University of Pennsylvania Perelman School of Medicine High-Throughput Screening Core

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Mission

➢ **Provide the PSOM community with HTS resources.**

- To <u>educate</u> and assist with HTS assay development, optimization, miniaturization, and validation

- To provide laboratory robotics <u>infrastructure</u> and technically trained staff for HTS

- To provide <u>libraries</u> of small molecule and genetic tools for HTS

- To facilitate small-scale screens from <u>user-defined</u> gene-sets

➢ **Develop novel technology**

➢ **Seed collaborative research programs and grants.**

➢ **Educate the SOM on utility and uses of HTS**

SOM Screening Core

› **Libraries**
  › Maintenance
  › Distribution
› **Liquid handling**
  › Janus MDT/Verispan 8-tip
  › Bulk reagent dispensors
  › ELx405 microplate washer
› **Assay Detection**
  › EnVision multi-mode microplate reader
  › ImageXpress Micro
  › FLIPR screening system
› **BSL2 Tissue Culture capabilities**
  › Hood
  › Incubators
› **Informatics**
  › Automated image analysis
  › Statistical analysis
  › Validation analysis

Automation: Screening

Automated pippeting station
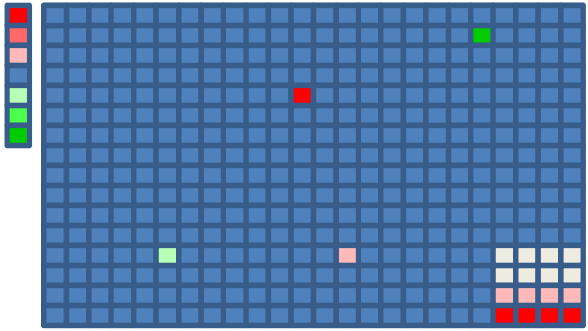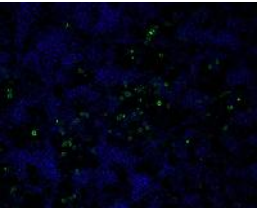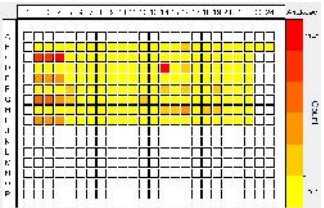
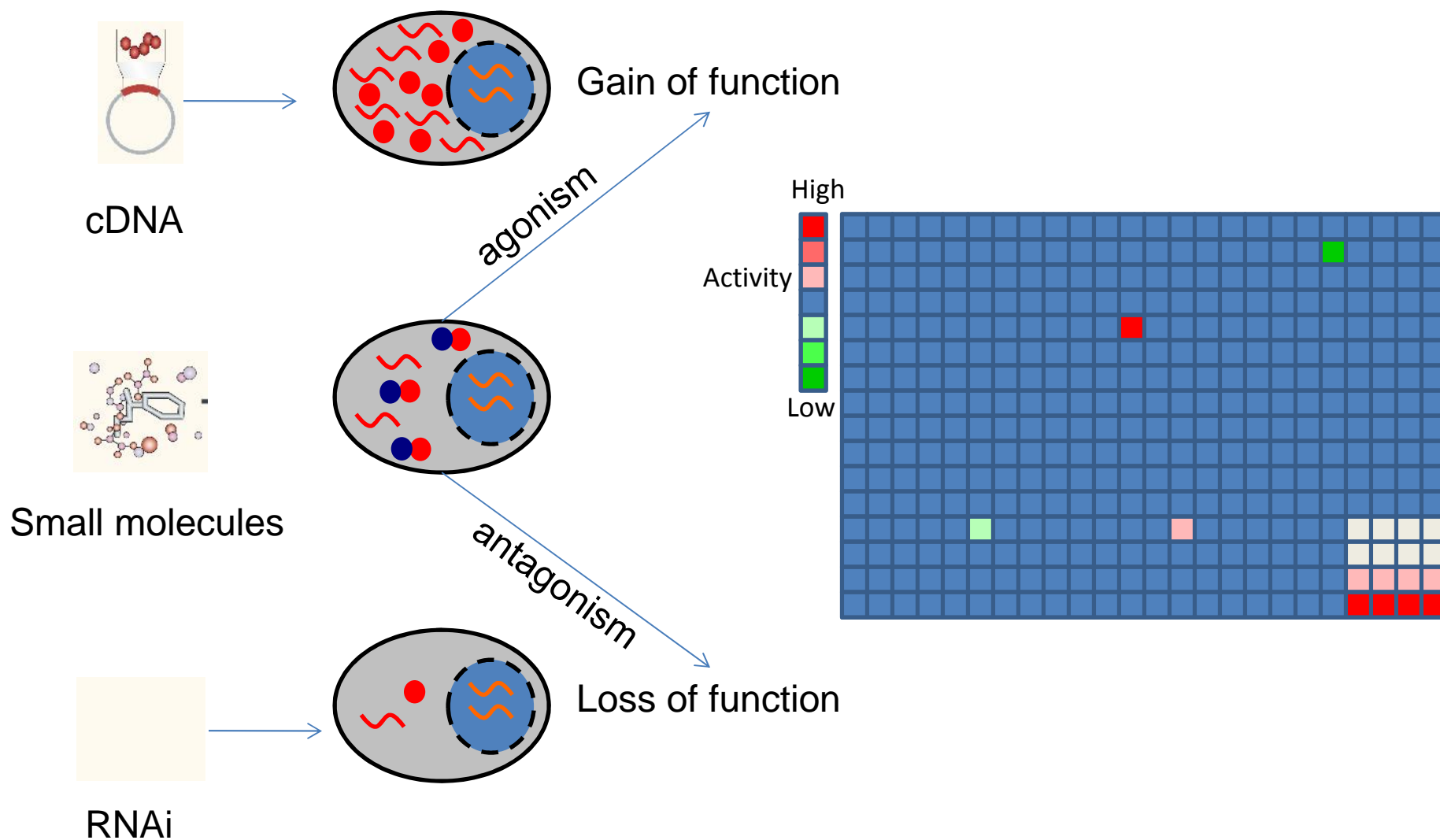384w plates spotted with cDNAs or siRNAs

Dispenser

HT Multimode Reader

Automated Microscopy

"Hit"

Slide courtesy of J.Hogenesch

Cell-based functional screening approaches

cDNA

Small molecules

RNAi

Gain of function

agonism

antagonism

Loss of function

High

Activity

Low

SOM Screening Core Chemical Libraries

Bioactives, FDA approved, and FDA-like compounds
- <u>SelleckChem Bioactives</u> (~2100)
  - Kinase Inhibitors, Epigenetic Inhibitors, Cancer compounds, GPCR/Ion Channel, Metabolism, Microbiology, FDA approved/FDA-like
- <u>LOPAC</u> (1280): Library of Pharmacologically Active Compounds

Natural Products
- <u>Microsource Purified Natural Products</u> (800)

