Rapid Identification of Monospecific Monoclonal Antibodies Using a Human Proteome Microarray*

Jun Seop Jeong, a Lizhi Jiang, b Edisa Albino, c Josean Marrero, c Hee Sool Rho, a Jianfei Hu, d Shaohui Hu, a Carlos Vera, c Diane Bayron-Poueymiroy, c Zully Ann Rivera-Pacheco, c Leonardo Ramos, c Cecil Torres-Castro, c Jiang Qian, d Joseph Bonaventura, c Jef D. Boeke, p Wendy Y. Yap, f Ignacio Pino, c,g Daniel J. Eichinger, h,i Heng Zhu, a,f,j and Seth Blackshaw b,d,f,k,l,m

To broaden the range of tools available for proteomic research, we generated a library of 16,368 unique full-length human ORFs that are expressible as N-terminal GST-His6 fusion proteins. Following expression in yeast, these proteins were then individually purified and used to construct a human proteome microarray. To demonstrate the usefulness of this reagent, we developed a streamlined strategy for the production of monospecific monoclonal antibodies that used immunization with live human cells and microarray-based analysis of antibody specificity as its central components. We showed that microarray-based analysis of antibody specificity can be performed efficiently using a two-dimensional pooling strategy. We also demonstrated that our immunization and selection strategies result in a large fraction of monospecific monoclonal antibodies that are both immunoblot and immunoprecipitation grade. Our data indicate that the pipeline provides a robust platform for the generation of monoclonal antibodies of exceptional specificity. Molecular & Cellular Proteomics 11: 10.1074/mcp.O111.016253, 1–10, 2012.

Protein affinity reagents are fundamental tools of both basic and applied biomedical research. They are used for a wide range of applications, including measurement of protein expression levels, detection of protein-protein and protein-nucleic acid interactions, and detection of disease biomarkers (1). Currently, the most widely used protein affinity reagents are polyclonal and monoclonal antibodies. Monoclonal antibodies (mAbs)1 are indefinitely renewable and recognize a single protein epitope, making them the more desirable of the two reagents for most applications (2, 3). Indeed, with over 20 mAb-based drugs now in use and over 100 in clinical trials, they have also become a central pillar of the biopharmaceutical industry (4). However, despite their widespread use, well characterized protein affinity reagents are not available for the great majority of human proteins. This lack of characterization has led to a major bottleneck in analyzing protein expression and function, often making interpretation of data obtained using any class of protein affinity reagent problematic (5, 6). Several recent studies have suggested that many commercially available mAbs may not even recognize their purported targets and cross-react extensively with other cellular antigens (7). Antibody cross-reactivity is an even more pressing problem in diagnostic and therapeutic applications, as underlined by the recent withdrawal of several mAb-based pharmaceuticals from the market (8, 9).

Several large scale efforts are now underway to systematically identify high grade antibodies against much of the human proteome (4, 10–12). These approaches, which are primarily directed toward validation of polyclonal antibodies, rely heavily on immunocytochemistry and immunohistochemistry for validation, using both cell lines and tissue microarrays. Although these efforts provide a great deal of useful information, they neither cover all tissues nor confirm that the antibody in question is actually recognizing its target antigen in all of the tissues examined. To address this, it would be necessary to comprehensively measure cross-reactivity of any given antibody against the full proteome, something that is in principle possible using microarray-based analysis of antibody specificity (13–15).

1 The abbreviations used are: mAb, monoclonal antibody; DMEM, Dulbecco’s modified Eagle’s medium; ICC, immunocytochemistry; shRNA, small hairpin RNA; IP, immunoprecipitation; F/B, foreground/background signal; mmAb, monospecific mAb; QC, quality control.
Rapid Identification of Monospecific mAbs

A protein microarray approach has been previously used to analyze the specificity of antibodies generated against viral (16), microbial (17, 18), and mammalian (19–22) proteins and is already used on a small scale as part of the Human Protein Atlas project (11). However, existing human protein microarrays either are protein family-specific (22) or are comprised of only a minority of the human proteome (20, 23, 24). Although Goshima et al. (25) described fabrication of a more comprehensive human protein microarray that contained a total of 13,364 human proteins, these proteins were not purified away from the in vitro translation reaction mixtures used for protein synthesis, a fact that severely limits the potential usefulness of this reagent.

