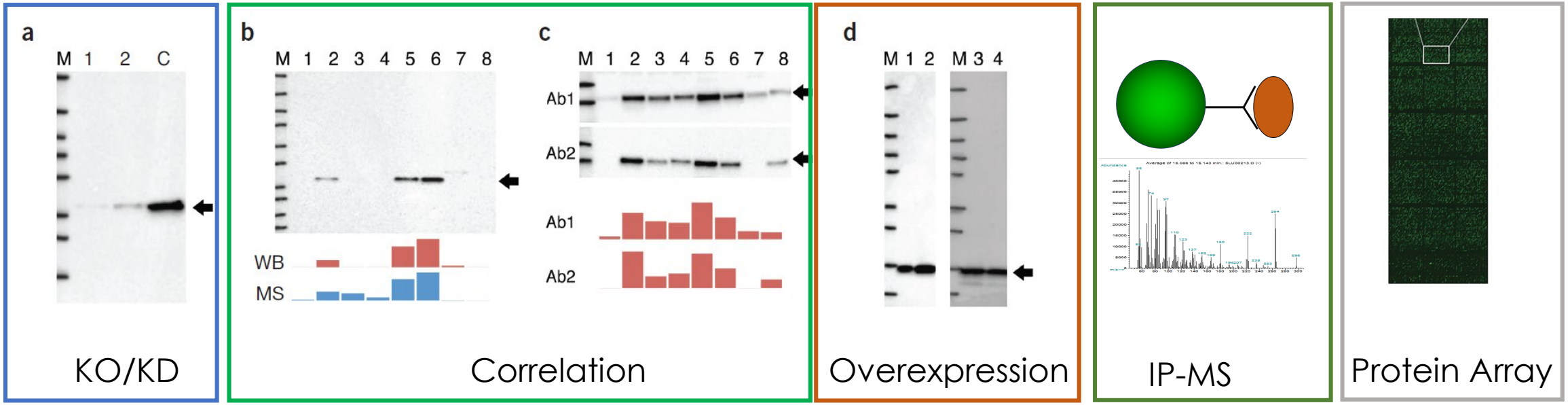
Immunoprecipitation-Mass Spectrometry in Validation

The Antibody Society Webcast series – Antibody Validation #8

Fridtjof Lund-Johansen Oslo University Hospital

ANTIBODY SOCIETY

"Pillars"



A proposal for validation of antibodies

Mathias Uhlen[1], Anita Bandrowski[2], Steven Carr[3], Aled Edwards[4], Jan Ellenberg[5], Emma Lundberg[1], David L Rimm[6], Henry Rodriguez[7], Tara Hiltke[7], Michael Snyder[8] & Tadashi Yamamoto[9]

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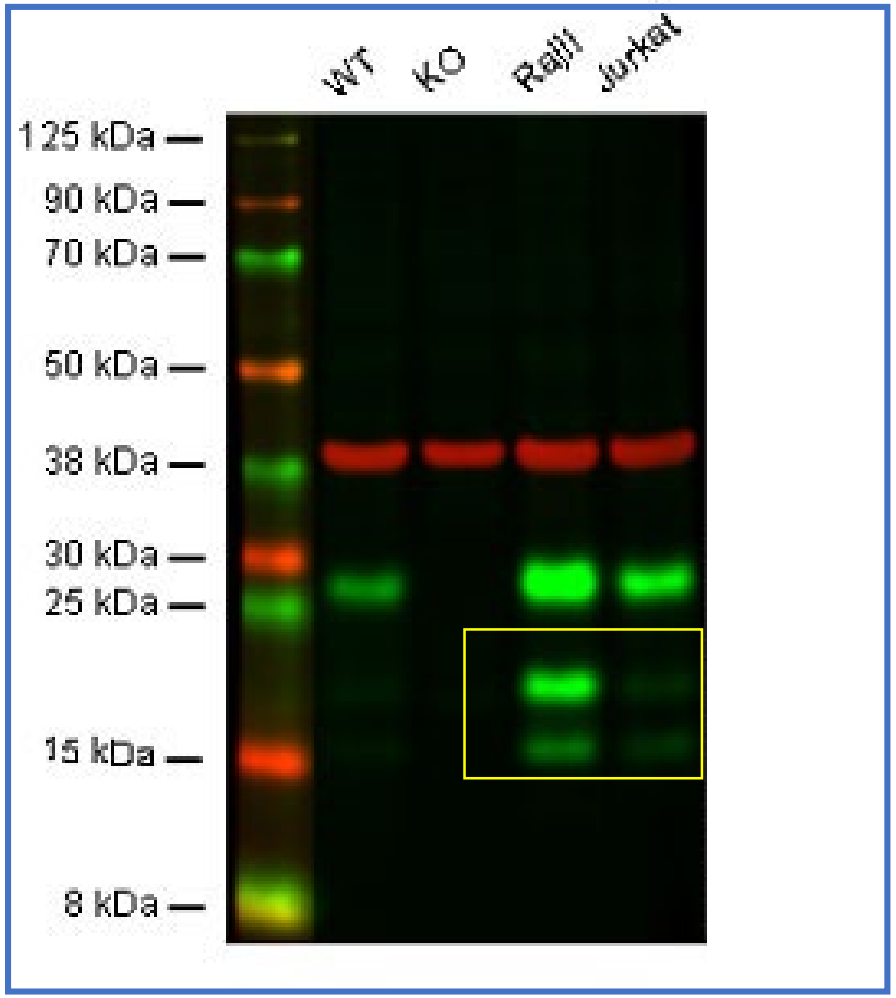
Knockout: is Consensus

