Identification of Aggressive Prostate Cancers: In-depth Proteomics of Tissues and Urines

Thomas Kislinger
Princess Margaret Cancer Centre
University of Toronto, Department of Medical Biophysics

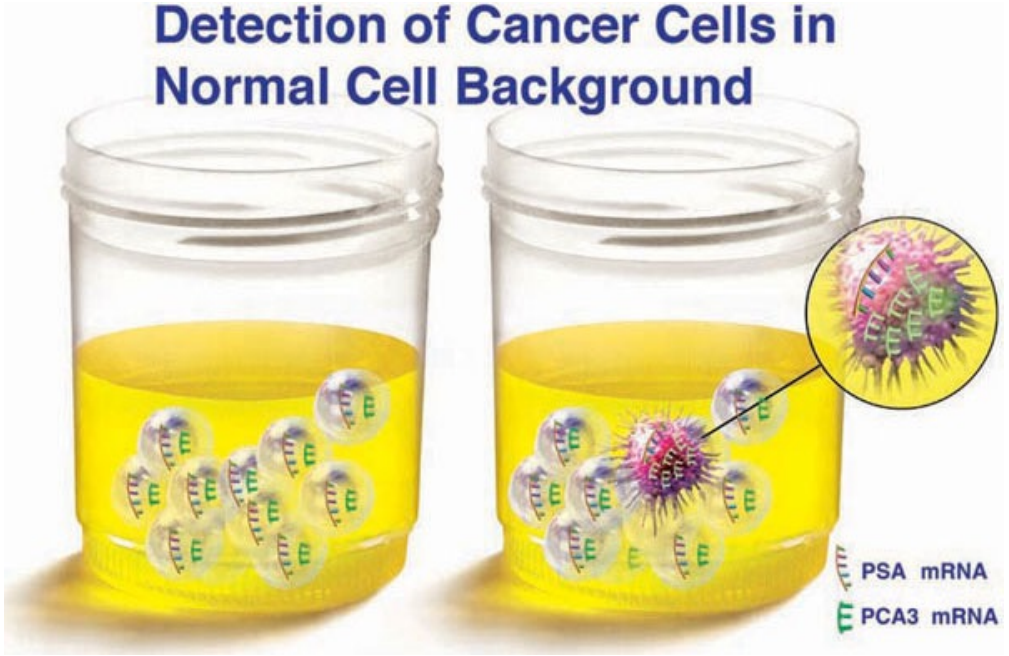
11th EDRN Scientific Workshop
September 9-12, 2019
Bethesda, USA

Introduction

1. Current diagnostic and prognostic protocols (PSA, DRE, biopsy) are inaccurate in predicting patients' risk
2. Molecular biomarkers and/or imaging approaches could improve the decision process
3. Molecular profiling of prostate cancer tissues and proximal fluids (post-DRE urine & dEPS) at the level of DNA, RNA and protein
4. Biomarkers for early detection of aggressive disease

Prostate Proximal Fluids

Direct EPS	post-DRE-urine
Proteins & cells shed by the gland	**Prostatic secretions in urine (DRE)**
Rich source of prostate biomarkers	**Can be collected frequently - longitudinal**
Collected prior to RP	**Applicable to routine collection**



http://www.usrf.org/news/PCA3

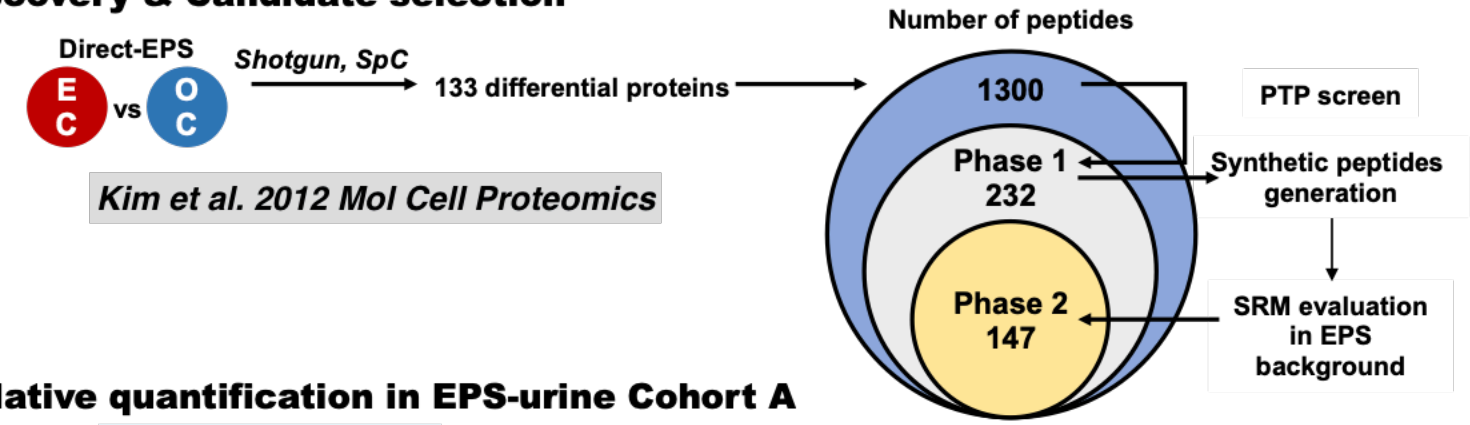
Kim Y, et al. Nat Commun. 2016
Principe S, et al. Proteomics. 2013
Kim Y, et al. Mol Cell Proteomics. 2012
Principe S, et al. J Proteome Res. 2012
Drake RR, et al. J Proteome Res. 2010

Proteomics Profiling of Prostate Proximal Fluids

Discovery
dEPS

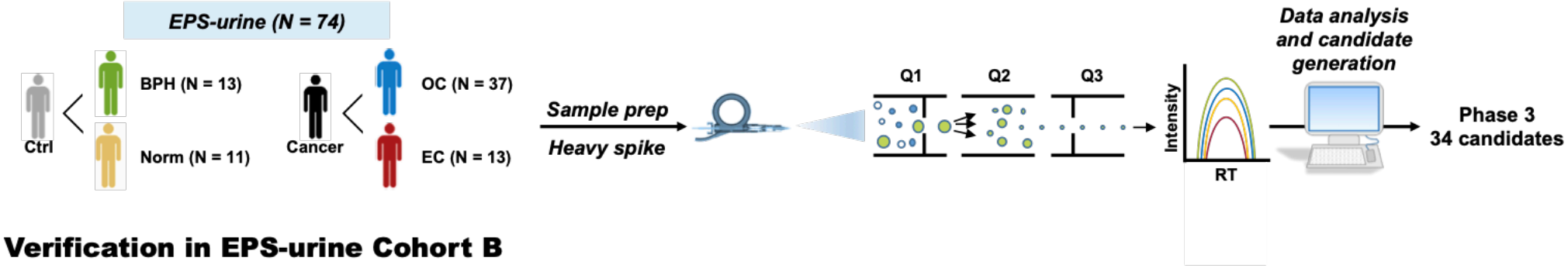
Verification
post-DRE urine

Discovery & Candidate selection

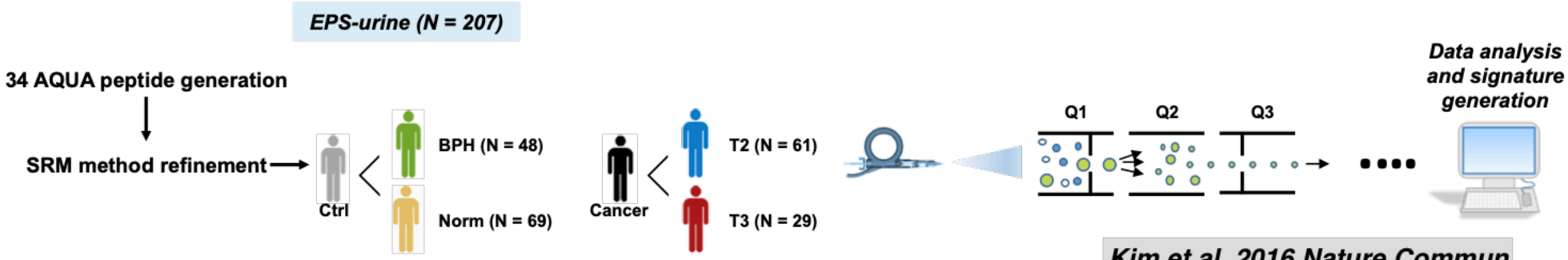


Kim et al. 2012 Mol Cell Proteomics

Relative quantification in EPS-urine Cohort A



Verification in EPS-urine Cohort B



Kim et al. 2016 Nature Commun

Optimization of Sample Processing

Previous

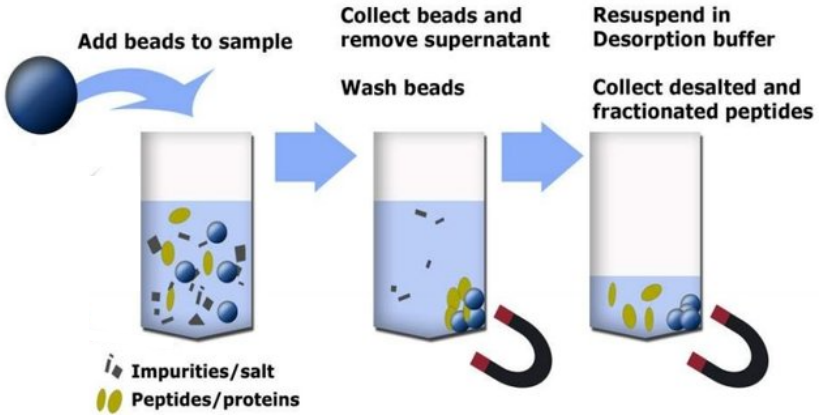
3KDa - TFE

Concentration
↓
MeOH precipitation
↓
TFE solubilization
↓
Trypsin digestion
↓
LC-MS

