PRODUCTS AND SERVICES GUIDE 2017-2018

CUSTOM OLIGONUCLEOTIDE SYNTHESIS

WE ♥ YOUR DNA

Biolegio

BIOLEGIO HAS OVER 20 YEARS OF **EXPERIENCE** WITH THE SYNTHESIS OF **OLIGOS**

Biolegio was founded in 1996 in Nijmegen, the Netherlands. The first production facility was located on the campus of the Radboud University in Nijmegen. After several years of growth the company needed more space to keep up with demands and moved to a new building. This new building, still located in Nijmegen, offered the space we needed to expand our endeavours. The product range has broadened from custom made oligonucleotides to highly modified oligonucleotides, fitted with every commercially available modification. Making them suitable for a wide range of applications including (Next Generation) Sequencing, PCR, Real-Time PCR, SNP detection, genotyping, gene expression and mutation detection.

Biolegio now has over 20 years of experience with the synthesis of oligos and has satisfied customers located all over the world. They use our products on a daily basis and benefit from our specialist knowledge within the field.

Our core values

Key element in satisfying the needs and wants of our customers are our core values as described below. By operating according to these values we do everything we can to maximize customer satisfaction.

Boundless Innovation

Optimized synthesis protocols, adopting new techniques and instruments result in key benefits: next day delivery, low pricing, consistent high-quality and oligo's up to 300 bases.

Approachable Flexibility

We take 'Custom Oligo Synthesis' to the next level. With our dispensing & packaging service you can have your oligo delivered in any format, quantity, concentration or combination.

Structured Efficiency

Biolegio's manufacturing facility is ISO 9001:2008 and ISO 13485:2012 certified. Reflecting strong product quality and manufacturing procedures and ongoing improvement in those areas.

Proven Experience

Over 20 years of experience, gives us the know-how and expertize that is needed to meet and exceed your expectations.

Trustable Convenience

A fully automated Laboratory Management System enables us to work faster and more efficient. Moreover, it gives you a user-friendly tool to order easy.

Successful Partnership

It is our pleasure to deliver the dedication and commitment that you expect from a successful partnership.

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WE ♥ YOUR DNA

OLIGONUCLEOTIDE SYNTHESIS FACILITY

- ✓ Stringent quality control system ensures high quality oligonucleotides
- ✓ In-house built equipment with latest advanced techniques, designed with over 20 years of expertise.
- ✓ Minimal error due to fully automated production process
- ✓ ISO 9001:2008 and ISO 13485:2012 certified quality management system

Chemistry

Biolegio uses optimized phosphoramidite chemistry and reagents of the highest quality. Order handling and oligo 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 of additional purification for most basic molecular biology applications, such as PCR, sequencing, hybridization studies and antisense studies. Additional purification by HPLC, PAGE or cartridge is available for more sensitive applications.

Backbones

We offer 4 distinct types of bases as backbones together with two different types of linkages.

Bases

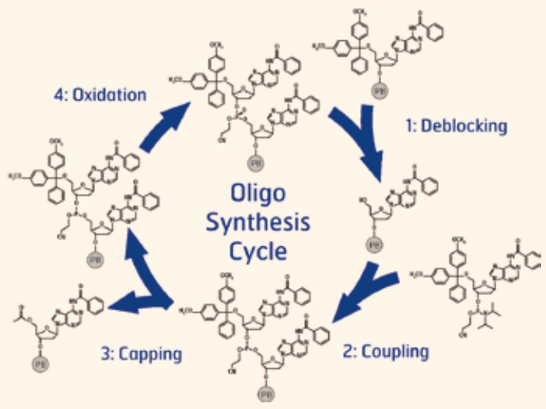
- ✓ DNA oligos
- ✓ RNA oligos
- ✓ LNA oligos
- ✓ 2'O-Me oligos

Linkages

Phosphodiester bonds, which is our default linkage is the linkage between the 3' carbon atom of one sugar molecule and the 5' carbon atom of another, deoxyribose in DNA and ribose in RNA. Phosphorothioate bonds substitute a sulphur atom for a non-bridging oxygen in the phosphate backbone of an oligo, making the oligo resistant to nuclease degradation.