Diversity set
Chembridge (32,000 total)
> 20,000 from Core set and 12,000 from Express Pick set
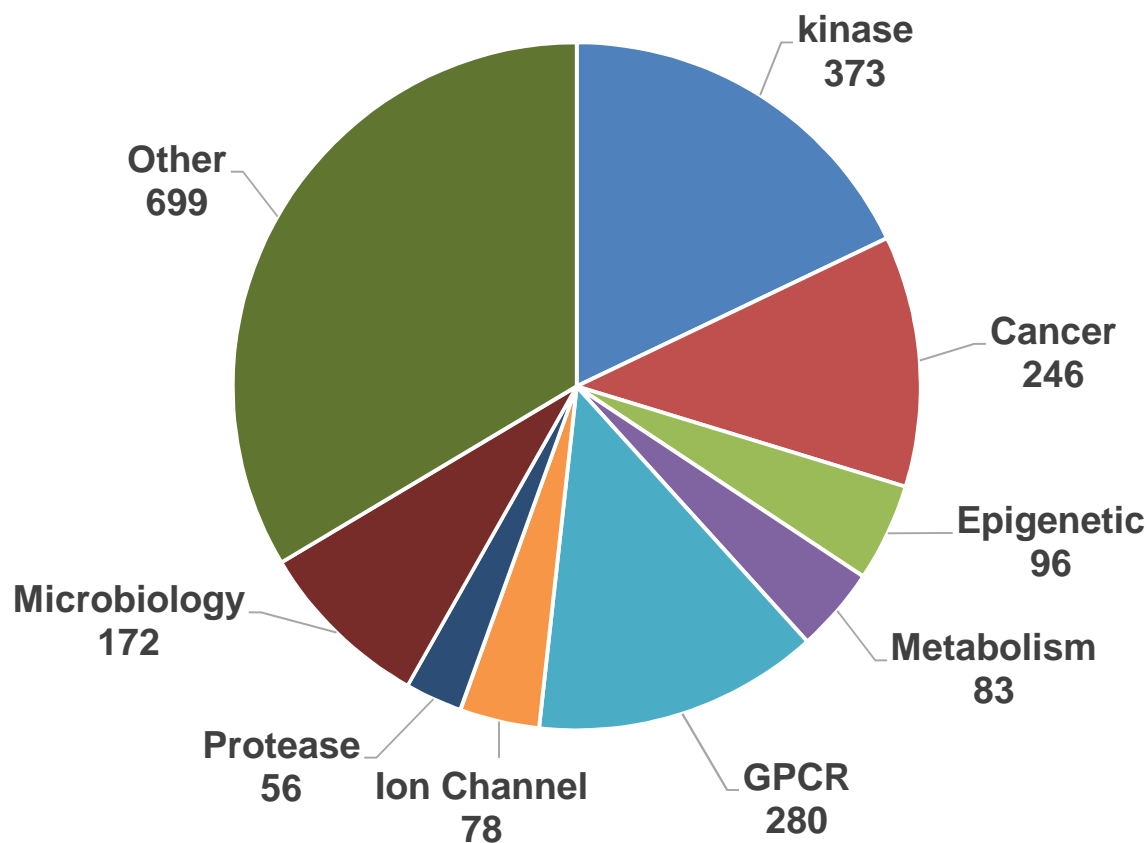
ChemDiv (12,000 total)
> SMART library

Chemical Libraries:
SelleckChem Bioactive Library (2083 cmpds)

•Kinase Inhibitors, Epigenetic Inhibitors, Cancer compounds, GPCR/Ion Channel, Metabolism, Microbiology, FDA approved/FDA-like

kinase
373

Cancer
246

Epigenetic
96

Metabolism
83

GPCR
280

Ion Channel
78

Protease
56

Microbiology
172

Other
699

http://www.selleckchem.com/screening/chemical-library.html

Chemical Libraries:
LOPAC[1280]: Library of Pharmacologically Active Compounds

1,280 FDA approved, marketed drugs with annotated biological activities, predictable activities and proven scaffolds directed against a wide range of drug targets


http://www.sigmaaldrich.com/catalog/product/sigma/lo1280?lang=en&region=US
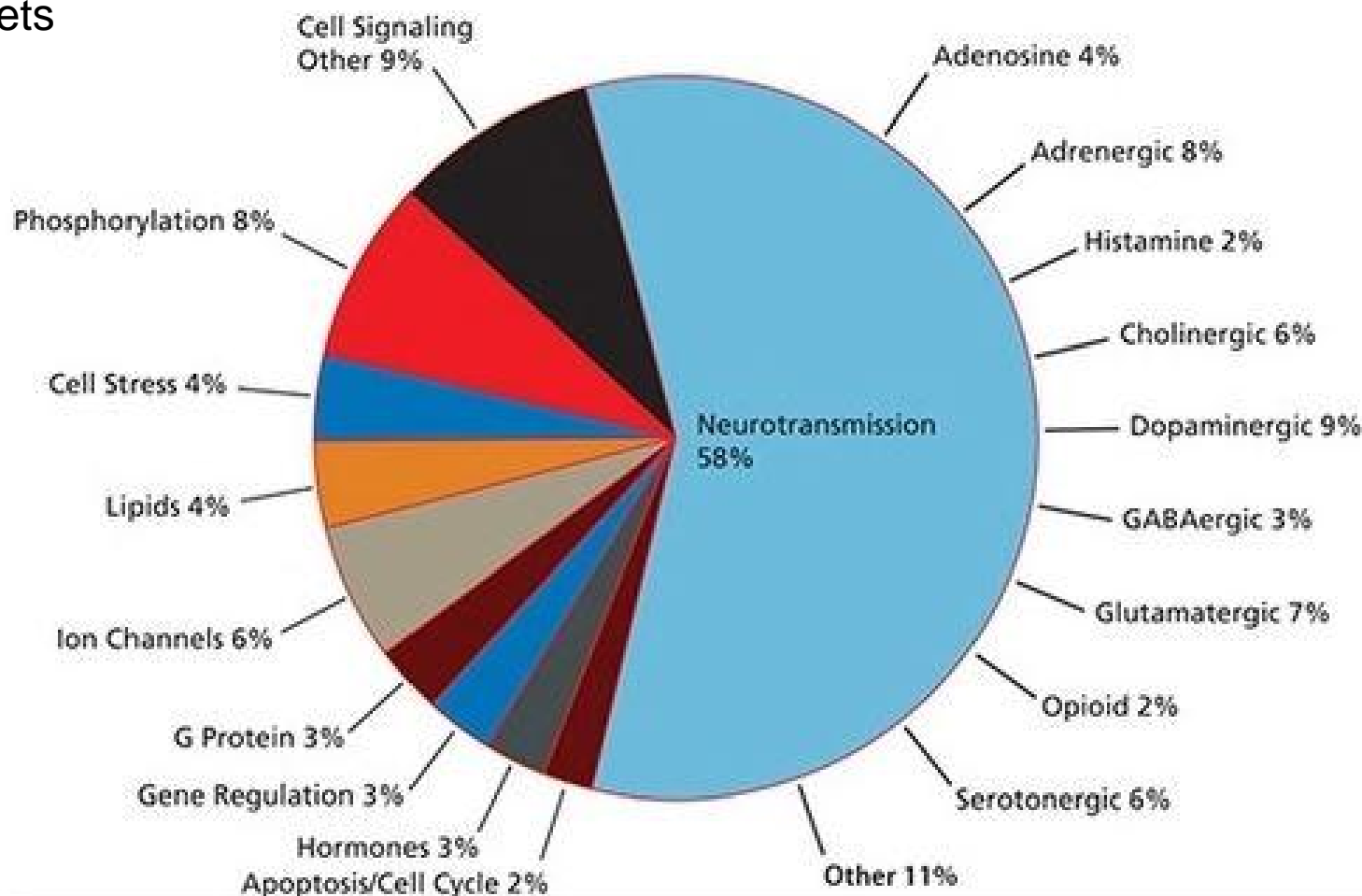
Diversity Library: cherry picked 44K from…

- Started with ~200,000 compounds

- Used modified Lipinski parameters to filter the set.

- Removed Reactive groups (e.g. Michael acceptors) and compounds with undesirable functionalities.

- Duplicates were removed.

- Lead Finder Clustering and MACCS fingerprints were generated.

- PAINS1, PAINS2 and PAINS3 substructure filters were applied.

- REOS filters were applied.

