

Protein Microarrays for Gene Expression and Antibody Screening

Angelika Lueking, Martin Horn, Holger Eickhoff, Konrad Büsow,
Hans Lehrach, and Gerald Walter

Max Planck Institute for Molecular Genetics, Ihnestrassse 73, D-14195 Berlin, Germany

Received December 17, 1998

Proteins translate genomic sequence information into function, enabling biological processes. As a complementary approach to gene expression profiling on cDNA microarrays, we have developed a technique for high-throughput gene expression and antibody screening on chip-size protein microarrays. Using a picking/spotting robot equipped with a new transfer stamp, protein solutions were gridded onto polyvinylidene difluoride filters at high density. Specific purified protein was detected on the filters with high sensitivity (250 amol or 10 pg of a test protein). On a microarray made from bacterial lysates of 92 human cDNA clones expressed in a microtiter plate, putative protein expressors could be reliably identified. The rate of false-positive clones, expressing proteins in incorrect reading frames, was low. Product specificity of selected clones was confirmed on identical microarrays using monoclonal antibodies. Cross-reactivities of some antibodies with unrelated proteins imply the use of protein microarrays for antibody specificity screening against whole libraries of proteins. Because this application would not be restricted to antigen-antibody systems, protein microarrays should provide a general resource for high-throughput screens of gene expression and receptor-ligand interactions.

© 1999 Academic Press

Key Words: microarray; robotics; protein; gene; expression; antibody; screening.

While 40–80% of the total number of human genes are now represented as expressed sequence tags (ESTs)¹ in the dbEST database (<http://www.ncbi.nlm.nih.gov/dbEST>) (1), only a minority have yet been as-

signed a function. Whole-genome expression screens are regarded an essential prerequisite for functional studies (2). Automated technology allows high-throughput gene activity monitoring by analysis of complex expression patterns (3), resulting in fingerprints of diseased versus normal or developmentally distinct tissues (4). Starting from high-density filter membranes (5), DNA microarrays have now been devised in chip format (6–8). Using microarrays, gene expression profiles were attained for *Arabidopsis thaliana* (9), *Saccharomyces cerevisiae* (10–15), and human lymphoid cells and tissues like bone marrow, brain, prostate, and heart (16). cDNA microarrays have already been used to profile complex diseases such as rheumatoid arthritis (17), melanoma (18, 19), and Ewing's sarcoma (20). Logically, the next step will be profiling of protein products encoded by differentially expressed cDNA clones. This requires a highly parallel approach to protein expression analysis, including the simultaneous expression of large numbers of cDNA clones in an appropriate vector system and high-speed arraying of protein products.

We have previously shown that cDNA libraries can be screened for protein expression on high-density filter membranes (21). Using robot technology (22), a human fetal brain cDNA expression library (hEx1) was arrayed in microtiter plates, and bacterial colonies were gridded onto polyvinylidene difluoride (PVDF) filters. *In situ* expression of recombinant fusion proteins was induced and detected using an antibody against a His₆ tag-containing epitope. We have now extended this approach to automated spotting of protein microarrays from liquid expression cultures using a new transfer stamp mounted onto a flat-bed spotting robot. These protein microarrays allow very sensitive protein expression and antibody specificity screening and should provide powerful tools for high-throughput ligand-receptor interaction studies.

¹ Abbreviations used: ESTs, expressed sequence tags; PVDF, polyvinylidene difluoride; IPTG, isopropyl- β -D-thiogalactopyranoside; BSA, bovine serum albumin; TBST, Tris-buffered saline with Tween 20; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RF, reading frames.

MATERIALS AND METHODS

Vector Constructs

pQE-30NST (GenBank Accession No. AF074376) has been described (21). pQE-BH6 was constructed using the polymerase chain reaction (PCR) for insertion of an oligonucleotide encoding the protein sequence LNDIFEAQKIEW between MRGS and His₆ of pQE-30 (Qiagen), thereby separating the two parts of the RGS-His₆ epitope.

Antibodies

Monoclonal antibodies of the following manufacturers were used at dilutions as indicated: mouse anti-RGS-His (Qiagen, 1:2000), mouse anti-rabbit GAPDH (Research Diagnostics Inc., clone 6C5, 1:5000), mouse anti-HSP90 (Transduction Laboratories, clone 68, 1:2000), rat anti- α tubulin (Serotec Ltd., clone YL1/2, 1:2000).

Secondary antibodies were F(ab')₂ rabbit anti-mouse IgG HRP (Sigma) and F(ab')₂ rabbit anti-rat IgG HRP (Serotec Ltd.), diluted 1:5000, for the detection of mouse and rat monoclonals, respectively.

