Biolegio

BUILDING YOUR BASES 30 YEARS OF DISCOVERY

LegiSeq™ NGS

Excellence In Every Read

Illumina® NGS Adapters & Indexing Primers

- ✓ Ready-to-use Unique Dual Index (UDI) Adapters & Primers
- ✓ Pre-configured sets for ligation (TruSeq™) & tagmentation (Nextera™) library prep
- ✓ Produced under optimized NGS conditions to minimize index hopping
- ✓ High-quality oligos: efficient ligation,low adapter-dimer formation
- ✓ Each batch NGS QC-checked (Ultra-low contamination)
- ✓ Compatible with all Illumina® platforms
- ✓ Indexes designed with edit distance ≥3,no duplicates with other suppliers

Manufactured under ISO9001:2015 & ISO13485:2016 standards

**Building
the Bases
of Discovery**

NGS Oligo Synthesis Re-Designed

Amongst the most powerful tools in Genomics science, Next Generation Sequencing (NGS) can sequence millions of DNA fragments simultaneously and provide comprehensive insights into genomic and genetic structure, variations, function and behaviour-furthering understanding and leading to it's increased use as a research and diagnostic tool.

Continuous advancements have delivered greater sequencing depth, accelerated more accurate sequencing, enhanced data interpretation and improved cost efficiencies.

However, NGS remains a method with significant cost investment with NGS Oligos being a contributor and one that may have an important impact on the success or failure and value of both the overall investment and data derived from your sample.

At Biolegio we carried out an extensive and comprehensive review of our entire NGS Oligo synthesis pipeline. With production and Quality Control procedures re-evaluated from the ground-up specifically for NGS, every aspect of production has been optimised.

Utilising custom built proprietary synthesis platforms and carefully designed handling protocols for post synthesis processing, dispensing and annealing, we ensure that every aspect has been considered and addressed. This delivers not only the highest quality and performance NGS Indexing Oligos but cross-contamination levels of co-synthesised unique dual indexed (UDI) adapters and primer pairs **always guaranteed at <0.01%.**

The sensitivity and specificity of Next Generation Sequencing make it the **ultimate quality control check** for the Oligos you use in your library preparation workflows.

Minimize Cross Contamination & Index-Hopping

The increasing capacity of the latest sequencing platforms provide ever increasing sample multiplexing capabilities. With the need to sort and identify each library following sequencing (demultiplexing), there is the requirement to add unique sequences- called sequencing Indexes (also known as simply Indexes or Barcodes)- to each end of the DNA sample fragment during the library preparation workflow.

During demultiplexing a misassigned Index can lead to sequencing reads being assigned to the wrong sample- often referred to as Index-Hopping.

At Biolegio, we have looked to minimize the risk of NGS Adapters and Indexing Primers as a potential source Index Hopping and Cross-Contamination and provide additional reassurance in the integrity of your sequencing results.

Unique Dual Indexing

In contrast to a Combinatorial Dual Index (CDI) strategy – where the same Indexes are repeated across a set so the Index combinations are unique, but the individual Indexes are not – the use of Unique Dual Indexes (UDIs) ensures a totally unique index sequence for every read.
This approach can mitigate Index-Hopping observed during downstream analysis by more clearly highlighting misassignments and labelling them as undetermined reads, allowing filtering and exclusion from the final analysis.

All Biolegio LegiSeq™ Ready-to-Use, pre-configured Adapter and Indexing Primer sets utilise a Unique Dual Indexing (UDI) strategy.

Unique Molecular Identifiers

LegiSeq™ Full Length UDI-UMI Adapters incorporate a 9bp Unique Molecular Identifier (UMI), to uniquely label the starting material of each library fragment.
This can allow any duplicate reads and amplification errors potentially introduced during library prep, enrichment or sequencing to be identified and filtered out during analysis and can be used for a variety of applications including, contamination detection, low-frequency variant identification, copy number variation and correcting for amplification bias.