- Property Based Selection of 50K cpds was performed

- Vendor re-supply validated

Joe Salvino & Dora Schnur, Drexel SOM

44K Diversity Set Characteristics
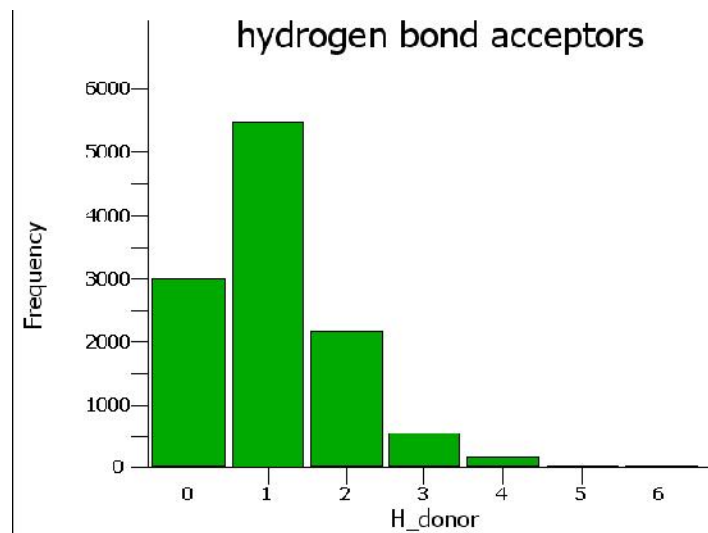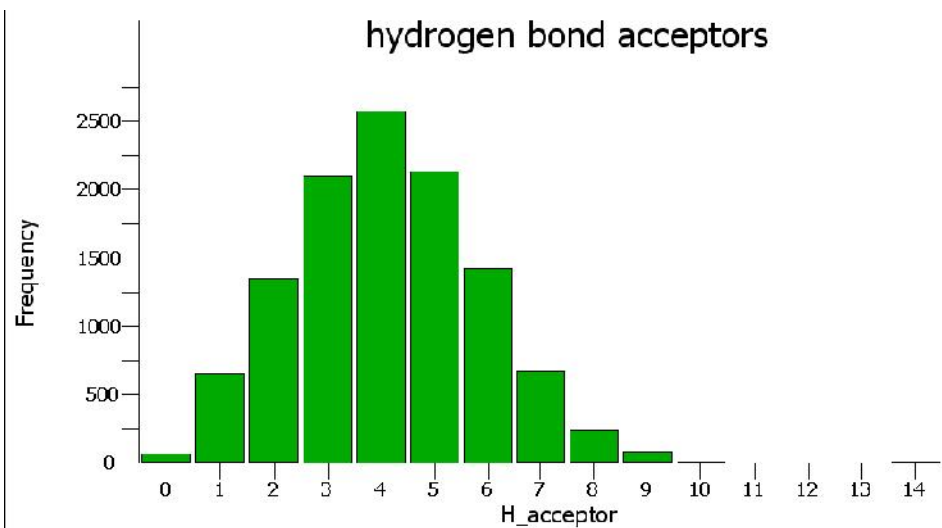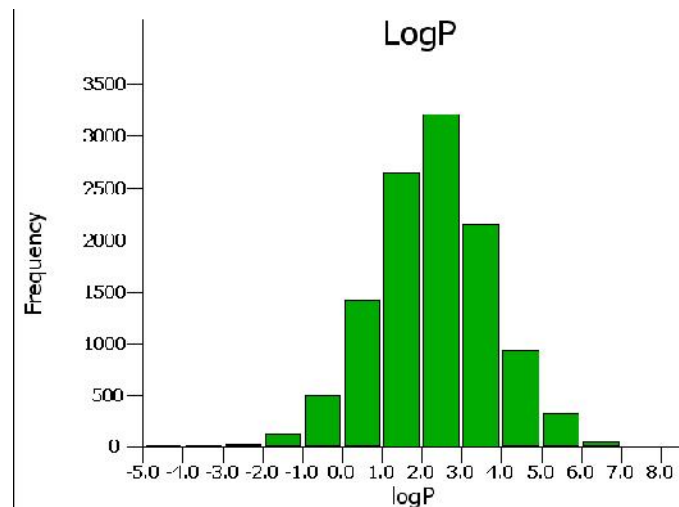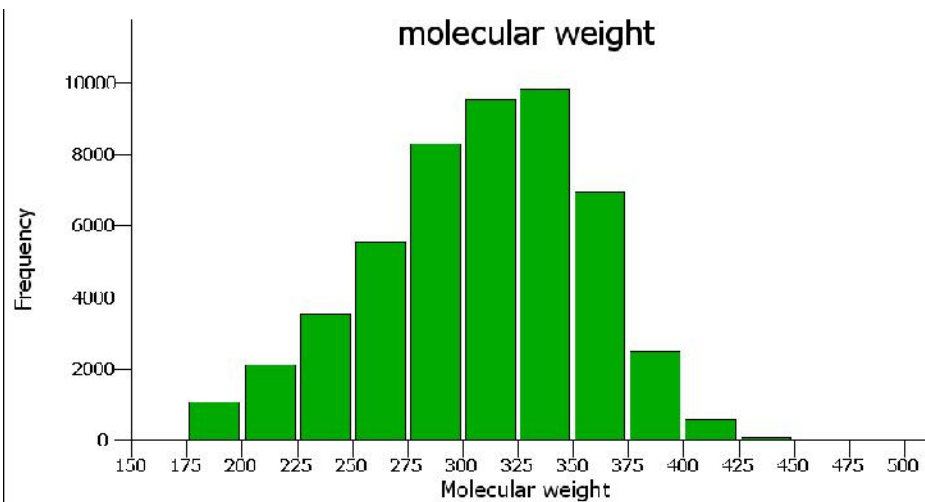
44K Diversity Set Characteristics-II
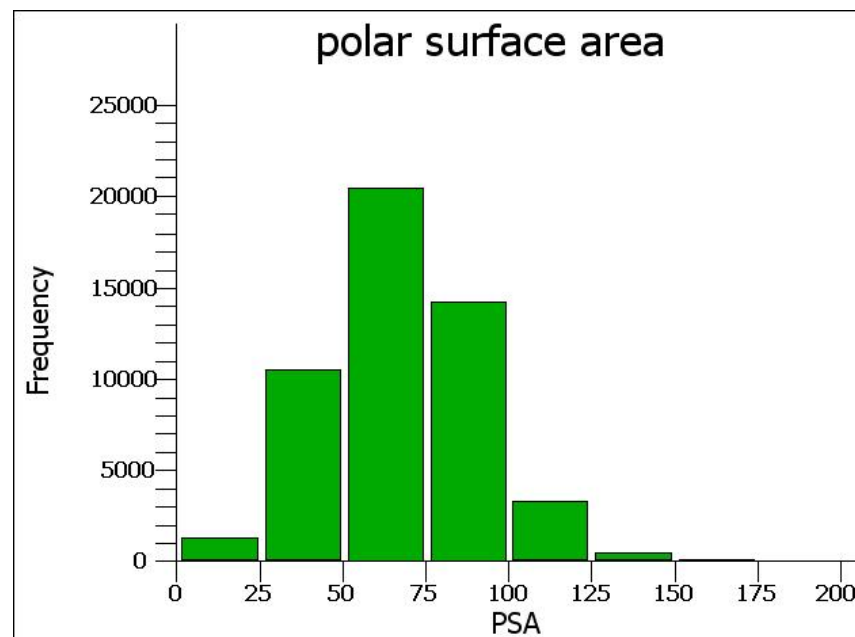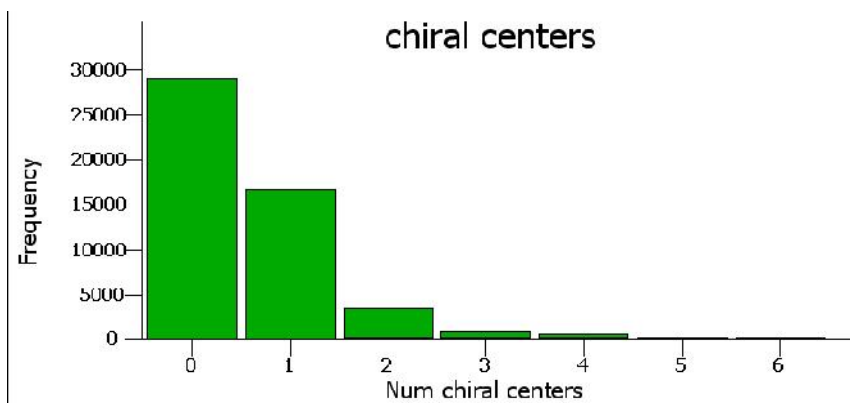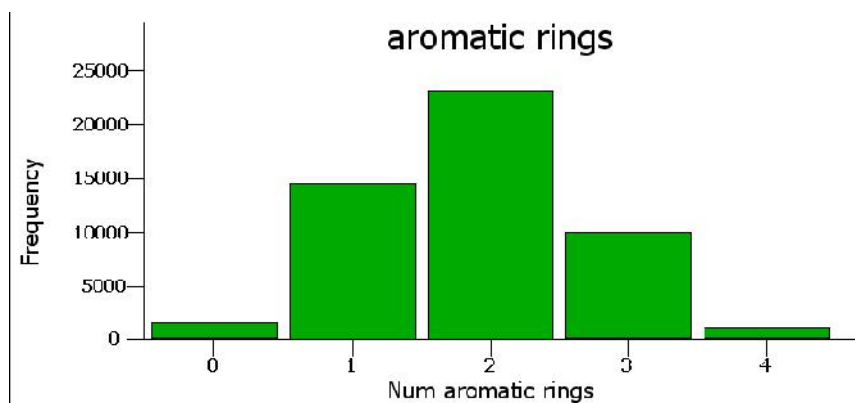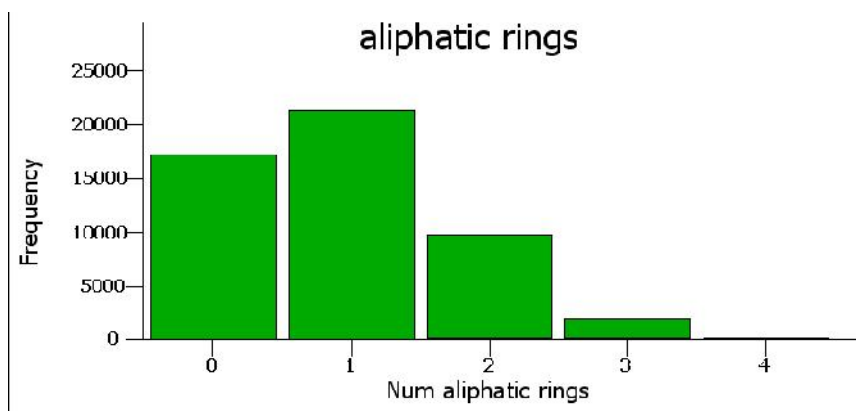
SOM Screening Core Genetic Libraries