Figure 1 | The oligonucleotide synthesis cycle

Oligonucleotide synthesis is carried out by a stepwise addition of nucleotide residues to the 5'- terminus of the growing chain until the desired sequence is assembled. Each addition is referred to as a synthetic cycle (figure 1) and consists of four chemical reactions: deblocking, coupling, capping and oxidation. At the end of the synthesis the oligo is released from the solid support and is eluted from the column or well. As solid support we use polystyrene beads (PB).



Quality control

The reactions involved in coupling the nucleotides to form an oligonucleotide are not 100% effective. This is inherent to these reactions and causes failure sequences which, for some applications, could make a purification necessary. Biolegio only uses high quality chemicals and robust optimized protocols to ensure the best possible coupling efficiency.

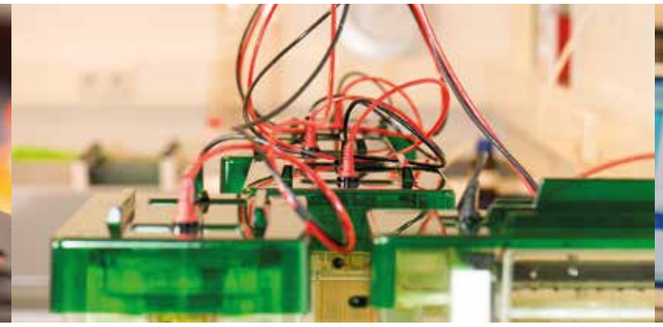
A stringent quality control system ensures that you can expect the quality of our oligonucleotides to be of the highest standard. During the fully automated oligo synthesis, all steps of the synthesis are monitored by multiple control functions on our DNA synthesizers. Synthesis is followed by further quality controls to guarantee the quality of the oligos. All oligos are routinely analysed by optical density (OD) measurement.

DNA OLIGONUCLEOTIDE SYNTHESIS FROM **2 TO 300 BASES**

In addition, oligos are randomly analysed by gel electrophoresis and Liquid Chromatography Mass Spectrometry (LCMS). If an oligo does not meet our requirement it's resynthesized. The result of our stringent QC process is a product of the highest standard. All oligos are delivered with our digital product specification sheet, which includes: % GC content, yield in nmol and µmicrogram, OD, melting temperature and molecular weight (MW).

CUSTOM DNA SYNTHESIS

- ✓ Your choice of tubes, plate or strips for most optimal flexibility in your laboratory
- ✓ Additional HPLC, PAGE or Cartridge purification available
- ✓ DNA oligonucleotide synthesis from 2 to 300 bases



Custom DNA synthesis in tubes

Oligos can be shipped dry or dissolved in a requested solution. They are usually packed in individual labelled micro centrifuge tubes.

Custom DNA synthesis in plates

For medium or high throughput applications Biolegio offers the possibility for the delivery of oligonucleotides in 96 wells plates. Biolegio offers different kind of 96 wells formats e.g. the deep well plate and the PCR plate. Delivery in cryotubes is also possible. To order your oligos in a 96 well format you can use our excel order form, which can be downloaded from our website. In the excel sheet (order form) please select the tab containing the format of your choice. Synthesis in plates starts from 48 oligos. Ordinarily, all oligos in PCR plate format are synthesized on a 10 or 40 nmol scale and are delivered in max. 100 µl water/buffer with a standard working concentration of 100 pmol/µl (= 10 nmol shipped). For larger volumes deep well plates or cryotubes are used.

Custom DNA synthesis in various formats and quantities

Other formats and working concentrations are available on request. Have a look at our oligo dispensing service (page 13). With this service you can order your primers and/or probes premixed, in any format and quantity that suits your workflow, for instance in 2D barcoded tubes.

Synthesis Scales

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.

Maximum DNA oligo length in relation to synthesis scale	
Synthesis scale	**Max. oligo length**
10 nmol standard oligo	40 bases
40 nmol standard oligo	150 bases
200 nmol standard oligo	300 bases
1000 nmol standard oligo	300 bases

(Oligos longer than 80-bases are synthesized with our B-pure protocol)

Guaranteed yield for non-labelled, non purified oligos up to 40 bases	
Synthesis scale	**Min. oligo yield**
10 nmol standard oligo	10 nmol
40 nmol standard oligo	20 nmol
200 nmol standard oligo	95 nmol
1000 nmol standard oligo	400 nmol

Delivery Schedule

- Custom oligos with maximum length 100 bases are shipped within 24 hours if ordered before 5:00 PM GMT.
- Purified & modified oligos are shipped within 3-4 business days.
- We also offer a 24 hour delivery service.