Index Design

LegiSeq™ Adapter and Indexing Primer sets all use 10bp Indexes with a nucleotide edit distance of ≥3 across our full sets of 16, 96 and 384 (Hamming distance -the number of nucleotide substitutions required to change one sequence into another).
LegiSeq™ sets have also been cross-referenced against those from other major suppliers to ensure there are no duplicate Indexes and an edit distance of ≥2 between Indexes to potentially allow end-users to combine libraries using different Index sets on the same sequencing run if required. We have also taken into account many other design criteria including color-balancing on both 2 & 4-channel instrument platforms.

Collectively these design considerations all minimise the risk that misread bases within the Index sequences occur during sequencing.

For alternative indexing strategies please contact us to discuss our custom NGS Oligo services.

Superior Quality Control for Superior NGS Oligos

Quality Control

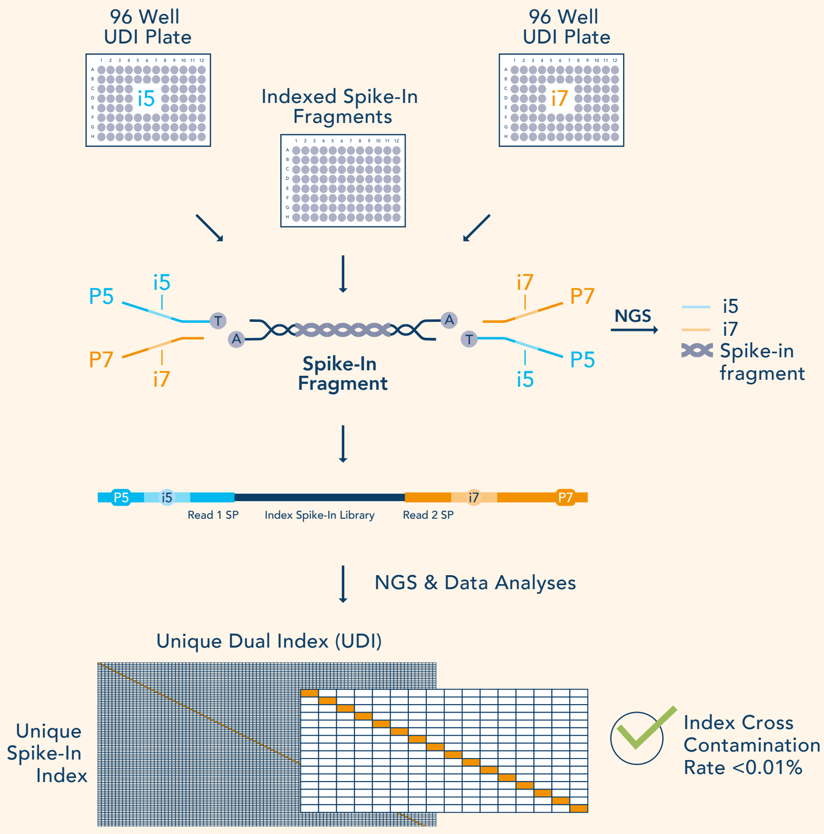
Every production batch of LegiSeq™ Ready-to-Use, pre-configured Adapter and Indexing Primer sets are subject to a dedicated NGS based Quality Control check developed In-House and based upon the **S**ample **A**ssurance **S**pike-**I**n Sequencing (**SASI-Seq**)[1] assay method.

This method uses unique, synthetic spike-in sequences as the sample, which undergo library preparation with LegiSeq™ Unique Dual Indexed Adapters or Primers.

During the analysis pipeline reads of the expected fragment provide confirmation of correct sample identity, while reads of unexpected fragments (from other samples in the same run) indicate cross-contamination or index hopping. By calculating the fraction of misassigned reads, the assay gives a quantitative contamination rate.

Biolegio analyses both contamination rates of individual i5 & i7 indexes and combined indexes to ensure every batch has a cross contamination rate <0.01%.