siRNA
- human genome-wide, human drugable genome, human GO categories
- <u>user-defined</u> human and mouse

Non-coding RNAs
- lncRNAs (human)
- miRNA mimics/antagonists (human V20)

Human TRC 2.0 and Mouse TRC1.0 Lentivirus shRNA library
- Screening pools: GO categories; <u>user-defined</u> sets
- Order groups/individuals

MGC cDNA collection (CMV-driven)
- 18,000 full length, sequenced, mouse and human (arrayed);
- <u>user-defined</u> sets
- Order groups/individuals

Genetic Libraries:
Ambion Silencer Select siRNA & miRNA mimics/inh.

A. Human genome Silencer Select siRNA

	Genes	siRNAs	siRNAs/GT	format	# plates*
Druggable genome^	9032	27093	3	pooled	26
Druggable genome Ext.	1383	4149	3	pooled	4
Rest of Genome	11170	33510	3	pooled	34

^ Further organized into GO categories (e.g. kinases, NHRs, GPCRs)

B. Human non-coding RNA siRNA (lncs) & miRNA mimics/inhibitors

	Targets	siRNAs	siRNAs/Tar	format	# plates*
long non-coding RNAs (lncs)	2220	6660	3	pooled	8
miRNA mimics (human V20)	2555	2555	1	individual	8
miRNA inhibitors (human V20)	2555	2555	1	individual	8

* All assay plates are pre-spotted with siRNAs/miRNAs, including controls

Control siRNAs

Control	siRNA_ID	Type	Library
Neg1	S813	Negative	Genome, lnc
Neg2	S814	Negative	Genome, lnc
GAPDH	S815	Positive mammalian	Genome, lnc
GFP	s229097	Positive Non-mammalian	genome
Luciferase	s229095	Positive Non-mammalian	genome
Kif11	S7903	Positive Mammalian (death)	Genome, lnc, mir
MALAT1	s239370	Positive Mammalian	Lnc

* Controls are pre-spotted in all assay plates

Custom siRNA Libraries

- ➢ Custom siRNA libraries are provided by Ambion/Life technologies

- ➢ Minimum order of 20 siRNAs REQUIRED

- ➢ 0.1 nmol of <u>Silencer Select</u> siRNA (human)

- ➢ 1 nmol of <u>Silencer</u> siRNAs (human and mouse)

- ➢ siRNAs are received in microtiter plates (96 or 384)

- ➢ **All orders are submitted through the HTSC**

Custom Library Ordering

- **Complete the order form**
  - ➤ Customer information (26 digit Penn fund no) (REQUIRED)
  - ➤ NCBI Gene ID, Gene Symbol, # of siRNAs/ gene target, species (REQUIRED)
  - ➤ Email to dschultz@upenn.edu

Product Line (select from dropdown list)	siRNA or miRNA ID	# for same target*	Catalog Number	Entrez Gene ID**	Gene Symbol
Silencer® Select siRNA		3	4427030	23468	CBX5
Silencer® Select siRNA		3	4427030	1660	DHX9
Silencer® Select siRNA		3	4427030	3069	HDLBP
Silencer® Select siRNA		3	4427030	27316	RBMX, RBMXL1
Silencer® Select siRNA		3	4427030	23435	TARDBP
Silencer® Select siRNA		3	4427030	5725	PTBP1

Custom Library Ordering (cont)

- ➢ **Costs**

    - • **HTS/Omics validation set**

        100 gene targets x 3 siRNAs is ~$4,500

    - • **Custom library** (Epigenetics/metabolism)

        500 gene targets x 3 siRNAs is ~$12,000

- ➢ **Turnaround**

Order size	Order to deliver
20-250 siRNAs	5-15 business days
251-500 siRNAs	16-25 business days
501-1000 siRNAs	20-30 business days
1001-2500 siRNAs	25-30 business days

Genetic libraries:
TRC human/mouse LV-shRNA library

		Gene Targets	clones	Validated	% val
Human	complete	20,018	129,695	43,470	34
Mouse	V1.0	15,960	77,819	20,564	26
Totals		35,978	207,514	64,034	

http://www.sigmaaldrich.com/life-science/functional-genomics-and-rnai/shrna/library-information.html
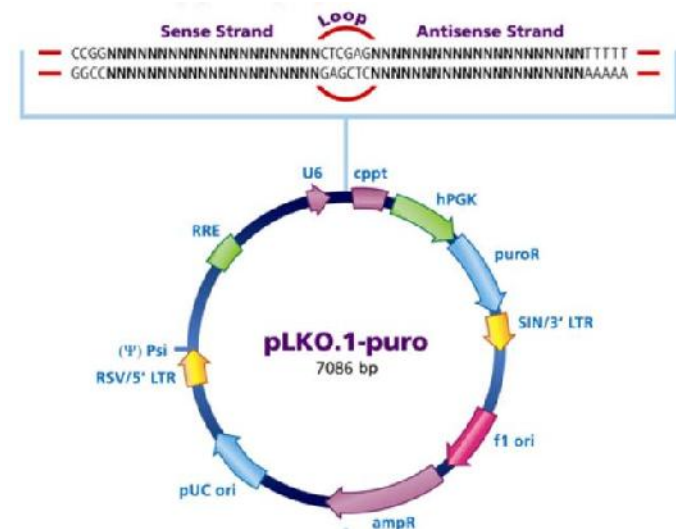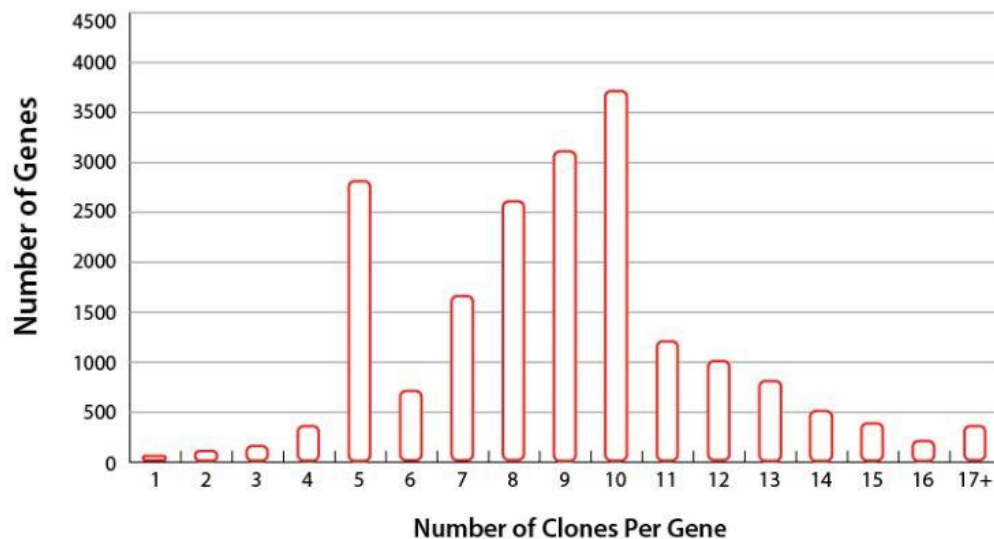
shRNA library services

