



ExpressCells

Better knock-ins, better cell lines, better science.

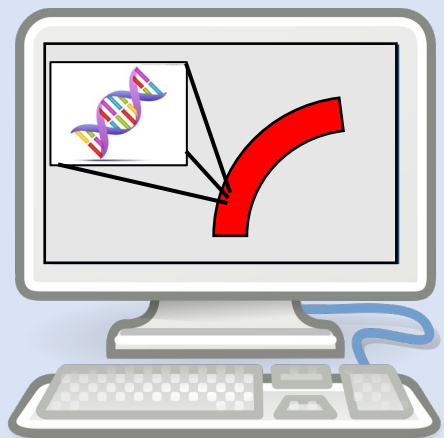
Faster. Way faster.

Types of Projects

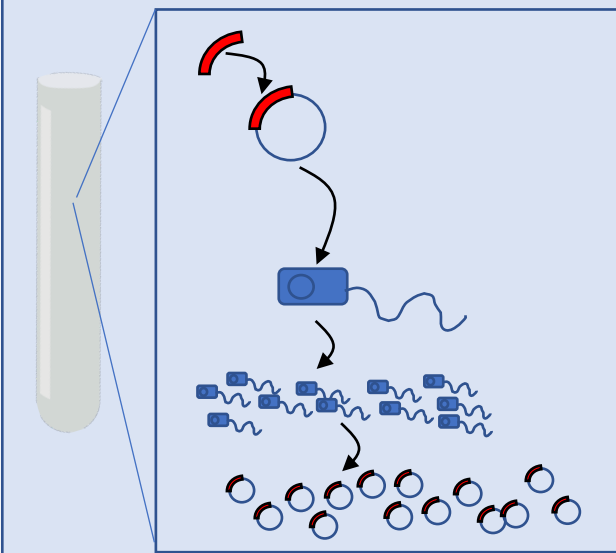
- Core technology: knock-in cell lines built through antibiotic selection and proprietary plasmid system
 - C-terminal tagging
 - Overexpressing cell lines
 - Point mutations—knock-out target portion of gene and replace with no sequence
- Can also produce knock-outs using standard CRISPR techniques



