General workflow for small molecule screening at ICCB-Longwood

Complete online small molecule screen application

http://iccb.med.harvard.edu/application-forms-and-fees/small-molecule-screening/

Assistant Director or Director will contact you for initial meeting

Jennifer Smith, Assistant Director, Jennifer_Smith@hms.harvard.edu
Caroline Shamu, Director, Caroline_Shamu@hms.harvard.edu

Discuss: specifics and goals of your screen
general screening process at ICCB-Longwood
steps to move forward

Sign and return small molecule user agreement

PDF on ICCB-L website (RNAi screening application page, Screening Documents page)
http://iccb.med.harvard.edu/faq/screening-documents/

Receive equipment training

This needs to occur prior to initiating work at ICCB-Longwood, but initial assay development frequently occurs outside ICCB-Longwood

Complete online request on ICCB-L website
http://iccb.med.harvard.edu/for-current-screeners/equipment-training-request/

Complete billing form

PDF on ICCB-L website (Screening Documents page, http://iccb.med.harvard.edu/faq/screening-documents/)

If screen readout is image-based, ICCB-L will set up meeting with Image Analyst

Tiao Xie, tiao_xie@hms.harvard.edu
Image and Data Analysis Core (IDAC) http://idac.hms.harvard.edu/

Assay Development and Optimization

This is an iterative process. Assay development and optimization may take as little as 2 weeks or as much as 2 years. Screeners generally start by developing an assay suitable for small molecule screening in their own lab and then work to optimize the assay using automated equipment.

Refer to Helpful Publications and Links on the ICCB-Longwood website
http://iccb.med.harvard.edu/screening-information/helpful-publications-links/
See Overview and Guidelines for essential information regarding ICCB-Longwood policies and procedures
http://iccb.med.harvard.edu/screening-information/overview-and-guidelines/

Important parameters to optimize:

- Plate type
  - Dependent on assay readout and reagent or cell type
- Cell-based assays
  - # cells/well
    - Determine robust way to consistently count cells during screen
  - Cell passage #: can impact transfection efficiency and phenotype
  - Tolerance to DMSO?
    - Typical % DMSO in assay is 0.3% (100 nl pin transfer into 30 ul total volume)
    - Recommend to test assay in up to 1% DMSO
- Reagent volume or concentration/well
  - In 384-well plate, typical assay volumes are 10 ul (low volume, biochemical assays) or 30 ul (cell-based assays)
  - Determine conditions that provide most robust and consistent phenotype
  - Batch-to-batch variability in reagents, proteins, bacteria, etc. may exist
    - Consider 1 batch (stock) for entire screen
  - Pin transfer volumes are 33 nl, 100 nl or 300 nl, with 100 nl standard transfer volume
- Choose positive and negative controls
  - Negative control typically DMSO, as ICCB-L compound libraries are dissolved in DMSO
  - Beneficial to have positive controls of different strengths – strong, medium
  - If small molecule positive control is not available, consider other options
- Temperature
  - Pin transfer occurs at ambient temperature
- Time of readout
  - Biochemical assay readouts may be as short as 30 min post pin-transfer
  - Cell-based assays typically range from 16 h – 72 h post pin-transfer
- Practice with instruments at ICCB-Longwood
- Automate protocol
  - Reagent volumes and concentrations
  - Timing (this may change as the assay moves from 1 or 2 library plates to 10+ library plates per pin transfer).
    - Allow $\geq$ 30 minutes between each step in assay
  - Addition of assay-specific controls
- Assay readout – can optimize this step independent of compound pin transfer

Success in assay optimization generally monitored by assessing assay robustness factors, including the Z' factor. See below for more details.

Please contact ICCB-Longwood staff at any point with questions or issues you would like to discuss.

