

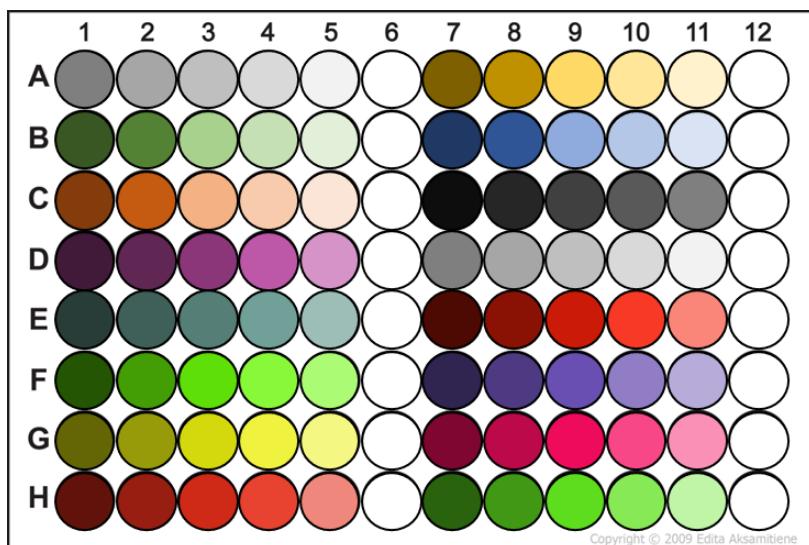
Validation - overview

Goal: systematic screening validation - false positives/negatives rate

1. Are we losing hits in our screening setup?
2. What are the influences of similar compound structures/functions on triggering promoters?

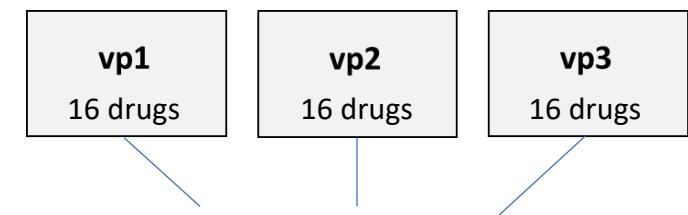
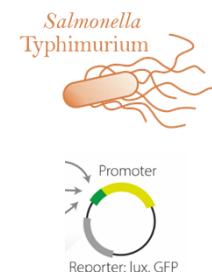
Concentrations: 0, 30, 60, 90, 120 and 150 µM

Compounds per plate: 16



96 wp format x 4 replicates = 384 wp

Validation setup:



48 selected compounds
Quadruplicate

- ✓ Growth inhibition and promoter expression
- ✓ Dose-response curves
- ✓ Higher quality data for selected stressors
- ✓ Faster covering of promoters

Validation final promoters overview

EVC	marR	acrAB	MicF	ramA	soxS
rob	RyhB1	PinT	lsrK	katE	GcvB
nc700	ArcZ	OmrB	iscR	OxyS	DsrA
CpxP	MicA	virK	OppX	SroC	GlmY
SdsR	nc1390	feoB	Tpke11	FnrS	lexA
Ion	RyeC	SroA	SSpA	lsrJ	CsrB
CsrC	nc 710	nc1430	nc1480	nc1640	nc4080
nc 520	nc 880	nc1280	nc2050		



KO-backgrounds

Δ marA	Δ ramA	Δ marA Δ ramA
EVC	EVC	EVC
marR	marR	marR
ramA	ramA	ramA
MicF	MicF	MicF
acrAB	acrAB	acrAB

