grill into a square Petri dish before pouring the agar, and the grill remains embedded in the media. Small glass beads can be added to each segment of the 48 grids to spread the solution. Colonies are picked by a colony picker, and are grown in 96-well deep-well blocks containing media and appropriate antibiotics. Using a liquid-handling robot and a colony picker for a single round of cloning, it is possible to achieve >80% efficiency in a 96-well format. The same robotic setup can be used for the subsequent recovery of recombinant plasmid before DNA sequencing and protein expression.

4.4.4. Protein expression and purification

Small-scale protein expression in an HT format enables evaluation of the expression, solubility and purification of target proteins or multiple constructs. Autoinduction allows many protein expression tests in parallel without having to monitor cell densities to optimize induction, and is thus ideal for HT approaches. Each protein in a 96-well plate will have different chemical properties and sizes, and thus affinity tags are the method of choice for parallel purification of multiple proteins on a single platform. Dot blots can be used to test expression or solubility, and miniaturized resin deposited in a 96-well plate can be used for a purification test. Although up to 90 µg of protein have been purified from 1 ml of *E. coli* cell culture (Scheich *et al.*, 2003), the amount of protein obtained from small-scale purification is generally not enough for initial crystallization trials, even with low-volume crystallization, and thus scaled-up protein production and purification is needed. The purification tags can be removed upon large-scale purification.

4.4.4.1. Autoinduction

Protein expression under control of the T7 *lac* promoter system can be induced either with the chemical inducer isopropyl-β-galactoside (IPTG) or by autoinduction using a mixture of glucose, glycerol and lactose during *E. coli* growth. In autoinduction, cells grow to relatively high density in a defined ratio of glucose-to-lactose media (Studier, 2005). Initially, glucose prevents induction by lactose. When available glucose is used up, lactose then induces target protein expression switching to lactose metabolism.

Cell cultures (1 ml) are grown in 2.2 ml deep-well 96-well blocks. Protein expression is autoinduced using commercially available glucose/lactose media (*e.g.* Overnight Express from EMD Biosciences). The following day, cells are lysed either by repeated freezing and thawing cycles, or by addition of a lysozyme solution. A combination of lysozyme and benzonase solutions (*e.g.* PopCulture reagent from Novagen) eliminates the need for cell harvesting prior to lysis and lysate clarification following lysis. The cell lysate can then be directly used for expression and purification tests. Alternatively, whole-cell lysates can be filtered though a 96-well filter plate, allowing for separation of inclusion bodies from the soluble fraction (filtrate) so that protein expression in the soluble fraction can be assessed.

4.4.4.2. Expression and solubility test: dot blot

Dot blot is a simple method that can be used to analyse either total protein expression in the cell lysate, or soluble protein expression following the separation of supernatant and cell pellet. Protein samples from the total cell lysate in a 96-well plate are dotted onto a nitrocellulose membrane by applying a vacuum. Target proteins are then probed with an antibody against the protein or against an affinity tag, *e.g.* anti-His-tag antibody for His-tagged protein detection (Fig. 4.4.3.1*c*). Alternatively, the cell lysates are analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) for protein expression using a pre-cast 48- or 96-well SDS gel (E-PAGE 48 or 96 from Invitrogen). The gel consists of 48 or 96 wells for samples, and an additional four or eight wells for protein markers. The protein can be loaded either with a liquid-handling robot or with a multichannel pipettor, and electrophoresis is completed within 15 min (Fig. 4.4.3.1*d*).

4.4.4.3. Small-scale purification

Affinity purifications are preferred in a 96-well format because the specific interaction between the protein and affinity resin allows for a simple 'bind–wash–elution' procedure. Purification using a His-tag or a GST-tag (where GST = glutathione S-transferase) is popular in structure determination projects, and appropriate resins are available as magnetic beads or agarose resin in a 96-well plate, *i.e.* magnetic Ni-NTA or GST beads, or Ni- or Co-linked agarose discs, respectively.

The soluble fraction or whole-cell lysate is transferred to a 96-well plate containing an affinity resin for protein binding. The beads are separated from unbound protein by placing them in a magnetic stand designed to accommodate a 96-well format, or by filtration using a 96-well filter plate that retains the beads but allows passage of the cell lysate. Beads are washed, and bound protein is then eluted with the appropriate elution buffer (*i.e.* imidazole and reduced glutathione for His-tagged and GST-tagged proteins, respectively) into a 96-well plate by applying a vacuum (either as part of an appropriately equipped liquid-handling robot, or by using a 96-well vacuum manifold) or by centrifugation. Purified proteins are analysed by SDS–PAGE using a pre-cast 48- or 96-well protein gel. The gels can then be stained either by protein staining or western blotting. A new system for protein and DNA analysis has been developed based on a microfluidic chip (Caliper Labchip, Caliper Life Science). These chips contain a network of miniaturized channels, through which fluids and chemicals are moved to separate DNAs or proteins. The DNA and protein signal is measured by laser-induced fluorescence. The instrument is capable of separating 2 ng–2 µg amounts of protein, one well after another; it takes about 1 h to analyse a 96-well plate.

4.4.5. Crystallization

HT crystallization using 96-well crystallization plates greatly reduces the total amount of protein required for screening and thus structure determination. Robotic crystallization systems are capable of dispensing nanolitre droplets (<100 nl) and hence substantially increase the number of conditions that can be screened with a fixed amount of protein sample, as well as reducing the time required for setting up a series of crystallization trials. A thousand conditions with different crystallization parameters (*e.g.* pH, salts, temperature) can be screened with 100 µl of protein sample. At the time of writing, commercially available crystallization robots include the Honey Bee (Cartesian Micro-array), Phoenix (Rigaku), Mosquito (Molecular Dimensions Limited) and Oryx (Douglas Instrumentation). These crystallization robots can set up either vapour-diffusion, microbatch or hanging-drop methods in 96-well plates within 15 min. Crystallization robots can also be integrated into a larger