“Isolation and Downstream Analysis of Extracellular Vesicles (EVs)/Exosomes Cargo: Products and Services Offerings”

SBI Webinar
May 19, 2022
Starts at 10:00am Pacific Time

Presenter: Enal Razvi, Ph.D., SBI

SBI System Biosciences
Harnessing innovation to drive discoveries

Topics Covered in this Webinar

- Isolation of Evs from Conditioned Cell Culture Media, Biofluids (Serum, Plasma, CSF, Urine)
- Types of Downstream Analyses on EVs/EV Cargo
  - Morphology
  - RNA, Protein
- SBI Products to Study EVs
- SBI Custom Services for EV Characterization:
  - Exo-RNA-Seq
  - Exo-Mass Spec

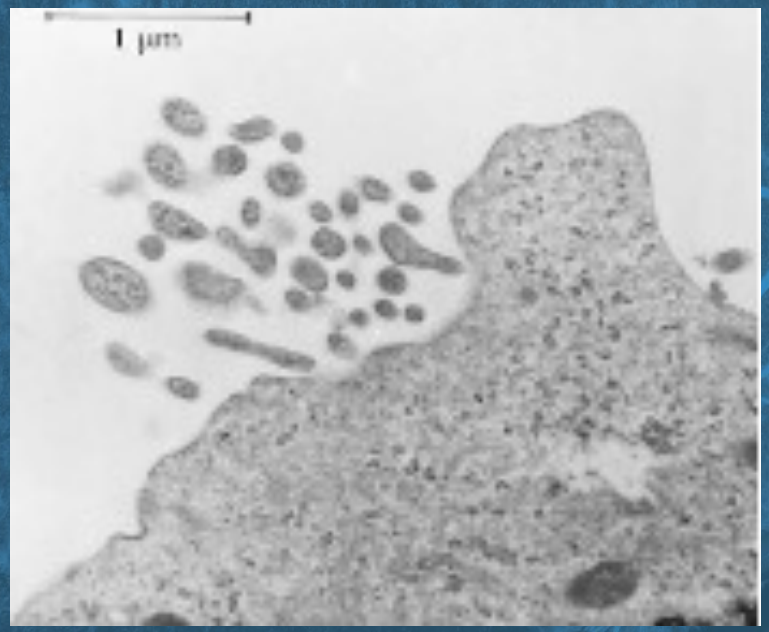
Overall Goal of this Webinar

- EVs Contain Information About the *In Vivo* State of a Cell and this Information can be Harvested and Studied
- Study of EVs as Circulating Biomarkers
- Biofluids as a Rich Source of Circulating Biomarkers
- An Overview of the Downstream Analyses that can be Performed on Isolated EVs
  - Classes of Downstream Analyses based on Analyte/Cargo Class
- SBI Portfolio of Products and Services for EV Downstream Analysis

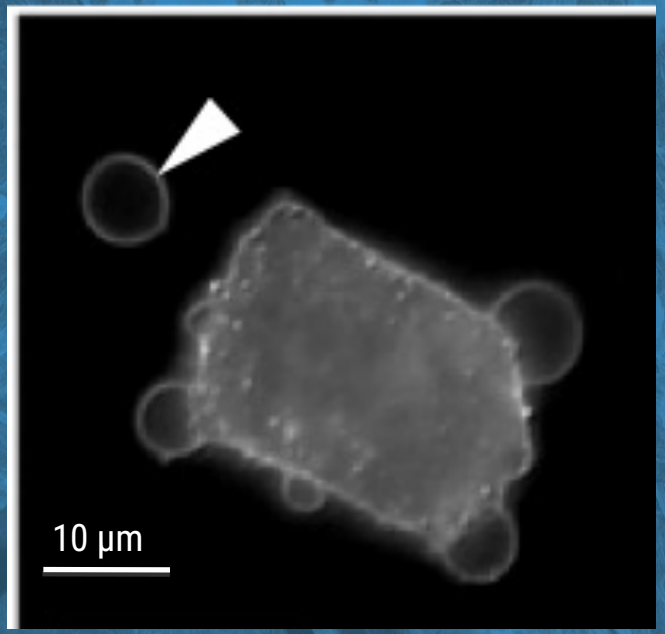
Wide Range of Extracellular Vesicle (EV) Sizes:

Therefore, a Biologically–Heterogeneous Population *In Vivo*

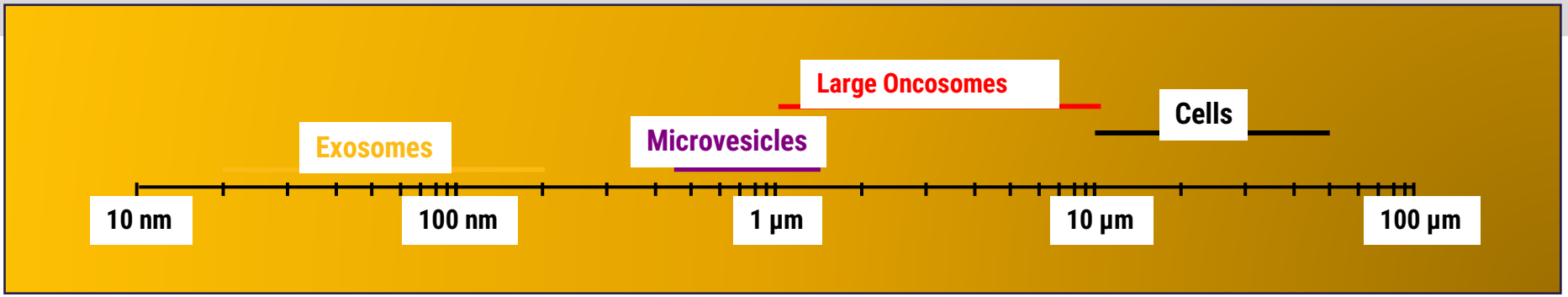
Information-Rich Content with Biomarker Potential



