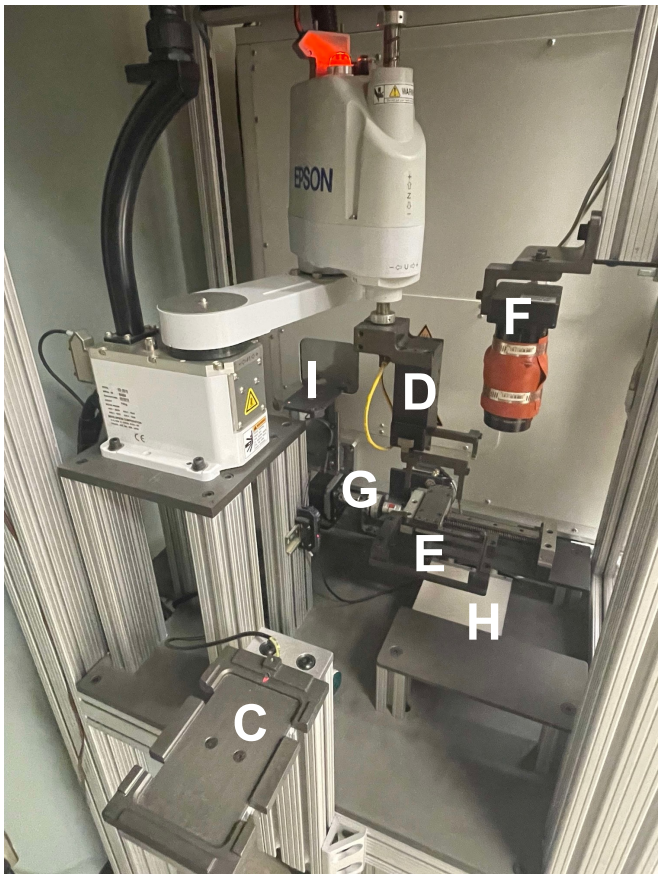
Yeast Aging Core

The UAB NSC Yeast Aging Core leverages custom quantitative high throughput cell array phenotyping (Q-HTCP) technology for yeast chronological lifespan analysis, which we call quiescence profiling. The assay is 384-well based with custom procedures designed to maximize reproducibility. Multiple projects can run concurrently. The max capacity for the system is 210 arrays x 384 cultures/array (80,640 cultures). The assay is modified from that described in the publication below. In brief, stationary phase cultures are rescued to fresh media periodically as they age chronologically, after which growth curve analysis is conducted to estimate relative survival. Additional assay details will be provided as needed for project design. See:

Santos SM, Laflin S, Broadway A, Burnet C, Hartheimer J, Rodgers J, Smith DL, Jr., Hartman IV JL: High-resolution yeast quiescence profiling in human-like media reveals complex influences of auxotrophy and nutrient availability. Geroscience 2021, 43:941-964.

https://link.springer.com/article/10.1007/s11357-020-00265-2



For Q-HTCP data collection a custom robotic scanner (Innovation, Inc) is integrated with a Cytomat 10C (ThermoFisher) robotic incubator. (**A**) The ThermoFisher Cytomat 10C is integrated with (**B**) a custom robotic imaging instrument, for automated growth curve analysis (Q-HTCP) of yeast cultures spotted in 384-array format onto agar media. The Q-HTCP Imager consists of (**C**) an Entry Transfer Station for new cell arrays to enter the system, (**D**) An Epson robotic arm, (**E**) Imaging Stage (**F**) Scanner, (**G**) a servomoter., (**H**) LED backlighting. Cell arrays are housed in the incubator and on

a defined schedule transferred robotically for imaging and returned for incubation. The series of images are subjected to growth curve analysis and analyzed for survival vs. culture age as illustrated below.

