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TRENDS IN CELL-BASED SCREENING

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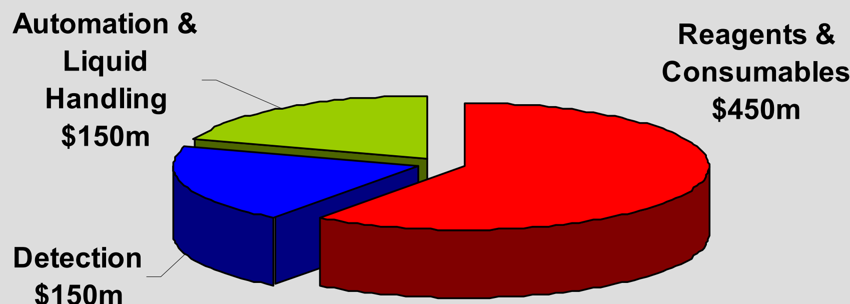
Definition: ***Assays where the fundamental unit of expression is the cell, either cell populations or single. These assays normally include three key elements i.e. an instrument to conduct and monitor the assay, a cellular component, e.g. primary cells and an informatics component to capture, manage and analyse data arising from the assay.***

Market Drivers

- Increased use of cell-based assays seen as way of overcoming hurdles in drug discovery
- Enables Screening under physiological conditions
- Avoids requirement for purification of target protein
- Generates leads that already have a degree of validation (saves time and cuts costs)

Market for Cell-based Screening in 2004 was Worth ~US$750m in 2004 (up 20% YoY)



Key Applications & Screening Technologies

- Cell-based assays make up approx. 50% of drug discovery screens
- Most cell-based screening is done in lead identification (primary and secondary screening) or hits2leads processes whilst most cell-based ADMET assays are done in lead optimization.
- Receptors and ion channels contribute to more than 50% of all drug targets screened; both target classes depend on cell-based assays. Nearly 1/3rd of all cell-based screens are second messenger readouts of GPCR activation.
- Fluorescence is the predominant detection technology, used in 2/3rd of primary screens. Moreover, 1 in 4 fluorescence-based cell assays are done using the FLIPR (Fluorometric Imaging Plate Reader). Manual and increasingly automated patch clamping are used in the secondary screening of ion channels.
- CHO and HEK 293 are the main cell lines used in pharma cell-based screening.

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Key Trends

- Use of HCS is becoming more widespread – in contrast to HTS, HCS collects multiple pieces of useful information from each well (most current interest in HCS centers on protein translocation assays).
- About half of all screening groups in lead discovery recently expressed an interest in obtaining primary, stem and progenitor cells for cell-based screening.
- There is growing evidence that 3-D cell cultures enable the discovery of patterns of gene expression and other biological activity that more closely mirror what happens in living organisms. The 3-D culture of primary cells is likely to create a much more relevant and valuable cell assay for drug discovery.
- The availability of humanised cells (i.e. incorporating human genes in primary cells) for metabolism studies and the use of new improved cryopreservation techniques have the potential to solve the problem of supply and variability that restrict the use of human hepatocytes for *in vitro* screening today.
- Novel labels for fluorescence and luminescence read-out continue to emerge providing alternatives to established flurophores and green fluorescent proteins. Emerging technologies include aequorin (another photoprotein), nanocrystals and nanoparticles. In addition microplate-based label free detection is expected to make an important contribution to the assay of difficult cell-based targets in the coming years.
- Vitally important hERG channel assays (manual and automated patch clamping) are far from ideal and there remains a significant opportunity for an automated high-throughput physiologically-relevant assay for studying cardiac toxicity (and cardiovascular drug discovery).

Challenges

- Resourcing and capacity to sub-clone and maintain cell lines; to pick clones; to setup cell-based assays (harvesting and plating); to visually (microscopically) inspect cells; are all viewed as activities that limit the productivity of cell-based screening today.
- Major challenges to the widespread adoption of HCS are the development and availability of assays and the limited availability of platform-independent user-friendly software applications (algorithms).

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Cell-Based Screening Supporting Information Contents:

- Introduction to Cell Based Assays
- Key Market Drivers
- Key Applications
- Current Screening Technologies
- Cell Screening Automation
- Key Market Trends
- Market Size & Dynamics
- Challenges
- Scottish Context
- Drug Discovery Terminology

Many of the market trends and estimates presented within this review are based on recent market surveys and reports conducted by HTStec Limited.

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Introduction to Cell-based Assays

Cell-based assays can be defined as: *assays where the fundamental unit of expression is the cell, either cell populations or single. These assays normally include three key elements: an instrument to conduct and monitor the assay; a cellular component, e.g. primary cells; and an informatics component to capture, manage and analyse data arising from the assay*

- With the development of techniques to identify genes and proteins, and increased insight into the molecular mechanisms underlying disease, a target-based approach to drug discovery using mechanism based screening assays has replaced the old non-specific assays.
- In order to enable the rapid analysis of large numbers of compounds for their effects on the function of specific targets, so-called high-throughput screening (HTS) assays have been developed. HTS assays are generally performed robotically and consequently applicable protocols require minimal manipulation, possibly in arrayed small volumes (e.g. in multi-well microtitre plates).
- Many types of cell-free assays that were originally developed to measure the biochemical activity of purified proteins, mostly enzymes, could be readily converted to HTS by applying detection systems such as fluorescence that do not require separation of the reaction product from the substrate.
- These automated assays allow rapid screens of large compound libraries to identify so-called ‘hits’, namely compounds that show the desired effect on the biochemical activity of the specific target in the isolated *in vitro* system. Hits are then subjected to chemical modifications and further screening through the HTS system to select more specific and potent derivatives called ‘lead’ compounds.
- In the classical drug discovery process, lead compounds are subsequently tested in various *in vivo* assays using cellular and animal models in order to select those that may become drug candidates for clinical trials.
- In the last few years, cell-based assays using engineered cells and micro-organisms have become an increasingly attractive alternative to *in vitro* biochemical assays for HTS in the early phase of the drug discovery process. The requirements for such *in vivo* assays are the ability to examine a specific cellular process triggered by a defined target and a means to readily measure its output in an HTS system.

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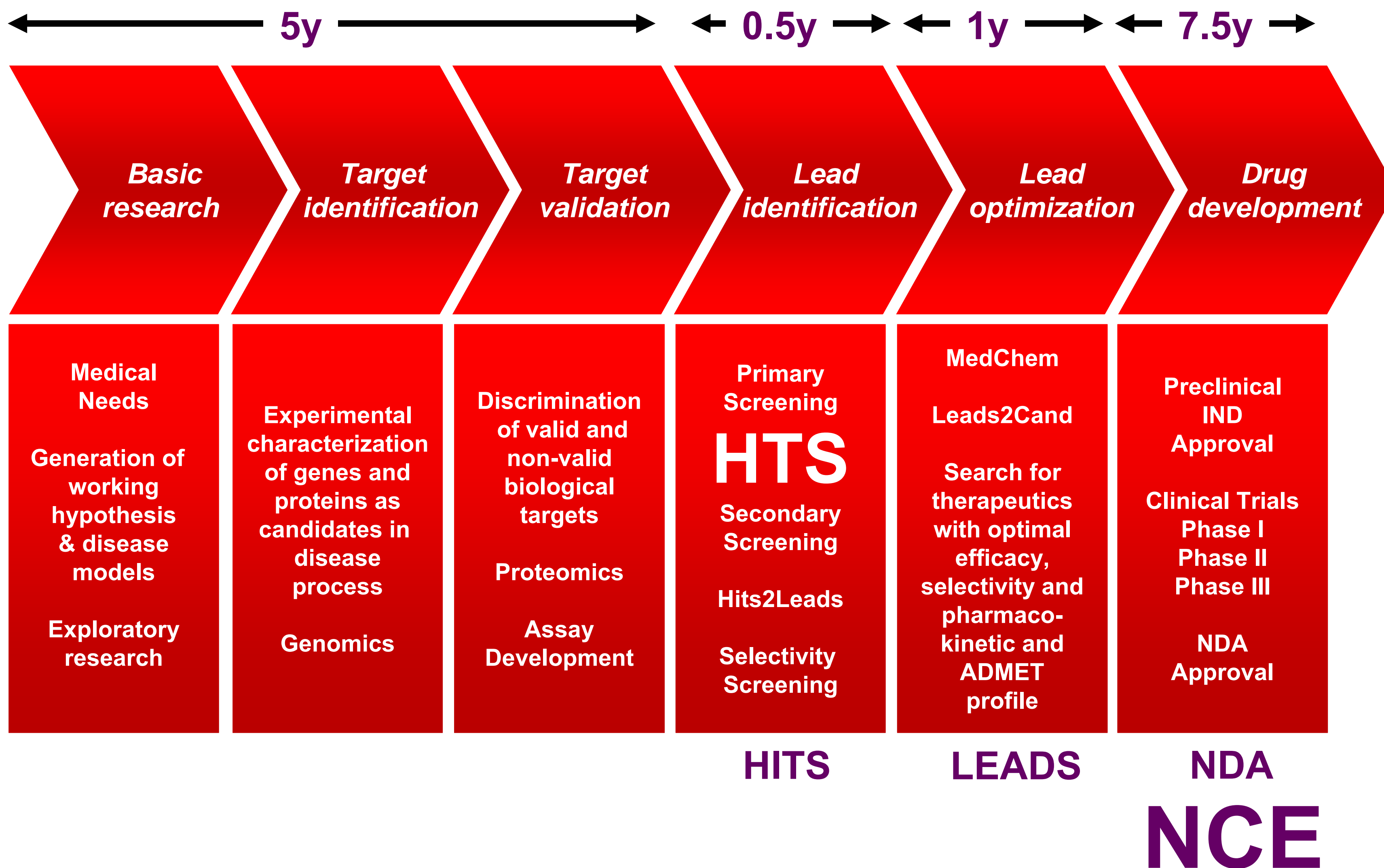
The Drug Discovery Process



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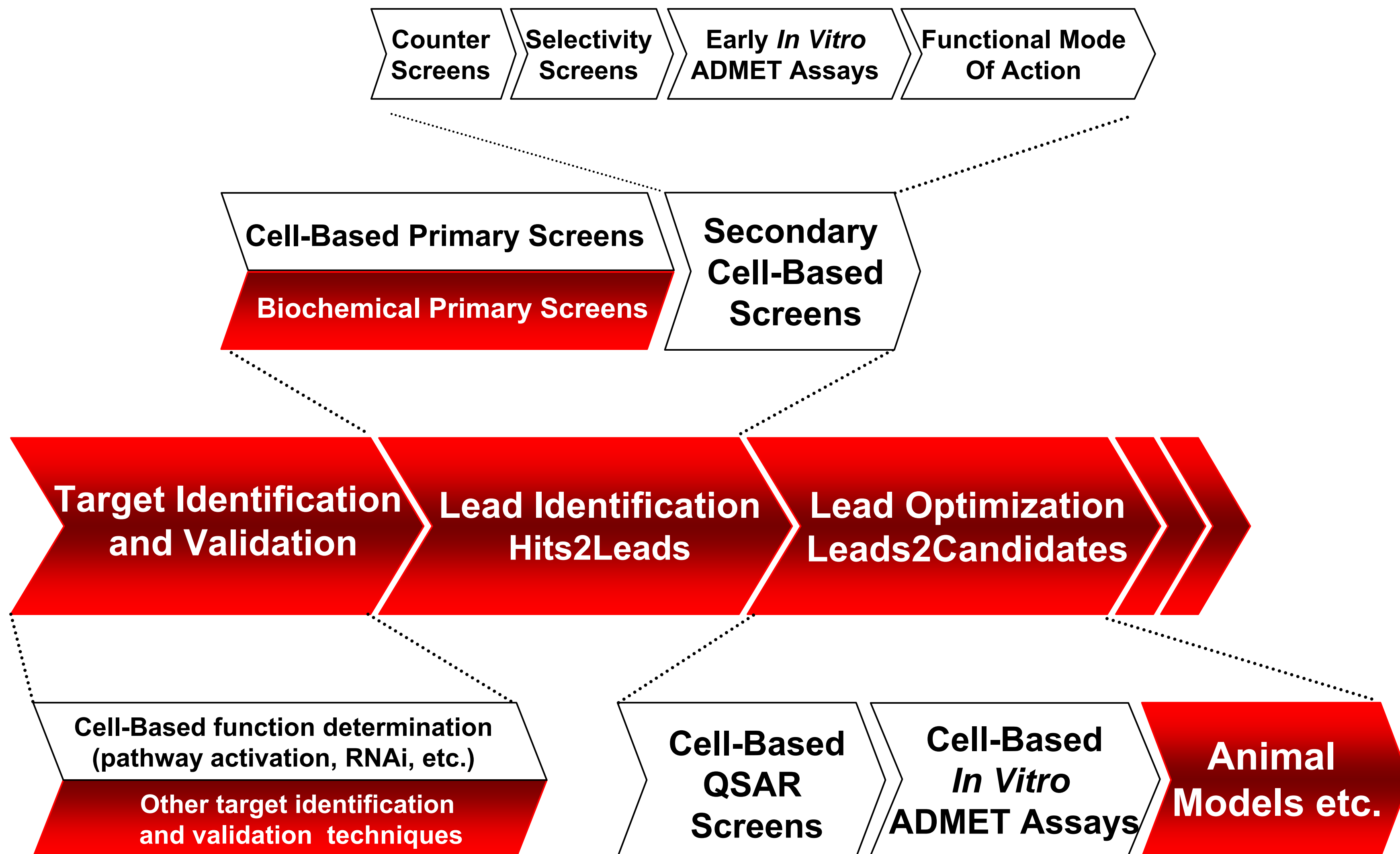
Where Does Cell-Based Screening Reside?