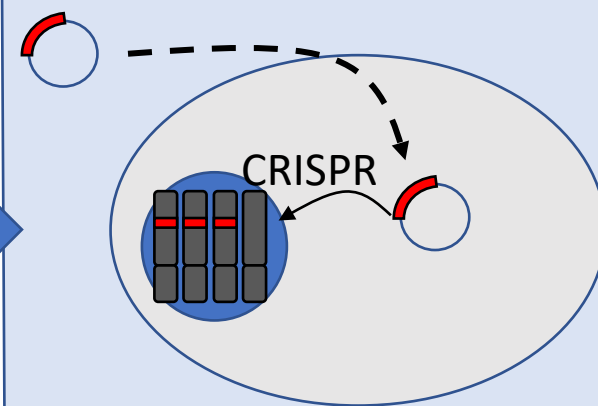
Phase 1: Design Work



Phase 2: Bacteria Work



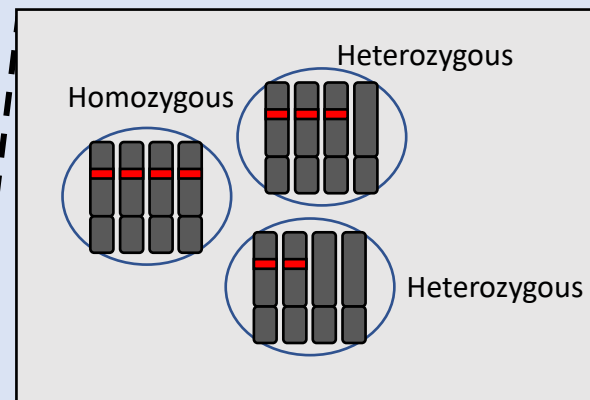
Phase 3: Cell Culture



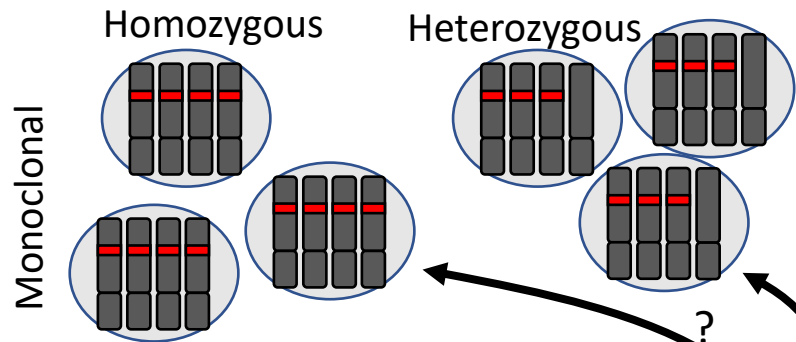
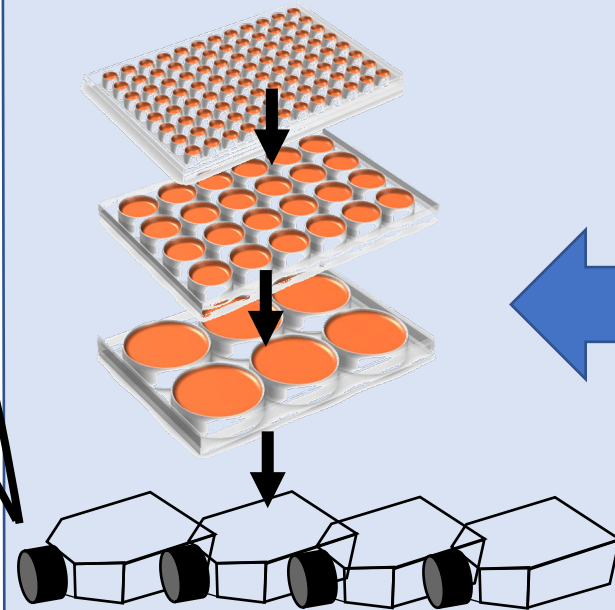
Antibiotic



Cell Pool (Polyclonal)

