## Workflow for Double CRISPRi sequencing data analysis

## fastq files

Seqlib11\_R1.fastq Seqlib11\_R2.fastq Seqlib12\_R1.fastq Seqlib12\_R2.fastq Seqlib13\_R1.fastq Seqlib13\_R2.fastq

## Process raw sequencing data

Code (for each sequencing lane run following scripts sequentially): pull\_tags\_double\_crispri\_v2\_seqlibXX.py parse\_discarded\_seqlibXX.py

#### Count data

Two files generated per lane from python scripts: all\_counts.txt recovered counts.txt

Normalize count data to remove PCR chimeras

Code (separate R markdown with QC on each lanes data): seglibXX.Rmd

### Fitness and GI Data:

Two files per condition, one with all information and one abbreviated:

ypd24\_data.txt ypd24\_short.txt ypd48\_data.txt ypd48\_short.txt ypeg\_data.txt ypeg\_short.txt ypd37\_data.txt ypd37\_short.txt ura\_data.txt ura\_short.txt

## Compute fitness and GI score estimates

Code (data from all lanes combined and analyzed in one R markdown file): fit gi v4.Rmd

# Visualize data

#### Code:

One R markdown file per figure and an additional file for all supplemental figures

## Count data (chimeras removed)

One file per lane: seqlibXX\_chimera\_normalized\_counts.Rdata

Also saved raw counts to RData objects: seglibXX raw counts.RData

## **Manuscript Figures**