Harvard Medical School
ICCB-Longwood Screening Facility

250 Longwood Avenue Boston, Massachusetts 02115

Instructions for Primary Screen Report

Screen Documentation

Please use the accompanying Metadata Template (Microsoft Excel) to document your screen, with particular attention to the items below. Do not delete any of the fields from Column A. Examples have been included in the template to provide guidance.

a. PMID/PMCID identifiers for any published references directly pertaining to your screen (e.g., assay or reagent preparation protocols, and reagents such as purified protein, cell lines, and bacterial or viral strains).

b. For small molecule screens designed to identify chemicals interacting with a protein target, such as enzyme inhibitors, please include the nucleotide or protein GI identifier of the target molecule.

c. Biosource Taxon ID is indicated with as much specificity as possible.

d. The exact assay protocol for your screen, including all experimental details (i.e., reagent names, cell line information, buffer composition and volumes, incubation times, equipment used). If you performed a counterscreen assay as part of your primary screen, please also include that protocol information at the same level of detail.

e. Description of your positive and negative controls, and their location on your assay plates.

f. Description of how you analyzed your data and how you identified your screening positives, including how the results were calculated and how to interpret them. For example, if you defined your screening positives as strong, medium, and weak using cut-off ranges, please give the signal ranges. It is important that the description of your data analysis and definition of positives is as specific and clear as possible.

g. If your data will be deposited into PubChem, you must include your definition of Activity Score.

Annotated Screening Data

Please submit appropriately annotated data for all compounds screened, including designation of screening positives. If your data will be deposited into PubChem, your annotated dataset must include an Activity Score for each well screened.

Contact Information

The metadata file, the annotated data file, and any questions regarding their preparation should be sent to:

David Wrobel
Seeley Mudd Building, Room 604, Harvard Medical School, 250 Longwood Avenue, Boston, MA 02115
david_wrobel@hms.harvard.edu
617-432-6266 (phone)
617-432-6424 (fax)

You must begin depositing appropriately annotated primary screen data within 3 months of starting to screen ICCB-L library plates.

Small Molecule Example

Field name	Category name				Additional information regarding this field
Screen Number	Screen Number	704			
Last Name	Contact	Walker	Swoboda		
First Name		Suzanne	Jonathan		
Institution		Harvard Medical School	Harvard Medical School		
Address		Dept. of Microbiology and Molecular Genetics, 200 Longwood Avenue	Dept. of Microbiology and Molecular Genetics, 200 Longwood Avenue		
City		Boston	Boston		
Country		USA	USA		
Email		suzanne_walker@hms.harvard.edu			
Screen Title	Screen Description	A screen for compounds that inhibit cell wall-associated teichoic acid synthesis in Staphylococcus aureus.			
Screen Type		Small molecule			
Target Process		Teichoic acid biosynthesis in Staphylococcus aureus			
Abstract		Both Gram-positive and Gram-negative bacteria contain bactoprenol-dependent biosynthetic pathways expressing non-essential cell surface polysaccharides that function as virulence factors. Although these polymers are not required for bacterial viability in vitro, genes in many of the biosynthetic pathways are conditionally essential: they cannot be deleted except in strains incapable of initiating polymer synthesis. We report a cell-based, pathway-specific screen for small molecule inhibitors of conditionally essential enzymes involved in wall teichoic acid (WTA) biosynthesis in Staphylococcus aureus. WTAs are anionic cell surface polysaccharides required for host colonization that have been suggested as targets for new antimicrobials. We identified a small molecule, 7-chloro-N,N-diethyl-3-(phenylsulfonyl)-[1,2,3]triazolo[1,5-a]quinolin-5-amine, that inhibits the growth of a panel of S. aureus strains (MIC = 1-3 microg mL(-1)), including clinical methicillin-resistant S. aureus (MRSA) isolates. Subsequent biochemical and genetic studies identified the molecular target as TarG, the transmembrane component of the ABC			

