



Pitt
Medicine

The Carvunis Lab

LI Detector: Measuring Small Fitness Effects in High Throughput

Saurin Parikh

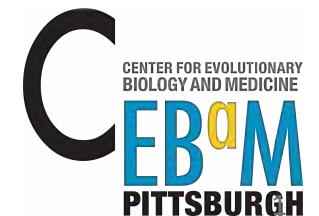
Carvunis Lab

Department of Computational and Systems Biology

Pittsburgh Center for Evolutionary Biology and Medicine

University of Pittsburgh School of Medicine

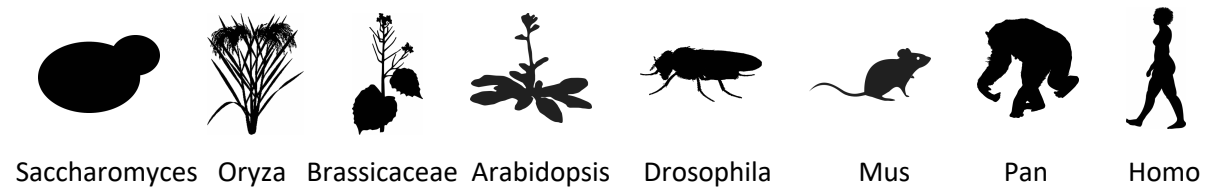
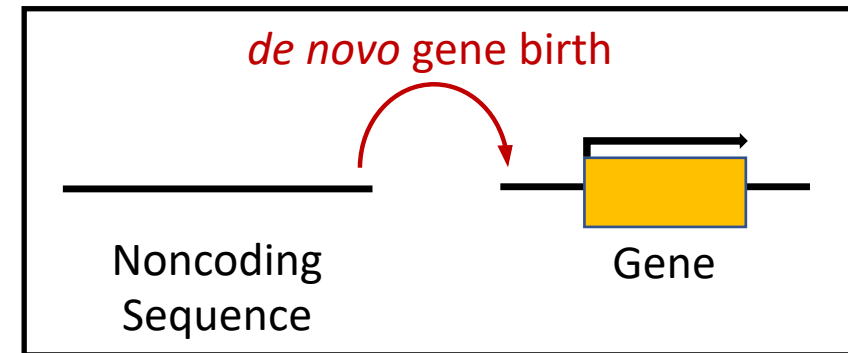
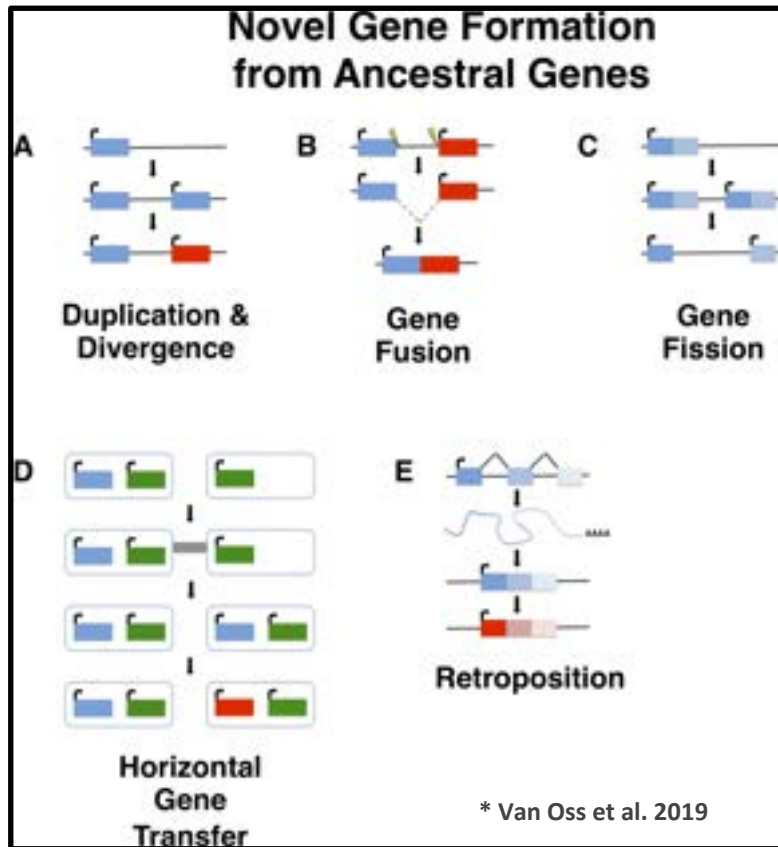
INTegrative **S**YSTEMS **B**IOLOGY



Overview

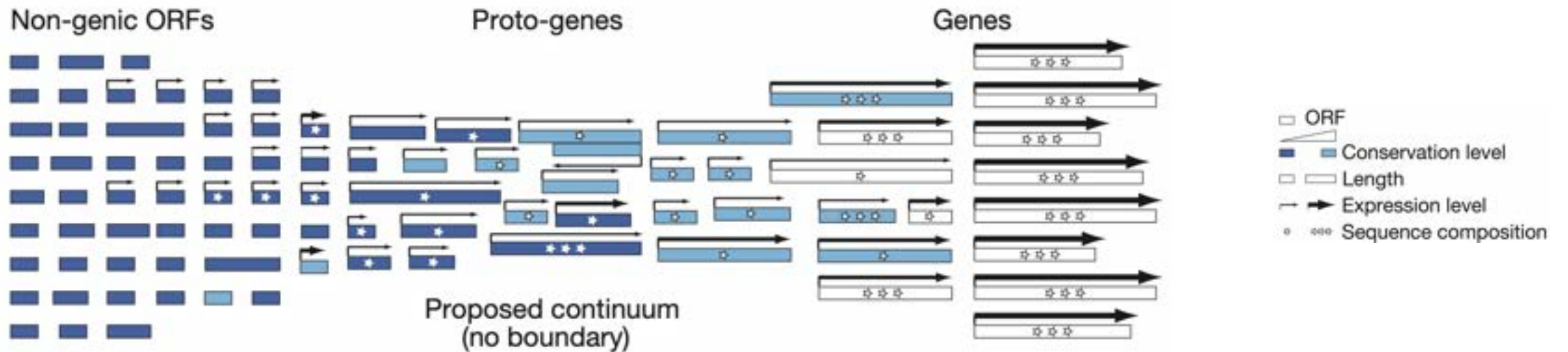
1. Background
2. Colony based high-throughput screens (**CBHTS**)
3. Problem of **spatial bias** in CBHTS
4. How does **LI Detector** address this issue?

Where do novel genes come from?



*inspired from Van Oss et al. 2019

Proto-gene model of de novo gene birth



*Carvunis et al. 2012

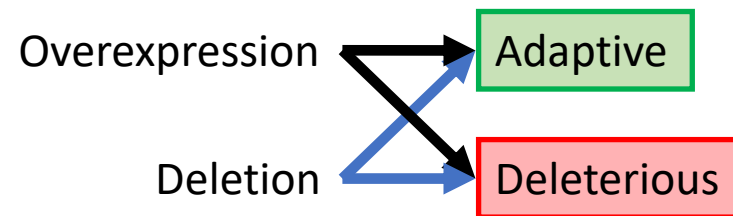
Proto-genes and yeast!

