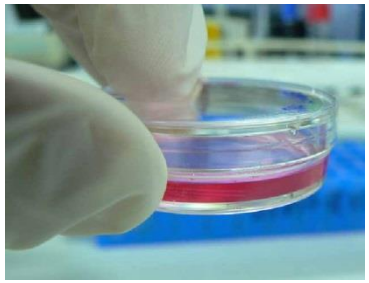
CPTAC Assay Portal: a repository for well-characterized quantitative targeted proteomic assays

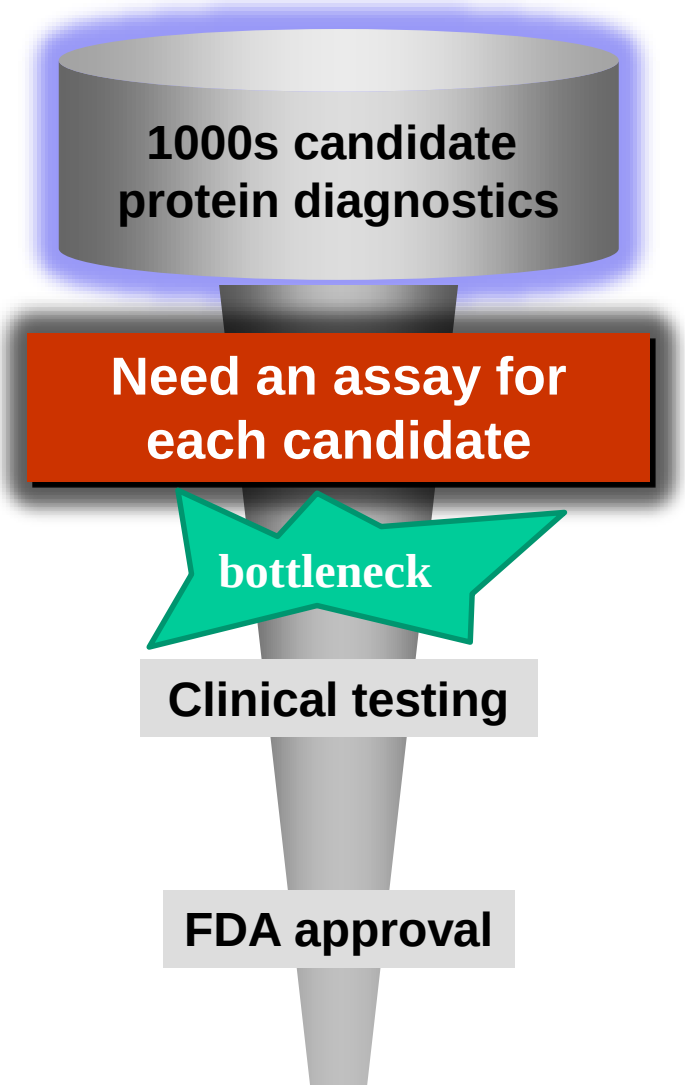
Jeff Whiteaker

Skyline Users Group Meeting, Baltimore, 2014

Targeted proteomics has great potential for impacting the protein diagnostics pipeline.

Basic / translational scientist looking at cellular responses





Clinical scientist looking for biomarkers (fluids, tissues)

A lack of standardized methods for quantifying proteins is explicitly cited as one cause for the irreproducibility of pre-clinical research.

BIOMEDICINE

NIH mulls rules for validating key results

US biomedical agency could enlist independent labs for verification.

BY MEREDITH WADMAN

In biomedical science, at least one thing is apparently reproducible: a steady stream of studies that show the irreproducibility of many important experiments.

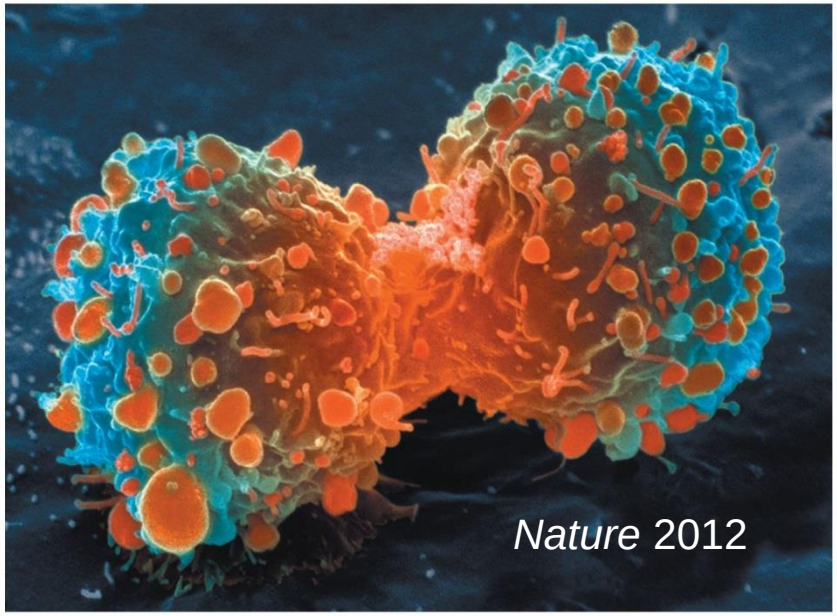
In a 2011 internal survey, pharmaceutical firm Bayer HealthCare of Leverkusen, Germany, was unable to validate the relevant preclinical research for almost two-thirds of 67 in-house projects. Then, in 2012, scientists at Amgen, a drug company based in Thousand Oaks, California, reported their failure to replicate 89% of the findings from 53 landmark cancer papers. And in a study published in May, more than half of the respondents to a survey at the MD Anderson Cancer Center in Houston, Texas, reported failing at least once in attempts at reproducing published data (see 'Make believe').