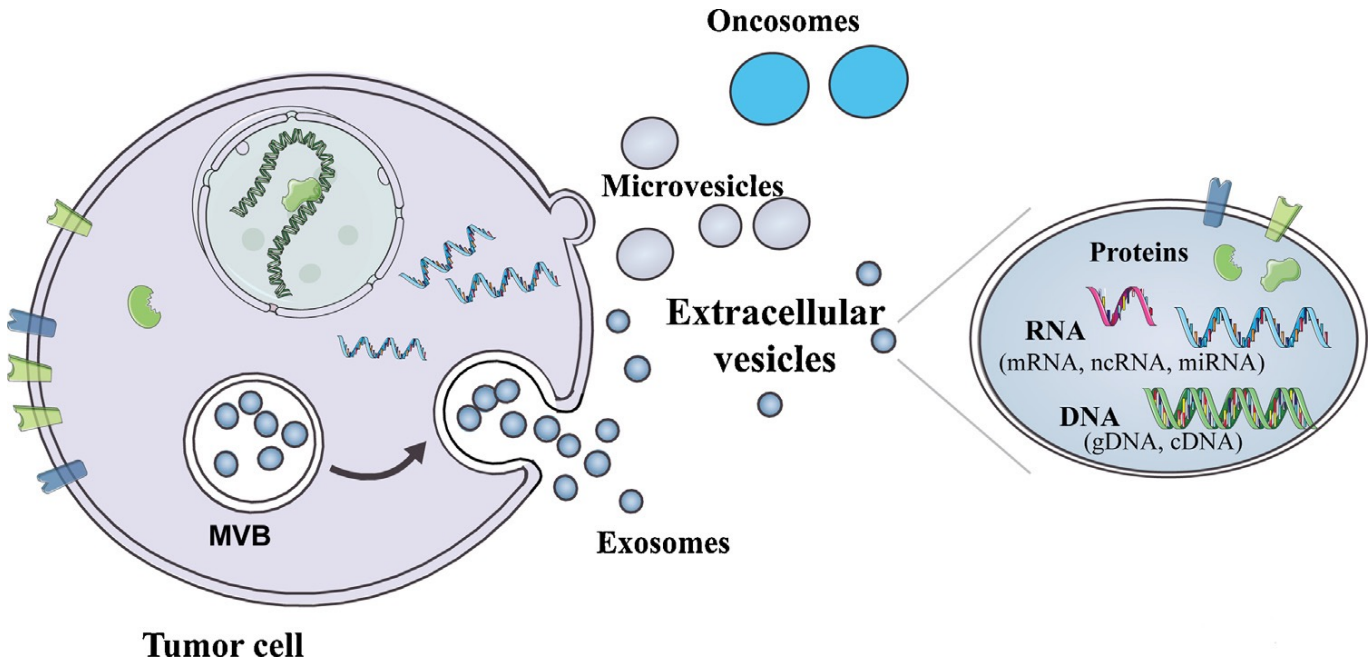
Cocucci et al., 2009



Di Vizio et al., 2012



EV Cargo

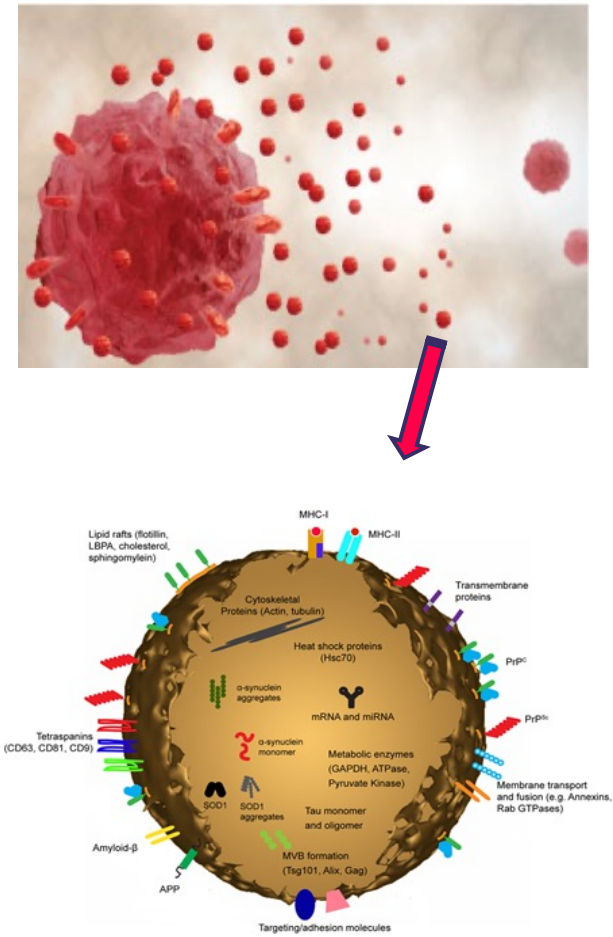


Exosomes are formed by the internalization of the endocytic membrane and formation of MVB inside the cell. The fusion of the MVBs with the plasma membrane results in the releaseof exosomes into the extracellular milieu. Microvesicles are formed by the outward budding of the plasma membrane and are directly released into the extracellular milieu. Oncosomes are a larger type of EV released by cancer cells through budding of the plasma membrane.

EVs contain a Variety of Proteins, RNA species and types of DNA →
Rich Source of Circulating Biomarkers

EVs: A Treasure Trove For Biomarkers

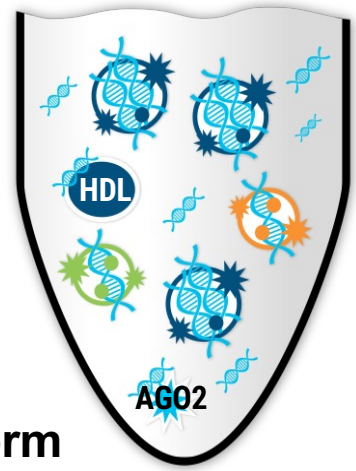
- Exosomes contain:
  - RNA
    - mRNA
    - microRNA
    - Non-coding RNAs (ncRNAs)
  - Proteins
    - Matrix metalloproteinases (MMPs)
    - Angiogenic proteins
    - Tumor specific proteins (EGFRvIII)
    - Tau protein
    - Alpha-synuclein protein
    - Prion protein
  - DNA
    - Embedded on the surface
    - Inside
- Exosomes are stable packages with disease specific macromolecules in the native configuration of the cell of origin



