

Systematic Development of Prostate Cancer Liquid Biopsies: Proteomics and Proteogenomics of Post-DRE Urines and Cancer Tissues

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On behalf of the team (John Semmes, EVMS and Paul Boutros, OICR)

Overview

1. How did we get here?

A. Proteomics of prostatic secretions and post-DRE urines

2. What will we do as part of the EDRN program?

A. Preliminary data

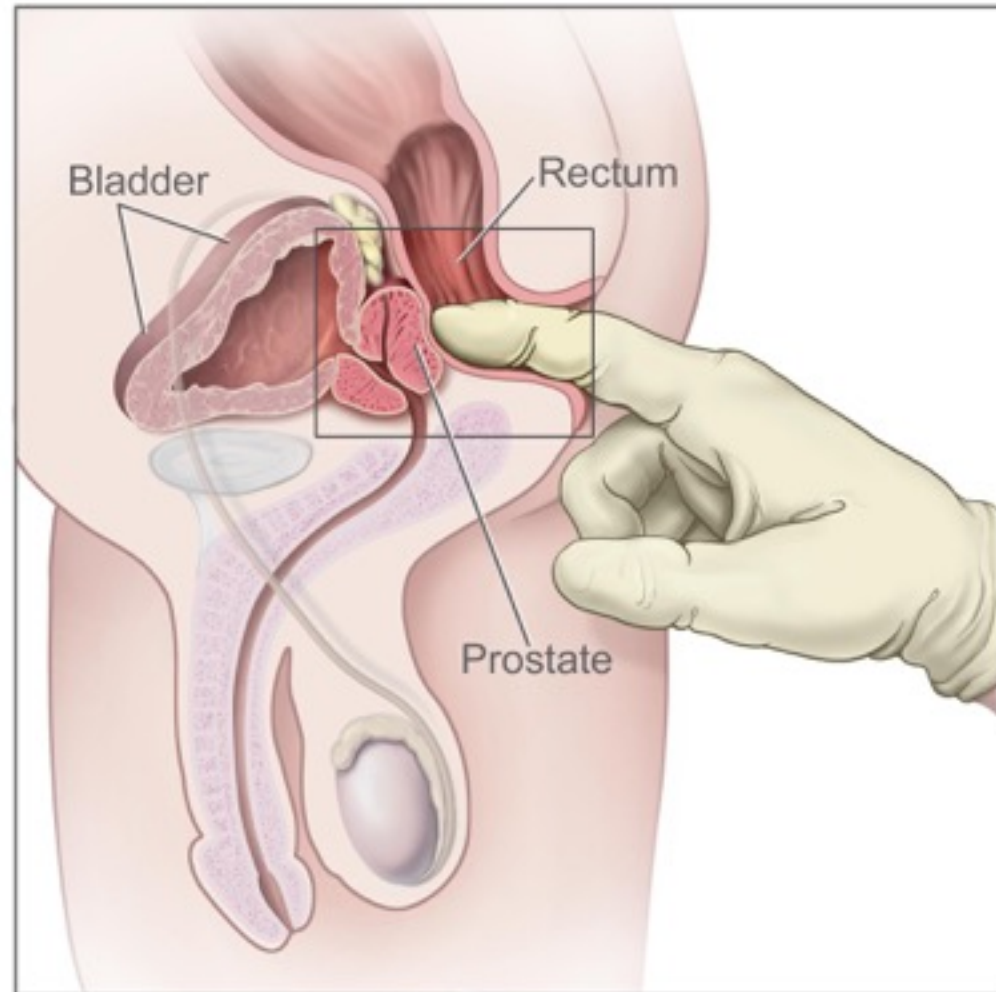
3. Additional prostate cancer proteomics data (PCC, OICR)

A. CPC-GENE genomics and proteogenomics

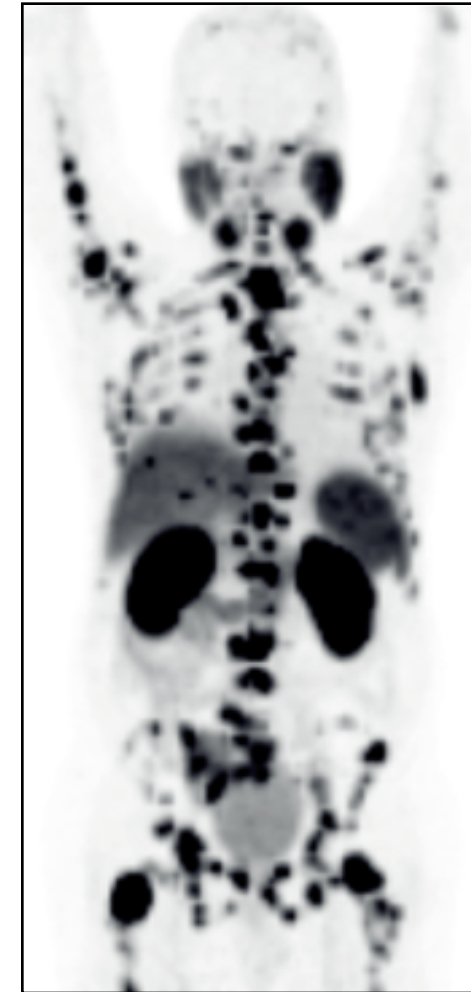
B. post-DRE urine (proteomics, lipidomics)