Kim Y, et al. Nat Commun. 2016
Kim Y, et al. Mol Cell Proteomics. 2012

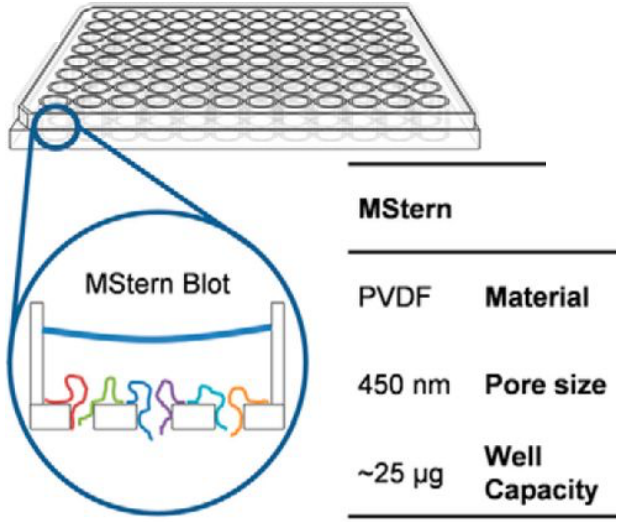
Tested

C8 beads

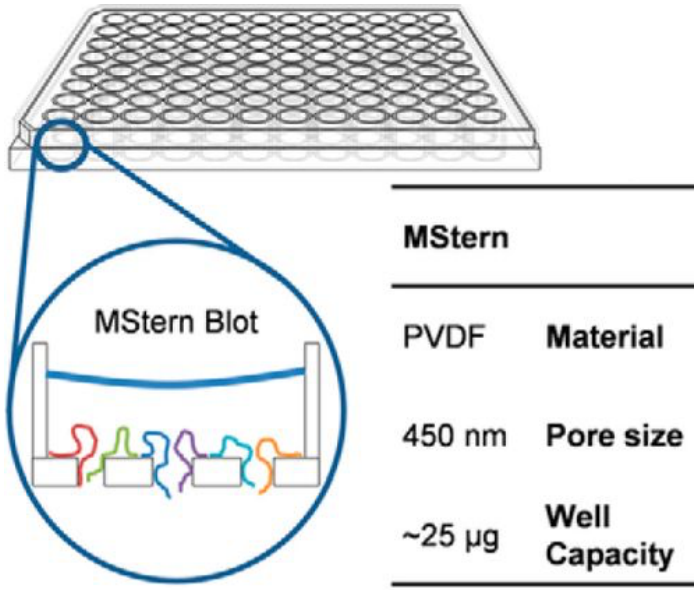


MStern

Berger et al. MCP 2015

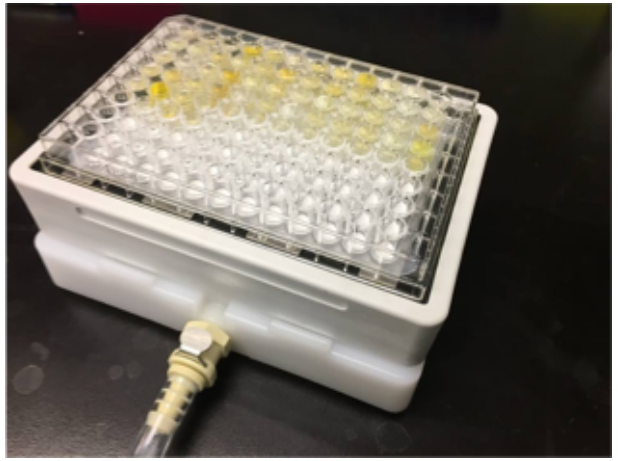


MStern Protocol



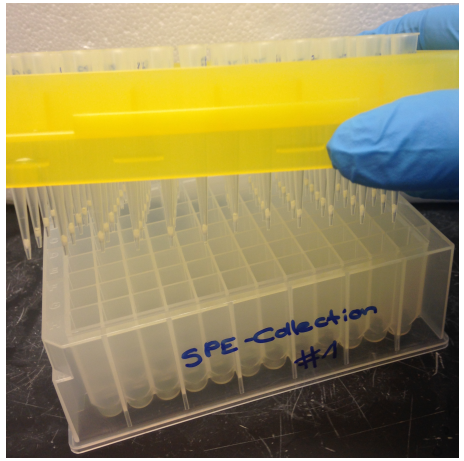
MStern	
PVDF	Material
450 nm	Pore size
~25 µg	Well Capacity

- **10 – 20 µg of protein**
- **short enzymatic digestion (4 h)**
- **Multiplexed sample preparation**



Vacuum Manifold

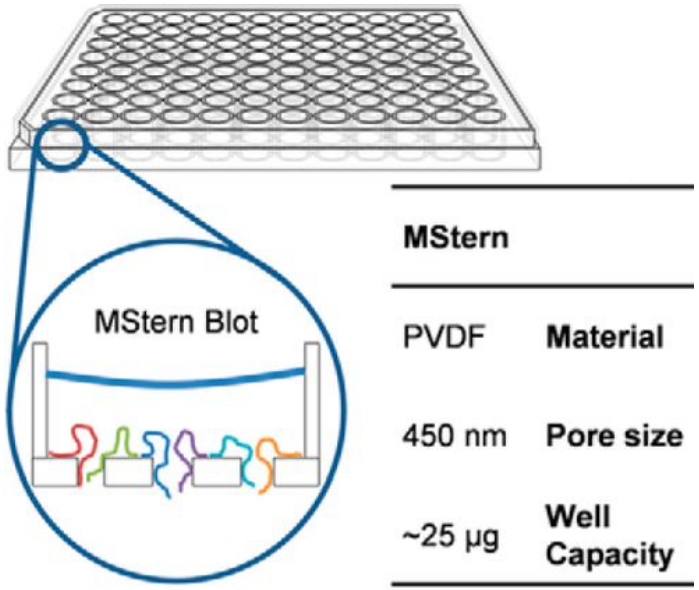
- Binding to PVDF membrane
- 200 uL input



Solid Phase Extraction

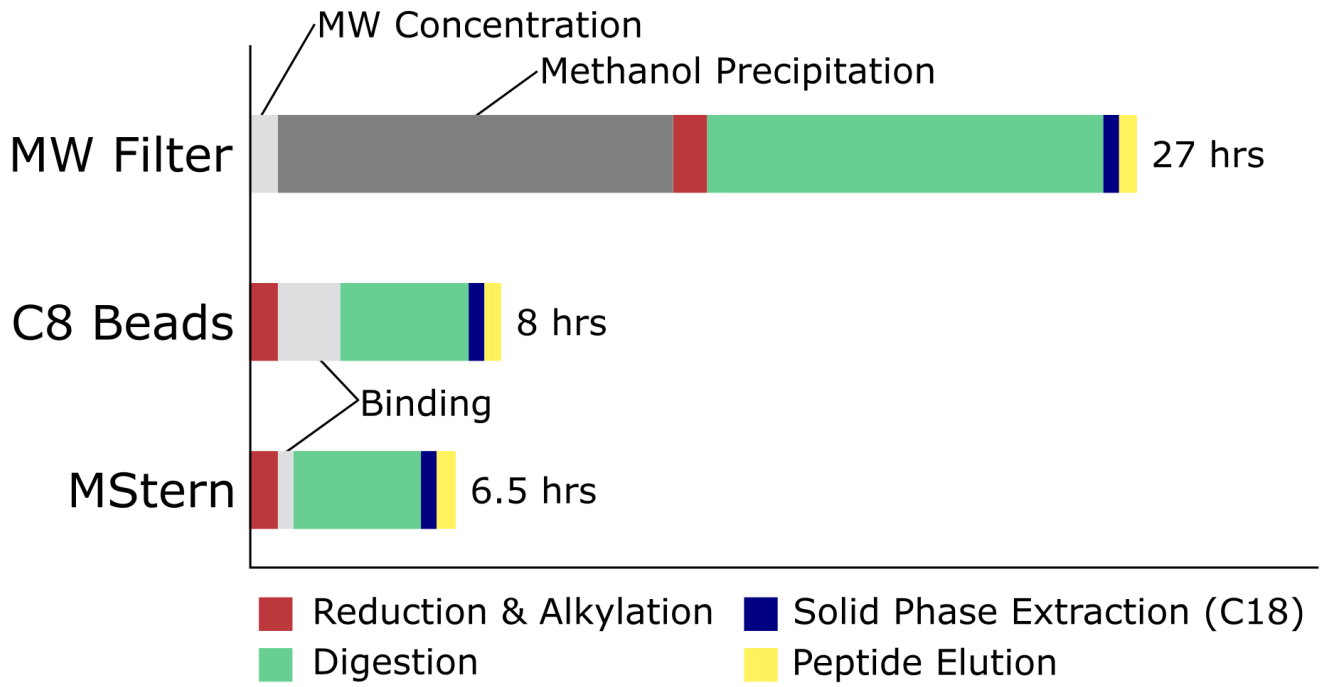
- in-house made stage tips
- Large-scale format

