

S1 File. The Poisson statistical analysis of dRPA results.

The Poisson equation:

$$p = (\lambda^k \cdot e^{-\lambda}) / k! \quad k=0, 1, 2, 3, \dots$$

where p is the probability of having k templates in a well given λ . λ is the average number of DNA templates per well. In the digital reaction, when the template number $k > 0$, the amplification reaction in the well will proceed, and its fluorescence intensity will increase, which is regarded as “positive” well; when the template number $k = 0$, there is no amplification reaction in the well, and its fluorescence intensity will not increase, which is regarded as “negative” well.

The equation simplifies to $\lambda = -\ln(1 - p)$ when $k > 0$. We can get the “measured” number of copies per well (cpw) λ_m by using poisson statistics, where $p = f/n$. f is the number of positive wells, detected by the optical setup through analyzing the increasing fluorescence intensity. n is the total number of wells on the chip.

The “expected” number of cpw is $\lambda_e = c_0 \cdot \nu \cdot x_{dil}$, and c_0 is the stock concentration of DNA templates, ν is the volume of each chamber, and x_{dil} is the dilution factor. Therefore, we can assess the performance of dRPA method by comparing the correlation between the “measured” cpw and the “expected” cpw.