Prostate Cancer Prognosis

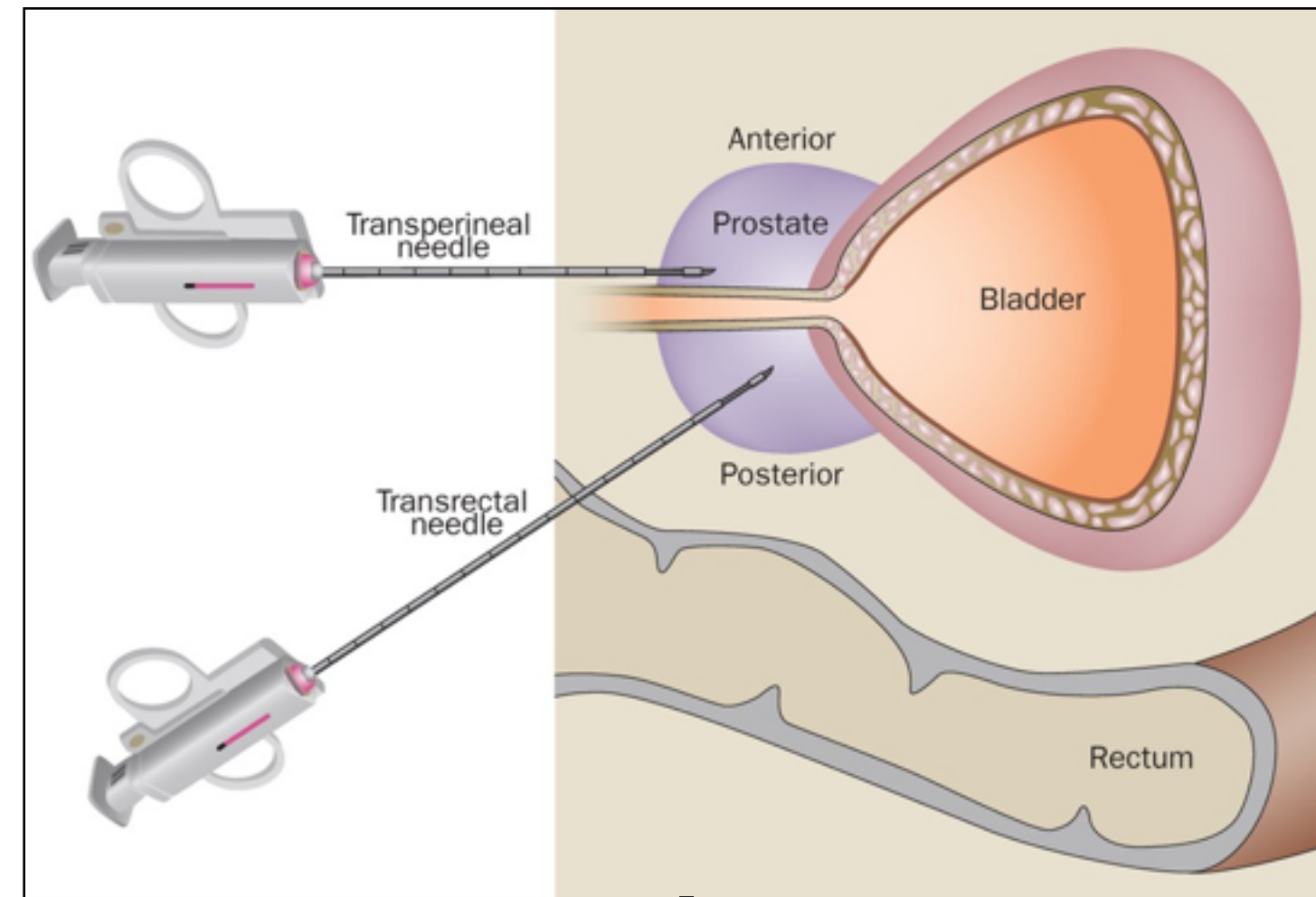
DRE



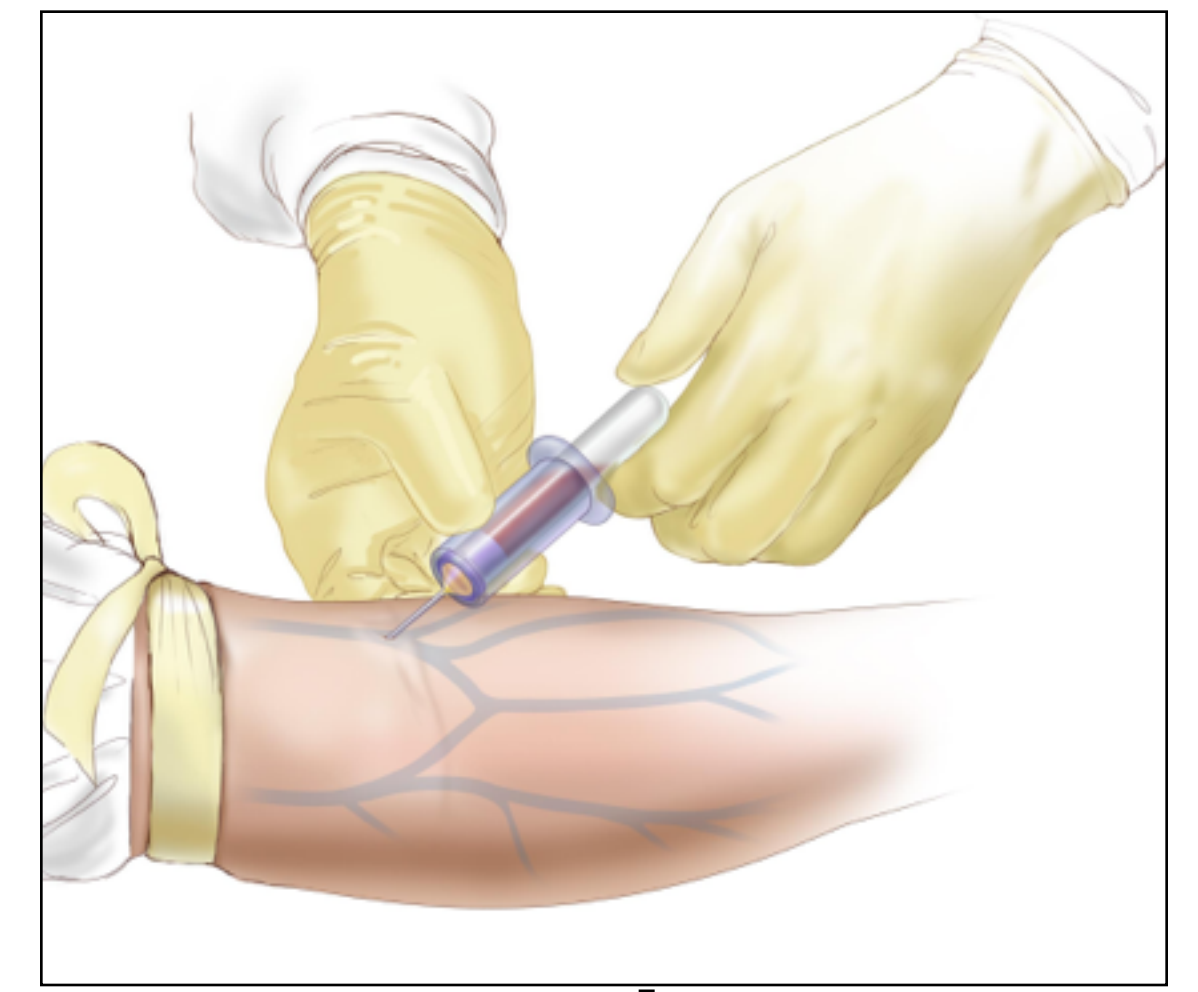
Imaging



Prostate biopsy



Blood test



TNM Stage

Tumour Grade

PSA

Low Risk

Intermediate Risk

High Risk

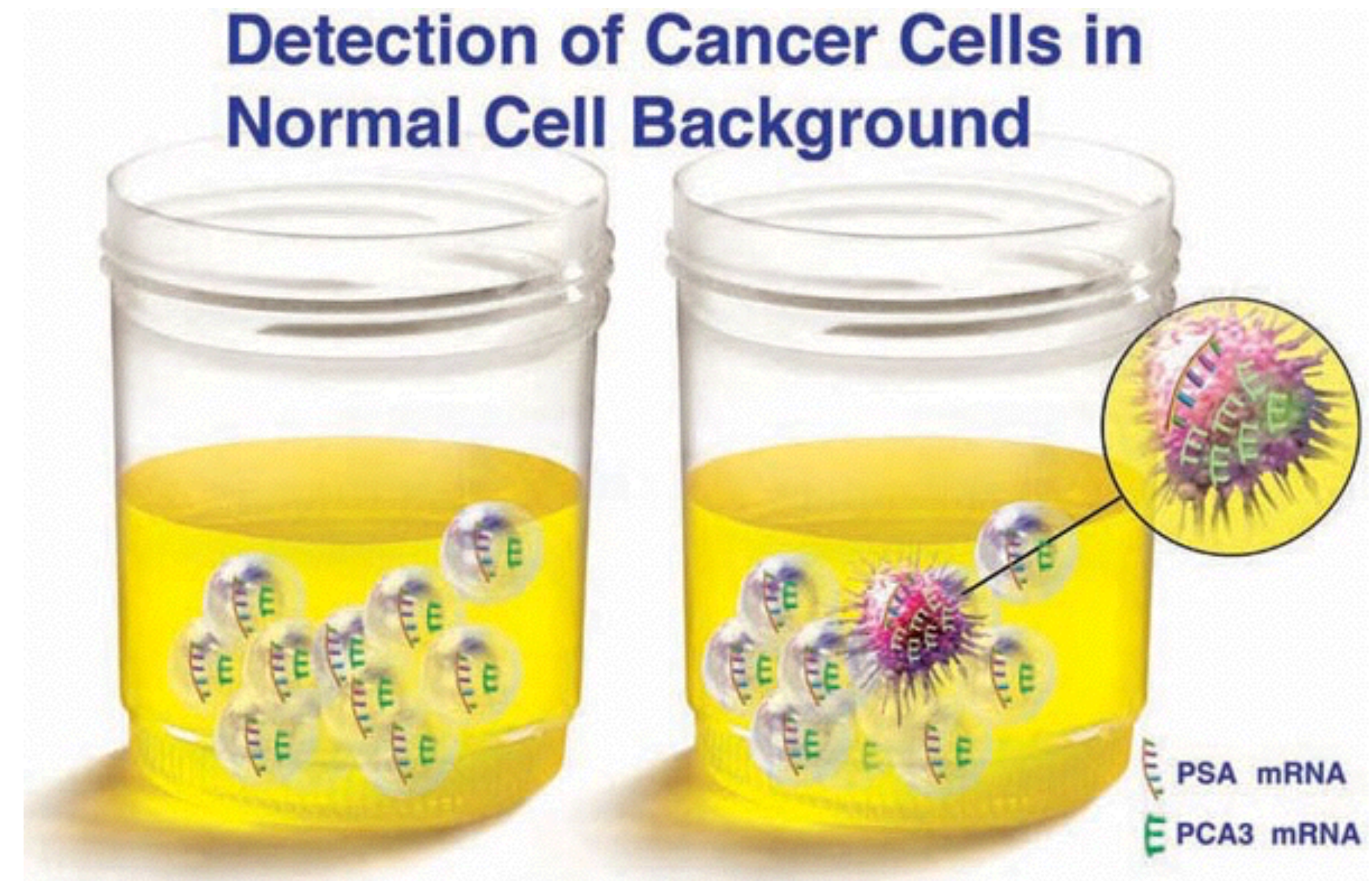
Metastatic

Localized Cancers

Expressed Prostatic Secretions

