XRNA Exosome RNA-Seq Library Kit

Cat# XRNAxxxA-1

User Manual

Store Kits at -20°C upon receipt

Contents

I. Introduction

A. Exosome Overview

Exosomes are 60 - 180 nm membrane vesicles secreted by most cell types in vivo and in vitro. These microvesicles are produced by the inward budding of multivesicular bodies (MVBs) and are released from the cell into the microenvironment following the fusion of MVBs with the plasma membrane. Exosomes are extracellular, nanoshuttle organelles that facilitate communication between cells and organs. Exosomes are found in blood, urine, amniotic fluid, breast milk, malignant ascites fluids and contain distinct subsets of RNAs and proteins depending upon the cell

type from which they are secreted, making them useful for biomarker discovery. SBI has engineered tools and provides services for exosome proteomic Mass spec analysis and next-generation sequencing of exosome RNA to accelerate the study of exosomes, exosome protein and exosome biomarkers.

B. The XRNA kits for exosome RNA-Seq NGS

The XRNA RNA-Seq Sample Preparation Kits are sourced from SeqMatic, LLC. The system offers a high sensitivity solution for generating exosome RNA (including miRNA) libraries from low concentration RNA samples. These kits enable the discovery and profiling of miRNAs from various organisms and tissues via the Illumina sequencing platform. The unique XRNA reagents and workflow have been developed for simplicity and reproducibility without sacrificing quality or yield. The kits work with exosomes isolated using ultracentrifugation as well as using ExoQuick (serum, plasma, ascites samples) or ExoQuick-TC (cell media, urine, spinal fluid) or immunopurify specific exosome subpopulations using SBI's Exo-Flow IP kits. The isolated exosomes are then lysed and the RNA purified using spin columns (we recommend **SBI's SeraMir kits**, see Table below).

Description	Size	Catalog#
SeraMir Serum/Plasma Exosome RNA Purification only kit (5ml ExoQuick and 20 exoRNA columns)	20 preps	RA806A-1
SeraMir Exosome RNA Purification only kit for Media, Urine and CSF (10ml ExoQuick-TC and 10 exoRNA columns)	10 preps	RA806TC-1
SeraMir Exosome RNA Purification Column kit (20 exoRNA columns)	20 preps	RA808A-1

XRNA Kit Features

- Wide dynamic range, requires as little as 1 ng of exosome RNA input and up to 5 ug RNA input.
- User friendly workflow, libraries can be prepared in a single day with less than one hour of hands on time.
- Comprehensive sample prep kit – most components are supplied as ready-to-use mixtures which improves consistency and reproducibility.
- The XRNA kits come complete to create barcoded exosome RNA libraries compatible with Illumina HiSeq and MiSeq instruments.
- SBI sources the XRNA kit components from SeqMatic, LLC.

C. XRNA Procedures

Materials provided:

Each XRNA Sample Preparation Kit contains one set of core reagents (8 samples), one set of 8 unique barcodes, and a gel purification kit. The core reagents and PCR barcode primers should be stored at -15°C to -25°C. The kit is designed to be stable for up to one year after the shipping date.

Set 1: Core Reagents

1.	Mix A300	5.	Mix E300
2.	Mix B300	6.	Mix F300
3.	Mix C300	7.	Mix G300
4.	Mix D300	8.	Mix H300

The RNA Control is provided as a positive control to monitor the performance of the XRNA Sample Preparation kit. The Control Library should contain a microRNA library peak at the 140-150 bp

region. The XRNA Sample Preparation Kit supports both single-end and paired-end sequencing on the Illumina platform. The Illumina built-in Read 1 primer is compatible with the XRNA libraries. A custom primer must be used for Read 2 of paired-end sequencing. The Read 2 Primer is provided in 100 µM stock in 10mM Tris.

Set 2: Oligo Set

Catalog # XRNA101A-1	Barcode Sequence	Catalog # XRNA102A-1	Barcode Sequence
PCR Primer		PCR Primer	
Custom Ladder	140,150, 500 bp	Custom Ladder	140,150, 500 bp
Barcode 1	ATCACG	Barcode 9	GATCAG
Barcode 2	CGATGT	Barcode 10	TAGCTT
Barcode 3	TTAGGC	Barcode 11	GGCTAC
Barcode 4	TGACCA	Barcode 12	CTTGTA
Barcode 5	ACAGTG	Barcode 13	AGTCAA
Barcode 6	GCCAAT	Barcode 14	AGTTCC
Barcode 7	CAGATC	Barcode 15	ATGTCA
Barcode 8	ACTTGA	Barcode 16	CCGTCC

Set 3: Gel Purification Kit

1. Gel Cutter Tool
2. Gel Breaker Tool

Consumables Preparation

The kit contains all necessary reagents to perform the experiment with the exception of common consumables and instruments. Please make sure all equipment is available before starting this experiment (see next table).