The growing problem is threatening the reputation of the US National Institutes of Health (NIH) based in Bethesda, Maryland, which funds many of the studies in question. Senior NIH officials are now considering adding requirements to grant applications to make experimental validations routine for certain types of science, such as the foundational work that leads to costly clinical trials. As the NIH pursues such top-down changes, one company is taking a bottom-up approach, targeting scientists directly to see if they are willing to verify their experiments.

There is the looming

➲ NATURE.COM
For more on the challenges of reproducibility:
go.nature.com/zqtrnp

S. GSCHMEISSNER/SPL



Many landmark findings in preclinical oncology research are not reproducible, in part because of inadequate cell lines and animal models.

Raise standards for preclinical cancer research

C. Glenn Begley and Lee M. Ellis propose how methods, publications and incentives must change if patients are to benefit.

The PCAST Report on Pharmaceutical Innovation: Implications for the FDA

J Woodcock[1]

In September 2012, the President's Council of Advisors on Science and Technology (PCAST) released the report "Propelling Innovation in Drug Discovery, Development and Evaluation." A product of discussions with many stakeholders, the report reiterates current problems in drug development, including diminished return on basic biomedical research. The report calls for doubling the current annual output of innovative new medicines—an ambitious goal. Recommendations and resulting initiatives will probably affect the FDA's drug regulatory programs.

trial costs, time to market, and regulatory uncertainty. Although the two are intertwined, the report discusses improvements in drug discovery and development (factors i, ii, and iii above) separately from factor iv, regulatory uncertainty. The following discussion touches on all four factors.

Recommendation 2 is the centerpiece of PCAST's prescription for improving drug discovery and development. The report calls for the creation of a broad-based "partnership to accelerate therapeutics" that would coordinate, facilitate, and possibly launch projects to address various roadblocks, but not carry them out on its own. The Institute of Medicine is evaluating this concept.

The report identifies three principal roadblocks to more efficient drug discovery and development: (i) knowledge gaps in the science, technology, and methodologies that underlie these processes, (ii)

We have a clinical, economic, and scientific imperative to standardize protein quantification to support the inter-laboratory reproducibility of preclinical results.

Using a fit-for-purpose approach to defining and standardizing assays

- Workshop held in June 2013
- Representatives from academia, clinical laboratories, diagnostic companies, biotech, biology

- What defines an assay?
- How reliable are the measurements?
- What performance criteria are essential?
- What information is needed to distribute or transfer assays?

MCP MOLECULAR & CELLULAR PROTEOMICS

QUICK SEARCH Author: Keyword: Year: Vol: Pa

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Targeted Peptide Measurements in Biology and Medicine: Best Practices for Mass Spectrometry-based Assay Development Using a Fit-for-Purpose Approach*

Steven A. Carr[‡,u], Susan E. Abbatiello[‡], Bradley L. Ackermann[§], Christoph Borchers[¶], Bruno Domon[‖], Eric W. Deutsch[**], Russell P. Grant[‡‡], Andrew N. Hoofnagle[§§], Ruth Hüttenhain[¶¶‖‖], John M. Koomen[a], Daniel C. Liebler[b], Tao Liu[c], Brendan MacLean[§§], DR Mani[‡], Elizabeth Mansfield[d], Hendrik Neubert[e], Amanda G. Paulovich[f], Lukas Reiter[g], Olga Vitek[h], Ruedi Aebersold[¶¶], Leigh Anderson[i], Robert Bethem[j], Josip Blonder[k], Emily Boja[k], Julianne Botelho[l], Michael Boyne[d], Ralph A. Bradshaw[‖‖], Alma L. Burlingame[‖‖], Daniel Chan[m], Hasmik Keshishian[‡], Eric Kuhn[‡], Christopher Kinsinger[k], Jerry S.H. Lee[k,m], Sang-Won Lee[n], Robert Moritz[**], Juan Oses-Prieto[‖‖], Nader Rifai[o], James Ritchie[p], Henry Rodriguez[k], Pothur R. Srinivas[q], R. Reid Townsend[r], Jennifer Van Eyk[m], Gordon Whiteley[s], Arun Wiita[‖‖] and Susan Weintraub[t]

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2014 Mar;13(3):907-17.

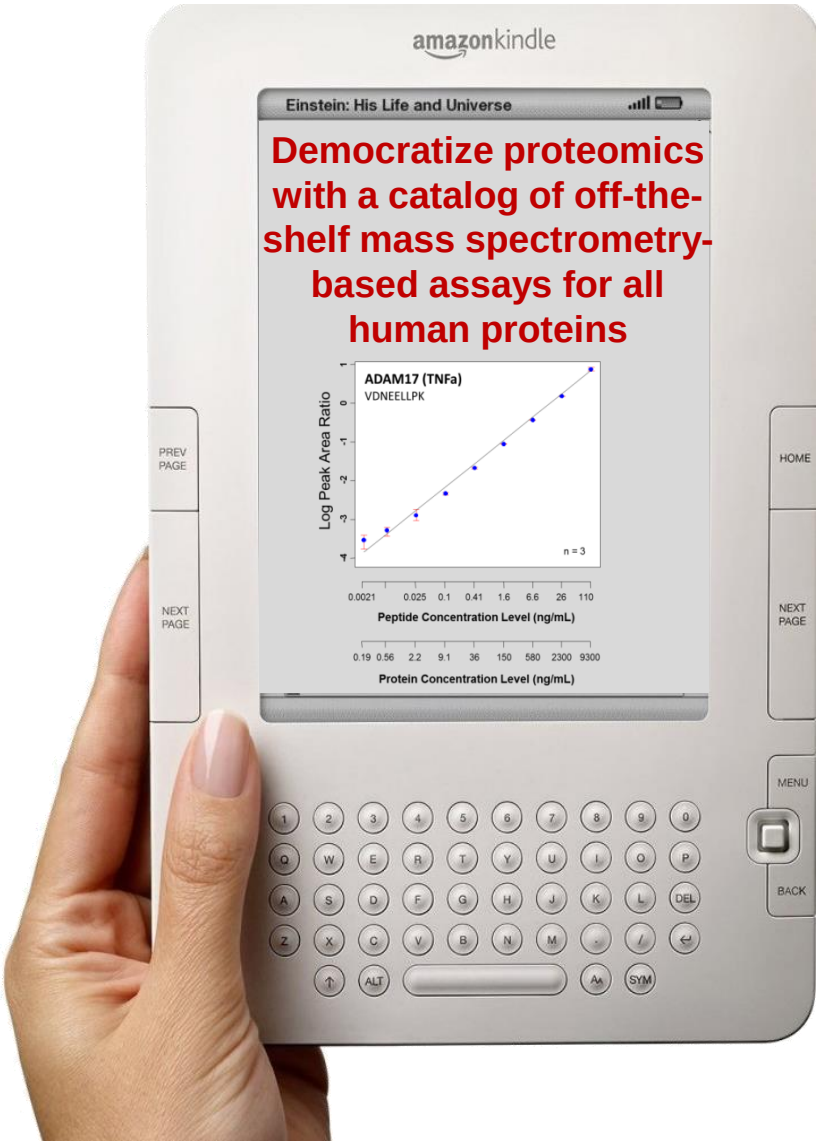
Assays divided into three tiers based on intended use

