

### Supplementary Note 1.

Under the same conditions, the fluorescence intensity ratio  $\lambda$  between each individual green ( $g$ ) and red ( $r$ ) dye is constant ( $\lambda = g/r$ ). The green and red fluorescence intensities ( $G$  and  $R$ , respectively) of a microbead bound with  $n$  nanobarcodes are calculated by formulas  $G = n \times (a \times g)$  and  $R = n \times (b \times r)$ , respectively. The total fluorescence intensity ratio ( $K$ ) between red and green fluorophores ( $K = R/G$ ) can be used to calculate the code number,  $a/b$ , using the formula  $a/b = 1/(K\lambda)$ . When  $G$  and  $R$  are measured using flow cytometry,  $K$  can be calculated with the equation:  $\log(G) = \log(R) - \log(K)$ , where  $\log(K)$  is the intercept of a two-color (green-red) flow plot. Thus the constant  $\lambda$  can be calculated with one known nanobarcode as a reference ( $a_{ref}/b_{ref}$  is known) using the formula:  $\lambda = (1/K_{ref}) \times (b_{ref}/a_{ref})$ . Once  $\lambda$  is determined, the code number of other nanobarcodes,  $a/b$ , can be obtained with the equation:  $a/b = 1/(K\lambda) = (K_{ref}/K) \times (a_{ref}/b_{ref})$ , where  $K$  is derived from the flow plot. In our experiment, a known target DNA from *Francisella tularensis*, coded by 2G1R (i.e.,  $a_{ref}/b_{ref} = 2$ ), was used as a reference to determine  $\lambda$ , as shown in **Figure 4a**. The measured value of  $K_{ref}$  (i.e.  $K_{2G1R}$ ) from **Figure 4a** was 22 and thus  $\lambda$  was equal to  $1/44$  ( $= 1/22 \times 1/2$ ). Based on the equation,  $a/b = 1/(K\lambda)$ , the code number,  $a/b$ , for any other nanobarcodes, was equal to  $44/K$ . Since  $K$  can be measured for each nanobarcode by the flow plot, the code number can be determined.