Direct-EPS	EPS-urine (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

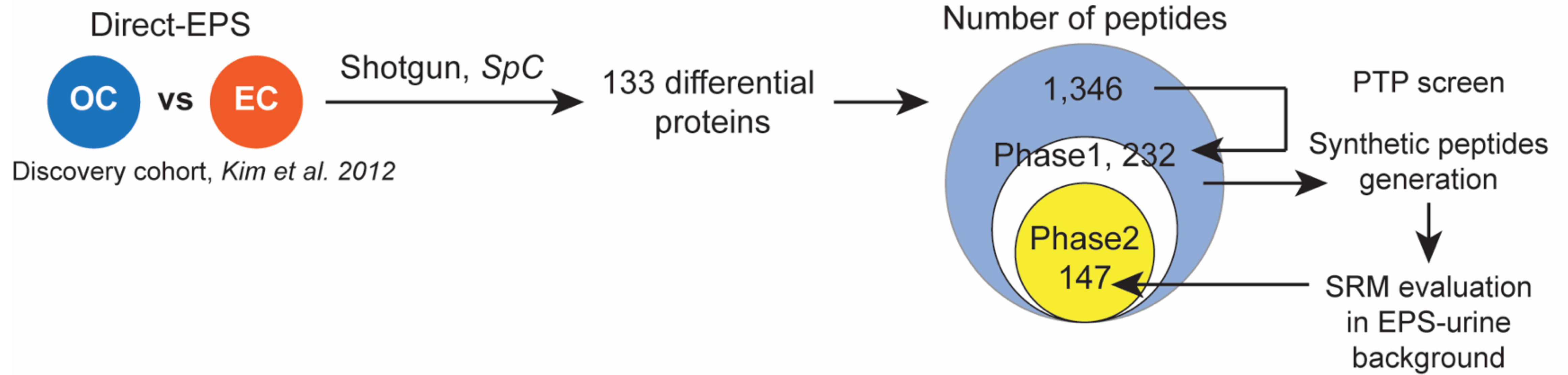
>2,000 proteins & ~350 patients



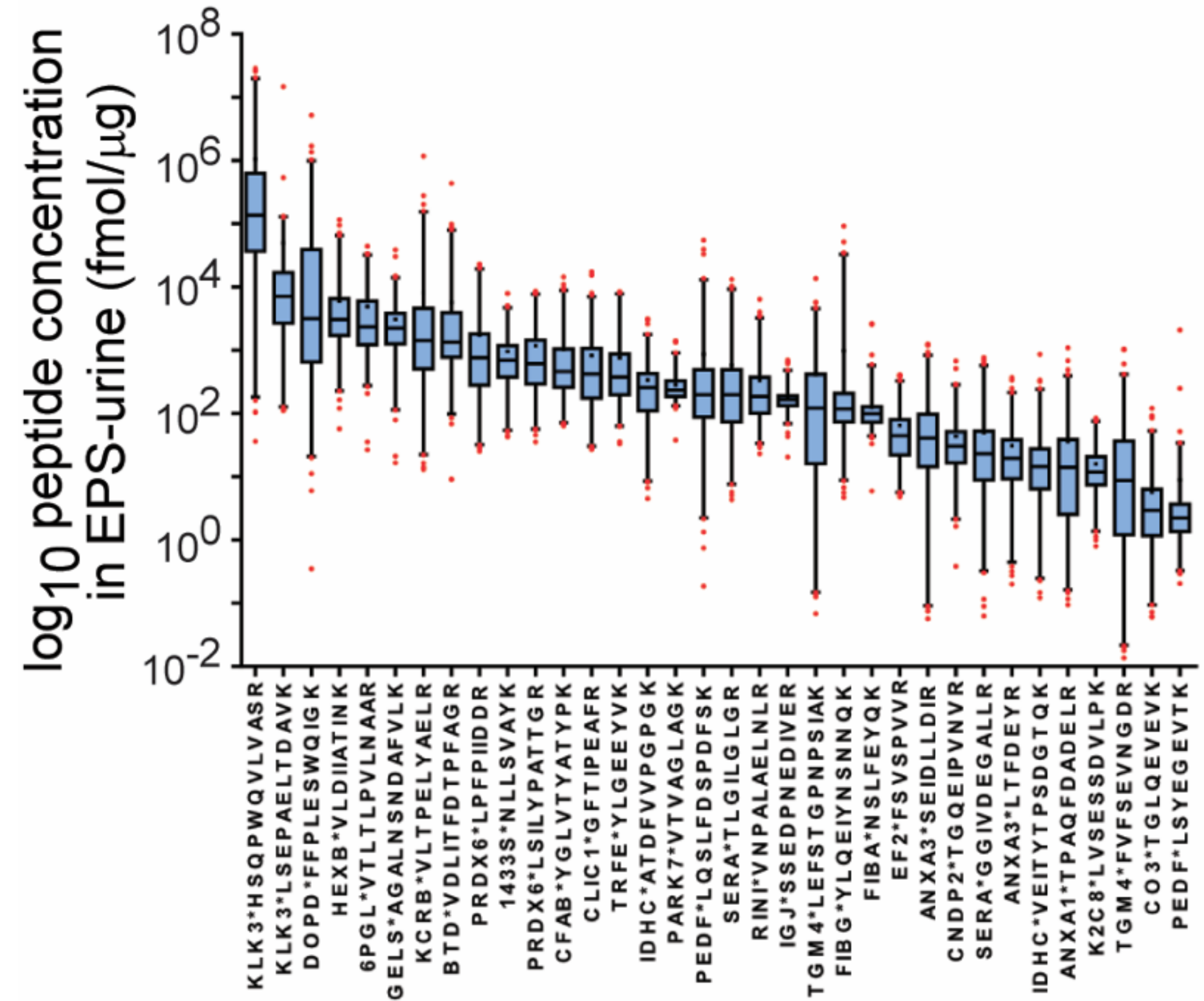
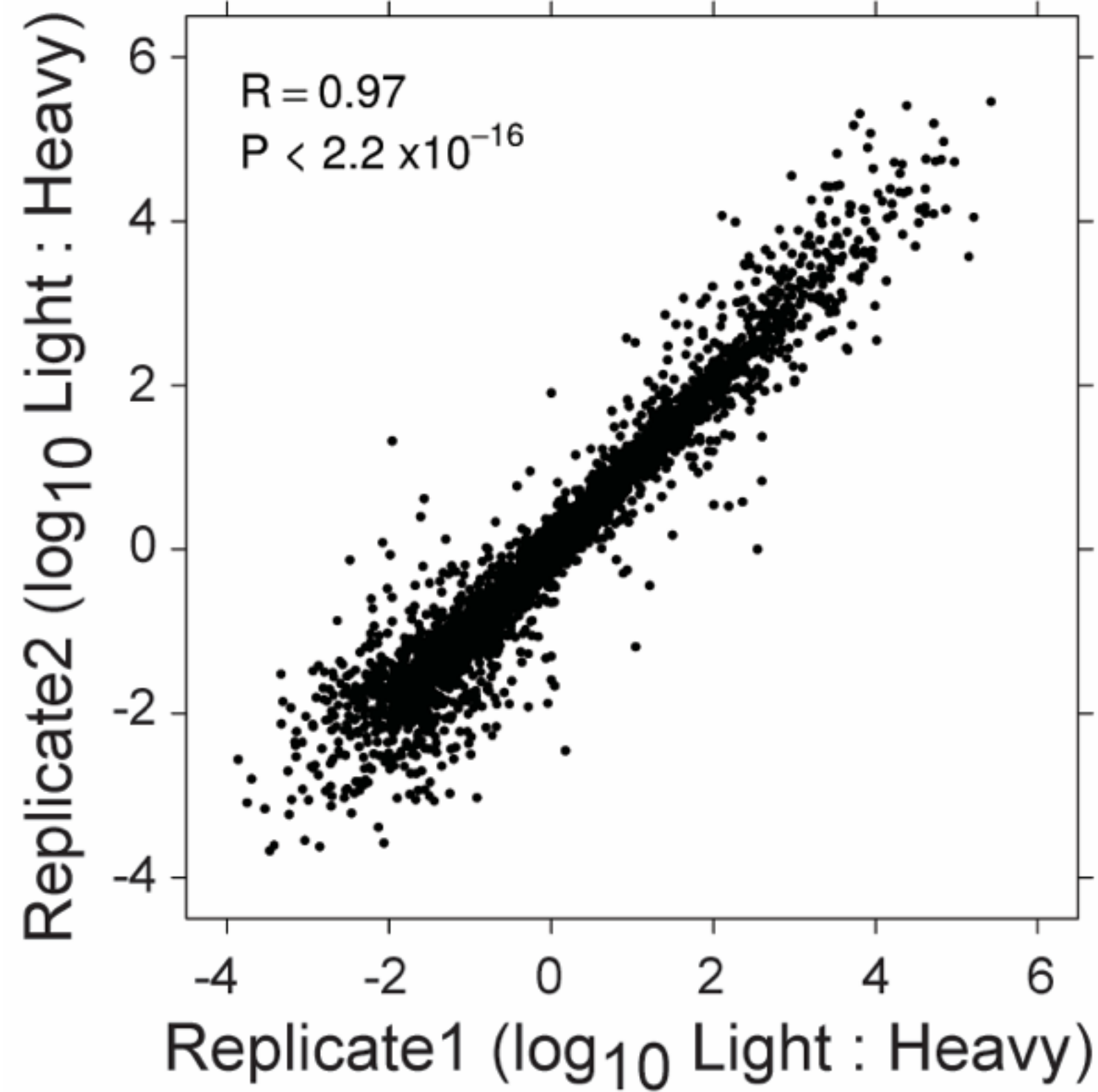
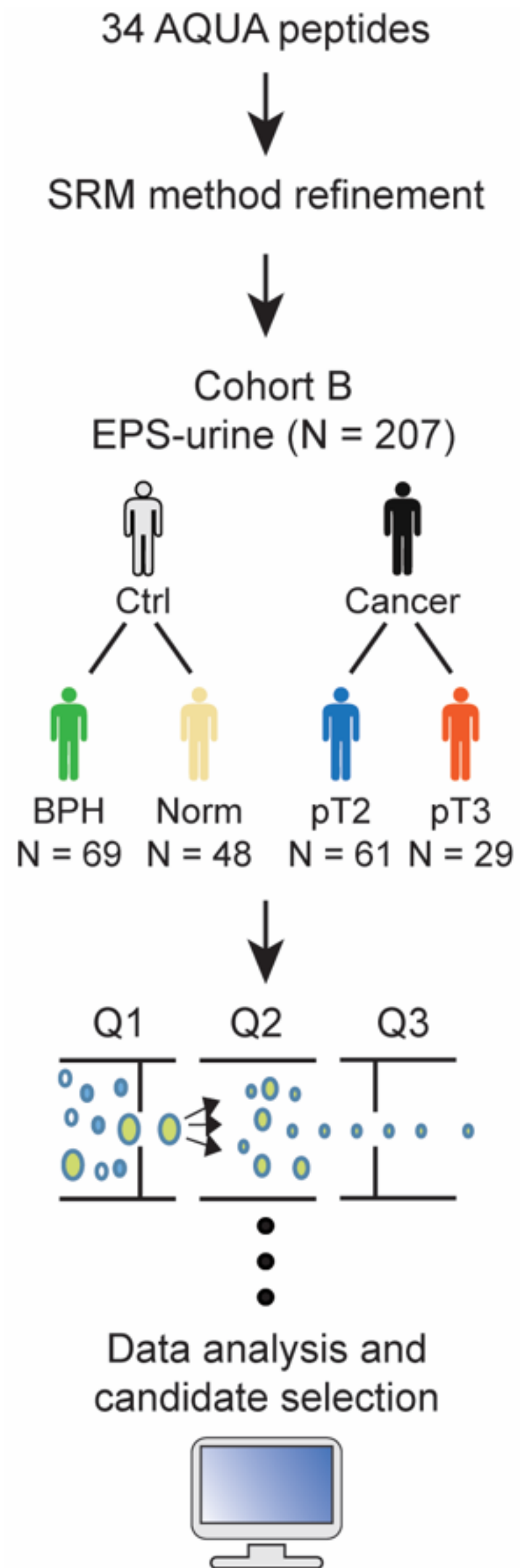
<http://www.usrf.org/news/PCA3>

