BioChain®
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User's Manual and Instructions

RapidSeq™ MasterMix Directional mRNA Sample Prep Kit

Catalog Number:

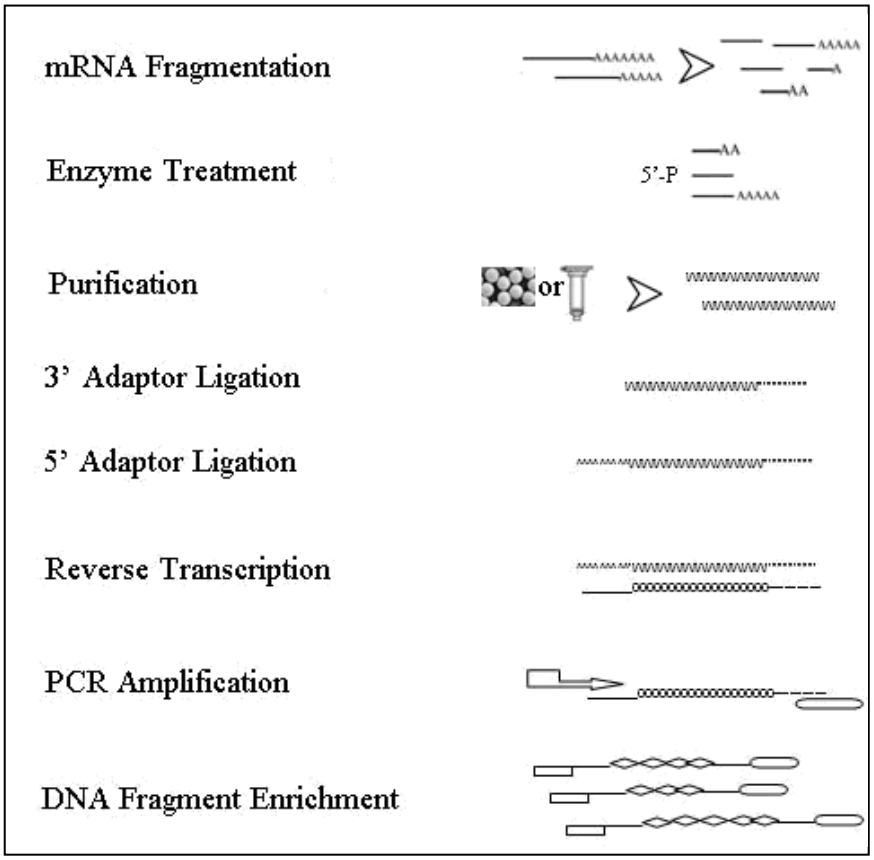
- **KS075012**
- **KS075012-I**
- **KS075012-II**
- **KS075012-III**
- **KS075012-IV**

Introduction

Analysis of differential RNA expression helps us to understand biological pathways and molecular mechanisms which involved in regulation of cell fate, development, and disease progression. Sequencing technologies provide a powerful tool for transcriptome analysis. Next-generation sequencing (NGS) has great advantages over conventional methods, such as tremendously reduced sequencing costs and increased coverage. Transcriptome sequencing (RNA-seq) is a novel method for gene expression studies by NGS of transcripts. Advantages of RNA-Seq are without bias toward known RNA molecules as with probe-based technologies, ability to detect novel rare transcripts, novel alternative splice isoforms and direct measurement of transcript abundance within biological samples.

This manual aims to prepare directional NGS libraries for subsequent cluster generation, using purified mRNA as start material. The protocol includes steps for mRNA fragmentation, purification, adapter ligation, reverse transcription, PCR amplification, and DNA fragment enrichment to generate strand specific library product compatible with illumina NGS platform (Figure 1).

Figure 1. Workflow Chart of Directional mRNA NGS Library Construction



BioChain also provides other tools and services to researchers interested in using NGS technologies. Please contact BioChain Technical Support for further details.

