

Everything **You** Need To Know About **Exosomes**

From Purification to Disease Monitoring

Presented By:

Dr. Abdalla, M.Sc, Ph.D

Senior Research Scientist

About the Speaker

Hi! My name is Dr. Abdalla

Dr Abdalla has a long-standing scientific and professional background in biological sciences focusing on exosomal research. He is the Director of Sales and Marketing at Norgen Biotek Corp. and acts as a Senior Research Scientist, developing many innovative technologies at Norgen.



Key Learning Objectives

01 Using liquid biopsies as a sample source for disease monitoring

02 The composition and capabilities of exosomes

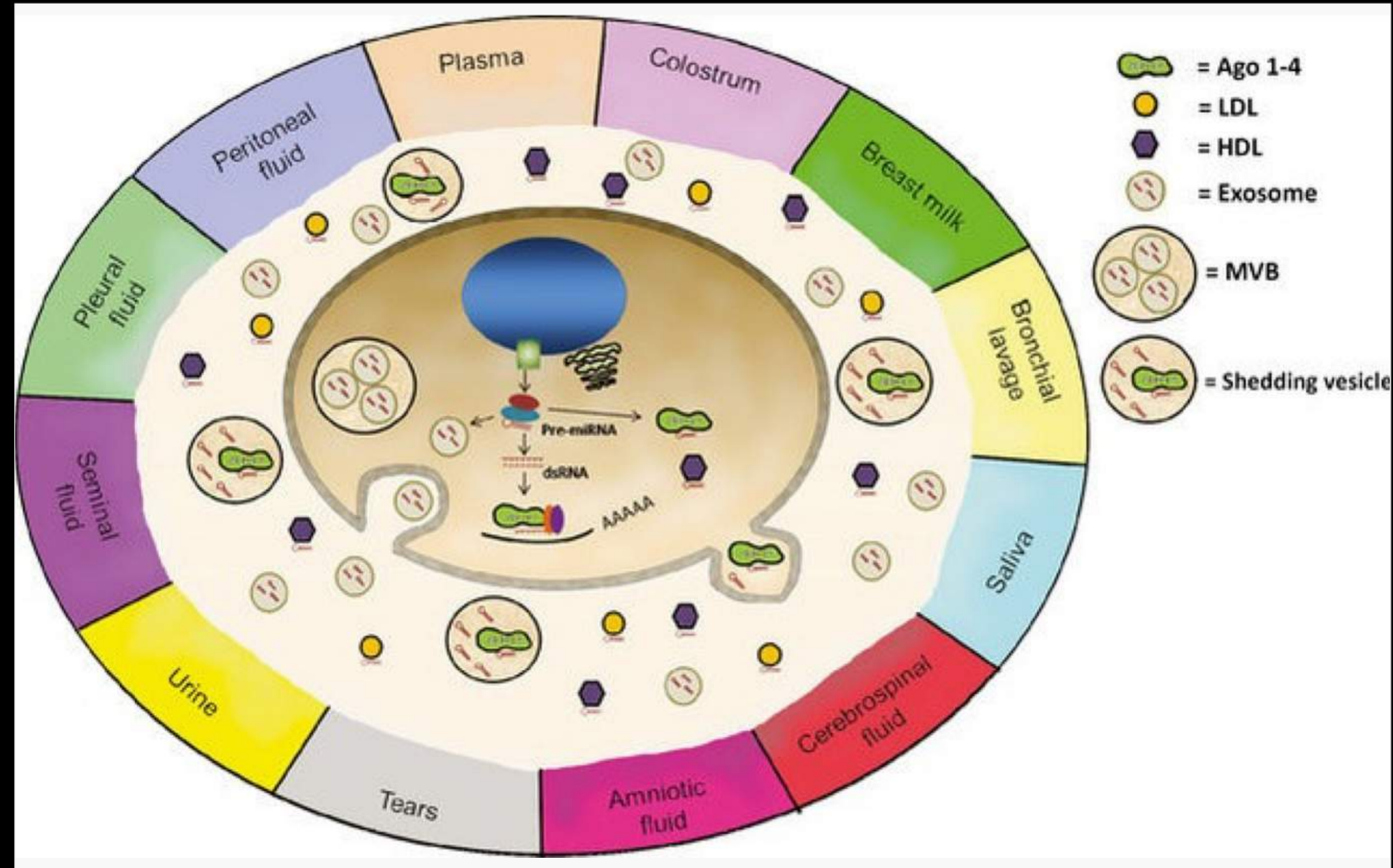
03 The differences between several exosome purification technologies

04 Which sample types should be used to isolate exosomes

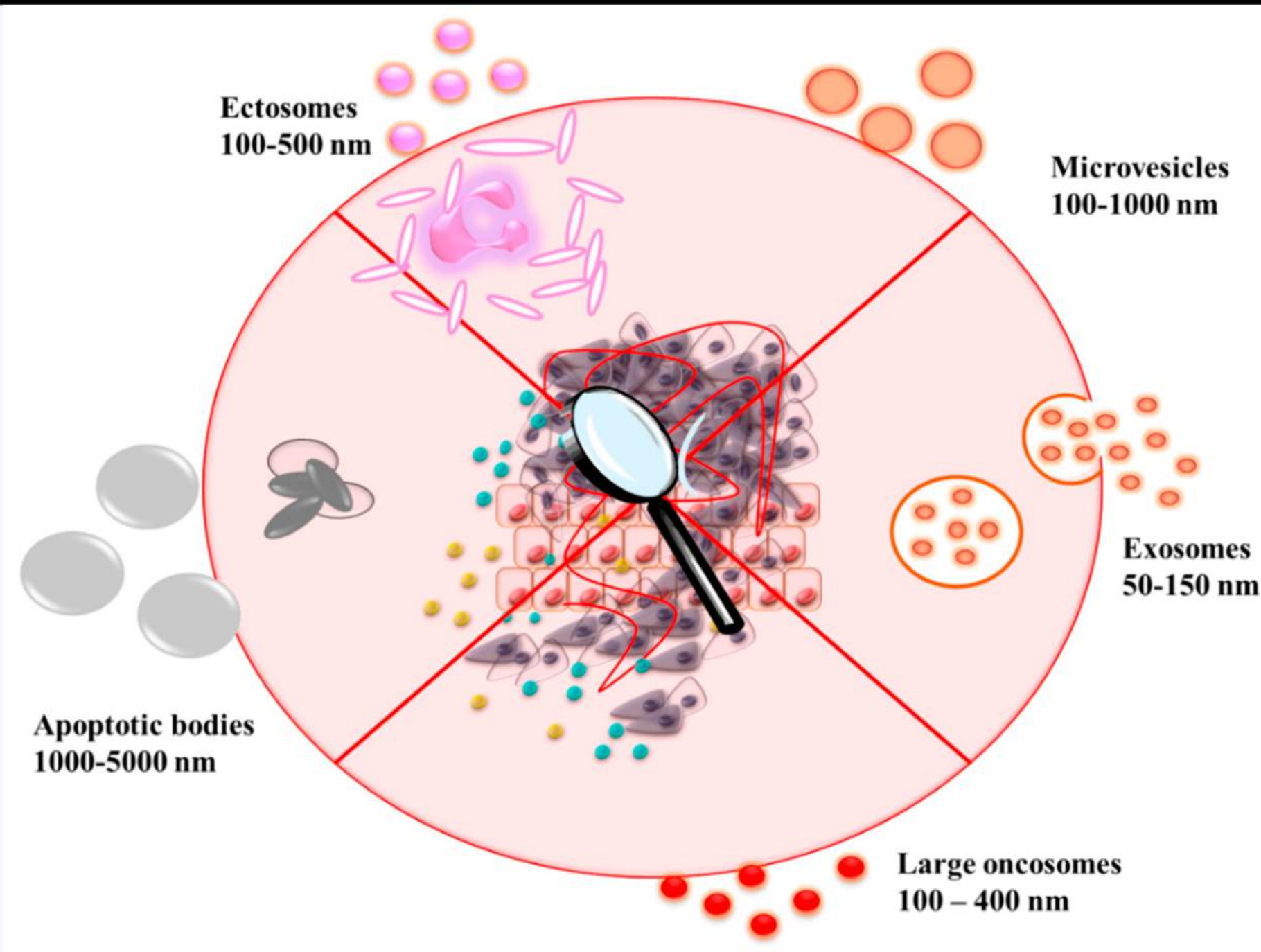
05 How exosomes can be used for disease monitoring and detection

06 How to interpret information found in exosomes

Liquid Biopsies and Cell-Free RNA



Extracellular Vesicles



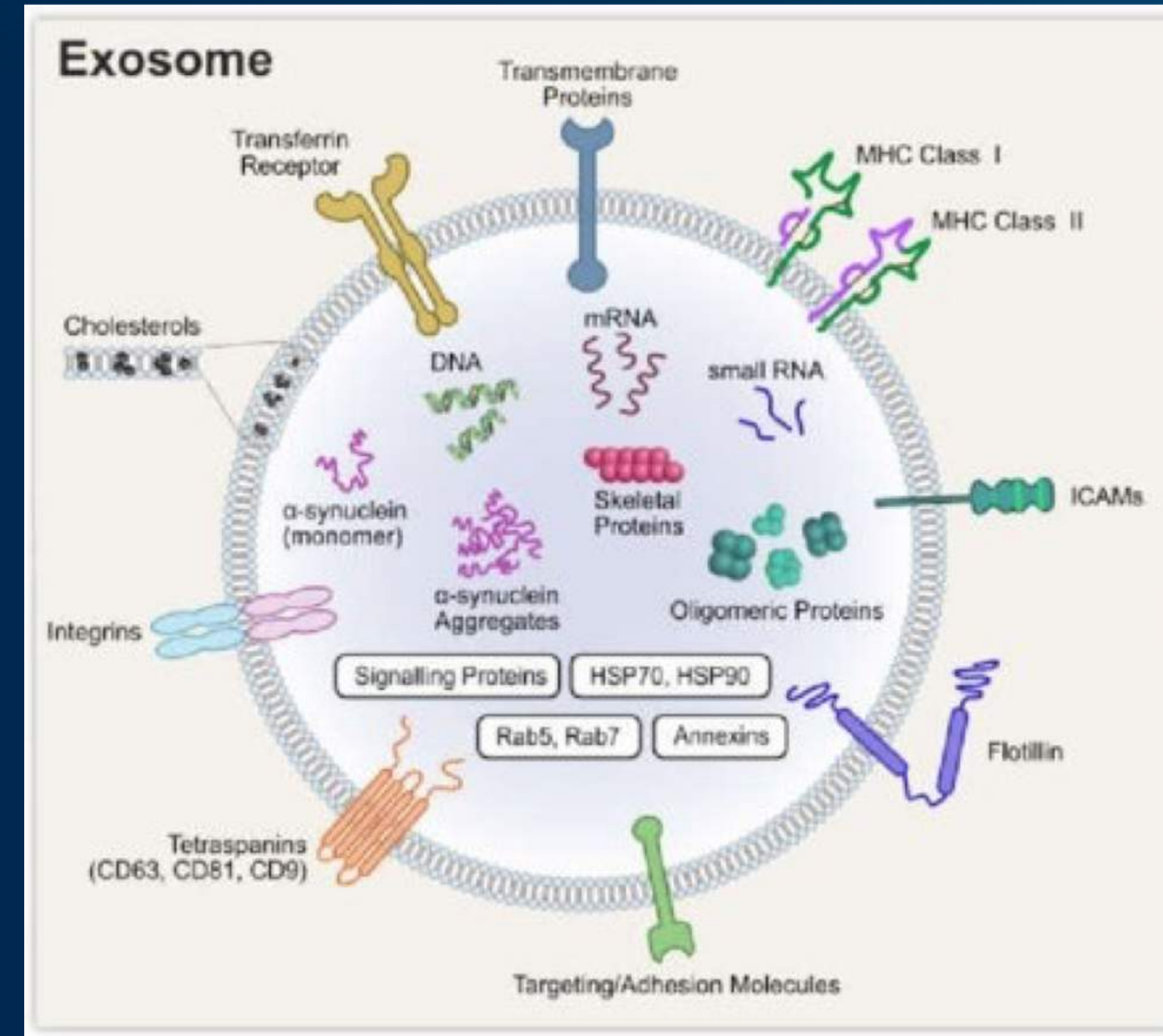
<https://doi.org/10.3390/cancers12020298>

