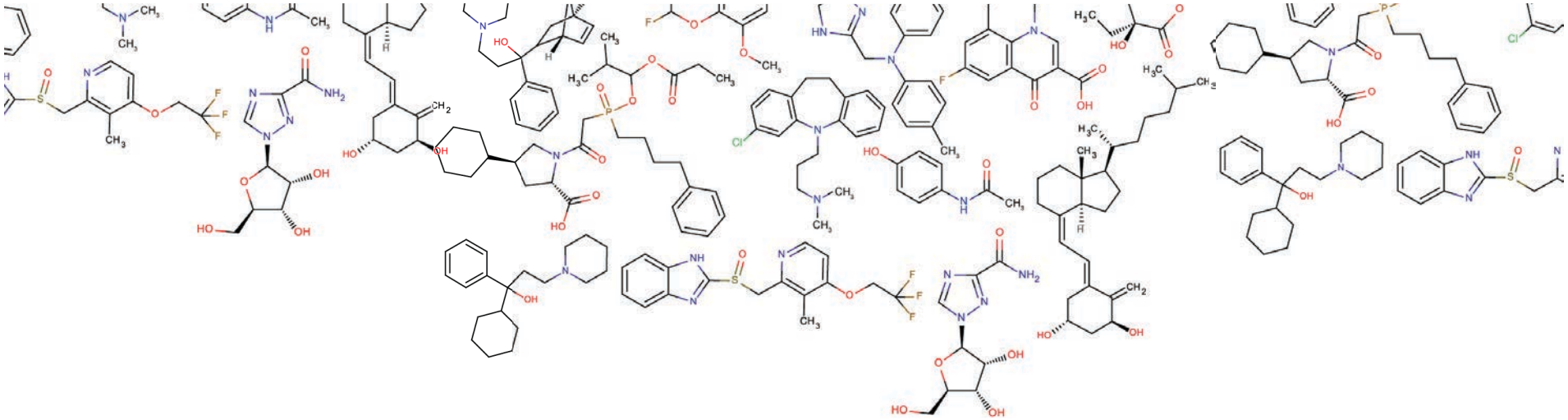

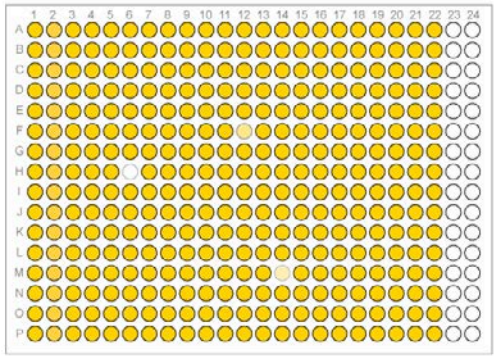

High-throughput screening

a short introduction

a process that leverages *automation* to rapidly *assay* the biological or biochemical activity of a large collection of drug-like *compounds*

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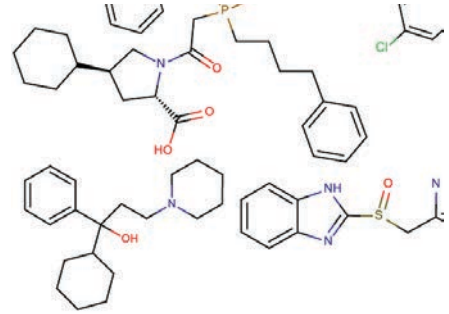
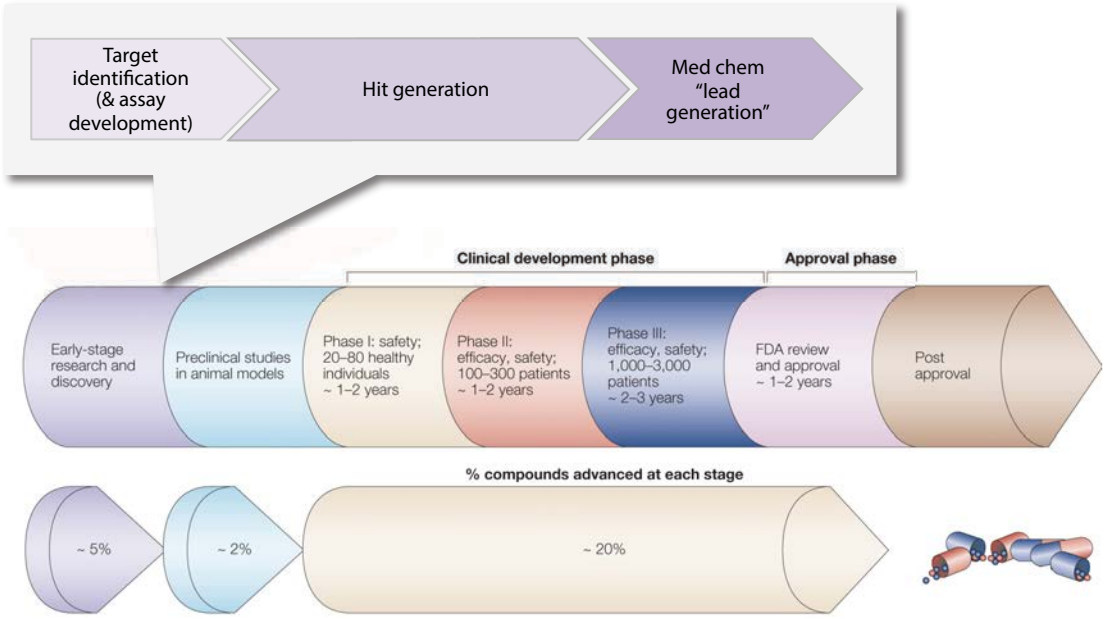
HTS & drug discovery



