Using *Drosophila* cell-based technologies as part of an *in vivo* study

Stephanie Mohr, PhD

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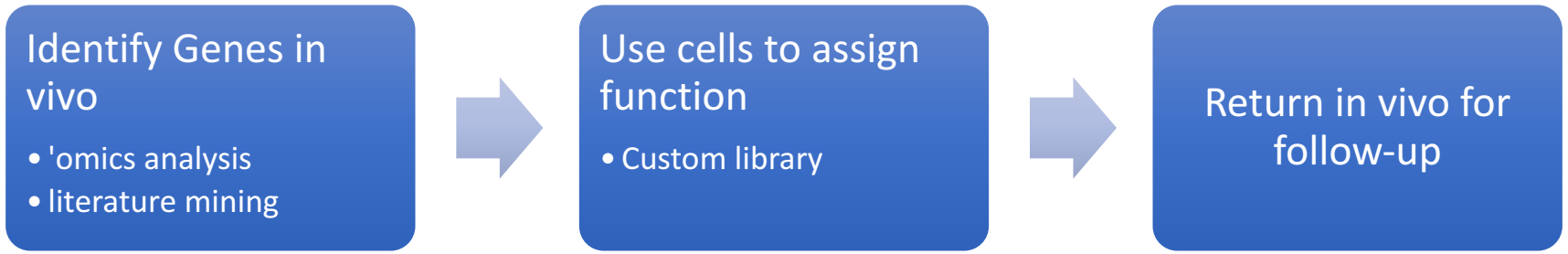
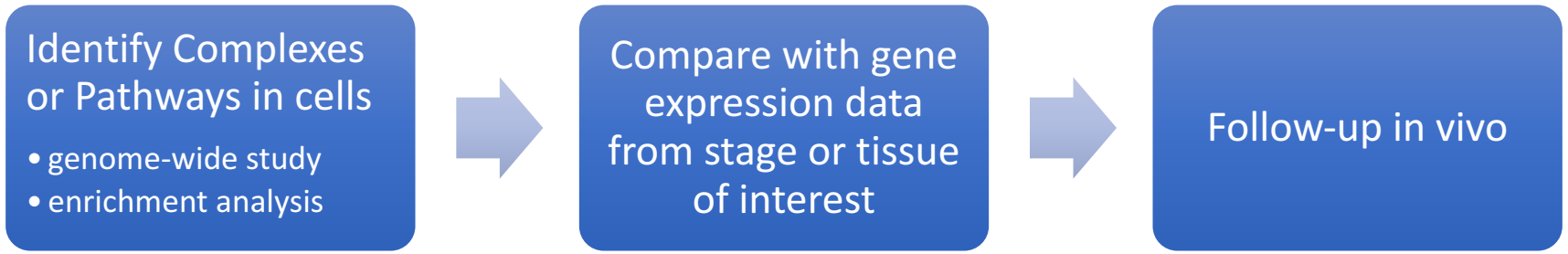

Identify Genes in vivo
• 'omics analysis
• literature mining
Use cells to assign function
• Custom library
Return in vivo for follow-up


Identify Complexes or Pathways in cells
• genome-wide study
• enrichment analysis
Compare with gene expression data from stage or tissue of interest
Follow-up in vivo

What kinds of screens and cell assays are possible?

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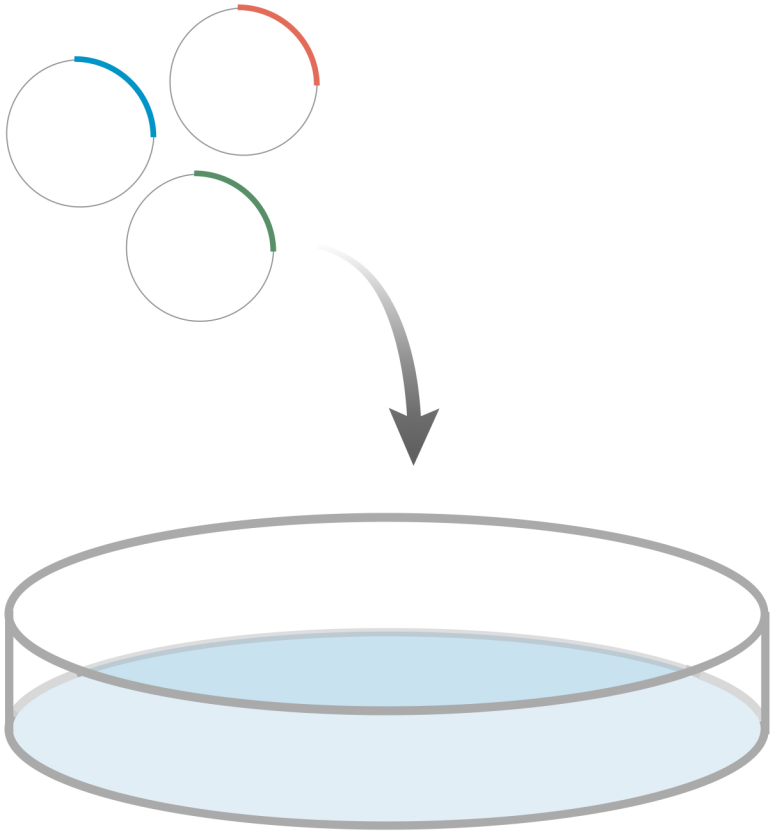
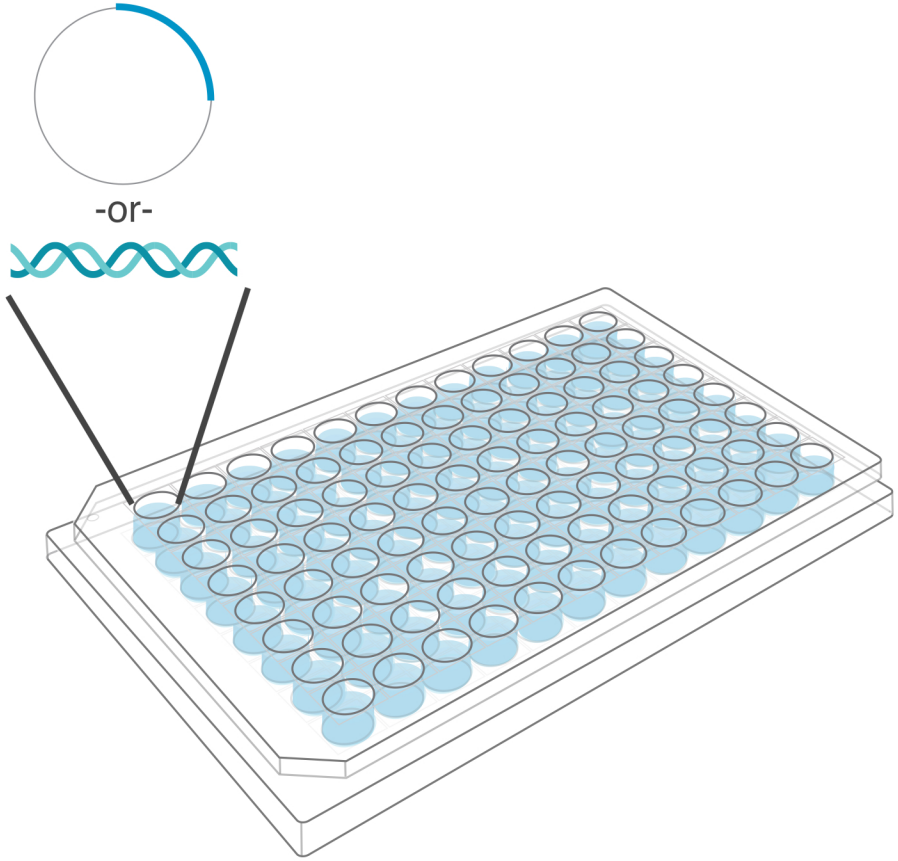
Publications page for specific examples