To remedy this situation, we have developed a microarray that includes nearly two-thirds of the annotated full-length human proteome. The proteins used to generate this microarray were purified under native conditions at low cost following galactose-induced expression from Saccharomyces cerevisiae (24, 26). Expressing recombinant eukaryotic proteins in yeast allows one to obtain higher success rate of purification and also improves the chances that proteins will retain biological activity relative to prokaryotic and in vitro-based expression systems (24, 26, 27). Furthermore, the use of an evolutionarily distant heterologous expression system like yeast minimizes the risk of contamination of recombinant human proteins with interacting cellular proteins, which is a potential complication that can result from the use of mammalian cells for protein expression.

A microarray with this level of coverage of the human proteome can potentially be used to identify antibodies that efficiently recognize proteins in their native conformation and that are thus useful for applications such as immunoprecipitation. We have used this tool as the backbone of an integrated platform that enables rapid and low cost identification of mAbs that both selectively bind a diverse assortment of human proteins and are useful in a wide range of experimental applications.

MATERIALS AND METHODS

Human Protein Microarray Construction—The cloning of human ORFs and protein purification was described previously (24). NanoPrint LM210 system (ArrayIT, Sunnyvale, CA) was used to print protein microarrays on Full Moon slides (Full Moon BioSystems, Sunnyvale, CA).

Generation of Hybridomas—Cultured human cells (SH-SY5Y, HepG2, HCT116, HeLa, HL-60, and MCF7) were grown to mid-log phase in appropriate media, collected from HepG2, HCT116, HeLa, HL-60, and MCF7) used for immunization (16), microbial (17, 18), and mammalian (19–22) proteins and are useful in a wide range of experimental applications.

Immunocytochemistry (ICC) Prescreening of mAbs—Cultured cell lines used for immunization were grown to 80% confluence in appropriate media, harvested, and plated with fresh media onto untreated (for HeLa and HCT116 cells) or poly-L-lysine-coated (for SH-SY5Y, HepG2, MCF7, and HL-60 cells) 16-well glass slides. Slides receiving HL-60 cells were additionally centrifuged for 2 min at 200 r.c.f. to improve attachment. All slides were incubated overnight at 37 °C with 5% CO2 atmosphere. The cells were washed twice with PBS and fixed with 100 μl of 4% paraformaldehyde in PBS for 15 min at room temperature. The wells were blocked with 100 μl of a mixture of 3% goat serum, 3% BSA, 0.1% Triton X-100 in PBS for 1 h at room temperature. mAb-containing solutions were adjusted to 10 μg/ml IgG in blocking buffer and incubated with cells for 2 h at room temperature or overnight at 4 °C. The wells were washed three times with 150 μl of PBS for 10 min. Secondary antibody (anti-mouse IgG:DyLight 488) was added to wells at room temperature for 2 h in the dark. Wells were washed three times with PBS, gaskets were removed, and the slides were mounted with antifade solution for viewing with a fluorescence microscope equipped with a computer-coupled camera.

Protein Microarray-based Analysis of mAb Specificity—Hybridoma supernatants (with antibodies at an average final concentration of 20 μg/ml in 1 × PBS) were screened in batches of 144 as two-dimensional pools. Twelve horizontal and vertical pools of 300-μl volumes were generated, consisting of equal mixtures of supernatants derived from 12 different hybridomas. These supernatants were combined such that each individual hybridoma was included in exactly one vertical and one horizontal pool, but such that no two hybridomas that are part of a given horizontal pool are found together in any vertical pool. The binding specificity of each horizontal and vertical pool was then profiled. Protein microarrays were then blocked for 2 h with 2% BSA in 1 × PBS at room temperature, incubated with antibody pools for 1 h at room temperature along with rabbit anti-GST (Millipore) at 1:5000, washed three times for 15 min in 1 × TBST, incubated with 1:800 Cy5-goat anti-mouse and 1:1000 Cy3 goat anti-rabbit (Invitrogen) in blocking buffer for 1 h at room temperature, washed three times for 15 min in 1 × TBST, rinsed once in double-distilled H2O, spun dry, and scanned using a GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA). Scanned images were analyzed using GenePix software, and proteins that bound to serum mouse IgG alone were excluded from analysis. Signal intensity was then calculated as the ratio of median foreground and median background signals in the Cy5 channel when analyzing bound mAb signal and the Cy3 channel when detecting GST.