Small Molecule Example

PMID		PMID: 19689117			Please provide PMID numbers for any publications directly relevant to your assay protocol or screen project (e.g. publication of your assay protocol, or publication of your primary or secondary screen results). Note that at the time of first submission of primary screen data, many screen projects will have no directly relevant publications to list in this field.
PMCID		PMC2787957			Please provide PMCID numbers (if available) for any publications listed in the preceding row.
Biosource Name	Biosource	Staphylococcus aureus RN4220	Staphylococcus aureus RN4220 ΔtarO		The term Biosource refers to any biological organism or product used in your screen, including purified proteins. If your screen used more than one Biosource (e.g., a virus and a mammalian cell line), please document each Biosource using a separate column.
Biosource Provider		Jonathan Swoboda	Jonathan Swoboda		
Biosource Provider ID(s		RN4220	RN4220 ΔtarO		
Biosource Contact		suzanne_walker@hms.harvard.edu	suzanne_walker@hms.harvard.edu		
Biosource Reference(s)		PMID: 6226876	PMID: 16547033		Please provide PMID or PMCID numbers for any publications describing the biosources used in your screen (e.g., construction of reporter fusion, development of purification protocol). For small molecule screens designed to identify chemicals interacting with a protein target, such as enzyme inhibitors, please include the NCBI nucleotide or protein GI identifier of the target molecule. For more information on GI identifiers, please see http://www.ncbi.nlm.nih.gov/Sitemap/sequenceIDs.html.
Biosource Genus/Species		Staphylococcus aureus	Staphylococcus aureus		
Biosource Taxon ID		Taxon ID: 561307			Use the NCBI Taxonomy Browser (http://www.ncbi.nlm.nih.gov/taxonomy) to derive the Taxonomy ID for all biosources listed previously. Please be as specific to species (and strain, if available) as possible. Note that the Taxon ID for humans (*Homo sapiens)* is 9606.
Biosource Tissue					
Biosource Modifications, Genotype, or Genetic Characteristics		Plasmid pMS182 introduced by electroporation into RN4220	Plasmid pMS183 introduced by electroporation into RN4220 ΔtarO		
Library Type		Small molecule			
Library Format (single/pool)		Single			

Small Molecule Example

Number of Perturbators per Well	Library	1			
Perturbator Target Genus/Species		Staphylococcus aureus			
Pre-perturbation Condition Description	Assay Protocol	Prior to screening, a wildtype strain of S. aureus RN4220 was freshly transformed with the plasmid pMS182 encoding constitutively expressed GFP and a ΔtarO strain of RN4220 was transformed with the plasmid pMS183 encoding constitutively expressed mCherry. Following transformation, both plasmids were maintained with chloramphenicol (Cm) at a concentration of 10 µg/mL. The day before screening, both strains were grown overnight (~24 h) in sterile-filtered tryptic soy broth (TSB) with 10 µg/mL chloramphenicol at 30 °C with shaking.			
Perturbation Condition Description		On the day of screening, columns 1-23 of assay plates (Corning 3710) were prefilled with 40 uL of TSB. Next, 300 nL of each compound were pin-transferred to each plate. For every compound library plate, there were four daughter plates (A & B containing mCherry-labeled ΔtarO and C & D containing GFP-labeled wildtype). To equilibrate the number of cells, the overnight cultures of RN4220 and RN4220 ΔtarO were diluted to an OD600 = 2 and then diluted 1000x into TSB (Cm 10). 40 uL of the diluted cultures were added to columns 1-22 of the corresponding assay plates. Wells in column 23 contained only medium and served as the negative controls. Column 24 was filled with the positive control (erythromycin at 20 ug/mL). Final assay well volume was 80 uL. Plates were stacked 5 high, covered with lids (Corning 3009), and incubated at 30 °C overnight (~18 h).			
Post-perturbation Condition Description		The following day, assay plates were read using a PerkinElmer EnVision (600 nm filter).			
Perturbator Delivery Method Name		Pin Transfer			
Perturbator Delivery Method Description		300 nL of compounds transferred by stainless steel pin array from library plate to each assay plate			
Amount of Perturbator Delivered		300 nL			
Final Assay Volume		80 uL			
Final Perturbator Concentration		18.75 ug/ml			

