INSTRUCTION MANUAL

NEBNext® Library Quant Kit for Ultima Genomics®

NEB #E3410S/L

100/500 reactions

Version 2.0_07/25

Table of Contents

The Kit Includes

The volumes provided are sufficient for preparation of up to 100 reactions (NEB #E3410S) and 500 reactions (NEB #E3410L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

Luna® Universal qPCR Master Mix

NEBNext Library Dilution Buffer

○ (white) NEBNext UG Library Quant Primer Mix

○ (white) NEBNext UG Library Quant Standard 1

● (green) NEBNext UG Library Quant Standard 2

● (blue) NEBNext UG Library Quant Standard 3

● (yellow) NEBNext UG Library Quant Standard 4

● (lilac) NEBNext UG Library Quant Standard 5

Required Materials Not Included

- Nuclease-free water
- qPCR machine
- qPCR plates and seals
- PCR strip tubes or microcentrifuge tubes
- Conical centrifuge tubes

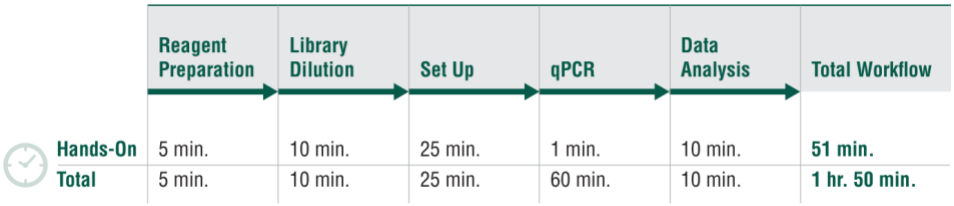
Introduction

The NEBNext Library Quant Kit for Ultima Genomics contains reagents optimized for the precise qPCR-based quantitation of PCR-free libraries prepared for the Ultima Genomics next-generation sequencing platform. Library quantitation is critical for proper loading onto the Ultima Genomics UG 100™ platform. This kit contains forward and reverse primers, which target the Ultima adapter sequences and a set of high-quality, pre-diluted DNA standards to enable reliable quantitation of diluted DNA libraries.

Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together.

For larger volume requirements, customized and bulk packaging is available for purchase through the Customized Solutions Team at NEB. Please contact custom@neb.com for further information.

Figure 1. NEBNext Library Quant Kit for Ultima Genomics (NEB #E3410) workflow



Section 1
Experimental Considerations

- The Luna Universal qPCR Master Mix in the NEBNext Library Quant Kit for Ultima Genomics includes a blue, non-interfering, visible tracking dye, which can also be used as a passive reference dye. Therefore, no additional ROX is required for real-time instruments that require ROX normalization.
- qPCR is a sensitive DNA detection method. Proper sterile techniques and careful pipetting should be used to avoid DNA contamination and ensure accurate quantitation results.
- The range of standard concentrations in the NEBNext Library Quant Kit for Ultima Genomics is 30–0.003 pM.
- Ensure that all kit components are thawed and mixed prior to use.
- We recommend including a no-template control (NTC) reaction using NEBNext Library Dilution Buffer (1X) in addition to the NEBNext UG Library Quant Standards 1-5. The Cq from the NTC will not be used in quantitation analysis but serves as a valuable control reaction to ensure performance of the kit and the absence of sample contamination.
- Accurate library quantitation relies on careful dilution of library DNA samples. Prepare and use a 1X concentration of NEBNext Library Dilution Buffer and carefully dilute samples using a serial dilution scheme between 1,000 and 10,000-fold dilutions.
- We recommend running triplicate reactions per sample; however, duplicates can also be run for each standard and library sample. This ensures the most accurate quantitation and enables exclusion of outlier traces due to bubbles, plate sealing or other problems.
- Multichannel pipettes can be used with the kit, but care should be taken to ensure consistency of volume when loading both the 8 µl of qPCR Master Mix (consisting of Luna Universal qPCR Master Mix (5 µl) and NEBNext UG Library Quant Primer Mix (3 µl) per reaction) and 2 µl of sample into the qPCR plate.
- When pipetting into the qPCR plate, it is advisable to avoid the formation of bubbles. Centrifugation of sealed qPCR plates for 2–5 minutes at ~2,500 rpm is recommended to collect samples at the bottom of wells but is not required. If 1–2 small bubbles are present at the top of the liquid after loading, the assay can proceed as the bubbles will be removed when the plate is heated for the denaturation step of the PCR cycling.
- Use the “SYBR® Green” or “SYBR/FAM” channel of the qPCR instrument. Only the single channel plate read is necessary for the NEBNext Library Quant Kit for Ultima Genomics and selecting a single read often results in faster experiment times.
- Denaturation or melt curves may be included in the qPCR cycling protocol, although this will add time to the workflow and not provide helpful information about library quality.
- Amplification efficiency estimates may vary across qPCR instruments due to differences in hardware and analysis software.

