OLIGOS & PROBES SERVICE

DNA OLIGOS

PROBES

NGS(Next Generation Sequence)
SPECIAL OLIGOS

MIPS

EXPRESSION, EDITING & GENE SYNTHESIS

- CR:tracRNA
- sgRNA
- customRNA
- HDR repair knok-inSeq
- SiRNA
- miRNA

DNA OLIGOS

- Custom DNA oligo synthesis from 2 to 300 bases
- Your choice of plate, strip or tube for optimal flexibility
- Standard DNA oligos are shipped within 24 hours
- Purified & modified oligos within 4 business days
- Over 300 modifications available

Synthesis & Scales

With Biolegio synthesis programs result in an average coupling efficiency above 99,5%. With this efficiency we are able to synthesize DNA oligos up to 300 bases. Synthesis is performed under low salt conditions, which avoids the need for additional purification for most basic molecular biology applications, such as PCR, sequencing, hybridization studies and antisense studies.
Biolegio offers four different synthesis scales for DNA oligos: 10 nmol, 40 nmol, 200 nmol and 1000 nmol. For each synthesis scale there is a restriction regarding the length of the oligo.

Purifications

Reverse-phase cartridge purifications
Level of purity typically 80%.
HPLC Reverse-phase purification
Level of purity typically 90%
PAGE Purification
Level of purity typically 95-99%

Long Oligos Synthesis

B-pure protocol enables high-quality oligonucleotides up to 300 bases

Coupling efficiency

up to 99,9%

Maximum DNA oligo length in relation to synthesis scale

Synthesis scale	Max. oligo length
10 nmol standard oligo	40 bases
40 nmol standard oligo	200 bases
200 nmol standard oligo	300 bases
1000 nmol standard oligo	300 bases

(oligos longer than 80 bases are synthetized with our B-pure protocol)

Guaranteed yeald for non-labelled, non purified oligos up to 40 bases

Synthesis scale	Min. oligo yeald
10 nmol standard oligo	10 nmol
40 nmol standard oligo	20 nmol
200 nmol standard oligo	95 nmol
1000 nmol standard oligo	400 nmol

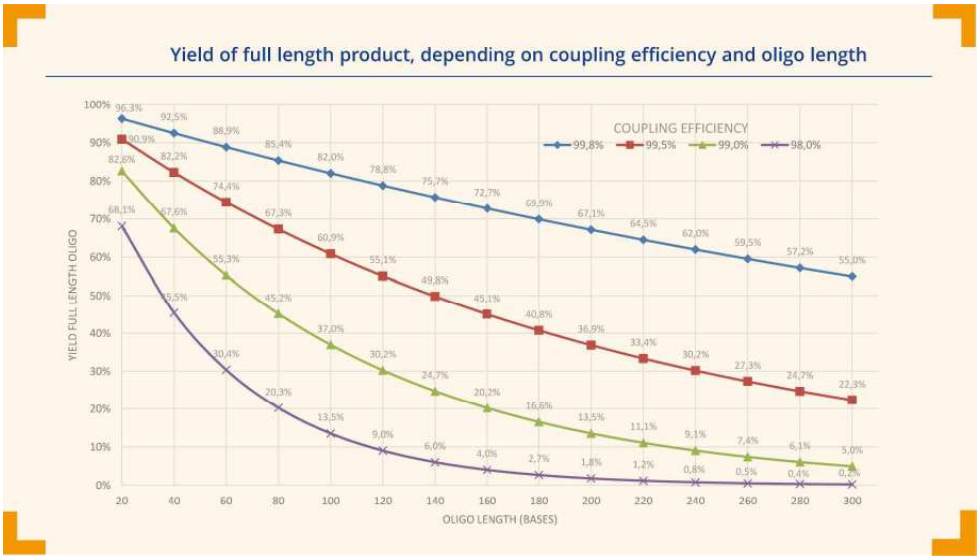
Ordering

DNA oligos in tubes

DNA oligos in 96- or 384-well plates

Premixed oligos (oligos dispensing service)

Oligo prepaid service



Modified Oligo Synthesis

- Over 300 modifications available
- available for diferents backbones (DNA,RNA 2'-O-Me,LNA)
- Dificult/Special designs, e.g. long haiprin oligo structures with 2 or 3 modifications

