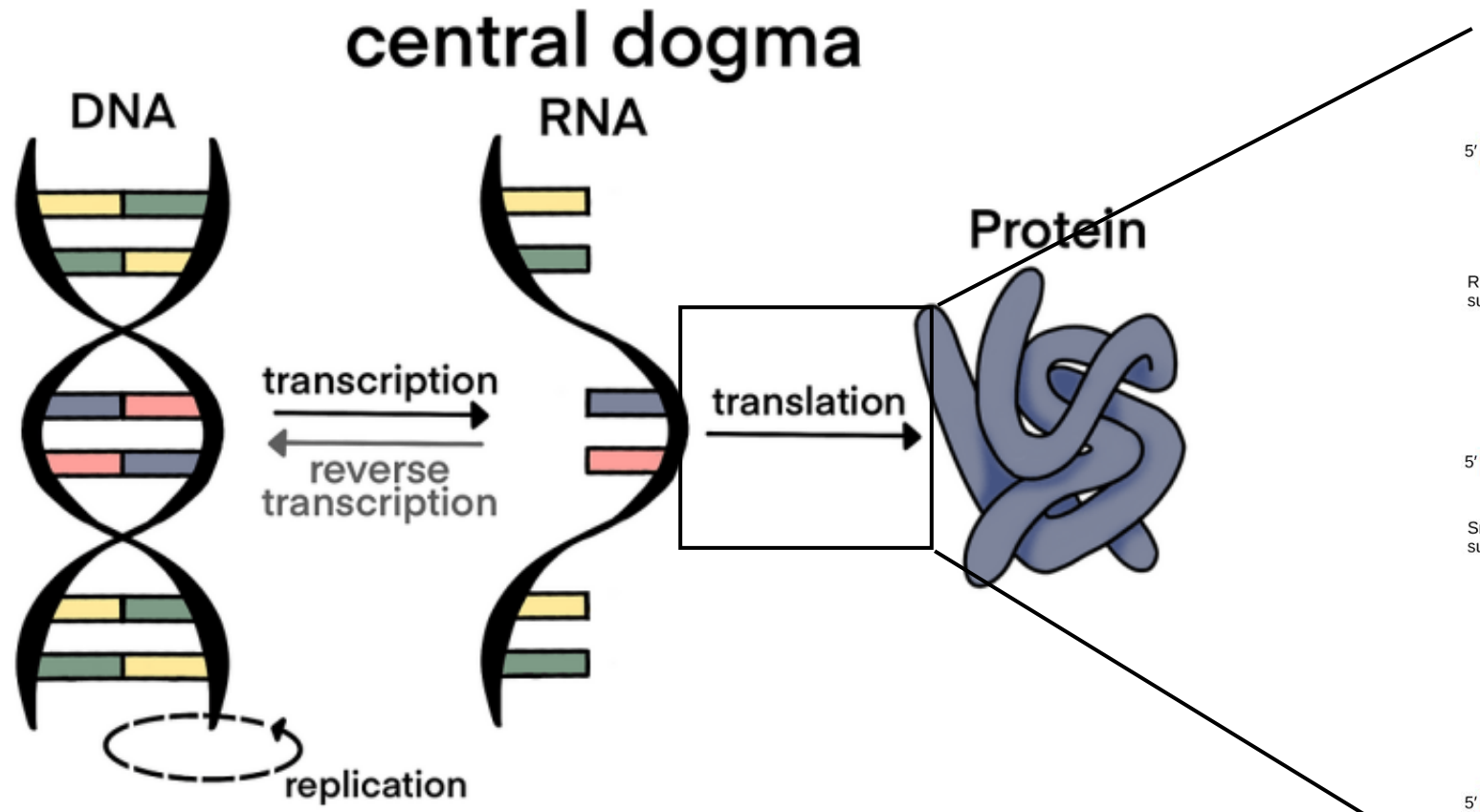
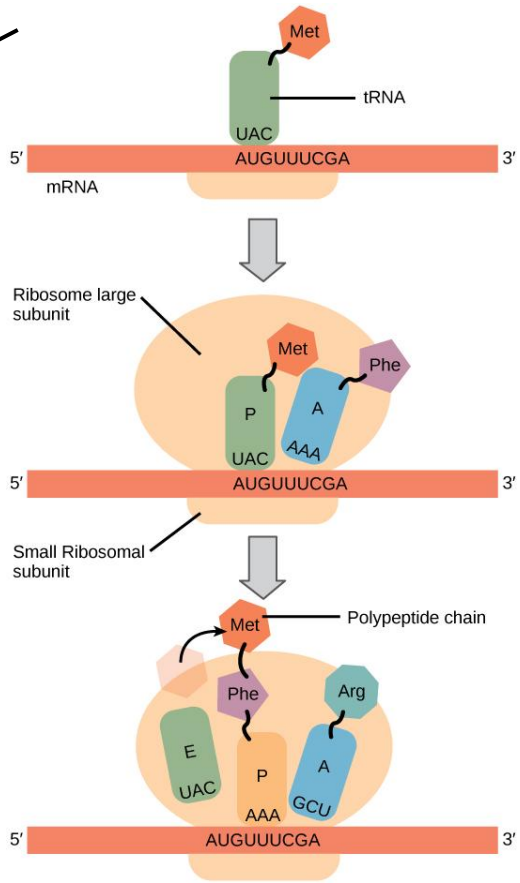
Meeting with Eileen