Consumables and Equipment	Supplier
1.5 ml nuclease-free microcentrifuge tubes	General lab supplier
200 µl, clean, nuclease-free PCR tubes	General lab supplier
Nuclease-free water	General lab supplier
TE buffer	General lab supplier
Tween-20	General lab supplier
Cooler block	SeqMatic, 6388-001
Thermal cycler	General lab supplier
Agencourt AMPure XP beads	Beckman Coulter Genomics, A63881
Ethanol	General lab supplier
Magnetic stand-96	Life Technologies, AM10027
5X TBE buffer	General lab supplier
8% TBE Gels 1.0 mm, 10 Well	LIFE Technologies, EC6215BOX
Hi-Density TBE Sample Buffer (5X)	LIFE Technologies, LC6678
50 or 100 bp DNA Ladder	General lab supplier

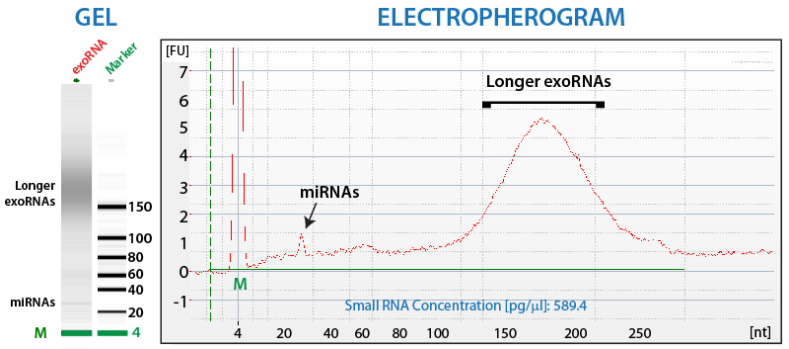
SYBR® Gold Nucleic Acid Gel Stain (Optional)	LIFE Technologies, S-11494
Ethidium Bromide 10mg/ml	BioRad, 161-0433
XCell SureLock® Mini-Cell	LIFE Technologies, EI0001
Electrophoresis power supply	General lab supplier
Dark reader transilluminator	General lab supplier
Bench top microcentrifuge	General lab supplier
Tube shaker or thermal mixer	General lab supplier
NanoDrop	Thermo Scientific
2100 Bioanalyzer	Agilent Technologies
High Sensitivity RNA and DNA chips	Agilent Technologies, 5067-4626

Best Practices

- Always wear gloves and use sterile technique.
- Set up reactions using sterile non-stick nuclease-free tubes.
- Place samples and reagents on ice at all times and avoid extended pauses.
- Reagents should be prepared using RNase-free components
- Prepare an extra 10% mixture when running multiple samples.
- Avoid repeated freeze/thaw cycles.

RNA Input

This protocol has been optimized using approximately 1 to 10 ng of purified exosome RNA. Typical exosome RNA ranges in very small sizes (20-30 nt, like miRNAs) and have a peak abundance of exoRNAs in the 150 – 250 nt size range. Due to this small exoRNA size range and low abundance, typical UV-based RNA quantitation methods like NanoDrop may not be sensitive enough to properly measure the purified exoRNA concentration. We recommend using an Agilent Bioanalyzer 2100 system with the Agilent RNA 6000 Pico Kit which is optimized for the analysis of low concentrated RNA samples down to 50 pg/µL of total RNA or 250 pg/µL of microRNA. An example of purified serum exosome RNA (1 ul of 15 ul total exoRNA from 500 ul starting serum) is shown below. SBI's ExoQuick and SeraMir kits were used to purify the exoRNA.



Serum exosomes tip: Use ExoQuick precipitation twice on a serum sample to remove some co-purifying serum proteins. To do this, take 500 ul serum, add 120 ul ExoQuick and incubate at 5°C for 30 minutes. Spin the tube for 3 minutes at highest speed.

Discard supernatant. Resuspend the exosome pellet in 250 ul 1x PBS and add 60 ul ExoQuick. Incubate at 5°C for 30 minutes and spin for 3 minutes at maximum speed to pellet the exosomes. These are now ready for exoRNA purification. If you have plasma samples, please defibrinate using SBI's cat# TMEXO-1 kit.

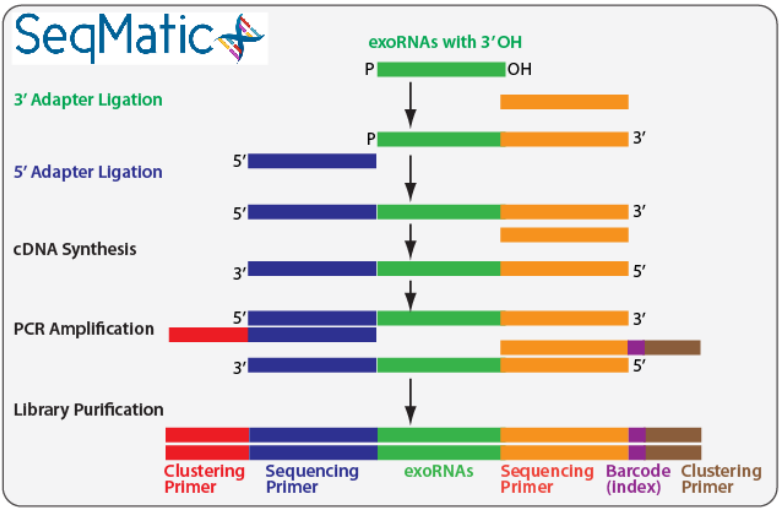
Media/Urine/CSF exosomes tip: For studying exosomes in media from cells in culture, you should grow your cells in the absence of bovine FBS. SBI offers bovine exosome-depleted FBS for this purpose (cat# EXO-FBS-50A-1). Urine and CSF samples should be pre-spun at 3,000 xg to pellet cellular debris prior to exosome isolation with SBI's ExoQuick-TC (cat# EXOTC10A-1).