- There are 1891 proto-genes in *S. cerevisiae* genome (Carvunis et al. 2012)

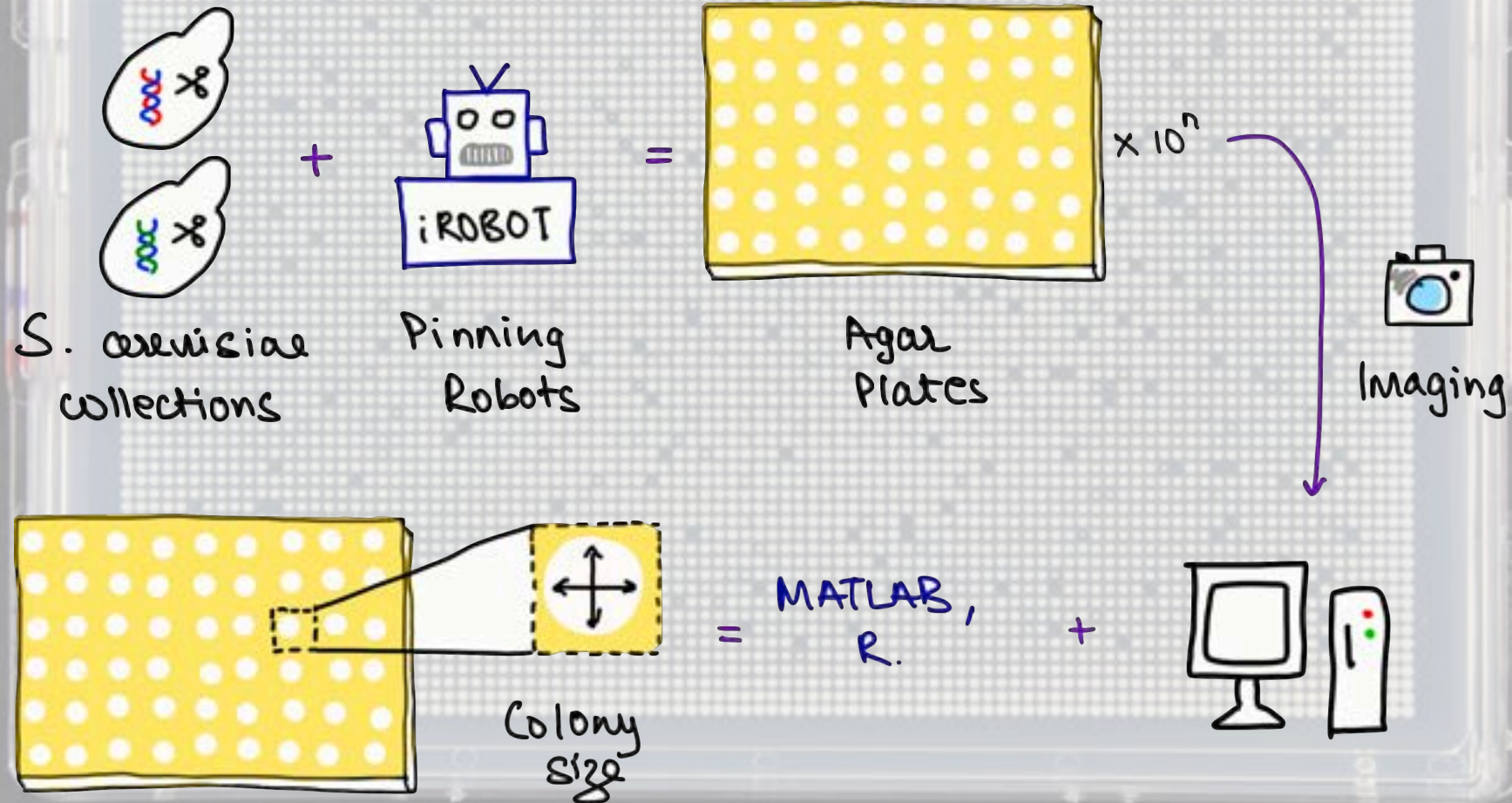


S. cerevisiae

- I want to characterize the phenotypic impact of these proto-genes!



Colony-based high-throughput screens (CBHTS)



CBHTS have been widely used

RESEARCH ARTICLE
Global Mapping of the Yeast Genetic Interaction Network
Amy Hin Yan Tong^{1,2,*}, Guillaume Lepage^{1,2}, Gary D. Bader¹, Huiming Ding¹, Hong Xu^{1,2}, Xiaofeng Xie^{1,2}, James Young¹, Gab...

Science 06 Feb 2004
Vol. 303, Issue 5695, pp. 808-813
DOI: 10.1126/science.1091317

RESEARCH ARTICLE
The Genetic Landscape of a Cell

Michael Costanzo^{1,2,*}, Anastasia Baryshnikova^{1,2,*}, Jeremy Bellay³, Yungil Kim³, Eric D. Spear⁴, Cam...

Science 22 Jan 2010
Vol. 327, Issue 5944, pp. 429-431
DOI: 10.1126/science.1188823

RESEARCH ARTICLE
A global genetic interaction network maps a diagram of cellular function

Michael Costanzo^{1,2}, Benjamin VanderSluis^{1,2,*}, Elizabeth M. Koch^{1,2}, Anastasia Baryshnikova^{1,2}, Cam...

Science 23 Sep 2010
Vol. 328, Issue 5966, pp. 1420-1423
DOI: 10.1126/science.1194230

RESEARCH ARTICLE
Conservation and Rewiring of Functional Modules Revealed by an Epistasis Map in Fission Yeast

Assen Rogen^{1,2}, Sourav Banerjee^{1,2}, Martin Zeflat¹, Ke Zhang¹, Tamas Fischer¹, Sean R. Collins^{1,2,3}, Hongling Qi^{1,2}, ...

Science 17 Oct 2008
Vol. 322, Issue 5900, pp. 405-410
DOI: 10.1126/science.1162609

A comprehensive analysis of protein-protein interactions in *Saccharomyces*



Article | [Open Access](#) | Published: 07 February 2020

De novo emergence of adaptive membrane proteins from thymine-rich genomic sequences

Nikolaos Vakirlis, Omer Acar, Brian Hsu, Nelson Castilho Coelho, S. Branden Van Oss, Aaron Wacholder, Kate Medetgul-Ernar, Ray W. Bowman II, Cameron P. Hines, John Iannotta, Saurin Bipin Parikh, Aoife McLysaght, Carlos J. Camacho, Allyson F. O'Donnell , Trey Ideker  & Anne-Ruxandra Carvunis 

Nature Communications 11, Article number: 781 (2020) | [Cite this article](#)

mapping and high-content screening to explore yeast spindle morphogenesis

Franco J. Vizeacoumar, Nydia van Dyk, Frederick S. Vizeacoumar, Vincent Cheung, Jingling Li, Yaroslav Sydorskiy, Nicole Case, Zhijian Li, Alessandro Datti, Corey Nislow, Brian Raught, Zhaoxi Zhang, Brendan Frey, Kerry Bloom, Charles Boone , Brenda J. Andrews 

+ Author and Article Information

J Cell Biol (2010) 188 (1): 69–81. | <https://doi.org/10.1083/jcb.200909013> | [Article history](#) 

Integration of chemical-genetic and genetic interaction data links bioactive compounds to cellular target pathways

Alastair R. Parsons, Renée L. Brost, Huiming Ding, Zhijian Li, Chaoying Zhang, Bilal Sheikh, Grant W. M. Kane, Timothy R. Hughes & Charles Boone 

Technology 22, 62–69 (2004) | [Download Citation](#) 



Vol. 22, 11 August 2004, Pages 611–625

Defining the Mode-of-Action of Bioactive Compounds by Chemical-Genetic Profiling in

Li, A. H., Andres Lopez^{1,2,3}, Inmar E. Givoni^{1,2,3,4,5,6,7,8}, David E. Williams¹, Christopher A. Gray¹, Gordon Chua¹, Michelle Sopko^{1,2}, Renée L. Brost¹, Cheuk-Hoi Ho^{1,2}, Jiyi Wang¹, Troy Ketela¹, ...