MStern Protocol



MStern	
PVDF	Material
450 nm	Pore size
~25 µg	Well Capacity

- **10 – 20 µg of protein**
- **short enzymatic digestion (4 h)**
- **Multiplexed sample preparation**



Prostate Cancer Program

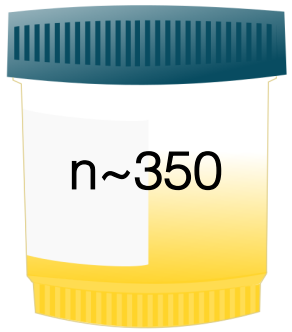
dEPS

MStern

Katharina Fritsch

Joe Otto

Post-DRE urine



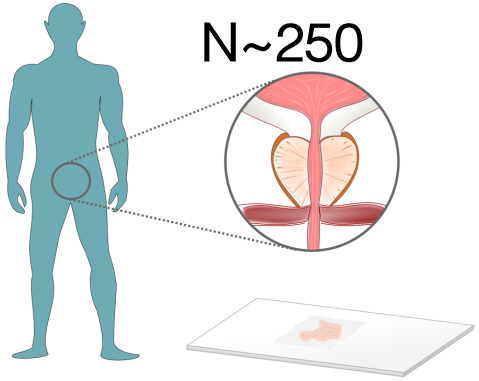
MStern

Andrew Macklin

EVs

Amanda Khoo

Tissue



Ankit Sinha

n=76

Amanda Khoo

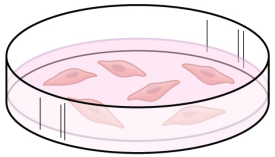
n=40 tumor
n=40 adj. normal

Lydia Liu

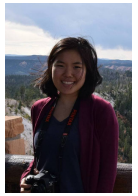
N~100

Sinha et al. *Cancer Cell* 2019

Cell lines



Natalie Kurganovs


Amanda Khoo

Vlad Ignatchenko

Direct EPS - Discovery

Eastern Virginia Medical Centre

direct-EPS

148 patients

GG1	GG2	GG3	GG4
n = 51	n = 47	n = 40	n = 10

Analyzed in Toronto