Section 2
Protocol for the NEBNext Library Quant Kit for Ultima Genomics

Symbols

This caution sign signifies a step in the protocol that has multiple paths leading to the same end point but is dependent on a user variable, like the kit sizes, the amount of primer added, and the qPCR instrument used.

2.1. Thaw and Mix Kit Reagents

2.1.1. Thaw the Luna Universal qPCR Master Mix and the NEBNext UG Library Quant Primer Mix at room temperature, then place on ice. After thawing completely, briefly mix each component by gentle vortexing for 5 seconds.

2.1.2. Thaw the NEBNext UG Library Quant Standards, tubes 1–5 at room temperature. Mix by pulse vortexing (approximately ~1 second) and spin briefly to collect material from the sides of the tubes. Place on ice.

2.1.3. Thaw the NEBNext Library Dilution Buffer (10X) at room temperature. Mix well by vortexing for 10 seconds. Centrifuge briefly to collect material from the sides of the tube. Place on ice.

2.2. Prepare the Universal qPCR Reaction Mix

2.2.1. Determine the total number of reactions needed to measure the following in duplicate or triplicate:

- Each of the five NEBNext UG Library Quant Standards
- Each library dilution being assayed
- A no-template control (NTC)

i.e., for duplicates:
Total number of reactions = (2 x 5 Standards) + (2 x 1 NTC) + (2 x # library samples)
= 12 + (2 x # library samples)

i.e., for triplicates:
Total number of reactions = (3 x 5 Standards) + (3 x 1 NTC) + (3 x # library samples)
= 18 + (3 x # library samples)

2.2.2. Prepare the Universal qPCR Reaction Mix for the number of required reactions calculated in step 2.2.1.

REAGENT	VOLUME PER SINGLE 10 µL REACTION
Luna Universal qPCR Master Mix (2X)	5 µl
NEBNext UG Library Quant Primer Mix	3 µl
Total Volume Reaction Mix Per Reaction	**8 µl**

Mix thoroughly but gently by pipetting or vortexing. Collect liquid to the bottom of the tube by brief centrifugation. Place the prepared master mix on ice until use.

2.3. Prepare the NEBNext Library Dilution Buffer (1X)

2.3.1. Dilute the NEBNext Library Dilution Buffer (10X) 1:10 with nuclease-free water. Mix by vortexing for 10 seconds. Prepare sufficient buffer for the desired number of libraries to be quantitated. Unused NEBNext Library Dilution Buffer (1X) can be stored at 4˚C.

COMPONENT	VOLUME
NEBNext Library Dilution Buffer (10X)	1 ml
Nuclease-free Water	9 ml
Total Volume	**10 ml**

2.4. Prepare Library Dilutions

Using a serial dilution scheme, we recommend diluting each library sample between 1,000 and 10,000-fold in prepared (1X) NEBNext Library Dilution Buffer. See example in 2.4.1–2.4.2 for a 1:10,000 dilution:

2.4.1. Add 1 µl of library to 99 µl NEBNext Library Dilution Buffer to create a 1:100 dilution.

2.4.2. Add 1 µl of the 1:100 dilution from step 2.4.1. to 99 µl NEBNext Library Dilution Buffer to create a 1:10,000 dilution.