11.22.24

Central dogma

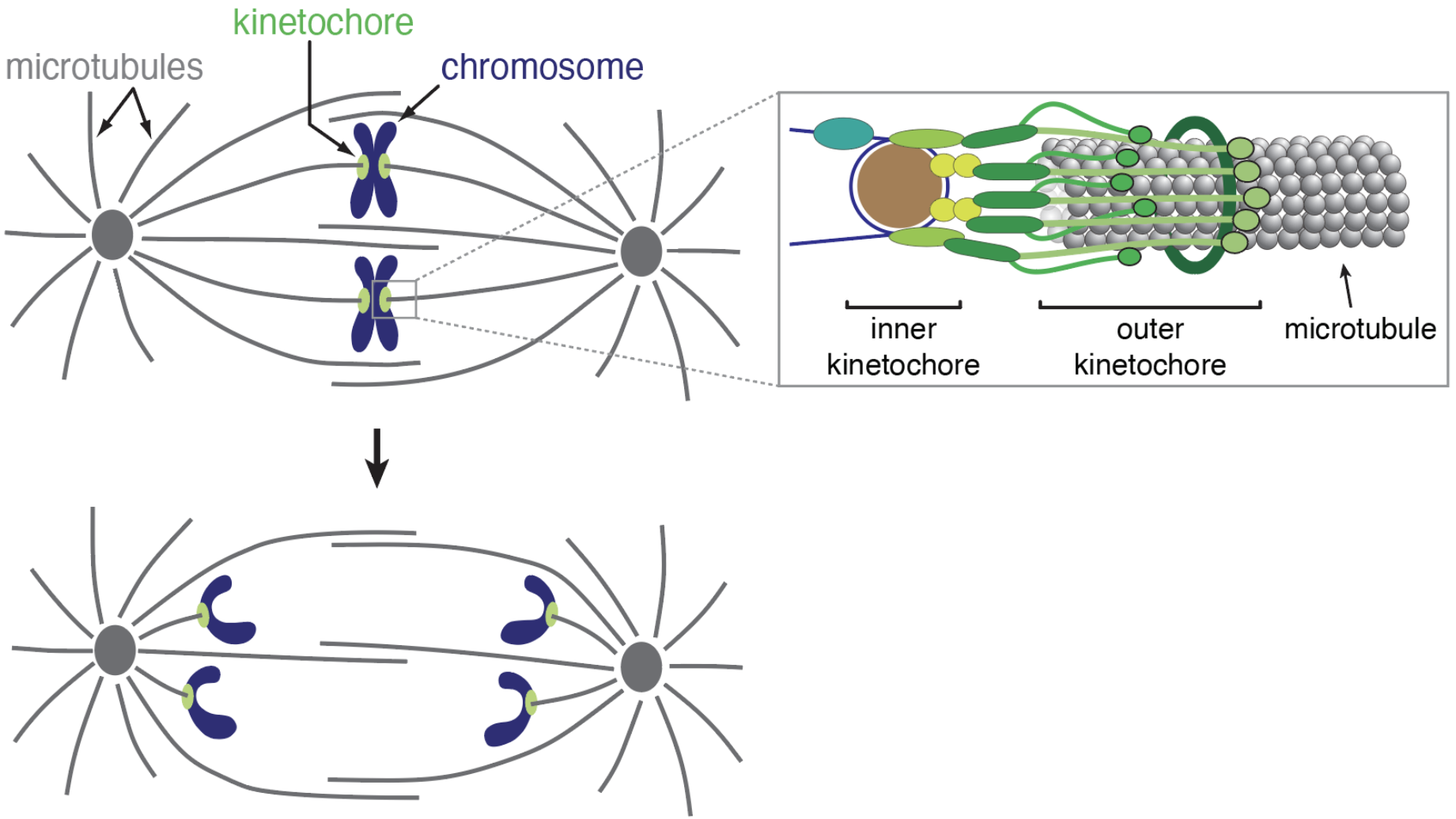




https://ensureias.com/blog/science-&-technology/central-dogma-and-gene-expression

https://opentextbc.ca/biology/chapter/9-4-translation/

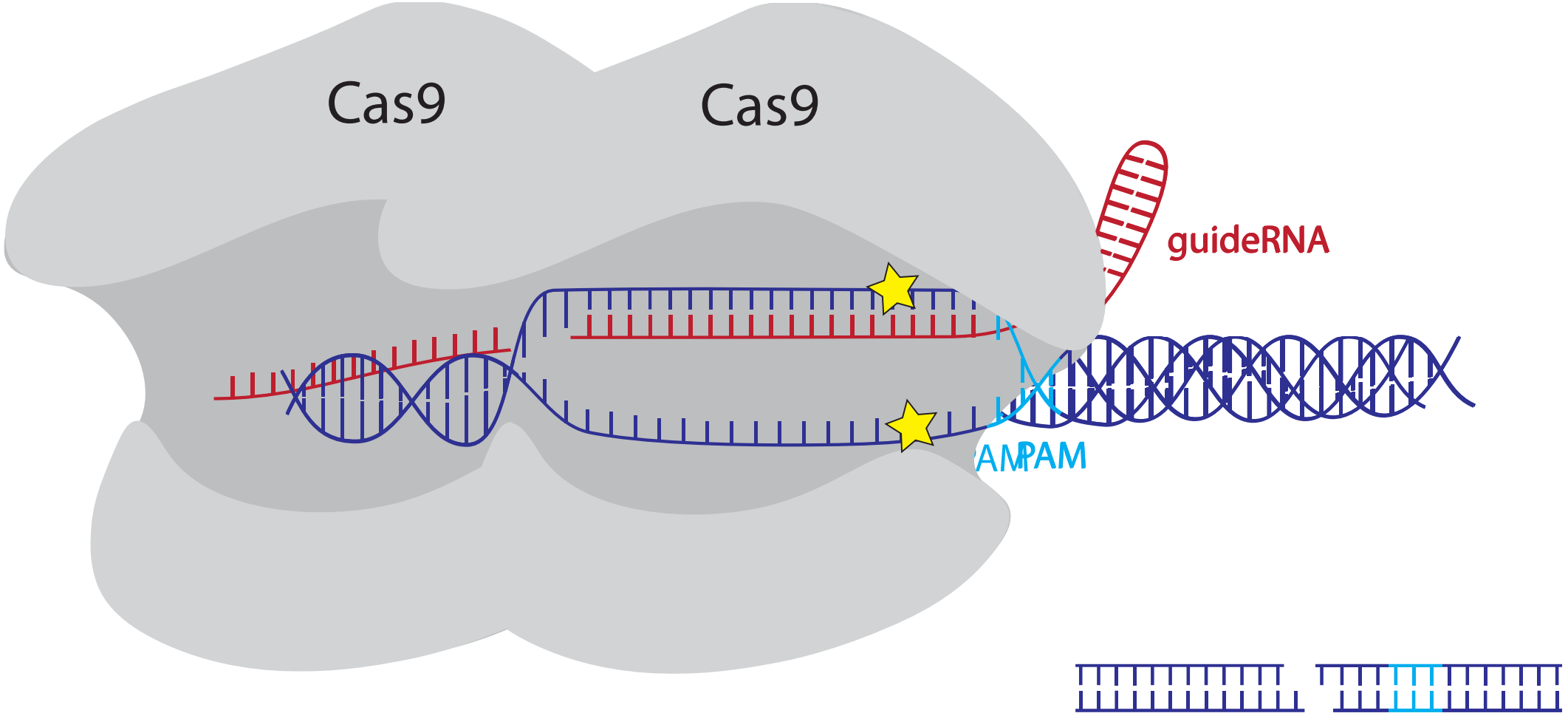
Kinetochores direct chromosome segregation