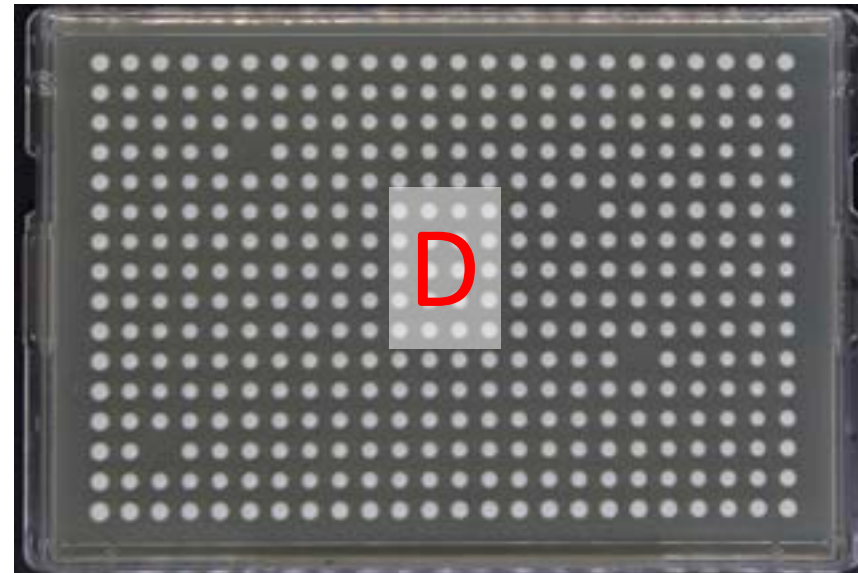
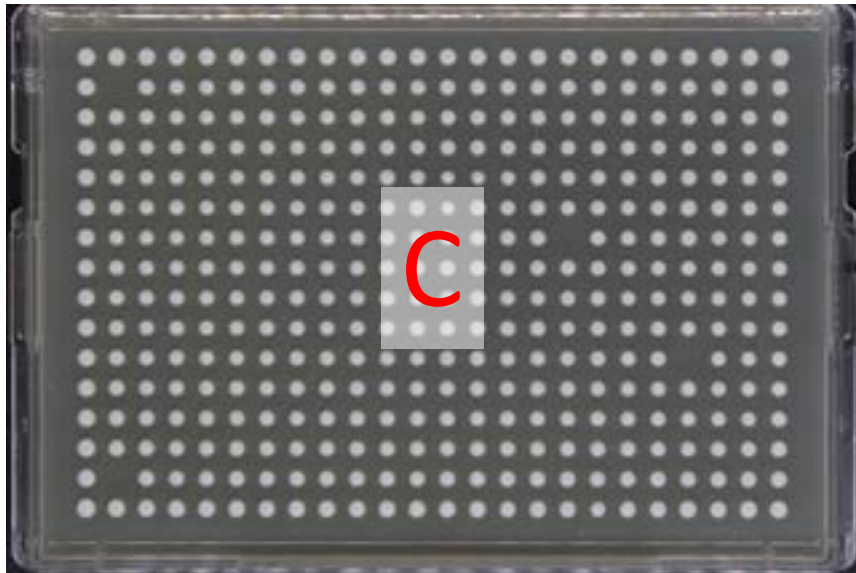
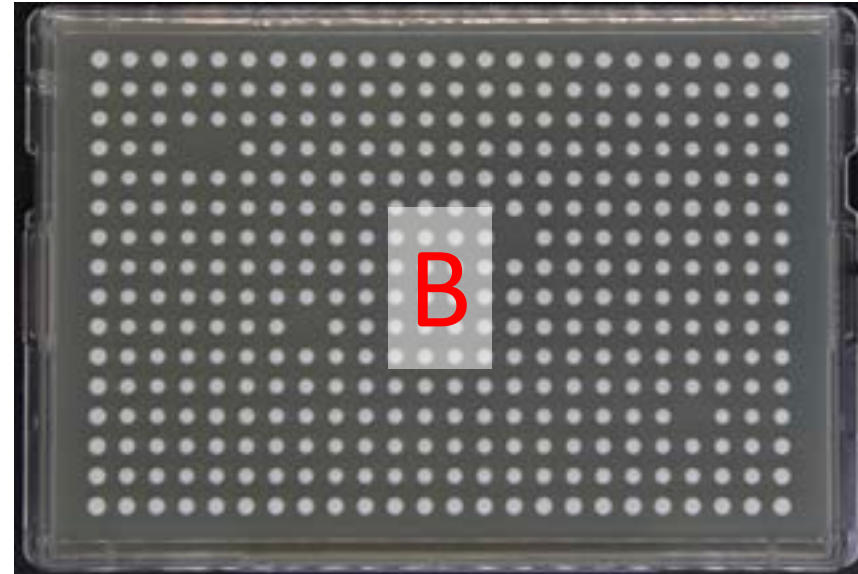
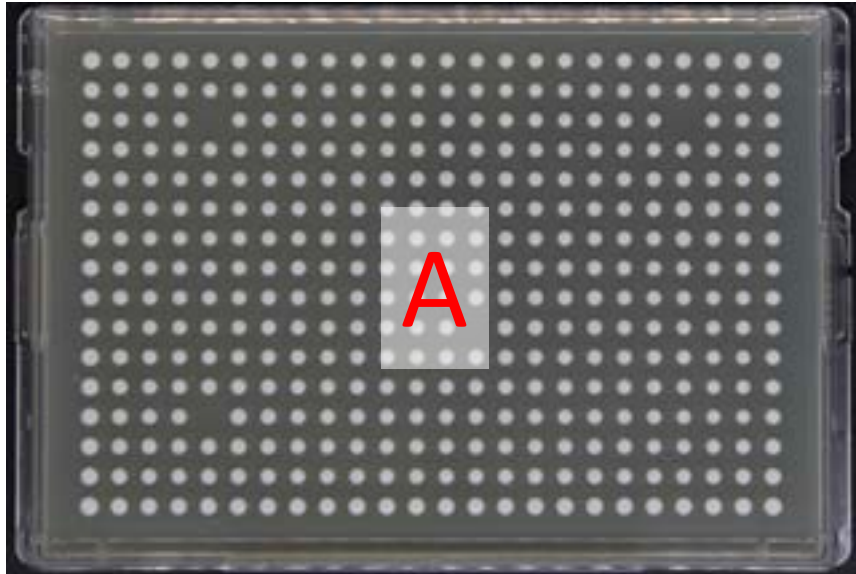
Published: 01 August 2008

eSGA: *E. coli* synthetic genetic array analysis

Gareth Butland, Mohan Babu, [...] Andrew Emili 

Nature Methods 5, 789–795(2008) | [Cite this article](#)