2.5. Prepare the qPCR Assays

For best results, we recommend running each DNA standard and library sample in triplicate. However, duplicates can also be run for each standard and library sample. Please refer to the examples below for plate layout with library in triplicates and duplicates.

2.5.1. Aliquot 8 µl of the pre-mixed Universal qPCR Reaction Mix (Step 2.2.2.) into qPCR tubes or plate. For best results, ensure accurate and consistent pipetting volumes and minimize bubbles.

2.5.2. Add 2 µl DNA Standard, Diluted Library, or Library Dilution Buffer to qPCR tubes or plate on ice. To avoid cross-contamination, add DNA standards from low to high concentration.

COMPONENT	VOLUME
Pre-mixed Universal qPCR Reaction Mix (from Step 2.2.2.)	8 µl
DNA standard, diluted library or Library Dilution Buffer (1X, as NTC)	2 µl
Total Volume	**10 µl**

Mix reactions carefully by pipetting at least 5 times. Try to minimize bubbles in plate wells, but 1–2 bubbles per well on top of the liquid will be removed by heating and not affect results.

2.5.3. Seal tubes with flat, optically transparent caps; seal plates with optically transparent film. Care should be taken to properly seal plate edges and corners to prevent artifacts caused by evaporation.

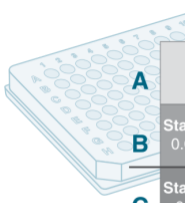
2.5.4. Spin tubes or plates briefly to remove bubbles and collect liquid (2–5 minutes at ~2,500 rpm).

2.5.3. **Load the qPCR Plate**

Prepare the qPCR plate as desired. Recommended conditions and layouts are listed below:

- Add standards from low to high concentration (as shown in layouts) to avoid cross contamination
- qPCR performed with two sample dilutions per library, assayed in triplicate (recommended)
- qPCR performed with two sample dilutions per library, assayed in duplicate
- qPCR performed with one sample dilutions per library, assayed in triplicate
- qPCR performed with one sample dilutions per library, assayed in duplicate

Layout for qPCR performed with two sample dilutions per library, assayed in triplicate (recommended):



	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	NTC	Library 1 1:4,000	Library 1 1:4,000	Library 1 1:4,000	Library 5 1:4,000	Library 5 1:4,000	Library 5 1:4,000	Library 9 1:4,000	Library 9 1:4,000	Library 9 1:4,000
B	Standard 5 0.003 pM	Standard 5 0.003 pM	Standard 5 0.003 pM	Library 1 1:10,000	Library 1 1:10,000	Library 1 1:10,000	Library 5 1:10,000	Library 5 1:10,000	Library 5 1:10,000	Library 9 1:10,000	Library 9 1:10,000	Library 9 1:10,000
C	Standard 4 0.03 pM	Standard 4 0.03 pM	Standard 4 0.03 pM	Library 2 1:4,000	Library 2 1:4,000	Library 2 1:4,000	Library 6 1:4,000	Library 6 1:4,000	Library 6 1:4,000	Library 10 1:4,000	Library 10 1:4,000	Library 10 1:4,000
D	Standard 3 0.3 pM	Standard 3 0.3 pM	Standard 3 0.3 pM	Library 2 1:10,000	Library 2 1:10,000	Library 2 1:10,000	Library 6 1:10,000	Library 6 1:10,000	Library 6 1:10,000	Library 10 1:10,000	Library 10 1:10,000	Library 10 1:10,000
E	Standard 2 3 pM	Standard 2 3 pM	Standard 2 3 pM	Library 3 1:4,000	Library 3 1:4,000	Library 3 1:4,000	Library 7 1:4,000	Library 7 1:4,000	Library 7 1:4,000	Library 11 1:4,000	Library 11 1:4,000	Library 11 1:4,000
F	Standard 1 30 pM	Standard 1 30 pM	Standard 1 30 pM	Library 3 1:10,000	Library 3 1:10,000	Library 3 1:10,000	Library 7 1:10,000	Library 7 1:10,000	Library 7 1:10,000	Library 11 1:10,000	Library 11 1:10,000	Library 11 1:10,000
G	—	—	—	Library 4 1:4,000	Library 4 1:4,000	Library 4 1:4,000	Library 8 1:4,000	Library 8 1:4,000	Library 8 1:4,000	Library 12 1:4,000	Library 12 1:4,000	Library 12 1:4,000
H	—	—	—	Library 4 1:10,000	Library 4 1:10,000	Library 4 1:10,000	Library 8 1:10,000	Library 8 1:10,000	Library 8 1:10,000	Library 12 1:10,000	Library 12 1:10,000	Library 12 1:10,000

