TriLink BIOTECHNOLOGIES
part of Maravai LifeSciences

CleanTag® Small RNA Library Prep for NGS Using Barcode Convert Sets with Torrent Suite Software

Application Note

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Introduction

Library preparation from small RNAs poses technical barriers not prevalent in transcriptomic library preparation for next generation sequencing (NGS). Small RNAs are generally smaller than 100 nucleotides, are not well-recovered from samples using common isolation methods and are too short for effective random priming. Additionally, ligation technology applied to small RNAs faces a widespread problem of adapter dimer formation that interferes with sequencing and must be purified away or otherwise ameliorated (1).

The CleanTag® Small RNA Library Prep Kit uses chemically modified adapters to convert small RNA to a library of larger RNA molecules by adapter ligations to the RNA termini, followed by reverse-transcription and then PCR to a corresponding DNA library for next-generation sequencing (NGS). CleanTag technology for adapter ligation is unique in that the problem of adapter-dimer 'contamination' otherwise encountered with small library preparation is strongly suppressed through the use of optimized structure modifications at the termini of the adapter molecules (2). This simplifies the library workflow and speeds the process, reducing hands-on and overall time.

The CleanTag Small RNA Library Prep Kit is designed for use with either Illumina® sequencers or the Ion Torrent™ sequencers using separately purchased RT Primer and PCR primers compatible with the two respective sequencer technologies. The Barcode Convert Sets each include the RT primer, 12 barcoded (indexed) forward-PCR primers and a reverse-PCR primer. A total of 24 barcodes are available in the two Barcode Convert Primer Sets (Table 1). Applicable Ion Torrent sequencers include the Ion Proton™, Ion Personal Genome Machine™ (PGM™), and IonS5™ and S5™ XL systems.

Table 1. Ion Torrent Compatible Kit and Primer Barcode Sets

Catalog No.	Product
L-3206-24	CleanTag® Small RNA Library Prep Kit
L-3210-24	Barcode Convert Primer Set 1 for Ion Torrent™
L-3211-24	Barcode Convert Primer Set 2 for Ion Torrent™

Chapter 1: Key Features of Adapter Ligations

Due to differences in the Ion Torrent and Illumina sequencing technologies, the Barcode Convert Primers were designed accordingly to be used with the NGS hardware and Torrent Suite software system. Figure 1 illustrates the key differences in generation of the amplicon structure for the two systems when using the CleanTag Small RNA Library Prep kit. Barcode Convert indexes are placed on the PCR amplicon when forward primer hybridizes to the complementary 5'-adapter sequence of cDNA generated by reverse-transcription of the adapter-modified library. The amplicons extend and replicate during the PCR step of library prep. The amplicon library is then purified for use in sequencing.

Figure 1. Index Incorporation by PCR into amplicon libraries

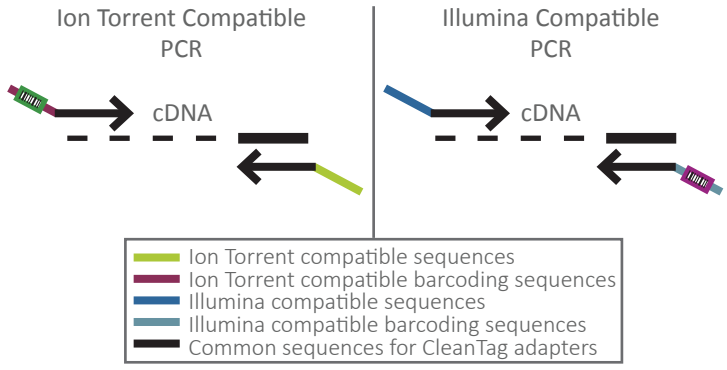

Figure 2, below, represents a single strand of the amplicon from library preparation using Index 1 included in Barcode Convert Primer Set 1. When using Ion Torrent sequencers with the CleanTag Small RNA Library Kit and Barcode Convert Sets the user must initially set up Torrent Suite software parameters specific to TriLink's adapter sequences and the related PCR primers.

Figure 2. Representative DNA strand of library amplicon

Chapter 2: Implementation

The CleanTag® Small RNA Library Prep Kit (L-3206) can be used with indexed primers Barcode Convert Primer Set 1 (L-3210) and Barcode Convert Primer Set 2 (L-3211) when sequencing will be carried out using Ion Torrent sequencers. To facilitate small RNA analysis of the sequencing data certain parameters in the Torrent Suite software that correspond to the use of TriLink's CleanTag Adapter and Barcode Convert Primer sequences must be input prior to sequencing run. In addition, a downloadable plug-in for use with Torrent Suite software is available from ThermoFisher for small RNA Analysis that aligns reads to mature micro RNAs using the tmap or bowtie2 alignment software. By making these analysis parameter changes, small RNA sequencing data may be routinely obtained using the CleanTag Small RNA Library Prep Kit and Barcode Convert Primers.

