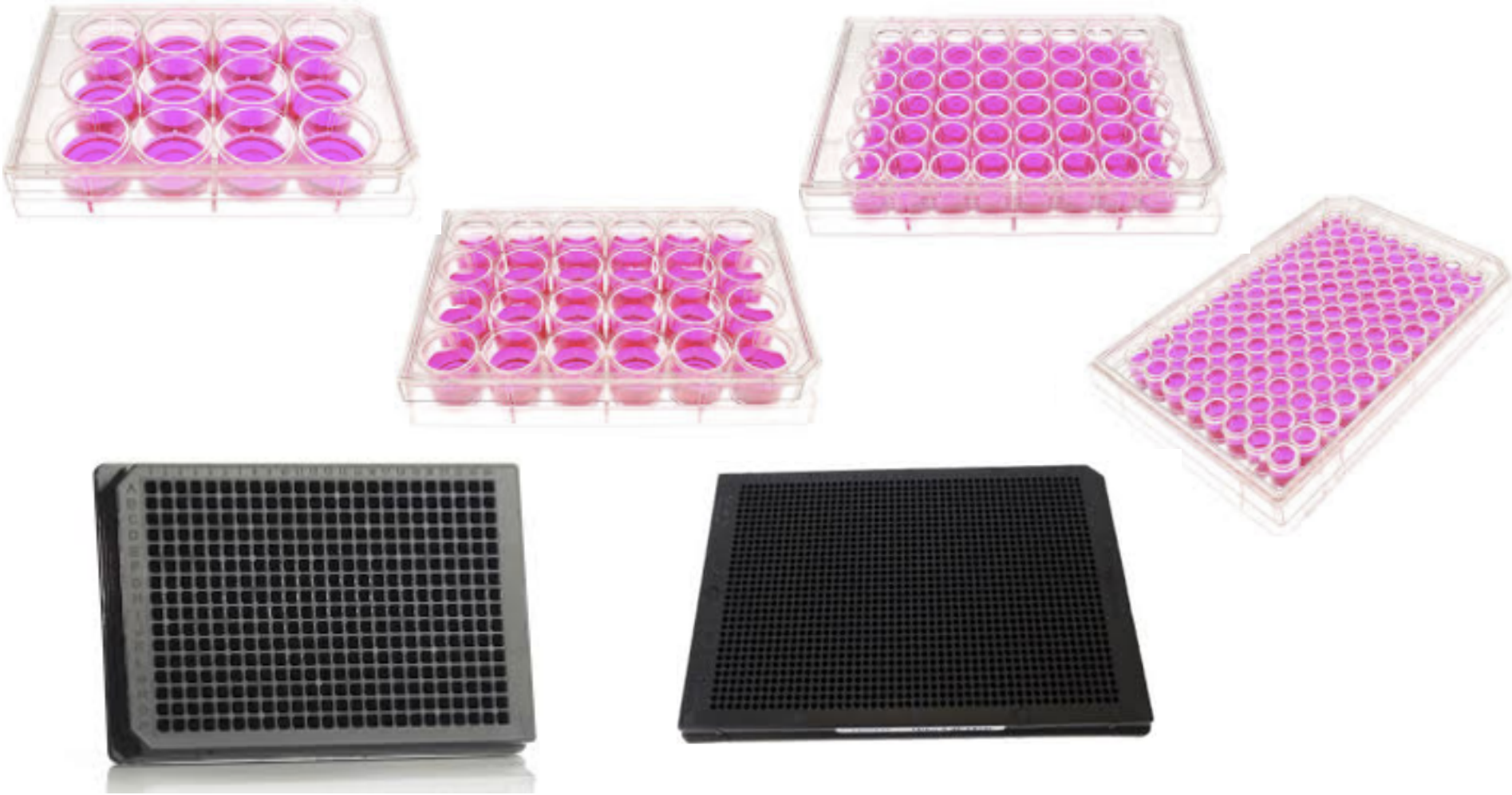
Assay Automation and Quantitation – From Benchtop to HTS



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HARVARD MEDICAL SCHOOL

http://iccb.med.harvard.edu

How can laboratory automation improve my experiments?

How many experimental samples am I working with at a time?

- Conditions
- Cell lines
- Dilution series

What number of replicates are needed for data analysis?

- Technical
- Biological

Which steps in the experiment contribute most to variability?

- Each additional step adds variability

What steps are the slowest?

- Reagent preparation
- Conducting the experiment
- Data acquisition
- Data analysis

What steps have flexible timing?

What type of small molecule screen(s) will best address my biological question(s)?

What resources are available to me?

- Compound libraries
- Laboratory automation

What am I interested in?

- Target/pathway identification
- Novel tool compounds
- Early stage drug discovery

How large of a screening campaign am I interested in conducting?

Is a phenotypic (cell-based) or biochemical assay most appropriate?

What is the desired assay readout?

- Plate reader
- Imaging
- FACS, qPCR, NMR

What functional genomics tools are (most) appropriate for my biological question(s)?

What resources are available to me?

- Libraries
- Laboratory automation

What am I interested in?

- Knockdown (siRNA, shRNA, CRISPRi)
- Knockout (CRISPR)
- Activation (CRISPRa)

Can my cells be transfected? Infected?

What incubation time is required to quantitate phenotype? Is it compatible with the assay?

- Short (siRNA, CRISPRi) – 3 to 5 days
- Long (shRNA, CRISPR) – 2+ weeks

What is the desired assay readout?

- Selection (pooled library – shRNA, CRISPR)
- Imaging, plate reader (arrayed library – siRNA, CRISPR, shRNA, CRISPRi)

Cell perturbations: small molecules vs. functional genomics

	Small Molecules	Functional Genomics
Availability	Not available for every target Significant screening effort often require to obtain initial active small molecule	Available against every target Options: siRNA, shRNA, CRISPR Multiple vendors
Mechanism	Inhibition, partial inhibition, activation of enzymes or other proteins	Gene editing RNA depletion Depletion or partial depletion of protein
Timing	Fast Often reversible	Slower RNAi is gradual depletion
Specificity	Non-specific effects possible	Off-target effects possible

Comparing RNAi and CRISPR

	RNAi (*siRNA*)	CRISPR
Modification	Knockdown Post-transcriptional RNA degradation	Knockout Transcription inhibition Transcription activation
Target	Transcript – coding and noncoding	DNA – ORF/promoter/enhancer (adjacent to PAM)
Site of action	Cytoplasm	Nucleus
Length of time	Transient – 2 to 6 days	Permanent (for knockout/knockin)
Off target effects?	Yes Strategies to address	Yes Fewer, *TBD*

What laboratory resources are available to me?

Fully automated platforms

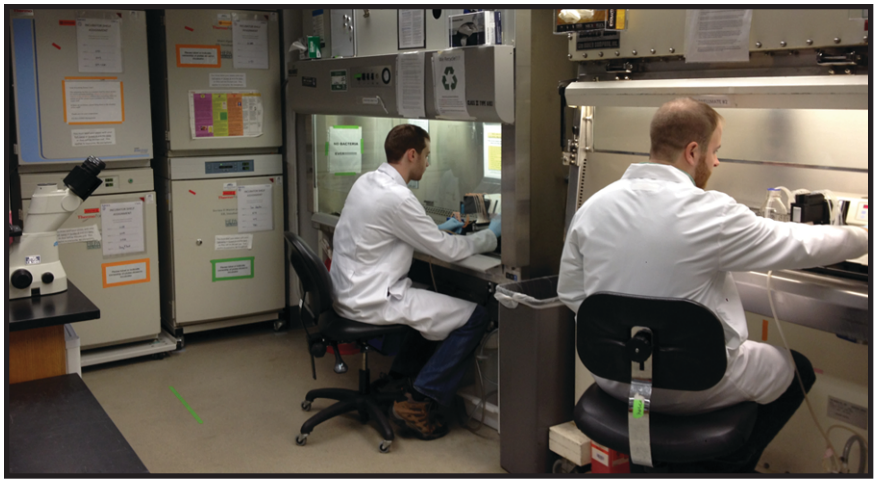
What laboratory resources are available to me?

Modular work stations

What laboratory resources are available to me?

Standard laboratory equipment – can conduct pooled screens

Hood, centrifuge, plate reader, qPCR instrument, microscope

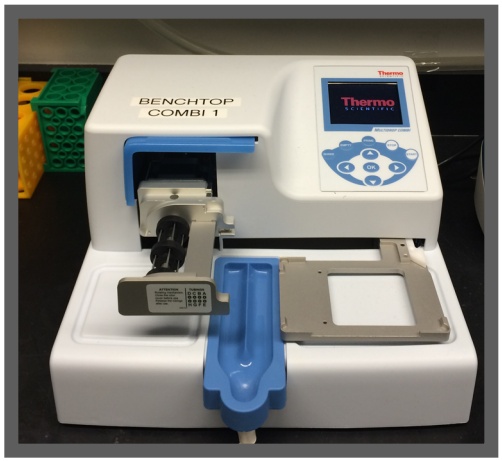


NGS for quantitating enriched or depleted shRNAs/crRNAs

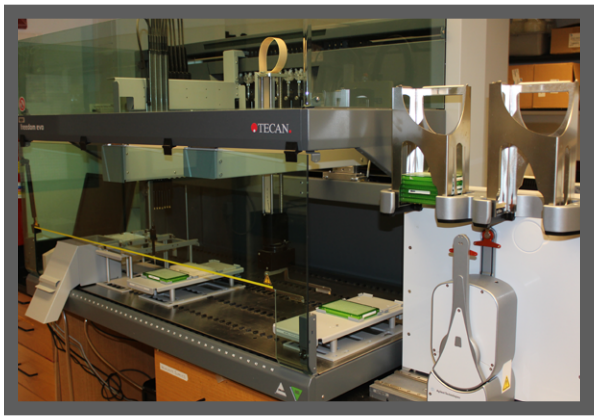
Analytical chemistry resources for compound QC – LCMS, NMR

Liquid handling: bulk dispensers, automated pipettors

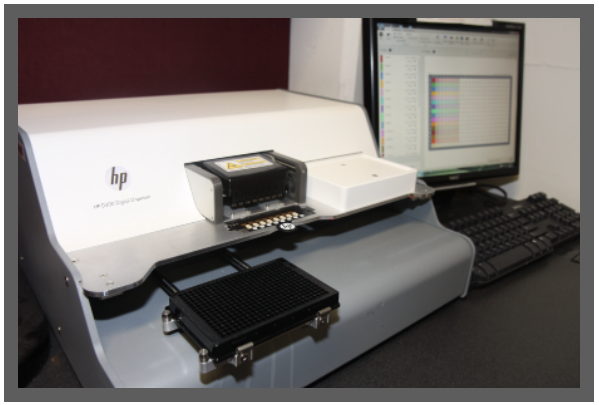
Labcyte Echo



Thermo Multidrop Combi

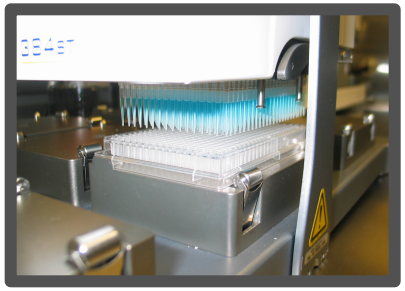


Tecan EVO150

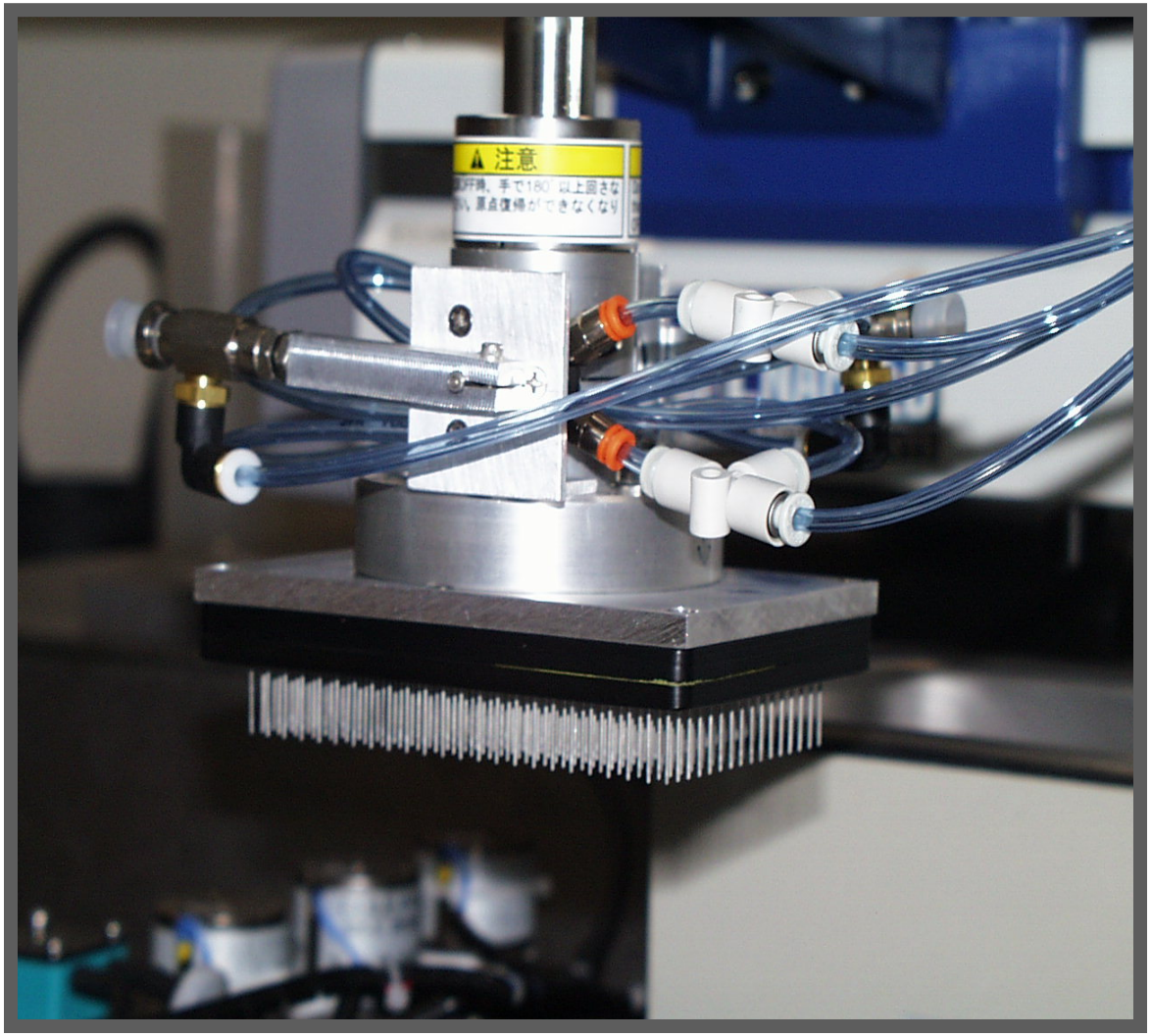


Hewlett Packard D300

Hamilton STARlet



Pin transfer of compounds to library plate



384-pins (V&P Scientific)

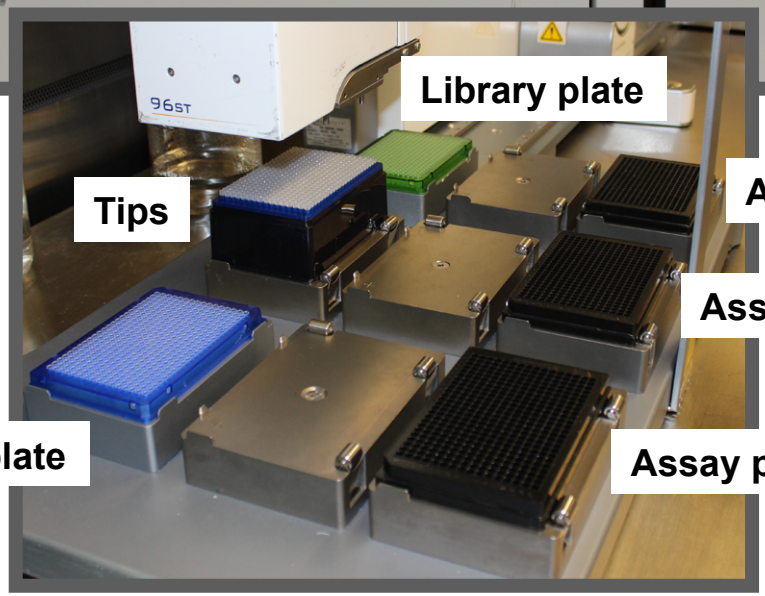
Common assay volumes:
30 or 10 ul/well

Typical pin transfer:
33 nl, 100 nl, 300 nl

Compounds dissolved in DMSO

Screening concentration dependent on:
- Library concentration
- Dilution

Tip-based transfections for RNAi to library plates

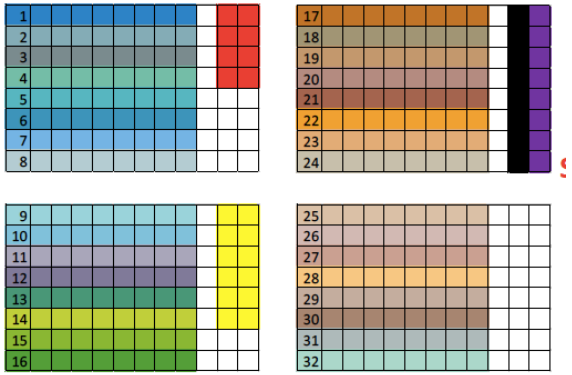


Creation of custom library plates

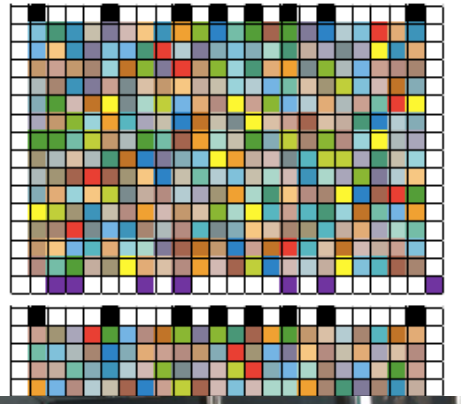
Perturbagens

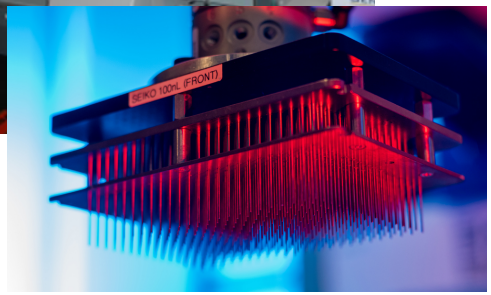
1	SJB3-019A
2	WP1130
3	IU1
4	spautin-1
5	b-AP15
6	HY-17541A
7	STK547622
8	SJB2-043
9	1247825-37-1
10	HY-50737A
11	LDN-57444
12	R140309
13	p22077
14	HY-50736
15	ML-323
16	HBX19818
17	Compound 2
18	HY-17542
19	z-VAE(OMe)-fmk
20	trifluoperazine
21	PB49673382

96-well master plate with dilutions

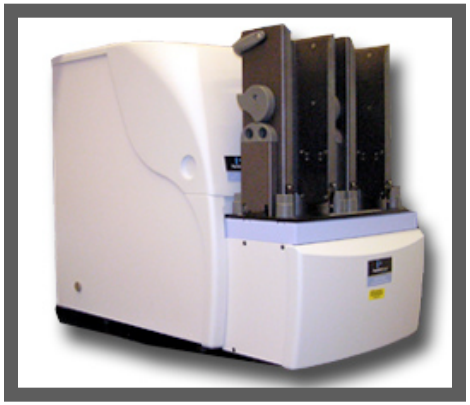


384-well library screening plates



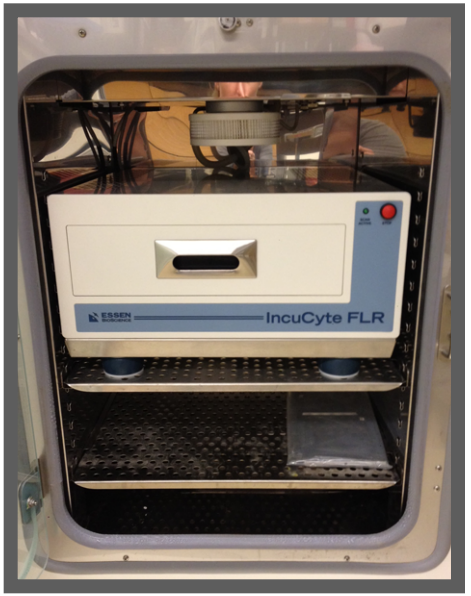


Assay readout: plate readers



PerkinElmer EnVision

Li-COR Aerius

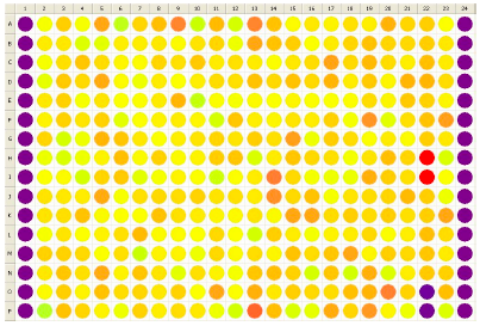


Essen Biosciences IncuCyte FLR

Hamamatsu FDSS7000EX

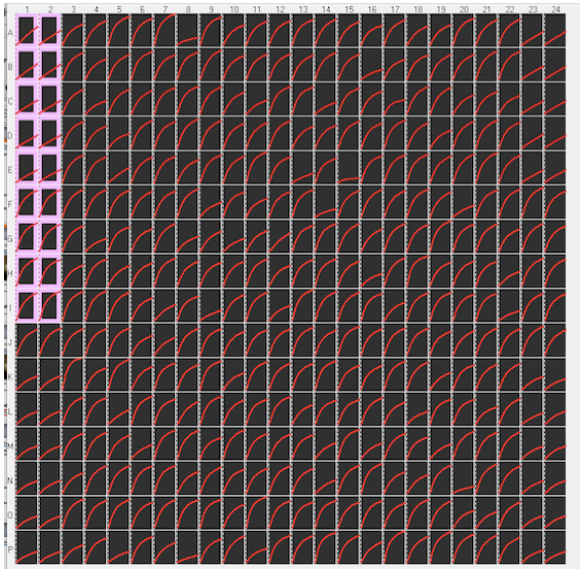
LifeTechnologies QS7

Plate reader-based assay readouts



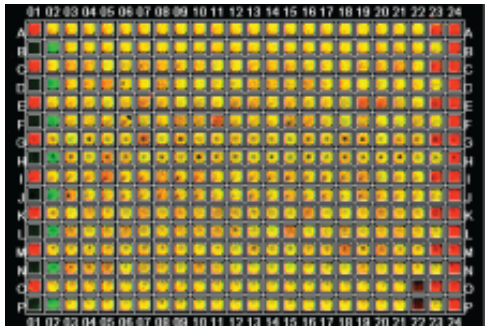
Uniform well read out

- Absorbance
  - Colorimetric enzyme assays
- Luminescence
  - Cell viability - *CellTiterGlo*
  - Luciferase as reporter for transcription
  - Luciferase as reporter for protein stability
- Fluorescence
  - In-Cell Western
  - Channel kinetics, membrane potential
  - Fluorescence polarization, HTRF, AlphaLISA



Advantages

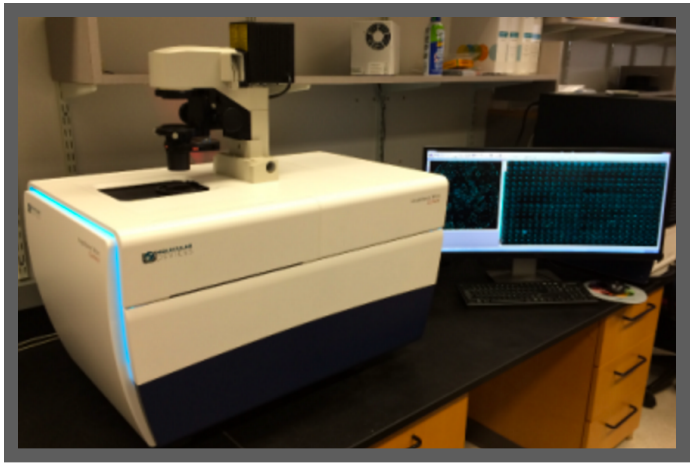
- Fast (1-5 minutes/384-well plate)
- Output is numerical data, quantitative



Disadvantages

- Limited information
- Reagent cost can be high

Screening by imaging: screening microscopes and image analysis



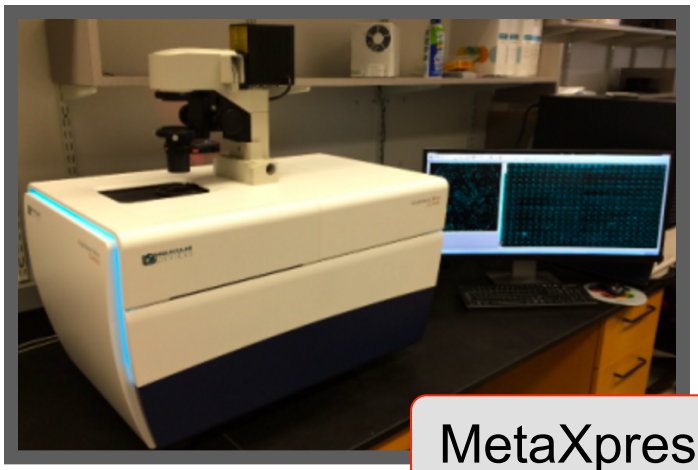
Molecular Devices IXM and IXM-C

GE InCell6000

Perkin Elmer Operetta

TTPLabTech Acumen eX3

Screening by imaging: screening microscopes and image analysis



MetaXpress

Molecular Devices IXM and IXM-C

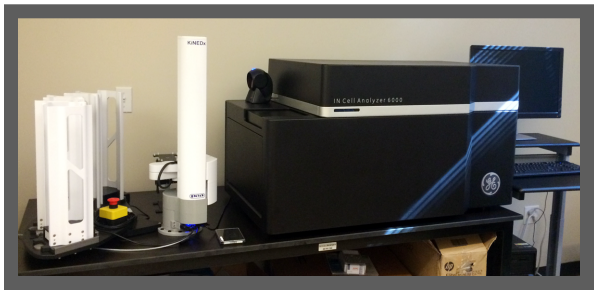
IDAC

IMAGE AND DATA ANALYSIS CORE

CellProfiler

cell image analysis software

BROAD INSTITUTE



GE InCell6000

Columbus

Perkin Elmer Operetta

Cellista

TTPLabTech Acumen eX3

Image-based assay readouts

Ability to monitor multiple parameters on a per cell basis – high content screening

