracrRNA and crRNA. To avoid time-consuming in vitro transcription processes or the hybridisation step of crRNA with tracrRNA, this sgRNA gives you the option for direct transfection or direct RNP formation.

Chemical Modifications

We offer 2'-O-methyl and 3' phosphorothioate internucleotide linkages at the first three 5' and 3' terminal RNA residues. The chemically modified RNA sequences has been shown to provide additional improvements in editing efficiency for particular cell types and genomic targets that prove otherwise challenging to edit, such as T-cells and stem cells.

Ongoing discoveries of CRISPR Cas systems

Besides many Cas9 orthologues multiple other CRISPR systems have been discovered and described in the last few years. Systems as CPF1 and C2C2 exhibit different functionalities and requirements for e.g. type of substrate, PAM regions, length of the required guide RNA and mode of substrate cleaving.

Synthego's Custom RNA is a flexible, fully custom sequence product that can be tailored to your research needs.

Custom RNA 50
Custom RNA 50 is perfect for designing short guide RNAs for nucleases that don't require long sequences, such as Cpf1. You can use it to design your own Cas9 crRNA and custom 3 linker to a tracrRNA.

Custom RNA 75
Custom RNA 75 is perfect for designing guide RNAs for nucleases that don't require guides as long as S. pyogenes Cas9. You can use it to design your own Cas9 tracrRNA and custom linker to a crRNA.

Custom RNA 100
Custom RNA 100 is perfect for designing full length sgRNA (100-mer) for CRISPR at a practical scale and price point. You can custom design your entire sgRNA sequence from 76-100 nucleotides.

HDR repair knock-in sequences

Our unrivalled expertise in synthesis of long DNA / RNA constructs give rise a multitude of advantages for CRISPR applications such like knock-in constructs containing barcodes or multiple cutting sites. Use our oligonucleotides as knock-in sequences for Homology Directed Repair applications.

IVT templates

With the longest, high quality, high purity DNA oligos commercially available - Up to 300 bases. Our Long DNA oligos perfectly complement our already extensive CRISPR product offering and can be used as direct templates for in vitro transcription of RNA. Please contact us for further details.

Unmatched custom synthesized oligonucleotides

Oligonucleotides reside at the heart of so many molecular biology techniques and are a crucial but often overlooked component of everyday research.

With over 20 years' experience with the synthesis of oligos, our boundless innovation, structured efficiency and approachable flexibility makes us your oligo partner of choice!

> "By making use of the synthetic guide RNA's provided by Biolegio, we now easily make a Cas9/gRNA (RNP) complex that together with or without a template (PCR product) can be transferred at high efficiencies into the eukaryotic cells by using lipofection methods. Compared to the plasmid based genome editing procedures we observed less toxicity, increased specificity and remove the introduction of foreign DNA (plasmid backbone) into the eukaryotic cells. This makes the genome editing procedure much cleaner and therefore our group encourage colleagues in the field to start using synthetic guide RNAs."
>
> Dr. Rogier Louwen

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CUSTOM SYNTHESIZED OLIGONUCLEOTIDES		
Examples of Purposes / Description	**Product**	**Length (nts)**
Cloning / plasmid based gRNA introduction, HR knock in constructs, IVT templates, PCR, sequencing, NGS	DNA Oligonucleotides	2 - 80
Long HR knock-in constructs, up to 300 nucleotides, internal PCR controls	DNA Longmers	81 - 300
crRNA, tracrRNA, sgRNA, siRNA, Aptamers	RNA Oligonucleotides	2 - 80
Chimeric sgRNA for Cas9/sgRNA RNP's	RNA Longmers	80 - 100

Contact us now to discuss how we can help you to overcome your challenges today: www.biologio.com

References

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