

Adapted from Luria and Delbruck, Genetics 28:491; 1943, Armitage, Jour. Hygiene 51:162, 1953, and Lea and Coulson, Jour. Genetics 49:264, 1949.

## 1. Assumptions

- a. Growth is synchronous and uniform for mutant and non mutant cells.
- b. Mutations occur at cell division, yielding one mutant, one non mutant offspring.
- c. All mutant cells are effectively counted precisely at time of assay.

## 2. Definitions

N Cell number per culture.

t Generation number. Defined by the law of geometric growth  $N = 2^t$  or  $t = \log_2 N$ .

r Number of mutant cells.

m Number of mutational events. d average mutant clone size =  $r/m$ .

a Probability that a division will yield one mutant offspring.

At each generation, N increases to 2N by means of N divisions. By summing the total divisions, it is evident that  $N - N_0$  divisions are involved in the increase from an inoculum  $N_0$  to a final value N. As  $N_0$  is usually negligible in comparison to N, we may usually write that N divisions have occurred in the growth of a culture of size N. Therefore:

$$(1) \quad m = aN$$

## Null fraction method

The probability that a mutation will not occur at a given division is  $1-a$ . Therefore the probability that no mutations will have occurred in a culture is

$$(2) \quad p_0 = (1-a)^N \text{ which can be shown to be closely approximated by}$$

$$(2a) \quad p_0 = e^{-aN} \text{ or } (2b) \log_e p_0 = -aN, \quad a = \frac{1}{N} \log_e \frac{1}{p_0} = \frac{2.3}{N} \log_{10} \frac{1}{p_0}$$

where  $p_0$  is that fraction of a series of cultures which contains no mutants.

Average number of mutants

(Warning; Carefully distinguish  $r$  from  $m$  !!) It will be shown that each generation contributes an equal number of mutants  $r$  to the final crop. At the  $i$ 'th generation,  $N_i$  cells are produced from  $N_i/2$  by means of  $N_i/2 = 2^i/2$  divisions. On the average, there will then be  $a \cdot 2^i/2$  mutations at this generation. Each of the cells of the  $i$ 'th generation will increase by a factor  $N/N_i = 2^{t-i}$  by the time of assay. The total crop of mutants from the  $i$ 'th generation will therefore be  $a \cdot 2^i/2 \cdot 2^{t-i}$  or  $a \cdot 2^t/2$ . Summing over all  $n$  generations we find the total mutant crop

$$(3) \quad r = at2^t/2 = atN/2 = a N \log_2 N/2$$

$$a = 2r/N \log_2 N = .602 r/N \log_{10} N.$$

$$(4) \quad \text{and } d = r/m = t/2.$$

Luria and Delbruck's likely average correction

L and D point out that (3) displays the average value of  $r$ , including the contribution of a great many mutants from rare, very early mutations. They set up another expression which they consider will give the "likely average" in any given experiment of  $C$  cultures, each size  $N$ .

It is assumed that no mutations are likely to have occurred prior to  $t = i$ .  $i$  is selected arbitrarily, as a function of mutation rate, so that in the entire experiment with  $C$  cultures there will have been one premature mutation, that is, so that  $a \cdot C \cdot N_i = 1$ , or  $2^i = 1/aC$  and  $i = -\log_2 aC$ . Thus it is assumed that the mutations in each culture have occurred throughout  $t-i$ , rather than all  $t$ , generations. (3) then becomes

$$(5) \quad r' = 1/2 \cdot (t-i) aN = (aN/2)(t-i) = (aN/2) (\log_2 N + \log_2 aC) = (aN/2) \log_2 aCN.$$

This cannot be solved explicitly for  $a$ , but may be handled numerically or with the help of a chart provided by L and D. This treatment has been criticized by Armitage; it has also been abused by workers who have pooled estimates of  $a$  from different experiments, rather than summing the pooled data. At best this technique does not mitigate the very high variance of  $r$ , which makes feasible estimates of its mean very difficult.

Use of the median and upper quartile

The (limited) solution of the theoretical distribution of  $r$  by Lea and Coulson allowed the development of two other measures, the median  $r_2$  and the upper quartile  $r_3$  (i.e. the values standing at the positions  $(n+1)/2$  and  $3(n+1)/4$  in a series of  $n$  observations ranked by size).

They have shown that  $r_2/m - \log_e m = 1.24$  and  $r_3/m - \log_e m = 4.09$ , respectively. Tables to assist the calculation of  $m (=aN)$ , and hence of  $a$ ) are given by these authors. These methods do not make full use of all the numerical data, but provide more stable estimates of  $r$  than methods based on the experimental mean.