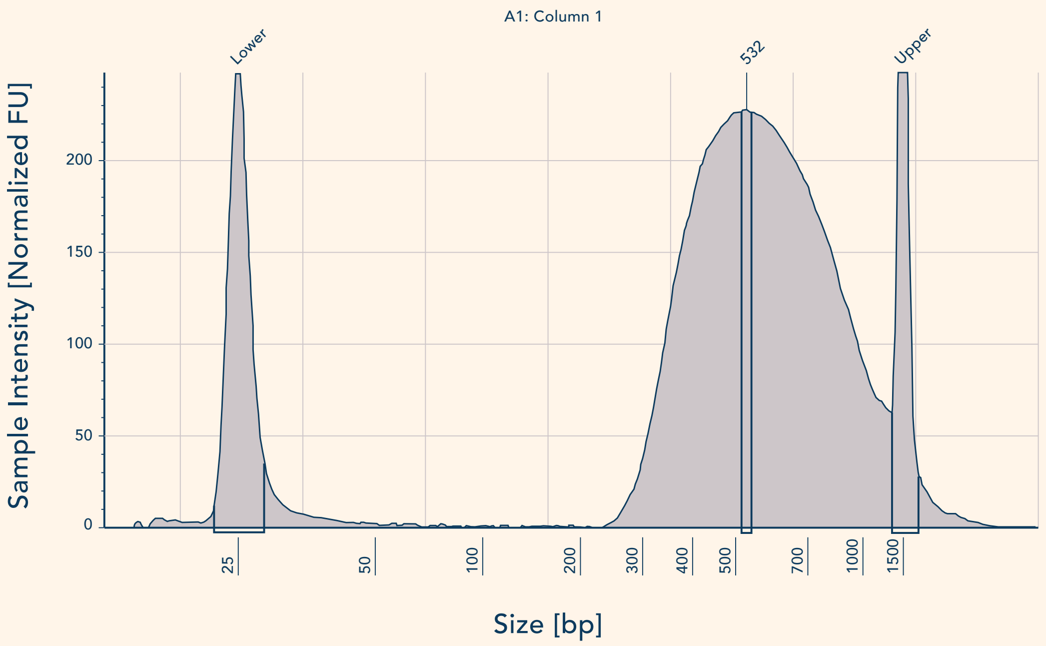
Figure 1 | Cross contamination QC- 96 Unique Dual Index Adaptors



Cross contamination QC- 96 Unique Dual Index Adaptors are ligated to samples containing unique Index Spike-In sequences in each well. The subsequent unique combination of sequencing indexes and spike-in sequence are defined in known co-ordinates in the final library. Following Sequencing during data analysis a 96 x 96 array is generated to confirm the presence of the barcode and spike-in in the expected position, with a QC clearance criteria that the indexes are detected in <0.01% of reads in any other position.

Additional functional QC is performed on every batch to demonstrate high ligation efficiency, consistent library yields and clustering efficiency and that our adapters meet the needs of your multiplexed sequencing applications.

Figure 2 | Analysis of Libraries Prepared with LegiSeq Full Length UMI-UDI Adapters

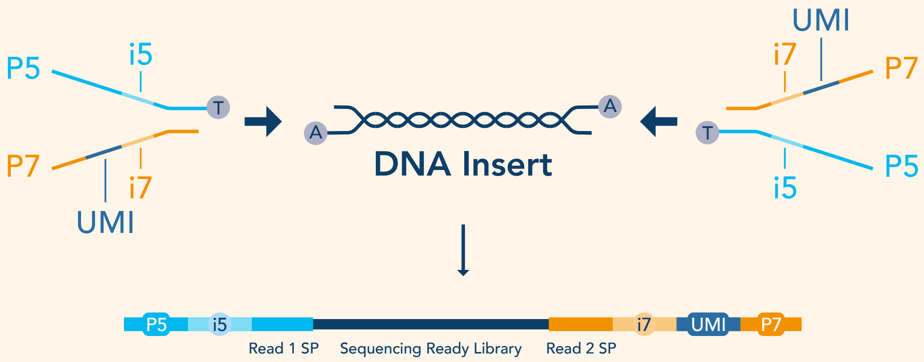


48 libraries were prepared with the first 48 Adaptors from the the LegiSeq Full Length UMI-UDI Adapters, 96 rxns set , libraries were quantified on an Agilent® TapeStation® 4000. TapeStation traces of the 96 libraries show the minimal presence of adaptor-dimers.

All design and QC considerations, **minimise the risk** of LegiSeq™ Oligos being a potential cause of **Index Hopping**.