➢ **Distribution of Individual clones**
  - $100.00 per Gene Target (5+ clones)
  - Investigator should sequence confirm shRNA sequence
  - Investigator responsible for validating knock-down

➢ **Preparation of Custom Gene Sets**
  - Cherry-pick and array clones of interest into new plates
    GO categories (e.g. Epigenetic targets)
    User-defined sets (e.g. 'omics validation)
 - Glycerol stock plate + pooled plasmid DNA
  - 150 gene Targets/625 clones: $2000
  - 600 gene Targets/3000 clones: $5000

Ordering shRNA clones

- **Complete the order form**
    - Customer information (26 digit Penn fund no) (REQUIRED)
    - NCBI Gen ID, Gene Symbol, Refseq no., species (REQUIRED)
    - Email to dschultz@upenn.edu

Instructions															
As shown in the example, please provide:				Price: $100.00 per complete set of clones for a Gene Target											
Entrez Gene_ID (required)															
Gene_symbol (required)															
RefSeq_number (required)															
species-human or mouse (required)															
Leave the remaining fields blank.															

EXAMPLE

#	GENE_ID	SYMBOL	REFSEQ_ID	species	clone_#	TRC_ID	TRC_ver	PLATE_NAME	Row	Col	Freezer	Freezer_loc	Sense_seq	VALIDATED	CELL_LINE
1	1654	DDX3X	NM_001356.3	human											
2	10155	TRIM28	NM_005762.2	human											
3	1029	CDKN2A	NM_058197.4	human											
4	7157	TP53	NM_001126116.1	human											

Order Form

#	GENE_ID	SYMBOL	REFSEQ_ID	species	clone_#	TRC_ID	TRC_ver	PLATE_NAME	Row	Col	Freezer	Freezer_loc	Sense_seq	VALIDATED	CELL_LINE
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															

Genetic libraries:MGC cDNA collection

- Obtained by J.Hogenesch

- 18,000 full length, sequenced, mouse and human cDNAs pre-cloned into pCMV-SPORT6
  - Insert is fully sequenced and guaranteed to match corresponding BC Accession Number
  - Expression-ready vectors eliminate additional cloning steps
  - Robust CMV promoters drive cDNA expression
  - Gateway sites flanking coding sequence allow for additional flexibility
  - Stored as bacterial glycerol stocks, arrayed in 96 well microtiter plates

Screen:

- Complete library
- user-defined sets (e.g. Interferon Stimulated Genes-ISGs)
- Order individual clones or groups
  - $50.00/clone
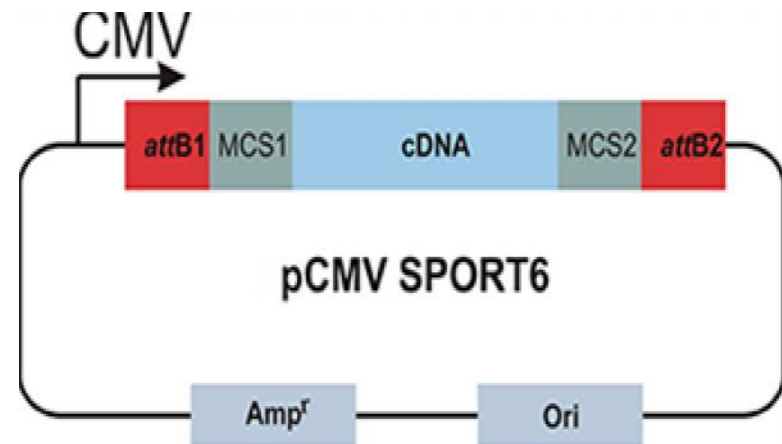
MCS1: KpnI,AgeI,EcoRI,SmaI,EcoRV, and SalI
MCS2: NotI,XbaI,XhoI,ApaI,HindIII, and MluI

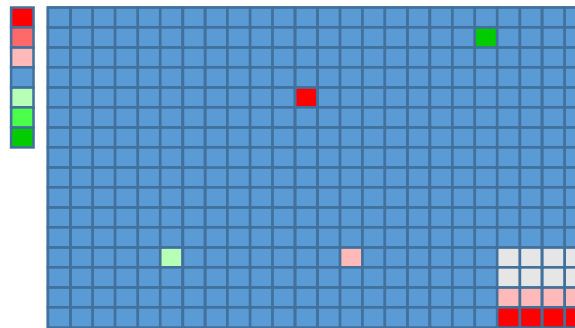# Automation: Screening

Automated
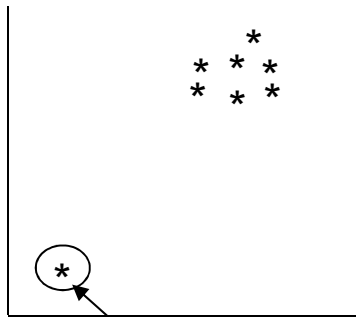pippeting station
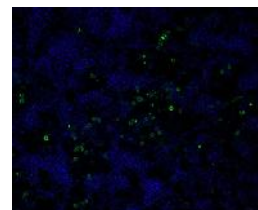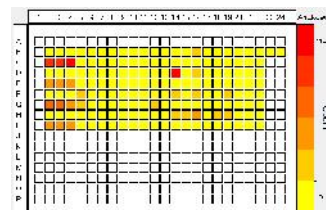
384w plates spotted
with cDNAs or siRNAs

Dispenser

HT Multimode Reader

Automated Microscopy

Slide courtesy of J.Hogenesch

"Hit"

Envision Xcite: multi-mode microplate reader

A. Measurement Technologies

- absorbance,
- flouresence intensity (FI),
- fluorescence polarization (FP),
- time-resolved fluorescence (HTRF)
- ultra-luminesence
- AlphaScreen (Amplified Luminescence Proximity Homogeneous Assay)

B. Assays

Anything that requires a plate reader!

- Luciferase Reporter Gene Assays (RGAs)
- Viability assays (e.g. Cell-titer Glo)
- Enzymatic assays (FI)
- Protein: nucleic acid interaction (FP, HTRF, alphascreen)
- Protein: protein interactions (FP, HTRF, alphascreen)
- AlphaLISA

ImageXpress Micro XLS

A. Enables automated acquisition of multi-channel fluorescence images in microtiter plates

- 5 colors

B. Phenotypic assays upto 40X

- Physical properties of cells (size, shape, etc.)

