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Well-less, gel-permeation formats for ultra-HTS

David J. Burns, James L. Kofron, Usha Warrior and Bruce A. Beutel

An alternative approach to miniaturization has been developed that does not rely upon increasing microtiter plate densities. Gel-permeation assays, originally developed for screening antibody–antigen interactions and anti-microbial screening, are a well-less technique. Newer approaches extending this technology to enable the screening of traditional compound-collections show exceptional promise and offer the potential to convert most (if not all) 96-well assays into this novel high-density format.

***David J. Burns, James L. Kofron, Usha Warrior and Bruce A. Beutel**
Abbott Laboratories
D4PN and D47P
100 Abbott Park Road
Abbott Park, IL 60064, USA
*fax: +1 847 935 4200
e-mail: david.j.burns@abbott.com

▼ Sample collections within most pharmaceutical companies contain approximately 200,000–1,000,000 unique chemical entities from a variety of sources[1]. The sheer volume of compounds has forced these companies to rely on one or more of the following strategies to streamline lead discovery:

- Pooling of compounds for screening
- Use of diversity algorithms to select specific subsets of the compound library for screening
- Miniaturization of assay components before screening.

Only by adopting the miniaturization strategy can a pharmaceutical company economically survey their entire compound-collection as single entities for potential leads. Most attempts to further miniaturize HTS have focussed on increasing plate densities (384, 1536 and 3456 wells), but numerous hurdles still exist for converting many traditional 96-well assays into these formats[2].

Origins of well-less assay formats

Early assays developed for immunological and antimicrobial screening used porous gel matrices (primarily agar or agarose) to detect antigen–antibody interactions or potential anti-bacterial agents[3]. In anti-microbial screening, compounds have generally been spotted directly onto a thin agarose matrix containing the appropriate cells; the compounds then diffuse into the gel and interact with the cells. Zones of inhibition correspond to lack of cell growth and indicate the presence of compounds with potential anti-microbial activity. Such assay formats are still in use today with minimal modifications. For example, American Cyanamide (Princeton, NJ, USA) uses solidified agar containing a variety of modified yeast strains for screening compounds that act on G-protein-coupled receptors (GPCRs)[4,5]. Aventis has designed a fully-automated robotic workstation to screen anti-microbial compounds using, primarily, positive-growth readout assays[6]. Relevant bacterial or yeast strains are incorporated into liquid agar medium before being poured into a Petri plate. Test compounds are then spotted directly onto the agar surface (96 compounds per plate) and evaluated for growth induction.

Ultra-HTS of combinatorial bead-based libraries

The use of agarose and/or agar matrices for ultra-HTS (uHTS) did not begin until the mid-90s with the rise of solid-phase synthesis and the generation of large combinatorial (mix-split) libraries. Mix-split libraries pose unique challenges to screening because they comprise vast mixtures of very small beads, where each bead contains a unique covalently-bound compound at a low quantity (typically <1 nmol)[7]. A major challenge for screening random mix-split libraries, therefore, is manipulation of the beads, release of bead-based compounds, and identification of the hits. For these reasons, conventional plate formats were not well-suited to the screening of bead-based combinatorial libraries and have therefore led to the development of new screening techniques. Methods developed to screen bead-based combinatorial libraries have variously been referred to as: zone format, free format, lawn format, field format, gel-permeation format, gel-diffusion format,

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combinatorial diffusion format and two-dimensional agar format. All of the assays described in the literature are simple homogeneous or mix-and-read type formats and can be divided into two main classes. First, those that incorporate cells (bacterial and mammalian among others) directly into the gel matrix, and second, those that use two-gel systems for assaying simple enzymatic activities.

Cell-based gel-permeation assays for screening combinatorial libraries

An early report documenting the use of a gel-based bioassay to screen a combinatorial peptide library was published in 1994 by Michael Lerner's lab (Yale School of Medicine, New Haven, CT, USA)[8]. In this simple assay system, cells derived from *Xenopus laevis* epithelium (melanophores) were used to identify peptide agonists for a specific GPCR. Experimentally, the melanophore cells were grown in circular plates and overlaid with low-melt agarose. After solidification, the agarose was overlaid (face-down) with a polyethylene film containing a random array of pre-cleaved combinatorial peptide beads. Peptides from specific beads diffuse through the gel matrix and interact with specific receptors on the melanophores. Following receptor activation, pigments in the cells will either aggregate (turning the cells lighter in colour) or disperse (turning the cells darker). In Lerner's paper, potential bombesin receptor agonists were identified as dark spots (pigment dispersion) in a field of clear cells. Recently, a group from GlaxoSmithKline (Research Triangle Park, NC, USA) successfully screened a 442,368 bead-based peptide library for receptor agonists using the melanophore technology[9]. These cells have also been used successfully to screen combinatorial peptide libraries for antagonists in the gel-permeation format (see Fig. 1)[10,11]. In summary, the use of recombinant melanophore cells in a well-less gel-permeation format is useful for rapidly and efficiently screening large, bead-based combinatorial libraries for GPCR agonists and antagonists. This is primarily because of the homogenous nature of the cell-based assay and the inherent simplicity of the assay readout (simple black-and-white images).