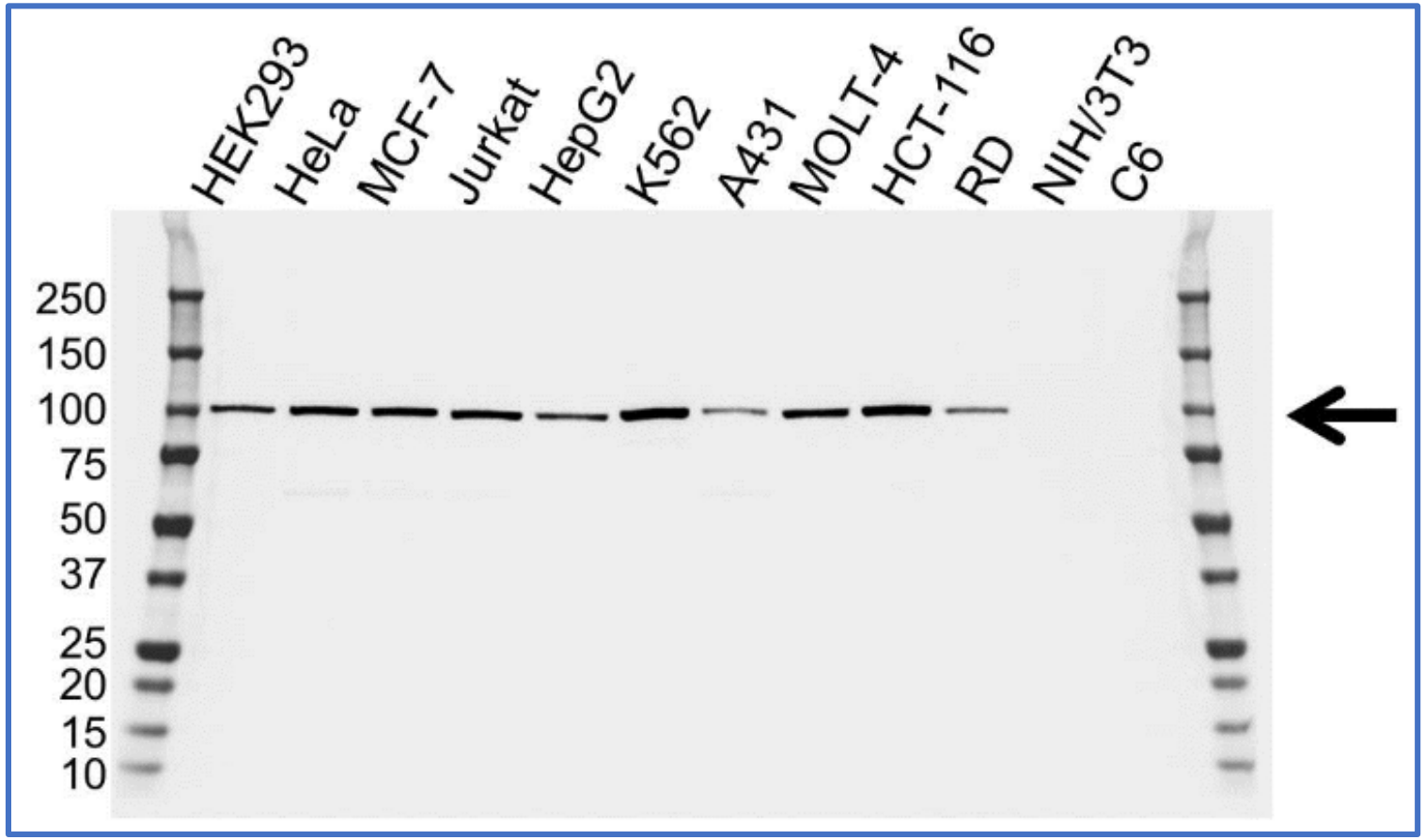


BUT:
The presence of extra bands is often sample-dependent

abcam®

ANTIBODY SOCIETY

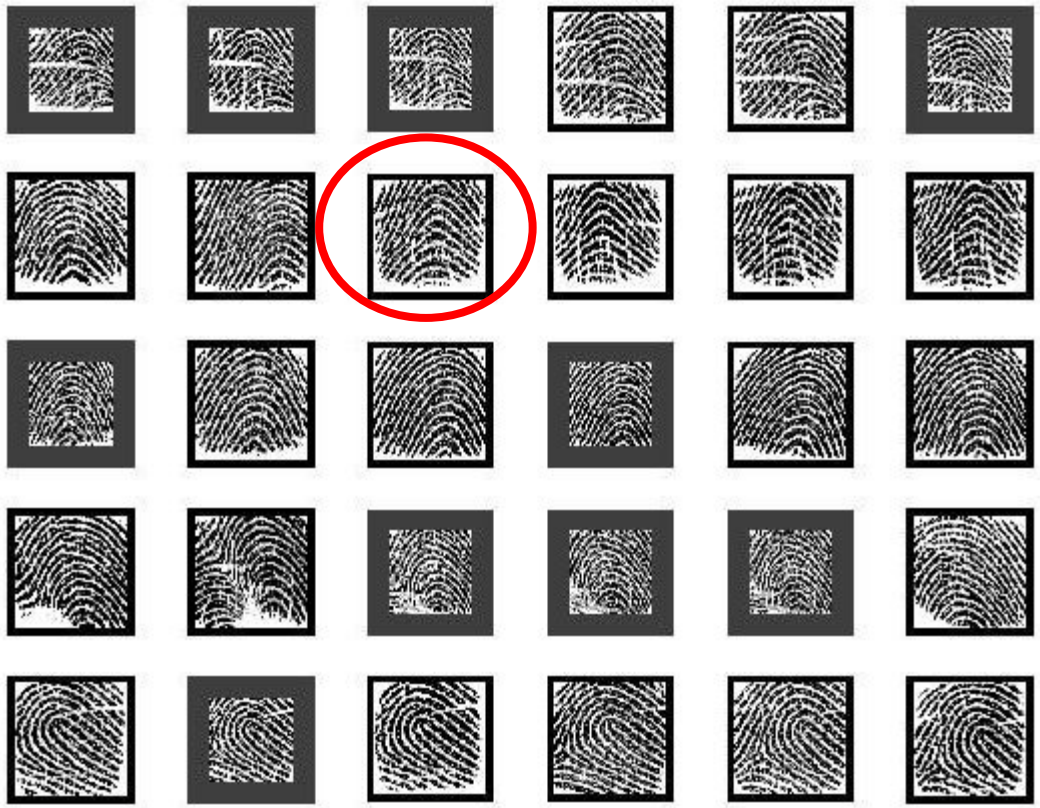
Knockout:
Should be combined with testing many samples



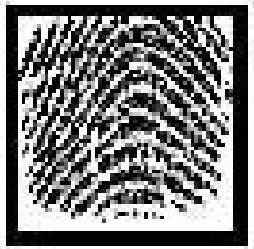
BIO-RAD

Correlation:
Analogous to fingerprint matching

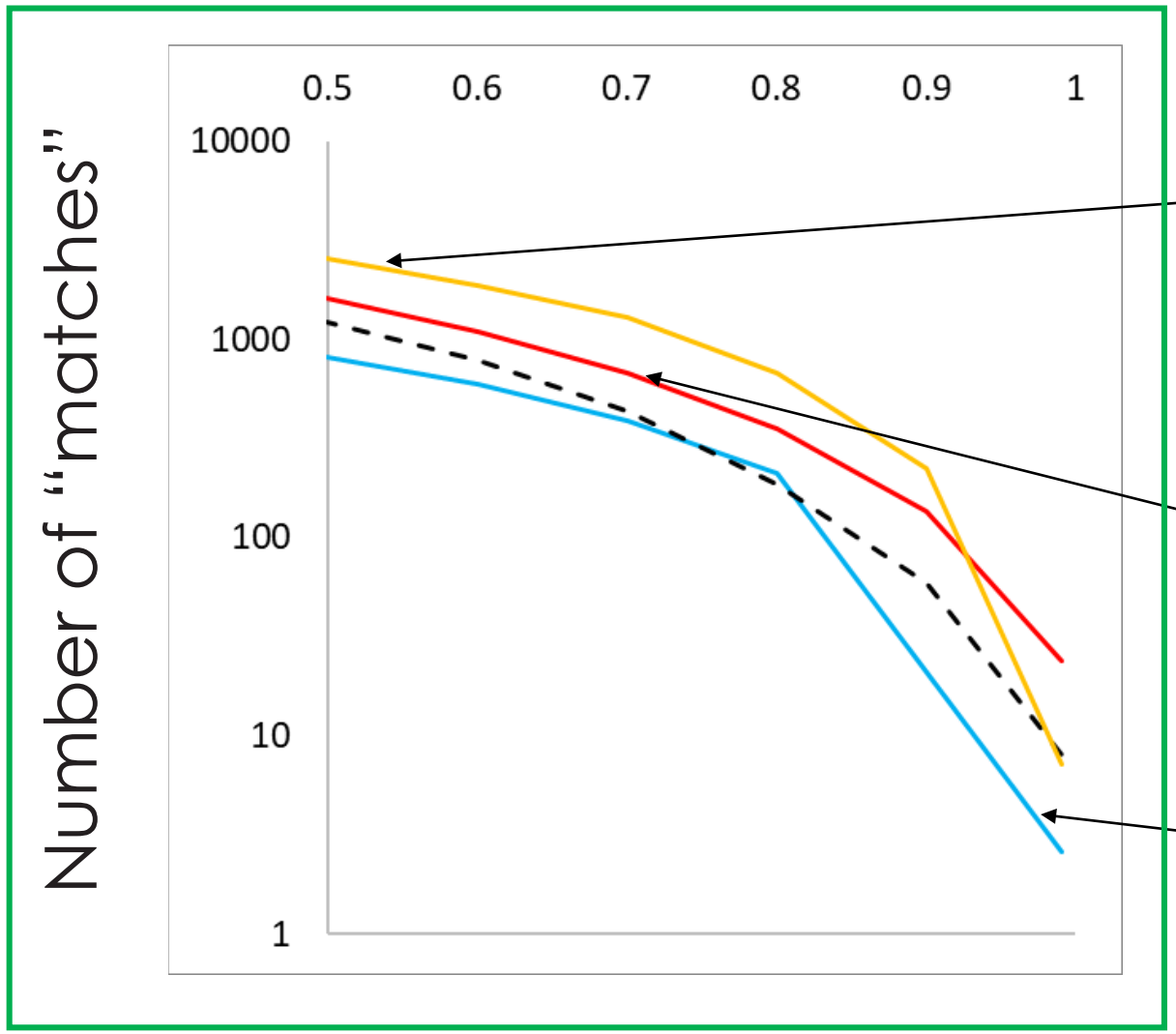
Mass spec data for sample series



Staining across
Series of samples



Correlation:
Currently too many matches for MS data



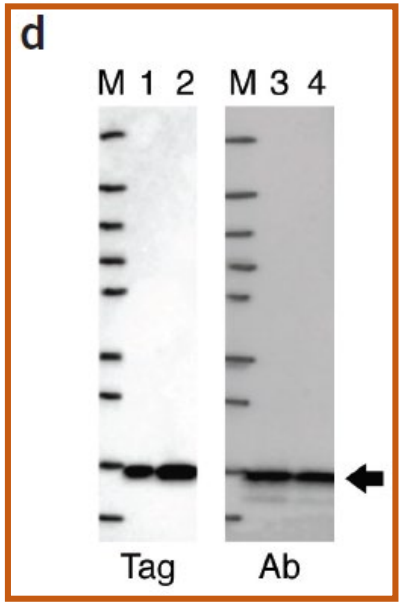
Edfors et al. :
MS data 8 cell lines
Nature Communications 2018

Sikorski et al
MS data
Nature Methods 2018

Sikorski et al
Antibody pairs
Nature Methods 2018

ANTIBODY SOCIETY

Overexpression: Typically validates antibodies that fail on realistic expression models



Research Article

Biotechnology Journal

www.biotechnology-journal.com

2014

Antibody performance in western blot applications is context-dependent

Cajsa Älgenäs[1], Charlotta Agaton[2], Linn Fagerberg[3], Anna Asplund[4], Lisa Björling[3], Erik Björling[1], Caroline Kampf[4], Emma Lundberg[3], Peter Nilsson[3], Anja Persson[1], Kenneth Wester[4], Fredrik Pontén[4], Henrik Wernérus[2], Mathias Uhlén[3], Jenny Ottosson Takanen[1] and Sophia Hober[5,]*

82% of 1544 antibodies failed WB of endogenously expressed proteins but passed "validation" on *over-expressed* lysates.