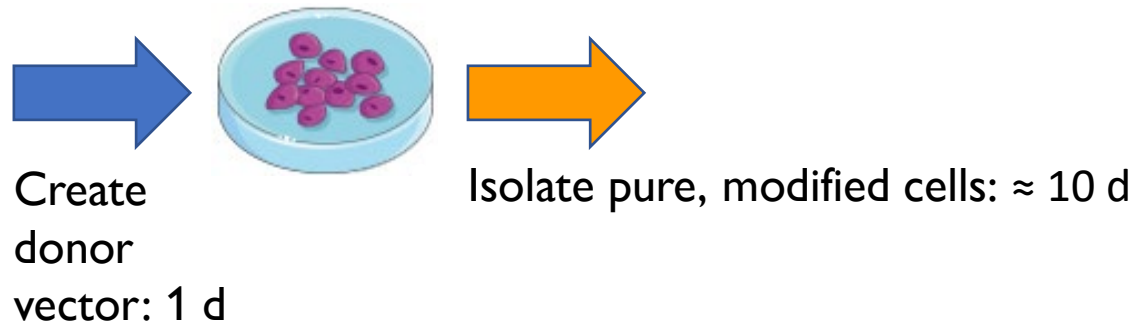


Single Cell Dilution

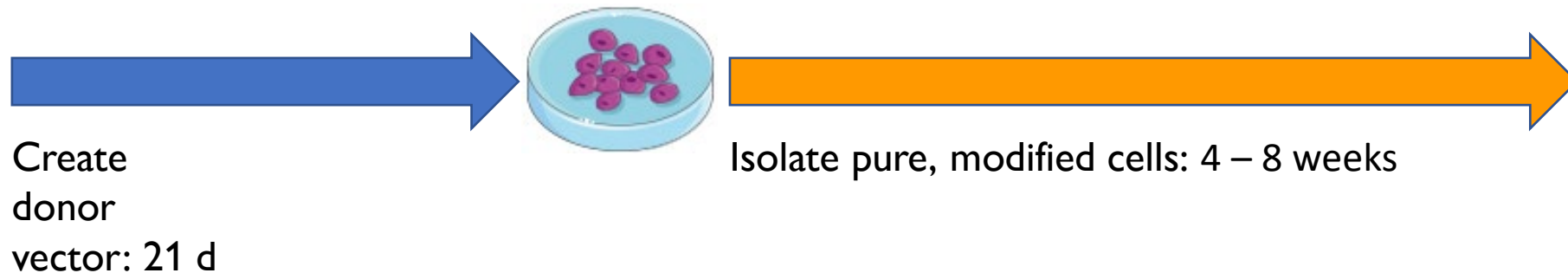


C-tagging Knock-in Cell Lines in < 14 Days

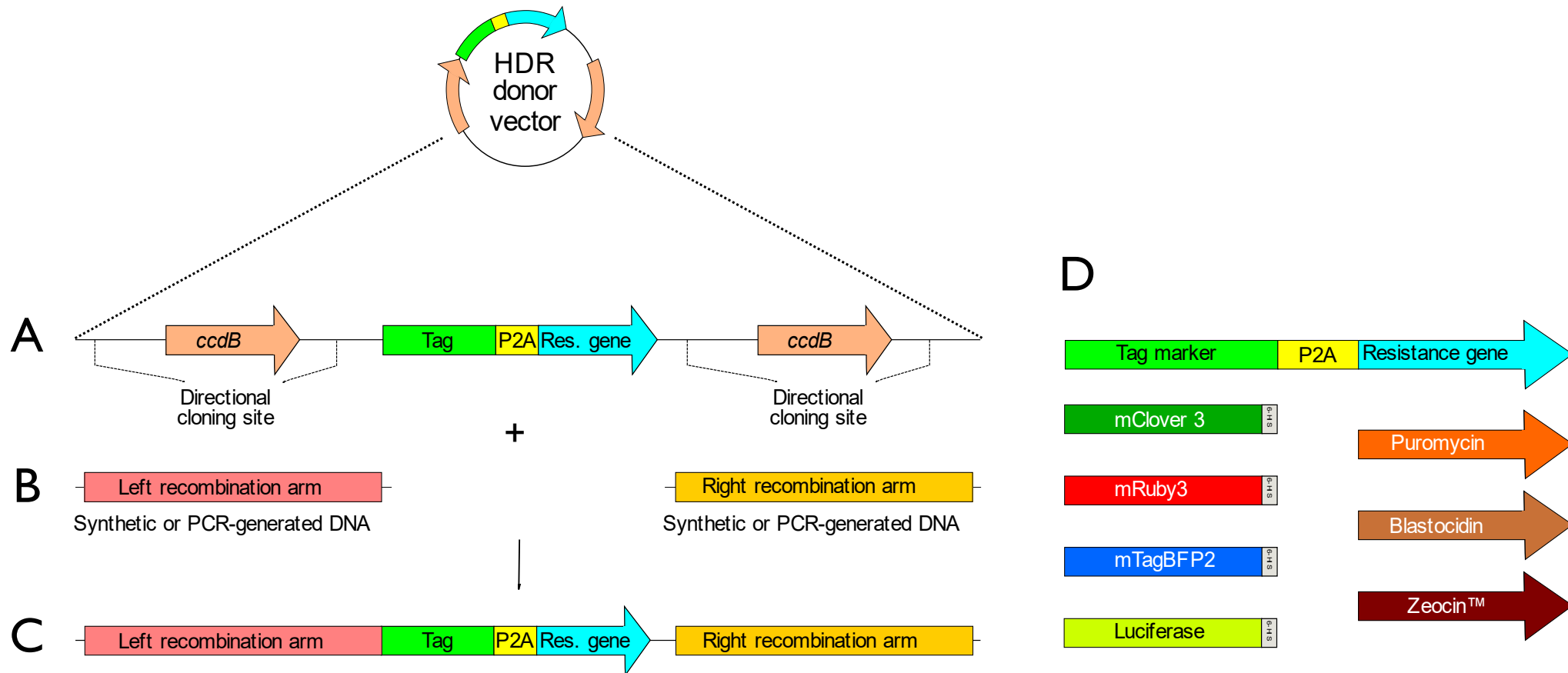