FLUOROPHORES

Modifications	5'	Internal	3'
6-FAM™	•		•
HEX™	•		•
TET™	•		
6-TAMARA™	•		•
Cyanine 3	•		•
Cyanine 5	•		•
ATTO™ Dye	•	•	•
Yakima Yellow™	•		
Dyomic™ Dyes	•	•	•
JOE™	•		•
Texas Red™	•		•
Quasar 570,647,705™	•		•
LC Replacement Dye	•		•
NED™ Replacement Dye	•	•	•
PET™ Replacement Dye	•	•	•
VIC™ Replacement Dye	•	•	•
Alexa Fluor™ Dyes	•	•	•
Calfluor™ Dyes	•		
ROX™	•	•	•
Fluorescein dT		•	

QUENCHERS

Modifications	5'	internal	3'
Black Hole Quencher 0	•		•
Black Hole Quencher 1	•		•
Black Hole Quencher 2	•		•
Black Hole Quencher 3	•		•
Black Hole Quencher 1dT		•	
Black Hole Quencher 2 dT		•	
Black Berry Quencher 650			•
Deep Dark Quencher 1			•
Deep Dark Quencher 2			•
Dabcyl™			•
TAMARA™	•		•
Eclipse	•		•

SPACERS

Modifications	5'	internal	3'
PC Spacer (Photocleavable)	*	*	*
Spacer C3/9/C12/18	*	*	*
d Spacer	*	*	*
3' Spacer C3-methyl	*	*	*

OTHER

Modifications	5'	Internal	3'
Phosphorilation	•		•
Amino Modifier C6	•	•	•
Amino Modifier C12	•		
Amino Modifier C3/C7			•
Thiol Modifier	•		•
Thiol Modifier S-S	•		•
Biotin	•	•	
Biotin TEG			•
Biotin Photocleavable	•		
Uracil™	•	•	•
Inosine	•	•	•
2'-O-Methyl RNA	•	•	•
Phosphorothiation (S-Oligos)		•	
DIG	•	•	•
Azide	•	•	•
Alkyne	•	•	•
Aldehyde	•		
Nitroindole	•	•	•
Methyl dC	•	•	•
DBCO-TEG	•		
Hexynyl	•		
Acrydite	•		

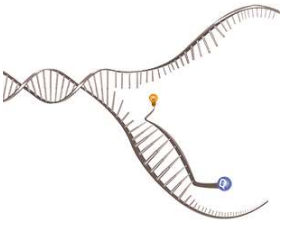
PCR Probes for REAL-TIME PCR

- Cost-effective alternatives for VIC, NED, PET and the MGB-moiety
- Wide variety of fluorophores - quencher combinations
- Order your primers and Real-time PCR probes premixed with the oligo dispensing service

Real-Time PCR probes
We have different kinds of Real-Time PCR probes. You can modify the DNA probe of your choice with our wide collection of quenchers and fluorescent dyes. Choose from a broad range of (alternative) modifications for all types of Real-Time PCR (qPCR) platforms.

Twice Dyed Probes

Twice dyed probes (TDP's) are Biolegio's version of dual-labeled probes. Our twice dyed probes are highly sensitive, dual-labeled DNA probes. We offer a wide variety of quenchers and fluorescent reporter dyes for all types of Real-Time PCR platforms.

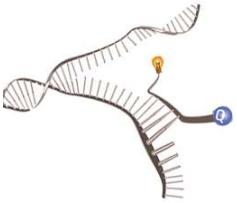


All Twice Dyed Probes include synthesis of maximum 40 nt, 5' modification, 3' quencher and HPLC purification.

- Wide variety quenchers and dyes available
- Cost-effective replacement for MGB probes: XS-probes
- Competitive priced alternative VIC, NED and PET dyes

XS-probes - MGB probe alternative

In addition to our twice dyed probes, Biolegio is offering the XS-probe. XS-probes mimic the features of the Minor Groove Binder (MGB probe) moiety: it gives greater stability to the hybridized probe. This raises its melting temperature. As a result, XS-probes can be effective at lengths shorter than traditional dual-labeled probes.

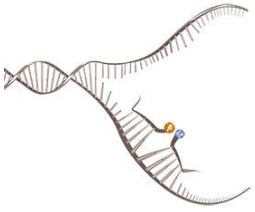


All Twice Dyed Probes include synthesis of maximum 40 nt, 5' modification, 3' quencher and HPLC purification.