ANTIBODY SOCIETY

IP-MS: Strong evidence for on-target binding. Off-target binding difficult to assess

NATURE METHODS
2015

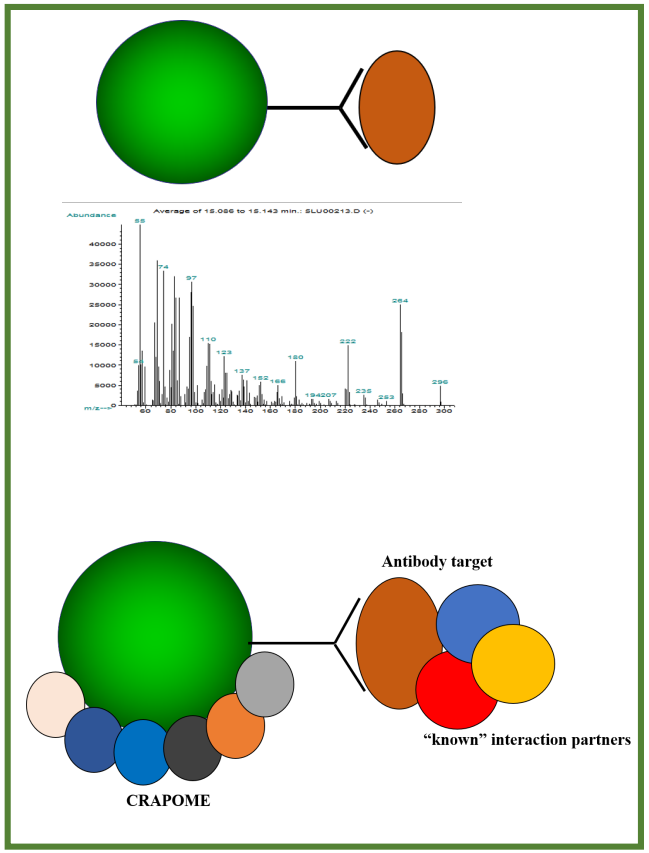
Assessment of a method to characterize antibody selectivity and specificity for use in immunoprecipitation

Edyta Marcon[1], Harshika Jain[2], Anandi Bhattacharya[2], Hongbo Guo[1], Sadhna Phanse[1], Shuye Pu[1,3], Gregory Byram[4], Ben C Collins[5], Evan Dowdell[6], Maria Fenner[2], Xinghua Guo[1], Ashley Hutchinson[2], Jacob J Kennedy[7], Bryan Krastins[4], Brett Larsen[8], Zhen-Yuan Lin[8], Mary F Lopez[4], Peter Loppnau[2], Shane Miersch[1], Tin Nguyen[9], Jonathan B Olsen[9], Marcin Paduch[6], Mani Ravichandran[2], Alma Seitova[2], Gouri Vadali[4], Maryann S Vogelsang[4], Jeffrey R Whiteaker[7], Guoqing Zhong[1], Nan Zhong[2], Lei Zhao[7], Ruedi Aebersold[5,10,11], Cheryl H Arrowsmith[2], Andrew Emili[1,9], Lori Frappier[9], Anne-Claude Gingras[8,9], Matthias Gstaiger[5,10], Amanda G Paulovich[7], Shohei Koide[6], Anthony A Kossiakoff[6], Sachdev S Sidhu[1], Shoshana J Wodak[3,9,12], Susanne Gräslund[2], Jack F Greenblatt[1,9] & Aled M Edwards[2]

Antibody evaluation

IP gold standard: intended antigen places in top three most abundant proteins

Inconclusive: intended antigen below top three most abundant proteins



Protein arrays:
We don't know yet.....

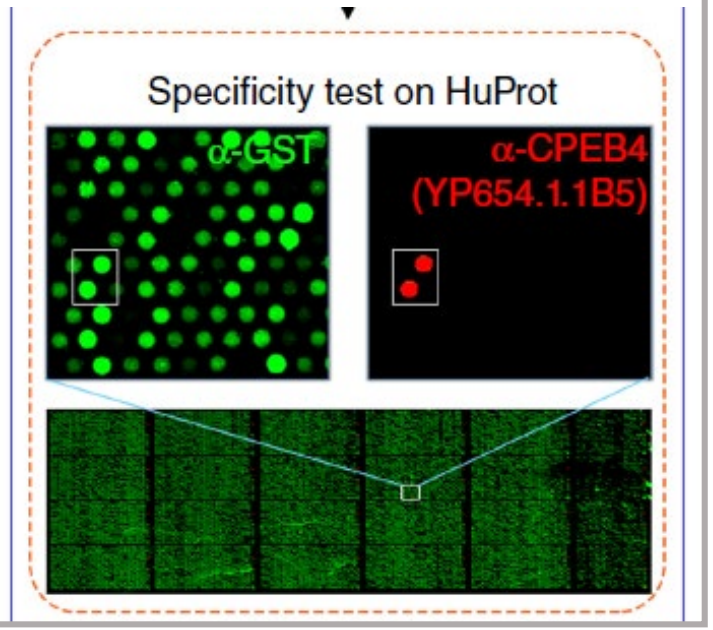
NATURE METHODS

2018

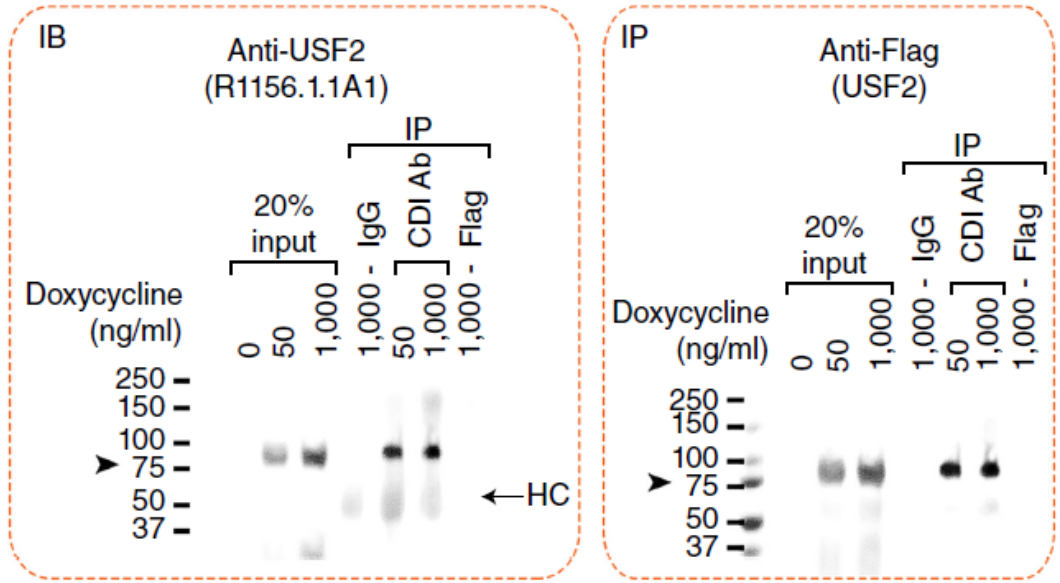
A toolbox of immunoprecipitation-grade monoclonal antibodies to human transcription factors

Anand Venkataraman[1], Kun Yang[2], Jose Irizarry[3], Mark Mackiewicz[4], Paolo Mita[5–7], Zheng Kuang[6,19], Lin Xue[1], Devlina Ghosh[1], Shuang Liu[8], Pedro Ramos[3], Shaohui Hu[3], Diane Bayron Kain[3,19], Sarah Keegan[5,6], Richard Saul[8], Simona Colantonio[9], Hongyan Zhang[8], Florencia Pauli-Behn[4], Guang Song[8], Edisa Albino[3], Lillyann Asencio[3], Leonardo Ramos[3], Luvir Lugo[3], Gloriner Morell[3], Javier Rivera[3], Kimberly Ruiz[3], Ruth Almodovar[3], Luis Nazario[3], Keven Murphy[3], Ivan Vargas[3], Zully Ann Rivera-Pacheco[3], Christian Rosa[3], Moises Vargas[3], Jessica McDade[7], Brian S Clark[1], Sooyeon Yoo[1], Seva G Khambadkone[10], Jimmy de Melo[1], Milanka Stevanovic[1], Lizhi Jiang[1], Yana Li[11], Wendy Y Yap[3], Brittany Jones[12], Atul Tandon[12], Elliot Campbell[13,14], Gaetano T Montelione[13,14], Stephen Anderson[13,14], Richard M Myers[4], Jef D Boeke[5–7], David Fenyö[5,6], Gordon Whiteley[9], Joel S Bader[2], Ignacio Pino[3], Daniel J Eichinger[3], Heng Zhu[8,15] & Seth Blackshaw[1,15–18]