Cell number and viability

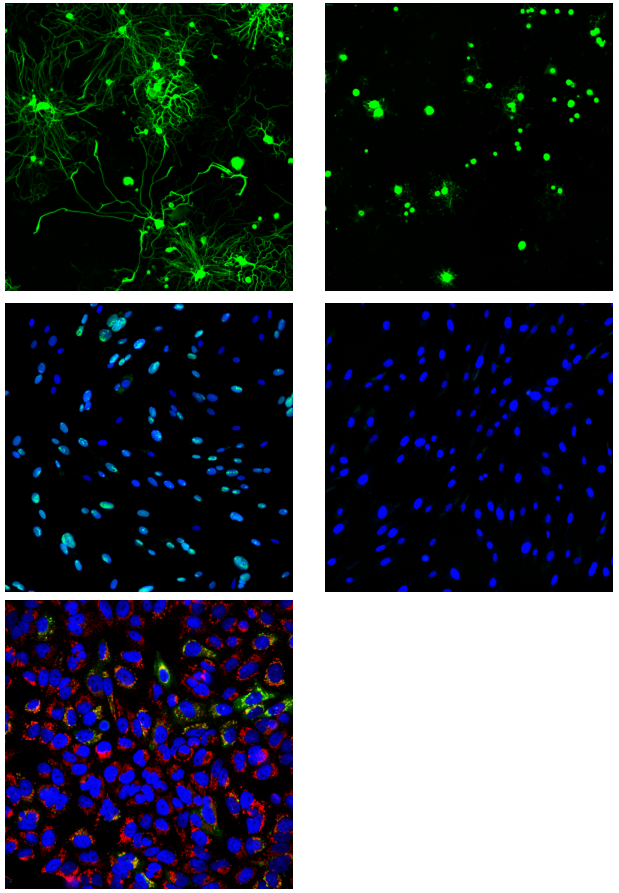
Presence/absence/intensity of probe

Changes in protein stabilization/localization

Changes in morphology – cell or organelle

Cell migration

Viral or bacterial replication, spread



Advantages

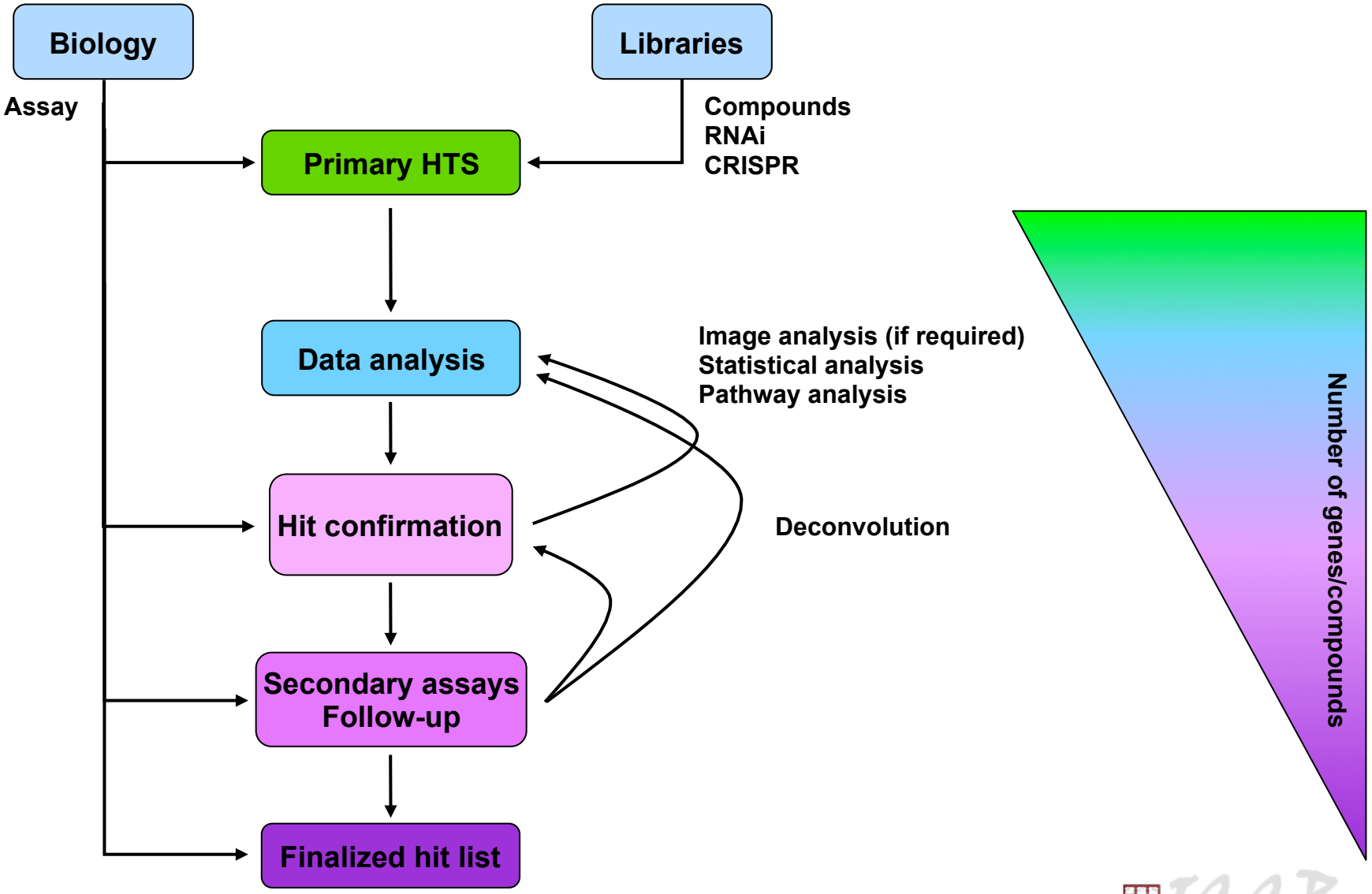
High content screening
Potentially less expensive reagents

Disadvantages

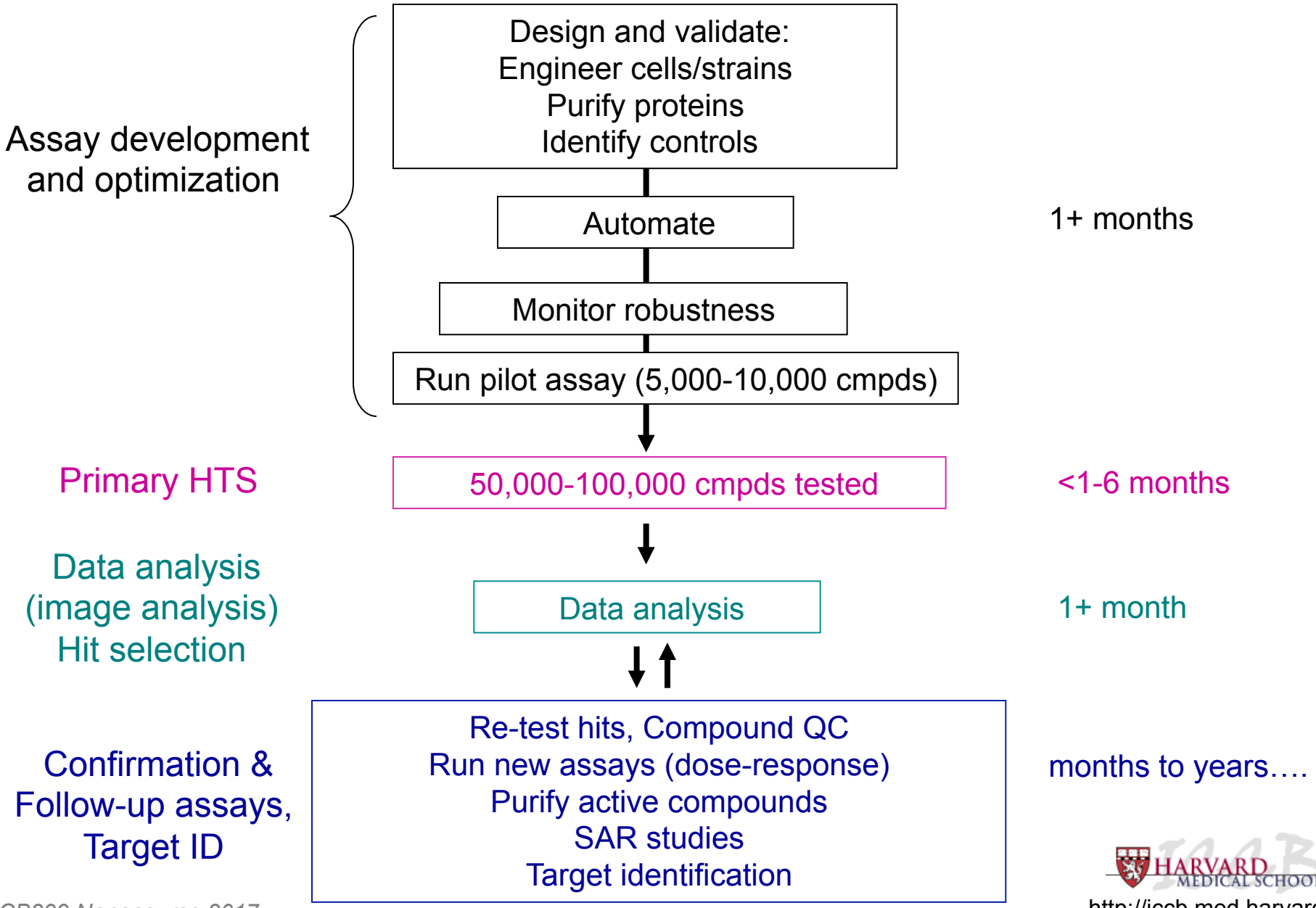
Image capture on microscopes/scanner can be slow (10 min – 2+ hours/384-well plate)

Analysis can be challenging, time intensive

General high throughput screening workflow



Timeline for small molecule assay development and screening



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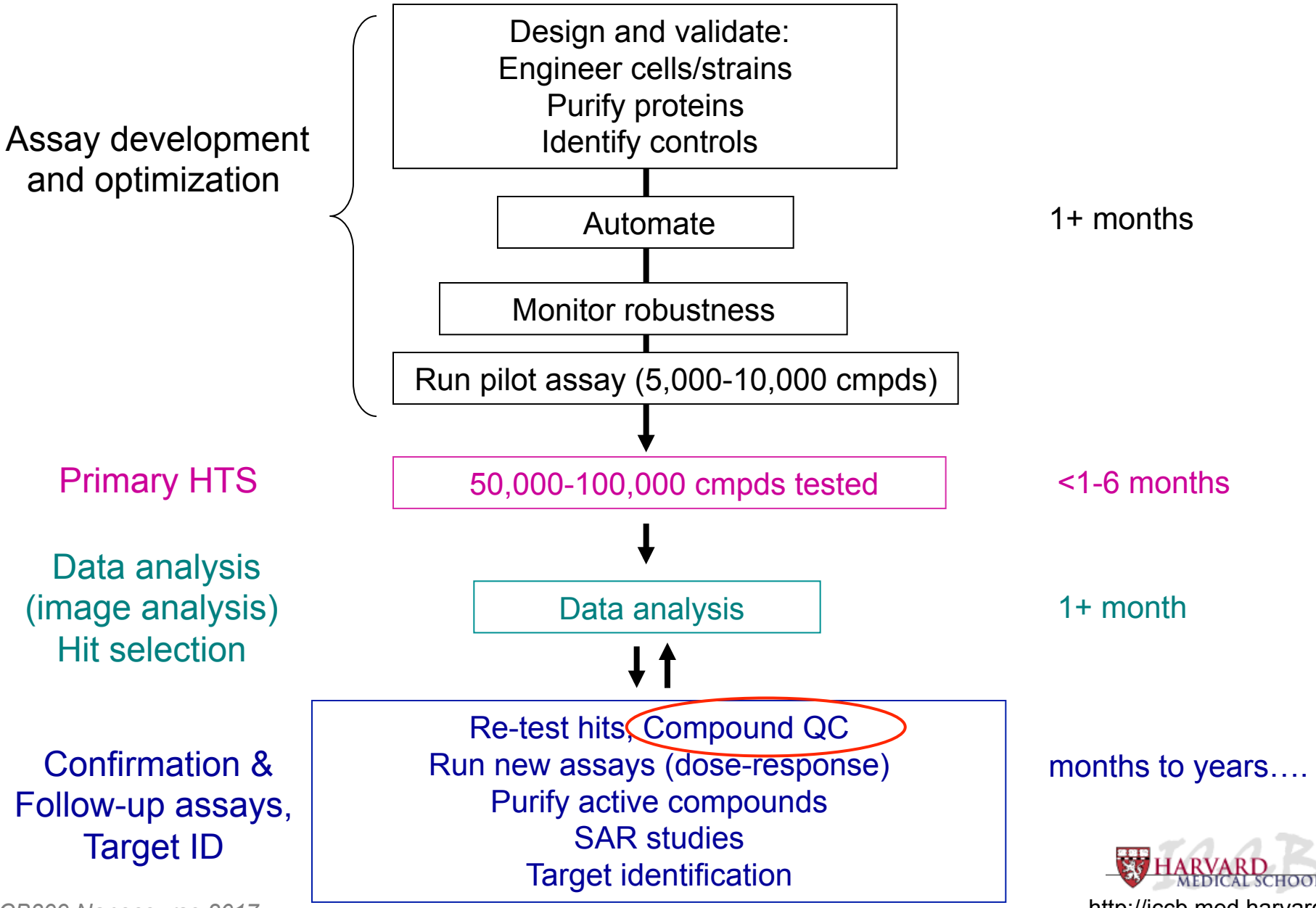
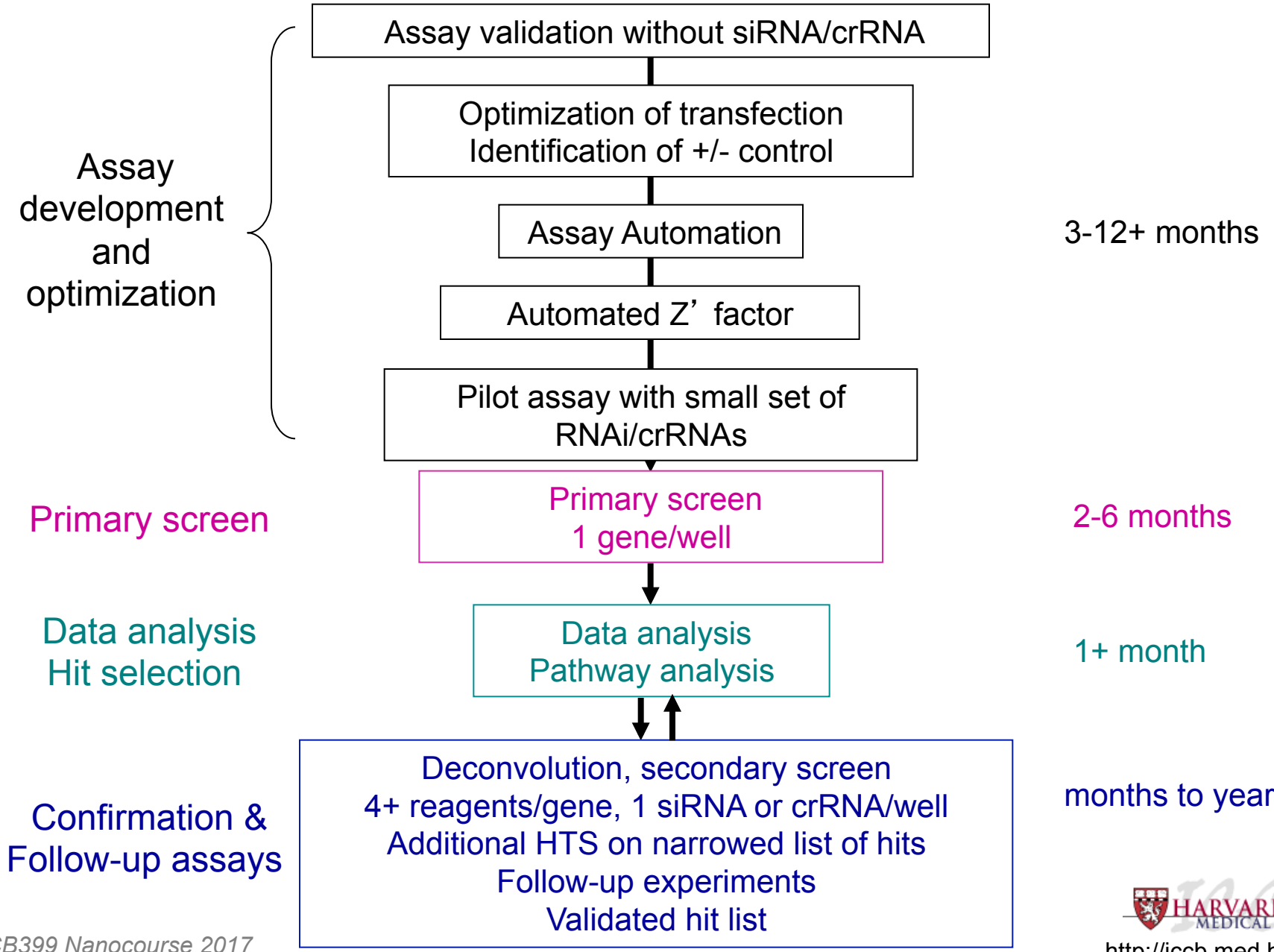


Timeline for RNAi/crRNA assay development & screening



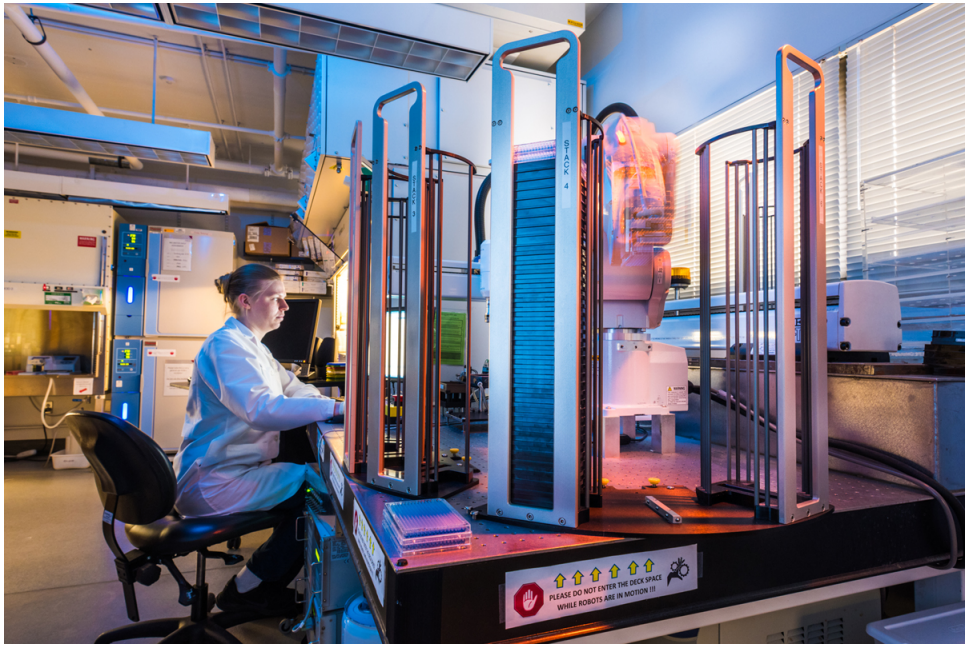
Considerations for assay development

Infrastructure

Reliable tools appropriate for scale of screen?

Informatics

-Ability to track data?
-Analyze data?
-Visualize data?
-Access to other screening results?



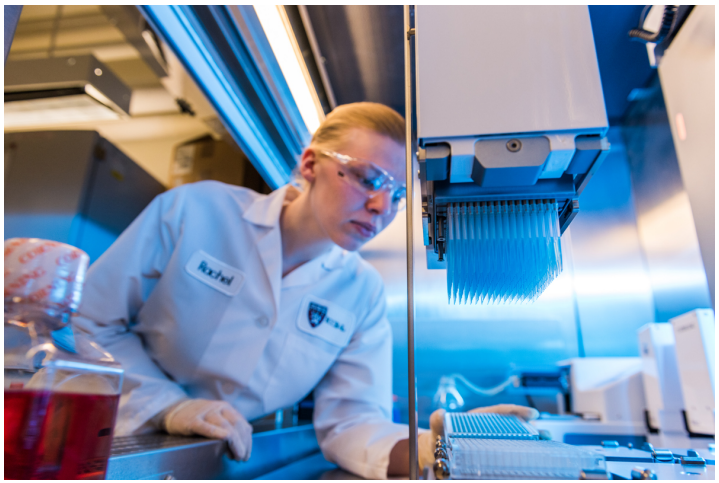
Choice of library

-Reagent type?
-Single vs pooled?
-Scale?

Considerations for assay development

Scale:

How high throughput do assay and readout need to be?
Microplate density– 96-well or 384-well? Higher?
Automation requirements
Controls
Impact on data analysis



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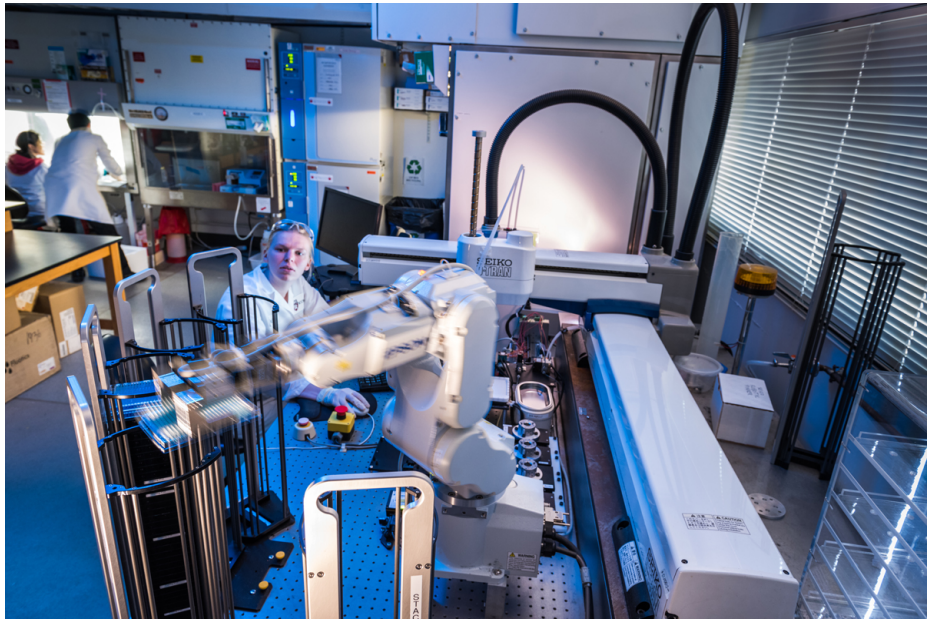


Primary screen assay

-Asking the appropriate question?

-Adaptable to screening?

-Controls?

-Counter screen to focus hit selection?

-Strategy to minimize false negatives?

Secondary assays

-How will potential hits identified in primary screen be narrowed to those most relevant to question being asked?

-Orthogonal assays

Challenge – establish a robust assay that is physiologically relevant

Specific

Sensitive

Reproducible

Scalable

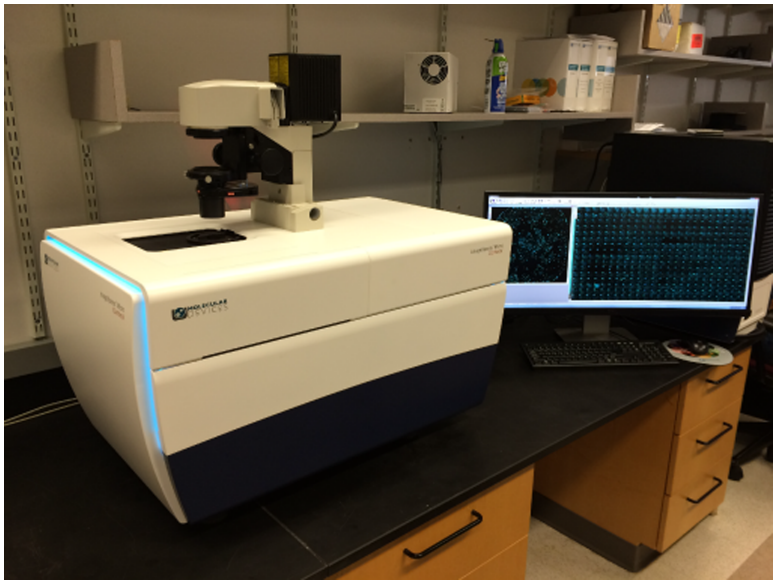
- Biochemical assays:

-Is there a reliable and sufficient supply of protein?
-Timing
-Assay temperature

- Cell-based assays:

-Cell lines vs primary cells
-Are cells transfectable?
-Stable cells lines for reporter assays or bulk transfection/infection of reporter

Challenge – establish a robust assay that is physiologically relevant



Assay readout

Plate type requirements

Optimize prior to including variable of compound/transfection/infection treatment:

- Protein concentrations
- Incubation times
- Antibody dilutions
- Washes
- Image analysis

Determine potential signal window

Use CRISPR to generate reporter cell line for screen

- Mutation or deletion to mimic disease
- Transcription reporter in endogenous context – GFP, luciferase
- Tag endogenous protein with fluorescent marker

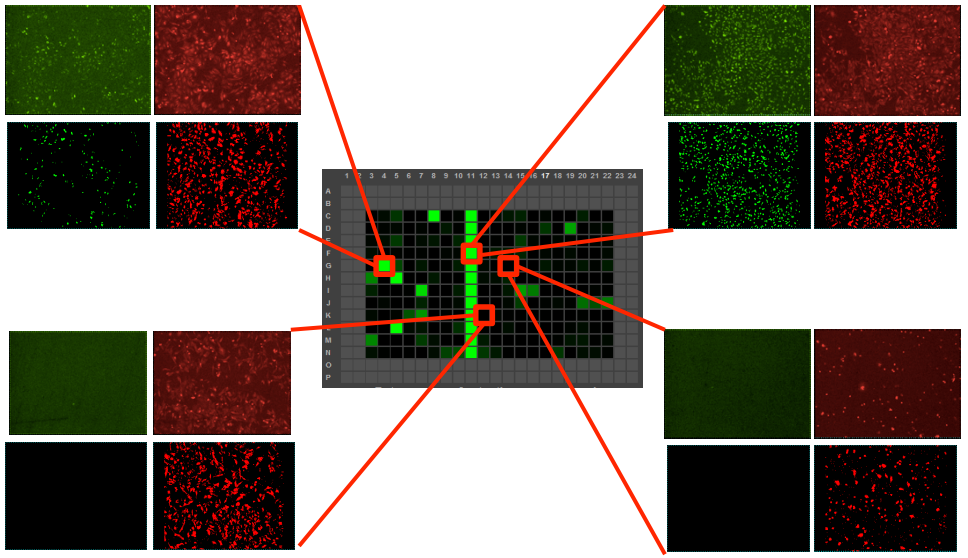
Identification of positive and negative controls

Compound screening – how does DMSO impact assay?