Key Features of the Main EVs Populations

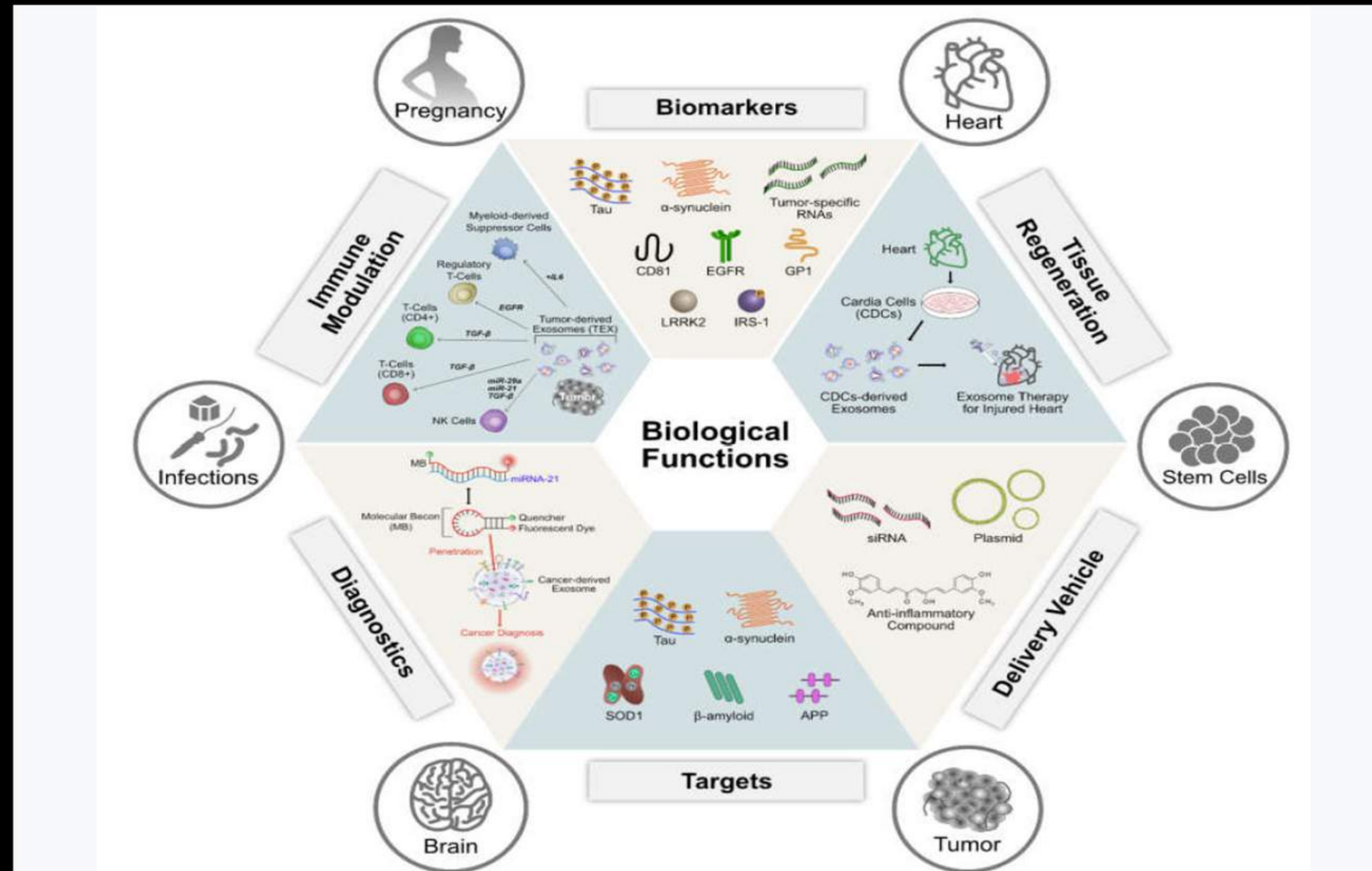
Feature	Exosomes	Microvesicles	Apoptotic Bodies
Size	40-100 nm	100-1000nm	1000-5000 nm
Markers	CD63, CD9, Alix, and TSG101	Annexin V, integrin, selectin, flotillim-2	Annexin , DNA, histones
Cell Shapes	Multivesicular bodies fusion with plasmatic membrane	Membrane Blebbing	Cell shrinkage and cell death
Contents	Proteins, RNA, and miRNA	Proteins, RNA, and miRNA Membrane permeable	Cell organelles, proteins, DNA, RNA, and miRNA
Detection Methods	FACS with CD68 capture, electron microscopy, Western blot for exosomes enriched markers	FACS and electron microscopy	FACS and electron microscopy
Mechanism of Release	Exocytosis of MVBs	Budding from plasma membrane	1000-5000 nm

Exosomes

- 01 Exosomes are small membranous vesicles secreted by a number of cell types including neurons and can be isolated from conditioned cell media or bodily fluids such as urine and plasma.
- 02 Exosomes contain an array of different proteins; some are specific to the cell type of origin, while others are common across all exosomes
- 03 They also contain heat shock proteins, adhesion molecules, metabolic enzymes, cytoskeletal proteins and are heavily enriched in tetraspanins such as CD63 and CD81
- 04 In addition to their protein content these vesicles have recently been shown to contain messenger RNA (mRNA) and microRNA (miRNA) species

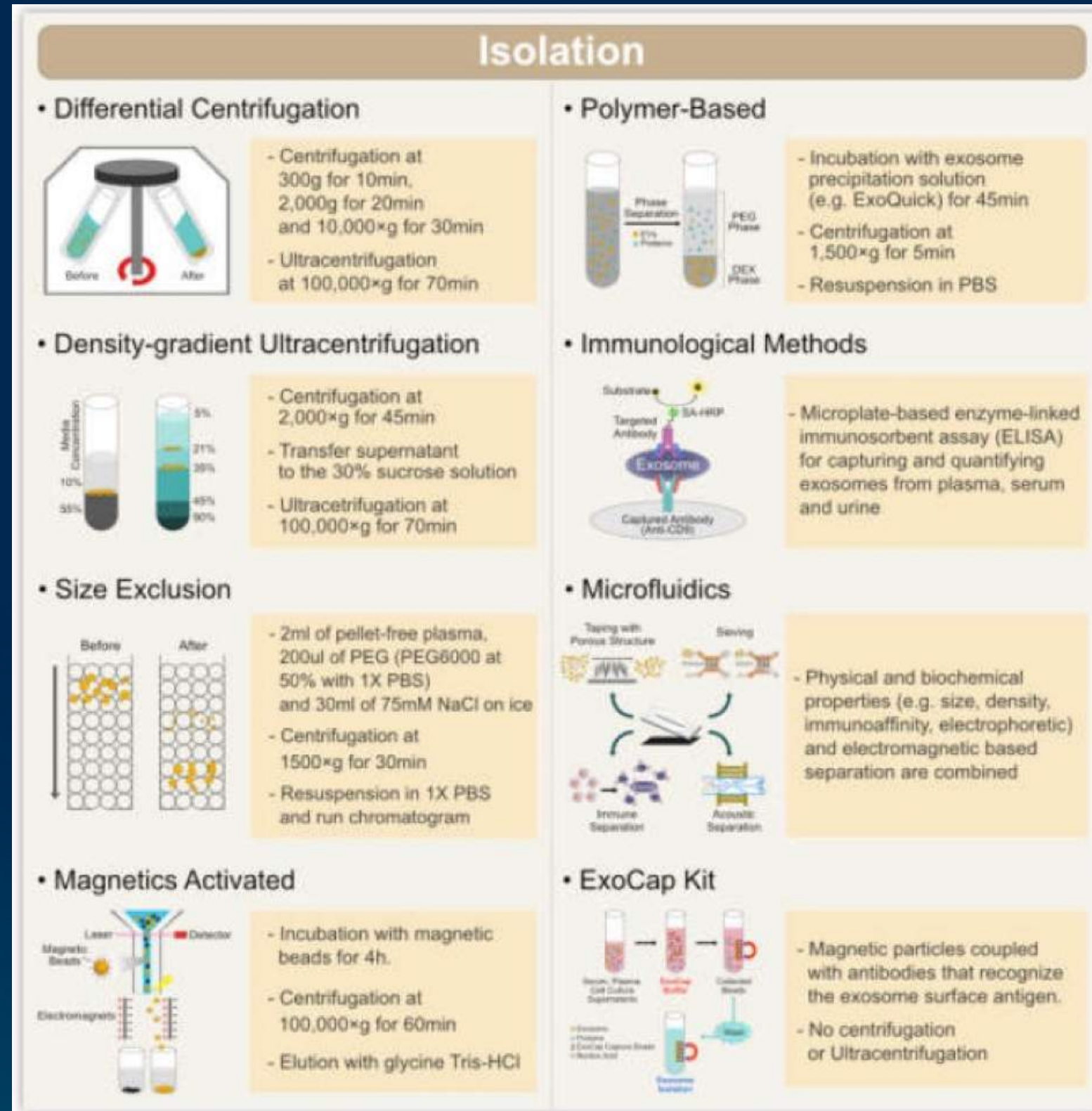


Biological Functions for Exosomes



DOI: 10.3390/cells8040307

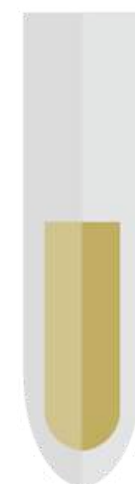
Methods for **Exosome / Extracellular Vesicles (EVs)** Purification