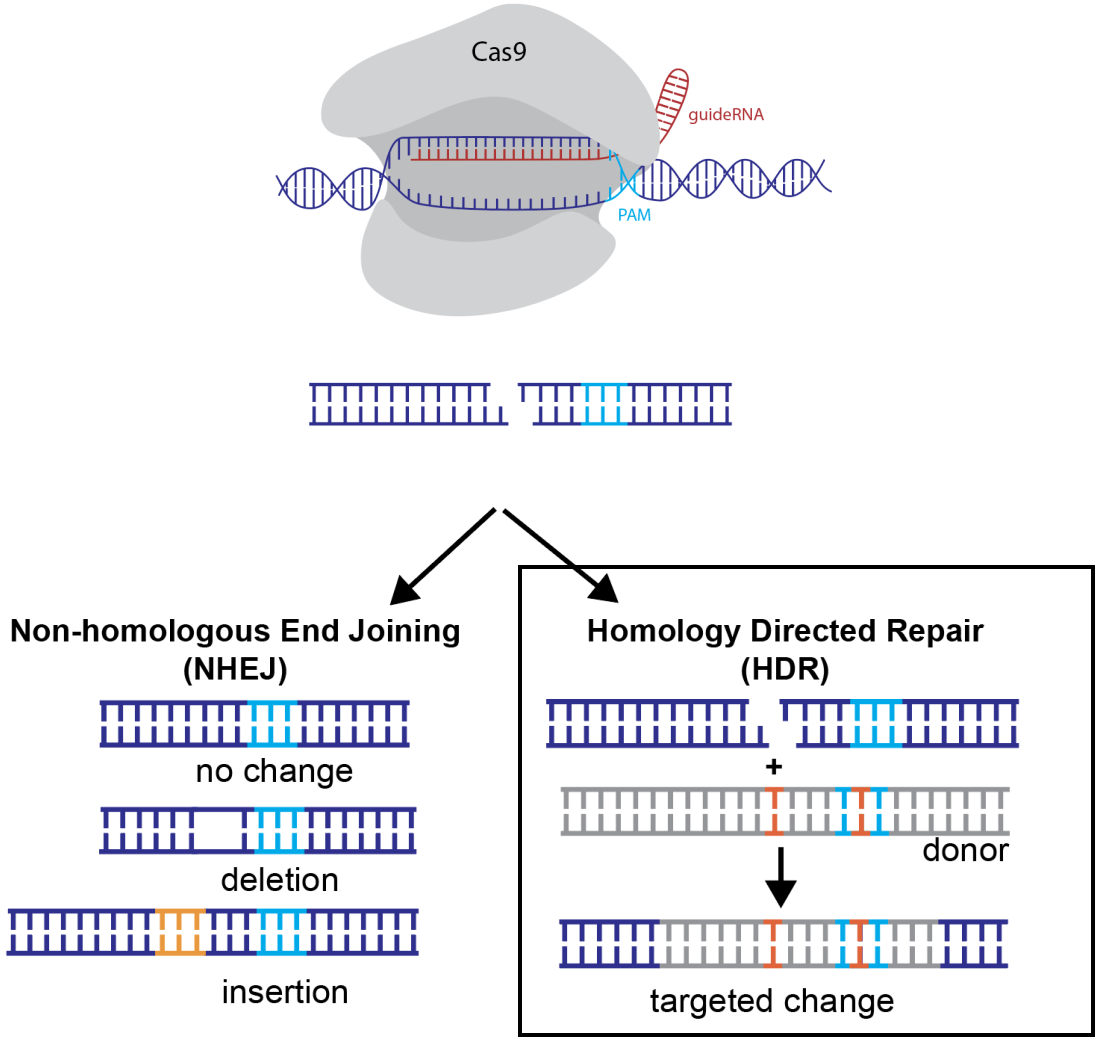
Tiling mutagenesis is effective strategy for uncovering uncharacterized function

Normally time consuming and difficult

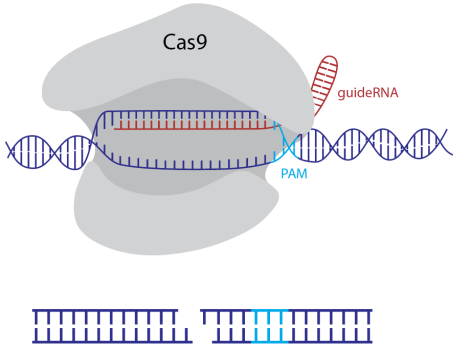
CRISPR-Cas9



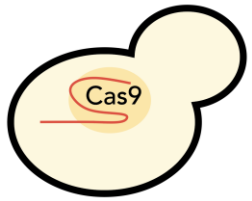
Cells use two pathways to repair DNA breaks