Mammalian cells have also been used in a gel-diffusion format to screen bead-based combinatorial libraries for anticancer agents[12]. Salmon and colleagues placed tumor cells directly into agarose gels in the presence of combinatorial beads and tissue culture medium. The neutral-pH environment of the tissue culture medium in the gel enables cleavage of the linkers that tether the compounds to the combinatorial beads. Compounds that are cytotoxic to the tumor cells are identified visually as zones of diminished cell growth. Although the technique is similar to the melanophore technology already described, it is not as versatile because only the agents that either kill cells or prevent their proliferation can be identified.

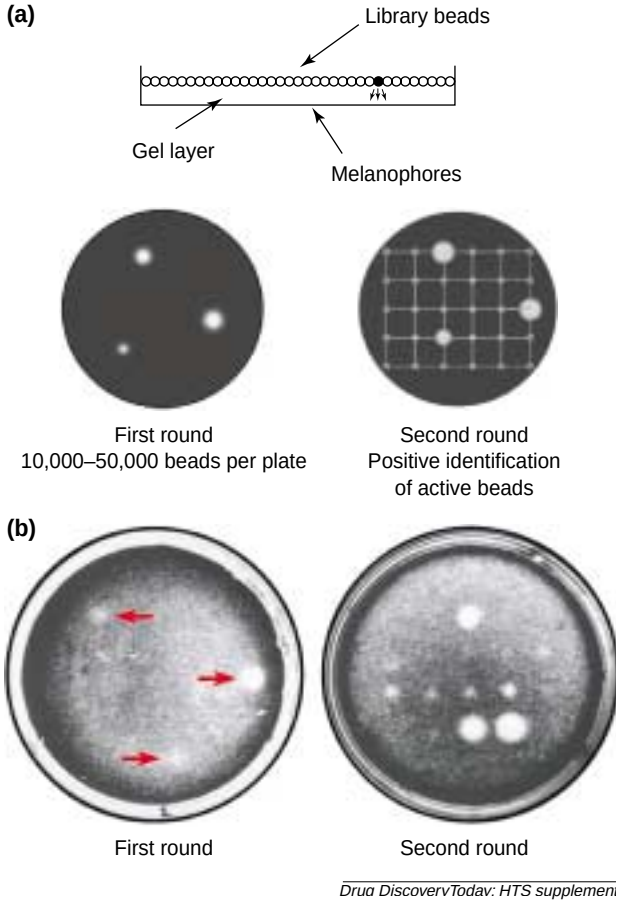


Figure 1. Melanophore antagonist screen. **(a)** A typical bead-based screen for the identification of melanocortin-receptor antagonists. Typically, when combinatorial libraries are screened, two rounds of screening are performed. In the first round, partially-cleaved active beads are first identified and then collected for a second round of screening. The second round of screening is necessary because, during the isolation of potential active beads from the first screen, some inactive (contaminating) beads will also be collected. The second round therefore ensures the positive identification of active beads. **(b)** In this experiment, melanophore cells (within an agarose layer) are first treated with a mixture of melatonin (1 nM) and melanocyte stimulating hormone (MSH) (2.5 nM) to keep the cells in a pigment dispersed state (dark background). This combination of compounds maintains the cells in a dispersed state as the effect of the G_s-coupled melanocortin receptor overrides that of the G_i-coupled melatonin receptor. After application of cleaved combinatorial beads from a 31,360-member combinatorial library based on α-MSH [5–13] peptide to the surface of the gel layer, the compounds diffuse into the gel and interact with receptors on the melanophore cell-surface. Antagonists that inhibit the melanocortin receptor appear as light zones on a dark background because the effect of melatonin (induction of pigment aggregation) in those areas of the gel is no longer blocked by the effect of MSH.

In addition to mammalian cells, gel-permeation formats employing whole bacterial and yeast cells have also been readily used to screen combinatorial libraries for antimicrobial activity. Silen and colleagues used *Bacillus subtilis* cells embedded in

an agarose matrix to screen a 46,656-member bead-based combinatorial library for novel anti-microbial compounds[13]. Again, the simple nature of the assay format (cells contained within a gel) coupled with a straightforward readout (inhibition of cell growth) make this a simple method for screening bead-based libraries. Oldenburg and colleagues used a dual-culture assay in an agar format to interrogate a bead-based combinatorial library for anti-bacterial and anti-fungal activity simultaneously[14]. In this instance, the bacterial cells were manipulated to express a reporter gene (green fluorescent protein) and the yeast cells were engineered with a gene that enabled growth to be monitored by the activity of an enzyme. Again, this format is a simple mix-and-read type of gel-permeation assay, but is more versatile in terms of readout and complexity.