Silicon Carbide as a Matrix for Exosome Purification



Sample



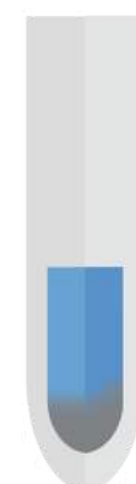
+ ExocC



+ Slurry E



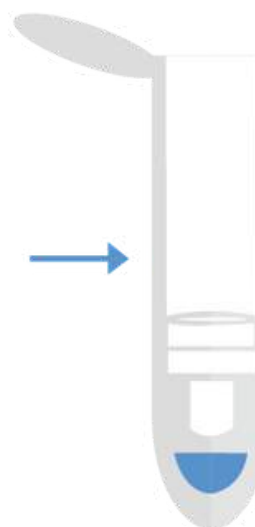
Slurry pellet



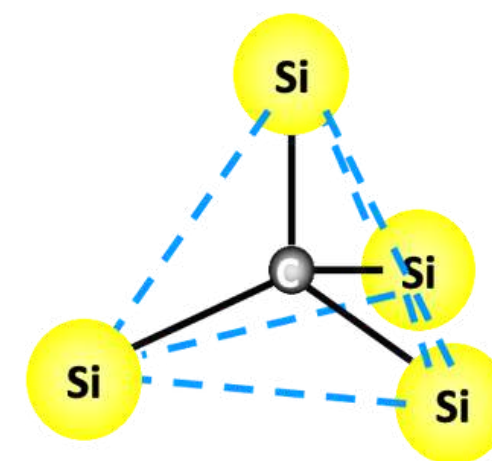
+ ExoR
5 min @ RT



+ ExoR
5 min @ RT



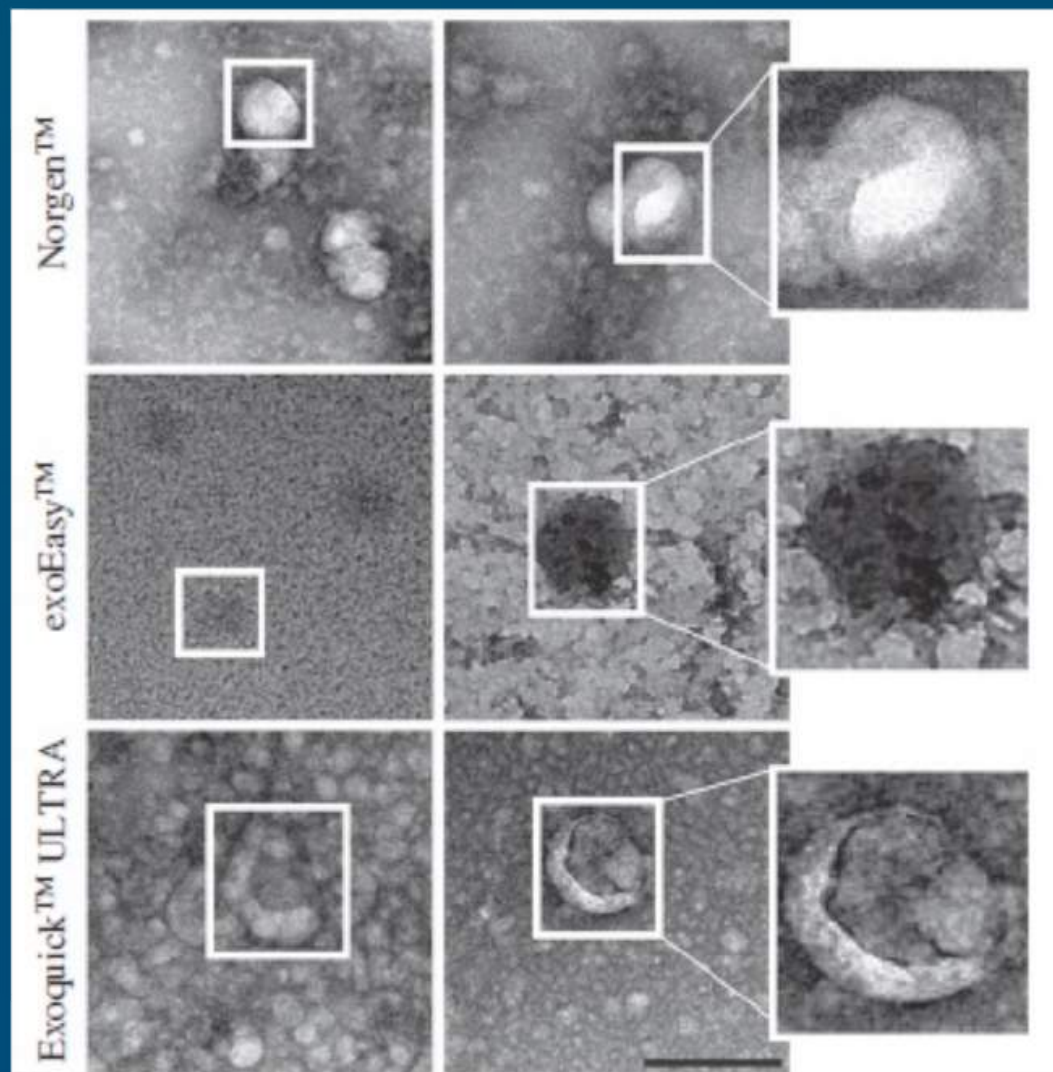
Purified Exosomes
Ready for further analyses!



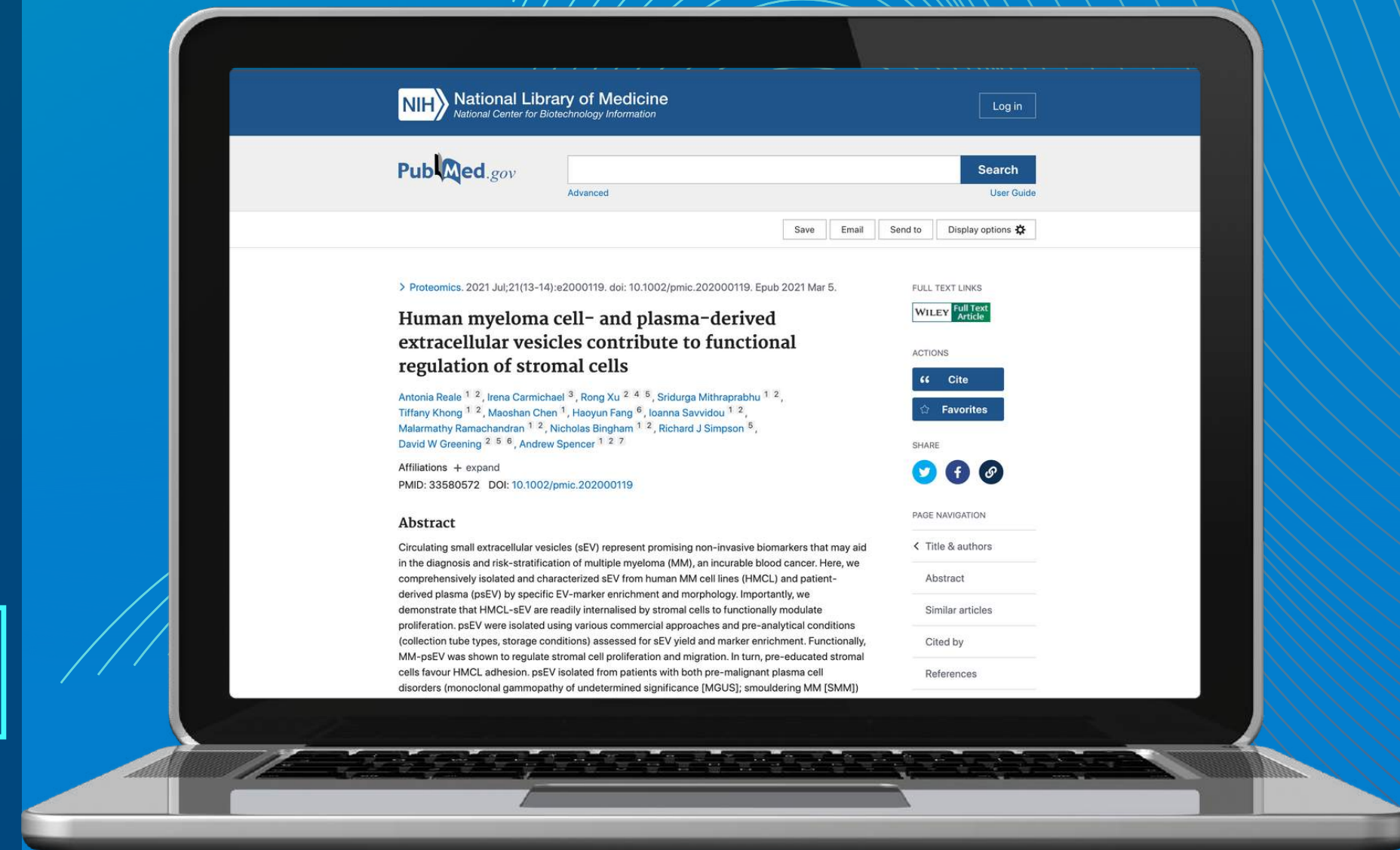


Important Exosomal Downstream Applications

Human myeloma cell- plasma derived extracellular vesicles contribute to functional regulation of stromal cells



Resin-based kit outperforms
isolations of small EVs



DOI: 10.1002/pmic.202000119

Exosomes Purified with High Quality and Quantity

Blood Plasma (PBPL)
(1 mL)

4°C

1800 x g, 10 min → Platelet Poor PBPL

2000 x g, 15 min → Platelet Free PBPL (PFP)

10 000 x g, 30 min → Large EVs (pLEV)
+PBS wash (x 3)

Norgen

exoEasy™

exoquick™ ULTRA

Membrane-base
Affinity

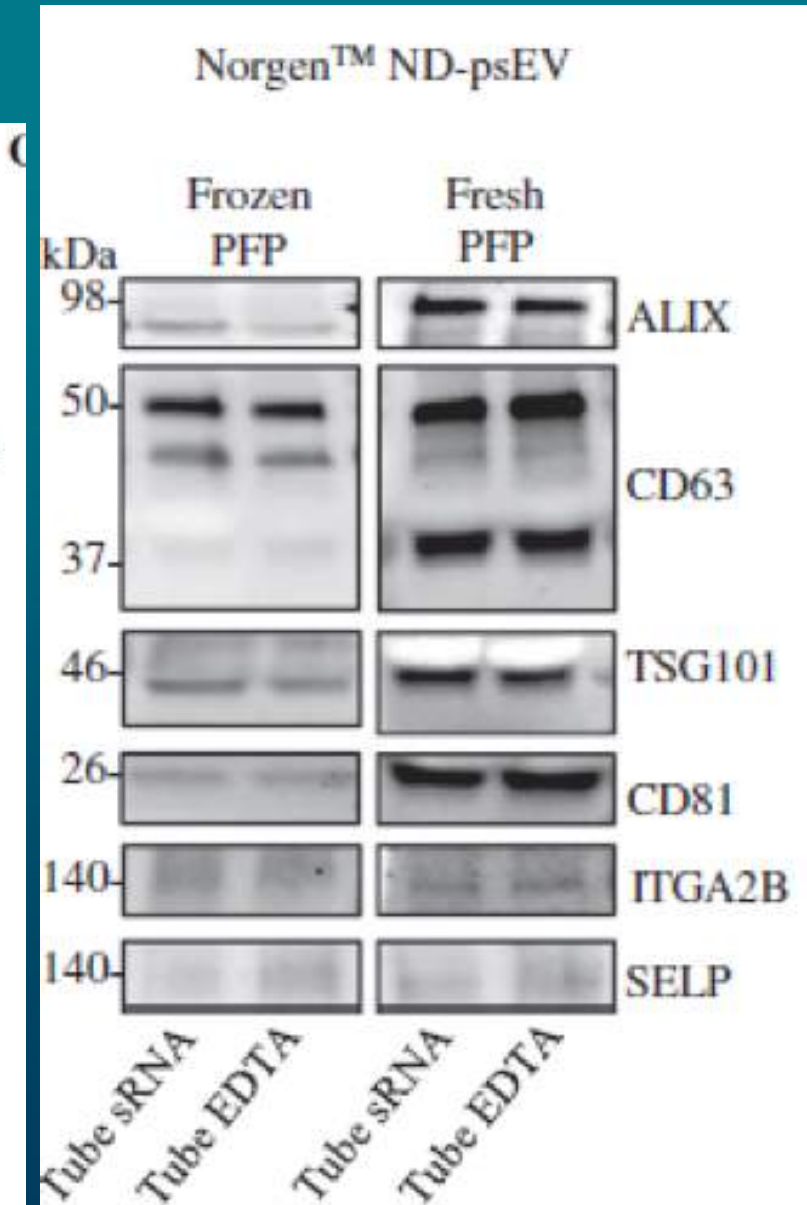
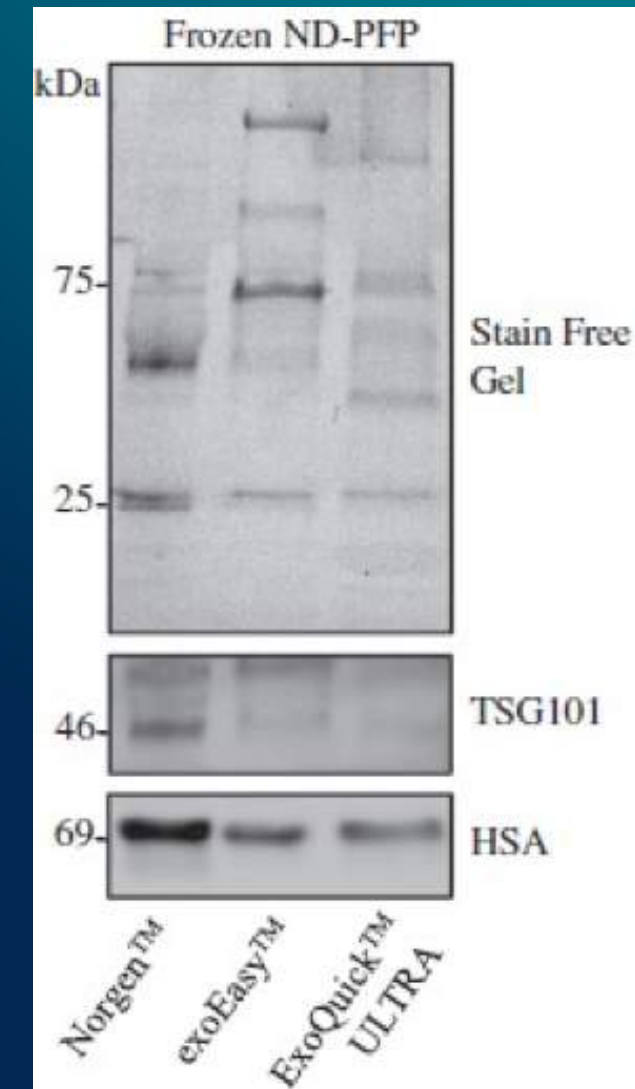
- Thrombin pre-treatment
- Precipitation
- Purification column

PFP small EVs eluates (psEV)

