Otto Lab Bioscreen Protocol

1) Grow up culture to stationary phase

For colony bioscreen:

- streak out to single colonies from frozen
- inoculate into the liquid medium that you will be using in the bioscreen
- grow to stationary phase (usually 24-48 hours)

For population bioscreen:

- inoculate frozen culture into the liquid medium you will be using in the bioscreen
- allow to grow to stationary phase

2) 1:100 (or 1:50) dilution into fresh medium in batch

Similar to a pcr reaction, decide how many wells you will fill and make up one batch of diluated culture (don't forget to add a couple extra wells). e.g., if you will fill 4 wells, make 800uL diluted culture.

3) Transfer 150uL from (2) into each bioscreen well

Things to keep in mind:

Decide well layout – I almost always put culture randomly across all 200 wells. I have an excel sheet where one column contains all the experimental tube information and I use a random number generator (e.g., http://www.psychicscience.org/random.aspx) to generate 200 random number without replacement. I copy and paste from there into MS Word, replace spaces with commas (find and replace) and save as a .csv or .txt file. I then import this into MS Excel as a row of numbers, which I then copy and paste special/transpose into a second column of my original excel sheet. If Excel hadn't taken away macros from my version this would be easier.

Number of technical replicates: I often do between 3-5 "technical replicates" i.e., replicates from the same original tube, as in step (2).

Media batch: The most important thing I've found that increases variation in results is the media batch; this may be specific to my media (YPD), but maybe not. I now always use the same medium within a single run, and try really hard to plan my experiments accordingly. If I want many replicates of each experimental tube I will do two bioscreen runs with fewer replicates within each (rather than two run with different experimental tubes).

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