Enzymatic gel-permeation assays for screening combinatorial libraries

Methods similar to cell-based gel-permeation formats have also been developed for simple enzymatic assays but, rather than using a single gel, the majority of these assays require two gel matrices. Generally, for these simple mix-and-read assays, the first gel contains the enzyme and the second gel contains a substrate that is specific to the enzyme. Beads containing the test compounds can be introduced directly into the enzyme gel and then cleaved, or the combinatorial beads can be arrayed (randomly or ordered) onto a plastic surface or culture dish and cleaved before addition of the enzyme gel. Scientists at Pharmacopia (Princeton, NJ, USA) have converted a plate-based enzymatic assay into a gel-permeation format to screen random, mix-split libraries[15]. The gel-permeation assay or 'field format' assay for the enzyme carbonic anhydrase was set up in two steps. First, enzyme and buffer were incorporated into low-melt agarose and then layered over cleaved combinatorial beads containing potential inhibitors in a circular tissue culture plate. After solidification of the enzyme gel, a second gel layer containing a fluorogenic substrate (fluorescein diacetate) was added. After an adequate reaction time, the esterase activity of the enzyme cleaves the substrate thus generating a fluorescent signal. Dark zones in this field of fluorescence are indicative of potential inhibitors of the enzyme[16]. Therefore, gel-permeation formats for simple enzyme assays are also possible and are similar to previously described cell-based assays. However, there are no reports in the literature documenting the conversion of more-complex plate-based assays (especially those involving several transfer and washing steps) into this gel-diffusion format.

Ultra-HTS of archived compound-collections

Essentially, the gel-permeation format is a method for miniaturizing microtiter plate assays to enable the screening of compounds that are synthesized on solid supports (e.g. beads). In theory, any assay that can be performed in a 96-well plate can be converted into such a format. As already described, simple enzyme assays and whole-cell-based assays are the easiest to adapt to the gel-permeation format because they are, typically, homogenous in nature. Extending the gel-diffusion technology to non-homogeneous (heterogeneous) assay formats is inherently more difficult and unprecedented. Scientists at Abbott Labs (Abbott Park, IL, USA) were the first to demonstrate that this convenient gel-permeation format was actually applicable to many different types of assays – heterogeneous as well as homogeneous[17,18] (Schurdak, M.E. *et al.*, unpublished results). More importantly, they also extended the technology to enable the screening of any test compound from archived compound collections (Dandliker, P.J. *et al.*, unpublished results), not just from bead-based combinatorial libraries.

Arrayed compound screening platforms

In Abbott's arrayed compound screening (ARCS) methodology, 8,640 compounds are arrayed onto a polystyrene sheet that has the same footprint as a microtiter plate. Compounds are spotted 1 mm apart using 40 nl of a 5 mM stock of compound (~200 pmol) delivered from a Pixsys nQuad eight-tip liquid dispenser [Humphrey, P. *et al.*, Nanoliter dispensing of compound libraries for use in continuous format high-throughput screening. *5th Annual Society for Biomolecular Screening Conference and Exhibition*, 13–16 September 1999, Edinburgh, UK (Poster 103)]. One advantage of this methodology over traditional plate-screening is that it enables the stockpiling of the library in a 'ready-to-assay' format: these compound sheets are dried under vacuum and stored at –80°C until used. Another major advantage of the ARCS gel technology over miniaturized plate assays is that it has been extended to a variety of non-homogeneous assays (e.g. ELISA-based assays), as well as being completely compatible with the more-traditional homogeneous assays. Gels contain most of the assay components and addition of gel layers to an 'assay sandwich' is analogous to a pipetting step in a traditional plate-based assay. Furthermore, gels can be manipulated by washing or soaking reagents into them, and additional materials, such as membranes with specialty coatings (streptavidin, WGA or antibody) or plastics, can be introduced throughout the assay. Typically, ARCS assays are developed in 96-well plates in a similar way to traditional assays; the concentrations of reagents in the gel are often identical to those used in the plate assay. ARCS has all of the advantages associated with miniaturization (low reagent consumption and low cost), and none of the disadvantages of high-density plate screening, such as complex liquid-handling requirements, evaporation problems, plate edge-effects and extensive automation requirements.

Examples of ARCS enzyme assays: proteases, kinases and polymerases

Assays that detect inhibitors of serine proteases (e.g. urokinase or cathepsin B) are examples of simple, homogeneous ARCS assays [Anderson, S. *et al.*, A novel continuous format high-throughput screen to identify inhibitors of cathepsin B. *5th Annual Society for Biomolecular Screening Conference and Exhibition*, 13–16 September 1999, Edinburgh, UK (Poster 102)]. In Fig. 2, the urokinase enzyme and its fluorogenic substrate were incorporated into 1% low-melt agarose gels, and the assay was initiated as shown in Fig. 2a. Fig. 2b shows a typical gel from the screening of 8,640 compounds that contains dark spots on a fluorescent field, indicating potential inhibitors of urokinase. Bright spots on the gel are compounds within the collection that fluoresce under the illumination conditions. These types of interfering compounds can be eliminated by using an alternative substrate with a time-resolved fluorescence (TRF) readout (Gopalakrishnan, S.M. *et al.*, Continuous format high-throughput screening for identification of inhibitors of caspase-3. *6th Annual Society for Biomolecular Screening Conference and Exhibition*, 6–9 September 2000, Vancouver, BC, Canada (Poster 3001)].

