

Video Article

Rapid Identification of Chemical Genetic Interactions in *Saccharomyces cerevisiae*

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URL: <https://www.jove.com/video/52345>

DOI: [doi:10.3791/52345](https://doi.org/10.3791/52345)

Keywords: Microbiology, Issue 98, chemical biology, high-throughput screen, *Saccharomyces cerevisiae*, yeast knock-out collection, deletion mutant array, chemical genetic interaction, synthetic lethal, synthetic sick, chemogenomics

Date Published: 4/5/2015

Citation: Dilworth, D., Nelson, C.J. Rapid Identification of Chemical Genetic Interactions in *Saccharomyces cerevisiae*. *J. Vis. Exp.* (98), e52345, doi:10.3791/52345 (2015).

BMM5828/BTC5819 - Utilização de *Saccharomyces cerevisiae* como organismo modelo em biologia molecular

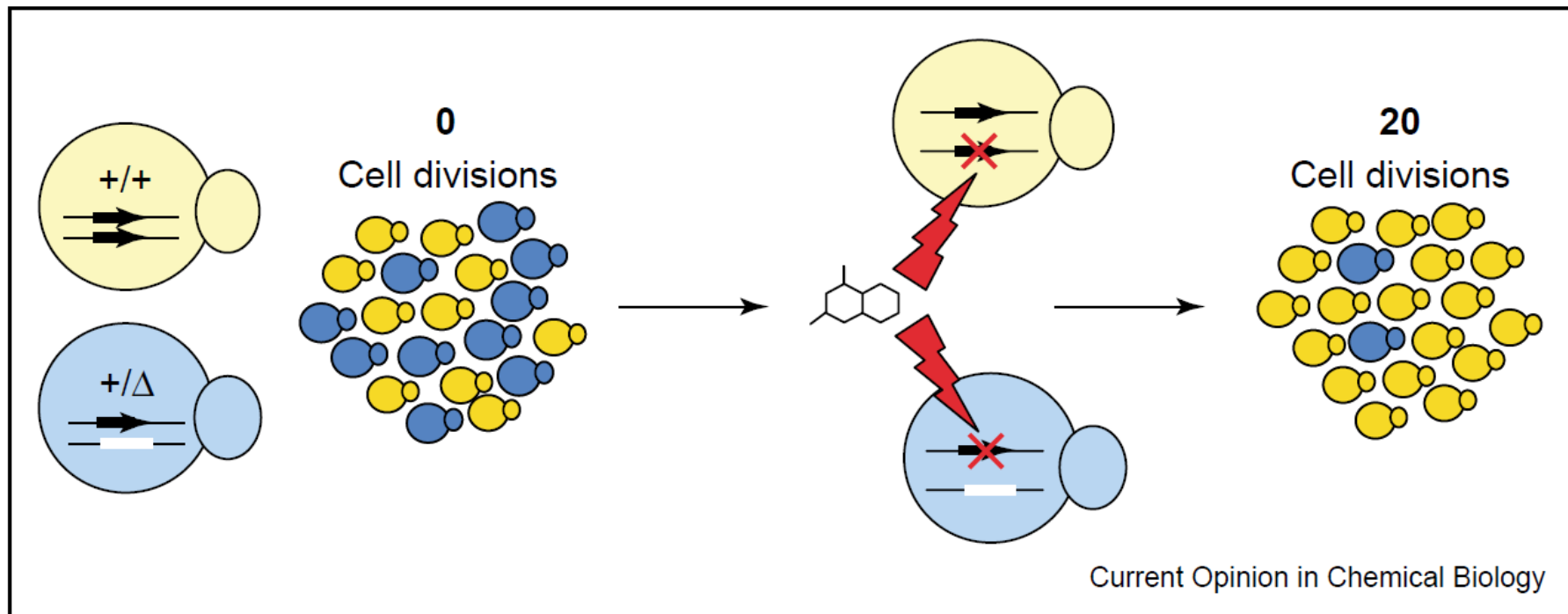
Uso de pequenas moléculas em sistemas biológicos



Estrutura da apresentação

1. Haploinsuficiência fármaco-induzida em meio líquido;
2. Rastreamento químico-genético em meio sólido por comparação de tamanho de colônias;
3. Sistema Duplo-Híbrido Reverso