Jen Smith, Assistant Director, Jennifer_Smith@hms.harvard.edu
Caroline Shamu, Director, Caroline_Shamu@hms.harvard.edu

Z' factor determination

http://iccb.med.harvard.edu/screening-information/overview-and-guidelines/#quantitative
Perform this test using automation protocol developed for your assay
Options are:
1/2 plate positive control and 1/2 plate negative control
1/3 plate each of 2 different strength positive controls, 1/3 plate negative control (***preferred***)
Same # of replicates as will be utilized in screen
Any sign of edge effect? Trending?
Z' factor acceptable? Ideally ≥ 0.5

Yes

Set up appointment with data team to discuss data deposition and templates
Jennifer_Nale@hms.harvard.edu
David_Wrobel@hms.harvard.edu

No

Consult Jen Smith
Jennifer_Smith@hms.harvard.edu
Reoptimize assay (page 2)

Pilot screen

2 library plates representative of what screen will include:
Known bioactive plates if cell-based
1 plate from commercial library or natural product extracts (if planning to screen these collections)

Image analysis (if necessary)

Send raw numerical data to Data Curator

Jennifer Nale, Jennifer_Nale@hms.harvard.edu

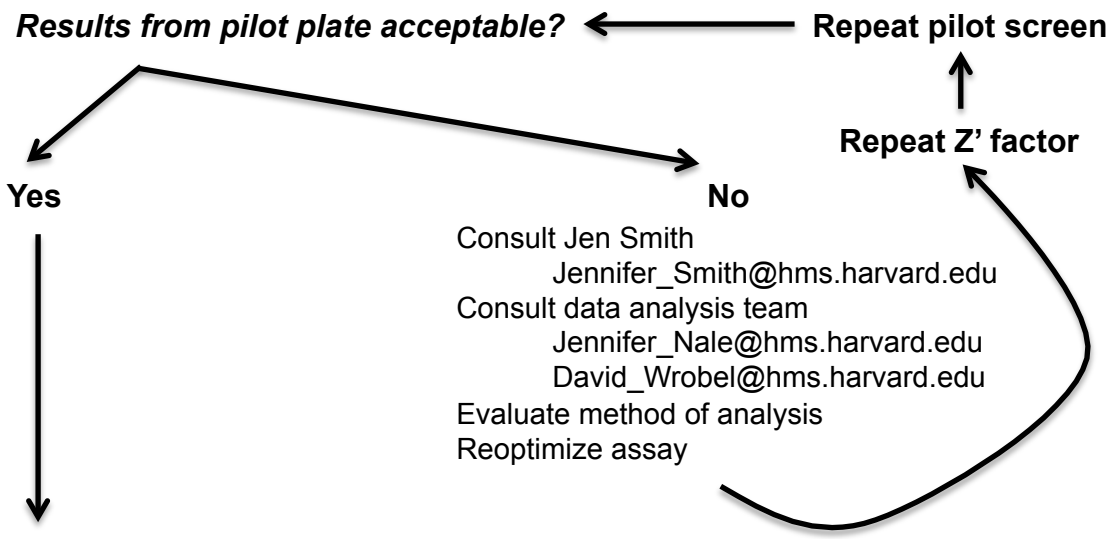
Jen will: format data into standard ICCB-Longwood format;
generate graphs to visualize quality control parameters (utilizing Dotmatics Vortex software);
calculate Z' factor based upon assay-specific positive and negative controls; and
email formatted data and graphs back to screener

Review and analyze data

See below for more information about data analysis

Analyze data
Acceptable Z' factor?
Potential hits?
Do the data make biological sense if known bioactives showed potential hits?

Examine graphs from Jen Nale
Good correlation of replicates?
Clear separation of positive and negative controls?
Any visible patterns or edge effects?
Are there trends from 1st plate to last plate in a given batch?



To ensure quality and consistency of data, use the same assay protocol throughout your entire screen!

Primary screen

Send data to Jen Nale regularly as each screening session is complete
Pay attention to Z' factor and positive/negative controls on each assay plate
Catch any problems or changes in screen robustness as soon as possible!
Analyze data on a regular basis
Cherry picks can be performed at any point throughout primary screen
A cherry pick after 10,000 to 50,000 compounds can be helpful

Analyze screen data: quality control and identifying potential screening positives

Helpful to categorize potential hits as strong, medium, weak

Potential methods of data analysis:

1. Z-score – frequently used and is an easily applied method for plate normalization. The raw data distribution of sample values is converted to a standard normal distribution with a mean of 0 and standard deviation of 1. Each z-score for a well replicate represents the number of standard deviations above or below the plate mean of the experimental wells

$z = (x - \mu)/\sigma$

x is the raw data value of the well to be standardized
μ is the mean of the experimental well population of plate
σ is the standard deviation of the experimental well population of plate

2. Percent of control (or sample) – divide each well value by the plate mean of either negative controls or positive controls, or by the plate average of samples (experimental wells). The plate median for experimental wells is typically used, in which case the average result value for all sample wells will be ~1.0 (unless the raw data distribution is very skewed). Calculating percent of control or plate sample average does not account for plate variation to the same extent as the z-score. This type of analysis should only be performed when there is an adequate number of controls and a small coefficient of variation (ideal < 10%).