Layout for qPCR performed with two sample dilutions per library, assayed in duplicate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	Library 1 1:4000	Library 1 1:4000	Library 5 1:4,000	Library 5 1:4,000	Library 9 1:4,000	Library 9 1:4,000	Library 13 1:4,000	Library 13 1:4,000	Library 17 1:4,000	Library 17 1:4,000
B	Standard 5 0.003 pM	Standard 5 0.003 pM	Library 1 1:10,000	Library 1 1:10,000	Library 5 1:10,000	Library 5 1:10,000	Library 9 1:10,000	Library 9 1:10,000	Library 13 1:10,000	Library 13 1:10,000	Library 17 1:10,000	Library 17 1:10,000
C	Standard 4 0.03 pM	Standard 4 0.03 pM	Library 2 1:4000	Library 2 1:4000	Library 6 1:4,000	Library 6 1:4,000	Library 10 1:4,000	Library 10 1:4,000	Library 14 1:4,000	Library 14 1:4,000	Library 18 1:4,000	Library 18 1:4,000
D	Standard 3 0.3 pM	Standard 3 0.3 pM	Library 2 1:10,000	Library 2 1:10,000	Library 6 1:10,000	Library 6 1:10,000	Library 10 1:10,000	Library 10 1:10,000	Library 14 1:10,000	Library 14 1:10,000	Library 18 1:4,000	Library 18 1:4,000
E	Standard 2 3 pM	Standard 2 3 pM	Library 3 1:4000	Library 3 1:4000	Library 7 1:4,000	Library 7 1:4,000	Library 11 1:4,000	Library 11 1:4,000	Library 15 1:4,000	Library 15 1:4,000	Library 19 1:10,000	Library 19 1:10,000
F	Standard 1 30 pM	Standard 1 30 pM	Library 3 1:10,000	Library 3 1:10,000	Library 7 1:10,000	Library 7 1:10,000	Library 11 1:10,000	Library 11 1:10,000	Library 15 1:10,000	Library 15 1:10,000	Library 19 1:10,000	Library 19 1:10,000
G	—	—	Library 4 1:4000	Library 4 1:4000	Library 8 1:4,000	Library 8 1:4,000	Library 12 1:4,000	Library 12 1:4,000	Library 16 1:4,000	Library 16 1:4,000	Library 20 1:4,000	Library 20 1:4,000
H	—	—	Library 4 1:10,000	Library 4 1:10,000	Library 8 1:10,000	Library 8 1:10,000	Library 12 1:10,000	Library 12 1:10,000	Library 16 1:10,000	Library 16 1:10,000	Library 20 1:10,000	Library 20 1:10,000