Assays with a radiometric readout and those that require separation and/or washing steps are, technically, very difficult to miniaturize. However, ARCS provides a convenient and cost-effective alternative for running these types of heterogeneous assays. For example, the activity of any serine/threonine or tyrosine protein kinase can be assayed by the incorporation (and subsequent detection) of ^{33}[P] into a biotinylated peptide substrate [Voorbach, M. *et al.*, Continuous format high-throughput screening of serine-threonine kinases. *6th Annual Society for Biomolecular Screening Conference and Exhibition*, 6–9 September 2000, Vancouver, BC, Canada (Poster 3002)]. In a prototypical serine/threonine protein kinase ARCS assay (Fig. 3), a solution-phase kinase assay is initiated by first placing the enzyme gel in contact with the compound sheet. After removal of the compound sheet, a peptide substrate ^{33}[P]–ATP gel is then added to the enzyme gel. The biotinylated peptide migrates through the gels and is captured by a streptavidin membrane (SAM™; Promega Corporation, Madison, WI, USA). After washing and exposure of the membrane to a PhosphorImager, light zones (owing to a lack of ^{33}[P] incorporation into the peptide substrate) in a dark field indicate possible inhibitors of the kinase enzyme.

Another advantage to the ARCS platform is that any of these kinase assays can be readily converted into a non-radioactive ELISA format if substrate-specific antibodies are available. Miniaturization of an ELISA assay into a higher-density-plate format (e.g. 1536) is extremely difficult owing to the difficulties in washing high-density microwell plates.

RNA-dependent DNA-polymerases are major targets for the treatment of diseases such as HIV infection and cancer[19,20]. In the presence of magnesium, these enzymes utilize an *in vitro* transcribed RNA fragment as a template and a DNA oligonucleotide as a primer to initiate polymerization. We have adapted a heterogeneous, multi-step protocol for the RNA-dependent DNA-polymerase assay to an ARCS format [Xuei, X. *et al.*, Use of SAM™ membrane in continuous format high-throughput screening for the identification of viral replication inhibitors. *5th Annual Society for Biomolecular Screening Conference and Exhibition*, 13–16 September 1999, Edinburgh, UK (Poster 104)]. This complex gel-permeation assay (Fig. 4) utilizes a variety of reagents (including RNA and protein), numerous assay components

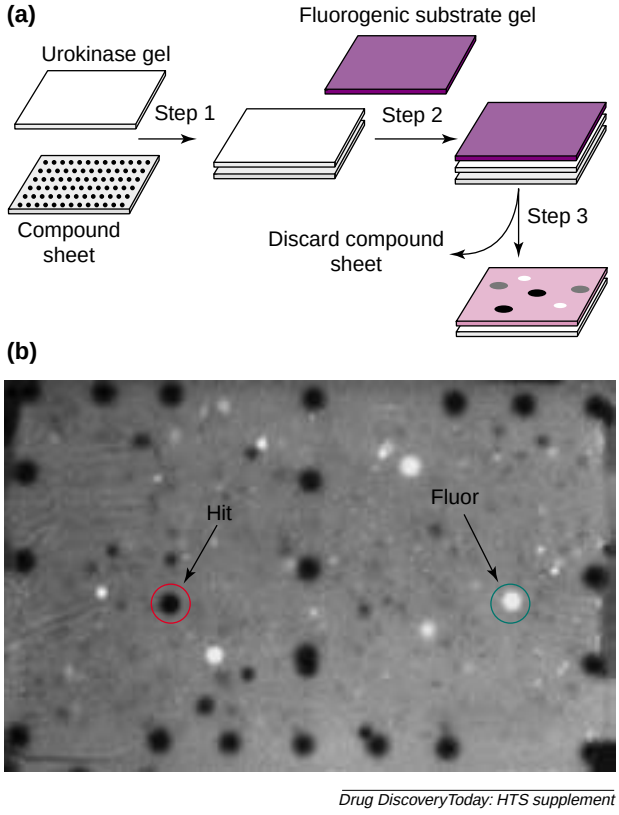


Figure 2. Urokinase arrayed compound screen (ARCS; Abbott Laboratories, Abbott Park, IL, USA). (a) In this assay protocol, the first step is to place the urokinase enzyme gel in contact with the compound sheet for 10 min to enable the compounds to diffuse into the gel matrix. A fluorogenic substrate gel [containing *N*-glutaryl-gly-arg-7-amido-4-methylcoumarin (*N*-glutaryl-GR-AMC)] is then placed on top of the sandwich in Step 2. After a 10 min reaction time, the gels are removed from the compound sheet and imaged under a charge-coupled device (CCD) camera using the appropriate excitation and emission wavelengths to detect the cleaved AMC (Step 3). (b) A sample image from an ARCS urokinase assay that has been incubated with a sheet containing 8640 compounds. The dark spots are potential inhibitors of urokinase and the light spots are fluorescent library compounds. The regular patterns of dark spots around the edges and down the center of the image are markers, which aid the software in the location of hits.