Functional genomics – essential to identify a negative control

Ideal – use different positive controls that have range of phenotypes in assay

- Monitor dynamic range
- siRNA/crRNA/compound
- Genetic or conditional



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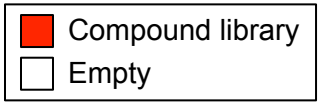
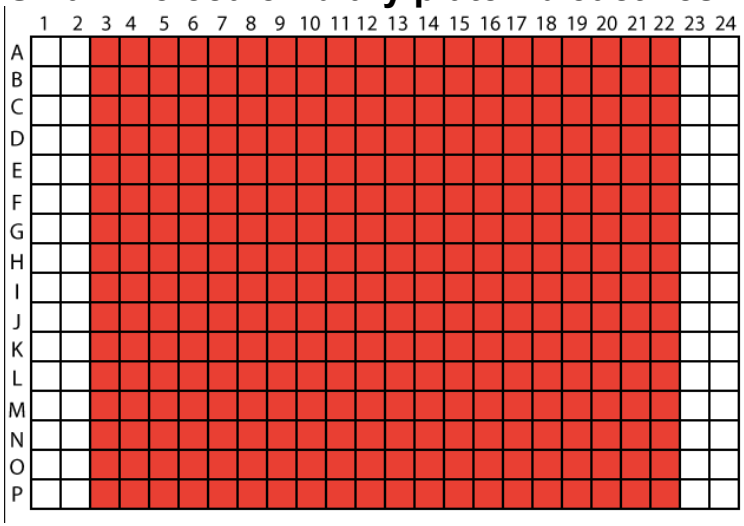
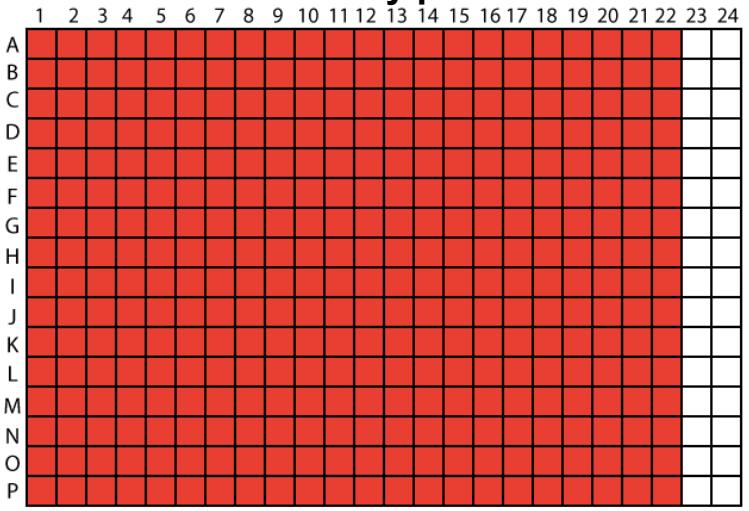


Small molecule library plate - bioactives



Small molecule library plate - commercial



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Compound screening – how does DMSO impact assay?

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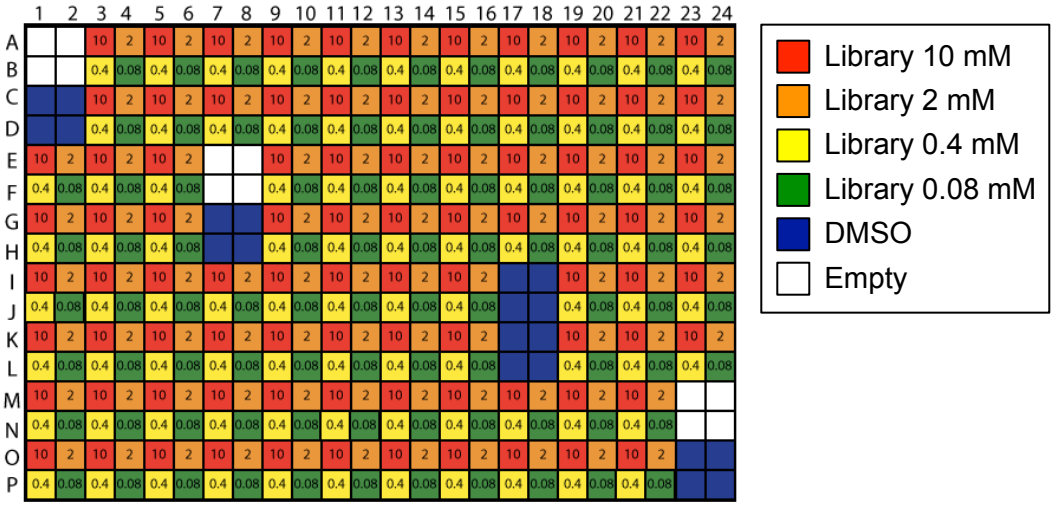
Small molecule library plate – bioactive, multiple [ ]



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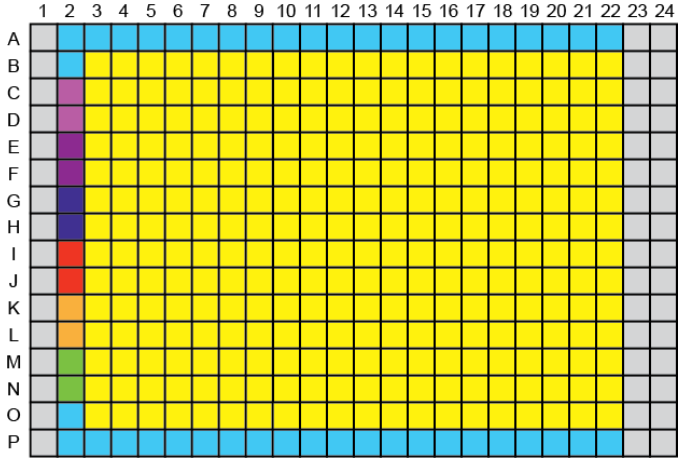
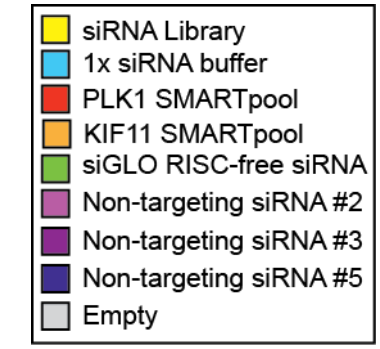
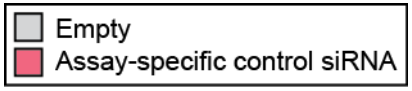
- Genetic or conditional

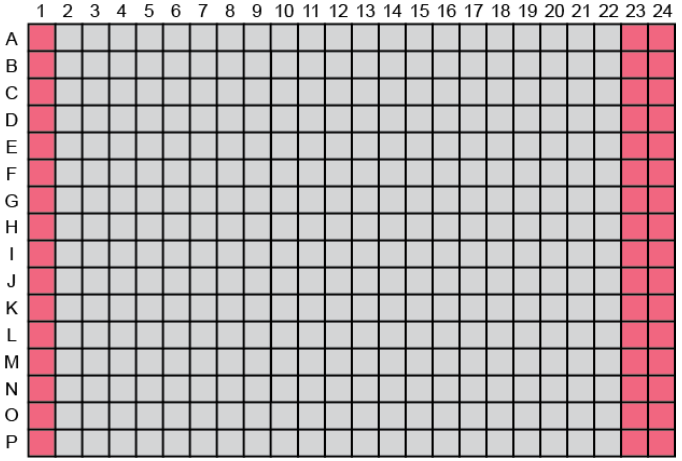
siRNA library plate





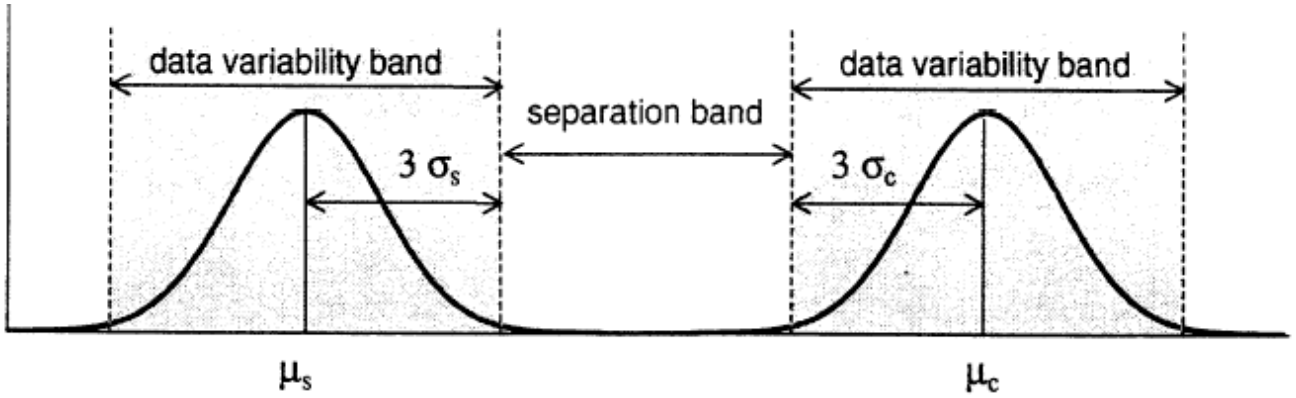


Assay specific controls plate



Assay optimization – automated Z' factor experiment

Measure of HTS robustness
Opportunity to mimic automation utilized in screen



$$\textbf{Z' factor} = 1 - \frac{3(\sigma p + \sigma n)}{|\mu p - \mu n|}$$

Assumes normality
Range 1 to - ∞

Biochemical small molecule > 0.75 ideal
Cell-based small molecule > 0.6 ideal
RNAi > 0.5 ideal

Zhang JH, et al. J Biomol Screen. 1999;4(2):67-73.

Assay optimization – pilot screen

How does assay perform when using a variety of compounds or siRNA/ crRNA?

Visualize and analyze data

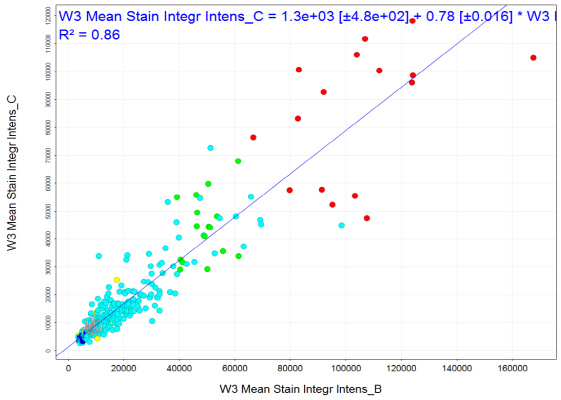
Acceptable Z' factor?
Potential hits?
If siRNA, do potential hits make biological sense?

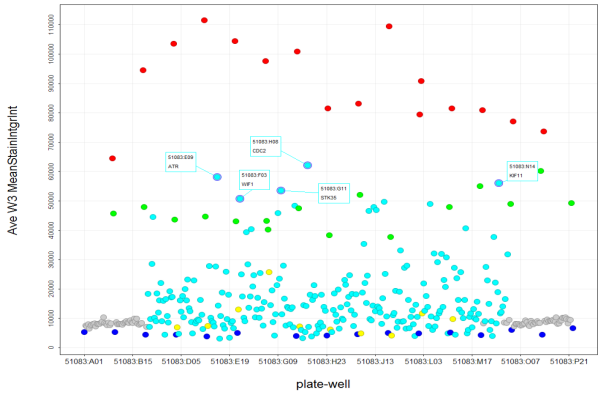
Good correlation of replicates?

Clear separation of positive and negative controls?

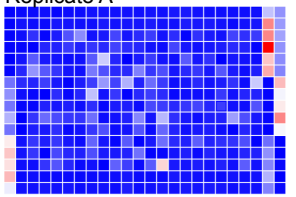
Any visible patterns or edge effects?

Utilize visualization software

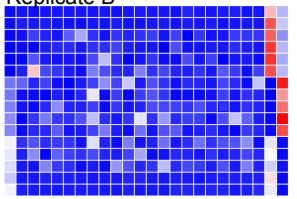




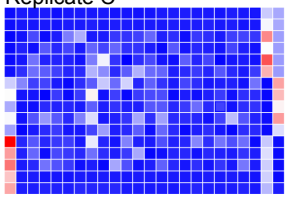
Replicate A



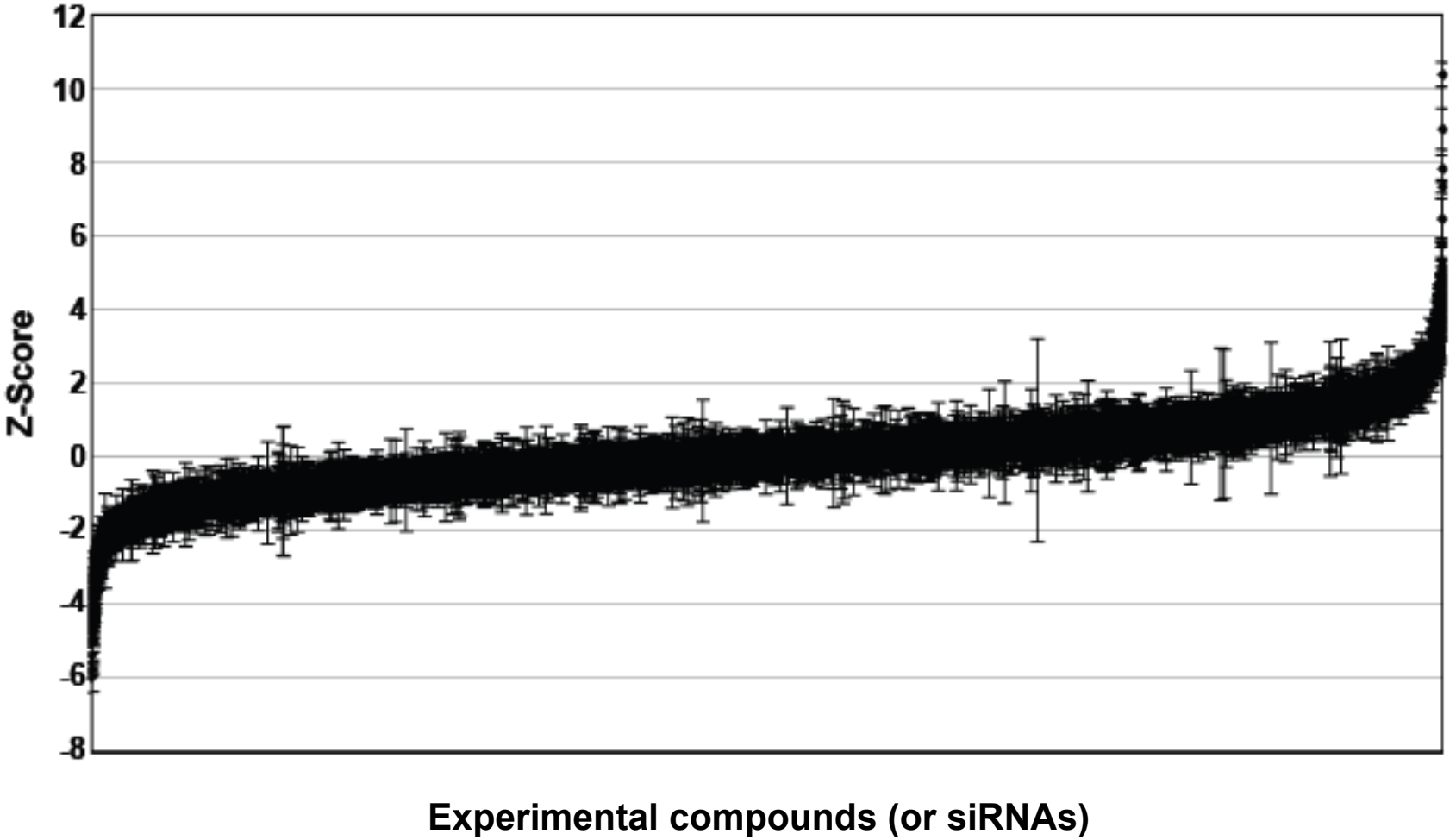
Replicate B



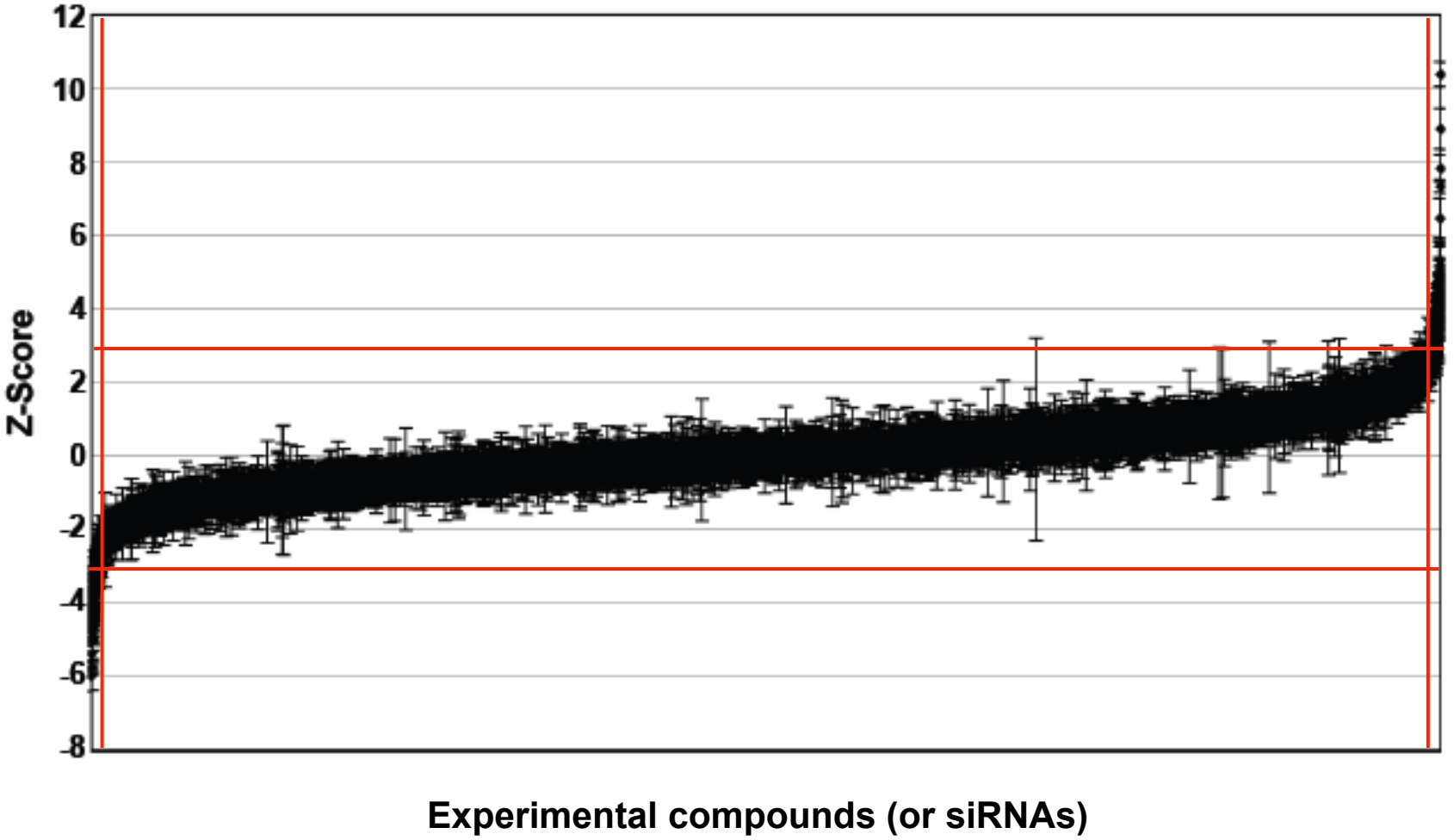
Replicate C



A continuum of activity/phenotype is observed in the primary screen

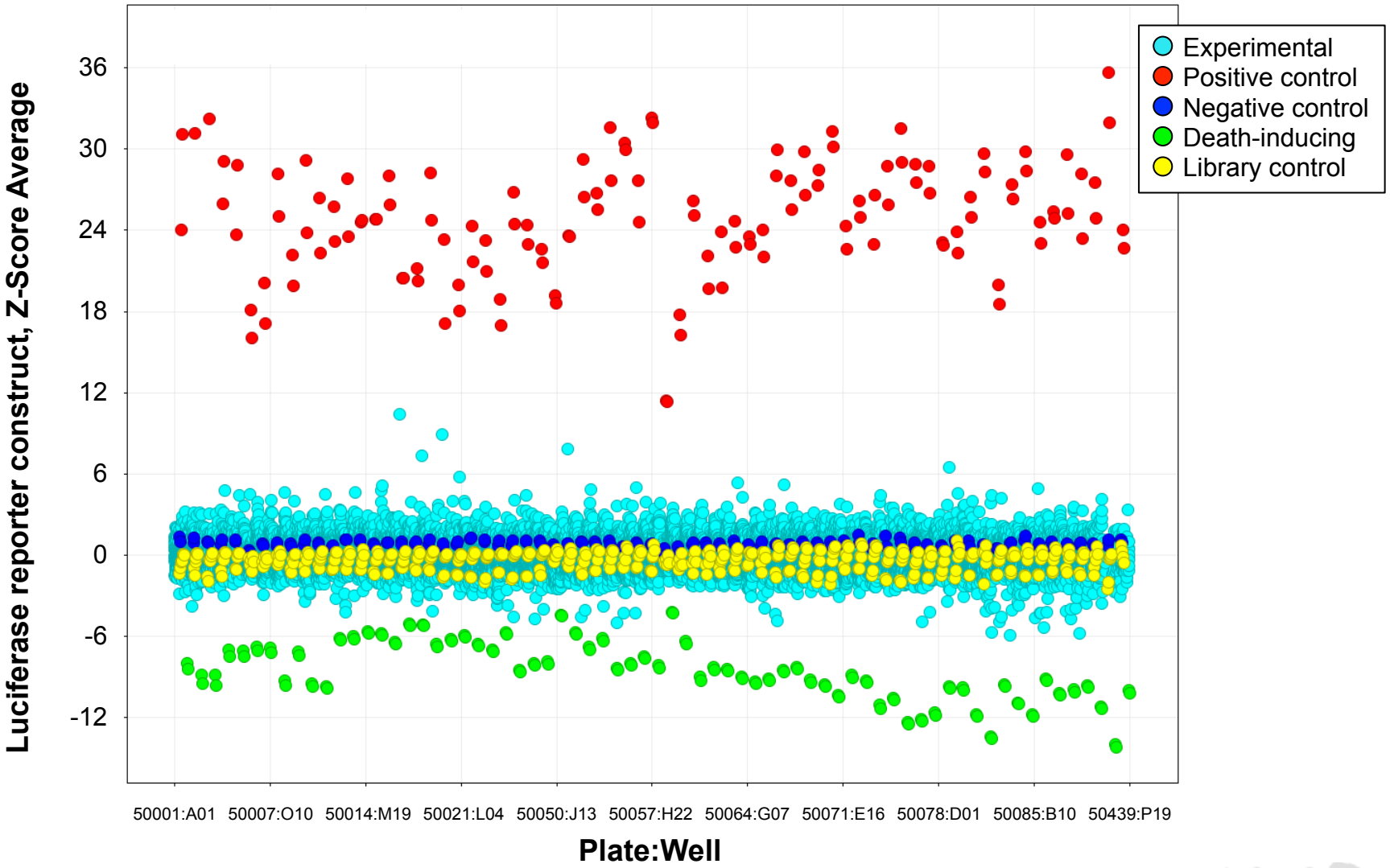


A continuum of activity/phenotype is observed in the primary screen

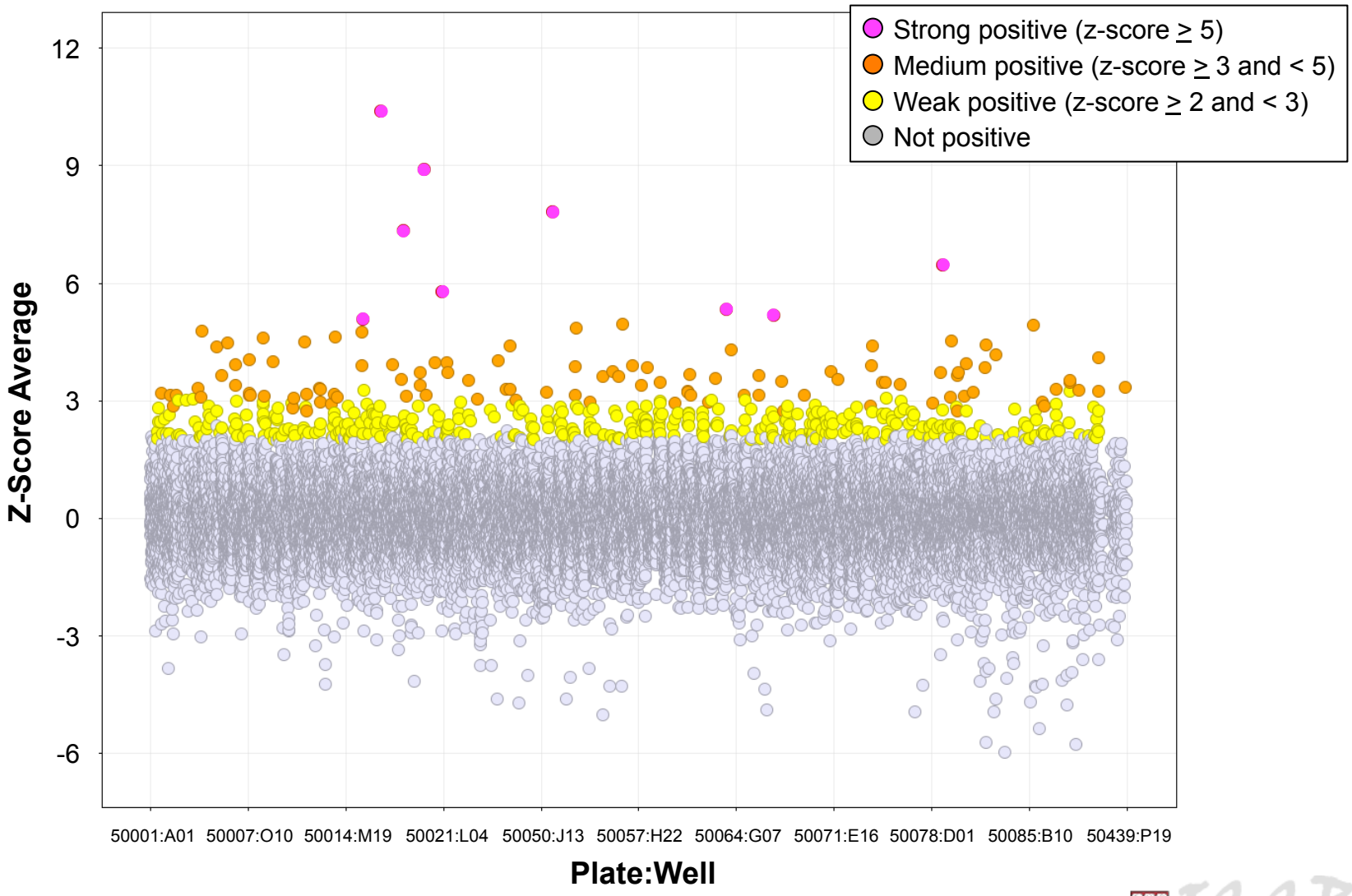


Follow the positives!