% Neg Control = x / Avg neg

% Exp Control = x / Avg sample

Analyze screen data (continued)

3. Normalized Percent Inhibition/Activation – can be used for plate normalization, but requires good performance from both positive and negative controls. The ideal positive control is a small molecule that produces a response that mimics the desired effect. The use of controls for calculating a numeric result should only be used if the Z' factor for each plate is $\geq$ 0.7, as it will be difficult to distinguish true positives from the background if there is an inadequate signal window based on poorly performing controls.

NPI =(Avg neg - x) / (Avg neg - Avg pos) x 100

Submit analyzed data from primary screen in ICCB-L format and primary screen report

Email to David Wrobel (David_Wrobel@hms.harvard.edu)

Cherry pick screen

Submit cherry pick request to David Wrobel (David_Wrobel@hms.harvard.edu)

A cherry pick of 0.3% of the number of compounds screened is included as part of the screen

Provided with 1 ul of each compound
Cherry pick experiments may be performed at ICCB-Longwood or in your own laboratory

Suggested to perform same assay as primary screen to identify compounds that reconfirm
Typical for ~50% of compounds to reconfirm phenotype of primary screen

Screeners may elect to dilute cherry pick with DMSO, growth medium or buffer to enable compounds to be tested in additional assays

Options for screening cherry picks at ICCB-Longwood:

1. HP D300
   Wide range of possibilities to perform dose-response curves
   ~300 nL used to prime dispense head, ~700 nL available for experiment
2. Pocket tip transfer
   50 nl, 100 nl or 250 nl transfer possible
   Limited retest from cherry pick
   (2 x 250 nl or 5 x 100 nl or 6 x 50 nl)
   If interested in dose-reponse and unable to utilize HPD300, recommended to dilute cherry pick samples
3. Dilute cherry picks with media and transfer
   Transfer can be via Vprep or by hand
   Once diluted, use all of the cherry pick samples immediately to ensure compound integrity

Analyze results of cherry pick
Negative controls important as many compounds tested will score as hits at this stage

Prioritize hits based on re-test results and bioinformatics analysis

Follow-up experiments and analysis

After hits from the primary screen are preliminarily validated via the cherry pick, additional experiments are required for hit prioritization and, for cell-based screens, target ID. This section describes a few strategies that can be undertaken.

Secondary assays may be high or low throughput.

Orthogonal assays can help determine specificity of hits or assist with target ID (if required).

Does another batch of the same compound produce the same phenotype? Compounds should be reordered from the same vendor or a new supplier prior to drawing firm conclusions about compound activity.

Compound validation using HPLC, LC/MS and/or NMR (refer to analytical chemistry support below) – confirm structure of active compound. Keep in mind that compound intermediates and/or degradation products can also produce phenotypes.

Dose response curves (suggested initial range from 100 nM to 100 uM, however may want to assay as low as 1 nM)

Structure-activity relationship (SAR) studies utilizing commercially available analogs and (almost inevitably) custom-synthesized analogs

Cell- or animal-based studies

Note: primary screening hits have some likelihood of becoming validated research probes, but an extremely low probability of becoming therapeutic leads in the absence of a strong medicinal chemistry effort. An exception might be hits identified in a drug-repurposing screen that only assays known drugs.

Chemistry Resources

Source for assistance with commercial SAR, identifying vendor sources of compounds, and ordering compounds:

eMolecules
http://academics.emolecules.com/

Local medicinal chemistry core:

The Medicinal Chemistry Core, Dana-Farber Cancer Institute
http://medchemcore.dfci.harvard.edu/

Local analytical chemistry support:

The Small Molecule Mass Spectrometry Facility, Harvard FAS Division of Science
http://massspec.fas.harvard.edu/

Please contact Jennifer Smith (Jennifer_Smith@hms.harvard.edu) or Caroline Shamu (Caroline_Shamu@hms.harvard.edu) if interested in depositing the data from your screen into a public repository (*e.g.* PubChem BioAssay)