Screening Assay Development: How to avoid pitfalls

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General Flow of Assay Development

1. Establish an assay in a research lab setting
2. Identify potential assay formats compatible with high-throughput screening (HTS)
3. Develop assay protocols and reagents
4. Adopt screening assay to automation and scale up
5. Statistically validate assay performance
6. Optimize steps 3–5.
7. Develop secondary assays to validate hits obtained from HTS.

What to know before coming to a HTS facility

- ❑ Know your choice of target for screen: disease relevance; chemical tractability; screenability.
- ❑ Have a good assay as a basis for developing an HTS assay.
- ❑ Know your data analysis strategies, hit selection tools and follow up assays.

Characteristics of good HTS assays

- ❑ High sensitivity
- ❑ Low variability (reproducibility) -- well to well; plate to plate; day to day
- ❑ High signal to background ratio (Z')
- ❑ Large dynamic range
- ❑ Simple steps (liquid-handling compatibility)
- ❑ DMSO Tolerance
- ❑ Positive controls

Web Resources for Assay Development

- ❑ Assay Guidance Manual from the National Center for Advancing Translational Sciences
  http://www.ncbi.nlm.nih.gov/pubmed/22553861
- ❑ General Enzyme Kinetics
  http://themedicalbiochemistrypage.org/enzyme-kinetics.php
  http://www.ultranet.com/~jkimball/BiologyPages/E/EnzymeKinetics.html
- ❑ A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays
  http://jbx.sagepub.com/content/4/2/67 (doi: 10.1177/108705719900400206)
- ❑ Other types of biochemical assays
  Thermal shift assays – for enzyme with no viable detection methods for assay development
  http://thermofluor.org/resources/PTI-FluorescenceBasedThermalShiftAssay.pdf
  Alpha-screen for Protein-Protein Interaction Assays
  http://www.perkinelmer.com/Catalog/Category/ID/AlphaScreen+Assays+and+Reagents
- ❑ Explanation of High Content Screening
  http://www.cellomics.com/home/three-worlds-buttons/what-is-high-content.html
- ❑ RNAi global initiative
  http://www.rnaiglobal.org/Home/