University of Wisconsin Biotechnology Center
Gene Expression Center

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Client:

Funding No. Direct Invoice
Sub.No. M007087
No BRC Analysis

NEBNext Ultra II Directional mRNA (Schistosoma mansoni; NovaSeq X plus 2x150bp 10B full lane)

RNA Extraction- In house w/ DNase
RNA to be picked upon project completion

NEB Ultra II Directional mRNA Sample Preparation Guide (Document # HB-2783-001, June 2020; manualE7760_E7765 ver4.0_4/21)

8/15/2024 KV
Samples quantified by RNA Qubit

8/26/2024 SMSB
Reached out to client and suggested NEBNext UltraII PolyA to help dilute out the phenol contamination.

8/26/2024
Client approved moving forward with NEBNext UltraII PolyA prep.

9/6/2024 KV
Samples thawed on ice.
Samples normalized by hand

9/6/2024 KV
Stranded rgts removed from 4C or -20C and thawed according to protocol directions
Samples removed from -80C, thawed and kept on ice

Protocol: (8)

10ng total RNA input	RNA Thermal Cycler#4
Poly(A)+ enrichment (oligo(dT) bead capture) & Fragmentation (10 time)	
First & Second strand cDNA Synthesis	DNA Thermal Cycler#1
Purification of ds cDNA (SPRI Select beads lot #20562700)	

Samples stored at -20C

9/7/2024 KV

Adenylate 3' Ends	DNA Thermal Cycler#1
Ligate Adapters (UDI) Lot # 10231569	
0.8X Purification of ds cDNA (SPRI Select beads lot #20562700)	
PCR Amplification (**17 cycles**)	
0.8X Final library purification (NEB beads lot #10223634)	

9/7/2024 KV

Library QC-

Each library quantified with Qubit HS DNA Assay and on the 4200 Tapestation on a HS D1000 and D1000 Screentape—all libraries except Liv-03 have >1% AD contamination.

Libraries stored @-20C

9/12/2024 SMSB

Client notified of AD contamination. "We completed the prep Tuesday with QC Wed. AM. We were able to generate library for all samples but 7/8 have adapter/dimer contamination >1%. Attached are two Agilent pdf files with the library profiles. The peak at ~155bp is AD. The small ADs will preferentially sequence over the library template on the XP Plus pattern flowcells. As you only need 1/2 10B lane (other 1/2 is shared with another set of libraries), we pinged the DNA Seq core to see if they would accept the libraries as is for sequencing. The yields were also low for 7/8, which correlates with input. The yields are to low to try another bead purification as it's likely recovery will to be low which results insufficient template for sequencing.

As soon we hear from the DNA Seq core we will be in touch. If they say no the AD is too high to be mixed on the lane, you will have the choice of 1) buying your own full lane ($1790), or 2) not proceeding with the libraries."—Waiting for response.

9/13/2024—Client would like to proceed with sequencing of all libraries on a full 10B lane.

9/16/2024 KV

Libraries submitted to the DNA Seq Core in a 96 well plate

Final Library Table

Prep Well ID	Sample Name	Sequence	bp
A1	Liv-01	TATCAGTAAT-AGTAGTAAAC	401
B1	Liv-02	CGAGTCAGAT-TACTAAGGAC	391
C1	Liv-03	TTTCCATCAT-CATTCGGAAC	449
D1	Liv-04	CTTTAACTAT-AATCGTCAAC	407
E1	Int-01	GCCTCTATAT-GCTGATTTAC	399
F1	Int-02	CCTCCTTTAT-CGCGAAAGAC	423
G1	Int-03	CAGTTCCCAT-TTGCCACTAC	436
H1	Int-04	TACCTTGTAT-TTCGTGGAAC	418

A visible measure of a core's impact is made through proper acknowledgement of usage in publications. To acknowledge our cores, please use the following statement:

The author(s) utilized the University of Wisconsin – Madison Biotechnology Gene Expression Center (Research Resource Identifier - RRID:SCR_017757) for RNA library preparation and the DNA Sequencing Facility (RRID:SCR_017759) for sequencing.