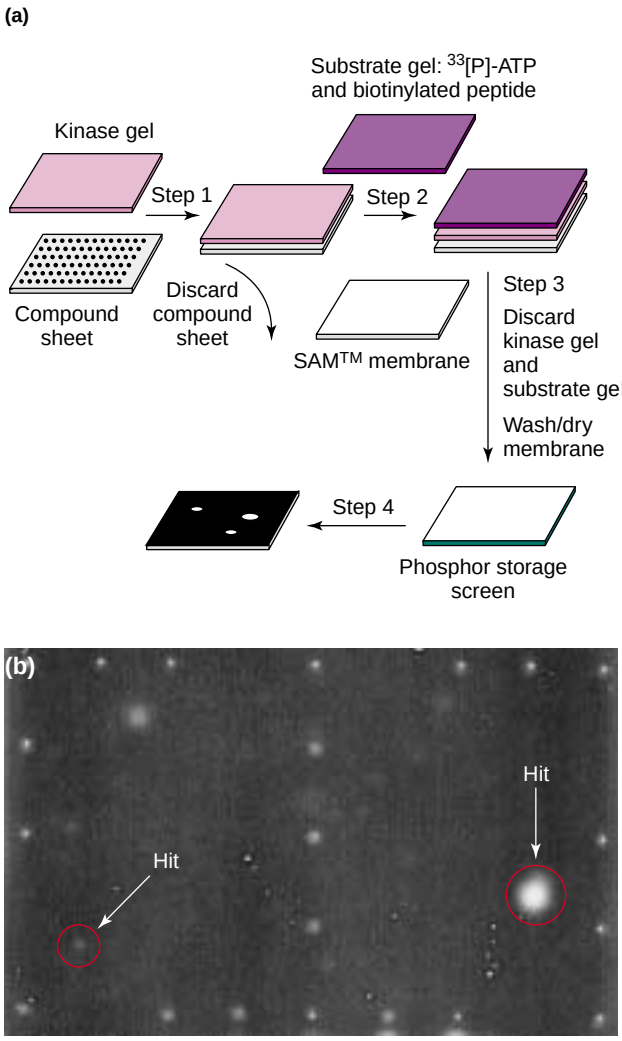


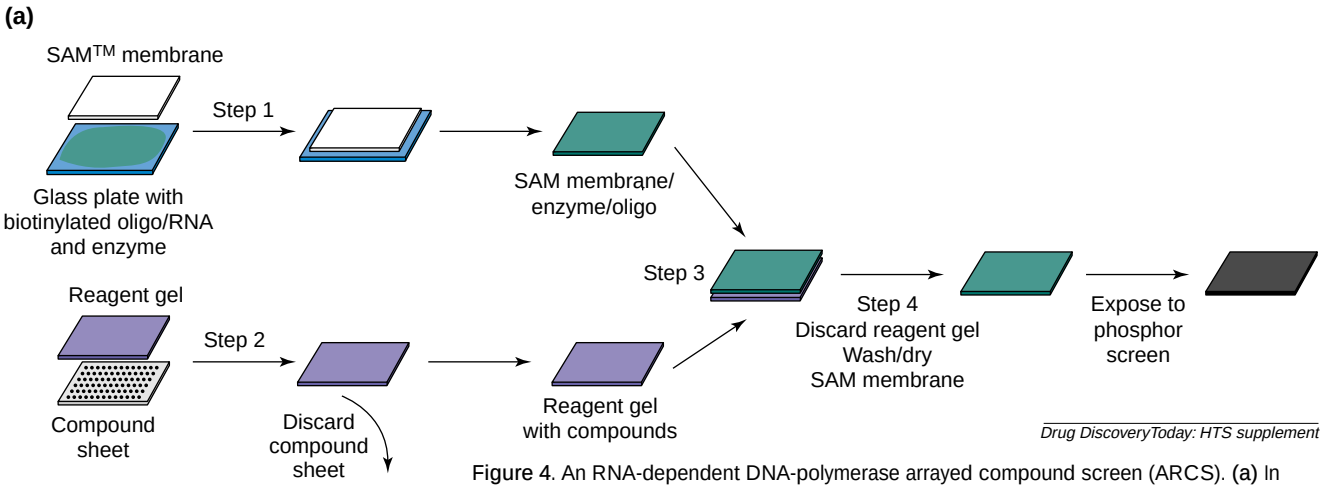
Figure 3. A prototypical serine/threonine protein-kinase arrayed compound screen (ARCS). **(a)** The enzyme gel containing a specific serine/threonine protein kinase was layered onto the compound sheets (Step 1) and the compounds were left to diffuse into the gel matrix for 10 min. After this step, the enzyme gel was removed from the compound sheets and transferred to a pre-wetted streptavidin membrane (SAM™; Promega, Madison, WI, USA). A substrate gel containing the biotinylated peptide substrate and ^{33}[P]-ATP was placed on top of the enzyme gel, initiating the reaction (Step 2). The substrates diffused through the enzyme gel and the biotinylated peptide was captured on the streptavidin membrane. This peptide was phosphorylated with ^{33}[P] throughout most of the gel area, but remained unphosphorylated in areas where there were inhibitors of kinase activity. After a 1 h reaction time, the enzyme and substrate gels were removed and discarded, and non-specifically bound radioactivity was removed from the streptavidin membrane by washing. This membrane was dried and placed in contact with a phosphor storage screen, which was read the next day using the Phosphor Imager (Molecular Dynamics, Sunnyvale, CA, USA). **(b)** A sample image from an ARCS kinase assay that has been incubated with a sheet containing 8,640 library compounds. White spots on a dark background represent potential kinase inhibitors. **(c)** Discovery of active compounds by screening the same 8,640 compounds in both ARCS and 96-well plates (as 10 compounds-per-well mixtures). In the ARCS screen, three compounds were discovered that had IC_{50} values less than 10 μM. The plate screen only identified one of these three compounds. For compounds with IC_{50} values greater than 10 μM, six were discovered in the plate screen, and five were discovered in the ARCS screen; four of these compounds were discovered in both formats.

