

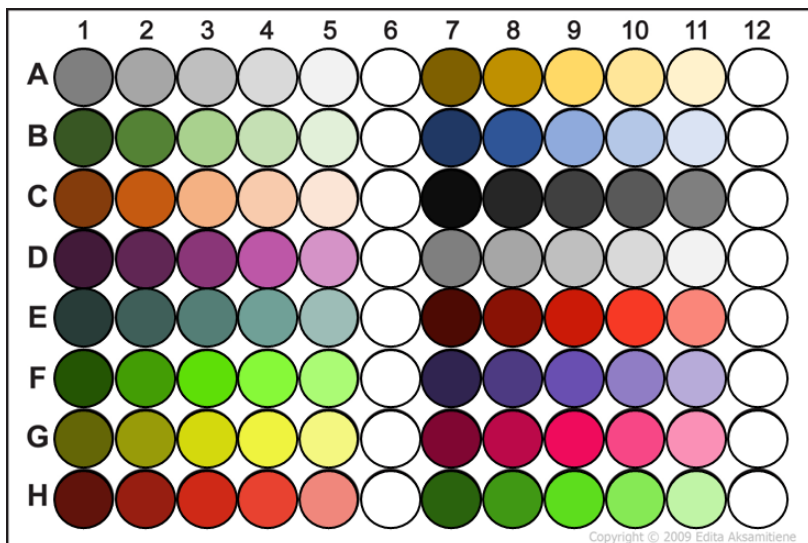
Validation - overview

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?

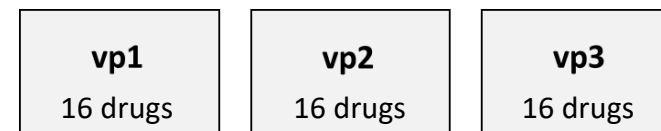
Concentrations: 0, 30, 60, 90, 120 and 150 μ M

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 | IsrK | katE | GcvB |
| nc700 | ArcZ | OmrB | iscR | OxyS | DsrA |
| CpxP | MicA | virK | OppX | SroC | GlmY |
| SdsR | nc1390 | feoB | Tpke11 | FnrS | lexA |
| lon | RyeC | SroA | SSpA | IsrJ | CsrB |
| CsrC | nc 710 | nc1430 | nc1480 | nc1640 | nc4080 |
| nc 520 | nc 880 | nc1280 | nc2050 | | |

KO-backgrounds

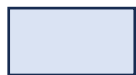
| Δ marA | Δ ramA | Δ marA Δ ramA |
|---------------|---------------|--------------------------------|
| EVC | EVC | EVC |
| marR | marR | marR |
| ramA | ramA | ramA |
| MicF | MicF | MicF |
| acrAB | acrAB | acrAB |

regulators

effectors

sRNA

Non-sRNA



Lp-screened, to test in KO backgrounds



Lp-screened



Not Lp-screened

Validation final compounds overview

| compound | srn_code | vp | max_cond |
|--------------------------------|----------|-----|----------|
| Isoconazole (nitrate) | lp6_I16 | vp1 | 150 |
| Zinc Pyrithione | lp3_B1 | vp1 | 100 |
| Thioridazine (hydrochloride) | lp7_O20 | vp1 | 150 |
| Phenazine (methylsulfate) | lp4_N4 | vp1 | 150 |
| Trimethoprim | lp6_M22 | vp1 | 50 |
| Erythromycin Ethylsuccinate | lp3_I1 | vp1 | 50 |
| Sulconazole (mononitrate) | lp7_H1 | vp1 | 150 |
| Dicyclomine (hydrochloride) | lp2_N12 | vp1 | 150 |
| Diiodohydroxyquinolone | lp5_B6 | vp1 | 150 |
| Benzethonium | lp2_L23 | vp1 | 150 |
| Metixene hydrochloride hydrate | lp4_L10 | vp1 | 150 |
| Doxylamine (succinate) | lp7_A15 | vp1 | 150 |
| Ciclopirox | lp7_K3 | vp1 | 150 |
| Benfluorex (hydrochloride) | lp3_A18 | vp1 | 150 |
| Methylene blue (trihydrate) | lp1_B17 | vp1 | 150 |
| Clindamycin (hydrochloride) | lp5_E6 | vp1 | 100 |
| Pinaverium bromide | lp2_C8 | vp2 | 150 |
| Tioconazole | lp3_F16 | vp2 | 150 |
| Ketoconazole | lp5_M23 | vp2 | 150 |
| Clotrimazole | lp2_L18 | vp2 | 150 |
| Gemcitabine | lp4_P17 | vp2 | 150 |
| Clemastine (fumarate) | lp5_D22 | vp2 | 150 |
| Terfenadine | lp7_L21 | vp2 | 150 |
| Fluoxetine hydrochloride | lp6_M3 | vp2 | 150 |