FAST-HDR



Conventional methods

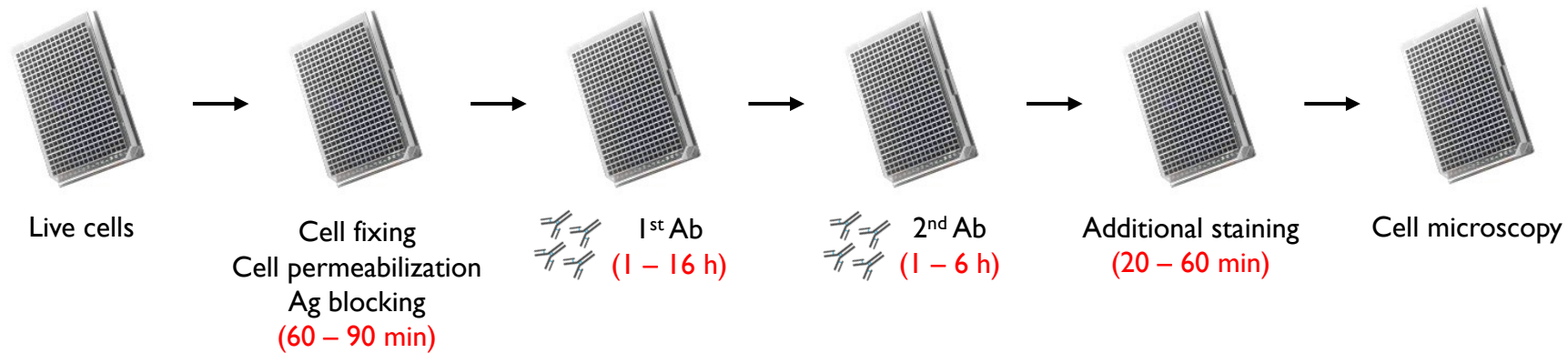


FAST-HDR Plasmid Vector System



Triple-Labeling With FAST-HDR Obviates the Need for Immunofluorescence and Staining

