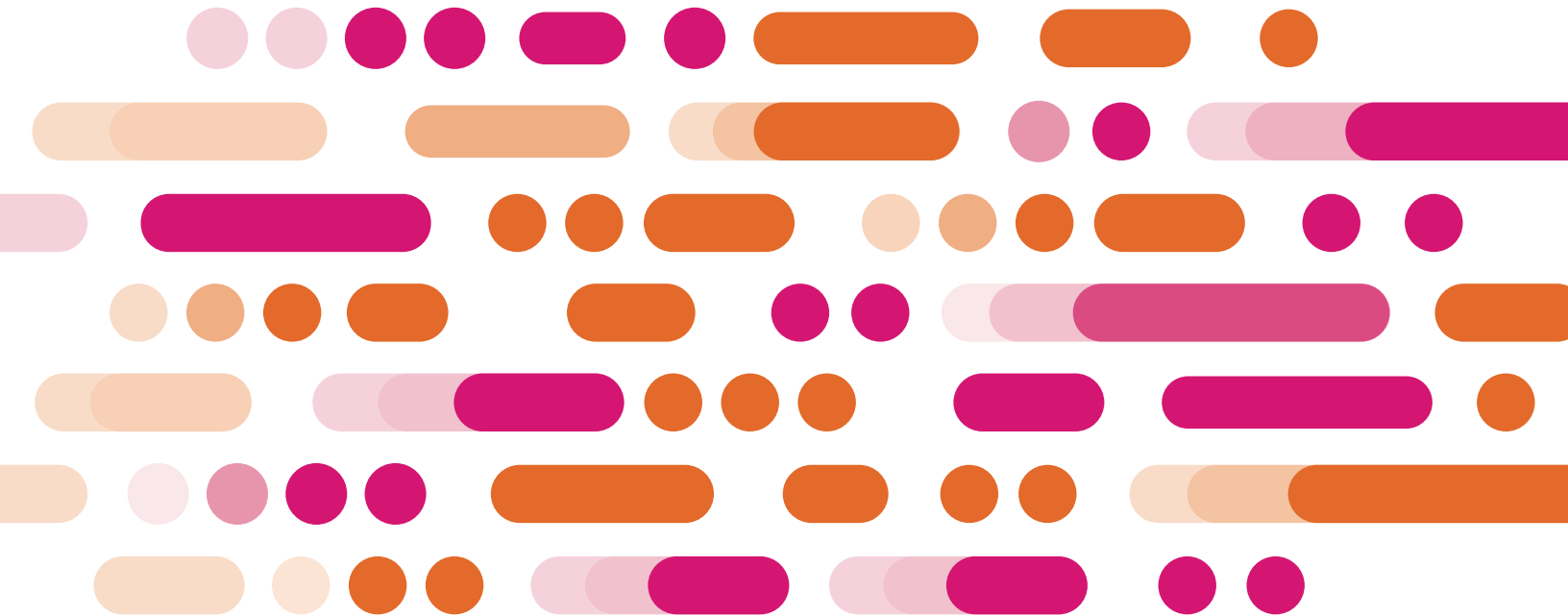


BioXp[®] NGS Library Prep kit for Plasmid & Whole Genome Sequencing User Guide



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Introduction to BioXp NGS library prep kit for plasmid sequencing

The BioXp NGS library prep kits allows for the generation of Illumina®-compatible, sequencing-ready Next Generation Sequencing (NGS) libraries from 10 to 20 ng of either purified plasmid or genomic DNA, depending on the type of kit. This kit is designed to work with the BioXp 3250 & the BioXp 9600 systems allowing for a fully automated, walk-away workflow comprised of an enzymatic fragmentation, end-repair, A-tailing, adaptor ligation, and a high-fidelity library amplification. The BioXp NGS library prep kits will create uniformly fragmented DNA-seq libraries allowing for the generation of high- quality sequencing data.

Overview of the BioXp NGS library prep workflow

The BioXp NGS library prep workflow is summarized in figure 1. The workflow demonstrates both the steps that occur on the benchtop as well as those that occur on the BioXp system.