115 patients

OC	EC
n = 59	n = 56

Analyzed in Virginia

59 are overlapping

GG = ISUP grade groups

Post-DRE Urine - Discovery

Post-DRE urine

Eastern Virginia Medical Centre

148 patients

GG1	GG2	GG3	GG4
n = 51	n = 47	n = 40	n = 10

75 patients

OC	EC
n = 38	n = 37

Sunnybrook Cancer Centre

166 patients

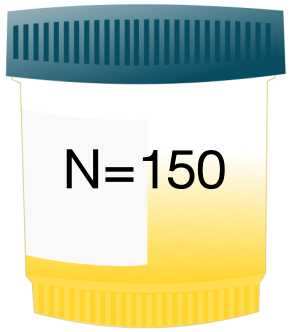
Normal	BPH
n = 20	n = 20

GG1	GG2	GG3	GG4	GG5
n = 49	n = 22	n = 20	n = 6	n = 14

10 patients: pre- and post-DRE
5 patients: longitudinal AS

Post-DRE Urine – Extracellular Vesicles

Post-DRE urine



Sunnybrook Cancer Centre

BPH n=25

GG1	GG2	GG3	GG4	GG5
n = 50	n = 22	n = 22	n = 8	n = 13

5 patients: pre- and post-DRE
5 patients: longitudinal AS

Cancer cell lines

RWPE1	PC3	DU145
normal	AR-	AR-

LNCaP	22Rv1
AR+	AR+

OVCAR3	OVCAR433
SKOV3	OVCAR5

