Technical Note

GENERAL AUTOMATION LAB TECHNOLOGIES

Poisson Distribution of Cells in the Microwells of the Prospector™ System Arrays

A key step in the GALT Prospector system workflow is the loading of a bacterial suspension of interest on the GALT array at concentrations that will result in the capture of individual cells in the nanoliter-scale wells of the array. This Technical Note describes how the Poisson distribution can be used to predict the number of cells per well on the array and help target the right concentrations of bacteria to use.

The Prospector system is a high-throughput platform for microbiome analysis that is designed to isolate and cultivate microbes from complex samples. The system relies on the use of microscope slide-sized arrays containing more than 6000 nanoliter-sized microwells to capture individual cells from complex populations. Since, there are neither valves or channels, nor imaging or scattering to find cells in the array, this is done simply by applying a cell suspension of known concentration to the array. Every microwell is loaded similarly and randomly with a tiny aliquot of the total volume through a stochastic process. The cells will disperse in the microwells across the array following a Poisson distribution, which is a description of the number of random events that occur in a fixed time or space assuming they are independent of each other.

Here we describe how Poisson distribution can be used to calculate the optimal cell concentration for a desired array performance.

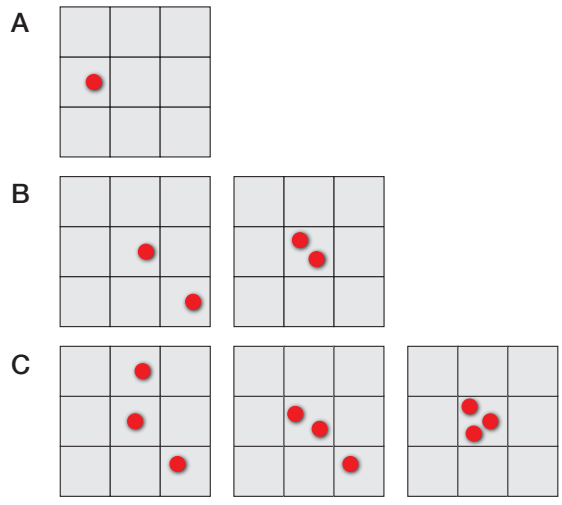

FIGURE 1: Distribution of 1, 2 and 3 cells in 9 wells.

Distribution of Cells in the Microwells of an Array

For a simple array of nine wells, if there is only one cell in enough volume to fill all nine wells then the loaded density is 1 cell / 9 wells or 0.11 cells per well (Fig. 1 A). The colony that grows up must be pure. If there are 2 cells / 9 wells or 0.22 cells per well (Fig. 1 B), once the array is loaded up, there are two possible outcomes: two wells containing a single cell each (good) or one well that contains both cells (bad). The two conditions do not occur with the same probability. The Poisson distribution is used to describe how likely one scenario is compared to the other. With an increasing number of cells, there are increasing numbers of possibilities. Three cells in nine wells can sort in many different ways (Fig. 1 C).

For a single well, the probability of a certain number of cells being in that well is described by:

$$P(n) = e^{-\lambda}\frac{\lambda^n}{n!}$$

Where λ is the average number of cells per well, *e* is Euler's constant, and *n* is the actual number of cells that end up in the well.

For nine wells and three cells, λ is 0.33 cells / well (3 cells / 9 wells). The probability of each event when the array is loaded is as follows:

Cells in Well	Probability
0	72%
1	24%
2	4%
3	0.4%

Most of the wells will end up with zero cells or one cell, which is what we want, but there are occasionally wells with two or more cells but the chance that this occurs is very low

compared with the zero cells or one cell events. However, there is still a 4% chance of getting more than one cell in a well. For some experiments that would be tolerable. If purity is a must, then we need to load fewer cells, in other words: a lower cell density.

A GALT array has 6109 microwells, so it is possible to load very dilute solutions and still have many 96-well plates' worth of isolates. For example, if we assume 10% of microwells are occupied, then λ is 0.1 cells / microwell leading to the probability of more than one cell per microwell occurring only 0.5% of the time. In this scenario, there are still 610 microwells likely to be occupied by one cell.

Higher loading densities might be advantageous for screening applications when the target is very rare. One might also target loading many cells per microwell in order to investigate community dynamics.