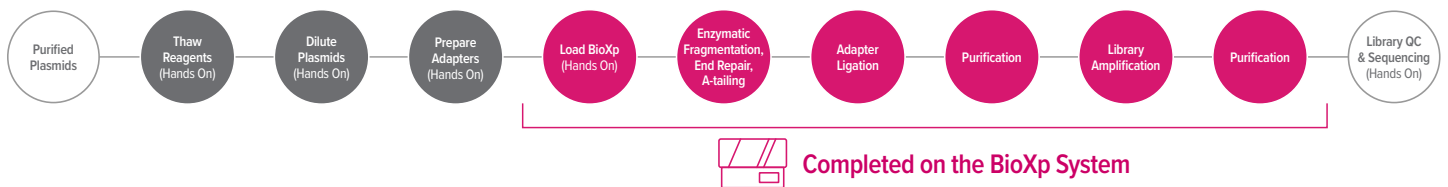


Figure 1. End to end workflow showing the generation of NGS libraries for plasmid sequencing.

Ordering Information

Types of libraries

BioXp NGS library prep kits are available to order through myBioXperience™. These kits can be selected from the NGS library prep category on the left-hand navigation panel (see figure 2). The kits are available for the BioXp 3250 and the BioXp 9600 as indicated in Table 1. There are two types of BioXp NGS library prep kits - BioXp NGS Library Prep kits for Plasmid Sequencing & BioXp NGS Library Prep kits for Whole Genome Sequencing. Please ensure you select the correct kit for your input type as results may be impacted by using the wrong combination.

System Compatibility	SKU	Kit Name
BioXp 3250	BX-NGS-PLA-08	BioXp® 3250 NGS library prep kit for plasmid sequencing, 08 rxn
	BX-NGS-PLA-24	BioXp® 3250 NGS library prep kit for plasmid sequencing, 24 rxn
BioXp 9600	BXN-NGS-PLA-08	BioXp® 9600 NGS library prep kit for plasmid sequencing, 08 rxn
	BXN-NGS-PLA-24	BioXp® 9600 NGS library prep kit for plasmid sequencing, 24 rxn
	BXN-NGS-PLA-48	BioXp® 9600 NGS library prep kit for plasmid sequencing, 48 rxn
	BXN-NGS-PLA-96	BioXp® 9600 NGS library prep kit for plasmid sequencing, 96 rxn
BioXp 3250	BX-NGS-WGS-08	BioXp® 3250 NGS Library Prep kit for Whole Genome Sequencing, 08 rxn
	BX-NGS-WGS-24	BioXp® 3250 NGS Library Prep kit for Whole Genome Sequencing, 24 rxn
BioXp 9600	BX9-NGS-WGS-08	BioXp® 9600 NGS Library Prep kit for Whole Genome Sequencing, 08 rxn
	BX9-NGS-WGS-24	BioXp® 9600 NGS Library Prep kit for Whole Genome Sequencing, 24 rxn
	BX9-NGS-WGS-48	BioXp® 9600 NGS Library Prep kit for Whole Genome Sequencing, 48 rxn
	BX9-NGS-WGS-96	BioXp® 9600 NGS Library Prep kit for Whole Genome Sequencing, 96 rxn

Table 1. BioXp NGS library prep kit for plasmid sequencing varieties.

The image shows a three-part screenshot of the myBioXperience website. The top part is the home page, which greets the user 'David Weiss!' and offers options to build on the BioXp® system, such as DNA and RNA. The middle part shows a search for 'BioXp® NGS Kits', displaying two product cards: 'BioXp® 9600 NGS library prep kit for plasmid sequencing' and 'BioXp® 3250 NGS library prep kit for plasmid sequencing'. The bottom part is a detailed view of the 'BioXp® 9600 NGS library prep kit for plasmid sequencing', featuring a magnifying glass icon with 'ATGC' inside, a description of the kit's use, and a list of four size options with their respective prices: 8 rxn (\$320.00), 24 rxn (\$960.00), 48 rxn (\$1,920.00), and 96 rxn (\$3,840.00). An 'Add to cart' button is visible at the bottom right of this section.

Figure 2. Ordering experience on myBioXperience showing the workflow for ordering a plasmid sequencing kit.

Storage and Handling

The BioXp NGS library prep kits are shipped on ice packs. Upon receipt, store Module A at RT and Module K at $-20 \pm 5^{\circ}\text{C}$.

Required Materials not included

Note: The BioXp NGS library prep kit for plasmid sequencing does not provide sequencing adapters. Telesis Bio recommends purchasing through IDT for Illumina – Truseq DNA UD Indexes v2 (96 indexes, 96 samples) from Illumina (part # 20040870) for the generation of NGS libraries on the BioXp system..