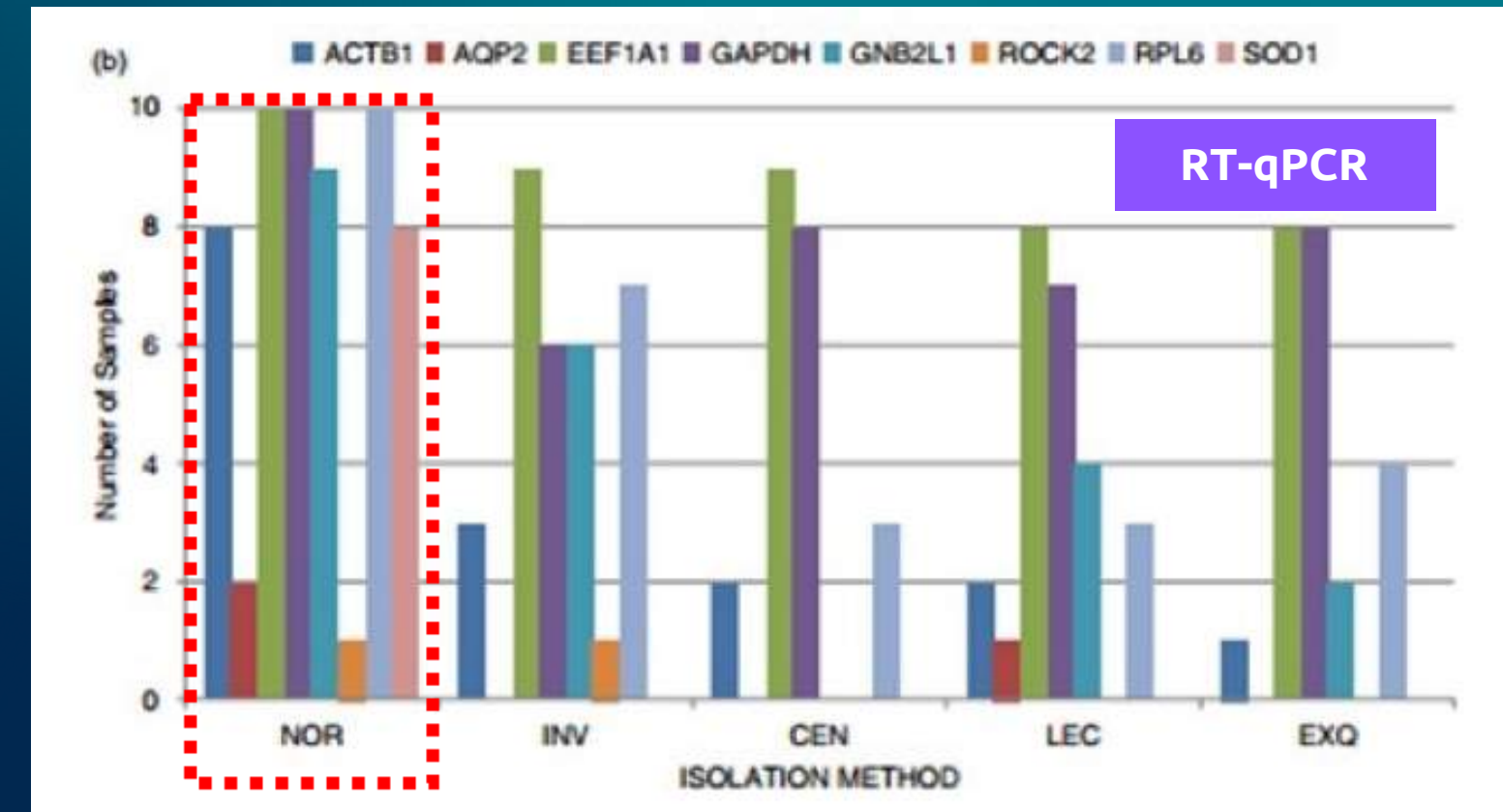
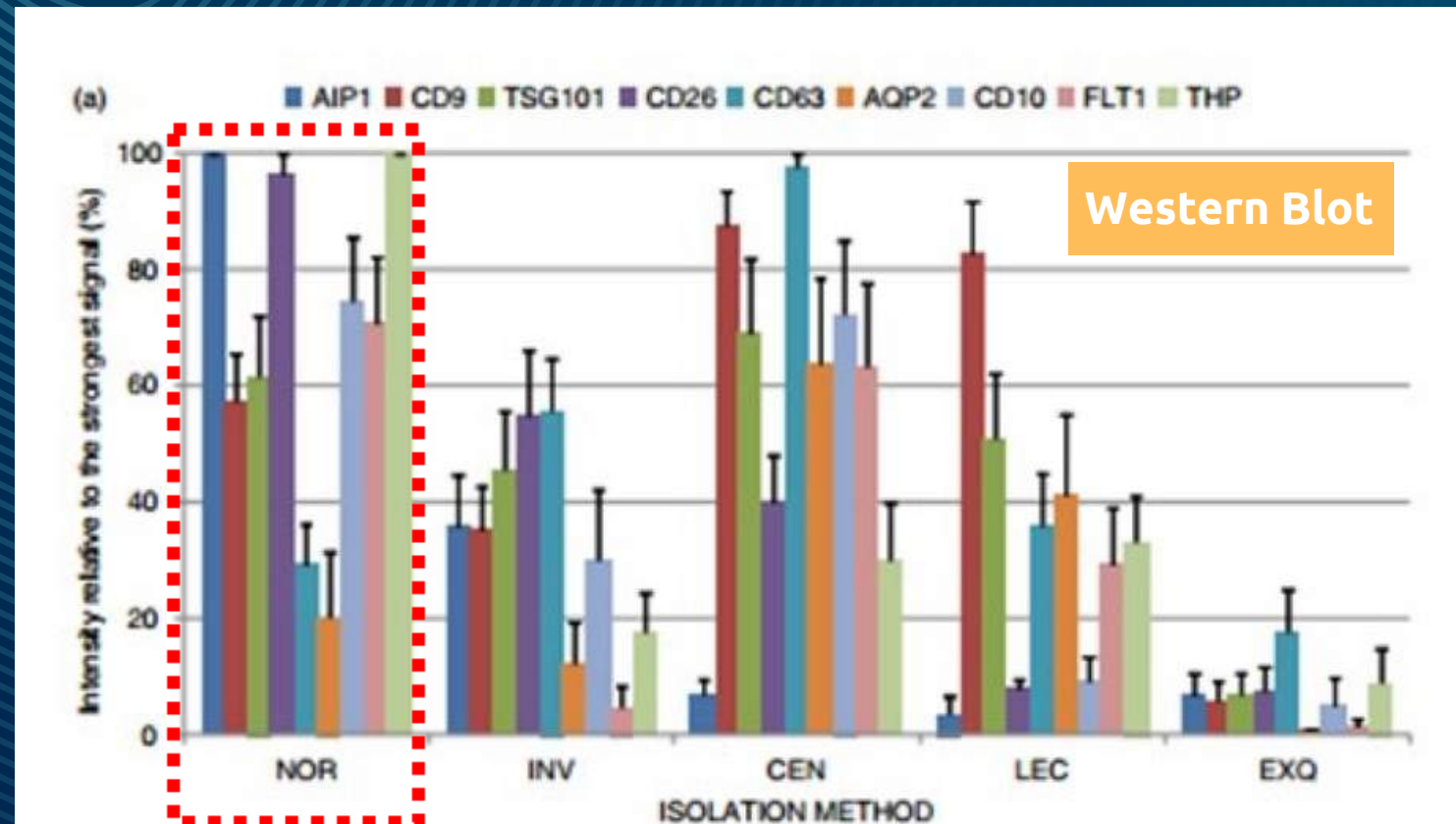
(1514 ± 220 µg)

(360 ± 170 µg)

(1430 ± 374 µg)



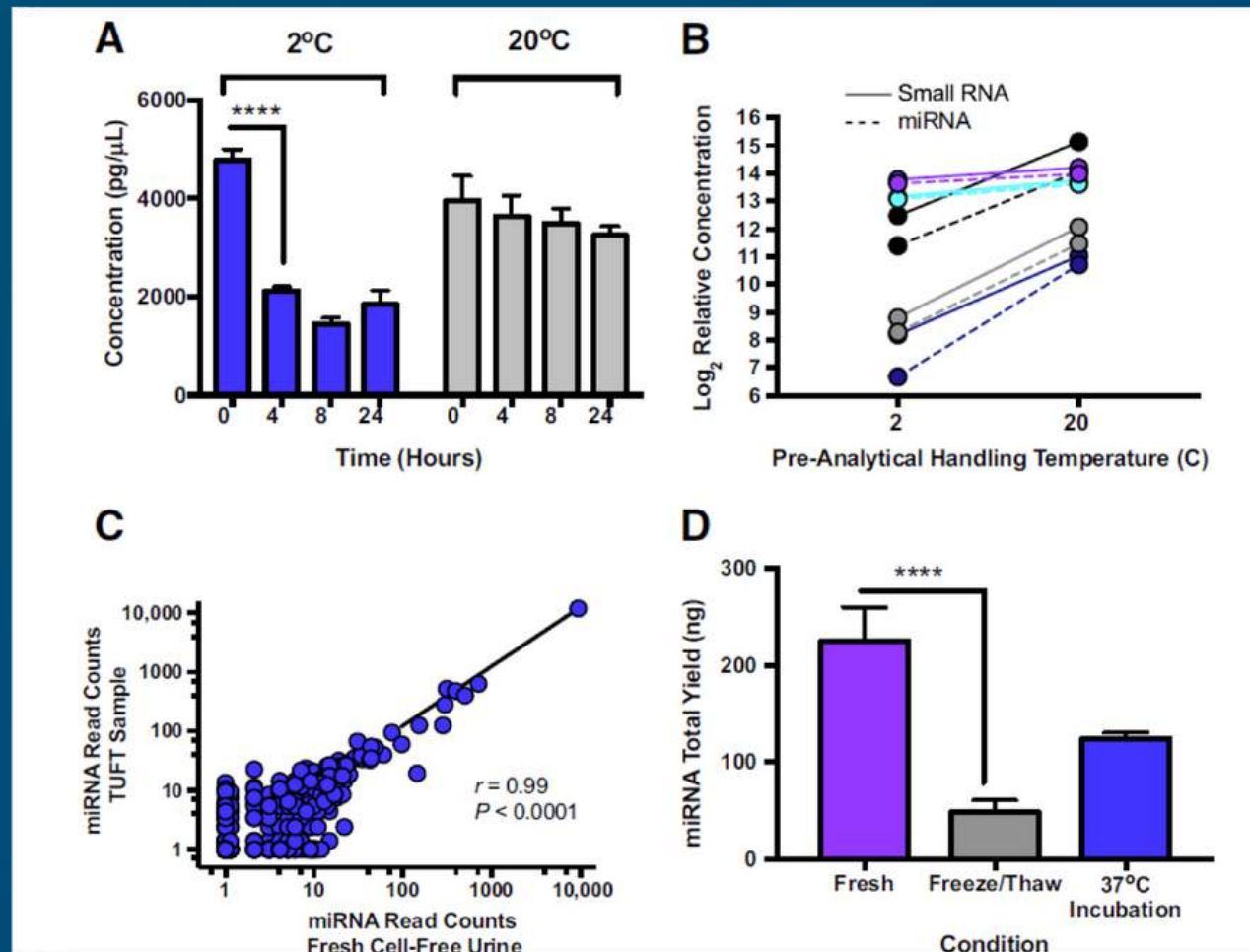
Simple and Rapid Exosome Purification Method



Enrichment of Exosomes using Norgen's technology resulted in Better Signals for Protein and RNA when compared to Ultracentrifugation, Lectin-Based Protocol or other Commercial Products.