NOTE:

ExoQuick and ExoQuick-TC for exosome isolation purposes are not provided in the XRNA kits and can be purchased separately. The following ExoQuick products are recommended for exosome concentration prior to Exo-Flow purification.

XRNA Library Workflow Overview

The XRNA kits have a user friendly workflow that allows the preparation of exosome RNA Illumina® NGS libraries for sequencing in a single day with minimal hands on time. All reagents are supplied as optimized, ready-to-use mixtures. The XRNA kits include specialized plastics and gel cutters to streamline gel purification of amplified NGS libraries. The kits are fully compatible with Illumina® sequencing platforms, including HiSeq, MiSeq, and Genome Analyzer II.



XRNA Sample Library Prep Protocol

3' Adapter Ligation

1. Allow Mix C300 to equilibrate to room temperature for 30 minutes before use.

2. Pre-heat the thermal cycler to 70°C and pre-heat another thermal cycler to 25°C if available.

3. Denature the RNA Sample by assembling the following components in a sterile 200 µl PCR tube:

Reagent	Volume (µl)
exoRNA Sample	6
Mix A300	2
Total	**8**

4. Gently pipette mix thoroughly and incubate at 70°C for 1 minute and then place the tube on ice.

5. Set up the following 3' Adapter Ligation reaction:

Reagent	Volume (µl)
Denatured RNA mix from step 4	8
Mix B300	2
Mix C300	6.5
Total	**16.5**

6. Gently pipette mix thoroughly and incubate at 25°C for 1 hour.

Ligation Product Clean Up

7. Vortex the AMPure XP beads until they are evenly resuspended.

8. Prepare 80% ethanol for wash steps.

9. Add 30 µl of AMPure XP beads to each sample. Gently pipette mix thoroughly and incubate at room temperature for 15 minutes.

10. Place the sample tube on the magnetic stand at room temperature for 5 minutes.

11. Remove and discard 40 µl of the supernatant.

12. Keep sample tube on the magnetic stand. Gently add 100 µl of 80% ethanol into each sample tube without disrupting the beads. Incubate at room temperature for 30 seconds.

13. Remove and discard 95 µl of the supernatant.

14. Repeat steps 10 and 11 once. Remove and discard all residual supernatant after the second 80% ethanol wash.

15. Air dry sample tube at room temperature for 15 minute or until the AMPure XP beads are dry.

16. Remove sample tube from the magnetic stand. Resuspend the dried AMPure XP beads in 8.5 µl of nuclease free water. Incubate resuspension at room temperature for 2 minutes.

17. Place the sample tube on the magnetic stand at room temperature for 5 minutes.

18. Transfer 7 µl of the supernatant into a fresh 200 µl PCR tube.

5' Adapter Ligation

19. Set up the following 5' Adapter Ligation reaction:

Reagent	Volume (µl)
3' Adapter Ligated RNA from step 18	7
Mix D300	3
Mix E300	2
Total	**12**

20. Gently pipette mix thoroughly and incubate at 25°C for 1 hour and then place the tube on ice.

cDNA Synthesis

21. Pre-heat the thermal cycler to 50°C.

22. Set up the following cDNA Synthesis reaction on ice.

Reagent	Volume (µl)
3' and 5' Adapter Ligated RNA from step 20	12
Mix F300	2
Mix G300	1
Total	**15**

23. Gently pipette mix thoroughly and incubate at 50°C for 1 hour and then place the tube on ice.

PCR Amplification

24. Set up the following PCR reaction in a fresh sterile 200 µl PCR tube on ice:

Reagent	Volume (µl)
cDNA from step 23	5
Mix H300	18
PCR Primer	1
Barcode Primer*	**1**
Total	**25**

Only one of the barcode primers is used for each sample.

25. Gently pipette mix thoroughly and amplify the samples in the thermal cycler using the following PCR cycling conditions:

1) 98°C for 30 seconds
2) 18-20 cycles of:
i. 98°C for 15 seconds
ii. 60°C for 15 seconds
iii. 72°C for 1 minute
3) 72°C for 5 minutes
4) Hold at 4°C

Library Purification

26. Determine the volume of TBE buffer needed and dilute 5X TBE Buffer to 1X for use in gel electrophoresis.

27. Assemble the gel electrophoresis apparatus.

28. Mix 2 µl of Custom ladder (140, 160 500 bp) with 2 µl of Hi-Density TBE Sample Buffer.

29. (Optional) Mix 2 µl of 50 or 100 bp DNA ladder with 2 µl of Hi-Density TBE Sample Buffer.

30. Add 2.5 µl of Hi-Density TBE Sample Buffer to 25 µl of PCR product and pipet mix thoroughly.

31. Load 25 µl of the PCR product into one well in the middle of the gel.

32. To ensure precise excision of the target region, load 2 µl of the Custome ladder and dye mix into two wells of the 8% PAGE gel,

bracketing each PCR product lane. Refer to the gel Figure in Step 40 for an example.

33. (Optional) Load 2 µl of the 50 or 100 bp DNA ladder and dye mix into a separate well for additional position references.