Primary screening



"Validation" on overexpressed lysates



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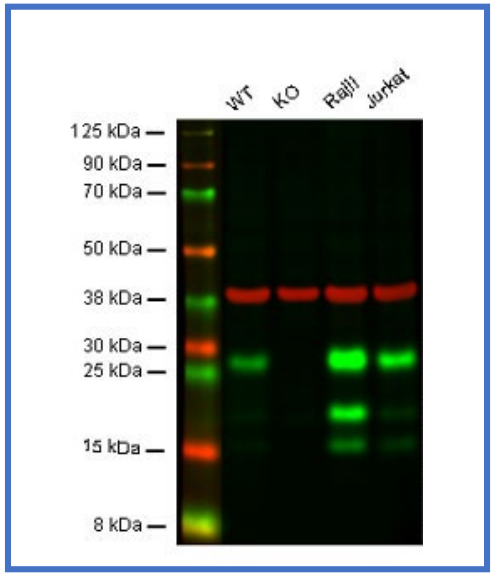
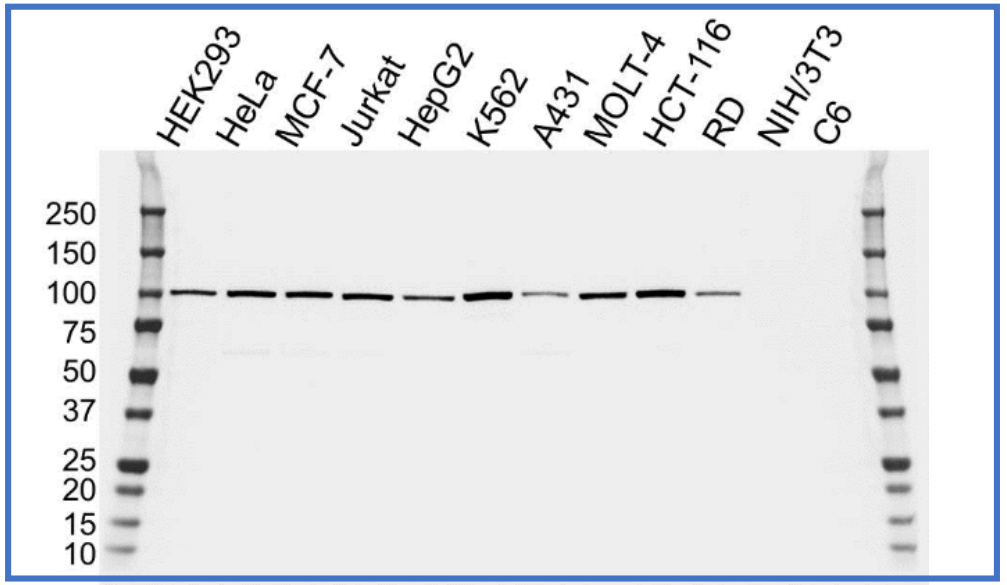
Consensus:
Knockout - BUT - Throughput? Cost?



+



+

IP-MS
IF
IHC

eLIFE

Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72

Carl Laflamme[1], Paul M McKeever[2,3], Rahul Kumar[1], Julie Schwartz[1], Mahshad Kolahdouzan[4], Carol X Chen[1], Zhipeng You[1], Faiza Benaliouad[1], Opher Gileadi[5], Heidi M McBride[1], Thomas M Durcan[1], Aled M Edwards[1,6], Luke M Healy[4], Janice Robertson[2,3], Peter S McPherson[1]*

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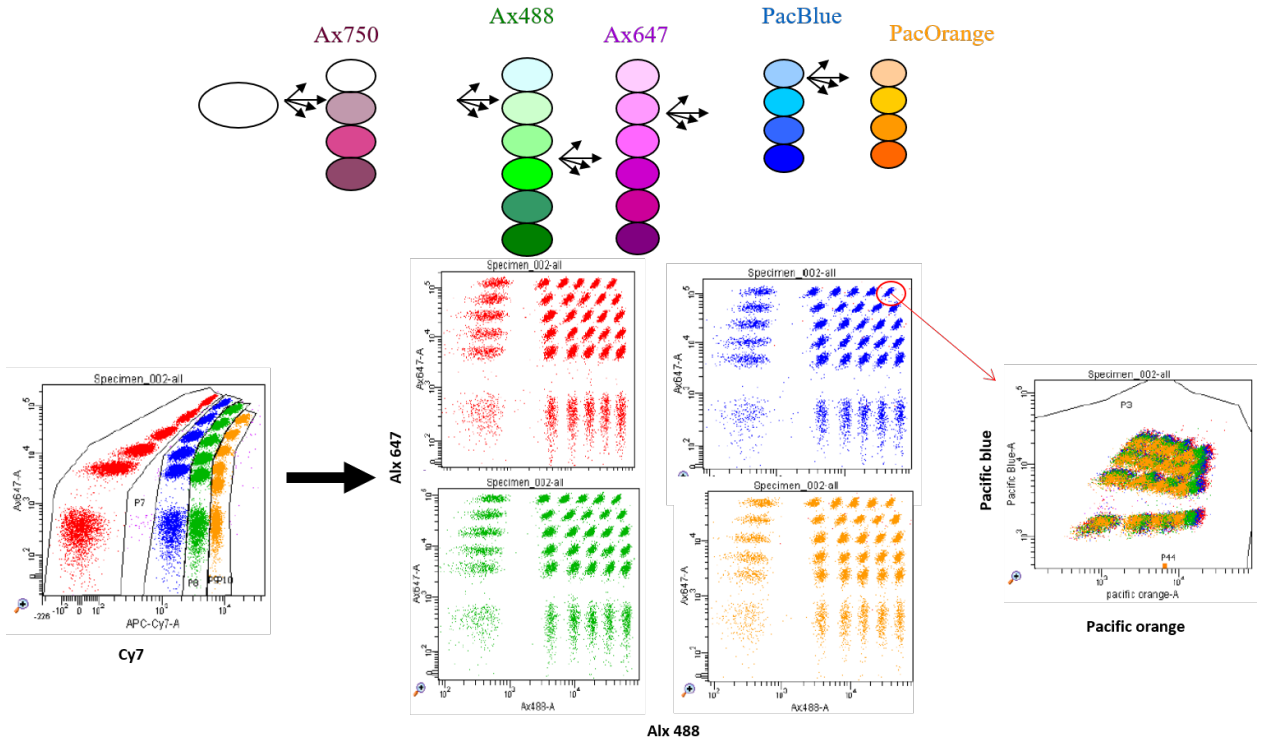
>2 M Antibodies:
How do we screen the whole haystack?

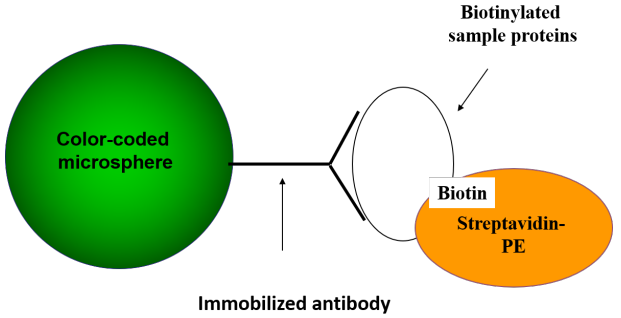
Parallel testing of thousands of antibodies



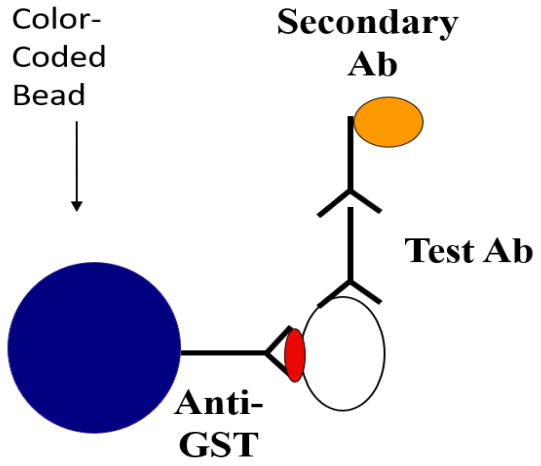
nature methods
BRIEF COMMUNICATION
https://doi.org/10.1038/s41592-018-0179-8

A high-throughput pipeline for validation of antibodies

Krzysztof Sikorski[1,2], Adi Mehta[1,3,4], Marit Inngjerdingen[1], Flourina Thakor[3,4], Simon Kling[5], Tomas Kalina[6], Tuula A. Nyman[1], Maria Ekman Stensland[1], Wei Zhou[7], Gustavo A. de Souza[8], Lars Holden[9], Jan Stuchly[6], Markus Templin[5] and Fridtjof Lund-Johansen[1,2]*



