Fitness Assay, a.k.a. Competitive Growth Rate Assay (2/19/09)

General Principles / Tips

Below is a list of tips I have learned during my experimenting—an astute observer will notice that I have not always followed my own advice, but how else would I have learned what not to do.

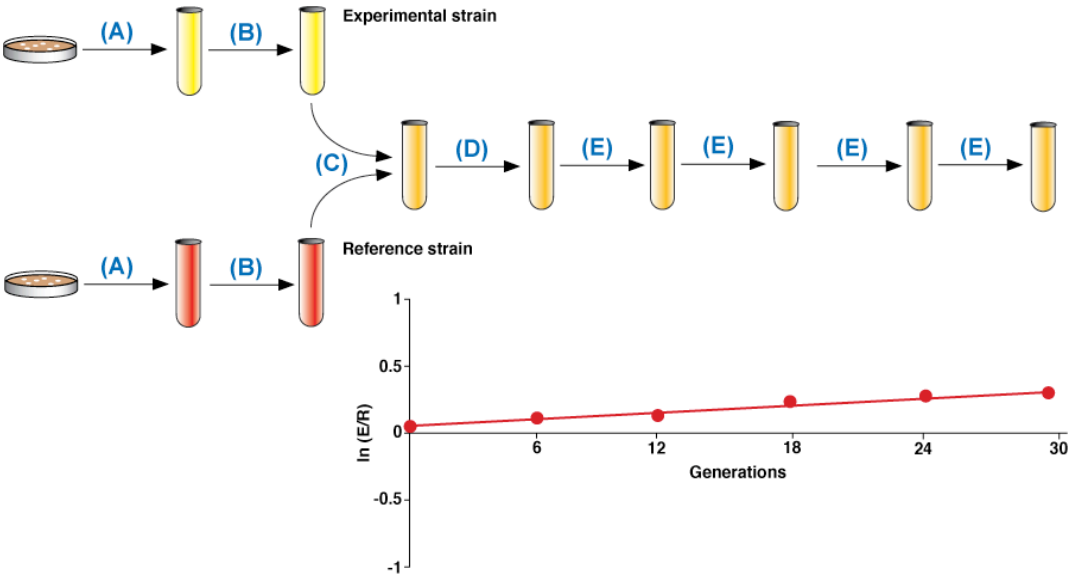
Compete only two strains at a time—it makes life (read analysis) much simpler.

Avoid *ade2* mutants—autofluorescence of a metabolic by-product in these mutants can cause issues (if you must use an *ade2* mutant, supplement sufficiently).

Like microarray experiments, it is good practice to use a common reference strain for each run in an experiment. That said, it is also good practice to compete two strains whose genotypes are similar (different genotypes will saturate at different densities or have different lag times). For this reason I have started doing two-color assays were both strains carry a unique fluorescent protein—as a bonus, two colors also aids in discriminating between the two populations.

Prewarm all media in the 30° room and use the same batch of media throughout. This will prevent cells from lagging following dilution into new media. For ease, I prepare YPD, dispense 10 ml into test tubes using a fixed volume dispenser and autoclave the racks of tubes.

The Experiment



Below is a general protocol for the fitness assays in YPD—different media will require different dilutions.

Day 1

Prepare media, start o/n cultures **(A)** (don't forget the Reference strain).

Day 2

Dilute o/n cultures 1:300 **(B)**. Make sure to do multiple tubes of the reference strain—this strain is needed for every competition. This usually puts me on schedule to do my first dilution 9 hours after the 1:300 dilutions.

Coulter count reference and several experimental strains to determine at what ratio to mix the strains. Total volume will be 500 µl. (If doing a one-color assay, I put the reference strain at a slight advantage (~60:40) since it carries the burden of a fluorescent protein).

Mix strains in microfuge tubes (total volume of 500 µl) **(C)**. Immediately dilute into 10 ml of prewarmed media and place tubes on rollerdrum **(D)**. Dilutions should be adjusted so that after the cultures return to the same density every 12 hours. I have found that the ideal dilution is ~1:600 (16 µl into 10 ml) for YPD and ~1:90 (110 µl into 10 ml) for YPGal.

Count mixed cultures in microfuge tubes to get an estimate of cell number (I often just count a subset).

Prepare samples for FACS by spinning microfuge tubes 1 min, aspirating off media, and resuspending in PBST (1 x PBS, 0.1% Tween 20). Place at 4° C until all samples are ready for FACS.