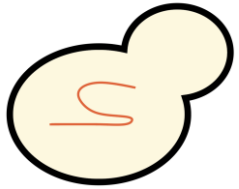


Not all guides work equally well

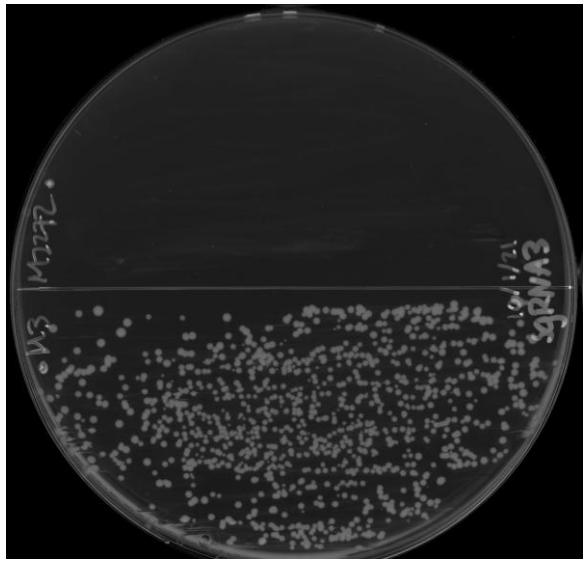


Constitutive Cas9

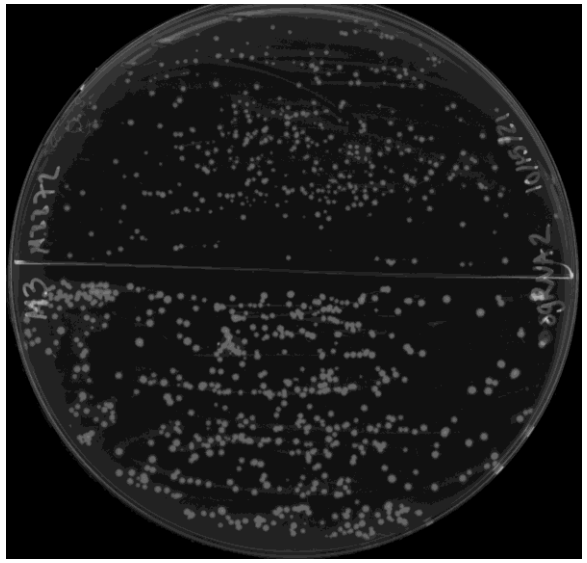




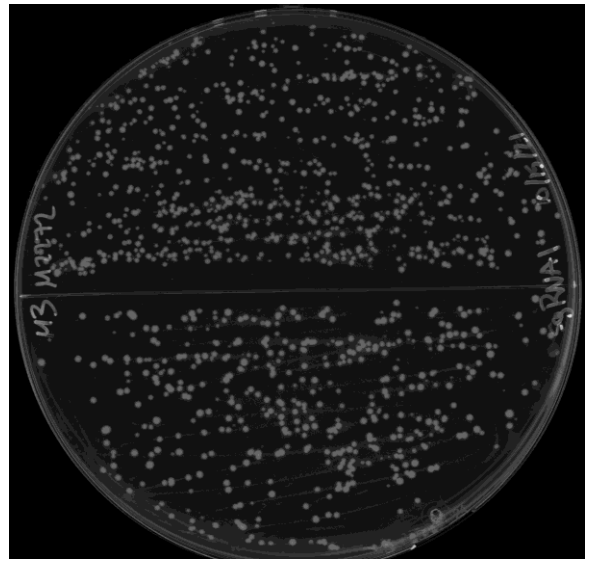
Guide A



Guide B

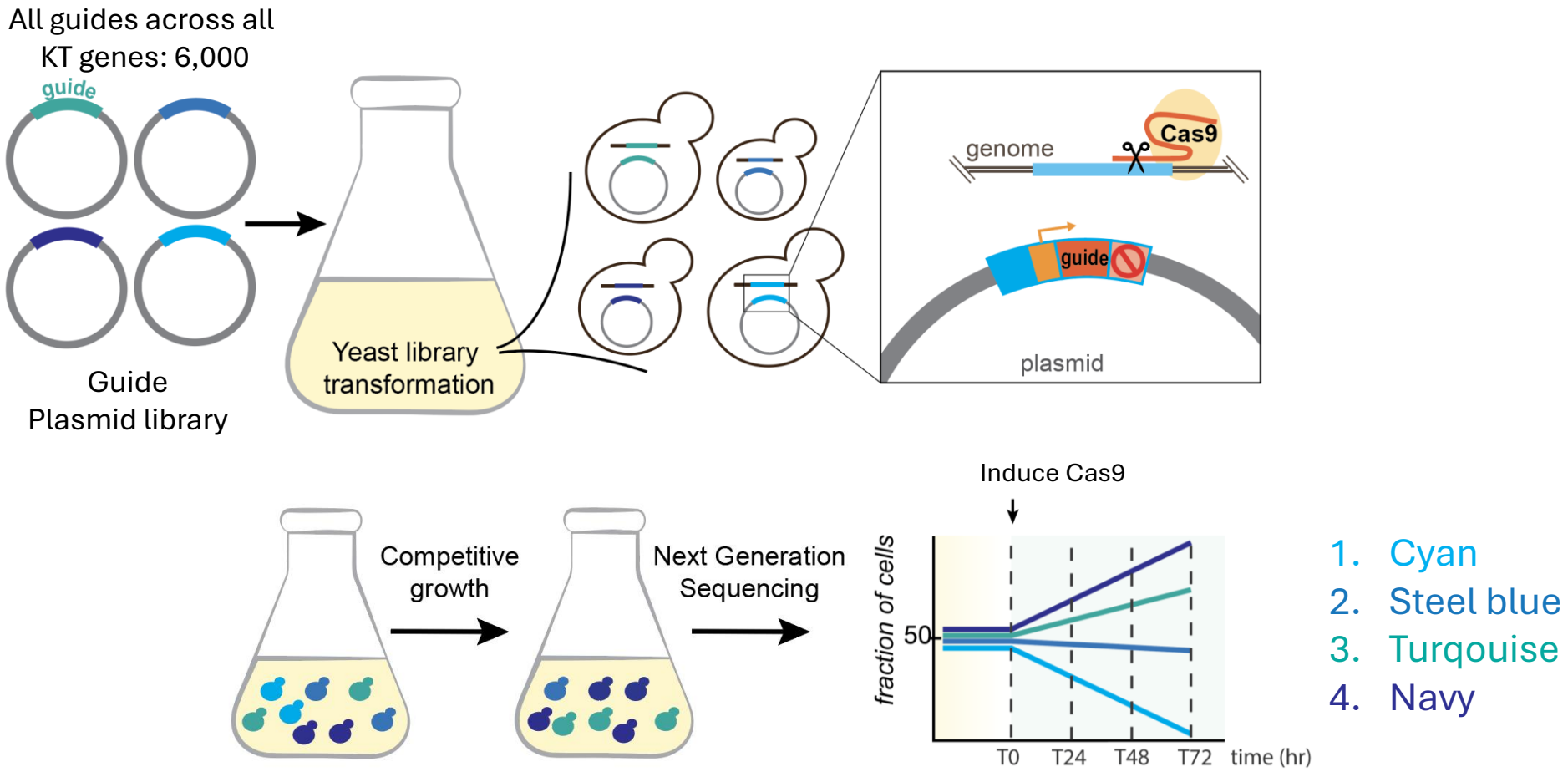


Guide C



All score equally well in online prediction tools

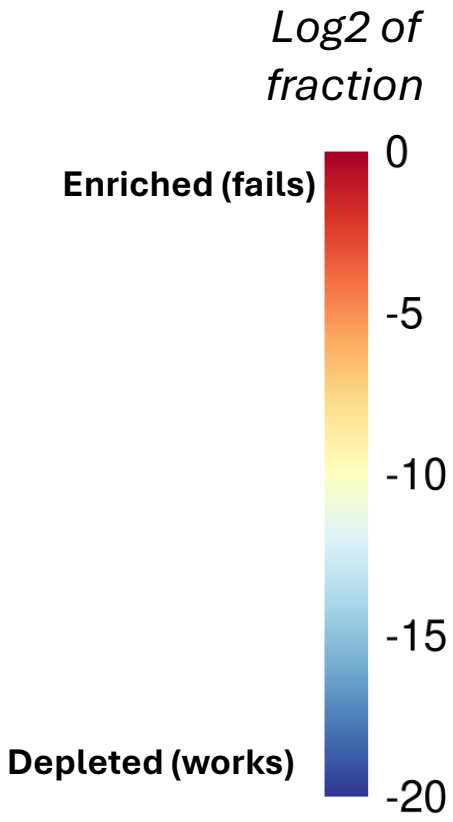
Testing guides alone will reveal best guides for use in screen