Antibody arrays



Protein arrays

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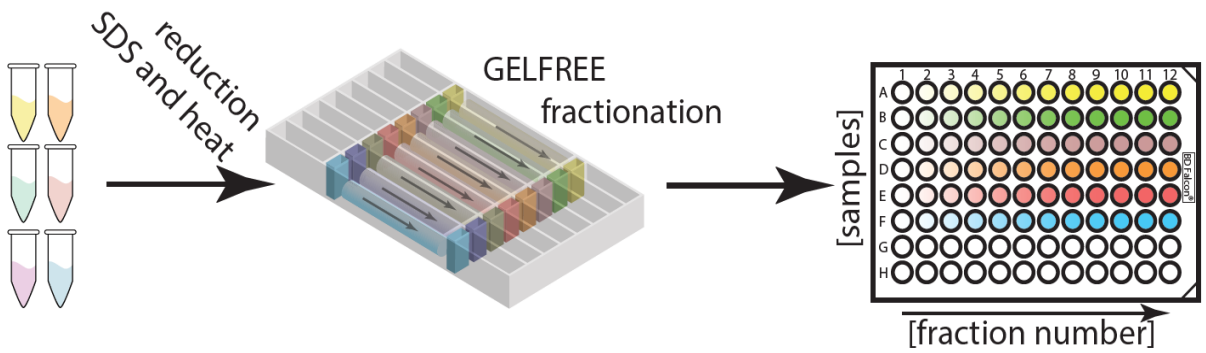
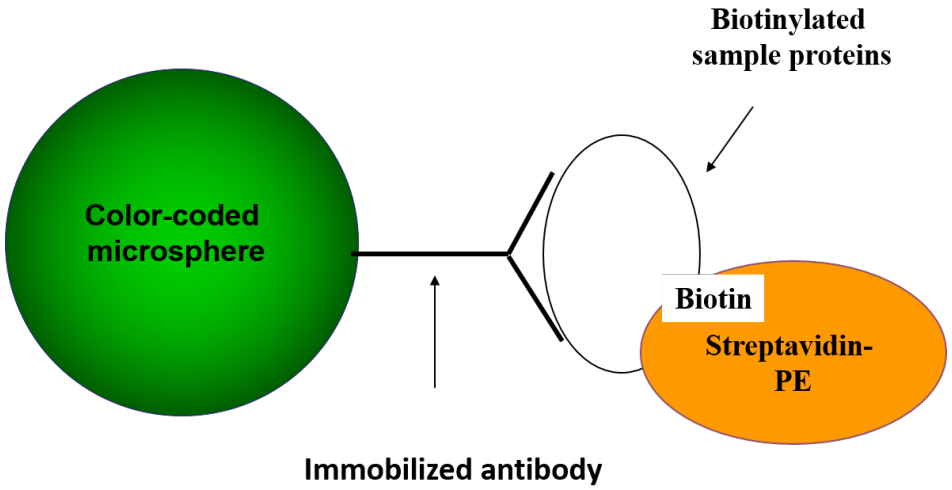
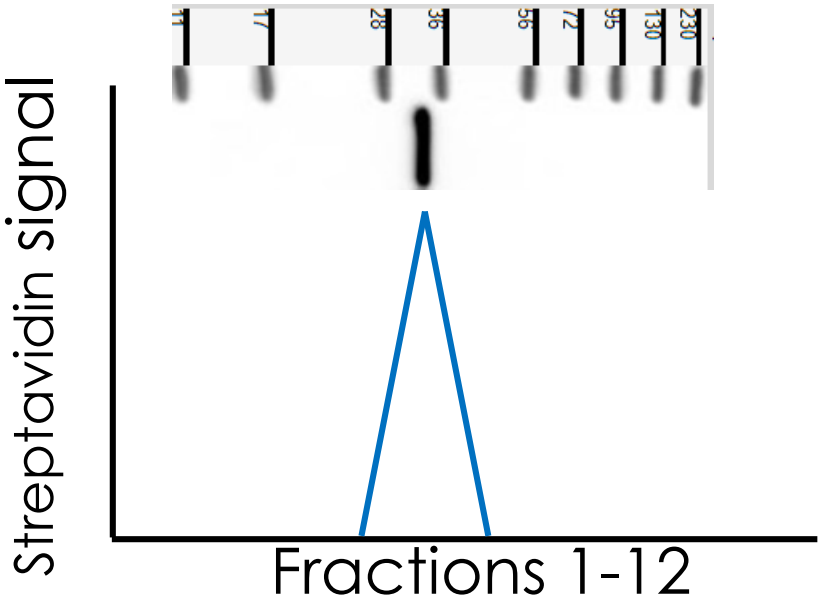
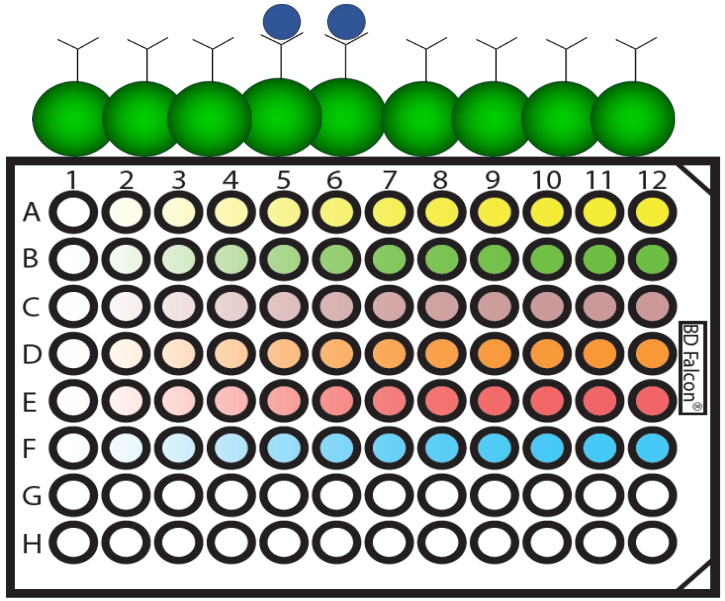
Western-MAP: "Capture WB"