34. Run the gel for 65 minutes at 145V and immediately remove the gel from the apparatus.

Recover Purified Library

35. Prepare TE buffer with 0.1% Tween-20.

Reagent	Volume (µl)
TE buffer	9,990
Tween-20	10
Total	**10,000**

36. Open the gel cassette and stain with 1µg/ml ethidium bromide solution according to the manufacturer's instructions.

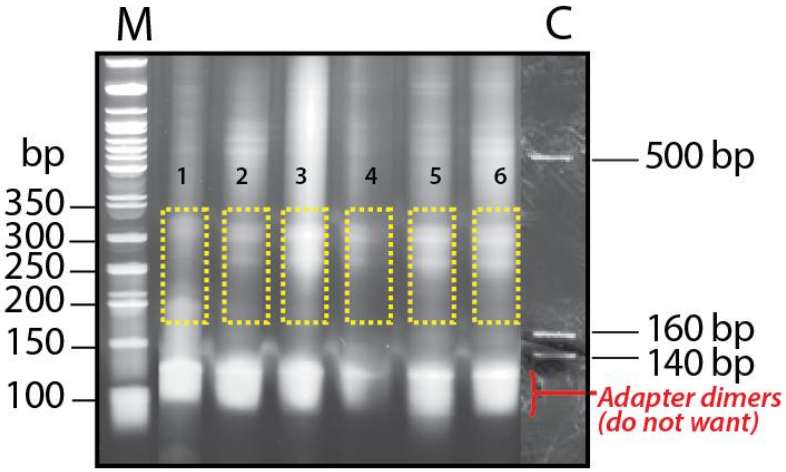
37. Place the gel on a UV Transilluminator and observe the banding pattern (see page 15).

38. (Alternative) Stain gel with SYBR Gold according to the manufacturer's instructions and observe the banding pattern on a Dark Reader Transilluminator.

39. Place the gel breaker tube into a sterile 1.5ml microcentrifuge tube or 2.0 ml collection tube.

40. Align the center of the gel cutter tool with the 160 bp band of the custom ladder on the sample lane to excise the libraries. We recommend a broader gel excision range starting from 160 bp to

about 400 bp. Press down firmly into the gel and excise the gel fragment. This can be repeated to include larger band sizes up to about 400 bp. Alternatively, use a clean scalpel and excise the desired gel region. Example of an 8% TBE gel stained with SYBR gold is shown below. The library material highlighted in by the yellow box was excised for gel-purification. **M** is a 50 bp ladder and **C** refers to the custom ladder.



41. Insert the gel cutter tool (or excised gel fragments) containing the gel slice into the gel breaker tube.

42. Briefly spin the gel cutter and gel breaker assembly. Make sure the gel slice is collected in the gel breaker tube. Discard gel cutter.

43. Add 50 µl of TE buffer with 0.1% Tween-20 to the gel breaker tube containing the gel slice. *If needed, add additional TE buffer with 0.1% Tween-20 to cover the broken gel fragments.*

44. Centrifuge the gel breaker assembly in a bench top centrifuge at maximum speed (approximately 13,000x g) for two minutes at room temperature. Ensure that all of the gel has moved through the holes into the collection tube.

45. Elute the DNA by shaking the tube at 1000 rpm at room temperature for at least one hour. The tube can be shaken overnight if desired.

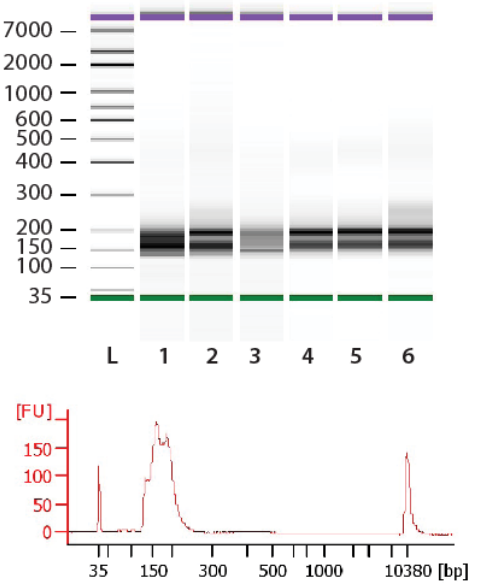
46. To collect the micro RNA library, spin the gel mix at maximum speed (approximately 13,000 xg) for 2 minutes.

47. With a P10 pipette, gently remove eluate from gel mix.

Library Validation

48. Use of an Agilent Technologies 2100 Bioanalyzer is recommended as a quality control analysis of your sample library. Use 1 µl of resuspended construct from step 45 on a High Sensitivity DNA chip to check the size, purity and concentration of the sample.

Sample Agilent Bioanalyzer 2100 High Sensitivity DNA Assay Electrophoresis File Run Summary shown to the right.



Sample Pooling

The XRNA exosome RNA NGS Sample Preparation kit is capable of multiplexing up to 16 samples into a single lane of an Illumina flow cell. Multiplexing of 6 to 8 libraries is recommended for each MiSeq v3 sequencing run. While processing multiple samples in parallel, use a unique barcode primer for each sample at the PCR step. Samples can be pooled before or after the library purification step.