Layout for qPCR performed with one sample dilutions per library, assayed in triplicate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	NTC	Library 1 1:10,000	Library 1 1:10,000	Library 1 1:10,000	Library 9 1:10,000	Library 9 1:10,000	Library 9 1:10,000	Library 17 1:10,000	Library 17 1:10,000	Library 17 1:10,000
B	Standard 5 0.003 pM	Standard 5 0.003 pM	Standard 5 0.003 pM	Library 2 1:10,000	Library 2 1:10,000	Library 2 1:10,000	Library 10 1:10,000	Library 10 1:10,000	Library 10 1:10,000	Library 18 1:10,000	Library 18 1:10,000	Library 18 1:10,000
C	Standard 4 0.03 pM	Standard 4 0.03 pM	Standard 4 0.03 pM	Library 3 1:10,000	Library 3 1:10,000	Library 3 1:10,000	Library 11 1:10,000	Library 11 1:10,000	Library 11 1:10,000	Library 19 1:10,000	Library 19 1:10,000	Library 19 1:10,000
D	Standard 3 0.3 pM	Standard 3 0.3 pM	Standard 3 0.3 pM	Library 4 1:10,000	Library 4 1:10,000	Library 4 1:10,000	Library 12 1:10,000	Library 12 1:10,000	Library 12 1:10,000	Library 20 1:10,000	Library 20 1:10,000	Library 20 1:10,000
E	Standard 2 3 pM	Standard 2 3 pM	Standard 2 3 pM	Library 5 1:10,000	Library 5 1:10,000	Library 5 1:10,000	Library 13 1:10,000	Library 13 1:10,000	Library 13 1:10,000	Library 21 1:10,000	Library 21 1:10,000	Library 21 1:10,000
F	Standard 1 30 pM	Standard 1 30 pM	Standard 1 30 pM	Library 6 1:10,000	Library 6 1:10,000	Library 6 1:10,000	Library 14 1:10,000	Library 14 1:10,000	Library 14 1:10,000	Library 22 1:10,000	Library 22 1:10,000	Library 22 1:10,000
G	—	—	—	Library 7 1:10,000	Library 7 1:10,000	Library 7 1:10,000	Library 15 1:10,000	Library 15 1:10,000	Library 15 1:10,000	Library 23 1:10,000	Library 23 1:10,000	Library 23 1:10,000
H	—	—	—	Library 8 1:10,000	Library 8 1:10,000	Library 8 1:10,000	Library 16 1:10,000	Library 16 1:10,000	Library 16 1:10,000	Library 24 1:10,000	Library 24 1:10,000	Library 24 1:10,000

Layout for qPCR performed with one sample dilutions per library, assayed in duplicate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	Library 1 1:10,000	Library 1 1:10,000	Library 9 1:10,000	Library 9 1:10,000	Library 17 1:10,000	Library 17 1:10,000	Library 25 1:10,000	Library 25 1:10,000	Library 33 1:10,000	Library 33 1:10,000
B	Standard 5 0.003 pM	Standard 5 0.003 pM	Library 2 1:10,000	Library 2 1:10,000	Library 10 1:10,000	Library 10 1:10,000	Library 18 1:10,000	Library 18 1:10,000	Library 26 1:10,000	Library 26 1:10,000	Library 34 1:10,000	Library 34 1:10,000
C	Standard 4 0.03 pM	Standard 4 0.03 pM	Library 3 1:10,000	Library 3 1:10,000	Library 11 1:10,000	Library 11 1:10,000	Library 19 1:10,000	Library 19 1:10,000	Library 27 1:10,000	Library 27 1:10,000	Library 35 1:10,000	Library 35 1:10,000
D	Standard 3 0.3 pM	Standard 3 0.3 pM	Library 4 1:10,000	Library 4 1:10,000	Library 12 1:10,000	Library 12 1:10,000	Library 20 1:10,000	Library 20 1:10,000	Library 28 1:10,000	Library 28 1:10,000	Library 36 1:10,000	Library 36 1:10,000
E	Standard 2 3 pM	Standard 2 3 pM	Library 5 1:10,000	Library 5 1:10,000	Library 13 1:10,000	Library 13 1:10,000	Library 21 1:10,000	Library 21 1:10,000	Library 29 1:10,000	Library 29 1:10,000	Library 37 1:10,000	Library 37 1:10,000
F	Standard 1 30 pM	Standard 1 30 pM	Library 6 1:10,000	Library 6 1:10,000	Library 14 1:10,000	Library 14 1:10,000	Library 22 1:10,000	Library 22 1:10,000	Library 30 1:10,000	Library 30 1:10,000	Library 38 1:10,000	Library 38 1:10,000
G	—	—	Library 7 1:10,000	Library 7 1:10,000	Library 15 1:10,000	Library 15 1:10,000	Library 23 1:10,000	Library 23 1:10,000	Library 31 1:10,000	Library 31 1:10,000	Library 39 1:10,000	Library 39 1:10,000
H	—	—	Library 8 1:10,000	Library 8 1:10,000	Library 16 1:10,000	Library 16 1:10,000	Library 24 1:10,000	Library 24 1:10,000	Library 32 1:10,000	Library 32 1:10,000	Library 40 1:10,000	Library 40 1:10,000

