UG Catalog: TM-581 & TM-582
Version: U23E15003

User Guide

TailorMix Dual Indexed PhiX Control Library (Non-Denatured)

Introduction

SeqMatic's dual indexed PhiX control library is used as a control for Illumina sequencing instruments. Our custom designed dual-indexed library eliminates contamination of PhiX sequencing reads in sample data, which enables the generation of cleaner raw data in multiplexed sequencing run.

Features

- **100% Compatibility:** Can be used in place of Illumina PhiX V3 in all Illumina systems, including platforms using **ExAmp cluster generation**.
- **High diversity:** Contains randomized inserts with 45% GC content and free of adapter dimers*. Can be used to supplement low diversity sequencing runs.
- **Unique dual index barcode:** Custom designed index sequences compatible with all Illumina sample prep kits.

Ordering Information

Catalog #	Supplied Volume
TM-581	Pack of 6 (10µL)
TM-582	Pack of 12 (10µL)

Related Product

TailorMix Ready-to-Use Dual-Indexed PhiX Control Library, Denatured

- Designed for MiSeq, MiniSeq, NextSeq and HiSeq 2500
- 20pM denatured library supplied in HT1 buffer

Catalog #	Supplied Volume [µL]
TM-503	Pack of 6 (200µL)
TM-504	Pack of 12 (200µL)

Visit SeqMatic.com to browse our entire line of NGS products and services.
*No bioinformatically detectable adapter dimer in sequencing data.

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Contents

- Sample type: Non-denatured Illumina adapter ligated library
- Buffer condition: TE, pH 8.0
- Concentration: 10 nM
- Average Insert size: 380 bp

Storage Recommendations

The TailorMix Dual-Indexed PhiX Control Library is stable when stored at -20°C. Storage at -80°C is recommended for long term storage.

Best Practices

- Do not store the TailorMix Dual-Indexed PhiX Control Library in frost-free freezers.
- Aliquots should be made to allow for use to minimize repeated freeze/thaw cycles.
- Determine the appropriate aliquot volume based on usage.
- Aliquots that are in use can be stable for up to one month if kept on ice at all times during use and stored at temperature of -20°C or lower when not in use.

Consumables Preparation

Please make sure all consumables and equipment are available before starting this experiment.

Consumables and Equipment	Supplier
Micropipettor	General lab supplier
Micropipettor Tips	General lab supplier
HT1 Buffer	Illumina
Microcentrifuge tubes	General lab supplier

Procedures

1. Spike in non-denatured Dual-Indexed PhiX Control Library to non-denatured sample libraries according to the desired target ratio.

2. Denature and dilute the library mix according to Illumina standard operating procedures.

3. Recommended ratio for the Dual-Indexed PhiX Control Library:

    1. **For standard TruSeq and Nextera libraries:**

        i. Add 1% of the denatured Dual-Indexed PhiX control library to your sample as a positive control for QC purposes.

    2. **For miRNA and Small RNA libraries:**

        i. Add 5% of the denatured Dual-Indexed PhiX control library to your sample as a positive control for QC purposes.

    3. **For low diversity amplicon libraries:**

        i. Add 15-25% of the denatured Dual-Indexed PhiX control library to your sample as a positive control for QC purposes and for increasing diversity of your samples.

        Note: *Optimal ratio of Dual-Indexed PhiX control library in the sequencing run could be different for each amplicon library.*

4. Load your sample onto the sequencing or clustering instrument according to Illumina standard operating procedures for your instrument.

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Sequencing Sample Sheet Setup

The TailorMix Dual-Indexed PhiX control library contains dual-indexed barcodes which can be demultiplexed with all Illumina compatible demultiplexing software. To monitor the percentage of PhiX reads in a sequencing run, include the TailorMix Dual-Indexed PhiX barcodes on the sequencing Sample Sheet for demultiplexing.

If the TailorMix Dual-Indexed PhiX barcodes are not included on the sequencing Sample Sheet, the PhiX reads will be parsed into a folder named "Unknown" or "Undetermined" along with any other reads with index sequences not defined in your sample sheet.

For individual downstream analysis of the Dual-Indexed PhiX library, your sample sheet can be modified to demultiplex the PhiX reads into its own individual set of FASTQ read files. Simply add a new line to your sample sheet file containing the sequences below as your index barcodes.

For single index read sequencing runs, only use the i7 Index 1 barcode sequence. The sequence length may also be adjusted as necessary to match the length of the index read.

	i7 Index 1 barcode	i5 Index 2 barcode		
	All Illumina systems	**Forward orientation for use with:** • MiSeq • HiSeq 2000/2500	**Forward orientation for use with*:** • NextSeq 1000/2000 • NovaSeq X	**Reverse orientation for use with:** • iSeq • MiniSeq • NextSeq 500 • HiSeq 3000/4000 • NovaSeq 6000 (V1.5 Reagents)
10 bp	TCGAATGATC	GACATGCGAC	ACGACATGCG	CGCATGTCGT
8 bp	TCGAATGA	GACATGCG	ACGACATG	CGCATGTC
6 bp	TCGAAT	GACATG	ACGACA	CGCATG

*The forward orientation should be used for NextSeq 1000/2000 and NovaSeq X using onboard DRAGEN demultiplexing. For demultiplexing with standalone pipelines, the reverse orientation should be used.

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