Urine Exosomes
Urine Exosome RNA Purification Kit (#47200)
 Royo et al (CIC bioGUNE, Spain)
 J. Extracellular Vesicles 2016, 5: 29497

Pre-Analytical Handling Conditions and Small RNA Recovery from Urine for miRNA Profiling



DOI:10.1016/j.jmoldx.2018.04.003



RNA Purification: Technology Consideration for Exosomes

- 1 - Exosomes or EVs are sub-populations of Liquid Biopsies (such as plasma/serum) - Low RNA Quantity
- 2 - There is still no universally accepted view in regards to the RNA content found inside Exosomes or EVs
 1. Large RNAs vs miRNAs
 2. Presence or Absence of rRNAs

Exosomal RNA Purification Technology Must be:

- Sensitive
- Diverse in size (Size Diversity)



Phenol:Chloroform Sample Prep has Base-Bias (Loss of Diversity)

Molecular Cell

Letter to the Editor

Cell
PRESS

Short Structured RNAs with Low GC Content Are Selectively Lost during Extraction from a Small Number of Cells

In our recent paper (Kim et al., 2011), we reported that a subset of microRNAs (miRNAs) including miR-141, miR-29b,

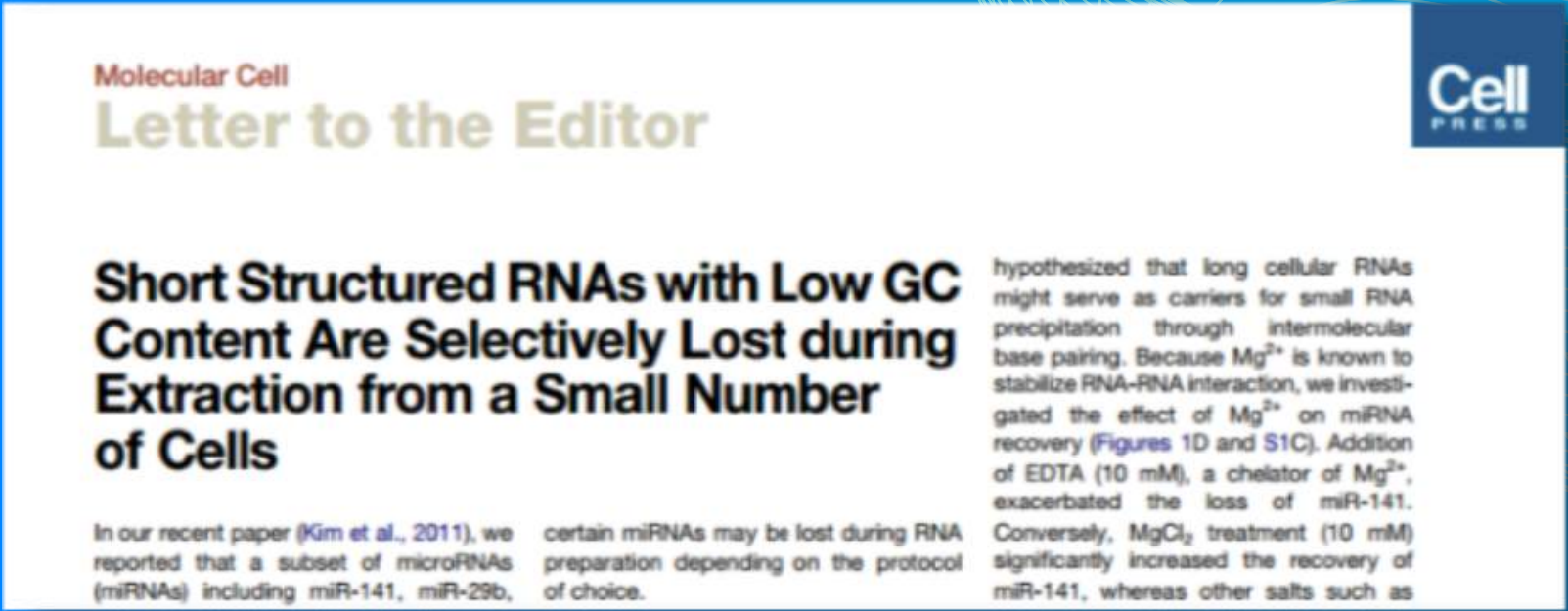
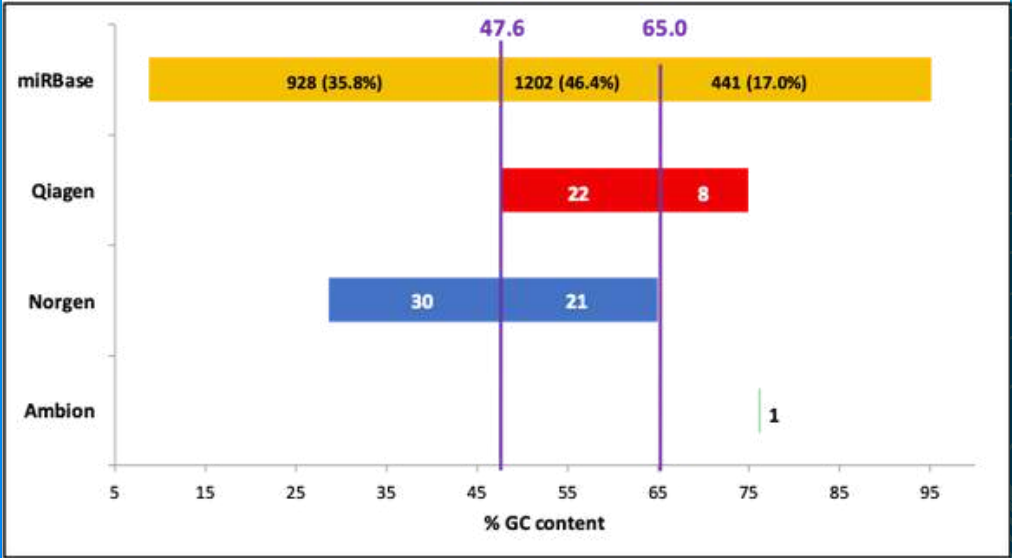
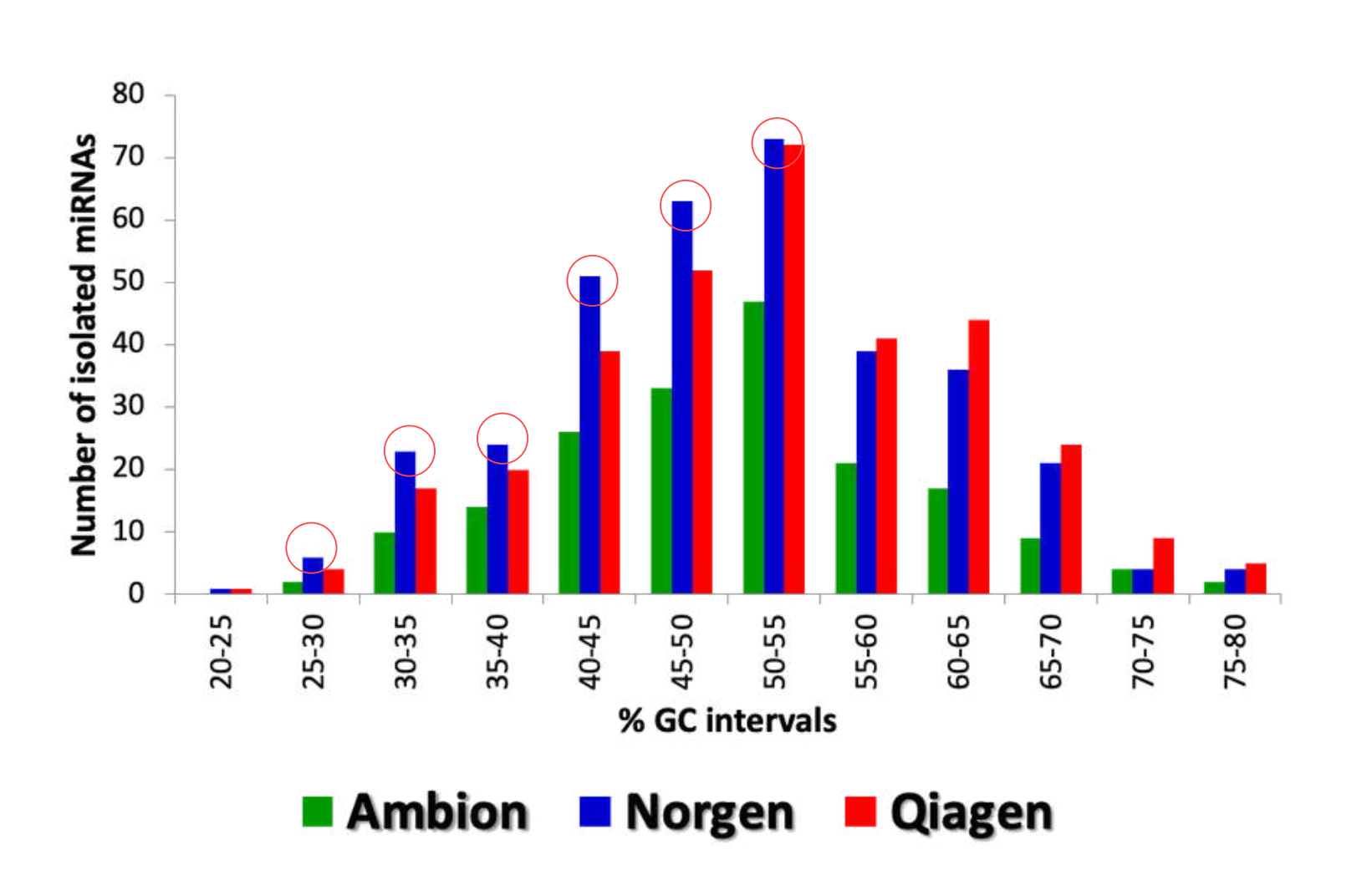
certain miRNAs may be lost during RNA preparation depending on the protocol of choice.

hypothesized that long cellular RNAs might serve as carriers for small RNA precipitation through intermolecular base pairing. Because Mg^{2+} is known to stabilize RNA-RNA interaction, we investigated the effect of Mg^{2+} on miRNA recovery (Figures 1D and S1C). Addition of EDTA (10 mM), a chelator of Mg^{2+} , exacerbated the loss of miR-141. Conversely, $MgCl_2$ treatment (10 mM) significantly increased the recovery of miR-141, whereas other salts such as

Phenol:Chloroform Extraction Procedures tend to have **bias in RNA (particularly miRNA) recovery** based on **GC contents**

Kim, Y.K., Yeo, J., Ha, M., Kim, B., and Kim, V.N. (2011). Cell adhesion-dependent control of microRNA decay. Mol. Cell 43, 1005–1014.

Silicon Carbide Technology recovers a Better Diversity of microRNAs, especially microRNAs with Low GC Contents



Molecular Cell 46, 893-895, June 29 2012

Simple and Rapid Exosome Purification Method

Flexible Entry Point of Workflow to Customize Individual's Needs

Urine

Plasma/Serum

Culture Media

Exosome Purification
(UC, Beads, Filters)

Exosome Purification
(Norgen Biotek Corp.)