To quantify the affinity of individual mAbs to specific proteins on the array, we first calculated the mean and standard deviation of the signal intensity across all spots on the chip. We obtain normalized signal intensity for any pair of spots, which we define as A, which is the mean z score for each duplicate pair of spotted proteins, where

\[
A = \frac{z_1 - m_z}{\sigma_z}
\]

Here, I is the ratio of median foreground and median background fluorescence for any given spot pair, m is the median value for I for all spots on the array, and \(\sigma\) is the standard deviation for I. mAbs found in one unique row pool and one column pool that showed z scores greater than 2.8 for both duplicate spots of any given protein were then flagged for individual analysis.

mAbs identified as potentially specific using this pooling strategy were then tested individually against the entire array, and A was
measured for each spotted protein. We next quantitatively evaluated the specificity of any individual mAb identified as potentially specific by means of this analysis. To do this, we calculated a value for specificity that we define as $S$, where $S = A_1 - A_2$. Here, $A_1$ represents the spot pair on the array that shows the highest value of $A$, and $A_2$ represents the spot pair with the second highest value of $A$.

As for the statistical analysis, we calculated a value for the specificity of any individual mAb identified as potentially specific by means of this analysis. The results indicated that although proteins localized to the cytoplasm ($p = 1.27E-40$) or mitochondria ($p = 7.56E-8$) showed significantly higher F/B ratios when compared with all proteins on the array, proteins localized to the plasma membrane are more challenging to produce. A similar trend was observed for commercially available mAbs.
observed when F/B ratios were analyzed for individual protein families; both G-protein coupled receptors and transcription factors exhibited lower level of expression among protein families examined (Fig. 2e). Not unexpectedly, these data imply that the physical and functional properties of target proteins affect observed protein yield but also show that the majority of proteins in all major functional classes can be efficiently expressed and purified using this yeast-based expression system. Finally, to evaluate whether protein length affects the success of protein purification, we applied a scatter plot analysis but only observed an insignificant negative correlation ($r = -0.196$) between protein length and signal intensity (Fig. 2f). Taken together, these analyses indicate that the yeast expression system is suitable for high throughput protein purification in higher eukaryotes. The microarray produced from these recombinant proteins represents the most comprehensive protein microarray comprised of individually purified proteins reported to date.

**Generation of Hybridomas and Identification of Target Antigens**—Ever since the early days of mAb production, highly complex mixtures of antigens such as whole cells or tissues have been used to generate ICC grade mAbs (30, 31). Generating mAbs in this so-called “shotgun” manner greatly increases the likelihood that they will recognize native protein epitopes, thus increasing the range of potential applications for the resultant mAbs. However, the major limitation of this approach has been the difficulty in identifying antigens preferentially recognized by a given mAb, which has typically only been possible through affinity purification coupled with mass spectrometry. By screening mAbs against human proteome microarrays, it becomes possible to directly identify the corresponding antigen recognized by a given mAb that has been generated by immunization with complex biological samples.

To generate mAbs that recognize a broad range of human proteins in their native conformations, we have immunized mice with a variety of different human cancer cell lines (Fig. 3). 2.5 x 10^6 cells were injected directly into footpad or hock areas, and 2 weeks later popliteal lymph nodes were collected, and lymphocytes were fused with a myeloma line. A total of 32,112 hybridomas were thus generated, of which 2326 both grew well and were IgG-positive. To enrich for useful mAbs, a high throughput ICC prescreening step against the cell line used for immunization was conducted, using supernatants from 2088 different IgG-positive hybridomas. A total of 643 ICC-positive hybridomas were then grown further, isotypes, and selected for microarray analysis. To determine whether this prescreening step indeed enriched for highly specific antibodies, a total of 332 randomly selected ICC-negative hybridomas. In addition, a total of 238 randomly selected hybridomas were used for microarray-based analysis of binding specificity without undergoing ICC prescreening. Individual supernatants were combined into sets of 12 x 12 two-dimensional pools, and these pools were then individually incubated on the human proteome microarrays. This was then followed by stringent washes and incubation with a Cy5-coupled anti-IgG secondary antibody to determine their binding profiles.