Haploinsuficiência fármaco-induzida

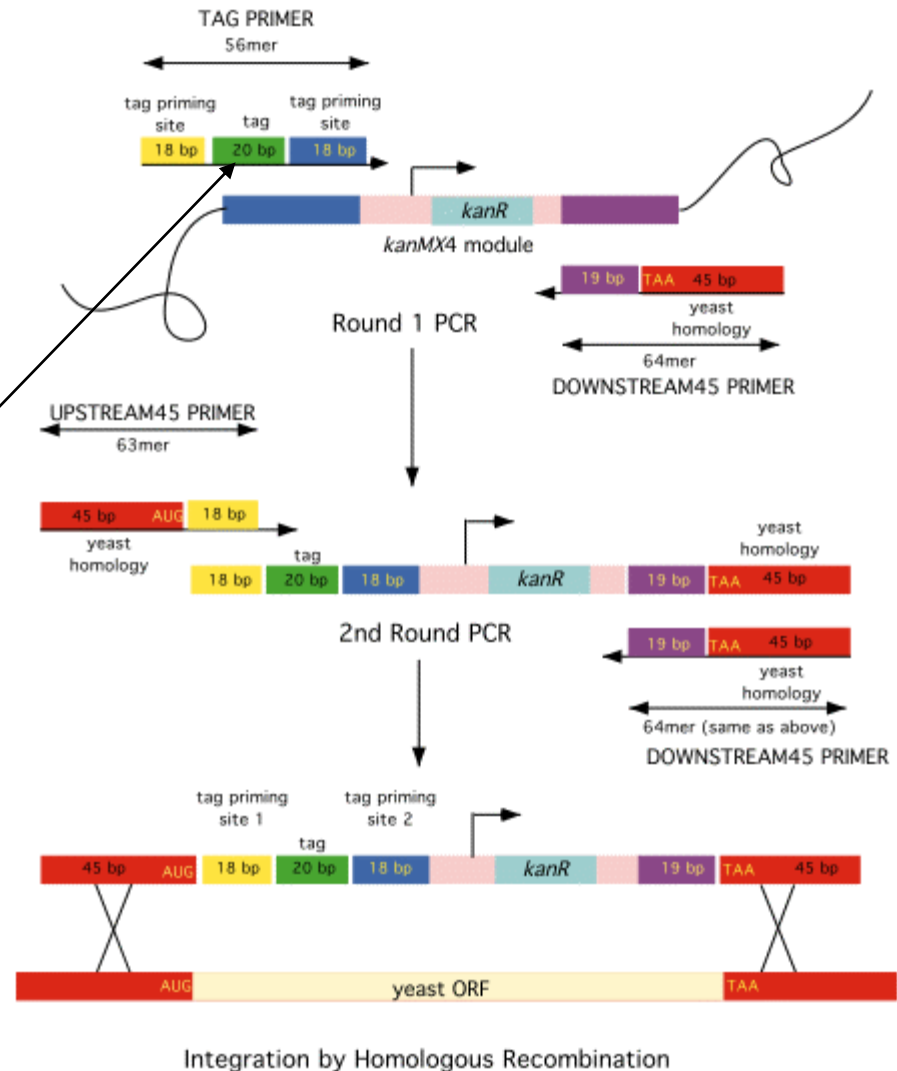


A levedura como modelo de estudo da ação de pequenas moléculas

≈6000 coleção de diplóides heterozigotos

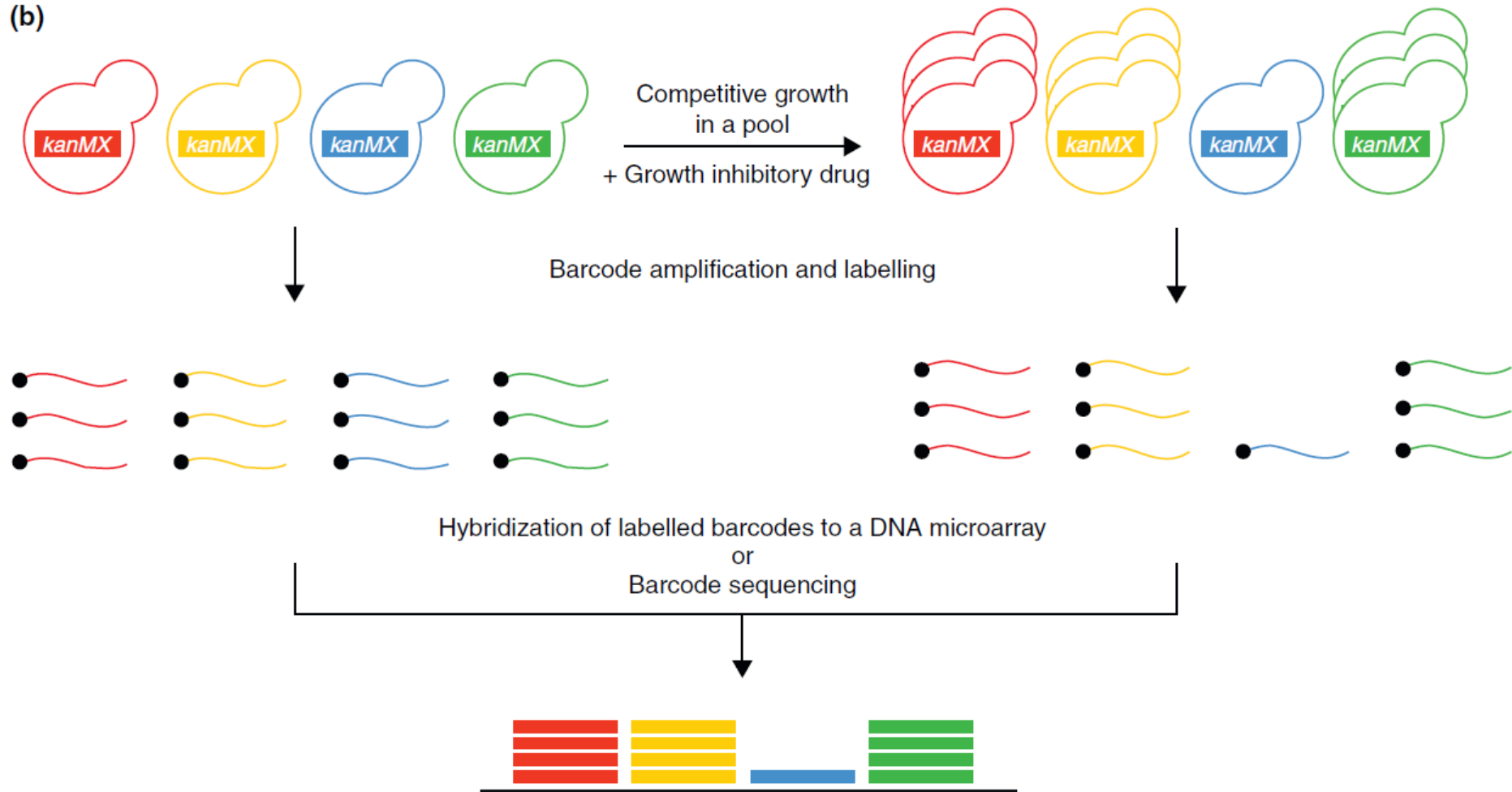
≈5000 haplóides viáveis

“código de barras” (20 pb)

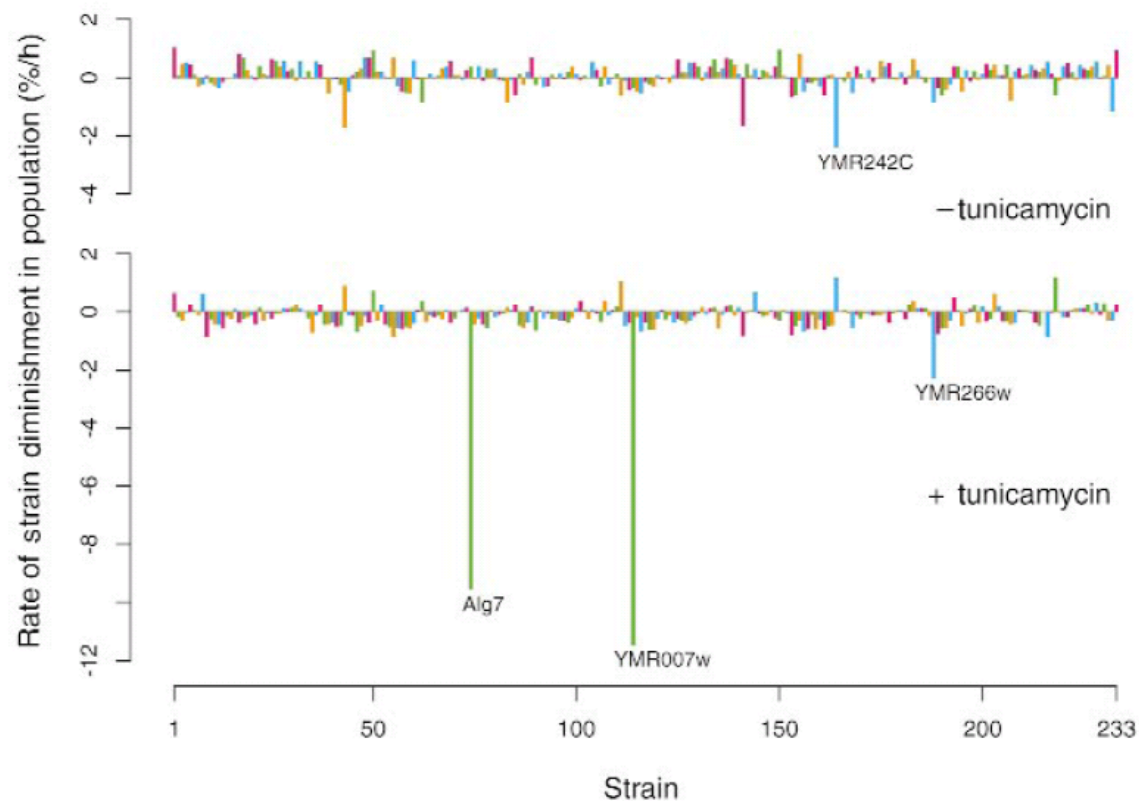
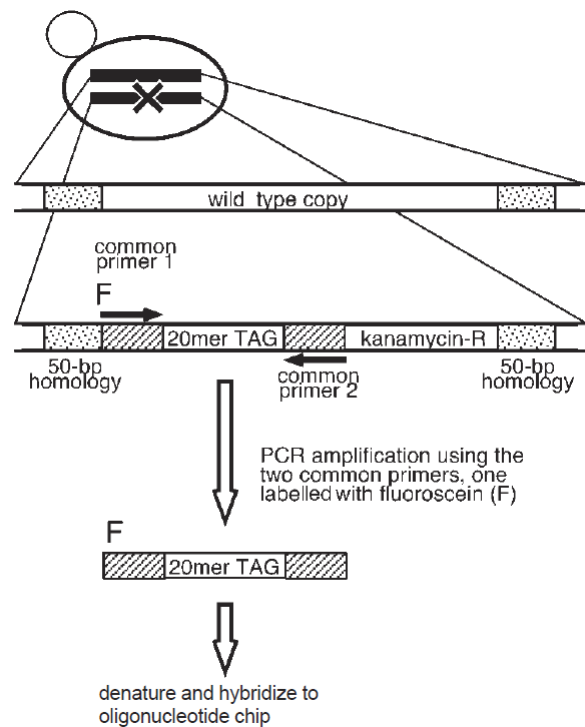