Kim Y, et al. Nat Commun. 2016
 Yang L, et al. Oncotarget. 2015
 Principe S, et al. Proteomics. 2013
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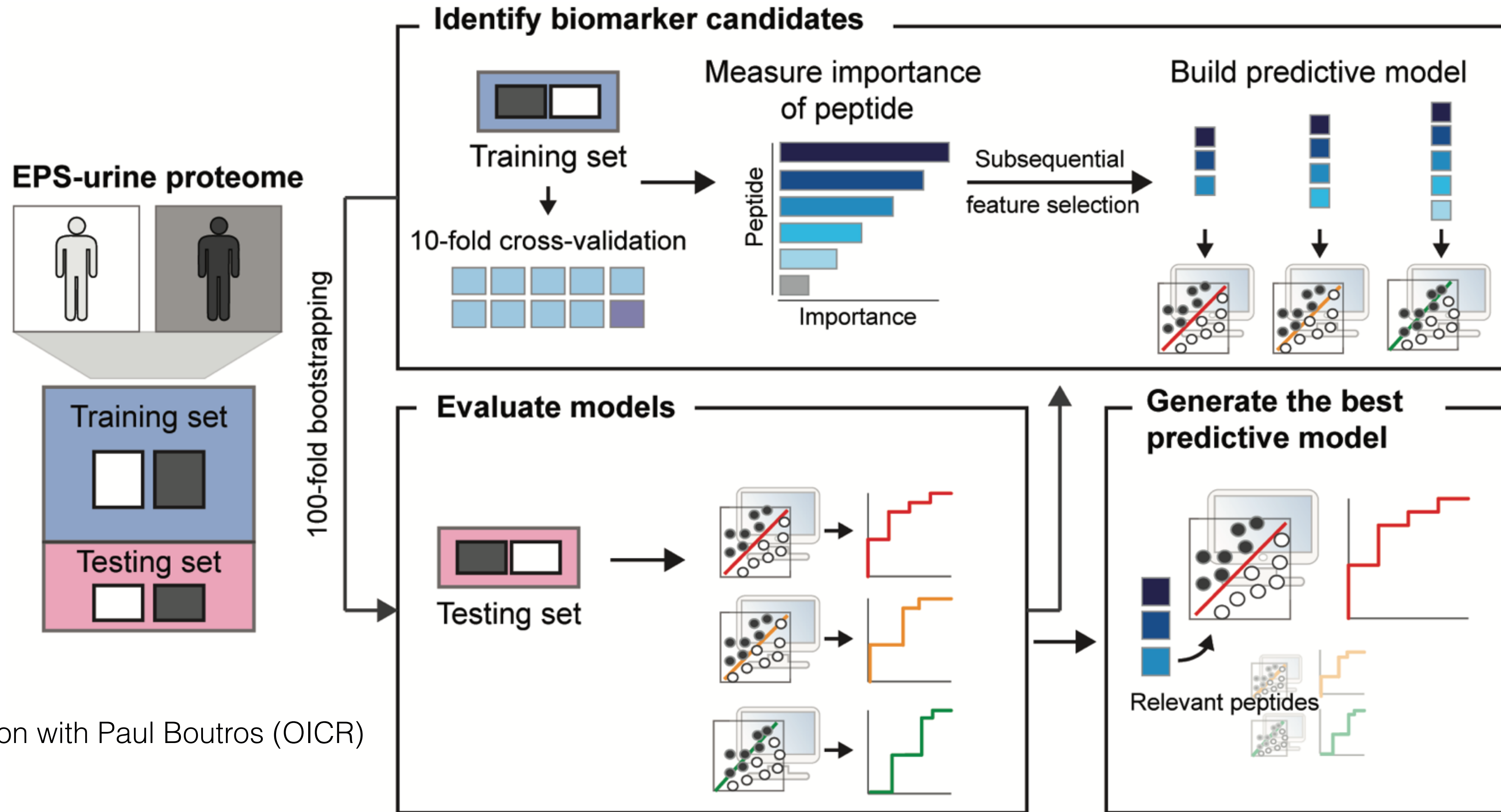
Development of Targeted Proteomics Assays



Tissue Proximal Fluids



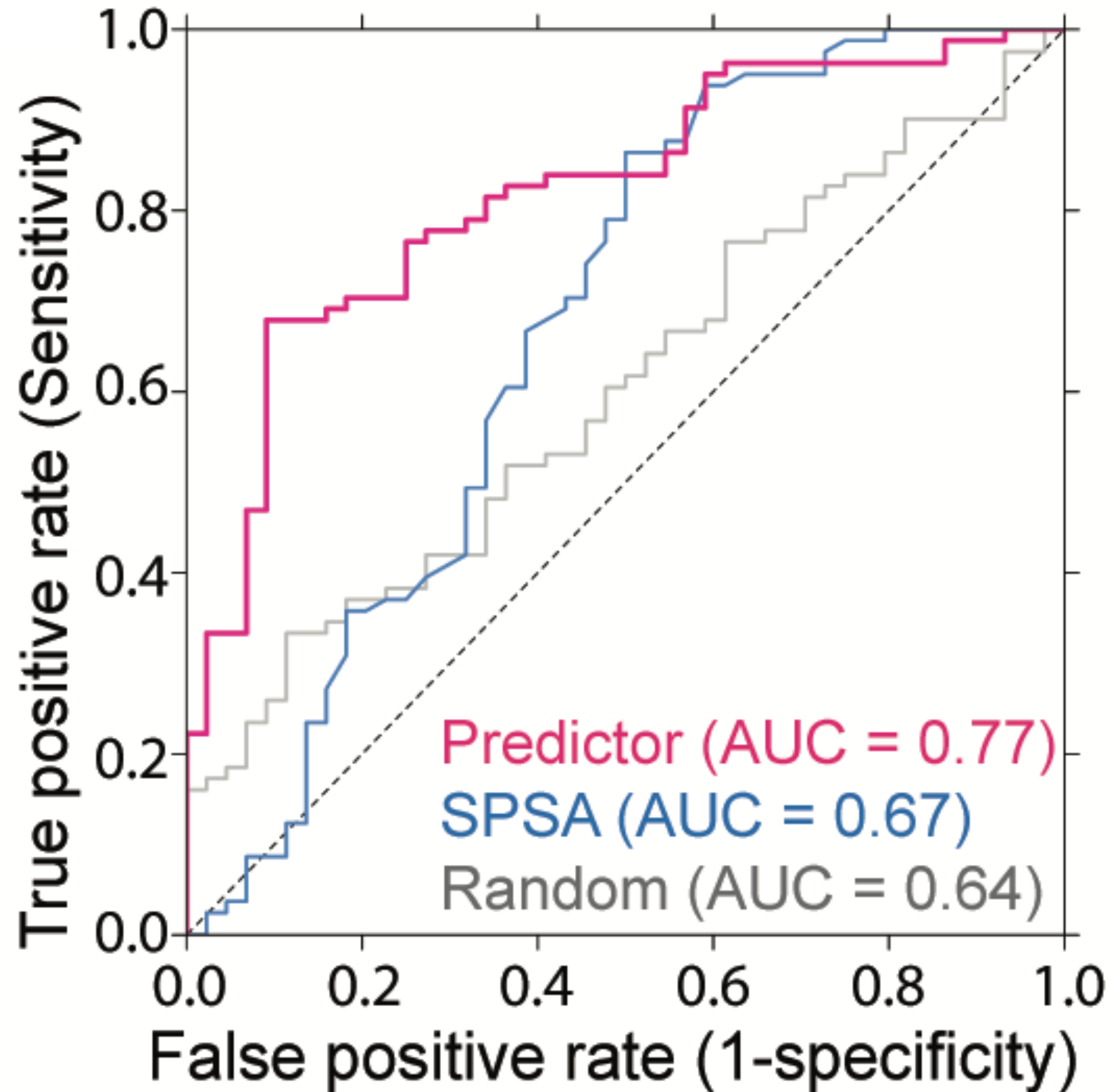
Tissue Proximal Fluids



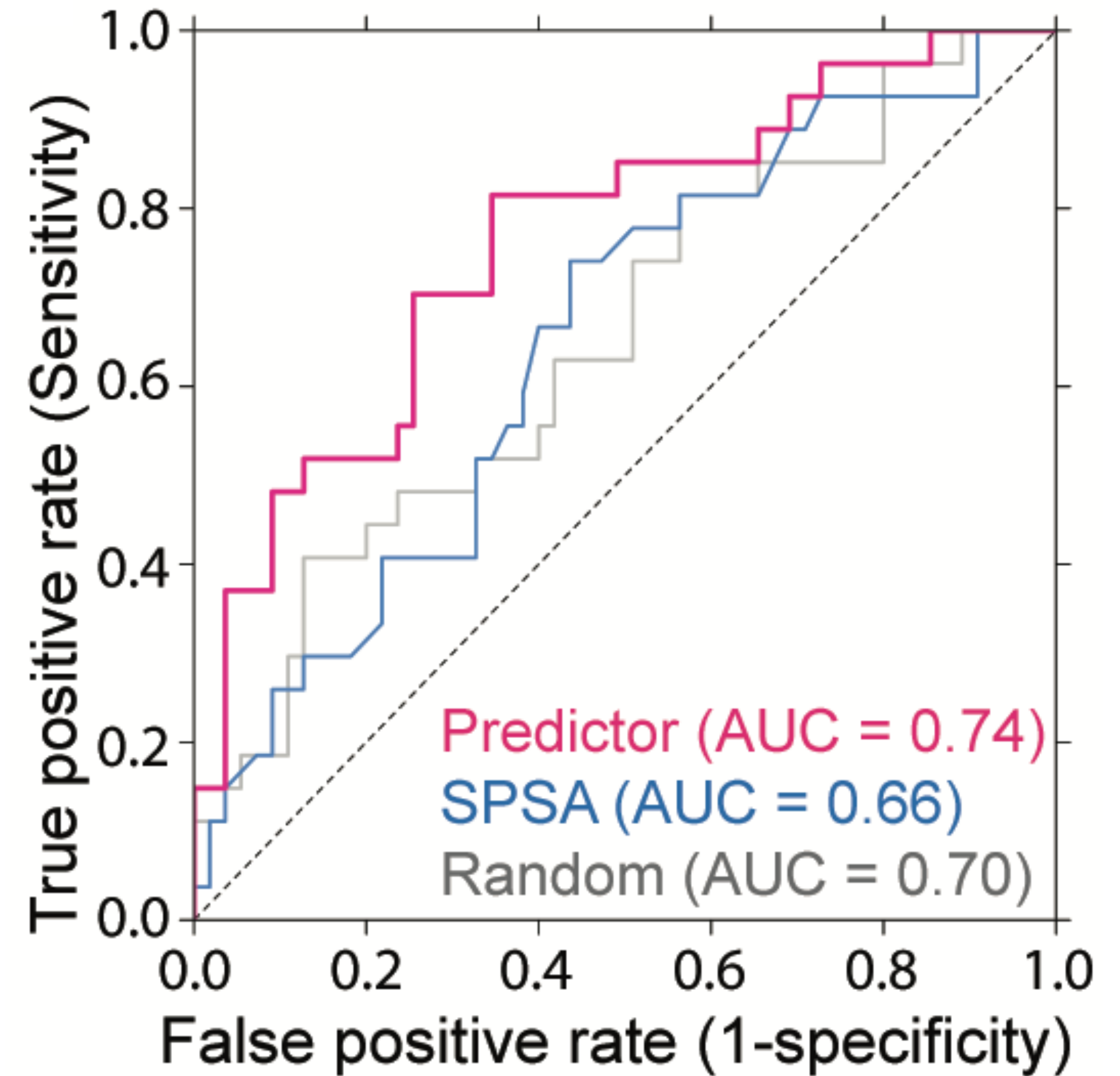
Collaboration with Paul Boutros (OICR)