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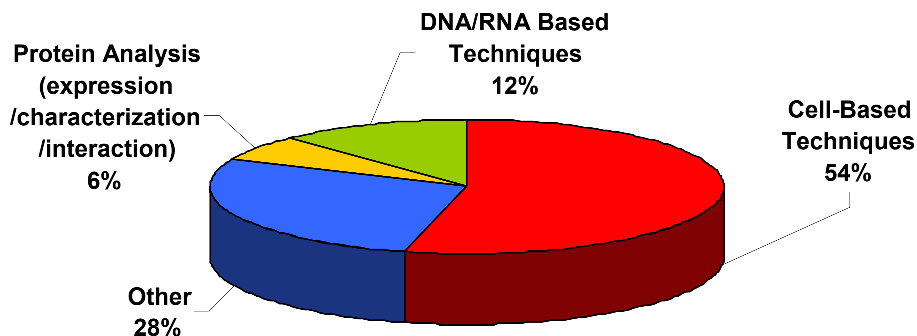
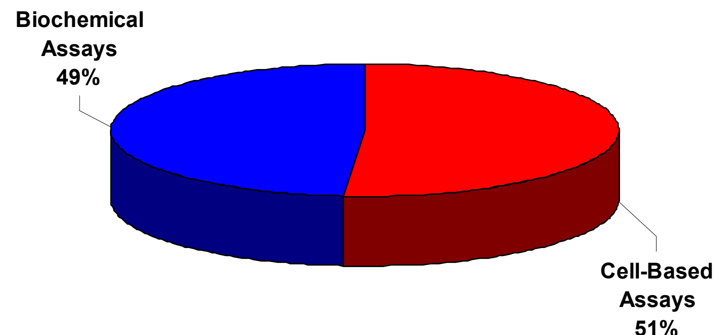
Rationale For Cell-Based Screening

Increased use of cell-based screening assays is seen as most important way of overcoming hurdles in drug discovery



At least 50% of all drug discovery assays are now cell-based



- Advantages of cell-based assays over biochemical assays :
  - the conformation and the activity of the target protein, as well as the read-out to monitor the effect of compounds, are examined in a cellular context that represents the natural physiological state more closely than biochemical assays.
  - assays do not require purification of the target protein and therefore eliminate investment of resources to gain the necessary knowledge for obtaining a biochemical active target
  - cell-based assays can immediately select against compounds that are generally cytotoxic, or that cannot permeate cellular membranes to reach intracellular targets. Thus hit and lead compounds that are identified through cell-based assays have passed important validation steps (saving valuable time and costs in the development of a drug).
  - visualization of all possible drug-target interactions e.g. activators, target interactions, allosteric modulators
  - discrimination between agonist and antagonist
  - enhanced assay sensitivity

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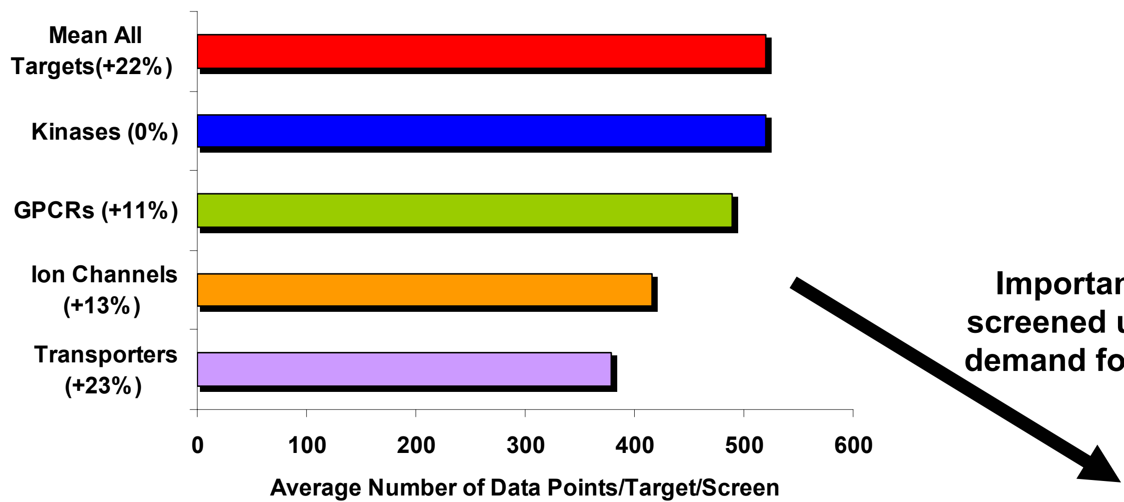
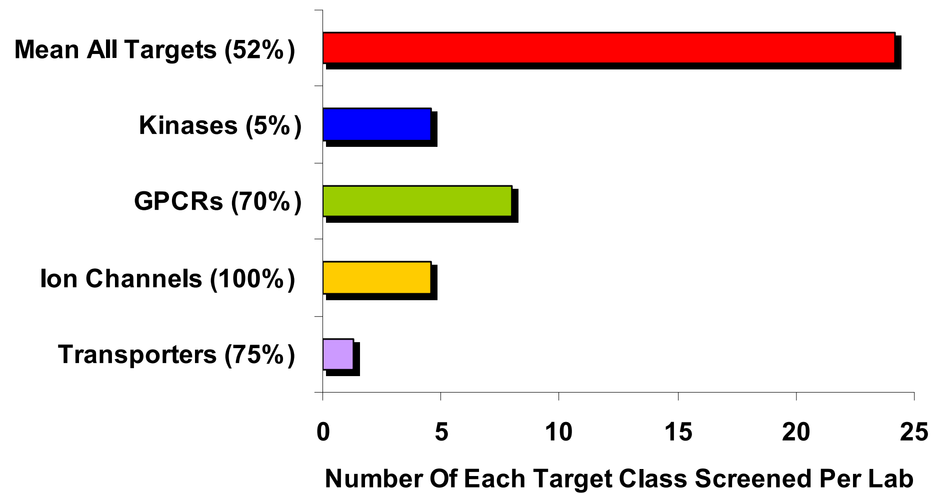
Factors Impacting On The Use Of Cell-Based Screens (1)

Number of compounds being screened is increasing -average number of drugs screened against each target in 2004 and 2003-2004 growth rate (in brackets)



Important as many compounds are being screened using cell-based assays and so the demand for these assays is expected to grow

Assays involving cells increasing -average number of each target screened per lab and % cell-based (in brackets)



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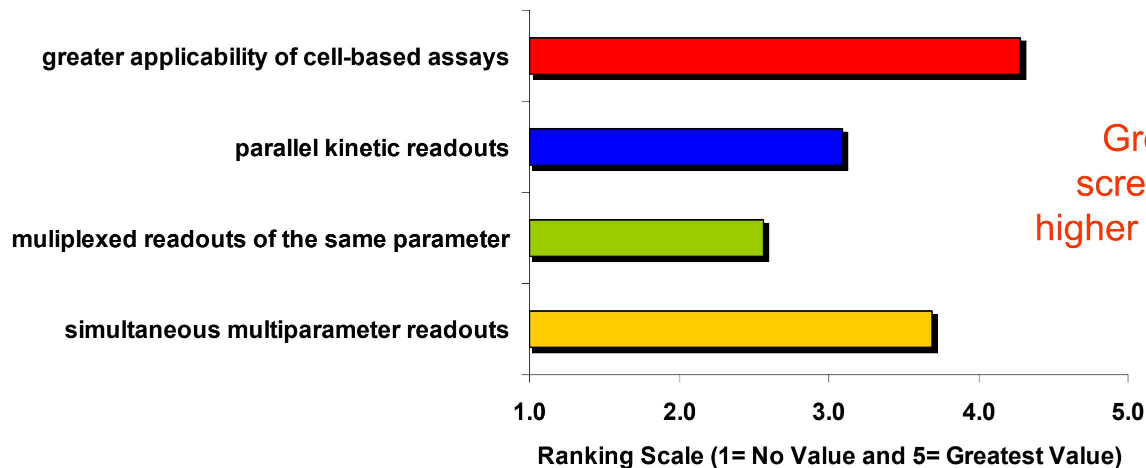
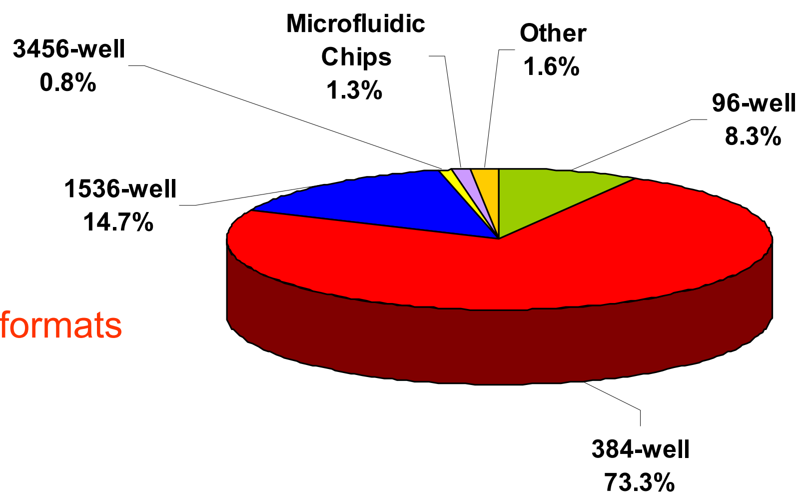
Factors Impacting On The Use Of Cell-Based Screens (2)



Growing demand for higher content screening – scientists were asked how higher content screening could be achieved

Shift towards higher density plate formats



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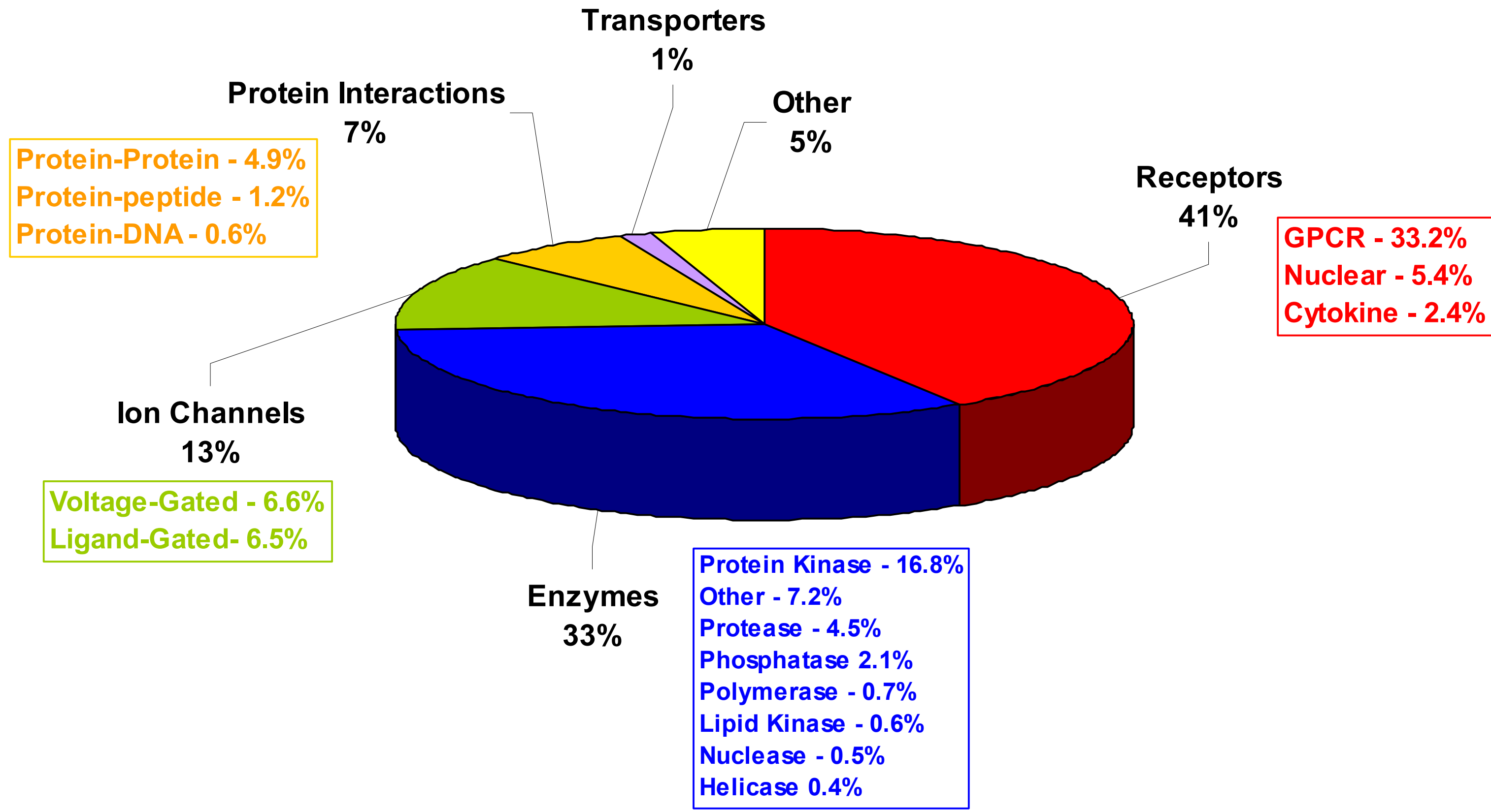
Major Drug Discovery Target Classes (assayed using both biochemical & cell-based screens)