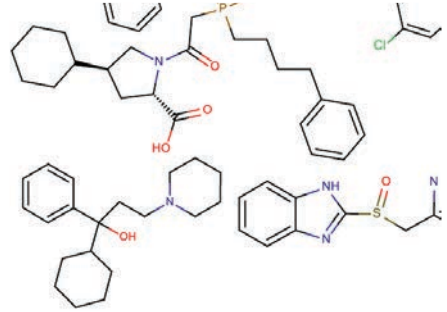


1. **Assay optimization (pre-screen)**
   - Plate format
   - Reagent stability, DMSO tolerance of the assay, order of addition and concentration of reagents, incubation times, etc.
   - Assay signal detection
   - Positive and negative controls
   - Pre-screen for hit rate
2. **High-throughput screening**
   - Full compound library or subset
3. **Hit validation (post-screen)**
   - Hit selection criteria
   - Reproduce hits
   - Measure potency in primary assay
   - Remove false positives in counterscreen (orthogonal assay)
   - Confirm activity in secondary assays

Adapted from: C. O'Driscoll. *A Virtual Space Odyssey*. Charting Chemical Space. Nature.com 2004

assay development:

Assay format & signal detection



Protein-based **biochemical assays** (cell-free)

- monitor enzyme activity (endpoint or kinetic assays)
- protein-protein, protein-ligand interactions
- higher throughput, smaller volumes
- + : known target
- - : not guaranteed to be biologically relevant

Cell-based assays

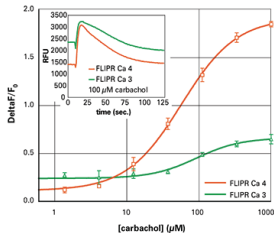
- Known target or phenotype-based (molecular mechanism unknown)
- Formats:
  - Uniform well readout: cell viability and reporter gene assays
  - High-content: imaging of cells and measurement of multiple signals

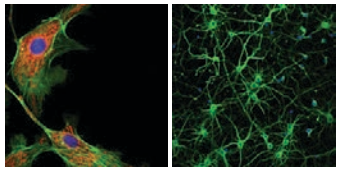
qPCR (differential scanning fluorimetry)

Absorbance
Fluorescence intensity
Fluorescence polarization
FRET, TR-FRET, HTRF
AlphaScreen
Luminescence

FLIPR (Ca^{2+} flux)

High-content imaging





recommended resource:
NIH Assay Guidance Manual
http://www.ncbi.nlm.nih.gov/books/NBK53196/