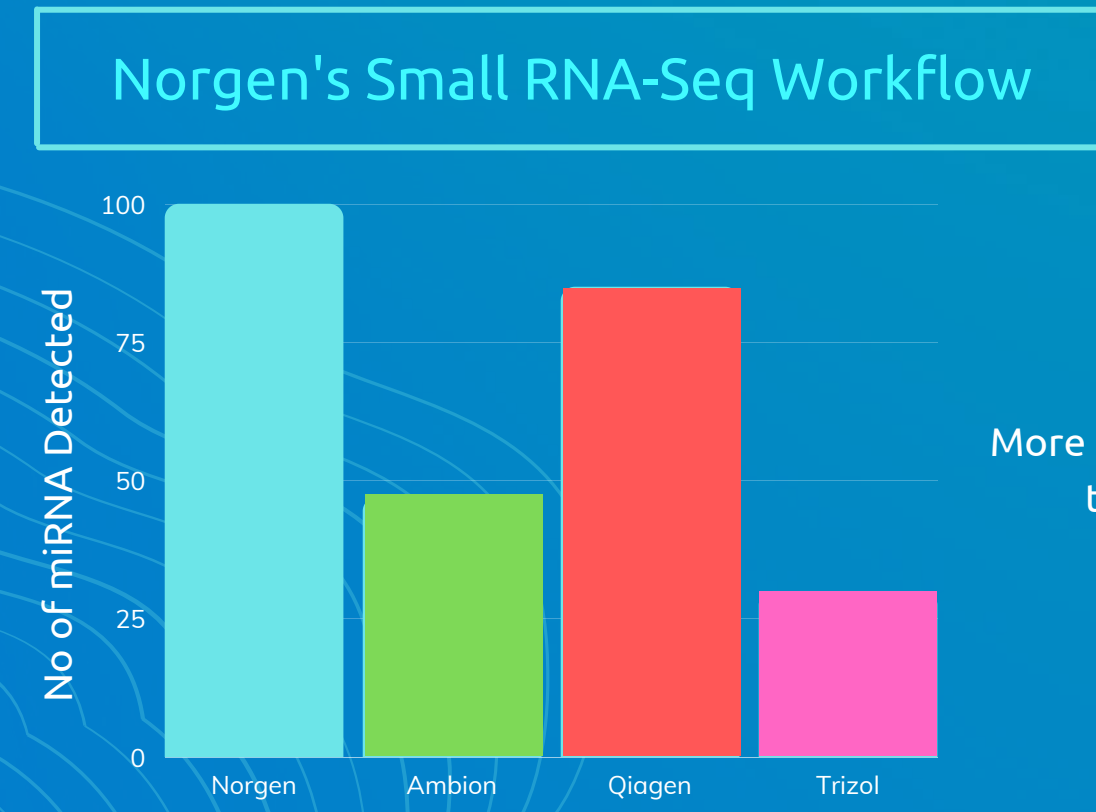
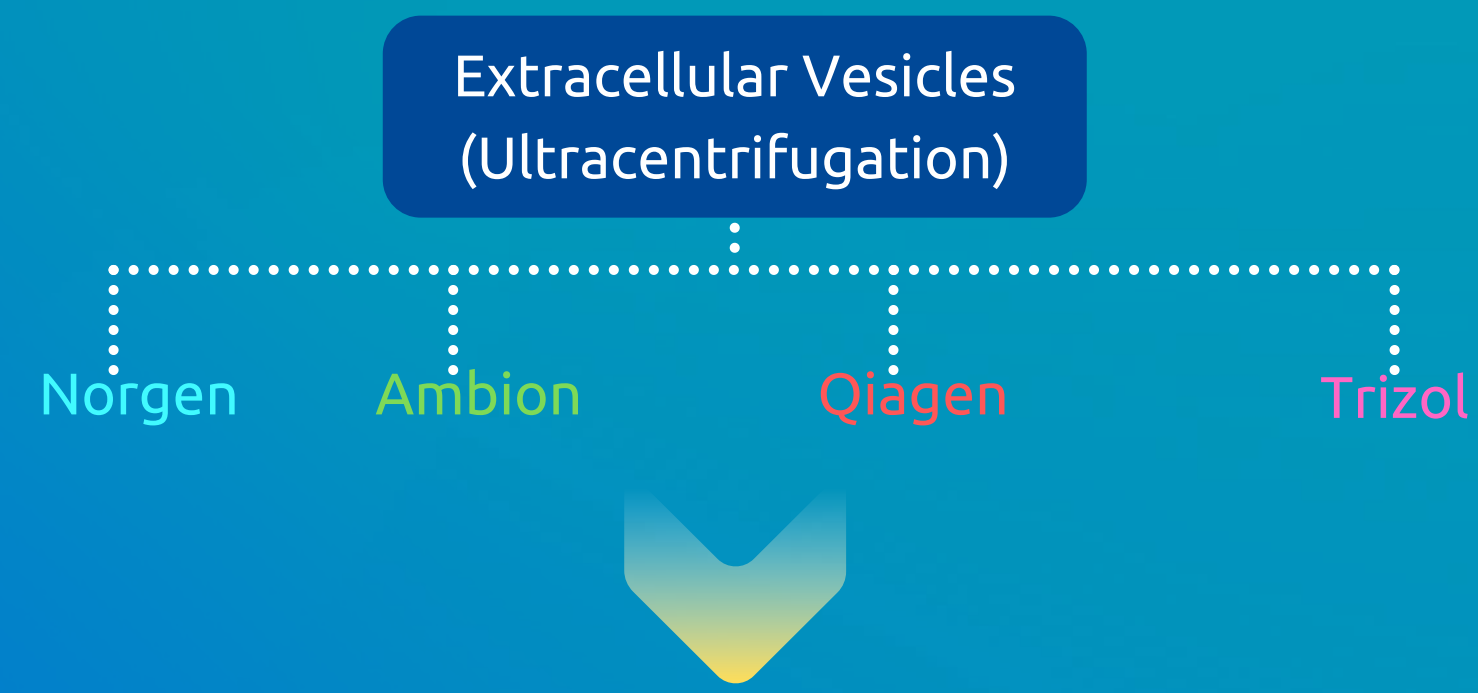
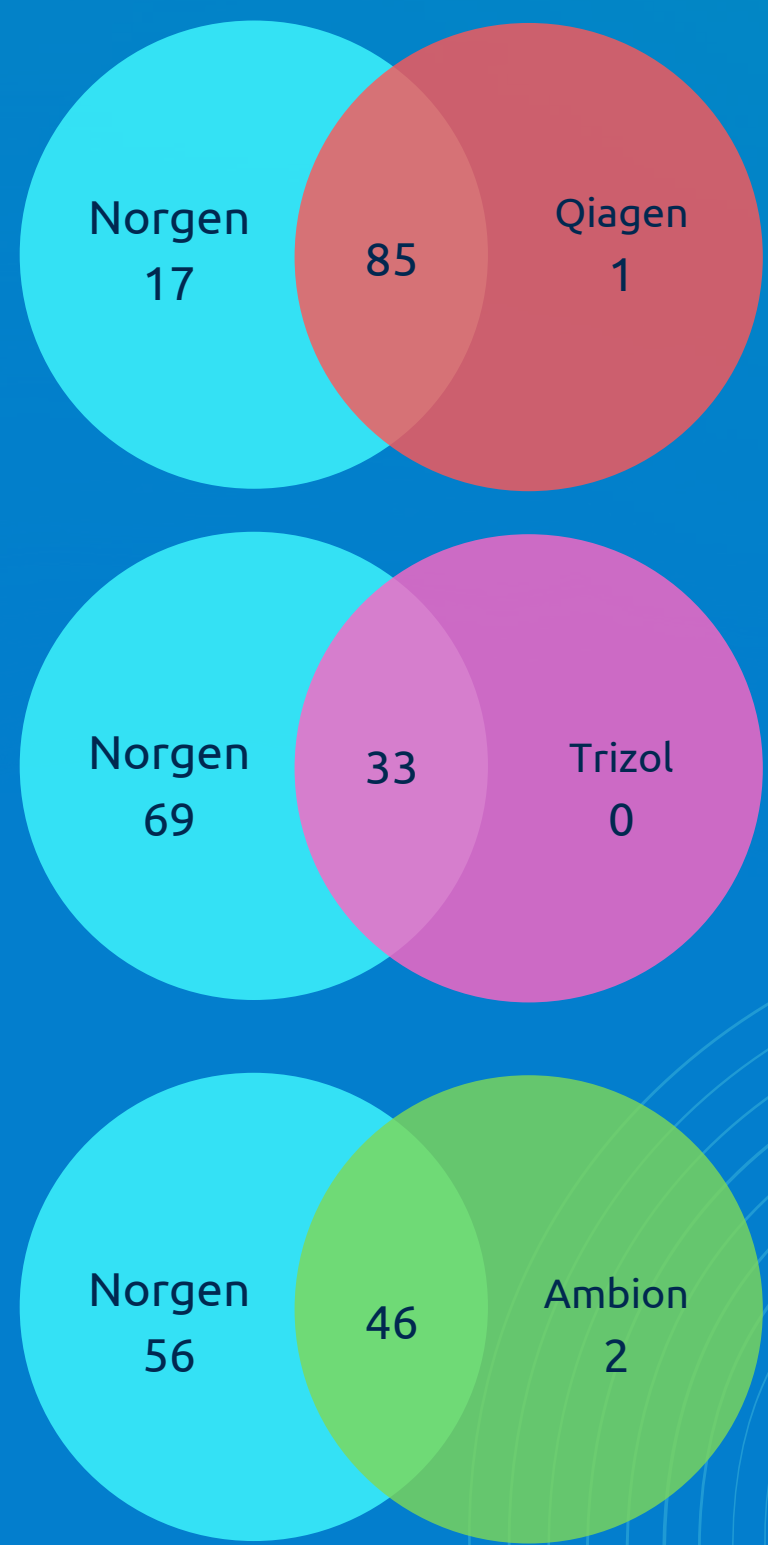
Norgen's Best-in-Class RNA Purification

Norgen's Small RNA-Seq Workflow



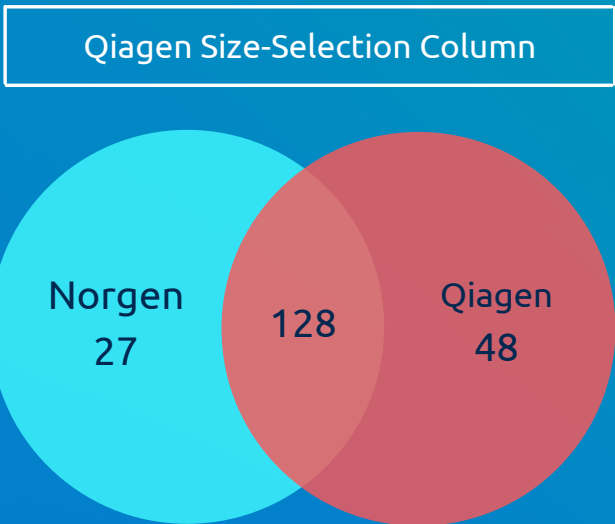
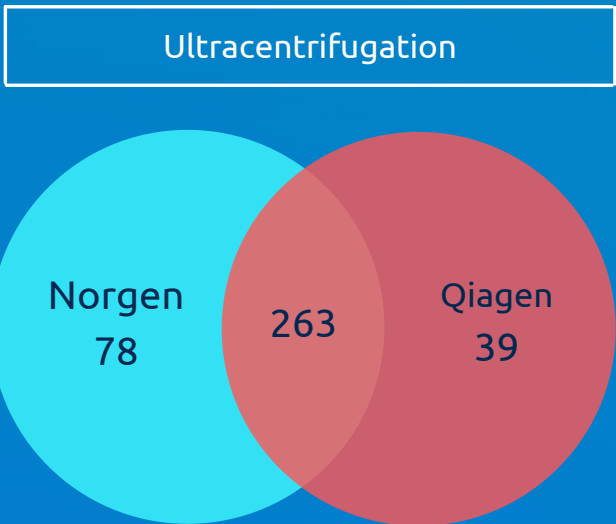
Illumina
Propel Certified™

Exosomes with Low RNA Content Requires Sensitive RNA Sample Preparation

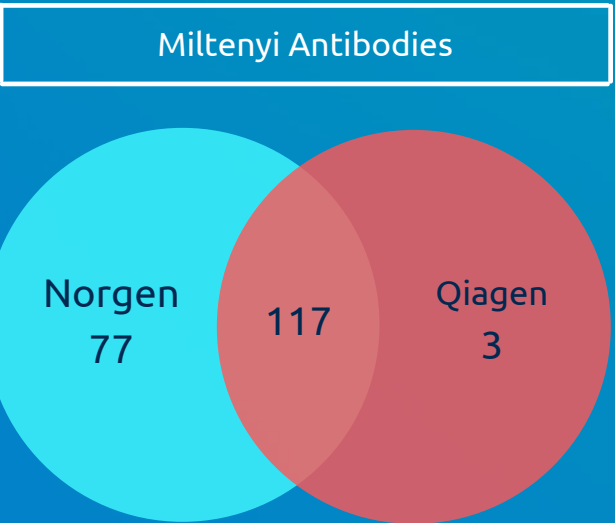
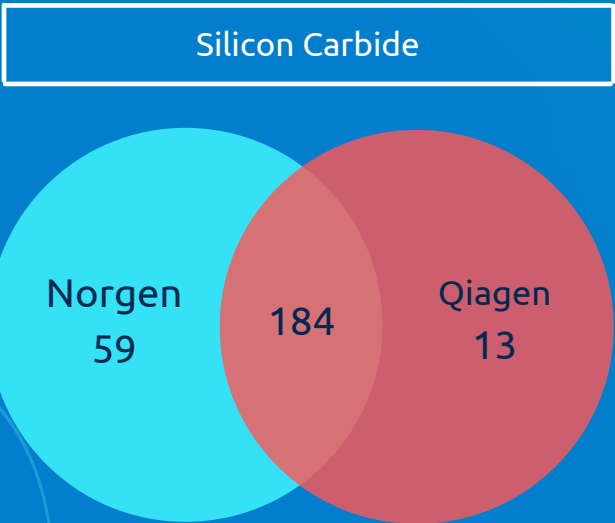
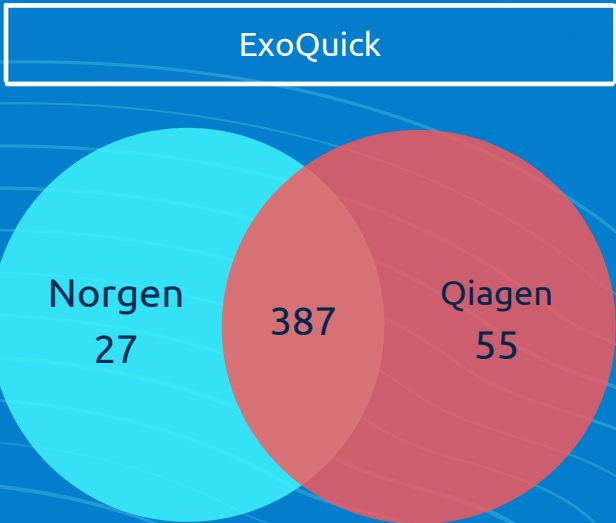


More miRNA significantly increases the chance for Discovery

Comparing microRNA Diversity Recovered among Different Exosome Purification Methods