Every 12 hours . . .

Dilute cells as before (put back on the rollerdurm as soon as possible) **(E)**.

Sample 10 µl for Coulter Counter

Sample 500 µl for FACS, spin 1 min, aspirate, resuspend in 1 ml PBST, and store at 4° C.

I typically do about 30 – 40 assays in parallel—the time commitment is about 1.5 hours every 12 hours. I typically do 3 (or 5) dilutions—that gives me 4 (or 6) data points through which to draw a line.

Preparation for FACS

Remove microfuge tubes from fridge, vortex, and transfer to a 5 ml polystyrene FACS tube. Sonicate. Sounds easy, but 200 tubes will take ~3 hours.

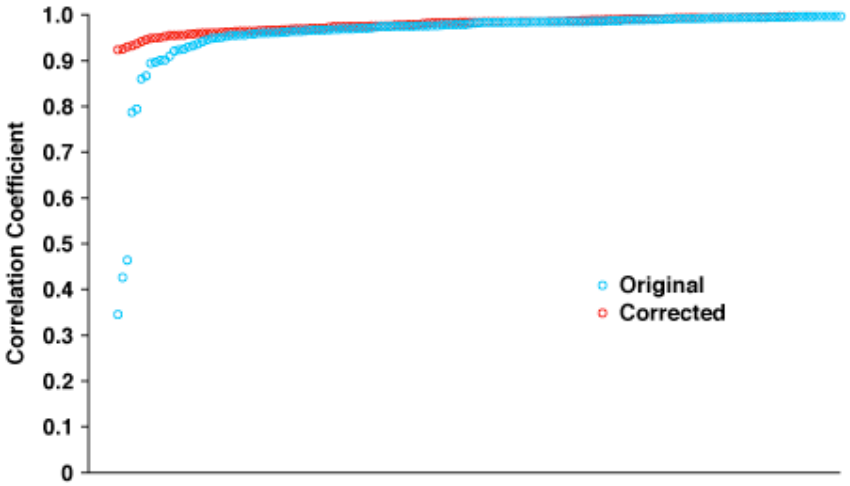
For FACS, I usually count 30,000 – 50,000 events for each sample. I have been using the LSRII (BD Biosciences) for analysis.

Data Analysis

From the FACS data, determine the ratio of Experimental to Reference strain for each sample. The selection coefficient is the change in the ln ratio versus generations.

Normalizing: If the reference strain has a different fitness from wild type, you can normalize the value of s using the equation $s_{Exp\ vs\ WT} = (1 + s_{Exp\ vs\ Ref})/(1 + s_{WT\ vs\ Ref}) - 1$. This is derived from the relation of $w_{Exp\ vs\ WT} = w_{Exp\ vs\ Ref}/w_{WT\ vs\ Ref}$, and substituting 1 + s for w.

Quality of data: Ideally a fitness assay will produce a straight line on a plot of the natural-log ratio of the strains versus generations. It is useful to do some kind of quality of data check. I have typically calculated the correlation coefficient (r^2), but this may not be the best method since a line with a slope ~0 will have a poor r^2 value. In a previous experiment, 13 out of 162 assays had an r^2 value for the correlation between *ln* ratio versus generations of less than 0.925. In all cases, one of the four data points deviated from the trend, most often due to one strain entering a lag phase after mixing. The offending data point was removed, and s and r^2 values were recalculated for each experiment.



Derivation of formula (from Hartl, Primer of Population Genetics, 2000)

s = selective coefficient
w = fitness
E/R = ratio of experimental to reference

$w = 1 + s$.

This implies that $s > 0$ is a beneficial mutation and $s < 0$ is deleterious. Some prefer the opposite.

Let's assume that the experimental strain has a per-generation fitness advantage s over the reference strain. Then after one generation of growth, the ratio of experimental to reference will have changed by (1 + s):

$(E/R)_1 = (E/R)_0 (1 + s)$.

After two generations,

$(E/R)_2 = (E/R)_0 (1 + s)^2$.

And after t generations,

$(E/R)_t = (E/R)_0 (1 + s)^t$.

Using the trick that as long as s is small, $(1 + s)^t \sim e^{st}$, we see that $(E/R)_t = (E/R)_0 e^{st}$.

Taking the natural log of both you can see that $ln(E/R)_t = ln(E/R)_0 - st$.

Therefore s is the change in the $ln(E/R)$ versus t in generations:

$s = [ln(E/R)_t - ln(E/R)_0]/t$