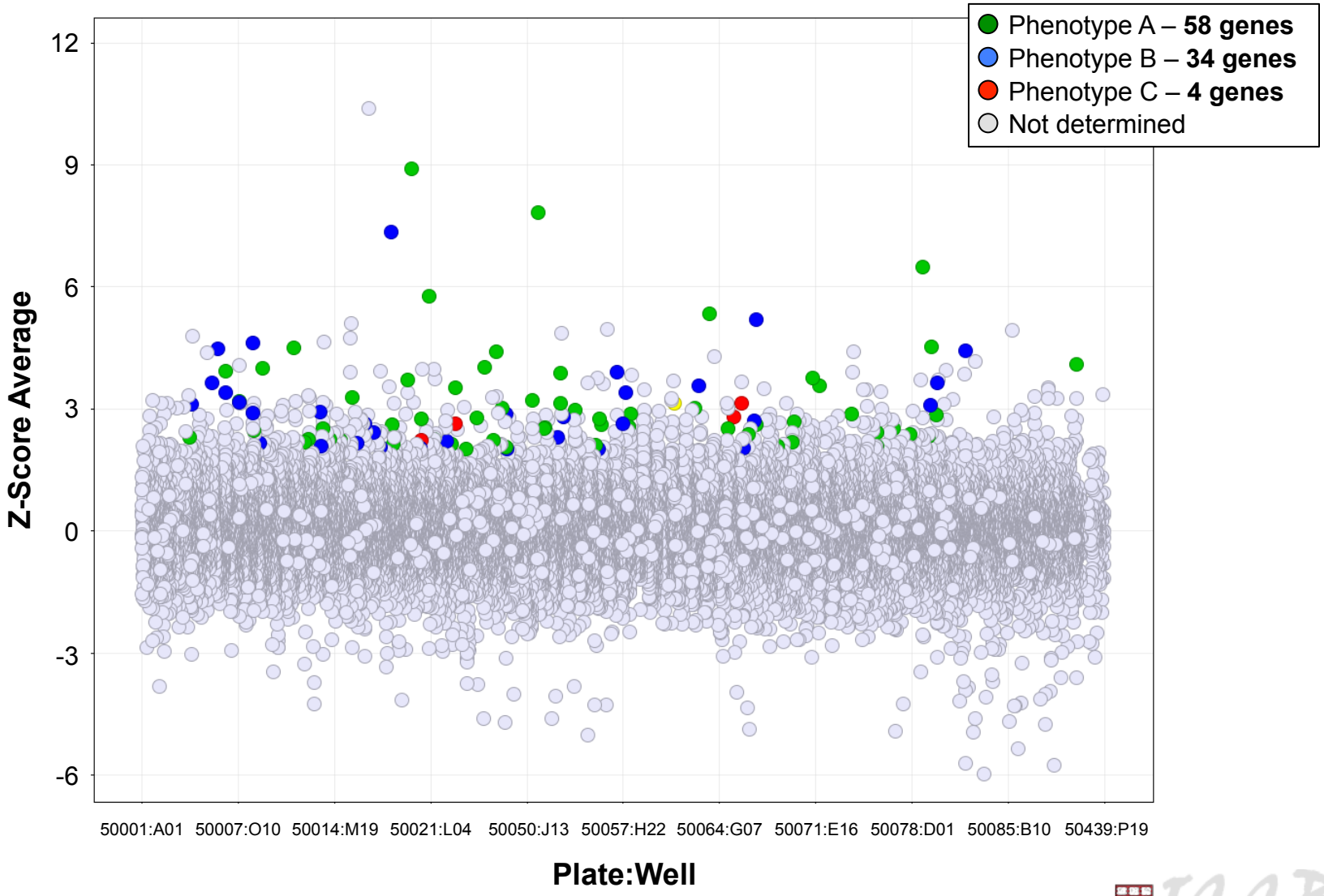
Visualization of a full genome siRNA screen, with library- and assay-specific controls included



Potential hits can be categorized by strength



Additional secondary high-throughput screens can limit and categorize hits



How do I visualize and analyze HTS data?

What resources are available to me?

- Software
- Coding capability

How many data points am I working with?

- Per experiment
- Overall

What question am I trying to answer?

- How do the data look across a specific experiment? Overall?
- How reproducible are the data?
- Are there positional effects? Biases?
- Is the data normally distributed?
- What is the potential hit rate? Within specific profile (multiple conditions)?
- Does the analysis fit with what is anticipated from the raw data?
- Are there trends in selected hits?

How will hits be identified?

What resources are available to me for data visualization?

Custom script– self, collaboration

The R Project for Statistical Computing

https://www.r-project.org

https://www.mathworks.com

https://card.niaid.nih.gov

spotfire.tibco.com

https://www.graphpad.com

What resources are available to me for data visualization?

dotmatics
knowledge solutions

https://www.dotmatics.com

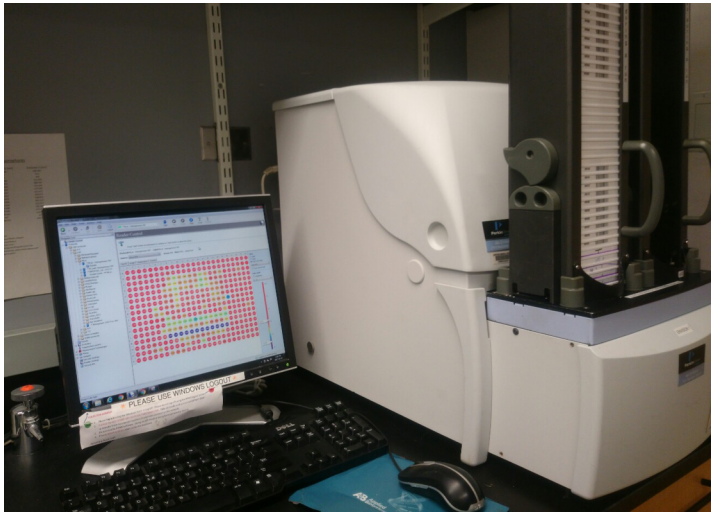
Vortex

Intuitive and versatile scientific data visualization and analysis

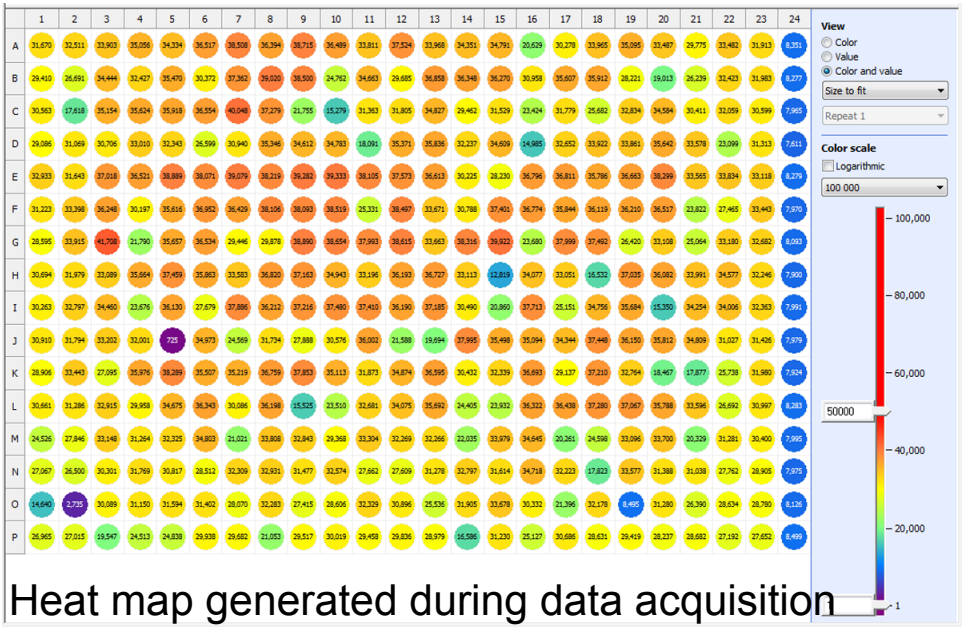
Vo

Laurie Martensen
laurie.martensen@dotmatics.com
619.306.3412

Data acquisition → analysis → visualization



Output file: csv



Heat map generated during data acquisition

Data acquisition → analysis → visualization

Output file: csv

Plate well location
Well identifier
1 row/compound
All values/well
All replicates/well

Formatted data file: Excel

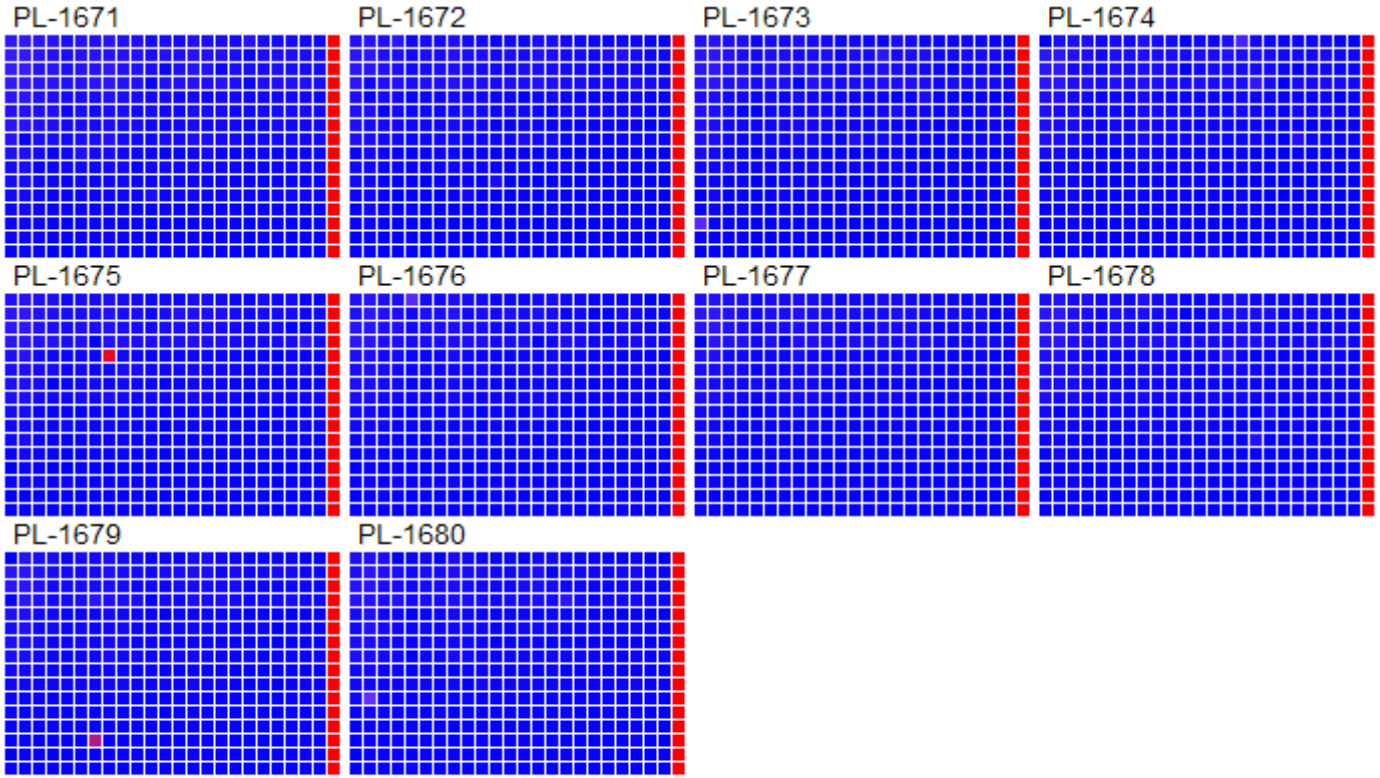
	A	B	C	D	E	F	G	H	I	J	K
1	Plate	Well	Type	Exclude	HTRF-Ch1 A	HTRF-Ch2 A	HTRF-Ratio A	HTRF-Ch1 B	HTRF-Ch2 B	HTRF-Ratio B	
2	1394	A01	X		20556	8648	23775	21061	8198	25690	
3	1394	A02	X		20322	8071	25179	20399	7357	27727	
4	1394	A03	X		21698	7764	27947	21896	7142	30658	
5	1394	A04	X		21855	7859	27809	22880	7506	30482	
6	1394	A05	X		22759	7943	28653	23457	7826	29973	
7	1394	A06	X		22996	8394	27396	23747	7884	30120	
8	1394	A07	X		23279	8270	28149	24415	7737	31556	
9	1394	A08	X		34126	13099	26052	36321	13646	26617	
10	1394	A09	X		23516	7675	30640	23413	7769	30136	
11	1394	A10	X		23500	7957	29534	24747	7787	31780	
12	1394	A11	X		24035	7999	30048	24647	7865	31338	
13	1394	A12	X		24042	8383	28679	24415	7906	30882	
14	1394	A13	X		23785	8044	29569	24821	7665	32382	
15	1394	A14	X		24389	8013	30437	24714	7788	31733	
16	1394	A15	X		23495	7950	29553	24186	7595	31845	
17	1394	A16	X		22729	7813	29091	24027	7709	31167	
18	1394	A17	X		23208	7781	29827	24414	7738	31551	
19	1394	A18	X		22820	7775	29350	23894	7716	30967	
20	1394	A19	X		23189	7615	30452	23897	7666	31173	
21	1394	A20	X		23806	7792	30552	23741	7610	31197	
22	1394	A21	X		22988	7481	30729	23298	7440	31315	
23	1394	A22	X		23117	8180	28260	22981	7667	29974	
24	1394	A23	P		22901	7774	29458	23107	7506	30785	
25	1394	A24	N		10190	8915	11430	9194	8751	10506	
26	1394	B01	X		20280	8129	24948	21590	7544	28619	
27	1394	B02	X		21807	7745	28156	21983	7411	29663	
28	1394	B03	X		21881	7831	27942	22290	7483	29788	
29	1394	B04	X		22567	7703	29296	23542	7647	30786	
30	1394	B05	X		23045	7691	29964	23451	7414	31631	
31	1394	B06	X		23272	7563	30771	24361	7507	32451	
32	1394	B07	X		22550	7498	30075	23427	7286	32153	
33	1394	B08	X		23904	7423	32203	24047	7553	31838	
34	1394	B09	X		24073	7649	31472	24215	7038	34406	
35	1394	B10	X		23363	7274	32119	24276	7084	34269	
36	1394	B11	X		23725	7957	29817	24717	7515	32890	
37	1394	B12	X		23759	7430	31977	24877	7203	34537	
38	1394	B13	X		23540	7749	30378	24277	7649	31739	
39	1394	B14	X		16987	3590	47318	17633	3765	46834	
40	1394	B15	X		24025	7417	32392	24335	7310	33290	
41	1394	B16	X		24366	7355	33128	25536	7026	36345	
42	1394	B17	X		23070	7209	32002	24538	7623	32189	
43	1394	B18	X		24637	8367	29445	25016	7760	32237	
44	1394	B19	X		23503	7225	32530	24260	7229	33559	
45	1394	B20	X		23254	7433	31285	24044	7223	33288	
46	1394	B21	X		22608	7639	29595	23201	7238	32054	
47	1394	B22	X		22947	7118	32238	22968	7135	32191	
48	1394	B23	P		22684	7250	31288	23192	7237	32046	
49	1394	B24	N		9376	8637	10856	9169	8437	10868	
50	1394	C01	X		21241	7625	27857	21461	7582	28305	
51	1394	C02	X		22261	7819	28470	22089	7494	29476	
52	1394	C03	X		22890	7704	29712	23495	7441	31575	
53	1394	C04	X		23396	7500	31195	24129	7284	33126	
54	1394	C05	X		23576	7521	31347	24453	7240	33775	
55	1394	C06	X		23843	7521	31702	24779	7765	31911	
56	1394	C07	X		24203	7191	33657	24777	7272	34072	
57	1394	C08	X		22920	7265	31549	23670	6950	34058	

Controls | 1394 | 1395 | 1396 | 1397 | 1398

Sent to ICCB-L data curator
Custom script
Screensaver

Analysis software
Visualization software

How do the data look across a specific experiment?

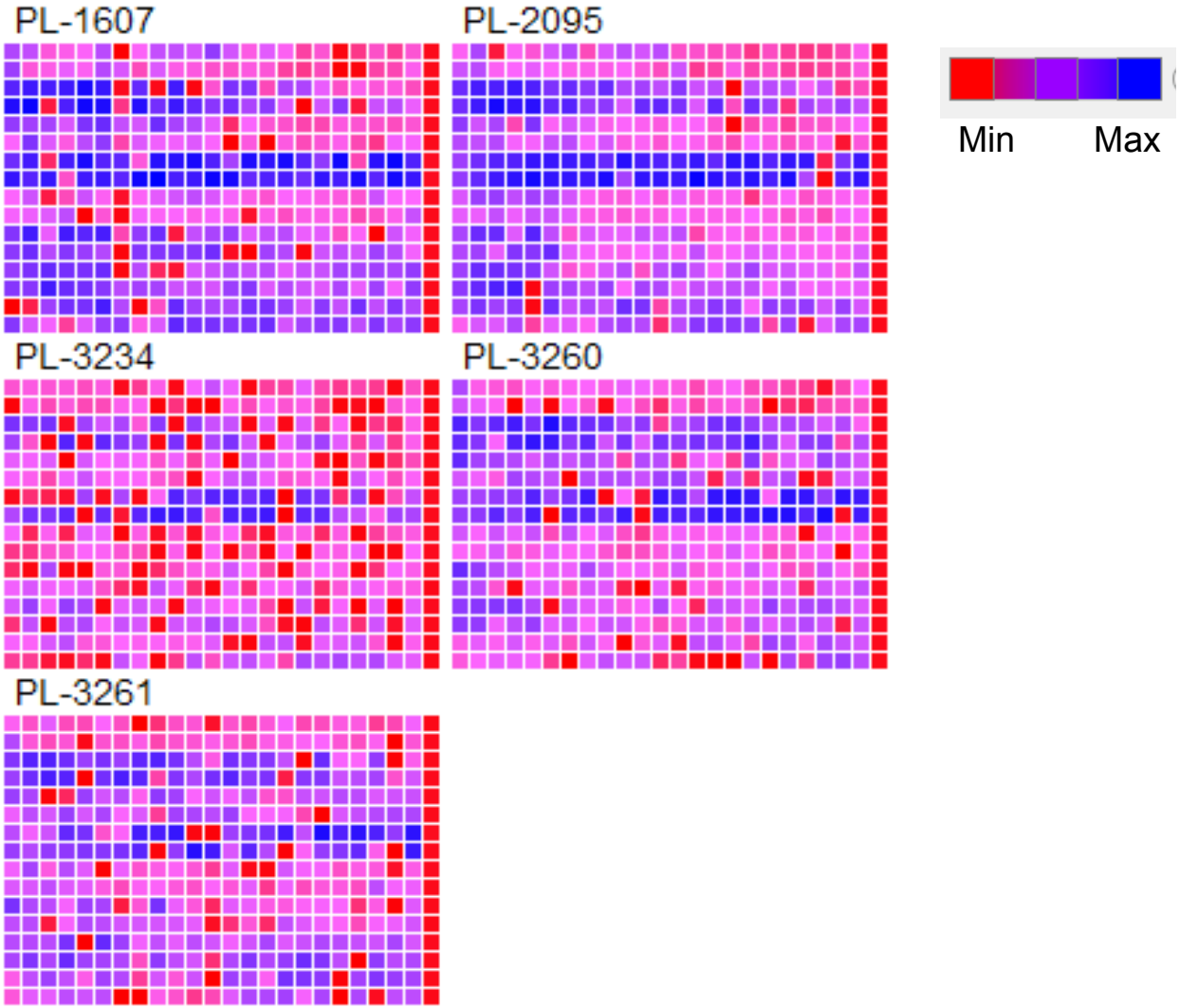


Visualize using heat map function

Controls and potential hits should be clearly identifiable

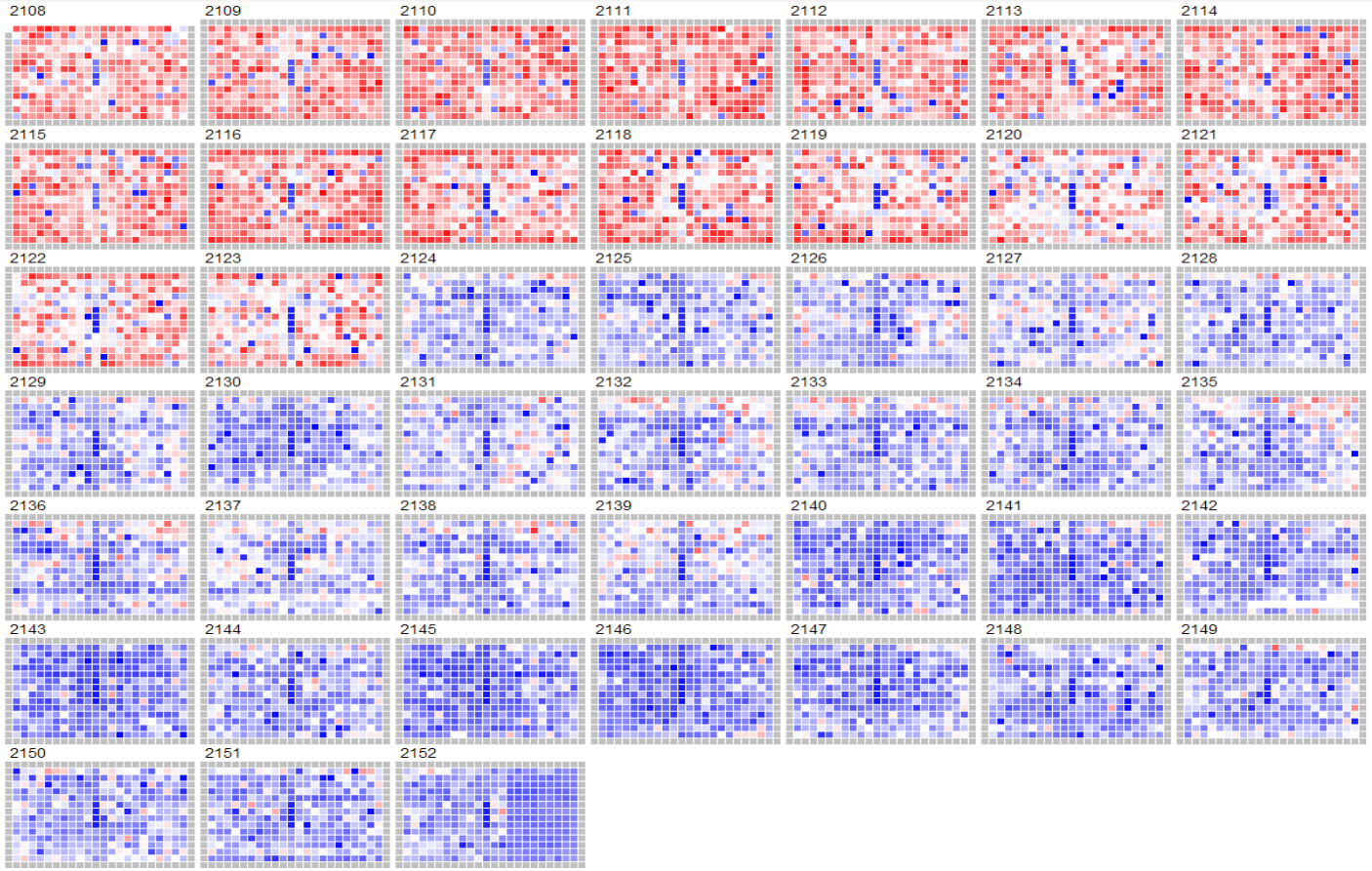
Can be used to distinguish bad plates in a run or trends

How do the data look across a specific experiment?



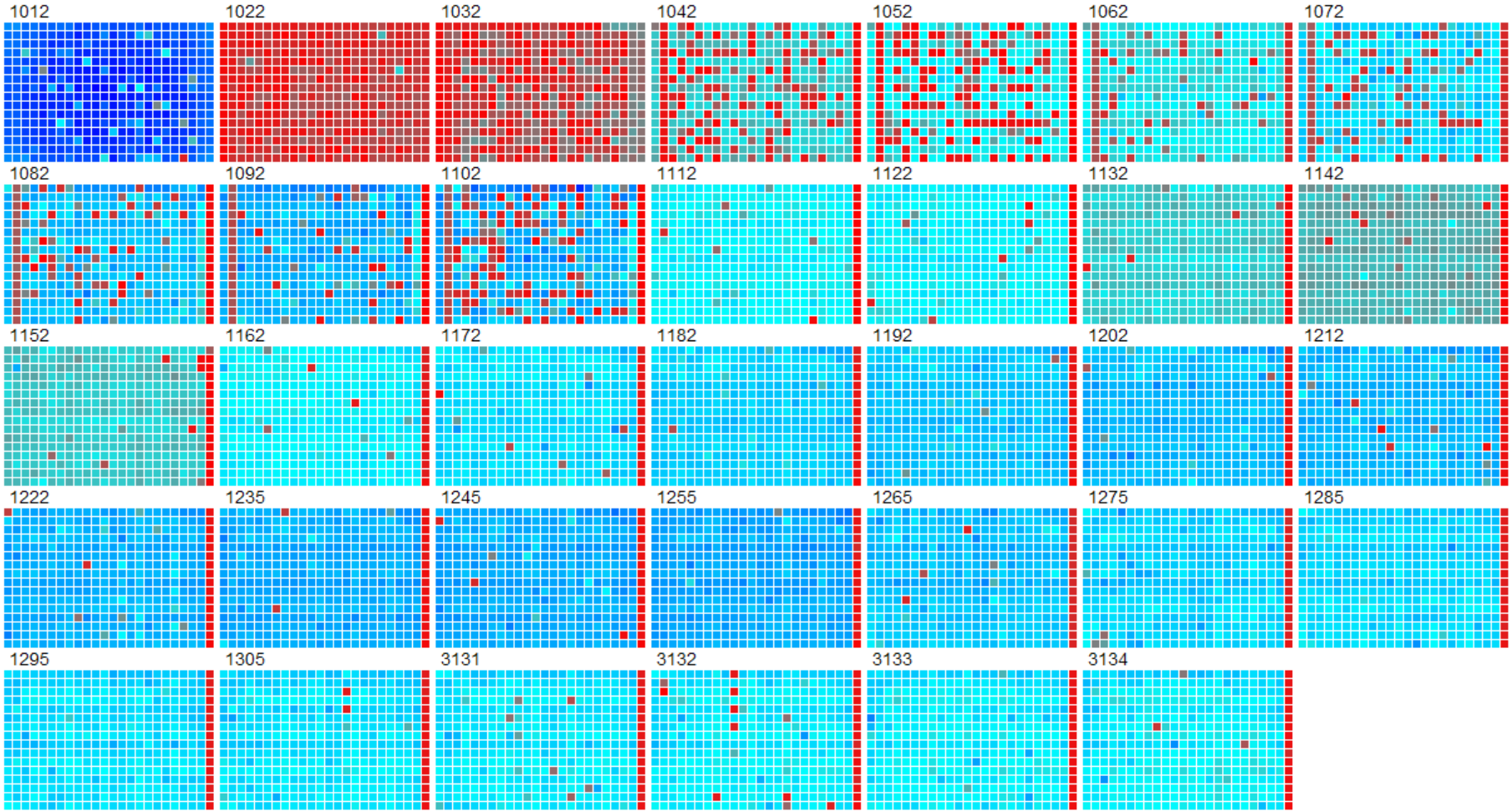
***Example*: row trends, atypical hit rate**

How do the data look across a specific experiment? Overall?