Pooled vs. Arrayed format screens



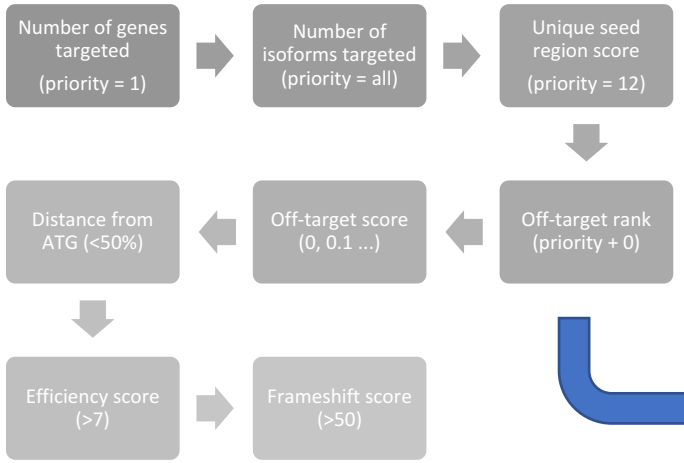
pooled format

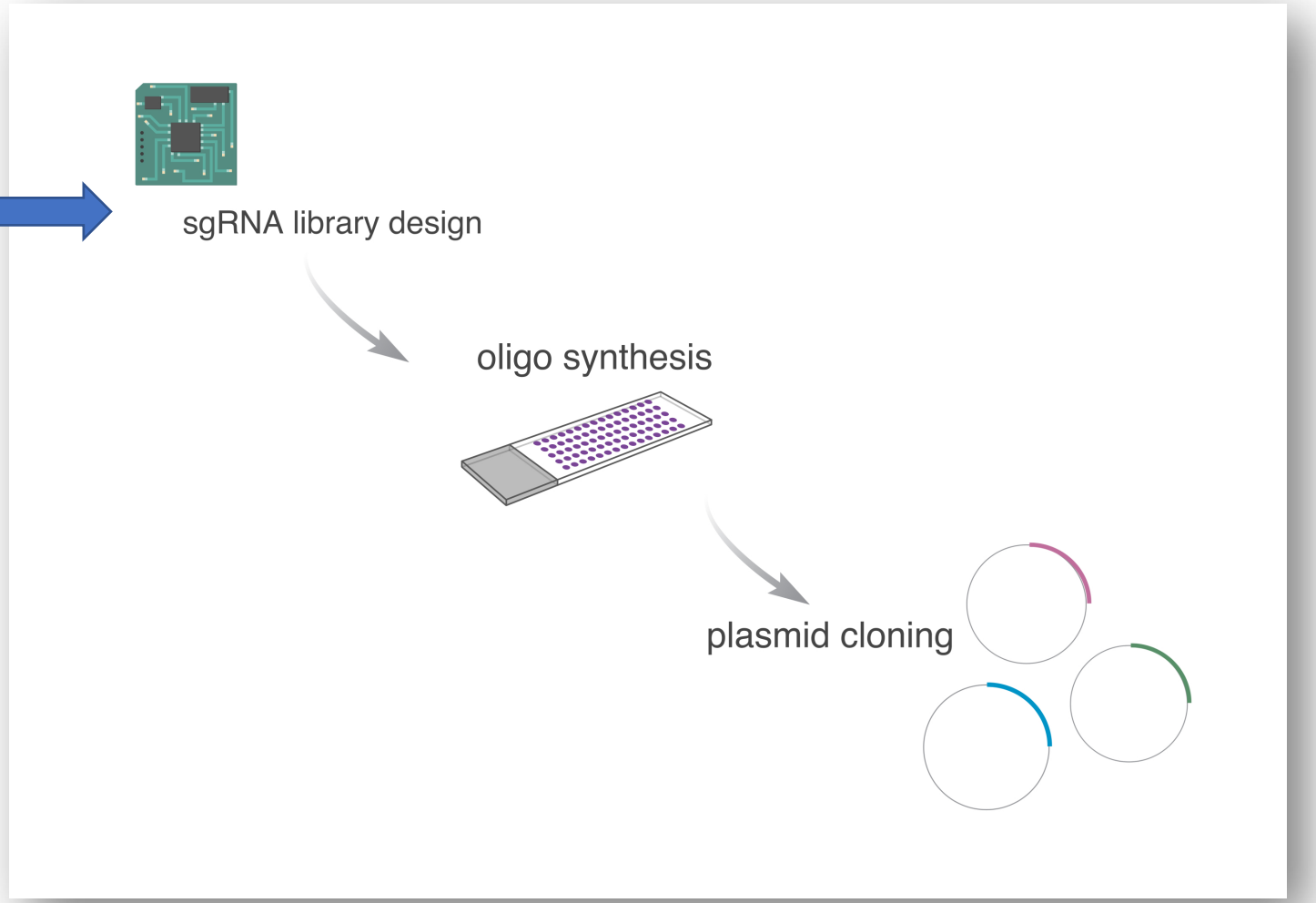


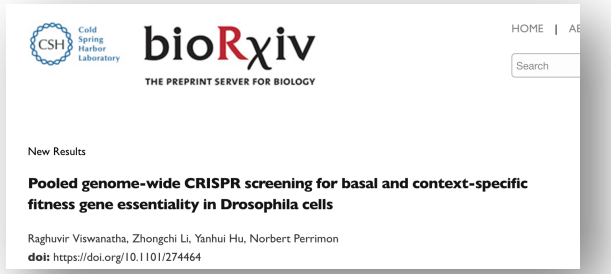
arrayed format cell screens

Pooled vs. Arrayed format screens

Pooled	Arrayed
Reagent library introduced in bulk, at random	Reagents arrayed in 96- or 384-well plates
Relatively easy to generate new libraries (synthesis on a chip)	Relatively difficult to generate new libraries (synthesis or cloning, benefits from automation)
Assay period of weeks	Assay period of days
Assays require separation between non-hits and hits, e.g. viability, selection, FACS	Assays range from well-level analyses (total ATP, transcription reporters, etc.) to imaging
Positive ‘hits’ identified by next-generation sequencing (compare population at start to endpoint)	Positive ‘hits’ identified by looking up the plate and well on a spreadsheet or database