Receptors continue to be the most important target class in drug discovery

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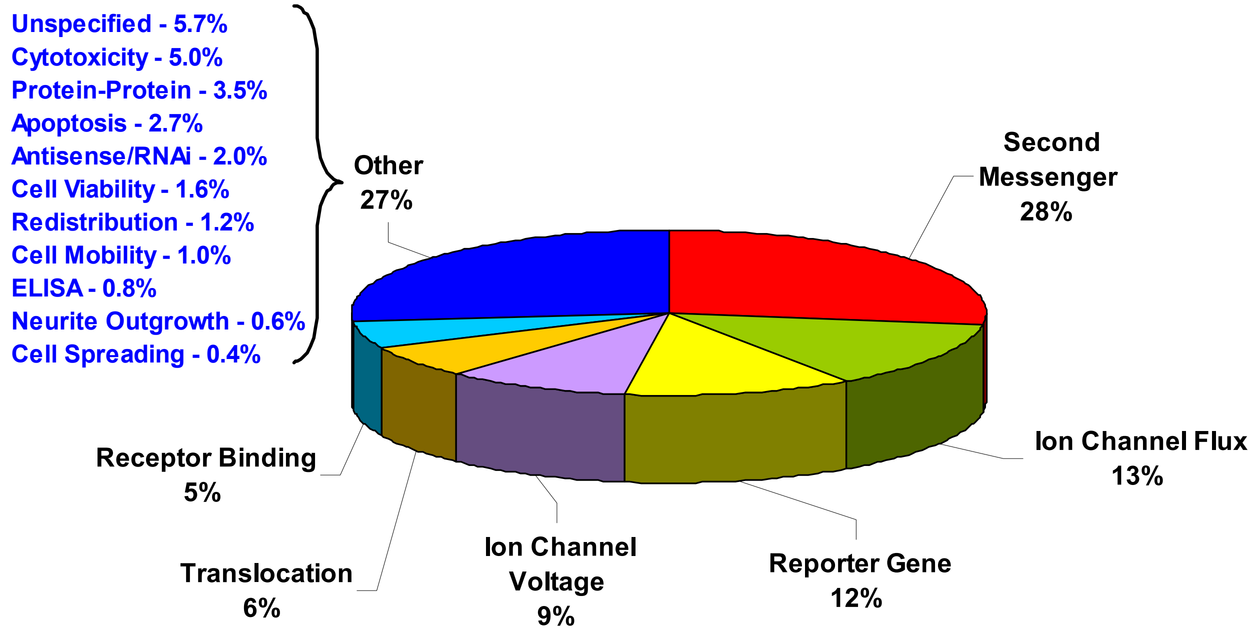
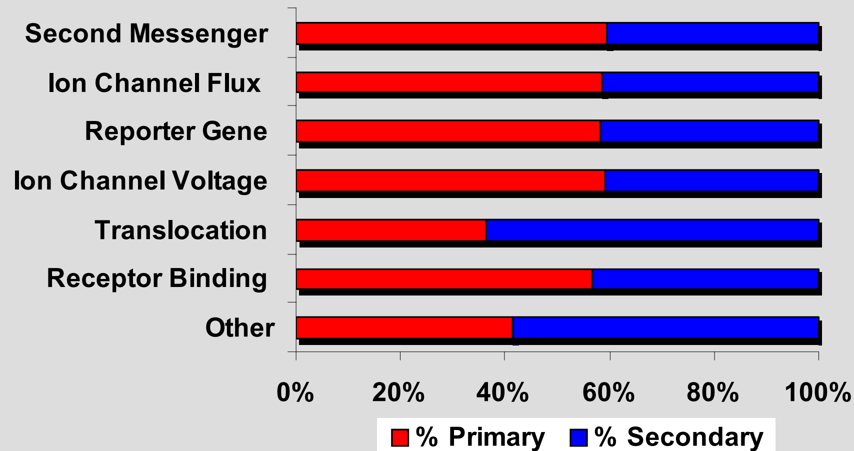
Major Cell-Based Assays Used in Lead Discovery



Relative use of various cell-based assays in primary & secondary screening



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Cell-Based *In Vitro* ADMET Assays

Absorption, Distribution, Metabolism, Excretion (ADME) Assays

- Absorption/Permeability
  - Caco-2 Permeability Assay
  - P-Glycoprotein Mediated Compound Efflux
- Metabolism
  - Hepatocytes
    - Metabolic Stability
    - Induction Of Cytochrome P450 Enzymes
  - Metabolic Activity (Cell Proliferation)

Toxicology Assays

- Genotoxicity/Mutagenesis
  - Ames Test
  - Genetic Screening Assays (using RNAi libraries)
- Cytotoxicity Assays
  - Plasma Membrane Leakage
    - Artificial Dye
    - Enzyme (LDH) Release
    - Dye Uptake
  - Apoptosis
    - DNA Fragmentation
    - Caspase Activity
    - ATP Measurement
  - PXR Reporter Gene
  - Cytochrome P450 Expression
  - Ion Channels
    - hERG Binding Assay for potassium channel toxicity and QT Prolongation

- **Technologies to watch:** the availability of humanised cells (i.e. incorporating human genes in non-human primary cells) for metabolism studies and the use of new improved cryopreservation techniques have the potential to solve the problem of supply and variability that restrict the use of human hepatocytes for *in vitro* screening today.

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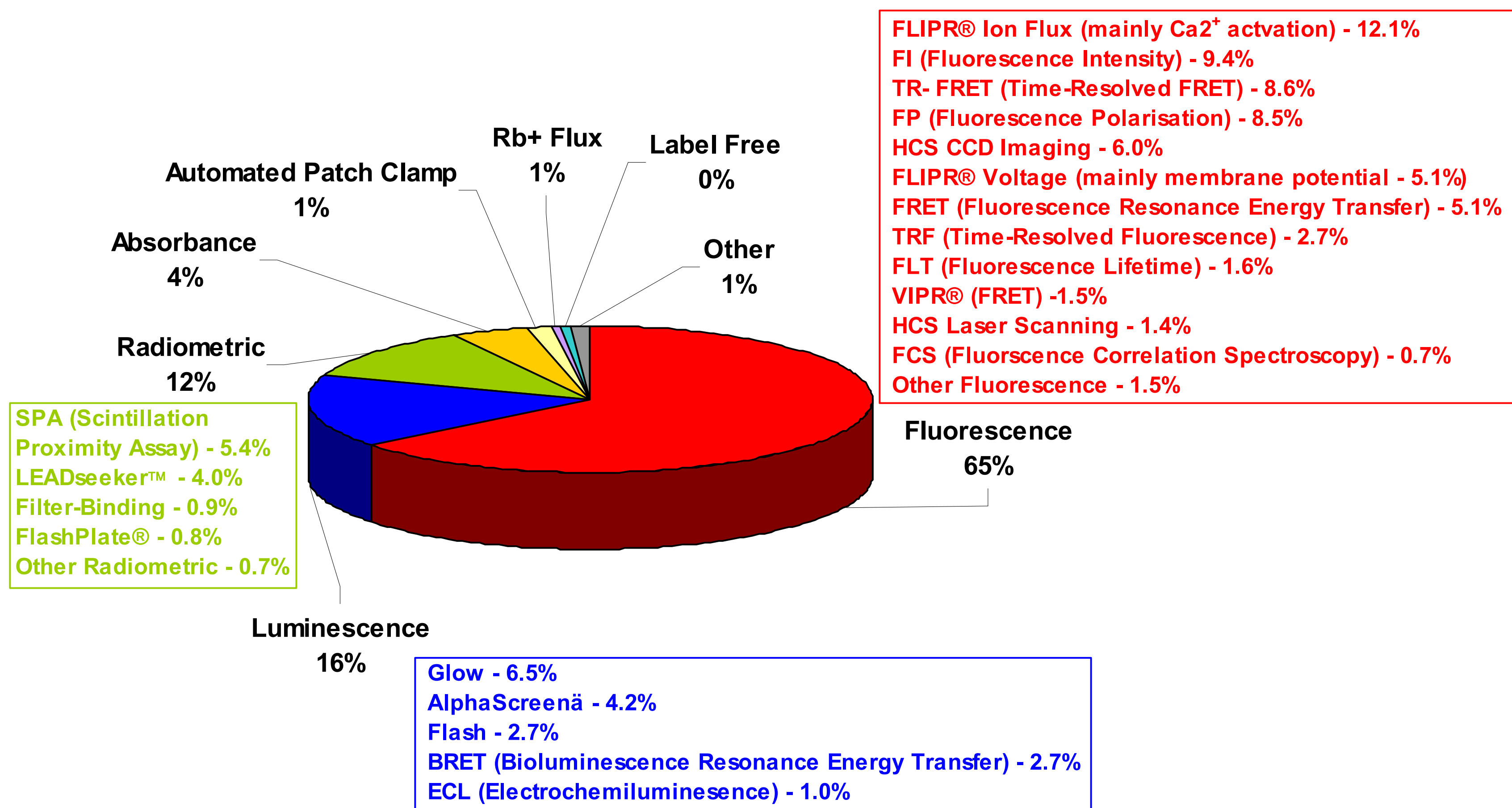
Detection Technologies – relative usage in primary screening

Cellular assays can be classified based on the method of detection for labelling. Most cellular assays use fluorescence or bio/chemiluminescence; however, many novel detection methods are currently under development and existing methods continue to be refined. Note that some cellular assay products can use a variety of detection methods.



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Detection Technologies

- Almost 2/3rd of all assays used in primary screening are fluorescence-based.
- Of the fluorescence-based assays 1 in 4 use FLIPR (Fluorometric Imaging Plate Reader).
- The majority of fluorescent cellular assays are performed using fluorescent dyes. These dyes can either be linked to specific target genes and proteins, or be free floating molecules in the cell.
- Green fluorescent proteins are naturally fluorescent and are used in cellular assays as reporter molecules. GFP is a powerful tool for visualizing proteins within living cells. Almost any cDNA or DNA sequence of interest can be fused with the gene for GFP to create a reporter protein that will fluoresce when excited with blue light. Unlike other reporters, GFP fluoresces in the absence of any other proteins or substrates.
- Bio/chemiluminescence detection is based on the principle of light emitted through a naturally occurring reaction within the cell. Luciferase is currently the preferred chemical for use with mammalian cells.
- Aequorin EuroScreen SA markets AequoScreen cell lines that express various GPCRs and aequorin, another photoprotein. EuroScreen uses its AequoScreen cellular assay platform to patent new GPCR targets and drug candidates (fluorescence indicates GPCR activation by test candidate). The AequoScreen system can be used in high-throughput screening of approximately 10,000 assays per hour.
- Novel labels including nanocrystals (such as the quantum dots developed by the Quantum Dot Corporation) and nanoparticles for cellular assay use, are now emerging. These alternative detection methods utilize smaller particles than the typical fluorescence or luminescence compounds. The smaller sized compounds are believed to decrease the interference in cellular reactions caused by larger compounds, allowing for more accurate studies of cellular behavior. In addition, they show improvement over fluorescent dyes in that they have brighter, more stable signals and enable multiplexing because they provide many more colors than fluorescent dyes.