Following scanning, we expressed the signal intensity for each spot as the ratio of median foreground to median background signals. To identify antigens bound by individual mAbs, a histogram of signal intensity of every protein spot on an array was plotted to determine the median signal intensity and S.D. value. Using a z score cutoff of $\approx 2.8$, for both replicate spots, positive antigens were identified; mAbs found at the intersection between one unique row pool and one column pool that recognize a particular positive antigen were then flagged for individual analysis. mAbs identified as potentially specific using this pooling strategy were then tested again individually using a new array to reconfirm the reactive antigens. Positive antigens were identified as described...
above and ranked based on their signal intensity. When signal intensity of a top antigen is $\geq 6$ S.D. above the median and $\geq 3$ S.D. higher than the second best antigen, it is termed a monospecific mAb (mmAb) (supplemental Fig. 2). A total of 76 mmAbs were identified (supplemental Table 4). In many cases, mmAbs showed extremely high selectivity, with 15
displaying an S-score of >50, intensity between the top and second best antigens (supplemental Fig. 3). We also identified 27 mAbs that bound almost equally well to two different antigens on the array (S < 3), which we termed dispecific mAbs. Because the top two target proteins may be readily distinguishable if they show divergent cellular or subcellular expression, these dispecific mAbs were also selected for further analysis. Notably, three highly specific mAbs selectively recognized proteins for which the F/B ratio of the GST signal was less than 1.3, demonstrating their ability to readily detect relatively small quantities of recombinant protein.

To further characterize the utility and specificity of the mAbs and dispecific mAbs identified in this initial screening step, we then purified antibodies from either hybridoma supernatants or, in some cases, ascites fluid from BALB/c mice injected with the hybridomas and used these to conduct a variety of different assays. We first repeated the ICC analysis on human cancer cell lines and collected images to assess the subcellular expression pattern of the endogenous target antigen. Of 79 tested mAbs, 70 produced specific staining in cultured cells (Fig. 4a and supplemental Fig. 3). Confirmation of the observed specificity of individual mAbs comes from the finding that 48 of 64 (75%) of all ICC patterns obtained in this study matched those previously reported for their target proteins, when these data were available (supplemental Table 5). The subcellular distribution of the proteins for which high quality mAbs were obtained showed no clear preference for any particular cellular compartment, confirming that shotgun immunization can generate mAbs to a wide range of cellular proteins (supplemental Fig. 4). A total of 19% (12 of 64) of the targets possessed a predicted/annotated transmembrane domain, as compared with 26% for all human proteins (32).

Validation of Identified mAbs—Next, we analyzed the ability of the newly identified mAbs to detect their top target protein using immunoblot analysis. Individual target proteins were overexpressed in HeLa cells as N-terminal V5-tagged fusion proteins, and expression was confirmed via immunoblotting with anti-V5. Of 50 purified mAbs tested that recognized a total of 47 different proteins, 28 (56%) detected their target protein by immunoblotting, whereas 12 (24%) recognized endogenous untagged protein in these cells (supplemental Fig. 5). To further confirm the accuracy of these results, we co-transfected the V5-tagged ORF with individual shRNA constructs targeting the gene under investigation. In all six cases tested, we observed a substantial reduction in immunoblot signal, confirming the fidelity of these mAbs (Fig. 4b).

We next tested whether these mAbs could work effectively for immunoprecipitation and thus recognize native proteins in cell homogenates. Remarkably, we found that of the 50 purified mAbs tested, 33 (66%) could efficiently precipitate transfected protein, which was then detected via immunoblotting with anti-V5 (Fig. 4c and supplemental Fig. 6). In all, 21 of 50 (42%) of all mAbs worked effectively for both immunoblotting
and immunoprecipitation. We next investigated whether these mAbs were useful for a broader range of applications compared with those already available from other commercial suppliers. We first directly compared mAbs from 12 different suppliers generated to the same set of 47 antigens as those in this study (Table I). In all, based on the supplier’s reports, 80% of commercially available mAbs work for immunoblotting, 55% are ICC or IHC grade, but only 18% are IP grade. In contrast, a much higher fraction of mAbs generated using the pipeline described here were ICC and IP grade, although a somewhat lower fraction were immunoblot grade. To eliminate any systematic bias introduced by selection of these antigens, we also investigated the performance of 100 randomly selected mAbs from a subset of major commercial suppliers and found that these numbers were very similar, with 86% of randomly chosen mAbs working for IB, 55% for IHC or ICC, 17% for IP, and 11% useful for all three applications (Table II). Furthermore, no commercially available antibodies were available for 10 of the proteins recognized by mmAbs identified in this study, whereas only a single antibody was available for six others (supplemental Table 5). We thus conclude that the protein microarray-based shotgun approach described here is able to generate highly specific mAbs to proteins that are both well and poorly targeted by the existing antibody repertoire and are useful for a broader range of applications than those currently available from commercial suppliers.