regulators

effectors

sRNA

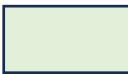


Lp-screened, to test in KO backgrounds

Non-sRNA



Lp-screened



Not Lp-screened

Validation final compounds overview

compound	srn_code	vp	max_cond
Isoconazole (nitrate)	Ip6_I16	vp1	150
Zinc Pyrithione	Ip3_B1	vp1	100
Thioridazine (hydrochloride)	Ip7_O20	vp1	150
Phenazine (methylsulfate)	Ip4_N4	vp1	150
Trimethoprim	Ip6_M22	vp1	50
Erythromycin Ethylsuccinate	Ip3_I1	vp1	50
Sulconazole (mononitrate)	Ip7_H1	vp1	150
Dicyclomine (hydrochloride)	Ip2_N12	vp1	150
Diiodohydroxyquinolone	Ip5_B6	vp1	150
Benzethonium	Ip2_L23	vp1	150
Metixene hydrochloride hydrate	Ip4_L10	vp1	150
Doxylamine (succinate)	Ip7_A15	vp1	150
Ciclopirox	Ip7_K3	vp1	150
Benfluorex (hydrochloride)	Ip3_A18	vp1	150
Methylene blue (trihydrate)	Ip1_B17	vp1	150
Clindamycin (hydrochloride)	Ip5_E6	vp1	100
Pinaverium bromide	Ip2_C8	vp2	150
Tioconazole	Ip3_F16	vp2	150
Ketoconazole	Ip5_M23	vp2	150
Clotrimazole	Ip2_L18	vp2	150
Gemcitabine	Ip4_P17	vp2	150
Clemastine (fumarate)	Ip5_D22	vp2	150
Terfenadine	Ip7_L21	vp2	150
Fluoxetine hydrochloride	Ip6_M3	vp2	150

Cinnarizine	Ip6_I12	vp2	150
Econazole (nitrate)	Ip7_M10	vp2	150
Oxiconazole (nitrate)	Ip4_F3	vp2	150
Nefazodone (hydrochloride)	Ip2_F13	vp2	150
Auranofin	Ip6_L23	vp2	50
Tannic acid	Ip5_G7	vp2	50
Novobiocin	Ip2_K2	vp2	150
Paraquat	not present	vp2	50
Menadione sodium bisulfite	Ip3_B9	vp3	150
Mupirocin	Ip1_P2	vp3	50
Pyrogallol	Ip3_B8	vp3	150
Clomipramine	Ip1_K1	vp3	150
Tryhexyphenidyl	Ip5_O3	vp3	150
Ajmaline	Ip5_L7	vp3	150
Alprenolol	Ip1_D3	vp3	150
Dantrolene	Ip1_O22	vp3	150
Eltrombopag	Ip1_J6	vp3	150
Clavulanate	Ip5_M17	vp3	150
Parthenolide	Ip3_I6	vp3	150
Nafamostat	Ip2_C1	vp3	150
Pentamidine	Ip5_J21	vp3	150
Etidronic acid	Ip7_K5	vp3	150
Didanosine	Ip1_J16	vp3	150
Visomitin	Ip5_L22	vp3	50

Direction: Validation final results overview

- Promoters in WT background: 46
 - Compounds: 48
 - Concentrations: 5 each compound
 - Quadruplicate
-
- Expected drug-gene pairs: 2208
 - 11040 conc_drug-gene pairs considering the concentrations

Direction: Validation final results overview

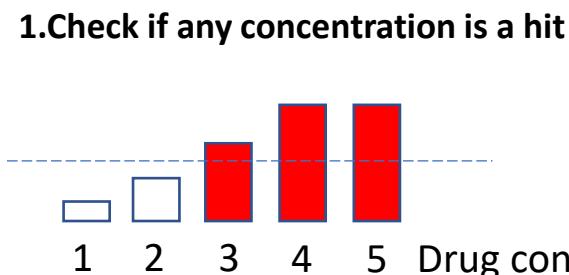
1. Qualitative: Determine if the given drug-gene pair is a **hit**