For more information about ordering and shipping, please go to page 19.

Purifications

Biolegio offers three options for purifying your oligo: HPLC purification, PAGE purification and Reverse-Phase Cartridge purification. Which kind of purification is selected largely depends on your demands concerning purity and yield of the end product. You must consider that purification results in less yield of the final product. PAGE purification, although resulting in a higher purity, generally gives lower yields than the HPLC purification. The kind of purification which is chosen also depends on the length of an oligo. For oligos with a length over 50 bases we recommend PAGE purification to guarantee purity of the final product.

Reverse-Phase cartridge purifications

Purification with Reverse-Phase cartridge offers the lowest level of purity (typically 80%). The basis of the separation is the difference in hydrophobicity between full length product (which contains a 5'-DMT) and truncated sequences (without DMT groups). Because the differences in hydrophobicity between the full length DMT product and non-DMT truncated sequences are reduced as the oligo length is increased, cartridge purification is not recommended for oligos > 50 bases.

STRINGENT CONDITIONS TO **PREVENT** CONTAMINATION DURING **PURIFICATION**

HPLC Reverse-phase purification

Reverse-phase HPLC operates on the same principle as the reverse-phase cartridges, but typical yields a product of 90% purity.
The capacity and resolving properties of HPLC columns are also much greater than cartridge devices. Therefore, HPLC is the method of choice for purifying larger quantities of oligos (i.e. >= 1 µmol). As with cartridges, reverse-phase HPLC is usually not recommended for purifying oligos longer than 50 bases.

PAGE Purification

With the excellent resolution of PAGE a 95-99% purity can be achieved. The basis of the separation is charge and molecular weight.
In most cases, a full length (n) oligo can be separated from oligos only one base shorter (n-1). PAGE is the recommended technique for purifying oligos over 50 bases long. Yields from PAGE are lower than from other methods due to the relative inefficient extraction of oligos from the gel.

Unmatched synthesis length: UP TO 300 BASES!

Long oligo synthesis

Our B-Pure oligonucleotide synthesis protocol enables custom synthesis of affordable, high-quality oligonucleotides up to 300 bases. Oligonucleotides longer than 80 bases are synthesized automatically on this protocol. The B-Pure oligonucleotide synthesis protocol results in oligos with a length not achieved by any other company.

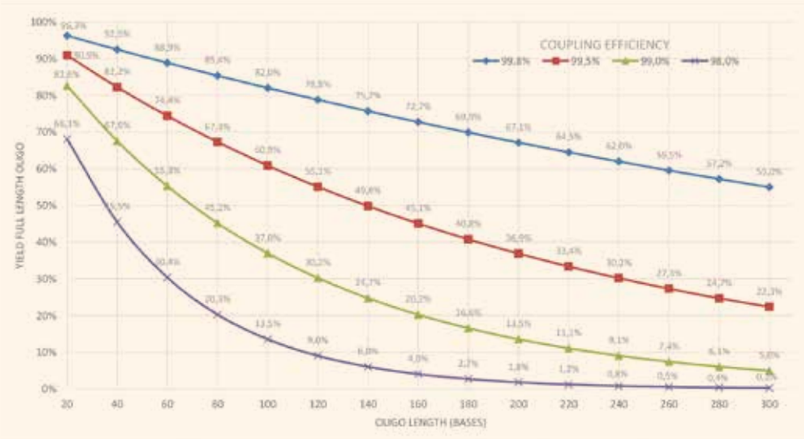
Coupling efficiency

By using the B-Pure synthesis protocol we achieve a coupling efficiency up to 99.9% through an entire 300 base synthesis reaction. A coupling efficiency of 99% or 98% seems to be very good, but on closer examination the full-length yield is only half for a 40 mer! For a 300 mer this will result in a crude yield of less than 5%, which is not acceptable. As demonstrated in figure 2, the only way to synthesize longmers up to 300 bases with an appreciable yield of full length material (>30%) is to have a coupling efficiency of more than 99.5%.