Additional Reagents	Recommended Supplier	Kit Name
IDT for Illumina-Truseq DNA UD Indexes v2 (96 indexes)	Illumina, Inc	Required
10 mM Tris-HCl, pH 8.0	-	Must use at least one
Tris low EDTA (0.1 mM EDTA) buffer	-	
Nuclease-free water	-	
96-well plate	-	Required
100% Ethanol	-	Required
50 ml conical tubes	-	Optional
Qubit dsDNA Quantitation, High Sensitivity	Thermo Fisher Scientific	Optional
HS D5000 ScreenTape, Reagents, & Ladder	Agilent Technologies	Optional
HS NGS Fragment Kit (1-6000bp), 500	Agilent Technologies	Optional
DNA 7500 Reagents	Agilent Technologies	Optional
NGS Fragment kit (1-6000 bp), 500	Agilent Technologies	Optional
qPCR NGS Library Quant Kit	-	Optional

Additional Equipment	Recommended Supplier	
Pipettes and tips	-	Required
Vortex	-	Required
Qubit Fluorometer	Thermo Fisher Scientific	Optional
4150/4200 TapeStation	Agilent Technologies	Optional
2100 Bioanalyzer	Agilent Technologies	Optional
5200/5300/5400 Fragment Analyzer	Agilent Technologies	Optional
qPCR System	-	Optional

Table 2. List of additional required reagents and equipment. Please note, multiple options are given for quantification and qualification. It is not necessary to have all systems and reagents. Please choose the system and reagents that best support your workflow.

Input DNA Requirements and Guidelines

These kits and the associated automated protocols have been optimized for 10 – 20 ng of input DNA. DNA can be purified using either established column-based or bead-based purification methods. It is not recommended to use this kit for other input amounts or qualities as yields and library uniformity may suffer.

Input DNA samples should be resuspended in nuclease-free water, 10 mM Tris-HCl pH 8, or Tris low EDTA (0.1 mM EDTA).

Higher concentrations of EDTA will significantly slow the enzymatic fragmentation reaction.

Samples should be quantitated using a fluorescence-based method, such as Qubit or PicoGreen, as these methods provide a more accurate quantitation as compared to spectrophotometric methods, such as NanoDrop.

System Compatibility

The BioXp NGS library prep kits are designed to run on the BioXp 3250 and 9600 systems.

Preparing the BioXp Prior to Run

It is important that the BioXp system is free from contaminants that could interfere with the generation of NGS libraries. Spray 70% ethanol (RNase-free) or 70% isopropanol (RNase-free) onto a lint-free wipe and clean all the exposed BioXp system surfaces, including the four pipettors of the pipette head. Do not spray the solution directly on the BioXp system as this may leave residual liquid which could interfere with proper functioning of the instrument including damaging the circuitry. Once the surfaces are dry, proceed with loading the reagents.

Reagent Preparation

Prepare the Reagent Block

1. When you are ready to generate NGS libraries, remove the reagent block from the -20°C and place at room temperature for 15 minutes to thaw.
2. Once the reagent block has been thawed for 15 minutes, check the bottom of the block to ensure reagents have thawed completely.
3. Spin the block down for 1 minute at 500 rcf to collect the reagents at the bottom of each well.
4. Place the reagent block on ice until ready to load the BioXp system.

Prepare the Sequencing Adapters

1. Completely thaw the source index/adaptor plate on ice. **Note: Please ensure that the adapters remain on ice and are prepared fresh for each run as issues with the adapters can significantly impact the outcome of the library prep protocol. It is not recommended to use the source index/adapters for more than two (2) freeze-thaws. It may be beneficial to aliquot the adapters into single-use aliquots upon initial thawing. Please see the adapter QC protocol recommendations in the troubleshooting section of the user guide.**
2. Quickly spin the plate to collect indexes at the bottom of the well. Place plate on ice.
3. While on ice, transfer 5 µl of each adapter to be used in the run to a clean, customer-provided 96-well plate.
4. To each well containing adapter, add 45 µl of 10 mM Tris-HCl, pH 8.0 or Tris low EDTA buffer and mix well by pipetting.
5. Briefly spin down the diluted adapter plate to collect the adapters at the bottom of each well.
6. Place the Telesis Bio-provided 96-well plate (no carrier for 8 & 24 reaction or green carrier for 48 & 96 reaction) on ice and transfer 2.5 µl of diluted adapter to the wells as defined in the loading map provided with your kit.
7. Carefully tap the covered plate on a solid surface to ensure all liquid is at the bottom of the wells.
8. Place the input plate on ice until ready to load onto the BioXp system..