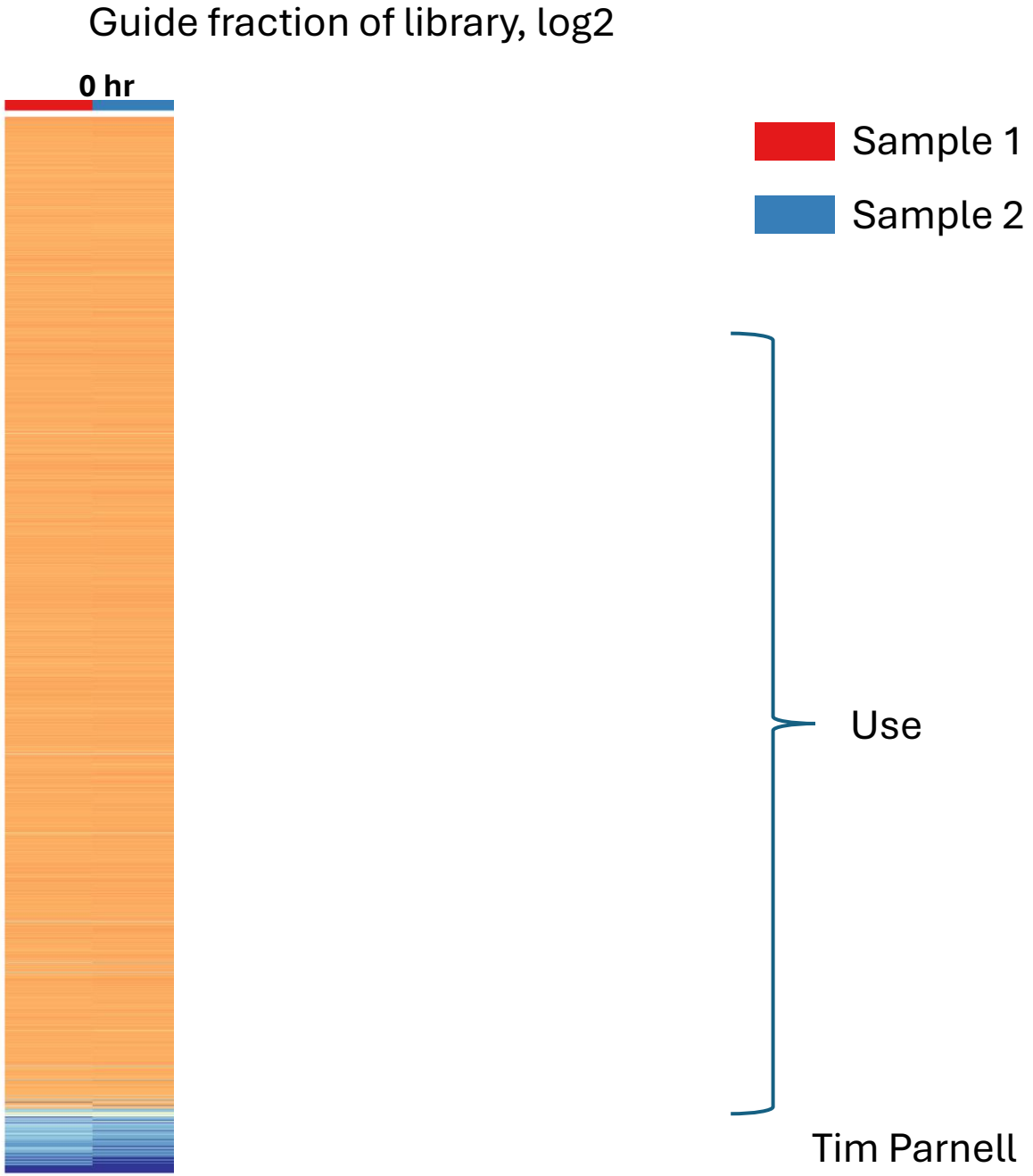


1. Cyan
2. Steel blue
3. Turqouise
4. Navy

At least 2/3 of guides work

60 genes
-6000 guides

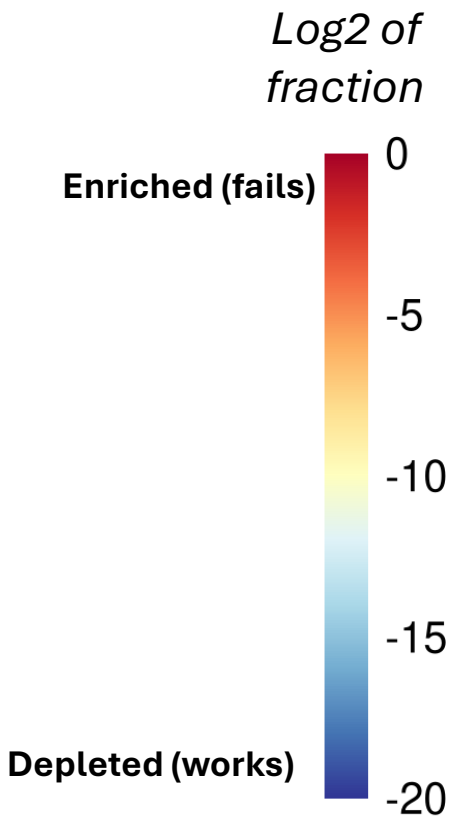


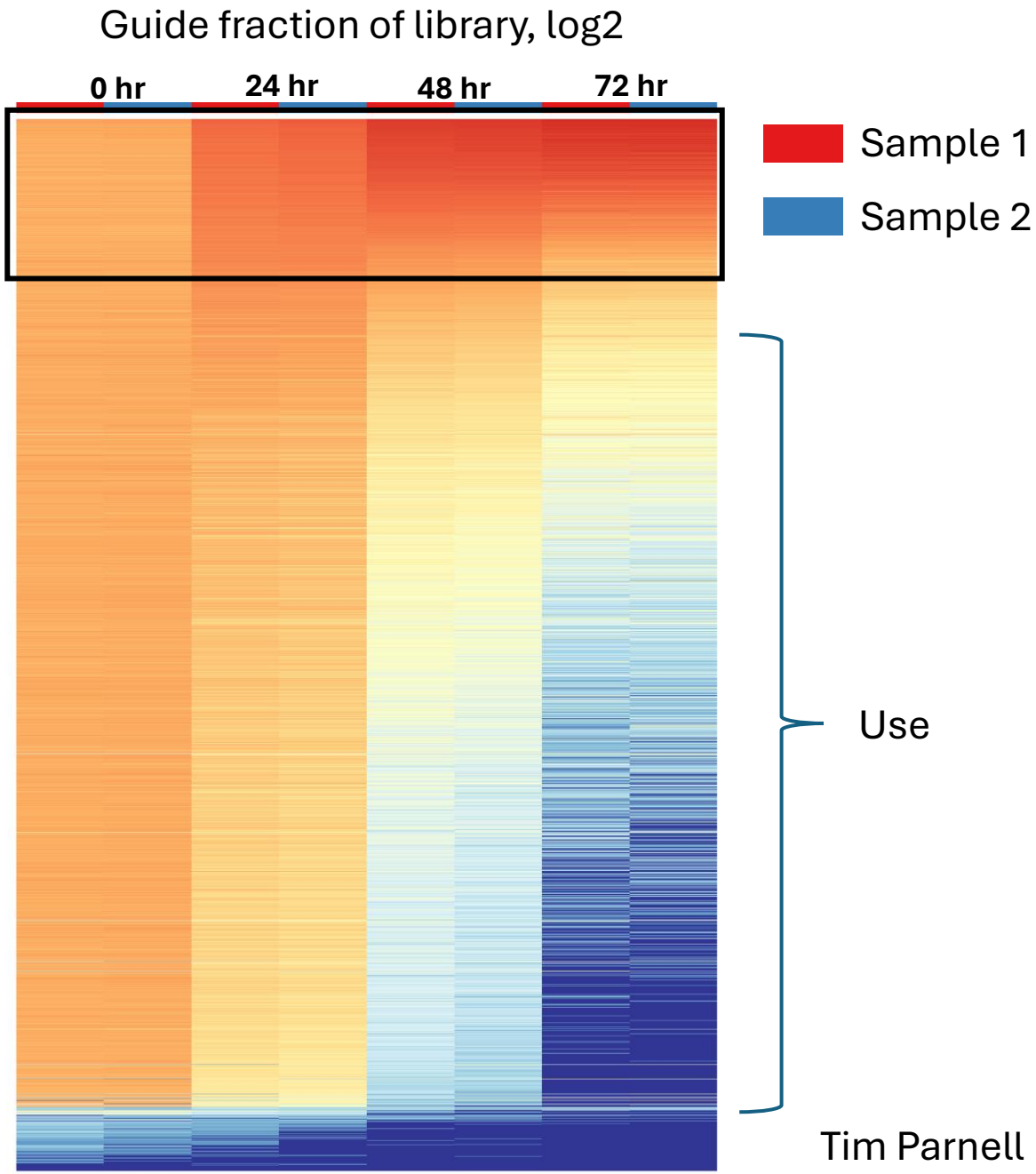


Tim Parnell

At least 2/3 of guides work

60 genes
-6000 guides



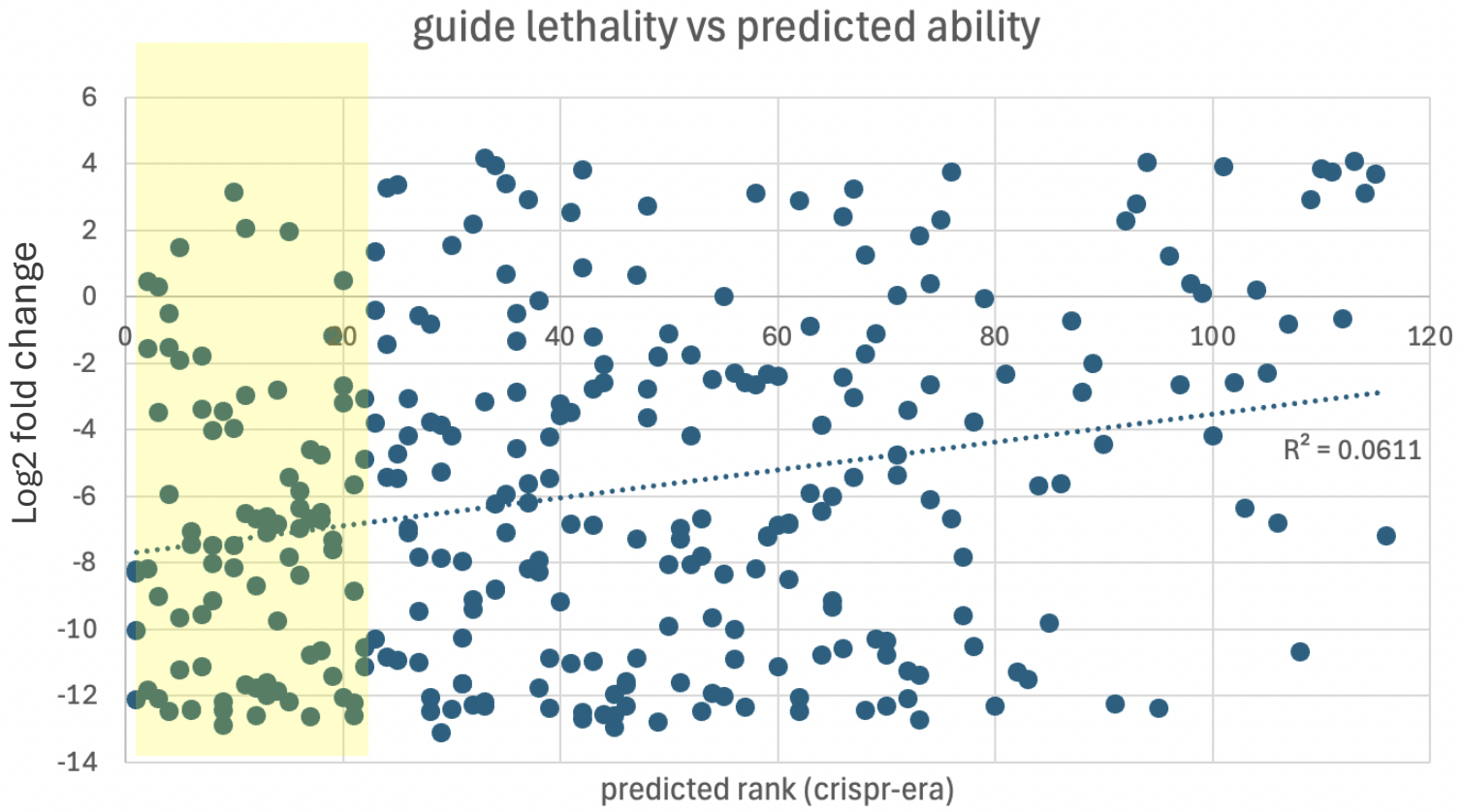


Tim Parnell