A levedura como modelo de estudo da ação de pequenas moléculas

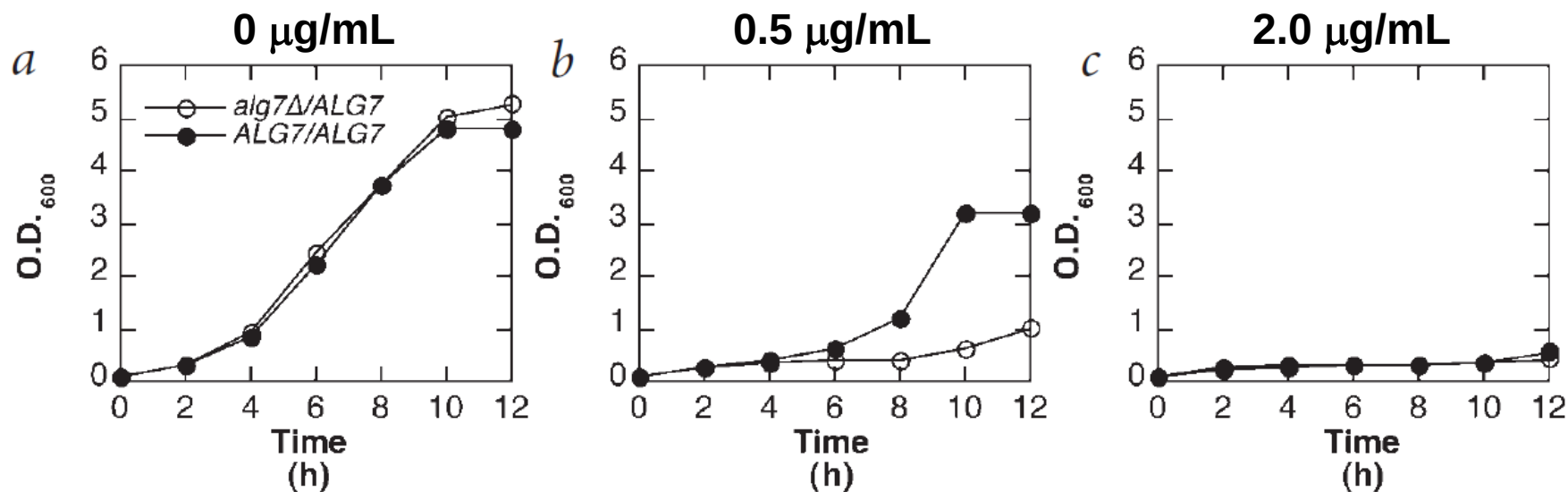
(b)