Finally, to determine whether the ICC-prescreening step enriched for highly specific and broadly usable mAbs, we compared the quality of the antibodies analyzed from the ICC-positive and the ICC-negative groups. Overall, of the
hybridomas that underwent ICC prescreening, we found that 10.3% (66 of 643) of ICC-positive hybridomas were highly specific, significantly higher than the 7.5% (25 of 332) positive rate observed in ICC-negative hybridomas (p < 0.03). Furthermore, we found that ICC-positive mAbs showed significantly higher signal intensity and specificity than did ICC-negative mAbs (p < 0.05). Although both pools generated similar proportions of IB and IP grade mAbs (supplemental Table 4), ICC prescreening results in a significant improvement in overall antibody quality and specificity.

**DISCUSSION**

We have constructed the most comprehensive human protein microarray to date that is comprised of individually purified proteins expressed in a eukaryotic cell system. A recent effort reported construction of a human proteome scale microarray using a wheat germ *in vitro* translation system for protein production (25). However, the use of crude translation reaction mixtures rather than individually purified proteins may limit the use of this type of protein microarray, because proteins in each spot are unavoidably contaminated with large amount of proteins present in the wheat germ system. Because assays conducted on protein microarrays are generally more sensitive than those performed in a traditional liquid-based format, it is harder to successfully carry out those assays that require high purity of the spotted proteins using such a reagent (33). The use of individually purified proteins to construct a functional protein microarray has been proven highly successful in characterizing a diverse range of protein functions over the past decade (11, 14, 15, 17, 22–24, 26, 27, 33). Furthermore, the fact that a majority of the full-length human proteome is printed on a single slide makes it possible

---

**Table I**

Summary of experimental uses for commercially available mAbs that recognize the same target antigens as the highly specific mAbs identified in this study

| Supplier | Number of mAbs | IB (%) | ICC/IHC (%) | IP (%) | Multiple (%) | All (%) |
|----------|----------------|--------|--------------|--------|--------------|--------|
| Supplier A | 57 | 77 | 46 | 16 | 37 | 7 |
| Supplier B | 12 | 83 | 50 | 17 | 42 | 17 |
| Supplier C | 31 | 65 | 71 | 6 | 58 | 6 |
| Supplier D | 19 | 84 | 53 | 5 | 47 | 5 |
| Supplier E | 19 | 63 | 47 | 0 | 26 | 0 |
| Supplier F | 15 | 93 | 73 | 47 | 80 | 33 |
| Supplier G | 44 | 84 | 70 | 39 | 59 | 25 |
| Supplier H | 8 | 88 | 50 | 0 | 38 | 0 |
| Supplier I | 20 | 95 | 20 | 10 | 20 | 0 |
| Supplier J | 9 | 100 | 44 | 22 | 44 | 22 |
| Supplier K | 11 | 91 | 0 | 18 | 9 | 0 |
| Supplier L | 22 | 73 | 86 | 14 | 55 | 9 |
| All commercial mAbs | 267 | 80 | 55 | 18 | 58 | 11 |
| This study | 50 | 56 | 90 | 66 | 74 | 42 |

**Table II**

Summary of experimental uses for 100 randomly chosen mAbs from different commercial suppliers

| mAbs by supplier | IB (%) | ICC/IHC (%) | IP (%) | Multiple (%) | All (%) |
|------------------|--------|-------------|--------|--------------|--------|
| Supplier A | 87 | 28 | 11 | 35 | 3 |
| Supplier B | 93 | 26 | 6 | 29 | 0 |
| Supplier C | 96 | 83 | 0 | 81 | 0 |
| Supplier D | 91 | 14 | 0 | 13 | 0 |
| Supplier E | 85 | 64 | 25 | 60 | 17 |
| Supplier F | 67 | 70 | 34 | 55 | 21 |
| Supplier G | 83 | 100 | 40 | 85 | 38 |
| Overall mean | 86 | 55 | 17 | 51 | 11 |
| This study | 56 | 90 | 66 | 74 | 42 |
to perform more comprehensive screens of protein function in a high throughput fashion.