Tissue Proximal Fluids

Cancer vs. Control



EC vs. OC



Aims of the EDRN Grant

Development of Protein Biomarkers in Post-DRE Urine for use in Liquid Biopsy of Prostate Cancer

Aim 1

Development of Protein Biomarker Signature from Post-DRE Urine (Kislinger)

- A) Validation and Assay Optimization of Liquid Biopsy Peptide Signature
- B) Discovery in Prostatic Secretions to Expand Utility of Existing Signature
- C) Discovery of Soluble/Secreted Glycoproteins in Prostatic Secretions

Aim 2

Exosomes as a Source of Liquid Biopsy Protein Biomarkers for Prostate Cancer (Semmes)

Aim 3

Integrating Biomarker Discovery through Bioinformatics (Boutros)

Work in Progress

Aim 1

Development of Protein Biomarker Signature from Post-DRE Urine (Kislinger)

A) Validation and Assay Optimization of Liquid Biopsy Peptide Signature

B) Novel Discovery in Prostatic Secretions to Expand Utility of Existing Signature

C) Targeted Discovery of Soluble/Secreted Glycoproteins in Prostatic Secretions

Current Protocol

- 4ml EPS-urine
- 3kDa filter concentration
- Methanol precipitation
- TFE solubilization/digestion
- C18 purification
- Peptide quantification
- 1 ug peptides on column
- 15 cm n-HPLC-SRM-MS (TSQ Vantage)

Protocol Optimization

- PVDF membrane
- Magnetic C8 beads
- Compare to old protocol
- Use shotgun proteomics
- Coverage, time & automation

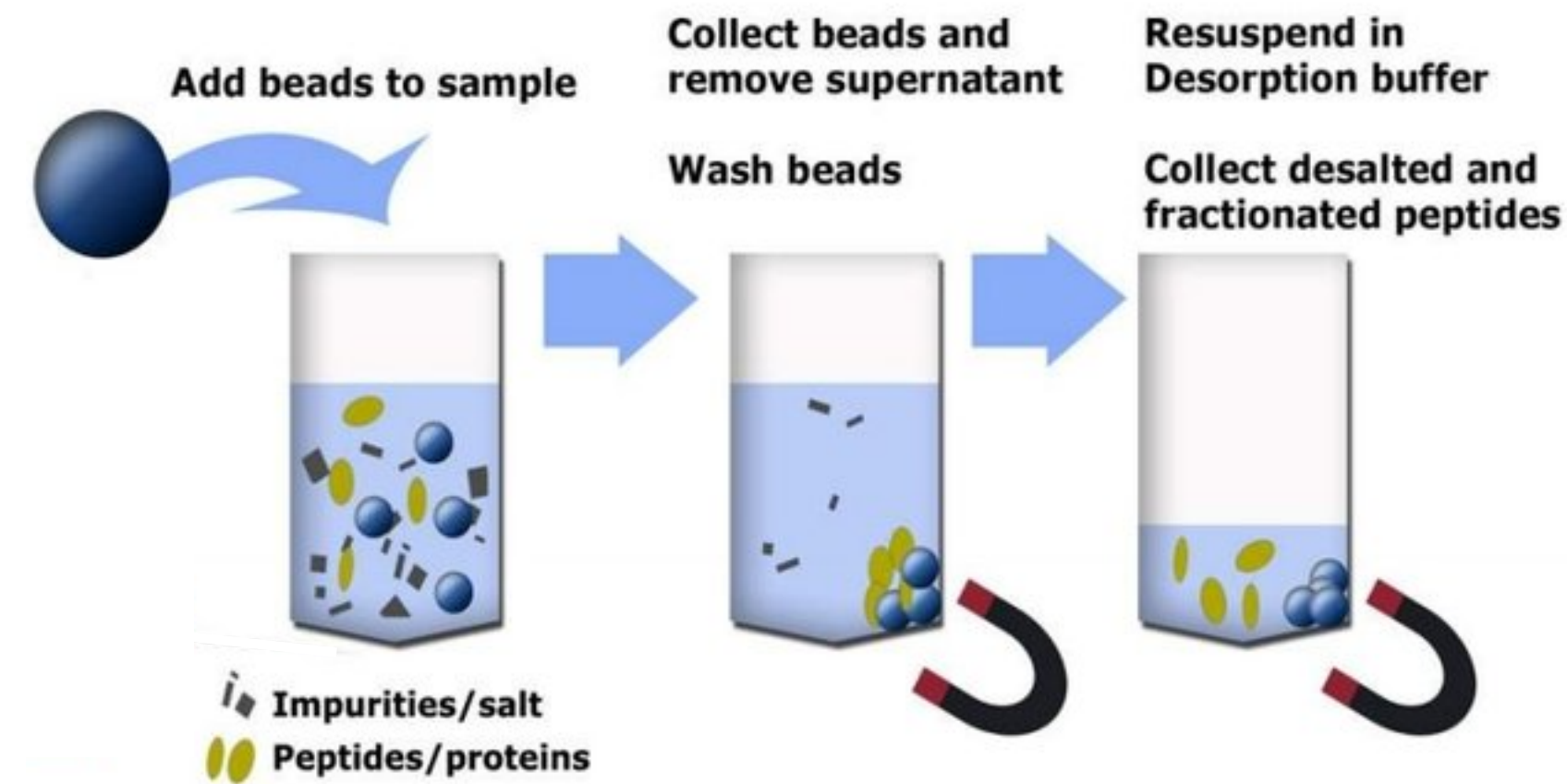
- Convert targeted assays
- 50 cm nUPLC-PRM-MS (QE HF)
- Assay metrics (LOD, LOQ, linearity, scheduling)