Bellingham SA et al. *Front. Physiol.* May 2012

Biofluids Contain Multiple Sources of Nucleic Acid
→ *Isolation Method Matters*

Serum/plasma



1

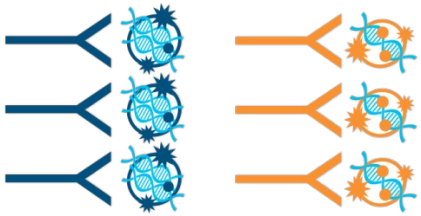
2

Exosome/EV platform

Enables sub-fractionation of RNA from different cellular processes

1. Total Exosome/EV Extraction
   a) Ultracentrifugation
   b) Precipitation
   c) Size Selection

2. Affinity Purification
   a) Magnetic beads
   b) Microfluidics
   c) Flow Cytometry

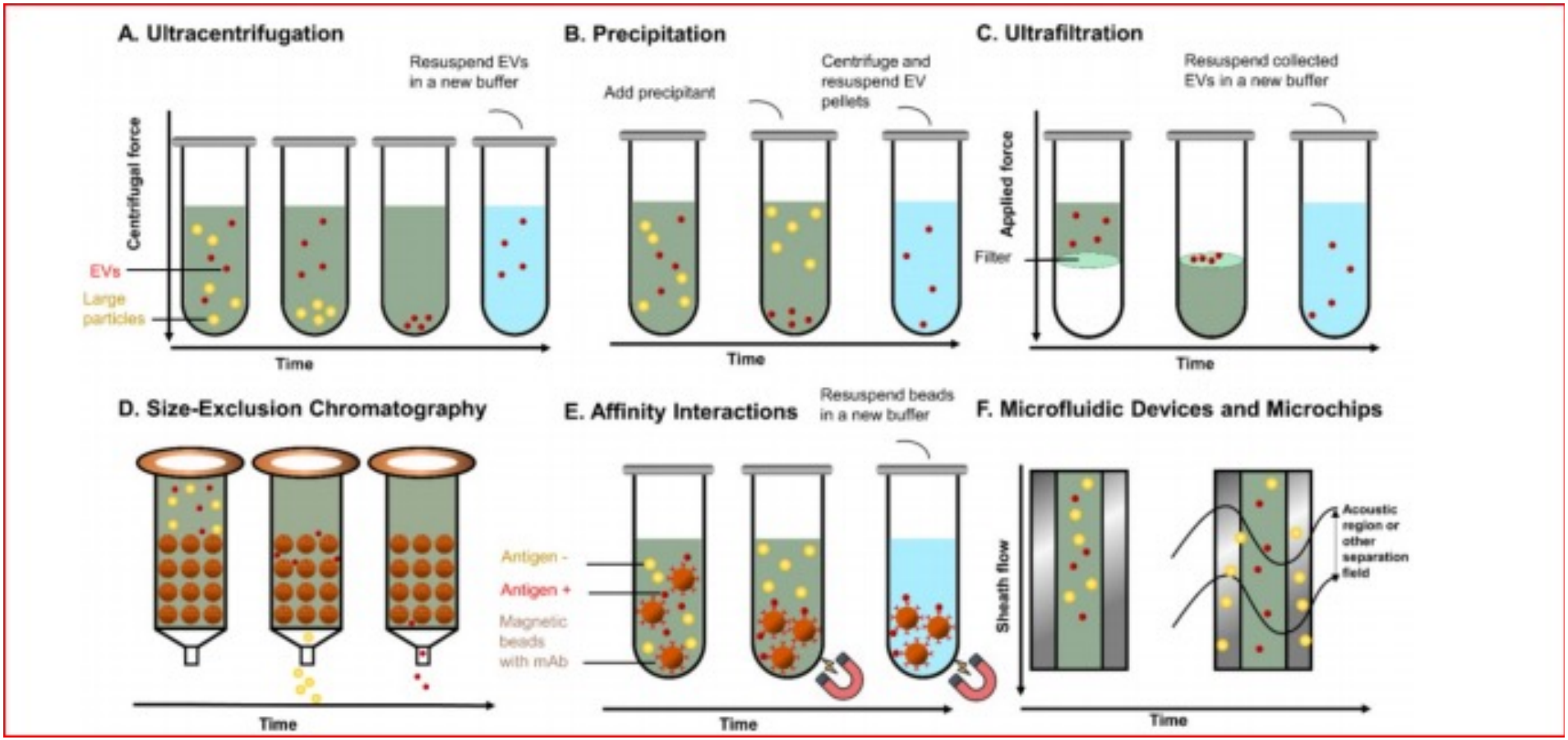


Whole plasma extraction

No Selectivity

SBI System Biosciences
Harnessing innovation to drive discoveries

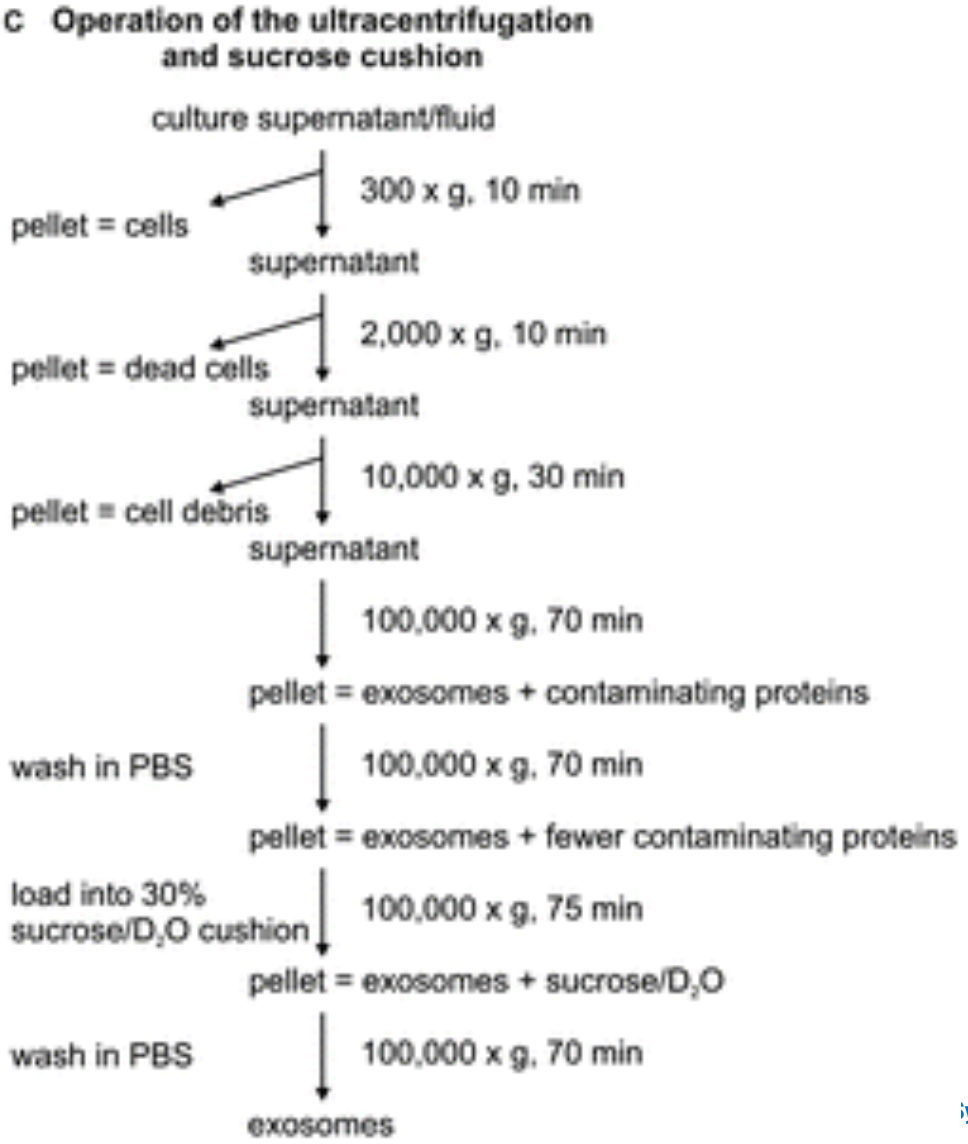
A Large Number of EV Isolation Methods → How to Choose the Optimal Method for Your Research Project?



From Ultracentrifugation to Microfluidics: A Huge Dynamic Range…
Source: Pang *et al.*, Theranostics 2020

Ultracentrifugation Protocol for EV Isolation

1. Very Hands-On
2. Many Steps
3. Time Consuming
4. Non Walk Away Operation
5. Low Yield
6. Not Amenable for Sample Scale



EV Research Workflow

Sample Prep

- Depends on Front-End Sample Studied
- Conditioned Cell Culture Media
- Biofluids – Serum, Plasma, CSF, Ascites Fluid, Urine

EV Isolation Step

- Choice of Methodology Depends on Sample Amount, Type, Downstream Analysis
- SBI SmartSEC® Chromatographic Separation Based on Size
- SBI ExoQuick® Precipitation-based

EV Cargo Downstream Analysis

- Depends on EV Cargo Under Investigation
- EV Size via NTA, fNTA – Important as a Tool for *Bona Fide* EVs
- RNA-Seq for Studies on Small or Large RNAs Found in EVs – Much of the literature is focused on small RNAs such as microRNAs
- Proteomic Analysis for the Protein Compartment in/on EVs
- Lipidomics/Metabolomics – Future Areas of Research

