Determination of Mutation Rate using Fluctuation Assay

Adaptation of Luria-Delbruck Protocol by Greg Lang

OPTIMIZATION OF CULTURE SIZE

In order to analyze the data using the p_0 method, ~10-80% of the cultures need to have zero mutation events. Multiple culture conditions are tested in this section that limit the growth of the culture (culture size and sugar concentration) for identifying optimal culture conditions that give rise to the number of generations that allows the correct fraction of zero-class events.

Four conditions are tested here (though many others could be tested to get the correct size of zero-class events):
a) YP + 2% Dextrose – 100 μL culture
b) YP + 0.2% Dextrose – 100 μL culture
c) YP + 2% Dextrose – 10 μL culture
d) YP + 0.2% Dextrose – 10 μL culture

1. Grow an overnight culture of the strain to be tested in permissive media.

2. Dilute culture 1:10,000 into 40 mL of YP + 2% Dextrose and 40 mL of YP + 0.2% Dextrose.

3. Into a 96-well plate, divide samples into wells (24-wells per condition) using the appropriate culture volume (either 100 μL or 10 μL).

4. Incubate 96-well plate at 30°C overnight without shaking for cultures to reach saturation (make sure to cover plate with a plate seal or the 10 μL cultures will evaporate and not grow as many generations as expected).

5. On the next day, add 90 μL of YPD to the 10 μL cultures so that there is enough volume to plate.

6. Plate the entire volume of each culture onto a dried plate. Using this dried plate technique, also developed by Greg Lang, ~9 – 100 μL cultures can be plated (as distinct spots) on a standard 10 cm plate.
   To dry the plates, place a sterilized 90 mm Whatman filter paper (Cat No. 1003-090) on the plate using a replica plating apparatus. Allow this to sit for at least 30 minutes before use. Immediately before use, use sterile forceps to remove the filter paper from the plate (which is now textured and has a decreased moisture content)

7. Incubate plates for 1-2 days at 30°C until colonies grow. Calculate fraction of zero-class events, and use the same condition for the actual fluctuation assay.

FLUCTUATION ASSAY

One 96-well plate is enough to estimate the mutation rate using the p_0 method (72 cultures will be plated to test for mutants, while the other 24 cultures will be pooled to get an accurate reading of cell density).

1. Grow an overnight culture of the strain to be tested in permissive media.

2. Dilute culture 1:10,000 into 40 mL of media determined in the “Optimization of Culture Size” section.

3. Divide samples into all wells of a 96-well plate, again using the culture volume determined in the “Optimization of Culture Size” section.

4. Incubate 96-well plate at 30°C overnight without shaking for cultures to reach saturation (make sure to cover plate with a plate seal).

5. Pool 24 of the cultures and measure their cell density three separate times. Average these measurements to get an accurate cell count.

6. Plate the entire volume of each remaining culture using dried plate technique described above (if using small volume cultures, bring volume up to 100 μL before plating).

7. Incubate plates for 1-2 days at 30°C until colonies grow. Calculate fraction of zero-class events, and use this data to calculate the mutation rate using the p_0 method (see below).

ANALYSIS OF RESULTS

There are multiple methods for determining mutation rate from the fluctuation assay. The simplest, used in the 1943 Luria and Delbruck paper, is the p_0 method. This method agrees with the others as long as the fraction of zero-class events is approximately 10 – 80%. For more details about other methods of data analysis, see Rosche and Foster, *Methods*, "Determining Mutation Rates in Bacterial Populations," 2000. **20**: 4-17.

1. Use the following formula to calculate the mutation rate:

$$u = \frac{-\ln(P0)}{N}$$

P0 = probability that a mutation does not occur in the entire culture (fraction of cultures with zero mutations divided by the total number of cultures tested)

N = number of generations that has occurred (technically this is the total number of cells at saturation minus the total number of cells at time zero… but because there is such a small number at time zero compared to the final number, we make the simplifying assumption that the number of generations is equal to the number of cells at saturation).

u = mutation rate (mutations of interest per genome per generation, can also be called mutations of interest per cell division)