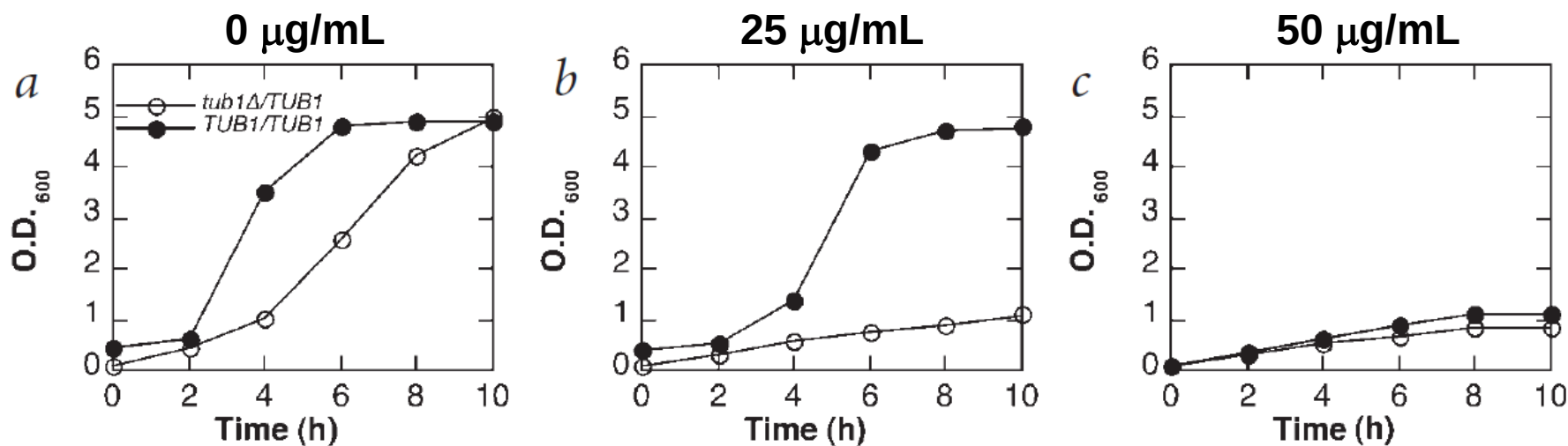
Haploinsuficiência fármaco-induzida



Tunicamycin



Benomyl



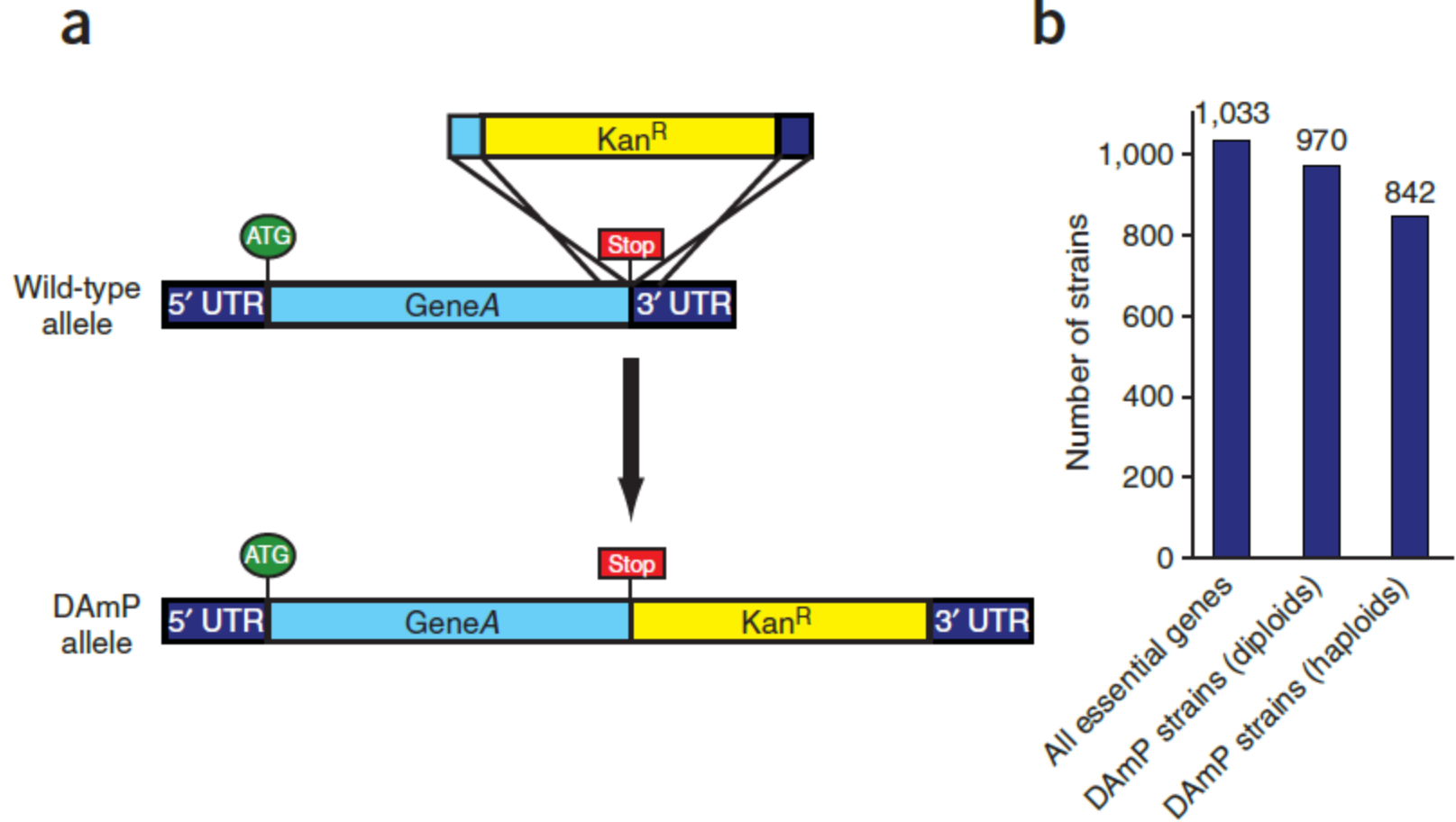
Haploinsuficiência fármaco-induzida

Table 1 • Genes encoding known drug targets used in individual strain analysis

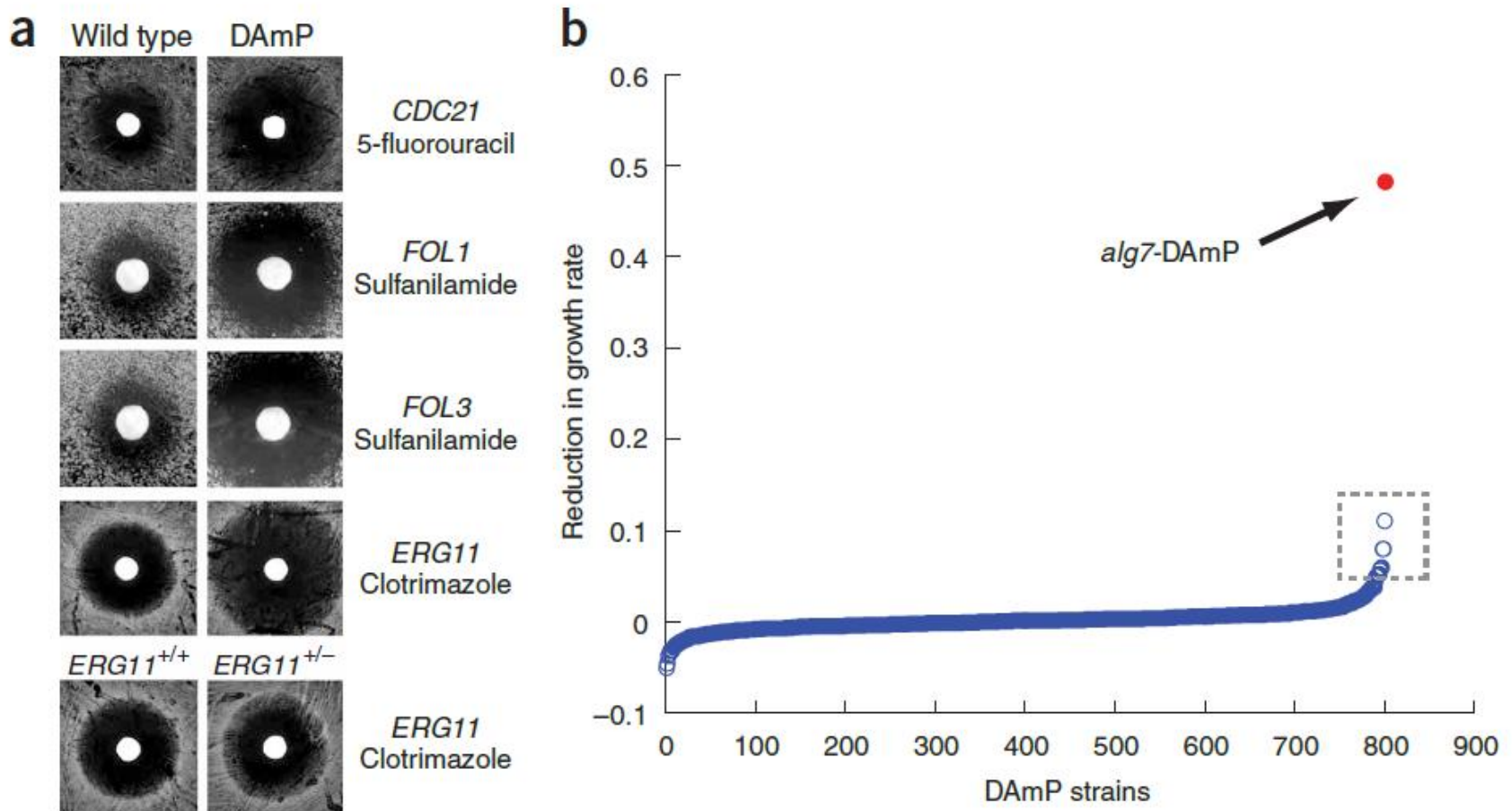
Gene	Protein; protein function	Inhibited by	Haplo-insufficiency*
<i>HIS3</i>	imidazoleglycerol-phosphate dehydratase; required for histidine biosynthesis	3-amino-triazole ²¹	100 mM
<i>ALG7</i>	Asn-linked glycosyl transferase; required for Asn-linked glycosylation	tunicamycin ⁹	0.6 µM
<i>RNR2</i>	small subunit of ribonucleotide reductase; required for nucleotide biosynthesis	hydroxyurea ²²	50 mM
<i>TUB1</i>	α-tubulin structural protein; essential component of mitotic apparatus	benomyl ⁷	85 µM
<i>TUB2</i>	β-tubulin structural protein; essential component of mitotic apparatus	benomyl ⁷	85 µM
<i>ERG11</i>	cytochrome P450 lanosterol 14α-demethylase; required for biosynthesis of ergosterol	fluconazole ²³	40 µM

*Haploinsufficiency: concentration of drug where O.D.₆₀₀ wild type/O.D.₆₀₀ heterozygote was greatest. Gene information obtained from the Saccharomyces Genome Database (<http://genome-www.stanford.edu/Saccharomyces>).