Features

- Simple workflow - most components are supplied as ready-to-use master mixtures which reduces setup time and liquid handling steps.
- Great performance - comparable quality with market leading products.
- Wide dynamic range - purified mRNA could be down to 50 ng.

Applications

- Expression of all coding RNAs.
- Identification of alternative splicing events.
- Detection of single nucleotide polymorphisms or mutations.
- Discretion of translocations and fusion transcripts.
- Discovering of allele specific expression patterns

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Description

Components in this kit are prepared with pure chemicals to construct NGS libraries compatible with Illumina's sequencing platform for subsequent cluster generation, using purified mRNA as input. Four sets of the kit with different 4 sets of 12 aligners are available, respectively.

Quality Control

At least one kit of each lot has been tested for directional mRNA NGS library construction using purified mRNA from Adult Normal Lung Tissue Total RNA (Cat# R1234152-50) processed by BioChain's MagSeq mRNA Purification Kit (Cat# K2012008). Good coverage and directionality are observed.

Components

One kit with aligner has 3 boxes listed in below; only one aligner box is included in one kit. (see table 1-3 below). Reagents are sufficient for 12 assays. The kit without aligner only includes 2 boxes.

Table 1. Contents List of RapidSeq™ MasterMix Directional mRNA Sample Prep Kit (Box 1 of 2, Store at -20ºC)

Cap Color	Item	Amount in kit	Part No.
Nature	Fragmentation Buffer	30 µl	KS073012-1
Amber	Fragmentation Enzyme Mix	70 µl	KS073012-2
Green	Tail Oligo	14 µl	KS071012-1
	Tail MasterMix	53 µl	KS071012-2
	Ligation Enhancer	13µl	KS071012-3
Red	Cap Oligo	14 µl	KS071012-4
	Cap MasterMix	26.5 µl	KS071012-5
Yellow	RT Oligo	14 µl	KS071012-6
	RT MasterMix	75 µl	KS071012-7
Blue	Universal Primer	28 µl	KS071012-8
	PCR MasterMix	340 µl	KS071012-9
Orange	DNA Storage Solution	1500 µl	LB3401010

Table 2. Contents List of RapidSeq™ MasterMix Directional mRNA Sample Prep Kit (Box 2 of 2, Store at -20º C)

KS072012-I

Item	Amount in kit (µl)	Part No.	Sequence
Aligner 1	10	KS072012-1	ATCACG
Aligner 2	10	KS072012-2	CGATGT
Aligner 3	10	KS072012-3	TTAGGC
Aligner 4	10	KS072012-4	TGACCA
Aligner 5	10	KS072012-5	ACAGTG
Aligner 6	10	KS072012-6	GCCAAT
Aligner 7	10	KS072012-7	CAGATC
Aligner 8	10	KS072012-8	ACTTGA
Aligner 9	10	KS072012-9	GATCAG
Aligner 10	10	KS072012-10	TAGCTT
Aligner 11	10	KS072012-11	GGCTAC
Aligner 12	10	KS072012-12	CTTGTA

KS072012-II

Item	Amount in kit (µl)	Part No.	Sequence
Aligner 13	10	KS072012-13	AGTCAA
Aligner 14	10	KS072012-14	AGTTCC
Aligner 15	10	KS072012-15	ATGTCA
Aligner 16	10	KS072012-16	CCGTCC
Aligner 17	10	KS072012-17	GTAGAG
Aligner 18	10	KS072012-18	GTCCGC
Aligner 19	10	KS072012-19	GTGAAA
Aligner 20	10	KS072012-20	GTGGCC
Aligner 21	10	KS072012-21	GTTTCG
Aligner 22	10	KS072012-22	CGTACG
Aligner 23	10	KS072012-23	GAGTGG
Aligner 24	10	KS072012-24	GGTAGC