Tier and Areas of Application	Degree of Analytical Validation	Labeled Internal Standards	Reference Standards	Specificity	Precision	Quantitative Accuracy	Repeat-ability	Comments and Suggested References
Tier 1 **Clinical bioanalysis/ diagnostic laboratory test; single analyte or small numbers of analytes**	High, including batch-to-batch QC	Yes, for every analyte	Yes	High	High (typically <20-25% CV achieved)	Defining accuracy is a goal; true accuracy difficult to demonstrate.	High	Precise, quantitative assays; established, high performance; may need comply with FDA and CLIA guidance depending on use of assay Refs. 30, 41, 42, 53
Tier 2 **Research use assays for quantifying proteins, peptides, and post-translational modifications; 10's to 100's of analytes**	Moderate-to-high	Yes, for every analyte	Limited use	High	Moderate-to-high (typically <20-35% CV achieved)	Not applicable	High	Precise, relative quantitative assays; established performance; suitable for verification Refs. 30, 31, 36, 37, 40, 51, 70, 71
Tier 3 **Exploratory studies; 10's to 100's of analytes**	Low-to-moderate	None-to-limited	No	Moderate-to-high	Low-to-moderate: similar to label-free discovery	Not applicable	Moderate-to-high	Discovery in a targeted mode; performance not defined; results require further verification using quantitative techniques Refs. 36, 37, 86-89

Tier 2 – precise, relative quantification

- Measure changes in expression levels for nonclinical purposes
- Biomarker verification
- Characterized assays with high transferability and reproducibility

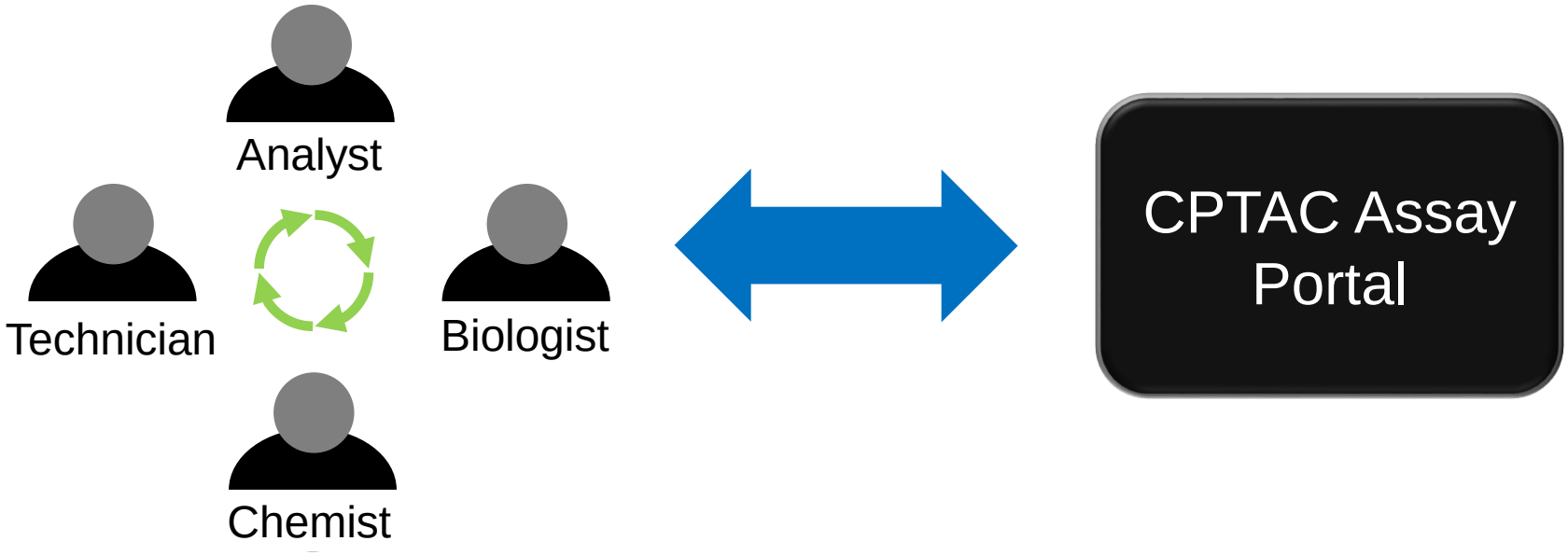
What will it take to for the targeted assay technology to meet its potential to promote rapid advances in protein-based biomedical research?