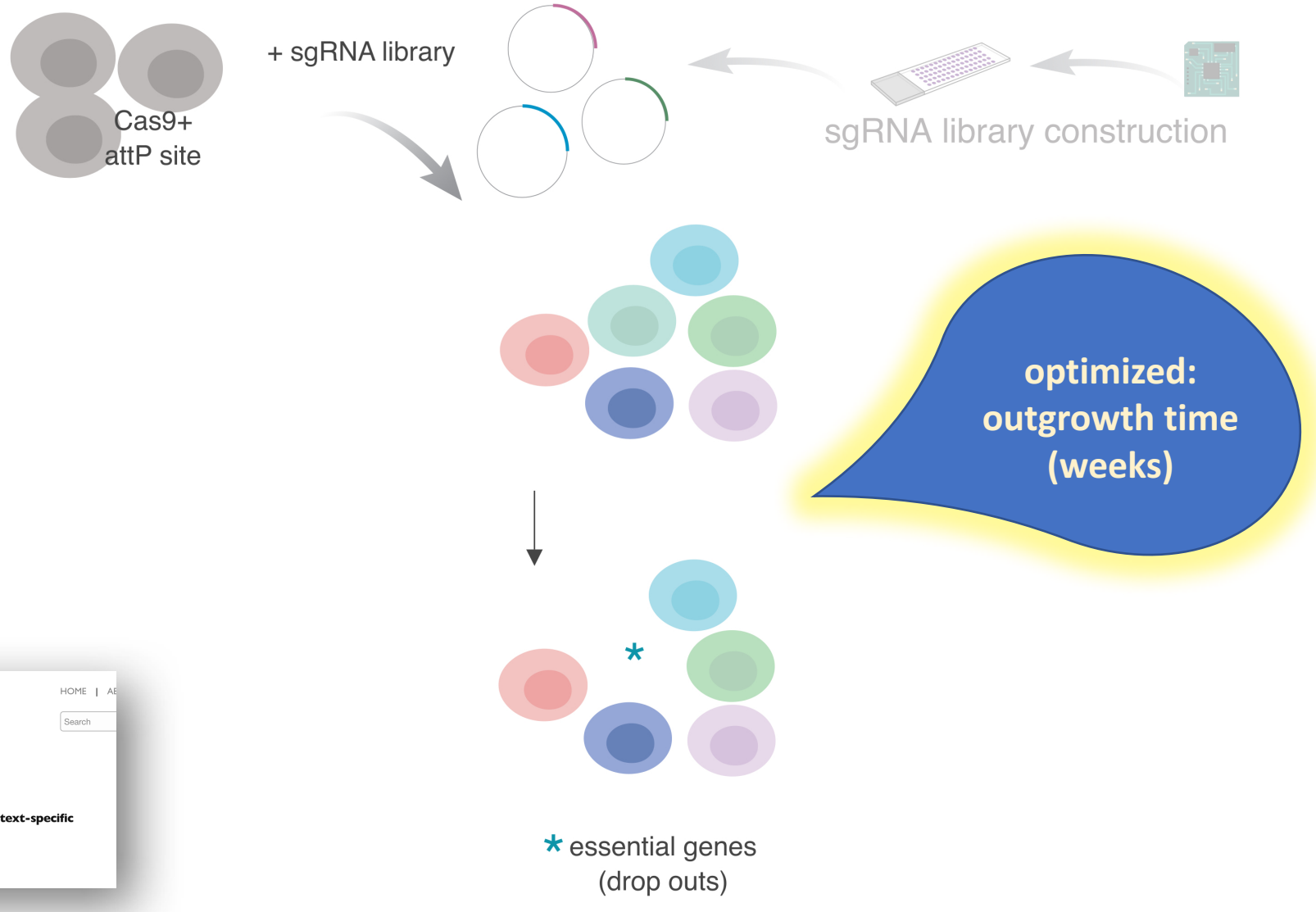


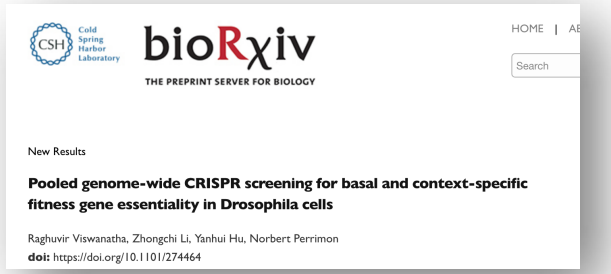




Hu & Viswanatha

pooled screen to detect essential genes




bioRχiv
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New Results

Pooled genome-wide CRISPR screening for basal and context-specific fitness gene essentiality in Drosophila cells

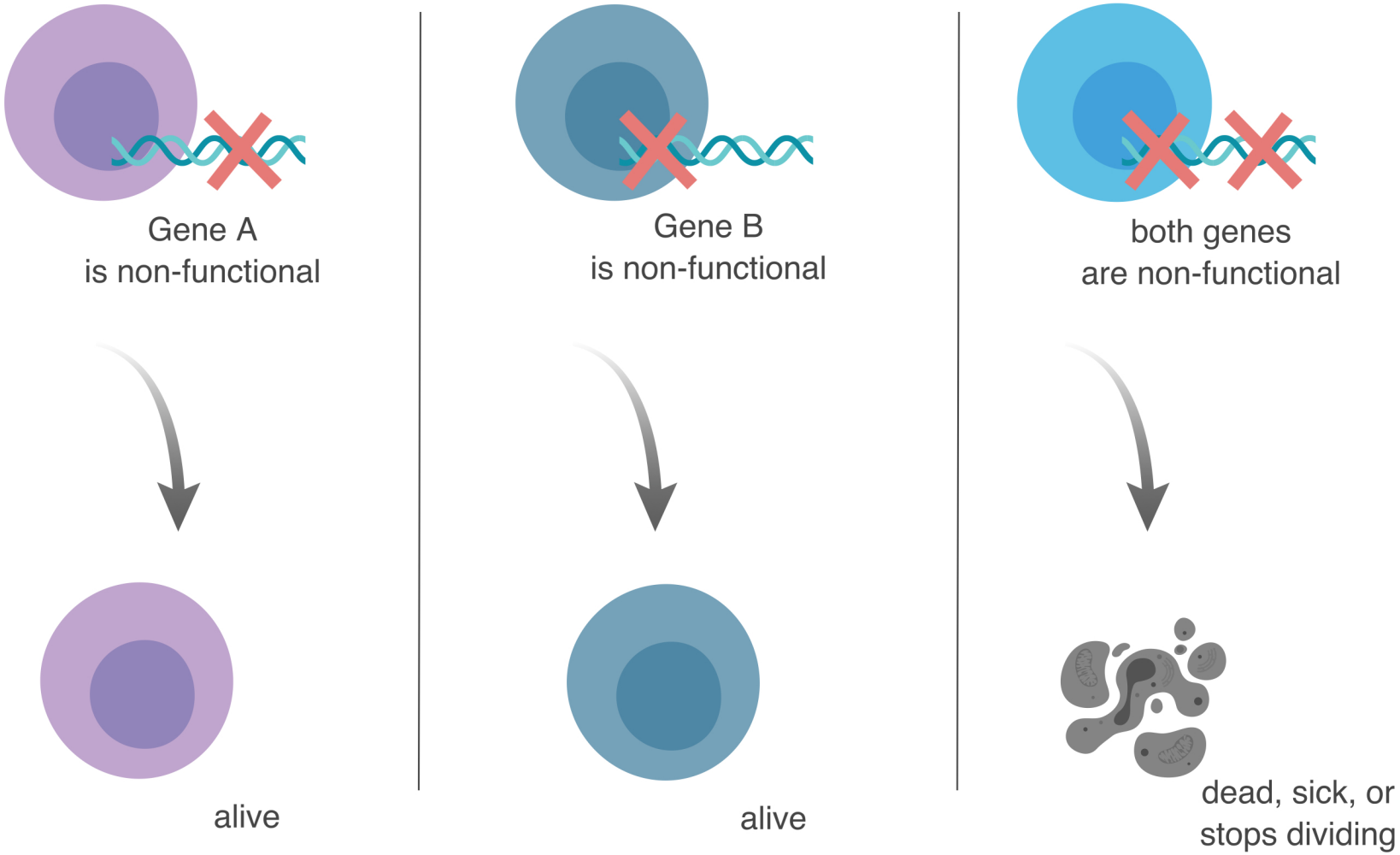
Raghuvir Viswanatha, Zhongchi Li, Yanhui Hu, Norbert Perrimon