- Cost-effective replacement for MGB probes
- Design shorter probes and increase your specificity
- Premixed assay delivery possible with the oligo dispensing service

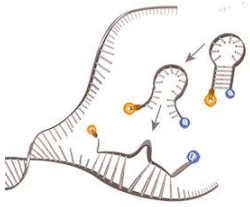
LightCycler FRET Probes

A LightCycler FRET probe system is a pair of single-stranded fluorescent-labeled oligonucleotides. They are sequence specific and highly sensitive.



Molecular beacons

Molecular beacons are highly sensitive, structured probes. They are used for sequence-specific detection in quantitative Real-Time PCR. Ideal for discriminating single nucleotide polymorphisms (SNPs).



Double Quenched Probes: 2Q-Probes

For optimal Quenching characteristics, use the 2Q-probes with an extra internally positioned Quencher to lower bacground and increase signal detection.

Synthesis scales and yield

Biolegio offers three different synthesis scales for your probes: 40 nmol, 200 nmol and 1000 nmol

Yeald for Real-Time Pcr Probes

Synthesis scale	yield
40 nmol probe	5-10 nmol
200 nmol probe	20-25 nmol
1000 nmol probe	50-60 nmol

Probes for Real-Time PCR

- Highly sensitive, dual-labelled fluorescent oligonucleotides.
- Specific probes for specific applications
- Wide variety of fluorophores-quencher combinations
- Alternative dyes available

NGS Oligo's

- **Dedicated and strictly monitored workflow for NGS oligo's**
- **Highly optimized cross contamination elimination State of the art LC-MS QC included in NGS-SP & NGS-P Services.**

Over the last decades NGS oligo technologies have evolved at a rapid pace, and concurrently so has the demand for high purity - high quality associated products like oligonucleotides. With the throughput of sequencing platforms increasing, multiplexing samples is now the common method for making sequencing increasingly economical. Even at very minimal amounts barcode cross contamination can be disastrous to a sequencing experiment. At Biolegio we dedicated ourselves to develop a workflow for NGS oligo's which has proven itself moreover for various NGS applications.

Biolegio NGS oligonucleotides are synthesized in an environment which is monitored by externally executed qPCR assays using swaps of the facility, hardware and technicians. Thus enables the detection of contaminating oligos and high risk areas.

Synthesis and Purification is performed on media used only once for every single oligonucleotide to rule out the possibility of cross contamination, therefore HPLC purification is not available for our NGS oligos. Based on the high coupling efficiency we reach due to our optimized synthesis protocols of 99,5% and higher, the purity of our NGS-S grade oligo's are fully compatible for most NGS applications.

At Biolegio we offer three NGS oligo services: NGS-S, NGS-SP and NGS-P. For all three categories we give the same attention to the synthesis workflow to exclude cross contamination on the highest level possible. You decide for the turnaround time, purity and quality control as well as documentation needs and importance of yields.

Within our NGS-SP & NGS-P service the purity and quality for every single NGS oligonucleotide is assessed using state of the art UPLC-MS to ensure purity and correct mass.

NGS Oligos Service Overview

	NGS-S (standard)	NGS-SP (Standard Plus)	NGS-P (Premium)
Quality	Highest synthesis Quality minimal reduced cross contamination	Highest Quality and Purity Maximal reduced cross contamination	Highest Quality and Purity maximal reduced cross contamination guarenteed yields up to 80 nts
Purification	Desalted	PAGE	PAGE
Purity	ca. 80%	ca. 95-99%	ca. 95-99%
Modification	Various modifications & all degenerated codes available for all NGS services		
QC	Standard	Standard + LCMS QC Report Optional	Standard + LCMS QC Report Included
Scales & Yields	Ca. Yields 40 nmol: 20-40 nmol 200 nmol: 40-100 nmol 1000 nmol: 150-300 nmol	Ca. Yields 40 nmol: 5-10 nmol 200 nmol: 20-40 nmol 1000 nmol: 50-100 nmol	Guaranteed yiealds 40 nmol: 10-20 nmol 200 nmol: 40-50 nmol 1000 nmol: 100-150 nmol
Shipment	After Order is complete	After Order is complete	Partial Shiment if necessary (e.g. resynthesis or modified shipped extra)
Documentation	Table format data sheet	Table format data sheet	Table format data sheet order overview LCMS Report per oligo (digital)
Shipping Within	20-30 NGS primes: 3 bisness days 30-60 NGS primes: 4 bisness days <100 NGS primers: 6 bisness days >100 NGS primers: on reuest	20-30 NGS primer: 6 bisness days 30-60 NGS primer: 8 bisness days <100 NGS primer: 12 bisness days >100 NGS primer: on request	20-30 NGS primer: 8 bisness days 30-60 NGS primer: 10 bisness days <100 NGS primer: 14 bisness days >100 NGS primer: on request

Molecular Inversion Probe (MIP)

- **Ideal as target enrichment technique for NGS application**
- **Extraordinary high specificity in multiplex reactions**
- **High capture reproducibility**
- **Bias reduction: no fragmentation and PCR needed**
- **Straight forward and easily automatable**

What is a Molecular Inversion Probe?