The ability to run mass spectrometry-based assays must be put in the hands of the <u>target user community</u> (basic, translational, and clinical scientists *without specialized training in proteomics*).

Purposes of a web-based assay portal

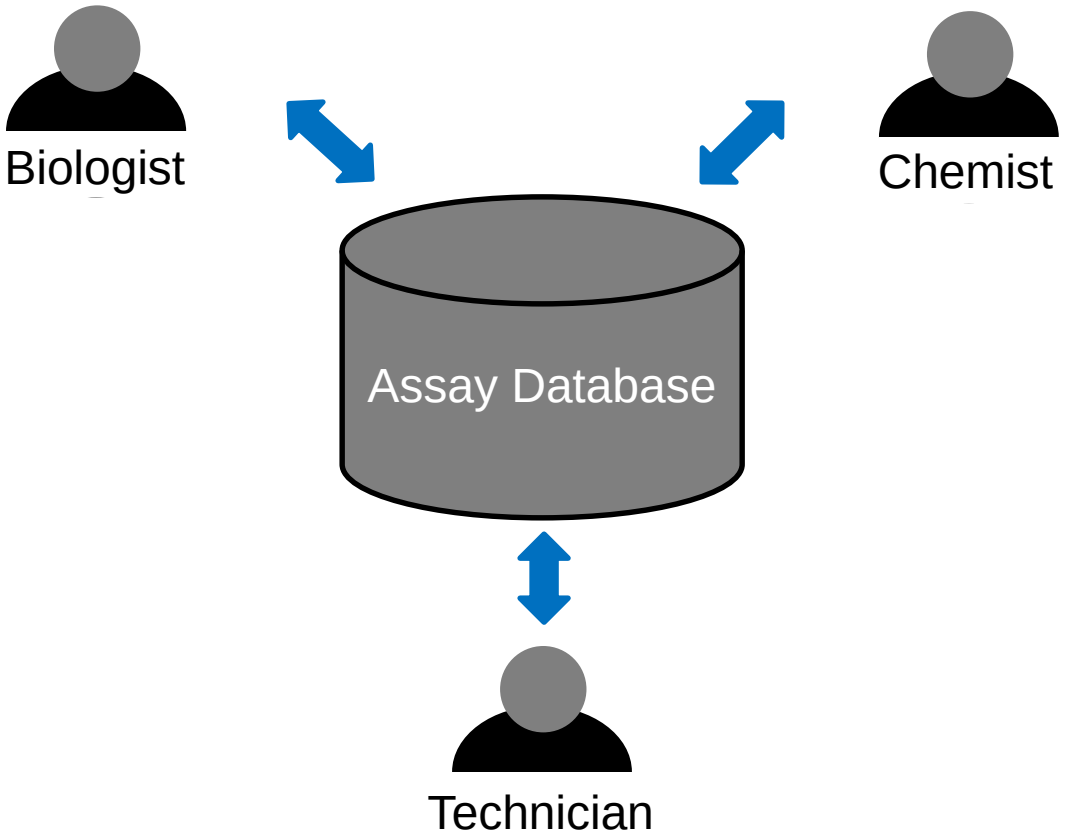
- to promote the development and dissemination of well characterized mass spectrometry-based proteomic assays
- link a broad user community to valuable assay resources



Assay portal features can be generalized into three areas

1. enable biological and clinical researchers to find protein targets or modifications of interest

2. present well characterized performance data for evaluation of assays



3. make available the data, assay parameters, reagent information, and SOPs for implementation

Assay Portal Features

assays.cancer.gov

Additional features of the assay portal and launch timeline

- High standards for inclusion of assays, supported by a validation guidance document
- Supports a variety of targeted proteomics technologies through Skyline and Panorama
- Expanded pathway and ontology integration coming soon

- Core features to launch in July
- Features will continue to be developed with user feedback
- Target date for acceptance of public contributions mid 2014

Acknowledgements

CPTAC Assay Development Working Group

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Vagisha Sharma
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Mike MacCoss

Browsing assays

Search the MRM Database by:

Pathways

Select Pathway(s)

Find assays to proteins encoded in a specific chromosomal region

Chromosome Number

Include All

Chromosomal Location

Start

Stop

Find assays to quantify proteins that interact with the following protein(s):