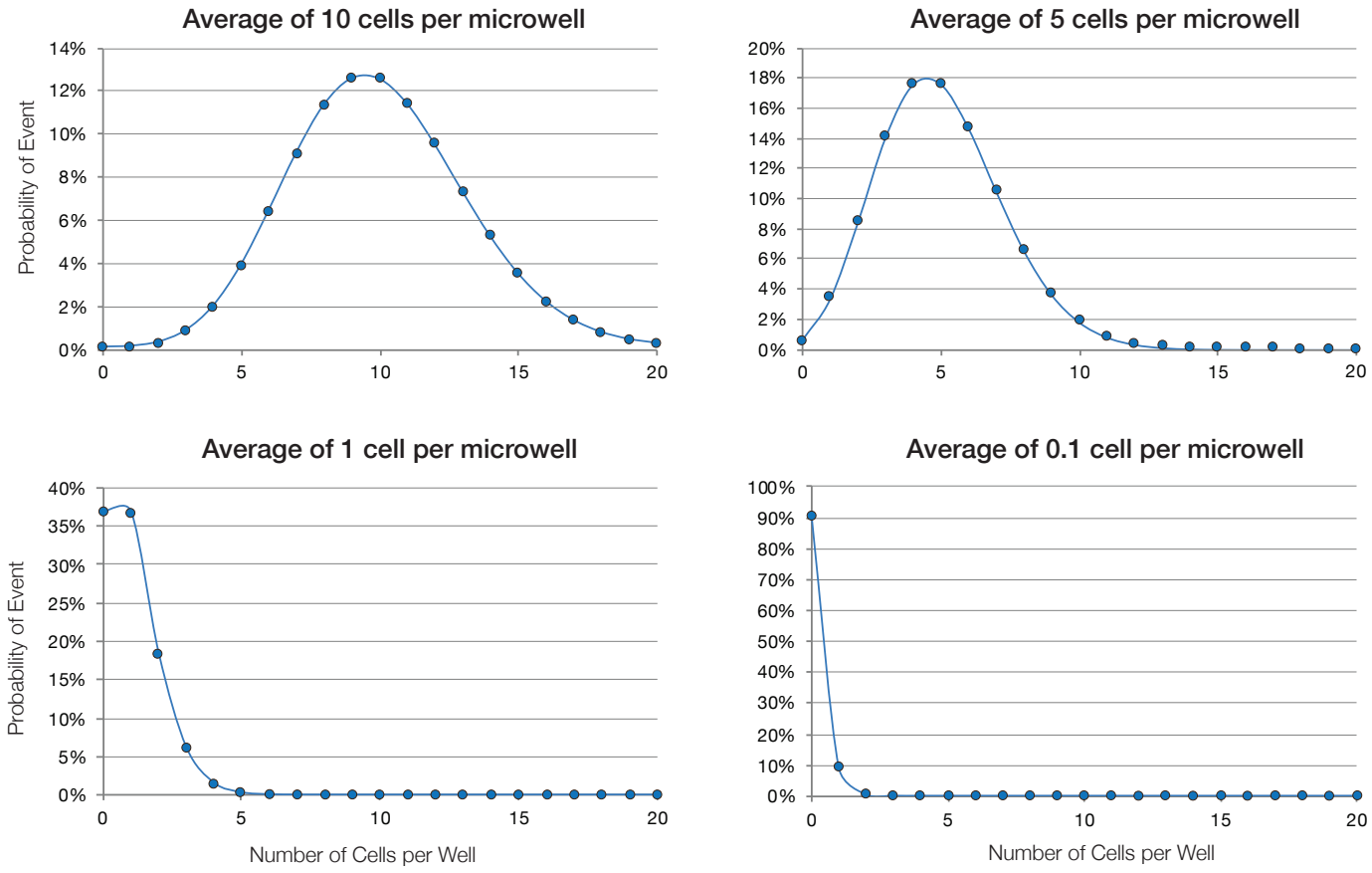

FIGURE 2: Poisson distribution of cells in the microwells of a GALT array at various loading concentrations.

Further Reading

- GALT Tech Note #TN3 *Determination of Cell Dilution Factor for Loading a Prospector™ Array*