doi: https://doi.org/10.1101/274464

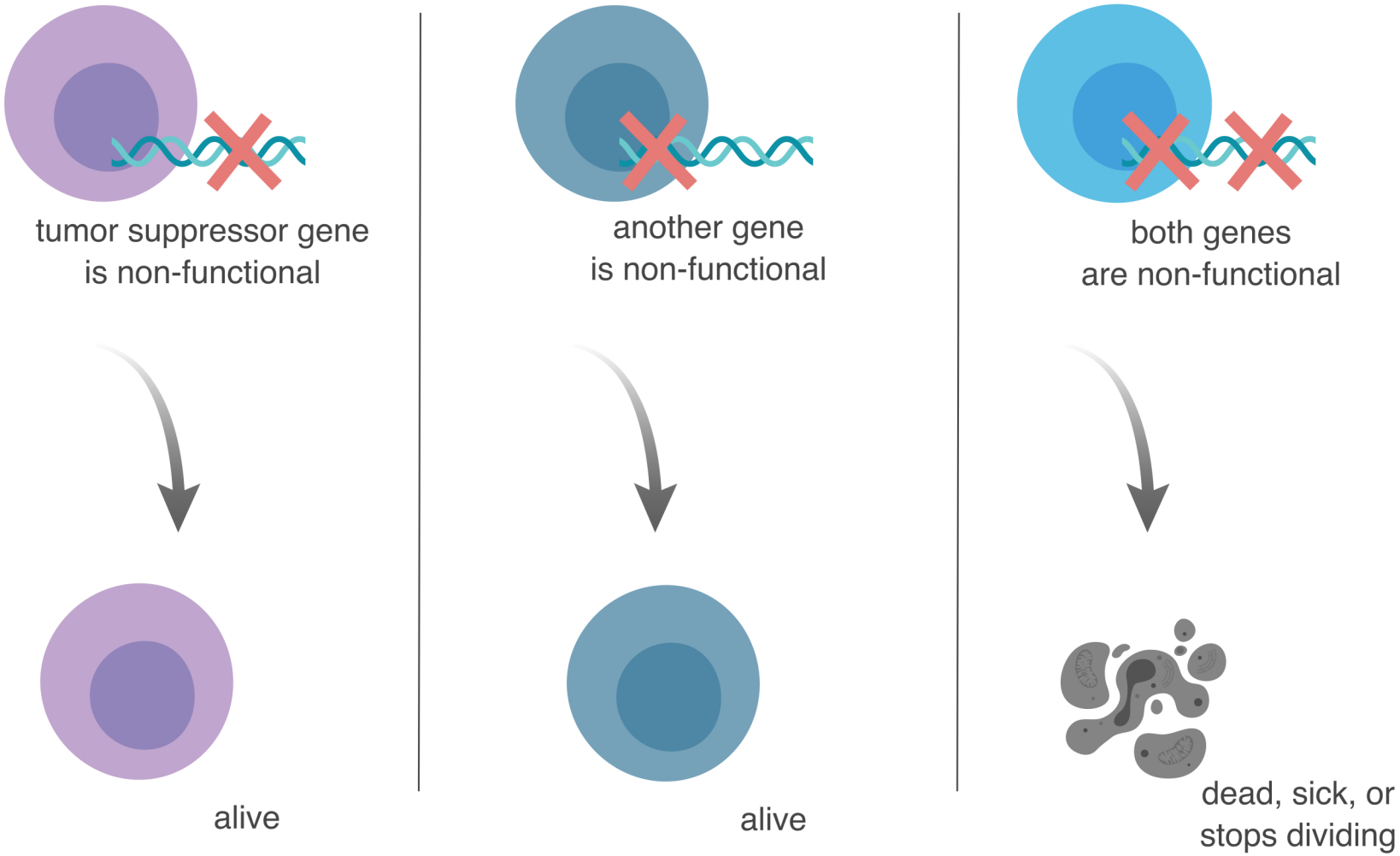
R. Viswanatha

Why pool? Use-case 1: cancer therapeutics

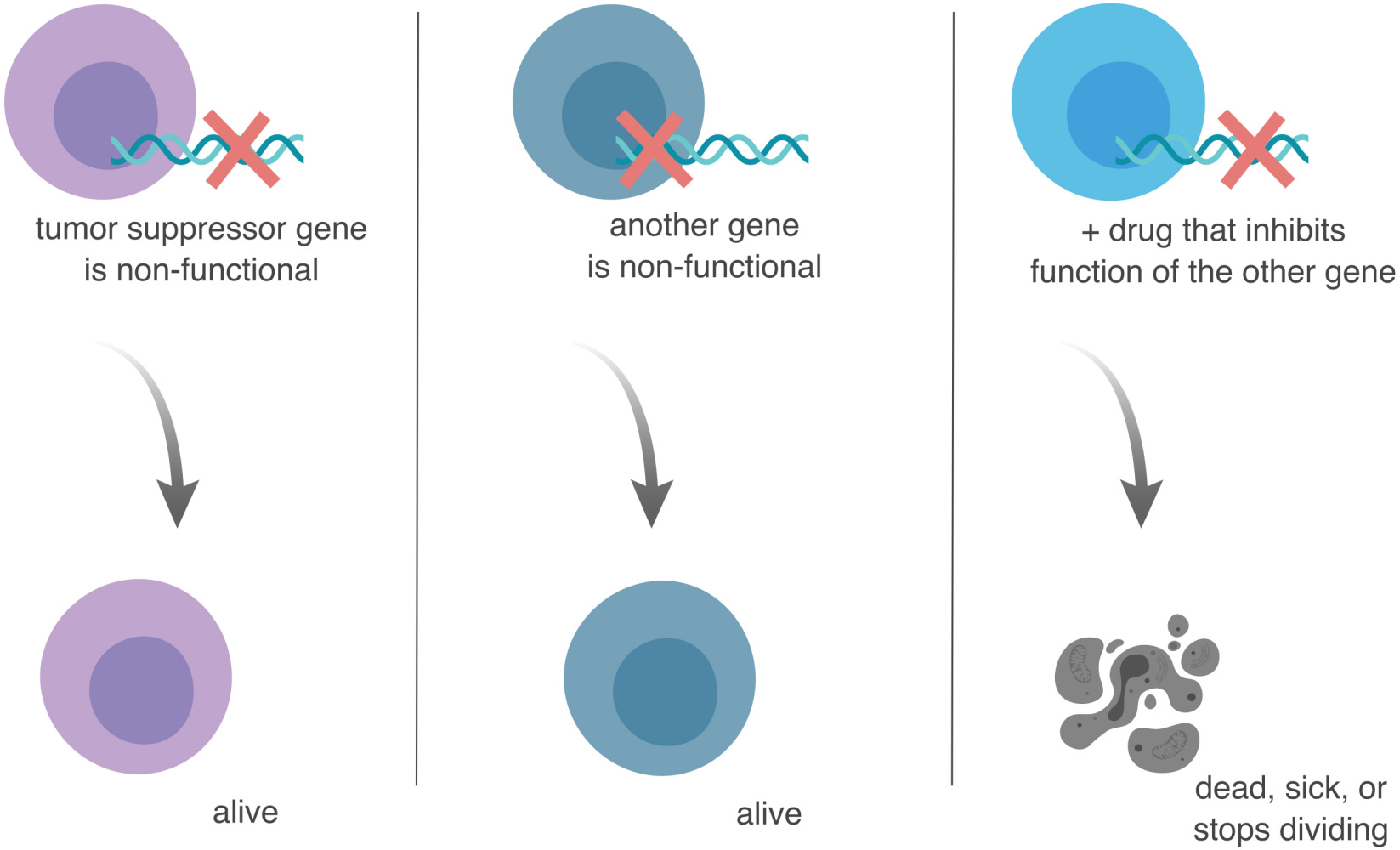
Concept:


Gene A
is non-functional
Gene B
is non-functional
both genes
are non-functional
alive
alive
dead, sick, or
stops dividing

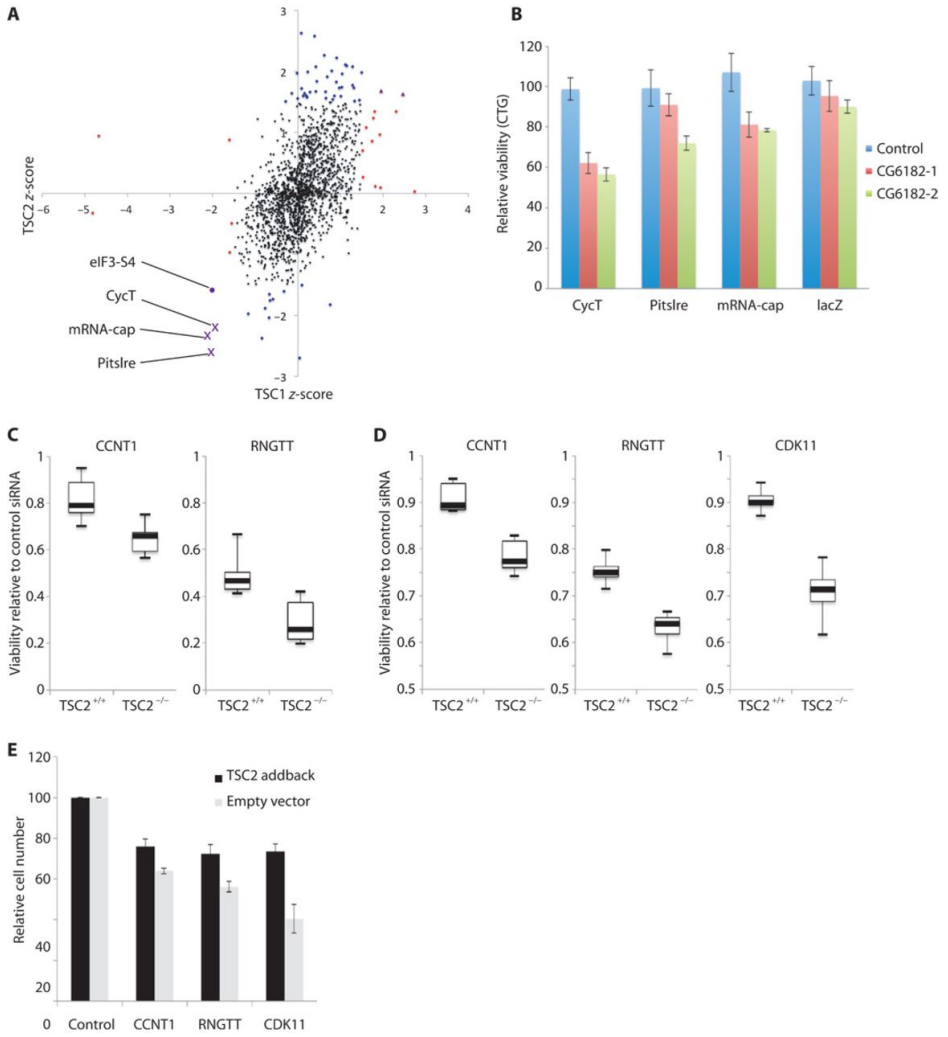
Approach:


tumor suppressor gene
is non-functional
another gene
is non-functional
both genes
are non-functional
alive
alive
dead, sick, or
stops dividing

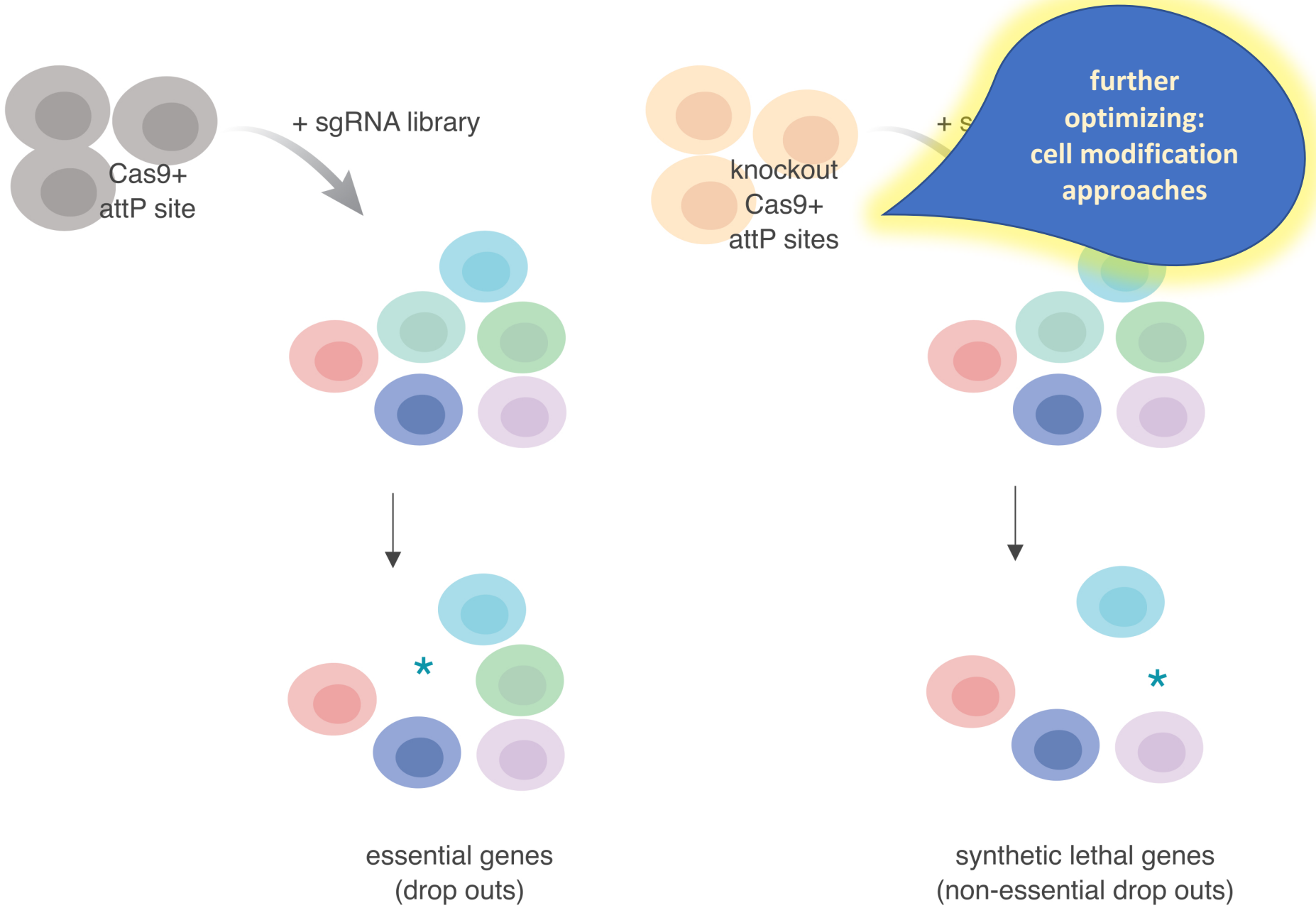
Goal:


tumor suppressor gene
is non-functional
alive
another gene
is non-functional
alive
+ drug that inhibits
function of the other gene
dead, sick, or
stops dividing