KS072012-III

Item	Amount in kit (µl)	Part No.	Sequence
Aligner 25	10	KS072012-25	ACTGAT
Aligner 26	10	KS072012-26	ATGAGC
Aligner 27	10	KS072012-27	ATTCCT
Aligner 28	10	KS072012-28	CAAAAG
Aligner 29	10	KS072012-29	CAACTA
Aligner 30	10	KS072012-30	CACCGG
Aligner 31	10	KS072012-31	CACGAT
Aligner 32	10	KS072012-32	CACTCA
Aligner 33	10	KS072012-33	CAGGCG
Aligner 34	10	KS072012-34	CATGGC
Aligner 35	10	KS072012-35	CATTTT
Aligner 36	10	KS072012-36	CCAACA

KS072012-IV

Item	Amount in kit (µl)	Part No.	Sequence
Aligner 37	10	KS072012-37	CGGAAT
Aligner 38	10	KS072012-38	CTAGCT
Aligner 39	10	KS072012-39	CTATAC
Aligner 40	10	KS072012-40	CTCAGA
Aligner 41	10	KS072012-41	GACGAC
Aligner 42	10	KS072012-42	TAATCG
Aligner 43	10	KS072012-43	TACAGC
Aligner 44	10	KS072012-44	TATAAT
Aligner 45	10	KS072012-45	TCATTC
Aligner 46	10	KS072012-46	TCCCGA
Aligner 47	10	KS072012-47	TCGAAG
Aligner 48	10	KS072012-48	TCGGCA

Storage and Stability

Upon receipt, store all reagents appropriately. Avoid repeated freeze/thaw cycles. This kit is stable for half a year after shipping date.

Protocol

Consumables Preparation

The kit has all key reagents to run experiment but not common consumables and instruments. Please make sure all needs are available before starting this protocol (Table 3).

Table 3. List of Consumables and Equipments

Consumables and Equipments	Supplier
0.2 ml, 1.5 ml, and 2 ml clean, nuclease - free microcentrifuge tubes	General lab supplier
200 µl, clean, nuclease - free PCR tubes	General lab supplier
Nuclease-free Water	General lab supplier
Ethanol	General lab supplier
100 mM EDTA	General lab supplier
RNA clean kit	Zymo Concentrator-5 (R1015) or Qiagen MinElute (74204)
DNA clean kit	Zymo Concentrator-5 (D4003) or Beckman Coulter Genomics Agencourt AMPure XP Beads (A63880/1/2)
Magnetic stand	General lab supplier
NanoDrop	Thermo Scientific
Thermal cycler	General lab supplier
Vortexer	General lab supplier
Benchtop microcentrifuge	General lab supplier
2100 Bioanalyzer	Agilent
DNA 1000 chip	Agilent, 5067 - 1504
High Sensitivity DNA chip (optional)	Agilent, 5067 - 4626

Cautions

1. This product is for ***Research Use Only***.
2. Close adherence to the protocol will assure optimal performance and reproducibility.
3. Set up reactions in sterile, nuclease - free tubes on ice.
4. Prepare 10% extra mixture when running multiple samples.
5. Care should be taken to ensure nuclease - free processing.
6. Due to the analytical sensitivity of this test, extreme care should be taken to avoid the contamination of reagents.
7. The assay kit should be used as a system. Do not substitute other manufacturer's reagents. Dilution, reducing reaction volumes, or other deviation in this protocol may affect the performance of this testing kit.
8. Do not mix or combine reagents from kits with different lot numbers.
9. Materials are stable until the labeled expiration date when stored and handled as directed. Do

not use kits beyond their expiration date.