Large-Scale Protein Expression and Purification

Proteins were expressed in *Escherichia coli* (strain SCS1) liquid cultures. Nine hundred milliliters of SB medium (12 g/liter bactotryptone, 24 g/liter yeast extract, 17 mM KH₂PO₄, 72 mM K₂HPO₄, 0.4% (v/v) glycerol) containing 100 μ g/ml ampicillin and 15 μ g/ml kanamycin was inoculated with 10 ml of an overnight culture and shaken at 37°C until an OD₆₀₀ of 0.8 was reached. Isopropyl- β -D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM. The culture was shaken for 3.5 h at 37°C and cooled to 4°C on ice. Cells were harvested by centrifugation at 2100g for 10 min, resuspended in 100 ml phosphate buffer (50 mM NaH₂PO₄, 0.3 M NaCl, pH 8.0) and centrifuged again. Cells were lysed in 3 ml per gram wet weight of lysis buffer (50 mM Tris, 300 mM NaCl, 0.1 mM EDTA, pH 8.0) containing 0.25 mg/ml lysozyme on ice for 30 min. DNA was sheared with an ultrasonic homogenizer (Sonifier 250, Branson Ultrasonics, Danbury, U.S.A.) for 3 $\times$ 1 min at 50% power on ice. The lysate was cleared by centrifugation at 10,000g for 30 min. Ni-NTA agarose (Qiagen) was added and mixed by shaking at 4°C for 1 h. The mixture was poured into a column which was subsequently washed with 10 bed volumes of lysis buffer containing 20 mM imidazole. Protein was eluted in lysis buffer containing 250 mM imidazole and was dialyzed against TBS (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) at 4°C overnight.

High-Throughput Small-Scale Protein Expression

Proteins were expressed from selected clones of the arrayed human fetal brain cDNA expression library

hEx1 (21). This library was directionally cloned in pQE-30NST for IPTG-inducible expression of His₆-tagged fusion proteins. The 96-well microtiter plates with 2-ml cavities (StoreBlock, Zinsser) were filled with 100 μ l SB medium, supplemented with 100 μ g/ml ampicillin and 15 μ g/ml kanamycin. Cultures were inoculated with *E. coli* SCS1 cells from 384-well library plates (Genetix, Christchurch, UK) that had been stored at -80°C. For inoculation, replicating devices carrying 96 steel pins (length 6 cm) were used. After overnight growth at 37°C with vigorous shaking, 900 μ l of prewarmed medium was added to the cultures, and incubation was continued for 1 h. For induction of protein expression, IPTG was added to a final concentration of 1 mM. All following steps, including centrifugations, were also performed in the 96-well format. Cells were harvested by centrifugation at 1900g (3400 rpm) for 10 min, washed by resuspension in phosphate buffer, centrifuged for 5 min, and lysed by resuspension in 150 μ l buffer A (6 M guanidinium-HCl, 0.1 M NaH₂PO₄, 0.01 M Tris-HCl, pH 8.0). Bacterial debris was pelleted by centrifugation at 4000 rpm for 15 min. Supernatants were filtered through a 96-well filter plate containing a non-protein-binding 0.65- μ m pore size PVDF membrane (Durapore MADV N 65, Millipore) on a vacuum filtration manifold (Multiscreen, Millipore).

Automated Filter Spotting

Precut (25 $\times$ 75 mm) PVDF filter strips (Immobilon P, Millipore) were soaked with 96% ethanol and rinsed in distilled water for 1 min. Five wet filter strips were fixed with tape onto a 230 $\times$ 230-mm plastic tray. The spotting was carried out by a motor-carried transfer stamp (Fig. 1) which can be positioned at a resolution of 5 μ m in x-y-z directions (Linear Drives, Basildon, UK). This allows densities of approximately 300 samples/cm², spotted in a duplicate pattern. The transfer stamp accommodates 4 $\times$ 4 = 16 individually mounted, spring-loaded pins at 4.5-mm spacing. Since the spacing is compatible to the spacing of 384-well plates, this tool enables high-density spotting out of 384-well microtiter plates. The size of the blunt-end tip of the stainless-steel pins is 250 μ m. Prior to each transfer, the spotting gadget was washed in a 30% ethanol bath and subsequently dried with a fan to prevent cross-contamination. For the experiments shown here, 4 $\times$ 4 patterns were spotted with each pin. Each pattern contains 4 ink guide spots surrounded by 6 samples spotted in duplicate (12 sample spots in total, Fig. 2A). Each spot was loaded five times with the same protein sample (5 nl each). Having adjusted the spotting height in advance, the spotting of 96 samples took approximately 20 min for the generation of five identical protein microarrays.

Antibody Detection and Image Analysis

After spotting, filters were soaked in ethanol for 1 min, rinsed in distilled water, washed in TBST (TBS, 0.1% Tween 20) for 1 min, blocked in 2% bovine serum albumin (BSA)/TBST for 60 min and incubated with monoclonal antibodies in 2% BSA/TBST for 1 h at room temperature, followed by two 10-min TBST washes and 1 h incubation with secondary antibodies in 2% BSA/TBST. Subsequently, filters were washed in 20 ml TBST overnight and incubated in 2 ml CN/DAB solution (Pierce) for 1–10 min, and positive reactions were detected as black spots. Images were acquired with a cooled CCD Camera (Fuji LHS, Raytest, Germany). Pictures were taken through a Fujinon objective (f: 0.8, 50 mm) with an integration time of 20 ms. Image analysis was carried out with the AIDA package (Raytest, Germany) for spot recognition and quantification. The resulting spot values were transferred to an Excel spreadsheet (Microsoft, U.S.A.) to display the diagrams of Figs. 2, 3, and 4.