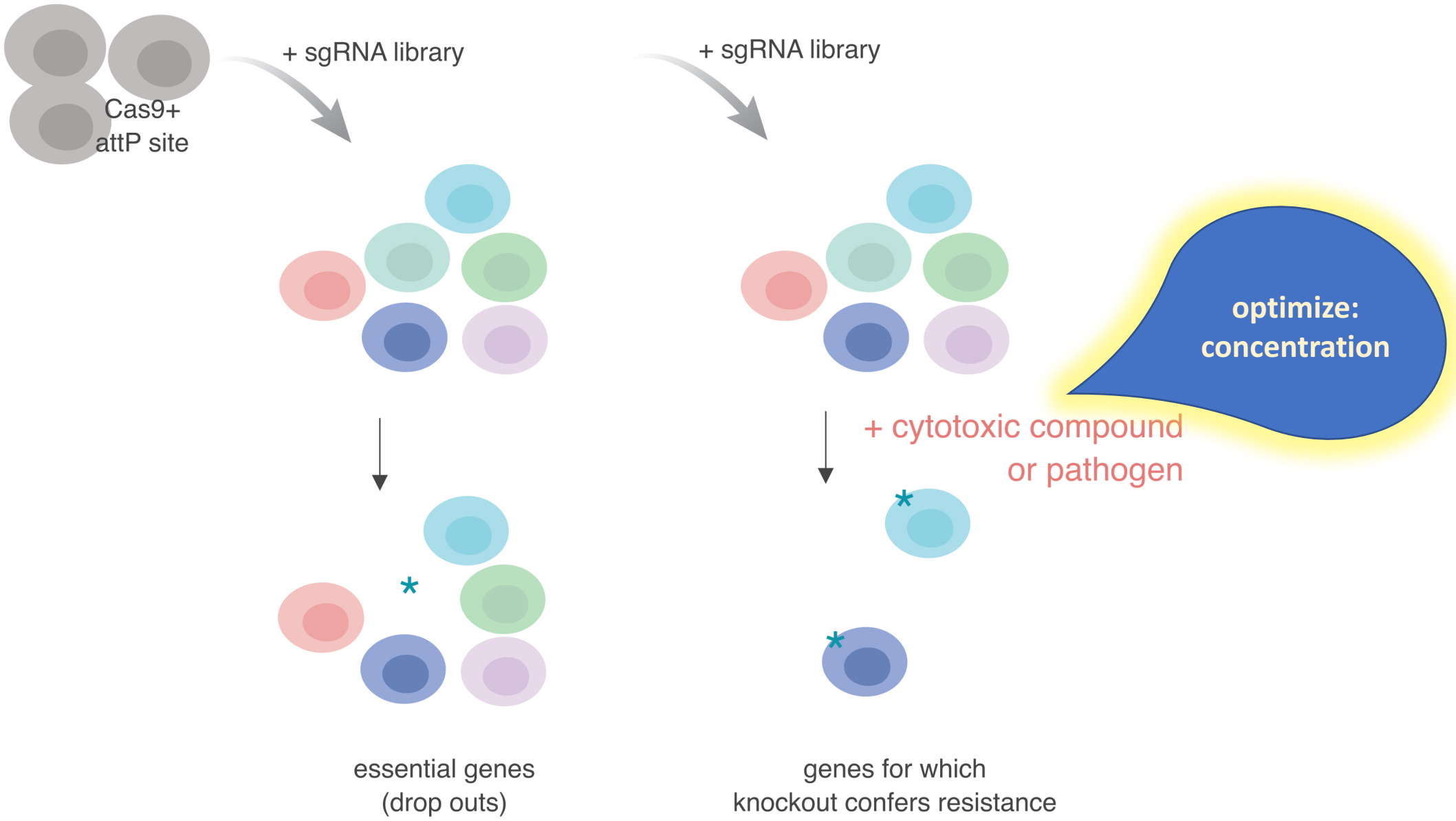
Identification of TSC-specific drug targets using synthetic screening.



Benjamin E. Housden et al., Sci. Signal. 2015;8:rs9


Cas9+
attP site
+ sgRNA library
knockout
Cas9+
attP sites
+ s
further
optimizing:
cell modification
approaches
*
*
essential genes
(drop outs)
synthetic lethal genes
(non-essential drop outs)

Why pool? Use-case 2: sensitivity or resistance to a treatment


Cas9+
attP site
+ sgRNA library
+ sgRNA library
optimize:
concentration
+ cytotoxic compound
or pathogen
essential genes
(drop outs)
genes for which
knockout confers resistance

Pooled assays

- Outgrowth—essential genes (wildtype)
- Outgrowth—essential genes (mutant cell line)
- Outgrowth in presence of mild dose of toxin—sensitivity
- Selection (toxin, pathogen, etc.)—resistance
- Sort cells (e.g. with a GFP marker)

Libraries available at the DRSC

- Pooled CRISPR sgRNA library for knockout screens
- Pooled CRISPR sgRNA library for activation screens
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  - focused (kinases, transmembrane, RNA binding, etc.)
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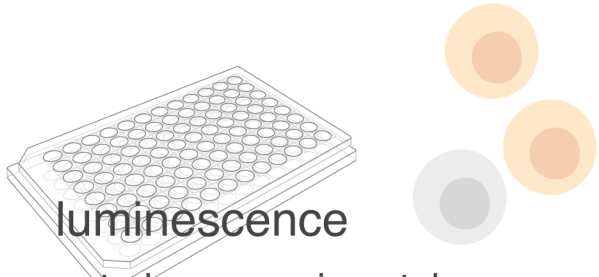
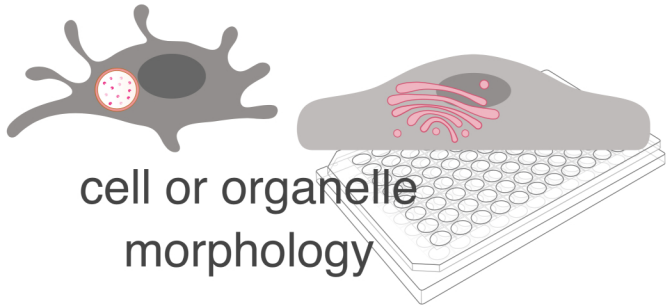
Positive 'hits' identified by next-generation sequencing (compare population at start to endpoint)	Positive 'hits' identified by looking up the plate and well on a spreadsheet or database