Sample Preparation Protocols

3KDa - TFE

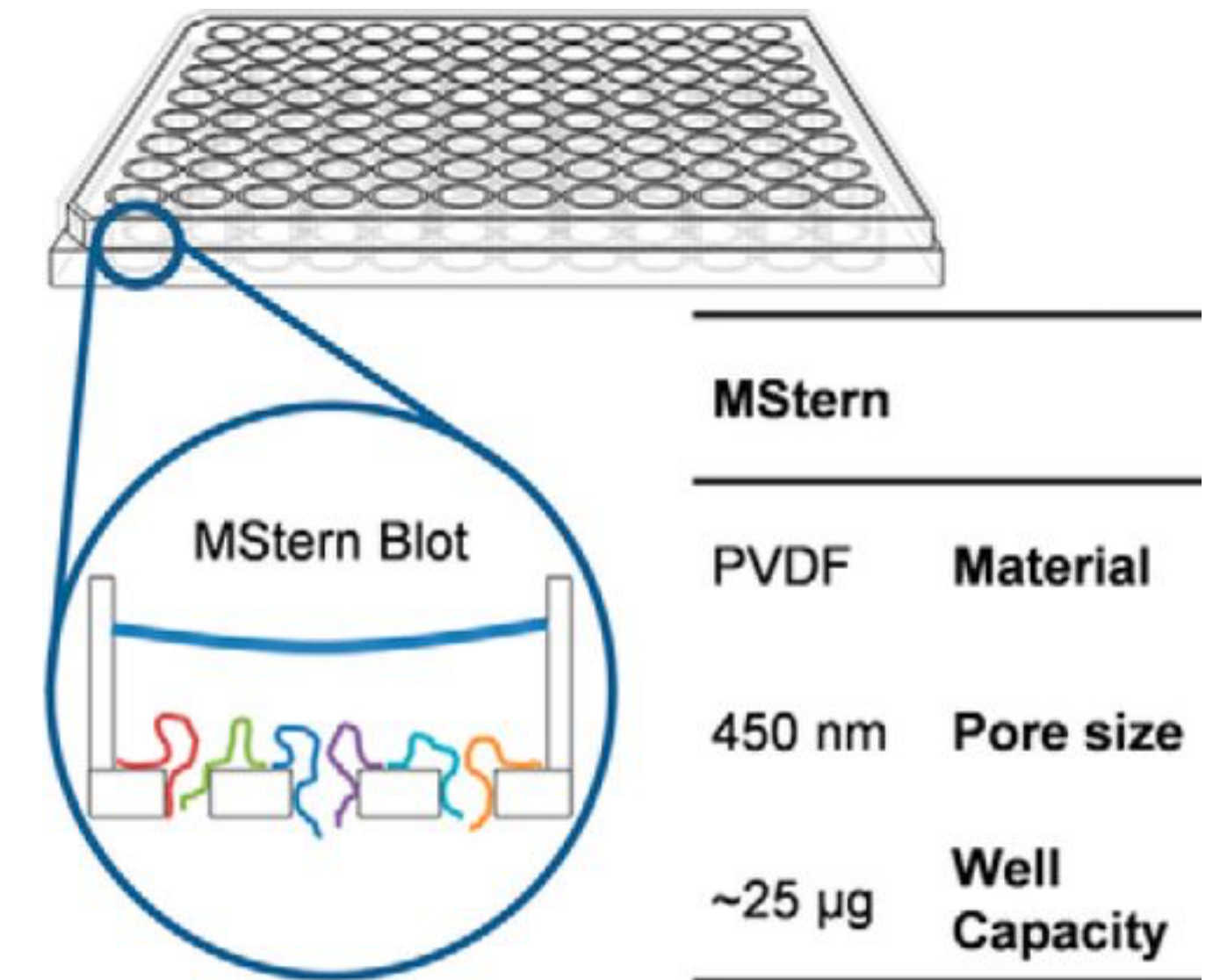


C8 beads



MStern

Berger et al. MCP 2015

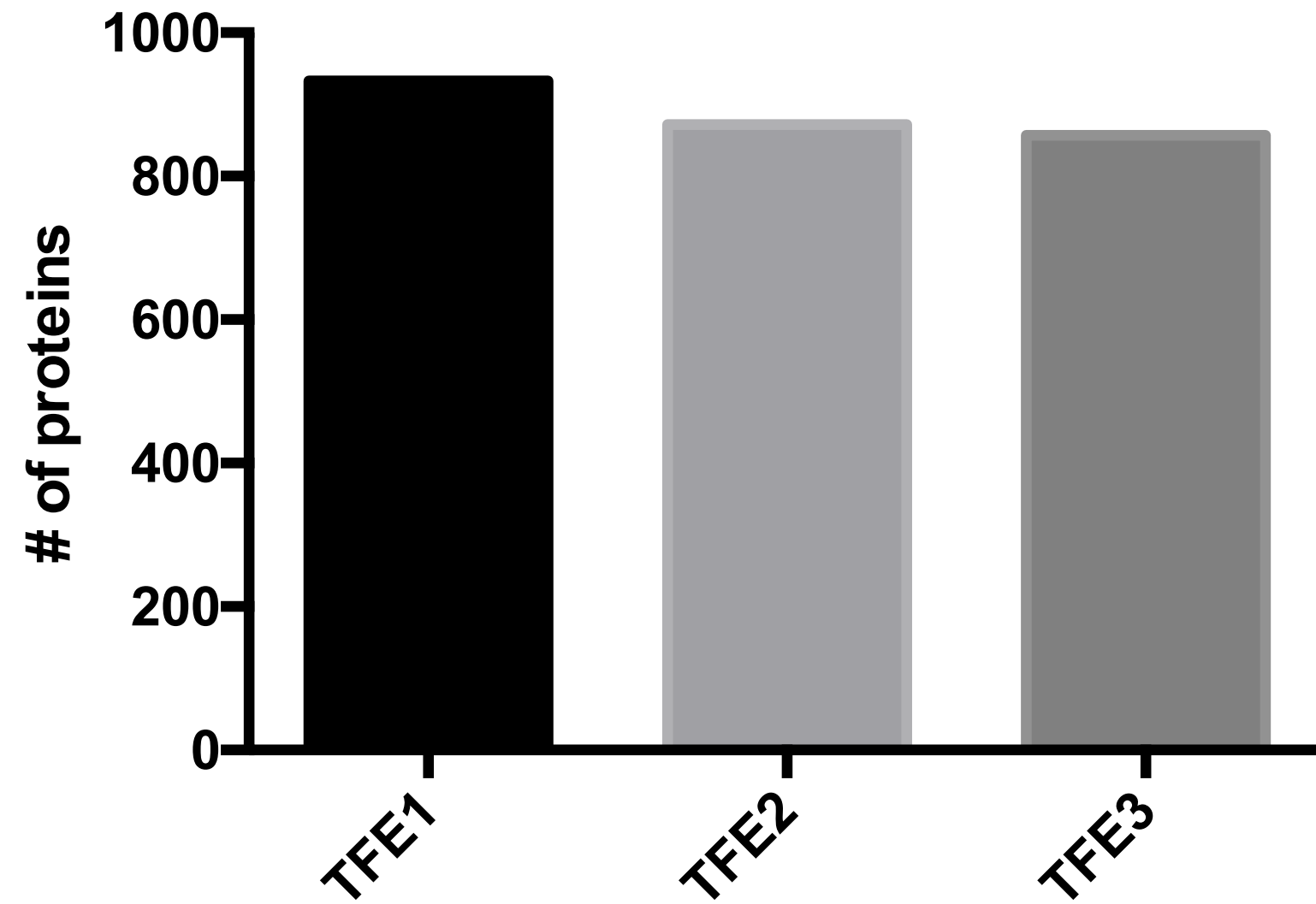


150 μ l of urine
~15 μ g of protein

Sample Preparation Protocols

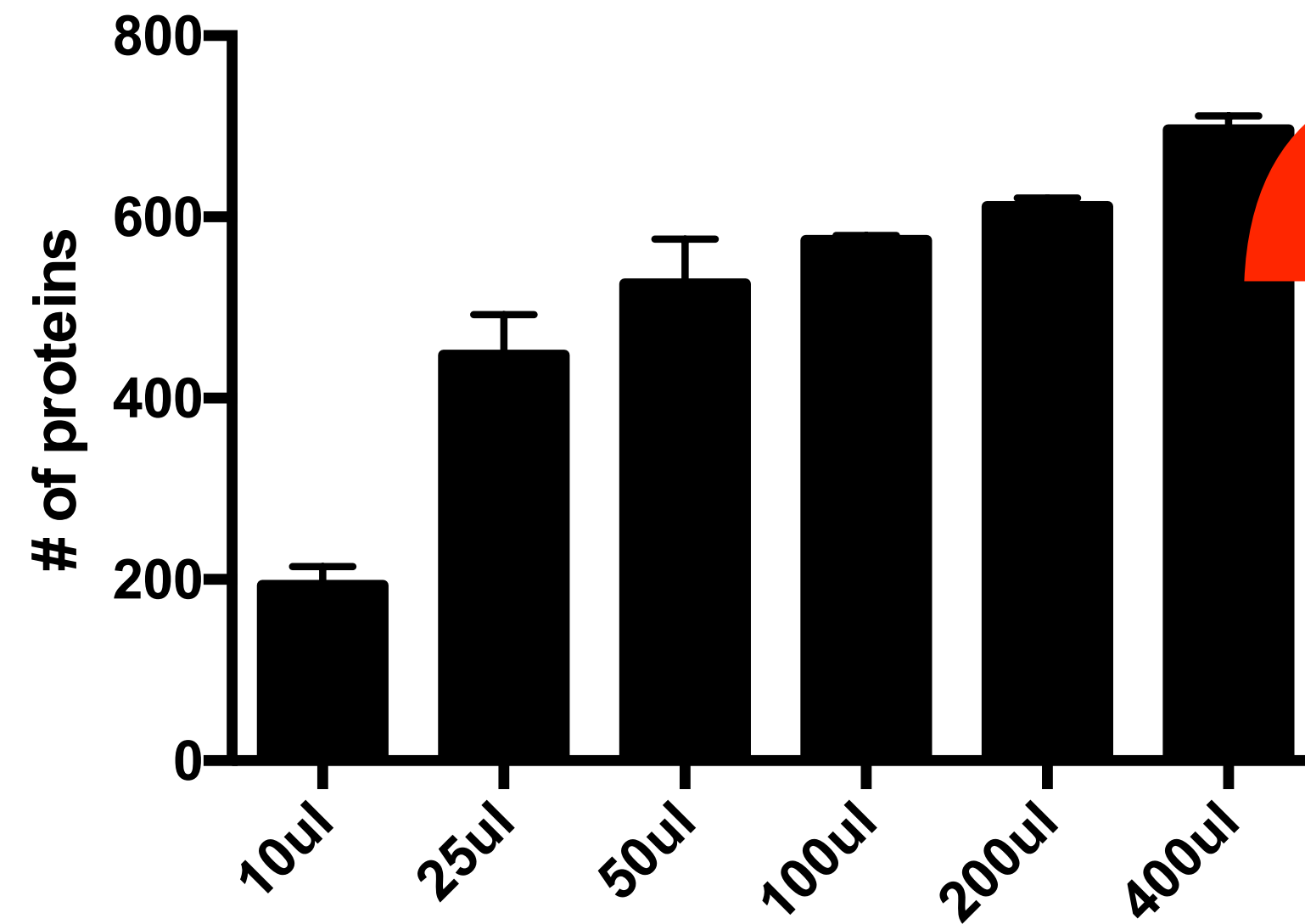
3KDa - TFE

Spin-filter TFE protocol



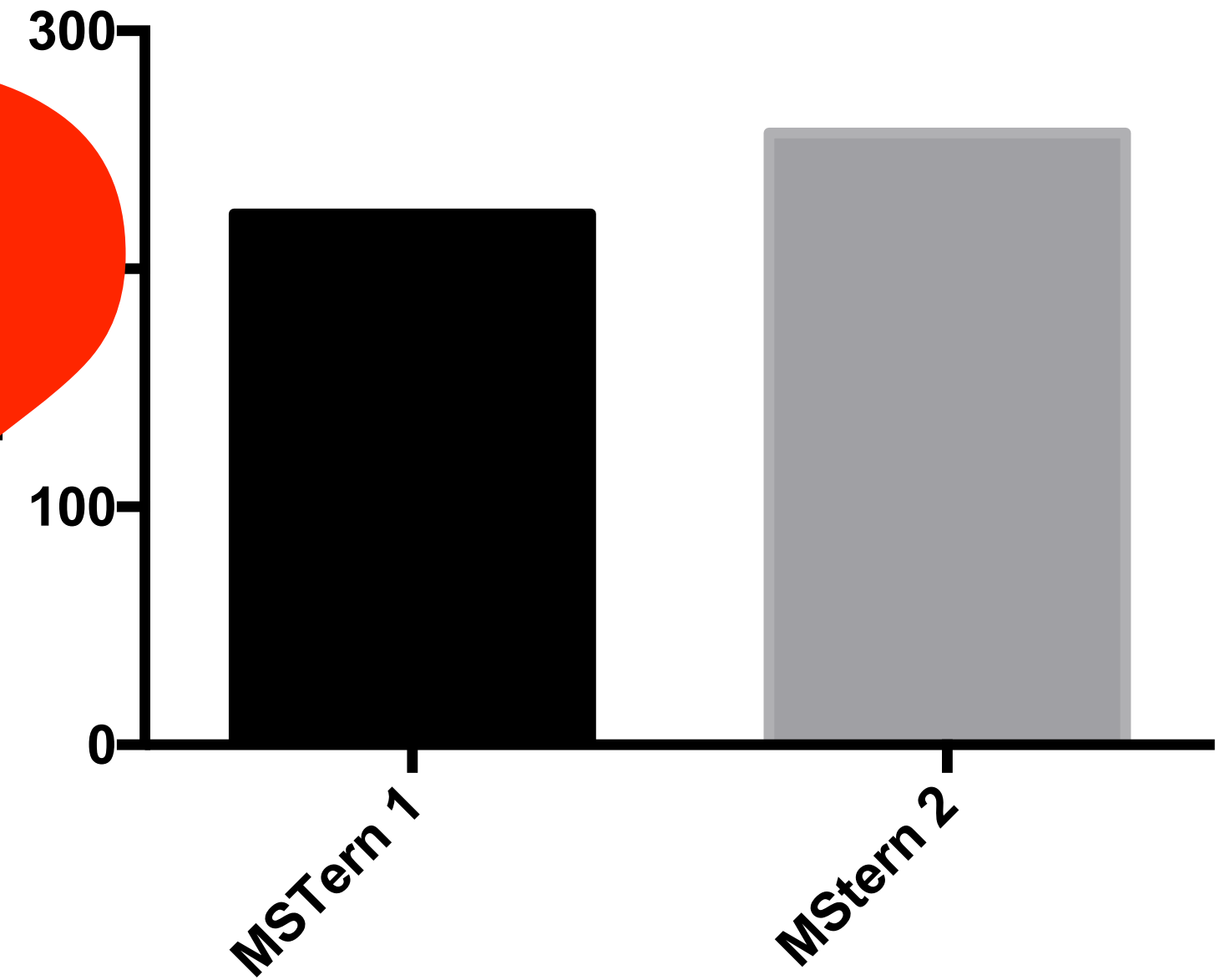
C8 beads

C8 magnetic beads



MStern