RESULTS AND DISCUSSION

Fabrication of Protein Microarrays

Proteins were expressed in liquid bacterial cultures, and solutions were spotted onto PVDF filters, either as crude lysates or after purification by Ni-NTA-immobilized metal affinity chromatography (IMAC) (23). PVDF filter membranes were used for their superior protein binding capacity and mechanical strength (compared to nitrocellulose) and satisfactory former performance (21). The new transfer stamp (Fig. 1) consists of pins with 250- μm tip size, which is nearly half the size of the 450- μm pins that have previously been used for the generation of *in situ* protein expression filters (21). Although Figs. 2, 3, and 4, as our first test results, show about the same spotting density as our *in situ* filters, the smaller pin tip diameter enables higher spotting densities. While an *in situ* filter of 222×222 mm accommodates 27,648 clones (5×5 duplicate spotting pattern with one guide spot), more than 100,000 samples could be placed onto the same area using the new transfer stamp. This allows a substantial reduction in total array size to a convenient microscopic slide format (25×75 mm holding 4800 samples, corresponding to 2400 duplicates). The miniaturized setup allows a very economic use and high concentrations of reagents in incubating solutions as a much smaller buffer volume is needed to cover the filters. In contrast to *in situ* filters, the signals obtained on microarrays are sharp and well localized. As the next step toward the fabrication of protein chips, we envisage a further increase in density by using high-speed picoliter spotting (inkjetting) onto modified glass surfaces.



FIG. 1. Transfer stamp for protein solution transfer from 384-well microtiter plates to PVDF membranes. Sixteen individual, spring-loaded, stainless-steel pins are mounted into a POM (polyoxymethylene, polyformaldehyde, polyacetate) corpus. The pin-to-pin distance is 4.5 mm. The blunt end tip size was measured to 250 μm .

Alternative approaches to protein microarrays have been reported using either photolithography of silane monolayers (24) or inkjetting onto polystyrene film (25, 26). In contrast to our library spotting technology, those advances have been focused on the fabrication of miniaturized immunoassay formats by patterning of single proteins (e.g., BSA, avidin, or anti-IgG monoclonal antibodies).

Sensitivity of Specific Protein Detection

The sensitivity of specific protein detection on microarrays was assessed by spotting different concentrations of three purified proteins, human glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Swiss-Prot P04406), a C-terminal fragment (40.3 kDa) of human heat shock protein 90 α (HSP90 α , Swiss-Prot P07900) and rat immunoglobulin heavy chain binding protein (BIP, Swiss-Prot P06761). Microarrays were subsequently incubated with a monoclonal anti-GAPDH antibody, rabbit anti-mouse IgG HRP, and HRP substrate CN/DAB (Fig. 2A). The sensitivity of detection, as the lowest concentration that delivered clearly visible, specific spots above background (detection threshold), was calculated to be 10 fmol/ μl , corresponding to 250 amol or 10 pg of GAPDH in 25 nl spotted (Fig. 2B).