Small Molecule Example

Number of Replicates		2			
Description of Replicates		Library plates were screened in duplicate, with both assay plates in a given set prepared on the same day. For every compound library plate, there were four daughter plates (A & B containing mCherry-labeled ΔtarO and C & D containing GFP-labeled wildtype).			
Control Name		Positive	Negative		
Control Description		Erythromycin (20 ug/mL) in all wells of columns 24	Growth medium only in all wells of column 23		
Instrument Name		Matrix WellMate	Epson	EnVision	
Instrument Model				2102 and 2103	
Instrument Vendor		Matrix Technologies (Thermo Fisher)	Epson	PerkinElmer	
Instrument Settings			300 nL	Photometric 600 filter (600 nm, 8 nm bandpass)	
Readout Type		Photometry			
Raw Data Type	Data Documentation	Numeric			
Raw Data Format		CSV			
Data Analysis Methods		OD600 values were normalized to the positive and negative controls to determine a normalized percent survival. Based on the normalized OD600 values, a substance was considered active with >50% survival for the ΔtarO strain and <10% survival for the wild type strain. PubChem activity scores were calculated using average % survival for each strain. Average % survival ≤ 0 was scored as 100 for activity; average % survival ≥ 100 was scored as 0 for activity. Average % survival between 0 and 100 was subtracted from 100 to generate activity scores for intermediate values (i.e. average % survival = 40 corresponds to an activity score of 60).			
Software		Excel			
Software Manufacturer		Microsoft			
Processed Data Type		Numeric			
Additional Comments					Please provide any additional comments regarding your screen that may not have been adequately captured elsewhere in the metadata template (e.g., problems that may have affected data quality)

RNAi Example

Field name	Category name				Additional information regarding this field
Screen Number	Screen Number	729			
Last Name	Contact	Howley	Smith		
First Name		Peter	Jennifer		
Institution		Harvard Medical School	Harvard Medical School		
Address		77 Avenue Louis Pasteur, Rm 950a	77 Avenue Louis Pasteur, Rm 950a		
City		Boston	Boston		
Country		USA	USA		
Email		peter_howley@hms.harvard.edu	jennifer_smith@hms.harvard.edu		
Screen Title	Screen Description	An siRNA screen for human genes that are involved in human papilloma virus (HPV) transcriptional repression.			
Screen Type		siRNA			
Target Process		E2-dependent regulation of human papillomavirus gene expression			
Abstract		An essential step in the pathogenesis of human papillomavirus (HPV)–associated cancers is the dysregulated expression of the viral oncogenes. The papillomavirus E2 protein can silence the long control region (LCR) promoter that controls viral E6 and E7 oncogene expression. The mechanisms by which E2 represses oncogene expression and the cellular factors through which E2 mediates this silencing are largely unknown. We conducted an unbiased, genome-wide siRNA screen and series of secondary screens that identified 96 cellular genes that contribute to the repression of the HPV LCR. In addition to confirming a role for the E2-binding bromodomain protein Brd4 in E2-mediated silencing, we identified a number of genes that have not previously been implicated in E2 repression, including the demethylase JARID1C/SMCX as well as EP400, a component of the NuA4/TIP60 histone acetyltransferase complex. Each of these genes contributes independently and additively to E2-mediated silencing, indicating that E2 functions through several distinct cellular complexes to repress E6 and E7 expression.			
Measurement Method		Luciferase readout to quantitate expression of an E2-repressible reporter in which luciferase expression is controlled by the HPV18 LCR.			
PMID		20133580			Please provide PMID numbers for any publications reporting results from your screen (e.g., publication of primary or secondary results, publication of assay protocol separately from screen results).
PMCID		PMC2840515			Please provide PMCID numbers (if available) for any publications listed in the preceding row.