NGS Data Analysis Overview

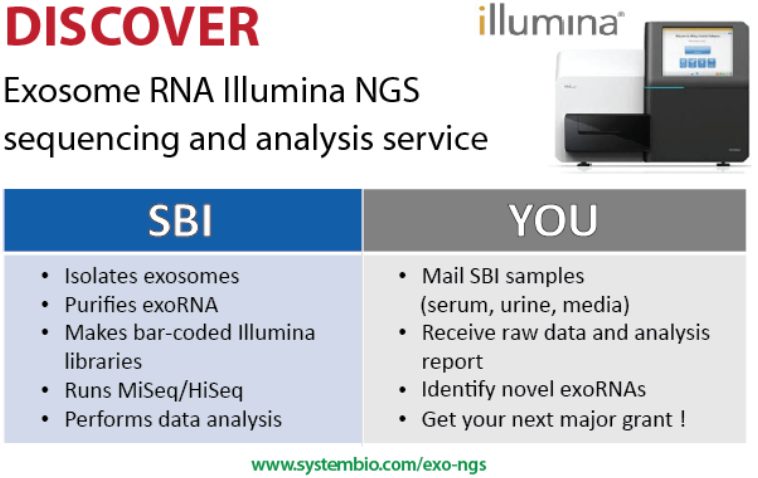
The Exosome RNA-seq Analysis (See Section D. Sample XRNA exosome RNA-Seq data) initiates with a data quality check of the input sequence using FastQC, an open-source quality control (QC) tool for high-throughput sequence data. FastQC runs analyses of the uploaded raw sequence reads that reveal the quality of the data and inform the subsequent preprocessing steps in the analysis.

Following the initial assessment, Bowtie2 is used to map the spike-in DNA before the analysis moves to the trimming and filtering steps where RNA-seq reads are preprocessed to improve the quality of data input for read mapping. The open-source tools used for trimming of adapters are FastqMcf, part of the ea-utils package, and cutadapt, with PRINSEQ used in the quality filtering step.

Maverix exosome RNA-Seq information link:
http://www.maverixbio.com/platform/

Exo-NGS Custom Services

SBI and Maverix Biomics have teamed up to provide a complete analytics solution on deep sequencing data of exosome associated RNAs. The analysis service includes library sequence quality control metrics, data analysis for relative RNA abundance and identity, differential expression analysis and visualization of the data in a cloud-based, private UCSC Genome Browser. Simplify and accelerate your exosome RNA biomarker discovery with the advanced bioinformatics analysis included in SBI's Exo-NGS service. Visit: www.systembio.com/exo-ngs



Isolating Exosome using ExoQuick, ExoQuick-TC and Exo-Flow Immunocapture kits.

Description	Size	Catalog #
ExoQuick Serum exosomes	75 rxns	**EXOQ5A-1**
ExoQuick Plasma Exosome prep	75 rxns	**EXOQ5TM-1**
Thrombin Plasma Exosome prep	100 rxns	**TMEXO-1**
ExoQuick Serum exosomes	300 rxns	**EXOQ20A-1**
ExoQuick-TC for Tissue Culture Media	10 rxns	**EXOTC10A-1**
ExoQuick-TC for Tissue Culture Media	50 rxn	**EXOTC50A-1**

Exosome isolation protocols using ExoQuick reagents

Combine your biofluid sample containing exosomes with ExoQuick or ExoQuick-TC using the guidelines shown in the Table below. Mix the ExoQuick precipitation reagent with the biofluid sample by inversion and place at 4°C for 30 minutes to overnight, then

recover the exosomes in a pellet with a low speed spin. Please refer to the ExoQuick or ExoQuick-TC User manuals for more details. Recommended amounts of exosomes provided in Table.

Biofluid	Sample volume	ExoQuick-TC volume	Resuspend exosome pellet	Volume to use in Exo-Flow
Urine	10 ml	2 ml	500 µL PBS	100 µL/rxn
Spinal fluid	10 ml	2 ml	500 µL PBS	100 µL/rxn
Culture media	10 ml	2 ml	500 µL PBS	100 µL/rxn

Biofluid	Sample volume	ExoQuick	Resuspend exosome pellet	Volume to use in Exo-Flow
Serum	**500 µL**	**120 µL**	**500 µL PBS**	**100 µL/rxn**
Plasma	**50 µL**	**120 µL**	**500 µL PBS**	**100 µL/rxn**
Ascites fluid	**500 µL**	**120 µL**	**250 µL PBS**	**100 µL/rxn**

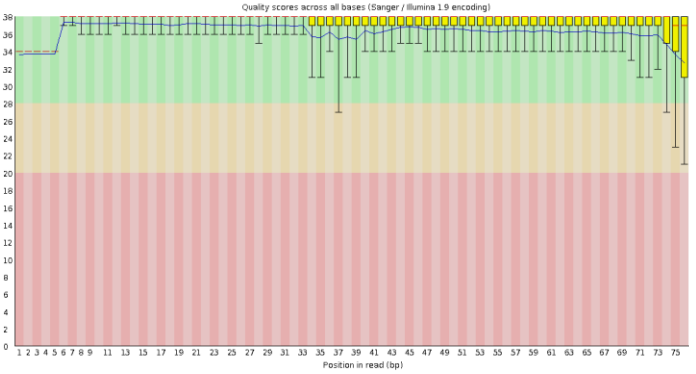
Amount of exosomes to use

The number of exosomes in a given biofluid will vary depending upon the sample itself. There are abundant levels of exosome in serum, less in cell culture medium and urine. Use the guidelines in the tables above as a starting point.