EV/Exosome Isolation Methodologies from SBI

SBI System Biosciences
Harnessing innovation to drive discoveries

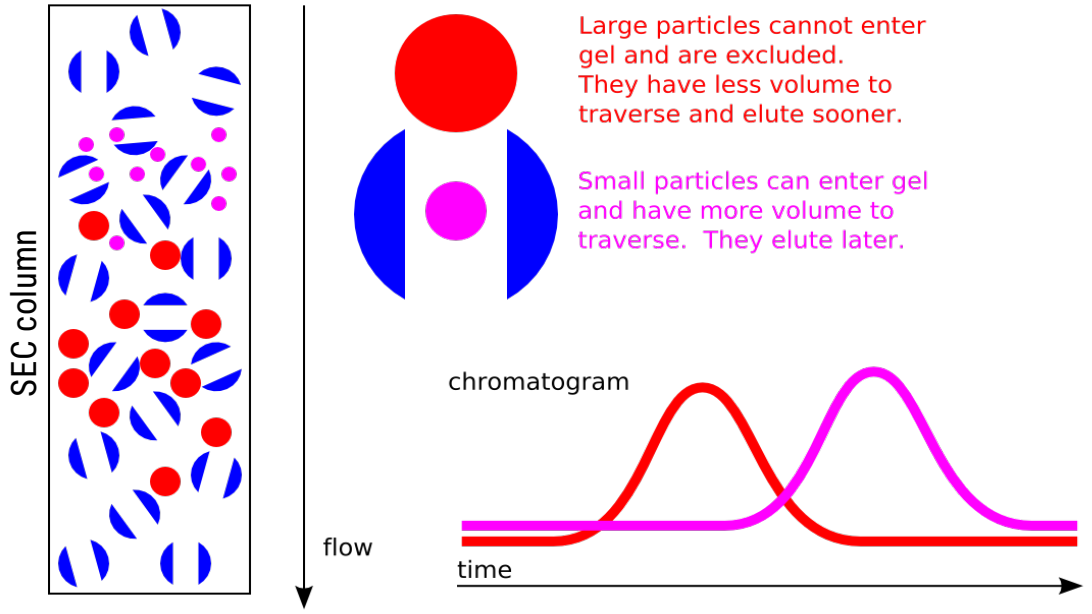
SBI SmartSEC → Size-based Isolation

SBI System Biosciences
Harnessing innovation to drive discoveries

SmartSEC Family of Products from SBI for EVs/Exosomes ("EV") Isolation

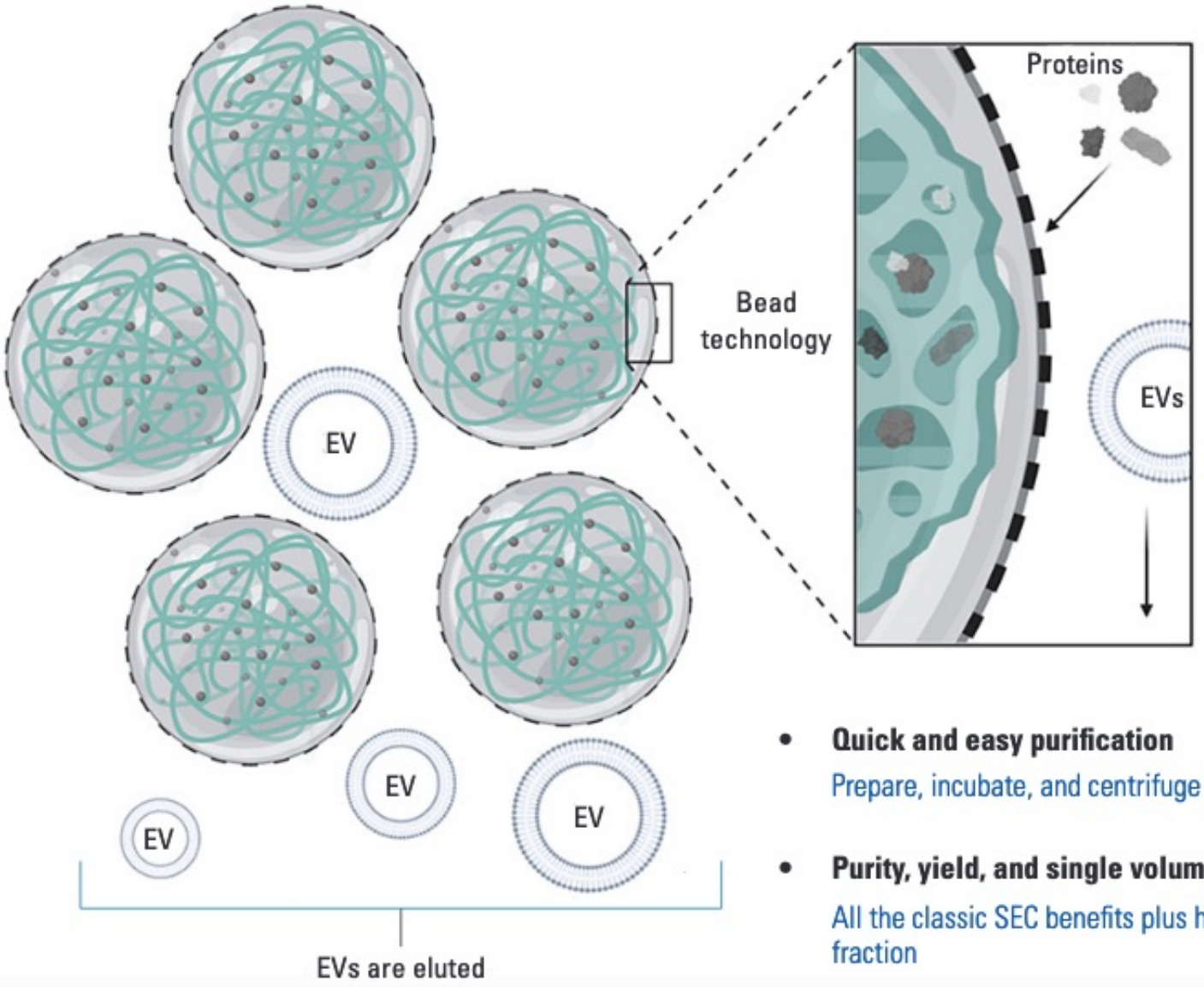
- Size-based Isolation of EV Using SBI's SmartSEC Technology
- Powerful Approach Because:
    1. Clean Preps for Downstream Studies of the EVs including their RNA or Protein Cargo Studies
    2. Many different sample classes as the starting materials without any front-end sample prep requirement [such as serum, plasma]
    3. Size Range that is captured allows for EV capture rather than other vesicles/contaminants
    4. Column-based approach enables scale-up in terms of number of samples

Size-Exclusion (SEC) Chromatography Enables Capture of EVs



- How it Works In Words
- Next Slide Illustrates Pictorially

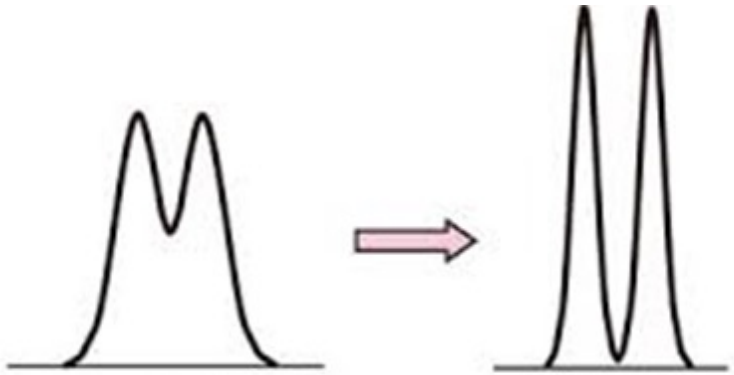
SBI SmartSEC



- **Quick and easy purification**
  Prepare, incubate, and centrifuge for EV isolation
- **Purity, yield, and single volume collection**
  All the classic SEC benefits plus highly concentrated EVs in one fraction

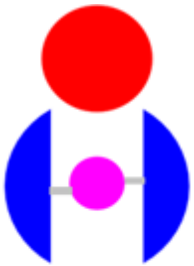
SmartSEC-Single:
Fast, Easy to Use and Compatible with Small Sample Volumes

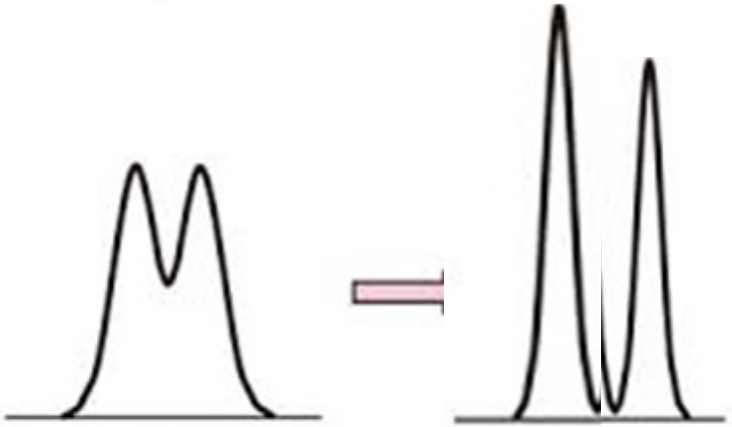
Traditional SEC

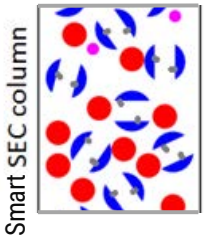


- Bulky
- Challenging
- Laborious
- Time-consuming
- Larger input volume
- Diluted output