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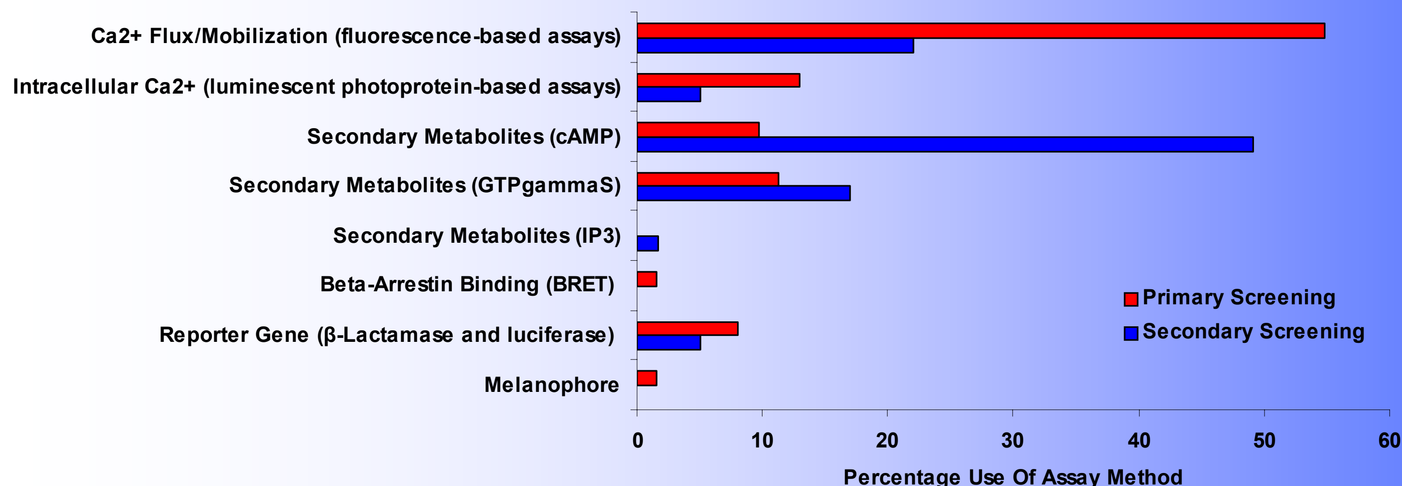
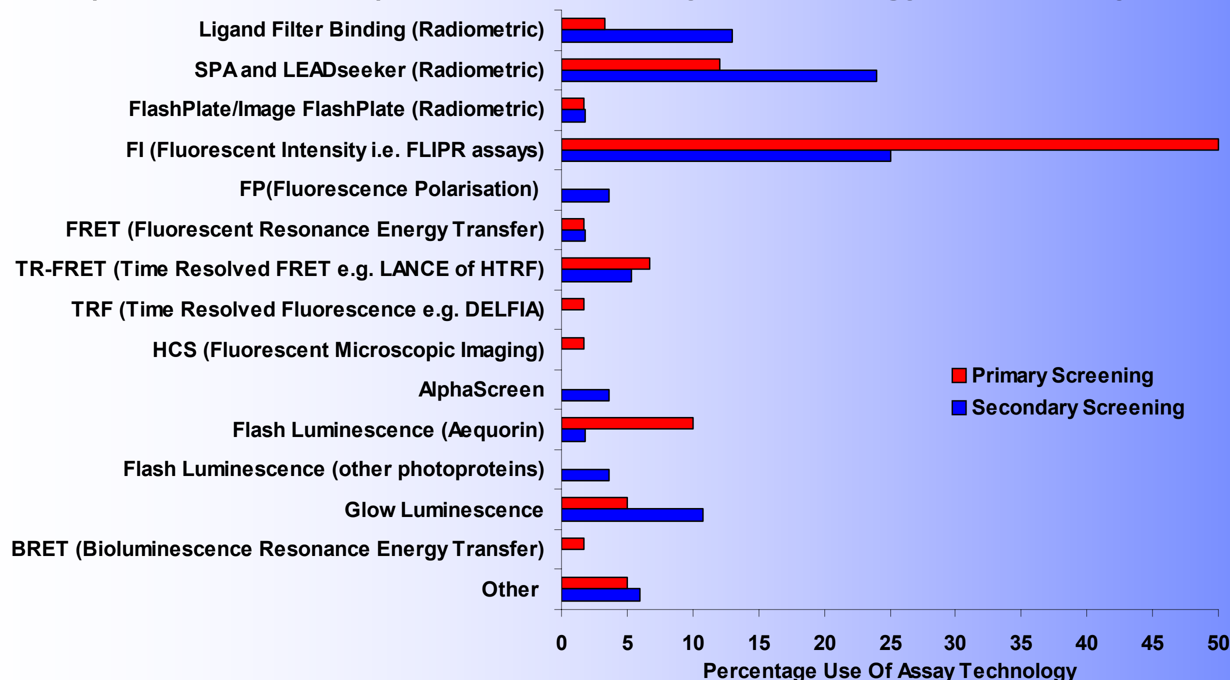
GPCRs (70% Cell-Based) - Preferred Method of Assaying GPCR Activation



GPCRs (70% Cell-Based) - Preferred Assay Technology for Primary & Secondary Screening



Kinases (5% Cell-Based) - Preferred Assay Technology for Primary & Secondary Screening

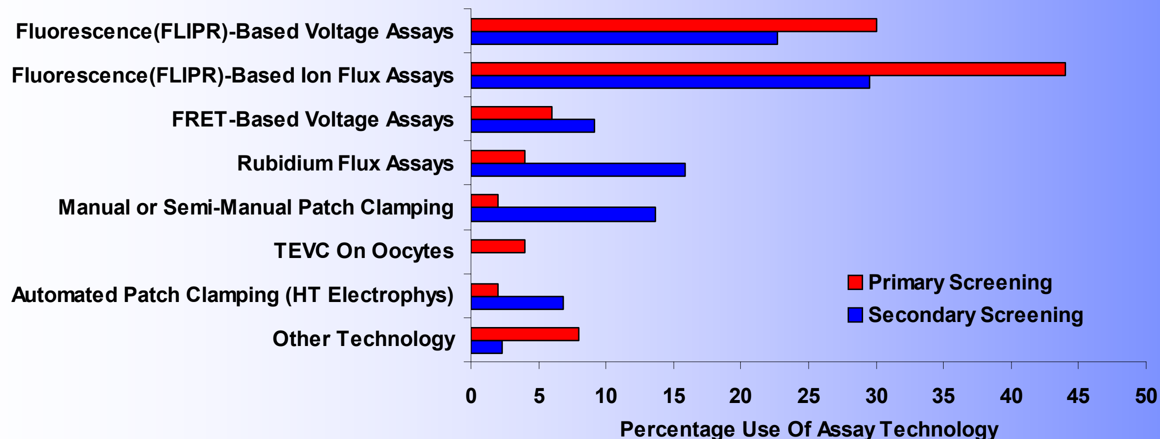
Radiometric Filter Binding (e.g. 33P incorporation)
SPA and LEADseeker (Radiometric)
FlashPlate (Radiometric)
FI (Fluorescent Intensity)
FP (Fluorescent Polarisation)/Antibody)
FP (Fluorescent Polarisation)/Ligand (i.e. NO antibody)
FRET (Fluorescent Resonance Energy Transfer)
TR-FRET (Time Resolved FRET e.g. LANCE of HTRF)
TRF (Time Resolved Fluorescence e.g. DELFIA)
FLT (Fluorescence Lifetime)
AlphaScreen
ECL (ElectroChemiLuminescence)
Glow Luminescence
Other

Primary Screening
Secondary Screening

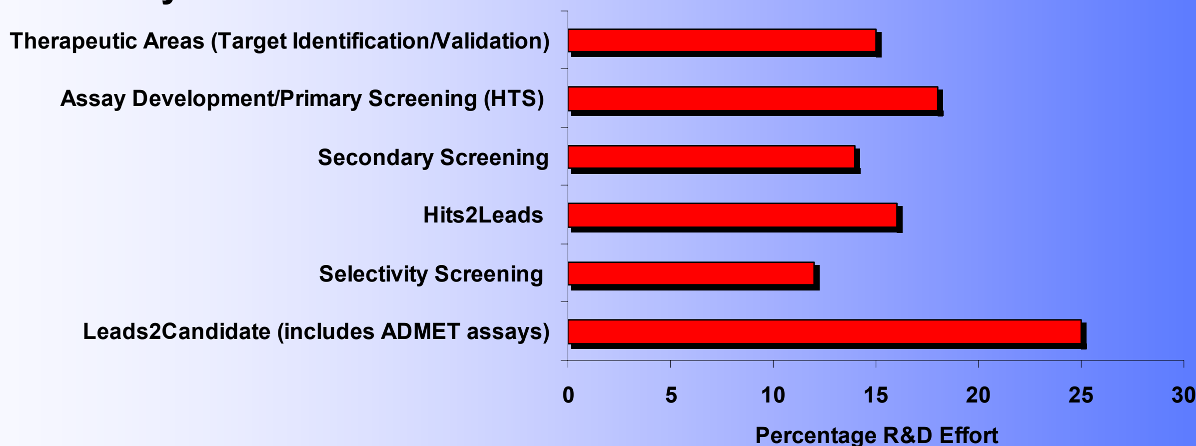
0 2 4 6 8 10 12 14 16

Percentage Use Of Assay Technology

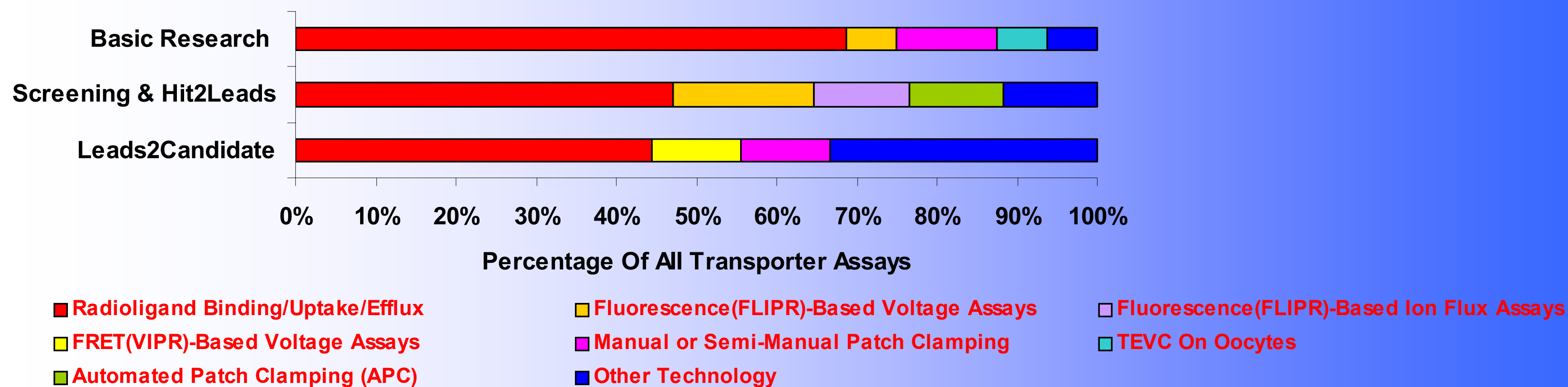
Ion Channels (100% Cell-Based) -Preferred Assay Technology for Primary & Secondary Screening



Transporters (~75% Cell-Based) - R&D Resourcing for Transporter Assays used in various stages of Drug Discovery



Transporters (~75% Cell-Based) - Preferred Technology for Transporter Assays



- **Most R&D on transporters is done in Lead2Candidates (ADMET), although increasingly the therapeutic potential of transporter targets is being investigated in primary screening.**
- **Radioligand binding assays are most widely used for transporters, although many view them as inadequate and are looking for new higher throughput alternatives more suited to primary screening**

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Key Cell-Based Platform Technologies (1)

Molecular Devices FLIPR (Fluorometric Imaging Plate Reader)

- combines microplate fluorescent imaging (laser excitation at 488nm with CCD imaging) with integrated on-line liquid dispensing (96 or 384 channels)
- allows for the simultaneous microplate monitoring of kinetic cellular responses (at ~1 sec intervals)
- angled optics give quasi-confocality, to minimize background interference from fluorescent dye in medium above cell layer
- used extensively in ion channel/GPCR HTS
- the most commonly used GPCR assay is based on Ca2+ mobilization/flux following GPCR activation
- FLIPR does not directly measure ion current, but measures membrane-potential-dependent or ion-concentration-dependent changes in fluorescent signal as a result of ionic flux
- the new MDC FLIPR Tetra has variable wavelength excitation and 1536 simultaneous dispensing

Patch Clamping

- manual electrophysiology technique, involving insertion of glass patch micropipette into cell, enabling analysis of ion channel function through direct measurement of ion current flowing through one or more ion channels
- definitive ‘gold-standard’ method for studying ion channel function
- yields info about voltage, rate- and use-dependence of compound binding

Planar or Automated Patch Clamping (APC)

- reverses the operating sequence of traditional patch clamping i.e. moves the cell to the patch pipette
- uses a porated flat substrate in glass, silicon, plastic or other materials
- high throughput (>2000dp/day) achieved by:
  - microfabrication of multiple planar patch structures
  - simultaneous parallel recordings
  - automation of cell and ligand liquid additions
- Examples: Molecular Devices IonWorks and Axon PatchXpress, Flyion FlyScreen

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Key Cell-Based Platform Technologies (2)

What Is High Content Screening (HCS)?