RNAi Example

Biosource Name	Biosource	C33A/BE2/18LCR c4			The term Biosource refers to any biological organism or product used in your screen, including purified proteins. If your screen used more than one Biosource (e.g., a virus and a mammalian cell line), please document each Biosource using a separate column.
Biosource Provider		Jennifer Smith			
Biosource Provider ID(s		C33A/BE2/18LCR c4			
Biosource Contact		peter_howley@hms.harvard.edu			
Biosource Reference(s)		PMC2840515			Please provide PMID or PMCID numbers for any publications describing the biosources used in your screen (e.g., construction of reporter fusion, development of purification protocol).
Biosource Genus/Species		Homo sapiens			
Biosource Taxon ID		Taxon ID: 9606			Use the NCBI Taxonomy Browser (http://www.ncbi.nlm.nih.gov/taxonomy) to derive the Taxonomy ID for all biosources listed previously. Please be as specific to species (and strain, if available) as possible. Note that the Taxon ID for humans (*Homo sapiens*) is 9606.
Biosource Tissue		Cervix			
Biosource Modifications, Genotype, or Genetic Characteristics		C33A cells engineered to stably express FLAG-HA-tagged bovine PV1 (BPV1) E2 from a bicistronic mRNA that also encodes the IL2 receptor α subunit (IL2Rα). These cells also contain an E2-repressible reporter in which luciferase expression is controlled by the HPV18 LCR.			
Library Type	Library	siRNA			
Library Format (single/pool)		Pool			
Number of Perturbators per Well		4			
Perturbator Target Genus/Species		Homo sapiens			
Pre-perturbation Condition Description		C33/BE2/18LCR c4 cells were maintained as subconfluent monolayers in high-glucose DMEM with 10% FBS, 50 units/mL penicillin G, 50 ug/mL streptomycin sulfate, and 0.75 ug/mL puromycin. Cells were depleted of antibiotics 1 day prior to transfection.			

RNAi Example

Perturbation Condition Description	Assay Protocol	On the day of transfection, siRNA buffer (1x; Dharmacon) was aliquoted into wells, siRNA was added so that the final concentration was 40 nM/well, and DharmaFECT2/OptiMEM was dispensed into wells. While the siRNA/lipid was allowed to complex, cells were trypsinized, counted, and resuspended to reach a plating density of 7 x 10E3 cells/well. Cells were seeded on top of the siRNA/lipid mixture, briefly centrifuged, and incubated at 37 C for 72 h.			
Post-perturbation Condition Description		At 72 h post-transfection, Steady Glo reagent (Promega) was added to each well and luciferase units/well measured using an EnVision plate reader.			
Perturbator Delivery Method Name		Transfection			
Perturbator Delivery Method Description		siRNA buffer (1x; Dharmacon) was aliquoted into wells, siRNA was added so that the final concentration was 40 nM/well, and DharmaFECT2/OptiMEM was dispensed into wells. While the siRNA/lipid was allowed to complex, cells were trypsinized, counted, and resuspended to reach a plating density of 7 x 10E3 cells/well. Cells were seeded on top of the siRNA/lipid mixture, briefly centrifuged, and incubated at 37 C for 72 h.			
Amount of Perturbator Delivered		2 x 10E-12 mole			
Final Assay Volume		50 uL			
Final Perturbator Concentration		40 nM			
Number of Replicates		3			
Description of Replicates		Library plates were screened in triplicate, which all three assay plates in a given set prepared on the same day.			
Control Name		Positive	Negative	Other	
Control Description		BPV E2 (BE2#3) siRNA added to some wells in column 22	USP15 siRNA added to some wells in column 22	G418 added to two wells in column 22 to induce cell death	
Instrument Name		Matrix WellMate	Velocity11 Bravo	EnVision	
Instrument Model				2102 and 2103	
Instrument Vendor		Matrix Technologies (Thermo Fisher)	Velocity11	PerkinElmer	
Instrument Settings				Crosstalk-corrected luminescence, luminescence aperture 9, measurement height 0 mm, measurement time 0.1 s	
Readout Type		Luminescence			
Raw Data Type		Numeric			
Raw Data Format		CSV			

RNAi Example

Data Analysis Methods	Data Documentation	The mean and SD for each plate, minus wells with no siRNA, positive control siRNA, or G418, were calculated. The z-score [z = (x - m)/s] was calculated for each SMARTpool on the plate, where x is the raw score to be standardized, m is the mean of the plate, and s is the standard deviation of the plate. The z-scores from the three replicates for each SMARTpool were averaged and the SD determined. A SMARTpool was considered a positive if the average z-score was ≥ 2. Weak positives were defined as having an average z-score ≥ 2 and < 3. Medium positives were defined as having an average z-score ≥ 3 and < 5. Strong positives were defined as having an average z-score ≥ 5.			
Software		Excel			
Software Manufacturer		Microsoft			
Processed Data Type		Numeric			
Additional Comments					Please provide any additional comments regarding your screen that may not have been adequately captured elsewhere in the metadata template (e.g., problems that may have affected data quality)