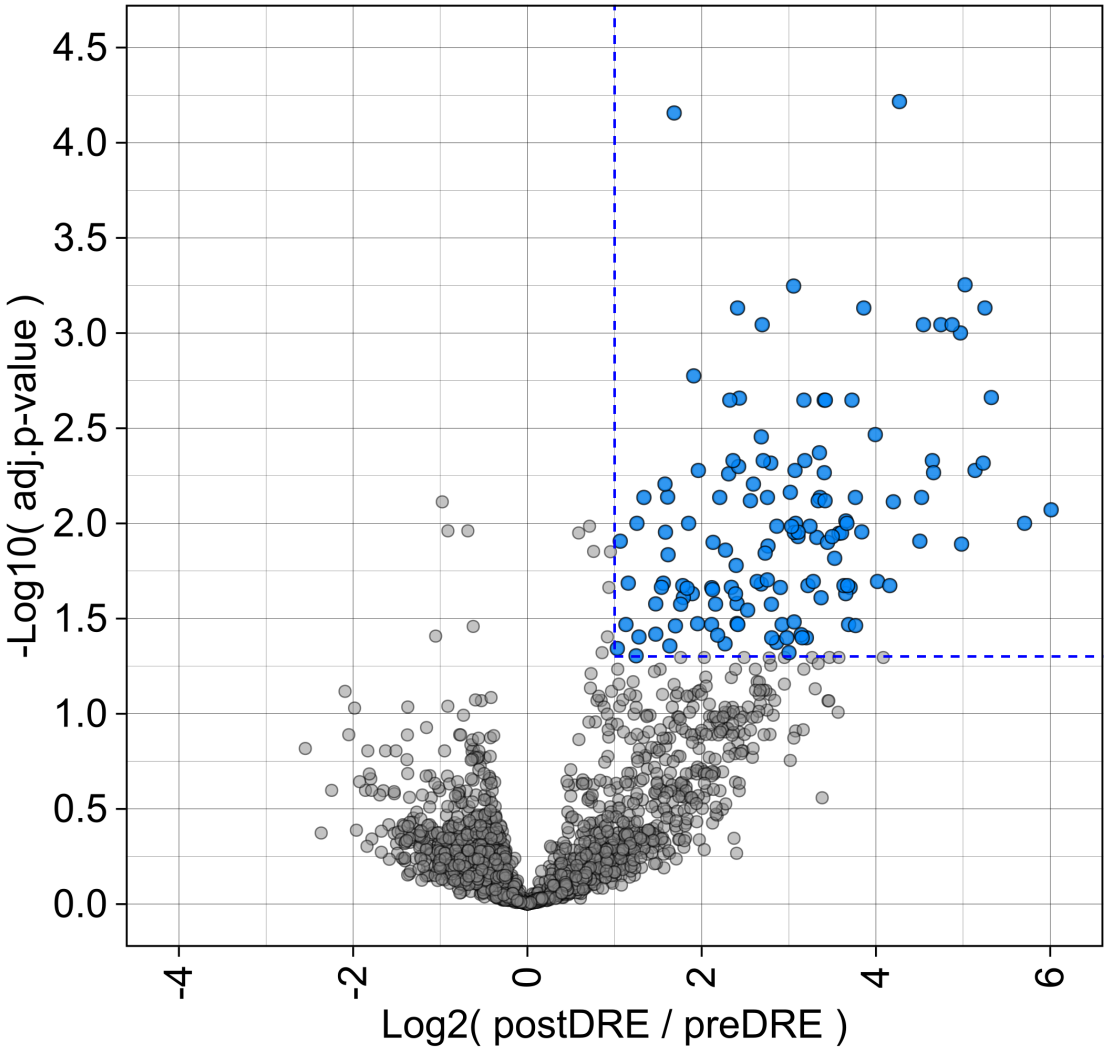
Sinha et al. *BBRC* 2014

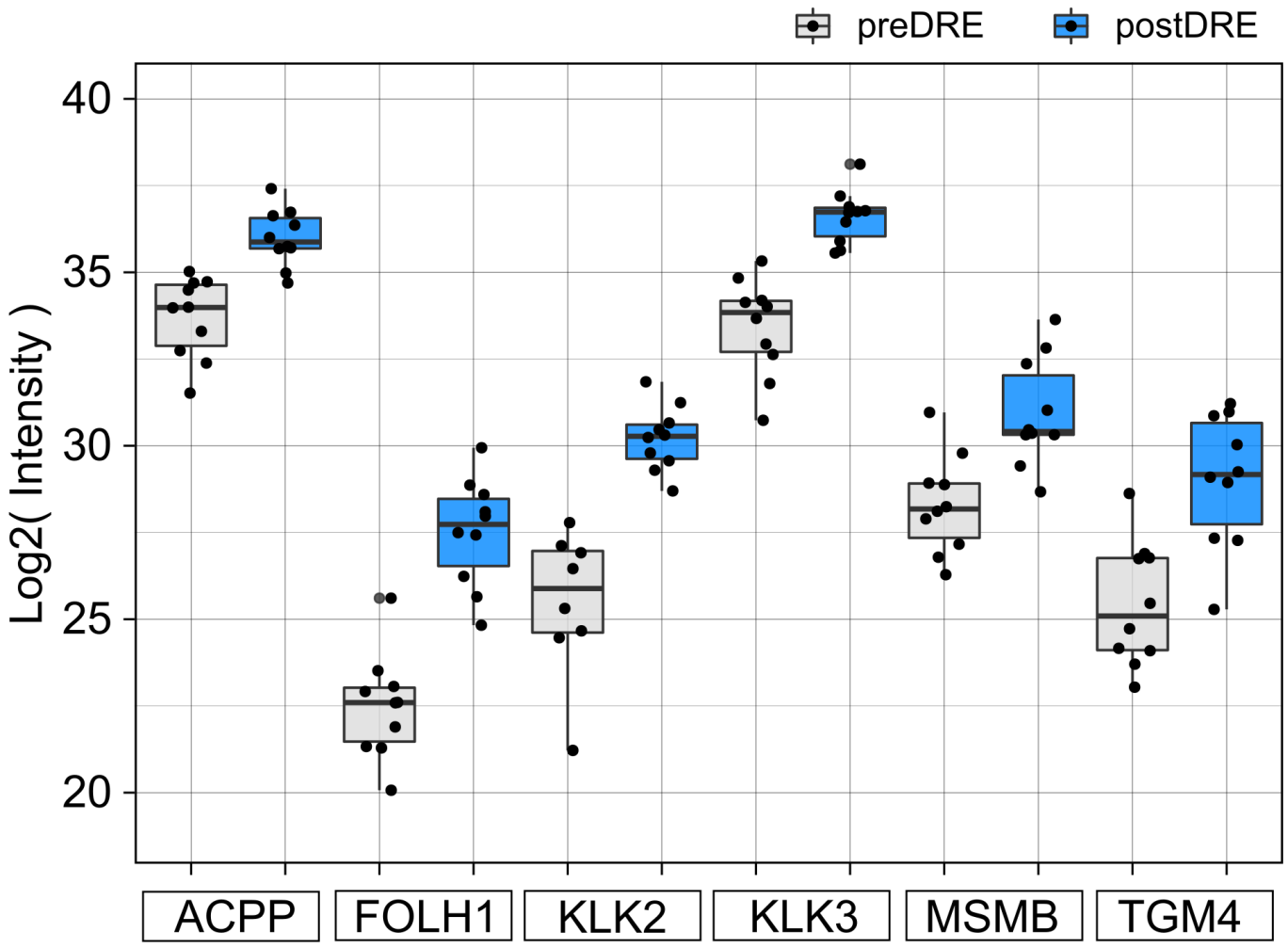
Primary Fibroblasts

4x CAFs	4x NAFs

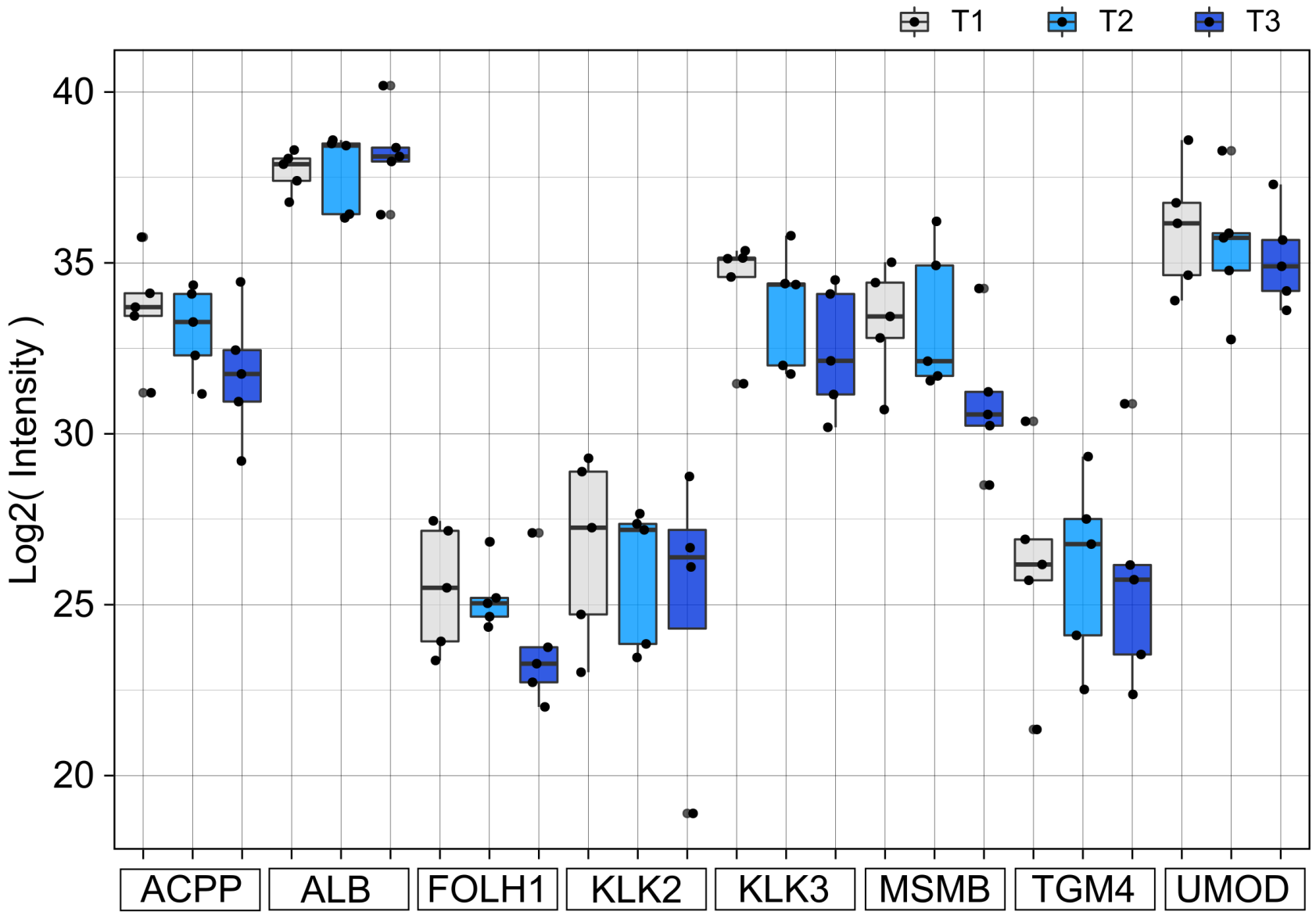
Principe et al. *JPR* 2018

pDRE-urine – pre *vs.* post-DRE

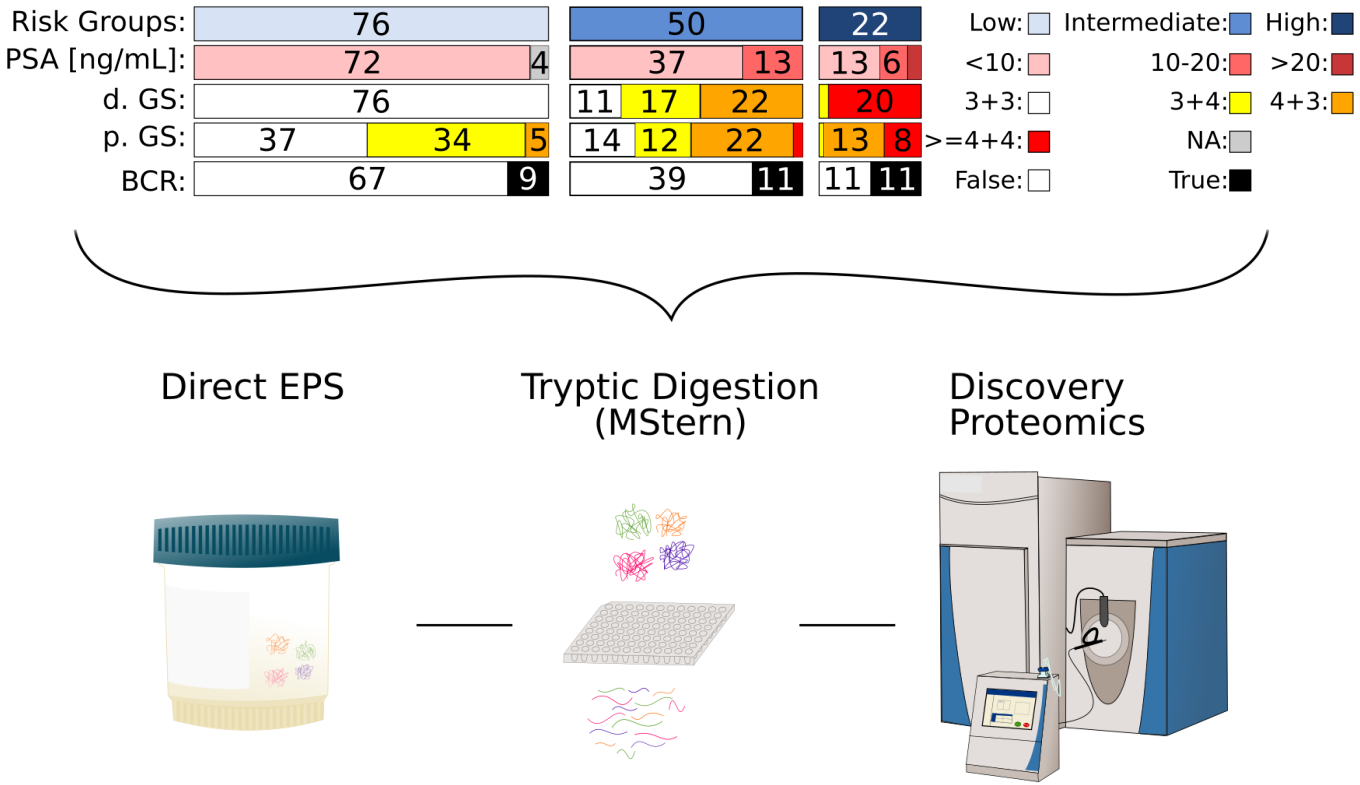


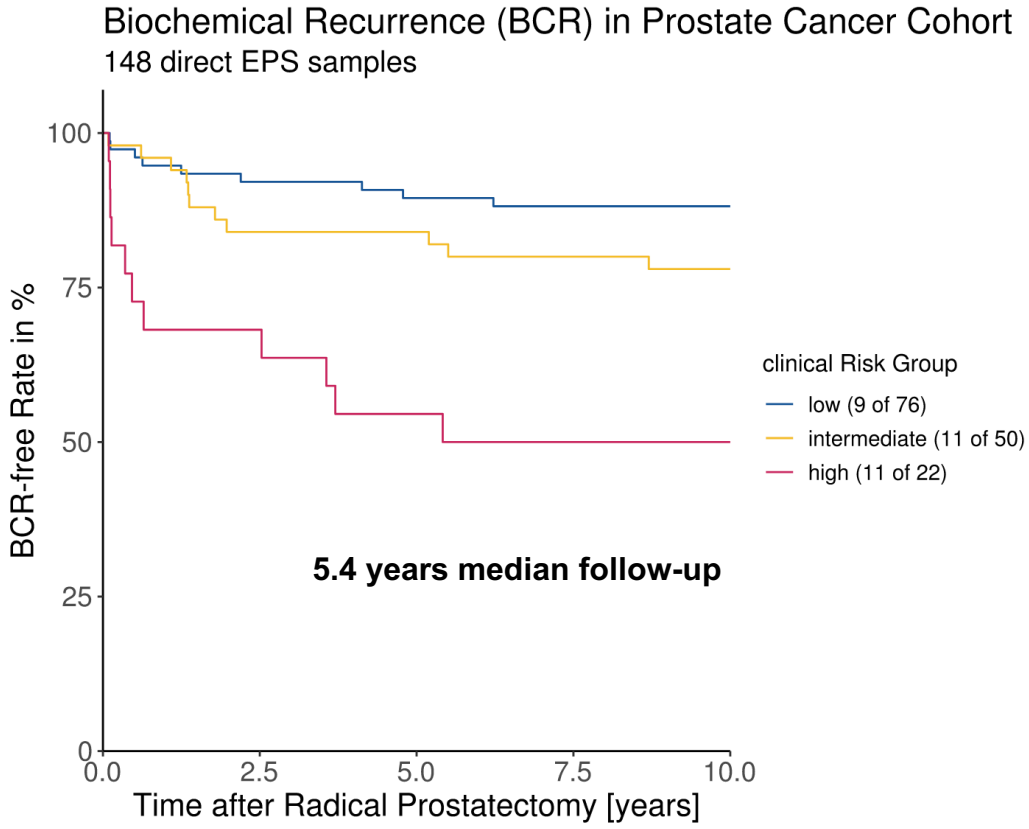


pDRE-urine - Longitudinal Samples



dEPS Patient Cohort





Sample preparation & Data evaluation

Direct-EPS (15ug/200ul)

↓

2 pmol SUC2 (yeast)

↓

Reduce & Alkylate

↓

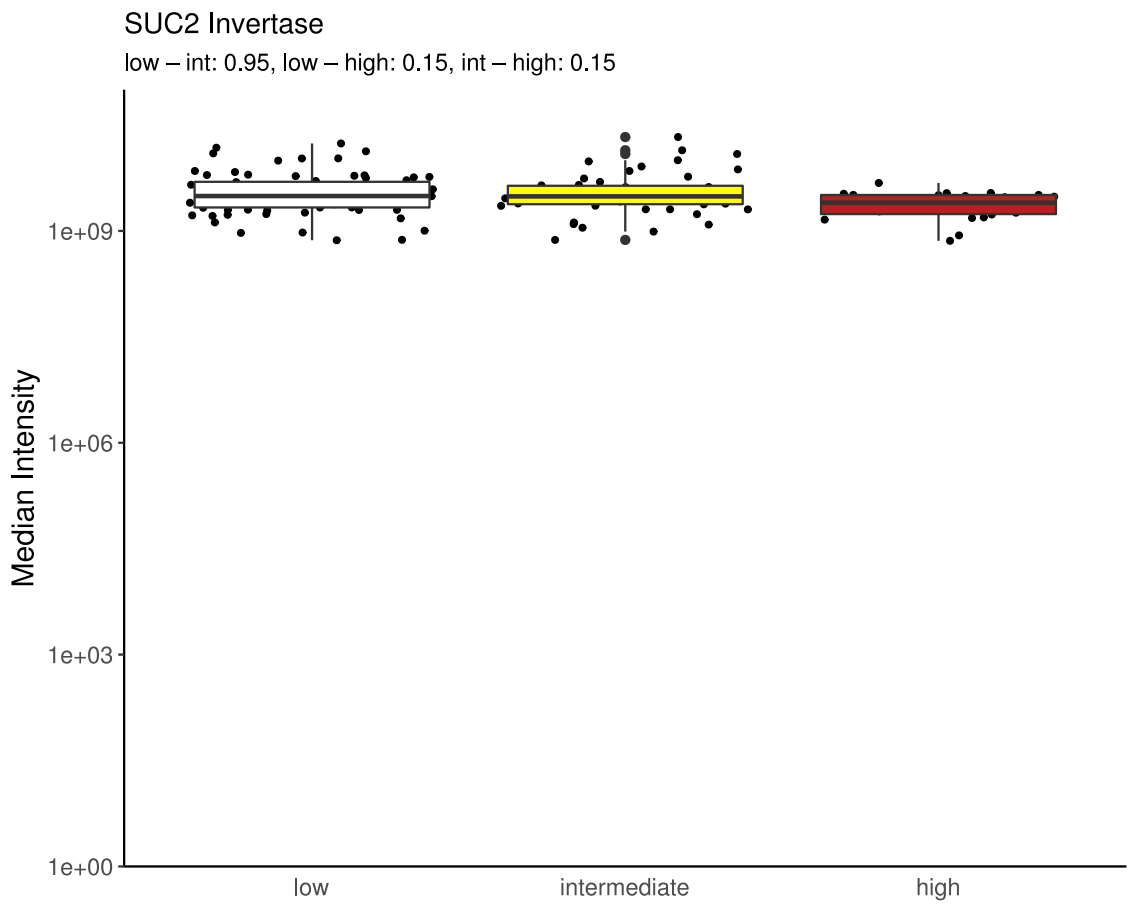
Trypsin-LysC
(25ul ABiC-5% ACN)

↓ *4 hrs*

Collect peptides &
C18 StageTips
iRT peptides

↓

Load aliquot



Sample preparation & Data evaluation

Direct-EPS (15ug/200ul)

↓

2 pmol SUC2 (yeast)

↓

Reduce & Alkylate

↓

Trypsin-LysC
(25ul ABiC-5% ACN)