- Puncta formation (autophagy, lipid droplets, etc)

- Activity (migration, invasion, neurite outgrowth)

- Expression and localization of native proteins

- Measurement of fluorescent labels (antibody stains, EdU/BrdU, Calcein, PI uptake, mitochondria, etc.)
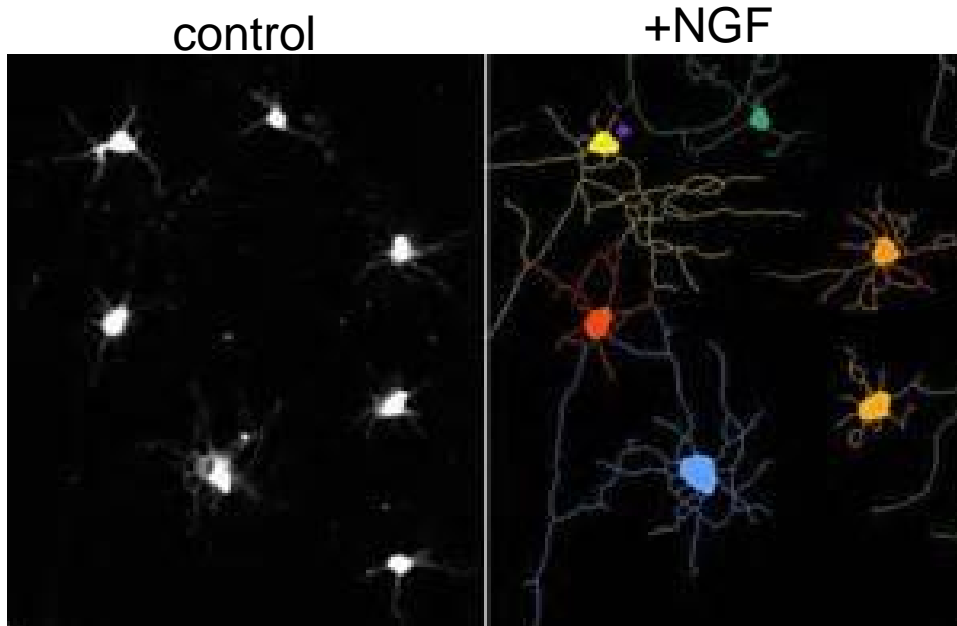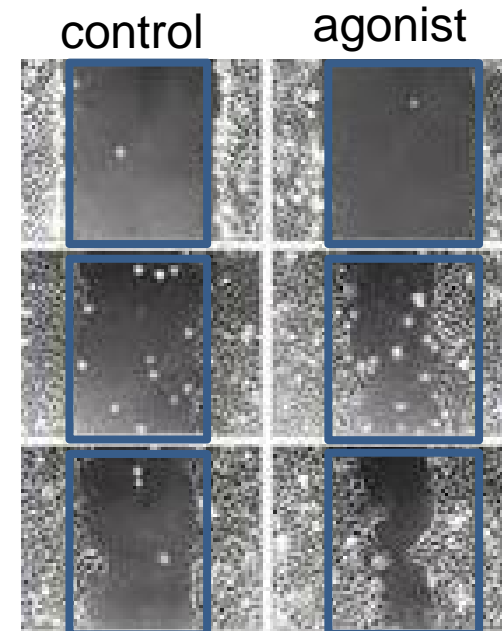
- FRET
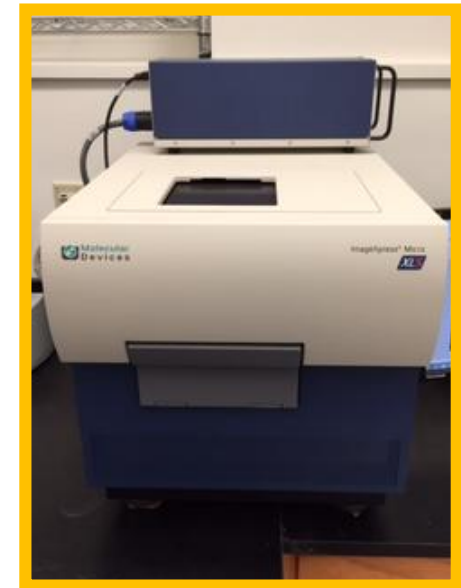
C. Image analysis: MetaXpress

- Easy analysis pipelines in-house

- Computational analysis of images enables measurement of multiple cellular properties of interest at the object/cell level and/or population level.
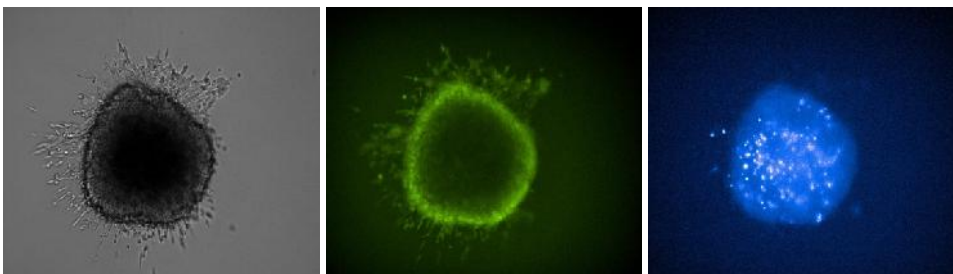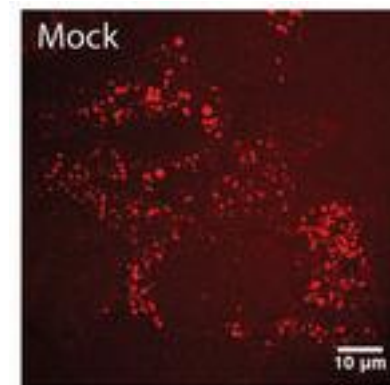
High-content Screening Assays

Neurite Outgrowth

control   +NGF

Migration/scratch assay

control   agonist

3D tumor spheroids (Invasion/viability)

Lipid droplets/puncta

Mock

10 µm

FLIPR Tetra: Flouresence Imaging Plate Reader

A. Measurement Technology:

- Integrates liquid handling with rapid whole plate imaging of <u>fluorescence</u> and <u>luminescence</u>

- Can read at sub-second intervals, which enables the <u>kinetics</u> of the response to be captured

- Integrated pipettor enables successive liquid additions, providing an opportunity to detect agonists, antagonists, and allosteric modulators all in one assay

B. Assays:
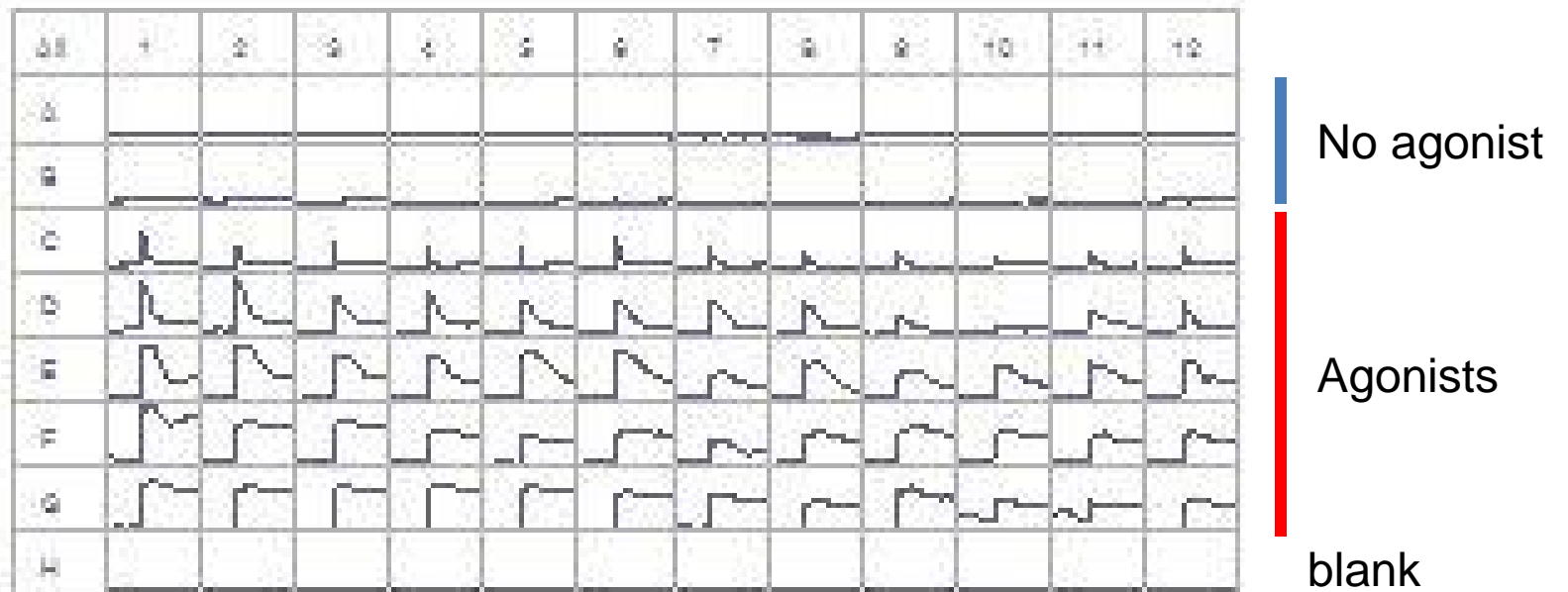
1. <u>Intracellular Ca2+ flux</u>: cell permeable Ca2+ sensitive fluorescent dye (e.g. cardiotoxicity);
2. <u>Fatty acid or neurotransmitter uptake</u> of fluorescence dyes.
3. <u>Membrane potential</u>: lipophilic, anionic, fluorescent dye that partitions across the cytoplasmic membrane of live cells based membrane potential.