Solutions For Ligation Based Library Prep

Figure 3 | LegiSeq™ Full-Length UDI/UMI Adapters for Illumina®



Full length Indexed Adapters for direct TA-ligation based library preparation for Illumina® sequencing (TruSeq™ Compatible).

Ordering Information

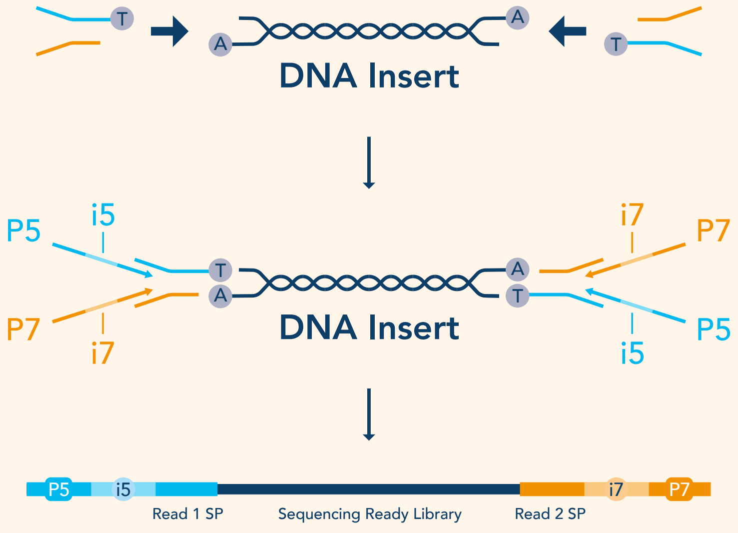
LegiSeq™ Full Length UDI-UMI Adapters

Full length UDI-UMI adapters for direct Illumina® TA-ligation based library preparation (TruSeq™ Compatible). Available as ready-to-use, single-use plates of 16, 96 or 384 adapters incorporating a 10 nt Unique Dual Index (UDI) & a 9nt Unique Molecular Identifier (UMI).
Bulk volumes also available for high throughput requirements, contact info@biolegio.com for further information.

LEGISEQ™ FULL LENGTH UDI-UMI ADAPTERS

Catalogue Nr	Description
647014	LegiSeq™ Full Length UMI-UDI Adapters, 16 rxns
647015	LegiSeq™ Full Length UMI-UDI Adapters, 96 rxns, Plate 1
647016	LegiSeq™ Full Length UMI-UDI Adapters, 96 rxns, Plate 2
647017	LegiSeq™ Full Length UMI-UDI Adapters, 96 rxns, Plate 3
647018	LegiSeq™ Full Length UMI-UDI Adapters, 96 rxns, Plate 4
647019	LegiSeq™ Full Length UMI-UDI Adapters, 384 rxns, Plates 1-4

Figure 4 | LegiSeq™ Universal Short Adapters and UDI Primers



The LegiSeq™ Universal Short Adapter is a truncated adapter for Illumina® NGS. The adapters are TA-ligated to library fragments following an A-tailing step and are used with the Biolegio UDI Primers to incorporate Unique (10 nucleotide) Indexes via a subsequent PCR step.

Ordering Information

LegiSeq™ Universal Short Adapter & UDI Indexing Primers

Universal Short Adapter and unique dual indexing primers provided together- For Illumina® sequencing TA-ligation based library preparation (TruSeq™ Compatible). Available as ready-to-use, single-use plates of 16 or 96 UDI primer pairs (10nt Indexes) and an accompanying tube of Universal Short Adapters with a corresponding number of reactions.

LEGISEQ™ UNIVERSAL SHORT ADAPTERS & UDI PRIMERS

Catalogue Nr	Description
647001	LegiSeq™ Universal Short Adapter & UDI Primers, 16rxns
647002	LegiSeq™ Universal Short Adapters & UDI Primers, 96 rxns Plate 1
647026	LegiSeq™ Universal Short Adapters & UDI Primers, 96 rxns Plate 2
647027	LegiSeq™ Universal Short Adapters & UDI Primers, 96 rxns Plate 3
647028	LegiSeq™ Universal Short Adapters & UDI Primers, 96 rxns Plate 4
647040	LegiSeq™ Universal Short Adapters & UDI Primers, 384 rxns

LegiSeq™ Universal Short Adapter Primers

Universal Short Adapter only- For Illumina® sequencing TA-ligation based library preparation (TruSeq™ Compatible). Sample Indexing/Barcoding performed via PCR using Indexing Primers (sold separately).