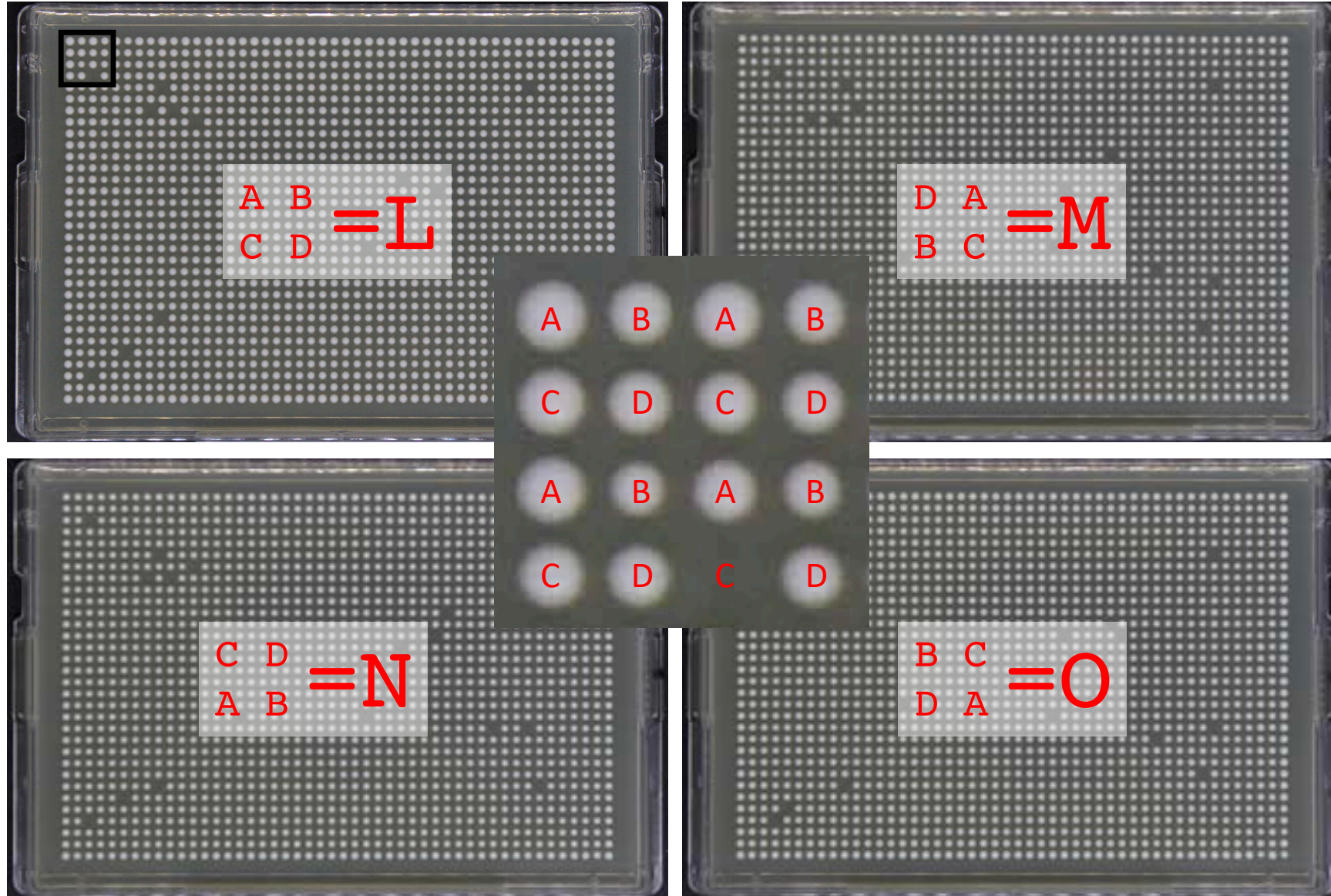
384 - Density Plates



Strain: FY4
Media: YPD

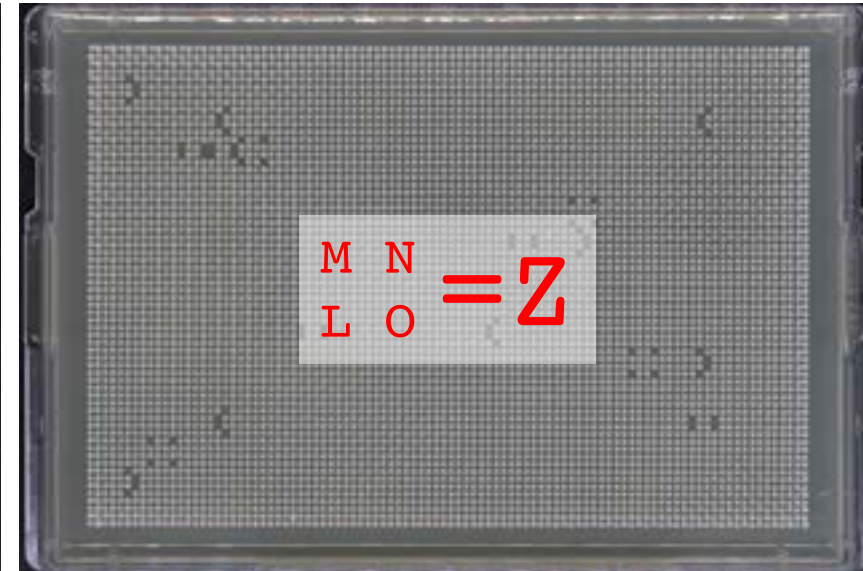
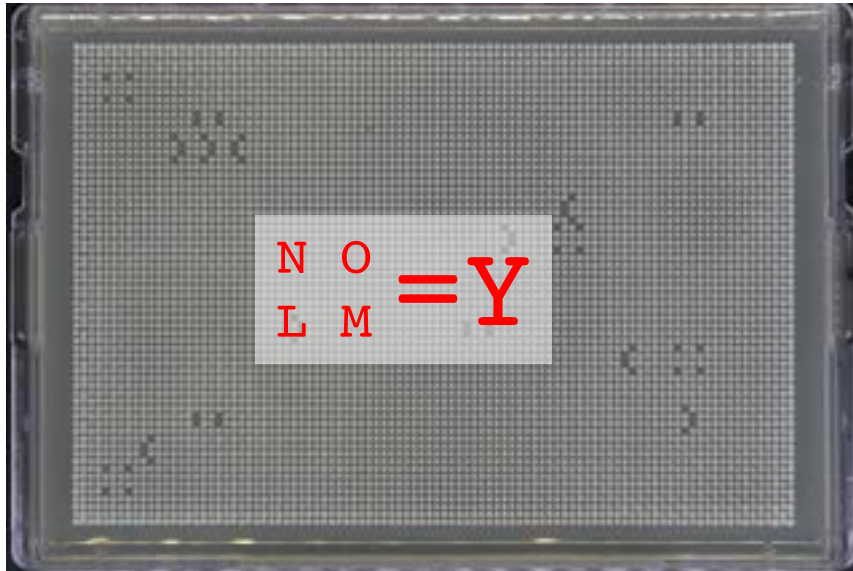
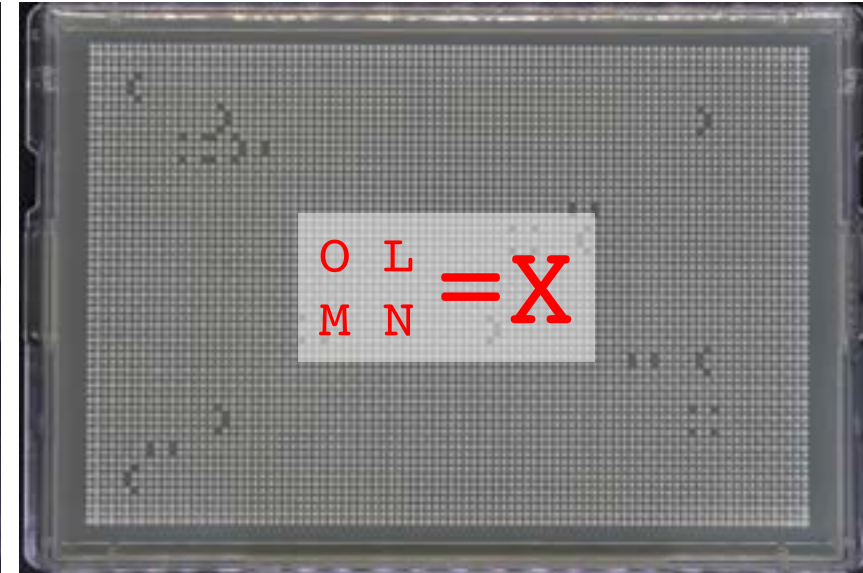
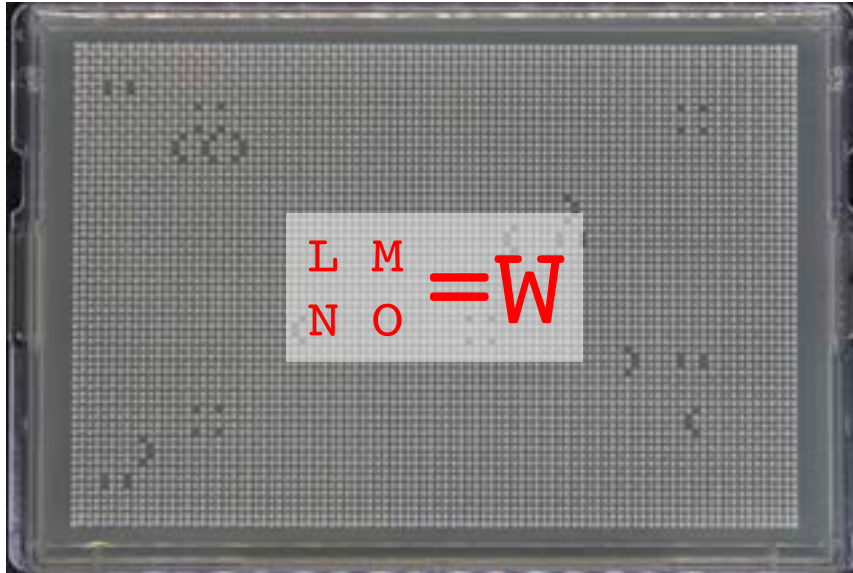
384-density glycerol stocks >> 384-density agar plates