DAmP (decreased abundance by mRNA perturbation) approach form construction of hypomorphic alleles of essential yeast genes

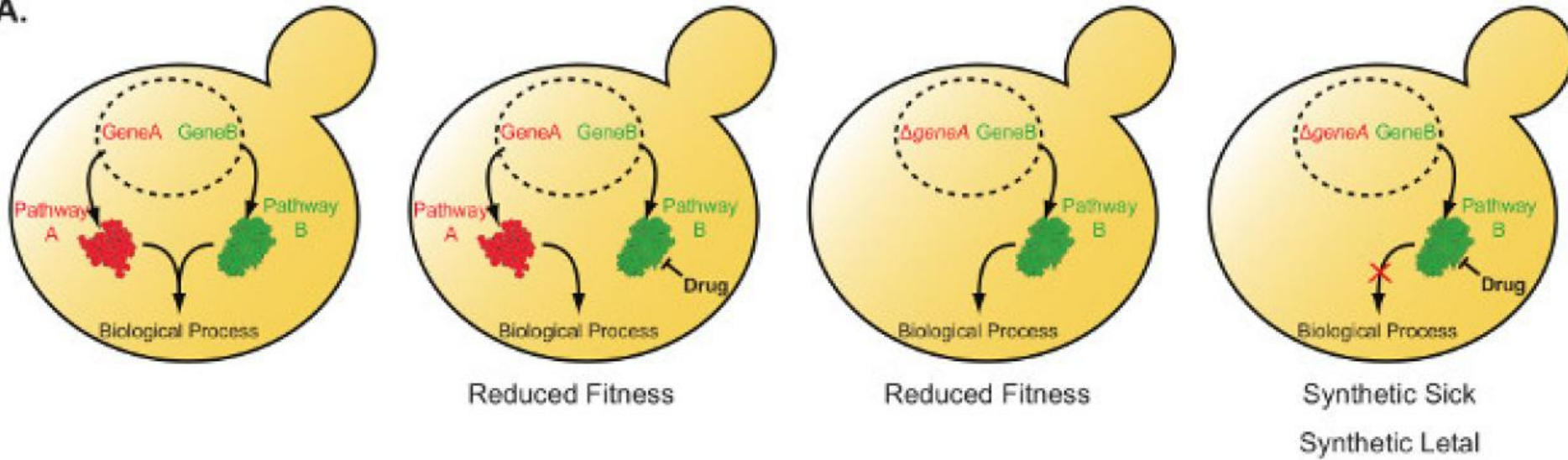


Utility of DAmP strains for identification of drug targets

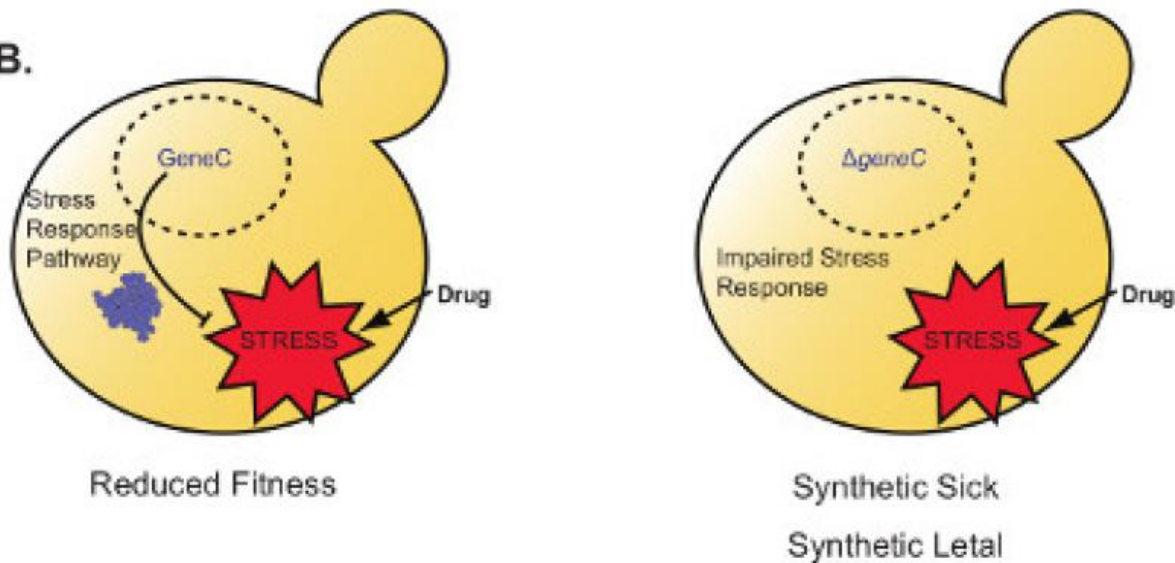


Schematic Representation of Chemical Genetic Interactions

A.

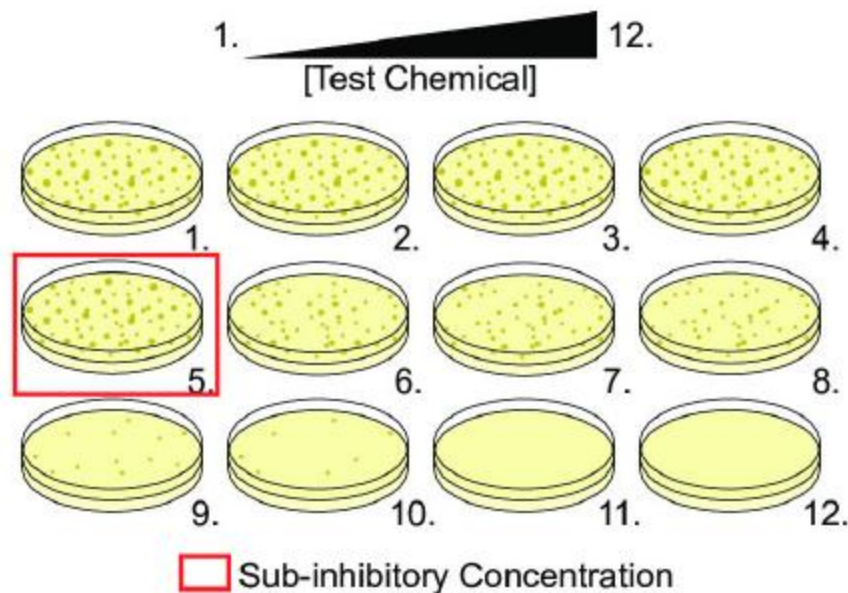


B.