SBI Smart SEC



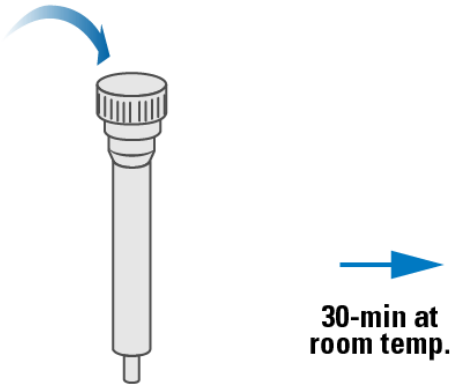




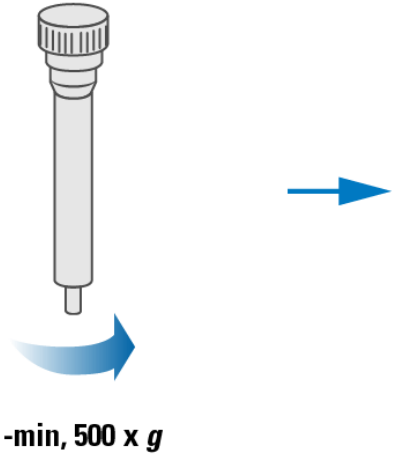
SmartSEC Single

SBI System Biosciences
Harnessing innovation to drive discoveries

SmartSEC-Single: Simple Experimental Protocol



1. Load biofluid into pre-washed SmartSEC Single column and incubate 30-min at room temperature

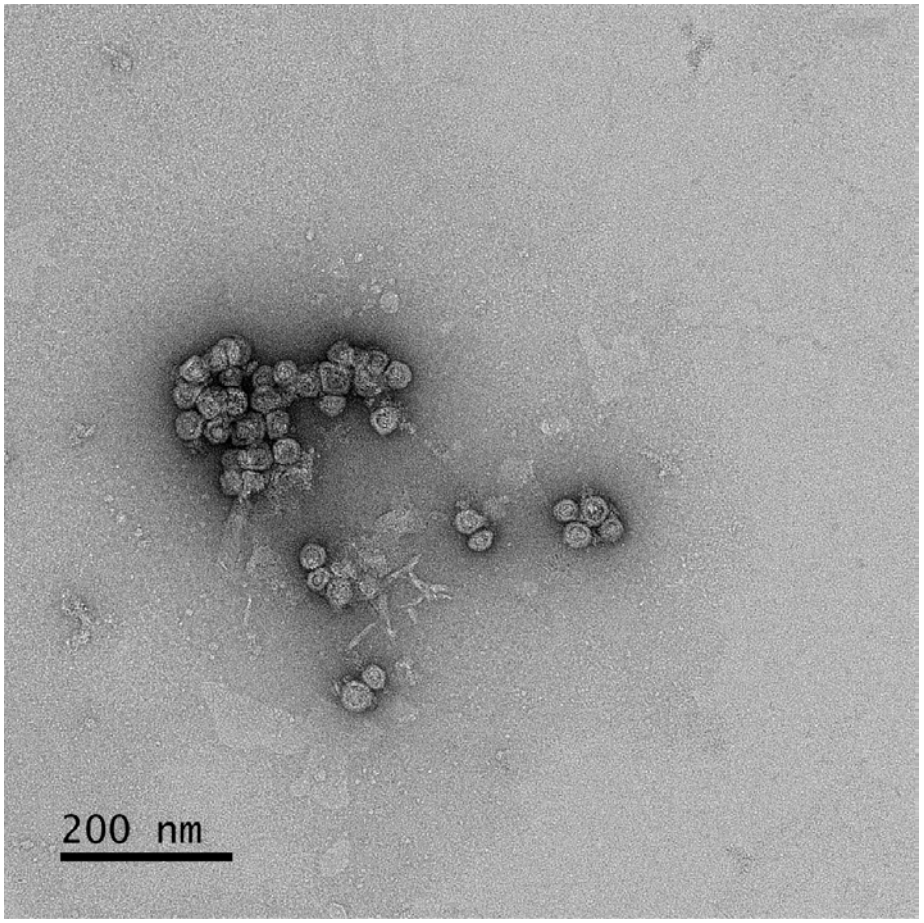


2. Centrifuge for 1-min at 500 x *g*

3. EVs are in the flow-through

SBI System Biosciences
Harnessing innovation to drive discoveries

EVs Isolated from Serum using SmartSEC Single Possess Typical EV Morphology



- Intact vesicles with a double layer of membranes
- Little visible background debris

SBI System Biosciences
Harnessing innovation to drive discoveries

SmartSEC™ Single for EV Isolation, SSEC200A-1, 10 Reaction Kit

Component	Qty/Volume
SmartSEC Single column	10 columns
Column buffer	50 mL

Samples Sizes:
100 µl – 250 µl of serum/plasma (No more than 250 µl)

Up to 4 mL for other biofluids (for example CSF, Urine)

****Not designed for Tissue Culture Media****

This is Your Starting Product Format to Start Using SmartSEC

SBI System Biosciences
Harnessing innovation to drive discoveries

SBI SmartSEC Platform Product Family:
→ Product Form Factors Scale with Your Research

Mini

Ideal for small samples as low as 10µl

Single

For the Majority of Researchers

100 µl – 250 µl of serum/plasma
Up to 4 mL for other biofluids (for example CSF, Urine)

HT

96-well Footprint
Up to 96 samples in parallel

SBI SmartSEC Platform

Fast, Easy to Use, Compatible with Small Volumes,
Scalable → Enables Many Samples to be Processed in Parallel

SmartSEC: Features, Benefits

- No Front-End Complicated Sample Prep
- Works with Many Biofluid Classes of Biological Relevance
  - Serum
  - Plasma
  - CSF
  - Urine
- Fast
- SmartSEC-HT Enables Parallel Processing of Multiple Samples → Scales-Up with your research as more samples processed in parallel
- Intact Purified EV Prep can be Eluted Downstream for Functional Experiments, RNA and Protein Cargo Studies since the Chromatographic Resin is Inert and Does Not Interact with the EVs
  - Compatible with most downstream applications such as mass spectrometry, western blotting, nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and RNA-sequencing (NGS)

SBI ExoQuick → Precipitation-based Isolation

SBI System Biosciences
Harnessing innovation to drive discoveries

Precipitation Is Actually an Excellent Way to Initiate Your EV/Exosome Studies

- A polymer net is inert and does not functionally affect or modulate the EVs/Exosomes
- Cast the Net Wide – Capture of Lots of Particles Easily
- Bulk Collection of EVs/Exosomes is
  - Fast
  - Uses Off-the-Shelf Reagents (ExoQuick Family of Products)
  - Samples are amenable to downstream studies on RNA Cargo and with an additional 'ULTRA' column purification step for Protein Cargo
  - Can use large volume sample sizes – such as Tissue Culture Media to Study EVs/Exosomes Secreted from Tissue Cultured Cells → A Start to Many Research Projects that then Proceed to Biofluids
  - Plus with Biofluids there is an array of particle sizes such as the Oncosomes so a "wide net" for biomarker studies

ExoQuick Family of Products from SBI → First Generation

Product	Starting Samples	Pros	Cons
ExoQuick	• Serum • Plasma • Ascites Fluid	• Widely-Used Methodology so Customer-Tested in Lots of Publications • Easy Workflow, Minimal Hands-on Time • Casts a Broad Net in terms of particle capture • Can study RNA cargo downstream	• Ppt brings down many carryover proteins not specific to EVs *per se* • Prep cannot be used for proteomics studies via Mass Spec • Plasma needs extra sample prep step
ExoQuick-TC	• Urine • Tissue Culture Media • CSF	• Scalable to Large Sample Sizes when Biological Sample is Dilute • Can be used for research on cells in culture since majority of life science research projects begin with tissue culture model systems	• Same as above

ExoQuick Family of Products from SBI → Second Generation

Product	Starting Samples	Pros	Cons
ExoQuick-ULTRA	• Serum • Plasma	• Clean Prep enables Downstream Proteomic Analyses • Fast Column-based Clean-Up • Increased EV Yield in Final Prep • **SmartSEC is an Excellent and Improved Platform Replacing EQ-ULTRA**	• Ppt brings down many particles not specific to EVs *per se* but column-based clean-up increases purity of EV preparation
ExoQuick-TC-ULTRA	• Tissue Culture Media • Other Biofluid Classes	• Clean Prep enables Downstream Proteomic Analyses • Fast Column-based Clean-Up • Increased EV Yield in Final Prep	• Same as above