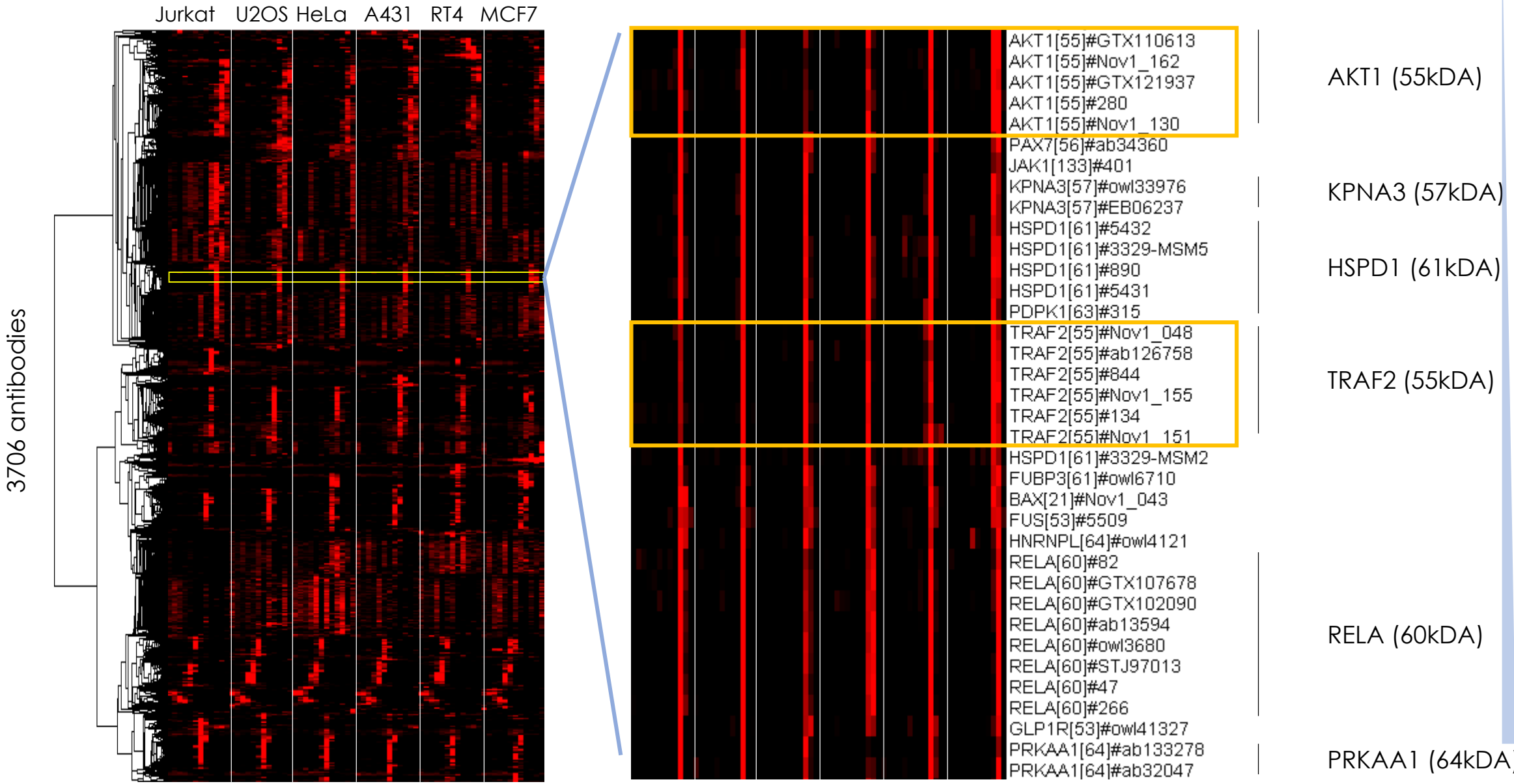




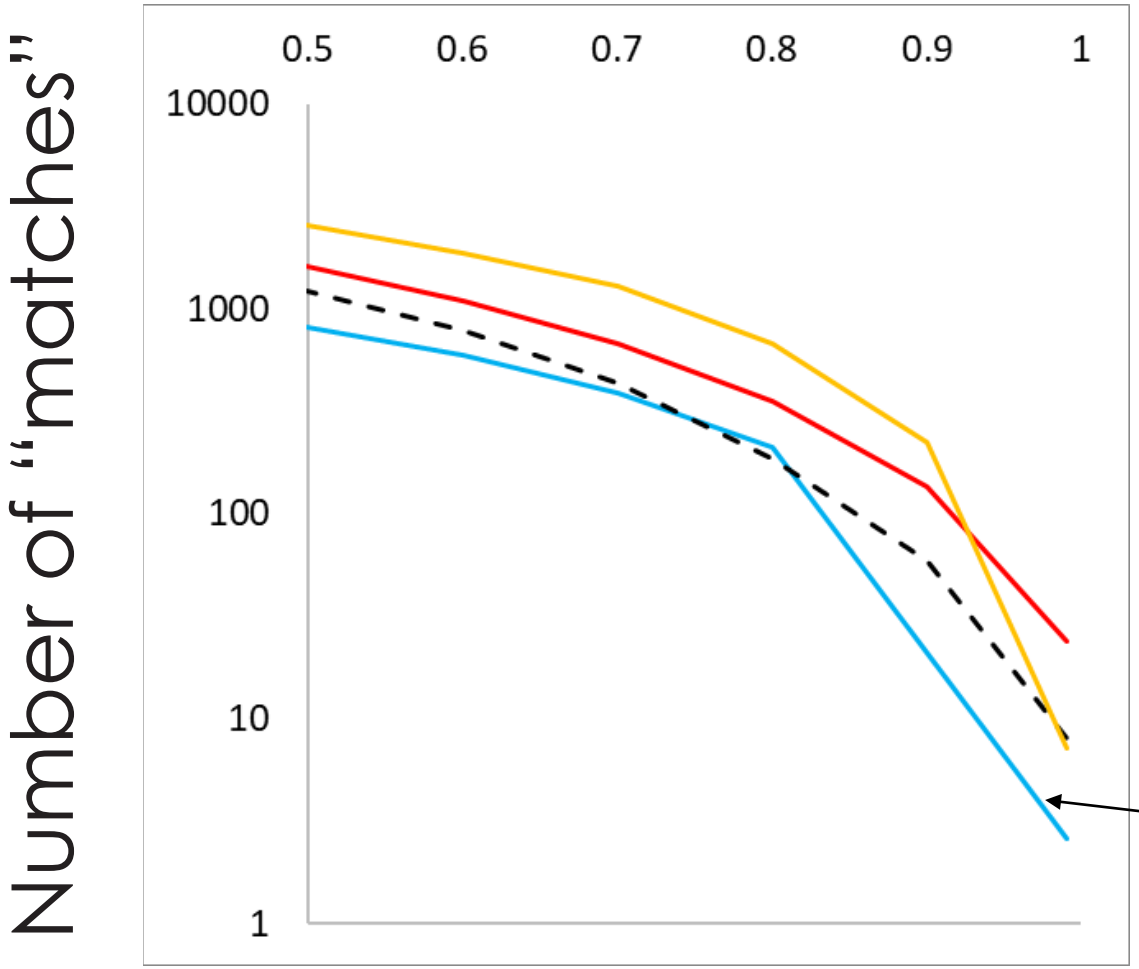




Indirect validation by correlation/clustering: r=0.95



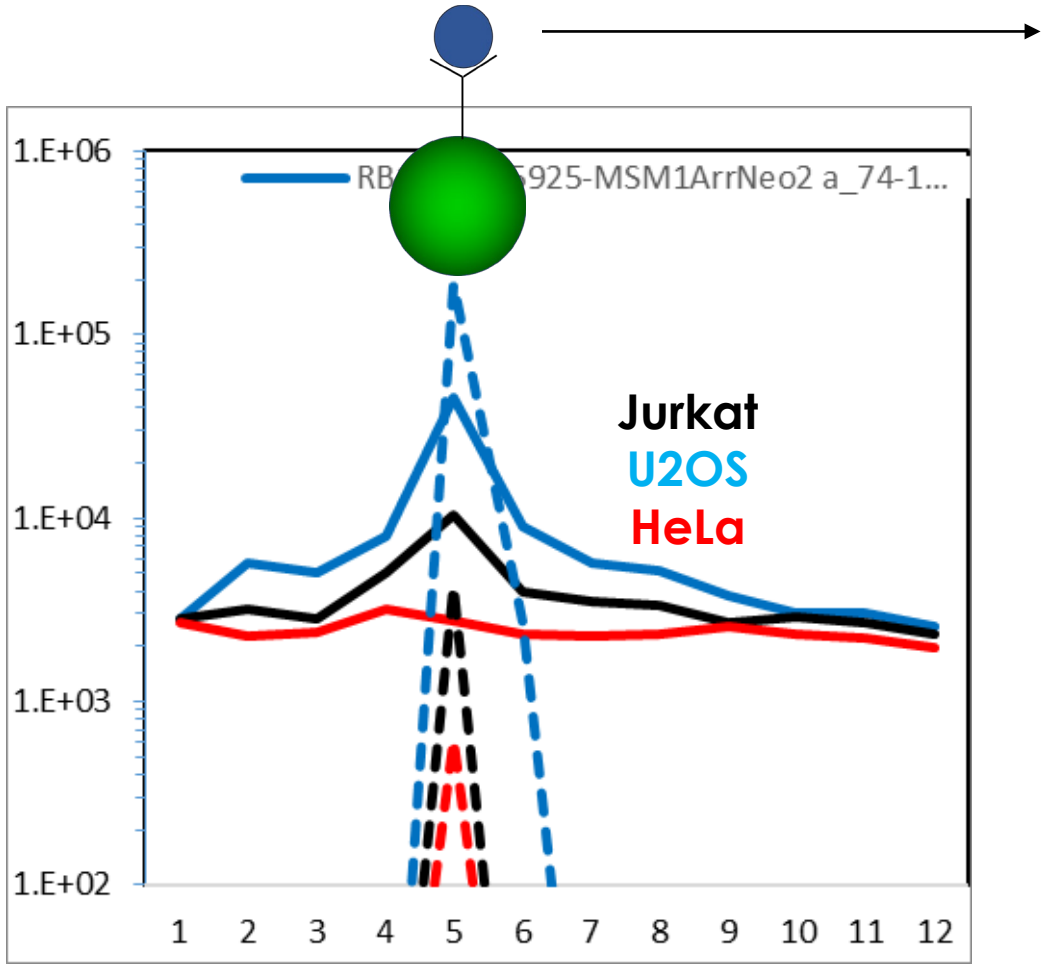
Correlation:
R=0.95 is good but not definitive