Workflow for Chemical Genetic Screen

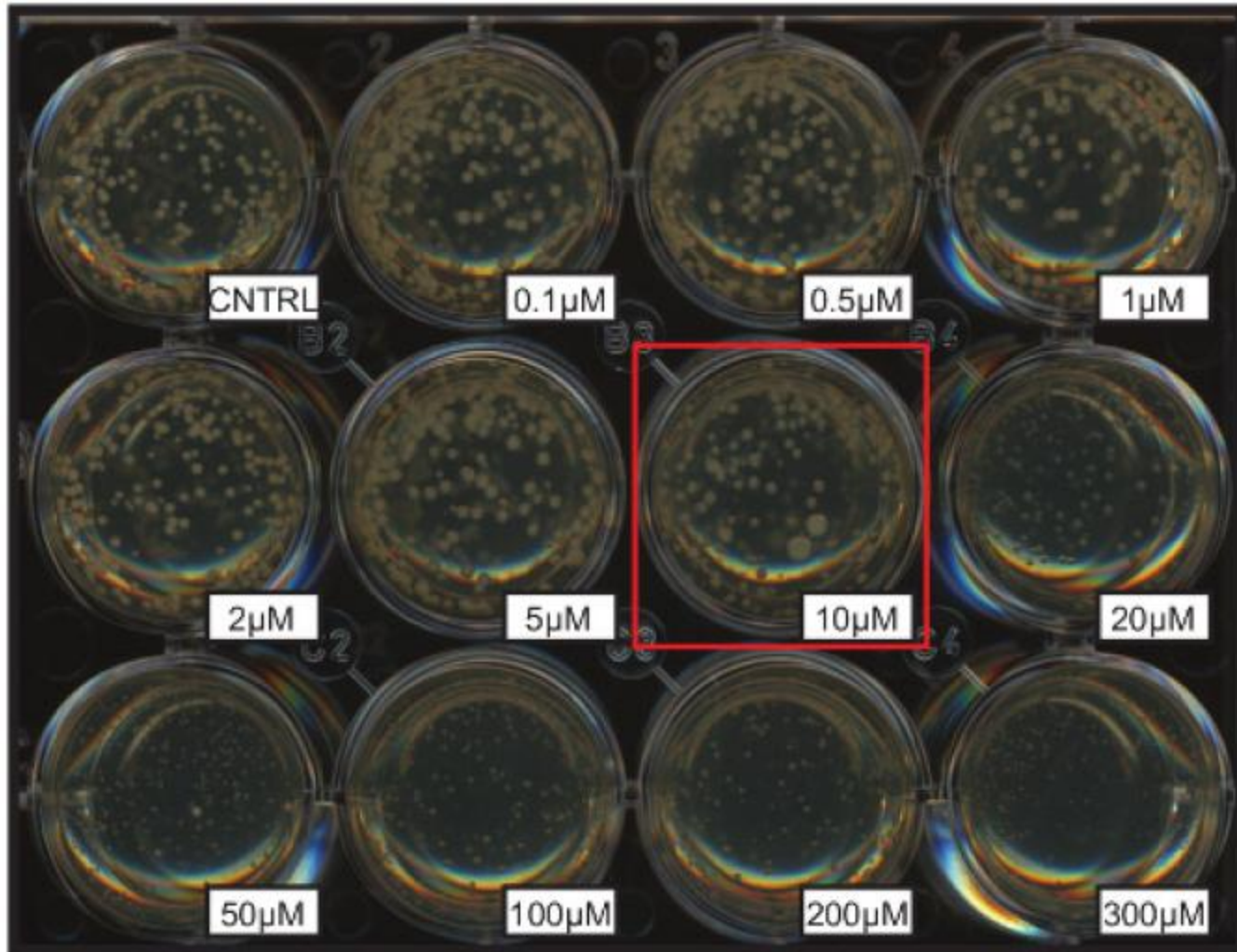
1. Determination of sub-inhibitory dose



Select a sub-lethal concentration of compound that does not inhibit growth by greater than 10%-15% to perform the screen.

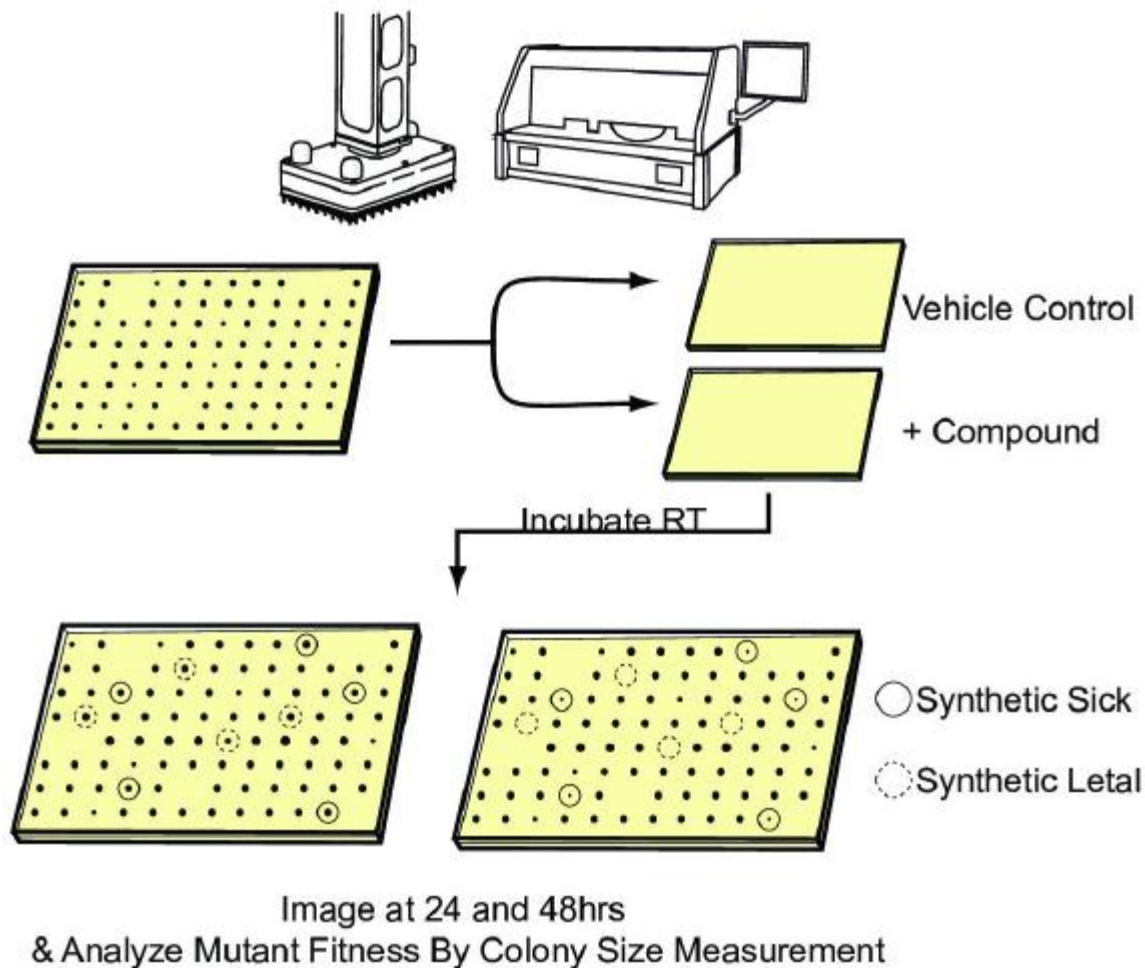
Determination of Sub-Inhibitory Concentration

A.



