1269-B

# Small Molecule Microarray Screening Scale-Up and Automation at Kronos Bio

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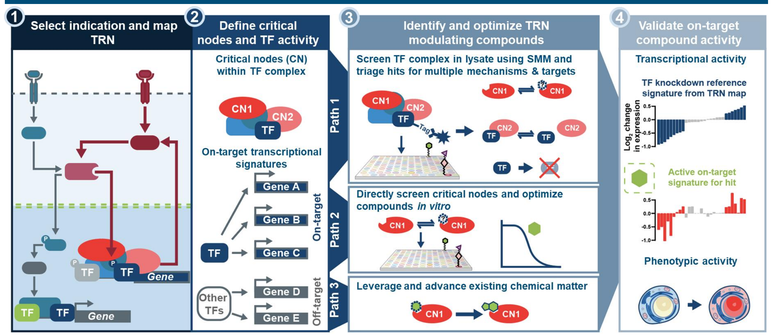
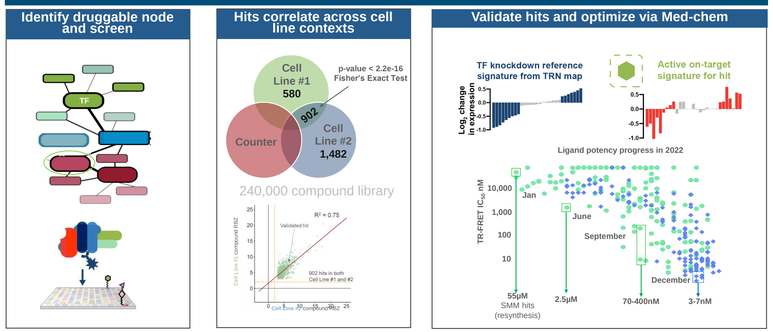
## Abstract

Transcriptional dysregulation is common among many types of cancers. Attempts to modulate transcriptional activity by directly drugging transcription factors remains broadly elusive. Transcription factors (TF) are disordered in isolation but become more structured and druggable upon recruiting co-factors in their native complexes. Our product engine involves mapping transcriptional regulatory networks (TRN) to unravel complex transcription factor interactomes and identify proteins that serve as critical nodes (CN) in these networks. We then screen for binders to these critical nodes, in either purified or lysate context, using our small molecule microarrays (SMM). These arrays are functionalized glass slides upon which a large number of drug-like small molecules have been robotically arrayed to allow for parallel high-throughput screening. As a means to further industrialize the process and increase throughput, we developed a scaled-up fabrication method to functionalize glass slides in preparation for array immobilization. Furthermore, we designed and built an automated screening workflow using a Hamilton Microlab STAR liquid handler. With the employment of both scaled and automated processes we reduced the time required to prepare slides for 18 full copies of our ~240,000 compound library from 80 days to 4 days, truncated screen times, and reduced user intervention.

