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PCR Add-on Kit for Ion Torrent

Instruction Manual

Catalog Numbers:
012 (QuantSeq 3′ mRNA-Seq Library Prep Kit for Ion Torrent)
022 (Purification Module with Magnetic Beads)

021IM066V0100

1. Overview

This instruction manual outlines the protocol for the PCR Add-on Kit for Ion Torrent (Cat. No. 021.96).

It contains a PCR Mix (**PCR** ●) including Ion Torrent platform specific primers, a thermostable polymerase (**E** ●) for 96 PCR reactions used to amplify QuantSeq 3′ mRNA-Seq libraries (Cat. No. 012.24A and 012.24B) for Ion Torrent. Cycle numbers for the endpoint PCR may differ depending on the RNA used. Therefore, we recommend diluting the sample and perform more PCR reactions. to determine the exact number of cycles for the endpoint PCR in order to prevent any under- or overcycling of your library. Undercycling may result in too little library and overcycling can lead to significant distortions in gene expression values. If any sample is undercycled, the PCR Add-on Kit can furthermore be used to add more cycles and reamplify your library.

2. Kit Components and Storage Conditions

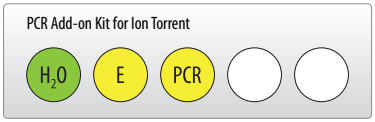

Figure 1. Location of kit components.

Kit Component	Tube Label	Volume*	Storage
PCR Mix	**PCR** ●	1267.2 µl	-20 °C
Enzyme Mix	**E** ●	105.6 µl	-20 °C
H_2O	**H_2O** ●	1500 µl	-20 °C

*including a 10 % surplus

NOTE: The Enzyme Mix (**E** ●) is the same as the Enzyme Mix 3 (**E3** ●) in the QuantSeq kits and can be interchangeably used.

3. PCR

PCR	
PCR **E**	– thawed at RT – keep on ice or at -20 °C **ATTENTION**: Spin down all solutions before opening tubes!
Thermocycler	98 °C, 30 sec 98 °C, 10 sec 65 °C, 20 sec 72 °C, 30 sec } 13 x for 500 ng RNA input see QuantSeq 3' mRNA-Seq User Guide for Ion Torrent for different input amounts, p.20 72 °C, 1 min 10 °C, ∞

1. Dilute the double stranded library from step 25 of the QuantSeq 3' mRNA-Seq protocol for Ion Torrent to 34 µl by adding 17 µl **H_2O** ● (1:2 dilution).

2. Prepare a mastermix containing 12 µl of PCR Mix (**PCR** ●) and 1 µl Enzyme Mix (**E** ●) per reaction.

3. Add 13 µl of this **PCR / E** mastermix to 17 µl of the eluted library. Mix well by pipetting. Seal the plate, and quickly spin down to make sure all liquid is collected at the bottom of the well.

4. Conduct 13 cycles of PCR with the following program: Initial denaturation at 98 °C for 30 seconds, 13 cycles of 98 °C for 10 seconds, 65 °C for 20 seconds and 72 °C for 30 seconds, and a final extension at 72 °C for 1 minute, hold at 10 °C. **NOTE:** A list of recommended cycle numbers for RNAs from a variety of organisms and tissues can be found at www.lexogen.com in the Frequently Asked Questions (FAQ) section of the QuantSeq webpage. When diluting your sample please keep in mind to add 1 more cycle for the 1:2 dilution.

5. Purify your sample using the Purification Module with Magnetic Beads (Cat. No. 022.96) following the instructions given in the QuantSeq 3'mRNA-Seq User Guide for Ion Torrent.

6. Check the quality of the libraries on a microfluidics device (such as Agilent 2100 Bioanalyzer), qPCR or gelelectrophoresis. If the libraries show a second peak in high molecular regions your sample is overcycled and you should use less PCR cycles for the remaining half of your sample. If the yield is too low, add 1 - 5 PCR cycles (yield approximately doubles with each cycle).

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