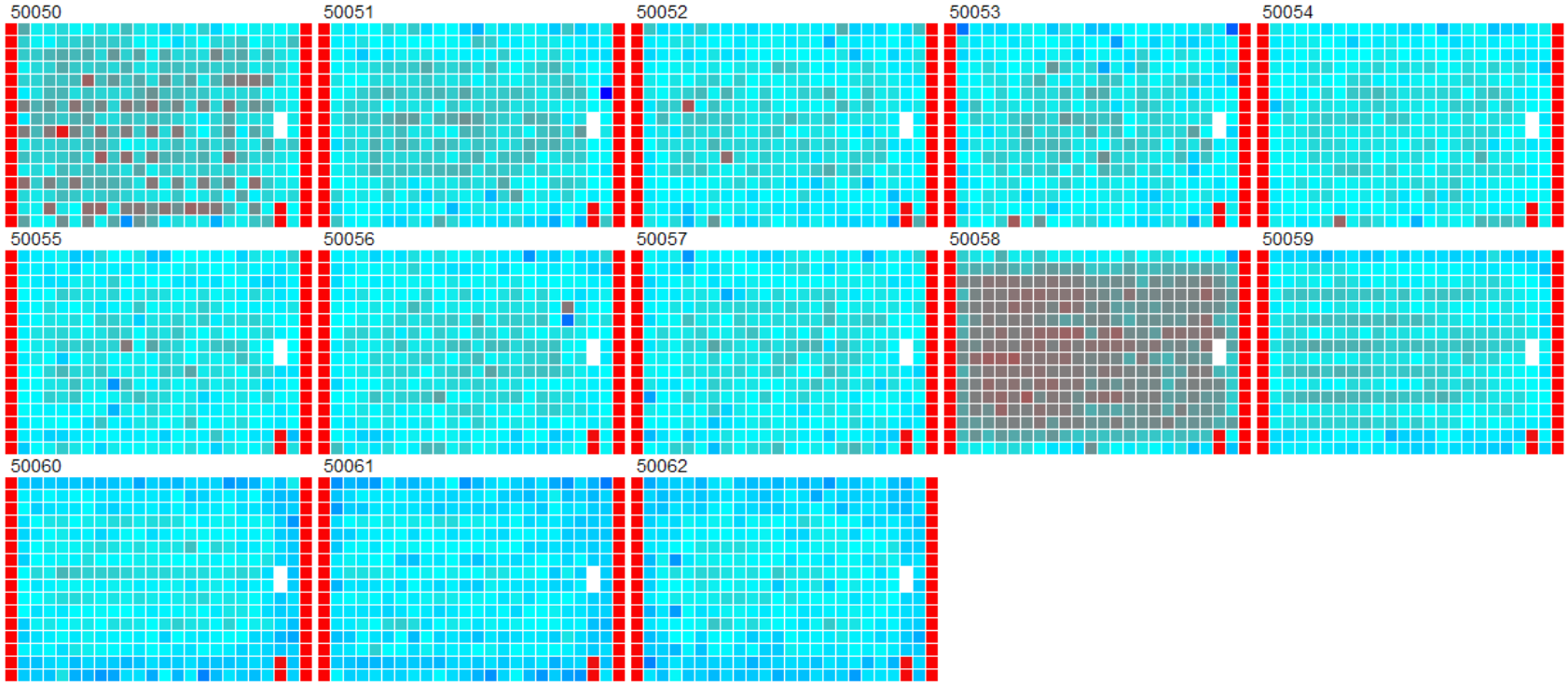
Confirmed what investigator had suspected – first 16 plates different from rest of screen (45 plates total)

How do the data look across a specific experiment? Overall?



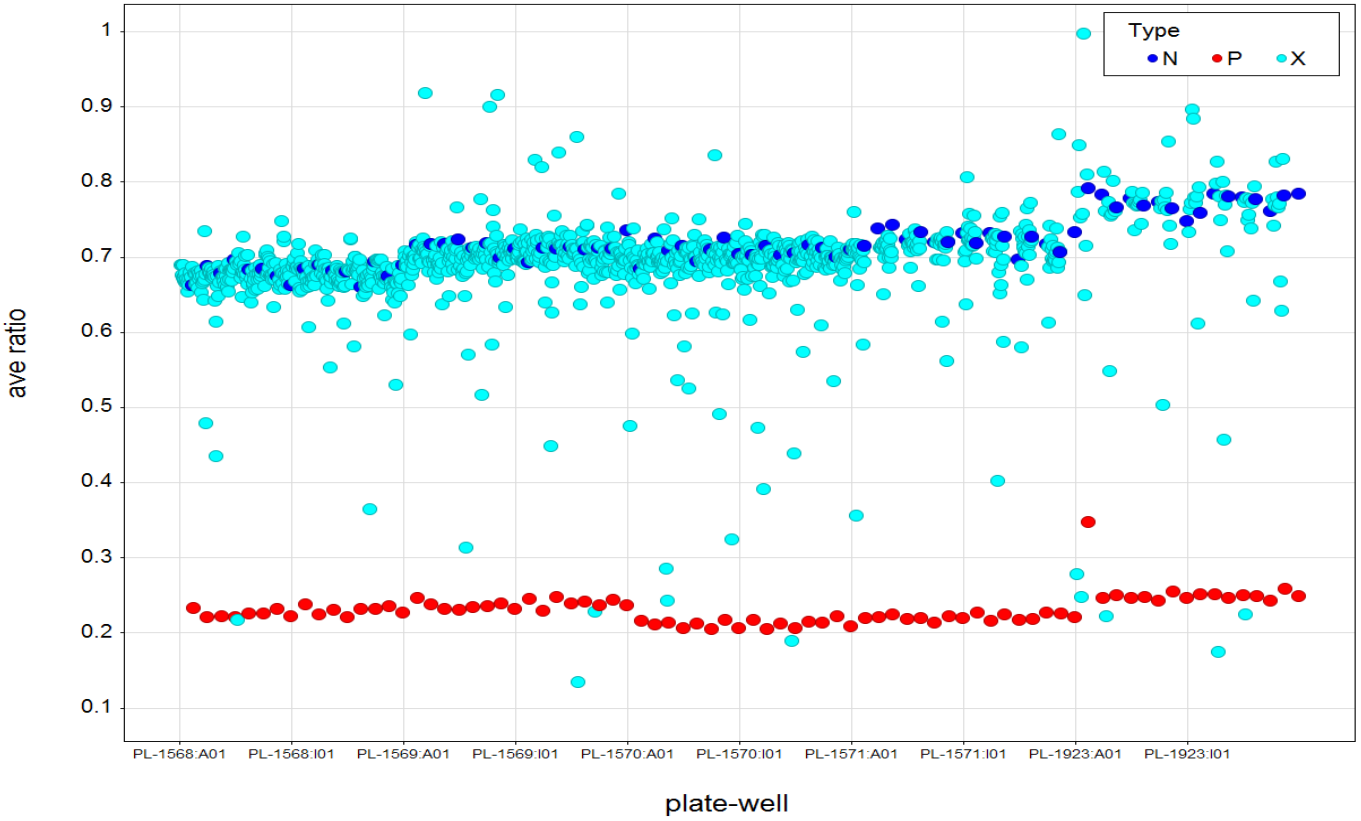
Highlighted issues quantitating cell numbers at the beginning of the screen. Screener paused, re-optimized, then continued.

How do the data look across a specific experiment? Overall?



Striping on two plates
Experimental wells on one plate atypical

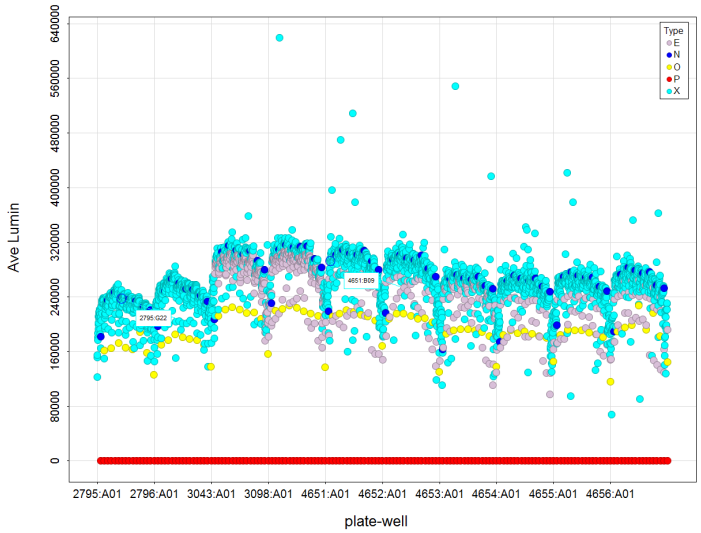
Basic scatterplot



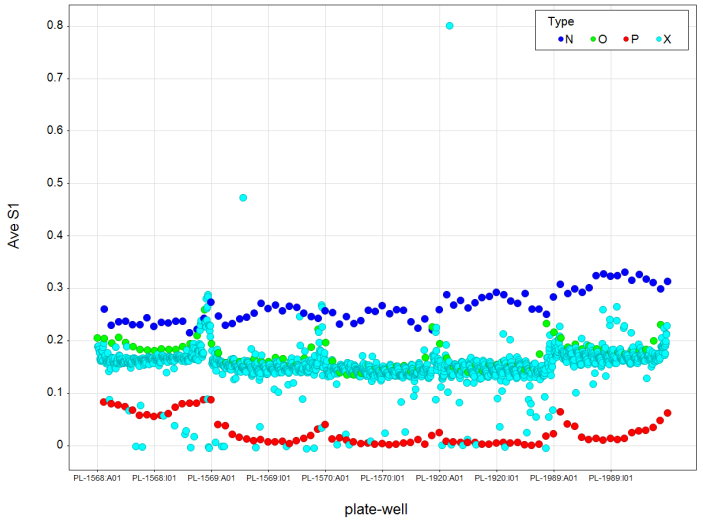
Most informative: basic scatter plot with a unique ID (plate-well, reagent ID) vs average data value. Color set by well 'type'

Enables visualization of: separation of controls, dynamic range
potential positives
positional effects
trends in data (positional, time)

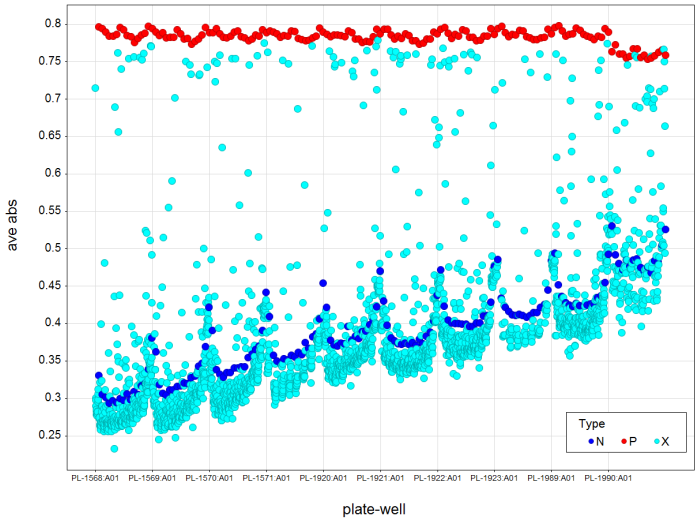
Basic scatterplot – problems!



Positional trend, periodicity



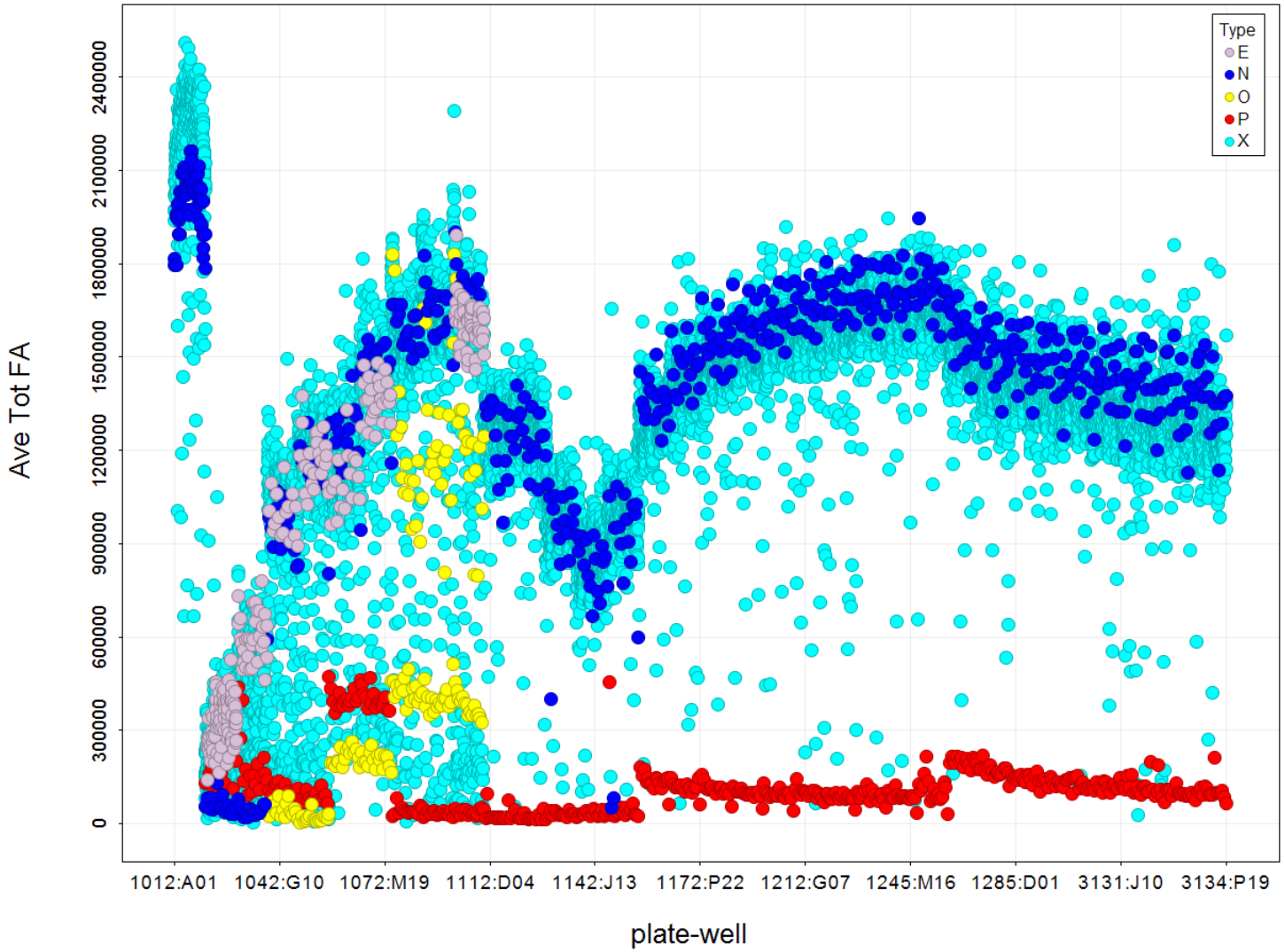
Low signal:background



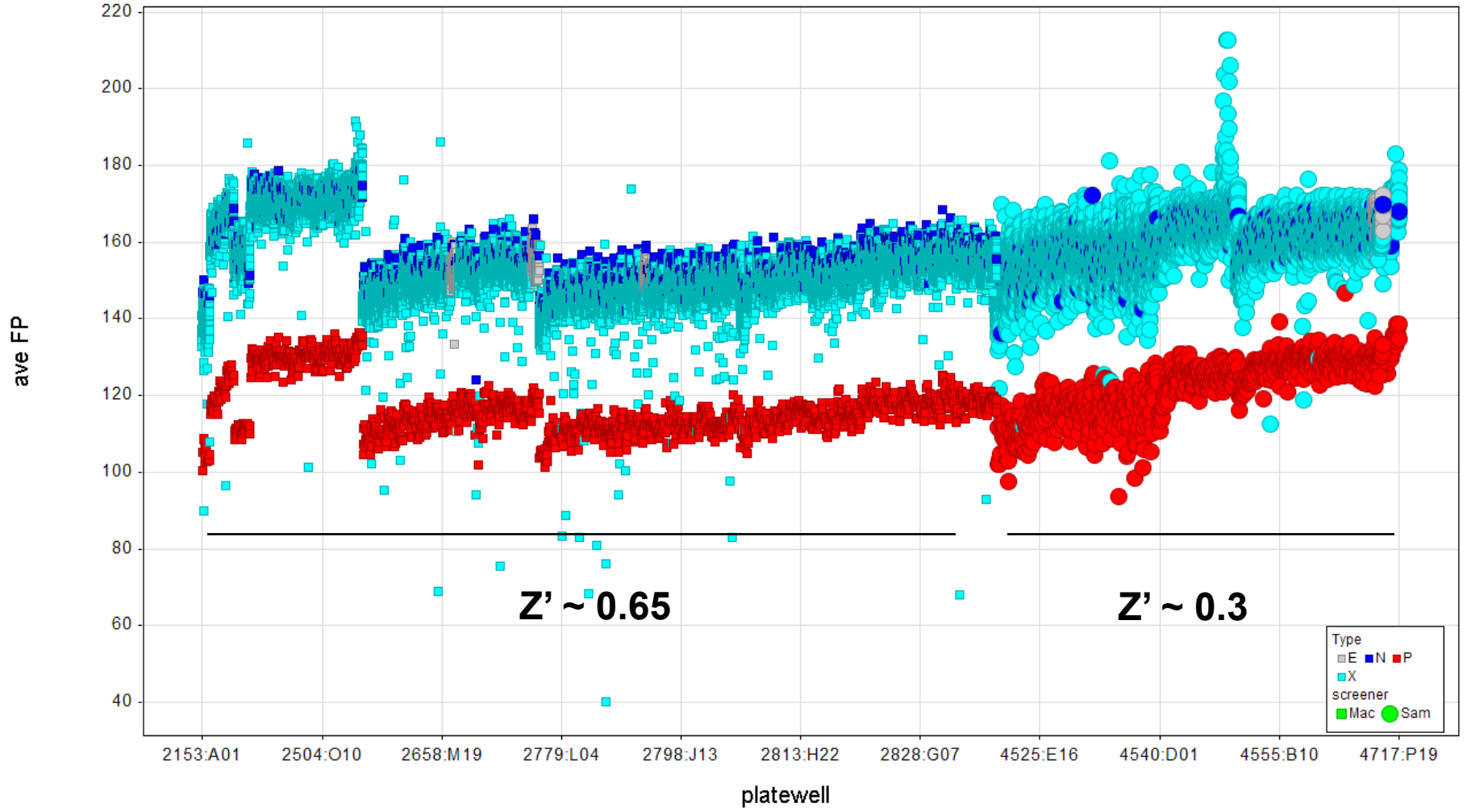
Time trend, drift

Excessive scatter
Unusually high or low plates
Issues with control
Possible saturation

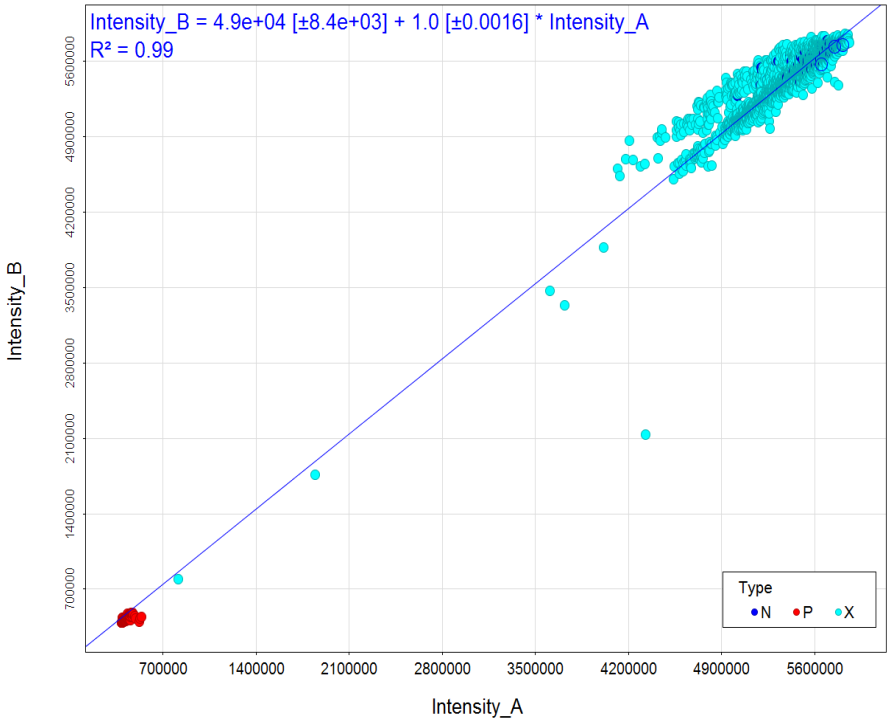
Basic scatterplot – *big* problems!

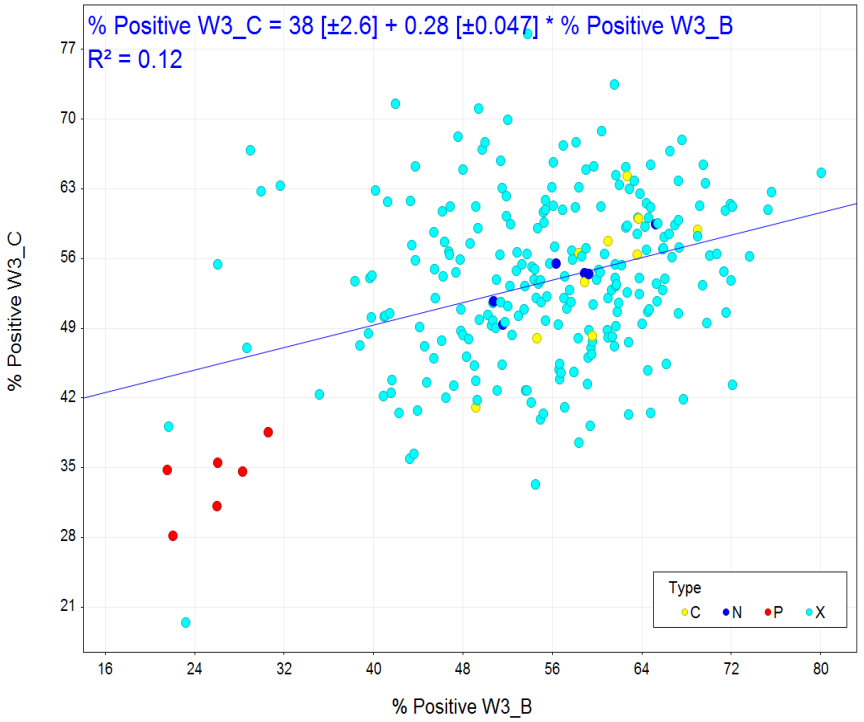


Basic scatterplot for biochemical screen



How reproducible are the data?



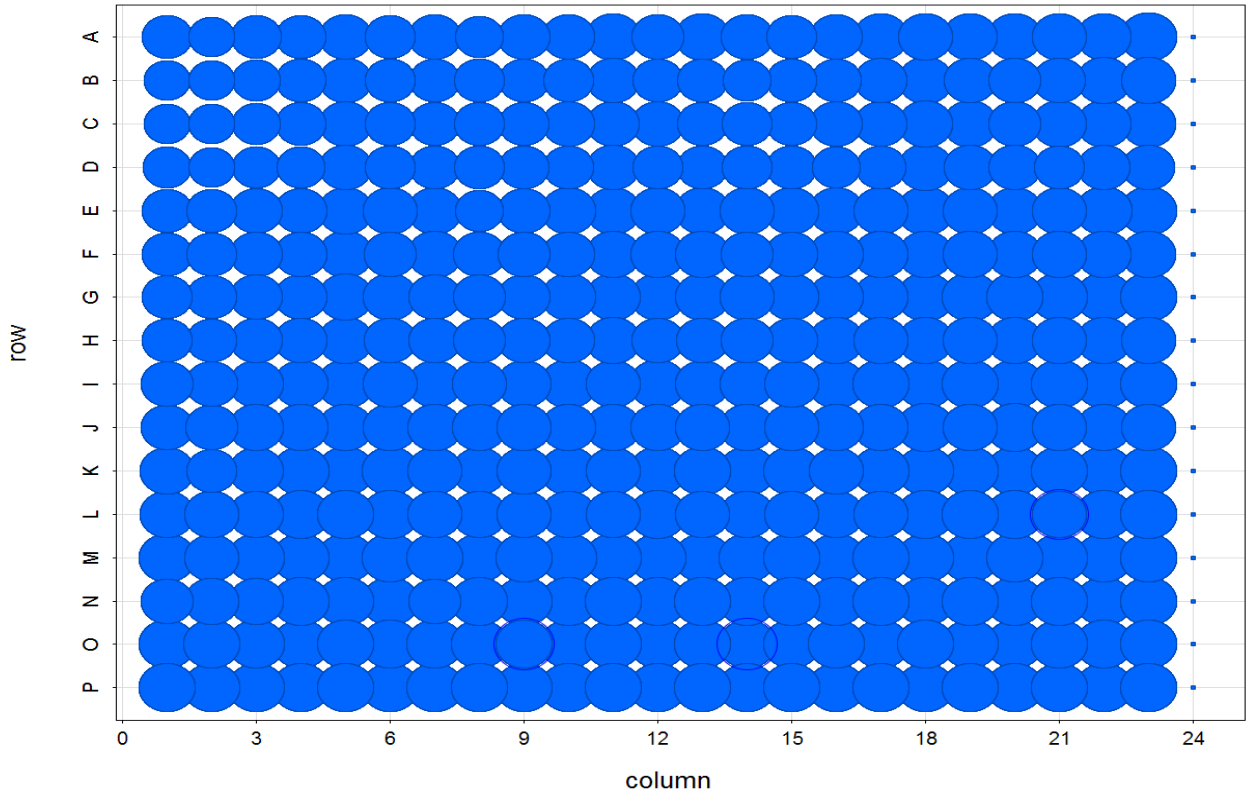


Use a scatter plot in Vortex to: compare replicates – A vs B, B vs C, C vs A
display a regression line
calculate R^2

Goal: $R^2 > 0.8$

Note: controls can skew data, artificially inflating R^2. Determine correlation +/- controls

Are there positional effects? Biases?