LEGISEQ™ UNIVERSAL SHORT ADAPTER	
Catalogue Nr	**Description**
647003	LegiSeq™ Universal Short Adapter, 16rxns
647004	LegiSeq™ Universal Short Adapter, 96 rxns
647041	LegiSeq™ Universal Short Adapters, 384 rxns

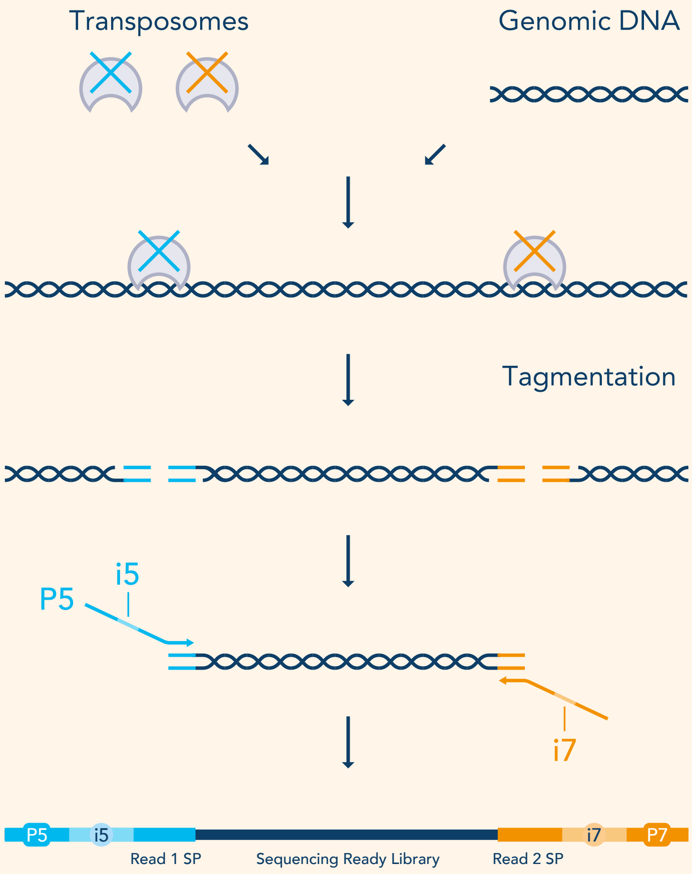
LegiSeq™ Indexing Primers for Ligation Based Library Prep

Premixed Primer Pairs, for use in combination with Universal Short Adapters (sold separately) for Illumina® sequencing TA-ligation based library preparation (TruSeq™ Compatible). Available as ready-to-use, single-use plates of 16, 96 or 384 primer pairs. 10 nt indexes with Unique Dual (UDI) format.
Bulk volumes also available for high throughput requirements, contact info@biolegio.com for further information.

LEGISEQ™ INDEXING PRIMERS FOR LIGATION BASED LIBRARY PREP	
Catalogue Nr	**Description**
647005	LegiSeq™ UDI Primers, 16 rxns
647006	LegiSeq™ UDI Primers, 96 rxns- Plate 1
647007	LegiSeq™ UDI Primers, 96 rxns- Plate 2
647008	LegiSeq™ UDI Primers, 96 rxns- Plate 3
647009	LegiSeq™ UDI Primers, 96 rxns- Plate 4
647010	LegiSeq™ UDI Primers, 384 rxns- Plates 1-4

Solutions For Tagmentation Based Library Prep

Figure 5 | LegiSeq™ Indexing Primers for Tagmented Libraries



LegiSeq™ Indexing Primers for Tagmented Libraries are pre-mixed 10nt indexed primer pairs compatible with the Illumina® Nextera™ adapters incorporated during a preceding transposase/tagmentation reaction.