Find assays that work in a particular species

Include All

Assay Type

Include All

Show / hide columns

Show 50 entries

Showing 1 to 50 of 870 entries

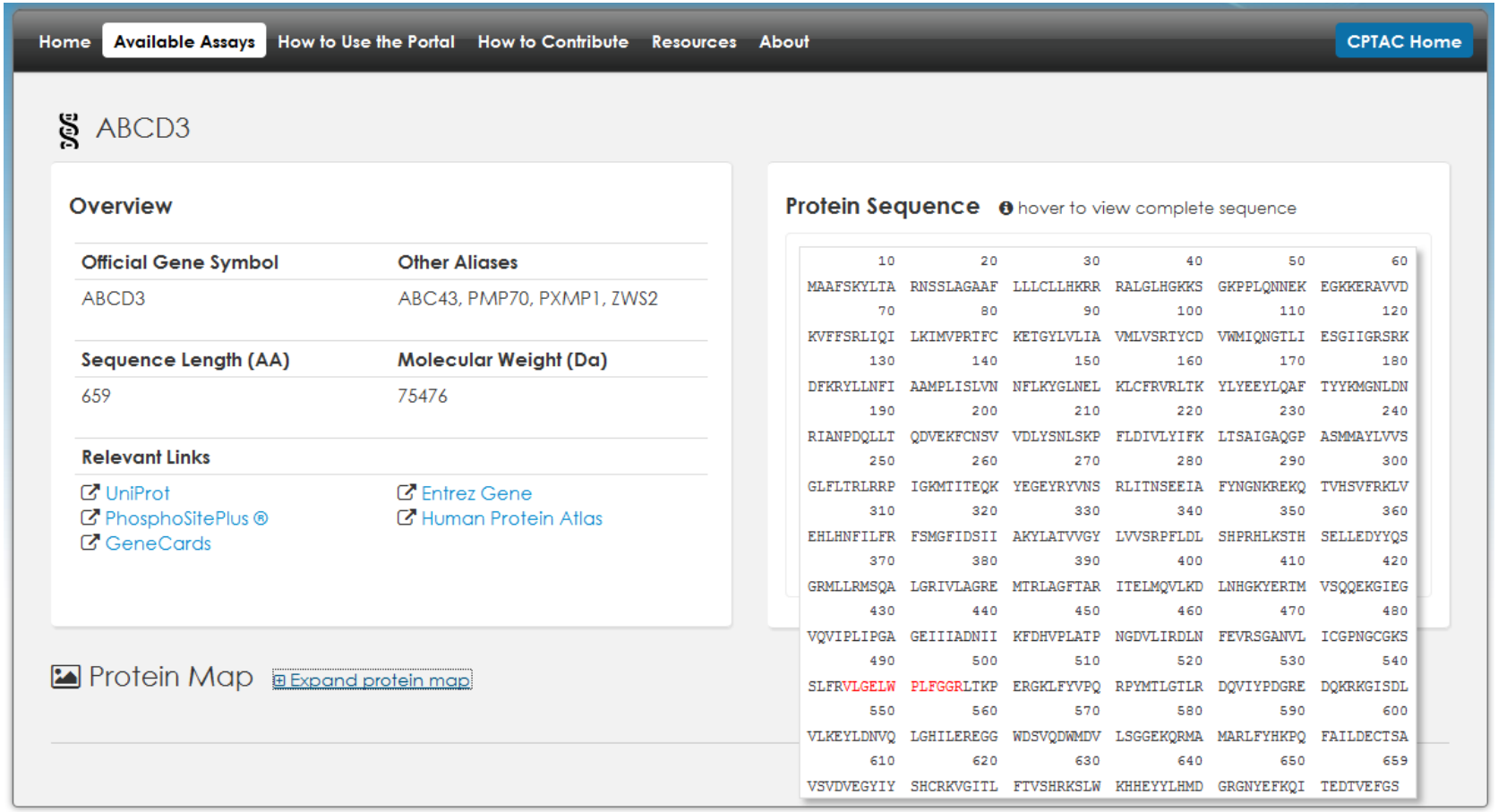
Search:

← Previous 1 2 3 4 5 Next →

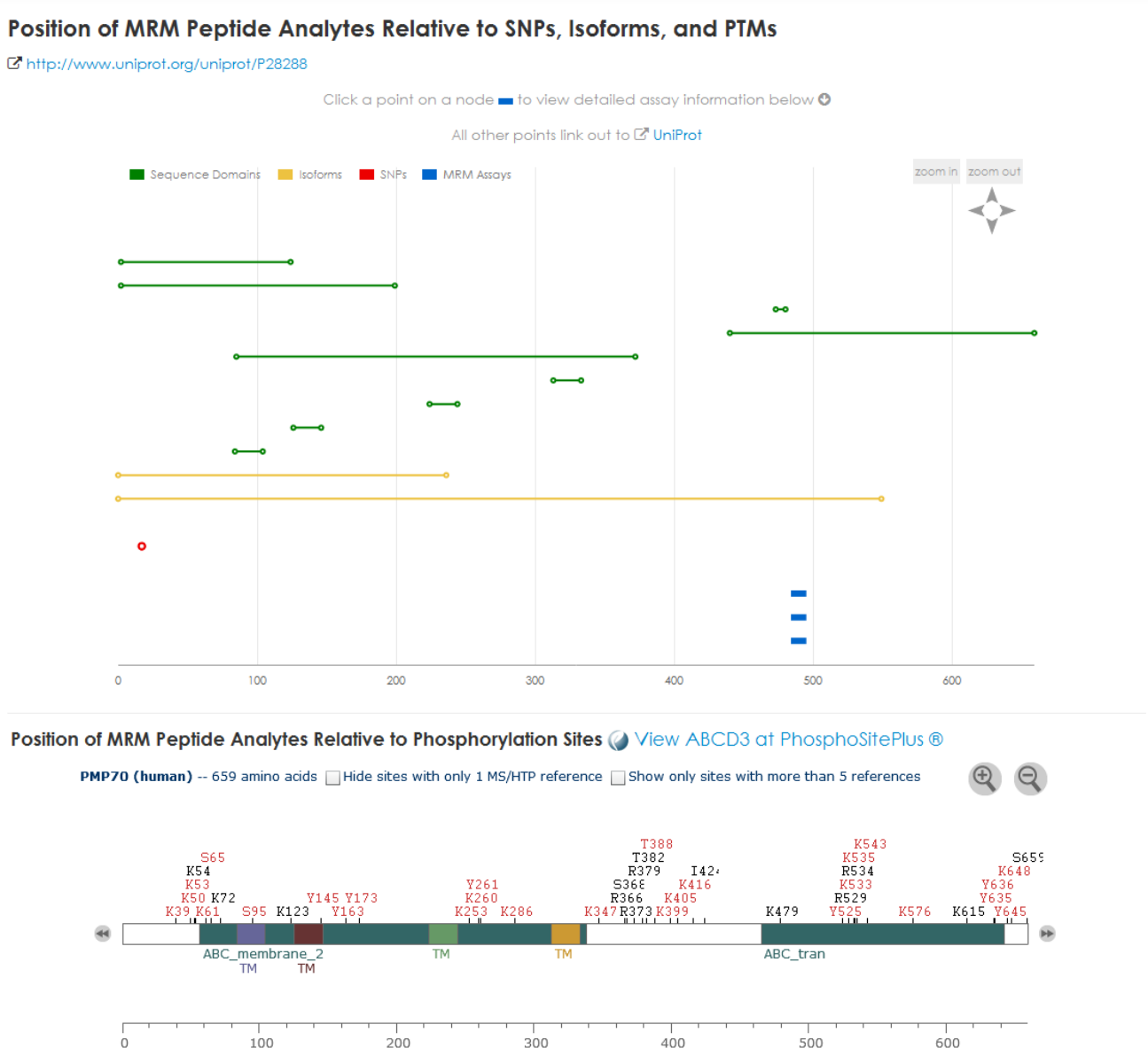
Proteins and peptides for which assays are available	Modification	Matrix
AARS - UniProt Accession ID: P49588		
AVFDETYPDPVR (Fred Hutchinson Cancer Research Center)	unmodified	cell line lysate pool
TITVALADGGRPDNTGR (Fred Hutchinson Cancer Research Center)	unmodified	cell line lysate pool
ABAT - UniProt Accession ID: P80404		
ALLTGLLDLQAR (Fred Hutchinson Cancer Research Center)	unmodified	cell line lysate pool
GTFCSFDTPDDSIR (Fred Hutchinson Cancer Research Center)	unmodified	cell line lysate pool
ABCD3 - UniProt Accession ID: P28288		
VLGELWPLFGGR (Broad Institute)	unmodified	cell line lysate pool