- there is little consensus over the definition of HCS, but it includes the following:
  - high resolution multi-colour fluorescence imaging of multiple independent or interacting targets or pathways within intact single (including live) cells
  - provides detailed information on sub-cellular temporal and molecular events
  - allows for the discrimination/analysis of different cell populations
  - collects multiple images on multi-parameters (multiplexed measurements) per microplate well
  - utilizes automated fluorescence microscope imaging systems, some with confocal optics, fluorescence-based reagents, as well as advanced bioinformatics tools

Factors Influencing HCS Implementation:

- current instrument throughputs lower than that needed by most HTS Hit finding labs
- further secondary assays may still be required for QSAR or lead optimization
- needs dedicated/expert assay development (AD) resource, otherwise AD is prolonged
- requires a philosophy change in the way most HTS groups work today
- complexity/data output currently not compatible with most HTS IT setups
- large amount of data from multi-parameter assays; only useful if it is converted into meaningful information
- limited by availability/validity of user friendly software applications (algorithms)
- requires strategy on image format, retention, sharing, access and generic algorithms

Advantages Of HCS:

- the assays collect multiple pieces of useful information from each well of microplates rather than HTS, which typically only makes only one measurement per well as a cell population average.
- allows walk-away automation of HTS targets impossible by other assay methods
- reduces cost of cell-based assays - minimises cell line development and validation
- simplifies design of cell-based assays
- enables cross-correlation of potency, specificity and toxicity in a single assay
- identifies cell sub-populations and sub-cellular compartments
- leads to identification of auto-fluorescent and cytotoxic compounds
- helps to understand mechanism of action of drug candidates

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HCS Imaging Technology

Laser Scanners

- have their origins in fluorescence cell sorting
- some systems have confocal optics
- laser excitation (1 or more fixed wavelengths)
- simultaneous multicolour emission
- point detection at varying resolutions (line width and sample interval)
- produces pseudo-images (i.e. reconstituted objects)
- particularly suited for whole cell and bead analysis (differentiating free from bound or internalised label)
- optimised for high speed HCS
- instruments examples:
  - TTP Labtech Acumen Explorer™
  - Applied Biosystems 8200
  - Compucyte iCyte™

CCD Imagers

- have their origins in fluorescence microscopy
- typically confocal optics or switchable optical modes, with autofocus
- white light (variable wavelength) or laser (fixed wavelength) excitation sources
- sequential (one CCD) or simultaneous multicolour emission (multiple CCDs)
- area detection, ability to drill down (zoom-in) to very high resolution
- optimized for high definition HCS
- instruments examples:
  - Cellomics ArrayScan Vti
  - GE Healthcare IN Cell Analyzer 3000
  - MDC Pathway and ImageXpress

Kinetic Cell Imagers

- are CCD Imagers with single well liquid handling capability
  - Cellomics Kinetics Scan
  - GE Healthcare IN Cell Analyzer 1000
  - Becton Dickinson Atto pathway HT

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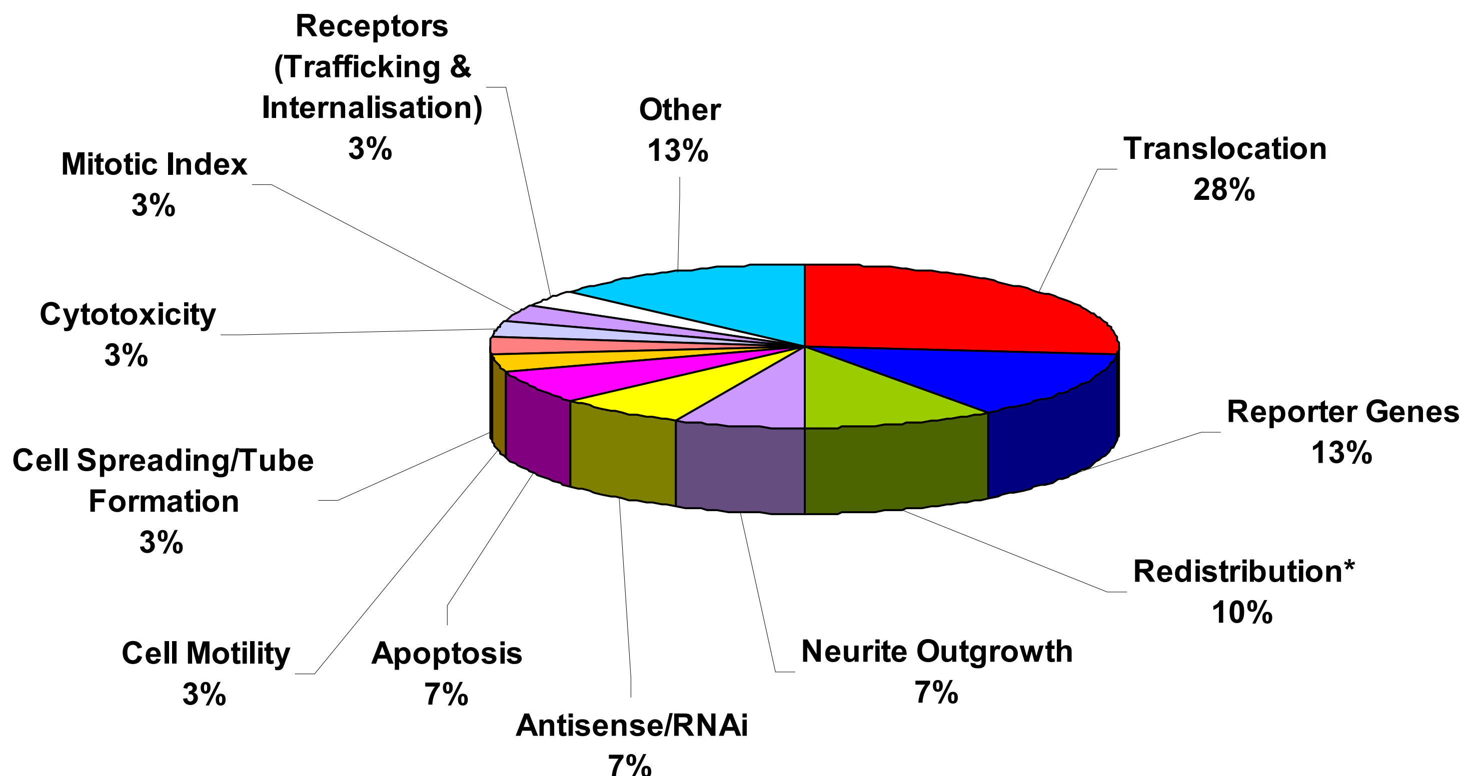
Relative Interest in HCS Applications



- Respondents view translocation assays (fixed or live) as one of the most important applications for HCS.
- Many believe that the biggest potential benefit in drug development to be provided by HCS is in secondary screening – evaluating hits from primary screening and focusing down the number of hits to identify "true" hits.
- *Refers to Bioimage's Redistribution® image-based technology for cellular (protein translocation) assays and pathway analysis, utilizing Aequorea victorea GFP (green fluorescent protein). GFP is available under licence from GE Healthcare for use in discovering translocation modulators. Most recent live cell applications include kinase pathway profiling assays and cell cycle status phase markers.

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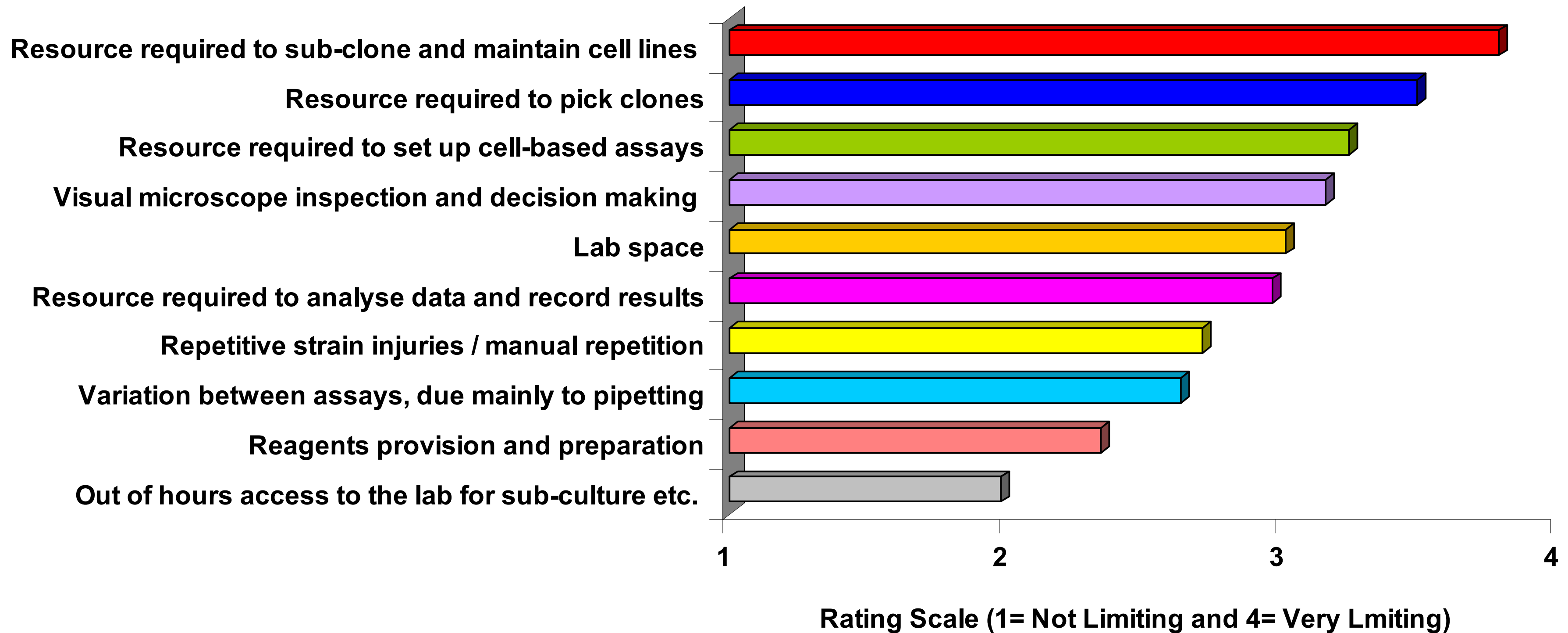
Major Limitations Of Cell-Based Assay Productivity