2.6. Run the qPCR Assay in a Real-time Thermal Cycler Using FAM/SYBR Setting

2.6.1. qPCR cycling conditions

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	95°C	1 minute	1
Denaturation Extension	95°C 60°C	15 seconds 60 seconds	30

Note: A denaturation/ melt curve (65–95°C) can be included if desired but is optional.

Section 3
Data Analysis

3.1. Use the Real-time Thermal Cycler Software to Annotate the Concentration of the DNA Standards as Follows:

SAMPLE NAME	CONCENTRATION
NEBNext UG Library Quant Standard 1	30 pM
NEBNext UG Library Quant Standard 2	3 pM
NEBNext UG Library Quant Standard 3	0.3 pM
NEBNext UG Library Quant Standard 4	0.03 pM
NEBNext UG Library Quant Standard 5	0.003 pM

Using the provided Standards, confirm that the efficiency for the NEBNext UG Library Quant Standards (derived from the slope of the linear fit of the DNA standard curve plotted on a log-scale x-axis) is 85–110%. The coefficient of determination (R^2) for the linear fit of the standards should be ≥ 0.99.

3.2. Calculate the Library Concentration

The NEB qPCR webtool can be used to assist with data analysis: https://nebiocalculator.neb.com/#!/qPCRlibQnt
Be sure to select NEBNext Library Quant Kit for Ultima Genomics as the kit you are using.

- Enter the Cq values for the standards into the standards boxes at the top of the page. The Cq average for each standard is then plotted against the known concentrations to calculate the PCR efficiency and standard curve for library concentration determination.
- Enter the Cq values for each library dilution and replicate. Adjust the sample concentration for size by entering the average library size. The following equation is used to adjust the concentration for size, where the size of the DNA standards is 200 bp.:
  Size-adjusted concentration = concentration * [size of DNA standards (bp) / library size (bp)]
- Alternatively, Cq data from the qPCR instrument can be uploaded using the provided template file for analysis with the NEB qPCR Library Quantification webtool.

3.3. Recommendations

- If one of the triplicates of a diluted library sample is an outlier (> 0.5 Cq from the other replicates), then the data from this outlier should be excluded from the data analysis.
- For accurate quantitation, it is essential to have at least one library dilution that falls within the range of the DNA Standards 1–5. If all qPCR traces for the diluted library sample fall outside the range of the standards, then the qPCR assay should be repeated with higher (if faster than Standard 1) or lower (if slower than Standard 5) dilution.
- The traces for the NTC may show unexpected amplification due to contamination of library targets, primer-dimers, or amplified products if the qPCR assays have been performed repeatedly. If the Cq value for the NTC is far enough away from the dynamic range of the DNA Standards (e.g., Cq of NTC should be > 30), the NTC amplification can be ignored as it will not have any effect on library quantitation. If the NTC shows faster amplification with a Cq value within 2 cycles of the last DNA standard, a new (1X) NEBNext Library Dilution Buffer should be prepared, and the qPCR assay should be repeated.