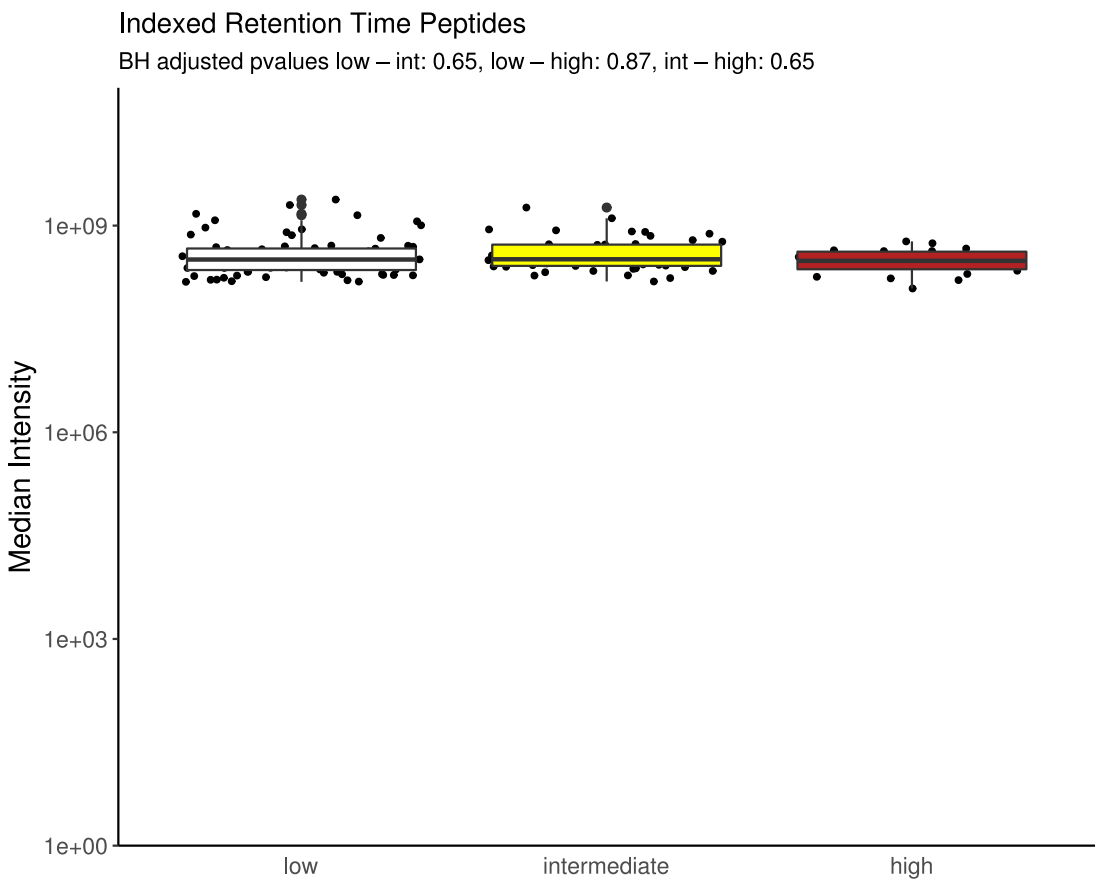
↓ ***4 hrs***

Collect peptides &
C18 StageTips
iRT peptides

↓

Load aliquot

←



Sample preparation & Data evaluation

Direct-EPS (15ug/200ul)

↓

2 pmol SUC2 (yeast)

↓

Reduce & Alkylate

↓

Trypsin-LysC
(25ul ABiC-5% ACN)

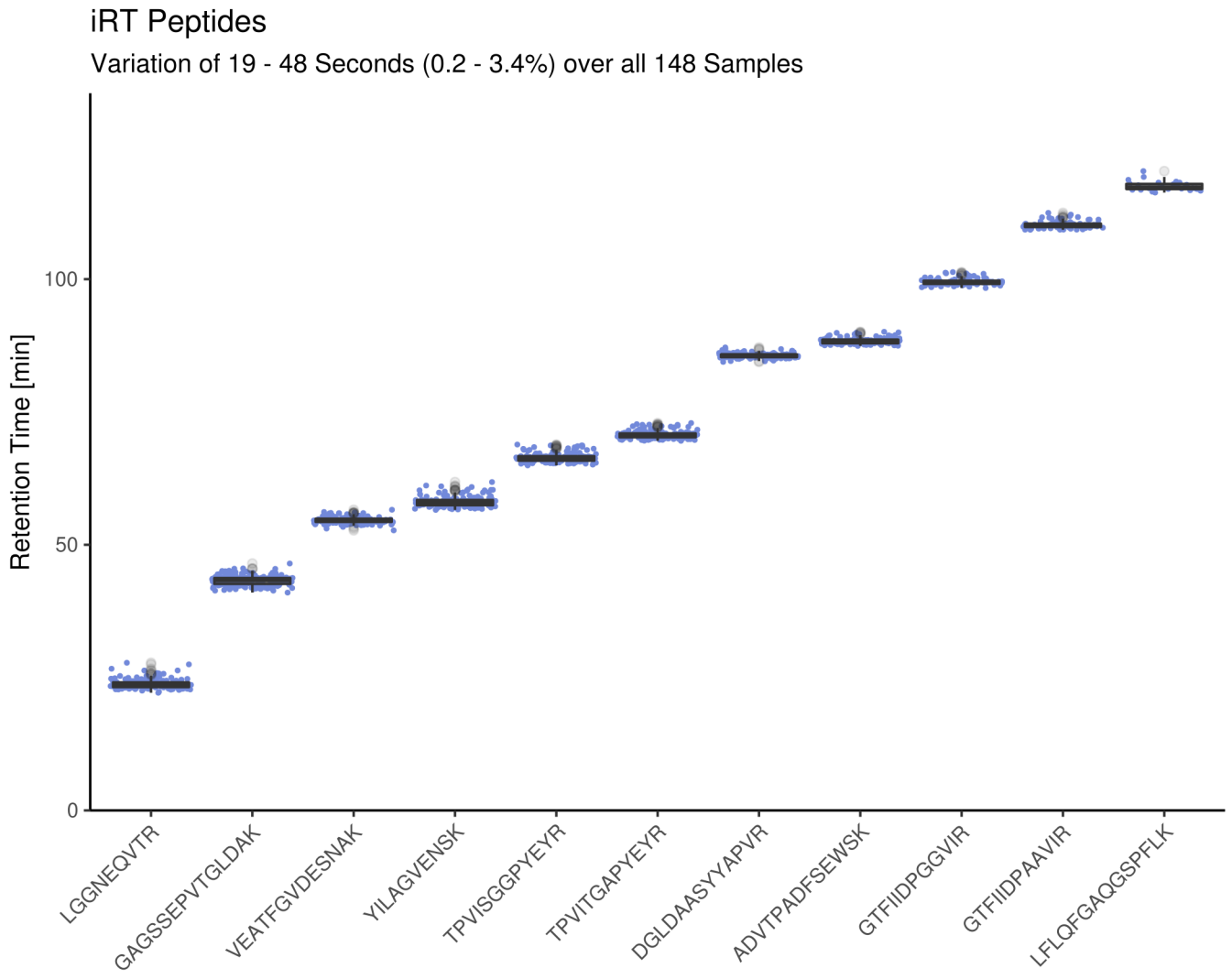
↓ ***4 hrs***

Collect peptides &
C18 StageTips
iRT peptides

←

↓

Load aliquot



Sample preparation & Data evaluation

Direct-EPS (15ug/200ul)

↓

2 pmol SUC2 (yeast)

↓

Reduce & Alkylate

↓

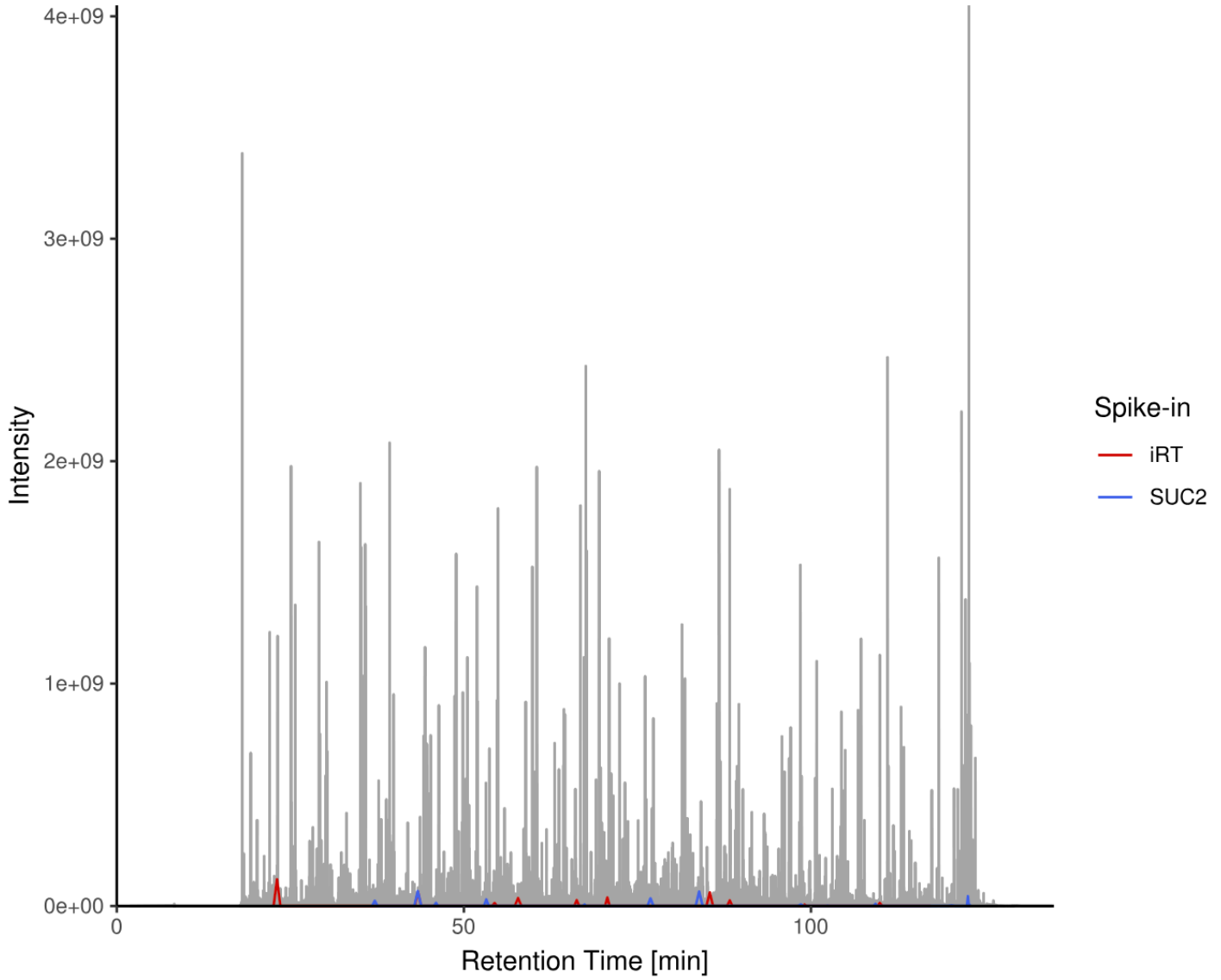
Trypsin-LysC
(25ul ABiC-5% ACN)