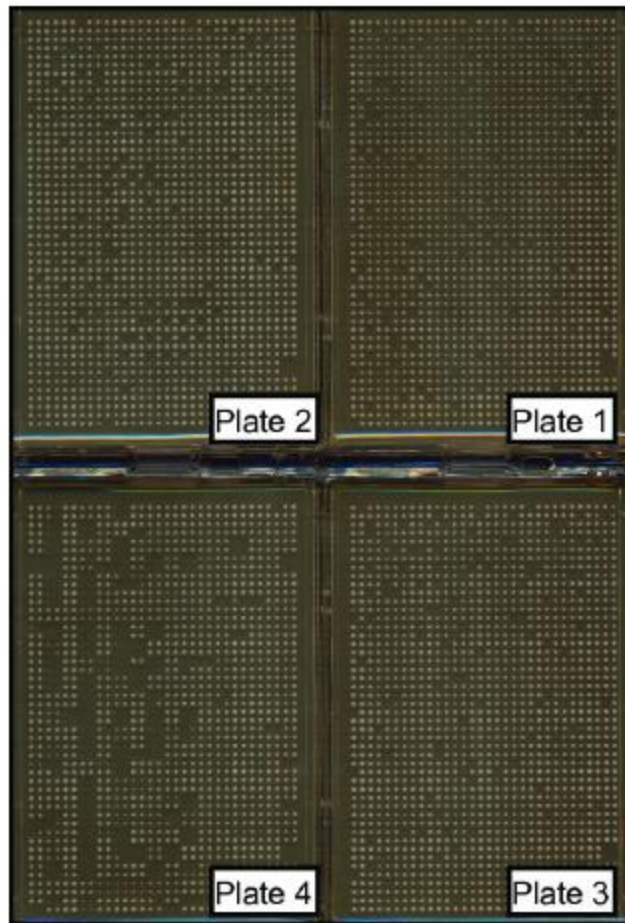
Workflow for Chemical Genetic Screen

2. Systematic Chemical Genetic Screen

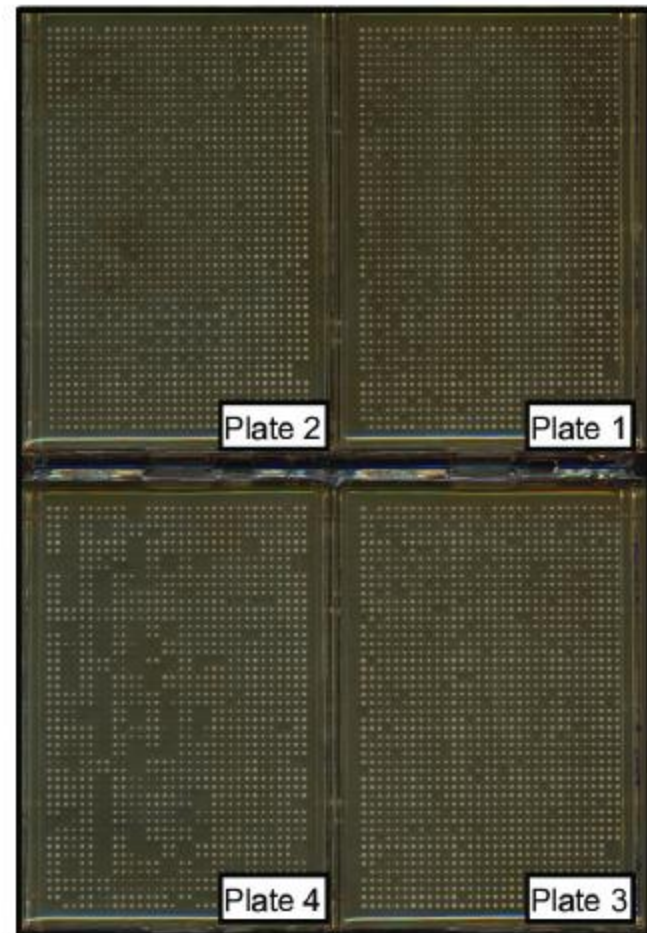


Chemical Genetic Screen of 5-Fluorouracil

A.

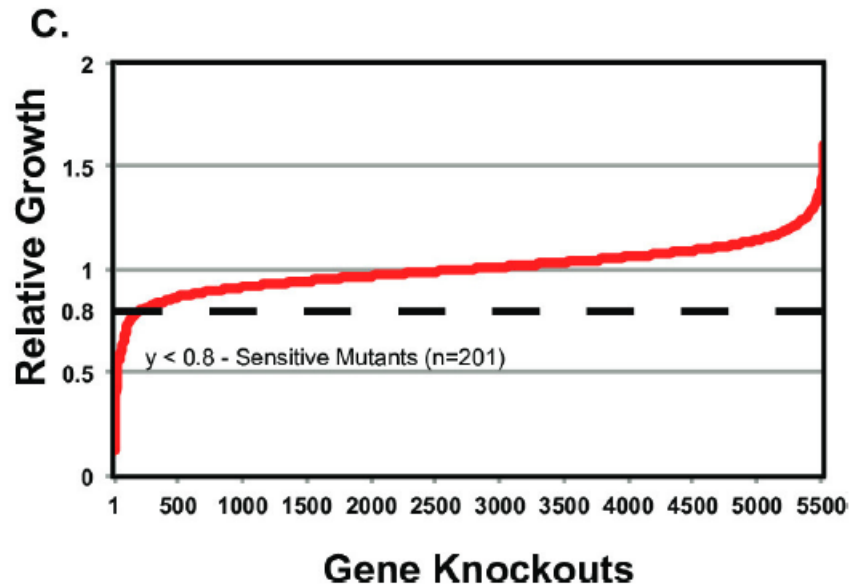
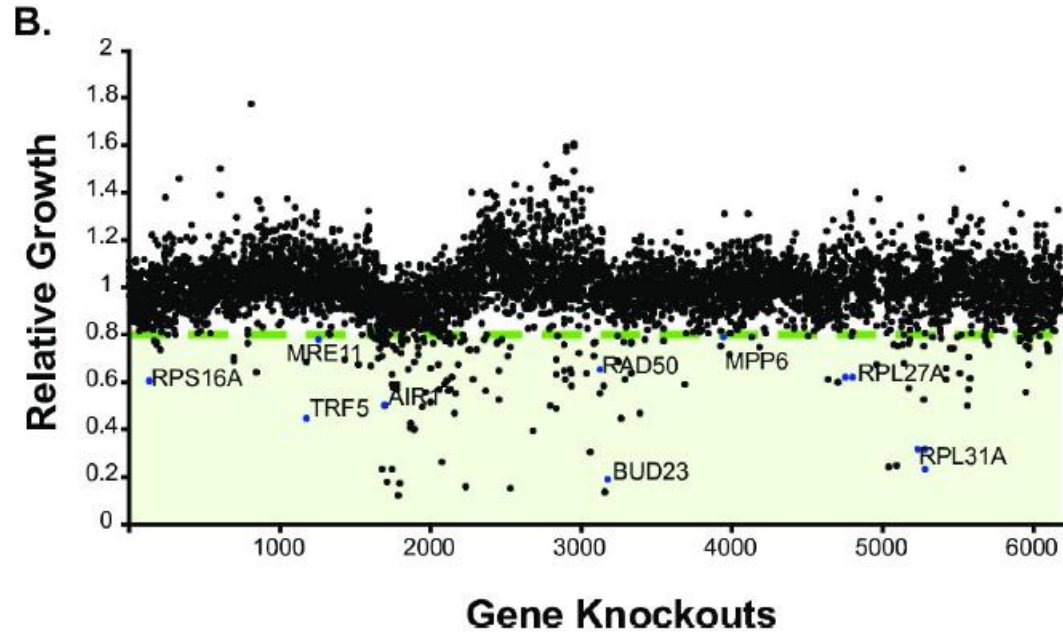