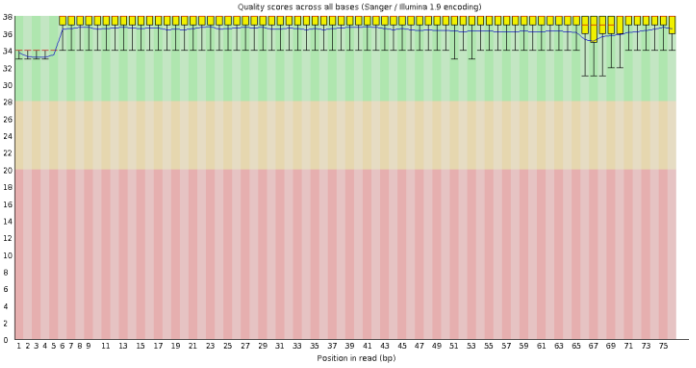
D. Sample XRNA exosome RNA-Seq data (PE 2x 75 bp)

DATA PREPROCESSING (examples)

Sequence QC Pre-trimming



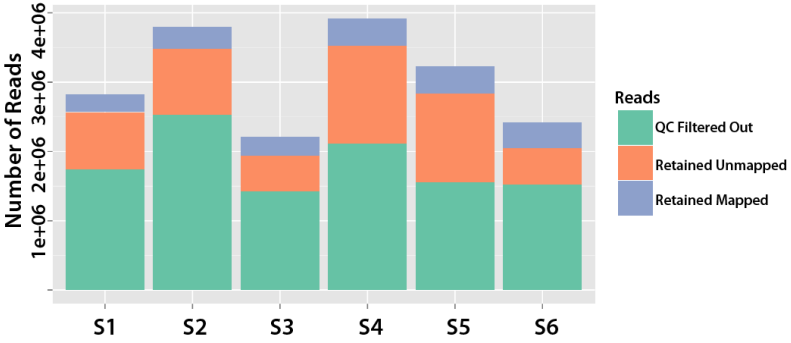
Sequence QC Post-trimming



Example Mapping Statistics summary

The improved set of sequence reads are merged, if needed, using

SeqPrep then mapped to the reference genome using Bowtie, an ultrafast, memory- efficient short read aligner, followed by generation of a mapping rate summary chart for review. Using the open-source software BEDTools and SAMtools, read alignment and read coverage tracks are generated and deployed to the genome browser.



Sequencing read mapping rate. The charts show the percentage and number of reads, respectively, for trimmed, mapped and unmapped reads for each of the samples. In most NGS runs, adapter dimers form during amplification and barcoding. These adapter sequences are filtered out and removed from the exoRNA sequences of interest before mapping to the genome of choice (i.e. Human, Mouse, Rat, etc.). A typical NGS sequence data set may filter out 50% of the reads due to QC and/or adapter sequences present.

What should I expect?

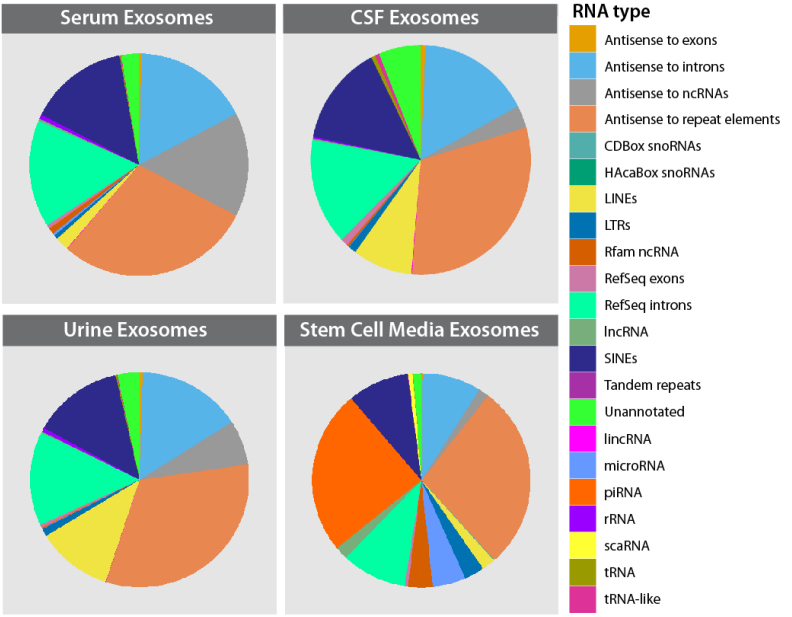
Cellular RNA-Seq versus exoRNA-Seq

A typical cellular RNA content is extremely complex, with miRNAs, piRNAs, tRNAs, mRNAs, lncRNAs, rRNAs and other ncRNAs. These types of cellular RNA-Seq NGS experiments require about 300M reads per sample to obtain a depth worth analyzing. In contrast, exosome are tiny nanovesicles averaging about 100nm in diameter. The cargo of an exosome is comprised of proteins and RNAs. Due to these size constraints, the exosome RNA complexity and diversity is estimated to be that of about 1% of a cellular RNA diversity. Exosome RNA population lengths are small to begin with (250 nt and smaller) as seen in the Agilent Bioanalyzer RNA chip analysis. We suggest targeting approximately 2-5 million reads per sample for these reasons to achieve a depth where expression differences can be easily observed. We also recommend performing paired-end 2x 75 bp reads to ensure enough read length to properly map a given RNA sequence to the genome. While NGS is not as quantitative as qPCR, expression patterns do clearly emerge of RNA sequence abundances when comparing across sample origins (different conditions, patients, etc.).