Products & Services for the Downstream Analysis of EV Cargo

SBI System Biosciences
Harnessing innovation to drive discoveries

SBI Offers Downstream Analyses as Both *Products* and *Custom Services*

- The **Products** are designed for studies best performed by the researchers in their lab as they conduct EV experiments in their own workflow
- Products Classes that fall under this category are:
  - EV Detection
  - EV Quantification
  - EV Labeling
  - EV Modification/Engineering

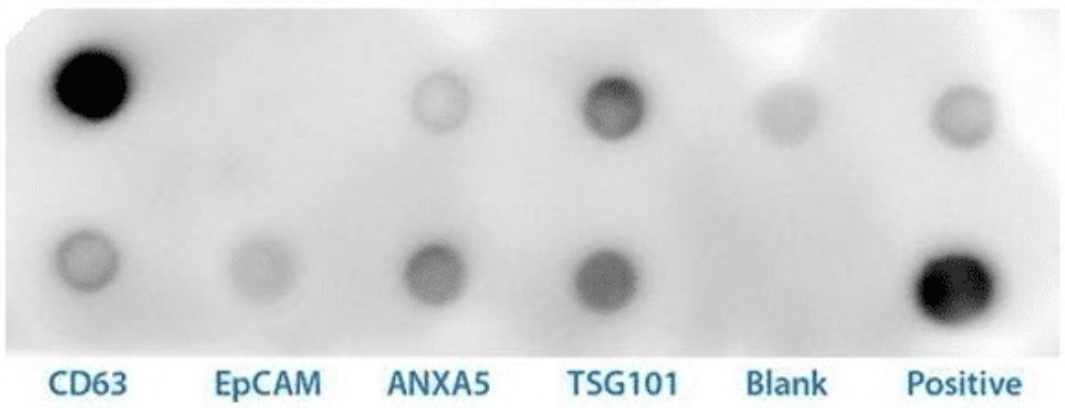
EV Detection - ExoCheck

- This is the **<u>Qualitative</u> Detection of EVs**
- Once you've performed your EV isolation, you want to ask the question – Do I have EVs in my prep?
- The ExoCheck Antibody Arrays are membranes with capture Abs which are specific for EVs per the literature → The Isolated EVs are added and then the signal developed

- Easy
- Fast
- Membrane-based Capture Assay
- Called Exo-Check → Check to see if your EVs are Present

Serum Exosomes

	1	2	3	4	5	6
A	Positive	GM130	FLOT-1	ICAM	ALIX	CD81
B	CD63	EpCAM	ANXA5	TSG101	Blank	Positive



SBI System Biosciences
Harnessing innovation to drive discoveries

EV Quantification – ELISA-based

- So you've isolated your EVs
- Now you want to quantify to be able to use them in your research studies
- How to quantify?
  - Use an Ab-Capture ELISA-based Methodology based on EV Surface Markers which are Well Established as Canonical EV Markers
    - CD63
    - CD81
  - Use an Enzyme Assay based on AChE – the literature is still conflicting on this so best to use a combination of methods to quantify the EVs in your prep

Exo-ELISA *vs.* Exo-ELISA Ultra

Exo-ELISA : CD9

- Slower – 2 days
- Input amount – 1-5mg protein
  - Will likely consume exosome sample
- Absorbance –based detection
- Multiple markers available

Exo-ELISA Ultra: CD63 /CD81

- Fast – 4 hours
- Input amount – <1µg protein
  - Samples remain for further study
- Absorbance –based detection
- CD63 and CD81 marker detection

SBI Offerings for EV Quantification

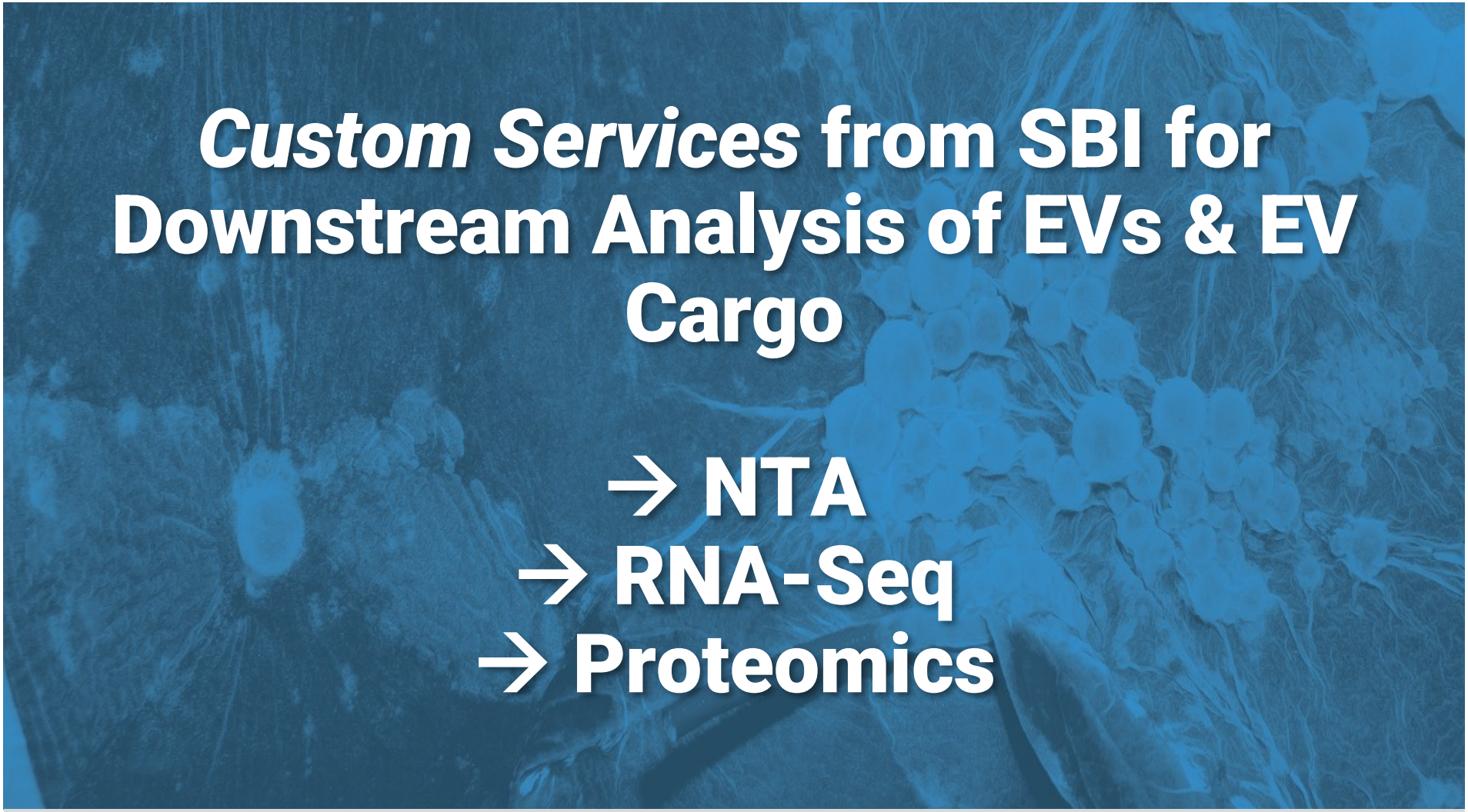
	EXOELISA-ULTRA CD63 EXOELISA-ULTRA CD81	EXOELISA CD9 EXOELISA CD63 EXOELISA CD81	EXOCET	FLUOROCET
Application:	For fast and sensitive antibody-based quantitation of exosomes	For sensitive quantitation of exosomes when time and input sample are not limiting	For fast quantitation of extracellular vesicles with moderate sample input requirements	For the most sensitive quantitation of extracellular vesicles with very low sample input requirements
Detection method	Antibody	Antibody	Enzymatic	Enzymatic
Quantitation chemistry	Enzymatic (HRP)	Enzymatic (HRP)	Colorimetric	Fluorescent
Total protocol time	4 hours (no overnight incubation)	24 hours	20 min	60 min
Input sample amount (protein equivalent)	1 – 200 µg	>500 µg	50 µg	<1 µg