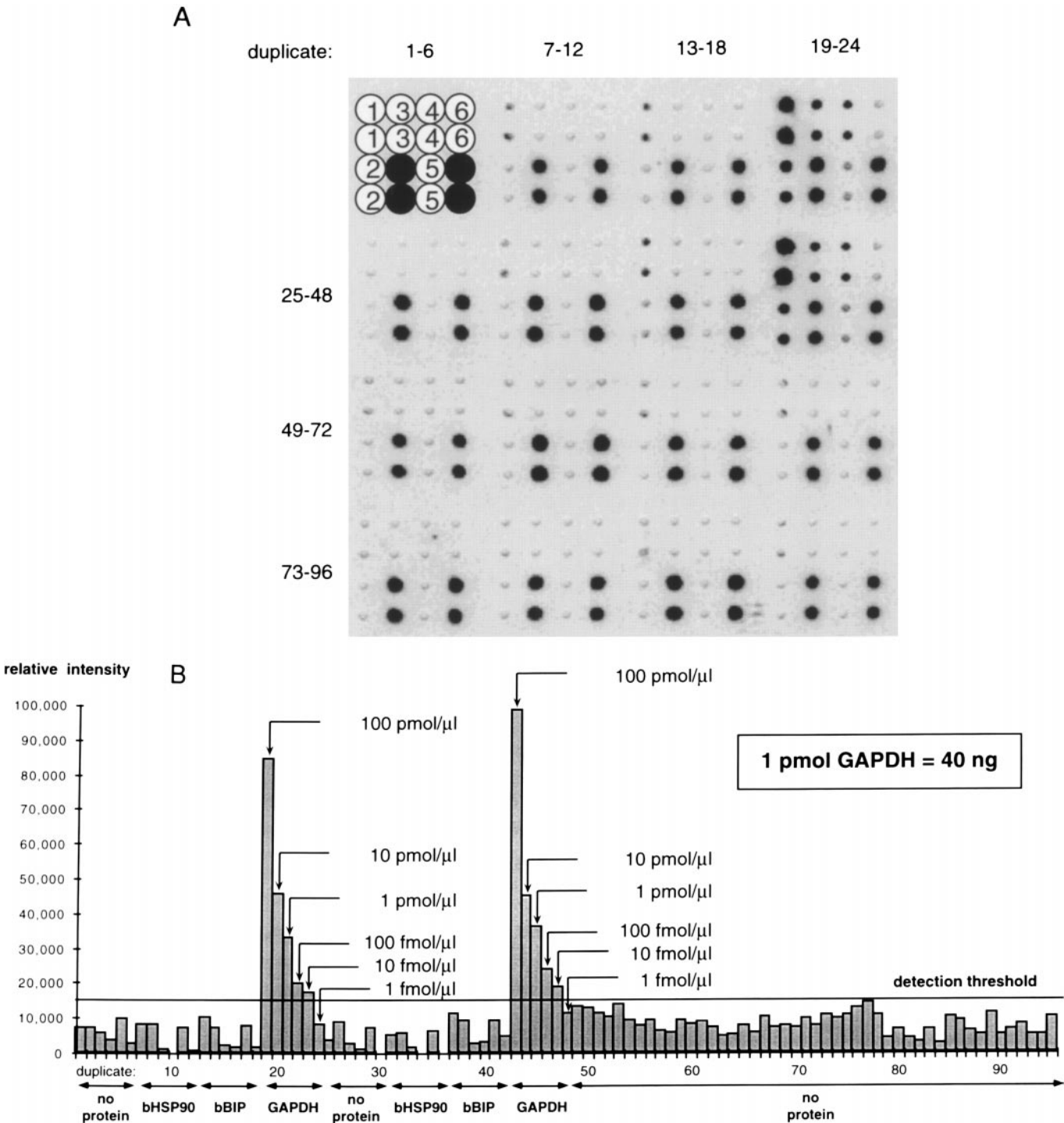


FIG. 2. Sensitivity of specific protein detection on microarrays. Equimolar concentrations (100 pmol/ μ l to 1 fmol/ μ l) of purified human GAPDH (duplicates 19–24 and 43–48), human bHSP90 α (duplicates 7–12 and 31–36), and rat bBIP (duplicates 13–18 and 37–42) were spotted (5 $\times$ 5 nl) in two identical series of duplicates and detected using a monoclonal anti-GAPDH antibody. (A) Spot array on PVDF filter membrane (1.9 $\times$ 1.9 cm holding 128 samples, 4 $\times$ 4 vertical duplicate spotting pattern, black duplicate guide spots, counting of duplicates as indicated). (B) Relative intensities of means of duplicates in A (guide spots excluded), indicating numbering of duplicates (as in A), name and amounts of protein spotted, and detection threshold.