Q-HTCP-generated growth curve data is used to estimate quiescent survival

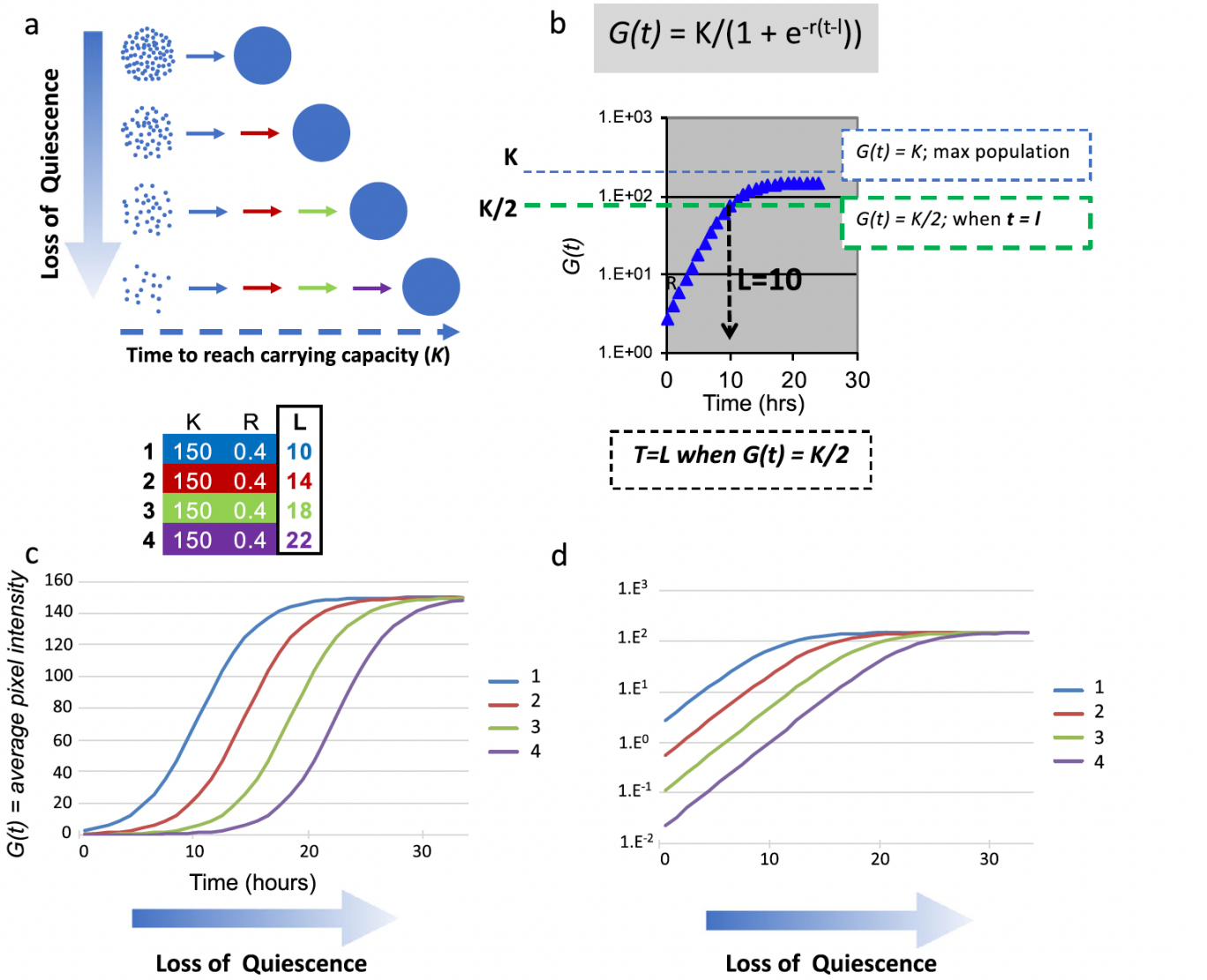


Fig. 1 The cell proliferation parameter, *L*, reports on colony-forming capacity and quiescence. **a** Quiescence is manifest as the maintenance of colony-forming capacity with age. As quiescence is lost with age (top to bottom), colony-forming capacity is reduced (indicated by blue microcolonies), and thus, a lower percentage of cells is able to reenter the cell division cycle, such that a spot culture requires more time to proliferate through additional generation times (blue, red, green, and purple arrows) to reach the same final carrying capacity, *K* (blue lawn). Q-HTCP captures time series images of yeast cultures after spotting liquid suspensions of aged stationary phase cultures onto fresh agar media (see Fig. 2). **b** The time series of images for each spot culture are analyzed for average pixel intensity over an area of the lawn and the data are fit to the logistic function, $G(t) = K/(1 + e^{-r(t-l)})$, to obtain the parameter *L*, which is the time required for the culture to reach half of carrying capacity (*K*/2). **c**, **d** Loss of colony-forming capacity, as illustrated in panel **a** results in a delay in the occurrence of the growth curve, and thus an increase in *L*. Thus, *L* increases with loss of quiescence, which is color-coded across panels **a**, **c**, and **d**. Panels **c** and **d** depict *L* on a (**c**) linear and (**d**) logarithmic scale for the y-axis. For illustration of *L* as a reporter of quiescence, using experimental data, see Fig. 2 and Online Resource 4-Fig. S1

Core Usage Fees:

(1) Yeast chronological lifespan and quiescence profiling: $300 per 384-well assay (fee includes assessment at 6 ages (typically 1, 3, 5, 7, 9 and 12 weeks).

The cost covers materials, supplies and personnel costs for quiescence profiling (e.g., requisite plastic ware, media, benchwork, robotic cell array imaging, image analysis and bioinformatics).

(2) Custom projects are also possible according to needs of individual investigators, leveraging a wide array of yeast genetic methods, including media or drug interventions, conditional (doxycycline-regulated) gene expression, CRISPR genome editing, Q-HTCP analysis of yeast gene deletion library strains, double-mutant construction by the synthetic genetic array method, and more. Please reach out to John Hartman (jhartman@uab.edu) with inquiries and customizations.

Examples of additional resources, approaches and technology that can be incorporated into OEC Yeast Core projects are demonstrated in publications below:

Santos SM, Hartman IV JL: A yeast phenomic model for the influence of Warburg metabolism on genetic buffering of doxorubicin. *Cancer Metab* 2019, 7:9.
DOI: 10.1186/s40170-019-0201-3

Santos SM, Icyuz M, Pound I, William D, Guo J, McKinney BA, Niederweis M, Rodgers J, Hartman IV JL: A Humanized Yeast Phenomic Model of Deoxycytidine Kinase to Predict Genetic Buffering of Nucleoside Analog Cytotoxicity. Genes (Basel) 2019, 10.
DOI: 10.3390/genes10100770