Digital PCR on a SlipChip

Feng Shen, Wenbin Du, Jason E. Kreutz, Alice Fok, and Rustem F. Ismagilov

Department of Chemistry and Institute for Biophysical Dynamics, The University of Chicago,

929 E 57th St, Chicago, Illinois 60637

Derivation of Equation 1 to estimate template concentration:

The Poisson equation (Equation S1),

$$p = \left(\lambda^k \cdot e^{-\lambda}\right)\!/k! \tag{S1}$$

simplifies to $p = e^{-\lambda}$ when $k=0$, where p is the probability of having k particles in a well given λ, the average concentration of particles per well. The average concentration is different at each dilution factor, but λ can be found using the expression $\lambda = c*x$, where x is the dilution factor of the original concentration, c. Using s/n as an estimator of p, where s is the number of negative wells, f is the number of positive wells, and n is the total number of wells (so $s = n - f$), then Equation S2 is generated:

$$\frac{(n-f)}{n} = e^{-c \cdot x} \tag{S2}$$

This equation can be linearized by taking the natural logarithm (ln) of both sides to give Equation S1 (also Equation 1 in the main text)

$$Y = \ln(n-f) = -c \cdot x + \ln n \tag{S3, 1}$$

The y-intercept then gives the number of wells, so can be used to test the suitability of the equation, or used to constrain the fit based on the known n. The slope then gives the negative concentration in terms of particles/well.

Table S1. Number of positive wells in each quadrant of the SlipChip for various concentrations of genomic DNA and agreement with the 95% CI.

	Number of positive wells in quadrant				Average # per quadrant	Score based 95% CI range		Rounded 95% CI	
1 fg/µL	Upper Left	Upper Right	Lower Left	Lower Right		Lower limit	Upper limit	Lower limit	Upper limit
Trial 1	0	1	1	1	0.75	0.11	5.17	0	5
Trial 2	0	2	0	0	0.50	0.05	4.73	0	5
Trial 3	0	0	1	0	0.25	0.01	4.28	0	4
Trial 4	0	0	1	1	0.50	0.05	4.73	0	5
10 fg/µL									
Trial 1	3	5	5	4	4.25	1.70	10.49	2	10
Trial 2	5	4	3	0*	3.00	1.02	8.70	1	9
Trial 3	5	3	3	0*	2.75	0.90	8.33	1	8
100 fg/µL									
Trial 1	24	21	22	16	20.75	13.66	31.14	14	31
Trial 2	18	22	19	26	21.25	14.06	31.73	14	32
Trial 3	19	13	15	24	17.75	11.28	27.59	11	28
1 pg/µL									
Trial 1	154	159	159	151	155.75	138.38	173.22	138	173
Trial 2	151	145	135	140	142.75	125.63	160.28	126	160
Trial 3	131	140	152	148	142.75	125.63	160.28	126	160

*These values fell outside the CI.