FLIPR Assays

Monitoring Intracellular Ca2+ Mobilization

The Assay Development Process

~ 1-6 months

Design
- Idea
  - Phenotypes
  - Reporters, cell types
  - Controls
  - Existing infrastructure

~ 1 hr to a month

Development
- Controls: Z-factors, S/N, CVs
- Solvent; Timing; TX reagents
- Cell type, numbers
- Day to day; Plate to plate

POC
- Test set (e.g. 1K set)
- Data analysis
- Scale up

Slide courtesy of J.Hogenesch
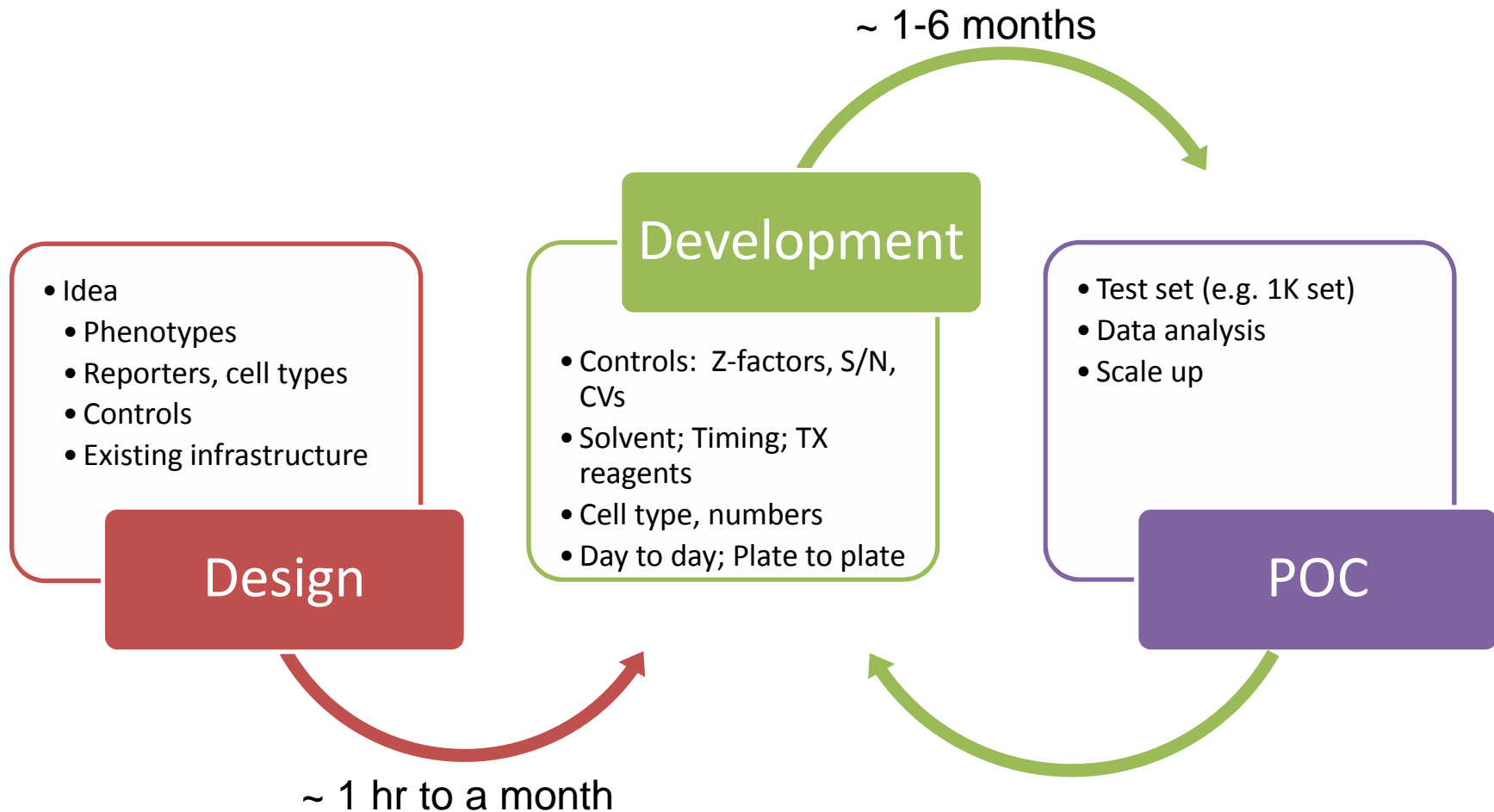
Assay Design and Development

- Is the nature of the response clearly defined?
- Is the response dependent only on the activity of the compound being tested or is it conditioned by another stimulus?
- What is the duration of the response?
- What sort of 'secondary assays' exist to confirm activity and determine selectivity of newly identified probes?
- What structural classes of known actives exist and what are the limitations to their use?

Goal: To design an assay that is robust and sensitive

What biology and assays are readily screened?

- Enzymatic

- Protein: protein interactions

- Protein: nucleic acid interactions

- Reporter Gene Assay

- Sensitivity to drugs

- Simple design and robust signals…

Biochemical Assays

- **Enzymatic assays**
  - Luminescence (e.g. ADP-Glo)
  - Fluorescence (e.g. QFRET, AmplexRed)
  - Transcreener
- **Protein-protein interactions**
  - ALPHAscreen
  - HTRF
  - ELISA (e.g. luminescence)
- **Protein-nucleic acid interactions**
  - ALPHAscreen
  - HTRF
- **Ligand binding**
  - Differential Scanning Flourimetry/Thermal Stability
  - SPR

Profiling and Sensitivities

- Phenotypic profiling of cell lines
  - FDA and FDA-likes
  - Annotated gene family (e.g. kinome)
  - Synthetic lethality screens
  - Synergy studies (combinations gene-gene; gene-drug; drug-drug)
- Differentiated iPSC from individuals
- Across tumors (e.g. melanomas)
- Of a particular patient tumor line (to define responsiveness)