Using the proteome microarray as a central component, we built a pipeline for mAb selection using random hybridomas generated by the shotgun approach. The pipeline for generation of mAbs described here has a number of unique advantages over existing approaches. First, the use of live cells as immunogens bypasses the cumbersome and costly step of antigen preparation and ensures that only proteins in their native conformation are detected by the host immune system. Second, the use of protein microarrays in the pipeline effectively allows ready determination of mAb specificity and also provides a direct measurement of specificity for every mAb analyzed. Third, the incorporation of an ICC prescreening step increases the likelihood of generating highly specific mAbs. Finally, mAbs identified using this approach are useful for a wide range of biochemical applications, most notably those requiring recognition of native epitopes such as immunoprecipitation, and outperform mAbs available from commercial suppliers. Because immunoprecipitation grade antibodies typically have $K_D$ values no higher than $50\text{ nm}$ ($34, 35$), these mAbs typically show both high affinity and high specificity. The microarray-based analysis described here is both relatively low cost and high throughput and can also readily be used to evaluate the binding specificity of any protein affinity reagents, including recombinant antibodies and aptamers. This platform may prove particularly useful in evaluating the binding specificity of the many thousands of mAbs already used by the research and clinical communities and could be readily adapted to identify highly specific mAbs obtained following immunization with individual proteins. The development of large numbers of mAbs made using a single, consistent pipeline and exhibiting high specificity using the approaches described here may have major implications for both basic and clinical research.

Acknowledgments—We thank Alan Long and Sarah Zheng for expert assistance with laboratory automation and managing clone collections, Yu-Yi Lin for help with siRNA screening, Sheng-Ce Tao for help with protein purification, and S. Chen and T. Shimoigori for comments on the manuscript.

S. B., H. Z., I. P., D. E., J. B., and J. D. B. are co-founders of CDI Laboratories and all hold stock in the corporation. I. P., J. B., E. A., D. B., L. R., Z. A. R., and C. T. are all current employees of CDI Laboratories.

* This work was supported by National Institutes of Health Grants R41MH088008 (to S. B. and I. P.) and 1R01EY017015 (to S. B.), by National Institutes of Health Common Fund Grant U54RR020839 (to H. Z. and J. D. B.), and by a grant from the Puerto Rico Science and Technology Trust. Additional support was obtained from the Institute for Cell Engineering and the Fund for Medical Discovery, Johns Hopkins University School of Medicine, and the Puerto Rico Science and Technology Trust. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

To whom correspondence may be addressed. E-mail: ignacio.pino@cdi-lab.com.

To whom correspondence may be addressed. E-mail: eichid01@nyu.edu.

To whom correspondence may be addressed. E-mail: hzhu4@jhmi.edu.

m W. M. Keck Distinguished Young Investigator in Medical Research. To whom correspondence may be addressed. E-mail: sbblack@jhmi.edu.

REFERENCES

1. Uhlen, M., (2008) Affinity as a tool in life science. BioTechniques 44, 649–654

2. Streblhardt, K., and Ullrich, A. (2008) Paul Ehrlich’s magic bullet concept: 100 years of progress. Nat. Rev. Cancer 8, 473–480

3. Köhler, G., and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256, 495–497

4. Stoevesandt, O., and Tausig, M. J. (2007) Affinity reagent resources for human proteome detection: Initiatives and perspectives. Proteomics 7, 2738–2750

5. Kalyuzhny, A. E. (2009) The dark side of the immunohistochemical moon: Industry. J. Histochem. Cytochem. 57, 1099–1101

6. Couchman, J. R. (2009) Commercial antibodies: The good, the bad, and really ugly. J. Histochem. Cytochem. 57, 1–6

7. Jensen, B. C., Swigt, P. M., and Simpson, P. C. (2009) Ten commercial antibodies for α1-adrenergic receptor subtypes are nonspecific. Nauyn-Schmiedeb ergs Arch. Pharmacol. 379, 409–412

8. Hughes, B., (2010) Antibody-drug conjugates for cancer: Pioneed to deliver? Nat. Rev. Drug Discov. 9, 665–667

9. Berger, J. R., Houff, S. A., and Major, E. O. (2009) Monoclonal antibodies and progressive multifocal leukoencephalopathy. mAbs 1, 583–589