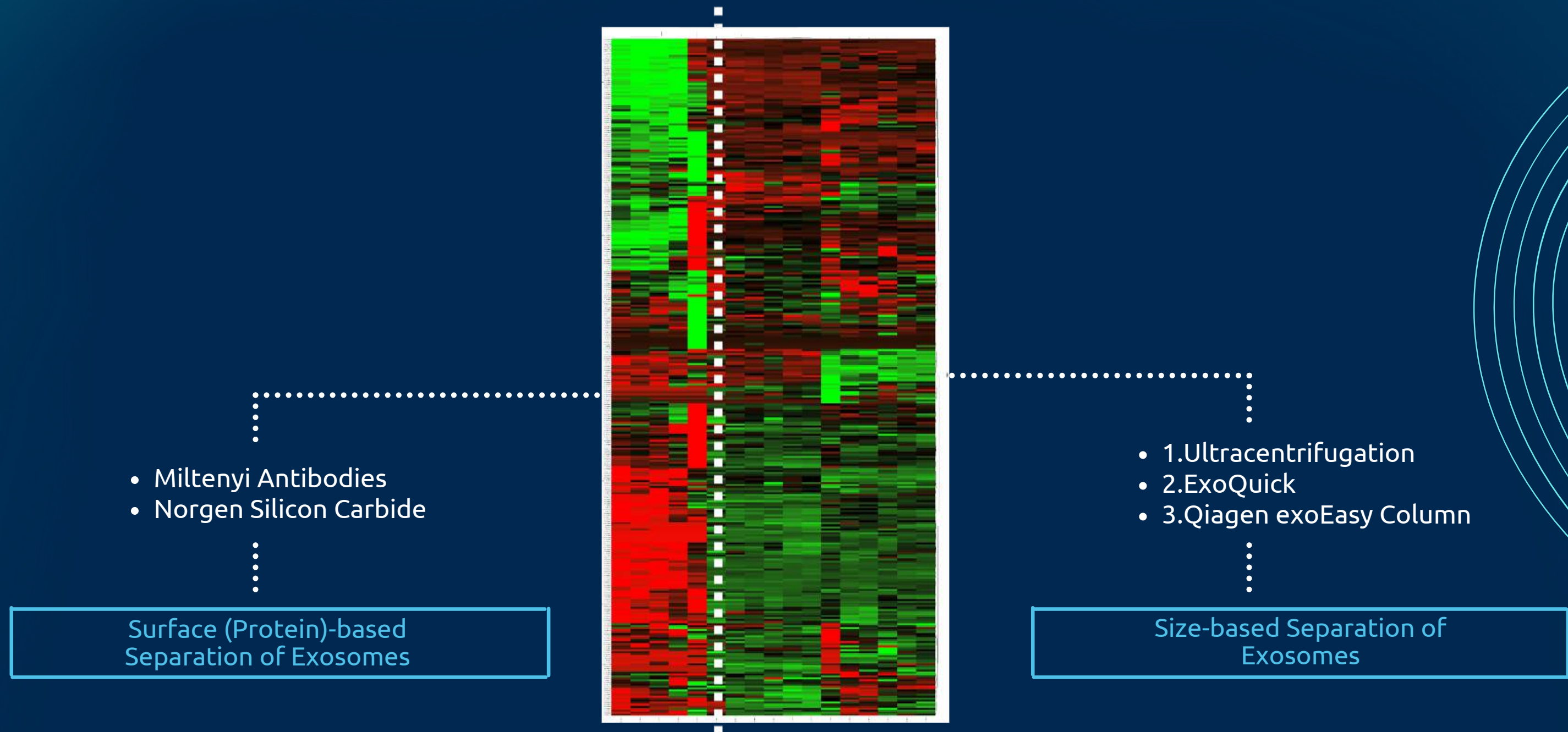


Only miRNAs detected in all replicates at >1 read are reported

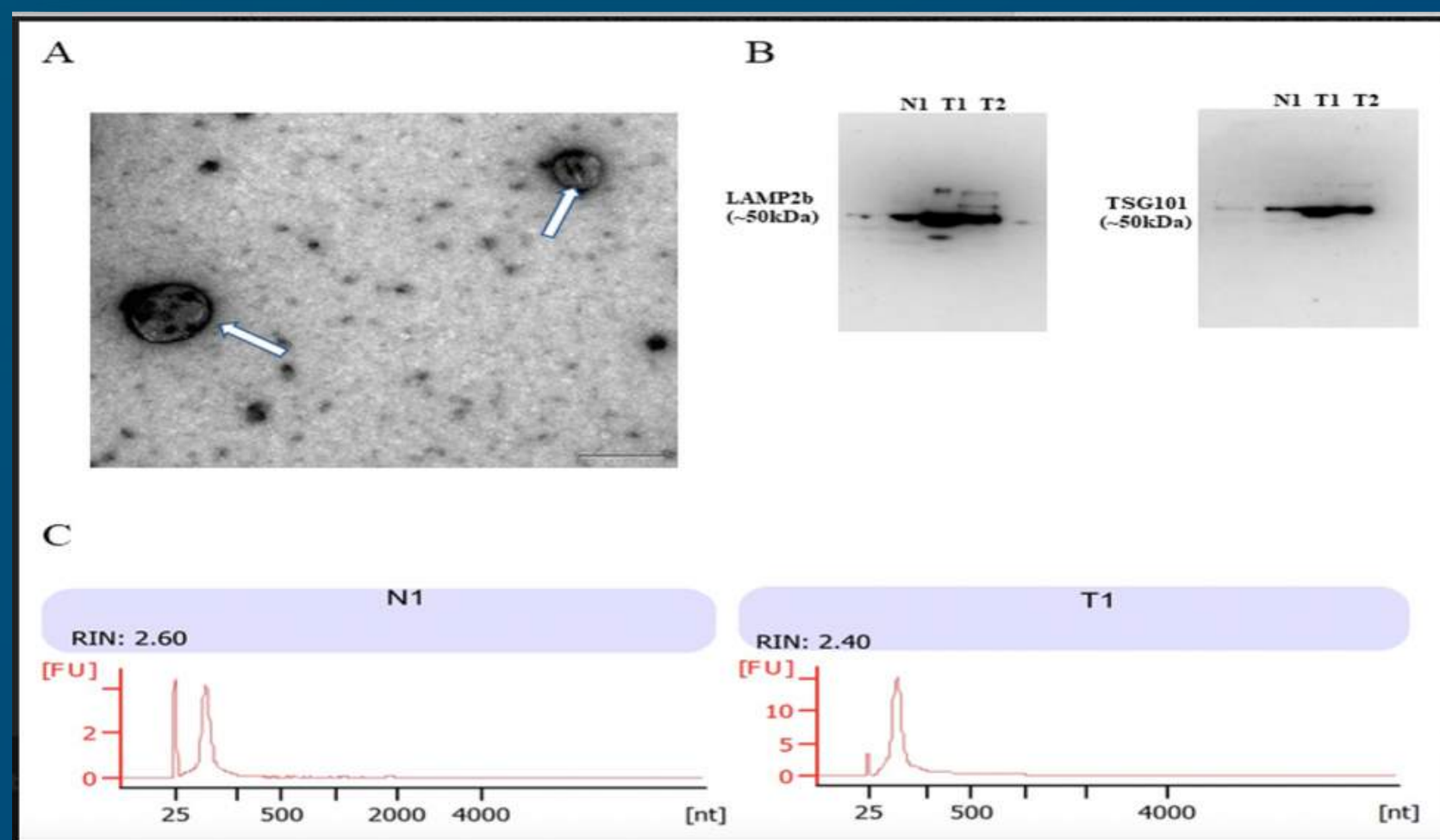


- 1.Exosome package a significant amount of microRNAs
- 2.Each exosome purification method may yield a different set of exosome or exosomal microRNA

Comparing **microRNA Diversity** Recovered among Different Exosome Purification Methods

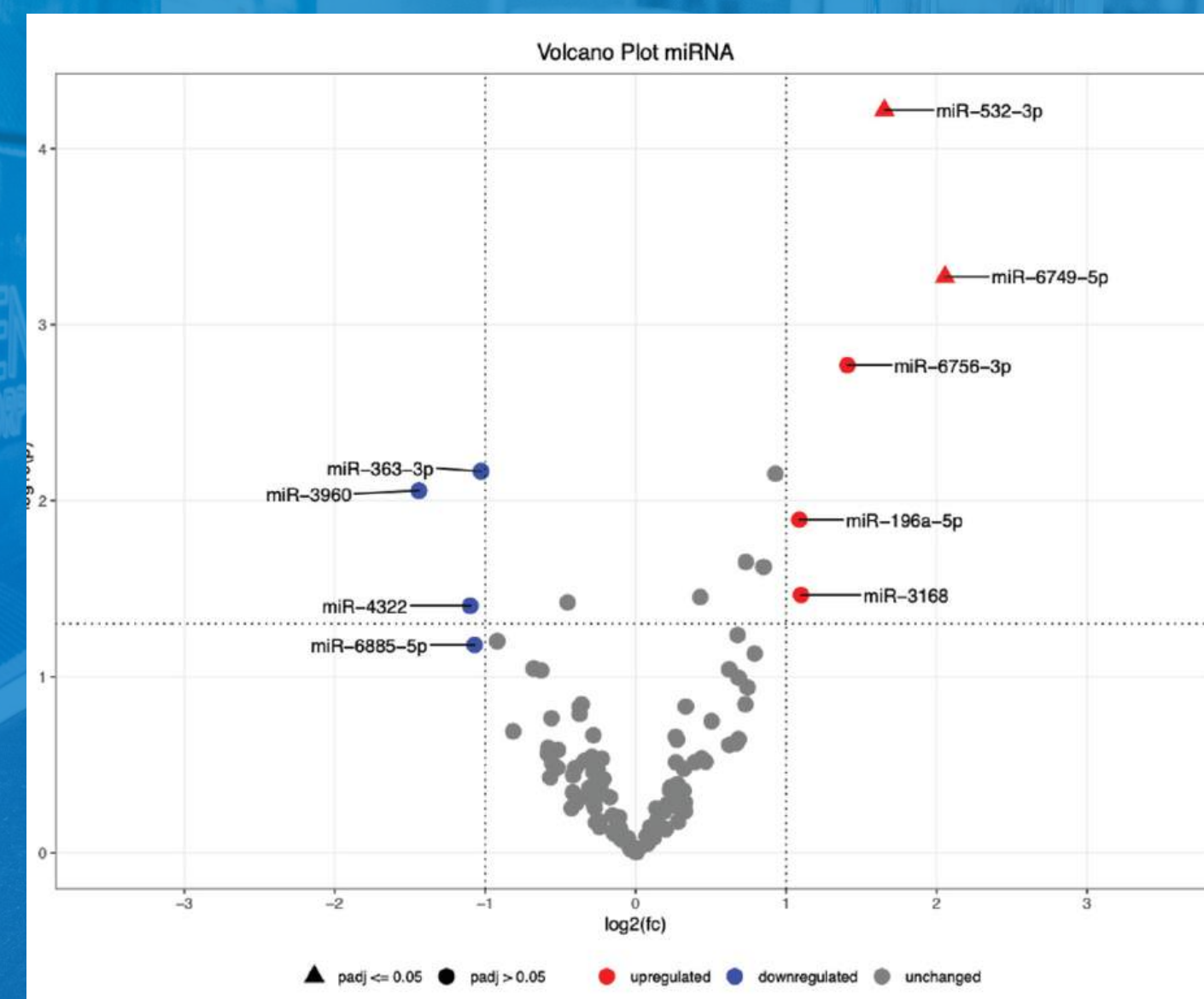


Identification of piRNA Targets in Urinary Extracellular Vesicles for the Diagnosis of Prostate Cancer