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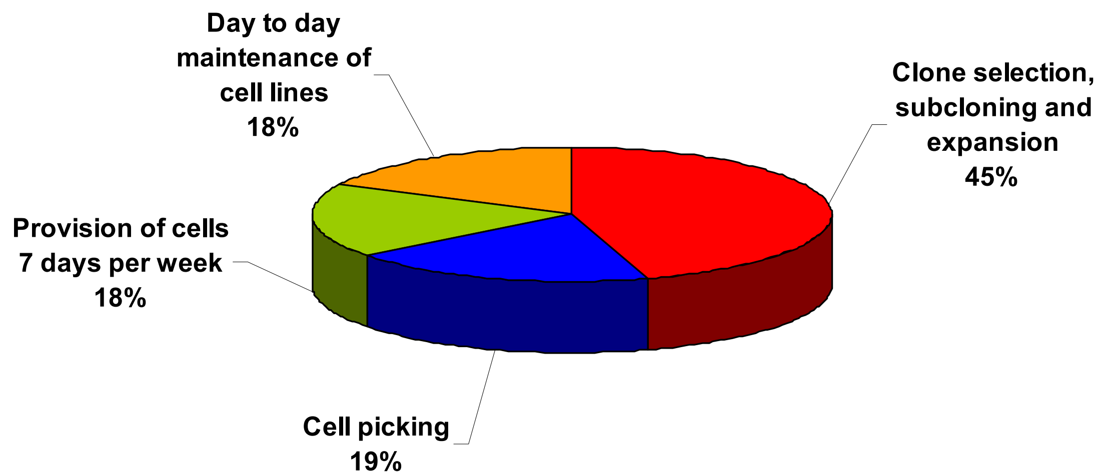
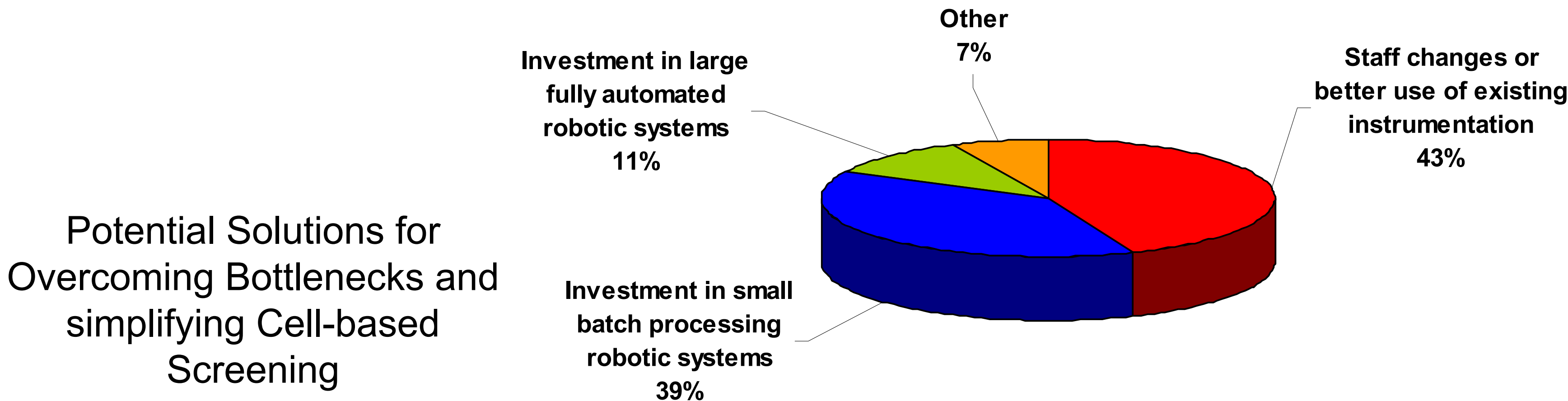
Cell Culture Bottlenecks & Potential Solutions

Bottlenecks in the Maintenance & Production of Tissue Culture Cell Lines





Potential Solutions for Overcoming Bottlenecks and simplifying Cell-based Screening

- Large fully automated robotic cell maintenance and culture systems are available from TAP (Cellmate,SelecT & Cello) and RTS Life Science International (ac*CELL*erator™), Velocity 11, Protedyne
- Small batch processing robotic workstations for cell-based screening are available from Beckman, CaliperLS, Hamiliton, Genetix, PerkinElmer, Tecan etc.

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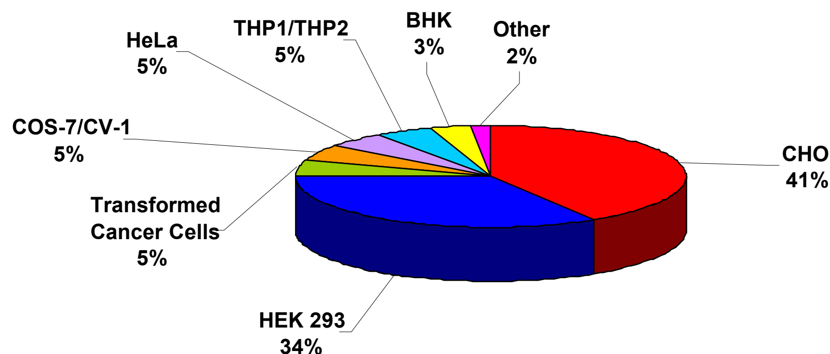
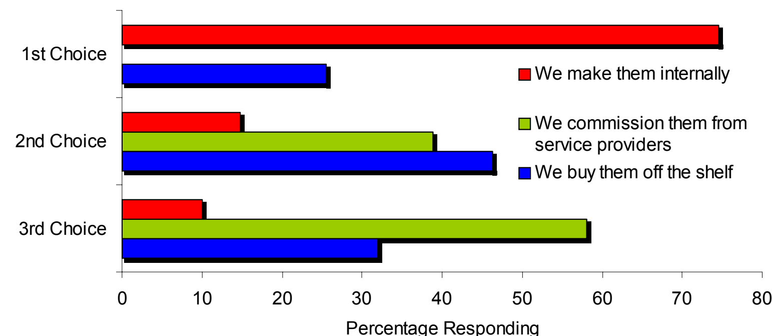
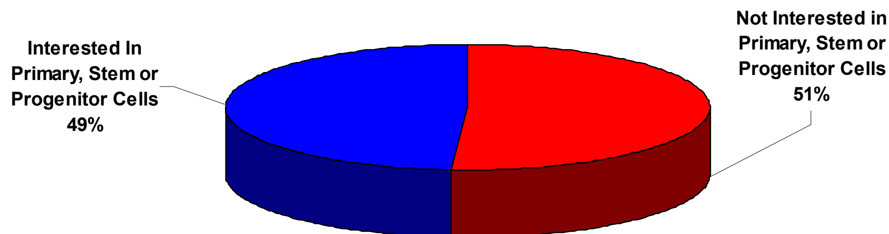
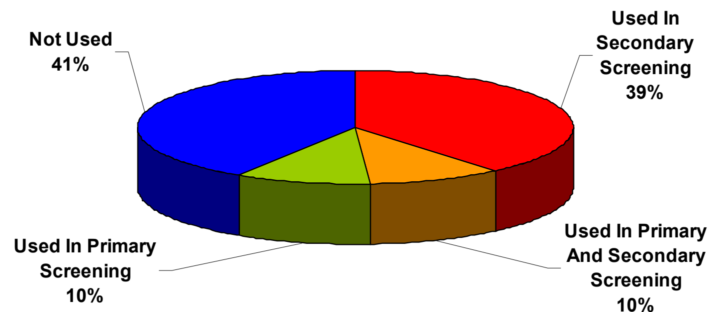
Trends in Cell Lines used in Screening









- Most cell-based screening today involves CHO and HEK 293 cells.
- The preferred source of new stable cell lines expressing targets of interest is to make them internally, although there is increasing willingness by Pharma to outsource.
- More than 50% of the individuals surveyed in lead discovery are interested in applying primary, stem and progenitor cells.

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3-D Cell Culture

- Limitations of screening in 2-D (monolayer) are becoming more apparent and there is growing evidence that 3-D cell cultures enable the discovery of patterns of gene expression and other biological activity that more closely mirror what happens in living organisms.
- To grow in 3-D culture, cells need to be embedded in a structure (matrix) that mimics the extracellular matrix of structural proteins. Ideally, these structures should be tissue-specific.
- Researchers are keen to move away from using materials derived from living tissues to create matrices as these are like to suffer from batch variation. Many experts believe that synthetic materials hold the most promise for creating tailor made matrices for 3-D cell culture.
- The 3-D culture of primary cells is likely to create a much more relevant and valuable cell assay for drug discovery.
- In 2003 the US National Cancer Institute (NCI) launched a new $40 million initiative on the cellular micro-environment which will include specific funding to spur the development of 3-D culturing techniques.

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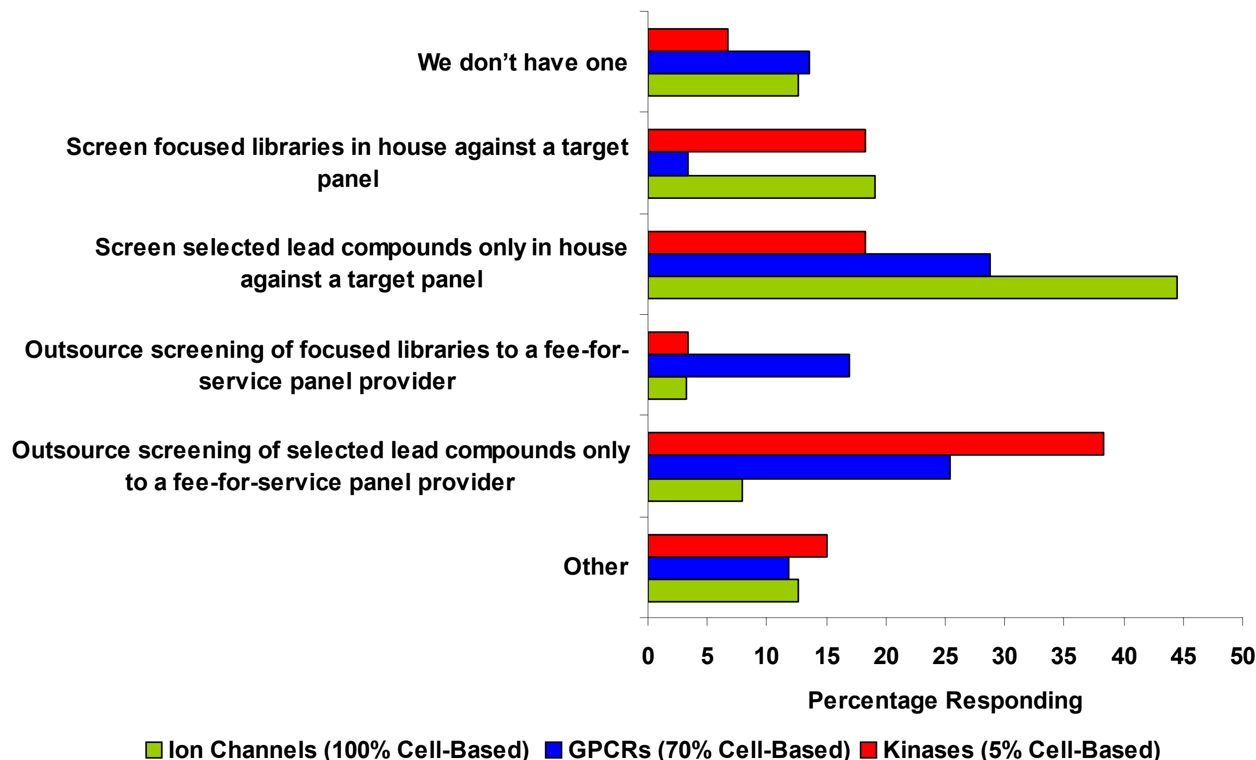
Selectivity Screening (Target Profiling)



Approach to Selectivity Screening

- This is the process of confirming and narrowing down hits
- Typically compounds are profiled against a panel (diverse range) of related targets to determine cross-reactivity (specificity and potency), but can be used to check out non-specific cell effects
- Increasingly profiling is being done earlier in the lead discovery process, on larger numbers of compounds, sometimes as part of primary screening
- Some big Pharma have their own target profiling groups
- Activity is frequently outsourced; significant opportunities for greater outsourcing

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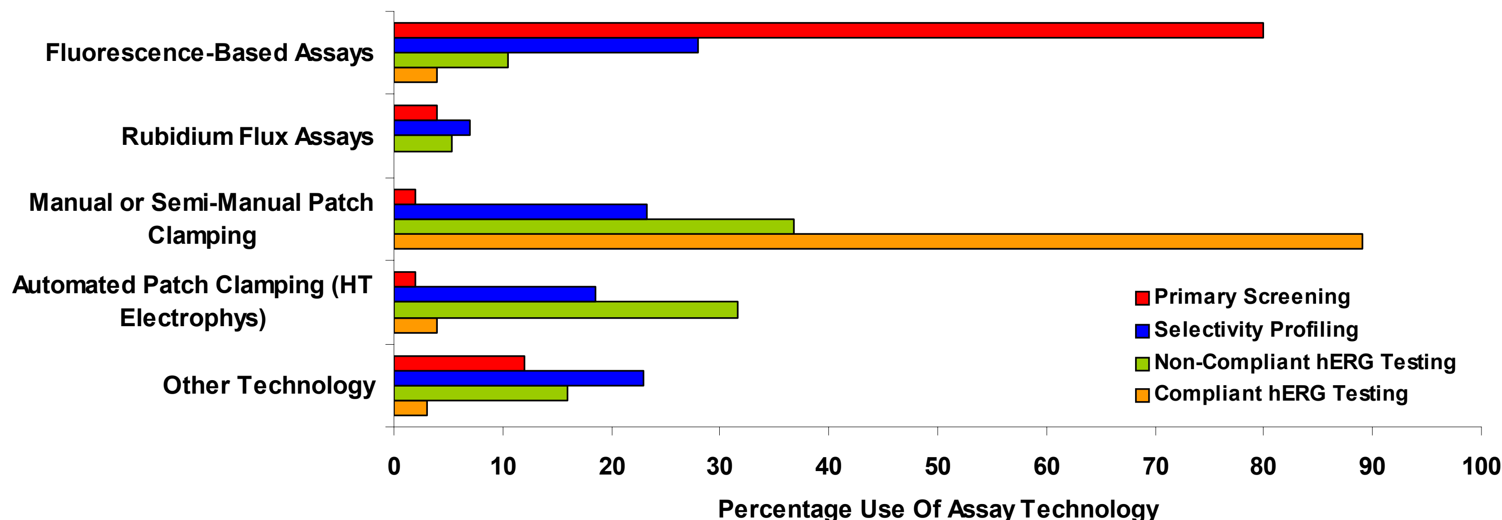
hERG Assays (1)



- Rapidly activating delayed rectifier K+ currents (IKr) critically contribute to cardiac repolarisation.
- hERG (human ether-a-go-go-related gene) is expressed in the heart and encodes the pore-forming α subunit for IKr
- Heterologously expressed hERG currents in mammalian cells, including HEK293 and CHO cells, are known to share pharmacological and biophysical properties with IKr.
- Mutations in hERG are characterized by delayed ventricular repolarisation, manifested on the electrocardiogram as a prolongation of QT interval (congenital long QT syndrome).
- Many drugs are known to cause QT prolongation by blocking IKr K+ channels (acquired long QT syndrome), which is the underlying cause of life-threatening torsade de pointes, a form of polymorphic ventricular arrhythmia, in susceptible individuals.
- Cardiac safety relating to IKr K+ channels has become a major concern of regulatory agencies; the FDA has stipulated a requirement for all drugs to be screened for cardiac side-effects, in particular the effects of cardiac electrophysiology. hERG channel inhibition has been identified as the firmest link to QT prolongation.