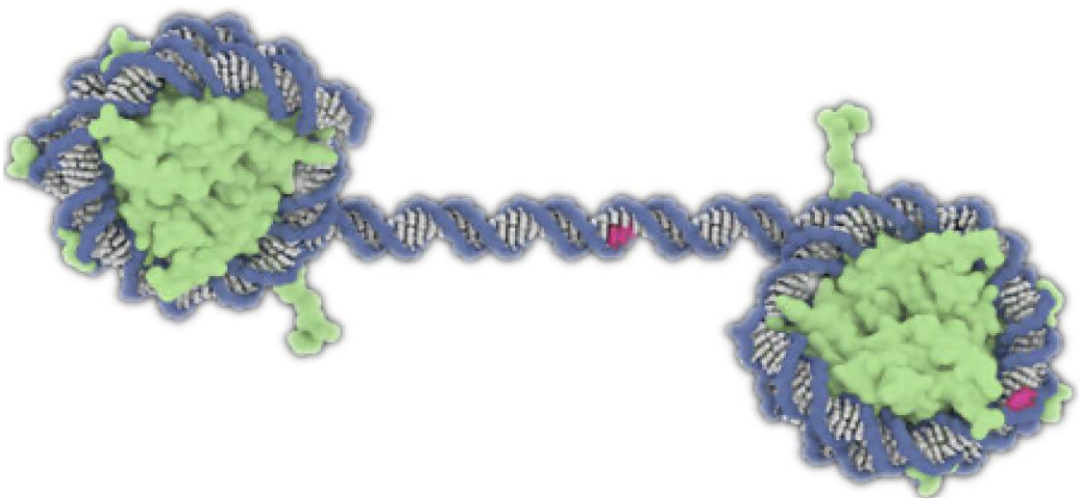
No correlation to online tool

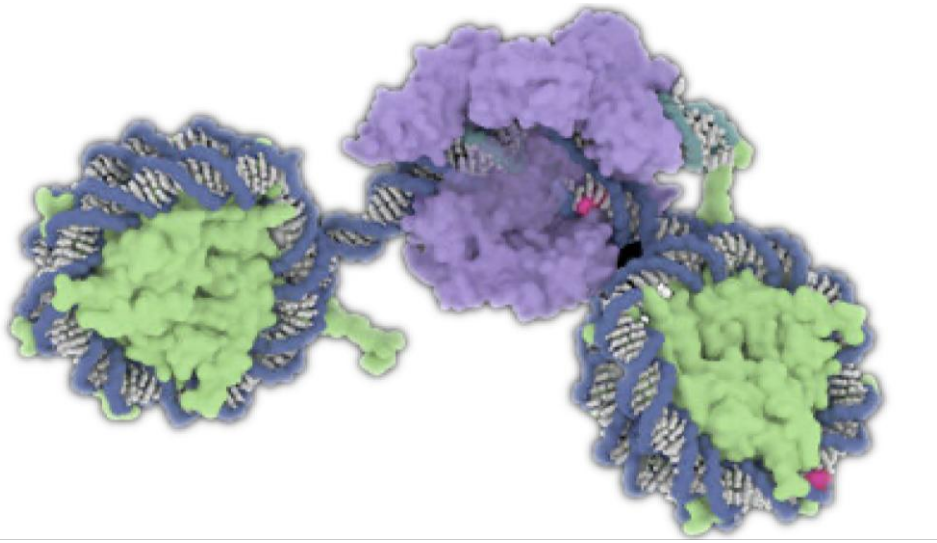
<u>Genes analyzed:</u>

- Ndc80
- Nuf2
- Spc24
- Spc25



Nucleosomes were first suspect

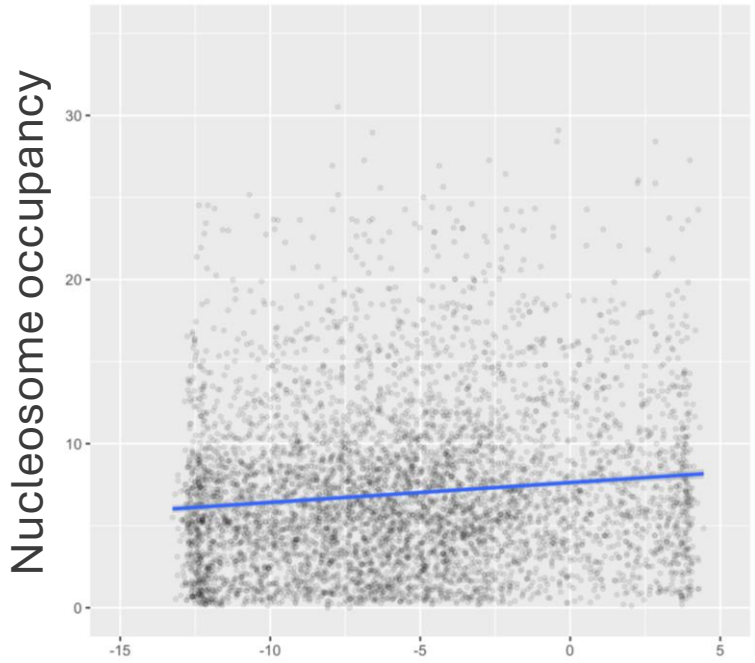




Expected correlation of nucleosome occupancy and guide depletion

Nucleosome occupancy does not predict guide depletion