A Molecular Inversion Probe is a single stranded oligonucleotide containing two annealing arms complimentary to the target of interest with a sequence gap in between. This sequence gap can target a SNP or a larger region of interest. In between the annealing arms of the MIP binding sites. Universal primers are included and other functionalities like index sequences or digestion sites can be incorporated depending on the experimental setup.

Multiple advantages

The advances in DNA analysis made a great leap forward with the emergence of Next Generation Sequencing (NGS). With these advances different target enrichment techniques have been developed to select the regions of interest for NGS analysis in a sensitive and cost-effective way. Amongst these techniques a solution phase “capture by circularization” method using “Molecular Inversion Probes” (MIPs) has gained increasing interest. Extensively used for research in Single Nucleotide Polymorphisms (SNPs) and Copy Number Variation (CNV), now the MIPs have shown multiple advantages as a Genomic partitioning technique allowing
enrichment for regions of interest at a scale that is matched by Next Generation Sequencing platforms.

MIPs Key Features

Specifity	High specificity compared to other genome partitioning techniques	Biolegio offers high quality MIPs produced with robust and sublime coupling efficiency
Multiplexing	Due to the high specificity MIPs are ideal for multiplexing reactions	MIPs are produced in the NGS workflow where cross-contamination is eliminated
Reproducability	Multiple experimental repeats with a balanced pool of MIPs exhibits high reproducability	order Biolegio MIPs at any custom concentration to facilate your workflow
ibrary prep	No need for fragmentation or PCR reducing blas.	isolate your DNA, add the MIP pool and you are ready to go! Use our flexible dispense service to recieve your oligo’s in any concentration pooled combination and any tube/plate format to optimize and standarize your workflow
Esay of use	Straight forward and automatable-workflow without the need of specialized instrumentation	see above

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.

Initially discovered as an "adaptive immune system" in prokaryotes 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.

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)

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.

cr:tracrRNA

Optimized for SpCas9, the cr:tracrRNA Kit is designed for researchers conducting basic CRISPR genome editing experiments. The CRISPR kit contains all the necessary components to successfully anneal, transfect, target and edit a gene using CRISPR/Cas9 and synthetic guide RNA.

crRNA

You provide the 17-20nt RNA sequence that binds to the DNA 17-20nt target sequence that is opposite to the PAM sequence. An optimized 22mer Linker is added on the 3' end of your target sequence. For example, you will receive a crRNA with this format:

5'-AAUUUCACAGCUGCACAUA+Synthego Linker-3'

tracrRNA

We have optimized the 72mer tracrRNA sequence based on S. pyogenes, and provide this with a proprietary linker to duplex with the crRNA. You do not need to provide a sequence for the tracrRNA.