MStern - 150ul



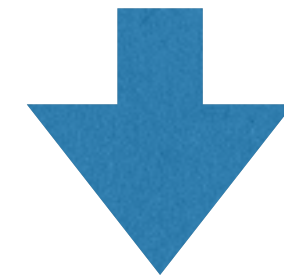
Prostate Cancer Proteomics

Prostate Proximal Fluids

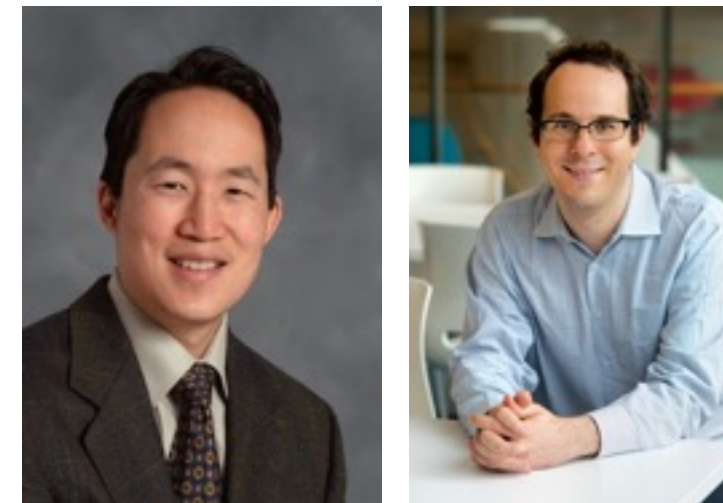
Prostatic Secretions
Post-DRE urines



EDRN

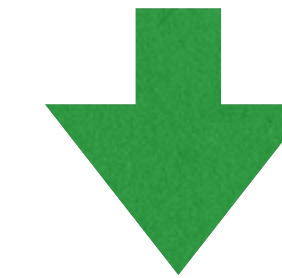


PCC, OICR

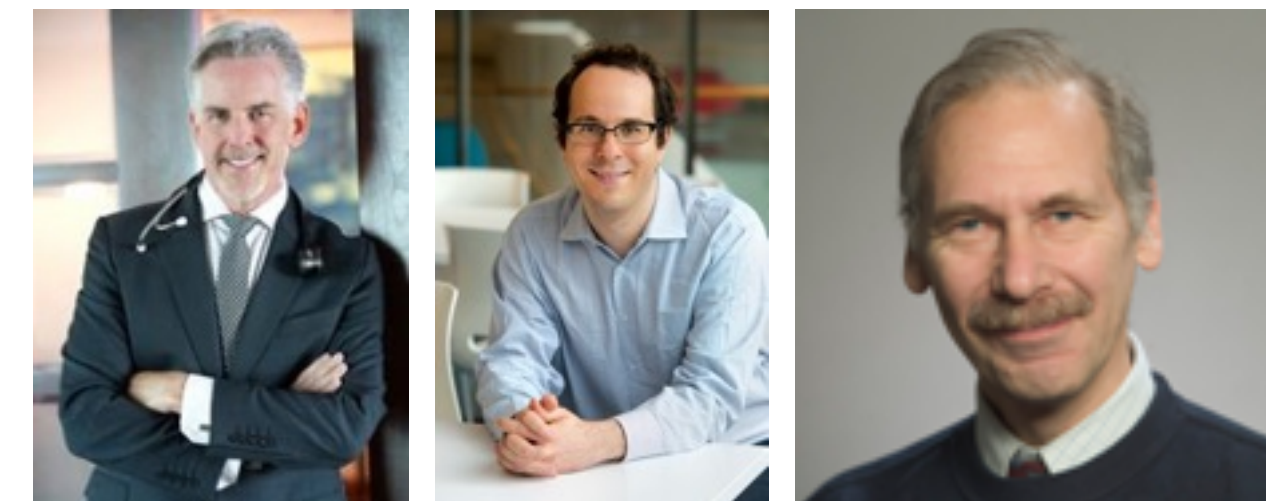


Prostate Cancer Tissues

CPC-GENE

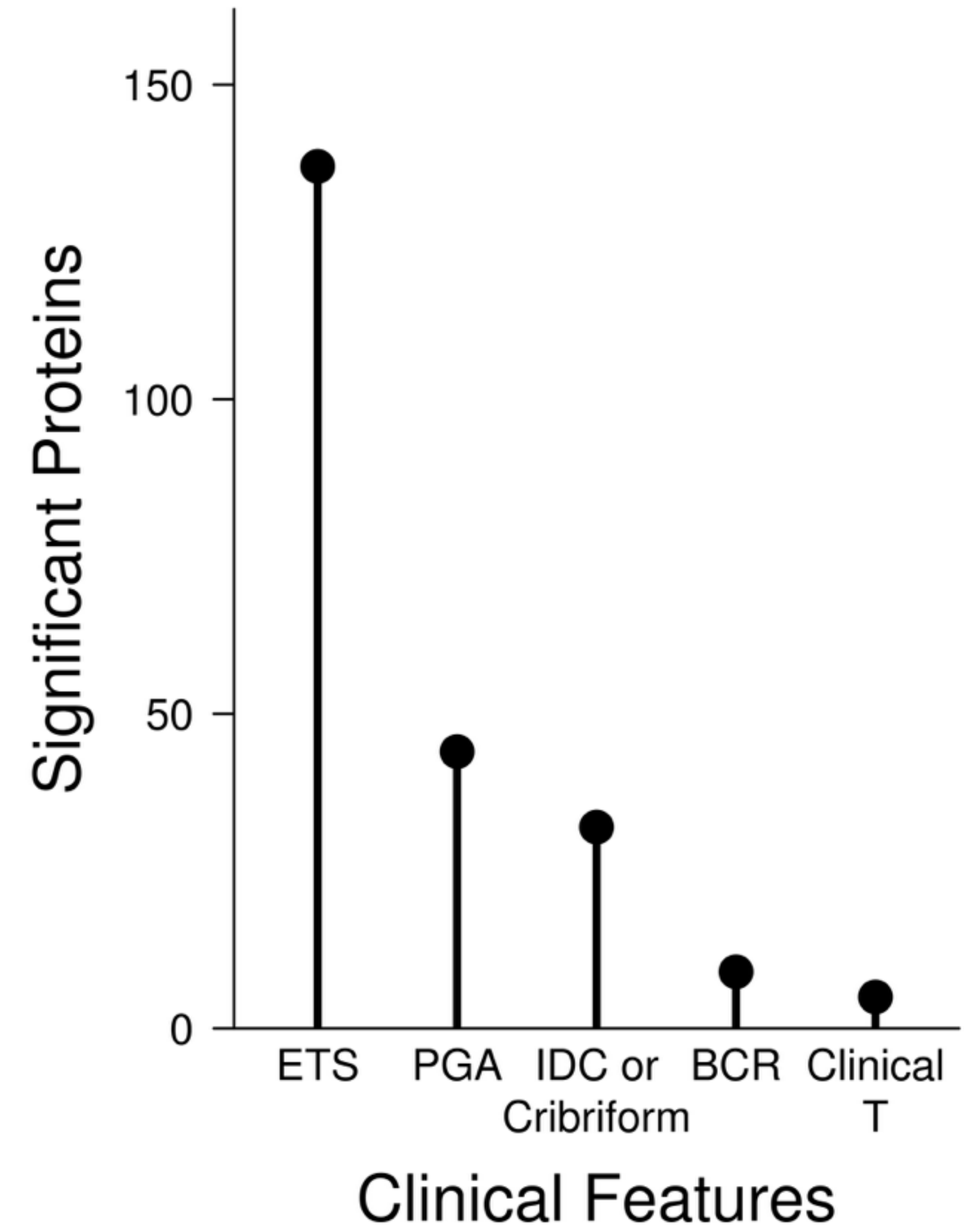
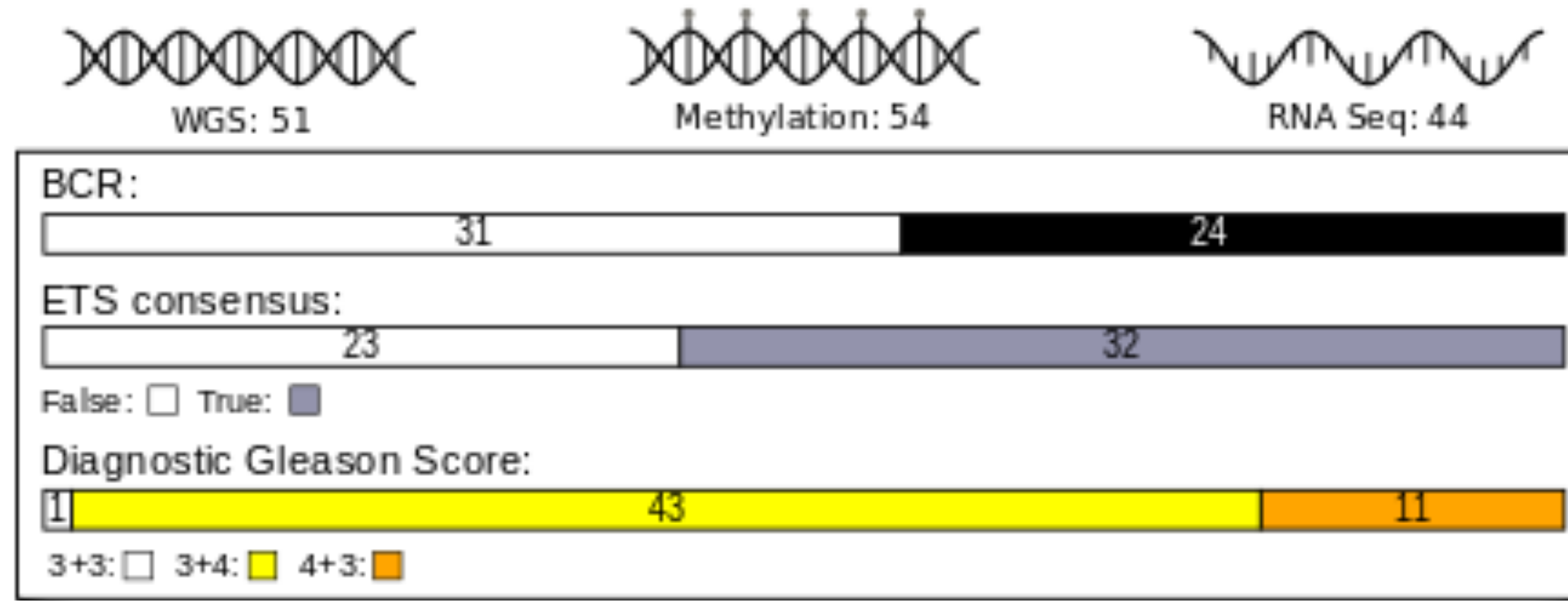