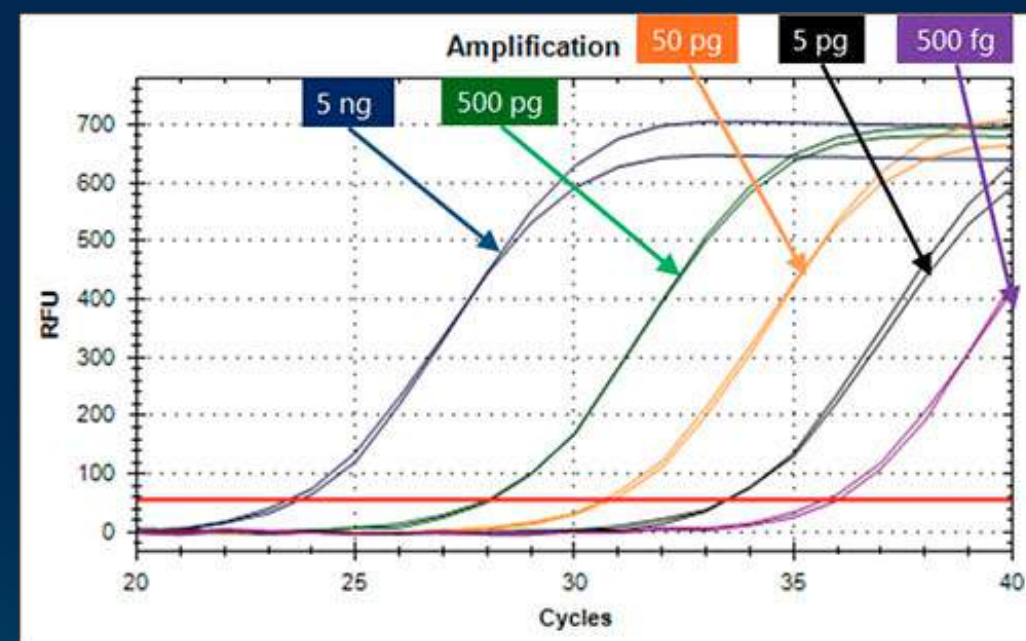
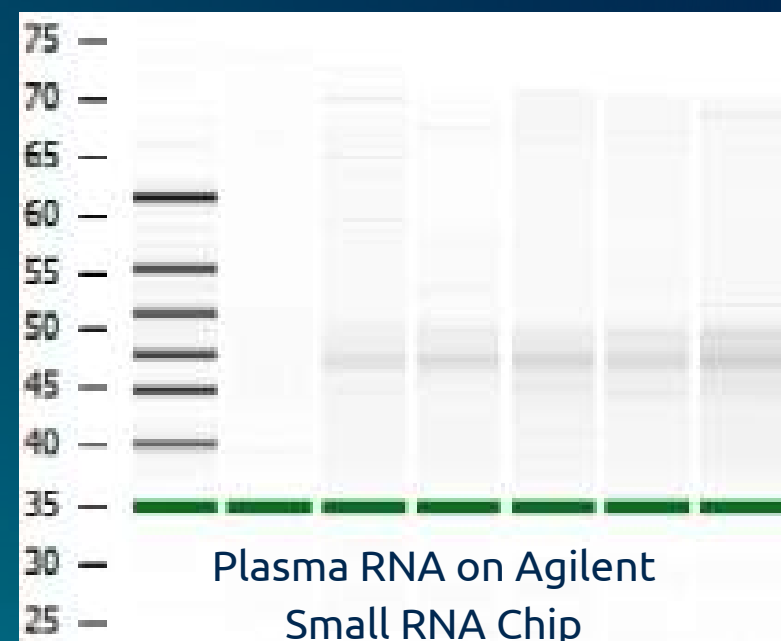
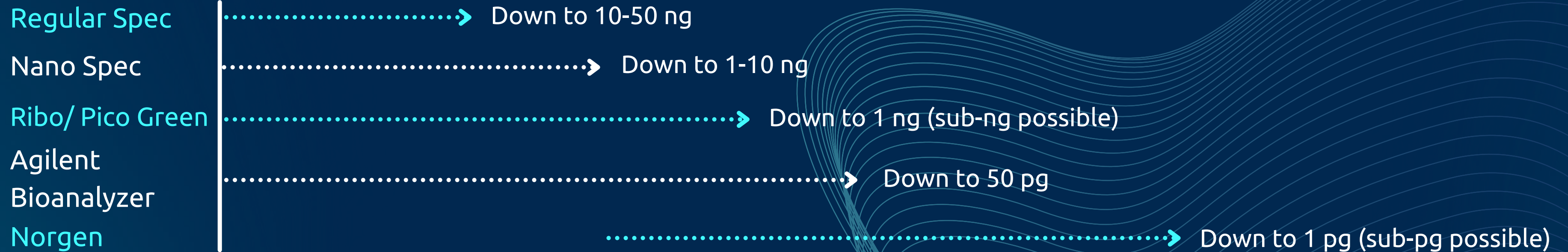
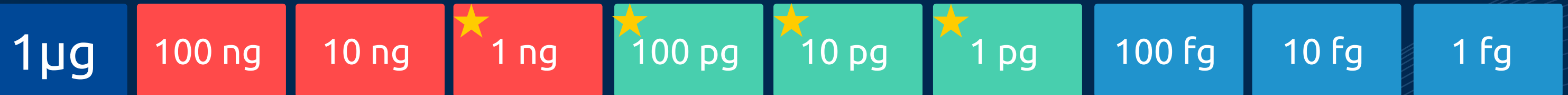
DOI: 10.3390/diagnostics11101828

MicroRNAs from urinary exosomes as alternative biomarkers in the differentiation of benign and malignant prostate diseases



DOI: 10.33393/jcb.2022.2317

Quantification of Nucleic Acids of Liquid Biopsies is Challenging

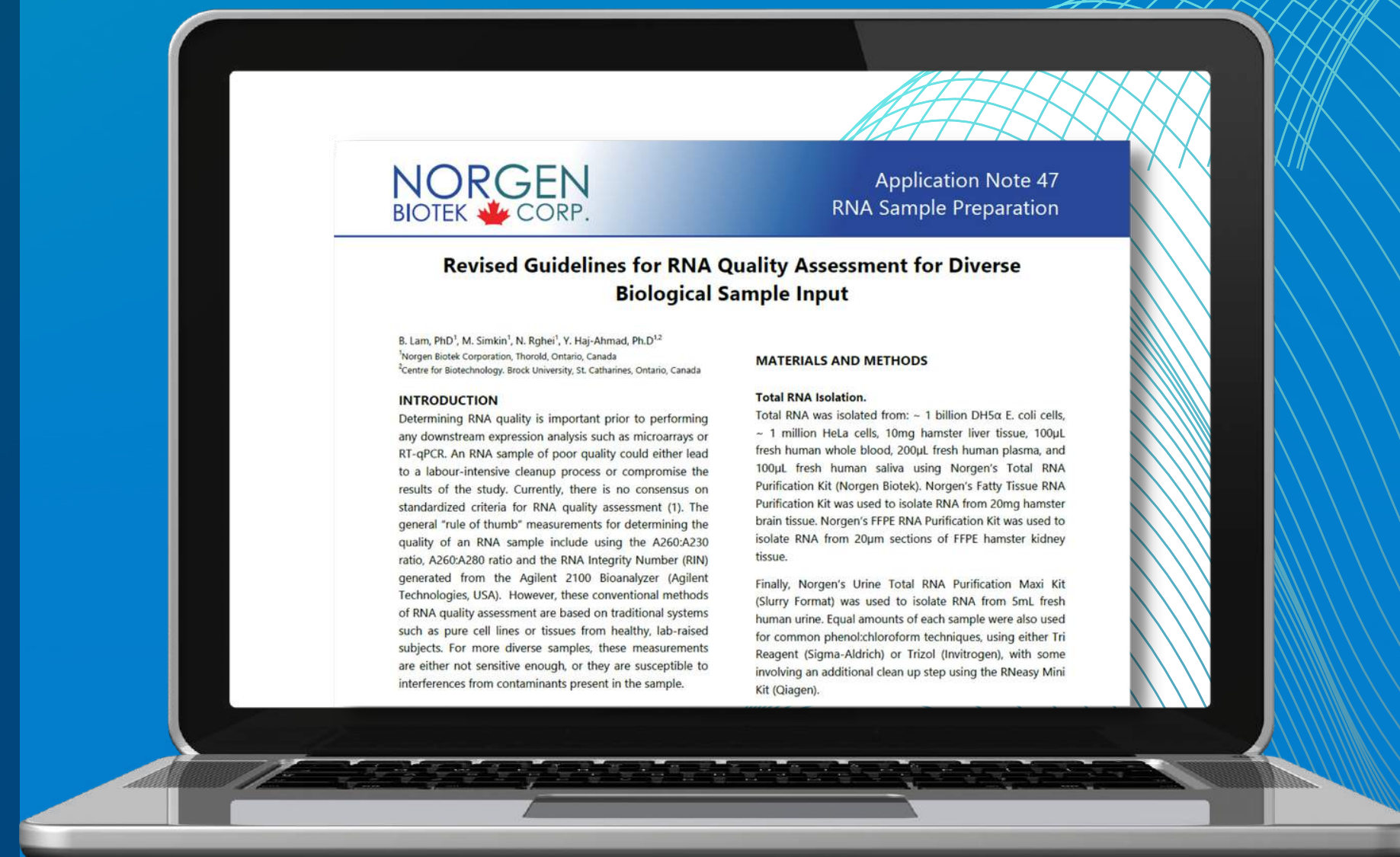
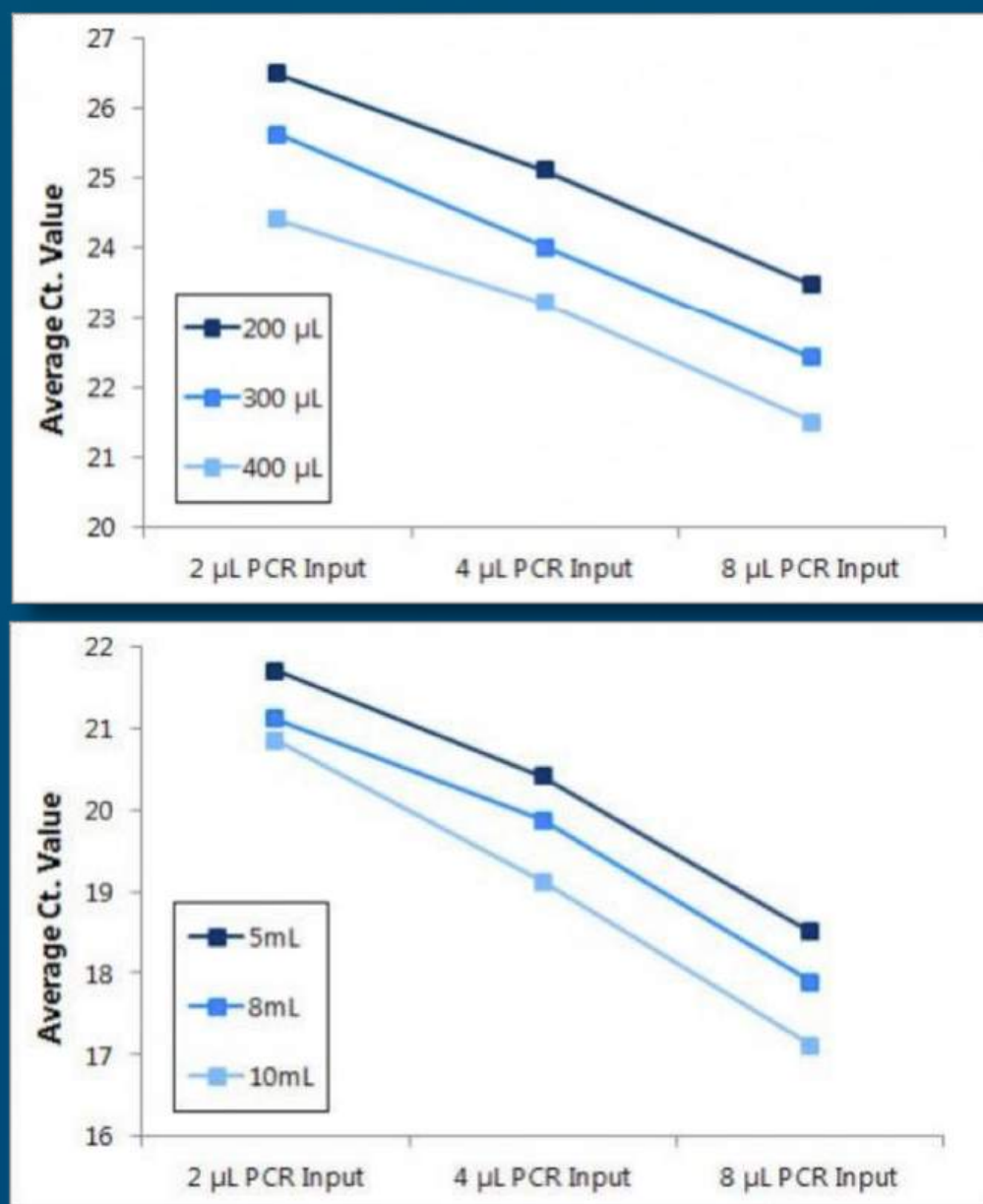




Application Note 47

RNA Sample Preparation

Revised Guidelines for RNA Quality Assessment for Diverse Biological Sample Input



Handout: Download App Note 47



Thank You

Call or email us for any inquiries regarding all of your Exosome needs!

Let us know how we did!

Complete the survey and **Receive 10% Off***

*Offer is valid with use of promo code for one use, for one Exosome product.

Request a Free Sample

Phone Number

905 227 8848

Email Address

info@norgenbiotech.com

Website

www.norgenbiotech.com