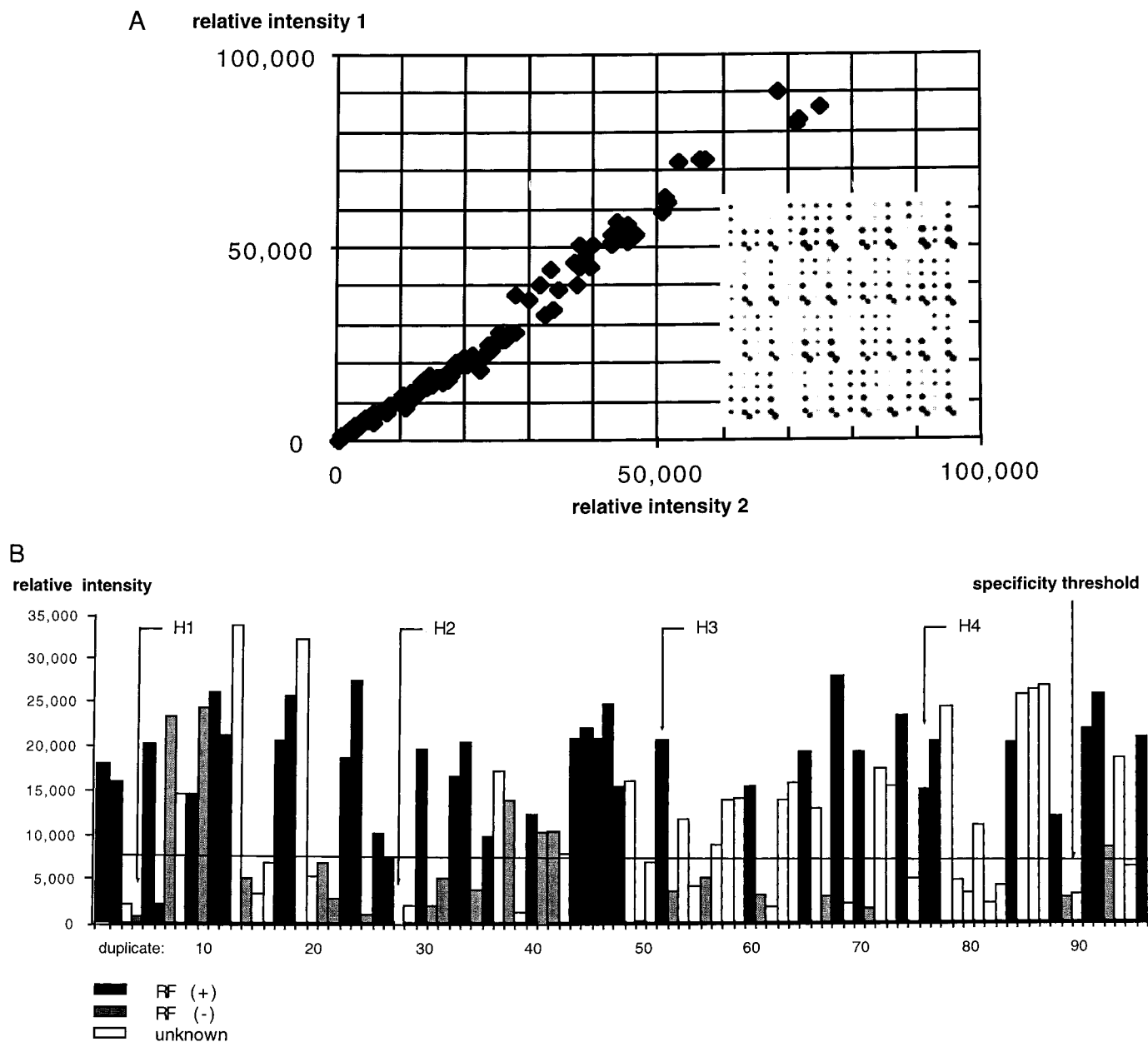


FIG. 3. High-throughput expression of RGS-His₆-tagged fusion proteins from clones of the arrayed hEx1 library as detected on a microarray using the monoclonal antibody RGS-His (Qiagen). Crude, filtered lysates of 92 clones were spotted from a 96-well microtiter plate, including 4 wells with control proteins (H1, vector pQE-30NST without insert; H2, bHSP90 α , clone N15170, vector pQE-BH6; H3, GAPDH, clone D215, vector pQE-30NST; H4, bBIP, vector pQE-BH6). (A) Reproducibility of detection as diagonal of relative intensities of duplicates. (Inset) Spot array on PVDF filter membrane (as in Fig. 2, lower guide spots doubled for orientation). (B) Diagram as in Fig. 2, indicating (+) or (-) reading frames of inserts if known (specificity threshold arbitrarily set to 7500 relative intensity).

High-Throughput Screening for Protein Expression

Crude lysates of 92 clones of the arrayed human fetal brain cDNA library hEx1 (21), previously identified as protein expressors by the monoclonal antibody RGS-His (Qiagen) on *in situ* filters, were spotted in duplicate, alongside with 4 control samples and ink guide spots. Microarrays were screened for expression of

RGS-His₆-tagged fusion proteins using the same antibody (Fig. 3A, inset). When relative intensities of duplicates (see Fig. 2A) are plotted against each other, the resulting diagonal indicates a good reproducibility of the detection method (Fig. 3A). Therefore, means of duplicates were plotted for all 96 samples, and an arbitrary specificity threshold for identification of positives was set to 7500 relative intensity (Fig. 3B). Un-