↓ *4 hrs*

Collect peptides &
C18 StageTips
iRT peptides

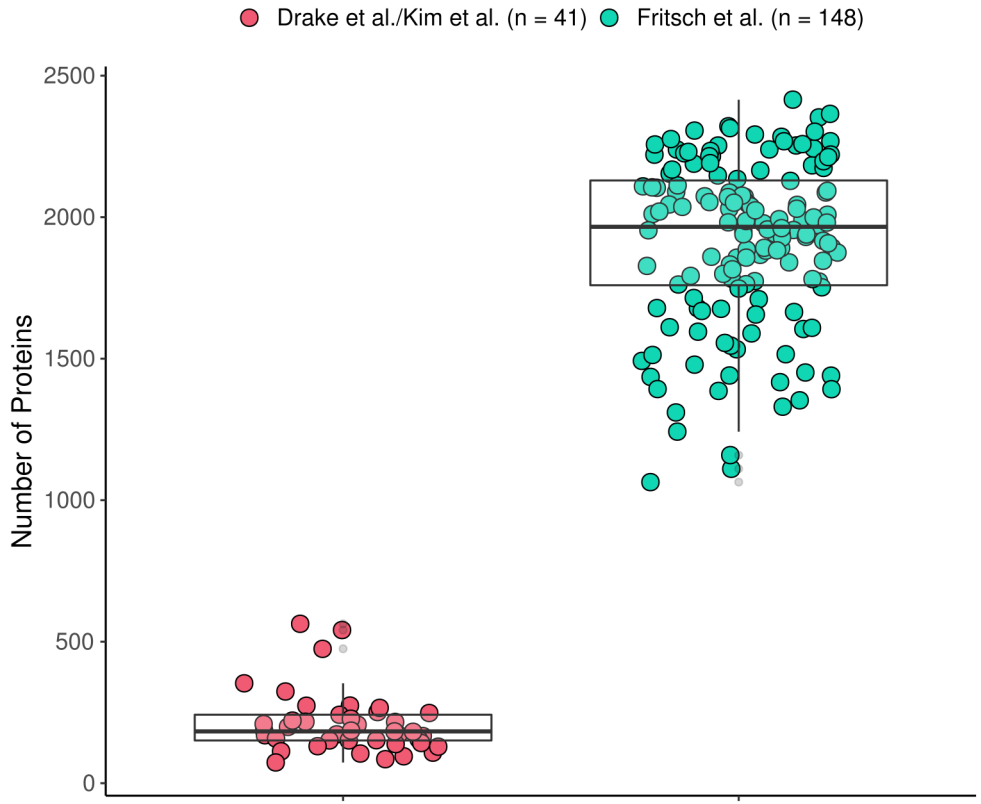
↓

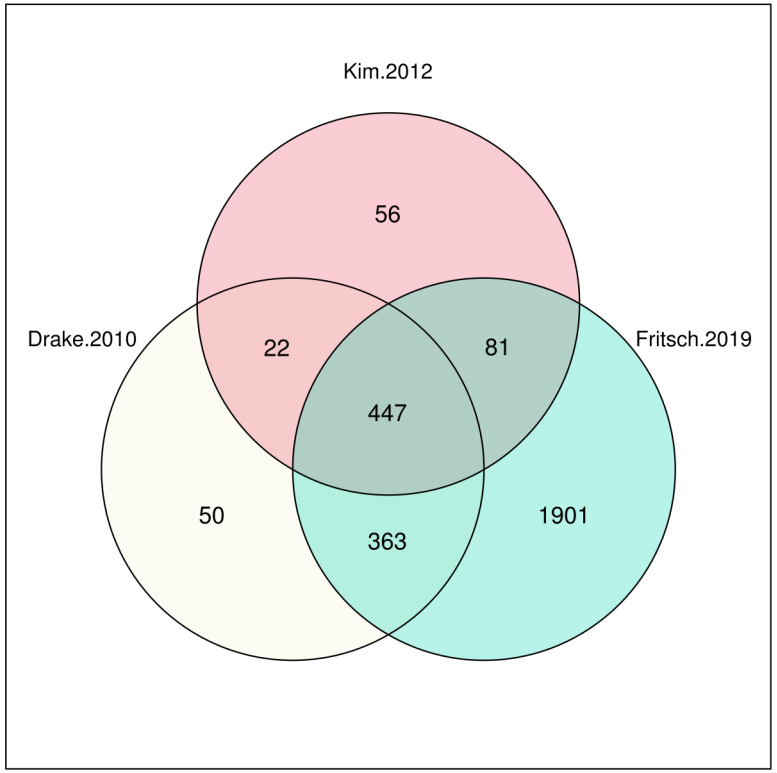
Load aliquot



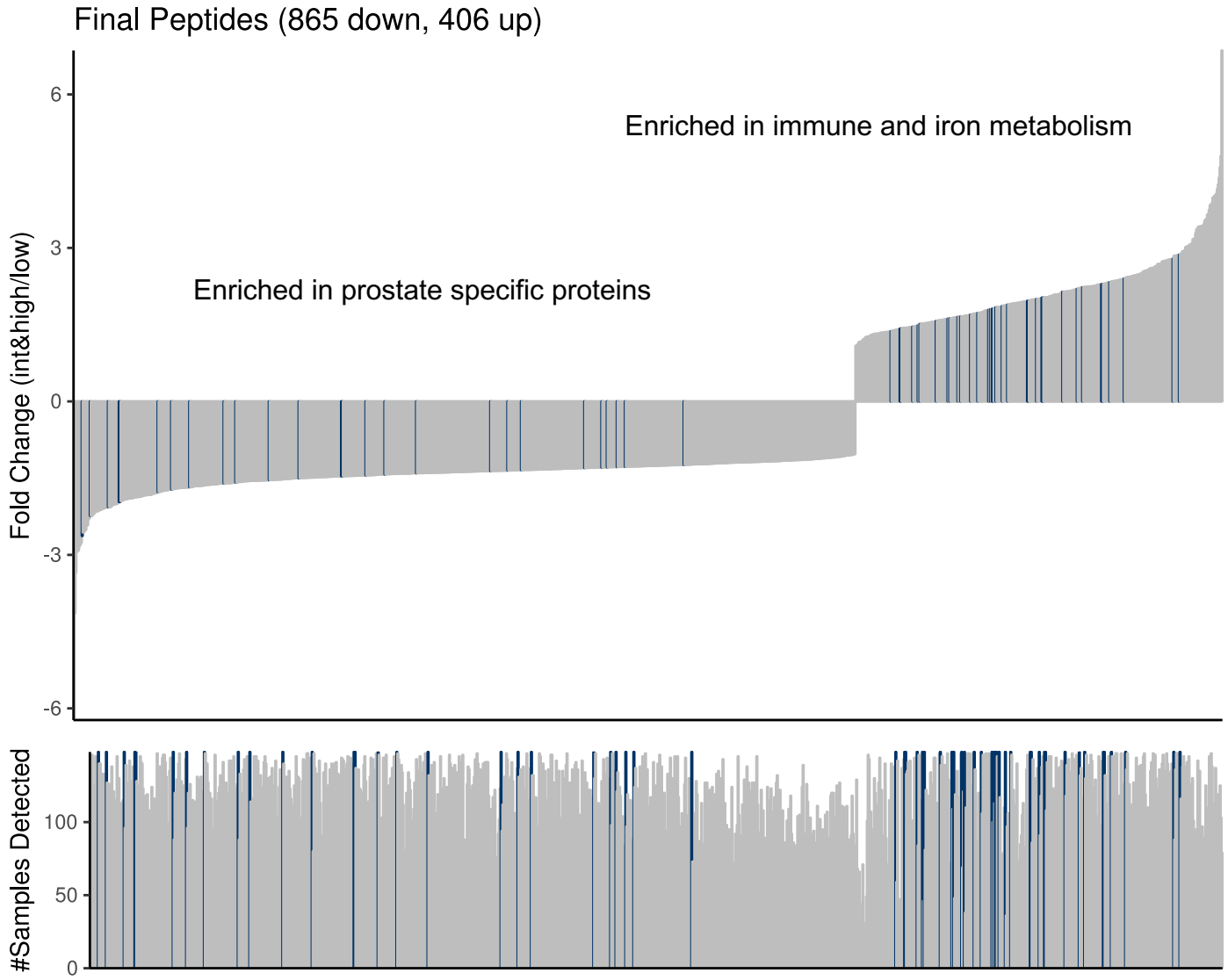
Technology Advances

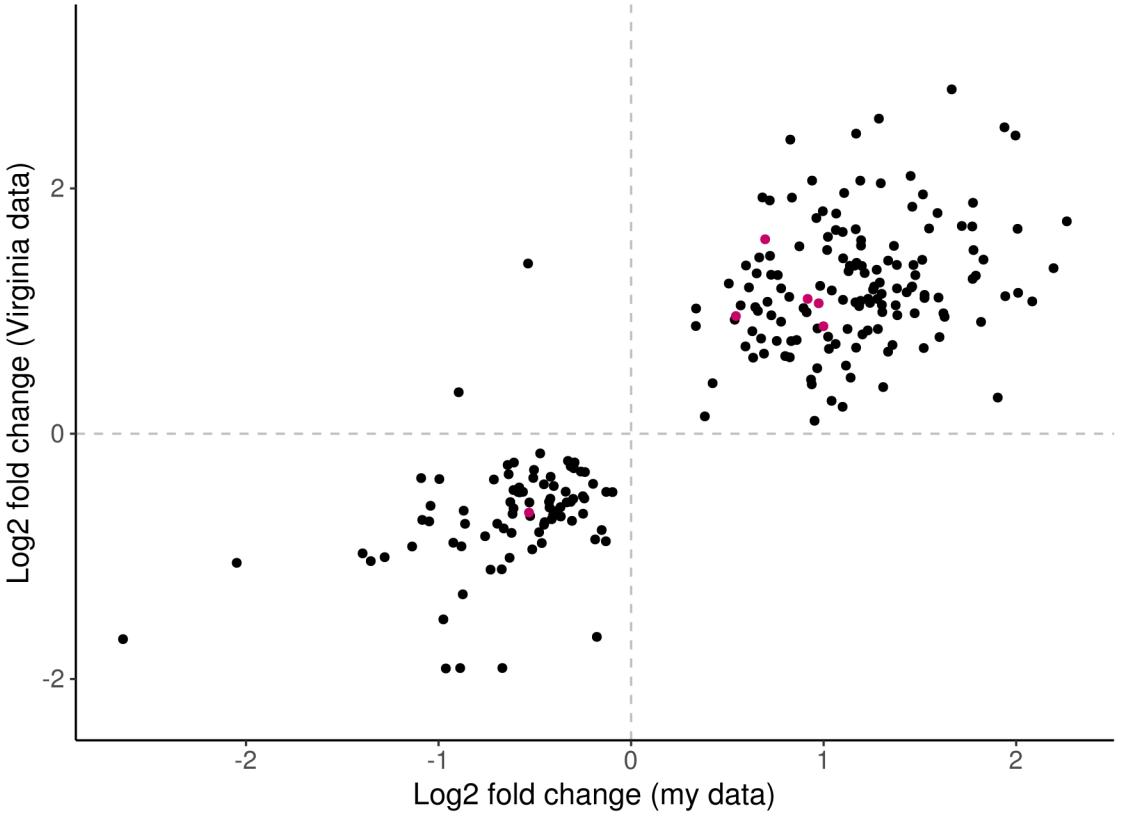
	Drake et al. 2010	Kim et al. 2012	Fritsch 2019 (unpublished)
Input material	100 ug	100 ug	15 ug
Sample processing (hrs)	27	27	6.5
Gradient length	9x120 min (18 hrs)	9x120 min (18 hrs)	135 min
Proteins detected	916	624	2852
Proteins/sample	366 +/- 127	166 +/- 47	1915 +/- 292