RNA Input

1. This protocol has been optimized using purified 100 ng of high quality human lung mRNA as input. Messenger RNA populations can vary significantly between different tissue types and species. Use of mRNA from other species, tissues, or qualities may require further optimization.
   For low amount and poor quality RNA sample, please choose RapidSeq™ High Yield Directional mRNA Sample Prep Kit (Cat# KS073012).
2. BioChain recommends using Adult Lung Tissue mRNA (catalog #M1234152) as a positive control sample for this protocol.

mRNA Fragmentation

Pre-heat the thermal cycler to 94°C.
1. Prepare mRNA sample (50~100 ng) in a sterile, nuclease-free 200 µl PCR tube as total volume at **16** µl.
2. Add **2** µl Fragmentation Buffer, Gently pipette the entire volume up and down 6–8 times to mix thoroughly, then centrifuge briefly.
3. Incubate the tube at 94°C for 5 minutes and then immediately place the tube on ice.
   (Note: The fragmentation time sometimes need to be optimized from shorter period since some of the fragile RNAs can be completely degraded or fragmented into very short sizes)
4. Immediately add **2** µl 100 mM EDTA to the tube.

Fragmentation Enzyme Treatment

1. Add **5** µl Fragmentation Enzyme Mix to the fragmentation reaction tube. Gently pipette the entire volume up and down 6–8 times to mix thoroughly, then centrifuge briefly.
2. Incubate the tube at 37°C for 60 minutes and then place the tube on ice.

Treated mRNA Purification

Recommend use Zymo RNA Clean & Concentrator-5 or Qiagen RNeasy MinElute Cleanup kit according manufacturer's instruction. Use 10 µl nuclease-free water for elution.

Pooling

Each RapidSeq™ MasterMix Directional mRNA Sample Prep Kit can be used to construct libraries that are compatible with illumina multiplexing. While processing samples in parallel, incorporate the index at the amplification step following reverse transcription. Samples could be pooled immediately prior to DNA fragment enrichment or make pools of samples after that.

Library Preparation

Pre-heat the thermal cycler to 70°C and pre-heat another thermal cycler to 28°C if available.
1. Briefly centrifuge the thawed reagents at 600 xg for 5 seconds, then place them on ice.
2. Prepare purified Fragmentation Enzyme treated mRNA sample for total volume at **5** µl (use Nuclease-free Water as dilution if necessary) in a sterile, nuclease-free 200 µl PCR tube on ice.
3. Add **1** µl Tail Oligo into RNA tube. Gently pipette the entire volume up and down 6–8 times to mix thoroughly, then centrifuge briefly.
4. Incubate the tube at 70°C for 2 minutes and then immediately place the tube on ice.

5. Add **4** µl of Tail MasterMix to the reaction tube. Gently pipette the entire volume up and down 6–8 times to mix thoroughly.
6. Incubate the tube at 28°C for 1 hour.
7. Directly add **1**ul Ligation Enhancer into each reaction tube remaining on the thermal cycler, gently pipette the entire volume up and down 6–8 times to mix thoroughly, continue incubate the tube at 28°C for 15 minutes and then place the tube on ice.
8. Aliquot **1** µl Cap Oligo into a separate, nuclease-free 200 µl PCR tube, incubate at 70°C for 2 minutes and then immediately place the tube on ice.
9. Add **2** µl of Cap MasterMix to Cap Oligo tube for each reaction. Gently pipette the entire volume up and down 6–8 times to mix thoroughly.
10. Transfer these **3** µl of the Cap mixture to the Tail reaction tube. Gently pipette the entire volume up and down 6–8 times to mix thoroughly.
11. Incubate at 28°C for 1 hour and then place the tube on ice.
12. Aliquot **6** µl of the whole reaction into a separate, sterile, nuclease-free, 200 µl PCR tube. (Left could be stored at -80°C.)
13. Add **1** µl RT Oligo. Gently pipette the entire volume up and down 6–8 times to mix thoroughly, then centrifuge briefly.
14. Incubate at 70°C for 2 minutes and then immediately place the tube on ice.
15. Pre-heat the thermal cycler to 50°C.
16. Add **5.5** µl of RT MasterMix. Gently pipette the entire volume up and down 6–8 times to mix thoroughly, then centrifuge briefly.
17. Incubate at 50°C for 1 hour and then place the tube on ice.
18. In a separate, sterile, nuclease-free, 200 µl PCR tube, set up PCR mixture as below.