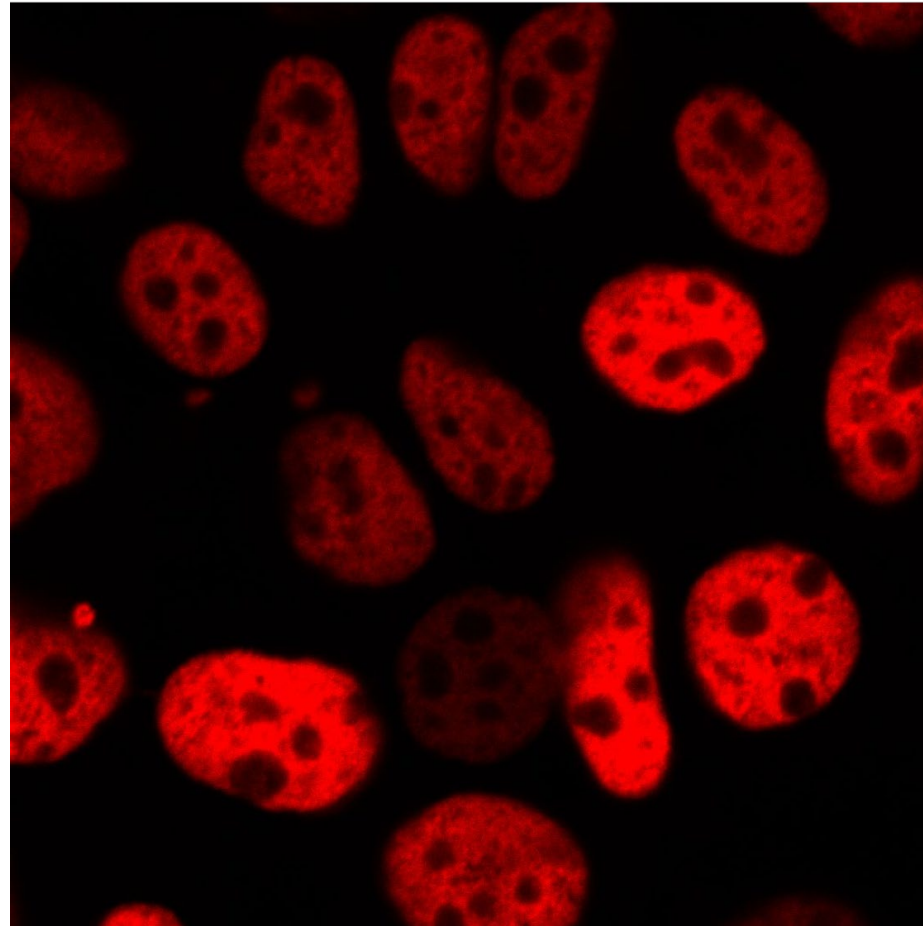
Traditional Screening



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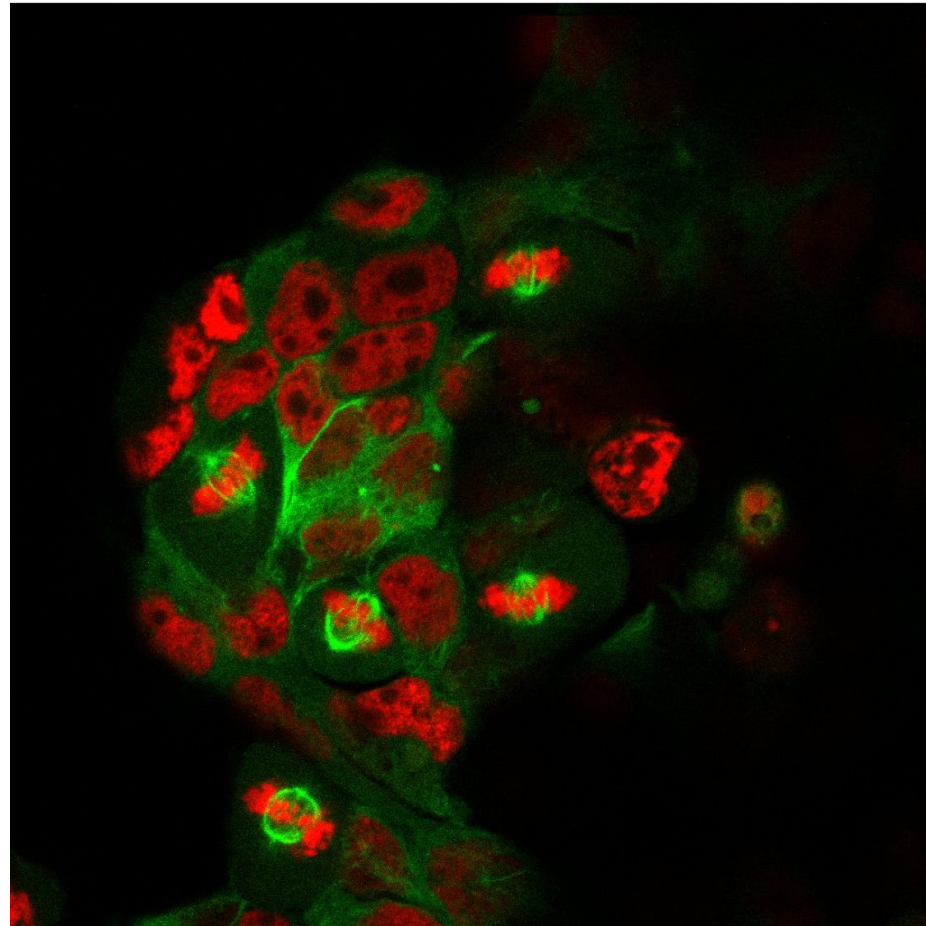


Endogenous, Single Gene Labeling Tagging With mRuby3, Selected With Zeocin™, Day 14 Following Transfection