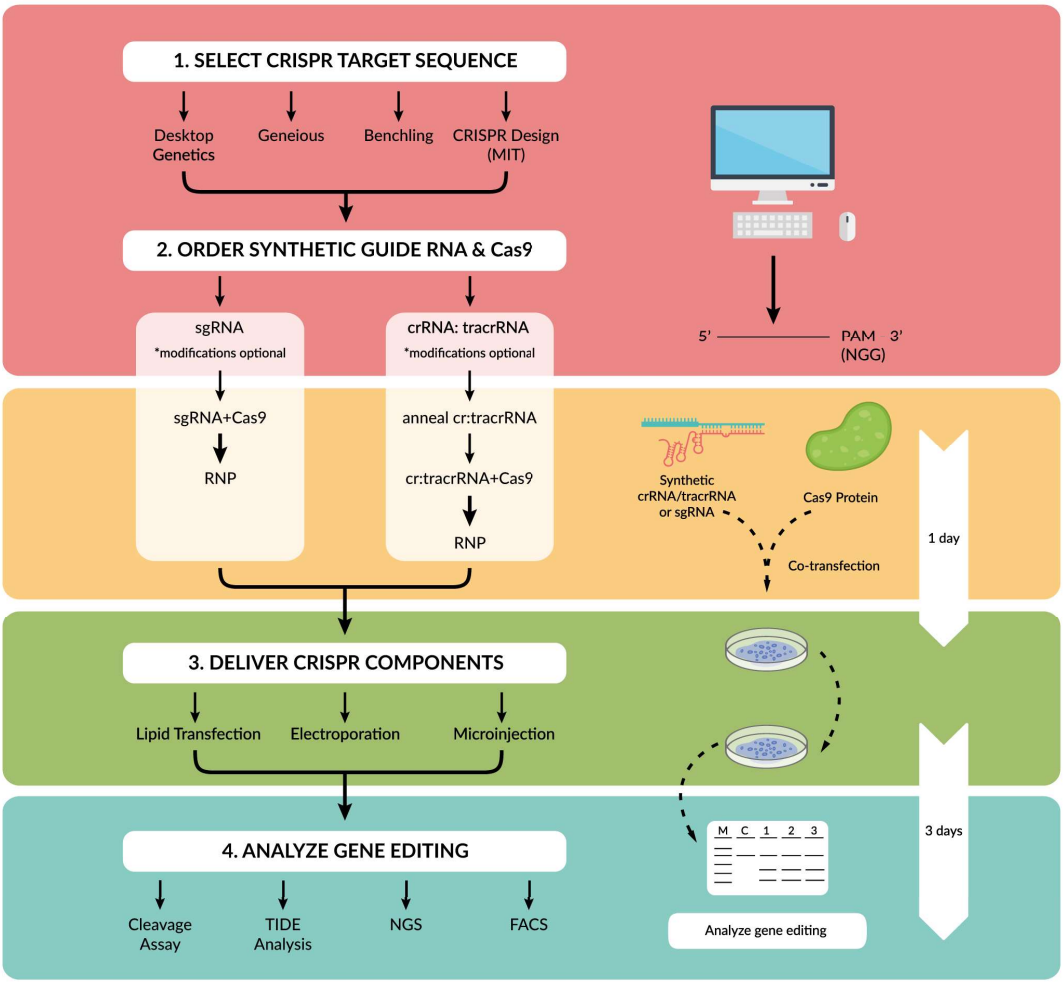
Synthetic sgRNA

Achieve up to 90% editing efficiency with the highest quality synthetic sgRNA in the market. Biolegio is the first company to deliver a production scale, full length 100-mer synthetic sgRNA product at a practical price and volume.
Unlike custom developed solutions that are expensive and can take weeks before transfection readiness, CRISPRevolution sgRNA ships up to 4X faster and 80% lower cost.

Benefits of Synthetic sgRNA

- No need to anneal crRNA:tracrRNA
  Higher efficiency and reduced lab time
- Better in vivo stability
  When duplexed with Cas9 nuclease
- Cost-effective
  Highly scalable for large numbers of experiments
- 100% DNA-free
  No risk of integrating foreign DNA into cell line
- A single oligonucleotide
  Ready for transfection out-of-the box
- Optimized for SpCas9
  Optimized 80mer scaffold for use with SpCas9

No need to anneal, the sgRNA format arrives ready for transfection. It uses a single strand of user-defined 17-20nt RNA target sequence that will bind to the DNA 17-20nt target sequence that is opposite to the PAM sequence.
An optimized 80mer Synthego Scaffold is added on the 3' end of your target sequence.



	Synthetic Guide RNA	Plasmid	IVT
Process	**sgRNA** 1. Choose target sequence 2. Order synthetic RNA **crRNA + tracrRNA** 1. Choose target sequence 2. Order Synthetic RNA 3. Anneal crRNA + tracrRNA	1. Choose target sequence 2. Design/order DNA primers 3. PCR insert 4. Ligate into plasmid 5. Transform into cells 6. Screen cells 7. Sequence verify plasmid 8. Purify plasmid DNA	1. Choose target sequence 2. Design/order DNA primers 3. Assemble guide by PCR 4. Perform IVT 5. Purify guide RNA
Time to Transfection	Ready for transfection	7-14 days	1-3 days
Transfection Labor Time	Minimal	Days of lab work	Full day of lab work
Off-target Effects	Lowest	Variable	Variable
Efficiency	Up to 90% efficiency	Variable	Variable
Consistency	Highest	Variable	Variable

Custom RNA

Custom RNA is tailored to how you want to do your research: Cpf1 (Cas12a), C2c2, S. aureus Cas9 or any novel nuclease. Or to alter the secondary structure of your tracrRNA.
Tell us the sequence you want and we'll make you the highest quality RNA – Synthego's Custom RNA is a fully custom target sequence guide RNA product for genome editing.