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hERG Assays (2)

- In order to prevent costly attrition, it has become a high priority in drug discovery to screen out inhibitory activity on hERG channels in lead compounds as early as possible.
- Electrophysiological study using the manual patch clamp technique in hERG-transfected mammalian cells generates the most definitive data on hERG inhibition. However, these assays are costly, time-consuming and labour intensive.
- Other functional assays for hERG include Rubidium (Rb+) flux or voltage-sensitive (fluorescent) dyes; both are inexpensive compared to electrophysiology. The correlation of Rb+ flux to patch clamp (although poor) is better than with membrane potential dyes, as the latter is associated with a high rate of false positives.
- Rb+ also offers sufficient throughput to support medium-sized HTS. As a consequence Rb+ based on AAS (Atomic Adsorption Spectroscopy) is used by ~40% of all Pharma ion channel labs in their ion channel discovery programmes.
- The other main technique used to ascertain cardiac safety is native cardiac cell work. This too has disadvantages: current equipment has a very low throughput, high maintenance and very expensive (with specialist research scientists required for operation).
- This lack of a rapid, standardised and biologically accurate assay preparation is significantly hampering toxicology screening processes and novel-drug development.
- Alternative assays have been developed including Automated patch clamping (APC), which is increasingly being adopted for non-compliant hERG assays of Hits, early in the Hit2Leads process.
- Although APC offers higher throughput, it is still not adequate for primary screening.
- Compliant hERG testing (for regulatory approval) still relies on manual patch clamping and is typically done early in the lead optimization process.

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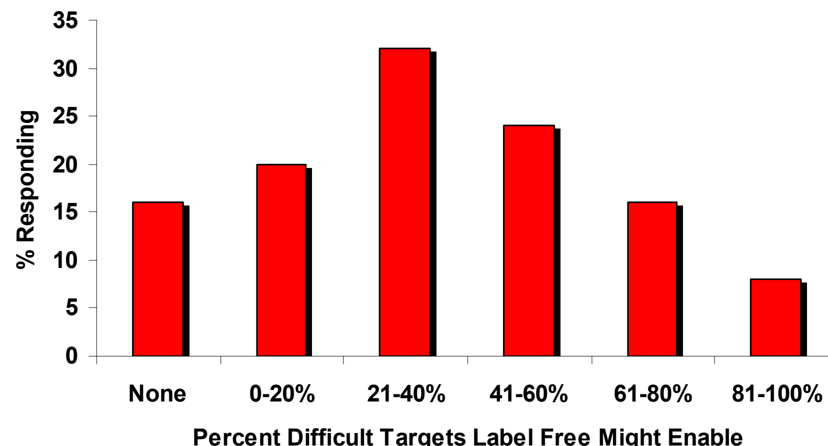
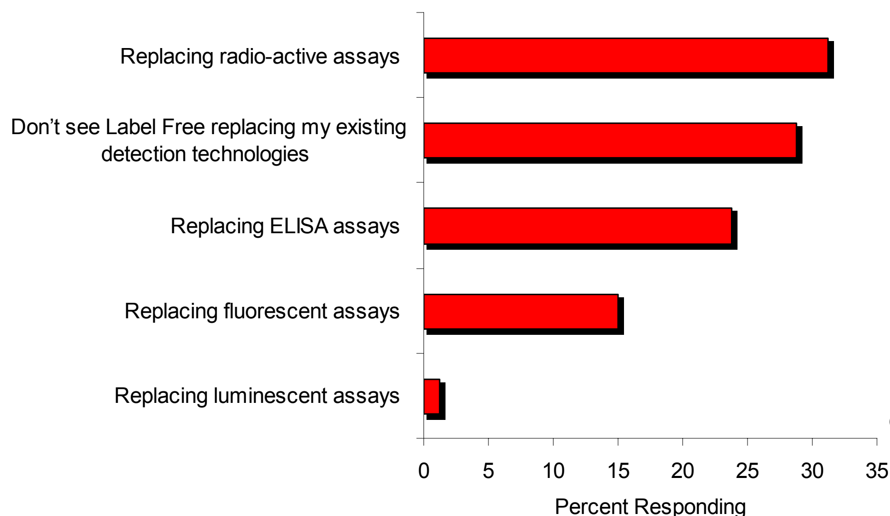
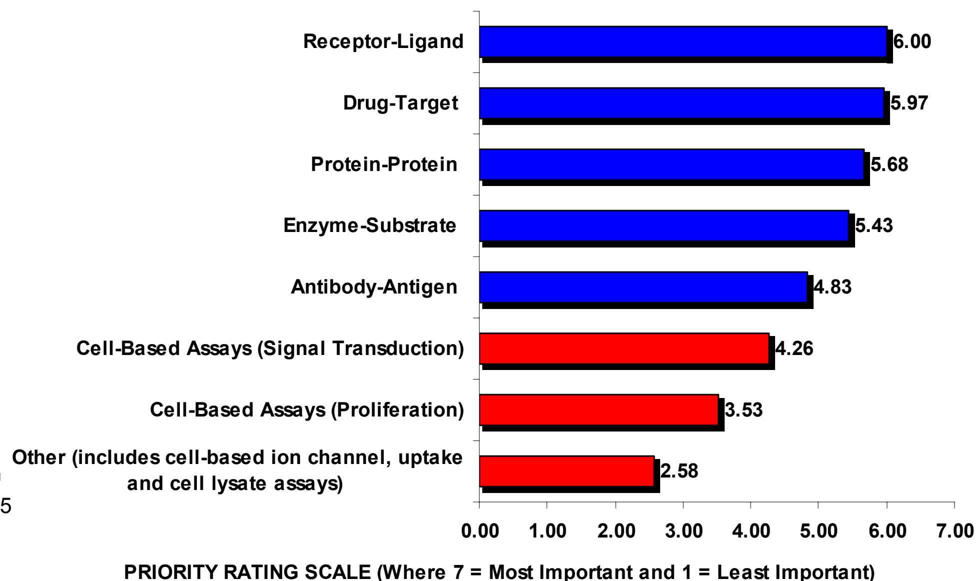
Label Free Detection

- Direct detection of binding or other biological interactions without the use of an external label
- Rapidly growing interest in higher throughput microplate-based alternatives to surface plasmon resonance (SPR and Biacore)
- Key drivers: access to difficult or new target classes (e.g. where no robust assay available); no influence of label on binding properties; no need to label ligand (reduced assay costs), generic assays; easier and simpler assay development
- **Emerging cell-based technologies to watch:** Cellular Dielectric Spectroscopy (MDS Sciex); Real-Time Cell Electronic Sensing (ACEA Biosciences); Optical Resonant Reflection (Corning EPIC™ or SRU Biosystems BIND™ systems)







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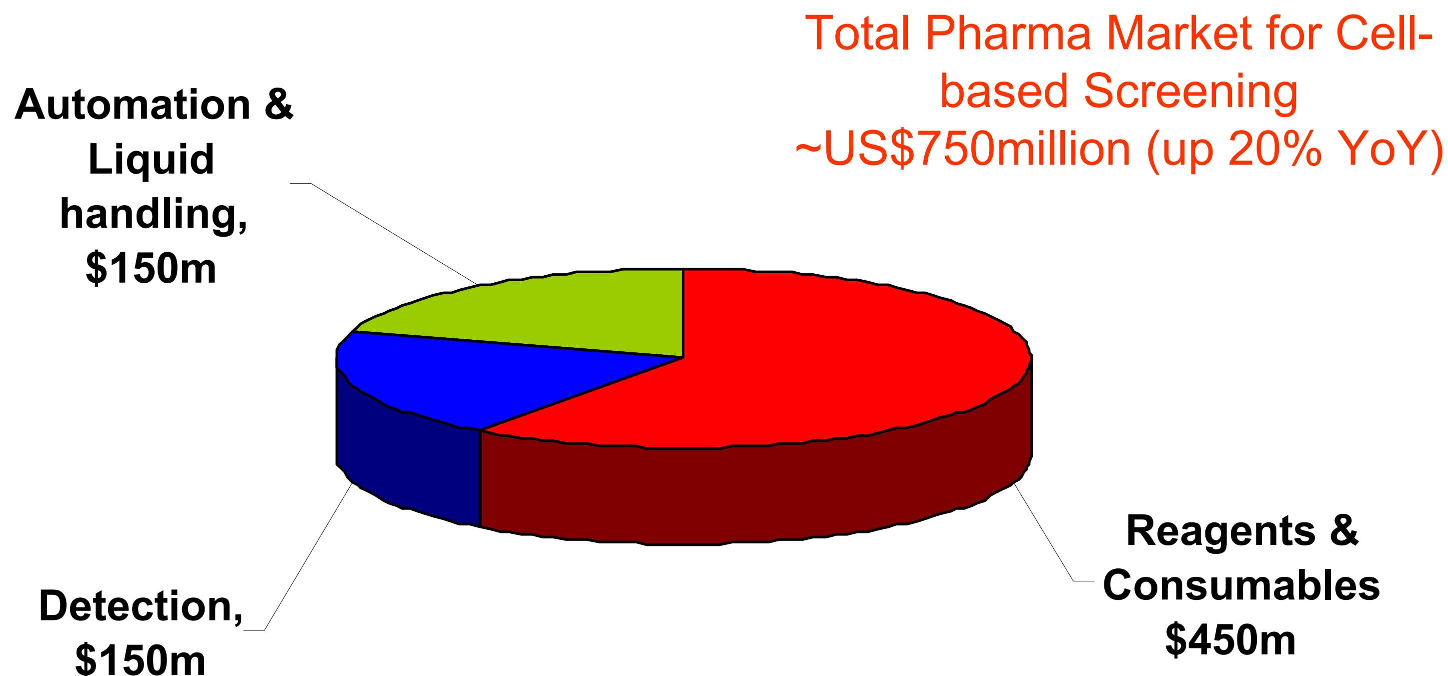
Market Size – Cell-Based Screening



- Estimates are for 2004 and are based on an amalgamation of recent published market survey and reports (mainly by www.htstec.com).
- Current market research does not account for the academic segment.