Sample exosome NGS data

The final steps of the Maverix exosome RNA-Seq analysis are abundance determination and differential expression analysis by DEseq. Abundance levels for ncRNAs (miRNAs, tRNAs, rRNAs, lincRNAs, piRNAs, snoRNAs), antisense transcripts, coding genes and repeat elements (LTR, LINE, SINE,and tandem repeats) are determined, then a summary of reads overlapping each of these annotations in the reference genome is created using SAMtools and provided for visualization in pie charts using R, a software environment for statistical computing and graphics.

Example RNA types and their relative abundance from Serum, CSF, Urine and Stem Cell Media exosomes.



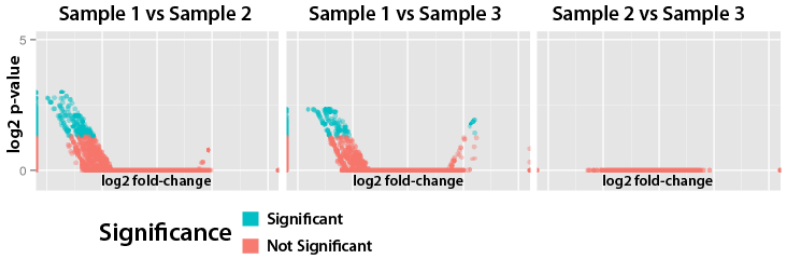
The types and relative abundances of serum, CSF and urine exosome RNAs appear to maintain and certain pattern between those biofluids. The stem cell secreted exosome RNA types and abundances seem to be very different from patient biofluid exosomes.

Differential expression analysis across samples

The differences in expression of ncRNA, antisense transcripts, and repeat elements between samples can be calculated based on how many times a given RNA sequence is read and calibrated to the total number of reads mapped for that given sample. Visual representation of the analysis results are provided in the online Maverix platform, including volcano plots showing an overview of significantly differentially expressed genes, as well as interactive tabular and heatmap views linked to the integrated UCSC

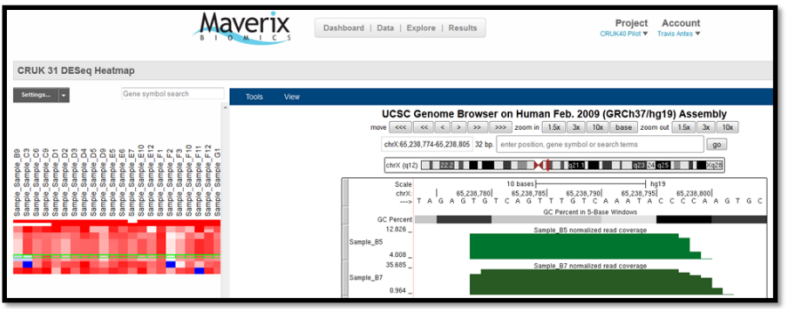
Genome Browser. The Maverix Exosome RNA-seq Analysis produces outputs that include read alignment and coverage tracks for the genome browser, annotations to the reference genome, as well as abundance determination and differential expression analysis. The Maverix Analytic Platform allows researchers to visualize their data and analytic results using an integrated UCSC Genome Browser, automatically configured for their specific organism of interest. With access to data in the UCSC Genome Browser directly from the platform, researchers can add custom tracks to the browser, securely surf their data, and easily share or publish their results.

Sample Volcano Plots showing fold-change and p-values of serum exosome RNA expression



The example volcano plots above show how the exosome RNA differential expression can be visualized and used to quickly identify RNA biomarkers of interest. Sample 1 is different from Sample 2 and 3, whereas Samples 2 and 3 are more similar.

Screenshot of interactive heatmap within the Maverix platform



The heatmap (above) is displayed on the left hand side of its visualization, with the integrated genome browser on the right. In our example, the first two columns are samples, with the third column identifying the chromosomal location of features, where chromosomes are classified by unique colors. Hovering over a region of the heatmap brings up a tooltip, as shown, with information about the mapped exosomal component, including name, chromosomal position on the reference genome, and differential expression values for each sample. Clicking on the row for a feature of interest on the heatmap will bring up the associated region in the integrated UCSC Genome Browser for visualization of exosome components in their genomic context and with public datasets. The identities of the microRNAs in the sample figures above have been purposefully hidden with a dark blue box.

For more information about the Maverix analytics platform, visit www.maverixbio.com.