- User interface to browse or query the assay database in a multitude of ways (e.g. gene symbol, acc num, chromosomal location, subcellular localization, assay type, figures of merit, modification site, etc.)

Browsing assay details



“Protein Map” display allows browsing of peptide assays within a protein



Browsing characterization data

- Assays fully characterized with respect to analytical figures of merit (sensitivity, linearity, reproducibility, selectivity)
- Full integration with Panorama for data browsing

Assay Parameters

⊟ Collapse assay parameters

Instrument
5500 QTRAP (ABSCIEX)

Internal Standard
peptide

Peptide Standard Purity
>95%

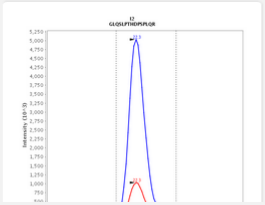
Peptide Standard Label Type
Panorama (what fieldname?)

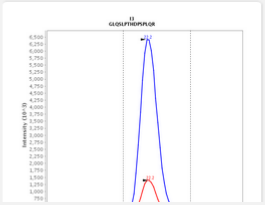
Parameter
ChromXP C18-CL, 3 um, 120A

Parameter
150 x 0.075 mm

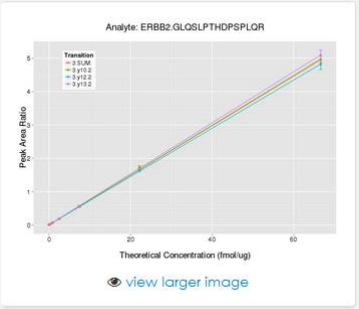
Parameter
500 nL / min

Chromatograms





Response Curves



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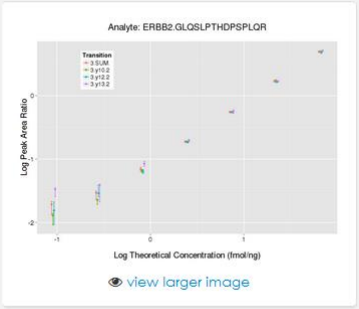

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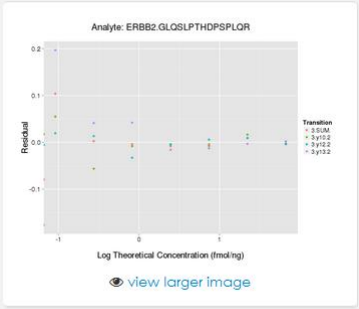


view larger image

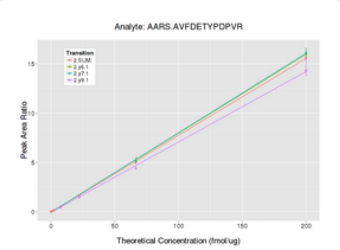
Peptide	Transition	LOD blank only	LOD blank+low-conc	LOD rsd limit	LLOQ blank only	LLOQ blank+low-conc	LLOQ rsd limit
GLQSLPTHDPSPLQR	Sum.tr	3.85	1.99	4.50	12.82	5.97	13.51
	3.y13.2	9.80	4.83	11.46	32.68	14.50	34.37
	3.y12.2	2.83	3.13	3.32	9.42	9.38	9.96
	3.y10.2	3.65	0.71	4.27	12.17	2.12	12.81

Assay characterization guidelines: Experiment 1 – response curves

- Multipoint response curve
- Used for determination of LOD, LLOQ, linearity
- Multiple replicates analyzed in matrix background

Response Curves

Data source: Panorama



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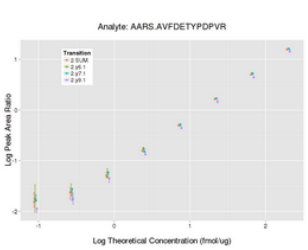

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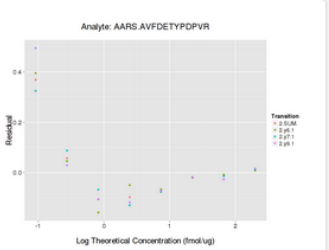