1536 - Density Plates



Strain: FY4
Media: YPD

6144 - Density Plates



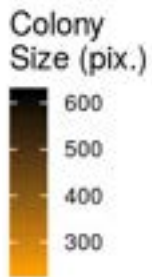
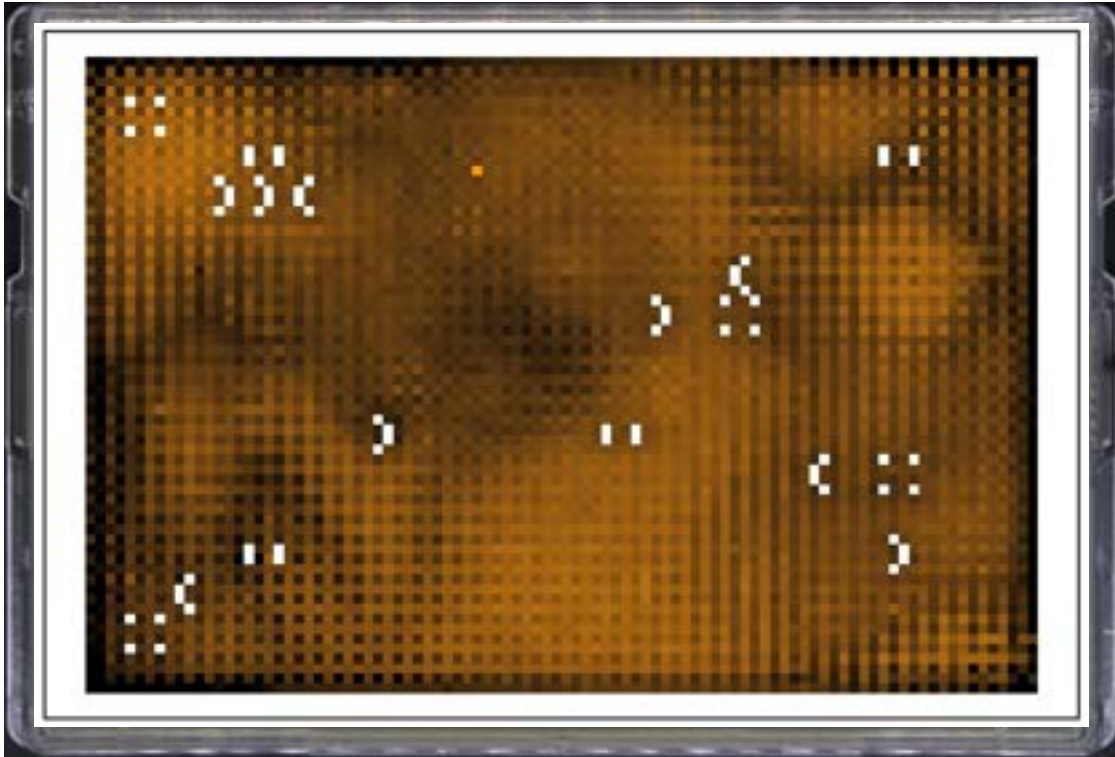
Strain: FY4
Media: YPD

Any questions so far?

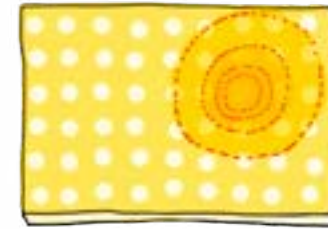
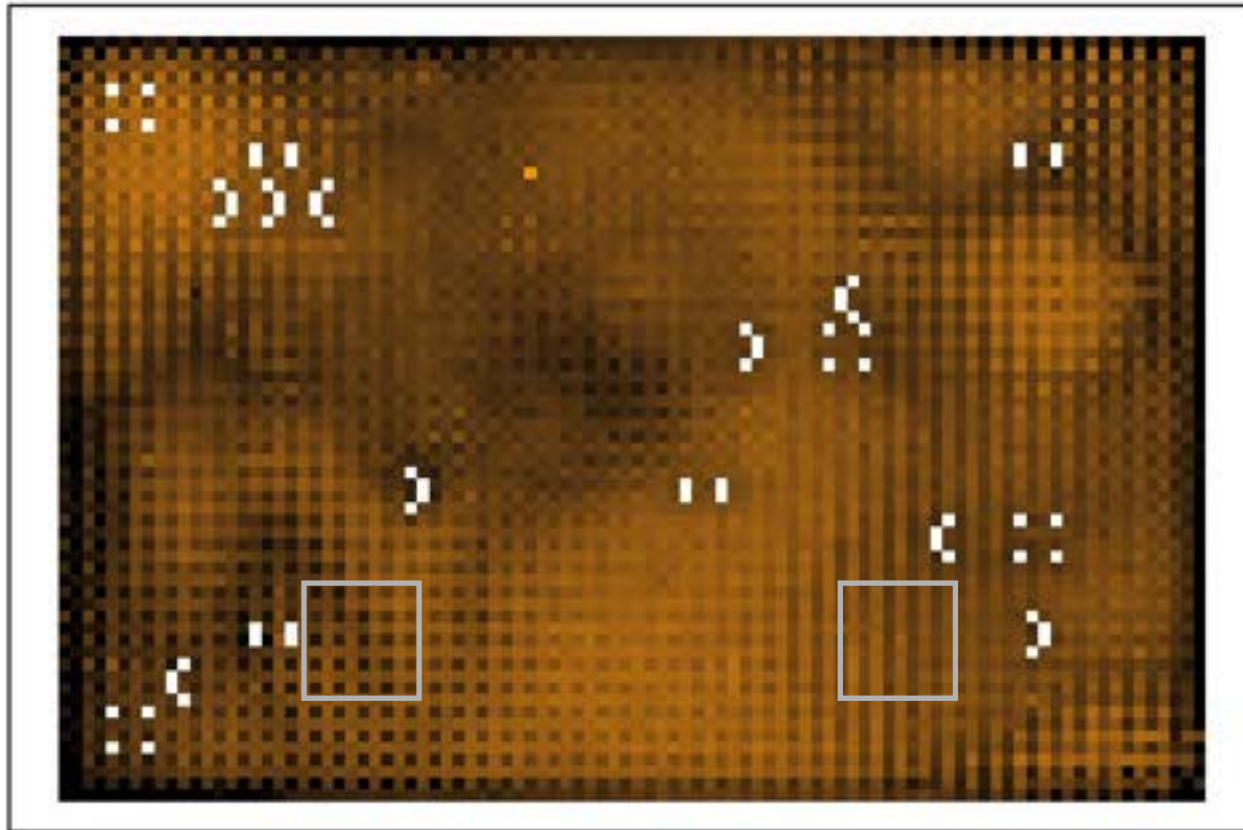
1. Background
2. Colony based high-throughput screens (**CBHTS**)
3. Problem of **spatial bias** in CBHTS
4. How does **LI Detector** address this issue?

Spatial Bias

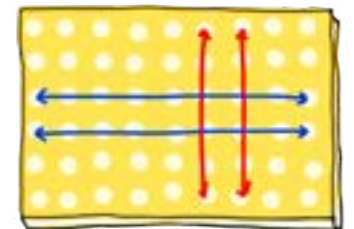
systematic differences in colony sizes that arise due to technical reasons



Varieties of spatial bias



Agar Surface



Row / Column

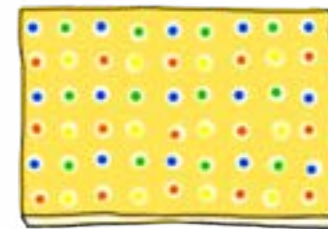
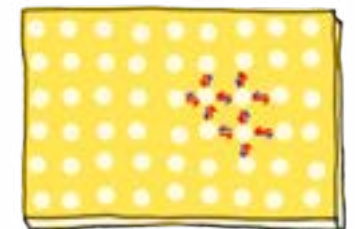


Plate History



Neighbor Colony

Correcting for spatial bias is a huge challenge

*“The environment can rarely be maintained constant ~~across plates~~”
on a plate”*


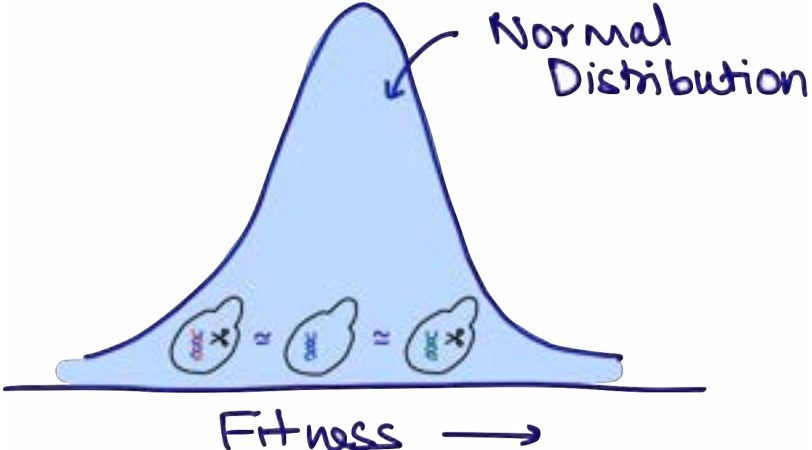
- Zackrisson et al. 2016

- Saurin Parikh

Existing Methods of Spatial Bias Correction

Assumptions

Limitations

1.	 <p><i>mutant #1</i> <i>wild type</i> <i>mutant #2</i></p>	<ul style="list-style-type: none"> • Small fitness effects cannot be observed
2.	 <p><i>Normal Distribution</i></p> <p><i>Fitness</i> →</p>	<ul style="list-style-type: none"> • Screens with fewer plates • Screens with fewer mutants • Conditions producing polarizing fitness effects • Testing the uncharacterized genome • Anything less than a genome-wide screen in 'normal' condition

Any questions?