72 hr post Cas9 induction



Tim Parnell

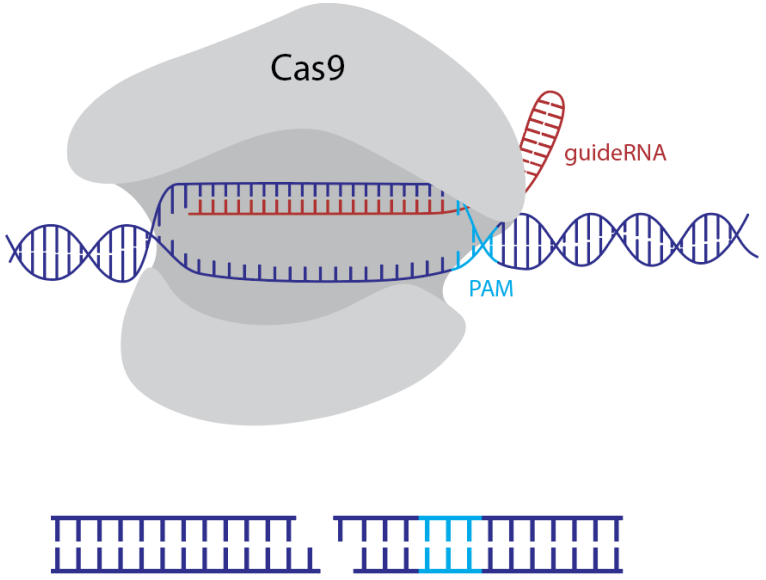
What makes for an efficient guide?

Something else?

? ? ?

Other Potential factors: gRNA issues

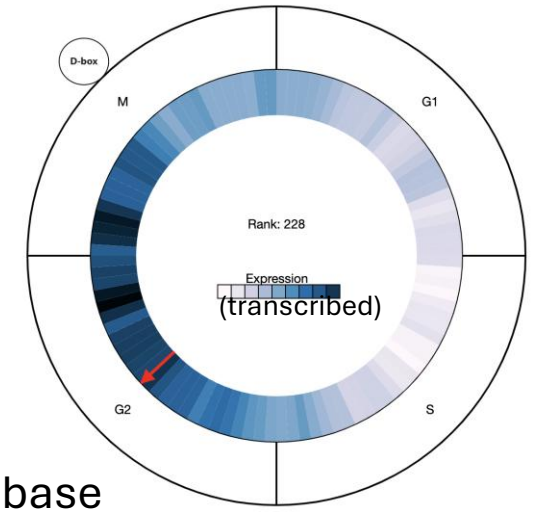
- gRNA binding other molecules in cell
- gRNA degraded by nuclease
- Secondary structures in gRNA
- Variants in lab strain (unlikely)