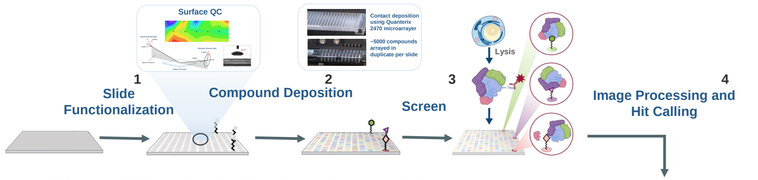
## Our Discovery Engine




## Success Case



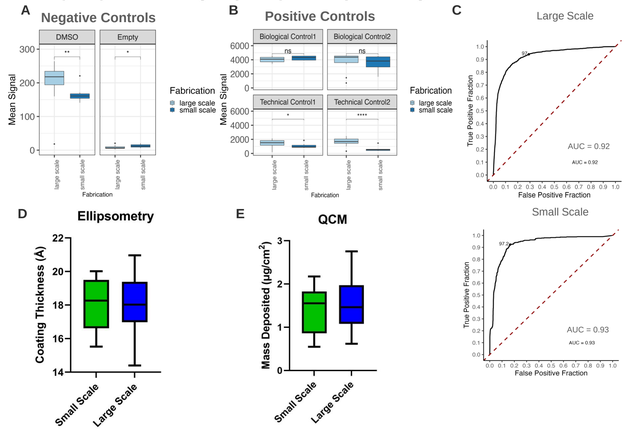

## SMM Process Overview




1. Build up of linkers terminating in an isocyanate to facilitate covalent capture of library compounds
2. Robotic spotting of compounds from library plates to slides followed by quenching of unreacted groups
3. Incubation with lysate or purified protein followed by incubation with relevant fluorophore-tag conjugate
4. Scan slides and determine hits based on metrics including fluorescent signal intensity, RBZ score, spot size, etc.

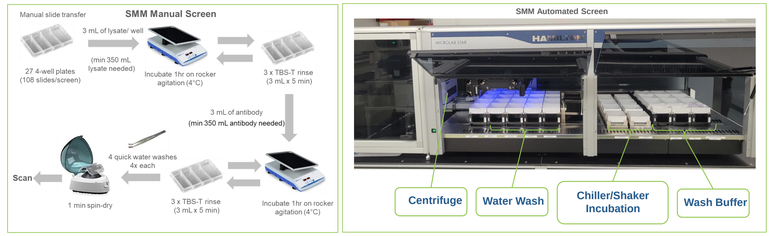
## SMM fabrication scale-up reduces labor and saves time while preserving slide quality and performance

**Scaled Up Fabrication process reduced total time of a typical production campaign (~2000 slides or 36 complete library copies) from 80-day effort to 4 days by increasing capacity and optimizing reaction times.**



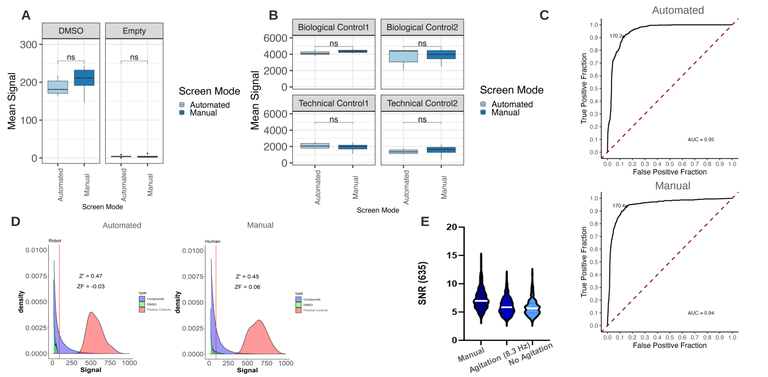

Screen results for slides produced by large and small-scale fabrication processes **A:** Negative control signal. DMSO and empty spots as negative controls. **B:** Positive control signal for two technical controls and two known ligands as positive controls. **C:** True positive fraction of large-scale produced slides (top) and small-scale produced slides (bottom). **D:** Coating thickness, in Å, determined by ellipsometry. **E:** Deposited mass, in μg/cm$^2$, determined by QCM.

## SMM Automated Screening Process




- Manual screening requires 9 hr with user intervention every 1 hr
- Automated screening is a higher throughput, lower labor process requiring 4 hr to complete a full library screen with little user intervention as opposed to manual process
  - Carriers proceed along tracks of troughs for each screening stage. Four tracks able to run up to 4 conditions in parallel (30 slide capacity per track)

## Comparison of manual vs automated screen results




Screen results for automated and manual screening processes **A:** Negative control signal . DMSO and empty spots as negative controls. **B:** Positive control signal for two technical controls and two known ligands as positive controls. **C:** True positive fraction of large-scale produced slides (top) and small-scale produced slides (bottom). **D:** Distribution of signal for different compound classes (DMSO, Positive control, Bulk compounds) showing good separation between negative and positive control populations. **E:** SNR of for screens with different agitation modes. Similarity of distributions suggests that more aggressive agitation of automated screening process does not disrupt TF ability to form necessary complexes

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