Prepare the Plasmid DNA Input

1. This kit is validated for an input range of 10 – 20 ng of purified DNA in nuclease free water.
2. Confirm the DNA concentration by analysis with a Qubit dsDNA Quantitation, High Sensitivity Assay on the Qubit Fluorometer. If a Qubit is not available, concentration can be determined by spectrophotometry on a Nanodrop spectrophotometer.
3. Dilute each DNA sample to 10 ng/μl or 20 ng/μl using nuclease-free water.
4. Remove the biosecurity cover from the Telesis Bio-provided DNA Input Plate (96-well plate with the white carrier) and add 19 μl of nuclease-free water to the appropriate wells as defined in the loading map provided with your kit.
5. Transfer 1 μl of diluted DNA sample to each of the wells containing 19 μl nuclease-free water.
6. Alternatively, directly dilute DNA input to either 0.5 ng/μl or 1 ng/μl in 20 μl in the DNA Input Plate.
7. Mix samples well by gentle pipetting. **Note: To ensure proper sample mixing, use >80% of the sample volume and mixing 10-15 times.**
8. Replace the biosecurity cover.
9. Carefully tap the covered plate on a solid surface to ensure all liquid is at the bottom of the wells.
10. Place the input plate on ice until ready to load onto the BioXp system.

Prepare the Purification Strips

1. Retrieve purification strip(s) from the kit and vortex for 10 seconds to ensure the beads are properly mixed.
2. Briefly quick spin the purification strip to ensure that the bead solution is collected at the bottom of the wells. **Note: Do not over spin the purification strip as this could cause the beads to pellet at the bottom of the wells and can cause reduced library yields.**
3. Store the purification strip(s) at room temperature until ready to load onto the BioXp system.

Loading the BioXp System

Every BioXp reagent kit ships with detailed loading instructions that are specific to the kit and the BioXp system it is intended to be loaded on. Please reference the appropriate **BioXp NGS library prep kit – loading map & checklist** shipped with your kit or download a copy from the [Telesis Bio resources webpage](#), under the “BioXp kits” document section.

Initiating the BioXp NGS library prep run

1. Once the BioXp system has been loaded according to the appropriate loading map, close the hood.
2. Select “Next” on the BioXp system touchscreen to begin the initialization process, including scanning of the run-specific barcodes and loading of the associated run scripts.
3. Once the initialization process is complete, select “Start” on the touchscreen to begin the run.

Retrieving your NGS Library and Completing the Run

1. Once the run is complete, the BioXp system will indicate a successful run with a green internal light. Additionally, the screen will read the time of run completion.
2. Select the unlock icon on the touchscreen to unlock the BioXp system hood.
3. Open the hood and remove the output plate from the thermal cycler and seal with an adhesive seal. This plate contains your prepared NGS libraries. NGS libraries will be located in wells A1-H3 on runs performed on the BioXp 3250 and will be located in A1-H12 on runs performed on the BioXp 9600.
4. Place the sealed plate on ice until ready to proceed with sample QC.
5. **If not proceeding directly to sample QC, store the sealed output plate at 2 °C to 8 °C for up to one week or at -20 °C for longer storage.**
6. Remove and discard all remaining components on the BioXp system. **Note: Please do not discard the plastic EtOH trough.**

QC steps to assess quantity and quality of the final NGS library

Quantification

1. qPCR

qPCR will provide the most accurate quantitation of sequence-able fragments in the NGS library. Quantitative PCR (qPCR) can be used as a quality control (QC) method for NGS libraries by measuring the concentration and quality of the library before sequencing. When using a qPCR-based method for quantitating samples, ensure that the most recent vendor-specific protocols are being used.

2. Fluorometric methods

When using a fluorometric method for quantitation of the NGS libraries, follow the most recent protocol from the vendor. Telesis Bio recommends using the Qubit fluorometer and the Qubit dsDNA Quantitation, High Sensitivity Assay from Thermo Fisher Scientific if using a fluorometric method.

Quality and Size Analysis

Electrophoretic methods, such as the Agilent Technologies TapeStation, Bioanalyzer, or Fragment Analyzer are recommended for assessing the quality and size distribution of the NGS libraries. Ensure that the most recent versions of the vendor protocols are being used.

Normalization and Pooling of NGS Libraries

The number of indexed libraries that may be multiplexed in a single sequencing lane is determined by the output specifications of the platform used, together with the amount of sequencing data required for your research. Telesis Bio recommends 50x average read depth for each plasmid being sequenced to ensure confidence in sequence confirmation. Calculate the number of indexes that can be combined per lane, according to the capacity of your platform and the amount of sequencing data required per sample.