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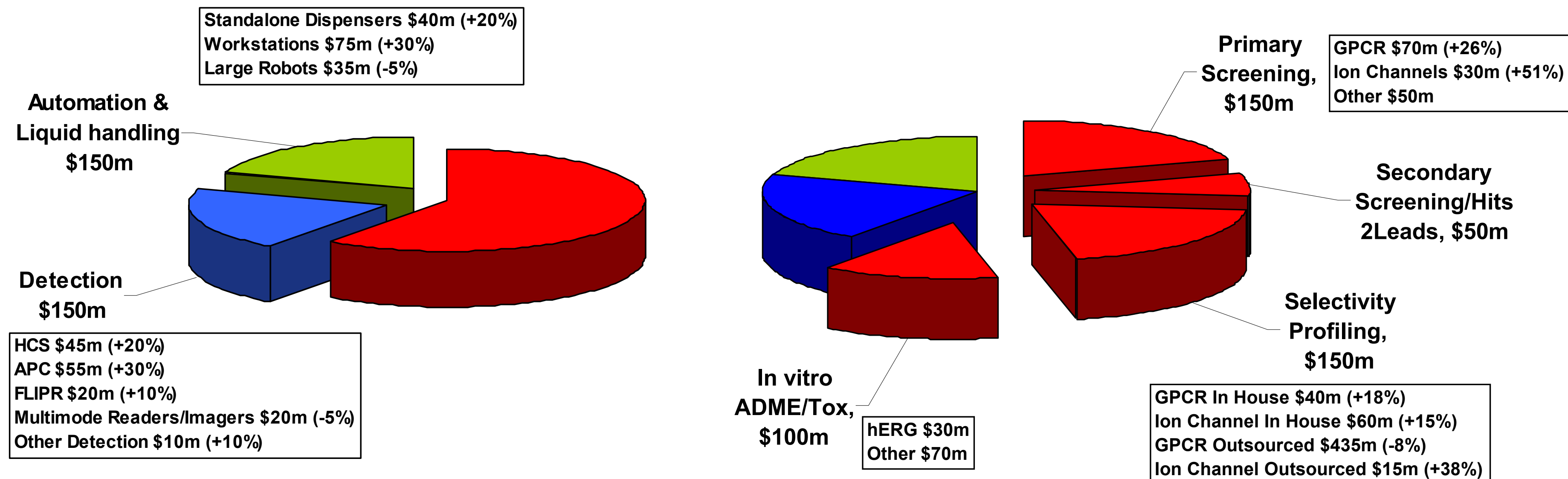
Break-down of Cell-Based Screening Market



- Ion channel and GPCR screening currently together represent around 2/3rd of all cell-based assays; no single assay technology or target class dominates the other segment.
- Stand-alone dispensers are split into 96/384 channel pipettors and non-contact bulk reagent dispensers. The latter are particularly important in adding cells suspensions to microplates.

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Cell-Based Assays Buying Considerations:

What Do We Mean By Quality In A Cell-Based/Reagent Product?

- **Stability over time, with good documentation of short term stability**
- **Stability, so that limits are based on real data**
- **A homogeneous product so that results are consistent between vials of the same batch**
- **A reproducible product so that the batches are consistent, and thereby results over the long term are consistent**
- **A commutable product that shows the same trends when assayed by different methods, by different labs on different occasions**

Choosing a New Assay Technology (in order of preference):

- **Time to validate and prove technology**
- **Cost to use (per data point)**
- **Cost to implement (training, licence fees and new capital investment)**
- **Positive cost/benefit over what already have, must bring something new, be enabling and offer improvement**
- **Demonstrated reliability**
- **Compatibility with existing/future automation (e.g. 1536)**

Choosing a New Screening Instrument (in order of preference):

- **Resulting data quality**
- **Instrument reliability, accuracy and precision (%CV)**
- **Instrument throughput**
- **Compatibility with existing/future automation (e.g. 1536)**
- **Capital investment (Instrument Cost)**
- **Service & support (Operating Cost)**

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Cell-Based Screening Bottlenecks, Challenges & Solutions (1)

Bottlenecks:

- **Assay development (especially secondary assays)**
- **Resources/capacity**
- **Cell supply for screening**
- **Automated solution for colony selection, cell picking**
- **Flexible instrumentation**
- **Lack of high throughput imaging-based solutions**
- **Sophisticated image analysis software**
- **Identification of quality leads**
- **Doing hERG assays earlier**

Potential Solutions:

- **More native-like cell lines and less reliance on rational approaches will improve overall success**
- **More multiplexed (analyse more than one parameter from a single sample) assays to permit screening of multiple targets and/or various toxicities**
- **More early predictive Tox using relevant cell types (primary cells)**
- **Better automated cell growth systems**
- **Treatments to get reproducible cell growth**
- **Technologies to stimulate growth**
- **Ability to freeze cells for consistency**
- **FACS sorting to improve cell line selection and consistency of cell culture**
- **Better understanding of immortalized cell growth**
- **Binding assays that confirm relevance of hits**
- **Generation, by suppliers, of stable cell lines already transformed with target**

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Cell-Based Screening Bottlenecks, Challenges & Solutions (2)

Challenges:

- Producing, growing & maintaining stable cells cultures
- Ensuring the bio-relevance of hits to the target
- Maintaining stable expression levels
- Obtaining selectivity across closely related targets whilst retaining potency and suitable physiochemical properties
- Generating a stable cell line
- Managing DMSO sensitivity, compound toxicity, long incubations
- Miniaturizing cell-based assays (dispensing)
- Maintaining precise protocols (automated cell factories)
- Developing robust assays

Potential Solutions:

- Better expression vectors
- Reduction in artefacts, non-specific interactions New reporter systems
- Higher throughput, user friendly imaging systems
- Pattern recognition algorithms
- Better ways of validating the performance of a cell
- Intelligent clone picking
- Automated counting of viable cell numbers based on non-destructive markers
- Automated measurement of confluence
- Fluorescent monitoring of expression markers or transfection markers in/and on the cell
- Fluorescent monitoring of excreted products
- Better fluorescent probes

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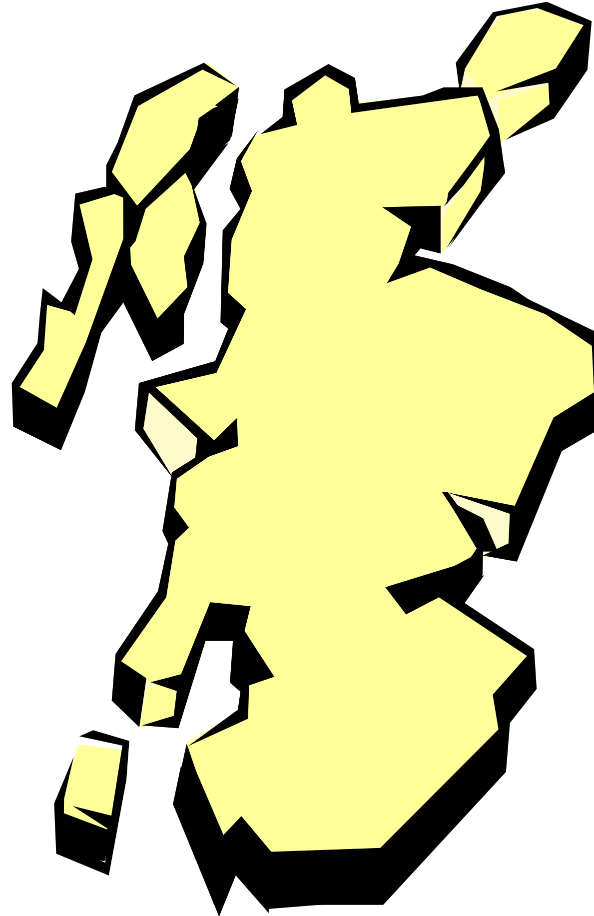
Academia

- **Glasgow University**
- **Strathclyde University**
  - Strathclyde Institute for Drug Research
- **Dundee University**



Commercial

- **Scottish Biomedical**
- **Organon** (part of Akzo Nobel)
- **Hannah InterActions**
- **Axiope**
- **Biopta**
- **LUX biotech**
- **Upstate** (part of Serologicals Corp.)
- **CXR**

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- **Assay** – analytical method to monitor a biological process
- **Screen –** an assay with the following attributes:
  - addresses relevant molecular/cellular interactions for hit identification
  - utilizes appropriate detection technology/demonstration of a specific signal
  - uses simplified methodology
  - optimized signal/background (S/B) ratio
  - fulfils criteria of reproducibility and acceptable robustness (usually Z'Factor > 0.5)
  - minimized reagent requirement (making it cheap)
  - minimized reaction time (making it quick)
  - reasonable throughput (making it suitable for HTS)
- **True Positive** – compound with activity that confirms on retest
- **Negative** – compound lacking activity in all screens
- **False Positive** – compound showing activity in first screen that is not confirmed on retest
- **False Negative** – compound with activity that was not identified (hidden) in first screen
- **IC50** – compound concentration causing 50% inhibition in a bioassay
- **% Inhibition** – proportional reduction in positive control activity in presence of compound after subtraction of background (negative control)
- **Z'-Factor** – a tool for comparison and evaluation of overall assay quality, without intervention of compounds. It is utilized in assay development and optimization and is a measure of assay control variation
- **S/B** – Signal-to-Background ratio = mean signal/mean background
- **Hit Rate** – the % compounds with positive activity in a screen above a preset threshold
- **Threshold** – the level of inhibition above which a compound is described as being active; the threshold is adjusted to minimise false positives and false negatives and to keep the number of actives within a reasonable (manageable) limit

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- **IND** – Investigational New Drug Application
- **NDA** – New Drug Application
- **NCE** – New Chemical Entity
- **HIT** – Compound with confirmed activity in a primary screen and known level of activity (% inhibition and IC50).
- **LEAD** – Compound or series derived from a hit, with confirmed activity in secondary screens, usually with enhanced activity over hit, preliminary QSAR, in vitro ADMET and preliminary in vivo pharmacokinetic (PK) profile.
- **QSAR** – Quantitative Structure Activity Relationship
- **ADMET** – Absorption Distribution Metabolism Excretion and Toxicity
- **Primary Screening** - The first large-scale screen (filter) for activity against target using the full diversity of the compound library. Can be biochemical or cell-based.
- **Secondary Screening** - Any screen conducted after the primary screen that provides additional information to move a hit towards a lead (Hits2Leads). Includes the confirmation of hits, selectivity, dose response or potency and functional cell-based assays to determine the mechanism of action.
- **Selectivity Screening** – The process of confirming and narrowing down hits. Typically compounds are profiled against a panel (diverse range) of related targets to determine cross-reactivity, but can be used to check out non-specific cell effects. May be performed at different stages in the lead discovery process. The current trend is to carry out this testing earlier, sometimes as part of primary screening. This activity is frequently outsourced.
- **Counter Screening -** A secondary screen that is performed on Hits to eliminate false positives and negatives and to confirm true positives. It is usually the same target run using a different or alternative assay technology. In some cases these screens may be cell-based versus biochemical primary screen. The outcome of a counter screen is usually a confirmed hit.
- **Lead Optimization** - The process by which leads progress towards candidates (Leads2Candidates). Involves SAR (Structure Activity Relations) work on one or more lead series, medicinal chemistry and demonstration of adequate ADMET and PK profile.

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KEY DECISION POINTS

- **IC50 Hit**
  - Dose-dependent potency in HTS assay
- **Validated Hit**
  - Identity and purity proven
  - Suitable structure
- **Qualified Hit**
  - Tested in cell-based assay
  - Evaluated in vitro selectivity
  - Suitable PhysChem (calc and logP exp) and Tox (calc) properties
  - Potential for chemical optimization
  - Early hERG testing shows no libility
- **Lead Structure**
  - Active in relevant cell-based assay
  - Suitable pharmacokinetic properties
  - Suitable PhysChem Properties (experimental)
  - Efficacy in vivo or in secondary in vitro model
  - Clear Patent strategy
  - Preliminary QSAR/clear optimization strategy

TYPICAL LEAD STRUCTURE CRITERIA

- **Molecular properties**
  - Molecular weight 200 to 500
  - Log P/log D -1 to 5
  - H-Donors 0 to 5
  - H-Acceptors < 10
  - Solubility (H2O, pH 7.4) > 5 mg/l (or 10xIC50)
- **Pharmacodynamics**
  - Potency in vitro (IC50) 100 to 1000 nM
  - Efficacy in vitro active in cell-based assay
  - Selectivity >10 (very project specific)
- **Pharmacokinetics**

  ***in vitro*:** **-** Permeability (Caco2) 100 cm/sec x 10^{-7}
  - Stability liver microsomes
  - Rat, mouse, human (%R30min) 50 to 80%

  ***in vivo* (rat): -** Plasma clearance (ml/min/kg) < 50
  - Distribution volume (l/kg) 1-10
  - Oral bioavailability (%) > 25
- **Structural optimization potential**
  - Synthetic accessibility
  - Preliminary QSAR
- **Patentability**
  - Clear patent strategy

EARLY MEDCHEM ACIVITIES (In Hits2Leads)

- Suggestion of hits for validation and qualification based on expected optimization potential
- Synthesis of qualified Hits
- Synthesis of first analogs for preliminary QSAR
- (Limited) Synthetic optimization of qualified Hits to evaluate optimization potential
- Synthesis of virtual Hits
- Synthesis of other compounds suggested by computational chemistry (de novo)
- Synthesis of tool compounds / competitor compounds

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