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		LOD (fmol/ug)			LLOQ (fmol/ug)		
Peptide	**Transition**	**blank only**	**blank+low-conc**	**rsd limit**	**blank only**	**blank+low-conc**	**rsd limit**
AVFDETYPDPVR	Sum.tr	2.5e-2	5.3e-2	1.6e-1	8.4e-2	1.6e-1	4.8e-1
	2.y9.1	3.6e-3	1.1e-2	3.0e-2	1.2e-2	3.3e-2	9.0e-2
	2.y7.1	3.6e-2	5.7e-2	9.2e-2	1.2e-1	1.7e-1	2.8e-1
	2.y6.1	3.4e-2	8.4e-2	2.8e-1	1.1e-1	2.5e-1	8.3e-1

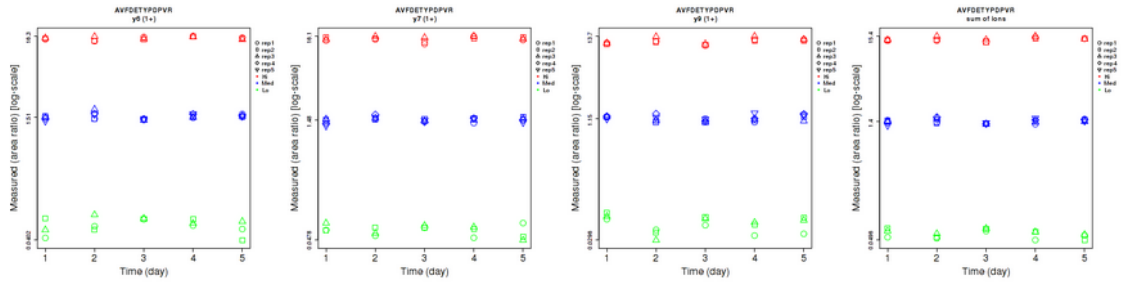
		Curve Fit		
Peptide	**Transition**	**slope**	**intercept**	**r squared**
AVFDETYPDPVR	Sum.tr	0.07	-0.00	1.00
	2.y6.1	0.08	-0.02	1.00
	2.y7.1	0.07	-0.00	1.00
	2.y9.1	0.06	-0.00	1.00

Assay characterization guidelines: Experiment 2 – Mini-validation of repeatability

- Assess repeatability by examining intra- and inter-day variability
- Use LLOQ from Experiment 1
- Evaluate over three concentrations, 5 days
- As close to sample process as possible

Repeatability

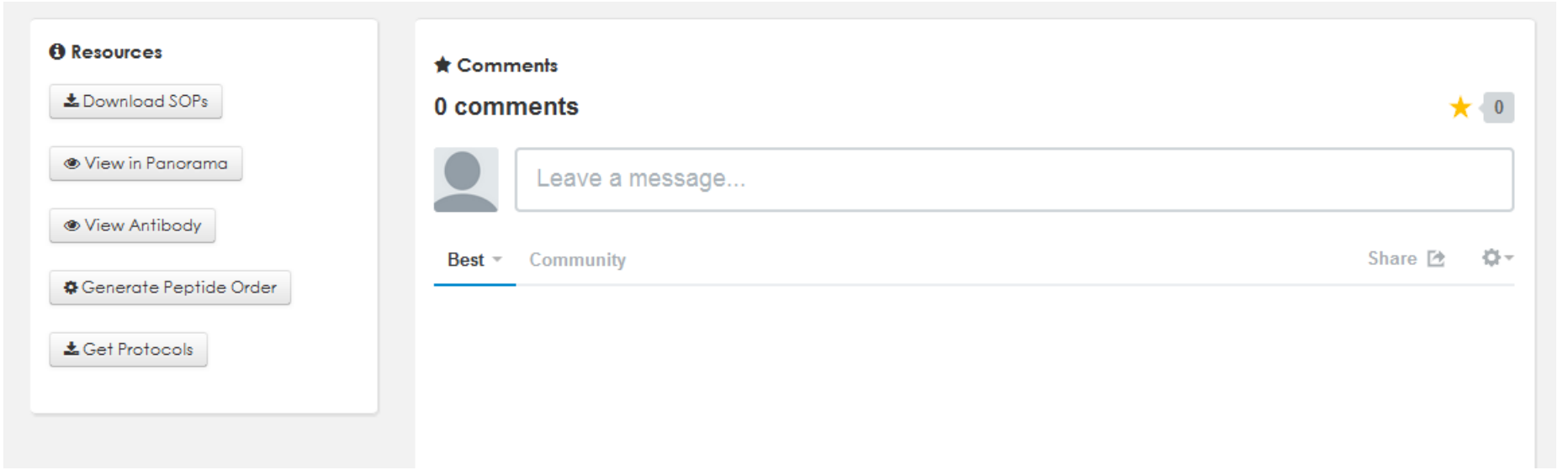
Data source: Panorama



view larger image

	Average intra-assay CV (within day CV)			Average inter-assay CV (between day CV)			Total CV $\sqrt{intraCV^2 + interCV^2}$			n=		
Fragment ion / Transition	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High
y6 (1+)	18.3	5.7	3.3	20.3	8	4.5	27.3	9.8	5.6	15	25	15
y7 (1+)	14	5.6	5.2	15.2	6.2	5.7	20.7	8.4	7.7	15	25	15
y9 (1+)	16.5	7.5	4.7	22.2	8.2	8	27.7	11.1	9.3	15	25	15
sum	8.9	4.7	3.1	10.4	6.3	4.8	13.7	7.9	5.7	15	25	15