(glass plates, agarose gel matrices and SAM™) and multiple transfer and washing steps to identify potential inhibitors of the enzyme. The image in Fig. 4b is from an 8640-compound ARCS screen. There are several white spots that could be potential inhibitors of the RNA-dependent DNA-polymerase. In addition, the white streaks in the gel photo correspond to areas in the membrane that did not absorb the enzyme/RNA oligonucleotide mixture well. This illustrates a unique advantage of well-less gel-permeation methodology; potential artifacts can be easily discerned from actual signal by the shape of the signal in question. Finally, this type of heterogeneous assay format can be used for other macro-molecular complex assays.

Examples of ARCS cell-based assays

Ion channels and GPCRs are important therapeutic targets for drug discovery. These targets are coupled to a variety of intracellular signaling events and their activity can be assessed by changes in the levels of intracellular Ca^{2+} or membrane potential. With the introduction of the Fluorometric Image Plate Reader (FLIPR) by Molecular Devices (Sunnyvale, CA, USA), it became feasible to run high-throughput functional assays for GPCRs and ion channels in 96- or 384-well plate formats[21]. We have adapted these cell-based assay formats to our 8,640 ARCS ultra-high-density platform to search for modulators of ion-channel and receptor-ligand targets.

Ligand-gated and voltage-gated ion channel assays can be readily converted into the ARCS format using either cells grown on a Transwell™ membrane (Corning, Acton, MA, USA) or by incorporating the cells of interest directly into an agarose matrix. For specific ligand-gated ion channels, the Ca^{2+}-sensitive dye Fluo-4, which monitors changes in intracellular calcium, can be used to identify agonists and antagonists. In one example, recombinant human embryonic kidney (HEK) cells containing a specific ligand-gated ion channel were grown to confluency on a Transwell™ membrane, loaded with Fluo-4 dye, washed with phosphate-buffered saline to remove the excess dye and



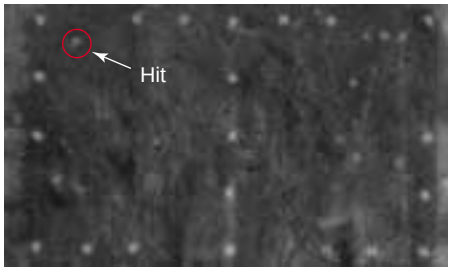


Figure 4. An RNA-dependent DNA-polymerase arrayed compound screen (ARCS). (a) In this assay protocol, a solution of a biotinylated oligo/RNA substrate and the enzyme was prepared and spread onto a clean, silanized glass plate using a 25 ml disposable pipette. Immediately, a streptavidin membrane (SAM™; Promega, Madison, WI, USA) was placed gently on top of the mixture solution, which gave uniform binding of the components to the SAM™ membrane. In the meantime, a reagent gel was prepared that contained the magnesium dideoxynucleotide triphosphates (dNTPs), ^{33}[P]-thymidine triphosphates (TTP) and buffer in 1% agarose; this gel was placed on top of a compound sheet to absorb the compounds into the gel. The enzymatic reaction was initiated by placing the reagent/compound gel on top of the SAM™ membrane (containing the immobilized RNA/enzyme). After an appropriate incubation period (to enable the reaction to occur), the membrane was washed, dried and exposed to a phosphor image storage screen for imaging. (b) The screen was scanned in a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA, USA). Inhibitors appear as white spots on a dark background.