PCC

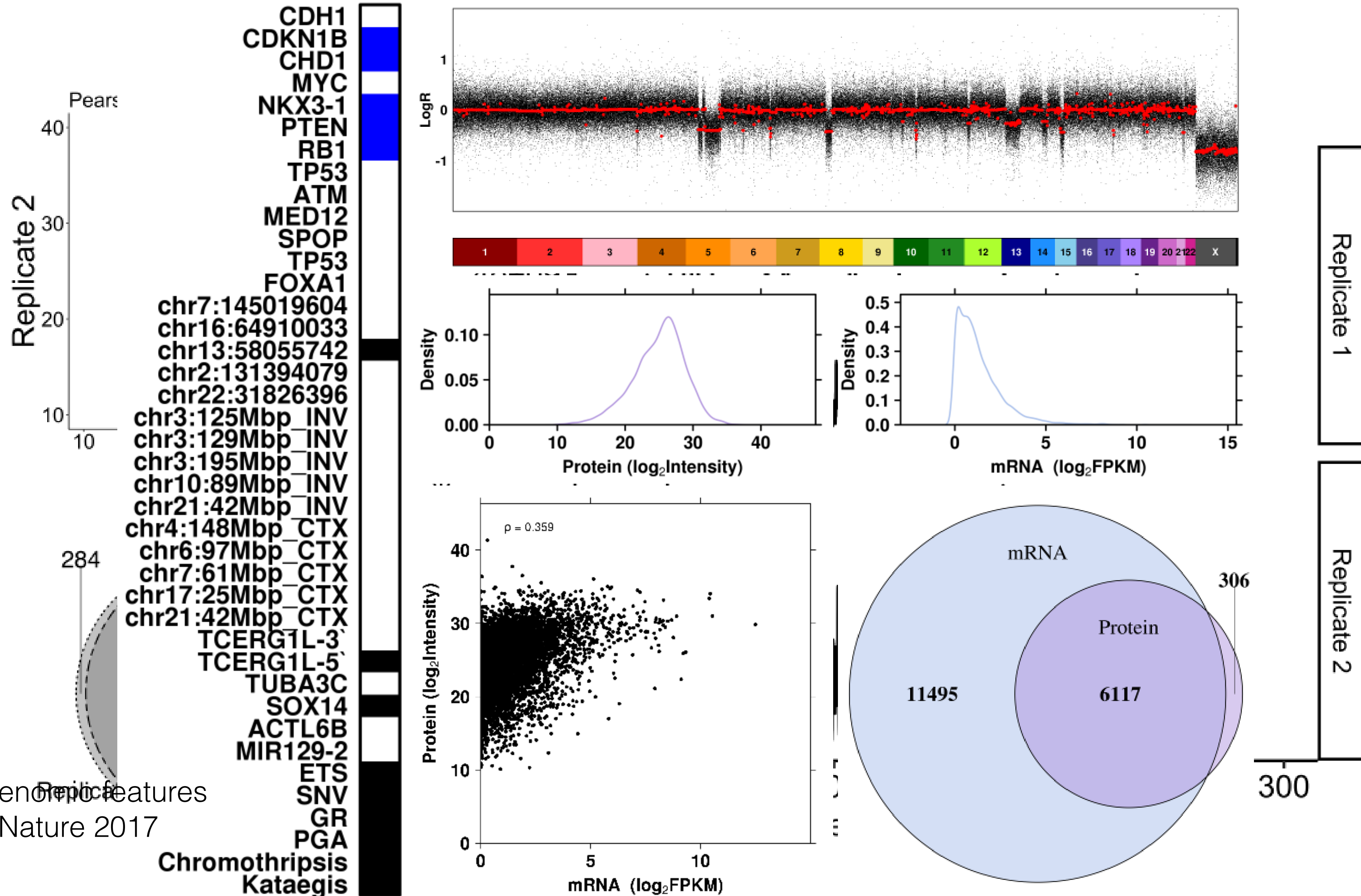


CPC-GENE Proteogenomics

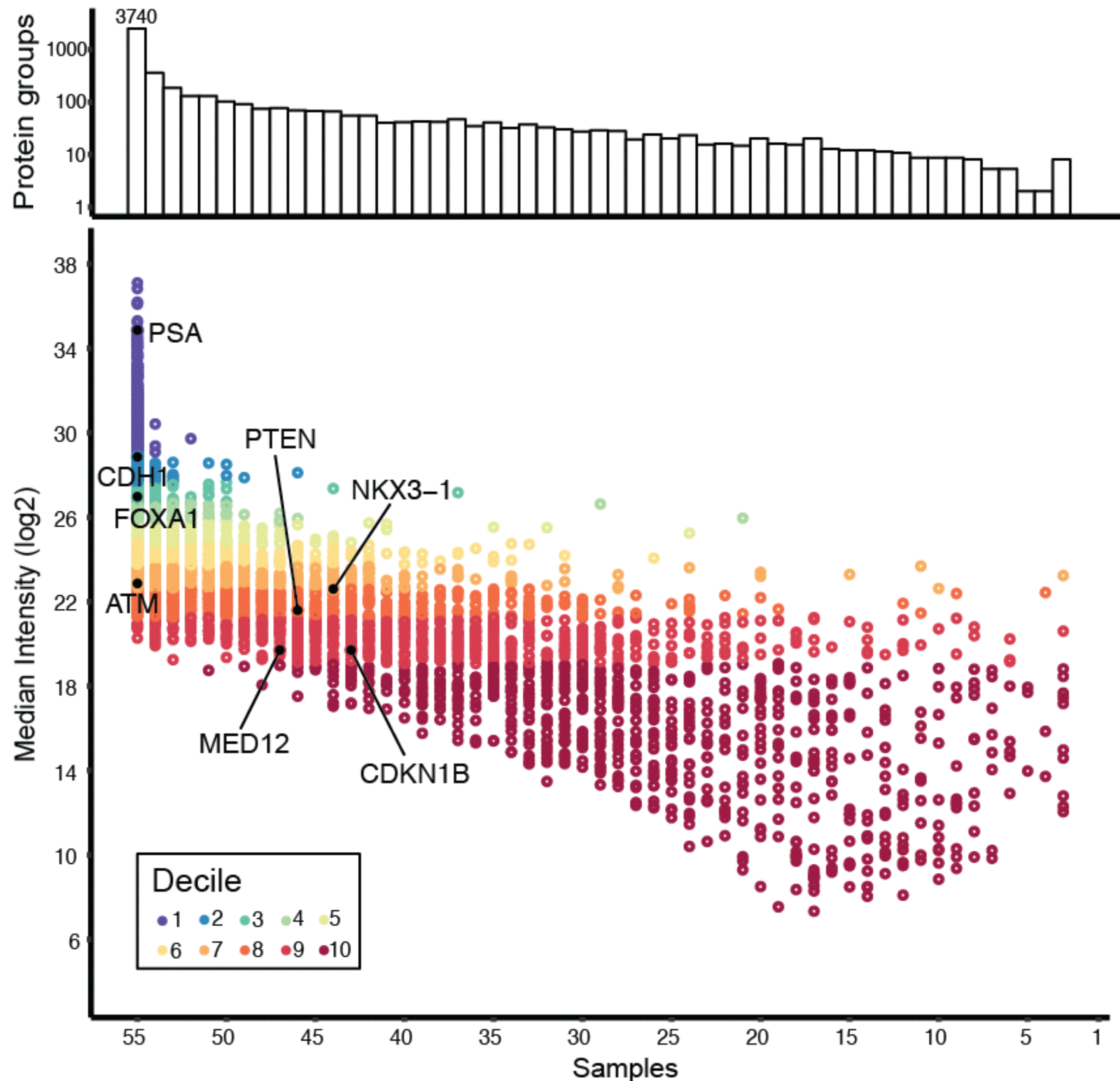
55 Prostate Tumour Samples



Patient-specific Proteogenomics



Prostate Cancer Proteogenomics



1. Proteins placed in abundance deciles
2. Span several orders of magnitude
3. 6050 proteins (85%) are in >40 tissues
4. Global correlation to mRNA abundance correlates with decile
5. Combination of protein and mRNA best predict BCR (preliminary!)

Summary

1. Proteomics of prostatic secretions is useful for identification of liquid biopsy signatures.

A. Optimize, validate and extend

2. Additional post-DRE urine proteomics & lipidomics (pre- vs. post-DRE urine)

3. Tissue-based proteogenomics (CPC-GENE)

A. Multi-omic based signatures to predict BCR

Acknowledgements

Generous donation by the **patients**

EDRN Team

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CPC-GENE

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Ankit Sinha
Vladimir Ignatchenko

Jacob Kagan

 National Cancer Institute

U.S. National Institutes of Health | www.cancer.gov

 **Early Detection Research Network**
Biomarkers: the key to early detection

DCP Division of
Cancer Prevention

 Prostate Cancer
Canada



A nighttime photograph of a city skyline, likely Toronto, with the CN Tower prominently featured on the left. The city lights are reflected in the water in the foreground. The sky is a deep blue with some light clouds.

Thank you

Questions?