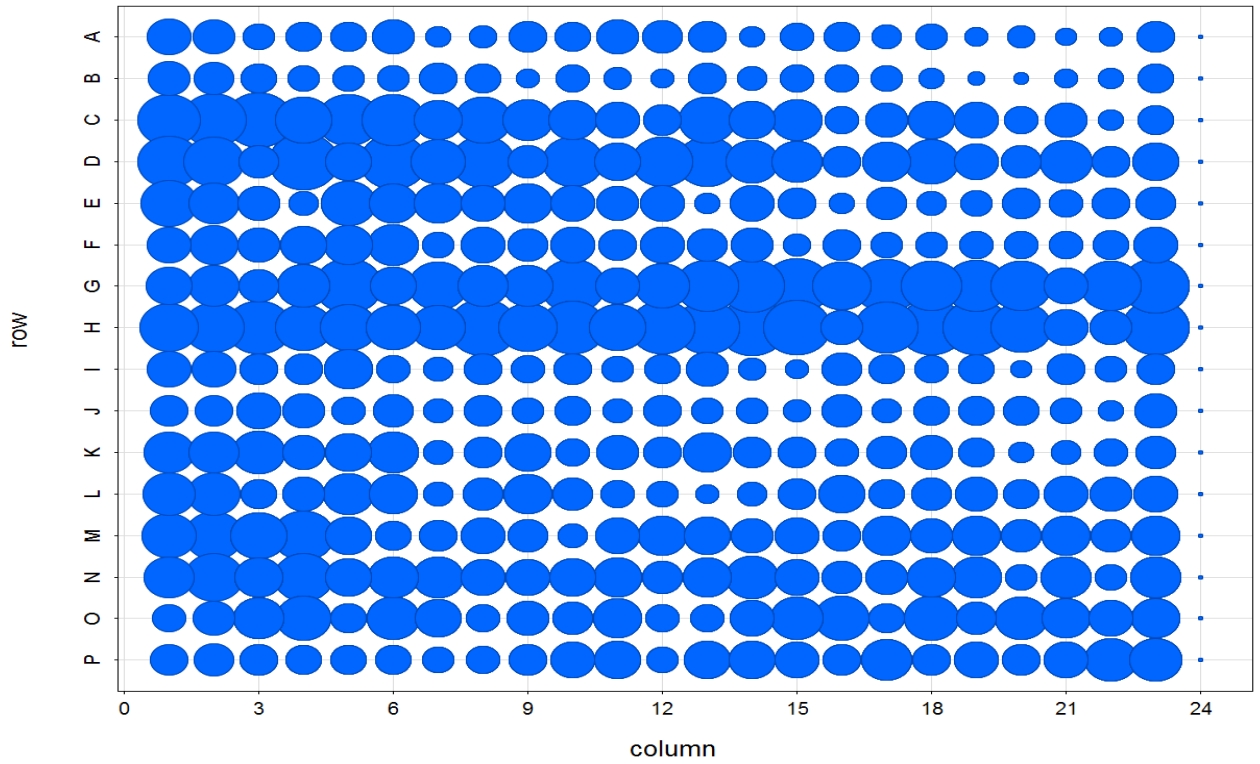


Needed solution to average raw data by well location across all plates in a run

Statistics feature: mean of raw data using a 'group by function of row and column'
Generates new data column "Z"
Graph: X = column, Y = row, Z = size

Goal: all wells (except positive control) are the same size

Are there positional effects? Biases?

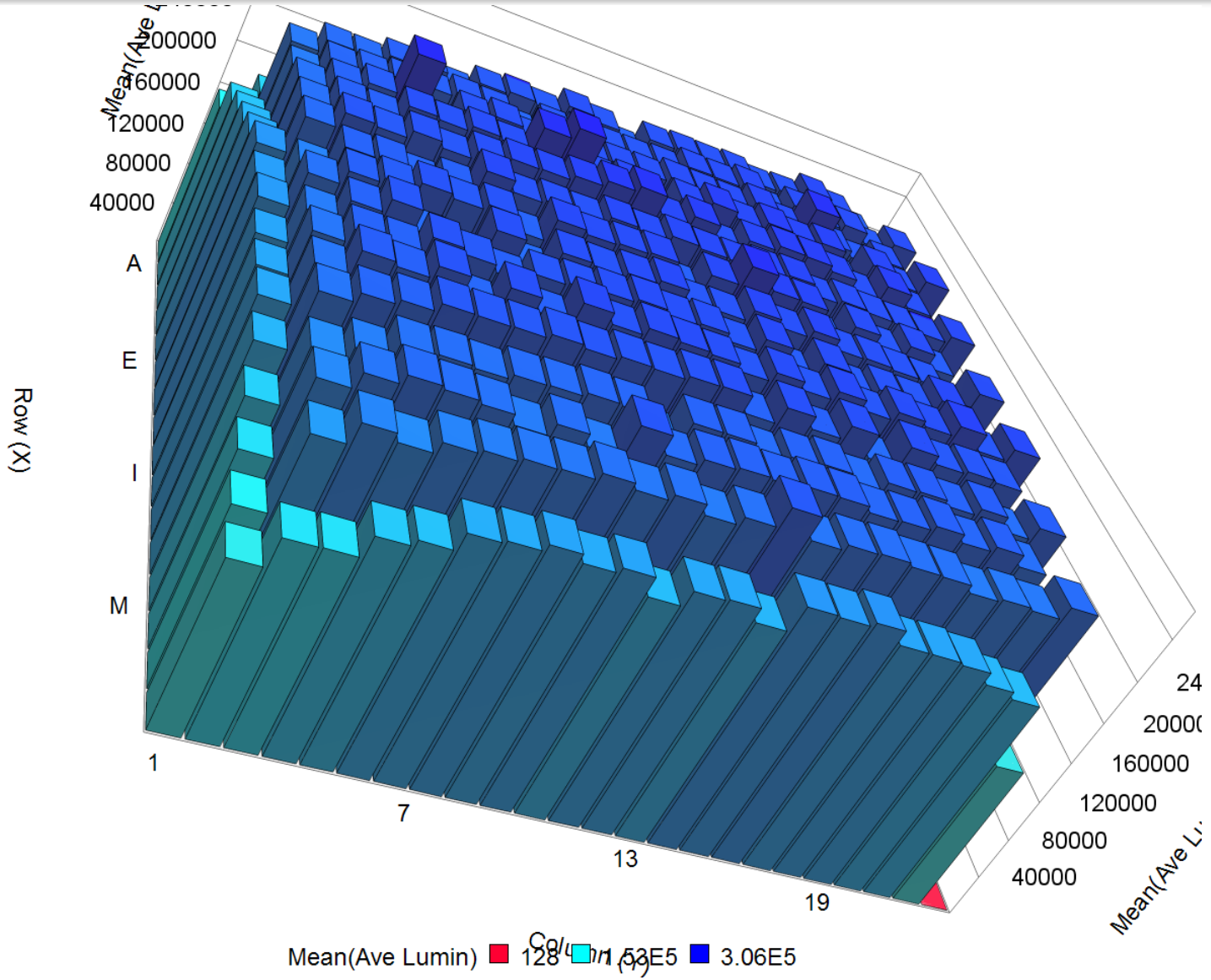


***Example*: Clear trends in rows C/D and G/H**

Technical issue with Combi?

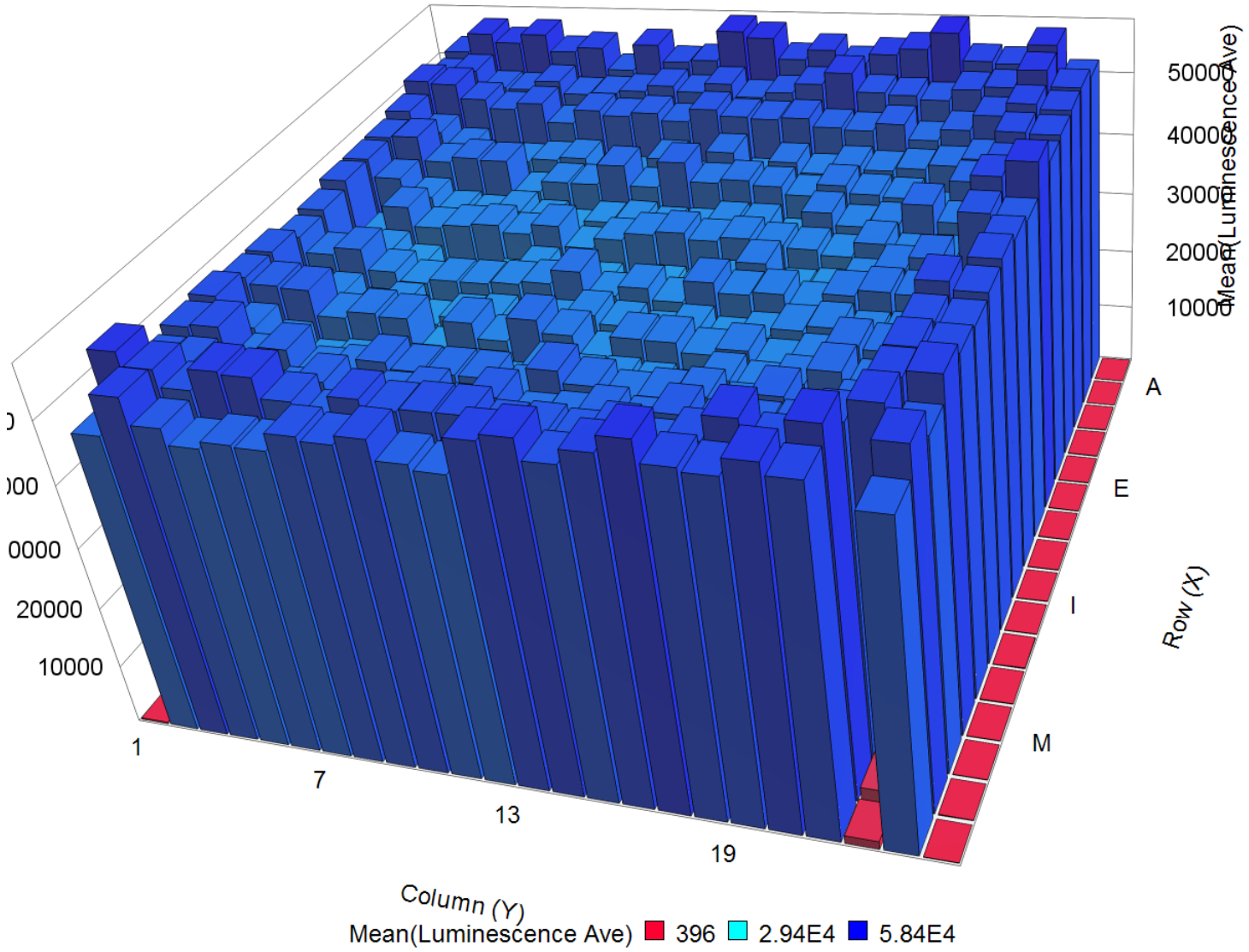
Paired row trends frequently consequence of peristaltic pump manifold utilized to fill assay plates because 1 nozzle fills 2 rows (A/B, C/D, *etc.*)

Are there positional effects? Biases?



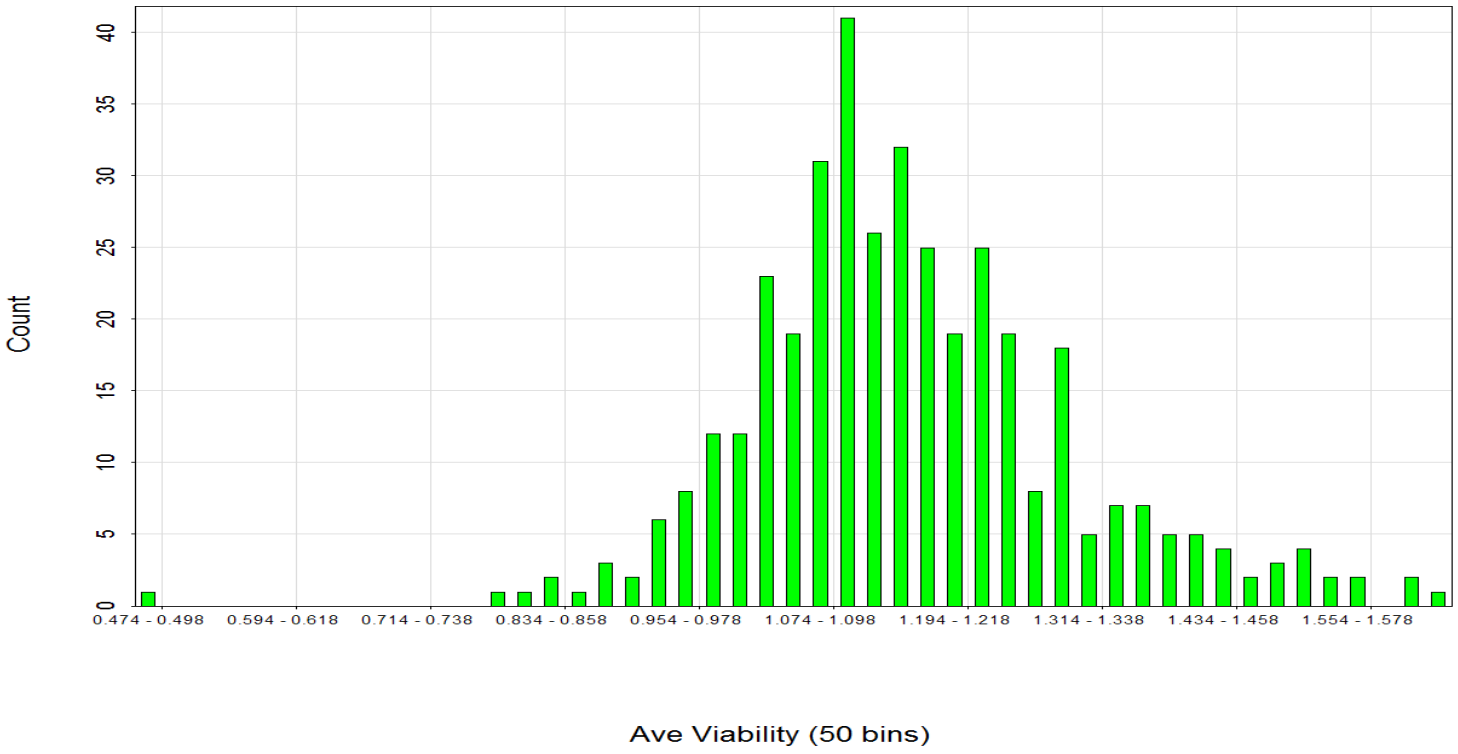
Edge effect – temperature gradient, evaporation

Are there positional effects? Biases?



Edge effect – temperature gradient, evaporation

Are the data normally distributed?



Built using bar graph

Ideal is bell-shaped curve, with both tails visible

Some assays only display one tail (signal issues, starting point of assay)

Distribution Important to know – influences data analysis methods

How will I analyze my data?

1. Triage

2. Normalization

- Performed per plate
- Removes systematic data effects
- Options: sample median, control median, log transformation, scores
- May have to exclude some plates/wells from analysis
- Enables comparison and combination of data from different plates

3. Quality check

4. Hit selection – statistical analyses

How will hits be identified?

Z-score

- Assumes normality
- Parametric (score implies a probability)
- Scale?

Robust Z-score

- Recommended for RNAi
- Robust to outliers
- Nonparametric

B-score

- Can take into account positional effects

Percent of control

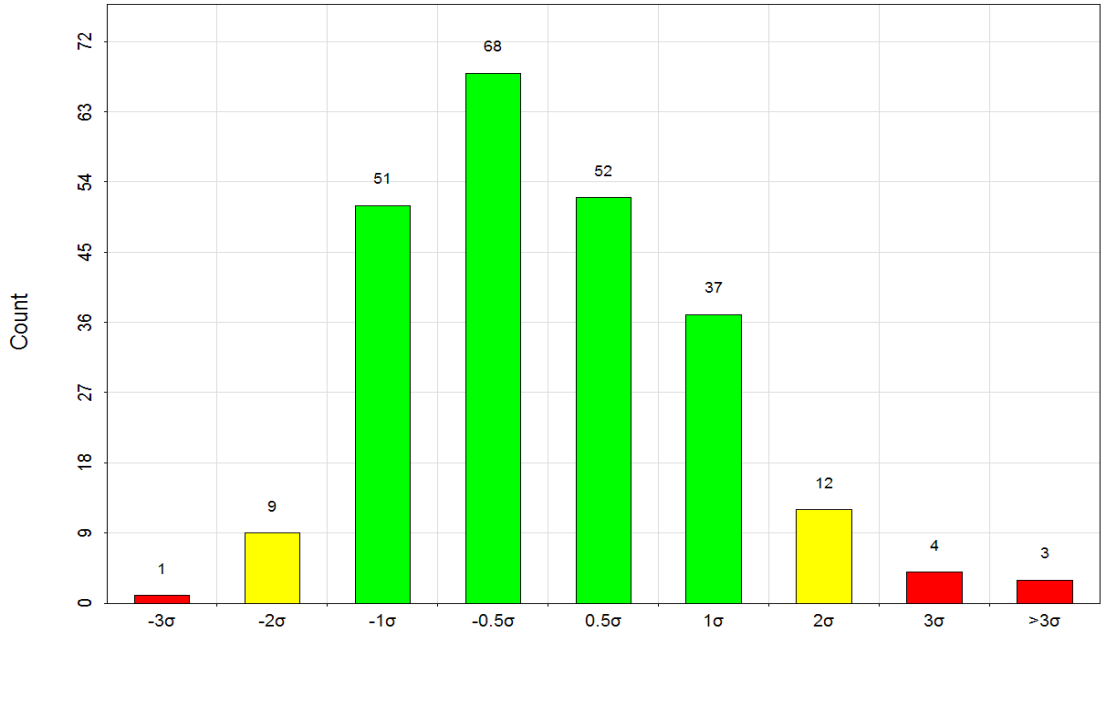
Normalized percent inhibition/activation

Rank product

SSMD – *S*trictly *S*tandardized *M*ean *D*ifference

RSA – *R*edundant *s*iRNA *A*ctivity

What is the potential hit rate?



Built using bar graph, bin by standard deviation option

Breakdown of experimental wells by standard deviations away from the average

Goal: observe majority of wells having minimal effect (green and yellow) and a few potential positives (red)

Provides estimated number of hits, how many enhancers and suppressors

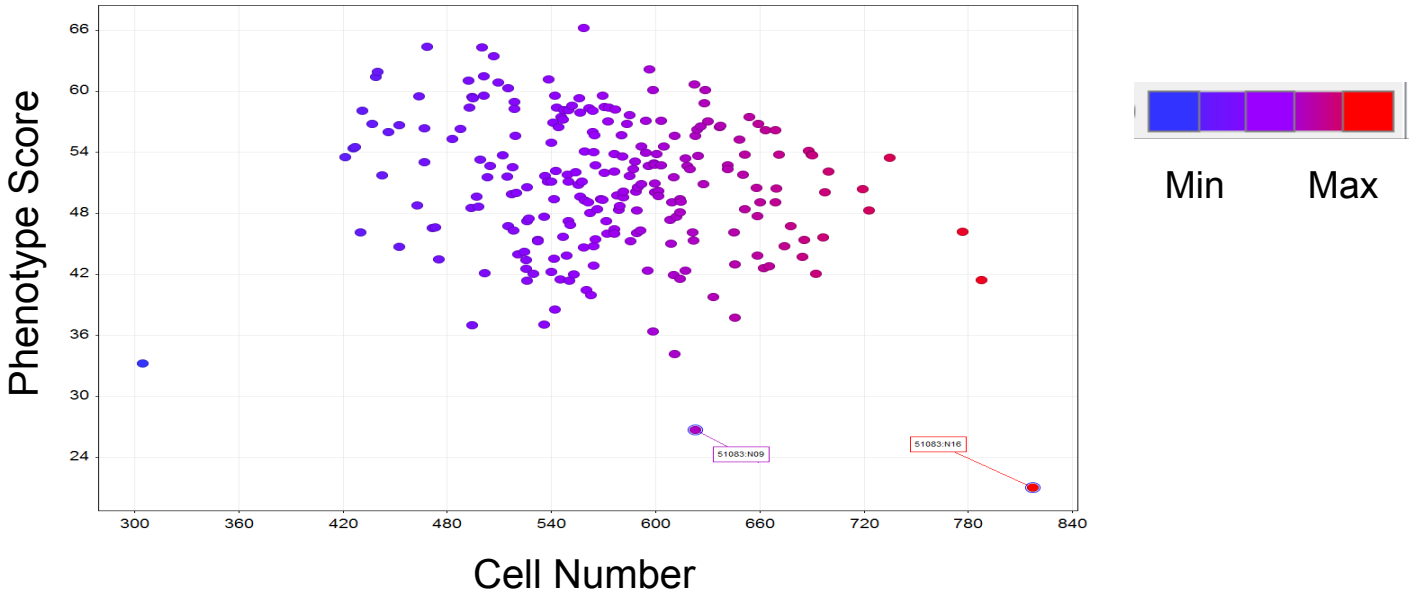
Accept there will be both false positives and false negatives

Follow the positives

What is the potential hit rate? Within specific profile (multiple conditions)?

Is the assay capable of identifying potential hits with specified profile?

Focused siRNA screen

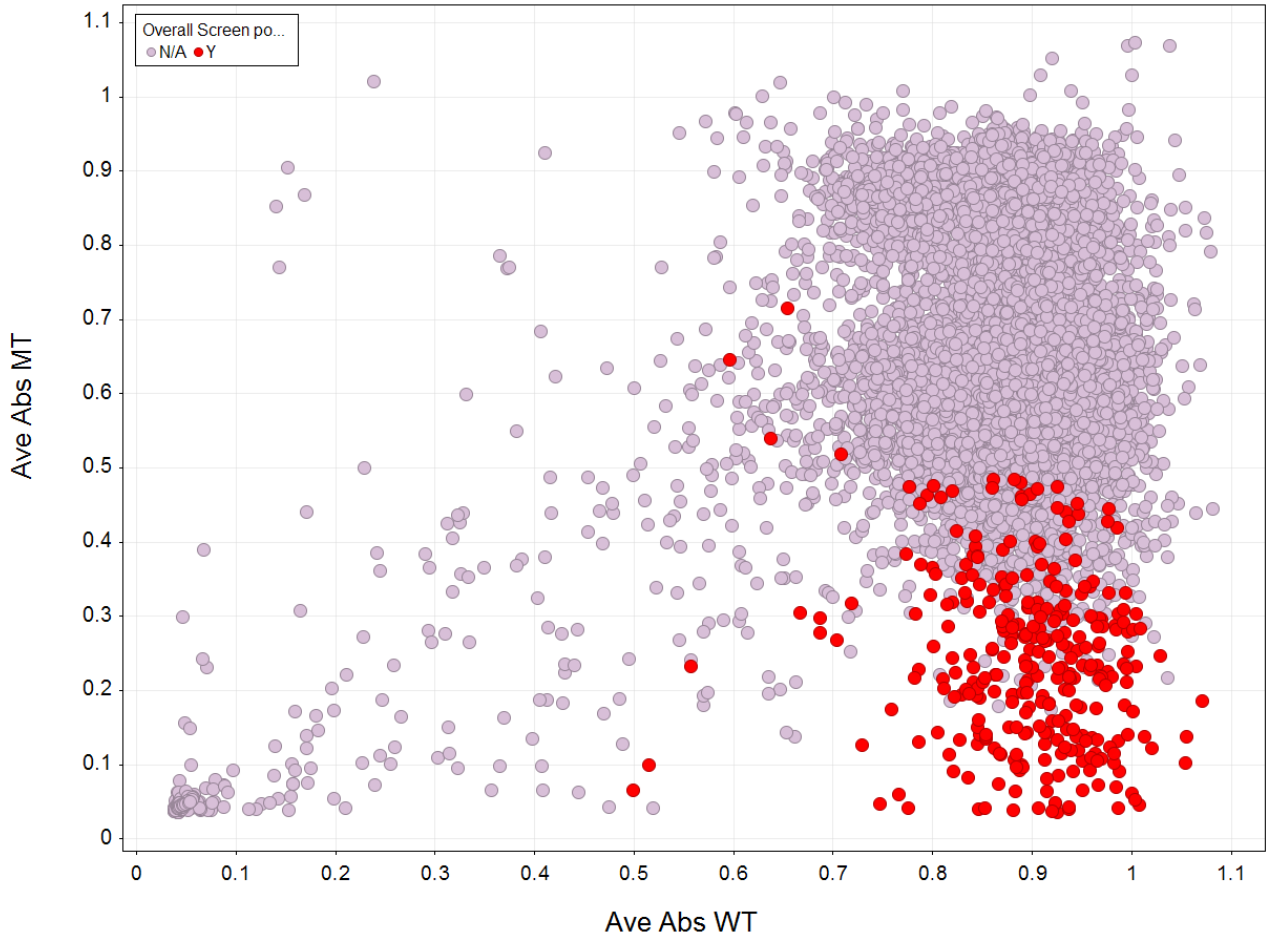


2 endpoints were measured: Phenotype score (screen)
Cell number (counter screen)

Goal: identify genes that result in low phenotypic score (y-axis), but are not generally toxic (x-axis)

Ideal: low on y-axis, high on the x-axis and red

Primary small molecule screen with 2 conditions

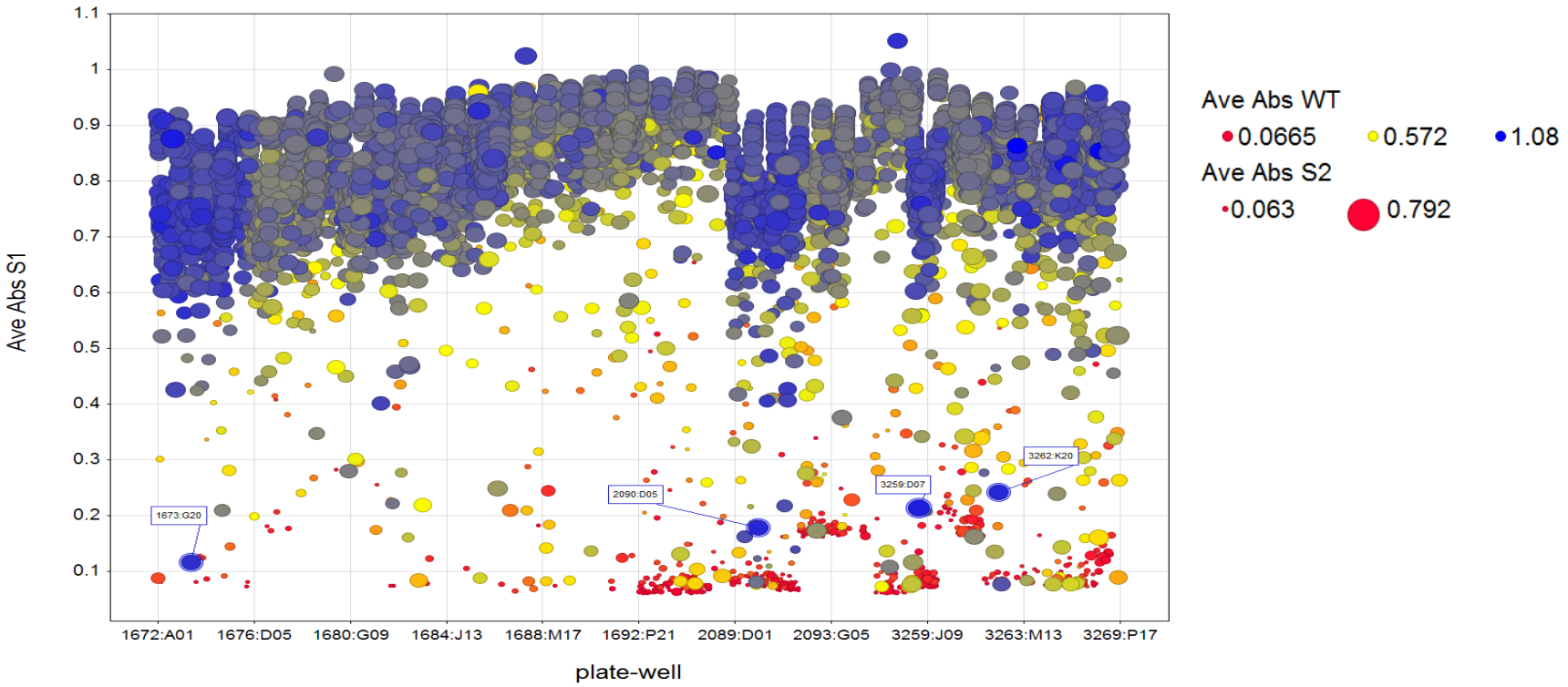


Same compound library is being run against wild-type cell and mutant cell line

Goal: identify compounds that kill mutant but not wt strain

Ideal: low on y-axis, high on x-axis

Primary small molecule screen with 3 conditions



Same compound library is being run against wild-type cell and 2 different genetically modified strains

Goal: identify compounds that hit in 1 modified strain, but not the other or wild-type

Wild-type: color by average Abs
Strain 1: position on y-axis by average Abs
Strain 2: size by average Abs

Ideal: low on y-axis, in blue, large size

Quality check – does the analysis fit?