| | | | |
|----------------------------|-------------|-----|-----|
| Cinnarizine | lp6_I12 | vp2 | 150 |
| Econazole (nitrate) | lp7_M10 | vp2 | 150 |
| Oxiconazole (nitrate) | lp4_F3 | vp2 | 150 |
| Nefazodone (hydrochloride) | lp2_F13 | vp2 | 150 |
| Auranofin | lp6_L23 | vp2 | 50 |
| Tannic acid | lp5_G7 | vp2 | 50 |
| Novobiocin | lp2_K2 | vp2 | 150 |
| Paraquat | not present | vp2 | 50 |
| Menadione sodium bisulfite | lp3_B9 | vp3 | 150 |
| Mupirocin | lp1_P2 | vp3 | 50 |
| Pyrogallol | lp3_B8 | vp3 | 150 |
| Clomipramine | lp1_K1 | vp3 | 150 |
| Tryhexyphenidyl | lp5_O3 | vp3 | 150 |
| Ajmaline | lp5_L7 | vp3 | 150 |
| Alprenolol | lp1_D3 | vp3 | 150 |
| Dantrolene | lp1_O22 | vp3 | 150 |
| Eltrombopag | lp1_J6 | vp3 | 150 |
| Clavulanate | lp5_M17 | vp3 | 150 |
| Parthenolide | lp3_I6 | vp3 | 150 |
| Nafamostat | lp2_C1 | vp3 | 150 |
| Pentamidine | lp5_J21 | vp3 | 150 |
| Etidronic acid | lp7_K5 | vp3 | 150 |
| Didanosine | lp1_J16 | vp3 | 150 |
| Visomitin | lp5_L22 | vp3 | 50 |

Direction: Validation final results overview

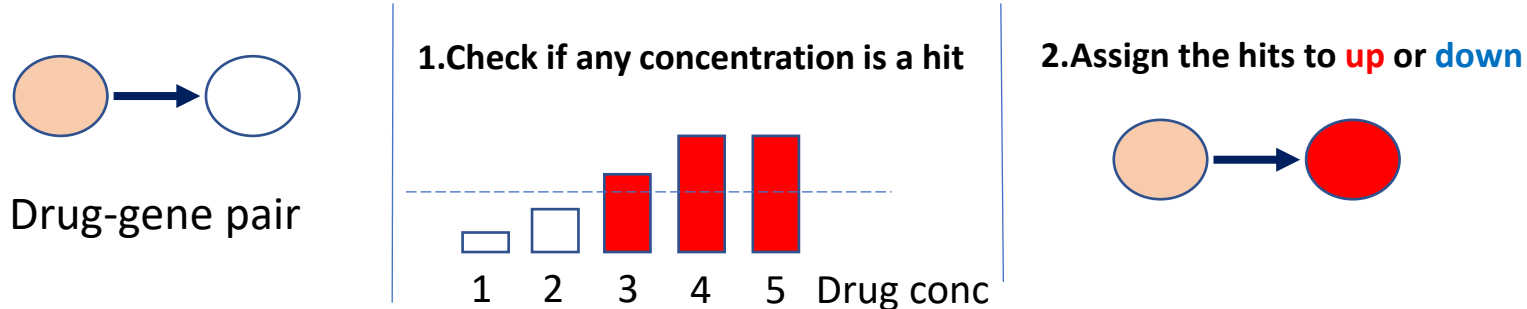
- Promoters in WT background: 46
 - Compounds: 48
 - Concentrations: 5 each compound
 - Quadruplicate
-
- Expected drug-gene pairs: 2208
 - 11040 conc_drug-gene pairs considering the concentrations

