

A REVOLUTION IN SEQUENCING: NGS OLIGOS

THE NEW ERA OF GENOME ANALYSIS

An introduction to Next-Generation Sequencing Technology

In the beginning of the 21st century Next Generation Sequencing (NGS) became a commercially available and increasingly used technique. In little over a decade the enormous quantities of data produced resulted in huge advances in the field of molecular biology. From the first sequenced tRNA molecule in 1964 to the ability to sequence a human genome in a day; technologies have evolved at a rapid pace, and concurrently so has the demand for high purity - high quality associated products like oligonucleotides.



The multiplexing ability of NGS using DNA barcodes in the library preparation gives researchers a valuable tool to sequence a multitude of samples in one run. Individual samples are barcoded with unique oligo adapters through ligation or PCR and pooled to be sequenced in one NGS run. Afterwards researchers can distinguish between the different fragments by identifying the used adapters. This valuable and highly potent workflow can easily be blurred or devaluated by cross contamination of the barcode oligo's leading to misinterpretation of sequenced fragments.

The oligonucleotide synthesis and purification process is critical in reducing NGS process bias or devaluation of results through barcode cross contamination. The production laboratory should be free of contaminating aerosols and thoroughly cleaned. Since oligo's are typically produced in batches containing 96 - 384 oligo's per batch, the hardware used for production and purification should be well maintained and free of oligonucleotide contamination. Also one of the main risks for barcode cross contamination is that the purification media (e.g. HPLC columns, RP-cartridges) are often used for multiple batches. Even stringent washing and regeneration of these media will not be sufficient to control cross contamination.

Unmatched Purity and Quality

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

- ▶ 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 for every single NGS-SP or NGS-P oligonucleotide is assessed using state of the art UPLC-MS to ensure purity and correct mass.

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.

Based on our customers' needs we established three categories of NGS oligo services.

NGS-S (Standard)

NGS-S is the fastest and most cost effective way to receive primers with lowest cross contamination possible (<0.1%). NGS-S service follows the same strict synthesis workflow as all NGS oligos to eliminate mix-up of samples identities by barcode cross contamination. NGS-S oligos are desalted to prevent any salt interference of your experiment. Typically purity for a desalted oligo is 80%.

NGS-SP (Standard Plus)

NGS-SP provides highest quality and purity for sensitive, difficult, crucial and multiplex applications, e.g. high multiplexing PCRs. Additionally to the dedicated synthesis workflow (to minimize cross contamination) your NGS primers are stringed PAGE purified to remove impurities like n-1 up to 99 %. To consider purity and quality every oligonucleotide is assessed by UPLC-MS and monitored by our experienced lab workers.

NGS-P (Premium)

You need highest quality, purity & safety for your critical, sensitive and /or multiplex PCR - but also reliable oligo amounts convenient to your project or to minimize validation purposes? With our NGS-P service you receive your NGS primer naturally synthesized to the dedicated workflow, highly stringed PAGE purified, UPLC-MS analyzed, including the full QC report for every oligo and not to forget with guaranteed yields.

Easy ordering of your NGS oligo service, just copy your sequence & oligo name to our web-shop screen or into our excel order template, choose scale and your NGS "method". You need additional modifications? Just choose your modification from our drop down menu.

Please find more information in the table below and feel free to contact us.

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
Purification	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)
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 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



Custom degeneracy ratios
and modifications available
please contact us for
additional information:
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