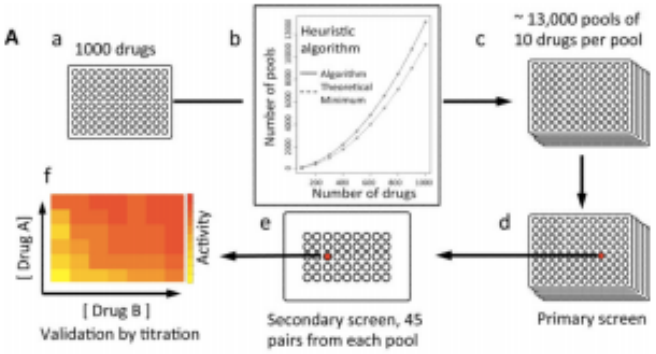
An investigator came back with an analyzed data set and specific questions:

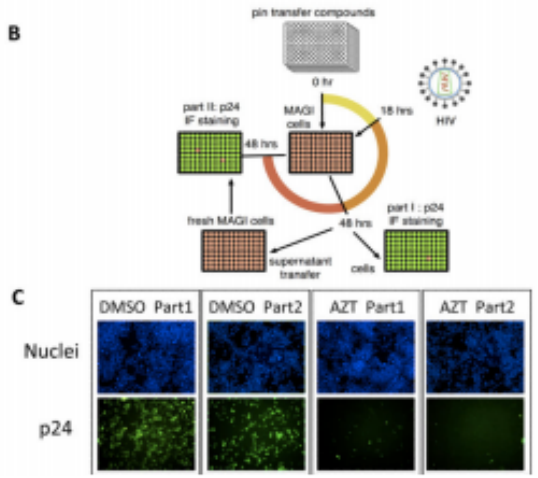
Does the math fit?

Check algorithm for hit selection to see if it visually looks as intended

How does the counter screen (viability) change the data set?

Can Vortex be used to spot errors or trends in the selected hits?





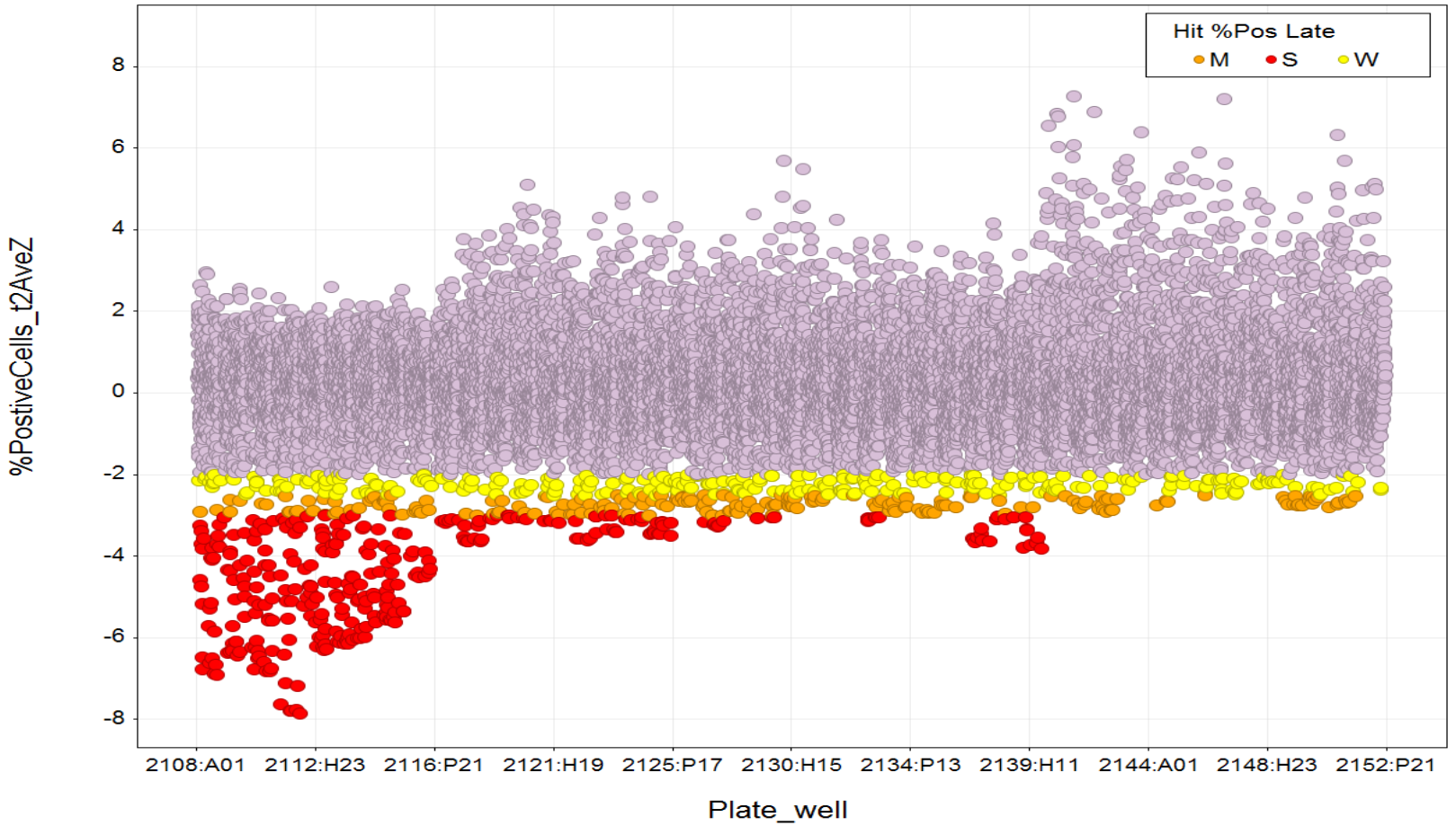
Note: cell-based assay with multiplexed known bioactive compounds – high primary hit rate

Systematic Identification of Synergistic Drug Pairs Targeting HIV

Xu Tan[1], Long Hu[2,#], Lovelace J. Luquette III[3,#], Geng Gao[1], Yifang Liu[2], Hongjing Qu[1], Ruibin Xi[3], Zhi John Lu[2,*], Peter J. Park[3,*], and Stephen J. Elledge[1]

Nat Biotechnol. 2012 November ; 30(11): 1125–1130.

Quality check – does the analysis fit?

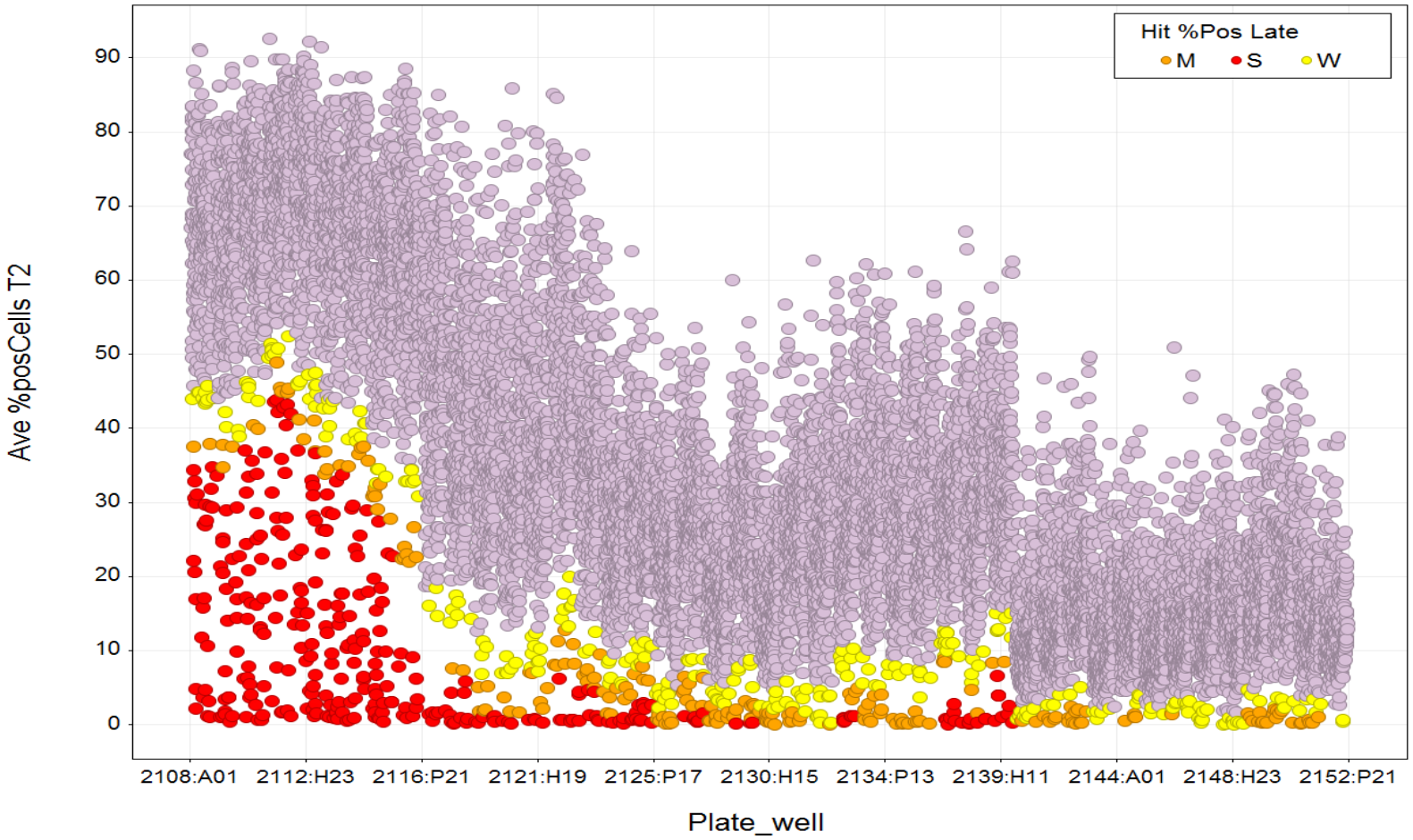


1. Does my algorithm for picking hits match what I would pick visually on the calculated data?

13230 number of experimental wells

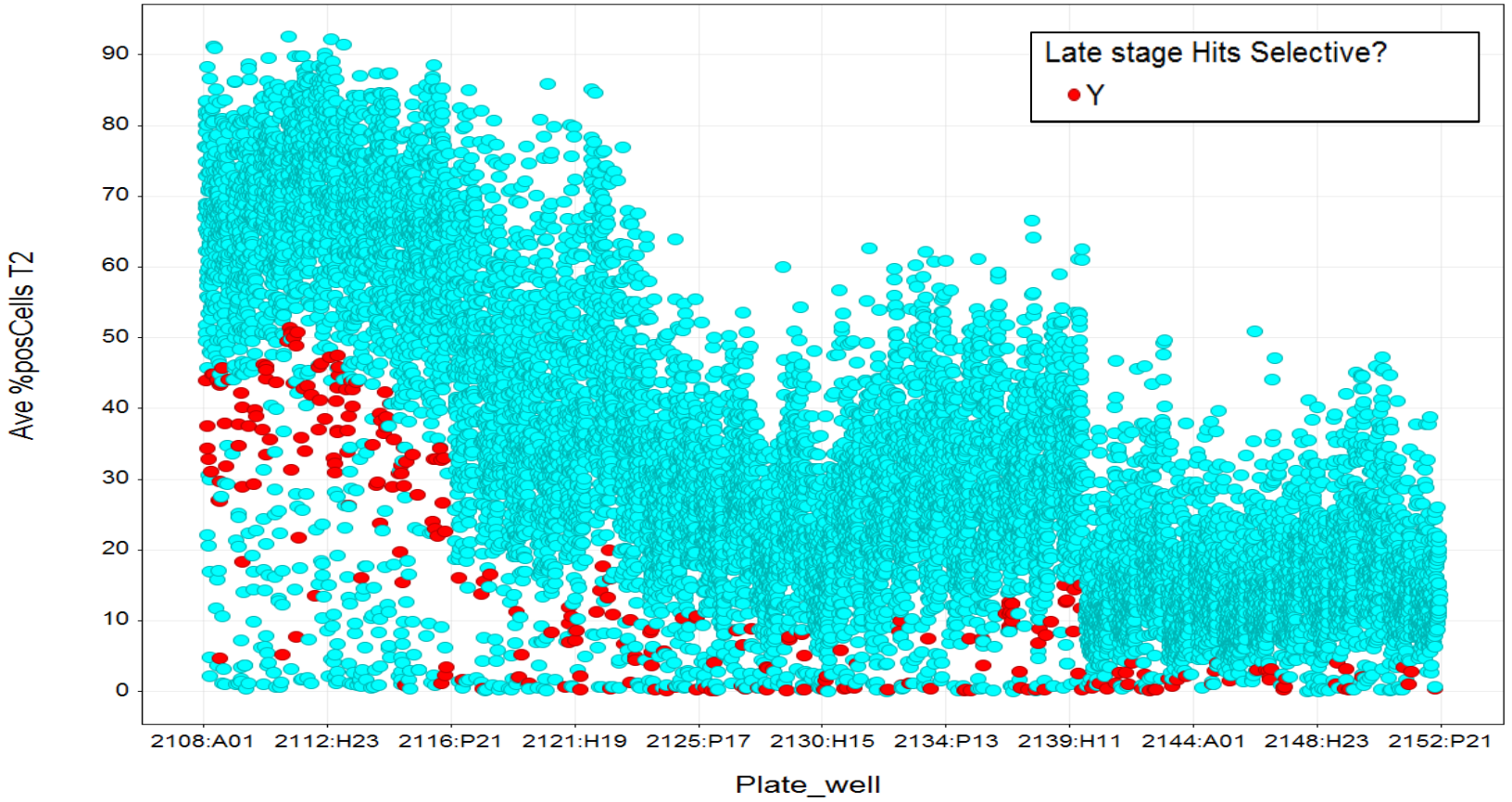
Potential hits categorized as Strong (333), Medium (211) and Weak (284) based upon z-score

Do the analyzed and raw data correspond?



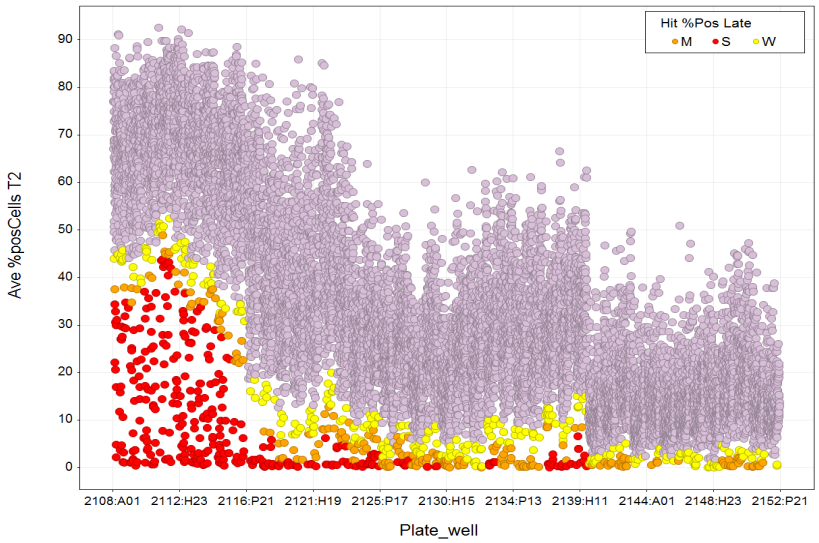
2. Does my algorithm for picking hits match what I would pick visually on the RAW DATA?

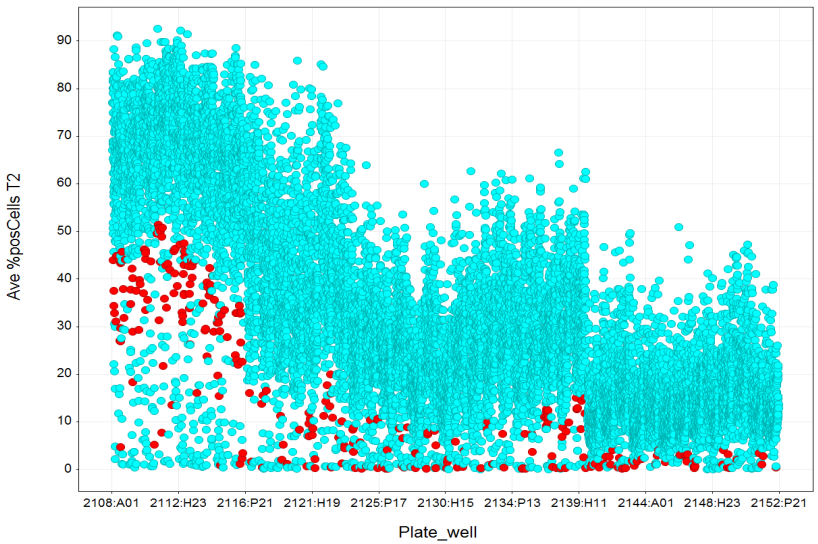
How does the counter screen change potential hits?



3. What does hit selection on the raw data look like after the counter screen filter (toxicity) is applied?

How does the counter screen change potential hits?

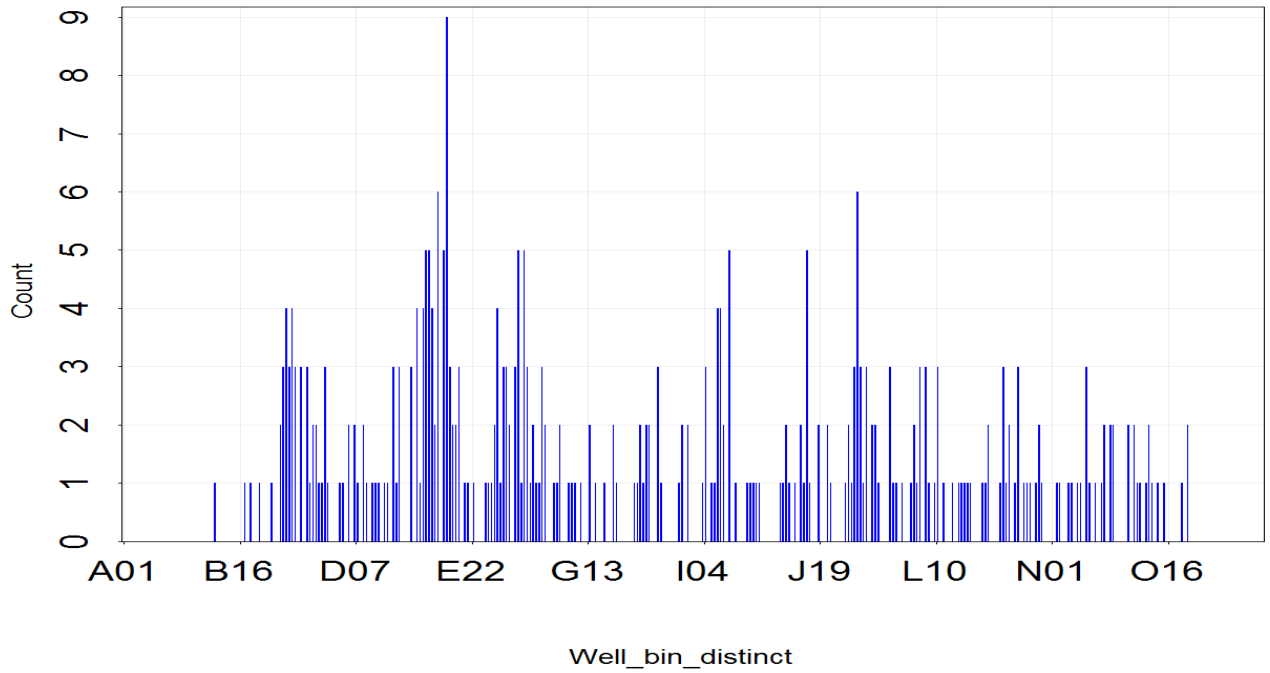




Counter screen enables follow up to be on the most relevant potential hits

Are there trends in the potential hits?

Which wells are my potential hits in?

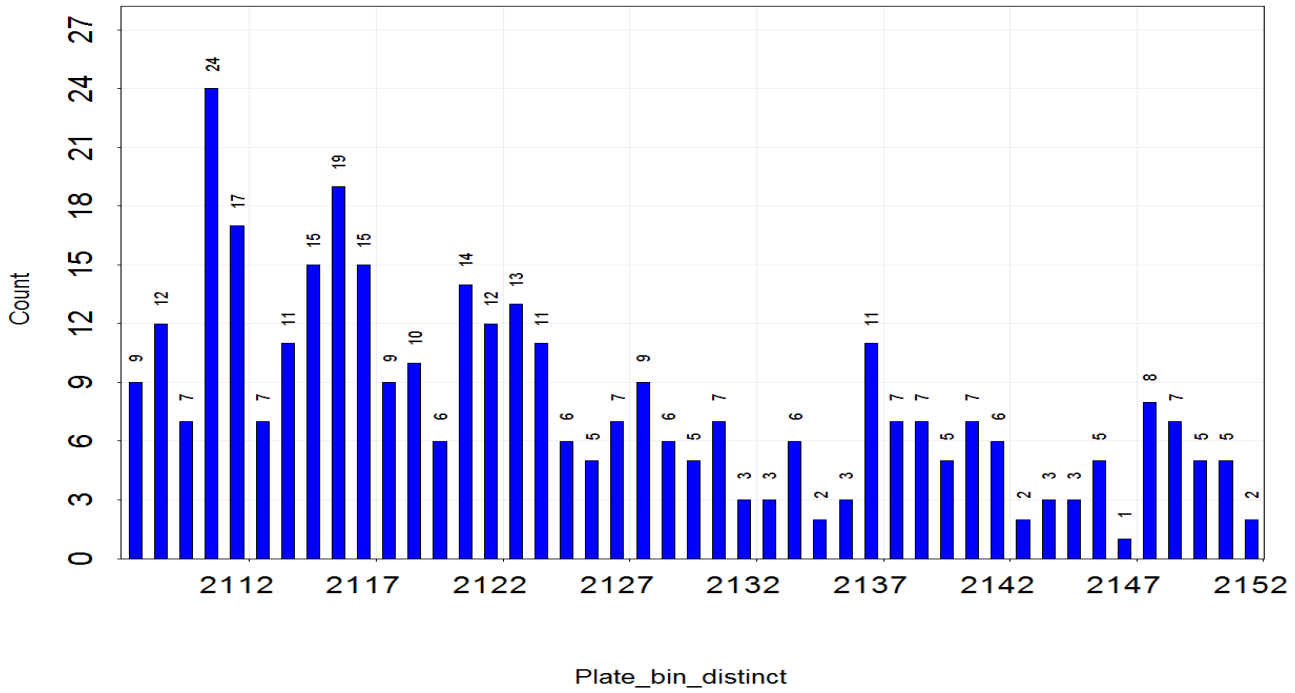


Utilize distribution graph to check which wells are picked and how often

This example: wells in row E (E14) frequently represented, periodicity

Are there trends in the potential hits?

Which library plates are my potential hits in?



Example: clear indication that the first 10 plates have a large number of hits

Library clustering of similar compounds, target type?

May justify more stringent criteria

Goals for academic HTS: Small molecule screening

Practical

Discovery of biological research tools

- Probes of pathways
- Discovery/validation of new 'druggable' targets
  - note: target ID can be challenging!

More Ambitious

Early-stage therapeutic lead discovery

- Requires significant medicinal chemistry
- Animal studies required to evaluate efficacy, PK/PD, toxicity....