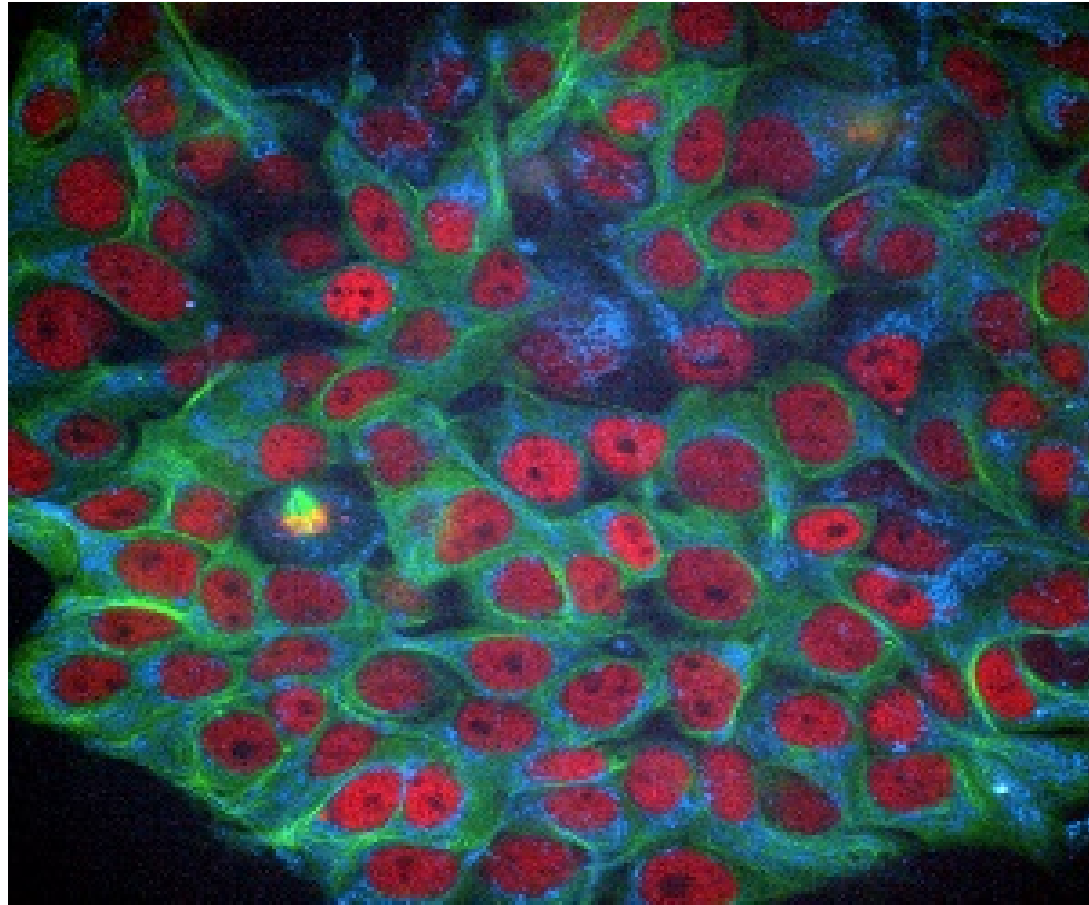
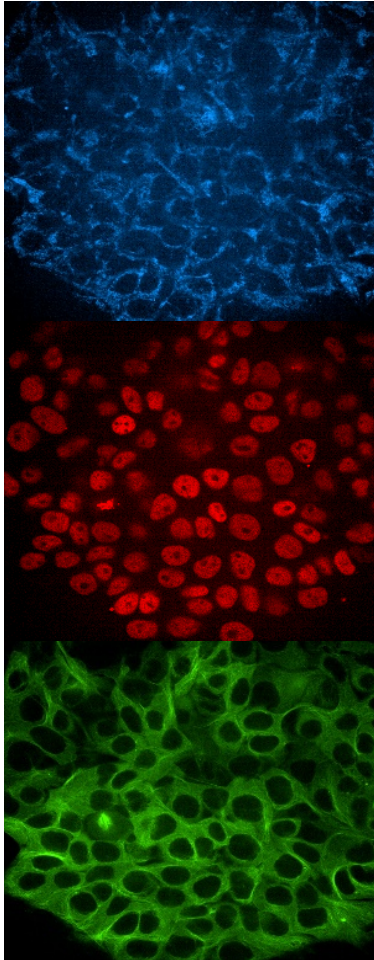
Double Gene Labeling