then exposed to a buffer gel that contained de-escalating doses of five different agonists [Warrior, U. *et al.*, Continuous format high-throughput screening for the identification of modulators of a ligand-gated ion channel. *5th Annual Society for Biomolecular Screening Conference and Exhibition*, 13–16 September 1999, Edinburgh, UK (Poster 107)]. The agonists interact with ion channels on the cell surfaces, triggering the release of intracellular Ca^{2+} and giving rise to a fluorescent signal that can be detected with a charge coupled device (CCD) imaging system (Fig. 5a). For voltage-gated ion channels, the fluorescent dye bisoxanol $DiBAC_4$ can be used to monitor changes in the membrane potential of the cell. Cells transfected with a specific voltage-gated ion channel were suspended in $DiBAC_4$ buffer and then incorporated directly into a 1% low-melt agarose matrix (Fig. 5b). After solidification, the gel was incubated at 37°C for 10 min and exposed to the sheet containing 8,640 compounds. Compounds that alter the membrane potential of the recombinant cells appear either as dark (hyperpolarizing) or bright (depolarizing) spots using a CCD imaging system.

Ultra-high-throughput functional assays that identify agonists and antagonists for GPCRs are also possible with the ARCS platform – even those with very fast response times (sec). In Fig. 5c, HEK cells were transfected with a specific GPCR. This particular receptor has a relatively fast response time and gives a maximum response at 10 sec when stimulated by an agonist in a typical FLIPR assay. However, in the ARCS format, because the diffusion of the compounds into the cell gel is time-dependent, the maximum response is observed after 1 min. After the cells were pre-loaded with Fluo-4 and cast into a 1% agarose gel, they were placed on top of the compound sheet and imaged immediately using a CCD system. Agonists appear as lighter, diffuse spots with a light exterior and a darker interior; the bright ring indicates the concentration of agonist that activates the cells along the diffusion front of the compound, and the darker interior reflects the speed of the desensitization of the cells to this agonist response. This is a distinct advantage because potentially false-positive compounds can be distinguished by their continuous fluorescence (tightly focussed white spots). With improvements in imaging systems, it might be possible in the future to measure other ion channel mediated responses, even those with fast response times and those that rapidly desensitize after agonist stimulation.

Future directions

Questions still remain as to the future direction of well-less gel-permeation formats. How many assays can be converted to gel-permeation formats? What are the limitations? At Abbott Laboratories, researchers are exploring two other methodologies