Goals for academic HTS: Functional genomics screens

Practical

Gene/target discovery in specific cell pathway or process

Small molecule target identification or elucidation of mechanism

- Perform in parallel with small molecule screen
- Screen for enhancers/suppressors of small molecule-induced phenotype

More Ambitious

Systematic annotation of a whole genome or gene family

Examples of high throughput screening projects

HOME / RESOURCES /

http://iccb.med.harvard.edu/small-molecule-publications

Small Molecule Publications

Below is a list of publicatio
notify us of your publicatio

Khan AS, Murray MJ, Ho C
GlaxoSmithKline Protein
Replication that Prevents
ahead of print:doi:10.1099

Beelontally R, Wilkie GS, L
Strang BL. **Identification**
of IE2 proteins. Antiviral F

Balasubramanian A, Manz
the identification of smal

Zervantonakis IK, Arvanitis
acoustofluidic 3D tumor

RNAi Publications

http://iccb.med.harvard.edu/rnai-publications

Below is a list of publications that have resulted from RNAi screening efforts at ICCB-Longwood. Please notify us of your publications that involve use of the ICCB-Longwood Screening Facility.

Nelms B, Dalomba NF, Lencer W. **A targeted RNAi screen identifies factors affecting diverse stages of receptor-mediated transcytosis.** J Cell Biol 2017;

Simpson KJ, Smith JA. **Knocking down the obstacles to functional genomics data sharing.** Sci Data 2017;4:170019 doi:10.1038/sdata.2017.19.

Dutta B, Azhir A, Merino L-H, Guo Y, Revanur S, Madhamshettiwar PB, Germain RN, Smith JA, Simpson KJ, Martin SE, Beuhler E, Fraser LDC. **An interactive web-based application for Comprehensive Analysis of RNAi-screen Data**. Nat Commun 2016;7:10578.

Polachek WS, Moshrif HF, Franti M, Coen DM, Streenu VB, Strang BL. **High-Throughput Small Interfering RNA Screening Identifies Phosphatidylinositol 3-Kinase Class II Alpha as Important for Production of Human Cytomegalovirus Virions.** J Virol 2016;90(18):8360-71.

Savidis G, McDougall WM, Meraner P, Perreira JM, Portmann JM, Trinucci G, John SP, Aker AM, Renzette N, Robbins DR, Guo Z, Green S, Kowalik TF, Brass AL. **Identification of Zika Virus and Dengue Virus Dependency Factors using Functional Genomics**. Cell Reports 2016;16(1):232-46.

Fluorescence Polarization (FP) Assays: protein-protein interactions

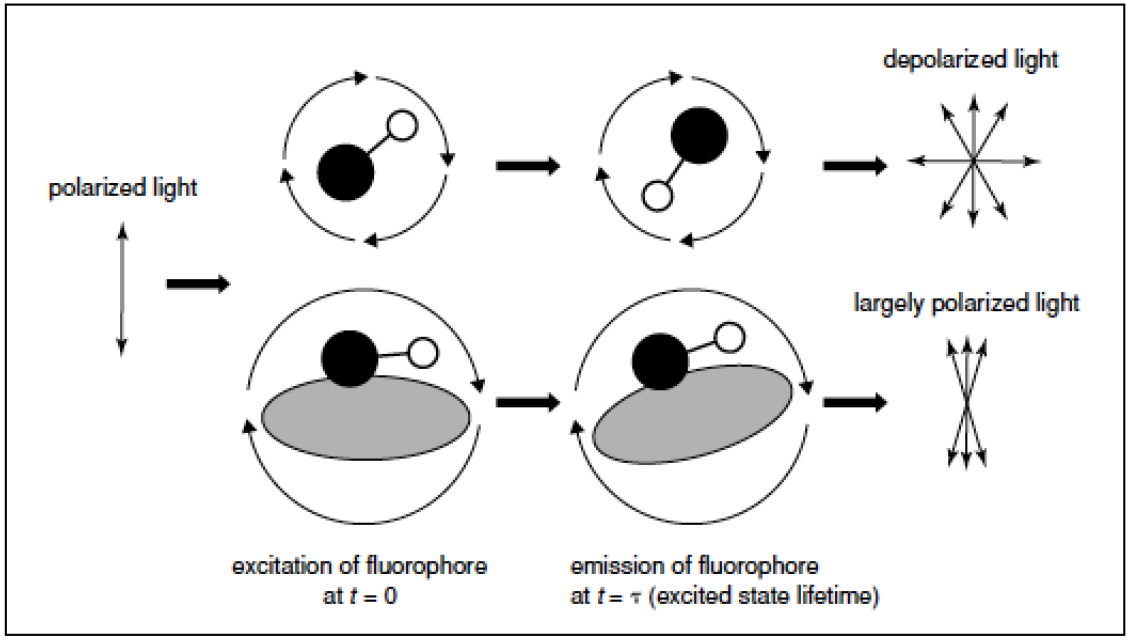


Figure 1 Schematic depicting the basic principle of fluorescence polarization. When a small peptide or nucleic acid ligand (dark circle) with a fluorescent label attached (white circle) is excited by polarized light at the excitation wavelength of the fluorophore, the ligand reorients to a significant degree due to molecular tumbling during the excited state lifetime of the fluorophore. This causes the emitted light to be largely depolarized. If the ligand is bound to a protein (gray ellipse), the resulting complex tumbles much slower, and the emitted light retains its polarization.

Moerke (2009) Current Protocols in Chem Biol *1*:1

Example ICCB-L FP screens:

Frey et al. (2006) PNAS *103*:13938– HIV gp41 conformational change

Moerke at al. (2007) Cell *128*:257– eIF4E/eIF4G interactions

Compound 4EGI-1 is a competitive inhibitor of the eIF4E/eIF4G interaction

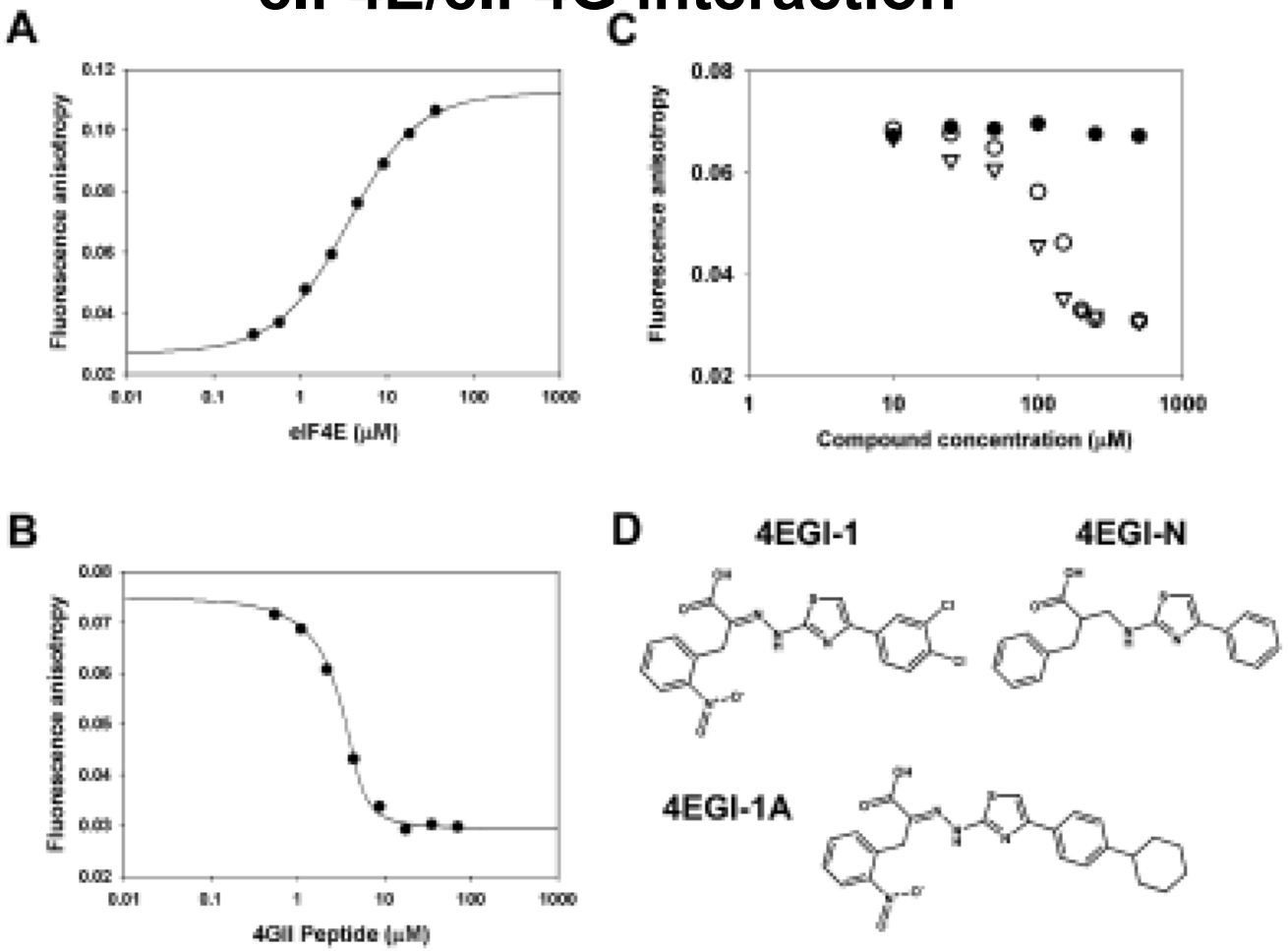


Figure 1. A FP Assay Identifies the Compound 4EGI-1 as a Competitive Inhibitor of the eIF4E/eIF4G Interaction

4EGI-1 Disrupts eIF4F Complex Formation and Inhibits Cap-Dependent Translation

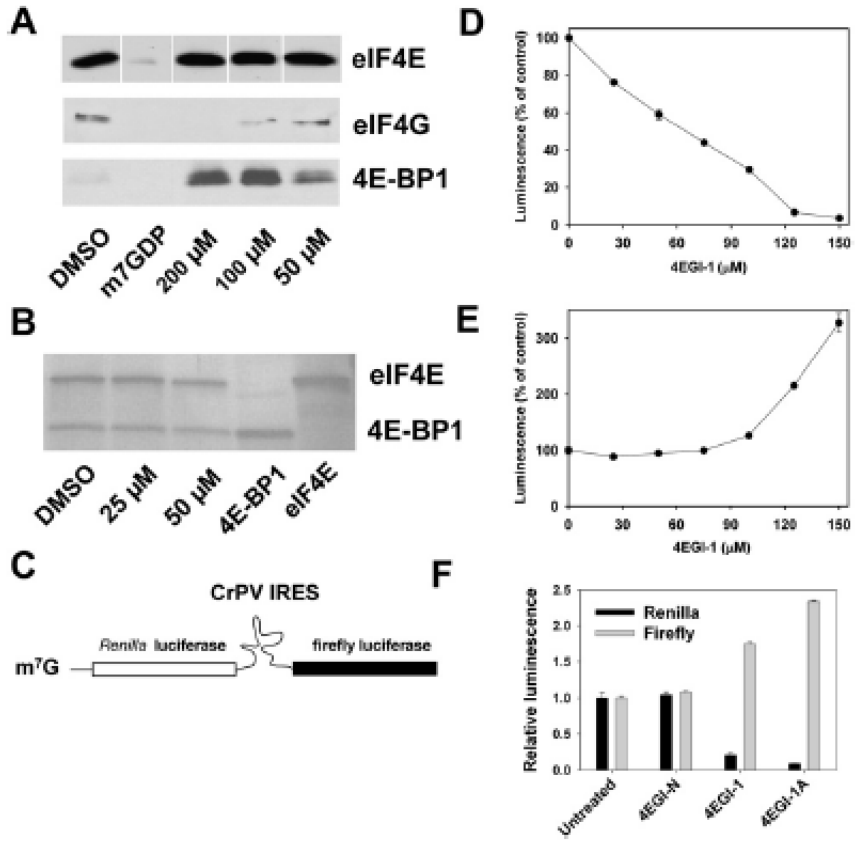


Figure 3. 4EGI-1 Disrupts eIF4F Complex Formation and Inhibits Cap-Dependent Translation

-retic lysate binding assay

-purified protein binding

-cap-dependent translation assay

Moerke at al. (2007) Cell *128*:257

4EGI-1 Disrupts the eIF4F Complex and Inhibits Expression of Oncogenic Proteins in Mammalian Cells

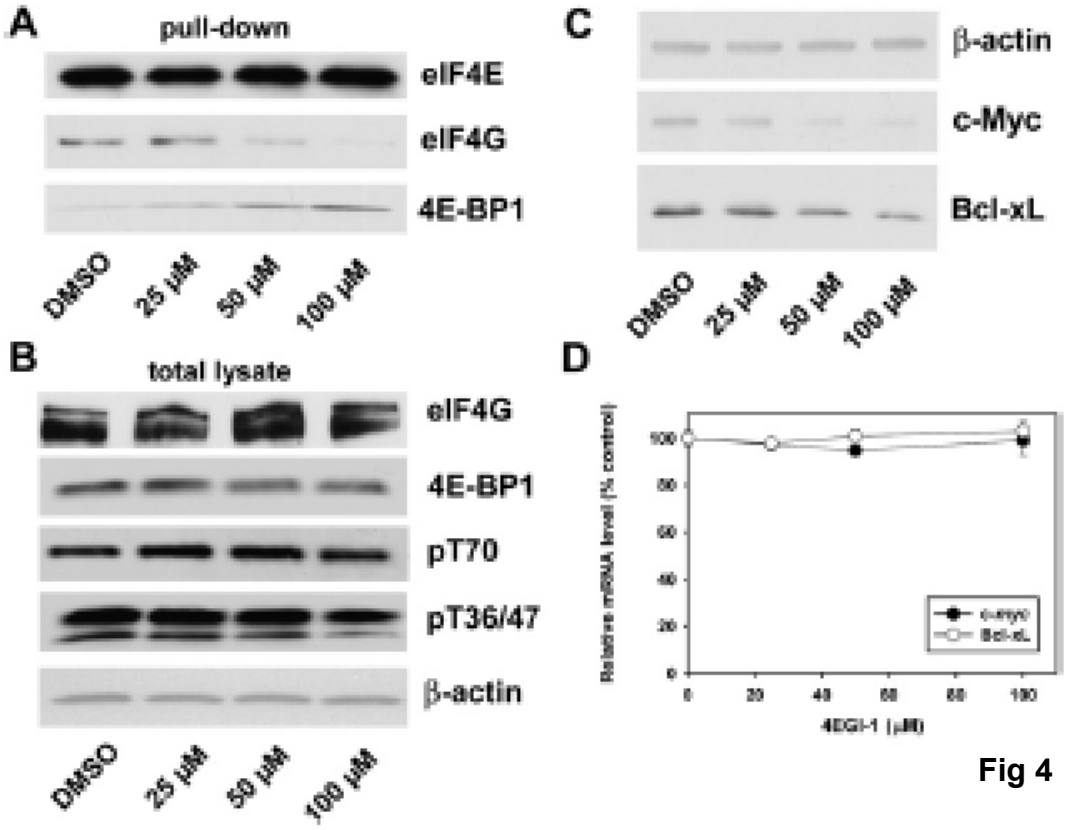


Fig 4

Moerke at al. (2007) Cell *128*:257

4EGI-1 Has Proapoptotic Activity and Inhibits the Growth of Multiple Cancer Cell Lines

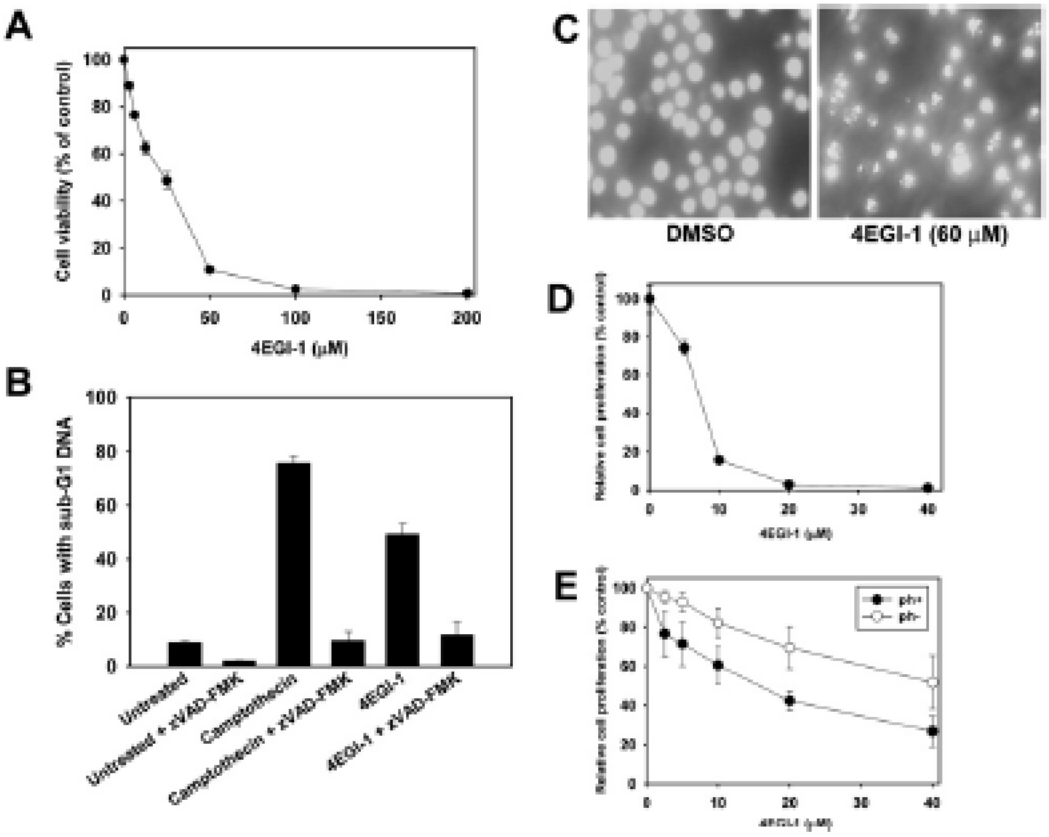


Figure 5. 4EGI-1 Has Proapoptotic Activity and Inhibits the Growth of Multiple Cancer Cell Lines

Moerke at al. (2007) Cell *128*:257

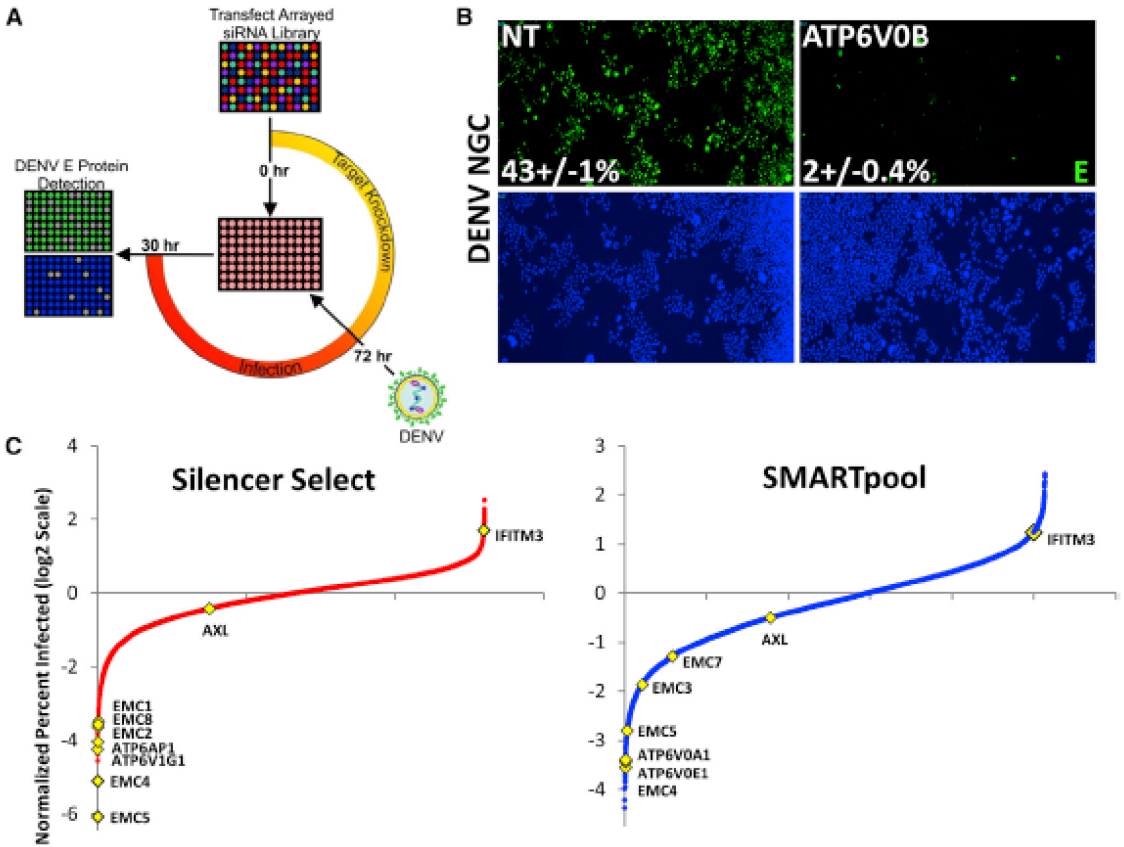
Functional genomics example - Abraham Brass

Identification of Zika Virus and Dengue Virus Dependency Factors using Functional Genomics

Cell Reports *16*, 232–246, June 28, 2016

George Savidis,[1,5] William M. McDougall,[1,5] Paul Meraner,[1,5] Jill M. Perreira,[1] Jocelyn M. Portmann,[1] Gaia Trincucci,[1] Sinu P. John,[2] Aaron M. Aker,[1] Nicholas Renzette,[1] Douglas R. Robbins,[1] Zhiru Guo,[3] Sharone Green,[3] Timothy F. Kowalik,[1] and Abraham L. Brass[1,4,*]

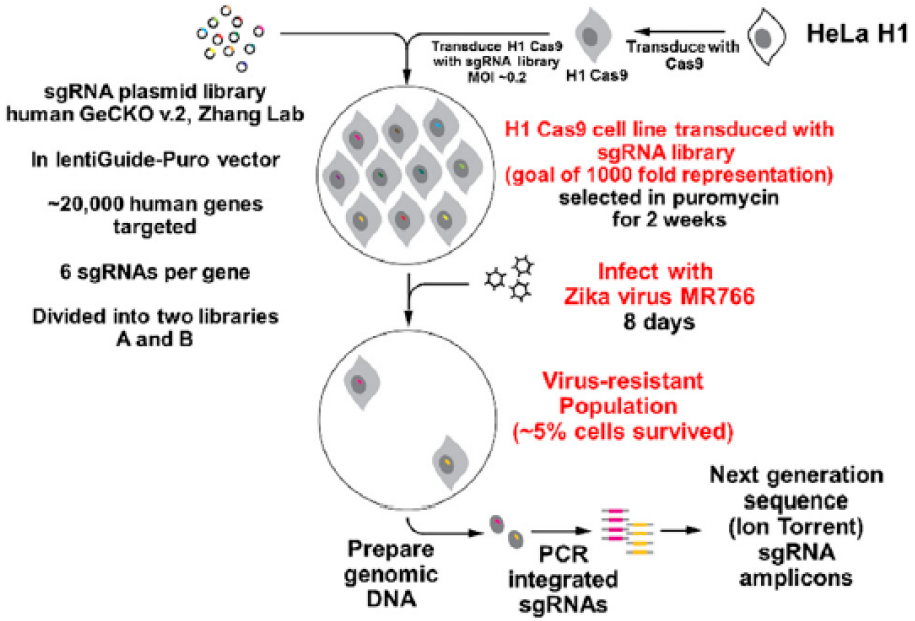
MORR – *M*ultiple *O*rthologous *R*NAi *R*eagent – Dharmacon, Ambion

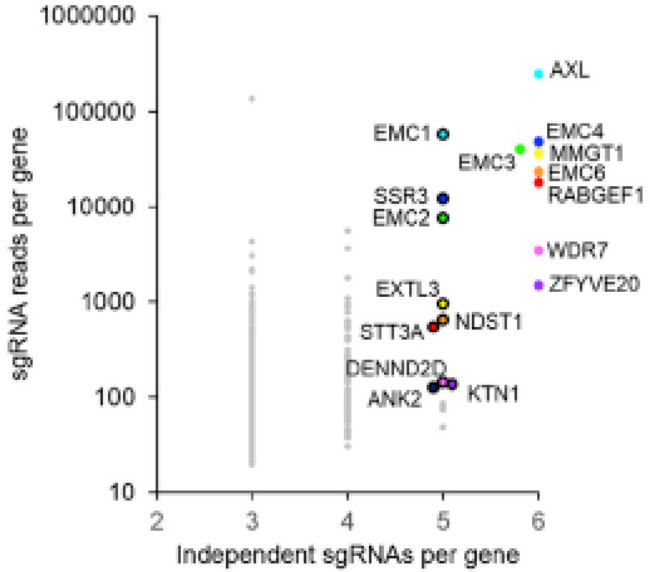


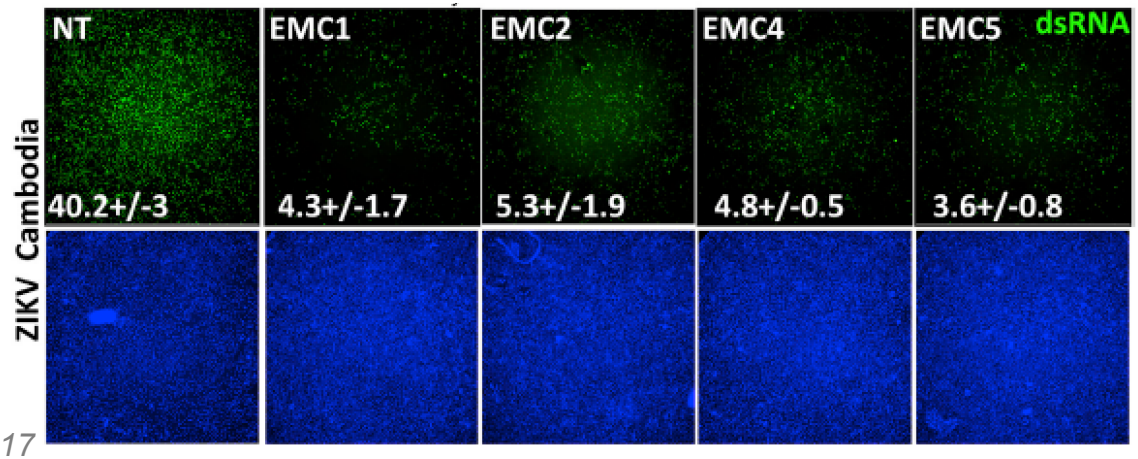
Functional genomics example - Abraham Brass

Pooled CRISPR/Cas9 survival enrichment screen

Cell Reports *16*, 232–246, June 28, 2016







Functional genomics example - Abraham Brass