Purification

With our B-Pure protocol we can reach a maximum yield of full length products as you can see in figure 2. However, due to increasing length, the total yield decreases. Furthermore, the likelihood of building loops, hairpins or other secondary structures during synthesis increases. This leads to a higher presence of deletions in the oligonucleotide sequence, which makes a PAGE purification strongly recommended for long oligos. With PAGE purification you can reach purities between 97% to 99.9% depending on oligo sequence and structure.

Figure 2 | Yield of full length product, depending on coupling efficiency and oligo length



MODIFIED OLIGO SYNTHESIS

- ✓ Over 300 modifications available
- ✓ Available for different backbones (DNA, RNA, 2'-O-Me, LNA)
- ✓ Difficult/special designs, e.g. long hairpin oligo structures with 2 or 3 modifications

Overview

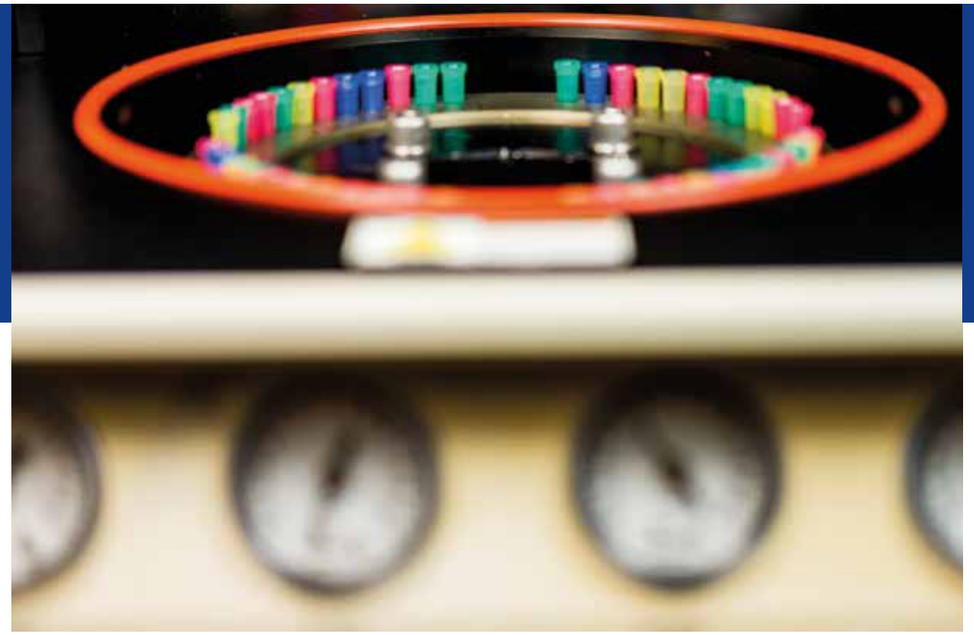
Oligos can be modified in several different ways by utilizing the active groups of the nucleotide or creating nucleotide analogues. We offer a wide range of modifications with new ones being added constantly.

FLUOROPHORES			
Modifications	**5'**	**Internal**	**3'**
6-FAM™	•		•
HEX™	•		•
TET™	•		•
6-TAMRA™	•		•
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 1 dT		•	
Black Hole Quencher 2 dT		•	
Black Berry Quencher 650			•
Deep Dark Quencher 1			•
Deep Dark Quencher 2			•
Dabcyl™			•
TAMRA™	•		•

More modifications are available, please contact us for additional information.

More modifications on next page



SPACERS			
Modifications	**5′**	**Internal**	**3′**
PC Spacer (Photocleavable)	•	•	•
Spacer C3/9/C12/18	•	•	•
d Spacer	•	•	•

OTHER			
Modifications	**5′**	**Internal**	**3′**
Phosphorylation	•		•
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	•	•	•
Phosphorothioation (S-oligos)		•	
DIG	•	•	•
Azide	•	•	•
Alkyne	•	•	•
Aldehyde	•		
Nitroindole	•	•	•
Methyl dC	•	•	•
DBCO-TEG	•		
Hexynyl	•		
Acrydite	•		

OVER **300 MODIFICATIONS** AVAILABLE!