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Hypothetical (!) Scenario

Spatial Context



Observation



20 mph



10 mph



10 mph



5 mph

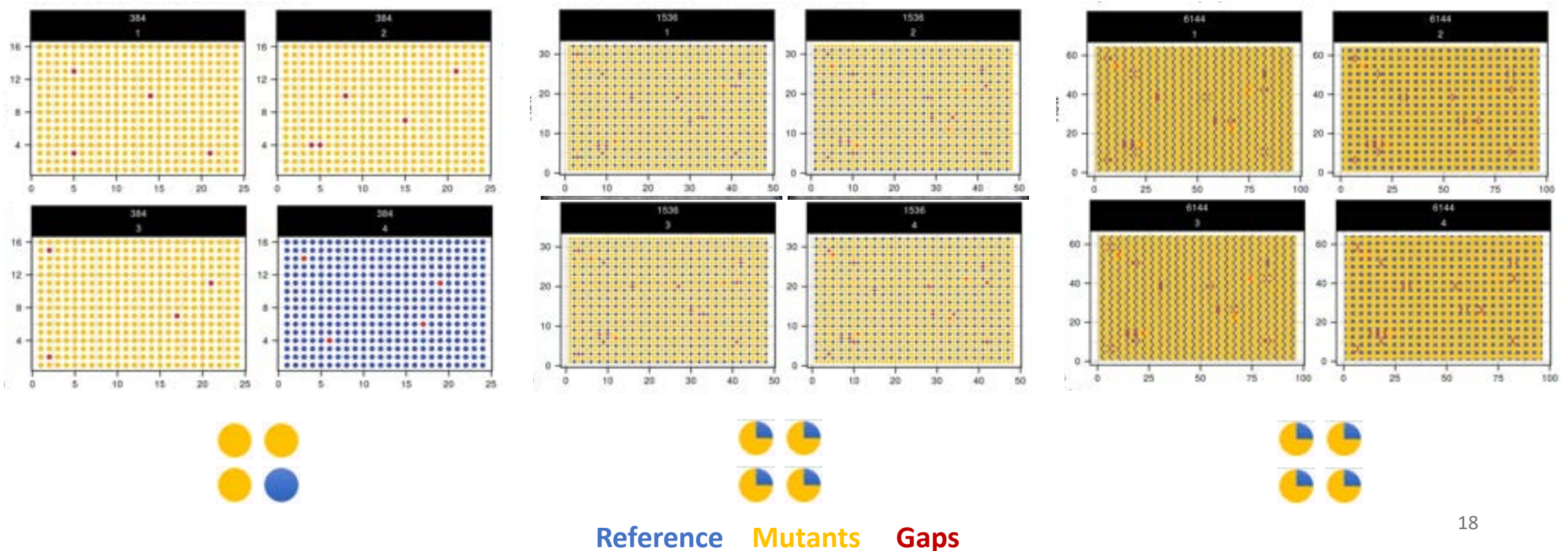
Relative Fitness

Saurin/Usain = 0.5

Nelson/Usain = 0.5

How to apply this concept in CBHTS?

1. Find a way to introduce a reference population

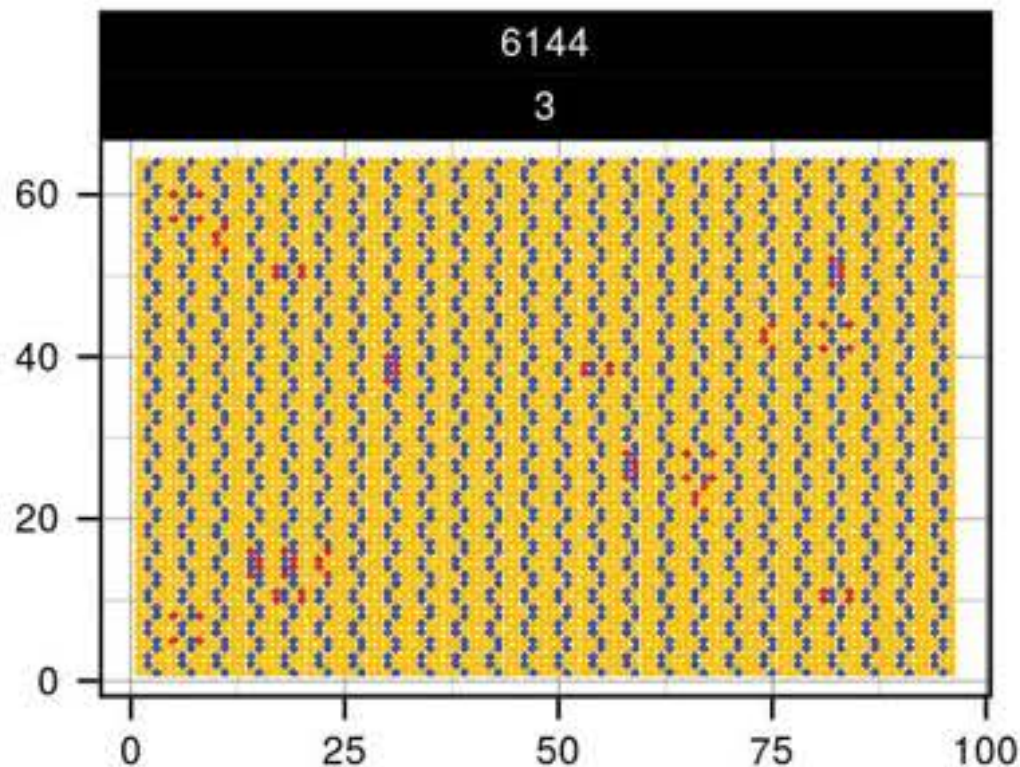


How to apply this concept in CBHTS?

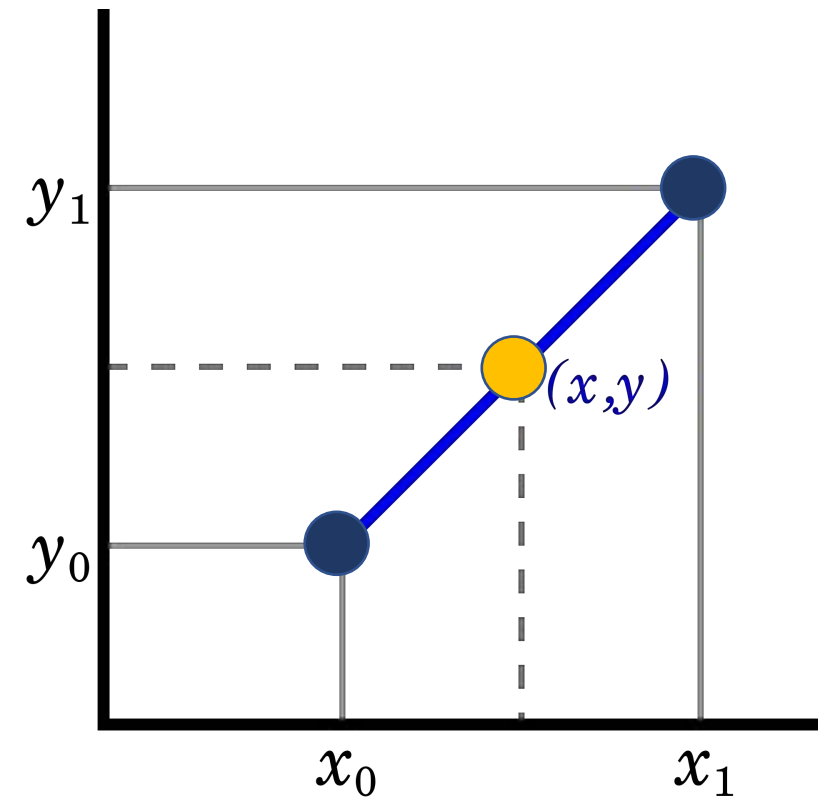
1. Find a way to introduce a reference population