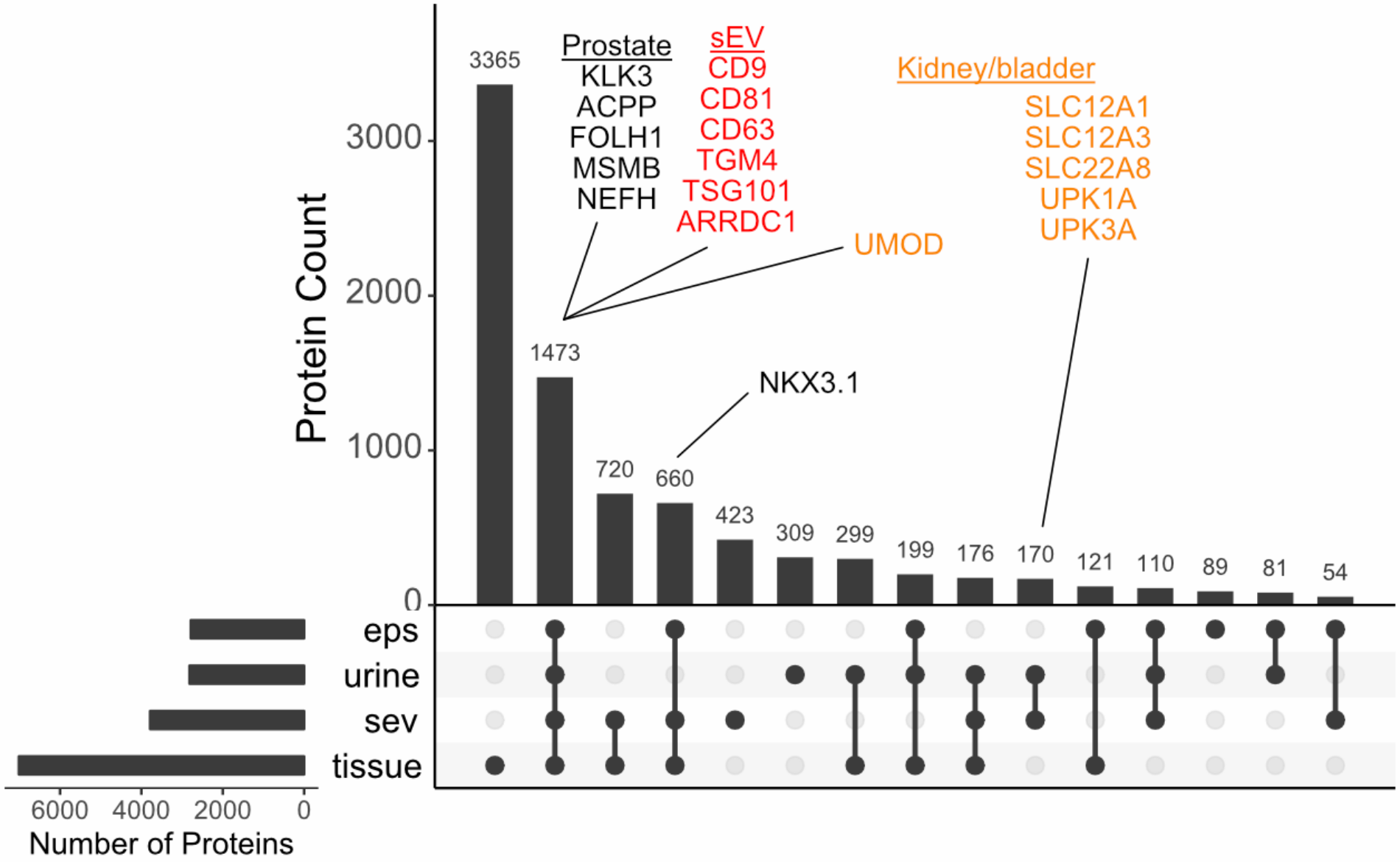


Differentially Expressed Peptides





Prostate Cancer Database



Summary

Proteomics of prostate cancer

A. Markers of aggressive disease

B. Integration of tissues and fluids

C. Combination of complementary biomolecules

D. Relational database

Acknowledgements

UCLA - Toronto

Vincent Huang
Julie Livingstone
Michael Fraser
Jouhyun Jeon

Toronto

Yunee Kim
Lydia Liu
Amanda Khoo
Kathi Fritsch
Andrew Macklin
Ankit Sinha
Salvador Mejia
Natalie Kurganovs

EVMS

Brian Main
Joseph Otto