Please contact us for additional information.

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

Twice Dyed Probes

Twice Dyed Probes (TDPs) are highly sensitive, dual-labelled fluorescent oligos that are designed to be sequence specific. They can be modified with a wide variety of fluorophores. TDPs can be used with quantitative Real-Time PCR instruments and multiplex analysis systems.

XS-probes

In addition to our twice dyed probes, Biolegio is offering the XS-probe. XS-probes have almost the same benefits as the Minor Groove Binder (MGB) moiety: it gives greater stability to the hybridized probe, by raising its melting temperature. As a result, XS-probes can be effective at lengths shorter than traditional dual-labelled probes. XS-probes can be labelled with the same modifications as dual-labelled probes.

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).

LightCycler FRET Probes

A LightCycler FRET probe system is a pair of single-stranded fluorescently-labelled oligonucleotides (fluorescent acceptor probe and a fluorescent donor probe). They are sequence specific and highly sensitive.

Double Quenched probes: 2Q-Probes

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

Synthesis scales and yield

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

YIELD 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

* Yield depends on sequence and label selection.

OLIGOS DISPENSING SERVICE

- ✓ No more cumbersome pipetting of primers and probes
- ✓ Your assay design - our synthesis and processing expertise
- ✓ All fluorophore - quencher combinations possible for Real-Time PCR purposes
- ✓ Reduce your batch-to-batch variation
- ✓ Minimize handling errors associated with manual reaction set-up
- ✓ Dried down, or in solution

Order your oligos premixed according to your specifications. The oligos may contain a variety of modifications. Ideal for simplifying your in-house (Real-Time) PCR assays and increasing your efficiency. Available in any format and quantity that suits your workflow: i.e. tubes, strips, 96/384 wells plates, (2D) bar-coded cryotubes or snap-in tubes for the BD-MAX platform*. All fluorophore - quencher combinations are possible.

Order your oligos premixed according to your specifications.

*More info about BD-MAX™ assays on page 14 .

AMBeR SEQUENCING PRIMERS

- ✓ Advanced Mobility
- ✓ Better Resolution in the small fragments

Overview

Optimize the results of your DNA sequencing reactions. Biolegio, in cooperation with NimaGen, now offers AMBeR Sequencing Primers. Use these modified sequencing primers to improve resolution and mobility of the first 50 bases in your sequencing chromatograms (figure 3 and 4).

Applications

AMBeR Sequencing Primers can be used for all different Sanger sequencing applications. Especially when it is critical to read directly after the primer.

Platforms

310, 3130, 3130XL, 3500, 3500XL, 3730 & 3730XL Genetic Analyzers.

Figure 3 | First bases of a sequencing chromatogram with a regular primer (Run with POP-7)

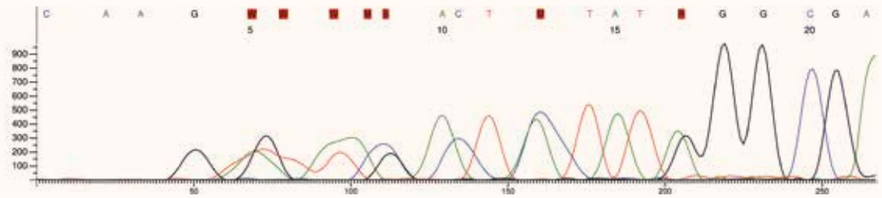
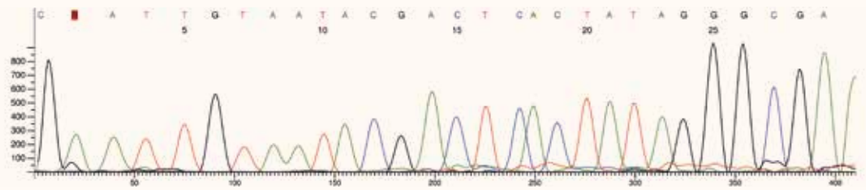


Figure 4 | First bases of a sequencing chromatogram with an AMBeR primer (Run with POP-7)