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.

- Your 76-100nt Sequence
- Material for 10-20 Transfections
- Quantity: 3 nmol by OD260
- Modified RNA Optional*
- Ships in 5-10 Business Days

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 5' linker to a crRNA.

- Your 51-75nt Sequence
- Material for 25-50 Transfections
- Quantity: 5 nmol by OD260
- Modified RNA Optional*
- Ships in 5-10 Business Days

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.

- Your 10-50nt Sequence
- Material for 25-50 Transfections
- Quantity: 5 nmol by OD260
- Modified RNA Optional*
- Ships in 5-10 Business Days

Custom RNA Specifications

- Custom RNA Specifications
- Product Type: Fully Synthetic RNA for CRISPR or other applications
- Purification: High-Quality Liquid Chromatography SPE
- Analysis Method: Electrospray Ionization Mass Spectrometry
- Shipping Format: Nuclease-free Tubes

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 commecialy 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.

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

Custom RNA Synthesis

RNA synthesis products have many uses, such as understanding the role of ribozymes (catalytic RNA) and cellular RNA as a target for antisense therapeutics. However the need for chemical RNA synthesis has become increasingly important since the advent of synthetic siRNA for use in siRNA-mediated RNA interference (RNAi), and the upcoming CRISPR applications.

- High quality custom synthetic RNA
- Long RNA Oligos possible

siRNA synthesis

Biolegio's siRNA is synthesized with high quality chemicals. Synthesis is performed under stringent computer controlled conditions. Internal control functions measure the base coupling efficiency and guarantee the siRNA oligo to be of the highest quality standard, as you can expect from all products of Biolegio.

siRNA-mediated RNA

Interference siRNA oligos are an easy and efficient way to achieve RNA interference (RNAi). RNAi is amechanism of gene silencing at the mRNA level. This phenomenon is triggered by small interfering (si)RNAs and micro (mi)RNAs. These RNAs are capable of inhibiting gene expression by eitherdirecting the degradation of homologous mRNA targets or inducing the repression of translation of mRNA targets, which have incomplete complementarity.

2'-OMe RNA synthesis

2'-O-Methyloligoribonucleotides are extremely useful reagents for a variety of molecular biology applications. The 2'-OMe RNA-RNA duplex is more thermally stable than the corresponding DNA-RNA one. This is not a substrate for RNase H. In addition, 2'-OMe-RNA is chemically more stable than either DNA or RNA and is resistant to degradation by RNA- or DNA-specific nucleases.
The enhanced RNase and DNase resistance, and the increased thermal stability of their duplexes and triplexes, have been examined in a number of ways. Applications range from the simple antigen type experiments to the correction of aberrant splicing. Researchers have also made use of biotinylated 2'-OMe RNA for the affinity selection of affinity depletion of ribonucleoprotein complexes, most notably in the field of RNA processing.

Synthesis scale and yield

Biolegio offers three different synthesis scales for your RNA oligos: 40 nmol, 200 nmol and 1000 nmol. The maximum length of a RNA oligo is 100 bases (largely sequence dependent).

APPROXIMATE YIELD TO SYNTHESIS SCALE (HPLC PURIFIED)

Synthesis scale	Approx. yield
40 nmol RNA/siRNA oligo	5-10 nmol
200 nmol RNA/siRNA oligo	25 nmol
1000 nmol RNA/siRNA oligo	60 nmol

ORDERING

You can order our custom synthesized product by sending a completed order form webshop at www.teknokorma.es

Webshop

Order your oligos quick and easy in our webshop. With your own account you can directly see the prices of oligos. Have direct access to your order history. All this is packaged in an easy-to-use on-line interface. We can integrate your in-house ordering system with our webshop by using a "punch-out" protocol.

Shipping and handling

Shipping and handling details are largely dependent on the shipping location. Please contact us for more information about shipping and handling.

Delivery schedule

> Standard oligos with maximum length < 100 bases are shipped within 48 hours, order before 5:00 PM GMT.
> Purified & modified oligos are shipped within 5 business days.
> Custom DNA probes are shipped within 10 business days.
> RNA oligos are shipped within 12 business days.