A. Components

CleanTag® Small RNA Library Prep Kit (L-3206)
Barcode Convert Primer Set 1 for Ion Torrent™ (L-3210)
Barcode Convert Primer Set 2 for Ion Torrent™ (L-3211)
IonExpress™ TriLink Index File (TriLinkBarcodeConvert_IonExpress.csv available at trilinkbiotech.com)

B. Instruction Guide

Raw sequence data from the Ion Proton™ system, Ion Personal Genome Machine™ (PGM™) and Ion S5™ and S5™ XL systems may be used. The parameter changes outlined below apply to small RNA sequence data analysis using Torrent Suite versions 5.2 through the current version, 5.10. Barcode sequences are provided in a comma delimited file, the IonExpress TriLink Index File, as described below.

1. Install the TriLink Barcode Convert Set. TriLink 5' sequences differ from Life Technologies small RNA adapter/primer sequences in that there is no universal overhang (GAT), so Torrent Suite software must be informed by uploading the new index primer information, even though the same index sequences are used by both companies. In the Torrent Browser, go to the upper right widget, References, Barcode Sets. Upload the barcode set IonExpress TriLink Index File. Note that this file set has 96 indexes, although at this time TriLink only offers 24 indexed primers for use with the CleanTag® Small RNA Library Prep Kit.
2. Create the CleanTag 3' Adapter for trimming the ends of the reads. Go to the admin panel. Widget > Config > Admin Control Panel > 3' Adapters. Create a new one and call it:
   TGGAATTCTCGGGTGCCAAGGATCACCGACTGCCCATAGAGAGGCTGAGAC
   Note: When you reanalyze the report, be sure to select this 3' adapter in the drop down.
3. Trim the 26-base 5' adapter from the sequence by a hard clip method. Add the information below to the Basecaller field when you analyze the run using the following syntax:
   --extra-trim-left 26--min-read-length
   You may decide that a larger minimum read length than 5 is more appropriate
4. Shift the polyclonal analysis window due to the homology early in the read. Thus, add the following string to the advanced Analysis window using the following syntax:
   --mixed-first-flow 71--mixed-last-flow 131

Chapter 3: Small RNA Bioinformatics Workflow

The small RNA bioinformatics pipeline has five major tasks to perform once sequences (reads) are acquired from the sequencing system, as described by Life Technologies (3).

1. 3'-end quality/adapter trimming preprocessing
2. Quality control/assessment
3. Mapping to a reference genome and or transcripts
4. Counting mapped reads
5. Generation of global mapping statistics

Some adjustments are needed depending on your specific sequencing needs. The CleanTag kit library will include small RNAs terminated with 5'-phosphate and 3'-OH. Thus, if you seek to map small RNAs other than miRNA, data preprocessing will need to increase the maximum read length set in the fastq quality trimmer tool longer than the prescribed 35 recommended for miRNA. Small RNA is typically considered to be <200 nt. Once FastQC has been run on the trimmed FASTQ file the alignment tool in Torrent Suite is used for sequence mapping against a suitable sequence database.

References

1. Francois Vigneault, Dmitry Ter-Ovanesyan, Shahar Alon, Seda Eminaga, Danos C. Christodoulou, J. G. Seidman, Eli Eisenberg, George M Church. High-throughput multiplex sequencing of miRNA. Curr Protoc Hum Genet. 2012 Apr; 0 11: Unit–11.1210. doi: 10.1002/0471142905.hg1112s73
2. Shore S, Henderson JM, Lebedev A, Salcedo MP, Zon G, McCaffrey AP, Paul N, Hogrefe RI. Small RNA Library Preparation Method for Next-Generation Sequencing Using Chemical Modifications to Prevent Adapter Dimer Formation. PLoS One. 2016 Nov 22;11(11):e0167009. eCollection 2016.
3. Methods, tools, and pipelines for analysis of Ion PGM™ Sequencer miRNA and gene expression data. 2012, Ion RNA-Seq white paper, 2012 Life Technologies Corporation
4. IonExpress™ TriLink Index File (TriLinkBarcodeConvert_IonExpress.csv) www.trilinkbiotech.com/cleantagkit

Contact Information

For further information on CleanTag® Small RNA Library Prep Kit, please visit our website or contact TriLink directly.
www.trilinkbiotech.com/cleantag
Phone: (800) 863-6801 or (858) 546-0004
email: support@trilinkbiotech.com