Ordering Information

LegiSeq™ Indexing Primers for Tagmented Libraries

Primers

Premixed Primer Pairs, for use with Illumina® Nextera™ based library preparation incorporating a 10 nt index with Unique Dual (UDI). Available as ready-to-use, single-use plates of 16, 96 or 384 primer pairs.
Bulk volumes also available for high throughput requirements, contact info@biolegio.com for further information.

LEGISEQ™ INDEXING PRIMERS FOR TAGMENTED LIBRARIES

Catalogue Nr	Description
647020	LegiSeq™ Tagmentation UDI Primers, 16 rxns
647021	LegiSeq™ Tagmentation UDI Primers, 96 rxns- Plate 1
647022	LegiSeq™ Tagmentation UDI Primers, 96 rxns- Plate 2
647023	LegiSeq™ Tagmentation UDI Primers, 96 rxns- Plate 3
647024	LegiSeq™ Tagmentation UDI Primers, 96 rxns- Plate 4
647025	LegiSeq™ Tagmentation UDI Primers, 384 rxns- Plates 1-4

Supplementary Products

LegiSeq™ Library Amplification Primer Mix

Pre-mixed primer pairs compatible with the P5/P7 sequences to amplify and enrich libraries to the required yield for sequencing. Available in single tubes for up to 16 & 96 rxns.

LEGISEQ™ LIBRARY AMPLIFICATION PRIMER MIX

Catalogue Nr	Description
647029	LegiSeq™ Library Amplification Primers, 16 rxns
647030	LegiSeq™ Library Amplification Primers, 96 rxns

LegiSeq™ Duplex Adapter Buffer

Buffer for the storage and dilution of Biolegio Full Length and Universal Short Adapters.

LEGISEQ™ DUPLEX ADAPTER BUFFER

Catalogue Nr	Description
647035	LegiSeq™ Duplex Adapter Buffer, 500ml

LegiSeq™ Custom Adapter & Indexing Primer Solutions

If our pre-configured Adapter & Indexing Primer products are not the exact fit for your Illumina® NGS library prep workflow, please contact a member of our team to discuss if a custom solution can address your needs.

Whether you are looking for:

- Alternative Index Lengths
- Custom Index Sequences
- Pre-configured sets with alternate Index formats
  - Single Index (SI), Unique Combinatorial Indexing (CDI), Unique Dual Indexing (UDI)- with or without a Unique Molecular Identifier (UMI)
- Methylated bases or alternative modifications
- Custom post synthesis purification options
- Higher yield or bulk requirements for High Throughput Sequencing or Commercial production requirements

1- Quail, M.A., Smith, M., Jackson, D. et al. SASI-Seq: sample assurance Spike-Ins, and highly differentiating 384 barcoding for Illumina sequencing. BMC Genomics 15, 110 (2014) https://doi.org/10.1186/1471-2164-15-110

Biolegio

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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.

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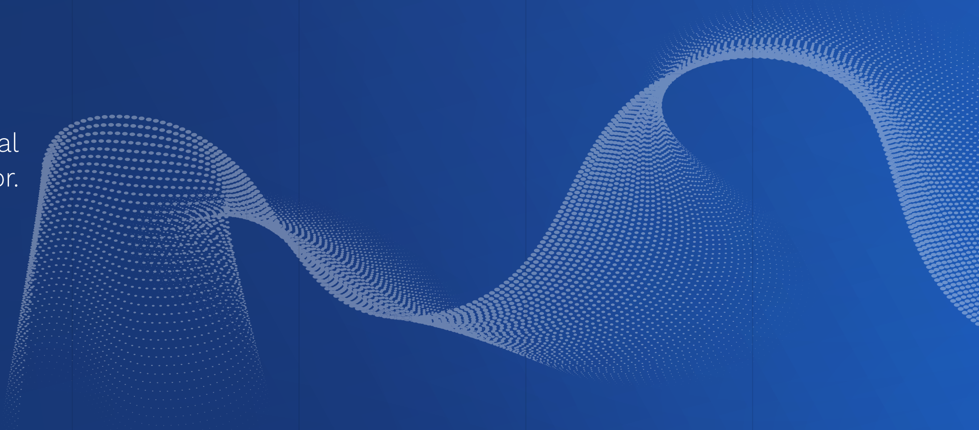

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