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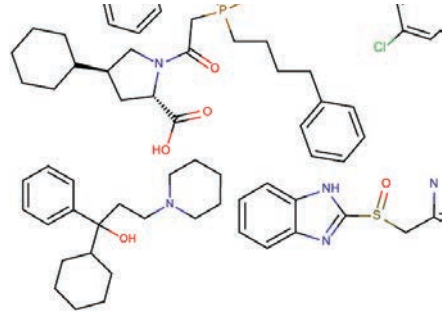
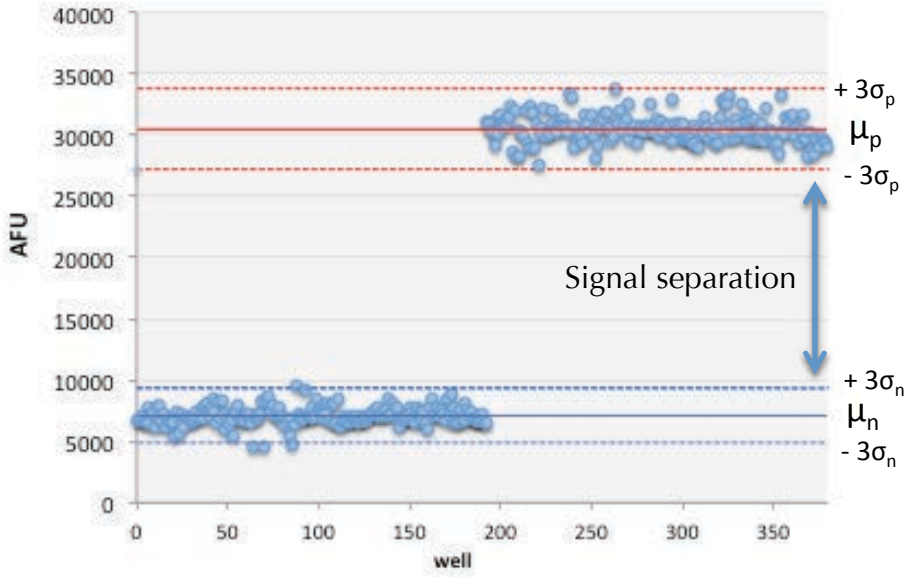
assay development:

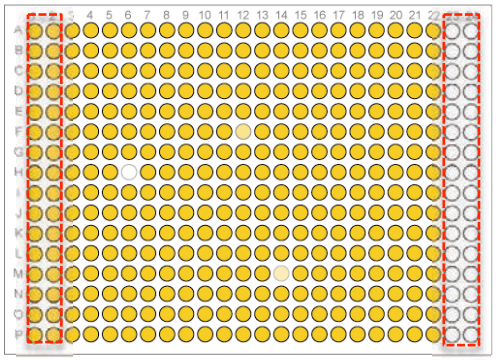
Assay quality: *Z'-factor*



$$Z' = 1 - \frac{3\sigma_p + 3\sigma_n}{|\mu_p - \mu_n|}$$

$Z' = 1.0$:	perfect assay
$0.5 < Z' < 1.0$:	excellent assay
$0 < Z' < 0.5$:	marginal assay
$Z' < 0$:	unsuitable assay





Positive and negative controls included on every HTS assay plate

Zhang JH, Chung TDY, Oldenburg KR (1999). "A simple statistical parameter for use in evaluation and validation of high throughput screening assays". *Journal of Biomolecular Screening* **4**: 67–73.

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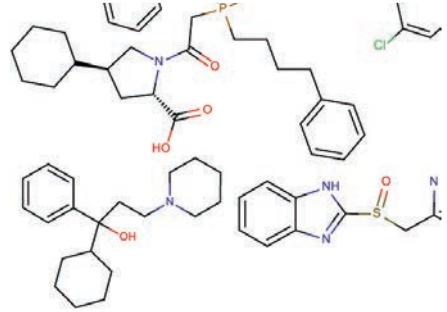
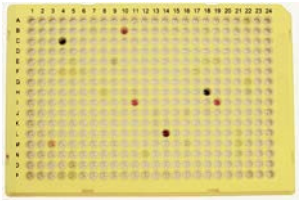
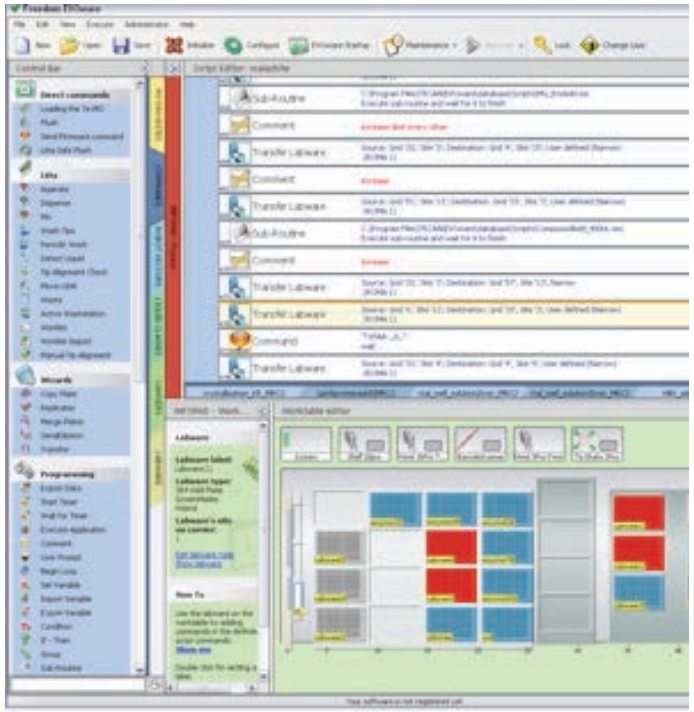
primary screen:

Robots, readers, and compounds



- Assemble assay with liquid-handling robots:
  - Dispense/mix reagents, plate cells, add compound, shake, incubate, wash, etc.
  - Track plates with barcodes and time addition of components and incubation times
- Measure the signal output, compile and analyze data





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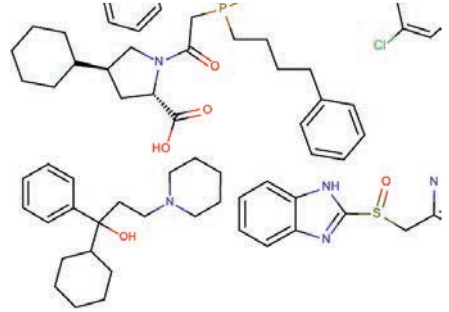
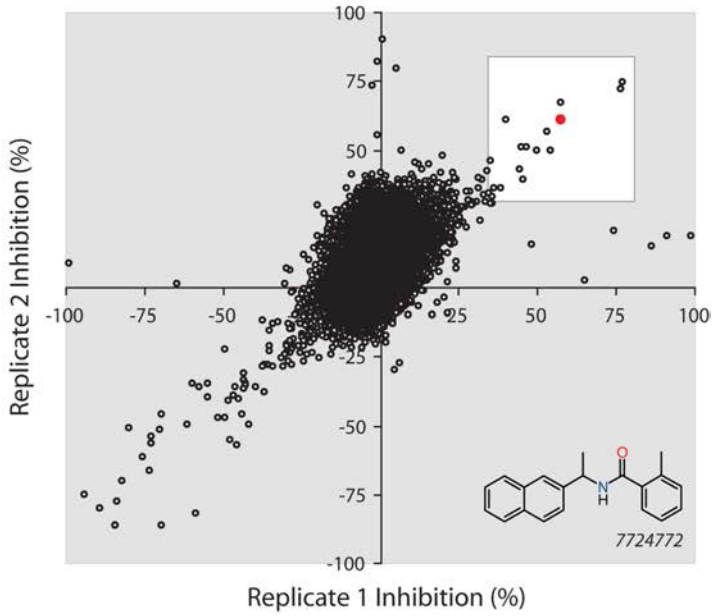
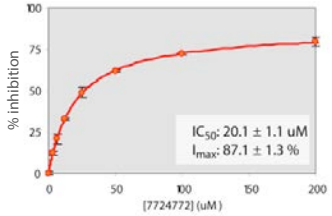
post-screen:

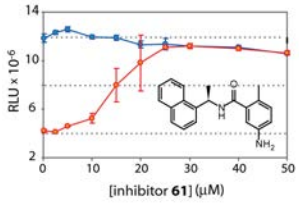
Hit identification & validation



- Rank and select hits (% activity cut-off, standard deviation from the mean, top 50 or top 0.5%, etc)
- Cherry-pick hits and retest in **primary assay**
- Run **orthogonal/counterscreen** to eliminate false positives
- Purchase hits, confirm activity, and run dose-response in primary assay
- Run **secondary assays** to confirm activity in a complementary assay to validate inhibition of the target
- Other: selectivity panels, SAR analysis







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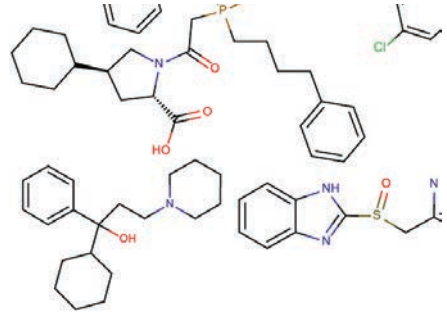
Keys to successful screens



strength of target and/or system

- has target been validated?
- is the target or approach (assay) novel?
- does the lab have sufficient expertise with target/system and have the tools to screen (proteins, specialty substrates or cell lines, etc)?

assay design

- is the assay developed and optimized?
- are the proper controls in place?
- Z′-factor > 0.5

post-screen

- what false positives does the investigator anticipate (compound interference, upstream effects, toxicity, reporter inhibition)?
- is there a good **counterscreen** in place to rule out false positives?
- Is there a **secondary assay** in place to confirm hits?
- Is **selectivity** a concern?