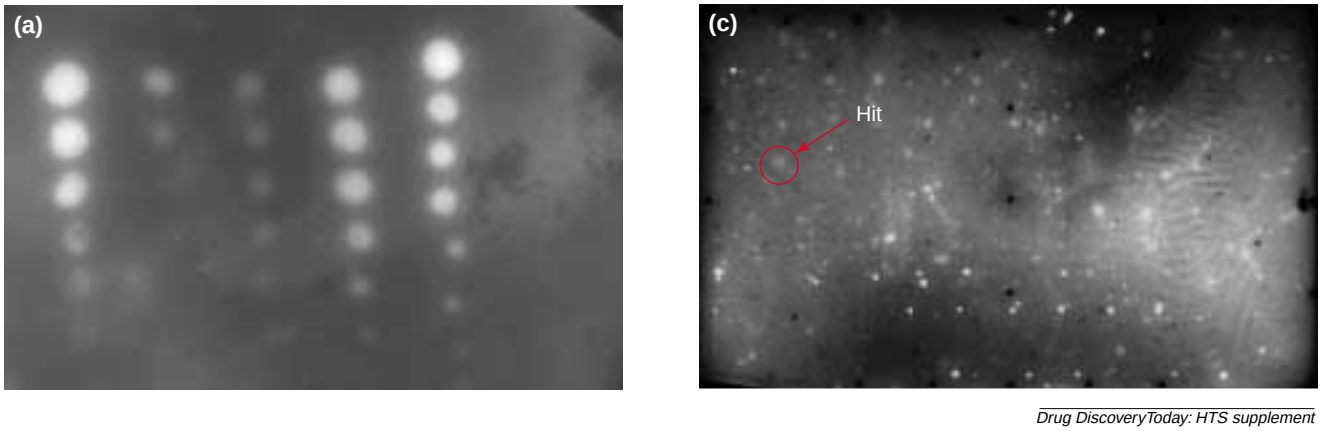


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Figure 5. Ion channel and G-protein-coupled receptor (GPCR) arrayed compound screens (ARCS). **(a)** For a ligand-gated ion-channel screen, recombinant human embryonic kidney (HEK) cells containing a specific ion-channel were seeded onto a Transwell™ plate (Corning, Acton, MA, USA), grown to confluency and loaded with Fluo-4. A phosphate buffered saline (PBS) buffer gel with 1% agarose was placed on top of the compound sheets for 10 min to enable the compounds to diffuse into the gel. This gel was then placed on the bottom of the Transwell™ plate to initiate the assay. Compounds diffuse from the agarose gel through the Transwell™ membrane to interact with the cells. Agonists were detected by fluorescent imaging using the Tundra™ charged coupled device (CCD) imaging system (Imaging Research, Ontario, Canada), and appear as bright spots on a dark background. To detect the antagonists, an agonist gel was prepared (1% agarose) and applied to the top of the compound gel. Cells exposed to antagonists appeared as dark spots on a bright background (data not shown). **(b)** A fibroblast cell line transfected with a voltage-gated ion-channel in an 8640-compound ARCS assay designed to detect changes in membrane potential. In this assay, the cells are loaded with the fluorescent dye $DiBAC_4$ and then cast directly into an agarose gel. The cell gel is then exposed to the compound sheet and subsequently imaged using ViewLux™ (Perkin Elmer LifeSciences, Turku, Finland). Active compounds appear as either a dark spot (hyperpolarizing) or a bright spot (depolarizing), depending on mechanism. **(c)** A HEK cell line transfected with a specific GPCR receptor in an 8640-compound ARCS assay designed to detect agonists of the receptor. In this example, cells were treated with Fluo-4 just before being cast into an agarose gel. The cells in the gel were then exposed to the compound sheet and imaged immediately using ViewLux™. Agonists appear as lighter, diffuse spots and fluorescent compounds appear as tightly focussed white spots.

that show promise in the ARCS format: detection of mRNA and protein levels in cell-based assays. In the case of mRNA, the level of a message in a cell can be modulated by the introduction of compounds. In the ARCS format, this can be performed at a high density (8640 compounds) using either radiolabeled or fluorescently-labeled probes. To assess the regulation of a specific cellular protein, the assay format requires an antibody to the protein of interest. The ARCS methodology thus, essentially, becomes an 8640-dot cytoblot technique. Furthermore, assays can be developed in the ARCS platform to screen for ADME (absorption, distribution, metabolism and excretion) and toxicological properties of compounds.

The hardware and software necessary to exploit the ARCS platform fully is also being developed. Casting systems that use disposable gel cassettes should increase screening throughput to the 500,000 compound-per-day level, while still maintaining the 100-fold reagent savings over 96-well screening. Efforts to create flexible imaging systems for time-resolved fluorescence and fluorescent polarization of gel assays have yielded positive results, thereby extending the usefulness of this technique. Software developments have focussed on creating a visually-based database/user system that tracks the location of compounds as well as handling the data processing for hit selection and re-test follow-up [Voorbach, M. *et al.*, Custom software for use in continuous format high-throughput screening. *5th Annual Society for Biomolecular Screening Conference and Exhibition*, 13–16 September 1999, Edinburgh, UK (Poster 106)]. Software enhancements such as 'autopicking' of hits will make the ARCS screening faster and more user-friendly.

Potential disadvantages of this well-less technology center around the question of diffusion. In the case of compound sheets with a high percentage (>1%) of potent inhibitors, a gel

assay can be overwhelmed by the large inhibitory signal owing to the diffusion of the signal. In the case of larger macromolecules, the diffusion rates through a gel can be much slower than the compounds. Although problematic, this effect can usually be overcome by redesigning the assay, and differences in diffusion could actually be exploited to design an assay that would not be able to be run in a traditional microtiter plate format. Most data collected at Abbott indicate that solubility and diffusion of small molecule compounds, and potential binding of these compounds to the plastic surface are not major issues and have not been a problem with ARCS to date.

In summary, the miniaturization of traditional high-throughput assays using gel-permeation methodology has some significant advantages over high-density (≥1536-well) microplates. The simplicity, speed and redundancy of compounds in a screen are all major advantages of ARCS. In addition, the ability to perform complex, heterogeneous assays in the gel format is a benefit that is unmatched in comparison to high-density microplates. The quality of data is very high because there is no well-to-well variation in terms of pipetting, and evaporation is not an issue. The low infrastructure cost and the flexibility of screening formats for ARCS make it an attractive alternative to fully-automated robotic screening platforms. The ability to store several copies of the screening library in a freezer in a ready-to-screen format enables rapid initiation of a screen: over 10 million compound samples can be stored on a single shelf in a freezer. Finally, the data that we have collected in our HTS group over the past two years on ARCS indicate that there is significant overlap in the compounds that are discovered through this technology versus traditional plate-screening. Several leads from ARCS screens now have significant medicinal chemistry effort, and we believe that this will continue as we transfer the majority of our HTS screens to the ARCS platform.

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