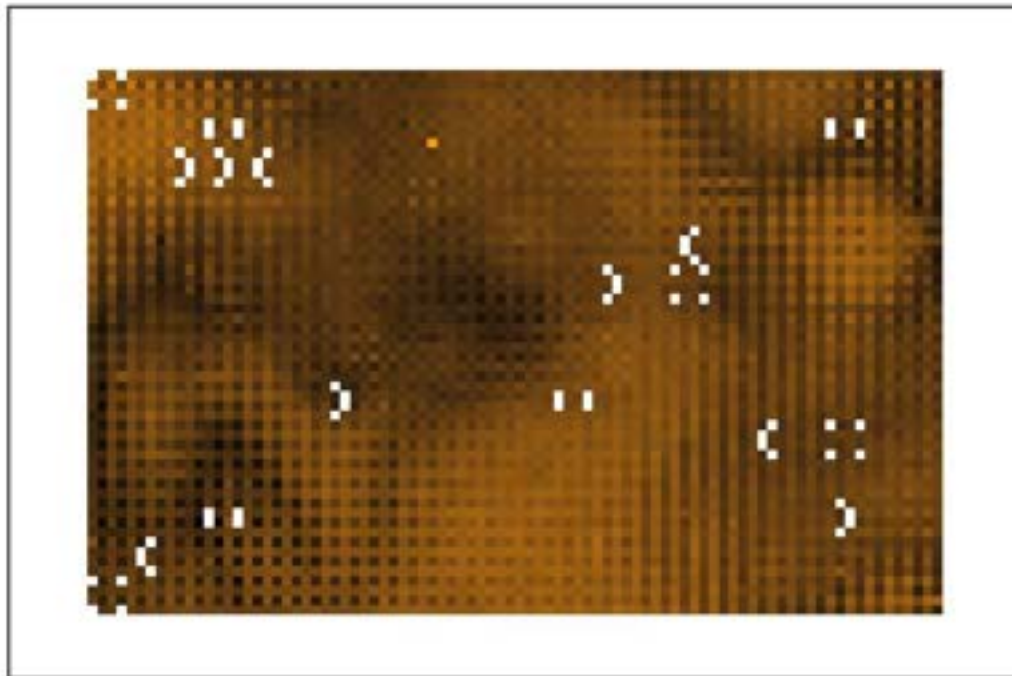
2. Find a way to use that to correct spatial bias



Reference
Mutants
Gaps

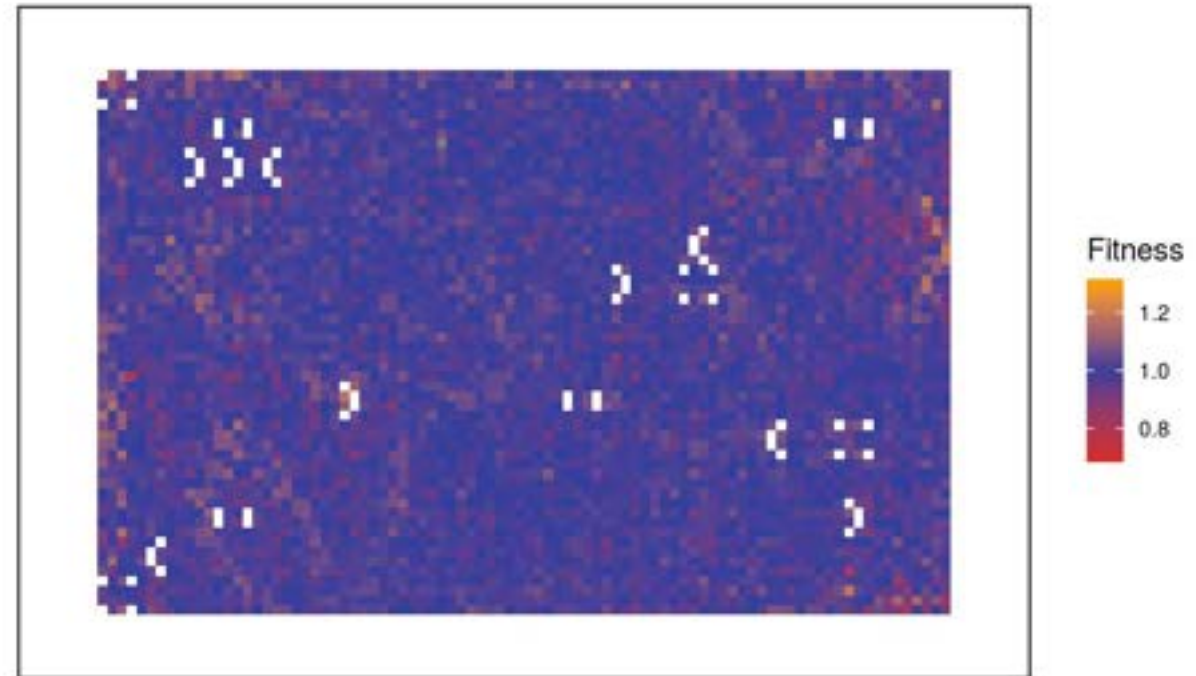


Removing Spatial Bias



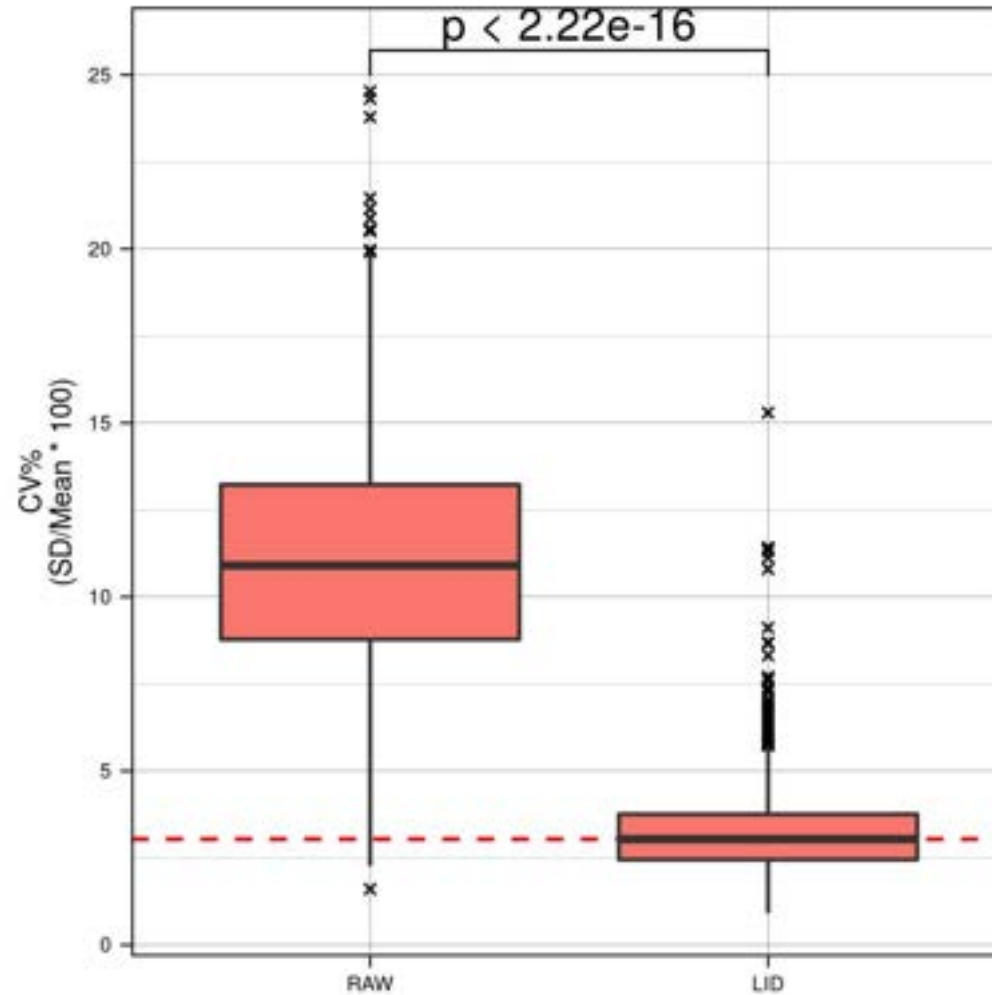
Variability in observation =
Spatial bias + Biological variability

>



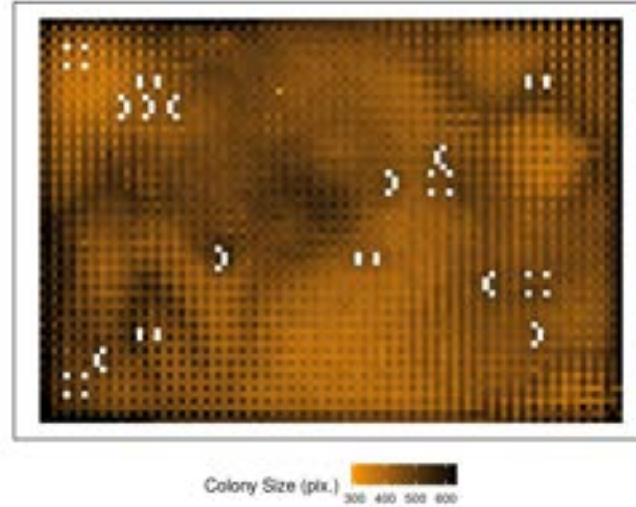
Variability in observation =
Biological variability

