Evolutionary pathways to collateral sensitivity

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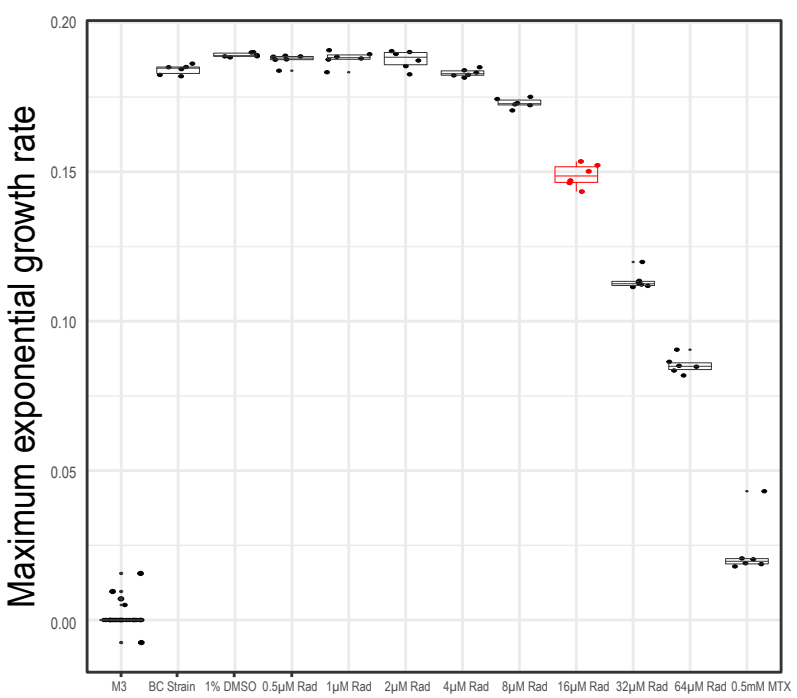
Abstract

Instances of drug resistance have been steadily increasing creating considerable human health and economic impacts. In the United States, the CDC reports that ~35,000 deaths and $55 billion can be attributed to drug resistant infections per year. With resistance outpacing new therapeutics it has become clear that understanding the products of evolution is not enough to develop effective treatment strategies that delay resistance. The process of evolution must also be considered.

Collateral sensitivity (CS), where developing resistance to drug A results in sensitivity to drug B, is a strategy that could be used to combat drug resistance. At present, studies in both cancer cells and bacteria populations have demonstrated that collateral sensitivity is unpredictable and nonrepeatable. This can be attributed to the wide array of mutations that can occur under the stress of the drug A and the low replicate size used in the experiments. Here, we propose using a barcoded yeast system to track a large population of yeast as they develop resistance to drug A then are subsequently challenged by drug B. This system tracks hundreds of thousands of replicate yeast lineages, thus revealing the full spectrum of adaptive mutants that protect against drug A while maintaining susceptiblity to drug B. Experiments using this system will provide a more quantitative understanding of the likelihood of collateral sensitivity, as well as the evolutionary paths that lead to collateral sensitivities.

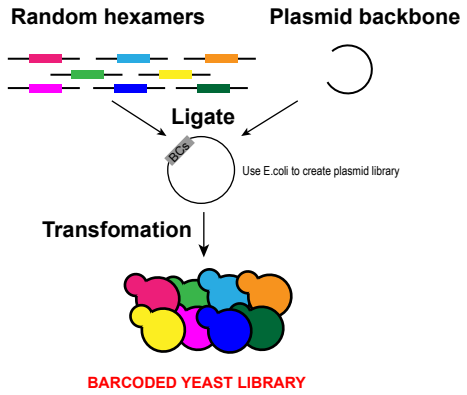
DRUG A DETERMINATION



Radicicol concentration for initial evolution 16μM

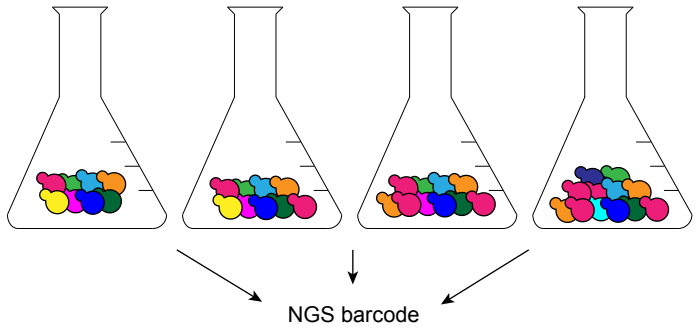
WORKFLOW

Starting barcoded population ~500,000 unique barcodes



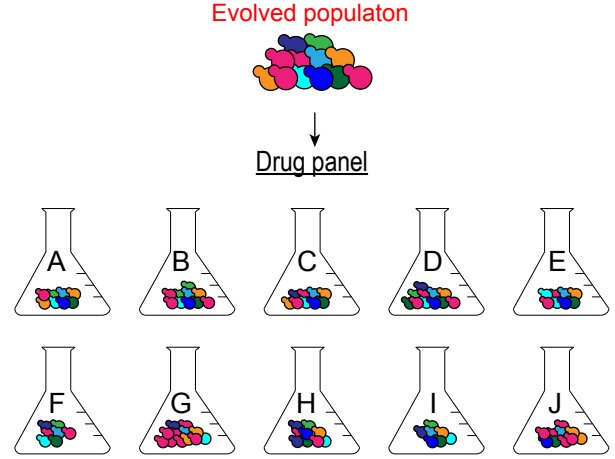
Evolve population in drug A

Transfer every 48 hours for 6 weeks



Assess sensitivity in drug B

Evolved populaton



Transfer every 48 hours for one week

Determine barcode frequencies and assess sensitivity

Drug B panel

	Drug	Mechanism of action
(A)	Cycloguanil	Dihydrofolate reductase inhibitor
(B)	Methotrexate	Dihydrofolate reductase inhibitor
(C)	Pyrimethamine	Dihydrofolate reductase inhibitor
(D)	Trimethoprim	Dihydrofolate reductase inhibitor
(E)	Flucytosine	DNA/RNA synthesis inhibitor
(F)	Benomyl	Fungicide
(G)	Geldanamycin	Hsp90 inhibitors
int	Radicicol	Hsp90 inhibitors
(H)	Amphotericin B	Membrane disruption
(I)	Fluconazole	Membrane synthesis inhibition
(J)	Cycloheximide	Protein synthesis inhibitor

EXPECTED OUTCOMES

Reveal spectrum of mutations that result in CS to drug B

Collateral sensitivity results from drug panel