E. Frequently asked questions.

Question?	Answer
How many reads do I need per sample?	Cellular RNA is complex, requires 300M reads per sample. Exosomes have ~ 1% of that complexity, need 3-5M reads per sample.
How many libraries can I multiplex on a MiSeq?	MiSeq v3 produces 25M reads. If you want 3-5M reads/sample, pool 6 libraries.
Should I do single or paired-end reads?	We recommend paired-end 2x 75bp NGS runs. Gives greater confidence of RNA sequence identity.
How long does a MiSeq run take?	The typical MiSeq run is 18 hours.
What file format does it generate?	You will only need the FASTQ files.
How much PhiX Control do I need for the MiSeq sequencing run?	A minimum of 5% PhiX Control spike-in is recommended.

F. Related Products and Services

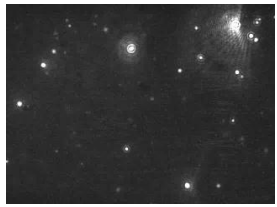
SBI offers a number of exosome research products. Review them here: http://www.systembio.com/exosomes

Isolate Exosomes with ExoQuick and ExoQuick-TC

One-step Exosome Isolation for Serum and Plasma, Tumor Ascites Fluid, Follicular fluid, Tissue Culture Media, Urine, Spinal fluid.

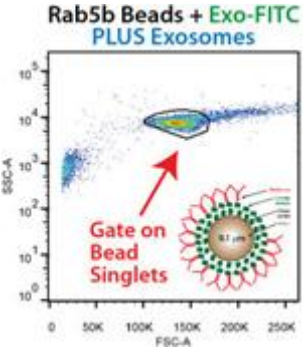
Purified human cancer exosomes and and mouse dexosomes

Use for RNA, Protein analysis, calibration standards and engineering cargo for delivery to target cells. All exosomes are characterized by NanoSight for size, intactness and concentration as well as tested to be CD63 positive by Western blot analysis. The purified exosomes are provided frozen with >1x10^6 exosomes (50 ug protein).



Fluorescently label exosome cargo

Label endogenous exosome RNAs Red and internal exosome Proteins Green to monitor exosome cargo delivery to cells in real-time.



Immunopurify Exosomes and use with FACS

Selectively capture distinct subpopulations of intact exosomes based on a particular surface marker and sort by FACS - **"Flow Exometry"**. Choose from the following tetraspanin, annexin, adhesion, fusion and immune presentation targets or customize your own capture system. **• CD9 • CD31 • CD63 • CD81 • Rab5b • HLA-G**

Exosome RNA-Seq and Mass Spec sample prep kits

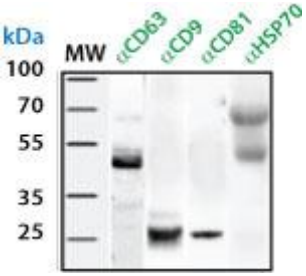
Exosomes are the future of biomarkers in medicine. The XRNA kits are specifically designed to make high-quality exosome RNA-Seq libraries for Illumina® Next-Gen sequencing using low RNA input amounts. Discover exosome protein biomarkers using the XPEP Mass Spec peptide library sample prep kit.

Culture Cells in Exosome-depleted FBS

Study exosomes from cultured cells and not from bovine exosomes in FBS itself. Exo-FBS has been stripped of bovine exosomes yet supports robust growth of cells in culture.

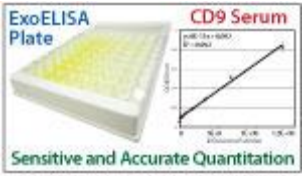
Verify Exosome Recovery with Antibodies and Antibody Arrays

Track exosomes by Western blots and Antibody Arrays using well-characterized exosome protein markers. Verify exosome recoveries after isolation with ExoQuick or ultracentrifugation using validated antibodies and arrays.



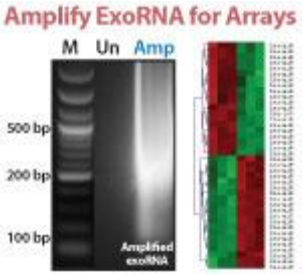
Quantitate Exosomes with ELISAs

Exo-ELISAs measure the levels of exosome particles with antibodies to detect CD9, CD63 or CD81. Highly sensitive and quantitative assays in a convenient 96-well format with validated exosome standards.



Amplify Exosome MicroRNAs with SeraMir for qPCR and microarrays

Purify exoRNAs with SeraMir columns and covert to cDNA for microRNA qPCR arrays or amplify exoRNAs for microarrays analysis.



Discover Novel Exosome RNA Biomarkers with Next-Gen Sequencing

Complete exosome RNA Next-Gen sequence analytics solution for researchers interested in identifying novel exosome-associated RNA biomarkers. Abundance, RNA type, expression heatmaps and genomic mapping all included in service.

Exosome RNA-Seq and Mass Spec sample prep kits

Exosomes are the future of biomarkers in medicine. The XRNA kits are specifically designed to make high-quality exosome RNA-Seq libraries for Illumina® Next-Gen sequencing using low RNA input amounts. Discover exosome protein biomarkers using the XPEP Mass Spec peptide library sample prep kit.

G. Shipping and Storage Conditions for Kits

The XRNA kits are shipped on dry ice and should be stored at -20°C. Avoid freeze-thawing the reagents. Shelf life of the product is 1 year after receipt if stored in -20°C.

II. References

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III. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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(888) 266-5066 (Toll Free)
Fax: (650) 968-2277
E-mails:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

VII. Licensing and Warranty information

Limited Use License

Use of the XRNA™ Kits (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

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- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a credit. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

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