Mixture	**µl**
PCR MasterMix	25
Universal Primer	2
Aligner*	2
Nuclease-free Water	8.5
Total	**37.5**

* For each reaction, only one of the 48 Aligners is used during this step.

Gently pipette the entire volume up and down 6–8 times to mix thoroughly, centrifuge briefly, then place the tube on ice.

19. Transfer this **37.5** µl mixture to the RT reaction tube. Gently pipette the entire volume up and down 6–8 times to mix thoroughly, then centrifuge briefly and place the tube on ice.
20. Amplify the tube in the thermal cycler using the following PCR cycling conditions: 1) 98°C for 30 seconds; 2) 12 cycles of: 98°C for 10 seconds, 60°C for 30 seconds, 72°C for 15 seconds; 3) 72°C for 10 minutes; 4) hold at 4°C.

Amplification products may vary based on RNA input amount, tissue type, and species. This process was optimized using 100 ng of Adult Lung Tissue mRNA. The number of PCR cycles can be adjusted to a maximum of 15 cycles if very low amount of product.

DNA Fragment Enrichment

Recommend use Zymo DNA Clean & Concentrator-5 or Beckman Coulter Genomics Agencourt AMPure XP Beads for PCR clean up. Use 11 µl DNA storage Solution for elution.

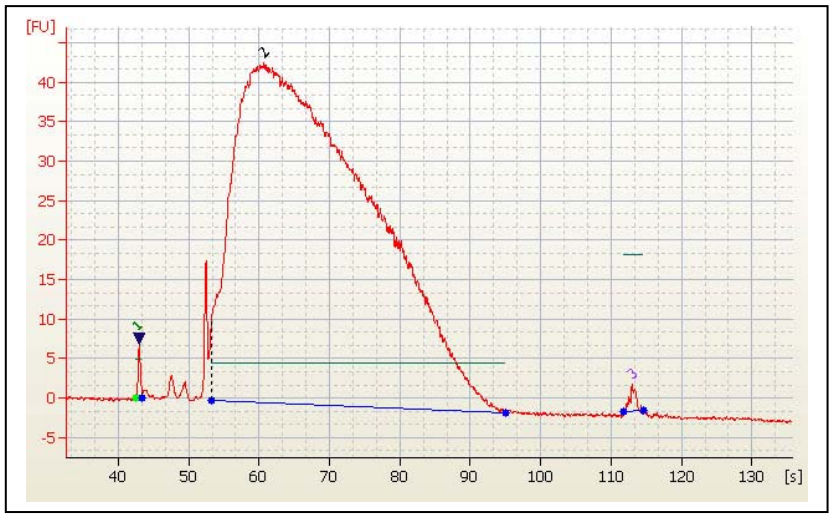
Library Validation

Agilent Technologies 2100 Bioanalyzer is highly recommended as quality control system to

validate DNA library generated from above.

1. Use **1** µl resuspended construct for DNA-1000 chip or High Sensitivity DNA chip.
2. Check the size, purity and concentration of the sample.

Figure 2. High Sensitivity DNA Chip Trace of the Final Library from an Adult Normal Lung Tissue mRNA Sample



* Peak 1: Lower Marker; Peak 2: mRNA NGS Library; Peak 3: Upper Marker

Related Products

RapidSeq™ High Yield Directional mRAN Sample Prep Kit (Cat# KS073012)
RapidSeq™ MasterMix Small RNA Sample Prep Kit (Cat# KS071012)
RapidSeq™ High Yield Small RNA Sample Prep Kit (Cat# KS074012)
MagSeq mRNA Purification Kit (Cat# K2012008)
Adult Normal Lung Tissue mRNA (Cat# M1234152)

References

1. Wang Z, *et al. Nature Reviews Genetics* 2009. 10:57- 63.
2. Ozsolak F. *et al. Nature* 2009. 461:814 - 818.
3. Labaj PP. *et al. ISMB* 2011. 27:i383 - i391.