10. Berglund, L., Börling, E., Oksvold, P., Fagerberg, L., Asplund, A., Szigyarto, C. A., Persson, A., Ottosson, J., Wernérus, H., Nilsson, P., Lundberg, E., Sivertsson, A., Navani, S., Wester, K., Kampf, C., Hober, S., Pontén, F., and Uhlen, M. (2008) A genecentric Human Protein Atlas for expression profiles based on antibodies. Mol. Cell. Proteomics 7, 2019–2027

11. Uhlen, M., and Hober, S. (2009) Generation and validation of affinity reagents on a proteome-wide level. J. Mol. Recognit. 22, 57–64

12. Colwill, K., and Graslund, S. (2011) A roadmap to generate renewable protein binders to the human proteome. Nat. Methods 8, 551–558

13. Mattoon, D. R., and Schweitzer, B. (2009) Antibody specificity profiling on functional protein microarrays. Methods Mol. Biol. 524, 213–223

14. Predki, P. F., Mattoon, D., Bangham, R., Schweitzer, B., and Michaud, G. (2005) Protein microarrays: A new tool for profiling antibody cross-reactivity. Hum. Antibodies 14, 7–15

15. Robinson, W. H. (2006) Antigen arrays for antibody profiling. Curr. Opin. Chem. Biol. 10, 67–72

16. Davies, D. H., Wyatt, L. S., Newman, F. K., Earl, P. L., Chun, S., Hernandez, J. E., Molina, D. M., Hirst, S., Moss, B., Frey, S. E., and Felgner, P. L. (2008) Antibody profiling by proteome microarray reveals the immunogenicity of the attenuated smallpox vaccine modified vaccinia virus ankara is comparable to that of Dryvax. J. Virol. 82, 652–663

17. Michaud, G. A., Salcius, M., Zhou, F., Bangham, R., Bonin, J., Guo, H., Snyder, M., Predki, P. F., and Schweitzer, B. I. (2003) Analyzing antibody specificity with whole proteome microarrays. Nat. Biotechnol. 21, 1509–1512

18. Keasey, S. L., Schmid, K. E., Lee, M. S., Meegan, J., Tomas, P., Minto, M., Tikhonov, A. P., Schweitzer, B., and Ulrich, R. G. (2009) Extensive antibody cross-reactivity among infectious Gram-negative bacteria revealed by proteome microarray analysis. Mol. Cell. Proteomics 8, 924–935

19. Kijanka, G., Ipcho, S., Baars, S., Chen, H., Hadley, K., Beveridge, A., Gould, E., and Murphy, D. (2009) Rapid characterization of binding specificity and cross-reactivity of antibodies using recombinant human protein arrays. J. Immunol. Methods 340, 132–137

20. Lueking, A., Possling, A., Huber, O., Beveridge, A., Horn, M., Eickhoff, H., Schuchardt, J., Lehrah, H., and Cahill, D. J. (2003) A nonredundant human protein chip for antibody screening and serum profiling. Mol. Cell. Proteomics 2, 1342–1349

