Follow-up of High Throughput Screening (HTS) Assays:

A Recommended Process for Collaborating with the Center for Chemical Genomics (CCG) and the Vahlteich Medicinal Chemistry Core (VMCC) in the Identification of Promising Leads

November 2010
Summary

Upon completion of an HTS assay, the principal investigator is left with a daunting task: how to begin with raw primary screening data on thousands of compounds and finish with the identification of a small set of promising leads for further development into probes or potential therapeutics. This process takes place on a regular basis in the pharmaceutical industry, but remains largely unfamiliar to most scientists in academia. The CCG and the VMCC, both of which include staff with extensive experience in the pharmaceutical industry, are available to assist academic researchers in navigating through this complex and challenging process. This brochure includes a concise summary of recommended key steps in the follow-up of an HTS assay, and clearly identifies where the CCG and the VMCC can be of assistance. A primary objective in providing this summary is to help scientists appreciate how many steps are involved in this process, and to understand what is being accomplished at each step, information that should be of great assistance in planning project budgets and timelines. There may be questions about intellectual property (IP): inventions, patents and disclosures. We have some links and general guidelines for those interested in contacting UM Tech Transfer Office (OTT).

Questions may be directed at any time to the individuals below:

CCG: http://lsi.umich.edu/ccg
David Sherman, Chemical Diversity Director (davidhs@umich.edu, 734-615-9907)
Rick Neubig, Cheminformatics Director (rneubig@umich.edu, 734-764-8165)
Martha Larsen, HTS Director (mjlarsen@umich.edu, 734-763-2340)

VMCC: http://pharmacy.umich.edu/mccsl/home
Hollis Showalter, Director (showalh@umich.edu, 734-764-5504)
Scott Larsen, Co-Director (sdlarsen@umich.edu, 734-323-1187)
Paul Kirchhoff, Informatics (pkircho@umich.edu, 734-277-2957)

OTT: http://www.techtransfer.umich.edu
Matt Bell, Technology Licensing specialist (msbell@umich.edu, 734-647-4738)
Recommended Steps

1) Run primary HTS assay (CCG)
2) Primary Triage Meeting (Paul Kirchhoff)
   a) Establish activity criteria
   b) Select compounds (“actives”) for follow-up
3) Re-test “actives” in primary assay (CCG)
4) Confirmation Triage Meeting (Paul Kirchhoff)
   a) Filter out undesirable compounds
   b) Select compounds (“confirmed actives”) for follow-up
5) Run dose response of “confirmed actives” in primary assay (CCG)
   a) Fit dose response curves
   b) Assign pAC50s
6) Dose Response Triage Meeting (Paul Kirchhoff/VMCC)
   a) Cluster analysis
   b) Med chem. Inspection
   c) Prioritize “confirmed actives” for follow-up
7) Re-mine CCG collection for structurally similar compounds for retesting in dose response (Paul Kirchhoff/CCG)
8) Establish confidence in structural identity/purity/activity of “confirmed actives”
   a) Select compounds for purchase (VMCC)
      i) If not available, consider re-synthesis (VMCC)
   b) Confirm primary assay activity with fresh powders
      i) Discuss solution prep strategies with CCG to reduce errors from solubility issues
      ii) many compounds have < 50µM solubility
      iii) reserve some powder for analytical purposes, eg. use d6-DMSO for sample prep
      iv) there are several strategies for solubility for difficult compounds, please use CCG resources for assistance
   c) Obtain stock samples from the CCG for several of the active compounds
      i) Run these as controls for the new fresh powder solutions
      ii) Obtain samples for analytical purposes
   d) Prove identity/purity by HPLC/NMR/MS (PI lab or VMCC)
9) Run “structurally confirmed actives” in secondary or functional assays
   a) Eliminate artifact-based actives
   b) Establish relative potency/efficacy
   c) Identify “hits” for continued follow-up (VMCC)
10) Select commercial analogs of “hits” for purchase (VMCC)
    a) Establish initial structure-activity relationships (SAR) or lack thereof in primary/secondary assays
11) Identify “hit” series for development (VMCC)
    a) Criteria:
       i. Activity/selectivity/toxicity
       ii. SAR
       iii. Ease of synthesis
       iv. Patentability
12) Options for further “hit” development in collaboration with VMCC
    a) Grant application assistance (co-PI or letter of support)
    b) Scale up of selected “hits” for additional studies
       i) Time/cost estimates provided
    c) Med chem design and synthetic analoging
Notes

1. The activity cutoff will typically be based on a percent inhibition (or activation) or the number of standard deviations from control or a combination of both.