1.1. Develop method/threshold to assign in a **yes/no** basis if the pair is considered a hit

1.2. If yes, assign if the drug **up- or downregulates** its target



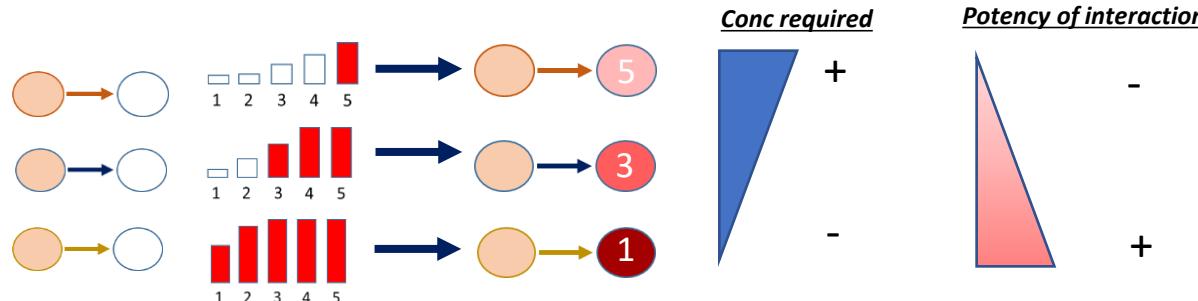
Drug-gene pair



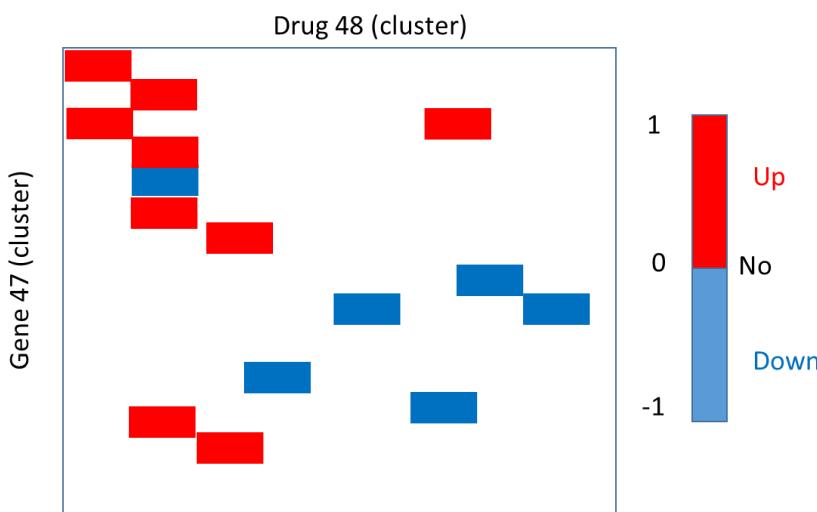
2. Assign the hits to **up** or **down**



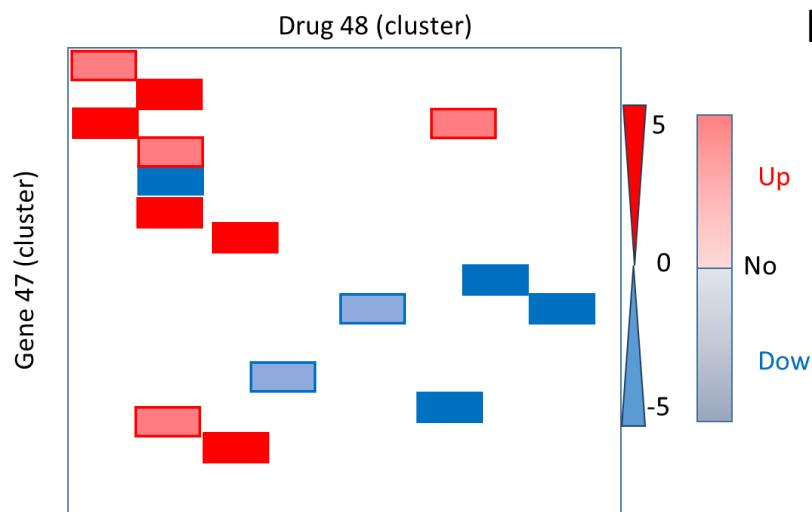
2. Quantitative: for a given drug-gene pair, what is the minimal triggering concentration?



Ideal: heatmap of gene-drug interactions



"qualitative" – yes/no



"quantitative"

"Fingerprinting" of Chemical triggering by a giving gene



Check the clusters to see how Chemical/genes associate



Compare 1344* drug-gene pairs
(yes/no for hits) from our 2 approaches:
lib screening and validation