Tagging With mRuby3 + mClover,
Selection With Zeocin™ + Puromycin

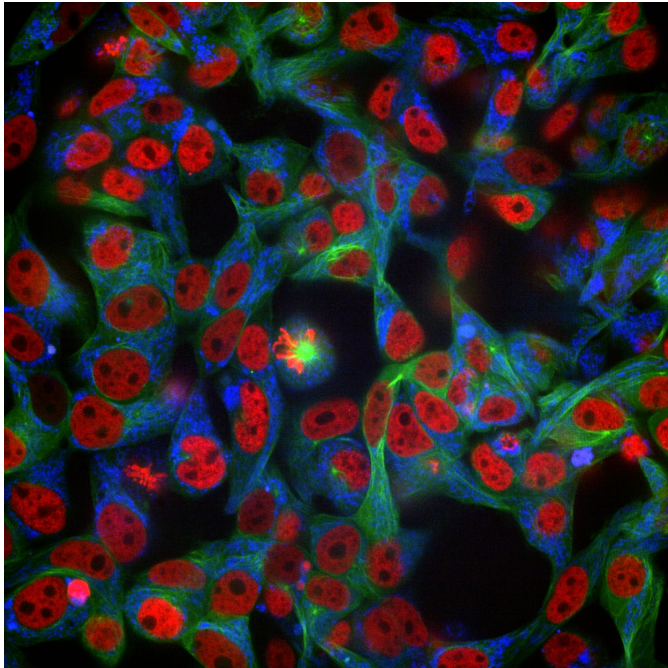


Triple Gene Labeling

ATP5B Tagging With mTagBFP2, β -tubulin With mClover3,
Histone H3.3 With mRuby3



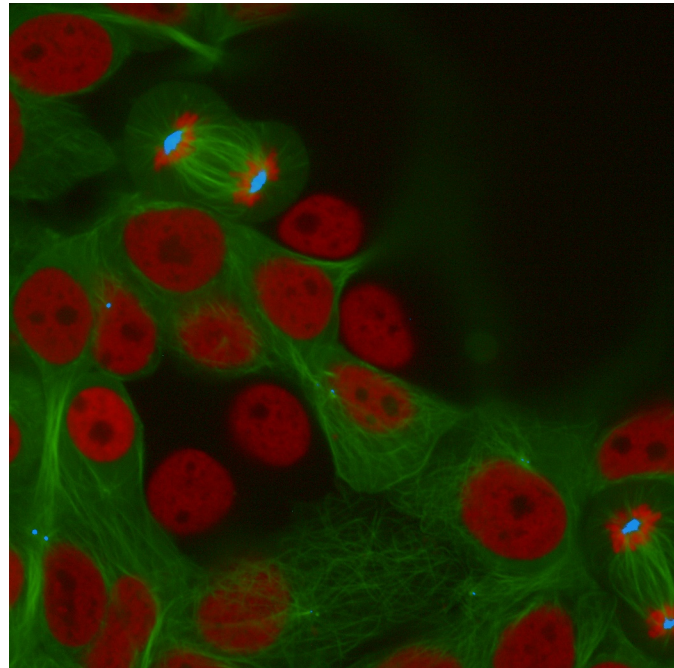
Flexible Approach



Mitochondria

Red: H3.3-mRuby3

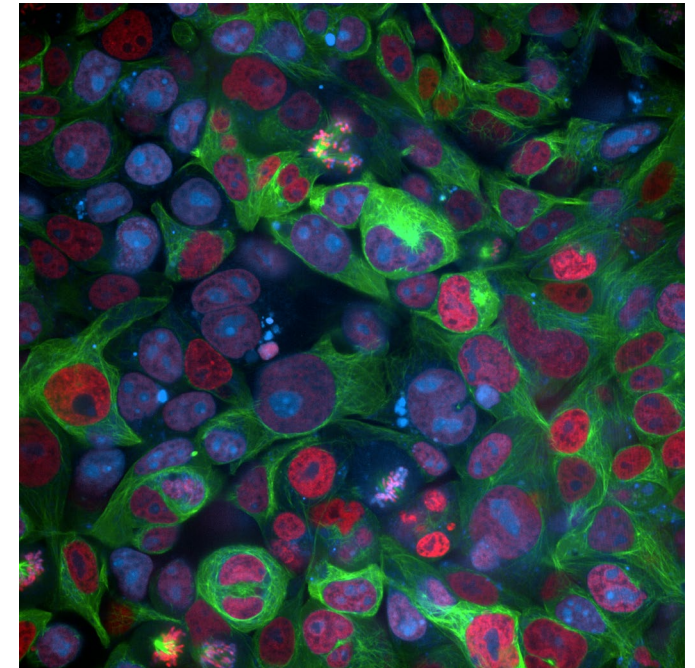
Green: β -tubulin



Spindle poles

Red: H3.3-mRuby3

Green: β -tubulin



Nucleus

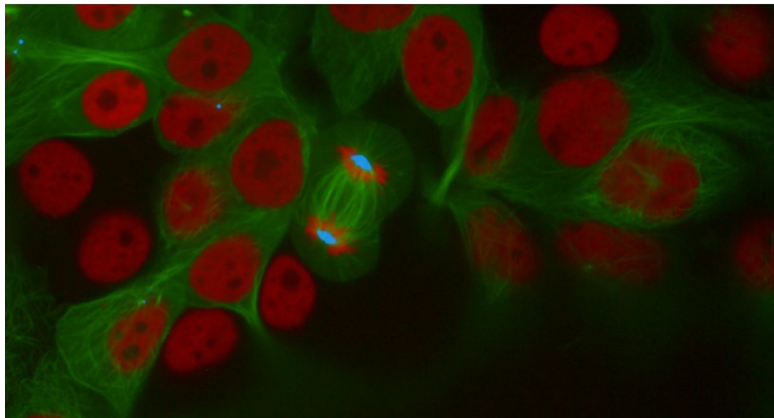
Red: H3.3-mRuby3

Green: β -tubulin

Two Case Studies

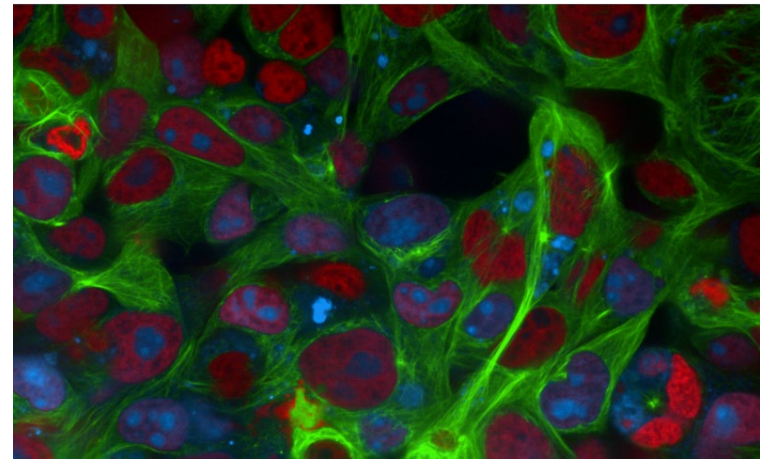
Problem: Does a New Oncology Agent Inhibit Mitosis?

Solution: Create cell line where cell division is readily identified via blue tag for protein expressed only during mitosis



Problem: Identify Impact of a Drug Candidate on Organelles

Solution: Insert three genes that clearly identify the cytoskeleton, nucleus, and mitochondria



Better High-Content Imaging With the FAST-HDR Plasmid Vector System

- Longitudinal compound library screening
 - Better target- or phenotype-based hit identification
- Longitudinal cell-based toxicology assays
- Longitudinal study of inhibitors of intracellular signaling pathways
- Discrimination among protein sequence variants
- Defining protein–membrane and protein–protein interactions without fixation and staining
- Rapid homozygous tagging of target genes





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