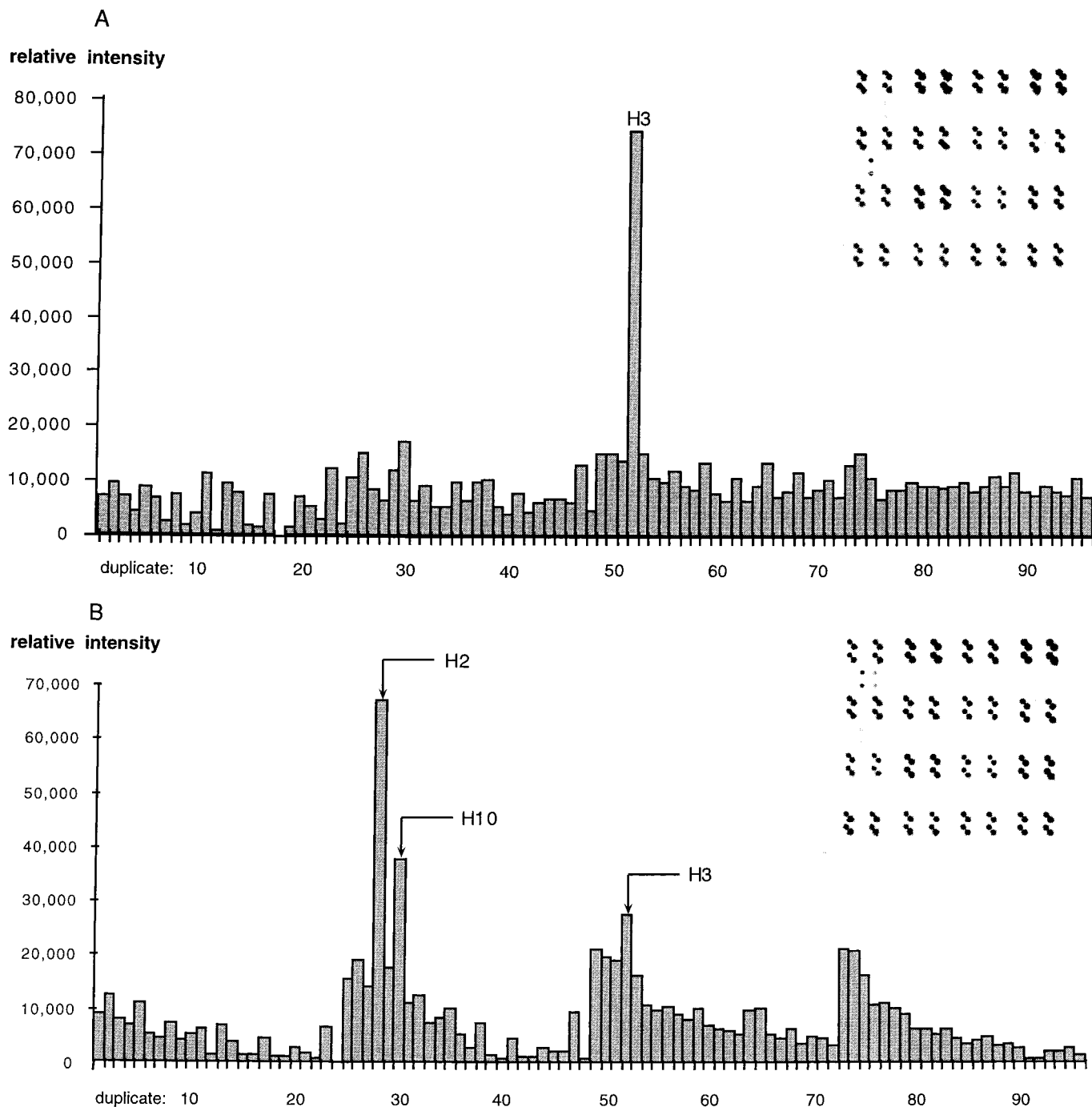


FIG. 4. Specificity testing of three monoclonal antibodies on identical microarrays of RGS-His₆-tagged fusion proteins expressed from clones of the arrayed hEx1 library as in Fig. 3. (A) Monoclonal anti-GAPDH (H3, GAPDH, clone D215, vector pQE-30NST); (B) monoclonal anti-HSP90 α (H2, bHSP90 α , clone N15170, vector pQE-BH6; H10, 60S ribosomal protein L18A; H3, GAPDH, clone D215, vector pQE-30NST); (C) monoclonal anti- α tubulin (F9 and A4, RF(+) α tubulin clones; C7, RF(-) α tubulin clone; B1 and B12, unknown genes; H3, GAPDH, clone D215, vector pQE-30NST; G1, RF(-) β tubulin clone; E5, RPL3 ribosomal protein L3; H10, RPL18A ribosomal protein L18A; E6 and D8, RPS2 ribosomal protein S2; F7, RPS3A ribosomal protein S3A; E3, RPS25 ribosomal protein S25); specificity threshold arbitrarily set to 25,000 relative intensity.

der these conditions, a negative control (H1, vector pQE-30NST without insert) was clearly negative (1500 relative intensity), as was an HSP90 α clone, featuring

a divided RGS-His₆ epitope (H2, vector pQE-BH6; 0 relative intensity). The lysate of an RGS-His₆-tagged GAPDH clone (H3, vector pQE-30NST) was used as a

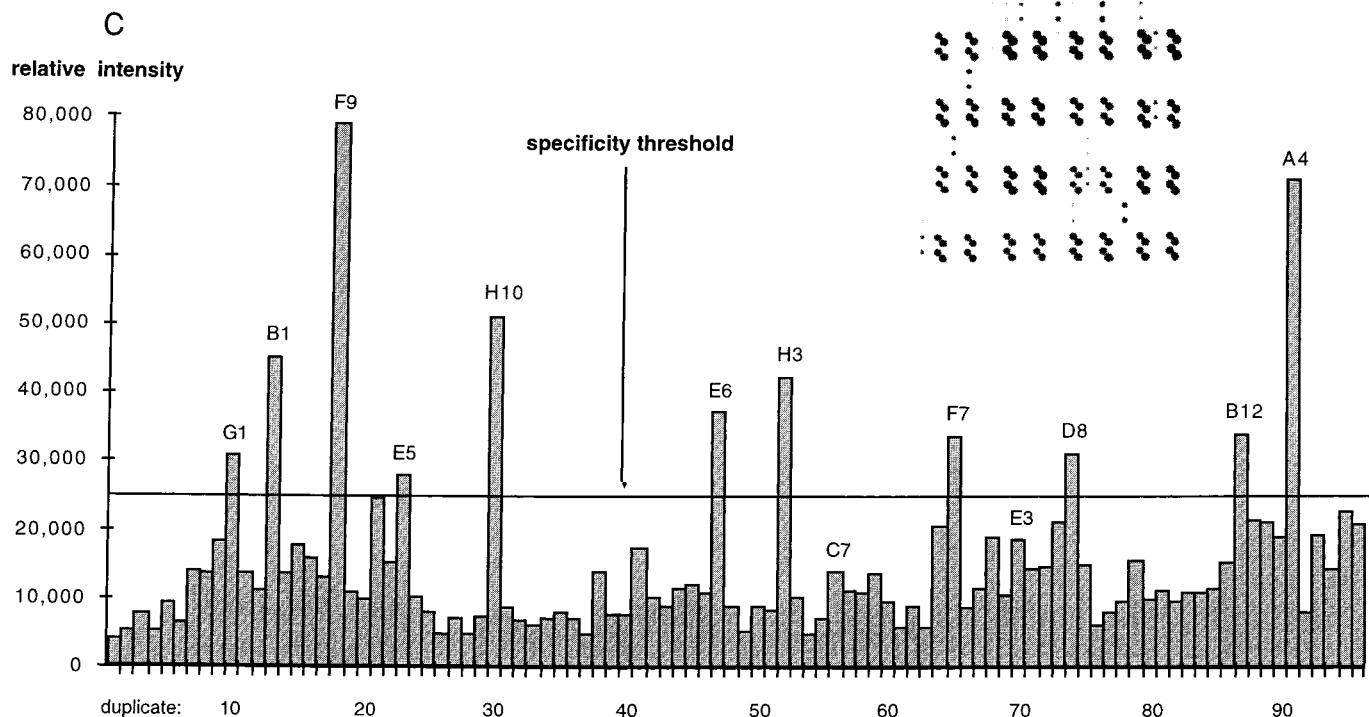


FIG. 4—Continued

positive control and delivered a signal of 21,000 relative intensity. The clearly positive result (15,000 relative intensity) obtained with a rat BIP clone (H4, vector pQE-BH6) is surprising because this clone also features a divided RGS-His₆ epitope. The reactivity might be explained by partial reconstitution of the RGS-His₆ epitope due to conformational characteristics of BIP.