can re-array the DRSC collection to customize

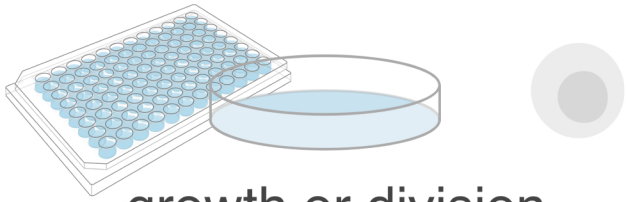


luminescence

e.g. control vs. experimental
transcription reporter

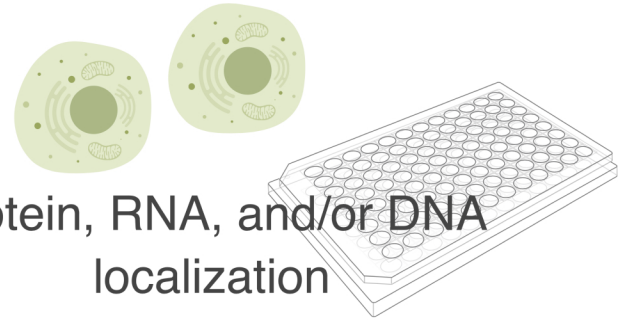


cell or organelle morphology

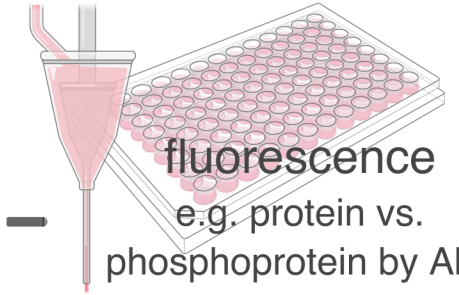


growth or division

e.g. total ATP,
survival over time

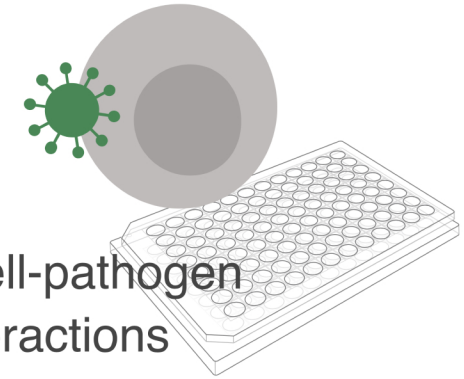


protein, RNA, and/or DNA localization



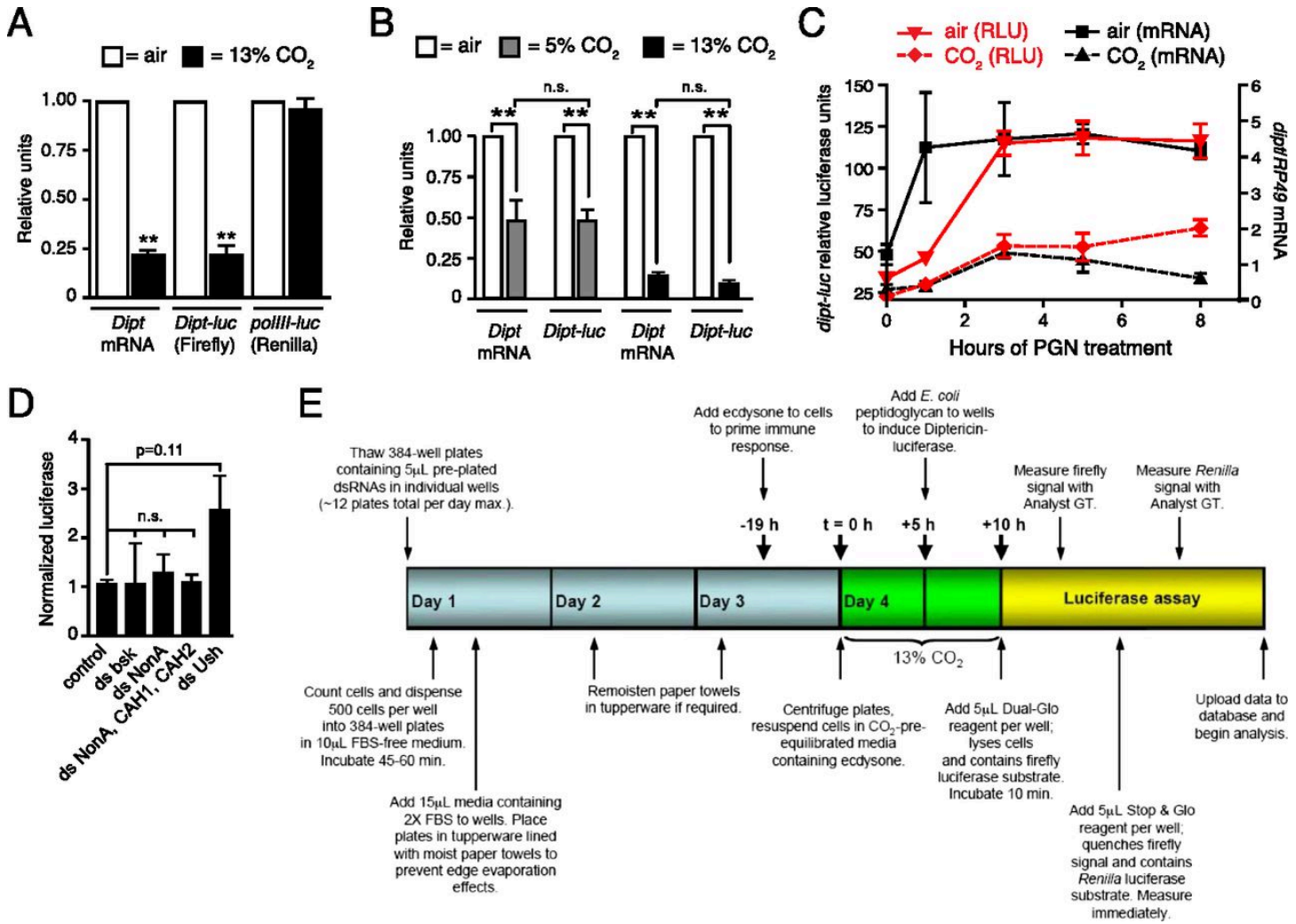
fluorescence

e.g. protein vs.
phosphoprotein by Abs



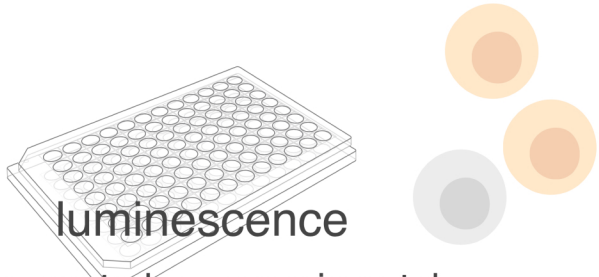
host cell-pathogen interactions

A Dipt-luc reporter construct enables a genome-wide screen for genes that mediate hypercapnic immune suppression.



Iiro Taneli Helenius et al. J Immunol 2016;196:655-667

The Journal of Immunology



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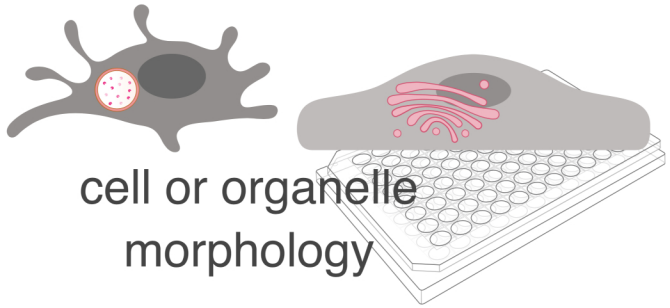

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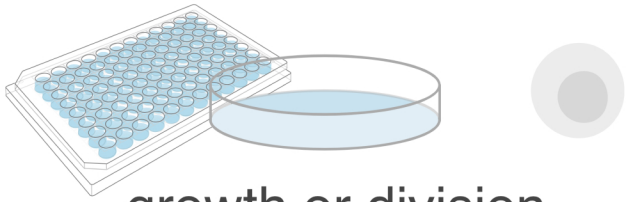

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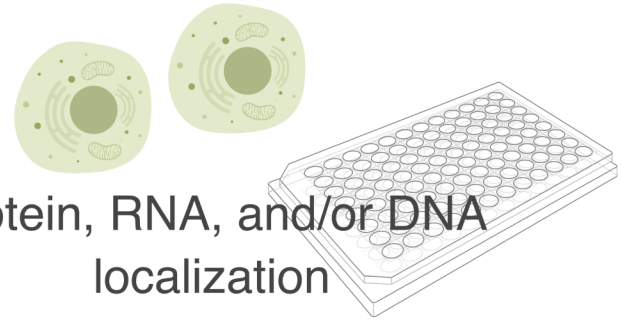