21. Büssow, K., Nordhoff, E., Lübbert, C., Lehrah, H., and Walter, G. (2000) A
human cDNA library for high-throughput protein expression screening. Genomics 65, 1–8
22. Hu, S., Li, Y., Liu, G., Song, Q., Wang, L., Han, Y., Zhang, Y., Song, Y., Yao, X., Tao, Y., Zeng, H., Yang, H., Wang, J., Zhu, H., Chen, Z. N., and Wu, L. (2007) A protein chip approach for high-throughput antigen identification and characterization. Proteomics 7, 2151–2161
23. Song, Q., Liu, G., Hu, S., Zhang, Y., Tao, Y., Han, Y., Zeng, H., Huang, W., Li, F., Chen, P., Zhu, J., Hu, C., Zhang, S., Li, Y., Zhu, H., and Wu, L. (2010) Novel autoimmune hepatitis-specific autoantigens identified using protein microarray technology. J. Proteome Res. 9, 30–39
24. Hu, S., Xie, Z., Onishi, A., Yu, X., Jiang, L., Lin, J., Rho, H. S., Woodard, C., Wang, H., Jeong, J. S., Long, S., He, X., Wade, H., Blackshaw, S., Qian, J., and Zhu, H. (2009) Profiling the human protein-DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling. Cell 139, 610–622
25. Goshima, N., Kawamura, Y., Fukumoto, A., Miura, A., Honma, R., Satoh, R., Wakamatsu, A., Yamamoto, J., Kimura, K., Nishikawa, T., Andoh, T., Iida, Y., Ishikawa, K., Ito, E., Kagawa, N., Kaminaga, C., Kanehori, K., Kavakami, B., Kenmochi, K., Kimura, R., Kobayashi, M., Kurotta, T., Kuwayama, H., Maruyama, Y., Matsuo, K., Minami, K., Mitsubori, M., Mori, M., Morishita, R., Murase, A., Nishikawa, A., Nishikawa, S., Okamoto, T., Sakagami, N., Sakamoto, Y., Sasaki, Y., Seki, T., Sono, S., Sugiyama, A., Sumiya, T., Takayama, T., Takayama, Y., Takeda, H., Togashi, T., Yahata, K., Yamada, H., Yanagisawa, Y., Endo, Y., Immunoto, F., Kikui, Y., Tanaka, S., Isogai, T., Imai, J., Watanabe, S., and Nomura, N. (2008) Human protein factory for converting the transcriptome into an in vitro-expressed proteome. Nat. Methods 5, 1011–1017
26. Zhu, H., Bilgin, M., Bangham, R., Hall, D., Casamayor, A., Bertone, P., Lan, N., Jansen, R., Bidlingmaier, J., Houfek, T., Mitchell, T., Miller, P., Dean, R. A., Gerstein, M., and Snyder, M. (2001) Global analysis of protein activities using proteome chips. Science 293, 2101–2105
27. Hall, D. A., Zhu, H., Zhu, X., Royce, T., Gerstein, M., and Snyder, M. (2004) Regulation of gene expression by a metabolic enzyme. Science 306, 482–484
28. Clamp, M., Fry, B., Kamal, M., Xie, X., Cuff, J., Lin, M. F., Kellis, M., Lindblad-Toh, K., and Lander, E. S. (2007) Distinguishing protein-coding and noncoding genes in the human genome. Proc. Natl. Acad. Sci. U.S.A. 104, 19428–19433
29. Liang, F., Matrubutham, U., Parvizi, B., Yen, J., Duan, M., Mirchandani, J., Hashima, S., Nguyen, U., Ubil, E., Loewenheim, J., Yu, X., Sipes, S., Williams, W., Wang, L., Bennett, R., and Carrino, J. (2004) ORFDB: an information resource linking scientific content to a high-quality open reading frame (ORF) collection. Nucleic Acids Res. 32, D595–D599
30. McMichael, A. J., Pitch, J. R., Gallifre, G., Mason, D. Y., Fabre, J. W., and Milstein, C. (1979) A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. Eur. J. Immunol. 9, 205–210
31. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein, C., Williams, A. F., and Ziegler, A. (1978) Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens—new tools for genetic analysis. Cell 14, 9–20
32. Fagerberg, L., Jonasson, K., von Heijne, G., Uhlén, M., and Berglund, L. (2010) Prediction of the human membrane proteome. Proteomics 10, 1141–1149
33. Smith, M. G., Jona, G., Ptacek, J., Devgan, G., Zhu, H., Zhu, X., and Snyder, M. (2005) Global analysis of protein function using protein microarrays. Mech. Ageing Dev. 126, 171–175
34. Osyon, M. R., Zheng, Y., Zhang, C., Colwill, K., Pershad, K., Kay, B. K., Pawson, T., and McCafferty, J. (2011) Mapping protein interactions by combining antibody affinity maturation and mass spectrometry. Anal. Biochem. 417, 25–35
35. Harlow, E., and Lane, D. (1998) Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

In order to cite this article properly, please include all of the following information: Jeong, J. S., Jiang, L., Albino, E., Marrero, J., Rho, H. S., Hu, J., Hu, S., Vera, C., Bayron-Poueymiroy, D., Rivera-Pacheco, Z. A., Ramos, L., Torres-Castro, C., Qian, J., Bonaventura, J., Boeke, J. D., Yap, W. Y., Pino, I., Eichinger, D. J., Zhu, H., and Blackshaw, S. (2012) Rapid Identification of Monospecific Monoclonal Antibodies Using a Human Proteome Microarray. Mol. Cell. Proteomics 11(6):O111.016253. DOI: 10.1074/mcp.O111.016253.