Removing Spatial Bias



Virtual Plates with Random Colony Size Distribution

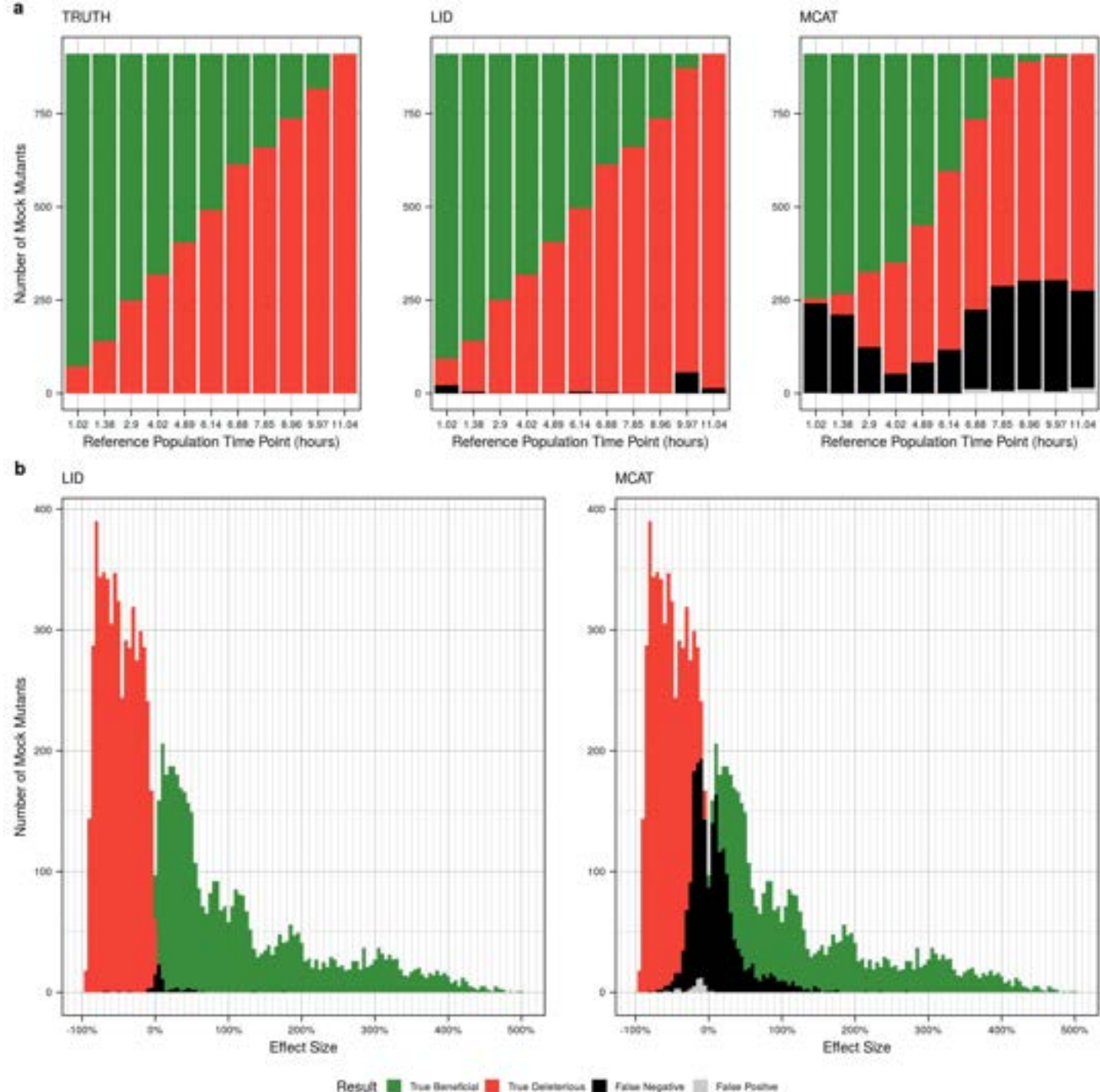
Time = 6104 hours



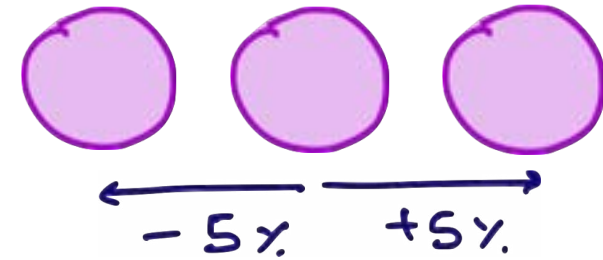
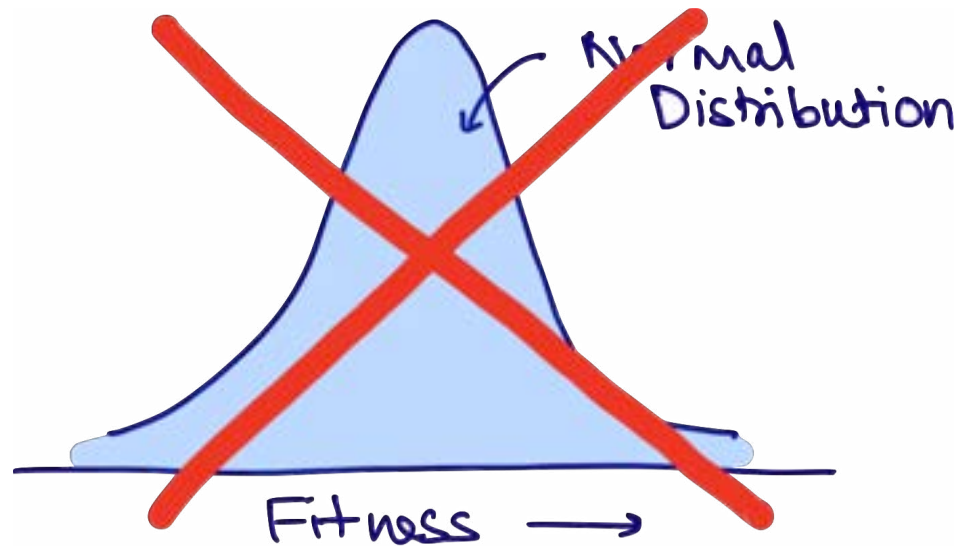
Results from Random Distribution

MCAT =
MATLAB Colony Analyzer Toolkit

$$\text{Effect Size} = \frac{\text{mutant colony size} - \text{reference colony size}}{\text{reference colony size}} \times 100$$



LI Detector improves upon existing methods



5 % Fitness effects
> 95 % sensitivity
> 95 % specificity

LI Detector can expand the use of CBHTS

1. Although developed in yeast, it can be applied to all colony-forming-microorganisms

LI Detector can expand the use of CBHTS

2. Free of scale and assumptions of underlying fitness distribution
 - a. Screen a handful of mutants of your favorite gene (YFG), each having a specific residue of its active site mutated to see which position is most important
 - b. Genome-wide yeast-two-hybrid screen to figure out protein-protein interaction of YFG

LI Detector can expand the use of CBHTS

3. High sensitivity and specificity in observing small changes in fitness would provide reliable and highly resolved phenotypic data
 - a. Can be used to improve upon the existing gene-gene, gene-environment and protein-protein interaction networks

LI Detector can expand the use of CBHTS

4. It is equally sensitive towards detecting both decreases and increases in fitness
 - a. Screens that examine gain-of-function mutations
 - b. Pharmacological screens of drug/chemical resistance
 - c. Explore questions of evolutionary biology

Proto-genes & LI Detector

- We have expanded the BarFLEX collection (Douglas et al., 2012) to include ~1800 proto-genes in total
- Screen for their phenotypic impact in a variety of conditions using CBHTS & LI Detector

Summary

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2. Colony based high-throughput screens (**CBHTS**)
3. Problem of **spatial bias** in CBHTS
4. How does **LI Detector** address this issue?

The Carvunis Lab



Thank You All!

Questions?



@sauriiin

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APPENDIX