A method for global protein expression and antibody screening on high-density filters of an arrayed cDNA library

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ABSTRACT

We have developed a technique to establish catalogues of protein products of arrayed cDNA clones identified by DNA hybridisation or sequencing. A human fetal brain cDNA library was directionally cloned in a bacterial vector that allows IPTG-inducible expression of His$_6$-tagged fusion proteins. Using robot technology, the library was arrayed in microtitre plates and gridded onto high-density in situ filters. A monoclonal antibody recognising the N-terminal RGS$_{H\alpha}$ sequence of expressed proteins (RGS-H$_\alpha$ antibody, Qiagen) detected 20% of the library as putative expression clones. Two example genes, GAPDH and HSP90$\alpha$, were identified on high-density filters using DNA probes and antibodies against their proteins.

For construction of the human expression library hEx1, cDNA was prepared from fetal brain poly(A)$^+$ RNA by oligo(dT)-priming (Superscript Plasmid System, Life Technologies). Products were size-fractionated by gel filtration and directionally ($SalI$–$NotI$) cloned into a modified pQE-30 (Qiagen) vector for IPTG-inducible expression of His$_6$-tagged fusion proteins (pQE-30NST, GenBank accession no. AF074376). *Escherichia coli* SCS1 cells (Stratagene) carrying the plasmid pSE111 with the lacI<sup>0</sup> repressor and the argU gene for a rare arginine tRNA (1) were transformed by electroporation. PCR analysis of 96 clones revealed an average insert size of $\sim$1.5 kb (range 0.5–5.0 kb).

The library was plated onto 2×YT-AKG agar plates (230 mm×230 mm Nunc Bio Assay Dishes containing 2×YT agar, 100 $\mu$g/ml ampicillin, 15 $\mu$g/ml kanamycin and 2% glucose) and grown at 37°C overnight. Using a picking/gridding robot (2), 193,536 colonies were picked into 384-well microtitre plates (Genetix). Colony size was inspected by gel filtration and directionally (SalI–NotI) cloned into a modified pQE-30 (Qiagen) vector for IPTG-inducible expression of His$_6$-tagged fusion proteins (pQE-30NST, GenBank accession no. AF074376). *Escherichia coli* SCS1 cells (Stratagene) carrying the plasmid pSE111 with the lacI<sup>0</sup> repressor and the argU gene for a rare arginine tRNA (1) were transformed by electroporation. PCR analysis of 96 clones revealed an average insert size of $\sim$1.5 kb (range 0.5–5.0 kb).

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For global protein expression, high-density filters were screened with the monoclonal antibody RGS-H$_\alpha$ (Qiagen). This antibody recognises the N-terminal sequence RGS$_{H\alpha}$ of fusion proteins over-expressed from pQE-30 vectors and labelled $\sim$20% of the hEx1 clones (Fig. 1A). Negative clones have inserts in incorrect reading frames with stop codons leading to short polypeptides that cannot fold into stable structures and are degraded within the host cell (4). Two example proteins, GAPDH (35.9 kDa, Swiss-Prot P04406) and HSP90$\alpha$ (84.5 kDa, Swiss-Prot P07900) were chosen for detailed analysis. A set of three DNA filters (80 640 clones) were screened with cDNA probes. Two hundred and six (0.26%) clones were positive with a human GAPDH probe (Fig. 1B), and 56 (0.07%) clones were identified with a human HSP90$\alpha$ probe. About 25% of these clones were positive with the RGS-H$_\alpha$ antibody. To confirm the expression of GAPDH or HSP90$\alpha$ proteins by these clones, protein filters were screened with antibodies against GAPDH (Fig. 1C) or HSP90$\alpha$, respectively. Fifty-seven percent of the GAPDH and 72% of the HSP90$\alpha$ clones detected by the RGS-H$_\alpha$ antibody were also positive with the protein-specific antibodies. Sequence analysis showed that the remaining clones had inserts in an incorrect reading frame or expressed truncated GAPDH which reacted poorly with the GAPDH antibody.

In turn, 100% of the anti-GAPDH- but only 35% of the anti-HSP90$\alpha$-positive clones were detected by the RGS-H$_\alpha$ antibody. All RGS-H$_\alpha$-negative HSP90$\alpha$ clones had inserts in incorrect reading frames but nevertheless expressed proteins detected by the HSP90$\alpha$ antibody on western blots (data not shown). This indicates HSP90$\alpha$ molecules without a His$_6$ tag, suggesting translational start sites within cDNA inserts. Three anti-HSP90$\alpha$ positive clones contained inserts that were not recognised by the cDNA probe and turned out to be unrelated.

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positive clones to be confirmed by sequencing and/or protein characterisation. Therefore, our approach is exclusively based on positive clones
arbitrary thresholds for manual or automated image analysis. Quantification of signal intensities on filters is largely based on
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We envisage two main fields of application for our method. First, catalogues of protein products can be established for
different tissues and developmental stages. As these proteins are expressed from arrayed cDNA clones, their identity can easily be
checked by high-throughput gene identification techniques (e.g. oligonucleotide fingerprinting; 6). Therefore, gene expression
patterns of normal and diseased tissues can be translated to the
protein level, keeping a direct link to already existing DNA
sequence data. Second, our method should also enable high-
throughput analysis of antibody specificity and other protein–protein
interaction or ligand-receptor systems (7), including non-protein
molecules.

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