READYMAX™ ASSAYS

- ✓ Use your own in-house developed assays as ready to use snap-in tubes
- ✓ No more cumbersome pipetting of primers and probes
- ✓ Reduce your batch-to-batch variation
- ✓ Minimize handling errors associated with manual reaction set-up
- ✓ Dried down, stable at room temperature
- ✓ Broad and growing selection of commercially available ReadyMax™ assays

YOUR ASSAY delivered as ready to use snap-in tube for direct use on BD MAX™ platforms

Introduction

ReadyMax™ Assays are custom synthesized assays for use on BD MAX™ platforms. The primers and probe(s) mixture of your own Real-Time PCR assays is synthesized according to your specifications. The oligonucleotides are dried down and delivered in a sealed conical tube, which is compatible with the BD MAX™ cartridges. No more cumbersome pipetting of primers and probes. With ReadyMax™ assays you just snap in your assay and start your run, making it possible to increase your laboratory throughput and efficiency.

Quality control

Our stringent quality control system ensures that you will get oligonucleotides of the highest standard. All oligos are HPLC purified and LCMS checked. After synthesis a small quantity of the assay will be sent for in-house testing, enabling you to verify the performance.

> "The ease of use of the BD MAX™ system makes assays applicable in a wide range of microbiological laboratories. The primers and probes for our assays are manufactured and marketed in ready to use snap-in tubes. Resulting in a more efficient workflow, which means the quality is further improved."
>
> Customer feedback

B-CAP ASSAY | Targets:
- Legionella pneumophila
- Mycroplasma pneumoniae
- Chlamydophila pneumoniae
- Chlamydophila psittaci

MTB ASSAY | Targets:
- Mycobacterium Tuberculosis (MTB)

HSV/VZV ASSAY | Targets:
- Herpes simplex virus types 1 & 2
- Varicella zoster-virus

AP-1 ASSAY | Targets:
- Legionella pneumophila
- Toxoplasma gondii
- Pneumocystis jirovecii

AP-2 ASSAY | Targets:
- Mycoplasma pneumoniae
- Chlamydia pneumoniae
- PAN-Chlamydiaceae

NP-1 ASSAY | Targets:
- Mycoplasma hominis
- Ureaplasma parvum
- Ureaplasma urealyticum

NP-2 ASSAY | Targets:
- Mycoplasma hominis
- Ureaplasma parvum
- Ureaplasma urealyticum
- Chlamydia trachomatis

MP-1 ASSAY | Targets:
- E. coli
- Streptococcus agalactiae
- Listeria monocytogenes

MP-2 ASSAY | Targets:
- Neisseria meningitidis
- Streptococcus pneumoniae

BB ASSAY | Targets:
- B. burgdorferi sensu lato complex (ospA- and p41 Flagellin genes)

EP-1 ASSAY | Targets:
- EHEC stx1
- EHEC stx 2
- EHEC eaeA
- EHEC hlya

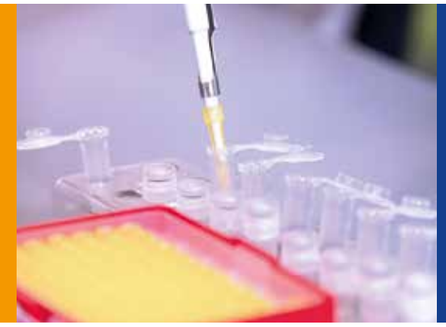
EP-2 ASSAY | Targets:
- EAEC
- aggR
- EPEC – eae and EAF
- EIEC – ipaH
- ETEC - LT

CARBA-1 ASSAY | Targets:
- KPC
- OXA48
- IMP 8-13 group ($bla_{IMP-2,-8,-13,-15,-19,-20,-24,-33,-37}$)
- GES

CARBA-2 ASSAY | Targets:
- NDM
- IMP 14-18 group ($bla_{IMP-14,-18}$)
- VIM ($bla_{VIM-1bis-34}$)
- GIM

CUSTOM RNA SYNTHESIS

- ✓ High quality custom synthetic RNA
- ✓ Long RNA Oligos possible



Overview

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.