EV Labeling

- For some experiments, such as EV NTAs you may need to label EVs with a Fluorescent Dye
- The labeling is specific to intact EVs and Enables you to Quantify *Bona Fide* EVs as opposed to Membrane Fragments, Apoptotic Blebs and other Debris
- Available for Malvern and Particle Metrix NTA Instrument Platforms

EV Modification/Engineering

- The Majority of EV Research is Focused on Basic EV Life Science Research + EVs as Circulating Biomarkers
- However, there is an emerging group of researchers and companies seeking to use **EVs as carriers for drug delivery or engineered to contain proteins such as biologics** for Therapeutic Applications
- This is a small group currently but is expected to grow over the coming years
- For these researchers we offer:
    - Deliver miRNA for knockdown studies with XMIRs and AXMIRs
    - Transfect nucleic acids, proteins, and even small molecules into exosomes using Exo-Fect
    - Tag proteins for loading into exosomes using XPack™
    - Facilitate uptake of exosomes by cells using EV Shuttle and EV Entry Kits
    - Target exosomes to specific tissues with XSTAMP



SBI System Biosciences
Harnessing innovation to drive discoveries

Custom Services for EV Researchers

- The EV Research *Products* presented in this webinar are designed for researchers to utilize in their lab and integrate into their research workflow
- However, some types of EV Downstream Analyses require sophisticated sample prep and/or downstream data interpretation and analysis → For these we recommend, *Custom Services* performed within SBI by our trained scientists
  - **NTA** for studying the EV size → Important for publications and therefore life science researchers who do not have access to NTA instruments use this Custom Service from SBI
  - **RNA-Seq** to interrogate EV RNA Cargo – typically small RNA but also larger RNAs and mRNAs
  - **Proteomics** via Mass Spectrometry – this is for the growing interest in the protein compartment of EVs which may be very crucial to their biological role *in vivo*

Exo-RNA-Seq Workflow

EV Isolation

- EVs were isolated from plasma following ExoQuick kit protocol. In brief, plasma was first centrifuged to remove debris and then treated with thrombin to remove fibrin. The fibrin was cleared with a second spin and the resulting supernatant was transferred to a fresh tube for EV precipitation by ExoQuick.

RNA Isolation

- Total EVs were lysed and crude RNA extracted using a spin column. RNA was washed and then eluted into 25 uL of nuclease-free water.
- RNA quality was checked on Bioanalyzer (Agilent) using the RNA Pico kit

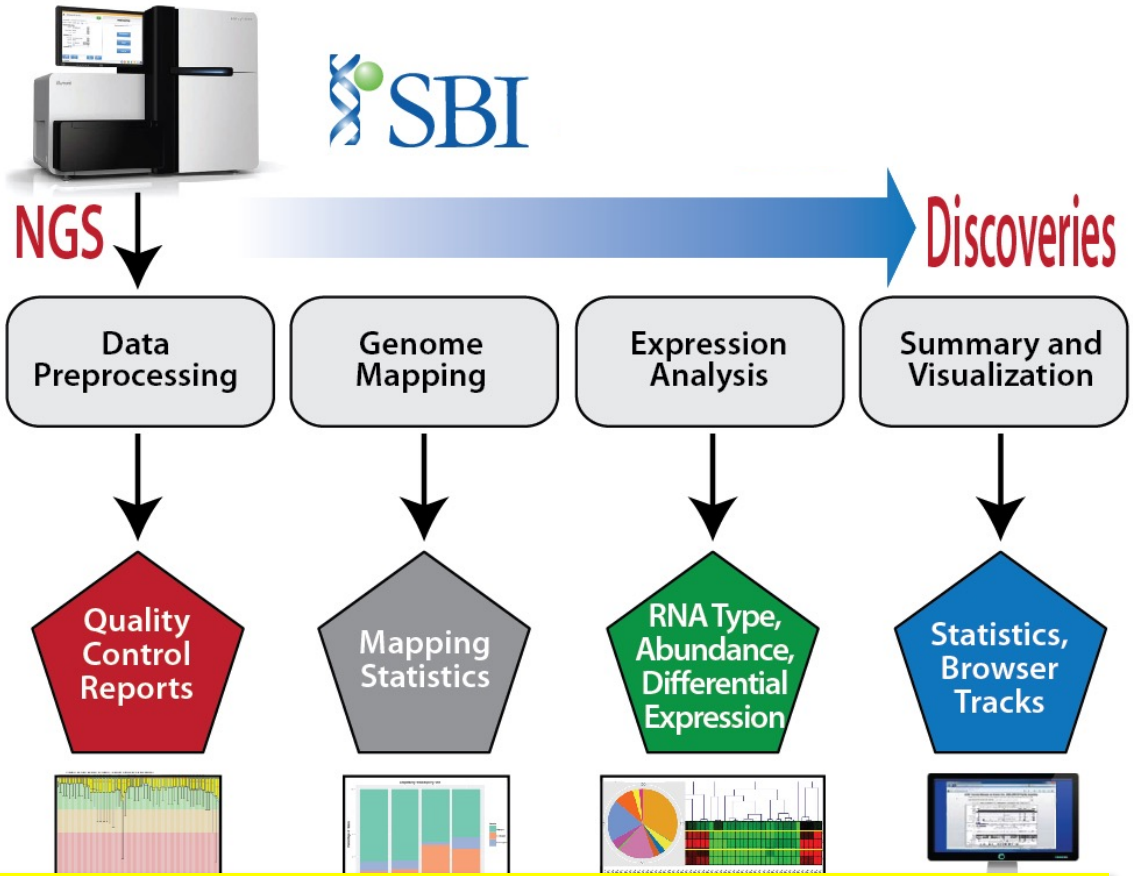
Library Prep

- Total RNA population, starting with as little as 1ng of total RNA, was entered into small RNA library prep for single-end sequencing. Successful libraries were purified by gel extraction to ensure the correct size was isolated. Libraries were amplified by qPCR and assessed for viability by the concentration (>1nM) to be moved on to sequencing.
- The sequencing is carried out on Illumina NextSeq instrument with single-read 1 X75 bp run with up to 10-15 million reads per sample
- Sequencing data is analyzed for quality and contamination. Raw reads are trimmed to remove adapters, then trimmed and filtered based on quality scores. The scores used for trimming and filtering are specific to the sequencing platform. The preprocessed reads are then assessed for quality and plots are generated of per base quality before and after trimming

Exo RNA-Seq Custom Service

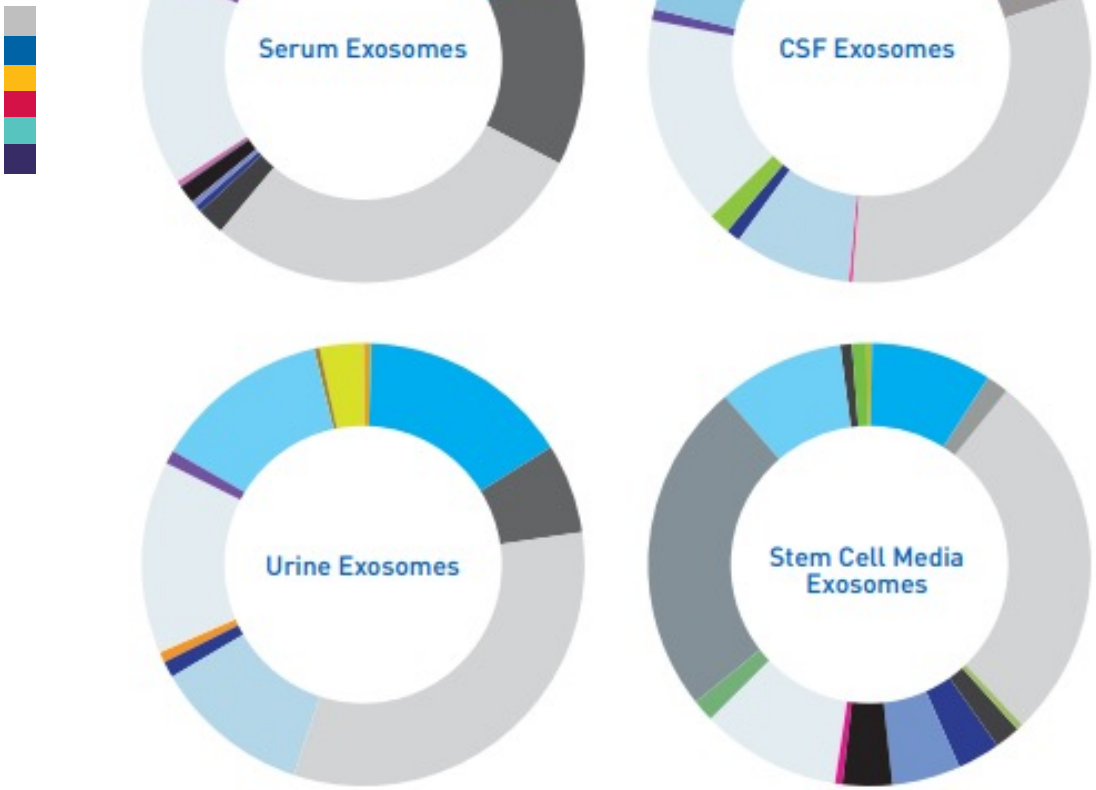
- Ship samples to SBI
- SBI isolates EV and extracts RNA
- Library Created
- Sequenced on Illumina platform
- 10-15 million reads depth