DMSO Control

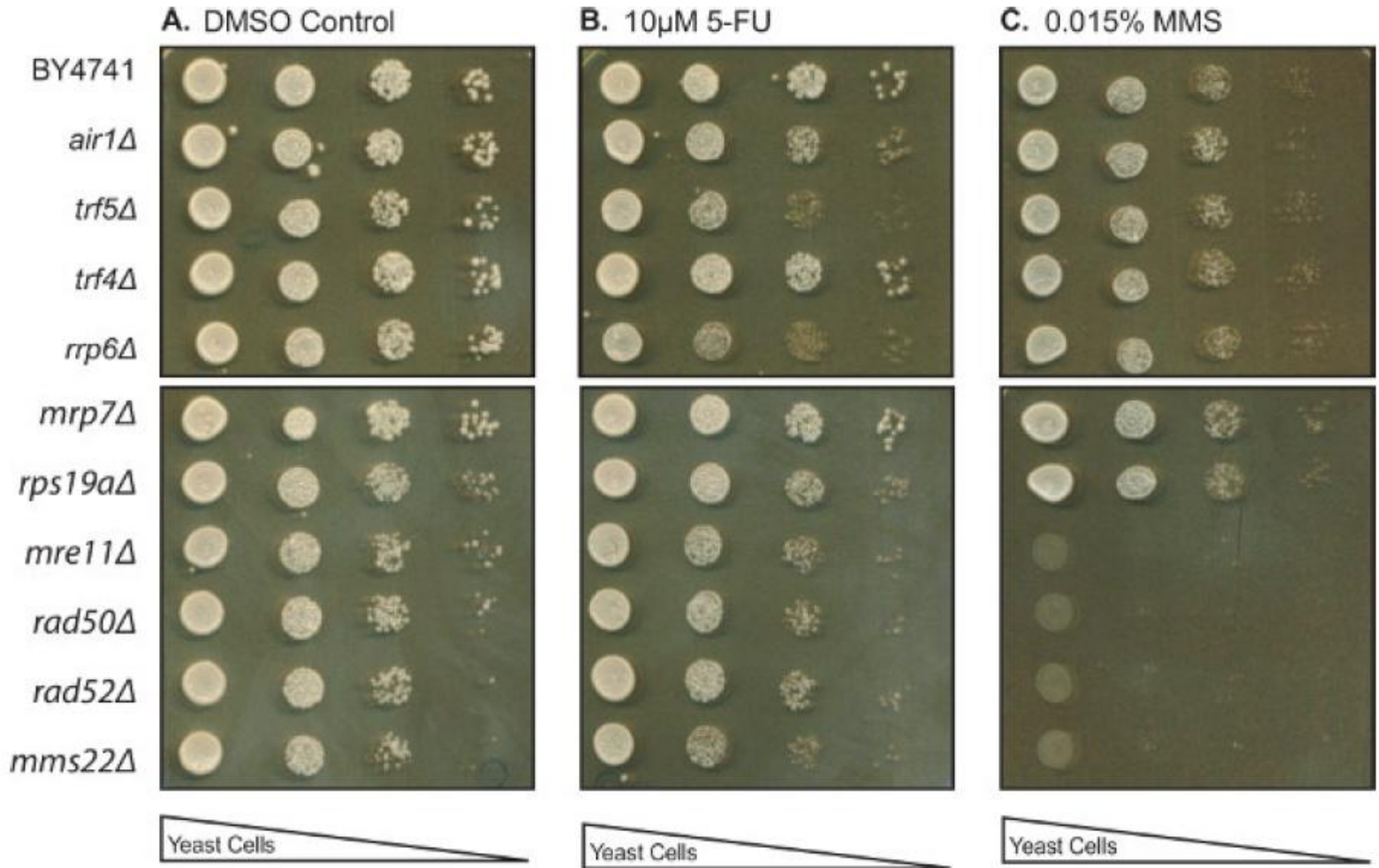


10 μ M 5-FU

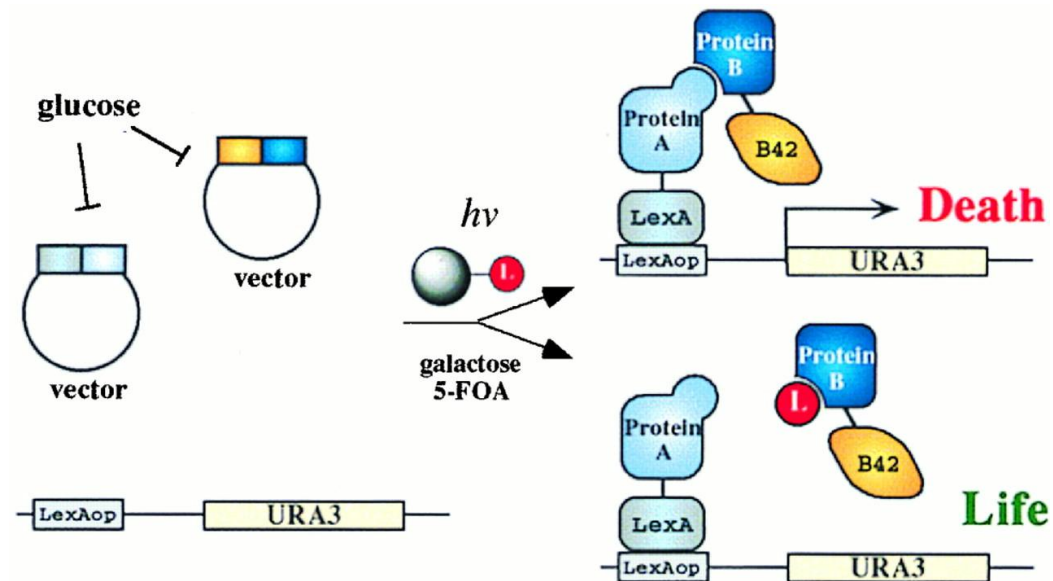
Chemical Genetic Screen of 5-Fluorouracil



Validation of Chemical Genetic Interactions

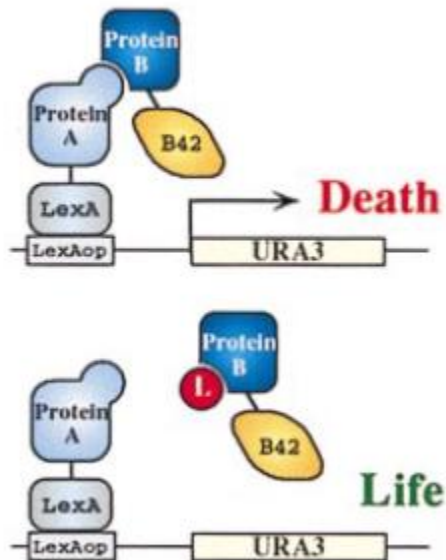
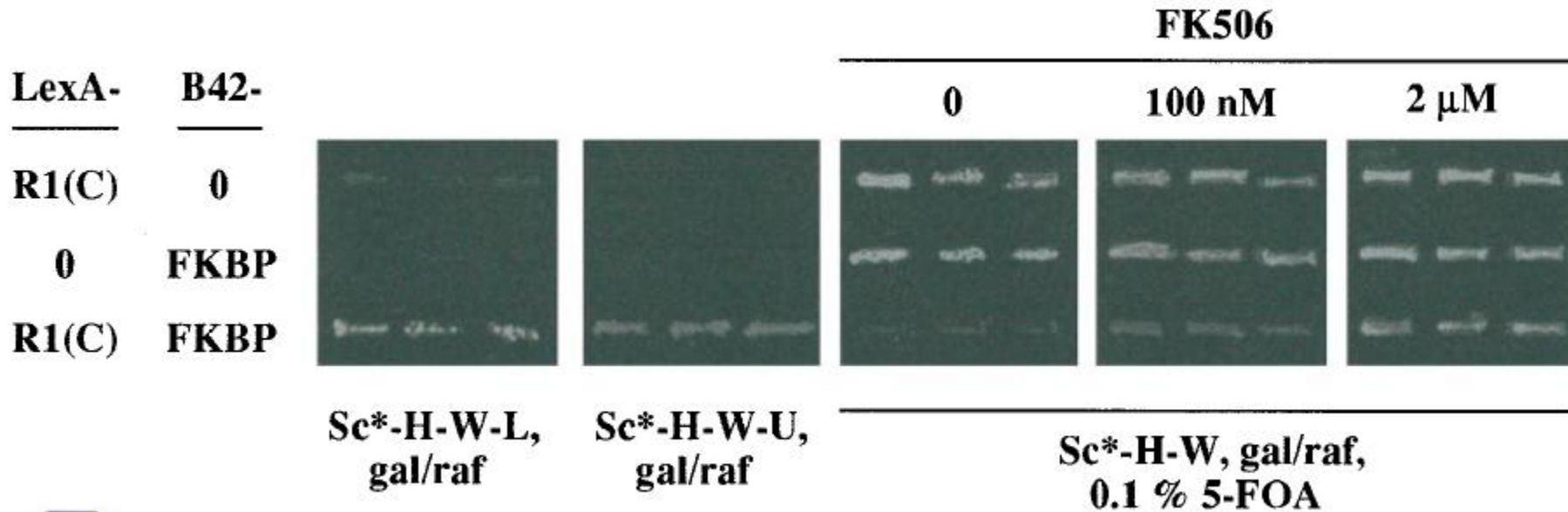


Duplo-híbrido reverso com pequenas moléculas



FK506 prevents interaction between FKBP12 and transforming growth factor β type I receptor R1

A



Resumo

1. Haploinsuficiência fármaco-induzida utiliza cepas diplóides, o código de barras das células, microarray/sequenciamento. Pode-se estudar todas as linhagens em uma única cultura;
2. Rastreamento químico-genético em meio sólido por comparação de tamanho de colônias permite estudar os mutantes individuais. Requer robô e software de análise de imagem.;
3. Sistema Duplo-Híbrido Reverso permite identificar as moléculas que impedem a interação entre duas proteínas.