Data downloads and commenting



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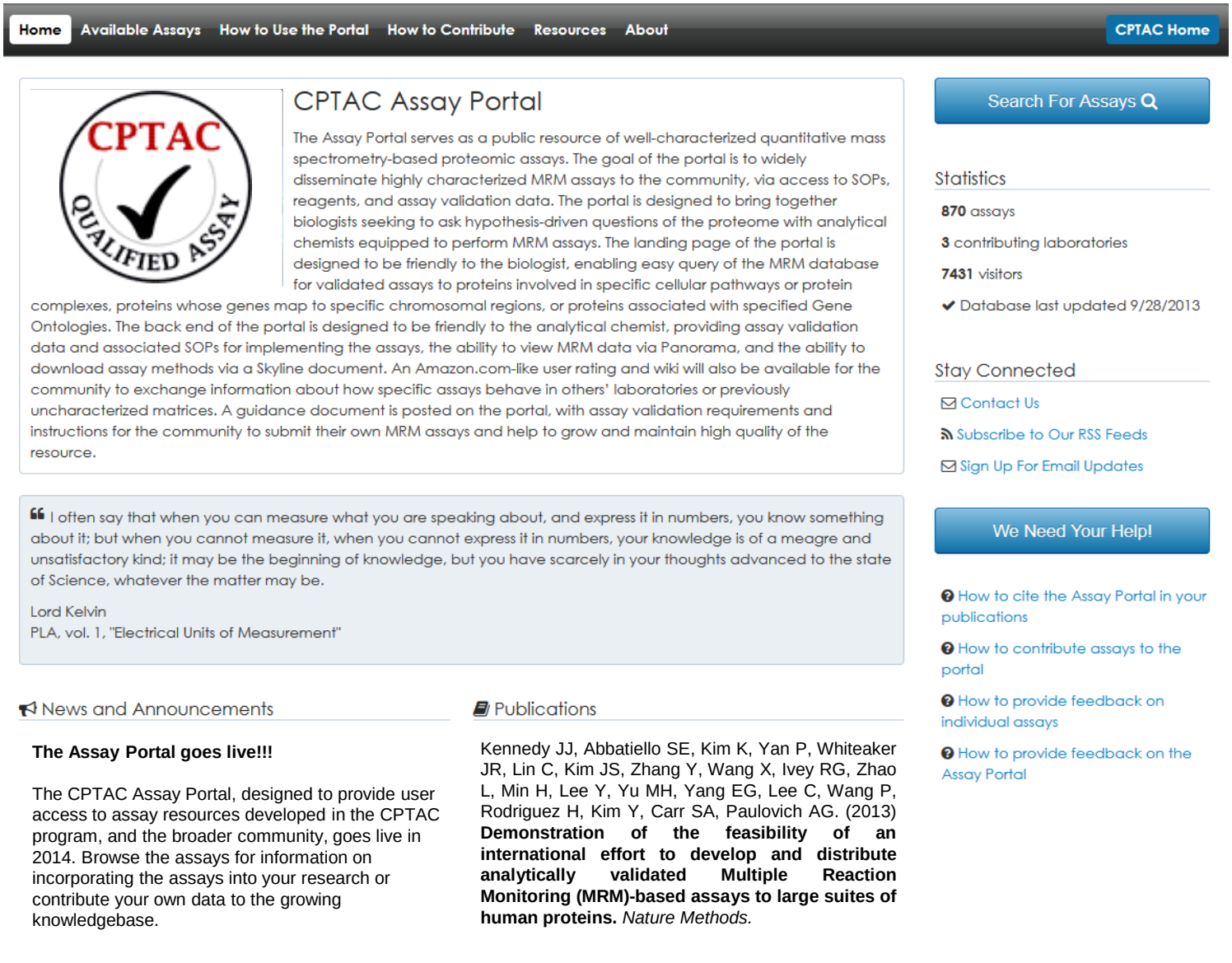
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QUALIFIED ASSAY
CPTAC Assay Portal
The Assay Portal serves as a public resource of well-characterized quantitative mass spectrometry-based proteomic assays. The goal of the portal is to widely disseminate highly characterized MRM assays to the community, via access to SOPs, reagents, and assay validation data. The portal is designed to bring together biologists seeking to ask hypothesis-driven questions of the proteome with analytical chemists equipped to perform MRM assays. The landing page of the portal is designed to be friendly to the biologist, enabling easy query of the MRM database for validated assays to proteins involved in specific cellular pathways or protein complexes, proteins whose genes map to specific chromosomal regions, or proteins associated with specified Gene Ontologies. The back end of the portal is designed to be friendly to the analytical chemist, providing assay validation data and associated SOPs for implementing the assays, the ability to view MRM data via Panorama, and the ability to download assay methods via a Skyline document. An Amazon.com-like user rating and wiki will also be available for the community to exchange information about how specific assays behave in others' laboratories or previously uncharacterized matrices. A guidance document is posted on the portal, with assay validation requirements and instructions for the community to submit their own MRM assays and help to grow and maintain high quality of the resource.
I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely in your thoughts advanced to the state of Science, whatever the matter may be.
Lord Kelvin
PLA, vol. 1, "Electrical Units of Measurement"
News and Announcements
The Assay Portal goes live!!!
The CPTAC Assay Portal, designed to provide user access to assay resources developed in the CPTAC program, and the broader community, goes live in 2014. Browse the assays for information on incorporating the assays into your research or contribute your own data to the growing knowledgebase.
Publications
Kennedy JJ, Abbatiello SE, Kim K, Yan P, Whiteaker JR, Lin C, Kim JS, Zhang Y, Wang X, Ivey RG, Zhao L, Min H, Lee Y, Yu MH, Yang EG, Lee C, Wang P, Rodriguez H, Kim Y, Carr SA, Paulovich AG. (2013) Demonstration of the feasibility of an international effort to develop and distribute analytically validated Multiple Reaction Monitoring (MRM)-based assays to large suites of human proteins. Nature Methods.
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