1. Dilute an aliquot of each sample to be sequenced to 4 nM using Tris low EDTA. The formula in figure 4 can be used to convert from ng/μl to nM.
2. Combine equal volumes of each diluted sample to create the final pool.
3. Telesis Bio recommends the inclusion of 1% PhiX (Illumina) as a sequencing control and to balance the lack of sequence diversity for plasmid sequencing.
4. Follow Illumina's recommended protocol for the specific instrument that will be used to sequence the samples.

$$\frac{(\text{Concentration in ng/}\mu\text{l})}{(660\text{g/mol} \times \text{average library size in bp})} \times 10^6 = \text{concentration in nM}$$

Figure 4. Calculation to convert ng/μl to nM.

Troubleshooting

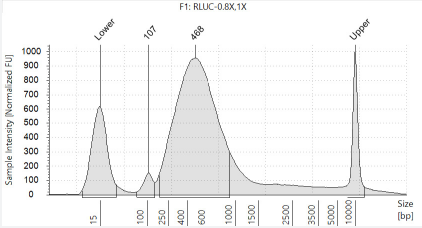
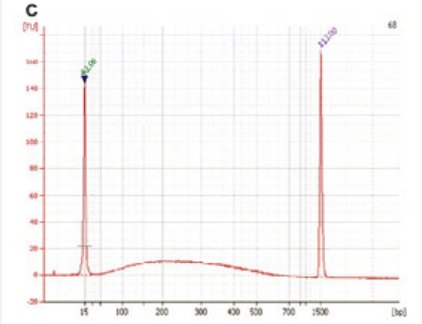
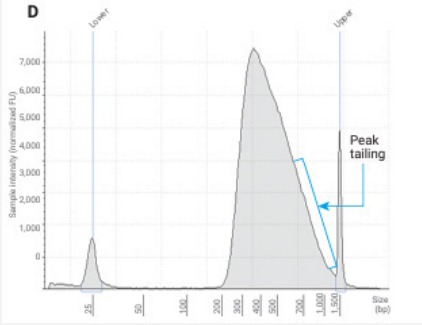
Phenotype	Possible Causes
<p>Presence of adapter dimer (~120bp peak)</p> 	<ul style="list-style-type: none"> • Input amount is lower than expected resulting in a higher adapter:insert ratio – re-quantitate using Qubit or qPCR • Quality of input material is not appropriate for adapter concentration used – re-assess input quality using a 1% agarose gel • Reduced efficiency of library preparation due to adapter quality – confirm adapter receipt/storage per manufacturer’s guidelines • Adapter stock is not diluted correctly – confirm adapter dilution
<p>Lower yield than expected</p> 	<ul style="list-style-type: none"> • Ensure DNA sample is pure (Absorbance (A260/280) using spectrophotometer ~1.8 • Reduce input DNA amount during library preparation to 30 - 50ng per ECL sample • Dilute pooled library to 1.5nM to 2nM before denaturing with NaOH • Load 6pM – 10pM for sequencing
<p>Lower or higher fragment size than desired</p> 	<ul style="list-style-type: none"> • Quantitation of input material – re-quantitate using Qubit or qPCR • Storage of reagents – Review reagent storage conditions

Table 3: Describes recommendations for troubleshooting when you have a problem with the intended use of the BioXp NGS Library Prep kit.

Recommended Adapter QC Protocol

The steps for QC of sequencing adapters are to measure the quality of the adapters to determine if the adapters can be applied for successful NGS library preparation using BioXp kits.

Required Materials

10 mM Tris-HCl, pH 8.0 (Teknova, T1173), nuclease-free water, NanoDrop photometer system

Protocol

- Make sure the purchased adapters are compatible with the ligation-based library preparation methods. We recommend using IDT for Illumina – Truseq DNA UD indexes v2 kit (96 indexes, 96 samples) (Illumina, 20040870) as the adapters for BioXp library prep automation. Tagmentation-based adapter does not work with all the BioXp NGS library-prep kits.
- Thaw the original/diluted adapter plate on ice.
- Once thawed, use 20 µl multi-channel pipette briefly mixing each adapter well 5 times. Place the adapter plate back on ice.
- Initiate the NanoDrop photometer system, using nuclease-free water to clean and dry the surface of each probe.
- Blank the system by loading 1.5 µl 10 mM Tris-HCl, pH 8.0/nuclease-free water on the probe.
- Clean the surface of the probe, set the system for dsDNA measurement and load 1.5 µl of each adapter to the probe for the assay.
- Record the A260, 260/280 and concentration to determine the quality of the adapter. Good quality adapters should have 260/280 between 1.8-2.0 with over 40 ng/µl in concentration.
- Once completed, clean and dry the NanoDrop photometer system using nuclease-free water for future usage.

For additional questions please contact Telesis Support at help@Telesisbio.com or 858.526.3080

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