The cDNA inserts of 54 of the 92 putative hEx1 expression clones show homology to GenBank entries of human genes (27). These inserts were checked for their reading frames (RF) in relation to the vector-encoded RGS-His₆ tag sequence. Thirty-four inserts (63%) were found to be cloned in the correct reading frame (RF+), while 20 (37%) were in an incorrect reading frame (RF-); hence, those clones could not be expected to express the predicted protein. However, all 92 clones were originally selected as protein expressors on *in situ* filters due to clearly positive signals with the monoclonal antibody RGS-His (intensity levels 2 and 3) (27). On microarrays, the number of incorrect reading frame clones identified as protein expressors was decreased by 70%, as only 6 RF(-) clones were still confirmed as positives (Fig. 3B). This indicates that the new microarray technology is a major advancement over *in situ* filters for its superior ability to exclude incorrect reading frame clones. On the other hand, only one RF(+) clone was clearly below the specificity threshold and would have been missed in this screen, probably due to an insufficient amount of protein ex-

pressed in the microtiter well. This stresses again the nature of our approach that is exclusively based on "positives" to be confirmed by sequencing and/or protein characterization (21).

In summary, our high-throughput protein expression screening on microarrays resulted in a false-negative rate of under 2% (1 undetected RF(+) clone per 54 clones total). The rate of false-positive clones, expressing proteins in incorrect reading frames, was down to 11%, compared to 37% on *in situ* filters (27). That makes protein microarrays an economical tool for very sensitive protein expression screening.

Antibody Specificity Screening

Protein microarrays featuring the same test set of 92 hEx1 expression clones and 4 controls (see above) were screened for the human proteins GAPDH, HSP90 α and α tubulin using monoclonal antibodies. While the anti-GAPDH antibody detected its target antigen exclusively (H3, Fig. 4A), anti-HSP90 α preferentially recognized its target antigen (H2, Fig. 4B) but showed some cross-reactivity with at least two other clones (H10, 60S ribosomal protein L18A and H3, GAPDH). Antibody cross-reactivity was even more pronounced in the anti- α tubulin screen (Fig. 4C). While the two RF(+) α tubulin clones in the test set (F9 and A4) were specifically recognized and the only RF(-) clone (C7) was left undetected, nine other clones showed anti- α tubulin

reactivity above the arbitrary specificity threshold. Two of these clones (B1 and B12) represent unknown genes, and G1 is an RF(−) β tubulin clone. H3 is the GAPDH positive control clone of Fig. 3 (see above), which to some extent seems to cross-react unspecifically (Figs. 4B and 4C), possibly due to an exceptionally high level of protein expression. Surprisingly, all other (five) clones above threshold express ribosomal proteins in a correct reading frame (E5, RPL3; H10, RPL18A; E6 and D8, RPS2; F7, RPS3A). Only one additional ribosomal protein in the test set (E3, RPS25) did not show an anti- α tubulin reactivity. The epitope recognized by the anti- α tubulin antibody (YL1/2) (28) was identified as the linear sequence spanning the carboxy-terminal residues of tyrosinated α tubulin (29). According to those authors, the minimal sequence requirements, as defined by dipeptide studies, are a negatively charged side chain in the penultimate position followed by an aromatic residue which must carry the free carboxylate group. Because none of the cross-reacting ribosomal proteins on our microarrays fulfill these requirements, other (e.g., structural) epitopes might mimic the antigenic specificity.

In summary, the detection of antibody cross-reactivities on protein microarrays is not surprising because antibodies are not usually tested against whole libraries of proteins. This suggests an interesting application of the described technology for screening antibodies against arrays of potential antigens to detect common epitopes. This seems particularly important for reagents that are to be used for immunohistochemistry or physiological studies on whole cells or tissues, where they face batteries of different structures. Using the same technology, antibodies with no known antigen specificity (e.g., lymphoma proteins) can be screened for binding to a highly diverse repertoire of protein molecules. Because all of these proteins are expressed from isolated clones of arrayed cDNA libraries, the corresponding inserts can easily be sequenced to identify antigen-encoding genes. Such an approach can be used for homology studies on protein families, defining binding domains and epitopes. Furthermore, the technique is not limited to antigen–antibody screening but should be applicable to any ligand–receptor system.

CONCLUSIONS

We have shown that protein microarrays provide the means for very sensitive gene expression and antibody specificity screening at high throughput. Using a new transfer stamp, 4800 samples can be placed onto a microscopic slide and simultaneously screened, applying minimal amounts of reagents. Sharp and well localized signals allow the detection of 250 amol or 10 pg of a spotted test protein (GAPDH). Protein expression clones of a cDNA library are reli-

ably detected, with a low rate (11%) of false-positive clones expressing proteins in incorrect reading frames. Antibodies can be screened for specificity against whole libraries of proteins.