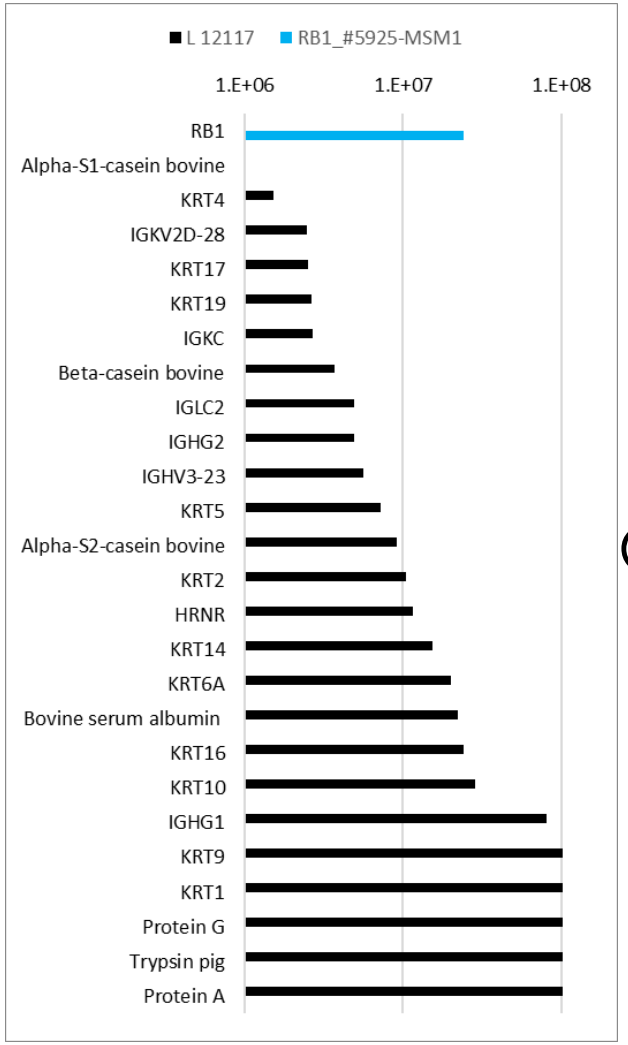


Sikorski et al
Antibody pairs
Nature Methods 2018

ANTIBODY SOCIETY

Direct specificity assessment by IP-MS

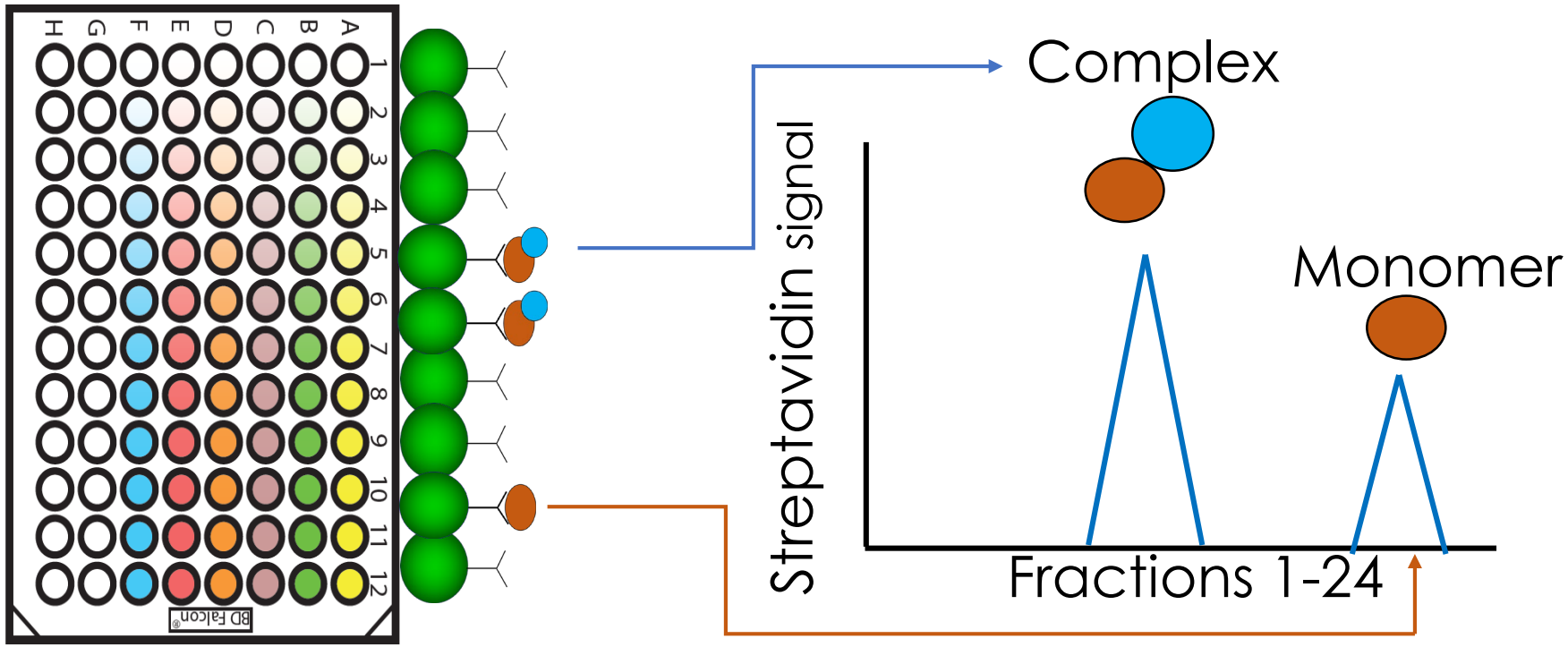




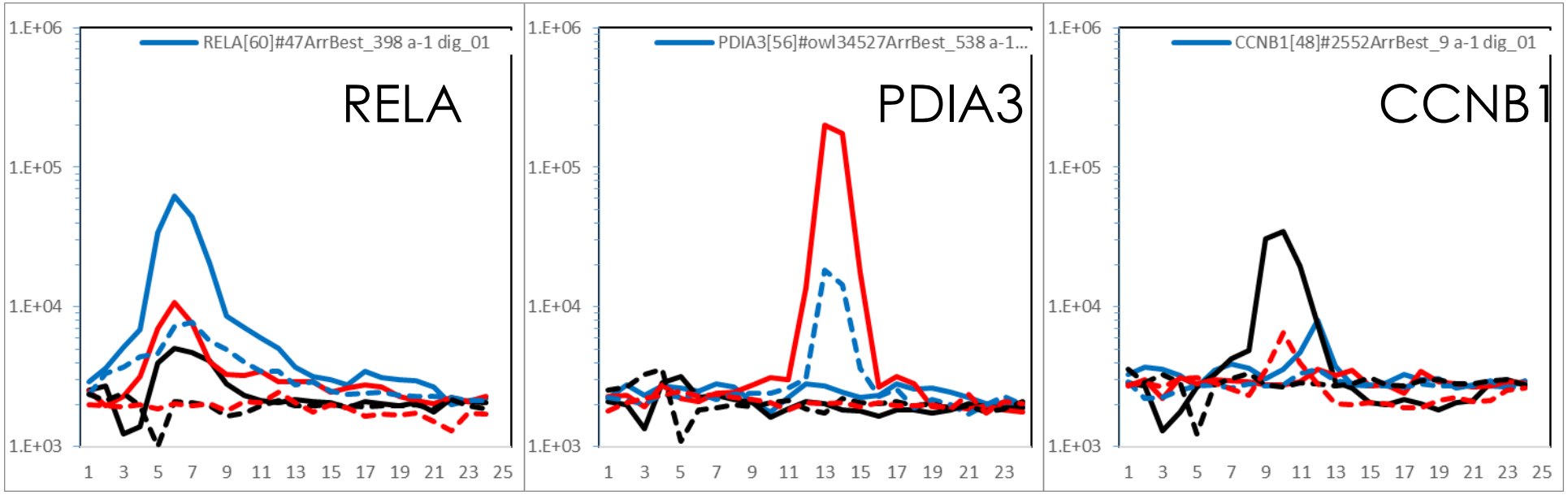
Sample

Contaminants

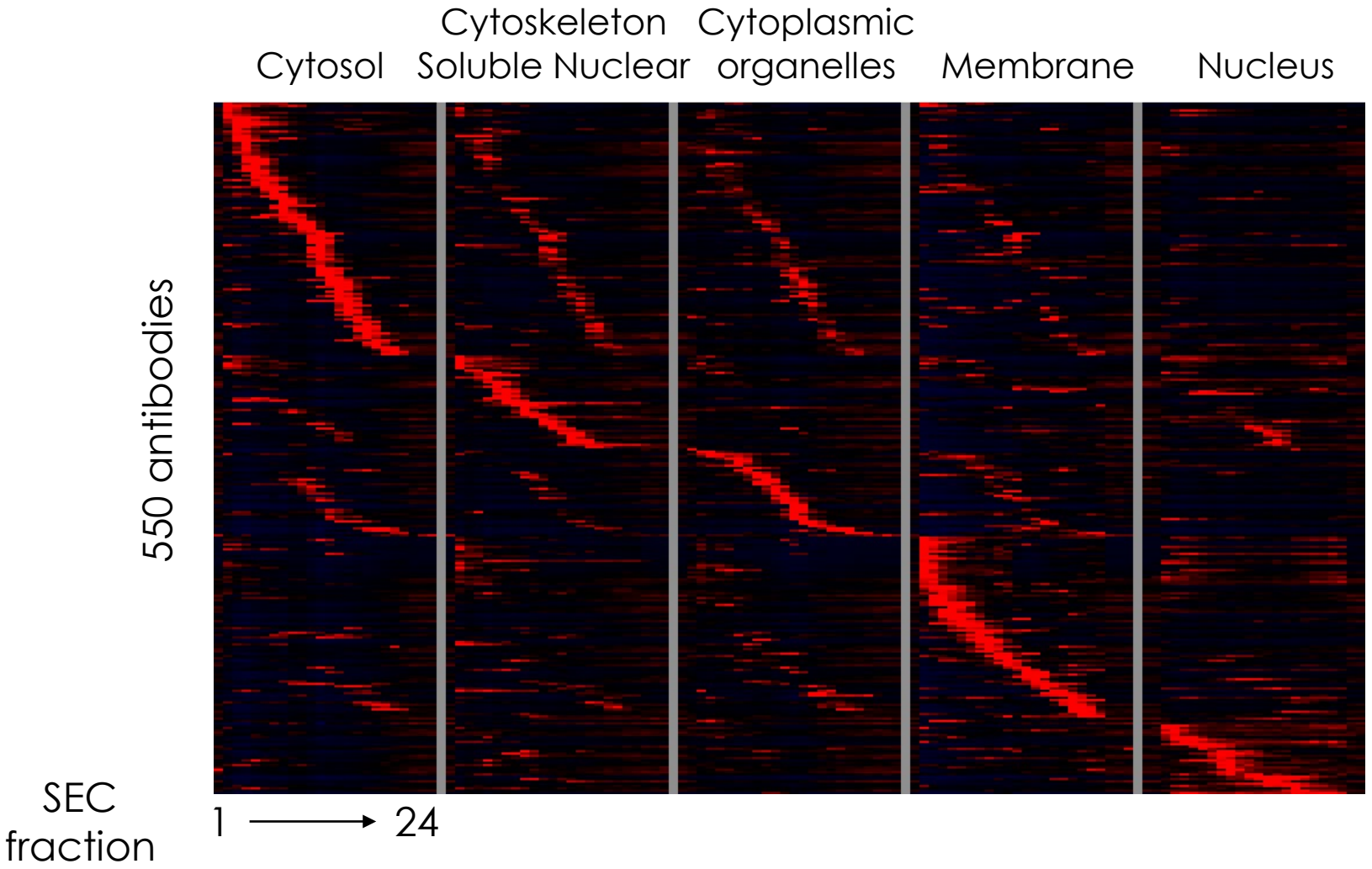
Native-MAP : Subcellular fractionation + Size Exclusion Chromatography