Other Potential factors: DNA state

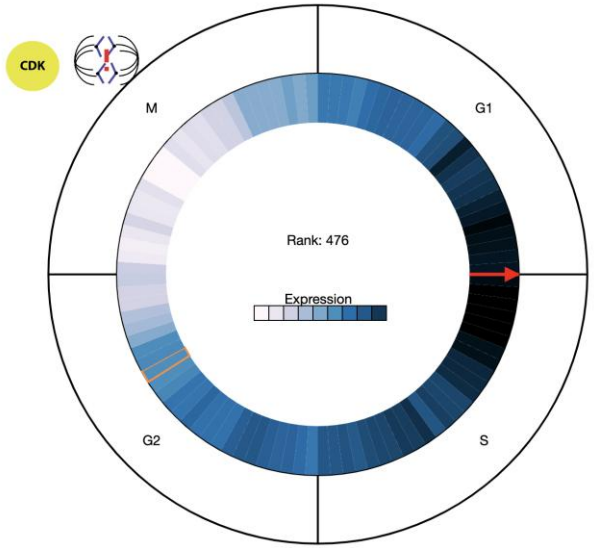
- Epigenetic marks
- Transcription levels (unlikely)

Nuf2

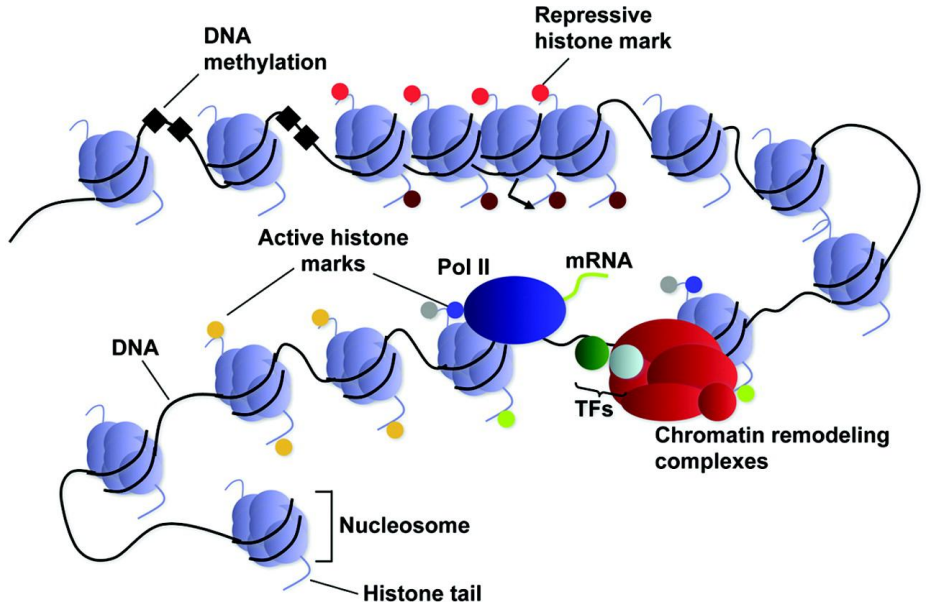


(transcribed)

Ipl1



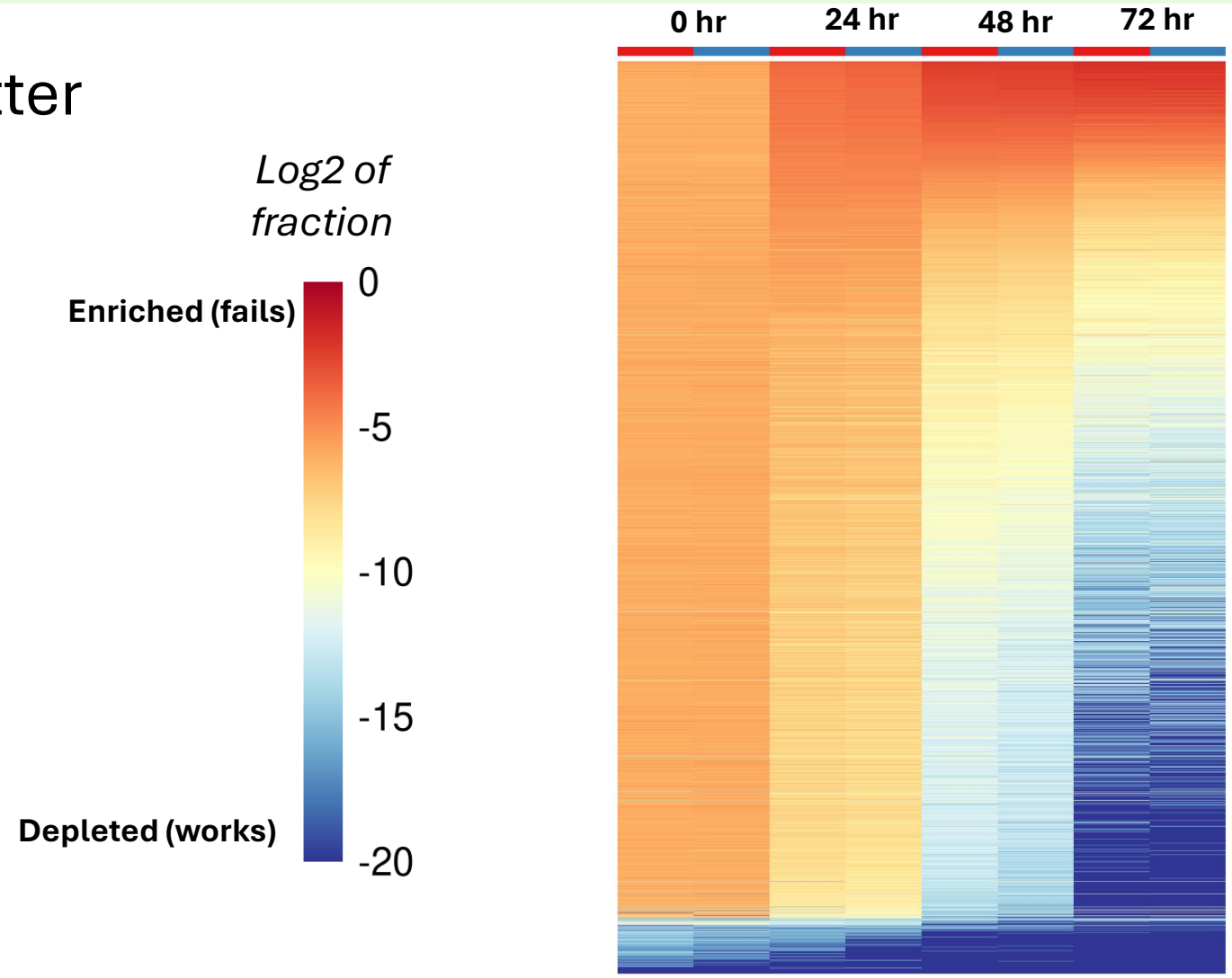
cyclebase



https://www.ahajournals.org/doi/10.1161/res.0b013e3181f17dfe

Other Potential factors: Kinetics

- Repaired better



Tim Parnell