Pharmacological Response of Genetically Defined Tumor Cells
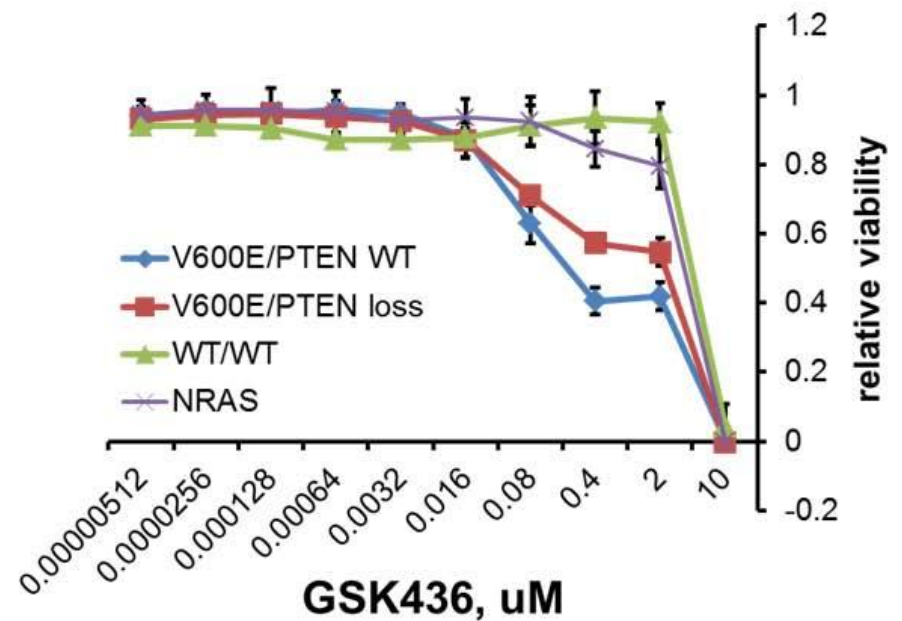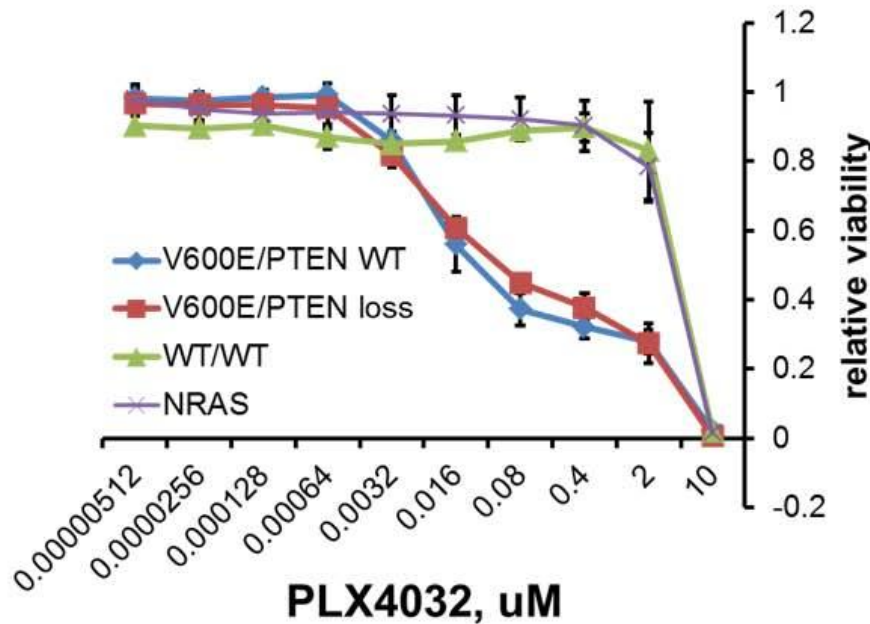
Cell-based Assays

- **Anything you can read in a plate reader**
  - Reporter Gene Assays (e.g. luciferase)
  - Survival (e.g. Cell-titer Glo, Alomar Blue, MTS)
  - Signaling (e.g. alphascreen, alphaLISA)
- **Microscopy assays**
  - EdU/BrDU incorporation
  - Autophagy (e.g. LC3-GFP/RFP)
  - Migration assays (e.g. scratch assay)
  - Neurite Outgrowth
  - Infection
  - Nuclear/cytoplasmic shuttling
  - Antigen localization/staining
- **Signaling** (e.g. Ca^{2+}, membrane potential)

Cell types

- **Chemical screens- all cells amenable**

- **Genetic screens**
  - **Transformed cells**
  - **Many primary cells**
    - **Fibroblasts, endothelial, epithelial, macrophages, etc**
    - **Lymphocytes challenging**
    - **Stem cells**
  - **Mixed cultures**
    - **Perturb one cell type and assay another**
    - e.g. siRNA in fibroblast but read tumor cell biology; siRNA in macrophage but read T cell biology

What scale is useful to you?

- Do you have a defined set of genes or pathways you are interested in?
  - Functional study of 'OMICS data
    - RNAseq
    - CHIPseq
    - GWAS
    - Exome
  - Interested in Kinases or another 'category'

Services

- **Consultations** (per hour)
  - Assay development
  - Assay optimization
  - Assay validation
  - Grant submissions
- **Equipment usage** (per hour)
  - With help
  - Without help
- **Small scale screens**
  - User-defined (siRNAs, shRNAs, cDNAs, chemicals)
  - Library plates (e.g. kinome)
- **Large scale screens**
  - Library plates

- **Data Analysis**
  - Normalization, annotation
  - HCA analysis sequence dev.
  - Screen reports
- **Reagents**
  - Transfection
  - Plastics
    - Tips
    - Plates
- **siRNAs, shRNAs, cDNAs**
  - User defined sets
  - Individual clones

Funding Opportunities

- **NIH**
  - PAR-13-364 Development of Assays for High-Throughput screening for use in Probe and Pre-therapeutic Discovery (R01)
  - PAR-14-283/PAR-14-284, High-Throughput Screening (HTS) to Discover Chemical Probes (R21/R01)
  - PAR-14-279, Discovery of *in vivo* Chemical Probes (R01)
  - PAR-13-049/PAR-13-048, Drug Discovery for Nervous System Disorders (RO1/R21)
  - PAR-13-007, Early-stage Pharmacological Validation of novel Targets and Accompanying Pre-therapeutic Leads for Diseases of Interest to the NIDDK (RO1)
  - PAR-14-006, Seeding Collaborations for Translational Research to Discover and Develop New Therapies for Diseases and Conditions within NIDDK's Mission (RO1)
  - PAR-15-056, Building on High Impact Neurobiology Through Assay Development: Advancing Tools for Therapeutic Development (RO1)
  - PAR-15-070, Innovation Grants to Nurture Initial Translational Efforts (IGNITE): Assay Development and Therapeutic Agent Identification and Characterization to Support Therapeutic Discovery (R21/R33)
  - PAR-15-071, Innovation Grants to Nurture Initial Translational Efforts (IGNITE): Pharmacodynamics and In vivo Efficacy Studies for Small Molecules and Biologics/Biotechnology Products (R21/R33)

Funding Opportunities (part II)

- **NIH** (continued)
  - PAR-13-267, Novel NeuroAIDS Therapeutics: Integrated Preclinical/Clinical Program (P01)
  - PAR-15-041, Targeting Persistent HIV Reservoirs (TaPHIR) (R21/R33)

- **NCAT/TRND opportunities**

- **Foundations (**e.g. Welcome Trust, Melanoma Research Foundation, Leukemia/Lymphoma Society, Gates, Cystic Fibrosis**)**

- **Commercial** (e.g. Bayer Grants4targets, Astrazeneca Openinnovation)

- **Institute/Center/Program Pilot project funds**

  - Institute for Immunology
  - Center for Orphan Disease Research
  - Institute for Regenerative Medicine
  - Epigenetics Program

Input from the Community

We are expanding based on SOM needs….

- FLIPR from Physiology

- cDNA library from J. Hogenesch (Pharm)

- Mouse shRNA library

  - Contributions: CHOP, Cancer Biology and IRM

- Small molecule libraries

  - Diversity library contributions: CDB, Biochemistry and Microbiology

  - LOPAC from J. Hogenesch (Pharm)

What libraries or functionality would be useful to you?

HOW TO GET STARTED?

- Contact David Schultz at dschultz@mail.med.upenn.edu or 215-573-9641 for an initial consultation
  - ➤ Define the project
  - ➤ Determine if the facility has relevant expertise/technology to pursue the project
  - ➤ Develop a management plan
  - ➤ Set expectations
  - ➤ Get started!