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 a mechanism 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 either directing 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

Purification

We strongly recommend purification for any RNA oligo longer than 40 bases. RNA oligos can be purified by PAGE and HPLC purification.

CRISPR RNA SYNTHESIS

Higly pure synthetic RNA for:

- ✓ **Less off-target effects**
- ✓ **Improved in vivo stability (through RNP formation or RNA modification)**
- ✓ **Improved editing effeciency**
- ✓ **No unwanted genomic foreign DNA integration**
- ✓ **Less toxicity**
- ✓ **Less hands on time (e.g. no cloning, reverse transcription)**

Our offer

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 fast evolving research around CRISPR systems are described and harnessed for genome editing in a rapid pace. Whether you prefer to use CPF1, C2C2 or other systems, the Custom RNA products will allow you to use high quality RNA for your research.

HDR repair knock-in sequences

Our unrivalled expertise in synthesis of long DNA / RNA constructs give rise a multitude of advantages for CRISPR applications like knock-in constructs containing barcodes or multiple cutting sites. Use our oligonucleotides up to 300 bases as knock-in sequences for Homology Directed Repair applications.

Please visit our website for a complete and up to date overview of all available CRISPR RNA Synthesis.

THE WIDE RANGE HIGH QUALITY SYNTHETIC OLIGONECLEOTIDES FOR CRISPR APPLICATION INCLUDE:

Product	Quantity	Description
cr:tracrRNA	5 nmol each	A 17-20nts variable RNA sequence (crRNA) complementary to the target site followed by the required *S. Pyogenes* sequence that interact with the tracrRNA. The tracrRNA, or scaffold sequence, is a long RNA that is optimized for *S. Pyogenes* Cas9 nuclease. Annealing required before RNP formation or transfection. sgRNA & cr:tracrRNA Kit includes both: nuclease free water, TE Buffer.
sgRNA	3 nmol	A single RNA chimera of cr:tracrRNA. Full length functional RNA for optimal editing efficiency. No annealing required before RNP formation or transfection. sgRNA & cr:tracrRNA Kit includes both: nuclease free water, TE Buffer
Custom RNA 50	5 nmol	A 10-50 nts custom RNA oligo, perfect for designing guide RNAs that don't require long sequences like CPF1 or for custom crRNA to be anealed with an alternative tracrRNA.
Custom RNA 75	5 nmol	A 51-75 nts custom RNA oligo, perfect for designing guide RNAs that don't require long sequences like CPF1 or for custom tracrRNA sequences.
Custom RNA100	3 nmol	76-100 nts custom RNA oligo, prefect for designing full length sgRNA for CRISPR at a practical scale and price point. You can design your entire sgRNA sequence from 76-100nts.

* This product will be delivered with annealing buffer and nuclease free water.
All RNA sequences can be synthesized with 2'-O-methyl and phosphorotioate internucleotide linkages at the first three 5' and 3' residues for improved exo-nuclease resistance.

NGS OLIGONUCLEOTIDES

- ✓ **NGS oligonucleotides are synthesized in an environment which is monitored by externally executed qPCR assays using swaps of the facility, hardware and technicians.**
- ✓ **Purification is performed on media used only once for one single oligonucleotide to rule out the possibility of cross contamination.**
- ✓ **The purity and quality of every Standard Plus (SP) or Premium NGS oligonucleotide is assessed using state of the art UPLC-MS to ensure purity and correct mass.**

Unmatched Purity and Quality

At Biolegio our NGS oligonucleotides production is subjected to a dedicated workflow.

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, wasting both money and hours of work. As with so many molecular biology techniques oligonucleotides are at the heart of this application and choosing the right oligo supplier can make the difference to the quality of your results.

Biolegio offers three NGS oligonuleotides services, NGS-S, NGS-SP and NGS-P all following the same dedicated workflow to minimize cross contamination but different purities and yields. Our NGS oligonucleotides are compatible with Ilumina, Ion Torrent™ or Pacbio platforms.