In the context of genomics and proteomics, protein microarrays are a useful tool for connecting gene expression analysis and molecular binding studies on a whole-genome level. If differentially expressed genes are identified using cDNA microarrays, the same clones can be analyzed simultaneously for protein expression in different cellular systems or by *in vitro* transcription/translation. On identical protein microarrays, expression clones can be screened for binding to other proteins (e.g., antibodies) or to a plethora of diverse molecules from nucleic acids to small-molecule ligands. This versatility should make protein microarrays a multipurpose tool for diagnostic use.

ACKNOWLEDGMENTS

This work was funded by the Bundesministerium für Bildung und Forschung (BMBF Grants 0311018 and 0311323) and the Max-Planck-Gesellschaft.

REFERENCES

1. Wolfsberg, T. G., and Landsman, D. (1997) *Nucleic Acids Res.* **25**, 1626–1632.
2. Strachan, T., Abitbol, M., Davidson, D., and Beckmann, J. S. (1997) *Nat. Genet.* **16**, 126–132.
3. Meier-Ewert, S., Maier, E., Ahmadi, A., Curtis, J., and Lehrach, H. (1993) *Nature* **361**, 375–376.
4. Meier, E. S., Lange, J., Gerst, H., Herwig, R., Schmitt, A., Freund, J., Elge, T., Mott, R., Herrmann, B., and Lehrach, H. (1998) *Nucleic Acids Res.* **26**, 2216–2223.
5. Lennon, G. G. and Lehrach, H. (1991) *Trends Genet.* **7**, 314–317.
6. Schena, M., Heller, R. A., Theriault, T. P., Konrad, K., Lachenmeier, E., and Davis, R. W. (1998) *Trends Biotechnol.* **16**, 301–306.
7. Jordan, B. R. (1998) *J. Biochem.* **124**, 251–258.
8. Ramsay, G. (1998) *Nat. Biotechnol.* **16**, 40–44.
9. Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. (1995) *Science* **270**, 467–470.
10. Shalon, D., Smith, S. J., and Brown, P. O. (1996) *Genome Res.* **6**, 639–645.
11. DeRisi, J. L., Iyer, V. R., and Brown, P. O. (1997) *Science* **278**, 680–686.
12. Lashkari, D. A., DeRisi, J. L., McCusker, J. H., Namath, A. F., Gentile, C., Hwang, S. Y., Brown, P. O., and Davis, R. W. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 13057–13062.
13. Cho, R. J., Campbell, M. J., Winzler, E. A., Steinmetz, L., Conway, A., Wodicka, L., Wolfsberg, T. G., Gabrielian, A. E., Landsman, D., Lockhart, D. J., and Davis, R. W. (1998) *Mol. Cell.* **2**, 65–73.
14. Wodicka, L., Dong, H., Mittmann, M., Ho, M. H., and Lockhart, D. J. (1997) *Nat. Biotechnol.* **15**, 1359–1367.
15. Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O., and Herskowitz, I. (1998) *Science* **282**, 699–705.

16. Schena, M., Shalon, D., Heller, R., Chai, A., Brown, P. O., and Davis, R. W. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 10614–10619.
17. Heller, R. A., Schena, M., Chai, A., Shalon, D., Bedilion, T., Gilmore, J., Woolley, D. E., and Davis, R. W. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 2150–2155.
18. DeRisi, J., Penland, L., Brown, P. O., Bittner, M. L., Meltzer, P. S., Ray, M., Chen, Y., Su, Y. A., and Trent, J. M. (1996) *Nat. Genet.* **14**, 457–460.
19. Trent, J. M., Bittner, M., Zhang, J., Wiltshire, R., Ray, M., Su, Y., Gracia, E., Meltzer, P., De, R. J., Penland, L., and Brown, P. (1997) *Clin. Exp. Immunol.* **1**, 33–40.
20. Welford, S. M., Gregg, J., Chen, E., Garrison, D., Sorensen, P. H., Denny, C. T., and Nelson, S. F. (1998) *Nucleic Acids Res.* **26**, 3059–3065.
21. Büsow, K., Cahill, D., Nietfeld, W., Bancroft, D., Scherzinger, E., Lehrach, H., and Walter, G. (1998) *Nucleic Acids Res.* **26**, 5007–5008.
22. Lehrach, H., Bancroft, D., and Maier, E. (1997) *Interdiscip. Sci. Rev.* **22**, 37–44.
23. Hochuli, E., Dobeli, H., and Schacher, A. (1987) *J. Chromatogr.* **411**, 177–184.
24. Mooney, J. F., Hunt, A. J., McIntosh, J. R., Liberko, C. A., Walba, D. M., and Rogers, C. T. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 12287–12291.
25. Ekins, R. P. (1998) *Clin. Chem.* **44**, 2015–2030.
26. Silzel, J. W., Cercek, B., Dodson, C., Tsay, T., and Obremski, R. J. (1998) *Clin. Chem.* **44**, 2036–2043.
27. Büsow, K. (1998) Arrayed cDNA Expression Libraries for Antibody Screening and Systematic Analysis of Gene Products. Thesis, Department of Chemistry, Free University Berlin.
28. Kilmartin, J. V., Wright, B., and Milstein, C. (1982) *J. Cell Biol.* **93**, 576–582.
29. Wehland, J., Schroder, H. C., and Weber, K. (1984) *EMBO J.* **3**, 1295–1300.