Section 4
Troubleshooting Guide

OBSERVATION	POSSIBLE CAUSES	SUGGESTED SOLUTIONS
qPCR traces fall outside the range of the NEBNext Library Quant DNA Standards 1–5	Library dilutions are not optimal for the range of DNA standards (30–0.003 pM)	• Omit data for qPCR traces that fall outside the dynamic range of the DNA standards • If all qPCR traces fall outside the range of DNA standards, use a different dilution scheme
All qPCR traces show low or no amplification	Reagent omitted from qPCR assay Reagent added improperly to qPCR	• Verify all steps of the protocol were followed correctly
	Incorrect cycling protocol	• Refer to the proper qPCR cycling protocol in this user manual
	Incorrect reporter dye selected for the qPCR thermal cycler	• Select FAM/SYBR on the qPCR instrument
	Reagents are contaminated or degraded	• Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in this manual • Rerun the qPCR assay with fresh reagents
qPCR trace for replicate data has different shape than the others	Improper pipetting during qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler. Exclude problematic trace(s) from data analysis
	Poor mixing of reagents during qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
qPCR traces for the DNA standards are as expected but those for the library samples show little or no amplification	Library samples are incompatible with this kit	• Confirm libraries are made with UG Adapters
	Library samples diluted incorrectly	• Re-dilute the library samples
DNA standard curve has a poor correlation coefficient/ efficiency of the DNA standard curve falls outside the 85–110% range	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers • Omit data from traces that fall outside the dynamic range of the DNA standards
	Improper pipetting during qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
No template control qPCR trace is within 4 cycles of the last DNA standard	Luna Universal qPCR Master Mix/ NEBNext UG Library Quant Primer Mix has been stored improperly	• Verify proper storage conditions provided in this manual
	NEBNext Library Dilution Buffer or another component is contaminated with amplified library	• Discard previously used 1X NEBNext Library Dilution Buffer, re-make with new aliquot of nuclease free-water, and repeat. If NTC is still with 4 Cq of Standard 5, a new NEBNext Library Quant Kit for Ultima Genomics should be used.

Kit Components

NEB #E3410S Table of Components

COMPONENT#	PRODUCT NAME	VOLUME
E3409AVIAL	Luna Universal qPCR Master Mix	0.5 ml
E3411AVIAL	NEBNext UG Library Quant Standard 1	0.01 ml
E3412AVIAL	NEBNext UG Library Quant Standard 2	0.01 ml
E3413AVIAL	NEBNext UG Library Quant Standard 3	0.01 ml
E3414AVIAL	NEBNext UG Library Quant Standard 4	0.01 ml
E3415AVIAL	NEBNext UG Library Quant Standard 5	0.01 ml
E3416AVIAL	NEBNext UG Library Quant Primer Mix	0.32 ml
E7633AVIAL	NEBNext Library Dilution Buffer	1.0 ml

NEB #E3410L Table of Components

COMPONENT#	PRODUCT NAME	VOLUME
E3409AAVIAL	Luna Universal qPCR Master Mix	2.5 ml
E3411AAVIAL	NEBNext UG Library Quant Standard 1	0.043 ml
E3412AAVIAL	NEBNext UG Library Quant Standard 2	0.043 ml
E3413AAVIAL	NEBNext UG Library Quant Standard 3	0.043 ml
E3414AAVIAL	NEBNext UG Library Quant Standard 4	0.043 ml
E3415AAVIAL	NEBNext UG Library Quant Standard 5	0.043 ml
E3416AAVIAL	NEBNext UG Library Quant Primer Mix	0.8 ml
E7633AAVIAL	NEBNext Library Dilution Buffer	7.5 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	04/25
2.0	Added "To avoid cross-contamination, add standards from low to high concentration." to page 5 step 2.5.2. Changed "https://nebiocalculatordev.neb.com/#!/qPCRlibQnt" to "https://nebiocalculator.neb.com/#!/qPCRlibQnt" on page 8 step 3.2. Added "The following equation is used to adjust the concentration for size, where the size of the DNA standards is 200 bp.: Size-adjusted concentration = concentration * (size of DNA standards (bp) / library size (bp))" to page 8 step 3.2 bullet 2.	07/25