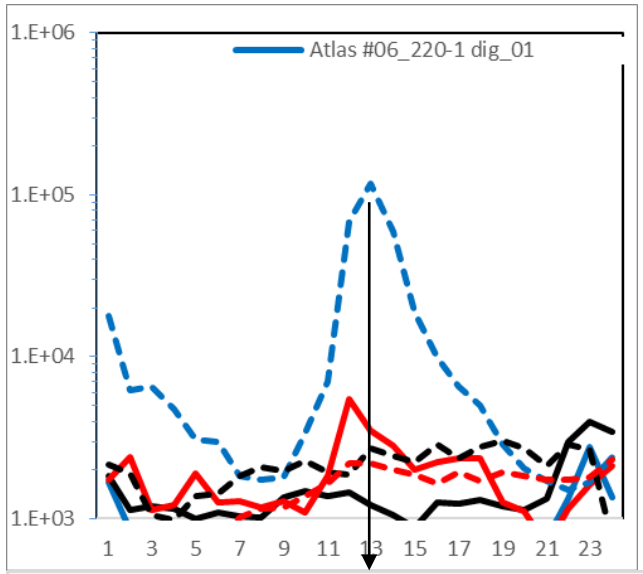
Chromatograms for individual antibodies

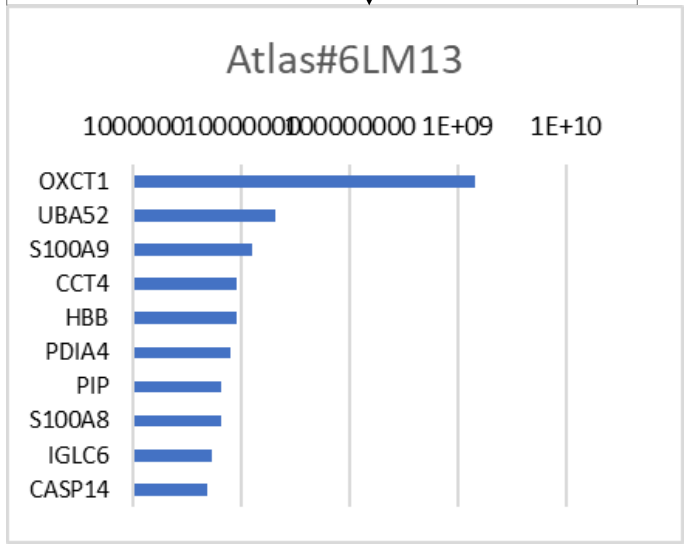


Chromatograms for the targets of hundreds of antibodies



Native



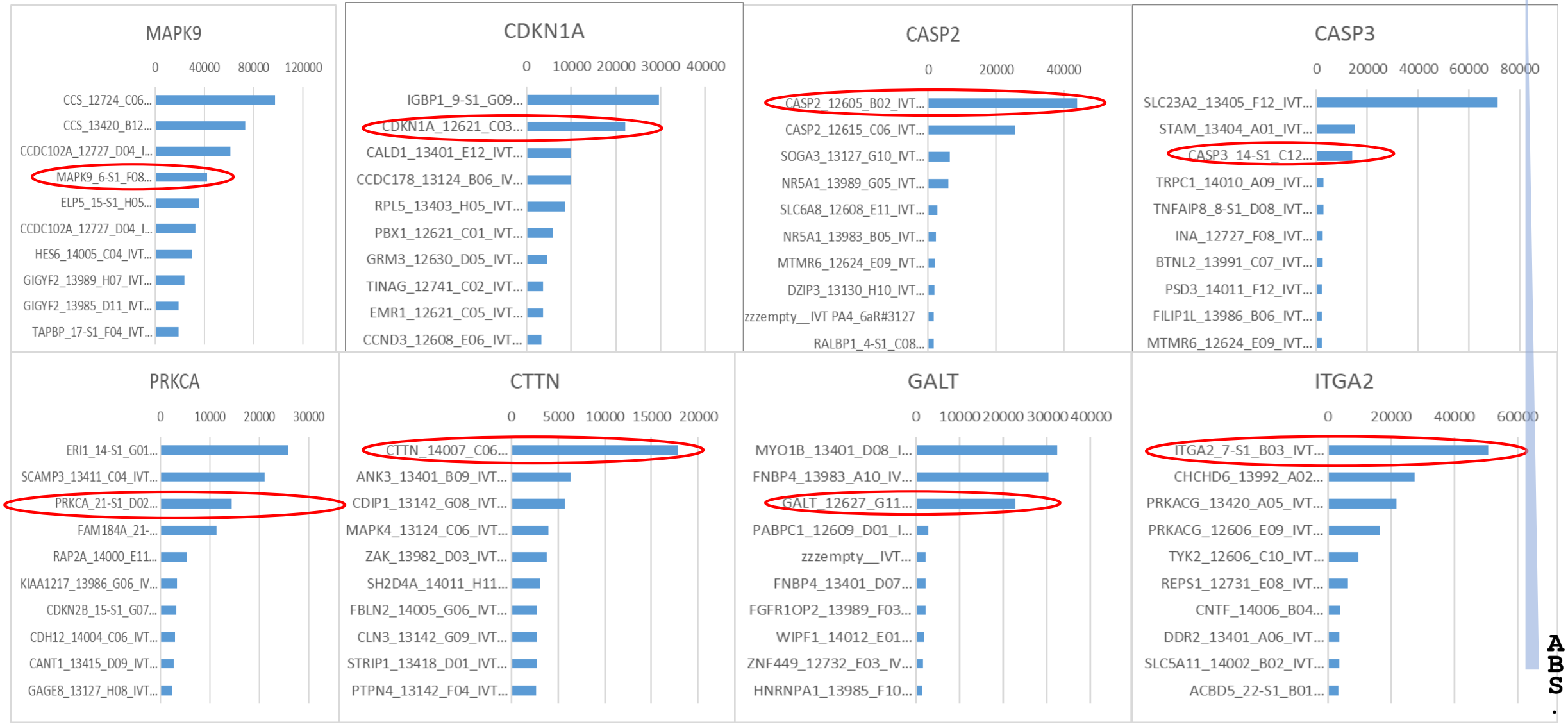


Cytosol
Cytoskeleton
Cytoplasmic organelles
Membrane
Nucleus
Pellet

IP-MS

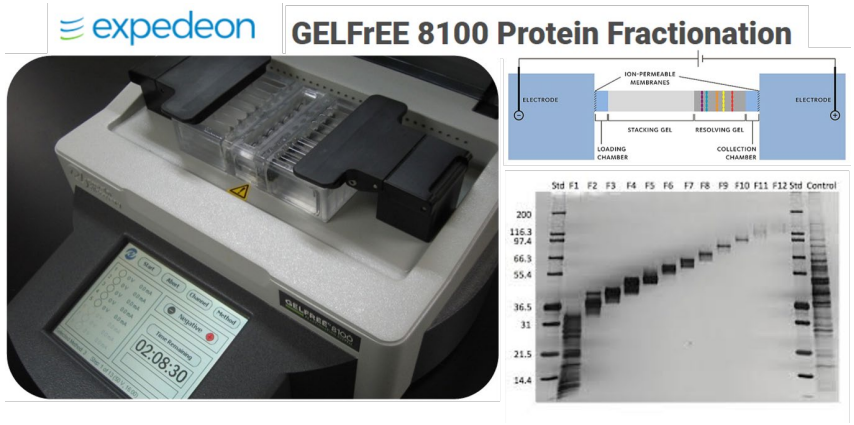
ANTIBODY SOCIETY

Protein arrays: Intended target usually in the top five top-ranked, But mono-specificity rare even for antibodies that stain single bands on WB.

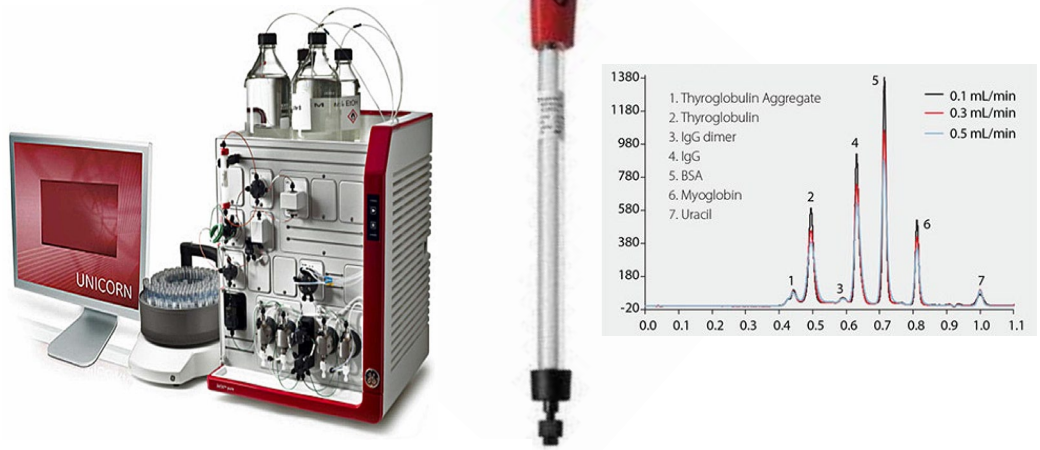


Antibody array pipeline

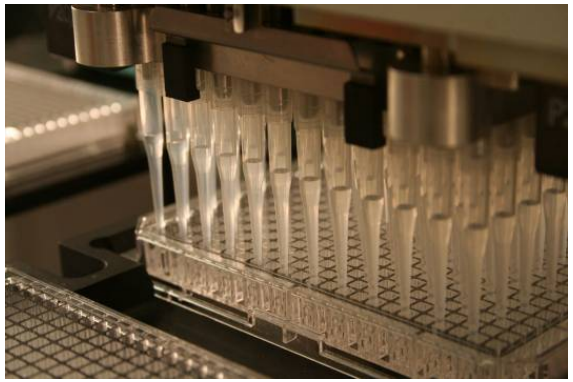
Denatured



Native



Liquid handling robotics



Automated flow cytometry

Mass spectrometry

ANTIBODY SOCIETY

Immunoprecipitation-Mass Spectrometry in Validation

The Antibody Society Webcast series – Antibody Validation #8

Fridtjof Lund-Johansen Oslo University Hospital

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