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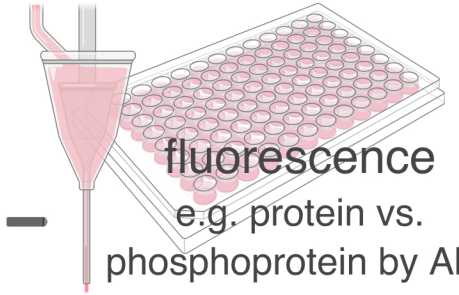

fluorescence

e.g. protein vs.
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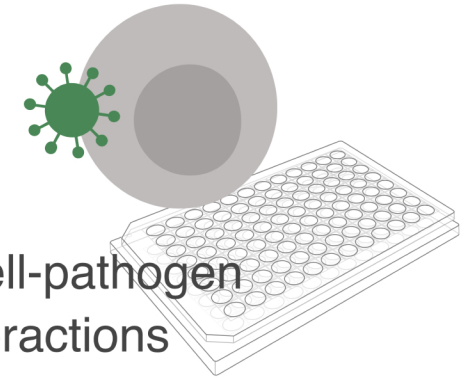
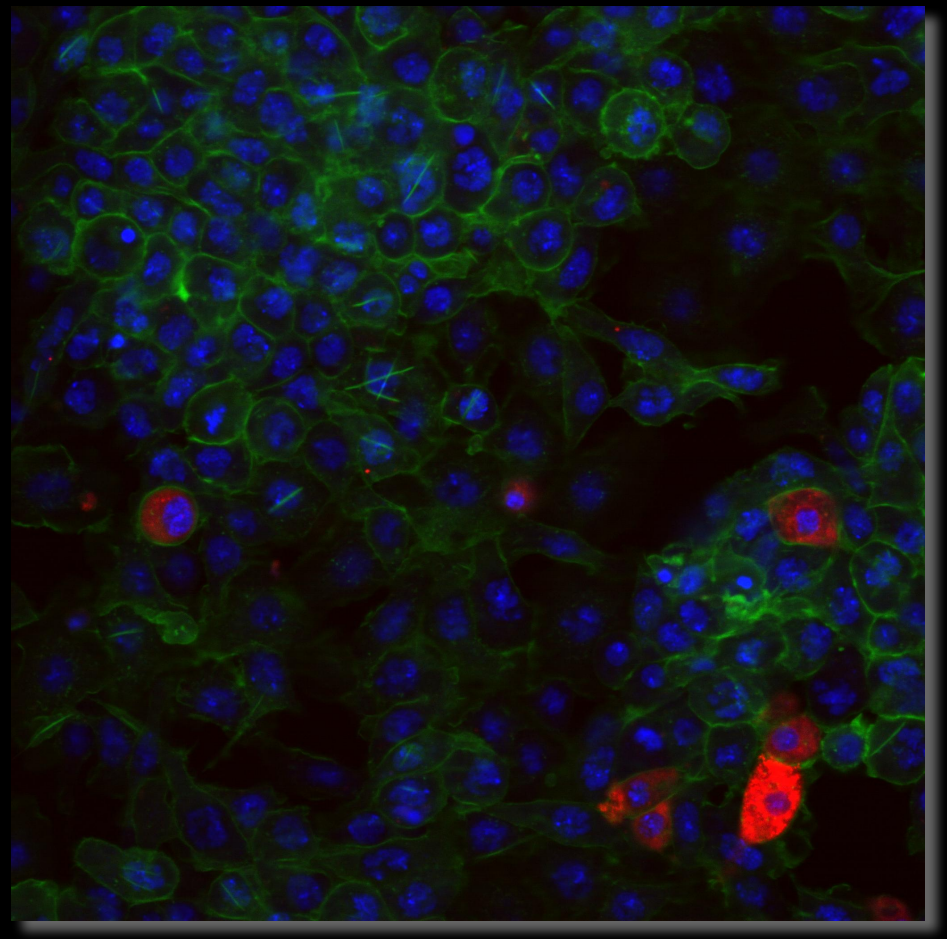


host cell-pathogen interactions

Image screens at DRSC: IN Cell 6000

- Bright field
- Epifluorescence
- Confocal



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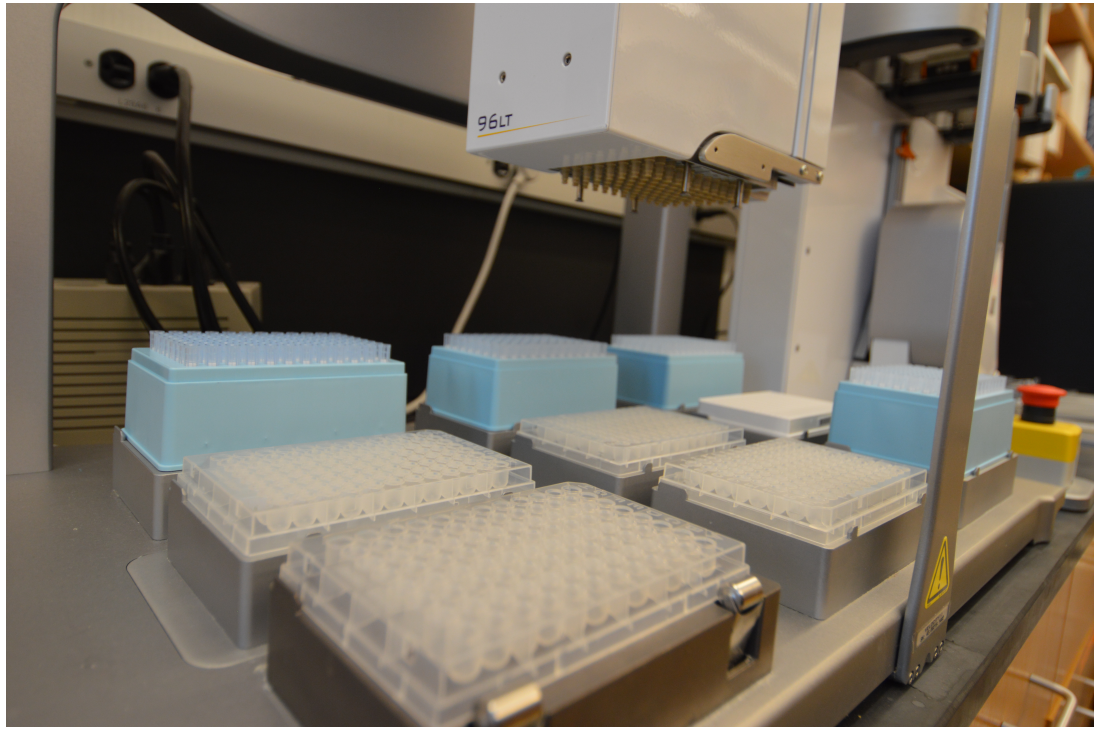

Summary

- Cell study → follow up *in vivo* -or- in mammalian cells
- in vivo study → use cells to assign function
- Pooled-format screens (CRISPR-KO, CRISPR-OE)
- Arrayed-format screens (dsRNA) including image-based screens
- DRSC has genome-wide, focused, and custom libraries

- *Contact us for help at any stage!*

Acknowledgements

Norbert Perrimon

Raghuvir Viswanatha

Claire Yanhui Hu

Jonathan Zirin

DRSC/TRiP staff

Gabriel Amador

Limmond Ayisi

Verena Chang

Ryan Colbeth

Aram Comjean

Colby Devereaux

Luping Lu

Rong Tao

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Donghui Yang-Zhou

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Howard Hughes Medical Institute

Dana Farber/Harvard Cancer Center

HMS Tools & Technology Program

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Hugo Bellen (CRIMIC Project)

Amanda Simcox (cell lines project)