Direction: Validation final results overview

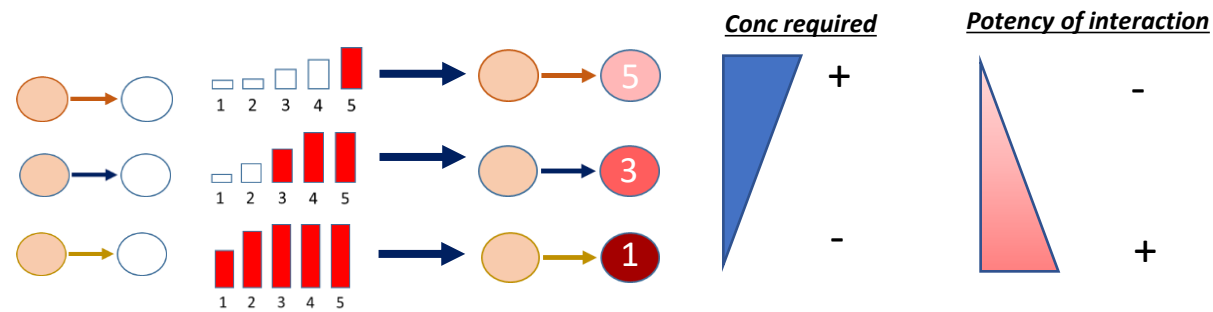
1. **Qualitative:** Determine if the given drug-gene pair is a **hit**

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

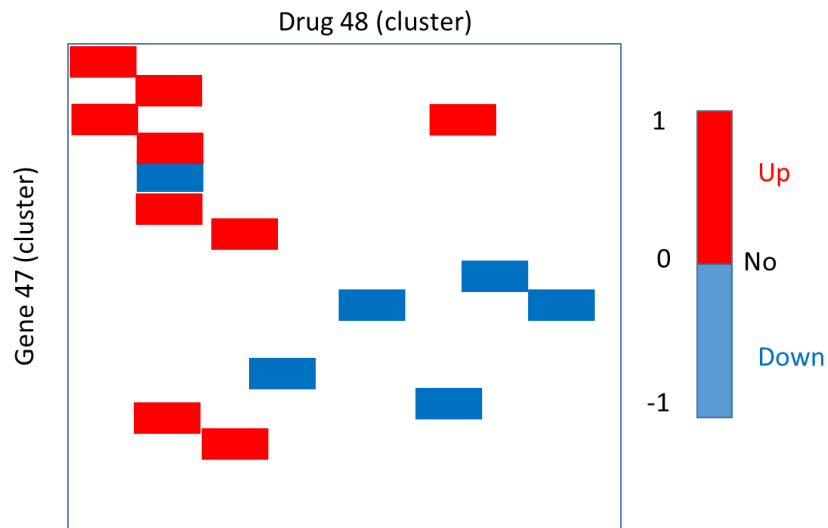
1.2. If **yes**, assign if the drug **up-** or **downregulates** its target



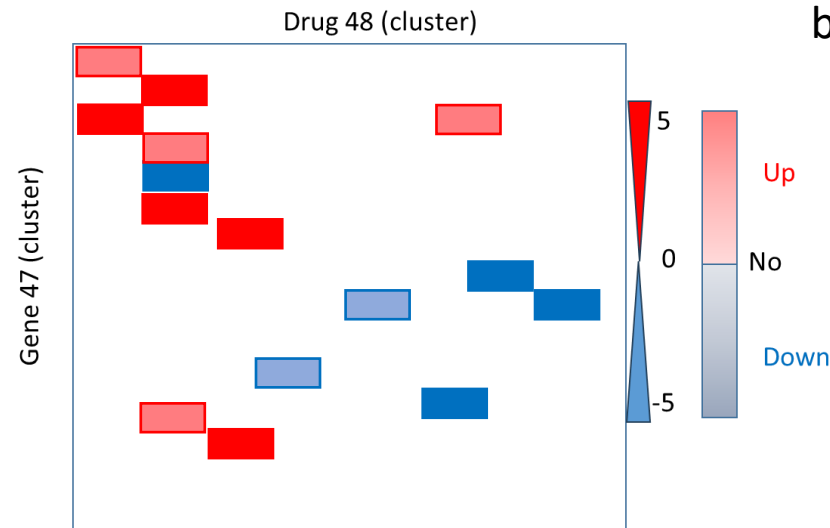
2. **Quantitative:** for a given drug-gene pair, what is the minimal triggering concentration?



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