Biofluid	Volume
Serum	500µl - 1ml
Plasma	500µl - 1ml
Cell Media	5ml - 10ml
Urine	5ml - 10ml
Spinal Fluid	5ml - 10ml
Ascites Fluid	500µl - 1ml
Other	Inquire



Exo-NGS: Complete End-to-End EV RNA-Seq Custom Service
→ Study the RNA Composition of Your EVs

RNA-Seq Provides a Deep Dive into the RNA Composition of EVs From Your Experiment



- Antisense to exons
- Antisense to introns
- Antisense to ncRNAs
- Antisense to repeat elements
- CDBox snoRNAs
- HAcaBox snoRNAs
- LINEs
- LTRs
- Rfam ncRNA
- RefSeq exons
- RefSeq introns
- lncRNA
- SINEs
- Tandem repeats
- Unannotated
- lincRNA
- microRNA
- piRNA
- rRNA
- scaRNA
- tRNA
- tRNA-like

Study the Types of RNAs Present in Your EVs and Their Relative Abundance

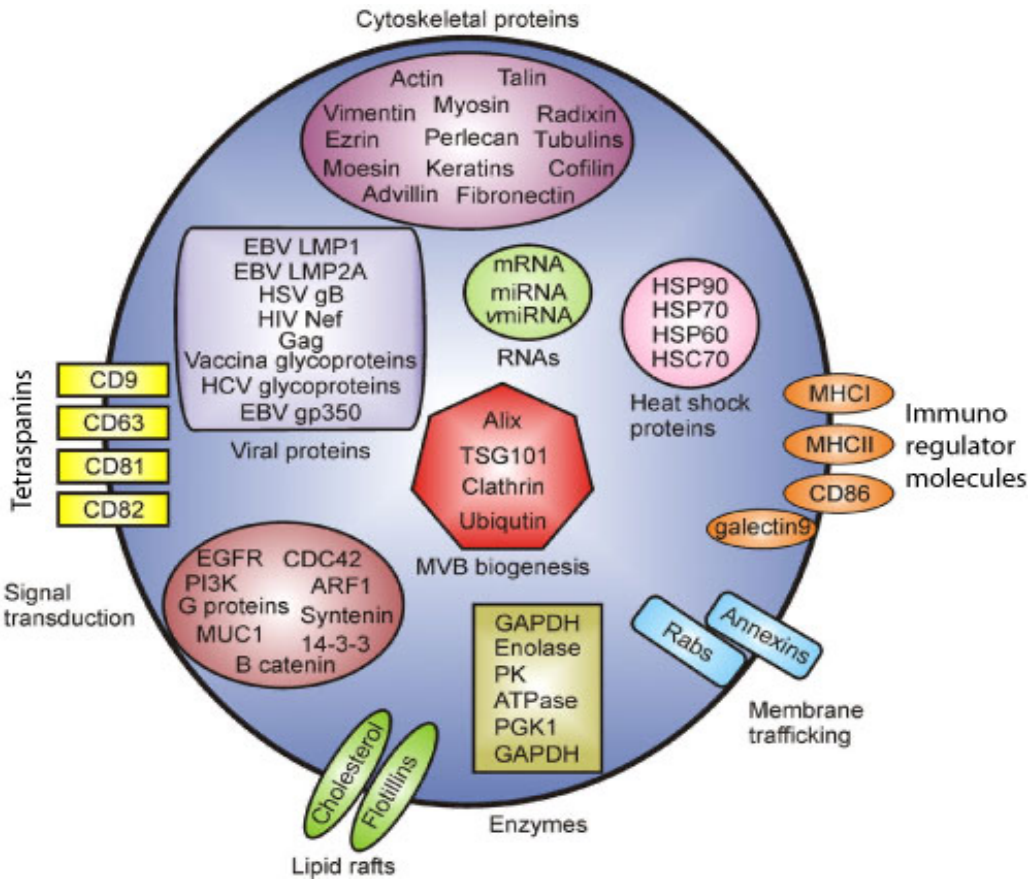
SBI System Biosciences
Harnessing innovation to drive discoveries

Protein Components of EVs: An Important Biomarker Class But Notoriously Difficult to Study

- Western blotting
  - Advantages: Easy to do, many Abs available
  - Disadvantages: Low-throughput, ID only known markers, quantitation bias
- Protein arrays
  - Advantages: Scalable throughput (low-mid), commercially available panels
  - Disadvantages: Cost, ID only known markers, quantitation bias, reproducibility issues
- Mass Spectrometry (LC/MS/MS)
  - Advantages: Unbiased protein quantification, ID all proteins
  - Disadvantages: Complex setup/expertise, high cost/sample, sensitive to contaminating proteins in sample

EV Mass Spec as a Custom Service is an Excellent Way for You to Study the Protein Composition of Your EVs

- Tedious Sample Prep Performed by SBI
- Housekeeping Proteins Removed
- Standard Protocol Removing Experimental Variation
- Available for Key Biofluid Classes
- High Information Content Useful for Studying Biomarker and for Research Activities on EV Biology
- Works for Total Protein Content of EVs or Surface-Membrane Proteins of EVs



SBI System Biosciences
Harnessing innovation to drive discoveries

Questions/Topics Addressed in this Webinar

- The potential to find circulating biomarkers in EVs
- Harvesting and studying EVs to learn about the *in vivo* state of the parent cell
- Studies on the different RNA classes carried by EVs—small RNAs/microRNAs to mRNAs and other large RNAs
- Studies on the protein and metabolomic content of EVs
- How the morphology and size distribution of EVs, from microvesicles to oncosomes, may hold clues to their *in vivo* functionality
- SBI's products and services that enable EV studies

In Summary

- Extracellular Vesicles, Exosomes Provide a Rich Source of Biomarkers for Research
- There is also Basic Research on Exosomes, EVs + Research on Therapeutic Applications of EVs
- All these Research Projects Require Isolation of Exosomes/EVs and then the Subsequent ***Downstream Analysis of the EV Cargo***?
- Which Methods Should I Use for Downstream Analysis?
- Which Off-the-Shelf Product or Custom Service Should I Use?
- This Webinar Seeks to Provide a Foundation for How to Make the Above Choice
- SBI with its 10-year Track Record in Developing and Commercializing Exosome Research Tools Offers Many Choices – Based on Your Research Question

More Information/Contact Details

- Full Details of SBI's Exosome/EV Products and EV Custom Services on SBI website
- Products can be ordered directly from SBI or its International Distributors (for customers outside the US)
    - Questions on SBI Products and Services: info@systembio.com
- SBI On-Site Technical Support is available to address questions - E-mail: tech@systembio.com

SBI Website: systembio.com

SBI System Biosciences
Harnessing innovation to drive discoveries