2. “Actives” may be single compounds (CCG-Nos) or natural product extract mixtures (SID-Nos). Follow-up of CCG-Nos and SID-Nos is essentially the same through Step 5, after which additional steps entailing separation and identification of the active component(s) of SID-Nos must be undertaken. Contact David Sherman, Chemical Diversity Director (davidhs@umich.edu, 734-615-9907) for assistance with follow-up of SIDs.

3. Compounds are typically eliminated at this stage due to the presence of structural features expected to impart toxicity, chemical reactivity or extremely poor pharmacokinetic properties.

4. Clustering involves computationally grouping molecules with similar structures. It is used to determine if a range of activity is present within a class (ideal), if only a single member of a class is active (sometimes a sign of an invalid active), or if all of the members have similar activity (a possible sign of false activity due to physicochemical interference with the assay).

5. Med chem inspection refers to an assessment of the likelihood that a compound is a valid active and amenable to development. Primary considerations at this stage are the presence (or absence) of potentially reactive functionality or perceived physicochemical properties (such as detergency) that might have interfered with the assay.

6. The cost of purchasing 1 mg of fresh library samples is typically $25-$50/compound; note that multiple samples for the same vendor results in large cost savings and assists in comparative samples retesting. Please consider ordering larger samples (>1mg) for reserve analytical and QC as well as secondary and tertiary testing; different lots from the same vendor will require re-confirmation. Vendors and compound numbers are available in MScreen (http://ccg.lsi.umich.edu/) or through the VMCC.

7. Activity in a secondary or functional assay eliminates the possibility that the primary assay activity was due to an off-target interaction (e.g. inhibition of a reporter enzyme).

Definitions of Compound Designations*

1. “Unknown” (designated by a “?”) is a compound that has been tested but the results are inconclusive: assay activity threshold not set, plate rejected.

2. “Inactive” (designated by a red X) is a compound that has been tested and is below the activity threshold set by PI. This is the designation accepted by IUPAC† and PubChem.

3. “Active” (designated by a green check mark) is a compound that has been tested (n=1) and meets the threshold set by PI. This is the designation accepted by IUPAC† and PubChem.

4. “Inactive” (designated by red X and green checkmark) is a compound that was active in the primary HTS, but not confirmed in a subsequent repeat primary assay.

5. “Confirmed active” (designated by two green checkmarks) is a compound that has been tested and confirmed (n>1). For many compounds, these will have pAC50s determined (note: pIC50 and pEC50 will now be referred to as pAC50 and the Hill slope will designate inhibitory or excitatory).

6. “Structurally confirmed active” (designated with three green checkmarks) is a compound that has been “confirmed active” and is structurally identified (a new lot of compound with analytical data available).

7. “Confirmed active/Unknown structure” (designated by red X and two green checkmarks) is a compound that has been “confirmed active” but is not structurally correct (a new lot of compound does not confirm activity).

8. “Hit” (designated by gold crown) is a compound that is a structurally confirmed active and has been determined not to be an artifact of the assay (usually by secondary assay activity). This is the designation accepted by IUPAC† and PubChem.
*Compound designations for individual assays are indicated in the MScreen database (http://ccg.lsi.umich.edu/) under “HTS Results Information”.

†SBS/IUPAC Definitions can be found at http://www.sbsonline.com/links/terms.php

**Estimated Attrition Rates from Screen to Hit**

**Screening Collection**

- 150,000 samples
- 0.1 – 2% “actives” 1500
- ~30% repeat 500
- ~50% titrate 250
- ~50% available 125
- ~50% titrate 60 –70
- 10-40% “hits” 5 – 30

**Primary Screen**

- Repeat Primary Screen
- Dose Response

**Obtain New Sample**

- Repeat Dose Response

**Secondary, Selectivity Screens**

Overall: Only ~2% of “actives” will become “hits”

Increasing Confidence

Same conditions, collection sample

Different conditions