BIOLEGIO NGS OLIGOS OVERVIEW

	NGS-S (Standard)	NGS-SP (Standard Plus)	NGS-P (Premium)
Quality	Highest synthesis Quality Maximal reduced cross contamination	Highest Quality and Purity Maximal reduced cross contamination	Highest Quality and Purity Maximal reduced cross contamination Guaranteed yields up to 80 nts
Purifica-tion	Desalted	PAGE	PAGE
QC	Standard	Standard + LCMS QC-Report optional	Standard + LCMS QC-Report included
Scales and yields	Ca. yields 40 nmol: 10-20 nmol 200 nmol: 40- 80 nmol 1000 nmol: 100 -200 nmol	Ca. yields 40 nmol: 5-10 nmol 200 nmol: 20-40 nmol 1000 nmol: 50-100 nmol	Guaranteed yields* 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 shipment if necessary (e.g. resynthesis or modified shipped extra)
Documen-tation	Table format data sheet	Table format data sheet	Table format data sheet Order overview LCMS report per oligo (digital)
Shipping within:	20-30 NGS primer: 3 business days 30-60 NGS primer: 4 business days ≤ 100 NGS primer: 6 business days > 100 NGS primer: on request	20-30 NGS primer: 6 business days 30-60 NGS primer: 8 business days ≤ 100 NGS primer: 12 business days > 100 NGS primer: on request	20-30 NGS primer: 8 business days 30-60 NGS primer: 10 business days ≤ 100 NGS primer: 14 business days > 100 NGS primer: on request

* for oligos up to 80 nts without modification. For modified oligos, we take care to ship highest yields expectable

MIPS: MOLECULAR INVERSION PROBES

✓ High Quality Target Enrichment Solution

An Introduction to MIPs

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 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. At Biolegio we have closely collaborated with customers to develop, optimize and validate our target enrichment solutions to introduce MIP assays of the highest quality and performance.

What is a MIP?

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.

The MIP technology has been combined with the "single molecule tagging" approach resulting in "Single Molecule Molecular Inversion Probes" or "smMIPs". These probes incorporate a stretch of random degenerate nucleotides creating probes with unique molecular tags enabling detection of low-frequency and sub clonal genetic variation. This resulted in an ultra-sensitive targeted sequencing method exhibiting the specificity and multiplexing advantage of the MIPs and the quantitation ability of the "single molecule tagging" approach.

These advantages combined with the practical workflow, flexible probe sequence/quantity adjustment and low per-sample costs, give science a valuable tool for future research.

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 facilitate your workflow.
Library prep	No need for fragmentation or PCR reducing bias.	Isolate your DNA, add the MIP pool and you are ready to go! Use our flexible dispense service to receive your oligo's in any concentration, pooled combination and any tube/plate format to optimize and standardize your workflow.
Easy of use	Straight forward and automatable-workflow without the need of specialized instrumentation.	See above.

ORDERING & SHIPPING

✓ Easy online ordering in our webshop
✓ Integrate your in-house order system with our webshop
✓ Eliminate possible delays with our oligo prepaid service

Ordering

You can order our custom synthesized product by sending a completed order form to info@biolegio.com or order in our webshop at www.biolegio.com.

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 24 hours, order before 5:00 PM GMT.
- Purified & modified oligos are shipped within 3-4 business days.
- Custom DNA probes are shipped within 5-7 business days.
- RNA oligos are shipped within 5-10 business days.
- We offer a 24 hours delivery service for custom oligo synthesis.

Next day delivery service

- Minimum of 10 oligos
- Maximum length of 25 bases
- 40 nmol scale
- Dissolved delivery
- Unpurified & unlabelled oligos
- Order before 1:00 PM GMT

Oligo prepaid service

With our oligo prepaid service we make oligo ordering easy. Credit can be easily transferred to your account. Oligos can then be ordered immediately, eliminating possible delays due to purchase authorization. Please contact Biolegio for more information.

Order quick and easy: www.biolegio.com

Contact details

Postal Address Biolegio BV
P.O. Box 91
6500 AB Nijmegen (the Netherlands)

Visiting address & contact details
Lagelandseweg 56
6545 CG Nijmegen (the Netherlands)

Phone: +31 (0)24 358 68 85
Fax: +31 (0)24 358 02 59
Email: info@biolegio.com
Website: www.biolegio.com

Please contact us for your local account manager or distributor.

Biolegio

WE ♥ YOUR DNA